# Supplementary Figures and Tables

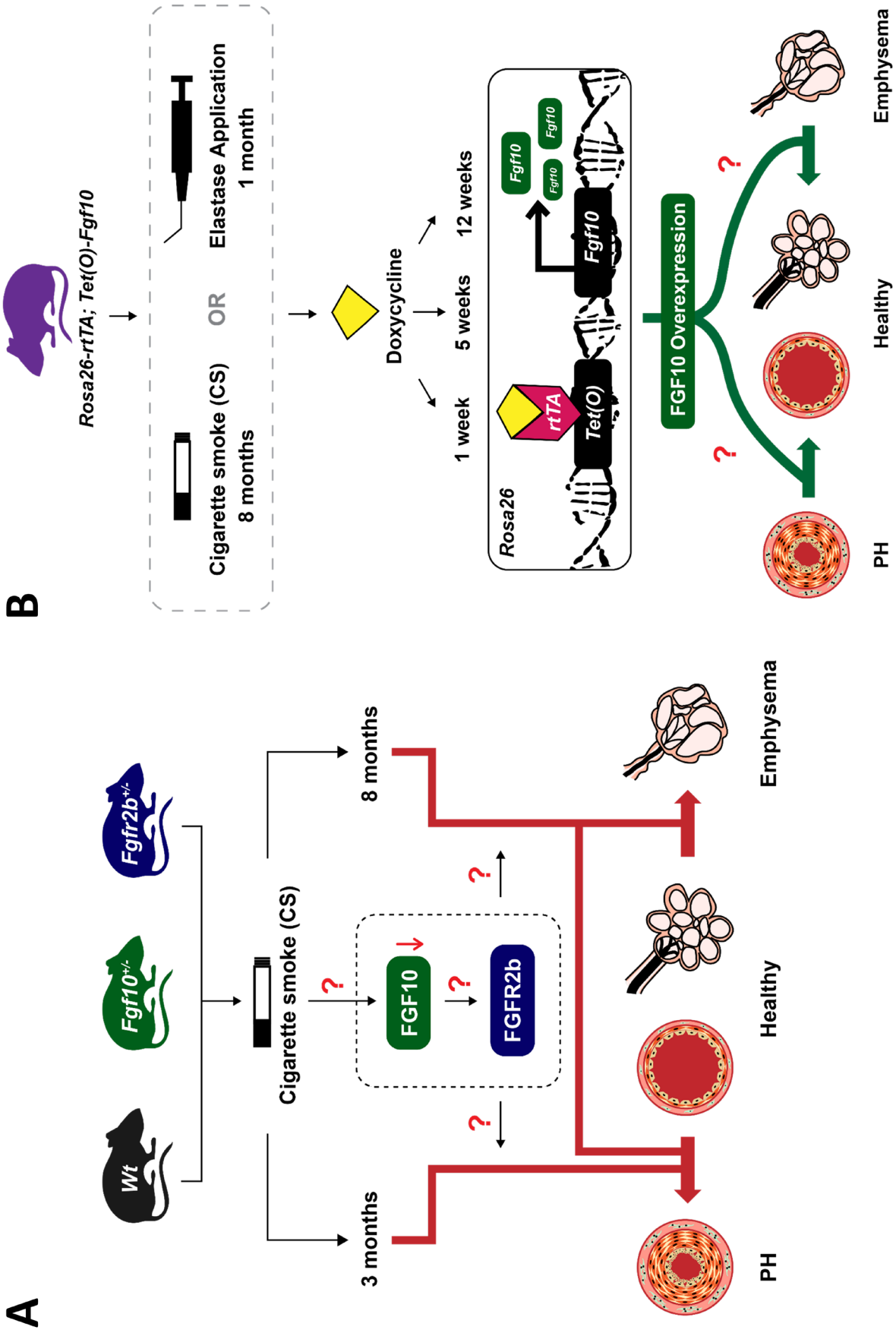

**Figure S1. Schematic drawing of the study design and hypothesis.**

**(A)** To elucidate the contribution of impaired FGF10 signalling in the development of cigarette smoke (CS)-induced emphysema and pulmonary hypertension (PH), *Fgf10*$^{+/-}$ and *Fgfr2b*$^{+/-}$ mice, together with wild-type (Wt) littermate controls, were exposed to CS or room air (RA) for 3 or 8 months. **(B)** Mice with inducible FGF10 expression were used to elucidate if restoration of FGF10 signalling can reverse CS- or elastase-induced emphysema and PH. In these transgenic animals, after the lung injury, FGF10 overexpression was induced by feeding mice with doxycycline-containing feed. Animals were sacrificed after 1, 5 or 12 weeks of treatment.

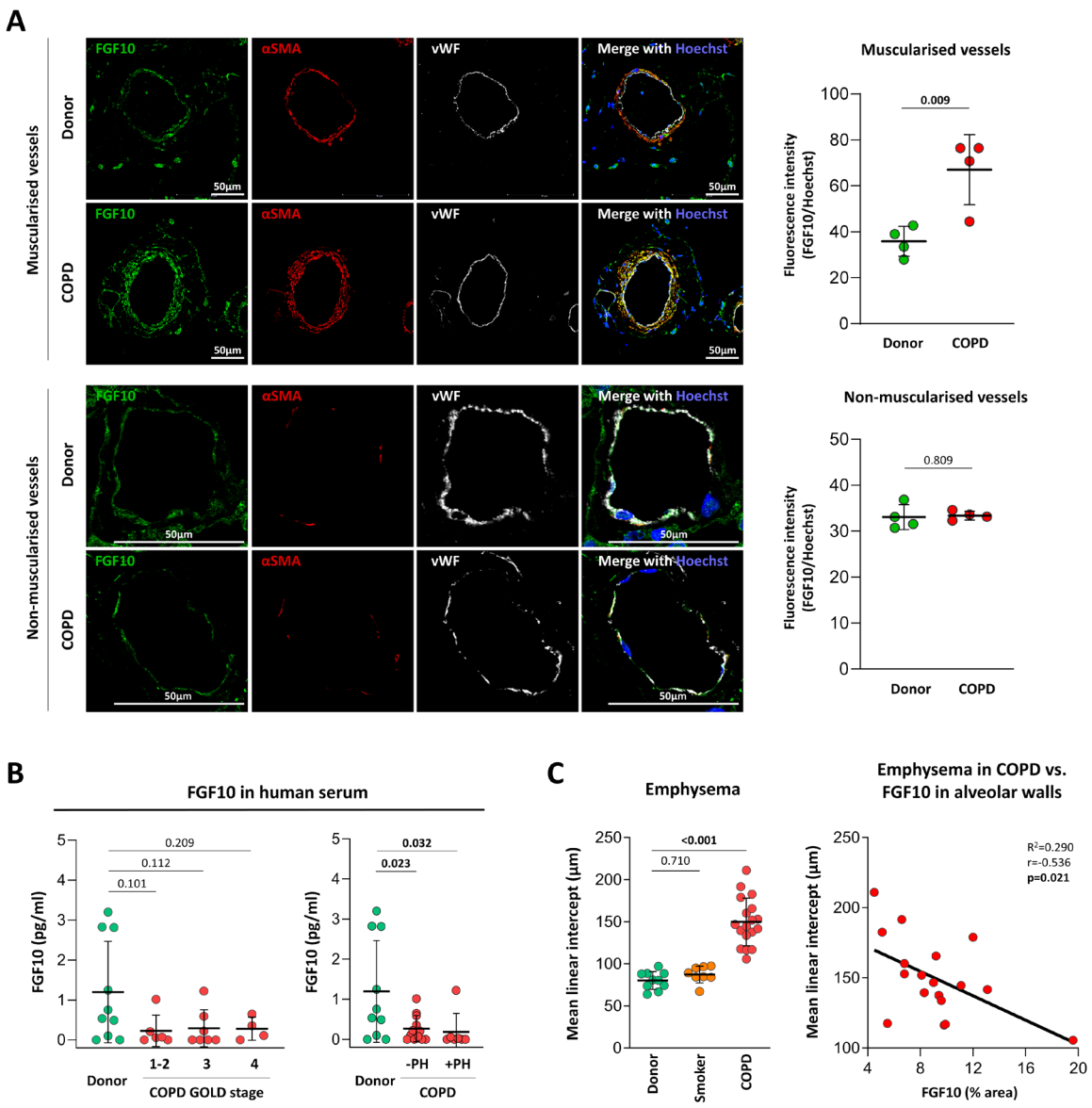


**Figure S2. FGF10 levels in human donor and COPD lungs and serum.**

**(A)** Immunofluorescence staining and quantification of FGF10 in proximal and distal vessels in donor (n=4) and COPD (n=4) lungs. **(B)** FGF10 levels in human serum from healthy donors (n=10) and from COPD patients in various stages of disease severity (GOLD stage 1-2: n=6; GOLD stage 3: n=7; GOLD stage 4: n=4). Among these COPD patients, n=12 were without and n=7 with diagnosed pulmonary hypertension (PH). **(C)** Histological quantification of emphysema (mean linear intercept) in paraffin cut sections of human donor (n=10), smoker without emphysema (n=8) and emphysematous COPD lungs (n=18) and correlation between emphysema severity and FGF10 protein expression in alveolar walls of COPD lungs (n=18).

