Supplemental Information:

Supplemental Methods:

Immunohistochemistry details:

Antigen retrieval was performed in EDTA buffer using a pressure cooker. One hour primary incubation with rabbit-antibodies against PLXND1 (1:200 Novus) or SEMA3D (1:200 Abcam) was followed by two washes and kit-supplied secondary amplifier antibody. After washing, ImmPRESS Excel polymer was added for 30 minutes. 3,3'-Diaminobenzidine hydrochloride (DAB) was added for development. All slides were counterstained with hematoxylin. PNI was scored directly by a blinded pathologist using H&E stained slides. Tumor presence in the perineurium/endoneurium region or a minimum of 120 degree encasement of the nerves by tumor cells was considered to be PNI.

Multiplex and duplex IHC was performed using the sequential staining and striping method on mouse and human tissue. In brief, slides were blocked and incubated with primary antibody for 1 hour for EpCam (1:500 Abcam), TUJ1 (1:1000 BioLegend), and PLXND1 (1:200 Novus), or 3 hours for SEMA3D (1:50 Abcam) and secondary antibody (Nacalai USA) for 30 minutes. Lastly, slides were developed with AEC (Vector Laboratories), scanned and stripped to allow for subsequent antibody staining.

Halo Image Analysis:

Stained slides were scanned using the Hamamatsu (Nano Zoomer) at 20-40x magnification and analyzed using the HALO 2.0 software. Regions of interest (tumor area and individual nerves) and any staining artifacts (excluded) were manually selected. Individual nuclei were determined by hematoxylin counterstain. Threshold weak, moderate, and strong positive expression values for SEMA3D/PLXND1 were applied to all slides. Spatial data was stored upon analysis. Distance analysis was determined by the amount of total positive (weak, moderate and strong) cells within 500 microns, 10 bins of 50 microns each, of all manually selected nerves in the tumor tissue. Cell number was normalized to tissue area and overall SEMA3D/PLXND1 staining to account

for inherent increase in SEMA3D/PLXND1 positive staining in PNI compared to no PNI samples. Total weak SEMA3D and PLXND1 staining per μm^2 tissue was used to compare tissue cellularity.

Supplemental Figure Legends:

Figure S1, Related to Figure 1 and 3: DRG co-culture increases tumor cell invasion. (A) KPC tumor cells were plated on the top chamber of a modified Boyden chamber and DRG or no DRG cells were plated on the bottom chamber. CCK8 was used to quantify the number of invading cells after 24 hours. Data are means ± SEM from 4 technical replicates. *$p<0.05$, (unpaired, two-tailed students t-test). (B) Western blot of KPC cells transfected with SEMA3D targeting shRNA or control shRNA blotted for SEMA3D and loading control β-actin. (C) Western blot of KPC cells transfected with ANXA2 targeting siRNA or scramble control siRNA blotted for ANXA2 and loading control β-actin.

Figure S2, Related to Figure 2: PLXND1 is expressed on postnatal murine DRG cells. Representative immunohistochemical images stained for PLXND1 on murine DRG nerve tissue. Higher magnification is shown in the panel the right.

Figure S3, Related to Figure 2: Tumor condition media increases neurite outgrowth. (A) DRG cells were treated with control media, KPC conditioned media or KPC control media + PLXND1 Ab. Phase contrast images were taken every three hours and using neurite outgrowth analysis, the neurite length per cell body cluster area and (B) the neurite branch points per cell body cluster area over time was analyzed. Data are means ± SEM from 4 technical replicates and representative of at least 2 experiments, Kruskal-Wallis test. n.s., not significant, ****$p<0.0001$.

