Novel 3D µtissues Mimicking the Fibrotic Stroma in Pancreatic Cancer to Study Cellular Interactions and Stroma-Modulating Therapeutics

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Supplementary Table S1: Gene abbreviations.

Gene Abbreviation	Full Name
αSMA	Alpha smooth muscle actin
COL1α1	Collagen, type 1, alpha 1
FN1	Fibronectin 1
VIM	Vimentin
CDH1	Cadherin 1 (E-cadherin)
TGFβR1	Transforming growth factor beta receptor 1
VEGFα	Vascular endothelial growth factor alpha
PDGFRβ	Platelet-derived growth factor receptor beta 1
POSTN	Periostin
ITGA5	Integrin alpha 5
PXN	Paxillin
VCL	Vinculin
TN-C	Tenascin-C
HIF1α	Hypoxia-inducible factor 1 alpha
MMP2	Matrix metallopeptidase 2
IL-6	Interleukin 6
CK19 (also known as KRT19)	Cytokeratin 19
FAP	Fibroblast activation protein
RPS18 (housekeeping)	Ribosomal protein 18

Supplementary Table S2: Primers used in real-time PCR.

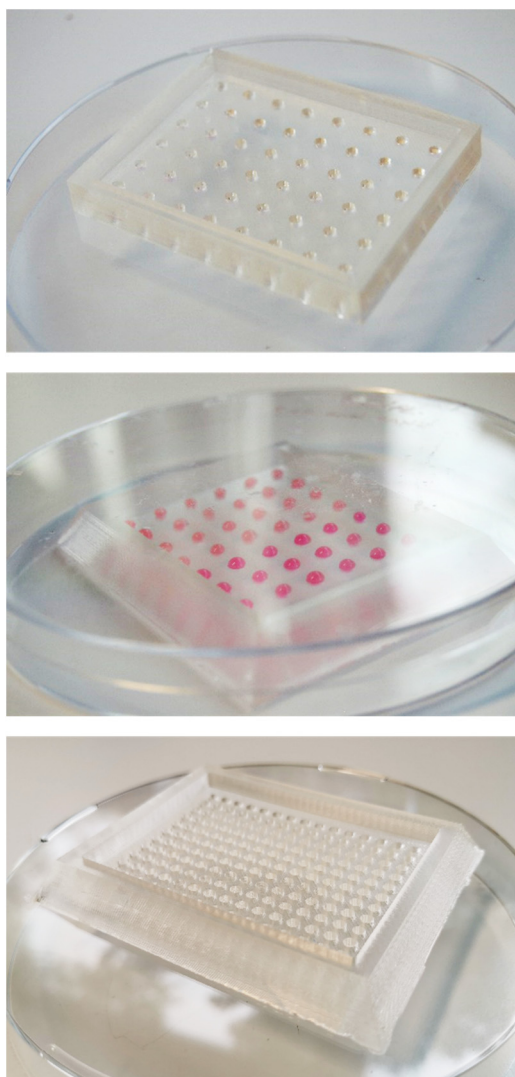| Gene	Forward Primer	Reverse Primer
αSMA	CCCCATCTATGAGGGCTATG	CAGTGGCCATCTCATTTTCA
COL1α1	GTACTGGATTGACCCCAACC	CGCCATACTCGAACTGGAAT
FN1	GTATACGAGGGCCAGCTCAT	CCCAGGAGACCACAAAGCTA
VIM	TCCAGAGAGAGGAAGCCGAA	AAGGTCAAGACGTGCCAGAG
CDH1	AACCCAAGCACGTATCAGGG	GAGTGTTGGGGGCATCATCA
TGFβR1	TCTTGGAACCTGCTCTCCTG	GTCCCACAACTTCCATCAGC
VEGFα	GCTCAGAGCGGAGAAAGCAT	GCAACGCGAGTCTGTGTTTT
PDGFRβ	AGGCAAGCTGGTCAAGATCT	GCTGTTGAAGATGCTCTCCG
POSTN	ACAAGAAGAGGTCACCAAGGTC	CTTGCAACTTCCTCACGGGT
ITGA5	CAACTTCTCCTTGGACCCCC	GTCCTCTATCCGGCTCTTGC
PXN	CATGGACGACCTCGACGC	CAAGAACACAGGCCGTTTGG
VCL	CAGAACTGGCTTGCAGATCC	CTCCTTTCCCCTGTCTTCGT
TN-C	GAAGAGCATTCCTGTCAGCG	CCCACTCCACTTCCACAGAT
HIF1α	ATGTAATGCTCCCCTCACCC	CAGGGTCAGCACTACTTCGA
MMP2	TTTCTATGGCTGCCCCAAGG	GTCAAGGTCACCTGTCTGGG
IL-6	TGATGCTGGTGACAACCACGGC	TAAGCCTCCGACTTGTGAAGTGGTA
CK19	CGCGACTACAGCCACTACTA	AGAGCCTGTTCCGTCTCAAA
FAP	GCAGTGTATCGAAAGCTGGG	AGGGCCAGTGATGAAACGTA
RPS18	TGAGGTGGAACGTGTGATCA	CCTCTATGGGCCCGAATCTT

