A microenvironment-inspired synthetic 3D model for pancreatic ductal adenocarcinoma organoids

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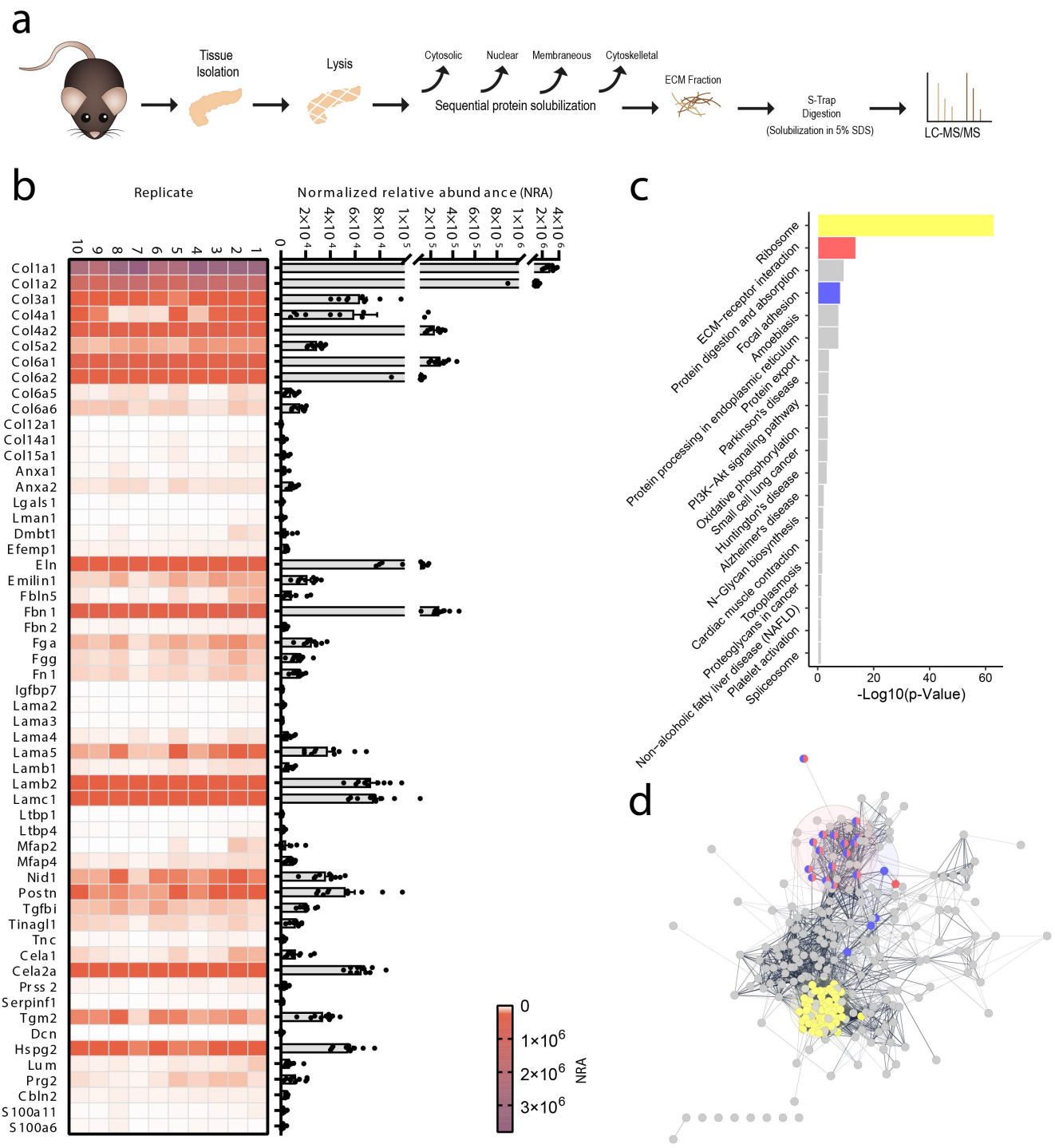
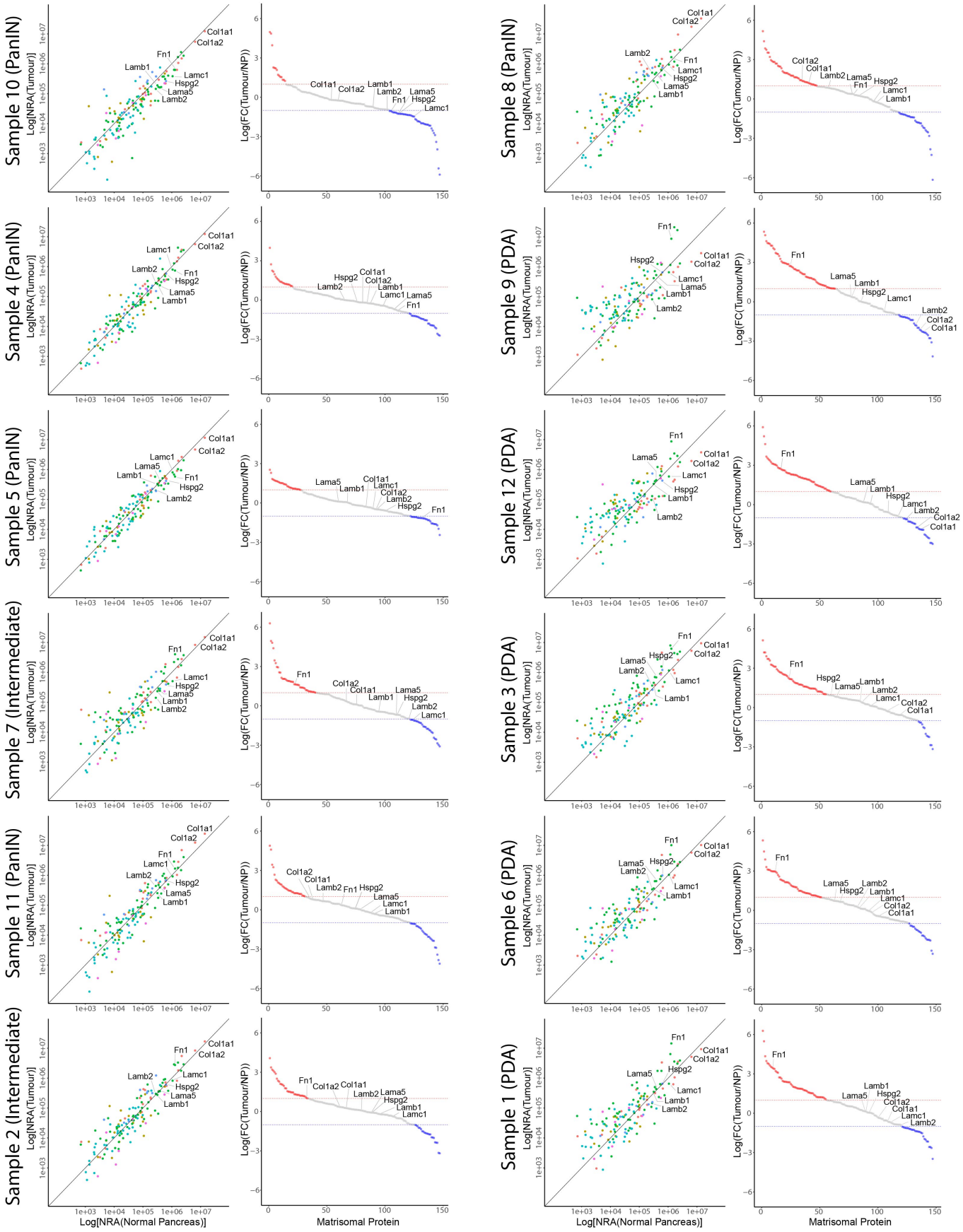
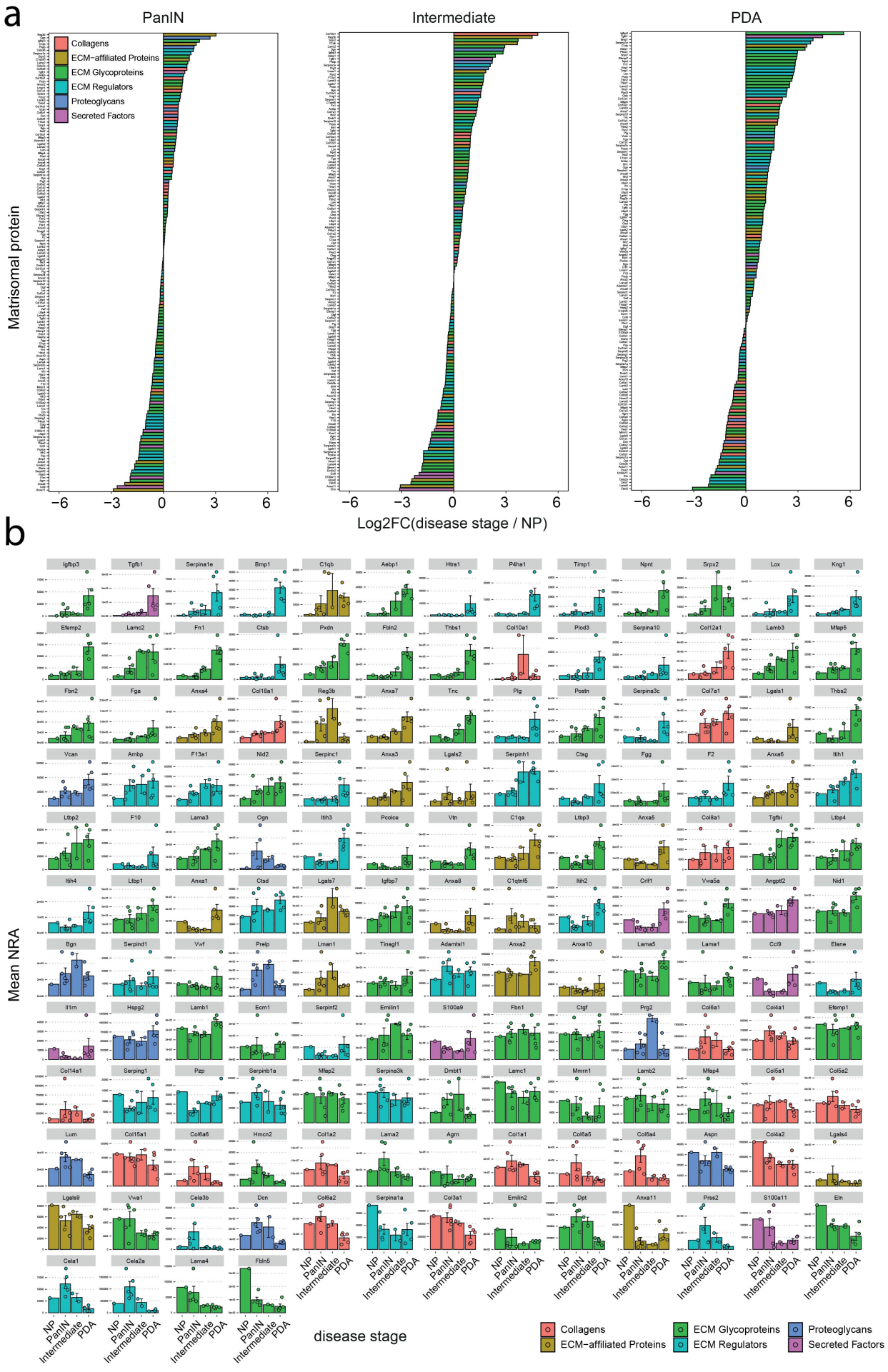

a
Tissue Isolation
Lysis
Cytosolic
Nuclear
Membraneous
Cytoskelletal
Sequential protein solubilization
ECM Fraction
S-Trap Digestion
(Solubilization in 5% SDS)
LC-MS/MS
b
Replicate
Normalized relative abundance (NRA)
Col1a1
Col1a2
Col3a1
Col4a1
Col4a2
Col5a2
Col6a1
Col6a2
Col6a5
Col6a6
Col12a1
Col14a1
Col15a1
Anxa1
Anxa2
Lgals1
Lman1
Dmbt1
Efemp1
Eln
Emilin1
Fbln5
Fbn1
Fbn2
Fga
Fgg
Fn1
Igfbp7
Lama2
Lama3
Lama4
Lama5
Lamb1
Lamb2
Lamc1
Ltbp1
Ltbp4
Mfap2
Mfap4
Nid1
Postn
Tgfbi
Tinagl1
Tnc
Cela1
Cela2a
Prss2
Serpinf1
Tgm2
Dcn
Hspg2
Lum
Prg2
Cbln2
S100a11
S100a6
NRA
c
Ribosome
ECM-receptor interaction
Protein digestion and absorption
Focal adhesion
Amoebiasis
Protein processing in endoplasmic reticulum
Protein export
Parkinson's disease
PI3K-Akt signaling pathway
Oxidative phosphorylation
Small cell lung cancer
Huntington's disease
Alzheimer's disease
N-Glycan biosynthesis
Cardiac muscle contraction
Toxoplasmosis
Proteoglycans in cancer
Non-alcoholic fatty liver disease (NAFLD)
Platelet activation
Spliceosome
-Log10(p-Value)
d

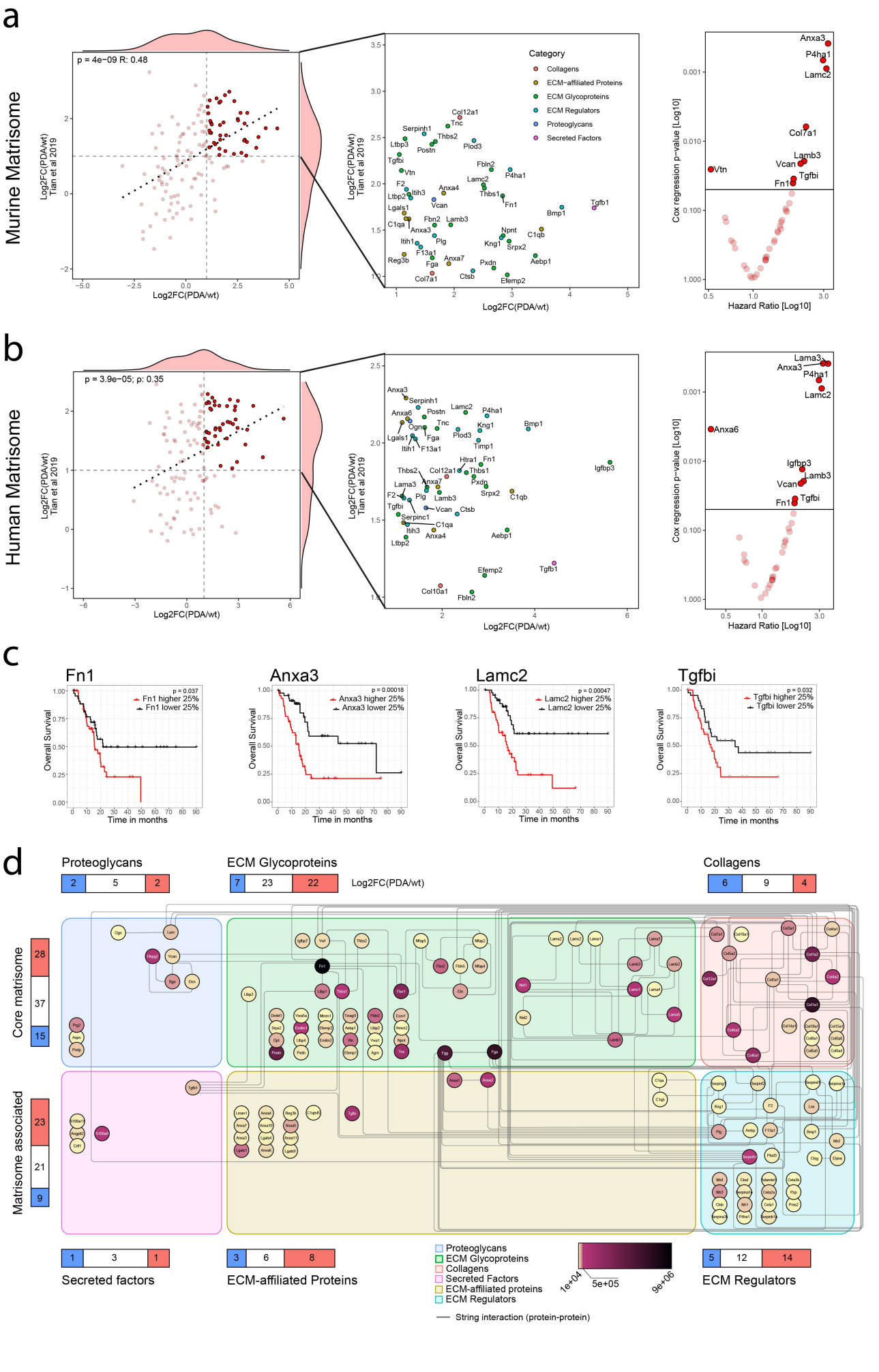
Supplementary Figure 1: a, Outline of ECM-isolation from healthy or tumour-bearing pancreas. **b,** Heatmap of normalized relative abundance (NRA) for each matrisomal protein across analytical replicates (left) and mean NRA (right). Each datapoint show median value from five analytical LC-MS/MS replicates (n=10, error bars: s.e.m.). **c,** Top 20 Kyoto encyclopedia for genes and genomes (KEGG)-based pathway annotations for pathways significantly enriched in matrisome datasets, demonstrating the purity of the proteomic dataset (Fisher's exact test corrected for multiple hypothesis testing). **d,** String-based visualization of pathways from **c**. Highlighted areas indicate three top MCODE-based clusters of the protein-protein interaction network.


Sample 10 (PanIN)
Sample 8 (PanIN)
Sample 4 (PanIN)
Sample 9 (PDA)
Sample 5 (PanIN)
Sample 12 (PDA)
Sample 7 (Intermediate)
Sample 3 (PDA)
Sample 11 (PanIN)
Sample 6 (PDA)
Sample 2 (Intermediate)
Sample 1 (PDA)
Log[NRA(Tumour)]
Log(FC(Tumour/NP))
Log[NRA(Normal Pancreas)]
Matrisomal Protein
Collagens
ECM Glycoproteins
Proteoglycans
ECM−affiliated Proteins
ECM Regulators
Secreted Factors

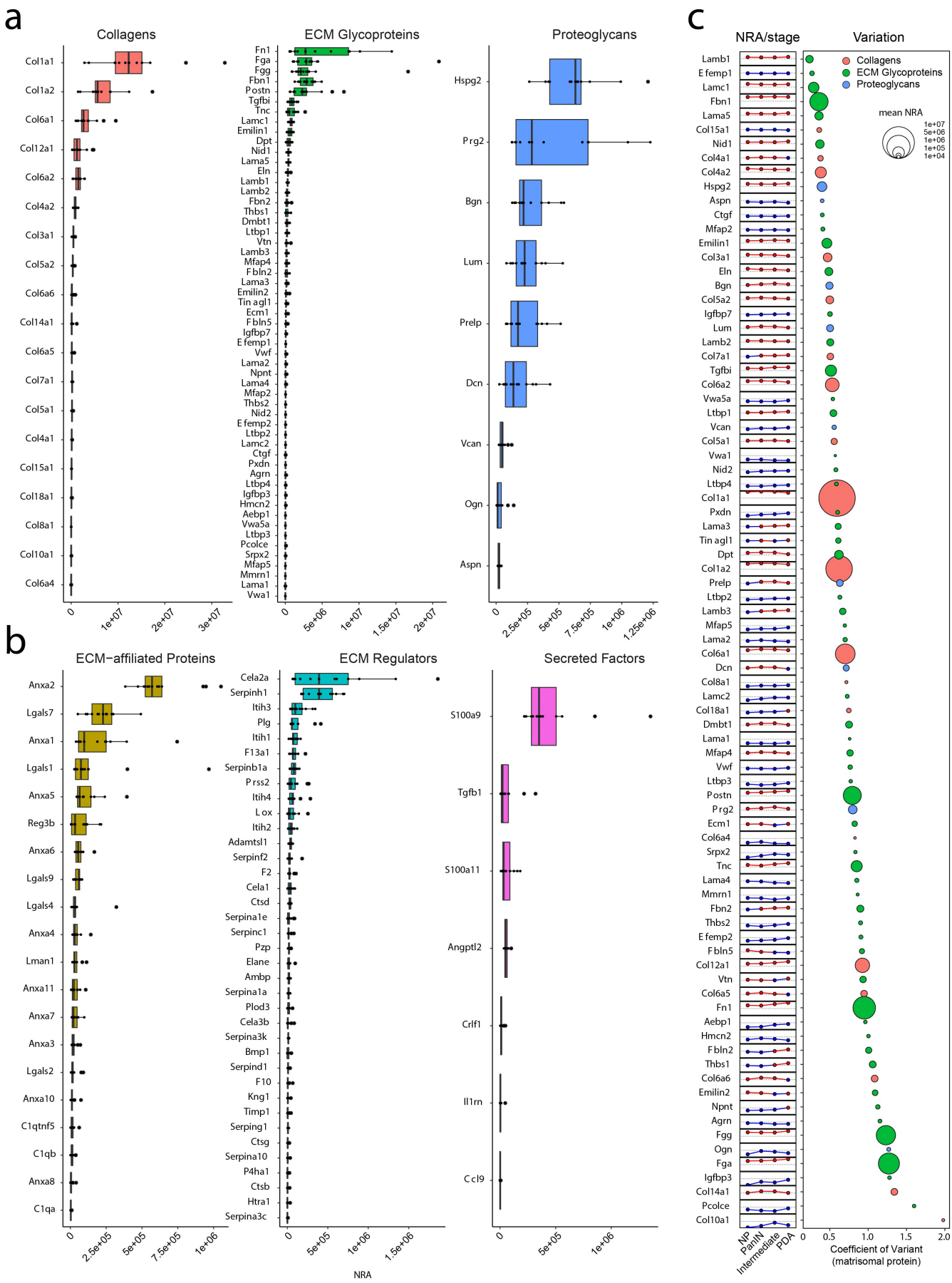
Supplementary Figure 2: Pairwise analysis of tumour and normal tissue samples. The normalised relative abundances (NRA) of identified and quantified matrisomal proteins from each murine tumour are plotted against normal pancreas (left) and the relative abundance of tumour matrisomal proteins is shown right. Dashed lines represent Log2 thresholds of 1 (red) or -1 (blue) respectively. Each datapoint represents the mean value from three replicates. Abbreviations: PanIN: Pancreatic intraepithelial neoplasia, PDA: Pancreatic ductal adenocarcinoma.


a
PanIN
Intermediate
PDA
Collagens
ECM−affiliated Proteins
ECM Glycoproteins
ECM Regulators
Proteoglycans
Secreted Factors
Matrisomal protein
−6
−3
0
3
6
Log2FC(disease stage / NP)
b
Mean NRA
NP
PanIN
Intermediate
PDA
disease stage
Collagens
ECM−affiliated Proteins
ECM Glycoproteins
ECM Regulators
Proteoglycans
Secreted Factors