Figure S4, Related to Figure 4: PLXND1 is expression is reduced in PLAC mice. (A) PLXND1 immunohistochemical staining on DRG cells isolated from control (PLXND1$^{f/f}$, PL) or PLXND1 nerve specific knock out mice (PLXND1$^{f/f}$ ADV-CRE$^{+/+}$, PLAC). (B) H&E and PLXND1 staining on nerves innervating PL

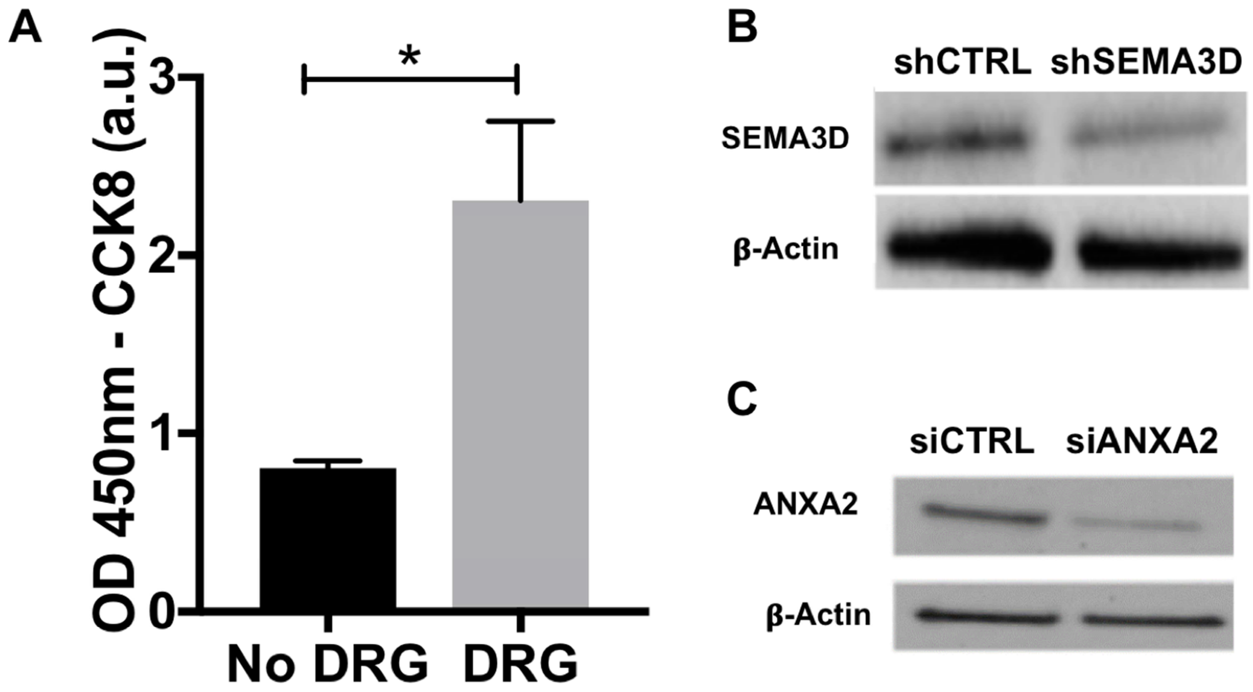
and PLAC mice pancreas tumor tissue. Scale bar= 50µm. (C) Western Blot of DRG cells isolated from PL and PLAC mice blotted for PLXND1 and GAPDH.

Figure S5, Related to Figure 5: SEMA3D and PLXND1 co-localize in murine tumor epithelia. Representative images from KPC PDA tumor tissue stained with multiplex IHC. A single slide of KPC PDA tissue was stained for Hematoxylin, SEMA3D and PLXND1 through sequential staining and stripping. SEMA3D and PLXND1 co-localization was analyzed and is shown in the merge column. Scale bar =300µm.

Figure S6, Related to Figure 5 and Table 1: SEMA3D and PLXND1 expression in PNI. Representative images (zoomed in from Figure 5C) of H&E, SEMA3D and PLXND1 staining in primary human PDA tissue with and without PNI. Arrows indicate nerves and arrowheads indicate tumor cells. Scale bar = 50µm.

Figure S7, Related to Figure 5: Method to determine distance analysis. (A) Representative image analysis of human primary tumor tissue slides identified without or with PNI were stained for PLXND1. Halo Image Analysis was used to determine the distance positively stained cells were positioned in proximity to intratumoral nerves (blue). PLXND1 positive tumor cells within 500 microns of nerves were highlighted in red; whereas, PLXND1 positive tumor cells located greater than 500 microns are indicated in green. (B) Human primary tumor tissue slides identified with weak SEMA3D or (C) PLXND1 cells normalized to the tissue area analyzed demonstrating no difference in tumor cellularity between samples with and without PNI.

