Supplementary information

Supplemental Methods

Human samples

Primary human (ph) ATII cells were isolated from non-IPF (N=4) or IPF (N=4) lung tissue biopsies from the Comprehensive Pneumology Center cohort of the BioArchive CPC-M at the University Hospital Grosshadern of the Ludwig Maximilian University. Participants provided written informed consent to participate in this study, in accordance with approval by the local ethics committee of the LMU, Germany (Project 333-10, 455-12). Additionally, formalin-fixed, paraffin-embedded lung tissue samples from 14 patients with sporadic IPF (mean age ± sd: 55.82 ± 9.74 years; 5 females, 9 males) and 5 non-diseased control subjects (organ donors; mean age ± sd: 56.60 ± 11.46 years; 4 females, 1 male) were immunohistochemically investigated. All lung tissue samples were collected in frame of the European IPF registry (eurIPFreg) and provided by the UGMLC Giessen Biobank (member of the DZL Platform Biobanking). The study protocol was approved by the Ethics Committee of the Justus-Liebig-University Giessen (No. 111/08 and 58/15).

Animal experiments

Six- to eight-week-old pathogen-free female C57BL/6N mice were obtained from Charles River and housed in rooms with constant humidity and temperature with 12h light cycles and free access to water and rodent chow. For the induction of experimental fibrosis, mice were subjected to intratracheal bleomycin (Bleomycin sulfate, Almirall, Barcelona, Spain, was dissolved in sterile PBS) instillation using the Micro-Sprayer Aerosolizer, Model IA-1C (Penn-Century, Wyndmoor, PA), as a single dose of 2 U/kg body weight in 50 µl PBS. Control mice were treated with 50 µl PBS. Mice were sacrificed at day 7, 14 or day 21 after instillation. Lungs were used for collection of whole lung tissue, ATII cells or 3D-LTCs. All animal studies were conducted under strict governmental and international guidelines and approved by the

local government for the administrative region of Upper Bavaria (Project 55.2-1-54-2532-88-12).

Isolation of primary murine alveolar epithelial cell type II (pmATII) cells

The pmATII cells were isolated from pathogen-free C57BL/6 mice treated with PBS/Bleomycin and sacrificed at day 14 after instillation as previously described [1, 2] with slight modifications. In brief, lungs were filled with dispase (BD Bioscience, San Jose, CA, US) and low gelling temperature agarose (Sigma Aldrich, Saint Louis, MO, USA) before tissue was minced and the cell suspension was filtered through 100-, 20-, and 10-µm nylon meshes (Sefar, Heiden, Switzerland). Negative selection of fibroblasts was performed by adherence on non-coated plastic plates. Macrophages and white blood cells were depleted with CD45 and endothelial cells were depleted with CD31 specific magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Cell purity was assessed routinely by analysis of endothelial (CD31), mesenchymal (α-SMA, CD90), epithelial (EpCAM, panCK and proSP-C), and hematopoietic cell (CD45) markers by immunofluorescence or flow cytometry.

Isolation of primary human alveolar epithelial type II (phATII) cells

Isolation of phATII cells was performed as previously described [1, 3], with some modifications. Briefly, lung tissue was minced and digested with dispase/collagenase (Roche, Basel, Switzerland) at 37°C for 2 h before filtering through nylon meshes and centrifugation at 400 g, 4°C for 10 min. Next, the cells were layered onto a discontinuous Percoll density gradient (1.04–1.09 g/ml) and centrifuged at 300 g for 20 min. The interphase containing macrophages and alveolar epithelial cells was recovered. Macrophages and white blood cells were depleted with CD45 specific magnetic beads (Miltenyi Biotec, Bergisch) according to the manufacturer's instructions.

Cell culture

In experiments using pmATII cells were seeded, cultured for 48h, then treated with senolytic agents Dasatinib (500 nM, Selleck Chemicals, Houston, TX, USA) and Quercetin (50 µM, Sigma Aldrich, St Louis, MO, USA) or respective DMSO control for 24 or 48 h in DMEM (Sigma Aldrich) containing 10% FCS (PAA Laboratories, Pasching, Austria), 2 mM l-glutamine, 100 U·mL−1 penicillin, 100 µg·mL−1 streptomycin (both Life Technologies, Carlsbad, CA), 3.6 mg/ml glucose (Applichem GmbH, Darmstadt, Germany) and 10 mM HEPES (PAA Laboratories). Senolytic treatment with ABT263 (10 µM, Cayman Chemical, Ann Arbor, MI) led to similar results (data not shown). Cells were counted 48 h after treatment with a Neubauer counting chamber. RNA was isolated isolated and reverse transcribed as described previously [2] and fibrotic gene expression was measured by quantitative (q) RT-PCR. Supernatants were collected, centrifuged at 14000 g for 10 min and stored at -80°C before analysis.

RNA isolation and Reverse Transcription Polymerase Chain Reaction (RT-PCR, qPCR)

Total RNA was extracted using the miRNeasy Mini kit (Qiagen, Hilden, Germany) for human tissue and Peqlab Total RNA extraction Kit (Peqlab, Erlangen, Germany) for mouse samples, according to the manufacturer's instructions. cDNAs were generated by reverse transcription using SuperScriptTM II (Invitrogen, Carlsbad, CA, USA) and for human tissue with iScript Advanced kit (BioRad, Hercules, CA, USA). Quantitative (q)RT-PCR was performed using Light Cycler 480 detection system and SYBR Green (Roche Diagnostics, Mannheim, Germany). Hypoxanthine phosphoribosyltransferase (HPRT) was used as a reference gene.

Relative gene expression is presented as ΔCt value (ΔCt =[Ct Hprt]-[Ct gene of interest]). Relative change in transcript level upon treatment is expressed as fold change 2^ΔΔCt value (ΔΔCt= ΔCt of treated sample-ΔCt of control).

Primers for *Col1a1, Fibronectin, Sftpc, Sftpa, T1a, Cdh1* and *MMP12* were as previously reported with *HPRT* as reference gene [2, 4]. Additional primers were

Gene	forward primer	reverse primer
hp16	ACCAGAGGCAGTAACCATGC	CCTGTAGGACCTTCGGTGAC
hp21	GTCAGTTCCTTGTGGAGCCG	TGGGTTCTGACGGACATCCC
mp16	CGGGGACATCAAGACATCGT	GCCGGATTTAGCTCTGCTCT
mp21	ACATCTCAGGGCCGAAAACG	AAGACACACAGAGTGAGGGC
mSpp1	AGCCAAGGACTAACTACGACC	TGGCTATAGGATCTGGGTGC
mMMP2	ATCCACGGTTTCAGGGTCC	ATCGAGACCATGCGGAAGC
mPai1	AGGTCAGGATCGAGGTAAACGAG	GGATCGGTCTATAACCATCTCCGT
mCol5a3	CCACCACTGTCACGATTGGA	GAGTCGTCTGCTCGGTTTCAG
mWisp1	GTCCTGAGGGTGGGCAACAT	GGGCGTGTAGTCGTTTCCTCT

Generation and treatment of 3D-LTCs

C57BL6/N mice of 8-12 weeks were instilled with 2 U/kg bleomycin and sacrificed at day 14 after instillation. 3D-LTCs were generated as previously described [4]. Briefly, lungs were flushed through the heart with sterile sodium chloride solution and filled with low gelling temperature agarose (2%, A9414; Sigma) in DMEM/Ham's F12 supplemented with 100 U·mL−1 penicillin, 100 µg·mL−1 streptomycin and 2.5 µg·mL−1 amphotericin B (Sigma Aldrich). Next, lobes were cut with a vibratome (Hyrax V55; Zeiss, Jena, Germany) to a thickness of 300 µm (speed 10–12 µm·s−1, frequency 80 Hz, amplitude of 1 mm). 3D-LTCs were treated with senolytic agents Dasatinib (500 nM, Selleck Chemicals, Houston, TX, USA) and Quercetin (50 µM, Sigma Aldrich) or respective DMSO control for 48 h in sterile cultivation medium containing 0.1% FCS. RNA was isolated and fibrotic gene expression was measured by qRT-PCR. Supernatants were collected, centrifuged at 14000 g for 10 min and stored at -80°C before analysis.

