Supplementary Material

Aguilera et al., Collagen signaling enhances tumor progression after anti-VEGF therapy in a murine model of PDA

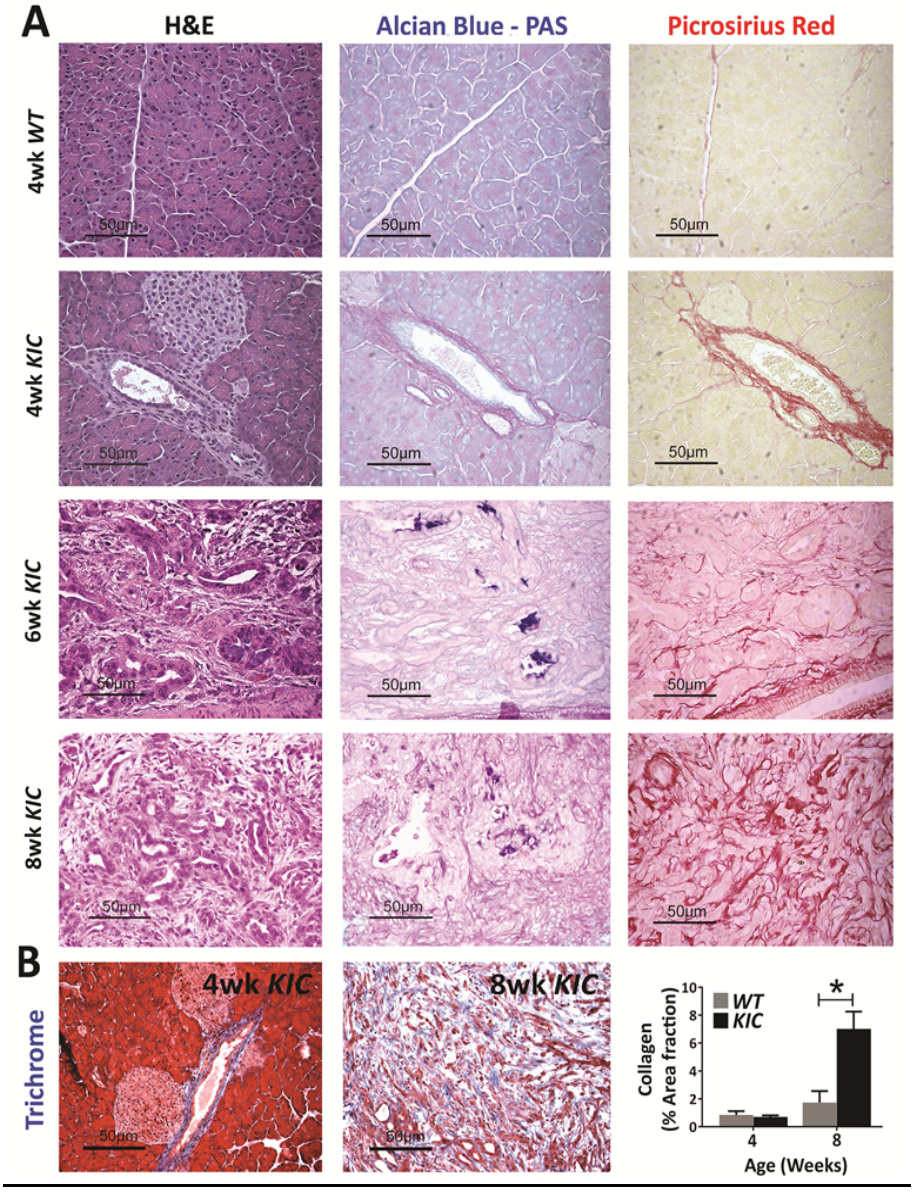
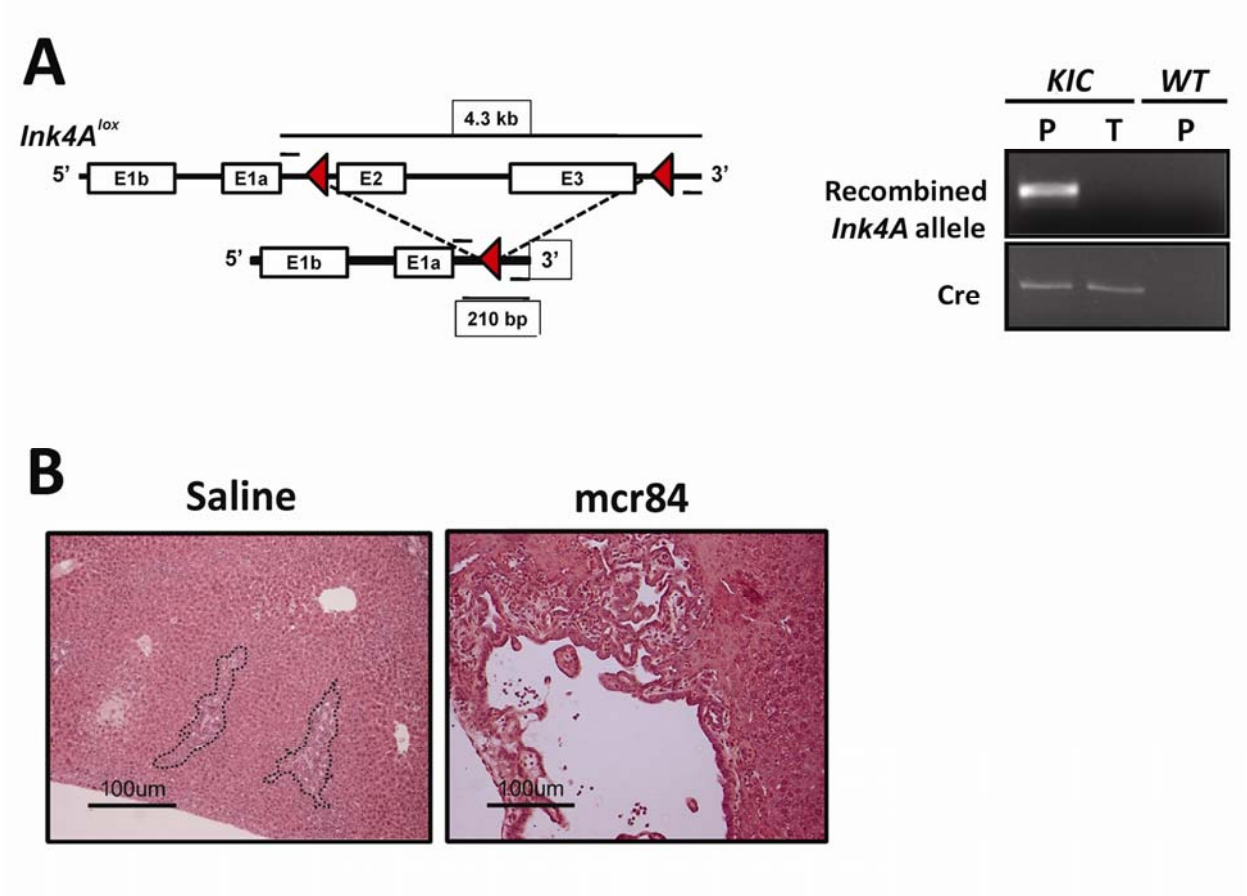
