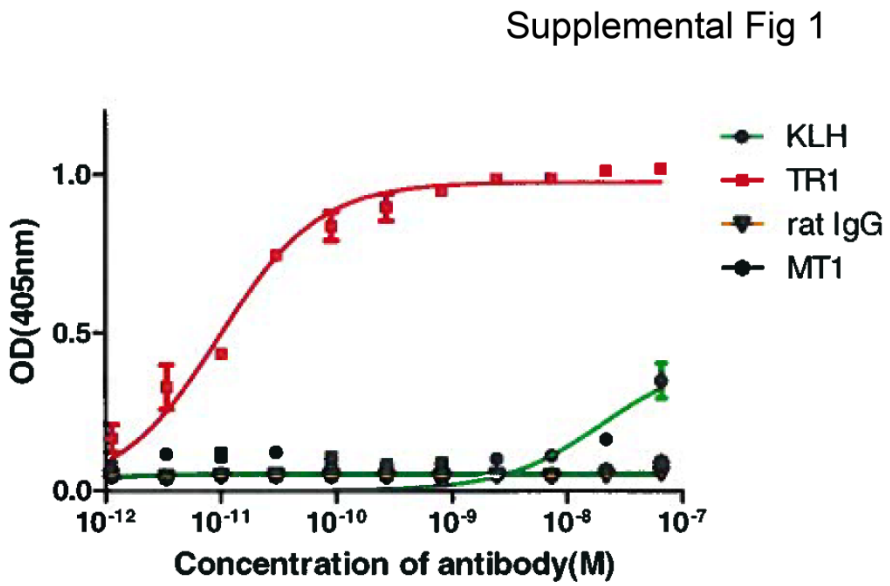
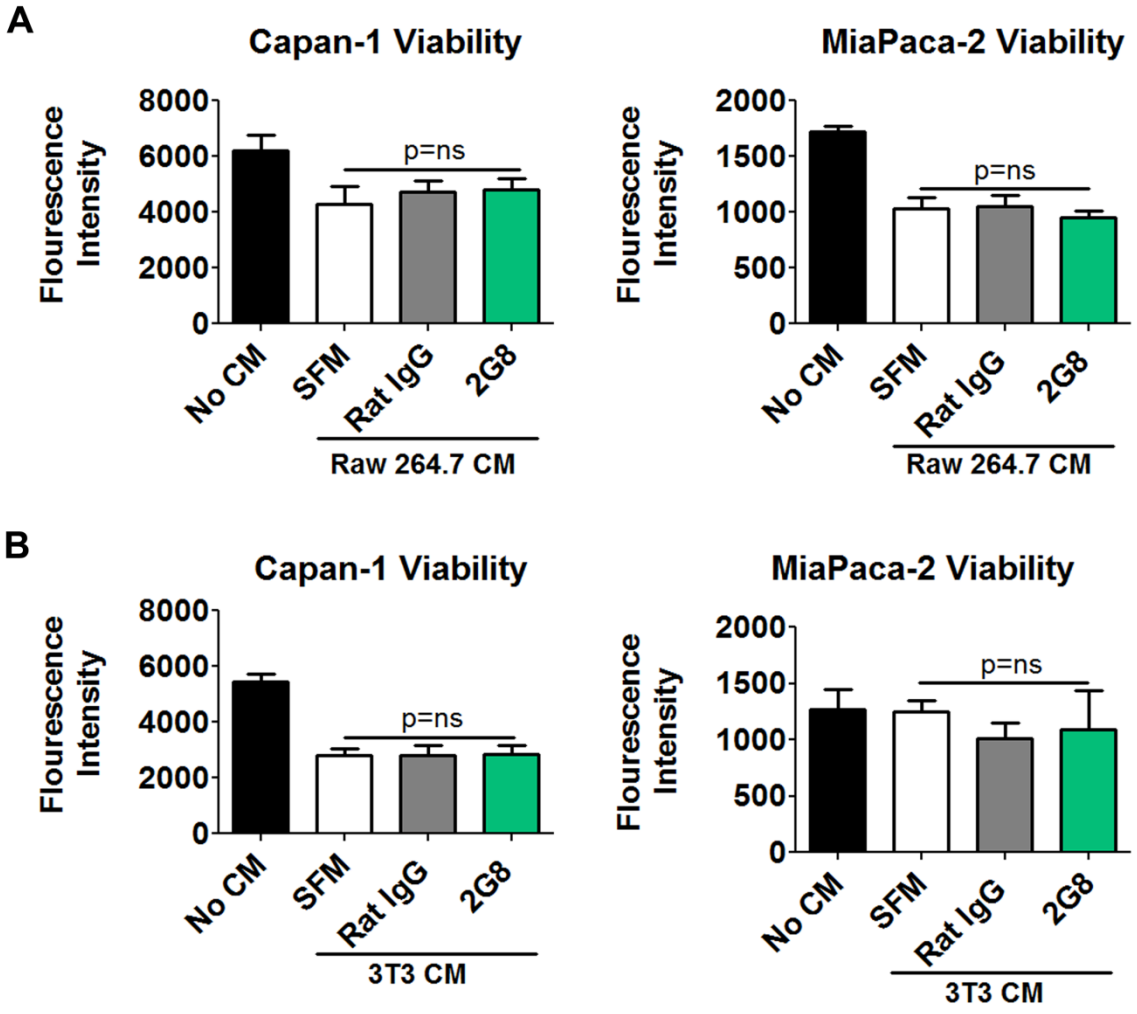
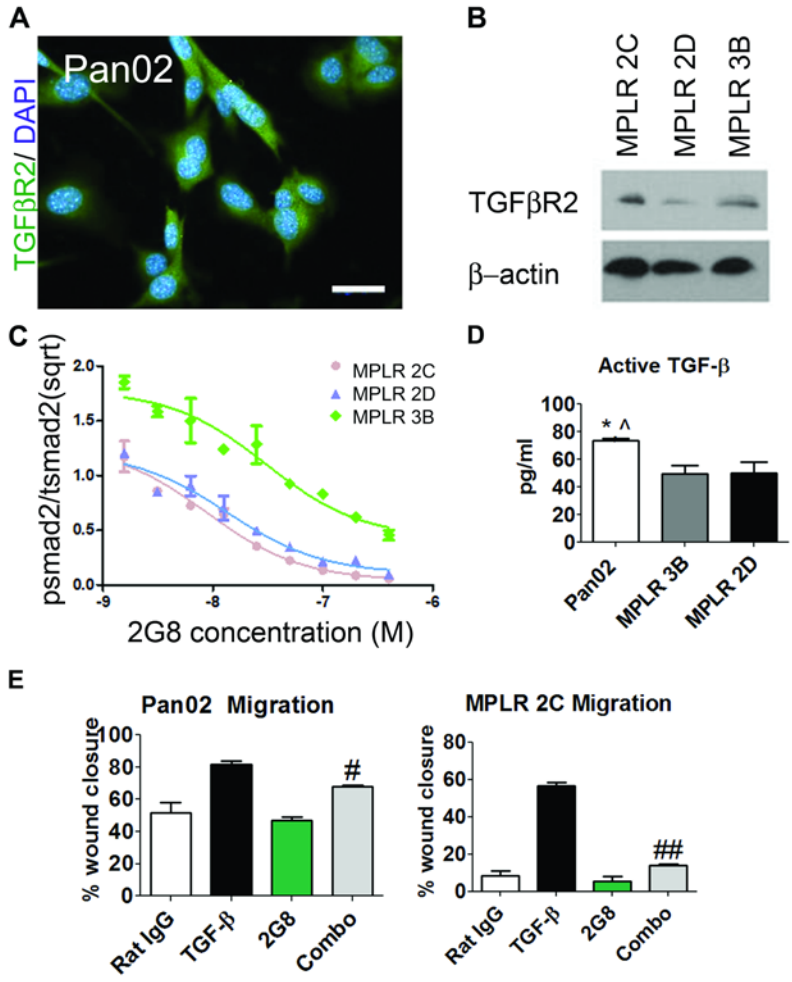
Supplemental Figures and Legends



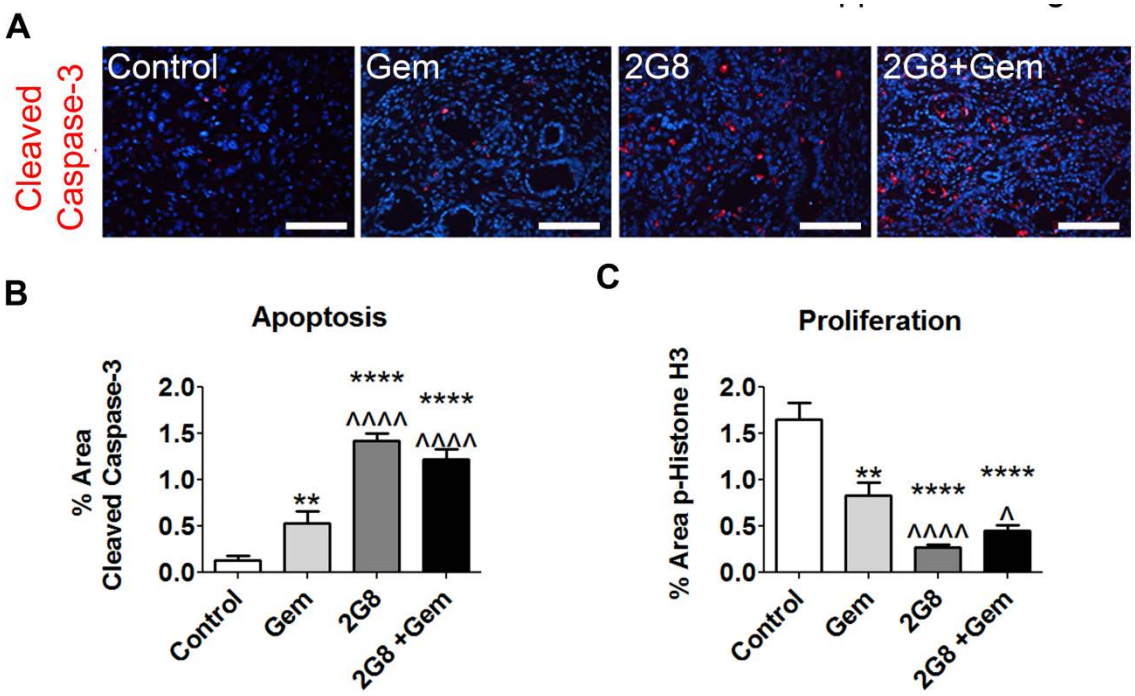
Supplemental Figure 1. 2G8 does not bind to human TGFβR2. ELISA of 2G8 (shown here as MT1), TR1 (anti-human TGFβR2) and two controls, anti-KLH and Rat IgG on human TGFβR2-Fc. TGFβR2-Fc was coated on to wells of an ELISA plate and the indicated antibodies were titrated. 2G8 displays no reactivity with human TGFβR2, even at high concentrations.