Western blotting

Cells or pulverized lung tissue were lysed with Tissue Protein Extraction Reagent (T-Per, Thermo Fisher) containing phosphatase and protease inhibitors (Roche Diagnostics,

Mannheim, Germany). Protein concentration was determined by BCA assay (Pierce, Thermo Fisher Scientific). Equal amounts of protein were loaded with 4× Laemmli loading buffer (150 mM Tris HCl [pH 6.8], 275 mM SDS, 400 nM dithiothreitol, 3.5% (w/v) glycerol, 0.02% bromophenol blue) and subjected to electrophoresis in 17% polyacrylamide gels and transferred to PVDF membranes. Supernatants from treated 3D-LTCs were collected and stored at -80°C. Samples were normalized to supernatant volume. 200 µL of supernatant from each sample was concentrated using Nanosep 10K OMEGA columns (Pall Corporation; Ann Arbor, MI, USA) at 15000 g for 20 min. Formed concentrate was diluted in 60 µL lysis buffer to form a 3:10 ratio. Western blotting was performed with 6% TRIS-based gels. Membranes were blocked with 5% non-fat dried milk solution in TRIS-buffered saline containing 0.01% (v/v) Tween (TBS-T) (Applichem) for 1h and incubated with primary antibodies (anti p21, MAB88058, Merck Millipore (Billerica, MA, USA); anti β-actin, A3854, Sigma Aldrich, anti proSP-C, ab40879, Abcam (Cambridge, UK), anti E-Cadherin BD 610181, anti Collagen1, 600-401-103, Rockland (Limerick, PA, USA) at 4°C overnight. Next, blots were incubated for 1 h at RT with secondary, HRP-conjugated, antibodies (GE-Healthcare) prior to visualization of the bands using chemiluminescence reagents (Pierce ECL, Thermo Scientific, Ulm, Germany), recording with ChemiDocTMXRS+ system and analysis using Image Lab 5.0 software (Biorad, Munich, Germany).

For analysis of protein expression, peripheral lung tissue samples from the lower lobe, from the subpleural region of the lung was used. Lung homogenates were prepared of shock-frozen lung tissue samples (size 1 cm^3) from IPF patients (N=16; mean age ± SD: 50.67 ± 12.010 years; 2 females, 14 males) and non-diseased control subjects (organ donors, N=11; mean age ± SD: 49.67 ± 7.615 years; 5 females, 5 males, 1 unknown) according to the protocol previously described [5]. The protein concentration in lung homogenates was determined according to the Pierce® BCA protein assay from Thermo Scientific.

For one-dimensional SDS-PAGE, lung homogenates were then diluted (1:3) in 4×SDS-sample buffer [leading to a final concentration 2% (w/v) SDS, 2.5% (v/v) β-mercaptoethanol, 10% (v/v) glycerol, 12.5 mmol/L tris-HCl [pH 6.8], 0.1% (w/v) bromophenol blue in samples] and heated for denaturation at 99°C for 15 min. Denaturated proteins from each sample (50 µg/lane) were then separated by 15% Laemmli-SDS-PAGE. Thereafter, the separated proteins were transferred to a PVDF membrane (Millipore) in a semi-dry blotting chamber according to the manufacturer´s protocol (Bio-Rad, Munich, Germany). Obtained immunoblots were then blocked by incubating at room temperature for 1 h in blocking buffer [1 × tris-buffered saline (TBS; 50 mmol/L tris-HCl, pH 7.5, 50 mmol/L NaCl) containing 5% (w/v) nonfat dried milk and 0.1 % (w/v) tween 20], followed by immunostaining for p16 (ab108349, abcam, diluted 1:250) or p21 (ab109520, abcam, diluted 1:500). Blots were incubated with primary antibody (diluted in blocking buffer) overnight at 4°C with gentle shaking. The blots were then washed four times in 1 × TBS containing 0.1 % (w/v) tween 20, and incubated with horseradish peroxidase-conjugated secondary swine anti-rabbit IgG (DakoCytomation, Hamburg, Germany; diluted 1:2000 in blocking buffer) for 2 h at rt. After four washes, blot membranes were developed with the Immobilon Western Chemiluminescent HRP substrate (Millipore), and emitted signals were detected with a chemiluminescence imager (Intas ChemoStar, Intas, Göttingen, Germany). Thereafter, blots were stripped using ″stripping buffer″ [2% (w/v) SDS and 50 mmol/L dithiothreitol in tris-buffered saline (TBS)] under gentle shaking at 55°C for 30 min, followed by reprobing the blots using antibodies against the loading control protein β-actin (ab8226, abcam, diluted 1:3000).

For quantification, band intensities in acquired TIFF-images were analyzed by densitometric scanning and quantified using ImageJ software (Version 1.46r, NIH). The band densities were normalized to β-actin.

ELISA

Supernatants were obtained from assays of primary mouse ATII cells or mouse 3D-LTCs, centrifuged at 14000g for 10 min and only cell-free supernatant was used for the assay. Samples were then transferred to the respective ELISA plate and the assays were performed according to the manufacturer's instructions (WISP1 – DY1627, R&D, Minneapolis, Minnesota, USA; IL6 – DY406, R&D, Minneapolis, Minnesota, USA, SP-C - CSB-E12639m; Cusabio, Washington D.C., Columbia, USA).

Immunohistochemistry (IHC)

ZytoChem-Plus AP Kit (Fast Red) (Zytomed Systems, Berlin, Germany) was used for immunohistochemical localization of P16, P21, proSP-C, cytokeratin-5 (KRT5) and α-SMA in formalin-fixed, paraffin-embedded lung tissue sections from patients with sporadic IPF (N=14) and organ donors (N=5), according to the manufacturer´s instructions and previous published work [6]. Human lungs were placed in 4% (w/v) paraformaldehyde after explantation (fixation was done for 12–24h), and processed for paraffin embedding. Sections (3 µm) were cut and mounted on positively charged glass slides (Super Frost Plus, Langenbrinck (Emmendingen, Germany)). Paraffin-embedded tissue sections of normal donor and IPF lungs were deparaffinized in xylene and rehydrated in graded alcohol. Antigens were retrieved by cooking the sections for 5 min in 10 mmol/L citrate buffer (pH 6.0) using microwave irradiation (800 W). Thereafter, sections cooled down for 20 min at RT, followed by repeated cooking (800 W, 5 min) and cooling (20 min at RT). This procedure was performed three times. Importantly, the citrate buffer was freshly prepared by mixing 18 mL 100mmol/L citric acid monohydrate and 82 mL 100mmol/L sodium citrate tribasic dihydrate with 900 mL distilled water.

For immunostaining, the streptavidin-biotin-alkaline phosphatase (AP) method with use of the ZytoChem-Plus AP Kit (Fast Red) [Zytomed Systems, Berlin, Germany], according to the

manufacturer´s protocol, was employed. In the following, the primary antibodies used for IHC are listed, including the sources and dilutions: rabbit polyclonal for human proSP-C (1:750, Millipore, AB3786), rabbit monoclonal for human cytokeratin-5 [KRT5] (1:200, Abcam, ab75869), rabbit monoclonal for human cytokeratin-7 [KRT7] (1:200, used from Epitomics, #2303-1 as well as from Abcam, ab68459), rabbit polyclonal for human smooth muscle actin [α-SMA] (1:100, Abcam, ab5694), rabbit monoclonal for human p16INK4a (1:75, Abcam, ab108349), rabbit monoclonal for human p21 (1:100, Abcam, ab109520) and rabbit monoclonal for gamma H2A.X (phospho-S139) (1:100, Abcam, ab81299).

In general, sections were incubated for 2h at RT with primary antibodies, which were diluted in PBS containing 2% (w/v) BSA. Control sections were treated with PBS-2%BSA alone to determine the specificity of the staining. Detection was performed with a polyvalent secondary biotinylated antibody (rabbit, mouse, rat, guinea pig, provided by the ZytoChem-Plus AP Kit, 20 min incubation) followed by incubation with AP-conjugated streptavidin (20 min). Sections were then developed with Fast Red substrate solution, and the reaction was terminated by washing in distilled water. The stained sections were counterstained with hemalaun (Mayers hemalaun solution, WALDECK Division CHROMA GmbH & CO KG, Münster, Germany) and mounted in Glycergel (DakoCytomation). Lung tissue sections were scanned with a scanning device (Nano-Zoomer, Hamamatsu), and examined histopathologically using the ´NDP.view2 software´ at 100×, 200×, 400× and 800× original magnification. IHC for mentioned antibodies was undertaken in 14 IPF- and 5 control-donor lung samples.

