Figure S1. After surgical resection, primary tumors from 5 patients (1-5) were used to establish PDX tumors. These PDX tissues were then used to generate PDX-derived organoids and PDX-derived 2D cell lines. In a second group of patients (6-10), organoids were established directly from resected primary. For primary tumor and models, morphological analysis, protein expression and DNA and RNA sequencing were performed.

Figure S2. Patient and PDX-derived organoids share protein expression features with the primary tumor. **(A)** Representative IHC staining patterns of CK19 and CEA of primary PDAC, PDX and PDX-derived organoid models (patients 1-5), and **(B)** patient-derived organoids (patients 6-10). Results are representative of 5 independent fields. Imaging at 20X and 40X (bar 50μm). **(C)** CK19 and CEA protein levels from normal and adjacent benign pancreas, primary tumor, PDX, and organoids were quantified. Expression was calculated by quantifying pixel intensity of 5 different fields per patient (n=10, individual patients reflected by color, filled shapes are PDX-derived and empty shapes are tumor-derived groups). The mean is indicated. Statistical analysis overall compared to benign tissue, *$p<.05$, ** $p<.01$.

Figure S3. Principal component analysis (PCA) score plot of the RNA sequencing data from tumor, normal, PDX and 2D cell lines from patient 1-4 and 7, compared to the adjacent-normal control.

Figure S4. Patient adjacent-normal pancreas tissue acquired during pancreas resection surgery is similar to normal pancreas from non-disease patients. RNA sequencing data from **(A)** brain, **(B)** muscle (GTEx database, y-axis) are distinct from adjacent-normal pancreas (study patients, x-axis). r=.002, R2=0.0000. **(C)** RNA expression from study patient adjacent normal tissues (x-axis) is similar to non-diseased, normal pancreas (GTEx, y-axis). r=.4375, R2=0.1914. **(D)** Study patient

PDAC tissue RNA expression (x-axis) is distinct from non-diseased, normal pancreas (GTEx, y-axis). r=.011, R2=0.0001. RNA TPM counts were Log2 transformed.

Figure. S5. Analysis of gene expression data from primary tumor, PDX, and cell lines indicates cancer-related and immune signaling pathways that are up- or down-regulated across all samples. RNAseq was performed using isolated RNA from five primary tumor samples and the corresponding PDX, and/or 2D cell lines. Differentially-expressed genes (2-fold up or down from pooled normal, $p<0.05$) were analyzed by Ingenuity Pathway Analysis to predict cancer related **(A)**, or immune **(B)**, signaling pathway activation according to gene expression. The Benjamini-Hochberg method was utilized and an FDR cutoff of $q<0.05$ was applied**.**

Figure. S6. Correlation between RNA-seq measurements, in log(1+TPM) pooled organoids and the primary tumor (left), PDX (middle) and 2D cell line (right). R-square values are indicated in the upper right corner.

Movies S1. Example of a Control and Gemcitabine 30nM during a 72h time-course apoptotic assay with Annexin V. Control.

List S1. Complete marker genes list for Dropseq.