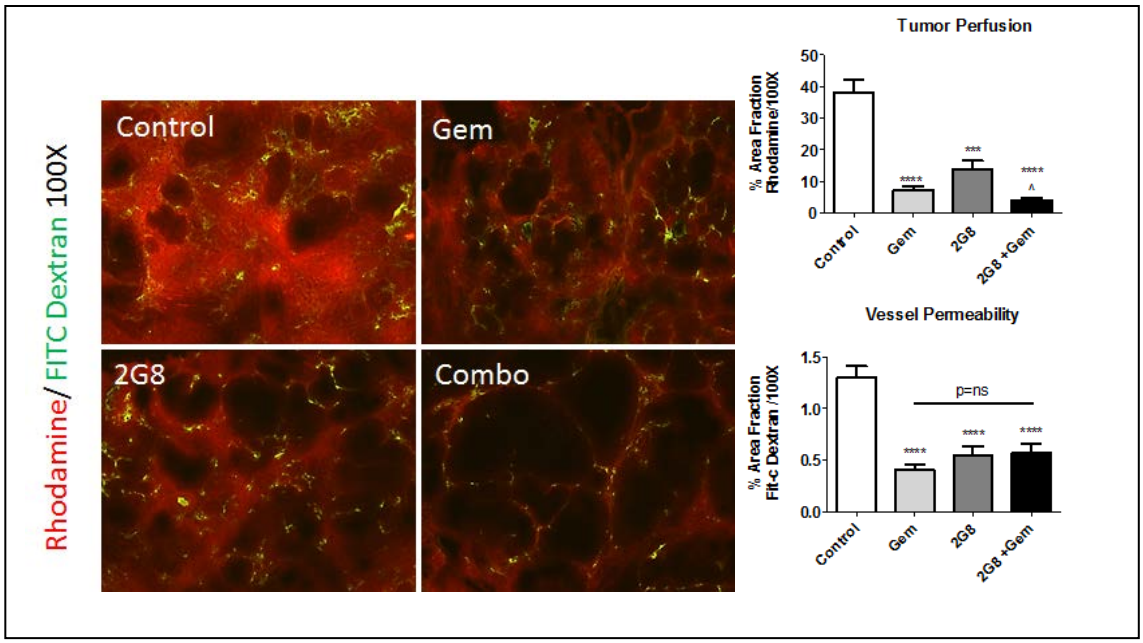
Supplemental Figure 2. 2G8 treated stromal cells do not induce tumor cell proliferation. Conditioned media from RAW 264.7 cells (RAW cells) and NIH 3T3 cells (3T3 cells) treated for 24 hours with serum-free media (SFM), Control Rat IgG (Rat IgG, 30 ng/mL) or 2G8 (30 ng/mL) was collected. Human pancreatic cancer cell lines Capan-1 and MiaPaCa-2 were treated with conditioned media for 24 hours and then cell viability was assessed. (**A**) 2G8 treated RAW CM had no effect on Capan-1 or MiaPaCa-2 tumor cell viability. (**B**) Conditioned media from 3T3 cells had no effect on Capan-1 or MiaPaCa-2 tumor cell viability. Bars represent mean+/- SEM of experiment performed in quadruplicate with similar results found over 3 independent experiments. Similar results were seen in Colo357 and C5LM2 cell lines (data not shown).



