

Supplementary Results

FGF10 haploinsufficient animals have increased respiratory system resistance

To further characterise pulmonary emphysema in experimental animals, we performed *in vivo* lung function tests. *Fgfr2b*^{+/-} mice exhibited an increase in static lung compliance already after 3 months of CS exposure compared to the corresponding controls (**Figure S5D**). As expected, only long-term CS exposure for 8 months led to a significant increase in lung compliance in Wt mice. At the 8-month time-point, RA-exposed *Fgfr2b*^{+/-} mice had significantly increased lung compliance without further increase upon CS exposure (**Figure S5D**). Interestingly, lung compliance in *Fgf10*^{+/-} mice remained unchanged upon 3 or 8 months of CS exposure (**Figure S5D**). Lung compliance in these animals was also not affected by ageing. Namely, the lung compliance results obtained from *Fgf10*^{+/-} mice did not correspond to the histologically quantified emphysema. To identify the cause of the observed discrepancy in the lung compliance data in *Fgf10*^{+/-} mice, we extensively analysed other parameters measured by the lung function test. *Fgf10*^{+/-} mice, after 3 or 8 months of CS exposure, indeed exhibited significantly increased overall respiratory system resistance (RRS) compared to corresponding Wt controls (**Figure S5E**). Overall respiratory system resistance could be increased due to increased airway and/or tissue resistance. Newtonian resistance, a resistance component related to the conductive airways, was significantly increased in CS-exposed *Fgf10*^{+/-} mice at 3- and 8-month time-points, compared to the corresponding CS-exposed Wt controls (**Figure S5F**). This could suggest airflow limitation in these animals. In contrast, RRS and Newtonian resistance in CS-exposed Wt mice were slightly lower compared to corresponding RA-exposed controls which could be a consequence of emphysema development. (**Figure S5E-F**). Moreover, tissue damping was also significantly increased in CS-exposed *Fgf10*^{+/-} mice at 3- and 8-month time-points, compared to the corresponding Wt controls (**Figure S5G**).

We further analysed the smooth muscle cells in the conductive airways in experimental mouse lung sections (**Figure S6**). In proximal airways, we observed an increase in the smooth muscle cells in CS-exposed Wt mice as well as in *Fgf10*^{+/-} mice regardless of the CS exposure (**Figure S6A**). However,

Fgf10^{+/-} mice had a pronounced amount of smooth muscle cells in the more distal and smaller airways. This was not the case in the small airways of CS-exposed Wt mice (**Figure S6B**). Therefore, increased muscularisation of small airways could be responsible for the increased airway resistance in the transgenic mice.

Since tissue damping corresponds to the stiffness of the distal lung parenchyma, we next investigated the collagen deposition in alveolar septal walls of the experimental animals. Even though we did not observe any typical signs of lung fibrosis, picosirius red staining revealed increased collagen deposition in Wt mice upon 8 months of CS exposure. In contrast, *Fgf10*^{+/-} and *Fgfr2b*^{+/-} results in an increase of collagen in alveolar septa in RA conditions at the 3 months and 8 months time-point compared to corresponding Wt controls, respectively (**Figure S7A**). Regardless of baseline differences, both transgenic animal lines reacted with a further increase in collagen deposition upon CS exposure. Despite the similar increase in the collagen amount in Wt CS-exposed mice at the 8 months time-point and in *Fgf10*^{+/-} RA-exposed mice at the 3 months time-point, we found different collagen organisation which we identified by high power view of the picosirius red staining (**Figure S7B**). While collagen in the lungs of CS-exposed Wt animals and *Fgfr2b*^{+/-} animals appeared organised in short fibres, collagen filaments seemed interconnected and spread through septal walls in *Fgf10*^{+/-} mouse lungs (**Figure S7B**). This could be the cause for increased tissue damping and respiratory system resistance (RRS). The discrepancy between mice with the different genetic context of impaired FGF10 signalling (*Fgf10*^{+/-} and *Fgfr2b*^{+/-}) regarding time course and organisation of collagen accumulation may be attributed to FGF10 signalling being mediated through other receptors or another ligand binding to FGFR2b.

Mice with impaired FGF10 signalling exhibit increased nitrotyrosine formation and cellular senescence in the lung

In addition, at the 8-month time-point, nitrotyrosine levels in RA-exposed *Fgf10*^{+/-} emphysematous mouse lungs were similar to those in CS-exposed Wt littermates and did not further increase upon CS exposure (**Figure S8A**). These results indicate that impaired FGF10 signalling may not only be a victim

of peroxynitrite but could also, in turn, contribute to the peroxynitrite formation, possibly due to a decreased antioxidant defence, as reported in several experimental models³⁷⁻³⁹. For example, FGF10 was also shown to induce heme oxygenase-1 (HO-1)-mediated defence, which scavenges both reactive oxygen and nitrogen species (ROS and RNS) and thus prevents peroxynitrite formation^{38,39}.

Moreover, we performed immunostaining for p16Ink4a, a marker of cell senescence, in lung sections of the experimental mice (**Figure S8B**). Pronounced cellular senescence was described as a major pathogenic factor in COPD³⁴. Indeed, we observed pronounced cell senescence in CS-exposed Wt mice, compared to corresponding RA controls at the 3 months time-point. *Fgf10*^{+/-} mice, regardless of the CS exposure, had a significantly higher p16Ink4a fluorescent signal in the distal lung parenchyma compared to Wt mice.

FGF10 overexpression could ameliorate elastase-induced airway resistance

Lung function tests revealed increased respiratory system resistance in elastase-treated animals (**Figure S17G**). The observed resistance seems to be caused by the Newtonian (airway) resistance (**Figure S17H**) which suggests airway limitation in this end-stage disease model. FGF10 overexpression did not increase airway resistance as one might expect from growth factor treatment. On the contrary, FGF10 could ameliorate elastase-induced airway obstruction (**Figure S17H**). This finding suggests that FGF10 treatment might have a beneficial effect against airway remodelling in COPD.

Supplementary Materials and Methods

Assessment of FGF10 levels in human serum

FGF10 levels were measured in human serum using a commercially available ELISA kit (ABIN6955812; Antibodies-online GmbH, Aachen, Germany) following the manufacturer's protocol.

Fgf10 or *Fgfr2b* haploinsufficient animals

Fgf10 haploinsufficient animals (B6;Cg-*Fgf10*^{tm1.3Sbel}/Sbel; abbreviated *Fgf10*^{+/-}) were obtained by crossing *Fgf10*^{tm1.2Sms}/J (Jackson lab reference 023729) with CMV-Cre mice. *Fgfr2b*^{+/-} (B6;Cg-*Fgfr2b*^{tm1.2Sbel}/Sbel) mice were obtained by crossing *Fgfr2b*^{flox/flox} from Clive Dickson with CMV-Cre mice.

