

1

## SUPPLEMENTARY METHODS

### 2 **Human Samples**

3 Deidentified frozen lung tissue from the patients diagnosed with IPF on the basis of  
4 appropriate clinical-radiographic features and usual interstitial pneumonia on  
5 histopathologic examination or normal tissues were obtained from the National Institutes  
6 of Health Lung Tissue Research Consortium. Demographics of the study population are  
7 provided in Table S4. The Institutional Review Board at the University of Vermont certified  
8 that the study did not constitute human subjects research.

9 The Lung Genomics Research Consortium (LGRC) cohort was used to determine gene  
10 expression. Data from individuals with IPF and controls were used for this analysis. The  
11 gene expression data are available from the GEO database  
12 (<http://www.ncbi.nlm.nih.gov/geo/>) under the accession GSE47460. R software (2.12.2)  
13 was used for data analysis. Gene expression in lung tissue was available for 160  
14 individuals with IPF and 132 controls from the LGRC cohort; demographic and  
15 physiological characteristics are presented in Supplementary Table S5. Lung function  
16 data (%DLCO) were available for 145 individuals with IPF and 92 controls from LGRC  
17 cohort.

18 Analysis of bulk RNA Seq gene expression data (GSE150910) was done in R software  
19 using the package “limma” (1). The FVC and %DLCO correlations with the gene  
20 expression were calculated using Pearson correlation. The significance of association  
21 was calculated using Fisher's asymptotic test. All the plots (figures S1B-D; S2D-L; S5D-  
22 F) were generated using the ggplot2 library in R.

23

## 24 **Inducible club cell-specific *Pdia3* ablation**

25 Age-matched male and female mice were used. Doxycycline-inducible-club cell  
26 (*Scgb1a1*) specific *Pdia3* knockout mice, referred to as  $\Delta$ Epi-*Pdia3* (genotype *Scgb1a1*-  
27 *rtTA/TetOP-Cre/Pdia3<sup>loxp/loxp</sup>*) were generated by breeding *Scgb1a1-rtTA*, *TetOP-Cre*  
28 mice with mice carrying the *Pdia3<sup>loxp/loxp</sup>* alleles (2, 3). Littermates containing either  
29 *Scgb1a1-rtTA/TetOP-Cre* or *Scgb1a1-rtTA/Pdia3<sup>loxp/loxp</sup>* and fed doxycycline-containing  
30 food (6 g/kg; Purina Diet Tech, St Louis, Mo) were used as control mice (*Ctr* mice). All  
31 mice were in C57BL6/NJ background. Mice were maintained on doxycycline-containing  
32 food from day 14 post BLM instillation until completion of the experiment as reported  
33 earlier (2).

34

## 35 **Administration of LOC14**

36 BLM instilled C57BL/6NJ male mice were treated therapeutically by oropharyngeal route  
37 at three different concentrations of LOC14 (Tocris, Cat. No. 5606; 0.015mg/kg, 0.15mg/kg  
38 and 1.5mg/kg weight of mice) from day 14 onward for five times at alternate day. On day  
39 24, mice were harvested, and BALF and lungs were collected for analysis.

40

## 41 **Blocking SPP1**

42 To examine the role of Osteopontin in bleomycin-induced lung fibrosis, bleomycin  
43 challenged mice were further treated with anti-Osteopontin antibody (R&D systems,  
44 AF808; 3 $\mu$ g and 30 $\mu$ g) or isotype-specific IgG (R&D systems, AB-108-C; 3 $\mu$ g and 30 $\mu$ g)  
45 by intraperitoneal route using the same regimen as LOC14 treatment, i.e., bleomycin  
46 instillation at day zero followed by five times treatment from day 14 at alternate day

47 followed by harvest at day 24. After the experiments, BALF and lungs were collected for  
48 analysis.