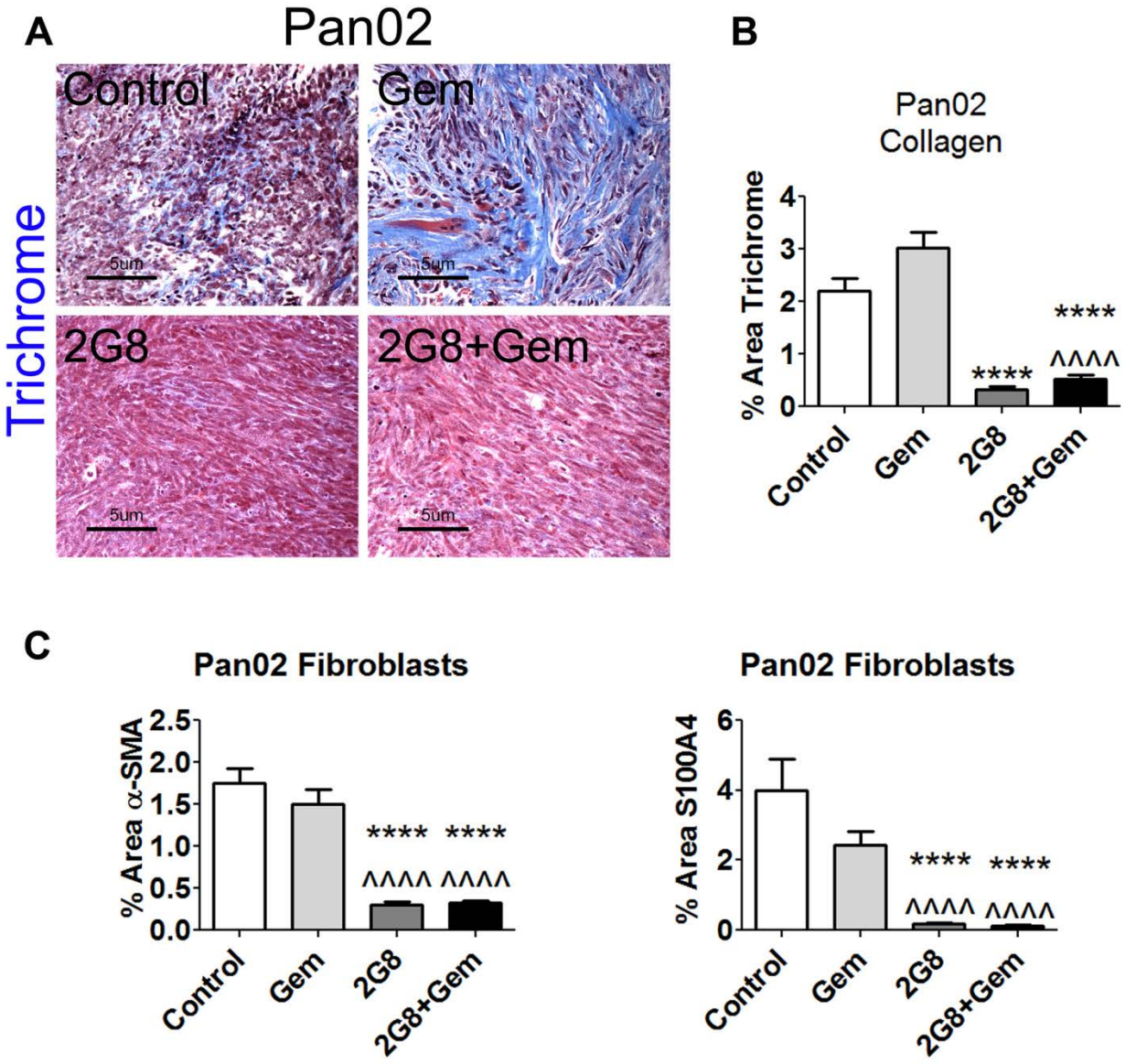
Supplemental Figure 3. 2G8 inhibits smad2 dependent signaling in a dose dependent fashion. (**A**) Pan02 cells express Tgfβr2 as demonstrated by immunocytochemistry. scale bar, 50 µm. (**B**) mPLR 2C, 2D and 3B cell lines express Tgfβr2 as demonstrated by western blotting. (**C**) 2G8 inhibits Smad2 phosphorylation in a dose-dependent fashion mPLR cells lines as determined by ELISA. (**D**) Pan02 cells at baseline secrete more active TGF-β than mPLR cell lines over 24 hours as determined by ELISA. (**E**) 2G8 significantly inhibited TGF-β induced tumor cell migration as assessed by wound closure assay. $^{\#\#}$, $p<0.01$ vs. Rat IgG; *, $p<0.05$; **, $p<0.01$ vs. TGF-β. Bar graphs represent mean +/- SEM of triplicates. Results representative of 3 independent experiments.



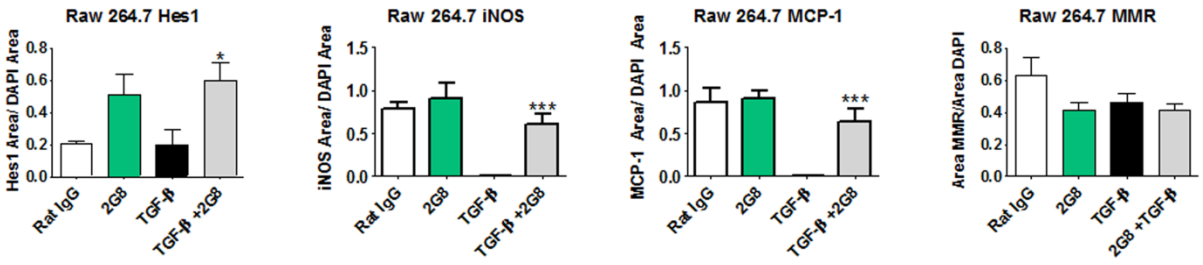
Supplemental Figure 4. Tgfβr2 inhibition induces apoptosis and inhibits proliferation in a murine transgenic model of PDAC. Tumors from *LSL-Kras^{G12D}; Cdkn2a$^{lox/lox}$; p48^{Cre}* (*KIC*) mice treated from age 4-8 weeks with Saline (Control), gemcitabine (Gem), 2G8 or Gem+2G8 were harvested and evaluated for apoptosis and proliferation by immunohistochemistry. (**A**) A significant increase in apoptosis (cleaved caspase-3, red) was noted in tumors treated with 2G8 or the combination. scale bar, 100 µm. (**B**) Quantification of cleaved caspase-3 immunohistochemistry. (**C**) A reduction in proliferation was noted in tumors treated with 2G8 or the combination (phospho-histone H3). Graphs represent Mean +/- SEM, n=5 mice/group. **, p<0.01; ****, p<0.0001 vs control; ^, p<0.05; ^^^^, p<0.0001 vs gem.