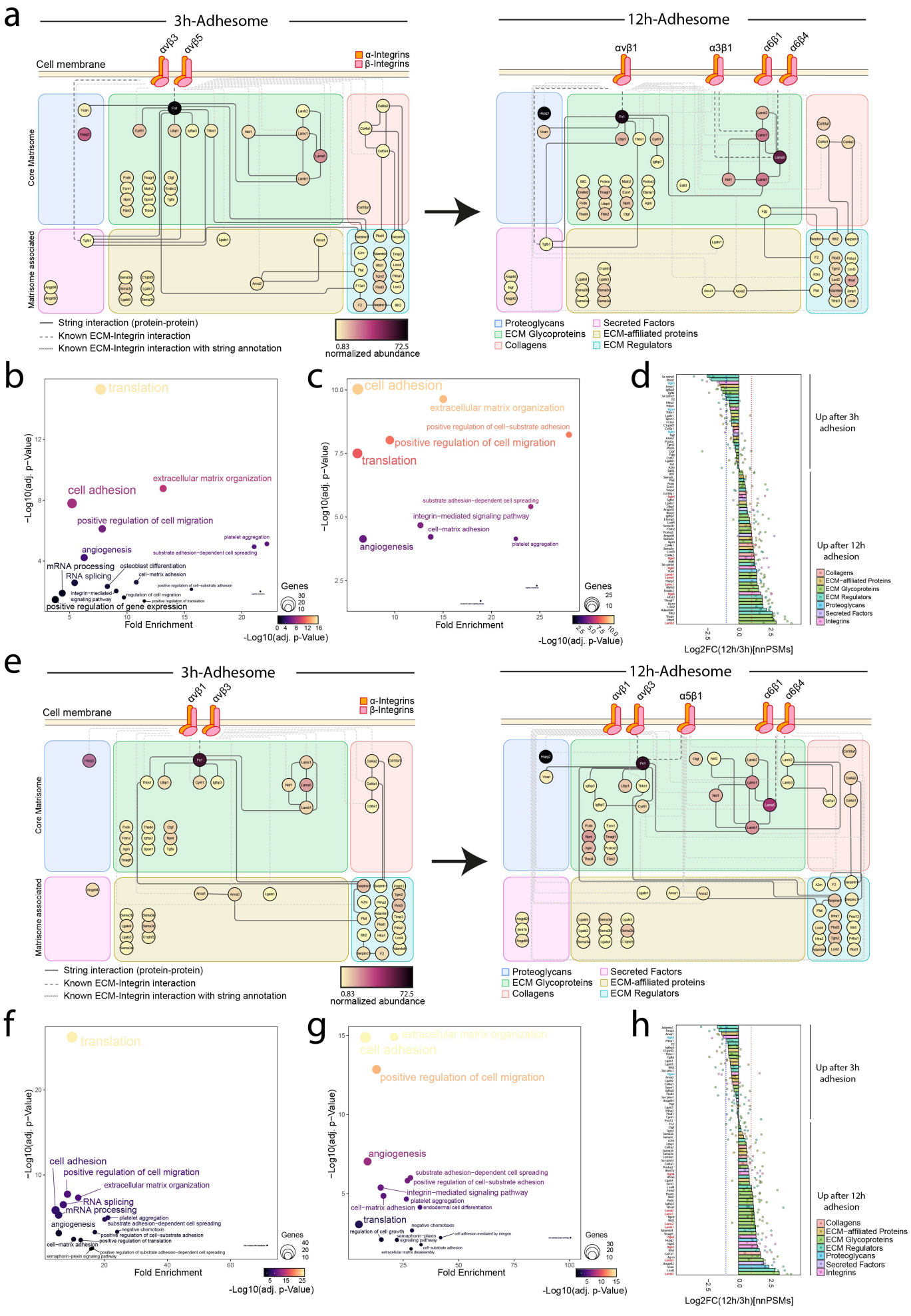
Supplementary Figure 3: a, Waterfall plot of mean Log2 (fold change) of matrisomal protein abundance relative to normal pancreas and the indicated disease stage. **b,** Barplots of mean matrisomal normalised relative protein abundance (NRA) across all disease stages. Datapoints show mean NRA for each sample (n=3, error bars: s.d.).


a
Murine Matrisome
p = 4e−09 R: 0.48
Log2FC(PDA/wt)
Tian et al 2019
Category
Collagens
ECM−affiliated Proteins
ECM Glycoproteins
ECM Regulators
Proteoglycans
Secreted Factors
Cox regression p−value [Log10]
Hazard Ratio [Log10]
b
Human Matrisome
p = 3.9e−05; ρ: 0.35
c
Fn1
Anxa3
Lamc2
Tgfbi
Overall Survival
Time in months
Fn1 higher 25%
Fn1 lower 25%
Anxa3 higher 25%
Anxa3 lower 25%
Lamc2 higher 25%
Lamc2 lower 25%
Tgfbi higher 25%
Tgfbi lower 25%
d
Proteoglycans
ECM Glycoproteins
Collagens
Log2FC(PDA/wt)
Core matrisome
Matrisome associated
Secreted factors
ECM-affiliated Proteins
ECM Regulators
String interaction (protein-protein)

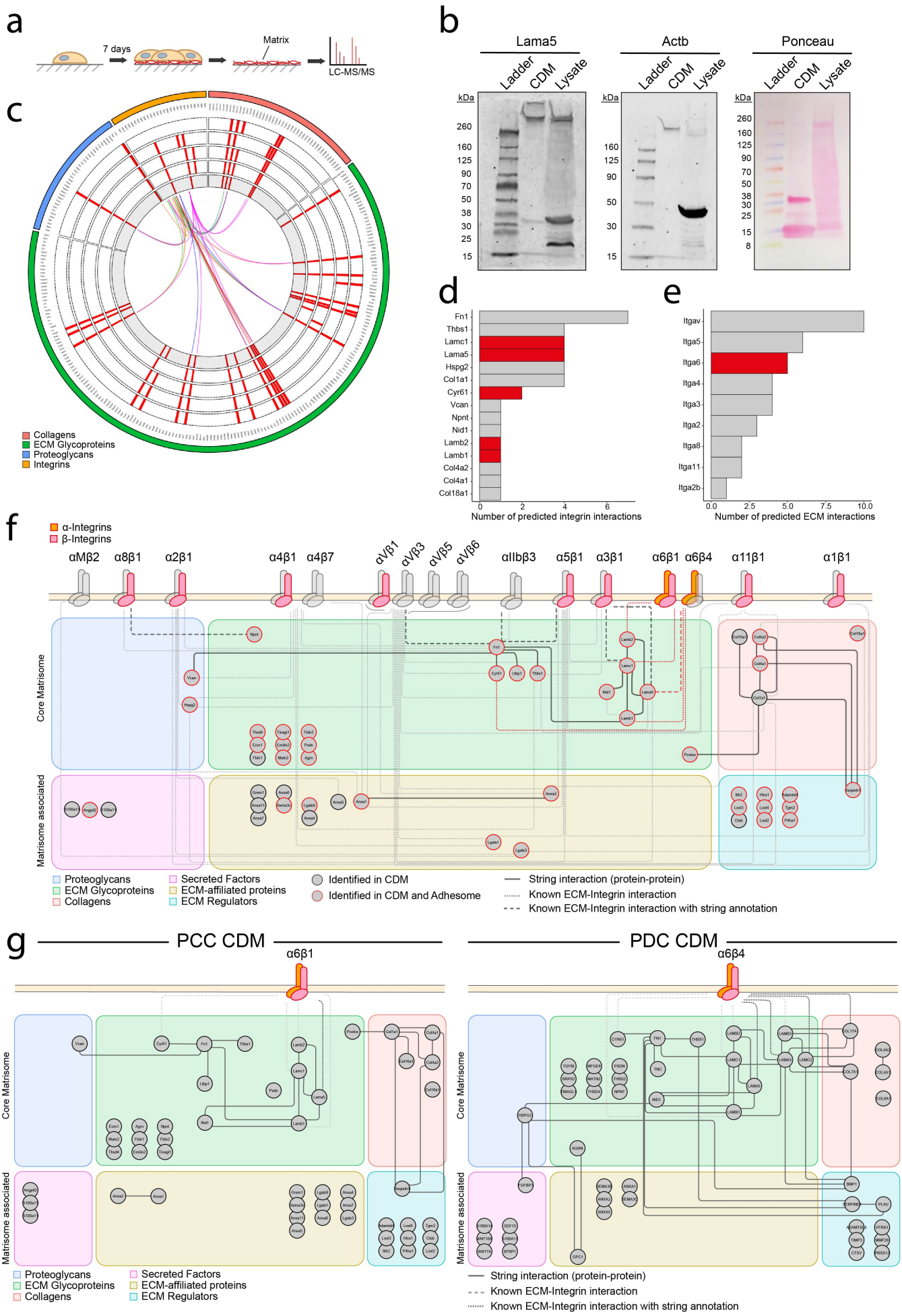
Supplementary Figure 4: a-b, Correlation analysis between all quantified matrisomal proteins from PDA samples (this study) and murine **a**, or human **b**, matrisomal proteins from Tian et al 2019 (left) and survival analysis (TCGA PAAD) of all matrisomal proteins with Log2 fold change over one in both this study and Tian et al 2019, quantile stratified. Matrisome protein abundance is displayed as Log2 (fold change PDA vs healthy normal pancreas). Lines represent a Log2 (fold change) cut off value of one and distribution of each dataset is represented as density plot orthogonal to the respective axis. Inlays depict the top right quadrant of the correlation plot. Correlation p-values and coefficients were calculated using Pearson- **a**, or Spearman- **b**, based rank correlation depending on normality of the dataset. For survival analysis, the hazard ratio for each matrisomal proteins is depicted over its cox regression p-value with significance levels adapted to 0.05 (horizontal line). Abbreviations: PanIN: Pancreatic Intraepithelial neoplasia, PDA: Pancreatic Ductal Adenocarcinoma. **c**, Kaplan-Meier survival plots of four selected matrisomal proteins with quantile stratified PDA patients. **d**, Consensus matrisome map for proteins identified in this study and in Tian et al 2019. Matrisomal proteins are colour-coded by the mean NRA across PDA samples (from this study). Bar charts indicate the relative proportion of the matrisomal proteins that are down- (blue) or upregulated (red) or are stable (white) in PDA compared to the healthy normal pancreas within each category of the matrisome.


a
Collagens
Col1a1
Col1a2
Col6a1
Col12a1
Col6a2
Col4a2
Col3a1
Col5a2
Col6a6
Col14a1
Col6a5
Col7a1
Col5a1
Col4a1
Col15a1
Col18a1
Col8a1
Col10a1
Col6a4
0
1e+07
2e+07
3e+07
ECM Glycoproteins
Fn1
Fga
Fgg
Fbn1
Postn
Tgfbi
Tnc
Lamc1
Emilin1
Dpt
Nid1
Lama5
Eln
Lamb1
Lamb2
Fbn2
Thbs1
Dmbt1
Ltbp1
Vtn
Lamb3
Mfap4
Fbln2
Lama3
Emilin2
Tinagl1
Ecm1
Fbln5
Igfbp7
Efemp1
Vwf
Lama2
Npnt
Lama4
Mfap2
Thbs2
Nid2
Efemp2
Ltbp2
Lamc2
Ctgf
Pxdn
Agrn
Ltbp4
Igfbp3
Hmcn2
Aebp1
Vwa5a
Ltbp3
Pcolce
Srpx2
Mfap5
Mmrn1
Lama1
Vwa1
0
5e+06
1e+07
1.5e+07
2e+07
Proteoglycans
Hspg2
Prg2
Bgn
Lum
Prelp
Dcn
Vcan
Ogn
Aspn
0
2.5e+05
5e+05
7.5e+05
1e+06
1.25e+06
b
ECM−affiliated Proteins
Anxa2
Lgals7
Anxa1
Lgals1
Anxa5
Reg3b
Anxa6
Lgals9
Lgals4
Anxa4
Lman1
Anxa11
Anxa7
Anxa3
Lgals2
Anxa10
C1qtnf5
C1qb
Anxa8
C1qa
0
2.5e+05
5e+05
7.5e+05
1e+06
ECM Regulators
Cela2a
Serpinh1
Itih3
Plg
Itih1
F13a1
Serpinb1a
Prss2
Itih4
Lox
Itih2
Adamtsl1
Serpinf2
F2
Cela1
Ctsd
Serpina1e
Serpinc1
Pzp
Elane
Ambp
Serpina1a
Plod3
Cela3b
Serpina3k
Bmp1
Serpind1
F10
Kng1
Timp1
Serping1
Ctsg
Serpina10
P4ha1
Ctsb
Htra1
Serpina3c
0
5e+05
1e+06
1.5e+06
NRA
Secreted Factors
S100a9
Tgfb1
S100a11
Angptl2
Crlf1
Il1rn
Ccl9
0
5e+05
1e+06
c
NRA/stage
Variation
Lamb1
Efemp1
Lamc1
Fbn1
Lama5
Col15a1
Nid1
Col4a1
Col4a2
Hspg2
Aspn
Ctgf
Mfap2
Emilin1
Col3a1
Eln
Bgn
Col5a2
Igfbp7
Lum
Lamb2
Col7a1
Tgfbi
Col6a2
Vwa5a
Ltbp1
Vcan
Col5a1
Vwa1
Nid2
Ltbp4
Col1a1
Pxdn
Lama3
Tinagl1
Dpt
Col1a2
Prelp
Ltbp2
Lamb3
Mfap5
Lama2
Col6a1
Dcn
Col8a1
Lamc2
Col18a1
Dmbt1
Lama1
Mfap4
Vwf
Ltbp3
Postn
Prg2
Ecm1
Col6a4
Srpx2
Tnc
Lama4
Mmrn1
Fbn2
Thbs2
Efemp2
Fbln5
Col12a1
Vtn
Col6a5
Fn1
Aebp1
Hmcn2
Fbln2
Thbs1
Col6a6
Emilin2
Npnt
Agrn
Fgg
Ogn
Fga
Igfbp3
Col14a1
Pcolce
Col10a1
Collagens
ECM Glycoproteins
Proteoglycans
mean NRA
1e+07
5e+06
1e+06
1e+05
1e+04
NP
PanIN
Intermediate
PDA
0
0.5
1.0
1.5
2.0
Coefficient of Variant
(matrisomal protein)

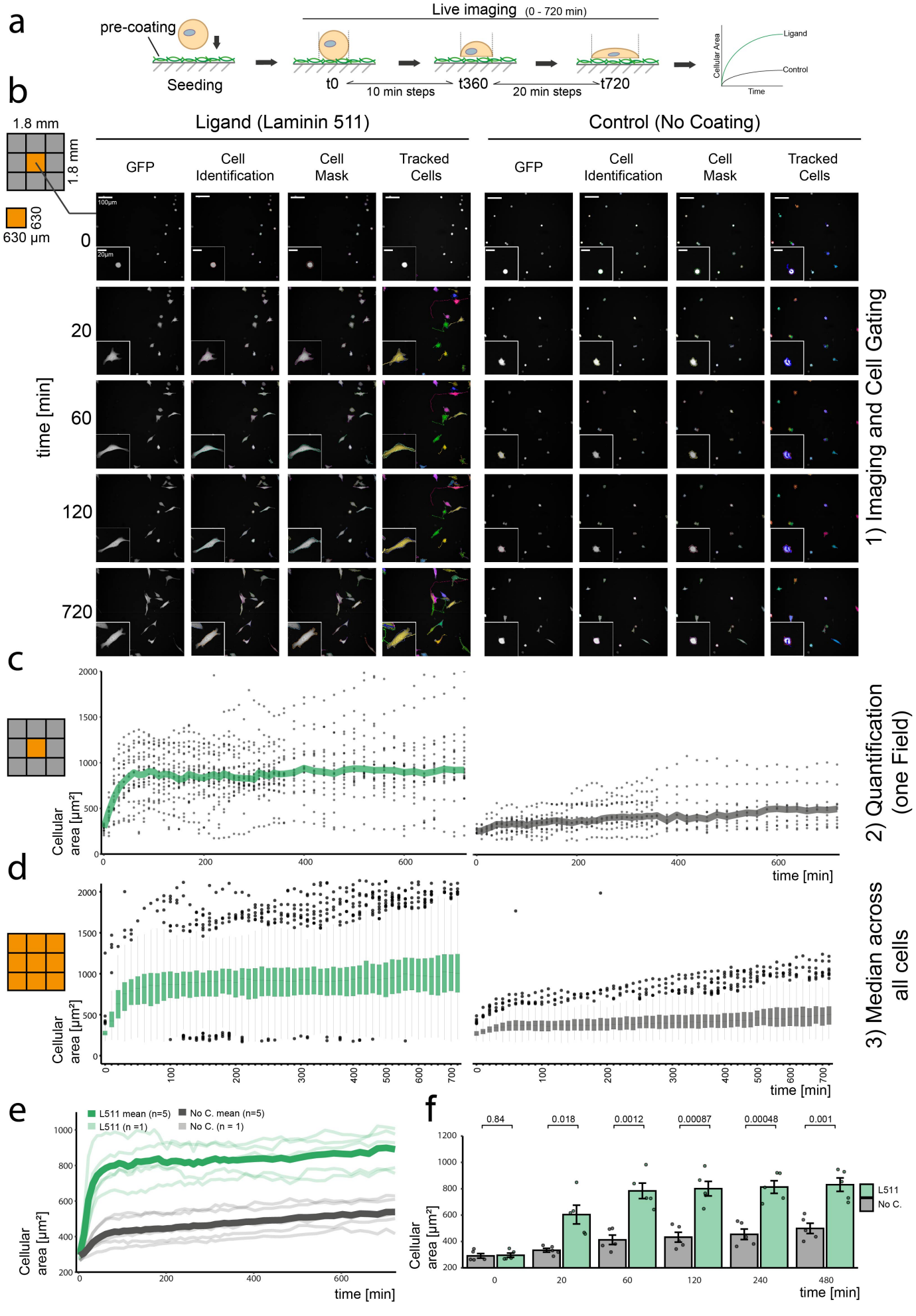
Supplementary Figure 5: a-b, Abundance distribution of core-matrisome **a**, or matrisome-associated **b**, proteins across all 13 pancreatic cancerous samples. Datapoints show mean value for individual samples. Boxes show first and third quartiles (25^{th} and 75^{th} percentiles) with median (50^{th} percentile) depicted and whiskers with 1.5 · IQR from hinges, outliers are depicted. **c,** Mean NRA of each core matrisome protein stratified by disease stage (left) and coefficient of variance (sd/mean) across all 13 samples (right). Horizontal line (left) shows the median NRA for the respective disease stages. Matrisomal proteins colour coded for high- (red nodes and edges) or low-abundance (blue nodes and edges). Size of the nodes (right) depicts the mean NRA across all 13 samples and colour code reflect matrisome category.


a
3h-Adhesome
12h-Adhesome
Cell membrane
αvβ3
αvβ5
α-Integrins
β-Integrins
αvβ1
α3β1
α6β1
α6β4
Core Matrisome
Matrisome associated
String interaction (protein-protein)
Known ECM-Integrin interaction
Known ECM-Integrin interaction with string annotation
0.83
72.5
normalized abundance
Proteoglycans
ECM Glycoproteins
Collagens
Secreted Factors
ECM-affiliated proteins
ECM Regulators
b
translation
extracellular matrix organization
cell adhesion
positive regulation of cell migration
platelet aggregation
angiogenesis
substrate adhesion−dependent cell spreading
mRNA processing
osteoblast differentiation
RNA splicing
cell−matrix adhesion
integrin−mediated signaling pathway
positive regulation of gene expression
−Log10(adj. p−Value)
Fold Enrichment
Genes
-Log10(adj. p-Value)
c
cell adhesion
extracellular matrix organization
positive regulation of cell−substrate adhesion
positive regulation of cell migration
translation
substrate adhesion−dependent cell spreading
integrin−mediated signaling pathway
cell−matrix adhesion
angiogenesis
platelet aggregation
d
Up after 3h adhesion
Up after 12h adhesion
Collagens
ECM−affiliated Proteins
ECM Glycoproteins
ECM Regulators
Proteoglycans
Secreted Factors
Integrins
Log2FC(12h/3h)[nnPSMs]
e
3h-Adhesome
12h-Adhesome
Cell membrane
αvβ1
αvβ3
α5β1
α6β1
α6β4
α-Integrins
β-Integrins
Core Matrisome
Matrisome associated
String interaction (protein-protein)
Known ECM-Integrin interaction
Known ECM-Integrin interaction with string annotation
0.83
72.5
normalized abundance
Proteoglycans
ECM Glycoproteins
Collagens
Secreted Factors
ECM-affiliated proteins
ECM Regulators
f
translation
cell adhesion
positive regulation of cell migration
extracellular matrix organization
RNA splicing
mRNA processing
platelet aggregation
angiogenesis
substrate adhesion−dependent cell spreading
negative chemotaxis
positive regulation of cell−substrate adhesion
cell−matrix adhesion
positive regulation of translation
positive regulation of substrate adhesion−dependent cell spreading
semaphorin−plexin signaling pathway
−Log10(adj. p−Value)
Fold Enrichment
Genes
-Log10(adj. p-Value)
g
extracellular matrix organization
cell adhesion
positive regulation of cell migration
angiogenesis
substrate adhesion−dependent cell spreading
positive regulation of cell−substrate adhesion
integrin−mediated signaling pathway
platelet aggregation
cell−matrix adhesion
endodermal cell differentiation
translation
negative chemotaxis
regulation of cell growth
semaphorin−plexin signaling pathway
cell adhesion mediated by integrin
cell−substrate adhesion
extracellular matrix disassembly
−Log10(adj. p−Value)
Fold Enrichment
Genes
-Log10(adj. p-Value)
h
Up after 3h adhesion
Up after 12h adhesion
Collagens
ECM−affiliated Proteins
ECM Glycoproteins
ECM Regulators
Proteoglycans
Secreted Factors
Integrins
Log2FC(12h/3h)[nnPSMs]

