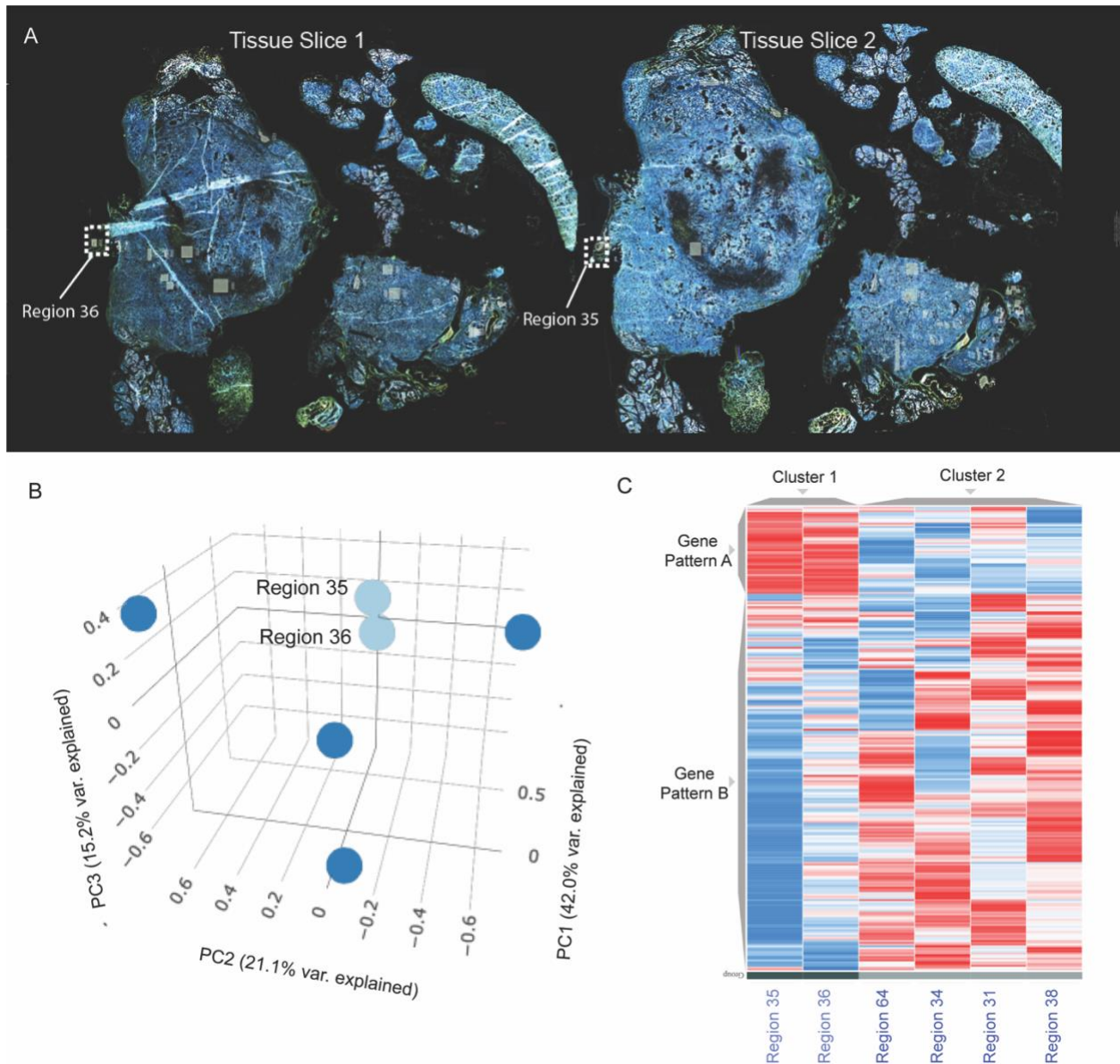
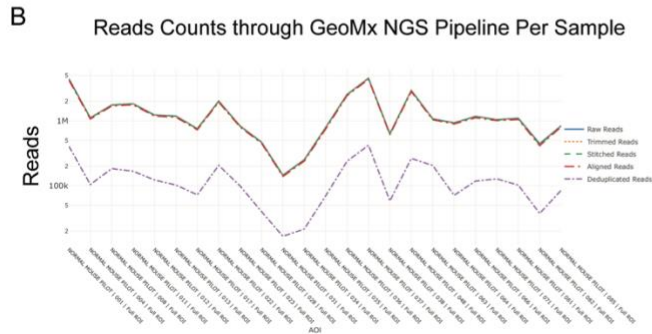
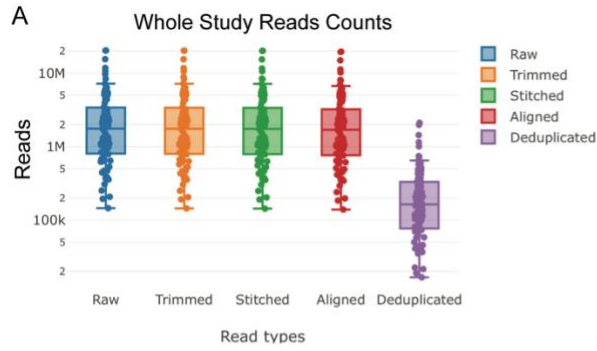