49

#### 50 **Immunoprecipitation**

51 Lung tissues were lysed in buffer containing 20mM Tris·HCl (pH 7.5), 150mM NaCl, 0.5%  
52 Nonidet P-40, 10% glycerol, and 1% protease inhibitor cocktail (Sigma-Aldrich, P8340)  
53 (v/v), 1% phosphatase inhibitor cocktails 1 and 2 (Sigma-Aldrich, P5726, P0044) (v/v).  
54 Insoluble proteins were pelleted via high centrifugation. PDIA3 was immunoprecipitated  
55 using anti-PDIA3 antibody (Enzo LifeSciences, ADI-SPA-585-F). Lung lysates from (PBS  
56 (n=3), BLM\_14d (n=3), BLM\_24d (n=3) and the IgG control BLM24d (n=1)) are mixed  
57 with PDIA3 antibody or IgG isotype control. PDIA3-bound proteins are  
58 immunoprecipitated using recombinant G agarose beads (Invitrogen,15920010).  
59 Samples were then suspended in loading buffer with dithiothrietol (DTT) and resolved by  
60 SDS-PAGE.

61

#### 62 **Western blot and densitometry analysis.**

63 Right lung lobes were flash-frozen, pulverized and lysed in buffer containing 20mM  
64 Tris·HCl (pH 7.5), 150mM NaCl, 0.5% Nonidet P-40, 10% glycerol, and 1% protease  
65 inhibitor cocktail (Sigma-Aldrich, P8340) (v/v), 1% phosphatase inhibitor cocktails 1 and  
66 2 (Sigma-Aldrich, P5726, P0044) (v/v). Insoluble proteins were pelleted via high  
67 centrifugation. Following protein quantitation of the supernatant using DC Protein Assay  
68 (Bio-Rad, 5000116) (4), samples were resuspended in loading buffer with dithiothrietol  
69 and resolved by SDS-PAGE and electrophoretically blotted onto Immun-Blot PVDF

70 membrane, and membranes were probed using a standard immunoblotting protocol.  
71 Western blots were performed using antibodies (Abs) directed against PDIA3 (LSBio;  
72 LS-B9768), SCGB1A1 (Santa Cruz Biotechnology; sc-390313),  $\beta$  actin (Sigma; A5441),  
73 GAPDH (BioLegend; 919501) and Osteopontin (R&D systems; AF808). The  
74 quantification of protein expression was performed by densitometry using ImageJ  
75 software (NIH, <https://imagej.nih.gov/ij/>). Details of the antibodies used for western blots  
76 can be found in table S6.

77

#### 78 **Measurement of lung hydroxyproline.**

79 Lung collagen in mice were quantified by measurements of hydroxyproline levels in the  
80 upper right lobe. The upper right lobe was used to sample lung collagen levels throughout  
81 this study. Lobe was homogenized with a handheld homogenizer followed by hydrolysis  
82 in an equal volume of 6 N HCl for 18 h at 110°C. After hydrolysis lung samples were  
83 neutralized with NaOH. Following neutralization hydroxyproline assay was performed  
84 with the samples as previously described (5, 6).

85

#### 86 **RNA extraction and qRT-PCR.**

87 Total RNA was extracted from lungs using Qiazol Lysis Reagent (Qiagen) and purified  
88 using the RNeasy kit (Qiagen). One microgram of RNA was reverse transcribed to cDNA  
89 (Promega) and qRT-PCR was performed for quantitative assessment of gene expression  
90 using SYBR green PCR Master Mix (Bio-Rad) as performed earlier in our lab (7).  
91 Expression values were normalized to the geometric mean of 3 housekeeping genes  
92 (*Gapdh*, *Pp1*, and *Rp2*) using the  $\Delta\Delta CT$  method. Primers used are listed in table S7.

