Matricytosis is a phenotype-specific ECM internalization program dysregulated across macrophages and fibroblasts in pulmonary fibrosis

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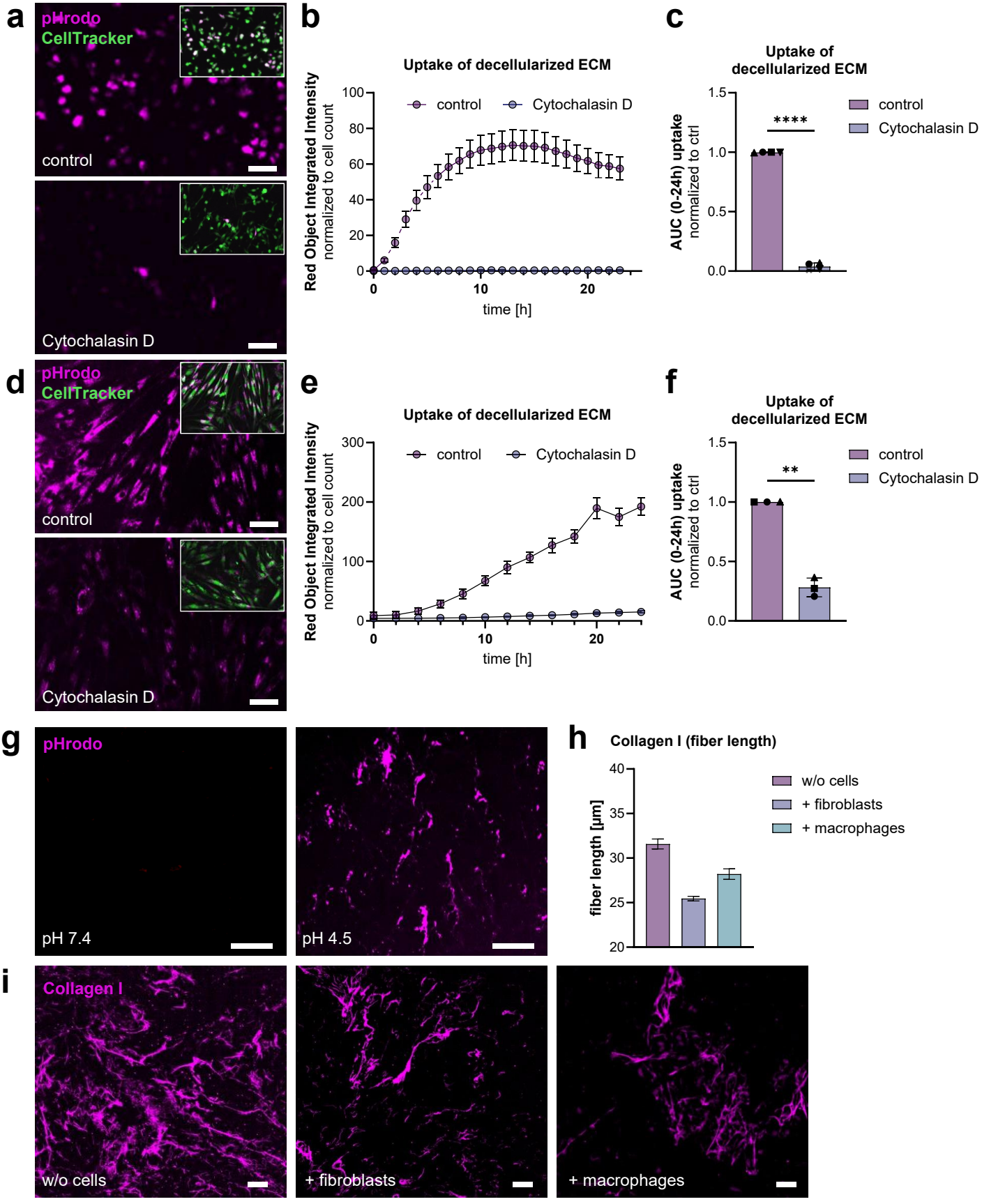
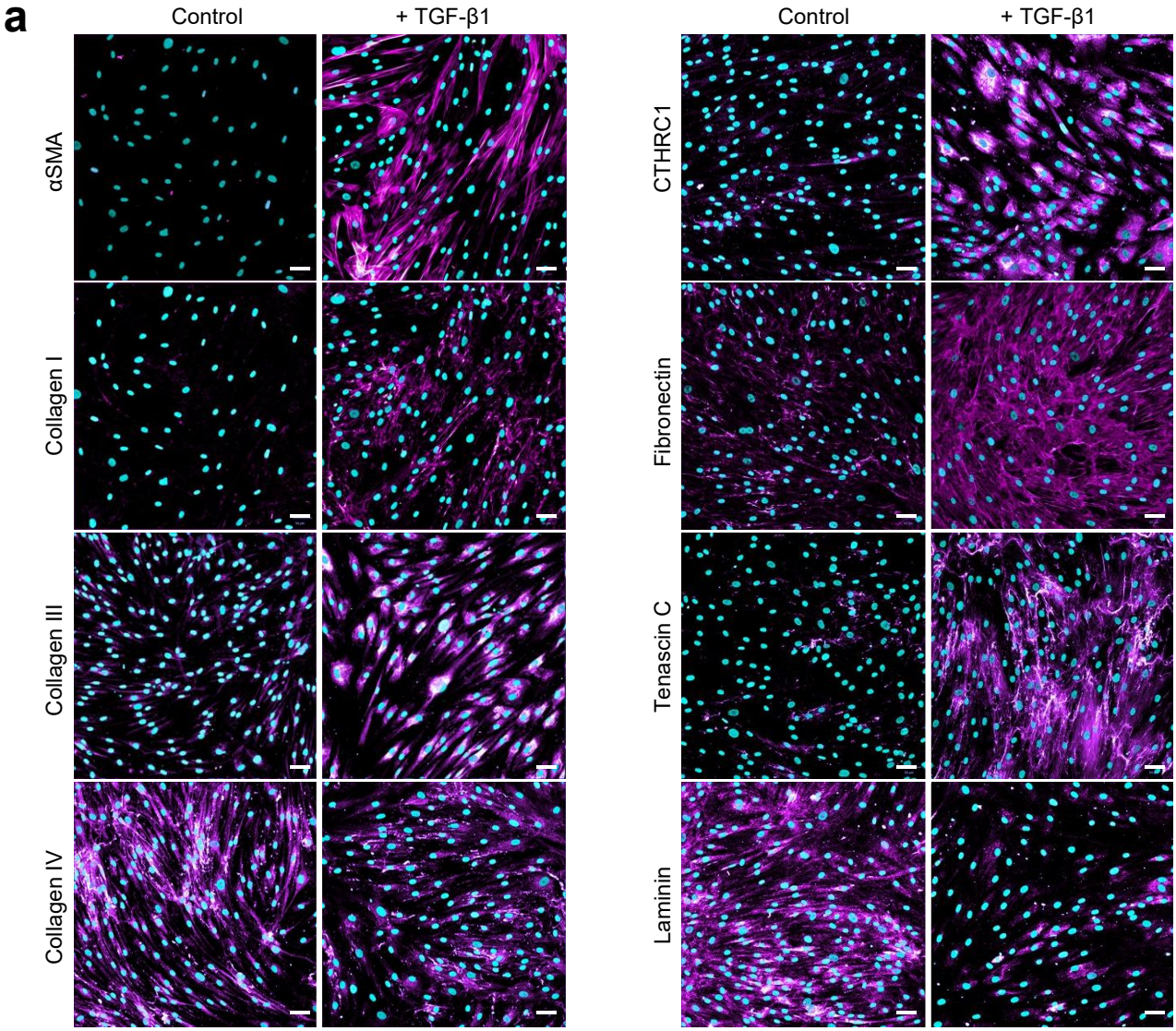
Supplementary Information