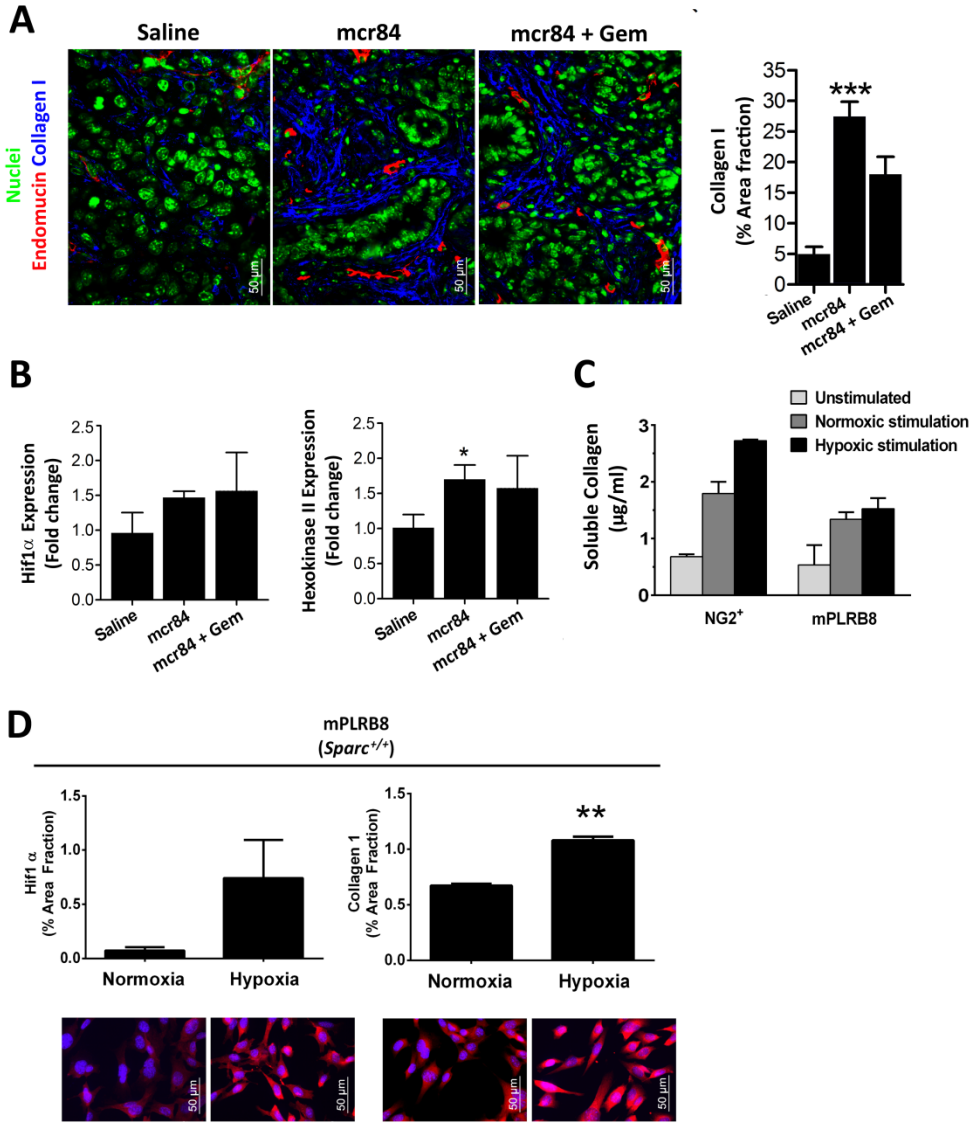


Figure 1. Murine PDA model mimics the human disease.

