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Metabolome characterization of the *FTO* genotype (rs1421085) in primary human preadipocytes of male donors across differentiation

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“Things happen for reasons.”

(Aristotle)

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Abbreviations

ACACA	Acetyl-CoA carboxylase alpha
ACSL1	Acyl-CoA synthetase long chain family member 1
ALDH7A1	Aldehyde dehydrogenase 7 family member A1
BMI	Body mass index
DI-FT-ICR MS	Direct infusion Fourier transform ion cyclotron mass spectrometry
DB	Data base
EC	Enzyme
EtOH	Ethanol
FCS	Fetal calf serum
FTO	Fat mass and obesity-associated
GLYAT	Glycine-N-acyltransferase
HADHA	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha
MeOH	Methanol
NOS2	Nitric oxide synthase 2
NOS3	Nitric oxide synthase 3
PACs	Preadipocytes
PARP1	Poly(ADP-ribose) polymerase 1
SLC27A5	Solute carrier family 27 member 5
SMPD1	Sphingomyelin phosphodiesterase 1
SNP	Single nucleotide polymorphism
TNFAIP3	Tumor necrosis factor alpha induced protein 3
TYMS	Thymidylate synthetase
T2D	Type 2 diabetes

I. Abstract

1. English

Globally, the obesity epidemic reached an appalling number of 650 million people in 2016. Adiposity contributes to non-communicable diseases such as type 2 diabetes and is the consequence of a positive energy balance. Even though unfavorable lifestyle changes have propelled the obesity epidemic, the genetic predisposition is an important parameter since some individuals are prone to develop obesity. Single nucleotide polymorphisms (SNPs) in the first intron of the Fat mass and obesity-associated gene (*FTO*) have been discovered by genome-wide association studies (GWAS) being linked to obesity-related characteristics. The discovery of the causal variant rs1421085 has provided novel insight into potential underlying mechanisms for the association of the *FTO* locus with obesity. Recently, the rs1421085 *FTO* regulatory circuitry in vivo at the whole organism level was shown to be dependent on nutritional challenges in males. A steroid metabotype as well as mitochondrial dysfunction and consequent increased lipid storage have been associated with this specific *FTO* variant. In order to further investigate the effect of this variant, the main aim of this work was the establishment of a cell culture protocol suiting the highly sensitive DI-FT-ICR MS and performing cell culture experiments to differentiate primary human preadipocytes (PACs) from male donors with an homozygous rs1421085 *FTO* genotype (CC vs. TT) to elucidate metabolite patterns in order to unveil the metabolic pathways connected to the genetically determined metabotype. I describe a complex picture by investigating cell lysates and corresponding media during differentiation by means of ultra-high-resolution metabolome analysis. Moreover, I confirm an rs1421085-specific related glycerolipid- and steroid metabotype which has been described within a male population following nutritional challenges. Additionally, I link the rs1421085 *FTO* genotype to a stagnation of the mitochondrial beta oxidation within the early differentiation phase. The results and the established cell culture protocol provide a technical basis for further in-depth analyses (e.g. multiomics) as well as replications with a bigger male cohort in order to better understand the altered genotype-dependent metabolic pathways.

2. German

Weltweit erreichte die Adipositas-Epidemie 2016 erschreckende 650 Millionen. Adipositas trägt zu Krankheiten wie Typ-2-Diabetes bei und ist die Folge einer positiven Energiebilanz. Obwohl ungünstige Lebensstilveränderungen die Adipositas-Epidemie antreiben, ist die genetische Veranlagung eine wichtige Determinante, da Personen unter gleichen Lebensbedingungen ein unterschiedliches Risiko zur Fettleibigkeit aufweisen. Einzelnukleotid-Polymorphismen im ersten Intron des *FTO* Gens wurden in genomweiten Assoziationsstudien mit Adipositasmerkmalen in Verbindung gebracht. Die Entdeckung der kausalen Variante rs1421085 hat neue Einblicke in mögliche zugrunde liegende Mechanismen für die Assoziation des *FTO*-Lokus mit Adipositas gebracht. Kürzlich wurde gezeigt, dass die Effekte der rs1421085 *FTO*-Variante in vivo auf der Ebene des gesamten Organismus von ernährungsphysiologischen Belastungen bei Männern abhängig sind. Ein Steroid-Metabotyp sowie mitochondriale Dysfunktion und daraus resultierende erhöhte Lipidspeicherung waren mit dieser *FTO*-Variante assoziiert. Daher war das Ziel der Arbeit die Etablierung eines Zellkulturprotokolls angepasst an die hochsensitive DI-FT-ICR MS und die Durchführung von Differenzierungsexperimenten an primären humanen PACs von männlichen Spendern mit einem homozygoten rs1421085 *FTO*-Genotyp (CC vs. TT) zur Aufklärung von Metabolitenmustern, um die mit dem genetisch bestimmten Metabotyp verbundenen Stoffwechselwege aufzudecken. Ich beschreibe ein komplexes Bild, indem Zelllysate und Medien während der Differenzierung mittels ultrahochauflösender Metabolomanalyse untersucht werden. Darüber hinaus bestätige ich einen rs1421085-spezifischen Glycerolipid- und Steroid-Metabotypen, der innerhalb unserer männlichen Population nach Ernährungsbelastung gefunden wurde. Zusätzlich verknüpfe ich den rs1421085 *FTO*-Genotyp mit einer verringerten mitochondrialen Beta-Oxidation während der frühen Phase der Differenzierung. Die Ergebnisse und das etablierte Zellkulturprotokoll bieten eine technische Grundlage für weitere Analysen (z.B. Multiomics) sowie Replikationen mit einer größeren männlichen Kohorte, um die genotyp-abhängig veränderten Stoffwechselwege besser zu verstehen.

II. Introduction

1. Obesity and type 2 diabetes

a. Background

Obesity carries on being a public health concern worldwide, as it is associated with an elevated risk of multiple non-communicable diseases such as type 2 diabetes (T2D), cardiovascular diseases (CVD), and hypertension **[1-4]**. The number of patients with obesity almost tripled between 1975 and 2016 **[4]**. Across the globe, 39 % of adults (18 years and older) were overweight in 2016, and 13% were obese. According to Y.C. Chooi et al., America and Europe have the highest prevalence of overweight and obesity. In America, an increase was noticed for obesity from 12.9 % (1980) to 28.3 % (2015), while the countries US and Mexico have the highest rates. In the European population, the prevalence of overweight increased from 48 % in 1980 to 59.6 % in 2015, whereas obesity elevated from 14.5 % to 22.9 % during the same time frame **[5]**. In Germany, two-thirds of the male population (67 %) and half of the women (53 %) are overweight. Moreover, a quarter of German adults (23 % males, 24 % females) are obese **[6]**. The World Health Organization (WHO) defined overweight and obesity as "abnormal or excessive fat accumulation that may impair health" **[4]**. The Body Mass Index (BMI) formula (kg/m^2) is usually used to define adults as overweight (BMI ≥ 25 kg/m^2) and obese (BMI ≥ 30 kg/m^2). Further classification of obesity by BMI has been applied as described in Table 1 **[7]**.

Table 1: Obesity classifications by WHO based on BMI values [kg/m²].

BMI value [kg/m^2]	Obesity classifications
30 to 34.9	obesity class I
35 to 39.9	obesity class II
≥ 40	obesity class III

The BMI is widely used to categorize populations during health screenings, even though it is not a true criterion for an individual's adiposity **[3, 4]**. Therefore, waist circumference is another

parameter that includes subcutaneous (SAT) and visceral adipose tissue (VAT), but also non-adipose organs such as skeletal muscle tissue. It is closely correlating with BMI, but is additionally adding and surpassing a prediction for disease risk **[3, 8]**. Indeed, waist circumference is more closely correlated with abdominal obesity which is metabolically more harmful **[9]**. In 2013, the American Heart Association (AHA), American College of Cardiology (ACC), and The Obesity Society (TOS) developed a guideline for the management of overweight and obesity. The guideline has suggested that waist circumference should be assessed in people with a BMI between 25 and 34.9 kg/m^2 to improve risk prediction, with waist circumference levels of > 88 cm and > 102 cm considered to be elevated in females and males, respectively **[10]**. The metabolic syndrome (MetSyn) can be developed through a combination of certain risk factors: visceral obesity, high blood pressure, increased fasting glucose as well as serum triglycerides, and low HDL cholesterol. It is known as the most common cause of cardiovascular diseases and T2D and is associated with morbidity as well as mortality risk. An accumulation of MetSyn risk factors has been found among subjects with glucose intolerance as well as T2D **[11]**.

Obesity is a multifactorial disease **[2, 8]** resulting from a chronic energy imbalance, where energy intake surpasses energy expenditure (EE) over an extended period **[5, 12]**. Total EE is composed of basal metabolic rate (BMR) as the main contributor accounting for 60 -75 %, the thermic effect of food (TEF) with 8 -12 %, and the most variable factor “activity EE" with 10 - 30 % of total energy expenditure **[12]**. The development of the chronic disease “obesity” is multi-factorial, including parameters such as the environment, socio-cultural conditions, physiology, medical care, behavior, epigenetic, genetic, as well as many others leading to weight gain and endurance **[2, 8, 13]**. Although the main constituent and promoter of the obesity pandemic can be attributed to the food industry advertising for energy-dense and poor/inadequate-nutrient foods and, therefore, encouraging passive overconsumption **[14].** Nevertheless, a shift from physical activity to a sedentary lifestyle in the modern world also plays an important role **[15]**. The surplus of energy is metabolized to triglycerides and stored

in the adipose tissue enlarging its dimensions, whereas increasing body fat causes weight gain **[5]**.

b. Management and treatment of patients with obesity

The therapy of the multifactorial and non-communicable disease “obesity” is demanding. Non-invasive treatments have a general low weight loss effect and only small success in terms of long-term outcomes. Moreover, aiming for weight maintenance seems to be challenging as well **[16]**.

i. Non-invasive interventions

The main focus of conservative treatments is behavioral changes.

Lifestyle intervention

In general, weight loss should be the number one recommendation for patients with obesity and overweight patients with comorbidities such as prediabetes, diabetes, dyslipidemia, and hypertension. An initial weight loss of 5 -10 % over a time frame of 6 months should be targeted for such patients **[10]**. Therefore, guidelines for the management of patients with obesity, therefore, focus on lifestyle interventions that involve dietary improvements, higher physical activity, and general behavioral modifications **[3]**. Besides a remarkable weight loss (5 -10 %), a broad and intensive lifestyle intervention with at least 14 personal visits within 6 months is necessary. In-person sessions have seemed to be most efficient compared to electronic as well as phone-based treatments **[10]**. All in all, the most coherent parameter that anticipates a successful long-term weight loss is the value of initial weight loss **[17]**. The Diabetes Prevention Program (DPP) study, a randomized controlled trial (RCT), has included 3,234 participants with prediabetes. The cohort has been randomized to the intensive lifestyle intervention (ILI)-, metformin-, or placebo group, demonstrating a reduction of 58 % (ILI group) and 31 % (metformin group) reduction of diabetes incidence compared to the control group **[18]**. Moreover, the study team has investigated the long-term effects after 10 years of follow-up. The diabetes incidence in the ILI group was reduced by 34 % and by 18 % for the metformin

group compared to the placebo group. Significantly, the ILI group has regained weight underlining the importance of losing as well as maintaining weight for patients at risk for obesity-related comorbidities such as diabetes **[19]**. Recently, the prediabetes lifestyle intervention study (PLIS) has reported that within the high-risk group an intensified LI has been superior to conventional LI. Moreover, during the 3 years' follow-up chances of reaching regular glucose tolerance has been higher within the ILI group as well **[20]**. If patients require further interventions, the following possibilities are available: pharmacotherapy, medical devices, and bariatric surgery **[3]**.

Pharmacotherapy

Especially for patients who have failed to benefit from lifestyle intervention and those who have not been able to maintain weight loss over a long period, pharmacotherapy is the next step **[3]**. Five medications have been approved for weight management in the US, whereas only 3 of them, namely orlistat, liraglutide, and naltrexone/bupropion have been approved in the European Union **[21]**. Besides, Amfepramon, a sympathomimetic agent, is explicitly approved for a short-term treatment only **[22]**.

All in all, obesity pharmacotherapies are supposed to be adjunct and supportive treatment options for patients to facilitate their long-term behavioral change that can improve cardiometabolic health **[16]**.

ii. Invasive interventions

In comparison to the conservative treatments of obesity, invasive interventions seem to be most potent **[16]**. Medical devices for the treatment of obesity are a new trend and considered as less invasive than bariatric surgery and potentially reversible. Those techniques are categorized into gastric- and duodenal therapies **[23]**. Bariatric surgery, an invasive intervention, has received popularity in the past decades due to the outstanding results regarding weight loss and improvements of obesity-related comorbidities compared to non-invasive interventions **[3, 24]**. But such a step will only be considered for patients who have

failed to lose weight with the conservative treatments. Criteria for bariatric surgery that patients should meet are: BMI ≥ 40 or BMI ≥ 35 kg/m^2 and severe obesity-related comorbid conditions. Patients with a BMI of 50 kg/m^2 and higher represent a primary indication for bariatric surgery without previous non-invasive intervention attempts. Moreover, primary indications for surgery are also applied to patients with severe comorbidities and obesity-related complications or those with low success chances regarding non-invasive interventions. More details are listed in the S3 national guideline from 2018 **[25]**. In the beginning, when the BMI has been the only determining parameter for the indication of surgical therapy, weight-related comorbidities, especially T2D, diminished distinctively after obesity surgery **[26]**. Studies with long-term follow-ups such as the "Swedish Obese Subjects" (SOS) trial have confirmed postoperative effects on T2D and reduction in cardiovascular-related deaths **[27]**. During the 2nd Diabetes Surgery Summit (DSS-II) conference, international diabetes organizations compiled global guidelines for metabolic surgery in the treatment algorithm of T2D. Metabolic surgery has focused on the therapy of metabolic-related comorbidities in addition to weight reduction **[28]**.

All in all, the development of obesity surgery in Germany has taken place slowly but well-structured. This is indicated by very low surgery-related complications and mortality rates of < 0.5 % **[29]**. The restrictive approach of the health insurance companies has delayed an adequate development of surgical treatments.

c. Health care costs

Global data have shown that a high BMI has been the cause for 4.0 million deaths in 2015, whereas 40 % of the people were not considered obese, but overweight. Moreover, cardiovascular diseases have accounted for more than two-thirds of the deaths related to high BMI **[30]**. Total health care expenses treating obesity-related ailments in adults (≥ 18 years) increased from 20.6 % in 2005 to 27.5 % in 2010 and 28.2 % in 2013 in the USA **[31]**. The main focus of the high spending on medical care for obesity is accounted for people with a BMI of 35 and higher (obesity level II), since quick rises in costs are statistically expected. Similar findings have been reported in Germany, where mean costs for general practitioners have

been risen from 78 EUR to 126 EUR (63 % increase) and 168 EUR (116 % increase) for individuals categorized as average weight, obese class II (BMI ≥ 35 - < 40 kg/m^2) and obese class III (BMI≥ 40 kg/m^2), respectively. Total annual direct medical expenses have risen with higher BMI and have been significantly lower in the regular weight class compared to the obesity group. For people considered as obese class II and obese class III, the total direct spendings have increased by 46 % and 104 % compared to the normal-weight group, respectively **[32]**.

In summary, it can be stated that the obesity pandemic is causing a burden in many perspectives. Therefore, it is necessary to investigate further in prevention and treatment of obesity focusing on the morbidly obese groups and its comorbidities.

2. Genetics

a. Background

Obesity is a multifactorial disease that is influenced by social and environmental determinants which have been mostly underrated **[33]**. Adverse lifestyle changes boosted the obesity epidemic, however the genetic predisposition is important, since some individuals under specific environmental influences tend to develop obesity **[34]**. In general, the heritability in obesity accounts for 40-70 % **[35]**. From 1999 until 2019 a 4-fold increase in the discovery of rare monogenic disease-causing genes took place due to novel high-throughput techniques and sequenced genomes of various citizens across the globe **[36]**. The achievement of the first human genome sequencing in 2000 boosted to overcome barriers in modern human genetics **[37]**. A growing uncovering of the genetic basis of rare diseases has supported the shift to genome-wide sequencing of larger populations with various phenotypes that has identified new diseases with more distinct clinical displaying **[38, 39]**. However, basically all non-communicable diseases with high frequencies in a population (e.g. obesity, T2D, cardiovascular diseases) are a) of polygenic nature and b) variants are frequently located in the noncoding areas of the genome **[40]**.

All in all, the detection of more than 60,000 genetic associations with human diseases and traits have been boosted by worldwide attempts of GWAS and sequencing investigations **[36, 41]**.

b. Genetic approaches for the discovery of obesity-related genes

The first methods approaching susceptibility-gene discovery and herewith linking genotypes and phenotypes have been the candidate-gene approach and genome-wide linkage analysis. Gene analyses such as candidate-gene studies have been limited to one or more genes and concentrated thereby only on those candidates that have had possible associations with the traits of the disorder based on their common biological function **[42]**. The genome-wide linkage analysis has been an unbiased method to map human disease loci and investigating their main impacts coming from family-based cohorts **[43]**.

i. Genome-wide association studies (GWAS)

Genome-wide association studies (GWAS) have investigated the association of hundreds of thousands of genetic variants (mostly single-nucleotide-polymorphisms - SNPs) and disease-related phenotypic traits (cases vs. controls) **[42]**. Moreover, GWAS as an unbiased approach has based thereby on linkage disequilibrium (LD) defined as the dependence of loci frequencies at two or more genes **[44]**. In 2005, the first full genome-wide association study (GWAS) was performed **[45]**. Shortly afterwards, European GWAS have discovered associations of loci with BMI as well as severe obesity **[46-48]**. The first GWAS of type 2 diabetes have been reported by Sladek et al. **[49]**. Discovered risk variants have the potential as determining causal associations of characteristics and disease progression provoked by circulating biomarkers as well as environmental influences **[50]**. The disposability of big prospective cohort data has been a substantial improvement. The first biobank approach took place already in 1948 with the recruitment start of the Framingham Heart Study **[51]**. Recently, the UK Biobank involving 500,000 subjects and providing whole-genome genotyping as well as phenotypic information has shared their data with research groups worldwide as an open

resource **[52]**. The Genotype-Tissue Expression (GTEX) project needs to be mentioned in this context, as here genome data and RNA sequencing (RNA-Seq) is available for a multitude of tissues enabling for expression quantitative trait loci (eQTL) analysis and the investigation of whether a variant is driving the expression of a certain gene in a specific tissue **[53]**. Availability of vast phenotypic information due to biobank efforts has enabled the way for phenome-wide association studies (PheWAS) which are evaluating various phenotypes to a single genetic variant. J.C. Denny et al. have demonstrated pleiotropism, meaning that one gene influences multiple non-related traits in different tissues, of different variants connected to diverse characteristics **[54, 55]**. However, there are certain associations of obesity-related SNPs with T2D and Non-alcoholic Fatty Liver Disease (NAFLD) that have been foreseeable **[56]**. Current attempts try to elucidate the mechanisms by connecting cellular events with organismal physiology based on genetic divergence linked to proteomic and metabolomic findings **[57, 58]**. The single nucleotide polymorphism is the principle of monogenic non-syndromic obesity which is indicating early onset accompanied by eating disorders **[59]**. Genes involved in these rare monogenic forms of obesity are mostly linked to the leptin/melanocortin pathway that is localized in the hypothalamus and involved in energy intake and expenditure. Besides, the hormone leptin is produced and secreted primarily by WAT **[60, 61]**. An *FTO* gene mutation is among others causing monogenic obesity **[62]**.

c. *FTO* locus and functions

In 1999, the *Fto* locus was identified in a mouse model for the first time and termed “Fatso” due to its magnitude **[63]**. FTO (FTO = Alpha-Ketoglutarate Dependent Dioxygenase) is a nuclear protein of the AlkB related non-haem iron and 2-oxoglutarate-dependent oxygenase superfamily. The human *FTO* locus is located on chromosome 16q12.2 downstream of the Iroquois gene family including *IRX3* and *IRX5* **[64]**. It is expressed mainly in adipose tissues and skeletal muscles, but with the highest values in the brain (hypothalamus) **[65, 66]**. Intriguingly, Sobreira et al. have shown that *FTO* acts in a pleiotropic manner in brain and adipose tissue **[67]**. *FTO* is able to attach to several kinds of RNAs, including mRNA, snRNA,

and tRNA **[68]**. Nonetheless, the N6-methyladenosine (m6A) is *FTO's* most convenient nucleobase substrate **[69]**. Already in 2011, Jia et al. have discovered m6A in nuclear RNA as substantial for *FTO* **[70]**. As the first RNA demethylase, *FTO* has received a lot of attention and the functions of its proteins have been unveiled step by step since then. Karra et al. have discovered that *FTO* overexpression has resulted in enhanced energy intake by a simultaneous reduction in ghrelin mRNA m^{6}A levels **[71]**. Preadipocyte differentiation is regulated by *Fto* through modulation of m^{6}A levels near splice sites and thereby regulating the exonic splicing of RUNX1T1 (adipogenic factor) **[72]**. Wu et al. have analyzed the *Fto* regulation of adipogenesis through cell cycle proteins (CCNA2, CDK2) via the m^{6}A-YTHDF2 pathway **[73, 74]**. Autophagy has a crucial function in obesity and its development responding to environmental stress signals as well as nutrition **[75, 76]**. The mammalian target protein rapamycin (mTOR) is a serine/threonine-protein kinase which is a fundamental regulator in cell metabolism and mRNA translation. The mTOR complex 1 (mTORC1) is able to influence the process of obesity via direct engagement of downstream pathways or autophagy **[77]**. Gulati et al. have identified a role of *Fto* in the linkage of certain amino acids and mTORC1 signaling *in vivo* **[78]**. Among others, mTOR can facilitate the production of obesity-related factors (e.g. leptin) **[79]**. All in all, the *FTO* locus can influence the obesity process through the mTOR pathway **[64]**.

i. Association of *FTO* SNPs with obesity

In 2007, only 2 years after the first GWAS, the *FTO* gene was discovered by a GWAS as a locus linked to obesity-related characteristics **[80-82]**. Since then it has been termed as the "fat mass and obesity-associated" (*FTO*) gene. As the first obesity-related locus, it has gotten tremendous attention since then. Frayling et al. have demonstrated that the *FTO* variant (rs9939609) in the subgroup of homozygous risk carriers accounted for a 1.67 times increased chance of developing obesity and a surplus of about 3 pounds in body weight **[82]**. The importance of the *FTO* locus and its variants (rs1121980, rs1421085, rs9930506, rs8050136) within the first intron has been discovered by many GWAS studies in the European population

[80, 81, 83, 84]. Moreover, there is a clear connection between *FTO* risk allele carriers and their role in food consumption. Risk carriers tend to an enhanced energy intake accompanied by an inactive lifestyle and a preference for fat-rich food, whereas this often results in unhealthy supernutrition **[85-88]**. However, the risk of developing obesity in adulthood can be encountered by physical activity even in homozygous risk allele carriers **[89]**. An analysis across 96 BMI-related variants, has shown the highest association of BMI and fat mass for the *FTO* SNP (rs1558902) **[90]**.

ii. SNP rs1421085

The causal variant rs1421085 is a SNP located in the first intron of the *FTO* locus on chromosome 16. The minor allele frequency for the global population accounts for C = 0.39113 (ALFA) as well as C = 0.419704 (ALFA) for the European population, whereas the basepairs CC indicate homozygous risk allele carriers and the non-risk allele carriers are characterized by the basepairs TT **[91, 92].** Obesity and abdominal obesity in male risk carriers (rs1421085) is already manifested in childhood and continues through adulthood. The linkage is associated to a greater degree by differences in energy metabolism of adipocytes than lifestyle parameters **[93].** In 2015, Claussnitzer et al. elucidated a possible mechanistic base for the association of the *FTO* locus with obesity **[94]**. Milestones have been considered the identification of the causal variant (rs1421085) out of the 89 SNPs in intron 1 and 2 as well as its underlying functions in PACs. The variant rs1421085 hereby disrupts ARID5B repressor binding and this leads to a derepression of long distant targets *IRX3* and *IRX5* during early adipocyte differentiation. Moreover, it results in a cell-autonomous translation towards lipid-storing white adipocytes over energy-consuming beige adipocytes by a decrease in mitochondrial thermogenesis (by factor 5). Claussnitzer et al. have demonstrated that rescuing the ARID5B motif in risk allele carriers by CRISPR-Cas 9 editing reconditioned *IRX3* and *IRX5* repression and activated thermogenesis (by factor 7) **[94]**. Above all, this study has provided a model for deciphering non-coding variants elucidated by GWAS that can help to investigate and translate GWAS information in the near future **[95]**. Based on these evolutional findings, Laber and

Forcisi et al. have linked recently the *FTO* causal variant (rs1421085) to cellular, metabolic as well as organismal phenotypes in vivo across species **[96]**. They have been aiming to recapitulate the *FTO* (rs1421085) regulatory circuitry in vivo with the support of a mouse model indicating a mutation at the murine homologous area adjacent to the human rs1421085 variant. Claussnitzer et al. **[94]** have been reporting in human PACs *IRX3* and *IRX5* as long distant target genes of the rs1421085 cis-regulatory module (CRM) and this has been verified in murine preadipocytes by Laber and Forcisi et al. **[96].** Moreover, they unveiled an effect with metabolic implications, e.g. fat mass, and skin thickness in mice under high-fat diet requirements. In addition, Laber and Forcisi et al. have discovered an rs1421085 related decrease of steroids and their derivatives in rs1420185-DEL82 mice under High-fat-diet (HFD) conditions compared to controls using untargeted, ultra-high-resolution metabolome analysis. Besides, two independent human cohorts following an oral glucose challenge have shown a significant increase of the steroids compound class in male risk carriers compared to non-risk carriers in the immediate response (0 h -1 h) and a subsequent decrease in the short-term (1 h -2 h). Further compound classes have been discovered by untargeted metabolomics involved (among others) flavonoids and glycerolipids **[96]**. The mitochondrial characteristics (number of mitochondria, marker genes for thermogenesis and browning) in murine adipose tissue of rs1421085-DEL82 have been in line with the findings by Claussnitzer et al. in human white adipose tissue **[94]**.

3. Metabolomics

a. Background

The fast-developing field of metabolomics aims at a comprehensive measurement of metabolites in cells and body fluids. In 2018, the latest Human Metabolome Database (HMDB) version reported 106,151 metabolite entries and has consequently almost tripled within 5 years **[97]**. Metabolites are defined as small molecules (< 1,500 Da) which undergo metabolic processes and thereby experience chemical transformation. They display functional cell conditions and can provide a direct and distinctive biochemical state making it simpler to link them to phenotypic traits **[98]**. This

is due to their downstream location of genomics, transcriptomics, and proteomics, respectively, in the *omics* cascade **[99]**. Moreover, the endophenotype concept represents an approach that is based on analyzing characteristical differences that are inheritable and more likely associated with the origin of a disease than unspecific clinical phenotypes **[100, 101]**. Overall, the bright sector of system biology aims to give a global view on biological systems decoding molecular components and enabling biological pathways by taking advantage of high-throughput technologies. Developments in bioinformatics, as well as computational biology, have made the analysis of these enormous data sets feasible **[102, 103]**. Metabolomics has been proven as a valuable instrument that can elucidate changes in the metabolome at the cellular stage in consequence of environmental and genetic disruptions **[104]**. The metabolome is hereby defined as the entire collection of metabolites of different biochemical classes within a biological sample **[105]**. In the metabolomics field, there are two different approaches: targeted and untargeted. Targeted metabolomics focus on a defined selection of metabolites, in terms of determined compound classes (e.g. lipids, nucleic acids and peptides), which is a rather hypothesis-motivated strategy **[106]**.

On the contrary, untargeted metabolomics aims to analyze a global metabolic profile of the sample with an unbiased approach and herewith generating hypotheses **[98]**. Many analytical platforms are applied in metabolome analysis such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) which is commonly paired with other analytical separation methods (FT-MS, GC-MS, LC-MS, etc.). The basis of the NMR spectroscopy is the absorption of energy and the re-emission of atom nuclei resulting from an applied magnet field **[107]**. On the other hand, MS produces spectral data (mass-to-charge ratio m/z) and allows assumptions about the intensity level of analyzed compound classes **[108]**. The main difference between both technologies “NMR and MS” is attributed to sensitivity.

Table 2: Analytical techniques in metabolomics research **[107, 109-114]**.

NMR spectroscopy	Mass spectrometry		
	DI-FTICR MS	GC-MS	LC-MS
Advantages			
✓ Fast ✓ Highly reproducible ✓ Non-destructive	✓ Ultra-high resolution ✓ Highly sensitive ✓ High through-put ✓ Low sample volume ✓ Precise mass detection	✓ High resolution capacity ✓ Good sensitivity ✓ High mass accuracy	✓ High resolution ✓ Very sensitive ✓ Reproducible ✓ High mass accuracy
Disadvantages			
- Low sensitivity - Less than 200 metabolites definable - Higher sample volume	- High costs - Ion suppression - No identification of isomers - Destructive	- Derivatiza-tion required to volatile compounds - Poor quantification - Matrix effect	- Ion suppression - Compound detection more problematic (lower number of libraries) - High costs - Analytical variability

NMR can detect organic compounds within a micro-molar concentration and at the same time quantify. Moreover, samples can be utilized for additional analysis afterwards (non-destructive). But the main limitation of NMR in terms of comprehensive metabolite profiling is the sensitivity which is low compared to MS techniques **[115]**. Therefore, NMR is restricted in biomarker identification in terms of complex matrices and when compound concentrations are low. In general, when performing high-throughput profiling higher sample volumes need to be considered **[114]**. In high-throughput metabolomic studies, the MS approach has been mostly selected on account of its high sensitivity and can therefore study biofluid samples that are constituted by a vast molecular variety **[116]**. Another advantage is the high-resolution which enables assignment to molecular formulas **[106]**.

Samples can be investigated with MS applying either direct-infusion mode allowing to measure an enormous number of metabolites or coupled with subsequent chromatography separation (e.g. gas chromatography, liquid-) **[115]**. However, FT-ICR MS works with direct infusion and is not connected to any other methods which would lower the resolution **[117]**. On the one hand, gas-chromatography mass spectrometry (GC-MS) involves derivatization products in terms of volatile metabolites and therefore restricts the utilization in the field of metabolomics. On the other hand, liquid-chromatography mass spectrometry (LC-MS) is considered a reproducible and sensitive method with a high resolution. This powerful tool for analyzing sophisticated sample matrices received much attention in regards to preterm clinical diagnostics of diseases beyond obesity and Type 2 Diabetes **[118]**. Due to the enormous variety of compound classes within the metabolome, additional analytical platforms are supporting a good coverage of metabolites. Fourier transform ion cyclotron resonance MS (FT-ICR MS) allows ultrahigh-resolution metabolome analyses with precise mass detection **[119, 120]**. Already in 1974, the FT mass analyzer was described by Comisarow and Marshall **[121]**. A detailed primer of the principles and applications for the high resolution mass analyzer FTMS has been reported by Marshall's group in a review about 25 years later **[122]**. A huge advantage of this mass analyzer is the simultaneous measurement of compound classes over the whole mass spectrum without the necessity of any separate scans **[123]**. However, the biggest advantage is the high-resolution. The ion cyclotron resonance thereby supports an enormously sensitive and semiquantitative analysis of metabolites. In particular, the non-targeted metabolomics approach requires the detection of as many compounds as possible in a biological sample **[117]**. Intending to investigate the complex picture of the preadipocytes and its environment, we applied non-targeted metabolomics via direct-infusion-electrospray-ionization (DI-ESI) FT-ICR MS. FT-ICR MS had been the technique of choice due to its high resolution and the consequent precise assignment of molecular formulas and annotation of different compound classes. This analysis is semiquantitative and considered as a discovery approach, whereas based on these findings a hypothesis can be generated. The next step in the future will be the integration of different platforms such as LC-MS to distinguish isomers.

In summary, metabolomics is a promising domain with high expectations of novel biomarker discovery for preventive and personalized medicine. However, the way to eligible biomarkers in clinical diagnosis

and utilization is long and challenging **[124].** The major challenge is the assignment of metabolites to biological pathways and the mechanistic elucidation of the respective disease **[125]**.

b. Metabolomics studies in obesity and type 2 diabetes

Obesity is routinely identified and classified by the BMI (kg/m^2) as described in Chapter II.1.a. above. Admittedly, this rather simple measurement does not reflect the disturbances in the metabolism that are linked to insulin resistance in tissues such as adipose tissue **[126].** Besides, the hypertrophy of adipocytes, such as an increase in volume of existing adipocytes, as a consequence of e.g., excess nutrient supply, activates inflammatory signaling pathways caused by endoplasmic reticulum (ER) stress. Hereby explaining only one of the possible factors as a basis for metabolic disease, since it is highly multifactorial (mitochondrial dysfunction, insulin resistance etc.) and stem from various organelles and cell types **[127].**
Among patients with obesity, the classification into metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUHO), can be applied. Lower levels of inflammatory markers for MHO subjects occur partly from a more beneficial blood lipid profile **[128].** Metabolomics is thereby a supportive tool in elucidating the differences of MHO and MUHO in response to e.g., nutritional challenges **[129].** However, MHO is often only a transient state with most individuals becoming MUHO sooner or later **[130].** Generally, the increase in metabolomics studies related to obesity and Type 2 Diabetes in humans as well as in animal models has been tremendous in the past decade **[131].** A milestone has been declared by Suhre et al. with the first multi-platform approach of metabolome-wide analyses in order to identify novel biomarkers and classify diseases (e.g. T2D) at an early stage **[132].** In the course of this, particular metabolic signatures in terms of compound classes have been demonstrated in the blood profile of subjects with obesity and/or T2D **[133, 134].** As substantial compound classes of impairment had been reported, lipids in general as well as the amino acids (AA), in particular branched-chain amino acids (BCAA) **[134, 135].** Cirulli et al. have found mainly lipids and amino acids being associated with the BMI. BMI-related metabolite pattern changes have been discovered and linked inversely to phospholipids. Also, a lower level of the steroid metabolite

cortisone has been reported in individuals with obesity **[136].** The biosynthesis of steroids and their related metabolome in body fluids have been of interest for decades **[137].**

A report of four different German population-based studies indicating an overweight BMI for each cohort has shown reproducible links to the compound classes of amino acids and phospholipids **[138].** However, general disturbances in lipid metabolism have been discovered for both IR as well as obesity **[139].** Floegel et al. have shown various phospholipids being linked independently to the risk of developing T2D, whereas lower levels of glycerophospholipids have been associated with T2D as well **[140].** The KORA cohort has linked alterations in the lysophospholipid metabolism for morbid obese subjects to diabetes **[141, 142].** Moreover, patients with obesity and undergoing post-bariatric surgery as well as post-dietary treatment (hypocaloric diet) have indicated a downregulation in the class of glycerophospholipids **[143].** Overall, the majority of human metabolomics studies addressing obesity and Type 2 Diabetes have been focusing on analyzing body fluids **[134]**.

i. Metabolomics in adipocytes and adipose tissue

There are fewer metabolomics studies in the field of obesity and T2D focusing on adipose tissue and adipocytes, although a lot of insights have been discovered particularly concerning molecular differences in the white adipose tissue of MHO and MUHO subjects **[144, 145]**. Consequently, a direct comparison of subcutaneous and paired visceral adipose tissue with obesity state and insulin sensitivity have been of general interest **[146].** In addition, Otto et al. have linked the outcomes of both fat depots with different body fluids by applying untargeted metabolomics and thereby enabling the identification of pathways associated with metabolic diseases **[147].** Besides, Rampler et al. have been investigating the formation of lipid droplets by differentiating mesenchymal stem cells and comparing primary human preadipocytes with adipocytes via multi-omics (lipidome, metabolome, and proteome) **[148].** Indeed, adipocyte precursors represent an attractive model for studying the underlying mechanisms and finding possible therapeutic targets in the early differentiation state by assessing the metabolic footprint **[149]**. However, adipocyte culture systems have limitations especially in regards to

high glucose and/or lipid deprived (without n-6 and n-3 FAs) media conditions that can alter metabolic profiles **[150, 151]**. Higher glucose concentrations can e.g. influence mitochondrial function **[151]** and n-3/n-6 fatty acids affect different pathways (e.g. eicosanoid metabolism). Moreover, those are substantial components of cell membranes in general **[150]**. Therefore, all limitations and strengths should be considered ahead of planning a cell culture study.

c. Genetic-driven metabolome

The description of the metabolome based on the genetic architecture has been a promising field in terms of personalized medicine. In 2008, Gieger et al. reported the first genome-wide association (GWA) study including metabolic phenotypes (metabotypes*)* supporting a better understanding of disease pathogenesis in terms of involved biochemical pathways **[152].** Only 3 years later, Suhre et al. published a substantial analysis of genetically determined metabotypes (GDMS) via GWAS and untargeted metabolomics in a European population **[153]**. The awakened hope for clinical relevance by revealing multilayered phenotypic traits such as the pathophysiology of T2D is high, but at the same time challenging **[154]**. However, there is a great availability of high-throughput technologies for linking the genome to the metabolome, and the knowledge gained from the initial metabolic GWAS (mGWAS) can be applied **[155].** Different compound class signatures based on genetic determinants were detected in the blood profile **[156, 157]**. Besides, metabolites in the circulating blood have been linked to the obesity status and T2D based on the *FTO* genotype by Yeon-Jung et al. **[158]**.
Overall, the combination of both, GWAS and metabolomics, is a powerful tool in enabling new findings leading us further to personalized healthcare **[159]**.

4. Cell culture

a. Background

The loose connective adipose tissue (AT) by count comprises lipid-filled adipocytes (25%) besides many other cell types (75 %) originating from the stromal-vascular fraction (SVF) such as preadipocytes, mesenchymal stem cells, immune cells, fibroblasts, and vascular cells among others. All cell types are encompassed by blood capillaries and an innervation

meshwork. In adults, adipocytes can differ tremendously in size (20 -200 µm in diameter) **[160, 161].** The main purpose of adipocytes is to store fat in the form of triglycerides as lipid droplets during times of calorie surplus and to release that energy when needed. In total, 90 % of the adipocyte cell volume is occupied by the lipid droplet and the rest is dedicated to the nucleus as well as the cytoplasm at the periphery **[162].** Mature adipocytes are equipped with all necessary enzymes and regulatory proteins for performing lipolysis as well as de novo lipogenesis. During embryonic development, these abilities are obtained to prepare for the postnatal phase. However, the expansion of white adipocytes is taking place just shortly after birth **[163].** At the stage of preadipocytes, there is no remarkable storage of lipids yet and additionally, they are appearing under the microscope with a fibroblast-like shape. They can be differentiated into adipocytes and applied as in-vitro models for metabolic studies. However, the number of adipocyte precursors that are able to differentiation deviate between species, age of the donor, and sex **[164].** Freshly isolated human preadipocytes can be derived from different sites of adipose depots, the subcutaneous- (SAT) and visceral adipose tissue (VAT) which are divided by the peritoneum in terms of abdominal SAT. The VAT is situated intra-abdominally and is, therefore, directly "connected" to other organs e.g., through the portal vein to the liver **[165].** On the other hand, the SAT is located below the skin layers and therefore ideal to isolate the body from cold as well as cushioning from physical harm **[166].** SAT is also the main energy storage site in the form of lipids and exceeds the volume of VAT by multiple folds **[165].** Both sites have crucial differences in metabolic functioning in terms of secretory products, inflammation markers, lipolysis, and thermogenesis **[167].** Especially, it is worth mentioning that VAT expansion is usually more metabolically harmful than SAT expansion **[168].** Moreover, the SAT/VAT categorization of adipose tissue is very crude and variations within these depots might exist **[169].**

i. Cell types: white, brown, and beige

Three different types of adipocytes were described in the literature: white, brite or beige, and brown adipocytes. The classic and most prevalent type in humans is the white adipocytes

which purpose is storing energy in the form of triglycerides and mobilizing those by lipolysis as described above. The second subtype are called brite or beige adipocytes that are found scattered among the white adipose tissue, mostly subcutaneous **[170, 171]**. They can additionally perform energy expenditure via thermogenesis due to the rich number of mitochondria. However, the third class of "brown adipocytes" are mainly conducting the so-called adaptive non-shivering thermogenesis via uncoupling protein-1 (UCP1) in the mitochondria by burning fatty acids and glucose **[172].** Brown adipocytes are found mainly in the interscapular depot of infants and are located paraspinal as well as supraclavicular in adults **[167].** Overall, the BAT is highly innervated with the sympathetic nervous system (SNS), and therefore more commonly nerve tubes are directly connecting to brown adipocytes **[172].** Besides, these three classes vary in their origin as well as by morphological characteristics. Brown adipocytes derive from an MYF5+ lineage shared with skeletal muscle, whereas white and brite/beige adipocytes origin from mesenchymal precursors. White adipocytes mainly constitute a unilocular lipid droplet and have an overall spheric appearance. The nucleus is located between the lipid droplet and the cell membrane. There are additionally very few other organelles such as mitochondria, endoplasmic reticulum, and Golgi apparatus **[173]**. On the other hand, mature brown adipocytes are high in mitochondria with the corresponding rich expression of the proton transporter UCP1 **[174].** Moreover, they demonstrate small multilocular lipid droplets as well as appearing normally with an ellipsoid shape **[175].** The center is occupied by the nucleus in between the cytoplasm and including the other organelles. The PR domain containing 16 *(Prdm16)* occurs in both adipocytes: white as well as brown and is a key factor in lineage plasticity by converting white into beige adipocytes **[176]**. *PRDM16* is able to influence repressors: C-terminal-binding protein-1 *(CtBP-1)* as well as *CtBP-2* and herewith deactivating WAT-related genes and/or activating browning-related genes via peroxisome proliferator-activated receptor-ɣ (PPARɣ) coactivator-1α (*PGC-1α*) **[177].** For a transformation of white into beige is a β3-adrenergic stimulation, permanent *PPARɣ* induction or cold exposure necessary. Overall, *PRDM16* is considered as a stimulant sustaining a beige/brite adipocyte stage. However, UCP1 can conduct thermogenesis and is, therefore, the

hallmark component of heat production **[178]**. The proton transporter is located in the inner membrane of the mitochondria and is only found among beige and brown adipocytes. Activated UCP1 enables the proton (H^+) reflux from the intermembrane space into the matrix of the mitochondrion. Thereby, the proton-motive force is uncoupled from ATP synthesis, maximum mitochondrial respiration is stimulated and chemical energy is dissipated in the form of heat **[179, 180]**. However, there are genetic-based indications that variants at the *FTO* (fat mass and obesity-associated gene) locus influence the thermogenic potential. Claussnitzer et al. have demonstrated that with carrying the risk allele within the specific SNP, a shift from browning to whitening and therefore a downregulation in thermogenesis has been shown **[94].**

ii. Differentiation process of white adipocytes

Adipogenesis is the process of cell differentiation whenpreadipocytes become adipocytes. Multipotent mesenchymal precursors get obliged to follow the adipocyte lineage and do not undergo the path of myoblasts, chondroblasts, or osteoblasts **[167, 181]**. It has been demonstrated that bone morphogenetic protein (BMP) members 2 and 4 (BMP2 and BMP4) are adequate for *in-vitro* adipocyte differentiation **[182, 183]**. Activation of "Mothers Against Decapentaplegic Homolog 4" (*SMAD4*) transcription factors are accomplished by enabling its heterodimeric members via binding of BMPs and consequently, signaling through their receptors **[184]**. Then *SMAD4* can boost terminal differentiation by induction of the key modulator of adipogenesis, the *PPARɣ*. Independently, the zinc-finger protease ZFP423 has been demonstrated as an important determination factor in white adipocytes **[185].** However, *PPARɣ* is essential for adipogenic differentiation *in-vitro* as well as *in-vivo* and considered as the "master regulator" of adipocyte differentiation **[186, 187].** It has been proven that the synchronic induction of dexamethasone's canonical pathways, through the glucocorticoid receptor and CCAAT-enhancer-binding proteins (*C/EBPs*) as well as rosiglitazone via *PPARɣ* is sufficient and even indispensable for inducing human mesenchymal stem cells (hMSC) adipocyte differentiation **[188]**. Overall, *PPARɣ's* downstream effect of inducing the transcription factor *C/EBPα* belongs to the most crucial events **[189]**. However, a complete

adipocyte differentiation program into mature adipocytes is only taking place when both are collaborating. The adipocyte differentiation can be regulated by endocrine hormones such as the class of steroids. Some steroid hormones, specifically estrogen and androgen classes, have been shown to mainly impact adipogenesis negatively. In contrast, the other classification of steroids "glucocorticoids" is boosting adipocyte differentiation **[173, 190]**.

b. Obesity: mechanistic and morphological alterations in adipocytes and adipose tissue

In humans, PACs develop during fetal development, whereas the number of adipocytes increases during early childhood. The proliferation potential diminishes when puberty is reached, whereas it stays stable during adulthood. Despite the fact that adipocyte number is mainly set during childhood, this does not mean that there is not any turnover and renewal in adulthood. Approximately, 10 % of the adipocyte population is renewed yearly **[191]**. However, from then adipose tissue mainly enlarges by growing in adipocyte size (hypertrophy) **[192]**. Obesity and T2D are linked to hypertrophy in adipose tissue based on a decreased adipogenesis capacity **[193]**. In particular, a larger adipocyte size in patients with obesity is accompanied by mitochondrial dysfunction, alterations in proteins within the cell membrane, increased inflammation processes, and cell death. All in all, these processes are related to metabolic disorders but humans who enlarge adipose tissue mainly by hyperplasia are metabolically favorable **[194]**. However, the capacity of proliferation and differentiation in the subcutaneous depot decreases with increasing age and/or obesity stage **[195]**.

i. Inflammation and insulin resistance

Recent research elucidates underlying disturbances in human adipose tissue. Chronic low-grade inflammation is induced by lipids and adipokines which are generally causing mitochondrial disorders, producing reactive oxygen species (ROS), and leading to cell death, inflammation as well as a disruption of metabolic homeostasis **[196]**. Excess nutrient supply in terms of obesity leads to adipocyte hypertrophy demonstrating a higher accumulation of triglycerides and elevated lipid droplet size. Already early hypertrophic adipocytes show an

altered mitochondrial oxygen consumption and oxidant production **[197]**. Thereby, the overloaded tricarboxylic acid (TCA) cycle and mitochondrial respiratory chain results in an increased ROS production leading to oxidative stress which in turn promotes inflammation **[198]**. ROS involving free oxygen and superoxides with unpaired electrons contribute to oxidative stress. The abnormal mitochondrial function causes the above-mentioned higher amounts of ROS and can at a certain point transcend the buffer limit of the cell. All together instigates the process of oxidative damage which is targeting DNA, RNA, amino acids as well as oxidized lipids. Oxidative damage to mitochondrial electron-transport-chain constituents in adipocytes causes inadequate production of ATP and metabolic failures **[199]**. However, ROS produced by mitochondria have also important purposes in eukaryotic cells for cellular signaling and maintaining function as e.g. the proliferation process **[200]**. Indeed, crosstalks have been described between adipogenesis, ROS, and insulin signaling at a molecular stage. The insulin signaling cascade produces ROS via NADPH oxidase (NOX4) which is blocking an intracellular protein phosphatase that indicates inhibitory effects on the pathways related to insulin **[201]**. The feature of ROS is hereby linked to an increase in insulin signaling and enhanced adipogenesis. One sequela of low-grade inflammation in the AT is considered to be insulin resistance **[173]**. For decades, macrophages are known as the major players in the adipose tissue inflammation processes and their content correlates with BMI as well as adipocyte size. They are considered as a key source for inflammatory factors **[202]**. There are two macrophage groups: the classical M1 and alternating enabled M2 macrophages. The products of the subgroup M1 are pro-inflammatory cytokines (e.g., TNF-α, interleukin IL-6 and MCP-1) that induce insulin resistance. In contrast, M2 macrophages generate anti-inflammatory cytokines that are predominantly available in fat tissues of lean subjects and support the preservation of tissue homeostasis **[203]**. However, there are also pro-inflammatory cytokines that improve insulin sensitivity (as e.g. IL-33) within the adipose tissue **[204]**. In addition, macrophages have housekeeping tasks and take care of the removal of dead adipocytes thereby forming characteristic “crown like structures” **[205]**. Besides the mentioned adipocyte size as a causal factor for macrophage recruitment in white adipose

tissue, clear depot-specific differences have been presented **[206]**. However, also during phases of caloric restriction such as in patients with anorexia nervosa, AT inflammation is occurring **[207]**. Overall, fast changes in adipose mass can cause inflammation processes **[208]**.

5. Aim

a. Thesis

The aim of the Thesis comprises 3 central goals.

- Firstly, the establishment of a preadipocytes (PACs) differentiation protocol suiting DI-FT-ICR MS.
- Secondly, it has been important to assess the optimal number of cells for the ultra-high-resolution metabolome analysis of cell lysates and corresponding media due to the limited highly valuable human biomaterial.
- Lastly, the accomplishment of cell culture experiments with preadipocytes from risk- (CC) and non-risk (TT) allele carriers (rs1421085) as a result of the 2 achieved goals above.

b. Study

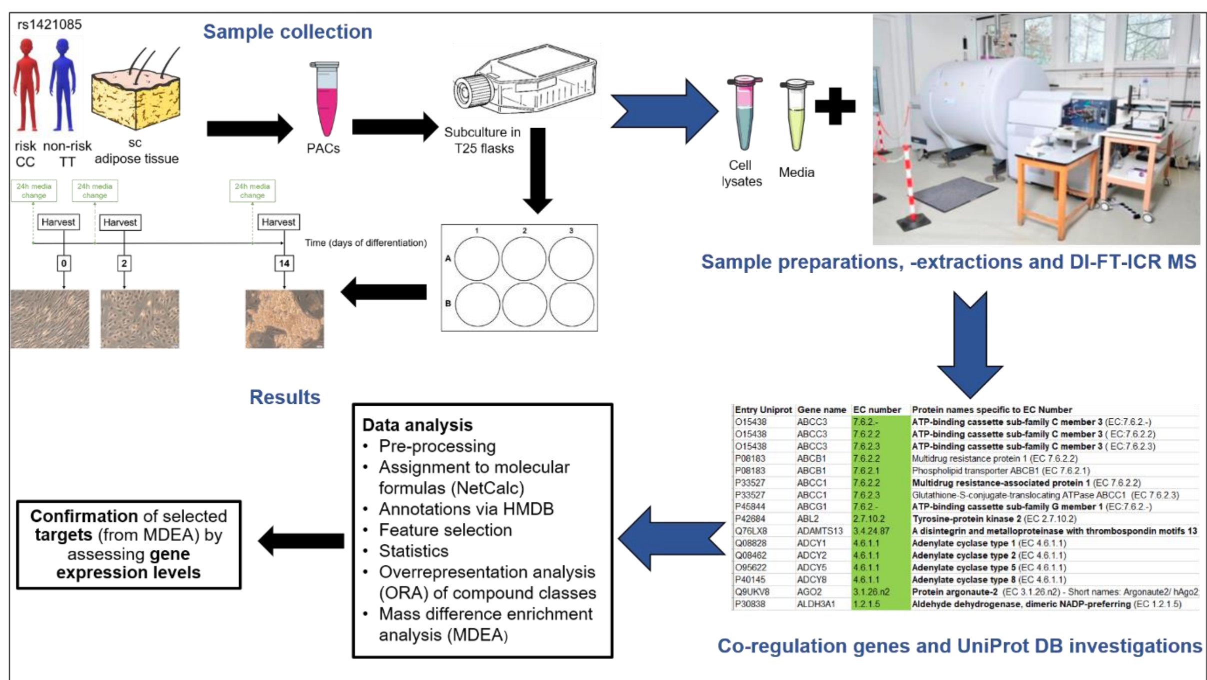
The overall aim of the study was the elucidation of the metabolic pathways of primary human PACs in male subjects over phases of differentiation with a homozygous *FTO* genotype (rs1421085 CC vs. TT) and linking these insights to our findings highlighted in the previous studies across species (mice and humans). Besides steroid metabolic patterns, a mitochondrial disruption and a subsequent increased fat accumulation were associated with this specific *FTO* variant **[96]**.

In order to further explore the involved biochemical pathways, we addressed the following issues by additional experimental studies with in-vitro differentiated primary human PACs to:

- Reveal which genes play a major role (as well as their encoded enzymes) integrating gene-driven mass-difference enrichment analysis (MDEA) with the main pathways shown in the previous study (mitochondrial function and lipid storage) **[96]**.

- Focus on the PACs differentiation days (day 0, day 2 and day 14) as emphasized by the main outcomes demonstrated by Claussnitzer et al. **[94]**.

- Recapitulate this study in male individuals homozygous for the equal *FTO* SNP (rs1421085) analyzing cell lysates and corresponding media via DI-FT-ICR MS.



Entry Uniprot	Gene name	EC number	Protein names specific to EC Number
O15438	ABCC3	7.6.2.-	**ATP-binding cassette sub-family C member 3** (EC:7.6.2.-)
O15438	ABCC3	7.6.2.2	**ATP-binding cassette sub-family C member 3** (EC:7.6.2.2)
O15438	ABCC3	7.6.2.3	**ATP-binding cassette sub-family C member 3** (EC:7.6.2.3)
P08183	ABCB1	7.6.2.2	Multidrug resistance protein 1 (EC 7.6.2.2)
P08183	ABCB1	7.6.2.1	Phospholipid transporter ABCB1 (EC 7.6.2.1)
P33527	ABCC1	7.6.2.2	**Multidrug resistance-associated protein 1** (EC 7.6.2.2)
P33527	ABCC1	7.6.2.3	Glutathione-S-conjugate-translocating ATPase ABCC1 (EC 7.6.2.3)
P45844	ABCG1	7.6.2.-	**ATP-binding cassette sub-family G member 1** (EC:7.6.2.-)
P42684	ABL2	2.7.10.2	**Tyrosine-protein kinase 2** (EC 2.7.10.2)
Q76LX8	ADAMTS13	3.4.24.87	**A disintegrin and metalloproteinase with thrombospondin motifs 13**
Q08828	ADCY1	4.6.1.1	**Adenylate cyclase type 1** (EC 4.6.1.1)
Q08462	ADCY2	4.6.1.1	**Adenylate cyclase type 2** (EC 4.6.1.1)
O95622	ADCY5	4.6.1.1	**Adenylate cyclase type 5** (EC 4.6.1.1)
P40145	ADCY8	4.6.1.1	**Adenylate cyclase type 8** (EC 4.6.1.1)
Q9UKV8	AGO2	3.1.26.n2	**Protein argonaute-2** (EC 3.1.26.n2) - Short names: Argonaute2/ hAgo2
P30838	ALDH3A1	1.2.1.5	**Aldehyde dehydrogenase, dimeric NADP-preferring** (EC 1.2.1.5)

Figure 1: Overall study workflow [209]. Indicating the major stages of the study with sample collection; sample preparations, extractions and DI-FTICR MS measurements; Investigations on the UniProt database (DB) of co-regulation genes with enzyme (EC) number and results in terms of evaluation.

III. Study cohort

1. Munich Obesity Biobank (MOBB) cohort

a. Study design and aim

The biobank has been established at the chair of Nutritional Medicine in Freising under the direction of Prof. H. Hauner (initially funded by the Federal Ministry of Research and Education with the Competence Network on Obesity). This research project aims to ascertain the differences in structure and function of visceral and subcutaneous fat tissue that could explain the increased risk of developing metabolic diseases for people with an increased abdominal fat mass. Additionally, the influence of the genome on the fat tissue and metabolic diseases will be explored by conducting investigations of the genetic material. The aim is the prediction and prevention of diabetes mellitus type 2 as well as cardiovascular diseases via the observed changes in the fat tissue.

For sample collection, the patient approved tissue donorship and participation by signing the informed consent document to donate additional 2 EDTA tubes (9 mL each) and 2 g to 5 g of fat tissue for each depot (subcutaneous and visceral). The participants of the MOBB cohort were recruited by surgeons of the Surgical Clinic Munich-Bogenhausen and underwent abdominal laparoscopic surgeries (sleeve gastrectomy, fundoplication or appendectomy) **[210]**. The biobank was approved by the ethic committee of the *MRI Klinikum rechts der Isar* (Technical University of Munich). All patients signed the informed consent prior to surgery.

b. Subjects

For our study, we obtained isolated primary human subcutaneous preadipocytes from 12 male subjects of the MOBB cohort with an homozygous genotype for rs1421085 (6 risk allele carriers CC, 6 non-risk allele carriers TT) (Appendix 11, SOP 21). Patients were either morbidly obese (n = 8) or overweight (n = 4) (Table 3).

Table 3: Selected study subjects among the MOBB cohort. *Information about gender, age, BMI and risk/non-risk alleles for rs1421085 are listed.*

Subject ID	Gender (male)	Age [years]	BMI [kg/m^2]	rs1421085 allele 1	rs1421085 allele 2
m142	m	64	26.83	T	T
m170	m	45	47.97	T	T
m187	m	67	25.65	T	T
m189	m	58	27.04	T	T
m252	m	72	51.36	T	T
m300	m	43	67.28	T	T
m123	m	60	39.08	C	C
m164	m	46	53.40	C	C
m186	m	52	29.01	C	C
m256	m	58	42.05	C	C
m258	m	17	60.37	C	C
m266	m	64	43.94	C	C

IV. Results and discussion

1. Pilot studies

I took advantage of the locally well-established differentiation protocol for human primary subcutaneous preadipocytes at the Chair of Nutritional Medicine in Freising, Weihenstephan **[211, 212]** (Appendix 6, SOP 9). However, adjustments were necessary to make the basic cell culture protocol suitable for the direct infusion Fourier transform ion cyclotron resonance mass spectrometry (DI-FT-ICR MS).

Pilot study 1

Several pilot studies were performed. The first pilot study focused mainly on the media ingredients and possible deletions of salts, colors, antibiotics, and proteins. Consequently, a different basal medium was chosen: DMEM-F12 without HEPES and without phenol red (Gibco™ DMEM/F-12, No Phenol Red; see Appendix 16, Table A3 for ingredients list). Also, harvesting in either the original medium or in a Krebs-Ringer-Henseleit (KRH) buffer was tested. Both solutions stayed for 24 h (before harvesting) on the cells in order to keep homogeneous intervals for comparison reasons. Our goal was to analyze cell lysates and media samples at different harvesting days across various subjects. Further establishment work was necessary to extract the metabolites and analyze those via DI-FT-ICR MS **[213]**. Two different protocols were established to extract the metabolites from the cell lysates and media samples (see Chapter VI.2.a for final protocols). For the cell lysates, a homogenization with ceramic beads followed by centrifugation was chosen. For the analysis of the media, a different protocol via solid-phase extraction (SPE) was applied (hydrophilic-lipophilic balance (HLB) cartridges and C18 Zip-Tip). In both cases, different dilutions (1:20, 1:50) of various collection days were tested. KRH buffer had been compared to the original cell culture media. The results showed high interferences of the buffer in comparison to the original media. We observed a good mass coverage by using the C18 Zip-Tip. This technique required a lower volume of the sample and the possibility of automatization via a pipetting robot (Eppendorf:

ep*Motion*® 96). All in all, the media samples indicated to be "poorer" in metabolites compared to cell lysates.

Concluding remarks of the pilot study 1 were as follows:

- Continuing the harvesting in the 24 h original media instead of 24 h KRH buffer.
- Due to the limited available and high valuable human biological material, the cell number in terms of wells of a 6 well plate was planned to be reduced for the non-targeted metabolomics approach (from originally 6 wells to 3 wells). If possible, the number was aimed to fall even below 3 wells.
- Testing of C4 chemistry in the Zip-Tip protocol (media sample preparation protocol) to appreciate the different kinds of molecules coverage in terms of polarity.
- A reduction of the 24 h media change volume from 2 mL to 1 mL. This had been confirmed to be the lowest possible volume (per well of 6 well plate) without expecting any problems, such as drying of the cells.

Pilot study 2

The 2nd pilot study was performed with the subject PAC 501 (BMI = 24.5 kg/m^2; *FTO* risk allele carrier (CC); female; age: 27 years). We mainly tested the following well numbers: 6 vs. 3 wells and harvested on days 0, 1, 2, 3, 6 and 14. Moreover, we tested different dilutions (1:20, 1:50) for the analysis via DI-FT-ICR MS. The evaluation of the spectra was done by Dr. Sara Forcisi (Research Unit Analytical BioGeoChemistry, Helmholtz Munich) and the outcomes indicated that the reduction to 3 wells of a 6 well plate is sufficient to analyze the cell lysates via DI-FT-ICR MS. Moreover, the 1:20 dilution of the cell lysates has been confirmed as optimal. The evaluation of the media-related spectra appeared to be more difficult, due to the presence of diverse ingredients such as amino acids, vitamins, glucose, and fatty acids. Nevertheless, this did not interfere with the major class of interest: steroids. The only and "at great cost" solution

was the compound labeling, therefore we compromised on not considering the compounds from the media ingredients list for the evaluation.

As already mentioned, metabolites in the media samples were highly diluted although the media volume was reduced to 1 mL. The intensity decreased obviously from 6 to 3 wells. We considered performing again the extraction technique (hydrophilic and lipophilic balance (HLB) cartridges) with an adjusted protocol to concentrate the metabolites of the secretome by increasing the quantity of the sorbent (100 mg before 30 mg) and the load (500 µL before 250 µL). Unluckily, the tested C4 Zip-Tip did not show any diversity compared to C18. Further, a major issue was the suppression effect of day 0 media samples. We decided to test harvesting on day 0 in proliferation medium without FCS including previous washing steps. Moreover, we considered to test an even lower number of wells (< 3), however the media samples indicated to be the limiting factor due to a high dilution. Also, we compromised on focusing on fewer harvesting days (day 0, day 2, and day 14), but at the same time increasing the available study subject number to a maximum to achieve an appropriate power of statistical calculation.

Pilot study 3

The 3rd pilot study was mainly performed to test a lower number of wells: 3 vs. 2 vs. 1 with the same subject PAC 501 (BMI = 24.5 kg/m^2; *FTO* risk allele carrier (CC); female; age: 27 years). Cell lysates were collected on harvesting days 0, 2 and 14. Due to the suppression effect of the media samples (day 0), we also tested the harvesting on day 0 in proliferation medium without fetal calf serum (FCS) and with additional washing steps (2x2 mL proliferation medium without fetal calf serum (FCS)) for the last 24 h media change. We assumed that the suppression effect was resulting from the media ingredient FCS. Additionally, an apoptosis test (Lactate dehydrogenase (LDH) measurement) was performed and the results [units/min] showed an adequate cell viability (if ≤15) for the PACs on day 0 cultured in proliferation media without FCS for 24 h (see Figure 2 and Table 4 below; Appendix 12, SOP 29). There were

barely differences shown between the different FCS concentrations and clearly in the range of an adequate cell viability.

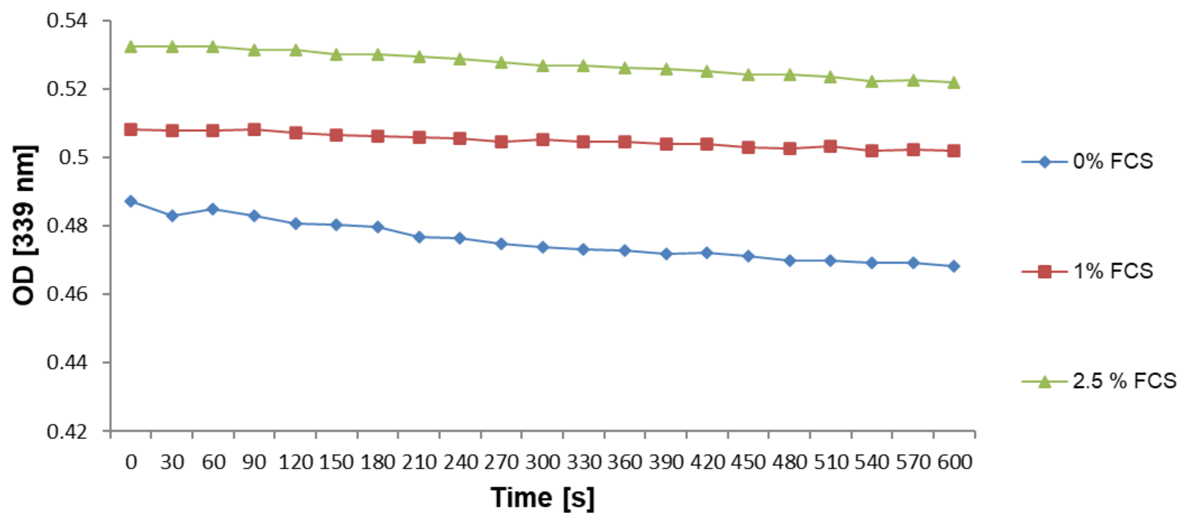


Figure 2: Cell viability test - by measuring LDH at an optical density (OD) of 339 nm and under different FCS concentrations over time (seconds).

Table 4: Results summary LDH measuring under different FCS concentrations.

Different FCS concentrations	Results [units/min]
0 %	9.52
1 %	3.10
2.5 %	5.23

Furthermore, the evaluation of the spectra performed by Dr. Sara Forcisi confirmed the removal of FCS as no suppression effect. Cell number confirmation had been achieved for 2 wells of a 6 well plate and showed to be the best compromise considering the secretome. However, issues in the handling during extraction steps of the cell lysates were faced (low final sample volume). We compromised on 3 wells as a final well number to be able to obtain an adequate sample volume after extraction. Moreover, in previous experiments we observed a strong background derived from Falcon® tissue culture-treated 6-well plates (product number 353224). After testing an Eppendorf brand 6 well plate (product number 0030720113), we did not see any differences in the growth pattern of PACs compared to the TC-treated Falcon® plates. However, the observed background noise was similar. On the other hand, the non-

tissue culture-treated Falcon® 6 well plates (product number 351146, applicable for suspension cell culture) were, as expected, not suitable for adherent PACs. Since only a few cells were able to grow on the plate surface, we decided to continue with the established brand and kind of 6 well plates that had been applied for differentiation experiments over years at the chair of Nutritional Medicine in Freising, Weihenstephan.

a. Setups of final technical experiment and study

For identifying the framework to perform the final study, we were running one concluding and comprehensive technical experiment on the complete variety of tested well numbers (6 well plate: 1 well vs. 2 wells vs. 3- vs. 6-) in order to confirm our previously gained knowledge. Figure 3 shows the setup of the final technical experiment as outcome of all pilot studies.

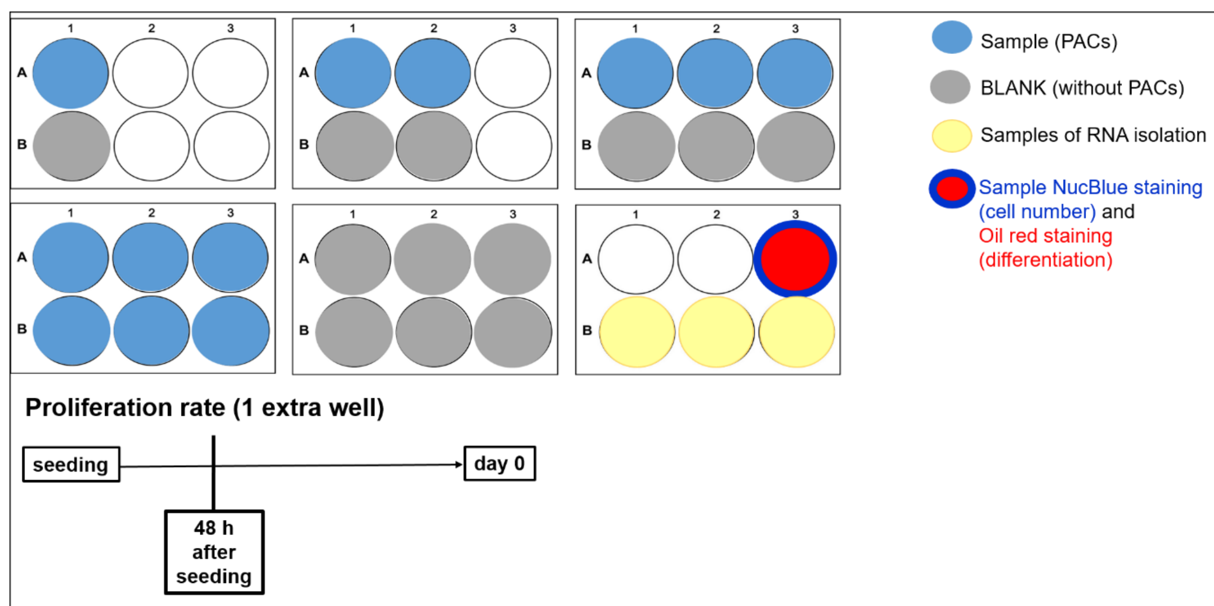


Figure 3: Setup of final technical experiment. 6 well plate layouts for each subject and harvesting day (0, 2, 14). Proliferation rate after 48 h was assessed with one additional well of a 6 well plate (48 h after seeding into 6 well plates). Samples were harvested for RNA and untargeted metabolomics (samples and blanks).

Two healthy male subjects with a normal weight BMI were selected (Table 5). Samples for RNA and untargeted metabolomics (samples and blanks) were harvested. Blanks did not contain any PACs, but were handled in the same manner as the samples. Those were obligatory to obtain environmental influences on the PACs themselves. Moreover, one well

was stained with NucBlue (nuclei) and OilRed O (lipid droplets) (Chapter VI.1.d.). The proliferation rate (after 48 h) of each subject was assessed via the CellProfiler pipeline **[214]**. Moreover, OilRed was quantified spectrophotometrically and the actual cell number was determined with the CellProfiler software for each harvesting day as well as well number **[214]** (Appendix 16, Table A2). However, our major focus was the successful realization of the methodological aspects and replication of the previous revealed insights from the pilot studies. Luckily, for the untargeted metabolomics measurements our decision for the number of 3 wells was confirmed.

Table 5: Subjects for final technical experiment and corresponding clinical parameters.

Male subjects (IDs)	PAC 614	PAC 657
BMI [kg/m^2]	23.6	22.1
Age [years]	33	37

After the approved completion of the final technical experiment, we planned the subsequent study, where the genotyped subjects were obtained from the MOBB cohort (Chapter III and Table 3).

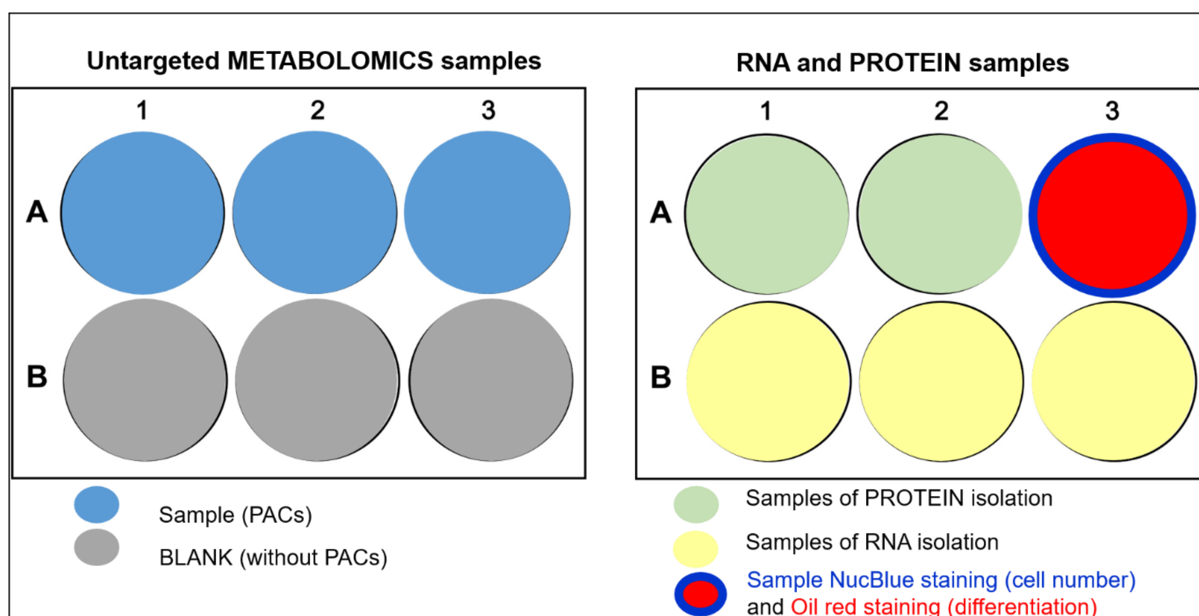


Figure 4: Setup of study. 6-well plate layouts for each subject and harvesting day (0,2,14). Samples were harvested for RNA, proteins and untargeted metabolomics (samples and blanks).

The study subjects were cultured simultaneously in equal numbers of risk allele carriers/non-risk allele carriers (e.g., 3 CC and 3 TT) per run to avoid any batch effect. Then, we decided for the final plate layout and harvesting of samples as follows. For the untargeted metabolomics measurements 3 wells for samples and blanks were considered. In addition, 3 wells for RNA and 2 wells as protein samples were reserved on the 6 well plate. Moreover, one well was utilized to stain nuclei (via NucBlue) and lipid droplets (OilRed) at once (Appendix 10, SOP 19).

2. Work scheme of final human study

The workflow of the study with *FTO* carriers is described as an overview in Figure 5. A total number of 12 male subjects were chosen and the cohort was evenly distributed between risk- (CC) and non-risk carriers (TT).

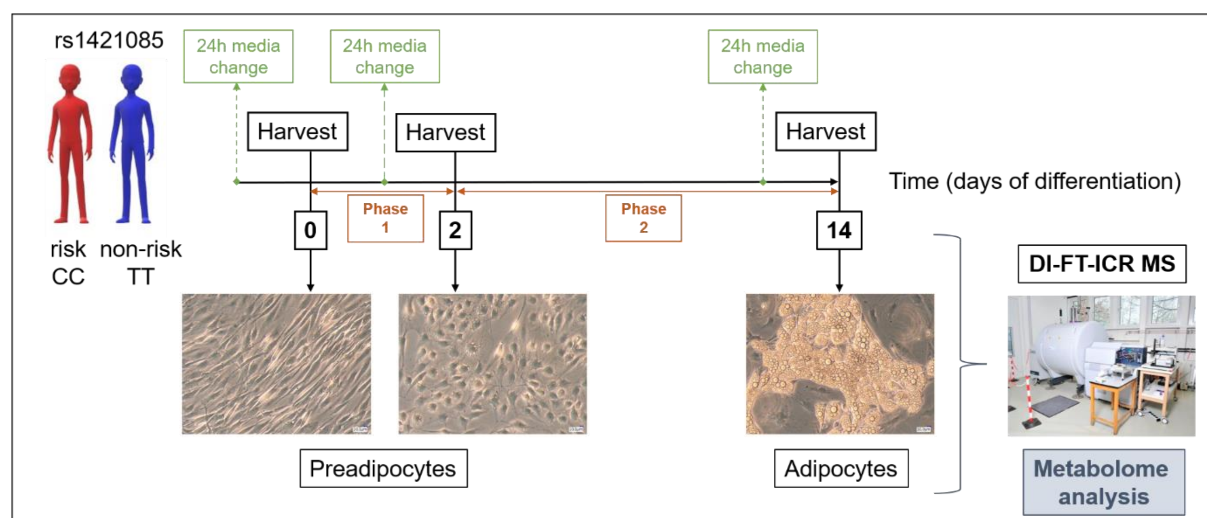


Figure 5: Work scheme of study with *FTO* risk and non-risk allele carriers. Microscope pictures show transmitted light single pictures (200x magnification) with scale bars = 10 µm.

We obtained primary human preadipocytes (PACs) from donors with a homozygous genotype for the *FTO* SNP (rs1421085). Moreover, we were running cell culture differentiation experiments and kept a homogeneous media change of 24 h before harvesting. We harvested on days 0, 2 and 14, where we distinguished between Phase 1 (day 2 -day 0) and Phase 2

(day 14 -day 2) as indicated with corresponding phenotypic transmitted light pictures (200x magnification). All samples were analyzed via DI-FT-ICR MS.

3. Study cohort characteristics

The study cohort showed an older population for non-risk carriers (58.17 ± 11.89 years [TT] vs. 49.50 ± 17.13 years [CC], p-value = 0.2424), whereas the BMI was higher in the risk carrier subcohort (44.64 ± 11.01 [CC] kg/m^2 vs. 41.02 ± 17.19 kg/m^2 [TT], p-value = 0.2944), but did not reach statistical significance for both features (Table 6). However, both groups were considered as populations with severe obesity according to their mean BMI (class 3, BMI > 40 kg/m^2). Within the study cohort 3 subjects were classified with T2D, where 2 individuals' origin from the non-risk- and 1 from the risk group. The morphological characteristics reported a shorter time frame of proliferation until confluence for risk carriers compared to non-risk carriers (7.0 ± 0.8944 days vs. 7.5 ± 0.5477 days, p-value = 0.1970). The total cell number showed highest numbers for day 0 and day 2 (approximately 1.9 M) without clear differences between the subgroups. Also, within the complete group, no differences could be assessed across the differentiation days (p-value = 0.3679). Nevertheless, the cell number almost halved on the harvesting day 14 (approximately 1.0 M) due to the detachment of cells most likely during media changes as well as because of high lipid accumulation and – probably - easy detachment of lipid-loaden cells. The complete list of cell numbers for each subject is listed in the Appendix 16 (Table A1).

Table 6: Clinical parameters and morphological characteristics of the study cohort (n = 12) divided into the subcohorts risk allele carriers (CC) and non-risk allele carriers (TT). *Mean (Ø) including standard deviation (SD). Total cell number is calculated for harvesting days: day 0 (= D0), day 2 (= D2) and day 14 (= D14).*

	Study cohort (males) n = 12	**rs1421085 risk allele carriers (CC) n = 6**	**rs1421085 non-risk allele carriers (TT) n = 6**
Clinical parameters			
Ø Age ± SD [years]	53.83 ± 14.77	49.50 ± 17.13	58.17 ± 11.89
Ø BMI ± SD [kg/m^2]	42.83 ± 13.89	44.64 ± 11.01	41.02 ± 17.19
T2D status [total number of subjects]	3	1	2
Morphology characteristics			
Ø Days until confluence in 6 well plates ± SD	7.25 ± 0.7538	7.0 ± 0.8944	7.5 ± 0.5477
Total cell number (3 wells) per harvesting day	**D0**= 1,865,995	**D0**= 1,800,267	**D0**= 1,931,724
	D2= 1,872,947	**D2**= 1,885,252	**D2**= 1,860,641
	D14= 1,061,363	**D14**= 1,126,869	**D14**= 995,856

a. Microscopical organization

The phenotypic microscope images are shown for the harvesting days in Figure 5. Thereby, during the proliferation phase preadipocytes maintain their spindle shape. From the time point of induction (= day 0) the cell shape changes from spindle- to round-shaped cells. After changing from the induction to the differentiation media, it takes on average up to 2 days until storage of lipid droplets is visible under the microscope. Adipocytes are considered as lipid-filled between 12 -16 days after induction of differentiation, would be able to continue differentiation for another 10 -14 days, while the multiple droplets would only grow further in size until they constitute of one single lipid droplet. Nevertheless, with increasing fat storage adipocytes detach easier depending on the amount of fat storage **[211]**. We observed that during differentiation between days 9 until 14 the lipid-containing cells were growing in size rather than in number.

