

Technische Universität München
TUM School of Medicine and Health

The Effect of Intrapancreatic Glial Cell Depletion on Tumor Progression
and Pain Perception in Pancreatic Cancer

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Vollständiger Abdruck der von der TUM School of Medicine and Health der
Technischen Universität München zur Erlangung einer Doktorin der Natur-
wissenschaften (Dr. rer. nat.) genehmigten Dissertation.

Vorsitz: Prof. Dr. Heiko Lickert

Prüfende der Dissertation:

1. apl. Prof. Dr. Ihsan Ekin Demir, Ph.D.
2. Prof. Angelika Schnieke, Ph.D.

Die Dissertation wurde am 05.03.2024 bei der Technischen Universität
München eingereicht und durch die TUM School of Medicine and Health
am 05.06.2024 angenommen.

Presentations & Publications

Publications related to this thesis include:

1. **Yurteri Ü**, Çifcibaşı K, Friess H, Ceyhan GO, Istvanffy R, Demir IE. Schwann Cells in Peripheral Cancers: Bystanders or Promoters? *Adv Biol (Weinh)*. 2022 Jun 3:e2200033. [doi: 10.1002/adbi.202200033](https://doi.org/10.1002/adbi.202200033). PMID: 35656739.
2. Besikcioglu HE, **Yurteri Ü**, Munkhbaatar E, Ye L, Zhang F, Moretti A, Mota Reyes C, Özoğul C, Friess H, Ceyhan GO, Istvanffy R, Demir IE. Innervated mouse pancreas organoids as an ex vivo model to study pancreatic neuropathy in pancreatic cancer. *STAR Protoc*. 2021 Nov 17;2(4):100935. [doi: 10.1016/j.xpro.2021.100935](https://doi.org/10.1016/j.xpro.2021.100935). PMID: 34841274; PMCID: PMC8605431.
3. Mota Reyes C, **Yurteri Ü**, Friess H, Ekin Demir I. Future directions of neoadjuvant therapy in pancreatic cancer. *Oncoscience*. 2020 May 14;7(7-8):44-46. [doi: 10.18632/oncoscience.506](https://doi.org/10.18632/oncoscience.506). PMID: 32923514; PMCID: PMC7458337.
4. Xiaobo Wang, Rouzanna Istvanffy, Linhan Ye, Steffen Teller, Melanie Laschinger, Kalliope N. Diakopoulos, Kıvanç Görgülü, Qiaolin Li, Lei Ren, Carsten Jäger, Katja Steiger, Alexander Muckenhuber, Baiba Vilne, Kaan Çifcibaşı, Carmen Mota Reyes, **Ümmügülsüm Yurteri**, Maximilian Kießler, Ibrahim Halil Gürçınar, Maya Sugden, Saliha Elif Yıldızhan, Osman Uğur Sezerman, Sümeyye Çilingir, Güldal Süyen, Maximilian Reichert, Roland M. Schmid, Stefanie Bärthel, Rupert Oellinger, Achim Krüger, Roland Rad, Dieter Saur, Hana Algül, Helmut Friess, Marina Lesina, Güralp Onur Ceyhan, and Ihsan Ekin Demir. Phenotype screens of murine pancreatic cancer identify a Tgfa-Ccl2-paxillin axis driving human-like neural invasion. *The Journal of Clinical Investigation*. 2023 August. doi.org/10.1172/JCI166333. PMID: 37607005

5. Hüseyin Erdinc Besikcioglu, **Ümmügülsüm Yurteri**, Linhan Ye, Fang Zhang, Alessandra Moretti, Ibrahim H. Gürcinar, Alper, Dogruöz, Didem Karakas, Helmut Friess, Güralp Onur Ceyhan, Rouzanna Istvanffy, Ihsan Ekin Demir. Protocol for Whole-mount immunofluorescence staining protocol of ECM gel-embedded innervated pancreatic organoids. *STAR Protoc.* 2024;5(2):103132. [doi:10.1016/j.xpro.2024.103132](https://doi.org/10.1016/j.xpro.2024.103132).

Parts of this thesis were presented at the following scientific meetings:

1. **Ümmügülsüm Yurteri**, H.Erdinc Besikcioglu, Guralp O. Ceyhan, Helmut Friess, Ih-san Ekin Demir. Intrapancreatic Glial Cells Orchestrate Tumor Progression in Pancreatic Cancer. Oral Presentation, UEG week virtual 2020, 11-13 October 2020.
2. **Ümmügülsüm Yurteri**, H. Erdinc Besikcioglu, Guralp O. Ceyhan, Helmut Friess, Ih-san Ekin Demir. The Effect of Intrapancreatic Glial Cell Depletion on Tumor Progression and Pain Perception in Pancreatic Cancer. Poster Presentation, Deutscher Pancresclub, 13-15 February 2020, Halle, Germany.
3. Besikcioglu E., **Yurteri Ü.**, Friess H., Demir I.E. Innervated Pancreas Organoids as an Ex-vivo Model to Study Pancreatic Neuropathy in Pancreas Cancer. Oral Presentation, UEG week virtual, 11-13 October 2020.
4. **Ümmügülsüm Yurteri**, H.Erdinc Besikcioglu, Guralp O. Ceyhan, Helmut Friess, Ih-san Ekin Demir. The Effect of Intrapancreatic Glial Cell Depletion on Tumor Progression and Pain Perception in Pancreatic Cancer. Oral Presentation, DGNM, 06-07 March 2021.

*****Disclaimer:** This thesis underwent grammar checking using Grammarly AI Premium version.

To my eternal guide M.K.A.

„Science is the most accurate guide for everything in the world, for materiality, spirituality, life, and success. To seek guidance outside of science is heedlessness, ignorance, and heresy.

However, it is essential to comprehend the development of the stages of science every minute we live and to follow their progress on time.

Attempting to apply the principles drawn by the language of science of a thousand, two thousand, thousands of years ago the same way today, after so many thousand years, is, of course, not being in science.”

Mustafa Kemal Atatürk

22 September 1924, from Atatürk's speech to teachers, Samsun

Atatürk's Complete Works Volume 17, p. 44

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Introduction

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is an invasive neoplasm with a high incidence of mortality due to the complexity of early diagnosis and restricted responsiveness to therapies (Kaur, Kumar, Momi, Sasson, & Batra, 2013). PDAC is the predominant pancreatic neoplasm and appears in the exocrine compartment, causing more than 90 per cent of pancreatic cancer incidents (Halbrook, Lyssiotis, di Magliano, & Maitra, 2023). Unfortunately, despite scientific advances in understanding PDAC tumor biology and developing new therapeutic regimens, the average 5-year survival rate remains below 10% (Khalaf, El-Serag, Abrams, & Thrift, 2021).

Despite the widespread use of traditional treatment modalities such as surgery, chemotherapy, and radiation, significant improvement in clinical outcomes has yet to be achieved (Adamska, Domenichini, & Falasca, 2017; Ansari, Gustafsson, & Andersson, 2015). For patients with advanced-stage pancreatic cancer, surgical resection and chemotherapy are not feasible, though they have enhanced the survival rate of individuals with early-stage pancreatic cancer (Vaccaro, Sperduti, & Milella, 2011). Innovative immunotherapy technologies have provided promising results in different solid tumors and, in some cases, have surpassed chemotherapy as a first-line treatment option (H. Chen et al., 2020). On the other hand, although immunotherapy has opened a new era in the field of cancer therapy, its application in the treatment of PDAC is challenging, as this kind of cancer has a non-immunogenic, immunosuppressive, and treatment-resistant microenvironment (Oberstein & Olive, 2013).

Although pancreatic cancer has been researched over the past years, the results are still insufficient (Fan et al., 2020). Therefore, a thorough understanding of the PDAC tumor microenvironment is vital for developing new and effective treatments. To this end, further research should be conducted to identify the potential of immunotherapies, such as checkpoint inhibitors, that target tumor-associated antigens in the microenvironment (Yurteri et al., 2022).

PDAC genetics

PDAC is characterized by marked genetic heterogeneity between patients and within the same primary tumor niche. This genetic heterogeneity contributes to the complexity of accurately diagnosing and treating PDAC, as specific genetic changes could account for differential treatment responses. Alterations in multiple signaling pathways may explain the presence of multiple different resistance mechanisms. The course of PDAC is determined by inherited (~ 2 -10%) or somatic mutations (Habbe, Langer, Sina-Frey, & Bartsch, 2006; Wescott & Rustgi, 2008).

Most of these are linked with high-frequency somatic mutations in a group of genes, containing those encoding the small GTPase protein *KRAS* and the tumor suppressors *CDKN2A*, *p53*, and *SMAD4* (Morris, Wang, & Hebrok, 2010; Samuel & Hudson, 2012). *KRAS* mutations that arise in PDAC result in a protein that can not hydrolyze GTP and is structurally blocked in an active state, thus ensuring continuous signal transduction to downstream effectors (Rodriguez-Viciano et al., 2005).

Another mutation seen in PDAC is the *TP53* mutation. Between 50-75% of human pancreatic ductal adenocarcinomas (PDAC) have a *TP53* gene mutation following an activation mutation in the *KRAS* gene (Oberstein & Olive, 2013). These *p53* mutations usually result in the expression of a stable protein rather than a complete loss of protein expression (Jennifer P. Morton et al., 2010). These often result in the deposition of mutant p53 protein with typically either potentially

gain-of-function or dominant-negative properties. However, in most cancers, *TP53* is mutated rather than deleted, suggesting that mutant *p53* may confer some advantage in tumor cell growth (Scarpa et al., 1993).

Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) is a tumor suppressor gene encoding the p16^{INK4A} protein. *CDKN2A* is responsible of negative regulation of cell cycle progression (G1 to S phase transition) by interfering with complex formation between *CDK4/6* and *cyclin D*. *CDKN2A* causes genetic alterations by homozygous deletion, point mutation, promoter hypermethylation, and is commonly found in an inactivated form in cancers (Serra & Chetty, 2018; Zhao, Choi, Lee, Bode, & Dong, 2016).

Loss of *Mothers Against Decapentaplegic Homolog 4* (*SMAD4*) gene, encoding a transcription factor involved in transforming growth factor- β (*TGF- β*) and bone morphogenetic protein (BMP) signaling, is a late step during PDAC progression, and *SMAD4* mutations occur in approximately 55% of PDAC patients (Dardare, Witz, Merlin, Gilson, & Harlé, 2020).

Pancreatic intraepithelial neoplasms (PanINs) are the most common precursor lesions of PDAC (Hezel, Kimmelman, Stanger, Bardeesy, & DePinho, 2006). They are classified into three stages based on their histologic appearance and progressive cellular and nuclear abnormality (Hruban et al., 2004). Molecular research has shown that the PanIN stage is associated with increased mutation frequency, diversity, and accumulation of mutations (Figure 1) (Feldmann, Beaty, Hruban, & Maitra, 2007; Orth et al., 2019). For instance, PanIN1 lesions generally have mutated *KRAS* and are less likely to have *p53* or *SMAD4* mutations. On the other hand, PanIN3 lesions are likely to express mutated *KRAS*, *p53* and *SMAD4* (Wilentz et al., 2000).

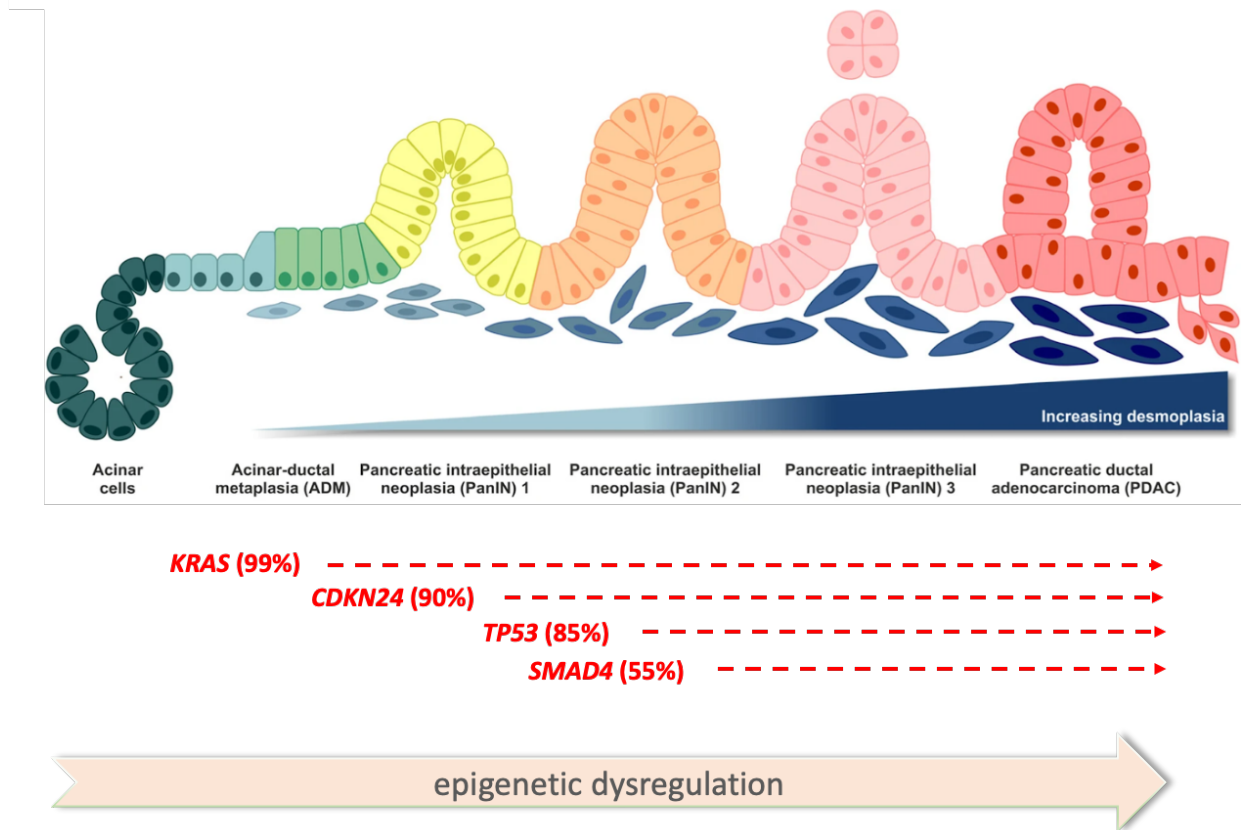


Figure 1. Involvement of KRAS as a catalyst for the emergence and spread of PDAC. Pancreatic ductal adenocarcinoma (PDAC) and pancreatic intraepithelial neoplasia (PanIN) can arise from *Kras*G12D or *Kras*G12V mutations. In humans, it has been discovered that PanINs contain an increasing number of mutations and are divided into three phases of increasing cellular atypia. Desmoplastic alterations correlate with epithelium alterations. By activating mutant KRAS in embryonic pancreatic progenitors, the human PanIN spectrum, coupled with progression to PDAC, has been replicated in animal models. PDAC latency is significantly reduced by removing tumor suppressors frequently inactivated in medical disorders. Insulin-positive cells and acini can develop into PanINs and, in some cases, into PDAC, depending on tissue injury and tumor suppressor inactivation, according to mouse models in which certain adult cell types have direct KRAS activation. To assume the PanIN-PDAC lineage, these cell types must be reprogrammed into 'ductal' cell types. The image is adopted from (Orth et al., 2019).

Beyond mutations at the *KRAS*, *CDKN2A*, *TP53*, and *SMAD4/DPC4* genes, other gene mutations at the *BRCA2*, *MUC16*, *BRAF* gene loci, are also involved in the expansion of pancreatic cancer, as shown in Figure 2 (Hu et al., 2021).

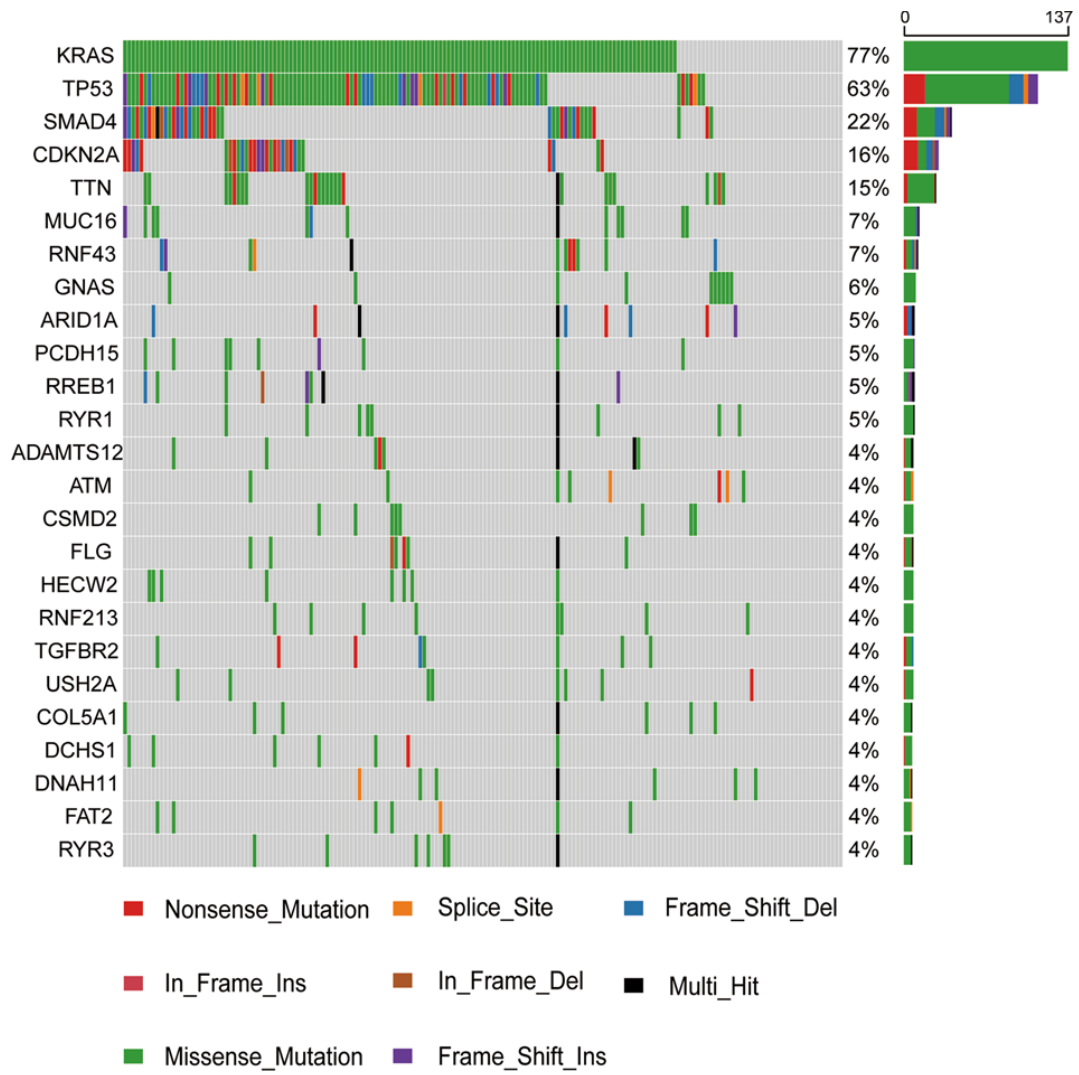


Figure 2. Mutation content of human pancreatic cancer. Hu et al. (2021) examined 178 pancreatic tumors from the TCGA dataset. The most frequently mutated genes in pancreatic cancer were localized to the *KRAS*, *CDKN2A*, *TP53*, and *SMAD4* genes, with corresponding mutation rates of 77%, 63%, 22%, and 16%. Moreover, the two main types of mutations are missense mutations and nonsense mutations. By permission of Springer Nature (Hu et al., 2021).

Neural invasion in pancreatic cancer

Perineural invasion (PNI) is a distinctive phenomenon in which cancer cells invade adjacent nerves and perineural sites (Ceyhan et al., 2008). The poor prognosis of PDAC is thought to be affiliated with PNI (Dai et al., 2007).

PNI is seen in about 90% of pancreatic cancer patients. The incidence of high PNI in pancreatic cancer limits radical resection and supports local recurrence (Nakao, Harada, Nonami, Kaneko, & Takagi, 1996). PNI is a directed process involving diverse signaling molecules from many signaling pathways, and these signaling molecules are generated by cancer cells and nerve cells. When cancer cells invade nerves, they can reproduce within neural spaces. This phenomenon provides an opportunity for cancer cells for dissemination to distant territories as an alternative metastatic route (Bapat, Hostetter, Von Hoff, & Han, 2011).

Studies have revealed that cancer cells enter the innermost parts of the nerve sheath, indicating that highly adaptive signaling activities connect cancer cells to peripheral nerves and promote cancer cell migration (Liebig, Ayala, Wilks, Berger, & Albo, 2009). Many signaling molecules are involved in PNI, including chemokines, cell surface ligands, neurotrophins, and receptors (Jingbo Li, Kang, & Tang, 2021). According to several studies, nerves emit ligands or neurotrophic factors that could serve as PDAC's potential intermediates of neuroplasticity and attract cancer cells to the nerves through chemoattraction (Dai et al., 2007). These neurotrophic factors, like NGF, pleiotrophin, and the GDNF family of ligands, also have a chemoattractive property, which is how they influence the "neurotropism" of PDAC cells (Ceyhan et al., 2008; Hirai et al., 2002).

Glial cells

Rudolf Virchow, Santiago Ramón y Cajal and Pió del Ro-Hortega, pioneering neuroscientists of the 19th century, were the first to recognize glial cells. At that time, the idea was put forward that glial cells should only function as "Nervenkitz", the German word for "nerve rod". It is often reflected in the term "glial cell", derived from the archaic Greek word "glia", which is translated in English as "glue" (Jäkel & Dimou, 2017).

Glial cells, particularly Schwann cells, produce myelin and wrap neurons to engage them in place (Jäkel & Dimou, 2017). The maintenance of the ionic environment of nerve cells, adjusting the rate of nerve impulse generation, balancing synaptic activity by regulating the uptake of neurotransmitters, providing a framework for some perspectives on neural development, and supporting (or, in some cases, preventing) recovery from neural injury are examples of well-known glial functions (Boerboom, Dion, Chariot, & Franzen, 2017; Kristján R. Jessen, Mirsky, & Lloyd, 2015). Additionally, glial cells also act as a scaffold for the formation of neuronal networks and play a role in synaptic plasticity by supporting the maturation and differentiation of neurons as well as controlling their growth and survival (Akiko Nishiyama, 2007).

Although glial cells are frequently confused with neurons, glial cells are distinctive from nerve cells (Araque & Navarrete, 2010). Glial cells do not straightforwardly take part in synaptic intuitive and electrical signaling. Instead, their supporting capacities offer assistance to characterize synaptic contacts and keep up the signaling capabilities of neurons (Rao, Noble, & Mayer-Pröschel, 1998).

Peripheral glial cells; Schwann cells

Schwann cells develop throughout embryological processes from neural crest cells (Salzer & Zalc, 2016). Schwann cells (SCs) surround neurons, keeping them viable and frequently integument them with a myelin sheath (Kristján R Jessen et al., 2015). They are the primary type of glial cell in the peripheral nervous system. Schwann cells are essential for the growth, maintenance, protection, and regeneration of peripheral nerves (Yurteri et al., 2022). In addition, these cells form a shield around the axons of peripheral nerve fibers, allowing nerve impulses to reach their targets efficiently (Lavdas & Matsas, 2014).

According to their myelin structure, Schwann cells (SCs) are divided into two main groups: myelinating and satellite cells. Myelinating SCs ensure that all large-diameter peripheral axons are wrapped in myelin (Lavdas & Matsas, 2014). They are responsible for transmitting electrical signals at the highest speed. On the other hand, non-myelinating Schwann cells (Satellite glial cells) cover the neuron bodies in the sensory, sympathetic, and parasympathetic ganglia (Matsas, Lavdas, Papastefanaki, & Thomaidou, 2008). They are considered to be functioning in a similar manner to astrocytes in the central nervous system (CNS) (Matsas et al., 2008; Salzer & Zalc, 2016).

Schwann cells are cells have extraordinary plasticity. This feature of SCs has profound importance in post-nerve injury or demyelinating neuropathies (Boerboom et al., 2017). As previously mentioned, Schwann cells derive from neural crest cells during the embryonic period. This transformation proceeds in two well-characterized intermediate phases. These are Schwann cell precursors (SCPs) in early embryonic nerves and immature Schwann cells (iSch) in late embryonic and perinatal nerves. In this context, lineage involves three main stages of developmental transition. First, SCPs form from crest cells, then SCPs give rise to iSch and finally iSchs develop into myelin and Satellite cells (Figure 3) (Kristján R Jessen et al., 2015; Monk, Feltri, & Taveggia, 2015)

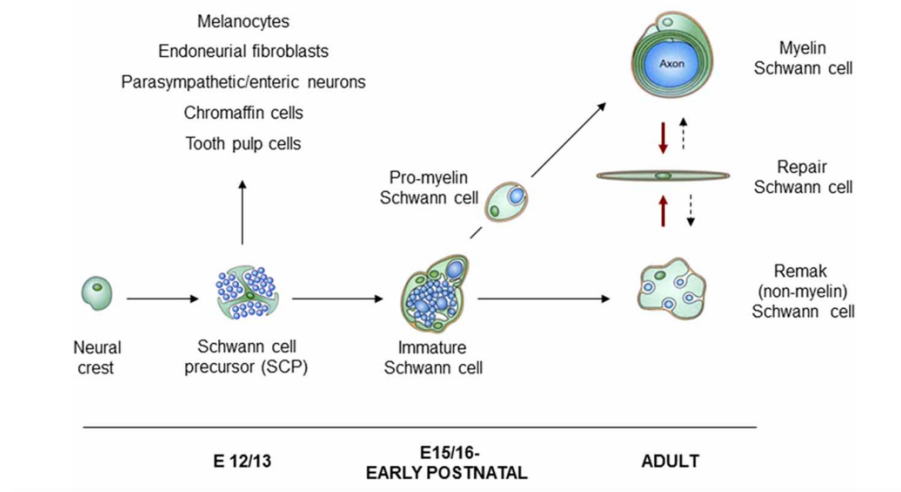


Figure 3. The principal crossovers in the genesis of Schwann cells. A representation of the principal cell subtypes participating in Schwann cell differentiation and neural repair. Immature Schwann cells (iSch) are also shown as alternate cells that evolve from Schwann cell precursors (SCP). Schwann cell precursors (SCP) have also been shown to give rise to other cells besides immature Schwann cells (iSch). Solid black arrows indicate regular transitions in developmental stages. Red arrows indicate changes in Schwann cell damage. Myelin and Remak cells regenerate after repair, as the striped arrows indicate. With permission from Oxford Academic Press (Kristjan R. Jessen & Mirsky, 2019).

An essential role of Schwann cells involved in neural degeneration is to stimulate axon regeneration (Bolívar, Navarro, & Udina, 2020). Following an injury or peripheral nerve damage, Schwann cells undergo a trans differentiation process, which includes the degradation of the myelin sheath and the expression of a series of genes that drive nerve growth (Kristján R Jessen et al., 2015). These differentiated Schwann cells then provide a supportive environment for nerve repair by directing the remodeling of axons (K. R. Jessen & Mirsky, 2016).

Schwann cells are additionally responsible for clearing cellular debris in the peripheral nervous system (PNS). Throughout neural degeneration, axons can sustain Wallerian degeneration, involving the disintegration and breakdown of axonal constituent (K. R. Jessen & Mirsky, 2016; Kristján R Jessen et al., 2015). Schwann cells are involved in phagocytosis and removing these residues from the neural milieu, resulting in effective nerve regeneration (Gaudet, Popovich, & Ramer, 2011).

Many substances that enhance tumor development and survival may be secreted by Schwann cells (Bunimovich, Keskinov, Shurin, & Shurin, 2017). For instance, nerve growth factor (NGF), a protein that has been demonstrated to promote the proliferation and survival of colon cancer cells both in vitro and in vivo, can be secreted by Schwann cells (S. Han et al., 2022). Moreover, NGF can stimulate the *PI3K-Akt* signaling pathway, which is involved in cell survival and growth, and release downstream growth factors that promote tumor formation (Jung et al., 2021).

Schwann cells may help create a tumor microenvironment that promotes the growth and spread of tumors (Bunimovich et al., 2017). Schwann cells may also interact with immune, cancer, and fibroblast cells as well as other cells in the tumor microenvironment, secreting substances that support angiogenesis and inflammation (Sun, Chen, & Chen, 2022).

The invasion and migration of cancer cells can be induced by Schwann cells through direct engagement (De Logu et al., 2021). Furthermore, laminin and fibronectin, expressed by Schwann cells, may interact with integrin receptors on cancer cells to facilitate cancer cells' adhesion and migration (Jain & Chauhan, 2022).

Schwann cells have been linked to GI cancer-related neuropathic pain generation in addition to their role in the formation and development of tumors (Gierthmühlen & Baron, 2016). Patients with GI malignancies may experience neuropathic pain due to changes in the enteric nervous system (ENS), including anomalies in Schwann cell function (Finnerup, Kuner, & Jensen, 2021). Recent studies demonstrated that SCs emerge early in the primary tumor during PDAC development and that their presence may be linked to an increase in the perineural invasion (Azam & Pecot, 2016; Roger et al., 2019).

Schwann cells direct cancer cells toward nerves by producing neural cell adhesion molecule 1 (NCAM1), which encourages perineural invasion (Sylvie Deborde et al., 2016). It has been demonstrated that SCs promote epithelial-mesenchymal transition and support metastatic dispersion (Sylvie Deborde & Wong, 2017). SCs have been shown to conceal cancer-related pain, prolonging the asymptomatic phase and delaying pain perception (Ihsan Ekin Demir et al., 2016).

Additionally, several cytokines and adhesion molecules have been identified that mediate gliacancer interactions. For example, SC-derived interleukin-6 enhances PDAC cell migration and invasion (Grothe et al., 2000). However, the all-molecular players secreted by SCs and their effects on PDAC still remain unclear.

The nature of the crosstalk between tumor and Schwann cells has not yet been elucidated. Schwann cells communicate with tumor cells mediated by plasma membrane proteins such as myelin-associated glycoprotein and neural cell adhesion molecule 1 (NCAM1) (Azam & Pecot, 2016). Mucin-1 further enhanced the adhesion of PDAC cells to Schwann cells and the invasion of sciatic nerve *in vivo* (Kaur et al., 2013). Additionally, Schwann cells undergo structural changes upon contact with PDAC cells, resulting in the development of projections of Schwann cells and their distribution among cancer cells (I. E. Demir, Friess, & Ceyhan, 2015). PNI develops gradually *in vitro* and *in vivo* because these structural changes depend on NCAM1 activation on the Schwann cell membrane (Kameda et al., 1999).

Strong commonalities exist between the Schwann cells involved in nerve repair and those involved in cancer invasion. When nerves are damaged, or cancer cells invade them, more Schwann cells express GFAP (Kristján R Jessen & Mirsky, 2008; Morgan, Jessen, & Mirsky, 1991).

The three-dimensional tumor cell spheres provide a better understanding of the interaction between Schwann cells and cancer cells. For example, it has been observed that GFAP positive Schwann cells promote tumor invasion of cancer cells within the tumor sphere (Sylvie Deborde et al., 2016). This process may amplify the progression and aggressiveness of cancer cells.

Glial fibrillary acidic protein (GFAP)

Glial fibrillary acidic protein (GFAP) is an intermediate filament (IF) type III protein. Its gene is located on *17q21.31*, mainly found in astrocytes in the central nervous system (CNS), non-myelinating Schwann cells in the peripheral nervous system (PNS), and enteric glial cells (Yang & Wang, 2015). *GFAP* gene activation and protein induction appear to play a critical role in astroglial cell activation (astrogliosis) following CNS injuries and neurodegeneration (Brenner, 2014). Several nuclear-receptor hormones, growth factors, and lipopolysaccharides regulate the expression of *GFAP* mRNA (Brenner, 2014). Following CNS injuries and neurodegeneration, *GFAP* gene activation and protein induction appear crucial for astroglial cell activation (astrogliosis) (Brenner, 2014). *GFAP* mRNA expression is a marker of astrocyte activation and glial scar formation by inducing astrogliosis (Yang & Wang, 2015). GFAP commands the blood-brain barrier, cytoskeletal structure, mechanical strength maintenance, and support of surrounding neurons (Yang & Wang, 2015). GFAP protein is highly homologous to non-epithelial intermediate filaments type III members like vimentin and peripherin and has a head, rod, and tail domain. All class III intermediate filament proteins exhibit this structural organization in a highly conserved manner (Middeldorp & Hol, 2011; Yang & Wang, 2015).

As previously mentioned, GFAP (glial fibrillary acidic protein) is a protein primarily expressed in astrocytes, a type of glial cell in the central nervous system (Janigro, Mondello, Posti, & Uden, 2022). However, GFAP expression has also been detected in peripheral tissues, including prostate (Yao et al., 2021), colon (von Boyen et al., 2011) and liver (Morini et al., 2005).

GFAP expression in peripheral cancers is of great interest to researchers as it is thought to be linked to many critical aspects of cancer pathology. The following reasons can be cited as examples to understand the link between GFAP expression and cancer biology;

1. Tumor aggressiveness: Several studies have reported that GFAP expression is associated with increased tumor aggressiveness in peripheral cancers, including gastrinoma and colon cancers. This is likely due to GFAP's role in regulating cell motility and invasion (K. J. Kim et al., 2011; A. Nishiyama et al., 1989; Sanz-Moreno et al., 2011).
2. Cancer stem cells: GFAP has been found to be a marker of cancer stem cells in glioblastoma (J. Wang et al., 2008). This finding suggests that it may also have a similar function in peripheral malignancies, which is an intriguing possibility.
3. Diagnosis and prognosis: Increased GFAP expression levels can be used as a diagnostically and prognostically important biomarker in different peripheral cancers such as colorectal (Yuan et al., 2020), breast (Momin, Rigler, & Chitralla, 2023), and hepatocellular carcinoma (Yu, Liu, Ji, & Liu, 2020).
4. Target for therapy: Studies have shown that GFAP may be a potential therapeutic target in some peripheral cancers. For instance, one study targeted GFAP expression with rapamycin in a mouse model of breast cancer and showed that tumor growth and metastasis could be significantly inhibited (Abdelaziz, Stone, & Komarova, 2015).

