

Supplementary Figures

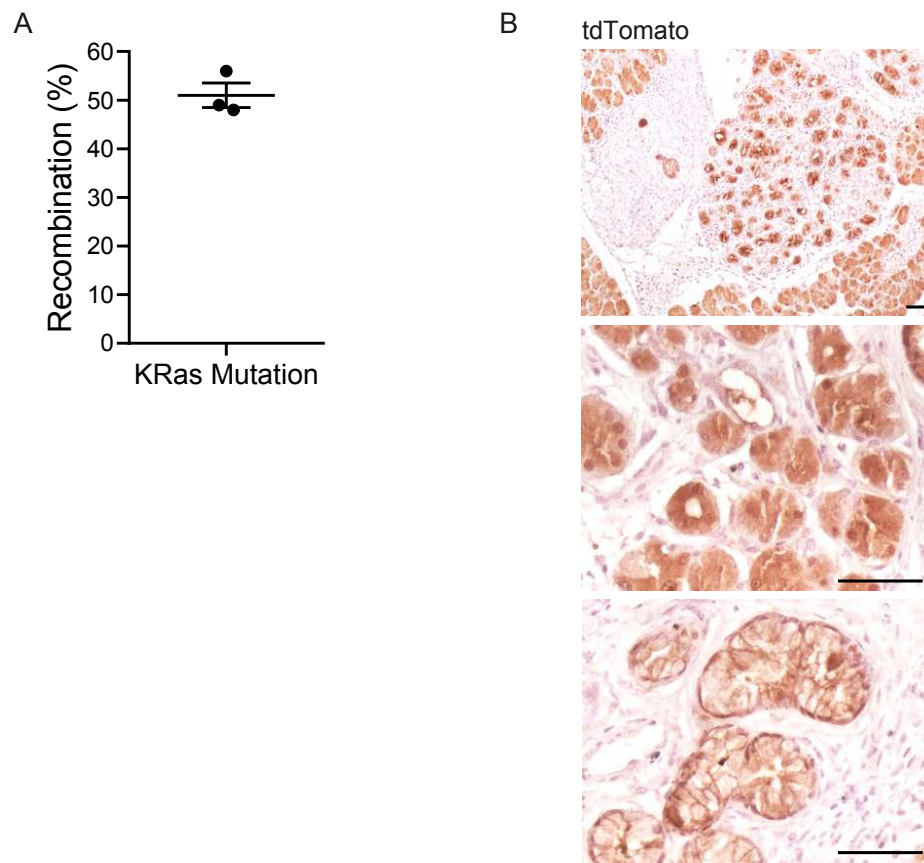


Figure S1. Recombination rates of *LSL-Kras*^{+/G12D} allele. **A.** Percentage of *LSL-Kras*^{+/G12D} alleles showing recombination, in tdTomato⁺ cells isolated by FACS from pancreata of 3 *Ptf1a-CreER*^{+/-}; *LSL-Kras*^{+/G12D}; *LSL-tdTomato*^{+/-} mice (dots) 5 days following tamoxifen treatment, as assessed by semi-quantitative PCR conducted on genomic DNA, comparing the ratio of recombined to non-recombined flox alleles. **B.** Representative images (low magnification – top, high magnification – middle and bottom) of PanIN tissues stained with an antibody against tdTomato, showing tdTomato expression in lesion cells and in surrounding acinar cells. Size markers = 50µm.