In the quantification panels, each dot represents a measurement obtained from an individual human subject. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – **(A)** unpaired t-test; **(B)** One-Way ANOVA (Dunnett´s multiple-to-one comparison); **(C)** One-Way ANOVA (Dunnett´s multiple-to-one comparison) and Pearson's correlation test ($R^2$ – coefficient of determination; r – correlation coefficient); p-value for each test is given in the graph. Abbreviations – GOLD: Global Initiative for Chronic Obstructive Lung Disease; αSMA: alpha smooth muscle actin; vWF: von Willebrand factor.

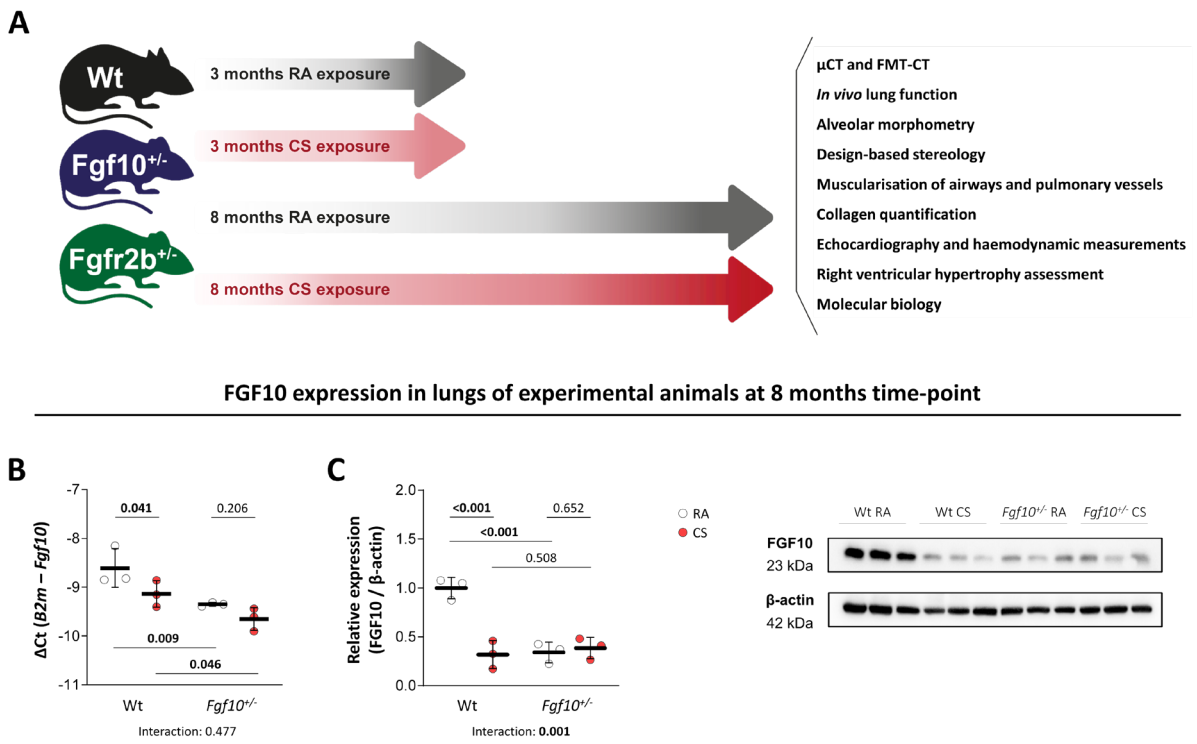


**Figure S3. Experimental design with FGF10 and FGFR2b haploinsufficient mice.**

**(A)** Experimental design: *Wt, Fgf10*$^{+/-}$ *and Fgfr2b*$^{+/-}$ mice were exposed to room air (RA) or cigarette smoke (CS) for 3 or 8 months and then examined for functional, structural and molecular alterations. Littermate mice with both functional alleles of *Fgf10* and *Fgfr2b* were used as Wt controls. **(B)** *Fgf10* mRNA expression in the lung homogenates from Wt and *Fgf10*$^{+/-}$ mice at 8-months time-point (n=3). mRNA was quantified using RT-qPCR and expression of the gene of interest related to the expression of *B2m* (reference gene). **(C)** Western blot quantification of FGF10 expression in lung homogenates from Wt and *Fgf10*$^{+/-}$ mice at 8-months time-point (n=3). Protein expression in western blot was quantified by band densitometry analysis and standardized to β-actin; relative expression was calculated by standardizing each value to the mean value of the Wt RA control group.

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs. Abbreviations – µCT: micro computed tomography; FMT: fluorescent molecular tomography; LMD: laser assisted microdissection.

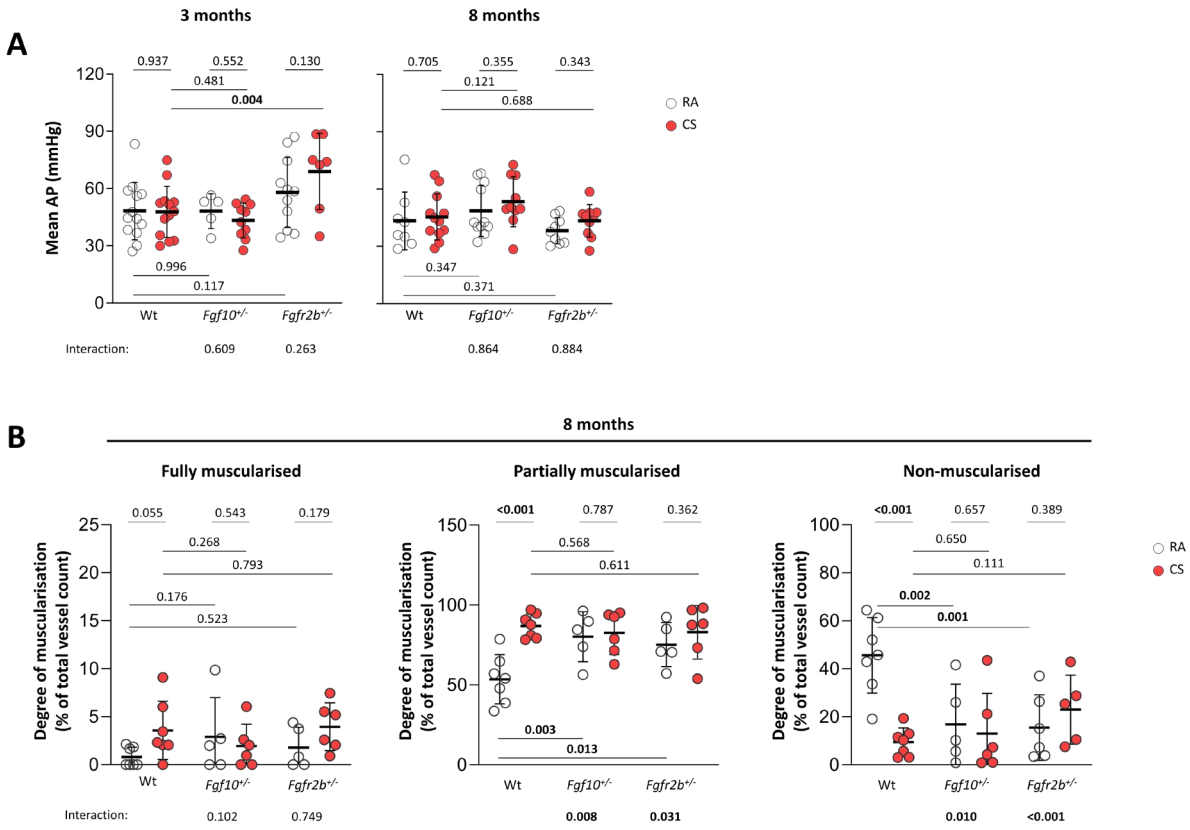


**Figure S4. Mice with impaired FGF10 signalling spontaneously develop pulmonary hypertension – extended data.**

**(A)** Mean arterial pressure (AP) measured in Wt, *Fgf10*$^{+/-}$ *or Fgfr2b*$^{+/-}$ mice exposed to room air (RA) or cigarette smoke (CS) for 3 or 8 months (n=5-12). **(B)** Histological quantification of fully, partially and non- muscularised pulmonary vessels (shown as percentage of total vessel count) in experimental mice after 8 months of RA or CS exposure (n=5-7).