Gene set enrichment analysis (GSEA)

Gene set enrichment analysis (GSEA) for senescence was performed using the GSEA Desktop Application software package from the Broad Institute [7] on previously published microarrays for whole mouse lungs of PBS- or bleomycin-treated animals (GSE16846) [8], fibroblasts isolated from PBS- or bleomycin-treated animals (GSE42564) [9] or isolated ATII

cells [1]. GSEA allows for computationally testing whether a defined set of genes, such as a list of genes associated with senescence, are significantly enriched in one of two biological conditions. Pre-ranked gene lists were generated from normalized data for the ATII cells based on log2 fold change whereas fold change was used for generating the fibroblast pre-ranked list. For whole lung homogenates, genes were ranked using the built-in Signal2Noise function. The senescence list was obtained from Fridman *et al.* [10].

Correlation analysis

Data for the analysis were extracted from Lung Genomics Research Consortium (GSE47460 GPL4680) and correlated to diffusion capacity of the lung for carbon monoxide (%DL_{CO}) and the forced vital capacity (FVC) in human patients as a measure of disease severity. Only normal control patients and patients with confirmed IPF were used from the dataset. For expression analysis of *P16* or *P21*, the expression data was extracted from the published datasets.

Immunofluorescence staining

For immunofluorescence staining experiments, ATII cells were seeded on poly-l-lysin treated coverslips. Cells were stopped at day 2 or after 48 h of treatment with senolytic drugs and fixed with ice-cold acetone-methanol (1:1) for 10 min and washed 3 times with 0.1% BSA in PBS. Next, cells were permeabilized with 0.1% Triton X-100 solution in PBS for 20 min, blocked with 5% BSA in PBS for 30 min at room temperature and incubated with primary antibodies (proSP-C 1:100 (AB3786, Millipore, Darmstadt, Germany), E-Cadherin 1:200 (610181, BD, Franklin Lakes, NJ, USA), Cytokeratin 1:500 (Dako, Glostrup, Denmark), Cleaved caspase 3 1:150 (9661, Cell Signaling, Danvers, Massachusetts, USA**)**, Histone H3K9me3 1:500 (8898, Abcam, Cambridge, MA 02139, USA), ZO-1 1:200 (400-2200, Invitrogen, Waltham, Massachusetts, USA), αSMA 1:500 (AB5228, Sigma Aldrich, St. Louis,

Missouri USA), followed by secondary antibodies, 1 h each. DAPI (Roche, Basel, Switzerland) staining for 10 min was used to visualize cell nuclei. Next, coverslips were fixed with 4% PFA for 10 min, mounted with fluorescent mounting medium (Dako, Glostrup, Denmark) and visualized with an Axio Imager microscope (Zeiss, Oberkochen, Germany) or confocal microscope (LSM 710; Zeiss, Oberkochen, Germany).

3D-LTCs were fixed with the mixture of acetone-methanol (1:1) for 20 min and punched to a 4 mm diameter. Tissue was blocked for 1h with 5% BSA in PBS and incubated overnight with primary antibodies in 4°C, followed by incubation with appropriate secondary antibodies for 1h and with DAPI for 5 min. Then tissue was fixed for 30 min with 4% PFA. Staining was evaluated via confocal microscopy (LSM 710; Zeiss, Oberkochen, Germany).

Secretome analysis

Eight- to ten-week-old, pathogen-free female C57BL/6N mice (Charles River Laboratories, Sulzfeld, Germany) were used for the isolation of pmATII cells for secretome analysis. Lung fibrosis was induced in the animals by intratracheal instillation of a single dose of bleomycin (5 U/kg body weight), dissolved in 80 µl sterile phosphate-buffered saline (PBS). Control mice were treated with 80 µl PBS. Mice were sacrificed at day 14 after instillation and 3 mice per treatment were pooled for the isolation of pmATII cells. ATII cells were seeded in 12-well plates in DMEM media without phenol red and conditioned media (4, 6, and 8 h, respectively) were harvested either on the first day of culture or after 2 days of culture. (day 2 4 h; day 2 6 h; day 2 8 h; Bleo ATII: n=3; PBS ATII: n=2). Samples were snap frozen in liquid nitrogen and subjected to mass spectrometry analysis as previously described [11]. Briefly, proteins in conditioned media were digested in solution with trypsin and LysC into peptides, which were analyzed on a Q-Exactive mass spectrometer (Thermo Fischer). Mass spec raw data was processed using the MaxQuant software [12] and proteins were quantified using the embedded label free quantification algorithm MaxLFQ [13]. Statistical data analysis was performed using the Perseus software suite [14].

Flow cytometry (FCM)-based apoptosis assay in pmATII cells

FCM-based apoptosis assay was performed according to the manufacturer's instructions (Annexin V apoptosis kit, eBioscience, San Diego, CA). Briefly, pmATII cells from PBS- and bleomycin-treated animals (day 2) were incubated with vehicle (DMSO) or senolytic drugs Dasatinib (500 nM, Selleck Chemicals, Houston, TX, USA) and Quercetin (50 µM, Sigma Aldrich, St Louis, MA, USA) for 48 hours and were subsequently incubated with Bafilomycin A1 (100 nM, Enzo Life Sciences, Farmingdale, NY; USA) and C_{12}FDG (20 nM, Life technologies, Carlsbad, CA; USA) for 1 and 2 h, respectively. The cells were harvested with trypsin/EDTA (Life technologies), washed once with calcium chloride-free and magnesium chloride-free PBS (Life technologies), and once with the Annexin V-binding buffer (eBioscience). Cells were incubated with APC-conjugated Annexin V (eBioscience) for 15 min, washed, and resuspended in the binding buffer. Cells without C_{12}FDG treatment were further stained with propidium iodide solution (PI, eBioscience). The groups of the cells stained with Annexin V+PI or Annexin V+C_{12}FDG were analyzed with a FACS LSRII cell analyzer (BD Bioscience).

Supplemental Figures

Figure S1: Expression of senescence markers is upregulated in IPF patients.

Gene expression of *P16* and *P21* in IPF versus donor specimens. N=91 donor, N=122 IPF. Data extracted from the LGRC GSE47460 GPL4680. Data is presented as mean ± s.e.m.. Means were compared using Mann-Whitney U test. **(B)** Correlation between *P16* expression in the lung and the lung function parameter %predicted DL_{CO} (N=194) showed a significantly negative linear correlation (dashed line = 95% CI; data extracted from LGRC GSE47460 GPL4680).

Figure S1

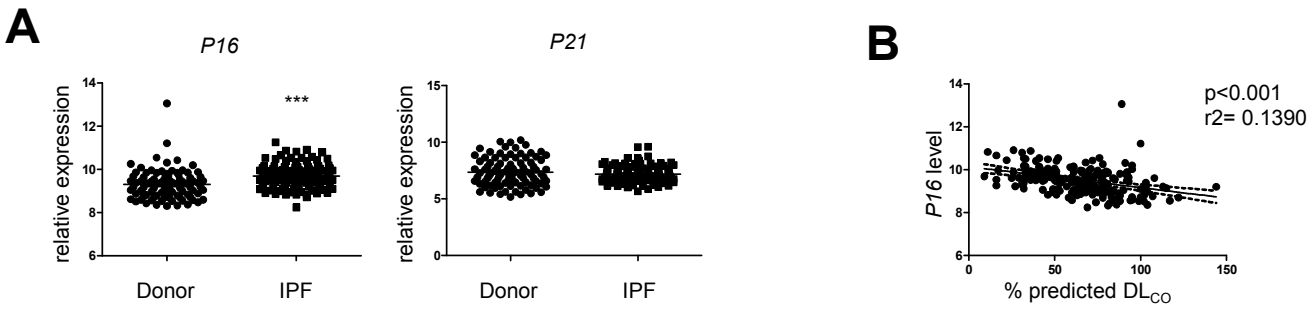

A
P16
P21
relative expression
***
Donor
IPF
B
P16 level
% predicted DLCO
p<0.001
r2= 0.1390

Figure S2: Expression and localization of P16 and P21 in IPF lungs.