93

94 **Histology and lung fixation.**

95 After euthanization left lobe of lungs was fixed with 4% paraformaldehyde (FIBROSISA)  
96 overnight, embedded in paraffin and cut into 5 µm sections and affixed to glass  
97 microscope slides for staining with Masson's trichrome or immunostaining. Lungs sections  
98 were prepared by deparaffinizing with xylene and rehydrating through a series of  
99 ethanols. Fibrotic remodeling was assessed in mouse lung sections by Masson's  
100 trichrome staining (8). Slides stained with trichrome were imaged using an Aperio VERSA  
101 8 Scanner Scope (Microscopy imaging Center, UVM).

102 For Immunostaining study antigen retrieval was done by heating slides for 20 min at 95  
103 °C in sodium citrate buffer with 0.05% TWEEN-20 then rinsed in dH<sub>2</sub>O. For  
104 Immunofluorescence studies sections were then blocked for 1 h in 1% BSA in PBS,  
105 followed by incubation using the following primary antibodies: PDIA3 (LSBio, LS-B9768),  
106 at 1:300 and SCGB1A1 (Santa Cruz Biotechnology, sc-390313), at 1:300; pro-SPC  
107 (Seven Hills Bioreagents, WRAB-9337), at 1:200 and human SCGB1A1 (Abnova,  
108 H00007356-MO1) at 1:200 overnight at 4 °C. Slides were then washed 3×5 min in PBS  
109 and incubated with species-specific Alexafluor-488– or Alexafluor-647–conjugated  
110 secondary antibodies, and counterstained with DAPI in PBS at 1:4000 for nuclear  
111 localization (7, 9). Sections were imaged using a Nikon A1R Confocal laser-scanning  
112 microscope. Images were captured at 20x and/or 40x magnification. The image files were  
113 converted to Tiff format. Brightness and contrast were adjusted equally in all images.

114 For immunohistochemistry after antigen retrieval tissue sections were blocked and  
115 processed further using Vector® Red Substrate Kit, Alkaline Phosphatase (AP) SK-5100

116 kit. The protocol was followed as suggested in the kit (10, 11). The antibody used for  
117 SCGB1A1 (Santa Cruz Biotechnology; sc-390313) at 1:200 and PDIA3 (LSBio, LS-  
118 B9768), at 1:150.

119

### 120 **Bronchoalveolar lavage (BAL) cell count and differential staining**

121 Bronchoalveolar lavage fluid (BALF) was collected by lavaging lungs with 1.0 mL of sterile  
122 PBS. Cells were isolated by centrifugation, and total cell counts were determined using a  
123 Guava easyCyte HT cytometer (Millipore) and analyzed using Flowjo (version 10.4.2,  
124 Ashland, OR: Becton, Dickinson and Company). Differential cell counts were obtained via  
125 cytopins using Hema3 stained (Fisher Scientific) total cells, on a minimum of 300  
126 cells/animal, or using a Guava easyCyte HT cytometer with a protocol adapted from van  
127 Rijt *et al* (12). Briefly, isolated cells were incubated with TruStain fcX (BioLegend 101320)  
128 to reduce background staining, then incubated in a mix containing 3 $\mu$ L CD3 PerCP/Cy5.5  
129 (BioLegend 100218), 3 $\mu$ L B220 PerCP/Cy5.5 (BioLegend 103236), 2 $\mu$ L CD11c APC  
130 (BioLegend 117310), 2 $\mu$ L I-A/I-E FITC (BioLegend 107616), and 1 $\mu$ L CCR3 PE  
131 (BioLegend 144506) per sample. Cells were identified based on forward and side scatter  
132 characteristics and differential staining for macrophages (CD11c+), lymphocytes (CD11c-  
133 , CD3/B220+), eosinophils (CD11c-, CD3/B220-, CCR3+) and neutrophils (CD11c-,  
134 CD3/B220-, CCR3-). Data was analyzed using Flowjo. Validity was tested against  
135 differential counts based on cytopins.