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

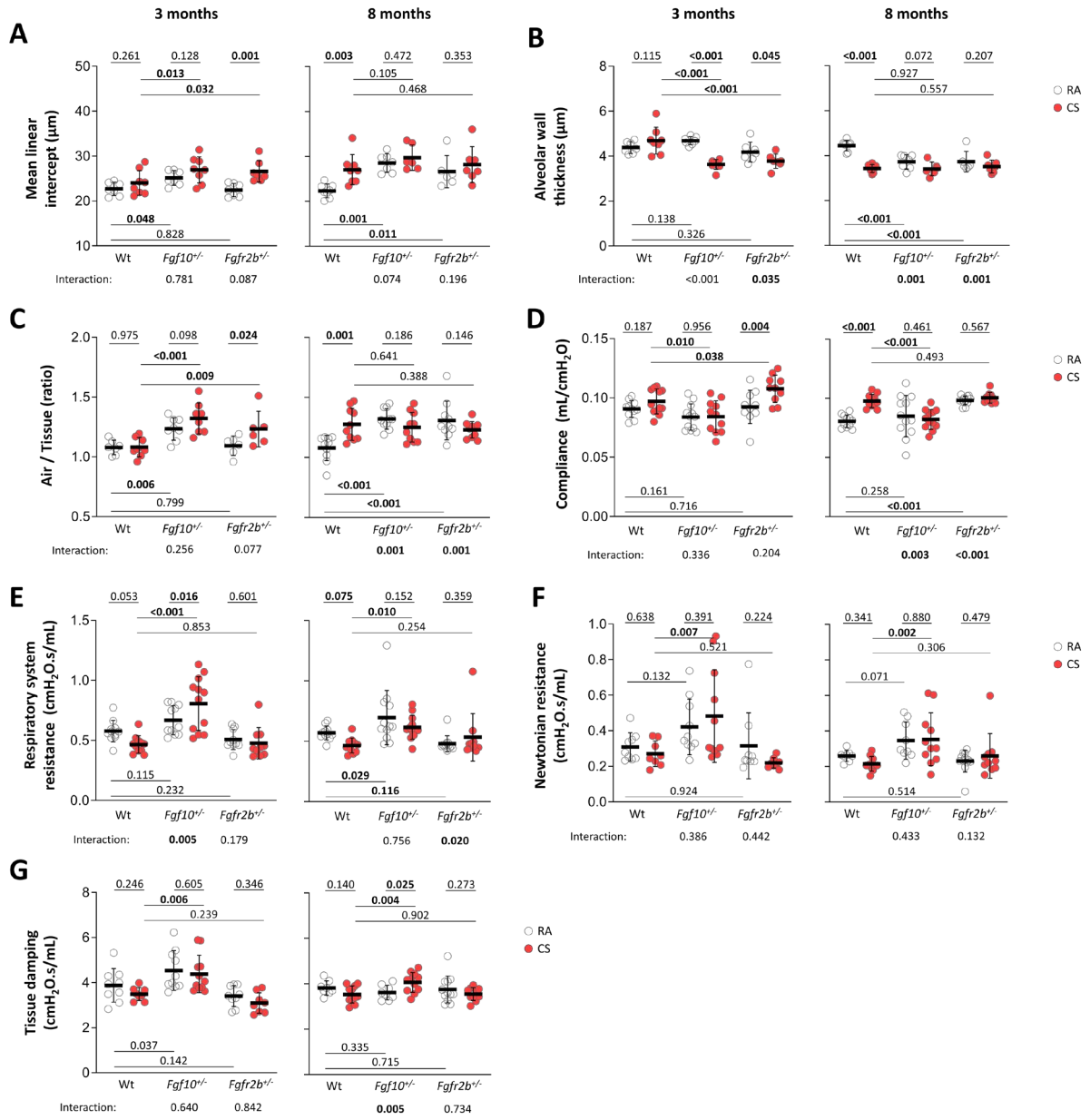


**Figure S5. Mice with impaired FGF10 signalling spontaneously develop emphysema – extended data.**

Histological quantification of **(A)** mean linear intercept and **(B)** alveolar wall thickness in lungs of in Wt, *Fgf10*$^{+/-}$ *or Fgfr2b*$^{+/-}$ mice exposed to room air (RA) or cigarette smoke (CS), exposure for 3 or 8 months (n=6-8). **(C)** Air to tissue volume ratio assessed *in vivo* by microcomputed tomography (µCT; n=6-10). **(D)** Static lung compliance (n=10-12), **(E)** overall respiratory system resistance (n=10-12), **(F)** conducting airway (Newtonian) resistance (n=8-12) and **(G)** stiffness of the distal lung parenchyma – tissue damping (n=8-12) measured by *in vivo* lung function tests in experimental mice.

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

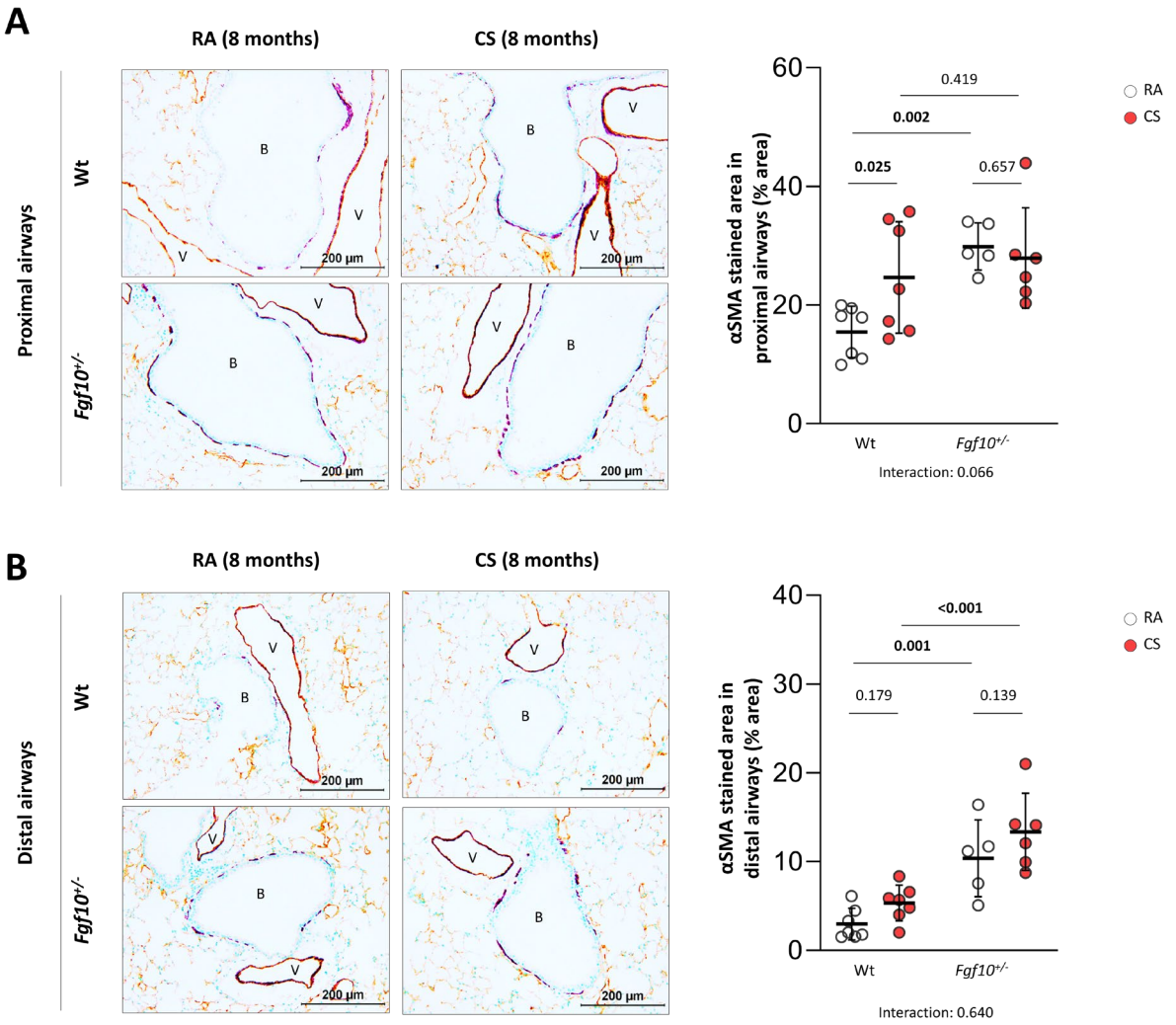


**Figure S6. Airway muscularisation in room air- or cigarette smoke-exposed Wt mice and mice with genetic FGF10 haploinsufficiency.**

Representative images and histological quantification of smooth muscle cells in walls of **(A)** proximal and **(B)** distal airways in lungs from Wt and *Fgf10*$^{+/-}$ mice after 8 months of room air (RA) or cigarette smoke (CS) exposure (n=5-7) by alpha smooth muscle actin staining (purple). Endothelial cells are visualised by von Willebrand factor staining (brown), nuclei are stained in green.

