Original research

IL-17A-producing CD8+ T cells promote PDAC via induction of inflammatory cancer-associated fibroblasts


ABSTRACT

Objective Pancreatic ductal adenocarcinoma (PDAC) is characterised by an abundant desmoplastic stroma composed of cancer-associated fibroblasts (CAF) and interspersed immune cells. A non-canonical CD8+ T-cell subpopulation producing IL-17A (Tc17) promotes autoimmunity and has been identified in tumours. Here, we evaluated the Tc17 role in PDAC.

Design Infiltration of Tc17 cells in PDAC tissue was correlated with patient overall survival and tumour stage. Wild-type (WT) or IL17ra-/- quiescent pancreatic stellate cells (pPSC) were exposed to tumoral media obtained from Tc17 cells (Tc17-CM); moreover, co-culture of Tc17-CM-induced inflammatory (i)CAF (Tc17-iCAF) with tumour cells was performed. IL-17A/F-, IL-17R-/-, RAG1-deficient and Foxn1nu mice were used to study the Tc17 role in subcutaneous and orthotopic PDAC mouse models.

Results Increased abundance of Tc17 cells highly correlated with reduced survival and advanced tumour stage in PDAC. Tc17-CM induced iCAF differentiation as assessed by the expression of iCAF-associated genes via synergism of IL-17A and TNF. Accordingly, IL-17RA controlled the responsiveness of pPSC to Tc17-CM. Pancreatic tumour cells co-cultured with Tc17-iCAF displayed enhanced proliferation and increased expression of genes implicated in proliferation, metabolism and protection from apoptosis. Tc17-iCAF accelerated growth of mouse and human tumours in Rag1-/- and Foxn1nu mice, respectively. Finally, IL17A-expressed by fibroblasts was required for Tc17-driven tumour growth in vivo.

Conclusions We identified Tc17 as a novel protumourigenic CD8+ T-cell subtype in PDAC, which accelerated tumour growth via IL-17A-dependent stroma modification. We described a crosstalk between three cell types, Tc17, fibroblasts and tumour cells, promoting PDAC progression, which resulted in poor prognosis for patients.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) belongs to the deadliest solid malignancies, with a 5-year overall survival (OS) rate of approximately 9%.1 Abundant desmoplastic stroma consisting of cancer-associated fibroblasts (CAFs) and immune cells, which constitute an inflammatory environment, supports the progression, heterogeneity and drug resistance in PDAC.2 CAF strongly influence tumour microenvironment (TME) through synthesis and remodelling of the extracellular matrix (ECM), and through production of factors, which direct cell infiltration and function.3 Depending on the subtype, CAFs are suggested to either promote or inhibit PDAC progression. CAF expressing α-smooth muscle actin (αSMA encoded by Acta2)
are termed myofibroblastic CAF (mCAF), and can exhibit a tumour-restricting or tumour-promoting function, dependent on the phenotype. TGFβ produced by tumour cells, myeloid cells and regulatory T cells (Treg) drives mCAF differentiation. Genetic depletion of Treg led to an accelerated tumour progression and differentiation of inflammatory CAF (iCAF) as well as myeloid cell infiltration in a mouse model of PDAC. Thus, iCAF differentiation is directed by tumour-secreting ligands including IL-1 and TNF, which are characterised by inflammatory gene expression, the marker Ly6c and a PDAC-promoting function.

IL-17A signals through the receptor (IL-17R) consisting of the specific IL-17RC and the common IL-17RA chains as a homodimer or IL-17RAF as heterodimer. Likewise, IL-17F homodimers bind to the same receptor, however, induce signalling with a lower potency. In non-haematopoietic cells, IL-17A induces production of CXCL1, CXCL2, CXCL8, IL-6 and G-CSF, which drive myeloid cell infiltration. In the context of PDAC, IL-17A supports early carcinogenesis (pancreatic intraepithelial neoplasia, PanIN). IL-17A regulates PDAC stem cell features, promotes tumour growth and mediates resistance to checkpoint inhibitors via induction of neutrophil extracellular traps. Furthermore, it has recently been shown that IL-17A modulates CAF transcriptome in KrasLSL-R172H/+; Trp53LSL-R172H/+; Pdx1-Cre (KPC)-derived tumours.

IL-17A can be produced by different lymphocyte populations including adaptive CD4+ and CD8+ T cells, termed Th17 and Tc17, respectively, but also by innate lymphocytes (ILC). In contrast to conventional IFNγ-producing and TNF-producing CD8+ cytotoxic T lymphocytes (CTL), Tc17 cells are non-cytotoxic; they express the transcription factor RORα and produce IL-17A/F and, to a lesser extent IFNγ, TNF, IL-21. IL-17A/F and IL-21. Tc17 cells play an important role in tissue homeostasis and protection from infections. During chronic inflammation, including autoimmunity of the skin (psoriasis), or the central nervous system (multiple sclerosis), Tc17 cells display a disease-promoting function. Likewise, in cancers of the gastrointestinal-tract, Tc17 cells are linked to poor survival suggesting a pathogenic contribution. However, a subpopulation of Tc17/IFNγ+ cells, displayed anti-glioma activity and cytotoxicity on IL-12 treatment. In PDAC, Tc17 presence and function has not been described so far.

Here, we provide data pointing to Tc17 as a new pathogenic cell population in the pancreatic TME, responsible for hitherto unknown stroma-modulating mechanisms that accelerates PDAC growth in mouse models and most likely in patients.

**MATERIALS AND METHODS**

Detailed methods are described in online supplemental information.

**RESULTS**

Increased Tc17 cell frequency correlates with advanced tumour stage and poor survival in PDAC

To evaluate the potential role of Tc17 cells in PDAC, we collected pancreatic tissue from 112 patients (online supplemental table 1) and identified the presence of two Tc17 markers, RORα and IL-17A, within infiltrating CD8+ cells (figure 1A-D, online supplemental figure 1A-B). Analysis of RORα+ or IL-17A+ CD8+ T-cells revealed a highly significant correlation between their enrichment and shorter OS (figure 1B,E). Increased Tc17 abundance was significantly associated with tumour size, lymph node metastases (N+), and advanced tumour stage and was confirmed for PDAC patients. Increased Tc17 abundance in PDAC in comparison to adjacent non-neoplastic tissue was detected using flow cytometry (online supplemental figure 1J).

Consistent with previous reports, enhanced accumulation of total CD8+ T cells, associated with longer OS (figure 1G), confirming their favourable role in PDAC. A high ratio of Tc17 to total CD8+ T cells correlated with a shorter OS (figure 1H), indicating an overriding protumourigenic Tc17 effect. Multivariate Cox-regression analysis confirmed Tc17 frequency as an independent prognostic marker for PDAC (online supplemental tables 2 and 3).

Interestingly, increased accumulation of Th17 cells (CD4+RORα+), (online supplemental figure 2A) failed to correlate with shorter OS, tumour size, lymph node metastases, or tumour-grading, but it associated with advanced PDAC stage (online supplemental figure 2B-D). Th17 outnumbered Tc17 cells and the frequencies of both populations moderately correlated in tumour tissues (online supplemental figure 2E-G). Multivariate Cox-regression analysis further confirmed the prevalence of Tc17, but not of Th17, as a prognostic marker for PDAC (online supplemental tables 4-6).

Recent transcriptome analysis in PDAC identified two major subtypes termed ‘classical’ and ‘basal-like’, with a prolonged and a shorter OS, respectively. To evaluate, whether presence of Tc17 cells associates with the PDAC subtype, we stained tumour tissues for GATA6 and cytokeratin 5 (CK5) as surrogate markers for the ‘classical’ and ‘basal-like’ subtype, respectively. Although GATA6 and CK5 expression was moderately inverse, they failed to correlate with OS and, consequently with Tc17 abundance in the PDAC tissues (online supplemental figure 3A-D).

Next, we examined Tc17 presence in KPC mice, and in a subcaneous model applying murine pancreatic tumour cells expressing a model antigen ovalbumin (Panc, NC). We found an infiltration of Tc17 cells in both KPC-derived and PancNC-derived PDAC tumours (online supplemental figure 3E-I).

Taken together, in contrast to total CD8+ T cells or Th17 cells, enrichment of Tc17 cells strongly associates with shorter OS and is an independent prognostic marker for PDAC. Moreover, Tc17 correlate with advanced tumour stage, increased tumour size, and metastases, suggesting their involvement in disease aggressiveness.
Figure 1  Increased Tc17 infiltration associates with shorter survival in PDAC. (A) Double immunostaining of PDAC tissue sections using antibodies against CD8α (green) and RORγt (brown), scale bar 100 µm. (B) Kaplan-Meier curve of overall survival (survival %) of patients with surgically resected PDAC showing ≤6/mm² vs >6/mm² CD8+ RORγt+ cell infiltration (n=71, p values determined by log-rank test). (C) CD8+ RORγt+ cell frequency per mm² in T1/T2 vs T3/T4 tumours (n=105), in tumours ≤4 cm vs >4 cm (n=105), No vs n+ tumours (n=109) and UICC stage I/II vs III/IV (n=106). Box-plots depict the lower and upper adjacent values (whiskers) and the upper and lower quartiles (top and bottom edges of the box). Horizontal lines inside boxes indicate median, p values determined by Mann-Whitney U test. Each dot represents one individual. (D) Triple immunostaining of PDAC tissue for CD8α, IL-17A, and alpha-smooth muscle actin (αSMA). Left, PDAC tissue areas are denoted with single-positive IL-17A+ cells (brown), single-positive CD8+ cells (magenta), double-positive CD8+IL-17A+ (magenta-brown) and single-positive αSMA+ cells (green), scale bar 200 µm. Right, image magnification of cells highlighted in the left panel, scale bar 20 µm. (E) Kaplan-Meier curve of overall survival (survival %) of patients with surgically resected PDAC showing ≤5/mm² vs >5/mm² CD8+IL-17A+ cell infiltration (n=71, p values determined by log-rank test). (F) Linear regression analysis of CD8+RORγt+ vs CD8+IL-17A+ cell frequencies in PDAC tissue. Linear regression line, Spearman’s correlation coefficient ρ, and respective p value is shown in the plot (n=111). (G–I) Kaplan-Meier curves of overall survival (survival %) of patients with surgically resected PDAC showing ≤38/mm² vs >38/mm² single-positive CD8+ cell infiltration (G), intratumoural CD8+IL-17A+/CD8 ratios ≤0.13 vs >0.13 (H) and intratumoural CD8+RORγt+/CD8+ ratios ≤0.15 vs >0.15 (I) (n=71, p values determined by log-rank test). PDAC, pancreatic ductal adenocarcinoma.
Pancreas

Tc17 cells enhance pancreatic tumour growth in a mouse model

To examine the function of Tc17 cells in PDAC, murine Tc17 and control (CTL) cells were generated in vitro. Their cytokine patterns were confirmed by flow cytometry (figure 2A). These cells were adoptively transferred into Panc⁶⁸⁵ tumour-bearing congenic (CD45.2⁺) mice (figure 2B). In comparison to mice without T-cell transfer, mice treated with CTLs rejected tumours as expected (figure 2C), while Tc17 significantly accelerated tumour growth. At the experimental endpoint, the transferred (CD45.2⁺) and endogenous (CD45.2⁻) intratumoral Tc17 cells were detectable, indicating that they invaded tumours and maintained their cytokine profile (figure 2D).

Figure 2  Tc17 cells enhance pancreatic tumour growth in vivo. (A) Purified CD8⁺ T cells isolated from CD45.2⁺ OT-I mice were stimulated with anti-CD3/CD28 antibodies in the presence of TGFβ+IL-6 (Tc17) or IL-12+IL-2 (CTL) for 4 days. Differentiation was confirmed by intracellular staining for IL-17A and IFNγ and subsequent FACS analysis. Representative FACS plots are shown. (B) Scheme of experimental design. Congenic CD45.2⁺ cells were subcutaneously injected with 10⁶ Panc⁶⁸⁵ cells. After 5 days, tumour-bearing mice were injected intraperitoneally (i.p.) with 10⁶ Tc17 or CTLs obtained from CD45.2⁺ OT-I mice or with PBS (no transfer). The analysis was performed at the indicated end of the experiment. (C) Tumour-growth curve of subcutaneous tumours is shown (tumour volume in mm³ (mean±SE, n=5–7 mice). *p<0.05 indicates the tumour volume comparisons between mice without transfer and mice with Tc17 or CTL transfer. (D) FACS analysis of IL-17A and IFNγ production after restimulation of tumour single-cell suspensions with PMA/Ionomycin in the presence of brefeldin A for 5 hours. Data from endogenous CD45.2⁺ or transferred CD45.2⁺ CD8⁺ T cells. Left, representative FACS plots are shown. Right, quantification of the frequency of IL-17A⁺ (top) or IFNγ⁺ (bottom) among endogenous (CD45.2⁻) or transferred (CD45.2⁺) CD8⁺ T cells with or without Tc17 transfer (n=4–5 tumours). (E) Quantification of cytokines (ng/mL) produced by in vitro differentiated Tc17 cells after restimulation with plate-bound anti-CD3 antibodies for 24 hours (n=5). (F) Scheme of the experimental design showing the relative titre of tumour cells cultured for 36 hours with WT CD8⁺ T cells or with Tc17-conditioned media (Tc17-CM). Tc17-CM were obtained after restimulation of differentiated Tc17 cells with plate-bound anti-CD3 for 24 hours. Bottom, the tumour-cell titre was obtained from Panc⁶⁸⁵ or KPC cells tagged with firefly luciferase (Panc⁶⁸⁵Luc or KPCLuc) and assessed as fold of luciferase activity, normalised to the control (0% FCS), which was arbitrarily set to 1. Tumour cells were cultured alone in 0% FCS (control) or 2% FCS (2% FCS), or in 2% FCS containing IL-17A (IL-17A), Tc17-CM (Tc17-CM) or Tc17 cells (Tc17) (n=3). (G) Top, scheme of the experimental design showing treatment of matrigel-embedded 3D organoid cultures with 50 ng/mL IL-17A. Bottom, organoid assay of mouse (Mm_Bu2548) or human (Hs_ACH0264T) PDAC organoids treated with recombinant murine (rm) or recombinant human (rh)IL-17A (n=3). The relative cell titre without IL-17A treatment (control) was arbitrarily set to 1. (D–G) Bars show mean±SD; biological replicates are plotted. In (C) *p<0.05 determined by mixed-effects model (REML), in (D) **p<0.01, ***p<0.001, ****p<0.0001 by t-test, in (F) statistics evaluated by two-way ANOVA followed by Tukey's HSD multiple comparison test, ns (non-significant) in (G) statistics evaluated by Mann-Whitney U test. ANOVA, analysis of variance; HSD, honestly significant difference; PBS, phosphate-buffered saline; PDAC, pancreatic ductal adenocarcinoma.
IL-17 but not IFNγ producing CD8⁺ T cells in tumours. Thus, transferred Tc17 cells are stable, migrate and accelerate endogenous Tc17 accumulation, and thereby promote tumour growth.

To understand, how Tc17 cells promote tumourigenesis, we examined the Tc17 secretome and detected mainly IL-17A, IL-17F, IFNγ and low TNF (figure 2E), suggesting an involvement of these cytokines. However, culturing PanOVA or KPC, or primary human PDAC cells together with IL-17A, or in condition media obtained from Tc17 cultures (Tc17-CM), or with Tc17 cells failed to enhance tumour cell growth (figure 2F,G), suggesting an indirect Tc17 effect.

