Cell Reports, Volume *29*

Supplemental Information

Modeling Progressive Fibrosis with Pluripotent Stem Cells Identifies an Anti-fibrotic Small Molecule

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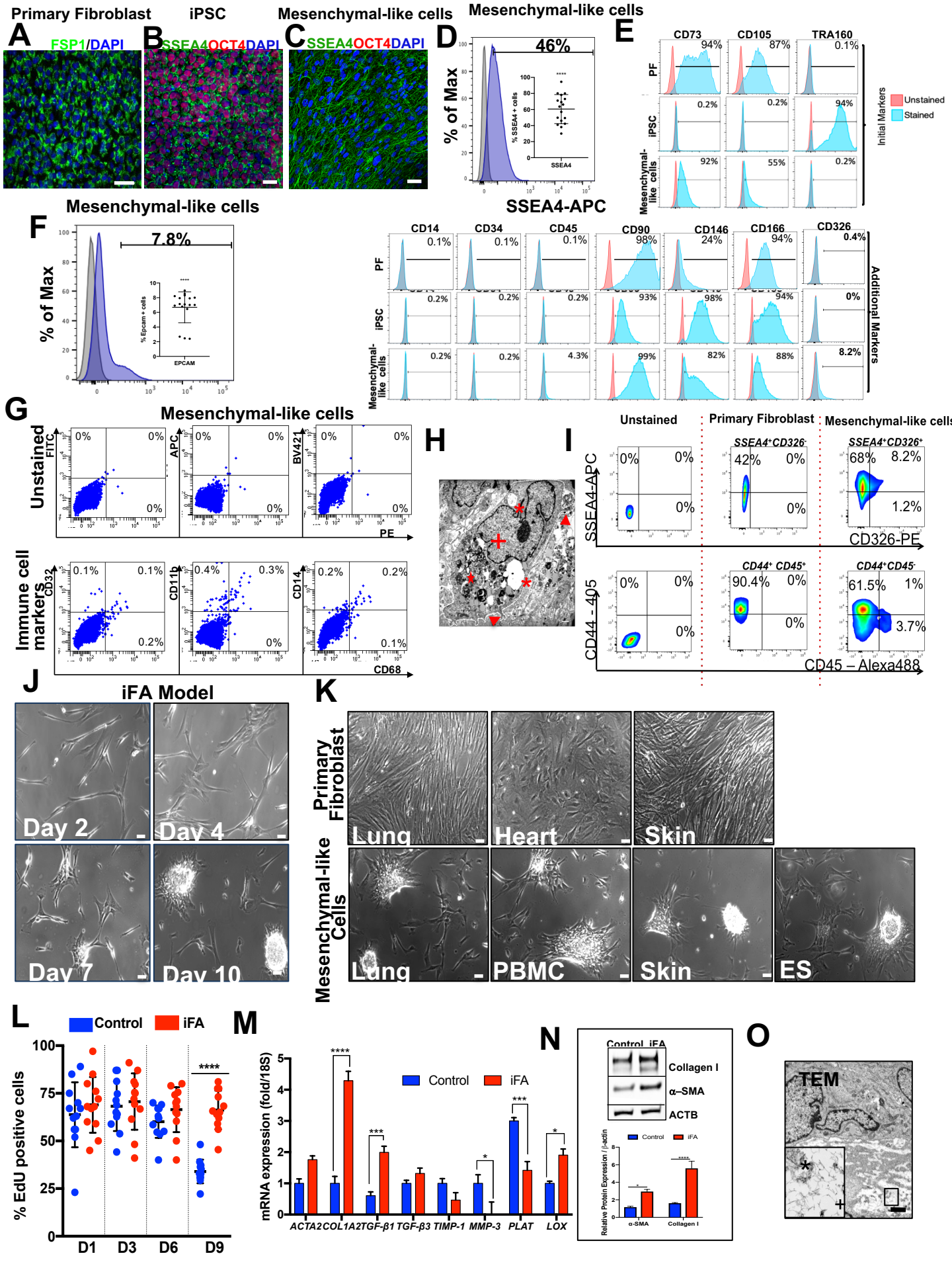
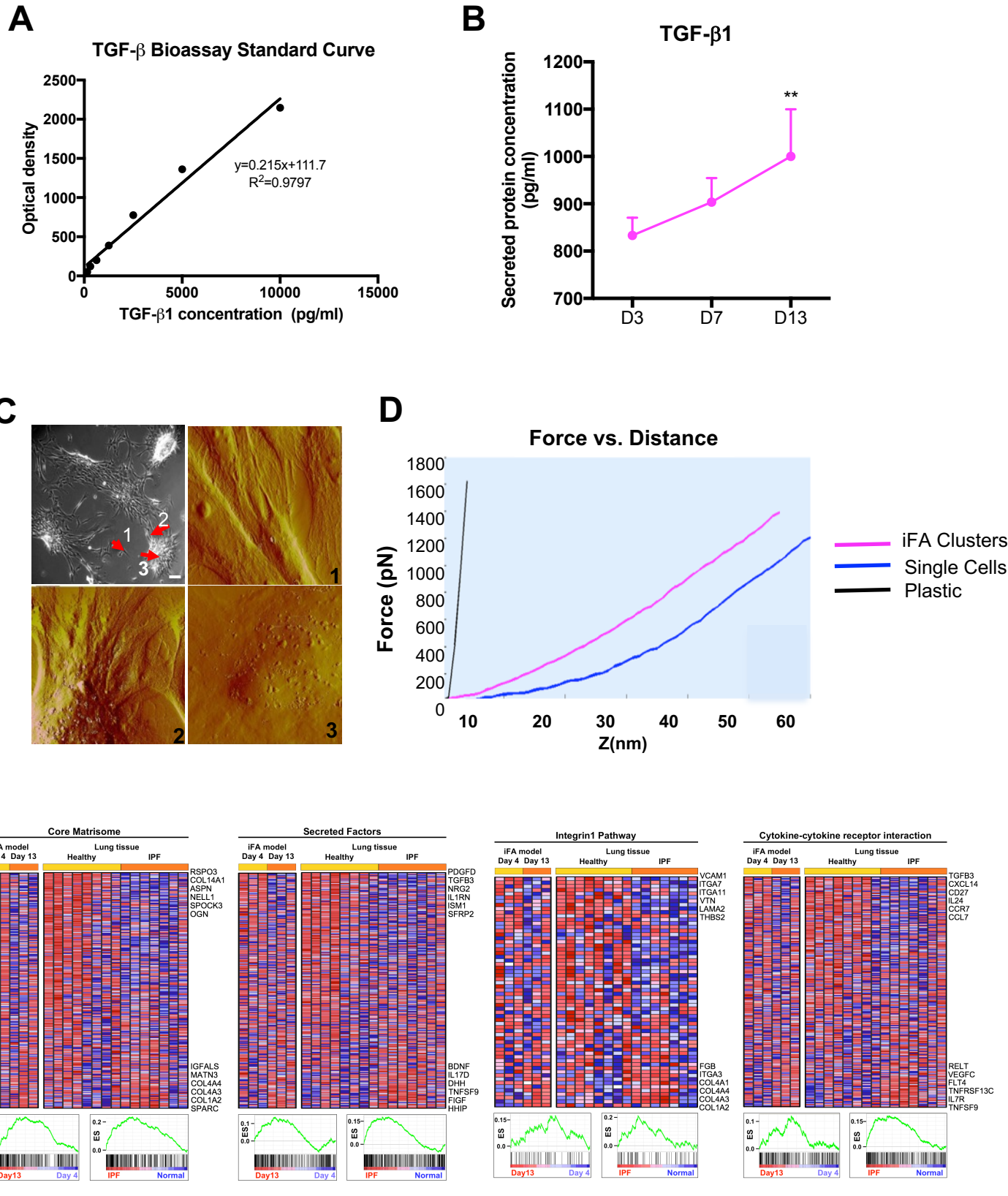