136

### 137 **ELISA.**

138 We used ELISA to measure concentrations of Osteopontin proteins in BALF and lung

139 tissue. For tissue ELISA right lung lobes were flash frozen, pulverized, and lysed in buffer  
140 containing 20mM Tris·HCl (pH 8.0), 150mM NaCl, 0.5% Nonidet P-40, 10% glycerol.  
141 Samples were normalized to total lung protein and assayed for Osteopontin proteins by  
142 ELISA (R&D Systems; DY441 for mouse and R&D Systems; DY1433 for human)  
143 according to manufacturer's instructions.

144

### 145 **Mass Spectrometry**

146 **Sample preparation and trypsin digestion:** Immunoprecipitates (PBS (n=3), BLM\_14d  
147 (n=3), BLM\_24d (n=3) and the IgG control BLM\_24d (n=1)) were run briefly onto the SDS-  
148 PAGE (1/3 of the whole length of the gel), which was then stained with silver. For each  
149 lane (sample), the gel regions containing the antibody heavy and light chains (labeled as  
150 "IgG") and the separated proteins were excised separately (labeled as "IP"). This allows  
151 the high abundant IgG and proteins with similar molecular weight and the relatively low  
152 abundant proteins in the other gel regions to be analyzed by separate mass spectrometry  
153 runs to increase the proteome coverage. The gel pieces were minced to 1 mm<sup>3</sup> cubes,  
154 combined, destained and subjected to disulfide reduction and alkylation, and trypsin  
155 digestion protocols, as described previously.

156

157 **Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based protein**  
158 **identification:** The tryptic peptides resuspended in 2.5% CH<sub>3</sub>CN and 2.5% formic acid  
159 (FA) in water were analyzed on the Q-Exactive Plus mass spectrometer coupled to an  
160 EASY-nLC 1200 (Thermo Fisher Scientific). Samples were loaded onto a 100 µm x  
161 350mm capillary column packed with UChrom C18 (1.8 µm particle size, 120 Å, Cat. No:

162 PN-80001; Nanolcms, CA) at a flow rate of 300 nl min<sup>-1</sup>. The column end was laser pulled  
163 to a ~3 µm orifice and packed with minimal amounts of 5µm Magic C18AQ before packing  
164 with the 1.8 µm particle size chromatographic materials. Peptides of immunoprecipitated  
165 proteins were separated by a gradient of 0-35% CH<sub>3</sub>CN/0.1% FA over 150 min, 35-80%  
166 CH<sub>3</sub>CN/0.1% FA in 1 min and then 80% CH<sub>3</sub>CN/0.1% FA for 8 min, followed by an  
167 immediate return to 0% CH<sub>3</sub>CN/0.1% FA and a hold at 0% CH<sub>3</sub>CN/0.1% FA. Peptides  
168 originating from heavy and light chains of IgG were separated by a gradient of 0-35%  
169 CH<sub>3</sub>CN/0.1% FA over 60 min, 35-80% CH<sub>3</sub>CN/0.1% FA in 1 min and then 80%  
170 CH<sub>3</sub>CN/0.1% FA for 8 min, followed by an immediate return to 0% CH<sub>3</sub>CN/0.1% FA and  
171 a hold at 0% CH<sub>3</sub>CN/0.1% FA. Samples were randomized in run order and peptide  
172 standards (bovine serum albumin) were run between samples. Mass spectrometry data  
173 was acquired in a data-dependent “Top 10” acquisition mode with lock mass function  
174 activated ( $m/z$  371.1012; use lock masses: best; lock mass injection: full MS), in which a  
175 survey scan from  $m/z$  350-1600 at 70, 000 resolution (AGC target 1e<sup>6</sup>; max IT 100 ms;  
176 profile mode) was followed by 10 higher-energy collisional dissociation (HCD) tandem  
177 mass spectrometry (MS/MS) scans on the most abundant ions at 17,500 resolution (loop  
178 count = 10; AGC target 5e<sup>4</sup>; max IT 100 ms; centroid mode). MS/MS scans were acquired  
179 with an isolation width of 1.6  $m/z$  and a normalized collisional energy of 26%. Dynamic  
180 exclusion was enabled (peptide match: preferred; exclude isotopes: on; underfill ratio:  
181 1%; exclusion duration: 15 sec). Minimum AGC target = 5 e2.