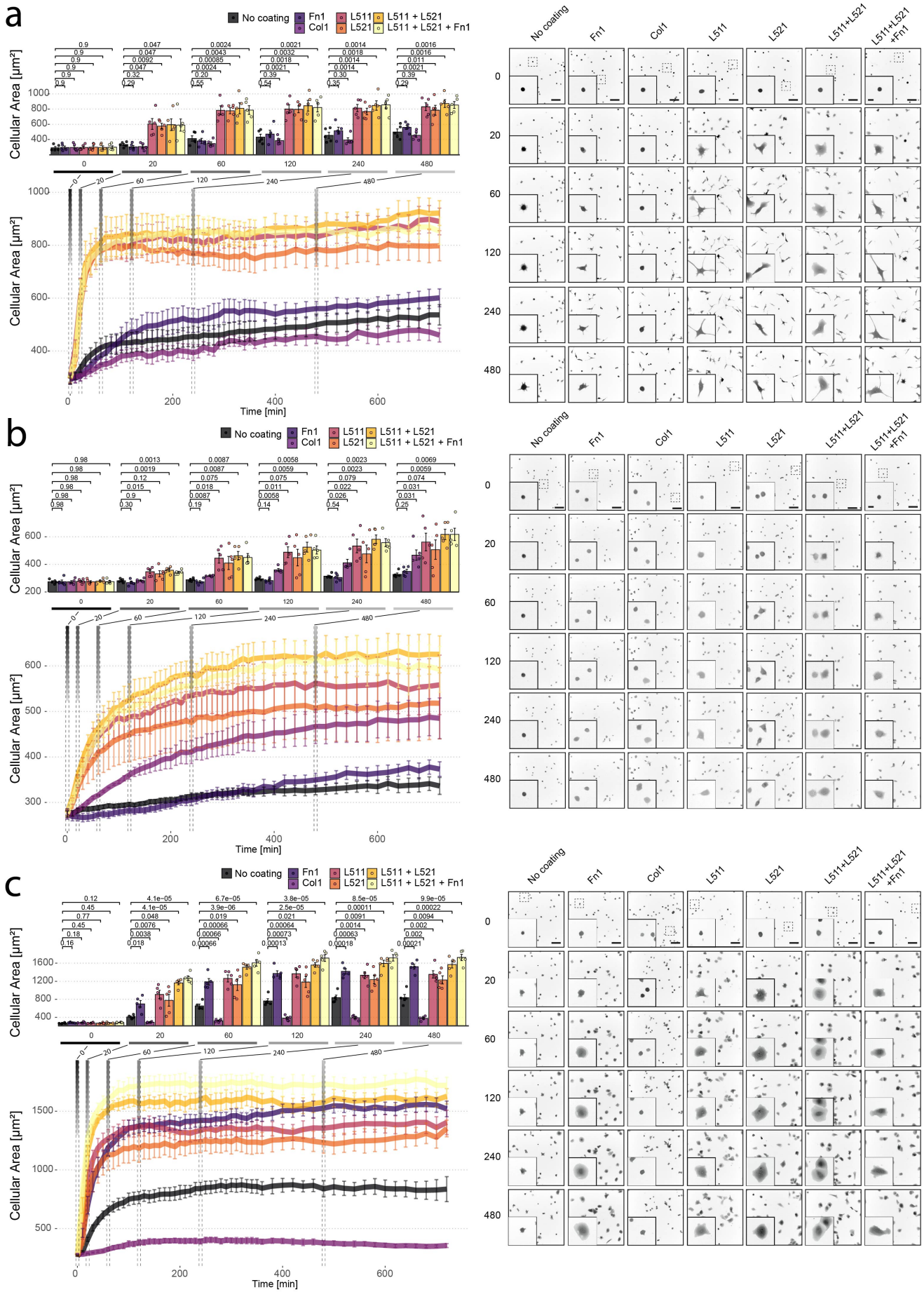
Supplementary Figure 6: a, String-based network of IACs isolated from KPC-1 cells after 3h (left) or 12h (right) adhesion to fibronectin. Matrisome proteins are stratified by matrisome category and are colour coded by their normalized abundance. Edges represent string-based protein-protein interactions or known ECM-integrin interactions. **b,c**, GO-term biological process enrichment of IACs after 3 **b**, or 12h **c**, of adhesion (Fisher's exact test corrected for multiple hypothesis testing). **d**, Log2 (fold change) of normalized abundance for matrisome proteins and integrins between 12h and 3h adhesion conditions. Bargraphs show mean across three biological replicates, error bars: s.e.m. Datapoints depict individual replicates. Vertical lines show Log2 (fold change) threshold of -1 (blue) or +1 (red). **e,f,g,h**) Same as in **a,b,c,d** but with IACs isolated from iKras cells.


a
7 days
Matrix
LC-MS/MS
b
Lama5
Actb
Ponceau
Ladder
CDM
Lysate
kDa
260
160
125
90
70
50
38
30
25
15
8
c
Collagens
ECM Glycoproteins
Proteoglycans
Integrins
d
Fn1
Thbs1
Lamc1
Lama5
Hspg2
Col1a1
Cyr61
Vcan
Npnt
Nid1
Lamb2
Lamb1
Col4a2
Col4a1
Col18a1
Number of predicted integrin interactions
e
Itgav
Itga5
Itga6
Itga4
Itga3
Itga2
Itga8
Itga11
Itga2b
Number of predicted ECM interactions
f
α-Integrins
β-Integrins
αMβ2
α8β1
α2β1
α4β1
α4β7
αVβ1
αVβ3
αVβ5
αVβ6
αIIbβ3
α5β1
α3β1
α6β1
α6β4
α11β1
α1β1
Core Matrisome
Matrisome associated
Proteoglycans
ECM Glycoproteins
Collagens
Secreted Factors
ECM-affiliated proteins
ECM Regulators
Identified in CDM
Identified in CDM and Adhesome
String interaction (protein-protein)
Known ECM-Integrin interaction
Known ECM-Integrin interaction with string annotation
g
PCC CDM
α6β1
PDC CDM
α6β4
Core Matrisome
Matrisome associated
Proteoglycans
ECM Glycoproteins
Collagens
Secreted Factors
ECM-affiliated proteins
ECM Regulators
String interaction (protein-protein)
Known ECM-Integrin interaction
Known ECM-Integrin interaction with string annotation

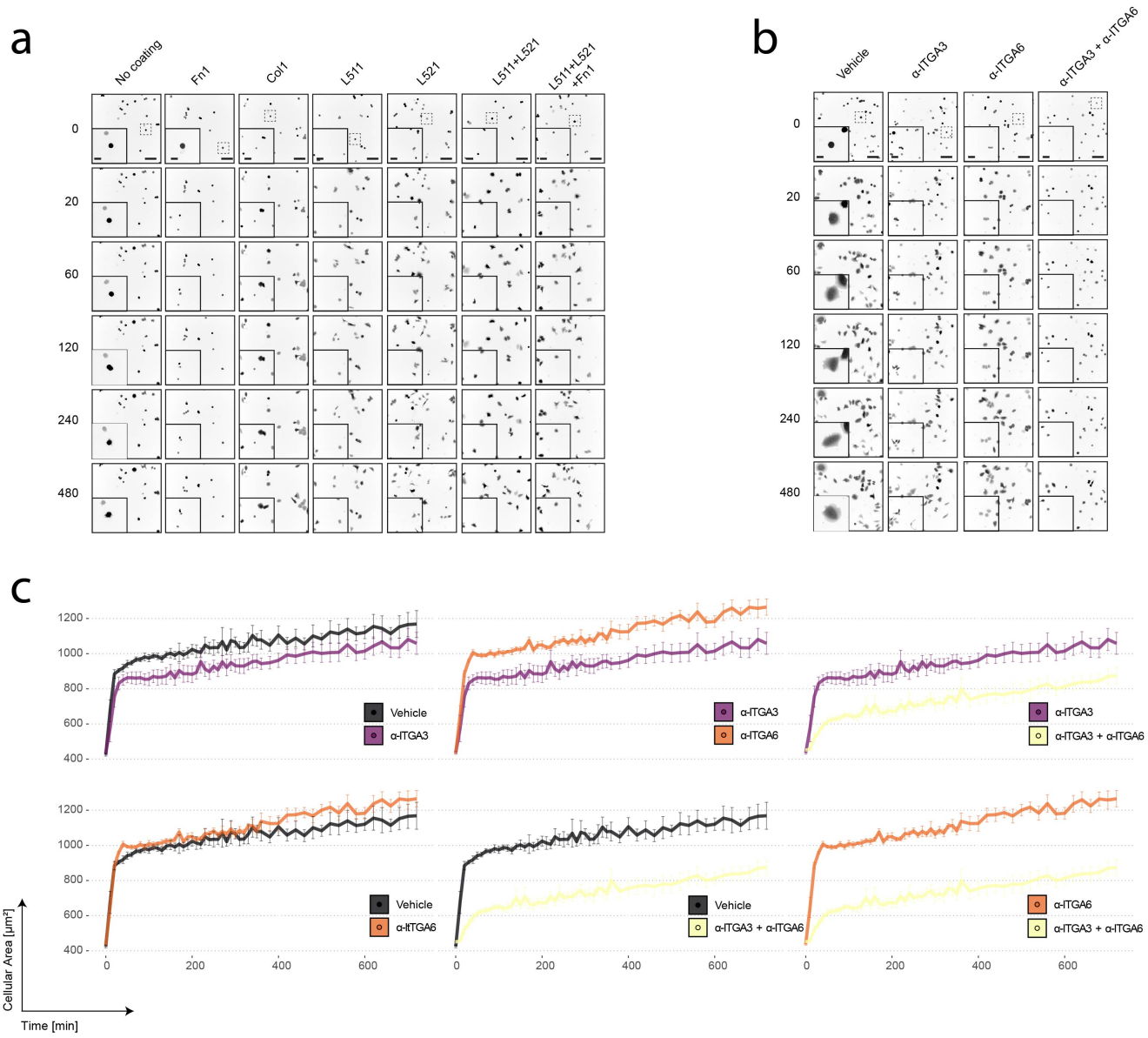
Supplementary Figure 7: a, Schematic illustration of cell-derived matrix (CDM) isolation and analysis. **b,** Western blot for Laminin alpha chain 5 (Lama5) or beta-Actin (Actb) or Ponceau-staining for CDM or whole-cell lysate to verify enrichment of matrisomal proteins (n=1). **c,** Matrisome-circos plot-based visualization of core matrisome proteins and interacting integrins from CDM isolations (outer tracks; white) and consensus CDM (inner track; grey) from KPC-1 cells (n=4). Red bars show identified proteins in respective samples. Integrin-ECM interactions are shown in the centre of the plot and connections of each alpha-integrin subunit have been colour-coded. **d,** Predicted number of interactions between detected core-ECM proteins from the consensus matrisome **c**, and all known integrin alpha subunits. Red bars indicate integrin α6 interactions. **e,** Predicted number of interactions between all alpha-integrin subunits and core-ECM proteins identified in the consensus matrisome **c**. Red bars indicate integrins that have been detected in the consensus CDM **c**. **f,** Protein-protein interaction network of all ECM proteins detected in the consensus CDM with all possible integrin ligands. Integrin subunits coloured in orange (alpha-subunits) or pink (beta-subunits) were experimentally identified. Red edges indicate ECM-integrin interactions between detected proteins. Configurations of experimentally identified integrins were assembled based on literature data. **g**, Sting-based network of CDM from pancreatic cancer cells (KPC-1, left) or normal pancreatic epithelial ductal cells (HPDE, right).


a
pre-coating
Seeding
Live imaging (0 - 720 min)
t0
10 min steps
t360
20 min steps
t720
Cellular Area
Ligand
Control
Time
b
1.8 mm
1.8 mm
630 µm
630
Ligand (Laminin 511)
Control (No Coating)
GFP
Cell Identification
Cell Mask
Tracked Cells
time [min]
0
20
60
120
720
1) Imaging and Cell Gating
c
Cellular area [µm²]
time [min]
2) Quantification (one Field)
d
Cellular area [µm²]
time [min]
3) Median across all cells
e
L511 mean (n=5)
L511 (n =1)
No C. mean (n=5)
No C. (n = 1)
Cellular area [µm²]
time [min]
f
0.84
0.018
0.0012
0.00087
0.00048
0.001
Cellular area [µm²]
time [min]
L511
No C.

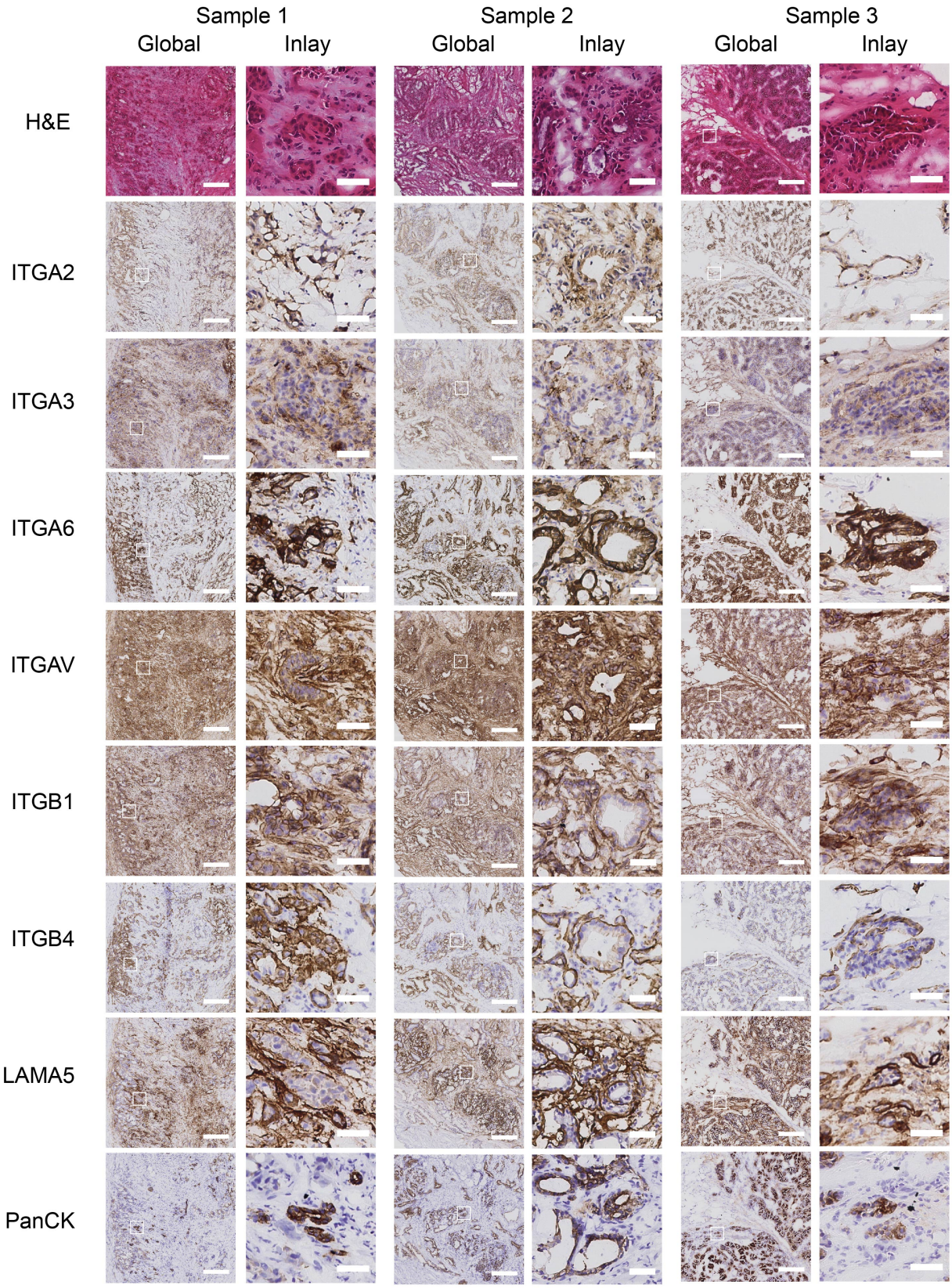
Supplementary Figure 8: a, Schematic outline of cell adhesion assay and quantification. **b,** GFP-labelled murine KPC-1 PCCs were imaged across 9 distinct 630 x 630 μm regions of interest (ROI, grey) per replicate. Representative time-lapse images of GFP-labelled KPC-1 cells adhering to an example ligand (left) or a non-coated surface (right) for one ROI (depicted in orange) are shown. Cells were first imaged, identified and a defined mask around the cell was used for cell area quantification. Cells were tracked during the entire course of the experiment to account for cell divisions. Scale bar: 100 μm; Inlay: 20 μm. To aid visual interpretation of the data, gamma was set to 2.3. **c,** Cellular area of individual cells and timepoint of the example ROI from **b** for a Laminin 511 pre- (left) or a non-coated (right) surface. Median across all cells for each timepoint is shown as green line. Individual cells (data points) are shown at each timepoint. **d,** Cellular area for all cells and timepoints across all profiled cells for the entire imaged area (orange) of one biological replicate depicted as boxplots for Laminin 511 (left) or a non-coated surface (right). Boxes were drawn between the first and third quartiles (25^{th} and 75^{th} percentiles) with median (50^{th} percentile) depicted and whiskers with 1.5 · IQR from hinges and outliers shown. **e,** Cellular area quantification from five individual adhesion assays (faint lines, computed as outlined in **a-d,** and mean values (bold lines) for KPC-1 PCCs adhering to either Laminin 511 (green) or a non-coated surface (grey). **f,** Mean cellular area of KPC-1 PCCs adhering to either Laminin 511 (green) or a non-coated surface (grey) for selected timepoints. Each barplot represents the mean cellular area across five independent experiments (as shown in **e**) with error bars: s.e.m. Significance was calculated from parametric two-sided Welch's t-test.


a
b
c
No coating
Fn1
Col1
L511
L521
L511 + L521
L511 + L521 + Fn1
Cellular Area [µm²]
Time [min]
0
20
60
120
240
480
200
400
600
L511+L521
L511+L521 +Fn1

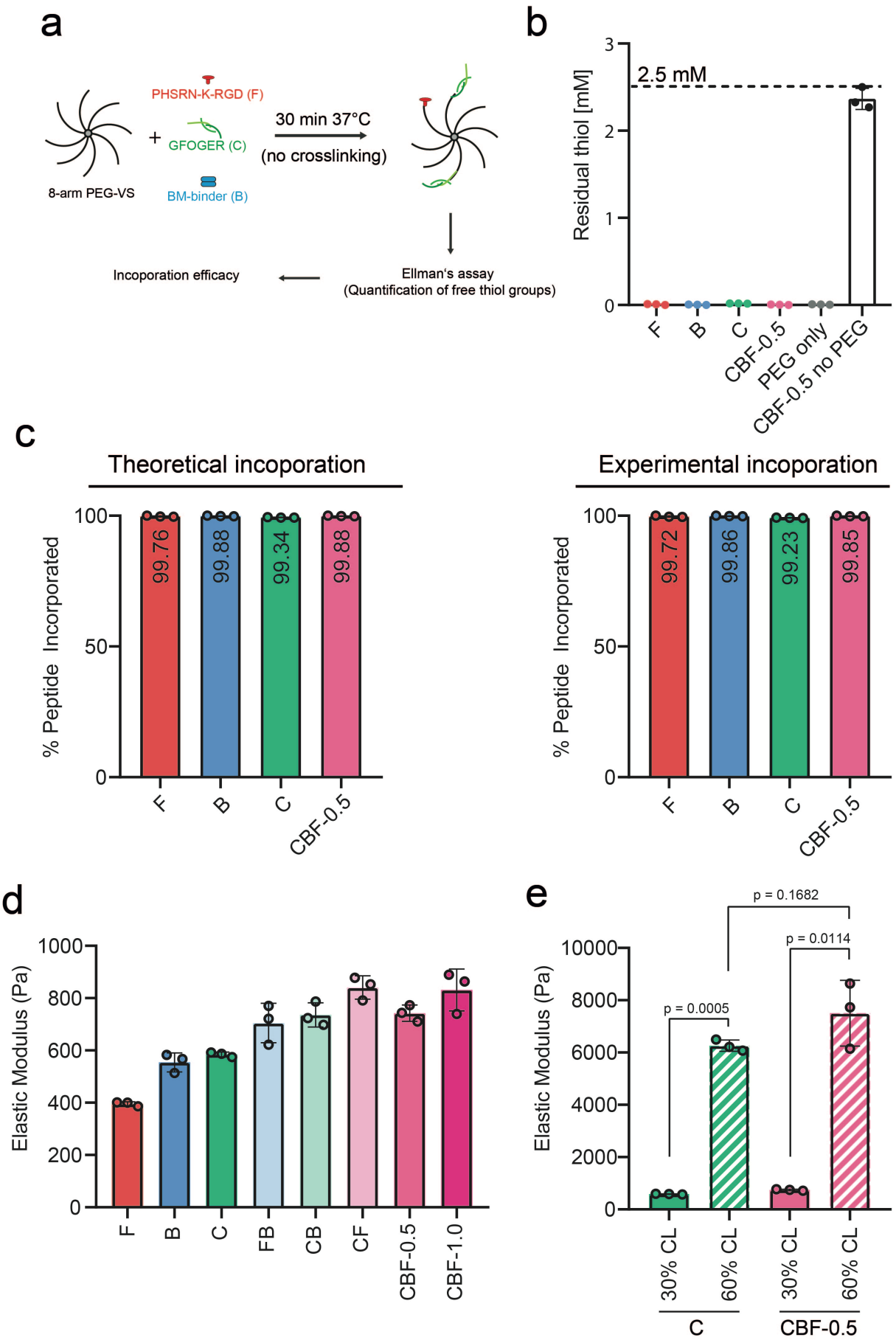
Supplementary Figure 9: a-c, Cell spreading assays for murine cancer cell lines KPC-1 **a**, KPC-43 **b**, as well as a murine pancreatic fibroblast (PaF) control cell line (**c**, left) with representative time-lapse images of cells plated on indicated ligands (right). To aid visual interpretation of the data, the gamma was set to 2.3 and images were greyscale-inverted. Scale bars: 100 μm. Each datapoint represents the mean value of five independent experiments, each measuring minimum 100 cells. Bar graphs display mean cellular area of five independent adhesion assays at selected timepoints with each datapoint representing single replicates. Significance was assessed using the two-sided parametric Welch's t-test between indicated conditions with Benjamini-Hochberg correction. Error bars: s.e.m.


a
No coating
Fn1
Col1
L511
L521
L511+L521
L511+L521 +Fn1
0
20
60
120
240
480
b
Vehicle
α-ITGA3
α-ITGA6
α-ITGA3 + α-ITGA6
c
Vehicle
α-ITGA3
α-ITGA6
α-ITGA3 + α-ITGA6
Cellular Area [µm²]
Time [min]

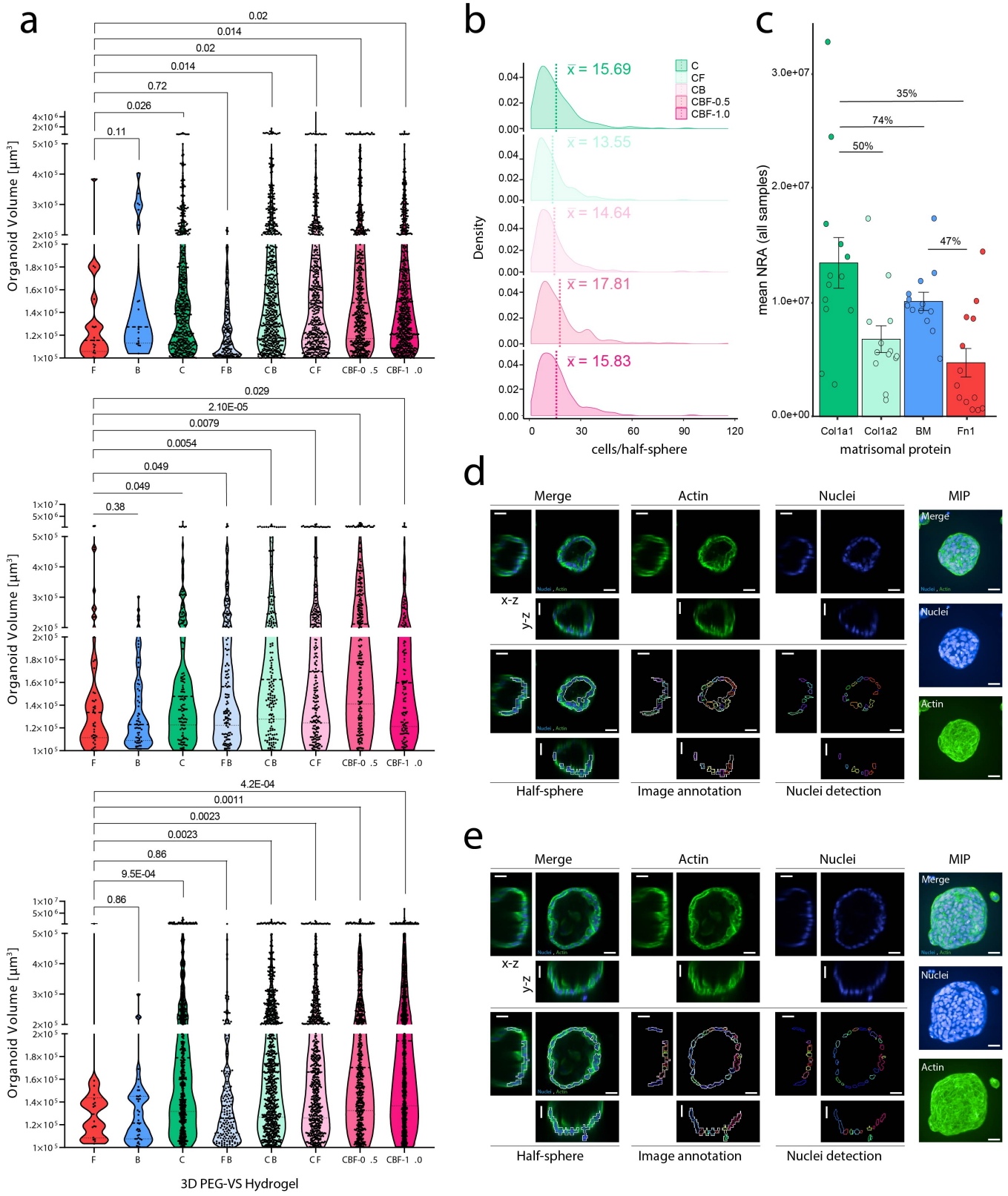
Supplementary Figure 10: a, Representative time-lapse images of GFP-labelled human Suit-2 PCCs plated on ligands as indicated. Images are representative of minimum 100 cells in each experiment from at least five independent adhesion experiments. To aid visual interpretation of the data, the gamma was set to 2.3 and images were inverted. **b**, Individual cell adhesion assays from individual treatments compared to the vehicle control. Each datapoint represents the mean value from five independent adhesion experiments with each quantifying > 100 cells. Scale bars: 100 μm, inlay: 20 μm.


