**SUPPLEMENTARY FIGURES AND FIGURE LEGENDS**

**Figure S1: Active NF-B signaling is associated with the iCAF phenotype. A.** Flow plots showing the gating strategy for sorting epithelial cells and CAFs from KPC tumors. **B.** Flow plots showing the gating strategy for epithelial cells and CAFs in KPC tumors, then used for the analysis of IL-1R1 expression. **C.** Violin plots showing single cell RNA-sequencing analysis of *Tnf*,*Tnfrsf1a*, *Cdh1* and *Pdpn* of a representative KPC tumor (n=2) in CAFs (orange) and epithelial cells (green). **D.** ELISA of TNF- from media of mouse 2D KPC cells (n=2), tumor (T) (n=5) and metastatic (M) (n=2) organoids, and controls that do not induce the iCAF phenotype (n=2 for each control). Results show mean ± SEM (standard error of the mean). **E.** Schematic illustration of the culture methods used to model quiescent PSCs, iCAFs and myCAFs. PSCs remain quiescent when cultured as monocultures in Matrigel in control media (i.e. 5% FBS DMEM). PSCs in Matrigel are activated to iCAFs when cultured with tumor organoid-conditioned media or in a transwell system with tumor organoids in 5% FBS DMEM. PSCs acquire myofibroblastic features when cultured in monolayer in 5% FBS DMEM. **F.** Visualization of *Col1a1*+ fibroblasts in a representative KPC tumor (n=2) by T-distributed stochastic neighbor embedding (tSNE), colored by subsets, as identified by graph-based clustering and marker gene expression (blue: myCAFs; orange: iCAFs). **G.** Heatmap of the top 25 differentially expressed genes *in vivo* in iCAFs and myCAFs in KPC tumors, with cells as columns and genes as rows. Color scheme represents Z-score distribution. **H.** Venn diagrams showing the overlap between the *in vitro* and *in vivo* signatures of iCAFs and myCAFs significantly upregulated genes compared to myCAFs and iCAFs, respectively, based on the single cell RNA-sequencing dataset of KPC tumors and on our previously published RNA-sequencing dataset of iCAFs and myCAFs cultured with our *in vitro* model system (19). **I.** Gene set enrichment analysis of single cell RNA-sequencing analysis of KPC tumors showing significantly upregulated and downregulated pathways *in vivo* in iCAFs compared to myCAFs. NES, normalized enrichment score; FDR, false discovery rate. **J.** Western blot analysis of phosphorylated p65 (p-p65), phosphorylated IB (p-IB) and IB in PSCs cultured in Matrigel in control media or tumor organoid-conditioned media (CM) for 10 min and 30 min. Loading control, HSP90.

**Figure S2: IL-1 signaling is the main pathway responsible for the induction of an inflammatory phenotype in CAFs**. **A.** qPCR analysis of iCAF (*Il1a* *Il6*, *Lif*, *Cxcl1* and *Csf3*) and myCAF (*Acta2* and *Ctgf*) markers in primary mouse PSC lines cultured in Matrigel in control media in the presence or absence of 1 ng/mL mouse IL-1for 4 days. Results show mean ± SD of 3 technical replicates. \*P<0.05, \*\*P<0.01, paired Student’s *t* test. **B.** qPCR analysis of *IL1*A*IL6*, *LIF*, *CXCL1*, *CSF3*, *ACTA2* and *CTGF* in human primary PSCs cultured in Matrigel with 1 ng/mL human IL-1for 4 days. Results show mean ± SD (standard deviation) of 2 technical replicates. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, paired Student’s *t* test. **C.** qPCR analysis of *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in PSCs cultured in Matrigel in control media in the presence or absence of 1 ng/mL mouse IL-1or 10 ng/mL mouse TNF-for 4 days. Results show mean ± SEM of 3 biological replicates. \*P<0.05, \*\*P<0.01, paired Student’s *t* test. **D.