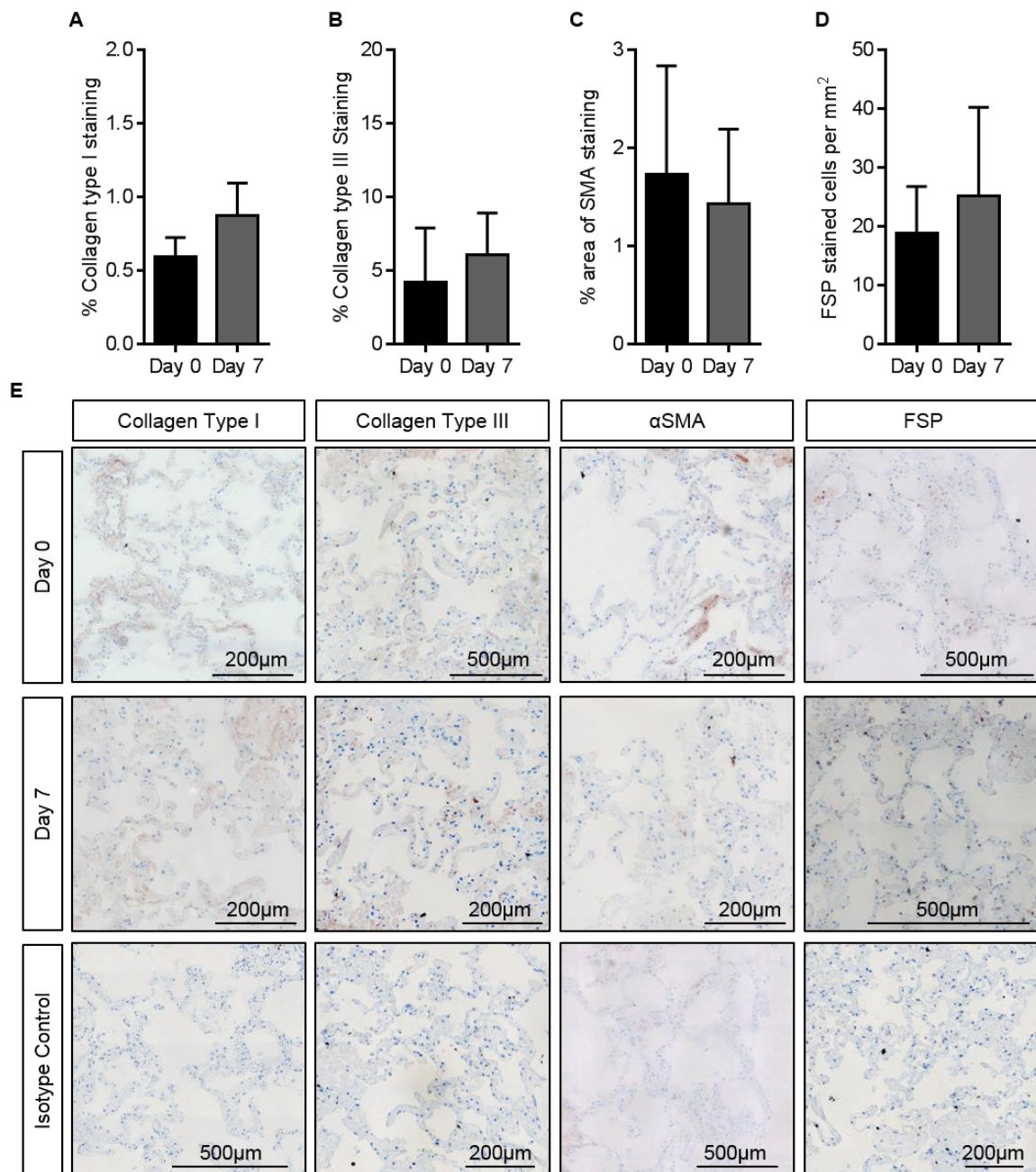


Supplementary material for

A model of human lung fibrogenesis for the assessment of anti-fibrotic strategies in idiopathic pulmonary fibrosis