Figure S1:



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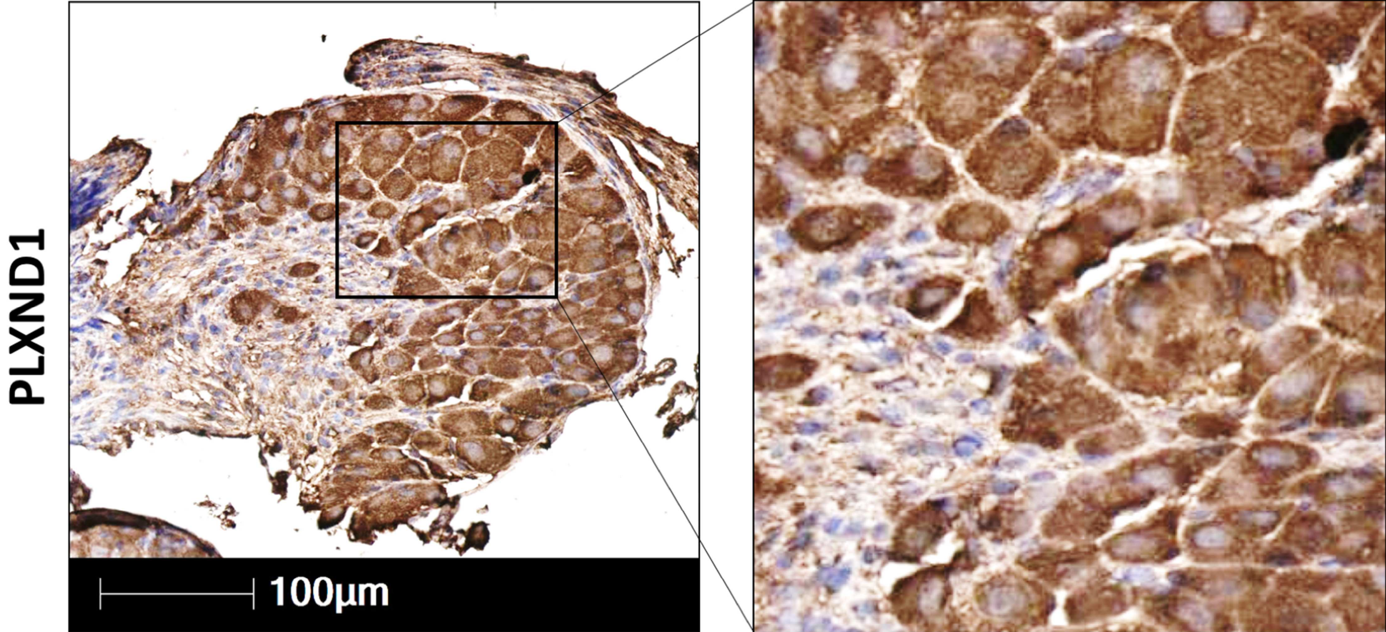