** qPCR analysis of *Il1a**Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in PSCs cultured in Matrigel with control media or tumor organoid-conditioned media in the presence of a neutralizing antibody targeting TNF- or an IgG control for 4 days. Results show mean ± SEM of 3 biological replicates. No statistical difference was found between PSCs cultured with tumor organoid-conditioned media in the presence or absence of anti-TNF-, as calculated by paired Student’s *t* test. **E.** Proliferation curves of PSCs cultured as myCAFs in the presence of a neutralizing antibody targeting IL-1 or an IgG control. Results show mean ± SEM of 2 biological replicates. **F.** ELISA of IL-1 from media of Rosa26-targeted controls and IL-1 knockout tumor organoids. Results show mean ± SD of 2 technical replicates. **G.** Western blot analysis of IL-1R1 in IL-1R1 knockout PSCs and Rosa26-targeted controls. Loading control, ACTIN. **H.** Western blot analysis of IL-1R1 in IL-1R1 knockout PSCs and Rosa26-targeted controls with or without ectopic expression of guide-resistant IL-1R1 constructs. Loading control, ACTIN. **I.** qPCR analysis of *Il1r1*, *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in Rosa26-targeted controls and IL-1R1 knockout PSCs with or without ectopic expression of guide-resistant IL-1R1 constructs cultured in Matrigel with control media or tumor organoid-conditioned media. Results show mean ± SEM of 5 biological replicates (for 3 knockout clones). \*P<0.05, \*\*P<0.01, paired Student’s *t* test. **J.** Western blot analysis of IL-1R1 in IL-1R1 wild-type PSCs and primary PSCs isolated from IL-1R1 knockout mice.Loading control, ACTIN. **K.** qPCR analysis of *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in primary IL-1R1 knockout PSCs (i.e. PSCs isolated from IL-1R1 knockout mice) cultured in Matrigel in control media in the presence or absence of 1 ng/mL IL-1 for 4 days. Results show mean ± SEM of 2 biological replicates. No statistical difference was found, as calculated by paired Student’s *t* test.

**Figure S3: Absence of IL-1 signaling shifts iCAFs to myCAFs *in vivo*. A.** Representative Masson’s trichrome stain in OGOs derived from transplantation of Rosa26-targeted controls and IL-1 knockout tumor organoids (n= 7 and 8, respectively). Scale bar, 200 m. **B.** Quantification of Masson’s trichrome stain in OGOs derived from transplantation of Rosa26-targeted controls and IL-1 knockout tumor organoids. Results show mean ± SEM of 7 and 8 biological replicates, respectively. No statistical difference was found, as calculated by paired Student’s *t* test. **C.** Representative immunohistochemistry of SMA stain in OGOs derived from transplantation of Rosa26-targeted controls and IL-1 knockout tumor organoids (n= 7 and 8, respectively). Scale bar, 200 m. **D.** Quantification of SMA stain in OGOs derived from transplantation of Rosa26-targeted controls and IL-1 knockout tumor organoids. Results show mean ± SEM of 7 and 8 biological replicates, respectively. No statistical difference was found, as calculated by paired Student’s *t* test. **E.** mRNA levels of *Il1b*relative to *Hprt* expressionin Rosa26-targeted controls and IL-1 knockout tumor organoids, and in EpCAM+ cells sorted from Rosa26-targeted controls and IL-1 knockout OGOs. Results show mean ± SEM of 4 (organoids), 12 (control OGOs) and 19 (IL-1 knockout OGOs) biological replicates. Different symbols identify tumors derived from transplantation of 3 distinct IL-1 knockout organoid lines. \*\*P<0.01, unpaired Student’s *t* test. **F.** mRNA levels of *Tnf*relative to *Hprt* expressionin Rosa26-targeted controls and IL-1 knockout tumor organoids, and in EpCAM+ cells sorted from Rosa26-targeted controls and IL-1 knockout OGOs. Results show mean ± SEM of 4 (organoids), 12 (control OGOs) and 19 (IL-1 knockout OGOs) biological replicates. Different symbols identify tumors derived from transplantation of 3 distinct IL-1 knockout organoid lines. \*P<0.05, \*\*P<0.01, unpaired Student’s *t* test. **G.