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

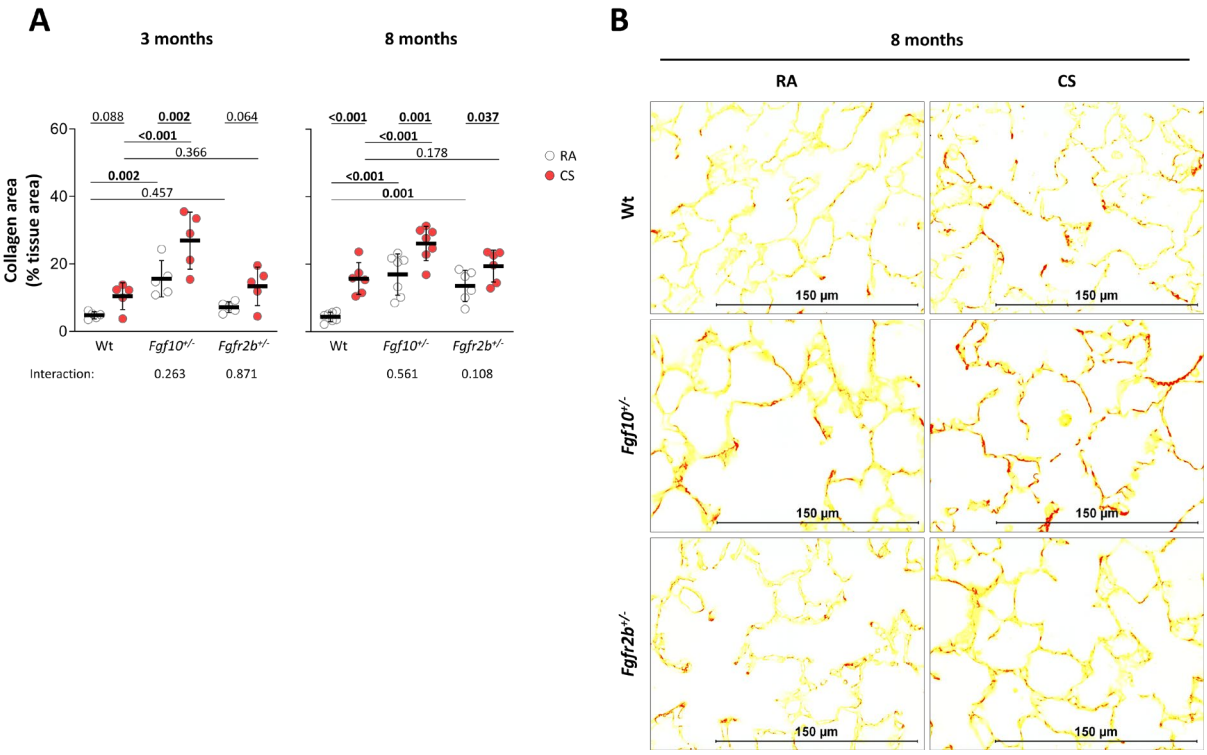


**Figure S7. Distal lung parenchyma stiffening is associated with cigarette smoke exposure and impaired FGF10 signalling.**

**(A)** Collagen quantification (n=5-7) in lung sections of Wt, *Fgf10*$^{+/-}$ *or Fgfr2b*$^{+/-}$ mice exposed to room air (RA) or cigarette smoke (CS) for 3 or 8 months. **(B)** Representative images of picrosirius red staining used to visualize collagen in the lung parenchyma of Wt, *Fgf10*$^{+/-}$ *or Fgfr2b*$^{+/-}$ mice after 8 months of RA or CS exposure. The collagen stained area (red) was quantified and standardised to the total stained area of alveolar septal walls (stained yellow).

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

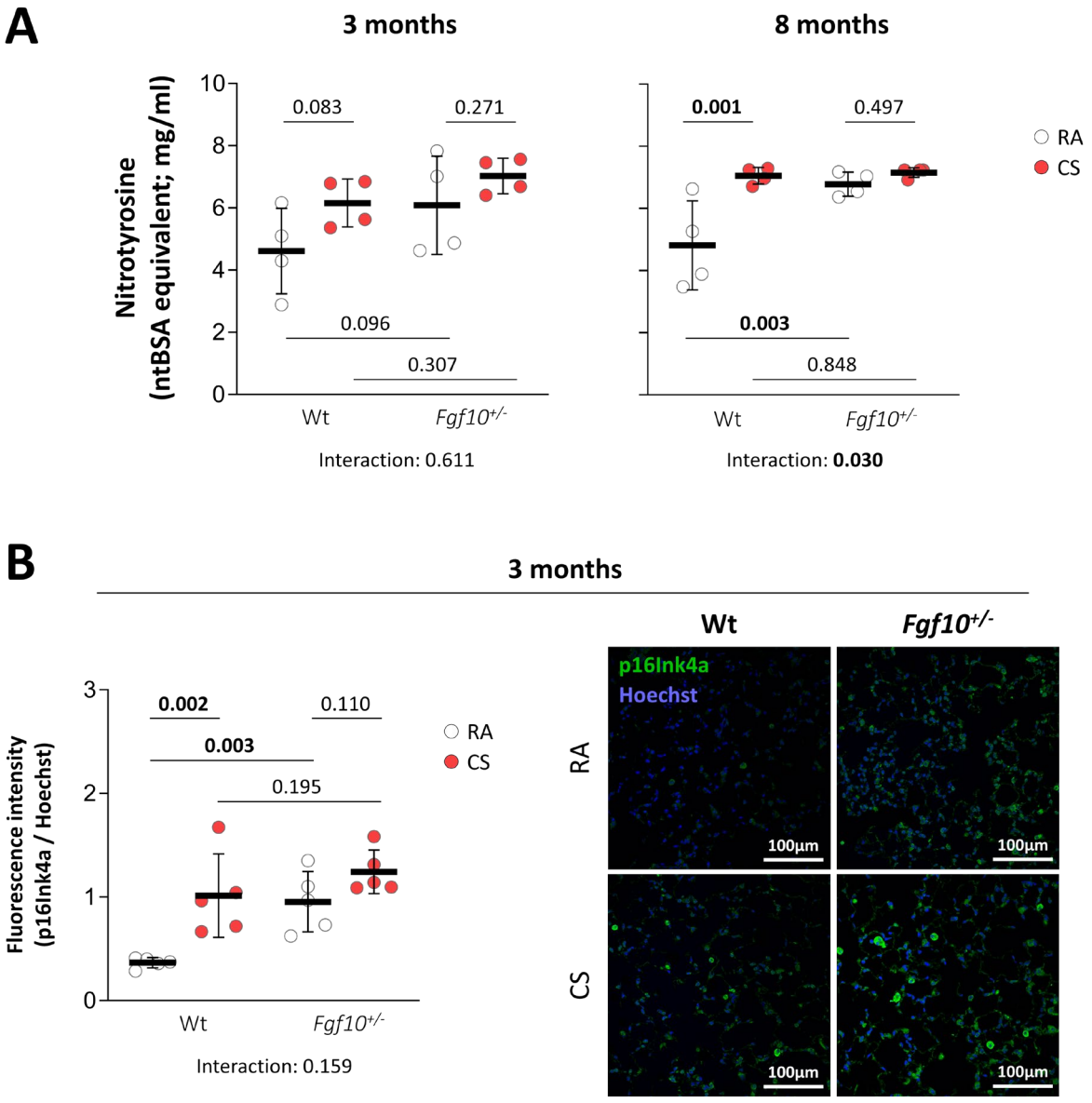


**Figure S8. Nitrotyrosine formation and cellular senescence in cigarette smoke-exposed Wt mice and mice with genetic FGF10 haploinsufficiency.**

**(A)** Nitrotyrosine quantified using ELISA in the lung homogenates (n=4) of Wt and *Fgf10*$^{+/-}$ mice exposed to room air (RA) or cigarette smoke (CS) for 3 or 8 months. The amount of nitrotyrosine was related to the nitrated bovine serum albumin (ntBSA) that was used as standard. **(B)** Cellular senescence determined by p16Ink4a immunostaining in lung sections of experimental animals (n=5) at 3 months time-point. Fluorescence intensity was quantified as ratio p16Ink4a / Hoechst. In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation.

Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

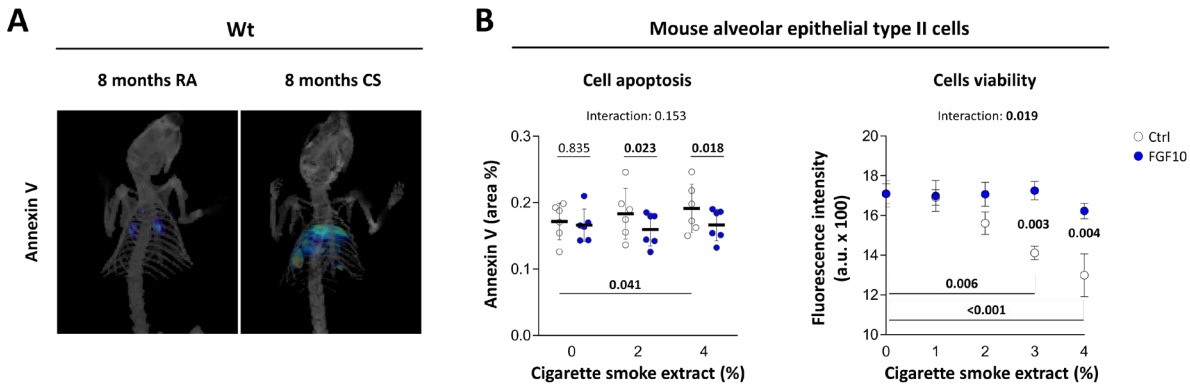


**Figure S9. Pulmonary cell apoptosis *in vivo* upon cigarette smoke exposure and apoptosis and viability of alveolar epithelial type 2 cells *in vitro* upon cigarette smoke extract exposure and FGF10 treatment.**

**(A)** Representative images showing the signal from Annexin-Vivo™ probe in lungs of experimental animals upon 8 months of room air (RA) or cigarette smoke (CS) exposure. The signal was captured *in vivo* via fluorescent molecular tomography (FMT) and merged with scans from microcomputed tomography (µCT). **(B)** Apoptosis, quantified as the surface of Annexin V-positive staining and cell viability estimated using AlamarBlue™ in alveolar epithelial type II cells isolated from n=6 Wt animals and *in vitro* treated with recombinant FGF10 and/or cigarette smoke extract (CSE).