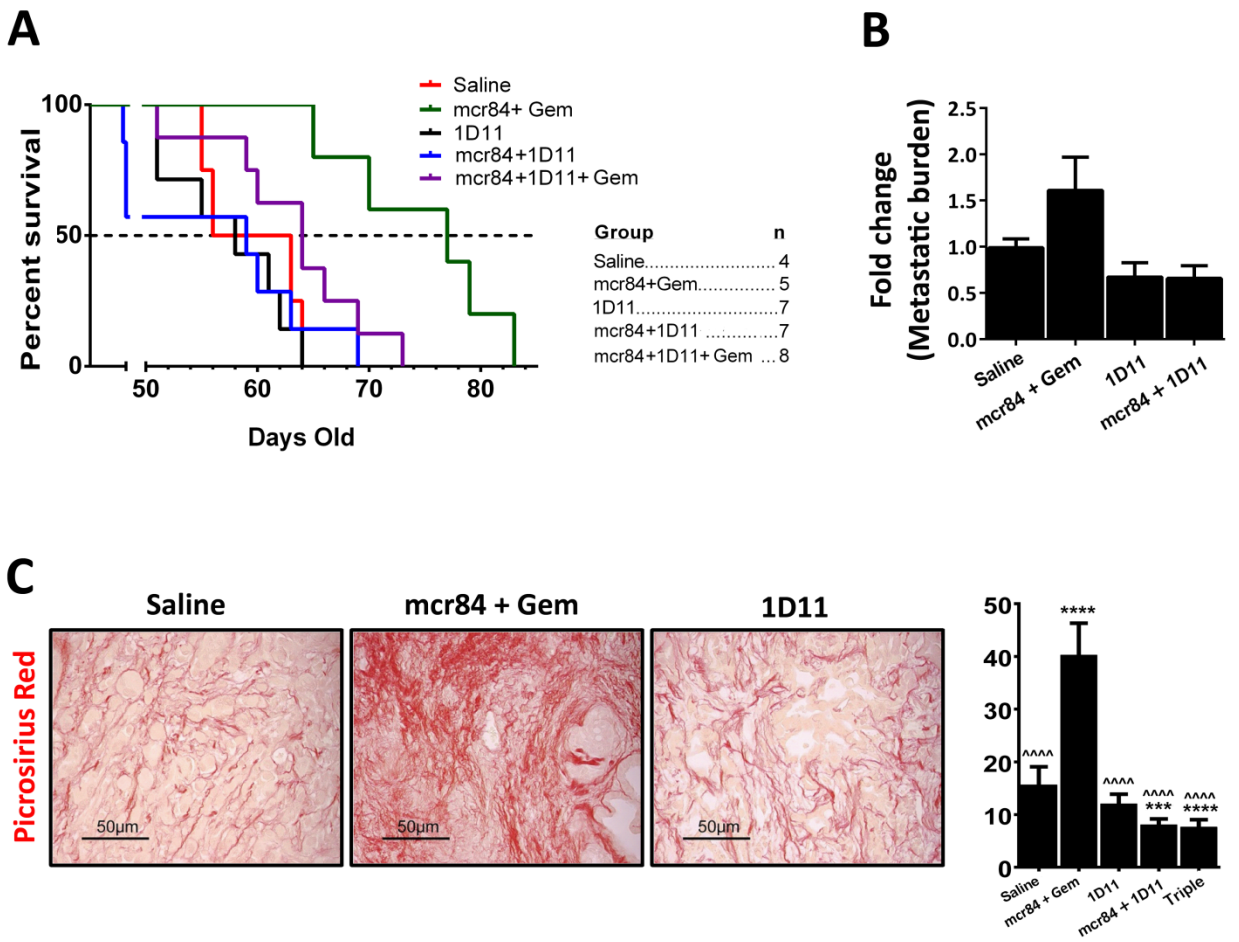
Characterization of tumor growth in *KIC* mice (*LSL-Kras*^{G12D}*; Cdkn2a*$^{lox/lox}$*; p48*^{Cre}). (A) *KIC* mice exhibit malignant adenocarcinoma as early as 4 weeks of age. Hematoxylin and eosin staining of 4Wk WT and *KIC* mice (4wk-8wk), characterization of *KIC* progression and malignancy through loss of Alcian Blue-PAS staining, and depiction of the fibrotic nature of *KIC* tumors with picrosirius red which stains fibrillar collagens red. (B) Fibrotic nature of *KIC* tumors. 4Wk and 8Wk *KIC* tissue were stained with trichrome which stains fibrillar collagens blue. Error bars represent SEM (*, $p < 0.05$).