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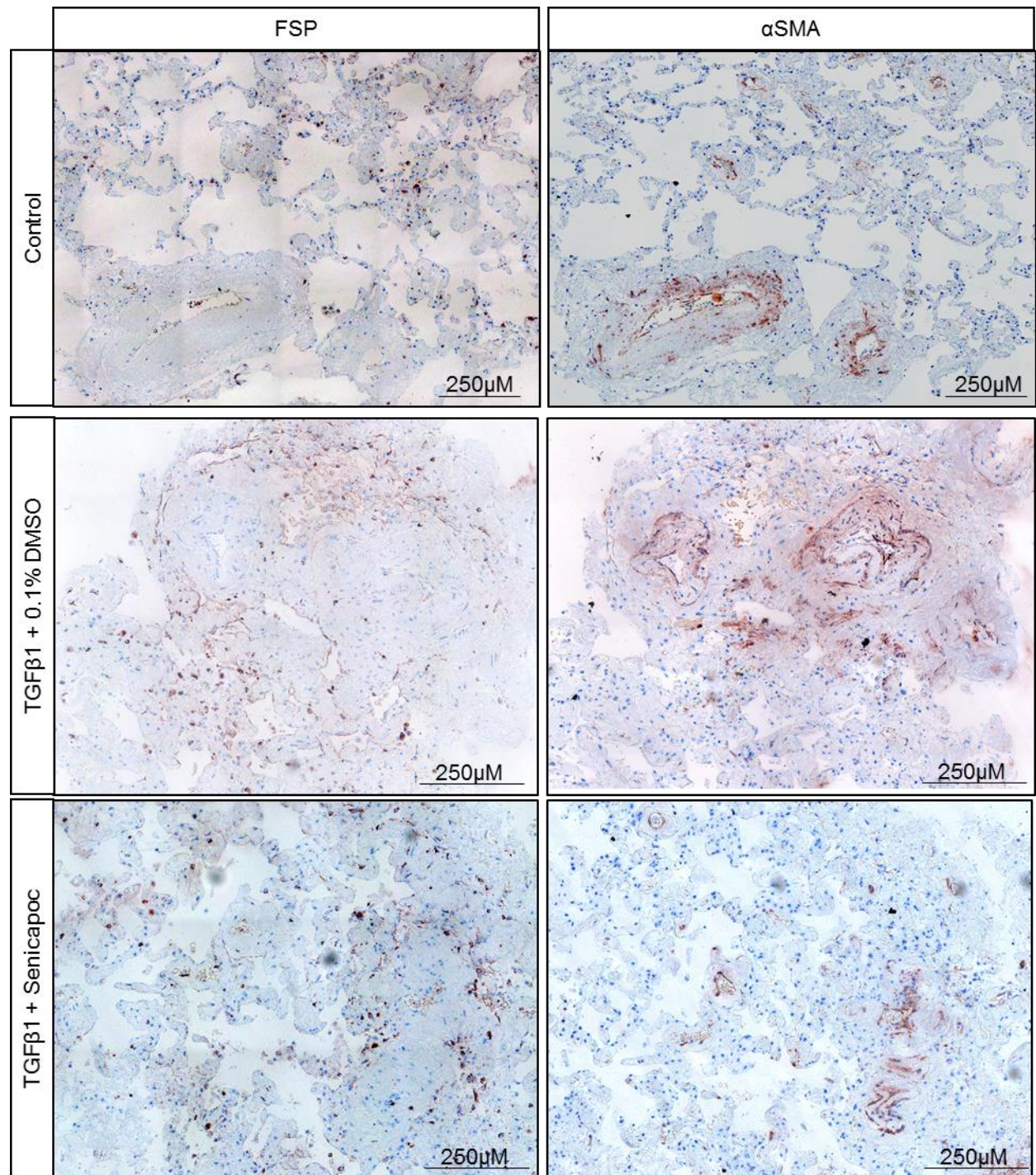


Supplementary figure 1 – Histological differences of cellular staining in tissue following 7 days of *ex vivo* culture in comparison to day 0.