182

183 **Database searches:** Raw files (.RAW) were analyzed using the Proteome Discoverer  
184 2.4 (Thermo Fisher Scientific), with the two raw files (“IgG” and “IP”) from each sample



185 imported as “fractions”. Product ion spectra were searched using the SEQUEST with the  
186 “Basic” Processing and Consensus workflows against a Uniprot *Mus musculus* protein  
187 database (UP000000589; downloaded Mar 3<sup>rd</sup>, 2020; 63,628 entries). Search  
188 Parameters were as follows: Full trypsin enzymatic activity; Mass tolerance at 10 ppm  
189 and 0.02 Da for precursor ions and fragment ions, respectively; dynamic modification on  
190 methionine (oxidation: +15.995 Da), and static modification on cysteines  
191 (carbamidomethylation: +57.021 Da). Percolator was included in the workflow to limit the  
192 false discovery rate to less than 1%. The resulting msf result files were incorporated into  
193 Q+S 4.11 (Proteome Software, OR) as mudPIT with “prefiltered mode” (Precalculated  
194 probability from Percolator was used) and Protein Cluster Analysis. FDR at protein and  
195 peptide levels at 1% and “Min number of peptides” = 2 were selected to achieve a 0.4%  
196 Protein Decoy FDR and 0.04% Peptide (PSM) Decoy FDR in the filtered dataset.

197

198 **Statistical Analysis of the mass spectrometry data:** The total spectral counts were  
199 statistically evaluated between “Control”, “BLM\_14d” and “BLM\_24d” with respect to  
200 specific protein isoforms/clustering. Statistical tools in Scaffold Quantitative Analysis  
201 Mode were used for pairwise comparisons (two-tailed t-test for BLM24d vs. PBS and  
202 BLM14d vs. PBS), and for the three-group comparison (ANOVA for BLM24d vs. BLM14d  
203 vs. PBS) with the following settings: No normalization was used; Min. Value = 0.5 (All  
204 spectral counts of 0 were replaced with a value of 0.5); and total spectra as quantitative  
205 method. “Spectral Counting Reports” were exported from Scaffold with “Show lower  
206 scoring peptides and <5% Probabilities” deactivated, and “Hide Decoy” selected. The  
207 total spectral counts of all proteins (including protein cluster members) and the

208 corresponding statistical analyses exported from Scaffold as “Spectrum Counting  
209 Reports” are included in Supplementary Tables S1, S2 and S3. Spectral counts of protein  
210 clusters (exported from “Current View” with “0”s replaced with “0.5”) were used to  
211 construct the heat maps and volcano plots for simplicity. One of the cluster members was  
212 selected to represent the cluster and the associated spectral counts were derived from  
213 the peptides of all the members (see Supplementary Tables for the spectral counts and  
214 p-values for all members/isoforms within clusters). Fold changes ( $\log_2$  ratio of the average  
215 number of peptides of the proteins between two groups) and p values ( $-\log_{10}$ ) were  
216 imported into Graph Pad Prism 8 (GraphPad Software Inc., CA) for constructing volcano  
217 plots. Heat maps were generated by first scaling the total spectral counts in individual  
218 samples so that all the counts in all samples summed up to the number of samples \*100  
219 (this allows the fold change of proteins of varying abundances be represented on the  
220 same color intensity scale) and then importing the scaled values into Graph Pad Prism 8  
221 (Dobule gradient; Baseline value set as 100). The mass spectrometry proteomics data  
222 have been deposited to the ProteomeXchange Consortium via the PRIDE (13) partner  
223 repository with the dataset identifier PXD021715 and 10.6019/PXD021715".