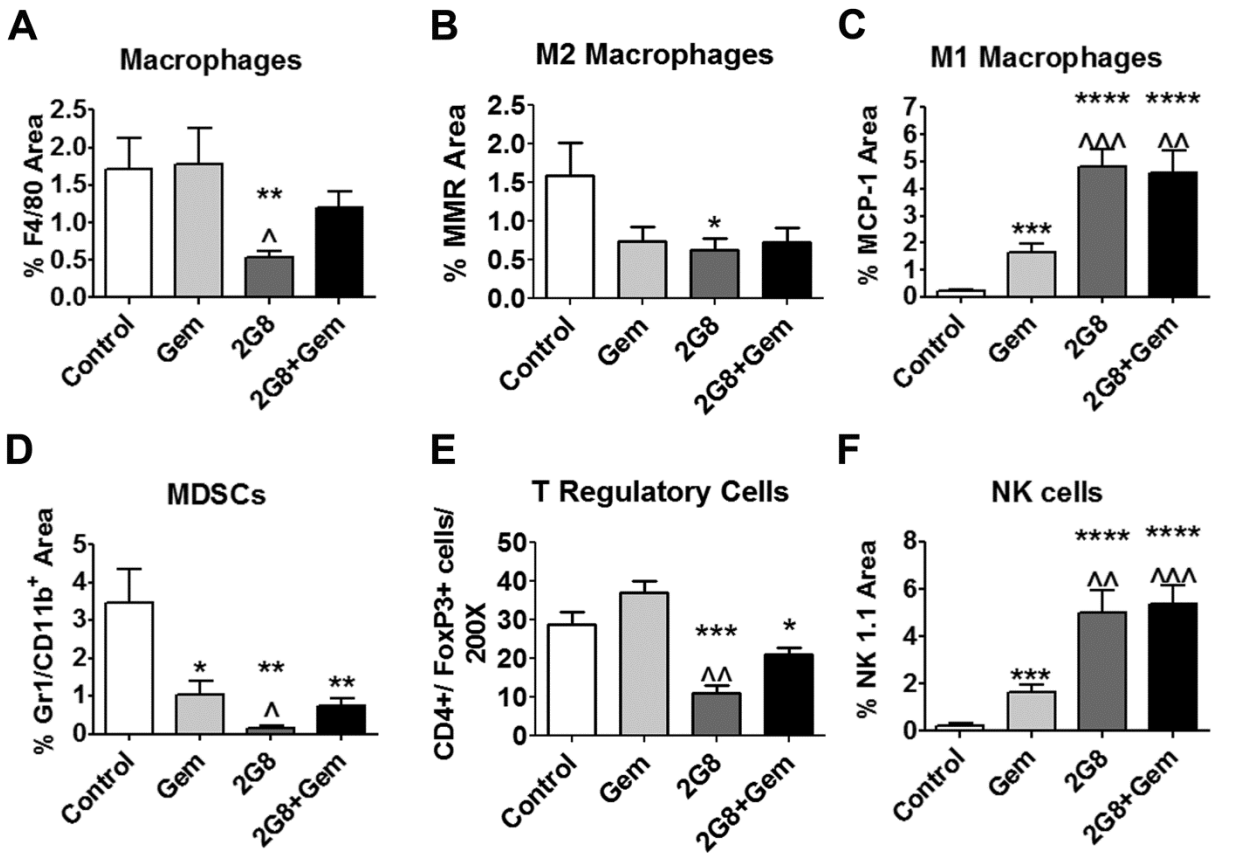
Supplemental Figure 5. Tgfβr2 inhibition reduces perfusion and permeability in KIC tumors. Tumors from *LSL-Kras^{G12D}; Cdkn2a$^{lox/lox}$; p48^{Cre}* (*KIC*) mice treated from age 4-8 weeks with Saline (Control), gemcitabine (Gem), 2G8 or Gem+2G8. **, p<0.01; ****, p<0.0001 vs control; ^, p<0.05; ^^^^, p<0.0001 vs gem.