**Tc17-CM promote iCAF differentiation via synergism of IL-17A and TNF**

Considering the highly desmoplastic character of PDAC with CAFs modulating tumourigenesis, we examined the impact of Tc17-CM on the differentiation of quiescent pancreatic stellate cells (qPSC), recently described to regulate stromal microenvironment associated with PDAC aggressiveness. We employed IL-17A or CM generated from IL-17A/F double-knockout Tc17 cells (DKOTc17, DKO-CM) as controls. DKOTc17 failed to produce any IL-17A/F, but secreted IFNγ and TNF, and expressed RORγt and T-bet to similar extent as WT Tc17 cells, indicating their bona fide Tc17 character (online supplemental figure 4A-C). Culture of qPSC with Tc17-CM upregulated Cxcl1, Il6, Lifi, Saa3, Csf3 and Ly6C expression, representing iCAF-associates markers, but not myCAF transcripts (figure 3B,C, online supplemental figure 4D). IL-17A upregulated the expression of some iCAF-specific genes to higher extent compared to DKO-CM, indicating qPSC reactivity to IL-17A. Accordingly, qPSC, TGFβ-myCAF and Tc17-induced-iCAF (Tc17-iCAF) expressed both receptor chains for IL-17A/F (IL-17RA and IL-17RC) (online supplemental figure 3E). CRISPR/Cas9-mediated deletion of IL-17RA in PSC, strongly restricted their responsiveness to Tc17-CM with respect to inflammatory gene induction (figure 3D,E, online supplemental figure 3F,G).

RNA-sequencing of qPSC, Tc17-iCAF and DKO/Tc17-induced-CM (DKO-CM) cells revealed differential gene expression (DEG=5639, padj<0.001). DEG were classified in nine modules, depending on the mutual upregulation or downregulation, and examined by Molecular Signatures Database (MSigDB Hallmark 2020). Pathways characteristic of epithelial-to-mesenchymal transition (EMT) and myogenesis (module 2) were downregulated in Tc17-iCAF and DKO-CM comparing to qPSC, indicating suppression of myCAF differentiation potential. Along with qPCR results, Tc17-iCAF comparing to DKO-CM strongly upregulated genes associated with inflammatory response and TNF-signalling (module 8), and with proliferation (modules 4, 6 and 9) (figure 3F,G), confirming their inflammatory character. Interestingly, the expression of Il1r1 mediating induction and maintenance of iCAF by tumour-derived factors, was significantly upregulated in Tc17-iCAF versus qPSC (online supplemental figure 4H). Further, gene set enrichment analysis (GSEA) using published gene signatures revealed that Tc17-iCAF transcriptome was enriched with genes expressed by tumour-driven iCAF comparing to DKO-CM or qPSC (figure 3H,I, online supplemental figure 4I), indicating that Tc17 and tumour-derived factors drive a similar inflammatory state in PSC.

To understand the contribution of Tc17-secreted cytokines in iCAF induction, we treated murine qPSC with IL-17A/F alone or in combination. In contrast to IL-17A, IL-17F only marginally affected the inflammatory gene expression (online supplemental figure 4J). Since TNF is produced by Tc17 and promotes iCAF differentiation, we examined its contribution. Neutralisation of TNF in Tc17-CM led to a significant reduction of iCAF gene expression; accordingly, combining it with IL-17A synergistically enhanced their levels (online supplemental figure 4K,L). Human PSC expressed the IL-17RA/C and responded to IL-17A/F with a similar inflammatory gene induction, indicating species-specific differences. However, as for mouse PSC, the IL-17A effect was synergistically upregulated by TNF also in human PSC (online supplemental figure 5A–C). Thus, Tc17-CM via synergism of IL-17A and TNF promotes iCAF differentiation.

The iCAF-promoting effect applied for both Tc17-CM and Th17-CM. However, Tc17-CM induced higher Il6 expression comparing to Th17-CM, while Th17-CM strongly enhanced Saa3 and Csf3 levels in PSC (online supplemental figure 5D). Analysis of 8 different cytokines revealed a significantly higher content of TNF and IFNγ in Tc17-CM vs Th17-CM (online supplemental figure 5E,F), suggesting that a specific cytokine-composition secreted by Tc17 versus Th17 cells affects the iCAF transcriptome. Considering that G-CSF encoded by Csf3 directs neutrophil-like cell infiltration and Saa3 was suggested to regulate metabolite supply for tumour cells, while IL-6 promotes angiogenesis, myeloid-derived suppressor cells (MDSCs), and PDAC invasiveness, one can speculate that Tc17 versus Th17 cells can differentially shape PDAC towards MDSCs accumulation, angiogenesis and invasiveness versus nutrient supply and neutrophilia, respectively.

To understand if Tc17-iCAF influence pancreatic tumour growth, we co-cultured them with PanOVA cells expressing luciferase. Besides Tc17-iCAF, we used qPSC, TGFβ-myCAF, IL-17A-driven-CAF or DKO-CM, as controls. This assay revealed that solely Tc17-iCAF promoted pancreatic cancer cell proliferation (figure 3J).

In CM obtained from Tc17-iCAF (Tc17-iCAF-CM) their marker cytokine IL-6 was readily detectable (online supplemental figure 5G). Consistent with the known Tc17 induction by IL-6 together with TGFβ, Th17-iCAF-CM in combination with TGFβ promoted IL-17, but not IFNγ production by CD8⁺ T cells in IL-6-dependent manner (figure 3K, online supplemental figure 5H,I), indicating a reciprocal crosstalk between Tc17 and iCAF positively enhancing each phenotype.

Thus, IL-17RA expression by PSC is required for Tc17-iCAF differentiation, which is mediated via synergism of IL-17A and TNF. Tc17-iCAF enhance tumour cell proliferation and promote Tc17 differentiation via IL-6 in a positive feedback loop.

**Tc17-iCAF promote tumour growth in vivo**

To investigate if Tc17-iCAF promote pancreatic tumour growth in vivo, we transduced PSC with a congenic marker CD90.1 to enable their separation from endogenous (CD90.2) CAFs. These transduced PSC were used to generate in vitro qPSC, CTL-CAF, Tc17-iCAF or DKO-CAF, which were co-injected subcutaneously with PanOVA cells into immunodeficient Rag2−/− mice (figure 4A). In samples with CAF co-injection, higher amounts of αSMA⁺ cells were detectible; however, there was only slight collagen deposition, probably due to fast tumour growth (figure 4B, online supplemental figure 6A,B). Tc17-iCAF strongly promoted tumour growth (figure 4C) and displayed Ly6c-high phenotype (figure 4D, online supplemental figure 6C,D). They expressed Il6, Csf3 and Saa3 at higher levels as compared to DKO-CAF or qPSC, while myCAF markers were rather downregulated (figure 4E, online supplemental
Figure 3  Reciprocal crosstalk between Tc17 cells and iCAF. (A) Scheme of the experimental design showing Tc17-CM production. Tc17-CM was used to stimulate matrigel-embedded quiescent murine quiescent pancreatic stellate cells (qPSC) and to evaluate mRNA expression of iCAF-specific transcripts (B) or Ly6c+ phenotype (C). (B) qPCR for indicated iCAF transcripts in PSC after a 48-hour incubation with control medium (-), +TGFβ(2 ng/mL) (TGFβ), +IL-17A(50 ng/mL) (IL-17A), 30% Tc17-CM (Tc17-CM) or 30% CM obtained from IL-17A/FDKO Tc17 cells (DKO-CM), respectively. All incubations were done in control medium supplemented with the respective compounds or media. Fold mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1; (n=3). (C) FACS analysis of Ly6c levels by PSC after 48 hours incubation as described in B; mean fluorescence intensity (MFI) is shown. Left, representative histograms. Right, quantification of Ly6c levels, (n=3). (D) FACS analysis of WT qPSC and Il17ra-/qPSC for IL-17RA levels, MFI is shown. Left, representative histograms. Right, quantification of IL-17RA levels (n=3). (E) qPCR analysis of the indicated iCAF transcripts in WT or Il17ra-/qPSC after incubation with Tc17-CM for 48 hours. Fold mRNA expression is shown, normalised to Il17ra-/qPSC, which was arbitrarily set to 1; (n=3). (F) Heatmap of differentially expressed genes (Z score normalised, FDR≤0.001) by PSC after incubation with control medium (qPSC) or with control medium containing 30% Tc17-CM (Tc17-iCAF) or 30% DKOt17-CM (DKO-CM) for 48 hours classified into modules based on the mutual upregulation or downregulation (n=4, biological replicates). (G) Pathway enrichment analysis for Molecular Signatures Database (MSigDB) Hallmark 2020. Bubble graph displays the three most significant enriched pathways by −log10 value (p adj) for nine modules established in (F). (H, I) Gene set enrichment analysis (GSEA) to identify differential expression of iCAF-associated genes based on raw data RNA-Seq GSE933134 (H) or GSE113615 (I) in Tc17-iCAF vs qPSC (left) or DKO-CMF (right). (J) Top, scheme of the experimental design showing Tc17-iCAF induction, thereafter co-culture with PancDNALuc cells for 36 hours. Bottom, tumour-cell titre was obtained from PancDNA cells tagged with firefly luciferase (PancDNA) after culture with control medium (-) or co-culture with TGFβ-myCAF (TGFβ-myCAF), IL-17A-iCAF (IL-17A-iCAF), Tc17-iCAF (Tc17-iCAF) or DKO-CMF (DKO-CMF). Tumour cell titre was assessed as fold of luciferase activity normalised to the control (-), which was arbitrarily set to 1, (n=4–5). (K) Top, scheme of the experimental design showing the production of CM from qPSC, TGFβ-myCAF, Tc17-iCAF, DKO-CMF, which were added to purified CD8+ T cells. Bottom, quantification of FACS analysis showing frequencies of IL-17A-producing CD8+ T cells after anti-CD3/CD28 activation in the presence or absence of TGFβ and with/without 50% CM obtained from qPSC (qPSC CM), TGFβ-myCAF (TGFβ-myCAF CM), Tc17-iCAF (Tc17-iCAF CM), Tc17-iCAF+αIL-6 (Tc17-iCAF CM+αIL-6) or DKO-CMF (DKO-CMF CM) after 72 hours (n=3). (B–E, J, K) Bars show mean±SD; biological replicates are plotted. In (B, C, J, K) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 evaluated by one-way ANOVA followed by Tukey’s HSD multiple comparison test, ns (non-significant) (D, E) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 evaluated by two-tailed, unpaired t-test. ANOVA, analysis of variance; HSD, honestly significant difference; iCAF, inflammatory cancer-associated fibroblast.
Figure 4  Tc17-iCAF promote pancreatic tumour growth in vivo. (A) Scheme of the experimental design. 5×10^5 PancOVA tumour cells ±5×10^5 in vitro differentiated CD90.1^+qPSC, CD90.1^+CTL-CAF, CD90.1^+Tc17-iCAF or CD90.1^+DKO-CAF were subcutaneously co-injected into immunodeficient Rag1^-/- mice. Histology and CAF analyses were performed at the indicated end of the experiment. (B) Quantification of αSMA staining in tumour tissue of mice injected with PancOVA cells alone (-) or co-injected with qPSC (qPSC), with CTL-CAF (CTL-CAF), with Tc17-iCAF (Tc17-iCAF) or with DKO-CAF (DKO-CAF), based on previously published scoring.17 (n=4–7). (C) Tumour-growth curve of subcutaneous tumours is shown (tumour volume in MM^3; mean±SEM, n=5–7, a representative of two independent experiments each with 5–7 mice). ***p<0.0001 indicates the tumour volume comparisons between mice with Tc17-iCAF vs qPSC injection. (D) FACS analysis of Ly6chigh cell frequency in gated EPCAM-CD45^+PDPN^+ fibroblasts in subcutaneous tumours (n=5–10). (E) qPCR analysis of the indicated genes expressed by transferred EPCAM CD45^+PDPN^+CD90.1^+ fibroblasts from subcutaneous tumours (mean±SD, n=3). Fold of mRNA expression is shown, normalised to the qPSC group, which was arbitrarily set to 1, (n=3). (F) Scheme of the experimental design. 2×10^4 KPC tumour cells±2×10^4 CD90.1^+qPSC, CD90.1^+Tc17-iCAF or CD90.1^+DKO-CAF were orthotopically co-injected into Rag1^-/- mice. The tumour volume and CAF phenotype were analysed at the indicated end of the experiment. (G) Tumour volume of orthotopic tumours of mice injected with KPC cells alone (-) or co-injected with qPSC (qPSC), with Tc17-iCAF (Tc17-iCAF) or with DKO-CAF (DKO-CAF) is shown (mean±SD, n=6 mice). (H) FACS analysis of Ly6chigh cell frequency in gated EPCAM CD45^+PDPN^+ cells in orthotopic tumours of mice treated as indicated (mean±SD, n=3–4). (I) Scheme of the experimental design. 5×10^5 PaTu8988T human PDAC cells together with 5×10^5 in vitro differentiated CD90.1^+qPSC or CD90.1^+Tc17-iCAF or CD90.1^+DKO-CAF or were co-injected subcutaneously into immunodeficient athymic Foxn1^nu/nu nude mice. CAF analysis was performed at the indicated end of the experiment. (J) Tumour-growth curve of subcutaneous tumours (MM^3) of mice co-injected with PaTu8988T cells and with qPSC (qPSC) or with Tc17-iCAF (Tc17-iCAF) or with DKO-CAF (DKO-CAF) on the right side of the graph indicates the comparison between mice with Tc17-iCAF vs DKO-CAF co-injection. **p<0.01, ***p<0.001, ****p<0.0001 on the right side of the graph indicates the comparison between mice with Tc17-iCAF vs qPSC co-injection. (K) FACS analysis of Ly6chigh cell frequency in gated EPCAM CD45^+PDPN^+ fibroblasts in subcutaneous tumours (n=4–6). (L) qPCR analysis of the indicated gene expression by sorted from subcutaneous tumours transferred EPCAM CD45^+PDPN^+CD90.1^+ fibroblasts (mean±SD, n=3). Fold of mRNA expression is shown, normalised to the qPSC group, which was arbitrarily set to 1 (n=3). (B, D, G, J) biological replicates are plotted. In (B, D, G, J) statistics by Kruskal-Wallis-Test, *p<0.05, ns (non-significant) (C, I) ***p<0.001, ****p<0.0001 determined by two-way ANOVA with Bonferroni post hoc test, (E, G, H, L) *p<0.05, **p<0.01 and p values by one-way ANOVA followed by Tukey’s HSD multiple comparison test. αSMA, a-smooth muscle actin; ANOVA, analysis of variance; HSD, honestly significant difference; iCAF, inflammatory cancer-associated fibroblasts; qPSC, quiescent pancreatic stellate cell.
figure 6E), indicating maintenance of an inflammatory phenotype in vivo. Interestingly, CTL-CAF promoted tumour growth to lower extent than Tc17-iCAF. Consistently, the CTL cytokine IFNγ, comparing to IL-17A alone or in combination with TNF, displayed a restricted proinflammatory effect on qPSC. Accordingly, Tc17-CM promoted stronger iCAF profile compared with CTL-CM (online supplemental figure 6f,G), indicating context-specific TNF effects modulating PSC differentiation.

We confirmed the Tc17-iCAF-driven tumour-growth function and maintenance of inflammatory Ly6c<sup>high</sup> phenotype in comparison to qPSC or DKO-CAF in the pancreatic tissue applying KPC tumour cells as described before<sup>11</sup> (figure 4F–H, online supplemental figure 6H,I). Likewise, Tc17-iCAF partially maintained the phenotype and strongly promoted growth of human PaTu8988T PDC cells in athymic nude mice comparing to qPSC or DKO-CAF (figure 4L, online supplemental figure 6J), indicating phenotype retention and growth-promoting function for mouse and human tumours.