In conclusion, GFAP expression in peripheral cancers is an exciting but under-researched area in terms of its association with tumor aggressiveness, cancer stem cells, diagnosis and prognosis, and as a potential target for therapy. Therefore, it is crucial to fully understand the precise role of GFAP in peripheral cancers and to explore more effective strategies to target this protein within cancer therapy.

IL-8, MDSCs and Schwann cells

Interleukin-8 (IL-8) is a pro-inflammatory cytokine, which is prominently implicated in the regulation of the immune response and the promotion of cancer (M. Zhang et al., 2020). One study suggested that IL-8 is involved in the crosstalk between Schwann cells and cancer cells in peripheral cancers (J. H. Kim, 2020).

IL-8 is a factor included in the interaction of Schwann cells and cancer cells in peripheral cancers (S. Chen & Chen, 2022). It acts as a chemoattractant to recruit Schwann cells to the tumor microenvironment, where they can secrete growth factors and cytokines that amplify cancer cell proliferation, invasion, and migration (Gundlach et al., 2022). In addition, IL-8 may also induce the production of pro-inflammatory cytokines and chemokines by Schwann cells, further amplifying the inflammatory response in the tumor microenvironment (Gregory, Powers, Jamshidi-Parsian, Griffin, & Song, 2023).

Peripheral cancers such as colorectal, pancreatic, and cholangiocellular carcinoma, IL-8 generated by Schwann cells can promote tumor growth, invasion, and metastasis (S. Chen & Chen, 2022; S. J. Chen et al., 2018). For instance, IL-8 may stimulate angiogenesis, providing oxygen and nutrients to the growing neoplasm. It may also enhance the migration and invasion of cancer cells by turning on signaling pathways implicated in cell movement (Storan, O'Gorman, McDonald, & Steinhoff, 2015).

Moreover, IL-8 may molecularly regulate the immune response in the tumor microenvironment. For example, it may recruit immune cells such as neutrophils and macrophages, which have a role in stimulating tumor growth and metastasis (Gundlach et al., 2022). IL-8 may also interfere with the function of CD8⁺ T cells, which are essential in the immune response toward cancer cells (S. Chen & Chen, 2022).

Myeloid-derived suppressor cells (MDSCs) are a diverse community of immune cells that drive from the bone marrow and have a prominent role in the development and spread of cancer

(Gabrilovich, 2017). MDSCs suppress immune responses through inhibitory molecules and immunosuppressive cytokines. By suppressing the activity of cytotoxic T cells, natural killer cells, and dendritic cells, they can create an immunosuppressive tumor microenvironment that facilitates the development and growth of cancer cells (Burke & Ostrand-Rosenberg, 2014). Furthermore, MDSCs enhance tumor angiogenesis by secreting pro-angiogenic factors that promote cancer cell growth and survival by improving vascularization in the cancer microenvironment. They also induce chronic tumor inflammation, promoting tumor cell proliferation, survival, and angiogenesis (Khaled, Ammori, & Elkord, 2013; Kumar, Patel, Tcyganov, & Gabrilovich, 2016).

Activation of MDSCs in the tumor microenvironment has been associated with IL-8 (Z. J. Han et al., 2021). IL-8 acts as a chemoattractant, allowing MDSCs to migrate to the tumor site. MDSCs express CXCR1 and CXCR2 receptors, the primary receptors for IL-8. Cross-talk between IL-8 and MDSC receptors facilitates the transport and accumulation of MDSCs into the tumor microenvironment (Nagaraj, Youn, & Gabrilovich, 2013; Ostrand-Rosenberg & Sinha, 2009).

Furthermore, IL-8 and MDSCs are both involved in tumor angiogenesis (Fousek, Horn, & Palena, 2021). IL-8 acts as a potent pro-angiogenic factor and can induce the production of vascular endothelial growth factor (VEGF) and other angiogenic factors (Gregory et al., 2023). MDSCs can also themselves produce factors that promote angiogenesis, including VEGF (Fousek et al., 2021). The interaction between IL-8 and MDSCs may thus mediate tumor growth and metastasis by exerting pro-angiogenic effects (Finke et al., 2011).

The IL-8 and MDSC association in cancer goes beyond immunosuppression and angiogenesis. Research has revealed that IL-8 triggers the proliferation and differentiation of MDSCs in the bone marrow, possibly contributing to increased cancer incidence (Matsushima, Yang, & Oppenheim, 2022).

In conclusion, IL-8 and MDSCs have a cross-talk in the cancer microenvironment. IL-8 promotes the recruitment, activation, and immunosuppressive functions of MDSCs, while MDSCs promote

IL-8 production and potentiate the pro-angiogenic characteristic of IL-8 (Matsushima et al., 2022; Ostrand-Rosenberg & Fenselau, 2018).

Targeting IL-8 or its receptors may offer therapeutic opportunities for regulating MDSC-mediated immunosuppression, suppressing angiogenesis, and enhancing anti-tumor immune responses. However, further research is required to elucidate the complex interactions between IL-8, MDSCs and Schwann cells and their influence on cancer development and therapeutic management.

Mouse models

Pancreatic cancer is a destructive disorder known for its invasive course. Researchers have extensively used animal models to gain a deeper insight into the molecular mechanisms governing pancreatic neoplasia and to develop targeted and effective therapies. Among these models, the KC, KPC mouse models derived from them were used in this project.

KC mouse model

The KC mouse model is a genetically engineered model designed to replicate crucial human pancreatic ductal adenocarcinoma (PDAC) features (Ariston Gabriel et al., 2020). This model involves activating the *Kras*^{G12D} oncogene in pancreatic epithelial cells. The *Kras*^{G12D} mutation increases the KRAS signaling pathway, a common characteristic of human PDAC cases. The Trp53^{R172H} mutation, when added, results in the loss of tumor suppressor function, which further promotes the progression and spread of the disease (Westphalen & Olive, 2012). The KC mouse model has proven to be a valuable tool for studying pancreatic cancer. By displaying the complete spectrum of pre-invasive PanIN and its progression to invasive and metastatic PDAC, this model has provided valuable insights into the molecular and cellular mechanisms involved in PDAC initiation, growth, and advancement (Pérez–Mancera, Guerra, Barbacid, & Tuveson, 2012).

KPC mouse model

The KPC pancreatic cancer mouse model is commonly employed as a preclinical model for researching pancreatic ductal adenocarcinoma (PDAC). "KPC" is an acronym for LSL-Kras^{G12D/+} ; LSL-Trp53^{R172H/+} ; Pdx1-Cre, which denotes the genetic changes present in this particular PDAC mouse model (Gopinathan, Morton, Jodrell, & Sansom, 2015). The KPC mouse model was explicitly designed to incorporate two mutations commonly found in human PDAC (Guerra & Barbacid, 2013). These mutations include an activating mutation (G12D) in the KRAS gene and a loss-of-function mutation in the TP53 (R172H) tumor suppressor gene. To ensure the precise insertion of these mutations into pancreatic cells, the Cre-loxP system is utilized, which is driven by Pdx1 or p48 promoters that are active during the early stages of pancreatic development (Guerra & Barbacid, 2013). These mutations result in pancreatic tumors that are very similar to human pancreatic cancer in terms of histopathological properties, gene expression profiles, and clinical behavior (Pérez-Mancera et al., 2012). The KPC mouse model has been a precious tool in revealing numerous facets of PDAC, such as tumor initiation, progression, dissemination, and treatment reactions (Ariston Gabriel et al., 2020). A similar and yet more aggressive phenotype was observed in KPC mice in which the floxed mutated p53 allele was replaced with two copies of a p53-floxed allele (p48-Cre; LSL-Kras^{G12D}; *Trp53*^{fl/fl}), which, in its homozygous form, leads to complete loss of the p53 function (Jennifer P Morton et al., 2010). These animals exhibit a more rapid tumor progression than the classical KPC mice with a *Trp53*^{R172H} allele.

The PDAC mice models utilized in this project are explained in more detail in the materials and methods chapter.

Aim of the present study

In many solid cancers, including pancreatic cancer, neural invasion (NI) is the leading cause of local recurrence and neuropathic pain. Schwann cells are hypothesized to initiate nerve invasion in gastrointestinal tumors by interacting with cancer cells. However, the role of peripheral glial cells in the progression and development of pancreatic cancer is controversial regarding early and prominent glial cell activation in PDAC and the interaction of glial cells and tumor cells. Therefore, glial cells should be considered to play a decisive role in the emergence of PDAC pathology at very early tumor stages, in PDAC-related neuroplastic changes in the pancreas and central nervous system, as well as in PDAC-induced neuropathic pain. However, in this context, previous studies have not evaluated the functional relevance of glial cells, and Schwann cells in particular, as potential interaction partners for tumor cells in PDAC.

The present study's primary and most significant aim was to determine how the absence of Schwann cells affects the tumor microenvironment and tumor growth in pancreatic cancer. Towards this aim, the following objectives were pursued in this study:

1. Investigate the effects of depletion of activated glial cells in murine PDAC (i.e. KC, KPC, and KC;GFAP-Tk, KPC;GFAP-Tk, and GFAP-Tk mice with orthotopic tumor implantation) on PDAC progression, tumor cell invasion, nerve constitution, and on the tumor microenvironment.
2. Compare the effect of glial cell depletion on abdominal mechanosensitivity (via Von Frey test) and pain perception in murine PDAC with glial cell depletion.
3. Identify the differentially regulated pathways in Schwann cells exposed to murine PDAC organoids or human PDAC cell lines.

This study, therefore, aimed to provide novel functional *in vivo* data on the contribution of Schwann cells to PDAC emergence and progression, PDAC microenvironment, and pain in PDAC.

Materials and Methods

Materials

Table 1. Primary antibodies

Antibody	Company	Catalogue number	Application
Anti-GFAP	Dako	M0761	IHC
Anti- α -SMA	abcam	ab5694	IHC
Anti-S100	abcam	EP1576Y	IHC
Anti-CD45	abcam	ab10558	IHC-IF
Anti-CD8	abcam	ab217344	IHC
Anti-F4/80	abcam	ab6640	IHC
Anti-P-Erk	abcam	ab201015	IHC
Anti-p-p38/38-MAPK14	abcam	ab170099	IF
Anti-Iba-1	abcam	ab178846	IF
Anti-CD31	abcam	ab124432	IHC
Anti-P75NTR	Sigma Aldrich	07-476	IF
Anti-GFAP	Invitrogen	PA1-10004	IF
Anti-c-fos	abcam	ab222699	IHC

Table 2. Secondary antibodies

Antibody	Company	Catalogue number	Ap- plic a- tion
Alexa Fluor goat anti- mouse IgG 488/594	Thermo Fisher Scientific	1834337/ 1830459	IF
Alexa Fluor goat anti- rabbit IgG 488/594	Thermo Fisher Scientific	1885240/ 1851471	IF
Goat HRP-Labelled Polymer anti-mouse Ab	Dako Deutschland GmbH	K4000	IHC
Goat HRP-Labelled Polymer anti-rabbit Ab	Dako Deutschland GmbH	K4003	IHC

Table 3. Chemicals and reagents

Chemical/Reagent	Company	Catalogue number
Gentle Cell Dissociation Reagent (GCDR)	Stemcell Technologies	07174
Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free	Corning	356231
DPBS without CaCl ₂ , without MgCl ₂	Sigma Aldrich	D8537
Y27632	Miltenyi Biotec	130-103-922
Advanced DMEM/F-12	Thermo Fisher/Gibco	12634010
GlutaMAX 1003	Thermo Fisher/Gibco	35050061
HEPES (1 M)	Thermo Fisher/Gibco	15630080
N-Acetylcysteine	Sigma-Aldrich	A9165-5G
Nicotinamide	Sigma-Aldrich	N0636-100G
Recombinant Murine Noggin	PeproTech	250-38

EGF Recombinant Mouse Protein	Thermo Fisher/Gibco	PMG8043
Recombinant Human FGF-10	PeproTech	100-26
Gastrin I (human)	Tocris	3006
A83-01	Tocris	2939
B27 supplement 50X (serum-free)	Thermo Fisher/Gibco	17504044
Minimum Essential Medium (MEM)	Thermo Fisher/Gibco	11095080
Buffer RLT	Qiagen	79216
2-Mercaptoethanol	Sigma	63689
Trypan Blue Solution, 0.4%	Thermo Fisher/Gibco	15250061
Bovine Serum Albumin Solution 30% in DPBS, sterile	Sigma-Aldrich	A9576-50ML
Gentamicin solution 50 mg/mL	Sigma-Aldrich	G1397
Albumin Factor V	Carl Roth	T844.3
Citric acid (Monohydrate)	Carl Roth	3958.4
Dulbecco's Phosphate Buffered Saline	Sigma	D8537
Ethanol 70 %	Otto Fischer	7078027
Ethanol 96 %	Otto Fischer	7138032
Ethanol absolute	Merck	64-17-5
Fetal Bovine Serum	Sigma-Aldrich	F7524
Fluorescence Mounting Medium	Dako	S3023
Glycin	Carl Roth	3908.3
Hydrogen Peroxide 30%	Carl Roth	9681.1
Methanol	Carl Roth	4627.5
Sodium Chloride (NaCl)	Carl Roth	3957.2
Normal Goat Serum	Life technologies	50062Z
Penicillin-Streptomycin	Sigma-Aldrich	P0781
Schwann Cell Medium	ScienCell	1701
Fetal Bovine Serum	ScienCell	0025
Penicillin-Streptomycin	ScienCell	0503
Schwann Cell Growth Supplement	ScienCell	1752
Phosphatase Inhibitor	Sigma-Aldrich	4906837001
Protease Inhibitor	Sigma-Aldrich	4693159001
RIPA Buffer	Sigma-Aldrich	R0278
Roticlear	Carl Roth	A538.1
Trisbase	Sigma-Aldrich	T1503
Triton X 100	Carl Roth	3051.2
Tween 20	Carl Roth	9127.2
Trypsin-EDTA	Sigma-Aldrich	T3924
Vecta Mount permanent mounting medium	Vector	H-5000
RPMI medium 1640	Gibco	21875-034
Silencer™ Select Negative Control No. 1	Silencer™ Select	4390843
Silencer™ Select Negative Control No. 2	Silencer™ Select	4390846
CXCL8 siRNAs human 1	Silencer™ Select	4390824
CXCL8 siRNAs human 2	Silencer™ Select	4390824
Lipofectamine™ RNAiMAX Transfection Rea- gent	Lipofectamine™ RNAiMAX	13778150

Opti-MEM medium	Thermo Fischer Scientific	31985070
Ganciclovir	Sigma-Aldrich	G2536-100 MG

Buffers and solutions

Immunohistochemistry and Immunofluorescence

Table 4. 10X Tris Buffered Saline (TBS)

Tris base	24.2 g
Sodium Chloride	85 g
Distilled water	800 mL
Adjust pH with	5 M HCl
Constant volume with distilled water to	1000 mL

Table 5. 20X Citrate buffer

Citric acid Monohydrate	21 g
Distilled Water	300 mL
Adjust pH to 7.4 with	5 M NaOH
Constant volume with distilled water to	1000 mL

Table 6. Washing buffer (1X PBST)

PBS	9.55 g
Tween 20	1 mL
Constant volume with distilled water to	1000 mL

Table 7. Tris-EDTA buffer

Tris base	1.21 g
EDTA	0.37g
Distilled water	1000 mL
Tween 20	0.5 mL

Table 8. Kits

Proteome Profiler Human Phospho-Kinase Array Kit	R&D systems (ARY003C)
RNeasy mini kit	Qiagen
Human Cytokine Array C5	RayBiotech (AAH-CYT-5-4)
BCA Assay kit	Sigma-Aldrich

Table 9. Softwares

Caseviewer	3DHISTECH 2.4
ImageJ	1.52v version
QuPath	0.3.2 version
Carl Zeiss	ZEN (blue Edition)
GraphPad Prism	Prism 10

Table 10. Consumables and laboratory equipment

Class II Biological Safety Cabinet	Thermo Scientific
CO2 Incubator	Eppendorf
Refrigerated Centrifuge	Eppendorf
37°C Water Bath	MEDAX
Pipette Boy	INTEGRA
Micro pipette set	Thermo Scientific
Hemocytometer	Sigma
Microscopes	Olympus IX50 inverse microscope and Zeiss Axioplan 2
200 µL filtered sterile pipette tip	Starlab
1000 µL filtered sterile pipette tip	Starlab
5 mL Serological pipette	Greiner Bio-one
10 mL Serological pipette	Greiner Bio-one
25 mL Serological pipette	Greiner Bio-one
15 mL Conical tube	Greiner Bio-one
50 mL Conical tube	Greiner Bio-one
6-well Plates	Corning
24-well Plates	Corning
35 mm petri dish	Corning
100 mm dish	Corning
Cell Scraper	Sarstedt
1.5 mL tubes	eppendorff
10 µL unfiltered sterile pipette tip	Starlab
10 µL filtered sterile pipette tip	Starlab
100 µL filtered sterile pipette tip	Starlab
Microscope	Olympus IX50 inverse microscope
Ph-meter	Beckman
Tissue embedding machine	Leica
Tissue processor	Leica
Cover slips	Plano
Magnetic mixer	IKA-COMBIMAG RET
Stereomicroscope	Zeiss Stemi 2000
Microtome	Leica JUNG RM2055
Scanner	Zeiss AxioScan.Z1
Gel Imager	Analytic Jena
Flow cytometer	Beckman Coulter

Methods

Mouse husbandry

For all experiments in this research, mice were housed under special pathogen-free conditions and guaranteed free access to food and water. Furthermore, all practices involving mice were reviewed and controlled by the Zentrum für Präklinische Forschung of the Technische Universität München, which follows the German federal guidelines for the ethical treatment of animals (Regierung von Oberbayern). The study was approved by the Ethics Committee of the Technical University of Munich, Germany (approval nr. 2017-352_1-S).

The animal experiments were approved by the Government of Upper Bavaria (nr. 55.2-2532-Vet-02-16-165 and 55.2-1-54-2532-223-2015).

GFAP-Tk mice

For ablating activated GFAP⁺ astroglia, Busch et al. (1998) developed transgenic mice expressing the herpes simplex virus thymidine kinase protein via the mouse glial fibrillary acidic protein (GFAP) promoter. The mice were then given ganciclovir, a phosphorylated antiviral agent activated by the herpes simplex virus thymidine kinase protein. In these mice, ganciclovir was specifically activated and only affected cells expressing the herpes simplex virus thymidine kinase protein (Bush et al., 1998).

Gfap-HSV-Tk fusion gene structure

The Gfap-lacZ plasmid fused the lacZ sequence with the HSV-Tk gene sequence as illustrated in Figure 4. To achieve this, a 1.7 kb XbaI-Bam HI fragment was used to introduce the HSV-Tk gene and a polyadenylation signal into the pBSII-KS vector from Stratagene. Following this, the first exon of the modified murine GFAP promoter (clone 445) was removed using Sal I and Not I and this custom fragment was inserted. The integrity of the HSV-Tk insert and its flanking regions was assured by single-stranded sequencing. The resulting Gfap-HSV-Tk fusion gene construct was purified by SfiI digestion and gel electrophoresis purification (Bush et al., 1998).

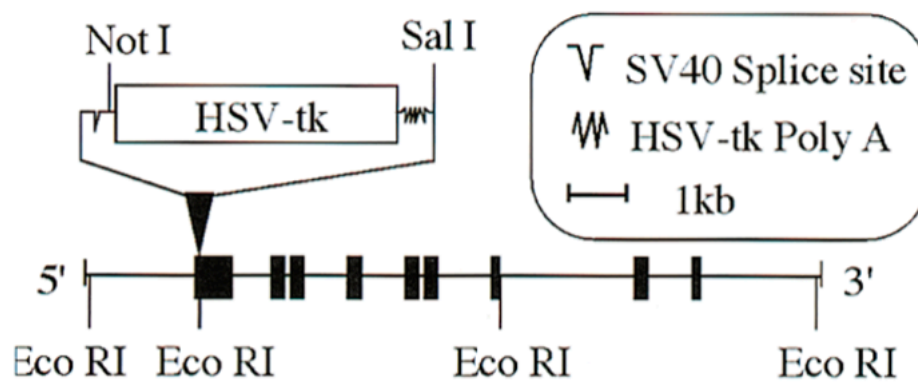


Figure 4. In Vivo Expression of the *Gfap-HSV-Tk* Transgene. *HSV-Tk* fusion gene construct inserted into exon 1 of the mouse *GFAP* gene (Bush et al., 1998).

The GFAP-Tk, B6. *Cg-Tg (Gfap-TK)7.1Mvs/J*, strain of the mice, used in this project, were obtained from The Jackson Laboratory, JAX stock #005698 and produced for further use and used in crossing with other mouse strains (KC: Ptf1a/p48-Cre ; LSL-Kras^{G12D} and KPC: Ptf1a-Cre; LSL-Kras^{G12D}; Trp53^{fl/fl}).

KC;GFAP-Tk mice

KC mice were bred by crossing the *Ptfla/p48-Cre* line with the *LSL-Kras^{G12D}* mouse strain, expressing Cre recombinase in exon 1 of the *Ptfla/p48* gene under the control of this pancreas-specific promoter. This combination of mouse lines results in mice expressing *Kras^{G12D}* in their pancreas, providing an ideal animal model for studying the effects of mutant *Kras^{G12D}* on pancreatic cancer development. The gene expressing Cre recombinase is located in exon1 of pancreatic transcription factor1 (*Ptfla*). *Ptfl* is a heterotrimeric protein complex that serves as a transcriptional regulator and is implicated in the appropriate development of the ventral pancreatic bud during the early stages of pancreatic organogenesis. The *Ptfla/p48-Cre* and *LSL-Kras^{G12D}* mouse lines alone do not have a phenotype different from *C57BL/6J* mice. However, crossing these mouse lines results in the KC (*Ptfla/p48-Cre; LSL-Kras^{G12D}*) mouse line (Hingorani, Petricoin III, et al., 2003). In this project, we crossed GFAP-Tk and KC mice to obtain mice with the *Ptfla/p48-Cre; LSL-Kras^{G12D}; Gfap-Tk* genotype (termed herein *KC;GFAP-Tk*). Ganciclovir was administered by intraperitoneal injection (i.p.) for five days at 25 mg/mL/kg when the mice reached the age of 19 weeks. The same dose of ganciclovir was administered to the KC and GFAP-Tk groups, and the results were compared. On the 14th day of ganciclovir administration, the von Frey mechanosensitivity test was applied on the abdomen, followed by euthanasia and organ harvesting (pancreas, spinal cord, liver and brain). The harvested tissues were fixed with 4% paraformaldehyde for 12-18 hours. The collected organs were embedded in paraffin for further histological analyses.

KPC;GFAP-Tk mice

The KPC model was first described in 2003 by Sunil Hingorani as a genetically engineered mouse model that recapitulates the development of pancreatic ductal adenocarcinoma (Hingorani, Petricoin, et al., 2003). Here, *LSL-Kras^{G12D}* knock-in, *Ptfla-Cre* and *Trp53^{fl/fl}* strains were cross-bred to generate *Ptfla-Cre; LSL-Kras^{G12D}; Trp53^{fl/fl}* mice. In *Trp53^{fl/fl}* mice, the exons 2–10 of the *Trp53* gene are flanked by loxP sites, enabling Cre-mediated recombination to remove this functional region. These *Ptfla-Cre; LSL-Kras^{G12D}; Trp53^{fl/fl}* mice rapidly create invasive PDAC as a result of this modification and have a median survival time of about 61 days as opposed to 25 weeks in *Ptfla-Cre; LSL-Kras^{G12D}; LSL-Trp53^{R172H/+}* mice. This drastic reduction in median survival time serves to illustrate the fact that the bi-allelic inactivation of *Trp53* significantly increases the aggressiveness of *Kras^{G12D}*-driven pancreatic cancers (Hingorani, Petricoin, et al., 2003).

In this project, we intercrossed four strains, i.e., *Ptfla-Cre; LSL-Kras^{G12D}; Trp53^{fl/fl}* and *Gfap-Tk* mice to obtain the *Ptfla-Cre; LSL-Kras^{G12D}; Trp53^{+/fl}; Gfap-Tk* genotype (herein termed KPC;GFAP-Tk). The 14-15 weeks-old, KPC;GFAP-Tk mice were first tested for abdominal mechanosensitivity (von Frey), and the von Frey scores were first evaluated before ganciclovir treatment. Then, KPC;GFAP-Tk mice were divided into two different groups. Mice in the first group were treated with PBS as a control group. KPC;GFAP-Tk mice in the second group were treated with 10 mg/m/kg ganciclovir i.p. for five days, and the pain responses of all two groups were evaluated by the von Frey test before and after therapy. After euthanasia, pancreas, spinal cord, brain, and spleen of the mice were placed in 4% paraformaldehyde for 12-18 hours. The collected organs were embedded in paraffin for further histological analyses.

Orthotopic implantation model

To exclude the possibility that the observed effects might be due to an ectopic expression of GFAP in the murine cancer cells, we also applied orthotopic implantation models using a *KPC-LacZ* cancer cell line in *Gfap-Tk* mice. As such, this orthotopic model can be considered a control model to investigate the effect of glial cell depletion.

5-6 weeks old littermates were housed under specific pathogen-free conditions and given free access to food and water. 2×10^5 *KPC-LacZ* tumor cells resuspended in Matrigel solution were orthotopically injected into the pancreas of *Gfap-Tk* mice. The suspension solution containing Matrigel was diluted 1/4 with DMEM without supplementation. Implanted mice were divided into four groups: the first and the second group was treated either with PBS or ganciclovir (10 mg/mL/kg i.p.) for five days starting from the 30th day of orthotopic implantation. The third group was treated with ganciclovir and FOLFIRINOX (Calcium Folate 5 mg/kg, Oxaliplatin 0.25 mg/kg, Irinotecan 2.5 mg/kg, 5-Fluorouracil 2.5 mg/kg). The fourth group treated only with FOLFIRINOX (Calcium Folate 5 mg/kg, Oxaliplatin 0.25 mg/kg, Irinotecan 2.5 mg/kg, 5-Fluorouracil 2.5 mg/kg). The experiment employed varying treatment schedules for mice that had orthotopic tumor implantation. The first and second cohorts were treated either with PBS or ganciclovir on day 30, while the third cohort received FOLFIRINOX from day 43 to day 50. The fourth cohort solely received FOLFIRINOX from day 43 to day 50. All groups were sacrificed at 60 days after implantation. Pancreas tissues were collected, fixed with 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Other organs, such as the spinal cord, liver, and spleen, were collected and processed similarly.

Table 11. Genotype, age, number and survival characteristics of the analyzed mice in this project

Annota- tion	Genotype	Treatment	Age/ weeks	Number (n=)
KC	<i>K-ras^{LSL.G12D/+};Ptf1a-Cre</i>	Ganciclovir	19-20	12
KC;GFAP-Tk	<i>K-ras^{LSL.G12D/+};Ptf1a-Cre; GFAP-Tk</i>	Ganciclovir	18-20	11
GFAP-Tk	<i>GFAP-Tk</i>	Ganciclovir	19-20	7
KPC	<i>K-ras^{LSL.G12D/+}; Trp53^{fl/+}; Ptf1a-Cre</i>	-	-	-
KPC;GFAP-Tk	<i>K-ras^{LSL.G12D/+}; Trp53^{fl/+}; Ptf1a-Cre; GFAP-Tk</i>	Ganciclovir/PBS	14-15	13
Control	<i>Trp53^{fl/fl}; Ptf1a-Cre; GFAP-Tk</i>	Ganciclovir/PBS	14-15	8
Orthotopic Implanta- tion	<i>GFAP-Tk</i>	Ganciclovir/ PBS / FOLFIRINOX/ GANCICLOVIR & FOLFIRINOX	5-6	n=9 (GCV) n= 6 (PBS) n= 7 (FOLFIRI- NOX) n= 11 (GCV & FOLFIRINOX)

Mouse genotyping

Genotyping of mice was performed by DNA extraction from ear clips of mice. Ear clips were obtained from mice at 4 weeks of age. The Wizard Genomic DNA Purification Kit was used for the extraction of genomic DNA. The ear sample was mixed with 3µl of RNase Solution after being lysed for 10 seconds in Nuclei Lysis Solution. The sample was then cooled to room temperature after being incubated at 37 °C for 15–30 minutes. The mixture was then aggregated with 200µl of Protein Precipitation Solution, vortexed, and cooled for 5 minutes on ice. Centrifugation at 13,000–16,000 g* for 4 minutes was then performed. The supernatant was transferred to a new tube containing 600 µl of room-temperature isopropanol for the precipitation step and centrifuged at 13,000-16,000 g* for 1 min. Then, 600 µl of 70% ethanol was added at room temperature after removing the supernatant. Then, a standard PCR protocol was performed. All primers were used at a final concentration of 10 pM.

Table 12. Genotyping PCR protocol for *p48-cre*

Step	Temperature	Time	Cycle number
Pre-incubation	94°C	1 min	1x
Amplification	94°C	2 min	40x
	58°C	30 sec	
	72°C	11 min	
Cooling	4°C	∞	1x

Table 13. Genotyping PCR protocol for *p53*, *GFAP-Tk*, *Kras*

Step	Temperature	Time	Cycle number
Pre-incubation	94°C	2 min	1x
Amplification	94°C	20 secs	10x
	65°C	15 secs	
	68°C	10 secs	
	94°C	15 secs	28X
	60°C	15 secs	
	72 °C	10 secs	
	72 °C	2 mins	
Cooling	4°C	∞	1x

Table 14. Genotyping primer sequences and product sizes

Name	Primer forward (5'-3')	Primer reverse (5'-3')	Product (bp)
<i>P53</i>	GGT TAA ACC CAG CTT GAC CA	GGA GGC AGA GAC AGT TGGAG	390
<i>Kras</i>	GCA GGT CGA GGG ACC TAA T	CTG CAT AGT ACG CTA TAC CCT GT	100
<i>p48</i>	GTC CAA TTT ACT GAC CGTACA CCA A	CCT CA AGG CGT CGT TGA TGG ACT GCA	1155
<i>GFAP-Tk</i>	Transgene: GGG GCC TCG GTC CTA GT	GTA GGC AGG GGT ACG AAG	Transgene: 290
	Internal control: GTC AAC GGG GGA CAT AAA AG	CTT CTA AGA GTT AAA CAA TAC CAG CTT	Internal control: 190

FOLFIRINOX

FOLFIRINOX is a chemotherapy regimen frequently used to treat pancreatic cancer. It is a combination of four drugs: 5-fluorouracil (5-FU), calcium folinate, irinotecan, and oxaliplatin (Tempero et al., 2017). Each drug has a unique role in the treatment of pancreatic cancer: 5-fluorouracil (5-FU) is a chemotherapy drug that attacks rapidly dividing cancer cells. It interferes with DNA and RNA synthesis in cancer cells, inducing their death. Calcium folinate is a type of folic acid administered together with 5-FU to reinforce its effectiveness. Calcium folinate helps 5-FU to infiltrate cancer cells and prevent them from growth and division. Irinotecan is a chemotherapy drug that targets an enzyme called topoisomerase I, which is required for DNA replication in cancer cells. By acting on this enzyme, irinotecan prevents cancer cells from dividing and growing. Oxaliplatin is a chemotherapy agent that damages the DNA in cancer cells and ultimately causes their death (Begg et al., 2020; Caparello et al., 2016).

Ganciclovir

Ganciclovir mimics guanine's structure, a vital DNA building block. It is closely related to acyclovir but has proven more effective against cytomegalovirus (Al-Badr & Ajarim, 2018). GFAP-Tk mice are genetically engineered mice expressing a viral *thymidine kinase* (Tk) gene that is under the control of the glial fibrillary acidic protein (GFAP) promoter. This gene drives the selective death of GFAP-expressing glial cells upon the administration of ganciclovir (Bush et al., 1998). Once ganciclovir is administered to GFAP-Tk mice, it is metabolized into a toxic metabolite by the viral Tk enzyme, which is exclusively produced in GFAP-expressing cells and in glial cells. This metabolite impairs DNA synthesis and leads to programmed cell death (Bush et al., 1998). In the GFAP-Tk mouse model, ganciclovir preferentially kills the activated GFAP-expressing cells, particularly astrocytes and some subtypes of oligodendrocytes, whereas preserving other cell types like neurons. This enables examining the particular functionalities of glial cell types *in vivo* (Bush et al., 1998). In this project, the KC group was administered ganciclovir at a dosage of 25 mg/kg for a period of five days. As ganciclovir might burden the animals through depletion of glial cells, the dosage of ganciclovir was reduced to 10 mg/kg for five days in the KPC and orthotopic implantation groups. Furthermore, it was considered that the mice in the orthotopic implantation group may face difficulties in tolerating a higher dosage of ganciclovir after surgery. In survival cohort 25 mg/kg of ganciclovir applied for five days.

von Frey Test

Since it is not possible to determine the pain response to a direct stimulus of the pancreas, we tested the so-called "referred hyperalgesia" in this experiment (Bonin, Bories, & De Koninck, 2014). The von Frey test is considered a standard test for the assessment of pain behavior in rodents (Deuis, Dvorakova, & Vetter, 2017). The von Frey test involves the application of calibrated nylon filaments of various thicknesses to the animal's hind paw or different parts of the body, e.g. to the abdomen from below, as performed in this study (Jeffrey, Sonya, & You, 2001). The filaments apply increasing amounts of pressure to the tissue surface until a withdrawal response is observed. The pressure required to produce a withdrawal response is measured and recorded as the animal's mechanical threshold (Deuis et al., 2017). To compare the effect of tumor growth on the pain sensation of GEMM, tactile stimuli with von Frey filaments of increasing size were applied ten times below a grid in the abdomen, repeatedly. For each filament, a positive response was recorded if the animal flinched or exhibited signs of withdrawal. The following reactions are regarded as pain reactions of the mouse: Pulling back, scratching and licking the abdomen, and getting up the mouse (Minett, Eijkelkamp, & Wood, 2014). In this project, von Frey filaments of different stiffness were applied to KC group mice right before euthanasia, and their withdrawal responses to pain were recorded for further evaluation. On the other hand, in the KPC and orthotopic implantation groups, the von Frey test was administered to the mice twice, right before ganciclovir administration and again right before euthanasia.