Sample 1
Global
Inlay
Sample 2
Global
Inlay
Sample 3
Global
Inlay
H&E
ITGA2
ITGA3
ITGA6
ITGAV
ITGB1
ITGB4
LAMA5
PanCK

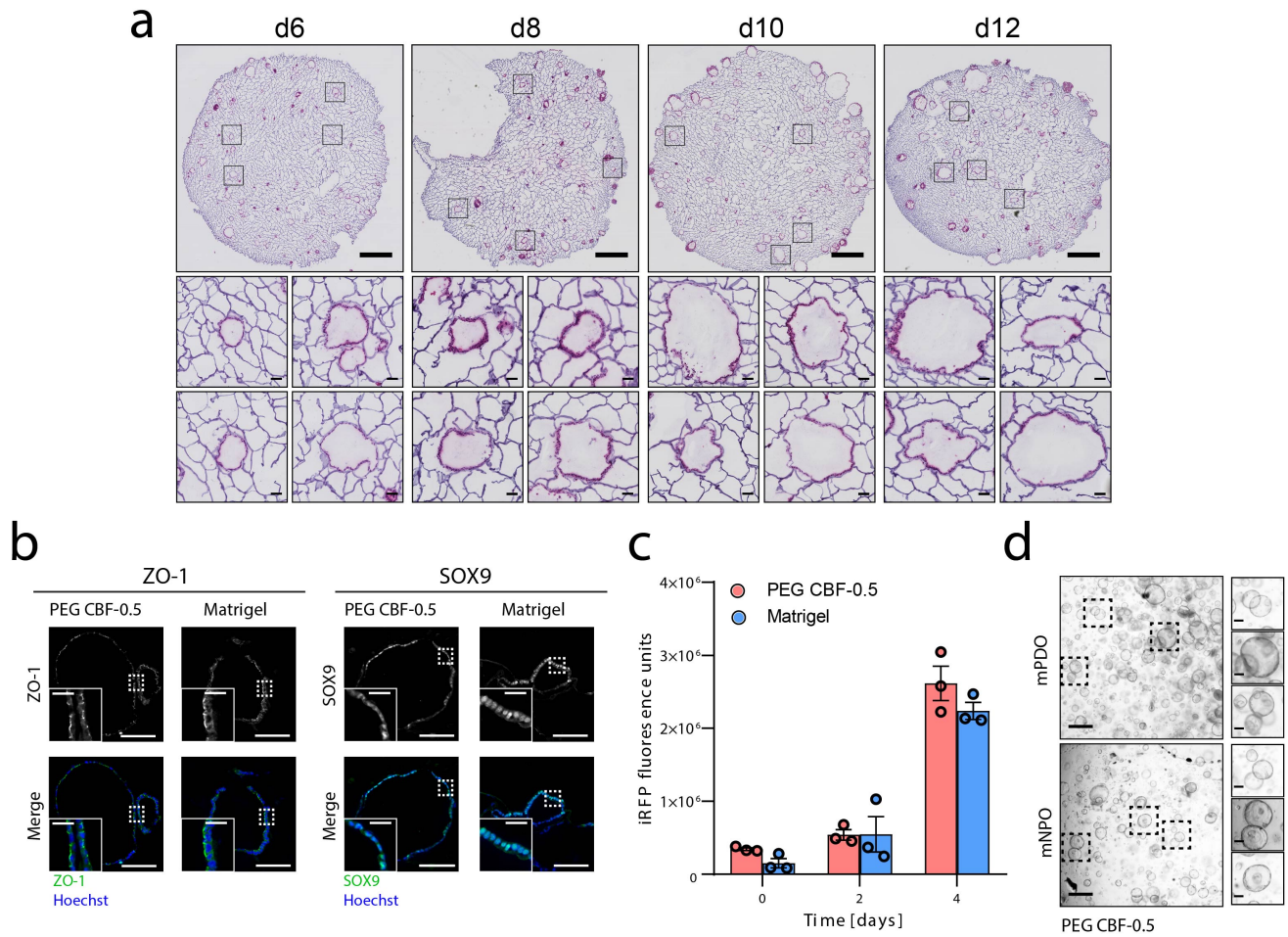
Supplementary Figure 11: Immunohistochemistry (IHC) for integrin alpha and beta subunits and Laminin alpha 5 chain (Lama5, common between L511 and L521) on three individual human resected surgical specimens with pathologically verified PDA. Scale bars: 300 μm, inlay: 50 μm. Representative images of the entire sample are shown.


a
PHSRN-K-RGD (F)
GFOGER (C)
BM-binder (B)
8-arm PEG-VS
30 min 37°C
(no crosslinking)
Incoporation efficacy
Ellman's assay
(Quantification of free thiol groups)
b
Residual thiol [mM]
2.5 mM
F
B
C
CBF-0.5
PEG only
CBF-0.5 no PEG
c
Theoretical incoporation
Experimental incoporation
% Peptide Incorporated
99.76
99.88
99.34
99.88
99.72
99.86
99.23
99.85
d
Elastic Modulus (Pa)
FB
CB
CF
CBF-0.5
CBF-1.0
e
p = 0.1682
p = 0.0114
p = 0.0005
30% CL
60% CL
C
CBF-0.5

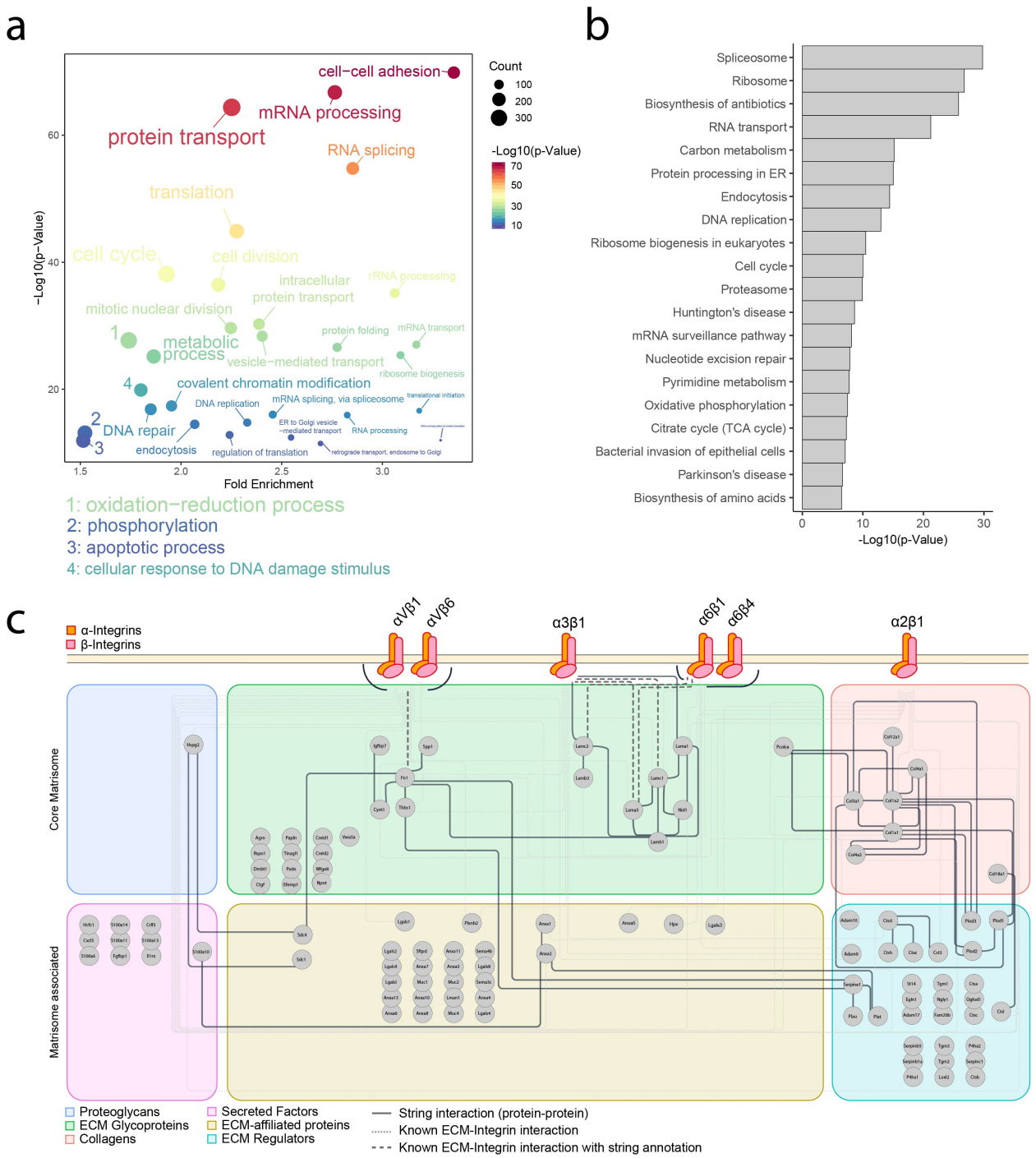
Supplementary Figure 12: a, Outline of the experimental approach quantifying peptide incorporation into the PEG scaffold. 8-arm PEG-VS was covalently functionalized with adhesion-mimetic peptides or BM-binder by Michael-type addition for 30 min at 37°C and amount of remaining free thiols (unincorporated peptide) was quantified using Ellman's assay. **b**, Residual thiol [mM] of F, B or C-peptides or CBF-0.5 (2.5mM final concentration across all conditions) upon functionalization of PEG molecules (n=3). PEG only and CBF-0.5 no PEG controls contain only PEG (no peptide) or only peptide (no PEG). Dashed line represents residual thiol quantity of 2.5 mM (total peptide input in CBF-0.5 formulation). Datapoints indicate individual replicates and barplots display mean value. Error bars: s.e.m. **c**, Theoretical (left) and experimentally quantified (right) peptide incorporation into 8-arm PEG-VS as quantified by the Ellman's assay from **b** (n=3). Datapoints indicate individual replicates and barplots show mean across all experiments. **d**, Rheology-based quantification of 3D PEG-gel elastic modulus. Gels were crafted with adhesion peptides as indicated (n=3). Datapoints indicate individual rheology measurements of independently crafted gels and barplots show mean across all experiments. Error bars: s.e.m. **e**, Rheology-based quantification of PEG-C or PEG-CBF-0.5 elastic modulus using 30% or 60% crosslinking (CL) density. Datapoints indicate individual rheology measurements of independently crafted gels and barplots show mean across all experiments. Error bars: s.e.m. Statistics from two-sided parametric Student's t-test without (comparison PEG-C 60% and PEG-CBF-0.5 60%) or with Welch correction (all other comparisons). Abbreviations: F – PHSRN-K-RGD, B – BM-binder, C – GFOGER, FB; CB; CF – combinations of indicated peptides, CBF-0.5; CBF-1.0 – combination of indicated peptides with PHSRN-K-RGD at 0.5 or 1 mM. All other peptides were used at 1 mM unless otherwise indicated.


a
b
c
d
e
Organoid Volume [µm³]
F
B
C
FB
CB
CF
CBF-0 .5
CBF-1 .0
3D PEG-VS Hydrogel
Density
cells/half-sphere
C
CF
CB
CBF-0.5
CBF-1.0
mean NRA (all samples)
matrisomal protein
Col1a1
Col1a2
BM
Fn1
Merge
Actin
Nuclei
MIP
x-z
y-z
Half-sphere
Image annotation
Nuclei detection

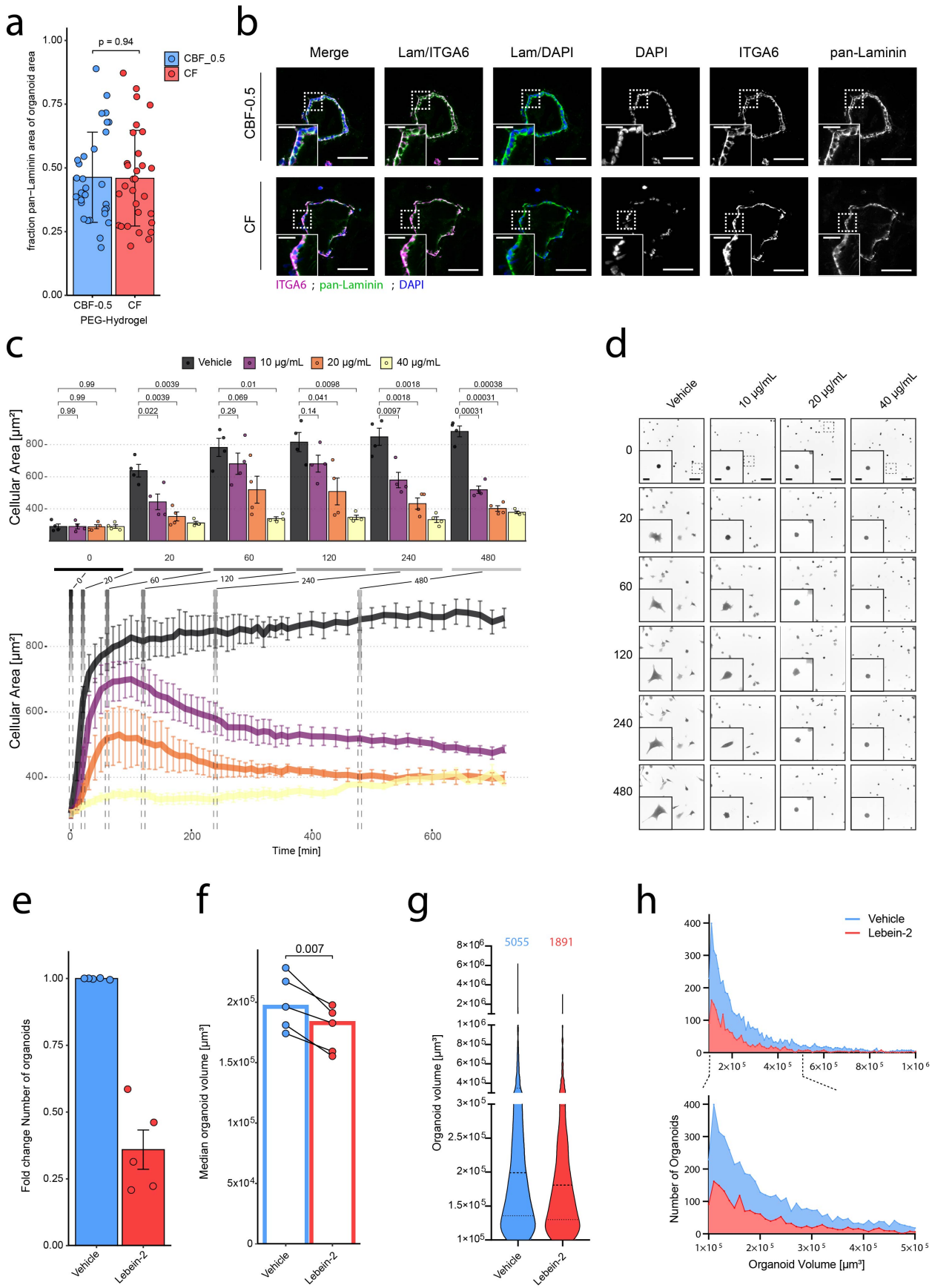
Supplementary Figure 13: a, Image analysis of KPC-derived mPCOs grown in 3D PEG hydrogels with adhesion peptides as indicated. Three independent experiments shown. Each datapoint represents individual organoids (d4, two-tailed Mann-Whitney test). **b,** Histograms displaying number of cells per profiled organoid half-sphere for all organoids with a volume > 150,000 μm^3 (n=3). Dashed lines show the mean volume of the population. **c,** Mean abundance of Col1a1, Col1a2, Fn1 or of 30 detected basement membrane (BM) proteins summarized for all profiled samples (n=13). Differences in abundances are displayed. Error bars: s.e.m. **d,e,** Representative single section IF and gated images of a scanned organoid with 27 **d**, or 72 **e**, single cells to verify analysis pipeline. For half-sphere images – masked regions indicate the component of the organoid with sufficient signal intensity used for nuclei detection. Maximum intensity projection (MIP) shows the scanned and gated organoid over the entire z-stack. Images are representative of at least 100 profiled organoids and three independent experiments. Scale bar: 25 μm. Abbreviations: F – PHSRN-K-RGD, B – BM-binder, C – GFOGER, FB; CB; CF – combinations of indicated peptides, CBF-0.5; CBF-1.0 – combination of indicated peptides with PHSRN-K-RGD at 0.5 or 1 mM. All other peptides were used at 1 mM unless otherwise indicated.


a
d6
d8
d10
d12
b
ZO-1
SOX9
PEG CBF-0.5
Matrigel
PEG CBF-0.5
Matrigel
ZO-1
Merge
SOX9
Merge
ZO-1
Hoechst
SOX9
Hoechst
c
4x10⁶
3x10⁶
2x10⁶
1x10⁶
0
iRFP fluorescence units
PEG CBF-0.5
Matrigel
0
2
4
Time [days]
d
mPDO
mNPO
PEG CBF-0.5

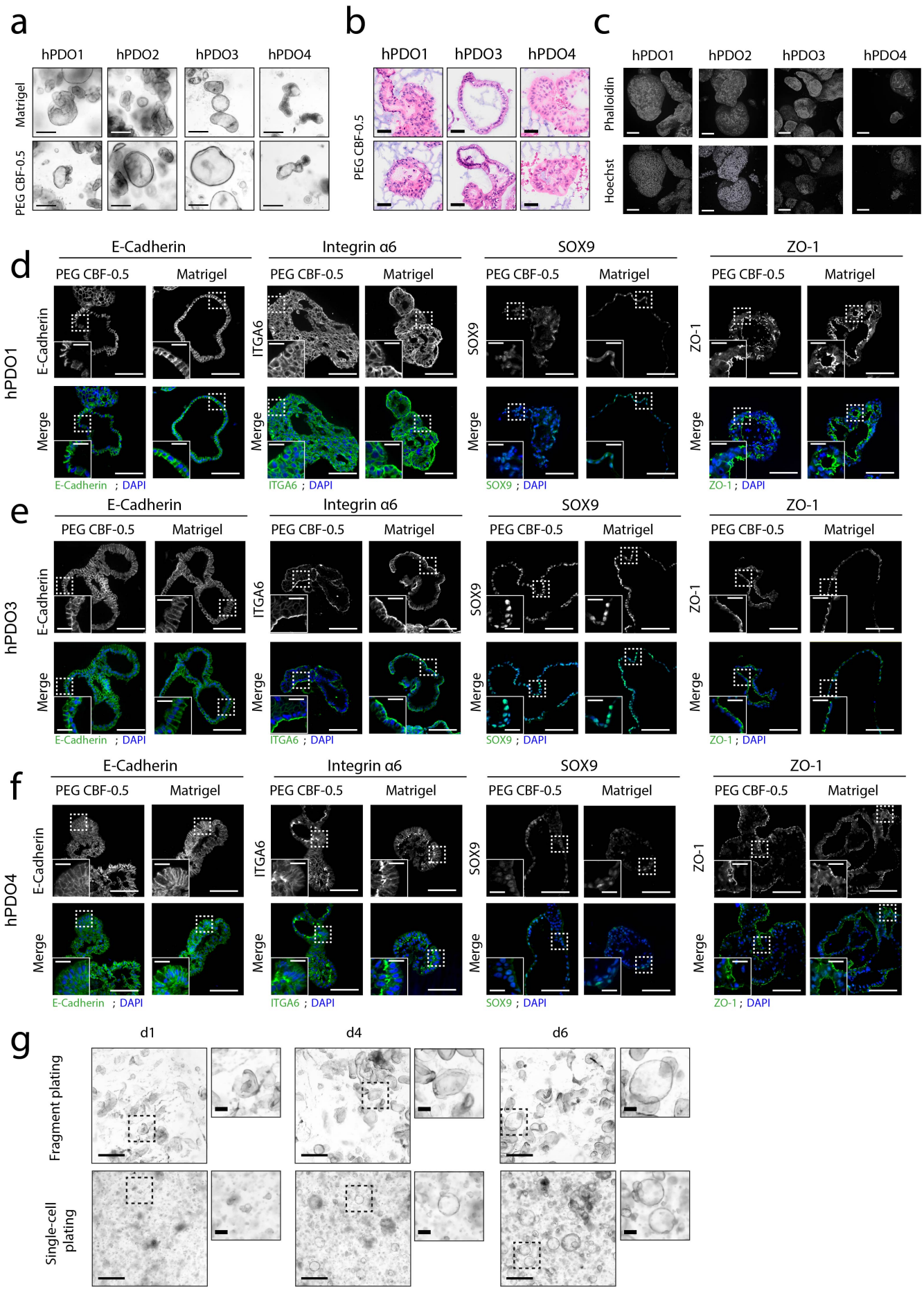
Supplementary Figure 14: a, H&E whole-gel images of mPCOs grown in 3D PEG CBF-0.5 gels as indicated. Inlays show four representative organoids for each timepoint. Scale bar: 1 mm, inlay: 50 μm. **b,** Representative IF images of mPCOs in PEG CBF-0.5 gels or Matrigel (d4, n=2). Scale bars: 100 μm, Inlay 25 μm. Images are representative of minimum 5 organoids in the analysed gels. **c,** iRFP-based quantification of organoids growth in 3D PEG CBF-0.5 gels or Matrigel (n=3). Bar graphs represent mean values, error bars: s.e.m. **d,** Representative brightfield images of murine KPC-derived organoids (mPCO) or murine normal organoids (mPNO) in 3D PEG CBF-0.5 hydrogels (d4). Scale bar: 500 μm, Inlay 100 μm.


