**Supplemental Materials**

**Supplementary Materials and Methods**

**Culture and co-culture**: Human pancreatic normal epithelial (HPNE cells) were cultured in a medium composed of 75% DMEM without glucose (Sigma), supplemented with 2 mM L-glutamine and 1.5 g/L sodium bicarbonate, and 25% Medium M3 Base (Incell Corp., San Antonio, TX). For complete growth medium, 5% FBS, 10 ng/ml human recombinant EGF, 5.5 mM D-glucose, and 750 ng/ml puromycin were added.

Co-culture experiments utilized 1x104 cells of GFP-HNDFs mixed with 5x103 PDA cells in 96 wells. GFP-HNDF proliferation was measured daily for 14 days by assessing fluorescence intensity (RFUs) using the SpectraMax Gemini XS microplate fluorometer (Molecular Devices, Sunnyvale, CA) at 485 nm excitation and 538 nm emission. To determine the effect of PDA cells on activation of fibroblasts and collagen deposition, 2.5x104 cells of NHF544 were cultured in a mixture with 2.5x104 cells of PDA cells transduced with shRNAs and plated on an 8-well Millicell® EZ SLIDE (Millipore, Billerica, MA). After 24 hours of co-culture, activation of fibroblasts and collagen deposition were analyzed by immunofluorescence by using α-SMA (Sigma) and collagen 1 (Abcam) antibodies, respectively. To study the proliferation of PDA cells in co-culture with hPSCs, 2x105 hPSCs were seeded in 12-well plates. 24 hours later, 6x104 (Panc1) or 9x104 (AsPC1) cells were added on top of the hPSCs feeder layer and were allowed to proliferate for up to 10 days.

**Immunohistochemistry (IHC)**:Paraffin-embedded de-identified PDA specimens from the IUSCC Tissue Bank were immunostained by using antibodies against TG2 (Neomarkers, 1:200) and isopeptide (Abcam, Cambridge, MA, 1:100), after sodium citrate antigen retrieval. Paraffin-embedded pancreatic xenografts were stained similarly, using a TG2 polyclonal antibody (NeoMarkers, 1:50) and ready to use α-SMA monoclonal antibody (IHC World) for myofibroblasts. Secondary labeling was based on the Avidin/Biotin system (Dako North America, Inc.). Slides were stained with 3-3’ diaminobenzidine (DAB) and counterstained with hematoxyllin. Negative controls were run in parallel, with isotype IgG control. H score was calculated as percentage of stained cells multiplied by intensity of staining (graded from 0 to 3+). All slides were reviewed by a board-certified pathologist (RE). In total, we stained 75 specimens, of which 52 tumors were part of a tissue microarray (TMA), 20 were identified as tumors associated with desmoplasia, and 3 were normal pancreas. The IU Institutional Review Board approved the use of de-identified human tissue specimens.

**Western Blot Analysis:** Cells were lysed in Radio-Immunoprecipitation Assay (RIPA) buffer containing Halt™ protease inhibitor cocktail (Thermo Scientific). Equal amounts of proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to Immuno-Blot polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Inc.). After blocking, membranes were blotted with primary antibodies followed by HRP-conjugated secondary antibodies. The protein-antibody complexes were visualized by enhanced chemiluminescence solution (Thermo Scientific), and images were captured by ImageQuant LAS 4000 mini imager with a Chemilux CCD camera (GE Healthcare, Uppsala, Sweden). All analyses were repeated at least 3 times.

**Immunofluorescence (IF)** used antibodies for TG2 (Neomarkers), α-SMA (Sigma), collagen 1 (Abcam), and YAP/TAZ (Cell Signaling) at a 1:100 dilution. PDA cells were cultured on collagen coated (StemCell Technologies, Inc., Vancouver, BC), fibronectin coated (BD Biosciences, Bedford, MA), or uncoated slides (Millicell® EZ SLIDE, Millipore). After fixation in 4% paraformaldehyde, cells were permeabilized using 0.1% Triton X-100 in PBS for 5 min and blocked for 1 hour with 3% goat serum in PBS-T (0.1% Tween 20). Subsequently, cells were incubated overnight at 4°C with antibodies for TG2 (Neomarkers), α-SMA (Sigma), collagen 1 (Abcam), and YAP/TAZ (Cell Signaling) at a 1:100 dilution, followed by 1 hour incubation with CyTM5-conjugated anti-mouse secondary antibody (Invitrogen Corporation, Camarillo, CA) and Alexa Fluor® 488 goat anti-rabbit IgG (Life Technologies, Carlsbad, CA). To detect matrix-bound TG2, cells were not permeabilized prior to incubation with the TG2 antibody (1:100 dilution), as previously described (23). Isotype-specific IgG was a negative control. Nuclei were visualized by 4’,6-diamidino-2-phenylindole (DAPI) staining (VECTASHIELD; Vector Laboratories, Burlingame, CA). Analysis used a Zeiss LSM 510 META confocal multiphoton microscope system (Carl Zeiss, Inc., Thornwood, NY, USA) under UV excitation at 635 nm for Cy5, 488 nm for Alexa Fluor® 488, and 405 nm for DAPI.