The mean value for each group is represented by a horizontal line or a dot +/- standard deviation. Statistical analysis – paired Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

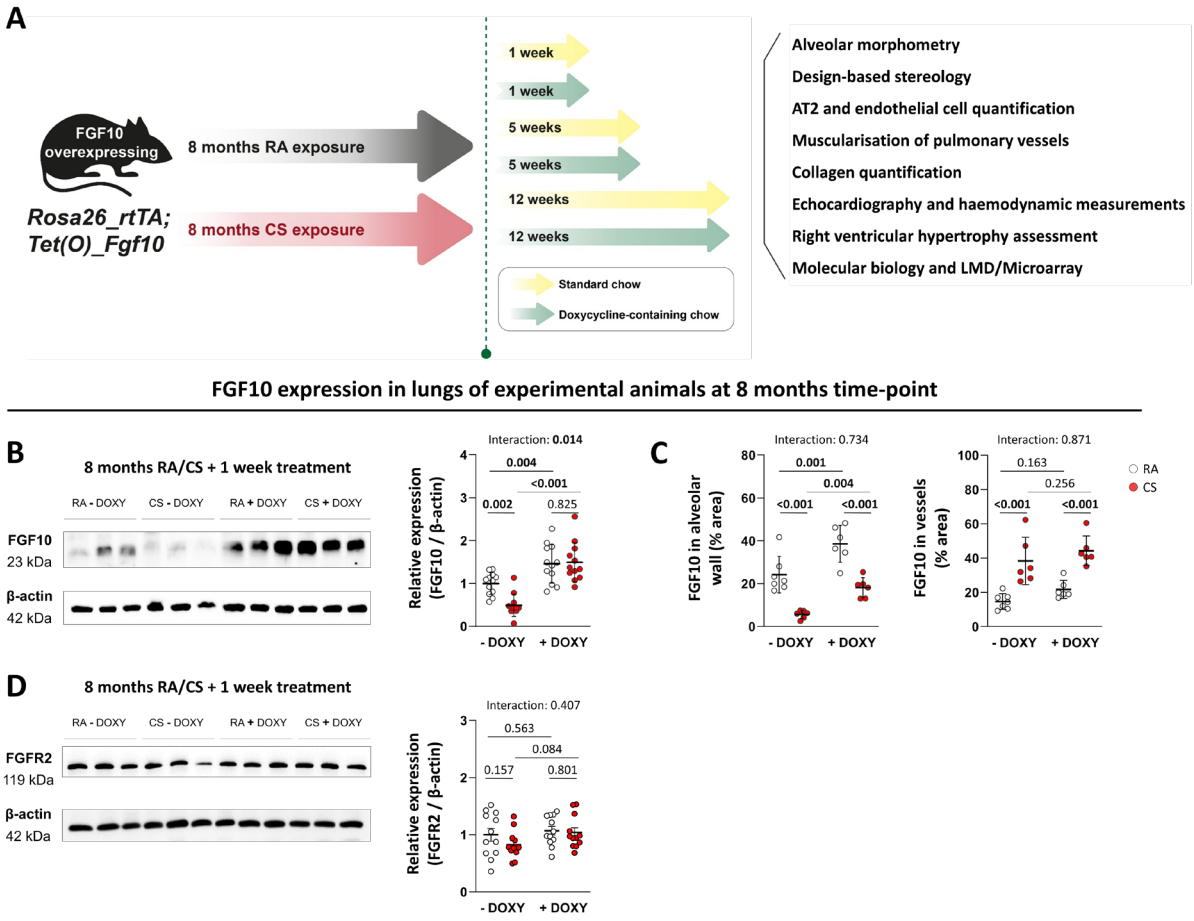


**Figure S10. Experimental design and FGF10/FGFR2 expression in FGF10 overexpressing mice.**

**(A)** Schematic drawing of the experimental design. Animals were exposed for 8 months to room air (RA) or cigarette smoke (CS) and subsequently fed with standard feed or feed containing doxycycline (DOXY) for 1, 5 or 12 weeks in RA conditions. **(B-C)** FGF10 overexpression confirmation by **(B)** Western blot in lung homogenates (n=12) and **(C)** histology (n=6-7) in alveolar septa and the pulmonary vasculature in lungs of experimental mice after 8 months of RA or CS exposure and subsequently fed with regular or doxycycline-containing (DOXY) feed for 1 week in RA condition. **(D)** Western blot quantification of FGF receptor 2 (FGFR2) in lung homogenates of experimental animals (n=12).

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented with a horizontal line +/- standard deviation. Statistics – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

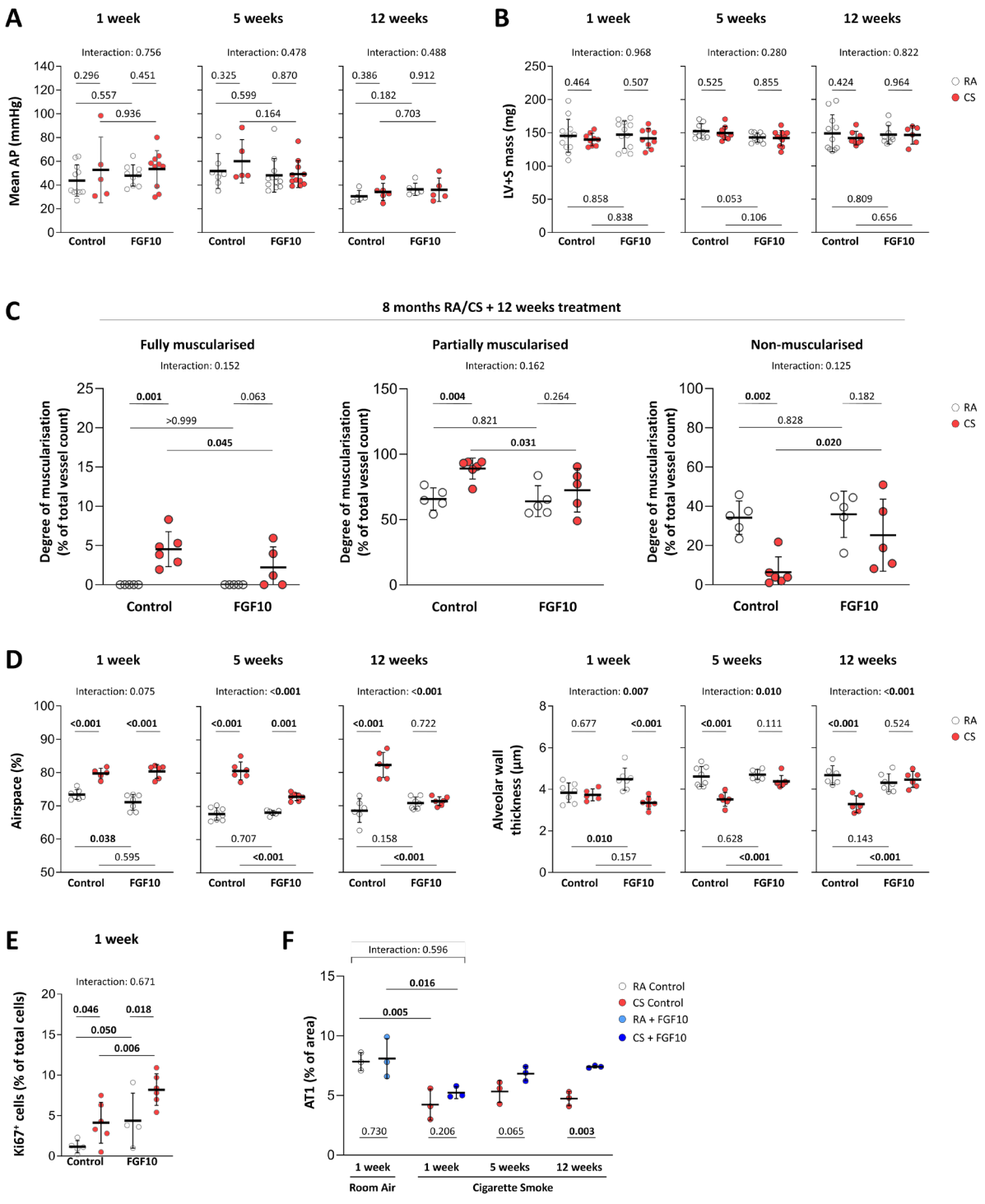


**Figure S11. FGF10 overexpression reverses cigarette smoke-induced pulmonary hypertension and emphysema in mice – extended data.**

**(A)** Mean arterial pressure (AP; n=5-12) and **(B)** weight of the left ventricle plus septum (LV+S; n=6-12) measured in experimental animals that were exposed for 8 months to room air (RA) or cigarette smoke (CS) and subsequently fed with standard feed (Control) or feed containing doxycycline for 1, 5 or 12

weeks (FGF10) in RA conditions. **(C)** Histological quantification of fully, partially and non- muscularised pulmonary vessels (shown as percentage of total vessel count) in experimental mice exposed for 8 months to RA or CS and subsequently fed with standard feed (Control) or feed containing doxycycline (FGF10) for 12 weeks (n=5-6). **(D)** Alveolar airspace percentage and alveolar wall thickness measured in the lung sections of experimental mice (n=5-8). **(E)** Percentage of proliferating (Ki67 positive) cells in lungs of experimental mice at 1 week time point (n=4-6). **(F)** Percentage of area covered by AT1 cells quantified by confocal microscopy in lung sections of experimental mice upon immunofluorescence staining against RAGE (Receptor for Advanced Glycation Endproducts) marker protein (n=3).