a
cell−cell adhesion
mRNA processing
protein transport
RNA splicing
translation
cell cycle
cell division
intracellular protein transport
rRNA processing
mitotic nuclear division
protein folding
mRNA transport
metabolic process
vesicle−mediated transport
ribosome biogenesis
covalent chromatin modification
mRNA splicing, via spliceosome
translational initiation
DNA replication
DNA repair
ER to Golgi vesicle −mediated transport
RNA processing
endocytosis
regulation of translation
retrograde transport, endosome to Golgi
1
2
3
4
−Log10(p−Value)
Fold Enrichment
Count
100
200
300
-Log10(p-Value)
70
50
30
10
1: oxidation−reduction process
2: phosphorylation
3: apoptotic process
4: cellular response to DNA damage stimulus
b
Spliceosome
Ribosome
Biosynthesis of antibiotics
RNA transport
Carbon metabolism
Protein processing in ER
Endocytosis
DNA replication
Ribosome biogenesis in eukaryotes
Cell cycle
Proteasome
Huntington's disease
mRNA surveillance pathway
Nucleotide excision repair
Pyrimidine metabolism
Oxidative phosphorylation
Citrate cycle (TCA cycle)
Bacterial invasion of epithelial cells
Parkinson's disease
Biosynthesis of amino acids
-Log10(p-Value)
c
α-Integrins
β-Integrins
αVβ1
αVβ6
α3β1
α6β1
α6β4
α2β1
Core Matrisome
Matrisome associated
Proteoglycans
ECM Glycoproteins
Collagens
Secreted Factors
ECM-affiliated proteins
ECM Regulators
String interaction (protein-protein)
Known ECM-Integrin interaction
Known ECM-Integrin interaction with string annotation

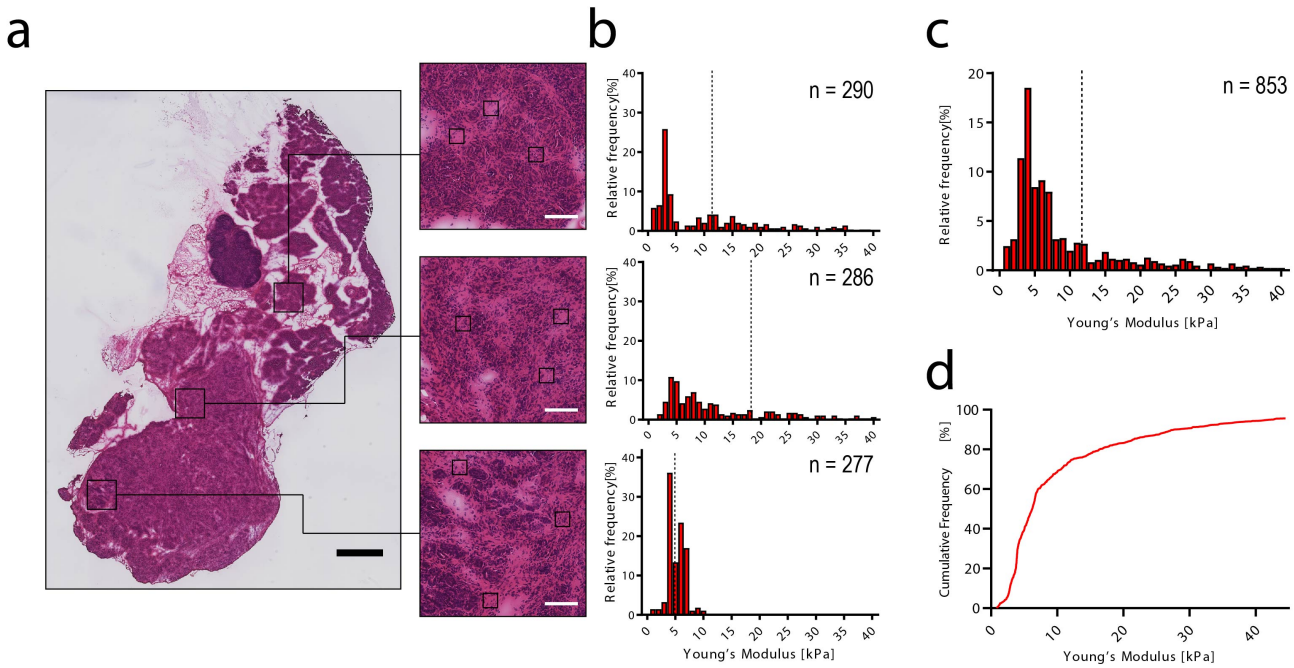
Supplementary Figure 15: a, Significantly enriched GO-BP-terms from proteomic analysis of mPCOs grown for four days in 3D PEG CBF-0.5 gels. **b,** Top 20 significantly enriched KEGG-pathways in dataset from **a**. **c,** Protein-Protein interaction network of identified integrin-ECM interactions. Integrin configurations were manually assembled from identified subunits based on literature data. **a**,**b**, Fisher's exact test corrected for multiple hypothesis testing.


a
p = 0.94
CBF_0.5
CF
fraction pan−Laminin area of organoid area
1.00
0.75
0.50
0.25
0.00
CBF-0.5
CF
PEG-Hydrogel
b
Merge
Lam/ITGA6
Lam/DAPI
DAPI
ITGA6
pan-Laminin
CBF-0.5
CF
ITGA6 ; pan-Laminin ; DAPI
c
Vehicle
10 µg/mL
20 µg/mL
40 µg/mL
0.99
0.99
0.99
0.0039
0.0039
0.022
0.01
0.069
0.29
0.0098
0.041
0.14
0.0018
0.0018
0.0097
0.00038
0.00031
0.00031
Cellular Area [µm²]
800
600
400
0
20
60
120
240
480
Cellular Area [µm²]
Time [min]
0
200
400
600
d
Vehicle
10 µg/mL
20 µg/mL
40 µg/mL
0
20
60
120
240
480
e
Fold change Number of organoids
1.00
0.75
0.50
0.25
0.00
Vehicle
Lebein-2
f
0.007
Median organoid volume [µm³]
2x10⁵
1.5x10⁵
1x10⁵
5x10⁴
0
Vehicle
Lebein-2
g
5055
1891
Organoid volume [µm³]
8×10⁶
6×10⁶
4×10⁶
2×10⁶
1×10⁶
8×10⁵
6×10⁵
4×10⁵
3×10⁵
2.5×10⁵
2×10⁵
1.5×10⁵
1×10⁵
Vehicle
Lebein-2
h
Vehicle
Lebein-2
400
300
200
100
0
2x10⁵
4x10⁵
6x10⁵
8x10⁵
1x10⁶
Number of Organoids
Organoid Volume [µm³]
1x10⁵
2x10⁵
3x10⁵
4x10⁵
5x10⁵

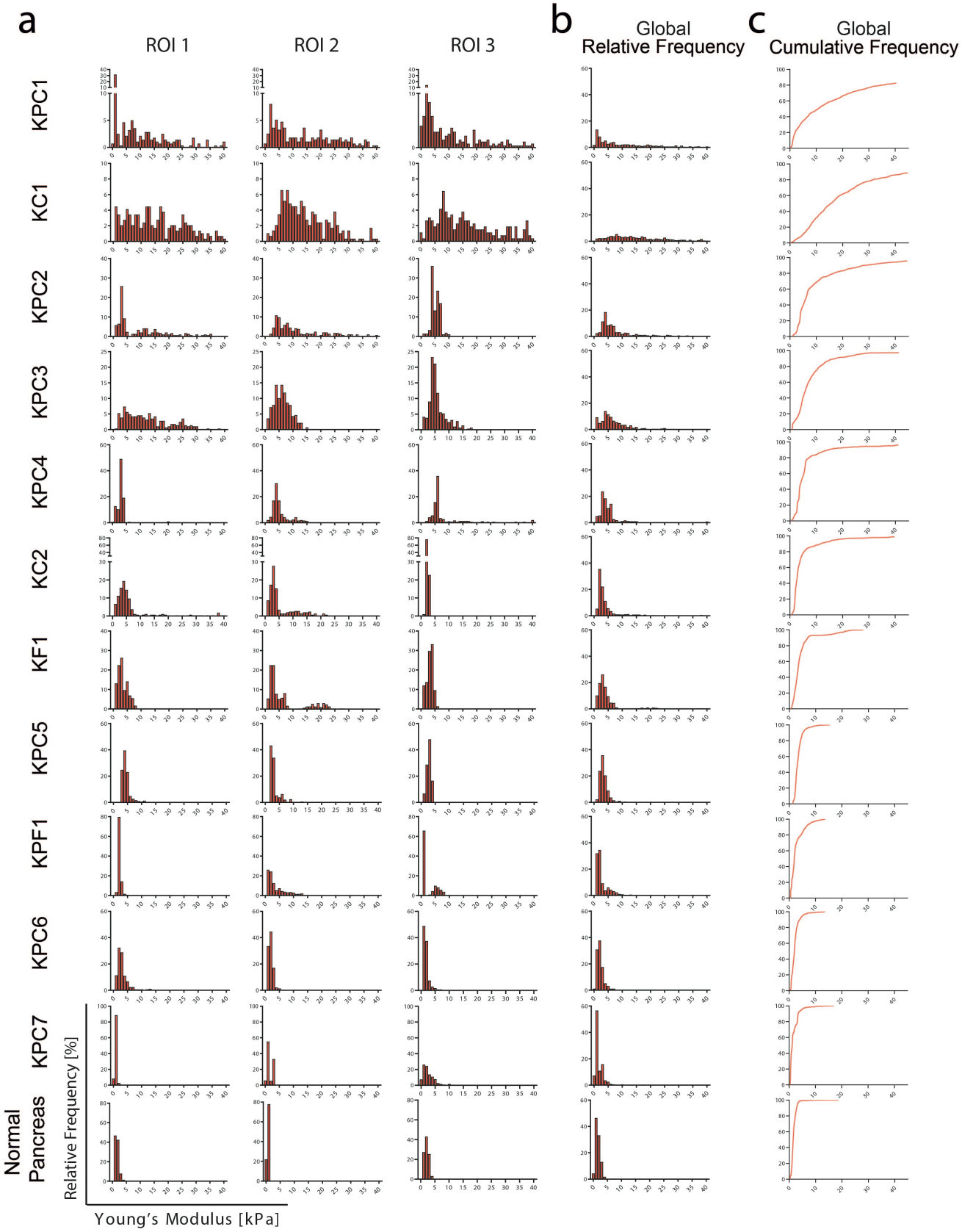
Supplementary Figure 16: a, Quantification of pan-Laminin area normalized to the total organoid area for 30 representative mPCOs grown in 3D PEG-VS CBF-0.5 or CF gels (d4). Datapoints show single organoids with mean pan-Laminin area displayed for all organoids. 10 organoids were analysed from each of n=3 experiments, error bars: s.d. two-sided Welch's t-test. **b,** Representative organoids IF images from 3D PEG CBF-0.5 or CF gels used for quantification in **a**. Scale bar: 100 μm, Inlay 25 μm. **c,d,** KPC-1 cell spreading assays **c**, and representative time-lapse images of cells **d**, adhering to L511+L521 in the presence of vehicle or lebein-2 at the indicated concentration. To aid visual interpretation of the data, gamma was adjusted to 2.3 and images were inverted. Scale bars: 100 μm, Inlay 25 μm. For cell adhesion assays, each datapoint represents the mean value of four independent adhesion experiments with minimum 100 cells analysed. Bar graphs show mean values from four independent adhesion assays with each datapoint representing the median cellular area from a single replicate at selected timepoints. Significance was assessed using the two-sided parametric student's t-test between indicated conditions with Benjamini-Hochberg correction. Error bars: s.e.m. **e,f,g,h,** Quantification of mPCOs grown for four days in PEG CBF-0.5 hydrogels treated with either vehicle control or 40 μg/mL lebein-2. **e,** Fold change of organoid formation between lebein-2 and vehicle treated organoids (n=5). Datapoints represent individual replicates normalized to vehicle treated organoids. Bargraphs show mean values across all normalized replicates. Error bars: s.e.m. **f,** Bargraph of median organoid volume across all profiled organoids from each replicate from **e**. Each datapoint represents the median organoid volume across the profiled organoid population and mean across all replicates being displayed. Statistics: paired parametric two-sided Welch's t-test. **g,** Violin plots of all five replicates combined. Dashed bold line indicates median organoid volume across all profiled organoids with faint dashed lines showing the 25^{th} or 75^{th} percentile respectively. Numbers above the plot show the number of profiled organoids from all replicates. **h,** Histogram of the organoid populations from **g** with organoid volume binned to 10,000 μm^3 bins between $1x10^5$-$1x10^6$ (above) or $1x10^5$-$5x10^5$ (below) μm^3 to aid visual interpretation of the data.


a
hPDO1
hPDO2
hPDO3
hPDO4
Matrigel
PEG CBF-0.5
b
hPDO1
hPDO3
hPDO4
PEG CBF-0.5
c
hPDO1
hPDO2
hPDO3
hPDO4
Phalloidin
Hoechst
d
E-Cadherin
Integrin α6
SOX9
ZO-1
PEG CBF-0.5
Matrigel
hPDO1
E-Cadherin
ITGA6
SOX9
ZO-1
Merge
E-Cadherin ; DAPI
ITGA6 ; DAPI
SOX9 ; DAPI
ZO-1 ; DAPI
e
hPDO3
f
hPDO4
g
d1
d4
d6
Fragment plating
Single-cell plating

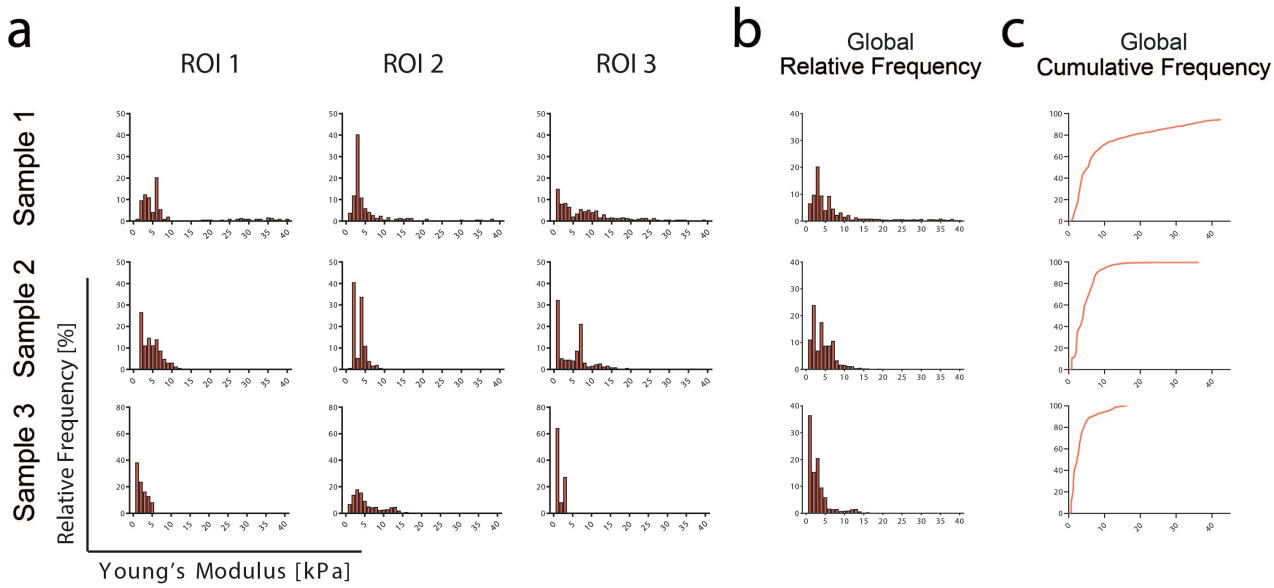
Supplementary Figure 17: a, Representative brightfield and **b,** H&E images of human patient-derived (hPDO) organoids grown in PEG CBF-0.5 hydrogels or Matrigel (d6, n=3). **a**, Scale bar: 250 μm. **b,** Scale bar: 200 μm. **c,d,e,** Representative IF images of selected markers in hPDO1 **c**, hPDO3 **d**, or hPDO4 **e**. Scale bars: 100 μm, Inlay 25 μm. Images are representative of at least 5 organoids in the respective gel across minimum two independent experiments. **f,** Representative brightfield images of hPDO3 organoids growing in 3D PEG-VS CBF-0.5 gels for the indicated time from fragment (top) or single-cell (bottom) plating (n=3). Scale bar: 500 μm, Inlay: 100 μm.


a
b
c
d
n = 290
n = 286
n = 277
n = 853
Relative frequency[%]
Young's Modulus [kPa]
Cumulative Frequency [%]

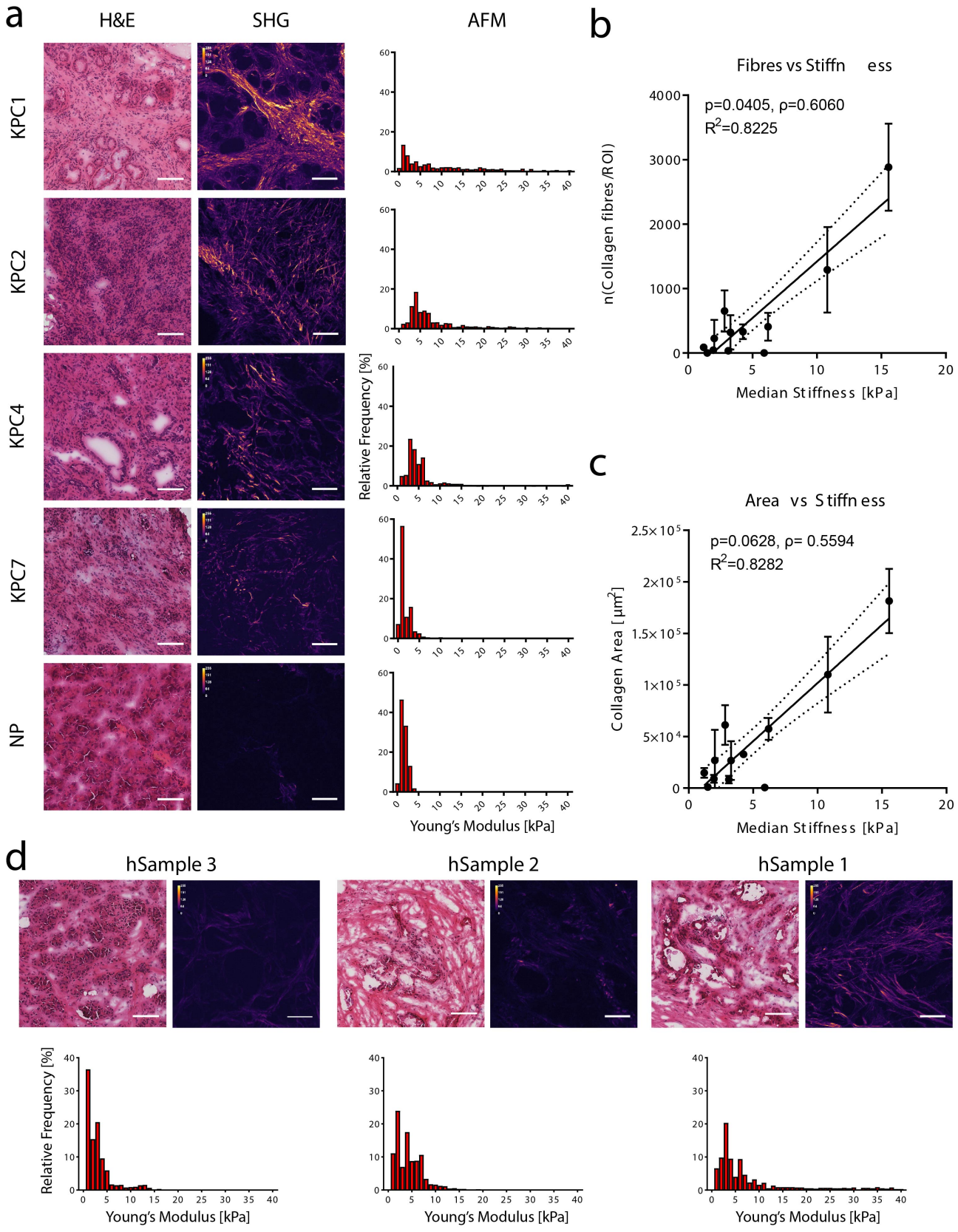
Supplementary Figure 18: a, Example H&E image of an AFM-profiled sample illustrating how individual stromal areas were analysed by AFM (inlay) (representative image from 11 cancerous sample shown). Marked areas show regions in which 100 AFM indentations were sampled. Scale bars: 1 mm, Inlay: 100 μm. **b,** Relative frequency of AFM measurements (Young's modulus). n - number of force curves sampled **c,** Stiffness plot of the entire sample from **a** combining all measurements from **b**. Dashed lines indicate the mean Young's modulus. n - number of force curves sampled. **d,** Cumulative frequency of Young's modulus measurements from **c**.


a
b
c
ROI 1
ROI 2
ROI 3
Global
Relative Frequency
Global
Cumulative Frequency
KPC1
KC1
KPC2
KPC3
KPC4
KC2
KF1
KPC5
KPF1
KPC6
KPC7
Normal
Pancreas
Relative Frequency [%]
Young's Modulus [kPa]

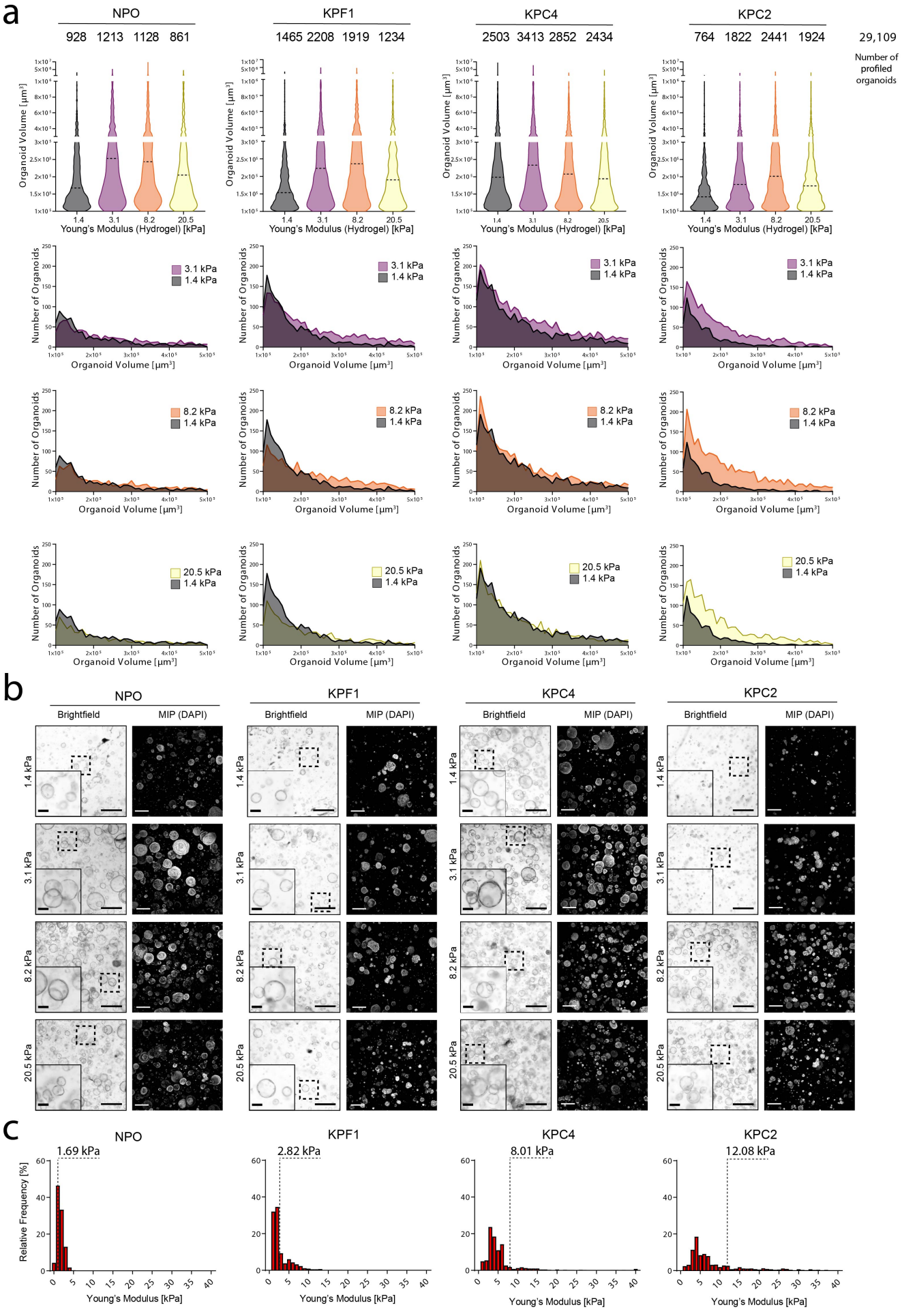
Supplementary Figure 19: a,b, Stiffness plots for each region of interest (ROI) **a**, and cumulated **b**, across analysed murine pancreatic cancerous samples. Data is displayed as relative frequency measurement (Young's modulus). **c,** Cumulative frequency of the Young's modulus measurements from **b**. For murine normal pancreas, each stiffness map from **a** display all stiffness measurements for one sample and **b** displays stiffness measurements for three normal pancreata.