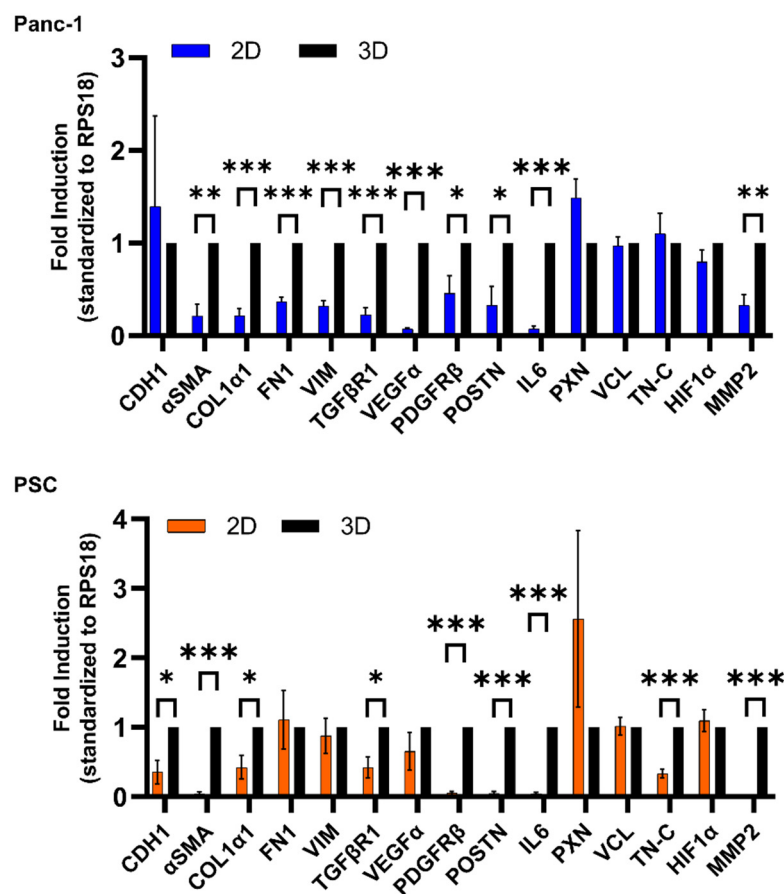**Figure S1: Custom-made PDMS well plates.**

Figure S2: Gene expression for 2D monolayer cultures versus 3D cultures. Statistical analysis was performed by two-tailed Student's *t*-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
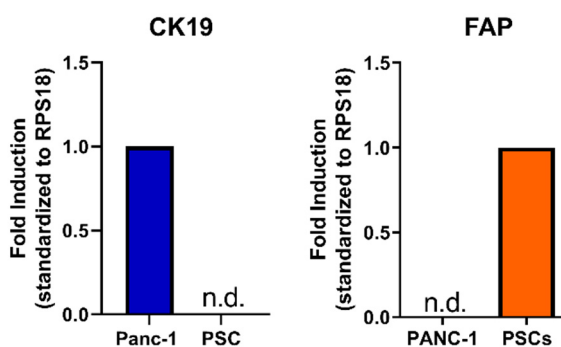

Figure S3: Specific expression of CK19 and FAP in Panc-1 and PSC 3D cultures.

Panc-1

	Panc-1 µTissue	Panc-1/ PSC Spheroids	Panc-1/ PSC µTissues
POSTN	1	10670.4	3058.136
PDGFRβ	1	1308.662	2859.181
ACTA2	1	52.90958	1031.916
FN1	1	1485.995	917.0333
COL1α1	1	0.977361	137.9807
MMP2	1	0.842493	33.16444
IL6	1	23.55897	19.60408
VIM	1	1.475845	10.44948
TGFβR1	1	1.830851	7.655798
TN-C	1	2.019891	6.150761
HIF1α	1	3.756505	3.151385
CDH1	1	0.50648	2.613294
VEGFα	1	0.530613	1.923361
PXN	1	1.768136	1.627317
VCL	1	1.558485	1.444101