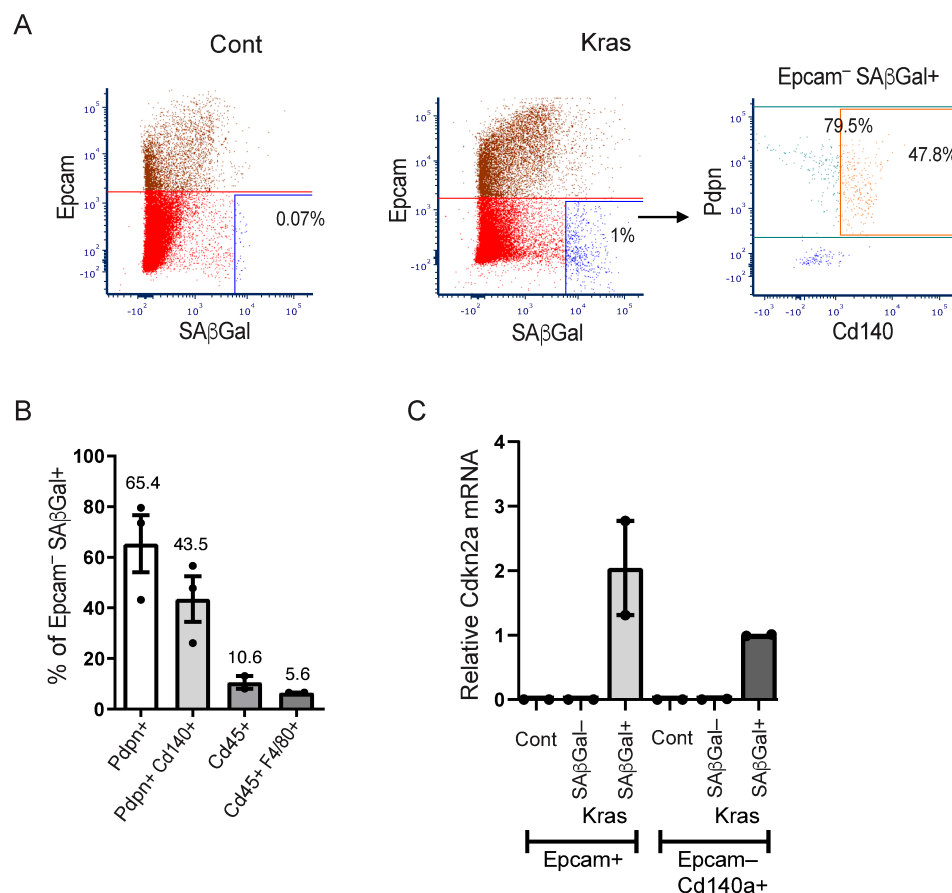
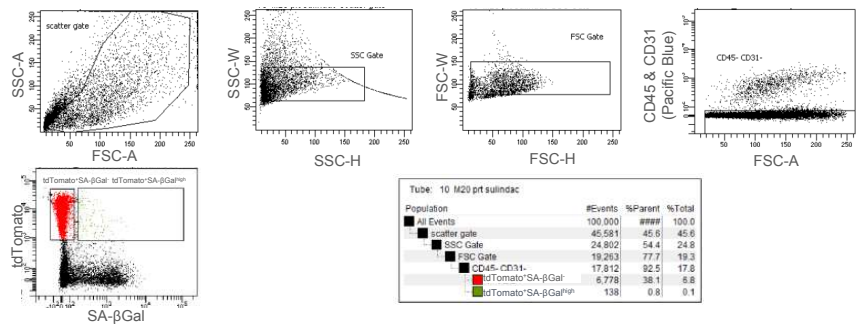


