**Supplementary Figure Legends**

**Figure S1. The neoplastic pancreas harbors a distinct microbiome that promotes PDA. (A)** Heatmap of the top 20 genera in the bacterial communities in pancreata of KC and WT mice.The relative abundance of bacterial genera was normalized by z-score as indicated by the color gradient. Double hierarchical linkage clustering of the samples was based on microbial composition and abundance. **(B)** Taxonomic composition of microbiota assigned to phylum level in pancreatic tumors of PDA patients based on average percent relative abundance and determined by 16S sequencing (n=12). **(C)** Heatmap of the top 20 genera in the bacterial communities in pancreatic tumors of PDA patients (n=12).The relative abundance of bacterial genera (z-score normalization) is indicated by the color gradient. Double hierarchical linkage clustering of the samples was based on microbial composition and abundance. **(D)** Cladogram showing differential taxonomic hierarchies in the pancreas of patients in the PDA (green, n=12) and NML (red, n=5) cohorts detected by LEfSe. Colors indicate the cohorts in which each differential clade was significantly abundant. **(E)** WT mice were treated with an ablative oral antibiotic regimen and then orthotopically inoculated with Pan02 cells. Tumor weights were recorded. This experiment was repeated twice with similar results (n=5/group; \*p<0.05). **(F)** The abundance of bacteria in the pancreas of control KC mice and mice treated with an ablative oral antibiotic regimen was determined by FISH. Representative images are shown.

**Figure S2. Temporal analysis of alterations in the gut microbiome during murine pancreatic oncogenesis. (A)** Taxonomic distribution of microbial phyla in the GI tract of KC and WT mice over time is shown (n=5/cohort; \*p<0.05; \*\*p<0.01). **(B)** Heat-map of top 20 genera showing longitudinal gut microbial diversity from week 3 to week 36 in KC and WT mice (n=5/group).Double hierarchical linkage clustering of the cohorts was based on composition (y-axis) and abundance (x-axis) of gut microbiota. Average abundances of each genus are row normalized (z-score) and are indicated by the color gradient. The dendrogram on the x-axis indicates the distinct clusters of each cohort. (**C**) Heat-map representing log2-transformed abundance of top 20 genera from week 3 to week 36 in KC mice (n=5/group).Double hierarchical linkage clustering of the cohorts was based on composition (y-axis) and abundance (x-axis) of gut microbiota. **(D)** LDA analysis identified differentially abundant taxa in KC (red bars) and WT (green bars) cohorts. **(E)** Relative abundance of *Bifidobacterium* species in the GI tract of KC and WT mice over time is shown (n=5/cohort). **(F)** Weighted PCoA plots of gut bacterial communities based on Unifrac distance matrix at specific time points. Each symbol represents a fecal sample from KC (red) or WT (blue) mice. Clusters were determined by pairwise PERMANOVA. X- and y- axes indicate percent variation and the ellipses indicate 95% CI. **(G)** Weighted PCoA plots of gut bacterial communities based on Unifrac distance matrix. Each symbol represents a cohort (n=5 each) of KC (red) and WT (blue) mice over time (weeks).Clusters were determined by pairwise PERMANOVA, p=0.002. Both x- and y- axes indicate percent variation and ellipses indicate the 95% CI.

**Figure S3. Longitudinal differences in the gut microbiome in KC mice based on alpha-diversity analyses. (A)** Analysis of longitudinal changes in Chao1, **(B)** Observed OTUs, **(C)** Shannon,and **(D)** PD diversity indices in KC cohorts over time. **(E)** Analysis of longitudinal changes in Chao1, **(F)** Observed OTUs, **(G)** Shannon, and **(H)** PD diversity indices between WT and KC cohorts from week 3 through week 36 of life are shown (n=5/group; \*p<0.05).

**Figure S4. *Proteobacteria* isenriched in human PDA tumors**. **(A)** Taxonomic distribution of gut microbiota in PDA patients (n=32) and age-, gender-, and BMI-matched non-cancer subjects (n=31) is shown. Listed phyla possess an average percentage relative abundance ≥ 0.1%. The remaining phyla are binned together as ‘Other’ (\*p<0.05, \*\*\*p<0.001). **(B)** Taxonomic distribution of microbial phyla in the gut and pancreas tumors of 9 PDA patients for which data from both compartments were available. Percentages indicate the relative abundance of *Proteobacteria* in each compartment. **(C)** Heatmap of the top 20 *Proteobacteria* genera in the gut and pancreas tumors of 9 PDA patients for which data from both compartments were available.The relative abundance of bacterial genera was normalized by z-score as indicated by the color gradient. Double hierarchical linkage clustering of the samples was based on microbial composition and abundance. **(D)** Cladogram showing differential taxonomic hierarchies in the pancreas (green) and duodenum (red) of KC mice by LEfSe (n=4/group).

**Figure S5. Analysis of the gut microbiome in human PDA and controls. (A)** Statistically significant differentially abundant genera in the gut of PDA patients (red) and healthy subjects (green) were identified by LDA effect size measurements. **(B)** The gut microbiome of PDA patients (n=32) and matched controls (n=31) were tested for differences in alpha-diversity indices including ACE, Chao1, Observed, Shannon, Simpson, and PD (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001).