**Supplemental Figure 1. GeoMX Nanostring validation and transcriptional change in distinct tissue regions of spleen and acinar pancreas.** (A) Principal component analysis of comparing spleen tissues and acinar tissues. spleen tissues (blue), acinar tissues (red). N = 9 Total ROIs from 6 acinar regions and 3 splenic regions. (B) Volcano plot of highlighting significant differential gene expression upregulated in acinar tissue (red) and downregulated in acinar tissue (blue; upregulated in spleen). (C) Gene ontology clustering and Kegg Pathways analysis of the most upregulated pathways based on differential gene expression analysis of spleen and acinar tissues.



**Supplemental Figure 2. GeoMx Nanostring validation and transcriptional change in matched tissue type and location regions from sequential tissue slices.** Regional analysis of sequential tissues from whole mount sections sent for GeoMx sequencing analysis. (A) Isolation of region 35 and region 36 (boxed white dotted line) from sequential sections of PDAC, acinar, and spleen tissues. Principal component analysis of comparing regional tissues 35 and 36 containing multiple cell types including F4/80+ cells and TH+ bundles and TH+ fibers. (C) Heatmap of expression values from spleen tissues (blue)

and acinar tissues (red). (B) Volcano plot of highlighting significant differential gene expression upregulated in acinar tissue (red) and downregulated in acinar tissue (blue; upregulated in spleen). (C) Differential gene expression matrix clustering of transcription profile cluster region 35 and 36 compared to four random, non-pairwise matched regions.

