# SUPPLEMENTARY INFORMATION

# Airway Basal Cells show a dedifferentiated KRT17highPhenotype and promote Fibrosis in Idiopathic Pulmonary Fibrosis

Benedikt Jaeger, Ph.D.[1,2], Jonas Christian Schupp, M.D.[2,3,4], Linda Plappert[1,2], Oliver Terwolbeck[1,2], Nataliia Artysh [1], Gian Kayser, M.D.[5], Peggy Engelhard, Ph.D.[6], Taylor Sterling Adams, B.S.[3], Robert Zweigerdt, Ph.D.[7], Henning Kempf, Ph.D.[7], Stefan Lienenklaus, Ph.D.[8], Wiebke Garrels, Ph.D.[8], Irina Nazarenko, Ph.D.[9,10], Danny Jonigk, M.D.[2,11], Malgorzata Wygrecka, Ph.D[12], Denise Klatt, Ph.D.[13], Axel Schambach, M.D. Ph.D.[13,14], Naftali Kaminski, M.D.[3], Antje Prasse, M.D.[1,2,4]

[1] Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany
[2] German Center for Lung Research, BREATH, Hannover, Germany
[3] Section of Pulmonary, Critical Care and Sleep Medicine, Yale School of Medicine, USA
[4] Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany
[5] Institute of Surgical Pathology, University Medical Center, Freiburg, Germany
[6] Department of Pneumology, University Medical Center Freiburg, Germany
[7] Leibniz Research Laboratories for Biotechnology and Artificial Organs, Medical School Hannover, Germany
[8] Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany.
[9] Institute for Infection Prevention and Hospital Epidemiology; Medical Center - University of Freiburg, Freiburg, Germany
[10] German Cancer Consortium (DKTK), Partner Site Freiburg and Cancer Research Center (DKFZ), Heidelberg, Germany
[11] Institute of Pathology, Medical School Hannover, Germany
[12] Department of Biochemistry, Faculty of Medicine, Justus Liebig University, Gießen, Germany
[13] Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany
[14] Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA

# Supplementary Tables

## Supplementary Table 1. Baseline Characteristics of IPF Patients and nonUIP ILD patients of the single cell sequencing cohort

| Supplementary Table 1. Baseline Characteristics of IPF and nonUIP ILD* | | |
|---|---|---|
| **Characteristic** | **IPF** N=9 | **NonUIP ILD** N=6 |
| Age -yr | 71 ± 7 | 64 ± 11 |
| Male/ female sex | 7/2 | 6/0 |
| FVC percent predicted value | 71 ± 9 | 69 ± 27 |
| DLCO percent predicted | 54 ± 13 | 53 ± 20 |
| Smoking Status | | |
| Never smoked -% | 3 | 3 |
| Former smoker-% | 6 | 3 |
| Current smoker-% | 0 | 0 |

* Plus–minus values are means ±SD. FVC denotes for forced vital capacity. DLCO denotes for diffusion capacity. NonUIP ILD denotes for patients with a fibrotic interstitial lung disease (ILD) who presented with a computer tomography (CT) scan that is not consistent with a definite Usual Interstitial Pneumonia (UIP) pattern. IPF denotes for idiopathic pulmonary fibrosis.

**Supplementary Table 2: Primary antibodies used for flow cytometry**

| Marker | Vendor | Dilution/ Conc. |
|---|---|---|
| Epcam APC | Miltenyi,<br># 130-11-000 | 1:50 |
| Cytokeratin 5 PE | abcam<br># ab224985 | 1:50 |
| Cytokeratin 17 A488 | abcam,<br># ab185032 | 1:50 |
| PROM1 APC | Miltenyi, # 130-113-668 | 1:100 |

**Supplementary Table 3: Primary antibodies used for IHC**

| Marker | Clone | Vendor | Procedure | Dilution/ Conc. | Incubation | Detection |
|---|---|---|---|---|---|---|
| C-src | polyclonal anti-src rabbit antibody | Abcam, #ab47405 | Manual | 1:50 | 30 min, RT | AP, (DAKO REAL Streptavidin Alkaline Phosphatase) |
| CK5/6 | monoclonal mouse anti-human CK5/6 (DAKO clone D5/16 B4) | Dako Omnis, #GA78061-2 | Manual | Ready to use | 30 min, RT | AP, (DAKO REAL Streptavidin Alkaline Phosphatase) |
| P63 | P63 polyclonal rabbit anti-human antibody | Calbiochem, #PC373 | Manual | 1:1000 | 30 min, RT | HRP, Peroxidase coupled secondary antibody (DAKO EnVision FLEX/HRP) |
| anti-human anti-αvβ6 | MAb6.3G9, primary monoclonal rat antibody | kindley provided by Shelia Violette Biogen Idec | Manual | 1:100 | 30 min, RT | HRP, Peroxidase coupled secondary antibody (DAKO EnVision FLEX/HRP) |
| negative control | Negative Control Mouse IgG1 antibody | Dako Omnis, #GA75066-2 | Manual | 1:20 | overnight,4°C | AP, (DAKO REAL Streptavidin Alkaline Phosphatase) |
| negative control | FLEX Universal Negative Control, Rabbit | Dako Omnis, #IS60061-2 | Manual | 1:20 | overnight,4°C | AP, (DAKO REAL Streptavidin Alkaline Phosphatase) |
| negative control | Negative Control Mouse IgG1 antibody | Dako Omnis, #GA75066-2 | Manual | 1:20 | overnight,4°C | AP, (DAKO REAL Streptavidin Alkaline Phosphatase) |

## Supplementary Table 4: Secondary antibodies used for IHC

| Clone | Vendor | Procedure | Dilution/ Conc. | Incubation |
|---|---|---|---|---|
| Goat IgG anti-Mouse IgG (H+L)-Biotin | Dianova, #115-065-003 | Manual | 1:800 | 30 min RT |
| Goat IgG anti-Rabbit IgG (H+L)-Biotin | Dianova, #111-065-003 | Manual | 1:800 | 30 min RT |

**Supplementary Table 5: Primary and secondary antibodies used for immunofluorescence staining**

| Sections | Marker | Clone | Vendor | Dilution | Detection Ab | Vendor |
|---|---|---|---|---|---|---|
| Human lung tissue | c-src | Src (36D10) Rabbit mAb | Cell Signaling #2109S | 1:400 | Donkey IgG anti-Rabbit IgG (H+L)-Cy5 | Dianova, #711-175-152 |
| Human lung tissue | CK5/6 | FLEX Monoclonal Mouse Anti-Human Cytokeratin 5/6 Clone D5/16 B4 | Dako Omnis, #GA78061-2 | Ready-to-Use | Donkey Fab anti-Mouse IgG (H+L)-Alexa Fluor 488 | Dianova, #715-547-003 |
| NSG-Venus Mice lung tissue | CK8 | Rabbit Anti-Cytokeratin 8 antibody [EP1628Y] | Abcam, #ab53280 | 1:100 | Donkey IgG anti-Rabbit IgG (H+L)-Cy3 | Dianova, #711-165-152 |
| NSG-Venus Mice lung tissue | CK5/6 | FLEX Monoclonal Mouse Anti-Human Cytokeratin 5/6 Clone D5/16 B4 | Dako Omnis, #GA78061-2 | Ready-to-Use | Donkey IgG anti-Mouse IgG (H+L)-Cy5 | Dianova, #715-175-150 |
| NRG mice lung tissue | AQP5 | Goat AQP5 Antibody (G-19) | Santa Cruz, #sc-9891 | 1:25 | Donkey IgG anti-Goat IgG (H+L)-Cy3 | Dianova, #705-165-147 |
| NRG mice lung tissue | eGFP | GFP (D5.1) XP® Rabbit mAb | Cell Signalling, #2956 | 1:75 | Donkey Fab anti-Rabbit IgG (H+L)-Alexa Fluor 488 | Dianova, #711-547-003 |
| NRG mice lung tissue | CK5/6 | FLEX Monoclonal Mouse Anti-Human Cytokeratin 5/6 Clone D5/16 B4 | Dako Omnis, #GA78061-2 | Ready-to-Use | Donkey Fab anti-Mouse IgG (H+L)-Alexa Fluor 488 | Dianova, #715-547-003 |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | CK5/6 | FLEX Monoclonal Mouse anti-human cytokeratin 5/6 Clone D5/16 B4 | Dako Omnis, #GA78061-2 | Ready-to-Use | Alexa Fluor® 488-conjugated AffiniPure Fab fragment donkey anti mouse IgG | Dianova, #715-547-003 |

