Figure S6

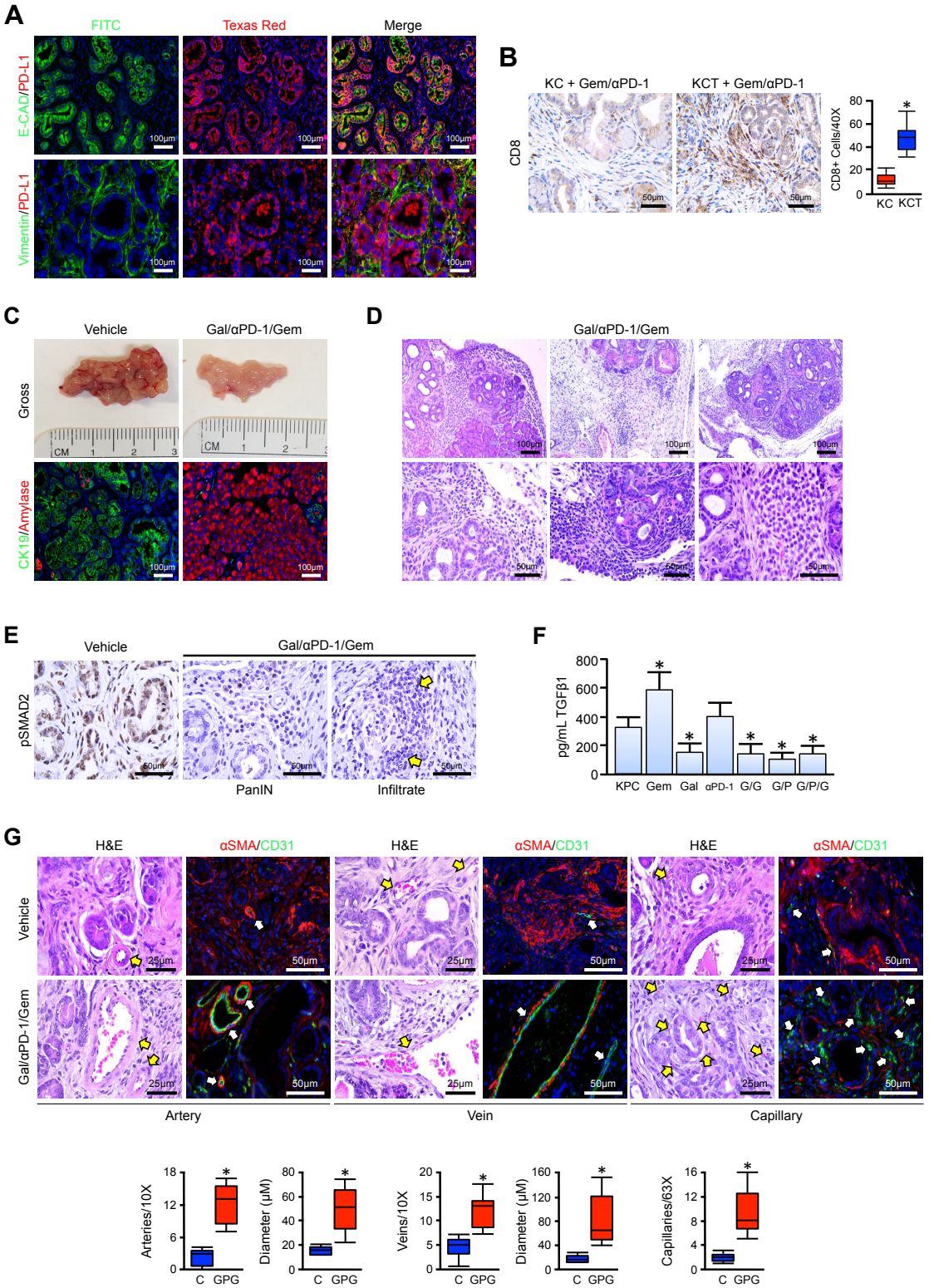

Figure S6. The combination of Gemcitabine, Galunisertib, and anti-PD-1 restores normal gland architecture and suppresses TGFβ signaling
(A) Tumor tissues from P48-Cre x LSL-*Kras*^{G12D} x *Tgfbr1*$^{+/-}$ (KCT) were stained with either PD-L1/E-Cadherin or PD-L1/Vimentin, showing strong PD-L1 expression in epithelial tissues and little PD-L1 expression in the tumor stroma. **(B)** KC and KCT mice were allowed to reach 12 weeks of age (N=8-14/group), and randomized at a 50:50 male to female ratio into one of two groups. Mice were either administered intraperitoneal injection every other day of either a PBS control (Vehicle) or 200μg of an anti-PD-1 neutralizing antibody (αPD-1) with twice-weekly doses of 100mg/kg Gemcitabine (Gem +αPD-1). The pancreas was collected at the conclusion of the study (100 days post enrollment) and stained for CD8 by immunohistochemistry. Tissue sections were quantified as described and displayed as box plot (*p < 0.05). **(C)** As described, Pdx1-Cre x LSL-KRAS^{G12D} x LSL-TP53^{R172H} (KPC) mice were used as a model of aggressive PDAC. At approximately 10 weeks of age, animals were randomized at a 50:50 male to female ratio into one of six groups. Mice were administered an intraperitoneal injection every other day of either saline (Vehicle) or 100mg/kg Gemcitabine (Gem) starting at 12 weeks, with the addition of 75mg/kg Galunisertib and 200μg anti-PD-1 at 14 weeks (Gal/αPD-1/Gem). The pancreas was then collected either when the animals were moribund or at the conclusion of the study (8 months), and representative gross pathology images displayed above. Tissue sections were also stained via immunohistochemistry for duct marker CK19 and acinar marker anti-pancreatic amylase, confirming the increase in normal tissue architecture in treated mice. **(D)** Additional representative images of H&E stained tissue from Galunisertib/anti-PD-1/Gemcitabine treated mice showing lymphocytosis and destruction of abnormal tissues. **(E)** Tissue sections were also stained for pSMAD2, affirming the reduction in TGFβ signaling in mice administered Galunisertib/anti-PD-1/Gemcitabine both in remaining areas of neoplastic tissues as well as in the lymphocyte infiltrate (arrows). **(F)** Tissue was collected from either vehicle treated KPC mice (KPC), or KPC mice treated with Gemcitabine (Gem), Galunisertib (Gem), anti-PD-1 (αPD-1), Gemcitabine and Galunisertib (GG), Galunisertib and anti-PD-1 (GP), or Galunisertib/anti-PD-1/Gemcitabine. Tissues were lysed, and 20μg of total protein was subjected to TGFβ1 ELISA (N=3/group, *p < 0.05). **(G)** Tissues were sectioned and stained either with H&E or via immunohistochemistry for vascular marker CD31 and αSMA. Arteries/arterioles, veins/venules, and capillaries are denoted with arrows. Both the number and size of vascular structures were quantified by three blinded investigators, or measured using Nikon NIS elements software and displayed as box plot.