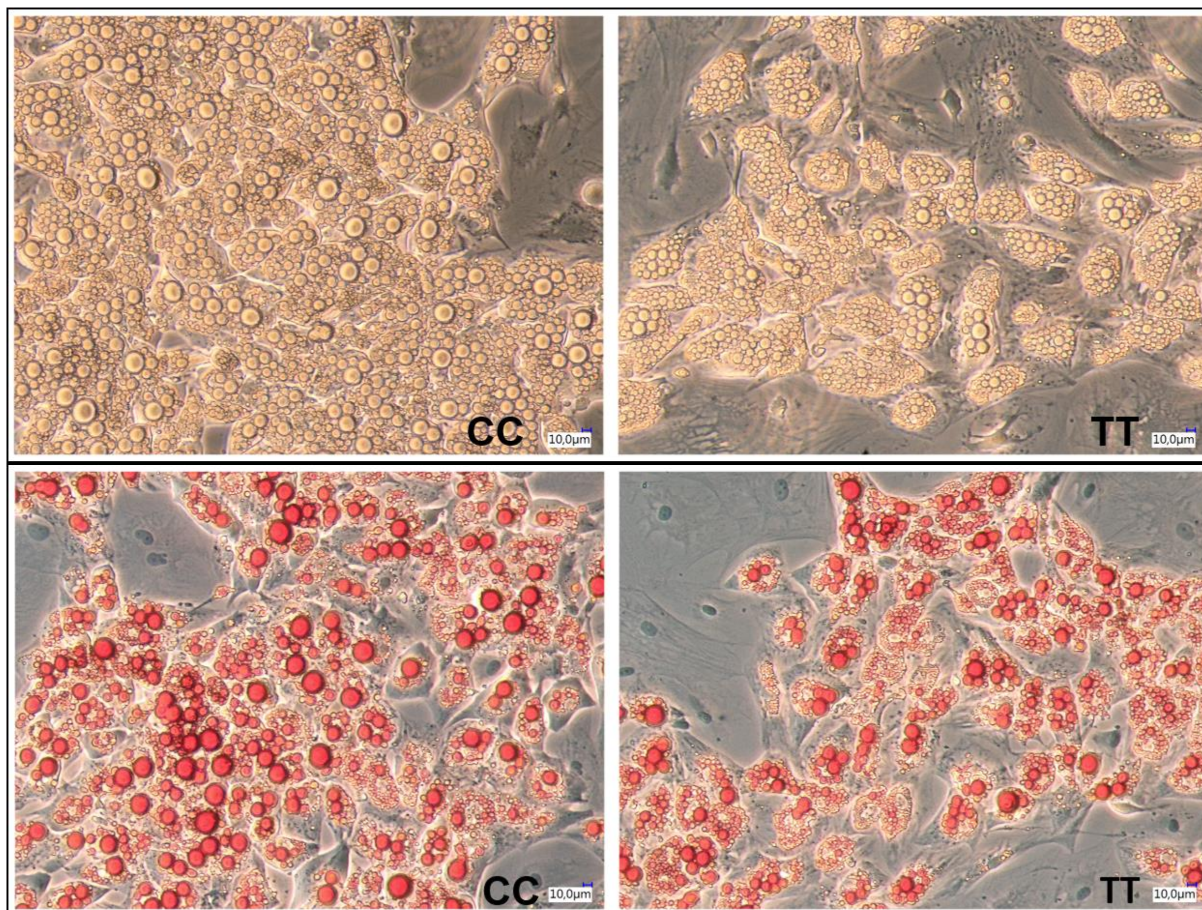


Figure 6: Microscopical pictures at day 14 comparing risk (CC) vs. non-risk (TT). Left: Adipocytes at day 14 from CC - risk allele carriers. Right: Adipocytes at day 14 of TT - non-risk allele carriers. Transmitted light single pictures (200x magnification) with scale bars = 10 µm are shown. Original appearance (unstained adipocytes, up) and stained with OilRed (down) is depicted.

Comparing the microscopical images of day 14 adipocytes from risk allele carriers (CC) and non-risk allele carriers (TT), an increased lipid droplet size is generally recognizable for risk carriers either stained with OilRed or unstained as shown in those exemplary microscope pictures (Figure 6). However, the lipid droplet size has not been measured and therefore, no significant differences can be described.

For calculation of total cell number, nuclei had been stained with NucBlue (original picture Figure 7, left) and then processed with a CellProfiler pipeline, where the counted nuclei were shown in different colors and the ones that failed the analysis were shown in grey (overlay picture Figure 7, right) **[214].** The cell number results for the whole group as well as risk/non-risk subgroups were already described above (Chapter IV.3. Study cohort characteristics).

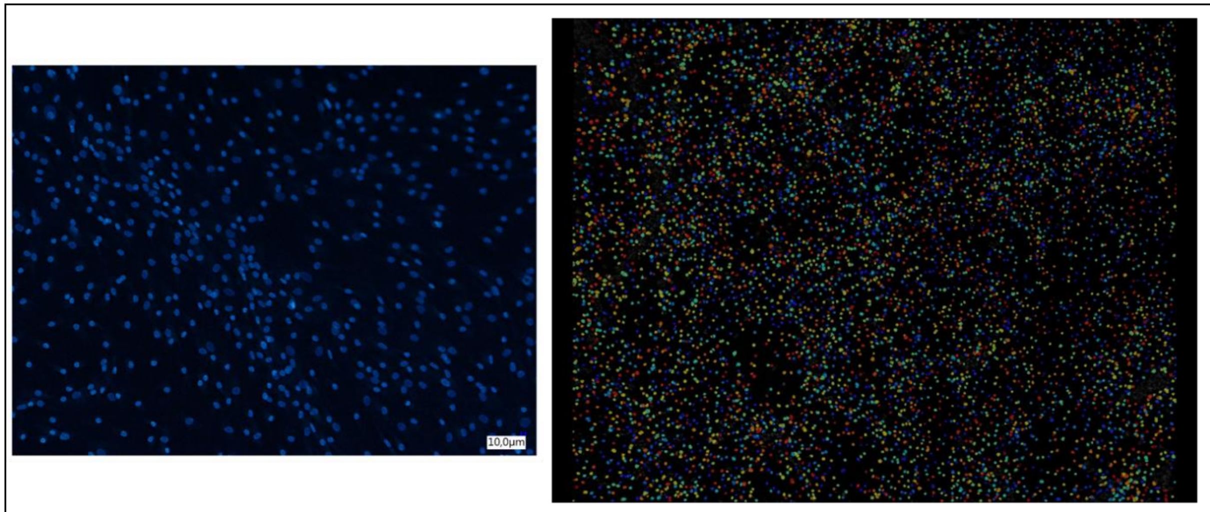


Figure 7: Risk allele carrier at day 0. *left:* UV light single shot image (300x magnification) Nuclei in blue color. Scale bar = 10 µm. *right:* CellProfiler overlay picture: UV light 5x5 stitches (300x magnification). Colored dots indicate counted cell nuclei.

For OilRed quantification of the accumulated lipids at day 14, the analysis was performed in two different ways. First, OilRed was dissolved from the stained cells and measured spectrophotometrically, whereas the intensity was calculated per 100,000 PACs involving the nuclei count (Chapter VI.1.e.). There was a tendency towards a higher OilRed intensity in the cultures from the risk allele carriers (Figure 8). On the other hand, the 5x5 stitch images at day 14 were analyzed via the QuPath 0.3.0 open source software **[215].** The area stained with OilRed within the well was named as "area positive" and displayed as percentage relative to the complete area (Figure 9). There was a clear tendency towards a higher percentage of positive area stained with OilRed. However, these calculations were not normalized to the cell number within the well. All in all, no significant differences were assessed for both approaches, respectively (p-value = 0.4686 and 0.1548).

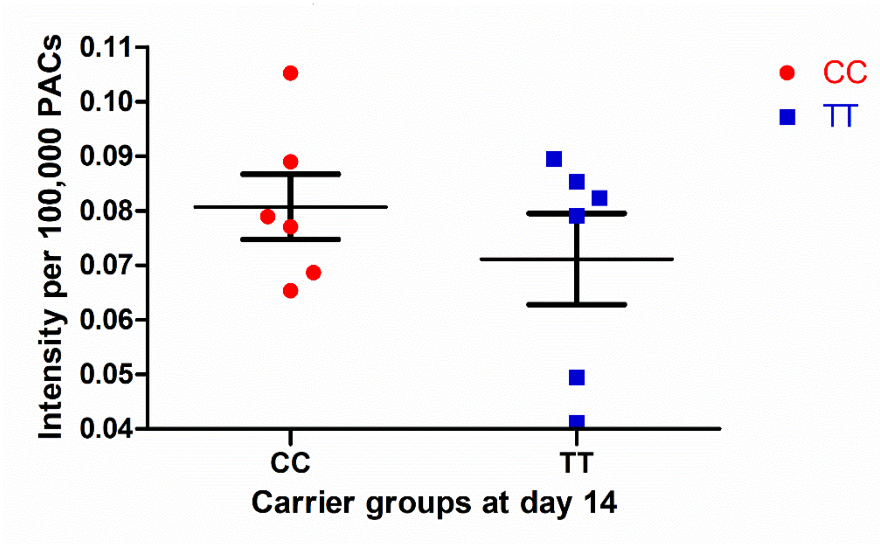


Figure 8: OilRed quantification at day 14 via spectrophotometer (A = 492 nm). Error bars show mean with standard error of the mean (SEM). Intensity of OilRed stain is shown per 100,000 preadipocytes (PACs) and grouped into risk carrier (CC, red) and non-risk carrier (TT, blue).

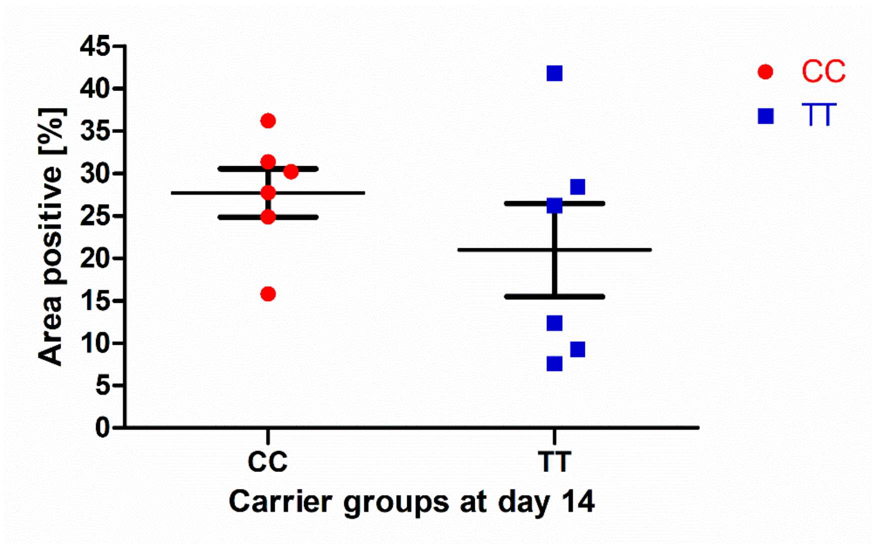


Figure 9: OilRed quantification at day 14 via QuPath software [215]. Error bars show mean with standard error of the mean (SEM). Area positive indicates neutral lipids stained with OilRed grouped into risk carrier (CC, red) and non-risk carrier (TT, blue).

4. Metabolome analysis

a. Compound classes

For analyzing the involved metabolites over the two differentiation phases, we performed untargeted metabolome analysis using DI-FT-ICR MS at the department of Analytical BioGeoChemistry (BGC, Helmholtz Munich) headed by Prof. Schmitt-Kopplin. The experimental MS features were assigned to high-confidence molecular formulas using mass difference network (MDiN) **[216]**. The molecular formulas were allocated to the known metabolites from the Human Metabolome database (HMDB) **[97]**. Dr. Sara Forcisi and Dr. Franco Moritz performed compound class overrepresentation analysis (ORA) of known metabolic features that distinguish between the rs1421085 risk vs. non-risk individuals (Figure 10-12) **[217]**.

i. Cell lysates

In the cells, during Phase 1 (day 2 -day 0) of differentiation, we observed in risk carriers compared to non-risk a significant decrease in glycerophospholipids (p-value = $8.62 * 10^{9}$), steroids and steroid derivatives (p-value = 0.036) and organonitrogen compounds (p-value = 0.019) (Figure 10). No significant scores were observed for the upregulation during this time window.

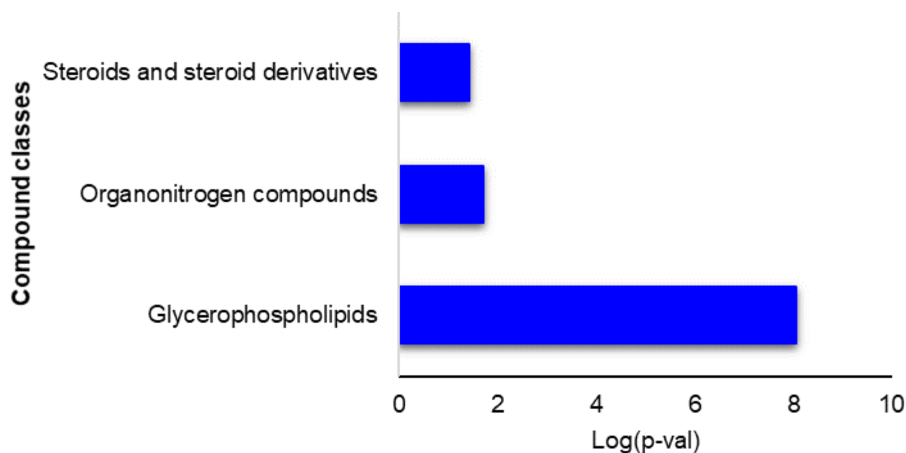


Figure 10: Compound classes downregulated at Phase 1 (day 0 -day 2) in cells of risk allele carriers in comparison to non-risk allele carriers.

During Phase 2 of differentiation (day 14 -day 2) we discovered in cells a decrease in glycerolipids (p-value = 0.014), phenols (p-value = 0.015) and endocannabinoids (p-value = 0.046) in risk carriers compared to non-risk and an increase in glycerophospholipids (p-value = 0.023) (Figure 11).

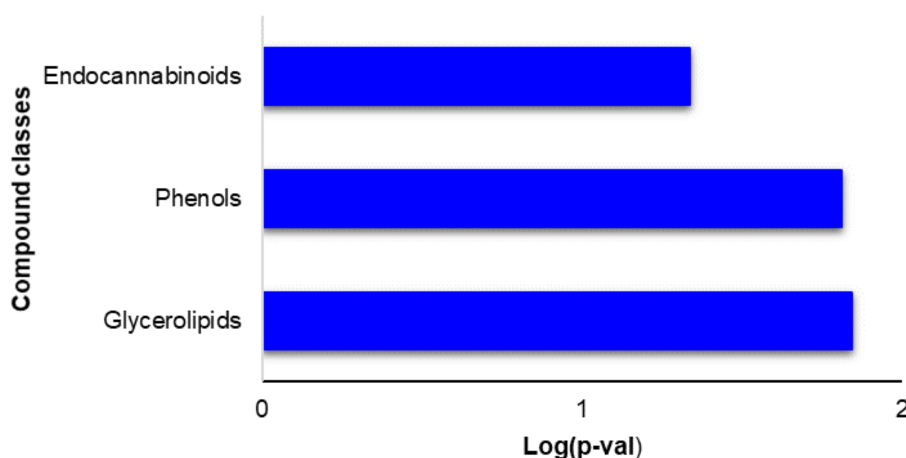


Figure 11: Compound classes downregulated at Phase 2 (day 14 -day 2) in cells of risk allele carriers compared to non-risk allele carriers.

ii. Media

In contrast, in the media during Phase 1 we could observe an increase in fatty acyls (p-value = $5.57 * 10^{-5}$), glycerolipids (p-value = 0.001), carboxylic acids and derivatives (p-value = 0.031)

as well as prenol lipids (p-value = 0.038) in risk carriers in comparison to non-risk (Figure 12). No significant differences were shown during Phase 2.

Figure 12: Compound classes upregulated at Phase 1 (day 2 –day 0) in the media of risk allele carriers compared to non-risk allele carriers.

b. Gene-driven mass difference enrichment analysis (MDEA) of cell lysates

A way to monitor (bio)chemical reactions (thereby the activity of genes with enzyme-encoding exons) is to investigate the mass differences between detected m/z-signals. One way to make use of this information is to perform mass difference enrichment analysis (MDEA) **[218]**. MDEA tests whether statistically important m/z peaks have a propensity to be associated with specific mass differences (screening of building blocks that make up a detected metabolome). The most important step in MDEA is the selection of mass differences that enable statements upon our hypotheses. Genetic products (proteins) can interact with small molecules (e.g. metabolites) by manipulating them directly (when a gene of interest encodes for an enzyme) or by ligand-protein interactions, which incite a specific activity that the protein is to perform or to not perform. Driven by the previous results, we decided to perform MDEA from the cell lysates data to investigate the underlying genes and pathways characteristic for the group of risk allele carriers across the two differentiation phases.

We obtained the list of genes co-expressed with *IRX3* and *IRX5* from the work of Prof. Claussnitzer published in 2015 **[94]**. Those genes were investigated on the UniProt database (DB) platform **[219]** which started a former colleague (Julia Petzold) in 2017. I updated and extended the information of these two lists (genes with and without enzyme (EC) number) in August/September 2020. The research included enzyme information such as EC number, protein names as well as gene name synonyms etc. (Appendix 16, Tables A8 and A9). The list "genes with EC number" included genes that encode for enzymes with an EC number and was utilized for the MDEA (Appendix 16, Table A8). Moreover, the second list contained the genes that encode for enzymes without an EC number (Appendix 16, Table A9). Further investigations had been done for these genes in order to include those into the analysis. Therefore, this list was extended with ligand information obtained from the Binding DB for 84 genes from our list that were present within the DB. However, this additional analysis did not lead to any further insights from the MDEA. The genes list with EC number contained 234 genes, whereas the list without EC number constituted 432 genes. Consequently, in total both lists encompassed 666 co-expressed genes (see Appendix 16, Tables A8-A9).

Those gene investigations were done in order to generate a literature-based list of the co-expressed genes, their encoded enzymes and consequently the biotransformation related to them (and the associated mass-differences). To define the link of each enzyme to all the reactants and the respective mass differences, we queried the Rhea DB which is a DB of chemical reactions **[220]**. We collected pairs of products and substrates and defined the mass-differences characteristic for each enzyme (average of Z-scores for each enzyme). From the original list, some genes were excluded because they did not catalyze any reaction or they were not found in the Rhea DB. Table 7 depicts the main results of the MDEA, highlighting the enzymes and the genes which obtained the highest scores in the analysis comparing risk- vs. non-risk allele carriers.

Table 7: Summary of MDEA for all targets with score > 3. *Enzymatic mass differences (MDs) including corresponding genes and UniProt ID's. Arrows indicate directions during Phase 1 (day 2 -day 0) and Phase 2 (day 14 -day 2).*

UniProt ID	Gene name	Protein names specific to EC number	Score [aggregate z-score]	Direction [Phase 1]	Direction [Phase 2]
P17405	***SMPD1***	Sphingomyelin phosphodiesterase (EC 3.1.4.12)	34.63	↓	↑
Q9Y2P5	***SLC27A5***	Bile acyl-CoA synthetase (EC 6.2.1.7) - Short name: BACS	9.02	↑	↓
P21580	***TNFAIP3***	Tumor necrosis factor alpha-induced protein 3 (EC 2.3.2.-) - Short name: TNF alpha-induced protein 3;	4.79	↓	↑
P09874	***PARP1***	Protein poly-ADP-ribosyltransferase PARP1 (EC:2.4.2.-)	4.14	↓	↑
P49419	***ALDH7A1***	Betaine aldehyde dehydrogenase (EC 1.2.1.8)	3.72	↓	↑
P40939	***HADHA***	Long-chain enoyl-CoA hydratase (EC 4.2.1.17)	3.69	↓	↑
P33121	***ACSL1***	Phytanate-CoA ligase (EC 6.2.1.24)	3.57	↑	↓
P04818	***TYMS***	Thymidylate synthase (EC 2.1.1.45) - Short names: TS/TSase	3.56	↓	↑
Q6IB77	***GLYAT***	Glycine N-benzoyltransferase (EC 2.3.1.71)	3.50	↑	↓
Q13085	***ACACA***	Acetyl-CoA carboxylase 1 (EC 6.4.1.2) - Short name: ACC1	3.39	↓	↑
P35228	***NOS2***	Nitric oxide synthase, inducible (EC 1.14.13.39)	3.07	↓	↑
P29474	***NOS3***	Nitric oxide synthase, endothelial (EC 1.14.13.39)	3.07	↓	↑

i. Overrepresentation analysis in Reactome database for target candidates

Only the MDEA results for genes with a magnitude greater than 3 were further investigated via overrepresentation analysis in the Reactome database **[221]**. The sum-up of the main results from the analysis are listed in Table 8 below, whereas only the significant pathways (p-value < 0.05) are shown **[221, 222]**. Moreover, the UniProt IDs were assigned to the gene names for identification. The involved pathways had been considered for the overall pathway schemes (Chapter IV.6) and are described in detail there (Figures 17, 18).

Table 8: Overrepresentation analysis of the targets (magnitude > 3) from the MDEA. *Genes underlined indicate an upregulaton at Phase 1 (day 2 -day 0) and a downregulation at Phase 2 (day 14 -day 2). All the other unmarked genes show an inverse behavior. Only entities with a significant p-value < 0.05 are shown.*

Pathway names	Entities p-value	Genes involved
Metabolism	4.26 E-05	*SLC27A5, NOS3, HADHA, ACACA, ACSL1, TYMS, SMPD1, ALDH7A1, GLYAT*
Nitric oxide stimulates guanylate cyclase	2.73 E-04	*NOS3, NOS2*
ROS and RNS production in phagocytes	6.63 E-04	*NOS3, NOS2*
Fatty acyl-CoA biosynthesis	7.00 E-04	*ACACA; ACSL1*
Metabolism of lipids	7.09 E-04	*SLC27A5, HADHA, ACACA, ACSL1, SMPD1*
Fatty acid metabolism	7.98 E-04	*HADHA, ACACA, ACSL1*
NOSIP mediated eNOS trafficking	0.0021	*NOS3*
Beta oxidation of palmitoyl-CoA to myristoyl-CoA	0.0032	*HADHA*
Beta oxidation of myristoyl-CoA to lauroyl-CoA	0.0032	*HADHA*
Platelet homeostasis	0.0038	*NOS3, NOS2*
Inhibition of nitric oxide production	0.0043	*NOS2*
NOSTRIN mediated eNOS trafficking	0.0053	*NOS3*
vRNA Synthesis	0.0053	*PARP1*
Beta oxidation of octanoyl-CoA to hexanoyl-CoA	0.0053	*HADHA*
Beta oxidation of lauroyl-CoA to decanoyl-CoA-CoA	0.0053	*HADHA*
Beta oxidation of hexanoyl-CoA to butanoyl-CoA	0.0053	*HADHA*
Conjugation of benzoate with glycine	0.0064	*GLYAT*
Mitochondrial fatty acid beta-oxidation of unsaturated fatty acids	0.0064	*HADHA*

Beta oxidation of decanoyl-CoA to octanoyl-CoA-CoA	0.0064	*HADHA*
Choline catabolism	0.0064	*ALDH7A1*
Acyl chain remodeling of CL	0.0064	*HADHA*
Defective HLCS causes multiple carboxylase deficiency	0.0075	*ACACA*
POLB-Dependent Long Patch Base Excision Repair	0.0085	*PARP1*
Conjugation of salicylate with glycine	0.0085	*GLYAT*
ChREBP activates metabolic gene expression	0.0085	*ACACA*
Defects in biotin (Btn) metabolism	0.0085	*ACACA*
Linoleic acid (LA) metabolism	0.0085	*ACSL1*
Amino Acid conjugation	0.0096	*GLYAT*
Conjugation of carboxylic acids	0.0096	*GLYAT*
HDR through MMEJ (alt-NHEJ)	0.0106	*PARP1*
Tetrahydrobiopterin (BH4) synthesis, recycling, salvage and regulation	0.0106	*NOS3*
Metabolism of steroids	0.0115	*SLC27A5*, *ACACA*
eNOS activation	0.0117	*NOS3*
Mitochondrial fatty acid beta-oxidation of saturated fatty acids	0.0117	*HADHA*
Biotin transport and metabolism	0.0117	*ACACA*
Lysine catabolism	0.0127	*ALDH7A1*
alpha-linolenic (omega3) and linoleic (omega6) acid metabolism	0.0138	*ACSL1*
alpha-linolenic acid (ALA) metabolism	0.0138	*ACSL1*
TNFR1-induced proapoptotic signaling	0.0149	*TNFAIP3*
Carnitine metabolism	0.0149	*ACACA*
Synthesis of bile acids and bile salts via 24-hydroxycholesterol	0.0149	*SLC27A5*
Metabolism of nitric oxide: NOS3 activation and regulation	0.0159	*NOS3*
Metabolism of vitamins and cofactors	0.0172	*NOS3, ACACA*
Recycling of bile acids and salts	0.0191	*SLC27A5*
Metabolism of cofactors	0.0201	*NOS3*
Defects in vitamin and cofactor metabolism	0.0232	*ACACA*
Suppression of phagosomal maturation	0.0243	*NOS2*
Synthesis of very long-chain fatty acyl-CoAs	0.0253	*ACSL1*
Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol	0.0253	*SLC27A5*
Resolution of AP sites via the multiple-nucleotide patch replacement pathway	0.0264	*PARP1*
VEGFR2 mediated vascular permeability	0.0285	*NOS3*

Downregulation of SMAD2/3:SMAD4 transcriptional activity	0.0285	*PARP1*
G1/S-Specific Transcription	0.0295	*TYMS*
TNFR1-induced NFkappaB signaling pathway	0.0316	*TNFAIP3*
Interconversion of nucleotide di- and triphosphates	0.0316	*TYMS*
Synthesis of bile acids and bile salts	0.0357	<u>*SLC27A5*</u>
Negative regulators of DDX58/IFIH1 signaling	0.0367	*TNFAIP3*
NOD1/2 Signaling Pathway	0.0378	*TNFAIP3*
DNA Damage Recognition in GG-NER	0.0398	*PARP1*
Mitochondrial Fatty Acid Beta-Oxidation	0.0398	*HADHA*
Ovarian tumor domain proteases	0.0409	*TNFAIP3*
Regulation of TNFR1 signaling	0.0409	*TNFAIP3*
Resolution of Abasic Sites (AP sites)	0.0409	*PARP1*
Response of Mtb to phagocytosis	0.0409	*NOS2*
Dual Incision in GG-NER	0.0429	*PARP1*
Formation of Incision Complex in GG-NER	0.0450	*PARP1*
Activation of gene expression by SREBF (SREBP)	0.0450	*ACACA*
Bile acid and bile salt metabolism	0.0470	<u>*SLC27A5*</u>
Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	0.0480	*PARP1*
Glycosphingolipid metabolism	0.0480	*SMPD1*

5. Gene expression levels and gene-metabolite correlation analysis

In order to confirm our metabolomics results, we assessed the gene expression levels of the most important target candidates from the MDEA. Moreover, we included the most relevant gene candidates as described in the previous work of Prof. Claussnitzer **[94]**. The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) revealed no significant differences across all selected targets (literature- and metabolomics-based) between risk allele- (CC) and non-risk allele carriers (TT) over all differentiation days (day 0, day 2 and day 14) by means of relative quantification (RQ) values adjusted for BMI and age (Appendix 16, Table A7.1). However, there was a slight tendency for a higher expression of the lipid storage marker leptin (*LEP*: -1.616 ± 0.7135 vs -2.060 ± 1.105, p-value = 0.2143) in the risk-group.

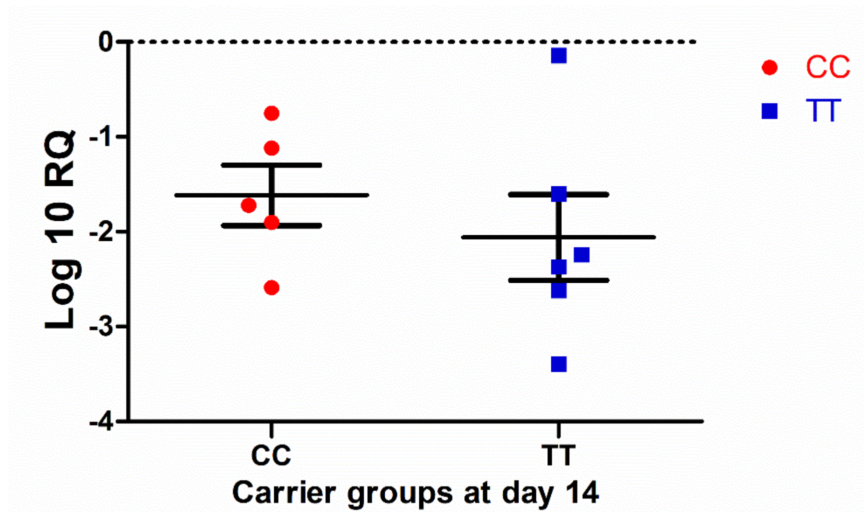


Figure 13: *LEP* gene expression levels as relative quantification (Log 10 RQ) at day 14 illustrated for both groups (CC and TT). All RQ values were adjusted for BMI and age. Scatter plots are shown with error bars indicating mean with standard error of the mean (SEM).

However, for comparing the metabolome results with the gene expression levels, a different approach was necessary. Therefore, comparisons were performed at the level of the matrices generated for Phase 1 (day 2 -day 0) and Phase 2 (day 14 -day 2) for the gene expression data as performed for the metabolomics data. But at first delta CT-values (target gene compared to reference genes) were multiplied with factor -1 and median centered as well as winsorized given the 5th and 95th percentile. Then it was computed RQ = $2^{-delta\ CT}$. Further, gene expression levels were transformed into data matrices for Phase 1 and Phase 2. The described modifications led to optimal comparability of the datasets. Since there was no significant influence given by BMI or age, it was not appropriate to adjust for it. Additionally, none of the metabolomics data in terms of compound classes and MDEA were adjusted either. Comparing those gene data (Appendix 16, Table A7.2) no significant differences could be discovered in regards to risk- vs. non-risk allele carriers and over phases of differentiation (Phase 1 and 2). However, the overall aim was to relate gene expression- to metabolite data. Therefore, it was important to link the gene expression to reactions potentially happening within the metabolomics data. More precisely, linking it to the difference between target (product) and

source (substrate) by means of the difference of effect in the target and effect in the source. Lastly, correlation coefficients and corresponding p-values (linear regression) were calculated.

Table 9: Correlation coefficients for all genes (analyzed via qPCR) with the metabolomics data during Phase 1 and Phase 2.

Gene names	Correlation coefficient (Phase 1)	p-values	Correlation coefficient (Phase 2)	p-values
SMPD1	-0.34310	< 0.0001	0.14721	< 0.0001
SLC27A5	-0.18640	< 0.0001	0.15896	< 0.0001
PARP1	0.37535	< 0.0001	-0.4160	< 0.0001
HADHA	0.45617	< 0.0001	0.13798	< 0.0001
ACACA	0.30261	< 0.0001	0.37105	< 0.0001
PPARG2	0.21574	< 0.0001	0.17833	< 0.0001
UCP1	1.8343 E-05	0.9986	0.16730	< 0.0001
PGC1A	-0.0653	< 0.0001	0.23018	< 0.0001
IRX3	0.03227	0.0018	0.36393	< 0.0001
IRX5	0.30715	< 0.0001	0.01132	0.2732
LEP	-0.26050	< 0.0001	-0.09860	< 0.0001

Considering the Phases (“1” = day 2 -day 0 and “2” = day 14 -day 2) and based on the correlation coefficients, only *SMPD1*, which had the highest score (34.63) by far, was able to confirm the directions observed from the MDEA (Table 7) in risk allele carriers compared to non-risk (Figure 14A, Table 9). Strongest positive correlations during Phase 1 (day 2 -day 0) could be discovered for *HADHA* (r = 0.45617, p-value < 0.0001) and *PARP1* (r = 0.37535, p-value < 0.0001) whereas *SMPD1* indicated a negative correlation (r = -0.34310, p-value < 0.0001). During Phase 2 (day 14 -day 2) *PARP1* showed a strong but inverse behavior (r = - 0.4160, p-value < 0.0001). Contrarily, *ACACA* (r = 0.37105, p-value < 0.0001) and *IRX3* (r = 0.36393, p-value < 0.0001) demonstrated high positive correlation coefficients for Phase 2 (day 14 -day 2). Moreover, *IRX3* and *IRX5* represent an alternating behavior and concurrently, positive correlations over the two phases (Phase 1 - *IRX5*: r = 0.30715, Phase 2 - *IRX3*: r = 0.36393, p-values < 0.0001). Generally, *ACACA* seems to be an important gene throughout the whole differentiation process (Phase 1: r = 0.30261 and Phase 2: r = 0.37105, p-values < 0.0001). Moreover, *UCP1* and *PGC1A* show distinct higher correlations during Phase 2

(day 14 -day 2) with solid coefficients in contrast to Phase 1 (*UCP1*: r = 1.8343*E-05 [n.s.] vs. r = 0.1673 [p-value < 0.0001] and *PGC1A*: r = - 0.0653 vs. r = 0.23018 [p-values < 0.0001]. All in all, a positive correlation coefficient as shown in Figure 14A for the gene *HADHA* during Phase 1 (day 2 -day 0) indicates an increase in risk allele carriers (compared to non-risk) in forward reaction towards the product over differentiation as effect for that specific gene. On the other hand, a negative correlation as represented in Figure 14B during Phase 1 (day 2 -day 0) indicates a decrease in the risk group (compared to non-risk) and away from the product (towards the substrate) over differentiation as effect for *SMPD1*. Moreover, it needs to be mentioned that the denser the cloud (scatter plot), the more significant is the correlation. Slight differences are visible in Figure 15 for I*RX5* and *IRX3* however, this could not be detected on the significance level (p-values < 0.0001) originating from linear regression analysis.

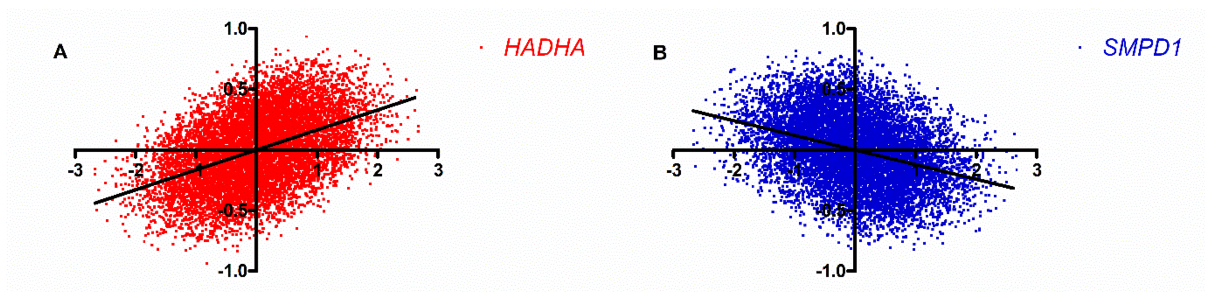


Figure 14: Positive correlation of *HADHA* (left, A) and negative correlation of *SMPD1* (right, B) with metabolite levels during Phase 1 (p-values < 0.0001).

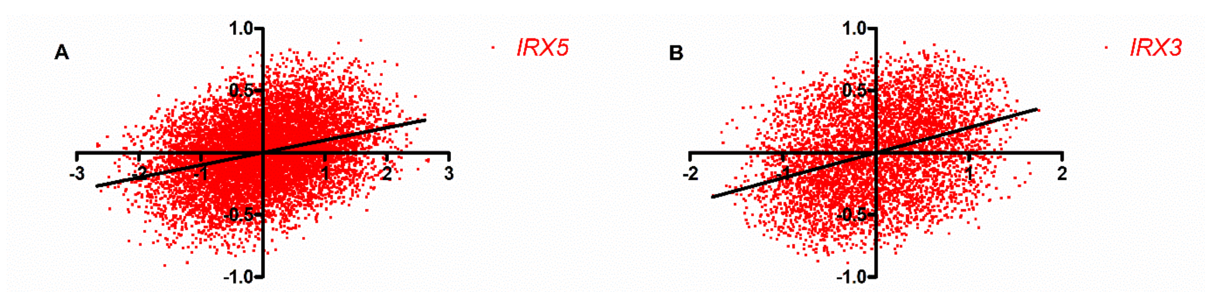
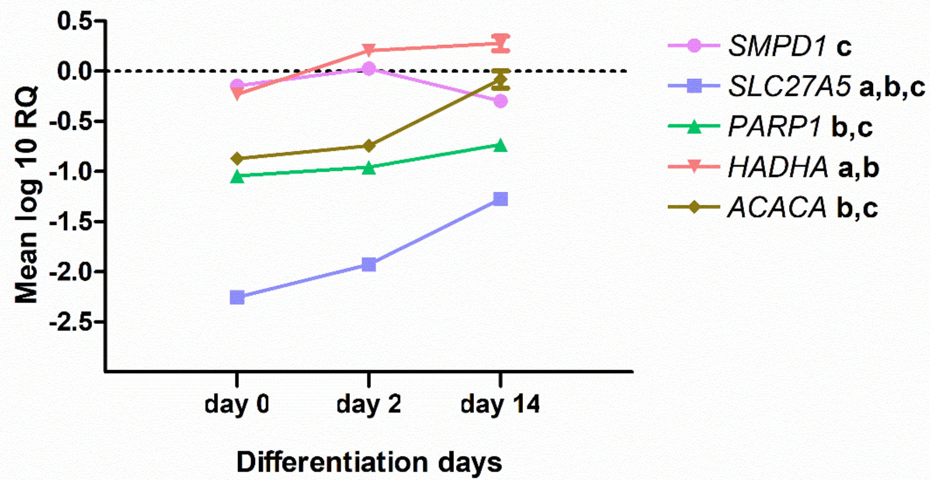


Figure 15: Positive correlations of *IRX5* (left, A) during Phase 1 and *IRX3* (right, B) during Phase 2 with metabolite levels (p-values < 0.0001).

In addition, the gene expression was demonstrated as course over the differentiation days for the complete cohort (n = 12, Figure 16) in order to test if statistical differences can be achieved across the harvesting days at least. Finally, we were able to observe significant differences on solely the gene expression level. Overall, the gene expression levels indicate to be low over the course of differentiation for all metabolomics-related targets. Moreover, all genes are increasing during the differentiation period, except for sphingomyelin phosphodiesterase (*SMPD1*). For this gene, a slight increase in expression from day 0 to day 2 was seen, while its lowest expression was found on day 14 as compared with day 2 (p-value < 0.01). The top 3 highest expressed genes are “hydroxyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha” (*HADHA*), *SMPD1* and “acetyl-CoA carboxylase alpha” (*ACACA*). The highest expressed gene *HADHA* among the study cohort is involved in the beta-oxidation of long chain fatty acids and cardiolipin remodeling. It reaches significance comparing day 0 and day 2 as well as day 0 and day 14 within the complete cohort (p-values < 0.001). A central gene in the fatty acid synthesis, the (*ACACA*), shows an overall increase in expression comparing day 0 vs. day 14 as well as day 2 vs. day 14 (p-values < 0.001). Interestingly, the solute carrier family 27 member 5 (*SLC27A5*) reaches significance across all differentiation days indicating a linear rise (p-value < 0.01 for day 0 vs. day 2; p-values < 0.001 for day 0 vs. day 14 and day 2 vs. day 14).



a, significant differences between day 0 and day 2
b, significant differences between day 0 and day 14
c, significant differences between day 2 and day 14

Figure 16: Metabolomic marker gene expression levels (mean log 10 RQ) depicted as course for the complete study cohort (n = 12) over differentiation days 0, 2 and 14. The mean is demonstrated with standard error of the mean (SEM). Exact p-values were *SMPD1*: c: $p < 0.01$; *SLC27A5*: a: $p < 0.01$; b: $p < 0.001$; c: $p < 0.001$; *PARP1*: b: $p < 0.01$; c: $p < 0.05$; *HADHA*: a: $p < 0.001$; b: $p < 0.001$; *ACACA*: b: $p < 0.001$; c: $p < 0.001$.

6. Discussion

The rs1421085 *FTO* variant indicates the highest polygenic risk for humans developing obesity. Moreover, it is associated with various obesity-linked phenotypes such as BMI, fat mass and basal metabolic rate (BMR). It was recently described by our group that this variant is shifting the differentiation of human adipocyte precursor cells towards a fat-storing phenotype and a decreased mitochondrial function **[94]**. The experiments described here served to further explore the functional consequences of the gene variant during adipose differentiation in human cells also exploiting the power of DI-FT-ICR mass spectrometry.

As a first step, I established a cell culture protocol that is suitable for the highly sensitive DI-FT-ICR MS. Subsequently, we performed ultra-high-resolution metabolome analysis in primary human preadipocytes of *FTO* risk allele carriers (rs1421085) during 2 phases of differentiation to elucidate metabolite patterns and linking this data to metabolic pathways and clinical characteristics of the previous human cohort study investigations. Laber and Forcisi et al. had linked the *FTO* variant (rs1421085) mechanism to whole-body physiology within a male population across different species **[96]**. In consideration and follow-up of that work, we decided to investigate a male study cohort with a homozygous genotype for the specific SNP (rs1421085) in comparison to a control group of homozygous non-risk allele carriers.

Our male donor group displayed an overall severe obesity defined by BMI. As expected, the group of risk allele carriers (CC) indicated tendencies of higher BMI values compared to the group of non-risk allele carriers (TT). This is in line with the findings by Frayling et al. who demonstrated that the *FTO* variant (rs9939609) of homozygous risk allele carriers resulted in a 1.67 times increased chance of developing obesity and a surplus of 3 pounds per allele in body weight **[82]**. However, it cannot be excluded that the difference in BMI was accidental, as no representative population was used for recruitment and as the sample size was rather small (n = 6 per subgroup). Moreover, it has to be considered that an analysis comparing 96 BMI-related variants indicated the highest association with BMI and fat mass for the *FTO* SNP (rs1558902) **[223]**.

On the metabolome niveau substantial differences were observed in terms of compound classes and the gene-driven MDEA comparing risk (CC)- and non-risk allele carriers (TT). All in all, this indicates that for distinguishing the metabolome fingerprint between risk- vs. non-risk allele carriers of this specific SNP (rs1421085), a smaller cohort size could be sufficient. We have described a decrease in steroids inside the cells of risk allele carriers compared to non-risk within Phase 1 (day 2 -day 0). In previous independent human cohort studies, when male subjects underwent an oral glucose tolerance test (OGTT), the class of steroids has been enhanced for the immediate response (0 -1 h) but decreased for the short-term response within risk allele carriers (CC) compared to non-risk (TT). Moreover, the subcutaneous white adipose tissue of male rs1421085-DEL82 mice on a high-fat diet has indicated a downregulation of steroids analyzed via untargeted ultrahigh-resolution metabolomics **[96]**. We were able to replicate a downregulation of steroids within Phase 1 (day 2 -day 0). An earlier downregulation can be possibly explained by the subculturing of PACs in medium with already high glucose concentrations (17.51 mM/3151.0 mg/L) for about 2 weeks before induction (= day 0). This time window between day 0 and day 2 could most likely represent the above-mentioned short-term response of an OGTT. Interestingly, the enzyme sphingomyelin phosphodiesterase (EC 3.1.4.12) encoded by *SMPD1* is mainly but not solely responsible for breaking down sphingomyelin into ceramide and phosphatidylcholine. Patients with Niemann-Pick disease indicate mutations in the enzyme sphingomyelin phosphodiesterase 1. They show accumulations of sphingomyelin in lysosomes and additional alterations in cholesterol signaling pathways, the so-called precursor for steroids. A plasma analysis via LC-MS demonstrated increased 7-ketocholesterol in sphingomyelinase-deficient subjects **[224]**. However, our MDEA findings indicated no deficiency but downregulation for *SMPD1* during Phase 1 (day 2 -day 0) which was confirmed by a definite negative gene-metabolite correlation coefficient ($r = -0.34310$, p-value < 0.0001; Table 9 and Figure 14B) in terms of a decrease in risk allele carriers compared to non-risk towards the substrate (sphingomyelin) as effect for *SMPD1* within the reaction. In literature, sphingolipids in general indicated linkage to cholesterol **[225]**. Further, the sphingolipid ceramide was demonstrated to play a critical role

in metabolic diseases **[226]**. Also, adipose tissue inflammation was connected to *SMPD1* expression levels, ceramide and liverfat **[227]**. Moreover, we can connect in this context the gene candidate *HADHA* which encodes for the enzyme long-chain enoyl-CoA hydratase that catalyzes the last three steps of the beta oxidation in the mitochondria **[228]**. The product acetyl-CoA deriving from the oxidization of fatty acids serves as the first substrate for cholesterol synthesis which is in line with our observations from the MDEA since *HADHA* represents a downregulation within Phase 1 (day 2 -day 0) and simultaneously, steroids are decreased inside the cell of risk allele carriers compared to non-risk, too.

However, during Phase 1 (day 2 -day 0) the highest downregulation has been achieved for glycerophospholipids in cells of risk allele carriers compared to the non-risk group. Contrarily, observing an inverse behavior with a clear shift towards an upregulation of glycerophospholipids during Phase 2 (day 14 -day 2). Glycerophospholipids are mainly constituted of two long-chain fatty acids and those are esterified with their -COOH groups to the hydroxyl groups at the 1- and 2- positions of glycerol 3-phosphate (G3P). Moreover, there is a residue connected to one of the phosphates from G3P. This residue can be ethanolamine, choline, inositol or serine and carries a positive charge, whereas the phosphate is negative. The hydrophobic part is represented by the fatty acids and the head with the different charges is hydrophilic. It is well known that glycerophospholipids are the major cell membrane components and create bilayers with a hydrophobic core. Phosphatidylcholine represents the main component as glycerophospholipid within the cell membrane. The glycerophospholipids originate from G3P that forms, with 2 fatty acyl-CoA, the phosphatidate and thereby, releases a phosphate resulting in diacylglycerol (DAG). At this point the DAG can continue building the triacylglycerol or synthesizing glycerophospholipids **[229]**. The inner membrane of the mitochondria (IMM) constitutes of a unique glycerophospholipid, the cardiolipin. It is synthesized in the inner leaflet of the IMM and will be subsequently remodeled in terms of fatty acids and finally integrated into the IMM. Generally, cardiolipin has a crucial role in influencing different protein complexes in the mitochondria by also getting incorporated into those **[230]**. Moreover, it is related to the respiratory chain and its various complexes which participate in

the transfer of electrons and the synthesis of ATP within the IMM **[231]**. Further, interactions with proteins, such as UCP1, were reported as well **[232]**. Nevertheless, cardiolipin still has a function as a membrane member dividing the cell and hereby, generating various states (e.g. pH) within the cell milieu **[231]**. In literature, it was stated that cardiolipin has a key position in the circuitry of apoptotic signaling in mitochondria in terms of inducing the procedure **[231]**. Moreover, changes in the constitution and substance of cardiolipin reported linkage to early stages of diabetes already more than a decade ago **[233]**. Cardiolipin synthase (*Crls1*) mRNA levels were shown to be significantly reduced in subcutaneous (sc) adipose tissue of diabetic subjects. This has indicated a negative correlation of *CRLS1* gene expression levels in human sc WAT with homeostasis model assessment 2 (HOMA2) measures of insulin resistance and positive correlations with HOMA2 measures of insulin sensitivity **[234]**. In our study, the strongest positive gene-metabolite correlation during Phase 1 (day 2 -day 0) was achieved by *HADHA* ($r = 0.45617$, p-value < 0.0001; Table 9 and Figure 14A), the gene that is responsible among others for the remodeling of cardiolipin **[230]**. The strong positive correlation indicated an increase in risk allele carriers in comparison to non-risk along the reaction over differentiation as effect for *HADHA*. However, the positive correlation was the opposite of what we expected based on the MDEA results. Intriguingly, the IMM is also the location where the synthesis of steroids begins with the step of converting cholesterol to pregnenolone via the cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1) in the adipocyte **[235]**. Hereby, we can link both compound classes, steroids and glycerophospholipids, that are decreased during Phase 1 (day 2 -day 0). In the literature, an upregulation of *IRX3* has been linked to an increased transcriptional activity of *UCP1* and this has been described in the process of browning as well as mitochondrial fission **[236, 237]**. On the other hand, the mitochondrial fusion is required for steroid biosynthesis **[238]**. Indeed, our results of the gene-metabolite correlation show a distinct positive correlation for *IRX3* ($r = 0.36393$, p-value < 0.0001) within Phase 2 (day 14 -day 2) whereas *UCP1* ($r = 0.16730$, p-value < 0.0001) also indicates a positive correlation and a change compared to Phase 1 (day 2 -day 0) within the risk group in relation to non-risk. These positive coefficients reveal an increase in the risk group

along the reactions over differentiation as effect of both genes. Moreover, a recent transcriptome analysis has described an upregulation of genes related to the organization of the extracellular matrix (ECM) in risk allele carriers (*FTO* rs1421085). Further, they demonstrated that ECM organization pathway is crucial for restraining the mitochondrial thermogenesis **[239]**. All in all, we can connect steroid production and non-shivering thermogenesis within the mitochondrial environment **[236, 238, 240, 241]**.

It is also noteworthy in this context that glycerophospholipids are markers for metabolic diseases such as obesity and T2D. The identification of metabolites significantly altered in obesity and T2D based on the *FTO* genotype (rs9939609) have shown highest effects for the compound class of glycerophospholipids in serum samples **[158]**. This compound class has been described as a T2D marker in blood for more than a decade **[242]**. In addition, recent research described the class of glycerophospholipids within the cell environment as a marker of metabolic disease **[243]**. Böhm et al. have shown that most phosphatidylcholines increased intracellularly (cell lysates) at day 20 of differentiation from metabolically unhealthy obese (MUHO) donors analyzed via targeted metabolomics. Furthermore, altered arachidonic acid (AA) metabolism has been described as higher among MUHO **[243]**. However, our findings indicate a change in reactive oxygen species (ROS) and nitric oxide (NO) within risk allele carriers considered as only a secondary effect of the *FTO* rs1421085 variant (Figure 18).
Interestingly, during Phase 1 (day 2 -day 0) when glycerophospholipids were downregulated comparing risk- with non-risk allele carriers, the pathways of beta oxidation, tricarboxylic acid (TCA) cycle and production of ROS were decreased, too. In contrast, an inverse behavior was observed for Phase 2 (day 14 -day 2). The ER, in general, has various cellular functions, whereas the smooth ER is mainly involved in the production of membrane lipids and their intermediates including glycerophospholipids, ceramides, and cholesterol. Overall, the ER is involved in inflammatory pathways and insulin signaling, nutrient metabolism as wells as cell proliferation and -death via the unfolded protein response (UPR). A chronic activation of this pathway was discovered in adipose tissue from diet-induced and genetic mouse models of obesity, but also in human obesity **[244]**. The locations of the ER that are in contact with

mitochondria are named as mitochondria-associated ER membrane (MAM). The MAM constitutes among others of calcium-processing proteins like IP_3 receptors (IP_3Rs) that are highly available in MAM and support reaching a fast interorganellar calcium balance. This is essential for signaling within the cell, adaptation, and viability. Calcium within the cytoplasm is usually taken up into both organelles, mitochondria and ER, where it changes the membrane potential and shifts mitochondrial pH, impacting the downstream production of ATP and reactive oxygen species (ROS) **[245]**. Principle fuels for the production of ATP in the mitochondrial environment are pyruvate and fatty acids among others. Before getting oxidized within the TCA cycle, they are transferred from the cytosol via the inner mitochondrial membrane into the matrix. On the one hand, calcium boosters the TCA cycle via calcium-dependent dehydrogenases and contrarily, an increased ATP/ADP ratio blocks it. ROS are known to be by-products of the fatty acid metabolism and corresponding oxidative phosphorylation in order to produce ATP **[229]**. Moreover, it has been demonstrated that ROS can activate a translocation of the enzyme sphingomyelinase interfered by lysosome exocytosis that in return depends on intracellular calcium levels **[246]**.

In general, changes in the ER cavity oxidizing setting can cause incorrect disulfide bond formation and aggregation of misfolded polypeptides, a state called as ER stress. Likewise, the ER cavity serves as a calcium depot location **[247]**. The coupling of both organelles (ER and mitochondria) and the corresponding transport of calcium within those, justifies an upregulation of the mitochondrial metabolism during short term ER stress. However, when the ER stress sustains, the metabolism of mitochondria is downregulated as a result of intolerable high calcium concentrations leading to apoptosis **[245]**. All in all, ER stress has been described as contributor to metabolic diseases such as obesity and T2D **[244, 245, 247]**.

In addition, we have observed a clear downregulation of glycerolipids during Phase 2 (day 14 -day 2) within cells of risk allele carriers in comparison to non-risk. This discovery can be underlined by our quantifications of lipid storage at day 14 with two independent methods (OilRed quantification and microscope image analysis) and the gene expression levels of the fat storage marker leptin. None of these results have reached statistical significance

comparing both subgroups (CC vs. TT), possibly also due to the relatively small cohort size (n = 12). All in all, only a slight trend towards higher lipid storage for risk allele carriers could be discovered and hereby, underlining the decreased glycerolipid levels and the missing replication of a higher lipid accumulation among risk allele carriers. A non-targeted lipidome analysis within subcutaneous AT supports this assumption. They indicated that major parts of the lipids (~ 97%) within a lipid droplet belong to the glycerolipid class which is followed by the glycerophospholipds **[248]**. Moreover, glycerophospholipids as well as glycerolipids act as second messenger molecules, whereby specific phospholipids can activate *PPARγ*, the key regulator of adipocyte differentiation **[229, 249]**. The Phosphatidylinositol bisphosphate (PIP_2) is located within the membrane. During the process of hydrolysis and with the help of a phospholipase, inositol trisphosphate (IP_3) and diacylglycerol (DAG) are produced. The glycerolipid DAG remains in the cell membrane and can activate inflammation processes **[250]**. This explanation is in contrast to a decrease of glycerolipids but indeed our results indicate increased inflammatory processes within Phase 2 of differentiation in risk allele carriers compared to non-risk (Figure 18). On the other hand, IP_3 proceeds to the endoplasmic reticulum (ER) and binds to receptors that release calcium ions which in return activate several processes within the cell as described above **[229]**.

However, according to an upregulation of glycerolipids in the medium of risk allele carriers during Phase 1 (day 2 -day 0), those must have been produced in the cell and consequently, transferred outside. Hereby, it needs to be emphasized that lipids were absent in the basal medium throughout the complete differentiation process and therefore, could not represent physiological plasma conditions either **[251]**. The original substrate for producing glycerol is glucose. This is in line with previous findings where adipocytes were incubated in different concentrations of glucose. Our basal medium (DMEM-F12) glucose concentration (3151 mg/L = 17.5 mM) is even exceeding the values of the high glucose incubation group (14 mM) **[252]**. Then glycerol got secreted into the medium via most likely aquaporin 7 (AQP7) transporters which are highly expressed in adipocytes. It was demonstrated that in obesity the expression of AQP7 is downregulated indicating that in our risk group the transporters still function within

the early Phase 1 (day 2 -day 0) **[253].** Nonetheless, a smaller contribution of the glycerolipids remained in the cell in order to proceed in the direction of glycerophospholipid synthesis with subsequent transport to e.g. the cell membrane. Hereby, possibly explaining the decreased glycerophospholipid levels observed inside the cell of risk- compared to non-risk allele carriers during Phase 1 (day 2 -day 0).

In the human prediabetes lifestyle intervention study (PLIS) cohort, the compound class of glycerolipids was demonstrated to be upregulated during the immediate response (0 -1 h) and to be downregulated for the short-term OGTT response (1 h - 2 h) **[96, 254]**. We have been able to replicate *in vitro* the glycerolipid metabolic phenotype (metabotype), in the media for Phase 1 (day 2 -day 0) as well as within the cell during Phase 2 (day 14 -day 2) among the risk group, that has been highlighted in male subjects undergoing a nutritional challenge. Intriguingly, it was demonstrated that among environmental influences, nutrition has the highest impact on developing obesity among *FTO* gene variant carriers **[255, 256].** Elevated hunger as well as increased emotional disinhibition score have been found among risk allele carriers (CT, CC) of the *FTO* rs1421085 SNP **[256-258]**. These features of risk allele carriers may promote an unhealthy eating behavior characterized by larger meal sizes and poorer food choices **[259]**. Interestingly, inverse correlations of obesity and coffee intake have been demonstrated for the phospholipid-family found in serum **[260]**.

Comprehensive genetic studies linking BMI-defined obesity to single nucleotide polymorphisms (SNPs), have indicated the central nervous system (CNS) as the principal biological tissue, as well as that the *FTO* haploid genotype seems to influence a manifestation of genes within the brain **[261, 262]**. Son et al. have described the mechanism and function of *Irx3* and *Irx5* in the hypothalamus on energy metabolic processes. Interestingly, they have linked a declined food intake to lower levels of *Irx3* and *Irx5* **[263]**. The *FTO* gene connects BMI and body composition but also the desire of favoring specific food **[264]**. This can be in consequence of a direct impact of the *FTO* gene on expression levels within the brain or due to an indirect genetic-driven metabotype. However, it could be a synergy of both.

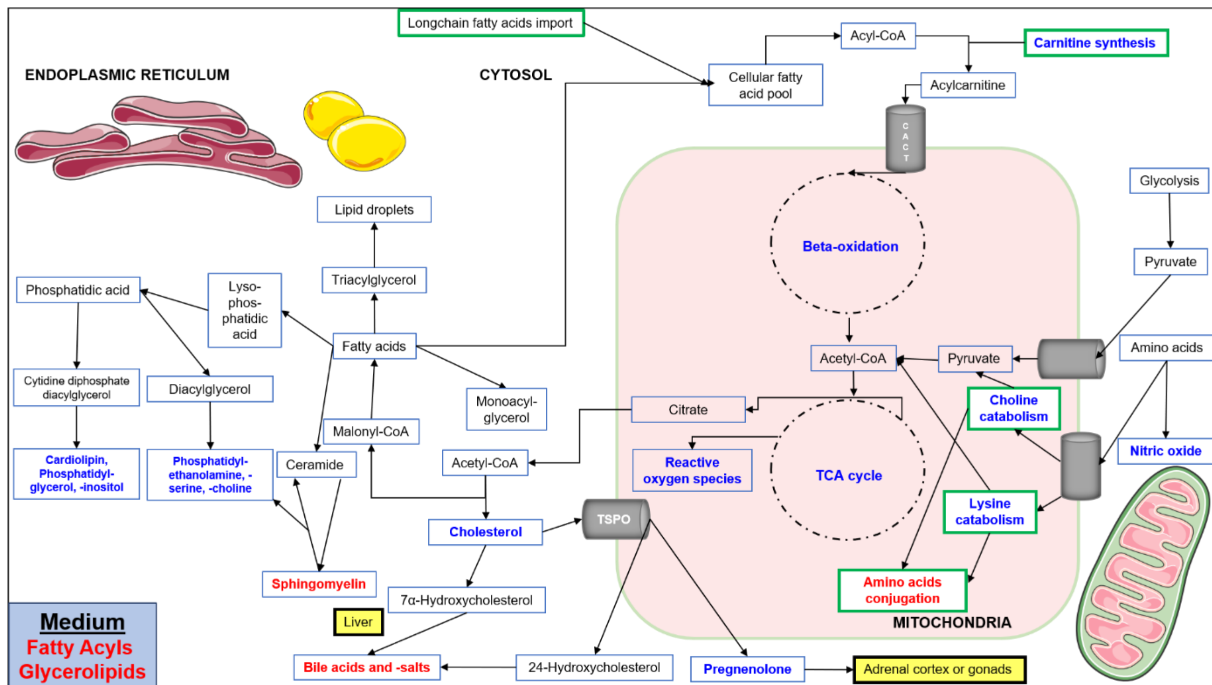


Figure 17: Scheme for pathways related to the cell and medium within Phase 1 of differentiation (day 2 -day 0) in rs1421085 *FTO* risk allele carriers compared to non-risk [209]. Legend: Green boxes: pathways; Blue boxes: compound classes; Bold colors: **up/downregulated**; Transporter for pyruvate: Pyruvate/H+-Symporter; CACT: Carnitin-Acylcarnitin-Transporter; TSPO: translocator protein.

The overall pathway schemes (Figures 17 and 18) represent the fat cell inside of risk allele carriers (CC) compared to non-risk (TT) during both phases of differentiation (Phase 1 and Phase 2) and the summary of results in terms of mass differences, compound classes as well as its interpretation. During Phase 1 of differentiation (day 2 -day 0) the overrepresentation analysis (ORA) on the Reactome database displayed a downregulation of mass differences for the carnitine synthesis and the corresponding decrease of beta-oxidation as well as the tricarboxylic acid (TCA) cycle, also known as Krebs cycle (Figure 17).

The stagnation within the preadipocyte phase may arise from a downregulation of mitochondrial thermogenesis characteristic for risk allele carriers (rs1421085) which is confirming previous work of Claussnitzer et al. **[94]**. They indicated a disruption of the ARID5B motif in risk allele carriers and herewith, leading to an impaired binding to its transcription factor binding site. This activates preadipocyte enhancers and, consequently, results in an increased expression of downstream targets *IRX3* and *IRX5* in the preadipocyte phase (between day 0

and day 2). Later, this disruption leads to an autonomous shift favoring lipid-storing white adipocytes over energy-consuming beige adipocytes. The mitochondrial thermogenesis has indicated a reduction with a factor of 5 in risk allele carriers **[94]**. Besides, Laber and Forcisi et al. have shown a 35% lower subcutaneous adipose layer thickness of rs1421085-DEL82 male mice. This has been supported by a lower body weight as well as overall fat mass **[96]**. However, an autonomous shift within Phase 2 (day 14 -day 2) and corresponding higher fat storage for risk allele carriers compared to non-risk was not recapitulated. But during Phase 1 (day 2 -day 0), we discovered a positive gene-metabolite correlation of *IRX5* in terms of upregulation in the risk group along with the reaction for *IRX5* and expectedly, no influence of the browning markers (*UCP1*: r = 1.8343 E-05 and *PGC1A*: r = - 0.0653). Further, the correlation analysis showed an absent influence of *IRX3* (r = 0.03227) within Phase 1 (day 2 -day 0) which might be one explanation for the missing detection of an autonomous shift in favoring lipid storing white adipocytes during Phase 2 (day 14 -day 2). Moreover, it needs to be emphasized that our subject number represented only a third of the cohort of Claussnitzer et al. **[94]**.

One of the products of the TCA cycle, citrate, has been shown to be converted into acetyl-CoA outside of the mitochondria and thereby taking two possible directions. On one hand, it can be converted into cholesterol and then transported back via the translocator protein (TSPO) into the mitochondria. This is considered as the first step of steroid hormone biosynthesis continuing outside of the mitochondria and whereby the steroids are downregulated during Phase 1 (day 2 -day 0) within the cell **[235].** The steroidogenic acute regulatory protein (StAR) is an important enzyme that monitors and navigates the process of steroidogenesis and its modulation by means of cholesterol import into mitochondria **[265]**. The product pregnenolone belonging to the steroids class continues to be processed by the adrenal cortex and/or gonads. On the other hand, the minor product 24-Hydroxycholesterol proceeds to the pathway of bile acid and -salt production which indicated an upregulation. This pathway continues in the liver thereby representing the major location for bile acid- and bile salt synthesis **[235]**.

In addition, acetyl-CoA can be converted into fatty acids via malonyl-CoA. Further, the fatty acids are conjugated to participate in the synthesis of glycerolipids (monoacylglycerol, diacylglycerol and triacylglycerol) and consequently, glycerophospholipids (e.g.: Cardiolipin and Phosphatidylcholine etc.) via phosphatidic acid indicating a downregulation within Phase 1 (day 2 -day 0). Moreover, fatty acids can enter the synthesis of sphingomyelin via the compound ceramide. During Phase 1 (day 2 -day 0) our gene-metabolite correlation analysis revealed a decrease in risk allele carriers in relation to non-risk towards the substrate (sphingomyelin) as effect for *SMPD1* ($r = - 0.34310$, $p\text{-value} < 0.0001$). Also, synthesized fatty acids contribute to the cellular pool and thereby, reentering the mitochondria which requires carnitine (CACT: Carnitin-Acylcarnitin-Transporter) and herewith, provide acyl-CoA for the beta oxidation taking place in the mitochondria. The long-chain fatty acid import via the plasma membrane is enabled by the bile-acyl-CoA synthetase (encoded by the gene *SLC27A5*) (Table 7). The number 2 selected target candidate *SLC27A5* (score 9.02) plays an important role in contributing to the cellular pool of fatty acids by importing long-chain fatty acids especially within Phase 1 (upregulation in MDEA).

Amino acids as basal media ingredients are able to enter the cell and subsequently the mitochondria. The downregulated lysine- and choline catabolism participate in beta oxidation as well as the TCA cycle via pyruvate and acetyl-CoA. On the other hand, amino acid conjugation is subsequently upregulated which is in line with the general anabolic state. The only significant differences in terms of compound classes in the extracellular milieu have been discovered for glycerolipids and fatty acyls during Phase 1 (day 2 -day 0) comparing risk- with non-risk allele carriers. Glycerol can exit the cell via aquaporin 7 which is expressed principally in adipocytes. **[253]**. The group of fatty acyls encompasses the substrates pyruvate, acylcarnitine, saturated- (SFA), monounsaturated- (MUFA), polyunsaturated (PUFA) fatty acids, and eicosanoids **[266].** Fatty acids can dissociate through the cell membrane without transporters although there is one supporting this process (CD36 fatty acid transporter). Outside of the cell, Triacylglycerol (TAG) is present with the transport protein “lipoprotein”.

However, this complex can be hydrolyzed with the support of the enzyme lipoprotein lipase into its components: non-esterified fatty acids (NEFAs), glycerol and the lipoprotein **[252].**

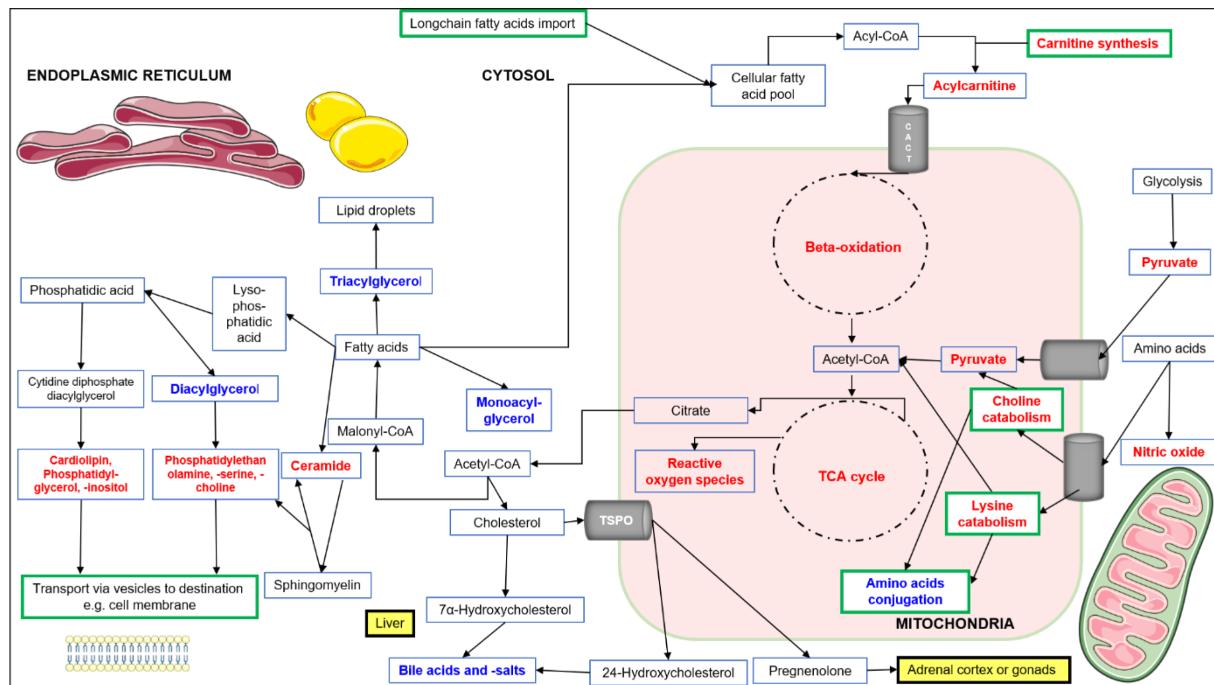


Figure 18: Scheme for pathways related to the cell during Phase 2 of differentiation (day 14 - day 2) in rs1421085 *FTO* risk allele carriers compared to non-risk [209]. Legend: Green boxes: pathways; Blue boxes: compound classes; **Bold colors: up/downregulated**; Transporter for pyruvate: Pyruvate/H+-Symporter; CACT: Carnitin-Acylcarnitin-Transporter; TSPO: translocator protein.

A shift towards energy-consuming pathways has been observed during Phase 2 of differentiation (day 14 -day 2) inside the adipocytes comparing risk- (CC) to non-risk allele carriers (TT). Contrarily to Phase 1 (day 2 -day 0), the carnitine synthesis as well as the following beta oxidation and TCA cycle are upregulated. Hereby, can be assumed that possibly a part of the increased levels of fatty acyls (e.g. acylcarnitine, pyruvate) from Phase 1 (day 2 - day 0) in the media had been taken up into the cell serving as substrates for the beta oxidation as well as the TCA cycle and thereby boosting those. Further, the choline- and lysine catabolism are upregulated and contrarily, the amino acid conjugation downregulated which are further indications for the catabolic state of the cell. Those pathways participate in beta oxidation as well as the TCA cycle via pyruvate and acetyl-CoA indicating another basis for the observed upregulation.

Evidence of oxidative stress and involved inflammatory pathways with regard to reactive oxygen species (ROS) and nitric oxide (NO) production are observed to be upregulated during Phase 2 of differentiation (day 14 -day 2). The already mentioned NO is produced by the inducible nitric oxide synthase taking place in activated macrophages which can impair mitochondrial dysfunction in PACs **[196]**. This enzyme is encoded by the gene *NOS2* that is one of our top selected target candidates (score 3.07) originating from the MDEA. Moreover, an overloaded tricarboxylic acid (TCA) cycle and, consequently, the mitochondrial respiratory chain results in an increased ROS production leading to oxidative stress which in turn promotes inflammation **[198]**. Moreover, contradictory results have been shown for the fatty acid metabolism whereas the central fatty acid synthesis enzyme “acetyl-CoA carboxylase 1” is upregulated according to MDEA during Phase 2 (day 14 -day 2), however, requires biotin for functioning. Investigations from the Reactome database indicated defects in biotin metabolism being upregulated during Phase 2 as well (Table 8). Additionally, a clear upregulation of the compound class of glycerophospholipids is demonstrated and those can be transported to destinations such as cell membranes (e.g. IMM). Hereby, linking the fatty acid metabolism with the acyl-chain remodeling of cardiolipin **[230]**. Moreover, the pool of glycerophospholipids will be increased by breaking down sphingomyelin into ceramide and phosphatidylcholine supporting the glycosphingolipid metabolism.

Due to the increased levels of energy-consuming processes, it could be speculated that this is related to an increased mitochondrial thermogenesis. Also, the gene-metabolite correlation analysis indicates an increase in thermogenesis marker such as *PGC1A* and *UCP1* for Phase 2 (day 14 -day 2) in the risk group compared to non-risk along the reaction pathway. However, this does not mean that browning markers such as the uncoupling protein 1 are active and therefore, cannot be equated to mitochondrial thermogenesis **[267]**. Moreover, within our MDEA we were not able to appreciate the role of the uncoupling protein *UCP1* or Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC1A*). *UCP1* encodes for proteins that do not indicate an enzyme number within the UniProt Database (Appendix 16, Table A9) and, consequently, could not be included in our analysis. Also, further investigations

within the Binding Database aiming to include ligand information, did not reach any more insights. Since there is no UCP1 activity expected within white adipocytes **[172]**, the role of mitochondrial thermogenesis within risk allele carriers (CC) could not be addressed in more detail.

Summarizing the overall pathway schemes and the involved processes originating from the MDE- and Reactome database OR analysis have indicated a stagnation of beta oxidation and TCA cycle among risk allele carriers compared to non-risk within Phase 1 (day 2 -day 0). Contrarily, the metabolic pathways of beta oxidation as well as the TCA cycle were enhanced and possibly overloaded indicating additional oxidative stress and inflammation as secondary outcome for *FTO* rs1421085 variant during Phase 2 (day 14 -day 2). The stagnation within Phase 1 (day 2 -day 0) may arise from a downregulation of mitochondrial thermogenesis characteristic for risk allele carriers (rs1412085) which is confirming previous work by Claussnitzer et al. **[94]**. The variant (rs1421085) hereby disrupts ARID5B repressor binding and this leads to a doubling of the expression of the distant targets *IRX3* and *IRX5* during early adipocyte differentiation. Moreover, it results in a cell-autonomous transformation towards lipid-storing white adipocytes over energy-consuming beige adipocytes by a decrease in mitochondrial thermogenesis by factor 5. Claussnitzer et al. have demonstrated that rescuing the ARID5B motif in risk allele carriers by CRISPR-Cas 9 editing reconditioned *IRX3* and *IRX5* repression and activated thermogenesis with factor 7 **[94]**. However, an autonomous shift and consequent higher fat storage in terms of e.g. glycerolipids within risk allele carriers compared to non-risk was not replicated during Phase 2 (day 14 -day 2).

V. Conclusion and outlook

The main aim of the Thesis was the establishment of a cell culture protocol suitable for the untargeted metabolomics approach using the highly sensitive DI-FT-ICR MS and performing experiments in adipocyte precursor cells from *FTO* risk/non-risk allele carriers with the lowest possible number of cells. The study specifically aimed to unveil metabolite patterns of primary human PACs from male donors during differentiation with the homozygous rs1421085 *FTO* genotype (CC vs. TT) in order to elucidate the metabolic pathways linked to the genetically driven metabotype discovered in former studies across species.

One limiting factor of our study is the missing confirmation of the target candidates with a proteomics approach. Moreover, we did not apply the high-cost compound labeling of the media ingredients in order to determine the secretome of the cells more accurately. In addition, we have a small number of study subjects due to the high selectivity of including homozygous carriers and males only. Therefore, on the gene expression level, it was not possible to reach any significant differences between the two groups and across the two phases of differentiation. It seems that for appreciating the differences on the mRNA level, a larger cohort is mandatory due to large interindividual variance.

However, we describe a sophisticated picture by analyzing the metabolome in terms of compound classes for cell lysates and the corresponding media using untargeted, ultra-high-resolution metabolome analysis via DI-FT-ICR MS which is a unique technique. Moreover, we performed a gene-driven mass difference enrichment analysis highlighting the included genes and corresponding encoded enzymes involved in the pathways characteristic for risk allele carriers (CC) during fat cell differentiation. In addition, we applied a gene-metabolite correlation analysis in order to deepen our assumptions. To our knowledge, the investigation of the metabolic pathways of primary human preadipocytes considering the specific *FTO* genotype (SNP rs1421085) in combination with the untargeted metabolomics approach is a novel and unique strategy to date.

Together, our data describe *FTO* genotype (rs1421085) dependent pathways of risk allele carriers indicating a stagnation of mitochondrial beta oxidation during the early phase of cell differentiation (day 2 -day 0) but without a significant favoring in lipid storage within Phase 2 (day 14 -day 2). We reveal an rs1421085-specific glycerolipid-, glycerophospholipid- and steroid metabotype within the two defined phases of differentiation (Phase 1 and Phase 2). Moreover, I established a robust PACs differentiation protocol suiting the highly sensitive DI-FT-ICR MS and being able to be implemented in future cell culture investigations in terms of metabolome analysis.

As outlook, it might be of interest to combine further multi-omics approaches such as transcriptomics (gene expression levels), proteomics (enzymes) or high-content imaging (cellular processes) in order to dissect and understand the altered pathways in more detail. Also, further follow-up studies should investigate in a cell culture differentiation protocol for visceral preadipocytes suiting the highly sensitive DI-FT-ICR MS which could provide further insight on the role of the visceral fat depot within the *FTO* genotype (rs1421085). Moreover, it would be highly recommended to increase the study group size for future investigations to reach significant differences on the gene expression level and integrate more mass spectrometry methods such as LC-MS in order to detect the specific compounds. Lastly, we would endorse a compound labeling approach for evaluating the media samples in terms of compound classes more specifically. All in all, the outcomes and the established protocol provide a great fundament for further in-depth analyses of genotype-driven changes in fat cell development and function.

VI. Methods

1. Cell culture in-vitro experiments

a. Fat tissue acquisition and isolation of primary human preadipocytes (PACs)

The collected subcutaneous tissue originated at the site of the surgical incision from beneath the skin **[210]** . The fat tissue collection and isolation were performed as described elsewhere **[211]**.

b. Differentiation of PACs into adipocytes

The primary human subcutaneous PACs had been isolated from fat tissues as described in SOP 05 (Appendix 5) and stored in vials à 500.000 cells/1 mL at -80 °C. Before initiating the thawing process under sterile conditions, the proliferation medium (Table 10) had been pre-warmed in a 37 °C water bath until hand-hot. Aliquots of proliferation medium (1 mL and 18 mL per vial) were prepared in separate tubes.

Thawing

In general, the thawing process of primary human PACs was considered as a critical step (Appendix 8, SOP 11). One vial à 1 mL contained 500,000 PACs and stayed on dry ice until starting the actual thawing procedure. Then the vial was placed into the 37 °C water bath until the sample was liquid. Moreover, the closed vial was disinfected with 80% ethanol (EtOH) before working under the sterile workbench.

The thawing procedure followed the subsequent scheme:

1) pipetting 1mL of the proliferation medium with a 5 ml serological pipette out of the 18 ml tube
2) taking 1 mL cell suspension out of the vial
3) adding 1 mL of proliferation medium

It was crucial to slow down the pipetting process as soon as any cells were in the tip. Afterward, the proliferation medium-PACs-solution-mix was carefully transferred into the 18 mL aliquot tube. Avoiding any further stress for the PACs, the pipet tip was always kept in contact with the tube wall during the transfer.

Making sure all cells were taken out of the cryotube, an additional rinsing step was applied, using the 1 mL proliferation medium aliquot and being transferred into the same tube. A mixing step was done before transferring 10 mL of the proliferation medium-PACs-suspension into each of the two T25 flasks. Both flasks were mixed carefully by 3 forward/backward as well as 3 left/right movements (in total 3 repetitions) making sure that all cells were spread evenly over the flask surface. The PACs stayed in the incubator at 37 °C and 5 % CO_2 conditions. A mandatory complete media change took place 24 hours later. Avoiding a draining of cells during the media change procedure, a leftover of about 1 mL had been left in each flask.