Primary Fibroblast
iPSC
Mesenchymal-like cells
A
FSP1/DAPI
B
SSEA4OCT4DAPI
C
SSEA4OCT4DAPI
Mesenchymal-like cells
D
46%
% of Max
% SSEA4 + cells
SSEA4
E
CD73
CD105
TRA160
PF
iPSC
Mesenchymal-like cells
94%
87%
0.1%
0.2%
0.2%
94%
92%
55%
0.2%
Unstained
Stained
Initial Markers
Mesenchymal-like cells
F
7.8%
% of Max
% Epcam + cells
EPCAM
SSEA4-APC
CD14
CD34
CD45
CD90
CD146
CD166
CD326
PF
iPSC
Mesenchymal-like cells
0.1%
0.1%
0.1%
98%
24%
94%
0.4%
0.2%
0.2%
0.2%
93%
98%
94%
0%
0.2%
0.2%
4.3%
99%
82%
88%
8.2%
Additional Markers
G
Mesenchymal-like cells
Unstained
FITC
APC
BV421
PE
0%
Immune cell markers
CD32
CD11b
CD14
CD68
0.1%
0.2%
0.4%
0.3%
0%
0.2%
0.1%
H
I
Unstained
Primary Fibroblast
Mesenchymal-like cells
SSEA4-APC
CD326-PE
SSEA4+CD326-
SSEA4+CD326+
42%
68%
8.2%
1.2%
CD44 - 405
CD45 – Alexa488
CD44+ CD45+
CD44+CD45-
90.4%
61.5%
1%
3.7%
J
iFA Model
Day 2
Day 4
Day 7
Day 10
K
Primary Fibroblast
Lung
Heart
Skin
Mesenchymal-like Cells
Lung
PBMC
Skin
ES
L
Control
iFA
% EdU positive cells
100
75
50
25
0
****
D1
D3
D6
D9
M
Control
iFA
mRNA expression (fold/18S)
****
***
***
*
*
ACTA2
COL1A2
TGF-β1
TGF-β3
TIMP-1
MMP-3
PLAT
LOX
N
Control
iFA
Collagen I
α–SMA
ACTB
Relative Protein Expression / β-actin
α-SMA
Collagen I
O
TEM

Figure S1. Generation and characterization of iPSC-derived mesenchymal-like cells. Related to Figure 1

(A-C) Steps involved in generation of mesenchymal-like cells. (A) Primary cultures of lung fibroblasts that expressed the fibroblast marker FSP1. (B) The primary fibroblasts were reprogrammed into iPSC and expressed pluripotency marker OCT4. (C) The iPSCs were differentiated into cells that expressed markers largely mesenchymal-like cells (SSEA4+). (**D**) Overlaid histogram plot that depicts the relative SSEA4 fluorescence intensity (blue) of the mesenchymal-like cells compared to the unstained controls (grey). Inset depicts % of positive cells (n=17). $p \leq 0.0001$ by Wilcoxon signed rank test. (**E**) Representative flow cytometric analysis results before and after differentiation of iPSCs. Representative flow cytometric profiles obtained from parent primary fibroblasts, undifferentiated iPSC and from mesenchymal stem cell (MSC)-like cells obtained after successful differentiation of iPSC to mesenchymal-like cells. The red histograms represent the isotype controls, while the blue histograms represent the individual markers being analyzed. **(F)** Overlaid histogram plot that depicts the relative CD326 (epithelial marker) fluorescence intensity (blue) of the mesenchymal-like cells compared to the unstained controls (grey). Inset depicts % positive cells (n=17). $p \leq 0.0001$ by Wilcoxon signed rank test. **(G)** Representative FACS plots revealing expression of monocyte/macrophage markers in the mesenchymal-like cells. The cells were co-stained for CD68 and CD32, CD11b or CD14, revealing 0.2-0.7% cells positive for monocyte/macrophage markers. Unstained controls are shown in top panel (n=7). $p=0.0156$ by Wilcoxon signed rank test. **(H)** Representative image of mesenchymal-like cell of a macrophage-like cell displaying heterochromatin (+), vacuolated cytoplasm (*), several microvilli (arrows) and whorls of phagocytosed matter (star). Scale bar, 1μm. **(I)** Characterization of primary fibroblasts (middle panel) and mesenchymal-like cells (right panel) using multi-color FACS. Unstained control plots are shown on the left panel. Representative FACS data with gating are shown for each marker. **(J)** Phase-contrast images demonstrating propagation of mesenchymal-like cells on 13 kPa hydrogels (iFA) over time that reveal progressively increasing scar-like aggregate size with progression from D2 to 10; Scale bar, 50μm. **(K)** Phase-contrast images demonstrating the development of the iFA phenotype only in cultures from mesenchymal-like cells generated from iPSCs of different sources and human ES cells. Primary fibroblasts failed to generate the phenotype irrespective of the parent source. Scale bar, 50μm. **(L)** Quantification of EdU positive cells in primary fibroblasts and

mesenchymal-like cells grown on 13kPa hydrogels related to Figure 1B. All data are presented as mean ± s.e.m; ****$P < 0.0001$ using 2-way ANOVA followed by Tukey's multiple comparisons test. **(M)** Fibrosis-related genes expression by qPCR in primary fibroblasts (control) and mesenchymal-like cells (iFA) cultured on 13kPa hydrogels (n=5). **(N)** Representative immunoblot analysis of the expression of Collagen I and α-SMA in primary fibroblasts (control) and mesenchymal-like cells (iFA) showing increased expression of the fibrosis-related proteins in the iFA cultures collected at day 13 (left panel). Beta actin was used as a loading control. Right panel shows quantitative data presented as mean ± s.e.m; ****$P < 0.0001$, *P<0.05 using 2-way ANOVA followed by Sidak's multiple comparisons test. **(O)** Representative transmission electron microscopic (TEM) images showing ultrastructure of cells in the iFA model amidst copious amounts of matricellular proteins (asterisk) and fibrillar proteins (plus). Inset is a higher magnification of the TEM image; Scale bar, 1μm.