**Tc17-iCAF alter the transcriptional programme of pancreatic tumour cells**

To identify the mechanisms by which Tc17-iCAF promote pancreatic tumour growth, we performed RNA-sequencing of sorted Panc<sup>OVA</sup> cells after co-culture with Tc17-iCAF, or TGFβ-myCAFs, or qPSC (online supplemental figure 7A). The comparison of Tc17-iCAF- with TGFβ-myCAF-induced tumour-cell transcriptome defined DEGs (1400, padj<0.1), which were classified into seven modules (1–7, based on the mutual upregulation or downregulation), and examined by gene ontology (figure 5A,B, online supplemental figure 7B). Most genes in modules 3, 4, 6 and 7 were upregulated by Tc17-iCAF comparing to TGFβ-myCAF. Gene module three captured the expression of genes associated with proliferation and cytokine-signalling, while module four transcripts were linked to cell–cell interactions and ECM organisation. Module 6 was enriched with genes related to metabolism. Finally, module 7 contained genes linked to negative regulation of apoptosis and inflammatory response. Thus, Tc17-iCAF imprinted pancreatic tumour cells with a unique transcriptional profile characterised by proliferation, ECM organisation, protection from apoptosis and metabolism comparing to TGFβ-myCAFs.

The CM obtained from Tc17 cells enhanced Lif expression in iCAF (figure 3B), and Lif promotes PSC-dependent tumourigenesis in KPC mice.<sup>42</sup> Comparison of our data set with Lif-dependent KPC tumour-cell signature<sup>42</sup> revealed enrichment of Tc17-iCAF upregulated genes versus TGFβ-myCAF in the Lif-dependent tumour transcriptome (figure 5C), indicating an overlap between Tc17-iCAF and PSC-Lif-mediated pancreatic tumour profile.

Furthermore, we compared our data set with human PDAC tumour cell transcriptome, split into two main subgroups, the classical-A/B associated with early disease and the Basal-like A with advanced stage.<sup>31</sup> In contrast to TGFβ-myCAF, Tc17-iCAF upregulated genes were significantly enriched in Classical-A and Basal-like A profile (figure 5D, online supplemental figure 7C).

In contrast, Tc17 cells caused only minor changes in Panc<sup>OVA</sup> gene expression comparing to Tc17-iCAF as visualised by principal component analysis and heatmap (online supplemental figure 8A–C). Among upregulated genes by Tc17-iCAF in Panc<sup>OVA</sup> cells, we found in module 3, besides transcripts associated with metabolism and ECM organisation, similarly to our results shown in figure 5A,B, also genes associated with hypoxia and extracellular vesicle production (module 4, online supplemental figure 8D). As before, the transcriptional profile of Panc<sup>OVA</sup> cells co-cultured with Tc17-iCAF versus Tc17, displayed similarities to the Lif-dependent tumour transcriptome as well as to human classical-A and Basal-like A profiles (online supplemental figure 8E,F).

Thus, Tc17-iCAF but not Tc17 cells, induce a specific transcriptional programme in tumour cells, which is enriched in LifR-sufficient KPC tumour profiles as well as in human classical-A and Basal-like A PDAC subtype signatures, indicating a potential involvement of Tc17-iCAF in human disease and the KPC model.

**IL-17RA-sufficient iCAF are required for Tc17 cell-driven tumour growth in vivo**

To investigate if Tc17 cells can induce iCAF during tumourigenesis, Tc17 cells from WT or DKO mice generated in vitro were adoptively transferred into WT mice, which were co-injected subcutaneously or orthotopically with Panc<sup>OVA</sup> or KPC tumour cells alone or together with qPSC (figure 6A,F, online supplemental figure 9A). As before, tumours with qPSC co-injection harboured higher frequency of dsMA<sup>+</sup> cells (figure 6B, online supplemental figure 9B,E), Tc17 cells significantly enhanced tumour growth as compared with DKO/Tc17 or no T-cell transfer (figure 6C,G, online supplemental figure 9C,J), confirming the tumour-promoting role of IL-17A-producing Tc17 cells in Panc<sup>OVA</sup> and KPC tumours in the subcutaneous and orthotopic setting. CAF obtained from mice injected with Tc17 cells displayed Ly6c<sup>high</sup> phenotype (figure 6D, online supplemental figure 9D,E) and higher inflammatory gene expression comparing to DKO/Tc17 or qPSC co-injection without T-cell transfer (figure 6E, online supplemental figure 9F). Consistent with the responsivity to Tc17 cells, ex vivo isolated CAF expressed both IL-17A/F receptor chains (online supplemental figure 9G). Thus, IL-17A/F-producing Tc17 cells promote iCAF phenotype and tumour growth in subcutaneous and orthotopic PDAC model.

Consistently, increased tumour growth and enhanced Ly6c expression by fibroblasts was detectable in WT, but not in Il17arf<sup>−/−</sup> mice, co-injected with qPSC comparing to the sole KPC-cell injection (figure 6I–K, online supplemental figure 10A), indicating that endogenously produced IL-17A/F enhance PSC-driven tumour growth and a Ly6c<sup>high</sup> fibroblast phenotype in the pancreatic tissue.

Finally, we analysed if IL-17RA expressed by fibroblasts contributes to Tc17-driven pancreatic tumour growth. Therefore, we adoptively transferred Tc17 cells into IL-17RA-deficient mice, which were co-injected orthotopically with KPC or subcutaneously with Panc<sup>OVA</sup> cells along with IL-17RA-sufficient (WT) or IL-17RA-deficient (Il17arf<sup>−/−</sup>) qPSC (figure 6L, online supplemental figure 10B). Along with the contribution of IL-17A/F to Tc17-mediated effects, WT PSC promoted tumour growth to a significantly higher extent comparing to Il17arf<sup>−/−</sup> PSC in orthotopic and subcutaneous settings. This was accompanied by significantly higher abundance of Ly6c<sup>high</sup> fibroblasts in tumours of mice injected with WT vs Il17arf<sup>−/−</sup> PSC (figure 6M,N, online supplemental figure 10C-F). Thus, IL-17RA expressed by fibroblasts is required for the Tc17-mediated iCAF phenotype and tumour-growth promoting function in vivo.

**DISCUSSION**

The hallmark of PDAC is the abundant desmoplastic stroma which accounts for up to 90% of tumour mass.<sup>43</sup> The stroma consists of CAF-producing ECM components and soluble factors, and of infiltrating immune cells. Within this microenvironment,
Pancreatic reciprocal interactions between immune and non-immune cells take place, which impact tumourigenesis. As graphically summarised in figure 6O, we demonstrated a reciprocal crosstalk between tumour-infiltrating Tc17 cells and CAF, in which Tc17 skewed the IL-17R+CAF towards an inflammatory phenotype via synergism of IL-17A and TNF. In turn, Tc17-iCAF via secreted IL-6 directed TGF-β-dependent Tc17 differentiation, indicating an amplification loop. Further, Tc17-iCAF evoked gene-regulatory events in PDAC cells, thereby promoting growth of mouse and human tumours.

Figure 5 Tc17-iCAF affect pancreatic tumour cell transcriptional profile. (A) Heatmap of differentially expressed genes (Z score normalised, FDR ≤ 0.1) by PancOVA cells after 36 hours of co-culture with Tc17-iCAF or TGFβ-myCAF classified into modules based on the mutual upregulation or downregulation (n=3, biological replicates). (B) Pathway enrichment analysis for gene ontologies (GO): Biological processes. Bubble graph displays the five most significantly enriched pathways by -log10 value (p adj) for seven modules established in (A). (C) GSEA for differential expression of LIF-dependent pancreatic cancer cell transcripts obtained from LifrWT KPf/fCL vs Lifrf/fKPf/fCL mice in PancOVA tumour cells after co-culture with Tc17-iCAF vs TGFβ-myCAF. (C, right), heatmap of color-coded z-scores from the rlog transformed expression values based on the GSEA. (D) GSEA for differential expression of classical-A or Basal-like A human PDAC transcripts in PancOVA tumour cells after co-culture with Tc17-iCAF vs TGFβ-myCAFs. CAF, cancer-associated fibroblasts; GSEA, pancreatic ductal adenocarcinoma; PDAC, pancreatic ductal adenocarcinoma.
Figure 6  Tc17 cells promote tumour growth in vivo via IL-17RA+ iCAF. (A) Scheme of the experimental design. 5 × 10^5 PancOVA tumour cells±5 × 10^6 CD90.1+ qPSC were subcutaneously co-injected into WT mice, which on the same day received i.p. injections of PBS or of 10^6 WT (Tc17) or IL-17A/FDKO Tc17 (DKOTc17) cells differentiated from WT or IL-17A/FDKO CD8+ T cells in the presence of TGFβ+IL-6. Histology and CAF analysis were performed at the indicated end of the experiment. (B) Quantification of αSMA staining in tumour tissue of mice injected with PancOVA cells alone (−) or co-injected with qPSC (qPSC), with qPSC+Tc17 cells (qPSC+Tc17) or with qPSC+DKOTc17 cells (qPSC+DKOTc17), based on previously published scoring.17 (n=4). (C) Tumour-growth curve of subcutaneous tumours is shown (tumour volume mm^3 mean±SEM, n=5 mice, one representative of two independent experiments each with 5–7 mice). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 indicate the tumour volume comparisons between the groups with qPSC+Tc17 versus qPSC. (D) FACS analysis of Ly6chigh cell frequency in gated EPCAM-CD45−PDPN+ fibroblasts (mean±SD, n=8–9). (E) FACS analysis of the indicated gene expression by sorted from subcutaneous tumours EPCAM-CD45−PDPN+ fibroblasts (mean±SD, n=5–8). Fold of mRNA expression is shown, normalised to the qPSC group, which was arbitrarily set to 1. (F) Scheme of the experimental design. 2×10^4 KPC tumour cells with/without 2×10^4 CD90.1+ qPSC were orthotopically injected into WT mice, which received on the next day i.p. injections of PBS or 10^6 WT (Tc17) or IL-17A/FDKO Tc17 (DKOTc17) cells or PBS. The tumour volume and CAF were analysed at the indicated end of the experiment. (G) Tumour volume of orthotopic tumours of mice injected with KPC cells alone (−) or co-injected with qPSC (qPSC), with qPSC+Tc17 cells (qPSC+Tc17) or with qPSC+DKOTc17 cells (qPSC+DKOTc17) is shown (tumour volume in MM^3; mean±SD, n=8–9 mice). (H) FACS analysis of Ly6chigh cell frequency in gated EPCAM-CD45−PDPN+ cells in orthotopic tumours of mice treated as indicated (mean±SD, n=8–9). (I) Scheme of the experimental design. 1.5×10^4 KPC tumour cells±6×10^4 CD90.1+ qPSC were orthotopically injected into WT or Il17af/− mice. The tumour volume and CAF were analysed at the indicated end of the experiment. (J) Tumour volume of orthotopic tumours of WT and Il17af/− mice injected with KPC cells alone (−) or co-injected with qPSC (qPSC) is shown (tumour volume in MM^3; mean±SD, n=6–7 mice). (K) FACS analysis of Ly6chigh cell frequency in gated EPCAM-CD45−PDPN+ cells in orthotopic tumours of mice treated as indicated (mean±SD, n=6–7). (L) Scheme of the experimental design. 2×10^4 KPC tumour cells±2×10^4 WT or Il17ra/−CD90.1+ qPSC were orthotopically injected into Il17ra/− mice, which on the next day i.p. injection of PBS or 10^5 Tc17 cells. The CAF analysis was performed at the indicated end of the experiment. (M) Tumour volume of orthotopic tumours of Il17ra/− mice injected with KPC cells alone (−) or co-injected with WT qPSC (WT qPSC+Tc17) or with Il17ra/− qPSC (Il17ra/− qPSC+Tc17) or with qPSC (qPSC+Tc17) (Il17ra/− qPSC+Tc17) is shown (tumour volume in MM^3; mean±SD, n=8–10 mice). (N) FACS analysis of Ly6chigh cell frequency in gated EPCAM-CD45−PDPN+ cells in tumours of mice treated as indicated (mean±SD, n=6–7). (O) Summary with the proposed mechanism of an indirect cancer-promoting role of Tc17 cells in PDAC. Tc17 cells via synergistic effect of secreted cytokines, IL-17A and TNF, shift PSC differentiation towards iCAF formation in an IL-17RA-dependent manner. In turn, Tc17-induced iCAF promote Tc17 differentiation via secreted IL-6 in combination with TGFβ. Furthermore, Tc17-induced iCAF imprint pancreatic tumour cells with a unique transcriptional profile characterised by the expression of genes involved in proliferation, signal transduction, metabolism and protection from apoptosis, thereby enhancing tumour growth. (B, D, E, G, H, J, K, M, N) Biological replicates are plotted. In (C) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 determined by two-way ANOVA with Bonferroni post hoc test, in (B, D, N) *p<0.05, **p<0.01 by Kruskal-Wallis-test, in (E, G, H, J, K, M) *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 and p values by one-way ANOVA followed by Tukey’s HSD multiple comparison test. ANOVA, analysis of variance; iCAF, inflammatory cancer-associated fibroblasts; PBS, phosphate-buffered saline; PDAC, pancreatic ductal adenocarcinoma.
CD8+ T-cell functional understanding in PDAC was hitherto linked to the conventional tumour-eradicating CTL, along with the association of CD8+ T-cell enrichment in tumour tissue with longer OS.\textsuperscript{31,32} We identified, for the first time in PDAC, Tc17 cells using two different markers, IL-17A and RORγ, thereby establishing reliability of our analysis. In contrast to the infiltration of total CD8+ T cells, increased Tc17 abundance strongly associated with shorter OS, indicating their disease-promoting function. Accordingly, increased frequency of Tc17 cells correlated with tumour size and with progressed stage. Thus, our findings extend the knowledge regarding the role of CD8+ T cells in PDAC, by revealing the presence of pathogenic Tc17 cells, which can be useful for patient stratification and selection of targeted treatments.

The prevalence of the second adaptive IL-17A-producing subpopulation, namely Th17 cells (CD4+RORγ+), associated with advanced tumour stage, however, it failed to correlate with shorter patient survival and to act as an independent prognostic marker for PDAC, further emphasising the unique Tc17 pathogenic role in PDAC. Besides Tc17 and Th17 cells, other populations including γδT17 cells, ILC3 and lymphoid tissue inducers, which express RORγ, a transcription factor regulating IL-17A, might contribute to PDAC with a differential extent. Further studies are necessary to address this issue in depth.

Tc17-CM directed the expression of typical iCAF-associated transcripts. Accordingly, RNA-seq analysis identified downregulated pathways associated with myCAF fate, while inflammatory genes were upregulated in Tc17-iCAF compared with qPSC or DKO-CAF. Furthermore, GSEA revealed similarities between Tc17-iCAF transcriptome and published profiles of iCAF induced by tumour-secreted ligands,\textsuperscript{3,6} indicating that besides tumour also Tc17-products can drive an iCAF-specific transcriptome. Thus, with Tc17 cells, we introduce a novel cell type modifying PDAC stroma towards tumour-promoting function. Interestingly, also Th17-CM induced iCAF-marker expression, however, with differences in specific genes as compared with Tc17-CM, which probably resulted from a unique secretome including higher IFNγ and TNF production by Tc17 vs Th17 cells. Therefore, we propose that Tc17 versus Th17 differentially modify stroma and thereby possibly tumourigenesis. That idea is supported by our histological analysis revealing a moderate correlation between Th17 and Tc17 infiltration in PDAC.

Tc17 cells induced iCAF-marker genes via synergism of IL-17A and TNF. We uncovered this mechanism by exposing qPSC to IL-17A and TNF, or to Tc17-CM with TNF-neutralisation. The synergistic proinflammatory effect of IL-17A and TNF is already known,\textsuperscript{44} but not for PSC, thus we add a new mechanism promoting human and mouse iCAF differentiation. In line, recent study has shown that TNF in the PDAC TME enforces baselike aggressive PDAC subtype.\textsuperscript{41} In contrast, the CTL-derived IFNγ displayed a restricted proinflammatory effect, pointing to T-cell subpopulation-specific cytokine effects modulating stroma.