Histology

Paraffin sections mouse tissue samples were fixed in 4% PFA /PBS for 12-18 hours, dehydrated, and embedded in paraffin. A microtome was used to cut 0.25- μ m-thick sections. Sections were mounted on adhesive-coated slides and dried at 37 °C for 18 hours. Sections were kept at 25°C until further analysis.

Immunohistochemistry, Immunofluorescence

Consecutively cut sections were taken from the paraffin-embedded tissues. After deparaffinization (3 x10 minutes) and dehydration steps (ethanol 100%, 96%, 70%; 3 minutes each and water 2 x 3 minutes) in Roticlear®, the sections were subjected to heat-induced (citrate or Tris-EDTA buffer) or enzymatic antigen retrieval protocols according to the recommendation of the antibody manufacturer. Subsequently, the tissues were incubated with normal goat serum for 1 hour at room temperature to eliminate nonspecific binding.

Afterward, the sections were incubated with the primary antibody at overnight 4°C. After incubation, the sections were washed with a washing buffer (3x 5 mins), kept at room temperature for 1 hour with the corresponding secondary antibody, and then washed again (3 x 10 mins). After this step, the tissue sections were subjected to color reaction with Dako Liquid DAB Substrate Chromogen System and followed by dehydration steps. For immunofluorescence staining, the slides were covered with fluorescence mounting media.

Cell culture

The human pancreatic cancer cell line SU86.86 was purchased from American Type Culture Collection (ATCC) and pancreatic cancer cell line T3M4 was a kind gift from Dr. Metzgar (Durham, North Carolina). The human Schwann cells were purchased from ScienCell. All cell lines were cultured according to the supplier's recommendations. Schwann cells thawed and seeded in Schwann cell Media supplemented with 10%, FBS (Fetal Bovine Serum), 1% Penicillin-Streptomycin (P/S) and Schwann cell growth supplements. On the other hand, the pancreatic cancer cell lines SU86.86 and T3M4 were seeded in RPMI medium supplemented with FBS 10% and P/S 1%. Cells were incubated at 37°C in 5% CO₂ until 80-90% reached confluency.

Organoid generation

Pancreatic ductal fragments were isolated according to an optimized version of the Tuveson Laboratory Murine and Human Organoid Protocols (Boj et al., 2015). After terminal anesthesia, the pancreas of 23 weeks old (KPC) and 12 days old (WT) mice was surgically removed and placed in mouse wash medium. The pancreas is then minced into pieces ~1-2 mm. After this dissection, the pancreas pieces were transferred to digestion medium (10 mL) and incubated for 1 hour at 37°C in a continuous shaker. Intra/interlobular ducts were collected by microdissection using a stereomicroscope.

Table 15. Mouse wash medium

DMEM + glucose, L-glutamine, sodium pyruvate	500 mL
100X Penicillin/Streptomycin	5 mL
100% FBS	5 mL

Table 16. Digestion medium

Collagenase Crude Type XI	25 mg
Dispase II	25 mg
Mause Wash Medium	200 mL
DNase I	10 mg/mL

After collection, the ducts were centrifuged (850 rpm for 5 minutes at 4°C), the pellet was resuspended in 125 µL of Matrigel to make 5 x 25 µL wells in a 48-well plate. Then the 48-well plate was kept at 37°C for 15 minutes to solidify the Matrigel. Finally, 250 µL of room temperature Rho Kinase Inhibitor-included Mouse Complete Feeding Media was added to each well and placed in an incubator at 37°C with 5% CO₂. Organoids were maintained with fresh Mouse Complete Feeding Medium without Rho Kinase Inhibitor every two days and passaged 24 well plate when 80% confluent.

Table 17. Mouse Basic medium

Advanced DMEM/F-12	485 mL
Glutamax (100X)	5 mL
Penicillin/Streptomycin (10.000 U/mL)	5 mL
HEPES (1M)	5 mL

Table 18. Mouse Complete Feeding Medium

Reagent	Final concentration	Amount
Mouse organoid basic medium	n/a	18 mL
1000X mEGF (50 mg/mL)	1X (0.05 mg/mL)	20µL
1000X hFGF-10 (100 mg/mL)	1X (0.1 mg/mL)	20µL
1000X A83-01 (0.21 mg/mL)	1X (0.21 mg/mL)	20µL
1000X mNoggin (100 mg/mL)	1X (0.1 mg/mL)	20µL
1000X hGastrin-I (0.021 mg/mL)	0.021 µg/mL	20µL
400X N-acetyl-cysteine (81.5 mg/mL)	0.2 mg/mL	50µL
100X Nicotinamide (122 mg/mL)	1.22 mg/mL	200 µL
50X B27 Supplement	1X	400 µL
10X RSpondin-1-conditioned medium	1X	2 mL
Total	n/a	20 mL

Transwell Assay

When the cells and organoids were 80% confluent, the old media were discarded, and the organoids and cells were washed with 1XPBS. To produce conditioned media, cells were cultured with serum-free media for 48 hours, and then the media was filtered with a 0.2-micrometer filter. To produce conditioned media for organoids, the co-culture media, described below, was used and incubated for 48 hours and filtered. Part of the media were separated for protein quantification via the bicinchoninic acid (BCA) assay, and the remaining media were stored at -20°C for further use.

Table 19. Co-Culture media for organoids

Mouse organoid basic medium	19.53 mL
50X B27 Supplement	400 μ L
400X N-acetylcysteine (81.5 mg/mL)	50 μ L
Y27632 (10 mM) ^a	20 μ L
Total	20 mL

In order to quantify the amount of protein in the conditioned media, the BCA assay was performed according to the protocol specified by the manufacturer. As a result of the BCA assay, the amount of protein in the conditioned media of Schwann cells, cancer cells, and organoids varied between 78- 80 μ g/mL. For this reason, it was decided to mix the media 1:1 (w/w) (1mL each) in the transwell assay protocol.

First, 80-90% confluent organoids were passaged and embedded in 6 well plates, and placed in a humidified incubator at 37°C and 5% CO₂ to allow the Matrigel to solidify. Meanwhile, 80-90% confluent Schwann cells and cancer cells were trypsinized and counted by a hemacytometer to obtain 2 million cells per well.

In the transwell assay protocol, cancer cells or organoids (2×10^6 per well) were first placed in 6 well plates. Then, Schwann cells were placed in the upper insert, and Schwann cell-cancer cell conditioned media mixture (w/w) or Schwann cell-organoid conditioned media mixture (w/w) were added to the wells. In addition, Schwann cells, SU86.86, T3M4 cancer cells and KPC, WT organoids were seeded as monoculture and used as a control group. All plates were kept in a 37°C, 5% CO₂ humidified incubator for 15 minutes or 6 hours. Protein isolation was performed 15 minutes after from the first set, and total RNA isolation was conducted 6 hours after the second set. Following 6 hours of incubation, total RNA was isolated from all samples by using the Qiagen RNeasy mini kit according to the manufacturer's protocol.

RNA extraction

RNA extraction was performed following the Transwell assay. Extraction was done from Schwann cells treated with pancreatic cancer cells and organoids and their mono-cultures. After incubation of Schwann cells with cancer cells and organoids for 6 hours, as described above, samples were immediately removed and homogenized in RLT lysis buffer supplemented with 1% β -mercaptoethanol and frozen. RNA was extracted from all specimens using the RNeasy Mini Kit. A NanoDrop™ 2000 spectrophotometer was used to determine RNA concentration and purity via absorbance ratios at 260 nm/280 nm and 260 nm/230 nm.

RNA Sequencing

RNA isolation, purity, and quantification were performed as described above. The samples were then sent to Novogene UK. Subsequent steps, sample preparation and library construction, quality control, sequencing, and data analysis, were performed by Novogene UK. According to the Novogene protocol, the preparation of the samples and construction of the library involved isolating the messenger RNA (mRNA) using magnetic beads linked with poly-T oligos. Depending on the library type, random hexamer primers or dUTP/dTTP was used for the first and second-strand cDNA synthesis. The library construction included end repair, A-tail, adaptor ligation, size selection, amplification, and purification according to established protocols. After construction, the quality of the libraries was assessed by quantifying them using a Qubit fluorometer and real-time PCR, followed by size distribution determination using a Bioanalyzer.

The libraries were then sequenced on Illumina platforms, and the resulting raw data underwent quality control steps to eliminate adapters, low-quality reads, and poly-N sequences. The clean reads were aligned to the reference genome, and gene expression levels were calculated. Differential expression analysis was conducted using various R packages, and enrichment analysis was

performed using the cluster Profiler R package and various databases. Alternative splicing and PPI analyses were also performed using specific software and databases. Finally, fusion gene analysis was conducted using STAR-Fusion software (v1.9.1).

Proteome Profiler Human Phospho-Kinase Array

The feasibility of analyzing the phosphorylation ratios of kinases and protein substrates is essential for a deep understanding of how cells perceive and respond to alterations in their milieu. Therefore, the present study aimed to compare Schwann cells co-cultured with cancer cells (SU86.86 and T3M4) or organoids (KPC, WT) to mono-cultured Schwann cells for identifying the differentially phosphorylated proteins and the activated kinases. In this project, the Proteome Profiler™ Array Human Phospho-Kinase Array Kit was used. The operating principle of this assay is to capture phosphoproteins in duplicate on two nitrocellulose membranes. Cell lysates are reconstituted and incubated overnight with the Human Phospho-Kinase Array to ensure antibody binding, followed by a series of washes to exclude any unbound proteins, and then incubated with a cocktail of biotinylated detection antibodies. Streptavidin-HRP and chemiluminescent detection reagents are used, and a signal is generated and related to the amount of phosphorylated protein bound at each capture point via densitometry.

The protein concentration of the sample was determined using a total protein assay (BCA). Lysis Buffer 6 was enhanced with 10 µg/mL Aprotinin, 10 µg/mL Leupeptin, and 10 µg/mL Pepstatin to create cell lysates. The cells were treated in lysis buffer at a concentration of 1×10^7 cells/mL and shaken for 30 minutes at 2-8 °C using a pipette. After microcentrifugation at 14,000 x g for 5 minutes, the supernatant was collected in a fresh test tube. The manufacturer's instructions were followed during the assay.

siRNA transfection

On day 0, human Schwann cells (HSc) were seeded in a 24-well plate at a density of 150,000 cells per well and cultured for 24 hours. A mixture of CXCL8 siRNA 1 and 2 or a mixture of Silencer Select negative control siRNA 1 and 2 was then added to the cells at a concentration of 45 pmol per well. Lipofectamine (9 μ l per well) was used as a transfection reagent to facilitate siRNA delivery to the cells. Cells were incubated for 24 hours to allow siRNA transfection and treatment, and at the end of 24 hours, the treatment was terminated, and cells were collected in the same well.

The collected cells were then incubated at 37°C for further 48 hours to produce conditioned media (CM). Following incubation, CM was collected, and BCA assay was performed to determine protein concentrations. SU86.86 and T3M4 cancer cells were seeded at 1.5 million per well, and after reaching 80% confluency, the medium was discarded and washed with 1XPBS. Each cancer cell type, SU86.86 and T3M4, was divided into two groups as siRNA treated and untreated. 1mL of each HSc CM (CXCL8 siRNA and Silencer Select negative control HSc CM) with 100 mg/mL protein concentrations was added to the first group and incubated at 37 °C for 15 minutes. Proteins were then isolated as previously described and stored at -80 degrees centigrade. After isolating the proteins, they were transferred to the Human Cytokine Array according to the manufacturer's instructions. The membranes were then exposed to the relevant specimen, allowing the target molecules to interact with the surface. Biotinylated detection antibodies were added to the membranes, which selectively bind to the target molecules. HRP-conjugated streptavidin was then added to amplify the detection signal, binding to the biotinylated antibodies. Detection buffers were used to immobilize and visualize the attached target molecules before imaging with a chemiluminescent imaging system.

Transwell migration assay & isolation of myeloid derived suppressor cells (MDSC)

In this investigation, a transwell migration method was applied to determine the migratory trend of myeloid-derived suppressor cells (MDSCs) toward Schwann cells. As illustrated on Figure 6, On Day 0, 2,250,000 human Schwann cells were seeded in 6 well plates at 150,000 cells per well, with 750,000 cells in each group. Three experimental groups were established: Group 1 (IL-8 siRNA treatment), Group 2 (negative control siRNA treatment), and Group 3 (no treatment). On day 2, after 22 hours of treatment, the media were removed from the cells, and cells were transferred into 24-well plates at 200,000 cells per well. Nine wells (3 replicates per group) were seeded using Schwann cell medium without supplementation.

Spleen dissociation and MDSC sorting from human spleen samples protocol requires several steps for the processing of spleen samples. First, three samples weighing 1 gram each were taken and minced in a petri dish. The minced tissue was then passed through a 70 μm cell filter in a Falcon tube, and the filter was washed twice with FACS buffer for proper filtration. Cell suspensions were then centrifuged, and the supernatant was discarded. Next, the cell pellets were suspended in ACK lysis buffer and briefly incubated. The suspension was then centrifuged again, and the pellet was resuspended in FACS buffer. Centrifugation was performed again, and two samples of 60×10^6 cells each were prepared and centrifuged again. The cells were then reconstituted in FACS buffer with the addition of FcR blocking reagent and incubated on ice. After this step, another round of centrifugation was performed. Color controls were prepared to contain an unstained sample and a sample stained with FACS PI only. The antibodies were added to the respective tubes containing the cells, and the antibodies were mixed to form a staining mixture added to each sample and incubated on ice. After incubation, final centrifugation was performed to collect the antibody mixture, and the samples were washed with FACS buffer, centrifuged, and the supernatant discarded. Samples were then transferred to the separator device. FACS PI was added to the probes except

for the unstained sample and filtered into FACS tubes to finalize the protocol. The unstained sample was filtered with FACS buffer instead of FACS PI. Sorting of MDSCs was done as shown in Figure 5.

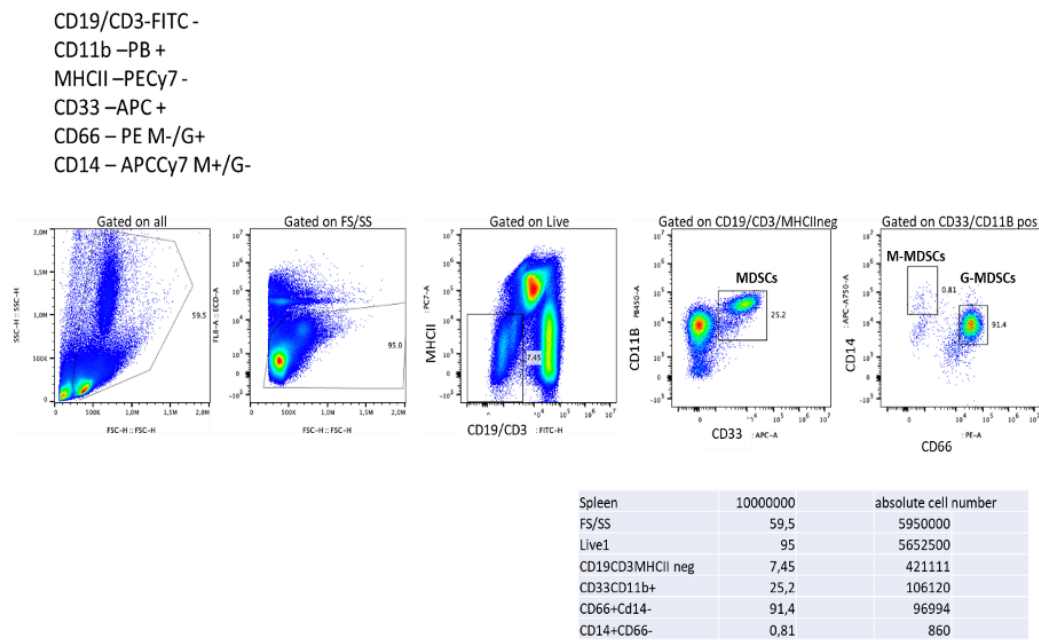


Figure 5. Sorting and gating schemes during MDSC isolation.

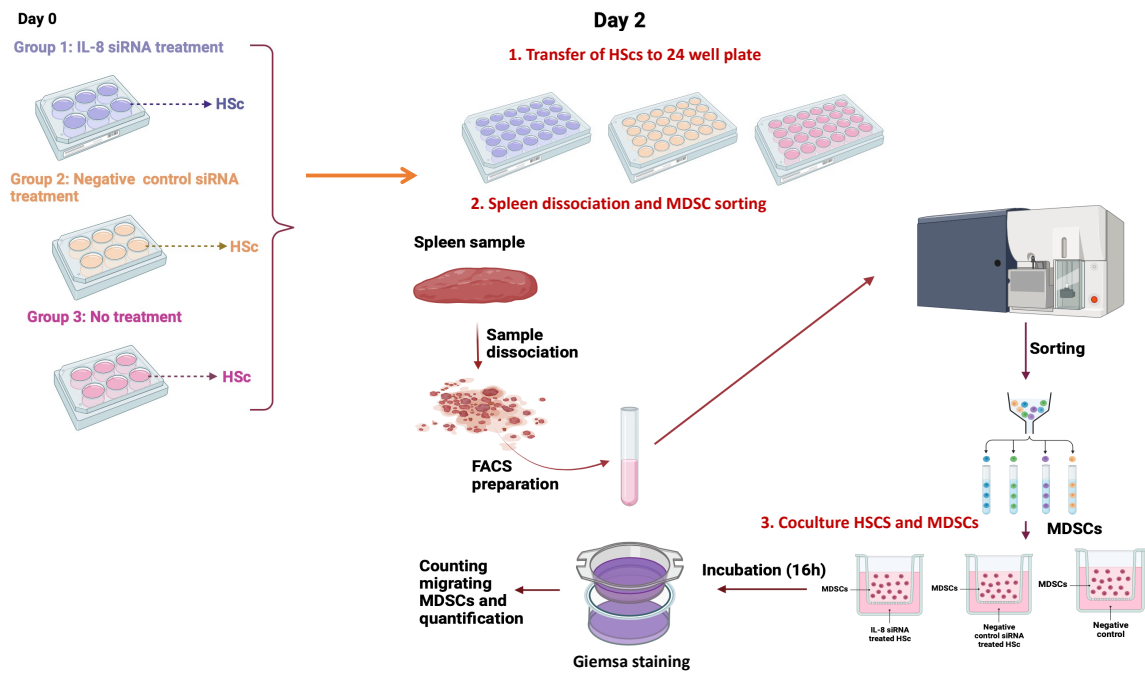


Figure 6. Schematic representation of experimental steps of transwell migration assay.

Meanwhile, in the second day, MDSCs isolated from the spleen were seeded in transwell inserts. The inserts had a pore width of 8 μm , and 100,000 MDSCs were seeded in each insert. A total of 900,000 MDSCs were used for the experiment. On day 3, the migration process was terminated after an average of 16 hours of incubation. Transwell inserts were stained with Giemsa solution (diluted 1:20) to visualize migrating cells. Six photographs were taken from each Transwell, migrating cells were counted using the analysis software.

Statistics

Data are presented as mean $\pm$ standard deviation (SD). Various t-tests were used to evaluate differences between the two groups. All statistical analyses were conducted using Prism 8 software (GraphPad Software, Inc.). The statistical significance levels, a key factor in interpreting the results, were set at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. The t-tests used included the Two-Sided Student's t-test, which assessed the mean differences between two independent groups, assuming normal distribution and equal variances between samples. The Unpaired t-test, which compared the means of data obtained from two different groups, also assuming equal variances for both groups. The Paired t-test was used to compare measurements from the same group at different time points, such as before and after treatment.

Results

The influence of GFAP+ glial cell depletion on pain response (von Frey Test)

The von Frey test was used in the present study to measure the pain response in the GEMM of PDAC after glial cell depletion. Von Frey filaments of different stiffness were applied to KC (Figure 7) group mice right before euthanasia, and their withdrawal responses to pain were recorded for further evaluation. On the other hand, in the KPC (Figure 8) and orthotopic implantation (Figure 9) groups, the von Frey test was performed to the mice twice, right before ganciclovir administration and again right before euthanasia. The pain responses were recorded for later evaluation.

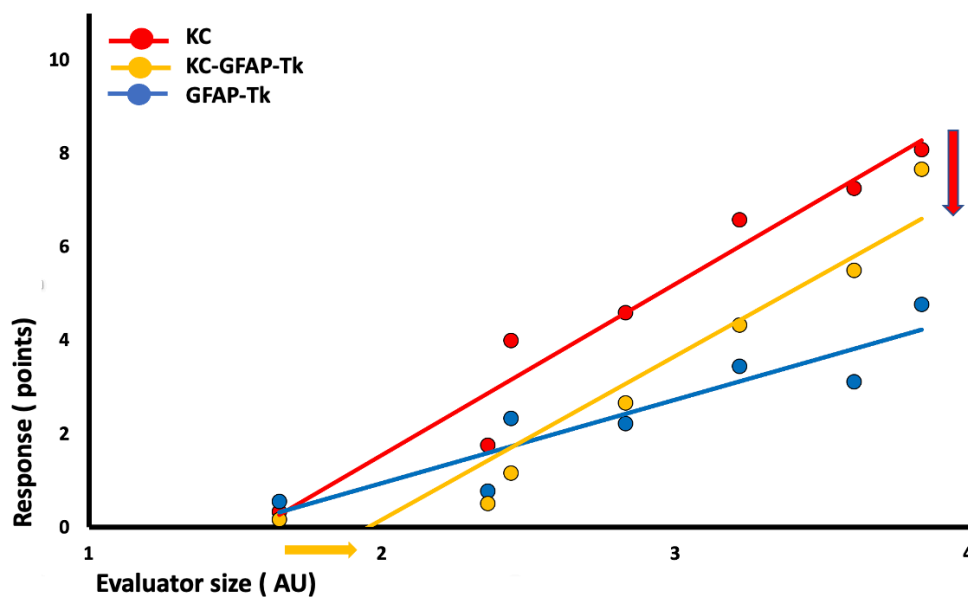


Figure 7. Pain reduction in KC mice after glial cell depletion. Three groups can be seen in the graph, each with its own pain threshold: KC mice (represented by the red line), KC-GFAP-Tk mice (represented by the yellow line), and GFAP-Tk mice (represented by the blue line). The red arrow indicates the difference in pain response between the KC and KC-GFAP-Tk groups. ($p=0.0061$). The yellow arrow represents mice with increased stiffness in response to alterations in evaluator size.

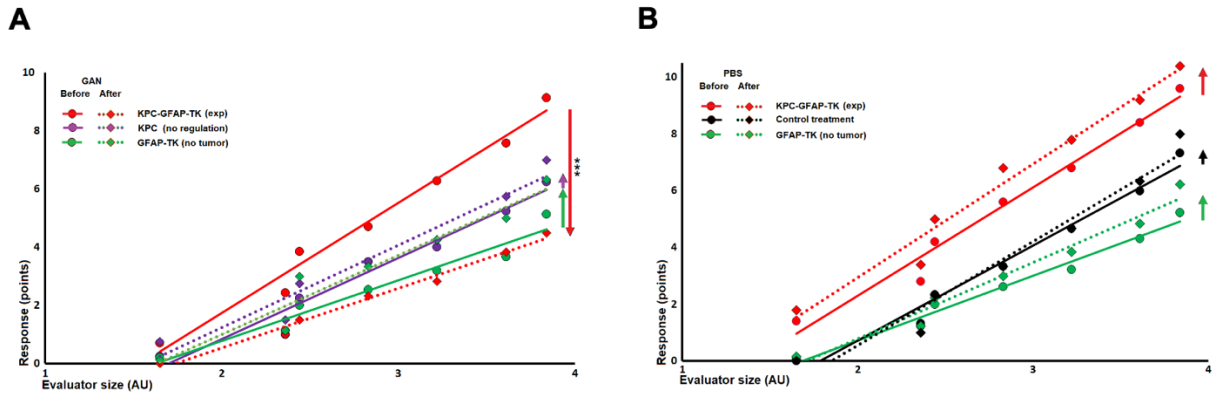


Figure 8. Pain reduction in KPC mice before and after glial cell depletion. (A) Assessments of pain response of KPC-GFAP-Tk, KPC, and GFAP-Tk mice before and after ganciclovir (GAN) administration. **(B)** Pain reaction of KPC-GFAP-Tk, KPC, and GFAP-Tk mice before and after PBS treatment. Exp.: the experimental group. PBS: phosphate buffered saline (p-value of KPC-GFAP-Tk group, before and after ganciclovir treatment, 0.008).

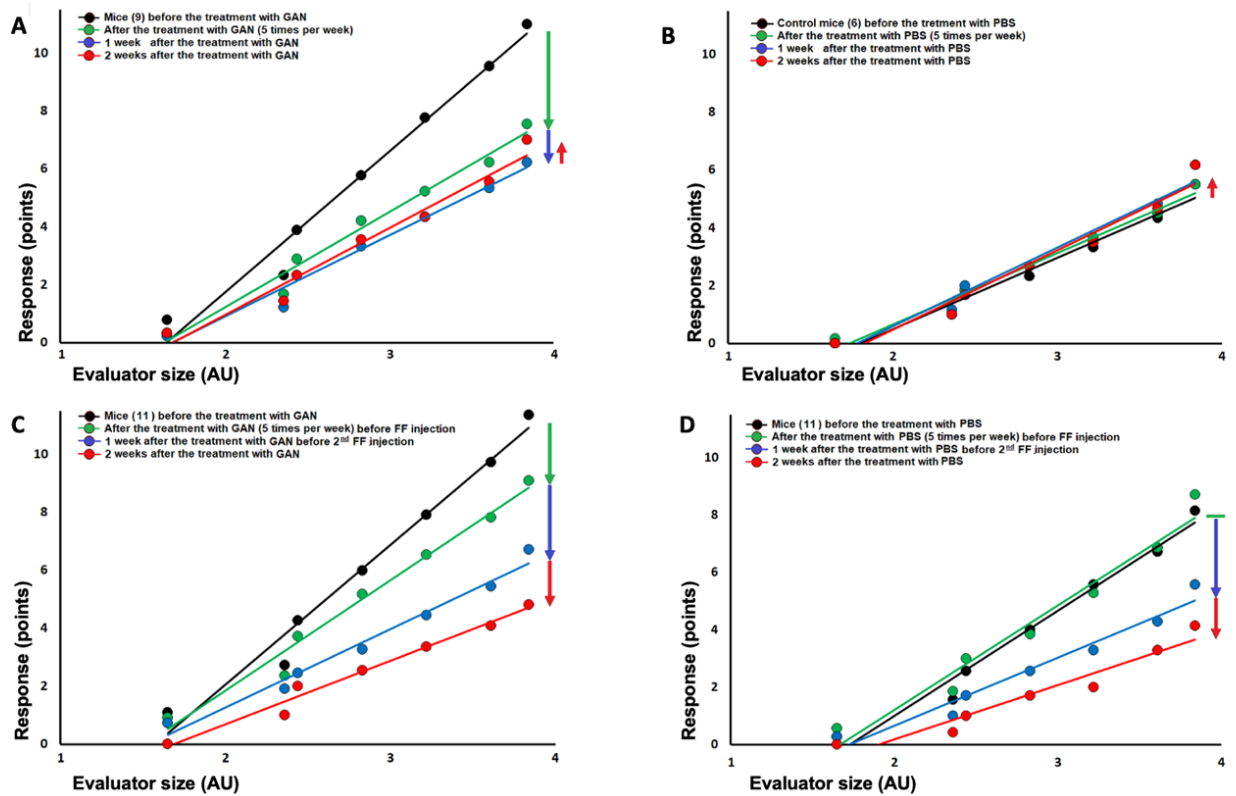


Figure 9. Pain perception before and after glial cell depletion in the orthotopic implantation model. (A) Quantifying pain perception in the orthotopic implantation model, focusing on the pain response of mice undergoing the orthotopic implantation procedure before and after administration of ganciclovir (GAN). **(B)** Pain evaluation in control mice, i.e., before and after treatment with PBS. **(C)** Determination of pain perception in orthotopically transplanted mice, analyzing pain perception before and after treatment with ganciclovir, FOLFIRINOX (FF), or the combination of ganciclovir and FOLFIRINOX (FF) **(D)** Measurements of pain response in orthotopically transplanted mice after FOLFIRINOX treatment (p=0.005, before and after ganciclovir administration).

In this part of the project, we observed that sensitivity to von Frey fibers was reduced in treatment groups with depleted glial cells (KC;GFAP-Tk, KPC;GFAP-Tk, GFAP-Tk and implantation) compared to control groups. In the KC cohort, glial cell depletion was observed in each group (KC, KC;GFAP-Tk, and GFAP-Tk). An attenuated pain response was observed in KC ($4,655 \pm 1,088$) and KC;GFAP-Tk ($2,968 \pm 0,7417$). However, there was no difference in pain response between KC;GFAP-Tk (2.968 ± 0.7417) and GFAP-Tk (3.143 ± 1.062) groups. KPC;GFAP-Tk (exp) mice exhibited a decrease in pain response before (GFAP+) and on day 14 (GFAP-) of ganciclovir administration compared with the PBS-treated group. Notably, the mean between the two treatments (before and after ganciclovir treatments) was 9.103 ± 1.184 . Conversely, no effect was observed in the PBS-treated KPC;GFAP-Tk mouse group before or after PBS administration, in which glial cell depletion did not occur.

As previously stated, the orthotopic implantation group was divided into four subgroups for treatment, namely: ganciclovir, vehicle (PBS), ganciclovir + FOLFIRINOX, and FOLFIRINOX. Before commencing the treatments, all groups underwent the von Frey test. To evaluate the effect of ganciclovir and FOLFIRINOX on pain response in mice, the pain test was conducted again on the 7th and 14th days following ganciclovir administration. During the first week, the group of mice treated with only ganciclovir (GFAP-) experienced a reduction in pain response. However, this reduction decreased (means $\pm$ SEM was calculated as 0.4521 ± 0.9123) during the second week, possibly due to the regeneration of glial cells after the first week. No difference in pain response was observed among the group that solely received vehicle treatment (PBS, GFAP+), when assessed before treatment, and in the first-, and second-weeks post-treatment. This experiment also assessed how mice responded to von Frey filaments before and during treatment with a combination of FOLFIRINOX and ganciclovir. Pain responses were recorded before ganciclovir administration, on the fifth day of treatment, immediately before FOLFIRINOX administration, and on the fifth and fourteenth days of ganciclovir treatment. Results showed a decrease (mean $\pm$

SEM, 8.542 ± 2.812) in pain response on day 14 compared to before the ganciclovir administration. These data imply that glial cells are likely to contribute to the pain hypersensitivity associated with murine pancreatic cancer.

The effect of GFAP+ cell depletion on tumor progression in PDAC

As PDAC progresses, glial cells exhibit an early and distinct migration to PanIN lesions, setting up the conditions for tumor cells to invade the neural tissue (Ceyhan et al., 2008). In our mouse models, GFAP-expressing glial cells were depleted in order to validate this theory. In the current project, ganciclovir was initially administered to deplete GFAP-expressing cells. Then pancreas sections from mice were stained with anti-Glial Fibrillary Acidic Protein (GFAP) antibodies to show that depletion had occurred. Finally, the results were evaluated to quantify and compare the change in GFAP expression between ganciclovir-treated and untreated mouse model groups (Figure 10-11 and 12). Lesion density pertains to the count and distribution of abnormal tissue areas (lesions) within the pancreas. These lesions are significant in determining the severity and advancement of pancreatic cancer. Typically, an elevated concentration of lesions is indicative that the disease has progressed to a more severe state with other malignant cells present within the pancreas.