| Sections | Marker | Clone | Vendor | Dilution | Detection Ab | Vendor |
|---|---|---|---|---|---|---|
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | Anti-alpha smooth muscle Actin | Rabbit monoclonal | Abcam,# ab124964 | 1:400 | Cy™5 AffiniPure DonkeyAnti-RabbitIgG (H+L) | Jackson immuno research, #711-175-152 |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | Vimentin | goat | Merck, #V4630 | 1:20 | Donkey IgG anti-Goat IgG (H+L)-Cy3 | Dianova, #705-165-147 |
| | Isotype control | Rabbit IgG, polyclonal – Isotype Control | Abcam, #ab37415 | 1:400 | Cy™5 AffiniPure DonkeyAnti-RabbitIgG (H+L) | Jackson immuno research, #711-175-152 |
| | Negative control | FLEX Universal Negative Control, Mouse | Dako Omnis, #GA75066-2 | Ready-to-Use | Donkey Fab anti-Mouse IgG (H+L)-Alexa Fluor 488 | Dianova, #715-547-003 |
| | Isotype Control | Goat IgG Isotype Control | Thermo Fisher Scientific, #31245 | 1:25 | Donkey IgG anti-Goat IgG (H+L)-Cy3 | Dianova, #705-165-147 |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | CK5 | Alexa Fluor® 647 Rabbit monoclonal [EP1601Y] to Cytokeratin 5 | Abcam, #ab 193895 | 1:200 | - | - |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | CK6 | Rabbit monoclonal [EPR1602Y] to Cytokeratin 6 | Abcam, # ab93279 | 1:50 | Cy™3 AffiniPure Donkey Anti-Rabbit IgG (H+L) | Jackson immuno research, #711-165-152 |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | MUC5AC | Rabbit Polyclonal Antibody, Cy3 Conjugated | Bioss Antibodies, # bs-7166R-Cy3 | 1:50 | - | - |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | Acetyl-α-Tubulin (Lys40) | Mouse monoclonal antibody | Cell signaling, #12152 | 1:500 | Cy™5 AffiniPure Donkey Anti-Mouse IgG (H+L) | Jackson immuno research, #715-175-150 |
| Cryo Slide/ 3D Organoids w/o IPF fibroblasts | CK17 | Mouse monoclonal antibody | NSJ Bioreagent s | 1:200 | Cy™3 AffiniPure DonkeyAnti-Mouse IgG (H+L) | Jackson immuno research, #715-165-150 |

## Supplementary Table 6: Primary antibodies used for Western Blot

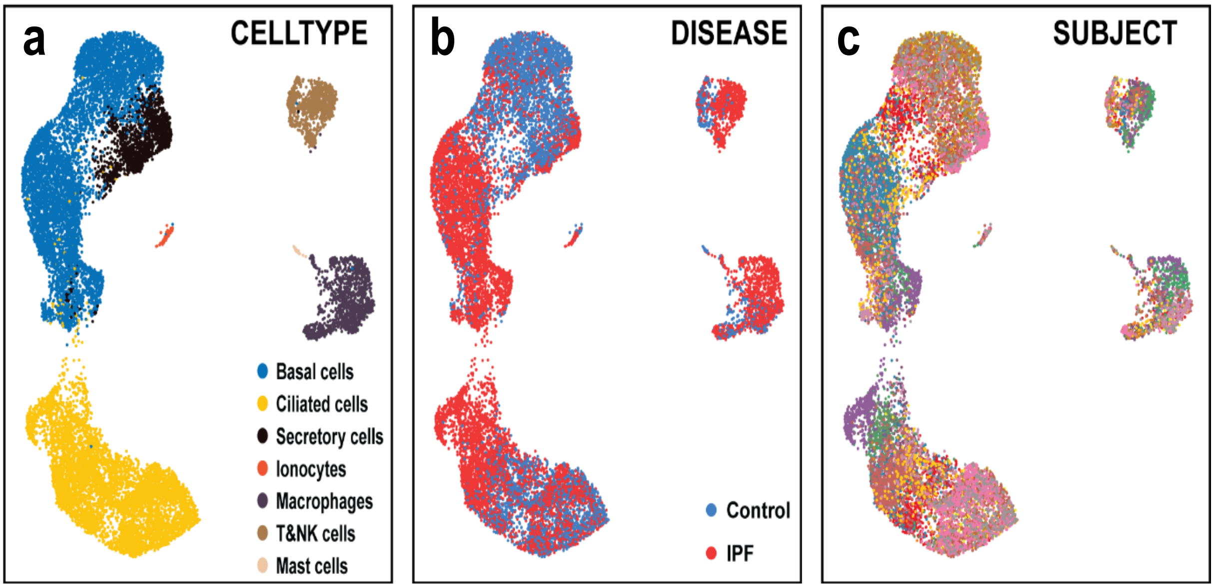
| Marker | Clone | Vendor | Dilution/ Conc. |
|---|---|---|---|
| c-src | polyclonal anti-src rabbit antibody | Abcam, #ab47405 | 1:500 |
| Phospho-Src | Phospho-Src (Tyr527) Antibody | Cell Signaling #2105S | 1:1000 |
| Type I Collagen | polyclonal goat anti-Type I Collagen | Southern Biotec, #SAB-1310-01 | 1:1000 |
| α-SMA | monoclonal mouse anti-α-SMA, ASM-1 | Merck Millipore, #CBL171 | 1:2000 |
| Fibronectin,cFn | monoclonal mouse anti-FN, DH1 | Enzo Life Sciences, #BML-FG6010 | 1:250 |
| p44/42 (Thr202/Tyr204) | mouse anti-phospho-p44/42 (Thr202/Tyr204) | Cell Signaling, #9106 | 1:500 |
| total p44/42 | rabbit mAb anti total p44/42 | Cell Signaling, #4695 | 1:1000 |
| phospho-EGFR (Tyr845) | rabbit phospho-EGFR (Tyr845) | Zytomed, #205-0235 | 1:500 |
| total EGFR | rabbit anti-total EGFR | Biomol | 1:500 |
| GAPDH (6C5) | mouse monoclonal antibody raised against GAPDH | Santa Cruz, #sc-32233 | 1:200 |
| α-Tubulin | Rabbit anti- α-tubulin antibody | Cell Signaling, #2125 | 1:1000 |

## Supplementary Table 7: Secondary antibodies used for Western Blot

| Clone | Vendor | Dilution/ Conc. |
|---|---|---|
| goat anti rabbit (H+L)-HRP conjugate | BioRad, #1706515 | 1:3000 |
| goat anti mouse (H+L)-HRP conjugate | BioRad, #1706516 | 1:3000 |