Cigarette smoke exposure

Animals were exposed to mainstream smoke from 3R4F cigarettes (Lexington, KY, USA) for 3 or 8 months (5 days/week, 6 hours/day) produced by a cigarette smoke generator (Burkhart, Wedel, Germany) as previously described^{5,6,22,23}. Age-matched controls were kept under identical conditions but without CS exposure.

Lung function test and hemodynamic measurements

Measurements of *in vivo* lung function and hemodynamic measurements were performed as previously described, with slight modifications^{5,22,23}.

Intratracheal elastase application

Animals were anaesthetised with 3% isoflurane in 100% O₂ and a plastic 20G tubus was placed in the trachea. In order to reach uniform distribution of the applied liquid, a MicroSprayer® Aerosolizer (Model IA-1C; Penn-Century, Inc., Wyndmoor, PA, USA) with a high-pressure syringe (Model FMJ-250; Penn-Century, Inc., Wyndmoor, PA, USA) was used. All animals received 100µl saline or elastase (E7885; Merck KGaA, Darmstadt, Germany) dissolved in saline (24U/kg body weight).

In vivo Fluorescent Molecular Tomography (FMT) and micro-computed tomography (µCT)

FMT imaging was performed using an FMT 2500 system (VisEn Medical, Bedford, MA, USA) as previously described ²³. FMT scanning was performed 6h after tail vein injection of 2nmol matrix metalloproteinase probe (MMPsense 750 FAST; PerkinElmer, Inc., Waltham, MA, USA) or 2h after injection of 2nmol of Annexin Vivo 750 (Perkin Elmer, Waltham, USA). Anaesthesia was induced with 3% isoflurane (Baxter Deutschland GmbH, Unterschleißheim, Germany) in 100% O₂ and maintained with 1.5-2% isoflurane during imaging with a gas-delivery system integrated into the multimodal-imaging cassette that holds the mouse during FMT and μ CT imaging. Micro CT (μ CT) was performed using a Quantum GX μ CT scanner (PerkinElmer, Inc., Waltham, MA, USA) as previously described ²². Reconstructed volumes were processed using Analyze Pro software (Analyze Direct, Mayo Clinic, Minnesota, USA).

Non-invasive echocardiography

Non-invasive *in vivo* transthoracic echocardiography was performed one day before the animal sacrifice, under isoflurane inhalation anaesthesia as previously described ^{5,22,23}. Imaging was done using a Vevo[®] 2100 high-resolution Imaging System (FUJIFILM VisualSonics, Toronto, Canada) equipped with the high-frequency M550D transducer (22-55MHz). Mice were initially anaesthetised with 3% isoflurane, and anaesthesia was maintained with 1-2% isoflurane supplemented in 100% O₂. Chest hair was removed before imaging, and body temperature was monitored using a rectal thermometer. Tricuspid annular plane systolic excursion (TAPSE) was obtained under RV-focused apical 4-chamber view and analysed using anatomical M-mode as previously described ^{5,22,23}.

Lung function test and hemodynamic measurements

Anaesthesia was induced with 3% isoflurane in 100% O₂. The mouse was then restrained on a thermoregulation plate with constant anaesthesia supply via a nose-only mask. ECG electrodes and a rectal thermometer were placed for monitoring the heart rate and body temperature. After tracheotomy, the animals were intubated with a 18 G metal tubus (SCIREQ Scientific Respiratory Equipment Inc., Montreal, Canada). The trachea was fixed with a thread and ventilated using a

FlexiVent system, equipped with an FX2 module (SCIREQ Scientific Respiratory Equipment Inc., Montreal, Canada), at a frequency of 150 breaths/minute and a tidal volume of 5ml/kg. Lung function tests were performed using the FlexiVent at a PEEP of 3 cmH₂O, with a consistent perturbation order, following the manufacturer's recommendations. The results are presented as an average of measurements with a coefficient of determination above 0.95.

For right ventricular systolic pressure (RVSP) measurements, the jugular vein was surgically prepared and catheterised by a micro-tip catheter (Millar Instruments, SPR 671, REF 8406719, Houston, TX, USA) that was then forwarded into the right ventricle. Systemic arterial and left ventricular systolic pressures were measured by a catheter inserted through the carotid artery. Hemodynamic measurements were recorded and analysed using the PowerLab system and LabChart 7.0 software (ADInstruments GmbH, Spechbach, Germany).

Lung fixation, organ harvest and heart ratio measurements

The lungs were perfused for 5 minutes through the pulmonary artery with saline solution (B. Braun Melsungen, Germany) under 22 cmH₂O pressure during continuous ventilation (Minivent, Hugo Sachs Elektronik, March, Germany). Right lung lobes were collected and frozen in liquid nitrogen for further analysis. Left lung lobes were either fixed with formalin (n=7 per group; Otto Fischar GmbH, Saarbrücken, Germany) or inflated with Tissue-Tek® O.C.T.™ (n=5 per group; Sakura Finetek Europe B.V., Alphen aan den Rijn, The Netherlands) and frozen. Fixation was performed under simultaneous vascular perfusion (22 cmH₂O) and inflation (12 cmH₂O) with formalin for 20 minutes before removing from the thoracic cavity. The left lung lobe was then incubated in formalin overnight at room temperature. Afterwards, lungs were washed in PBS for 24 h at 4 °C. The fixed tissue was put in agarose, cut in 3 mm thick sections and dehydrated as previously described²². The right ventricle (RV) was separated from the left ventricle and septum (LV+S) for right-heart hypertrophy measurements.

Lung vessel casting and *ex vivo* μCT imaging

After sacrifice, mouse lungs were perfused through the pulmonary artery with a vasodilation buffer – PBS containing 4 mg/l papaverine (Linden Arzneimittel GmbH, Heuchelheim, Germany), 1 g/l adenosine (Merck KGaA, Darmstadt, Germany) and 1000 i.e./l heparin (Ratiopharm GmbH, Ulm, Germany), using an Ismatec® roller-pump (Cole-Parmer GmbH, Wertheim Germany) at the flow rate of 5ml/min. The lung was then perfused with freshly prepared Microfil® (MV122 YELLOW; Flow Tech, Inc., Carver, MA) solution, following the manufacturer's recommendations. Subsequently, the lung was inflated via the trachea with formalin solution (Otto Fischar GmbH, Saarbrücken, Germany) under a pressure of 20 cmH₂O. After 30 minutes, the fixed lung was gently removed from the chest cavity and kept in formalin solution until further processing. The lungs were then embedded in an agarose block, and images were acquired using a Quantum GX µCT scanner (PerkinElmer Inc., Waltham, MA, USA). The scanner's complementary metal-oxide-semiconductor X-ray flat-panel detector was set to allow image acquisition with an X-ray tube voltage of 90kV and current of 80 µA. µCT data were collected in list-mode over a single complete gantry rotation with a total rotation time of 57 minutes. Raw projection images were reconstructed, and then reconstructed volumes were loaded into Analyze 12.0 software (Analyze Direct, Mayo Clinic).