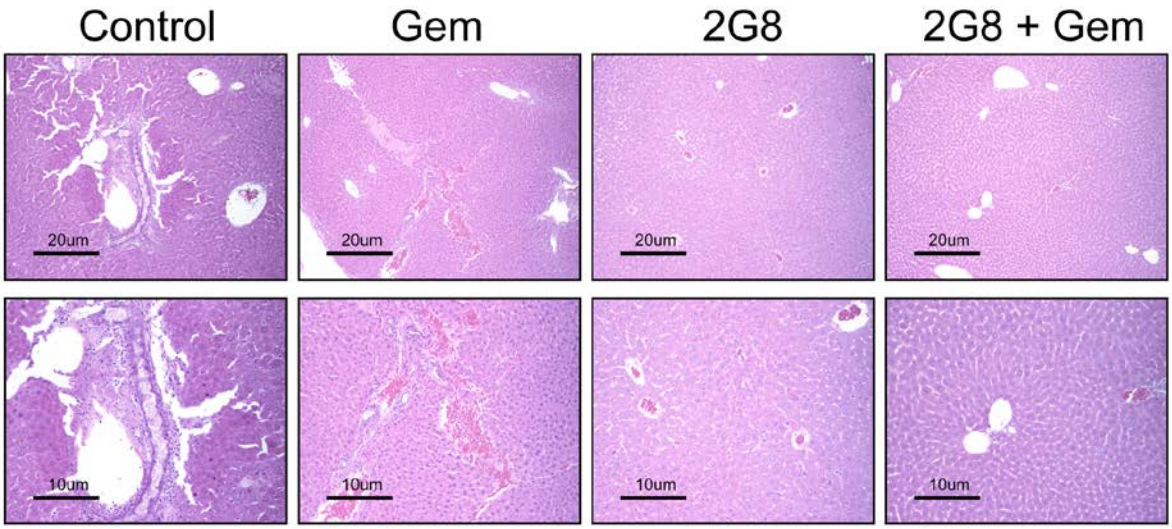
Supplemental Figure 6. Collagen deposition and fibroblast infiltration is reduced after Tgfβr2 inhibition in PDAC. TGF-β drives collagen deposition within PDAC tumors. (**A**) Pan02 tumors were assessed for collagen deposition by Masson's Trichrome (Trichrome, blue). scale bar, 5 µm. (**B**) Collagen deposition was quantitated by area fraction trichrome. (C) 2G8 reduced fibroblasts in Pan02 tumors as demonstrated by immunohistochemistry for α-SMA and S100A4 (FSP-1). 5 animals/group were analyzed with 5 representative pictures taken and analyzed per animal. Results are expressed as mean+/-SEM. ****, p<0.0001 vs. control; ^^^^, p<0.0001 vs. gem.



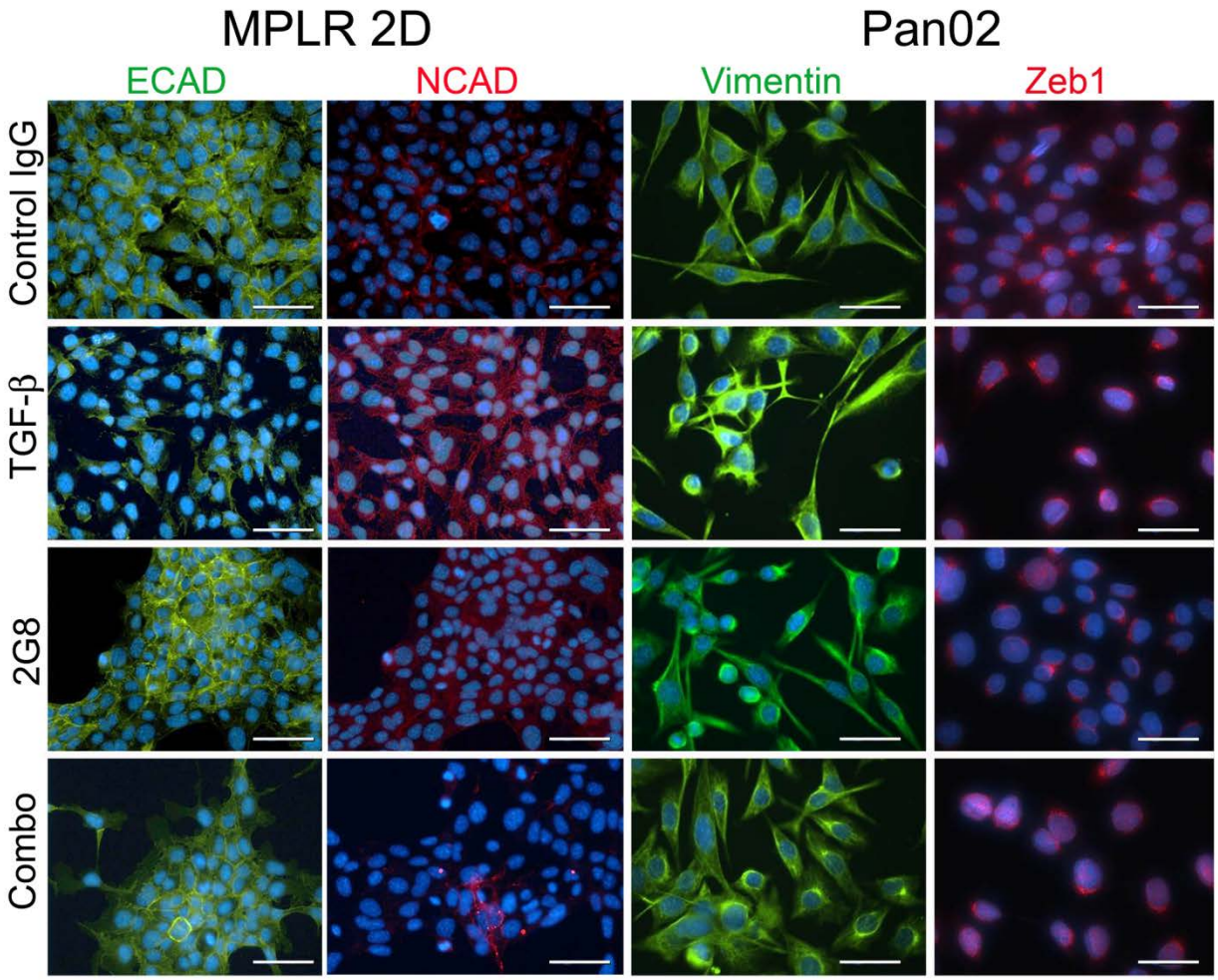
Supplemental Figure 7. 2G8 preserves M1 macrophage phenotype in the presence of exogenous TGF-β. RAW 264.7 cells (RAW cells) were treated with Control rat IgG (30 ng/mL), 2G8 (30 ng/mL), TGF-β (10 ng/mL) or 2G8+TGF-β for 24 hours. The expression level of inflammatory markers Hes1, iNOS, and MCP-1 and the anti-inflammatory marker MMR were assessed by immunocytochemistry. *, $p<0.05$; ***, $p<0.001$ vs. TGF-β treatment.