Splitting/subculturing

The cell proliferation procedure (6 -7days until confluence) was important to build an extracellular matrix (ECM). According to the needed number of cells for seeding, the splitting step took place between 75 % -100 % confluence of the T25 flasks (at confluence ca. 0.8 -1 million available cells per T25 flask). Herefore, medium was removed and PACs were two times washed with 10 mL phosphate-buffered saline (PBS). After removing the PBS completely, 1 mL of trypsin/EDTA was added to each flask. The flasks were incubated at 37 °C for 5 -10 min. The cell detaching progress was checked by eye or under the microscope. If necessary, physical support had been applied. Afterwards, 9 mL of pre-warmed proliferation medium were added to each flask, well rinsed, and transferred into a collection tube. After mixing the cell suspension, a 50 µL aliquot was taken and pipetted into a 1.5 mL tube. Moreover, 50 µL of Trypan blue had been added to count cells via the 'Neubauer Chamber' under the microscope (Appendix 7, SOP 10).

After assessing the cell number, the count had been inserted into the following formula:

$$\frac{\text{Counted cells}}{8} * 2 * 10\,000 = \mathbf{x}\ \text{Available cell number}/_{\text{mL}}$$

The calculated cell number (per mL) was projected to the total volume to assess the maximum of available cells. Further calculations of cell suspension and proliferation medium were done before seeding PACs into the plates and/or T25 flasks (see formulas below). If possible, preservation cell culture flasks were included as backup.

- <u>6 Well plates</u>: seeding of 250,000 PACs/6 well plate

$\frac{250{,}000}{\mathbf{x}} = \mathbf{y}\ \text{mL cell suspension}$ <u>Total volume</u>: 12.5 mL – y mL = z mL proliferation medium

- <u>T25 flasks</u>: seeding of 125,000 PACs/flask

$\frac{125{,}000}{\mathbf{x}} = \mathbf{y}\ \text{mL cell suspension}$ <u>Total volume</u>: 10.5 mL – y mL = z mL proliferation medium

A volume of 2 mL of the final proliferation medium-PACs-suspension was transferred into each well of a 6 well plate (see Figures 3 and 4 for plate layouts). For an even distribution of PACs within the well: 3 forward/backward, 3 left/right movements, and additional 3 left/right circle motions were performed in total 3 times before placing those plates into the incubator. When PACs had been seeded into wells, the general media change kept the following accurate and homogenous rhythm: "Monday/Thursday", "Tuesday/Friday", "Wednesday/Saturday" as well as the same time of a day by differentiating between "before noon" or "afternoon". Moreover, it was necessary to keep about 300 µL of the old medium during the change in the well to avoid draining of the cells.

Differentiation

A 24 h media change with proliferation medium without fetal calf serum (FCS; 1 mL/well, including 2x2 mL washing steps) for plates at about 95 % confluence had been performed for harvesting day 0 samples only. Due to the absence of FCS, a proliferation of an additional 2-5 % was expected for a 24 h time frame after testing that in previous pilot studies. The induction (= day 0) of the other plates (confluence 97 -100 %) was performed separately by removing the complete proliferation medium and replacing it with induction media (2 mL/well). On day 1

the medium of the harvesting day 2 samples had been removed completely in a 24 h media change and replaced with 1 mL of freshly prepared induction medium. On day 3, the obligatory replacement of induction medium with differentiation medium (DM) for the rest of the plates took place. The general media change rhythm was kept accurately as described above. On day 13, the differentiation medium was changed (1 mL/well) to a 24 h medium used for the harvesting day 14 samples. For all 24 h media changes the exact time had been noted.

Table 10: Media conditions of primary human subcutaneous PACs for untargeted metabolomics. *Legend: P/S – Penicillin and Streptomycin, B/P – Biotin and Pantothenate, T3 – Triiodothyronine, EGF/FGF – epidermal growth factor/fibroblast growth factor, IBMX – 3-isobutyl-1-methylxanthine.*

Proliferation medium with 2.5 % FCS				
Ingredients	Working solution	Final concentration	250 ml volume	500 ml volume
DMEM-F12		/	240.23 ml	480.46 ml
P/S	0	0 %	0	0
B/P	3.3 mM/1.7 mM	1 %	2.5 ml	5 ml
FCS		2.50%	6.25 ml	12.5 ml
EGF	5.0 µg/ml	0.010 µg/ml	500 µl	1000 µl
FGF	0.50 µg/ml	0.001 µg/ml	500 µl	1000 µl
Insulin	1.722 mM	0.000132 mM	19.2 µl	38.4 µl
Proliferation medium without FCS				
Ingredients	Working solution	Final concentration	250 ml volume	500 ml volume
DMEM-F12		/	246.48 ml	492.96 ml
P/S	0	0 %	0	0
B/P	3.3 mM/1.7 mM	1 %	2.5 ml	5 ml
FCS	0	0.00 %	0	0
EGF	5.0 µg/ml	0.010 µg/ml	500 µl	1000 µl
FGF	0.50 µg/ml	0.001 µg/ml	500 µl	1000 µl
Insulin	1.722 mM	0.000132 mM	19.2 µl	38.4 µl
Induction medium				
Ingredients	Working solution	Final concentration	250 ml volume	500 ml volume
Differentiation medium		/	246.5 ml	493 ml
Rosiglitazone	2.0 mM	1.0 µM	125 µl	250 µl
Dexamethasone	25 µM	25 nM	250 µl	500 µl
IBMX	20 mM	0.25 mM	3.125 ml	6.25 ml
Differentiation medium				

Ingredients	Working solution	Final concentration	250 ml volume	500 ml volume
DMEM-F12		/	244.5 ml	489 ml
P/S	0	0 %	0	0
B/P	3.3 mM/1.7 mM	1 %	2.5 ml	5 ml
Insulin	1.722 mM	0.861 µM	125 µl	250 µl
T3	2 µM	1.0 nM	125 µl	250 µl
Cortisol	100 µM	0.10 µM	250 µl	500 µl
Transferrin	1.0 mg/ml	0.01 mg/ml	2.5 ml	5 ml

For the ingredients list of the basal medium (DMEM-F12, Appendix 16, Table A3) and for preparation steps of working solutions see the Appendix enclosed (Appendix 6, SOP 09).

Table 11: Storage life of the applied media varieties.

Proliferation medium	Good for 2 weeks after preparation
Proliferation medium without FCS	Good for max. 3 weeks after preparation
Differentiation medium	Good for max. 3 weeks after preparation
Induction medium	Good for 24 h after preparation

c. Harvesting

For a detailed overview of the harvesting steps as well as harvesting buffers see Appendix 2 (Guideline F). The 6 well plates were taking out of the incubator and placed on ice. Exactly 24 h after media change, the harvesting took place in the same order. The starting point of harvesting had been considered the time point of the first plate from the 24 h media change. The following steps were applied for each of the harvesting days: 0, 2, and 14.

Direct infusion Fourier transform ion cyclotron resonance mass spectrometry (DI-FT-ICR MS) plate

The harvesting for the DI-FT-ICR MS samples was performed with 3 wells of a 6 well plate which had been the best compromise for obtaining an adequate cell extraction volume (Chapter IV.1.). The media pool of 3 sample- and blank wells were collected in separate 15 mL tubes. The aliquots of media for the DI-FT-ICR MS samples were 2x1000 µL.

All wells were washed two times with 2 mL ice-cold ultrapure water (LC-MS grade, ultrapure water system Type I PURELAB flex 2). After removing the water during the last washing step with a vacuum pump, the cell lysates (blank and samples) were harvested with 500 µL MeOH (LC-MS grade, Chromasolv^{TM}).

The harvesting volume of MeOH was tested in previous pilot studies (Chapter IV.1.). The cell lysates were scratched off the well with sterile cell scrapers. Every sample was transferred into a 1.5 mL tube. Moreover, all samples were immediately snap frozen in liquid nitrogen (N_2) and stored at -20 °C for the meanwhile until all samples of the day were collected. Finally, those were stored within 5 h at -80 °C.

RNA/protein/NucBlue (NB) & OilRed (OR) plate

The media samples had been collected as described above with the same aliquots of 2x1000 µL (per harvesting day and subject). The 3 RNA- and 2 protein wells of the plate were washed two times with 2 mL ice-cold PBS. Firstly, the RNA cell lysates were harvested with 350 µL of RLT-β-Mercaptoethanol (1 %) and subsequently cleaned with 80 % EtOH to erase evaporation of remaining β-mercaptoethanol before continuing harvesting. Secondly, the protein samples had been quenched with 250 µL radioimmunoprecipitation assay (RIPA) buffer - 0.5 M Phenylmethylsulfonylfluorid (PMSF) solution. All samples were also snap frozen in liquid N_2 and harvested at -80 °C as described above.

d. NucBlue and OilRed staining

Lastly, the cells of the NucBlue and OilRed well were fixed for 1 hour with 2 mL/well 4 % Formaldehyde (Appendix 10, SOP 19). Then the Histofix (4 %) was discarded and 2 mL OilRed solution was added to the well for a 1 h incubation. The OilRed solution was removed and cells were washed two times with 2 mL PBS. Finally, the ready-to-use NucBlue solution was added (4 drops/2 mL PBS). After the incubation of 25 min in the dark and at room temperature, the fluorescence microscope images had been taken (Chapter VI.1.f.). The NucBlue- & OilRed plates were wrapped in parafilm and placed in an optically opaque box at 4 °C for a long-term

storage of the wells. A refill with PBS should be applied every 5 -6 months to avoid draining of the wells.

e. OilRed quantification

The quantification of OilRed had been applied as described in SOP 19 (Appendix 10). However, different to the SOP 19, the OilRed intensity was demonstrated as intensity per 100,000 cells obtained from the nuclei staining.

f. Microscopy

Transmitted light-, reflected light- and fluorescence pictures had been taken (Appendix 10, SOP 19). For spotting constantly the same position on the plate, a grid including a coordinate system had been attached to the plate while taking pictures on harvesting days.

The Keyence microscope (VHX-6000 series) was used for pictures on harvesting days only. For fluorescence pictures, the sizes were determined as “REC format 1600x1200 pixel” to easily evaluate the cell number via the CellProfiler software and with a faster processing time (Chapter VI.1.f.i.). Before starting, a manual adjustment of the scale “reticle” with the center of the well (at 100x magnification) was necessary. Afterward, the magnification was switched to 300x as the optimal zoom for counting the nuclei via CellProfiler. The establishment work of the NucBlue staining protocol had been challenging in terms of finding the right level of the cells under the microscope. In general, the “depth of focus” of 12,200 µm (3D depth constitution) had been proven to be a bottom limit for any study subject. The following pictures were taken on the harvesting days (0, 2, 14) and at five representative positions of the well (Chapter VI.1.f.i.):

1) Reflected light image
2) Fluorescence single image
3) Fluorescence 5x5 stitches image

Moreover, 5x5 stitch images of harvesting day 14 plates were taken randomly on the well (300x magnification, REC format 1600x1200 pixel).

i. Imaging and statistical analysis (cell number and differentiation)

Five representative positions (Origin, A-D) on the well were selected (Table 11).

Table 12: Five independent positions on the well with corresponding coordinates in µm.

Well positions	Coordinates (X µm, Y µm)
Origin	X: 0, Y: 0
A	X: 5965 µm, Y: 6035 µm
B	X: 6035 µm, Y: -5965 µm
C	X: -5965 µm, Y: -6035 µm
D	X: -6035 µm, Y: 5965 µm

Image analysis was done automated by the CellProfiler pipeline (Nuclei_Pipeline_EccentricityFilter.cpproj). The CellProfiler software (Version 3.1.9, BROAD Institute, USA) is commonly used to count cells or other objects, by measuring the staining intensity (per cell) **[214]**. The 5x5 stitch images taken on 5 representative positions (per harvesting day and subject, 300x magnification) of the well were uploaded and the number of cells per picture was assessed and exported to an EXCEL spreadsheet. The average of nuclei per image (formula a) was calculated and multiplied with the number of images (formula b) to cover a complete well (formula c). The scaling factor was assessed as 2.857 (2000 µm divided by 700 pixels). For calculations of the cell number per well, only 1500x1200 were considered due to the black stripe on each side (left /right) of the image that counted for 50 pixels each (Appendix 16, Table A1-A2).

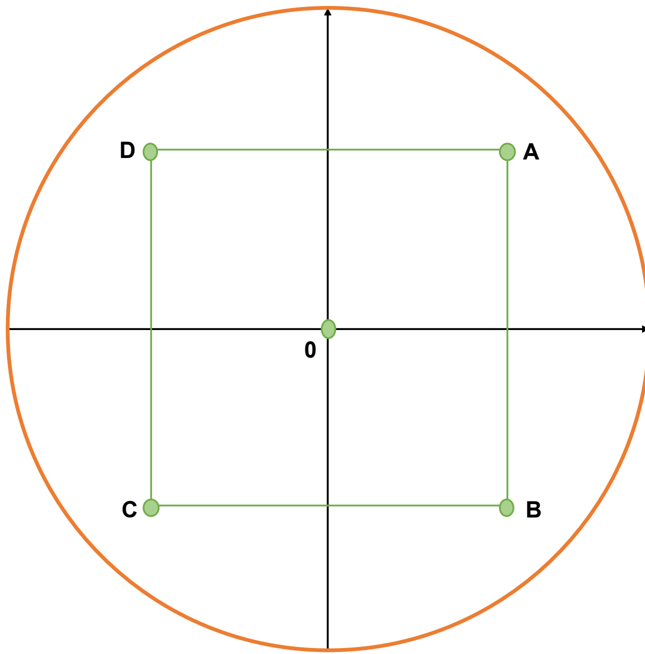


Figure 19: Layout of well with 5 selected spots. Legend: orange: well; black: X/Y- axis; green points: picture positions

a) $\text{Number of nuclei per image} = \frac{\text{Total nuclei counts}}{\text{Number of images}}$

b) $\text{Images to cover well} = \frac{9.60 * 10^{8}\ (\text{area well})\mu\text{m}^{2}}{13{,}714{,}285.71\ \mu\text{m}^{2}} = 70.0$

c) $\text{Cells per well} = \text{Number of nuclei per image} * 70$

Differentiation

The Image analysis was done automated by the open source software QuPath 0.3 **[215]**. For each subject one representative 5x5 stitch picture at day 14 (300x magnification) was evaluated. Herefore, a training data set of 5 different (500x500 pixel) segments was created. In each picture 5 negative and 5 positive spots had been marked. The software performed the image analysis based on the marked spots. The same scaling factor as for the CellProfiler analysis was applied. The results were demonstrated as percentage of positive area (equivalent to OilRed-stained lipid droplets) in comparison to total area. The total area encompassed 12,915,141 µm^2 which excluded scale bar and edges of the picture.

2. Untargeted metabolomics

a. Sample preparations

The sample preparation of cell lysates and media samples for the DI-FT-ICR MS took place at the laboratory of Prof. Dr. Schmitt-Kopplin's research unit, the Analytical BioGeoChemistry (BGC) department (Helmholtz, Munich). The establishment of the sample preparation protocols had been led and supervised by my mentor Dr. Sara Forcisi (BGC).

i. Media

After testing different sample preparation protocols for media samples (Chapter IV.1.), the following ZipTip protocol (C18), as described by Forcisi et al., was applied **[213]**.

All sample preparation steps took place under the laminar flow cabinet. An acronym list had been created in advance to minimize the labeling at the glass vial bottom and avoid contaminations. An important preparation step was the cleaning of tubes, vials, 96 well plates, and reservoirs with MeOH. In addition, the solutions 2 % Formic Acid (HCOOH) and 2 % Phosphoric acid (H_3PO_4) were prepared as well, before starting the sample extraction procedure.

Table 13: Cleaning of 96 well plates/reservoirs by rinsing with MeOH (repetition of 3 times).

96 Well plates [mL/per well]	**Reservoirs**
1x Loading plate [0.5 mL]	1x Pretreatment (H_3PO_4 2%)
3x Washing plates [2 ml]	1x Conditioning (MeOH)
1x Elution plate [0.5 mL]	1x Equilibration (HCOOH 2%)
1x Equilibration plate (HCOOH 2%) [2 mL]	/

The half-automatic Eppendorf robot (see Chapter IV.1. for model) was set up under the laminar flow cabinet. All information of the pipetting volumes (e.g., for dispensing) was saved as a protocol on the device for convenience. Any necessary adjustments of tips and corresponding plates had been done manually. After thawing the samples (1 mL each) for 2 h on ice and subsequent vortexing (10 s), the media sample preparation workflow had been published earlier and applied as follows **[213]**.

1) Pre-treatment: 50 µl media + 50 µl H_3PO_4 [2 %] (on dry ice)

The Pre-treatment took place in the 96 well plate (0.5 mL) for later loading. Firstly, 50 µL of 2 % Formic Acid were pipetted into the wells, and then each media sample was added in equivalent amounts. The mixing step was performed with the robot. Then tips had been changed to the C18 model (Omix C18, 100 µL tips, Varian) and the following steps continued:

2) Conditioning in MeOH (x10)
3) Equilibration in HCOOH [2 %] (x10)
4) Loading with sample (x50)
5) Washing with HCOOH [2 %], 500 µl/well (3 x10)

   The washing of samples continued until the foam was gone.

6) Elution in MeOH (100 µl/well) x30

The samples were considered as a 1:2 dilution. Afterward, samples were transferred from the elution plate into 0.5 mL tubes on dry ice. A total media elution sample had been recovered of approximately 90 µL for each subject and harvesting day.

7) Dilutions

The dilutions were prepared manually on dry ice. Herefore, aliquots of 250 µL had been prepared in glass vials. Afterward, the amount of MeOH (depending on the dilution) had been removed from the aliquot in the vial and replaced with the equivalent amount of sample. An important step during diluting had been the wetting of the tip to make sure of removing the exact amount of MeOH and sample.

Table 14: Dilution of extracted media samples.

Dilution 1:20
300 µl MeOH
- 30 µl MeOH
+ 30 µl Elution sample

The vials of choice were “max recovery conical vials” and closed with a magnetic cap. Moreover, 10 s of vortex mixing followed the preparation of dilutions. In addition, the system of the DI-FT-ICR MS vortex mixed immediately before injection of the sample. The order of

samples for measurements ranged from low concentrated to high concentrated samples considering the harvesting time points (day 0, day 2, etc.) and corresponding metabolites. In this regards a carry-over confounder had been minimized. Immediately after preparations, the samples had been inserted into the DI-FT-ICR MS according to the right order and by keeping a continuous cool chain.

ii. Cell lysates

The second sample preparation protocol had been performed as follows. After warming up the cell samples on ice for about 10 min (quenched in MeOH) and subsequent vortex mixing (10 s), those had been transferred into cryotubes with ceramic beads (2 mL). The original cell samples constituted of the same volumes (à 500 µL) for each subject and harvesting day. The cell lysate samples were extracted as in the following scheme:

1) Tissue lyzer (cooled with liquid N_2), (program: 2 cycles of 6,000 RPM; cycle 3 x 10 s; break: 20 s)
2) Centrifugation (program: 2 cycles of 12,000 RPM; 10 min; 4 °C)
3) Dilution in MeOH (on dry ice)

Table 15: Dilution of extracted cell samples.

Dilution 1:50
600 µl MeOH
- 12 µl MeOH
+ 12 µl Extraction sample

Subsequently to tissue lyzing, the samples went directly into the centrifuge. After the 1st centrifugation the supernatant had been transferred into a new tube (1.5 mL). This step followed the 2nd centrifugation whereas the supernatant (final extract) had been again carried over into a new 1.5 mL tube. After the first centrifugation, the pellet was hardly seen but after the 2nd centrifugation, the pellet was visible and a final extraction volume of 250 -300 µL had been collected. The dilution procedure, as well as the transport to the DI-FT-ICR MS, was performed as described for the media samples above.

Two separate runs one dedicated for the media samples and one for the cell lysates were performed. All media eluates and cell lysate extracts had been stored at -80 °C.

Table 16: List of prepared diluted acids.

Solution name	Volume MilliQ H_2O [mL]	Volume pure acid [mL]
2 % HCOOH	196	4
2 % H_3PO_4	196	4

b. DI-FT-ICR MS measurements

The extracts were analyzed in positive electrospray ionization mode (ESI) via direct infusion Fourier transform ion cyclotron resonance mass spectrometry (DI-FT-ICR MS), using a Bruker SolariX instrument equipped with a 12-Tesla magnet and an Apollo II ESI source (Bruker Daltonik GmbH, Bremen, Germany). The instrument was externally calibrated on clusters of arginine (1 mg/mL in methanol/water: 80/20) with calibration errors below 0.1 ppm. The injection flow rate was set to 120 µL/h. Three hundred scans were acquired and averaged for each spectrum within the interval from 147.4 to 1,000.0 *m*/*z* and with a time domain of 4 mega words (MWs). The voltages of capillary and spray shield were set to 3,800 V and −500 V, respectively. The ion accumulation time was set to 200 ms and the time of flight to detector was set to 1 ms. The nebulizer gas flow rate was set at 1 bar and the drying gas flow rate was set to 4 L/min with a temperature of 250 °C.

c. Data analysis

Data preprocessing

The acquired spectra were processed using Data Analysis 4.4 software (Bruker Daltonik, GmbH, Bremen, Germany). Peak-picking algorithm was conducted with a signal-to-noise ratio (S/N) of 4 and a minimum intensity threshold of 1.5×10^6 counts. All spectra were exported as tab-separated asc files and loaded into the Kernel Calibrator **[268]**. In the generated matrix,

m/z features that occurred in less than 10 % of all samples were discarded. Molecular formula assignment was performed following the mass difference network approach **[216]**.

Matrix generation

To perform peak alignment and generate data matrices for cell lysates and media, all the corresponding mass spectra were subjected to an in-house written matrix generator algorithm that was set to align peaks within a 1-ppm error window.

3. DNA

a. DNA isolation and genotyping (*FTO* rs1421085)

DNA of the MOBB subjects had been isolated from whole blood samples (DNeasy Blood&Tissue Kit by Qiagen) and quantified spectrophotometrically (Infinite M200, Tecan; Appendix 9, SOP 16). Genotyping was performed using the LightCycler 480 system (Roche, Basel Switzerland) for the rs1421085 *FTO* gene variant using RT-qPCR (Simple Probe Assay; Appendix 4 for LightSNiP Manual Version 150605 © 2015 TIB MOLBIOL) with 50 ng DNA as described in SOP 21 (Appendix 11).

4. RNA

a. RNA isolation of cell lysates

The cells were lyzed with 350 µL RLT-β-Mercaptoethanol (1 %) harvesting buffer and isolated with the Quiagen kit (RNeasy Mini Kit) according to the manufactures' instructions (Appendix 3, Quick-Start Protocol). However, the lysis buffer was not added additionally. Harvesting day 14 samples did not achieve an adequate concentration of RNA with the Qiagen Kit. Therefore, the backup day 14 samples were isolated according to the Trizol Reagent protocol (Appendix 15, SOP 39) All RNA concentrations were assessed spectrophotometrically (Infinite M200, Tecan).

b. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

All the samples had been checked on the BioAnalyzer (Agilent Technologies, Inc©; USA) for integrity (RNA Nano Chip) according to the protocol "Guideline D" (Appendix 1). The total RNA samples (2,000 ng, 500 ng, 150ng) were reverse transcribed to cDNA according to SOP 32 (Appendix 13). The RT-qPCR was performed as described in the established protocol (Appendix 13, SOP 32) at the chair of Nutritional Medicine in Freising. The human primers we applied and their forward/reverse sequence information are listed in Table 17 below. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and importin 8 (*IPO8*) were used as reference genes. Expression levels relative to *GAPDH* and *IPO8* are shown.

Table 17: Human primer list including forward/reverse sequence information.

Gene name	Primers forward	Primers reverse
PPARG2	5'-GAAAGCGATTCCTTCACTGAT-3'	5'- TCAAAGGAGTGGGAGTGGTC-3'
LEP	5'-TTTGGCCCTATCTTTTCTATGTCC-3'	5'-TGGAGGAGACTGACTGCGTG-3'
UCP1	5'-GGAAAGAAACAGCACCTAGTTTAGGAAGCA-3'	5'-CGTCAAGCCTTCGGTTGTTGCTATTATTCTG-3'
*PGC1*A	5'- TGCCCTGGATTGTTGACATGA-3'	5'- TTTGTCAGGCTGGGGGTAGG-3'
IRX3	5'- AGACAGACACCGACACACAC-3'	5'- GGGCTAAGTAAGGCAGCCAA-3'
IRX5	5'- CCGTGTGTGGCCATGTCCTAT-3'	5'- CTGGAGGTGCGAGTTGTAGC-3'
SMPD1	5'-GCTGGCTCTATGAAGCGATGGC-3'	5'-AGAGCCAGAAGTTCTCACGGGA-3'
SLC27A5	5'-GGAAGTCTACGGCTCCACAGAA-3'	5'-GTCGAACTGCACCAGCTCAAAG-3'
PARP1	5'-CCAAGCCAGTTCAGGACCTCAT-3'	5'-GGATCTGCCTTTTGCTCAGCTTC-3'
HADHA	5'-GCCGACATGGTGATTGAAGCTG-3'	5'-GGAGAGCAGATGTGTTACTGGC-3'
ACACA	5'-TTCACTCCACCTTGTCAGCGGA-3'	5'-GTCAGAGAAGCAGCCCATCACT-3'
GAPDH	5'-GATCATCAGCAATGCCTCCTGC-3'	5'-ACAGTCTTCTGGGTGGCAGTGA-3'
IPO8	5'-CGGATTATAGTCTCTGACCATGTG -3'	5'-TGTGTCACCATGTTCTTCAGG-3'

Table 18: Literature-based target list.

Marker for	Genes
Adipocyte differentiation	Peroxisome Proliferator-Activated Receptor gamma *(PPARG2)*
Lipid storage	Leptin *(LEP)*
Mitochondrial function	Uncoupling Protein 1 *(UCP1)* Peroxisome proliferator-activated receptor gamma coactivator 1-alpha *(PGC1A)*
FTO	Iroquois homeobox 3 *(IRX3)* Iroquois homeobox 5 *(IRX5)*

Table 19: FTMS-based target list.

Marker for	Genes
Glycosphingolipid metabolism	Sphingomyelin phosphodiesterase 1 *(SMPD1)*
Fatty acid elongation or complex lipid synthesis	Solute carrier family 27 member 5 *(SLC27A5)*
DNA repair	Poly (ADP-ribose) polymerase 1 *(PARP1)*
Mitochondrial beta oxidation	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha *(HADHA)*
Fatty acid synthesis	Acetyl-CoA carboxylase alpha *(ACACA)*

5. Statistical analysis

Scatterplots, boxplots and trajectory were created by GraphPad Prism 4. Prior to that, Shapiro-Wilk- and D'Agostino & Pearson omnibus normality test were done which failed due to the small group size (n = 6) and followed by testing for significance using Mann-Whitney U-Test (2 groups, non-parametric) and Kruskal-Wallis test (> 2 groups). For non-linear regression, groups were merged and the progression over differentiation days were displayed. Before that, Shapiro-Wilk- and D'Agostino & Pearson omnibus normality test were done in order to test for/against normality and 1-way ANOVA was performed, subsequently. In order to compare the different harvesting days a Bonferroni Multiple Comparison test was applied. Correlation coefficients originated from Pearson correlation and p-values were calculated via linear regression. Bar graphs were created with EXCEL 2016. For each analysis p-values < 0.05 were considered as significant.

The following list includes all points that had been conducted by myself.

Cell culture experiments (pilot studies, technical experiment and study):

- Differentiation of preadipocytes into adipocytes
- Harvesting
- Staining with NucBlue and OilRed
- OilRed quantification
- Microscopy including imaging and statistical analysis

Untargeted metabolomics (pilot studies, technical experiment and study):

- Sample preparations for media- and cell samples
- UniProt Database investigations of 666 genes
- Overrepresentation analysis of target candidates (score >3) on the Reactome database

DNA:

- Isolation and genotyping of a small subset of patients of the Munich Obesity Biobank

RNA (study):

- Isolation and RT-qPCR including statistical analysis

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VIII. Acknowledgements

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A special thank you goes to my second parents in U.S. who always supported me without borders. You made my time in Gainesville unique. I miss you both. Love, Katharina.

IX. APPENDIX

Thesis

Metabolome characterization of the *FTO* genotype (rs1421085) in primary human preadipocytes of male donors across differentiation

Katharina Antonia Kappo

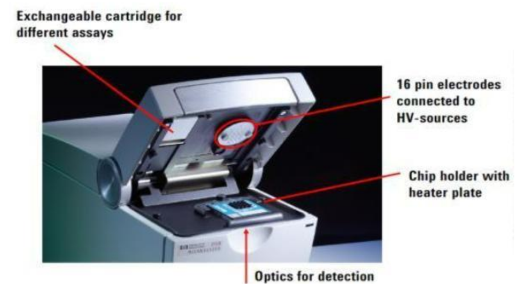
Appendix 1

Guideline D

Title: General Manual Bioanalyzer	
Version: 7	**Date: 29.05.19**
Author: CS2	
Reviewer: Maria Hidrobo, Tanja Krauss	

Principle

For the integrity check, the Bioanalyzer device can perform a Nano gel electrophorese via Chip. Only 1 – 5 µl are required, run takes 30 min. **Costs per chip 50 €.**



Regarding RNA/DNA depending on the concentration, different Chips are available:

CHIP	Range	Assay Name	Samples
RNA Nano	5-500ng/µl	assays\RNA\ Eukaryote Total RNA Nano Series II	**12**
RNA Pico	0.25-5ng/µl	assays\RNA\ Eukaryote Total RNA Pico Series II	**12**
RNA Small	Total RNA 1-100ng/µl / **Enriched small RNA (e.g. miRNA) 1-20ng/µl** Oligonucleotides 0.1-2 ng/µl	assays\RNA\ Small RNA Series II	**11**
DNA high sensitive	5-500 pg/µl	assays\dsDNA\ High Sensitivity DNA	**11**

Device location: Weihenstephaner Berg 3/I, Chair of Animal Physiology, room 02/3.21.

One day in advance contact Christian Grätz. (0176 438 59 776, chris.graetz@tum.de) to verify availability of device & materials.

1. Evaluate concentration of your samples via Nanodrop or Tecan plate reader
2. **If your samples are out of the range of the chip, make a dilution!**
3. Aliquot 2.5 µl of your sample in 0.2 ml caps/ strips (can be stored -80°C)
4. Call 5550 & ask for equilibration of the relevant kit (needs 30 min, see table above)

<u>For RNA a denaturation step is required</u>

1. Defrost RNA (2µl) & centrifuge tubes/stripes shortly down
2. Turn on the Eppendorf Master Cycler & check settings of program “BIOANALY” (Room 2.62)
3. Press Start, choose the program “BIOANALY” & press enter
4. After heating block & lid program rest on “HOLD”, add tubes & press enter to run
5. Afterwards program rest on “HOLD” at 25°C, press enter and remove tubes
6. Potentially centrifuge 0.2 ml tubes shortly, store on cool rack & go to the Bioanalyzer *(samples can be stored -20°C for 24h before running the BA)*

<u>At the device</u>

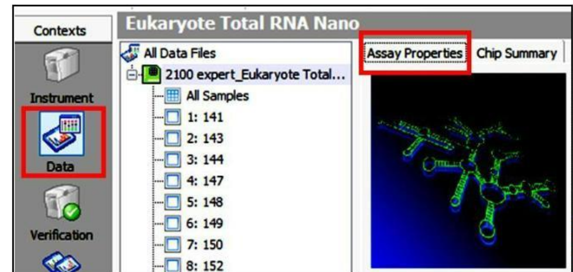
1. Turn on the device & the computer (PW: physio2000)
2. Open the program 2100 Expert
3. Add 400 µl nuclease free H_2O in the relevant cleaning chip (in the drawer), put in the device, close lid & incubate for 5 min
4. Open the lid of the device, remove chip & led lid open for 10 sec
5. Empty the cleaning chip by turning it on a paper towel
6. Take ladder from freezer **(room 02/3.18)**, heat up 2 min at 70°C (cap in cap) & centrifuge shortly
7. Check syringe and gasket of the priming station and run a “TEST Chip” (see page 5)
8. Follow the instructions from the relevant “**Kit Quick Start Guide**”
9. **Check positions of the priming station (different between Chips)**
10. Initiate the software by select relevant chip and enter sample names
11. During the run, put the sign **“Vibrations sensitive. Instrument is running”** on the door

Afterwards RESTORE remaining ladder at -80°C and the kit at 4°C

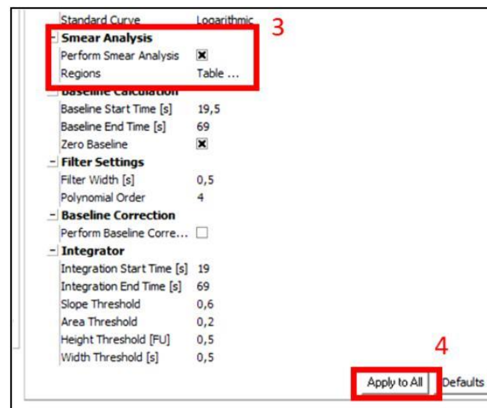
12. After the run repeat step 3-5, clean the work place & turn everything off
13. After the run save file ones by file save as .xad file
14. Second press file print as pdf to your USB stick

Result interpretation:

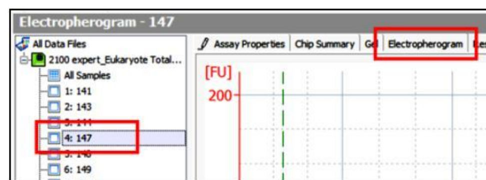
For the nano and pico RNA chip a RNA Integrity Number (RIN) is generated by the program (= ratio of the area under the 18S and 28S rRNA peaks to the total area under the graph) Further the DV_{200} value (distribution value; percentage of RNA fragments > 200 nucleotides) can be extracted of the software as follows:



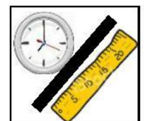
1. Open the program 2100 Expert
2. Load the relevant xad.file
3. Choose Data and the tab Assay Properties
4. Choose Global (1), change Normal to Advanced (2), tick Perform Smear Analysis (3) under **Smear Analysis** and click Apply to All (4)



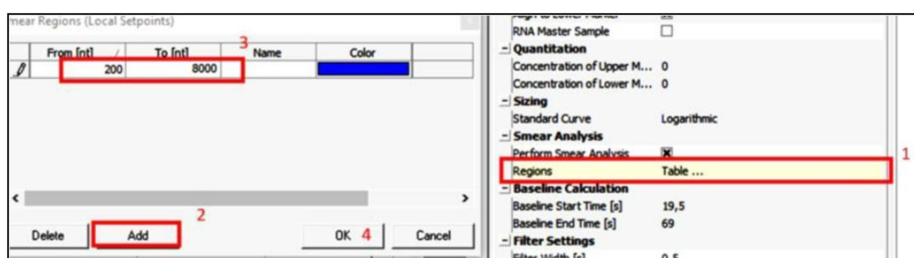
5. Choose the Electropherogram



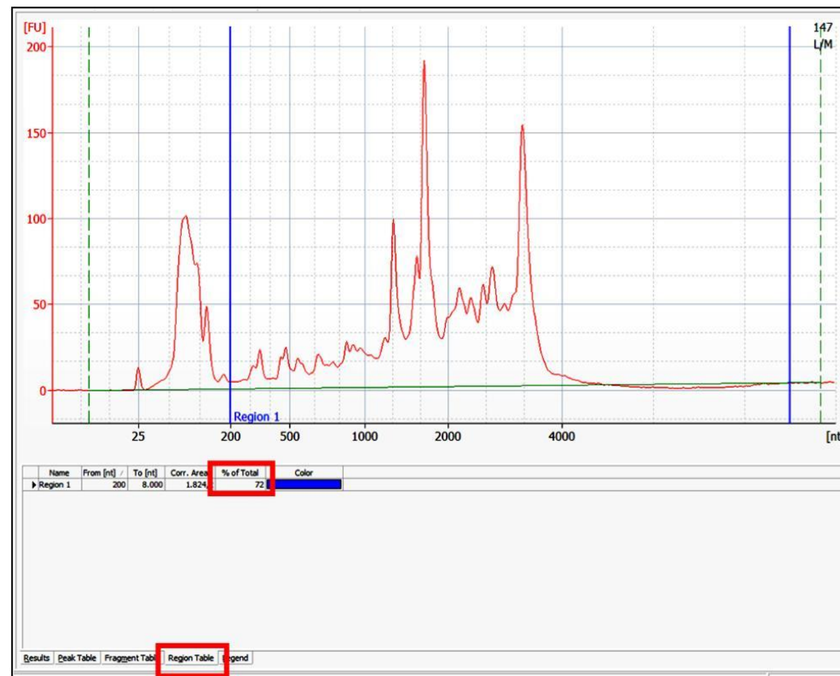
6. Switch in the electropherogramm from sec to nt values (clock / ruler symbol)



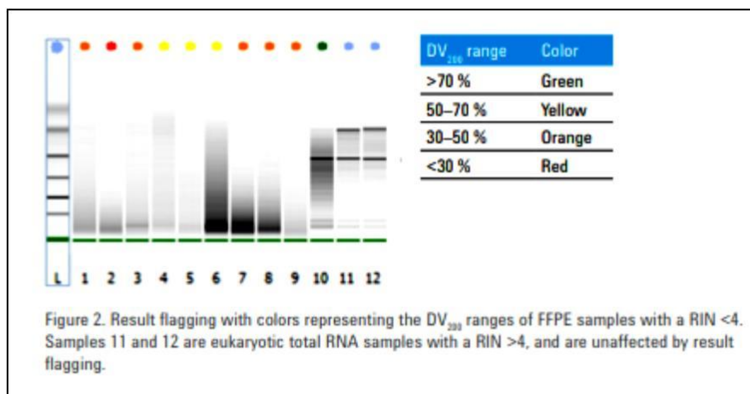
7. Double click Table under **Smear Analysis,** click Add and limit the Region from 200 nt to 8000 nt



8. Select the Region Table tab in the trace window to display the results (% un-degraded RNA of Total)



Recommandation



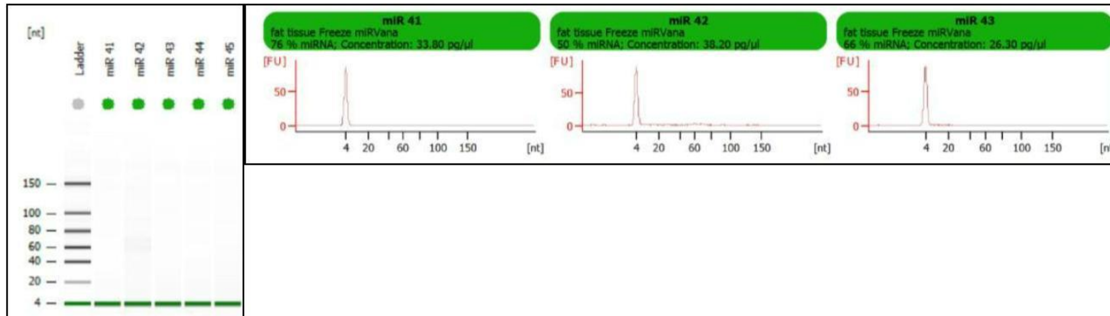
DV_{200} range	Color
>70 %	Green
50–70 %	Yellow
30–50 %	Orange
<30 %	Red

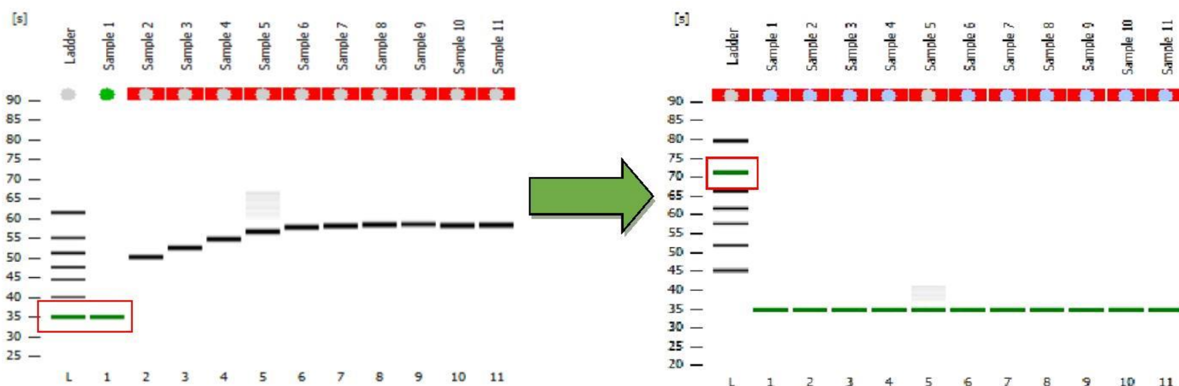
Figure 2. Result flagging with colors representing the DV_{200} ranges of FFPE samples with a RIN <4. Samples 11 and 12 are eukaryotic total RNA samples with a RIN >4, and are unaffected by result flagging.

RIN value should be best > 7 / by lower RIN values extract the DV_{200} value, by maximum of 30 % non-degraded RNA sample can be RT and qPCR of housekeeping genes can be runned.

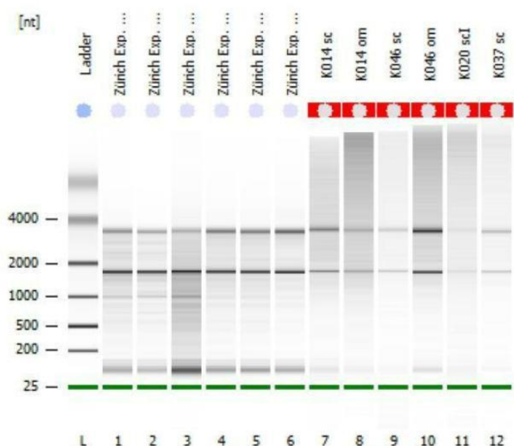
Examples

Perfect Positive results of samples run by the Small RNA Kit



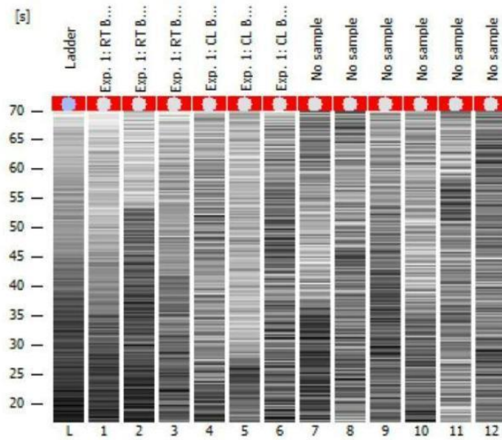


Run is fine, but from sample 2 the flagging failed. Adjust first ladder peak for flagging manually.



Last six samples are a bit degraded. Evaluate the RWT value in the software and by ¿? % undegraded RNA the samples can be RT and checked in the qPCR with the housekeeping genes.

Troubleshooting:



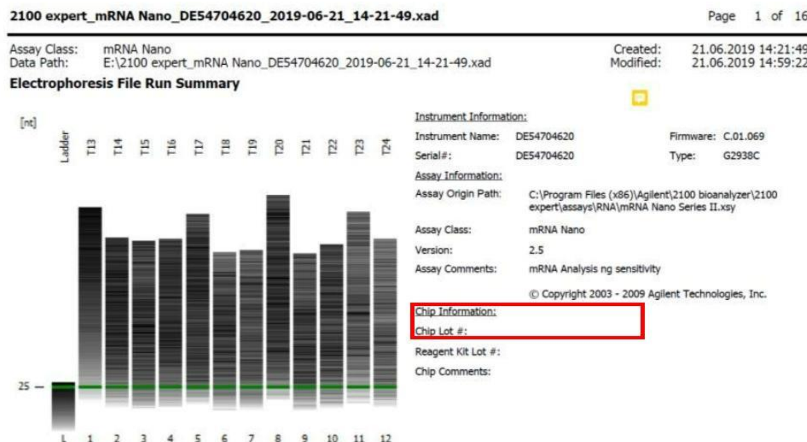
Priming the chip with gel was insufficient!

Air column in the syringe was to low > pressure t low > no gel distribution in the capillaries

Check Syringe and gasket and possible exchange both (check maintenance sheet on th cupboard, new syringes > chip storage, new gasket > BA pipettes/ cleaning chips)

Priming the chip with gel was insufficient!

Assay class is incorrect (Eukaryote Total RNA Nano see table 1 page 1).



Appendix 2

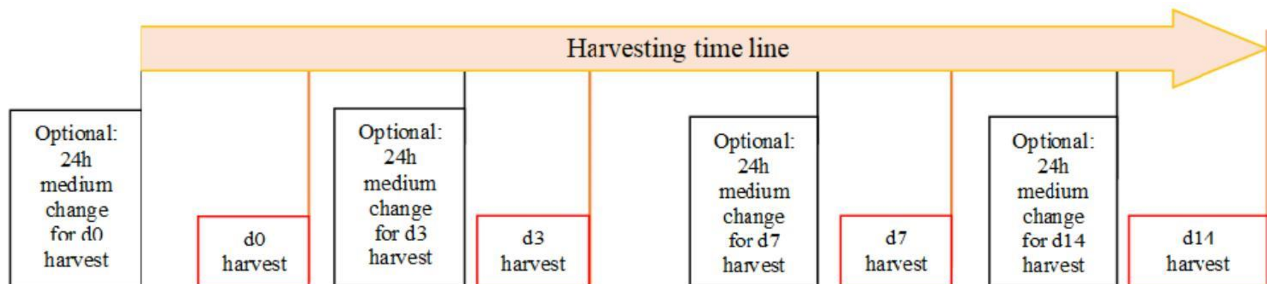
Guideline F

Title: Cell Harvesting	
Version: 1	Date: 14.08.2020
Author: Simone Heisz Reviewer: CS2	

Principle

By harvesting cells during different time points in the process of differentiation, differences between cell type/individuals/diseases/depots can be investigated. E.g. the average differentiation of PAC towards an adipocyte takes about 14 days. During this process, different time points for harvesting may be selected, but start (day 0) and terminus (day 14) are recommended.

Example of experimental setup:



Materials

	company	storage	order no.
β-Mercaptoethanol	Sigma	RT, hood, room 2.62	M3148
complete Mini	Roche	Fridge 5, room 2.65	11836153001
(Sodium) deoxycholate	Sigma	RT, room 2.65	D-6750
EDTA with sodium	Roth	RT	8043.2
EtOH	Fisher Scientifi	RT, room 2.65	E/0650DF/C17
$MgCl_2$	Fluka	RT	63063
NaCl	Sigma-Aldrich	RT	S7653-5KG
Nonidet P-40	Fluka	RT, room 2.65	74385
PBS (w/o) Ca2+ & Mg2+	Merck	RT, room 2.65	182-50

PhosphoStop	Roche via Sigma	Fridge 5, room 2.65	4906845001
PMSF	Sigma	RT poison cupboard	P7626
RLT buffer	Qiagen	RT, room 2.62	
SDS	Omnilab	RT, room 2.65	2.700131
Tris HCl	Sigma	RT	T3253
Tween	Sigma	RT, room 2.65	P1379-500ML

Solutions

1. ATAC lysis buffer

Stock solutions

1 M TRIS-HCl solution, pH 7.4
121 g TRIS
Dissolve in 800ml ddH_2O & adjust pH to 7.4. fill up to 1l

5 M NaCl
292 g NaCl, dissolve in 1l ddH_2O

1 M $MgCl_2$
20.3 g $MgCl_2$, dissolve in 100ml ddH_2O

10 % Tween
1 ml Tween (viscous, use positive displacement pipette), mix with 9ml ddH_2O

10 % Nonidet
1 ml Nonidet (viscous, use positive displacement pipette), mix with 9ml ddH_2O

ATAC Lysis buffer			
	Stock	Final Concentration	Volume
Tris-HCl	1 M	10 mM pH 7.4	100 µl
NaCl	5 M	10 mM	20 µl
$MgCl_2$	1 M	3 mM	100 µl
Fill up to 9.8 ml			
Add before experiment			
Tween	10 %	0.1 %	100 µl
Nonidet	10 %	0.1 %	100 µl
total 10ml			

2. RNA harvesting solution

RLT buffer	1ml	2ml	3ml	5ml	10ml	15ml	20ml
β-mercaptoethanol	10µl	20µl	30µl	50µl	100µl	150µl	200µl

Mix both components under fume hood and store dark (aluminium foil) at RT, MHD: 4 week

3. RIPA STOCK solution

1M Tris-Base, pH 8

121 g Tris base, dissolve in 800ml ddH_2O, adjust pH to 8.0, fill up to 1l

10% SDS solution

5g SDS Pellets, dissolve in 50 ml ddH2O

Vortex and use a shaker until pellets are solved. CAVE: do NOT use SDS powder

Stock Solution	C_{final}
5 ml 1M Tris-HCl pH 8	50 mM
877mg NaCl	150 mM
2 ml 10% SDS	0,2 %
1ml Nonidet P-40	1 %
0.5 g Deoxycholat	0,5 %

Fill up with ddH_2O to 100 ml, store fridge 5, room 2.65

(can be used as long as no precipitations are visible)

4. RIPA Storage Solution

Mix 10 ml RIPA Stock solution 1 with 1 x pill PhosphoStop and 1x pill complete Mini, aliquot à 500 µl and store at -20°C, freezer, room 2.65, MHD 5 years

5. PMSF Solution

	C_{final}
0.875g PMSF	0.5M

dissolve in 10 ml ddH_2O, aliquot a 0.5 ml, Freezer 1 BOX WB

6. RIPA HARVESTING solution

Defrost relevant RIPA Storage solution and add 1 µl PMSF/500 µl,

prepare fresh the day of usage

7. GPDH Harvesting Solution

	C_{final}
788 mg Tris/HCl	0.05M
29.2 mg EDTA W/O sodium	1mM
7µl Mercaptoethanol abs.	1mM

dissolve in 80 ml ddH2O, pH 7.4, fill up to 100ml, store fridge 5, room 2.65

(can be used as long as no precipitations are visible)

Procedure

During the whole procedure, wear lab coat and gloves

All steps containing β-mercaptoethanol have to be done under the fume hood

Make sure PBS is ready to use in the fridge

Label relevant 2 ml tubes

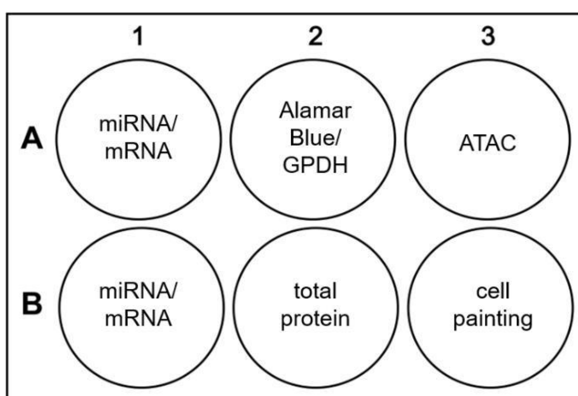
Get liquid nitrogen (alternative dry ice)

Chronological order – what to harvest/do first

1. ATAC → below, further procedure see SOP 20
2. miRNA/mRNA → below, further procedure see SOP 15
3. total protein → below, further procedure see SOP 18
4. Alamar Blue staining → see SOP 31
5. GPDH → below, further procedure see SOP 22
6. Cell painting → below, further procedure see SOP 34

Possible distribution of a 6 well plate working with PACs see below.

For cell lines like Hep1-6 /HepG2 also smaller formats as 12 well plates (3wells/ RNA/ protein are sufficient) can be used. Also for the cell painting special Eppendorf Cell Imaging Plates (glass bottom, 96 well plate; Cat. No. 0030741030) can be used.



- miRNA/mRNA: at least 2 x 6 wells
- GPDH: more than 1 x6 well is optional
- AlamarBlue: 1 x 6well is sufficient
- total protein: more than 1 x 6well is optional
- ATAC: if possible, 2 x wells are recommended
- cell painting: 1 x well is sufficient

ATAC (nuclei harvesting), 1 x6well is recommended, better are 2

a. Pre-cool centrifuge to 4 °C

b. Prechill on ice:

- DNA LoBind tubes (2 & 1.5 ml)
- ATAC Lysis buffer (add 100 µl Tween and 100 µl Nonidet to 9.8 ml buffer → vortex)
- Tagmentation buffer (provided with ATAC kit; allow to thaw on ice → Dilute 1:2 with PCR grade water)

Protect other harvesting purposes wells with a styrofoam plate to isolate them from the ice.

c. Transfer cells on ice and wash 2 x with cold PBS

d. Add 500 µl ATAC lysis working solution to each well and tilt plate to distribute homogenously. Leave plate on ice for 15 min.

e. **Continue by using SOP 20**

2. miRNA/mRNA harvesting

You can protect other 6 wells with a styrofoam plate to isolate RNA on ice.

f. Transfer cells on ice and wash 2 x with cold PBS

g. Relocate cells to fume hood and add relevant **RNA harvesting solution**

	cells	Seed	ml medium	confluence	Harvest
25er flask	PAC human	125.000/ flask	10 ml	85-90%	250 µl
75er flask	PAC human	250.000/ flask	10 ml	85-90%	500 µl
6 er	PAC human	250.000/ plate	2 ml / well	85%	350µl for 2 x 6 wells
6 er	cell line	500.000/ plate	2 ml / well	100%	1 well (350 µl at d0+d3, 500 µl d10, 700 µl d21)
12 er	cell line	250.000/ well	1 ml/ well	85%	350 µl for 3 wells
24er	cell line	125.000/ well	0.5 ml/well	85 %	250 µl for 3 wells

h. Harvest (first well) with a cell scraper, transfer buffer-cell-suspension in second well and harvest as well
i. Transfer into 2 ml tube
j. Check emptiness of the wells under the microscope
k. Vortex or pipet to mix and ensure that no cell clumps are visible
l. Put tubes directly in liquid nitrogen (alternative on dry ice)
m. Freeze immediately at -80°C
n. Wash empty 6 wells 3 times with 70 % EtOH to erase evaporation of remaining β-mercaptoethanol
o. Freeze the cell lysate at -80°C at least for 24 hours before RNA isolation

3. Total protein harvesting

p. Transfer cells on ice and wash 2 x with cold PBS
q. Relocate cells to fume hood and add relevant **Protein harvesting solution**

	cells	Seed	ml medium	confluence	Harvest
25er flask	PAC human	125.000/ flask	10 ml	85-90%	250 µl
75er flask	PAC human	250.000/ flask	10 ml	85-90%	500 µl
6 er	PAC human	250.000/ plate	2 ml / well	85%	250µl for 1 well
6 er	cell line	500.000/ plate	2 ml / well	100%	1 well (350 µl at d0+d3, 500 µl d10, 700 µl d21)
12 er	cell line	250.000/ well	1 ml/ well	85%	350 µl for 3 wells
24er	cell line	125.000/ well	0.5 ml/well	85 %	250 µl for 3 wells

r. Harvest with a cell scraper
s. Transfer via pipetting into 2 ml tube
t. Put tubes directly in liquid nitrogen (alternative on dry ice)
u. Freeze the cell lysate at -80°C at least for 24 hours before isolation

4. Alamar blue measurement

Transfer plate for 10 min in the incubator to reach again 37°C in the cells and use SOP 20 for continuing

5. GPDH harvesting

a. Transfer cells on ice and wash 2 x with cold PBS
b. Relocate cells to fume hood and add relevant **Protein harvesting solution**

	cells	Seed	ml medium	confluence	Harvest
6 er	PAC human	250.000/ plate	2 ml / well	85%	800µl for 1x 6 wells
6 er	cell line, e.g. HU7	500.000/ plate	2 ml / well	85%	800µl for 1x 6 wells

c. Harvest with a cell scraper
d. Transfer via pipetting into 2 ml tube
e. Put tubes directly in liquid nitrogen (alternative on dry ice)
f. Freeze the cell lysate at -80°C at least for 24 hours before isolation

6. Cell painting

For preparation for cell painting, discard medium and wash cells two times with cold PBS. For detailed staining protocol, see SOP 34.

Appendix 3

RNeasy Mini Handbook - Qiagen

Appendix 4

LightSNiP
rs1421085 FTO

Preparation of parameter-specific reagents (96 reactions):

One reagent vial contains all primers and probes to run 96 LightCycler reactions.

Spin vial before opening to ensure the yellow pellet is located at the base of the reaction tube.

Add 100 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

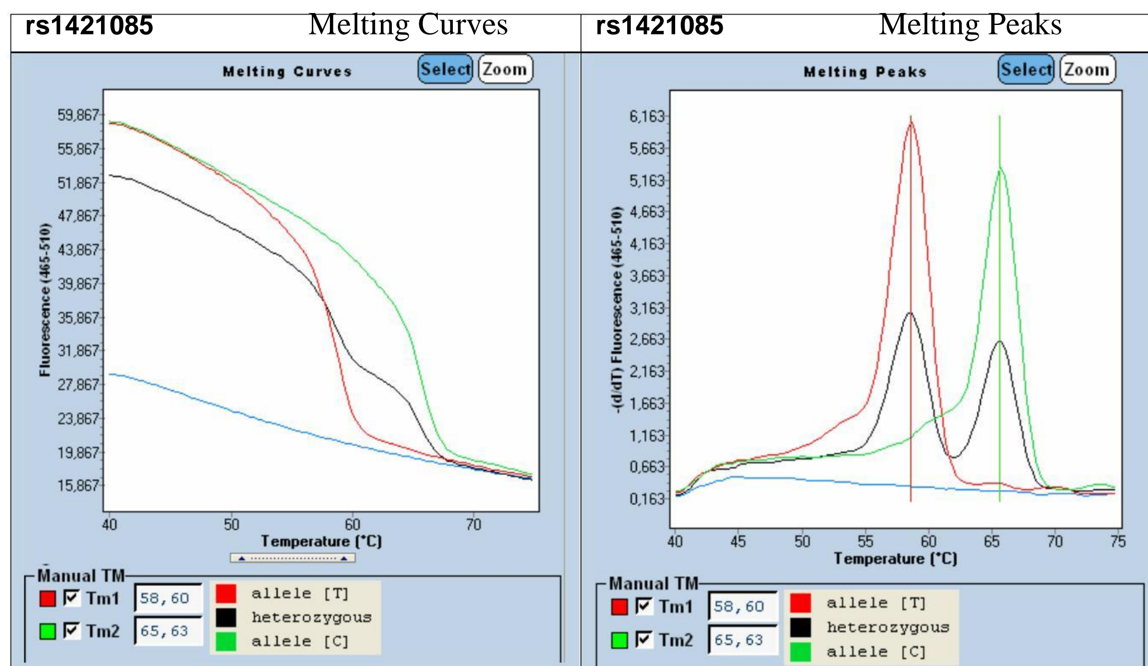
►Use 1 µl **Reagent Mix** for a 20 µl PCR reaction.

Preparation of the reaction mix:		**Settings:**	
20 µl reaction mixture		**LightCycler® 480 Instrument**	
H_2O	14.4 – 10.4 µl	**Block Type:** 384 or 96	
Reagent Mix	1.0 µl	**Detection Format:** Simple Probe	
FastStart DNA Master[1]	2.0 µl	LightCycler® 480 Instrument I:	483-533
$MgCl_2$ (25 mM)	1.6 µl	LightCycler® 480 Instrument II:	465-510
~~DNA~~	~~1.0 – 5.0 µl (~ 50 ng)~~		
Final $MgCl_2$ conc.:	3.0 mM		

[1]LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics)

Programming LightCycler 480 Instrument:

Program:	*Denaturation*	*Cycling*			*Melting*			*Cooling*
Parameter								
Analysis Mode	*None*	*Quantification*			*Melting Curves*			*None*
Cycles	*1*	*45*			*1*			*1*
Segment	*1*	*1*	*2*	*3*	*1*	*2*	*3*	*1*
Target [°C]	95	95	60	72	95	40	75	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s] ***384***	*4.6*	*4.6*	*2.4*	*4.6*	*4.6*	*2.0*	*-*	*2.0*
Ramp Rate [°C/s] ***96***	*4.4*	*4.4*	*2.2*	*4.4*	*4.4*	*1.5*	*-*	*1.5*
Acquisition Mode	None	None	Single	None	None	None	Continu.	None
Acquisitions [per °C]							3	



Reference: GTAGCAGTTCAGGTCCTAAGGCATGA[C/T]ATTGATTAAGTGTCTGATGAGAATT

http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1421085

Version 150605 2015 TIB MOLBIOL

Preparation of the reaction mix:	Settings:
20 µl reaction mixture	**LightCycler® 1.x / 2.0 Instruments**
H_2O 14.4 – 10.4 µl Reagent Mix 1.0 µl FastStart DNA Master[1] 2.0 µl $MgCl_2$ (25 mM) 1.6 µl DNA 1.0 – 5.0 µl (~ 50 ng) **Final $MgCl_2$ conc.:** 3.0 mM	LightCycler® 1.x Instrument: channel F1 LightCycler® 2.0 Instrument: channel 530

[1]LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics)

Programming LightCycler® 1.x / 2.0 Instruments:

Program:	*Denaturation*	*Cycling*			*Melting*			*Cooling*
Parameter								
Analysis Mode	*None*	*Quantification*			*Melting Curves*			*None*
Cycles	*1*	*45*			*1*			*1*
Segment	*1*	*1*	*2*	*3*	*1*	*2*	*3*	*1*
Target [°C]	95	95	60	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]	*20.0*	*20.0*	*20.0*	*20.0*	*20.0*	*20.0*	*0.2*	*20.0*
Acquisition Mode	None	None	Single	None	None	None	Continu.	None

Important Notes:

Temperatures reported in the manual are obtained with a LightCycler® 480 instrument version II. The Tm values may differ by up to 4°C when run on other instruments while the ΔTm values are relative constant.

LightSNiP assays are developed based on synthetic targets and verified using a few genomic DNA samples only, representing at least one of two possible genotypes but have not been validated on a larger number of samples. The amplified region is checked for other published polymorphisms (NCBI) at time of the design to avoid the interference due to other SNPs covered by primers or the probe, however, results may be influenced by other SNPs in the region.

The product is intended for research use only and must be validated on samples with a known genotype.

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.
SimpleProbe® probes produced under license from Roche Diagnostics GmbH.

Appendix 5

SOP No. 5

Title: Isolation of pre-Adipocyte cells (PAC) from fat tissue	
Version: 3	**Date: 01.03.18**
Author: CS2 Reviewer: YL / MH	

Materials

	company	order no.
BSA	Sigma Fridge 5	A 7906
(+) D-Biotin	Roth Fridge 5	3822.1
$CaCl_2$ x 2 H_2O	Roth	5239.1
Cell strainer 70µm	BD Falcon	352360
Collagenase 0.2 U	Serva Fridge 5	17454.01
DMEM/F12	Gibco	31330-038
EGF rh	R & D Systems Freezer 3	236-EG
FCS F	SigmaFreezer 2	F7524
FGF rh	R & D Systems	233-FB
Insulin	SigmaFridge 5	19278
KCl	Roth	6781.1
$MgSO_4$ x 7 H_2O	Merck	1.05886
NaCl	Sigma	S7653
NaH_2PO_4 x 1 H_2O	Merck	1.06346
D-Pantothenic acid hemicalcium salt	Sigma Fridge 5	P5155
PBS	Merck	L 182-50
Pen/Strep (**P/S**)	Sigma Freezer 3 *10.5ml/15ml tube*	P0781
sieve cloth	Merck	intern

Solutions

1. PAC Isolation medium — C_{final}

	C_{final}
500 ml DMEM/F12	/
5 ml Pen/Strep	1%
50 ml FCS F	10%

2. NaH_2PO_4 Buffer — C_{final}

13.79g NaH_2PO_4 x 1 H_2O — 0.1 mM
dissolve in 200 ml pure dest. H_2O
add 17 ml 5 M NaOH > pH 7.4,fill up to 1l, RT, MHD 2-3 month

3. PBS solution — C_{final}

47.75 g PBS — 0.995 %
dissolve in 5 l pure dest. H_2O, autoclave, MHD 2-3 month

4. KRP STOCK Solution 0.1%

Total volume (l)		1	2	4	C_{final} NaCl
g 7.41 14.81 29.64		126.7 mM			
KCl	g	0.38	0.76	1.52	5.07 mM
$CaCl_2$ x 2 H_2O	g	0.195	0.39	0.78	1.36 mM
$MgSO_4$ x 7 H_2O	g	0.31	0.62	1.24	1.27 mM
dissolve in pure dest. H_2O	ml	0.8	1.5	2.5	
very slowly add					
NaH_2PO_4 Buffer	ml	123.5	247	494	12.3 Mm
adjust pH to 7.4 (e.g. 0.5 M KOH) **add**					
BSA	g	1	2	4	0.1 %
adjust pH 7.4, fill up to relevant volume, sterile filtrate, 4°C, MHD 2-3 month					

5. 4% KRP Working Solution (depending on Fat volume) — C_{final}

8 g BSA — 4 %

dissolve in 200 ml KRP STOCK solution, pH 7.4, sterile filtrate, 4°C

6. Collagenase Solution **5ml /1g fat** **35ml/liposuction tube** — C_{final}

330 mg Collagenase — 200U/ml

dissolve in 200 ml 4% KRP working solution, adjust pH 7.4, sterile filtrate,
Residuals can be stored -20°C > reuse 1:1 with new collagenase solution

7. Erythrocytes - Lysis buffer — C_{final}

8.29 g NH_4Cl — 155 mM

0.99 g K_2HPO_4 — 5.7mM

0.037 g EDTA x $2H_2O$ — 0,1mM

dissolve in 0.7l pure dest. H_2O, pH 7.4, fill up to 1l, sterile filtrate, 4°C, MHD 2-3 month

8. Proliferation medium (100ml): — C_{final}

Total volume (ml)		100	200	300	500	C_{final}
DMEM/F12	ml	95.1	190.2	285.3	475.5	/
B/P	ml	1	2	3	5	Panth: 17 µM /Biotin: 33 µM
P/S	ml	1	2	3	5	1%
FCS F	ml	2.5	5	7.5	12.5	2.5%
Insulin solution	µl	7.7	15.4	23.1	38.5	0.13 µM
EGF WORKING	µl	200	400	600	1000	10ng/ml
FGF WORKING	µl	200	400	600	1000	1ng/mL

mix well, can be stored at 4°C for up to 4 weeks

9. BSA Solution	C_{final}
0.1g BSA	0.1 %

dissolve in 100 ml PBS, sterile filtrate, 4°C

10. Biotin/ D-Pantothenic acid Solution (**B/P**)	C_{final}
400 mg Biotine	3.3mM
200 mg D-Pantothenic acid hemicalcium salt	1.7mM

dissolve in 500 ml DMEM-F12 (solution will turn yellow), vortex thoroughly sterile filtrate, aliquot a 10ml > Freezer 3

11. EGF STOCK Solution	C_{final}
200 µg EGF lyophilized	50µg/ml

dissolve in 0.5ml BSA solution + 3.5ml BSA Solution, aliquot à 500µl > Freezer 3

12. EGF WORKING Solution (EGF 5µg/ml)	C_{final}
500 µl EGF STOCK solution	5µg/ml

mix with 4.5ml BSA Solution, aliquot à 200µl > Freezer 3

13. FGF STOCK Solution (FGF Stock 5µg/ml)	C_{final}
25 µg FGF lyophilized	5µg/ml

dissolve in 0.5ml BSA solution, add 4.5ml BSA Solution, aliquot a 500µl > Freezer 3

14. FGF WORKING Solution (FGF 0.5µg/ml)	C_{final}
500 µl FGF STOCK solution	0.5µg/ml

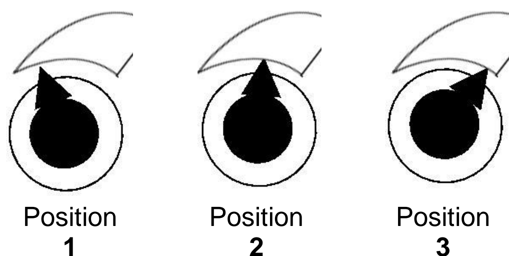
mix with 4.5ml BSA Solution, aliquot à 200µl > Freezer 3

FAT TISSUE

During the whole procedure, wear your S2 lab coat and gloves.

For each sample, new scissors and forceps are required!

1. Check filling level of the water bath & switch it on (37°C)
2. Mix & pre-warm required Medium & collagenase solution
3. Label & **weight** required tubes (**orange lid**)
4. Transfer fat tissue in a petri dish
5. Dissect the fat depots and discard blood vessels and connective tissue
6. Mash fat via scissors and forceps (mushy)
7. Transfer into the tubes (**max. 10 ml each**)
8. Weigh tubes > calculate fat > add relevant collagenase solution (**5ml/g fat**)
9. Close tubes properly with Parafilm & incubate horizontal at 37 °C in the water bath depending on the isolation (see table below)



Isolation	Conditions
Mature Ad.	60 min Pos.1
Pre-Ad-MSCs + Size analysis of mature Ad.	30 min Pos.2 > take 100 µl fat layer 30 min Pos.3
Pre-Ad-MSCs	60 min Pos.3

Isolation for Cachexia	Conditions
Pre-Ad-MSCs + Size analysis of mature Ad.	30 min Pos.3 >take 100 µl fat layer > 30 min Pos.3

10. For mature adipocytes size measurement take after 30 min at position two 100 µl fat layer with special cut 200µl tips. Size analysis see **SOP 6**
11. Close tubes again properly with Parafilm & incubate horizontal at 37 °C in the water bath regarding the conditions (see table above)
12. Take tubes out of the bath, dry lid
13. Centrifuge 10min, 200g, RT, 9/9 brakes (**pellet = PAC**)
14. Meanwhile pre-warm **isolation medium**
15. Clean tubes with 80% EtOH and transfer under the hood

16. Pipet fat layer and supernatant in the waste bottle
17. Add pre-warmed required isolation medium
18. Dissolve pellet via pipetting and transfer through a cell strainer (70µm)
19. Transfer cells to flask/ plate > Incubate cells at 37 °C, 5 % CO_2

Tissue [g]	Plate	Number of wells	Medium (ml)
< 0.5	12 well	2	1 per well >2.5
0.5 – 1.0	12 well	4	1 per well >4.5
1.0 – 1.5	6 well	2	2 per well > 13
1.5 – 2.0	6 well	3	2 per well > 19
2.0 – 2.5	6 well	4	2 per well > 25
2.5 – 3.0	6 well	5	2 per well > 31
3.5 – 5.0	1 x T 25 from Falcon blue lid		10.5 ml
6.0 – 10	2 x T 25 from Falcon blue lid		21 ml
11 – 12	T 75 from Falcon blue lid		10.5 ml

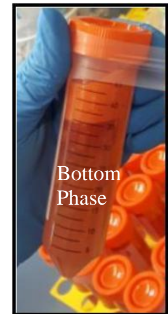
1. Next day**:**
   a. Remove non-adherent cells (e.g. erythrocytes)
   b. Shake culture flask gently to stir up non-adherent cells
   c. Aspirate culture supernatant & wash maximum three times with PBS

2. Add **proliferation medium** (37 °C)
3. Change medium twice a week until 80 - 90 % confluence

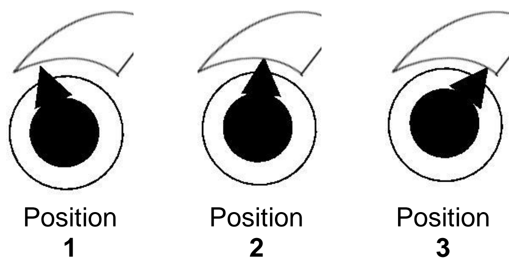
Liposuction:

During the whole procedure, wear your S2 lab coat and gloves

For this isolation, special cut and autoclaved sieve cloths (2000µm, 250µm, 150µm glass filter) and sterile plastic beaker are required!

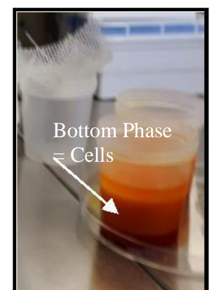


1. **Take liposuction material out of the fridge and rewarm to RT (1h)**
2. Pre-warm KRP STOCK Solution 0.1%
3. Check filling level of the water bath & switch it on (37°C)
4. Calculate and prep collagenase solution (5 tubes > 200ml)
5. Pipet 12.5 ml fat layer per 50 ml tube (50ml pipette)
6. Add 35 ml collagenase solution per tube
7. Close tubes properly with Parafilm & incubate horizontal, 37 °C



Isolation	Conditions
Mature Ad.	60 min Pos.1
Pre-Ad + analysis of mature Ad.	30 min Pos.2 > Size take 100 µl fat layer 30 min Pos.3
Pre-Ad	60 min Pos.3

8. Remove residual water from the tubes lid
9. Clean tubes with 70% EtOH and transfer under the hood
10. Open lids (need to stay sterile!)
11. Set a sterile 2000µm filter on a sterile beaker & pipet (50ml pipette) upper fat phases on it



The bottom liquid reddish phases include relevant PAC

12. Combine all bottom phases (no fat layer) from 50 ml tubes & beaker in tubes (max. 50ml)
13. Centrifuge 10min, 200 g/rcf, RT, 9/9 brakes
14. Meanwhile pre-warm **Erythrocytes - Lysis buffer** and **isolation medium**
15. Carefully discard supernatant

16. Resolve pellets from each 50 ml tube with pre-warmed Erythrocytes - Lysis buffer (3ml per tube >>> 4 tubes digested = 12 ml / 5 = 15ml)
17. Pool all tubes in a new one (blue lid)
18. Incubate 10 min at RT
19. Resuspend again and filtrate over a 250µm filter in a beaker (use a 2000µm filter as holder)
20. Filtrate flow through over a 150µm glass filter in a fresh 50 ml tube
21. Centrifuge flow through 10min, 200 g/rcf, RT, 9/9 brakes
22. Aspirate supernatant
23. Resolve pellet in relevant warm **PAC isolation medium** and transfer to flask (**blue lid**) (e.g. 4 Tubes digested > 6-10x**T75** => 60-100mL)
24. Incubate cells at 37 °C, 5 % CO_2

1. Next day**:**
   d. Remove non-adherent cells (e.g. erythrocytes)
   e. Shake culture flask gently to stir up non-adherent cells
   f. Aspirate culture supernatant & wash maximum three times with PBS

2. Add **proliferation medium** (37 °C)
3. Change medium twice a week until 80 - 90 % confluence

Used dishes / liquids > S2 waste // Used scissors & forceps > bath of disinfection

Appendix 6

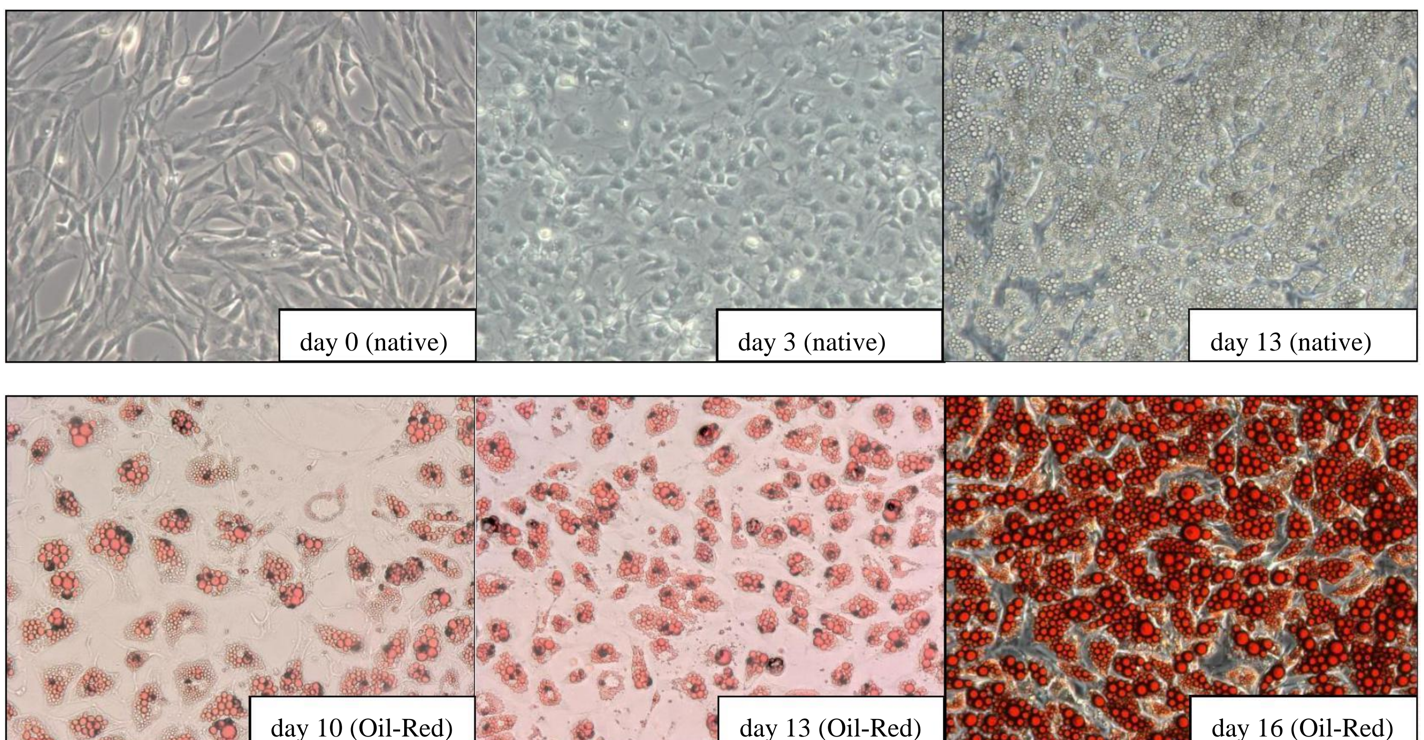
SOP No. 9

Title: Adipogenic differentiation of pre-Adipocyte cells (PAC)	
Version: 6	Date: 26.03.18
Author: KK	
Reviewer: CS2, MH, SH	

Principle

For the induction of adipogenic differentiation, cells are stimulated with a medium containing the antidiabetic agent Rosiglitazone, the glucocorticoid Dexamethasone and the phosphodiesterase inhibitor IBMX aiming a short cultivation time [4].

It was demonstrated that the exposure to Rosiglitazone induced proliferation inhibition in a dose-dependent manner [2]. Moreover, rosiglitazone alone was unable to induce adipocyte differentiation from hMSCs. However, rosiglitazone appears to enhance human mesenchymal stem cells (hMSC) adipogenesis in the presence of other hormones and/or compounds, such as methylisobutylxanthine [4]. *Contador & Ezquer et al.* demonstrated that the simultaneous activation of dexamethasone's canonical signaling pathways, through the glucocorticoid receptor and C/EBPs and rosiglitazone through PPAR-gamma is sufficient yet necessary for inducing hMSC adipogenic differentiation [3]. The differentiation protocol used in this SOP was developed intern and published see Ref. [1].