Along with the inflammatory phenotype, Tc17-iCAF boosted growth of murine and human tumours as compared with qPSC, CTL-CAF or DKO-CAF in Rag1\textsuperscript{-} and Foxn1\textsuperscript{null} mice. The injected qPSC or CTL-CAF enhanced tumour growth in comparison to sole tumour-cell injection and displayed an inflammatory phenotype, but to a lower extent as compared with Tc17-iCAF, suggesting that human and mouse TME can stabilise and probably further promote the iCAF phenotype already induced by Tc17. Considering an enhanced Ibr1 expression by Tc17-iCAF in comparison to qPSC in vitro, it is conceivable that tumour-derived factors stabilise Tc17-iCAFs via IL-1-dependent signalling in vivo.

Besides in vitro effects, Tc17 and IL-17A/F promoted iCAF phenotype and tumour growth in mouse orthotopic and subcutaneous models including Panc\textsuperscript{OVA} and KPC-derived tumour cells. Accordingly, qPSC and CAF expressed both IL-17R chains. IL-17RA-expression by qPSC was required to sense Tc17 signals mediating iCAF differentiation, and thereby to promote tumour growth as uncovered by reconstitution experiments in IL-17RA-deficient mice. Thus, we reveal an indirect IL-17RA-dependent iCAF tumour-promoting mechanism mediated by IL-17A/F-producing Tc17 cells. This evidence is supported by the finding that Tc17 cells had a less impact on pancreatic cancer-cell transcriptome in the co-culture system. Although our study is limited to adoptive Tc17 transfers, dissecting of Tc17 character and their antigen-specificity in PDAC will be important for understanding of their biology to develop new targeting strategies in the future.

IL-17A directly enhances early carcinogenesis,\textsuperscript{11-13} and mediates neutrophil driven resistance to checkpoint inhibitors.\textsuperscript{46} Moreover a recent report analysing IL-17A-deficient versus IL-17A-sufficient KPC mice demonstrated differences in the transcriptional profile of CAFs obtained from these models.\textsuperscript{15} We confirm the contribution of IL-17A to stroma modification; however, our data differ, likely due to the complexity of multiple Tc17-secreted factors comparing to a single-cytokine deletion.

Analysis of pancreatic cancer-cell transcriptome after co-culture with Tc17-iCAF versus TGFβ3-myCAF revealed that Tc17-iCAF upregulated gene-pathways associated with regulation of proliferation, adhesion, metabolism, apoptosis and inflammation. This was accompanied by enhanced expression of factors associated with epithelial-to-mesenchymal transition (Twist1, Prrx1, Loxl1,2, Cdh2, Cdh17),\textsuperscript{15} 46 aggressive phenotype (Lox, Gliz2, Meep2),\textsuperscript{47-49} stemness (Dclk, Tbx3)\textsuperscript{13} 50 pancreatic lineage differentiation (Gata4),\textsuperscript{33} canonical NF-kB signalling (Rel) and calcium/calcineurin signalling (Nfac2).\textsuperscript{51} Furthermore, transcripts involved in cholesterol biosynthesis and glycosylation were upregulated in consistency with the involvement of cholesterol\textsuperscript{52} 53 and glycoysis\textsuperscript{45,53} in PDAC progression.

Despite the limitation elicited by the co-culture, including soluble and cell-associated factors, the relevance of the RNA-Seq data for the in vivo tumourigenesis was confirmed by the enrichment of Tc17-iCAF upregulated genes among the Lif-dependent KPC tumour-cell transcripts.\textsuperscript{42} Regarding the LIF contribution to a paracrine PDAC progression and increased Lif levels expressed by Tc17-iCAF versus qPSC, our data suggest a possible LIF involvement in the Tc17-mediated tumourigenesis in vivo. Furthermore, transcripts upregulated by Tc17-iCAF versus TGFβ3-myCAF were enriched among human PDAC genes with a Basal-like A and a Classical-A profile, which coexist in the same tumours,\textsuperscript{15} suggesting a Tc17-iCAF participation in human disease.

In conclusion, our study reveals a crucial pathogenic role of a newly described Tc17-cells, which sustain a self-perpetuating communication with iCAF, thereby mediating PDAC progression. Considering that Tc17 cells, via synergism of IL-17A and TNF, trigger the protumourigenic iCAF function, the neutralisation of this cytokine combination may hinder PDAC from establishing tumour-favouring niches and, therefore, could be beneficial in personalised cancer therapy.

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**IL-17-producing CD8+ T cells promote PDAC via induction of inflammatory cancer-associated fibroblasts**

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**Supplementary Information**

1. **Supplementary Methods and References**

2. **Supplementary Tables 1-6**

3. **Supplementary Figures 1-10**

1. **Supplementary Methods and References**

**Human and Murine Tissue sample histology**

For histological analysis, human PDAC tumor tissue samples were provided by the tissue bank of the University Medical Center Mainz in accordance with the regulations of the tissue biobank and the approval of the ethics committee of University Medical Center Mainz (2019-14390; Landesärztekammer RLP). Tissue samples of 112 (supplementary table 1) patients with therapy naive pancreatic ductal adenocarcinoma (PDAC), who underwent surgical resection, were spotted on microarrays to obtain paraffin tissue sections for further analysis. To overcome heterogeneity, 4 array spots of each tissue were generated (2 central, 2 peripheral; diameter: 1 mm).

Murine tissue samples were taken from tumors and healthy pancreatic tissue of KPC mice, and from PancOVA-induced subcutaneous tumors, and then embedded in paraffin.

After deparaffinization, rehydration, and antigen retrieval in pH 6 (human triple and double staining) or pH 9 (murine triple staining) conditions, the following antibodies were used for human triple staining: anti-human(h)IL-17A (Biotechne, MAB3171), anti-hCD8 (DAKO, GA62361-2), and anti-hαSMA (DAKO, GA61161-2). For human double staining following antibodies were applied: anti-hCD8 (DAKO, GA62361-2), and anti-hRORγt (Biozol, LS-
B4659-100) or anti-hCD4 (Agilent, IR649). DAKO Envision Flex system (brown) and permanent Green (Zytomed) were used for visualization. For human CK5 and GATA6 double staining following antibodies were applied: anti-hCK5 (Leica, CK5-L-CE), anti-hGATA6 (Biotechne, AF1700). DAKO Envision Flex system (brown) and permanent Magenta Chromogen (red) were used for visualization. For murine triple staining following antibodies were applied: anti-mouse(m)IL17A (abcam, ab91649), anti-mCD8 (LSBio, LS-C414175), anti-mαSMA (Cell Signalling, #19245). For the signal amplification of primary antibodies, EnVision FLEX+ Mouse and Rabbit (LINKER) were used (#K8002, #K8009, Agilent, DAKO). DAKO Envision Flex system (brown), permanent Magenta Chromogen (red) and permanent Green (Zytomed) were used for visualization. CD8\(^+\)IL-17A\(^+\), CD8\(^+\)ROR\(\gamma\)t\(^+\), CD4\(^+\)RORgt\(^+\), total CD8\(^+\), total IL-17A\(^+\) cells were counted per mm\(^2\). The amounts of CK5\(^+\) or GATA6\(^+\) tumor cells are expressed as percent of total tumor cells as previously described.\(^1\)

Images were taken using the Gryphax Subra camera (Jenoptik, Jena, Germany). For confirmation of results, quantification was done using the ImageJ software.

For Masson-Goldner trichrome staining, paraffin tissues were deparaffinized in xylene and re-hydrated with graded alcohols. Standard staining procedure was performed using hematoxylin, followed by staining with Mallory Red, Phosphomolybdic Acid (1%), light green, and acidic acid (all chemicals provided by Merck, Darmstadt, Germany). Slides were dehydrated in 100% ethanol and xylene and mounted for further microscopy.

**Isolation, Stimulation and staining of human TILs from PDAC patients**

For FACS analysis, human PDAC tumor and healthy tissues were collected by the CBBMR (Comprehensive Biomaterials Bank Marburg). Tumor and control tissues were digested by 200 U ml\(^-1\) Collagenase IV (Worthington), 10 µg ml\(^-1\) DNAse 1(Roche) in HBSS, 37°C, 300 rpm agitation and filtered through 100 µm cell strainers (Sysmex). Single cell suspensions were restimulated for 5 h with PMA (50 ng ml\(^-1\)), Ionomycin (1 µg ml\(^-1\); Sigma-Aldrich), after 3 h in the presence of brefeldin A (5 µg ml\(^-1\); BioLegend). The cells were stained with: Zombie NIR fixable viability kit (Zombie NIR™ Fixable Viability Kit, BioLegend # 423106), anti-hCD3 (AF488, BioLegend #317310), anti-hCD4 (PacificBlue, BioLegend #300521) and anti-hCD8 (BV510, BioLegend #344732) for 45 min at 4°C. After fixation (2% paraformaldehyde 20 min, 4°C), intracellular staining in Saponin Buffer (0.1% Saponin, 1% BSA in PBS) was done for hIL-17A (PE #512306 BioLegend) and hIFN\(\gamma\) (APC, #502512
BioLegend) for 45 min at 4°C. Samples were measured and analyzed on an FACS Aria III (BD Biosciences), with FACS Diva and FlowJo V10.1.

**Patient involvement**

Tissue samples of 112 (supplementary table 1) patients with therapy naive pancreatic ductal adenocarcinoma (PDAC), who underwent surgical resection, were provided by the tissue bank of the University Medical Center Mainz in accordance with the regulations of the tissue biobank and the approval of the ethics committee of University Medical Center Mainz (2019-14390; Landesärztekammer RLP).

**Mice**

WT C57BL/6 CD45.2°C57BL/6 CD45.2 CD45.1° mice were bred in-house (Animal Facility Philipps-University Marburg, BMFZ). Rag1° (B6.129S7-Rag1tm1Mom/J) mice were received from Dr. H.D. Chang, TU Berlin, Germany. OT-I (B6.Cg-Tg(TcraTcbr)1100Mjb) mice were obtained from Jackson Laboratories. To obtain KPC mice, LSL-Kras^G12D/+LSL-Trp53^R172H/+ were crossed with Pdx-1-Cre mice to yield triple-mutant mice LSL-Kras^G12D/+LSL-Trp53^R172H/+Pdx-1-Cre, the F1 was genotyped by PCR. KPC° animals underwent weekly palpation and ultrasound screening starting from week 10. IL-17A/F DKO (B6.Cg-Il17a/Il17ftm1.1Impr Thy1a/J) mice were received from Dr. I. Prinz, UKE Hamburg, Germany. Il17ra° (IL-17ra^tm1.2Kurs)^2 mice were received from Dr. R. Savai, Max Planck Institute, Bad Nauheim, Germany, and Dr. A. Waismann, Johannes Gutenberg University Medical Center, Mainz, Germany. Athymic nude mice (athym-FoxJ^null^), which are T-cell- but not NK-cell-deficient, and C57BL/6J mice for orthotopic tumor transplantation were obtained from Janvier Labs. All studies were approved by the regional agency on animal experimentation (Regierungspräsidium Giessen).

**Murine T cell purification and in vitro differentiation**

CD8° T cells were obtained from lymph nodes (LNs) and spleens of WT or IL-17A/F DKO C57BL/6 mice (for all CM production or for adoptive transfer experiments depicted in figure 6), or of OT-I mice (for adoptive transfer depicted in figure 2A,C) using a negative selection kit (130-104-075, Miltenyi Biotec). For Tc17 differentiation, naïve CD8° T cells were cultured in RPMI (10% FCS) and stimulated with plate-bound anti-mCD3 mAb (5 µg ml⁻¹, clone 145-2C11, Biolegend) and soluble anti-mCD28 (0.5 µg ml⁻¹, clone 37.51, Biolegend) in the presence of rhIL-2 (50 U ml⁻¹, Novartis), rhTGF-β (0.5 ng ml⁻¹, Peprotech) rmIL-6 (30 ng ml⁻¹, Novartis), and rhIL-17A/F (Novartis).
Peprotech) and anti-mIFNγ (5 µg ml⁻¹, clone XMG1.2, Biolegend). CTL skewing conditions were achieved in RPMI (10% FCS) with plate-bound anti-mCD3 mAb (3 µg ml⁻¹) and soluble anti-mCD28 (0.5 µg ml⁻¹), rhIL-2 (50 U ml⁻¹), rmIL-12 (10 ng/ml⁻¹, Peprotech) and anti-mIFNγ (5 µg ml⁻¹). For intracellular cytokine staining, cells were restimulated after 72h of culture with PMA (50 ng ml⁻¹), Ionomycin (1 µg ml⁻¹, both from Sigma-Aldrich) in the presence of Brefeldin A (5 µg ml⁻¹; Biolegend) for 4h. After live/dead staining (Zombie NIR™ Fixable Viability Kit (# 423106 BioLegend), surface staining for CD8 molecule expression (anti-mCD8α FITC #11008182 BD Bioscience) was done for 20 min in cold PBS+1% FCS at 4°C, prior to fixation with 2% para-formaldehyde for 20 min at room temperature (RT). After fixation, intracellular staining in Saponin Buffer (0.1% Saponin, 1% BSA in PBS) was done for IL-17A (PE #12-7177-81 eBioscience or APC #17-7177-81 eBioscience) IL-17F (PE, #12-7471 eBioscience) and IFNγ (PerCp-Cy5.5, #505822 BioLegend) for 45 min at 4°C. Staining of transcription factors was performed without restimulation using the FOXP3/Fixation-Kit (eBioscience, 00-5521-00). For intranuclear staining, anti-m/hT-bet (eFluor660 #50582582 eBioscience) and anti-mRORγt (PE #12-6981-82 eBioscience) antibodies were diluted in 0.1% Saponin in PBS. The cells were acquired on the Attune Nxt Cytometer (Thermofisher Scientific).

**Conditioned Media (CM) production**

For CM production, in vitro differentiated CTLs, Tc17 or Tc17-DKO cells generated from purified CD8⁺ T cells obtained from spleens and LN of WT or IL-17A/FDKO C57BL/6 mice were harvested, washed with PBS, re-seeded in 24 well-plate 1 × 10⁶ cells in 1 ml fresh DMEM (2% FCS) and restimulated with plate-bound anti-CD3 mAb (5µg ml⁻¹). Supernatants were harvested after 24h of restimulation, then centrifuged at 400× g, 10 min, 4°C and stored at -80°C for further use. CM from fibroblast were harvested after 48h of skewing with medium or respective CM obtained from WT or IL-17A/FDKO Tc17 cells or with TGFβ.

**Bead assay**

Cytokine levels from cell-culture supernatants were assessed by LEGENDPLEX MU Th17 v2 (#740749 741048 Biolegend) according to manufacturer’s protocol.

**Generation of stable PancOVA/Luc and KPCloc cells**

PancOVA cells³ and KPC cells were transfected (Trans-IT-2020 (Mirus Bio)) with a firefly luciferase-expressing construct (PGK-Luc) and with an empty pEF6/V5-His plasmid (=EF-
MCS; Invitrogen) to confer Blasticidin resistance (ratio 9:1). Subsequently, cells were selected with Blasticidin (5µg ml⁻¹) and pooled, resistant clones were used in the experiments.

**Culture of human and murine pancreatic tumor cell lines**

Adherent PaTu8988T⁴, Panc⁰V⁰ (derived from Panc02), Panc⁰V⁰/Luc and pancreatic adenocarcinoma cells derived from a spontaneous KPC mouse tumor (termed KPC tumor cells), KPC⁴Luc cells were grown in T75 flasks (Sarstedt) with DMEM (10% FCS) and split every 2-4 days upon reaching 70% confluence, harvested by trypsinization (1x Trypsin/EDTA, Sigma T-4174) and reseeded (5 x 10⁵ cells / 20 ml Medium / T75 flask).