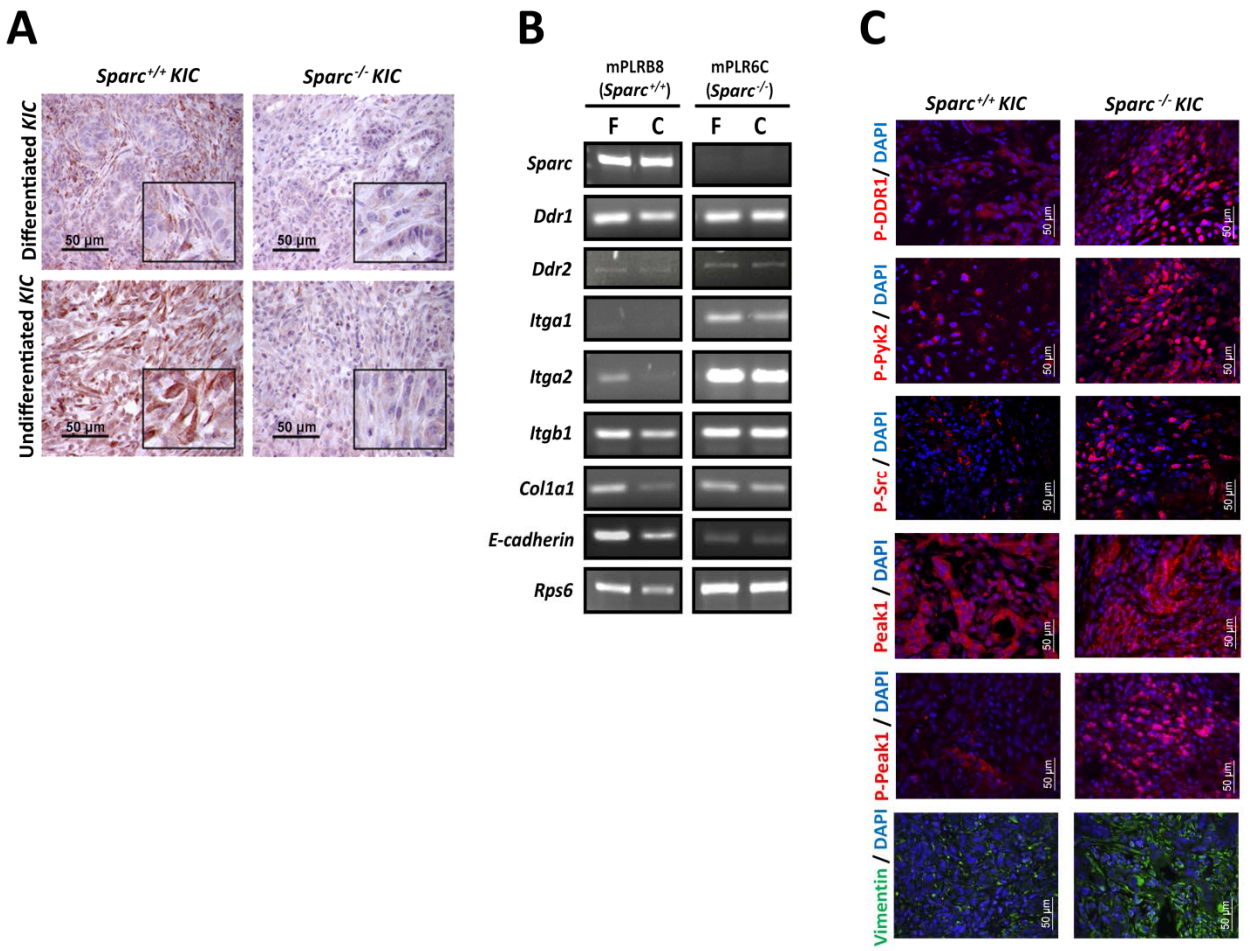
Supplementary Figure 2. Metastatic burden in the liver assessed by PCR and histology. Quantitative PCR for the recombined *Ink4a/Arf* allele (A) and histological analysis (B) provided an accurate measure of tumor burden in liver and demonstrated that mice receiving mcr84 had a higher burden than saline treated mice.