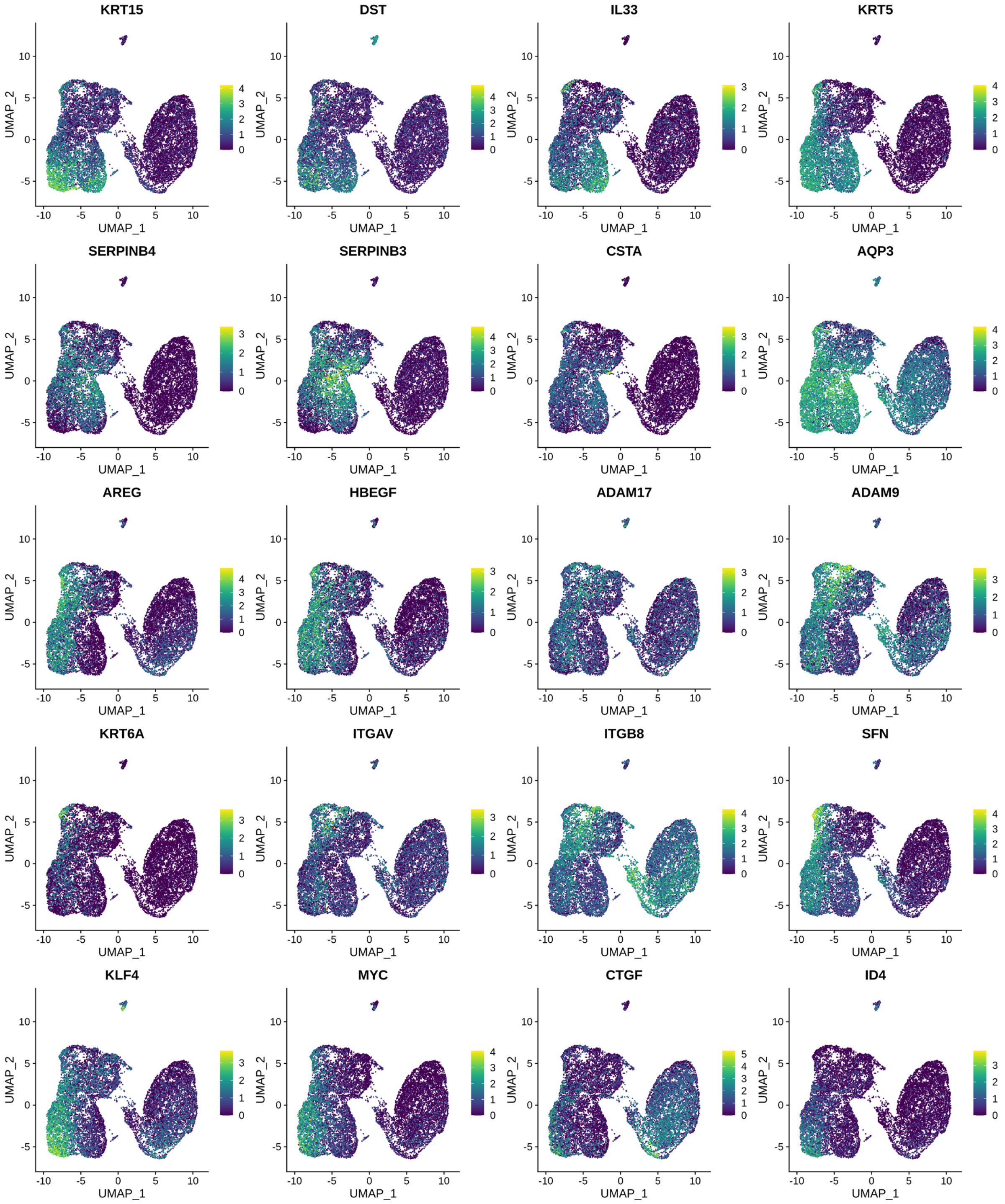
# Supplementary Figures

## Supplementary Figure 1



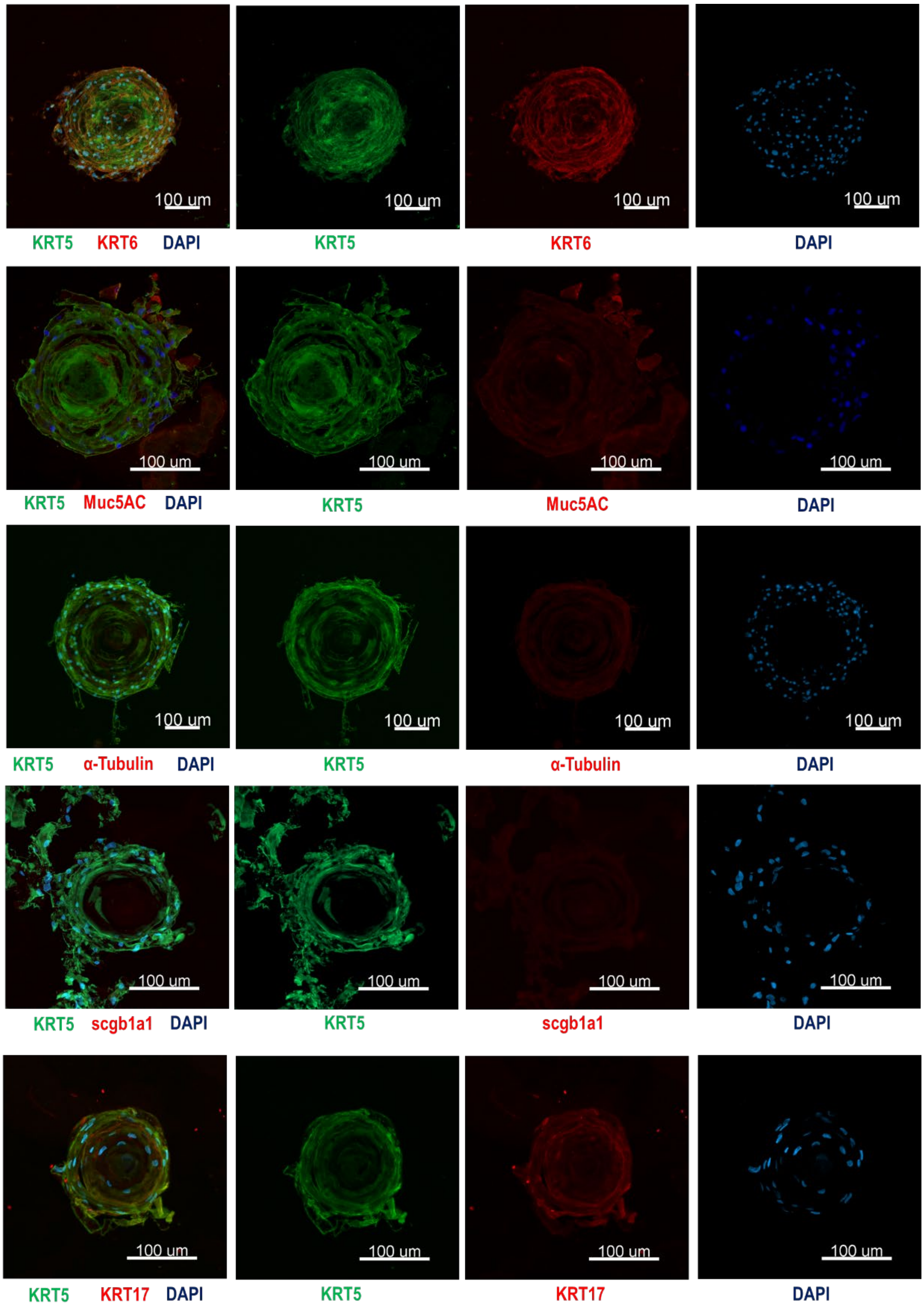

***Supplementary Fig. 1.*** *Uniform Manifold Approximation and Projection (UMAP) of the full dataset of 17,339 single cell transcriptomes visualizes the seven major discrete cell types detected.* ***a,*** *UMAPs colored by cell types.* ***b,*** *UMAPs colored by disease states.* ***c,*** *UMAPs colored by subjects.*

