





Supplemental Figure S4. KPC subcutaneous syngeneic allograft tumors are acidic and express GPR68.

(A) Heat map of GABA receptor expression by cell type from the Peng et al. (22) human pancreatic ductal adenocarcinoma tumor single cell sequencing dataset. (B) Structure of lorazepam (left) and alprazolam (right), pink circle denotes the n-unsubstitution and the teal circle denotes the n-substitution. (C) Dot plot visualization of Gpr68 gene expression level (color intensity) and frequency (size of dot) in different cell populations of murine PDAC samples from Kemp et al. (26). (D) Representative images of Gpr68+/Acta2+ cells by RNAscope in the two-week vehicle (left) treated subcutaneous KPC tumors and the KPC spontaneous tumor (right). (E) Representative images of Gpr68+/Pdgfrβ+ cells by RNAscope in the two-week vehicle treated subcutaneous syngeneic allograft tumors (F-H) UMAP plots of (F) GPR68, (G) RGS5 (pericyte marker), and (H) DCN (pan-CAF marker) in human PDAC CAF cluster reprocessed from the Steele et al. (25) single cell sequencing dataset. (I-K) UMAP plots of (I) DCN, (J) RGS5, and (K) GPR68 expression in (left to right) normal human pancreas, PDAC metastasis, and primary PDAC tumors from the Steele et al. (25) single cell sequencing dataset. (L, M) Representative extracellular pH tracing of (L) Normal C57BL/6 murine pancreas and (M) Murine PDAC tumors isolated from C57BL/6 mouse subcutaneously implanted bilaterally with KPC tumor chunks. (N) Scatterplot with bar of the extracellular pH values from normal pancreas (n=2 biological replicates) and KPC subcutaneous tumors (n=4 biological replicates), dots represent independent pH readings. (O) Representative 10x H&E image of the subcutaneous KPC tumor from the pH experiment. (P,Q) Representative extracellular pH tracing of (P) adjacent normal pancreas from C57BL/6 mice with orthotopically implanted KPC tumor pieces and (Q) Murine PDAC tumors isolated from C57BL/6 mouse orthotopically implanted bilaterally with KPC tumor pieces. (R) Scatterplot with bar of the extracellular pH values from normal pancreas (n=2 biological replicates) and KPC orthotopic tumors (n=2 biological replicates), dots represent independent pH readings. (S) Representative 10x H&E image of the orthotopic KPC tumor from the pH experiment. Statistics: pH values were compared using unpaired one-tailed t-tests.