Histology and alveolar morphometry

For alveolar morphometry, paraffin-cut mouse and human lung sections were stained with haematoxylin and eosin (H&E), following routine protocol and as previously described^{6,22}. Mean linear intercept (MLI), airspace percentage and alveolar wall thickness were assessed under a light microscope using uniform random sampling and Qwin alveolar morphometry software (Leica, Wetzlar, Germany) as previously described^{5,6,22}. Only the alveolar compartment was considered for the measurements; all visible vessels or airways were excluded from each analysed image.

Design-based stereology

The density of alveoli was estimated using a physical disector method, as previously described^{22,23}. Briefly, two alternate 3µm thick paraffin cut lung sections, with a 3 µm distance between each other,

were placed on the same slide and stained according to Weigert's elastin staining protocol as previously described^{22,23}. Slides from the experimental animals were then scanned and analysed using a light microscope (CTR6000; Leica, Wetzlar, Germany) equipped with newCast software for stereology (Visiopharm, Aarhus, Denmark). Respective measurements of density were then related to the left lung volume, and the total alveoli number was calculated (n=4-6 per group).

Immunohistochemistry and staining quantification

Paraffin-embedded mouse or human lungs were cut in 2 µm thick sections, deparaffinised and rehydrated following routine protocols. Antigen retrieval was then performed by cooking slides in decloaking solution. Rodent Decloaker (RD913; Biocare Medical LLC, Pacheco, CA, USA) solution was used for mice and Diva Decloaker (Biocare Medical LLC, Pacheco, CA, USA) for human lung sections. The slides were washed in TBS buffer (ZUC052; Zytomed Systems GmbH, Berlin, Germany) and unspecific binding was blocked using 10% BSA solution. The slides were incubated with primary antibody solution diluted in antibody diluent (ZUC025; Zytomed Systems GmbH, Berlin, Germany) overnight at 4 °C. Following primary antibodies were used: Anti-FGF10 (1:200; ABIN360398; Antibodies Online, Aachen, Germany; RRID:AB_2715513) – for human tissue; Anti-FGF10 (1:200; ABN44; Merck KGaA, Darmstadt, Germany; RRID:AB_11204345) – for mouse tissue; Anti-Bek/FGFR2 (1:200; sc-6930; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; RRID:AB_669015); Anti-BrdU (1:100; 339808; Biolegend, San Diego, CA, USA; RRID:AB_10895898). ZytoChem Plus phosphatase polymer kit (POLAP-100; Zytomed Systems GmbH, Berlin, Germany) and Warp Red Chromogen substrate kit (WR806; Biocare Medical, Pacheco, CA, USA) were used, following the manufacturer's protocol, to visualise staining. Counterstaining was performed using CAT Haematoxylin solution (CATHE-M; Biocare Medical LLC, Pacheco, CA, USA). The stained area or number of cells was quantified in randomly selected fields using Qwin software (Leica, Wetzlar, Germany). Quantification was separately performed for alveolar wall and vessel compartments. To avoid the effects of the alveolar wall (respiratory surface) loss or vascular remodelling, the stained area was always standardised to the overall tissue surface in each given picture.

Assessment of the vessel and airway muscularisation degree

The degree of muscularisation was determined histologically as previously described^{5,6,23}. Briefly, 2µm thick paraffin cut lung sections of experimental animals were subjected to double immunohistochemistry staining for von Willebrand factor (vWF; 1:500; GA527; DAKO GmbH, Jena, Germany; RRID:AB_2810304) and alpha smooth muscle actin (α SMA; 1:400; A2547; Merck KGaA, Darmstadt, Germany; RRID:AB_476701). Degree of muscularisation of small pulmonary vessels (outer diameter 20–70 µm) was calculated using customised Leica Qwin software (Leica, Wetzlar, Germany) as previously published^{5,6,23}. For this analysis at least 100 pulmonary vessels per animal lung were counted. Data are given as average muscularisation (average percentage of vessel circumference positive for alpha smooth muscle actin staining) or percentages of total vessel count for fully muscularised, partially muscularised and non-muscularised vessels in lung sections.

Airway muscularisation was measured using the customised Leica Qwin software (Leica, Wetzlar, Germany). Briefly, the α SMA-stained area was measured and related to the overall tissue area. Quantification for each animal was based on the measurement from 5 randomly selected proximal and 5 randomly selected distal bronchi. Proximal and distal bronchi were distinguished by size and position in the lung sections.

Immunofluorescence staining and confocal microscopy

Paraffin-embedded mouse or human lungs were cut in 3µm thick sections, deparaffinised and rehydrated following routine protocols. Tissue sections were examined by laser scanning confocal microscopy (Leica TCS SP5) as previously described²³. Antigen retrieval for mouse lung sections was performed by cooking slides in Rodent Decloaker (RD913; Biocare Medical LLC, Pacheco, CA, USA), whereas human lung sections were cooked in HIER TRIS-EDTA Buffer pH 9 (ZUC029; Zytomed Systems GmbH, Berlin, Germany). Unspecific binding was blocked using a 10% BSA solution. The slides were incubated with a primary antibodies: Rabbit Anti von Willebrand factor (vWF; 1:200; GA527; DAKO GmbH, Jena, Germany; RRID:AB_2810304), Goat Anti-FGF10 (1:75; AF345; R&D Systems, Minneapolis,

MN), Rabbit Anti-ProSPC (1:150; ab90716; Abcam, Cambridge, UK; RRID:AB_10674024), Goat Anti-Receptor for Advanced Glycation Endproducts (RAGE; 1:200; AF1145; R&D Systems, Minneapolis, MN, USA; RRID:AB_354628), Rabbit Anti-p16INK4a (1:200; PA5-20379; Thermo Fisher Scientific Inc. Waltham, MA; RRID:AB_11157205), Rat Anti-Ki67 (1:100; 14-5698-82; Thermo Fisher Scientific Inc. Waltham, MA; RRID: AB_10854564) overnight at 4°C. The next day, slides were washed in PBS and incubated with adequate secondary antibodies: Goat Anti-Rat Alexa Fluor Plus 488 (Thermo Fisher Scientific Inc. Waltham, MA, USA), Goat Anti-Rabbit Alexa Fluor Plus 488 (Thermo Fisher Scientific Inc. Waltham, MA, USA), Donkey Anti-Rabbit Alexa Fluor Plus 488/555/647 (1:400; Thermo Fisher Scientific Inc. Waltham, MA, USA), Donkey Anti-Goat 488/555/647 (1:400; Thermo Fisher Scientific Inc. Waltham, MA, USA) and/or fluorescently labelled primary antibody against alpha-smooth muscle actin (Mouse anti- α SMA Cy3; 1:400; C6198; Merck KGaA, Darmstadt, Germany; RRID:AB_476856) for 3 hours at room temperature. After washing and staining with 0.1 μ g/ml DAPI (D9542; Merck KGaA, Darmstadt, Germany) or Hoechst 33342 (1:2000; H3570; Thermo Fisher Scientific Inc. Waltham, MA, USA) slides were covered using an anti-fade mounting medium (FP001G10; Biocare Medical LLC, Pacheco, CA, USA). Tissue sections were examined by laser scanning confocal microscopy (Leica TCS SP5). Series of confocal optic sections were taken using a Leica Plan Apo \times 63/1.32 objective lens. Each recorded image was taken using multichannel scanning and consisted of 1.024 \times 1.024 pixels. To improve image quality and to obtain a high signal/noise ratio, each image from the series was signal-averaged and was deconvoluted using AutoQuant X2 software (Bitplane). Quantification for each animal was based on the average fluorescence in 5 randomly selected fields.