FIGURE S2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TGF-β Bioassay Standard Curve
Optical density
TGF-β1 concentration (pg/ml)
y=0.215x+111.7
R2=0.9797
B
TGF-β1
Secreted protein concentration (pg/ml)
**
D3
D7
D13
C
1
2
3
D
Force vs. Distance
Force (pN)
Z(nm)
iFA Clusters
Single Cells
Plastic
E
Core Matrisome
Secreted Factors
Integrin1 Pathway
Cytokine-cytokine receptor interaction
iFA model
Day 4 Day 13
Lung tissue
Healthy
IPF
RSPO3
COL14A1
ASPN
NELL1
SPOCK3
OGN
IGFALS
MATN3
COL4A4
COL4A3
COL1A2
SPARC
PDGFD
TGFB3
NRG2
IL1RN
ISM1
SFRP2
BDNF
IL17D
DHH
TNFSF9
FIGF
HHIP
VCAM1
ITGA7
ITGA11
VTN
LAMA2
THBS2
FGB
ITGA3
COL4A4
COL4A1
COL4A3
COL1A2
TGFB3
CXCL14
CD27
IL24
CCR7
CCL7
RELT
VEGFC
FLT4
TNFRSF13C
IL7R
TNFSF9
ES
Day13
Day 4
IPF
Normal

Figure S2. Characterization of the iFA phenotype. Related to Figure 2

(A) PAI-1 promoter/luciferase construct-transfected mink lung epithelial cells were incubated with various concentrations of human recombinant(r) TGF- β1 at 37°C for 20 h. The standard curve showed a dose-dependent increase in luciferase activity (relative light units, RLU) by rTGF-β1 between 0 and 10 ng/ml. The standard curve was used to determine the bioactivity of TGF-β in the iFA model. **(B)** Time-dependent levels of secreted TGF-β1 during the development of the iFA phenotype (D4 to 13). A significant increase in secreted TGF-β1 protein in the iFA model was observed over time similar to that reported in fibrotic organs (n=6). Data represent mean ± s.e.m; $^{**}P<0.01$ using 2-way ANOVA and Sidak's multiple comparisons test. **(C)** Representative phase contrast image (top left) of the iFA model at D13 that was used to measure the elastic modulus. Arrows point to representative regions of the culture where the measurements were made. 1 refers to single cells in the dish. 2 refers to the cells on the periphery of the iFA phenotype. 3 refers to the center of the iFA phenotype. 3D rendering of the AFM amplitude channel that shows representative areas of 1,2 and 3 from panel (top right and bottom panels). **(D)** Force versus distance curves measured on cells from (c). Black line depicts the curve obtained on the stiff petri dish. Magenta line represents curve obtained from cells in the iFA and blue line represents single cells. The measured indentation was fitted to the Sneddon model. Elastic moduli of iFA cells and single cells were calculated as 30kPa and 15kPa, respectively. **(E)** Heatmaps showing expression of genes (red/blue are up/down-regulated), encoding for selected canonical pathways (core matrisome, selected factors, integrin 1 and cytokine-cytokine receptor interaction) in iFA model and previously published IPF lung tissue. On the right of each heatmap, genes at the extremes of the lists are indicated. At the bottom of each panel, a plot showing running enrichment score (ES) across the ranked list in each pathway is indicated, where the score at the peak of the plot is the final enrichment of the given gene set.

Figure S5. Secondary Screening using the iFA model. Related to Figure 5.

(A) Schematic to represent the timeline of prevention and resolution of the iFA phenotype in the iFA model. **(B)** Representative still frames from a time-lapse series showing an invasive and progressive phenotype in the disease model (upper panel), which resolved on addition of AA5 (lower panel); Scale bar, 100µm. **(C)** Quantitative data presented as mean ± s.e.m depicting the expression of Collagen I and α-SMA in the iFA model and AA5-presolution cultures collected at day 16. Beta actin was used as a normalization control. n=3, $^{**}P < 0.01$, *P<0.05 using 2-way ANOVA followed by Sidak's multiple comparisons test. **(D)** Representative IF staining for HMGB1 in DMSO-treated, AA5-prevention- and AA5-resolution-treated iFA model revealing the absence or significantly reduced cytoplasmic HMGB1 in the AA5-treated cultures; Scale bar, 50µm. Bottom panels are higher magnification of insets; Scale bar, 25µm. **(E)** Overlaid histogram plot that depict the relative SSEA4 fluorescence intensity in the iFA model with DMSO (blue), AA5-prevention (orange) and AA5-reversal (green) treatments. Unstained control is depicted in red. Inset depicts % of positive cells (n=6). $^{**}P < 0.01$, *P<0.05 using two-tailed paired t-test. The percentage of SSEA4+ cells was significantly reduced in the iFA model with AA5 treatment. **(F-H)** The Rank Rank Hypergeometric Overlap (RRHO) analysis shows statistically significant hypergeometric overlap between differentially expressed genes in the iFA model post AA5-prevention (n=2) and post AA5-resolution (n=2) treatments, as compared to the untreated control, iFA (n=2). This suggests a similar mechanism of action between AA5 in preventing (**G**) and resolving (**H**) fibrosis, and both produce a shift in the gene expression levels that is comparable to the differential expression of genes in the lung IPF tissue (n=7) in contrast to healthy lung controls (n=8), (**G,H**). The signed, $\log_{10}$-transformed t-test *P*-values are indicated in the color scale bar. At the bottom and on the left, ranked gene lists are indicated. **(I-J)** The list of significantly enriched terms using ClueGO analysis of AA5-mediated prevention (I) and resolution (J) of iFA phenotype in the iFA model. Terms are grouped according to the functional group that they belong to. Terms that are part of more than one functional group are shown in purple. The group p-values (corrected with Bonferoni step down) are indicated in between the bar charts. The bars indicate the percentage of the up- (red) or down-regulated (blue) genes per each term. The numbers outside the bars show the actual number of genes associated with the specific terms, while the numbers in the parenthesis show the individual term p-value. Related to Figure 5d-e. **(K)** Representative immunoblot analysis of the expression of p-SMAD2/3 in iFA