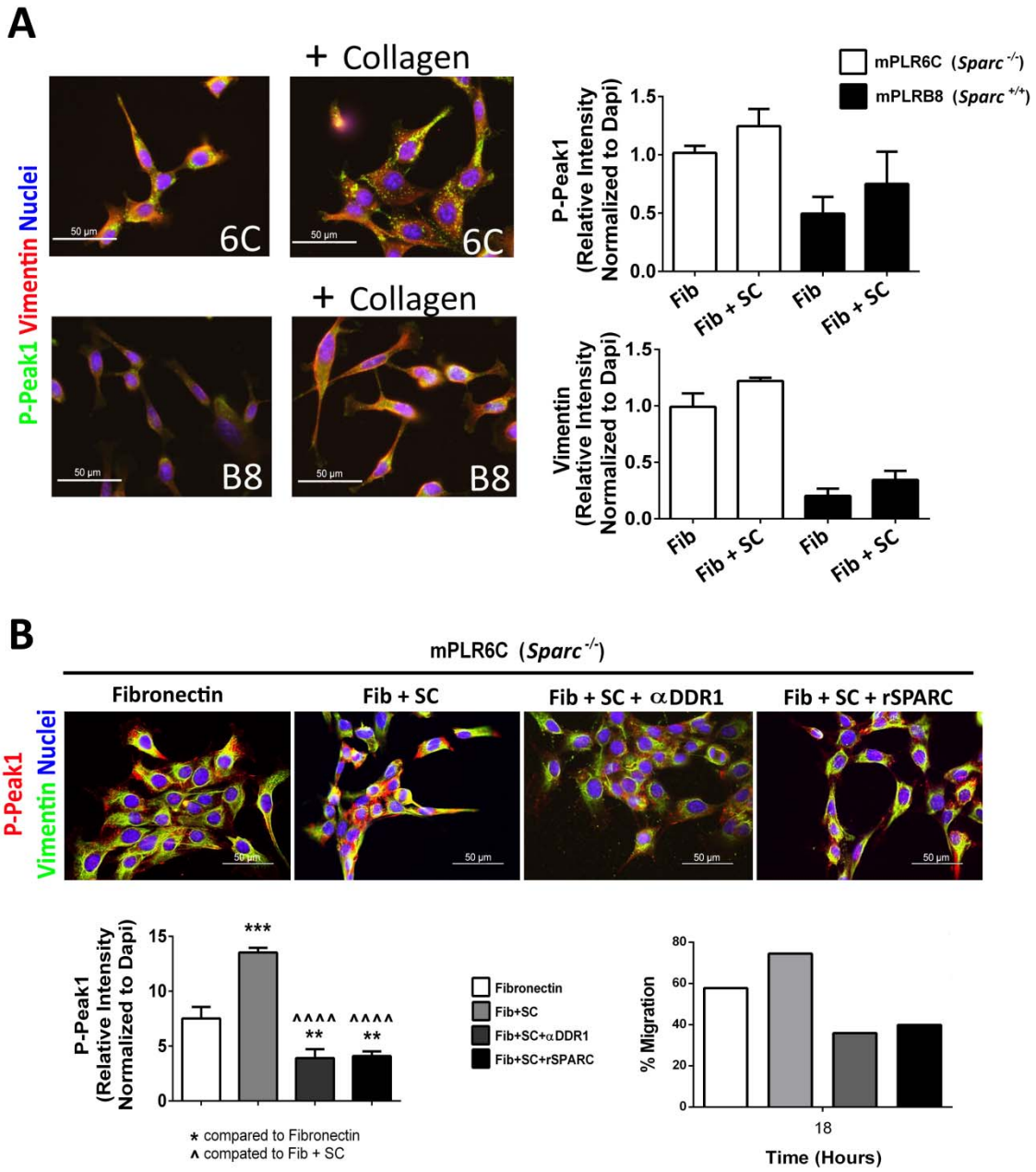
Supplementary Figure 3. Concordant with the induction of hypoxia, the expression of collagen I, Hif1α, and Hexokinase II were elevated in tumors from mice treated with mcr84 or mcr84 plus Gem. (A) Tumors from *KIC* mice following a 4-week treatment regimen were sectioned and stained with anti-collagen I antibody (blood vessels were visualized with anti-endomucin antibody). Collagen I positive area was quantitated and is presented in the right panel. (B) Expression of HIF1α and hexokinase II was assessed in these tumors as readouts of the hypoxic microenvironments. (C) Collagen is secreted by tumor-associated NG2+ cells. Collagen secretion was assessed in vitro under normoxic and hypoxic (2% O_2) conditions and compared between a immunomagetically separated NG2+ PDA stromal cell line and a PDA clone isolated from *KIC* mice. (D) Primary murine *KIC* PDA cells were plated on collagen under normoxic and hypoxic conditions. Hypoxia stimulated Hif1α, as well as an induction collagen secretion from PDA cells. Error bars represent SEM (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$).