Interstitial lung fibroblast from human patients

Interstitial lung fibroblasts were isolated from lungs that were explanted from healthy donors (n=6) or subjects with COPD (n=6). Distal lung tissue (cleared of pleura and visible bronchi/vessels) was placed in a falcon tube with DPBS (P04-36503, PAN-Biotech, Aidenbach, Germany) containing 2% penicillin-streptomycin (15070063, Lonza, Basel, Switzerland). Using the 3-scissors technique, the tissue was cut into small pieces and washed thoroughly with PBS. The pieces were then centrifuged at 240g for 5

minutes, resuspended in Dulbecco's Modified Eagle Medium (DMEM, 41965039, Gibco, Thermo Fischer, Massachusetts, USA) containing 10% FBS (F4135, Sigma-Aldrich, Germany) and 1% penicillin-streptomycin and plated in a T75 flask. The fibroblasts were left to outgrow for 1-2 weeks, changing the media every 3 days. Cells were then amplified and after the first passage cryopreserved until further use. For the treatments, cells were recovered and, in the following passage, seeded in 6-well plates. After reaching 80% confluence, cells were treated for 6h with 1% CSE. Protein samples were collected by scraping cells in the presence of Cell Lysis Buffer (9803; Cell Signaling Technology, MA, USA) containing 1mM PMSF and stored at -20°C until further processing.

Mouse alveolar epithelial type 2 cell isolation

Mice were anesthetised by intraperitoneal injection containing xylazine (20mg/kg body weight), ketamine (100mg/kg body weight) and heparin (50,000IU heparin/kg body weight). The mice were subsequently sacrificed by exsanguination, and lungs were perfused through the pulmonary artery with saline solution (B. Braun Melsungen, Germany), inflated through the trachea with 1ml dispase (354235, Corning Inc., USA) and put in a tube containing dispase. After 45 min of incubation at 37°C, lung lobes were separated and chopped using two scalpels. The suspension was then washed with DMEM and filtered through 70µm and 40µm strainers (83.3945.070; 83.3945.040; Sarstedt, Nümbrecht, Germany). Following centrifugation at 300g for 10 minutes, cell pellets were resuspended in DMEM medium, plated on a 10-cm dish which was pre-coated with rat anti-mouse CD31 (1:1000; 550274, BD Biosciences San Diego, CA, USA; RRID:AB_393571) rat anti-mouse CD45 (1:1000; 550539, BD Biosciences, San Diego, CA, USA; RRID:AB_2174426) and rat anti-mouse CD16/CD32 (1:1000; 553142, BD Biosciences, San Diego, CA, USA; RRID:AB_394657) antibodies. After 30-min incubation at 37°C, non-adherent cells were gently washed out and plated on cell culture dishes to negatively select fibroblasts. Following 1h of incubation at 37°C, the remaining cells were collected and centrifuged. The number of alveolar type 2 (AT2) cells was determined by the Nile red (Merck KGaA, Darmstadt, Germany) staining and counting in a Neubauer chamber (0640010, Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). AT2 cells were further cultured on fibronectin-coated (F1141, Merck

KGaA, Darmstadt, Germany) 96-well plates in DMEM medium containing 10% FBS and 1% penicillin-streptomycin.

Cigarette smoke extract preparation

Cigarette smoke extract (CSE) was always freshly prepared by burning a 3R4F cigarette (Lexington, KY, USA) in one minute and bubbling the smoke in 10ml of basal cell culture medium as previously described ²³. The medium was then sterile-filtered through a 0.2µm pore diameter (83.1826.001; Sarstedt, Nümbrecht, Germany) filter. The resulting CSE was considered as 100% and was further diluted with culture medium for treatment.

Apoptosis and viability assays

Into well in a 96-well plate, 35000 AT2 cells were seeded. Cells were incubated with or without 250 ng/ml recombinant human FGF10 (rhFGF10; R&D Systems, Minneapolis, MN, USA) for 24h. Afterwards, CSE was added in the medium for 6 h followed by measurement of apoptosis or viability. For assessment of apoptosis, an annexin V-based polarity-sensitive probe from Kinetic Apoptosis Kit (ab129817; Abcam, Cambridge, UK) was used according to the manufacturer's instructions. The images were obtained using the IncuCyte ZOOM (Essen BioScience, Ltd. Ann Arbor, MI, USA) system. Results are presented as a ratio between green fluorescence signal from annexin V probe and cell surface scanned in bright field. The viability of AT2 cells was measured using AlamarBlue Cell Viability Reagent (DAL1100; Thermo Fisher Scientific Inc. Waltham, MA, USA), following the manufacturer's protocol.

Precision cut lung slices (PCLS)

A piece of human lung tissue was inflated with 2% low melt agarose (6351, Carl Roth, Karlsruhe, Germany) solution through bronchi and left in ice-cold PBS until the agarose solidified. The COPD PCLS were cut in 400µm thick sections using a vibratome (Microm HM650V, Thermo Fisher Scientific Inc., Waltham, MA, USA) and cultured in RPMI 1640 medium containing 10% human serum (Seraclot; P40-3011; PAN-Biotech GmbH, Aidenbach, Germany), 1% Penicillin-Streptomycin, and 100µg/ml Normocin™ (ant-nt-1; InvivoGen, Toulouse, France). The medium was changed hourly in the first 6

hours to remove dead cells. In this study we included PCLS from 11 different COPD lungs (6 were used for assessment of FGF10-mediated cell proliferation and protein expression analysis and additional 5 for immunofluorescence staining). To better control our experiments, COPD PCLS were serially cut and used as control or for treatment with recombinant FGF10.

For assessment of proliferating cells, PCLS from 6 different COPD lungs were incubated for 24h in a medium containing bromodeoxyuridine (BrdU, 11647229001; Roche Holding AG, Basel, Switzerland) with or without 250ng/ml recombinant human FGF10 (rhFGF10; 345-FG-250; R&D Systems, Minneapolis, MN, USA). At the end of the treatment, PCLS were washed in cold PBS, fixed with 4% paraformaldehyde (sc-281692, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) for 2h at room temperature. Fixed PCLS were then embedded in paraffin and sectioned for immunostaining against BrdU, as described above. Number of BrdU positive cells were counted in control and treated COPD PCLS sections and related to the area of the tissue in all analysed fields.