FIGURE S3

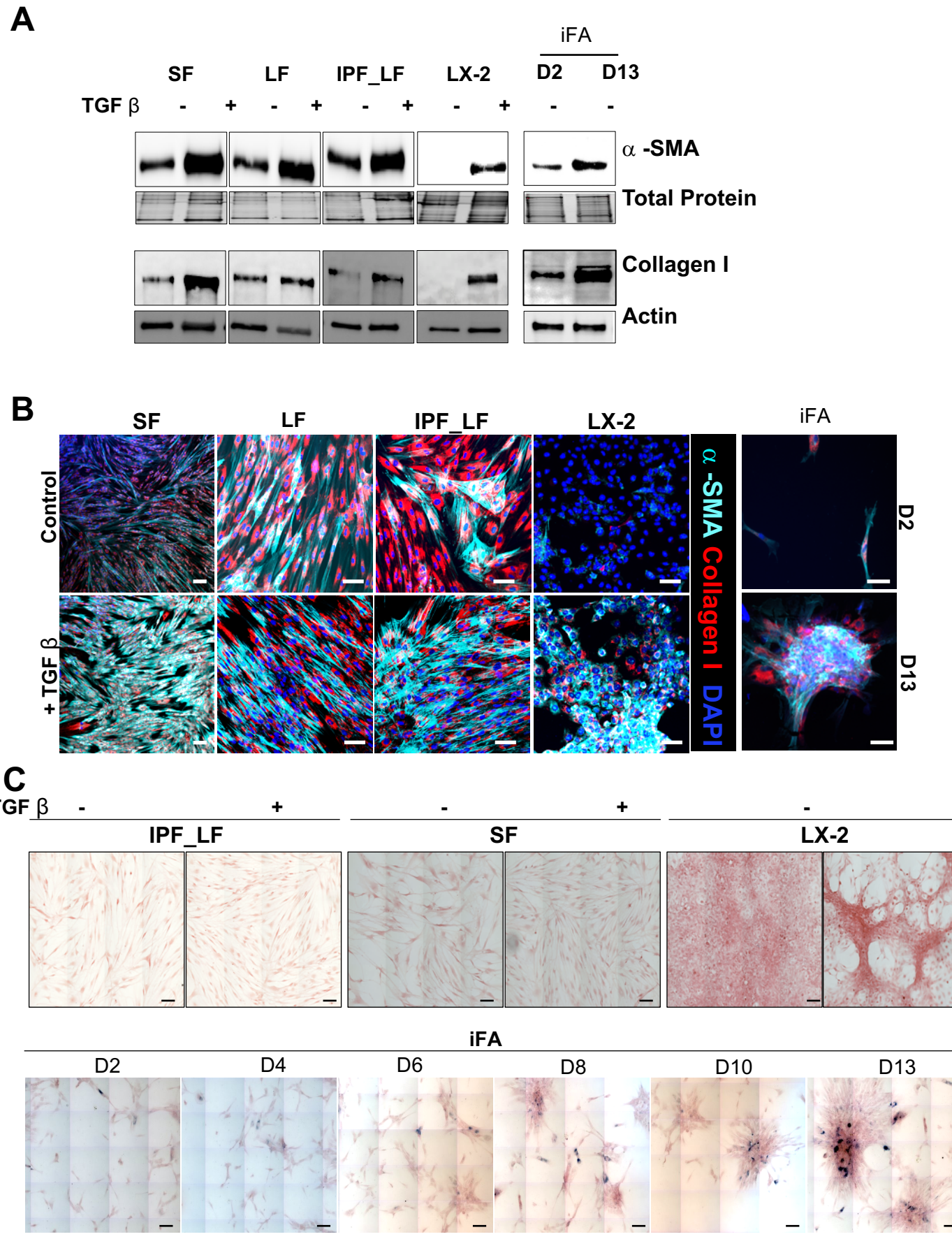
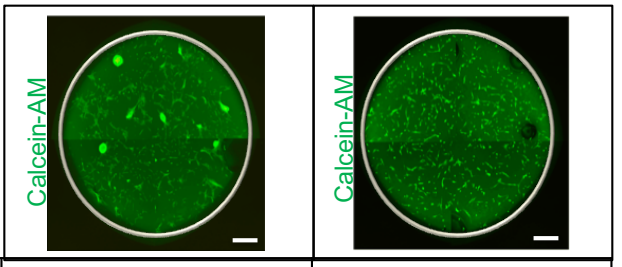

A
iFA
SF
LF
IPF_LF
LX-2
D2
D13
TGF β
- + - + - + - + - -
α -SMA
Total Protein
Collagen I
Actin
B
SF
LF
IPF_LF
LX-2
iFA
Control
+ TGF β
α -SMA Collagen I DAPI
D2
D13
C
TGF β
- + - + -
IPF_LF
SF
LX-2
iFA
D2
D4
D6
D8
D10
D13

Figure S3. Comparison of iFA Model with TGF-β-Induced Fibrosis Models. Related to Figure 3

(A) Representative immunoblot analysis of the expression of Collagen I and α-SMA in exogenously TGF-β-treated fibrosis models of skin (SF), lung (LF, IPF_LF) and liver (LX-2) at 48 hours compared to the iFA model at Day (D) 2 and 13 with no addition of TGF-β. Total protein (α-SMA) and ACTB (Collagen I) were used as a loading controls. **(B)** Representative IF images of Collagen I and α-SMA in exogenously TGF-β-treated fibrosis models of skin (SF), lung (LF, IPF_LF) and liver (LX-2) compared to the iFA model at Day (D) 2 and 13 with no addition of TGF-β. Scale bars, 50μm. **(C)** Representative images of exogenously TGF-β-treated fibrosis models of skin, lung and liver (48 hrs) compared to the iFA model during the progression of the fibrotic phenotype the iFA model at day (D) 2 to 13 of culture, demonstrating senescent cells with SA-β-Gal staining; Scale bars, 50μm.

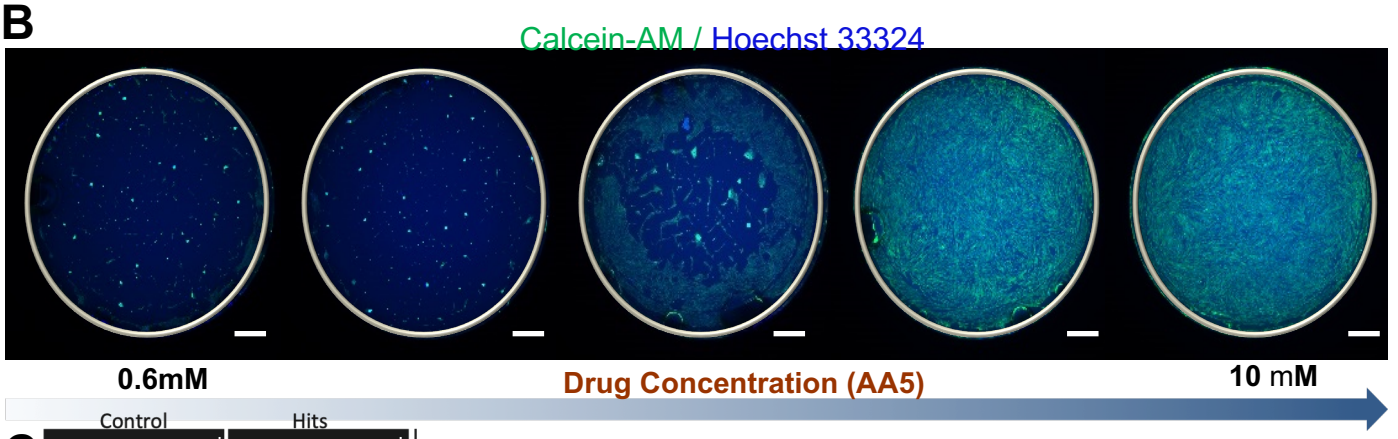
FIGURE S4

A

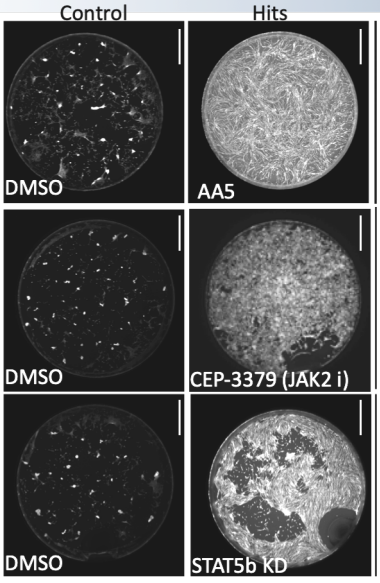


	Parameters to identify cellular aggregates	Parameters to identify single live cells
Approximate minimum width	60 μm	15 μm
Approximate maximum width	200 μm	100 μm
Intensity above local background	200 gray level	70 gray level