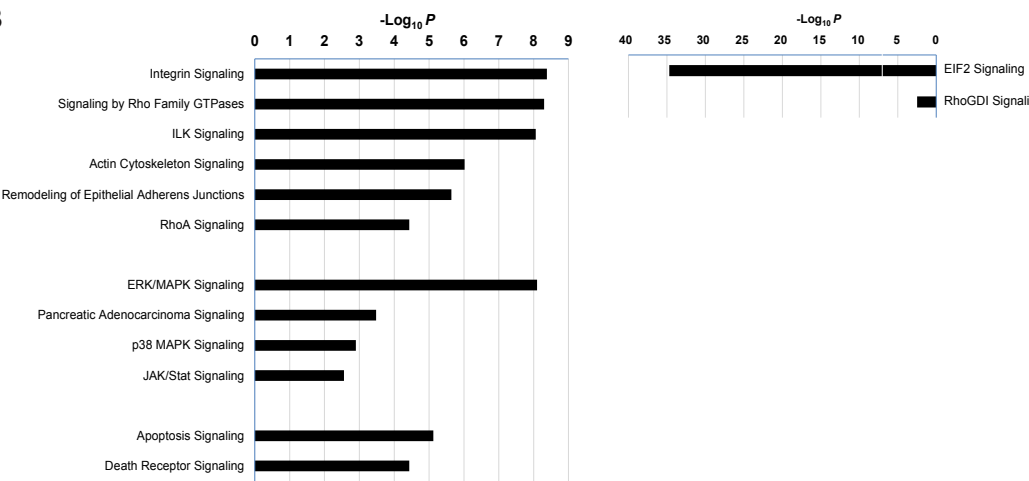
Figure S2. PanIN stroma contains senescent fibroblasts. A. Representative FACS analysis of cells from *Ptf1a-CreER^{+/+}; LSL-Kras^{G12D}* mice 3 months after Kras activation (Kras, right panels) and control *Ptf1a-CreER^{+/+}* mice (left panel, Cont). The tdTomato transgene was replaced by staining for the epithelial marker Epcam. Co-staining of Epcam and SA-βGal, conducted using the fluorescent substrate C₁₂FDG, allowed the characterization and isolation of Epcam⁻ SA-βGal^{high} cells, whose numbers were substantially increased in the Kras-expressing mice versus control mice (gate labeled blue). Staining with the cancer-associated fibroblast markers Pdpn and Cd140a (Pdgfra)⁴³ (right panel) revealed that the majority of Epcam⁻ SA-βGal^{high} cells are fibroblasts. Percentages on right panel dot plot are of total Pdpn⁺, or of Cd140a⁺, out of Epcam⁻ SA-βGal^{high} cells. **B.** Percentages of indicated subpopulations out of Epcam⁻ SA-βGal^{high} cells. The Cd140a⁺ fibroblast subpopulation is included in the Pdpn⁺ population; the F4/80⁺ macrophage subpopulation is included in the Cd45⁺ immune population. Values represent mean across individual mice (dots) ± S.E.M. **C.** Expression levels of the *Cdkn2a* senescence marker measured by qRT-PCR in SA-βGal positive and negative subpopulations of Epcam⁺ epithelial compartment and Cd140⁺ fibroblasts, isolated from Kras activated mice, or in corresponding SA-βGal-negative cells isolated from control mice (Cont). Values indicate average of cells sorted from two mice (dots).

43. Elyada E, Bolisetty M, Laise P, et al. Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. *Cancer Discov* 2019;9:1102-23.

A



B



C

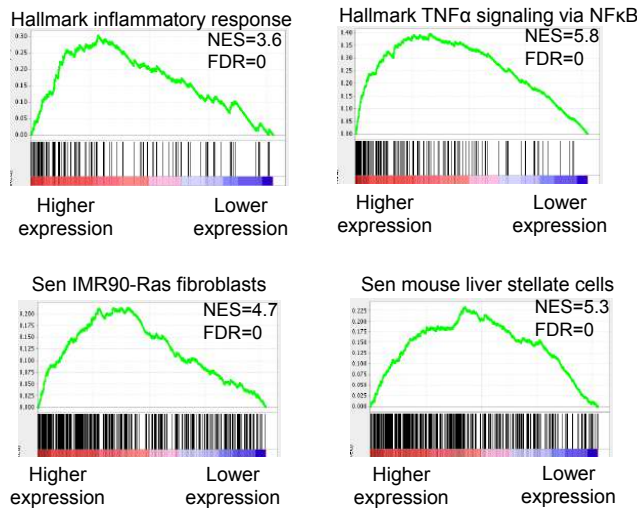


Figure S3. Isolation and profiling of the tdTomato⁺ SA-βGal^{high} population. **A.** Sorting procedure for the isolation of senescent and non-senescent PanIN cells, shown on a representative FACS of cells from triple-transgenic *Ptf1a-CreER^{+/+}*; *LSL-Kras^{+/-G12D}*; *LSL-tdTomato^{+/+}* mice 3 months after Kras activation. Following FSC and SSC gating to eliminate cell doublets, cells were gated to exclude CD45⁺ (hematopoietic) and CD31⁺ (endothelial) fractions; tdTomato⁺ SA-βGal⁻ cells (red, top left), and tdTomato⁺ SA-βGal^{high} (green, top right) were gated for isolation as indicated. **B.** Additional gene sets upregulated (left) and downregulated (right) in SA-βGal⁺ versus SA-βGal⁻ PanIN cells isolated from Kras-activated mice 3 months after tamoxifen treatment. Values indicate $-\log_{10}$ of Adj *P* value as determined by Ingenuity Pathway Analysis. **C.** Gene set enrichment analysis (GSEA) of expression profiles of SA-βGal⁺ versus SA-βGal⁻ PanIN cells indicating enrichment for inflammatory (top) and senescent cell (bottom) gene sets.