For protein expression analysis PCLS from 6 COPD lungs were incubated for 24h in a medium with or without 250ng/ml recombinant human FGF10 and then homogenised for protein extraction.

For immunofluorescence staining, PCLS from 5 COPD lungs were incubated for 24h or 48h in medium with or without 250ng/ml recombinant human FGF10. At the end of the treatment, the PCLS were washed in cold PBS, fixed with 4% paraformaldehyde and stained following the protocol described below.

Immunofluorescence staining in PCLS

After fixation, PCLS were washed in PBS and cell membranes were permeabilised by incubation in 0.1% Triton-X in PBS for 10 minutes. After washing and blocking, the PCLS were incubated with primary antibodies diluted in blocking buffer: Goat Anti-CD31/PECAM1 (1:75; AF3628; R&D Systems, Minneapolis, MN, USA; RRID:AB_2161028) or Mouse Anti HT2-280 (1:300; TB-27AHT2-280; Terrace Biotech, San Francisco, CA, USA; RRID:AB_2832931) overnight at +4°C. Blocking buffer contained BSA (2%; 9048-46-8; Merck KGaA, Darmstadt, Germany), skimmed milk (2%; 70166; Merck KGaA,

Darmstadt, Germany) and Tween-20 (0.1%; P1379; Merck KGaA, Darmstadt, Germany) in PBS. The following day, PCLS were washed and incubated with adequate fluorescently labelled secondary antibodies (1:400; mentioned above) or primary fluorescently labelled mouse Anti- α SMA Cy3 (1:400; mentioned above) overnight at +4°C. Nuclei were stained with Hoechst 33342 (1:1000; H3570; Thermo Fisher Scientific Inc. Waltham, MA, USA). After staining and washing, PCLS were transferred to a microscope glass where spacers were placed, immersed in mounting medium (CS70330; DAKO GmbH, Jena, Germany) and covered with coverslips. Tissue sections were examined by laser scanning confocal microscopy as described above.

mRNA expression analysis by RT-qPCR

mRNA expression levels were assessed by a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories GmbH, Hercules, CA, USA). Mice lungs were homogenised with RNA lysis buffer using Precellys® 24-bead beating Tissue Homogenizer (Bertin Corp, Thermo Fischer Scientific Inc., Waltham, Massachusetts, USA). Total RNA was further purified using the RNeasy Mini Kit (74106, QIAGEN, Hilden, Germany), following the manufacturer's recommendations. Then, total RNA was reverse-transcribed using the iScript cDNA Synthesis Kit (1708891, Bio-Rad Laboratories GmbH, Hercules, CA, USA), before plating on Hard-Shell® 96-Well PCR Plates (HSP9601, Bio-Rad Laboratories GmbH, Hercules, CA, USA) with the iQ SYBR Green Supermix (1708885, Bio-Rad Laboratories GmbH, Hercules, CA, USA) and the primers for the target genes. All Primers used in this article are listed in the Supplement Table 1.

Protein expression analysis by western blot

Mouse lungs or human COPD PCLS were homogenised in commercially available lysis buffer (Cell Signaling Technology, Danvers, MA, USA) containing 1mM PMSF, using Precellys® 24-bead beating Tissue Homogenizer (Bertin Corp, Thermo Fisher Scientific Inc., Waltham, MA, USA) Thermo Fischer, Massachusetts, USA). Lysates were separated on 12% polyacrylamide gels by electrophoresis (100V, 400mA, 150W for 1.5 hours) and then transferred to a polyvinylidene difluoride (PVDF) membrane

(Pall Corporation, Dreieich, Germany) using a semi-dry blotting system (Keutz, Germany). After blocking, membranes were incubated overnight at 4°C with a primary antibody and for 1 hour at room temperature with a secondary antibody conjugated with horseradish peroxidase. Antibodies were diluted in blocking buffer, in the dilution ratio mentioned below. Protein bands were visualised with the ECL kit (Clarity™, Bio-Rad Laboratories GmbH, Hercules, CA, USA) using a ChemiDoc MP Imaging System (Bio-Rad Laboratories GmbH, Hercules, CA, USA). Densitometry was performed using the Image Lab™ Software (Bio-Rad Laboratories GmbH, Hercules, CA, USA). Each band was standardised to the intensity of β -actin that was used as a loading control. Expression was shown as a regulation factor, calculated by standardising each sample to the mean value of the expression in the room air control group. Following primary antibodies were used: Anti-FGF10 (1:500; AP14882PU-N; Acris Antibodies GmbH, Herford, Germany; RRID:AB_1752406); Anti-p-Akt (1:500; 9271; Cell Signaling Technology, Danvers, MA, USA; RRID:AB_329825); Anti-Bek/FGFR2 (1:1000; sc-6930; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; AB_669015); Anti-Akt (1:1000; 9272; Cell Signaling Technology, Danvers, MA, USA; RRID:AB_329827); Anti-Sp1 (1:1000; 9389; Cell Signaling Technology, Danvers, MA, USA; RRID:AB_11220235); Anti- β -catenin (1:1000; 8480; Cell Signaling Technology, Danvers, MA, USA; RRID:AB_11127855); Anti-Wnt3a (1:500; 2721; Cell Signaling Technology, Danvers, MA, USA; RRID:AB_2215411) Anti- β -actin (1:10000; ab8226; Abcam, Cambridge, UK; RRID:AB_306371). Anti-rabbit (1:5000; W4011; Promega, Madison, WI, USA; RRID:AB_430833) and anti-mouse (1:5000; W4021; Promega, Madison, WI, USA, RRID:AB_430834) secondary HRP conjugated antibodies were used.

Nitrotyrosine quantification via ELISA

Proteins were isolated from lung homogenate and protein concentration was determined using the Bradford assay (#5000113-5; Bio-Rad Laboratories GmbH, Hercules, CA, USA). For each sample, 50 μ g of protein was loaded per well and nitrotyrosine was quantified using a nitrotyrosine ELISA kit (17-376; Merck KGaA, Darmstadt, Germany) following the manufacturer's protocol.

Laser-assisted microdissection

Laser microdissection was done as described previously with subtle modifications^{5,23}. Cryopreserved lungs were cut into 12µm thick sections using a cryotome (CM1850; Leica, Wetzlar, Germany) and placed on a glass slide that was covered with a polyethylene naphthalate (PEN) membrane (11505158; Leica, Wetzlar, Germany). Slides were then dipped in hematoxylin solution and washed in 100% ethanol. Microdissection was performed using LMD 6000 system (Leica, Wetzlar, Germany) equipped with a laser, as previously described²³. Pulmonary vessels and alveolar wall compartment were collected separately in tubes containing RNA lysis buffer (79216; Qiagen, Inc., Hilden, Germany) supplemented with 1% 2-mercaptoethanol (M3148; Merck KGaA, Darmstadt, Germany).