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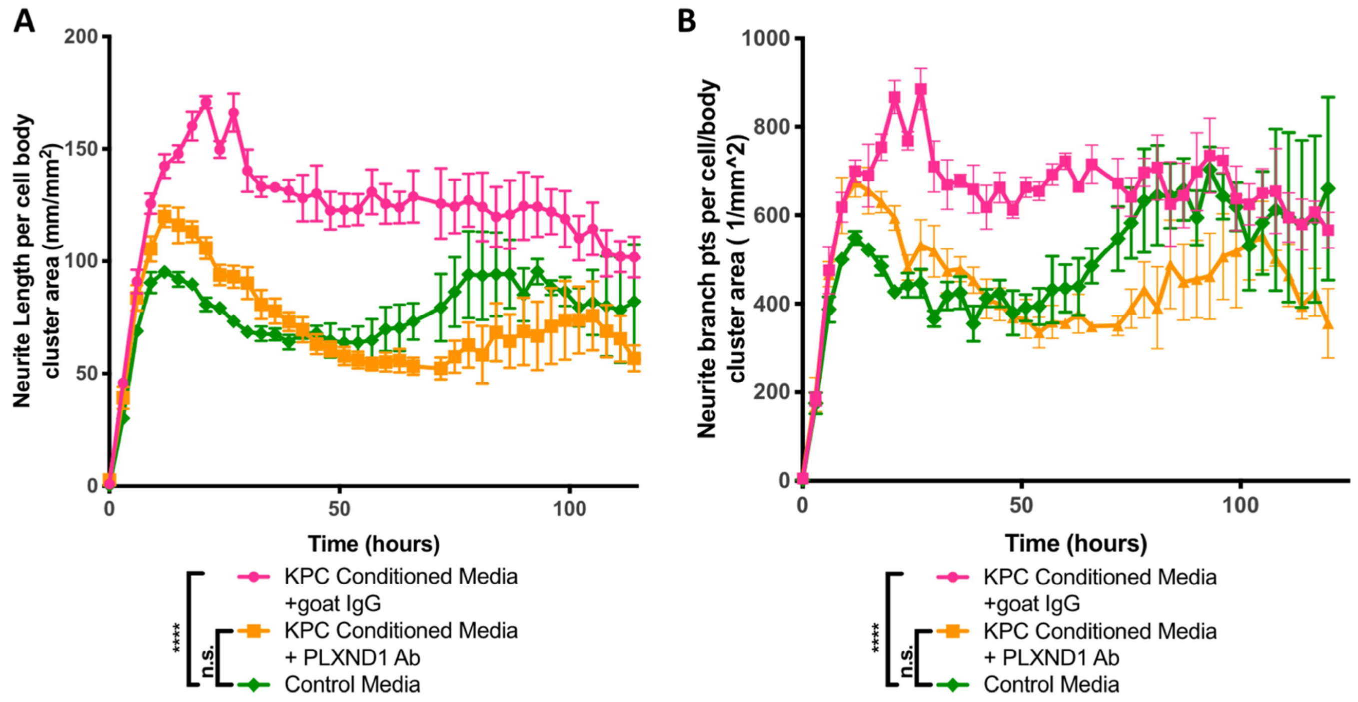

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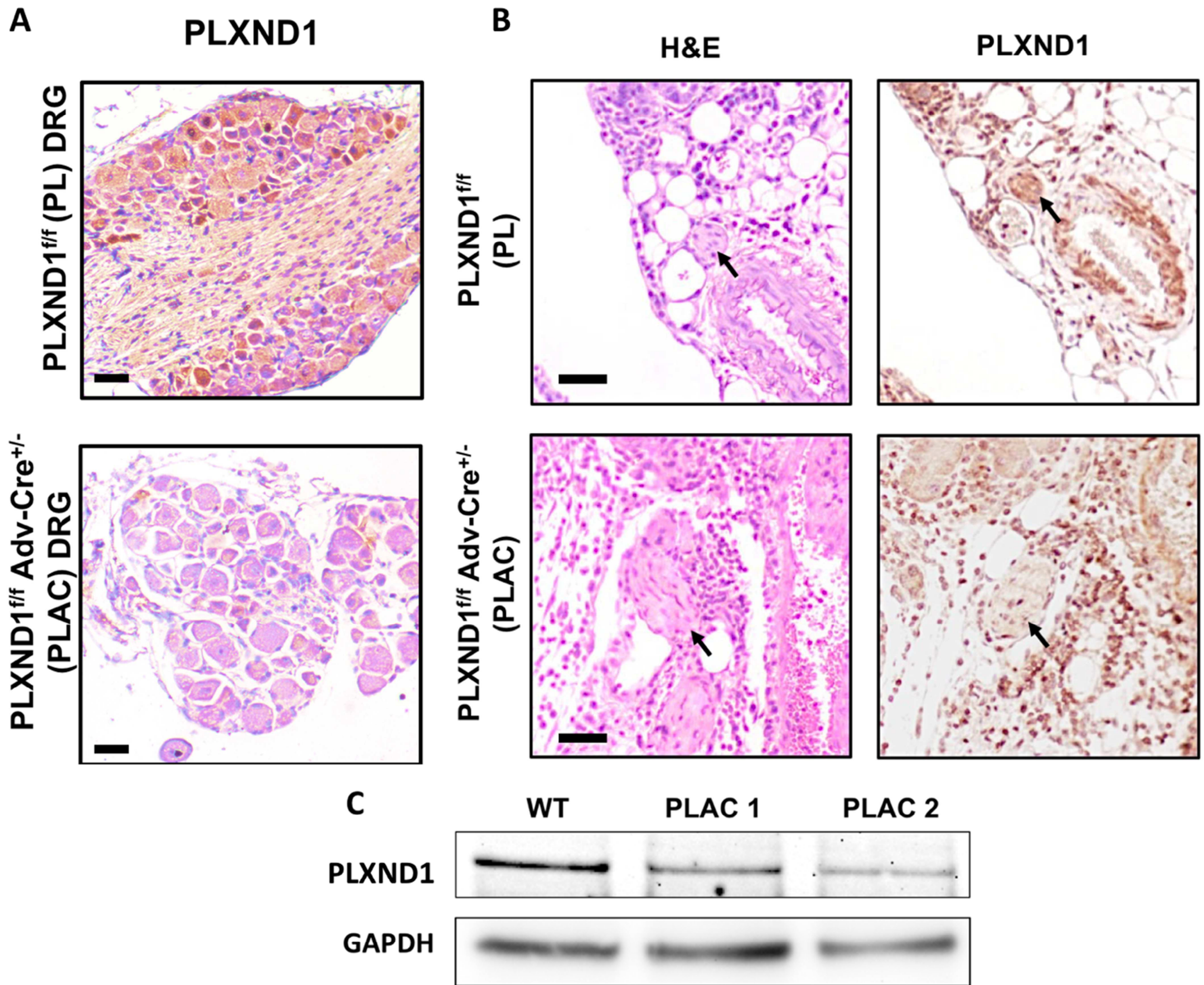