Materials

	company	storage	order no.
(+)D-Biotin	Roth	Fridge 5	3822.1
BSA (Bovine Serum Albumin)	Sigma	Fridge 5	A 7906
Hydrocortisone	Sigma	Fridge 5	H4001
Dexamethasone	Sigma	Fridge 5	D4902
DMEM/F-12	Gibco	Fridge 3	31330-038
DMSO (Dimethyl sulfoxide)	Merck	RT	1.02931
D-Pantothenic acid hemicalcium salt	Sigma	Fridge 5	P5155
EGF (Epidermal Growth Factor human)	R & D Systems	Freezer 3	236-EG
EtOH abs.	VWR	RT	20821.330
FCS F (Lot: charge: 045M3270)	Sigma	Freezer 2	F7524
FGF (Fibroblast Growth Factor)	R & D Systems	Freezer 3	233-FB
IBMX (3-Isobutyl-1-methylxanthine)	SERVA	RT	26445
Insulin, human	Sigma	Fridge 5	19278
NaCl	Merck	RT	6404
NP-40 (4-Nonylphenyl-polyethylene glycol)	Fluka	RT	74385
PBS (w/o) Ca^{2+} and Mg^{2+}	Merck	RT	182-50
Penicillin-Streptomycin, 10.000units/10mg/ml	Sigma	Freezer 2	P0781
Rosiglitazone	Sigma	Fridge 5	R2408
SDS (Sodium dodecyl sulfate)	Omnilab	RT	2.700131
T3 (3,3'-5-Triiodo-L-Thyronin Sodium Salt)	Sigma	Freezer 2	T-6397
apo-Transferrin human	Sigma	Fridge 5	T2252
Tris-HCl	Sigma	RT	T3253
Trypan Blue solution 0.4%	Sigma	RT	T8154
Trypsin/EDTA	Sigma	Freezer 2	T3924

Abbreviations on the tubes are in parentheses

Solutions

1. BSA Solution — C_{final}

0.1 g BSA — 0.1 %

dissolve in 100 ml PBS, sterile filter 0.2 µm, 4°C

2. Biotin/Pantothenate Solution (B/P) — C_{final}

400 mg Biotin — 3.3 mM

200 mg Pantothenate — 1.7 mM

dissolve in 500 ml DMEM-F12 (mix at room temp. for at least 30 min.)
Solution might turn slightly yellow/orange. sterile filter 0.2 µm, aliquot a 10 ml > Freezer 3
no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

3. Dexamethasone STOCK Solution — C_{final}

9.81 mg Dexa — 25 mM

dissolve in 1 ml EtOH **95%** (9.62 ml EtOH abs + 0.38 ml H_2O), sterile filter 0.2 µm, avoid unnecessary freeze/thaw cycles

4. Dexamethasone WORKING Solution (D) — **C_{final}**

1:1000 dilution of stock solution — 25 µM

15 µl Dexa STOCK solution

+ 14985 µl EtOH **50%** (10 ml EtOH abs + 10 ml H_2O), mix, sterile filter 0.2 µm

aliquot à 1 ml > Freezer 3

no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

5. EGF STOCK Solution — **C_{final}**

200 µg EGF lyophilized — 50 µg/ml

dissolve in 0.5ml BSA solution

transfer everything to 15 ml tube + 3.5 ml BSA Solution, aliquot à 500 µl > Freezer 3

no freeze/thaw cycles

6. EGF WORKING Solution (EGF 5 µg/ml) — **C_{final}**

1:10 dilution of stock solution — 5 µg/ml

500 µl EGF STOCK solution

mix with 4.5 ml BSA Solution, aliquot à 500-1000 µl > Freezer 3

no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

7. FGF STOCK Solution (FGF Stock 5 µg/ml) — **C_{final}**

25 µg FGF lyophilized — 5 µg/ml

dissolve in 0.5 ml BSA solution

transfer everything to 15 ml tube + 4.5 ml BSA Solution, aliquot a 500 µl > Freezer 3

no freeze/thaw cycles

8. FGF WORKING Solution (FGF 0.5 µg/ml) — **C_{final}**

1:10 dilution of stock solution — 0.5 µg/ml

500µl FGF STOCK solution

mix with 4.5ml BSA Solution, aliquot à 500-1000µl > Freezer 3

no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

9. Hydrocortisone STOCK Solution — **C_{final}**

3.625 mg Hydrocortisone — 10 mM

dissolve in 1 ml EtOH 100 %, sterile filter 0.2 µm, avoid unnecessary freeze/thaw cycles

10. Hydrocortisone WORKING Solution (C) — **C_{final}**

500 µl Hydrocortisone STOCK solution — 100 µM

mix with 49.5 ml 50%EtOH, sterile filter 0.2 µm, aliquot à 500-1000 µl > Freezer 3

no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

11. IBMX STOCK solution Solution (IBMX) **C_{final}**

220 mg IBMX 20 mM

dissolve with 50 ml ddH_2O & 1 big tip of a spatula Na_2CO_3, sterile filter 0.2 µm, aliquot à 5ml > Fridge 3

12. Rosiglitazone STOCK solution Solution **C_{final}**

10 mg Rosiglitazone 10 mM

dissolve in 2.797 ml DMSO, sterile filter 0.2 µm, > Freezer 3, avoid freeze/thaw cycles

13. Rosiglitazone WORKING solution Solution (R) **C_{final}**

1 ml Rosiglitazone STOCK solution 2 mM

+ 4 ml DMSO, aliquot à 100-250 µl > Freezer 3, no freeze/thaw cycles;
as soon as thawed: 2 weeks at 4°C

14. T3 STOCK Solution **C_{final}**

5 mg T3 50 µM

dissolve in 7.4 ml EtOH abs. with 2 drops 1M NaOH (ultrasonic)> dilute 1:20 in EtOH (e.g. 500 µl with 9500 µl EtOH abs.), sterile filter, aliquot à 1000µl > -20°C Freezer 3, avoid freeze/thaw cycles

15. T3 WORKING Solution (T3) **C_{final}**

500 µl T3 STOCK solution 2 µM

+ 12 ml 50 % EtOH, sterile filter 0.2 µm, aliquot a 500 µl > -20°C Freezer 3
no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

16. Transferrin Solution (T) **C_{final}**

100 mg Transferrin 1 mg/ml

dissolve in 100 ml ddH_2O, sterile filter 0.2 µm, aliquot a 5ml > Freezer 3
no freeze/thaw cycles; as soon as thawed: 2 weeks at 4°C

Media

1. Proliferation medium (PM)

Total volume (ml)		100	200	300	500	C_{final}
DMEM/F12	ml	95.1	190.2	285.3	475.5	/
B/P	ml	1	2	3	5	Pant: 17 µM / Biotin: 33 µM
P/S	ml	1	2	3	5	1 %
FCS F	ml	2.5	5	7.5	12.5	2.5 %
Insulin solution	µl	7.7	15.4	23.1	38.5	0.13 µM
EGF WORKING	µl	200	400	600	1000	10ng/ml
FGF WORKING	µl	200	400	600	1000	1ng/ml

mix well, can be stored at 4°C for up to 4 weeks.

2. Differentiation medium (DM)

Total volume (ml)		100	200	300	500	C_{final}
DMEM/F12	ml	96.8	193.6	290.4	484	/
B/P	ml	1	2	3	5	Panth: 17 µM / Biotin: 33 µM
P/S	ml	1	2	3	5	1%
Transferrin **T**	ml	1	2	3	5	0.01 mg/ml
Insulin solution	µl	50	100	150	250	0.86 µM
T3 WORKING	µl	50	100	150	250	1 nM
Hydrocortisone **C**	µl	100	200	300	500	0.1 µM

mix well, can be stored at 4°C for max. 3 weeks

3. Induction medium (IM)

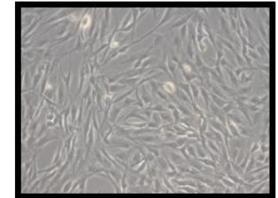
Total volume (ml)		25	50	100	200	C_{final}
Differentiation medium	ml	24.7	49.3	98.6	197.2	/
Rosiglitazone (**R**)	µl	12.5	25	50	100	1 µM
Dexamethasone **(D)**	µl	25	50	100	200	25 nM
IBMX	µl	312.5	625	1250	2500	0.25 mM

mix well, always prepare fresh!

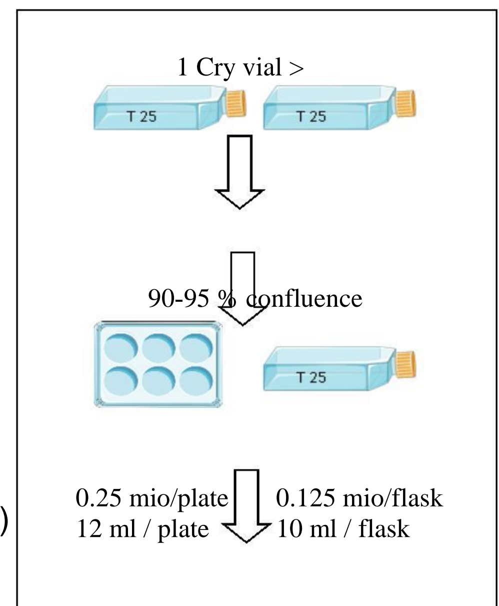
During the whole procedure, wear your S2 lab coat and gloves.
For working with cryopreserved cells, see **SOP 11** for Freezing/ Thawing cells.
Only use plastics from **BD Falcon tissue culture treated = TC treated**

Passaging & sub culturing

Splitting/Passaging



1. Let cells proliferate until ca. 90-95 % of confluence with medium changes twice a week, but keep approx. 300µL of old medium every time
2. Aspirate and discard the medium
3. Wash the cells twice with 10 ml PBS
4. Add 1ml **TRY**/T25 flask directly on to the surface & incubate for 5-7 min (max. 10 min) at 37°C, make sure trypsin covers the complete surface!
5. Further detach cells by agitating the culture flask
6. Check detachment of cells (by eye or microscope)
7. Add 9 ml pre-warmed proliferation medium (**PM**)
8. Transfer everything to a 50 ml tube (gently pipette up and down to rinse flask 2-3 times with 7-8ml of cell suspension)
9. Gently resuspend cells
10. Count cells via Neubauer Chamber (SOP. 10)



100 % confluence > Start of differentiation

Subculture in proliferation medium:

The subculture process (6-7days until confluence) is important to build ECM & give certain sub clones the chance to proliferate quicker than others.

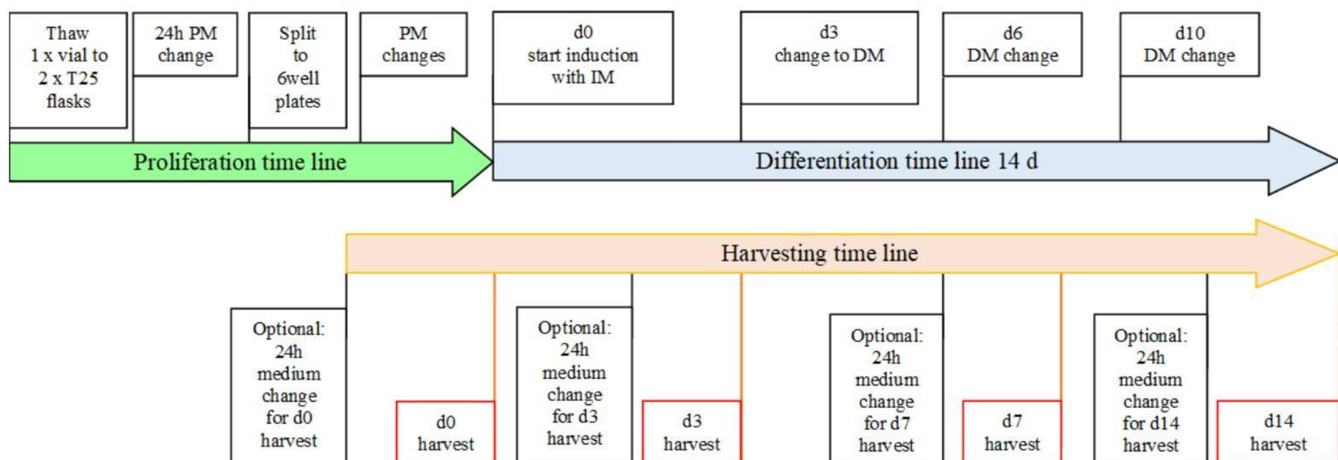
1 x cry vial > 2 x T25 flasks > confluence (ca. 0.8 – 1mio. in total) >

Split to 6-well plates or additional T25 flasks > 0.25 mio/ 6 well plate / 0.125mio/flask

> confluence (6-7days, medium change twice a week, but leave approx. 300µL of old medium) > **Start differentiation**

Differentiation

1. When changing proliferation medium, keep some rest liquid (approx. 300 µl) in the well > keeps "cell produced growth factors" for further proliferation
2. When 95-100 % confluence is reached, remove all (!) proliferation medium
3. Add induction medium (**IM**) (=day 0) (2 ml/ 6 well)
4. On day 3 **IM** has to be completely replaced by differentiation medium (**DM**)
5. Afterwards change medium twice a week, keep some rest liquid (approx. 300 µl) in the well (2 ml/ 6 well; usual schedule: Tuesday/Friday or Monday/Thursday)
6. Incubate in accordance to the time points (e.g. d3, d10 and d24)
7. See below a time frame for an experiment



For Harvesting procedure see Guideline F

References

[1] Protein Normalization in Different Adipocyte Models and Dependence on Cell Size Horm Metab Res. 2013 Aug;45(8):572-80. doi: 10.1055/s-0033-1341429. Epub 2013 Apr 2
[2] Huang, H. W., et al. (2009). "Rosiglitazone and all-trans retinoic acid inhibit human myeloma cell proliferation via apoptosis signaling pathway modulation." Zhonghua Xue Ye Xue Za Zhi 30(4): 242-246.
[3] Contador, D., et al. (2015). "Featured Article: Dexamethasone and rosiglitazone are sufficient and necessary for producing functional adipocytes from mesenchymal stem cells." Experimental Biology and Medicine 240(9): 1235-1246
[4] Ninomiya, Y., et al. (2010). "Development of a rapid culture method to induce adipocyte differentiation of human bone marrow-derived mesenchymal stem cells." Biochem Biophys Res Commun 394(2): 303-308

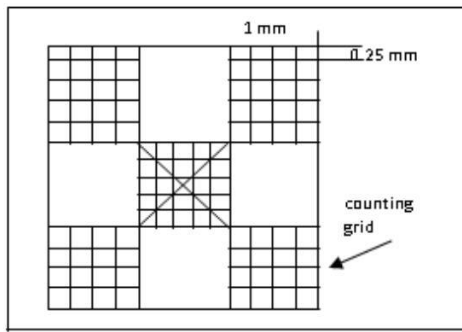
Appendix 7

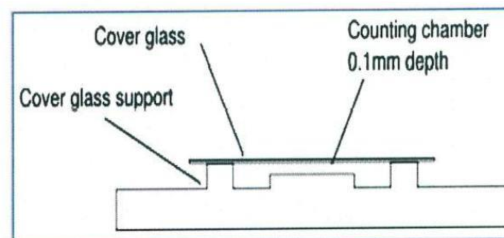
SOP No. 10

Title: Cell count and viability Trypan blue	
Version: 1	Date: 02.05.2018
Author: CS2 Reviewer: KK	

Principle

The **haemocytometer** (Neubauer chamber) is a device originally designed for counting blood cells. Nowadays also used to count other types of cells as well as other microscopic particles.





The diazo dye Trypan Blue is a vital stain used to selectively color dead tissues or cells in blue.

Materials

	company	order no.
Ethanol 70 %	intern	/
Trypan Blue solution 0.4%	Sigma	T8154

Procedure

1. **During the whole procedure, wear your S2 lab coat and gloves**
2. Clean the haemocytometer and the cover slip with Ethanol 70%
3. Breathe on the two bars of the haemocytometer and put the cover glass on until you can see the Newton's rings
4. Mix the cell suspension thoroughly, take out 50 µl and mix it with 50 µl Trypan Blue working solution
5. Transfer 10 µl of the mixture into each counting chamber

6. Count the cells in the eight outer counting grids (*Note: For cells on the sides of the squares in the counting grid, count in an L-shape* (only 2 of 4 sides) to avoid double counting!)
7. Calculate average cell number (total number of cells / amount of counting grids)

Calculation

1. Total number of cells = n * Vt * V * 10^4
2. Number of living cells = m * Vt * V * 10^4
3. Viability [%] = number of living cells / total number of cells * 100 [%] = m / n * 100

n: average of counted cells
m: average number of living cells
Vt: dilution factor (in this case 2)
V: volume of cell suspension
10^4: factor of the cell-counting chamber

<u>Example</u>

- 20 ml cell suspension
- counted average 82 (total number of cells / number of counting grids)
- living cell average 76 (total living cells / number of counting grids)
- dilution factor 2

> Total number of cells: 82 * 2 * 20 * 10^4 = 32.8 million cells
> Number of living cells: 76 * 2 * 20 * 10^4 = 30.4 million cells
> Viability: 30.4 million / 32.8 million * 100 = 92.7%

Appendix 8

SOP No. 11

Title: Cryopreservation of cells	
Version: 2	Date: 02.05.2018
Author: CS2 Reviewer: KK/SH	

Materials

	company	order no.
(+)D-Biotin	Roth Fridge 5	3822.1
CryoTubes 1ml	Thermo Scientific	3777224
Culture medium	(depending on the cell type)	
DMSO	Roth	4720.1
EGF rh	R & D Systems Freezer 3	236-EG
FCS F	Sigma Freezer 2	F7524
FGF rh	R & D Systems Freezer 3	233-FB
Inserts	Thermo Scientific	/
Isopropanol (Propan-2-ol)	Fisher Chemicals	P7500
MrFrosty	intern	/
Pen/Strep (P/S)	Sigma Freezer 2	P0781
Panthothenate (D-Pantothenic acid hemicalcium salt)	SigmaFridge 5	P5155
Trypan Blue solution 0.4%	Sigma	T8154
Trypsin/EDTA (TRY)	Sigma Freezer 3	T3924

Solutions

1. Proliferation medium PAC (100ml):

		Cfinal
96.1	ml DMEM/F12	/
1 ml	B/P	Panth: 33 µM /Biotin: 17 µM
1 ml	P/S	1%
2.5 ml	FCS F	2.5%
7.7 µl	Insulin solution Sigma	0.13 µM
200 µl	EGF WORKING solution EGF 5µg/ml	10ng/ml
200 µl	FGF WORKING solution FGF 0.5µg/ml	1ng/mL

mix well, can be stored at 4°C for up to 2 weeks.

2. Freezing medium (100ml):

	Cfinal
90 ml Culture Medium (PAC = Proliferation medium)	90%
10 ml DMSO	10%

mix freshly and store at 4°C before use

- **Store MrFrosty at 4°C, check Isopropanol, if precipitations visible replace Isopropanol (250 ml / MrFrosty)**
- **Prepare Freezing medium and store at 4°C**
- **Warm up PBS and Culture medium, defrost Trypsin (TRY) and store at RT**

Freezing cells:

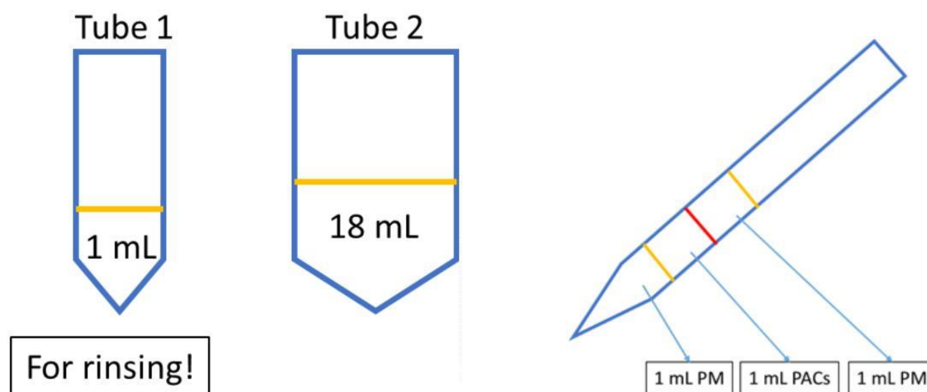
During the whole procedure, wear your S2 lab coat and gloves

1. Cell requirements (> 80 % confluence):
2. PACs: 0.5x10^6 cells / ml freezing medium
3. Insert corresponding inserts (red = human)
4. Aspirate culture medium from the adherent cells
   (for suspensions cell lines skip to point 12 of this SOP)
5. Once wash cells carefully with PBS
6. Add 1 x Trypsin-EDTA (TRY)
7. Incubate for 5 - 10 min at 37 °C, 5 % $C0_2$
8. Detach cells by agitating the culture flask
9. Check detachment of cells under the microscope
10. Add pre-warmed culture medium (1:10 see table)
11. Transfer cell suspension to 50 ml tubes, remove 50 µl aliquot for cell counting
12. Centrifuge: 200 g/rcf, 10 minutes, RT, 9/9 brakes
13. Meanwhile count cell number by adding 50 µl Trypan Blue > Count cells (SOP No. 10)
14. **Label required cryotubes with name of cell, concentration, date and name of the Operator *(*label marker plus program*)***
15. Meanwhile transfer cryotubes under the hood and open them
16. Aspirate supernatant
17. Resuspend pellet with required cold freezing medium (e.g. 20Mio cells + 40ml)
18. Immediately distribute the cells into the cryotubes (1ml / tube)
19. Immediately put the cryotubes in the Cryo-Safe Cooler (MrFrosty)
20. Put the Cryo-Safe Cooler to -80 °C for up to 3 days
21. Transfer cryotubes to the nitrogen tank

Thawing cells:

During the whole procedure, wear your S2 lab coat and gloves

1. Prepare and label 2 x T25 flasks with name, ID, passage, date
2. Warm up required medium up to 37 °C (PAC PM = Proliferation medium) and aliquot medium in tube 1 and 2
3. Rapidly thaw cells in cryotubes at 37°C water bath & clean with 80 % EtOH
4. Use a 5 ml pipette and adjust the pipette boy on the slowest mode
5. Take 1ml medium out of tube 2; than 1ml of cells (very slowly, dilution of DMSO) and again 1ml medium out of tube 2, so that there are 3 phases in the pipette



6. Very carefully pipette all three phases back in tube 2
7. Rinse cryo-vial with 1ml fresh medium of tube 1 & carefully add to the cell suspension
8. Transfer cell suspension into the 2 x T25 flasks (0.25 Mio / T25er flask)
9. After 24 h, check if cells attached to culture plastics
10. Change medium to remove dead cells and DMSO
11. Change medium twice a week until cells reach confluence (ca. 4-6 days)

Appendix 9

SOP No. 16

Title: DNA isolation from blood/ tissue/ cell lysates	
Version: 4	**Date:.16.04.18**
Author: CS Reviewer: JH,MH	

Principle

The DNeasy Blood&Tissue Kit by Qiagen provides a silica-based DNA purification**.** After isolation approximately 500ng DNA are expected. For genotyping, usually 1500 ng qPCR product in 15 µl are required.

Materials

	company	**order no.**
1.5ml DNA Low Bind tubes	Eppendorf	0030 108.051
2 ml DNA Low Bind tubes	Eppendorf	0030.108.078
Beads silica-zirconia	Roth	11079105
DNeasy Blood&Tissue Kit	Qiagen	69504
Ethanol 100% p.A.	VWR	20821.330
FastPrep 24 homogenizer	MP Biomedicals	
Microtubes	Sarstedt	72.693.465
PBS	Merck	L 182-50
PCR-Filter Tips	Sarstedt	
Proteinase K	Qiagen	19131
RNase A (17.500U)	Qiagen	19101
Trypan Blue solution 0.4%	Sigma RT	T8154
Trypsin/EDTA (TRY)	SigmaFreezer 2	T3924

Solutions

1. PBS solution — C_{final}

47.75 g PBS — 0.995 %

dissolve in 5 l pure dest. H_2O, autoclave, MHD 2-3 month

2. AW1 (component of the DNeasy kit)
Add EtOH p.A. according to the volume stated on the flask

3. AW2 (component of the DNeasy kit)
Add EtOH p.A. according to the volume stated on the flask

4. ATL & AL buffer ready to use (precipitates? > 56°C until disintegration)

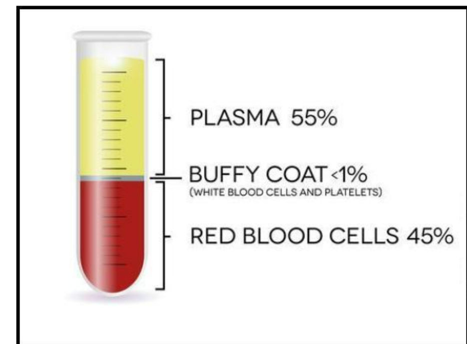
5. Ethanol/AL mixture (only for tissue) per sample mix 200µl EtOH abs.
+ 200µl AL buffer

Sampling:

Wear your S2 lab coat and gloves. Clean work surface with RNase Zap.
Preheat the thermo shaker with the according insert (1.5 ml or 2 ml) to 56°

Whole blood fresh:

1. Centrifuge 2.500g/rcf, 10min, RT
2. Remove plasma
3. Use 100µl of the buffy coat for isolation



Whole blood frozen after centrifugation:

1. Defrost on ice (Attention: 9ml tubes need 2h)
2. Don't shake or vortex
3. Remove plasma
4. Slowly collect buffy coat (grey slurry) in a swirling motion
5. Use 100µl of the buffy coat for isolation

Whole blood frozen without centrifugation:

1. Defrost on ice (Attention: 9ml tubes need 2h)
2. Do not centrifuge!
3. Use 200µl whole blood from the upper third for isolation

Fat tissue:

1. Weight approximately 200 mg **Beads** in a microtube
2. Fill tubes in a bag, autoclave & put bag under the UV cleaner for 5 h
3. Transfer fat tissue (max. 100 mg > 50ng/µl, more overload the column) in a microtube
4. Label tubes on top & side
5. Add **180µl ATL Puffer** & close tubes and seal with parafilm
6. Fast prep samples (MP2x24, Speed 6, 30s) > store samples 30 seconds on ice
7. Repeat fast prep for 2 more times (between samples on ice)
8. Centrifuge 12.000g/rcf, 10 min, 4°C
9. Transfer lower phase with an insulin syringe in a new 2ml DNA LoBind tube (don´t transfer fat!) alternative use long 200 µl Sorenson tips

Cells (PACs 1x T75er flask = $2x10^6$ / maximum $5x10^6$ cells):

1. Aspirate culture medium & wash cells once with PBS
2. Add 1 ml **TRY**/T75 flask directly on to the surface
3. Incubate for 5-7 min (max. 10 min) at 37°C
4. Further detach cells by agitating the culture flask
5. Check detachment of cells (by microscope)
6. Add 9 ml pre-warmed proliferation medium (PM see SOP 5)
7. Transfer everything to a 50 ml tube
8. Centrifuge 300 g/rcf / RT/ 10 min
9. Gently resuspend cells in 200µl PBS in a new 1.5 ml DNA LoBind tube
10. Freeze at -80°C until further procedure

Lysis:

1. Heat up Eppendorf thermos block 1.5 ml tubes, 56°C
2. Pipette components into a 1.5 ml DNA LoBind tube:

	Buffy coat	**Whole blood**	Cell lysate	Tissue
Sample	100 µl	200 µl	200µl in PBS	lower phases
Proteinase K	20 µl	40 µl	20 µl	20 µl
RNase A	4 µl	8 µl	4 µl	
PBS	100 µl	200 µl	/	
	Vortex & incubate 2 min RT			
AL-buffer	200 µl	400 µl	200 µl	
Vortex & incubate 10 min 56°C				
RNase A				4 µl
AL-buffer				200 µl vortex
EtOH	200 µl	400 µ	200 µl	200 µl
Vortex & transfer everything onto the spin columns				
Spin columns	one	**two**	one	one
Spin through ≥ 6.000g/rcf (8.000 rpm), 1min, RT				

After lysis, cool down the thermo shaker to 37°C and pre heat AE buffer

Washing

1. Discard flow through
2. Transfer spin column on a new collection tube (liquids transfer in waste bottle)
3. Add 500µL AW1 buffer / column
4. Centrifuge :≥ 6.000g/rcf (8.000rpm), 1min, RT
5. Transfer spin column on a new collection tube (liquids transfer in waste bottle)
6. Wash column with 500µL AW2 buffer
7. Centrifuge: **14.000g/rcf,** 3min, RT
8. Transfer spin column on a new DNA LoBind tube

Elution for 100µl buffy coat & tissue; one spin column

1. Add 60µL AE-buffer (**37°C for BC / 50°C for tissue**) directly (!) on the membrane
2. Centrifuge: 8.000g, 1min, RT (place the lid of the tube on the rotor)
3. Add 40µL AE-buffer directly (!) on the membrane
4. Centrifuge: 8.000g/rcf, 1min, RT (place the lid of the tube on the rotor)

Elution for 200µl whole blood; two spin columns

1. Transfer the first and second spin column onto two separate DNA LoBind tubes
2. Add 60µL AE-buffer (37 °C) directly (!) on the membrane of column 1
3. Centrifuge: 8.000g/rcf, 1min, RT (place the lid of the tube on the rotor)
4. Add 40µL AE-buffer (37 °C) directly (!) on the membrane of column 1
5. Centrifuge: 8.000g/rcf, 1min, RT (place the lid of the tube on the rotor)
6. Use the eluate from column 1 to eluate column 2 in the same manner, this approximately doubles your yield

Quality Control (Tecan or Nano Drop)

1. Use the Tecan photometer with the NanoQuant Plate (260/280 & (260/230))
2. In the software select the applications tab & choose dsDNA as sample type
3. Blank with AE-buffer
4. Use a sample volume of 1.5 µl and measure in duplicates
5. When highly accurate concentrations are required (e.g. array based analysis) a fluorescent dye should be used (e.g. PicoGreen or Qubit)
6. For the rs1421085, Simple Probe (SOP No.21) assay ~50 ng are required!
7. Samples can be stored at -4°C for 2 month / long time -20°C

References

[1] Qiagen DNeasy handbook
[2] Thermo fisher nucleic acid quantitation and quality control

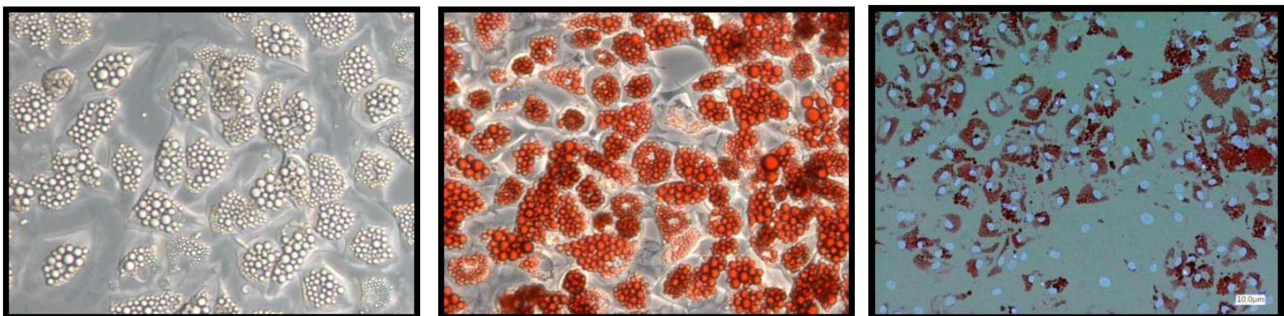
Appendix 10

SOP No. 19

Title: Oil red staining ± counter staining NucBlue	
Version: 2	Date:.04.07.18
Author: KK, CS2 Reviewer: JH	

Principle

Oil Red O staining is used to detect neutral lipids (red) in cultured cells / spongiosa. For cell-permeant nuclear counterstain NucBlue (Hoechst 33342) is used. It emits blue fluorescence when bound to DNA.



Unstained, Oil red stained & NucBlue + OR stained differentiated hPAC

Materials

	company	order no.	storage
NucBlue (Hoechst)	Thermo Scientific	R37605	RT
Oil-Red-O	Sigma	O0625	RT
Isopropanol	Roth	CP41.3	RT
PBS	Merck	L182-50	RT
Histofix (Formaldehyde 4 % buffered)	Roth	P087	RT
Glycerin	Roth	3783.1	RT
Kaiser- Gelatine	Merck	1.09242	RT

Solutions

1. Oil red Working Solution — C_{final}

0.3 g Oil red O — 0.3 %

dissolve in 60 ml Isopropanol & 40 ml H2O, **always filtrate before use!**

2. PBS solution — C_{final}

47.75 g PBS — 0.995 %

dissolve in 5 l pure dest. H_2O, autoclave, MHD 2-3 month

Procedure

1. Carefully aspirate culture medium
2. Wash cells twice with PBS, RT
3. Add relevant volume Histofix
4. Incubate for minimum 20min at RT
5. Aspirate Histofix & cover cells with Oil Red O Working Solution
6. Incubate for 1h at RT
7. Wash cells extensively with PBS to remove unbound stain
8. Add relevant volume PBS per well (6-well: 2ml/well)
9. For counter staining with **NucBlue** see page 3
10. Take a picture before resolving the stain for quantification
11. For storage wrap plate with paraffin > 4°C
12. Long term storage > add 87% Glycerin or Kaiser- Gelatine solution (ml equal to oil red staining solution), wrap plate with paraffin > 4°C

Quantification

1. Remove all water/PBS from the wells and let them dry on air for 10 min
2. Cover cells with relevant µl isopropanol (6-well: 800 µl/well)
3. Incubate for 10-20 minutes at RT (shaker) till all stain is resolved
4. Transfer 75µl/ well in a flat bottom 96well plate (3x determination) from Brand
5. Measure the OD of the resolved stain at "A = 492 nm" with the TECAN plate reader (5 blitz, settle time: 100 ms, no shake, no heating).
6. For absolute quantification (Oil red intensity (nm) /mg protein) add RIPA buffer per well (200µl/ 6 well) and determine protein content (see SOP 18)

Counter Stain with NucBlue

For evaluating the exact cell number as well as differentiation rate of the cells a counter staining with NucBlue **after the OR staining** is recommended.

1. Carefully aspirate PBS of the Oil red stained cells
2. Add relevant volume PBS (e.g. 6er 2 ml/well)
3. Add relevant drops NucBlue

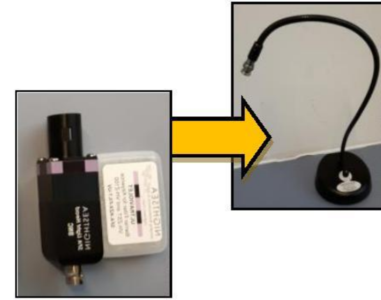
Well format	wash/ fix/ staining	NucBlue drops
96-well	50 µl / well	/
48 well	300 µl / well	1
24 well	500 µl / well	1
12 well	1 ml / well	2
6-well	2 ml / well	4

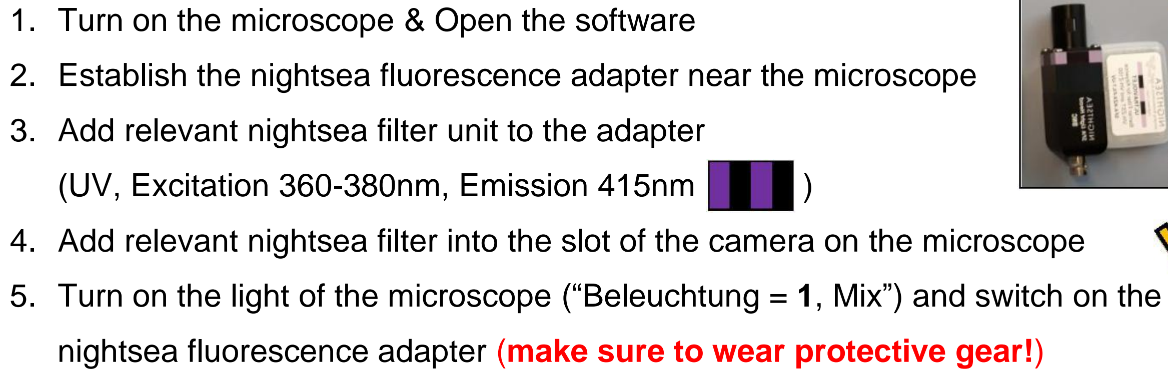
4. Incubate at 15min at 37°C, 5% CO_2 (RT in the dark also works)
5. Aspirate PBS and NucBlue solution
6. Wash cells twice with PBS to remove unbound stain
7. Add PBS per well (see table above)
8. Take a picture
9. Optional is a quantification (see **Quantification** page 2)
10. For storage wrap plate with parafilm > 4°C *(Fluorescence remains up to 3 days)*

References

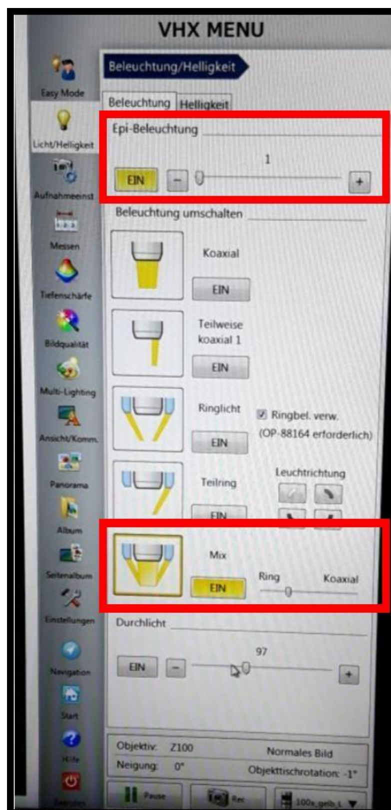
[1] Ramirez-Zacarias, J.L., et al., Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red 0. Histochemistry, 1992. 97(6):493-497.

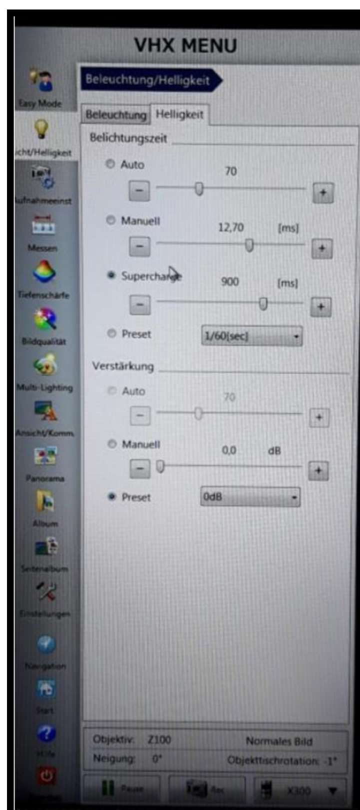
Imaging via Keyence microscope





1. Turn on the microscope & Open the software
2. Establish the nightsea fluorescence adapter near the microscope
3. Add relevant nightsea filter unit to the adapter
   (UV, Excitation 360-380nm, Emission 415nm)
4. Add relevant nightsea filter into the slot of the camera on the microscope
5. Turn on the light of the microscope ("Beleuchtung = **1**, Mix") and switch on the nightsea fluorescence adapter **(make sure to wear protective gear!)**





Parameter	ms
"Supercharge"	400-1200 ms
"Verstärkung (Gain)"	Manual 3-6 db

6. Add your cell plate and focus your cells
7. Change to the Register "Helligkeit" and change from "Auto" to "Supercharge"
8. Turn on "BILDSTABILISATOR"
9. Take one pic & transfer it

Alternatively: Take one pic in the bright field modus and one in the fluorescence set up (only one with scale bar!) Transfer pic to a stick and create an overlay with e.g. adobe Photoshop

Appendix 11

SOP No. 21

Title: FTO – rs1421085 Genotyping using Roche Simple Probes	
Version: 4	Date:.05.07.2018
Author: JH Reviewer: LM, CS2	

Principle

Roche SimpleProbes are molecular hybridization beacons designed to emit fluorescence when binding to complementary DNA. The probe sequence either matches to the homozygous wildtype or mutant [1]. In a melting curve analysis where temperature is increased gradually the probe dissociates at higher T_M when it fully matches the complementary sequence. If a mismatch/SNP is present, the probe dissociates earlier resulting in a decrease of fluorescent signal at lower T_M. Therefore, each variant results in a unique melting curve that is further explained in Figure 1 (WT, Heterozygous, Mutant).

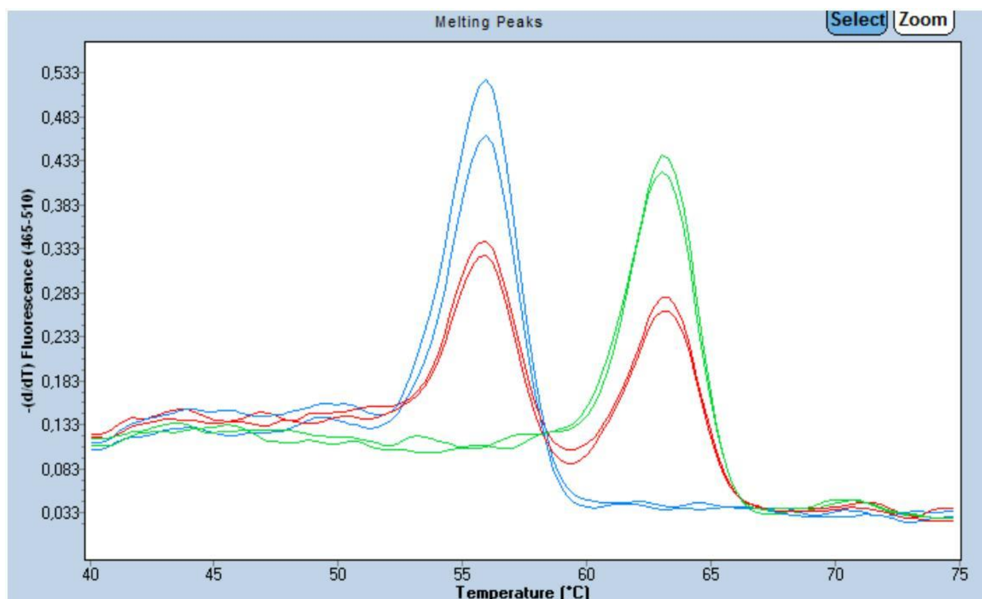


Figure 1: Melting curves for rs1421085 (FTO). 2 WT (Blue-TT), 2 Heterozygous (Red-CT), 2 Mutant (Green-CC)

The probe is designed to match the mutant variant [CC]. As the sequence fully matches the mutant the probe dissociates from the template at the highest melting temperature (green peak ~ 64°C). For the heterozygous variant the probe matches the mutant allele [C] and has a mismatch in the WT allele [T]. Therefore 2 peaks are visible at ~ 64 °C and ~55 °C (depicted in red). As the probe mismatches for both WT alleles [TT] this results in a single peak at the low T_M of ~55 °C (depicted in blue).

Materials & reagents

	Company	Order Nr	Storage
FrameStar® 384 Well Skirted PCR Plate, white wells,contains according seal	4titude	4ti-0382	
Matrix™ Equalizer Electronic Multichannel Pipette	Thermo Scientific	2032	
Matrix™ Pipette tips, 30 µl	Thermo Scientific	7431	
Eppendorf Multistepper	Eppendorf	-	
Eppendorf Combitips 100 µl	Eppendorf	0030089405	
Roche lightcycler 480	Roche	-	
Centrifuge with swing out rotor for plates	Eppendorf	-	Room 2.62
Vortexer	-	-	
Benchtop Centrifuge	-	-	
PCR grade water	any	-	RT
$MgCl_2$ 25 mM	any	-	RT
MyTaq DNA Polymerase 1 JH	Bioline	BIO-21106	Freezer
5x MyTaq Reaction Buffer 1 JH	Bioline	BIO-21106	Freezer
LightSNip rs1421085 FTO	TIB MOLBIOL	-	Fridge 5

Preparations

Light Cycler & Run Conditions

1. Enter your name, chair and phone number in the LC480 google calendar in advance (Login:lightcycler.480.witt@gmail.com pw: lc480witt), 1 run ~ 1.5 hours
2. User and password for PC and Light Cycler Software are listed on the screen of the computer

Programming LightCycler® 480 Instrument:

Program:	*Denaturation*	*Cycling*			*Melting*			*Cooling*
Parameter								
Analysis Mode	*None*	*Quantification*			*Melting Curves*			*None*
Cycles	*1*	*45*			*1*			*1*
Segment	*1*	*1*	*2*	*3*	*1*	*2*	*3*	*1*
Target [°C]	95	95	60	72	95	40	75	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s] ***384***	*4.6*	*4.6*	*2.4*	*4.6*	*4.6*	*2.0*	-	*2.0*
Ramp Rate [°C/s] ***96***	*4.4*	*4.4*	*2.2*	*4.4*	*4.4*	*1.5*	-	*1.5*
Acquisition Mode	None	None	Single	None	None	None	Continu.	None
Acquisitions [per °C]							3	

To start the experiment, select new experiment from existing template and open the prewritten template (**User 10 > Templates > JH_SimpleProbeFTO_MyTaq**)

SimpleProbe

1. The lyophilized probe is stored at room temperature protected from light (Manus roll container)
   - Never freeze the lyophilized SimpleProbe!
2. Spin down the vial before opening to ensure the yellow pellet is located at the bottom of the tube
3. Add 100 µl PCR grade water to the lyophilized probe
4. Mix the solution by vortexing and spin down shortly
5. Always keep the solution protected from light and store in the fridge 5 main lab **pink cardboard box** (Simple Probe FTO rs1421085)

Preparation of the reaction mix

1. To calculate the total amount of reaction mixture needed for your sample number refer to the attached excel sheet [2] (**for a whole 384 plate overhang = 35**).

10 µl Reaction mixture			
Reagent	Stock Concentration	Volume [µl]	Final Concentration
5x MyTaq Reaction Buffer	5x	2	1x
Reagent Mix Simple Probe Cont. Primer & Probe	N/A	0,5	N/A
MyTaq Polymerase	N/A	0,2	N/A
DNA	-	2	-
Water	N/A	5,3	
Final Volume	-	10	-

2. The reaction mixture can be stored on ice until used when protected from light

Pipetting

1. Vortex each DNA samples (Isolation see SOP 16)
2. For the assay ~50 ng are required!
3. Place 1.5 ml DNA sample tube in a rack and open

4. Set the Matrix pipette to 3 µl aspiration and 2 µl dispensing (Single determination)
   A maximum of eight samples can be transferred at once with the matrix pipette
5. Mark your pipetting scheme with permanent marker on the plate
6. In the widest setting aspirate 3 µl from the sample tube
7. Set the width between the pipette tips to the smallest setting and dispense 2 µl sample into the very bottom of the well (residuals pipet back in the original tube)
8. As no template control (NTC) pipet 2 µl PCR grade water in a well
9. Spin the plate down shortly (Eppendorf centrifuge Room 2.62)
10. Dispense 8 µl reaction mixture into each well (Multistepper Eppendorf)
11. Spin the plate down shortly (Eppendorf centrifuge Room 2.62)
12. Seal with the according adhesive foil and protect from light with aluminum foil
13. Put plate into the device and run the assay

Genotype calling

1. In the analysis tab the software automatically analyzes which genotype is present and displays the genotype in each well in a different color (mutant, heterozygous, WT)
2. Wells where the auto call did not work are depicted in a fourth color and need to be assigned manually
3. Mark a couple of samples where the calling worked as a comparison and assign the unknown sample to its correct genotype
4. If the signal is too weak or not assignable the experiment needs to be repeated
5. Export your results by right click into the results table
6. If desired take screenshots of the melting curves

Associated files:

[1] LightSNiP rs1421085 FTO Manual
(EKFZ - Labor 2. Stock\MATERIAL & METHODs\SOPs\Company Manuals)
[2] SOP_21_Simple Probe_Genotyping_V1_Calculation
(EKFZ - Labor 2. Stock\MATERIAL & METHODs\SOPs)
[3] 20180612_Vortrag_Simple Probe_JH
(EKFZ - Labor 2. Stock\LAB-SEMINAR\Presentations)

Troubleshooting:

- If in the first run some samples show a low signalling not enough for genotyping, rerun the plate again with the same program.

Appendix 12

SOP No. 29

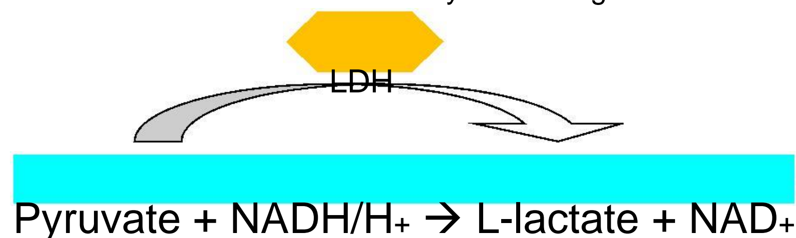
Title: LDH Measurement	
Version: 1	Date: 08.05.19
Author: SH Reviewer: CS2	

Principle

Lactat dehydrogenase (LDH) is an enzyme that appears in almost all cell types and is rarely found in blood under normal conditions. When cells die, LDH reaches bloodstream *in vivo* or supernatant *in vitro* and for that reason functions as a marker for cell apoptosis or cell stress.

LDH catalyzes the reversible conversion of pyruvate to L-lactate with NADH as a cofactor. The enzyme is located in the cytoplasm and its activity significantly increases during adipocyte preparation and can *in vitro* be reduced by a change of cell culture media. In order to achieve a precise progression of experiments with adipocytes, a marginal LDH activity has previously to be ensured. The activity of the enzyme is measured as the amount of pyruvate is consumed, by continuously monitoring the decrease in absorbance due to the oxidation of NADH at 339 nm using a UV reader.

LDH activity is determined as decrease in NADH by measuring the decrease of absorbance at 339 nm.



Materials

	company	storage	Room	order no.
NADH Disodium Salt	AppliChem	-20°C	2.62	A1393, 1000
Sodiumpyruvate	AppliChem	RT	2.65	A4859,0050
Tris	AppliChem	RT	2.65	A2264, 1000
NaCl	AppliChem	RT	2.65	A4661, 1000
Streifenplatte 96-well (12 x F8), medium binding	Brand		Storage 2	782300
Plate Reader Tecan Infinite 200 PRO	Tecan Group		2.65	

Solutions

1. Tris/NaCl solution pH 7,2 — **c_{final}**

4,92 g Tris (MW 121,14 g/mol) — 81,22 mM
5,95 g NaCl (MW 58,44 g/mol) — 200 mM
Dissolve in approx. 400 ml bidest. H_2O, adjust pH to 7,2 & fill up to 500 ml. Store at 4°C.

2. NADH — **c_{final}**

17 mg NADH (MW 709,41 g/mol) — 0,24 mM
Dissolve in 100 ml Tris/NaCl solution pH 7,2. Prepare 3 ml aliquots and store at -20°C.
Attention: aliquots can only be refrozen one time. Freezer 4, Tray, 3

3. Pyruvate — **c_{final}**

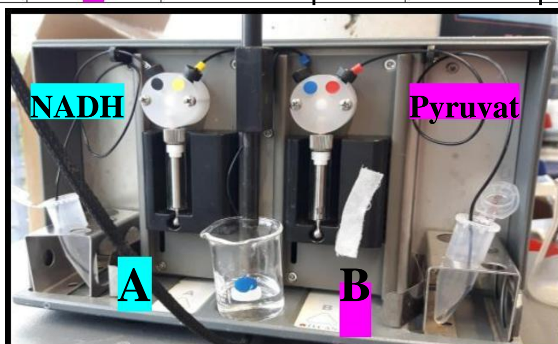
107 mg Sodiumpyruvate (MW 110,04 g/mol) — 9,72 mM
Dissolve in 100 ml Tris/NaCl solution pH 7,2. Prepare 3 ml aliquots and store at -20°C.
Attention: aliquots can only be refrozen one time. Freezer 4, Tray, 3

Procedure

Aliquot 50 µl supernatant of your samples in 0.2 ml tubes. Samples can be stored at 4°C up to 24 – max. 48 h before measurement is carried out.

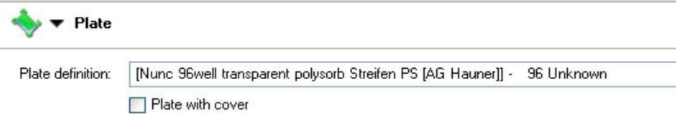
1. Unfreeze two aliquots (à 3 ml) of NADH and one aliquot Pyruvate at RT
   (sufficient for 16 wells (=8 samples in duplicates) on a 96-well plate)

Reagent	Injector	Filling Injector	Priming Injector	Injection µl/ Well
NADH	A	1000 µl	750 µl	156 µl
Pyruvate	B	1000 µl	750 µl	31 µl

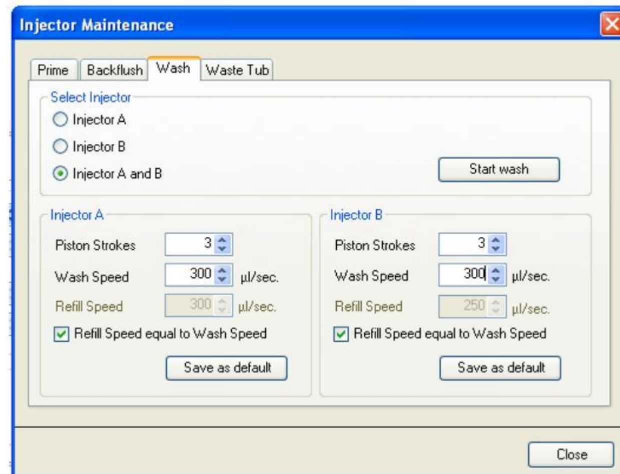


2. Switch on Tecan plate-reader first
3. Start Software i-control1.7
4. Select Script SOP_29_LDH.mdfx (in folder "SOPs_Hauner" on desktop)
5. **Clean and refill washing injector bottles with dest. H_2O**

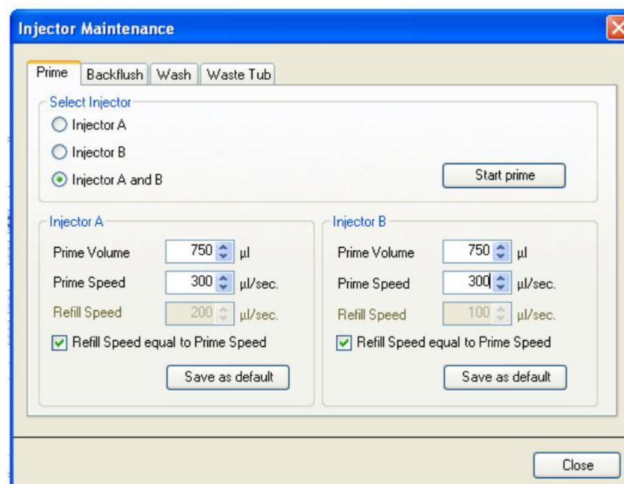
6. **Check Plate definition!**



7. Wash priming injectors by Settings→injectors→wash
8. Choose injector A & B
9. Click "Start wash"



10. Combine 5 ml NADH in one 5 ml tube
11. Add your NADH (A left) and Pyruvate tube (B right) to the corresponding injectors
12. Prime both injectors with adequate volume of reagent by clicking on Settings→injectors→prime
13. Choose injector A & B
14. Click "Start prime"



15. **Check the selected program file** SOP_29_LDH.mdfx

Dispense

Select Injector

Injector A: Volume: 156 µl Speed: 200 µl/sec.

Refill Speed equal to Dispense Speed Refill Speed: 200 µl/sec.

Injector B: Volume: 100 µl Speed: 200 µl/sec.

Refill Speed equal to Dispense Speed Refill Speed: 100 µl/sec.

Refill mode

Standard

Refill for every dispense

Injector A add 156 µl (NADH) in each well

Shaking

Parameter

Duration: 2 sec Amplitude: 1 mm

Mode: Linear Frequency: 173.9 rpm

Temperature

Parameter

On: Temperature: 37.0 °C

Off

Wait for Temperature

Parameter

Minimum: 36.5 °C Maximum: 37.5 °C

Further steps are set back, until reaction temperature of 37°C is reached

Dispense

Select Injector

Injector A: Volume: 100 µl Speed: 200 µl/sec.

Refill Speed equal to Dispense Speed Refill Speed: 100 µl/sec.

Injector B: Volume: 31 µl Speed: 200 µl/sec.

Refill Speed equal to Dispense Speed Refill Speed: 200 µl/sec.

Refill mode

Standard

Refill for every dispense

Injector B add 31 µl (Pyruvate) in each well

Shaking

Parameter

Duration: 2 sec Amplitude: 1 mm

Mode: Linear Frequency: 173.9 rpm

Move Plate

Move plate

In

Out

User Request

Text: Luftblasen?

Move Plate

Move plate

In

Out

Plate will move out; check & remove air bubbles in every well *(use ethanol vapor of spray bottle to eliminate)*

= o.k.

EtOH

Kinetic Cycle

Cycles

Number of cycles: 21

Duration

Kinetic Interval

Use kinetic interval:

Time: 00:00:30 (hh:mm:ss)

Time: 30000 ms

Wells are measured every 30 seconds for 21 cycles *eliminate)*

Absorbance

Wavelength

Measurement: 339 nm (9)

Reference

Read

Number of flashes: 5

Settle time: 100 ms

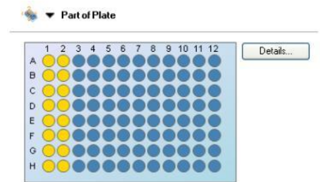
Multiple Reads per Well

Multiple reads per well

Label

Name: Label1

16. Open lid & place the injector in the main unit (click!)
17. Choose wells to be measured by clicking details
18. **Only use complete plates with all stripes set!**

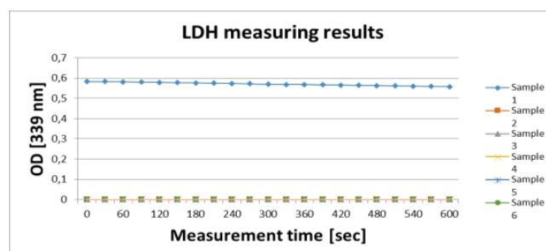


19. Pipet **6 µl of each sample** *(double determination)* in the corresponding well of the 96-well plate *repeats are rendered in wells side by side, i.e. same sample in well A1 and A2*
20. Place the 96-well plate into the Tecan plate reader & click Start
21. An excel sheet opens automatically

22. After Tecan has finished, save the data in an excel file as it is displayed
23. Remove plate & the injector from the main unit & close the lid
24. Replace NADH & Pyruvate with the corresponding H_2O bottles
25. Wash priming station as described in point 7-9

For final calculation of LDH activity in mU/ml the saved data is processed with the excel sheet "SOP_29_LDH_calculation_sheet". Handling of sheet:

1. Copy Tecan data into calculation excel sheet
2. Sample means, rates (dAbs/min), results (units/min) & graph are generated automatically
3. If "Results units/min" is not more than +/- 15, cell viability is adequate
4. Measured data can only be used, if minimum 50% of the curve is linear
   In case not: dilute samples & measured again (e.g. 1:5 or 1:10)



In case of linearity in parts of the curve: calculation time frame has to be chosen manually to calculate rates (dAbs/min); default in the sheet: $(OD_{10min}-OD_{0min})/10$ min

Note: during usage of induction medium, slope of curve can be found between 40 and 50; should decrease again after starting to use differentiation medium

For a standard evaluation:

1. Solve 2µl recombinant LDH in 10 ml **Tris/NaCl solution pH 7,2**
2. Measure 6µl 3 x determination with the samples in the Tecan plate reader

$$\Delta c/min = \frac{\Delta E * V\ (\mathbf{0.7625ml}) * 1000}{\varepsilon * d\ (1) * min * v\ (\mathbf{0.0125\ ml})}$$

Factor: 4984 for the tecan plate reader(factor in the SOP 29 excel sheet) (ε_{340} = 6.22)

LDH-solution: 5270U/ml => 5.27U/µL => 1:1000 dilution > 5.27mU/µL
66mU/eingesetzten 762.5µL => 85mU/mL im 1. Ansatz (Im 2. Ansatz 2x, im 3.Ansatz 3x soviel)

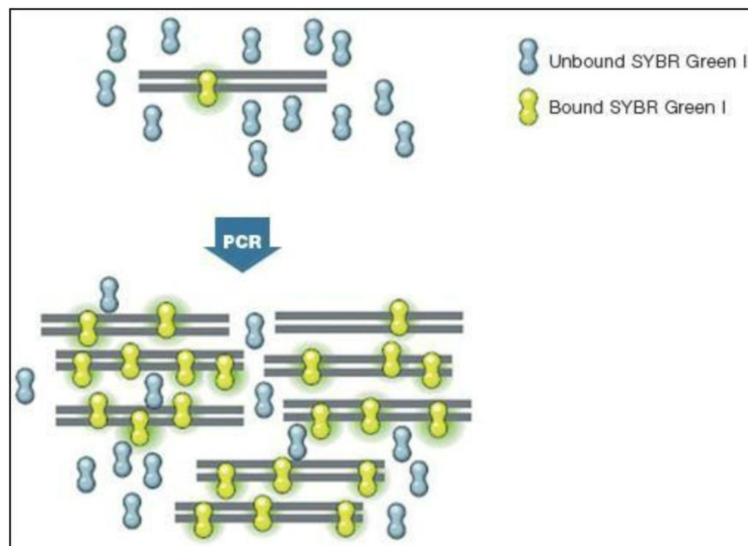
Appendix 13

SOP No. 32

Title: RNA & RT & qPCR via SybrGreen	
Version: 1	Date: 02.07.19
Author: CS2 viewer: BN	

Principle

A DNA-binding dye like SYBR Green binds to all the double-stranded (ds) DNA in a PCR reaction, causing fluorescence of the dye. An increase in DNA product during PCR, therefore leads to an increase in fluorescence intensity. The intensity is measured in each cycle, allowing DNA concentrations to be quantified.



Because the dye binds to all the double-stranded nucleic acids, including primer dimers, it is necessary to perform a melting curve analysis at the end of the run for SYBR Green assays.

Materials

	company	order no.	storage
Maxima 2 x SYBRGreen Master mix	Thermo	K0223	Freezer 5
High capacity cDNA RT Kit	Applied Biosystems	4368814	Freezer 5
q-PCR Primer	Eurofins/ Sigma		-20ºC
Nano RNA Chip + Kit + Ladder	Agilent	5067	4ºC
Ultrapure RNase free H_20	Roth	T143	RT
Ultrapure RNase free H_20	Qiagen	217204	RT
4titude Plates & Seal	4Titude	4ti-0832	/
Tips Multi channel pipette Witt	VWR	613-6027	/

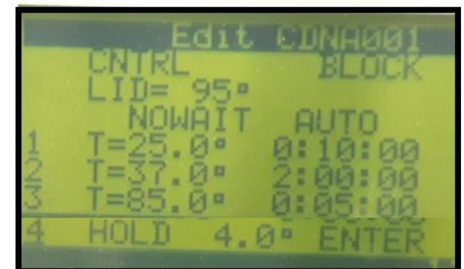
- **Wear laboratory gloves at all times during this procedure and change them frequently!**
- **Every time clean the lab bench**
- **Use RNase-free filter pipette tips**
- **NEVER put used tips into the kit reagents!**

Relevant RNA should be checked via Bioanalyzer or gel run to guarantee integrity (see Guideline D). Also RNA concentration is required (Tecan or NanoDrop)

With this reverse Transcription Kit up to 2 µg of RNA can be reverse transcribed!

1. Thaw template RNA & relevant RT kit solutions on a cool rack (-20°C)
2. Immediately before use, remove the Multi Scribe RTase enzyme from the freezer
3. Prepare relevant volume of RT master mix on ice (overhang 1 sample) in a 1.5.ml tube

High capacity cDNA RT Kit	***Per RNA sample***
10x RT Reaction buffer	2 µl RNase-free
water 4.2 µl	
25 x dNTP Mix (100 mM)	0.8 µl 10 x RT
random primer	2 µl
MultiScribe RTase	1 µl
Total	10µl



4. Mix gently and place the mix on ice
5. Turn on the Eppendorf Mastercycler (Room 2.62) & open the program “CDNA001”
6. Check the settings of the program
7. Pipette relevant volume of RNA sample (0.5-1 µg is recommended) in each 0.2 ml tube/ strip (immediately freeze RNA samples back!)
8. Add relevant volume of Ultrapure RNase free H_20 up to 10 µl
9. Add 10µl master mix & shortly spin down the strip or 0.2 ml tubes
10. Open the lid of the cycler, place the 0.2 ml strip tubes & press enter to run
11. Afterwards program rest on “HOLD” at 4°C,
12. Press enter & remove tubes
13. Centrifuge 0.2 ml tubes shortly & store on a cool rack

14.Dilute samples directly in the 0.2 ml tube or transfer everything in fresh RNase free 1.5. ml tube *(required concentration of 5 ng/µl)*

0.5 µg RNA > c-DNA 1:5 (20 µl RT-mix + 80 µl Ultrapure RNase free H_20)

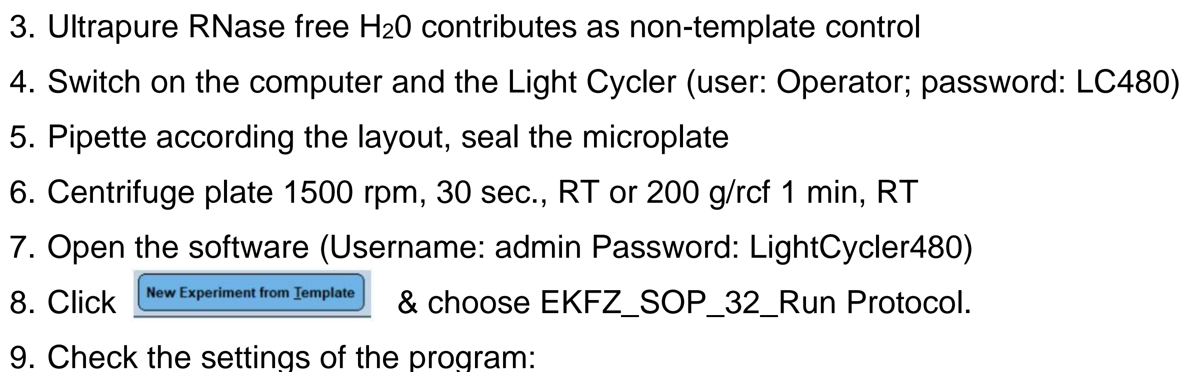
1 µg RNA > c-DNA 1:10 (20 µl RT-mix + 180 µl Ultrapure RNase free H_20)

15.Mix by vortexing & store samples at -20ºC

qPCR Assay via Roche Light Cycler 480

1. In advance, book the Cycler (Google-Account PW: lightcycler480witt)
2. Create a plate layout in an Excel sheet & calculate volume for the master mix (+ overhang)

	per sample
Maxima 2 x SYBRGreen Master mix	5 µl
Primer forward (10µM)	0.3 µl
Primer reverse (10µM)	0.3 µl
Ultrapure RNase free H_20	2.4 µl
c-DNA Sample (diluted)	2 µl = 10ng
Total	10µl



3. Ultrapure RNase free H_20 contributes as non-template control
4. Switch on the computer and the Light Cycler (user: Operator; password: LC480)
5. Pipette according the layout, seal the microplate
6. Centrifuge plate 1500 rpm, 30 sec., RT or 200 g/rcf 1 min, RT
7. Open the software (Username: admin Password: LightCycler480)
8. Click New Experiment from Template & choose EKFZ_SOP_32_Run Protocol.
9. Check the settings of the program:

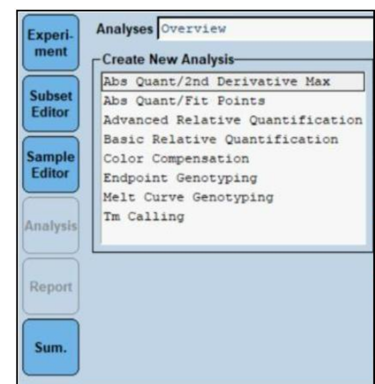
	Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp rate (°C/s)	Acquisition (per °C)	Sec target (°C)	Step size (°C)	Step delay (cycles)	Cycles	Analysis mode
Pretreatment	50	None	00:02:00	4.8		0	0	0	1	None
Initialdenaturation	95	None	00:10:00	1		0	0	0	1	None
Cycle	95	None	00:00:15	4.8		0	0	0	**40**	Quan-tification
	60*	None	00:00:30	2.5		0	0	0		
	72	Single	00:00:30	4.8		0	0	0		
Melting Curve	95	None	00:00:10	1					1	Melting Curves
	60	None	00:00:01	2.5						
	95	Continuous		0.29	2					
	95	None	00:00:20	4.8						

- Annealing temperature can vary depending on the used primers!

10. Add the plate and press start run
11. Add RUN name + date under the folder 10 experiments, your folder
12. Once the run finished, transfer the **ixo. file** to a USB stick

Do the analysis

1. Therefore, first select desired wells in the Subset Editor & click on apply
2. Second, go to Sample Editor, configure properties & add target name (primer)



3. Click on the analysis tab and perform:
   a) Abs Quant/2nd Derivative Max
   b) Tm calling for the melting curve

4. Select your subset & press ☑
5. By right mouse click results can be transferred to a txt. file

References

[1] Applied Biosystems High capacity cDNA RT Kit Handbook (2019)

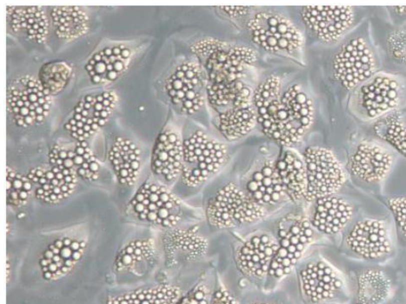
Appendix 14

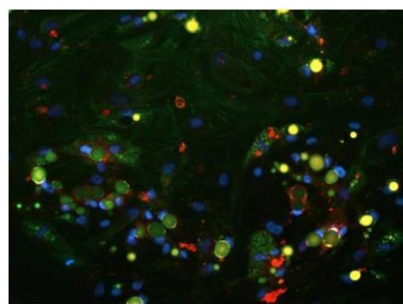
SOP No. 34

Title: Cell Painting PAC	
Version: 1	**Date:20.06.2020**
Author: TK Reviewer: CS2	

Principle

MitoTracker Red CMXRos staining is used for labeling active mitochondria in cultured cells. For cell-permeant nuclear counterstain NucBlue (Hoechst 33342) is used. Alexa Fluor® 488 phalloidin is a high-affinity filamentous actin (F-actin) probe conjugated to a bright, photostable, green-fluorescent Alexa Fluor® 488 dye. Additionally, BODIPY® 505/515 can be used as a stain for stored neutral lipids and as a tracer for oil and other nonpolar lipids.





Unstained and stained differentiated PAC

Overview fluorescent dyes and matching filter cubes:

FILTER CUBE	EXCITATION FILTER	SUPPRESSION FILTER
L5	480/40	527/30
A	340-380	425 **Y3**
545/30	610/75	
YFP	500/20	535/30

DYE	EXCITATION (NM)	EMISSION (NM)	FILTER CUBE
PHALLOIDIN	495	518	L5
NUCBLUE	360	450	A
MITOTRACKER	579	599	Y3
BODIPY	505	515	YFP

Materials

	company	order no.	storage
Alexa Fluor® 488 phalloidin	Thermo Scientific	A12379	≤-20°C, dark
BODIPY™ 505/515	Thermo Scientific	D3921	≤-20°C, dark
BSA	Sigma	A7906	4°C, Fridge 5
DMSO	Merck	1.02931.0500	RT
Growth Medium	Invitrogen	individual	4°C
Histofix (Formaldehyde 4 % buffered)	Roth	P087	RT, 2.65
Methanol	Roth	4627.5	RT, 2.65
MitoTracker Red CMXRos	Invitrogen	M7512	≤-20°C, dark
NucBlue (Hoechst)	Thermo Scientific	R37605	RT
PBS	Sigma	P441	4°C, cell culture
Triton X-100	Sigma	T9284	RT, 2.65

Solutions

For all working steps: Attention! Toxic material, wear nitrile gloves!

1. **PBS solution**
   Ready to use

2. **NucBlue solution**
   Ready to use

3. **1%-BSA solution**
   0.1 g of BSA (found at 4°C) in 10 ml PBS. Store the Solution at 4°C

4. **Permeabilisation solution (0.1% Triton X-100)**
   Dilute 100 µl of Triton X-100 in 100 ml PBS (be careful: Triton is slabby). Warming it for a better dilution

5. **MitoTracker**
   a. **Stock solution**
   Before opening a vial, allow to warm to RT. Dissolve the lyophilized product in DMSO to a final concentration of 1 mM (molecular weight is indicated on the product lable). Store the solution frozen at ≤ -20°C and protected from light.
   b. **Working solution**
   Dilute 1 mM Stock Solution to the final working concentration of 500 nM in prewarmed (37°C) growth medium (e.g. 2.5 µl Stock solution + 5 ml growth medium). Protected from light !

6. **Phalloidin**
   a. **Stock solution**
   Dissolve the vial contents in 1.5 ml of methanol to yield a 40x stock solution at a concentration of 2,000 assays/ml, which is equivalent to approximately 66 µM. Store the solution frozen at ≤ -20°C and protected from light.
   b. **Working solution**
   Add 10 µl Phalloidin Stock solution in 2 ml 1%-BSA solution. Protected from light!

7. **Bodipy**
   a. **Stock solution**
   Make a stock solution of 1 mg/mL (3.8 mM) in either absolute ethanol or anhydrous DMSO (e.g. 10 mg Bodipy powder + 10 ml DMSO). After resolubilizing, store the stock solution ≤ -20°C & protected from light
   b. **Working solution**
   Add 1.6 µl Bodipy stock solution to 2 ml PBS. Protected from light!

Staining Procedure

1. Carefully aspirate culture medium
2. Take out corresponding amount of MitoTracker, Bodipy, Phalloidin srocks & keep in the dark
3. Prepare MitoTracker working solution
4. Gently remove media from cells
5. Add 2 ml (pre-warmed) MitoTracker working solution per well (6er well)
6. Add relevant drops NucBlue in the same well

Well format	wash/ fix/ staining	NucBlue drops
96-well	50 µl / well	1/2
48 well	300 µl / well	1
24 well	500 µl / well	1
12 well	1 ml / well	2
6-well	2 ml / well	4

7. Incubate the plates for 30 min in the incubator, 37°C, 5% CO_2

8. Remove MitoTracker staining via vacuum pump
9. Wash cells 2x with PBS (RT) to remove unbound stain (6er 2 ml/well)
10. For fixation of cells add Histofix (6er 2 ml/well)
11. Incubate for 20 min in the dark, RT
12. Remove Hisofix
13. Wash cells 1x with PBS (RT) (6er 2 ml/well)
14. For permeabilization of cells add permabilization solution (6er 2 ml/well)
15. Incubate for 15 min in the dark, RT
16. Remove permabilization solution
17. Wash cells 2x with PBS (RT) (6er 2 ml/well)
18. Add 2 ml Phalloidin working solution (6er)
19. Incubate for 30 min in the dark, RT
20. Remove the staining solution
21. Wash cells 2x with PBS (RT) (6er 2 ml/well)
22. Add 2 ml Bodipy working solution (6er)
23. Incubate for 15 min in the dark, RT
24. Wash 3x with PBS, leaving the last wash in
25. For storage wrap plate with parafilm > 4°C & dark (**up to 1 weeks**)
26. Image plate with a fluorescent microscope

References

[1] Invitrogen; MitoTracker® Mitochondrion-Selective Probes
[2] Invitrogen; Alexa Fluor™ 488 Phalloidin
[3] Invitrogen; Molecular Probes™ NucBlue Live ReadyProbes™ Reagenz
[4] Protocol “Adipocyte Profiler”, Lab M. Claussnitzer; Boston

Appendix 15

SOP No. 39

Title: mRNA/miRNA Isolation TRI Reagent	
Version: 1	Date: 01.02.2021
Author: CS2	
Reviewer: SH, Annie Naujoks	

Principle

One of the most commonly used methods for isolating RNA from cells and tissues in aqueous solutions is phenol chloroform extraction followed by ethanol precipitation. During organic extraction, protein contaminants are denatured and either enter the organic phase or the interface between organic and aqueous phases, while nucleic acids remain in the aqueous phase.

Materials

	company	order no.	storage
Chloroform	Roth	3313.1	RT 2.62 under hood
EtOH absolute 96%	Fisher Scientific	10048291	RT 2.62 under hood
Isopropanol = Propan-2-ol	Fisher Scientific	P/7500/PC17	RT 2.65 under hood
Trizol Reagent	Ambion/Qiagen		RT 2.62 under hood
RNaseZAP	Sigma	R2020	RT
Ultrapure RNase free H_20	Qiagen/ Roth / water device 2.65 with ultrapure filter		

Solutions

1. **10 mM NaOH solution**

   Mix 500 µl 1 M NaOH with 49.5 ml distilled H_20

2. **70% ETOH**

   Mix 35 ml ETOH abs. with 15 ml Ultrapure RNase free H_20

Chloroform: weekly freshly aliquot in corresponding glass vials with glass pipette!

- **During the whole procedure, wear your lab coat.**
- **Wear gloves at all times during this procedure and change them frequently!**
- **Every time clean the lab bench, and pipettes with RNase ZAP**
- **Use RNase-free filter pipette tips**
- **NEVER put used tips into the kit reagents!**

Sample collection of cells e.g. PACs

1. At least 2 wells of a 6 well plate are recommended
2. Aspirate culture medium from the cells
3. Wash twice with cold PBS
4. Add TRI Reagent onto cells > **250µl/ 6er well** > 2x6er = 500µl
5. Collect cells by using a cell scraper
6. Transfer the cell suspension into a 2 ml LoBind Eppendorf tube
7. vortex the tube and freeze on dry ice
8. Transfer all samples to -80°C for at least 24h before isolation takes place

In case you harvested in RLT buffer + Mercaptoethanol defrost your samples and add same amount Trizol Reagent as RLT buffer

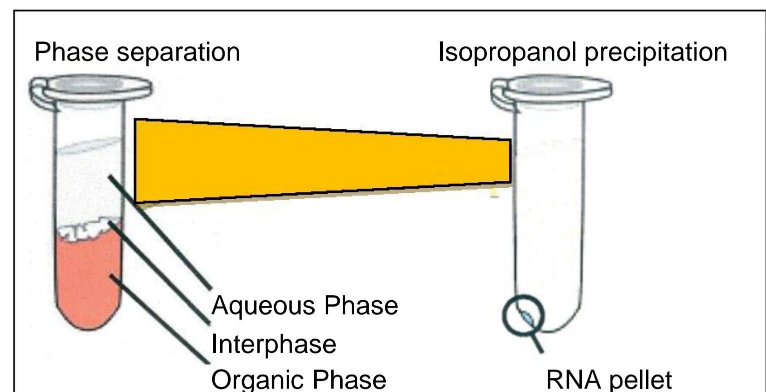
Isolation

1. Clean the fume hood and RNA-pipettes with RNAse ZAP
2. Pre-cool centrifuge to 4°C and collect ice
3. Take samples from -80°C & thaw them on ice

   If harvested in RLT +Mercapto add TRI Reagent

   (equal amount to lysate; e.g. 350 µl TRI Reagent to 350 µl RLT)
4. Incubate the suspension for 5 minutes on ice.
5. Add 100 µl of chloroform (per 500 µl TRI Reagent) to the suspension.
6. Vortex shortly
7. Incubate 10 minutes on ice
8. Centrifuge the samples at 14,000 g/rcf for 10 minutes at 4 °C.
9. During centrifugation, pipet 250 µl of isopropanol (per 500 µl TRI Reagent) into a fresh 1.5 ml tube for each sample.
10. After centrifugation, carefully transfer the RNA containing aqueous (upper / clear) phase into the prepared tubes.