Transcriptome analysis via microarray

Transcriptome analysis was performed using microarray technology as previously described^{5,23}. Briefly, RNA from microdissected vessels and alveolar walls was purified using RNeasy Micro Kit (74004; Qiagen, Hilden, Germany) and amplified using the Ovation PicoSL WTA System V2 kit (3312-24; NuGEN Technologies, San Carlos, CA, US). For each sample, 2µg amplified cDNA was cyanine-labelled using the SureTag DNA Labelling Kit (5190-3400; Agilent Technologies, Inc., Santa Clara, CA, US). Hybridisation to oligonucleotide spotted microarray slides (mouse genome 8 × 60 K, Design ID 028005; Agilent Technologies, Inc., Santa Clara, CA, US) and subsequent washing and drying of the slides were performed following the Agilent hybridisation protocol in Agilent hybridisation chambers, with the following modifications: 2µg labelled cDNA were hybridised for 22h at 65°C, and the cDNA was not fragmented before hybridisation. The dried slides were scanned at 2µm per pixel resolution using the InnoScan is900 (Innopsys, Chicago, IL, USA). Image analysis was performed with Mapix 6.5.0 software (Innopsys, Chicago, IL, USA; RRID SCR_002723), and calculated values for all spots were saved as GenePix results files. Stored data were evaluated using R software (The R Foundation, Iowa City, IA, USA; RRID SCR_001905) and the limma package from BioConductor (RRID SCR_010943). log₂ mean spot signals were calculated for further analysis. Data were background-corrected using the NormExp

procedure on the negative control spots and quantile-normalised before averaging. log₂ signals of replicate spots were averaged, and, of several different probes addressing the same gene, only the probe with the highest average signal was used. Genes with the $-\log p \geq 1.30$ (2.00 for the pulmonary vasculature) for the analysed comparison were considered as regulated. Interactions between genes and pathway analysis were performed using STRING Database (STRING-DB; Search Tool for the Retrieval of Interacting Genes/Proteins) ²⁸.

Data processing and statistical analysis

GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA) was used for graph assembly and data analysis. For comparison between two groups, t-test was used; for three groups, One-Way ANOVA was used; for comparison between four or more groups or for interaction analysis, Two-Way ANOVA was used. For correlation analysis, Pearson's test was performed. Details and further statistical procedures are described in the figure legends. Each difference, when $p \leq 0.05$, was considered statistically significant.

References

- 1 Barnes P. J., Burney P. G. J., Silverman E. K., Celli B. R., Vestbo J., Wedzicha J. A. and Wouters E. F. M. Chronic obstructive pulmonary disease. *Nature Reviews Disease Primers* **1**, 15076, doi:10.1038/nrdp.2015.76 (2015).
- 2 Barnes P. J. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clin Chest Med* **35**, 71-86, doi:10.1016/j.ccm.2013.10.004 (2014).
- 3 Hadzic S., Wu C. Y., Avdeev S., Weissmann N., Schermuly R. T. and Kosanovic D. Lung epithelium damage in COPD - An unstoppable pathological event? *Cell Signal* **68**, 109540, doi:10.1016/j.cellsig.2020.109540 (2020).
- 4 Gredic M., Blanco I., Kovacs G., Helyes Z., Ferdinandy P., Olschewski H., Barbera J. A. and Weissmann N. Pulmonary hypertension in chronic obstructive pulmonary disease. *Br J Pharmacol*, doi:10.1111/bph.14979 (2020).
- 5 Pichl A., Sommer N., Bednorz M., Seimetz M., Hadzic S., Kuhnert S., Kraut S., Roxlau E. T., Kojonazarov B., Wilhelm J., Gredic M., Gall H., Tello K., Richter M. J., Pak O., Petrovic A., Hecker M., Schermuly R. T., Grimminger F., Seeger W., Ghofrani H. A. and Weissmann N. Riociguat for treatment of pulmonary hypertension in COPD: a translational study. *Eur Respir J* **53**, doi:10.1183/13993003.02445-2018 (2019).
- 6 Seimetz M., Parajuli N., Pichl A., Veit F., Kwapiszewska G., Weisel F. C., Milger K., Egemnazarov B., Turowska A., Fuchs B., Nikam S., Roth M., Sydykov A., Medebach T., Klepetko W., Jaksch P., Dumitrascu R., Garn H., Voswinckel R., Kostin S., Seeger W., Schermuly R. T., Grimminger F., Ghofrani H. A. and Weissmann N. Inducible NOS inhibition reverses tobacco-smoke-induced