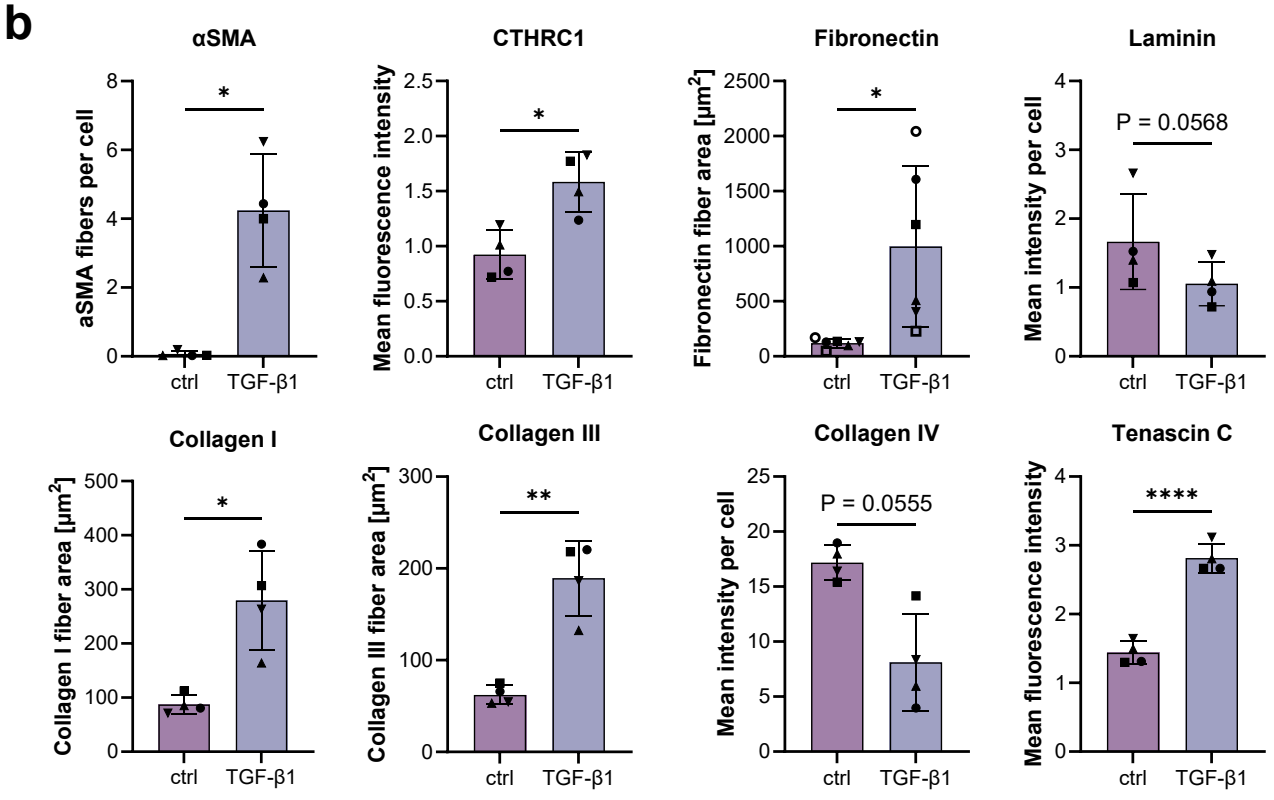
Fig S1


a
pHrodo
CellTracker
control
Cytochalasin D
b
Uptake of decellularized ECM
Red Object Integrated Intensity
normalized to cell count
control
Cytochalasin D
time [h]
c
Uptake of
decellularized ECM
AUC (0-24h) uptake
normalized to ctrl
****
control
Cytochalasin D
d
pHrodo
CellTracker
control
Cytochalasin D
e
Uptake of decellularized ECM
Red Object Integrated Intensity
normalized to cell count
control
Cytochalasin D
time [h]
f
Uptake of
decellularized ECM
AUC (0-24h) uptake
normalized to ctrl
**
control
Cytochalasin D
g
pHrodo
pH 7.4
pH 4.5
h
Collagen I (fiber length)
fiber length [µm]
w/o cells
+ fibroblasts
+ macrophages
i
Collagen I
w/o cells
+ fibroblasts
+ macrophages

Supplementary Figure S1. Actin orchestrates matrix uptake, while pH conditions regulate fluorescence activation across cellular and environmental settings. (a) Live-cell imaging of macrophages internalizing pHrodo™ labeled dECM under control conditions vs. Cytochalasin D treatment. Engulfed matrix (pHrodo, magenta) co-localizes with CellTracker™ labeled cells (green; representative images of *n* = 4 independent experiments; scale bar, 50 µm). **(b)** Time-course quantification of integrated fluorescence intensity over 24 h in control and Cytochalasin D-treated macrophages (representative data of *n* = 4 independent experiments). **(c)** Quantification of macrophage uptake kinetics (AUC; *n* = 4 independent experiments). **(d)** Live-cell imaging of TGF-β1-treated fibroblasts internalizing pHrodo™ labeled dECM under control conditions vs. Cytochalasin D treatment. Engulfed matrix (pHrodo, magenta) co-localizes with CellTracker™ labeled cells (green; scale bar, 50 µm). **(e)** Time-course quantification of integrated fluorescence intensity in control and Cytochalasin D-treated fibroblasts over 24 h (representative images of *n* = 3 donors). Fibroblasts were pre-treated with TGF-β1 to enhance assay sensitivity. **(f)** Quantification of fibroblast uptake kinetics (AUC; *n* = 3 donors). **(g)** Fluorescence images of the pH sensitive pHrodo™ probe (magenta) at pH 7.4 and pH 4.5, demonstrating fluorescence increase in acidic environments mimicking the lysosomal compartment (scale bar, 50 µm). **(h, i)** High-resolution imaging of Collagen I (magenta) in dECM and quantification of fiber length [µm] in the absence of cells, or with fibroblasts/ macrophages (*n* = 1). Images were taken with a 20x objective of an Opera Phenix® (scale bar, 50 µm). Data are presented as mean ± s.e.m. (standard error mean; b, e, h) or mean ± s.d. (e, f); **p* <0.05 is considered to be statistically significant (Paired Student‘s t-test).