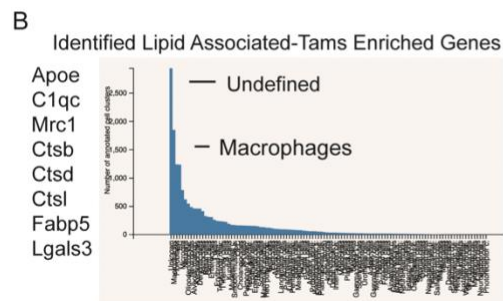
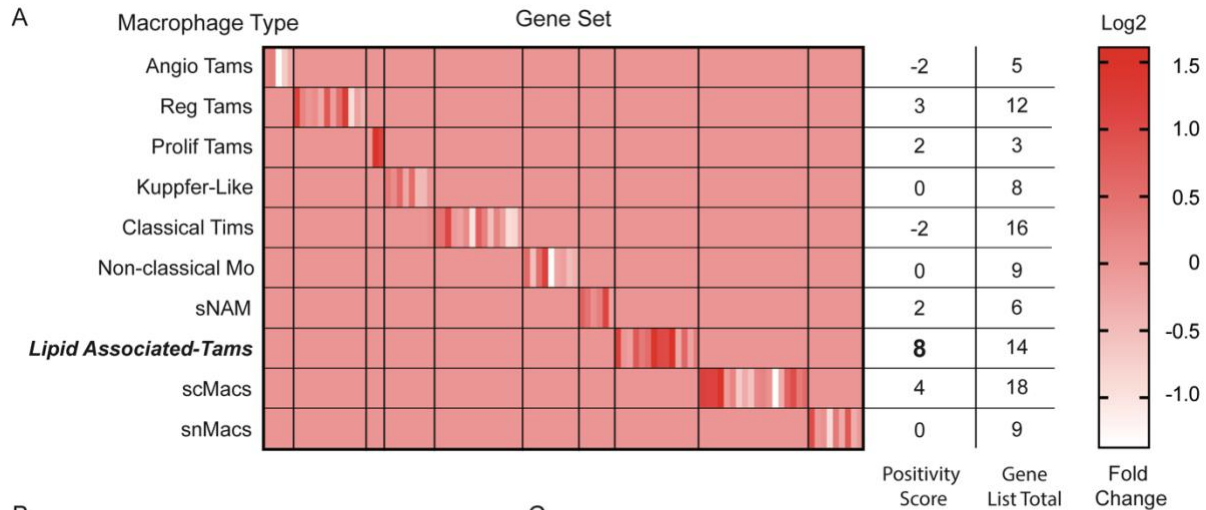


**C ROI Information**

ROI name	Surface area	Nuclei count	Sequencing s	UMI Q30
1	31752.9295	263	90.4372064	0.9962
4	4087.75458	18	90.2429185	0.9961
8	13757.1407	122	89.1744512	0.9965
12	5963.30325	71	89.6391844	0.9961
13	11650.1798	112	90.9969537	0.9966
17	6197.72702	53	90.071278	0.9968
22	15071.7525	139	89.3758369	0.9964
23	9605.03451	128	87.64789	0.9967
28	3637.61023	26	91.1479642	0.9966
31	1538.2574	11	88.0346492	0.9961
34	4580.53586	26	91.0425289	0.9968
35	38902.6167	163	90.6473235	0.9961
36	28671.5011	126	90.277333	0.9966
38	13907.7172	70	90.4567775	0.9965
48	19713.9454	224	90.7015608	0.9959
63	18904.6366	149	80.2056751	0.9962
64	6897.51129	60	92.0560033	0.9964
66	5795.29162	38	89.4391708	0.9964
71	10412.4413	104	87.3626744	0.9962
81	11927.2405	58	90.291956	0.9964
82	3529.35368	23	90.9565868	0.9964
85	18327.057	83	89.4520973	0.996

