Supplementary information

Fibulin-1 regulates the pathogenesis of tissue remodeling in respiratory diseases

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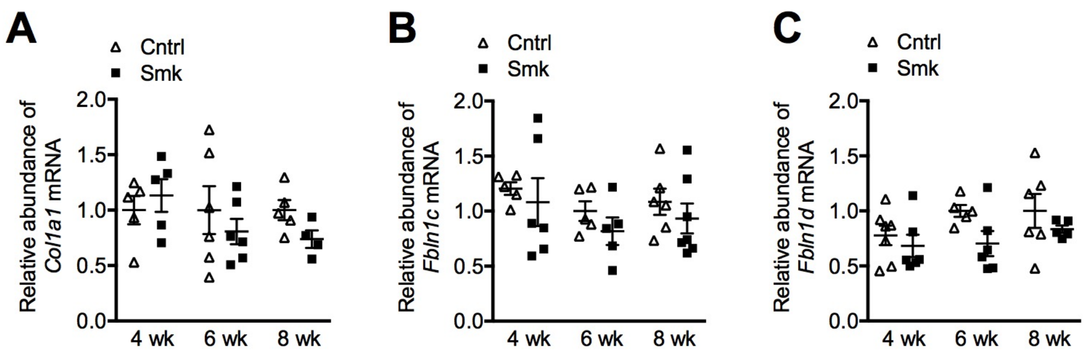
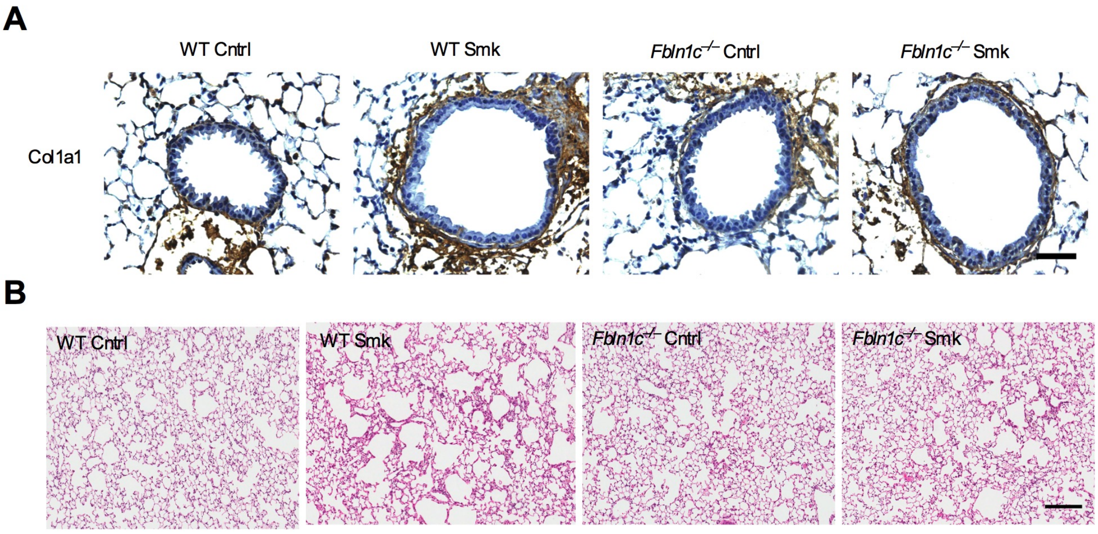
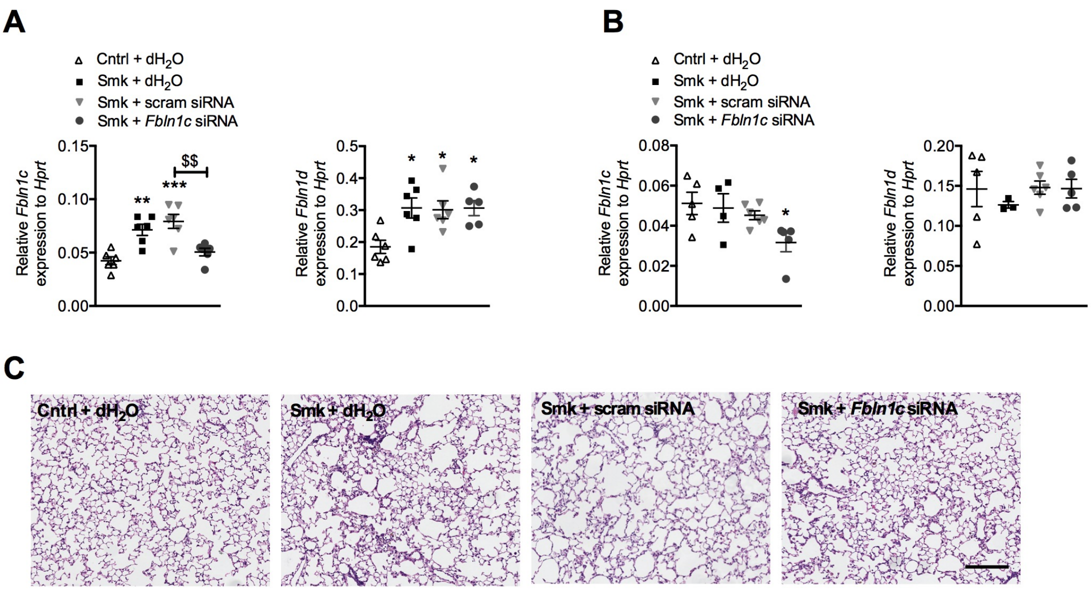
Authorship note: G. Liu, M.A. Cooley and A.G. Jarnicki contributed equally to this work.