(A, B) Immunohistochemical staining of serial sections of IPF lung tissue for α-SMA (marker for myofibroblasts/mesenchymal cells), proSP-C (marker for alveolar epithelial type II [ATII] cells), cytokeratin 5 (KRT5, marker for bronchiolar basal cells), cytokeratin 7 (KRT7, marker for simple epithelia) and P16 protein. $KRT5^+$ $KRT7^+$ basal cells expressing P16 are indicated by asterisks; α-SMA expressing mesenchymal cells which revealed no pronounced expression for P16 are indicated by hashmarks; proSP-C^+ $KRT7^+$ positive ATII cells expressing P16 **(B)** are indicated by arrows; proSP-C^- $KRT5^-$ $KRT7^+$ epithelial cells expressing P16 are indicated by arrowheads; proSP-C^+ $KRT5^+$ $KRT7^+$ positive epithelial cells are indicated by dashed arrows. The lung region shown in **(A)** did not exhibit ATII cells. The lung region shown in the left panel of **(B)** exhibited aberrant epithelial structures indicating KRT5 expressing basal cells (with abnormal superficial localization) in close proximity to proSP-C expressing ATII cells, as well as abnormal epithelial cells with co-expression of KRT5 and proSP-C, which all expressed P16. In contrast, the alveolar airspace shown in the middle panel of **(B)** indicated only a sparse amount of P16 expressing ATII. **(C, D)** Immunohistochemical staining of serial sections of IPF lung tissue for α-SMA (marker for myofibroblasts/mesenchymal cells), proSP-C (marker for alveolar epithelial type II [ATII] cells), cytokeratin 5 (KRT5, marker for bronchiolar basal cells), cytokeratin 7 (KRT7, marker for simple epithelia) and P21 protein. $KRT5^+$ $KRT7^+$ positive basal cells expressing P21 (**C**) are indicated by asterisks; α-SMA expressing mesenchymal cells which revealed no pronounced expression for P21 are indicated by hashmarks; proSP-C^+ $KRT7^+$ positive ATII cells expressing P21 are indicated by arrows; proSP-C^- $KRT5^-$ $KRT7^+$ epithelial cells expressing P21 **(C)** are indicated by arrowheads. The lung region shown in **(C)** exhibited aberrant epithelial structures indicating KRT5 expressing bronchiolar basal cells in close proximity to proSP-C expressing ATII cells, as well as epithelial cells with co-expression of KRT5 and proSP-C.

Figure S2

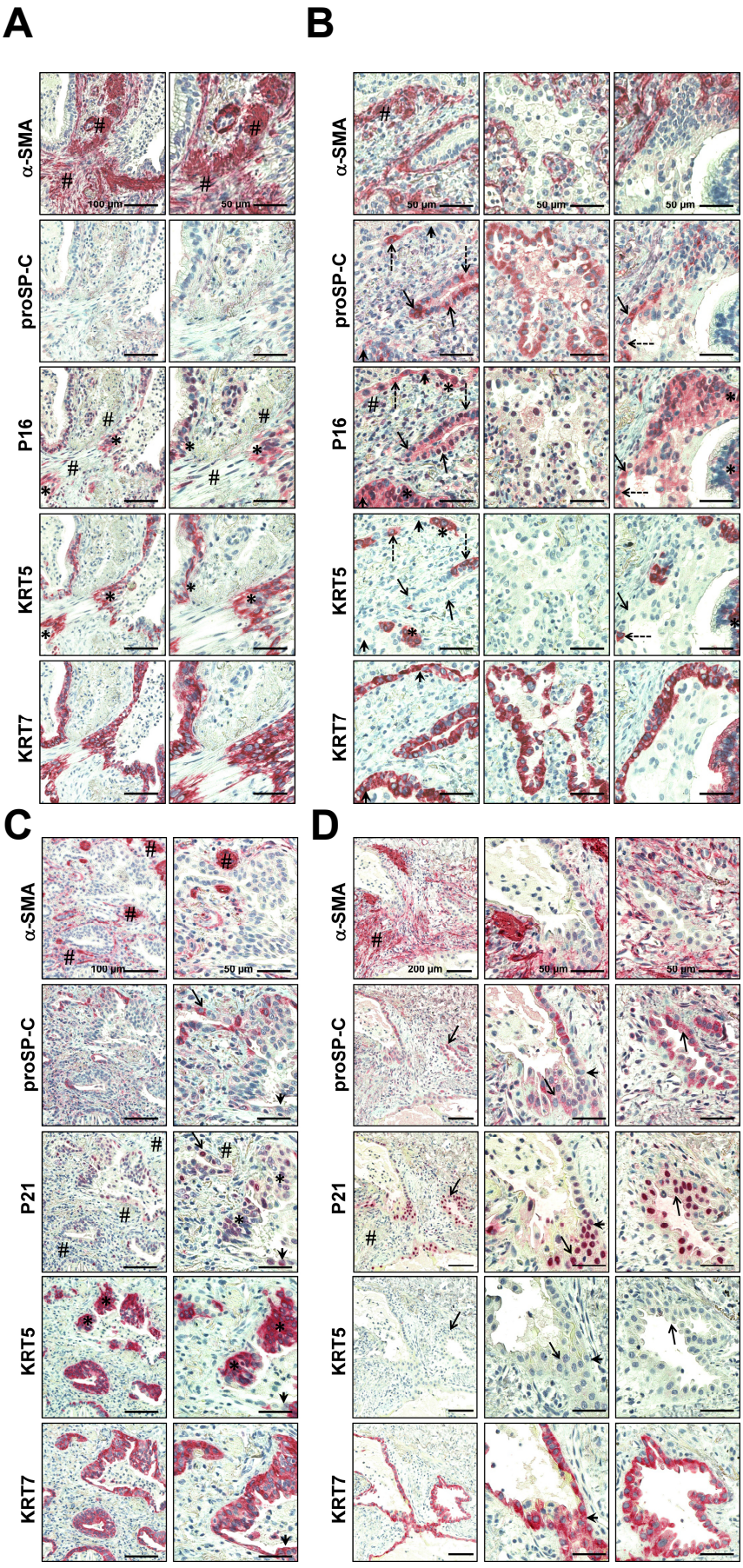


Figure S3: Induction and upregulation of γH2A.X (phospho-S139) in epithelial cells of IPF lungs.

(**A**-**E**) Immunohistochemical staining of serial sections of IPF (**A**, **B**, **D**, **E**) or donor lung tissue (**C**) for proSP-C (marker for alveolar epithelial type II [ATII] cells), cytokeratin 5 (KRT5, marker for bronchiolar basal cells), cytokeratin 7 (KRT7, marker for simple epithelia) and senescence markers γH2A.X (p-S139) and P16. In IPF (**A**, **B**, **E**), proSP-C^+ $KRT7^+$ ATII cells indicated robust nuclear staining for γH2A.X (p-S139) which co-localized with P16 overexpression (indicated by arrows). The same observations were made in proSP-C^- $KRT5^-$ $KRT7^+$ epithelial cells of alveolar spaces (indicated by arrowheads), whereas no substantial immunostaining could be observed in the fibrotic interstitium in IPF lungs **(C).**

The IPF-lung regions shown in **B**, **D** and **E** exhibited aberrant epithelial structures indicating KRT5 expressing basal cells (with abnormal superficial localization) in close proximity to proSP-C expressing ATII cells, which also revealed in part γH2A.X (p-S139) expression in co-localization with P16 (indicated by asterisks). In addition, proSP-C^+ $KRT5^+$ $KRT7^+$ positive epithelial cells in areas of aberrant re-epithelialization (**E**) indicated also concomitant expression of γH2A.X and P16 (indicated by dashed arrows). In general, induction of nuclear γH2A.X (p-S139) or P16 expression was frequently observed in bronchiolar basal cells in areas of bronchiolization in IPF **(B, D, E)**, but also in normal bronchioles of IPF lungs (not shown). In some instances, P16 overexpressing bronchiolar basal cells in IPF lungs did not indicate pronounced co-expression of γH2A.X (p-S139) (right panel of **B**, left panel of **D**). This phenomenon was also in part observed in proSP-C^+ $KRT7^+$ ATII cells as well as in other described epithelial cells (not shown). In age-matched normal donor lungs (**C**), minimal staining for P16 was observed; and no pronounced immunostaining for γH2A.X (p-S139) could be detected in any cells of donor lungs.