# Supplementary Figure 2



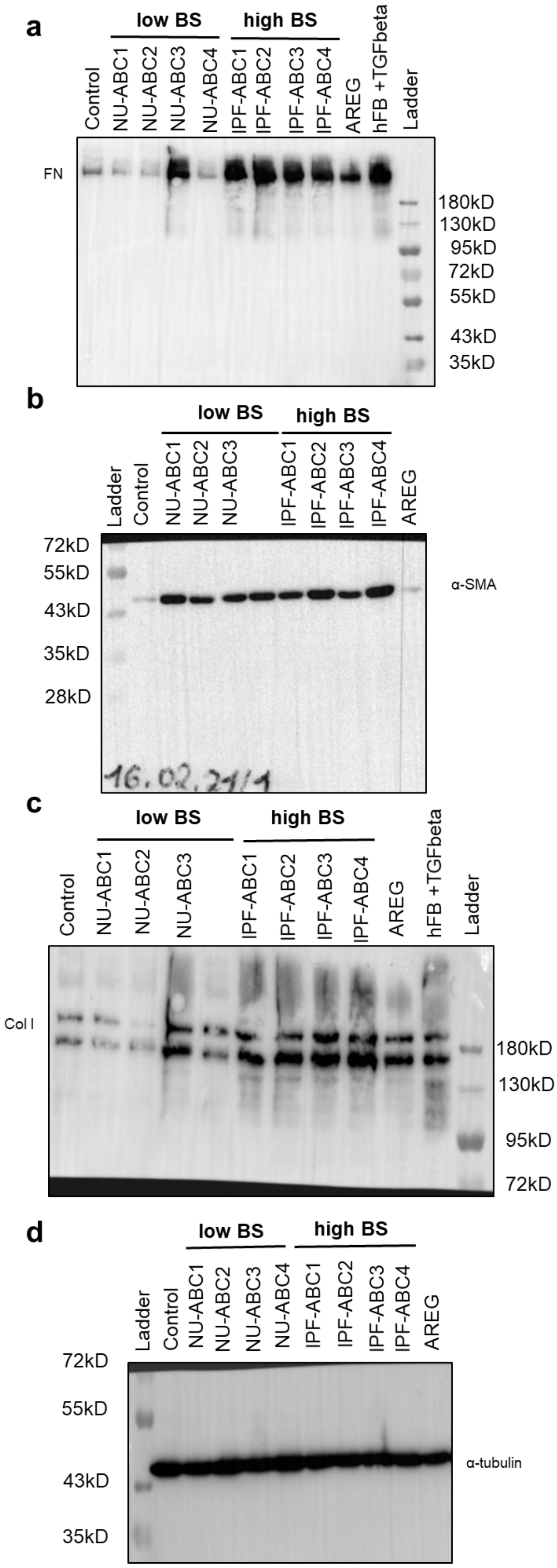

***Supplementary Fig. 2****: Expression of selected key genes of (IPF-)ABCs. UMAPs of epithelial cells colored by gene expression values of features that are associated with basal cells or with increased expression in IPF-ABCs compared to controls.*

## Supplementary Figure 3



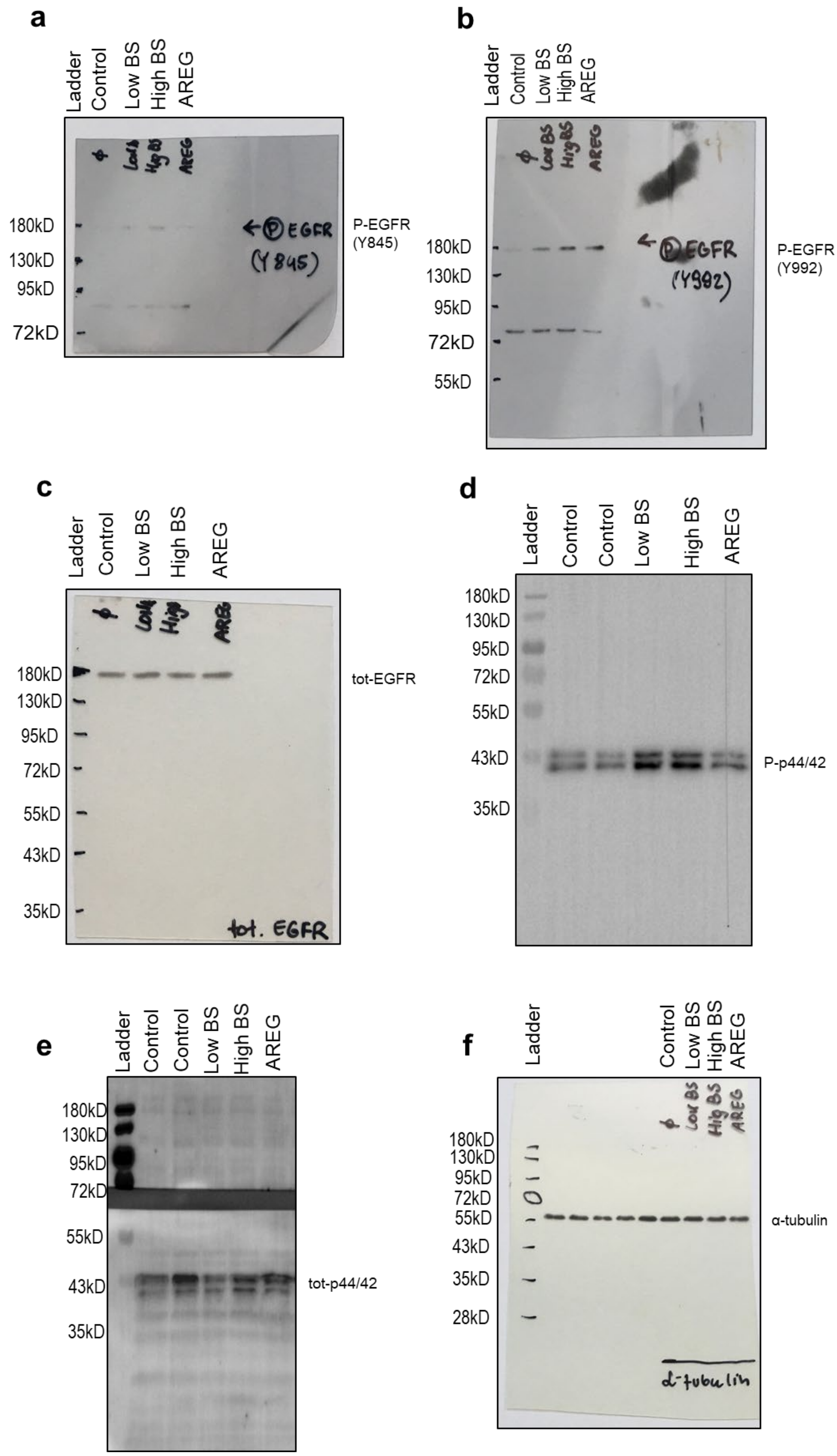

***Supplementary Fig. 3. Cellular composition of bronchospheres generated by IPF-ABCs.*** IPF-ABCs were cultured in matrigel applying a transwell system for 21 days as described*. Overlay and single original registrations of the generated bronchospheres obtained by confocal laser microscopy are depicted. At day 21 the bronchospheres consisted mainly out of KRT5+KRT6+KRT17+ basal cells. Only few cells expressed Muc5AC, a marker for secretory cells. Using our conditions, we did not observe differentitation into a-tubulin positive ciliated cells or scgb1A1 positive club cells. Representative registrations of five independent experiments are shown.*