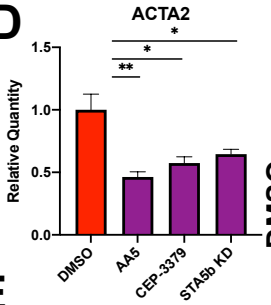
B



C



D



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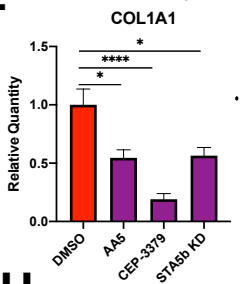

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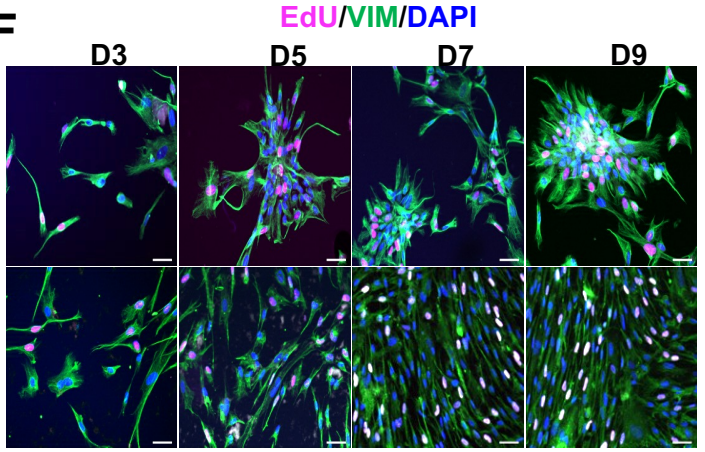

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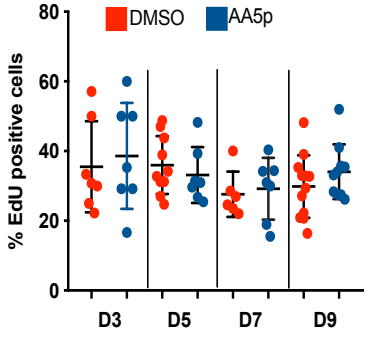
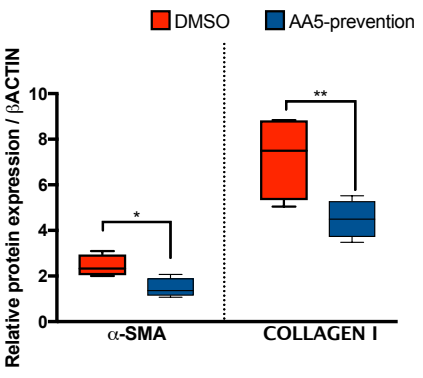


H



I

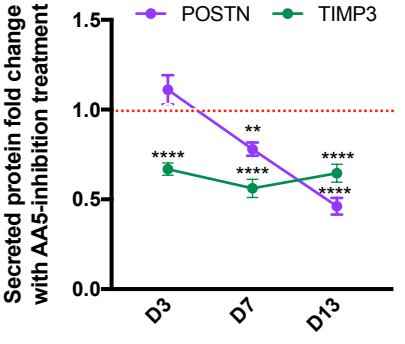
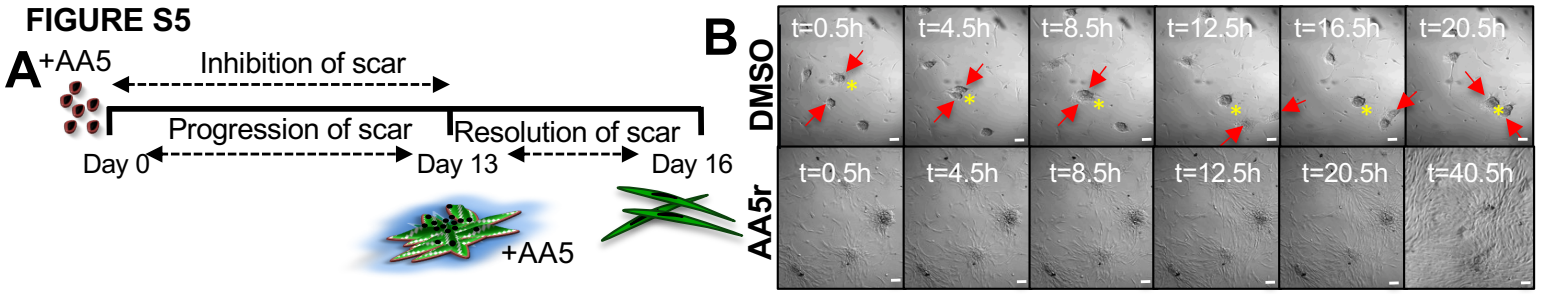
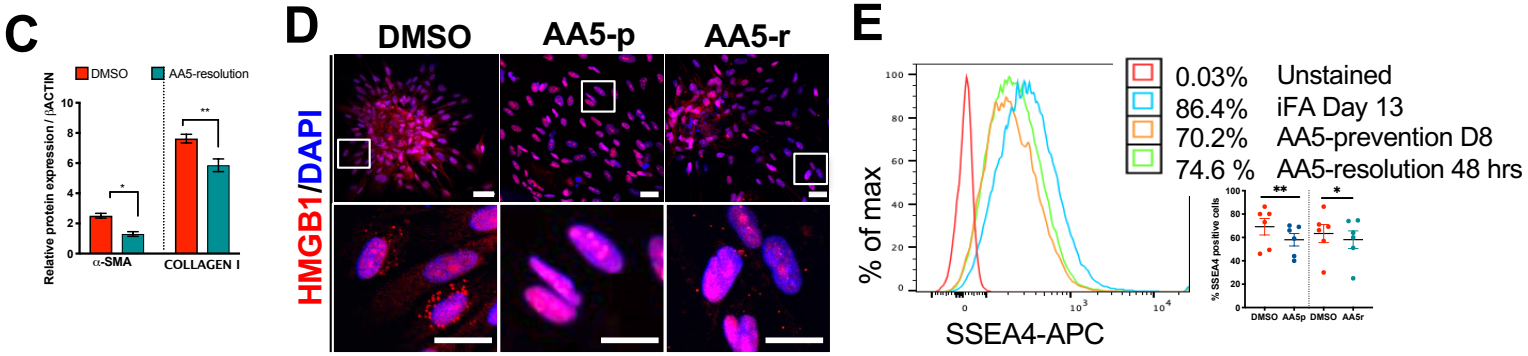


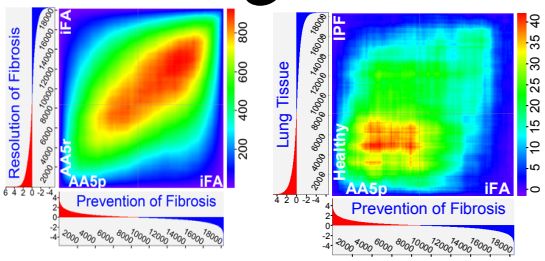
Figure S4. HTS using the iFA model identifies a primary hit molecule. Related to Figure 4.