** Representative Masson’s trichrome stain in tumors grown in B6J or IL-1R1 knockout hosts (n= 8 and 9, respectively). Scale bar, 200 m. **H.** Quantification of Masson’s trichrome stain in tumors grown in B6J or IL-1R1 knockout hosts. Results show mean ± SEM of 8 and 9 biological replicates, respectively. No statistical difference was found, as calculated by unpaired Student’s *t* test. **I.** Representative immunohistochemistry of SMA stain in tumors grown in B6J or IL-1R1 knockout hosts (n= 8 and 9, respectively). Scale bar, 200 m. **J**. Quantification of SMA stain in tumors grown in B6J or IL-1R1 knockout hosts. Results show mean ± SEM of 8 and 9 biological replicates, respectively. No statistical difference was found, as calculated by unpaired Student’s *t* test. **K.** Violin plots showing single cell RNA-sequencing analysis of the myCAF marker *Acta2,* and the iCAF markers *Cxcl1* and *Ly6c1* in myCAFs (blue) and iCAFs (orange) in a representative KPC tumor (n=2). **L.** tSNEshowing *Ly6c1* normalized gene expression in *Col1a1*+ fibroblasts in a representative KPC tumor (n=2). The black line delimits the iCAF population, as identified in Supplementary Figure S1F. **M.** Representative flow cytometric analysis of Ly6C+ iCAFs and Ly6C-myCAFs in tumors derived from orthotopic transplantation of 2 tumor organoid lines in B6J or IL-1R1 knockout hosts (n=5). The values shown represent the percentages from the parental gate. **N.** Representative flow cytometric analysis of Ly6C+ iCAFs and Ly6C- myCAFs in tumors derived from orthotopic transplantation of 2 tumor organoid lines in B6J or IL-1R1 knockout hosts (n=5).­

**Figure S4: IL-1-mediated induction of autocrine LIF in PSCs activates JAK/STAT signaling and promotes iCAF formation. A.** Western blot analysis of p-JAK1, p-JAK2, p-STAT3 and p-STAT1 in myCAFs, iCAFs and quiescent PSCs. Loading control, ACTIN. **B.** qPCR analysis of iCAF (*Il1a* *Il6*, *Lif*, *Cxcl1* and *Csf3*) and myCAF (*Acta2* and *Ctgf*) markers in PSCs cultured in Matrigel in control media or tumor organoid-conditioned media in the presence of a neutralizing antibody targeting G-CSF or an IgG control for 4 days. Results show mean ± SEM of 2 biological replicates. No statistical difference was found between PSCs cultured with tumor organoid-conditioned media in the presence or absence of anti-G-CSF, as calculated by paired Student’s *t* test. **C.** qPCR analysis of *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in PSCs cultured in Matrigel in control media or tumor organoid-conditioned media in the presence of a neutralizing antibody targeting IL-6 or an IgG control for 4 days. Results show mean ± SEM of 5 biological replicates. No statistical difference was found between PSCs cultured with tumor organoid-conditioned media in the presence or absence of anti-IL-6, as calculated by paired Student’s *t* test. **D.** Western blot analysis of p-JAK1, p-JAK2, p-STAT3 and p-STAT1 of controls and IL-6 knockout PSCs cultured in Matrigel with control media or tumor organoid-conditioned media (CM) for 4 days. Loading control, ACTIN. **E.** ELISA of LIF from media of PSCs cultured in Matrigel in control media in the presence or absence of 1 ng/mL mouse IL-1 for 4 days. Results show mean ± SD of 4 and 3 technical replicates for PSC4 and PSC5, respectively. **F.** ELISA of LIF from media of Rosa26-targeted controls and LIF knockout PSC clones cultured with 1 ng/mL mouse IL-1 for 4 days. Results show mean ± SD of 2 technical replicates. **G.** Western blot analysis of p-JAK1, p-JAK2, p-STAT3 and p-STAT1 of Rosa26-targeted controls and LIF knockout PSCs cultured in Matrigel with control media or tumor organoid-conditioned media (CM) for 4 days. Loading control, ACTIN.