Figure S3

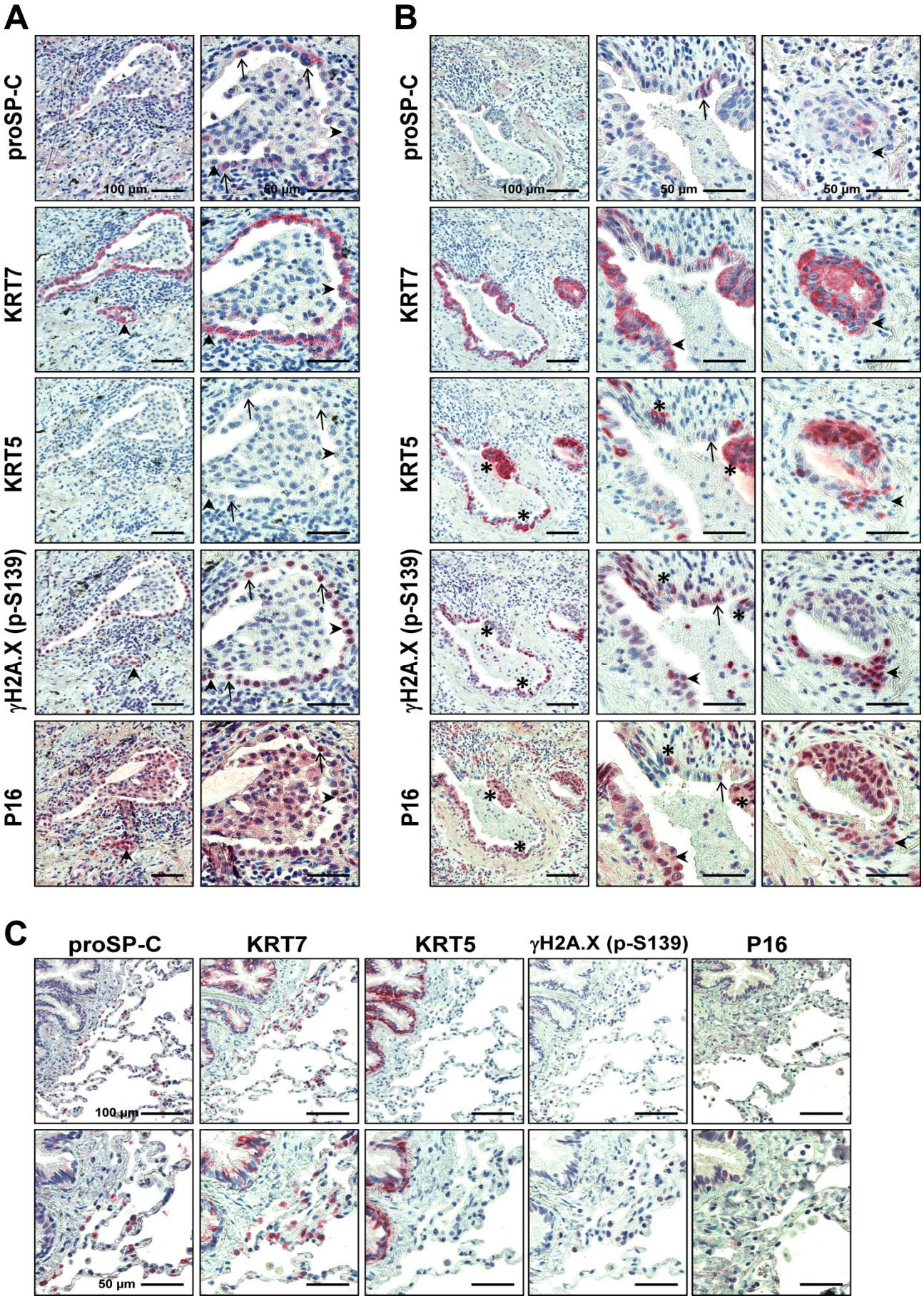


Figure S3 (continued)

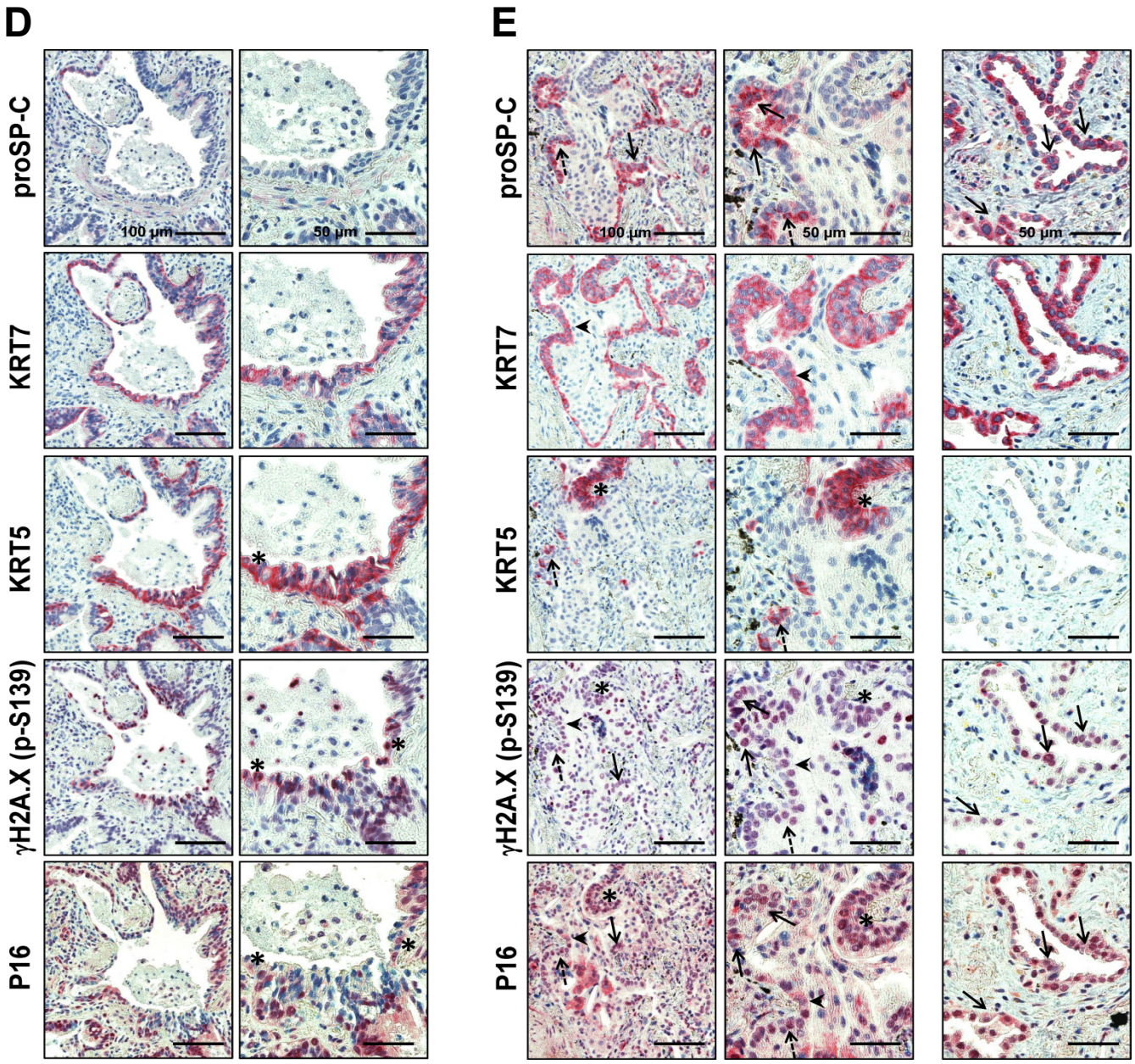
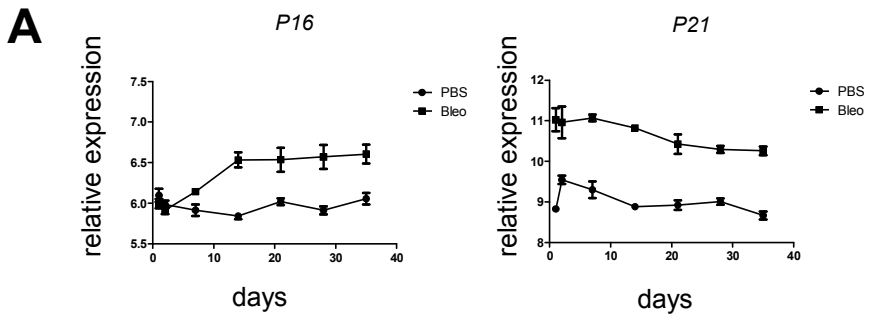
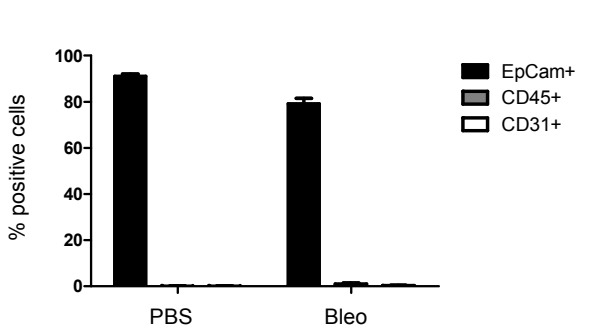