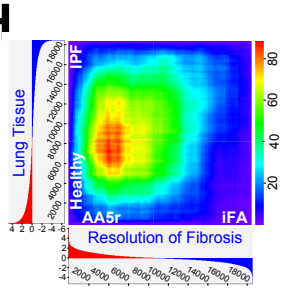
(A) High content staining discrimination of iFA phenotype vs individual spindle-shaped live cells. Cells are stained with Calcein-AM vital dye. Table shows the parameters that were used to identify and count the number of areas displaying the iFA phenotype and total number of individual spindle-shaped live cells. Scale bar, 750µm. **(B)** Representative images from wells of the iFA model treated with 0.6µM – 10µM AA5. Cells were stained with viability dye Calcein AM and the nuclei were counterstained with Hoechst 33342. Note full prevention of phenotype at low micromolar concentrations; Scale bar, 750µm. **(C)** Representative images from wells of the iFA model treated with DMSO control (left panel), hit molecules (right panel) stained with Calcein AM. No inhibition of the iFA phenotype was observed in the controls. The right panel shows partial or complete inhibition of the iFA phenotype. Scale bar, 750µm. **(D-E)** Relative expression of gene expression of *ACTA2 (D) and COL1A1 (E)* in controls, hits and non-hits using the iFA model. ****$P < 0.0001$ * $P<0.05$ using two-way ANOVA and Sidak's multiple comparison test. **(F)** Comparative IF images of DMSO (top panel) and AA5-prevention (bottom panel) treated iFA model from days (D) 3, 5, 7, and 9 in culture labeled with EdU for 6 hours. The cells were counterstained with VIM and DAPI. Scale bar, 50µm**. (G)** EdU positive DAPI cells from (c) was quantified for each time point. Treatment with AA5 did not display any significant difference in the proliferation rate when compared to the DMSO treated cells. All data are presented as the mean ± s.e.m; non-significant data using one-way ANOVA and Tukey's multiple comparison test. **(H)** Quantitation of immunoblot analysis of the expression of Collagen I and α-SMA in the DMSO and AA5-prevention samples at D8. ACTB was used as a loading control. **(I)** Time-dependent fold change in secreted POSTN and TIMP-3 in response to AA5-prevention treatment versus DMSO in the development of the iFA phenotype (D3 to D13).

FIGURE S5



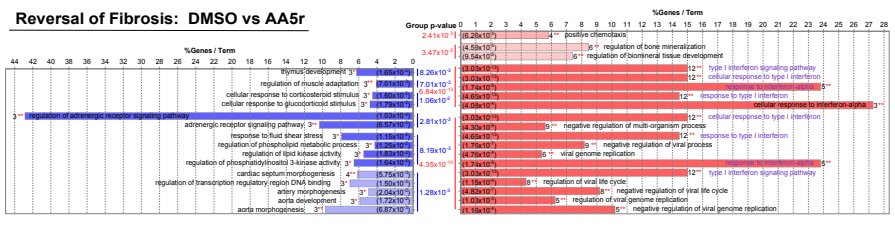






I Prevention of iFA phenotype (DMSO vs AA5 prevention

J Resolution of iFA phenotype (DMSO vs AA5-resolution)



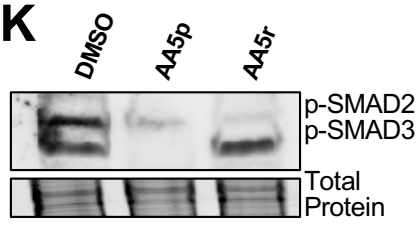


Figure S5. Secondary Screening using the iFA model. Related to Figure 5.

(A) Schematic to represent the timeline of prevention and resolution of the iFA phenotype in the iFA model. **(B)** Representative still frames from a time-lapse series showing an invasive and progressive phenotype in the disease model (upper panel), which resolved on addition of AA5 (lower panel); Scale bar, 100µm. **(C)** Quantitative data presented as mean ± s.e.m depicting the expression of Collagen I and α-SMA in the iFA model and AA5-presolution cultures collected at day 16. Beta actin was used as a normalization control. n=3, $^{**}P < 0.01$, *P<0.05 using 2-way ANOVA followed by Sidak's multiple comparisons test. **(D)** Representative IF staining for HMGB1 in DMSO-treated, AA5-prevention- and AA5-resolution-treated iFA model revealing the absence or significantly reduced cytoplasmic HMGB1 in the AA5-treated cultures; Scale bar, 50µm. Bottom panels are higher magnification of insets; Scale bar, 25µm. **(E)** Overlaid histogram plot that depict the relative SSEA4 fluorescence intensity in the iFA model with DMSO (blue), AA5-prevention (orange) and AA5-reversal (green) treatments. Unstained control is depicted in red. Inset depicts % of positive cells (n=6). $^{**}P < 0.01$, *P<0.05 using two-tailed paired t-test. The percentage of SSEA4+ cells was significantly reduced in the iFA model with AA5 treatment. **(F-H)** The Rank Rank Hypergeometric Overlap (RRHO) analysis shows statistically significant hypergeometric overlap between differentially expressed genes in the iFA model post AA5-prevention (n=2) and post AA5-resolution (n=2) treatments, as compared to the untreated control, iFA (n=2). This suggests a similar mechanism of action between AA5 in preventing (**G**) and resolving (**H**) fibrosis, and both produce a shift in the gene expression levels that is comparable to the differential expression of genes in the lung IPF tissue (n=7) in contrast to healthy lung controls (n=8), (**G,H**). The signed, $\log_{10}$-transformed t-test *P*-values are indicated in the color scale bar. At the bottom and on the left, ranked gene lists are indicated. **(I-J)** The list of significantly enriched terms using ClueGO analysis of AA5-mediated prevention (I) and resolution (J) of iFA phenotype in the iFA model. Terms are grouped according to the functional group that they belong to. Terms that are part of more than one functional group are shown in purple. The group p-values (corrected with Bonferoni step down) are indicated in between the bar charts. The bars indicate the percentage of the up- (red) or down-regulated (blue) genes per each term. The numbers outside the bars show the actual number of genes associated with the specific terms, while the numbers in the parenthesis show the individual term p-value. Related to Figure 5d-e. **(K)** Representative immunoblot analysis of the expression of p-SMAD2/3 in iFA

model treated with either DMSO, AA5-prevention (8 days) or AA5-resolution (48hrs) treatments. Total protein was used as a loading control.