## Supplementary Figure 4



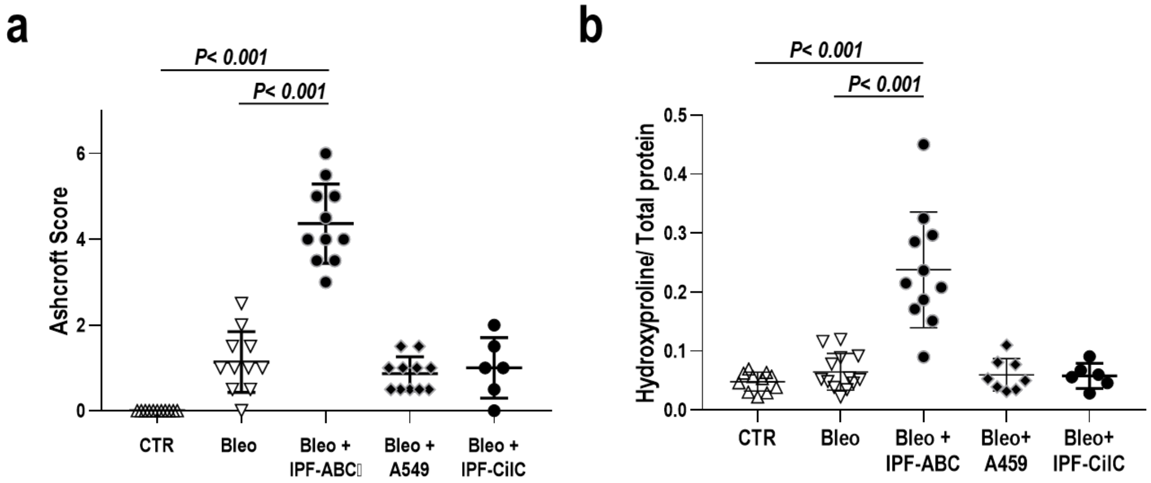

***Supplementary Fig. 4.*** *Uncropped version of the blots shown in* ***Fig. 2m*** *of the manuscript. Uncropped blot depicting* ***a,*** *fibronectin,* ***b,*** *α-SMA and* ***c,*** *collagen I and* ***d,*** *α-tubulin expression of lung fibroblasts, which were stimulated with conditioned media obtained from NU-ABC (low bronchosphere counts) or IPF-ABC (high bronchosphere counts) harvested at day 14.*

## Supplementary Figure 5



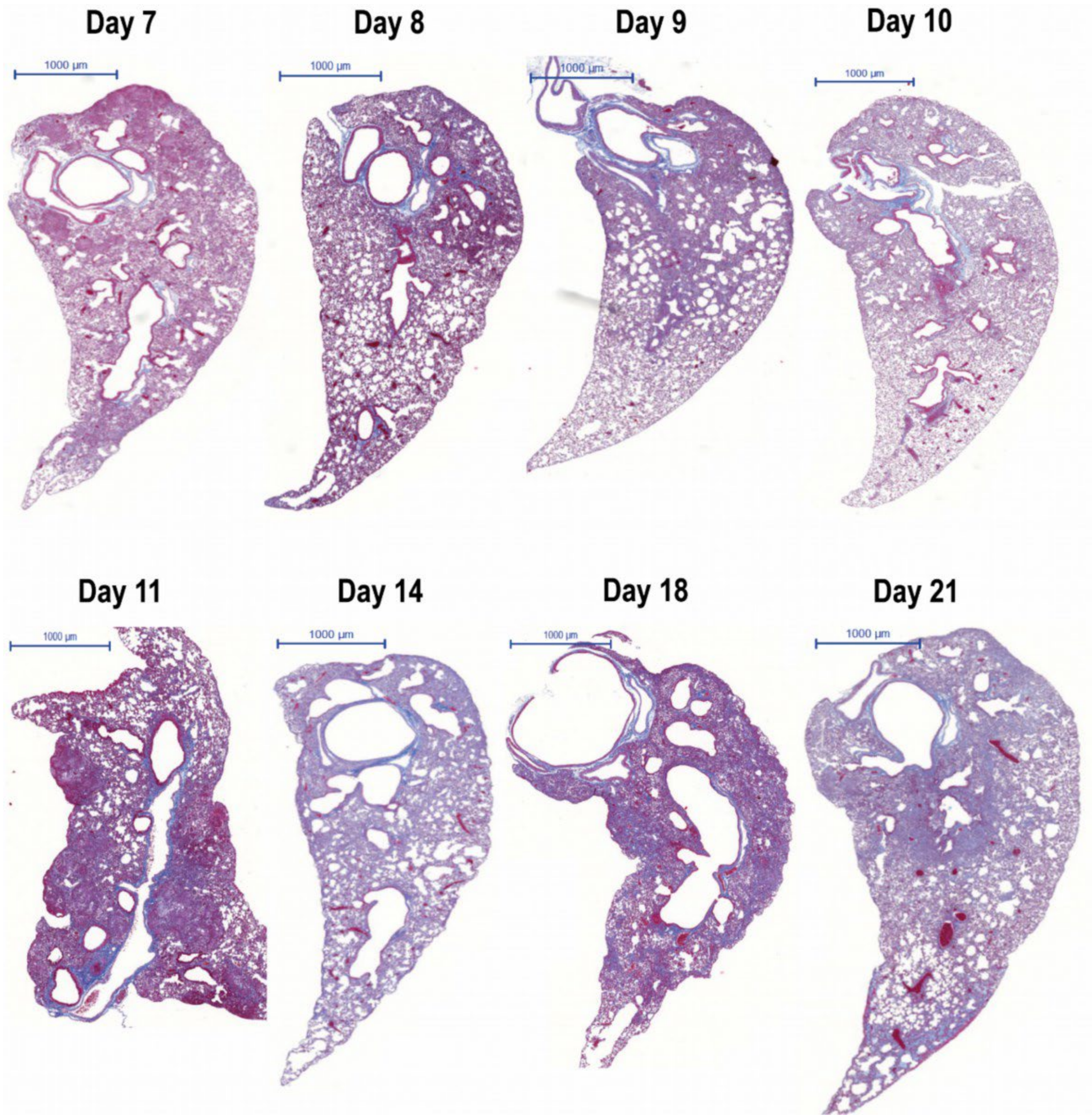

***Supplementary Fig. 5.*** *Uncropped version of the blot shown in* ***Fig. 2p*** *of the manuscript.* ***a,b,c,d,e*** *EGFR phosphorylation and* ***f,*** *α-tubulin expression in lung fibroblasts which were cultured in pooled conditioned media of bronchospheres derived from IPF-ABCs (High BS) in comparison to conditioned media of bronchospheres derived from NU-ABCs (Low BS).*

