Electronic Supplementary Material (ESI) for Biomaterials Science.

PDAC-on-chip for *in vitro* modeling of stromal and pancreatic cancer cells crosstalk

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SUPPLEMENTARY INFORMATION

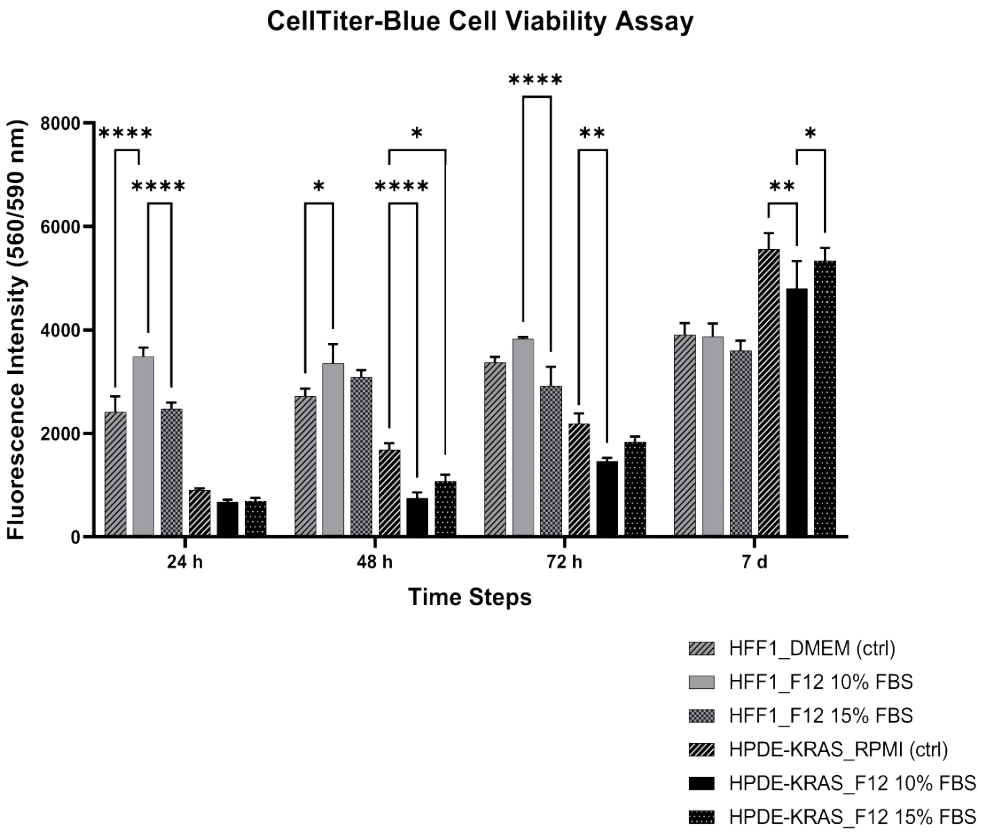

Fig. S1 Viability assay to identify the appropriate concentration of Fetal Bovine Serum (FBS) in the co-culture medium. The bar graph shows the fluorescence intensity measured for HFF1 cultured in DMEM (HFF1_DMEM) and in DMEM/F-12 supplemented with 10% FBS (HFF1_F12 10%) or 15% FBS (HFF1_F12 15%) and for HPDE-KRAS cultured in RPMI (HPDE-KRAS_RPMI) and in DMEM/F-12 supplemented with 10% FBS (HPDE-KRAS_F12 10%) or 15% FBS (HPDE-KRAS_F12 15%). Tukey's multiple comparisons test: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

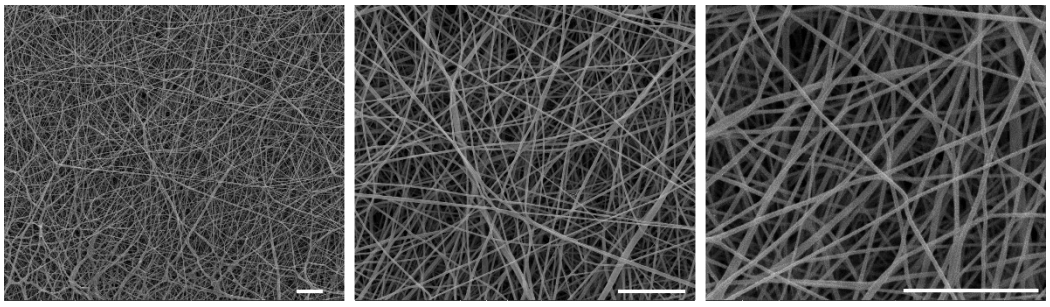

Fig. S2 Images from Scanning Electron Microscopy (SEM) analyses at different magnifications showing the PCL/Gel nanofibrous membrane architecture. Scale bars 5 µm.