FIGURE S6

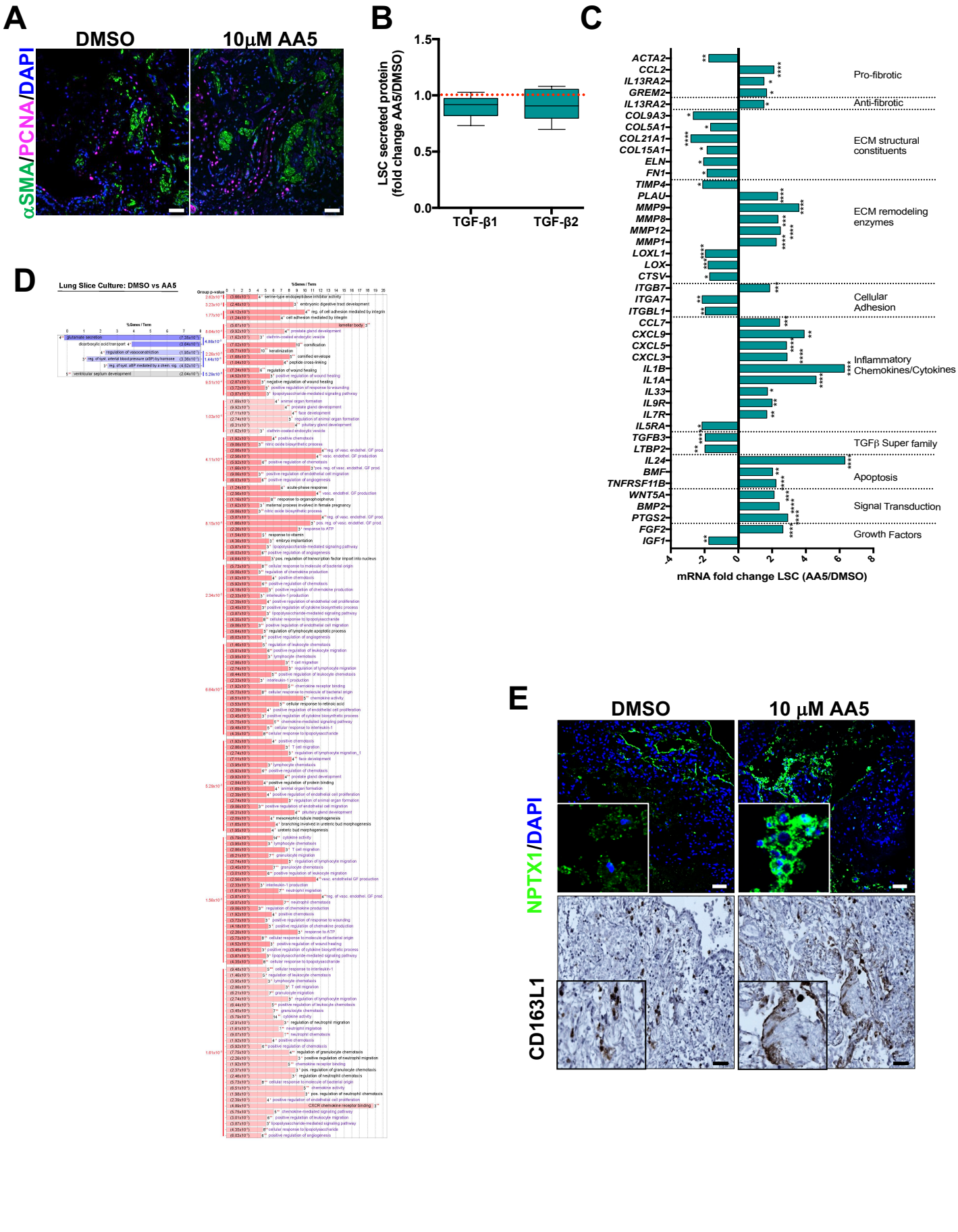

A
DMSO
10μM AA5
αSMA/PCNA/DAPI
B
LSC secreted protein (fold change AA5/DMSO)
1.5
1.0
0.5
0.0
TGF-β1
TGF-β2
C
ACTA2
CCL2
IL13RA2
GREM2
IL13RA2
COL9A3
COL5A1
COL21A1
COL15A1
ELN
FN1
TIMP4
PLAU
MMP9
MMP8
MMP12
MMP1
LOXL1
LOX
CTSV
ITGB7
ITGA7
ITGBL1
CCL7
CXCL9
CXCL5
CXCL3
IL1B
IL1A
IL33
IL9R
IL7R
IL5RA
TGFB3
LTBP2
IL24
BMF
TNFRSF11B
WNT5A
BMP2
PTGS2
FGF2
IGF1
Pro-fibrotic
Anti-fibrotic
ECM structural constituents
ECM remodeling enzymes
Cellular Adhesion
Inflammatory Chemokines/Cytokines
TGFβ Super family
Apoptosis
Signal Transduction
Growth Factors
-4
-2
0
2
4
6
8
mRNA fold change LSC (AA5/DMSO)
D
Lung Slice Culture: DMSO vs AA5
E
DMSO
10 μM AA5
NPTX1/DAPI
CD163L1

Figure S6. *Ex vivo* anti-fibrotic effect of AA5. Related to Figure 6

(A) Representative image of DMSO- and AA5-treated lung slice cultures (LSCs) stained for the proliferation marker PCNA 72 hours after treatment depicting viability of tissue. Samples were counterstained for α-SMA and DAPI. (**B)** Relative secreted levels of TGF-β proteins in supernatants of AA5-treated LSCs compared to DMSO-treated controls, 48 hours after treatment (n=9). Data represent min-max and median protein abundance in AA5-treated LSCs relative to DMSO treated controls (red line). **(C)** Gene expression analysis showing relative expression levels of fibrosis-related genes in LSCs treated with AA5 compared with DMSO treatment depicting the fibro-protective effect of AA5 (n=6). TMM (trimmed mean of M-values) was used to normalize the gene expression; ****$P < 0.0001$ *** $P<0.001$ ** $P<0.01$ * $P<0.05$. **(D)** The list of significantly enriched terms in the ClueGO analysis from the pairwise comparison between AA5 and DMSO treated LSCs. Terms are grouped according to the functional group that they belong to. Terms that are part of more than one functional group are shown in purple. The group p-values (corrected with Bonferoni step down) are indicated in between the bar charts. The bars indicate the percentage of the up- (red) or down-regulated (blue) genes per each term. The numbers outside the bars show the actual number of genes associated with the specific terms, while the numbers in the parenthesis show the individual term p-value. **(E)** Upper panel shows representative images of DMSO- and AA5 (10μM)-treated LSCs stained for NPTX1 revealing excessive NPTX1 staining in the honey comb cyst areas of the IPF lung in the AA5-treated samples within 48 hours of treatment in comparison to the DMSO controls. The samples were counterstained for DAPI. Insets are higher magnified images. Scale bar, 50μm. Lower panel shows representative images of DMSO- and AA5 (10μM)-treated LSCs stained for scavenger receptor protein CD163L1 by immunohistochemistry and revealed prominent staining on macrophages in both treatments, but a higher expression of shed receptor staining was seen on AA5 treatment suggesting activation of the phagocytic cells. Insets are higher magnified images. Scale bar, 100μm.