**Figure S5: JAK/STAT signaling mediates the induction of the iCAF phenotype. A.** Western blot analysis of p-STAT3, p-STAT1 and SMA in PSCs cultured in Matrigel in the presence or absence of 1 ng/mL mouse IL-1 and 500 nM JAK inhibitor (JAKi) for 4 days. Loading control, ACTIN. **B.** Western blot analysis of p-STAT3, p-STAT1 and SMA in PSCs cultured with control media or tumor organoid-conditioned media (CM) in the presence or absence of 500 nM JAKi for 4 days. Loading control, ACTIN. **C.** ELISA of IL-6, G-CSF and LIF from media of PSCs cultured in Matrigel with control media or tumor organoid-conditioned media in the presence or absence of 500 nM JAKi for 4 days. Results show mean ± SEM of 9, 6 and 5 biological replicates, respectively. \*\*P<0.01, \*\*\*P<0.001, paired Student’s *t* test. **D.** qPCR analysis of iCAF (*Il1a* *Il6*, *Lif*, *Cxcl1* and *Csf3*) and myCAF (*Acta2* and *Ctgf*) markers in PSCs cultured in Matrigel in the presence or absence of 1 ng/mL mouse IL-1 and 500 nM JAKi for 4 days. Results show mean ± SEM of 3 biological replicates. \*P<0.05, \*\*\*P<0.001, paired Student’s *t* test. **E.** Proliferation curves of PSCs cultured as myCAFs in the presence or absence of 500 nM JAKi. Results show mean ± SD of 5 technical replicates. \*\*P<0.01, unpaired Student’s *t* test calculated for the last time point. **F.** Gene set enrichment analysis showing significantly downregulated and upregulated pathways in PSCs cultured in Matrigel with tumor organoid-conditioned media and JAKi for 4 days compared to untreated PSCs cultured in tumor organoid-conditioned media. NES, normalized enrichment score; FDR, false discovery rate. **G.** Western blot analysis of p-STAT3 and p-STAT1 in PSCs cultured in Matrigel with control media or tumor organoid-conditioned media (CM) in the presence or absence of 500 nM JAKi for the last 24 h following 4 days in culture with conditioned media alone. Loading control, ACTIN. **H.** Proliferation curves of PSCs cultured in Matrigel with control media or tumor organoid-conditioned media in the presence or absence of 500 nM JAKi for the last 24 h. Results show mean ± SEM of 2 biological replicates. The arrow indicates the time the drug was added to the media. **I.** Western blot analysis of STAT1 in Rosa26-targeted controls and STAT1 knockout PSC clones. Loading control, ACTIN. **J.** qPCR analysis of *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in controls and STAT1 knockout PSCs cultured in Matrigel in transwell with tumor organoids for 4 days. Results show mean ± SEM of 2 biological replicates. No statistical difference was found between controls and STAT1 knockout PSCs, as calculated by paired Student’s *t* test. **K.** Western blot analysis of STAT3 in Rosa26-targeted controls and STAT3 knockout PSCs. Loading control, ACTIN. **L.** qPCR analysis of *Il1r1* in iCAFs compared to myCAFs. Results show mean ± SEM of 4 biological replicates. \*P<0.05, paired Student’s *t* test. **M.** Western blot analysis of IL-1R1 in PSCs cultured in Matrigel with control media or tumor organoid-conditioned media (CM) in the presence or absence of 500 nM JAKi for 4 days. Loading control, ACTIN. **N.** Western blot analysis of IL-1R1 in Rosa26-targeted controls and STAT3 knockout PSCs. Loading control, ACTIN. **O.** Representative immunofluorescence co-stains of p-STAT3 (green) and SMA (red) in human PDAC sections (n= 5). Counterstain, DAPI (blue). Arrows indicate examples of SMA+ p-STAT3- myCAFs, arrow-heads indicate examples of SMA- p-STAT3+ cells, the asterisk indicates examples of SMA+ p-STAT3+ cells. Scale bar, 100 m. **P.** Quantification of SMA+ p-STAT3- cells and SMA+ p-STAT3+ cells in human PDAC sections. Results show mean ± SEM of 5 biological replicates. \*\*\*P<0.001, ­­­­­­paired Student’s *t* test.