Legend: 20, 15, 10, 7.5, 5, 2.5, 1.25, 1, 0.8, 0.6, 0.5

PSC

	PSC µTissue	Panc-1/ PSC Spheroids	Panc-1/ PSC µTissues
COL1α1	1	2.217327	18.13842
POSTN	1	0.299006	10.04996
FN1	1	1.776217	9.981029
IL6	1	0.17308	4.923643
MMP2	1	3.478194	2.875902
VCL	1	0.526974	2.763158
PDGFRβ	1	0.414906	2.574895
HIF1α	1	1.113096	2.005294
ACTA2	1	0.297566	1.864036
TGFβR1	1	0.347521	1.820489
VIM	1	0.112112	1.805876
TN-C	1	0.632633	1.716668
CDH1	1	0.944998	1.6475
PXN	1	2.016267	1.237862
VEGFα	1	0.407759	1.125917

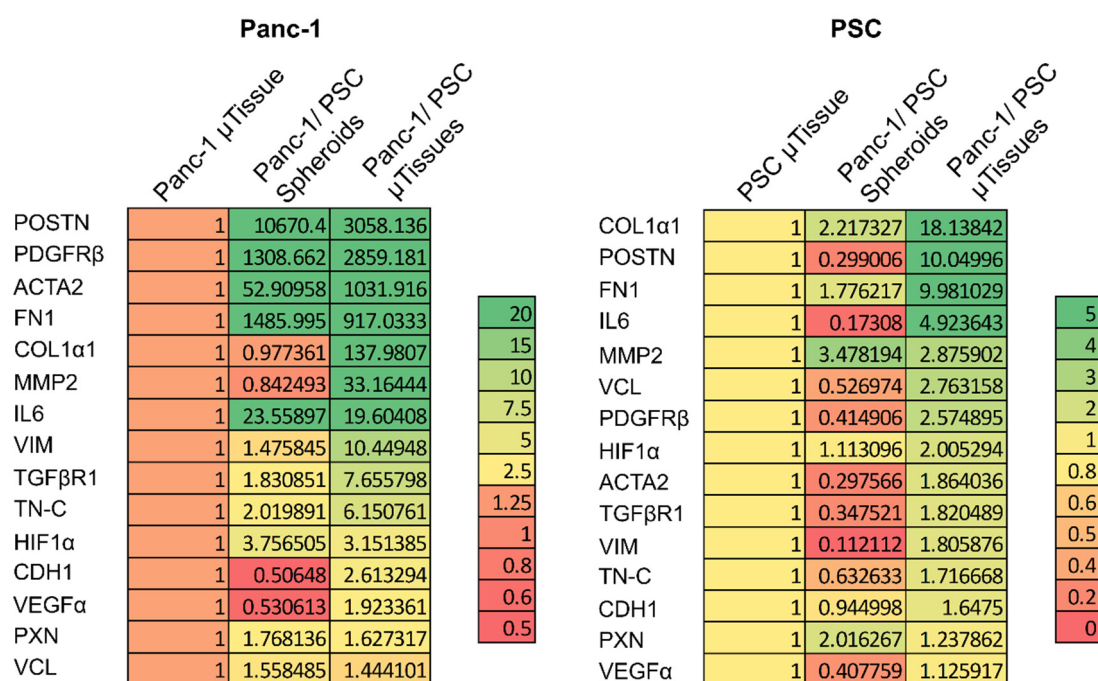Legend: 5, 4, 3, 2, 1, 0.8, 0.6, 0.5, 0.4, 0.2, 0