Figure S4: Senescent phenotype of ATII cells after bleomycin challenge. (A) Data for *P16* and *P21* was extracted from GSE40151 [15] for PBS/bleomycin timecourse. **(B)** Mice were instilled with PBS or Bleomycin. At day 14 after instillation mice were sacrificed and pmATII cells were isolated. Cells were stained for EpCAM, CD45 and CD31 and analyzed by FACS. Shown are mean ± s.e.m., n=3-6. **(C)** Representative images of immunofluorescence staining for senescence associated heterochromatic foci marker H3K9me3 and epithelial cell marker E-Cadherin in fibrotic and non-fibrotic pmATII cells at day 2 of culture. Fluorescent images represent a 630× magnification. Scale bar represents 20 µm. Representative of n=2.

Figure S4


A
P16
P21
relative expression
days
PBS
Bleo


B
% positive cells
EpCam+
CD45+
CD31+
PBS
Bleo

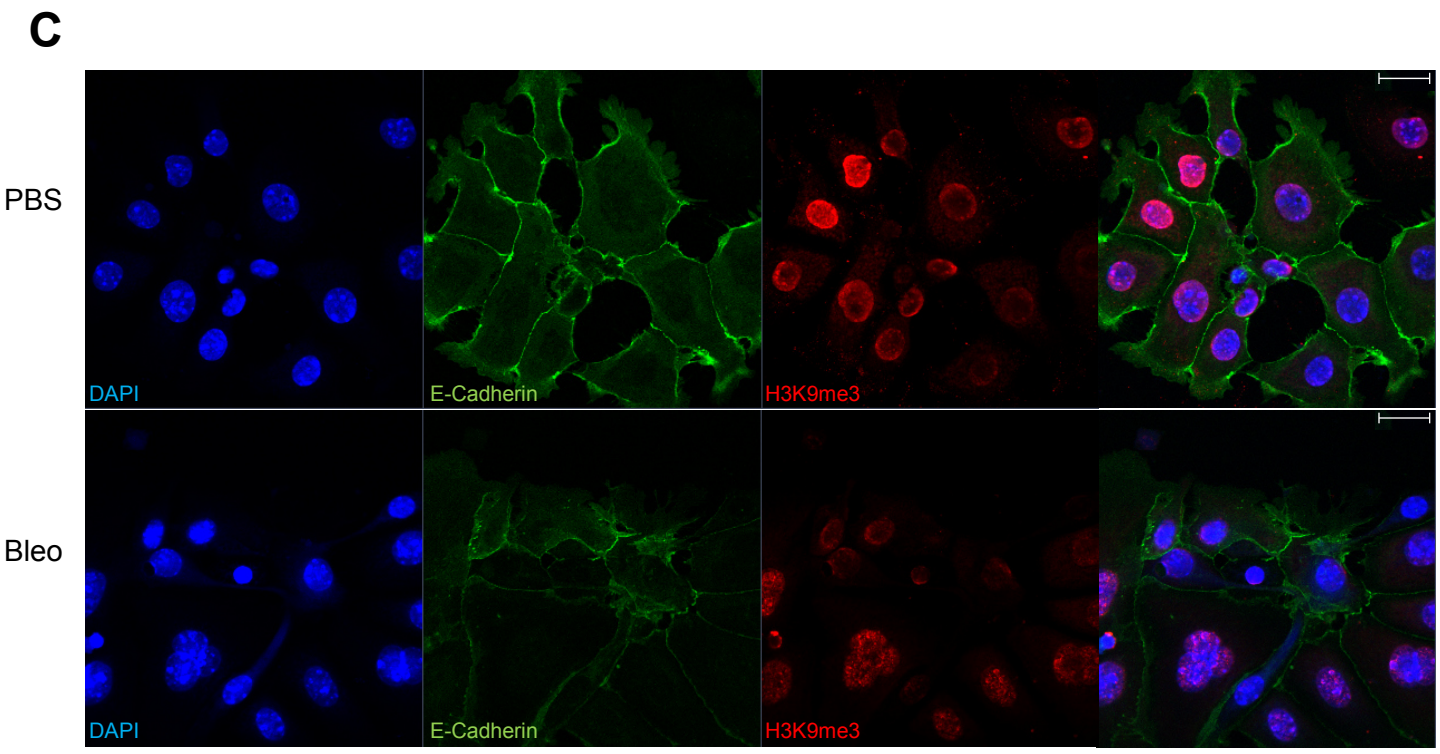

C
PBS
Bleo
DAPI
E-Cadherin
H3K9me3

Figure S5: Depletion of senescent cells in fibrotic pmATII cells

Mice were instilled with either PBS or bleomycin (Bleo). At day 14 after instillation mice were sacrificed and pmATII cells were isolated. **(A)** Gene expression analysis of fibrotic markers after 24/48 h of mock treatment was performed by qPCR. Data were normalized to *Hprt*. ΔCt is presented as mean ± s.e.m.. Significance was assessed with one-way Anova followed by Newman-Keuls's multiple comparison test, n=4. **(B-D)** Fibrotic pmATII cells were cultured in the presence of senolytic drugs Dasatinib (D; 200 nM) and Quercetin (Q; 50 µM). After 24 hours, expression of **(B)** *P16* **(C)** fibrotic markers and **(D)** epithelial cell markers was analyzed by qPCR. Data were normalized to *Hprt* levels. ΔCt is presented as mean ± s.e.m.. Significance was assessed with paired Student's t-test, n=4. Significance: *p<0.05, **p<0.01; ***p<0.001.