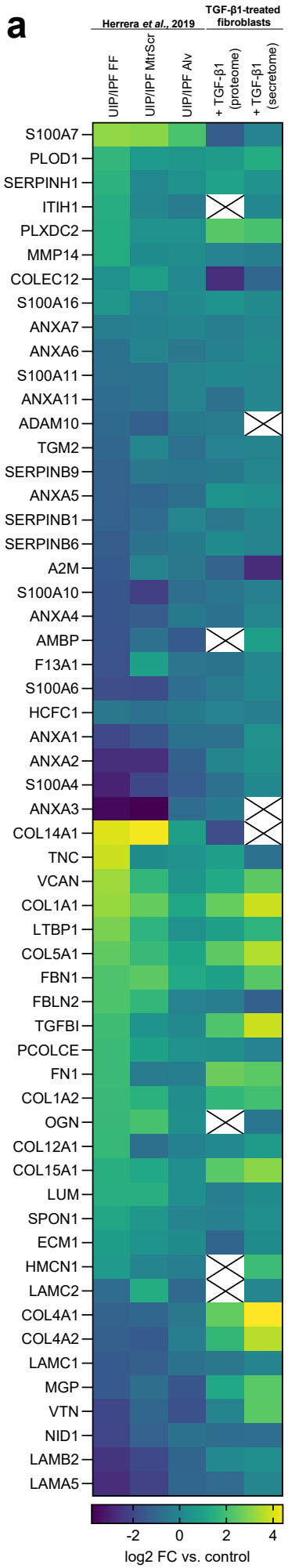
Fig S2


a
Control
+ TGF-β1
αSMA
Collagen I
Collagen III
Collagen IV
CTHRC1
Fibronectin
Tenascin C
Laminin


b
αSMA
aSMA fibers per cell
CTHRC1
Mean fluorescence intensity
Fibronectin
Fibronectin fiber area [µm²]
Laminin
Mean intensity per cell
P = 0.0568
Collagen I
Collagen I fiber area [µm²]
Collagen III
Collagen III fiber area [µm²]
Collagen IV
Mean intensity per cell
P = 0.0555
Tenascin C
Mean fluorescence intensity
ctrl
TGF-β1

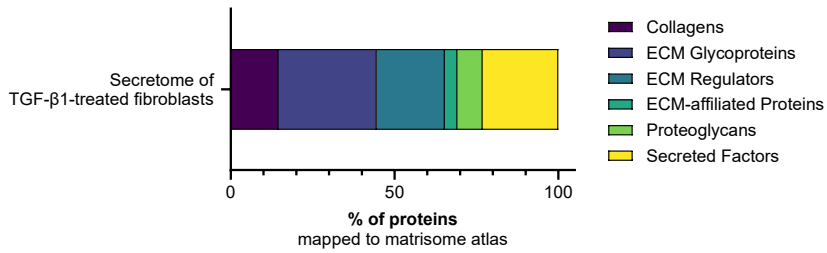
Supplementary Figure S2. Fibrotic stimulation induces heterogeneous patterns of ECM proteins in fibroblasts. (a) Representative high-resolution fluorescence images of fibroblasts stained for cytoskeletal (αSMA), IPF-associated (CTHRC1) and ECM markers (collagen type I, III and IV, fibronectin, tenascin C and laminin; magenta) stimulated with TGF-β1 vs. control (*n* = 4-6 donors). Images were acquired using the 20x objective on an Opera Phenix® (scale bar, 50 µm). (**b**) Quantification of markers corresponding to the imaging panels in (**a**), shown as fiber area/cell [μm^2] or mean fluorescence intensity/cell (*n* = 4-6 donors). Bar graphs show mean ± s.d. (Paired Student‘s t-test).

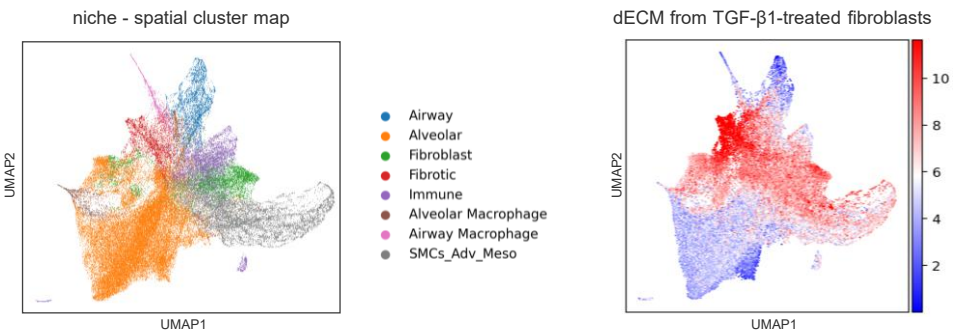
Fig S3

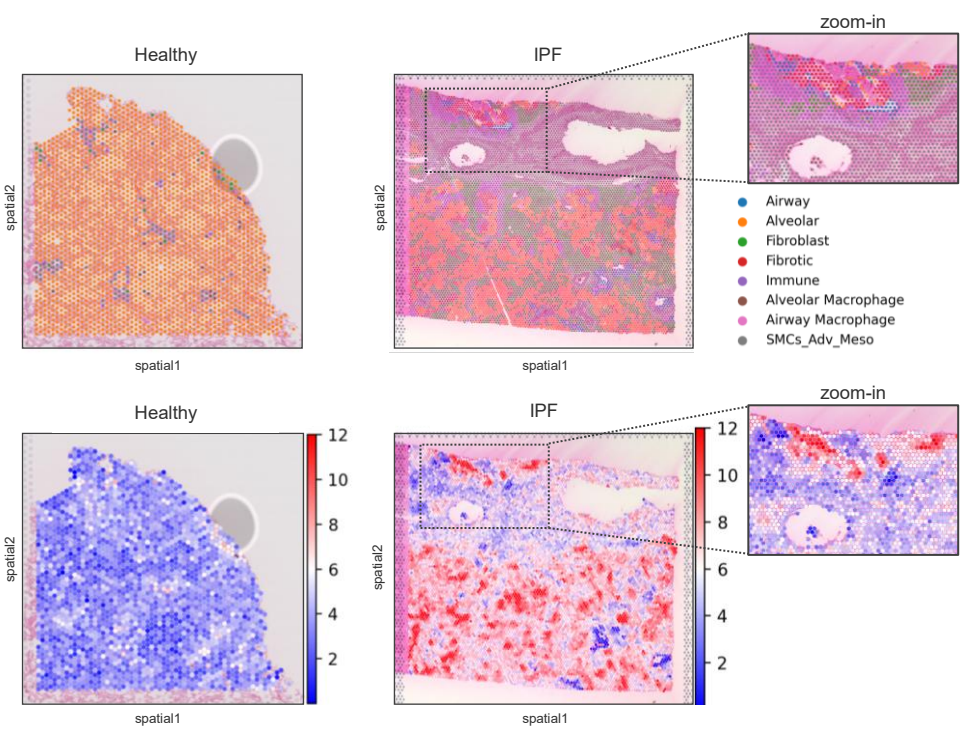


b

Pathway name	Reactome pathway identifier (R-HSA)
Assembly of collagen fibrils and other multimeric structures	R-HSA-2022090
ECM proteoglycans	R-HSA-3000178
Collagen formation	R-HSA-1474290
Collagen degradation	R-HSA-1442490
Extracellular matrix organization	R-HSA-1474244
Collagen biosynthesis and modifying enzymes	R-HSA-1650814
Non-integrin membrane-ECM interactions	R-HSA-3000171
Degradation of the extracellular matrix	R-HSA-1474228
Integrin cell surface interactions	R-HSA-216083
Collagen chain trimerization	R-HSA-8948216