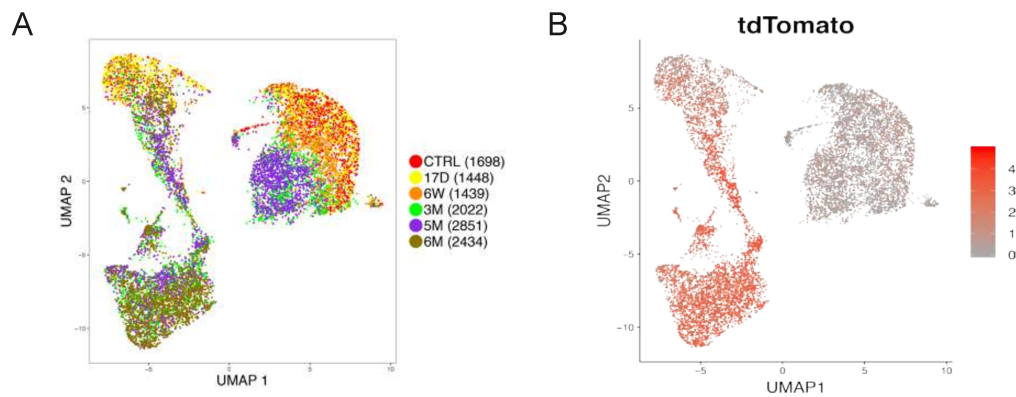


Figure S4. scRNA-Seq of Kras-driven PanINs. **A.** Uniform manifold approximation and projection (Umap) cell clustering of the scRNA-seq expression profile of epithelial cells from *Ptf1a-CreER*^{+/-}; *LSL-Kras*^{+/*G12D*}; *LSL-tdTomato* mice, as shown in Fig. 2I,J. Colors indicate different time points after Kras activation in which cells were collected, as indicated. Cells from *Ptf1a-CreER*^{+/-}; *LSL-tdTomato* control mice (CTRL) are labeled red. Numbers of sequenced cells at each time point are indicated in parentheses. **B.** Expression of tdTomato across cells (red – high, grey – low), observed in normal acinar cells as well as PanIN cells.

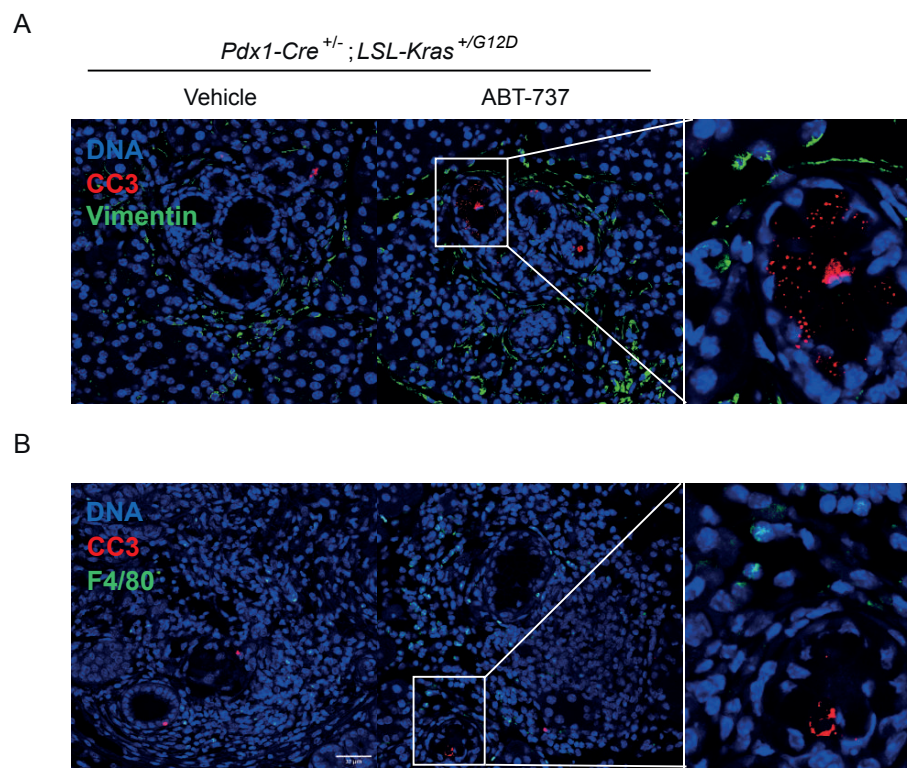


Figure S5. Bcl2-family protein inhibition by ABT-737 does not induce apoptosis in stromal cells. *Pdx1-Cre^{+/-};LSL-Kras^{+G12D}* mice were treated by caerulein at 2.5 months of age, followed by treatment with ABT-737 two weeks subsequently, and analyzed two days later. **A.** Co-staining of pancreas sections from vehicle and ABT-737 treated mice for CC3 (red), and the fibroblast marker vimentin (green), showing no overlap in expression, with CC3 stain detected only within PanIN epithelial ductal structures. **B.** Co-staining of pancreas sections from vehicle and ABT-737 treated mice for CC3 (red), the macrophage marker F4/80 (green) showing no overlap in expression.