a
ROI 1
ROI 2
ROI 3
b
Global
Relative Frequency
c
Global
Cumulative Frequency
Sample 1
Sample 2
Sample 3
Relative Frequency [%]
Young's Modulus [kPa]

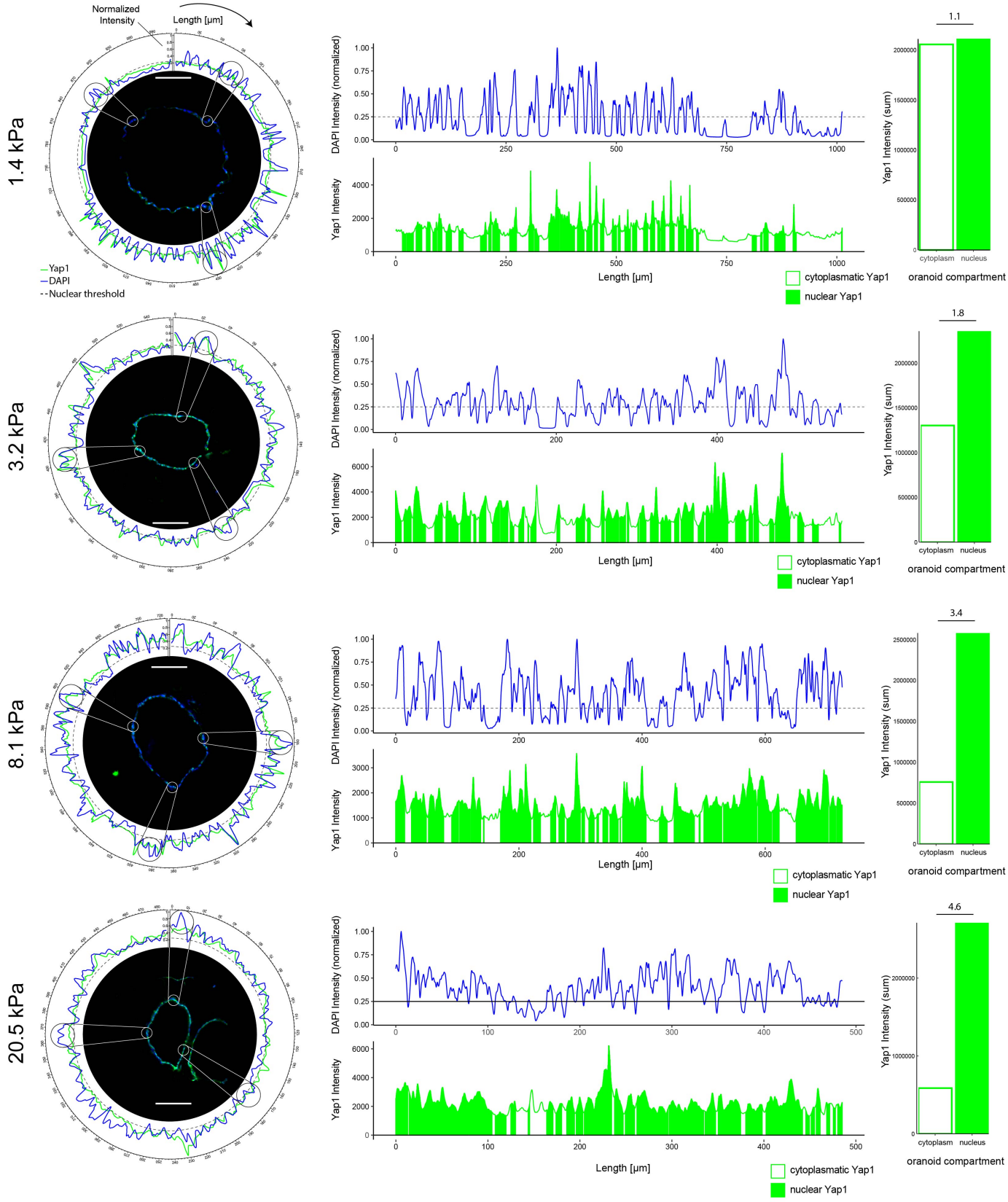
Supplementary Figure 20: a,b, Stiffness maps for each selected stromal region of interest **a**, and cumulated **b**, for profiled human pancreatic cancerous samples (n=3). Data is displayed as relative frequency measurements (Young's modulus). **c,** Cumulative frequency of Young's modulus measurements from **b**.


a
H&E
SHG
AFM
KPC1
KPC2
KPC4
KPC7
NP
Relative Frequency [%]
Young's Modulus [kPa]
b
Fibres vs Stiffn ess
p=0.0405, ρ=0.6060
R2=0.8225
n(Collagen fibres/ROI)
Median Stiffness [kPa]
c
Area vs Stiffn ess
p=0.0628, ρ= 0.5594
R2=0.8282
Collagen Area [µm2]
Median Stiffness [kPa]
d
hSample 3
hSample 2
hSample 1
Relative Frequency [%]
Young's Modulus [kPa]

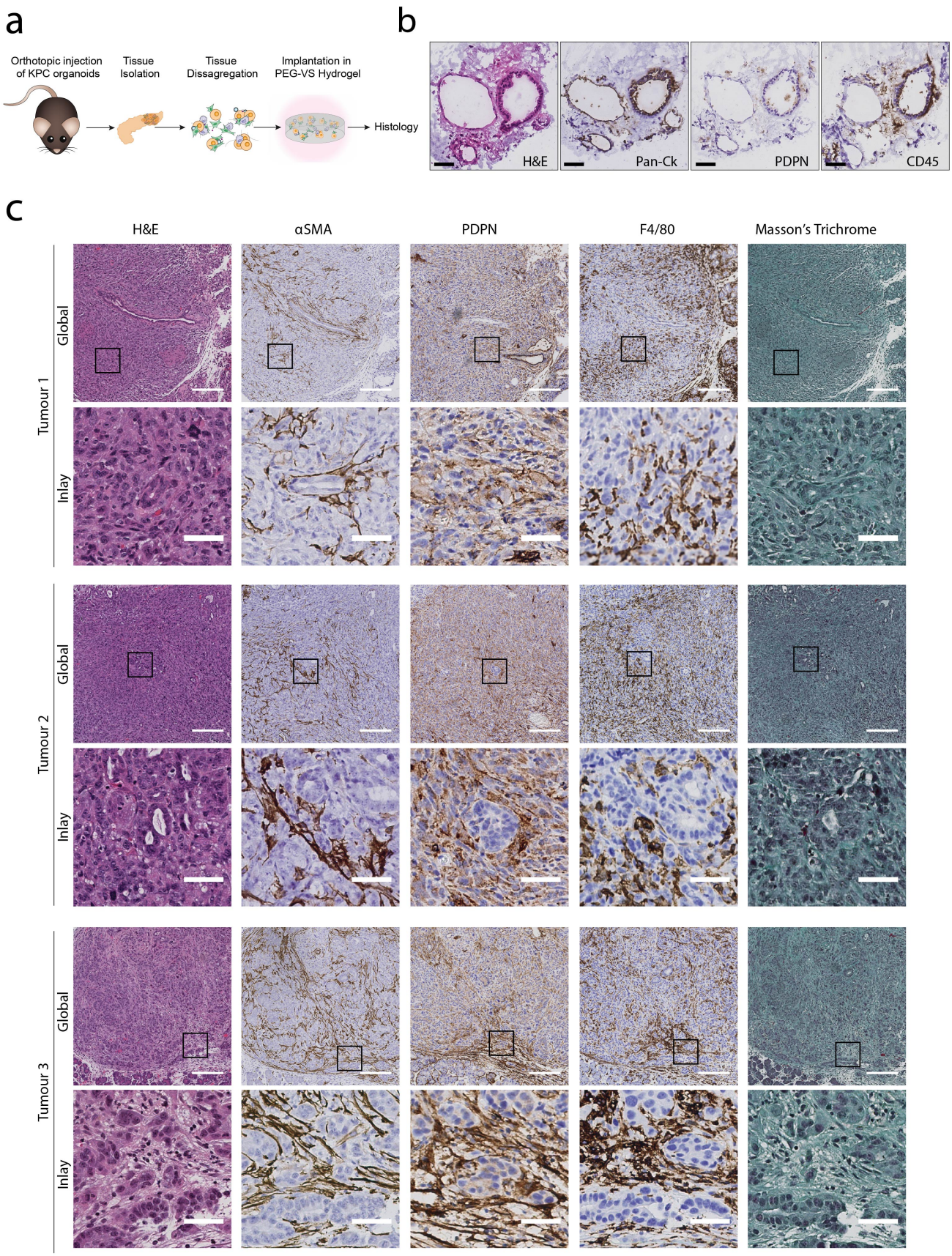
Supplementary Figure 21: a, H&E sections and second-harmonic generation (SHG) images of stromal regions from murine PDAs analysed by AFM. Scale bar: 100 μm. **b,c,** Correlation analysis of median stiffness (kPa) and mean number of collagen fibres **b**, or mean collagen area **c**, for each sample. Three independent regions were analysed in each of n=11 samples. Correlations were evaluated using Spearman for **b**, R^2=0.8225, ρ=0.6060, p=0.0405 and **c**, R^2=0.8282, ρ=0.5595, p=0.0628. Linear regression is shown in solid lines and dashed lines indicate 95% confidence interval Error bars: s.d. **d,** H&E sections and second-harmonic generation (SHG) images of stromal regions from human PDA analysed by AFM. For SHG, images are representative over all three profiled regions within each sample. Scale bar: 100 μm. Abbreviations: NP – Normal pancreas, hSample – human Sample, ROI – Region of interest.


a
NPO
KPF1
KPC4
KPC2
928 1213 1128 861
1465 2208 1919 1234
2503 3413 2852 2434
764 1822 2441 1924
29,109
Number of profiled organoids
Organoid Volume [µm³]
Young's Modulus (Hydrogel) [kPa]
1.4
3.1
8.2
20.5
Number of Organoids
3.1 kPa
1.4 kPa
8.2 kPa
20.5 kPa
b
Brightfield
MIP (DAPI)
c
Relative Frequency [%]
Young's Modulus [kPa]
1.69 kPa
2.82 kPa
8.01 kPa
12.08 kPa

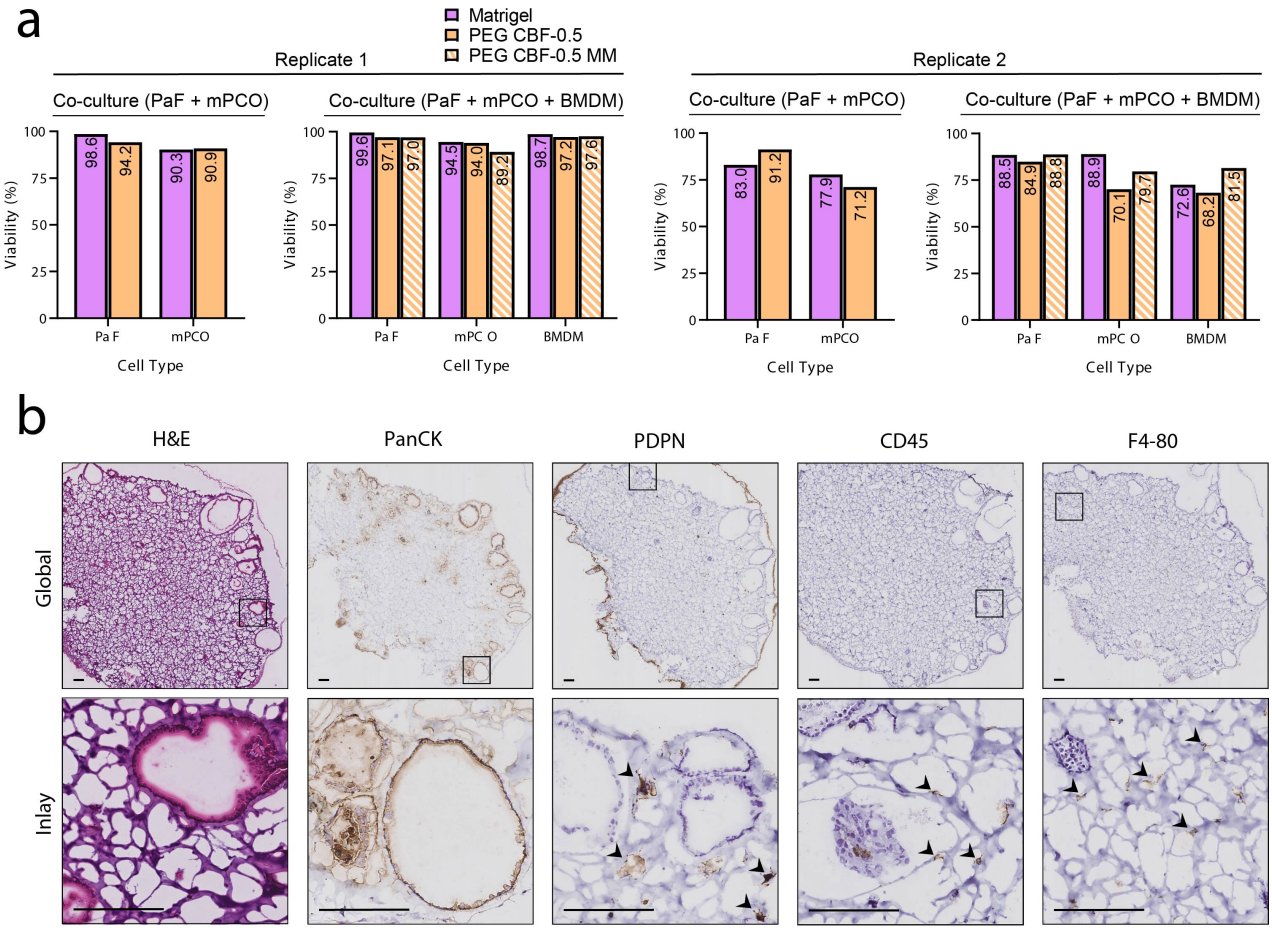
Supplementary Figure 22: a,b, Quantification of pancreatic organoids (POs) in 3D PEG-VS CBF-0.5 hydrogels at different stiffnesses. **a,** Violin plots of four individual replicates of POs grown in 3D PEG CBF-0.5 gels at the indicated stiffness (top), and histogram of the organoid populations with organoid volume in 10,000 μm^3 bins between $1x10^5$-$5x10^5$ μm^3 (bottom) to aid visual interpretation of the data. Dashed bold line indicates median organoid volume across all profiled organoids. Numbers above the plot indicate the number of profiled organoids. **b,** Representative brightfield images (left) and maximum intensity projections (MIP) of DAPI signal over the entire height of a profiled gel (right). Scale bar: Left: 500 μm, left inlay: 100 μm, Right: 200 μm. **c,** Stiffness plots of the tissues from which the organoids used in **a**,**b**, were isolated. Data is displayed as relative frequency measurement (Young's modulus). Dashed line indicated the mean stiffness for the profiled sample.


1.4 kPa
3.2 kPa
8.1 kPa
20.5 kPa
Normalized Intensity
Length [µm]
Yap1
DAPI
Nuclear threshold
DAPI Intensity (normalized)
Yap1 Intensity
Length [µm]
cytoplasmatic Yap1
nuclear Yap1
Yap1 Intensity (sum)
cytoplasm
nucleus
oranoid compartment
1.1
1.8
3.4
4.6

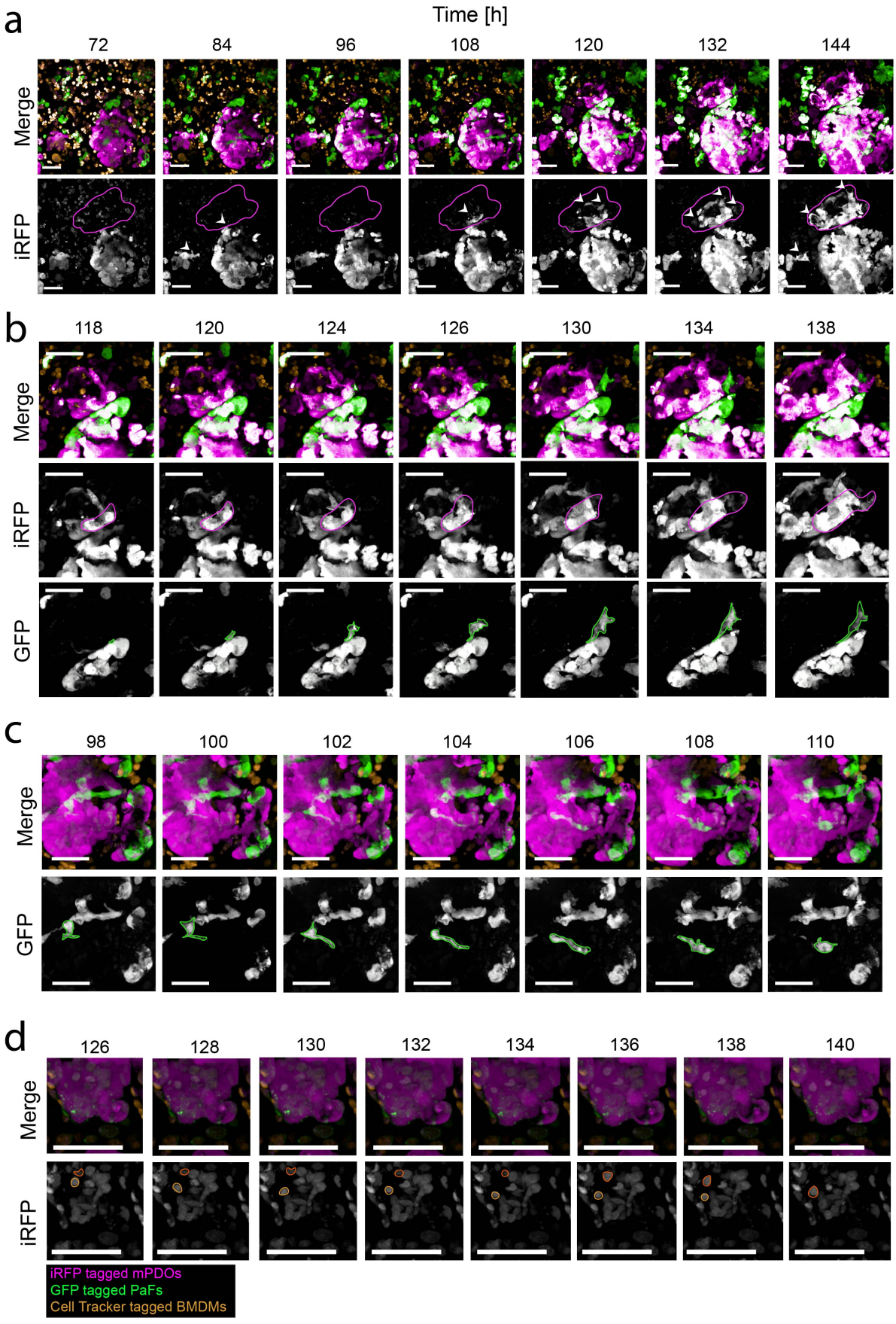
Supplementary Figure 23: Quantification of YAP1 translocation in mPCOs growing in PEG CBF-0.5 hydrogels at the indicated stiffness. Left: Normalized intensity profiles of DAPI or YAP1 plotted around the profiled organoid. Three ROIs are highlighted to aid the visual interpretation of the data. Depicted and profiled organoids are representative for minimum 30 organoids from three independent experiments. Scale bar: 100 μm. Middle: Intensity profiles with nuclear and cytoplasmatic regions being gated based on the DAPI stain. The dashed line indicates a normalized DAPI intensity of 0.25 which has been used as nuclear cut off in this study. YAP1 intensities are depicted as nuclear (green bar) or cytoplasmatic (white bar). Right: Nuclear and cytoplasmatic YAP1 intensity from middle panel summarized and nuclear/cytoplasmatic ratio shown.


a
Orthotopic injection of KPC organoids
Tissue Isolation
Tissue Dissagregation
Implantation in PEG-VS Hydrogel
Histology
b
H&E
Pan-Ck
PDPN
CD45
c
H&E
αSMA
PDPN
F4/80
Masson's Trichrome
Tumour 1
Tumour 2
Tumour 3
Global
Inlay