- emphysema and pulmonary hypertension in mice. *Cell* **147**, 293-305, doi:10.1016/j.cell.2011.08.035 (2011).
- 7 Peinado V. I., Pizarro S. and Barberà J. A. Pulmonary vascular involvement in COPD. *Chest* **134**, 808-814, doi:10.1378/chest.08-0820 (2008).
- 8 Kruk D., Wisman M., Bruin H. G., Lodewijk M. E., Hof D. J., Borghuis T., Daamen W. F., van Kuppevelt T. H., Timens W., Burgess J. K., Ten Hacken N. H. T. and Heijink I. H. Abnormalities in reparative function of lung-derived mesenchymal stromal cells in emphysema. *Am J Physiol Lung Cell Mol Physiol* **320**, L832-L844, doi:10.1152/ajplung.00147.2020 (2021).
- 9 Kojonazarov B., Hadzic S., Ghofrani H. A., Grimminger F., Seeger W., Weissmann N. and Schermuly R. T. Severe Emphysema in the SU5416/Hypoxia Rat Model of Pulmonary Hypertension. *American journal of respiratory and critical care medicine* **200**, 515-518, doi:10.1164/rccm.201902-0390LE (2019).
- 10 Yildirim A. O., Muyal V., John G., Muller B., Seifart C., Kasper M. and Fehrenbach H. Palifermin induces alveolar maintenance programs in emphysematous mice. *American journal of respiratory and critical care medicine* **181**, 705-17, doi:10.1164/rccm.200804-573OC (2010).
- 11 Klar J., Blomstrand P., Brunmark C., Badhai J., Hakansson H. F., Brange C. S., Bergendal B. and Dahl N. Fibroblast growth factor 10 haploinsufficiency causes chronic obstructive pulmonary disease. *J Med Genet* **48**, 705-9, doi:10.1136/jmedgenet-2011-100166 (2011).
- 12 Ornitz D. M. and Itoh N. The Fibroblast Growth Factor signaling pathway. *Wiley interdisciplinary reviews. Developmental biology* **4**, 215-266, doi:10.1002/wdev.176 (2015).
- 13 Bellusci S., Grindley J., Emoto H., Itoh N. and Hogan B. L. Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* **124**, 4867-78 (1997).
- 14 Yuan T., Volckaert T., Redente E. F., Hopkins S., Klinkhammer K., Wasnick R., Chao C. M., Yuan J., Zhang J. S., Yao C., Majka S., Stripp B. R., Günther A., Riches D. W. H., Bellusci S., Thannickal V. J. and De Langhe S. P. FGF10-FGFR2B Signaling Generates Basal Cells and Drives Alveolar Epithelial Regeneration by Bronchial Epithelial Stem Cells after Lung Injury. *Stem Cell Reports* **12**, 1041-1055, doi:10.1016/j.stemcr.2019.04.003 (2019).
- 15 Gupte V. V., Ramasamy S. K., Reddy R., Lee J., Weinreb P. H., Violette S. M., Guenther A., Warburton D., Driscoll B., Minoo P. and Bellusci S. Overexpression of fibroblast growth factor-10 during both inflammatory and fibrotic phases attenuates bleomycin-induced pulmonary fibrosis in mice. *American journal of respiratory and critical care medicine* **180**, 424-36, doi:10.1164/rccm.200811-1794OC (2009).
- 16 Ahmadvand N., Khosravi F., Lingampally A., Wasnick R., Vazquez-Armendariz I., Carraro G., Heiner M., Rivetti S., Lv Y., Wilhelm J., Gunther A., Herold S., Al Alam D., Chen C., Minoo P., Zhang J. S. and Bellusci S. Identification of a novel subset of alveolar type 2 cells enriched in PD-L1 and expanded following pneumonectomy. *Eur Respir J*, doi:10.1183/13993003.04168-2020 (2021).
- 17 Volckaert T., Dill E., Campbell A., Tiozzo C., Majka S., Bellusci S. and De Langhe S. P. Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. *J Clin Invest* **121**, 4409-19, doi:10.1172/jci58097 (2011).
- 18 Volckaert T., Yuan T., Chao C. M., Bell H., Sitaula A., Szimtenings L., El Agha E., Chanda D., Majka S., Bellusci S., Thannickal V. J., Fässler R. and De Langhe S. P. Fgf10-Hippo Epithelial-Mesenchymal Crosstalk Maintains and Recruits Lung Basal Stem Cells. *Dev Cell* **43**, 48-59.e5, doi:10.1016/j.devcel.2017.09.003 (2017).
- 19 Moiseenko A., Vazquez-Armendariz A. I., Kheirollahi V., Chu X., Tata A., Rivetti S., Günther S., Lebrigand K., Herold S., Braun T., Mari B., De Langhe S., Kwapiszewska G., Günther A., Chen C., Seeger W., Tata P. R., Zhang J. S., Bellusci S. and El Agha E. Identification of a Repair-Supportive Mesenchymal Cell Population during Airway Epithelial Regeneration. *Cell Rep* **33**, 108549, doi:10.1016/j.celrep.2020.108549 (2020).
- 20 Jackson V. E., Latourelle J. C., Wain L. V., Smith A. V., Grove M. L., Bartz T. M., Obeidat M., Province M. A., Gao W., Qaiser B., Porteous D. J., Cassano P. A., Ahluwalia T. S., Grarup N., Li

- J., Altmaier E., Marten J., Harris S. E., Manichaikul A., Pottinger T. D., Li-Gao R., Lind-Thomsen A., Mahajan A., Lahousse L., Imboden M., Teumer A., Prins B., Lyytikäinen L. P., Eiriksdóttir G., Franceschini N., Sitlani C. M., Brody J. A., Bosse Y., Timens W., Kraja A., Loukola A., Tang W., Liu Y., Bork-Jensen J., Justesen J. M., Linneberg A., Lange L. A., Rawal R., Karrasch S., Huffman J. E., Smith B. H., Davies G., Burkart K. M., Mychaleckyj J. C., Bonten T. N., Enroth S., Lind L., Brusselle G. G., Kumar A., Stubbe B., Kahonen M., Wyss A. B., Psaty B. M., Heckbert S. R., Hao K., Rantanen T., Kritchevsky S. B., Lohman K., Skaaby T., Pisinger C., Hansen T., Schulz H., Polasek O., Campbell A., Starr J. M., Rich S. S., Mook-Kanamori D. O., Johansson A., Ingelsson E., Uitterlinden A. G., Weiss S., Raitakari O. T., Gudnason V., North K. E., Gharib S. A., Sin D. D., Taylor K. D., O'Connor G. T., Kaprio J., Harris T. B., Pederson O., Vestergaard H., Wilson J. G., Strauch K., Hayward C., Kerr S., Deary I. J., Barr R. G., de Mutsert R., Gyllenstein U., Morris A. P., Ikram M. A., Probst-Hensch N., Glaser S., Zeggini E., Lehtimäki T., Strachan D. P., Dupuis J., Morrison A. C., Hall I. P., Tobin M. D. & London S. J. Meta-analysis of exome array data identifies six novel genetic loci for lung function. *Wellcome Open Res* **3**, 4, doi:10.12688/wellcomeopenres.12583.3 (2018).
- 21 Smith B. M., Traboulsi H., Austin J. H. M., Manichaikul A., Hoffman E. A., Blecker E. R., Cardoso W. V., Cooper C., Couper D. J., Dashnaw S. M., Guo J., Han M. K., Hansel N. N., Hughes E. W., Jacobs D. R., Jr., Kanner R. E., Kaufman J. D., Kleerup E., Lin C. L., Liu K., Lo Cascio C. M., Martinez F. J., Nguyen J. N., Prince M. R., Rennard S., Rich S. S., Simon L., Sun Y., Watson K. E., Woodruff P. G., Bagloli C. J. and Barr R. G. Human airway branch variation and chronic obstructive pulmonary disease. *Proc Natl Acad Sci U S A* **115**, E974-e981, doi:10.1073/pnas.1715564115 (2018).
- 22 Hadzic S., Wu C. Y., Gredic M., Kojonazarov B., Pak O., Kraut S., Sommer N., Kosanovic D., Grimminger F., Schermuly R. T., Seeger W., Bellusci S. and Weissmann N. The effect of long-term doxycycline treatment in a mouse model of cigarette smoke-induced emphysema and pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* **320**, L903-L915, doi:10.1152/ajplung.00048.2021 (2021).
- 23 Seimetz M., Sommer N., Bednorz M., Pak O., Veith C., Hadzic S., Gredic M., Parajuli N., Kojonazarov B., Kraut S., Wilhelm J., Knoepp F., Henneke I., Pichl A., Kanbagli Z. I., Scheibe S., Fysikopoulos A., Wu C.-Y., Klepetko W., Jaksch P., Eichstaedt C., Grünig E., Hinderhofer K., Geiszt M., Müller N., Rezende F., Buchmann G., Wittig I., Hecker M., Hecker A., Padberg W., Dorfmueller P., Gattenlöhner S., Vogelmeier C. F., Günther A., Karnati S., Baumgart-Vogt E., Schermuly R. T., Ghofrani H. A., Seeger W., Schröder K., Grimminger F., Brandes R. P. and Weissmann N. NADPH oxidase subunit NOXO1 is a target for emphysema treatment in COPD. *Nature Metabolism* **2**, 532-546, doi:10.1038/s42255-020-0215-8 (2020).
- 24 El Agha E., Schwind F., Ruppert C., Gunther A., Bellusci S., Schermuly R. T. and Kosanovic D. Is the fibroblast growth factor signaling pathway a victim of receptor tyrosine kinase inhibition in pulmonary parenchymal and vascular remodeling? *Am J Physiol Lung Cell Mol Physiol* **315**, L248-L252, doi:10.1152/ajplung.00140.2018 (2018).
- 25 Jones M. R., Chong L. and Bellusci S. Fgf10/Fgfr2b Signaling Orchestrates the Symphony of Molecular, Cellular, and Physical Processes Required for Harmonious Airway Branching Morphogenesis. *Frontiers in Cell and Developmental Biology* **8**, doi:10.3389/fcell.2020.620667 (2021).
- 26 Diaz A. A., Maselli D. J., Rahaghi F., Come C. E., Yen A., Maclean E. S., Okajima Y., Martinez C. H., Yamashiro T., Lynch D. A., Wang W., Kinney G. L., Washko G. R. and San José Estépar R. Pulmonary vascular pruning in smokers with bronchiectasis. *ERJ Open Research* **4**, 00044-2018, doi:10.1183/23120541.00044-2018 (2018).
- 27 Basson J., Sung Y. J., Schwander K., Kume R., Simino J., de las Fuentes L. and Rao D. Gene-education interactions identify novel blood pressure loci in the Framingham Heart Study. *Am J Hypertens* **27**, 431-44, doi:10.1093/ajh/hpt283 (2014).
- 28 Szklarczyk D., Gable A. L., Lyon D., Junge A., Wyder S., Huerta-Cepas J., Simonovic M., Doncheva N. T., Morris J. H., Bork P., Jensen L. J. and Mering C. V. STRING v11: protein-protein association

- networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* **47**, D607-d613, doi:10.1093/nar/gky1131 (2019).
- 29 Fysikopoulos A., Seimetz M., Hadzic S., Knoepp F., Wu C. Y., Malkmus K., Wilhelm J., Pichl A., Bednorz M., Tadele Roxlau E., Ghofrani H. A., Sommer N., Gierhardt M., Schermuly R. T., Seeger W., Grimminger F., Weissmann N. and Kraut S. Amelioration of elastase-induced lung emphysema and reversal of pulmonary hypertension by pharmacological iNOS inhibition in mice. *Br J Pharmacol*, doi:10.1111/bph.15057 (2020).
- 30 Antunes M. A. and Rocco P. R. Elastase-induced pulmonary emphysema: insights from experimental models. *An Acad Bras Cienc* **83**, 1385-96, doi:10.1590/s0001-37652011005000039 (2011).
- 31 Limjunyawong N., Craig J. M., Lagassé H. A., Scott A. L. and Mitzner W. Experimental progressive emphysema in BALB/cJ mice as a model for chronic alveolar destruction in humans. *Am J Physiol Lung Cell Mol Physiol* **309**, L662-76, doi:10.1152/ajplung.00214.2015 (2015).
- 32 Woo K. V., Shen I. Y., Weinheimer C. J., Kovacs A., Nigro J., Lin C. Y., Chakinala M., Byers D. E. and Ornitz D. M. Endothelial FGF signaling is protective in hypoxia-induced pulmonary hypertension. *J Clin Invest* **131**, doi:10.1172/jci.141467 (2021).
- 33 Tang H., Chen J., Fraidenburg D. R., Song S., Sysol J. R., Drennan A. R., Offermanns S., Ye R. D., Bonini M. G., Minshall R. D., Garcia J. G. N., Machado R. F., Makino A. and Yuan J. X. J. Deficiency of Akt1, but not Akt2, attenuates the development of pulmonary hypertension. *American journal of physiology. Lung cellular and molecular physiology* **308**, L208-L220, doi:10.1152/ajplung.00242.2014 (2015).
- 34 Houssaini A., Breau M., Kebe K., Abid S., Marcos E., Lipskaia L., Rideau D., Parpaleix A., Huang J., Amsellem V., Vienney N., Validire P., Maitre B., Attwe A., Lukas C., Vindrieux D., Boczkowski J., Derumeaux G., Pende M., Bernard D., Meiners S. and Adnot S. mTOR pathway activation drives lung cell senescence and emphysema. *JCI Insight* **3**, doi:10.1172/jci.insight.93203 (2018).
- 35 Bozinovski S., Vlahos R., Hansen M., Liu K. and Anderson G. P. Akt in the pathogenesis of COPD. *Int J Chron Obstruct Pulmon Dis* **1**, 31-38, doi:10.2147/copd.2006.1.1.31 (2006).
- 36 Conlon T. M., John-Schuster G., Heide D., Pfister D., Lehmann M., Hu Y., Ertüz Z., Lopez M. A., Ansari M., Strunz M., Mayr C., Angelidis I., Ciminieri C., Costa R., Kohlhepp M. S., Guillot A., Günes G., Jeridi A., Funk M. C., Beroshvili G., Prokosch S., Hetzer J., Verleden S. E., Alsafadi H., Lindner M., Burgstaller G., Becker L., Irmeler M., Dudek M., Janzen J., Goffin E., Gosens R., Knolle P., Pirotte B., Stoeger T., Beckers J., Wagner D., Singh I., Theis F. J., de Angelis M. H., O'Connor T., Tacke F., Boutros M., Dejardin E., Eickelberg O., Schiller H. B., Königshoff M., Heikenwalder M. and Yildirim A. Ö. Inhibition of LT β R signalling activates WNT-induced regeneration in lung. *Nature* **588**, 151-156, doi:10.1038/s41586-020-2882-8 (2020).
- 37 Tan X., Yu L., Yang R., Tao Q., Xiang L., Xiao J. and Zhang J.-S. Fibroblast Growth Factor 10 Attenuates Renal Damage by Regulating Endoplasmic Reticulum Stress After Ischemia–Reperfusion Injury. *Frontiers in Pharmacology* **11**, doi:10.3389/fphar.2020.00039 (2020).
- 38 Li S., Zhu Z., Xue M., Pan X., Tong G., Yi X., Fan J., Li Y., Li W., Dong Y., Shen E., Gong W., Wang X., Yu Y., Maeng Y. J., Li X., Lee K. Y., Jin L. and Cong W. The protective effects of fibroblast growth factor 10 against hepatic ischemia-reperfusion injury in mice. *Redox biology* **40**, 101859-101859, doi:10.1016/j.redox.2021.101859 (2021).
- 39 Li Y.-H., Yang L.-Y., Chen W., Li Y.-K. and Yuan H.-B. Fibroblast growth factor 10 protects neuron against oxygen–glucose deprivation injury through inducing heme oxygenase-1. *Biochem Biophys Res Commun* **456**, 225-231, doi:10.1016/j.bbrc.2014.11.063 (2015).