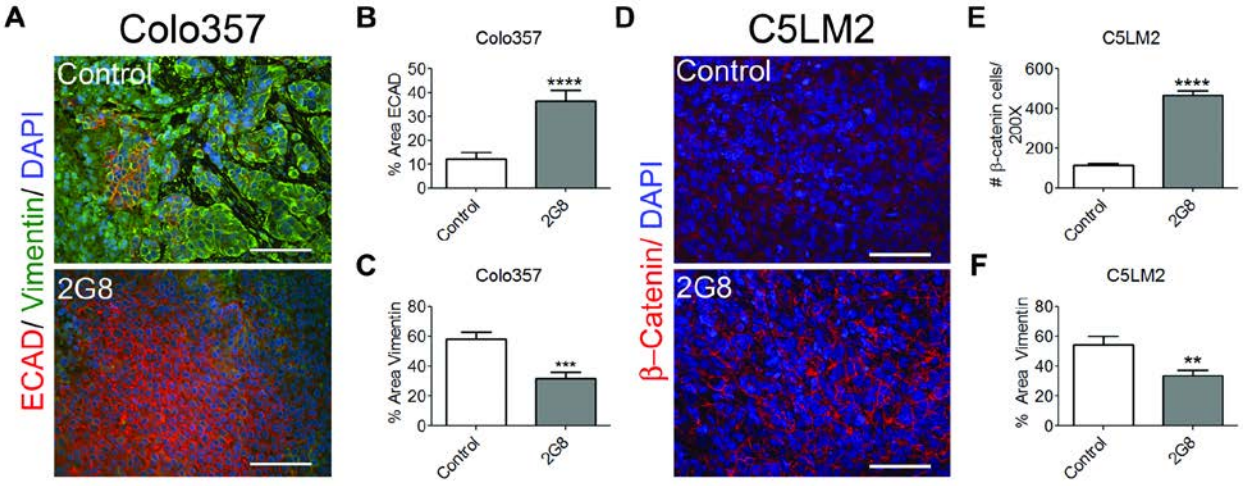
Supplemental Figure 8. 2G8 promotes a proinflammatory immune cell phenotype in an immunocompetent model of PDAC. Pan02 tumors were evaluated by immunohistochemistry for immune cell infiltration. Treatment with 2G8 alone or in combination with Gemcitabine (Gem) reduced F4/80 (**A**), and M2 (anti-inflammatory) macrophages (MMR, **B**) but elevated the level of M1 (pro-inflammatory) macrophages (MCP-1, **C**) in orthotopic Pan02 tumors. (**D, E**) 2G8 alone or in combination with Gem also reduced the level of myeloid deprived suppressor cells (MDSCs, $Gr1^{+}CD11b^{+}$, **D**) and T regulatory cells (T reg, $CD4^{+}FoxP3^{+}$, **E**). (**F**) Conversely 2G8 alone or in combination with Gem elevated the level of Natural Killer cells (NK cells, NK1.1, **F**). 5 animals/group were analyzed with 5 representative pictures taken and analyzed per animal. Results are expressed as mean+/-SEM. **, $p<0.01$; ****, $p<0.0001$ vs. control; ^, $p<0.05$; ^^^^, $p<0.0001$ vs. gem.