**Supplemental Figure 3. Quality control analysis of GeoMx Nanostring samples.**

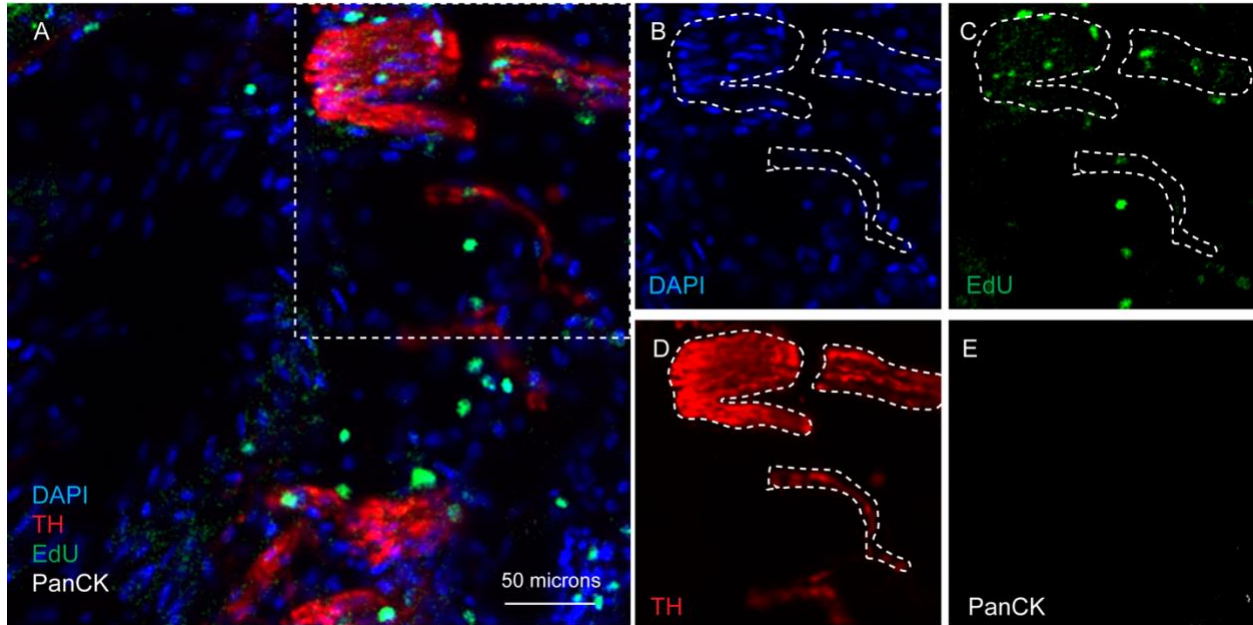
(A) Read counts averaged from all samples for the whole study during raw, trimming, stitching, alignment and deduplications. (B) Breakdown of read counts per sample. (C) ROI information containing surface area, nuclei count, sequencing saturation and UMIQ30.



**C**

Reactome pathways	# expected	Fold Enrichment	+/-	Δ raw P value	FDR
<a href="#">Immune System</a>	7	.63	11.19	+	1.35E-07 2.30E-04
<a href="#">Innate Immune System</a>	6	.38	15.76	+	3.05E-07 2.59E-04
<a href="#">Collagen degradation</a>	3	.02	> 100	+	6.51E-07 3.69E-04
<a href="#">MHC class II antigen presentation</a>	3	.04	72.36	+	8.05E-06 3.42E-03
<a href="#">Trafficking and processing of endosomal TLR</a>	2	.00	> 100	+	1.05E-05 3.57E-03
<a href="#">Degradation of the extracellular matrix</a>	3	.05	65.47	+	1.08E-05 3.06E-03
<a href="#">Neutrophil degranulation</a>	4	.19	20.91	+	2.16E-05 5.24E-03
<a href="#">Adaptive Immune System</a>	4	.29	13.73	+	1.11E-04 2.35E-02
<a href="#">Extracellular matrix organization</a>	3	.10	29.36	+	1.14E-04 2.14E-02
<a href="#">Assembly of collagen fibrils and other multimeric structures</a>	2	.02	90.15	+	2.23E-04 3.80E-02