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276 **Supplementary Figures Legends**

277 **Figure S1. Various PDIs along with UPR markers are up regulated in IPF patients.**

278 (A) *PDIA3* mRNA levels in Control (n=132) and IPF (n=160) patients (*PDIA3* is marked in  
279 red). (B) Box plots displaying the distribution (five-point summary) of expression levels of  
280 *PDIA3* gene in healthy controls and IPF. Each point in the plot represents a sample and  
281 the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th  
282 percentiles) of each distribution. The upper whisker extends from the hinge to the largest  
283 value no further than  $1.5 * IQR$  and the lower whisker extends from the hinge to the  
284 smallest value at most  $1.5 * IQR$  of the hinge, where IQR or the inter-quartile range is  
285 distance between the first and third quartiles. Points beyond the end of the whiskers are  
286 potential outlier samples in each case. (C-E) *PDIA3* mRNA expression and correlation  
287 with % DLCO and FVC in IPF and controls. The FVC/DLCO measurements are on the y-  
288 axis and mRNA expression levels on the x-axis. Each circle/point represents a patient  
289 sample within the study and the blue-colored points are healthy controls while the red-  
290 colored points represent IPF samples. The FVC and %DLCO correlations with the gene  
291 expression were calculated using Pearson correlation. The significance of association  
292 was calculated using Fisher's asymptotic test. All the plots were generated using the  
293 ggplot2 library in R. (F-H) mRNA levels of UPR markers *HSPA5*, *HSP90B1*, and *XPB1* in  
294 Control (n=132) and IPF (n=160) patients. Unpaired two-tailed non-parametric Mann-  
295 Whitney *t* test. Error bars represent  $\pm STDEV$ .

296

297 **Figure S2. UPR markers and club cell markers in fibrotic mice and IPF. (A and B)**

298 measurement of *Hspa5* and *Xbp1s* (spliced form) in BLM or PBS challenged mice

299 harvested at various time points. \* $p < 0.05$  as compared to PBS group and # $p < 0.05$  as  
300 compared to 4 day-BLM group by ANOVA, error bars represent  $\pm$ SEM (n=5-9  
301 mice/group). (C) IHC staining (red) for PDIA3 and SCGB1A1 in serial (5 $\mu$ m) sections of  
302 the lungs, the arrow indicates PDIA3 and SCGB1A1 staining in the same cells (arrows).  
303 Secondary antibody staining on fibrotic mouse lungs is used as a negative control. Scale  
304 bar 400 $\mu$ m. (D-F) Club cell markers in control and IPF lungs. Box plots displaying the  
305 distribution (five-point summary) of expression levels of club cell markers in healthy  
306 controls and IPF. Each point in the plot represents a sample and the lower and upper  
307 hinges correspond to the first and third quartiles (the 25th and 75th percentiles) of each  
308 distribution. The upper whisker extends from the hinge to the largest value no further than  
309 1.5 \* IQR and the lower whisker extends from the hinge to the smallest value at most 1.5  
310 \* IQR of the hinge, where IQR or the inter-quartile range is distance between the first and  
311 third quartiles. Points beyond the end of the whiskers are potential outlier samples in each  
312 case. (G-L) Correlation of club cell markers with % DLCO and FVC in IPF and controls.  
313 The FVC/DLCO measurements are on the y-axis and mRNA expression levels on the x-  
314 axis. Each circle/point represents a patient sample within the study and the blue-colored  
315 points are healthy controls while the red-colored points represent IPF samples. The FVC  
316 and %DLCO correlations with the gene expression were calculated using Pearson  
317 correlation. The significance of association was calculated using Fisher's asymptotic test.  
318 All the plots were generated using the ggplot2 library in R.