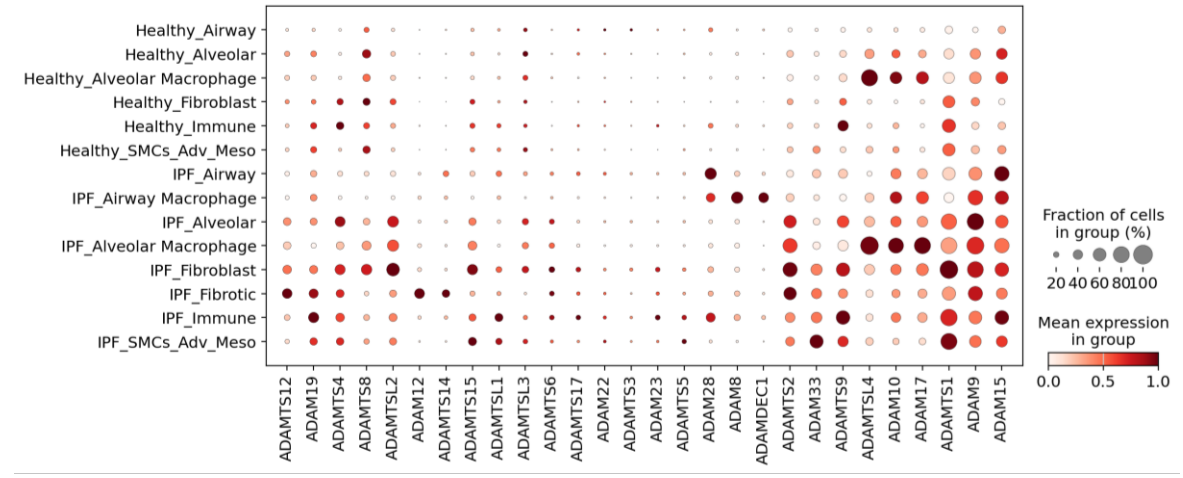


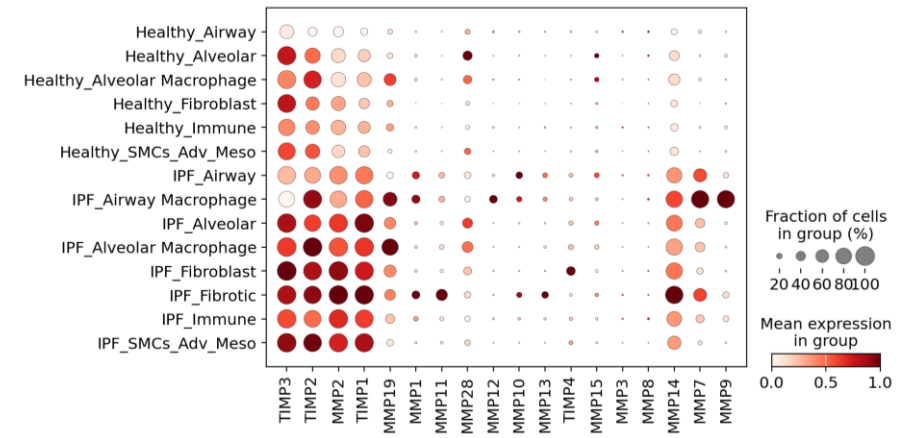


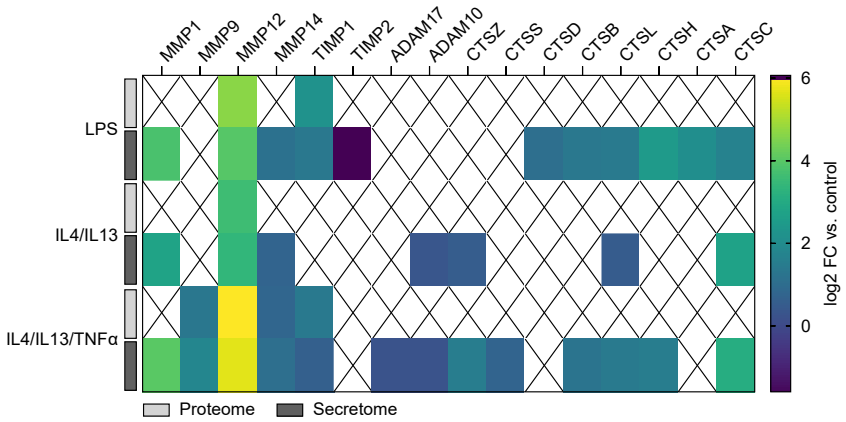


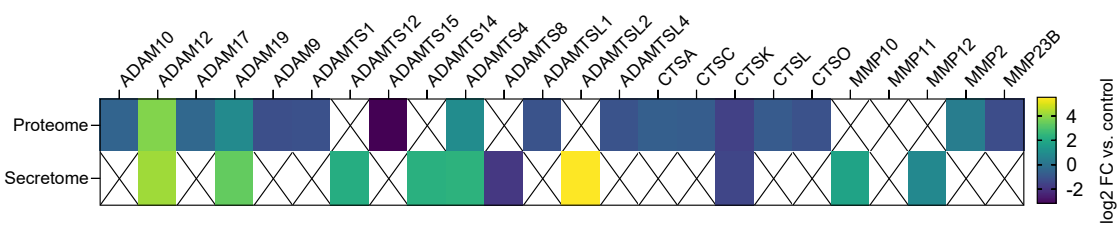
Supplementary Figure S3. Fibrotic stimulation of fibroblasts induces an IPF-like ECM. (a) Heatmap showing expression levels of ECM-associated proteins, comparing proteome and secretome of TGF-β1-treated fibroblasts (significantly deregulated compared to control fibroblasts, *n* = 6 donors) with published data set (Herrera *et al.* 2019[1]). UIP/IPF: Usual Interstitial Pneumonia/ Idiopathic Pulmonary Fibrosis, FF: fibroblast foci, MtrScr: mature scar, Alv: fibrotic alveoli. **(b)** Reactome pathway enrichment analysis of ECM-associated proteins (significantly deregulated secretome of TGF-β1-treated fibroblasts, *n* = 6 donors) with a false discovery rate of 7.66E-15 each. **(c)** Bar chart summarizing the proportional distribution of the significantly deregulated secretome of TGF-β1-treated fibroblasts (*n* = 6 donors) to Naba matrisome[2]. **(d, e)** Proteome signature of dECM from TGF-β1-treated fibroblasts (significantly deregulated compared to control fibroblasts; *n* = 4 for control dECM, *n* = 6 for TGF-β1 dECM) mapped on a spatial transcriptomic atlas from healthy and IPF lungs[3] shown as UMAP (**d**) and on exemplary spatial plots (**e**). SMCs_Adv_Meso: smooth muscle/adventitial/mesothelial niche.