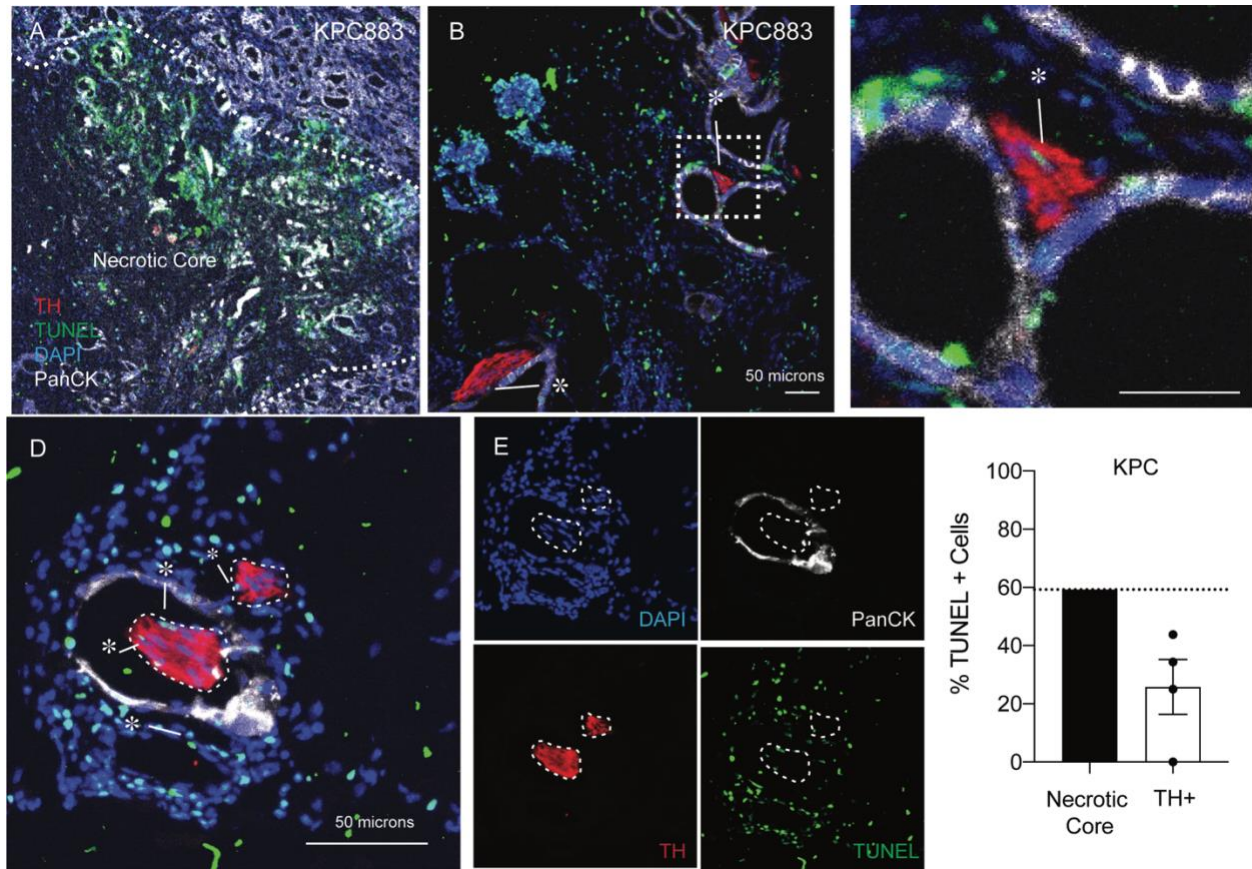
**Supplemental Figure 4. Nerve associated macrophages are enriched for genes expressed by lipid-associated TAMs.** (A) Gene enrichment positivity analysis for nerve associated macrophages compared to angio-TAMs, reg-TAMs, proliferating TAMs, Kupffer-like, classical TIMs, non-classical macrophages, sympathetic nerve associated macrophages, lipid associated TAMs, and sciatic nerve associated macrophage subsets (sn and scMacs). (B) Identified lipid associated-TAM signature genes were clustered in Panglao DB. (C) Functional gene ontology analysis of significantly upregulated pathways identified using nerve associated macrophage containing lipid signature genes.