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented with a horizontal line +/- standard deviation. Statistics – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

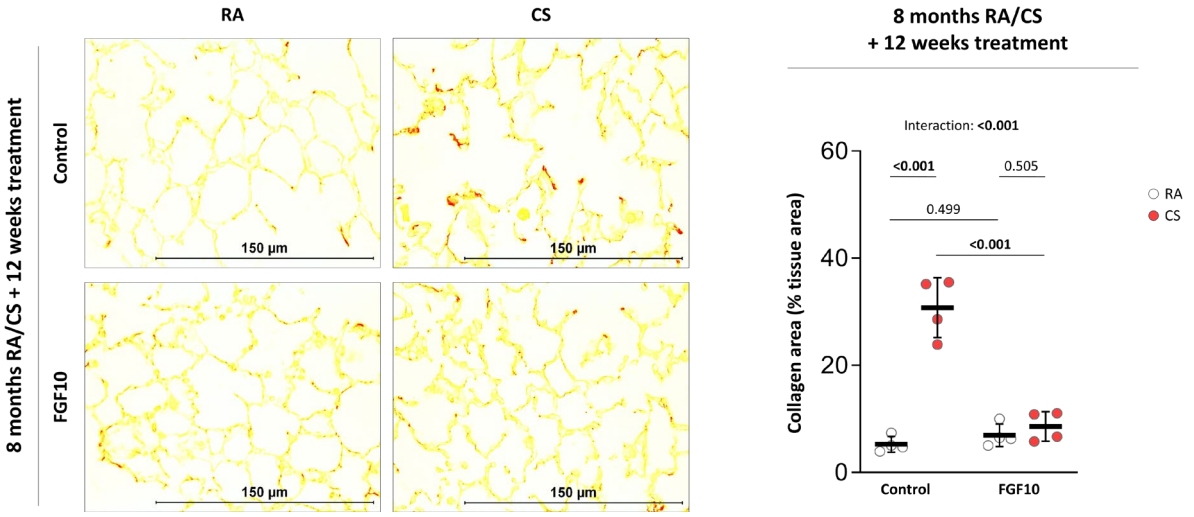


**Figure S12. FGF10 overexpression affects extracellular matrix in cigarette smoke-exposed mice lungs.**

Representative images of picrosirius red staining and collagen quantification (n=4) in lung sections of FGF10 overexpressing experimental mice exposed for 8 months to room air (RA) or cigarette smoke (CS) and subsequently fed with standard feed (Control) or feed containing doxycycline (FGF10) for 12 weeks. Collagen-stained area (red) was quantified and standardised to the total stained area of alveolar septal walls (stained yellow).

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

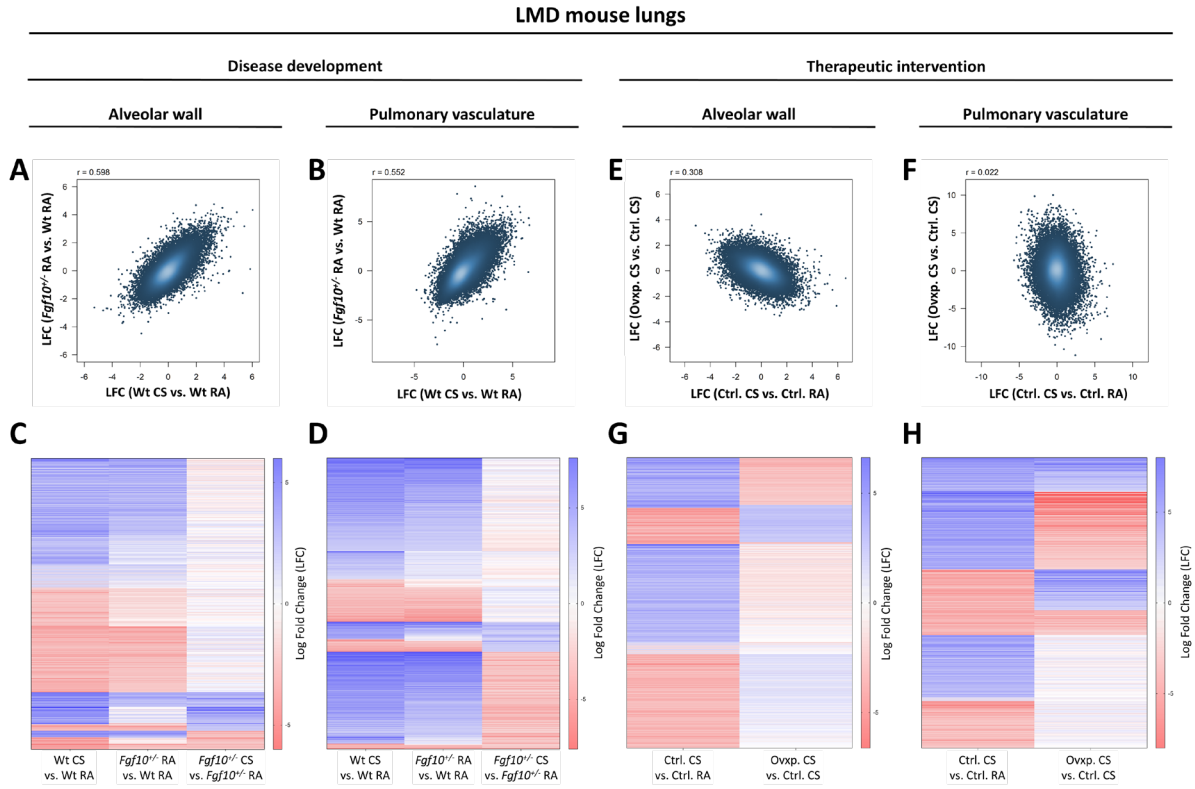


**Figure S13. Cigarette smoke - and FGF10-related gene expression patterns involved in the development or reversion of emphysema and PH – extended data.**

Global gene expression comparison between **(A, B)** Wt CS vs. Wt RA and *Fgf10*$^{+/-}$ RA vs. Wt RA during the disease development and **(E, F)** between Ctrl. CS vs. Ctrl. RA and FGF10 ovxp. CS vs. Ctrl. CS during the therapeutic intervention in **(A, E)** alveolar septa and **(B, F)** pulmonary vasculature. Expression pattern of all genes altered upon CS exposure during **(C, D)** disease development in or **(G, H)** therapeutic intervention by FGF10 overexpression in **(C, G)** the alveolar wall or **(D, H)** the pulmonary vasculature. The overlap regions show genes that are in common for the compared groups. Genes that are commonly regulated were further investigated in comet plots. **(C-D, G-H).** Abbreviations: RA – room air; CS – cigarette smoke; Ctrl. – control; Ovxp. – overexpression.