Fig S4


a
Healthy_Airway
Healthy_Alveolar
Healthy_Alveolar Macrophage
Healthy_Fibroblast
Healthy_Immune
Healthy_SMCs_Adv_Meso
IPF_Airway
IPF_Airway Macrophage
IPF_Alveolar
IPF_Alveolar Macrophage
IPF_Fibroblast
IPF_Fibrotic
IPF_Immune
IPF_SMCs_Adv_Meso
ADAMTS12 ADAM19 ADAMTS4 ADAMTS8 ADAMTSL2 ADAM12 ADAMTS14 ADAMTS15 ADAMTSL1 ADAMTSL3 ADAMTS6 ADAMTS17 ADAM22 ADAMTS3 ADAM23 ADAMTS5 ADAM28 ADAM8 ADAMDEC1 ADAMTS2 ADAM33 ADAMTS9 ADAMTSL4 ADAM10 ADAM17 ADAMTS1 ADAM9 ADAM15
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
0.0 0.5 1.0

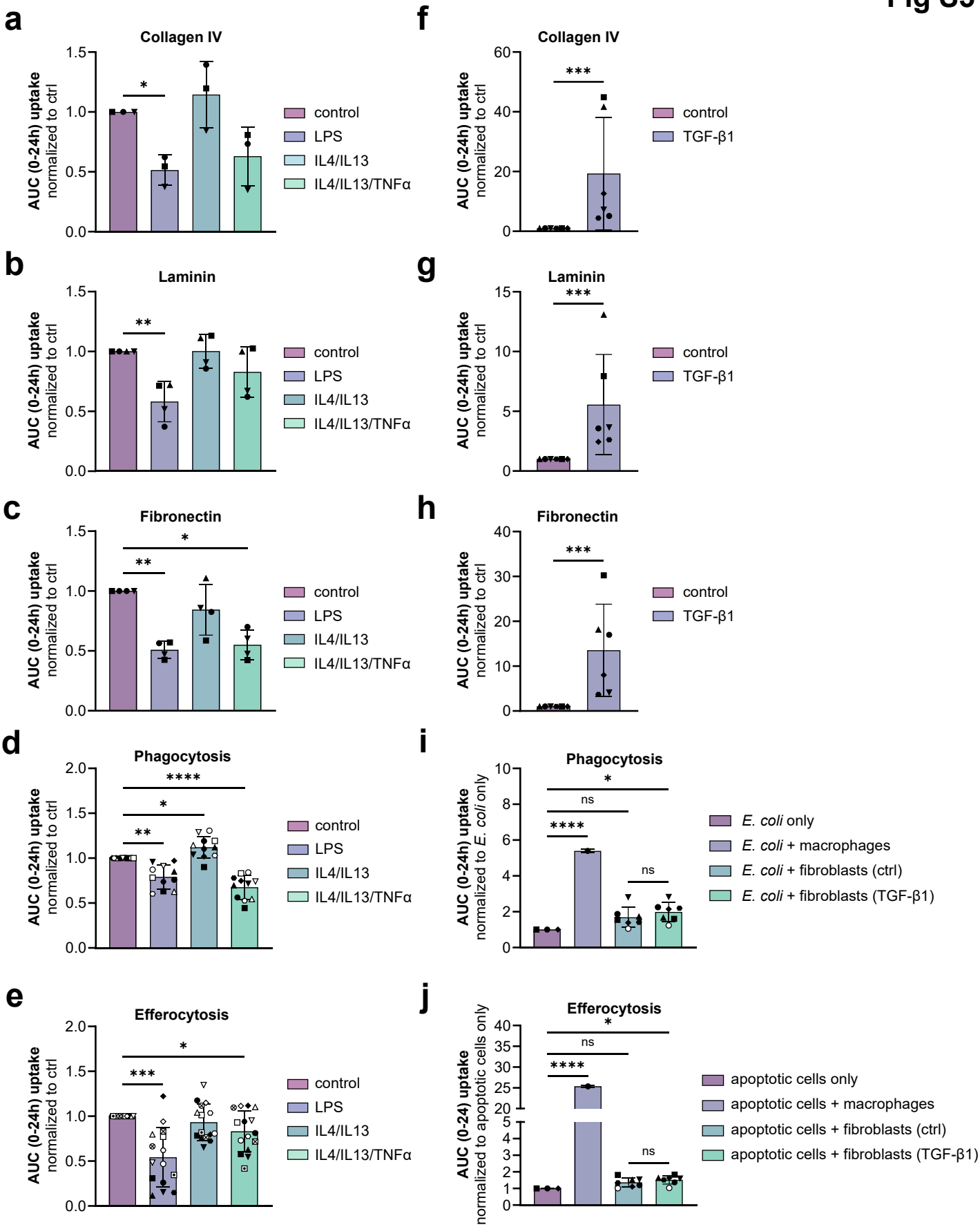

b
Healthy_Airway
Healthy_Alveolar
Healthy_Alveolar Macrophage
Healthy_Fibroblast
Healthy_Immune
Healthy_SMCs_Adv_Meso
IPF_Airway
IPF_Airway Macrophage
IPF_Alveolar
IPF_Alveolar Macrophage
IPF_Fibroblast
IPF_Fibrotic
IPF_Immune
IPF_SMCs_Adv_Meso
TIMP3 TIMP2 MMP2 TIMP1 MMP19 MMP1 MMP11 MMP28 MMP12 MMP10 MMP13 TIMP4 MMP15 MMP3 MMP8 MMP14 MMP7 MMP9
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
0.0 0.5 1.0


c
MMP1 MMP9 MMP12 MMP14 TIMP1 TIMP2 ADAM17 ADAM10 CTSZ CTSS CTSD CTSB CTSL CTSH CTSA CTSC
LPS
IL4/IL13
IL4/IL13/TNFα
log2 FC vs. control
6
4
2
0
Proteome
Secretome


d
ADAM10 ADAM12 ADAM17 ADAM19 ADAM9 ADAMTS1 ADAMTS12 ADAMTS15 ADAMTS14 ADAMTS4 ADAMTS8 ADAMTSL1 ADAMTSL2 ADAMTSL4 CTSA CTSC CTSK CTSL CTSO MMP10 MMP11 MMP12 MMP2 MMP23B
Proteome
Secretome
log2 FC vs. control
4
2
0
-2