FIGURE S7

A

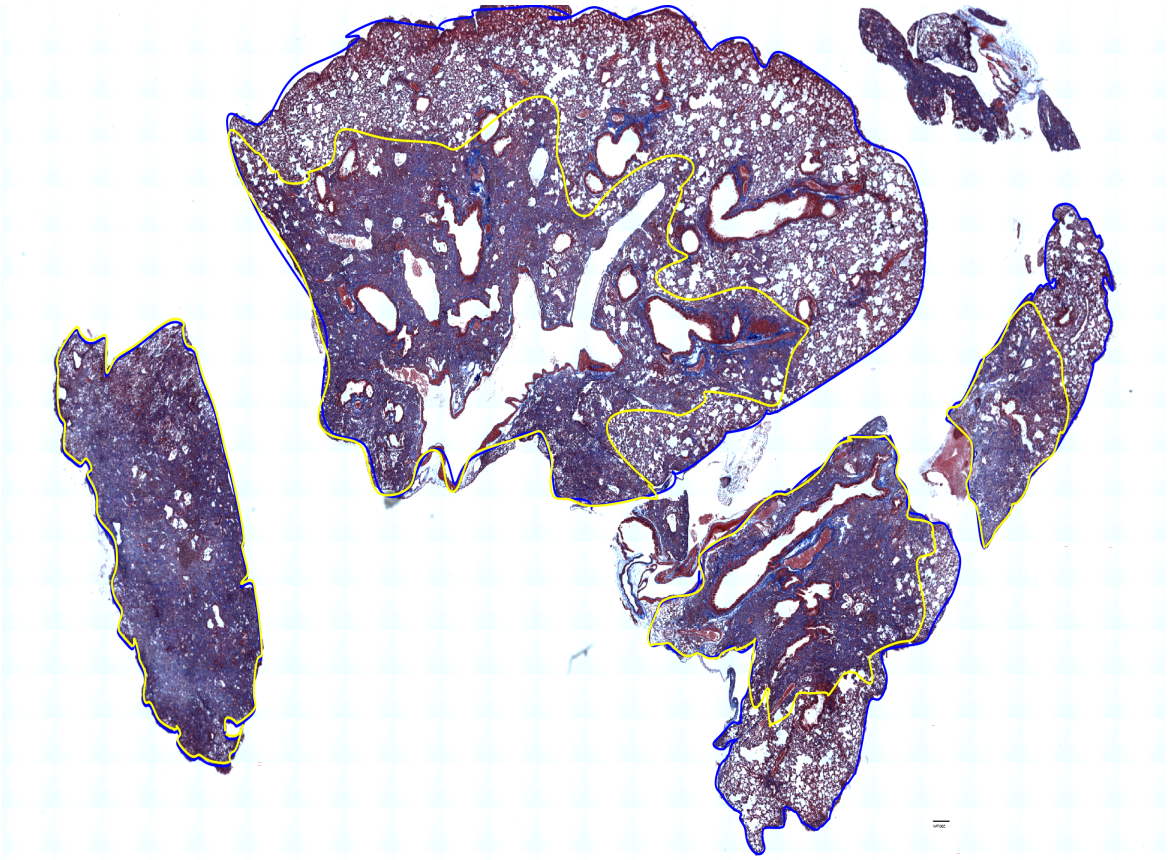


Figure S7. *In vivo* anti-fibrotic effect of AA5. Related to Figure 7.

(A) Representative Masson trichrome stained section of bleomycin treated lungs with the borders of the lung and fibrotic areas marked using the spline contour tool. Percent fibrotic area was calculated for each lobe (related to Figure 7J) Scale bars, 100 μm.

S.No	Patients	Tissue source	Age	Gender
1	IPF patient 1	Lung, skin	65	F
2	IPF patient 2	Lung, skin	55	M
3	IPF patient 3	Lung, skin	62	M
4	IPF patient 4	Lung, skin	50	F
5	IPF patient 5	Lung, skin	64	M
6	No prior disease patient 1	lung	NK	NK
7	No prior disease patient 2	lung	NK	NK
8	No prior disease patient 3	lung	NK	NK
9	No prior disease patient 4	lung	NK	NK
10	No prior disease patient 5	lung	NK	NK
11	No prior disease patient 6	lung	NK	NK
12	No prior disease patient 7	blood	NK	M
13	No prior disease patient 8	skin	NK	M
14	No prior disease patient 9	skin	NK	M
15	Patient with NKx2.1 mutation #1	skin	NK	M
16	Patient with NKx2.1 mutation #2	Skin	NK	F
17	Patient with NKx2.1 mutation #3	Skin	NK	F

NK – not known

Table S1: Information of subjects from whom samples were collected for IPSC derivation. Related to Figures 1 and S1.

Oligonucleotides		
TGFB3	Thermo Fisher Scientific	Hs01086000_m1
TIMP1	Thermo Fisher Scientific	Hs01092512_g1
MMP3	Thermo Fisher Scientific	Hs00968305_m1
PLAT	Thermo Fisher Scientific	Hs00263492_m1
LOX	Thermo Fisher Scientific	Hs00942480_m1
PTX3	Thermo Fisher Scientific	Hs00173615_m1
NPTX1	Thermo Fisher Scientific	*Hs00982601_m1*
CD163L1	Thermo Fisher Scientific	*Hs00264549_m1*
ACTA2	Thermo Fisher Scientific	Hs00426835_g1
COL1A2	Thermo Fisher Scientific	Hs01028956_m1
TGFB1	Thermo Fisher Scientific	Hs00998133_m1

Table S3: List of TaqMan probes used in this study. Related to Methods.

Table S2: Canonical pathway enrichment analysis of IFA model compared to previously published fibrotic lung, liver and kidney tissues. Related to Figures 2H and S2E.

Supplemental Videos:

Video S1. Time-lapse movie showing pre-iFA phenotype in the model. Related to Figure 1.

Transmitted light time-lapse images of iFA model at Day 4 revealing the initial stages of the formation of the iFA phenotype. The time-lapse covers a period of about 17 hours, imaged every 8 minutes and exported at 12 frames per second.

Video S2. Time-lapse movie showing iFA phenotype in the model. Related to Figures 1 and S5B.

Transmitted light time-lapse images of iFA model at Day 13 revealing fully established iFA phenotype at Day 13. The time-lapse covers a period of about 20 hours, imaged every 8 minutes and exported at 12 frames per second. The clusters show directionality, likely a consequence of the chemokine/cytokine secretion, combing of clusters accounting for the progressively increasing size of the clusters.

Video S3. Time-lapse movie showing the iFA-resolution effect of AA5. Related to Figures 5 and S5B.

Transmitted light time-lapse images of iFA model at Day 13, 5 hours after addition of 10μM AA5 revealing full resolution of the iFA phenotype within 48 hours. The time-lapse covers a period of about 20 hours, imaged every 8 minutes and exported at 12 frames per second.