Avoid the transfer of any interphase!



11. Sway cautiously until all streaks (=”Schlieren”) disappear
12. Incubate 10 minutes on ice
13. Centrifuge the samples at 14,000 g/rcf for 10 minutes at 4 °C
14. Decant supernatant carefully from RNA pellet (gel-like).
15. Wash the RNA pellet with 1 ml of 70 % ethanol.
16. Centrifuge the samples at 14,000 g/rcf for 10 minutes at 4 °C.

17. Decant ethanol carefully from the RNA pellet and repeat step 15 and 16 once again
18. Decant supernatant carefully from the RNA pellet and place the tubes upside down on a paper towel for 5 min
19. Put tubes in the rack and leave open for 5 min. under the closed fume hood (ETOH evaporates)
20. Re-suspend the pellet with **30µl ultrapure RNase free H_20**
21. Investigate RNA yield via Tecan or Nano drop
22. Aliquot each 2 µl of 2-3 samples in a 0.2 ml strip for the Bioanalyzer

Immediately store all RNA samples at -80ºC!

miRNA / mRNA integrity check via Bioanalyzer

Device location: Weihenstephaner Berg 3/I, Chair of Animal Physiology, room 02/3.21.

See Guideline D for processing

miRNA Reverse Transcription (miRCURY LNA RT Kit) (c-DNA) See **SOP17**

mRNA Reverse Transcription (Applied bio systems) (c-DNA) See **SOP32**

References

[1] Chomczynski, P., *A reagent for the single-step simultaneous isolation of RNA, ONA and proteins from ce/1 and tissue samples.* Biotechniques 15, 532-34,536-37, 1993.

Appendix 16

Figures and Tables

Table A1: Cell numbers of study subjects across differentiation days and described within the groups of risk allele-/non-risk allele carriers (rs1421085) and complete study cohort.

	Risk carriers (CC)				**Non-risk carriers (TT)**		
	Total cell number (3 wells)				Total cell number (3 wells)		
Subject ID	**Day 0**	**Day 2**	**Day 14**	**Subject ID**	**Day 0**	**Day 2**	**Day 14**
m123	1954826	2037146	1049737	m142	3076102	2397198	990702
m164	1859334	1843537	1325078	m170	1918487	2053923	1414571
m186	1856002	1993947	1031940	m187	1334917	1352165	786783
m256	1470510	1666862	1184428	m189	2000925	1834090	1259104
m258	1471372	1547420	1143503	m252	1391757	1509278	905050
m266	2189555	2222601	1026530	m300	1868154	2017193	618929
Mean	1800267	1885252	1126869	**Mean**	1931724	1860641	995856
Mean total cell number (3 wells) all study subjects							
			d0	1865995			
			d2	1872947			
			d14	1061363			

Table A2: *Subjects of final technical experiment with corresponding cell number and OilRed intensity.*

Cell number				
Total number	**48 h**	**Day 0**	**Day 2**	**Day 14**
PAC 614				
Cells per well	148882	261007	291165	155781
Cells for 2 wells	n/a	522013	582329	311562
Cells for 3 wells	n/a	783020	873494	467342
Cells for 6 wells	n/a	1566040	1746987	934685
PAC 657				
Cells per well	558038	367265	414997	319820
Cells for 2 wells	n/a	734530	829995	639639
Cells for 3 wells	n/a	1101794	1244992	959459
Cells for 6 wells	n/a	2203589	2489984	1918918
OilRed quantification				
Days	**Intensity per well**	**Cell number per well**	**Scalefactor**	**Intensity per 100,000 PACs**
PAC 614				
Day 0	0.09	261007	2.61	0.03
Day 2	0.09	291165	2.91	0.03
Day 14	0.35	155781	1.56	0.22
PAC 657				
Day 0	0.08	367265	3.67	0.02
Day 2	0.09	414997	4.15	0.02
Day 14	0.81	319820	3.2	0.25

Table A3: *DMEM-F12 ingredients list.*

https://www.fishersci.de/shop/products/gibco-dmem-f-12-no-phenol-red/11580546?searchHijack=true&searchTerm=11580546&searchType=RAPID&matchedCatNo=11580546

***Table A4**: Compound class overrepresentation analysis (ORA) of known features that differ in the rs1421085 risk carriers (CC) compared to non-risk carriers (TT) among the male cohort.*

Metabolome Analysis				
Time point	**Compound classes**	**Total counts**	**Subset counts Up ↑ /Down ↓ regulated**	**p-value**
Cells				
Phase 1 (day 2-day 0)	Glycerophospholipids	98	35 ↓	$8.62 * 10^{-9}$
	Organonitrogen compounds	22	7 ↓	0.019
	Steroids and steroid derivatives	82	17 ↓	0.036
Phase 2 (day 14-day 2)	Glycerolipids	80	18 ↓	0.014
	Phenols	26	8 ↓	0.015
	Endocannabinoids	11	4 ↓	0.046
	Glycerophospholipids	102	21 ↑	0.023
Media				
Phase 1 (day 2-day 0)	Fatty acyls	457	65 ↑	$5.57 * 10^{-5}$
	Glycerolipids	73	15 ↑	0.001
	Carboxylic acids and derivatives	128	18 ↑	0.031
	Prenol lipids	353	41 ↑	0.038

Table A5: *Results of all targets from the mass difference enrichment analysis (MDEA) including internal ID, UniProt ID, Gene name, protein names, magnitude as well as the direction of regulation (arrows) for Phase 1 (day 0 vs. day 2) and Phase 2 (day 2 vs. day 14).*

Internal ID	Entry Uniprot	Gene name	Protein names specific to EC Number	Magnitude of Change	Direction [Phase 1] [Phase 2]
130_EC	**P17405**	***SMPD1***	Sphingomyelin phosphodiesterase (EC 3.1.4.12)	34.63	↓↑
129_EC	**Q9Y2P5**	***SLC27A5***	Bile acyl-CoA synthetase (EC 6.2.1.7) - Short name: BACS	9.02	↑↓
137_EC	**P21580**	***TNFAIP3***	Tumor necrosis factor alpha-induced protein 3 (EC 2.3.2.-) - Short name: TNF alpha-induced protein 3;	4.79	↓↑
262_EC	**P09874**	***PARP1***	Protein poly-ADP-ribosyltransferase PARP1 (EC:2.4.2.-)	4.14	↓↑
180_EC	**P49419**	***ALDH7A1***	Betaine aldehyde dehydrogenase (EC 1.2.1.8)	3.72	↓↑
220_EC	**P40939**	***HADHA***	Long-chain enoyl-CoA hydratase (EC 4.2.1.17)	3.69	↓↑
162_EC	**P33121**	***ACSL1***	Phytanate--CoA ligase (EC 6.2.1.24)	3.57	↑↓
141_EC	**P04818**	***TYMS***	Thymidylate synthase (EC 2.1.1.45) - Short names: TS/TSase	3.56	↓↑
209_EC	**Q6IB77**	***GLYAT***	Glycine N-benzoyltransferase (EC 2.3.1.71)	3.50	↑↓
152_EC	**Q13085**	***ACACA***	Acetyl-CoA carboxylase 1 (EC 6.4.1.2) - Short name: ACC1	3.39	↓↑
106_EC	**P35228**	***NOS2***	Nitric oxide synthase, inducible (EC 1.14.13.39)	3.07	↓↑
107_EC	**P29474**	***NOS3***	Nitric oxide synthase, endothelial (EC 1.14.13.39)	3.07	↓↑
181_EC	**P49189**	***ALDH9A1***	4-trimethylaminobutyraldehyde dehydrogenase (EC 1.2.1.47) - Short names: TMABA-DH/TMABALDH	2.77	↓↑
34_EC	**Q07973**	***CYP24A1***	1,25-dihydroxyvitamin D(3) 24-hydroxylase, mitochondrial (EC 1.14.15.16) - Short names: 24-Ohase/Vitamin D(3) 24-hydroxylase;	2.61	↑↓
169_EC	**P00352**	***ALDH1A1***	Retinal dehydrogenase 1 (EC 1.2.1.-) - Short names: RALDH 1/RalDH1;	2.46	↓↑
183_EC	**P49189**	***ALDH9A1***	Gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19)	2.45	↓↑
149_EC	**P42765**	***ACAA2***	Acetyl-CoA acyltransferase; Acyl-CoA hydrolase, mitochondrial (EC 3.1.2.-)	2.42	↑↓

110_EC	P30613	*PKLR*	Pyruvate kinase PKLR (EC 2.7.1.40)	2.40	↓↑
272_EC	P41247	*PNPLA4*	Patatin-like phospholipase domain-containing protein 4 (EC 3.1.1.3)	2.39	↑↓
161_EC	P33121	*ACSL1*	Arachidonate--CoA ligase (EC 6.2.1.15)	2.29	↑↓
218_EC	Q16836	*HADH*	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (EC 1.1.1.35) - Short name: HCDH	1.92	↑↓
219_EC	P40939	*HADHA*	Monolysocardiolipin acyltransferase (EC:2.3.1.-)	0.606	↑↑
105_EC	P43490	*NAMPT*	Nicotinamide phosphoribosyltransferase (EC 2.4.2.12) - Short names: NAmPRTase/Nampt	1.81	↑↓
151_EC	P42765	*ACAA2*	Acetyl-CoA acyltransferase; Acyl-CoA hydrolase, mitochondrial (EC:3.1.2.2)	1.78	↓↑
148_EC	P42765	*ACAA2*	Acetyl-CoA acetyltransferase (EC:2.3.1.9)	1.52	↓↑
150_EC	P42765	*ACAA2*	Acetyl-CoA acyltransferase; Acyl-CoA hydrolase, mitochondrial (EC 3.1.2.1)	1.52	↓↑
170_EC	P00352	*ALDH1A1*	Retinal dehydrogenase 1 (EC 1.2.1.36) - Short names: RALDH 1/RalDH1;	1.60	↑↓
179_EC	P49419	*ALDH7A1*	Aldehyde dehydrogenase family 7 member A1 (EC 1.2.1.3)	1.55	↑↓
178_EC	P49419	*ALDH7A1*	Alpha-aminoadipic semialdehyde dehydrogenase (EC 1.2.1.31) - Short name: Alpha-AASA dehydrogenase	1.53	↑↓
37_EC	O15528	*CYP27B1*	25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial (EC 1.14.15.18)	1.42	↑↓
197_EC	P08684	*CYP3A4*	1,8-cineole 2-exo-monooxygenase (EC 1.14.14.56)	0.208	↑↓
199_EC	P08684	*CYP3A4*	Quinine 3-monooxygenase (EC 1.14.14.55)	1.26	↑↓
55_EC	P49327	*FASN*	Fatty acid synthase (EC 2.3.1.85)	1.25	↑↓
56_EC	P49327	*FASN*	[Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38)	1.25	↑↓
57_EC	P49327	*FASN*	[Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39)	1.25	↑↓
58_EC	P49327	*FASN*	3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41)	1.25	↑↓
59_EC	P49327	*FASN*	3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100)	1.25	↑↓

60_EC	**P49327**	***FASN***	3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59)	1.25	↑↓
61_EC	**P49327**	***FASN***	Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39)	1.25	↑↓
62_EC	**P49327**	***FASN***	Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)	1.25	↓↑
133_EC	**Q06520**	***SULT2A1***	Bile salt sulfotransferase (EC 2.8.2.14)	1.21	↑↓
182_EC	**P49189**	***ALDH9A1***	Aldehyde dehydrogenase family 9 member A1 (EC 1.2.1.3)	1.17	↑↓
196_EC	**P08684**	***CYP3A4***	Cytochrome P450 3A4 (EC 1.14.14.1)	1.12	↑↓
229_EC	**P26440**	***IVD***	Isovaleryl-CoA dehydrogenase, mitochondrial (EC 1.3.8.4) - Short name: IVD	1.10	↑↓
42_EC	**P22680**	***CYP7A1***	24-hydroxycholesterol 7-alpha-hydroxylase (EC 1.14.14.26)	1.05	↑↓
43_EC	**P22680**	***CYP7A1***	Cholesterol 7-alpha-monooxygenase (EC 1.14.14.23)	1.05	↑↓
14_EC	**P16050**	***ALOX15***	Arachidonate 12-lipoxygenase, leukocyte-type (EC:1.13.11.31) - Short name: 12-LOX	1.03	↑↓
15_EC	**P16050**	***ALOX15***	Arachidonate 15-lipoxygenase (EC 1.13.11.33) - Short names: 15-LOX/15-LOX-1	1.03	↑↓
16_EC	**P16050**	***ALOX15***	Hepoxilin A3 synthase Alox15 (EC 1.13.11.-)	1.03	↑↓
17_EC	**P16050**	***ALOX15***	Linoleate 13S-lipoxygenase (EC 1.13.11.12)	1.03	↑↓
70_EC	**P04062**	***GBA***	Lysosomal acid glucosylceramidase (EC 3.2.1.45) - Short name: Lysosomal acid Gcase	1.03	↑↓
71_EC	**P04062**	***GBA***	Cholesterol glucosyltransferase (EC 2.4.1.-) - Short name: SGTase	1.03	↑↓
72_EC	**P04062**	***GBA***	Cholesteryl-beta-glucosidase (EC 3.2.1.104)	1.03	↑↓
27_EC	**P06732**	***CKM***	Creatine kinase M-type (EC 2.7.3.2)	0.98	↓↑
284_EC	**P35610**	***SOAT1***	Sterol O-acyltransferase 1 (EC 2.3.1.26)	0.92	↓↑
31_EC	**P19099**	***CYP11B2***	Corticosterone 18-monooxygenase,CYP11B2 (EC:1.14.15.5)	0.801	↓↓
32_EC	**P19099**	***CYP11B2***	Steroid 11-beta-hydroxylase, CYP11B2 (EC:1.14.15.4)	0.801	↓↑
245_EC	**O14880**	***MGST3***	Glutathione peroxidase MGST3 (EC:1.11.1.-)	0.741	↑↓
35_EC	**O43174**	***CYP26A1***	Cytochrome P450 26A1 (EC 1.14.13.-)	0.670	↑↓

75_EC	P09211	*GSTP1*	Glutathione S-transferase P (EC 2.5.1.18)	0.670	↓↑
44_EC	**Q9UNU6**	***CYP8B1***	7-alpha-hydroxycholest-4-en-3-one 12-alpha-hydroxylase (EC 1.14.18.8)	0.677	↓↓
216_EC	**Q03013**	***GSTM4***	Glutathione S-transferase Mu 4 (EC 2.5.1.18)	0.654	↑↑
174_EC	**P51648**	***ALDH3A2***	Aldehyde dehydrogenase family 3 member A2 (EC 1.2.1.94)	0.624	↓↓
144_EC	**P47989**	***XDH***	Xanthine dehydrogenase (EC 1.17.1.4) - Short name: XD	0.618	↓↑
33_EC	**P11511**	***CYP19A1***	Aromatase (EC 1.14.14.14)	0.532	↑↑
145_EC	**P47989**	***XDH***	Xanthine oxidase (EC 1.17.3.2) - Short name: XO	0.494	↓↓
173_EC	**P51648**	***ALDH3A2***	Aldehyde dehydrogenase family 3 member A2 (EC 1.2.1.3)	0.472	↓↓
157_EC	**P24752**	***ACAT1***	Acetyl-CoA acetyltransferase, mitochondrial (EC 2.3.1.9)	0.448	↑↑
127_EC	**Q9NRC8**	***SIRT7***	NAD-dependent protein deacetylase sirtuin-7 (EC 2.3.1.286)	0.439	↓↓
222_EC	**P55084**	***HADHB***	3-ketoacyl-CoA thiolase (EC 2.3.1.155)	0.363	↓↓
73_EC	**P38435**	***GGCX***	Vitamin K-dependent gamma-carboxylase (EC 4.1.1.90)	0.347	↑↓
171_EC	**Q3SY69**	***ALDH1L2***	Mitochondrial 10-formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) - Short names: Mitochondrial 10-FTHFDH/mtFDH	0.330	↑↑
132_EC	**Q8IWU5**	***SULF2***	Extracellular sulfatase Sulf-2 (EC 3.1.6.-) - Short name: hSulf-2	0.311	↓↑
1_EC	**P08183**	***ABCB1***	Multidrug resistance protein 1 (EC 7.6.2.2)	0.300	↓↑
2_EC	**P08183**	***ABCB1***	Phospholipid transporter ABCB1 (EC 7.6.2.1)	0.300	↓↑
185_EC	**P06576**	***ATP5B***	ATP synthase subunit beta, mitochondrial (EC 7.1.2.2)	0.300	↓↑
18_EC	**P16615**	***ATP2A2***	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (EC 7.2.2.10) - Short names: SERCA2/SR Ca(2+)-ATPase 2	0.300	↓↑
3_EC	**P33527**	***ABCC1***	Multidrug resistance-associated protein 1 (EC 7.6.2.2)	0.300	↓↑
206_EC	**P19447**	***ERCC3***	General transcription and DNA repair factor IIH helicase subunit XPB (EC 3.6.4.12) - Short name: TFIIH subunit XPB	0.300	↓↑
4_EC	**P33527**	***ABCC1***	Glutathione-S-conjugate-translocating ATPase ABCC1 (EC 7.6.2.3)	0.300	↓↑

20_EC	**Q9BX63**	***BRIP1***	Fanconi anemia group J protein (EC 3.6.4.13) - Short name: Protein FACJ	0.300	↓↑
242_EC	**P33993**	***MCM7***	DNA replication licensing factor MCM7 (EC 3.6.4.12)	0.300	↓↑
225_EC	**Q14527**	***HLTF***	Helicase-like transcription factor (EC 3.6.4.-)	0.300	↓↑
283_EC	**P51531**	***SMARCA2***	Probable global transcription activator SNF2L2 (EC 3.6.4.-)	0.300	↓↓
77_EC	**P56524**	***HDAC4***	Histone deacetylase 4 (EC 3.5.1.98) - Short name: HD4	0.284	↓↓
232_EC	**Q92831**	***KAT2B***	Spermidine acetyltransferase KAT2B (EC 2.3.1.57)	0.284	↑↓
191_EC	**P23786**	***CPT2***	Carnitine O-palmitoyltransferase 2, mitochondrial (EC 2.3.1.21)	0.277	↑↓
287_EC	**P50416**	***CPT1A***	Carnitine O-palmitoyltransferase 1, liver isoform (EC 2.3.1.21) - Short name: CPT1-L	0.277	↑↓
175_EC	**P51649**	***ALDH5A1***	Succinate-semialdehyde dehydrogenase, mitochondrial (EC 1.2.1.24)	0,259	↓↓
13_EC	**P30838**	***ALDH3A1***	Aldehyde dehydrogenase, dimeric NADP-preferring (EC 1.2.1.5)	0.243	↓↑
80_EC	**P00492**	***HPRT1***	Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) - Short names: HGPRT/HGPRTase	0.242	↑↑
82_EC	**P14778**	***IL1R1***	Interleukin-1 receptor type 1 (EC 3.2.2.6) - Short names: IL-1R-1/IL-1RT-1/IL-1RT1	0.231	↑↑
136_EC	**O60603**	***TLR2***	Toll-like receptor 2 (EC 3.2.2.6)	0.231	↑↑
84_EC	**Q01638**	***IL1RL1***	Interleukin-1 receptor-like 1 (EC 3.2.2.6)	0.231	↑↑
83_EC	**Q9NPH3**	***IL1RAP***	Interleukin-1 receptor accessory protein (EC 3.2.2.6) - Short names: IL-1 receptor accessory protein/IL-1RAcP	0.231	↑↑
85_EC	**Q9HB29**	***IL1RL2***	Interleukin-1 receptor-like 2 (EC 3.2.2.6)	0.231	↓↓
166_EC	**Q8IWW8**	***ADHFE1***	Hydroxyacid-oxoacid transhydrogenase, mitochondrial (EC 1.1.99.24) - Short name: HOT	0.197	↑↓
38_EC	**P51589**	***CYP2J2***	Cytochrome P450 2J2 (EC 1.14.14.-)	0.178	↓↓
39_EC	**P51589**	***CYP2J2***	Albendazole monooxygenase (hydroxylating) (EC 1.14.14.74)	0.178	↓↓
40_EC	**P51589**	***CYP2J2***	Albendazole monooxygenase (sulfoxide-forming) (EC 1.14.14.73)	0.178	↓↓
41_EC	**P51589**	***CYP2J2***	Hydroperoxy icosatetraenoate isomerase (EC 5.4.4.7)	0.178	↓↓

81_EC	**P01112**	***HRAS***	GTPase HRas (EC 3.6.5.2)	0.157	↑↑
234_EC	**Q5S007**	***LRRK2***	Leucine-rich repeat serine/threonine-protein kinase 2 (EC 3.6.5.-)	0.157	↑↑
67_EC	**P35575**	***G6PC***	Glucose-6-phosphatase (EC 3.1.3.9) - Short names: G-6-Pase/G6Pase	0.123	↑↑
68_EC	**Q9NQR9**	***G6PC2***	Glucose-6-phosphatase 2 (EC 3.1.3.9) - Short name: G-6-Pase 2/G6Pase 2	0.123	↑↑
74_EC	**P07203**	***GPX1***	Glutathione peroxidase 1 (EC 1.11.1.9) - Short names: GPx-1/GSHPx-1	0.114	↑↑
211_EC	**P36969**	***GPX4***	Phospholipid hydroperoxide glutathione peroxidase (EC 1.11.1.12) - Short name: PHGPx	0.114	↑↑
212_EC	**Q96SL4**	***GPX7***	Glutathione peroxidase 7 (EC 1.11.1.9) - Short names: GPx-7/GSHPx-7	0.114	↑↑
202_EC	**P00374**	***DHFR***	Dihydrofolate reductase (EC 1.5.1.3)	0.101	↑↑
159_EC	**Q15067**	***ACOX1***	Peroxisomal acyl-coenzyme A oxidase 1 (EC 1.3.3.6) - Short name: AOX	0.075	↓↑
172_EC	**P05091**	***ALDH2***	Aldehyde dehydrogenase, mitochondrial (EC 1.2.1.3)	0.075	↓↑
177_EC	**Q02252**	***ALDH6A1***	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (EC 1.2.1.27) - Short names: MMSDH/Malonate-semialdehyde dehydrogenase [acylating]	0.0541	↓↓
5_EC	**P45844**	***ABCG1***	ATP-binding cassette sub-family G member 1 (EC:7.6.2.-)	0.035	↑↑
176_EC	**Q02252**	***ALDH6A1***	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (EC 1.2.1.18) - Short names: MMSDH/Malonate-semialdehyde dehydrogenase [acylating]	0.011	↑↑
241_EC	**O95243**	***MBD4***	Methyl-CpG-binding domain protein 4 (EC 3.2.2.-)	0.027	↑↑
290_EC	**Q8IZV5**	***RDH10***	Retinol dehydrogenase 10 (EC 1.1.1.300)	0.019	↓↑
164_EC	**P00325**	***ADH1B***	All-trans-retinol dehydrogenase [NAD(+)] ADH1B (EC 1.1.1.105)	0.014	↑↑
243_EC	**P48163**	***ME1***	NADP-dependent malic enzyme (EC 1.1.1.40) - Short name: NADP-ME	0.009	↑↑

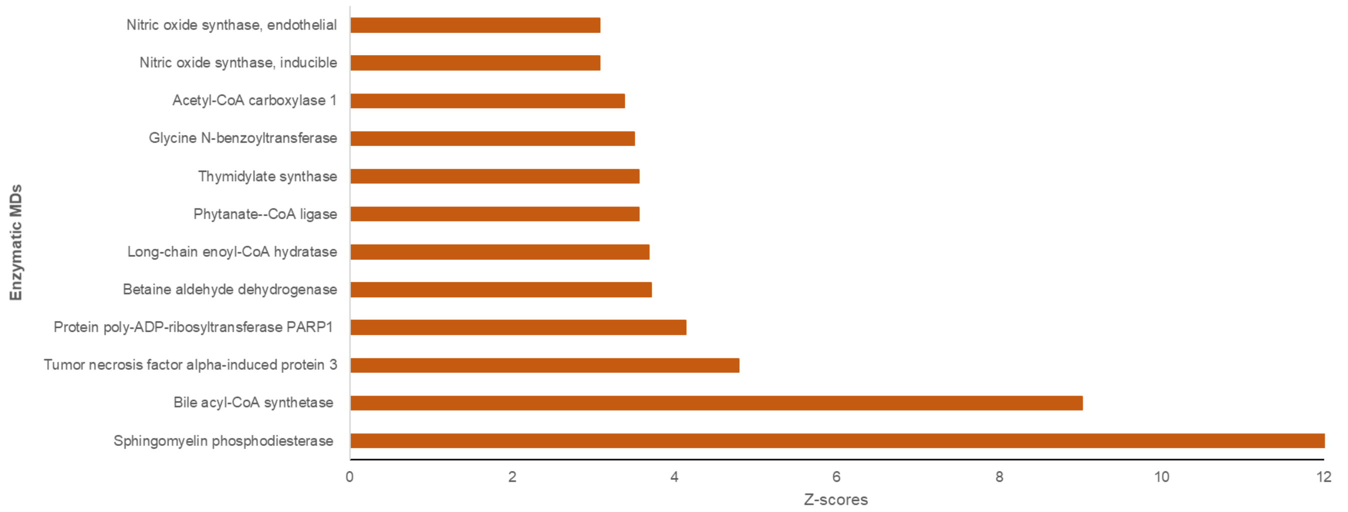


Figure A1: All selected target candidates (magnitude >3) from the MDEA during the two phases of differentiation.

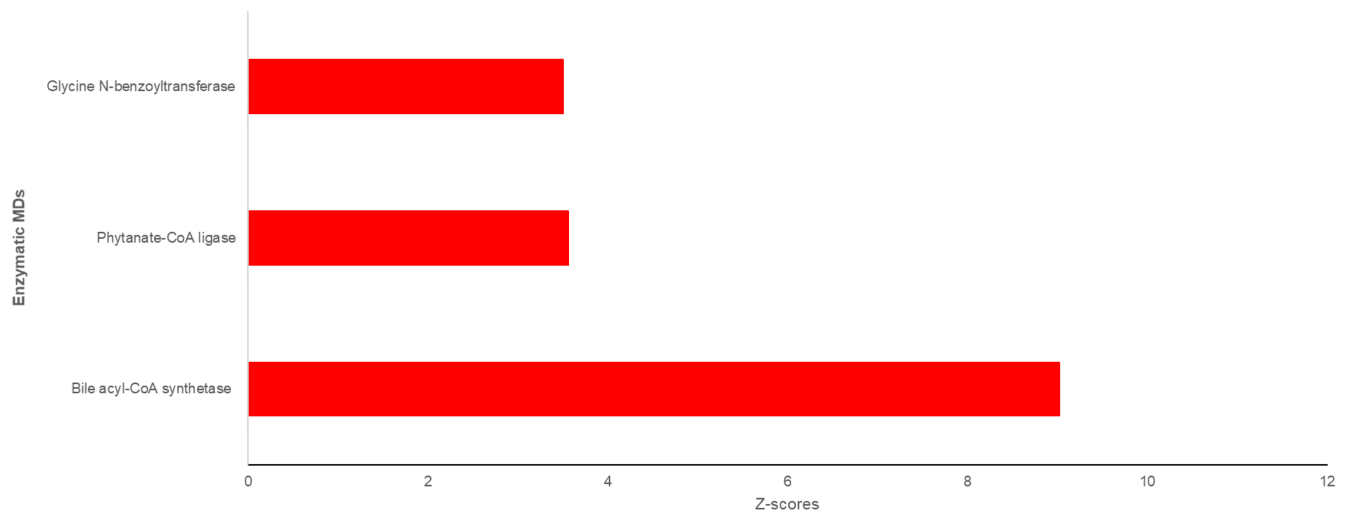


Figure A2: Targets (magnitude >3) from the MDEA **upregulated** in risk allele carriers during Phase 1 of differentiation.

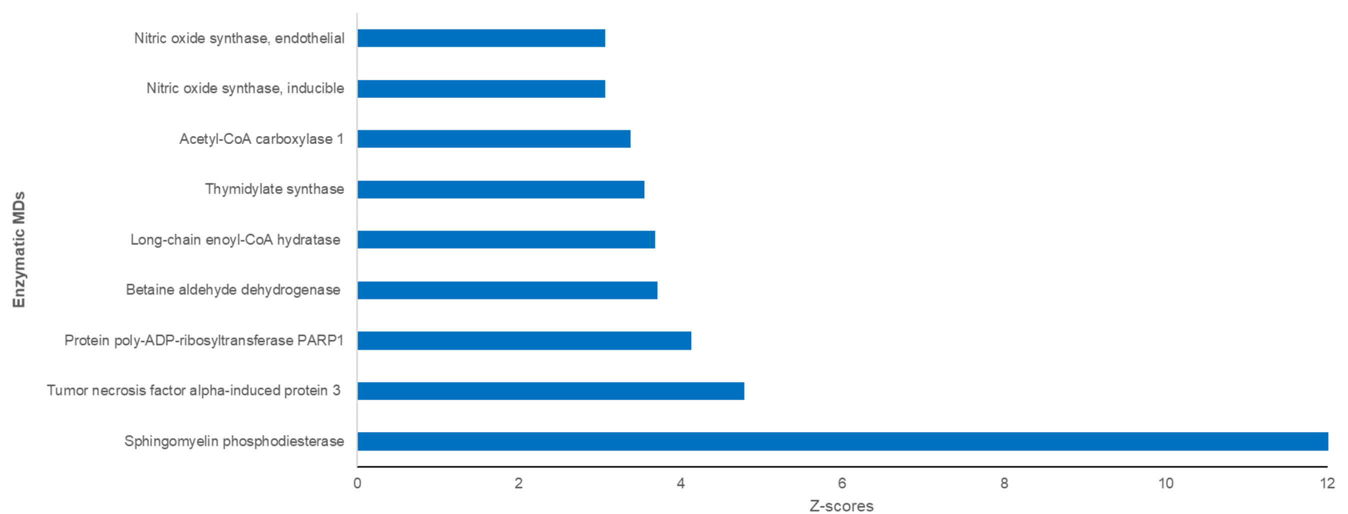


Figure A3: Targets (magnitude >3) from the MDEA **downregulated** in risk allele carriers during Phase 1 of differentiation.

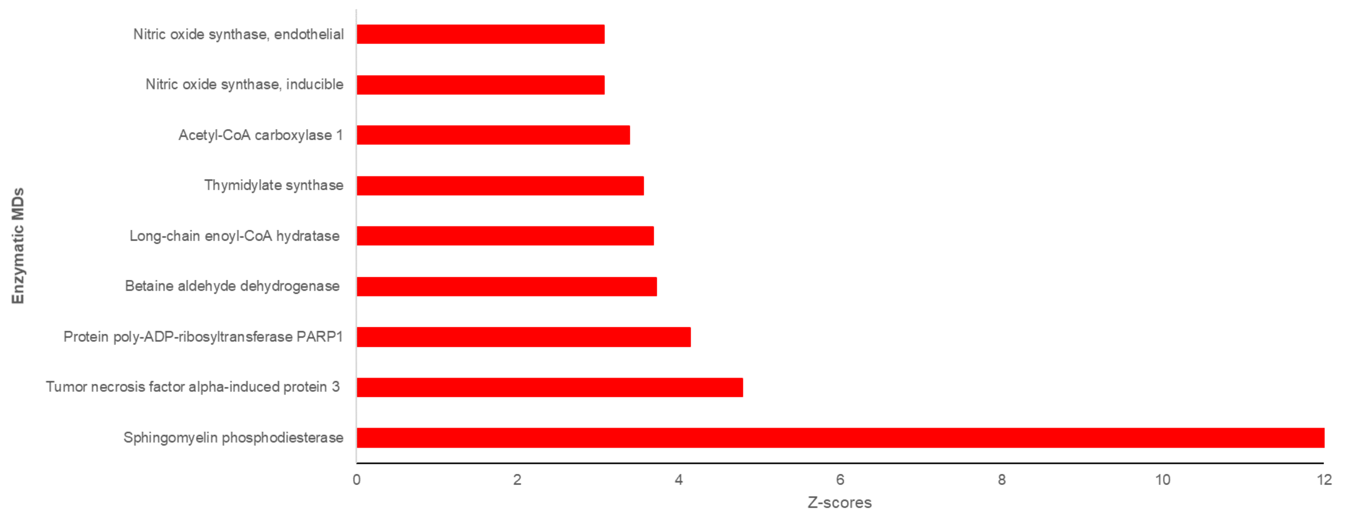


Figure A4: Targets (magnitude >3) from the MDEA **upregulated** in risk allele carriers during Phase 2 of differentiation.

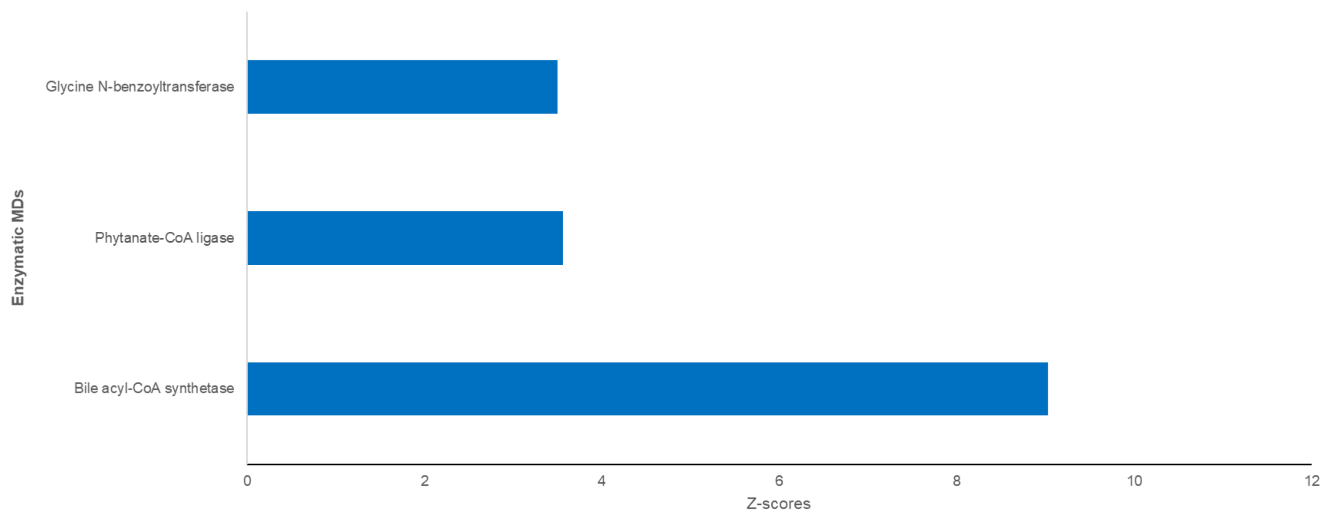


Figure A5: Targets (magnitude >3) from the MDEA **downregulated** in risk allele carriers during Phase 2 of differentiation.

Table A6: *Overrepresentation analysis of selected targets (magnitude > 3) from the MDEA on the Reactome Database. All pathways and corresponding UniProt IDs are listed including p-values.*

Pathway names	Entities pValue	Submitted entities (UniProtIDs) found
Metabolism	4.26E-05	Q9Y2P5;P29474;P40939;Q13085;P33121;P04818;P17405;P49419;Q6IB77
Nitric oxide stimulates guanylate cyclase	2.73E-04	P29474;P35228
ROS and RNS production in phagocytes	6.63E-04	P29474;P35228
Fatty acyl-CoA biosynthesis	7.00E-04	Q13085;P33121
Metabolism of lipids	7.09E-04	Q9Y2P5;P40939;Q13085;P33121;P17405
Fatty acid metabolism	7.98E-04	P40939;Q13085;P33121
NOSIP mediated eNOS trafficking	0.00213371185527389	P29474
Beta oxidation of palmitoyl-CoA to myristoyl-CoA	0.003199002040564114	P40939
Beta oxidation of myristoyl-CoA to lauroyl-CoA	0.003199002040564114	P40939
Platelet homeostasis	0.003841870682712467	P29474;P35228
Inhibition of nitric oxide production	0.004263249635790944	P35228
NOSTRIN mediated eNOS trafficking	0.0053264555686465265	P29474
vRNA Synthesis	0.0053264555686465265	P09874
Beta oxidation of octanoyl-CoA to hexanoyl-CoA	0.0053264555686465265	P40939
Beta oxidation of lauroyl-CoA to decanoyl-CoA-CoA	0.0053264555686465265	P40939
Beta oxidation of hexanoyl-CoA to butanoyl-CoA	0.0053264555686465265	P40939
Conjugation of benzoate with glycine	0.006388620766085706	Q6IB77
Mitochondrial fatty acid beta-oxidation of unsaturated fatty acids	0.006388620766085706	P40939
Beta oxidation of decanoyl-CoA to octanoyl-CoA-CoA	0.006388620766085706	P40939
Choline catabolism	0.006388620766085706	P49419
Acyl chain remodeling of CL	0.006388620766085706	P40939
Defective HLCS causes multiple carboxylase deficiency	0.007449746154315262	Q13085
POLB-Dependent Long Patch Base Excision Repair	0.00850983265880556	P09874
Conjugation of salicylate with glycine	0.00850983265880556	Q6IB77
ChREBP activates metabolic gene expression	0.00850983265880556	Q13085
Defects in biotin (Btn) metabolism	0.00850983265880556	Q13085
Linoleic acid (LA) metabolism	0.00850983265880556	P33121
Amino Acid conjugation	0.0095688812042799	Q6IB77
Conjugation of carboxylic acids	0.0095688812042799	Q6IB77
HDR through MMEJ (alt-NHEJ)	0.01062689271472661	P09874
Tetrahydrobiopterin (BH4) synthesis, recycling, salvage and regulation	0.01062689271472661	P29474
Metabolism of steroids	0.011455033304306417	Q9Y2P5;Q13085
eNOS activation	0.01168386811338773	P29474

Mitochondrial fatty acid beta-oxidation of saturated fatty acids	0.01168386811338773	P40939
Biotin transport and metabolism	0.01168386811338773	Q13085
Lysine catabolism	0.012739808322771329	P49419
alpha-linolenic (omega3) and linoleic (omega6) acid metabolism	0.013794714264640628	P33121
alpha-linolenic acid (ALA) metabolism	0.013794714264640628	P33121
TNFR1-induced proapoptotic signaling	0.014848586860023993	P21580
Carnitine metabolism	0.014848586860023993	Q13085
Synthesis of bile acids and bile salts via 24-hydroxycholesterol	0.014848586860023993	Q9Y2P5
Metabolism of nitric oxide: NOS3 activation and regulation	0.0159014270292116	P29474
Metabolism of vitamins and cofactors	0.017195616218282583	P29474;Q13085
Recycling of bile acids and salts	0.019053762171417077	Q9Y2P5
Metabolism of cofactors	0.020102481823959795	P29474
Defects in vitamin and cofactor metabolism	0.02324247742948804	Q13085
Suppression of phagosomal maturation	0.0242870912310863	P35228
Synthesis of very long-chain fatty acyl-CoAs	0.025330680855952914	P33121
Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol	0.025330680855952914	Q9Y2P5
Resolution of AP sites via the multiple-nucleotide patch replacement pathway	0.026373247217025608	P09874
VEGFR2 mediated vascular permeability	0.028455313795863524	P29474
Downregulation of SMAD2/3:SMAD4 transcriptional activity	0.028455313795863524	P09874
G1/S-Specific Transcription	0.029494815835841792	P04818
TNFR1-induced NFkappaB signaling pathway	0.03157076196695763	P21580
Interconversion of nucleotide di- and triphosphates	0.03157076196695763	P04818
Synthesis of bile acids and bile salts	0.03571044786809152	Q9Y2P5
Negative regulators of DDX58/IFIH1 signaling	0.03674283164177916	P21580
NOD1/2 Signaling Pathway	0.037774202145735125	P21580
DNA Damage Recognition in GG-NER	0.03983390696044253	P09874
Mitochondrial Fatty Acid Beta-Oxidation	0.03983390696044253	P40939
Ovarian tumor domain proteases	0.04086224307737618	P21580
Regulation of TNFR1 signaling	0.04086224307737618	P21580
Resolution of Abasic Sites (AP sites)	0.04086224307737618	P09874
Response of Mtb to phagocytosis	0.04086224307737618	P35228
Dual Incision in GG-NER	0.042915887240422945	P09874
Formation of Incision Complex in GG-NER	0.0449654999806105	P09874
Activation of gene expression by SREBF (SREBP)	0.0449654999806105	Q13085
Bile acid and bile salt metabolism	0.04701108849369351	Q9Y2P5
Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer	0.04803237591058174	P09874
Glycosphingolipid metabolism	0.04803237591058174	P17405
TNF signaling	0.05007194154976191	P21580
Regulation of cholesterol biosynthesis by SREBP (SREBF)	0.058190212719966605	Q13085

Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways	0.06222545336669072	P21580
Peroxisomal protein import	0.06624483988870822	P35228
Base Excision Repair	0.0762243156963831	P09874
Signaling by TGF-beta Receptor Complex	0.07721686681354656	P09874
Extra-nuclear estrogen signaling	0.08216497494128894	P29474
SUMOylation of DNA damage response and repair proteins	0.08315167319407901	P09874
DDX58/IFIH1-mediated induction of interferon-alpha/beta	0.08315167319407901	P21580
Infection with Mycobacterium tuberculosis	0.08413739902561557	P35228
Global Genome Nucleotide Excision Repair (GG-NER)	0.08610593690944424	P09874
Sphingolipid metabolism	0.09296531608681735	P17405
VEGFA-VEGFR2 Pathway	0.09977747187936103	P29474
Metabolism of nucleotides	0.10268258442807676	P04818
Signaling by TGFB family members	0.10557910081997934	P09874
Signaling by VEGF	0.10942779084554177	P29474
Integration of energy metabolism	0.11038758836514961	Q13085
Nucleotide Excision Repair	0.11134643759609886	P09874
Phase II - Conjugation of compounds	0.11230433939020734	Q6IB77
Interleukin-4 and Interleukin-13 signaling	0.11230433939020734	P35228
PPARA activates gene expression	0.11898319512583244	P33121
Homology Directed Repair	0.12088296108917795	P09874
Regulation of lipid metabolism by PPARalpha	0.12088296108917795	P33121
Metabolism of water-soluble vitamins and cofactors	0.12655975101544092	Q13085
Innate Immune System	0.12663230983752116	P29474;P21580;P35228
Glycerophospholipid biosynthesis	0.12938552694747407	P40939
G1/S Transition	0.1312647192569032	P04818
Death Receptor Signalling	0.1470883798399496	P21580
DNA Double-Strand Break Repair	0.1480109034733268	P09874
Mitotic G1 phase and G1/S transition	0.1480109034733268	P04818
Influenza Viral RNA Transcription and Replication	0.15077298918225046	P09874
Protein localization	0.1635545196419621	P35228
Influenza Infection	0.16897813855085442	P09874
SUMO E3 ligases SUMOylate target proteins	0.1707788352750237	P09874
SUMOylation	0.17615948732560982	P09874
Hemostasis	0.17945766572984523	P29474;P35228
ESR-mediated signaling	0.1903518121262625	P29474
Phospholipid metabolism	0.20431969692428342	P40939
Biological oxidations	0.21379410586113712	Q6IB77
Diseases of metabolism	0.2357785228839242	Q13085
Signaling by Nuclear Receptors	0.2555701963136816	P29474
Deubiquitination	0.26367716563061816	P21580
DNA Repair	0.2882972671346483	P09874
Metabolism of amino acids and derivatives	0.3352884976656635	P49419
Infectious disease	0.369265686909256	P35228;P09874

Disease	0.3700025945708416	Q13085;P35228;P09874
Signaling by Interleukins	0.3923960901290876	P35228
Immune System	0.4421587323519006	P29474;P21580;P35228
Cell Cycle, Mitotic	0.44435071242306556	P04818
Signaling by Receptor Tyrosine Kinases	0.44497355028745644	P29474
Post-translational protein modification	0.4603740553192832	P21580;P09874
Cell Cycle	0.5217930113641549	P04818
Signal Transduction	0.5419382527802737	P29474;P21580;P09874
Cytokine Signaling in Immune system	0.5896958418941857	P35228
Metabolism of proteins	0.6413485793169579	P21580;P09874
Generic Transcription Pathway	0.7594309788263349	P09874
RNA Polymerase II Transcription	0.7924453305760891	P09874
Gene expression (Transcription)	0.8260453291506424	P09874

Table A7.1: *Gene expression markers and-levels (Log10 RQ) of the study cohort (n=12) depicted for the different groups of risk allele- and non-risk allele carriers (rs1421085) over differentiation days (day0-D0; day2-D2 and day14-D14). PPARG2-peroxisome proliferator activated receptor gamma 2, LEP-leptin, UCP1-uncoupling protein 1, PGC1A-PPARG coactivator 1 alpha, IRX3/5-iroquois homeobox 3/5, SMPD1-sphingomyelin phosphodiesterase 1, SLC27A5- solute carrier family 27 member 5, PARP1- poly (ADP-ribose) polymerase 1, HADHA- hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha, ACACA-acetyl-CoA carboxylase alpha. The following targets and harvesting were below n=6 for subcohorts: LEP (n=5, day14-CC),UCP1 (n= 4, day0-TT and day2-TT) IRX3 (n=5, day14-CC), IRX5 (n=5, day14-TT), SLC27A5 (n=5, day14).*

Gene expression				
Marker for	**Gene name**	**rs1421085 risk allele carriers (CC) n = 6 mean (log 10 RQ) ± standard deviation**	**rs1421085 non-risk allele carriers (TT) n = 6 mean (log 10 RQ) ± standard deviation**	**p - value**
Adipocyte Differentiation	*PPARG2*	**D0**=-3.383 ± 0.4258	**D0**=-3.372 ± 0.8178	n.s.
		D2=-1.463 ± 0.2837	**D2**=-1.588 ± 0.4822	n.s.
		D14=-0.6533 ±1.047	**D14**= -0.7567 ± 1.022	n.s.
Lipid storage	*LEP*	**D0**=4.678 ± 4.203	**D0**=1.488 ± 5.919	n.s.
		D2=-1.015 ± 3.760	**D2**=-2.642 ± 0.3432	n.s.
		D14=-1.616 ± 0.7135	**D14**= -2.060 ± 1.105	n.s.
Mitochondrial function	*UCP1*	**D0**=-3.908 ± 0.2494	**D0**=-3.703 ± 0.5657	n.s.
		D2=-3.723 ± 0.3398	**D2**=-3.505 ± 0.2339	n.s.
		D14=-1.460 ± 1.467	**D14**=-1.362 ± 1.159	n.s.
	PGC1A	**D0**=-2.258 ± 0.3690	**D0**=-2.343 ± 0.6908	n.s.
		D2=-0.8950 ± 0.1021	**D2**=-0.9050 ± 0.3494	n.s.
		D14=-0.4700 ± 1.112	**D14**=-0.4767 ± 1.074	n.s.
FTO marker	*IRX3*	**D0**=-1.862 ± 0.2424	**D0**=-1.727 ± 0.6159	n.s.
		D2=-1.742 ± 0.2569	**D2**=-1.727 ± 0.3104	n.s.
		D14=-1.816 ± 1.165	**D14**=-1.857 ± 1.437	n.s.
	IRX5	**D0**=-1.667 ± 0.1499	**D0**=-1.528 ± 0.4793	n.s.
		D2=-1.433 ± 0.2321	**D2**=-1.508 ± 0.2139	n.s.
		D14=-1.415 ± 0.8258	**D14**=-1.078 ± 0.7937	n.s.
Metabolomics marker	*SMPD1*	**D0**=-0.1533 ± 0.2803	**D0**=-0.1367 ± 0.1954	n.s.
		D2=0.00333 ± 0.2280	**D2**=0.0500 ± 0.1115	n.s.
		D14=-0.2733 ±0.2111	**D14**=-0.3217 ± 0.2522	n.s.
	SLC27A5	**D0**=-2.242 ± 0.1713	**D0**=-2.270 ± 0.2292	n.s.
		D2=-1.923 ± 0.2437	**D2**=-1.932 ± 0.2464	n.s.
		D14=-1.316 ± 0.2089	**D14**=-1.236 ± 0.1997	n.s.
	PARP1	**D0**=-1.003 ± 0.1684	**D0**=-1.083 ± 0.1886	n.s.
		D2=-0.8883 ± 0.1983	**D2**=-1.027 ± 0.1300	n.s.
		D14=-0.7133 ±0.2231	**D14**=-0.7533 ± 0.2407	n.s.
	HADHA	**D0**=-0.2033 ± 0.1678	**D0**=-0.2600 ± 0.2058	n.s.
		D2=0.2717 ± 0.2305	**D2**=0.1383 ± 0.1634	n.s.
		D14=0.3300 ± 0.2382	**D14**=0.2233 ± 0.2672	n.s.
	ACACA	**D0**=-0.8683 ± 0.1990	**D0**=-0.8783 ± 0.2199	n.s.
		D2=-0.6883 ± 0.1937	**D2**=-0.8000 ± 0.1826	n.s.
		D14=-0.0050 ±0.2470	**D14**=-0.1617 ± 0.3391	n.s.

Table A7.2: *Gene expression markers and-levels as relative quantification (RQ) for correlation analysis of the study cohort (n=12) depicted for the different groups of risk allele- and non-risk allele carriers (rs1421085) for Phase 1 and Phase 2. Delta CT-values were multiplied by -1, median centered and winsorized -given the 5th and 95th percentiles- and backtransformed into RQ. PPARG2-peroxisome proliferator activated receptor gamma 2, LEP-leptin, UCP1-uncoupling protein 1, PGC1A-PPARG coactivator 1 alpha, IRX3/5-iroquois homeobox 3/5, SMPD1-sphingomyelin phosphodiesterase 1, SLC27A5- solute carrier family 27 member 5, PARP1- poly (ADP-ribose) polymerase 1, HADHA- hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha, ACACA-acetyl-CoA carboxylase alpha*

Phase 1	**Individuals risk group (subject with ID)**						**Individuals non-risk group (subject with ID)**						
Genes	**CC**	**CC**	**CC**	**CC**	**CC**	**CC**	**TT**	**TT**	**TT**	**TT**	**TT**	**TT**	p-value
	123	164	186	256	258	266	142	170	187	189	252	300	
SMPD1	0.33582	0.70534	-0.42542	0.81840	0.56844	0.01946	0.20153	0.97413	0.03612	0.49146	0.86279	0.76749	0.197
SLC27A5	-0.03754	0.37544	0.28937	0.15233	0.39694	0.42636	0.45960	0.01682	0.08653	0.41514	0.46044	0.37522	0.294
PARP1	0.63999	0.09481	0.44012	0.28425	0.48452	-0.36913	0.08238	0.23646	0.11276	-0.13134	0.32804	0.05676	0.120
HADHA	0.51943	0.45963	0.56953	0.54598	0.85457	0.59147	0.20221	0.52637	0.33564	0.67067	0.54985	0.38588	0.120
ACACA	0.03502	0.13129	0.18008	0.10827	0.26394	0.39258	-0.12087	0.02568	-0.09485	0.30664	0.12432	0.24142	0.155
PPARG2	0.44266	0.49091	0.53120	0.43261	0.49331	0.63779	0.41389	0.23294	0.25405	0.66502	0.51269	0.59022	0.350
UCP1	0.06241	0.14151	0.29193	0.08163	-0.09365	-0.06061	0.07832	-0.23813	0.16991	0.21647	0.07947	0.09796	0.409
PGC1A	0.43285	0.53317	0.42856	0.46303	0.28202	0.62279	0.61193	0.10520	0.22427	0.76028	0.54387	0.56185	0.350
IRX3	-0.36902	0.35009	0.24393	0.23365	-0.02918	0.66557	0.44510	-1.82713	0.07374	0.74193	0.33071	0.44401	0.294
IRX5	0.45317	0.69850	0.94992	0.61173	1.57478	0.24632	-0.04417	-4.69247	0.51190	1.95474	0.56379	0.24682	0.155
LEP	-1.21105	-1.16255	0.10959	0.03784	-1.12833	-1.13995	0.05217	-1.22667	-1,21477	-1.15539	0.09381	0.07898	0.469
Phase 2	**Individuals risk group (subject with ID)**						**Individuals non-risk group (subject with ID)**						
Genes	**CC**	**CC**	**CC**	**CC**	**CC**	**CC**	**TT**	**TT**	**TT**	**TT**	**TT**	**TT**	p-value
	123	164	186	256	258	266	142	170	187	189	252	300	
SMPD1	-0.23727	-1.11973	-0.4981	-1.24972	-0.79795	0.38125	-0.40203	-1.48189	-0.34704	-0.68043	-1.11949	-1.40384	0.242
SLC27A5	1.09731	0.79507	0.73327	0.48809	0.76158	-0.52465	0.65195	1.26664	1.19804	0.48770	-0.4973	0.58773	0.500
PARP1	0.59197	1.01249	0.89537	0.62312	0.72402	0.12077	1.06153	0.75853	1.32990	1.12705	0.18062	0.87509	0.090
HADHA	0.32996	0.40639	0.07452	0.19896	-0.01994	-0.30654	-0.02267	0.04194	0.45323	0.24694	0.06016	-0.06935	0.409
ACACA	1.24462	1.50199	0.96748	1.61939	1.42829	0.63593	0.60110	1.50126	1.55909	1.48185	0.81323	0.52443	0.294
PPARG2	0.46160	0.45692	0.19776	0.56515	0.37396	-0.34597	0.15748	0.43530	0.87571	0.23494	0.25313	-0,20824	0.350
UCP1	1.55731	1.75809	1.22592	2.18393	1.53526	-0.08128	1.42510	1.56000	2.13444	1.42622	0.76311	0.63941	0.350
PGC1A	0.23139	0.23617	0.21184	0.59815	0.34200	-0.54078	0.06888	0.34093	0.78527	0.06987	-0.09316	-0.28858	0.294
IRX3	1.14918	0.71602	0.54138	-0.11212	0.64006	-1.10674	-1.39437	1.27251	2.01856	0.08461	-0.77404	-0.72568	0.409
IRX5	0.66952	-0.41115	-0.43446	4.40044	-0.46875	-0.81027	0.59739	2.36271	4.59288	-1.77821	0.04345	-0.12687	0.242
LEP	0.03274	0.06863	0.06666	-1.04092	0.06602	0.89861	0.01337	0.03518	0.26769	0.03430	0.03425	-0.05312	0.242

Co-expressed GENE lists

(UniProt Database investigations):

Table A8: *Co-expressed genes with enzyme (EC) number.*

Internal ID	Entry Uniprot	Gene name	Origin	EC number	Protein names specific to EC Number	Protein names	Comments
0_EC	O15438	ABCC3	M	7.6.2.-	**ATP-binding cassette sub-family C member 3** (EC:7.6.2.-)	(Alternative names: Canalicular multispecific organic anion transporter 2; Multi-specific organic anion transporter D - Short name: MOAT-D; Multidrug resistance-associated protein 3)	EC number updated and added to the list; Gene names synonyms:CMOAT2, MLP2, MRP3
0_EC	O15438	ABCC3	M	7.6.2.2	**ATP-binding cassette sub-family C member 3** (EC:7.6.2.2)	(Alternative names: Canalicular multispecific organic anion transporter 2; Multi-specific organic anion transporter D - Short name: MOAT-D; Multidrug resistance-associated protein 3)	EC number updated and added to the list; Gene names synonyms:CMOAT2, MLP2, MRP3
0_EC	O15438	ABCC3	M	7.6.2.3	**ATP-binding cassette sub-family C member 3** (EC:7.6.2.3)	(Alternative names: Canalicular multispecific organic anion transporter 2; Multi-specific organic anion transporter D - Short name: MOAT-D; Multidrug resistance-associated protein 3)	EC number updated and added to the list; Gene names synonyms:CMOAT2, MLP2, MRP3
1_EC	P08183	ABCB1	M	7.6.2.2	Multidrug resistance protein 1 (EC 7.6.2.2)	**ATP-dependent translocase ABCB1**; (Alternative names: ATP-binding cassette sub-family B member 1; P-glycoprotein 1; CD antigen CD243)	EC Number updated; Gene names synonyms:MDR1, PGY1
2_EC	P08183	ABCB1	M	7.6.2.1	Phospholipid transporter ABCB1 (EC 7.6.2.1)	**ATP-dependent translocase ABCB1**; (Alternative names: ATP-binding cassette sub-family B member 1; P-glycoprotein 1; CD antigen CD243)	EC Number updated; Gene names synonyms:MDR1, PGY1
3_EC	P33527	ABCC1	M	7.6.2.2	**Multidrug resistance-associated protein 1** (EC 7.6.2.2)	(Alternative names: ATP-binding cassette sub-family C member 1; Leukotriene C(4) transporter - Short name: LTC4 transporter)	EC Numbers updated;Gene names synonyms:MRP, MRP1
4_EC	P33527	ABCC1	M	7.6.2.3	Glutathione-S-conjugate-translocating ATPase ABCC1 (EC 7.6.2.3)	(Alternative names: ATP-binding cassette sub-family C member 1; Leukotriene C(4) transporter - Short name: LTC4 transporter)	EC Numbers updated;Gene names synonyms:MRP, MRP1
5_EC	P45844	ABCG1	M	7.6.2.-	**ATP-binding cassette sub-family G member 1** (EC:7.6.2.-)	(Alternative names: ATP-binding cassette transporter 8; White protein homolog)	EC Number updated; Gene names synonyms:ABC8, WHT1
6_EC	P42684	ABL2	M	2.7.10.2	**Tyrosine-protein kinase 2** (EC 2.7.10.2)	(Alternative names: Abelson murine leukemia viral oncogene homolog 2; Abelson tyrosine-protein kinase 2; Abelson-related gene protein; Tyrosine-protein kinase ARG)	Protein names updated; Gene names synonyms:ABLL, ARG

7_EC	Q76LX8	ADAMTS13	M	3.4.24.87	**A disintegrin and metalloproteinase with thrombospondin motifs 13** (EC 3.4.24.87) - Short names : ADAM-TS 13/ ADAM-TS13/ ADAMTS-13	(Alternative names: von Willebrand factor-cleaving protease - Short names: vWF-CP/ vWF-cleaving protease)	Gene names synonyms:C9orf8
8_EC	Q08828	ADCY1	M	4.6.1.1	**Adenylate cyclase type 1** (EC 4.6.1.1)	(Alternative names: ATP pyrophosphate-lyase 1; Adenylate cyclase type I; Adenylyl cyclase 1; Ca(2+)/calmodulin-activated adenylyl cyclase)	0
9_EC	Q08462	ADCY2	M	4.6.1.1	**Adenylate cyclase type 2** (EC 4.6.1.1)	(Alternative names: ATP pyrophosphate-lyase 2; Adenylate cyclase type II; Adenylyl cyclase 2)	Gene names synonyms:KIAA1060
10_EC	O95622	ADCY5	M	4.6.1.1	**Adenylate cyclase type 5** (EC 4.6.1.1)	(Alternative names: ATP pyrophosphate-lyase 5; Adenylate cyclase type V; Adenylyl cyclase 5 - Short name: AC5)	0
11_EC	P40145	ADCY8	M	4.6.1.1	**Adenylate cyclase type 8** (EC 4.6.1.1)	(Alternative names: ATP pyrophosphate-lyase 8; Adenylate cyclase type VIII; Adenylyl cyclase 8 - Short name:AC8; Ca(2+)/calmodulin-activated adenylyl cyclase)	0
12_EC	Q9UKV8	AGO2	M	3.1.26.n2	**Protein argonaute-2** (EC 3.1.26.n2) - Short names: Argonaute2/ hAgo2;	(Alternative names: Argonaute RISC catalytic component 2; Eukaryotic translation initiation factor 2C 2 - Short names: eIF-2C 2/ eIF2C 2; PAZ Piwi domain protein - Short name: PPD; Protein slicer)	Gene names synonyms:EIF2C2
13_EC	P30838	ALDH3A1	M	1.2.1.5	**Aldehyde dehydrogenase, dimeric NADP-preferring** (EC 1.2.1.5)	(Alternative names: ALDHIII; Aldehyde dehydrogenase 3; Aldehyde dehydrogenase family 3 member A1)	Gene names synonyms:ALDH3
14_EC	P16050	ALOX15	M	1.13.11.31	Arachidonate 12-lipoxygenase, leukocyte-type (EC:1.13.11.31) - Short name: 12-LOX	**Polyunsaturated fatty acid lipoxygenase ALOX15**; (Alternative names: 12/15-lipoxygenase; Arachidonate omega-6 lipoxygenase)	Gene names synonyms:LOG15; EC number updated; protein names updated
15_EC	P16050	ALOX15	M	1.13.11.33	Arachidonate 15-lipoxygenase (EC 1.13.11.33) - Short names: 15-LOX/15-LOX-1	**Polyunsaturated fatty acid lipoxygenase ALOX15**; (Alternative names: 12/15-lipoxygenase; Arachidonate omega-6 lipoxygenase)	Gene names synonyms:LOG15; EC number updated; protein names updated
16_EC	P16050	ALOX15	M	1.13.11.-	Hepoxilin A3 synthase Alox15 (EC 1.13.11.-)	**Polyunsaturated fatty acid lipoxygenase ALOX15**; (Alternative names: 12/15-lipoxygenase; Arachidonate omega-6 lipoxygenase)	Gene names synonyms:LOG15; EC number updated; protein names updated

17_EC	P16050	ALOX15	M	1.13.11.12	Linoleate 13S-lipoxygenase (EC 1.13.11.12)	**Polyunsaturated fatty acid lipoxygenase ALOX15**; (Alternative names: 12/15-lipoxygenase; Arachidonate omega-6 lipoxygenase)	Gene names synonyms:LOG15; EC number updated; protein names updated
18_EC	P16615	ATP2A2	M	7.2.2.10	**Sarcoplasmic/endoplasmic reticulum calcium ATPase 2** (EC 7.2.2.10) - Short names: SERCA2/SR Ca(2+)-ATPase 2	(Alternative names: Calcium pump 2;Calcium-transporting ATPase sarcoplasmic reticulum type, slow twitch skeletal muscle isoform; Endoplasmic reticulum class 1/2 Ca(2+) ATPase)	EC Number updated; Gene names synonyms:ATP2B
19_EC	O43520	ATP8B1	M	7.6.2.1	**Phospholipid-transporting ATPase IC** (EC 7.6.2.1)	(Alternative names: ATPase class I type 8B member 1; Familial intrahepatic cholestasis type 1; P4-ATPase flippase complex alpha subunit ATP8B1)	EC Number updated; Gene names synonyms:ATPIC, FIC1, PFIC
20_EC	Q9BX63	BRIP1	M	3.6.4.13	**Fanconi anemia group J protein** (EC 3.6.4.13) - Short name: Protein FACJ	(Alternative names: ATP-dependent RNA helicase BRIP1; BRCA1-associated C-terminal helicase 1; BRCA1-interacting protein C-terminal helicase 1 - Short name: BRCA1-interacting protein 1)	Additionally another EC Number under "Enzyme and pathway databases" (BRENDA EC: 3.6.4.12) found; Gene names synonyms:BACH1, FANCJ
21_EC	O43683	BUB1	M	2.7.11.1	**Mitotic checkpoint serine/threonine-protein kinase BUB1** (EC 2.7.11.1) - Short name: hBUB1	(Alternative name: BUB1A)	Gene names synonyms:BUB1L
22_EC	O60566	BUB1B	M	2.7.11.1	**Mitotic checkpoint serine/threonine-protein kinase BUB1 beta** (EC 2.7.11.1)	(Alternative names: MAD3/BUB1-related protein kinase - Short name:hBUBR1; Mitotic checkpoint kinase MAD3L; Protein SSK1)	Gene names synonyms:BUBR1, MAD3L, SSK1
23_EC	P00918	CA2	M	4.2.1.1	**Carbonic anhydrase 2** (EC 4.2.1.1)	(Alternative names: Carbonate dehydratase II; Carbonic anhydrase C - Short name: CAC; Carbonic anhydrase II - Short name:CA-II)	0
24_EC	P24941	CDK2	M	2.7.11.22	**Cyclin-dependent kinase 2** (EC 2.7.11.22)	(Alternative names: Cell division protein kinase 2; p33 protein kinase)	Gene names synonyms:CDKN2
25_EC	O15111	CHUK	M	2.7.11.10	**Inhibitor of nuclear factor kappa-B kinase subunit alpha** (EC 2.7.11.10)- Short names: I-kappa-B kinase alpha/IKK-A/IKK-alpha/IkBKA/IkappaB kinase;	(Alternative names: Conserved helix-loop-helix ubiquitous kinase; I-kappa-B kinase 1 - Short name: IKK1; Nuclear factor NF-kappa-B inhibitor kinase alpha - Short name: NFKBIKA; Transcription factor 16 - Short name: TCF-16)	Gene names synonyms:IKKA, TCF16
26_EC	O14578	CIT	M	2.7.11.1	**Citron Rho-interacting kinase** (EC 2.7.11.1) - Short name: CRIK	(Alternative names: Serine/threonine-protein kinase 21)	Gene names synonyms:CRIK, KIAA0949, STK21

27_EC	P06732	CKM	M	2.7.3.2	**Creatine kinase M-type** (EC 2.7.3.2)	(Alternative names: Creatine kinase M chain; Creatine phosphokinase M-type - Short name: CPK-M; M-CK	Gene names synonyms:CKMM
28_EC	Q9Y5Q5	CORIN	M	3.4.21.-	**Atrial natriuretic peptide-converting enzyme** (EC 3.4.21.-)	(Alternative names: Corin; Heart-specific serine proteinase ATC2; Pro-ANP-converting enzyme; Transmembrane protease serine 10) [Cleaved into: Atrial natriuretic peptide-converting enzyme, N-terminal propeptide; Atrial natriuretic peptide-converting enzyme, activated protease fragment; Atrial natriuretic peptide-converting enzyme, 180 kDa soluble fragment; Atrial natriuretic peptide-converting enzyme, 160 kDa soluble fragment; Atrial natriuretic peptide-converting enzyme, 100 kDa soluble fragment]	Gene names synonyms:CRN, TMPRSS10
29_EC	Q96IY4	CPB2	M	3.4.17.20	**Carboxypeptidase B2** (EC 3.4.17.20)	(Alternative names: Carboxypeptidase U - Short name: CPU; Plasma carboxypeptidase B - Short name: pCPB; Thrombin-activable fibrinolysis inhibitor - Short name: TAFI)	0
30_EC	P08311	CTSG	M	3.4.21.20	**Cathepsin G** (EC 3.4.21.20) - Short name: CG	0	0
31_EC	P19099	CYP11B2	M	1.14.15.5	Corticosterone 18-monooxygenase,CYP11B2 (EC:1.14.15.5)	**Cytochrome P450 11B2, mitochondrial**; (Alternative names: Aldosterone synthase - Short name: ALDOS; Aldosterone-synthesizing enzyme; CYPXIB2; Cytochrome P-450Aldo; Cytochrome P-450C18; Steroid 18-hydroxylase)	protein names updated
32_EC	P19099	CYP11B2	M	1.14.15.4	Steroid 11-beta-hydroxylase, CYP11B2 (EC:1.14.15.4)	**Cytochrome P450 11B2, mitochondrial**; (Alternative names: Aldosterone synthase - Short name: ALDOS; Aldosterone-synthesizing enzyme; CYPXIB2; Cytochrome P-450Aldo; Cytochrome P-450C18; Steroid 18-hydroxylase)	protein names updated
33_EC	P11511	CYP19A1	M	1.14.14.14	**Aromatase** (EC 1.14.14.14)	(Alternative names: CYPXIX; Cytochrome P-450AROM; Cytochrome P450 19A1; Estrogen synthase)	Gene names synonyms:ARO1, CYAR, CYP19
34_EC	Q07973	CYP24A1	M	1.14.15.16	**1,25-dihydroxyvitamin D(3) 24-hydroxylase, mitochondrial** (EC 1.14.15.16) - Short names: 24-Ohase/Vitamin D(3) 24-hydroxylase;	(Alternative names: Cytochrome P450 24A1; Cytochrome P450-CC24)	Gene names synonyms:CYP24

35_EC	O43174	CYP26A1	M	1.14.13.-	**Cytochrome P450 26A1** (EC 1.14.13.-)	(Alternative names: Cytochrome P450 retinoic acid-inactivating 1 - Short names: Cytochrome P450RAI/hP450RAI; Retinoic acid 4-hydroxylase; Retinoic acid-metabolizing cytochrome)	Gene names synonyms:CYP26, P450RAI1
36_EC	Q6V0L0	CYP26C1	M	1.14.-.-	**Cytochrome P450 26C1** (EC 1.14.-.-)	0	0
37_EC	O15528	CYP27B1	M	1.14.15.18	**25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial** (EC 1.14.15.18)	(Alternative names: 25-OHD-1 alpha-hydroxylase; 25-hydroxyvitamin D(3) 1-alpha-hydroxylase - Short name: VD3 1A hydroxylase; (Calcidiol 1-monooxygenase; Cytochrome P450 subfamily XXVIIB polypeptide 1; Cytochrome P450C1 alpha; Cytochrome P450VD1-alpha; Cytochrome p450 27B1)	Gene names synonyms:CYP1ALPHA, CYP27B
38_EC	P51589	CYP2J2	M	1.14.14.-	**Cytochrome P450 2J2** (EC 1.14.14.-)	(Alternative name: Arachidonic acid epoxygenase; CYPIIJ2)	EC numbers updated and protein names updated
39_EC	P51589	CYP2J2	M	1.14.14.74	Albendazole monooxygenase (hydroxylating) (EC 1.14.14.74)	(Alternative name: Arachidonic acid epoxygenase; CYPIIJ2)	EC numbers updated and protein names updated
40_EC	P51589	CYP2J2	M	1.14.14.73	Albendazole monooxygenase (sulfoxide-forming) (EC 1.14.14.73)	(Alternative name: Arachidonic acid epoxygenase; CYPIIJ2)	EC numbers updated and protein names updated
41_EC	P51589	CYP2J2	M	5.4.4.7	Hydroperoxy icosatetraenoate isomerase (EC 5.4.4.7)	(Alternative name: Arachidonic acid epoxygenase; CYPIIJ2)	EC numbers updated and protein names updated
42_EC	P22680	CYP7A1	M	1.14.14.26	24-hydroxycholesterol 7-alpha-hydroxylase (EC 1.14.14.26)	**Cytochrome P450 7A1**; (Alternative names: CYPVII; Cholesterol 7-alpha-hydroxylase;)	EC number updated and protein names updated; Gene names syonyms: CYP7
43_EC	P22680	CYP7A1	M	1.14.14.23	Cholesterol 7-alpha-monooxygenase (EC 1.14.14.23)	**Cytochrome P450 7A1**; (Alternative names: CYPVII; Cholesterol 7-alpha-hydroxylase;)	EC number updated and protein names updated; Gene names syonyms: CYP7
44_EC	Q9UNU6	CYP8B1	M	1.14.18.8	**7-alpha-hydroxycholest-4-en-3-one 12-alpha-hydroxylase** (EC 1.14.18.8)	(Alternative names: 7-alpha-hydroxy-4-cholesten-3-one 12-alpha-hydroxylase; CYPVIIIB1; Cytochrome P450 8B1; Sterol 12-alpha-hydroxylase)	Gene names synonyms:CYP12
45_EC	P55073	DIO3	M	1.21.99.3	**Thyroxine 5-deiodinase** (EC 1.21.99.3)	(Alternative names: 5DIII; DIOIII; Type 3 DI; Type III iodothyronine deiodinase)	Gene names synonyms:ITDI3, TXDI3
46_EC	P28562	DUSP1	M	3.1.3.16	**Dual specificity protein phosphatase 1** (EC 3.1.3.16)	(Alternative names: Dual specificity protein phosphatase hVH1; Mitogen-activated protein kinase phosphatase 1 - Short name: MAP kinase phosphatase 1/MKP-1) ; Protein-tyrosine phosphatase CL100)	Gene names synonyms:CL100, MKP1, PTPN10, VH1

47_EC	P28562	DUSP1	M	3.1.3.48	**Dual specificity protein phosphatase 1** (EC 3.1.3.48)	(Alternative names: Dual specificity protein phosphatase hVH1; Mitogen-activated protein kinase phosphatase 1 - Short name: MAP kinase phosphatase 1/MKP-1) ; Protein-tyrosine phosphatase CL100)	Gene names synonyms:CL100, MKP1, PTPN10, VH1
48_EC	Q16690	DUSP5	M	3.1.3.16	**Dual specificity protein phosphatase 5** (EC 3.1.3.16)	(Alternative name: Dual specificity protein phosphatase hVH3)	Gene names synonyms:VH3
49_EC	Q16690	DUSP5	M	3.1.3.48	**Dual specificity protein phosphatase 5** (EC 3.1.3.48)	(Alternative name: Dual specificity protein phosphatase hVH3)	Gene names synonyms:VH3
50_EC	P0DPD6	ECE2	M	3.4.24.71	**Endothelin-converting enzyme 2** (EC 3.4.24.71) - Short name: ECE-2	0	Entry Uniprot updated; EC Number updated; protein names updated; gene names synonyms:KIAA0604
51_EC	Q14249	ENDOG	M	3.1.30.-	**Endonuclease G, mitochondrial** (EC 3.1.30.-) - Short name: Endo G	0	0
52_EC	P11678	EPX	M	1.11.1.7	**Eosinophil peroxidase** (EC 1.11.1.7) - Short name: EPO	[Cleaved into the following 2 chains: Eosinophil peroxidase light chain; Eosinophil peroxidase heavy chain]	Gene names synonyms:EPER, EPO, EPP
53_EC	P03951	F11	M	3.4.21.27	**Coagulation factor XI** (EC 3.4.21.27) - Short name: FXI	(Alternative name: Plasma thromboplastin antecedent - Short name: PTA [Cleaved into the following 2 chains: Coagulation factor XIa heavy chain; Coagulation factor XIa light chain]	0
54_EC	P00734	F2	M	3.4.21.5	**Prothrombin** (EC 3.4.21.5)	(Alternative name: Coagulation factor II) [Cleaved into the following 4 chains: Activation peptide fragment 1; Activation peptide fragment 2; Thrombin light chain; Thrombin heavy chain]	0
55_EC	P49327	FASN	M	2.3.1.85	**Fatty acid synthase** (EC 2.3.1.85)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS

56_EC	P49327	FASN	M	2.3.1.38	[Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS
57_EC	P49327	FASN	M	2.3.1.39	[Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS
58_EC	P49327	FASN	M	2.3.1.41	3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS
59_EC	P49327	FASN	M	1.1.1.100	3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC	Protein names updated; Gene names Synonyms:FAS

						1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	
60_EC	P49327	FASN	M	4.2.1.59	3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS
61_EC	P49327	FASN	M	1.3.1.39	Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS
62_EC	P49327	FASN	M	3.1.2.14	Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)	(Alternative names: Type I fatty acid synthase); [Including the following 7 domains: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Acyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	Protein names updated; Gene names Synonyms:FAS
63_EC	P09769	FGR	M	2.7.10.2	**Tyrosine-protein kinase Fgr** (EC 2.7.10.2)	(Alternative names: Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog; Proto-oncogene c-Fgr; p55-Fgr; p58-Fgr; p58c-Fgr)	Gene names synonyms:SRC2

64_EC	P62942	FKBP1A	M	5.2.1.8	**Peptidyl-prolyl cis-trans isomerase FKBP1A** (EC 5.2.1.8) - Short name: PPIase FKBP1A	(Alternative names: 12 kDa FK506-binding protein - Short name: 12 kDa FKBP/FKBP-12; Calstabin-1; FK506-binding protein 1A - Short name: FKBP-1A; Immunophilin FKBP12; Rotamase)	Gene names synonyms:FKBP1, FKBP12
65_EC	P68106	FKBP1B	M	5.2.1.8	**Peptidyl-prolyl cis-trans isomerase FKBP1B** (EC 5.2.1.8) - Short name: PPIase FKBP1B	(Alternative names: 12.6 kDa FK506-binding protein - Short name: 12.6 kDa FKBP/FKBP-12.6; FK506-binding protein 1B - Short name: FKBP-1B; Immunophilin FKBP12.6; Rotamase; h-FKBP-12	Gene names synonyms:FKBP12.6, FKBP1L, FKBP9, OTK4
66_EC	P17948	FLT1 FLT	M	2.7.10.1	**Vascular endothelial growth factor receptor 1** (EC 2.7.10.1) - Short name: VEGFR-1	(Alternative names: Fms-like tyrosine kinase 1 - Short name: FLT-1; Tyrosine-protein kinase FRT; Tyrosine-protein kinase receptor FLT - Short name: FLT; Vascular permeability factor receptor	Gene names synonyms:FLT, FRT, VEGFR1
67_EC	P35575	G6PC	M	3.1.3.9	**Glucose-6-phosphatase** (EC 3.1.3.9) - Short names: G-6-Pase/G6Pase	(Alternative names: Glucose-6-phosphatase alpha - Short name: G6Pase-alpha)	Gene names synonyms:G6PT
68_EC	Q9NQR9	G6PC2	M	3.1.3.9	**Glucose-6-phosphatase 2** (EC 3.1.3.9) - Short name: G-6-Pase 2/G6Pase 2	(Alternative names: Islet-specific glucose-6-phosphatase catalytic subunit-related protein)	Gene names synonyms:IGRP
69_EC	P10253	GAA	M	3.2.1.20	**Lysosomal alpha-glucosidase** (EC 3.2.1.20)	(Alternative names: Acid maltase; Aglucosidase alfa); [Cleaved into the following 2 chains: 76 kDa lysosomal alpha-glucosidase; 70 kDa lysosomal alpha-glucosidase]	0
70_EC	P04062	GBA	M	3.2.1.45	**Lysosomal acid glucosylceramidase** (EC 3.2.1.45) - Short name: Lysosomal acid Gcase	(Alternative names: Acid beta-glucosidase; Alglucerase; Beta-glucocerebrosidase - Short name: Beta-GC; D-glucosyl-N-acylsphingosine glucohydrolase; Imiglucerase)	EC numbers updated and protein names updated; Gene names synonyms:GC, GLUC
71_EC	P04062	GBA	M	2.4.1.-	Cholesterol glucosyltransferase (EC 2.4.1.-) - Short name: SGTase	(Alternative names: Acid beta-glucosidase; Alglucerase; Beta-glucocerebrosidase - Short name: Beta-GC; D-glucosyl-N-acylsphingosine glucohydrolase; Imiglucerase)	EC numbers updated and protein names updated; Gene names synonyms:GC, GLUC
72_EC	P04062	GBA	M	3.2.1.104	Cholesteryl-beta-glucosidase (EC 3.2.1.104)	(Alternative names: Acid beta-glucosidase; Alglucerase; Beta-glucocerebrosidase - Short name: Beta-GC; D-glucosyl-N-acylsphingosine glucohydrolase; Imiglucerase)	EC numbers updated and protein names updated; Gene names synonyms:GC, GLUC