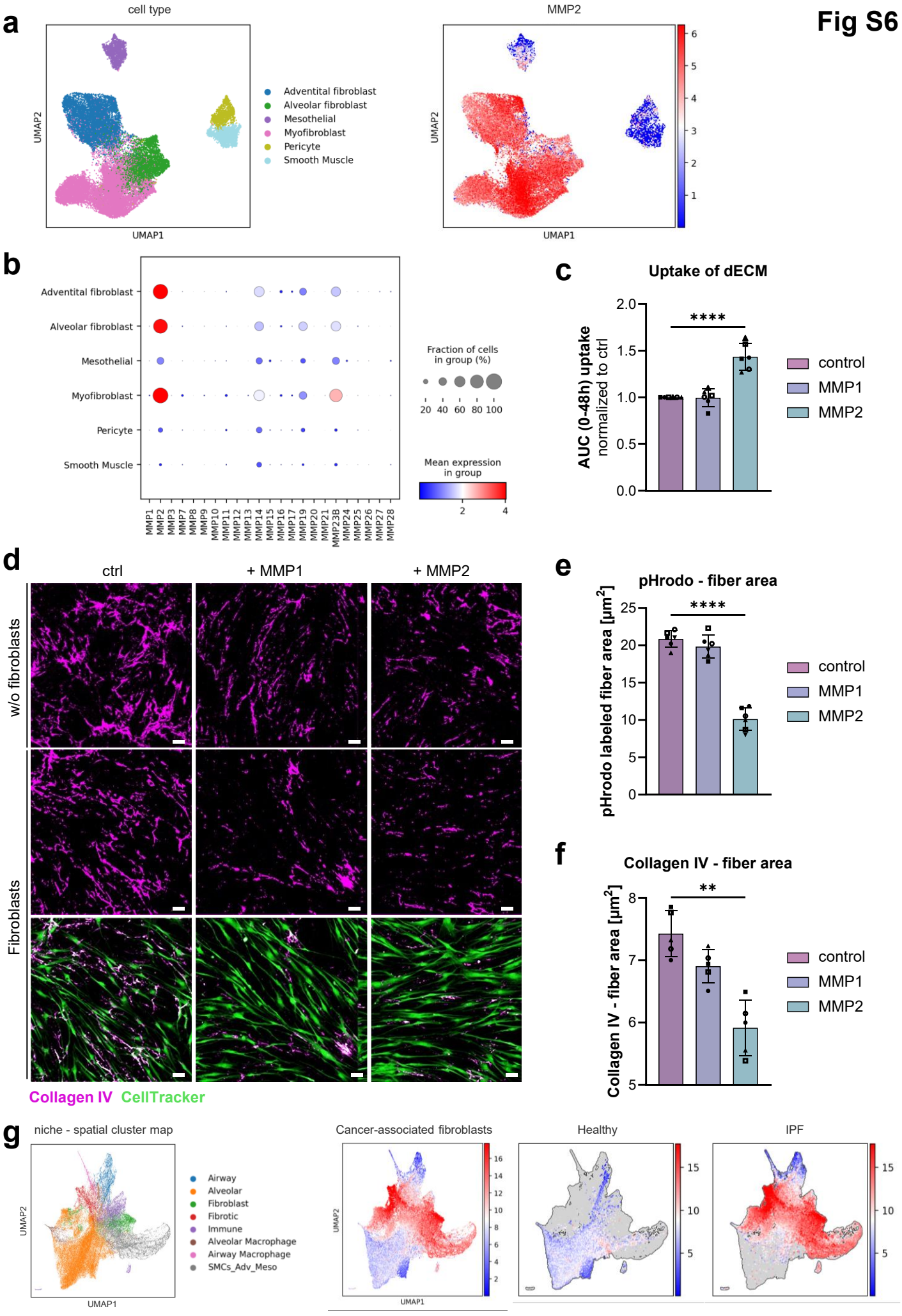
Supplementary Figure S4. Spatial transcriptomics and *in vitro* cell states reveal specific expression of matrix-cleaving enzymes altered in IPF and in disease-associated phenotypes. (a) Dot plot showing expression of *ADAMTS* family genes across major stromal, immune, airway, and mesenchymal niches mapped on a spatial transcriptomic atlas from healthy and IPF lungs[26]. Dot size indicates the fraction of expressing cells (%); colour intensity reflects mean expression levels, highlighting disease- and cell-type-specific patterns. **(b)** Dot plot of *MMP* gene expression across the same cell niches revealing distinct protease signatures associated with fibrotic remodeling. **(c, d)** Heatmaps of significantly deregulated (p <0.05) matrix-cleaving enzymes (ADAMs, MMPs, cathepsins (CTS)) across *in vitro* macrophage (n = 3 independent experiment; **c**) and fibroblast (n = 6 donors; **d**) subsets, measured in proteome and secretome. Colour intensity represents relative expression and crossed-out entries denote proteins not detected or not significantly expressed in the respective populations.