**Figure S6: TGF- signaling antagonizes IL-1-induced JAK/STAT signaling and inhibits the iCAF phenotype. A.** qPCR analysis of iCAF markers (*Il1a**Il6*, *Lif*, *Cxcl1* and *Csf3*) and TGF- responsive genes (*Ctgf* and *Col1a1*) in mouse PSCs cultured in Matrigel in control media in the presence or absence of 20 ng/mL mouse TGF-for 4 days. Results show mean ± SEM of 3 biological replicates. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, paired Student’s *t* test. **B.** Proliferation curves of mouse PSCs cultured in Matrigel in control media in the presence or absence of 20 ng/mL mouse TGF-. Results show mean ± SD of 5 technical replicates. \*\*\*P<0.001, unpaired Student’s *t* test calculated for the last time point. **C.** Bright field images of PSCs cultured in Matrigel in control media in the presence or absence of 20 ng/mL mouse TGF-for 6 days. Bar, 100 m. **D.** Representative immunofluorescence co-stains of p-SMAD2 (green) and E-cadherin (ECAD, red) in KPC tumor sections (n=2). Counterstain, DAPI (blue). Arrows indicate examples of SMA+ p-SMAD2+ cells. Scale bars, 100 m. **E.** Representative immunofluorescence co-stains of p-SMAD2 (green) and SMA (red) in human PDAC sections (n=3). Counterstain, DAPI (blue). Arrowheads indicate examples of SMA+ p-SMAD2+ cells. Scale bar, 100 m. **F.** Quantification of p-SMAD2+ SMA- cells and p-SMAD2+ SMA+ cells in human PDAC sections. Results show mean ± SEM of 3 biological replicates. \*P<0.05, paired Student’s *t* test. **G.** qPCR analysis of *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in PSCs cultured in Matrigel with control media or tumor organoid-conditioned media in the presence or absence of 1 M TGF- signaling inhibitor A83-01 for 4 days. Results show mean ± SEM of 4 biological replicates. \*\*P<0.01, \*\*\*P<0.001, paired Student’s *t* test. **H.** qPCR analysis of *Il1a* *Il6*, *Lif*, *Cxcl1*, *Csf3*, *Acta2* and *Ctgf* in mouse PSCs cultured in Matrigel in control media in the presence or absence of 0.1 ng/mL mouse IL-1 and 20 ng/mL mouse TGF- for 4 days. Results show mean ± SEM of 2 biological replicates. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, paired Student’s *t* test**. I.** qPCR analysis of *IL1A* *IL6*, *LIF*, *CXCL1*, *CSF3*, *ACTA2* and *CTGF* in human PSCs cultured in Matrigel with 0.1 ng/mL human IL-1 for 4 days in the presence or absence of 20 ng/mL human TGF-. Results show mean ± SD of 4 technical replicates. \*P<0.05, \*\*P<0.01, paired Student’s *t* test**. J.** Western blot analysis of p-JAK1, p-JAK2, p-STAT1, p-STAT3, p-SMAD2, CTGF, SMA in mouse PSCs cultured in Matrigel with 0.1 ng/mL mouse IL-1 and 20 ng/mL mouse TGF- for 4 days. Loading control, ACTIN. **K.** Proliferation curves of quiescent PSCs, iCAFs and myCAFs. Results show mean ± SD of 5 technical replicates. \*\*\*P<0.001, unpaired Student’s *t* test calculated for the last time point. **L.** qPCR analysis of *Il1r1* in mouse and human PSCs cultured in Matrigel with control media in the presence or absence of 0.1 ng/mL mouse or human IL-1 and 20 ng/mL mouse or human TGF- for 4 days. Results show mean ± SEM of 3 and 2 biological replicates for mouse and human PSCs, respectively. \*P<0.05, \*\*P<0.01, paired Student’s *t* test. **M.** Western blot analysis of IL-1R1 in mouse PSCs cultured in Matrigel with 0.1 ng/mL mouse IL-1 and 20 ng/mL mouse TGF- for 4 days. Loading control, ACTIN.