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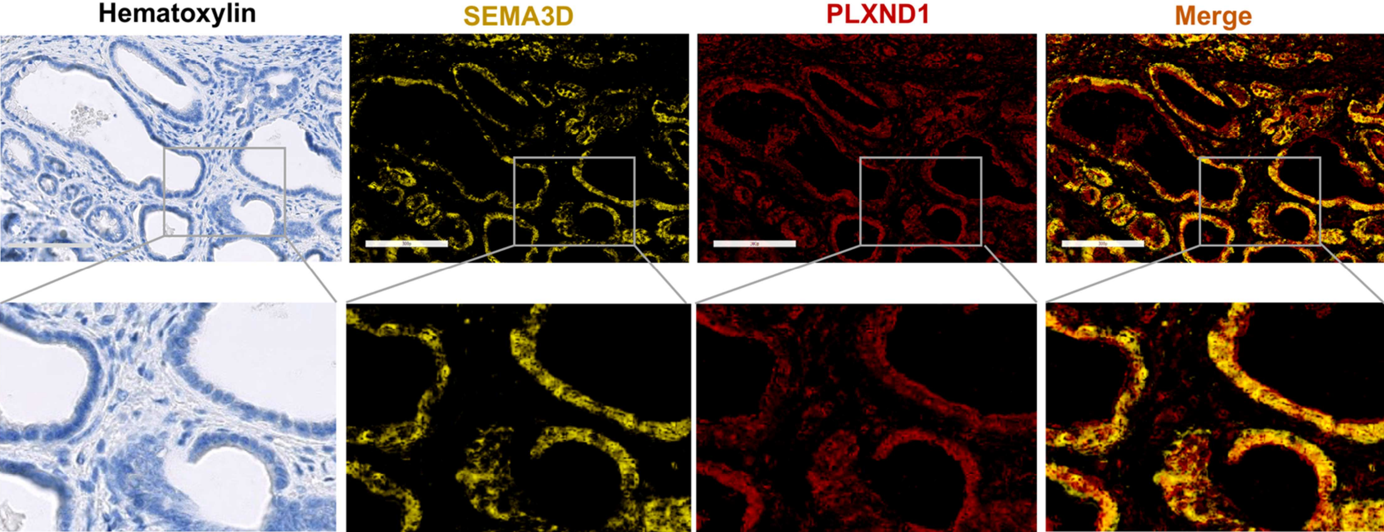