73_EC	P38435	GGCX	M	4.1.1.90	**Vitamin K-dependent gamma-carboxylase** (EC 4.1.1.90)	(Alternative names: Gamma-glutamyl carboxylase; Peptidyl-glutamate 4-carboxylase; Vitamin K gamma glutamyl carboxylase)	Gene names synonyms:GC
74_EC	P07203	GPX1	M	1.11.1.9	**Glutathione peroxidase 1** (EC 1.11.1.9) - Short names: GPx-1/GSHPx-1	(Alternative name:Cellular glutathione peroxidase)	0
75_EC	P09211	GSTP1	M	2.5.1.18	**Glutathione S-transferase P** (EC 2.5.1.18)	(Alternative names: GST class-pi; GSTP1-1)	Gene names synonyms:FAEES3, GST3
76_EC	P25092	GUCY2C	M	4.6.1.2	**Heat-stable enterotoxin receptor** (EC 4.6.1.2) - Short names: STA receptor/hSTAR	(Alternative names: Guanylyl cyclase C - Short name: GC-C; Intestinal guanylate cyclase)	Gene names synonyms:GUC2C, STAR
77_EC	P56524	HDAC4	M	3.5.1.98	**Histone deacetylase 4** (EC 3.5.1.98) - Short name: HD4	0	Gene names synonyms:KIAA0288
78_EC	P30519	HMOX2	M	1.14.14.18	**Heme oxygenase 2** (EC 1.14.14.18) - Short name: HO-2	0	Gene names synonyms:HO2
79_EC	P05981	HPN	M	3.4.21.106	**Serine protease hepsin** (EC 3.4.21.106)	(Alternative name:Transmembrane protease serine 1) [Cleaved into the following 2 chains: Serine protease hepsin non-catalytic chain; Serine protease hepsin catalytic chain]	Gene names synonyms:TMPRSS1
80_EC	P00492	HPRT1	M	2.4.2.8	**Hypoxanthine-guanine phosphoribosyltransferase** (EC 2.4.2.8) - Short names: HGPRT/HGPRTase	0	Gene names synonyms:HPRT
81_EC	P01112	HRAS	M	3.6.5.2	**GTPase HRas** (EC 3.6.5.2)	(Alternative names: H-Ras-1; Ha-Ras; Transforming protein p21; c-H-ras; p21ras); [Cleaved into the following chain: GTPase HRas, N-terminally processed]	EC Number updated; Molecular function: Hydrolase; Ligand: GTP-binding, Nucleotide-binding; Gene names synonyms:HRAS1
82_EC	P14778	IL1R1	M	3.2.2.6	**Interleukin-1 receptor type 1** (EC 3.2.2.6) - Short names: IL-1R-1/IL-1RT-1/IL-1RT1	(Alternative names: CD121 antigen-like family member A; Interleukin-1 receptor alpha - Short name: IL-1R-alpha; Interleukin-1 receptor type I; p80; CD_antigen: CD121a); [Cleaved into the following 2 chains: Interleukin-1 receptor type 1, membrane form - Short names: mIL-1R1/mIL-1RI; Interleukin-1 receptor type 1, soluble form - Short names: sIL-1R1/sIL-1RI]	EC Number updated; Molecular function: Hydrolase, Receptor; Biological process:Inflammatory response; Ligand: NAD; Gene names synonyms:IL1R, IL1RA, IL1RT1
83_EC	Q9NPH3	IL1RAP	M	3.2.2.6	**Interleukin-1 receptor accessory protein** (EC 3.2.2.6) - Short names: IL-1 receptor accessory protein/IL-1RAcP	(Alternative names: Interleukin-1 receptor 3 - Short names: IL-1R-3/IL-1R3)	EC Number updated; Molecular function: Hydrolase, Receptor;Biological process: Immunity, Inflammatory response, Innate

							immunity; Ligand: NAD; Gene names synonyms:C3orf13, IL1R3
84_EC	Q01638	IL1RL1	M	3.2.2.6	**Interleukin-1 receptor-like 1** (EC 3.2.2.6)	(Alternative name: Protein ST2)	EC Number updated; Molecular function: Hydrolase, Receptor; Ligand: NAD; Gene names synonyms:DER4, ST2, T1
85_EC	Q9HB29	IL1RL2	M	3.2.2.6	**Interleukin-1 receptor-like 2** (EC 3.2.2.6)	(Alternative names: IL-36 receptor - Short name: IL-36R; Interleukin-1 receptor-related protein 2 - Short names: IL-1Rrp2/IL1R-rp2)	EC Number updated; Molecular function: Hydrolase, Receptor; Biological process: Immunity, Inflammatory response, Innate immunity; Ligand: NAD; Gene names synonyms:IL1RRP2
86_EC	P51617	IRAK1	M	2.7.11.1	**Interleukin-1 receptor-associated kinase 1** (EC 2.7.11.1) - Short name: IRAK-1	0	Gene names synonyms:IRAK
87_EC	Q9Y616	IRAK3	M	2.7.11.1	**Interleukin-1 receptor-associated kinase 3** (EC 2.7.11.1) - Short name: IRAK-3	(Alternative name: IL-1 receptor-associated kinase M -Short name: IRAK-M)	0
88_EC	P08519	LPA	M	3.4.21.-	**Apolipoprotein(a)** (EC 3.4.21.-) - Short names: Apo(a)/Lp(a)	0	0
89_EC	P29376	LTK	M	2.7.10.1	**Leukocyte tyrosine kinase receptor** (EC 2.7.10.1)	(Alternative name: Protein tyrosine kinase 1)	Gene names synonyms:TYK1
90_EC	Q02750	MAP2K1	M	2.7.12.2	**Dual specificity mitogen-activated protein kinase kinase 1** (EC 2.7.12.2) - Short names: MAP kinase kinase 1/MAPKK 1/MKK1	(Alternative names: ERK activator kinase 1; MAPK/ERK kinase 1 - Short name: MEK 1)	Gene names synonyms:MEK1, PRKMK1
91_EC	P46734	MAP2K3	M	2.7.12.2	**Dual specificity mitogen-activated protein kinase kinase 3** (EC 2.7.12.2) - Short names: MAP kinase kinase 3/MAPKK 3	(Alternative names: MAPK/ERK kinase 3 - Short name: MEK 3; Stress-activated protein kinase kinase 2 - Short names: SAPK kinase 2/SAPKK-2/SAPKK2)	Gene names synonyms:MEK3, MKK3, PRKMK3, SKK2
92_EC	Q99558	MAP3K14	M	2.7.11.25	**Mitogen-activated protein kinase kinase kinase 14** (EC 2.7.11.25)	(Alternative names: NF-kappa-beta-inducing kinase - Short name: HsNIK; Serine/threonine-protein kinase NIK)	Gene names synonyms:NIK
93_EC	P41279	MAP3K8	M	2.7.11.25	**Mitogen-activated protein kinase kinase kinase 8** (EC 2.7.11.25)	(Alternative names: Cancer Osaka thyroid oncogene; Proto-oncogene c-Cot; Serine/threonine-protein kinase cot; Tumor progression locus 2 - Short name: TPL-2)	Gene names synonyms:COT, ESTF
94_EC	Q15759	MAPK11	M	2.7.11.24	**Mitogen-activated protein kinase 11** (EC 2.7.11.24) - Short	(Alternative names: Mitogen-activated protein kinase p38 beta - Short names: MAP kinase p38	Gene names synonyms:PRKM11, SAPK2, SAPK2B

					names: MAP kinase 11/MAPK 11	beta/p38b; Stress-activated protein kinase 2b - Short name: SAPK2b; p38-2)	
95_EC	P53778	MAPK12	M	2.7.11.24	**Mitogen-activated protein kinase 12** (EC 2.7.11.24) - Short names: MAP kinase 12/MAPK 12	(Alternative names: Extracellular signal-regulated kinase 6 - Short name: ERK-6; Mitogen-activated protein kinase p38 gamma - Short name: MAP kinase p38 gamma; Stress-activated protein kinase 3)	Gene names synonyms:ERK6, SAPK3
96_EC	Q13164	MAPK7	M	2.7.11.24	**Mitogen-activated protein kinase 7** (EC 2.7.11.24) - Short names: MAP kinase 7/MAPK 7	(Alternative names Big MAP kinase 1 - Short name: BMK-1; Extracellular signal-regulated kinase 5 - Short name: ERK-5)	Gene names synonyms:BMK1, ERK5, PRKM7
97_EC	P49137	MAPKAPK 2	M	2.7.11.1	**MAP kinase-activated protein kinase 2** (EC 2.7.11.1) - Short names: MAPK-activated protein kinase 2/MAPKAP kinase 2/MAPKAP-K2/MAPKAPK-2/MK-2/MK2)	0	0
98_EC	P14174	MIF	M	5.3.2.1	**Macrophage migration inhibitory factor** (EC 5.3.2.1) - Short name: MIF	(Alternative names: Glycosylation-inhibiting factor - Short name: GIF; L-dopachrome isomerase; Phenylpyruvate tautomerase)	Gene names synonyms:GLIF, MMIF
99_EC	P14174	MIF	M	5.3.3.12	**L-dopachrome tautomerase** (EC 5.3.3.12)	(Alternative names: Glycosylation-inhibiting factor - Short name: GIF; L-dopachrome isomerase; Phenylpyruvate tautomerase)	Gene names synonyms:GLIF, MMIF
100_EC	P03956	MMP1	M	3.4.24.7	**Interstitial collagenase** (EC 3.4.24.7)	(Alternative names: Fibroblast collagenase; Matrix metalloproteinase-1 - Short name: MMP-1) [Cleaved into the following 2 chains: 22 kDa interstitial collagenase; 27 kDa interstitial collagenase]	Gene names synonyms:CLG
101_EC	P09237	MMP7	M	3.4.24.23	**Matrilysin** (EC 3.4.24.23)	(Alternative names: Matrin; Matrix metalloproteinase-7 - Short name: MMP-7; Pump-1 protease; Uterine metalloproteinase)	Gene names synonyms:MPSL1, PUMP1
102_EC	P14780	MMP9	M	3.4.24.35	**Matrix metalloproteinase-9** (EC 3.4.24.35) - Short name: MMP-9	(Alternative names: 92 kDa gelatinase; 92 kDa type IV collagenase; Gelatinase B - Short name: GELB) [Cleaved into the following 2 chains: 67 kDa matrix metalloproteinase-9; 82 kDa matrix metalloproteinase-9]	Gene names synonyms:CLG4B
103_EC	P05164	MPO	M	1.11.2.2	**Myeloperoxidase** (EC 1.11.2.2) - Short name: MPO	[Cleaved into the following 5 chains: Myeloperoxidase; 89 kDa myeloperoxidase; 84 kDa myeloperoxidase; Myeloperoxidase light chain; Myeloperoxidase heavy chain]	0

104_EC	Q32MK0	MYLK3	M	2.7.11.18	**Myosin light chain kinase 3** (EC 2.7.11.18)	(Alternative name: Cardiac-MyBP-C-associated Ca/CaM kinase- Short name: Cardiac-MLCK)	Gene names synonyms:MLCK
105_EC	P43490	NAMPT	M	2.4.2.12	**Nicotinamide phosphoribosyltransferase** (EC 2.4.2.12) - Short names: NAmPRTase/Nampt	(Alternative names: Pre-B-cell colony-enhancing factor 1 - Short name: Pre-B cell-enhancing factor; Visfatin)	Gene names synonyms:PBEF, PBEF1
106_EC	P35228	NOS2	M	1.14.13.39	**Nitric oxide synthase, inducible** (EC 1.14.13.39)	(Alternative names: Hepatocyte NOS - Short name: HEP-NOS; Inducible NO synthase - Short name: Inducible NOS/iNOS; NOS type II; Peptidyl-cysteine S-nitrosylase NOS2)	Gene names synonyms:NOS2A
107_EC	P29474	NOS3	M	1.14.13.39	**Nitric oxide synthase, endothelial** (EC 1.14.13.39)	(Alternative names: Constitutive NOS - Short name: cNOS; EC-NOS; Endothelial NOS - Short name: eNOS; NOS type III - Short name: NOSIII)	0
108_EC	Q8NBP7	PCSK9	M	3.4.21.-	**Proprotein convertase subtilisin/kexin type 9** (EC 3.4.21.-)	(Alternative names: Neural apoptosis-regulated convertase 1 - Short name: NARC-1; Proprotein convertase 9 - Short name: PC9; Subtilisin/kexin-like protease PC9)	Gene names synonyms:NARC1
109_EC	P11309	PIM1	M	2.7.11.1	**Serine/threonine-protein kinase pim-1** (EC 2.7.11.1)	0	0
110_EC	P30613	PKLR	M	2.7.1.40	**Pyruvate kinase PKLR** (EC 2.7.1.40)	(Alternative names: Pyruvate kinase 1; Pyruvate kinase isozymes L/R; R-type/L-type pyruvate kinase; Red cell/liver pyruvate kinase)	Gene names synonyms:PK1, PKL
111_EC	P00750	PLAT	M	3.4.21.68	**Tissue-type plasminogen activator** (EC 3.4.21.68) - Short names: t-PA/t-plasminogen activator/tPA	(Alternative names: INN: Alteplase; INN: Reteplase) [Cleaved into the following 2 chains: Tissue-type plasminogen activator chain A; Tissue-type plasminogen activator chain B]	0
112_EC	P00747	PLG	M	3.4.21.7	**Plasminogen** (EC 3.4.21.7)	[Cleaved into the following 5 chains: Plasmin heavy chain A; Activation peptide; Angiostatin; Plasmin heavy chain A, short form; Plasmin light chain B]	0
113_EC	P53350	PLK1	M	2.7.11.21	**Serine/threonine-protein kinase PLK1** (EC 2.7.11.21)	(Alternative names: Polo-like kinase 1 - Short name: PLK-1; Serine/threonine-protein kinase 13 - Short name: STPK13)	Gene names synonyms:PLK
114_EC	Q8N490	PNKD	M	3.-.-.-	**Probable hydrolase PNKD** (EC 3.-.-.-)	(Alternative names: Myofibrillogenesis regulator 1 - Short name: MR-1; Paroxysmal nonkinesiogenic dyskinesia protein; Trans-activated by hepatitis C virus core protein 2)	Gene names synonyms:KIAA1184, MR1, TAHCCP2
115_EC	P30405	PPIF	M	5.2.1.8	**Peptidyl-prolyl cis-trans isomerase F, mitochondrial**	(Alternative names: Cyclophilin D - Short names: CyP-D/CypD; Cyclophilin F;	Gene names synonyms:CYP3

					(EC 5.2.1.8) - Short name: PPIase F	Mitochondrial cyclophilin - Short name: CyP-M; Rotamase F)	
116_EC	P62140	PPP1CB	M	3.1.3.16	**Serine/threonine-protein phosphatase PP1-beta catalytic subunit** (EC 3.1.3.16) - Short name: PP-1B; PPP1CD	0	0
117_EC	P62140	PPP1CB	M	3.1.3.53	**Serine/threonine-protein phosphatase PP1-beta catalytic subunit** (EC 3.1.3.53) - Short name: PP-1B; PPP1CD	0	0
118_EC	P22612	PRKACG	M	2.7.11.11	**cAMP-dependent protein kinase catalytic subunit gamma** (EC 2.7.11.11) - Short name: PKA C-gamma	0	0
119_EC	Q02156	PRKCE	M	2.7.11.13	**Protein kinase C epsilon type** (EC 2.7.11.13)	(Alternative name: nPKC-epsilon)	Gene names synonyms:PKCE
120_EC	P05129	PRKCG	M	2.7.11.13	**Protein kinase C gamma type** (EC 2.7.11.13) - Short name: PKC-gamma	0	Gene names synonyms:PKCG
121_EC	Q04759	PRKCQ	M	2.7.11.13	**Protein kinase C theta type** (EC 2.7.11.13)	(Alternative name: nPKC-theta)	Gene names synonyms:PRKCT
122_EC	Q05513	PRKCZ	M	2.7.11.13	**Protein kinase C zeta type** (EC 2.7.11.13)	(Alternative name: nPKC-zeta)	Gene names synonyms:PKC2
123_EC	Q14289	PTK2B	M	2.7.10.2	**Protein-tyrosine kinase 2-beta** (EC 2.7.10.2)	(Alternative names: Calcium-dependent tyrosine kinase - Short name: CADTK; Calcium-regulated non-receptor proline-rich tyrosine kinase; Cell adhesion kinase beta - Short names: CAK-beta/CAKB; Focal adhesion kinase 2 - Short name: FADK 2; Proline-rich tyrosine kinase 2; Related adhesion focal tyrosine kinase - Short name: RAFTK)	Gene names synonyms:FAK2, PYK2, RAFTK
124_EC	P18031	PTPN1	M	3.1.3.48	**Tyrosine-protein phosphatase non-receptor type 1** (EC 3.1.3.48)	(Alternative name: Protein-tyrosine phosphatase 1B - Short name: PTP-1B)	Gene names synonyms:PTP1B
125_EC	P12724	RNASE3	M	3.1.27.-	**Eosinophil cationic protein** (EC 3.1.27.-)- Short name: ECP	(Alternative name: Ribonuclease 3 - Short name: RNase 3)	Gene names synonyms:ECP, RNS3
126_EC	P31350	RRM2	M	1.17.4.1	**Ribonucleoside-diphosphate reductase subunit M2** (EC 1.17.4.1)	(Alternative names: Ribonucleotide reductase small chain; Ribonucleotide reductase small subunit)	Gene names synonyms:RR2

127_EC	Q9NRC8	SIRT7	M	2.3.1.286	**NAD-dependent protein deacetylase sirtuin-7** (EC 2.3.1.286)	(Alternative name: Regulatory protein SIR2 homolog 7; SIR2-like protein 7)	EC Numbers updated and protein names updated; Gene names synonyms:SIR2L7
128_EC	Q9NRC8	SIRT7	M	2.3.1.-	NAD-dependent protein deacylase sirtuin-7 (EC:2.3.1.-)	(Alternative name: Regulatory protein SIR2 homolog 7; SIR2-like protein 7)	EC Numbers updated and protein names updated; Gene names synonyms:SIR2L7
129_EC	Q9Y2P5	SLC27A5	M	6.2.1.7	**Bile acyl-CoA synthetase** (EC 6.2.1.7) - Short name: BACS	(Alternative names: Bile acid-CoA ligase - Short names: BA-CoA ligase/BAL; Cholate--CoA ligase; Fatty acid transport protein 5 - Short name: FATP-5; Fatty-acid-coenzyme A ligase, very long-chain 3; Solute carrier family 27 member 5; Very long-chain acyl-CoA synthetase homolog 2 - Short names: VLCS-H2/VLCSH2; Very long-chain acyl-CoA synthetase-related protein - Short names: VLACS-related/VLACSR)	Gene names synonyms:ACSB, ACSVL6, FACVL3, FATP5
130_EC	P17405	SMPD1	M	3.1.4.12	**Sphingomyelin phosphodiesterase** (EC 3.1.4.12)	(Alternative name: Acid sphingomyelinase - Short name: aSMase)	Gene names synonyms:ASM
131_EC	P04179	SOD2	M	1.15.1.1	**Superoxide dismutase [Mn], mitochondrial** (EC 1.15.1.1)	0	0
132_EC	Q8IWU5	SULF2	M	3.1.6.-	**Extracellular sulfatase Sulf-2** (EC 3.1.6.-) - Short name: hSulf-2	0	Gene names synonyms:KIAA1247
133_EC	Q06520	SULT2A1	M	2.8.2.14	**Bile salt sulfotransferase** (EC 2.8.2.14)	(Alternative names: Dehydroepiandrosterone sulfotransferase - Short name: DHEA-ST; Hydroxysteroid Sulfotransferase - Short name: HAST; ST2; ST2A3; Sulfotransferase 2A1 - Short name: ST2A1)	Gene names synonyms:HST, STD
134_EC	O14746	TERT	M	2.7.7.49	**Telomerase reverse transcriptase** (EC 2.7.7.49)	(Alternative names: HEST2; Telomerase catalytic subunit; Telomerase-associated protein 2 - Short name: TP2)	Gene names synonyms:EST2, TCS1, TRT
135_EC	P37173	TGFBR2	M	2.7.11.30	**TGF-beta receptor type-2** (EC 2.7.11.30) - Short name: TGFR-2	(Alternative names: TGF-beta type II receptor; Transforming growth factor-beta receptor type II - Short name: TGF-beta receptor type II/TbetaR-II)	0
136_EC	O60603	TLR2	M	3.2.2.6	**Toll-like receptor 2** (EC 3.2.2.6)	(Alternative names: Toll/interleukin-1 receptor-like protein 4; CD_antigen: CD282)	EC Number updated; Molecular function: Hydrolase, Receptor; Biological process: Immunity, Inflammatory response, Innate

							immunity; Ligand: NAD; Gene names synonyms:TIL4
137_EC	P21580	TNFAIP3	M	2.3.2.-	**Tumor necrosis factor alpha-induced protein 3** (EC 2.3.2.-) - Short name: TNF alpha-induced protein 3;	(Alternative names: OTU domain-containing protein 7C; Putative DNA-binding protein A20; Zinc finger protein A20) [Cleaved into the following 2 chains: A20p50; A20p37]	EC Number updated; Gene names synonyms:OTUD7C
138_EC	P21580	TNFAIP3	M	3.4.19.12	**Tumor necrosis factor alpha-induced protein 3** (EC 3.4.19.12) - Short name: TNF alpha-induced protein 3;	(Alternative names: OTU domain-containing protein 7C; Putative DNA-binding protein A20; Zinc finger protein A20) [Cleaved into the following 2 chains: A20p50; A20p37]	EC Number updated; Gene names synonyms:OTUD7C
139_EC	Q12933	TRAF2	M	2.3.2.27	**TNF receptor-associated factor 2** (EC 2.3.2.27)	(Alternative names: E3 ubiquitin-protein ligase TRAF2; RING-type E3 ubiquitin transferase TRAF2; Tumor necrosis factor type 2 receptor-associated protein 3)	Gene names synonyms:TRAP3
140_EC	Q969Q1	TRIM63	M	2.3.2.27	**E3 ubiquitin-protein ligase TRIM63** (EC 2.3.2.27)	(Alternative names: Iris RING finger protein; Muscle-specific RING finger protein 1 - Short names: MuRF-1/MuRF1; RING finger protein 28; RING-type E3 ubiquitin transferase TRIM63; Striated muscle RING zinc finger protein; Tripartite motif-containing protein 63)	Gene names synonyms:IRF, MURF1, RNF28, SMRZ
141_EC	P04818	TYMS	M	2.1.1.45	**Thymidylate synthase** (EC 2.1.1.45) - Short names: TS/TSase	0	Gene names synonyms:TS
142_EC	Q16739	UGCG	M	2.4.1.80	**Ceramide glucosyltransferase** (EC 2.4.1.80)	(Alternative names: GLCT-1; Glucosylceramide synthase - Short name: GCS; UDP-glucose ceramide glucosyltransferase; UDP-glucose:N-acylsphingosine D-glucosyltransferase)	0
143_EC	O75604	USP2	M	3.4.19.12	**Ubiquitin carboxyl-terminal hydrolase 2** (EC 3.4.19.12)	(Alternative names: 41 kDa ubiquitin-specific protease; Deubiquitinating enzyme 2; Ubiquitin thioesterase 2; Ubiquitin-specific-processing protease 2)	Gene names synonyms:UBP41
144_EC	P47989	XDH	M	1.17.1.4	Xanthine dehydrogenase (EC 1.17.1.4) - Short name: XD	**Xanthine dehydrogenase/oxidase** [Including the following 2 domains: Xanthine dehydrogenase (EC 1.17.1.4) - Short name: XD; Xanthine oxidase (EC 1.17.3.2) - Short name: XO]; (Alternative names: Xanthine oxidoreductase - Short name: XOR)	Gene names synonyms:XDHA
145_EC	P47989	XDH	M	1.17.3.2	Xanthine oxidase (EC 1.17.3.2) - Short name: XO	**Xanthine dehydrogenase/oxidase** [Including the following 2 domains: Xanthine	Gene names synonyms:XDHA

						dehydrogenase (EC 1.17.1.4) - Short name: XD; Xanthine oxidase (EC 1.17.3.2) - Short name: XO]; (Alternative names: Xanthine oxidoreductase - Short name: XOR)	
146_EC	P09110	ACAA1	M	2.3.1.16	**3-ketoacyl-CoA thiolase, peroxisomal** (EC 2.3.1.16)	(Alternative names: Acetyl-CoA acyltransferase; Beta-ketothiolase; Peroxisomal 3-oxoacyl-CoA thiolase)	Gene names synonyms:ACAA, PTHIO
147_EC	P42765	ACAA2	M	2.3.1.16	**3-ketoacyl-CoA thiolase, mitochondrial** (EC 2.3.1.16)	(Alternative names: Beta-ketothiolase ;Mitochondrial 3-oxoacyl-CoA thiolase ;T1)	EC Numbers updated and Protein names updated
148_EC	P42765	ACAA2	M	2.3.1.9	Acetyl-CoA acetyltransferase (EC:2.3.1.9)	(Alternative names: Beta-ketothiolase ;Mitochondrial 3-oxoacyl-CoA thiolase ;T1)	EC Numbers updated and Protein names updated
149_EC	P42765	ACAA2	M	3.1.2.-	Acetyl-CoA acyltransferase; Acyl-CoA hydrolase, mitochondrial (EC 3.1.2.-)	(Alternative names: Beta-ketothiolase ;Mitochondrial 3-oxoacyl-CoA thiolase ;T1)	EC Numbers updated and Protein names updated
150_EC	P42765	ACAA2	M	3.1.2.1	Acetyl-CoA acyltransferase; Acyl-CoA hydrolase, mitochondrial (EC 3.1.2.1)	(Alternative names: Beta-ketothiolase ;Mitochondrial 3-oxoacyl-CoA thiolase ;T1)	EC Numbers updated and Protein names updated
151_EC	P42765	ACAA2	M	3.1.2.2	Acetyl-CoA acyltransferase; Acyl-CoA hydrolase, mitochondrial (EC:3.1.2.2)	(Alternative names: Beta-ketothiolase ;Mitochondrial 3-oxoacyl-CoA thiolase ;T1)	EC Numbers updated and Protein names updated
152_EC	Q13085	ACACA	M	6.4.1.2	**Acetyl-CoA carboxylase 1** (EC 6.4.1.2) - Short name: ACC1	(Alternative name: Acetyl-Coenzyme A carboxylase alpha - Short name: ACC-alpha)	EC Number updated and protein name updated; Gene names synonyms:ACAC, ACC1, ACCA
153_EC	Q9UKU7	ACAD8	M	1.3.8.-	**Isobutyryl-CoA dehydrogenase, mitochondrial** (EC 1.3.8.-) - Short name: IBDH	(Alternative names: Activator-recruited cofactor 42 kDa component - Short name: ARC42; Acyl-CoA dehydrogenase family member 8 - Short name: ACAD-8)	EC Number updated; Gene names synonyms:ARC42, IBD
154_EC	P28330	ACADL	M	1.3.8.8	**Long-chain specific acyl-CoA dehydrogenase, mitochondrial** (EC 1.3.8.8) - Short name: LCAD	0	0
155_EC	P11310	ACADM	M	1.3.8.7	**Medium-chain specific acyl-CoA dehydrogenase, mitochondrial** (EC 1.3.8.7) - Short name: MCAD	0	0
156_EC	P16219	ACADS	M	1.3.8.1	**Short-chain specific acyl-CoA dehydrogenase, mitochondrial** (EC 1.3.8.1) - Short name: SCAD	(Alternative name: Butyryl-CoA dehydrogenase)	0

157_EC	P24752	ACAT1	M	2.3.1.9	**Acetyl-CoA acetyltransferase, mitochondrial** (EC 2.3.1.9)	(Alternative names: Acetoacetyl-CoA thiolase; T2)	Gene names synonyms:ACAT, MAT
158_EC	Q99798	ACO2	M	4.2.1.3	**Aconitate hydratase, mitochondrial** (EC 4.2.1.3) - Short name: Aconitase	(Alternative name: Citrate hydro-lyase)	0
159_EC	Q15067	ACOX1	M	1.3.3.6	**Peroxisomal acyl-coenzyme A oxidase 1** (EC 1.3.3.6) - Short name: AOX	(Alternative names: Palmitoyl-CoA oxidase; Straight-chain acyl-CoA oxidase - Short name: SCOX); [Cleaved into the following 3 chains: Peroxisomal acyl-CoA oxidase 1, A chain; Peroxisomal acyl-CoA oxidase 1, B chain; Peroxisomal acyl-CoA oxidase 1, C chain]	Protein names updated; Gene names synonyms:ACOX
160_EC	P33121	ACSL1	M	6.2.1.3	**Long-chain-fatty-acid--CoA ligase 1** (EC 6.2.1.3)	(Alternative names: Acyl-CoA synthetase 1 - Short name: ACS1; Long-chain acyl-CoA synthetase 1 - Short name: LACS 1; Long-chain acyl-CoA synthetase 2 - Short name: LACS 2; Long-chain fatty acid-CoA ligase 2; Palmitoyl-CoA ligase 1; Palmitoyl-CoA ligase 2)	EC numbers updated and Protein names updated; Gene names synonyms:FACL1, FACL2, LACS, LACS1, LACS2
161_EC	P33121	ACSL1	M	6.2.1.15	Arachidonate--CoA ligase (EC 6.2.1.15)	(Alternative names: Acyl-CoA synthetase 1 - Short name: ACS1; Long-chain acyl-CoA synthetase 1 - Short name: LACS 1; Long-chain acyl-CoA synthetase 2 - Short name: LACS 2; Long-chain fatty acid-CoA ligase 2; Palmitoyl-CoA ligase 1; Palmitoyl-CoA ligase 2)	EC numbers updated and Protein names updated; Gene names synonyms:FACL1, FACL2, LACS, LACS1, LACS2
162_EC	P33121	ACSL1	M	6.2.1.24	Phytanate--CoA ligase (EC 6.2.1.24)	(Alternative names: Acyl-CoA synthetase 1 - Short name: ACS1; Long-chain acyl-CoA synthetase 1 - Short name: LACS 1; Long-chain acyl-CoA synthetase 2 - Short name: LACS 2; Long-chain fatty acid-CoA ligase 2; Palmitoyl-CoA ligase 1; Palmitoyl-CoA ligase 2)	EC numbers updated and Protein names updated; Gene names synonyms:FACL1, FACL2, LACS, LACS1, LACS2
163_EC	P07327	ADH1A	M	1.1.1.1	**Alcohol dehydrogenase 1A** (EC 1.1.1.1)	(Alternative name: Alcohol dehydrogenase subunit alpha)	Gene names synonyms:ADH1
164_EC	P00325	ADH1B	M	1.1.1.105	**All-trans-retinol dehydrogenase [NAD(+)] ADH1B** (EC 1.1.1.105)	(Alternative names: Alcohol dehydrogenase 1B; Alcohol dehydrogenase subunit beta)	EC number updated and protein name updated; Gene names synonyms:ADH2
165_EC	P00326	ADH1C	M	1.1.1.1	**Alcohol dehydrogenase 1C** (EC 1.1.1.1)	(Alternative name: Alcohol dehydrogenase subunit gamma)	Gene names synonyms:ADH3
166_EC	Q8IWW8	ADHFE1	M	1.1.99.24	**Hydroxyacid-oxoacid transhydrogenase,**	(Alternative names: Alcohol dehydrogenase iron-containing protein 1 - Short name: ADHFe1; Fe-containing alcohol dehydrogenase)	0

					mitochondrial (EC 1.1.99.24) - Short name: HOT		
167_EC	O95831	AIFM1	M	1.6.99.-	**Apoptosis-inducing factor 1, mitochondrial** (EC 1.6.99.-)	(Alternative name: Programmed cell death protein 8)	EC number updated; Gene names synonyms:AIF, PDCD8
168_EC	P31751	AKT2	M	2.7.11.1	**RAC-beta serine/threonine-protein kinase** (EC 2.7.11.1)	(Alternative names: Protein kinase Akt-2; Protein kinase B beta - Short name: PKB beta; RAC-PK-beta)	0
169_EC	P00352	ALDH1A1	M	1.2.1.-	**Retinal dehydrogenase 1** (EC 1.2.1.-) - Short names: RALDH 1/RalDH1;	(Alternative names: ALDH-E1; ALHDII; Aldehyde dehydrogenase family 1 member A1; Aldehyde dehydrogenase, cytosolic)	Gene names synonyms:ALDC, ALDH1, PUMB1
170_EC	P00352	ALDH1A1	M	1.2.1.36	**Retinal dehydrogenase 1** (EC 1.2.1.36) - Short names: RALDH 1/RalDH1;	(Alternative names: ALDH-E1; ALHDII; Aldehyde dehydrogenase family 1 member A1; Aldehyde dehydrogenase, cytosolic)	Gene names synonyms:ALDC, ALDH1, PUMB1
171_EC	Q3SY69	ALDH1L2	M	1.5.1.6	**Mitochondrial 10-formyltetrahydrofolate dehydrogenase** (EC 1.5.1.6) - Short names: Mitochondrial 10-FTHFDH/mtFDH	(Alternative name: Aldehyde dehydrogenase family 1 member L2)	0
172_EC	P05091	ALDH2	M	1.2.1.3	**Aldehyde dehydrogenase, mitochondrial** (EC 1.2.1.3)	(Alternative names: ALDH class 2; ALDH-E2; ALDHI)	Gene names synonyms:ALDM
173_EC	P51648	ALDH3A2	M	1.2.1.3	**Aldehyde dehydrogenase family 3 member A2** (EC 1.2.1.3)	(Alternative names: Aldehyde dehydrogenase 10; Fatty aldehyde dehydrogenase; Microsomal aldehyde dehydrogenase)	EC Number updated and protein name updated; Gene names synonyms:ALDH10, FALDH
174_EC	P51648	ALDH3A2	M	1.2.1.94	**Aldehyde dehydrogenase family 3 member A2** (EC 1.2.1.94)	(Alternative names: Aldehyde dehydrogenase 10; Fatty aldehyde dehydrogenase; Microsomal aldehyde dehydrogenase)	EC Number updated and protein name updated; Gene names synonyms:ALDH10, FALDH
175_EC	P51649	ALDH5A1	M	1.2.1.24	**Succinate-semialdehyde dehydrogenase, mitochondrial** (EC 1.2.1.24)	(Alternative names: Aldehyde dehydrogenase family 5 member A1; NAD(+)-dependent succinic semialdehyde dehydrogenase)	Gene names synonyms:SSADH
176_EC	Q02252	ALDH6A1	M	1.2.1.18	**Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial** (EC 1.2.1.18) - Short names: MMSDH/Malonate-semialdehyde dehydrogenase [acylating]	(Alternative name: Aldehyde dehydrogenase family 6 member A1)	Gene names synonyms:MMSDH
177_EC	Q02252	ALDH6A1	M	1.2.1.27	**Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial** (EC	(Alternative name: Aldehyde dehydrogenase family 6 member A1)	Gene names synonyms:MMSDH

					1.2.1.27) - Short names: MMSDH/Malonate-semialdehyde dehydrogenase [acylating]		
178_EC	P49419	ALDH7A1	M	1.2.1.31	**Alpha-aminoadipic semialdehyde dehydrogenase (EC 1.2.1.31)** - Short name: Alpha-AASA dehydrogenase	(Alternative names: Antiquitin-1; Delta1-piperideine-6-carboxylate dehydrogenase - Short name: P6c dehydrogenase)	Gene names synonyms:ATQ1
179_EC	P49419	ALDH7A1	M	1.2.1.3	Aldehyde dehydrogenase family 7 member A1 (EC 1.2.1.3)	(Alternative names: Antiquitin-1; Delta1-piperideine-6-carboxylate dehydrogenase - Short name: P6c dehydrogenase)	Gene names synonyms:ATQ1
180_EC	P49419	ALDH7A1	M	1.2.1.8	Betaine aldehyde dehydrogenase (EC 1.2.1.8)	(Alternative names: Antiquitin-1; Delta1-piperideine-6-carboxylate dehydrogenase - Short name: P6c dehydrogenase)	Gene names synonyms:ATQ1
181_EC	P49189	ALDH9A1	M	1.2.1.47	**4-trimethylaminobutyraldehyde dehydrogenase** (EC 1.2.1.47) - Short names: TMABA-DH/TMABALDH	(Alternative names: Aldehyde dehydrogenase E3 isozyme; R-aminobutyraldehyde dehydrogenase); [Cleaved into the following chain: 4-trimethylaminobutyraldehyde dehydrogenase, N-terminally processed]	Gene names synonyms:ALDH4, ALDH7, ALDH9
182_EC	P49189	ALDH9A1	M	1.2.1.3	Aldehyde dehydrogenase family 9 member A1 (EC 1.2.1.3)	(Alternative names: Aldehyde dehydrogenase E3 isozyme; R-aminobutyraldehyde dehydrogenase); [Cleaved into the following chain: 4-trimethylaminobutyraldehyde dehydrogenase, N-terminally processed]	Gene names synonyms:ALDH4, ALDH7, ALDH9
183_EC	P49189	ALDH9A1	M	1.2.1.19	Gamma-aminobutyraldehyde dehydrogenase (EC 1.2.1.19)	(Alternative names: Aldehyde dehydrogenase E3 isozyme; R-aminobutyraldehyde dehydrogenase); [Cleaved into the following chain: 4-trimethylaminobutyraldehyde dehydrogenase, N-terminally processed]	Gene names synonyms:ALDH4, ALDH7, ALDH9
184_EC	Q13315	ATM	M	2.7.11.1	**Serine-protein kinase ATM** (EC 2.7.11.1)	(Alternative name: Ataxia telangiectasia mutated - Short name: A-T mutated)	0
185_EC	P06576	ATP5B	M	7.1.2.2	**ATP synthase subunit beta, mitochondrial** (EC 7.1.2.2)	(Alternative name: ATP synthase F1 subunit beta)	EC Number updated and protein name updated; Gene name: ATP5F1B; Gene names Synonyms:ATP5B, ATPMB, ATPSB
186_EC	Q13825	AUH	M	4.2.1.18	**Methylglutaconyl-CoA hydratase, mitochondrial** (EC 4.2.1.18)	(Alternative name: AU-specific RNA-binding enoyl-CoA hydratase - Short name: AU-binding protein/enoyl-CoA hydratase)	EC Number updated and protein name updated

187_EC	Q13825	AUH	M	4.2.1.56	Itaconyl-CoA hydratase (EC 4.2.1.56)	(Alternative name: AU-specific RNA-binding enoyl-CoA hydratase - Short name: AU-binding protein/enoyl-CoA hydratase)	EC Number updated and protein name updated
188_EC	P55211	CASP9	M	3.4.22.62	**Caspase-9** (EC 3.4.22.62) - Short name: CASP-9	(Alternative names: Apoptotic protease Mch-6; Apoptotic protease-activating factor 3 - Short name: APAF-3; ICE-like apoptotic protease 6 - Short name: ICE-LAP6); [Cleaved into the following 2 chains: Caspase-9 subunit p35; Caspase-9 subunit p10]	Gene names synonyms:MCH6
189_EC	P04040	CAT	M	1.11.1.6	**Catalase** (EC 1.11.1.6)	0	0
190_EC	Q00534	CDK6	M	2.7.11.22	**Cyclin-dependent kinase 6** (EC 2.7.11.22)	(Alternative names: Cell division protein kinase 6; Serine/threonine-protein kinase PLSTIRE)	Gene names synonyms:CDKN6
191_EC	P23786	CPT2	M	2.3.1.21	Carnitine O-palmitoyltransferase 2, mitochondrial (EC 2.3.1.21)	(Alternative name: Carnitine palmitoyltransferase II - Short name: CPT II)	Gene names synonyms:CPT1
192_EC	P68400	CSNK2A1	M	2.7.11.1	**Casein kinase II subunit alpha** (EC 2.7.11.1) - Short name: CK II alpha	0	Gene names synonyms:CK2A1
193_EC	P19784	CSNK2A2	M	2.7.11.1	**Casein kinase II subunit alpha'** (EC 2.7.11.1) - Short name: CK II alpha'	0	Gene names synonyms:CK2A2
194_EC	P08574	CYC1	M	7.1.1.8	**Cytochrome c1, heme protein, mitochondrial** (EC 7.1.1.8)	(Alternative names: Complex III subunit 4; Complex III subunit IV; Cytochrome b-c1 complex subunit 4; Ubiquinol-cytochrome-c reductase complex cytochrome c1 subunit - Short name: Cytochrome c-1)	EC Number updated; Molecular function: Translocase; Biological process: Electron transport, Respiratory chain, Transport; Ligand: Heme, Iron, Metal-binding
195_EC	P33260	CYP2C18	M	1.14.14.1	**Cytochrome P450 2C18** (EC 1.14.14.1)	(Alternative names: CYPIIC18; Cytochrome P450-6b/29c)	0
196_EC	P08684	CYP3A4	M	1.14.14.1	**Cytochrome P450 3A4** (EC 1.14.14.1)	(Alternative names: 1,4-cineole 2-exo-monooxygenase; Albendazole sulfoxidase; CYPIIIA3; CYPIIIA4; Cholesterol 25-hydroxylase; Cytochrome P450 3A3; Cytochrome P450 HLp; Cytochrome P450 NF-25; Cytochrome P450-PCN1; Nifedipine oxidase)	Gene names synonyms:CYP3A3
197_EC	P08684	CYP3A4	M	1.14.14.56	1,8-cineole 2-exo-monooxygenase (EC 1.14.14.56)	(Alternative names: 1,4-cineole 2-exo-monooxygenase; Albendazole sulfoxidase; CYPIIIA3; CYPIIIA4; Cholesterol 25-hydroxylase; Cytochrome P450 3A3; Cytochrome P450 HLp;	Gene names synonyms:CYP3A3

						Cytochrome P450 NF-25; Cytochrome P450-PCN1; Nifedipine oxidase)	
198_EC	P08684	CYP3A4	M	1.14.14.73	Albendazole monooxygenase (sulfoxide-forming) (EC 1.14.14.73)	(Alternative names: 1,4-cineole 2-exo-monooxygenase; Albendazole sulfoxidase; CYPIIIA3; CYPIIIA4; Cholesterol 25-hydroxylase; Cytochrome P450 3A3; Cytochrome P450 HLp; Cytochrome P450 NF-25; Cytochrome P450-PCN1; Nifedipine oxidase)	Gene names synonyms:CYP3A3
199_EC	P08684	CYP3A4	M	1.14.14.55	Quinine 3-monooxygenase (EC 1.14.14.55)	(Alternative names: 1,4-cineole 2-exo-monooxygenase; Albendazole sulfoxidase; CYPIIIA3; CYPIIIA4; Cholesterol 25-hydroxylase; Cytochrome P450 3A3; Cytochrome P450 HLp; Cytochrome P450 NF-25; Cytochrome P450-PCN1; Nifedipine oxidase)	Gene names synonyms:CYP3A3
200_EC	Q9HB55	CYP3A43	M	1.14.14.1	Cytochrome P450 3A43 (EC 1.14.14.1)	0	0
201_EC	P40126	DCT	M	5.3.3.12	**L-dopachrome tautomerase** (EC 5.3.3.12) - Short names: DCT/DT	(Alternative names: L-dopachrome Delta-isomerase; Tyrosinase-related protein 2 - Short names: TRP-2/TRP2)	Gene names synonyms:TYRP2
202_EC	P00374	DHFR	M	1.5.1.3	**Dihydrofolate reductase** (EC 1.5.1.3)	0	0
203_EC	Q13011	ECH1	M	5.3.3.-	**Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial** (EC 5.3.3.-)	0	0
204_EC	P30084	ECHS1	M	4.2.1.17	**Enoyl-CoA hydratase, mitochondrial** (EC 4.2.1.17)	(Alternative names: Enoyl-CoA hydratase 1; Short-chain enoyl-CoA hydratase - Short name: SCEH)	0
205_EC	O75521	ECI2	M	5.3.3.8	**Enoyl-CoA delta isomerase 2** (EC 5.3.3.8)	(Alternative names: DRS-1; Delta(3),delta(2)-enoyl-CoA isomerase - Short name: D3,D2-enoyl-CoA isomerase; Diazepam-binding inhibitor-related protein 1 - Short name: DBI-related protein 1; Dodecenoyl-CoA isomerase; Hepatocellular carcinoma-associated antigen 88; Peroxisomal 3,2-trans-enoyl-CoA isomerase - Short name: pECI; Renal carcinoma antigen NY-REN-1)	Gene names synonyms:DRS1, HCA88, PECI
206_EC	P19447	ERCC3	M	3.6.4.12	**General transcription and DNA repair factor IIH helicase**	(Alternative names: Basic transcription factor 2 89 kDa subunit - Short name: BTF2 p89; DNA excision repair protein ERCC-3; DNA repair	Protein names updated; Gene names synonyms:XPB, XPBC

					subunit XPB (EC 3.6.4.12) - Short name: TFIIH subunit XPB	protein complementing XP-B cells; TFIIH basal transcription factor complex 89 kDa subunit - Short names: TFIIH 89 kDa subunit/TFIIH p89; Xeroderma pigmentosum group B-complementing protein)	
207_EC	P00742	F10	M	3.4.21.6	**Coagulation factor X** (EC 3.4.21.6)	(Alternative names: Stuart factor; Stuart-Prower factor); [Cleaved into the following 3 chains: Factor X light chain; Factor X heavy chain; Activated factor Xa heavy chain]	0
208_EC	Q6IB77	GLYAT	M	2.3.1.13	**Glycine N-acyltransferase** (EC 2.3.1.13)	(Alternative names: Acyl-CoA:glycine N-acyltransferase - Short name: AAc; Aralkyl acyl-CoA N-acyltransferase; Aralkyl acyl-CoA:amino acid N-acyltransferase; Benzoyl-coenzyme A:glycine N-acyltransferase; HRP-1(CLP))	Gene names synonyms:ACGNAT, CAT, GAT
209_EC	Q6IB77	GLYAT	M	2.3.1.71	Glycine N-benzoyltransferase (EC 2.3.1.71)	(Alternative names: Acyl-CoA:glycine N-acyltransferase - Short name: AAc; Aralkyl acyl-CoA N-acyltransferase; Aralkyl acyl-CoA:amino acid N-acyltransferase; Benzoyl-coenzyme A:glycine N-acyltransferase; HRP-1(CLP))	Gene names synonyms:ACGNAT, CAT, GAT
210_EC	P43304	GPD2	M	1.1.5.3	**Glycerol-3-phosphate dehydrogenase, mitochondrial** (EC 1.1.5.3) - Short names: GPD-M/GPDH-M)	(Alternative names: mitohondrial glycerophosphate dehydrogenase gene - Short name: mGDH; mtGPD)	0
211_EC	P36969	GPX4	M	1.11.1.12	**Phospholipid hydroperoxide glutathione peroxidase** (EC 1.11.1.12) - Short name: PHGPx	(Alternative name: Glutathione peroxidase 4 - Short names: GPx-4/GSHPx-4)	0
212_EC	Q96SL4	GPX7	M	1.11.1.9	**Glutathione peroxidase 7** (EC 1.11.1.9) - Short names: GPx-7/GSHPx-7	(Alternative name: CL683)	Gene names synonyms:GPX6
213_EC	O15217	GSTA4	M	2.5.1.18	**Glutathione S-transferase A4** (EC 2.5.1.18)	(Alternative names: GST class-alpha member 4; Glutathione S-transferase A4-4)	0
214_EC	Q9Y2Q3	GSTK1	M	2.5.1.18	**Glutathione S-transferase kappa 1** (EC 2.5.1.18)	(Alternative names: GST 13-13; GST class-kappa; GSTK1-1 - Short name: hGSTK1; Glutathione S-transferase subunit 13)	0
215_EC	P21266	GSTM3	M	2.5.1.18	**Glutathione S-transferase Mu 3** (EC 2.5.1.18)	(Alternative names: GST class-mu 3; GSTM3-3 - Short name: hGSTM3-3)	Gene names synonyms:GST5
216_EC	Q03013	GSTM4	M	2.5.1.18	**Glutathione S-transferase Mu 4** (EC 2.5.1.18)	(Alternative names: GST class-mu 4; GST-Mu2; GSTM4-4)	EC number updated and protein name updated

217_EC	Q03013	GSTM4	M	4.4.1.20	Leukotriene C4 synthase GSTM4 (EC 4.4.1.20)	(Alternative names: GST class-mu 4; GST-Mu2; GSTM4-4)	EC number updated and protein name updated
218_EC	Q16836	HADH	M	1.1.1.35	**Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial** (EC 1.1.1.35) - Short name: HCDH	(Alternative names: Medium and short-chain L-3-hydroxyacyl-coenzyme A dehydrogenase; Short-chain 3-hydroxyacyl-CoA dehydrogenase)	Gene names synonyms:HAD, HADHSC, SCHAD
219_EC	P40939	HADHA	M	2.3.1.-	Monolysocardiolipin acyltransferase (EC:2.3.1.-)	**Trifunctional enzyme subunit alpha, mitochondrial**; (Alternative names: 78 kDa gastrin-binding protein; TP-alpha); [Including the following 2 domains: Long-chain enoyl-CoA hydratase (EC 4.2.1.17); Long chain 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211)]	EC Number updated and Protein name updated; Gene names synonyms:HADH
220_EC	P40939	HADHA	M	4.2.1.17	Long-chain enoyl-CoA hydratase (EC 4.2.1.17)	**Trifunctional enzyme subunit alpha, mitochondrial**; (Alternative names: 78 kDa gastrin-binding protein; TP-alpha); [Including the following 2 domains: Long-chain enoyl-CoA hydratase (EC 4.2.1.17); Long chain 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211)]	EC Number updated and Protein name updated; Gene names synonyms:HADH
221_EC	P40939	HADHA	M	1.1.1.211	Long chain 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211)	**Trifunctional enzyme subunit alpha, mitochondrial**; (Alternative names: 78 kDa gastrin-binding protein; TP-alpha); [Including the following 2 domains: Long-chain enoyl-CoA hydratase (EC 4.2.1.17); Long chain 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211)]	EC Number updated and Protein name updated; Gene names synonyms:HADH
222_EC	P55084	HADHB	M	2.3.1.155	3-ketoacyl-CoA thiolase (EC 2.3.1.155)	**Trifunctional enzyme subunit beta, mitochondrial**; (Alternative name: TP-beta; [Including the following 1 domains: 3-ketoacyl-CoA thiolase (EC 2.3.1.155; EC 2.3.1.16); (Alternative names: Acetyl-CoA acyltransferase; Beta-ketothiolase)]	EC number updated
223_EC	P55084	HADHB	M	2.3.1.16	3-ketoacyl-CoA thiolase (EC 2.3.1.16)	**Trifunctional enzyme subunit beta, mitochondrial**; (Alternative name: TP-beta; [Including the following 1 domains: 3-ketoacyl-CoA thiolase (EC 2.3.1.155; EC 2.3.1.16); (Alternative names: Acetyl-CoA acyltransferase; Beta-ketothiolase)]	EC number updated

224_EC	Q14527	HLTF	M	2.3.2.27	**Helicase-like transcription factor** (EC 2.3.2.27)	(Alternative names: DNA-binding protein/plasminogen activator inhibitor 1 regulator; HIP116; RING finger protein 80; RING-type E3 ubiquitin transferase HLTF; (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 3; Sucrose nonfermenting protein 2-like 3)	Gene names synonyms:HIP116A, RNF80, SMARCA3, SNF2L3, ZBU1
225_EC	Q14527	HLTF	M	3.6.4.-	**Helicase-like transcription factor** (EC 3.6.4.-)	(Alternative names: DNA-binding protein/plasminogen activator inhibitor 1 regulator; HIP116; RING finger protein 80; RING-type E3 ubiquitin transferase HLTF; (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 3; Sucrose nonfermenting protein 2-like 3)	Gene names synonyms:HIP116A, RNF80, SMARCA3, SNF2L3, ZBU1
226_EC	P51659	HSD17B4	M	1.1.1.n12	(3R)-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.n12)	**Peroxisomal multifunctional enzyme type 2** - Short name: MFE-2; (Alternative names: 17-beta-hydroxysteroid dehydrogenase 4 - Short name: 17-beta-HSD 4; D-bifunctional protein - Short name: DBP; Multifunctional protein 2 - Short name: MPF-2; Short chain dehydrogenase/reductase family 8C member 1); [Cleaved into the following 2 chains: (3R)-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.n12); Enoyl-CoA hydratase 2 (EC 4.2.1.107; EC 4.2.1.119); (Alternative name: 3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholest-24-enoyl-CoA hydratase)]	Gene names synonyms:EDH17B4, SDR8C1
227_EC	P51659	HSD17B4	M	4.2.1.107	Enoyl-CoA hydratase 2 (EC 4.2.1.107)	**Peroxisomal multifunctional enzyme type 2** - Short name: MFE-2; (Alternative names: 17-beta-hydroxysteroid dehydrogenase 4 - Short name: 17-beta-HSD 4; D-bifunctional protein - Short name: DBP; Multifunctional protein 2 - Short name: MPF-2; Short chain dehydrogenase/reductase family 8C member 1); [Cleaved into the following 2 chains: (3R)-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.n12); Enoyl-CoA hydratase 2 (EC 4.2.1.107; EC 4.2.1.119); (Alternative name: 3-	Gene names synonyms:EDH17B4, SDR8C1

						alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholest-24-enoyl-CoA hydratase)]	
228_EC	P51659	HSD17B4	M	4.2.1.119	Enoyl-CoA hydratase 2 (EC 4.2.1.119)	**Peroxisomal multifunctional enzyme type 2** - Short name: MFE-2; (Alternative names: 17-beta-hydroxysteroid dehydrogenase 4 - Short name: 17-beta-HSD 4; D-bifunctional protein - Short name: DBP; Multifunctional protein 2 - Short name: MPF-2; Short chain dehydrogenase/reductase family 8C member 1); [Cleaved into the following 2 chains: (3R)-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.n12); Enoyl-CoA hydratase 2 (EC 4.2.1.107; EC 4.2.1.119); (Alternative name: 3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholest-24-enoyl-CoA hydratase)]	Gene names synonyms:EDH17B4, SDR8C1
229_EC	P26440	IVD	M	1.3.8.4	**Isovaleryl-CoA dehydrogenase, mitochondrial** (EC 1.3.8.4) - Short name: IVD	0	EC number updated and Protein name updated
230_EC	P26440	IVD	M	1.3.8.1	Butyryl-CoA dehydrogenase (EC 1.3.8.1)	0	EC number updated and Protein name updated
231_EC	Q92831	KAT2B	M	2.3.1.48	**Histone acetyltransferase KAT2B** (EC 2.3.1.48)	(Alternative names: Histone acetyltransferase PCAF - Short name: Histone acetylase PCAF; Lysine acetyltransferase 2B; P300/CBP-associated factor - Short name: P/CAF)	EC Number updated and Protein name updated; Gene names synonyms:PCAF
232_EC	Q92831	KAT2B	M	2.3.1.57	Spermidine acetyltransferase KAT2B (EC 2.3.1.57)	(Alternative names: Histone acetyltransferase PCAF - Short name: Histone acetylase PCAF; Lysine acetyltransferase 2B; P300/CBP-associated factor - Short name: P/CAF)	EC Number updated and Protein name updated; Gene names synonyms:PCAF
233_EC	Q5S007	LRRK2	M	2.7.11.1	**Leucine-rich repeat serine/threonine-protein kinase 2** (EC 2.7.11.1)	(Alternative name: Dardarin)	EC Number updated; Gene names synonyms:PARK8
234_EC	Q5S007	LRRK2	M	3.6.5.-	**Leucine-rich repeat serine/threonine-protein kinase 2** (EC 3.6.5.-)	(Alternative name: Dardarin)	EC Number updated; Gene names synonyms:PARK8
235_EC	P27338	MAOB	M	1.4.3.4	**Amine oxidase [flavin-containing] B** (EC 1.4.3.4)	(Alternative name: Monoamine oxidase type B - Short name: MAO-B)	0
236_EC	P45985	MAP2K4	M	2.7.12.2	**Dual specificity mitogen-activated protein kinase kinase 4** (EC 2.7.12.2) - Short	(Alternative names: JNK-activating kinase 1; MAPK/ERK kinase 4 - Short names: MEK 4; SAPK/ERK kinase 1 - Short name: SEK1; Stress-	Gene names synonyms:JNKK1, MEK4, MKK4, PRKMK4, SEK1, SERK1, SKK1

					names: MAP kinase kinase 4/MAPKK 4	activated protein kinase kinase 1 - Short names: SAPK kinase 1/SAPKK-1/SAPKK1; c-Jun N-terminal kinase kinase 1 - Short name: JNKK)	
237_EC	P53779	MAPK10	M	2.7.11.24	**Mitogen-activated protein kinase 10** (EC 2.7.11.24) - Short names: MAP kinase 10/MAPK 10	(Alternative names: MAP kinase p49 3F12; Stress-activated protein kinase 1b - Short name: SAPK1b; Stress-activated protein kinase JNK3; c-Jun N-terminal kinase 3)	Gene names synonyms:JNK3, JNK3A, PRKM10, SAPK1B
238_EC	Q16539	MAPK14	M	2.7.11.24	**Mitogen-activated protein kinase 14** (EC 2.7.11.24) - Short names: MAP kinase 14/MAPK 14	(Alternative names: Cytokine suppressive anti-inflammatory drug-binding protein - Short names: CSAID-binding protein/CSBP; MAP kinase MXI2; MAX-interacting protein 2; Mitogen-activated protein kinase p38 alpha - Short name: MAP kinase p38 alpha; Stress-activated protein kinase 2a - Short name: SAPK2a)	Gene names synonyms:CSBP, CSBP1, CSBP2, CSPB1, MXI2, SAPK2A
239_EC	P45983	MAPK8	M	2.7.11.24	**Mitogen-activated protein kinase 8** (EC 2.7.11.24) - Short names: MAP kinase 8/MAPK 8	(Alternative names: JNK-46; Stress-activated protein kinase 1c - Short name: SAPK1c; Stress-activated protein kinase JNK1; c-Jun N-terminal kinase 1)	Gene names synonyms:JNK1, PRKM8, SAPK1, SAPK1C
240_EC	P45984	MAPK9	M	2.7.11.24	**Mitogen-activated protein kinase 9** (EC 2.7.11.24) - Short names: MAP kinase 9/MAPK 9	(Alternative names: JNK-55; Stress-activated protein kinase 1a - Short name: SAPK1a; Stress-activated protein kinase JNK2; c-Jun N-terminal kinase 2)	Gene names synonyms:JNK2, PRKM9, SAPK1A
241_EC	O95243	MBD4	M	3.2.2.-	**Methyl-CpG-binding domain protein 4** (EC 3.2.2.-)	(Alternative names: Methyl-CpG-binding endonuclease 1; Methyl-CpG-binding protein MBD4; Mismatch-specific DNA N-glycosylase)	Gene names synonyms:MED1
242_EC	P33993	MCM7	M	3.6.4.12	**DNA replication licensing factor MCM7** (EC 3.6.4.12)	(Alternative names: CDC47 homolog; P1.1-MCM3)	Gene names synonyms:CDC47, MCM2
243_EC	P48163	ME1	M	1.1.1.40	**NADP-dependent malic enzyme** (EC 1.1.1.40) - Short name: NADP-ME	(Alternative name: Malic enzyme 1)	0
244_EC	P10620	MGST1	M	2.5.1.18	**Microsomal glutathione S-transferase 1** (EC 2.5.1.18) - Short name: Microsomal GST-1	(Alternative name: Microsomal GST-I)	Gene names synonyms:GST12, MGST
245_EC	O14880	MGST3	M	1.11.1.-	Glutathione peroxidase MGST3 (EC:1.11.1.-)	**Microsomal glutathione S-transferase 3** - Short name: Microsomal GST-3; (Alternative name: Microsomal glutathione S-transferase III - Short name: Microsomal GST-III)	EC Number updated and protein names updated

246_EC	O14880	MGST3	M	4.4.1.20	LTC4 synthase MGST3 (EC:4.4.1.20)	**Microsomal glutathione S-transferase 3** - Short name: Microsomal GST-3; (Alternative name: Microsomal glutathione S-transferase III - Short name: Microsomal GST-III)	EC Number updated and protein names updated
247_EC	P00395	MT-CO1	M	7.1.1.9	**Cytochrome c oxidase subunit 1** (EC 7.1.1.9)	(Alternative name: Cytochrome c oxidase polypeptide I)	EC Number updated; Gene names synonyms:COI, COXI, MTCO1
248_EC	P00403	MT-CO2	M	EC 7.1.1.9	**Cytochrome c oxidase subunit 2** (EC 7.1.1.9)	(Alternative name: Cytochrome c oxidase polypeptide II)	EC Number updated; Molecular function: Translocase; Biological process: Electron transport, Respiratory chain, Transport; Ligand: Copper, Magnesium, Metal-binding; Gene names Synonyms:COII, COX2, COXII, MTCO2
249_EC	P00414	MT-CO3	M	EC 7.1.1.9	**Cytochrome c oxidase subunit 3** (EC 7.1.1.9)	(Alternative name: Cytochrome c oxidase polypeptide III)	EC Number updated; Molecular function: Translocase; Gene names synonyms:COIII, COXIII, MTCO3
250_EC	P03886	MT-ND1	M	7.1.1.2	**NADH-ubiquinone oxidoreductase chain 1** (EC 7.1.1.2)	(Alternative name: NADH dehydrogenase subunit 1)	EC Number updated; Gene names synonyms:MTND1, NADH1, ND1
251_EC	P03897	MT-ND3	M	7.1.1.2	**NADH-ubiquinone oxidoreductase chain 3** (EC 7.1.1.2)	(Alternative name: NADH dehydrogenase subunit 3)	EC Number updated; Gene names synonyms:MTND3, NADH3, ND3
252_EC	P03915	MT-ND5	M	7.1.1.2	**NADH-ubiquinone oxidoreductase chain 5** (EC 7.1.1.2)	(Alternative name: NADH dehydrogenase subunit 5)	EC Number updated; Gene names synonyms:MTND5, NADH5, ND5
253_EC	Q15788	NCOA1	M	2.3.1.48	**Nuclear receptor coactivator 1** (EC 2.3.1.48) - Short name: NCoA-1	(Alternative name: Class E basic helix-loop-helix protein 74 - Short name: bHLHe74; Protein Hin-2; RIP160; Renal carcinoma antigen NY-REN-52; Steroid receptor coactivator 1 - Short name: SRC-1)	Gene names synonyms:BHLHE74, SRC1
254_EC	P28331	NDUFS1	M	7.1.1.2	**NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial** (EC 7.1.1.2)	(Alternative name: Complex I-75kD - Short name: CI-75kD)	EC Number updated
255_EC	O75489	NDUFS3	M	7.1.1.2	**NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial** (EC 7.1.1.2)	(Alternative names: Complex I-30kD - Short name: CI-30kD; NADH-ubiquinone oxidoreductase 30 kDa subunit)	EC Number updated
256_EC	P19404	NDUFV2	M	7.1.1.2	**NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial** (EC 7.1.1.2)	(Alternative name: NADH-ubiquinone oxidoreductase 24 kDa subunit)	EC Number updated

257_EC	P16083	NQO2	M	1.10.5.1	**Ribosyldihydronicotinamide dehydrogenase [quinone]** (EC 1.10.5.1)	(Alternative names: NRH dehydrogenase [quinone] 2; NRH:quinone oxidoreductase 2; Quinone reductase 2 - Short name: QR2)	Gene names synonyms:NMOR2
258_EC	Q96L73	NSD1	M	2.1.1.357	**Histone-lysine N-methyltransferase, H3 lysine-36 specific** (EC 2.1.1.357)	(Alternative names: Androgen receptor coactivator 267 kDa protein; Androgen receptor-associated protein of 267 kDa; H3-K36-HMTase; (Lysine N-methyltransferase 3B; Nuclear receptor-binding SET domain-containing protein 1 - Short name: NR-binding SET domain-containing protein)	EC Number updated and protein names updated; Gene names synonyms:ARA267, KMT3B
259_EC	Q02218	OGDH	M	1.2.4.2	**2-oxoglutarate dehydrogenase, mitochondrial** (EC 1.2.4.2)	(Alternative names: 2-oxoglutarate dehydrogenase complex component E1 - Short name: OGDC-E1; Alpha-ketoglutarate dehydrogenase)	0
260_EC	O60260	PARK2	M	2.3.2.31	**E3 ubiquitin-protein ligase parkin** (EC 2.3.2.31) - Short name: Parkin	(Alternative names: Parkin RBR E3 ubiquitin-protein ligase; Parkinson juvenile disease protein 2 - Short name: Parkinson disease protein 2)	EC Number updated and Protein names updated; Gene name: PRKN; Gene names synonym: PARK2
261_EC	P09874	PARP1	M	2.4.2.30	**Poly [ADP-ribose] polymerase** 1 (EC 2.4.2.30) - Short name: PARP-1	(Alternative names: ADP-ribosyltransferase diphtheria toxin-like 1 - Short name: ARTD1; DNA ADP-ribosyltransferase PARP1 (EC:2.4.2.-); NAD(+) ADP-ribosyltransferase 1 - Short name: ADPRT 1; Poly[ADP-ribose] synthase 1)	EC Number updated and Protein names updated; Gene names Synonyms:ADPRT, PPOL
262_EC	P09874	PARP1	M	2.4.2.-	Protein poly-ADP-ribosyltransferase PARP1 (EC:2.4.2.-)	(Alternative names: ADP-ribosyltransferase diphtheria toxin-like 1 - Short name: ARTD1; DNA ADP-ribosyltransferase PARP1 (EC:2.4.2.-); NAD(+) ADP-ribosyltransferase 1 - Short name: ADPRT 1; Poly[ADP-ribose] synthase 1)	EC Number updated and Protein names updated; Gene names Synonyms:ADPRT, PPOL
263_EC	Q13370	PDE3B	M	3.1.4.17	**cGMP-inhibited 3',5'-cyclic phosphodiesterase B** (EC 3.1.4.17)	(Alternative names: CGIPDE1 - Short name: CGIP1; Cyclic GMP-inhibited phosphodiesterase B - Short name: CGI-PDE B)	0
264_EC	P08559	PDHA1	M	1.2.4.1	**Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial** (EC 1.2.4.1)	(Alternative name: PDHE1-A type I)	Gene names synonyms:PHE1A
265_EC	O15530	PDPK1	M	2.7.11.1	**3-phosphoinositide-dependent protein kinase 1** (EC 2.7.11.1) - Short name: hPDK1	0	Gene names synonyms:PDK1

266_EC	O00750	PIK3C2B	M	2.7.1.154	**Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta** (EC 2.7.1.154) - Short names: PI3K-C2-beta/PtdIns-3-kinase C2 subunit beta	(Alternative names: C2-PI3K; Phosphoinositide 3-kinase-C2-beta)	0
267_EC	Q8NEB9	PIK3C3	M	2.7.1.137	**Phosphatidylinositol 3-kinase catalytic subunit type 3** (EC 2.7.1.137) - Short names: PI3-kinase type 3/PI3K type 3/PtdIns-3-kinase type 3	(Alternative names: Phosphatidylinositol 3-kinase p100 subunit; Phosphoinositide-3-kinase class 3; hVps34)	Gene names synonyms:VPS34
268_EC	P42336	PIK3CA	M	2.7.1.153	**Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform** (EC 2.7.1.153) - Short names: PI3-kinase subunit alpha/PI3K-alpha/PI3Kalpha/PtdIns-3-kinase subunit alpha	(Alternative names: Phosphatidylinositol 4,5-bisphosphate 3-kinase 110 kDa catalytic subunit alpha - Short names: PtdIns-3-kinase subunit p110-alpha/p110alpha; Phosphoinositide-3-kinase catalytic alpha polypeptide)	Additionally another EC Number under "Enzyme and pathway databases" (BRENDA EC: 2.7.1.137)
269_EC	P42336	PIK3CA	M	2.7.11.1	**Serine/threonine protein kinase PIK3CA** (EC 2.7.11.1)	(Alternative names: Phosphatidylinositol 4,5-bisphosphate 3-kinase 110 kDa catalytic subunit alpha - Short names: PtdIns-3-kinase subunit p110-alpha/p110alpha; Phosphoinositide-3-kinase catalytic alpha polypeptide)	Additionally another EC Number under "Enzyme and pathway databases" (BRENDA EC: 2.7.1.137)
270_EC	P42338	PIK3CB	M	2.7.1.153	**Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform** (EC 2.7.1.153) - Short names: PI3-kinase subunit beta/PI3K-beta/PI3Kbeta/PtdIns-3-kinase subunit beta	(Alternative name: Phosphatidylinositol 4,5-bisphosphate 3-kinase 110 kDa catalytic subunit beta - Short names: PtdIns-3-kinase subunit p110-beta/p110beta)	Additionally another EC Number under "Enzyme and pathway databases" (BRENDA EC: 2.7.1.137) found; Gene names synonyms:PIK3C1
271_EC	Q99570	PIK3R4	M	2.7.11.1	**Phosphoinositide 3-kinase regulatory subunit 4** (EC 2.7.11.1) - Short name: PI3-kinase regulatory subunit 4	(Alternative names: PI3-kinase p150 subunit; Phosphoinositide 3-kinase adaptor protein)	Gene names synonyms:VPS15
272_EC	P41247	PNPLA4	M	3.1.1.3	**Patatin-like phospholipase domain-containing protein 4** (EC 3.1.1.3)	(Alternative name: Protein GS2)	Gene names synonyms:DXS1283E, GS2

273_EC	P09884	POLA1	M	2.7.7.7	**DNA polymerase alpha catalytic subunit** (EC 2.7.7.7)	(Alternative name: DNA polymerase alpha catalytic subunit p180)	Gene names synonyms:POLA
274_EC	P30048	PRDX3	M	1.11.1.24	**Thioredoxin-dependent peroxide reductase, mitochondrial** (EC 1.11.1.24)	(Alternative names: Antioxidant protein 1 - Short name: AOP-1; HBC189; Peroxiredoxin III - Short name: Prx-III; Peroxiredoxin-3; Protein MER5 homolog; Thioredoxin-dependent peroxiredoxin 3)	EC Number updated and Protein name updated; Gene names synonyms:AOP1
275_EC	P17612	PRKACA	M	2.7.11.11	**cAMP-dependent protein kinase catalytic subunit alpha** (EC 2.7.11.11) - Short name: PKA C-alpha	0	Gene names synonyms:PKACA
276_EC	P22694	PRKACB	M	2.7.11.11	**cAMP-dependent protein kinase catalytic subunit beta** (EC 2.7.11.11) - Short name: PKA C-beta	0	0
277_EC	P17252	PRKCA	M	2.7.11.13	**Protein kinase C alpha type** (EC 2.7.11.13) - Short names: PKC-A/PKC-alpha)	0	Gene names Synonyms:PKCA, PRKACA
278_EC	Q05655	PRKCD	M	2.7.11.13	**Protein kinase C delta type** (EC 2.7.11.13)	(Alternative name: nPKC-delta); [Cleaved into the following 2 chains: Protein kinase C delta type regulatory subunit; Protein kinase C delta type catalytic subunit; (Alternative name: Sphingosine-dependent protein kinase-1 - Short name: SDK1)]	0
279_EC	Q05655	PRKCD	M	2.7.10.2	Tyrosine-protein kinase PRKCD (EC 2.7.10.2)	(Alternative name: nPKC-delta); [Cleaved into the following 2 chains: Protein kinase C delta type regulatory subunit; Protein kinase C delta type catalytic subunit; (Alternative name: Sphingosine-dependent protein kinase-1 - Short name: SDK1)]	0
280_EC	O94806	PRKD3	M	2.7.11.13	**Serine/threonine-protein kinase D3** (EC 2.7.11.13)	(Alternative names: Protein kinase C nu type; Protein kinase EPK2; nPKC-nu)	Gene names synonyms:EPK2, PRKCN
281_EC	Q15185	PTGES3	M	5.3.99.3	**Prostaglandin E synthase 3** (EC 5.3.99.3)	(Alternative names: Cytosolic prostaglandin E2 synthase - Short name: cPGES; Hsp90 co-chaperone; Progesterone receptor complex p23; Telomerase-binding protein p23)	Gene names synonyms:P23, TEBP
282_EC	P31040	SDHA	M	1.3.5.1	**Succinate dehydrogenase [ubiquinone] flavoprotein**	(Alternative name: Flavoprotein subunit of complex II- Short name: Fp)	Gene name synonyms:SDH2, SDHF

					subunit, mitochondrial (EC 1.3.5.1)		
283_EC	P51531	SMARCA2	M	3.6.4.-	**Probable global transcription activator SNF2L2** (EC 3.6.4.-)	(Alternative names: ATP-dependent helicase SMARCA2; BRG1-associated factor 190B - Short name: BAF190B; Protein brahma homolog - Short name: hBRM; SNF2-alpha; SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 2)	Gene names synonyms:BAF190B, BRM, SNF2A, SNF2L2
284_EC	P35610	SOAT1	M	2.3.1.26	**Sterol O-acyltransferase 1** (EC 2.3.1.26)	(Alternative names: Acyl-coenzyme A:cholesterol acyltransferase 1 - Short name: ACAT-1; Cholesterol acyltransferase 1)	Gene names synonyms:ACACT, ACACT1, ACAT, ACAT1, SOAT, STAT
285_EC	O15164	TRIM24	M	2.3.2.27	**Transcription intermediary factor 1-alpha** (EC 2.3.2.27) - Short name: TIF1-alpha	(Alternative names: E3 ubiquitin-protein ligase TRIM24; RING finger protein 82; RING-type E3 ubiquitin transferase TIF1-alpha; Tripartite motif-containing protein 24)	Gene names synonyms:RNF82, TIF1, TIF1A
286_EC	P47985	UQCRFS1	M	7.1.1.8	**Cytochrome b-c1 complex subunit Rieske, mitochondrial** (EC 7.1.1.8)	(Alternative names: Complex III subunit 5; Cytochrome b-c1 complex subunit 5; Rieske iron-sulfur protein - Short name: RISP; Ubiquinol-cytochrome c reductase iron-sulfur subunit); [Cleaved into the following chain: Cytochrome b-c1 complex subunit 9 - Short names: Su9/Subunit 9; (Alternative names: 8 kDa subunit 9; Complex III subunit IX; Cytochrome b-c1 complex subunit 11; UQCRFS1 mitochondrial targeting sequence - Short name: UQCRFS1 MTS; Ubiquinol-cytochrome c reductase 8 kDa protein)]	EC Number updated and protein names updated
287_EC	P50416	CPT1A	S	2.3.1.21	**Carnitine O-palmitoyltransferase 1, liver isoform** (EC 2.3.1.21) - Short name: CPT1-L	(Alternative names: Carnitine O-palmitoyltransferase I, liver isoform - Short names: CPT I/CPTI-L; Carnitine palmitoyltransferase 1A)	Gene names synonyms:CPT1
288_EC	Q92813	DIO2	S	1.21.99.4	**Type II iodothyronine deiodinase** (EC 1.21.99.4)	(Alternative names: 5DII; DIOII; Type 2 DI; Type-II 5'-deiodinase)	Additionally another EC Number under "Enzyme and pathway databases" (BRENDA EC 1.97.1.10) found; Gene names synonyms:ITDI2, TXDI2
289_EC	Q9HAZ2	PRDM16	B	2.1.1.-	**Histone-lysine N-methyltransferase PRDM16** (EC 2.1.1.-)	(Alternative names: PR domain zinc finger protein 16; PR domain-containing protein 16; Transcription factor MEL1 - Short name: MDS1/EVI1-like gene 1)	EC Number updated; Molecular function: Activator, DNA-binding, Methyltransferase, Repressor, Transferase; Biological process: Differentiation, Transcription, Transcription

							regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:KIAA1675, MEL1, PFM13
290_EC	Q8IZV5	RDH10	S	1.1.1.300	**Retinol dehydrogenase 10** (EC 1.1.1.300)	(Alternative name: Short chain dehydrogenase/reductase family 16C member 4)	Gene names synonyms:SDR16C4

Table A9: *Co-expressed genes without enzyme (EC) number.*

Internal ID	Entry Uniprot	Gene names	Ori-gin	Type of Protein	Protein names	Comments
1_nEC	P04217	A1BG	M	0	**Alpha-1B-glycoprotein** (Alternative name: Alpha-1-B glycoprotein)	GO - Biological process: neutrophil degranulation, platelet degranulation
2_nEC	Q9NUT2	ABCB8	M	T, Mito, Trans	**Mitochondrial potassium channel ATP-binding subunit**; (Alternative names: ATP-binding cassette sub-family B member 8, mitochondrial - Short name: ABCB8; Mitochondrial ATP-binding cassette 1 - Short name: M-ABC1; Mitochondrial sulfonylurea-receptor - Short name: MITOSUR	BRENDA EC Number updated; Gene names synonyms:MABC1, MITOSUR
3_nEC	O15438	ABCC3	M	T, Trans	**ATP-binding cassette sub-family C member 3**; (Alternative names: Canalicular multispecific organic anion transporter 2 ; Multi-specific organic anion transporter D - Short name: MOAT-D; Multidrug resistance-associated protein 3)	Gene names synonyms:CMOAT2, MLP2, MRP3
4_nEC	O15439	ABCC4	M	T, Trans	**Multidrug resistance-associated protein 4**; (Alternative names: ATP-binding cassette sub-family C member 4; MRP/cMOAT-related ABC transporter; Multi-specific organic anion transporter B - Short name: MOAT-B)	Gene names synonyms:MRP4
5_nEC	Q9H172	ABCG4	M	T, Trans	**ATP-binding cassette sub-family G member 4**	Gene names synonyms:WHITE2
6_nEC	P62736	ACTA2	M	0	**Actin, aortic smooth muscle**; (Alternative names: Alpha-actin-2; Cell growth-inhibiting gene 46 protein); [Cleaved into the following chain: Actin, aortic smooth muscle, intermediate form]	Protein name updated; Gene names synonyms:ACTSA, ACTVS