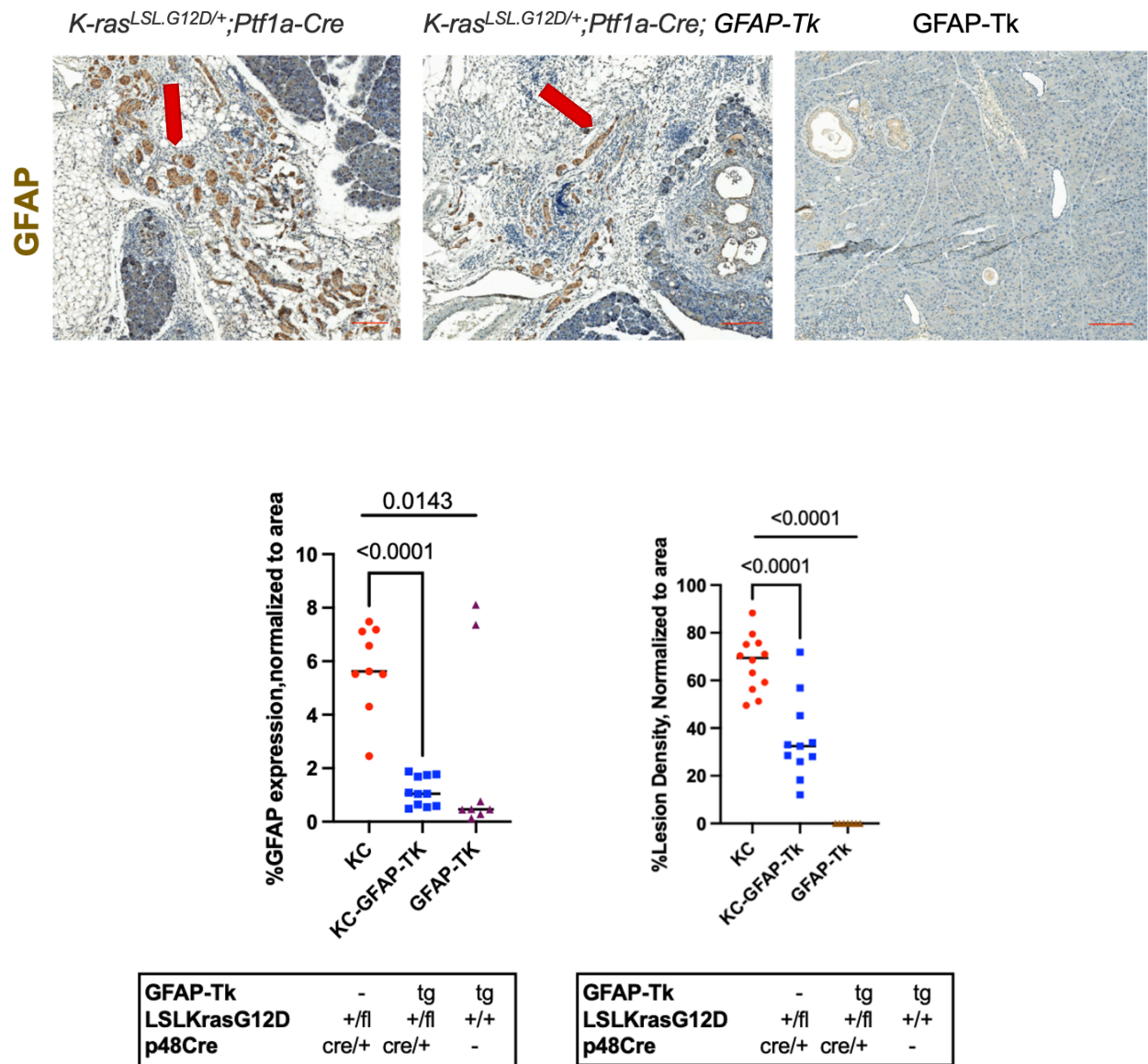


Figure 10. The effect of Ganciclovir-induced glial cell depletion on KC cohort. In the upper panel, the intensity of glial cells and lesions in the KC, KC;GFAP-Tk, and GFAP-Tk groups following ganciclovir administration are monitored by staining with anti-GFAP antibody. Brown colors and red arrows indicate that glial cells within pancreatic nerves. The bottom panel presents the quantification of glial cell GFAP expression and lesion density (unpaired t-test). Scale bars indicate 200 μ m.

For the KPC group, mice in the treatment group possessed the heterozygous *trp53*^{fl/+} genotype, whereas those in the internal control group exhibited the homozygous genotype.

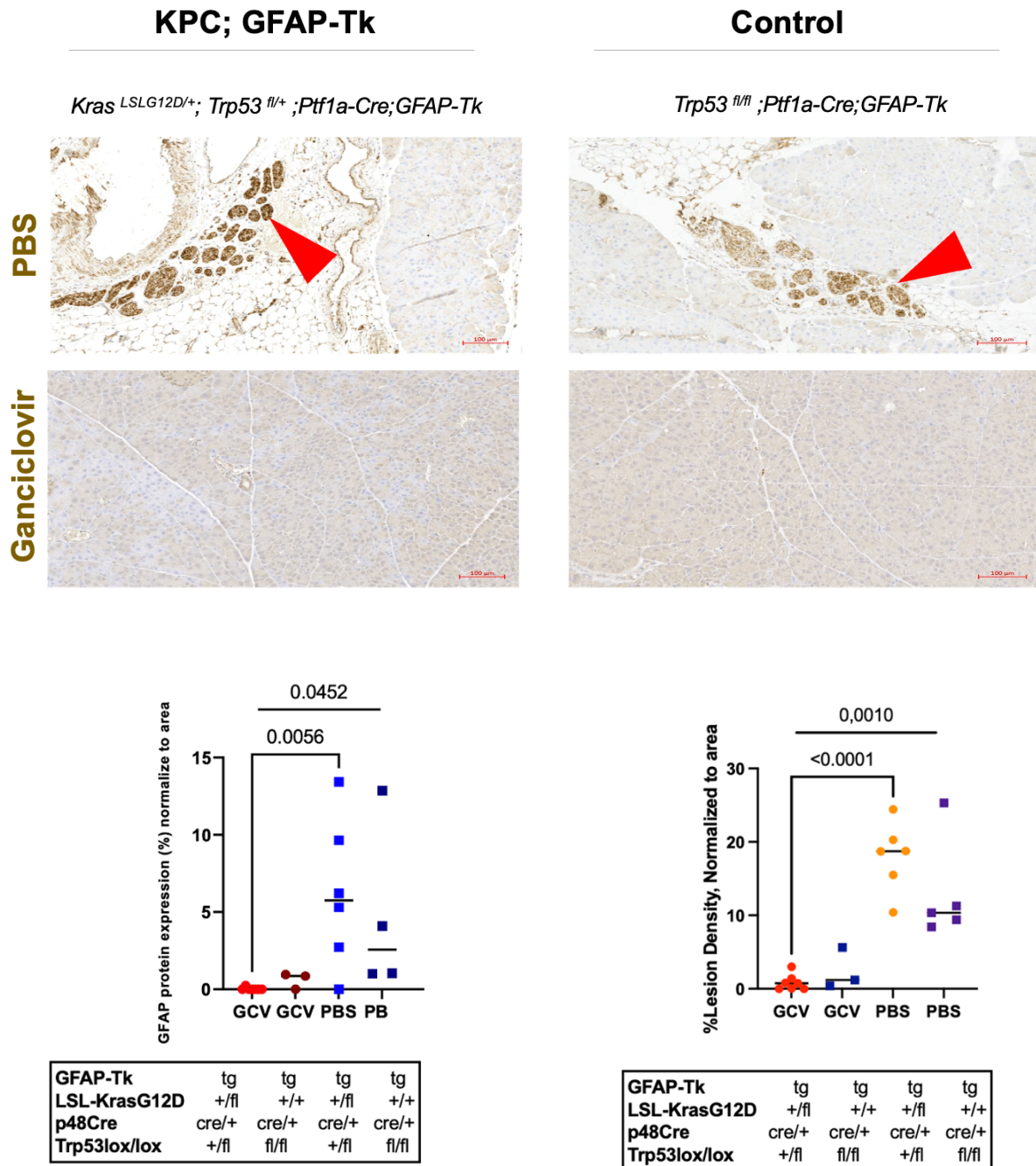


Figure 11. The effect of GFAP positive cell depletion on KPC cohort. In the upper panel, the brown color indicates the expression of glial cells upon ganciclovir and PBS applications in both the treatment and internal control groups. Red triangles point to the particular location of glial cells. The quantified data of glial cell expression are presented on the bottom panel. Scale bars: 100 μm (unpaired t-test).

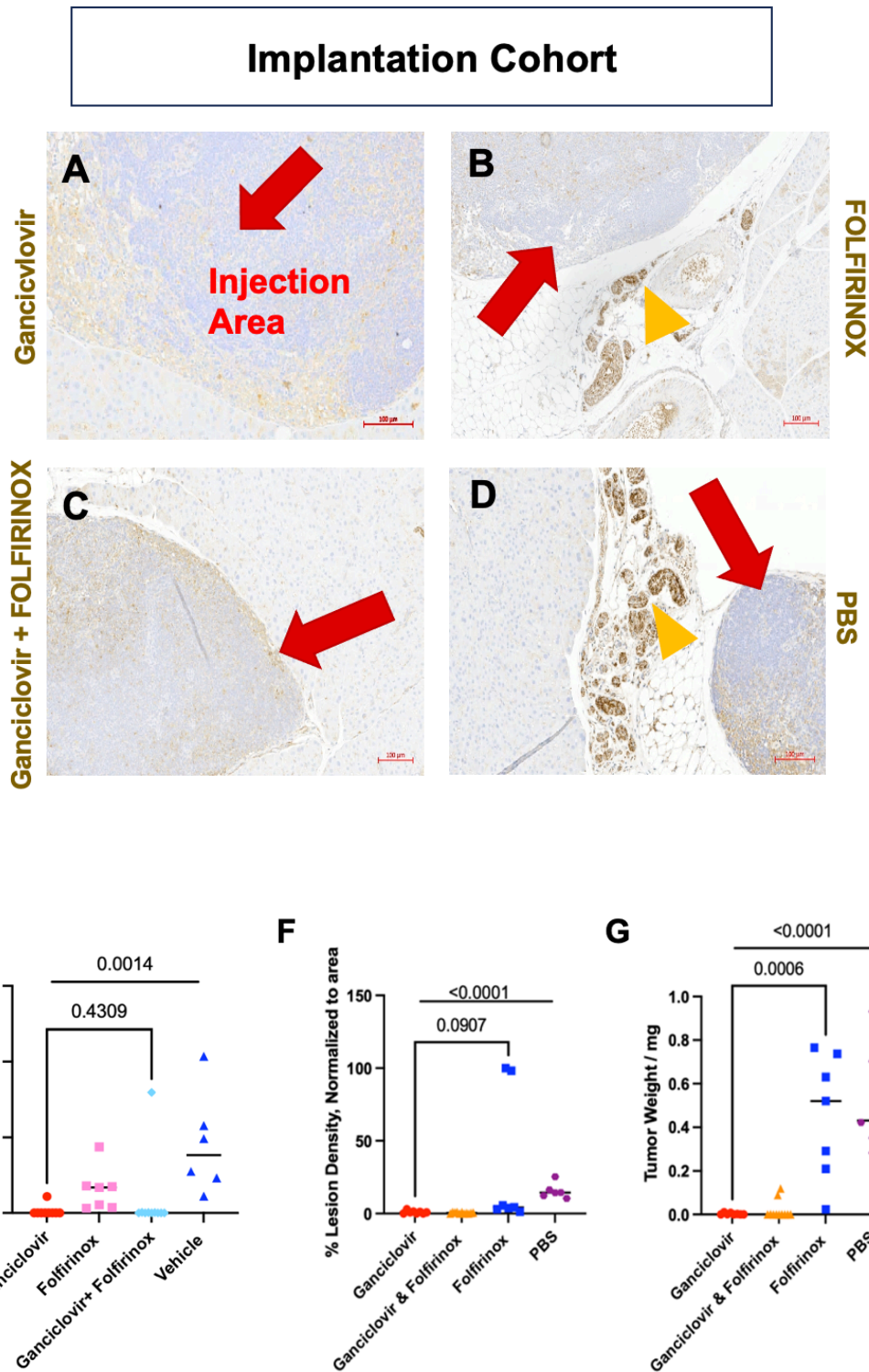


Figure 12. The effect of glial cell depletion in the orthotopic implantation cohort. Representative images of pancreas sections of the GFAP-Tk mice, in which KPC-LacZ cells were orthotopically implanted. The implanted mice were treated with ganciclovir (A), FOLFIRINOX (B), ganciclovir + FOLFIRINOX (C), or vehicle/PBS (D), (E), (F), (G) Quantification of the GFAP protein expression, lesion density and tumor weight. Scale bar: 100 μ m (unpaired t-test).

In the KC cohort, TK cassette activation leading to glial cell accumulation was observed in the KC;GFAP-Tk and GFAP-Tk subgroups despite ganciclovir treatment in all groups. In Figure 10, it is shown that the expression of the GFAP protein was higher in the KC subgroup in comparison to the KC;GFAP-Tk subgroup (KC $5,752 \pm 1.067$ and KC;GFAP-Tk $1,139 \pm 0.5438$, $p < 0.0001$). Besides, there is a significant difference in the GFAP-Tk subgroup when compared to the KC subgroup (2.255 ± 3.395 , $p = 0.2942$).

For the next experiment, two groups were used: the experimental group, KPC;GFAP-Tk (*K-ras^{LSL.G12D/+}; Trp53^{fl/+}; Ptfla-Cre; GFAP-Tk*), and the control group, KPC;GFAP-Tk (*Trp53^{fl/fl}; Ptfla-Cre; GFAP-Tk*). In the KPC;GFAP-Tk group GFAP expression exhibited differences between the experimental groups treated with ganciclovir and PBS (mean $\pm$ SEM: 4.710 ± 2.027 , $p=0.0452$: Figure 11). After conducting a comparison between the ganciclovir-treated and the vehicle (PBS)-treated groups in the orthotopic implantation group, we found that GFAP expression decreased noticeably in the ganciclovir-treated group (with a mean of 0.1226 and standard deviation of 0.3640, $p=0.0014$: Figure 12). However, when we compared the ganciclovir-treated (GFAP-) group with the FOLFIRINOX alone treated group in terms of GFAP expression, we found an unexpected result (with a mean of 1.547 and standard deviation of 1.413, $p=0.0014$), which led us to believe that FOLFIRINOX does not have a direct effect on GFAP expression (Figure 12). We measured lesion densities in the pancreas using the same sections and staining. The results showed that GFAP-Tk group had the lowest lesion density, while the KC group had the highest density (67.33 ± 11.75). Additionally, a reduction in lesion density was observed in the KC;GFAP-Tk group compared to the KC group (35.12 ± 17.14 , $p < 0.0001$: Figure 10), as determined by a two-tailed t-test.

Here we aimed to examine lesion density after glia depletion in pancreatic cancer. In KPC cohort, two groups were used - the experimental group, KPC;GFAP-Tk (*K-ras^{LSL.G12D/+}; Trp53^{fl/+}; Ptfla-Cre; GFAP-Tk*) and the control group, KPC;GFAP-Tk (*Trp53^{fl/fl}; Ptfla-Cre; GFAP-Tk*) and both groups had ganciclovir and/or PBS treatment. We compared the groups treated with ganciclovir

and PBS to assess the difference in lesion densities among the KPC cohort. The results revealed a considerable reduction in lesion density between the experimental and control groups treated with ganciclovir. When compared to the ganciclovir-treated subgroup the highest lesion density was observed in the PBS-treated experimental group (17.16 ± 1.827 , $p < 0.0001$; Figure 11).

In the orthotopic implantation cohort, lesion densities were quantified using a two-tailed t-test. When the ganciclovir-treated group (0.9074 ± 0.007) was compared with the vehicle (PBS)-treated group (15.52 ± 5.201 , $p < 0.0001$) the lesion density of the ganciclovir-treated group was lower than that of the PBS-treated group. However, there was no difference between the groups treated with PBS or FOLFIRINOX (29.96 ± 16.40 , $p = \text{ns}$). The results of tumor weight suggested that the FOLFIRINOX dose was rather insufficient for downsizing the tumor as shown in Figure 12.

These results showed that the ganciclovir-induced glial cell depletion in PDAC GEMM led to a reduction in lesion density compared to the control group this implies that glial cells promote tumor formation in the pancreas. Furthermore, the observation of a decrease in lesion density in mice depleted of glial cells indicates that the abundance of these cells is associated with a higher number of tumor lesions, suggesting that glial cells may contribute to developing or maintaining a tumor microenvironment favorable to lesion formation in the pancreas.

The effect of ganciclovir-induced glial cell depletion on angiogenesis and pancreatic neuritogenesis

PDAC is an angiogenic tumor since it encourages the growth of new blood vessels to supply the expanding tumor with nutrients and oxygen (Ren et al., 2018). As a result, pancreatic cancer may become even more aggressive and challenging to treat. For these reasons, this study aimed to evaluate the effect of GFAP⁺ cell depletion on angiogenesis in pancreatic cancer. The vascular density of the KC, KC;GFAP-Tk, GFAP-Tk, KPC, KPC;GFAP-Tk, and orthotopic implantation groups was compared regarding angiogenesis between the groups treated and not treated with ganciclovir. In the KC and KPC groups, we were able to visualize both vessels and nerves in the same pancreatic sections by IHC double staining. In the orthotopic implantation group, we performed two separate immunohistochemical staining for nerves (S100) and vessels (CD31). As figure 13 shows a significant difference in vascular density between KC and KC;GFAP-Tk groups (KC: 0.07 ± 0.022 , KC;GFAP-Tk: 0.029 ± 0.001 , $p < 0.0001$). In overt PDAC, the glia depleted mice (KPC;GFAP-Tk +ganciclovir: 1.484 ± 1.551) exhibited a lower vascular density than the control (PBS-treated) mice without glia depletion (KPC;GFAP-Tk + PBS: 10.76 ± 3.503 , $p < 0.0001$: Figure 14).

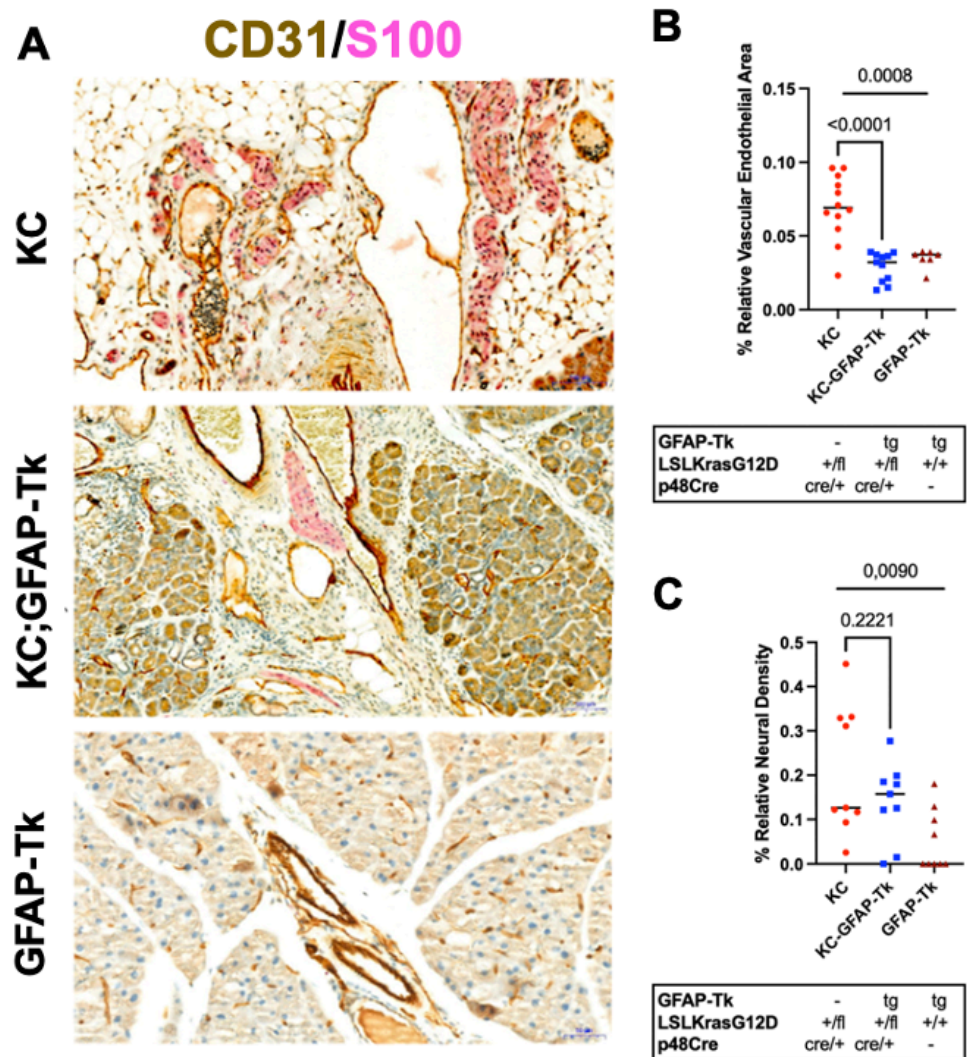


Figure 13. Effect of GFAP+ cell depletion on vascular and neural marker expressions in the KC cohort. (A) Representative images of CD31 and S100 staining of KC cohort vessels (brown) and nerves (pink) after ganciclovir administration in the KC, KC;GFAP-Tk, and GFAP-Tk groups. **(B-C)** The quantification of the vascular endothelial marker (CD31) and neural marker (S100). Scale bars: 200 μ m and 50 μ m.

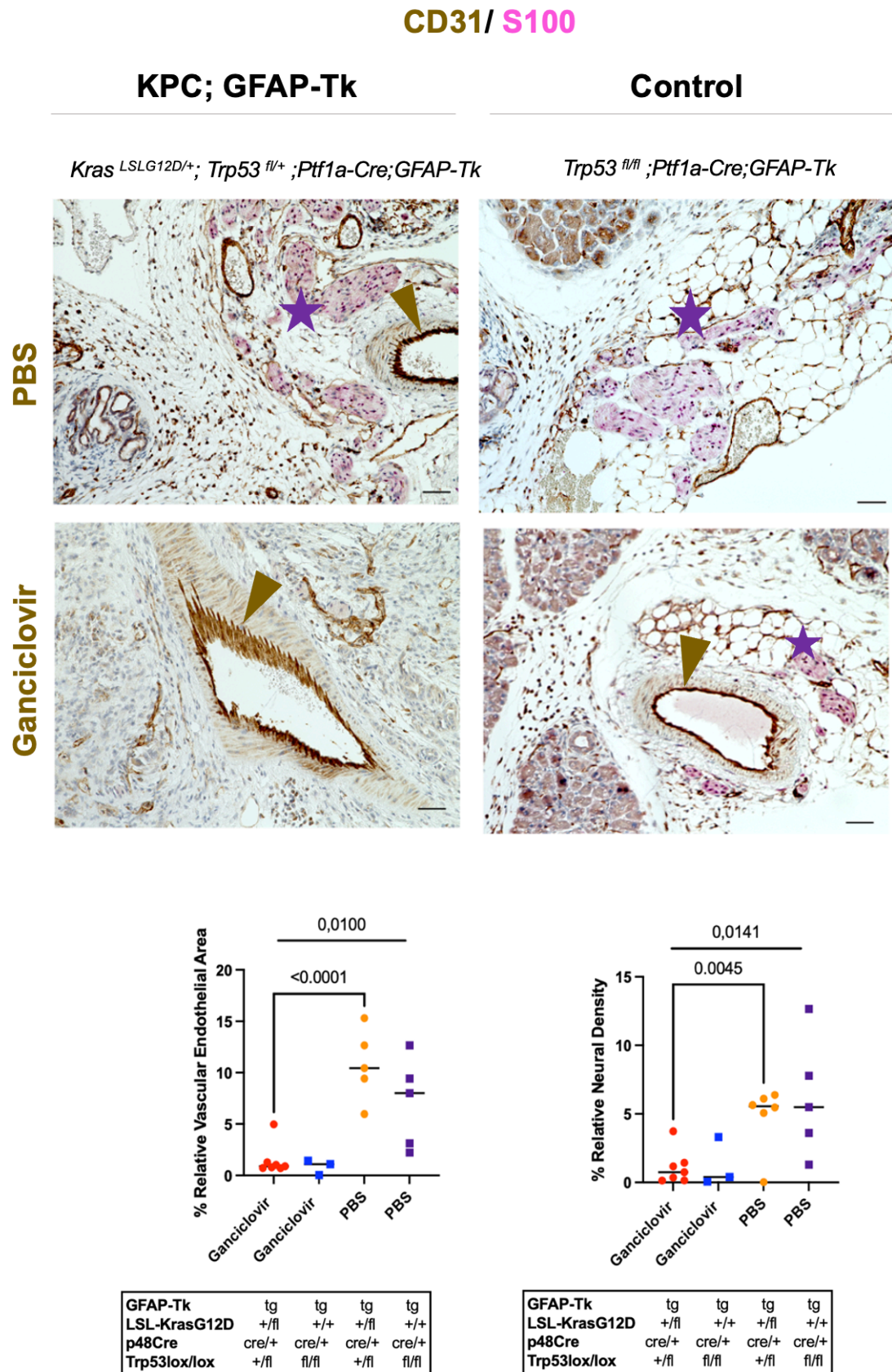


Figure 14. Effect of GFAP positive cell depletion on vascular and neural marker expression in the KPC cohort.

The upper panel presents representative images showing vessel and nerve locations after ganciclovir and PBS administration in KPC;GFAP-Tk, and control groups. The brown triangle indicates the vessels, and the purple star points to the nerves. The bottom panel demonstrates the quantification of CD31 and S100 protein expression after ganciclovir and PBS administration in KPC; GFAP-Tk and control groups-scale bars: 50 μ m.

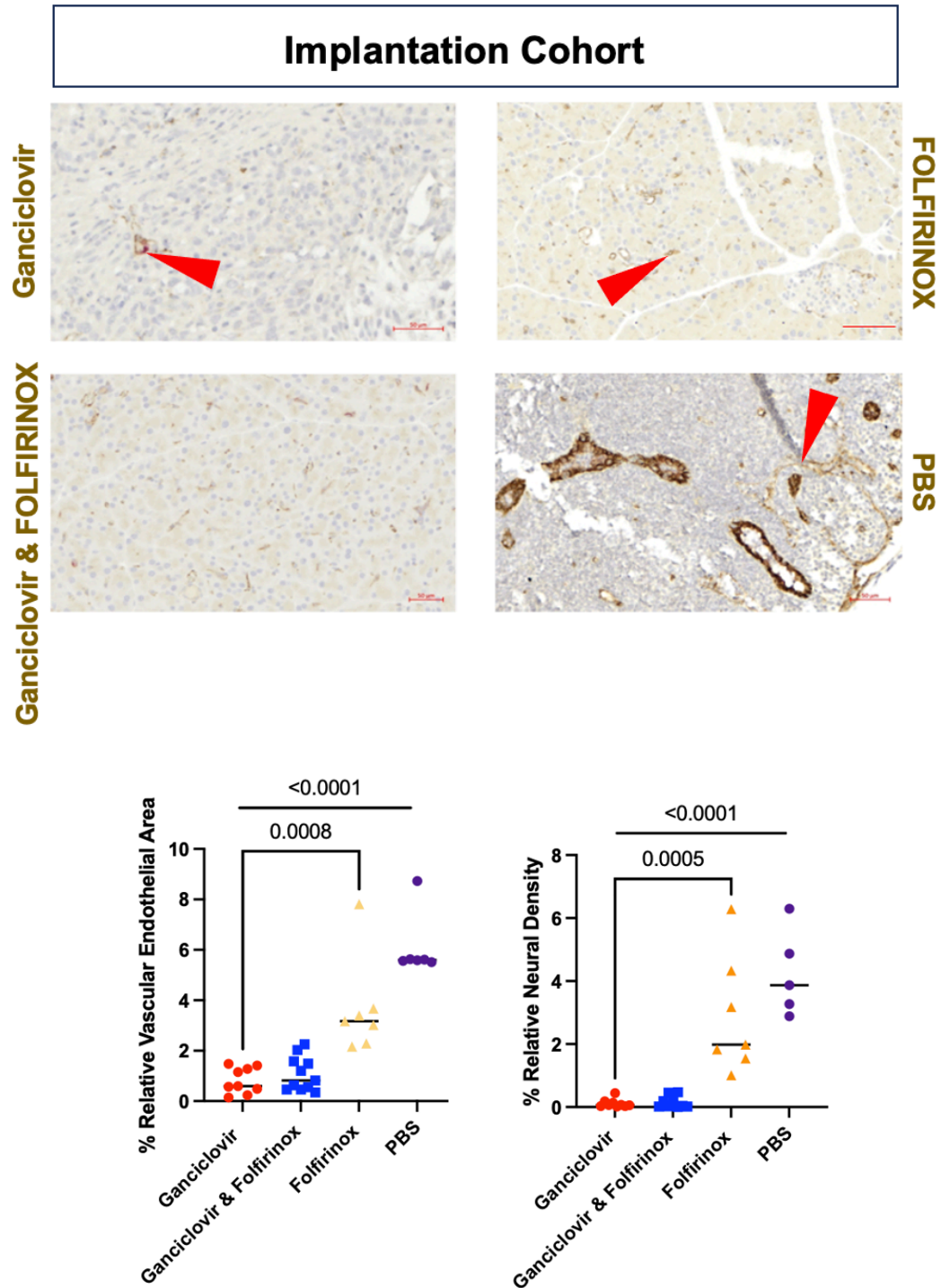


Figure15. The impact of GFAP+ cell depletion on vascular and neural marker expression in the orthotopic implantation cohort. The top part of the image shows CD31 expression (brown) in a GFAP-Tk mouse pancreas that has received an orthotopic injection of KPC cells and treated with different medications - ganciclovir, vehicle (PBS), ganciclovir+ FOLFIRINOX, and FOLFIRINOX. The bottom part of the image measures the expression of vascular endothelial marker (CD31) and neural marker (S100). The red arrow indicates the CD31 expression, and the scale bars show 50 μ m.

Upon comparing the groups of mice that were treated with ganciclovir and those treated with vehicle (PBS) in the orthotopic implantation group, we observed a significant reduction in CD31 expression in the ganciclovir-treated group (with a mean of 0.8173 and a standard deviation of 0.5156, $p < 0.0001$). Furthermore, when we compared the CD31 expression between the ganciclovir-treated and FOLFIRINOX-only-treated groups, we found a significant difference (with a mean of 2.828, a standard deviation of 0.6630, and a p-value of 0.0008, as shown in Figure 15). Glial cells play a pivotal role in the trophic maintenance of neuronal outgrowth and regulation of neurite density (Jäkel & Dimou, 2017). Therefore, determining the effect of glial cell depletion on PDAC-specific pancreatic neuropathy is paramount. Previous studies have shown that nerve invasion into gastrointestinal tumors is not due to active invasion of nerves by tumor cells but rather to glial cell migration into tumor precursor lesions, contrary to the traditional assumption (S. Deborde & Wong, 2022). Therefore, in this study, one aim was to investigate whether glial cell depletion affects the level of nerve invasion in this GEMM. For this purpose, we compared nerves from representative tissue sections after immunohistochemical staining with S100 (astrocyte marker). Thus, it was possible to compare the nerve density between the control and treatment groups, which were depleted in GFAP, another astrocyte marker.

After comparing the KC subgroup (0.2119 ± 0.1450) with the KC;GFAP-Tk subgroup (0.1401 ± 0.08807 , $p = 0.2221$; Figure 13), we observed a significant decrease in nerve density in the KC group. Here, we found a substantial reduction in nerve density. Indeed, the glia depleted mice (KPC;GFAP-Tk + ganciclovir) exhibited a lower density (1.103 ± 1.262) than the control (PBS-treated) mice without glia depletion (KPC;GFAP-Tk + PBS: 4.781 ± 2.381 , $p = 0.0045$; Figure 14). A two-tailed t-test was used to calculate the nerve density in the implantation cohort. The nerve density in the ganciclovir-treated group (mean $\pm$ SEM, 0.1143 ± 0.1344) was substantially lower than that of the vehicle (PBS)-treated group, (4.239 ± 1.373 , $p < 0.0001$; Figure 15) compared to the PBS-treated group. On the other hand, a difference was found between the ganciclovir-treated group and the FOLFIRINOX-treated group (2.766 ± 0.6191 , $p = 0.0005$; Figure

15). The neural density of the tumor was reduced in the treatment group with depleted glial cells compared to the control group in pancreatic cancer. The decrease in neural density could be associated with less secretion by glial cells of growth factors and cytokines in the tumor microenvironment, which are already known to enhance neuronal survival and growth. In addition, lower neural density results from reduced expression of the S100 protein, as it is primarily expressed in both neural and glial cells.

Analysis of stromal activity and inflammatory cell infiltration in PDAC GEMM after glial cell depletion

Studies have indicated that stromal cell activation leads to selective immune cell suppression during PDAC development (Sperb, Tsesmelis, & Wirth, 2020). In addition, downregulation of myofibroblasts in the PDAC stroma was associated with decreased immune cell infiltration in a PDAC mouse model (Özdemir et al., 2014). Neural and glial cells may mediate between mesenchymal cells and act as signaling agents in the communication of immune cells with mesenchymal cells. At this point, an essential point of investigation is how the depletion of glial cells in the GEMM of PDAC may affect the level of stromal response and immune cell infiltration in PDAC. To investigate this, the level of stromal activity in all mouse models used in the project was compared by immunohistochemistry against alpha-smooth muscle-actin (α -SMA) as a marker of activated myofibroblasts. In the second part of this experimental section, the degree of intratumoral immune cell infiltration in these GEMMs were quantitatively compared by immunohistochemistry against the following marker molecules: CD45⁺ for leukocytes, CD8⁺ for cytotoxic T lymphocytes and F4/80 for macrophages.