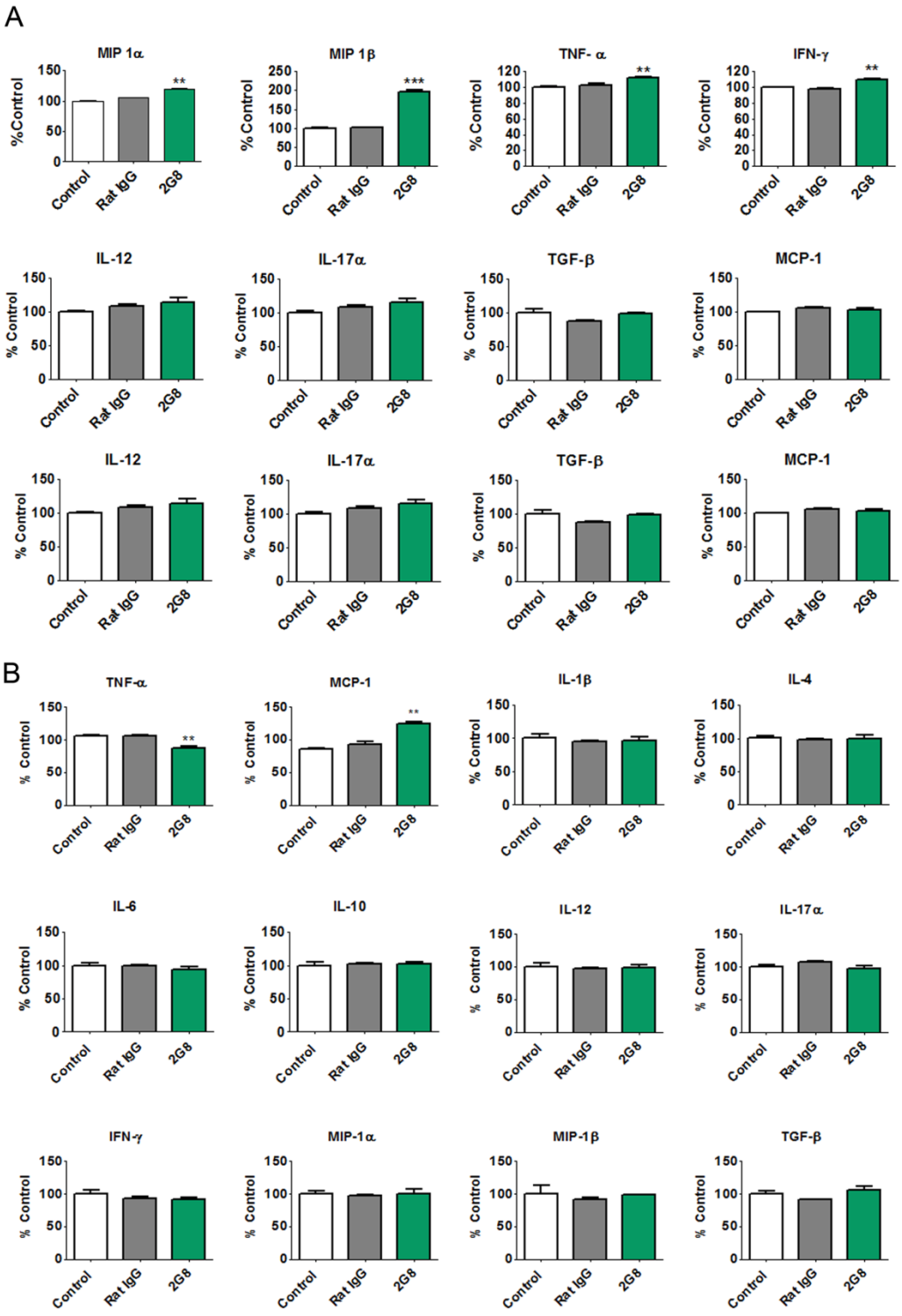
Supplemental Figure 9. 2G8 reduces metastatic burden in *KIC* mice. Representative liver histology from *KIC* mice treated with control, gemcitabine, 2G8 or 2G8 + gem. Histological evaluation of liver tissue was performed by a pathologist blinded to treatment groups.



Supplemental Figure 10. 2G8 inhibition of tumor cell Tgfβr2 alone is insufficient to alter tumor cell epithelial/mesenchymal phenotype in vitro. Murine pancreatic cancer cells (mPLR 2D and Pan02) were cultured on chamber slides and treated with Control Rat IgG, TGF-β (10 ng/mL), 2G8 (30 ng/mL) or the combination for 24-72 hours. The expression level of E-cadherin (ECAD) and N-cadherin (NCAD) or vimentin and Zeb1 were evaluated in mPLR 2D and Pan02 cells respectively. Representative images are shown. scale bar, 100 µm.



Supplemental Figure 11. 2G8 inhibits EMT in multiple in vivo human xenograft models. Colo357 and C5LM2 orthotopic xenograft tumors were established and mice were randomized to receive Control (saline) or 2G8 (30 mg/kg/weekly). (**A-C**) Immunohistochemistry of Colo357 tumors were evaluated for E-cadherin (ECAD, red) and vimentin (green). DAPI was used as a nuclear counterstain. (**A**) Representative images are shown. scale bar, 100 µm. Quantification of ECAD (**B**) and vimentin (**C**) are shown. (**D-F**) C5LM2 xenografts were evaluated by immunohistochemistry for β-catenin (red) and vimentin. (**D**) Representative images are shown of β-catenin depicting an increase in membranous β-catenin staining in C5LM2 tumors treated with 2G8. scale bar, 100 µm. (E) Quantification of membranous β-catenin staining cells/200X and vimentin (F) is shown. 5 animals/group were analyzed with 5 representative pictures taken and analyzed per animal. Results are expressed as mean+/-SEM. **, $p<0.01$; ****, $p<0.0001$ vs control.



Supplemental Figure 12. Cytokine changes in stromal cells in vitro after inhibition of Tgfβr2. The effect of 2G8 on cytokine expression of RAW 264.7 (A) and NIH 3T3 (B) cells was determined protein cytokine analysis of conditioned media. Results are expressed as mean+/- SEM of control. **, p<0.01; ***, p<0.001 vs control.