7_nEC	P68032	ACTC1	M	0	**Actin, alpha cardiac muscle 1**; (Alternative name: Alpha-cardiac actin); [Cleaved into the following chain:Actin, alpha cardiac muscle 1, intermediate form]	Protein name updated; Gene names synonyms:ACTC
8_nEC	P29274	ADORA2A	M	R	**Adenosine receptor A2a**	Gene names synonyms:ADORA2
9_nEC	P08913	ADRA2A	M	R	**Alpha-2A adrenergic receptor** (Alternative names: Alpha-2 adrenergic receptor subtype C10; Alpha-2A adrenoreceptor - Short names: Alpha-2A adrenoceptor/Alpha-2AAR)	Gene names synonyms:ADRA2R, ADRAR
10_nEC	P18825	ADRA2C	M	R	**Alpha-2C adrenergic receptor** (Alternative names: Alpha-2 adrenergic receptor subtype C4; Alpha-2C adrenoreceptor - Short names: Alpha-2C adrenoceptor/Alpha-2CAR)	Gene names synonyms:ADRA2L2, ADRA2RL2
11_nEC	P50052	AGTR2	M	R	**Type-2 angiotensin II receptor**; (Alternative name: Angiotensin II type-2 receptor - Short name: AT2)	0
12_nEC	P35869	AHR	M	R	**Aryl hydrocarbon receptor** - Short names: Ah receptor/AhR); (Alternative name: Class E basic helix-loop-helix protein 76 - Short name: bHLHe76)	Gene names synonyms:BHLHE76
13_nEC	P02765	AHSG	M	0	**Alpha-2-HS-glycoprotein**; (Alternative nmes: Alpha-2-Z-globulin; Ba-alpha-2-glycoprotein; Fetuin-A); [Cleaved into the following 2 chains: Alpha-2-HS-glycoprotein chain A; Alpha-2-HS-glycoprotein chain B]	Gene names synonyms:FETUA
14_nEC	Q12802	AKAP13	M	0	**A-kinase anchor protein 13** - Short name: AKAP-13; (Alternative names: AKAP-Lbc; Breast cancer nuclear receptor-binding auxiliary protein; Guanine nucleotide exchange factor Lbc; Human thyroid-anchoring protein 31; Lymphoid blast crisis oncogene - Short name: LBC oncogene; Non-oncogenic Rho GTPase-specific GTP exchange factor; Protein kinase A-anchoring protein 13 - Short name: PRKA13; p47)	Molecular function: Guanine-nucleotide releasing factor; Ligand: Metal-binding, Zinc; Gene names synonyms:BRX, HT31, LBC
15_nEC	P02760	AMBP	M	0	**Protein AMBP** [Cleaved into the following 3 chains: Alpha-1-microglobulin - Short name: Protein HC; (Alternative names: Alpha-1 microglycoprotein; Complex-forming glycoprotein heterogeneous in charge); Inter-alpha-trypsin inhibitor light chain - Short name: ITI-LC; (Alternative names: Bikunin; EDC1; HI-30; Uronic-acid-rich protein); Trypstatin]	Molecular function: Protease inhibitor, Serine protease inhibitor; Biological process: Host-virus interaction; Ligand: Chromophore; Gene names synonyms:HCP, ITIL
16_nEC	P03971	AMH	M	0	**Muellerian-inhibiting factor**; (Alternative names: Anti-Muellerian hormone - Short name: AMH; Muellerian-inhibiting substance - Short name: MIS)	Molecular function: Growth factor; Biological process: Differentiation, Gonadal differentiation; Gene names synonyms:MIF
17_nEC	O15123	ANGPT2	M	0	**Angiopoietin-2** - Short name: ANG-2	Molecular function: Developmental protein; Biological process: Angiogenesis, Differentiation; Ligand: Calcium, Metal-binding

18_nEC	Q15327	ANKRD1	M	0	**Ankyrin repeat domain-containing protein 1**; (Alternative names: Cardiac ankyrin repeat protein; Cytokine-inducible gene C-193 protein; Cytokine-inducible nuclear protein)	Gene names synonyms:C193, CARP, HA1A2
19_nEC	P02743	APCS	M	0	**Serum amyloid P-component** -Short name: SAP; (9.5S alpha-1-glycoprotein) [Cleaved into the following chain: Serum amyloid P-component(1-203)]	Ligand: Calcium, Lectin, Metal-binding; Gene names synonyms:PTX2
20_nEC	Q9ULZ1	APLN	M	0	**Apelin**; (Alternative name: APJ endogenous ligand); [Cleaved intothe following 4 chains: Apelin-36; Apelin-31; Apelin-28; Apelin-13]	Molecular function: Developmental protein, Hormone;Biological process: Angiogenesis, Gastrulation, Host-virus interaction ;Gene names synonyms:APEL
21_nEC	P02647	APOA1	M	T	**Apolipoprotein A-I** - Short names: Apo-AI/ApoA-I; (Alternative name: Apolipoprotein A1); [Cleaved into the following 2 chains: Proapolipoprotein A-I - Short name: ProapoA-I; Truncated apolipoprotein A-I (Alternative name: Apolipoprotein A-I(1-242))]	Biological process: Cholesterol metabolism, Lipid metabolism, Lipid transport, Steroid metabolism, Sterol metabolism, Transport
22_nEC	P02652	APOA2	M	T	**Apolipoprotein A-II** - Short names: Apo-AII/ApoA-II; (Alternative name: Apolipoprotein A2) [Cleaved into the following 2 chains: Proapolipoprotein A-II - Short name: ProapoA-II; Truncated apolipoprotein A-II; (Alternative name: Apolipoprotein A-II(1-76))]	Biological process: Host-virus interaction, Lipid transport, Transport
23_nEC	P06727	APOA4	M	T	**Apolipoprotein A-IV** -Short names: Apo-AIV/ApoA-IV; (Alternative name: Apolipoprotein A4)	Biological process: Lipid transport, Transport
24_nEC	Q6Q788	APOA5	M	T	**Apolipoprotein A-V** - Short names: Apo-AV/ApoA-V; (Alternative names: Apolipoprotein A5; Regeneration-associated protein 3)	Biological process: Lipid transport, Transport; Gene names synonyms:RAP3
25_nEC	P02655	APOC2	M	T	**Apolipoprotein C-II** -Short names: Apo-CII/ApoC-II; (Alternative name: Apolipoprotein C2); [Cleaved into the following chain: Proapolipoprotein C-II - Short name: ProapoC-II)]	Biological process: Lipid degradation, Lipid metabolism, Lipid transport, Transport; Ligand Sialic acid; Gene names synonyms:APC2
26_nEC	P02656	APOC3	M	T	**Apolipoprotein C-III** - Short names: Apo-CIII/ApoC-III; (Alternative name: Apolipoprotein C3)	Biological process: Lipid degradation, Lipid metabolism, Lipid transport, Transport; Ligand: Sialic acid
27_nEC	P55056	APOC4	M	T	**Apolipoprotein C-IV** - Short names: Apo-CIV/ApoC-IV); (Alternative name: Apolipoprotein C4)	Biological process: Lipid transport, Transport
28_nEC	Q13790	APOF	M	T	**Apolipoprotein F** - Short name: Apo-F; (Alternative name: Lipid transfer inhibitor protein - Short name: LTIP)	Biological process: Cholesterol metabolism, Lipid metabolism, Lipid transport, Steroid metabolism, Sterol metabolism, Transport
29_nEC	P02749	APOH	M	0	**Beta-2-glycoprotein 1**; (Alternative names: APC inhibitor; Activated protein C-binding protein; Anticardiolipin cofactor; Apolipoprotein H - Short name: Apo-H; Beta-2-glycoprotein I - Short names: B2GPI/Beta(2)GPI)	Molecular function: Heparin-binding; Gene names synonyms:B2G1

30_nEC	P18847	ATF3	M	0	**Cyclic AMP-dependent transcription factor ATF-3** - Short name: cAMP-dependent transcription factor ATF-3; (Alternative name: Activating transcription factor 3)	Molecular function: DNA-binding, Repressor; Biological process: Transcription, Transcription regulation
31_nEC	O14867	BACH1	M	0	**Transcription regulator protein BACH1**; (Alternative names: BTB and CNC homolog 1; HA2303)	BRENDA EC Number updated
32_nEC	Q99933	BAG1	M	0	**BAG family molecular chaperone regulator 1** - Short name: BAG-1; (Alternative name: Bcl-2-associated athanogene 1)	Molecular function: Chaperone; Biological process: Apoptosis; Gene names synonyms:HAP
33_nEC	Q07812	BAX	M	Mito	**Apoptosis regulator BAX**; (Alternative name: Bcl-2-like protein 4 - Short name: Bcl2-L-4)	Biological process: Apoptosis, Host-virus interaction; Gene names synonyms:BCL2L4
34_nEC	Q96PG8	BBC3	M	0	**Bcl-2-binding component 3, isoforms 3/4**; (Alternative names: JFY-1; p53 up-regulated modulator of apoptosis)	Function: Isoform 3: Does not affect cell growth.; GO - Molecular function: ATPase binding; Gene names synonyms:PUMA
35_nEC	Q9BXH1	BBC3	M	0	**Bcl-2-binding component 3, isoforms 1/2**; (Alternative names: JFY-1; p53 up-regulated modulator of apoptosis)	Function: Essential mediator of p53/TP53-dependent and p53/TP53-independent apoptosis. Functions by promoting partial unfolding of BCL2L1 and dissociation of BCL2L1 from p53/TP53. Regulates ER stress-induced neuronal apoptosis ; Biological process: Apoptosis; Gene names synonyms:PUMA
36_nEC	Q07817	BCL2L1	M	Mito	**Bcl-2-like protein 1** - Short name: Bcl2-L-1; (Alternative name: Apoptosis regulator Bcl-X)	Biological process: Apoptosis, Endocytosis; Gene names synonyms:BCL2L, BCLX
37_nEC	P21810	BGN	M	0	**Biglycan**; (Alternative names: Bone/cartilage proteoglycan I; PG-S1)	Function: May be involved in collagen fiber assembly.; Gene names synonyms:SLRR1A
38_nEC	P55957	BID	M	0	**BH3-interacting domain death agonist**; (Alternative name: p22 BID - Short name: BID); [Cleaved into the following 3 chains: BH3-interacting domain death agonist p15 (Alternative name: p15 BID); BH3-interacting domain death agonist p13 (Alternative name: p13 BID); BH3-interacting domain death agonist p11 (Alternative name: p11 BID)]	Biological process: Apoptosis
39_nEC	O15392	BIRC5	M	0	**Baculoviral IAP repeat-containing protein 5**; (Alternative names: Apoptosis inhibitor 4; Apoptosis inhibitor survivin)	Molecular function: Protease inhibitor, Repressor, Thiol protease inhibitor; Biological process: Apoptosis, Cell cycle, Cell division, Chromosome partition, Mitosis, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:API4, IAP4
40_nEC	O95393	BMP10	M	0	**Bone morphogenetic protein 10** - Short name: BMP-10	Molecular function: Cytokine, Developmental protein, Growth factor; Biological process: Cell adhesion
41_nEC	P51587	BRCA2	M	0	**Breast cancer type 2 susceptibility protein**; (Alternative name: Fanconi anemia group D1 protein)	Molecular function: DNA-binding; Biological process: Cell cycle, DNA damage, DNA recombination, DNA repair; Gene names synonyms:FACD, FANCD1

42_nEC	P0C0L4	C4A	M	0	**Complement C4-A**; (Alternative names: Acidic complement C4; C3 and PZP-like alpha-2-macroglobulin domain-containing protein 2); [Cleaved into the following 6 chains: Complement C4 beta chain; Complement C4-A alpha chain; C4a anaphylatoxin; C4b-A; C4d-A; Complement C4 gamma chain]	Molecular function: Blood group antigen; Biological process: Complement pathway, Immunity, Inflammatory response, Innate immunity; Gene names synonyms:CO4, CPAMD2
43_nEC	P0C0L5	C4B	M	0	**Complement C4-B**; (Alternative names: Basic complement C4; C3 and PZP-like alpha-2-macroglobulin domain-containing protein 3) [Cleaved into the following 6 chains: Complement C4 beta chain; Complement C4-B alpha chain; C4a anaphylatoxin; C4b-B; C4d-B; Complement C4 gamma chain]	Molecular function: Blood group antigen; Biological process: Complement pathway, Immunity, Inflammatory response, Innate immunity; Gene names synonyms:CO4, CPAMD3 AND Name:C4B_2
44_nEC	P02748	C9	M	0	**Complement component C9**; [Cleaved into the following 2 chains: Complement component C9a; Complement component C9b]	Biological process: Complement alternate pathway, Complement pathway, Cytolysis, Immunity, Innate immunity
45_nEC	Q9Y6J0	CABIN1	M	0	**Calcineurin-binding protein cabin-1**; (Alternative name: Calcineurin inhibitor - Short name: CAIN)	Molecular function: Chromatin regulator; Gene names synonyms:KIAA0330
46_nEC	Q13936	CACNA1C	M	C	**Voltage-dependent L-type calcium channel subunit alpha-1C**; (Alternative names: Calcium channel, L type, alpha-1 polypeptide, isoform 1, cardiac muscle; Voltage-gated calcium channel subunit alpha Cav1.2)	Molecular function: Calcium channel, Calmodulin-binding, Ion channel, Voltage-gated channel; Biological process: Calcium transport, Host-virus interaction, Ion transport, Transport; Ligand: Calcium, Metal-binding; Gene names synonyms:CACH2, CACN2, CACNL1A1, CCHL1A1
47_nEC	O95180	CACNA1H	M	C	**Voltage-dependent T-type calcium channel subunit alpha-1H**; (Alternative names: Low-voltage-activated calcium channel alpha1 3.2 subunit; Voltage-gated calcium channel subunit alpha Cav3.2)	Molecular function: Calcium channel, Ion channel, Voltage-gated channel; Biological process: Calcium transport, Ion transport, Transport; Ligand: Calcium, Metal-binding, Zinc
48_nEC	Q08289	CACNB2	M	C	**Voltage-dependent L-type calcium channel subunit beta-2** - Short name: CAB2; (Alternative names: Calcium channel voltage-dependent subunit beta 2; Lambert-Eaton myasthenic syndrome antigen B - Short name: MYSB)	Molecular function:Calcium channel, Ion channel, Voltage-gated channel; Biological process: Calcium transport, Ion transport, Transport; Ligand: Calcium; Gene names synonyms:CACNLB2, MYSB
49_nEC	P01258	CALCA	M	0	**Calcitonin**; [Cleaved into the following 2 chains: Calcitonin; Katacalcin ;(Alternative names: Calcitonin carboxyl-terminal peptide - Short name: CCP; PDN-21)]	Molecular function: Hormone; Gene names synonyms:CALC1
50_nEC	P06881	CALCA	M	0	**Calcitonin gene-related peptide 1**; (Alternative names: Alpha-type CGRP; Calcitonin gene-related peptide I - Short name: CGRP-I)	Molecular function: Hormone; Gene names synonyms:CALC1
51_nEC	Q96S95	CAMK2N2	M	0	**Calcium/calmodulin-dependent protein kinase II inhibitor 2**; (Alternative name: CaM-KII inhibitory protein - Short name: CaM-KIIN)	Molecular function: Protein kinase inhibitor
52_nEC	P04632	CAPNS1	M	0	**Calpain small subunit 1** - Short name: CSS1; (Alternative names: Calcium-activated neutral proteinase small subunit - Short name:	BRENDA EC Number updated; Gene names synonyms:CAPN4, CAPNS

					CANP small subunit; Calcium-dependent protease small subunit - Short name: CDPS; Calcium-dependent protease small subunit 1; Calpain regulatory subunit	
53_nEC	P41180	CASR	M	R	**Extracellular calcium-sensing receptor** - Short names: CaR/CaSR/hCasR; (Alternative name: Parathyroid cell calcium-sensing receptor 1 - Short name: PCaR1)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Ligand: Calcium, Metal-binding ; Gene names synonyms:GPRC2A, PCAR1
54_nEC	P56539	CAV3	M	0	**Caveolin-3** ;(Alternative name: M-caveolin)	Function: May act as a scaffolding protein within caveolar membranes.
55_nEC	Q99731	CCL19	M	0	**C-C motif chemokine 19**; (Alternative names: Beta-chemokine exodus-3; CK beta-11; Epstein-Barr virus-induced molecule 1 ligand chemokine - Short names: EBI1 ligand chemokine/ELC; Macrophage inflammatory protein 3 beta - Short name: MIP-3-beta; Small-inducible cytokine A19)	Molecular function:Cytokine; Biological process: Chemotaxis, Inflammatory response; Gene names synonyms:ELC, MIP3B, SCYA19
56_nEC	P13500	CCL2	M	0	**C-C motif chemokine 2**; (Alternative names: HC11; Monocyte chemoattractant protein 1; Monocyte chemotactic and activating factor - Short name: MCAF; Monocyte chemotactic protein 1 - Short name: MCP-1; Monocyte secretory protein JE); Small-inducible cytokine A2)	Molecular function: Cytokine; Biological process: Chemotaxis, Inflammatory response; Gene names synonyms:MCP1, SCYA2
57_nEC	P80098	CCL7	M	0	**C-C motif chemokine 7**; (Alternative names: Monocyte chemoattractant protein 3; Monocyte chemotactic protein 3 - Short name: MCP-3; NC28; Small-inducible cytokine A7)	Molecular function: Cytokine, Heparin-binding; Biological process: Chemotaxis, Inflammatory response; Gene names synonyms:MCP3, SCYA6, SCYA7
58_nEC	P24385	CCND1	M	0	**G1/S-specific cyclin-D1**; (Alternative names: B-cell lymphoma 1 protein - Short name: BCL-1; BCL-1 oncogene; PRAD1 oncogene)	Molecular function: Cyclin, Repressor; Biological process: Cell cycle, Cell division, DNA damage, Transcription, Transcription regulation; Gene names synonyms:BCL1, PRAD1
59_nEC	P15391	CD19	M	R	**B-lymphocyte antigen CD19**; (Alternative names: B-lymphocyte surface antigen B4; Differentiation antigen CD19; T-cell surface antigen Leu-12; CD antigen CD19)	Biological process: Adaptive immunity, Immunity
60_nEC	P29965	CD40LG	M	0	**CD40 ligand** - Short name: CD40-L; (Alternative names: T-cell antigen Gp39; TNF-related activation protein - Short name: TRAP; Tumor necrosis factor ligand superfamily member 5; CD_antigen: CD154); [Cleaved into the following 2 chains: CD40 ligand, membrane form; CD40 ligand, soluble form - Short name: sCD40L]	Molecular function: Cytokine; Gene names synonyms:CD40L, TNFSF5, TRAP
61_nEC	P16070	CD44	M	R	**CD44 antigen**; (Alternative names: CDw44; Epican; Extracellular matrix receptor III - Short name: ECMR-III; GP90 lymphocyte homing/adhesion receptor; HUTCH-I; Heparan sulfate proteoglycan; Hermes antigen; Hyaluronate receptor; Phagocytic glycoprotein 1 - Short name: PGP-1; Phagocytic glycoprotein I - Short name: PGP-I; CD_antigen: CD44)	Molecular function: Blood group antigen, Receptor; Biological process: Cell adhesion; Gene names synonyms:LHR, MDU2, MDU3, MIC4

62_nEC	P38936	CDKN1A	M	0	**Cyclin-dependent kinase inhibitor 1**; (Alternative names: CDK-interacting protein 1; Melanoma differentiation-associated protein 6 - Short name: MDA-6; p21)	Molecular function: Protein kinase inhibitor; Biological process: Cell cycle; Ligand: Metal-binding, Zinc; Gene names synonyms:CAP20, CDKN1, CIP1, MDA6, PIC1, SDI1, WAF1
63_nEC	P49450	CENPA	M	0	**Histone H3-like centromeric protein A**; (Alternative names: Centromere autoantigen A; Centromere protein A - Short name: CENP-A)	Molecular function: DNA-binding; Biological process: Cell cycle, Cell division, Host-virus interaction, Mitosis
64_nEC	P10645	CHGA	M	0	**Chromogranin-A** - Short name: CgA; (Alternative name: Pituitary secretory protein I - Short name: SP-I); [Cleaved into the following 18 chains: Vasostatin-1; (Alternative name: Vasostatin I); Vasostatin-2 (Alternative name: Vasostatin II); EA-92; ES-43; Pancreastatin; SS-18; WA-8; WE-14; LF-19; Catestatin (Alternative name: SL21); AL-11; GV-19; GR-44; ER-37; GE-25; Serpinin-RRG; Serpinin; p-Glu serpinin precursor]	Molecular function: Antibiotic, Antimicrobial, Fungicide; Ligand:Calcium
65_nEC	P34972	CNR2	M	R	**Cannabinoid receptor 2** - Short names: CB-2/CB2/hCB2; (Alternative name:CX5)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Biological process: Inflammatory response; Gene names synonyms:CB2A, CB2B
66_nEC	Q01955	COL4A3	M	0	**Collagen alpha-3(IV) chain**; (Alternative name: Goodpasture antigen); [Cleaved into the following chain: Tumstatin]	Biological process: Cell adhesion
67_nEC	Q14055	COL9A2	M	0	**Collagen alpha-2(IX) chain**	Function: Structural component of hyaline cartilage and vitreous of the eye.; GO - Molecular function: extracellular matrix structural constituent, extracellular matrix structural constituent conferring tensile strength; GO - Biological process: extracellular matrix organization, skeletal system development
68_nEC	P02741	CRP	M	0	**C-reactive protein** [Cleaved into the following chain: C-reactive protein(1-205)]	Biological process: Acute phase; Ligand: Calcium, Metal-binding; Gene names synonyms:PTX1
69_nEC	P09603	CSF1	M	0	**Macrophage colony-stimulating factor 1** - Short names: CSF-1/M-CSF/MCSF; (Alternative name: Lanimostim); [Cleaved into the following chain: Processed macrophage colony-stimulating factor 1]	Molecular function: Cytokine, Growth factor; Biological process: Immunity, Inflammatory response, Innate immunity
70_nEC	P09919	CSF3	M	0	**Granulocyte colony-stimulating factor** - Short name: G-CSF; (Alternative names: Pluripoietin; INN: Filgrastim; INN: Lenograstim)	Molecular function: Cytokine, Growth factor; Gene names synonyms:C17orf33, GCSF
71_nEC	Q14406	CSHL1	M	0	**Chorionic somatomammotropin hormone-like 1** - Short name: Chorionic somatomammotropin-like; (Alternative name: Lactogen-like)	Molecular function: Hormone; Ligand: Metal-binding, Zinc; Gene names synonyms:CSHP1, CSL
72_nEC	P50461	CSRP3	M	0	**Cysteine and glycine-rich protein 3**; (Alternative name: Cardiac LIM protein; Cysteine-rich protein 3 - Short name: CRP3; LIM domain protein, cardiac; Muscle LIM protein)	Molecular function: Actin-binding, Developmental protein; Biological process: Differentiation, Myogenesis, Transcription,

						Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:CLP, MLP
73_nEC	P29279	CTGF	M	0	**CCN family member 2**; (Alternative names: Cellular communication network factor 2; Connective tissue growth factor; Hypertrophic chondrocyte-specific protein 24; Insulin-like growth factor-binding protein 8 - Short names: IBP-8/IGF-binding protein 8/IGFBP-8)	Molecular function: Heparin-binding; Biological process: Cell adhesion, DNA synthesis; Gene names:CCN2; Gene names synonyms:CTGF, HCS24, IGFBP8
74_nEC	P78310	CXADR	M	R, Trans	**Coxsackievirus and adenovirus receptor** - Short names: CAR/hCAR; (Alternative names: CVB3-binding protein; Coxsackievirus B-adenovirus receptor; HCVADR)	Molecular function: Host cell receptor for virus entry, Receptor; Biological process: Cell adhesion, Host-virus interaction; Gene names synonyms:CAR
75_nEC	P19876	CXCL3	M	0	**C-X-C motif chemokine 3**; (Alternative names: GRO-gamma(1-73); Growth-regulated protein gamma - Short name: GRO-gamma; Macrophage inflammatory protein 2-beta - Short name: MIP2-beta; [Cleaved into the following chain: GRO-gamma(5-73)]	Molecular function: Cytokine; Biological process: Chemotaxis, Inflammatory response; Gene names synonyms:GRO3, GROG, SCYB3
76_nEC	P42830	CXCL5	M	0	**C-X-C motif chemokine 5**; (Alternative names: ENA-78(1-78); Epithelial-derived neutrophil-activating protein 78; Neutrophil-activating peptide ENA-78; Small-inducible cytokine B5) [Cleaved into the following 2 chains: ENA-78(8-78); ENA-78(9-78)]	Molecular function: Cytokine; Gene names synonyms:ENA78, SCYB5
77_nEC	P10145	CXCL8	M	0	**Interleukin-8** - Short name: IL-8; (Alternative names: C-X-C motif chemokine 8; Chemokine (C-X-C motif) ligand 8; Emoctakin; Granulocyte chemotactic protein 1 - Short name: GCP-1; Monocyte-derived neutrophil chemotactic factor - Short name: MDNCF; Monocyte-derived neutrophil-activating peptide - Short name: MONAP; Neutrophil-activating protein 1 - Short name: NAP-1; Protein 3-10C; T-cell chemotactic factor); [Cleaved into the following 7 chains: MDNCF-a; (Alternative names: GCP/IL-8 protein IV; IL8/NAP1 form I); Interleukin-8 (Alternative names: (Ala-IL-8)77; GCP/IL-8 protein II; IL-8(1-77); IL8/NAP1 form II; MDNCF-b); IL-8(5-77); IL-8(6-77); (Alternative names: (Ser-IL-8)72; GCP/IL-8 protein I; IL8/NAP1 form III; Lymphocyte-derived neutrophil-activating factor - Short name: LYNAP; MDNCF-c; Neutrophil-activating factor - Short name: NAF); IL-8(7-77); (Alternative names: GCP/IL-8 protein V; IL8/NAP1 form IV); IL-8(8-77); (Alternative names: GCP/IL-8 protein VI; IL8/NAP1 form V); IL-8(9-77); (Alternative names: GCP/IL-8 protein III; IL8/NAP1 form VI)]	Molecular function: Cytokine; Biological process: Chemotaxis, Inflammatory response; Gene names synonyms:IL8

78_nEC	P61073	CXCR4	M	R	**C-X-C chemokine receptor type 4** - Short names: CXC-R4/CXCR-4; (Alternative names: FB22; Fusin; HM89; LCR1; Leukocyte-derived seven transmembrane domain receptor - Short name: LESTR; Lipopolysaccharide-associated protein 3 - Short names: LAP-3/LPS-associated protein 3; NPYRL; Stromal cell-derived factor 1 receptor - Short name: SDF-1 receptor; CD_antigen: CD184)	Molecular function: G-protein coupled receptor, Host cell receptor for virus entry, Receptor, Transducer; Biological process: Host-virus interaction
79_nEC	O00622	CYR61	M	0	**CCN family member 1**; (Alternative names: Cellular communication network factor 1; Cysteine-rich angiogenic inducer 61; Insulin-like growth factor-binding protein 10 - Short names: IBP-10/IGF-binding protein 10/IGFBP-10; Protein CYR61; Protein GIG1)	Molecular function: Growth factor binding, Heparin-binding; Biological process: Cell adhesion, Chemotaxis; Gene names synonyms:CYR61, GIG1, IGFBP10
80_nEC	Q9NYF0	DACT1	M	0	**Dapper homolog 1** - Short name: hDPR1; (Altenative names: Dapper antagonist of catenin 1; Hepatocellular carcinoma novel gene 3 protein)	Protein names updated; Molecular function: Developmental protein; Biological process: Neurogenesis, Wnt signaling pathway; Gene names synonyms:DPR1, HNG3
81_nEC	Q9UER7	DAXX	M	0	**Death domain-associated protein 6**; (Alternative names: Daxx - Short name: hDaxx; ETS1-associated protein 1 - Short name: EAP1; Fas death domain-associated protein)	BRENDA EC Number updated; Gene names synonyms:BING2, DAP6
82_nEC	P17661	DES	M	0	**Desmin**	Molecular function: Muscle protein
83_nEC	Q9Y4J8	DTNA	M	0	**Dystrobrevin alpha** - Short name: DTN-A; (Alternative names: Alpha-dystrobrevin; Dystrophin-related protein 3)	Function: May be involved in the formation and stability of synapses as well as being involved in the clustering of nicotinic acetylcholine receptors.; Ligand: Metal-binding, Zinc; Gene names synonyms:DRP3
84_nEC	Q01094	E2F1	M	0	**Transcription factor E2F1** - Short name: E2F-1; (Alternative names: PBR3; Retinoblastoma-associated protein 1 - Short name:RBAP-1; Retinoblastoma-binding protein 3 - Short name: RBBP-3; pRB-binding protein E2F-1)	Molecular function: Activator, DNA-binding; Biological process: Apoptosis, Cell cycle, Host-virus interaction, Transcription, Transcription regulation; Gene names synonyms:RBBP3
85_nEC	O00716	E2F3	M	0	**Transcription factor E2F3** - Short name: E2F-3	Molecular function: Activator, DNA-binding ;Biological process: Cell cycle, Transcription, Transcription regulation; Gene names synonyms:KIAA0075
86_nEC	P24530	EDNRB	M	R	**Endothelin B receptor** - Short names: ET-B/ET-BR; (Alternative name: Endothelin receptor non-selective type)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Gene names synonyms:ETRB
87_nEC	P29692	EEF1D	M	0	**Elongation factor 1-delta** - Short name: EF-1-delta; (Alternative name: Antigen NY-CO-4)	Molecular function: DNA-binding, Elongation factor; Biological process: Protein biosynthesis, Transcription, Transcription regulation; Gene names synonyms:EF1D
88_nEC	P18146	EGR1	M	0	**Early growth response protein 1** - Short name: EGR-1; (Alternative names: AT225; Nerve growth factor-induced protein A - Short name: NGFI-A; Transcription factor ETR103;	Molecular function: Activator, DNA-binding; Biological process: Biological rhythms, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:KROX24, ZNF225

					Transcription factor Zif268; Zinc finger protein 225; Zinc finger protein Krox-24)	
89_nEC	P78545	ELF3	M	0	**ETS-related transcription factor Elf-3**; (Alternative names: E74-like factor 3; Epithelial-restricted with serine box; Epithelium-restricted Ets protein ESX; Epithelium-specific Ets transcription factor 1 - Short name: ESE-1)	Molecular function: Activator, Developmental protein, DNA-binding, Repressor; Biological process: Differentiation, Inflammatory response, Transcription, Transcription regulation; Gene names synonyms:ERT, ESX, JEN
90_nEC	P15502	ELN	M	0	**Elastin**; (Alternative name: Tropoelastin)	Function: Major structural protein of tissues such as aorta and nuchal ligament, which must expand rapidly and recover completely. Molecular determinant of the late arterial morphogenesis, stabilizing arterial structure by regulating proliferation and organization of vascular smooth muscle.
91_nEC	Q99814	EPAS1	M	0	**Endothelial PAS domain-containing protein 1** - Short name: EPAS-1; (Alternative names: Basic-helix-loop-helix-PAS protein MOP2; Class E basic helix-loop-helix protein 73 - Short name: bHLHe73; HIF-1-alpha-like factor - Short name: HLF; Hypoxia-inducible factor 2-alpha - Short names: HIF-2-alpha/HIF2-alpha; Member of PAS protein 2; PAS domain-containing protein 2)	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Angiogenesis, Differentiation, Transcription, Transcription regulation; Gene names synonyms:BHLHE73, HIF2A, MOP2, PASD2
92_nEC	P01588	EPO	M	0	**Erythropoietin**; (Alternative name: INN: Epoetin)	Molecular function: Hormone; Biological process: Erythrocyte maturation
93_nEC	P03372	ESR1	M	R	**Estrogen receptor** - Short name: ER; (Alternative name: ER-alpha; Estradiol receptor; Nuclear receptor subfamily 3 group A member 1)	Molecular function:Activator, DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Lipid-binding, Metal-binding, Steroid-binding, Zinc; Gene names synonyms:ESR, NR3A1
94_nEC	P55085	F2RL1	M	R	**Proteinase-activated receptor 2** - Short name: PAR-2; (Alternative names: Coagulation factor II receptor-like 1; G-protein coupled receptor 11; Thrombin receptor-like 1); [Cleaved into the following 2 chains: Proteinase-activated receptor 2, alternate cleaved 1; Proteinase-activated receptor 2, alternate cleaved 2]	Molecular function: G-protein coupled receptor, Receptor, Transducer; Biological process: Immunity, Inflammatory response, Innate immunity; Gene names synonyms:GPR11, PAR2
95_nEC	P13726	F3	M	0	**Tissue factor** - Short name: TF; (Alternative names: Coagulation factor III; Thromboplastin; CD_antigen: CD142)	Biological process: Blood coagulation, Hemostasis
96_nEC	P51161	FABP6	M	T	**Gastrotropin** - Short name: GT; (Alternative names: Fatty acid-binding protein 6; Ileal lipid-binding protein - Short name: ILBP; Intestinal 15 kDa protein - Short name: I-15P; Intestinal bile acid-binding protein - Short name: I-BABP)	Biological process: Lipid transport, Transport; Ligand: Lipid-binding; Gene names synonyms:ILBP, ILLBP
97_nEC	P25445	FAS	M	R	**Tumor necrosis factor receptor superfamily member 6**; (Alternative names: Apo-1 antigen; Apoptosis-mediating surface antigen FAS; FASLG receptor; CD_antigen: CD95)	Molecular function: Calmodulin-binding, Receptor; Biological process: Apoptosis; Gene names synonyms:APT1, FAS1, TNFRSF6

98_nEC	Q9UGM5	FETUB	M	0	**Fetuin-B**; (Alternatve names: 16G2; Fetuin-like protein IRL685; Gugu)	Molecular function: Metalloenzyme inhibitor, Metalloprotease inhibitor, Protease inhibitor; Biological process: Fertilization
99_nEC	P02671	FGA	M	0	**Fibrinogen alpha chain**; [Cleaved into the following 2 chains: Fibrinopeptide A; Fibrinogen alpha chain]	Biological process: Adaptive immunity, Blood coagulation, Hemostasis, Immunity, Innate immunity; Ligand: Calcium, Metal-binding
100_nEC	O95750	FGF19	M	0	**Fibroblast growth factor 19** - Short name: FGF-19	Molecular function: Growth factor
101_nEC	Q9GZV9	FGF23	M	0	**Fibroblast growth factor 23** - Short name: FGF-23; (Alternative names: Phosphatonin; Tumor-derived hypophosphatemia-inducing factor); [Cleaved into the following 2 chains: Fibroblast growth factor 23 N-terminal peptide; Fibroblast growth factor 23 C-terminal peptide]	Molecular function: Growth factor; Biological process: Differentiation; Gene names synonyms:HYPF
102_nEC	P08620	FGF4	M	0	**Fibroblast growth factor 4** - Short name: FGF-4; (Alternative names: Heparin secretory-transforming protein 1 - Short names: HST/HST-1/HSTF-1; Heparin-binding growth factor 4 - Short name: HBGF-4; Transforming protein KS3)	Molecular function: Developmental protein, Growth factor, Heparin-binding, Mitogen; Biological process: Differentiation; Gene names synonyms:HST, HSTF1, KS3
103_nEC	P02679	FGG	M	0	**Fibrinogen gamma chain**	Biological process: Blood coagulation, Hemostasis; Ligand: Calcium, Metal-binding
104_nEC	Q14192	FHL2	M	0	**Four and a half LIM domains protein 2** - Short name: FHL-2; (Alternative names: LIM domain protein DRAL; Skeletal muscle LIM-protein 3 - Short name: SLIM-3)	Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:DRAL, SLIM3
105_nEC	P21333	FLNA	M	0	**Filamin-A** - Short name: FLN-A; (Alternative names: Actin-binding protein 280 - Short name: ABP-280; Alpha-filamin; Endothelial actin-binding protein; Filamin-1; Non-muscle filamin)	Molecular function: Actin-binding; Biological process: Cilium biogenesis/degradation
106_nEC	P15407	FOSL1	M	0	**Fos-related antigen 1** -Short name: FRA-1	Molecular function: DNA-binding; Gene names synonyms:FRA1
107_nEC	P55317	FOXA1	M	0	**Hepatocyte nuclear factor 3-alpha** - Short names: HNF-3-alpha/HNF-3A; (Alternative names: Forkhead box protein A1; Transcription factor 3A - Short name: TCF-3A)	Molecular function: Activator, Chromatin regulator, Developmental protein, DNA-binding, Repressor; Biological process: Transcription, Transcription regulation; Gene synonyms:HNF3A, TCF3A
108_nEC	Q9Y261	FOXA2	M	0	**Hepatocyte nuclear factor 3-beta** - Short names: HNF-3-beta/HNF-3B; (Alternative names: Forkhead box protein A2; Transcription factor 3B - Short name: TCF-3B)	Molecular function: Activator, Chromatin regulator, Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:HNF3B, TCF3B
109_nEC	P55318	FOXA3	M	0	**Hepatocyte nuclear factor 3-gamma** - Short names: HNF-3-gamma/HNF-3G; (Alternative names: Fork head-related protein FKH H3; Forkhead box protein A3; Transcription factor 3G - Short name: TCF-3G)	Molecular function: Activator, Chromatin regulator, Developmental protein, DNA-binding; Biological process: Differentiation, Spermatogenesis, Transcription, Transcription regulation; Gene names synonyms:HNF3G, TCF3G

110_nEC	Q08050	FOXM1	M	0	**Forkhead box protein M1**; (Alternative names: Forkhead-related protein FKHL16; Hepatocyte nuclear factor 3 forkhead homolog 11 - Short names: HFH-11; HNF-3/fork-head homolog 11; M-phase phosphoprotein 2; MPM-2 reactive phosphoprotein 2; Transcription factor Trident; Winged-helix factor from INS-1 cells)	Molecular function: Activator, DNA-binding; Biological process: Cell cycle, DNA damage, DNA repair, Transcription, Transcription regulation; Gene names synonyms:FKHL16, HFH11, MPP2, WIN
111_nEC	Q12778	FOXO1	M	0	**Forkhead box protein O1**; (Alternative names: Forkhead box protein O1A; Forkhead in rhabdomyosarcoma)	Molecular function: Activator, DNA-binding; Biological process: Apoptosis, Autophagy, Differentiation, Transcription, Transcription regulation; Gene names synonyms:FKHR, FOXO1A
112_nEC	O43524	FOXO3	M	0	**Forkhead box protein O3**; (Alternative names: AF6q21 protein; Forkhead in rhabdomyosarcoma-like 1)	Molecular function: Activator, DNA-binding; Biological process: Apoptosis, Transcription, Transcription regulation; Gene names synonyms:FKHRL1, FOXO3A
113_nEC	Q12841	FSTL1	M	0	**Follistatin-related protein 1**; (Alternative name: Follistatin-like protein 1)	Function: May modulate the action of some growth factors on cell proliferation and differentiation.; Molecular function: Heparin-binding; Gene names synonyms:FRP
114_nEC	O75293	GADD45B	M	0	**Growth arrest and DNA damage-inducible protein GADD45 beta**; (Alternative names: Myeloid differentiation primary response protein MyD118; Negative growth regulatory protein MyD118)	Molecular function: Developmental protein; Biological process: Apoptosis, Differentiation; Gene names synonyms:MYD118
115_nEC	P22466	GAL	M	0	**Galanin peptides**; [Cleaved into the following 2 chains: Galanin; Galanin message-associated peptide - Short name: GMAP)]	Molecular function: Hormone, Neuropeptide; Gene names synonyms:GAL1, GALN, GLNN
116_nEC	P43694	GATA4	M	0	**Transcription factor GATA-4**; (Alternative name: GATA-binding factor 4)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc
117_nEC	Q92908	GATA6	M	0	**Transcription factor GATA-6**; (Alternative name: GATA-binding factor 6)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc
118_nEC	P02774	GC	M	T	**Vitamin D-binding protein** - Short names: DBP/VDB; (Alternative names: Gc protein-derived macrophage activating factor - Short names: Gc-MAF/GcMAF; Gc-globulin; Group-specific component - Short name: Gc; Vitamin D-binding protein-macrophage activating factor - Short name: DBP-maf)	Molecular function: Actin-binding; Biological process: Transport; Ligand: Vitamin D
119_nEC	P01275	GCG	M	0	**Pro-Glucagon**; [Cleaved into the following 8 chains: Glicentin; Glicentin-related polypeptide - Short name: GRPP; Oxyntomodulin - Short names: OXM/OXY; Glucagon; Glucagon-like peptide 1 - Short name: GLP-1; (Alternative name: Incretin hormone); Glucagon-like peptide 1(7-37)- Short name: GLP-1(7-	Molecular function: Hormone

					37); Glucagon-like peptide 1(7-36) - Short name: GLP-1(7-36); Glucagon-like peptide 2 (GLP-2)]	
120_nEC	P47871	GCGR	M	R	**Glucagon receptor** - Short name: GL-R	Molecular function: G-protein coupled receptor, Receptor, Transducer
121_nEC	Q8N6F7	GCSAM	M	0	**Germinal center-associated signaling and motility protein**; (Alternative names: Germinal center B-cell-expressed transcript 2 protein; Germinal center-associated lymphoma protein - Short name: hGAL)	GO - Molecular function: actin binding, myosin II binding, protein kinase binding; GO - Biological process: negative regulation of lymphocyte migration, regulation of B cell receptor signaling pathway; Gene names synonyms:GAL, GCET2
122_nEC	Q9UK05	GDF2	M	0	**Growth/differentiation factor 2** - Short name: GDF-2; (Alternative name: Bone morphogenetic protein 9 - Short name: BMP-9)	Molecular function: Cytokine, Growth factor; Biological process: Angiogenesis; Gene names synonyms:BMP9
123_nEC	P01241	GH1	M	0	**Somatotropin**; (Alternative names: Growth hormone - Short names: GH/GH-N; Growth hormone 1; Pituitary growth hormone)	Molecular function: Hormone; Ligand: Metal-binding, Zinc
124_nEC	P01286	GHRH	M	0	**Somatoliberin**; (Alternative names: Growth hormone-releasing factor - Short name: GRF; Growth hormone-releasing hormone - Short name: GHRH; Somatocrinin; Somatorelin; INN: Sermorelin)	Function: GRF is released by the hypothalamus and acts on the adenohypophyse to stimulate the secretion of growth hormone.; Gene names synonyms:GHRF
125_nEC	Q9UBU3	GHRL	M	0	**Appetite-regulating hormone**; (Alternative names: Growth hormone secretagogue; Growth hormone-releasing peptide; Motilin-related peptide; Protein M46); [Cleaved into the following 3 chains: Ghrelin-27; Ghrelin-28 - Short name: Ghrelin; Obestatin]	Molecular function: Hormone; Gene names synonyms:MTLRP
126_nEC	P04899	GNAI2	M	Trans	**Guanine nucleotide-binding protein G(i) subunit alpha-2**; (Alternative name: Adenylate cyclase-inhibiting G alpha protein)	Molecular function: Transducer; Biological process: Cell cycle, Cell division; Ligand: GTP-binding, Magnesium, Metal-binding, Nucleotide-binding; Gene names synonyms:GNAI2B
127_nEC	P07359	GP1BA	M	0	**Platelet glycoprotein Ib alpha chain** - Short names: GP-Ib alpha/GPIb-alpha/GPIbA/Glycoprotein Ibalpha); (Alternative name: Antigen CD42b-alpha; CD_antigen: CD42b); [Cleaved into the following chain: Glycocalicin]	Biological process: Blood coagulation, Cell adhesion, Hemostasis
128_nEC	P62993	GRB2	M	0	**Growth factor receptor-bound protein 2**; (Alternative names: Adapter protein GRB2; Protein Ash; SH2/SH3 adapter GRB2)	Biological process: Host-virus interaction; Gene names synonyms:ASH
129_nEC	O96004	HAND1	M	0	**Heart- and neural crest derivatives-expressed protein 1**; (Alternative names: Class A basic helix-loop-helix protein 27 - Short name: bHLHa27; Extraembryonic tissues, heart, autonomic nervous system and neural crest derivatives-expressed protein 1 - Short name: eHAND)	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHA27, EHAND
130_nEC	Q99075	HBEGF	M	R	**Proheparin-binding EGF-like growth factor**; [Cleaved into the following chain: Heparin-binding EGF-like growth factor - Short	Molecular function: Growth factor, Heparin-binding, Receptor; Gene names synonyms:DTR, DTS, HEGFL

					names: HB-EGF/HBEGF; (Alternative name: Diphtheria toxin receptor -Short name: DT-R)]	
131_nEC	Q16665	HIF1A	M	0	**Hypoxia-inducible factor 1-alpha** - Short names: HIF-1-alpha/HIF1-alpha; (Alternative names: ARNT-interacting protein; Basic-helix-loop-helix-PAS protein MOP1; Class E basic helix-loop-helix protein 78 - Short name: bHLHe78; Member of PAS protein 1; PAS domain-containing protein 8)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHE78, MOP1, PASD8
132_nEC	P17096	HMGA1	M	0	**High mobility group protein HMG-I/HMG-Y** - Short name: HMG-I(Y); (Alternative names: High mobility group AT-hook protein 1 - Short name: High mobility group protein A1; High mobility group protein R)	Molecular function: DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:HMGIY
133_nEC	O75330	HMMR	M	R	**Hyaluronan mediated motility receptor**; (Alternative names: Intracellular hyaluronic acid-binding protein; Receptor for hyaluronan-mediated motility; CD_antigen: CD168)	Ligand: Hyaluronic acid;Gene names synonyms:IHABP, RHAMM
134_nEC	P20823	HNF1A	M	0	**Hepatocyte nuclear factor 1-alpha** - Short names: HNF-1-alpha/HNF-1A; (Alternative names: Liver-specific transcription factor LF-B1 - Short name: LFB1; Transcription factor 1 - Short name: TCF-1)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:TCF1
135_nEC	P41235	HNF4A	M	R	**Hepatocyte nuclear factor 4-alpha** -Short name: HNF-4-alpha; (Alternative names: Nuclear receptor subfamily 2 group A member 1; Transcription factor 14 - Short name: TCF-14; Transcription factor HNF-4)	Molecular function: Activator, DNA-binding, Receptor, Repressor; Biological process: Biological rhythms, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:HNF4, NR2A1, TCF14
136_nEC	Q9BPY8	HOPX	M	0	**Homeodomain-only protein**; (Alternative names: Lung cancer-associated Y protein; Not expressed in choriocarcinoma protein 1; Odd homeobox protein 1)	Molecular function: Developmental protein, Repressor; Biological process: Transcription, Transcription regulation; Gene names synonyms:HOD, HOP, LAGY, NECC1, OB1
137_nEC	P25021	HRH2	M	R	**Histamine H2 receptor** - Short names: H2R/HH2R); (Alternative name: Gastric receptor I)	Molecular function: G-protein coupled receptor, Receptor, Transducer
138_nEC	P28222	HTR1B	M	R	**5-hydroxytryptamine receptor 1B** - Short names: 5-HT-1B/5-HT1B; (Alternative names: S12; Serotonin 1D beta receptor - Short name: 5-HT-1D-beta; Serotonin receptor 1B)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Biological process: Behavior; Gene names synonyms:HTR1DB
139_nEC	P28223	HTR2A	M	R	**5-hydroxytryptamine receptor 2A** - Short names: 5-HT-2/5-HT-2A; (Alternative name: Serotonin receptor 2A)	Molecular function: G-protein coupled receptor, Host cell receptor for virus entry, Receptor, Transducer; Biological process: Behavior, Host-virus interaction; Gene names synonyms:HTR2
140_nEC	P28335	HTR2C	M	R	**5-hydroxytryptamine receptor 2C** - Short names: 5-HT-2C/5-HT2C/5-HTR2C; (Alternative names: 5-hydroxytryptamine receptor 1C - Short names: 5-HT-1C/5-HT1C; Serotonin receptor 2C)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Biological process: Behavior; Gene names synonyms:HTR1C

141_nEC	P05362	ICAM1	M	R, Trans	**Intercellular adhesion molecule 1** - Short name: ICAM-1; (Alternative names: Major group rhinovirus receptor; CD_antigen: CD54)	Molecular function: Host cell receptor for virus entry, Receptor; Biological process: Cell adhesion, Host-virus interaction
142_nEC	Q9Y6W8	ICOS	M	0	**Inducible T-cell costimulator**; (Alternative names: Activation-inducible lymphocyte immunomediatory molecule; CD_antigen: CD278)	GO - Biological process: cell-cell adhesion, immune response, positive regulation of protein kinase B signaling, T cell costimulation, T cell tolerance induction; Gene names synonyms:AILIM
143_nEC	P46695	IER3	M	0	**Radiation-inducible immediate-early gene IEX-1**; (Alternative names: Differentiation-dependent gene 2 protein - Short name: Protein DIF-2; Immediate early protein GLY96; Immediate early response 3 protein; PACAP-responsive gene 1 protein - Short name: Protein PRG1)	GO - Biological process: anatomical structure morphogenesis, apoptotic process, negative regulation of apoptotic process, regulation of phosphatidylinositol 3-kinase signaling, regulation of response to DNA damage stimulus; Gene names synonyms:DIF2, IEX1, PRG1
144_nEC	P01579	IFNG	M	0	**Interferon gamma** - Short name: IFN-gamma; (Alternative name: Immune interferon)	Molecular function: Cytokine; Biological process: Antiviral defense, Growth regulation
145_nEC	P08833	IGFBP1	M	0	**Insulin-like growth factor-binding protein 1** - Short names: IBP-1/IGF-binding protein 1/IGFBP-1; (Alternative name: Placental protein 12 - Short name: PP12)	Molecular function: Growth factor binding; Gene names synonyms:IBP1
146_nEC	P18065	IGFBP2	M	0	**Insulin-like growth factor-binding protein 2** - Short names: IBP-2/IGF-binding protein 2/IGFBP-2)	Molecular function: Growth factor binding; Biological process: Growth regulation; Gene names synonyms:BP2, IBP2
147_nEC	P22692	IGFBP4	M	0	**Insulin-like growth factor-binding protein 4** - Short names: IBP-4/IGF-binding protein 4/IGFBP-4)	Molecular function: Growth factor binding; Gene names synonyms IBP4
148_nEC	P22301	IL10	M	0	**Interleukin-10** - Short name: IL-10; (Alternative name: Cytokine synthesis inhibitory factor - Short name: CSIF)	Molecular function: Cytokine
149_nEC	P20809	IL11	M	0	**Interleukin-11** - Short name: IL-11; (Alternative names: Adipogenesis inhibitory factor - Short name: AGIF; INN: Oprelvekin)	Molecular function: Cytokine, Growth factor
150_nEC	P29459	IL12A	M	0	**Interleukin-12 subunit alpha** - Short name: IL-12A; (Alternative names: Cytotoxic lymphocyte maturation factor 35 kDa subunit - Short name: CLMF p35; IL-12 subunit p35; NK cell stimulatory factor chain 1 - Short name: NKSF1)	Molecular function: Cytokine, Growth factor; Biological process: Host-virus interaction; Gene names synonyms:NKSF1
151_nEC	P29460	IL12B	M	0	**Interleukin-12 subunit beta** - Short name: IL-12B; (Alternative names: Cytotoxic lymphocyte maturation factor 40 kDa subunit - Short name: CLMF p40; IL-12 subunit p40; NK cell stimulatory factor chain 2 - Short name: NKSF2)	Molecular function: Cytokine; Gene names synonyms:NKSF2
152_nEC	P40933	IL15	M	0	**Interleukin-15** - Short name: IL-15	Molecular function: Cytokine
153_nEC	Q16552	IL17A	M	0	**Interleukin-17A** - Short names: IL-17/IL-17A; (Alternative name: Cytotoxic T-lymphocyte-associated antigen 8 - Short name: CTLA-8)	Funtion: Ligand for IL17RA and IL17RC. ; Molecular function:Cytokine; Gene names synonyms:CTLA8, IL17

154_nEC	Q9UHF5	IL17B	M	0	**Interleukin-17B** - Short name: IL-17B; (Alternative name: Cytokine Zcyto7; Interleukin-20 - Short name: IL-20; Neuronal interleukin-17-related factor)	Function: Stimulates the release of tumor necrosis factor alpha and IL-1-beta from the monocytic cell line THP-1.; Molecular function: Cytokine; Gene names synonyms:IL20, NIRF, ZCYTO7
155_nEC	Q9P0M4	IL17C	M	0	**Interleukin-17C** - Short name: IL-17C; (Alternative name: Cytokine CX2)	Molecular function: Cytokine; Biological process: Inflammatory response
156_nEC	Q8TAD2	IL17D	M	0	**Interleukin-17D** - Short name: IL-17D; (Alternative name: Interleukin-27 - Short name: IL-27)	Function: Induces expression of IL6, CXCL8/IL8, and CSF2/GM-CSF from endothelial cells.; Molecular function: Cytokine
157_nEC	Q96PD4	IL17F	M	0	**Interleukin-17F** - Short name: IL-17F; (Alternative name: Cytokine ML-1)	Function: Ligand for IL17RA and IL17RC; Molecular function: Cytokine
158_nEC	Q96F46	IL17RA	M	R	**Interleukin-17 receptor A** - Short names: IL-17 receptor A/IL-17RA; (Alternative names: CDw217; CD_antigen: CD217)	Molecular function: Receptor; Gene names synonyms:IL17R
159_nEC	P01583	IL1A	M	0	**Interleukin-1 alpha** - Short name: IL-1 alpha; (Alternative name: Hematopoietin-1)	Molecular function: Cytokine, Mitogen, Pyrogen; Biological process: Inflammatory response; Gene names synonyms:IL1F1
160_nEC	Q8WWZ1	IL1F10	M	0	**Interleukin-1 family member 10** - Short name: IL-1F10; (Alternative names: Family of interleukin 1-theta - Short name: FIL1 theta; Interleukin-1 HY2 - Short name: IL-1HY2; Interleukin-1 theta - Shortname: IL-1 theta; Interleukin-38 - Short name: IL-38)	Function: Cytokine with immunomodulatory activity.; Molecular function: Cytokine; Gene names synonyms:FIL1T, IL1HY2, IL38
161_nEC	P27930	IL1R2	M	R	**Interleukin-1 receptor type 2** - Short names: IL-1R-2/IL-1RT-2/IL-1RT2; (Alternative names: CD121 antigen-like family member B; CDw121b; IL-1 type II receptor; Interleukin-1 receptor beta - Short name: IL-1R-beta; Interleukin-1 receptor type II; CD_antigen: CD121b); [Cleaved into the following 2 chains: Interleukin-1 receptor type 2, membrane form - Short names: mIL-1R2/mIL-1RII; Interleukin-1 receptor type 2, soluble form - Short names: sIL-1R2/ sIL-1RII]	Function: Non-signaling receptor for IL1A, IL1B and IL1RN. Reduces IL1B activities.; Molecular function: Receptor; Gene names synonyms:IL1RB
162_nEC	Q9HBE5	IL21R	M	R	**Interleukin-21 receptor** - Short names: IL-21 receptor/IL-21R); (Alternative names: Novel interleukin receptor; CD_antigen: CD360)	Function: This is a receptor for interleukin-21.; Molecular function: Receptor; Gene names synonyms:NILR
163_nEC	Q9H293	IL25	M	0	**Interleukin-25** - Short name: IL-25; (Alternative name:Interleukin-17E - Short name: IL-17E)	Function: Induces activation of NF-kappa-B and stimulates production of the proinflammatory chemokine IL-8. Proinflammatory cytokine favoring Th2-type immune responses.; Molecular function: Cytokine; Gene names synonyms:IL17E
164_nEC	P08700	IL3	M	0	**Interleukin-3** - Short name: IL-3; (Alternative names: Hematopoietic growth factor; Mast cell growth factor - Short	Molecular function: Cytokine, Growth factor

					name: MCGF; Multipotential colony-stimulating factor; P-cell-stimulating factor)	
165_nEC	Q9UHA7	IL36A	M	0	**Interleukin-36 alpha**; (Alternative names: FIL1 epsilon; Interleukin-1 epsilon - Short name: IL-1 epsilon; Interleukin-1 family member 6 - Short name: IL-1F6)	Molecular function: Cytokine; Biological process: Immunity, Inflammatory response, Innate immunity; Gene names synonyms:FIL1E, IL1E, IL1F6
166_nEC	Q9UBH0	IL36RN	M	0	**Interleukin-36 receptor antagonist protein** - Short name: IL-36Ra; (Alternative names: FIL1 delta; IL-1-related protein 3 - Short name: IL-1RP3; Interleukin-1 HY1 - Short name: IL-1HY1; Interleukin-1 delta - Short name: IL-1 delta; Interleukin-1 family member 5 - Short name: IL-1F5; Interleukin-1 receptor antagonist homolog 1 - Short name: IL-1ra homolog 1; Interleukin-1-like protein 1 - Short name: IL-1L1)	Molecular function: Cytokine; Biological process: Immunity, Innate immunity; Gene names synonyms:FIL1D, IL1F5, IL1HY1, IL1L1, IL1RP3
167_nEC	P24394	IL4R	M	R	**Interleukin-4 receptor subunit alpha** - Short names: IL-4 receptor subunit alpha/IL-4R subunit alpha/IL-4R-alpha/IL-4RA; (Alternative name: CD_antigen: CD124); [Cleaved into the following chain: Soluble interleukin-4 receptor subunit alpha - Short names: Soluble IL-4 receptor subunit alpha/Soluble IL-4R-alpha/sIL4Ralpha/prot; (Alternative name: IL-4-binding protein - Short name:IL4-BP)]	Molecular function: Receptor; Biological process: Immunity; Gene names synonyms:IL4RA
168_nEC	P05231	IL6	M	0	**Interleukin-6** - Short name: IL-6; (Alternative names: B-cell stimulatory factor 2 - Short name: BSF-2; CTL differentiation factor - Short name: CDF; Hybridoma growth factor; Interferon beta-2 - Short name: IFN-beta-2)	Molecular function: Cytokine, Growth factor; Biological process: Acute phase; Gene names synonyms:IFNB2
169_nEC	P08887	IL6R	M, B	R	**Interleukin-6 receptor subunit alpha** - Short names: IL-6 receptor subunit alpha/IL-6R subunit alpha/IL-6R-alpha/IL-6RA); (Alternative names: IL-6R 1; Membrane glycoprotein 80 - Short name: gp80; CD_antigen: CD126) [Cleaved into the following chain: Soluble interleukin-6 receptor subunit alpha - Short name: sIL6R]	Molecular function: Receptor
170_nEC	P05111	INHA	M	0	**Inhibin alpha chain**	Molecular function: Growth factor, Hormone
171_nEC	P08476	INHBA	M	0	**Inhibin beta A chain**; (Alternative names: Activin beta-A chain; Erythroid differentiation protein - Short name: EDF)	Molecular function: Growth factor, Hormone
172_nEC	Q14653	IRF3	M	0	**Interferon regulatory factor 3** - Short name: IRF-3	Molecular function: Activator, DNA-binding; Biological process: Antiviral defense, Host-virus interaction, Immunity, Innate immunity, Transcription, Transcription regulation
173_nEC	P78413	IRX4	M	0	**Iroquois-class homeodomain protein IRX-4**; (Alternative names: Homeodomain protein IRXA3; Iroquois homeobox protein 4)	Function: Likely to be an important mediator of ventricular differentiation during cardiac development.; Molecular function: DNA-binding; Gene names synonyms:IRXA3

174_nEC	Q9Y6Y0	IVNS1ABP	M	0	**Influenza virus NS1A-binding protein** - Short names: NS1-BP/NS1-binding protein; (Alternative names: Aryl hydrocarbon receptor-associated protein 3; Kelch-like protein 39)	Protein names updated; Biological process: Host-virus interaction; Gene names synonyms:ARA3, FLARA3, KIAA0850, KLHL39, NS1, NS1BP
175_nEC	P17535	JUND	M	0	**Transcription factor jun-D**	Function: Transcription factor binding AP-1 sites.; Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation
176_nEC	Q14654	KCNJ11	M	C, R	**ATP-sensitive inward rectifier potassium channel 11**; (Alternative names: IKATP; Inward rectifier K(+) channel Kir6.2; Potassium channel, inwardly rectifying subfamily J member 11)	Molecular function: Ion channel, Voltage-gated channel; Biological process: Ion transport, Potassium transport, Transport; Ligand: Potassium
177_nEC	Q9Y5W3	KLF2	M	0	**Krueppel-like factor 2**; (Alternative name: Lung krueppel-like factor)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:LKLF
178_nEC	P18428	LBP	M	0	**Lipopolysaccharide-binding protein** - Short name: LBP	Molecular function: Antibiotic, Antimicrobial; Biological process: Immunity, Innate immunity, Lipid transport, Transport
179_nEC	P80188	LCN2	M	T	**Neutrophil gelatinase-associated lipocalin** - Short name: NGAL; (Alternative names: 25 kDa alpha-2-microglobulin-related subunit of MMP-9; Lipocalin-2; Oncogene 24p3; Siderocalin LCN2; p25)	Biological process: Apoptosis, Immunity, Innate immunity, Ion transport, Iron transport, Transport; Ligand: Iron; Gene names synonyms:HNL, NGAL
180_nEC	P01130	LDLR	M	R	**Low-density lipoprotein receptor** - Short name: LDL receptor	Molecular function: Host cell receptor for virus entry, Receptor; Biological process: Cholesterol metabolism, Endocytosis, Host-virus interaction, Lipid metabolism, Lipid transport, Steroid metabolism, Sterol metabolism, Transport
181_nEC	P41159	LEP	M	0	**Leptin**; (Alternative names: Obese protein; Obesity factor)	Function: Key player in the regulation of energy balance and body weight control. Once released into the circulation, has central and peripheral effects by binding LEPR, found in many tissues, which results in the activation of several major signaling pathways.; Gene names synonyms:OB, OBS
182_nEC	P15018	LIF	M	0	**Leukemia inhibitory factor** - Short name: LIF; (Alternative names: Differentiation-stimulating factor - Short name: D factor; Melanoma-derived LPL inhibitor - Short name: MLPLI; INN: Emfilermin)	Molecular function: Cytokine, Growth factor; Gene names synonyms:HILDA
183_nEC	Q7Z4I7	LIMS2	M	0	**LIM and senescent cell antigen-like-containing domain protein 2**; (Alternative names: LIM-like protein 2; Particularly interesting new Cys-His protein 2 - Short name: PINCH-2)	Function: Adapter protein in a cytoplasmic complex linking beta-integrins to the actin cytoskeleton, bridges the complex to cell surface receptor tyrosine kinases and growth factor receptors. Plays a role in modulating cell spreading and migration.; Ligand: Metal-binding, Zinc; Gene names synonyms:PINCH2

184_nEC	Q9NZU5	LMCD1	M	0	**LIM and cysteine-rich domains protein 1**; (Alternative name: Dyxin)	Molecular function: Repressor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc
185_nEC	P02545	LMNA	M	0	**Prelamin-A/C** [Cleaved into the following chain: Lamin-A/C; (Alternative names: 70 kDa lamin; Renal carcinoma antigen NY-REN-32)]	Function: Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. ; Gene names synonyms:LMN1
186_nEC	P01374	LTA	M	0	**Lymphotoxin-alpha** - Short name: LT-alpha; (Alternative names: TNF-beta; Tumor necrosis factor ligand superfamily member 1)	Function : Cytokine that in its homotrimeric form binds to TNFRSF1A/TNFR1, TNFRSF1B/TNFBR and TNFRSF14/HVEM. In its heterotrimeric form with LTB binds to TNFRSF3/LTBR. Lymphotoxin is produced by lymphocytes and is cytotoxic for a wide range of tumor cells in vitro and in vivo.; Molecular function: Cytokine; Gene names synonyms:TNFB, TNFSF1
187_nEC	Q06643	LTB	M	0	**Lymphotoxin-beta** - Short name: LT-beta; (Alternative names: Tumor necrosis factor C - Short name: TNF-C; Tumor necrosis factor ligand superfamily member 3)	Function: Cytokine that binds to LTBR/TNFRSF3.; Molecular function: Cytokine; Gene names synonyms:TNFC, TNFSF3
188_nEC	Q9HBG7	LY9	M	R	**T-lymphocyte surface antigen Ly-9**; (Alternative names: Cell surface molecule Ly-9; Lymphocyte antigen 9; SLAM family member 3 - Short name: SLAMF3; Signaling lymphocytic activation molecule 3; CD_antigen: CD229)	Biological process: Adaptive immunity, Cell adhesion, Immunity, Innate immunity
189_nEC	Q9ULX9	MAFF	M	0	**Transcription factor MafF**; (Alternative names: U-Maf; V-maf musculoaponeurotic fibrosarcoma oncogene homolog F)	Molecular function: DNA-binding, Repressor; Biological process: Stress response, Transcription, Transcription regulation
190_nEC	P55145	MANF	M	0	**Mesencephalic astrocyte-derived neurotrophic factor**; (Alternative names: Arginine-rich protein; Protein ARMET)	Molecular function: Growth factor; Biological process: Stress response, Unfolded protein response; Ligand: Lipid-binding, Sialic acid; Gene names synonyms:ARMET, ARP
191_nEC	P21941	MATN1	M	0	**Cartilage matrix protein**; (Alternative name: Matrilin-1)	Function: Cartilage matrix protein is a major component of the extracellular matrix of non-articular cartilage. It binds to collagen.; Gene names synonyms:CMP, CRTM
192_nEC	P02144	MB	M	T	**Myoglobin**	Function: Serves as a reserve supply of oxygen and facilitates the movement of oxygen within muscles.; Molecular function: Muscle protein; Biological process: Oxygen transport, Transport; Ligand: Heme, Iron, Metal-binding
193_nEC	Q07820	MCL1	M	C	**Induced myeloid leukemia cell differentiation protein Mcl-1**; (Alternative names: Bcl-2-like protein 3 - Short name: Bcl2-L-3; Bcl-2-related protein EAT/mcl1; mcl1/EAT)	Molecular function: Developmental protein; Biological process: Apoptosis, Differentiation; Gene names synonyms:BCL2L3

194_nEC	P21741	MDK	M	0	**Midkine** - Short name: MK; (Alternative name: Amphiregulin-associated protein - Short name: ARAP; Midgestation and kidney protein; Neurite outgrowth-promoting factor 2; Neurite outgrowth-promoting protein)	Molecular function: Developmental protein, Growth factor, Heparin-binding, Mitogen; Biological process: Differentiation; Gene names synonyms:MK1, NEGF2
195_nEC	Q969V6	MKL1	M	0	**Myocardin-related transcription factor A** - Short name: MRTF-A; (Alternative name: MKL/myocardin-like protein 1; Megakaryoblastic leukemia 1 protein; Megakaryocytic acute leukemia protein)	Molecular function: Actin-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:KIAA1438, MAL, MKL1
196_nEC	Q99583	MNT	M	0	**Max-binding protein MNT**; (Alternative names: Class D basic helix-loop-helix protein 3 - Short name: bHLHd3; Myc antagonist MNT; Protein ROX)	Molecular function: DNA-binding, Repressor; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHD3, ROX
197_nEC	P04731	MT1A	M	0	**Metallothionein-1A** - Short name: MT-1A; (Alternative name: Metallothionein-IA - Short name: MT-IA)	Function: Metallothioneins have a high content of cysteine residues that bind various heavy metals; these proteins are transcriptionally regulated by both heavy metals and glucocorticoids; Ligand: Cadmium, Copper, Metal-binding, Metal-thiolate cluster, Zinc; Gene names synonyms:MT1S
198_nEC	Q14896	MYBPC3	M	0	**Myosin-binding protein C, cardiac-type** - Short name: Cardiac MyBP-C; (Alternative name: C-protein, cardiac muscle isoform)	Molecular function: Actin-binding, Muscle protein; Biological process: Cell adhesion; Ligand: Metal-binding, Zinc
199_nEC	P01106	MYC	M	0	**Myc proto-oncogene protein**; (Alternative names: Class E basic helix-loop-helix protein 39 - Short name: bHLHe39; Proto-oncogene c-Myc; Transcription factor p64)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHE39
200_nEC	P04198	MYCN	M	0	**N-myc proto-oncogene protein**; (Alternative name: Class E basic helix-loop-helix protein 37 - Short name: bHLHe37)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHE37, NMYC
201_nEC	P13533	MYH6	M	0	**Myosin-6**; (Alternative names: Myosin heavy chain 6; Myosin heavy chain, cardiac muscle alpha isoform - Short name: MyHC-alpha)	Molecular function: Actin-binding, Calmodulin-binding, Motor protein, Muscle protein, Myosin; Ligand: ATP-binding, Nucleotide-binding; Gene names synonyms:MYHCA
202_nEC	P12883	MYH7	M	0	**Myosin-7**; (Alternative names: Myosin heavy chain 7; Myosin heavy chain slow isoform - Short name: MyHC-slow; Myosin heavy chain, cardiac muscle beta isoform - Short name: MyHC-beta)	Molecular function: Actin-binding, Calmodulin-binding, Motor protein, Muscle protein, Myosin; Ligand: ATP-binding, Nucleotide-binding; Gene names synonyms:MYHCB
203_nEC	P24844	MYL9	M	0	**Myosin regulatory light polypeptide 9**; (Alternative names: 20 kDa myosin light chain - Short name: LC20; MLC-2C; Myosin RLC; Myosin regulatory light chain 2, smooth muscle isoform; Myosin regulatory light chain 9; Myosin regulatory light chain MRLC1)	Molecular function: Motor protein, Muscle protein, Myosin; Ligand: Calcium, Metal-binding; Gene names synonyms:MLC2, MRLC1, MYRL2
204_nEC	Q8IZQ8	MYOCD	M	0	**Myocardin**	Molecular function: Activator; Biological process: Transcription, Transcription regulation; Gene names synonyms:MYCD

205_nEC	P15172	MYOD1	M	0	**Myoblast determination protein 1**; (Alternative names: Class C basic helix-loop-helix protein 1 - Short name: bHLHc1; Myogenic factor 3 - Short name: Myf-3)	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Differentiation, Myogenesis, Transcription, Transcription regulation; Gene names synonyms:BHLHC1, MYF3, MYOD
206_nEC	P60321	NANOS2	M	0	**Nanos homolog 2** - Short name: NOS-2)	Molecular function: Developmental protein, RNA-binding; Biological process: Differentiation, Spermatogenesis, Translation regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NOS2
207_nEC	P60323	NANOS3	M	0	**Nanos homolog 3** - Short name: NOS-3	Molecular function: Developmental protein, RNA-binding; Biological process: Differentiation, Oogenesis, Spermatogenesis, Translation regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NOS3
208_nEC	Q9BPX3	NCAPG	M	0	**Condensin complex subunit 3**; (Alternative names: Chromosome-associated protein G; Condensin subunit CAP-G - Short name: hCAP-G; Melanoma antigen NY-MEL-3; Non-SMC condensin I complex subunit G; XCAP-G homolog)	Biological process: Cell cycle, Cell division, DNA condensation, Mitosis; Gene names synonyms:CAPG, NYMEL3
209_nEC	Q9Y618	NCOR2	M	R	**Nuclear receptor corepressor 2** - Short name: N-CoR2; (Alternative names: CTG repeat protein 26; SMAP270; Silencing mediator of retinoic acid and thyroid hormone receptor - Short name: SMRT; T3 receptor-associating factor - Short name: TRAC; Thyroid-, retinoic-acid-receptor-associated corepressor)	Molecular function: DNA-binding, Repressor; Biological process: Transcription, Transcription regulation; Gene names synonyms:CTG26
210_nEC	O75380	NDUFS6	M	Ca, Mito	**NADH dehydrogenase [ubiquinone] iron-sulfur protein 6, mitochondrial**; (Alternative names: Complex I-13kD-A - Short name: CI-13kD-A; NADH-ubiquinone oxidoreductase 13 kDa-A subunit)	Biological process: Electron transport, Respiratory chain, Transport
211_nEC	Q92692	NECTIN2	M	R	**Nectin-2**; (Alternative names: Herpes virus entry mediator B - Short names: Herpesvirus entry mediator B/HveB; Nectin cell adhesion molecule 2; Poliovirus receptor-related protein 2; CD_antigen: CD112)	Molecular function: Host cell receptor for virus entry, Receptor; Biological process: Cell adhesion, Host-virus interaction; Gene names synonyms:HVEB, PRR2, PVRL2
212_nEC	O95644	NFATC1	M	0	**Nuclear factor of activated T-cells, cytoplasmic 1** - Short names: NF-ATc1/NFATc1; (Alternative name: NFAT transcription complex cytosolic component - Short names: NF-Atc/NFATc)	Molecular function: Activator, DNA-binding, Repressor; Biological process: Transcription, Transcription regulation; Gene names synonyms:NFAT2, NFATC
213_nEC	Q13469	NFATC2	M	0	**Nuclear factor of activated T-cells, cytoplasmic 2** - Short names: NF-ATc2/NFATc2; (Alternative names: NFAT pre-existing subunit - Short name: NF-Atp; T-cell transcription factor NFAT1)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:NFAT1, NFATP
214_nEC	Q14934	NFATC4	M	0	**Nuclear factor of activated T-cells, cytoplasmic 4** - Short names: NF-ATc4/NFATc4; (Alternative name: T-cell transcription factor NFAT3 - Short name: NF-AT3)	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Differentiation, Transcription, Transcription regulation; Gene names synonyms:NFAT3

215_nEC	Q00653	NFKB2	M	0	**Nuclear factor NF-kappa-B p100 subunit**; (Alternative names: DNA-binding factor KBF2; H2TF1; Lymphocyte translocation chromosome 10 protein; Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2; Oncogene Lyt-10 - Short name: Lyt10); [Cleaved into the following chain: Nuclear factor NF-kappa-B p52 subunit]	Molecular function: Activator, DNA-binding, Repressor; Biological process: Biological rhythms, Transcription, Transcription regulation; Gene names synonyms:LYT10
216_nEC	P25963	NFKBIA	M	0	**NF-kappa-B inhibitor alpha**; (Alternative names: I-kappa-B-alpha - Short names: IkB-alpha/IkappaBalpha; Major histocompatibility complex enhancer-binding protein MAD3)	Function: Inhibits the activity of dimeric NF-kappa-B/REL complexes by trapping REL dimers in the cytoplasm through masking of their nuclear localization signals. On cellular stimulation by immune and proinflammatory responses, becomes phosphorylated promoting ubiquitination and degradation, enabling the dimeric RELA to translocate to the nucleus and activate transcription.; Biological process: Host-virus interaction; Gene names synonyms:IKBA, MAD3, NFKBI
217_nEC	Q15653	NFKBIB	M	0	**NF-kappa-B inhibitor beta** - Short name: NF-kappa-BIB; (Alternative names: I-kappa-B-beta - Short names: IkB-B/IkB-beta/IkappaBbeta; Thyroid receptor-interacting protein 9 - Short names: TR-interacting protein 9/TRIP-9)	GO - Molecular function: transcription coactivator activity; GO - Biological process: cellular response to lipopolysaccharide, cytoplasmic sequestering of NF-kappaB, signal transduction, transcription, DNA-templated; Gene names synonyms:IKBB, TRIP9
218_nEC	O00221	NFKBIE	M	0	**NF-kappa-B inhibitor epsilon** - Short name: NF-kappa-BIE; (Alternative names: I-kappa-B-epsilon - Short names: IkB-E/IkB-epsilon/IkappaBepsilon)	Function: Inhibits NF-kappa-B by complexing with and trapping it in the cytoplasm. Inhibits DNA-binding of NF-kappa-B p50-p65 and p50-c-Rel complexes.; GO - Biological process: cytoplasmic sequestering of transcription factor, D-serine transport; Gene names synonyms:IKBE
219_nEC	P08138	NGFR	M	R	**Tumor necrosis factor receptor superfamily member 16**; (Alternative names: Gp80-LNGFR; Low affinity neurotrophin receptor p75NTR; Low-affinity nerve growth factor receptor - Short name: NGF receptor; p75 ICD; CD_antigen: CD271)	Molecular function: Developmental protein, Receptor; Biological process: Apoptosis, Biological rhythms, Differentiation, Neurogenesis; Gene names synonyms:TNFRSF16
220_nEC	P52952	NKX2-5	M	0	**Homeobox protein Nkx-2.5**; (Alternative names: Cardiac-specific homeobox; Homeobox protein CSX; Homeobox protein NK-2 homolog E)	Molecular function: Developmental protein, DNA-binding; Gene names synonyms:CSX, NKX2.5, NKX2E
221_nEC	P01160	NPPA	M	R	**Natriuretic peptides A**; (Alternative names: Atrial natriuretic factor prohormone -Short name:proANF; Atrial natriuretic peptide prohormone -Short names: preproANP/proANP; Atriopeptigen; Cardiodilatin - Short name: CDD; preproCDD-ANF; [Cleaved into the following 12 chains:Long-acting natriuretic peptide - short name: LANP; (Alternative names: Long-acting natriuretic hormone -Short name: LANH; Pro atrial natriuretic factor 1-30 -Short name: proANF 1-30; Pro atrial natriuretic	Protein names updated; Molecular function: Hormone, Vasoactive, Vasodilator; Gene names synonyms:ANP, PND

					peptide 1-30 - Short name: proANP 1-30); Vessel dilator - Short name: VSDL; (Alternative names: Pro atrial natriuretic factor 31-67 -Short name: proANF 31-67; Pro atrial natriuretic peptide 31-67 -Short name:proANP 31-67); Kaliuretic peptide -Short name:KP; (Alternative names: Pro atrial natriuretic factor 79-98 - Short name: proANF 79-98; Pro atrial natriuretic peptide 79-98 - Short name: proANP 79-98); Urodilatin -Short name: URO; (Alternative names: CDD 95-126, CDD-ANP (95-126), Pro atrial natriuretic peptide 95-126 -Short name: proANP 95-126); Auriculin-C; (Alternative names: Atrial natriuretic factor 1-33 - Short name: ANF 1-33); Auriculin-D; (Alternative names:Atrial natriuretic factor 3-33 -Short name: ANF 3-33); Atrial natriuretic peptide - Short name: ANP; (Alternative names:Alpha-atrial natriuretic peptide; Alpha-hANP; Atrial natriuretic factor -Short name: ANF; CDD-ANF; CDD-ANP (99-126); Cardionatrin; Pro atrial natriuretic factor 99-126 -Short name: proANF 99-126); Auriculin-B; (Alternative name: Atrial natriuretic factor 8-33 - Short name: ANF 8-33); Auriculin-A; Atriopeptin-1; (Alternative name: Atriopeptin I); Atriopeptin-2; (Alternative name: Atriopeptin II); Atriopeptin-3; (Alternative name: Atriopeptin III)	
222_nEC	P23582	NPPC	M	0	**C-type natriuretic peptide**; [Cleaved into the following 3 chains: CNP-22; CNP-29; CNP-53]	Molecular function: Hormone, Vasoactive; Biological process: Osteogenesis; Gene names synonyms: CNP2
223_nEC	Q15466	NR0B2	M	R	**Nuclear receptor subfamily 0 group B member 2**; (Alternative names: Orphan nuclear receptor SHP; Small heterodimer partner)	Molecular function: Receptor, Repressor; Biological process: Biological rhythms, Transcription, Transcription regulation; Gene names synonyms:SHP
224_nEC	Q14994	NR1I3	M	R	**Nuclear receptor subfamily 1 group I member 3**; (Alternative names: Constitutive activator of retinoid response - Short name: Constitutive active response; Constitutive androstane receptor - Short name: CAR; Orphan nuclear receptor MB67)	Molecular function: Activator, DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:CAR
225_nEC	Q02297	NRG1	M	0	**Pro-neuregulin-1, membrane-bound isoform** - Short name: Pro-NRG1; [Cleaved into the following chain: Neuregulin-1; (Alternative names: Acetylcholine receptor-inducing activity - Short name: ARIA; Breast cancer cell differentiation factor p45; Glial growth factor; Heregulin - Short name: HRG; Neu differentiation factor; Sensory and motor neuron-derived factor)]	Molecular function: Growth factor; Gene names synonyms:GGF, HGL, HRGA, NDF, SMDF
226_nEC	O95631	NTN1	M	0	**Netrin-1**; (Alternative name: Epididymis tissue protein Li 131P)	Biological process: Apoptosis; Gene names synonyms:NTN1L
227_nEC	P02763	ORM1	M	T	**Alpha-1-acid glycoprotein 1** - Short name: AGP 1; (Alternative name: Orosomucoid-1 - Short name: OMD 1)	Biological process: Acute phase, Transport; Gene names synonyms:AGP1