**Cell titer assays**

Pancreatic cancer organoids (human: from PDAC patient; mouse: from KPC model⁵ were established according to Boj et al⁶ and treated with rh IL-17 (IL-17A, 50 ng ml⁻¹, Miltenyi Biotec, #130-093959) for 5d in organoid medium. Subsequently, cell titers were measured using the Cell Titer 3D GLO kit (Promega) (figure 2G).

Panc⁰V⁰/Luc or KPC⁴Luc cells (figure 2F) were seeded at 1x10⁴ cells per well in 24-well TC-plate (Sarstedt) and starved for 24h with FCS-free DMEM. After 24h the medium was exchanged and tumor cells were cultured alone in DMEM 0% FCS (control) or in DMEM 2% FCS, or in DMEM 2% FCS containing IL-17A (50 ng ml⁻¹), Tc17-CM (20%) or Tc17 cells (1x10⁵) per well. After 36h cells were washed with PBS and lysed in 100 µl of lysis buffer. Firefly luciferase activity was measured in 10µl lysate with 50µl luciferase solution (Beetle-Juice Luciferase assay Firefly, P.J.K.Biotech #102511) using OrionL luminometer (Berthold detection systems).

Co-cultures of primed PSC and Panc⁰V⁰/Luc cells (figure3J) were set up by harvesting single matrigel droplets of TGFβ-myCAF, IL-17A-iCAF, Tc17-iCAF or DKO-CAF, removing the matrigel by incubation with ice-cold DMEM and centrifugation (1000 rpm, 4°C, 5 min), and removing the supernatant. Panc⁰V⁰/Luc (2 x 10⁵) cells alone or with TGFβ-myCAF, IL-17A-iCAF, Tc17-iCAF or DKO-CAF (4 x 10⁵) were re-seeded in 70 µl in fresh matrigel and mixed at a 1:1 ratio with DMEM (10% FCS) on a 3.5 cm suspension dish (Sarstedt). The matrigel-embedded PSC and Panc⁰V⁰/Luc cells were covered with 0.5% FCS-containing DMEM. After 36h cells were washed with PBS and lysed in 100µl of lysis buffer. Firefly luciferase activity was measured in 10µl lysate with 50µl luciferase solution (Beetle-Juice Luciferase assay Firefly, P.J.K.Biotech #102511) using OrionL luminometer (Berthold detection systems).
Culture and priming of human and murine pancreatic stellate cells (PSC)

Pancreatic stellate cells (mPSCs, kindly obtained from Dr. A Neese and hPSCs, kindly obtained from Dr. M Löhr) were cultured in T75 flasks or TC-treated dishes (Sarstedt) in DMEM (10% FCS) and split every 4-7 days, upon reaching 70% confluence. They were harvested by trypsinization (1x Trypsin/EDTA, Sigma T-4174) and reseeded (2.5 × 10^4 cells/ml). To obtain quiescent (q)PSC, 6 ×10^5 PSC were seeded in a 70 µl matrigel drop (Growth Factor Reduced (GFR) Basement Membrane Matrix, Corning, 356230) and mixed at a 1:1 ratio with DMEM (10% FCS) on a 3.5 cm suspension dish (Sarstedt). The matrigel-embedded PSC were covered with 0.5% FCS-containing DMEM and incubated for 48h. Thereafter, the medium was removed. qPSC were treated in 0.5% FCS-containing DMEM with either rhTGFβ1 (2 ng ml⁻¹; Peprotech, #100-21), rmIL-17A (50 ng ml⁻¹; Peprotech, #210-17), rhIL-17A (50 ng ml⁻¹; Peprotech, #200-17), rmIL-17F (50 ng ml⁻¹; Peprotech, #210-17F), rhIL-17F (50 ng ml⁻¹; Peprotech, #200-25), rmTNF (5 ng ml⁻¹; Peprotech, #300-01A-10), rmαTNF (5 µg ml⁻¹; ImmunTools, #12343014), rmIFNγ (50 ng ml⁻¹; ImmunTools, #12343536), rhIL-1α (5 ng ml⁻¹; Peprotech, #200-01A) or in 30% conditioned media (CM) as in each experiment indicated. After 48 h of differentiation, qPCR-based phenotyping was performed. For this, medium was removed and drops were collected in eppendorf tubes mixed with ice cold PBS. After centrifugation (1000 rpm, 4°C, 5 min) and removing of PBS, cells were resuspended in ice cold PBS and incubated for 30 min at 4°C. Cells were centrifuged (1000 rpm, 4°C, 5 min), and pellets were frozen at -80°C for later RNA preparation.

PSC transfection with CD90.1 marker

MSCV-IRES-Thy1.1 DEST vector (Addgene #17442) viral supernatants were generated from Phoenix cells and used for polybrene (8 µg/ml) assisted spin-infection according to Campos et al. Resting mPSCs at ~65% density was used as recipient cells, grown for 72 h post-spin-infection and sorted for CD90.1 expression after surface staining (anti-CD90.1 BV510, BioLegend #202535). Surface marker expression stability was assessed by subsequent CD90.1 staining and FACS analysis, when cell cultures were split or re-thawed.

IL-17RA CRISPR/Cas9 knockout in mPSC

To knockout IL-17RA in PSC LentiCRISPRv2 (Addgene no. 52961) constructs were used. Mouse PSC were transfected with a single guide (sg)RNA targeting mIl17ra (CAGAAGCAGCCATCCCAGCG) using Opti-MEM (Thermo Fisher Scientific; 31985062) and polyethylenemine (PolyScience; 23966). After selection with 10 µg ml⁻¹ blasticidin (Gibco;
R21001) single cells were picked and plated as single clones in 96-well plates. Knockout was verified via FACS and WB. For FACS staining PSC were harvested, matrigel dissolved by incubation with Collagenase D (5 mg ml\(^{-1}\), Roche) and DNase 1 (10 μg ml\(^{-1}\), Roche) in HBSS at 37°C for 5-8 min. The cells were washed with PBS and Live/Dead staining performed (Zombie NIR™ Fixable Viability Kit, BioLegend # 423106). After washing with PBS/1%FCS and centrifugation (400xg, 4°C, 5 min) surface staining for Ly6c expression (anti-mLy6c BV421 #48593182 eBioscience) was done for 20 min in cold PBS+1%FCS at 4°C, prior to fixation with 2% para-formaldehyde. Then intracellular receptor staining in Saponin Buffer was done for IL-17RA (PE #12-7182-82 eBioscience) and IL-17RC (APC #FAB2270A R&D Systems) for 45 min on ice. Following matching isotype controls were used: Goat IgG Isotype Control (#403006 BioLegend) and Rat IgG2a, κ Isotype Control (#554689 BD Biosciences). For human fibroblasts the receptor staining was performed as described above with the following antibodies: anti-hIL-17RA PE (#FAB177P R&D Systems), anti-hIL-17RC APC (#FAB22691A R&D Systems), Mouse IgG1 κ Iso Control PE (#12-4714-42 eBiosciences) and Mouse IgG2b κ Iso Control (#555745 BD Pharmingen).

**Western Blotting**

Whole cell lysates were generated using RIPA buffer. Protein concentration was measured via Lowry assay. Proteins were electrophoresed, after boiling, on 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and blotted on PVDF membrane. After blocking with 5% milk powder for 1 h, membranes were incubated with primary antibodies over night at 4 °C (anti-IL-17RA R&D Systems AF488, 1:1000; anti-Tubulin Sigma-Aldrich MAB3408, 1:10000). Membranes were washed 3 times for 10 min with TBST and incubated 1h with respective secondary antibodies (anti-goat-HRP Santa Cruz sc2020, 1:1000; anti-mouse GE healthcare NA931V, 1:20000). After three washing steps with TBST proteins were visualized in a ChemiDoc system (BioRad) after incubation with a chemiluminescence detection reagent (Santa Cruz sc2048; Thermo Fisher 34095).

**Tumor cell and CAF co-cultures**

For co-culture and subsequent sorting, murine qPSC\(^{CD90.1}\) were primed for either Tc17-iCAF, TGFβ-myCAF or qPSC control condition as described above. Panc\(^{OVA}\) cells were harvested from adherent cell cultures, counted, and stained with Cell Proliferation Dye eFluor 670 (#65-0840-90 eBioscience) according to manufacturer’s protocol.
Co-cultures of primed PSC and labelled Panc<sup>OVA</sup> cells (figure 5) were set up by harvesting single matrigel droplets of qPSC, Tc17-iCAF or TGFβ-myCAF, removing the matrigel by incubation with ice-cold DMEM and centrifugation (1000 rpm, 4°C, 5 min), and removing the supernatant. Labeled Panc<sup>OVA</sup> (2.5 × 10<sup>5</sup>) cells and qPSC, Tc17-iCAF or TGFβ-myCAF (3.5 × 10<sup>5</sup>) were re-seeded in 70 µl of fresh matrigel and mixed at a 1:1 ratio with DMEM (10% FCS) on a 3.5 cm suspension dish (Sarstedt). The matrigel-embedded PSC were covered with 0.5% FCS-containing DMEM.

Co-cultures of iCAF, Tc17 cells and labelled Panc<sup>OVA</sup> cells (supplementary figure 8) were set up by harvesting single matrigel droplets of Tc17-iCAF followed by removing the media and the matrigel by incubation with ice-cold DMEM and centrifugation (1000 rpm, 4°C, 5 min). Labeled Panc<sup>OVA</sup> (2.5 × 10<sup>5</sup>) cells alone, mixed with Tc17-iCAF (3.5 × 10<sup>5</sup>) or mixed with (2.5 × 10<sup>4</sup>) differentiated Tc17 cells were re-seeded in 70 µl of fresh matrigel and mixed at a 1:1 ratio with DMEM (10% FCS) on a 3.5 cm suspension dish (Sarstedt). The matrigel-embedded PSC were covered with 0.5% FCS-containing DMEM.

After 48h, the matrigel droplets were harvested, dissolved by incubation with Collagenase D (5 mg/ml, Roche) and DNAse 1 (10 µg/ml, Roche) in HBSS at 37°C for 45 min. The cells washed twice with cold MACS buffer (2% FCS, 1 mM EDTA in PBS), centrifuged (400x g, 4°C, 5 min), resuspended in MACS buffer, stained with CD90.1 antibody (BV510, BioLegend #202535) and sorted into eFluor670<sup>+</sup> fractions on a FACS ARIA II. The eFluor670<sup>-</sup>CD90.1<sup>+</sup> fibroblast population was subsequently stained and phenotype was analyzed by FACS on an Attune Nxt (ThermoFisher). The eFluor670<sup>+</sup> tumor cell fraction was resuspended in RLTplus buffer (Qiagen) and snap-frozen for RNA isolation and RNAseq.

cDNA synthesis and qPCR analysis
RNA was isolated from in vitro cultured fibroblasts with the NucleoSpin RNA kit (Macherey-Nagel) or from FACS-sorted murine fibroblasts and/or tumor cells with RNeasy Plus kit (Qiagen) according to manufacturer’s protocol. For cDNA synthesis, the RevertAid first strand cDNA synthesis kit (ThermoFisher Scientific) was used according to manufacturer’s protocol. For qPCR analysis of Fibroblast marker genes, iTaq Universal SYBR Green (BioRad) on a StepOne (ThermoFisher Scientific) was used, with primers optimized for the standard qPCR reaction setup.
RNA-Seq analysis and bioinformatics

RNA integrity was assessed on an Experion StdSens RNA Chip (Bio-Rad). RNAseq libraries were prepared using the TruSeq Stranded mRNA Library Prep kit (Illumina) and sequenced on an Illumina NextSeq550 platform, High Output Kit v2.5, 50 bases single-reads according to the manufacturer’s instructions. The paired-end reads were mapped using salmon v1.3.0 against the mouse transcriptome (Ensembl - Mus musculus - release 100) with decoys. The quantifications from salmon were imported into R version 4.0.1 using the tximeta package (version 1.6.2). The transcript counts were summarized to gene level. The resulting gene level quantifications served as input to DESeq2 v1.28.1. Gene expression levels in qPSC, Tc17-iCAF and DKO-CAF were compared against each other respectively, using default parameters. Gene comparisons with padj < 0.001 were considered differentially expressed. Correction for base gene expression was performed.
by subtracting the mean expression values of the qPSC from both Tc17-iCAF and DKO-CAF. All differentially expressed genes (DEG) were stratified into 9 modules dependent on the mutual up- or downregulation in PSC vs Tc17-iCAF vs DKO-CAF. Gene expression profiles were plotted as heatmaps (color-coded z-scores for the regularized logarithm (rlog) transformed, corrected expression values with the R programming language and the pheatmap package (version 1.0.12). Sequencing data has been uploaded to GEO archives under accession number GSE202377.

Gene expression levels in PancOVA cells after the Tc17-iCAF, TGFβ-myCAF and qPSC co-cultures were compared against each other respectively, using default parameters. Gene comparisons with padj < 0.1 were considered differentially expressed. Correction for base gene expression was performed by subtracting the mean expression values of the qPSC control co-culture from both Tc17-iCAF and TGFβ-myCAF cocultures. All DEG were stratified into 7 defined modules dependent on the mutual up- or downregulation in Tc17-iCAF and TGFβ-myCAF tumor-cell co-culture. Gene expression profiles were plotted as heatmaps (color-coded z-scores for the regularized logarithm (rlog) transformed, corrected expression values with the R programming language and the pheatmap package (version 1.0.12). Sequencing data has been uploaded to GEO archives under accession number GSE182126.

Gene expression levels in PancOVA cells after the Tc17-iCAF and Tc17 co-cultures were compared against each other respectively, using default parameters. Gene comparisons with padj < 0.001 were considered differentially expressed. Correction for base gene expression was performed by subtracting the mean expression values of the PancOVA control from both Tc17-iCAF and Tc17 cocultures. All DEG were stratified into 4 defined modules dependent on the mutual up- or downregulation in Tc17-iCAF and Tc17 tumor-cell co-culture. Gene expression profiles were plotted as heatmaps (color-coded z-scores for the regularized logarithm (rlog) transformed, corrected expression values with the R programming language and the pheatmap package (version 1.0.12). Sequencing data has been uploaded to GEO archives under accession number GSE218816.

**Molecular Signatures Database Hallmark, Gene Ontology and STRING analysis**

Pathway enrichment analysis for Molecular Signatures Database (MSigDB) was performed on all 9 gene modules employing EnrichR\textsuperscript{13} referencing MSigDB Hallmark 2020. Top 3 significantly enriched (-log10 padj) MSigDB Hallmark 2020 terms for each module are plotted as bubble graphs in Prism v9. Gene ontology analysis was performed on all gene modules employing EnrichR\textsuperscript{13} referencing GO Biological processes. Top significantly enriched (-log10
padj) biological processes GO terms for each module are plotted as bubble graphs in Prism v9.
To predict an iCAF coculture-driven interactome, the same, significantly upregulated genes of Tc17-iCAF cocultured Panc\textsuperscript{OVA} cells were used as input dataset to STRING v11\textsuperscript{14}, set to medium confidence, only query proteins, no disconnected nodes, MCL clustering, inflation=3.
For further visualization, underlying coloring was added manually.

Gene set enrichment analysis (GSEA)
To validate iCAF specific signatures in Tc17-iCAF vs qPSC vs DKO-CAF cross-mapping to upregulated or downregulated genes (p adj=0.001) in iCAF, which were induced by tumor-derived ligands, comparing to qPSC\textsuperscript{15,16} was performed using GSEA (V 4.3.2 Broad Institute, San Diego). To validate tumor specific Tc17-iCAF vs TGF\(\beta\)-myCAF or Tc17-iCAF vs Tc17 co-culture dependent expression patterns, cross-mapping to LIFR-dependent KPC mouse tumor\textsuperscript{17} and human PDAC signatures\textsuperscript{18} was performed in GSEA (V 4.3.2 Broad Institute, San Diego). Signatures for murine LIFR-dependent tumor transcripts were extracted from GSE119694\textsuperscript{17} and used as reference gene set. The reference expression set for Classical-A or Basal-like A human PDAC tumors was taken from\textsuperscript{18}. Enriched genes were plotted as heatmaps (color-coded z-scores for the regularized logarithm (rlog) with the R programming language and the pheatmap package (version 1.0.12).