Fig S5

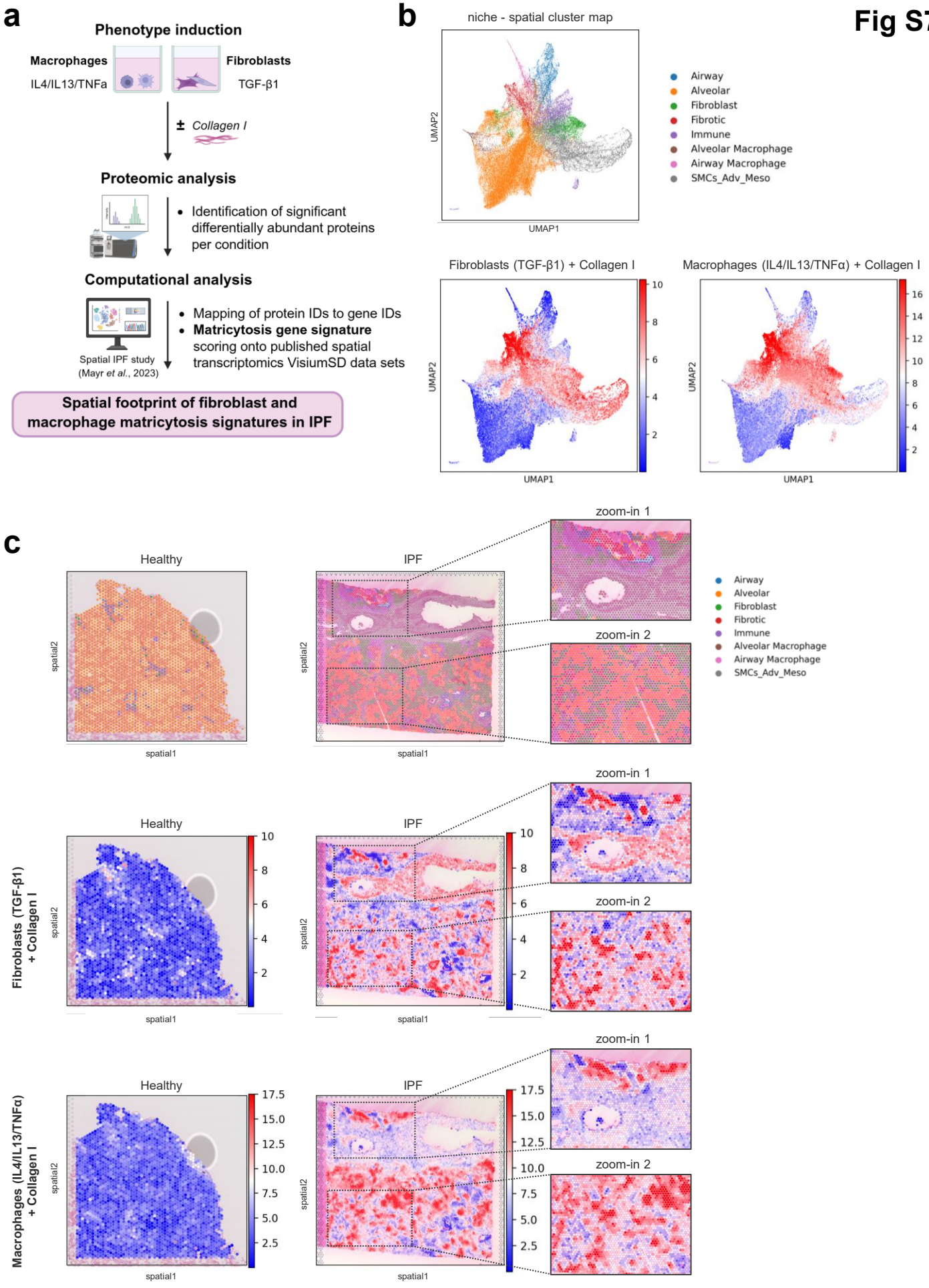

a
Collagen IV
AUC (0-24h) uptake
normalized to ctrl
control
LPS
IL4/IL13
IL4/IL13/TNFα
b
Laminin
c
Fibronectin
d
Phagocytosis
e
Efferocytosis
f
Collagen IV
TGF-β1
g
Laminin
h
Fibronectin
i
Phagocytosis
AUC (0-24h) uptake
normalized to E. coli only
E. coli only
E. coli + macrophages
E. coli + fibroblasts (ctrl)
E. coli + fibroblasts (TGF-β1)
j
Efferocytosis
AUC (0-24) uptake
normalized to apoptotic cells only
apoptotic cells only
apoptotic cells + macrophages
apoptotic cells + fibroblasts (ctrl)
apoptotic cells + fibroblasts (TGF-β1)
ns

Supplementary Figure S5. Distinct ECM-uptake programs differentiate matricytosis from macrophage-specific efferocytotic and phagocytotic function. (a-c) Quantification of uptake kinetics (AUC) using pHrodo™-labeled collagen IV (**a**), laminin (**b**) and fibronectin (**c**) in macrophage subsets measured via IncuCyte® live-cell imaging for 24 h (*n* = 3-4 independent experiments). **(d, e)** Quantification of phagocytosis (**d**) and efferocytosis (**e**) in macrophage subsets measured via IncuCyte® live-cell imaging over 24 h (AUC; *n* = 11-15 independent experiments). **(f-h)** Quantification of uptake kinetics (AUC) using pHrodo™-labeled collagen IV (**f**), laminin (**g**) and fibronectin (**h**) in fibroblasts for 24 h (*n* = 6 donors). Fibroblasts were pre-treated with TGF-β1 to enhance assay sensitivity **(i, j)** Quantification of phagocytosis (**i**) and efferocytosis (**j**) in fibroblasts. Shown is a direct comparison with pHrodo™-labeled *Escherichia coli* (*E. coli*) or apoptotic cells only and macrophage uptake windows after 24 h (AUC, *n* = 3-7 donors). Fibroblasts were pre-treated with TGF-β1 to enhance assay sensitivity. Data are presented as mean ± s.d; **p* <0.05 is considered to be statistically significant. (**a-c**) ANOVA/Tukey's correction, (**d**, **e**) ANOVA/Dunnett's correction, (**f-h**) one-tailed unpaired student's t test, (**i, j**) ANOVA/ Šidák correction.