228_nEC	P13725	OSM	M	0	**Oncostatin-M** - Short name: OSM	Molecular function: Cytokine, Mitogen; Biological process: Growth regulation
229_nEC	Q99650	OSMR	M	R	**Oncostatin-M-specific receptor subunit beta**; (Alternative name: Interleukin-31 receptor subunit beta - Short names: IL-31 receptor subunit beta/IL-31R subunit beta/IL-31R-beta/IL-31RB)	Molecular function: Receptor; Gene names synonyms:OSMRB
230_nEC	Q15116	PDCD1	M	R	**Programmed cell death protein 1** - Short names: Protein PD-1/hPD-1; (Alternative names: CD_antigen: CD279)	Biological process: Adaptive immunity, Apoptosis, Immunity; Gene names synonyms:PD1
231_nEC	P04085	PDGFA	M	0	**Platelet-derived growth factor subunit A** - Short name: PDGF subunit A; (Alternative names: PDGF-1; Platelet-derived growth factor A chain; Platelet-derived growth factor alpha polypeptide)	Molecular function: Developmental protein, Growth factor, Mitogen; Gene names synonyms:PDGF1
232_nEC	O00330	PDHX	M	Mito	**Pyruvate dehydrogenase protein X component, mitochondrial**; (Alternative names: Dihydrolipoamide dehydrogenase-binding protein of pyruvate dehydrogenase complex; E3-binding protein - Short name: E3BP; Lipoyl-containing pyruvate dehydrogenase complex component X; proX)	GO - Molecular function: transferase activity, transferring acyl groups; GO - Biological process: mitochondrial acetyl-CoA biosynthetic process from pyruvate, pyruvate metabolic process; Gene names synonyms:PDX1
233_nEC	P52945	PDX1	M	0	**Pancreas/duodenum homeobox protein 1** - Short name: PDX-1; (Alternative name: Glucose-sensitive factor - Short name: GSF; Insulin promoter factor 1 - Short name: IPF-1; Insulin upstream factor 1 - Short name: IUF-1; Islet/duodenum homeobox-1 - Short name: IDX-1; Somatostatin-transactivating factor 1 - Short name: STF-1	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:IPF1, STF1
234_nEC	P49763	PGF	M	0	**Placenta growth factor** - Short name: PlGF	Molecular function: Developmental protein, Growth factor, Heparin-binding, Mitogen; Biological process: Angiogenesis, Differentiation; Gene names synonyms:PGFL, PLGF
235_nEC	O75364	PITX3	M	0	**Pituitary homeobox 3**; (Alternative names: Homeobox protein PITX3; Paired-like homeodomain transcription factor 3)	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:PTX3
236_nEC	O60664	PLIN3	M	0	**Perilipin-3**; (Alternative names: 47 kDa mannose 6-phosphate receptor-binding protein - Short name: 47 kDa MPR-binding protein; Cargo selection protein TIP47; Mannose-6-phosphate receptor-binding protein 1; Placental protein 17 - Short name: PP17)	Biological process: Transport; Gene names synonyms:M6PRBP1, TIP47
237_nEC	Q03181	PPARD	M	R	**Peroxisome proliferator-activated receptor delta** - Short name: PPAR-delta; (Alternative names: NUCI; Nuclear hormone receptor 1 - Short name: NUC1; Nuclear receptor subfamily 1 group C member 2; Peroxisome proliferator-activated receptor beta -Short name: PPAR-beta)	Molecular function: Activator, DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR1C2, PPARB
238_nEC	O75807	PPP1R15A	M	0	**Protein phosphatase 1 regulatory subunit 15A**; (Alternative names: Growth arrest and DNA damage-inducible protein	Biological process: Apoptosis, Stress response, Translation regulation; Gene names synonyms:GADD34

					GADD34; Myeloid differentiation primary response protein MyD116 homolog)	
239_nEC	Q5VV67	PPRC1	M	Mito	**Peroxisome proliferator-activated receptor gamma coactivator-related protein 1**; (Alternative name: PGC-1-related coactivator - Short name: PRC)	Molecular function: Activator, RNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:KIAA0595
240_nEC	P31321	PRKAR1B	M	0	**cAMP-dependent protein kinase type I-beta regulatory subunit**	Function: Regulatory subunit of the cAMP-dependent protein kinases involved in cAMP signaling in cells.; Ligand: cAMP, cAMP-binding, Nucleotide-binding
241_nEC	P07225	PROS1	M	0	**Vitamin K-dependent protein S**	Biological process: Blood coagulation, Fibrinolysis, Hemostasis; Ligand: Calcium; Gene names synonyms:PROS
242_nEC	P34995	PTGER1	M	R	**Prostaglandin E2 receptor EP1 subtype** - Short names: PGE receptor EP1 subtype/PGE2 receptor EP1 subtype; (Alternative : Prostanoid EP1 receptor)	Molecular function: G-protein coupled receptor, Receptor, Transducer
243_nEC	P43119	PTGIR	M	R	**Prostacyclin receptor**; (Alternative names: Prostaglandin I2 receptor - Short names: PGI receptor/PGI2 receptor; Prostanoid IP receptor)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Gene names synonyms:PRIPR
244_nEC	P26022	PTX3	M	0	**Pentraxin-related protein PTX3**; (Alternative names: Pentaxin-related protein PTX3; Tumor necrosis factor alpha-induced protein 5 - Short name: TNF alpha-induced protein 5; Tumor necrosis factor-inducible gene 14 protein - Short name: TSG-14)	Function: Plays a role in the regulation of innate resistance to pathogens, inflammatory reactions, possibly clearance of self-components and female fertility.; Gene names synonyms:TNFAIP5, TSG14
245_nEC	O95398	RAPGEF3	M	0	**Rap guanine nucleotide exchange factor 3**; (Alternative names: Exchange factor directly activated by cAMP 1; Exchange protein directly activated by cAMP 1 - Short name: EPAC 1; Rap1 guanine-nucleotide-exchange factor directly activated by cAMP; cAMP-regulated guanine nucleotide exchange factor I - Short name:cAMP-GEFI)	Molecular function: Guanine-nucleotide releasing factor; Biological process: Angiogenesis; Ligand: cAMP, cAMP-binding, Nucleotide-binding; Gene names synonyms:CGEF1, EPAC, EPAC1
246_nEC	P10276	RARA	M	R	**Retinoic acid receptor alpha** - Short name: RAR-alpha; (Alternative name: Nuclear receptor subfamily 1 group B member 1)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR1B1
247_nEC	Q9NS23	RASSF1	M	R, Mito	**Ras association domain-containing protein 1**	Biological process: Cell cycle; Ligand: Metal-binding, Zinc; Gene names synonyms:RDA32
248_nEC	P53805	RCAN1	M	0	**Calcipressin-1**; (Alternative names: Adapt78; Down syndrome critical region protein 1; Myocyte-enriched calcineurin-interacting protein 1 - Short name: MCIP1; Regulator of calcineurin 1)	Function: Inhibits calcineurin-dependent transcriptional responses by binding to the catalytic domain of calcineurin A. Could play a role during central nervous system development ; Gene names synonyms:ADAPT78, CSP1, DSC1, DSCR1
249_nEC	Q14206	RCAN2	M	0	**Calcipressin-2**; (Alternative names: Down syndrome candidate region 1-like 1; Myocyte-enriched calcineurin-interacting protein 2 - Short name: MCIP2; Regulator of calcineurin 2; Thyroid hormone-responsive protein ZAKI-4)	Function: Inhibits calcineurin-dependent transcriptional responses by binding to the catalytic domain of calcineurin A. Could play a role during central nervous system development.; Gene names synonyms:DSCR1L1, ZAKI4

250_nEC	Q04206	RELA	M	0	**Transcription factor p65**; (Alternative names: Nuclear factor NF-kappa-B p65 subunit; Nuclear factor of kappa light polypeptide gene enhancer in B-cells 3)	Molecular function: Activator, DNA-binding; Biological process: Host-virus interaction, Transcription, Transcription regulation; Gene names synonyms:NFKB3
251_nEC	Q01201	RELB	M	0	**Transcription factor RelB**; (Alternative name: I-Rel)	Molecular function: Activator, DNA-binding, Repressor; Biological process: Biological rhythms, Transcription, Transcription regulation
252_nEC	Q9HD89	RETN	M	0	**Resistin**; (Alternative names: Adipose tissue-specific secretory factor - Short name: ADSF; C/EBP-epsilon-regulated myeloid-specific secreted cysteine-rich protein; Cysteine-rich secreted protein A12-alpha-like 2; Cysteine-rich secreted protein FIZZ3)	Molecular function: Hormone; Gene names synonyms:FIZZ3, HXCP1, RSTN
253_nEC	P41220	RGS2	M	0	**Regulator of G-protein signaling 2** - Short name: RGS2; (Alternative names: Cell growth-inhibiting gene 31 protein; G0/G1 switch regulatory protein 8)	Molecular function: GTPase activation, Signal transduction inhibitor; Biological process: Cell cycle, Translation regulation; Gene names synonyms:G0S8
254_nEC	Q9Y3P4	RHBDD3	M	0	**Rhomboid domain-containing protein 3**	GO - Molecular function: serine-type endopeptidase activity; Gene names synonyms:C22orf3
255_nEC	P55042	RRAD	M	C	**GTP-binding protein RAD**; (Alternative names: RAD1; Ras associated with diabetes)	Molecular function: Calmodulin-binding; Ligand: GTP-binding, Nucleotide-binding; Gene names synonyms:RAD;
256_nEC	P06703	S100A6	M	Trans	**Protein S100-A6**; (Alternative names: Calcyclin; Growth factor-inducible protein 2A9; MLN 4; Prolactin receptor-associated protein - Short name: PRA; S100 calcium-binding protein A6)	Function: May function as calcium sensor and modulator, contributing to cellular calcium signaling.; Ligand: Calcium, Metal-binding; Gene names synonyms:CACY
257_nEC	O95136	S1PR2	M	R	**Sphingosine 1-phosphate receptor 2** - Short names: S1P receptor 2/S1P2; (Alternative names: Endothelial differentiation G-protein coupled receptor 5; Sphingosine 1-phosphate receptor Edg-5 - Short name: S1P receptor Edg-5)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Gene names synonyms:EDG5
258_nEC	Q9UPU9	SAMD4A	M	0	**Protein Smaug homolog 1** - Short names: Smaug 1/hSmaug1; (Alternative name: Sterile alpha motif domain-containing protein 4A - Short name: SAM domain-containing protein 4A)	Molecular function: Repressor; Biological process: Translation regulation; Gene names synonyms:KIAA1053, SAMD4, SMAUG1
259_nEC	P51168	SCNN1B	M	C	**Amiloride-sensitive sodium channel subunit beta**; (Alternative names: Beta-NaCH; Epithelial Na(+) channel subunit beta - Short names: Beta-ENaC/ENaCB; Nonvoltage-gated sodium channel 1 subunit beta; SCNEB)	Molecular function: Ion channel, Sodium channel; Biological process: Ion transport, Sensory transduction, Sodium transport, Taste, Transport; Ligand: Sodium
260_nEC	P18827	SDC1	M	0	**Syndecan-1** - Short name: SYND1; (Alternative name: CD_antigen: CD138)	Cell surface proteoglycan that bears both heparan sulfate and chondroitin sulfate and that links the cytoskeleton to the interstitial matrix. Regulates exosome biogenesis in concert with SDCBP and PDCD6IP.; Gene names synonyms:SDC
261_nEC	P16581	SELE	M	Trans	**E-selectin**; (Alternative names: CD62 antigen-like family member E; Endothelial leukocyte adhesion molecule 1 - Short name:ELAM-1; Leukocyte-endothelial cell adhesion molecule 2 - Short name: LECAM2; CD_antigen: CD62E)	Biological process: Cell adhesion; Ligand: Calcium, Lectin, Metal-binding; Gene names synonyms:ELAM1

262_nEC	P49908	SELENOP	M	0	**Selenoprotein P** - Short name: SeP	Function: Might be responsible for some of the extracellular antioxidant defense properties of selenium or might be involved in the transport of selenium. May supply selenium to tissues such as brain and testis.; Ligand: Selenium; Gene names synonyms:SELP, SEPP1
263_nEC	P16109	SELP	M	R	**P-selectin**; (Alternative names: CD62 antigen-like family member P; Granule membrane protein 140 - Short name: GMP-140; Leukocyte-endothelial cell adhesion molecule 3 - Short name: LECAM3; Platelet activation dependent granule-external membrane protein - Short name: PADGEM; CD_antigen: CD62P)	Biological process: Cell adhesion; Ligand: Calcium, Lectin, Metal-binding; Gene names synonyms:GMRP, GRMP
264_nEC	P05121	SERPINE1	M	0	**Plasminogen activator inhibitor 1** - Short names: PAI/PAI-1; (Alternative names: Endothelial plasminogen activator inhibitor; Serpin E1)	Molecular function: Protease inhibitor, Serine protease inhibitor; Gene names synonyms:PAI1, PLANH1
265_nEC	P08697	SERPINF2	M	0	**Alpha-2-antiplasmin** - Short name: Alpha-2-AP; (Alternative names: Alpha-2-plasmin inhibitor - Short name: Alpha-2-PI; Serpin F2)	Molecular function: Protease inhibitor, Serine protease inhibitor; Biological process: Acute phase; Gene names synonyms:AAP, PLI
266_nEC	P29353	SHC1	M	Trans	**SHC-transforming protein 1**; (Alternative names: SHC-transforming protein 3; SHC-transforming protein A; Src homology 2 domain-containing-transforming protein C1 - Short name: SH2 domain protein C1)	Biological process: Angiogenesis, Growth regulation, Host-virus interaction; Gene names synonyms:SHC, SHCA
267_nEC	Q6IA17	SIGIRR	M	R	**Single Ig IL-1-related receptor**; (Alternative names: Single Ig IL-1R-related molecule; Single immunoglobulin domain-containing IL1R-related protein; Toll/interleukin-1 receptor 8 - Short name: TIR8)	Function: Acts as a negative regulator of the Toll-like and IL-1R receptor signaling pathways. Attenuates the recruitment of receptor-proximal signaling components to the TLR4 receptor, probably through an TIR-TIR domain interaction with TLR4. Through its extracellular domain interferes with the heterodimerization of Il1R1 and IL1RAP.
268_nEC	Q14973	SLC10A1	M	T, R	**Sodium/bile acid cotransporter**; (Alternative names: Cell growth-inhibiting gene 29 protein; Na(+)/bile acid cotransporter; Na(+)/taurocholate transport protein; Sodium/taurocholate cotransporting polypeptide; Solute carrier family 10 member 1)	Molecular function: Host cell receptor for virus entry, Receptor; Biological process: Host-virus interaction, Ion transport, Sodium transport, Symport, Transport; Ligand: Sodium; Gene names synonyms:NTCP
269_nEC	Q12908	SLC10A2	M	T	**Ileal sodium/bile acid cotransporter**; (Alternative names: Apical sodium-dependent bile acid transporter - Short name: ASBT; Ileal Na(+)/bile acid cotransporter; Ileal sodium-dependent bile acid transporter - Short names: IBAT/ISBT; Na(+)-dependent ileal bile acid transporter; Sodium/taurocholate cotransporting polypeptide, ileal; Solute carrier family 10 member 2)	Biological process: Ion transport, Sodium transport, Symport, Transport; Ligand: Sodium; Gene names synonyms:ASBT, ISBT, NTCP2
270_nEC	Q8WUM 9	SLC20A1	M	T, R, Trans	**Sodium-dependent phosphate transporter 1**; (Alternative names: Gibbon ape leukemia virus receptor 1 - Short name: GLVR-1; Leukemia virus receptor 1 homolog; Phosphate	BRENDA EC Number updated; Molecular function: Receptor; Biological process: Host-virus interaction, Phosphate

					transporter 1 - Short name: PiT-1; Solute carrier family 20 member 1)	transport, Symport, Transport; Gene names synonyms:GLVR1, PIT1
271_nEC	Q4U2R8	SLC22A6	M	T, Ca, Trans	**Solute carrier family 22 member 6**; (Alternative names: Organic anion transporter 1 - Short name: hOAT1; PAH transporter - Short name: hPAHT; Renal organic anion transporter 1 - Short name: hROAT1)	GO - Molecular function: anion:anion antiporter activity, chloride ion binding, identical protein binding, inorganic anion exchanger activity, organic anion transmembrane transporter activity, sodium-independent organic anion transmembrane transporter activity; GO - Biological process: alpha-ketoglutarate transport, organic anion transport, renal tubular secretion, response to methotrexate, sodium-independent organic anion transport; Gene names synonyms:OAT1, PAHT
272_nEC	Q9Y694	SLC22A7	M	T, Ca, Trans	**Solute carrier family 22 member 7**; (Alternative names: Novel liver transporter; Organic anion transporter 2 - Short name: hOAT2)	Biological process: Ion transport, Transport; Gene names synonyms:NLT, OAT2
273_nEC	Q9H2B4	SLC26A1	M	T, C, Trans	**Sulfate anion transporter 1** - Short name: SAT-1; (Alternative name: Solute carrier family 26 member 1)	Molecular function: Ion channel; Biological process: Anion exchange, Antiport, Ion transport, Transport; Gene names synonyms:SAT1
274_nEC	P02730	SLC4A1	M	T, Trans	**Band 3 anion transport protein**; (Alternative names: Anion exchange protein 1 - Short names: AE 1/Anion exchanger 1; Solute carrier family 4 member 1; CD_antigen: CD233)	Molecular function: Blood group antigen; Biological process: Anion exchange, Ion transport, Transport; Gene names synonyms:AE1, DI, EPB3
275_nEC	Q86UW1	SLC51A	M	T	**Organic solute transporter subunit alpha** - Short name: OST-alpha; (Alternative name: Solute carrier family 51 subunit alpha)	Function: Essential component of the Ost-alpha/Ost-beta complex, a heterodimer that acts as the intestinal basolateral transporter responsible for bile acid export from enterocytes into portal blood. Efficiently transports the major species of bile acids.; Biological process: Transport; Gene names synonyms:OSTA
276_nEC	Q86UW2	SLC51B	M	T, Trans	**Organic solute transporter subunit beta** - Short name: OST-beta; (Alternative name: Solute carrier family 51 subunit beta)	Function: Essential component of the Ost-alpha/Ost-beta complex, a heterodimer that acts as the intestinal basolateral transporter responsible for bile acid export from enterocytes into portal blood. Efficiently transports the major species of bile acids. Modulates SLC51A glycosylation, membrane trafficking and stability activities; Biological process: Transport; Gene names synonyms:OSTB
277_nEC	P31645	SLC6A4	M	T, Trans	**Sodium-dependent serotonin transporter** - Short name: SERT; (Alternative names: 5HT transporter - Short name: 5HTT; Solute carrier family 6 member 4)	Biological process: Neurotransmitter transport, Symport, Transport; Ligand: Metal-binding, Sodium; Gene names synonyms:HTT, SERT
278_nEC	Q01650	SLC7A5	M	T, Trans	**Large neutral amino acids transporter small subunit 1**; (Alternative names: 4F2 light chain - Short names: 4F2 LC/4F2LC; CD98 light chain; Integral membrane protein E16 - Short name:	Biological process: Amino-acid transport, Transport; Gene names synonyms:CD98LC, LAT1, MPE16

					E16; L-type amino acid transporter 1 - Short name: hLAT1; Solute carrier family 7 member 5; y+ system cationic amino acid transporter)	
279_nEC	P46721	SLCO1A2	M	T, Ca, Trans	**Solute carrier organic anion transporter family member 1A2**; (Alternative names: OATP-A; Organic anion-transporting polypeptide 1 - Short name: OATP-1; Sodium-independent organic anion transporter; Solute carrier family 21 member 3)	Biological process: Ion transport, Transport; Gene names synonyms:OATP, OATP1, OATP1A2, SLC21A3
280_nEC	Q9UIG8	SLCO3A1	M	T, Ca, Trans	**Solute carrier organic anion transporter family member 3A1** - Short name: OATP3A1; (Alternative name: Organic anion transporter polypeptide-related protein 3 - Short names: OATP-RP3/OATPRP3; Organic anion-transporting polypeptide D - Short name: OATP-D; PGE1 transporter; Sodium-independent organic anion transporter D; Solute carrier family 21 member 11)	Biological process: Ion transport, Transport; Gene names synonyms:OATP3A1, OATPD, SLC21A11
281_nEC	P84022	SMAD3	M	0	**Mothers against decapentaplegic homolog 3** - Short names: MAD homolog 3/Mad3/Mothers against DPP homolog 3/hMAD-3; (Alternative names: JV15-2; SMAD family member 3 - Short names: SMAD 3/Smad3/hSMAD3)	Molecular function DNA-binding Biological process: Host-virus interaction, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:MADH3
282_nEC	P53814	SMTN	M	0	**Smoothelin**	Function: Structural protein of the cytoskeleton.; GO - Molecular function: actin binding, structural constituent of muscle; GO - Biological process: actin cytoskeleton organization, muscle organ development, smooth muscle contraction; Gene names synonyms:SMSMO
283_nEC	O95863	SNAI1	M	0	**Zinc finger protein SNAI1**; (Alternative name: Protein snail homolog 1 - Short name: Protein sna)	Molecular function: Developmental protein, DNA-binding; Ligand: Metal-binding, Zinc; Gene names synonyms:SNAH
284_nEC	O15524	SOCS1	M	0	**Suppressor of cytokine signaling 1** - Short name: SOCS-1; (Alternative names: JAK-binding protein - Short name: JAB; STAT-induced STAT inhibitor 1 - Short name: SSI-1; Tec-interacting protein 3 - Short name: TIP-3)	Molecular function: Signal transduction inhibitor; Biological process: Growth regulation, Ubl conjugation pathway; Gene names synonyms:SSI1, TIP3
285_nEC	O14543	SOCS3	M, B	0	**Suppressor of cytokine signaling 3** - Short name: SOCS-3; (Alternative names: Cytokine-inducible SH2 protein 3 - Short name: CIS-3; STAT-induced STAT inhibitor 3 - Short name: SSI-3)	Molecular function: Signal transduction inhibitor; Biological process: Growth regulation, Ubl conjugation pathway; Gene names synonyms:CIS3, SSI3
286_nEC	P35321	SPRR1A	M	0	**Cornifin-A**; (Alternative names: 19 kDa pancornulin; SPRK; Small proline-rich protein IA - Short name: SPR-IA)	Function: Cross-linked envelope protein of keratinocytes. It is a keratinocyte protein that first appears in the cell cytosol, but ultimately becomes cross-linked to membrane proteins by transglutaminase. All that results in the formation of an insoluble envelope beneath the plasma membrane.; Biological process: Keratinization
287_nEC	P61278	SST	M	0	**Somatostatin**; (Alternative name: Growth hormone release-inhibiting factor); [Cleaved into the following 3 chains:	GO - Molecular function:hormone activity; GO - Biological process: cell-cell signaling, cell surface receptor signaling

					Somatostatin-28; Somatostatin-14 - Short name: SST-14; Neuronostatin - Short name: NST]	pathway, chemical synaptic transmission, digestion, G protein-coupled receptor signaling pathway, hormone-mediated apoptotic signaling pathway, hyperosmotic response, negative regulation of cell population proliferation, regulation of cell migration, response to acidic pH, response to amino acid, response to drug, response to heat, response to nutrient, response to steroid hormone; Molecular function: Hormone
288_nEC	P49675	STAR	M	Mito, T	**Steroidogenic acute regulatory protein, mitochondrial** - Short name: StAR; (Alternative name: START domain-containing protein 1 - Short name: StARD1)	Biological process: Lipid transport, Steroidogenesis, Transport; Ligand: Lipid-binding; Gene names synonyms:STARD1
289_nEC	P40763	STAT3	M, B	0	**Signal transducer and activator of transcription 3**; (Alternative name: Acute-phase response factor)	Molecular function: Activator, DNA-binding; Biological process: Host-virus interaction, Transcription, Transcription regulation; Gene names synonyms:APRF
290_nEC	P25103	TACR1	M	R	**Substance-P receptor** - Short name: SPR; (Alternative names: NK-1 receptor - Short name: NK-1R; Tachykinin receptor 1)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Gene names synonyms:NK1R, TAC1R
291_nEC	Q92844	TANK	M	0	**TRAF family member-associated NF-kappa-B activator**; (Alternative name: TRAF-interacting protein - Short name: I-TRAF)	Biological process: DNA damage, Host-virus interaction; Ligand: Metal-binding, Zinc; Gene names synonyms:ITRAF, TRAF2
292_nEC	Q9UL17	TBX21	M	0	**T-box transcription factor TBX21** - Short name: T-box protein 21; (Alternative names: T-cell-specific T-box transcription factor T-bet; Transcription factor TBLYM)	Molecular function: Activator, DNA-binding, Repressor; Biological process: Transcription, Transcription regulation; Gene names synonyms:TBET, TBLYM
293_nEC	P21731	TBXA2R	M	R	**Thromboxane A2 receptor** - Short name: TXA2-R; (Alternative name: Prostanoid TP receptor)	Molecular function: G-protein coupled receptor, Receptor, Transducer
294_nEC	O15273	TCAP	M	0	**Telethonin**; (Alternative name: Titin cap protein)	Function: Muscle assembly regulating factor. Mediates the antiparallel assembly of titin (TTN) molecules at the sarcomeric Z-disk.; Miscellaneous: The C-terminal domain appears to be unstructured in solution. It may promote the assembly of higher-order TTN complexes.
295_nEC	Q12870	TCF15	M	0	**Transcription factor 15** - Short name: TCF-15; (Alternative names: Class A basic helix-loop-helix protein 40 - Short name: bHLHa40; Paraxis; Protein bHLH-EC2)	Molecular function: Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHA40, BHLHEC2
296_nEC	P01135	TGFA	M	0	**Protransforming growth factor alpha**; [Cleaved into the following chain: Transforming growth factor alpha - Short name: TGF-alpha; (Alternative name: EGF-like TGF - Short name: ETGF; TGF type 1)]	Function: TGF alpha is a mitogenic polypeptide that is able to bind to the EGF receptor/EGFR and to act synergistically with TGF beta to promote anchorage-independent cell proliferation in soft agar.; Molecular function: Growth factor, Mitogen
297_nEC	P10600	TGFB3	M	0	**Transforming growth factor beta-3 proprotein**; [Cleaved into the following 2 chains: Latency-associated peptide - Short name:	Protein name updated; Molecular function: Growth factor, Mitogen

					LAP; Transforming growth factor beta-3 - Short name: TGF-beta-3]	
298_nEC	Q03167	TGFBR3	M	R	**Transforming growth factor beta receptor type 3** - Short names: TGF-beta receptor type 3/TGFR-3; (Alternative names: Betaglycan; Transforming growth factor beta receptor III - Short name: TGF-beta receptor type III)	Function: Binds to TGF-beta. Could be involved in capturing and retaining TGF-beta for presentation to the signaling receptors.; Molecular function: Receptor
299_nEC	P07204	THBD	M	R, Trans	**Thrombomodulin** - Short name: TM; (Alternative names: Fetomodulin; CD_antigen: CD141)	Molecular function: Receptor; Biological process: Blood coagulation, Hemostasis; Gene names synonyms:THRM
300_nEC	P07996	THBS1	M	0	**Thrombospondin-1**; (Alternative name: Glycoprotein G)	Molecular function: Heparin-binding; Biological process: Cell adhesion, Unfolded protein response; Ligand: Calcium; Gene names synonyms:TSP, TSP1
301_nEC	P35442	THBS2	M	0	**Thrombospondin-2**	Function: Adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions. Ligand for CD36 mediating antiangiogenic properties.; Molecular function: Heparin-binding; Biological process: Cell adhesion; Ligand: Calcium; Gene names synonyms:TSP2
302_nEC	Q8IUC6	TICAM1	M	0	**TIR domain-containing adapter molecule 1** - Short name: TICAM-1; (Alternative names: Proline-rich, vinculin and TIR domain-containing protein B; Putative NF-kappa-B-activating protein 502H; Toll-interleukin-1 receptor domain-containing adapter protein inducing interferon beta - Short names: MyD88-3/TIR domain-containing adapter protein inducing IFN-beta)	Biological process: Antiviral defense, Apoptosis, Host-virus interaction, Immunity, Inflammatory response, Innate immunity; Gene names synonyms:PRVTIRB, TRIF
303_nEC	P01033	TIMP1	M	0	**Metalloproteinase inhibitor 1**; (Alternative names: Erythroid-potentiating activity - Short name: EPA; Fibroblast collagenase inhibitor - Short name: Collagenase inhibitor; Tissue inhibitor of metalloproteinases 1 - Short name: TIMP-1)	Molecular function: Growth factor, Metalloenzyme inhibitor, Metalloprotease inhibitor, Protease inhibitor; Ligand: Metal-binding, Zinc; Gene names synonyms:CLGI, TIMP
304_nEC	P35625	TIMP3	M	0	**Metalloproteinase inhibitor 3**; (Alternative names: Protein MIG-5; Tissue inhibitor of metalloproteinases 3 - Short name: TIMP-3)	Molecular function: Metalloenzyme inhibitor, Metalloprotease inhibitor, Protease inhibitor; Biological process: Sensory transduction, Vision; Ligand: Metal-binding, Zinc
305_nEC	P31314	TLX1	M	0	**T-cell leukemia homeobox protein 1**; (Alternative names: Homeobox protein Hox-11; Proto-oncogene TCL-3; T-cell leukemia/lymphoma protein 3)	Function: Controls the genesis of the spleen. Binds to the DNA sequence 5'-GGCGGTAAGTGG-3'.; Molecular function: Developmental protein, DNA-binding; Gene names synonyms:HOX11, TCL3
306_nEC	P24821	TNC	M	0	**Tenascin** - Short name: TN; (Alternative names: Cytotactin; GMEM; GP 150-225; Glioma-associated-extracellular matrix antigen; Hexabrachion; JI; Myotendinous antigen; Neuronectin; Tenascin-C - Short name: TN-C)	Function: Extracellular matrix protein implicated in guidance of migrating neurons as well as axons during development, synaptic plasticity as well as neuronal regeneration. Promotes neurite outgrowth from cortical neurons grown on a monolayer of astrocytes. Ligand for integrins alpha-8/beta-1,

						alpha-9/beta-1, alpha-V/beta-3 and alpha-V/beta-6. In tumors, stimulates angiogenesis by elongation, migration and sprouting of endothelial cells; Biological process: Cell adhesion; Gene names synonyms:HXB
307_nEC	O00300	TNFRSF11 B	M	R	**Tumor necrosis factor receptor superfamily member 11B**; (Alternative names: Osteoclastogenesis inhibitory factor; Osteoprotegerin)	Molecular function: Receptor; Biological process: Apoptosis; Gene names synonyms:OCIF, OPG
308_nEC	Q9NP84	TNFRSF12 A	M	R	**Tumor necrosis factor receptor superfamily member 12A**; (Alternative names: Fibroblast growth factor-inducible immediate-early response protein 14 - Short name: FGF-inducible 14; Tweak-receptor - Short name: TweakR; CD_antigen: CD266)	Molecular function: Developmental protein, Receptor; Biological process: Angiogenesis, Apoptosis, Cell adhesion, Differentiation; Gene names synonyms:FN14
309_nEC	Q9Y5U5	TNFRSF18	M	R	**Tumor necrosis factor receptor superfamily member 18**; (Alternative names: Activation-inducible TNFR family receptor; Glucocorticoid-induced TNFR-related protein; CD_antigen: CD357)	Function: Receptor for TNFSF18. Seems to be involved in interactions between activated T-lymphocytes and endothelial cells and in the regulation of T-cell receptor-mediated cell death. Mediated NF-kappa-B activation via the TRAF2/NIK pathway.; Molecular function: Receptor; Biological process: Apoptosis; Gene names synonyms:AITR, GITR
310_nEC	P19438	TNFRSF1A	M	R	**Tumor necrosis factor receptor superfamily member 1A**; (Alternative names: Tumor necrosis factor receptor 1 - Short name: TNF-R1; Tumor necrosis factor receptor type I - Short names: TNF-RI/TNFR-I; p55; p60; CD_antigen: CD120a); [Cleaved into the following 2 chains: Tumor necrosis factor receptor superfamily member 1A, membrane form; Tumor necrosis factor-binding protein 1 - Short name: TBPI]	Molecular function: Receptor; Biological process: Apoptosis, Host-virus interaction; Gene names synonyms:TNFAR, TNFR1
311_nEC	P20333	TNFRSF1B	M	R	**Tumor necrosis factor receptor superfamily member 1B**; (Alterntive names: Tumor necrosis factor receptor 2 - Short name: TNF-R2; Tumor necrosis factor receptor type II - Short names: TNF-RII/TNFR-II; p75; p80 TNF-alpha receptor; CD_antigen: CD120b; INN: Etanercept); [Cleaved into the following 2 chains: Tumor necrosis factor receptor superfamily member 1b, membrane form; Tumor necrosis factor-binding protein 2; (Alternative names: TBP-2/TBPII)]	Molecular function: Receptor; Biological process: Apoptosis; Gene names synonyms:TNFBR, TNFR2
312_nEC	O43557	TNFSF14	M	0	**Tumor necrosis factor ligand superfamily member 14**; (Alternative names: Herpes virus entry mediator ligand - Short names: HVEM-L/Herpesvirus entry mediator ligand; CD_antigen: CD258); [Cleaved into the following 2 chains: Tumor necrosis	Function: Cytokine that binds to TNFRSF3/LTBR. Binding to the decoy receptor TNFRSF6B modulates its effects. Acts as a ligand for TNFRSF14/HVEM. Upon binding to TNFRSF14/HVEM, delivers costimulatory signals to T cells, leading to T cell proliferation and IFNG production.;

					factor ligand superfamily member 14, membrane form; Tumor necrosis factor ligand superfamily member 14, soluble form]	Molecular function: Cytokine; Gene names synonyms:HVEML, LIGHT
313_nEC	P63316	TNNC1	M	0	**Troponin C, slow skeletal and cardiac muscles** - Short name: TN-C	Function: Troponin is the central regulatory protein of striated muscle contraction. Tn consists of three components: Tn-I which is the inhibitor of actomyosin ATPase, Tn-T which contains the binding site for tropomyosin and Tn-C. The binding of calcium to Tn-C abolishes the inhibitory action of Tn on actin filaments.; Molecular function: Muscle protein; Ligand: Calcium, Metal-binding; Gene names synonyms:TNNC
314_nEC	P19429	TNNI3	M	0	**Troponin I, cardiac muscle**; (Alternative name: Cardiac troponin I)	Molecular function: Actin-binding, Muscle protein; Ligand: Calcium, Metal-binding; Gene names synonyms:TNNC1
315_nEC	P45379	TNNT2	M	0	**Troponin T, cardiac muscle** - Short name: TnTc; (Alternative name: Cardiac muscle troponin T - Short name: cTnT)	Function: Troponin T is the tropomyosin-binding subunit of troponin, the thin filament regulatory complex which confers calcium-sensitivity to striated muscle actomyosin ATPase activity.; Molecular function: Muscle protein
316_nEC	Q9BYV2	TRIM54	M	0	**Tripartite motif-containing protein 54**; (Alternative names: Muscle-specific RING finger protein - Short name: MuRF; Muscle-specific RING finger protein 3 - Short names: MuRF-3/MuRF3; RING finger protein 30)	Molecular function Developmental protein Biological process: Differentiation; Ligand: Metal-binding, Zinc; Gene names synonyms:MURF, MURF3, RNF30
317_nEC	Q9BYV6	TRIM55	M	0	**Tripartite motif-containing protein 55**; (Alternative names: Muscle-specific RING finger protein 2 - Short names: MuRF-2/MuRF2; RING finger protein 29)	Function: May regulate gene expression and protein turnover in muscle cells.; Molecular function: Muscle protein; Ligand: Metal-binding, Zinc; Gene names synonyms:MURF2, RNF29
318_nEC	P02766	TTR	M	T	**Transthyretin**; (Alternative names: ATTR; Prealbumin; TBPA)	Function: Thyroid hormone-binding protein. Probably transports thyroxine from the bloodstream to the brain.; Molecular function: Hormone, Thyroid hormone; Biological process: Transport; Gene names synonyms:PALB
319_nEC	P10599	TXN	M	0	**Thioredoxin** - Short name: Trx; (Alternative names: ATL-derived factor - Short name: ADF; Surface-associated sulphydryl protein - Short name: SASP; Allergen: Hom s Trx)	Molecular function: Activator; Biological process: Electron transport, Transcription, Transcription regulation, Transport; Gene names synonyms:TRDX, TRX, TRX1
320_nEC	P55089	UCN	M	0	**Urocortin**	Function: Acts in vitro to stimulate the secretion of adrenocorticotropic hormone (ACTH). Binds with high affinity to CRF receptor types 1, 2-alpha, and 2-beta. Plays a role in the establishment of normal hearing thresholds. Reduces food intake and regulates ghrelin levels in gastric body and plasma; Molecular function: Hormone; Biological process: Hearing
321_nEC	Q96RP3	UCN2	M	0	**Urocortin-2**; (Alternative names: Stresscopin-related peptide; Urocortin II - Short name: Ucn II; Urocortin-related peptide)	Function: Suppresses food intake, delays gastric emptying and decreases heat-induced edema. Might represent an endogenous ligand for maintaining homeostasis after stress.;

						Molecular function: Hormone; Gene names synonyms:SRP, URP
322_nEC	P52735	VAV2	M	0	**Guanine nucleotide exchange factor VAV2** - Short name: VAV-2	Function: Guanine nucleotide exchange factor for the Rho family of Ras-related GTPases. Plays an important role in angiogenesis. Its recruitment by phosphorylated EPHA2 is critical for EFNA1-induced RAC1 GTPase activation and vascular endothelial cell migration and assembly; Molecular function: Guanine-nucleotide releasing factor; Biological process: Angiogenesis; Ligand: Metal-binding, Zinc
323_nEC	P19320	VCAM1	M	0	**Vascular cell adhesion protein 1** - Short names: V-CAM 1/VCAM-1; (Alternative names: INCAM-100; CD_antigen: CD106)	Function: Important in cell-cell recognition. Appears to function in leukocyte-endothelial cell adhesion. Interacts with integrin alpha-4/beta-1 (ITGA4/ITGB1) on leukocytes, and mediates both adhesion and signal transduction. The VCAM1/ITGA4/ITGB1 interaction may play a pathophysiologic role both in immune responses and in leukocyte emigration to sites of inflammation.; Biological process: Cell adhesion
324_nEC	P11473	VDR	M	R	**Vitamin D3 receptor** - Short name: VDR; (Alternative names: 1,25-dihydroxyvitamin D3 receptor; Nuclear receptor subfamily 1 group I member 1)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR1I1
325_nEC	P15692	VEGFA	M	0	**Vascular endothelial growth factor A** - Short name: VEGF-A; (Alternative name: Vascular permeability factor - Short name: VPF)	Molecular function: Developmental protein, Growth factor, Heparin-binding, Mitogen; Biological process: Angiogenesis, Differentiation; Gene names synonyms:VEGF
326_nEC	P04004	VTN	M	0	**Vitronectin** - Short name: VN; (Alternative names: S-protein; Serum-spreading factor; V75); [Cleaved into the following 3 chains: Vitronectin V65 subunit; Vitronectin V10 subunit; Somatomedin-B]	Molecular function: Heparin-binding; Biological process: Cell adhesion
327_nEC	O95388	WISP1	M	0	**CCN family member 4**; (Alternative names: WNT1-inducible-signaling pathway protein 1 - Short name: WISP-1; Wnt-1-induced secreted protein)	Biological process: Cell adhesion, Wnt signaling pathway; Gene name: CCN4; Gene names synonym: WISP1
328_nEC	P04628	WNT1	M	Trans	**Proto-oncogene Wnt-1** ;(Alternative name: Proto-oncogene Int-1 homolog)	Molecular function: Developmental protein; Biological process: Wnt signaling pathway; Gene names synonyms:INT1
329_nEC	P56704	WNT3A	M	Trans	**Protein Wnt-3a**	Molecular function: Developmental protein; Biological process: Wnt signaling pathway
330_nEC	P17861	XBP1	M	0	**X-box-binding protein 1** - Short name: XBP-1; (Alternative name: Tax-responsive element-binding protein 5 - Short name: TREB-5); [Cleaved into the following 2 chains: X-box-binding protein 1, cytoplasmic form; X-box-binding protein 1, luminal form]	Function: Functions also as a major regulator of the UPR in obesity-induced insulin resistance and type 2 diabetes for the management of obesity and diabetes prevention; Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Angiogenesis, Apoptosis, Autophagy, Differentiation, Lipid biosynthesis, Lipid metabolism,

						Myogenesis, Protein transport, Stress response, Transcription, Transcription regulation, Transport, Unfolded protein response; Gene names synonyms:TREB5, XBP2
331_nEC	Q702N8	XIRP1	M	0	**Xin actin-binding repeat-containing protein 1**; (Alternative name: Cardiomyopathy-associated protein 1)	Function: Protects actin filaments from depolymerization.; Molecular function: Actin-binding; Gene names synonyms:CMYA1, XIN
332_nEC	Q15942	ZYX	M	0	**Zyxin**; (Alternative name: Zyxin-2)	Biological process: Cell adhesion, Host-virus interaction; Ligand: Metal-binding, Zinc
333_nEC	P13945	ADRB3	M, B	R	**Beta-3 adrenergic receptor**; (Alternative name: Beta-3 adrenoreceptor - Short name: Beta-3 adrenoceptor)	Function: Beta-adrenergic receptors mediate the catecholamine-induced activation of adenylate cyclase through the action of G proteins. Beta-3 is involved in the regulation of lipolysis and thermogenesis.; Molecular function: G-protein coupled receptor, Receptor, Transducer; Gene names synonyms:ADRB3R, B3AR
334_nEC	P27540	ARNT	M	0	**Aryl hydrocarbon receptor nuclear translocator** - Short name: ARNT protein; (Alternative names: Class E basic helix-loop-helix protein 2 - Short name: bHLHe2; Dioxin receptor, nuclear translocator; Hypoxia-inducible factor 1-beta -Short names: HIF-1-beta/HIF1-beta)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:BHLHE2
335_nEC	P56381	ATP5E	M	T, C, Mito, Trans	**ATP synthase subunit epsilon, mitochondrial** - Short name: ATPase subunit epsilon; (Alternative name: ATP synthase F1 subunit epsilon)	Molecular function: Hydrolase; Biological process: ATP synthesis, Hydrogen ion transport, Ion transport, Transport; Gene name: ATP5F1E; Gene names synonyms:ATP5E
336_nEC	P24539	ATP5F1	M	T, C, Mito, Trans	**ATP synthase F(0) complex subunit B1, mitochondrial**; (Alternative names: ATP synthase peripheral stalk-membrane subunit b; ATP synthase proton-transporting mitochondrial F(0) complex subunit B1; ATP synthase subunit b - Short name: ATPase subunit b)	Proteine name updated;Biological process: Hydrogen ion transport, Ion transport, Transport; Gene name: ATP5PB; Gene names synonym: ATP5F1
337_nEC	O75947	ATP5H	M	T, C, Mito, Trans	**ATP synthase subunit d, mitochondrial** - Short name: ATPase subunit d; (Alternative name: ATP synthase peripheral stalk subunit d)	Proteine name updated; Biological process: Hydrogen ion transport, Ion transport, Transport; Gene name: ATP5PD; Gene names synonyms:ATP5H
338_nEC	P18859	ATP5J	M	T, C, Mito, Trans	**ATP synthase-coupling factor 6, mitochondrial** - Short name: ATPase subunit F6; (Alternative name: ATP synthase peripheral stalk subunit F6)	Protein name updated; Biological process: Hydrogen ion transport, Ion transport, Transport; Gene name:ATP5PF; Gene names synonyms:ATP5A, ATP5J, ATPM
339_nEC	P56134	ATP5J2	M	T, C, Mito, Trans	**ATP synthase subunit f, mitochondrial**; (Alternative name: ATP synthase membrane subunit f)	Protein name updated; Biological process: ATP synthesis, Hydrogen ion transport, Ion transport, Transport; Gene name:ATP5MF; Gene names synonyms:ATP5J2, ATP5JL
340_nEC	Q7Z4Y8	ATP5L2	M	T, C, Mito, Trans	**Putative ATP synthase subunit g 2, mitochondrial** - Short name:ATPase subunit g 2; (Alternative name: ATP synthase membrane subunit g-like protein)	Protein names updated; Biological process: ATP synthesis, Hydrogen ion transport, Ion transport, Transport; Gene name: ATP5MGL; Gene names synonyms:ATP5K2, ATP5L2

341_nEC	P48047	ATP5O	M	T, C, Mito, Trans	**ATP synthase subunit O, mitochondrial**; (Alternative names: ATP synthase peripheral stalk subunit OSCP; Oligomycin sensitivity conferral protein - Short name: OSCP)	Protein name updated; Biological process: ATP synthesis, Hydrogen ion transport, Ion transport, Transport; Gene name: ATP5PO; Gene names synonyms:ATP5O, ATPO
342_nEC	Q99766	ATP5S	M	T, Mito, Trans	**ATP synthase subunit s, mitochondrial**; (Alternative names: ATP synthase-coupling factor B - Short name: FB; Distal membrane arm assembly complex 2-like protein; Mitochondrial ATP synthase regulatory component factor B)	Protein name updated; Biological process: ATP synthesis, Hydrogen ion transport, Ion transport, Transport; Ligand: Magnesium, Metal-binding; Gene name: DMAC2L; Gene names synonyms:ATP5S, ATPW
343_nEC	Q5TC12	ATPAF1	M	Mito	**ATP synthase mitochondrial F1 complex assembly factor 1**; (Alternative name: ATP11 homolog)	Function: May play an essential role for the assembly of the mitochondrial F1-F0 complex.; GO - Biological process: mitochondrial proton-transporting ATP synthase complex assembly ;Gene names synonyms: ATP11
344_nEC	P51636	CAV2	M	0	**Caveolin-2**	Function: May act as a scaffolding protein within caveolar membranes. Interacts directly with G-protein alpha subunits and can functionally regulate their activity. Acts as an accessory protein in conjunction with CAV1 in targeting to lipid rafts and driving caveolae formation. The Ser-36 phosphorylated form has a role in modulating mitosis in endothelial cells. Positive regulator of cellular mitogenesis of the MAPK signaling pathway. Required for the insulin-stimulated nuclear translocation and activation of MAPK1 and STAT3, and the subsequent regulation of cell cycle progression.
345_nEC	P46527	CDKN1B	M	0	**Cyclin-dependent kinase inhibitor 1B**; (Aternative names: Cyclin-dependent kinase inhibitor p27; p27Kip1)	Molecular function: Protein kinase inhibitor; Biological process: Cell cycle; Gene names synonyms:KIP1
346_nEC	P49715	CEBPA	M	0	**CCAAT/enhancer-binding protein alpha** - Short name: C/EBP alpha	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Host-virus interaction, Transcription, Transcription regulation; Gene names synonyms:CEBP
347_nEC	Q99967	CITED2	M	0	**Cbp/p300-interacting transactivator 2**; (Alternative names: MSG-related protein 1 - Short name: MRG-1; P35srj)	Molecular function: Activator, Developmental protein, Repressor; Biological process: Differentiation, Stress response, Transcription, Transcription regulation; Gene names synonyms:MRG1
348_nEC	Q9Y6N1	COX11	M	Mito, Ca	**Cytochrome c oxidase assembly protein COX11, mitochondrial**	Function: Exerts its effect at some terminal stage of cytochrome c oxidase synthesis, probably by being involved in the insertion of the copper B into subunit I.; GO - Molecular function: copper ion binding, electron transfer activity; GO - Biological process: metal ion homeostasis negative regulation of glucokinase activity; Ligand: Copper

349_nEC	Q7KZN9	COX15	M	0	**Cytochrome c oxidase assembly protein COX15 homolog**	Function: May be involved in the biosynthesis of heme A. Pathway heme A biosynthesis: This protein is involved in step 1 of the subpathway that synthesizes heme A from heme O.
350_nEC	P13073	COX4I1	M	Mito	**Cytochrome c oxidase subunit 4 isoform 1, mitochondrial**; (Alternative names: Cytochrome c oxidase polypeptide IV; Cytochrome c oxidase subunit IV isoform 1 - Short name: COX IV-1)	Molecular function: Oxidoreductase; Gene names synonyms:COX4
351_nEC	P24310	COX7A1	M	Mito	**Cytochrome c oxidase subunit 7A1, mitochondrial**; (Alternative names: Cytochrome c oxidase subunit VIIa-heart - Short name: Cytochrome c oxidase subunit VIIa-H; Cytochrome c oxidase subunit VIIa-muscle - Short name: Cytochrome c oxidase subunit VIIa-M)	Function: Component of the cytochrome c oxidase, the last enzyme in the mitochondrial electron transport chain which drives oxidative phosphorylation.; Molecular function: Oxidoreductase; Gene names synonyms:COX7AH
352_nEC	O14548	COX7A2L	M	Mito	**Cytochrome c oxidase subunit 7A-related protein, mitochondrial**; (Alternative names: COX7a-related protein; Cytochrome c oxidase subunit VIIa-related protein; EB1)	Function: Involved in the regulation of oxidative phosphorylation and energy metabolism (By similarity). Necessary for the assembly of mitochondrial respiratory supercomplex.; GO - Molecular function: cytochrome-c oxidase activity; GO - Biological process: mitochondrial electron transport, cytochrome c to oxygen, mitochondrial respirasome assembly, regulation of oxidative phosphorylation; Gene names synonyms:COX7AR, COX7RP
353_nEC	P24311	COX7B	M	Mito	**Cytochrome c oxidase subunit 7B, mitochondrial**;(Alternative name: Cytochrome c oxidase polypeptide VIIb)	GO - Molecular function: cytochrome-c oxidase activity; GO - Biological process: central nervous system development, mitochondrial electron transport, cytochrome c to oxygen
354_nEC	P15954	COX7C	M	Mito	**Cytochrome c oxidase subunit 7C, mitochondrial**; (Alternative name: Cytochrome c oxidase polypeptide VIIc)	GO - Molecular function: cytochrome-c oxidase activity; GO - Biological process: generation of precursor metabolites and energy, mitochondrial electron transport, cytochrome c to oxygen
355_nEC	Q8N907	DAND5	M	0	**DAN domain family member 5**; (Alternative names: Cerberus-like protein 2 - Short name: Cerl-2; Cysteine knot superfamily 1, BMP antagonist 3; Gremlin-3)	Function: Seems to play a role in the correct specification of the left-right axis. May antagonize NODAL and BMP4 signaling. Cystine knot-containing proteins play important roles during development, organogenesis, tissue growth and differentiation; Gene names synonyms:CER2, CKTSF1B3, GREM3, SP1
356_nEC	P03372	ESR1	M	R	**Estrogen receptor** - Short name: ER; (Alternative names: ER-alpha; Estradiol receptor; Nuclear receptor subfamily 3 group A member 1)	Molecular function Activator, DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Lipid-binding, Metal-binding, Steroid-binding, Zinc; Gene names synonyms:ESR, NR3A1
357_nEC	Q9BQ89	FAM110A	M	0	**Protein FAM110A**	Gene names synonyms:C20orf55, F10

358_nEC	P84996	GNAS	M	0	**Protein ALEX**; (Alternative name: Alternative gene product encoded by XL-exon)	Function: May inhibit the adenylyl cyclase-stimulating activity of guanine nucleotide-binding protein G(s) subunit alpha which is produced from the same locus in a different open reading frame.; Gene names synonyms:GNAS1
359_nEC	O95467	GNAS	M	0	**Neuroendocrine secretory protein 55** - Short name: NESP55; [Cleaved into the following 2 chains: LHAL tetrapeptide; GPIPIRRH peptide]	Gene names synonyms:GNAS1
360_nEC	Q5JWF2	GNAS	M	0	**Guanine nucleotide-binding protein G(s) subunit alpha isoforms Xlas**; (Alternative names: Adenylate cyclase-stimulating G alpha protein; Extra large alphas protein - Short name: Xlalphas)	Molecular function: Transducer; Ligand: GTP-binding, Magnesium, Metal-binding, Nucleotide-binding; Gene names synonyms:GNAS1
361_nEC	P63092	GNAS	M	0	**Guanine nucleotide-binding protein G(s) subunit alpha isoforms short**; (Alternative name: Adenylate cyclase-stimulating G alpha protein)	Molecular function: Transducer; Ligand: GTP-binding, Magnesium, Metal-binding, Nucleotide-binding; Gene names synonyms:GNAS1, GSP
362_nEC	Q13889	GTF2H3	M	0	**General transcription factor IIH subunit 3**; (Alternative names: Basic transcription factor 2 34 kDa subunit - Short name: BTF2 p34; General transcription factor IIH polypeptide 3; TFIIH basal transcription factor complex p34 subunit)	Biological process: DNA damage, DNA repair, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc
363_nEC	Q6ZYL4	GTF2H5	M	0	**General transcription factor IIH subunit 5**; (Alternative names: General transcription factor IIH polypeptide 5; TFB5 ortholog; TFIIH basal transcription factor complex TTD-A subunit)	Biological process: DNA damage, DNA repair, Transcription, Transcription regulation; Gene names synonyms:C6orf175, TTDA
364_nEC	Q15648	MED1	M	0	**Mediator of RNA polymerase II transcription subunit 1**; (Alternative names: Activator-recruited cofactor 205 kDa component - Short name: ARC205; Mediator complex subunit 1; Peroxisome proliferator-activated receptor-binding protein - Short names: PBP/PPAR-binding protein; Thyroid hormone receptor-associated protein complex 220 kDa component - Short name: Trap220; Thyroid receptor-interacting protein 2 - Short names: TR-interacting protein 2/TRIP-2; Vitamin D receptor-interacting protein complex component DRIP205; p53 regulatory protein RB18A)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:ARC205, CRSP1, CRSP200, DRIP205, DRIP230, PBP, PPARBP, PPARGBP, RB18A, TRAP220, TRIP2
365_nEC	P51948	MNAT1	M	0	**CDK-activating kinase assembly factor MAT1**; (Alternative names: CDK7/cyclin-H assembly factor; Cyclin-G1-interacting protein; Menage a trois; RING finger protein 66; RING finger protein MAT1; p35; p36)	Biological process: Cell cycle, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:CAP35, MAT1, RNF66
366_nEC	P00156	MT-CYB	M	Ca, Mito	**Cytochrome b**; (Alternative names: Complex III subunit 3; Complex III subunit III; Cytochrome b-c1 complex subunit 3; Ubiquinol-cytochrome-c reductase complex cytochrome b subunit)	Biological process: Electron transport, Respiratory chain, Transport; Ligand: Heme, Iron, Metal-binding, Ubiquinone; Gene names synonyms:COB, CYTB, MTCYB

367_nEC	Q15596	NCOA2	M	0	**Nuclear receptor coactivator 2** - Short name: NCoA-2; (Alternative nams: Class E basic helix-loop-helix protein 75 - Short name: bHLHe75; Transcriptional intermediary factor 2 - Short name: hTIF2)	Molecular function: Activator; Biological process: Biological rhythms, Transcription, Transcription regulation; Gene names synonyms:BHLHE75, SRC2, TIF2
368_nEC	Q13772	NCOA4	M	0	**Nuclear receptor coactivator 4** - Short name: NCoA-4; (Alternative names: Androgen receptor coactivator 70 kDa protein - Short names: 70 kDa AR-activator/70 kDa androgen receptor coactivator; Androgen receptor-associated protein of 70 kDa; Ret-activating protein ELE1)	Molecular function: Activator; Biological process: Transcription, Transcription regulation; Gene names synonyms:ARA70, ELE1, RFG
369_nEC	Q14686	NCOA6	M	0	**Nuclear receptor coactivator 6**; (Alternative names: Activating signal cointegrator 2 - Short name: ASC-2; Amplified in breast cancer protein 3; Cancer-amplified transcriptional coactivator ASC-2; Nuclear receptor coactivator RAP250 - Short name: NRC RAP250; Nuclear receptor-activating protein, 250 kDa; Peroxisome proliferator-activated receptor-interacting protein - Short names: PPAR-interacting protein/PRIP; Thyroid hormone receptor-binding protein)	Molecular function: Activator; Biological process: Transcription, Transcription regulation; Gene names synonyms:AIB3, KIAA0181, RAP250, TRBP
370_nEC	O95299	NDUFA10	M	Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondria**l; (Alternative names: Complex I-42kD - Short name: CI-42kD; NADH-ubiquinone oxidoreductase 42 kDa subunit)	Biological process: Electron transport, Respiratory chain, Transport; Ligand: FAD, Flavoprotein
371_nEC	Q9UI09	NDUFA12	M	Ca, Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12**; (Alternative names: 13 kDa differentiation-associated protein; Complex I-B17.2 - Short names: CI-B17.2/CIB17.2; NADH-ubiquinone oxidoreductase subunit B17.2)	Biological process: Electron transport, Respiratory chain, Transport; Gene names synonyms:DAP13
372_nEC	O43678	NDUFA2	M	Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2**; (Alternative names: Complex I-B8 - Short name: CI-B8; NADH-ubiquinone oxidoreductase B8 subunit)	Biological process: Electron transport, Respiratory chain, Transport
373_nEC	Q16718	NDUFA5	M	Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5**; (Alternative names: Complex I subunit B13; Complex I-13kD-B - Short name: CI-13kD-B; NADH-ubiquinone oxidoreductase 13 kDa-B subunit)	Biological process: Electron transport, Respiratory chain, Transport
374_nEC	P56556	NDUFA6	M	Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 6**; (Alternative names: Complex I-B14 - Short name: CI-B14; LYR motif-containing protein 6; NADH-ubiquinone oxidoreductase B14 subunit)	Biological process: Electron transport, Respiratory chain, Transport; Gene names synonyms:LYRM6, NADHB14
375_nEC	P51970	NDUFA8	M	Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8**; (Alternative names: Complex I-19kD - Short name: CI-	Biological process: Electron transport, Respiratory chain, Transport

					19kD; Complex I-PGIV - Short name: CI-PGIV; NADH-ubiquinone oxidoreductase 19 kDa subunit)	
376_nEC	Q16795	NDUFA9	M	Mito	**NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial**; (Alternative names: Complex I-39kD - Short name: CI-39kD; NADH-ubiquinone oxidoreductase 39 kDa subunit)	Biological process: Electron transport, Respiratory chain, Transport; Ligand: FAD, Flavoprotein; Gene names synonyms:NDUFS2L
377_nEC	O14561	NDUFAB1	M	Ca, Mito	**Acyl carrier protein, mitochondrial** - Short name: ACP; (Alternative names: CI-SDAP; NADH-ubiquinone oxidoreductase 9.6 kDa subunit)	Biological process: Electron transport, Fatty acid biosynthesis, Fatty acid metabolism, Lipid biosynthesis, Lipid metabolism, Respiratory chain, Transport
378_nEC	Q9Y375	NDUFAF1	M	Ca, Mito	**Complex I intermediate-associated protein 30, mitochondrial**; (Alternative name: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 1)	Function: Chaperone protein involved in early stages of the assembly of mitochondrial NADH:ubiquinone oxidoreductase complex (complex I).; Molecular function: Chaperone; Gene names synonyms:CIA30
379_nEC	O96000	NDUFB10	M	Mito	**NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10**; (Alternative names: Complex I-PDSW - Short name: CI-PDSW; NADH-ubiquinone oxidoreductase PDSW subunit)	Biological process: Electron transport, Respiratory chain, Transport
380_nEC	O43674	NDUFB5	M	Mito	**NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial**; (Alternative names: Complex I-SGDH - Short name: CI-SGDH; NADH-ubiquinone oxidoreductase SGDH subunit)	Biological process: Electron transport, Respiratory chain, Transport
381_nEC	O95139	NDUFB6	M	Mito	**NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 6**; (Alternative names: Complex I-B17 - Short name: CI-B17; NADH-ubiquinone oxidoreductase B17 subunit)	Biological process: Electron transport, Respiratory chain, Transport
382_nEC	O95169	NDUFB8	M	Mito	**NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial**; (Alternative names: Complex I-ASHI -Short name: CI-ASHI; NADH-ubiquinone oxidoreductase ASHI subunit)	Biological process: Electron transport, Respiratory chain, Transport
383_nEC	O43181	NDUFS4	M	Mito	**NADH dehydrogenase [ubiquinone] iron-sulfur protein 4, mitochondrial**; (Alternative names: Complex I-18 kDa - Short name: CI-18 kDa; Complex I-AQDQ - Short name: CI-AQDQ; NADH-ubiquinone oxidoreductase 18 kDa subunit)	Biological process: Electron transport, Respiratory chain, Transport
384_nEC	Q12857	NFIA	M	0	**Nuclear factor 1 A-type** -Short names: NF1-A/Nuclear factor 1/A; (Alternative names: CCAAT-box-binding transcription factor - Short name: CTF; Nuclear factor I/A - Short names: NF-I/A; NFI-A; TGGCA-binding protein)	Molecular function: Activator, DNA-binding; Biological process: DNA replication, Transcription, Transcription regulation; Gene names synonyms:KIAA1439
385_nEC	O00712	NFIB	M	0	**Nuclear factor 1 B-type** - Short names: NF1-B; Nuclear factor 1/B; (Alternative names: CCAAT-box-binding transcription factor - Short name: CTF; Nuclear factor I/B - Short names: NF-I/B; NFI-B; TGGCA-binding protein)	Molecular function: Activator, DNA-binding; Biological process: DNA replication, Transcription, Transcription regulation
386_nEC	Q9BQI9	NRIP2	M	0	**Nuclear receptor-interacting protein 2**	Biological process: Transcription, Transcription regulation

387_nEC	Q9UBK2	PPARGC1 A	M, B	0	**Peroxisome proliferator-activated receptor gamma coactivator 1-alpha** - Short names: PGC-1-alpha/PPAR-gamma coactivator 1-alpha/PPARGC-1-alpha; (Alternative name: Ligand effect modulator 6)	Molecular function: Activator, RNA-binding; Biological process: Biological rhythms, Transcription, Transcription regulation ; Gene names synonyms:LEM6, PGC1, PGC1A, PPARGC1
388_nEC	P54619	PRKAG1	M	0	**5'-AMP-activated protein kinase subunit gamma-1** - Short names: AMPK gamma1/AMPK subunit gamma-1/AMPKg	Biological process: Fatty acid biosynthesis, Fatty acid metabolism, Lipid biosynthesis, Lipid metabolism; Ligand: ATP-binding, Nucleotide-binding
389_nEC	P31323	PRKAR2B	M	0	**cAMP-dependent protein kinase type II-beta regulatory subunit**	Function: Regulatory subunit of the cAMP-dependent protein kinases involved in cAMP signaling in cells. Type II regulatory chains mediate membrane association by binding to anchoring proteins, including the MAP2 kinase.; Ligand: cAMP, cAMP-binding, Nucleotide-binding
390_nEC	Q8WV60	PTCD2	M	Mito	**Pentatricopeptide repeat-containing protein 2, mitochondrial**	Function: Involved in mitochondrial RNA maturation and mitochondrial respiratory chain function.; Biological process: mRNA processing
391_nEC	P13631	RARG	M	R	**Retinoic acid receptor gamma** - Short name: RAR-gamma; (Alternative name: Nuclear receptor subfamily 1 group B member 3)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR1B3
392_nEC	P28749	RBL1	M	0	**Retinoblastoma-like protein 1**; (Alternative names: 107 kDa retinoblastoma-associated protein - Short name: p107; pRb1)	Molecular function: Chromatin regulator, Repressor; Biological process: Cell cycle, Host-virus interaction, Transcription, Transcription regulation
393_nEC	Q08999	RBL2	M	0	**Retinoblastoma-like protein 2**; (Alternative names: 130 kDa retinoblastoma-associated protein - Short name: p130; Retinoblastoma-related protein 2 - Short name: RBR-2; pRb2)	Molecular function: Chromatin regulator, DNA-binding, Repressor; Biological process: Cell cycle, Transcription, Transcription regulation; Gene names synonyms:RB2
394_nEC	P02753	RBP4	M	T	**Retinol-binding protein 4**; (Alternative names: Plasma retinol-binding protein - Short names: PRBP/RBP); [Cleaved into the following 4 chains: Plasma retinol-binding protein(1-182); Plasma retinol-binding protein(1-181); Plasma retinol-binding protein(1-179); Plasma retinol-binding protein(1-176)]	Biological process: Sensory transduction, Transport, Vision; Ligand: Retinol-binding, Vitamin A
395_nEC	P28702	RXRB	M	R	**Retinoic acid receptor RXR-beta**; (Alternative names: Nuclear receptor subfamily 2 group B member 2; Retinoid X receptor beta)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR2B2
396_nEC	P48443	RXRG	M	R	**Retinoic acid receptor RXR-gamma**; (Alternative names: Nuclear receptor subfamily 2 group B member 3; Retinoid X receptor gamma)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR2B3
397_nEC	Q99643	SDHC	M	Ca, Mito	**Succinate dehydrogenase cytochrome b560 subunit, mitochondrial**; (Alternative names: Integral membrane protein CII-3; QPs-1 - Short name: QPs1; Succinate dehydrogenase	BRENDA EC Number updated; Biological process: Electron transport, Transport, Tricarboxylic acid cycle; Ligand: Heme, Iron, Metal-binding; Gene names synonyms:CYB560, SDH3

					complex subunit C; Succinate-ubiquinone oxidoreductase cytochrome B large subunit - Short name: CYBL)	
398_nEC	O14521	SDHD	M	Ca, Mito	**Succinate dehydrogenase [ubiquinone] cytochrome b small subunit, mitochondrial** - Short name: CybS; (Alternative names: CII-4; QPs3; Succinate dehydrogenase complex subunit D; Succinate-ubiquinone oxidoreductase cytochrome b small subunit; Succinate-ubiquinone reductase membrane anchor subunit)	Biological process: Electron transport, Transport, Tricarboxylic acid cycle; Ligand: Heme, Iron, Metal-binding; Gene names synonyms:SDH4
399_nEC	Q15796	SMAD2	M, B	0	**Mothers against decapentaplegic homolog 2** - Short names: MAD homolog 2/Mothers against DPP homolog 2; (Alternative names: JV18-1; Mad-related protein 2 - Short name: hMAD-2; SMAD family member 2 - Short names: SMAD 2/Smad2/hSMAD2)	Molecular function: DNA-binding; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:MADH2, MADR2
400_nEC	Q13485	SMAD4	M	0	**Mothers against decapentaplegic homolog 4** - Short names: MAD homolog 4/Mothers against DPP homolog 4; (Alternative names: Deletion target in pancreatic carcinoma 4; SMAD family member 4 - Short names: SMAD 4/ Smad4/hSMAD4)	Molecular function: DNA-binding; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:DPC4, MADH4
401_nEC	Q99717	SMAD5	M	0	**Mothers against decapentaplegic homolog 5** - Short names: MAD homolog 5/Mothers against DPP homolog 5; (Alternative names: JV5-1; SMAD family member 5 - Short names: SMAD 5/Smad5/hSmad5)	Molecular function: DNA-binding; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:MADH5
402_nEC	Q13573	SNW1	M	0	**SNW domain-containing protein 1**; (Alternative names: Nuclear protein SkiP; Nuclear receptor coactivator NCoA-62; Ski-interacting protein)	Biological process: Host-virus interaction, mRNA processing, mRNA splicing, Transcription, Transcription regulation; Gene names Synonyms:SKIIP, SKIP
403_nEC	P08047	SP1	M	0	**Transcription factor Sp1**	Molecular function: Activator, DNA-binding, Repressor; Biological process: Biological rhythms, Host-virus interaction, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:TSFP1
404_nEC	P36956	SREBF1	M	0	**Sterol regulatory element-binding protein 1 -** Short name: SREBP-1; (Alternative names: Class D basic helix-loop-helix protein 1 - Short name: bHLHd1; Sterol regulatory element-binding transcription factor 1); [Cleaved into: Processed sterol regulatory element-binding protein 1; (Alternative name: Transcription factor SREBF1]	Protein name updated; Molecular function: Activator, DNA-binding; Biological process: Cholesterol metabolism, Lipid metabolism, Steroid metabolism, Sterol metabolism, Transcription, Transcription regulation;Gene names synonyms:BHLHD1, SREBP1
405_nEC	Q96SI9	STRBP	M	0	**Spermatid perinuclear RNA-binding protein**	Molecular function: Developmental protein, DNA-binding, RNA-binding; Biological process: Differentiation, Spermatogenesis; Gene names synonyms: SPNR
406_nEC	Q9BZK7	TBL1XR1	M	0	**F-box-like/WD repeat-containing protein TBL1XR1**; (Alternative names: Nuclear receptor corepressor/HDAC3 complex subunit	Molecular function: Activator, Chromatin regulator, Repressor; Biological process: Transcription, Transcription

					TBLR1; TBL1-related protein 1; Transducin beta-like 1X-related protein 1)	regulation, Ubl conjugation pathway; Gene names synonyms:IRA1, TBLR1
407_nEC	Q00059	TFAM	M	Mito	**Transcription factor A, mitochondrial** - Short name: mtTFA; (Alternative names: Mitochondrial transcription factor 1 - Short name: MtTF1; Transcription factor 6 - Short name: TCF-6; Transcription factor 6-like 2)	Molecular function: Activator, DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:TCF6, TCF6L2
408_nEC	P10827	THRA	M	R	**Thyroid hormone receptor alpha**; (Alternative names: Nuclear receptor subfamily 1 group A member 1; V-erbA-related protein 7 - Short name:EAR-7; c-erbA-1; c-erbA-alpha)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:EAR7, ERBA1, NR1A1, THRA1, THRA2
409_nEC	P10828	THRB	M	R	**Thyroid hormone receptor beta**; (Alternative names: Nuclear receptor subfamily 1 group A member 2; c-erbA-2; c-erbA-beta)	Molecular function: DNA-binding, Receptor; Biological process: Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:ERBA2, NR1A2, THR1
410_nEC	Q92748	THRSP	M	0	**Thyroid hormone-inducible hepatic protein**; (Alternative name: Spot 14 protein - Short names: S14/SPOT14)	Biological process: Lipid biosynthesis, Lipid metabolism, Transcription, Transcription regulation
411_nEC	Q15643	TRIP11	M	0	**Thyroid receptor-interacting protein 11** - Short names: TR-interacting protein 11/TRIP-11); (Alternative names: Clonal evolution-related gene on chromosome 14 protein; Golgi-associated microtubule-binding protein 210 - Short name: GMAP-210; Trip230)	Function: Is a membrane tether required for vesicle tethering to Golgi. Has an essential role in the maintenance of Golgi structure and function. It is required for efficient anterograde and retrograde trafficking in the early secretory pathway, functioning at both the ER-to-Golgi intermediate compartment (ERGIC) and Golgi complex. Binds the ligand binding domain of the thyroid receptor (THRB) in the presence of triiodothyronine and enhances THRB-modulated transcription.; Gene names synonyms:CEV14
412_nEC	Q99757	TXN2	M	Mito	**Thioredoxin, mitochondrial** - Short names: MTRX/Mt-Trx; (Alternative name: Thioredoxin-2)	Biological process: Electron transport, Transport; Gene names synonyms:TRX2
413_nEC	P25874	UCP1	M	T, Mito, Ca, Trans	**Mitochondrial brown fat uncoupling protein 1** - Short name: UCP 1; (Alternative names: Solute carrier family 25 member 7; Thermogenin)	Molecular function: Ion channel; Biological process: Ion transport, Transport; Gene names synonyms:SLC25A7, UCP
414_nEC	P55851	UCP2	M, B	Ca, Mito, T	**Mitochondrial uncoupling protein 2** - Short name: UCP 2; (Alternative names: Solute carrier family 25 member 8; UCPH)	Function: UCP are mitochondrial transporter proteins that create proton leaks across the inner mitochondrial membrane, thus uncoupling oxidative phosphorylation from ATP synthesis. As a result, energy is dissipated in the form of heat.; Biological process: Transport; Gene names synonyms:SLC25A8
415_nEC	Q9UDW1	UQCR10	M	Mito	**Cytochrome b-c1 complex subunit 9**; (Alternative names: Complex III subunit 9; Complex III subunit X; Cytochrome c1 non-	Biological process: Electron transport, Respiratory chain, Transport; Gene names synonyms:UCRC

					heme 7 kDa protein; Ubiquinol-cytochrome c reductase complex 7.2 kDa protein)	
416_nEC	O14957	UQCR11	M	Mito	**Cytochrome b-c1 complex subunit 10**; (Alternative names: Complex III subunit 10; Complex III subunit XI; Ubiquinol-cytochrome c reductase complex 6.4 kDa protein)	Biological process: Electron transport, Respiratory chain, Transport; Gene names synonyms:UQCR11
417_nEC	P14927	UQCRB	M	Mito	**Cytochrome b-c1 complex subunit 7**; (Alternative names: Complex III subunit 7; Complex III subunit VII; QP-C; Ubiquinol-cytochrome c reductase complex 14 kDa protein)	Biological process: Electron transport, Respiratory chain, Transport; Gene names synonyms:UQBP
418_nEC	P22695	UQCRC2	M	Mito	**Cytochrome b-c1 complex subunit 2**, mitochondrial; (Alternative names: Complex III subunit 2; Core protein II; Ubiquinol-cytochrome-c reductase complex core protein 2)	Biological process: Electron transport, Respiratory chain, Transport
419_nEC	O14949	UQCRQ	M	Mito	**Cytochrome b-c1 complex subunit 8**; (Alternative names: Complex III subunit 8; Complex III subunit VIII; Ubiquinol-cytochrome c reductase complex 9.5 kDa protein; Ubiquinol-cytochrome c reductase complex ubiquinone-binding protein QP-C)	Biological process: Electron transport, Respiratory chain, Transport
420_nEC	P45880	VDAC2	M	C, Mito	**Voltage-dependent anion-selective channel protein 2** - Short names: VDAC-2/hVDAC2; (Alternative name: Outer mitochondrial membrane protein porin 2)	Molecular function: Porin; Biological process: Ion transport, Transport; Ligand: NAD, Nucleotide-binding
421_nEC	Q9Y277	VDAC3	M	C, Mito	**Voltage-dependent anion-selective channel protein 3** - Short names: VDAC-3/hVDAC3; (Alternative name: Outer mitochondrial membrane protein porin 3)	Molecular function: Porin; Biological process: Ion transport, Transport; Ligand: NAD, Nucleotide-binding
422_nEC	P61160	ACTR2	B	0	**Actin-related protein 2**; (Actin-like protein 2)	Molecular function: Actin-binding; Ligand: ATP-binding, Nucleotide-binding; Gene names synonyms:ARP2
423_nEC	P18075	BMP7	B	0	**Bone morphogenetic protein 7** - Short name: BMP-7; (Alternative names: Osteogenic protein 1 - Short name: OP-1; INN: Eptotermin alfa)	Molecular function: Cytokine, Developmental protein, Growth factor; Biological process: Chondrogenesis, Differentiation, Osteogenesis; Gene names synonyms:OP1
424_nEC	P34820	BMP8B	B	0	**Bone morphogenetic protein 8B** - Short names: BMP-8/BMP-8B; (Alternative name: Osteogenic protein 2 - Short name: OP-2)	Molecular function: Cytokine, Developmental protein, Growth factor; Biological process: Chondrogenesis, Differentiation, Osteogenesis; Gene names synonyms:BMP8
425_nEC	O60543	CIDEA	B	0	**Cell death activator CIDE-A**; (Alternative name: Cell death-inducing DFFA-like effector A)	Molecular function: Activator; Biological process: Apoptosis, Transcription, Transcription regulation
426_nEC	Q99966	CITED1	B	0	**Cbp/p300-interacting transactivator 1**; (Alternative name: Melanocyte-specific protein 1)	Molecular function: Activator, Developmental protein; Biological process: Apoptosis, Differentiation, Transcription, Transcription regulation; Gene names synonyms:MSG1
427_nEC	P05230	FGF1	B	0	**Fibroblast growth factor 1** - Short name: FGF-1; (Alternative names: Acidic fibroblast growth factor - Short name: aFGF; Endothelial cell growth factor - Short name: ECGF; Heparin-binding growth factor 1 - Short name: HBGF-1)	Molecular function: Developmental protein, Growth factor, Heparin-binding, Mitogen; Biological process: Angiogenesis, Differentiation; Gene names synonyms:FGFA

428_nEC	P41134	ID1	B	0	**DNA-binding protein inhibitor ID-1**; (Alternative names: Class B basic helix-loop-helix protein 24 - Short name: bHLHb24; Inhibitor of DNA binding 1; Inhibitor of differentiation 1)	Molecular function: Developmental protein, Repressor; Biological process: Biological rhythms, Transcription, Transcription regulation; Gene names synonyms:BHLHB24, ID
429_nEC	P37231	PPARG	B	R	**Peroxisome proliferator-activated receptor gamma** - Short name: PPAR-gamma; (Alternative name: Nuclear receptor subfamily 1 group C member 3)	Molecular function: Activator, DNA-binding, Receptor; Biological process: Biological rhythms, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:NR1C3
430_nEC	Q86YN6	PPARGC 1B	B	Mito	**Peroxisome proliferator-activated receptor gamma coactivator 1-beta** -Short names: PGC-1-beta/PPAR-gamma coactivator 1-beta/PPARGC-1-beta; (Alternative name: PGC-1-related estrogen receptor alpha coactivator)	Molecular function: Activator, RNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:PERC, PGC1, PGC1B, PPARGC1
431_nEC	Q03431	PTH1R	B	R	**Parathyroid hormone/parathyroid hormone-related peptide receptor**; (Alternative names: PTH/PTHrP type I receptor - Short names: PTH/PTHr receptor; Parathyroid hormone 1 receptor - Short name: PTH1 receptor)	Molecular function: G-protein coupled receptor, Receptor, Transducer; Gene names synonyms:PTHR, PTHR1
432_nEC	O60902	SHOX2	B	0	**Short stature homeobox protein 2**; (Alternative names: Homeobox protein Og12X; Paired-related homeobox protein SHOT)	Function: May be a growth regulator and have a role in specifying neural systems involved in processing somatosensory information, as well as in face and body structure formation.; Molecular function: Developmental protein, DNA-binding; Gene names synonyms:OG12X, SHOT
433_nEC	O43435	TBX1	B	0	**T-box transcription factor TBX1** - Short name: T-box protein 1; (Alternative name: Testis-specific T-box protein)	Molecular function: Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation
434_nEC	Q96SF7	TBX15	B	0	**T-box transcription factor TBX15** - Short name: T-box protein 15; (Alternative name: T-box transcription factor TBX14 - Short name: T-box protein 14)	Molecular function: DNA-binding; Biological process: Transcription, Transcription regulation; Gene names synonyms:TBX14
435_nEC	Q99593	TBX5	B	0	**T-box transcription factor TBX5** - Short name: T-box protein 5	Molecular function: Developmental protein, DNA-binding; Biological process: Transcription, Transcription regulation
436_nEC	Q6ZUK4	TMEM26	B	Trans	**Transmembrane protein 26**	0
437_nEC	Q15915	ZIC1	B	0	**Zinc finger protein ZIC 1**; (Alternative names: Zinc finger protein 201; Zinc finger protein of the cerebellum 1)	Molecular function: Activator, Developmental protein, DNA-binding; Biological process: Differentiation, Neurogenesis, Transcription, Transcription regulation; Ligand: Metal-binding, Zinc; Gene names synonyms:ZIC, ZNF201

positive regulation IRX3 and IRX5

negative regulation IRX3 and IRX5

EC number found - additionally added to list "Genes with EC#"

M -MicroArray Melina

B - Beiging genes from Sophie

M,B - gene of Sophies list already in Melinas file

Ca - Carrier
C - Channel
Mito - mitochondrial related
R - Receptor
Trans - transmembrane activity
T - Transporter

recommended protein name in bold