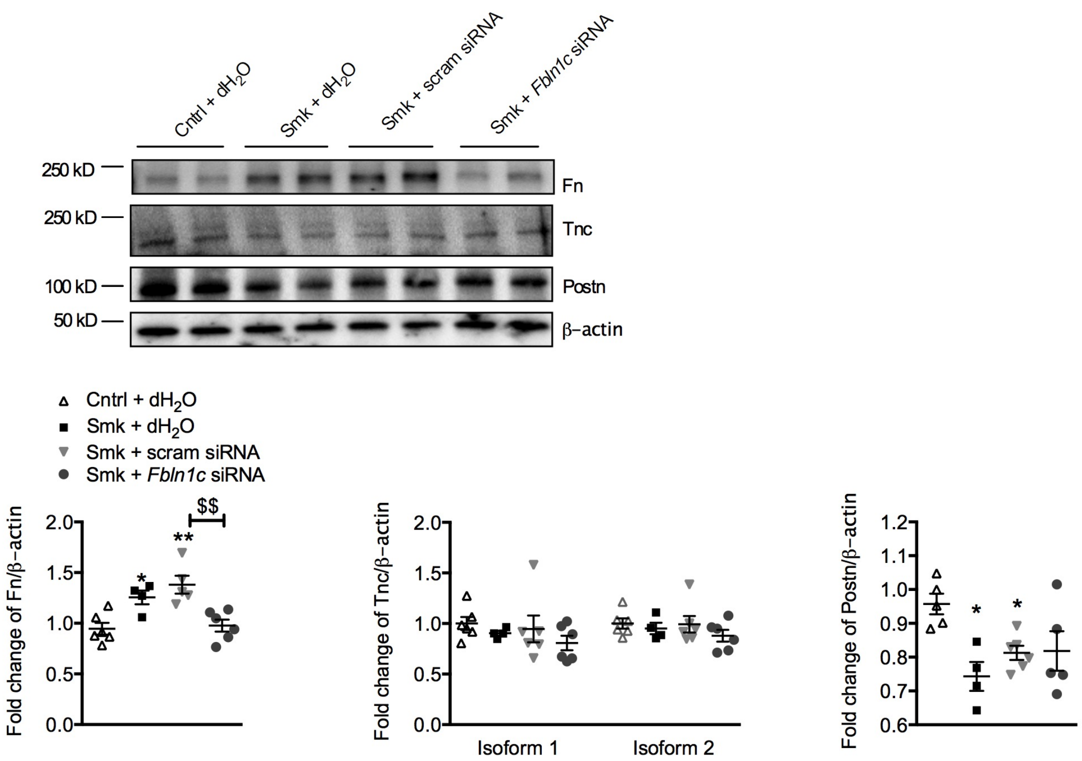
Supplementary Figure 1. *Col1a1*, *Fbln1c* and *Fbln1d* mRNA levels in cigarette smoke (CS)-induced experimental COPD. WT mice were exposed to CS for eight-weeks to induce experimental COPD, controls were exposed to normal air. Relative abundance of *Col1a1* (**A**), *Fbln1c* (**B**), and *Fbln1d* (**C**) mRNA levels in whole lungs after four-, six-, and eight-weeks of CS-exposure, determined by qPCR, n=5-6. Results are mean ± SEM. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.