Figure S5

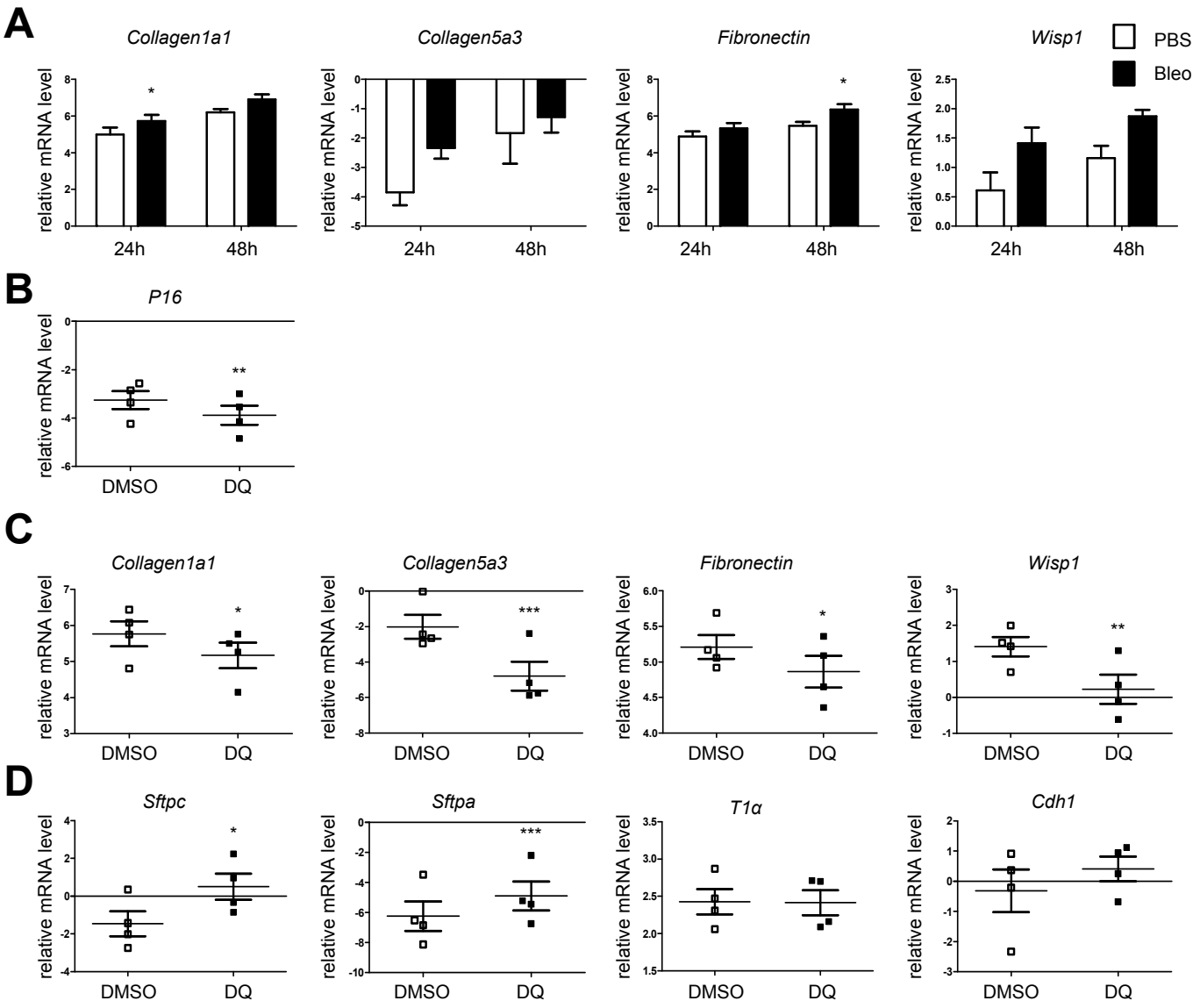

A
Collagen1a1
Collagen5a3
Fibronectin
Wisp1
PBS
Bleo
relative mRNA level
24h
48h
B
P16
DMSO
DQ
C
Collagen1a1
Collagen5a3
Fibronectin
Wisp1
D
Sftpc
Sftpa
T1α
Cdh1

Figure S6: Depletion of senescent cells in non-fibrotic pmATII cells

Mice were instilled with PBS. At day 14 after instillation mice were sacrificed and pmATII cells were isolated. Non-fibrotic pmATII cells were cultured for 48 h in the presence of senolytic drugs Dasatinib (D; 200 nM) and Quercetin (Q; 50 µM) and assessed for **(A)** cell numbers. Data are presented as normalized to DMSO control and as mean ± s.e.m. Significance was assessed with paired Student's t-test, n=3. **(B)** *P16* expression. Data were normalized to *Hprt* levels. ΔCt is presented as mean ± s.e.m, Significance was assessed with paired Student's t-test, n=6. **(C)** Representative images of immunofluorescence staining for the apoptotic marker cleaved caspase 3 and E-Cadherin. Fluorescent images represent a 630× magnification. The scale bar represents 20 µm. **(D-F)** qPCR analysis of non-fibrotic pmATII cells treated with senolytic drugs for the expression of **(D)** SASP markers, **(E)** fibrotic markers, **(F)** epithelial cell markers. Data were normalized to *Hprt* levels. ΔCt is presented as mean ± s.e.m. Significance was assessed with paired Student's t-test, n=6. *p<0.05, ***p<0.001.

Figure S6

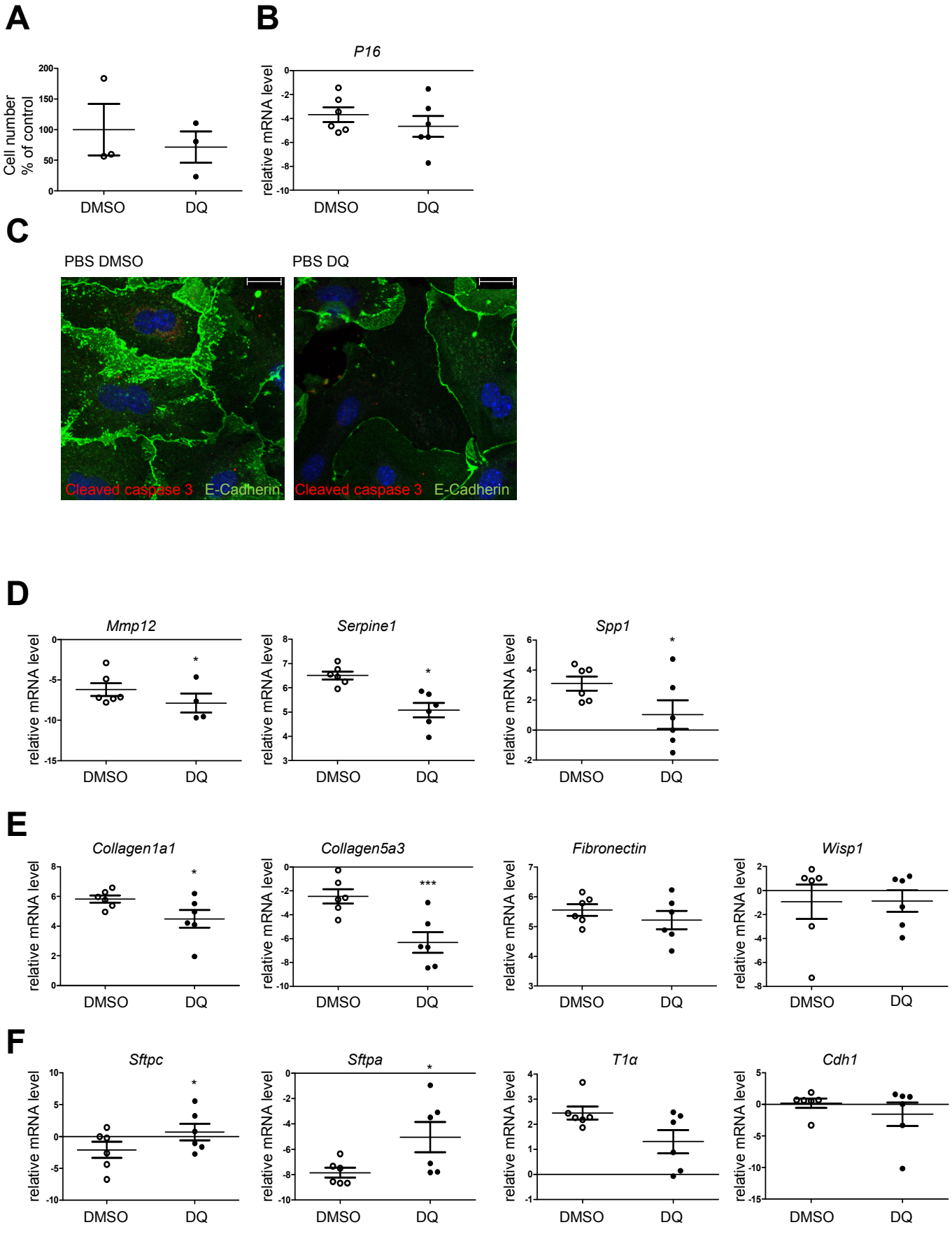

A
B
P16
Cell number
% of control
relative mRNA level
DMSO
DQ
C
PBS DMSO
PBS DQ
Cleaved caspase 3
E-Cadherin
D
Mmp12
Serpine1
Spp1
E
Collagen1a1
Collagen5a3
Fibronectin
Wisp1
F
Sftpc
Sftpa
T1α
Cdh1

Figure S7: Characterization of fibrotic markers in PBS/Bleo 3D-LTCs.