## Supplementary Figure 6



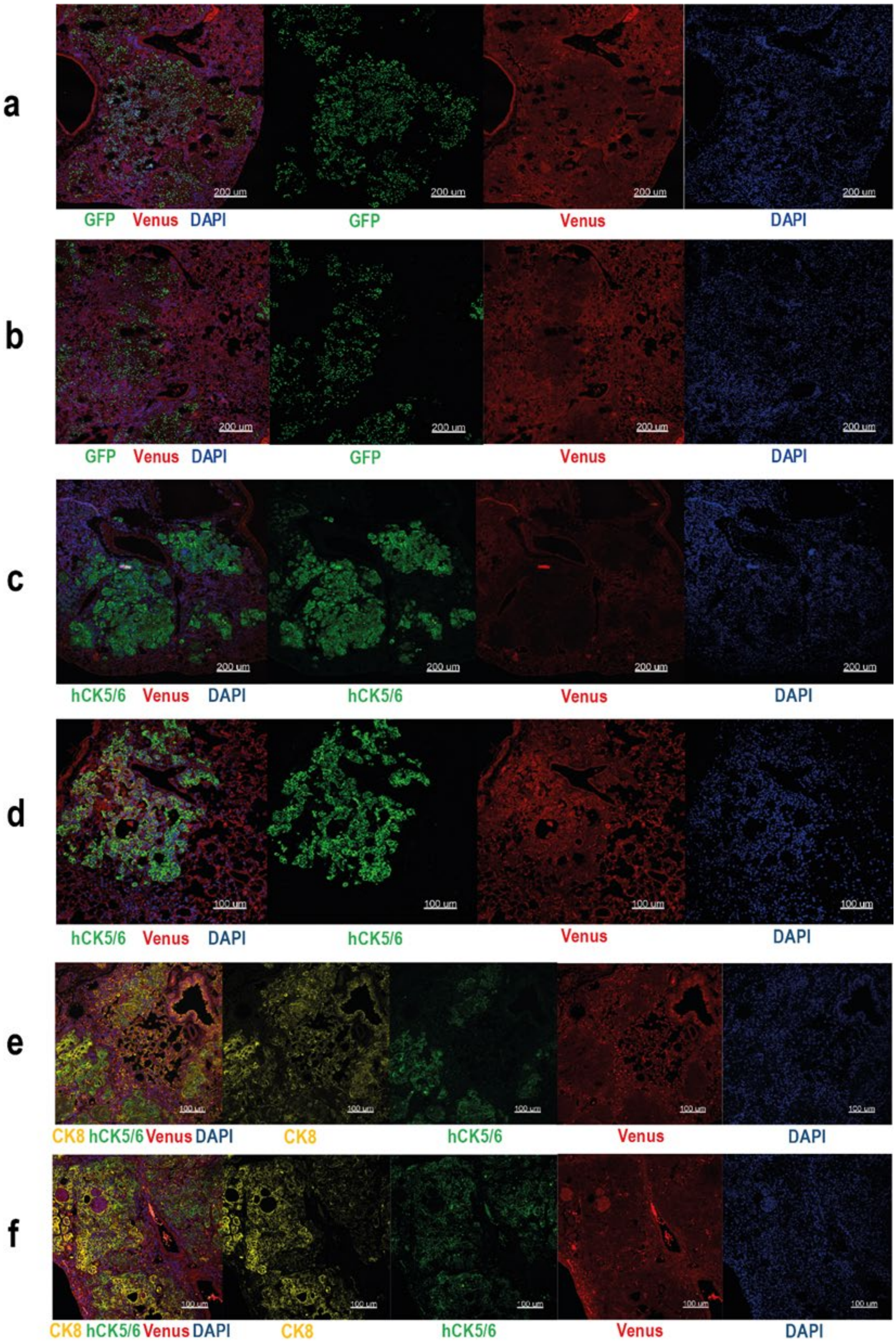

***Supplementary Fig. 6. Airway basal cells of IPF patients (IPF-ABC) but not A549 or ciliated cells augment fibrosis in bleomycin challenged NRG mice.*** *NRG mice received either PBS (CTR) or bleomycin (Bleo) intratracheally and some mice (Bleo + IPF-ABC) additionally three days later 200.000 IPF-ABCs, A549 or ciliated epithelial cells (CilC) intratracheally. Ciliated cells were isolated from air liquid interface (ALI) cultures derived from IPF-ABCs. Lungs of mice were harvested for either histopathological scoring (Ashcroft (mean ± SD),* ***a,*** *or hydroxyproline measurements (mean ± SD)* ***b,*** *CTR denotes for control mice; Bleo denotes for bleomycin; IPF-ABC denotes for airway basal cells derived from IPF patients. IPF-CilC denotes for ciliated epithelial cells derived from IPF patients. One-way ANOVA with Tukey correction for multiple testing (a,b).*

## Supplementary Figure 7



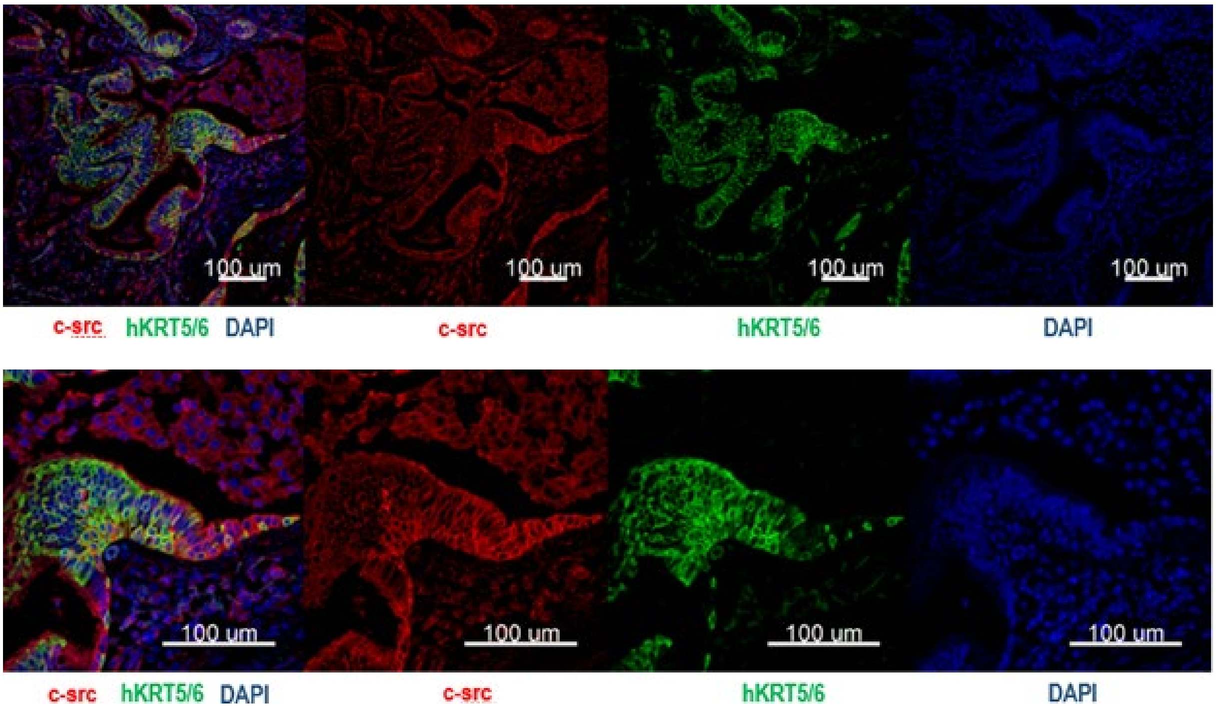

***Supplementary Fig. 7: Severely affected mice had to be euthanized at different time points and lungs of these mice suggest that fibrotic remodeling is visible from day 8.*** *NRG mice received bleomycin per intratracheal (i.t.) injection and human IPF-ABCs derived from different patients were administered i.t. three days later. There is heterogeneity in regards to the induced fibrotic remodeling, evolution of cystic lesions and traction bronchiectasis as well as evolution of dysplastic epithelial lesions between the human IPF-ABC lines. No systematic experiment with no replicates. Shown are lungs from mice that had to be euthanized prior to day 21.*

# Supplementary Figure 8



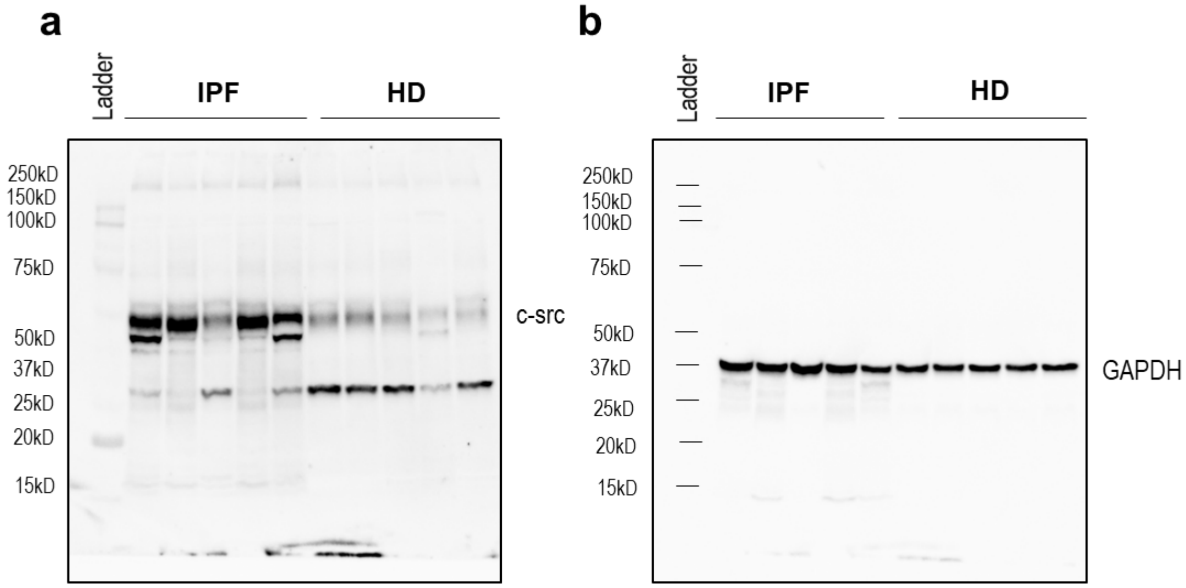

***Supplementary Fig. 8. Overlay and single original registrations obtained by confocal laser microscopy which are depicted in Fig. 2i.*** *NSG mice received bleomycin i.t. at day 0 and three days later human IPF-ABCs which were transduced with a lentiviral vector encoding for eGFP. Lungs were harvested at day 21.* ***a,b,*** *Shown are eGFP staining in green, Venus expression in red, and nuclei staining by DAPI in blue.* ***c,d,*** *Shown are human cytokeratin 5/6 (hCK5/6) staining in green, Venus expression in red and nuclei staining by DAPI in blue* ***e,f,*** *Shown are human cytokeratin 5/6 (hCK5/6) staining in green, cytokeratin 8 (CK8) staining in yellow, Venus expression in red and nuclei staining by DAPI in blue. Scale bars, 50µm. Representative registrations of six independent experiments with similar results are shown.*

## Supplementary Figure 9



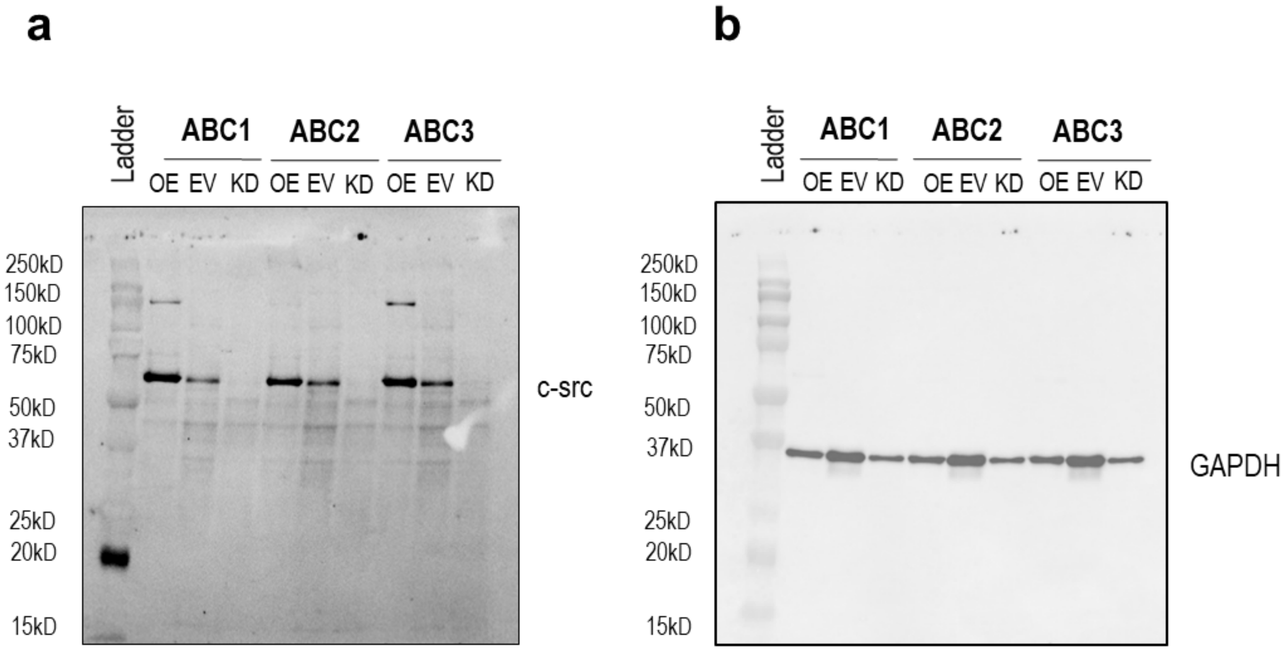

***Supplementary Fig. 9. C-SRC is highly expressed in human lung tissues derived from patients with IPF.*** *Overlay and single original registrations of human IPF lung tissue obtained by confocal laser microscopy which are depicted in Fig. 4B. Additional original registrations with higher magnification are depicted in the lower panel. KRT5/6 staining signal in green, c-src staining in red and nuclei staining by DAPI in blue. Representative registrations of ten independent experiments with similar results are shown.*