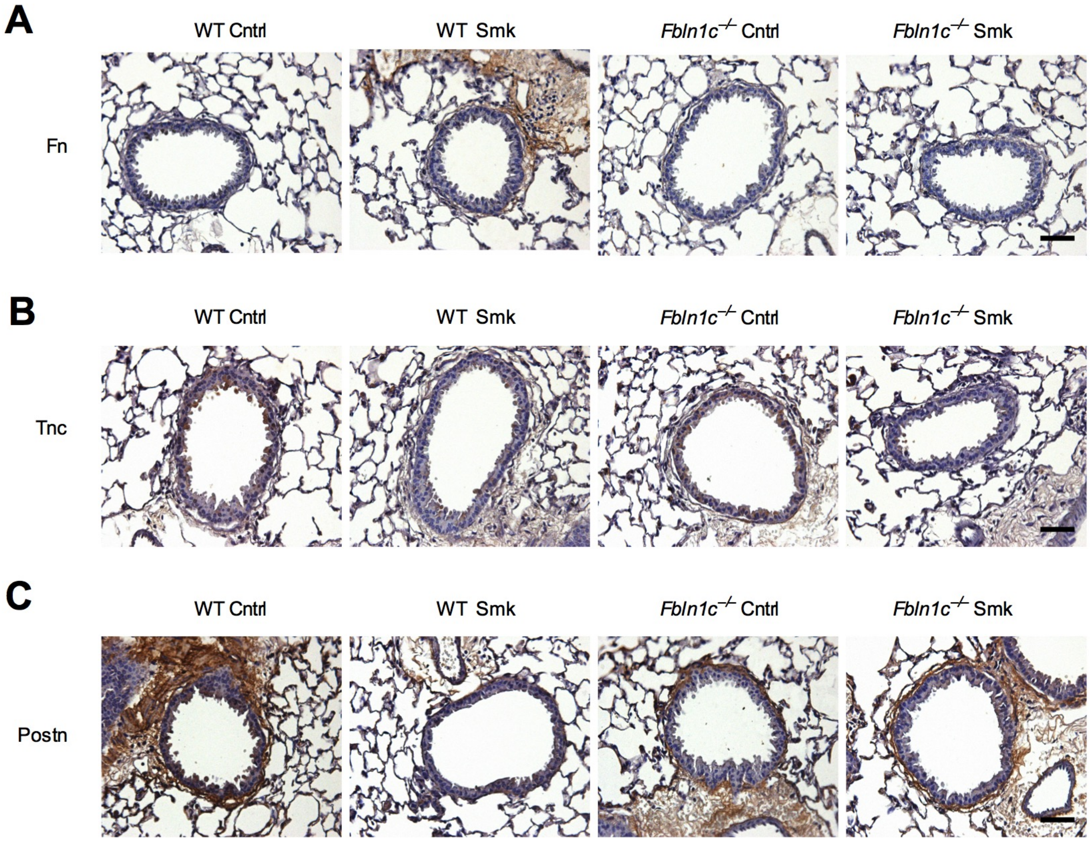
Supplementary Figure 2. Absence of *Fbln1c* protects against Col1a1 deposition around small airways and emphysema in experimental COPD. WT and *Fbln1c* deficient ($^{-/-}$) mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. (**A**) Col1a1 around mouse small airways (scale bar, 50 μm) assessed by immunohistochemistry. (**B**) Lung sections stained with hematoxylin and eosin (scale bar, 200 μm). n=5-6 mice per group.