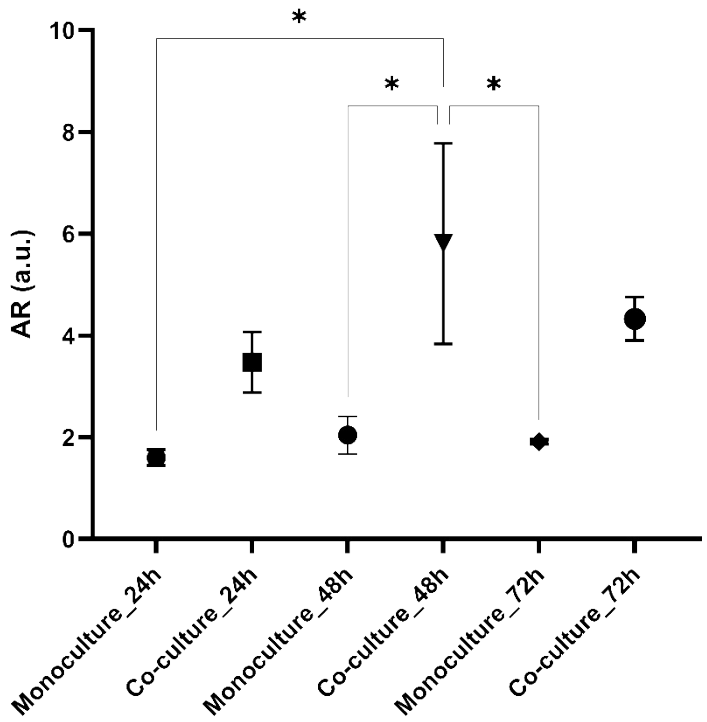

Fig. S3 Change in HFF1 aspect ratio under co-culture with HPDE-KRAS cells. Elongated fibroblasts show a mean aspect ratio above 3.9 while the round shape of HFF1 is reflected by an aspect ratio ~ 2. Tukey's multiple comparisons test: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. 60 cells in three images per condition (n=3) were analyzed. Representative images are reported in Fig.2 (first row).

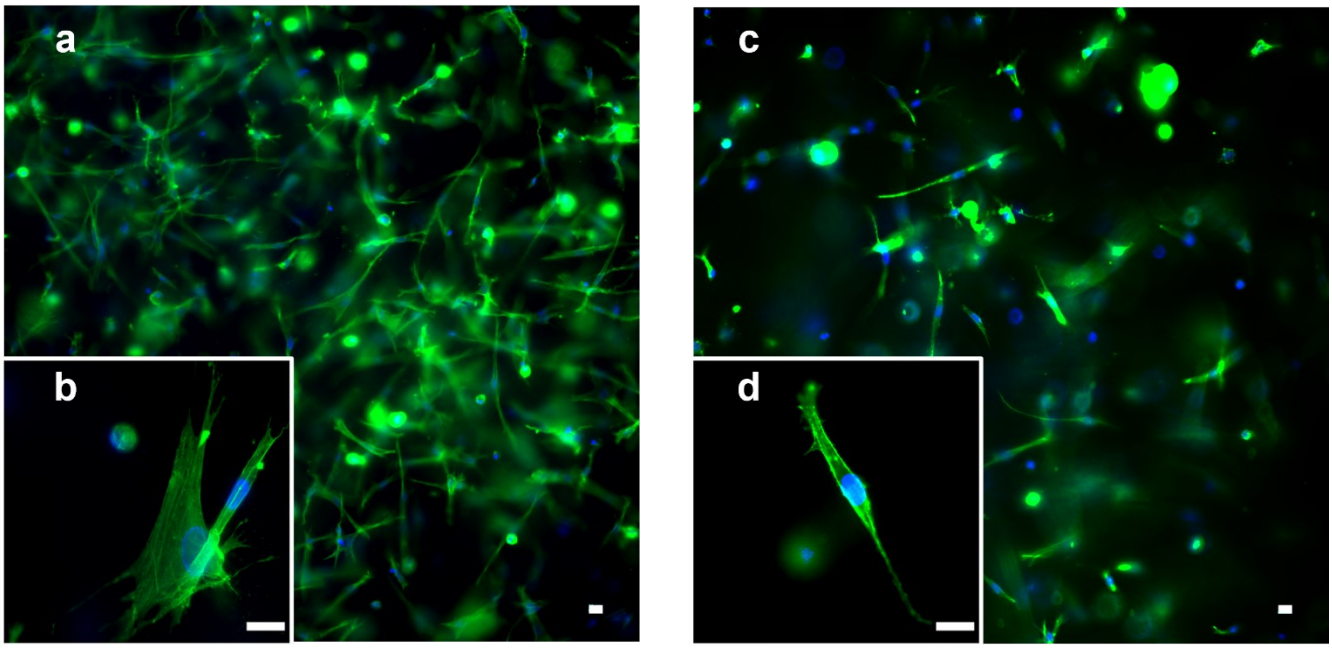

Fig. S4 Activation of human fibroblasts upon culture with HPDE-KRAS supernatant. (a-d) Fluorescent images of HFF1 embedded in type I collagen hydrogel and stained with Alexa Fluor™ 488 Phalloidin (F-actin) and DAPI (nuclei) to highlight the differences between the fibroblasts' cytoskeletons before (a-b) and after (c-d) the incubation with tumor supernatant. Cells were grown for 48h before the staining. Scale bars 20 µm.