Tumor models and adoptive T-cell transfer
For the subcutaneous (s.c.) in vivo tumor/T cell interaction model (figure 2A-D), 8–12-week-old WT mice were injected with 1 \times 10^6 Panc\textsuperscript{OVA} cells s.c. into the left flank. 5 days post-injection, tumor-bearing animals were treated with 1 \times 10^6 Tc17 or CTLs, which had been differentiated for 4 days in vitro from naïve CD8\(^+\) T cells isolated from OT-1 mice.

For the s.c. in vivo tumor cell/primed CAF interaction model (figure 4A-E, I-L)), qPSC, CTL-CAF, Tc17-iCAF and DKO-CAF were generated as described above. Each matrigel droplet, harboring 5 \times 10^5 CAF, was dissolved in 50 \(\mu\)l of ice-cold PBS, mixed with 5 \times 10^5 Panc\textsuperscript{OVA} cells and injected into the flanks of a Rag\textsuperscript{1\textlife} mouse, or mixed with 5 \times 10^5 PaTu8988T cells and injected s.c. into athymic Foxn\textsuperscript{nu/nu} mice.

For the s.c. in vivo CAF priming model (figure 6A-E, supplementary figure 9A-G, supplementary figure 10A-D), a mixture of 5 \times 10^5 WT or Il17ra\textsuperscript{-} qPSC and 5 \times 10^5 Panc\textsuperscript{OVA}, or 5 \times 10^5 KPC tumor cells was applied. Each matrigel droplet harboring 5 \times 10^5 WT or Il17ra\textsuperscript{-} qPSC was dissolved in 50 \(\mu\)l of ice-cold PBS, mixed with 5 \times 10^5 Panc\textsuperscript{OVA} or 5 \times 10^5 KPC-
derived tumor cells and injected into the flanks of either WT or \( I17ra^{-/-} \) mice, followed by an i.p. injection of \textit{in vitro} differentiated Tc17 cells from either WT or \( I17af^{-/-} \) CD8+ T cells. Tumor growth was measured daily and was visualized by volume approximation (V=\text{length} \times \text{width}^2/2 \text{ in cubic millimeters}).

For the orthotopic in vivo tumor cell/primed CAF interaction model (figure 4F-H, supplementary figure 6H,I) CD90.1+qPSC and CD90.1+Tc17-iCAF and were generated as described above, and a mixture of \( 2 \times 10^4 \) CD90.1+qPSC, CD90.1+Tc17-iCAF or CD90.1+DKO-CAF and \( 2 \times 10^5 \) KPC cells were orthotopically implanted into Rag1\(^{-/-} \) mice as previously described\(^19 \). Mice were kept anesthetized during all surgical procedures. A 1.5 cm abdominal incision was made, and the pancreas was pulled out from the abdominal cavity. 15\( \mu \)l of DMEM containing \( 2 \times 10^4 \) KPC tumor cells alone or with \( 2 \times 10^4 \) CD90.1+qPSC, CD90.1+Tc17-iCAF or CD90.1+DKO-CAF was injected into the pancreas. After injection, the peritoneum was closed using absorbable sutures, and the skin was sealed using a clip. Tumors were monitored weekly using ultrasound (Vevo 2100 Imaging System).

For the orthotopic in vivo CAF priming model (figure 6F-H, supplementary figure 9H-J) a mixture of \( 2 \times 10^4 \) KPC tumor cells \( \pm \) \( 2 \times 10^4 \) WT CD90.1+qPSC -were orthotopically implanted into WT C57BL/6J mice. On the next day the mice were i.p. injected with \textit{in vitro} differentiated \( 1 \times 10^6 \) Tc17 cells obtained from either WT or \( I17af^{-/-} \) CD8+ T cells.

For the orthotopic genetic CAF priming model WT and \( I17a/f^{-/-} \) mice (figure 6I-K, supplementary figure 10A) a mixture of \( 1.5 \times 10^4 \) KPC tumor cells \( \pm \) \( 6 \times 10^4 \) CD90.1+qPSC were orthotopically implanted into WT or \( I17af^{-/-} \) C57BL/6J mice.

For the orthotopic in vivo CAF priming model (figure 6L-N, supplementary figure 10E,F) a mixture of \( 2 \times 10^4 \) KPC tumor cells \( \pm \) \( 2 \times 10^4 \) WT or \( I17ra^{-/-} \) CD90.1+qPSC -were orthotopically implanted into \( I17ra^{-/-} \) C57BL/6J mice. On the next day the mice were i.p. injected with \textit{in vitro} differentiated \( 1 \times 10^6 \) Tc17 cells obtained from WT CD8+ T cells.

Mice were sacrificed upon poor outcome. Then, tumors were surgically removed and the tumor mass and volume were measured. The tumor volume expressed in cubic millimeters was determined by multiplying the length, the width and the height of surgically removed tumors. After surgical removal, tumors were digested into single-cell solutions by 200 \( \mu \)g/ml Collagenase IV (Worthington), 10 \( \mu \)g/ml DNase I (Roche) in HBSS, 37\(^\circ\)C, 300 rpm agitation and filtered through 100 \( \mu \)m cell strainers (Sysmex). Subsequently staining using TIL and fibroblasts panels were performed. Based on the expression of fibroblast markers and CD90.1 the CAF populations were sorted on FACSaria III (BD Biosciences) into endogenous or transferred populations and then analyzed for subtype-specific genes by qPCR.
Antibodies for FACS stainings:

**Human TIL Panel** (supplementary figure 1H,I): Zombie NIR™ Fixable Viability Kit (#423106 BioLegend), anti-hCD3 AF488 (#317310 BioLegend), anti-hCD4-Pacific Blue (#300521 BioLegend), anti-hCD8 (BV510, BioLegend #344732), hIL-17A (PE #512306 BioLegend) and hIFNγ (APC, #502512 BioLegend).

**Murine TIL Panel** (figure 2D, supplementary figure 3F,H,I): Zombie NIR™ Fixable Viability Kit (#423106 BioLegend), anti-mCD8α (AF488 #557668 BD Bioscience), anti-mCD4 (Pacific Blue #558107 BD Bioscience), anti-mCD45.2 APC (#109814 BioLegend), anti-mIL-17A (PE #12-7177-81 or APC #17-7177-81 eBioscience), and IFNγ (PerCp-Cy5.5 #505822 or APC #502810 BioLegend).

**Fibroblast/Sort Panel** (Figure 4D,E,K,L, 6D,E,H,K,N, supplementary figure 6C,D,E,H 9D,E,F, 10D,E): 7-Aminoactinomycin D (7-AAD) PerCP (#A1310 Thermo Fisher), anti-mEPCAM AF488 (#118210 BioLegend), anti-mCD45 PE (#103106 Biolegend), anti-mPDPN APC (#127410 BioLegend), anti-mCD90.1 BV500 (#202535 Biolegend), anti-mLy6c BV421 (48593182 eBioscience).

**Fibroblast IL-17 Receptor Panel** (Figure 3D, supplementary figure 3E,G,7G): Zombie NIR™ Fixable Viability Kit (#423106 BioLegend), anti-mIL-17RA PE (eBioscience #12-7182-82), anti-mIL-17RC APC (#FAB2270A R&D Systems), Rat IgG2a, κ Isotype Control PE (#554689 BD Biosciences), Goat IgG Isotype Control APC (#403006 BioLegend), for human PSC (Figure 4A); anti-hIL-17RA PE (#FAB177P R&D Systems), anti-hIL-17RC APC (#FAB22691A R&D Systems), Mouse IgG1 κ Iso Control PE (#12-4714-42 eBiosciences) and Mouse IgG2b κ Iso Control APC (#555745 BD Pharamingen) for human PSC.

**Statistics**

For statistical analysis, GraphPad Prism v10 was used. Data are presented as bar graphs (mean ± s.d) or as tumor growth curves (mean ± s.e.m.). Each data point represents one biological replicate except for Figure 2F in which 6 technical replicates representative for 3 independent experiments are shown. Normality of distribution and homogeneity of variances was evaluated by Shapiro-Wilk test and Brown-Forsythe, respectively for all datasets. Statistical significance to compare two groups was evaluated using two-tailed/unpaired t-tests. In case of datasets not conforming to normality criteria, nonparametric Mann-Whitney tests were used. The chosen confidence interval for all test was 95%. For multiple groups and/or multiple condition comparisons, one-way or two-way analysis of variance (ANOVA) was performed followed by a Tukey’s HSD, Dunnett’s or Bonferroni post hoc test, respectively. Datasets lacking
continuous values were analyzed by mixed-effects model (REML) with Tukey’s HSD post-test. For datasets not conforming to normality criteria, nonparametric Kruskal-Wallis test was used. A critical value for significance of $P < 0.05$ (*) was used throughout the study, and statistical thresholds of $P < 0.01$ (**), $P < 0.001$ (***) as well as $P < 0.0001$ (****) are indicated in the figures by asterisks.

**Patient data analysis**

Statistical data analysis was performed using MedCalc Version 20.006 (MedCalc Software, Ostend, Belgium). Mann-Whitney U test was used for comparison of independent continuous variables (Figures 1C, S1C-E, S2C,D,F, S3C,D, table 1 and 4, Age and Tumor size). Chi-square test was used for comparison of frequencies between groups (table 1 and 4 Gender, T, N, G and UICC stage). Correlation analyses were performed using Spearman's rank correlation test. Survival curves were estimated using the Kaplan–Meier method and compared by log-rank test (n=71 PDAC patients with known survival time). A Cox proportional hazard model was employed to identify the independent predictors of survival. The variables CD8$^+$IL-17A$^+$ cell infiltration ($\leq 5$ versus $> 5$ CD8$^+$IL-17A$^+$ cells/mm$^2$) or CD8$^+$ROR$\gamma^+$ cell infiltration ($\leq 6$ versus $> 6$ CD8$^+$ROR$\gamma^+$ cells/mm$^2$) or CD4$^+$ROR$\gamma^+$ cell infiltration ($\leq 9$ versus $> 9$ CD4$^+$ROR$\gamma^+$ cells/mm$^2$), T (extent of primary tumor, T1-2 versus T3-4), N (regional lymph node metastases, N0 versus N+), and G (grading, G1-2 vs. G3-4) were entered in the Cox model in one single step (enter method, n=68 PDAC patients with known Tc17, T, N, G strata), (enter method, n=71 PDAC patients with known Th17, T, N, G strata). Values of $P < 0.05$ were considered statistically significant. For analyses of CD8$^+$ROR$\gamma^+$, CD8$^+$IL-17A$^+$, CD8$^+$, IL-17A$^+$, CD4$^+$ROR$\gamma^+$, cells as well as for ratios CD8$^+$ROR$\gamma^+$/CD8$^+$ and CD8$^+$IL-17A$^+$/CD8$^+$ median values were used as cut-off. For tumor size the value of 4 cm was chosen as a cut-off, because this is the difference in tumor diameter between T2 vs T3 tumors according to the 8th TNM AJCC/UICC classification. For GATA6 cut-off 80 was chosen because of a very high median value (93.3333) and for CK5 cut-off 5 was chosen because of a very low median value (1.2500).

**Study approval**

The murine study was approved by the regional agency on animal experimentation (Regierungspräsidium Giessen). For human samples, approval was given by local Ethics Committee responsible (Landesärztekammer RLP for TMAs, Landesärztekammer Hessen for fresh PDAC samples).
References:


Table 1. Characteristics of PDAC patients: CD8^+IL-17A^+ and CD8^+RORγ^t^+ cells

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<th>CD8^+RORγ^t^+ (N=59)</th>
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<th>CD8^+RORγ^t^+ &gt; 5 mm²</th>
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<td>8/59 (14%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &lt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &lt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/51 (98%)</td>
<td>45/59 (76%)</td>
<td>44/59 (74%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.85</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &gt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &gt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/51 (8%)</td>
<td>4/59 (7%)</td>
<td>5/59 (8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &lt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &lt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/51 (50%)</td>
<td>25/59 (43%)</td>
<td>24/59 (41%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.65</td>
<td>0.75</td>
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</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &gt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &gt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/51 (2%)</td>
<td>1/59 (2%)</td>
<td>1/59 (2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &lt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &lt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>50/51 (98%)</td>
<td>49/59 (83%)</td>
<td>48/59 (81%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.95</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &gt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &gt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/51 (8%)</td>
<td>4/59 (7%)</td>
<td>4/59 (7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>CD8^+IL-17A^+ &lt; 5 mm²</th>
<th>CD8^+RORγ^t^+ &lt; 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>24/51 (47%)</td>
<td>24/59 (41%)</td>
<td>23/59 (40%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.75</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Table 2. Multivariate Cox-regression analysis of PDAC patients of overall survival for CD8⁺IL-17A⁺ cells (Overall Model Fit significance level $P=0.0015$, n=69)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Strata</th>
<th>$P$-value</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8⁺IL-17A⁺</td>
<td>&gt; 5/mm² vs. ≤ 5/mm²</td>
<td>0.0033</td>
<td>2.44 (1.35-4.41)</td>
</tr>
<tr>
<td>T</td>
<td>≥3 vs. ≤2</td>
<td>0.4060</td>
<td>1.29 (0.71-2.33)</td>
</tr>
<tr>
<td>N</td>
<td>+ vs. 0</td>
<td>0.7814</td>
<td>1.08 (0.62-1.89)</td>
</tr>
<tr>
<td>Grading</td>
<td>≥3 vs. ≤2</td>
<td>0.2189</td>
<td>1.45 (0.80-2.62)</td>
</tr>
</tbody>
</table>

Table 3. Multivariate Cox-regression analysis of PDAC patients of overall survival for CD8⁺RORγt⁺ cells (Overall Model Fit significance level $P=0.0032$, n=68)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Strata</th>
<th>$P$-value</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8⁺RORγt⁺</td>
<td>&gt; 6/mm² vs. ≤ 6/mm²</td>
<td>0.0053</td>
<td>2.22 (1.26-3.89)</td>
</tr>
<tr>
<td>T</td>
<td>≥3 vs. ≤2</td>
<td>0.4255</td>
<td>1.27 (0.71-2.28)</td>
</tr>
<tr>
<td>N</td>
<td>+ vs. 0</td>
<td>0.6709</td>
<td>1.13 (0.64-1.98)</td>
</tr>
<tr>
<td>Grading</td>
<td>≥3 vs. ≤2</td>
<td>0.2104</td>
<td>1.45 (0.81-2.58)</td>
</tr>
</tbody>
</table>