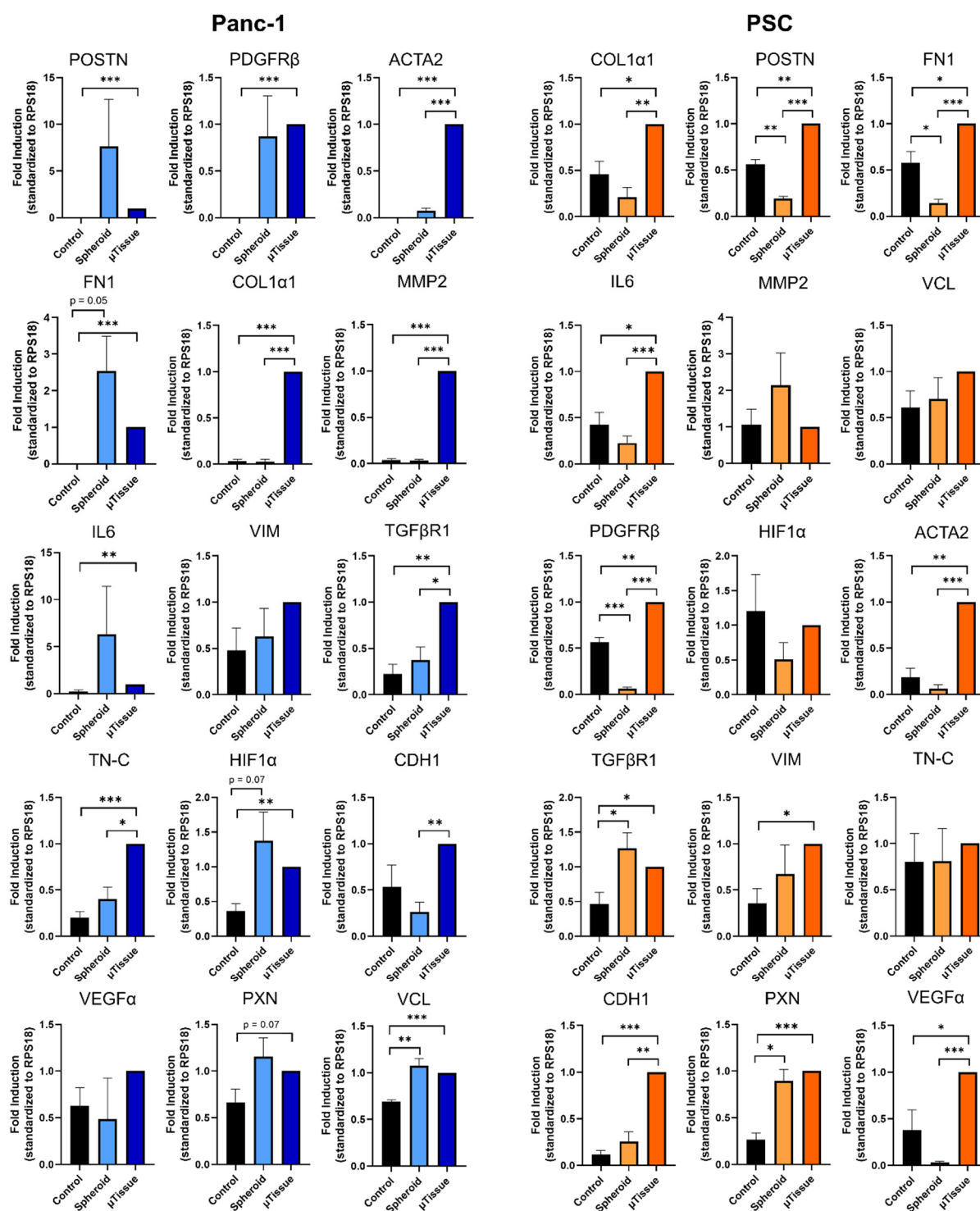**Figure S4: Gene expression profile with exact numbers.**

Figure S5: Gene expression comparing 3D single culture of Panc-1, Panc-1/ PSC heterospheroids and Panc-1/ PSC µtissues. Standardized to CK19 (left, blue graphs) and comparing 3D single culture of PSC, Panc-1/ PSC heterospheroids and Panc-1/ PSC µtissues standardized to FAP (right, orange graphs). Statistical analysis was performed by two-tailed Student's *t*-test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.
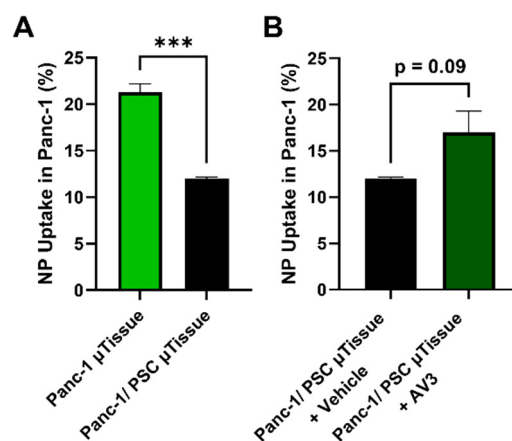
Figure S6: Nanoparticle Uptake in Panc-1 cell core. (A) Nanoparticle uptake in Panc-1 for Panc-1 μtissue versus Panc-1/ PSC μtissues. (B) Nanoparticle uptake in Panc-1 for Panc-1/ PSC μtissue treated with vechicle (DMSO) or 50 μM AV3. Statistical analysis was performed by two-tailed Student's *t*-test. ****p* < 0.001.