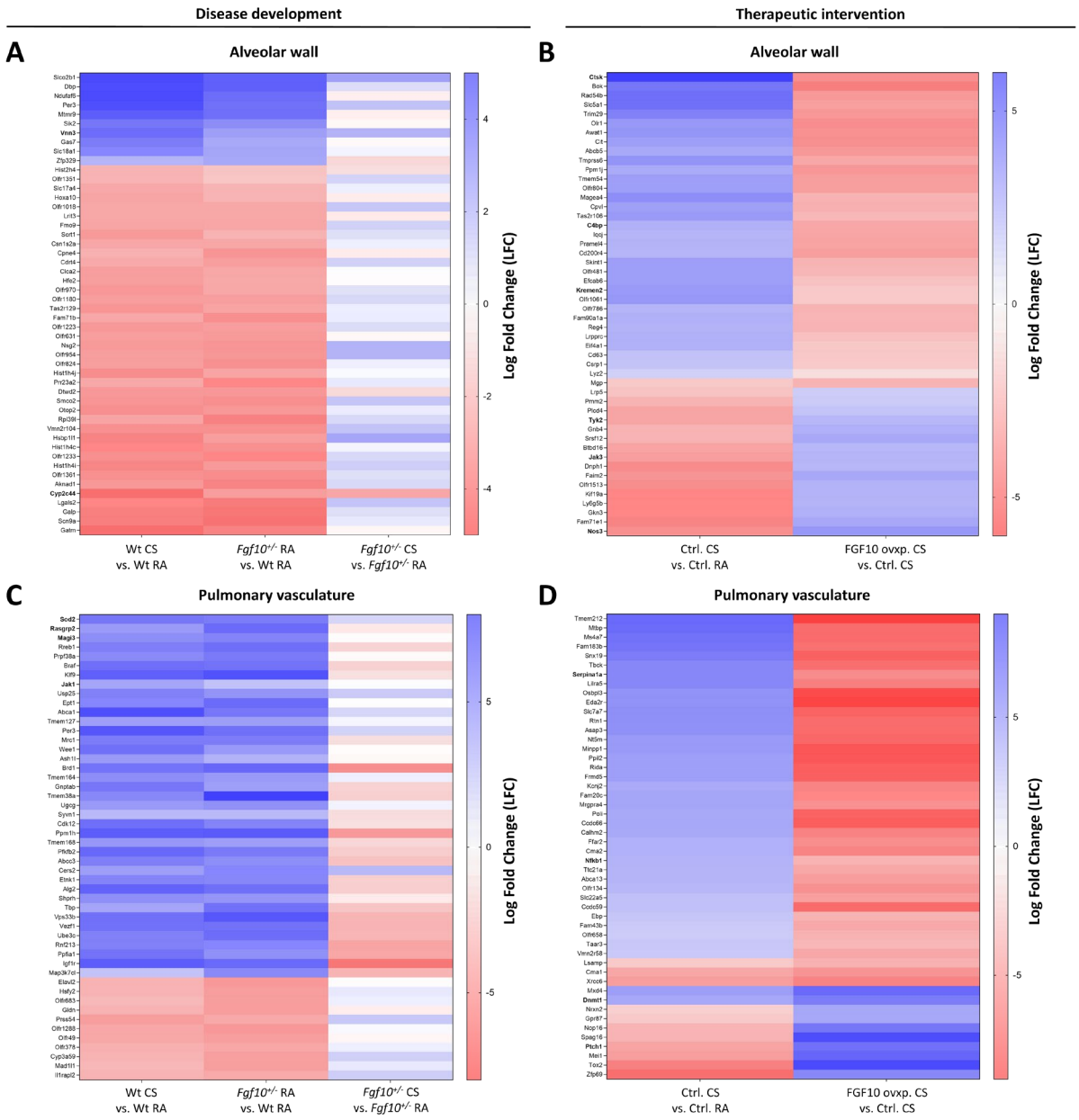


**Figure S14. Top 50 Cigarette smoke - and FGF10-regulated genes during the development or reversion of emphysema and pulmonary hypertension in mice.**

Heat maps show the top 50 most genes in **(A, C)** Wt CS vs. Wt RA and *Fgf10*$^{+/-}$ RA vs. Wt RA during the disease development or **(B, D)** in Control CS vs. Control RA and FGF10 overexpressing CS vs. Control CS during the therapeutic intervention. Top 50 genes are shown separately for **(A, B)** the alveolar wall compartment and **(C, D)** pulmonary vasculature. Red – downregulated; Blue – upregulated. Abbreviations: RA – room air; CS – cigarette smoke; Ctrl. – control; Ovxp. – overexpression.

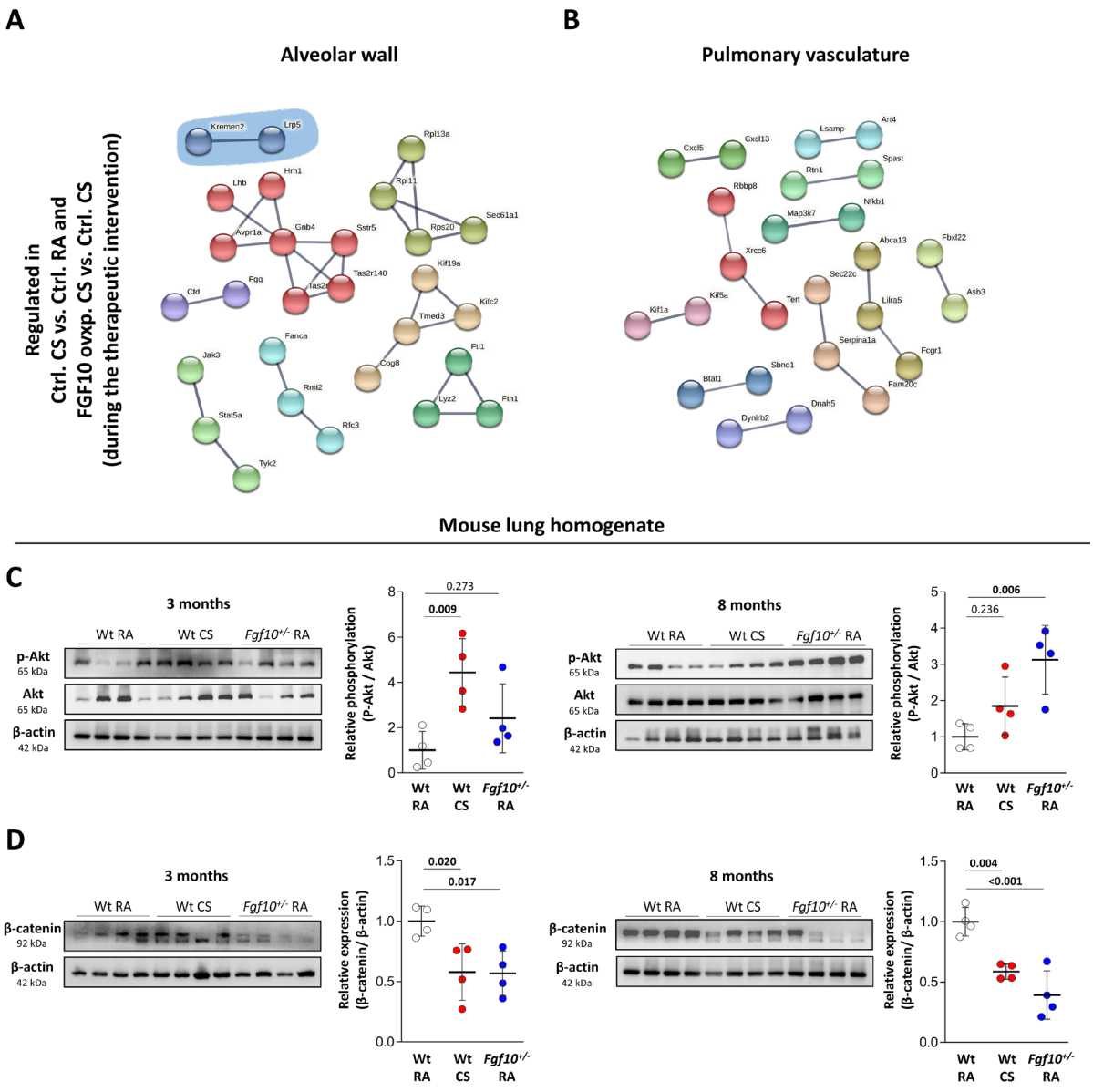


**Figure S15. Cigarette smoke- and FGF10-related molecular pathways during the development or reversion of emphysema and pulmonary hypertension in mice – extended data.**

**(A-B)** Genes that are regulated in Control CS vs. Control RA and FGF10 overexpressing CS vs. Control CS during the therapeutic intervention are investigated using functional protein association networks (STRING-DB database). The analysis shows only genes whose products are connected in **(A)** the alveolar wall compartment and **(B)** pulmonary vessels. Marked blue are the genes that play a role in the Wnt signalling pathway. **(C-D)** Western blot quantifications (n=4) of **(C)** p-Akt (phosphorylation at

serine 473) / total Akt and **(D)** of β-catenin / β-actin in lung homogenates from RA/CS-exposed Wt and RA-exposed *Fgf10*$^{+/-}$ animals exposed to RA and CS-exposed Wt mice at 3- and 8-month time-points. In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – One-Way ANOVA (Dunnett´s multiple-to-one comparison); *p*-value for each comparison is given in the graphs.

Abbreviations: Kremen2 – kringle containing transmembrane protein 2; Lrp5 – LDL receptor related protein 5. RA – room air; CS – cigarette smoke; Ctrl. – control; Ovxp. – overexpression.