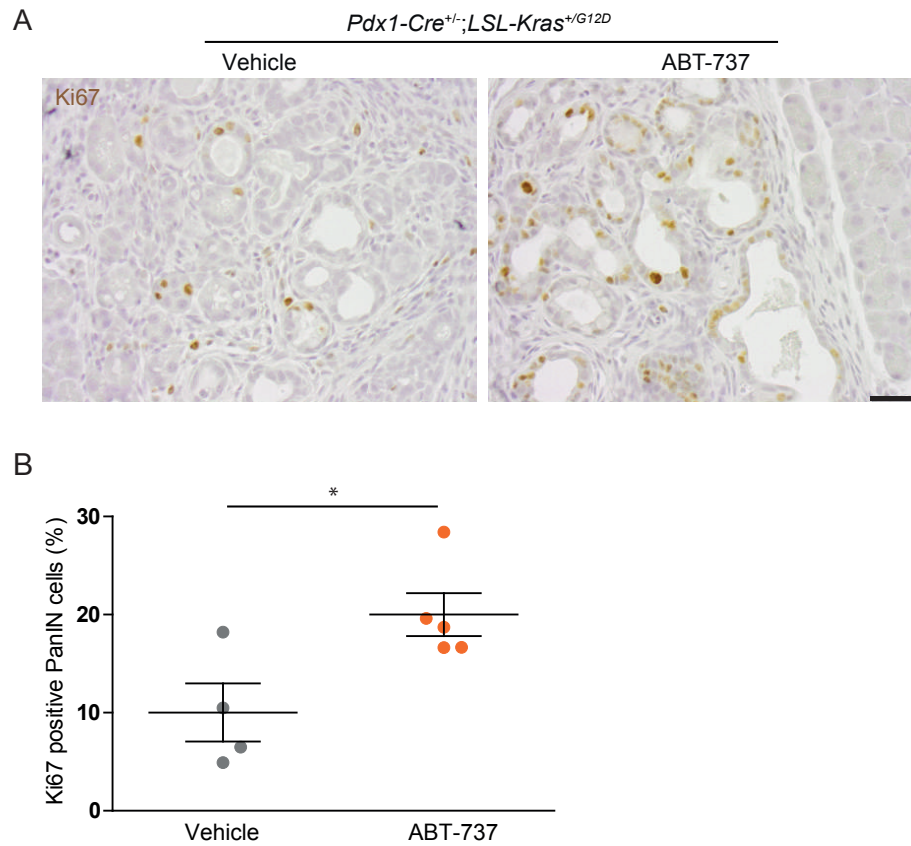


Figure S6. Bcl2-family protein inhibition by ABT-737 increases the percentage of Ki67⁺ cells in PanINs. *Pdx1-Cre^{+/+};LSL-Kras^{+/G12D}* mice were treated by caerulein at 2.5 months of age, followed by treatment with ABT-737 two weeks subsequently, and analyzed two days later. **A.** Representative images of pancreas sections from vehicle or ABT-737 treated mice stained for Ki67 (brown). **B.** Percentage of Ki67⁺ cells in PanINs from same mice. *, $P < 0.05$, **, $P < 0.005$, t -test.