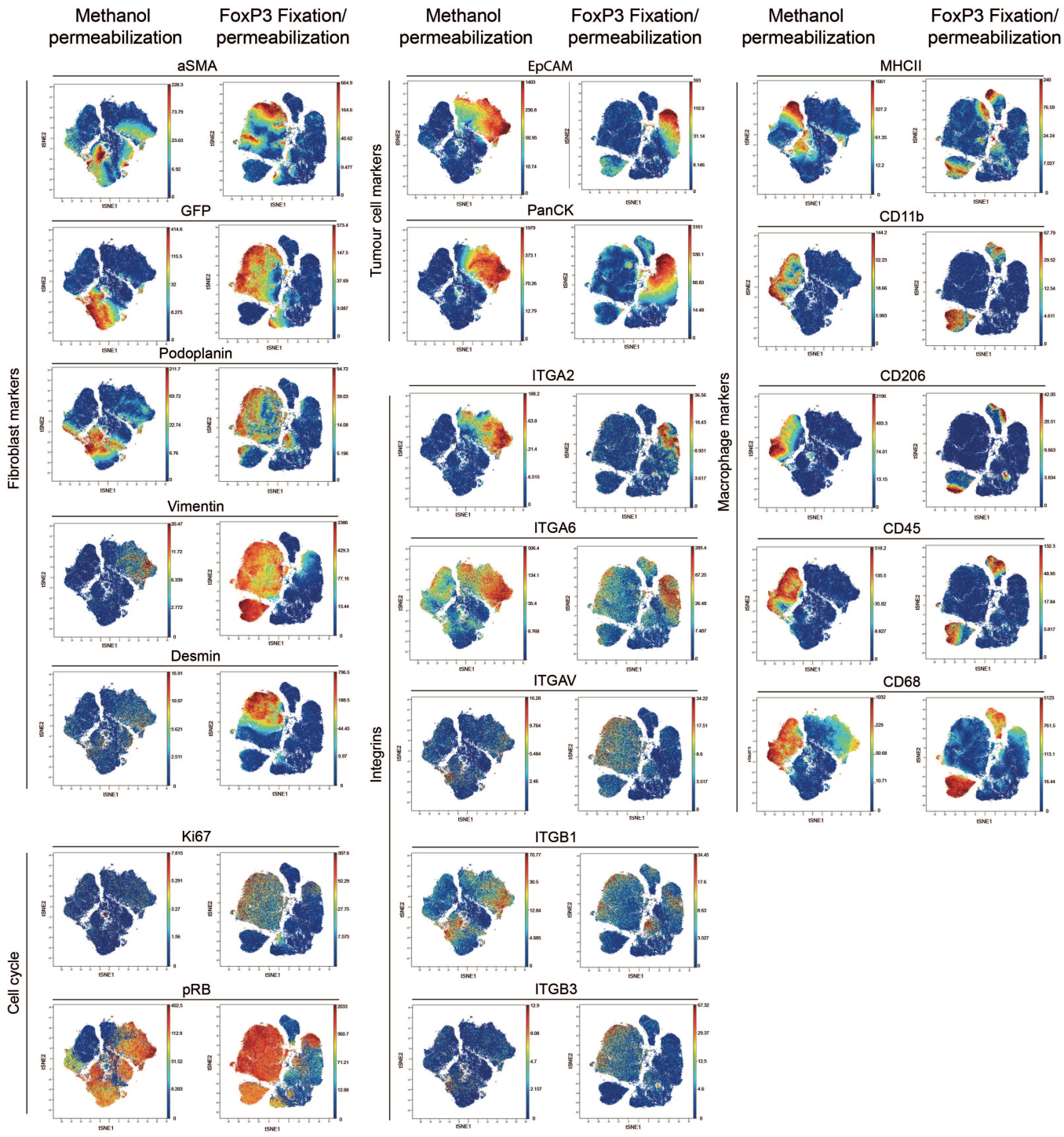
Supplementary Figure 24: a, Overview of direct implantation of murine tumour material into PEG-CBF-0.5 gels. **b,** H&E and immunohistochemical analysis of day 8 cultures. Sections stained for tumour cells (Pan-CK), cancer-associated fibroblasts (PDPN) and immune cells (CD45) shown. Data are representative of histological analysis of tumours from 3 mice. Scale bar: 50 μm. **c,** H&E, IHC and Masson's Trichrome staining of orthotopically generated tumours from mPCO injections (n=3). Images are representative for the entire tumour. Scale bars: Global: 200 μm, Inlay: 50 μm.


a
Matrigel
PEG CBF-0.5
PEG CBF-0.5 MM
Replicate 1
Replicate 2
Co-culture (PaF + mPCO)
Co-culture (PaF + mPCO + BMDM)
Co-culture (PaF + mPCO)
Co-culture (PaF + mPCO + BMDM)
Viability (%)
100
75
50
25
0
98.6
94.2
90.3
90.9
99.6
97.1
97.0
94.5
94.0
89.2
98.7
97.2
97.6
83.0
91.2
77.9
71.2
88.5
84.9
88.8
88.9
70.1
79.7
72.6
68.2
81.5
Pa F
mPCO
mPC O
BMDM
Cell Type
b
H&E
PanCK
PDPN
CD45
F4-80
Global
Inlay

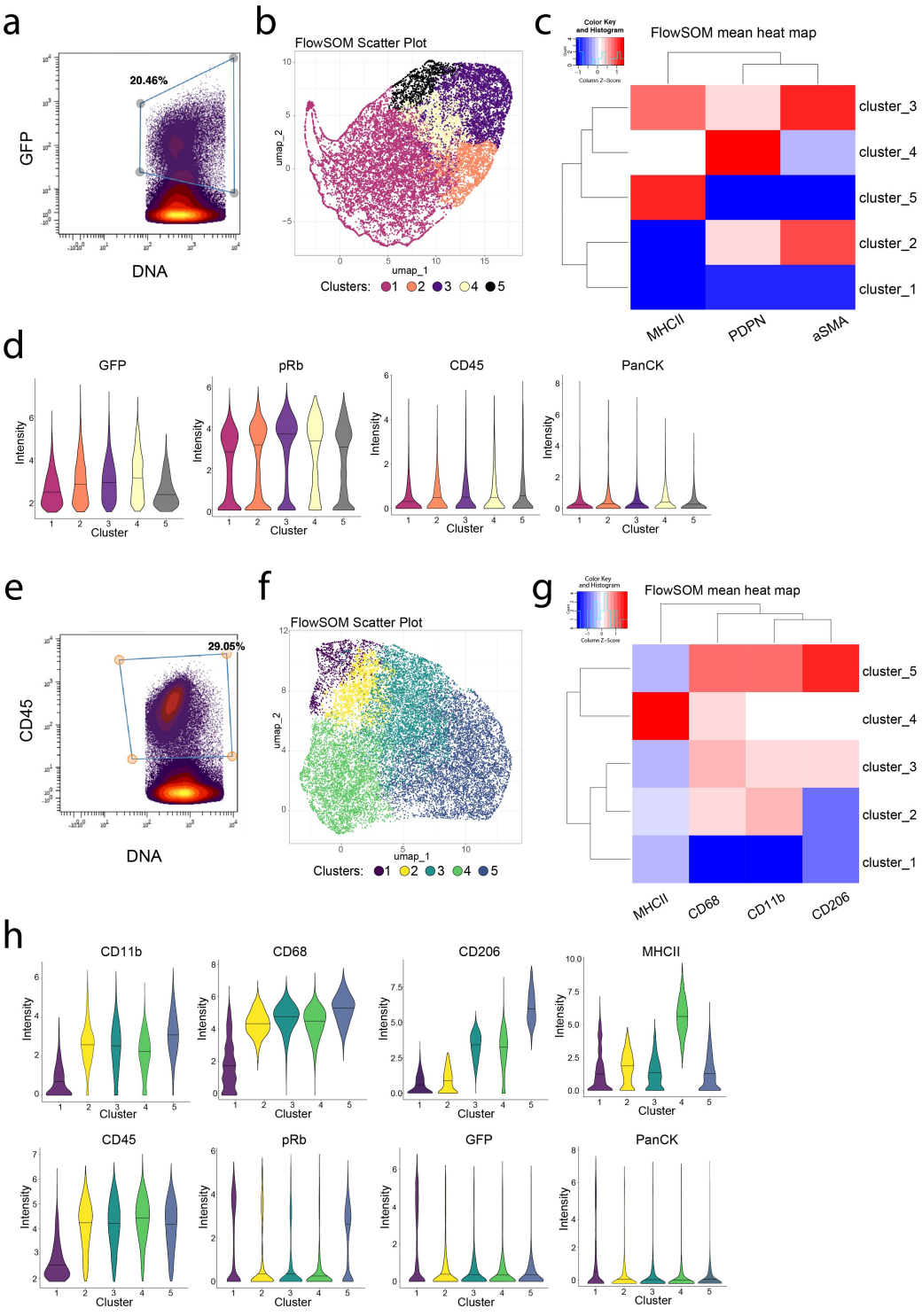
Supplementary Figure 25: a, Viability of mPCO, PaF or BMDMs liberated from dual (PaF+mPCO) or triple (PaF+BMDM+mPCO) co-cultures grown for six days in Matrigel or PEG CBF-0.5 hydrogels in full hPOCM media formulation or a reduced organoid media containing only essential growth factors (minimal media, MM). Two independent biological replicates are shown, barplots represent mean viability across an entire cell population gated by FACS. Numbers within barplots indicate viability. **b,** H&E and immunohistochemical analysis of 3-way co-culture of mPCOs, PaFs and BMDM in PEG-VS CBF-0.5 hydrogels after 6 days (n=2). Sections stained for Pan-CK, PDPN, CD45 and F4-80 shown. Scale bar: 200 μm.


a
Time [h]
72
84
96
108
120
132
144
Merge
iRFP
b
118
120
124
126
130
134
138
Merge
iRFP
GFP
c
98
100
102
104
106
108
110
Merge
GFP
d
126
128
130
132
134
136
138
140
Merge
iRFP
iRFP tagged mPDOs
GFP tagged PaFs
Cell Tracker tagged BMDMs

Supplementary Figure 26: a-c) Representative images of co-cultures in 3D PEG CBF-0.5 hydrogels at indicated time after seeding **a**, or selected regions to show fibroblast migration **b**, and/or cancer-cell migration **c**. Images are representative of at least five individual co-culture regions centred around mPCOs. Scale bar: 60 μm. See also Supplementary Videos 12-15.


Methanol permeabilization
FoxP3 Fixation/ permeabilization
Methanol permeabilization
FoxP3 Fixation/ permeabilization
Methanol permeabilization
FoxP3 Fixation/ permeabilization
Fibroblast markers
aSMA
GFP
Podoplanin
Vimentin
Desmin
Cell cycle
Ki67
pRB
Tumour cell markers
EpCAM
PanCK
Integrins
ITGA2
ITGA6
ITGAV
ITGB1
ITGB3
Macrophage markers
MHCII
CD11b
CD206
CD45
CD68
tSNE1
tSNE2

Supplementary Figure 27: Mass cytometry analysis co-cultures grown for six days in PEG CBF-0.5 hydrogels prepared using two different fixation methods, visualised by tSNE. Integrins, pRB and Ki67 were not used during clustering. Colour overlay shows relative quantification of selected markers. Range of colorimetric scale is indicated for individual markers.


a
20.46%
GFP
DNA
b
FlowSOM Scatter Plot
umap_2
umap_1
Clusters: 1 2 3 4 5
c
Color Key and Histogram
Column Z-Score
FlowSOM mean heat map
cluster_3
cluster_4
cluster_5
cluster_2
cluster_1
MHCII
PDPN
aSMA
d
GFP
pRb
CD45
PanCK
Intensity
Cluster
e
29.05%
CD45
DNA
f
FlowSOM Scatter Plot
umap_2
umap_1
Clusters: 1 2 3 4 5
g
Color Key and Histogram
Column Z-Score
FlowSOM mean heat map
cluster_5
cluster_4
cluster_3
cluster_2
cluster_1
MHCII
CD68
CD11b
CD206
h
CD11b
CD68
CD206
MHCII
CD45
pRb
GFP
PanCK
Intensity
Cluster

Supplementary Figure 28: a,b, Flow-based gating-approach for selection of fibroblasts from CyTOF analysis of co-cultures (left) and UMAP-based visualization of fibroblast sub-clusters (right). **c,** FlowSOM-based heatmap of mean intensity signal for MHCII, αSMA and PDPN across all fibroblast clusters from **b**. **d,** Violin plots of all five fibroblast clusters from **b,c** with intensity distribution for indicated markers. **e,f,g,h,** Same as in **a-d** but for a CD45+ cell population (myeloid cells).

Supplementary Videos:

Supplementary Video 1: Time-lapse image series of KPC-1 PCCs adhering to Laminin 511 (top left) with visualization of cell-masking (top right) to demonstrate the cellular adhesion measurement approach. Cellular migration (bottom left) is visualized along with the cellular generation (bottom left) to indicate that cellular division events are accounted for. Scale bar: 100 μm.

Supplementary Video 2: Time-lapse image series of KPC-1 PCCs adhering to a non-coated surface (top left) with visualization of cell-masking (top right) to demonstrate the cellular adhesion measurement approach. Cellular migration (bottom left) is visualized along with the cellular generation (bottom left) to indicate that cellular division events are accounted for. Scale bar: 100 μm.

Supplementary Video 3: Time-lapse image series of KPC-1 PCCs adhering to Laminin 511. Scale bar: 100 μm.

Supplementary Video 4: Time-lapse image series of KPC-1 PCCs adhering to Laminin 521. Scale bar: 100 μm.

Supplementary Video 5: Time-lapse image series of KPC-1 PCCs adhering to a combination of Laminin 511 and Laminin 521. Scale bar: 100 μm.

Supplementary Video 6: Time-lapse image series of KPC-1 PCCs adhering to a combination of Laminin 511, Laminin 521 and Fibronectin. Scale bar: 100 μm.

Supplementary Video 7: Time-lapse image series of KPC-1 PCCs adhering to Fibronectin. Scale bar: 100 μm.

Supplementary Video 8: Time-lapse image series of KPC-1 PCCs adhering to Collagen-1. Scale bar: 100 μm.

Supplementary Video 9: Time-lapse image series of KPC-1 PCCs adhering to a non-coated glass surface. Scale bar: 100 μm.

Supplementary Video 10: 3D reconstruction of a representative mPDO from Supplementary Figure 12d containing 27 single-cells (half-sphere). Scales as indicated.

Supplementary Video 11: 3D reconstruction of a representative mPDO from Supplementary Figure 12e containing 71 single-cells (half-sphere). Scales as indicated.

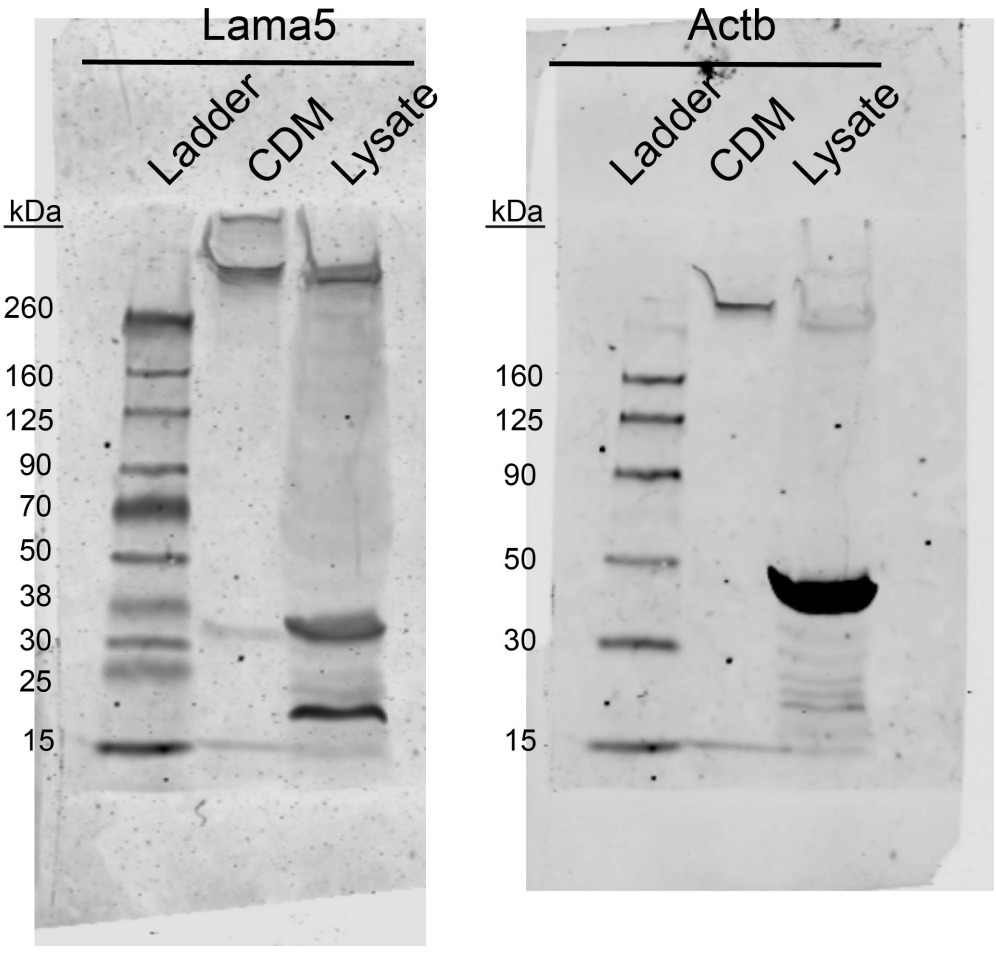
Supplementary Video 12: Maximum intensity projection (MIPs) videos of co-cultures from Supplementary Figure 25 grown in PEG-VS CBF-0.5 gels for three days from 72h-144h post seeding. Video is representative of at least 20 ROIs. Pseudo colouring as follows: Green: GFP-labelled PaFs; Purple: iRFP labelled mPCOs; Orange: Cell-tracker dye labelled BMDMs.

Supplementary Video 13: Maximum intensity projection (MIPs) videos of co-cultures from Supplementary Figure 25 grown in PEG-VS CBF-0.5 gels for three days from 72h-144h post seeding. Only iRFP channel (mPCOs) shown. Video is representative of at least 20 ROIs.

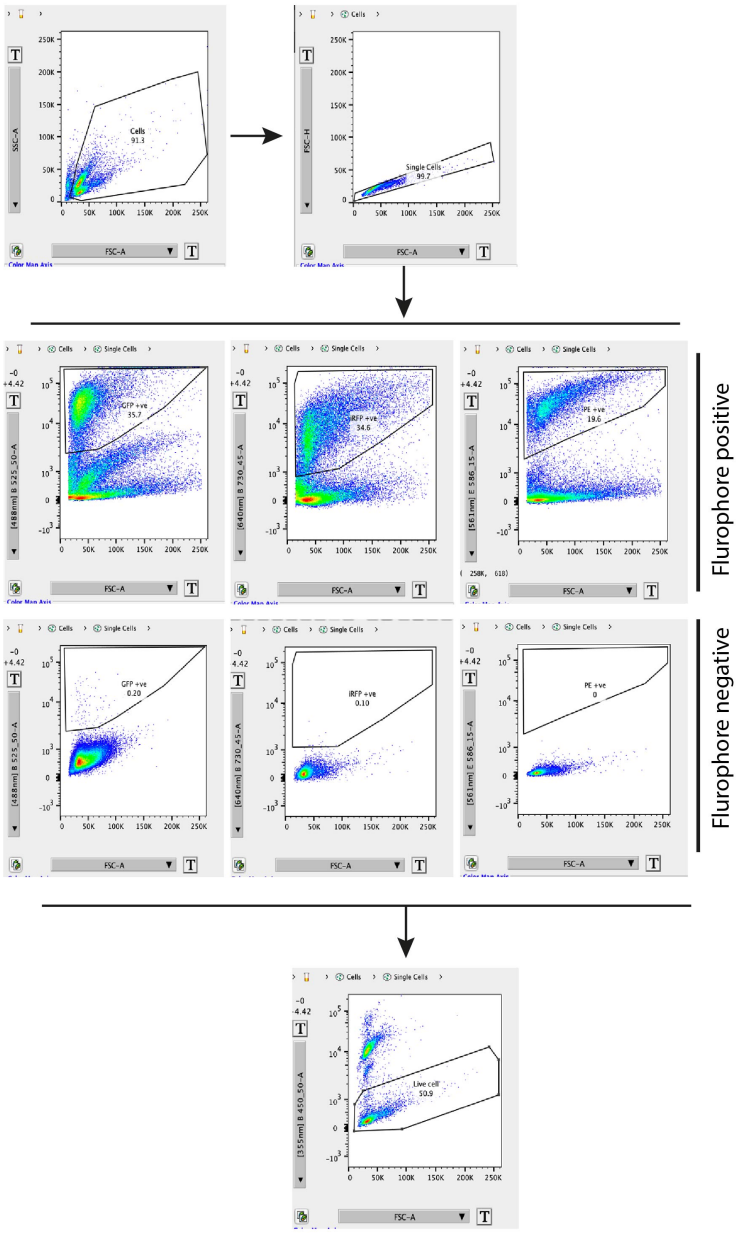
Supplementary Video 14: Maximum intensity projection (MIPs) videos of co-cultures from Supplementary Figure 25 grown in PEG-VS CBF-0.5 gels for three days from 72h-144h post seeding. Only GFP channel (PaFs) shown. Video is representative of at least 20 ROIs.

Supplementary Video 15: Maximum intensity projection (MIPs) videos of co-cultures from Supplementary Figure 25 grown in PEG-VS CBF-0.5 gels for three days from 72h-144h post seeding. Only cell-tracker orange channel (BMDMs) shown. Video is representative of at least 20 ROIs.

Full blots for Supplementary figure 7b



FlowSOM Gating strategy for supplementary figure 25a



Gating hierarchy

Group

- All Samples
  - Cells
    - Single Cells
      - GFP +ve
        - Live cell
      - iRFP +ve
        - Live cell
      - PE +ve
        - Live cell

Supplementary Materials and Methods:

Cell Culture:

Pancreatic fibroblasts (PaFs). Murine PaFs were isolated from pancreata of 8 week old C57-Bl/6 animals. Normal healthy pancreata were washed with RPMI (RPMI, gibco) and mechanically disrupted in freshly prepared sterile digestion buffer (2 mg/mL Collagenase IV (Thermo), 1 mg/mL DNAse1 (Thermo), 0.5 mg/mL hyaluronidase (Sigma Aldrich) in RPMI) until pieces of 1-2 mm diameter were obtained. Tissue pieces were then further enzymatically separated in digestion buffer for 10 minutes at 37°C in a humidified incubator to obtain a single-cell suspension which was thereafter carefully separated from larger, undigested tissue pieces, centrifuged and re-suspended in DMEM 10% v/v FBS supplemented with 1x Primocin. Cells were then seeded in T225 flasks in DMEM 20% v/v FBS supplemented with Primocin and media replenished every 2nd day. Purification of the fibroblast population and validation of fibroblast identity was conducted using fluorescent activated cell sorting (FACS) after cells were grown for at least six days following immortalization with SV40-LT.

PaF immortalization. Primary sorted PaFs were immortalized after seven days in culture using the pCL-Eco vector (pCL-Eco was a gift from Inder Verma (Addgene plasmid #12371; https://www.addgene.org/12371/)) and pBABE-puro SV40 LT (pBABE-puro SV40 LT was a gift from Thomas Roberts (Addgene plasmid #13970, https://www.addgene.org/13970/)) plasmids. In brief, viral particles were produced in 7.5x10^6 Phoenix cells following formation of complexes using Lipofectamine2000 (Thermo Fisher) reagent together with the pCL-Eco and pBABE-puro SV40-LT at a 50:50 ratio in 18 mL OptiMEM (gibco) for 20 minutes at RT according to the manufacturer's instructions. Cells were transfected for 6 hours in 150 mm dishes before the media was aspired to collect viral particles. After 24h, the media was collected again and sterile filtered using a 0.2 μm cell strainer (BD) to harvest another batch of viral particles. Fresh culture media was added and after another 24h of incubation, the media was collected, filtered and combined with the first harvest. PaFs were transduced by gently adding viral particles in the presence of 8 μg/mL polybrene (Thermo Fisher) and incubated for 24h at 37°C with constant agitation in 75 cm^2 Flasks (Corning). After culture for three days, cells were selected for one week using DMEM 10% v/v FBS supplemented with 1 μg/mL puromycin (Thermo Fisher) and FACS sorted as previously described. Cells were then re-sorted after another 7 days in culture to ensure high purity.

Lentiviral transduction of PaFs and PCCs. For labelling immortalized PaFs and PCCs with green fluorescent protein (GFP), a 2nd generation lentiviral packaging system was utilized [1]. In

brief, viral particles were generated by combing Polyethylenimine hydrochloride (PEI, Sigma Aldrich) at a volumetric ratio of 3:1 (PEI:plasmids) with pCMV delta R8.2 (Addgene plasmid #12263 was a gift from Didier Trono; http://n2t.net/addgene:12263) packaging-, pMD2.G (Addgene plasmid #12259 was a gift from Didier Trono; http://n2t.net/addgene:12259) envelope- and pEGFP-SEW (the plasmid was a gift from Tim Somervaille [2] in which the EGFP cassette was subcloned into pHR'SIN.cPPT-SEW; a gift from Dr Adrian Thrasher) plasmids in 1 mL DMEM at RT for 30 minutes. $4.5 \cdot 10^6$ HEK293-FT cells were then transfected with the resulting mixture overnight and virus-containing media was collected 24 and 48h after transduction. Lentiviral particles were concentrated to 100x using the PEG-IT reagent (Stratech, LV810A-1-SBI). Cells were transduced with the virus containing media supplemented with 8 μg/mL polybrene (Sigma Aldrich) for 24h at 37ºC. GFP-expressing cells were isolated using FACS.