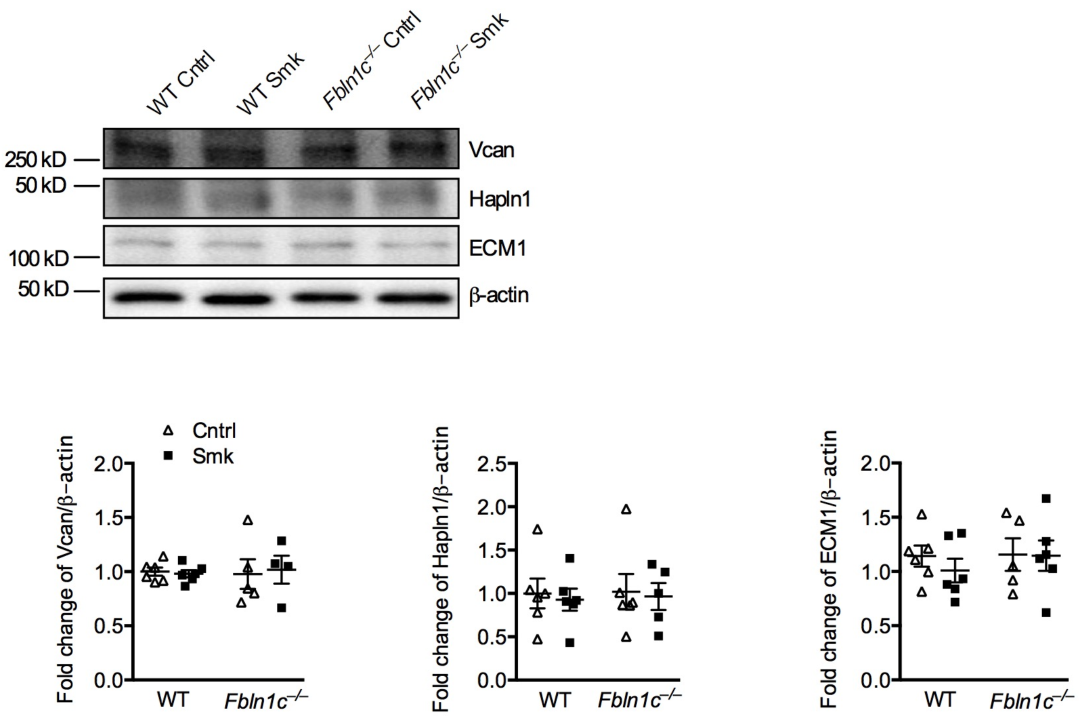
Supplementary Figure 3. *Fbln1c* siRNA reduces target mRNA expression in lungs and protects against emphysema in experimental COPD. WT mice were exposed to cigarette smoke (CS) for four-days or eight-weeks to induce experimental COPD and were treated with *Fbln1c* or scrambled siRNA. (**A**) *Fbln1c* and *Fbln1d* mRNA levels after siRNA treatment every two-days during four-days of CS-exposure, n=5-6. (**B**) *Fbln1c* and *Fbln1d* mRNA levels after siRNA treatment from weeks 6-8 of eight-weeks of CS-exposure, n=5-6. (**C**) Lung sections stained with hematoxylin and eosin (scale bar, 200 μm). Results are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 compared to normal air-exposed WT controls. $$P<0.01 compared to CS-exposed controls treated with scrambled siRNA. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.



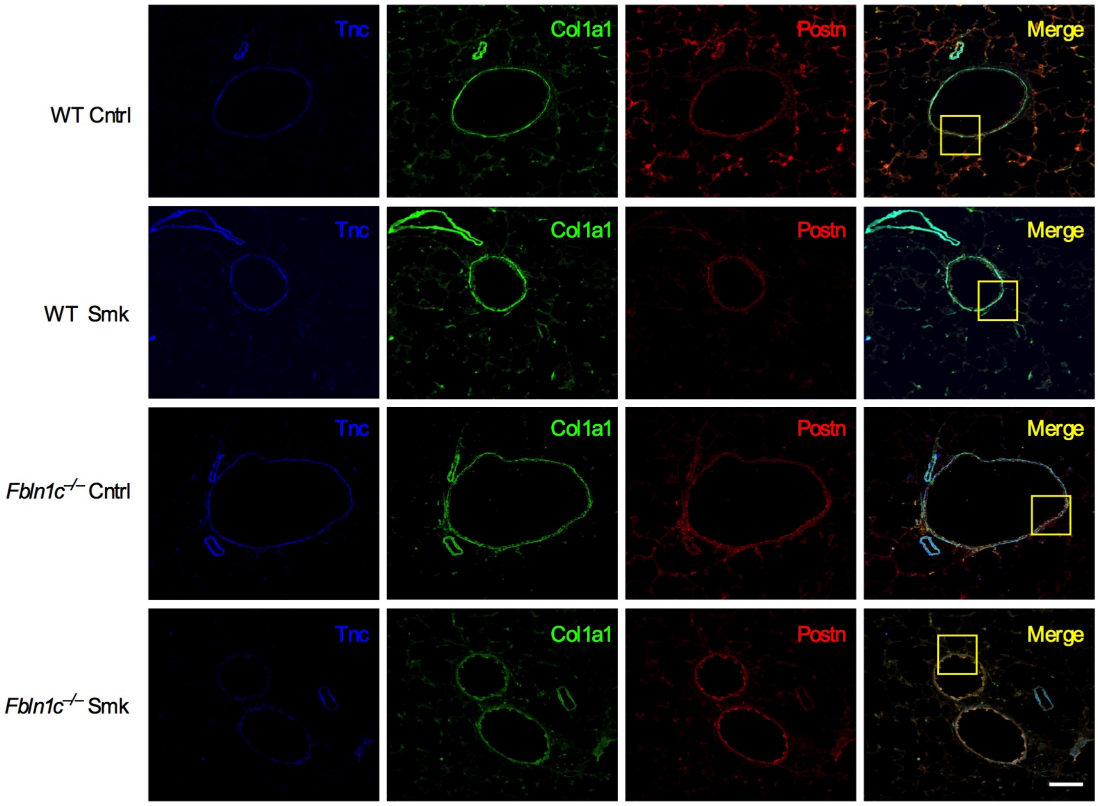
Supplementary Figure 4. *Fbln1c* siRNA alters Fbln1c binding proteins in whole lungs in experimental COPD. WT mice were exposed to cigarette smoke (CS) for eight-weeks to induce experimental COPD and were treated with *Fbln1c* or scrambled siRNA from weeks 6-8 of eight-weeks CS exposure. Fibronectin (Fn), tenascin-c (Tnc) and periostin (Postn) protein levels in whole lungs assessed by immunoblot (top), and fold change of protein densitometry normalized to β-actin (bottom, n=5-6). Results are mean ± SEM. *P<0.05, **P<0.01 compared to normal air-exposed WT controls. $$P<0.01 compared to CS-exposed controls treated with scrambled siRNA. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.