319

320 **Figure S3. PDIA3 levels after Dox treatment in mouse lung and expression of**  
321 **various PDIA3s with LOC14 treatment.** (A-B) Representative images from confocal

322 microscopy stained for SCGB1A1 (green), PDIA3 (red), and nucleus (blue) and  
323 quantitation of SCGB1A1 and PDIA3 staining in PBS challenged *ctr* and  $\Delta Epi-Pdia3$  mice  
324 lungs. Unpaired t-test, (n=4 mice/group); error bars  $\pm$ SEM. (C-I) Analysis of mRNAs for  
325 various *PDIA*s and ER stress markers (*Hspa5* & *Xbp1*) in the lungs challenged with BLM  
326 or PBS and treated with DMSO or LOC14. Outlier removed in *Pdia3* BLM + DMSO (n=1)  
327 \*p<0.05 as compared to PBS group and #p<0.05 as compared to BLM+DMSO group by  
328 ANOVA, error bars represent  $\pm$ SEM (n=5-7 mice/group).

329

330 **Figure S4. Inflammatory cells and ALT measurement in LOC14 treated mice. (A-E)**

331 Measurement of various inflammatory cells in the BALF by flow cytometry. Outliers  
332 removed in Total cells PBS+L14 0.15mg/kg (n=1), BLM+L14 0.015mg/kg (n=3),  
333 BLM+L14 0.15mg/kg (n=3) and BLM+L14 1.5mg/kg (n=2); Macrophages PBS+L14  
334 0.15mg/kg (n=1), BLM+L14 0.015mg/kg (n=2), BLM+L14 0.15mg/kg (n=2) and BLM+L14  
335 1.5mg/kg (n=1); Neutrophils PBS+L14 0.15mg/kg (n=2), BLM (n=2), BLM+L14  
336 0.015mg/kg (n=2), BLM+L14 0.15mg/kg (n=1) and BLM+L14 1.5mg/kg (n=1); Eosinophils  
337 PBS+L14 0.15mg/kg (n=1), BLM (n=1), BLM+L14 0.015mg/kg (n=1), BLM+L14  
338 0.15mg/kg (n=4) and BLM+L14 1.5mg/kg (n=1); Lymphocytes PBS+L14 0.15mg/kg  
339 (n=1), BLM (n=1), BLM+L14 0.015mg/kg (n=2), BLM+L14 0.15mg/kg (n=1) and BLM+L14  
340 1.5mg/kg (n=1). (F) Measurement of liver Alanine Aminotransferase (ALT) in the serum.  
341 \*p<0.05 as compared to PBS group and #p<0.05 as compared to BLM+DMSO group by  
342 ANOVA, error bars represent  $\pm$ SEM (n=5-9 mice/group).

343

344 **Figure S5. Analysis of PDIA3 interacting partners.** (A) Heat map of interacting partners  
345 of PDIA3 in fibrotic mouse lung, analyzed 24-day post BLM challenge (n=3  
346 samples/group, “C” immunoprecipitation using non-specific IgG used as a control).  
347 Identified proteins with a p value < 0.05 (two-tailed t-test: 14-day post BLM challenge vs.  
348 PBS) are represented. The number next to the gene symbol indicates the members in  
349 the cluster associated with that protein. The scale of the color intensity is arbitrary. (B)  
350 Volcano plot depicting the significance of interactions of PDIA3 post immunoprecipitation  
351 and mass spectrometry. Fold changes of 2 and p value at 0.05 (two-tailed t-test: 24-day  
352 post BLM challenge vs. PBS) are indicated by dotted lines on the x- and y-axis,  
353 respectively. (C) Silver stain of bands excised and analyzed by mass spectrometry. (D)  
354 Box plots displaying the distribution (five-point summary) of expression levels of *SPP1*  
355 gene in healthy controls and IPF. Each point in the plot represents a sample and the lower  
356 and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles)  
357 of each distribution. The upper whisker extends from the hinge to the largest value no  
358 further than  $1.5 * IQR$  and the lower whisker extends from the hinge to the smallest value  
359 at most  $1.5 * IQR$  of the hinge, where IQR or the inter-quartile range is distance between  
360 the first and third quartiles. Points beyond the end of the whiskers are potential outlier  
361 samples in each case. (E-F) *SPP1* expression and correlation of *SPP1* with % DLCO and  
362 FVC in IPF and controls. The FVC/DLCO measurements are on the y-axis and mRNA  
363 expression levels on the x-axis. Each circle/point represents a patient sample within the  
364 study and the blue-colored points are healthy controls while the red-colored points  
365 represent IPF samples. The FVC and %DLCO correlations with the gene expression were