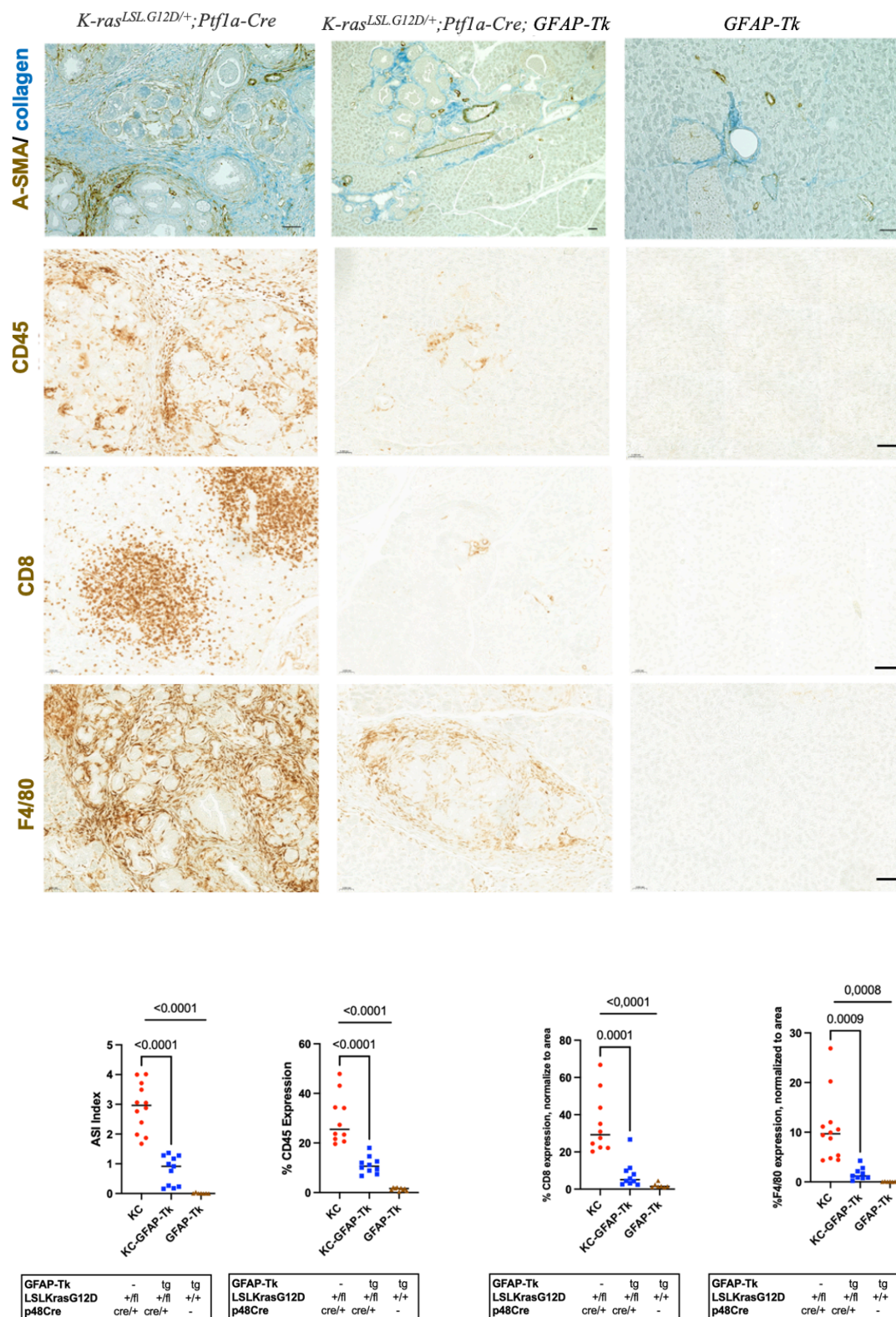


Figure 16. The effect of ganciclovir-induced GFAP positive cell depletion in stromal activation and immune cell infiltration in the KC cohort. The upper panel represents the differential expression of stromal activation, CD45, CD8, and F4/80 markers of GFAP-positive glial cell depletion after ganciclovir administration. The bottom panel shows the quantification of these markers. Scale bars indicate 50 μ m, 100 μ m, and 200 μ m.

Ganciclovir

PBS

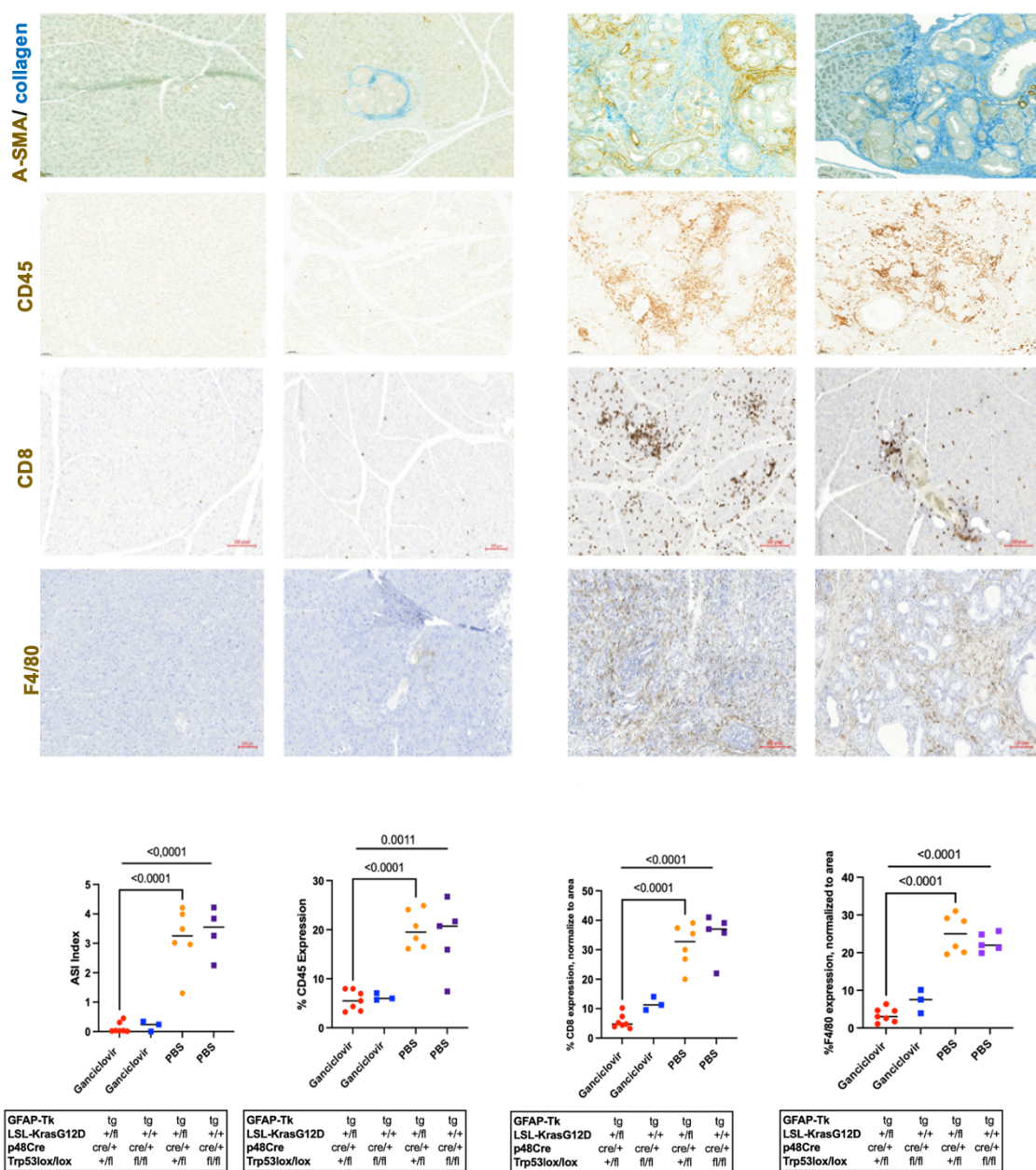


Figure 17. The effect of ganciclovir-induced GFAP-positive cell depletion in stromal activation and immune cell infiltration in the KPC cohort. The upper panel represents altered stromal activation, CD45, CD8, and F4/80 markers following ganciclovir treatment in GFAP-positive glial cell depletion. The lower panel indicates the quantification of these markers are presented. Scale bars indicate 50 μ m, 100 μ m, and 200 μ m.

Implantation Cohort

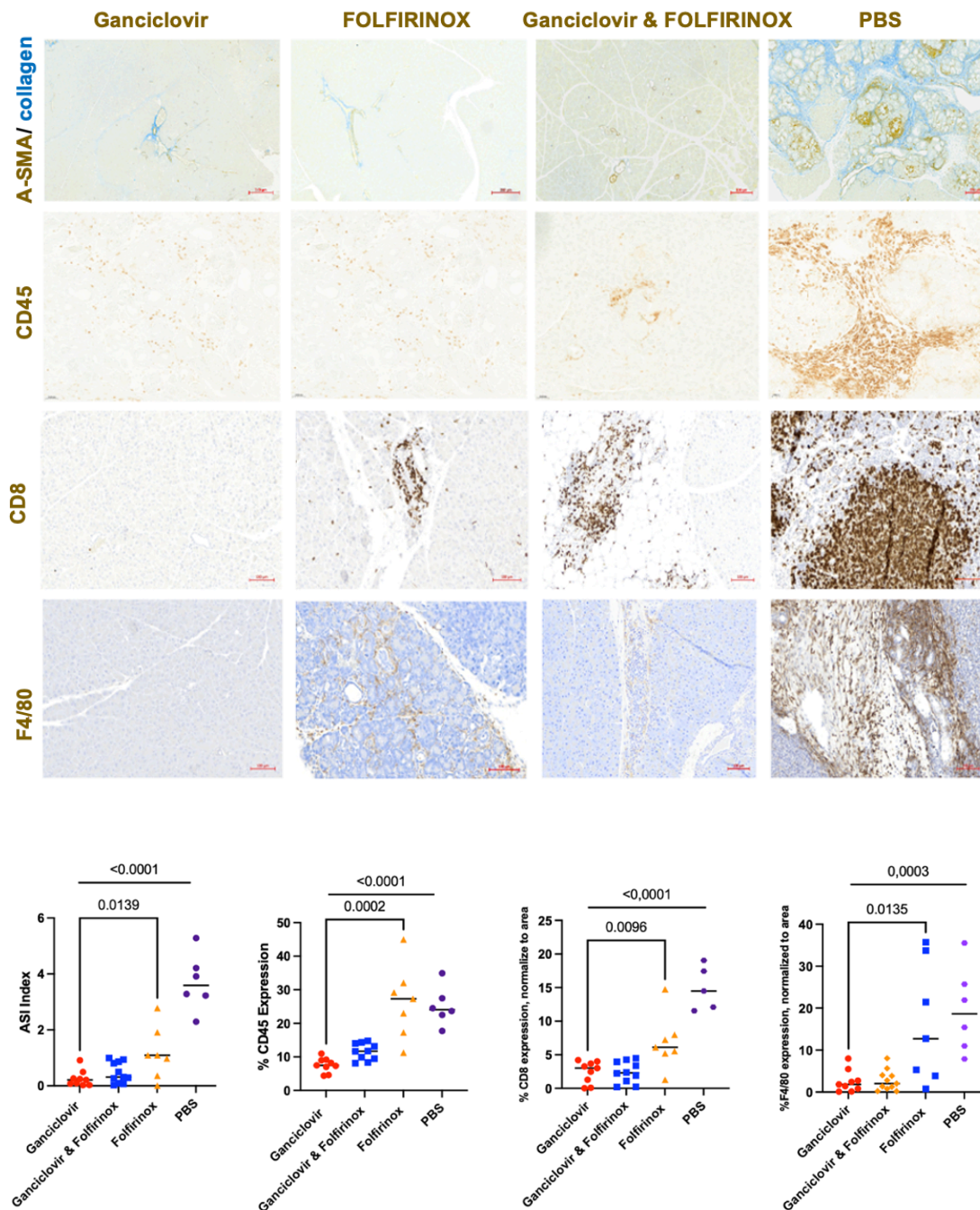


Figure 18. The effect of ganciclovir-induced depletion of GFAP-positive cells on stromal activation and immune cell infiltration in the orthotopic implantation cohort. The upper panel demonstrates changes in markers of stromal activation, CD45, CD8, and F4/80 after ganciclovir treatment of GFAP-positive glial cell depletion. The lower panel indicates the quantification of these markers. Scale bars indicate 50 μ m, 100 μ m, and 200 μ m.

Stromal activation index (ASI) was calculated by the formula total α -SMA area/total collagen area in all groups. When we evaluated the KC group in terms of stromal activation, we found a remarkable change in the stromal activation index between KC (mean $\pm$ SEM; 2.906 ± 0.81) and KC;GFAP-Tk (0.7814 ± 0.5 , $p < 0.0001$). A similar result was found between KC and GFAP-Tk subgroups (GFAP-Tk mean $\pm$ SEM, 0.0111 ± 0.016 , $p < 0.0001$; Figure 16). To determine the number of leukocytes in the KC cohort after ganciclovir administration, IHC with CD45+ marker was performed on pancreas sections and histopathological evaluation was performed. We observed a drastic decrease in CD45+ expression in the KC;GFAP-Tk subgroup (11.09 ± 3.415) compared to the KC subgroup (29.57 ± 9.910 , $p < 0.0001$). The GFAP-Tk subgroup showed a similar trend (1.421 ± 0.6217 , $p < 0.0001$; Figure 16). CD8+ IHC staining was performed to observe how cytotoxic T lymphocytes were affected by glial cell depletion. We observed a decrease in CD8+ expression in the KC;GFAP-Tk (7.886 ± 7.307) subgroup compared to the KC (mean $\pm$ SEM, 34.91 ± 15.77 , $p = 0.0001$; Figure 16) group. Another important immune cell type present in the tumor microenvironment is macrophages. F4/80 IHC staining was used to quantify macrophage numbers after glial cell depletion in the KC cohort. When the results were analyzed, F4/80 expression was observed a lower in the KC;GFAP-Tk (1.650 ± 1.257) subgroup compared to the KC (10.65 ± 6.775 , $p = 0.0009$; Figure 16) subgroup. In the second part, two groups were used - the experimental group, KPC;GFAP-Tk (*K-ras*^{LSLG12D/+}; *Trp53*^{f/f}; *Ptfla-Cre*; *GFAP-Tk*) and the control group, KPC;GFAP-Tk (*Trp53*^{f/f}; *Ptfla-Cre*; *GFAP-Tk*). Furthermore, the ganciclovir-treated KPC;GFAP-Tk group was compared with the PBS-treated KPC;GFAP-Tk group using an unpaired t-test (two-tailed). First, we wanted to assess the effect of glial cell depletion on stromal activation, so we measured the Stromal Activation Index (ASI). As shown in Figure 17, we observed that the ganciclovir-treated mice showed lesser stromal activation than the PBS-treated ones (3.036 ± 0.3973 , $p < 0.0001$).

Next, we checked leukocyte expression levels by performing IHC against CD45⁺. The ganciclovir-treated mice revealed lower CD45⁺ expression than the PBS-treated mice (20.11 ± 3.785 , $p < 0.0001$: Figure 17). Then, we checked out the CD8⁺ marker of cytotoxic T cells. Interestingly, the PBS-treated mice exhibited compared to the ganciclovir-treated mice (25.89 ± 2.895 , $p < 0.0001$: Figure17). Finally, we examined another group of immune cells, called macrophages, using a marker called F4/80. The ganciclovir-treated mice indicated lower F4/80 expression than the PBS-treated mice (21.55 ± 2.052 , $p < 0.0001$: Figure 17). After performing a comparison between the ganciclovir-treated (GFAP-) and vehicle (PBS)-treated groups in the orthotopic implantation group, we found that the stromal activation index was reduced in the ganciclovir-treated group (mean 0.2582 and standard deviation 0.2856, $p < 0.0001$: Figure 18). Additionally, we found a difference in stromal activation between the ganciclovir-treated group (0.2582 ± 0.2856) and the FOLFIRINOX-treated group (1.174 ± 0.9303 , $p = 0.0139$). When analyzing the orthotopic implantation cohort in terms of leukocyte numbers, the ganciclovir and PBS-treated groups were first evaluated. CD45⁺ expression was decreased in the ganciclovir-treated group (7.579 ± 2.124) compared to the PBS-treated group (25.13 ± 5.760 , $p < 0.0001$). In comparison of ganciclovir and FOLFIRINOX-treated groups, resulting in with a mean 26.44 and a standard deviation 10.88 and p value as 0.0002, indicating that CD45⁺ expression was statistically lower in the ganciclovir-treated group. The amount of cytotoxic T cells was determined using the CD8⁺ biomarker. The p value calculated for comparing the ganciclovir and PBS-treated groups was $p < 0.0001$ (ganciclovir-treated group 2.441 ± 1.619 and PBS-treated group 14.93 ± 3.279). The number of macrophages was defined using the anti-F4/80 antibody. The results showed that F4/80 expression was statistically decreased in the ganciclovir-treated group compared to the PBS-treated group, (ganciclovir-treated group 3.426 ± 1.855 and PBS-treated group 24.97 ± 5.079 , $p < 0.0001$) when comparing the ganciclovir and FOLFIRINOX-treated groups, F4/80 expression exhibited a less pronounced downward trend (FOLFIRINOX-treated 16.22 ± 14.36 , $p = 0.0135$), as shown in Figure 18.

Determination of activation of primary and secondary nociceptive neurons and spinal microglia & astroglia after glial cell depletion in PDAC GEMM

Previous research has shown that pain in experimental chronic pancreatitis is associated with increased c-fos and p-Erk (p44/42 MAPK) activity in the spinal cord's dorsal spinal ganglia and spinal dorsal horn cells (Gierthmühlen & Baron, 2016; Harris, 1998). To confirm the related activity in the GEMM of PDAC, immunohistological examinations were first performed against c-fos and p-Erk in mice spinal cord. The study aimed to identify activated primary and secondary nociceptive neurons and determine whether there is a possible difference in the different treatment groups. For this purpose, tissues were immunohistologically stained with c-fos and p-Erk antibodies, and the activation of primary and secondary nociceptive neurons were compared between different groups. Spinal cords (Th8-11) of all animal groups were immunohistologically stained against p-p38/38 GFAP, p75NTR, and Iba-1 to determine the activity of potentially reactive spinal glia. Expression levels of the corresponding protein levels were compared between different groups. To assess the activation of primary and secondary nociceptive neurons in spinal posterior horn cells and spinal ganglia, we evaluated c-fos and p-ERK (phosphorylated extracellular signal-regulated kinase) expression. Immunohistological examinations were conducted to measure c-fos and p-ERK activity.

In the KC cohort, analysis of c-fos expression revealed a decrease in the KC; GFAP-Tk subgroup compared to the KC subgroup (KC;GFAP-Tk 6.253 ± 2.581 and KC;GFAP-Tk 1.134 ± 0.9789 , $p < 0.0001$: Figure 19). Within the KPC cohort, c-fos expression was lower in both the ganciclovir-treated and PBS-treated groups compared to the experimental group (KPC;GFAP-Tk+ ganciclovir 0.7600 ± 0.7353 and KPC;GFAP-Tk+ PBS 7.119 ± 3.836 , $p = 0.0012$: Figure 19). And the orthotopic implantation cohort, the ganciclovir-treated group was compared to both the PBS-treated group (ganciclovir-treated 0.1688 ± 1.801 and PBS-treated 4.664 ± 1.825 , $p < 0.0001$) and the

FOLFIRINOX-treated group (2.526 ± 1.060 , $p < 0.0001$). As shown in Figure 19, in both comparisons, c-fos expression was lower in the ganciclovir-treated group. As shown in Figure 20, we found that in the KC group, there was a decrease in p-ERK expression in the KC; GFAP-Tk subgroup compared to the KC subgroup (KC;GAP-Tk 1.555 ± 1.086 and KC: 10.04 ± 4.521 , $p < 0.0001$). In addition, in the KPC cohort, p-ERK expression was lower in the ganciclovir-treated (0.3031 ± 0.3307) than the PBS-treated (7.988 ± 4.201 , $p = 0.0029$) groups. We also compared the ganciclovir-treated group (1.524 ± 1.999) in the orthotopic implantation cohort with both the PBS-treated group (8.157 ± 5.741 , $p = 0.6922$) and the FOLFIRINOX-treated group (3.225 ± 0.3899 , $p < 0.0001$). In both comparisons, p-ERK expression was lower in the ganciclovir-treated group.

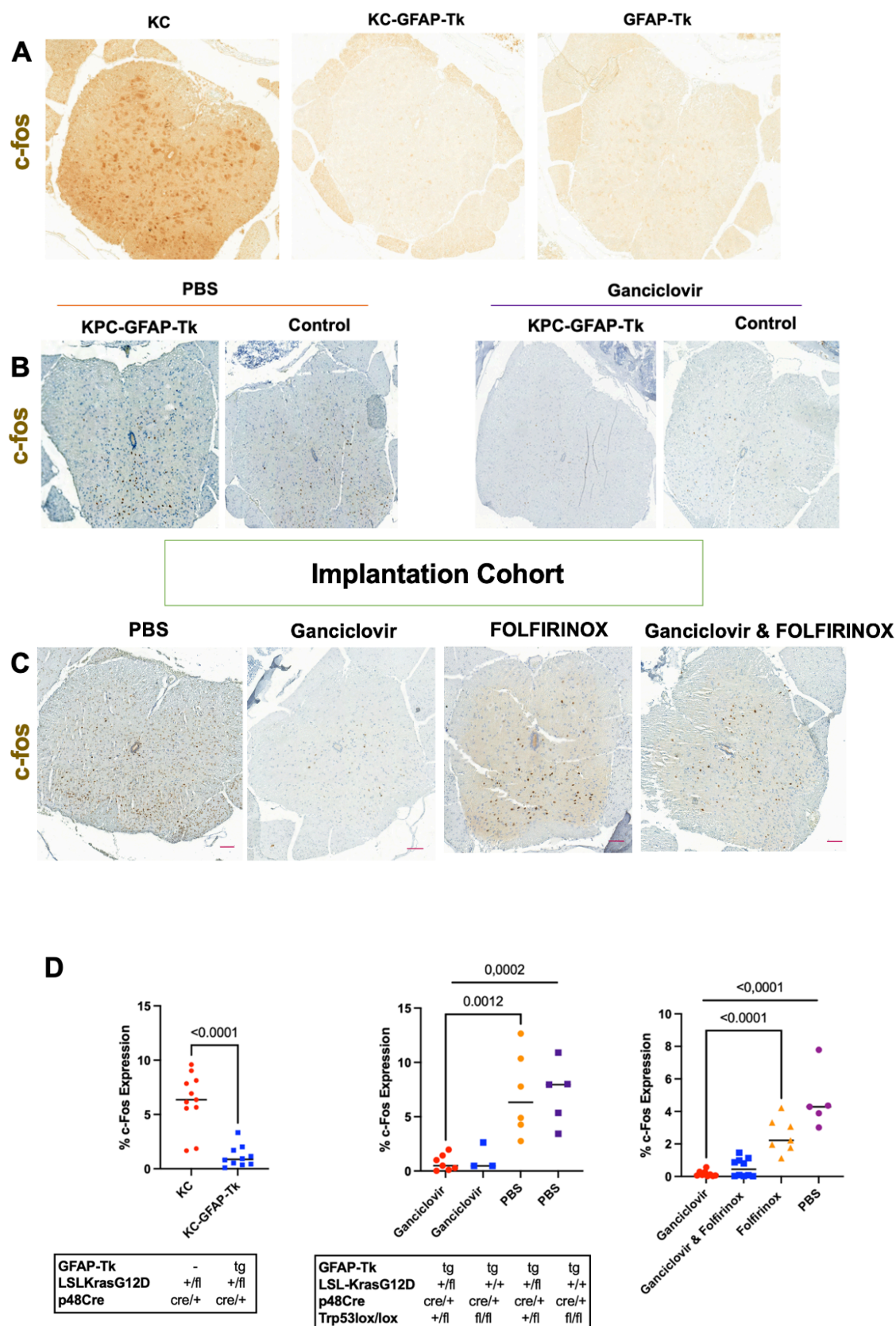


Figure 19. The effect of GFAP⁺ cell depletion on the c-fos proto-oncogene in KC, KPC, and orthotopic implantation cohorts. Representative images of c-fos expression in (A) KC cohort, (B) KPC cohort, (C) orthotopic implantation cohort. (D) Differential quantification of c-fos expression in experiments with different genotypes and treatment groups. Scale bars 100µm.

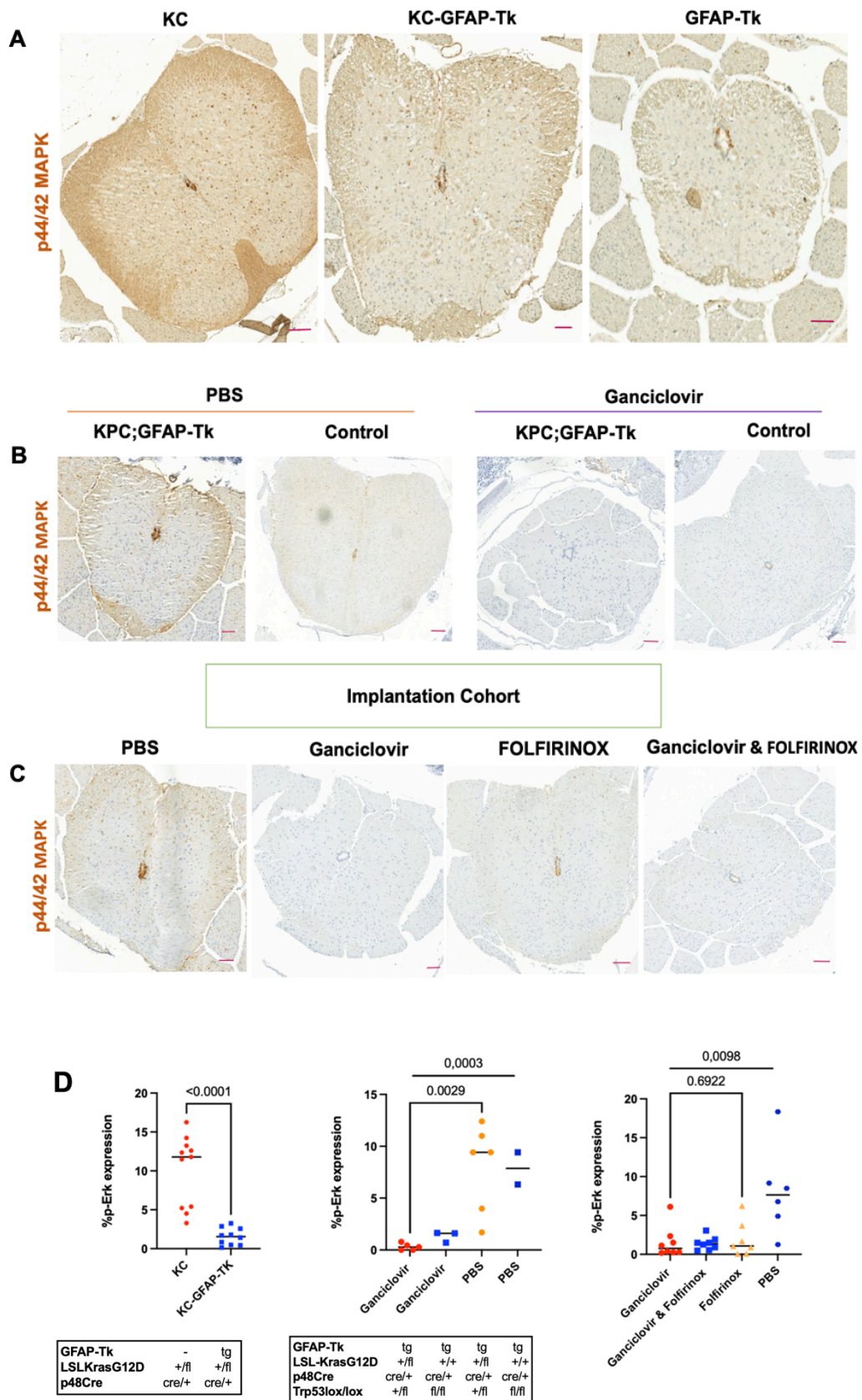
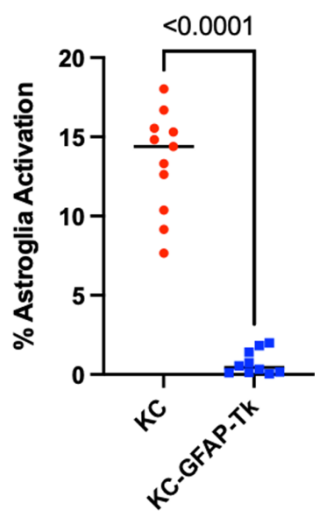
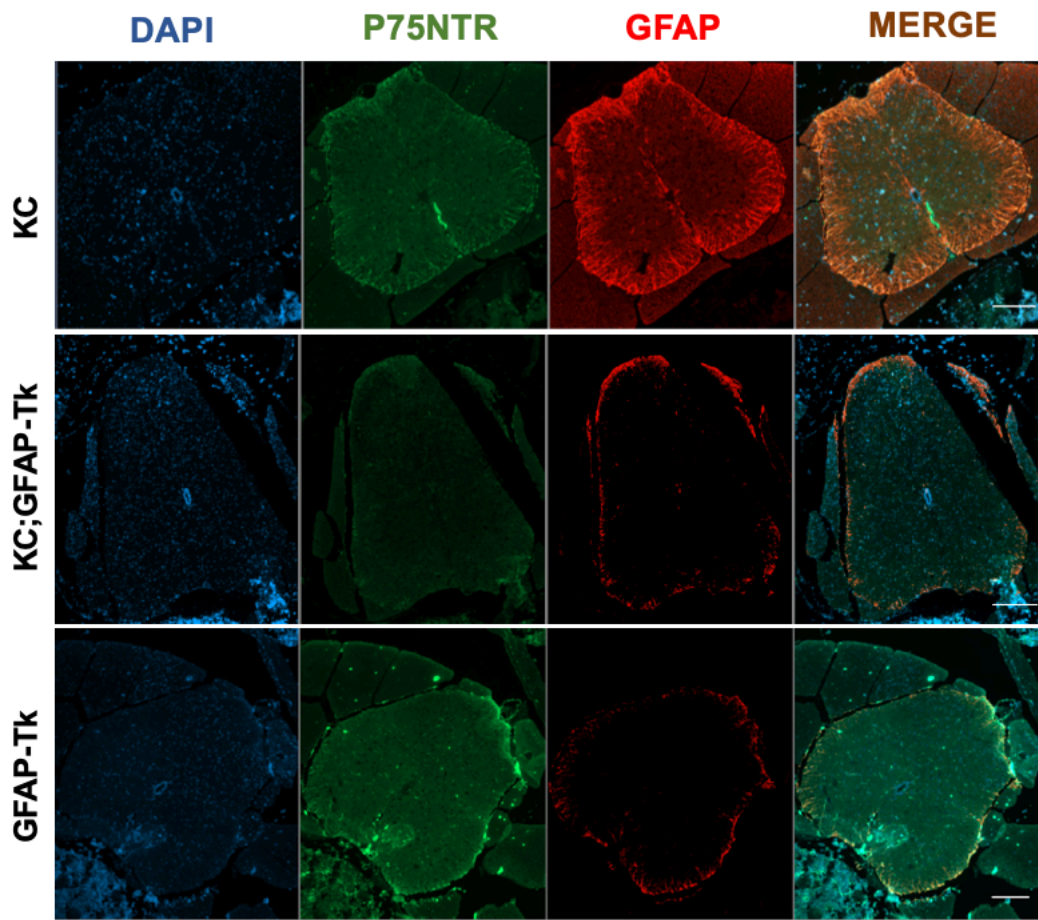


Figure 20. The effect of GFAP⁺ cell depletion on nociception in KC, KPC, and orthotopic implantation cohorts. Representative images of p-Erk expression in (A) KC cohort, (B) KPC cohort, (C) orthotopic implantation cohort (D) Differential quantification of p-Erk expression in experiments with different genotypes and treatment groups. Scale bars indicate 100µm.

The effect of ganciclovir-induced glial cell depletion on astroglia activation



GFAP-Tk	-	tg
LSLKrasG12D	+/-	+/-
p48Cre	cre/+	cre/+

Figure 21. The effect of GFAP positive cell depletion on astroglia activation in KC cohort. Blue DAPI, green p75NTR, red GFAP. Scale bars 200 μm.

Astroglia activation was calculated as follows: Total GFAP area / total p75NTR area * 100.