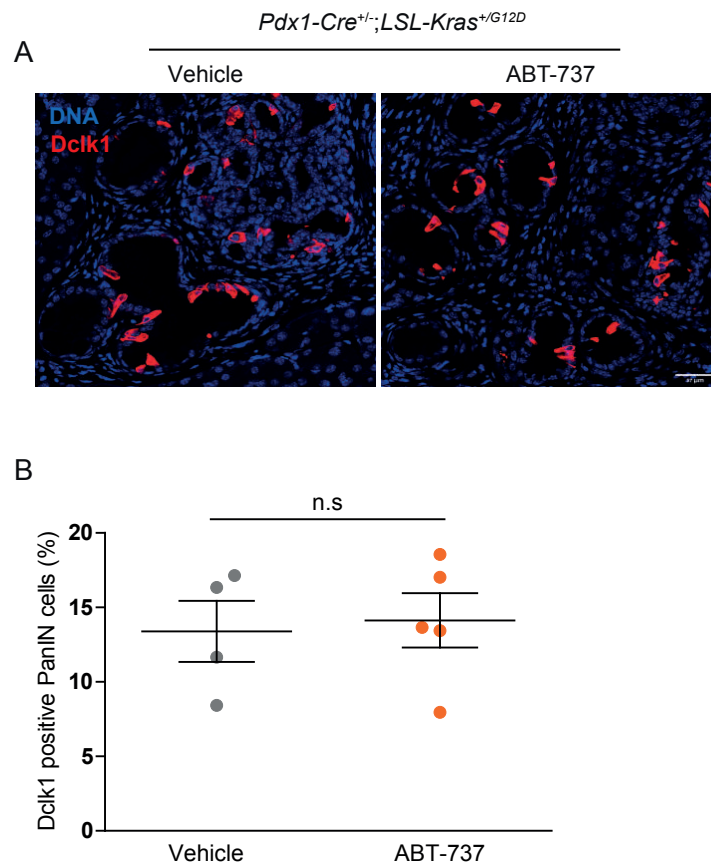


Figure S7. Bcl2-family protein inhibition by ABT-737 does not eliminate Dclk1⁺ cells. *Pdx1-Cre^{+/-};LSL-Kras^{+G12D}* mice were treated by caerulein at 2.5 months of age, followed by treatment with ABT-737 two weeks subsequently, and analyzed two days later. **A.** Representative staining for Dclk1 in pancreas sections from vehicle and ABT-737 treated mice. **B.** Percentage of Dclk1⁺ cells in PanINs from same mice. *, $P < 0.05$, **, $P < 0.005$, *t*-test.

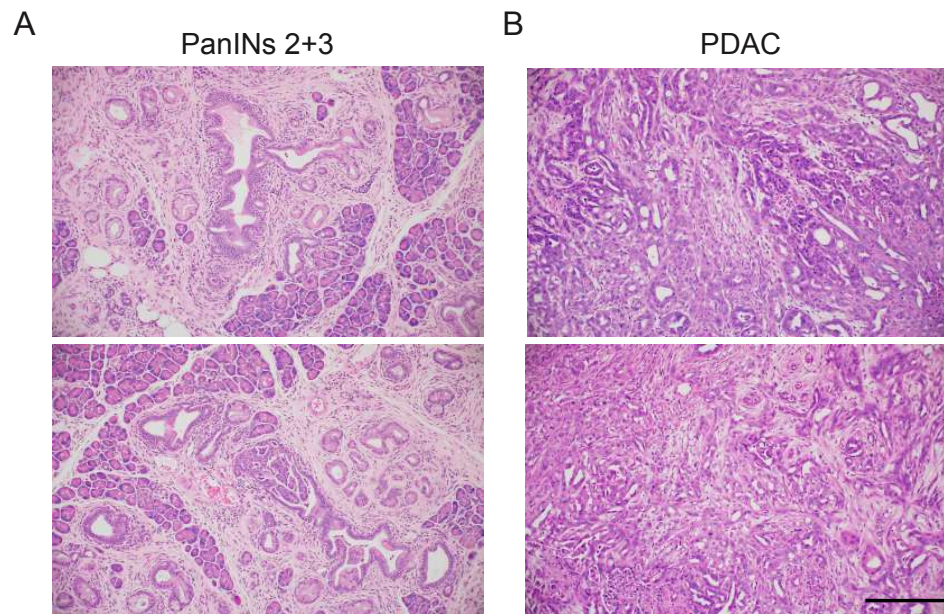


Figure S8. High grade PanINs and pancreatic carcinoma in *Pdx1-Cre^{+/-};LSL-Kras^{+/-}G12D* mice. Representative images of PanIN2 and PanIN3 lesions (panel A) and pancreatic ductal carcinoma (PDAC, panel B) observed in caerulein-treated *Pdx1-Cre^{+/-};LSL-Kras^{+/-}G12D* mice at 9 months of age. Lesions are from mice that did not receive ABT-737. Size marker = 200µm.