Lentiviral transduction of pancreatic organoids. mPCOs were transduced using spinoculation [3]. In brief, PGK_iRFP720-WPRE lentiviral particles (pRRLsinPGK_IRFP720_WPRE plasmid was a gift from Beverly Torok-Storb (Addgene plasmid #91785; http://n2t.net/addgene:91785)) were generated in HEK293-FT cells (ATCC) using the pMD2.G envelope and psPAX2 packaging plasmids, collected, filtered using a 0.45 um filter (BD) and concentration using the Lenti-X concentrator (Takara Bio Inc). Virus aliquots were stored at -80°C. 25,000 organoids were resuspended as single cells in 250 μL of virus-containing AdF-base medium supplemented with 4 μg/mL polybrene (Sigma Aldrich), centrifuged for 1 hour at 600 x g at room temperature and then incubated for 4-6 hrs at 37°C with occasional agitation. Cells were subsequently seeded in Matrigel and cultured in hPOCM media with daily media changes. After 1-2 weeks of expansion, organoid cells were FACS sorted twice to purify the iRFP-positive cell population.

Cell sorting. For fluorescent activated cell sorting (FACS) of murine PaFs, a cell suspension was generated by gently lifting the cells using Accutase (Thermo Fisher Scientific) for 5 min at 37°C and following neutralization with DMEM 10% v/v FBS and centrifugation, PaFs were re-suspended in PBS supplemented with 1% v/v FBS, hereafter termed FACS buffer (FB). Cells were then washed and re-suspended in FB supplemented with Fc-block and subjected to surface staining with brilliant violet 421 (BV-421) conjugated anti-CD45 (1:20, BioLegend Clone 30-F11); BV-421 conjugated CD-31 (1:20, BioLegend, clone MEC13.3); BV-421 conjugated Anti-EpCAM (1:20, BioLegend clone G8.8); Phycoerythrin (PE) conjugated CD-90 (1:20 Thermo, clone G7); PE-Cyanine7 (PE-Cy7) conjugated anti-podoplanin (PDPN, 1:20, BioLegend, clone 8.1.1) for 30-45 min on ice, shaking. Following staining, excess antibody

was removed using FB, cells were passed through a 70 μm filter (BD) to remove cell-clumps, re-suspended at a concentration of 5 x 10^6 cells/mL in FB and sorted for $BV421^-$ and PE^+ on an Aria III instrument (Becton Dickinson) according to the manufacturer's instructions.

For FACS of murine iRFP-labelled mPCOs, cells were prepared as single cell suspension using TrypLE, filtered using a 70 μm cell strainer (BD) and retained in AdF Base medium till sorting. iRFP-labelled mPCOs were sorted using an BDAria 3 SORP (Becton Dickinson) sorter using the 640 laser and 730/45 filter set. The sort was repeated after 7 days of growth to obtain a pure cell population.

Murine bone-marrow derived macrophage (BMDM) cultures. BMDMs were isolated as previously described [4]. In brief, under sterile conditions, femurs and tibias were harvested from 8-10 week old C57BL/6 mice. Bones were washed in 70% ethanol followed by 2 subsequent washes in ice-cold sterile PBS. In a sterile petri dish, epiphyses of the bones were removed, and bone marrow was flushed with DMEM media (DMEM, 10% FCS and 1% penicillin/streptomycin) using a 25-gauge needle and were passed through 70 μm cell strainer. Cells were washed and cultured in DMEM media supplemented with 20 ng/mL macrophage colony-stimulating factor (M-CSF) for 3 days. Media was replaced after 3 days and cells were further cultured for 3 additional days till used for seeding in 3D PEG-VS gels.

Orthotopic transplantation of mPCOs. For transplantation single-cell suspension of mPCOs were prepared and stored in Matrigel on ice. Mice were anesthetized using Isoflurane and subcutaneously injected with Buprenorphine (20 μL/10g body weight, Buprecare, Royal Veterinary College (RVC)) and Carprofen (60 μL/30g body weight, Rimadyl, RVC). Prior to surgery, fur was clipped, the skin cleaned using Chlorhexidine surgical scrub and the animal was draped for surgery to maintain aseptic conditions. Following incision at the left abdominal flank, the pancreas was isolated and 10,000 cells injected in 20 μL ice-cold Matrigel into the tail region of the pancreas. The success of the injection was then verified by the appearance of a clean bubble within the pancreas without any signs of leakage. The pancreas containing the injected cells was then carefully moved back into the peritoneal pocket and the abdominal wall and skin were sutured with absorbable Vicryl suture (IMS euro). Prior to suturing the skin, Bupivacaine Hydrochloride (1:20 in sterile saline, Marcain Polyamp 0.5%, RVC) were administered locally to aid in local anaesthesia and animals were injected subcutaneously with 0.3 mL sterile saline. Animals were carefully monitored after surgery and orally administered Carprofen (60 μL/30g body weight) in jelly for up to three days following surgery. Mice were euthanized four weeks after injection unless otherwise stated.

Sortase Expression and purification. Sortase A pentamutant (P94R/D160N/D165A/K190E/K196T) (plasmid eSrtA in pET29 was a gift from Dr. David Liu (Addgene plasmid #75144), http://n2t.net/addgene:75144)) expression and purification was conducted as previously described [5]. In brief, pET29 transformed BL21 *E.Coli* glycerol stocks grown over night at 37°C in 5 mL of LB media supplemented with 30 μg/mL kanamycin was transferred to 1 L of LB supplemented with 30 μg/mL kanamycin and cultured for 3-5 hours at 37°C until an OD of 0.4-0.6. Protein expression was induced by adding 1 mM IPTG overnight at RT, and cells were harvested by centrifugation. Cells were washed in lysis buffer (50 mM Tris-HCl 150 mM NaCl, 10% glycerol, 10 mM imidazole, 1 mg/mL lysozyme, 0.25% β-mercaptoethanol, EDTA-free protease inhibitor cocktail set III (Calbiochem)) and sonicated. Lysate was then centrifuged and proteins purified from the clarified supernatant using Ni-NTA agarose columns. Purity of elution fractions was ensured using SDS-Page and clean fractions were consolidated and dialyzed against 50 mM Tris-HCl, 150 mM NaCl and 10% v/v glycerol (pH 7.5) with subsequent concentration using Ultra-15 centrifugal filter units (Amicon) and sterile filtration using a 0.1 μm filter. Final aliquots of 500 uL typically had a concentration of 1 mg/mL and were flash frozen for long term storage at -80°C. Enzyme concentration was calculated using the extinction coefficient of 17420 $M^{-1}cm^{-1}$ after measuring the A_{280}.

Peptide incorporation analysis. 8-arm PEG-VS was functionalized with adhesion peptides using Michael-type reaction chemistry inside a 1 mL syringe (BD) modified by removal of the tip as described previously. 1.5 mg of a 8-arm 20kDa-PEG-VS macromer (2.4 mM PEG-VS (19.7 mM -VS) at 98% substitution) with the corresponding thiol-containing (-SH) adhesion and/or BM-binding peptides, in 1 x PBS/1 M HEPES (pH 7.8, gibco) buffer. After functionalization for 30 minutes at 37°C, the amount of unbound thiols (quantification of non-incorporated) peptide was quantified using the Ellman's reagent (Thermo) according to the manufacturer's instructions. The reported theoretical efficiencies (η) are based on the ratio of measured residual thiol and the expected amount of thiolated peptide added to the gels (2.5 mM), where η = 100* (2.5-[residual thiol])/2.5).

PEG gel rheology. 25 μL volume 8-arm PEG-VS hydrogels were formed as previously described with the indicated functional peptide and crosslinker concentrations in 1 mL syringes (BD 309659) with the tip cut off at the 0.1 mL mark. Hydrogels were allowed to polymerize for 30 minutes at 37°C before transfer to wells containing 1x PBS. Gels were allowed to swell at room temperature overnight before rheological testing. Elastic modulus was measured as previously described, with minor modifications [6,7]. Briefly, frequency sweeps were performed between 0.1 and 1 Hz at a fixed strain rate of 5% and a gap height of 1 mm on an MCR702

rheometer with an 8 mm sandblasted plate geometry and base (AntonPaar). Measurements were performed at room temperature, and 100 μL 1x PBS was added to the base around the gel to prevent drying during measurements. Storage modulus (G') was averaged over the measured regime and used to calculate the reported elastic moduli (E), assuming E = 2G'(1+v) and v is taken as 0.5.

Immunocytochemistry of human tumour samples and histology. Tumour samples were embedded in O.C.T medium (TissueTek) and snap frozen in a dry ice / isopentane (Fisher Scientific, 10407010) mix for 5-10 minutes. Following preparing 7 μm thick sections samples were fixed in PFA for 15 minutes at RT and washed in PBS. For Itga3 and Itgb4 staining, tissues were fixed in 1:1 Acetone/Methanol for 15 minutes at 4°C. Blocking was carried out with 3% H_2O_2 in PBSt for 10 minutes at RT followed by blocking with 10% casein for 20 minutes at RT. Tissues were then incubated for 1 hour at RT with primary rabbit anti-Itga3 (1:1000, Merck Millipore, AB1920), rat anti-Itga6 (1:1000, Merck Millipore, Clone NKI-GoH3), rabbit anti-Itga2 (1:2000, Merck Millipore, AB1936), mouse anti-Itgav (1:3000 abcam, ab16821), rat anti-Itgb1 (1:3000, BD Biosciences, 550531, Clone 9EG7), mouse anti-Itgb4 (1:250, Merck Millipore, MAB2059, Clone ASC-8), mouse anti-Lama5 (1:1000, Merck Millipore, MAB1924, Clone 4C7) and mouse anti-PanCK (1:5000, Sigma Aldrich, C2931) antibodies diluted in TBST. Tissues were then extensively washed and incubated with HRP conjugated goat anti-Rabbit (RTU, Dako, K4003), HRP conjugated goat anti-Rat (RTU, Vector Laboratories, MP-7444) secondary antibodies or EnVision HRP-conjugated anti-mouse polymer (Dako, K4001) for 30 minutes at RT. Liquid DAB+ (Dako, K3468) chromogen was applied for 5 minutes at RT followed by counterstaining in Gills I haematoxylin (Thermo Fisher, 72411) for 30 seconds. Following dehydration and coverslipping in pertex mounting medium, slides were imaged using the Leica SCN400 instrument. Hematoxylin and eosin (Shandon Gill II haematoxylin, Thermo Fisher, 6765008; Shandon Eosin Y, Thermo Fisher, 6766008) staining was conducted according to the manufacturer's instructions. For murine and clinical studies, the disease stage was scored by a pathologist (LF) blinded to the study and/or genotype of the animals or any experimental data.

Immunohistochemistry of murine tumour samples. Tumours were harvested and fixed in formalin for 24hours at RT followed by processing and embedding in paraffin. Sections were cut at 4 μm thickness. The Leica Bond RX automated IHC stainer was used to perform F4/80 staining. Primary rabbit anti-F4/80 (1:100, Cell Signalling Technology, 70076) was applied for 15 minutes at RT. The Bond polymer refine detection kit (Leica microsystems, DS9800) contains all reagents for endogenous blocking, detection, visualisation and counterstain. The

kit includes a mouse secondary option, which was omitted for staining. Non-specific antibody binding was blocked with 10% casein for 30 minutes at RT. Manual IHC was performed for mouse anti-αSMA (1:10000, Sigma Aldrich, A5228) using the ARK (Animal Research Kit)/HRP (Dako, K3954) to prevent non-specific mouse-on-mouse staining. Slides underwent deparaffinization, rehydration and heat induced antigen retrieval for 15 minutes in a pressure vessel (BioCare Medical) in Dako TRS buffer pH 6.0 (Dako, S2369) at 110°C. Staining followed manufacturer's guidelines for the ARK kit. Biotinylated primary antibody αSMA was incubated for 30 minutes at RT, followed by streptavidin conjugated HRP (15 minutes) and visualization by DAB. Samples were counterstained with Gills I haematoxylin for 30 seconds before dehydration and coverslipping in Pertex mounting medium.

Two photon second harmonic generation imaging. Second-harmonic generation (SHG) imaging was carried out on a Leica SP8 TCS inverted confocal microscope with a HCX IRAPO L 25x (numerical aperture (NA) 0.95) water-immersion objective (Leica) using a Leica HyD-RLD, a non-descanned detector with a SHG440/CFP filter cube (Leica). Samples were exposed to 880nm pulsed infrared laser line from a Chameleon Vision II tuneable laser source, 690-1080nm (Coherent) and applying a 25x/0.95 GPD Curve correction. A broadband dielectric mirror (d = 20.4 mm, λ = 400 – 750 nm, Thorlabs) was placed on the sample to enhance SHG signal. Fresh tumour tissue samples were embedded in O.C.T. medium, sectioned and cut at 14 μm sections and re-hydrated in ultrapure water 5 minutes prior to image acquisition. Images of representative stromal sites were captured with a resolution of 0.454 μm/pixel as a z-stack over the entire thickness of the sample with a z-step size of 1 μm. Individual planes were then combined to generate a maximum-intensity projection (MPI) of the field of view and converted to 8-bit. Collagen area was quantified by applying a common threshold (45 – 255) which was kept constant between all samples and measurements were conducted using the Fiji software suite (ref as above) Pseudo-colouring of representative MIPs was performed in Fiji using the inferno look up table (LUT). Collagen fibres were quantified using the CT-FIRE open-source software package (CT-FIRE V2.0, [8]) on default settings after application of a common threshold for all images (75 - 255) to remove background noise.

Cell-derived matrix isolation. CDM-generation was conducted as previously described [9]. In brief, KPC-1 or HPDE cells were passaged and 500,000 single cells were seeded in a 10 cm dish (Corning) and grown for seven days to allow for matrix secretion. Following washing of the cells gently with warm PBS, cells were then lysed in pre-warmed extraction buffer (0.5% (v/v) Triton X-100 (Sigma-Aldrich); 20 mM NH_4OH (Sigma-Aldrich) in PBS) and plates were incubated 5 minutes at room temperature. Cell bodies and cytosolic proteins were then

obtained in liquid phase by careful aspiration and 5 mL of PBS^{++} were added to the dish and matrix-lysate was collected and spun at 3000 x g for 5 minutes, RT. The supernatant containing soluble non-ECM proteins, was then carefully removed, and the pellet was resuspended in 5 mL of PBS^{++} supplemented with 25 U/mL Benzonase (Sigma-Aldrich) and samples were incubated for 10 minutes at 37ºC to digest genomic DNA. Samples were spun at 3000 x g for 5 minutes and the remaining pellet was washed twice with 5 mL PBS^{++}. For western blot, pellets were resuspended in 200 μL of 6x Laemmli buffer supplemented with 0.6 M DTT. For LC-MS/MS, samples were resuspended in 50 μL 8M urea (Sigma Aldrich) in 100 mM Ammonium Bicarbonate (ABC, Sigma Aldrich) supplemented with 10 mM DTT (Sigma Aldrich) and subjected to in-solution digestion.

In-solution digestion of CDM proteins and LC-MS/MS. CDM samples in 8M urea, 100 mM ABC were reduced for 2 h at 37ºC by adding 10 mM DTT (Sigma Aldrich) under continuous agitation at 1400 rpm using a Thermomix Comfort (Thermo Fisher Scientific) following alkylation by adding iodoacetamide (IAA, Sigma Aldrich) at 25 mM final concentration and incubation for 30 min in the dark. Subsequently, samples were de-glycosylated by first diluting the urea to 2 M using a 100 mM ABC solution (pH 8) and adding 1kU of PNGaseF (New England BiolabsL). Deglycosylation was conducted for 2 h at 37ºC under continuous agitation (1400 rpm) and proteins were subsequently digested using LysC (Wako) and Trypsin (Promega; Cat.-No.: V5111). For digestion, 1 μg of endopeptidase LysC in 100 mM ABC were added and proteins were incubated for 2 h at 37ºC under continuous agitation (1400 rpm). Thereafter, 3 μg of Trypsin (0.5 μg/μL) were added, and samples were incubated overnight at 37ºC followed by the addition of another 1.5 μg of Trypsin (0.5 μg/μL) the next day with an additional incubation for 2h further at 37ºC in 200 μL total volume. Peptides were then acidified using 50% TFA in HPLC-grade water (Rathburns Chemicals), spun at 16,000 g for 5 min, RT and dried down using a vacuum concentrator (Thermo; Savant SC250EXP Speedvac Concentrator)) at 60ºC for about 2 hours. Peptides were then desalted and cleaned-up using HLB Cartridges (Oasis HLB Cartridge; Waters; Cat.-No.: WAT094225) on SPE vacuum manifold according to the manufactures' instructions. In brief, samples were bound to the cartridge in 0.1% FA in HPLC-grade water (Thermo), washed and eluted in 35% Acetonitrile (Thermo) in HPLC-grade water. Cleaned peptides were then dried down using a vacuum concentrator and resuspended in 50 μL 1% FA, 2% Acetonitrile and 6 μL of a 1:10 dilution were injected. Samples were analysed using an Ultimate 3000 RSLCnano system (Thermo Scientific) coupled to an LTQ OrbiTrap Velos Pro (Thermo Scientific) operating in positive ion mode and used in data-dependent acquisition mode. Peptides were trapped on an Acclaim

PepMap 100 (C18, 100 μM x 2 cm) and then separated on an Easy-Spray PepMap RSLC C18 column (75 μM x 50 cm) (Thermo Scientific) at 300 nL/min using following gradient profile (minutes:%B); 0:2, 6:5, 130:35, 132:98, 152:98, 153:2, 170:2. The buffers used were: buffer A: water + 0.1% formic acid and buffer B: 80% acetonitrile + 0.08% formic acid (v/v). The eluent was directed into an Easy-Spray source (Thermo Scientific) with temperature set at 50˚C and a source voltage of 1.9 kV. Data was acquired on OrbiTrap Velos Pro with precursor scan ranged from 335 to 1800 m/z at 60,000 resolution and automatic gain control (AGC) target of 3e6. The isolation window was set to 2 Th. The top 15 most intense multiply charged precursors were selected for Collision-induced dissociation (CID) with Normalized coll. Energy set at 35.0% using wide band activation mode for MS2. For accurate mass measurement, the "lock mass" function (lock mass = 445.120024 Da) was enabled for MS scan modes.

The resulting data were searched against the SwissProt database with species set to Mus musculus (PCC CDM) or Homo sapiens (HPDE CDM) on an in house Mascot server (Matrix Science; 2016) in Proteome Discoverer (Thermo Fisher Scientific, V. 2.1). Search parameters included peptide modifications for carbamidomethylation (C) as static modification and oxidation (M, P and K) as well as deamination (N, Q) as dynamic modification. A decoy database search was performed to determine the peptide FDR with the Percolator module. A 1% peptide FDR threshold was applied, and peptides were filtered for high peptide confidence, minimum peptide length of 6, and finally peptides without protein reference were removed. Protein grouping was performed by applying strict parsimony principles. All proteins that exhibited a confidence of less than ‘high’ and with less than two uniquely identified peptides were excluded from further analysis.

Immunoblotting. CDM pellets were resuspended in 200 μL of 6x Laemmli buffer (47% v/v Glycerol (Sigma-Aldrich); 0.4 mM SDS (Sigma-Aldrich); 0.9 mM Bromphenol blue (Sigma-Aldrich); 60 mM Tris pH = 6.8 (Sigma-Aldrich) supplemented with 0.6 M DTT (Sigma-Aldrich) and electrophoretically separated by their molecular weight in 4 – 15% precast TGX polyacrylamide gradient gels (4-15% Mini-PROTEAN TGX gels; Bio-Rad) at 100 volts for about 45 – 60 minutes in 1x Tris/Glycerine/SDS (TGS: 20 mM Tris; 192 mM glycine; 0.1% v/v SDS; pH = 8.3; Bio-Rad) running buffer at room temperature. 20 μL of the enriched CDM material or whole cell lyse were run on the polyacrylamide gel. For whole cell lysate generation, cells were lysed in modified RIPA buffer followed by centrifugation to clear the lysate, sonication and benzonase treatment. A protein molecular size ladder (Chameleon Dup Prestained Protein Ladder; Licor) was used to distinguish protein bands and separated proteins were blotted onto a nitrocellulose membrane (Amersham Protran 0.2 NC; GE

Healthcare) for 90 minutes in 1 x transfer buffer (20 mM Tris; 192 mM glycine; 0.1% v/v SDS; pH = 8.3; 20% v/v Methanol (Thermo Fisher Scientific) at 90 volts with subsequent evaluation of transfer efficiency by visualizing with Ponceau S Staining (Sigma Aldrich; 0,1% Ponceau S (w/v) in 5% acetic acid). Blockage of non-specific binding was performed by incubation in blocking solution (Roti-Block; Roth) for 60 minutes at room temperature following probing with a specific primary antibody: mouse anti-Lama5 (1:1000, Mybiosource MBS820658) or rabbit anti-Gapdh (1:5000, abcam, ab181602) diluted in blocking solution overnight at 4ºC. Following extensive washing in TBS-T (TBS supplemented with 0.1% w/w Tween-20 (Sigma Aldrich) for 15 minutes each were membranes incubated for 1h at RT with species-specific DyLight 800 (1:15,000, Cell Signalling Technologies) or 680 (1:15,000, Cell Signalling Technologies) 4xPEG pe-conjugated secondary antibodies diluted in blocking buffer. Following washing were membranes developed using the Odyssey CIX (LiCor) imaging system according to the manufacturer's instructions. Images were analysed using the Image Studio Lite software suit (V 5.2; Licor).

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Supplementary Tables:

Supplementary Table 1: Primer sequence and validation parameters.

Gene	Primer	Sequence (5'->3')	Length	Product Length	Efficiency	R^2
Ctgf	mCcn_F	AAGGGACACGAACTCATTAGAC	22	121	86.753	0.98
	mCcn_R	TAGTTCCTCCCACGGTAGTT	20			
Actinb	mActin_F	CAGCCTTCCTTCTTGGGTATG	21	99	102.406	0.999
	mActin_R	GGCATAGAGGTCTTTACGGATG	22			

Supplementary Table 2: Antibodies used for Mass Cytometry experiments in this study.

Target	Tag	Supplier	Clone
CD68 (internal)	115In	Custom*	FA-11
CD68 (extracellular)	115In	Custom*	FA-11
CC3	142 Nd	Fluidigm	D3E9
CD11b	143Nd	Fluidigm	M1/70
ITGA2	147 Sm	Custom*	HMa2
Podoplanin	149Sm	Custom*	8.1.1
Pan Cytokeratin	152 Sm	Custom*	C11
Vimentin	154 Sm	Fluidigm	D21H3
Desmin	158Gd	Custom*	Y66
αSMA	159 Tb	Custom*	1A4
Ki67	162 Dy	Fluidigm	SolA15
ITGA6	164Dy	Custom*	GoH3
EpCam	166Er	Fluidigm	G8.8
CD206	169Tm	Fluidigm	Co68C2
ITGB1	172Yb	Custom*	HMB1-1
GFP	174 Yb	Custom*	FM264G
CD45	175Lu	Fluidigm	30-F11
pRb	198 Pt	Custom*	J112-906
MHC2	209 Bi	Fluidigm	M5/114.15.2

*Custom antibodies were conjugated using the Maxpar X8 antibody labelling kit (Fluidigm) as per manufacturer's instructions.