Supplementary Figure 4. Anti-TGFβ inhibits mcr84-induced collagen deposition. *KIC* mice were treated with saline (n=4), mcr84 plus Gem (n=5), 1D11 (n=7), 1D11+mcr84 (n=7), or the triple combination (n=8) starting at 4 weeks of age (A-C). (C) Blockade of TGFβ with 1D11 dramatically reduced hypoxia-induced collagen deposition. Error bars represent SEM ***, $p < 0.005$; ****, $p < 0.00005$ vs saline; ^^^^, $p < 0.00005$ vs mcr84 + Gem; One way ANOVA with Tukey's MCT.



Supplementary Figure 5. PDA cells express collagen receptors and Sparc. (A) Expression of Sparc in murine *KIC*. Tumors were harvested from moribund *Sparc*$^{+/+}$*:KIC* and *Sparc*$^{-/-}$*:KIC* mice, sectioned and stained for Sparc. Insets are enlarged to show stromal staining pattern in both differentiated and undifferentiated regions of the tumor. *Sparc*$^{-/-}$*:KIC* was used as a negative control for staining. (B) Collagen receptor expression profile of PDA clones mPLRB8 (*Sparc*$^{+/+}$) and mPLR6C (*Sparc*$^{-/-}$) on fibronectin-coated (F) and collagen I-coated (C) conditions. RT-PCR was performed on cDNA using primers sets for *Sparc*, discoidin domain receptor 1 (*Ddr1*), discoidin domain receptor 2 (*Ddr2*), integrin-α1 (*Itga1*), integrin-α2 (*Itga2*) integrin-β1 (*Itgb1*), collagen 1α1 (*Col1a1*), and ribosomal protein S6 (*Rps6*). (C) Tissue from *Sparc*$^{-/-}$*:KIC* and *Sparc*$^{+/+}$*:KIC* animals was analyzed for Ddr1 phosphorylation, as well as activation of downstream targets. The lack of Sparc leads to an enhanced induction of Vimentin and Ddr1 activation, as well as the activation of Pyk2, Src, and Peak1.



Supplementary Figure 6. Peak1 activation by collagen is sensitive to Ddr1 blockade. (A) PDA cells differentially express Sparc, which mediates differential induction of Peak1 activation. mPLRB8 (*Sparc*$^{+/+}$) and mPLR6C (*Sparc*$^{-/-}$) were plated on fibronectin in the presence or absence of 10ug/ml soluble collagen (+ Collagen). The presence of soluble collagen enhances the phosphorylation of Peak1 and the induction of a mesenchymal marker, vimentin. Collagen-induced Peak1 activation is elevated in cells that lack Sparc. (B) mPLR6C (*Sparc*$^{-/-}$) was plated on fibronectin-coated (Fib) chamber slides in the presence or absence of 10ug/ml soluble collagen (SC), a DDR1-blocking antibody (αDDR1), or recombinant SPARC (rSPARC). Ddr1 inhibitors reduced Peak1 activation as well as migration on each respective condition. Migration was measured with a scratch (wound healing) assay. Error bars represent SEM (**, $p < 0.005$; ***, $p < 0.0005$; ^^^^, $p < 0.00005$).