Table 4. Characteristics of PDAC patients: CD4⁺RORγt⁺ cells

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total (N=111)</th>
<th>CD4⁺RORγt⁺ ≤ 9/mm² (N=47)</th>
<th>CD4⁺RORγt⁺ &gt; 9/mm² (N=64)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>56 (50.0%)</td>
<td>26 (45.0%)</td>
<td>30 (56.9%)</td>
<td>0.3815</td>
</tr>
<tr>
<td>female</td>
<td>56 (50.0%)</td>
<td>21 (55.0%)</td>
<td>34 (43.1%)</td>
<td></td>
</tr>
<tr>
<td>Age [years]*</td>
<td>69 (62 – 74)</td>
<td>70 (61.25 – 73)</td>
<td>67.5 (62 – 75)</td>
<td>0.7335</td>
</tr>
<tr>
<td>T category</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT 1</td>
<td>10 (8.9%)</td>
<td>2 (4.3%)</td>
<td>8 (12.5%)</td>
<td>0.2585</td>
</tr>
<tr>
<td>pT 2</td>
<td>66 (58.9%)</td>
<td>30 (63.8%)</td>
<td>35 (54.7%)</td>
<td></td>
</tr>
<tr>
<td>pT 3</td>
<td>29 (25.9%)</td>
<td>11 (23.4%)</td>
<td>18 (28.1%)</td>
<td></td>
</tr>
<tr>
<td>pT 4</td>
<td>1 (0.9%)</td>
<td>1 (2.1%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>6 (5.4%)</td>
<td>3 (6.4%)</td>
<td>3 (4.7%)</td>
<td></td>
</tr>
<tr>
<td>N category</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN 0</td>
<td>37 (33.0%)</td>
<td>19 (40.0%)</td>
<td>17 (26.6%)</td>
<td>0.0060</td>
</tr>
<tr>
<td>pN 1</td>
<td>41 (36.6%)</td>
<td>21 (38.3%)</td>
<td>20 (31.3%)</td>
<td></td>
</tr>
<tr>
<td>pN 2</td>
<td>32 (28.6%)</td>
<td>6 (18.3%)</td>
<td>26 (40.6%)</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>2 (1.8%)</td>
<td>1 (3.3%)</td>
<td>1 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>5 (4.5%)</td>
<td>1 (6.7%)</td>
<td>4 (6.3%)</td>
<td>0.4328</td>
</tr>
<tr>
<td>G2</td>
<td>62 (55.4%)</td>
<td>29 (55.0%)</td>
<td>32 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>42 (37.5%)</td>
<td>16 (33.3%)</td>
<td>26 (40.6%)</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>1 (0.9%)</td>
<td>0 (16.7%)</td>
<td>1 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>2 (1.8%)</td>
<td>1 (3.3%)</td>
<td>1 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>UICC stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UICC I</td>
<td>29 (25.9%)</td>
<td>15 (33.3%)</td>
<td>13 (20.3%)</td>
<td>0.0120</td>
</tr>
<tr>
<td>UICC II</td>
<td>43 (38.4%)</td>
<td>22 (38.3%)</td>
<td>21 (32.8%)</td>
<td></td>
</tr>
<tr>
<td>UICC III</td>
<td>30 (26.8%)</td>
<td>7 (18.3%)</td>
<td>23 (35.9%)</td>
<td></td>
</tr>
<tr>
<td>UICC IV</td>
<td>5 (4.5%)</td>
<td>0 (3.3%)</td>
<td>5 (7.8%)</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>5 (4.5%)</td>
<td>3 (6.7%)</td>
<td>2 (3.1%)</td>
<td></td>
</tr>
<tr>
<td>Tumor size [cm]*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.4 (2.5 – 4.5)</td>
<td>3.2 (2.5 – 4.2)</td>
<td>3.5 (2.5 – 4.5)</td>
<td>0.4721</td>
</tr>
</tbody>
</table>

*Presented as median with (interquartile range). $P$ values are from Chi-square test (Gender, T, N, G, UICC stage) or Mann-Whitney test (Age, Tumor size).
Table 5. Multivariate Cox-regression analysis of PDAC patients of overall survival for CD4+RORγt+ cells (Overall Model Fit significance level $P=0.0176$, n=68)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Strata</th>
<th>P-value</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+RORγt+</td>
<td>&gt; 9/mm$^2$ vs. ≤ 9/mm$^2$</td>
<td>0.0512</td>
<td>1.74 (1.00-3.04)</td>
</tr>
<tr>
<td>T</td>
<td>≥3 vs. ≤2</td>
<td>0.1067</td>
<td>1.62 (0.90-2.90)</td>
</tr>
<tr>
<td>N</td>
<td>&gt; vs. 0</td>
<td>0.5789</td>
<td>1.17 (0.67-2.04)</td>
</tr>
<tr>
<td>Grading</td>
<td>≥3 vs. ≤2</td>
<td>0.1161</td>
<td>1.60 (0.89-2.86)</td>
</tr>
</tbody>
</table>

Table 6. Multivariate Cox-regression analysis of PDAC patients of overall survival for CD4+RORγt+ and CD8+RORγt+ cells (Overall Model Fit significance level $P=0.0050$, n=68)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Strata</th>
<th>P-value</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+RORγt+</td>
<td>&gt; 9/mm$^2$ vs. ≤ 9/mm$^2$</td>
<td>0.3387</td>
<td>1.35 (0.73-2.47)</td>
</tr>
<tr>
<td>CD8+RORγt+</td>
<td>&gt; 6/mm$^2$ vs. ≤ 6/mm$^2$</td>
<td>0.0307</td>
<td>1.96 (1.06-3.61)</td>
</tr>
<tr>
<td>T</td>
<td>≥3 vs. ≤2</td>
<td>0.3261</td>
<td>1.35 (0.74-2.46)</td>
</tr>
<tr>
<td>N</td>
<td>&gt; vs. 0</td>
<td>0.6555</td>
<td>1.14 (0.65-1.99)</td>
</tr>
<tr>
<td>Grading</td>
<td>≥3 vs. ≤2</td>
<td>0.1991</td>
<td>1.47 (0.82-2.63)</td>
</tr>
</tbody>
</table>
3. Supplementary Figures 1-10

A

B

100 μm

C

D

E

F

G

H

I

J
Supplementary Figure 1. Tc17 cells associate with tumor size, metastases and staging in PDAC. (A) Double immunostaining of PDAC tissue sections with beginning anaplasia was performed using antibodies against CD8α (green) and RORγt (brown), scale bar 100 µm. (B) Control staining with secondary antibody only of PDAC tissue, scale bar 100 µm. (C) CD8+ RORγt+ cell frequency per mm² in tumors with grading G1/G2 vs G3/G4 (n=109). (D) CD8+ IL-17A+ cell frequency per mm² in T1/2 vs T3/T4 tumors (n=106). (E) CD8+ IL-17A+ cell frequency per mm² in tumors ≤ 4 cm vs > 4 cm (n=106), N0 vs N+ tumors (n=110), UICC stage I/II vs III/IV (n=107) and tumor grading G1/G2 vs G3/G4 (n=110). (C-E) Box-plots depict the lower and upper adjacent values (whiskers) and the upper and lower quartiles (top and bottom edges of the box). The median is identified by a horizontal line inside the box, P value by Mann-Whitney U test. Each dot represents one individual. (F) Kaplan-Meier curve of overall survival (survival %) of patients with surgically resected PDAC showing ≤ 20/mm² vs > 20/mm² IL-17A+ cell infiltration (n=71, P values determined by log-rank test). (G,H) Linear regression analysis for frequencies CD8*RORγt+ vs IL-17A+ cells (G) and CD8*IL-17A+ vs IL-17A+ (H) in PDAC tissue. Linear regression line, Spearman’s correlation coefficient (rho), and respective P values are shown on the plot (n=111). (I) FACS analysis of IL-17+ and IFNγ+ CD8+ T cells, after restimulation with PMA/Ionomycin for 5h, brefeldin A was added for the last 3h, in adjacent or malignant pancreatic tissue, gated CD3+ CD8+ T cells are shown (mean ± s.d n=5-6). Left, representative FACS plots, right, quantification of IL-17A+ or IFNγ+ CD3+CD8+ T cell frequency is shown. (J) Gating strategy for FACS analysis of human fresh PDAC tissue samples. Acquired cells were first gated for exclusion of debris (SSC-A vs FSC-A), following by gating for living cells (L/D vs SSC-A) and then for single cells (FSC-H vs FSC-A). CD8+ T cells were identified based on the expression of CD3, then on CD8 marker (CD4 vs CD8). % of calculated CD8+ cells is based on the CD3+ gate. CD8+ T cells were then analyzed for the intracellular production of IFNγ- and IL-17A after restimulation with PMA and ionomycin in the presence of brefeldin A. In (I) each dot represents one individual, statistic evaluated by Mann-Whitney-U test. **P<0.01.
Supplementary Figure 2. Th17 prevalence associates with advanced tumor stage but not with shorter survival in PDAC. (A) Double immune staining of PDAC tissue was performed for CD4 (green) and RORγt (brown), scale bar 100 μm. (B) Kaplan-Meier curve of overall survival (survival %) of patients with surgically resected PDAC showing ≤9/mm² vs > 9/mm² CD4+RORγt+ cell infiltration (n=71, P values determined by log-rank test). (C) CD4+RORγt+ cell number per mm² in T1/2 vs T3/T4 tumors (n=105). (D) CD4+RORγt+ cell frequency per mm² in tumors ≤ 4 cm vs > 4 cm (n=105), N0 vs N+ tumors (n=109) and UICC stage I/II vs III/IV (n=106) and in tumors with grading G1/G2 vs G3/G4 (n=109). (C, D) Box-plots depict the lower and upper adjacent values (whiskers) and the upper and lower quartiles (top and bottom edges of the box). The median is identified by a horizontal line inside the box, P value by Mann-Whitney U test. Each dot represents one individual. (E) Numbers of CD4+ vs CD8+ RORγt+ cells per mm² tumor tissue from patients with surgically resected PDAC (n=111). (F) Numbers of CD4+RORγt+ cells per mm² in tumors with ≤ 6 vs > 6 CD8+RORγt+ cells/mm² or...
of CD8⁺RORγt⁺ cells per mm² in tumors with ≤ 9 vs > 9 CD4⁺RORγt⁺ cells/mm² from patients with surgically resected PDAC (n=111). (E, F) Box-plots depict the lower and upper adjacent values (whiskers) and the upper and lower quartiles (top and bottom edges of the box). The median is identified by a horizontal line inside the box. (E) P value by Wilcoxon test (paired samples). (F) P value by Mann-Whitney U test. Each dot represents one individual. (G) Linear regression analysis of CD8⁺RORγt⁺ vs CD4⁺RORγt⁺ cell frequencies in PDAC tissue. Linear regression line, Spearman’s correlation coefficient rho, and respective P values are shown on the plot (n=111).
Supplementary Figure 3. Tc17 are present in murine cancer models. (A) Double-staining of PDAC tissue was performed for CK5 (red) and GATA6 (brown); scale bar 200 µm. (B) Left, linear regression analysis of CK5+ vs GATA6+ tumor cell frequencies expressed as percentage of total tumor cells in PDAC tissue as described previously. Linear regression line, Spearman’s correlation coefficient (rho) and respective P values are shown on the plot (n=110). Right, Kaplan-Meier curve of overall survival (survival %) of patients with surgically resected PDAC showing ≤ 80 vs > 80 GATA6+ or of ≤ 5 vs > 5 CK5+ tumor cell frequency expressed as percentage of total tumor cells in PDAC tissue as described previously (n=71, P values determined by log-rank test). (C) CD8+ IL-17A+ cell frequency per mm² in GATA6+ ≤ 80 vs ≥ 80 (n=110) or in CK5+ ≤ 5 vs ≥ 5 tumors (n=110). (D) CD8+RORγ+ cell frequency per mm² in CK5+ ≤ 5 vs ≥ 5 (n=110) or in GATA6+ ≤ 80 vs ≥ 80 tumors (n=110). (E, D) Box-plots depict the lower and upper adjacent values (whiskers) and the upper and lower quartiles (top and bottom edges of the box). The median is identified by a horizontal line inside the box, P value by Mann-Whitney U test. Each dot represents one individual. (E, G) Double immune staining for CD8+ (red) and IL-17A+ (brown) cells in pancreatic tumors from KPC mice (E) or in subcutaneous PancOVA tumors (G), scale bar 200 µm. (F,H) FACS analysis of IL-17A+ and IFNγ+ CD4+ and CD8+ T cells in adjacent healthy or malignant pancreatic tissue from KPC mice (mean ± s.d, n=4) (F) and in subcutaneous PancOVA tumors from WT mice (mean ± s.d, n=9) (H). Gated CD8+ or CD4+ T cells after restimulation with PMA/ionomycin in the presence of brefeldin A for 4 h are shown. Left, representative FACS plots, right, quantification of IL-17A+ CD4+ or IL-17A+ CD8+ T cell frequency is shown. (I) Gating strategy for FACS analysis of murine tumor tissue samples. Acquired cells were first gated for exclusion of debris (SSC-A vs FSC-A), followed by gating for living cells (L/D vs SSC-A) and then for single cells (FSC-H vs FSC-A). CD8+ T cells were identified based on the expression of CD8 marker (CD4 vs CD8). % of calculated CD4+ and CD8+ cells is based on the single-cell gate. CD8+ T cells were then analyzed for the intracellular production of IFNγ and IL-17A after restimulation with PMA/ionomycin in the presence of brefeldin A. In (F,H) biological replicates are plotted. In (F,H) statistic was evaluated by two-tailed, unpaired t-test *P<0.05, ns (not significant).
Supplementary Figure 4. Tc17 cells via synergism of IL-17A and TNF promote murine iCAF differentiation. (A, B) Purified CD8+ T cells isolated from WT or IL-17A/F DKO mice were activated with anti-CD3/CD28 antibodies in the presence of TGFβ+IL-6 for four days. Tc17 differentiation was confirmed by intracellular staining for IL-17A, IL-17F and IFNγ. Representative FACS plots are shown (A) and by intranuclear staining for RORγt and Tbet. Representative histograms are shown (B). The numbers within the histograms indicate mean fluorescence intensity (MFI). (C) Quantification of cytokines (ng/ml) produced by in vitro differentiated IL-17A/F DKO Tc17 cells after restimulation with anti-CD3 antibodies for 24h (n=3). (D) qPCR for indicated transcripts were performed from qPSC incubated with control medium (-), control medium +2 ng/ml TGFβ (TGFβ), 50 ng/ml anti-IL-17A (IL-17A), 30% of Tc17-CM (Tc17-CM) or 30% CM obtained from IL-17A/F DKO Tc17 cells (DKO-CM) for 48h (mean ± s.d., n=3). Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1. (E) FACS analysis showing IL-17RA and IL-17RC levels (MFI) of PDPN+ qPSC, TGFβ-myCAF and Tc17-iCAF (mean ± s.d., n=3). Left, representative histograms, right, quantification of IL-17RA and IL-17RC levels is shown. (F) Western blot analysis of IL-17RA in IL-17RA knockout PSC and WT controls. Loading control, Tubulin (one representative of three independent experiments). (G) FACS analysis for IL-17RC levels (MFI) by WT and Il17ra−/− qPSC (mean ± s.d., n=6). Left, representative histograms, right, quantification of IL-17RC levels is shown. (H) z-score for Il1r1 of qPSC and Tc17-iCAF from RNA-Seq data from figure 3F (mean ± s.d., n=4). (I) Gen set enrichment analysis (GSEA) for differential expression of genes downregulated in iCAF- on raw data RNA-Seq GSE93313 or GSE113615 in Tc17-iCAF vs qPSC. (J) qPCR analysis for indicated gene expression by qPSC incubated with control medium (-), 50 ng/ml IL-17A (IL-17A), 50 ng/ml IL-17F (IL-17F) or 50 ng/ml IL-17A + 50 ng/ml IL-17F (IL-17A+F) for 48h (mean ± s.d., n=3). Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1. (K) qPCR analysis for indicated gene expression by qPSC incubated with control medium (-), 30% of Tc17-CM (Tc17CM) 30% Tc17-CM+ 5µg/ml anti-TNF antibodies (Tc17-CM+αTNF), 30% CM obtained from IL-17A/F DKO Tc17 cells (DKO-CM) or 30% DKO-CM+ 5µg/ml anti-TNF antibodies (DKO-CM+αTNF) for 48h (mean ± s.d., n=3). Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1. In (C-E,G,H,J-L) biological replicates are plotted. Bars show mean ± s.d, n=3. In (D,J,L) statistic was evaluated by one-way ANOVA followed by Dunnett’s post hoc test *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, in (E,G,H) by two-tailed, unpaired t-test *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, in (L) *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 by one-way ANOVA followed by Tukey’s HSD multiple comparison test.
A human PSC