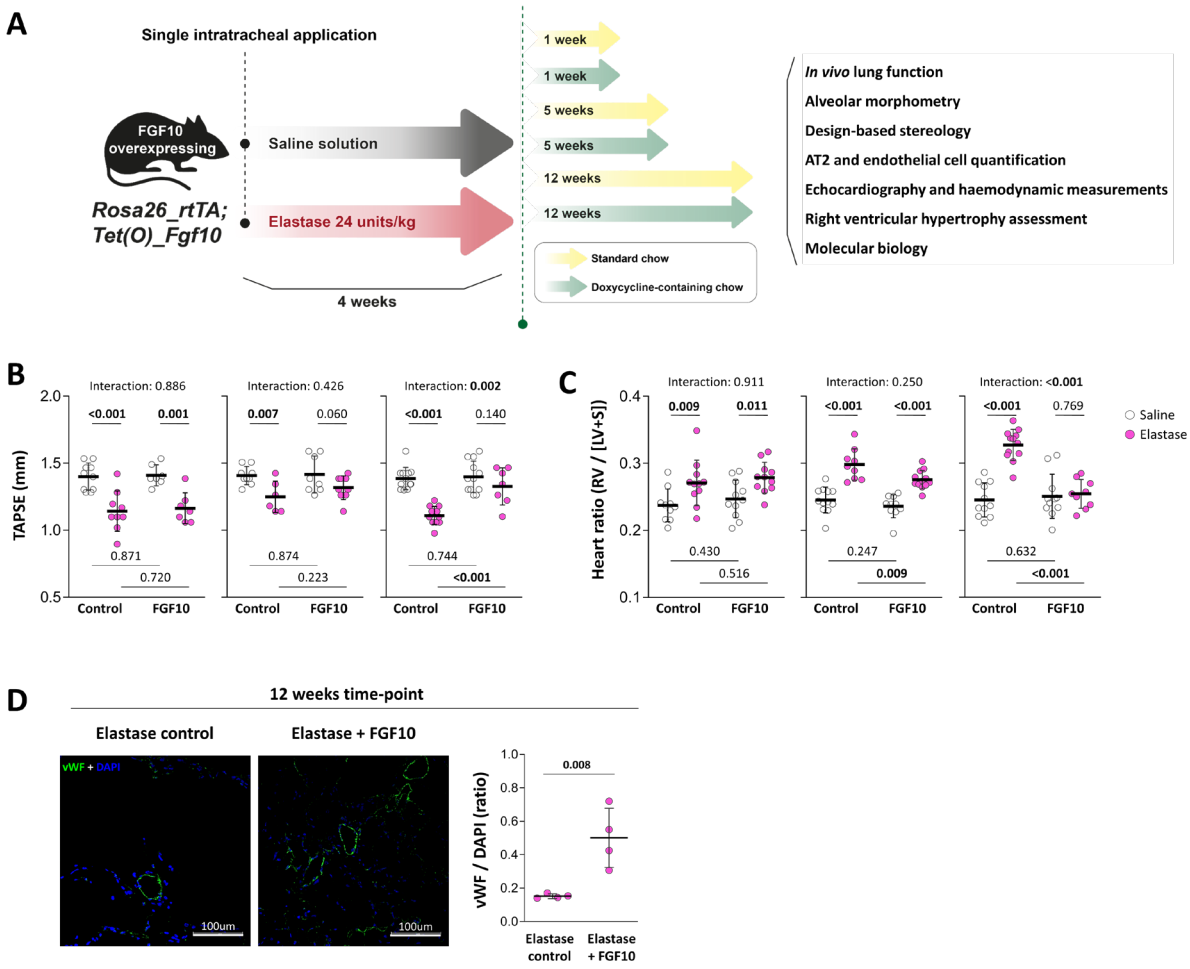


**Figure S16. FGF10 overexpression ameliorates elastase-induced pulmonary hypertension – extended data.**

**(A)** Schematic of the experimental design. Animals received a single intra-tracheal application of either saline or elastase solution; 4 weeks later, animals were fed with standard feed (Control) or feed containing doxycycline (FGF10) for 1, 5 or 12 weeks. **(B)** Echocardiographic assessment of tricuspid annular plane systolic excursion (TAPSE; n=6-12) and **(C)** the weight ratio between right ventricle (RV) and left ventricle plus septum (RV+S) mass (n=9-12). No significant changes in LV+S mass were detected between analysed groups. **(D)** Immunofluorescence staining and quantification for von Willebrand factor (vWF) in lungs from mice that received elastase and were subsequently fed with standard feed or feed containing doxycycline for 12 weeks (n=4).

In the quantification panels, each dot represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – **(B-C)** Two-Way ANOVA; **(D)** t-test; *p*-values for each comparison and interaction are given in the graphs.

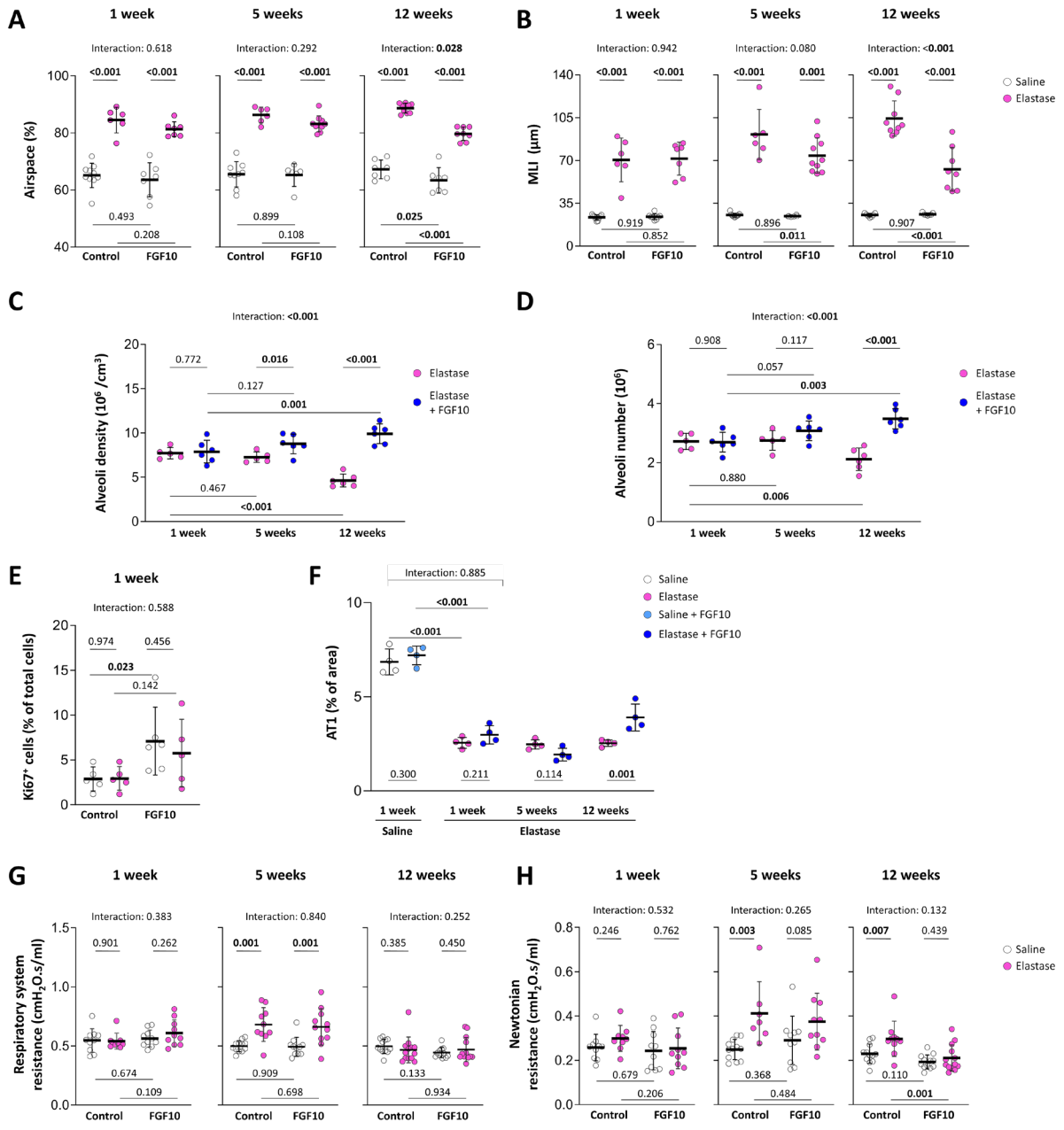


**Figure S17. FGF10 overexpression ameliorates elastase-induced emphysema and airway resistance.**

**(A-D)** Emphysema quantification by determination of **(A)** airspace enlargement (n=6-10), **(B)** mean linear intercept (MLI; n=6-10) from lung sections of saline- or elastase-treated mice that were subsequently fed with standard feed (Control) or feed containing doxycycline (FGF10) for 1, 5 or 12 weeks. **(C)** Design-based stereology was performed to determine alveolar density (n=5-6) in the left lung lobe of experimental animals treated with elastase and subsequently fed with standard feed or feed containing doxycycline for 1, 5 or 12 weeks. **(D)** Design-based stereology was performed to determine alveoli number, calculated by relating the alveoli density to the measured left lung volume

(n=5-6). **(E)** Percentage of proliferating (Ki67 positive) cells in lungs of experimental mice at 1 week time point (n=5-6). **(F)** Percentage of area covered by AT1 cells quantified by confocal microscopy in lung sections of experimental mice upon immunofluorescence staining against RAGE (Receptor for Advanced Glycation Endproducts) marker protein (n=4). **(G-H)** *In vivo* lung function measurements showing **(G)** respiratory system resistance (n=9-12) and **(H)** Newtonian (airway) resistance (n=7-13) in the experimental mice.

Each dot in the graphs represents a measurement obtained from one individual experimental animal. The mean value for each group is represented by a horizontal line +/- standard deviation. Statistical analysis – Two-Way ANOVA; *p*-values for each comparison and interaction are given in the graphs.

**Table S1 – primer sequences.**

| Gene abbreviation | Sequence |
|---|---|
| Mouse *Scgb1a1* | Forward: 5' ATGAAGATCGCCATCACAATCAC 3' |
| | Reverse: 5' GGGAGGGTATCCACCAGTCT 3' |
| Mouse *Sftpc* | Forward: 5' ATGGACATGAGTAGCAAAGAGGT 3' |
| | Reverse: 5' CACGATGAGAAGGCGTTTGAG 3' |
| Mouse *Fgf10* | Forward: 5' CGGGACCAAGAATGAAGACT 3' |
| | Reverse: 5' GCAACAACTCCGATTTCCAC 3' |
| Mouse *B2m* | Forward: 5' AGCCCAAGACCGTCTACTGG 3' |
| | Reverse: 5' AGCCCAAGACCGTCTACTGG 3' |

Abbreviations: *Scgb1a1* – Secretoglobin family 1A member 1; *Sftpc* – Surfractant protein C*; Fgf10* – Fibroblast growth factor 10*; B2m* – Beta 2 Microglobulin.