Figure S6

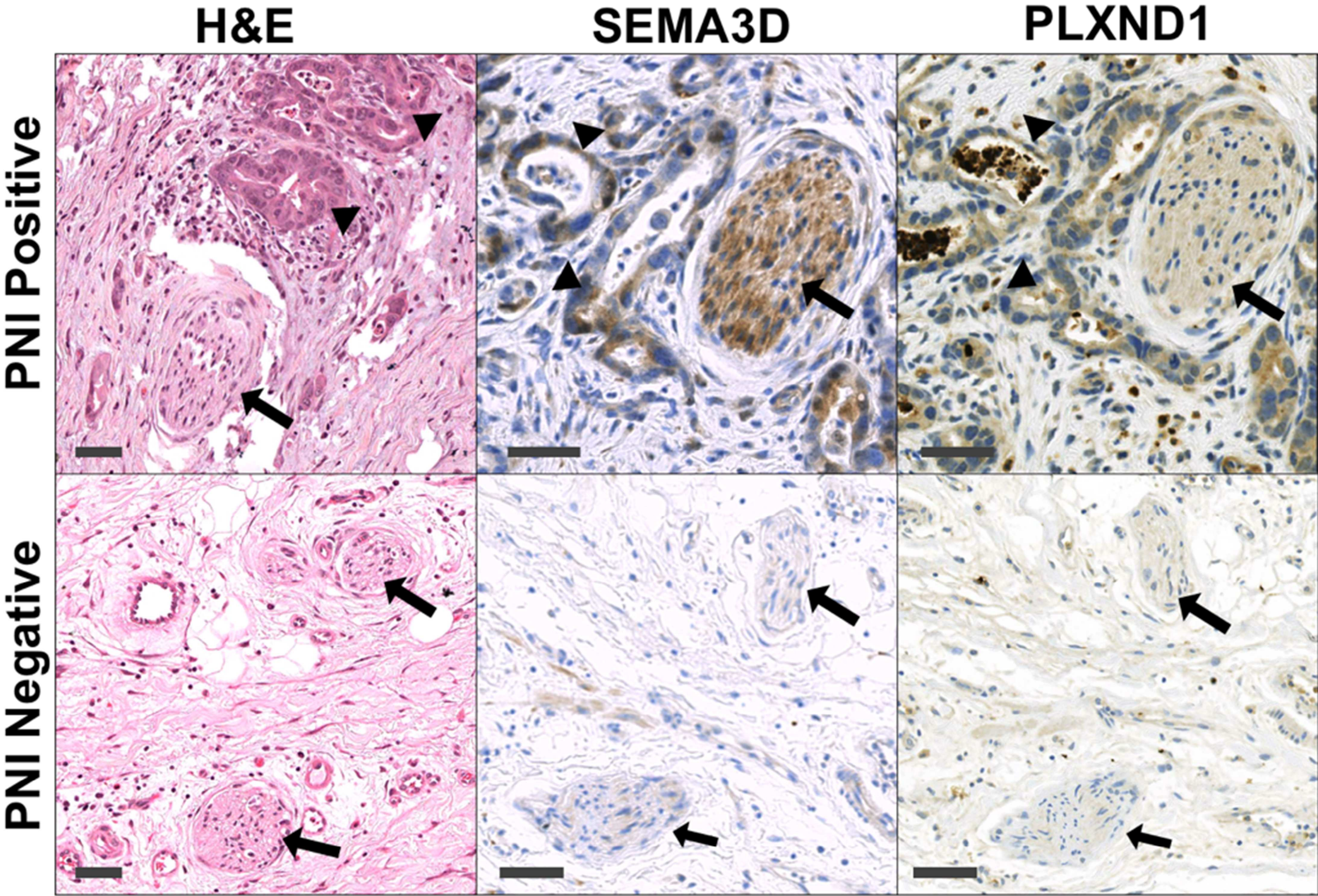

Figure S7: