Supplementary data for:

IL-11 disrupts alveolar epithelial progenitor function

Rosa K. Kortekaas[1,2*], Kerstin E. Geillinger-Kaestle[3], Kaoutar Belharch[1,2], Megan Webster[3**], Wim Timens[2,4], Janette K. Burgess[2,4***], Reinoud Gosens[1,2***]

1: Department of Molecular Pharmacology, University of Groningen, Groningen, the Netherlands

2: University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, the Netherlands

3: Department of Immunology and Respiratory Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany.

4: University of Groningen, University Medical Center Groningen, Department of Medical Biology and Pathology, Groningen, the Netherlands

*: corresponding author: r.k.kortekaas@rug.nl

Department of Molecular Pharmacology, University of Groningen

Antonius Deusinglaan 1

9713 AV Groningen, the Netherlands

**: current affiliation: Newcells Biotech, Newcastle upon Tyne, United Kingdom

***: authors contributed equally

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Methods

Animal handling

Pathogen-free wild type C57BL/6J mice (>8 weeks of age, both male and female) were used in this study. Animals were housed under a 12 hour light/dark cycle with controlled humidity at room temperature (24 ± 1 °C). Water and food were provided ad libitum. All experiments were performed according to the national guidelines and upon approval of the experimental procedures by the local Animal Care and Use committee of the University of Groningen.

Immunohistochemistry

The immunohistochemical stainings performed in this manuscript were conducted as part of the HOLLAND (HistopathOLogy of Lung Aging aNd COPD) project executed at the department of Pathology and Medical Biology of the University Medical Center Groningen. Lung tissue was derived from left-over lung tissue derived from lung resection and lung transplant surgeries. The study protocol was consistent with the Research Code of the University Medical Center Groningen (https://umcgresearch.org/nl/w/research-code-umcg), as well as Dutch national guidelines on ethics and professionalism (https://www.coreon.org/). Lung tissue from tumor resection surgery was taken as far away as possible from the tumor site and all tissues were checked by an experienced pathologist to ensure no abnormalities were present. For this manuscript histologically normal control lung tissue (hereafter termed 'control') and tissue from individuals with IPF was included.

Paraffin-embedded human lung tissue of control and IPF donors was cut with a Microm HM355S microtome (Thermo Fisher Scientific, Waltham, MA, USA) into serial sections of 3 µm. In the clinical characteristics of the donors (table 1 of manuscript) we included the best FEV1/FVC value that was available from either the pre or post BD measurements. Immunohistochemistry for the detection of either IL-11 or IL-11R was performed in one batch for all sections. Tissue sections were deparaffinised in xylene and rehydrated through a serial dilution of ethanol. Heat-induced epitope retrieval was performed in a Pascall S2800 DakoCytomation pressure cooker using 0.1M Tris/HCl buffer pH=9.0.

Sections were washed in PBS and incubated overnight at 4°C with primary antibodies rabbit anti-IL-11 (LSBio, Seattle, WA, USA, #LS-B15705) or rabbit anti-IL-11R (Abcam, Cambridge, UK, #ab125015, RRID: AB_109750018), diluted 1:100 (final concentration 17.1 µg/ml) or 1:400, respectively, in 1% (w/v) BSA 2% donkey serum (Jackson Immunoresearch, West Grove, PA, USA, #017-000-121, RRID: AB_2337258) in PBS. Sections were then washed with PBS and incubated with 0.3% H2O2 in PBS for 30 min. Subsequently, sections were incubated with the secondary antibody peroxidase-conjugated donkey anti-rabbit (Jackson Immunoresearch, #711-035-152, RRID: AB_10015282) diluted 1:500 in 1% BSA 2% donkey serum in PBS for 30 min at room temperature. Finally, the immunohistology reaction product was developed with NovaRED (Vector Laboratories, Burlingame, CA, USA #SK-4800, RRID: AB_2336845) for 10 min, followed by hematoxylin counterstain. They were then dehydrated through a series of ethanol-xylene and covered with xylene and Tissue-Tek® coverslipping film (Sakura Finetek, Tokyo, Japan, #4770). Negative control slides were included which were handled equally, except no primary antibody was added. Slides were scanned with a Hamamatsu NanoZoomer 2.0HT digital slide scanner (Hamamatsu Photonic K.K., Hamamatsu City, Japan) at magnification 40x. Aperio ImageScope V.12.4.3 (Leica Biosystems, Wetzlar, Germany) was used to view digital sections and to obtain descriptive images. For quantitative analysis, artefacts such as tar, air bubbles and fibers were removed from images, which were otherwise unaltered. Per immunohistochemical staining, representative images of weak to strong staining were used to set a color deconvolution vector which adequately separated the blue (hematoxylin) and red (NovaRED) channel, and to set a threshold for both the blue (hematoxylin) and red (NovaRED) channels separately. Fiji/ImageJ software (ImageJ 2.1.0) (1) was used to quantify the mean intensity and percentage of positive stained area for each protein. Data analyses were performed using R software V.4.0.0 (Boston, Massachusetts, USA) using the following formulas:

$$Area\ (\%) = \frac{Number\ of\ pixels\ positive\ for\ NovaRed}{number\ of\ pixels\ in\ total\ tissue} * 100$$

$$Mean\ intensity = 255 - \frac{Sum\ of\ intensities\ of\ pixels\ positive\ NovaRed}{Total\ number\ of\ pixels\ positive\ for\ NovaRed}$$

Fibroblast isolation and cell culture

Primary human lung fibroblasts of control and IPF donors were isolated from resected tissue and from lungs that were excised during organ transplantation respectively. Lung tissue was cut into blocks of 1 mm^3 and 2 blocks were transferred into 1 well of a 12-well plate, allowed to attach and cultured with 1 ml Ham's F12 with 10% (v/v) fetal bovine serum (FBS, PAA Laboratories, Pasching, Austria), 100 U/ml penicillin/streptomycin (Gibco under Thermo Fisher Scientific, Waltham, MA, USA, #15070-063) and 1% glutamax (Gibco, #35050-061) until sufficiently expanded. Mouse CCL206 lung fibroblasts (CCL-206; ATCC, Wesel, Germany, RRID: CVCL_0437) were cultured in DMEM (Lonza, Basel, Switzerland, #42430):Ham's F12 (Gibco, #21765) (1:1) supplemented with 10% FBS, 2 mM L-glutamine (Life Technologies under Thermo Fisher Scientific, Waltham, MA, USA, #35050–061), 100 U/ml penicillin/streptomycin, and 1% amphotericin B (Gibco, #15290-026). All cells were maintained at 37°C with 5% CO_2 and confirmed to be mycoplasma negative when used.

Organoid immunofluorescence

Organoid cultures were fixed and stained according to published protocols (2) using rabbit anti-Prosurfactant Protein C (pro-SPC) (MilliporeSigma, Burlington, MA, USA, #AB3786, RRID: AB_91588) and mouse anti-Acetylated α Tubulin (ACT) (Santa Cruz Biotechnology, Dallas, TX, USA, #sc-23950, RRID: AB_628409) diluted 1:200 in 0.1% BSA 0.1% Triton-X100 in PBS, and secondary antibodies donkey anti-rabbit Alexa fluor 488 (Thermo Fisher Scientific #A21206, RRID: AB_2535792) and donkey anti-mouse Alexa fluor 568 (Thermo Fisher Scientific #A10037, RRID: AB_2534013) diluted 1:200 in 0.1% BSA 0.1% Triton-X100 in PBS.

Mouse organoids for RNA-sequencing

Epithelial ($CD31^-$/$CD45^-$/$EpCam^+$) cells were isolated as previously described (2), after which 300,000 epithelial cells were mixed with 300,000 proliferation inactivated CCL206 fibroblasts and seeded into

6 well plates in 1 ml growth factor-reduced Matrigel diluted 1:1 with DMEM/Ham's F12 containing 10% FBS, 2 mM L-glutamine, and antibiotics. After 72 hours, Matrigel was digested with dispase for 45 minutes at 37 °C, and organoids were further disrupted with trypsin for 5 minutes at 37 °C. Cells were resorted into EpCam+ and CCL206 cells by using CD326 (EpCam) microbeads (Miltenyi #130-105-958) on LS columns (Miltenyi #130-091-051). Finally, RNA was isolated from both cell fractions separately using NucleoSpin® RNA kit (Macherey-Nagel, Düren, Germany, #740955.250) according to manufacturer's instructions, and subjected to bulk RNA-sequencing as described below.

Western blot

CCL206 fibroblasts were seeded in a 6-wells plate at a density of 500,000 cells per well and allowed to settle for 24 hours, after which they were serum-starved for 24 hours with 0.5% FBS in DMEM:Ham's F12 (1:1) supplemented with 2 mM L-glutamine, 100 U/ml penicillin/streptomycin, and 1% amphotericin B. They were treated with 1-100 ng/ml recombinant mouse IL-11 (Peprotech, Cranbury, NJ, USA, #220-11) or rhIL-11 (R&D systems, #218-IL) for 30 minutes, then washed with ice-cold PBS and lysed in modified RIPA buffer with protease inhibitors aprotinin, leupeptin, and pepstatin, and phosphatase inhibitors β-glycerolphosphate (Ser/Thr phosphatase inhibitor), sodium orthovanadate (Tyr phosphatase inhibitor) and sodium fluoride (Ser/Thr phosphatase inhibitor), and finally subjected to sonification. 30 µg of protein and 5 µl PageRuler Plus Protein Ladder (Thermo Scientific, #26620) were loaded on a 10% SDS-PAGE gel and run using 1x ELFO (0.025 M Tris, 0.25 M glycine and 0.1% SDS in UP water). Proteins were transferred to a nitrocellulose membrane using transfer buffer (0.025 M Tris, 0.192 M glycine, 0.1% SDS and 20% methanol (v/v) in UP), after which the membrane was cut between the 70 and 55 kDa markers. Membranes were blocked for 1.5 hours with 1x ROTI block (Carl Roth, Karlsruhe, Germany, #A151.2) for α-tubulin, ERK and p-ERK, 5% milk for STAT3 and 5% BSA for p-STAT3. Membranes were incubated with the primary antibodies mouse α-tubulin (final concentration 0.5 µg/ml, Sigma-Aldrich, #T6074, RRID: AB_477582), rabbit ERK1/2 (1:1000, Cell Signaling, Danvers, MA, USA, #9102, RRID: AB_330744), rabbit p-ERK1/2 (1:1000, Cell

Signaling, #9101, RRID: AB_331646), mouse STAT3 (1:1000, Cell Signaling, #9139, RRID: AB_331757) and rabbit p-STAT3 (1:2000, Cell Signaling, #9145, RRID: AB_2491009) overnight at 4 °C with rocking. Subsequently, membranes were incubated with secondary peroxidase-conjugated antibodies goat anti-rabbit (final concentration 0.33 µg/ml, Sigma-Aldrich, #12-348, RRID: AB_390191) or rabbit anti-mouse (1:3000, Sigma-Aldrich, #A9044, RRID: AB_258431) for 2 hours at room temperature with rocking. Protein bands were visualized using ECL Western Blotting Substrate in a GBox iChemi XR system (Syngene, Bangalore, India) using GeneSnap ver 7.12 and quantified using GeneTools ver 4.01 software. Band intensity of the protein of interest was normalized to the intensity of α-tubulin.

Precision cut lung slices

C57BL/6J mice were anesthetized by subcutaneous injection of 400 mg/kg Ketamidor® and 1 mg/kg Dexdomitor®. After anesthesia was confirmed, the animal was euthanized by exsanguination, after which the lungs were inflated with 1.5% low-melting point agarose (Gerbu Biotechnik GmbH, Wieblingen, Germany) solution in $CaCl_2$ (0.9 mM), $MgSO_4$ (0.4 mM), KCl (2.7 mM), NaCl (58.2 mM), NaH_2PO_4 (0.6 mM), glucose (8.4 mM), $NaHCO_3$ (13 mM), HEPES (12.6 mM), sodium pyruvate (0.5 mM), glutamine (1 mM), MEM-amino acids mixture (1:50) and MEM-vitamins mixture (1:100) pH=7.2. After inflation, lungs were removed and placed on ice for 15 minutes to allow the agarose to solidify. Subsequently, the lungs were separated in lobes and placed into cores. A tissue slicer (CompresstomeTM VF-300 microtome, Precisionary Instruments, San Jose, CA, USA) was used to cut precision cut lung slices (PCLS) of 250 µm in thickness in $CaCl_2$ (1.8 mM), $MgSO_4$ (0.8 mM), KCl (5.4 mM), NaCl (116.4 mM), NaH_2PO_4 (1.2 mM), glucose (16.7 mM), $NaHCO_3$ (26.1 mM), HEPES (25.2 mM), pH=7.2. Slices were washed 4 times for 30 minutes (2 hours total). Three slices were placed in one well of a 12-well plate and incubated with DMEM (Lonza, #42430) supplemented with 0.6% amphotericin B, 1 mM sodium pyruvate (HyClone Laboratories, Logan, UT, USA, #SH30239.01), 1% MEM non-essential amino acids (Gibco, #11130036), 0.6% gentamycin (Sigma-Aldrich, #G1272) and

100 U/ml penicillin/streptomycin, and 100 ng/ml rhIL-11 for 48 hours. The slices were collected and stored at -80 °C until further use.

Gene expression analyses

For gene expression studies, 300,000 primary fibroblasts of control and IPF donors of passage 5 until 7 were seeded in 6-well culture plates with low glucose DMEM (Biowest, Nuaillé, France, #L0064-500), supplemented with 10% FBS, 100 U/ml penicillin/streptomycin and 1% glutamax. The cells were allowed to settle for 24 hours and were subsequently serum deprived for 24 hours with low glucose DMEM with 0.1% BSA, glutamax and antibiotics. Cells were then exposed to rhIL-11 for 24 hours, after which they were collected in TRIzol (Invitrogen under Thermo Fisher Scientific, Waltham, MA, USA, #15596018). Total RNA was extracted from primary human lung fibroblasts according to manufacturer's instructions. The Maxwell simplyRNA tissue kit (Promega, Madison, WI, USA, #AS1280) and Maxwell 16 Instrument (Promega) were used to isolate RNA from mouse PCLS according to manufacturer's instructions. Total RNA concentrations were determined with a NanoDrop ND-1000 spectrophotometer. Equal amounts of total mRNA were then reverse transcribed (Promega). Real time PCR was performed with SYBR green as the DNA binding dye (Roche Applied Science, Mannheim, Germany) on a 7900HT Fast Real-Time PCR System (Applied Biosystems under Thermo Fisher Scientific, Waltham, MA, USA), with denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds for 40 cycles followed by 5 minutes at 72°C. Gene expression was normalized to *B2M*, *SDHA* and *HMBS* for human samples (primary fibroblasts) and to *Rpl13a*, *B2m* and *Actb* for mouse samples (PCLS). Fold changes were calculated using the $2^{-\Delta\Delta CT}$ method. The primers used are listed in tables S1 and S2.

RNA-sequencing

Primary human lung fibroblasts of control donors of passage 5 until 7 were seeded at a density of 300,000 cells per well in 6-well culture plates, allowed to settle for 24 hours, serum-starved for 24

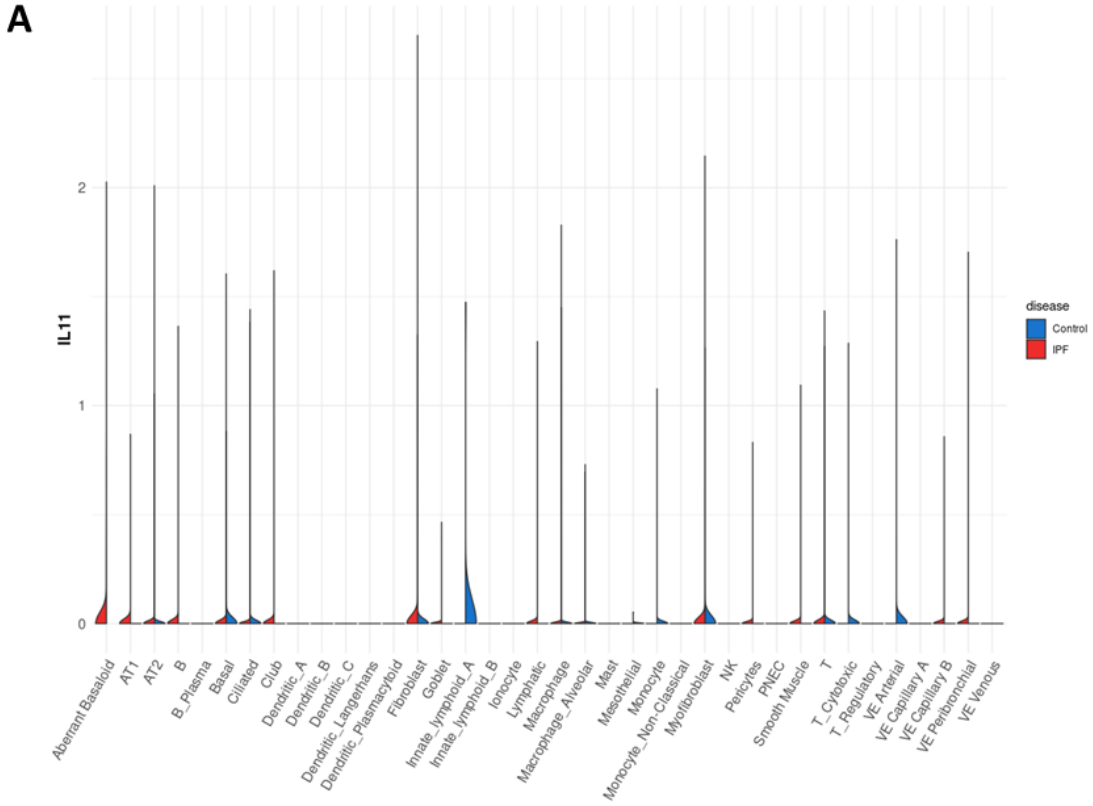
hours and then exposed to 100 ng/ml rhIL-11 for 24 hours. The fibroblasts were then lysed in 1 mL of TRIzol reagent and total RNA was isolated according to the manufacturer's instructions.

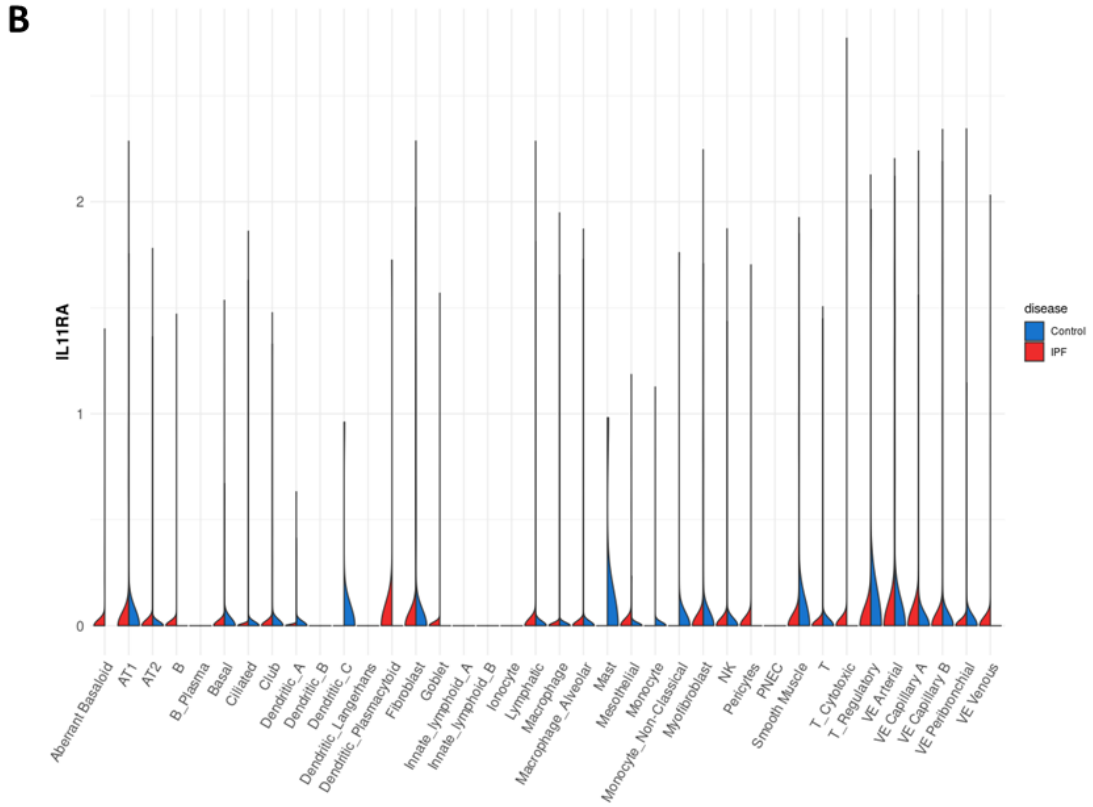
Total RNA concentrations were initially determined with a NanoDrop ND-1000 spectrophotometer and quantified in detail using Bio-analyzer fragment analyser from Agilent. An Illumina NovaSeq 6000 sequencer was used for bulk RNA-seq data analysis by GenomeScan (the Netherlands). The procedure included data quality control, adapter trimming, alignment of short reads and feature counting. Library preparation was checked by calculating ribosomal (and globin) content. Checks for possible sample and barcode contamination were performed and a set of standard quality metrics for the raw data set was determined using quality control tools (FstQC v0.34 and FastQA). Prior to alignment, the reads were trimmed for adapter sequences using Trimmomatic v0.30. To align the reads of each sample, the human reference Ensembl GRCh37.75 was used. Gene duplicates were removed. Differentially expressed genes (DEGs) were determined using paired-sample analysis in the DESeq2 package 1.34.0 in R 4.1.0, which were considered significant when padj<0.05. All significant and non-significant DEGs were used for pathway enrichment analysis using the fgsea package 1.20.0 in R, and pathways were considered significant when padj<0.05. A volcano plot was generated using the Enhanced Volcano package in R, with a cut-off value of 0.5 for Log2Fold change and 0.05 for padj value.

Data analyses

For sequencing data, statistical analyses were performed in R as described above. Statistical evaluation of all other data were performed using GraphPad Prism 8. Data are presented with mean and standard deviation unless described otherwise in figure legends, and N refers to the number of biological replicates (number of animals or individual donors). Prior to the studies, power calculations were performed using organoid number as primary read-out parameter at α 0.05 and power 0.8. A total of 10 biological replicates were found to be necessary. No exclusion criteria were set, and as such no animals or data points were excluded from any studies described here. Data were checked

for normality using the Shapiro-Wilk test, and log transformed if necessary. Data that were normalized to vehicle control were always log transformed. For normally distributed data, a two-tailed t-test, a one-way ANOVA or a two-way ANOVA was performed, whereas a Wilcoxon test or a Kruskall-Wallis test was used for nonparametric data, where appropriate. The statistical tests used are also specified in the figure legends. Differences were considered statistically significant when $p<0.05$.


A
IL11
2
1
0
disease
Control
IPF
Aberrant Basaloid
AT1
AT2
B
B_Plasma
Basal
Ciliated
Club
Dendritic_A
Dendritic_B
Dendritic_C
Dendritic_Langerhans
Dendritic_Plasmacytoid
Fibroblast
Goblet
Innate_lymphoid_A
Innate_lymphoid_B
Ionocyte
Lymphatic
Macrophage
Macrophage_Alveolar
Mast
Mesothelial
Monocyte
Monocyte_Non-Classical
Myofibroblast
NK
Pericytes
PNEC
Smooth Muscle
T
T_Cytotoxic
T_Regulatory
VE Arterial
VE Capillary A
VE Capillary B
VE Peribronchial
VE Venous


B
IL11RA
2
1
0
disease
Control
IPF
Aberrant Basaloid
AT1
AT2
B
B_Plasma
Basal
Ciliated
Club
Dendritic_A
Dendritic_B
Dendritic_C
Dendritic_Langerhans
Dendritic_Plasmacytoid
Fibroblast
Goblet
Innate_lymphoid_A
Innate_lymphoid_B
Ionocyte
Lymphatic
Macrophage
Macrophage_Alveolar
Mast
Mesothelial
Monocyte
Monocyte_Non-Classical
Myofibroblast
NK
Pericytes
PNEC
Smooth Muscle
T
T_Cytotoxic
T_Regulatory
VE Arterial
VE Capillary A
VE Capillary B
VE Peribronchial
VE Venous

Figures

Figure S1: the gene expression pattern of IL-11 and IL-11R in the human lung. *RNA sequencing data of the human lung adapted from the IPF cell atlas* (3,4)*, showing the normalized gene expression values of* IL11 *(A) and IL-11 receptor (*IL11RA*) (B) in the various cell types of the lung.*

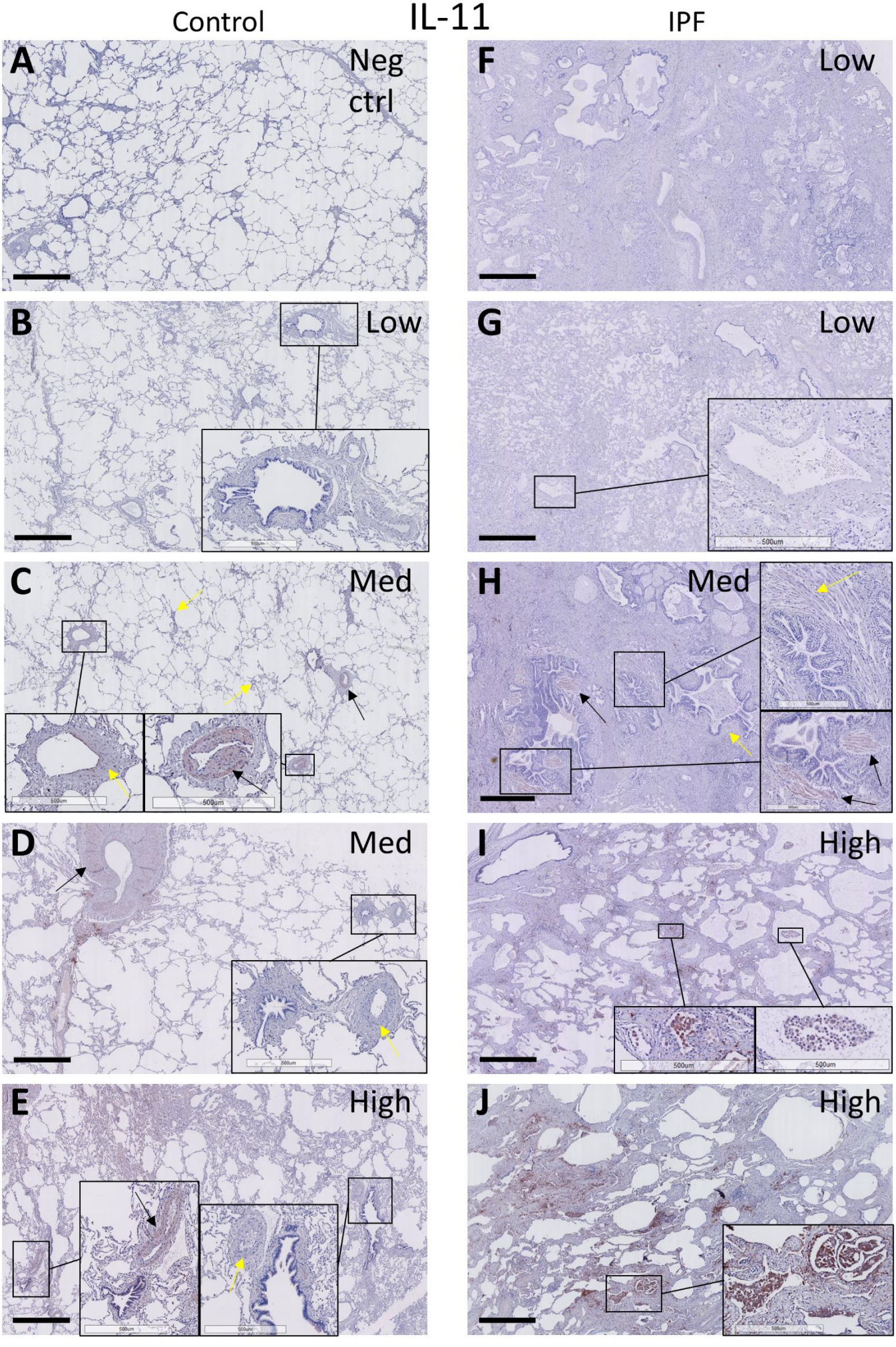
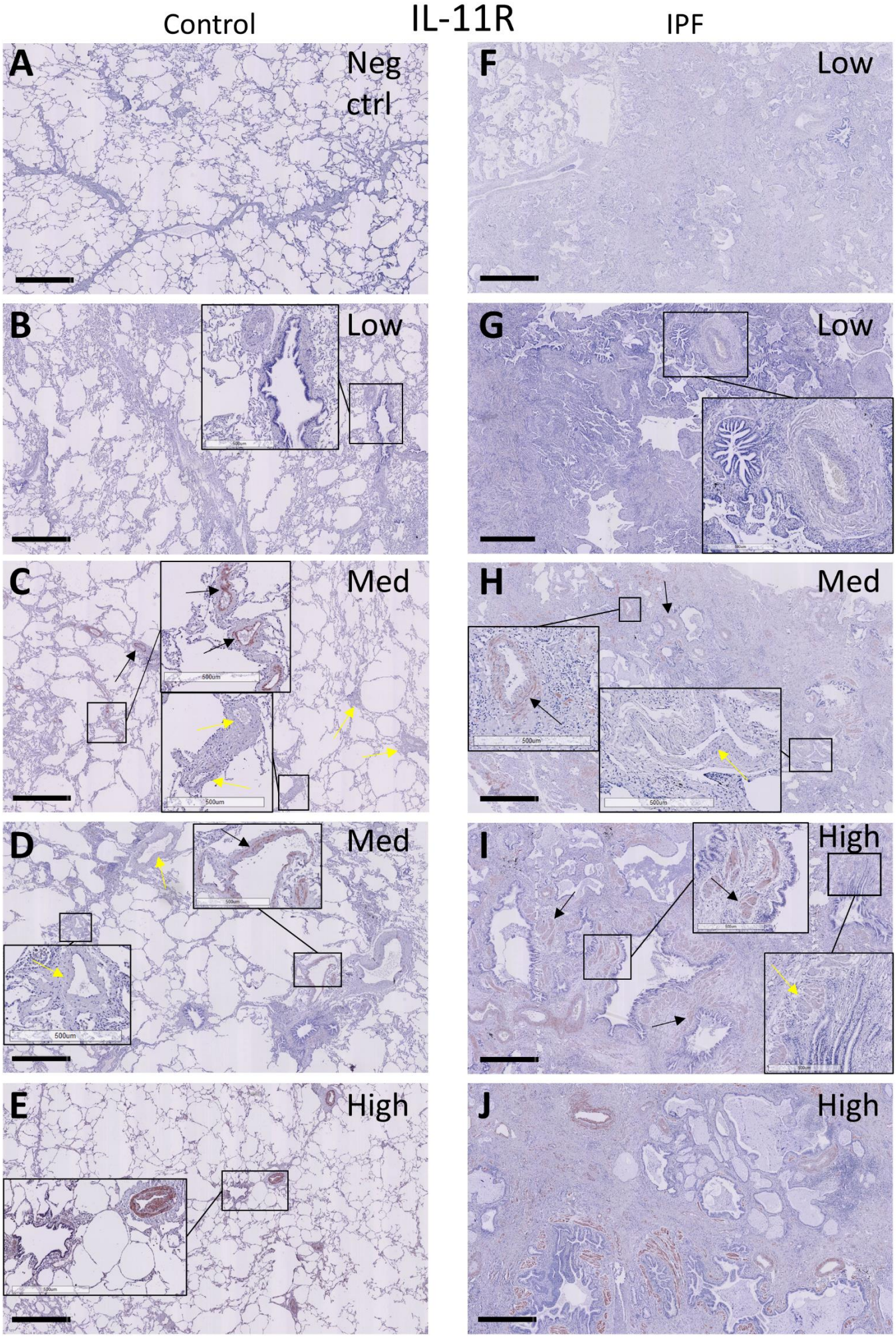

IL-11
Control
IPF
A
Neg ctrl
B
Low
C
Med
D
Med
E
High
F
Low
G
Low
H
Med
I
High
J
High

Figure S2: IL-11 staining in the human lung is variable amongst individuals, but also within single tissue sections. *IL-11 immunohistochemistry was performed on human lung tissue sections of control and idiopathic pulmonary fibrosis (IPF) donors, which was visualized with NovaRED (red). Sections were counterstained with hematoxylin (blue). Labels show whether the staining was relatively low, medium (med), or high. Black arrows indicate positively stained structures, whereas yellow arrows indicate corresponding structures in the same tissue section that appear negative or stained to a lesser extent. (A) shows the negative control. Example images (B-E) are from the control group, whereas (F-I) are from IPF donors. All scale bars in the overview images are 1 mm, scale bars in enlarged images are 500 µm.*


IL-11R
Control
IPF
A
Neg ctrl
B
Low
C
Med
D
Med
E
High
F
Low
G
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Med
I
High
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High
500um

Figure S3: inter- and intra-individual variability of IL-11 receptor (IL-11R) staining in the human lung. *Human lung tissue of control and idiopathic pulmonary fibrosis (IPF) donors were stained for IL-11R, developed with NovaRED (red), and counterstained with hematoxylin (blue). Labels indicate relative level of staining; low, medium (med), or high, compared to other sections. Black arrows show structures positive for IL-11R, and yellow arrows signal the same structures within the tissue section that are negative or stained to a lesser extent. (A) shows the negative control. Representative images (B-E) belong to the control group, and (F-I) are from IPF donors. The scale bars in overview images are 1 mm, in magnified images they are 500 µm.*

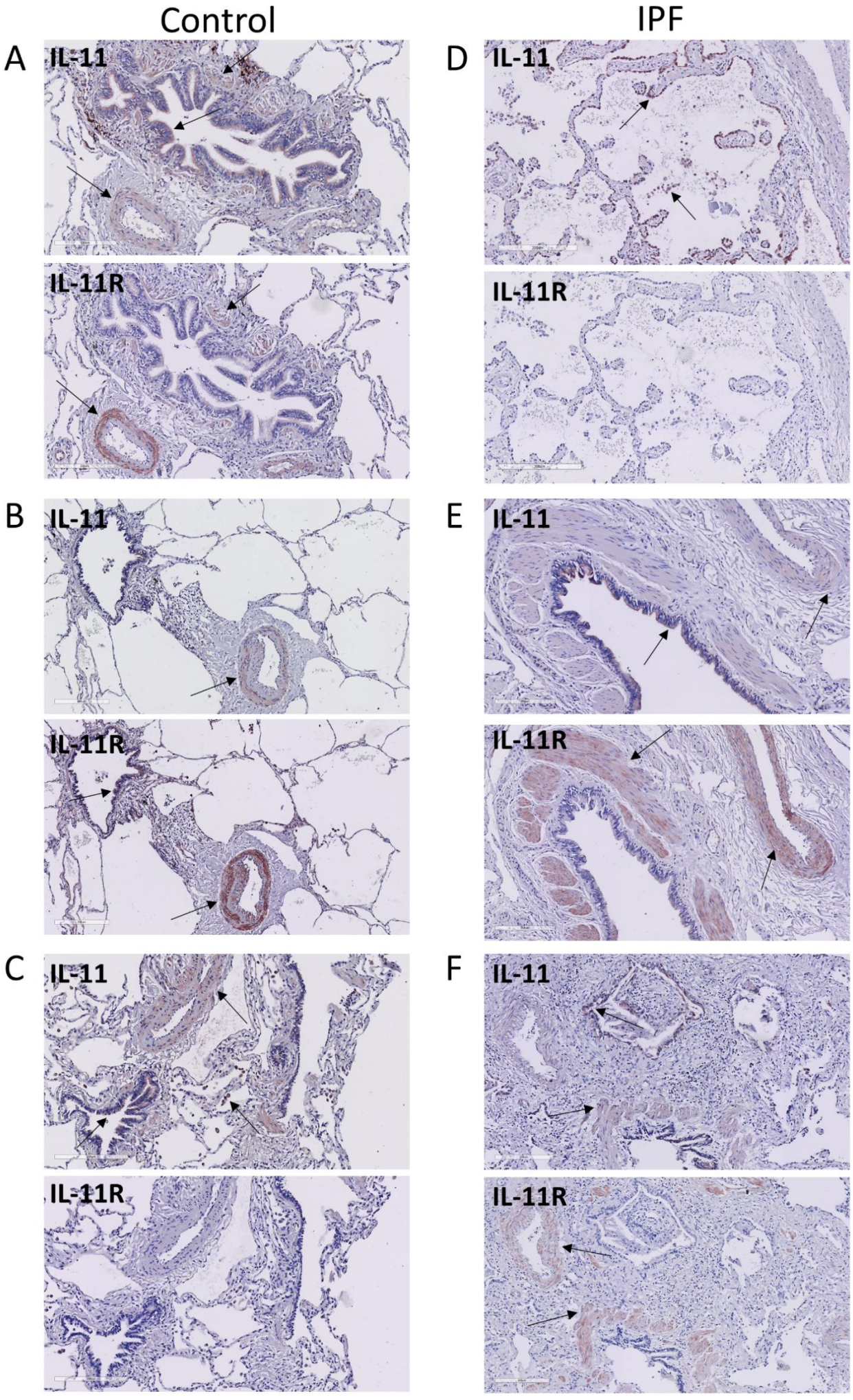
Control
IPF
A
IL-11
IL-11R
D
IL-11
IL-11R
B
IL-11
IL-11R
E
IL-11
IL-11R
C
IL-11
IL-11R
F
IL-11
IL-11R

Figure S4: comparison of IL-11 and IL-11R staining patterns in serial sections. *IL-11 and IL-11R stainings were performed on serial sections from human lung tissue of control and idiopathic pulmonary fibrosis (IPF) donors. Stainings were developed with NovaRED (red), and counterstained with hematoxylin (blue). Black arrows show structures positive for IL-11 or IL-11R staining. (A-C) serial sections are from control tissue, and (D-F) are from IPF donors.*

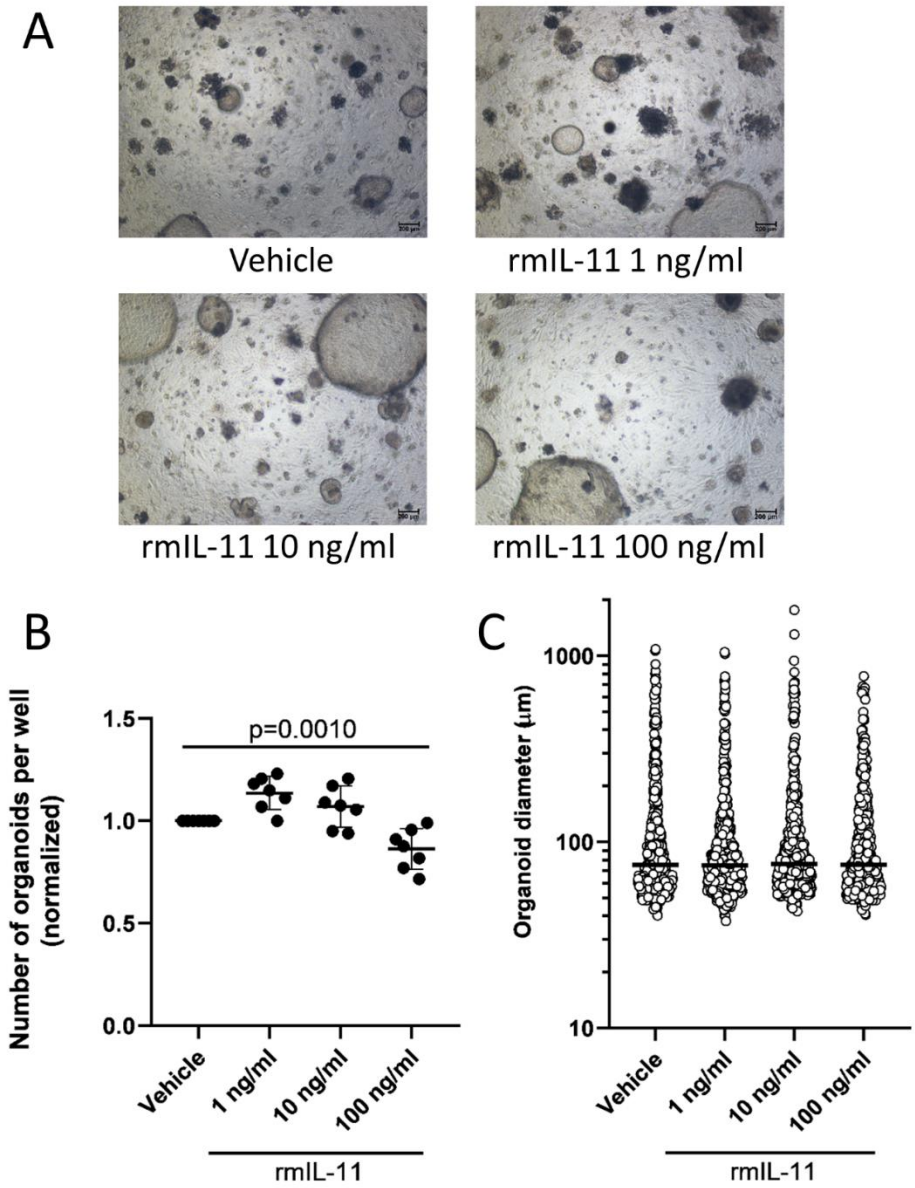

Figure S5: rmIL-11 influences mouse organoid formation. *(A) Representative brightfield images of mouse organoids exposed to a dose curve of rmIL-11. Scale bar = 200 µm. (B) Normalized number of organoids formed in response to 14 day rmIL-11 treatment (N=7, One-way ANOVA with Sidak's post hoc test on log transformed data). (C) Organoid size on day 14 after rmIL-11 exposure, median is shown (N=7, Kruskall-Wallis test with Dunn's post hoc test).*

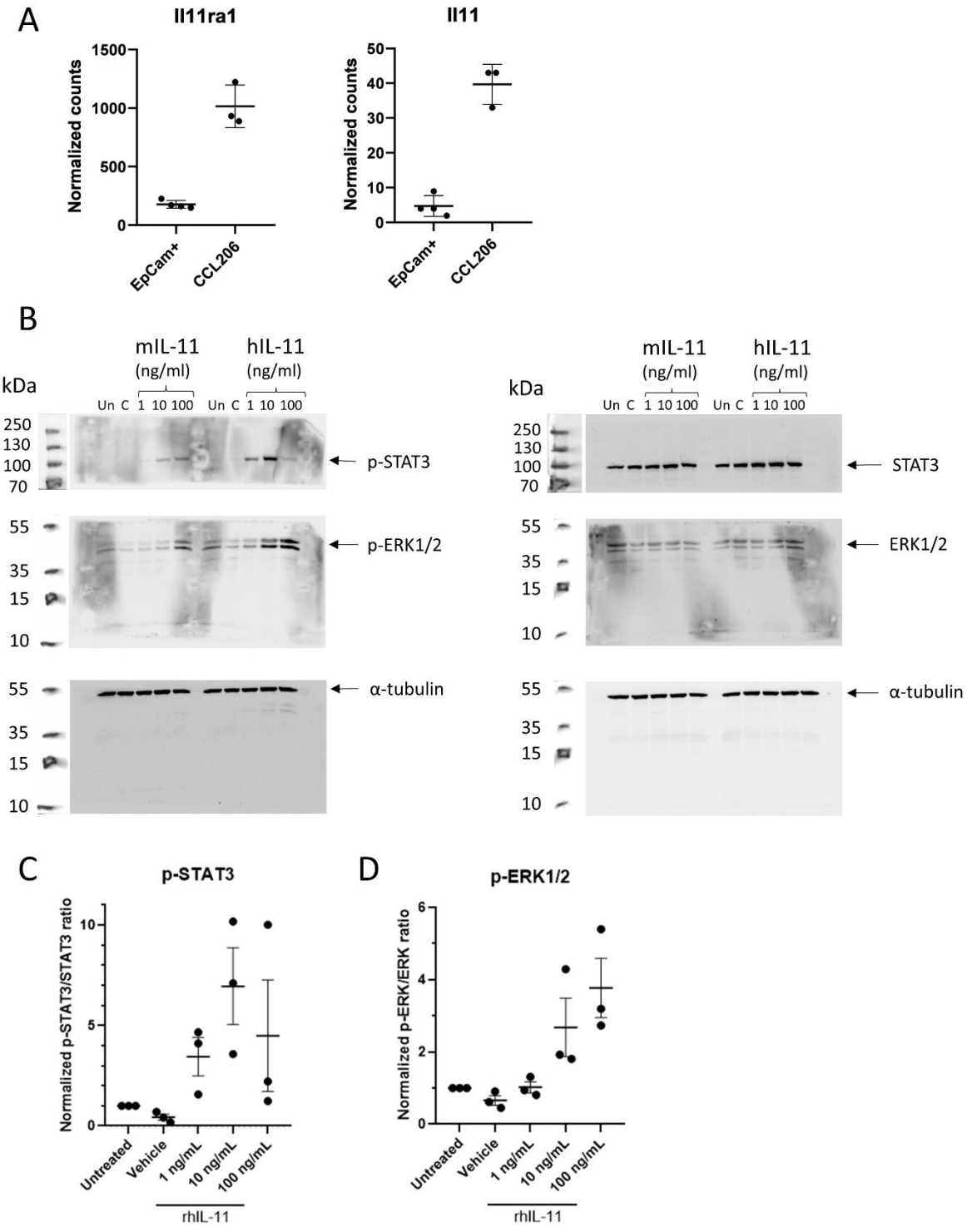
A
Il11ra1
Normalized counts
1500
1000
500
0
EpCam+
CCL206
Il11
Normalized counts
50
40
30
20
10
0
EpCam+
CCL206
B
mIL-11 (ng/ml)
hIL-11 (ng/ml)
kDa
Un C 1 10 100 Un C 1 10 100
250
130
100
70
p-STAT3
55
35
15
10
p-ERK1/2
α-tubulin
mIL-11 (ng/ml)
hIL-11 (ng/ml)
kDa
Un C 1 10 100 Un C 1 10 100
STAT3
ERK1/2
α-tubulin
C
p-STAT3
Normalized p-STAT3/STAT3 ratio
10
5
0
Untreated
Vehicle
1 ng/mL
10 ng/mL
100 ng/mL
rhIL-11
D
p-ERK1/2
Normalized p-ERK/ERK ratio
6
4
2
0
Untreated
Vehicle
1 ng/mL
10 ng/mL
100 ng/mL
rhIL-11

Figure S6: recombinant human IL-11 activates downstream signaling pathways JAK/STAT3 and MEK/ERK in CCL206 fibroblasts. *CCL206 mouse lung fibroblasts were exposed to 1-100 ng/ml rhIL-11 and rmIL-11 for 30 minutes, after which they were lysed in modified RIPA buffer and used for Western Blot experiments of STAT3 and ERK1/2 activation. (A) Normalized gene counts of* Il11ra1 *and* Il11 *in the EpCam+ and CCL206 fraction of control mouse organoids on day 3. (B) Representative uncropped western blots of untreated CCL206 cells (Un), CCL206 cells exposed to vehicle (C) and CCL206 cells in response to 1-100 ng/ml IL-11. Protein bands of total STAT3 and ERK1/2, their phosphorylated forms, and respective α-tubulin loading controls are shown. Samples were loaded onto 2 gels, of which 1 was used for STAT3 and ERK1/2, and the other for p-STAT3 and p-ERK1/2. Membranes were cut between the 70 and 55 kDa marker, after which the 250-70 kDa membrane was incubated with STAT3 or p-STAT3 antibody, and the 55-10 kDa membrane was incubated with ERK1/2 or p-ERK1/2 antibody and subsequently reprobed for α-tubulin. (C) Quantification of the phosphorylation of STAT3 (N=3) in response to rhIL-11 where the p-STAT3/STAT3 band intensity ratio (corrected for α-tubulin band intensity) is shown. (D) Quantification of the phosphorylation of ERK1/2 (N=3) after rhIL-11 exposure, showing the band intensity ratio of p-ERK1/2/ERK1/2 (corrected for α-tubulin band intensity).*

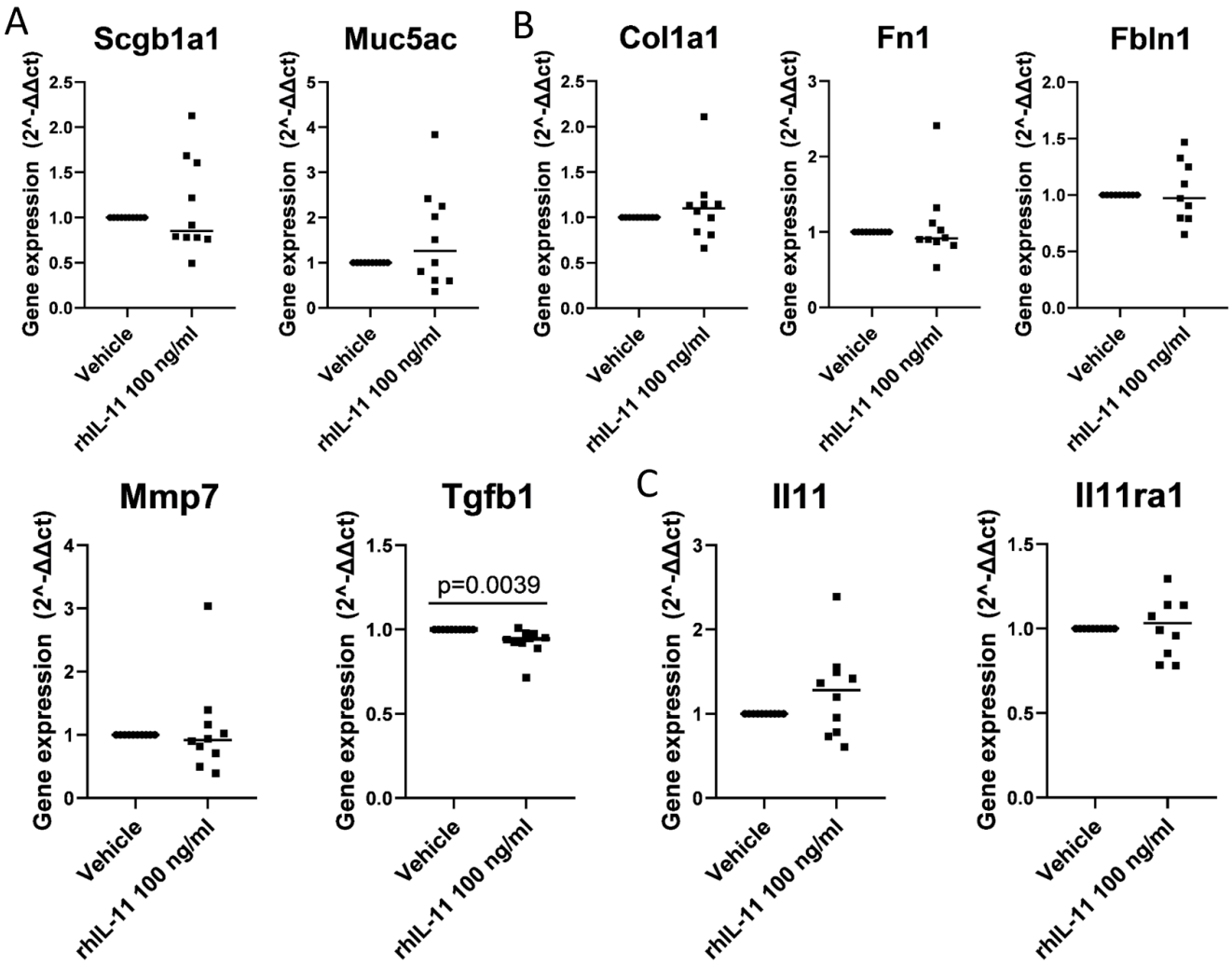

Figure S7: fibrosis marker genes are unaffected by IL-11 in lung slices. *Precision cut lung slices (PCLS) of wild type mice were treated with 100 ng/ml rhIL-11 for 48 hours, after which RNA was isolated from whole slices and gene expression studies were performed using PCR. (A) Gene expression of club cell marker* (Scgb1a1) *and goblet cell marker* (Muc5ac)*. (B) Expression of fibrosis-associated genes collagen 1* (Col1a1), *fibronectin 1* (Fn1), *fibulin 1* (Fbln1)*, MMP7* (Mmp7) *and TGFβ* (Tgfb1)*. (C) IL-11* (Il11) *and IL-11 receptor* (Il11ra1) *gene expression*. (A-C) *N=9 for* Fbln1, *N=10 for all other genes. For all genes: paired samples Wilcoxon test on delta Ct values (median is shown).*

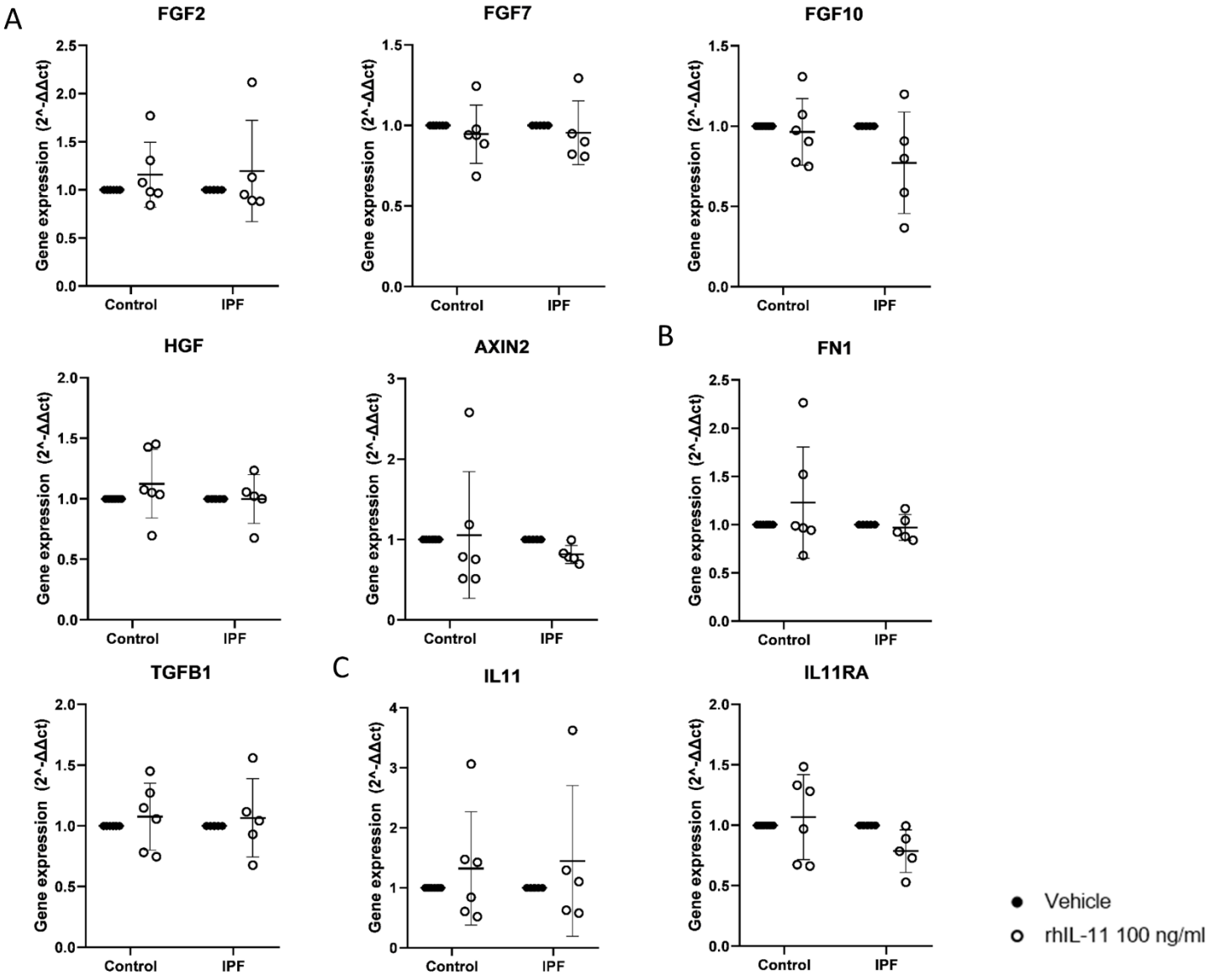

***Figure S8**: **IL-11 does not influence gene expression of established organoid supporting factors in primary human fibroblasts.** Primary human fibroblasts of either control or idiopathic pulmonary fibrosis (IPF) donors were exposed to 100 ng/ml rhIL-11 for 24 hours, after which RNA was isolated and PCR studies were performed. (A) Gene expression of the epithelial cell supporting factors* FGF2*,* FGF7*,* FGF10*, hepatocyte growth factor* (HGF) *and* AXIN2 *(Wnt signaling). (B) Expression of fibrosis marker genes fibronectin 1* (FN1) *and TGFβ* (TGFB1). *(C) Gene expression of IL-11 related genes IL-11* (IL11) *and IL-11R* (IL11RA). *(A-C) Control donors (N=6), IPF donors (N=5), Two-way ANOVA with Sidak's post hoc test on delta Ct values.*

Tables

Table S1: primer sequences used for analyses on human samples

Gene		**Sequence (5'-3')**
B2M	Fw	TGG AGG CTA TCC AGC GTA CT
	Rv	CGG ATG GAT GAA ACC CAG ACA
SDHA	Fw	GCA TGC CAG GGA AGA CTA CA
	Rv	ACG GGT CTA TAT TCC AGA GTG AC
HMBS	Fw	TGGACCTGGTTGTTCACTCCTT
	Rv	CAACAGCATCATGAGGGTTTTC
FGF2	Fw	AAA AAC GGG GGC TTC TTC CT
	Rv	TGT AGC TTG ATG TGA GGG TCG
FGF7	Fw	CCC TGA GCG ACA CAC AAG A
	Rv	CCA CAA TTC CAA CTG CCA CTG
FGF10	Fw	ATG TCC GCT GGA GAA AGC TA
	Rv	CCC CTT CTT GTT CAT GGC TA
HGF	Fw	CTG GTT CCC CTT CAA TAG CA
	Rv	CTC CAG GGC TGA CAT TTG AT
AXIN2	Fw	TGT GAG GTC CAC GGA AAC TG
	Rv	CTG CCC ACA CGA TAA GGA GG
FN1	Fw	AAT GCA CCA CAG CCA TCT CA
	Rv	GTC ACT TCT TGG TGG CCG TA
TGFB1	Fw	GTA CCT GAA CCC GTG TTG CT
	Rv	GAA CCC GTT GAT GTC CAC TT
IL11	Fw	GAG TTT CCC CAG ACC CTC GG
	Rv	GTA GGA CAG TAG GTC CGC TC
IL11RA	Fw	CCA GCC AGA TCA GCG GTT TA
	Rv	CCA GTG GGT TCA CCT CAG TC

Table S2: primer sequences used for analyses on mouse samples

Gene		**Sequence (5'-3')**
Rpl13a	Fw	AGA AGC AGA TCT TGA GGT TAC GG
	Rv	GTT CAC ACC AGG AGT CCG TT
B2m	Fw	ATG GGA AGC CGA ACA TAC TG
	Rv	CAG TCT CAG TGG GGG TGA AT
Actb	Fw	ATC GTG CGT GAC ATC AAA GA
	Rv	ATG CCA CAG GAT TCC ATA CC
Hopx	Fw	CGACTTTCAGTGGTTCCTGC
	Rv	GTGTGGAAGTCTGGGCGAG
Sftpc	Fw	GGAGCACCGGAAACTCAGAA
	Rv	GGAGCCGCTGGTAGTCATAC
Scgb1a1	Fw	GGCCCTCCTCATGGAATCAG

	Rv	GCATTTTGCAGGTCTGAGCC
Muc5ac	Fw	GAG ATG GAG GAT CTGG
	Rv	GCA GAA GCA GGG AGT GGT AG
Foxj1	Fw	CGG CCA TCT ACA AGT GGA TCA
	Rv	CTT GAA GGC CCC ACT GAG CA
Fn1	Fw	ACCACCCAGAACTACGATGC
	Rv	GGAACGTGTCGTTCACATTG
Col1a1	Fw	CACCCTCAAGAGCCTGAGTC
	Rv	GTTCGGGCTGATGTACCAGT
Fbln1	Fw	AGAACTATCGCCGCTCCGCA
	Rv	CCACCGCTGGCACTTGGATG
Mmp7	Fw	GGT GTG GAG TGC CAG ATG TT
	Rv	TAT CCG CAG TCC CCC CAA CTA
Tgfb1	Fw	GGACTCTCCACCTGCAAGAC
	Rv	GACTGGCGAGCCTTAGTTTG
Il11	Fw	TGT TCT CCT AAC CCG ATC CCT
	Rv	CAG GAA GCT GCA AAG ATC CCA
Il11ra1	Fw	ATC CGT ACC TGG TTA CCC GA
	Rv	CCC AGC CAC AGC ATC TGT TA

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