**Proliferation Assays:** Theproliferation of NHF544 on native or crosslinked collagen used the cell counting kit (CCK)-8 (Dojindo Molecular Technologies, Inc., Rockville, MD), according to the manufacturer’s instructions. Absorbance at 450 nm was measured using EL800 microplate reader (BioTek Instruments, Inc., Winooski, VT). The bromodeoxyuridine (BrdU) cell proliferation ELISA kit (Abcam) was used to measure proliferation of PDA cells. Absorbance at 370 nm was measured by SPECTRAmax® Plus 384 microplate spectrophotometer (Molecular Devices). Crystal violet staining was utilized to stain the PDA cells grown on the hPSCs feeder layer. Cells were fixed and stained for 20 min at room temperature with Fixing/Staining solution, which contains 0.05% crystal violet (w/v), 1% formaldehyde, 1X PBS, and 1% methanol. The dye was solubilized in methanol, and absorbance at 540 nm was measured on the SPECTRAmax® Plus 384 spectrophotometer (Molecular Devices). Experiments were performed in 4 replicates and were repeated 2-3 times.

**Reverse Transcription PCR (RT-PCR) and Quantitative Real-Time PCR (qRT-PCR)**:Total RNA was extracted using RNA STAT-60 Reagent (Tel-Test Inc., Friendswood, TX) and reverse-transcribed using iScript cDNA synthesis kit (Bio-Rad Laboratories, Inc.). The reverse transcriptase product (1 μl) and primers were heated at 94°C for 90-s followed by 24 rounds of amplification for GAPDH and 29 cycles for TG2 (30-s denaturing at 94°C, 30-s annealing at 57°C, and 30-s extension at 72°C) followed by a 10 min final extension at 72°C on a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc.). The RT-PCR product was visualized on an ImageQuant LAS 4000 mini imager (GE Healthcare) after fractionation on a 1% agarose gel. For qRT-PCR, the iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc.) on an ABI Prism 7900 platform (Applied Biosystems, Waltham, MA) was used. Primers used for RT-PCR were: *TG2 forward*: ACC CGC GTC GTG ACC AAC TAC AAC and *reverse*: GGT GAT ATC CTC CCG CTC GTC TCG; GAPDH *forward*: GAT TCC ACC CAT GGC AAA TTC C and *reverse*: CAC GTT GGC AGT GGG GAC. The primers for qRT-PCR were: *CTGF* *forward*: AGG AGT GGG TGT GTG ACG A and *reverse:* CCA GGC AGT TGG CTC TAA TC and *GAPDH* *forward*: AGC CAC ATC GCT CAG ACA C and *reverse*: GCC CAA TAC GAC CAA ATC C. Relative target gene expression was calculated using Ct method, 2-ΔCt=2-[Ct(CTGF)-Ct(GAPDH)], where Ct is the cycle threshold value defined as the fractional cycle number at which the target fluorescent signal passes a fixed threshold. All experiments were performed in duplicates in three independent experiments and results are presented as means ± standard error (SE) of replicates.

**Picrosirius Red Staining Quantification:** Original 12-bit images were transformed to 24-bit images by separating colors to 8-bit RGB images and recombining into a 24-bit image. The 24-bit images were resolved into the hue definitions as previously described (26); red 2-9 and 230-256, orange 10-38, yellow 39-51, and green 52-128. The proportion of different colored collagen fibers were calculated by dividing the number of pixels within each hue range by the total number of pixels in all five different hue ranges and expressed as a percentage by multiplying 100. The collagen content was quantified by dividing the total number of pixels in all five different hue ranges by total pixels of the image and calculated to percentages.

**Sircol Assay:** Frozen tumors (n=6 per group) were homogenized on ice in an extraction buffer containing 50 mM Tris-HCl (pH7.4), 150 mM NaCl, and 0.5% NP40. The homogenates were mixed by rotation for 2 hours at 4°C, followed by sonication on ice followed by 15 min of centrifugation. Protein concentration was measured by Bradford assay, and 500 μg of total protein diluted into PBS were loaded into 96-well plates and dried overnight at 37°C. 150 μl of Sircol dye reagent was added into each well and incubated for 1.5 hour at room temperature. Excess dye was removed, and the plate was washed with 5% acidified water before solubilization in 150 μl of 0.1 M NaOH. Absorbance was measured by BioPhotometer plus (Eppendorf, Hamburg, Germany) at a wavelength of 550 nm.

**Hydroxyproline Assay:** Hydroxyproline colorimetric assay kit was purchased from BioVison (Zurich, Switzerland). Frozen tumors (n=6 per group) were homogenized in dH2O (10 mg of tissue/per 100 μl of dH2O), according to manufacturer’s protocol. Absorbance was measured by SPECTRAmax® Plus 384 microplate spectrophotometer (Molecular Devices) at a wavelength of 560 nm.