**Figure S6. Microbial ablation induces immunogenic reprogramming in PDA. (A)** Schematic indicating that KC mice treated with an ablative oral antibiotic regimen beginning at 8 weeks of life were repopulated at 14 weeks of life with feces from either 3-month-old WT or KPC mice or sham-repopulated (vehicle only). Animals were sacrificed at 22 weeks of life. **(B)** Schematic indicating that germ-free KC were repopulated at 6 weeks of life or sham-repopulated. Animals were sacrificed at 14 weeks of life. Repopulation experiments were repeated more than 3 times with separate cohorts. **(C, D)** Control and ablative oral antibiotic-treated WT mice were orthtopically implanted with KPC-derived tumor cells and sacrificed at 3 weeks. (C) The percentages of tumor-infiltrating CD3+ T cells (n=15/group) and (D) Gr1+CD11b+ MDSC (n=8/group) among CD45+ intra-tumoral leukocytes were calculated. Our strategy for gating on live, singlet, and CD45+ intra-tumoral leukocytes is shown. These experiments were repeated more than 3 times with similar results. **(E)** TAMs were harvested from tumors of control or antibiotic-treated PDA-bearing mice and cultured overnight. Supernatant was assayed for an array of chemokines (n=7/group). **(F-J)** Control and ablative oral antibiotic-treated WT mice were orthotopically implanted with KPC-derived tumor cells. (F) The percentages of tumor-infiltrating CD4+ and CD8+ T cells as a subset of CD3+ cells were determined by flow cytometry (n=10/group). (G) CD4+ and CD8+ T cells were gated and tested for expression of IFN-γ (n=4/group) and (H) CD38 (n=3/group). (I) CD4+ T cells were gated and tested for expression of ICOS (n=5/group) and LFA-1 (n=9/group). (J) FoxP3 expression was determined in CD4+ T cells in each cohort. Immune-phenotyping experiments were repeated more than 5 times (n=5/group). **(K-M)** WT mice pre-treated with an ablative oral antibiotic regimen or vehicle for 6 weeks werechallenged with orthotopic Pan02 cells and sacrificed 5 weeks later. (K) Tumor-associated macrophage expression TNF-α, (L) the fraction of intra-tumoral TCRβ+ T cells, (M)and CD4+ T cell expression of CD44 were determined by flow cytometry.This experiment was repeated twice (n=4/group). **(N-P)** WT mice pre-treated with an ablative oral antibiotic regimen or vehicle for 6 weeks were repopulated with feces from 3 month-old KPC mice or sham-repopulated. Mice were then challenged with orthotopic KPC cells and sacrificed 3 weeks later. (N) The fraction of intra-tumoral MDSC, (O) TCRβ+ T cells, and (P) CD8+ T cell expression of CD44 was recorded. This experiment was performed twice with similar results (n=4-8/group); \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001).

**Figure S7. Microbial ablation modulates inflammatory signaling in PDA and ablation of gut bacteria synergizes with PD-1 targeted therapy. (A)** A heat map was constructed based on nanostring analysis of inflammatory gene expression in orthotopic KPC tumors in mice treated with an ablative oral antibiotic regimen (n=3) vs controls (n=2). Tumors were harvested at 3 weeks. Array-based analyses of expression of inflammatory mediators was repeated twice in separate cohorts of mice.**(B-E)** WT mice were treated with αPD-1, an ablative oral antibiotic regimen, or both. Mice were challenged with orthotopic KPC tumor and sacrificed at 3 weeks. Treatments were started before tumor implantation and continued until the time of sacrifice. (B) CD44 expression in tumor-infiltrating CD4+ and (C) CD8+ T cells was determined by flow cytometry. (D) The percentage of tumor-infiltrating CD4+ T cells expressing CXCR3 and (E) CD8+ T cells expressing LFA-1 are shown for each cohort. This experiment was repeated 4 times (n=5-9/group; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001).

**Figure S8. Stage-specific microbial signatures in murine and human PDA. (A)** Representative H&E-stained sections of pancreata of 3 month-old KPC mice with early (ea-KPC; left) and advanced (adv-KPC; right) PDA are shown (scale bar = 100µm). **(B)** Heat map showing distribution of top 20 bacterial genera infecal samples of 12-week-old WT (n=6), ea-KPC (n=8), and adv-KPC (n=4) mice.Hierarchical dendrogram linkage clustering is based on composition and abundance of genera in the samples. Average abundances are shown as z-score normalization. **(C-E)** LDA analysis was used to determine differentially enriched genera between WT, ea-KPC, and adv-KPC cohorts.All data shown are statistically significant by theKruskal-Wallis test. **(F)** Relationships between microbial communities for the WT, ea-KPC, and adv-KPC cohorts were analyzed by PCoA. The data indicate three distinct clusters representing each cohort. Variations are shown on the x- and y-axes. Ellipses indicate a 95% CI (p=0.009). **(G)** The gut microbiome of WT, ea-KPC, and adv-KPC mice were tested for differences in phylogenetic diversity (\*p<0.05). **(H)** LDA analysis was used to determine the differentially enriched genera between patients with Stage I/II and Stage IV PDA. LDA data shown are statistically significant by theKruskal-Wallis test.