## Supplementary Figure 10



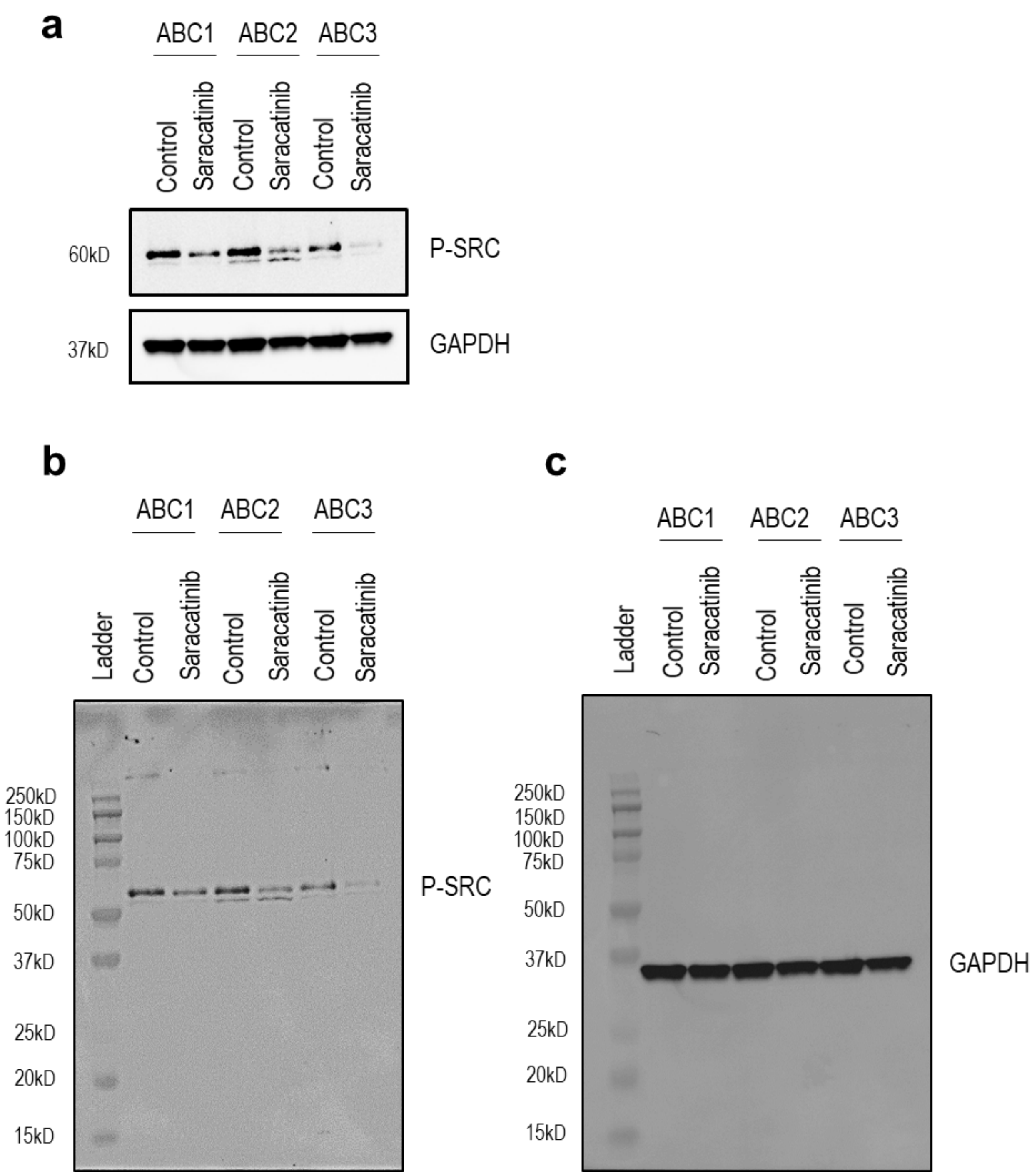

***Supplementary Fig. 10.*** *Uncropped version of the cropped blot shown in* ***Fig. 4d****. of the manuscript.* ***a,*** *un-cropped blot depicting c-src protein expression and* ***b,*** *uncropped blot detecting GAPDH expression in lung tissue homogenates of IPF-patients compared to healthy donors.*

## Supplementary Figure 11



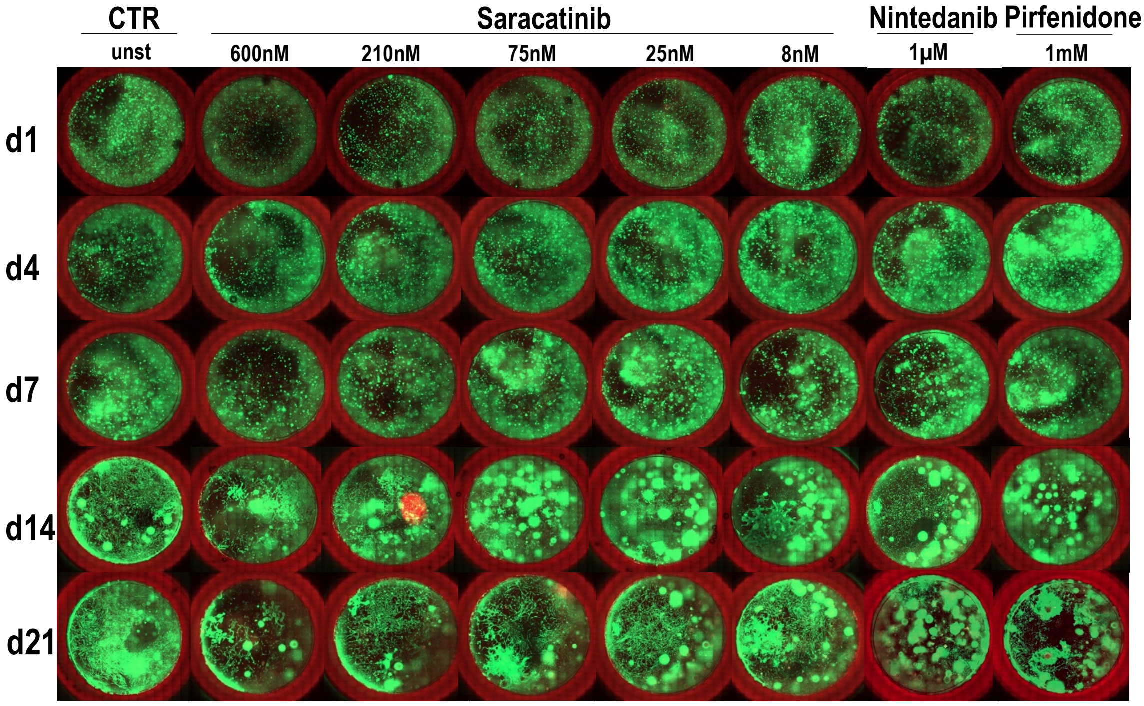

***Supplementary Fig. 11.*** *Uncropped version of the cropped blot shown in* ***Fig. 4f*** *of the manuscript.* ***a,*** *uncropped blot of c-src protein expression and* ***b,*** *uncropped blot of GAPDH expression in untouched, lentiviral vector transduced or knockdown of human IPF-ABCs.*

## Supplementary Figure 12




***Supplementary Fig. 12.*** *Down-regulation of phosphorylated c-src by saracatinib treatment in IPF-ABCs. Depicted are cropped and uncropped blots of IPF-ABCs which were either untreated or saracatinib treated.* ***a,*** *cropped version of the blot which shows down-regulation of phosphorylated c-src in IPF-ABCs by saracatinib treatment. A representative blot of two independent experiments with similar results is shown.* ***b,*** *uncropped version of the blot which depicts pospho-src.* ***c,*** *uncropped version of the blot which depicts GAPDH expression.*

## Supplementary Figure 13




***Supplementary Fig. 13. Live death staining of bronchosphere cultures with IPF lung fibroblasts.*** *Green staining detects vivid cells while red staining detects dead cells. In all bronchosphere cultures w/wo treatment with saracatinib, pirfenidone or nintedanib hardly any dead cells were detected at day 4 (d4), day 7 (d7), day 14 (d14), and day 21 (d21). Representative microphotographs of three independent experiments with similar results are depicted.*