A) Collagen type I staining was not significantly changed after 7 days in *ex vivo* culture, $P=0.25$ $n=3$. **B)** Similarly no differences in collagen type III staining was observed, $P=0.75$ $n=3$. **C)** The percentage area of α SMA staining was not affected following 7 days of *ex vivo* culture, $P=0.75$ $n=3$. **D)** No significant differences in the number of fibroblasts stained with fibroblast surface protein (FSP) at day 0 and day 7 were observed, $P=0.5429$ $n=3$. **E)** Representative images of collagen type I, collagen type III, α SMA, and FSP staining in day 0 and day 7 tissue. No significant differences were observed and relevant isotype controls were negative. Results are presented as mean $\pm$ SEM.

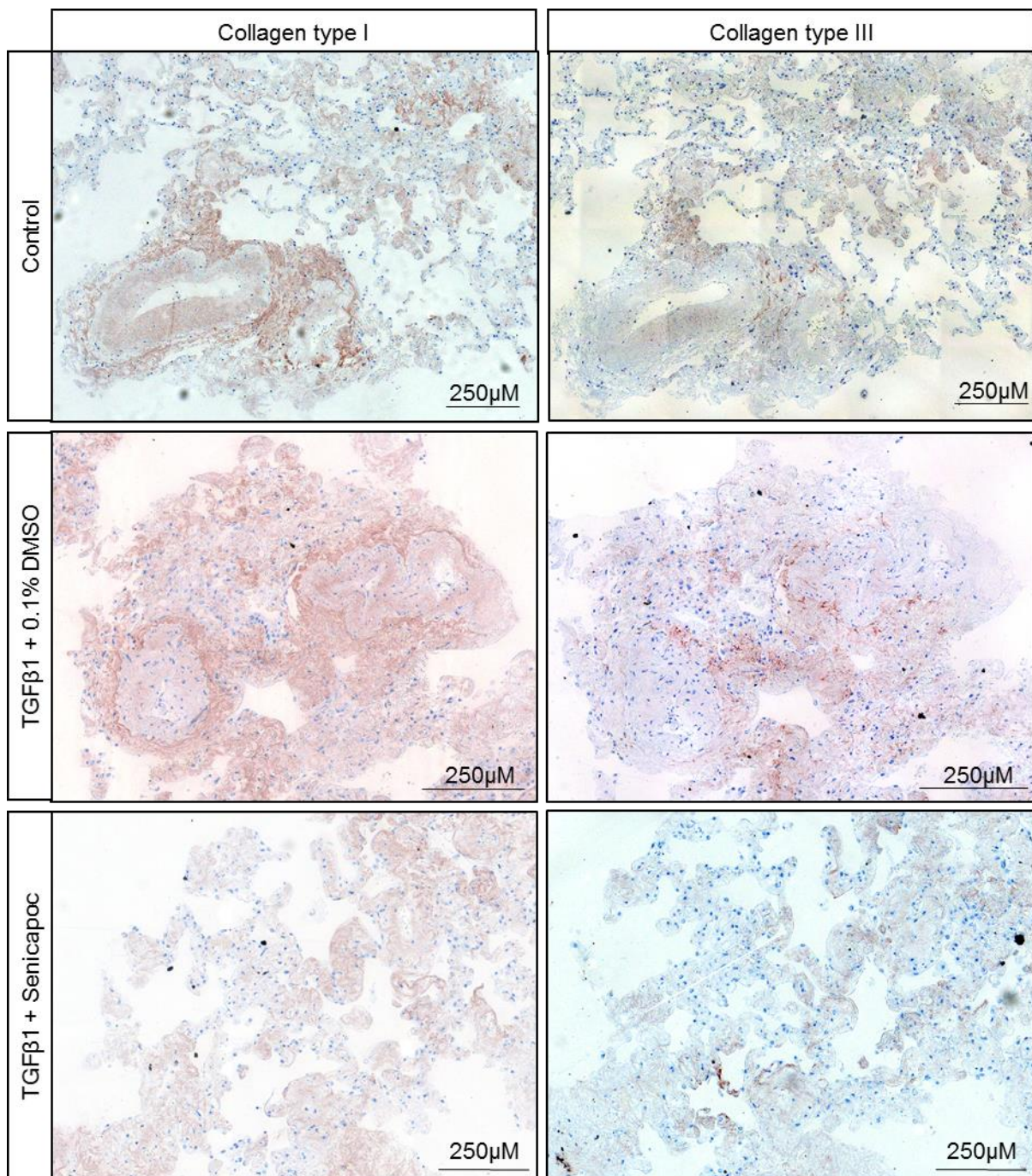
Supplementary figure 2 – Histological sequential FSP and α SMA staining in *ex vivo* cultured tissue following 7 days of with TGF β 1 and Senicapoc 100nM.