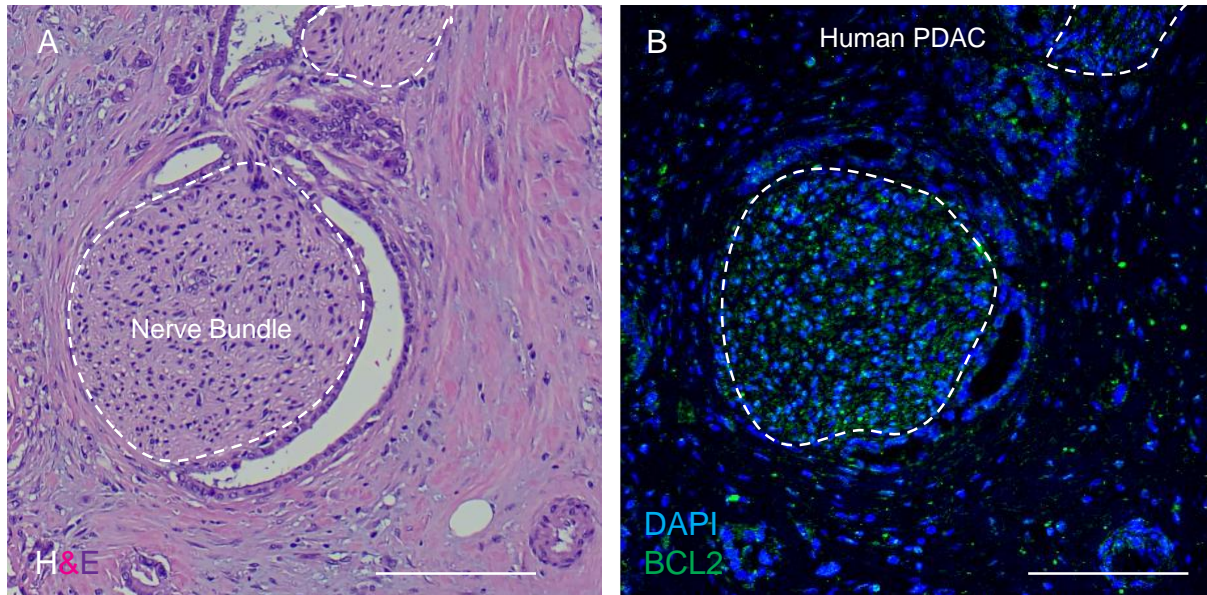
**Supplemental Figure 5. Colocalization of EdU positive nuclei within TH+ bundles.**

(A) Merged images from confocal microscopy immunofluorescence labeling TH (red), DAPI (blue), PanCK (white; negative), and EdU (green). Scale bar 50 microns. (B-E) Split images of merged figure A of DAPI [B], EdU [C], TH [D] and PanCK [E].





**Supplemental Figure 6. TUNEL positive cells are found in tumor nerve bundles within pancreatic tumors of KPC mice.** (A) Immunofluorescence analysis of KPC tumors marking TH+ bundles (red), TUNEL+ cells (green), PanCK+ epithelial cells (white), and cell nuclei (DAPI). (B-D) low and high magnification of KPC mice containing TH+ bundles with TUNEL+ cells inside. (E) Split fluorescence channel images of D. (F) Quantification of the percentage of TUNEL+ cells within TH+ bundles of KPC mice compared to TUNEL+ cells within necrotic core tissue.



**Supplemental Figure 7. BCL-2 immunofluorescence of human PDAC tissues. (A-B)** Sequential PPFE sections containing staining for (A) H&E and (B) immunofluorescence of BCL2 (green) and DAPI (blue; nuclei) in human PDAC tissue. Scale bar 200 microns.



**Supplemental Table 1. Differentially expressed genes between nerve bundles and fibers.** Differential expression of genes upregulated in ROIs from nerve bundles compared to ROIs from nerve fibers.

**Supplemental Table 2. Differentially expressed genes between spleen and acinar tissues.** Differential expression of genes upregulated in ROIs from acinar tissues compared to ROIs from spleen tissues.

**Supplemental Table 3. Differentially expressed genes between TH+ bundles and TH+, F4/80+ bundles.** Differential expression of genes upregulated in ROIs from acinar tissues compared to ROIs from spleen tissues.

**Supplemental Movie 1. Calcium imaging of the tumor nerve microenvironment in living PDAC organotypic tissue slices.**