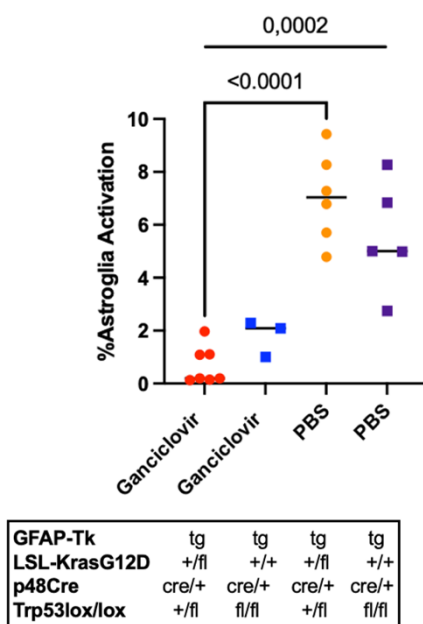
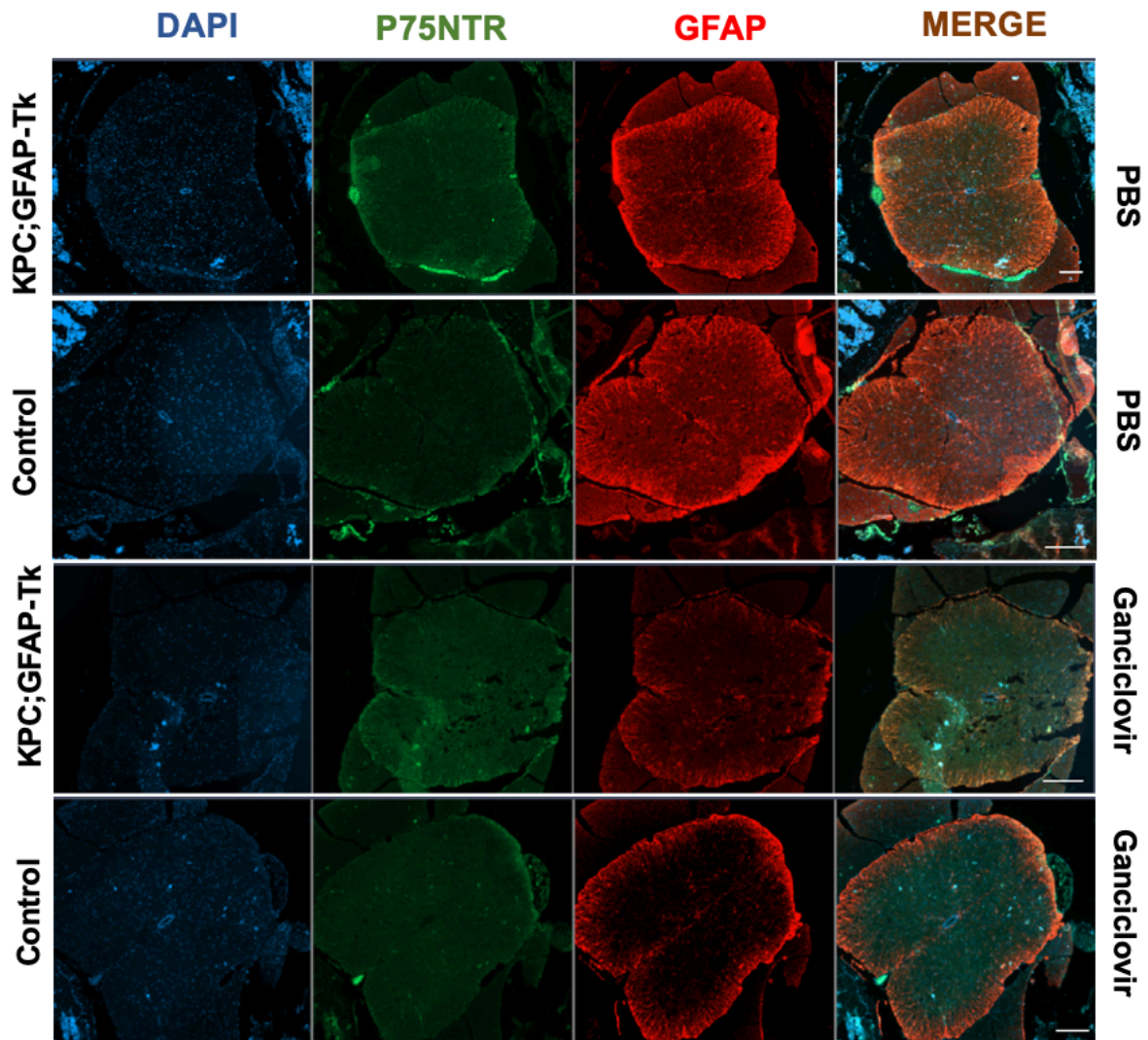


Figure 22. Ganciclovir-induced GFAP+ cell depletion affects astroglia activation in the KPC cohort. The left side of the figure shows the genotypes and the right side shows the treatments.

** KPC;GFAP-TK (*K-ras*^{LSLG12D/+}; *Trp53*^{fl/+}; *Ptfla-Cre*; *GFAP-Tk*) and the control group, KPC;GFAP-TK (*Trp53*^{fl/fl}; *Ptfla-Cre*; *GFAP-Tk*). Scale bars 200μm.

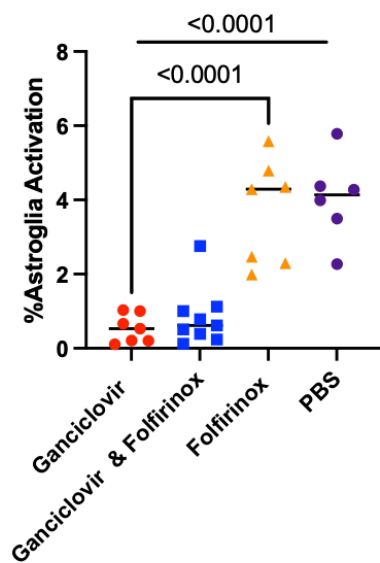
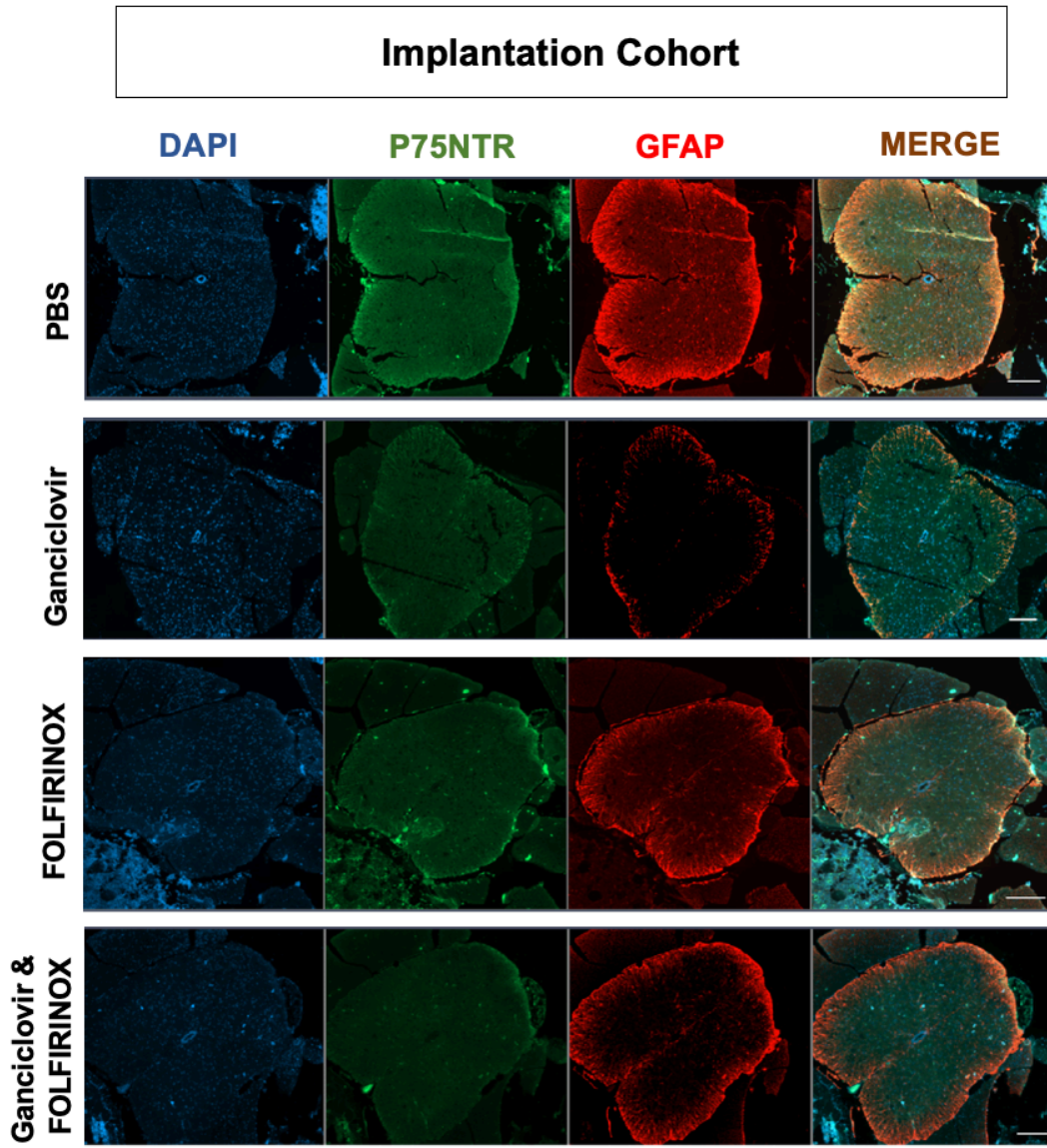


Figure 23. The effect of ganciclovir-induced GFAP+ cell depletion on astroglia activation in orthotopic implantation cohort. Blue DAPI, green p75NTR, red GFAP. Scale bars 200µm.

Astroglia activation was calculated as follows; Total GFAP area / total p75NTR area * 100.

Research has proven that astroglia plays a vital role in supporting neurons, regulating the extra-cellular environment, and aiding the immune response in the central nervous system. Changes in the number of astroglia can impact neuronal health, inflammatory responses, and developmental stage (Guo et al., 2007). In this project, spinal cord tissues in all groups were immunofluorescently stained with GFAP, p75NTR, and DAPI, and the immune response between different groups was compared using ImageJ-based colorimetry. Quantifications were performed using GraphPad Prism with an unpaired (two-tailed) t-test. Astroglia activation in the KC cohort was compared between the KC and KC;GFAP-Tk groups and shown in Figure 21 and demonstrated a dramatic decrease in astroglia activation in the KC;GFAP-Tk group (KC 13.44 ± 0.98 and KC;GFAP-Tk 0.7279 ± 0.2358 , $p < 0.0001$: Figure 21). The KPC cohort showed a decrease in astroglia activation between the ganciclovir and PBS-treated groups (KPC;GAP-Tk + ganciclovir 0.6910 ± 0.7150 and KPC;GFAP-Tk+ PBS 7.042 ± 1.685 : $p < 0.0001$: Figure 22). In the orthotopic implantation cohort, a decrease was observed between the ganciclovir and PBS-treated groups, (ganciclovir-treated 0.5351 ± 0.1444 and PBS-treated 4.026 ± 0.3974 , $p < 0.0001$), as well as between the ganciclovir and FOLFIRINOX-treated groups, (FOLFIRINOX- treated 3.681 ± 0.5329 : $p < 0.0001$: Figure 23).

Determination of microglia activation after glial cell depletion

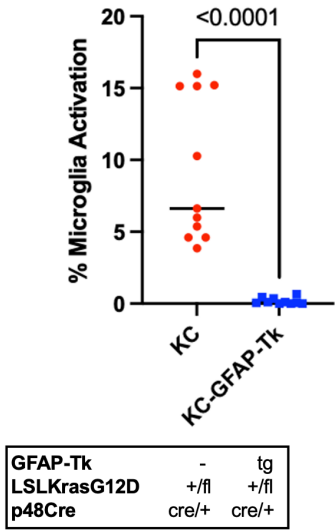
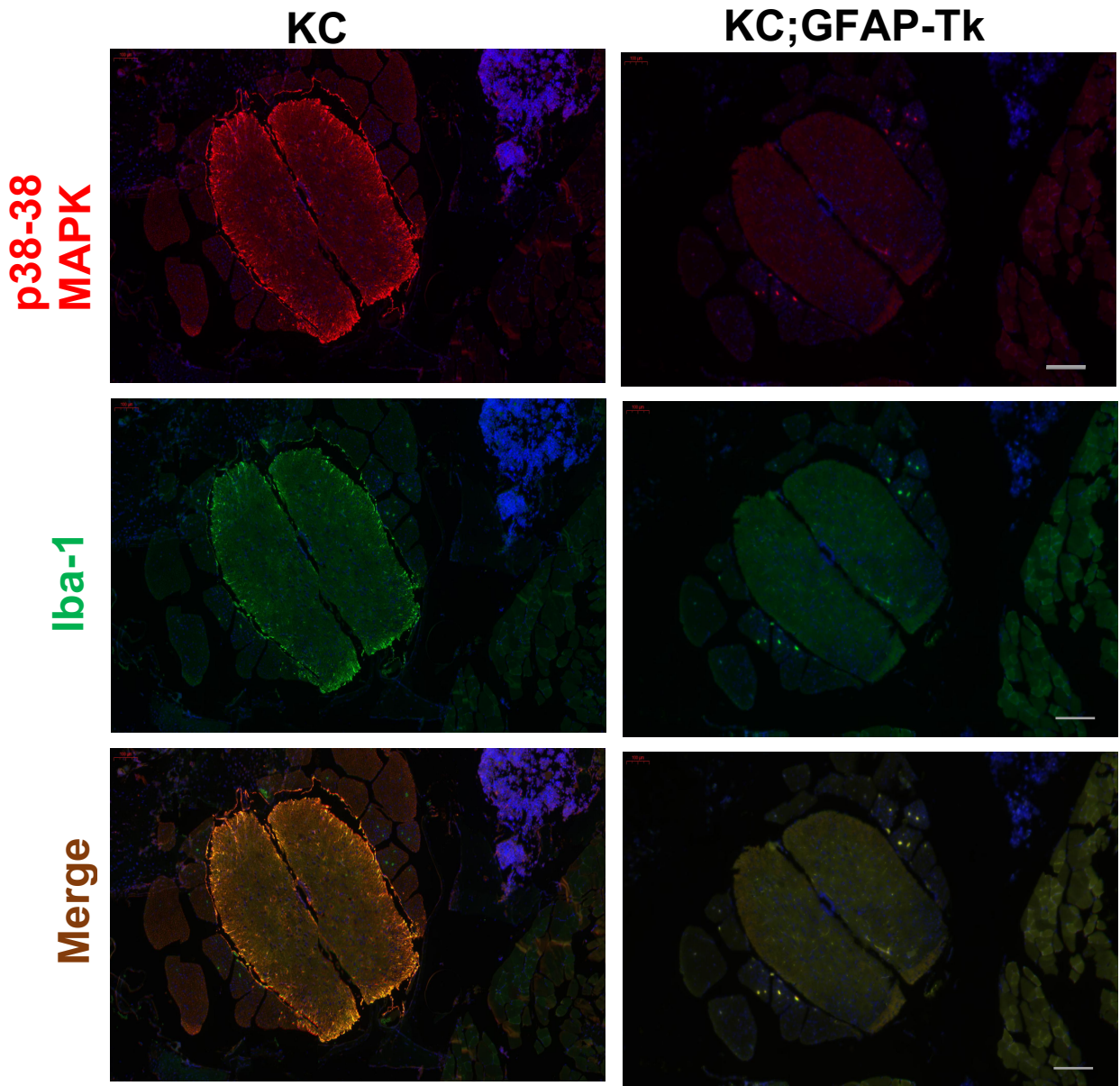
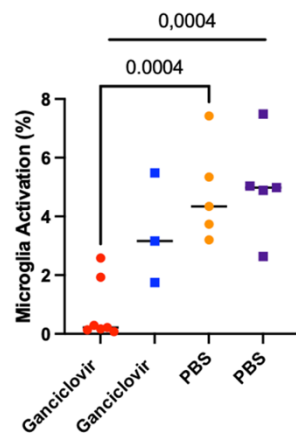
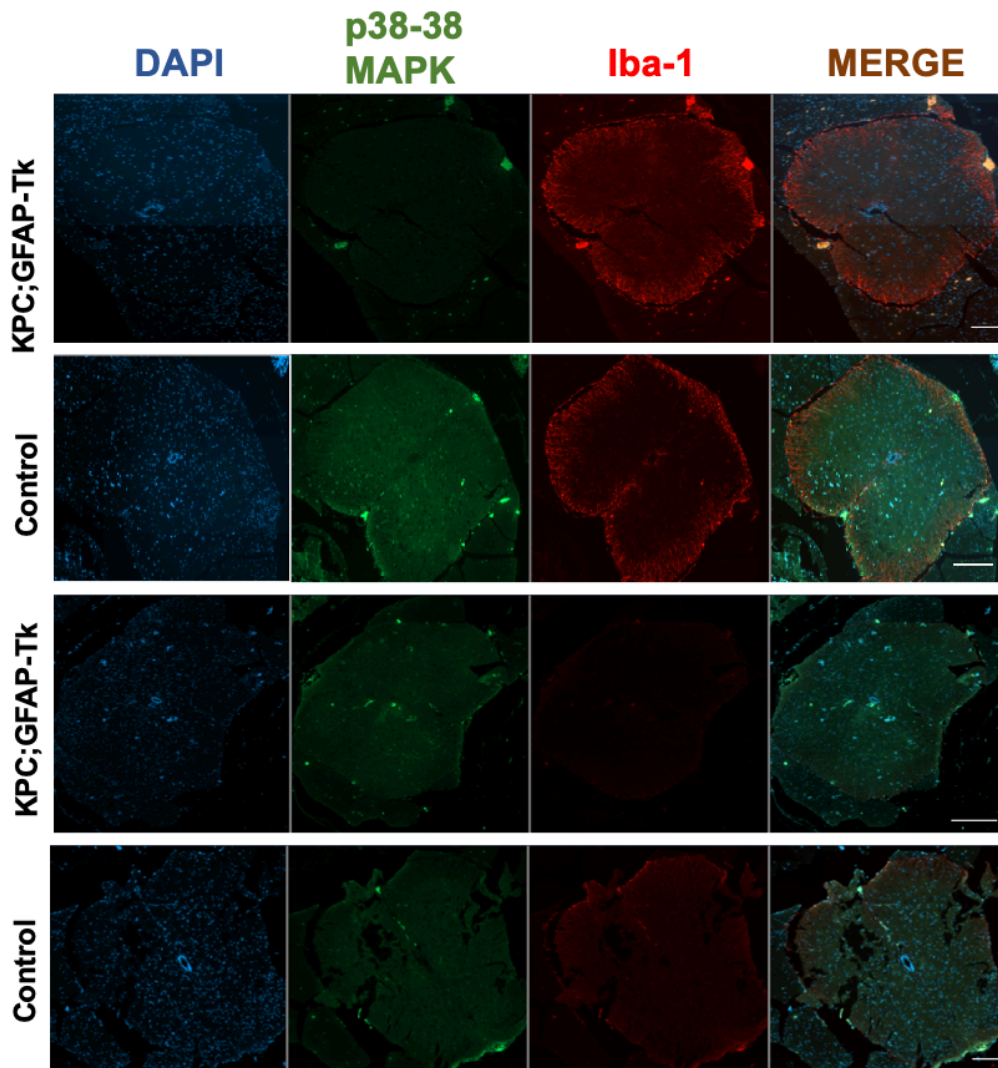


Figure 24. The impact of glial cell depletion on microglia activation in the KC cohort. Scale bars indicate 100µm.

Microglia activation was calculated as follows;
Total p-p38 MAPK area / total Iba-1 area * 100



GFAP-Tk	tg	tg	tg	tg
LSL-KrasG12D	+/-	+/+	+/-	+/+
p48Cre	cre/+	cre/+	cre/+	cre/+
Trp53lox/lox	+/-	fl/fl	+/-	fl/fl

Figure 25. The effect of ganciclovir-induced GFAP+ cell depletion on microglia activation in the KPC cohort. The left side of the figure shows the genotypes and the right side shows the treatments.

* KPC;GFAP-TK (*K-ras*^{LSLG12D/+}; *Trp53*^{fl/+}; *Ptfla-Cre*; *GFAP-Tk*) and the control group, KPC;GFAP-TK (*Trp53*^{fl/fl}; *Ptfla-Cre*; *GFAP-Tk*). Scale bars 100μm.

Implantation Cohort

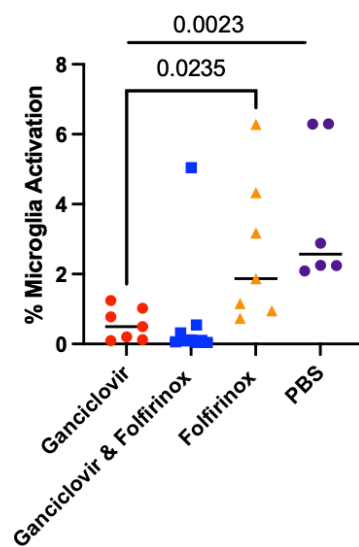
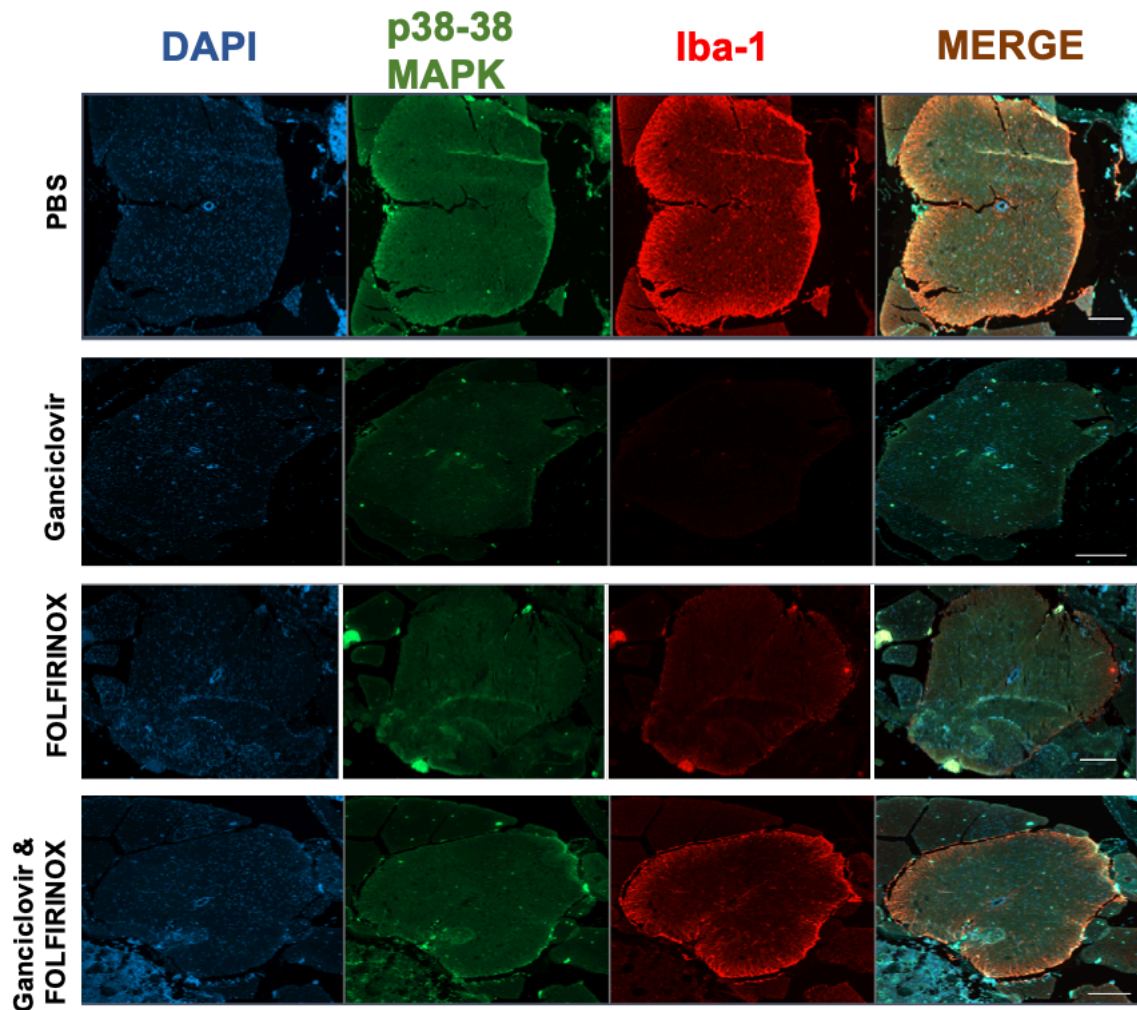


Figure 26. The effect of ganciclovir-induced GFAP+ cell depletion on microglia activation in the orthotopic implantation cohort.
Scale bars indicate 200 μm.

Microglia activation was calculated as follows;
Total p-p38 MAPK area / total Iba-1 area * 100

Microglia assume important functions in the central nervous system, such as immune surveillance, inflammation, synaptic pruning, tissue repair, and neuroprotection (R. R. Ji, Nackley, Huh, Terrando, & Maixner, 2018). Changes in the number of microglia can have a significant impact on these processes, and it can lead to neurological disorders (Feldman, 2022). In this project, spinal cord tissues from all groups were immunofluorescently stained with the microglia biomarker Iba-1 (Ionized calcium binding adaptor molecule 1), p38 MAPK (Mitogen-Activated Protein Kinase), and DAPI. Microglia activation in all groups was calculated as p38-38 MAPK total area/ Iba-1 total area *100. As represented in Figure 24, microglia activation in the KC cohort was compared between the KC (9.345 ± 1.526) and KC;GFAP-Tk (0.1828 ± 0.73 , $p < 0.0001$: Figure 24) groups, showing a decrease in microglia activation in the KC;GFAP-Tk group. KPC cohort showed a reduction in microglia activation between the ganciclovir and PBS-treated groups (KPC;GFAP-Tk +ganciclovir 0.7729 ± 1.033 and KPC;GFAP-Tk +PBS 4.810 ± 1.664 , $p = 0.0004$: Figure 25). In the orthotopic implantation cohort, a slight reduction was observed between groups treated with ganciclovir and PBS with a p value of 0.0023 (ganciclovir-treated 0.5657 ± 0.1738 and PBS-treated 3.677 ± 0.8354) and between groups treated with ganciclovir and FOLFIRINOX (FOLFIRINOX-treated 2.644 ± 0.8354 : $p = 0.0235$: Figure 26). The data from histopathological examinations of spinal cords from mice from ganciclovir- induced PDAC GEMM indicate that depletion of glial cells is likely correlated with a decreased expression of reactive glial cell markers. GFAP, p75NTR, and Iba-1 are markers of activated or reactive glial cells known to participate in neuroinflammation and neuropathic pain. GFAP is a marker of astrocytes, p75NTR, or p75 neurotrophin receptor, is a marker of Schwann cells. Iba-1, or ionized calcium-binding adaptor molecule 1, is a marker of microglia, a type of immune cell that functions as resident macrophages of the central nervous system. Overall, the present results suggest that the depletion of glial cells in the ganciclovir-treated group may lead to attenuated neuroinflammation and neuropathic pain in the spinal cord of GFAP-Tk mice. These results are consistent with previous studies suggesting

that glial cells are implicated in the induction of neuropathic pain and neuroinflammation in several chronic pain models.

RNA Sequencing Results

In this project, we also aimed to comprehend the molecular mechanisms and signaling pathways involved in the interactions of human Schwann cells (HSCs), pancreatic organoids, and cancer cell lines *in vitro*. Here, we used RNA Seq was used to identify differentially expressed genes in Schwann cells and pancreatic cancer cells (either as cell lines or as organoids) in mono- vs. co-culture. To conduct our study, we cultured human Schwann cells with different pancreatic organoids (KPC or WT) or pancreatic cancer cell lines (SU86.86 or T3M4) using transwell assays. After incubating for 6 hours, we collected whole RNA, measured their amount and purity, and sent to Novogene UK for sequencing.

The differential gene expression analysis revealed that 841 gene sets were up-regulated and 491 gene sets were down-regulated when Schwann cells were co-cultured with KPC organoids, when compared to Schwann cells co-cultured with WT organoids (Figure 27-A). It was discovered that there were 10143 common gene sets when Differentially Expressed Genes (DEGs) were compared between these two groups using a Venn diagram (Figure 27-C). Similar to this, 558 gene sets were up-regulated and 863 gene sets were down-regulated when HSCs were co-cultured with KPC organoids and compared to HSCs cultured alone (Figure 27-B). In the comparison of the SCs co-cultured with KPC organoids to HSCs co-cultured with WT organoids, we also performed a GO enrichment analysis (Figure 27-D). Angiogenesis, secondary active transmembrane transporter, glycosaminoglycan binding, and other processes associated with the development and progression of PDAC all revealed a significant enrichment.

One important process is angiogenesis, where new blood vessels are formed. The increase in gene sets related to angiogenesis implies that the presence of KPC organoids prompts the growth of new blood vessels due to the interaction of Schwann cells and HSCs. This aligns with the known role of angiogenesis in the development and spread of tumors. The activity of transporting molecules across cell membranes and binding with glycosaminoglycans (GAGs) was enriched. These findings indicate potential changes in cellular processes and the extracellular matrix, as GAGs play a role in various interactions.

GO enrichment analysis also emphasized cell-cell junctions, extracellular matrix organization, blood vessel formation, chemotaxis, and leukocyte migration. These mechanisms are recognized to be involved in cancer progression, implying that the interactions of organoids and Schwann cells affect the pathways associated with the development of PDAC.

Differential gene analysis revealed that 1801 gene sets were up-regulated and 3630 gene sets were down-regulated when SU86.86 cancer cells were co-cultured with Schwann cells and compared to SU86.86 monoculture cells (Figure 28-A). In addition, 10942 common gene sets were discovered using a Venn diagram to compare distinctive and frequent Differentially Expressed Genes (DEGs) between these two groups (Figure 28-B).

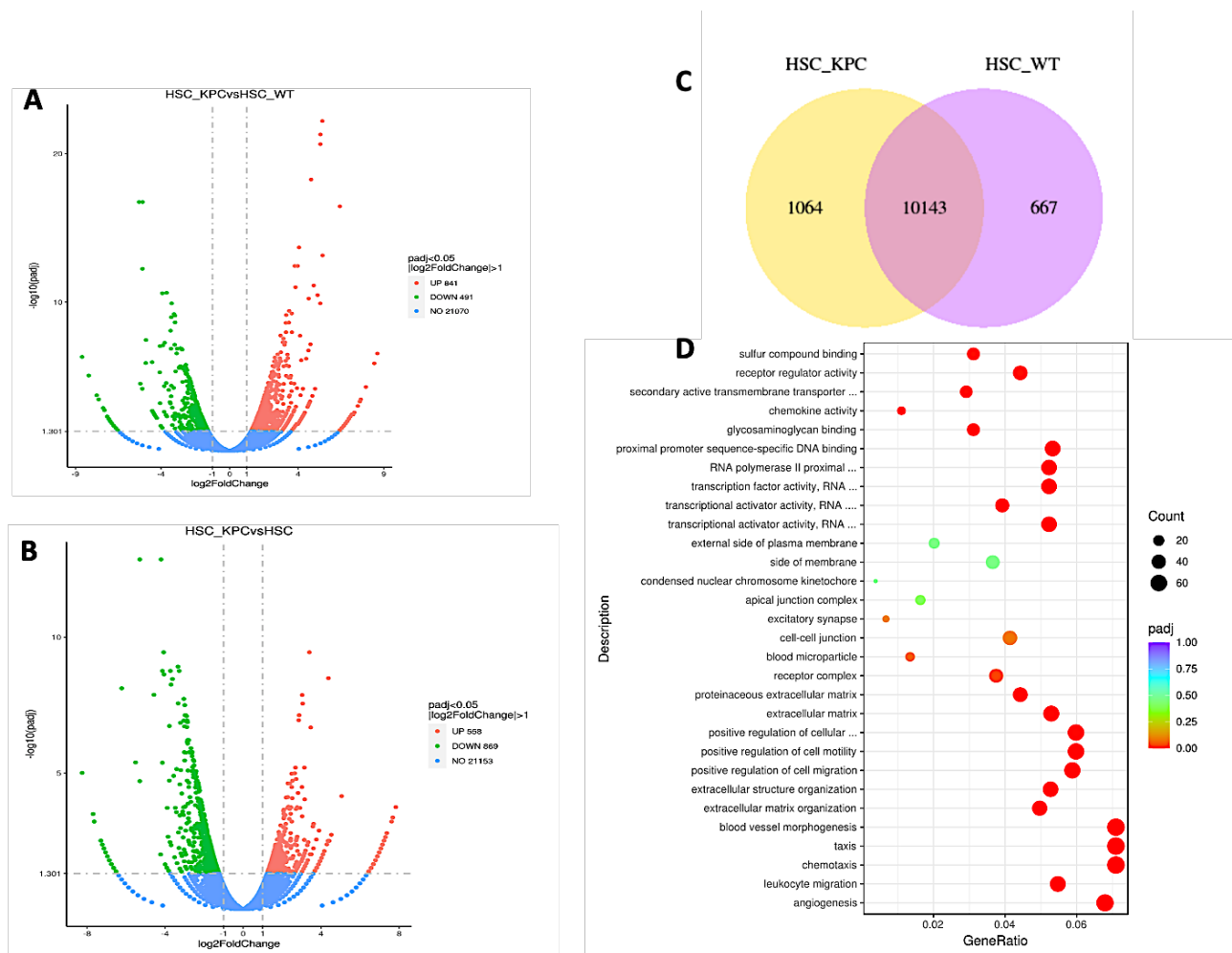


Figure 27. Differential gene expression analysis and GO (Gene Ontology) enrichment analysis of human Schwann cells in mono and co-cultures. (A) Differential Gene Volcano Map of human Schwann cells co-cultured with KPC organoids (HSC– KPC) versus human Schwann cells co-cultured with WT organoids (HSC-WT). (B) Differential Gene Volcano Map of human Schwann cells co-cultured with KPC organoids (HSC– KPC) versus human Schwann cells monoculture. (C) The co-expression Venn diagram of human Schwann cells co-cultured with KPC organoids (HSC– KPC) versus human Schwann cells co-cultured with WT organoids (HSC-WT). (D) GO Enrichment Analysis Scatter Plot of human Schwann cells co-cultured with KPC organoids (HSC– KPC) versus human Schwann cells co-cultured with WT organoids (HSC-WT). The size of a point represents the number of genes annotated to a specific GO Term, and the color from red to purple represents the level of the enrichment.