**Figure S7: Inhibition of TGF- signaling targets myofibroblasts *in vivo*. A.** Representative immunohistochemistry of phosphorylated SMAD2 (p-SMAD2) stain of vehicle- and TGFBR inhibitor (TGFBRi)- treated KPC tumor sections (n= 6 and 11, respectively). Scale bar, 200 m. **B.** Quantification of p-SMAD2 stain in vehicle- and TGFBRi- treated KPC tumors. Results show mean ± SEM of 6 and 11 biological replicates, respectively. \*P<0.05, unpaired Student’s *t* test. **C.** Representative sequential immunohistochemistry of phosphorylated H3 (p-H3, brown) and E-cadherin (ECAD, purple) stains of vehicle- and TGFBRi- treated KPC tumor sections (n= 6 and 11, respectively). Scale bar, 200 m. **D.** Quantification of p-H3 stain in vehicle- and TGFBRi- treated KPC tumors. Results show mean ± SEM of 6 and 11 biological replicates, respectively. No statistical difference was found, as calculated by unpaired Student’s *t* test. **E.** Representative flow cytometric analysis of iCAFs and myCAFs in vehicle- and TGFBRi- treated KPC tumors (n= 3 and 7 per cohort, respectively). The values shown represent the percentages from the parental gate. **F.** Quantification of Ly6C-myCAF/Ly6C+ iCAF ratio in vehicle- and TGFBRi- treated KPC tumors, as assessed by flow cytometry. Results show mean ± SEM of 3 and 7 biological replicates, respectively. No statistical difference was found, as calculated by unpaired Student’s *t* test.

**Figure S8: JAK inhibition shifts iCAFs to a myofibroblastic phenotype *in vivo*. A.** Representative immunohistochemistry of phosphorylated STAT3 (p-STAT3) stain of vehicle- and JAK inhibitor (JAKi)- treated KPC tumor sections (n=7). Scale bar, 200 m. **B.** Quantification of p-STAT3 stain in vehicle- and JAKi- treated KPC tumors. Results show mean ± SEM of 7 biological replicates. \*\*\*P<0.001, unpaired Student’s *t* test. **C.** Representative sequential immunohistochemistry of phosphorylated H3 (p-H3, brown) and E-cadherin (ECAD, purple) stains of vehicle- and JAKi- treated KPC tumor sections (n=7). Scale bar, 200 m. **D.** Quantification of p-H3 stain in vehicle- and JAKi- treated KPC tumors. Results show mean ± SEM of 7 biological replicates. \*P<0.05, unpaired Student’s *t* test. **E.** Representative flow cytometric analysis of CAFs in vehicle- and JAKi- treated KPC tumors (n=4 per cohort). The values shown represent the percentages from the single cell gate. **F.** Violin plots showing single cell RNA-sequencing analysis of the cell cycle genes *Cks2* and *Ccnb2*, and the proliferation marker *Mki67* in myCAFs (blue) and iCAFs (orange) in a representative KPC tumor (n=2). **G.** Representative flow cytometric analysis of iCAFs and myCAFs in vehicle- and JAKi- treated KPC tumors (n=3 per cohort). The values shown represent the percentages from the parental gate. **H.** Representative flow cytometric analysis of Ly6C+ iCAFs and Ly6C- myCAFs in vehicle- and JAKi- treated KPC tumors (n=3 per cohort).