Mice were instilled with either PBS or bleomycin. At day 14 after instillation mice were sacrificed and 3D-LTCs were generated. **(A)** Gene expression of fibrotic markers of 3D-LTCs after 48 h of culture was analyzed by qPCR. Data were normalized to *Hprt*. ΔCt is presented as mean ± s.e.m.. Significance was assessed with Student's t-test, n=8.

Figure S7

A

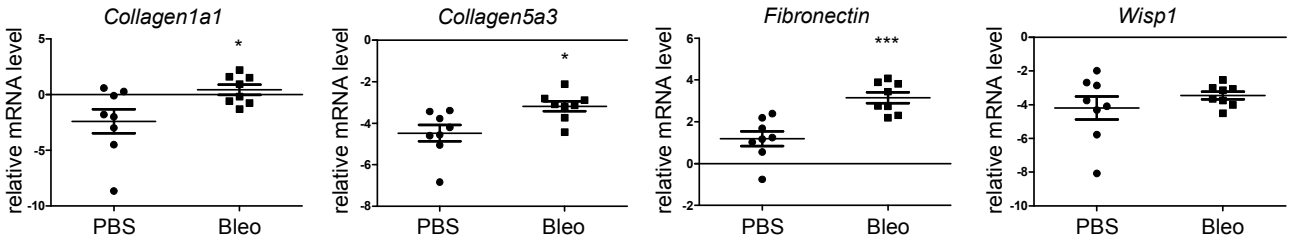
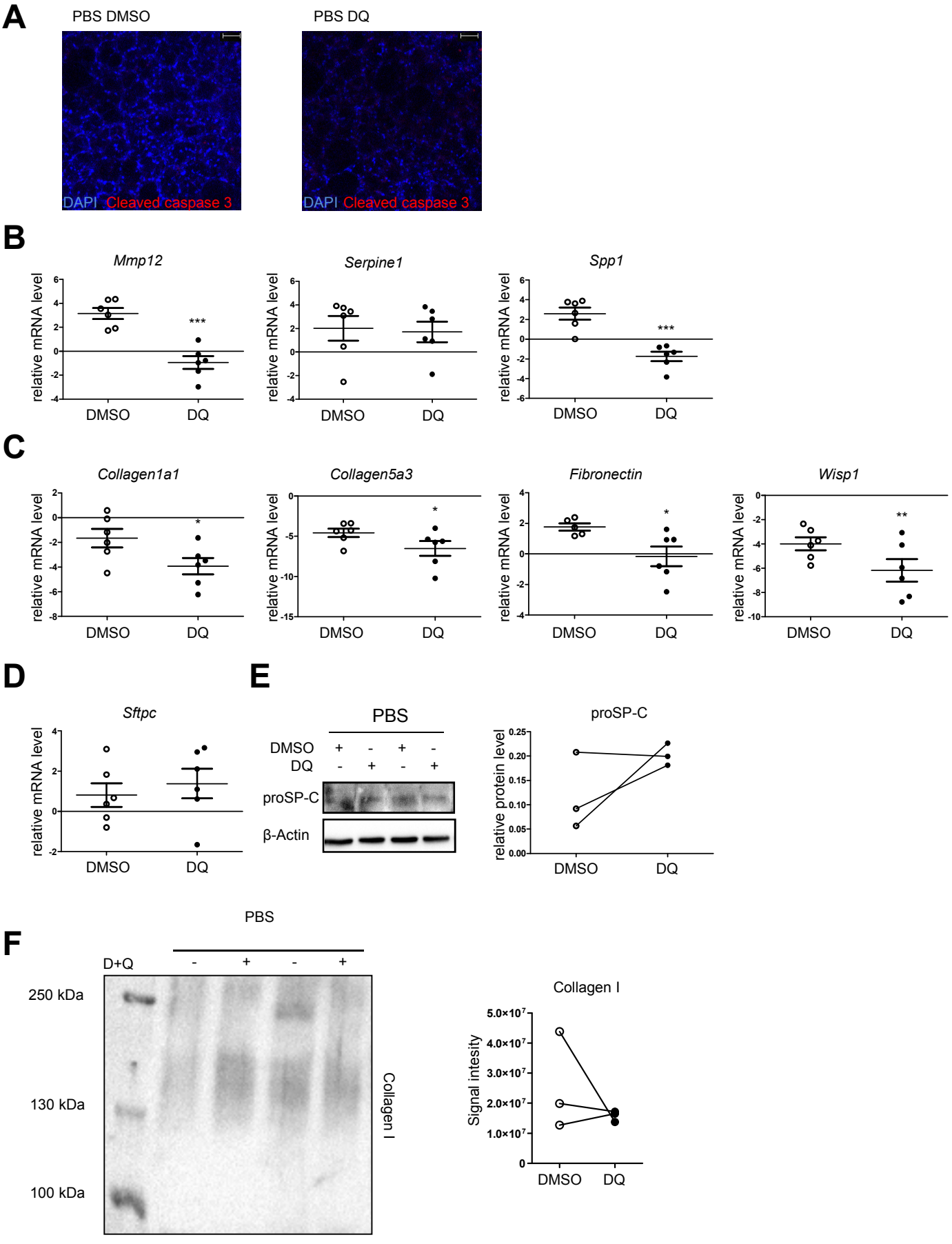

Collagen1a1
relative mRNA level
5
0
-5
-10
*
PBS
Bleo
Collagen5a3
relative mRNA level
0
-2
-4
-6
-8
*
PBS
Bleo
Fibronectin
relative mRNA level
6
4
2
0
-2
***
PBS
Bleo
Wisp1
relative mRNA level
0
-2
-4
-6
-8
-10
PBS
Bleo

Figure S8: Depletion of senescent cells in non-fibrotic 3D-LTCs.

Mice were instilled with PBS. At day 14 after instillation mice were sacrificed and 3D-LTCs were generated. 3D-LTCs were cultured for 48 h in the presence of senolytic drugs Dasatinib (D; 200 nM) and Quercetin (Q; 50 µM). **(A)** Representative images of immunofluorescence staining for apoptotic marker cleaved caspase 3. Fluorescent images represent a 200× magnification. The scale bar represents 50 µm. **(B-D)** 3D-LTCs from PBS animals treated with senolytic drugs were analyzed by qPCR for **(B)** components of the SASP **(C)** fibrotic markers **(D)** or *Sftpc***.** Data were normalized to *Hprt*. ΔCt is presented as mean ± s.e.m.. Significance was assessed with paired Student's t-test, n=6. **(E)** ProSP-C expression was assessed by Immunoblot. β-Actin was used as a loading control. Quantification of proSP-C protein relative to β-Actin. n=3. **(F)** Secreted Collagen I was assessed by Immunoblot. Quantification of secreted Collagen I normalized to supernatant volume. Significance was assessed with paired Student's t-test.n=3. Significance: *p<0.05, **p<0.01; ***p<0.001.

Figure S8


A
PBS DMSO
PBS DQ
DAPI Cleaved caspase 3
DAPI Cleaved caspase 3
B
Mmp12
Serpine1
Spp1
relative mRNA level
***
DMSO
DQ
C
Collagen1a1
Collagen5a3
Fibronectin
Wisp1
*
**
D
Sftpc
E
PBS
DMSO + - + -
DQ - + - +
proSP-C
β-Actin
proSP-C
relative protein level
F
PBS
D+Q - + - +
250 kDa
130 kDa
100 kDa
Collagen I
Collagen I
Signal intesity
5.0×10^7
4.0×10^7
3.0×10^7
2.0×10^7
1.0×10^7
0

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