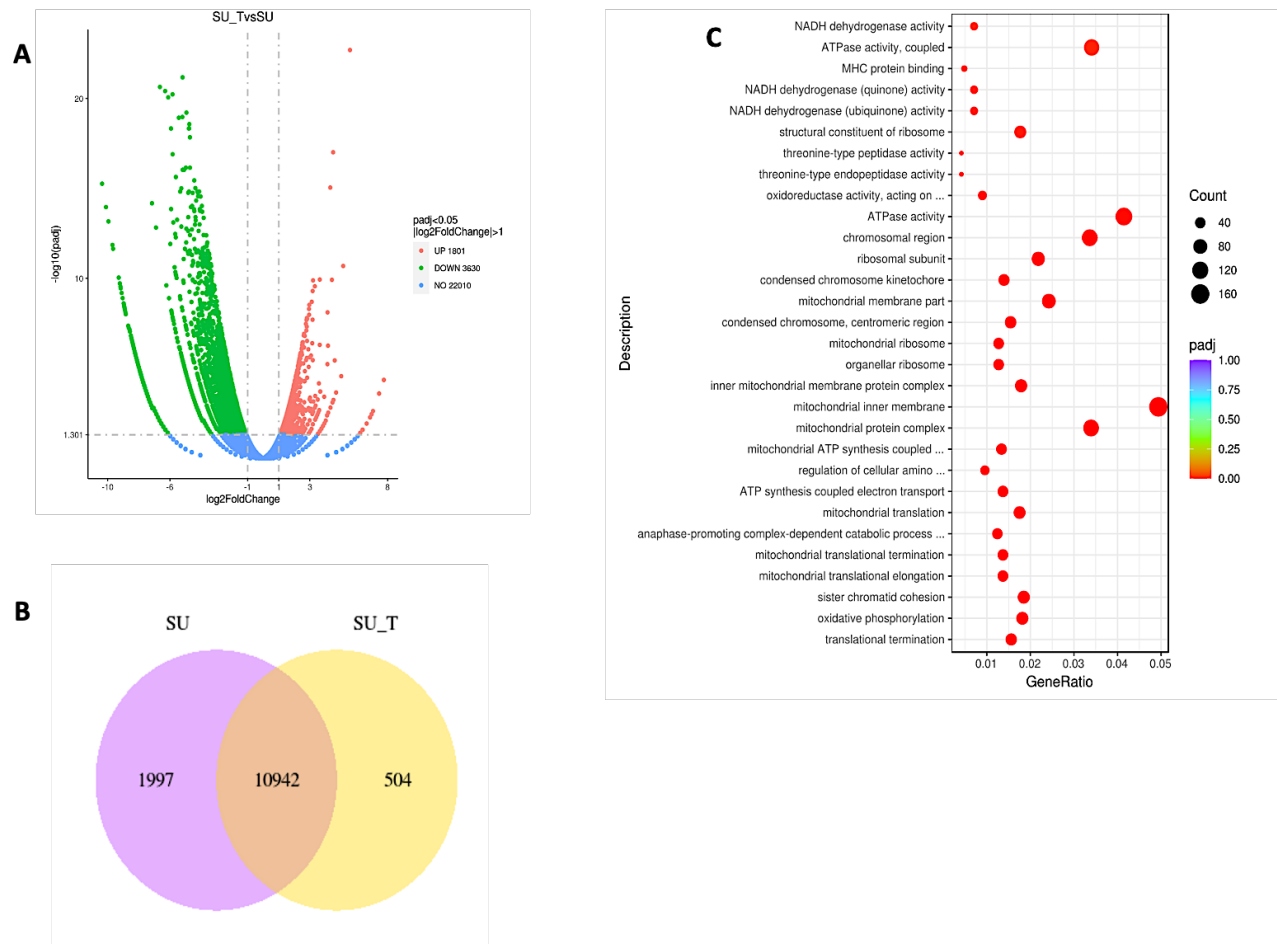


Figure 28. Differential gene expression analysis and GO (Gene Ontology) enrichment analysis of SU86.86 cells with or without human Schwann cell co-culture. (A) Differential Gene Volcano Map of SU86.86 cancer cells co-cultured with human Schwann cells versus SU86.86 monoculture. **(B)** The co-expression Venn diagram of SU86.86 cancer cells co-cultured with human Schwann cells versus SU86.86 monoculture. **(C)** GO Enrichment Analysis Scatter Plot of SU86.86 cancer cells co-cultured with human Schwann cells versus SU86.86 monoculture. The point size represents the number of genes annotated to a specific GO Term, and the color from red to purple represents the significant level of enrichment.

In the GO enrichment analysis (Figure 28-C), when SU86.86 cancer cells were co-grown with Schwann cells and compared to SU86.86 monoculture cells, we found ATPase activity, coupled, MHC protein binding, NADH dehydrogenase (quinone) activity, NADH dehydrogenase (ubiquinone) activity, structural constituent of ribosome declined. Significant gene enrichment has been linked to several pathways that contribute to the propagation of cancer. Common roles of these molecules in cancer progression are their participation in cellular energy consumption, protein synthesis, and chromosomal organization. Cancer progression can be accelerated by the dysregulation of several processes, which can affect cellular metabolism. Differential gene analysis showed that 118 gene sets were significantly up-regulated and 22 gene sets were down-regulated when T3M4 cancer cells were co-cultured with Schwann cells and in comparison, with T3M4 monoculture cells (Figure 29-A). Furthermore, 10842 common gene sets were identified using a Venn diagram to compare the distinctive and frequent Differentially Expressed Genes (DEGs) between these two groups (Figure 29-B). In the GO enrichment analysis (Figure 29-C), when T3M4 cancer cells were co-cultured with Schwann cells and compared to T3M4 monoculture cells, the following pathways were differentially regulated: platelet-derived growth factor binding, chemokine activity, integrin binding, cytokine receptor binding, collagen binding, extracellular matrix structural component, regulation of angiogenesis, collagen metabolic process, extracellular structure organization, and ECM organization. Significant gene enrichment has been associated with several pathways that contribute to the spread of cancer.

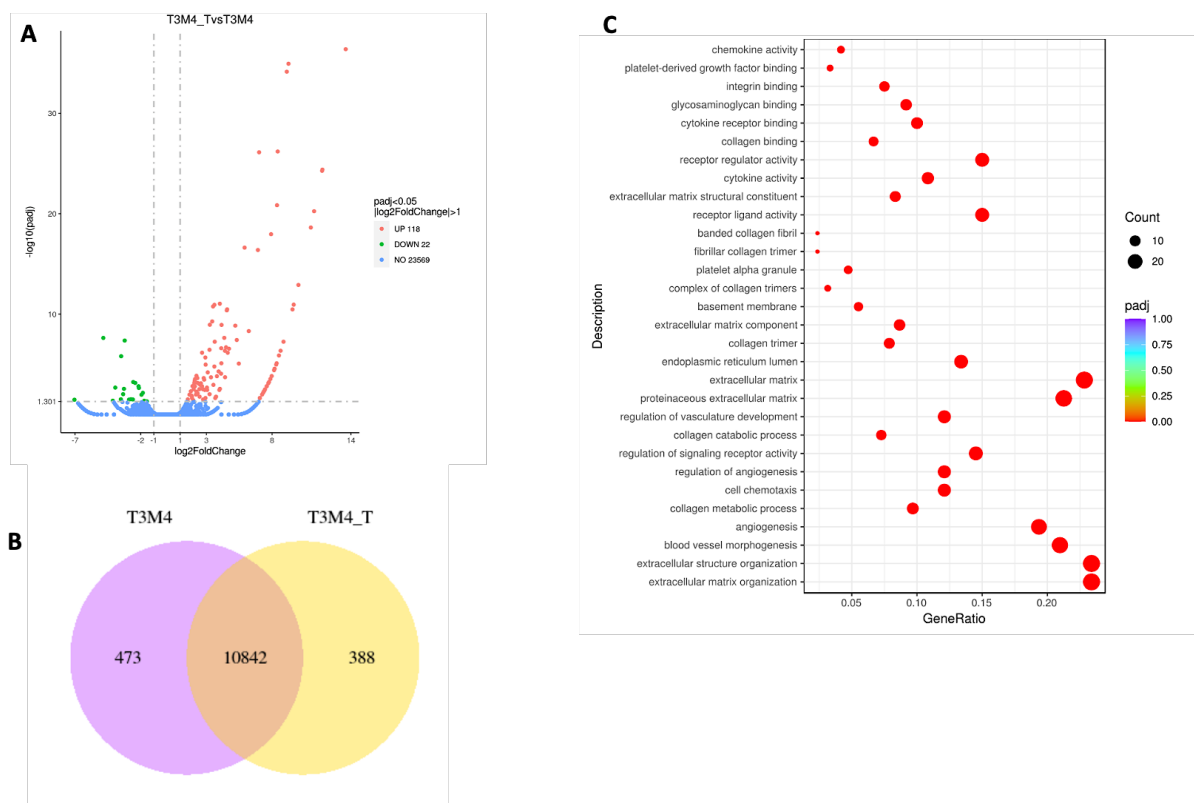


Figure 29. Differential gene expression analysis and GO (Gene Ontology) enrichment analysis of T3M4 cells with or without human Schwann cell co-culture. (A) Differential Gene Volcano Map of T3M4 cancer cells co-cultured with human Schwann cells versus T3M4 monoculture. **(B)** The co-expression Venn diagram of T3M4 cancer cells co-cultured with human Schwann cells versus T3M4 monoculture. **(C)** GO Enrichment Analysis Scatter Plot of T3M4 cancer cells co-cultured with human Schwann cells versus T3M4 monoculture. The point size represents the number of genes annotated to a specific GO Term, and the color from red to purple represents the significant level of the enrichment.

Proteome Profiler Human Phospho-Kinase Array Results

Studying the phosphorylation levels of kinases and protein substrates is essential to understand how cells respond to environmental changes. In this context, this study aimed to co-culture Schwann cells with cancer cells (SU86.86 and T3M4) or organoids (KPC, WT) and compare them with mono-cultured Schwann cells to determine the differences in phosphorylated kinases and protein substrates. The Proteome Profiler™ Array Human Phospho-Kinase Array Kit was used as described in the Methods section.

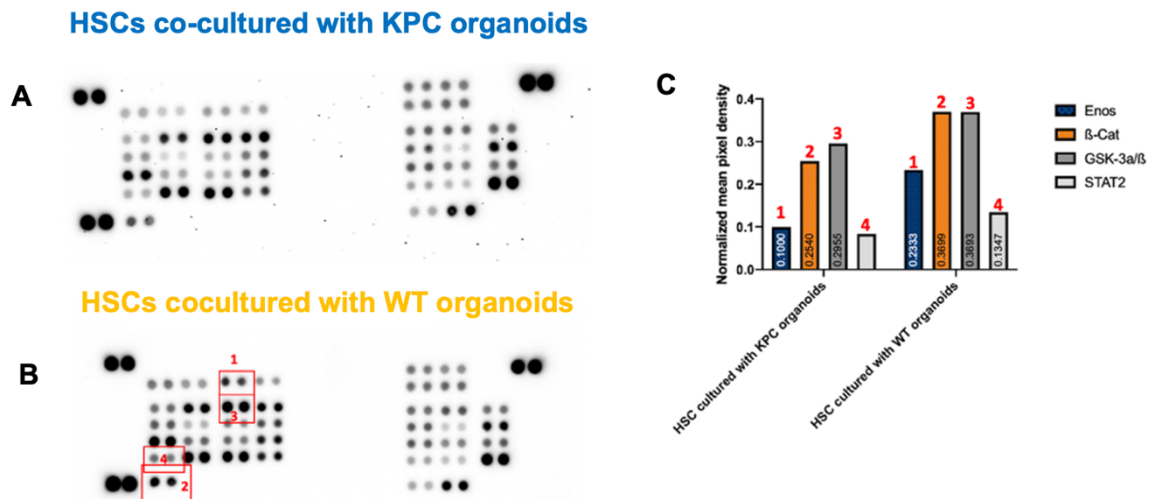


Figure 30. Differentially phosphorylated kinases and protein substrates between human Schwann cells (HSCs) co-cultured with KPC organoids or WT organoids. (A) Membrane image of phosphorylated kinases human Schwann cells co-cultured with KPC organoids. **(B)** Membrane image of phosphorylated kinases and protein substrates of human Schwann cells (HSCs) co-cultured with Wild Type (WT) organoids. **(C)** Quantification of differentially phosphorylated kinases and protein substrates. Numbers and red squares were used to point the locations of differentially phosphorylated molecules on membranes.

Using the Proteome Profiler Human Phospho-Kinase array, we discovered that different kinases and protein substrates in human Schwann cells had varying phosphorylation levels when co-cultured with KPC organoids compared to wild-type organoids (Figure 30). Specifically, Enos had a lower phosphorylation level in Schwann cells treated with KPC organoids (mean 0.1) than those co-cultured with wild-type organoids (mean 0.2333). Beta-catenin had higher phosphorylation in Schwann cells co-cultured with wild-type organoids (mean 0.3699) than in those treated with KPC organoids (mean 0.2540). GSK3α/β also showed increased phosphorylation in Schwann cells co-cultured with wild-type organoids (mean 0.3693) compared to those treated with KPC organoids (mean 0.2955). Furthermore, STAT2 phosphorylation was higher in Schwann cells co-cultured with wild-type organoids (mean 0.1347) than in those cultured with KPC organoids (mean 0.084). These different phosphorylation patterns indicate that the presence of KPC organoids or wild-type organoids may affect the activation or regulation of these kinases and their protein substrates in Schwann cells. This could potentially interfere with the co-culture system's signaling pathways and cellular processes. The findings demonstrated that Enos phosphorylation was decreased in

Schwann cells treated with KPC organoids, which raises the possibility that nitric oxide generation may be compromised. However, beta-catenin showed elevated levels of phosphorylation in wild-type organoid co-cultures, demonstrating a functional Wnt signaling pathway. In addition, GSK3a/β displayed increased phosphorylation in co-cultures of wild-type organoids, indicating a modification of its activity and downstream signaling. Additionally, wild-type organoid co-cultures had higher STAT2 phosphorylation levels than control co-cultures, which may be a marker of enhanced STAT2 signaling and possible effects on immunological responses and cell proliferation. These results underline the divergent cellular processes between the two co-culture environments and show the dynamic nature of kinase signaling, emphasizing the necessity for more investigation and functional studies.

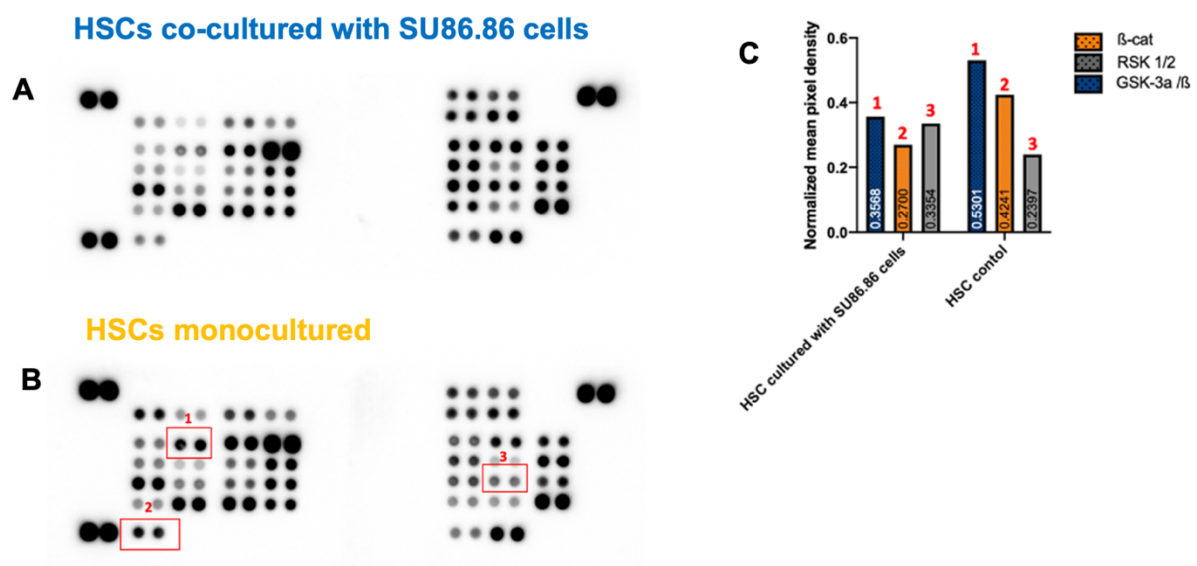


Figure 31. Differentially phosphorylated kinases and protein substrates between human Schwann (HSC) cells co-cultured with SU86.86 organoids or monoculture. (A) Membrane image of phosphorylated kinases human Schwann cells co-cultured with SU86.86 cells. **(B)** Membrane image of phosphorylated kinases and protein substrates of human Schwann cells (HSCs). **(C)** Quantifying differentially phosphorylated kinases and protein substrates between HSCs co-cultured with SU86.86 and HSCs monoculture. Numbers and red squares used to point the locations of differentially phosphorylated molecules on membranes.

Human Schwann cells co-cultured with the human SU86.86 cancer cells were also compared to mono-cultured Schwann cells using the Proteome Profiler Human Phospho-Kinase array (Figure 31), highlighting significant differences in the phosphorylation levels of key kinases and protein substrates. The mean value of GSK-3 α/β was found to be lower in co-cultured Schwann cells (0.3568) compared to monoculture Schwann cells (0.5301), pointing to putative moderation of GSK-3 α/β activity in the presence of cancer cells. This suggests that the existence of cancer cells can influence GSK-3 α/β activity and may potentially interfere with downstream signaling events. Likewise, beta-catenin exhibited diminished phosphorylation in Schwann cells exposed to SU86.86 cancer cells (0.27) versus monoculture Schwann cells (0.4241), implicating the alteration of Wnt signaling. This implies that interaction with cancer cells may modulate Wnt signaling, which plays a pivotal role in cell proliferation and tumor progression (Morris et al., 2010). Furthermore, RSK 1/2 showed increased phosphorylation in Schwann cells treated with SU86.86 cancer cells (0.3354) over that in monoculture Schwann cells (0.2397), highlighting the potentially enhanced activation of downstream signaling pathways. High phosphorylation of RSK 1/2 suggests activating this signaling pathway upon interacting with cancer cells, which might promote cellular responses and tumor advancement.

Overall, these results suggest that the presence of KPC organoids influences the phosphorylation levels of Enos, beta-catenin, GSK3 β , and STAT2 in co-cultured Schwann cells. These modifications in kinase phosphorylation may have significant consequences on cellular processes, including nitric oxide production, Wnt signaling, cell adhesion, proliferation, and immune responses.

Interaction of Schwann cells and MDSCs via transwell migration assay

During the analysis of RNA sequencing, a significant change in chemokine-cytokine signaling was detected when human Schwann cells (HSCs) were cultured with T3M4 cancer cells. One of the most notable chemokines observed in this context was IL-8, which can attract myeloid cells (MDSCs). As a result, we conducted additional investigations into the chemokine-cytokine signaling in this context. A transwell migration assay was designed to understand the migratory capacity of MDSCs in response to human Schwann cells. The migration of MDSCs may shed light on the possible role of Schwann cells in modulating the immune response and neuroinflammatory processes. This experiment quantifies MDSCs migrating through transwell inserts, providing an assessment of their migratory potential (Figure 32). Isolation and sorting of MDSCs from spleen and plate design for transwell assay were performed as described in the methods section.

The results of the transwell migration assay provided valuable insights into the migratory behavior of MDSCs versus Schwann cells. Quantification of migrating cells allowed a comparative analysis between experimental groups. The transwell migration assay demonstrated the ability of MDSCs to migrate in the presence of Schwann cells, and the results indicate that this ability to migrate is associated with the activation and release of IL-8 cytokine by Schwann cells. In addition, the experiment resulted in the quantification of migrating MDSCs on transwell inserts. There was a considerable difference in the migration of MDSCs (Myeloid-derived suppressor cells) between the siRNA control group and the IL-8 silenced group (mean $\pm$ SEM, 25.39 ± 3.82 , $p < 0.0001$). On the other hand, cell migration was lower in the IL-8 silenced group compared to the untreated group (mean $\pm$ SEM, 39.94 ± 3.372).

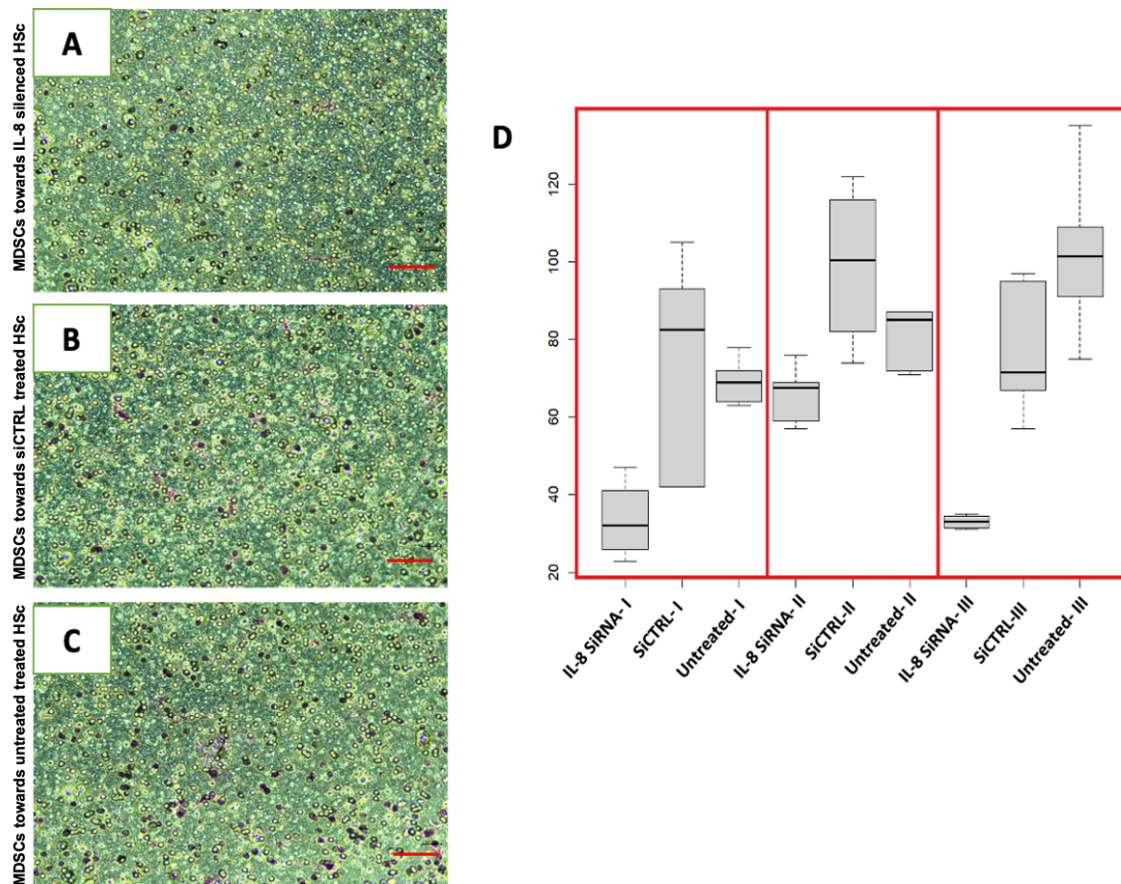


Figure 32. Interaction of Schwann Cells (HSCs) and MDSCs by Transwell Migration Assay. (A) MDSCs seeded on transwell insert to migrate towards IL-8 silenced human Schwann cells. (B) MDSCs seeded on transwell insert to migrate towards Silence control (siCTRL) human Schwann cells. (C) MDSCs seeded on transwell insert to migrate towards untreated human Schwann cells (D) Quantification of MDSC transwell migration to IL-8 silenced human Schwann cells (n=3, $p < 0.05$, one tailed-t test). Scale bars 200 μm .

Finally, MDSC migration was compared between the siRNA control group and the untreated control group, and it was found that MDSC migration was higher in the untreated group (14.56 ± 3.303 , $p=0.0028$). These results contribute to a better understanding of Schwann cell-MDSC interactions and their possible effects on the peripheral nervous system.

Silencing IL-8 in vitro results in distinct expression patterns of cytokines

Through co-culturing, this work aimed to investigate how silenced IL-8 expression in Schwann cells affects their interaction with PDAC cancer cells. We aimed to study how IL-8 affects cytokine signaling in pancreatic cancer cells. To achieve this, we screened multiple phosphorylated proteins using a cytokine array.

Quantification of dot plot pixel intensity revealed up-regulation of cytokines present in SU86.86 cells (Figure 33). In the IL-8 siRNA treated HSCs group, up-regulation of CXCL5, IGFBP-2, MIP-3 alpha, and FGF-9 were noted in comparison to the negative control group. Conversely, IL-8, IL-1 beta, BDNF, OPG, OPN, TIMP-1, and Angiogenin were down-regulated. These findings suggest that IL-8 plays an important role in modulating the expression of these cytokines in the context of SU86.86 cancer cells-Schwann cells crosstalk. The results of the SU86.86, cultured with IL-8 silenced HSC CM, showed differences in the expression of various cytokines compared to the negative control group. Up-regulation of CXCL5, IGFBP-2, MIP-3 alpha, and FGF-9 indicates that silencing of IL-8 in human Schwann cells and culturing with SU86.86 cancer cells affects the expression of these cytokines. On the other hand, down-regulation of IL-8, IL-1 beta, BDNF, OPG, OPN, TIMP-1, and angiogenin suggests that these molecules are also affected by the absence of IL-8. Based on the findings, the interaction between SU86.86 and Schwann cells initiates various biological responses such as inflammation, cell survival, proliferation, migration, and angiogenesis. These responses are believed to be triggered by the IL-8 signaling pathways. In particular, CXCL5, IGFBP-2, MIP-3 alpha, and FGF-9 are acknowledged to be up-regulated and activate signaling pathways such as MAPK/ERK and PI3K/Akt (L. Wang, Luo, Fu, & He, 2006). Furthermore, these signaling pathways are linked to cell survival, proliferation, and migration, indicating that they promote tumor progression. However, down-regulated molecules, including IL-8, IL-1 beta, BDNF, OPG, OPN, TIMP-1, and angiogenin, are linked to various signaling pathways affected by IL-8.

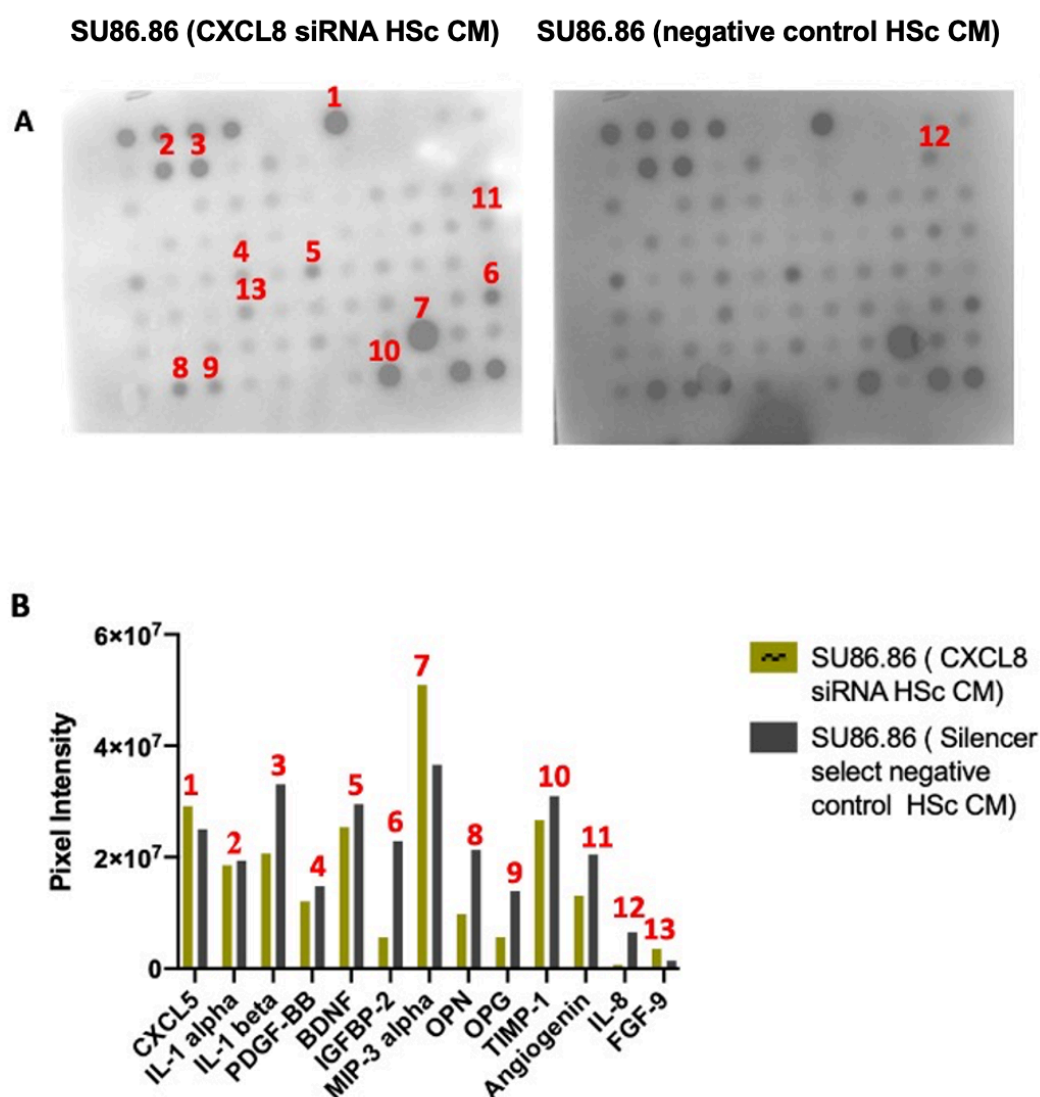


Figure 33. Differentially phosphorylated cytokines between SU86.86 co-cultured with CXCL8 silenced human Schwann cell conditioned media and SU86.86 silencer select negative control human Schwann cell conditioned media. (A) Membrane images of SU86.86 co-cultured with CXCL8 silenced human Schwann cell conditioned media (CM) and SU86.86 silencer select negative control human Schwann cell conditioned media. **(B)** Quantification of differentially phosphorylated cytokines. Numbers point to the locations of corresponding cytokines on the membranes (n=1).

Moreover, the down-regulation of angiogenin also suggests that IL-8 may be involved in the promoter of angiogenesis, forming new blood vessels. Quantification of dot plot pixel density revealed up-regulation of cytokines present in T3M4 cells (Figure 34) in the IL-8 siRNA silenced HSC treated group, up-regulation of IL-1 beta, OSM, BDNF, TIMP-1, and angiogenin was noted in the IL-8 silenced group compared to the negative control group. In contrast, CXCL5, IL-8, IL-1 alpha, IGF-1, FGF-9, IGFBP-2 were down-regulated. Based on the findings, it seems that IL-8

significantly impacts how these cytokines are expressed during the interaction between T3M4 cancer cells and Schwann cells. IL-1 beta is a cytokine that promotes inflammation and is implicated in immunological response (Weber, Wasiliew, & Kracht, 2010). Silencing of IL-8 may have sparked a pro-inflammatory response, as seen by the up-regulation of IL-1 beta in the silenced group. A cytokine called OSM (Oncostatin M) involves in several biological processes, such as inflammation, cell division, and proliferation (Masjedi et al., 2021). OSM's upregulation indicates a potential role in the cellular response to IL-8 suppression. Brain-derived neurotrophic factor, or BDNF, is a neurotrophic factor that supports both the development and survival of neurons (Allen, Watson, Shoemark, Barua, & Patel, 2013). Its increased expression in IL-8 silenced group raises the possibility that it participates in neural signaling or neuroprotective mechanisms. TIMP-1 (Tissue Inhibitor of Metalloproteinase 1) is an enzyme that prevents matrix metalloproteinases (MMPs), which modify the extracellular matrix, from operating (Schoeps, Frädrieh, & Krüger, 2023). Because of the silencing of IL-8, there may be a possible modification of MMP activity. Angiogenin is a protein that plays a part in angiogenesis (Detmar, 2000). The up-regulation of IL-8 in the silenced group points to a putative role in supporting angiogenesis in this respect. This evidence supports the hypothesis that IL-8 may inhibit the expression or secretion of angiogenin. However, the down-regulation of CXCL5, IL-8, IL-1 alpha, IGF-1, FGF-9, and IGFBP-2 in the IL-8 silencing group implies that IL-8 silencing lowers the expression or release of these cytokines. Chemokine CXCL5 plays a role in the development of tumors and inflammation. Its down-regulation suggests that, in response to IL-8 silencing, the inflammatory response or the tumor microenvironment may be modulated. In addition, the pro-inflammatory chemokine IL-8 is implicated in inflammation and the recruitment of immune cells (W. Zhang et al., 2020). The concept that IL-8 plays a function in modifying the inflammatory response is further supported by the down-regulation of IL-8 in the silenced group.