Sequential staining of FSP and α SMA following 7 days *ex vivo* culture with TGF β 1 stimulated tissue and TGF β 1 + Senicapoc.



Supplementary figure 3 – Histological sequential collagen type I and collagen type III staining in *ex vivo* cultured tissue following 7 days of with TGFβ1 and Senicapoc 100nM.

Sequential staining of collagen type I and III following 7 days *ex vivo* culture with TGFβ1 stimulated tissue and TGFβ1 + Senicapoc.



Supplementary table 1 – Differentially expressed genes in day 7 vs. day 0 lung tissue

Upregulated genes in day 7 explanted tissue compared to day 0 tissue				
Gene Symbol	Gene Name	Log ₂ FC	P-Value	Pass FDR
MMP1	Matrix metalloproteinase 1	4.0	0.0100	*
TIMP1	TIMP metalloproteinase inhibitor 1	3.0	0.0011	*
THBS2	Thrombospondin 2	2.9	0.0005	*
LOX	Lysyl oxidase	2.8	0.0022	*
MMP9	Matrix metalloproteinase 9	2.7	0.0374	
MMP14	Matrix metalloproteinase 14	2.7	0.0000	*

Downregulated genes in day 7 explanted tissue compared to day 0 tissue				
Gene Symbol	Gene Name	Log ₂ FC	P-Value	Pass FDR
IL13RA2	Interleukin 13 receptor, alpha 2	1.9744	0.0083	*
CCL2	Chemokine (C-C motif) ligand 2	-1.414	0.0059	*
CCL3	Chemokine (C-C motif) ligand 3	-1.559	0.0082	*
IL5	Interleukin 5	-1.964	0.0259	
SMAD6	SMAD family member 6	-2.14	0.0295	
CCL11	Chemokine (C-C motif) ligand 11	-3.831	0.0066	*
CTGF	Connective tissue growth factor	-4.211	0.0320	

Genes with a fold change ≥ 1.5 , and John D. Storey adjusted p-value < 0.05 in day 0 tissue compared to day 7 (n=9) are listed below. Using q-value of 0.05 (5% FDR).

Supplementary table 2 – Gene expression results from the 7 day ex vivo human lung fibrogenesis model were comparable to that previously published in IPF tissue.