366 calculated using Pearson correlation. The significance of association was calculated  
367 using Fisher's asymptotic test. All the plots were generated using the ggplot2 library in R.

368

369 **Figure S6. Inflammatory cells measurement in SPP1 blocking antibody treated**  
370 **mice.** (A) Measurement of total inflammatory cells in the BALF by flow cytometry. (B)  
371 Measurement of various types of inflammatory cells in the BALF by flow cytometry.  
372 \* $p < 0.05$  as compared to PBS+VC group by ANOVA, error bars represent  $\pm$ SEM (n=10-  
373 16 mice/group).

374

375 **Figure S7. Expression of *PDIA3* and *SPP1* in various cell types in control and IPF**  
376 **lungs.** Bar plots showing the *PDIA3* (A and B) and *SPP1* (C and D) expression in different  
377 cell types in control and IPF lungs from two publicly available single cell RNA-seq  
378 datasets. The bar plots are downloaded from the IPF single cell atlas  
379 (<https://p2med.shinyapps.io/IPFCellAtlas/>)(14). Boxes highlight *PDIA3* or *SPP1*  
380 expression in club cells.

381 **Figure S8. Expression of proSP-C, SCGB1A1 and PDIA3 in mouse model of fibrosis**  
382 **and/or IPF and normal lungs.** (A and B) Representative images from confocal  
383 microscopy stained for proSP-C (green), PDIA3 (red), and nucleus (blue) and quantitation  
384 of proSP-C and PDIA3. Secondary antibody (without primary) staining on fibrotic mouse  
385 lungs is used as the negative control. n=3-4 mice/group; error bars  $\pm$ SEM. Scale bar  
386 50 $\mu$ m. (C and D) Representative images from confocal microscopy on IPF and normal  
387 lungs stained for proSP-C (green), PDIA3 (red), and nucleus (blue) and quantitation of  
388 proSP-C and PDIA3. Secondary antibody (without primary) staining on IPF lung is used



389 as the negative control. n=4-5 samples/group; error bars  $\pm$ SEM. Scale bar 50 $\mu$ m. (E and  
390 F) Representative images of from confocal microscopy stained for SCGB1A1 (green),  
391 PDIA3 (red), and nucleus (blue) and quantitation of SCGB1A1 and PDIA3. Secondary  
392 antibody (without primary) staining on IPF lung is used as the negative control. n=4-5  
393 samples/group; error bars  $\pm$ SEM. Scale bar 50 $\mu$ m.

394 Supplementary Table S1. Proteins identified in IP and mass spectrometry-based  
395 proteomics in the BLM 24d, BLM 14d, and PBS challenged groups. (<1% FDR).

396

397 Supplementary Table S2. Protein identified with a differential abundance in BLM 14d vs.  
398 PBS challenged (p < 0.05, two-tailed t-test).

399

400 Supplementary Table S3. Protein identified with a differential abundance in BLM 24d vs.  
401 PBS challenged (p < 0.05, two-tailed t-test).

402

403 Supplementary Table S4: Patient demographic data between control and IPF patients in  
404 the 2014/2015 Cohorts from LTRC.

405

406 Supplementary Table S5: Patient demographic data of non-IPF and IPF patients from the  
407 Lung Genomics Research Consortium.

408

409 Supplementary Table S6: Antibodies used in this research study.

410

411 Supplementary Table S7: Primers sets to detect mouse mRNAs.