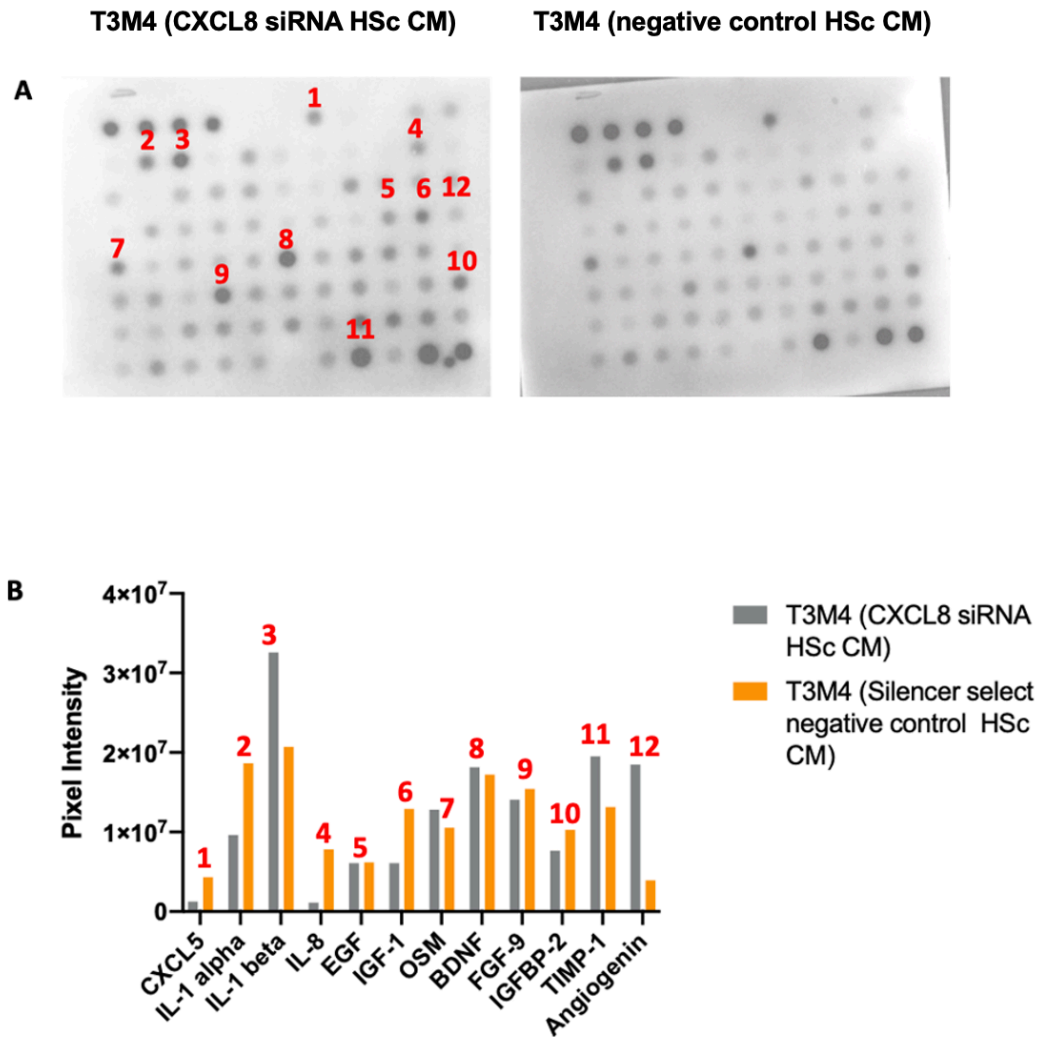


Figure 34. The differences in phosphorylated cytokines between T3M4 cells co-cultured with CXCL8-silenced conditioned media from human Schwann cells and negative control conditioned media from human Schwann cells. (A) The images show the difference in cell membranes between T3M4 cells co-cultured with CXCL8-silenced human Schwann cell conditioned media (CM), and T3M4 cells silencer select negative control human Schwann cell conditioned media. **(B)** The data present the quantification of the levels of cytokines that are differentially phosphorylated. The numbered locations on the membrane correspond to specific cytokines (n=1).

Another pro-inflammatory cytokine involved in immunological reactions and inflammation is IL-1 alpha. It's down-regulation reinforces the possibility that IL-8 and IL-1 beta collaborate to control the inflammatory cascade (Xu, Mu, & Wei, 2019). IGF-1, also known as insulin-like growth factor 1, is a growth factor that affects cell survival and proliferation. Its downregulation might indicate a possible connection between the IGF-1 and IL-8 signaling pathways (Kasprzak, 2021).

Ultimately, these findings show how intricate and intertwined cytokine signaling pathways are, and they imply that silencing IL-8 in HSC cells may substantially affect the production and secretion of different cytokines involved in angiogenesis, cell survival, inflammation, and growth factor signaling.

Discussion

This project aimed to investigate the effect of glial cell depletion on the progression of pancreatic cancer in PDAC mouse models. Here, for the first time, we performed a functional knockout of Schwann cells *in vivo*, thus demonstrating each stage of tumor development in two different models (KC and KPC PDAC GEMMs). We used a glial cell-depleted model to observe the effect of glial cell depletion on pain perception in PDAC GEMM, in which glial cells were selectively depleted in the mice. This project demonstrated that depletion of intrapancreatic glial cells leads to a reduction in PanIN progression, angiogenesis, neural density, immune cell infiltration, as well as astroglia and microglia activation, resulting in diminished pain response during the development of pancreatic cancer.

In central nervous system, glial cells have essential functions, including modulating and regulating pain processing (Araque & Navarrete, 2010). Thus, it is intriguing that the pain response was diminished in the glial cell-depleted mice compared to the control group. These findings support earlier research that showed glial cells were involved in controlling pain. Astrocytes and microglia can get activated in response to tissue damage or inflammation and release various chemicals, including cytokines and chemokines, which can sensitize pain receptors (K. R. Jessen & Mirsky, 2016). Glial cell activation or neuroinflammation are terms used to describe this process. As a result, it has been noted that inhibiting or eradicating glial cells diminishes pain perception in multiple pain models (R.-R. Ji, Berta, & Nedergaard, 2013). Our findings may suggest that pancreatic cancer pain in PDAC GEMMs could be reduced by glial cell depletion or targeting glial cell activation. In the context of our study, the observed decrease in pain response in mice lacking glial cells raises the possibility that glial cells play a role in the emergence or maintenance of pain in PDAC GEMMs. The neuroinflammatory response was likely impaired by glial cell depletion, which resulted in a reduction in pain sensitivity.

In the second part of the study, we analyzed GFAP expression and the lesion density that may develop accordingly. Based on the results of our study, glial cell depletion caused by ganciclovir in PDAC GEMMs was linked to a significant reduction in lesion density compared to the control group. This finding strongly suggests that glial cells are involved in promoting pancreatic tumorigenesis. The decrease in lesion density seen in mice lacking glial cells suggests a correlation between the abundance or presence of these cells and a higher number of neoplastic lesions. Importantly, this finding supports the idea that glial cells might help the PDAC create or maintain a tumor microenvironment conducive to lesion development.

These findings are consistent with earlier research linking glial cells to the development of tumors and their ability to influence the microenvironment around them. It has been established that glial cells interact with cancer cells and affect various aspects of tumor growth, invasion, and metastasis (S. Chen & Chen, 2022). They can secrete substances that can promote tumor cells' growth, migration, and survival, including cytokines, chemokines, growth factors, and extracellular matrix elements (Gregory et al., 2023). The association between glial cells and tumor development in pancreatic cancer is intriguing. Many stromal cells, including fibroblasts and immune cells, are present in the pancreatic cancer tumor microenvironment, which is highly desmoplastic (Târtea et al., 2017). Glial cells may support this desmoplastic response and foster the growth of tumor lesions in the pancreas.

Vascular density assessment can provide critical information about tumor angiogenesis and tumor progression. In addition, it is essential to understand the interplay between cancer cells and the surrounding vasculature. Therefore, in this project, we compared the tumor vasculature of glial cell-depleted mice and controlled mice to understand how glial cell depletion affects vascular density in PDAC GEMM. In addition, we wanted to evaluate the effect of glial cell depletion on other neural cells in the tumor microenvironment. In this part, the fact that the vascular density of tumors was significantly reduced in the glial cell-depleted cohort compared to the control group strongly indicates that glial cells may have a role in boosting tumor angiogenesis and forming new blood

vessels to supply oxygen and nutrients to the expanding tumor. Previous studies have suggested that glial cells, namely astrocytes, may enhance tumor angiogenesis mediated by the secretion of pro-angiogenic factors (Loeffler, Fayard, Weis, & Weissenberger, 2005).

One study showed that astrocytes promote angiogenesis in gliomas, a type of brain cancer, by expressing vascular endothelial growth factor (VEGF) through IL-6, leading to the growth of new blood vessels (Loeffler, Fayard, Weis, & Weissenberger, 2005). In another study, researchers have shown that astrocytes in the breast cancer microenvironment are likely to regulate angiogenesis by expressing interleukin-8 (IL-8), a chemokine that induces migration and proliferation of endothelial cells. They also identified tumor-associated macrophages (TAMs) as the primary source of CCL18, which attracts immune cells to the tumor microenvironment and promotes angiogenesis. The authors suggested that targeting the IL-8/CXCR2 pathway or TAMs could be a promising treatment strategy for breast cancer (Lin et al., 2015). These findings suggest that glial cells may promote tumor angiogenesis by secreting pro-angiogenic factors. However, the depletion of glial cells may reduce the release of these pro-angiogenic factors, resulting in reduced vascular density in neoplasms. This idea aligns with the finding that vascular density was significantly reduced in the treatment group with depleted glial cells compared to the control group in this set of experiments.

Regarding the cross-talk of glial cells and pancreatic cancer, the depletion of glial cells in the GFAP-Tk mouse model can lead to a decrease in S100 expression and a consequent decrease in the neural density of the tumor. This may be due to the role of glial cells in supporting neural cells in the tumor microenvironment and their interaction with cancer cells. The S100 protein family modulates various cellular pathways influencing pancreatic cancer progression and metastasis (Janigro et al., 2022). They have diverse roles inside and outside cells, in the cytoskeleton, in the modulation of protein phosphorylation and calcium levels. S100 proteins also interact with molecules such as RAGE, p53, and p21, which play an essential role in extracellular matrix (ECM) degradation and metastasis. They also promote pancreatic cancer progression and metastasis by

mediating interactions with cytoskeletal proteins and plasma membranes (Ji, Huang, Jiang, Ni, & Xiao, 2014).

In a recent study by Zhou et al. (2018), the function of S100A4 in promoting and progressing pancreatic cancer was investigated. The study revealed that S100A4 regulates the expression of matrix metalloproteinases (MMPs), enzymes that degrade the extracellular matrix and promote tumor invasion, thereby enhancing pancreatic cancer cell migration and invasion. Researchers also reported that S100A4 expression is significantly increased in pancreatic cancer tissues compared to normal pancreatic tissues and is associated with poor prognosis in pancreatic cancer patients (Zhou et al., 2018). Although there is, as yet, no convincing evidence that S100 β and S100A4 directly interact with each other, their shared regulatory functions and roles in driving cancer cell migration and invasion suggest that they may coexist in downstream pathways that promote pancreatic cancer progression and metastasis. Overall, the reduced neural density of the tumor in the glial cell depletion treatment group compared to the control group, as determined by S100 expression, suggests a potential function of glial cells in promoting tumor growth and invasion through their interactions with neural cells. However, the mechanisms behind this link and potential therapeutic implications need to be fully understood through additional research.

Studies show that the stroma plays an important role in tumor development, progression and spread and that targeting the stroma could lead to new therapeutic strategies for cancer treatment. The decrease of α -SMA in the glial cell-depleted cohort may indicate that glial cells may participate in the proliferation or activation of fibroblasts or myofibroblasts in the tumor stroma. In addition, the depletion of glial cells may impair the signaling pathways that guide fibroblast and myofibroblast mobilization. One hypothesis for the reduced expression of α -SMA in the glial cell-depleted line is the involvement of glial cells in the activation of fibroblasts and myofibroblasts. These glial cells might secrete growth factors, cytokines, or extracellular matrix proteins that promote the differentiation and proliferation of fibroblasts and myofibroblasts. Depletion of glial cells may be perturbing the signaling pathways that result in the forming of the stroma.

Another explanation for the reduced expression of α -SMA in the glial cell-depleted cohort is that glial cells may interfere with other cell types or signaling pathways implicated in stroma development. For example, glial cells may engage with immune or endothelial cells to favor stroma remodeling. Roger et al., 2019 demonstrated that high levels of TGF β signaling activation in human PDAC samples were positively associated with perineural invasion. Mass spectrometry analyses of proteins expressed alone or in combination of Schwann cells and pancreatic cancer cells highlighted the essential role of TGF β in neuro-epithelial interactions, where TGF β is also known to stimulate the production of extracellular matrix components by stromal cells and has a role in Epithelial Mesenchymal Transition (EMT). These results shed light on understanding the direct and indirect interactions of glial cells with stromal cells in the tumor microenvironment (Roger et al., 2019).

The applied GFAP-Tk mouse model is a useful modality in pancreatic cancer research as it enables selective targeted depletion of glial cells, essential constituents of the tumor microenvironment. In our experiments, we observed that depletion of glial cells resulted in a substantial reduction in the expression a number of immune cell markers, namely CD45, CD8 and F4/80, in the tumor microenvironment of pancreatic cancer. These findings suggest that glial cells may play essential roles in establishing and perpetuating the tumor microenvironment in pancreatic cancer. In addition, glial cells may molecularly modulate immune cell infiltration and function in the tumor microenvironment, which may impact the result of cancer therapy. One possibility for explaining the effectiveness of glial cell depletion in attenuating pancreatic cancer progression is that glial cells might have a role in inducing inflammation. Inflammation is well known to be an important factor in cancer initiation and prognosis (Storan et al., 2015), and glial cells likely contribute to this mechanism. Another explanation might be that glial cells may stimulate pancreatic cancer progression by secreting growth factors or other signaling molecules that propagate cancer cell proliferation and survival. It is conceivable that depleting glial cells may disrupt these signaling pathways and may slow the growth of pancreatic cancer cells. These results constitute an essential

baseline for conducting further studies on the role of glial cells in pancreatic cancer and the potentially therapeutic efficacies of targeting these cells.

The lower expression of immune markers CD45, CD8, and F4/80 in the treatment cohort indicates that glial cells may potentially have a role in regulating the immune response to pancreatic cancer. CD45 is a marker of leukocytes, including T cells, B cells, and natural killer cells, while CD8 is a marker of cytotoxic T cells, and F4/80 is a marker of macrophages. These immune cells are essential in recognizing and eliminating cancer cells, and their reduced presence in the tumor microenvironment may contribute to tumor progression and resistance to treatment (Hanahan & Weinberg, 2011).

Our results indicate that glial cells may participate in modeling the immune response in pancreatic cancer. Nevertheless, it is essential to emphasize that CD45 is not specific to a single type of immune cell. Therefore, the reduction observed in CD45 expression is likely driven by a decrease in a specific subset of immune cells instead of an overall decrease in immune cells in general. Further research will be required to determine this.

Peripheral cancers are well known to induce an immune response, including activating CD8⁺ T cells that recognize and eradicate cancer cells. Therefore, the reduction in CD8 expression in the treatment group may suggest that glial cells directly or indirectly mediate the modulation of the immune response to PDAC. One suggestion is that glial cells in the peripheral nervous system (PNS) may interfere with immune cells, e.g., CD8⁺ T cells, involved in the immune response against peripheral cancers. A deficit of glial cells in the PNS may disrupt this particular interaction, resulting in a lower expression of CD8.

One scenario is that glial cells in the central nervous system (CNS) may interact with immune cells in the PNS to modify their function. For example, depleting glial cells in the CNS could decrease the activation of CD8⁺ T cells in the PNS, thereby downregulating their expression. Understanding these findings also requires consideration of the possible limitations of the GFAP-Tk mouse model. Thus, this model's depletion of glial cells may not be specific to a particular glial

cell type, and other cell types may also be affected. In addition, using ganciclovir to induce glial cell depletion may cause off-target effects that may alter the immune response.

The decrease in CD8 expression in the treatment group compared to the control group suggests that glial cells may be an influential factor in the immune response to PDAC. However, further research is needed to clarify the mechanisms behind this effect and to determine its potential contribution to cancer progression.

In this set of experiments, it was found that the depletion of glial cells resulted in a decrease in the expression of the immune marker F4/80 in the tumor microenvironment of pancreatic cancer. F4/80 is a glycoprotein expressed by a niche cluster of macrophages, including tumor-associated macrophages (TAMs). TAMs have been demonstrated to exert a sophisticated role in cancer, with both pro- and anti-tumor effects based on their phenotype and state of activation (Dallavalasa et al., 2021). A probable interpretation of these findings is that glial cells may drive the recruitment and activation of TAMs in the tumor microenvironment.

One other possible elucidation is that glial cells modulate the expression of certain contributing factors that contribute to the activation and polarization of TAMs. In summary, our results present preliminary evidence that glial cells may mediate the immune response in pancreatic cancer, possibly by mediating interactions with immune cells. However, additional research is warranted to identify these results' specific mechanisms and putative clinical implications.

Additionally, we were interested in learning more about how glial cells affect pain perception and how glial cell depletion affects the expression of c-fos and P-Erk in pain processing. Here are some broad conclusions from the existing literature: Activation of glial cells, particularly astrocytes, and microglia, has been linked to the emergence and maintenance of pain sensitivity, according to some studies (J. Li et al., 2022). Additionally, increased c-fos and P-Erk expressions in the spinal cord are connected to the activation of glial cells and point to enhanced neuronal activity related to pain (Harris, 1998).

Chronic pain conditions have been linked to glial cell activation, through which ongoing pain signals cause sustained glial cell activation and subsequent changes in neuronal activity (R.-R. Ji et al., 2013). Furthermore, chronic pain states frequently involve elevated c-fos and P-Erk expressions in the spinal cord, pointing to a function for glial cells in sustaining chronic pain (J. Li et al., 2022). Our findings add to the growing body of research supporting the involvement of glial cells in pain sensitivity and further emphasize the potential impact of glial cell depletion on the expressions of c-fos and P-Erk during pain processing.

The observation of decreased astroglia and microglia activation in the spinal cord sections of the glial cell-depleted cohort compared to the control groups, in our study, has important implications for understanding glial cell involvement in pain processing. These results are consistent with prior research showing that glial cells, particularly astroglia and microglia, are involved in pain processing. Reduced astroglial and microglial activations have been shown to have analgesic effects and reduce pain sensitivity in studies using glial cell modulation methods or depletion models (J. Li et al., 2022). These findings imply that glial cells play a significant role in pain modulation and that glial cell depletion can reduce pain-related processes by affecting glial-neuronal interactions. Our findings contribute to research showing that glial cells are involved in pain perception. Furthermore, reduced activation of astroglia and microglia in mice with depleted glial cells suggests impaired glial-mediated inflammatory responses and modulation of neuronal activity, potentially leading to reduced pain sensitivity.

We also aimed to comprehend the molecular mechanisms and signaling pathways involved in interactions with human Schwann cells, pancreatic organoids, and cancer cell lines *in vitro*. For this purpose, we performed RNA-Sequencing. Co-culture of SU86.86 cancer cells and/or KPC organoids with Schwann cells demonstrated significant enrichment of gene sets involved in angiogenesis, glycosaminoglycan binding, cell-cell junctions, extracellular matrix organization, and immune-related processes. KPC organoids facilitated tumor growth and metastasis, potentially by

enhancing angiogenesis. These results contribute new insights into the molecular mechanisms behind PDAC. A detailed analysis of the RNA-Seq data emphasizes substantial alterations in gene expression and enrichment of pathways associated with cellular energy consumption, protein synthesis, chromosomal organization, and immune response. These results provide significant insights into the molecular mechanisms underlying the interactions between SU86.86 cancer cells and Schwann cells, thus potentially advancing our knowledge of cancer progression and therapeutic targets. On the other hand, GO enrichment analysis of T3M4 cancer cells co-cultured with Schwann cells exposed various gene enrichments, including chemokine activity, cytokine receptor binding, collagen binding, ECM structural component, receptor-ligand activity, regulation of angiogenesis, collagen metabolic process, extracellular structure organization, and ECM organization. These data are in concordance with the current literature on the role of these molecules in cancer progression, contributing to various aspects of cancer development. Understanding diverse molecular mechanisms is necessary for the targeted generation of cancer therapeutics and for the comprehension of the sophisticated relationship between cancer cells and their milieu.

Examining the degree of phosphorylation of kinases and their protein substrates is essential to comprehend how cells react to environmental changes entirely. To ascertain the differences in phosphorylated kinases and protein substrates between co-cultured Schwann cells and cancer cells (SU86.86 and T3M4) or organoids (KPC, WT), this study compared co-cultured Schwann cells to mono-cultured Schwann cells via the Proteome Profiler, Human Phospho-Kinase Array. Results indicated distinct phosphorylation levels of various kinases in Schwann cells co-cultured with KPC organoids compared to those co-cultured with wild-type organoids. The enhanced phosphorylation of Enos implies that this enzyme is being activated in the presence of KPC organoids. Enos produces nitric oxide, which has diverse roles, including neurotransmission, vasodilatation, and immune responses (Charles et al., 2010). The increased phosphorylation of Enos may imply an overproduction of nitric oxide in Schwann cells co-cultured with KPC organoids and may affect cellular communication and vascular dynamics.

Increased phosphorylation of beta-catenin (β -Cat) suggests activation of the Wnt signaling pathway in Schwann cells co-cultured with KPC organoids. Beta-catenin is an essential constituent of cell adhesion complexes and a transcriptional co-activator (Duchartre, Kim, & Kahn, 2016). Activation of the Wnt pathway and beta-catenin stabilization may impact cell adhesion, proliferation, and differentiation (Z. Wang, Li, & Ji, 2021). The enhancement of beta-catenin phosphorylation points to potential modifications in these cellular events in the existence of KPC organoids. Increased phosphorylation of GSK3 β (glycogen synthase kinase three beta) indicates its inactivation in Schwann cells co-cultured with KPC organoids. GSK3 β is found to be involved in numerous cellular processes, including glycogen metabolism, cell cycle regulation, and gene expression (Cheng & Merz, 2016). Inhibition of GSK3 β through phosphorylation may interfere with several downstream pathways, including the Wnt signaling pathway and cellular survival (Duchartre et al., 2016). Increased phosphorylation of GSK3 β signifies potential alterations in these cellular processes and may further contribute to alterations in Schwann cell behavior when co-cultured with KPC organoids.

Increased phosphorylation of STAT2 (signal transducer and activator of transcription 2) suggests its activation in Schwann cells co-cultured with KPC organoids. STAT2 is a transcription factor activated explicitly in response to cytokines and viral infections. Activation of STAT2 regulates immune responses and the interferon signaling pathway (Erdogan et al., 2022). The increased phosphorylation of STAT2 indicates possible modifications in immune response mechanisms in Schwann cells when co-cultured with KPC organoids. The observed alterations in Enos, beta-catenin, GSK3 β , and STAT2 phosphorylation levels in Schwann cells co-cultured with KPC organoids might influence pancreatic cancer development and progression. Even though the precise effects are context-dependent and may need additional research, there may be some potential implications presented here: Enos phosphorylation and enhanced nitric oxide (NO) synthesis correlate with tumor promotion and angiogenesis in many cancers. In pancreatic cancer, increased NO production is associated with tumor growth, invasiveness, and resistance to therapy (L. Wang

& Xie, 2010). The increased Enos phosphorylation in Schwann cells co-cultured with KPC organoids may suggest an altered NO signaling milieu, possibly influencing tumor-stromal interactions and cancer advancement. The activation of the Wnt/ β -catenin signaling pathway is commonly implicated in pancreatic cancer and is correlated with increased cell proliferation, invasion, and immunomodulation (Du et al., 2023). Increased phosphorylation of beta-catenin in Schwann cells co-cultured with KPC organoids indicates enhanced pathway stimulation. This may contribute to modified cellular behavior in the tumor microenvironment, possibly promoting pancreatic cancer progression.

By co-culturing MDSCs with IL-8 silenced Schwann cells, we aimed to reveal the role of IL-8 in cancer progression, which can be used to develop targeted therapies to disrupt IL-8 signaling and inhibit cancer cell migration and metastasis. The transwell migration assay demonstrated the ability of MDSCs to migrate in the presence of Schwann cells, and the results indicate that this ability to migrate is associated with the activation and release of IL-8 cytokine by Schwann cells. In addition, the experiment resulted in the quantification of migrating MDSCs on transwell inserts. These results contribute to a better understanding of Schwann cell-MDSC interactions and their possible effects on the peripheral nervous system. These findings are essential to explain how cancer cells and glial cells are related. On the other hand, a proof of concept is necessary, for example, by administering IL-8 to a glial-cell depleted model to monitor whether the tumor expands and the amount of MDSC in the tumor increases.

Ultimately, these findings show how intricate and intertwined cytokine signaling pathways are, and they imply that silencing IL-8 in human Schwann cells may substantially affect the production and secretion of different cytokines involved in angiogenesis, cell survival, inflammation, and growth factor signaling.

Finally, we wanted to examine the impact of Schwann cells' silenced IL-8 expression on their relationship with PDAC cancer cells to clarify the function of IL-8 in controlling cellular communication as well as how it affects the tumor microenvironment in PDAC. The main reasons why

both SU86.86 and T3M4 cancer cells phosphorylated different molecules despite the fact that both SU86.86 and T3M4 cancer cells were exposed to the same IL-8 silenced Schwann cell conditioned media for the same amount of time could be explained by the genetic and phenotypic differences between these two cell lines: SU86.86 cells carry mutations in the *KRAS* oncogene and *TP53* tumor suppressor gene, commonly found in pancreatic adenocarcinoma.

In addition, T3M4 cells also have *KRAS* and *TP53* mutations, indicating similarities in genetic profile with SU86.86 cells. However, additional mutations in *SMAD4*, *CDKN24*, and their frequencies may explain the difference. SU86.86 and T3M4 cells show different phenotypic features. SU86.86 cells have been reported to have invasive and migratory properties that favor their metastatic potency. T3M4 cells are known to manifest a mesenchymal phenotype. Therefore, they are used as a model to examine epithelial-mesenchymal transition (EMT) in pancreatic cancer. In addition, differences in the molecular signaling pathways followed by these two lines may lead to different phosphorylated cytokines. For example, the PI3K/Akt and MAPK/ERK pathways are commonly dysregulated in pancreatic cancer.

Research on pancreatic cancer has significantly benefited from orthotopic implantation models and genetically engineered mouse models like KC and KPC. These models do, however, have some limitations. The KC and KPC models' ability to accurately represent the disease's heterogeneity is constrained by their focus on particular genetic mutations frequently observed in pancreatic cancer (Westphalen & Olive, 2012). However, these models may not accurately reflect the genetic diversity in patient tumors. Furthermore, these models might not accurately reflect the complex tumor microenvironment present in human pancreatic cancer, which may impact the tumor's behavior and its response to treatment. There may be differences between the timing and manner of tumor initiation in these models and how pancreatic cancer develops naturally in humans, the modest metastatic spread in these models does not accurately represent the aggressive metastasis in patients. Moreover, how tumors react to anticancer treatments in these models might

not precisely mimic how patients' clinical responses manifest themselves. Despite these drawbacks, these models are still helpful for research in pancreatic cancer and evaluating novel treatment options.

It is important to note that these results are specific to how our study was conducted, involving PDAC GEMMs treated with ganciclovir to induce glial cell depletion. Depending on experimental models, genetic modifications, and other elements, the exact mechanisms by which glial cells promote the development of pancreatic tumors may vary.

Conclusion

This study employed ganciclovir-induced glial cell depletion in a GFAP-Tk mouse model to investigate its impact on pancreatic cancer progression. Our results indicated a significant reduction in pain response, immune infiltration, and vascular and nerve density in the treatment group compared to controls, suggesting a potential efficacy of glial cell depletion in attenuating pancreatic cancer progression.

The observed decrease in stromal and immune infiltration hints at a potential reduction in cancer-associated inflammation, while the reduced vascular and nerve density may imply a suppression of angiogenesis and nerve growth. These findings shed light on the role of glial cells in pancreatic cancer initiation and progression, highlighting glial cell depletion as a promising therapeutic strategy, albeit requiring further validation and research.

In-depth analysis of RNA-Seq data revealed significant changes in gene expression and enrichment of pathways related to cellular energy consumption, protein synthesis, chromosomal organization, and immunological response, providing valuable insights into the intricate molecular mechanisms underlying cancer development and potential therapeutic interventions.

Functional in vitro assays unveiled the active role of IL-8 secretion by Schwann cells, triggering angiogenesis, migration, and inflammatory pathways in the tumor microenvironment upon interaction with cancer cells. While acknowledging the limitations of using animal models, cell lines, and organoids, this study serves as a foundational exploration into the significance of Schwann cells in the tumor microenvironment, paving the way for future innovative treatment development in pancreatic cancer research. In this project, we used mouse models, cell lines, and organoids as simplified representations of cancer. However, it is crucial to acknowledge that these models may only partially reflect the complexity of human malignancies. While glial cell depletion is safe and feasible in mice, organoids, and cell lines, it is not applicable to humans with current technology. This study serves as a preliminary investigation to explore the significance of Schwann cells in

the tumor microenvironment and deepen our understanding of cancer development. It also lays the groundwork for the future development of innovative treatments.

Summary

This project aimed to investigate the role of glial cells in the progression of pancreatic cancer (PDAC) by studying mice models of pancreatic ductal adenocarcinoma. In order to investigate the various phases of tumor development in two PDAC mouse models, we carried out an *in vivo* functional knockout of Schwann cells, a subtype of glial cells.

In order to investigate the impact of glial cell depletion on PDAC mouse models' pain perception, we used a glial cell-depleted model. It is known that glial cells in the central nervous system, including astrocytes and microglia, regulate how pain is processed. Glial cells have been shown to play a role in pain regulation in the past, and our discovery that pain response was reduced in the glial cell-depleted mice compared to the control group supports this.

We also examined the expression of GFAP, a glial cell marker, and the density of lesions in the tumor microenvironment. We observed a significant decrease in lesion density in the glial cell-depleted mice, which suggests that glial cells boost the growth of neoplastic lesions and pancreatic tumorigenesis.

The study also investigated the impact of glial cell depletion on other neural cells and tumor vasculature in the tumor microenvironment. We observed that the glial cell-depleted mice had significantly lower vascular density, suggesting that glial cells may promote tumor angiogenesis and the growth of new blood vessels to support tumor development.

According to the findings, the expression of immune markers such as CD45, CD8, and F4/80 was also found to be lower in the tumor microenvironment of the glial cell-depleted mice. As a result, glial cells may influence how the immune system reacts to pancreatic cancer.

Overall, the results of this functional *in vivo* study suggest that glial cells influence the immune system, tumor growth, angiogenesis, pain perception, and tumor progression in pancreatic cancer.

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Acknowledgments

I am filled with a complex mixture of emotions as I write this chapter. While there is an undeniable sense of bitterness, I also experience profound feelings of accomplishment and happiness, having successfully navigated the numerous challenges of my Ph.D. journey. At this moment, I express my deepest gratitude to all those who have supported me throughout the completion of my thesis.

First and foremost, I would like to extend my sincere thanks to my thesis advisor, Prof. Dr. İhsan Ekin Demir. Throughout the Ph.D. process, Ekin has been an exemplary mentor, consistently providing guidance and feedback, irrespective of the time of day. His exceptional scientific insight and expertise were pivotal to the successful completion of my thesis. I am profoundly grateful for his unwavering support and understanding. It has been a privilege and an honor to work under his mentorship.

I also sincerely appreciate Prof. Dr. Güralp Onur Ceyhan for welcoming me into his research group and placing his trust in my ability to realize my academic aspirations.

Meeting and working with my second thesis supervisor, Prof. Dr. Angelika Schnieke, has been a privilege. Her significant contributions to my thesis and her role as a scientific role model have been invaluable. The opportunity to engage in scholarly discussions with Prof. Schnieke has greatly enriched my research experience.

I want to extend my gratitude to my colleagues, whose consistent support has been vital throughout this study: our former laboratory director Dr. Steffen Teller; our former technician Ulrike Bourquan, Dr. Sergey Tolalov, Carsten Jager, Linhan Ye, Dr. Xiaobo Wang, Dr. Hossam Tahe Dr. Pavel Stopakov, Haoyu Quhan; Dr. Okan Safak, Jasmine Reich, Alper Dogruöz, and Erdinç Besikcioglu. Erdinç, and Linhan have been esteemed colleagues and trusted friends, offering unwavering support, reliability, and camaraderie. It has been a pleasure to collaborate with each of you.

I am also profoundly thankful to Prof. Dr. Hana Algül, who graciously accepted me into his laboratory and treated me as a valued member of his research team. I want to express my gratitude to the esteemed members of AG Algül, including Dr. Kivanc Görgülü and Dr. Derya Kabacaoğlu and Nan Wu for their continuous support whenever needed. Your sincere assistance was truly invaluable.

Prof. Dr. Maximilian Reichert has demonstrated extraordinary humility as a respected expert in his field. His scientific insights and advice have been of immense value, and I am deeply grateful for his support. I also want to convey my most profound appreciation to Aristeidis Papagryrou, a member of the AG Reichert team, for his cooperation and unwavering support during challenging times. Furthermore, I am grateful to Joanna Madej for her friendship and valuable organoid assistance.

My master's thesis supervisor, Prof. Dr. Faruk Bozoglu, has upported me throughout my doctoral journey. His unwavering moral support and guidance have illuminated my path since my master's studies, guiding me in the right direction. Similarly, I extend my heartfelt appreciation to Prof. Dr. Seyhun Yurdugül, who has been a true mentor since my undergraduate years. I sincerely thank both of you for your invaluable mentorship throughout my academic journey.

I am deeply grateful to my current employer, Prof. Dr. Thomas Korn, thank you for providing me with the opportunity to pursue this academic endeavor. Prof. Korn's unwavering support, understanding, and flexibility were instrumental in helping me navigate the challenges of balancing work and academia. I extend my heartfelt thanks to the esteemed members of AG Korn, including Dr. Sylvia Heink for her mentorship, Christopher Sie for his professional technical support, Dr. Andreas Muschaweckh, Sofia Tyystjärvi, Lea Seeholzer, Lisa Richter, Hsin-Hsiang Chen, Diego Emiliano Navarro Ruiz, Dr. Meike Mitsdörffer, Dr. Anne Schnelzer, Dr. Luise Eckardt, Dr. Ingrid Stas, Dr. Ali Afzali and Dr. Patrizia Leopold for fostering a warm, collaborative environment and offering unwavering support. I am grateful for the continuous encouragement of my excellent office mate, Dr. Marlene Spitzel.

Finally, I express my deepest gratitude to my beloved family—my mother, İlhan Meryem Yurteri; my father, Hamdi Yurteri; and my sister, Şule Yurteri. You are the greatest blessing in my life. Thank you for your unwavering support, respecting my decisions, and standing by me through all circumstances. This thesis would not have been possible without your presence and support.

“If one day, my words are against science, choose science.”

Mustafa Kemal Atatürk