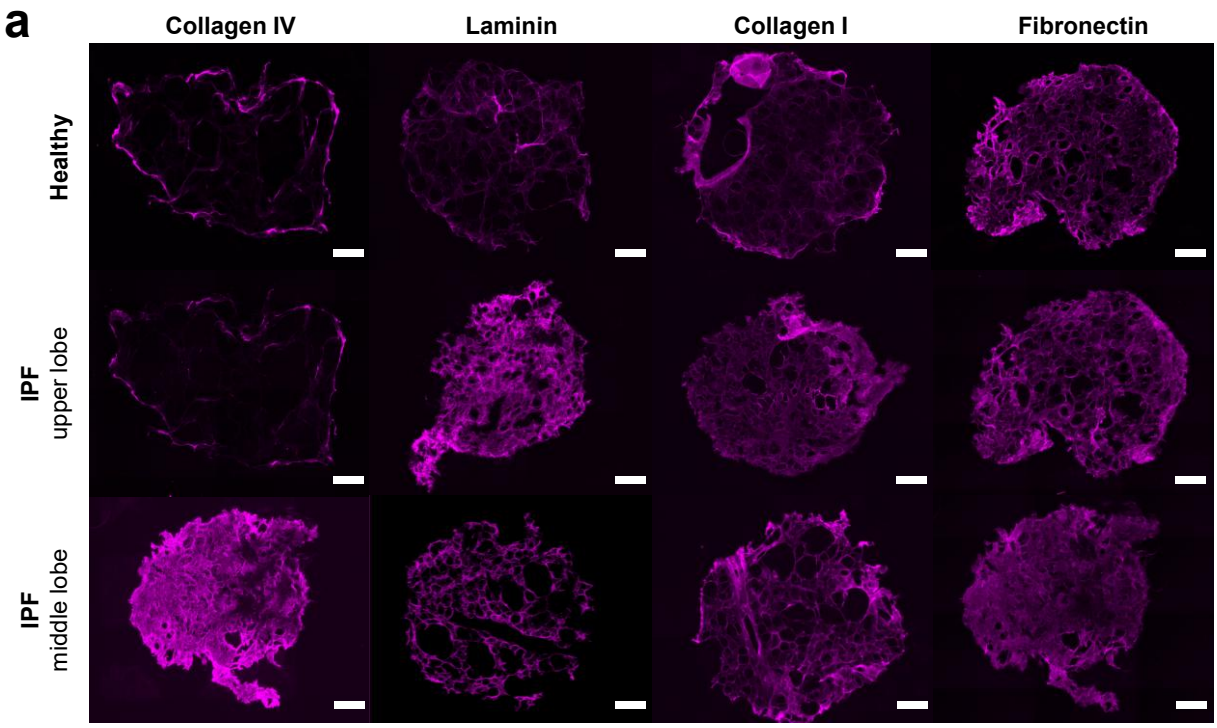
Fig S6

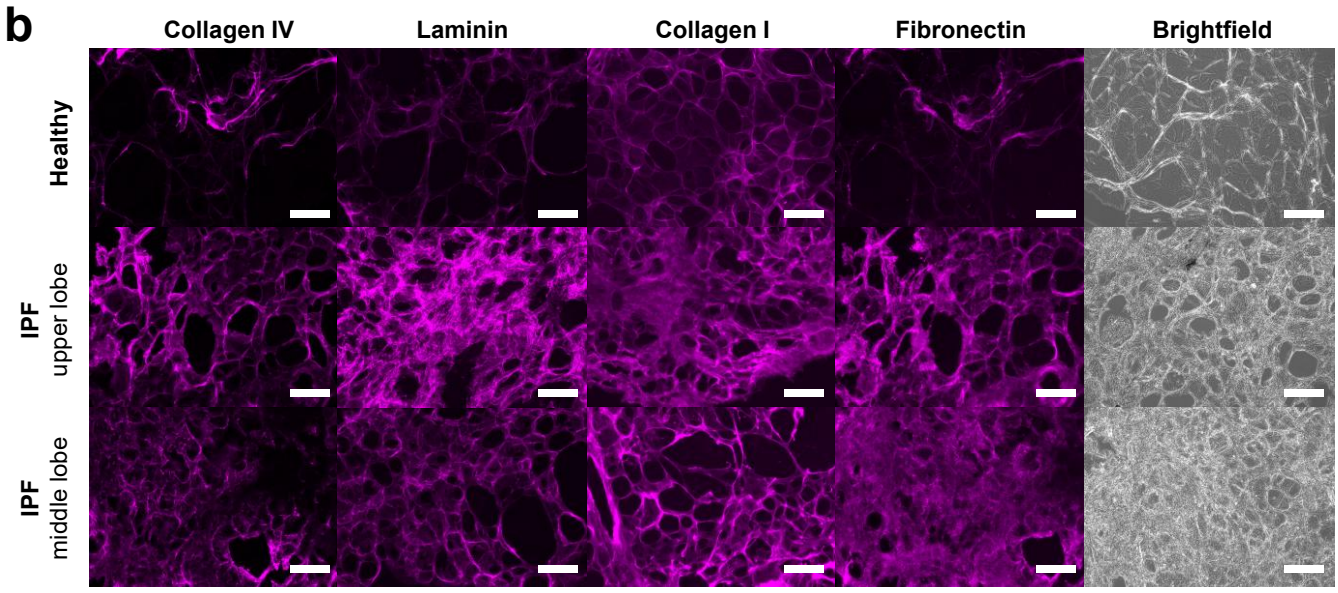

a
cell type
Adventital fibroblast
Alveolar fibroblast
Mesothelial
Myofibroblast
Pericyte
Smooth Muscle
UMAP1
UMAP2
MMP2
b
Fraction of cells in group (%)
Mean expression in group
MMP1 MMP2 MMP3 MMP7 MMP8 MMP9 MMP10 MMP11 MMP12 MMP13 MMP14 MMP15 MMP16 MMP17 MMP19 MMP20 MMP21 MMP23B MMP24 MMP25 MMP26 MMP27 MMP28
c
Uptake of dECM
AUC (0-48h) uptake normalized to ctrl
****
control
MMP1
MMP2
d
ctrl
+ MMP1
+ MMP2
w/o fibroblasts
Fibroblasts
Collagen IV CellTracker
e
pHrodo - fiber area
pHrodo labeled fiber area [µm²]
****
f
Collagen IV - fiber area
Collagen IV - fiber area [µm²]
**
g
niche - spatial cluster map
Airway
Alveolar
Fibroblast
Fibrotic
Immune
Alveolar Macrophage
Airway Macrophage
SMCs_Adv_Meso
Cancer-associated fibroblasts
Healthy
IPF

Supplementary Figure S6. MMP2 supports matricytosis and matrix remodeling *in vitro*. **(a)** UMAP visualization of *MMP2* expression across the mesenchymal compartment in a published PF-ILD atlas, highlighting cell-subtype-specific enrichment[3]. **(b)** Dot plot analysis of *MMP* family gene expression (MMP1-MMP29) across stromal subsets. Dot size indicates the fraction of expressing cells (%); colour denotes mean expression. **(c)** Quantification of dECM uptake (AUC) by fibroblasts with MMP1 or MMP2 pre-incubated dECM vs. control dECM, measured via IncuCyte® live-cell imaging over 48 h (n = 6 donors). **(d)** Representative high-resolution images of Collagen IV stained dECM (magenta) incubated without cells or with fibroblasts (green), and upon MMP1 or MMP2 pre-incubation of dECM, using a 20x objective of an Opera Phenix® (n = 5 donors; scale bar, 50 µm). **(e)** Confocal imaging and analysis of pHrodo™-labeled fiber area [μm^2] of dECM upon pre-incubation with MMP1 or MMP2 (n = 6 donors). **(f)** Confocal imaging and analysis of Collagen IV-labeled fiber area [μm^2] of dECM upon pre-incubation with MMP1 or MMP2 (n = 5 donors). **(g)** Signature of cancer-associated fibroblasts mapped on a spatial transcriptomic atlas from healthy and IPF lungs[3] shown as UMAP. Data are presented as mean ± s.d.; *p <0.05 is considered to be statistically significant (ANOVA/Dunnett's correction). SMCs_Adv_Meso: smooth muscle/adventitial/mesothelial niche.


a
Phenotype induction
Macrophages
IL4/IL13/TNFa
Fibroblasts
TGF-β1
± Collagen I
Proteomic analysis
• Identification of significant differentially abundant proteins per condition
Computational analysis
Spatial IPF study (Mayr et al., 2023)
• Mapping of protein IDs to gene IDs
• Matricytosis gene signature scoring onto published spatial transcriptomics VisiumSD data sets
Spatial footprint of fibroblast and macrophage matricytosis signatures in IPF
b
niche - spatial cluster map
UMAP1
UMAP2
Airway
Alveolar
Fibroblast
Fibrotic
Immune
Alveolar Macrophage
Airway Macrophage
SMCs_Adv_Meso
Fibroblasts (TGF-β1) + Collagen I
Macrophages (IL4/IL13/TNFα) + Collagen I
c
Healthy
IPF
zoom-in 1
zoom-in 2
spatial1
spatial2
Fibroblasts (TGF-β1) + Collagen I
Macrophages (IL4/IL13/TNFα) + Collagen I

Supplementary Figure S7. Spatial transcriptomic analysis reveals mapping of matricytosing cells to fibrotic niches in IPF. (a) Workflow showing generation of samples after matricytosis of Collagen I, proteomic analysis of cell lysates, matricytosis signature scoring based on deregulated proteins per cell type and subsequent analysis including mapping to the corresponding genes and integration to a spatial transcriptomic atlas from healthy and IPF lungs[3]. **(b, c)** Proteome signature of cells after matricytosis of Collagen I (*n* = 1, 4 technical replicates) mapped on a spatial transcriptomic atlas from healthy and IPF lungs[3] shown as UMAP (**b**) and on exemplary spatial plots (**c**). SMCs_Adv_Meso: smooth muscle/adventitial/mesothelial niche.





Supplementary Figure S8. ECM architecture and composition differ between healthy and IPF lung tissue. (a, b) Brightfield and fluorescent microscopy images (collagen I, IV, fibronectin and laminin) showing structural features, fiber organization and density of dPCLS from human healthy and IPF tissue. Shown is the whole tissue slice (**a**; scale bar, 1 mm) or structures with higher magnification (**b**; scale bar, 500 µm; upper and lower lobe lung sections; *n* = 1 donor, respectively).

Supplementary References

1. Herrera, J. A. *et al.* The UIP/IPF fibroblastic focus is a collagen biosynthesis factory embedded in a distinct extracellular matrix. *JCI Insight* **7**, (2022).

2. Naba, A. *et al.* The Matrisome: In Silico Definition and In Vivo Characterization by Proteomics of Normal and Tumor Extracellular Matrices. *Molecular & Cellular Proteomics* **11**, (2012).

3. Mayr, C. H. *et al.* Spatial transcriptomic characterization of pathologic niches in IPF. *Sci Adv* **10**, eadl5473 (2024).