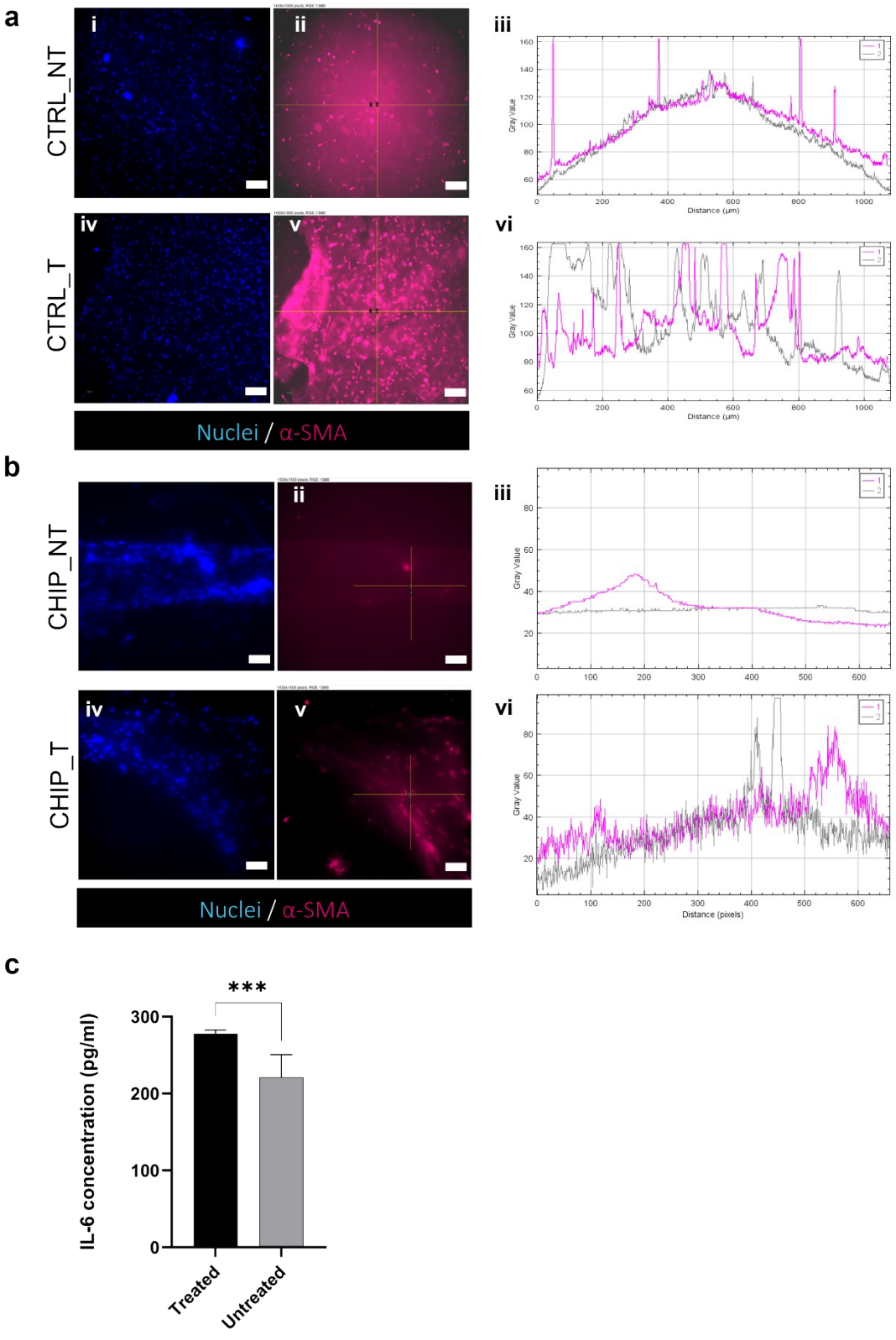

Fig. S5 Activation of human fibroblasts upon culture with HPDE-KRAS supernatant. (a-b) Confocal images showing the expression of α-SMA by fibroblasts embedded in type I collagen hydrogel (a) and seeded in the bottom layer of the microfluidic device (b) before (i-iii) and after (iv-vi) the treatment with HPDE-KRAS supernatant. The plots ($a_{iii,vi}$-$b_{iii,vi}$) report the intensity values along the two lines drawn in the images $a_{ii,v}$ and $b_{ii,v}$ (vertical line=1; horizontal line=2). Scale bars 100 μm. (c) IL-6 cytokines concentration in supernatants collected from the bottom chamber of the microfluidic device where the fibroblasts were cultured for 48h with (Treated) and without (Untreated) the HPDE_KRAS-derived supernatant. Bar plots of the data obtained from ELISA test IL-6 analysis for each culture condition (n=2). Tukey's multiple comparisons test: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.