B human PSC

C human PSC

D Cxcl1, Il6, Lf, Sox3, Csf3

E Th17-CM

F IFNγ

G TNFα

H TGFβ+

I IL-17A-APC

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Supplementary Figure 5. IL-17A and TNF synergistically promote human iCAF differentiation. (A) FACS analysis of human PSC differentiated to qPSC, myCAF (induced from qPSC by addition of 5 ng/ml rhTGFβ to control medium for 48h) or IL-1α-iCAF (induced from qPSC by addition of 5 ng/ml rhIL-1α to control medium for 48h) for IL17RA and IL-17RC levels (n=3). (B,C) qPCR analysis for indicated gene expression by human qPSC incubated with control medium (-), 50 ng/ml rhIL-17A (IL-17A), 50 ng/ml rhIL-17F (IL-17F) or 50 ng/ml IL-17A + 50 ng/ml rhIL-17F (IL-17A+F) (n=3) (B) or with 2 ng/ml rhTGFβ (TGFβ), 50 ng/ml IL-17A (IL-17A), 5 ng/ml rhTNF (TNF) or 50 ng/ml IL-17A + rhTNF (IL-17A+TNF) (n=3) (C) for 48h. Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1. (D) qPCR analysis for indicated marker expression by mouse qPSC incubated with control medium (-), 30% of Tc17-CM (Tc17-CM), 30% CM obtained from IL-17A/F DKO Tc17 cells (Tc17DKO-CM) 30% of Th17-CM (Th17-CM) or 30% CM obtained from IL-17A/F DKO Th17 cells (Th17DKO-CM) for 48h. Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1 (n=3). (E) Quantification of cytokines (ng/ml) produced by in vitro differentiated Th17 cells after restimulation with anti-CD3 antibodies for 24h (n=7). (F) Comparison of IFNγ and TNF secretion by Th17 vs Tc17 cells after restimulation with anti-CD3 antibodies for 24h (n=5-6) (G) ELISA for IL-6 from CM obtained from qPSC, TGFβ-myCAF, Tc17-iCAF and DKO-CAF after 48h of differentiation (n=3). (H,I) FACS analysis for frequency of IFNγ⁺ and IL-17A⁺ CD8⁺ T cells differentiated in CM obtained from qPSC, myCAF, Tc17-iCAF, Tc17-iCAF + αIL-6 and DKO-CAF for 72 hours in the presence or absence of 2 ng/ml TGFβ after restimulation with PMA/Ionomycin for 4 h in the presence of brefeldin A (n=3). Left, representative FACS plots, right, quantification of the frequency of CD8⁺IFNγ⁺ cells is shown. In (A-G,I) biological replicates are plotted, bars show mean ± s.d. In (A,F) statistic were determined by two-tailed, unpaired t-test *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, in (B,C,D) by one-way ANOVA followed by Dunnett’s post hoc test *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, ns (not significant), in (G) by Kruskal-Wallis-Test*P<0.05.
Supplementary Figure 6. Context-specific cytokine effects modulate PSC differentiation.

(A) αSMA staining performed on tumor tissue obtained from \textit{Rag1}−/− mice, which were
subcutaneously co-injected with \textit{in vitro} differentiated CD90.1*qPSC, CD90.1*CTL-CAF, CD90.1*Tc17-iCAF or CD90.1*DKO-iCAF and PanC\textsubscript{OVA} tumor cells. The \(\alpha\)SMA\textsuperscript{+} cell quantification (brown staining) was performed based on previously published scoring\textsuperscript{20}; scale bar 200 \(\mu\)m. (B) Masson-Goldner Trichrome staining to detect collagen (red spots) in tumor tissues; scale bar 200 \(\mu\)m. (C) Gating strategy for FACS analysis of subcutaneous murine tumor tissue samples. Acquired cells were first gated for debris exclusion (SSC-A vs FSC-A), followed by gating for living cells (L/D vs SSC-A) and then for single cells (FSC-H vs FSC-A). Fibroblasts were identified based on gating of CD45\textsuperscript{-} and EPCAM\textsuperscript{-} cells (EPCAM vs CD45, \% of calculated EPCAM CD45\textsuperscript{-} cells was based on the single-cell gate) and the expression of PDPN (PDPN vs CD90.1). In gated PDPN\textsuperscript{+} cells Ly6c expression was determined (Ly6c vs SSC-A). (D) FACS analysis of Ly6c\textsuperscript{high} cell frequency in gated EPCAM CD45 PDPN\textsuperscript{+} fibroblasts in subcutaneous tumors of mice co-injected with qPSC, CTL-CAF, Tc17-iCAF or DKO-CAF. Representative FACS plots are shown. (E) qPCR analysis for indicated gene expression by EPCAM CD45 PDPN\textsuperscript{+}CD90.1\textsuperscript{+} cells sorted from subcutaneous PanC\textsubscript{OVA} tumors of mice co-injected with qPSC, Tc17-iCAF and DKO-iCAF. Fold of mRNA expression is shown, normalised to qPSC group, which was arbitrarily set to 1 (n=3). (F) qPCR analysis for indicated gene expression by qPSC incubated with control medium (-), 50ng/ml IFN\(\gamma\) (IFN\(\gamma\)), 50 ng/ml IL-17A (IL-17A), 5 ng/ml TNF (TNF), 50ng/ml IFN\(\gamma\) + 5 ng/ml TNF (IFN\(\gamma\)+TNF) and 50 ng/ml IL-17A + 5 ng/ml TNF (IL-17A+TNF) for 48h. Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1 (n=3). (G) qPCR analysis for indicated gene expression by qPSC incubated with control medium (-), 30% of Tc17-CM (Tc17-CM) or 30% of CTL-CM (CTL-CM) for 48h. Fold of mRNA expression is shown, normalised to the control (-), which was arbitrarily set to 1 (n=3). (H) Quantification of \(\alpha\)SMA staining in tumor tissue obtained from \textit{Rag}1\textsuperscript{-/-} mice (n=4-5) orthotopically injected with KPC cells alone (-) or together with qPSC (qPSC), Tc17-iCAF (Tc17-iCAF) or DKO-CAF (DKO-CAF) based on previously published scoring\textsuperscript{20}. (I) Tumor weight of orthotopic tumors from \textit{Rag}1\textsuperscript{-/-} mice is shown (n=5-6 mice). (J) qPCR analysis for indicated gene expression by EPCAM CD45 PDPN\textsuperscript{+}CD90.1\textsuperscript{+} cells sorted from subcutaneous PaTu8988T tumors of mice co-injected with qPSC, Tc17-iCAF and DKO-CAF. Fold of mRNA expression is shown, normalised to qPSC group, which was arbitrarily set to 1 (n=3). (E-J) Biological replicates are plotted. Bars show mean ± s.d. In (E,G,H,I,J) statistic was evaluated by one-way ANOVA followed by Tukey’s HSD multiple comparison test \(*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, \) in (F) by one-way ANOVA followed by Dunnett’s post hoc test \(*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.\)
Supplementary Figure 7. Transcriptional signatures of tumor cells after co-culture with Tc17-iCAF vs TGFβ-myCAF. (A) Scheme of the experimental design showing RNA-sequencing of sorted PancOVA tumor cells after matrigel embedded co-culture with qPSC, TGFβ-myCAF or Tc17-iCAF. (B) STRING protein expression mapping of significantly upregulated genes in PancOVA cells (FDR<0.1) after co-culture with Tc17-iCAF. Only experimentally verified (confidence level 0.4) interactions between query proteins are shown. The distance between genes and the orientation is random. Highlighted are protein networks corresponding to the identified GO pathways in gene modules 3-7 in Figure 5B. (C) Heatmaps of color-coded z-scores from the rlog transformed expression values based on the GSEA for differential expression of Basal-like A or Classical-A human PDAC transcripts in PancOVA cells after 36 h coculture with Tc17-iCAF vs TGFβ-myCAF.
Supplementary Figure 8. Transcriptional signatures of tumor cells after co-culture with Tc17 cells vs Tc17-iCAF. (A) Scheme of the experimental design showing RNA-sequencing of sorted PanC<sup>OVA</sup> tumor cells after matrigel embedded co-culture with Tc17-iCAF or Tc17 cells for 36h. (B) Principal component analysis (PCA) of the top 500 genes with highest row variance expressed by PanC<sup>OVA</sup> cells cultured alone (red) or co-cultured with Tc17-iCAF (green) or with Tc17 cells (blue). (C) Heatmap of differentially expressed genes (Z score normalized, FDR≤0.001) by PanC<sup>OVA</sup> cells after 36h of co-culture with Tc17 cells or Tc17-iCAF classified into modules based on the mutual up- or down-regulation (n=4, biological replicates). (D) Pathway enrichment analysis for Gene Ontologies (GO): Biological processes. Bubble graph displays the three significantly enriched pathways by −log10 value (p adj) for four modules established in (C). (E) GSEA for differential expression of LIF-dependent pancreatic cancer cell transcripts obtained from Lifr<sup>WT</sup> KIP<sup>f/f</sup> CL vs Lifr<sup>f/f</sup> KIP<sup>f/f</sup> CL mice<sup>17</sup> in PanC<sup>OVA</sup> tumor cells ± Tc17-iCAF co-culture. (F) GSEA for differential expression of Classical-A or Basal-like A human PDAC transcripts<sup>18</sup> in PanC<sup>OVA</sup> tumor cells ± Tc17-iCAF co-culture.
**Supplementary Figure 9. Tc17 cells promote pancreatic tumor growth *in vivo*.** (A) Scheme of the experimental design. $5 \times 10^5$ KPC tumor cells ± $5 \times 10^5$ CD90.1+$\text{qPSC}$ were subcutaneously co-injected into WT mice, which on the same day received i.p. injections of PBS or $10^6$ WT (Tc17) or IL-17A/TKO Tc17 (DKOTc17) cells. Histology and FACS analysis were performed at the indicated end of the experiment. (B) Quantification of αSMA staining in tumor tissue of mice injected with KPC cells alone (-) or co-injected with qPSC (qPSC), with qPSC +Tc17 (qPSC+Tc17) or with qPSC + DKOTc17 (qPSC+DKOTc17), based on previously published scoring\(^{20}\), (mean ± s.d., n=5-6). (C) Tumor growth curve of subcutaneous KPC tumors is shown (mean ± sem, n=6-8 mice). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 indicate the tumor volume comparisons between the groups qPSC+Tc17 vs qPSC. (D) FACS analysis of Ly6c\(^{\text{high}}\) cell frequency in gated EPCAM-CD45-PDPN+CD90.1+ fibroblasts in subcutaneous KPC tumors after injection of PBS, Tc17 or DKOTc17 cells (mean ± s.d., n=3 tumors). Left, quantification of the frequency of Ly6c\(^{\text{high}}\) EPCAM CD45 PDPN+ cells, right, representative FACS plots are shown. (E) FACS analysis of Ly6c\(^{\text{high}}\) cell frequency in gated EPCAM CD45 PDPN+ cells in subcutaneous Panc\(^{\text{OVA}}\) tumors after injection of PBS, Tc17 or DKOTc17. Representative FACS plots are shown. (F) qPCR analysis for indicated gene expression by sorted EPCAM-CD45-PDPN+ cells obtained from subcutaneous Panc\(^{\text{OVA}}\) tumors after injections as indicated (mean ± s.d., n=5-8). Fold mRNA expression is shown, normalised to the DKOTc17 group, which was arbitrarily set to 1. (G) FACS analysis of EPCAM CD45 PDPN+ fibroblasts in subcutaneous Panc\(^{\text{OVA}}\) tumors for IL17RA and IL-17RC levels. Left, representative histograms of IL-17RA and IL-17RC levels expressed by EPCAM-CD45 PDPN+ fibroblasts in subcutaneous Panc\(^{\text{OVA}}\) tumors after co-injection of qPSC, dashed line indicates isotype control. Right, quantification of IL-17RA and IL-17RC levels expressed by gated EPCAM CD45 PDPN+ fibroblasts in subcutaneous Panc\(^{\text{OVA}}\) tumors after injections as indicated (mean ± s.d., n=3-4 tumors). (H) αSMA staining in tumor tissue from WT mice, which were orthotopically injected with KPC tumor cells and ± CD90.1+$\text{qPSC}$, followed by intraperitoneal adoptive transfer of WT (Tc17) or DKO Tc17 (DKOTc17) cells. The αSMA+ cell quantification (brown staining) was performed based on previously published scoring\(^{20}\), scale bar 200 µm. (I) Quantification of αSMA staining in tumor tissue of WT mice (mean ± s.d., n=8-9) orthotopically injected as indicated based on previously published scoring\(^{20}\). (J) Tumor weight of orthotopic tumors in WT mice is shown (tumor volume in mm\(^3\); mean ± sd, n=8-9 mice). (B,D,F,G,I,J) biological replicates are plotted. In (B) statistics was evaluated by Kruskal-Wallis-Test, **P<0.01, in (C) by two-way ANOVA followed by Bonferroni post-hoc test *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, in (D,J) by one-way ANOVA followed by Tukey's HSD multiple comparison test, *P<0.05, **P<0.01, ***P<0.001, in (F,I) by one-way ANOVA followed by Dunnett’s post hoc test, *P<0.05, **P<0.01, ****P<0.0001, in (G) two-tailed, unpaired t-test, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
Supplementary Figure 10. IL-17RA expressed by PSC is required for Tc17-driven tumor growth in vivo. (A) Tumor weight of orthotopic tumors from WT vs Il17ar<sup>−/−</sup> mice is shown (tumor weight in mg; mean ± sd, n=6-7 mice). (B) Scheme of the experimental design. 5 × 10<sup>5</sup> Panc<sup>OVA</sup> tumor cells with/without 5 × 10<sup>5</sup> WT or Il17ar<sup>−/−</sup>CD90<sup>+</sup>qPSC were subcutaneously injected into Il17ar<sup>−/−</sup> mice, which at the same received i.p. injections of PBS or 10<sup>6</sup> Tc17 cells or PBS. The CAF analysis was performed at the indicated end of the experiment. (C) Tumor
growth curve of subcutaneous tumors of mice injected with Panc\textsuperscript{OVA} cells alone (-) or co-injected with WT qPSC + Tc17 cells (WT qPSC+Tc17) or with \textit{Il17ra}\textsuperscript{-/-} qPSC + Tc17 cells (\textit{Il17ra}\textsuperscript{-/-} qPSC+Tc17) (mean ± sem, \(n=4-6\) mice). *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\), ****\(P<0.0001\) indicate the tumor volume comparisons between the groups WT qPSC+Tc17 vs \textit{Il17ra}\textsuperscript{-/-} qPSC+Tc17. (D) FACS analysis of Ly6c\textsuperscript{high} cell frequency in gated EPCAM CD45\textsuperscript{-} PDPN\textsuperscript{+} fibroblasts in subcutaneous Panc\textsuperscript{OVA} tumors of \textit{Il17ra}\textsuperscript{-/-} mice treated as indicated. (mean ± s.d., \(n=5\)). Left, quantification of the frequency of Ly6c\textsuperscript{high} EPCAM CD45 PDPN\textsuperscript{+} cells. Right, representative FACS plots are shown. (E) Tumor weight of orthotopic tumors from \textit{Il17ra}\textsuperscript{-/-} mice is shown (tumor volume in mm\textsuperscript{3}; mean ± sd, \(n=6-7\) mice). (F) Representative FACS plots of Ly6c\textsuperscript{high} cell frequency in gated EPCAM CD45 PDPN\textsuperscript{+} fibroblasts in orthotopic KPC tumors of \textit{Il17ra}\textsuperscript{-/-} mice treated as indicated. (A, C-E) the results from individual mice are plotted. In (A,E) ***\(P<0.001\), ****\(P<0.0001\) and \(P\) values by one-way ANOVA followed by Tukey’s HSD multiple comparison test, in (C) *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\), ****\(P<0.0001\) determined by two-way ANOVA with Bonferroni post-hoc test, in (D) two-tailed, ***\(P<0.001\) by unpaired \(t\)-test.