Gene Symbol	Gene Name	Fibrotic Model Tissue Log₂(FC)	IPF Tissue (DePianto et al. 2015) Log₂(FC)
THBS1	Thrombospondin 1	2.1	0.9
CCL11	Chemokine (C-C motif) ligand 11	1.9	4.1
COL3A1	Collagen, type III, alpha 1	1.6	1.5
COL1A2	Collagen, type I, alpha 2	1.4	2.1
GREM1	Gremlin 1	1.4	4.2
MMP13	Matrix metalloproteinase 13	1.3	4.1
TIMP1	TIMP metalloproteinase inhibitor 1	1.2	0.9
SMAD7	SMAD family member 7	1.1	-0.7
ITGB3	Integrin, beta 3	1.0	1.7
MMP3	Matrix metalloproteinase 3	0.9	2.3
ENG	Endoglin	0.9	-0.6
ITGA2	Integrin, alpha 2	0.9	1.2
SMAD6	SMAD family member 6	0.8	-1.6
TIMP4	TIMP metalloproteinase inhibitor 4	0.8	-2.7
THBS2	Thrombospondin 2	0.8	2.0
TIMP2	TIMP metalloproteinase inhibitor 2	0.7	0.6
PLAU	Plasminogen activator, urokinase	0.6	1.7
TGFB1	Transforming growth factor, beta 1	0.6	0.8
CCL2	Chemokine (C-C motif) ligand 2	0.6	3.1
LTBP1	Latent transforming growth factor beta binding protein 1	0.6	1.2
TIMP3	TIMP metalloproteinase inhibitor 3	0.5	-1.7
TGFB2	Transforming growth factor, beta receptor II	-0.3	-0.8
CAV1	Caveolin 1, caveolae protein, 22kDa	-0.4	-1.2
IL13RA2	Interleukin 13 receptor, alpha 2	-1.5	5.5

Supplementary table 3 – Differentially expressed genes in TGFβ1 + Senicapoc stimulated tissue in comparison to control tissue.

Upregulated expressed genes in TGFβ1 + Senicapoc treated tissue vs control tissue				
Gene Symbol	Gene Name	Log ₂ FC	P-Value	Pass FDR
THBS1	Thrombospondin 1	2.3	7.70E-04	*
SNAI1	Snail homolog 1 (Drosophila)	2.1	4.20E-03	*
SERPINE1	Serpin peptidase inhibitor, clade E	2.1	3.00E-03	*
CTGF	Connective tissue growth factor	2.0	1.54E-02	*
PDGFA	Platelet-derived growth factor alpha polypeptide	1.3	4.57E-03	*
TIMP1	TIMP metalloproteinase inhibitor 1	1.3	1.73E-03	*
PDGFB	Platelet-derived growth factor beta polypeptide	1.1	3.81E-02	
ITGB1	Integrin, beta 1	1.0	1.18E-02	*
SMAD7	SMAD family member 7	1.0	1.56E-02	*
LOX	Lysyl oxidase	0.9	2.05E-02	*
VEGFA	Vascular endothelial growth factor A	0.7	4.48E-02	
IL1B	Interleukin 1, beta	0.6	2.53E-02	*

Downregulated expressed genes in TGFβ1 + Senicapoc treated tissue vs control tissue				
Gene Symbol	Gene Name	Log ₂ FC	P-Value	Pass FDR
IL13RA2	Interleukin 13 receptor, alpha 2	-0.9	3.06E-03	*
MMP9	Matrix metalloproteinase 9	-1.3	6.32E-03	*

References

- (1) Livak K, Schmittgen T. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* 2001;25:402-408.
- (2) Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JPA, Hunt TC, Okayama Y, Heusser CH, Bullock GR, Church MK, Holgate ST. Interleukin-4 is Localized to and Released by Human Mast-Cells. *J Exp Med* 1992;176:1381-1386.
- (3) Siddiqui S, Mistry V, Doe C, Doe C, Roach KM, Morgan A, Wardlaw A, Pavord I, Bradding P, Brightling C. Airway hyperresponsiveness is dissociated from airway wall structural remodeling. *J All Clin Immunol* 2008;122:335-341e3.
- (4) SPSS, IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, America.