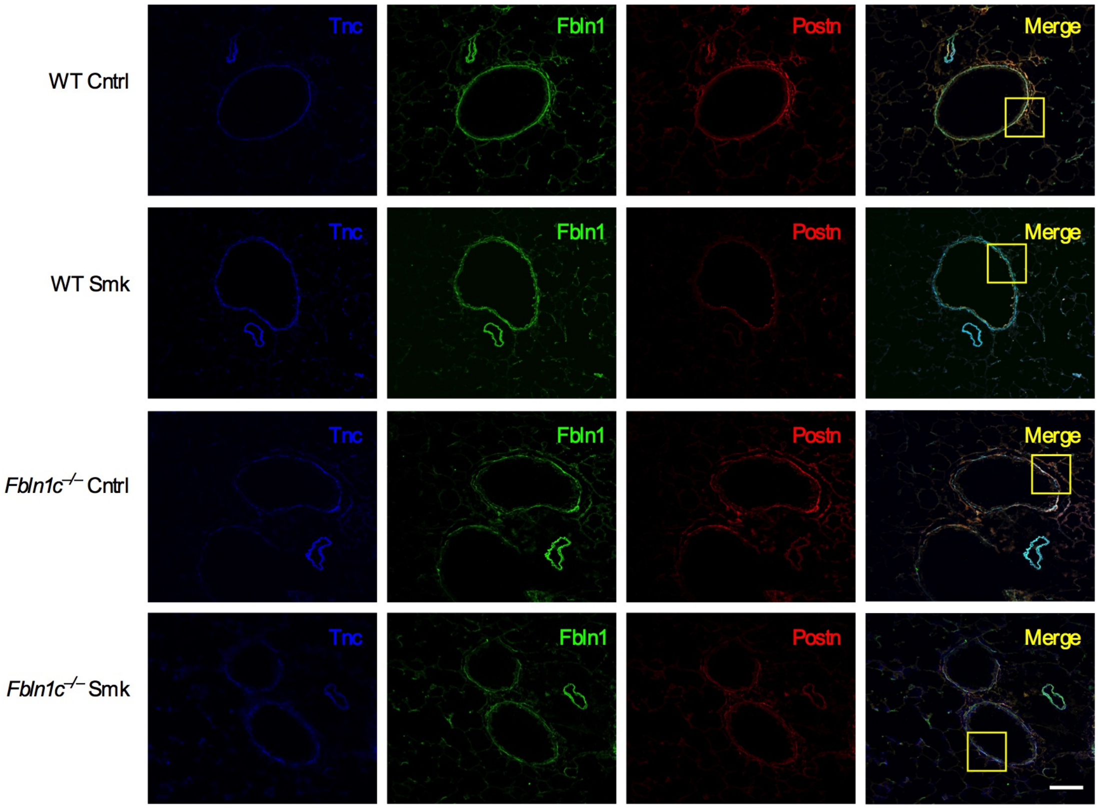
Supplementary Figure 5. **Fbln1c binding proteins are altered in whole lungs and around small airways in WT and *Fbln1c*$^{-/-}$ mice with experimental COPD.** WT and *Fbln1c*$^{-/-}$ mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. (**A**) Fibronectin (Fn), (**B**) tenascin-c (Tnc) and (**C**) periostin (Postn) around mouse small airways by immunohistochemistry (scale bar, 50 µm). n=5-6 mice per group.



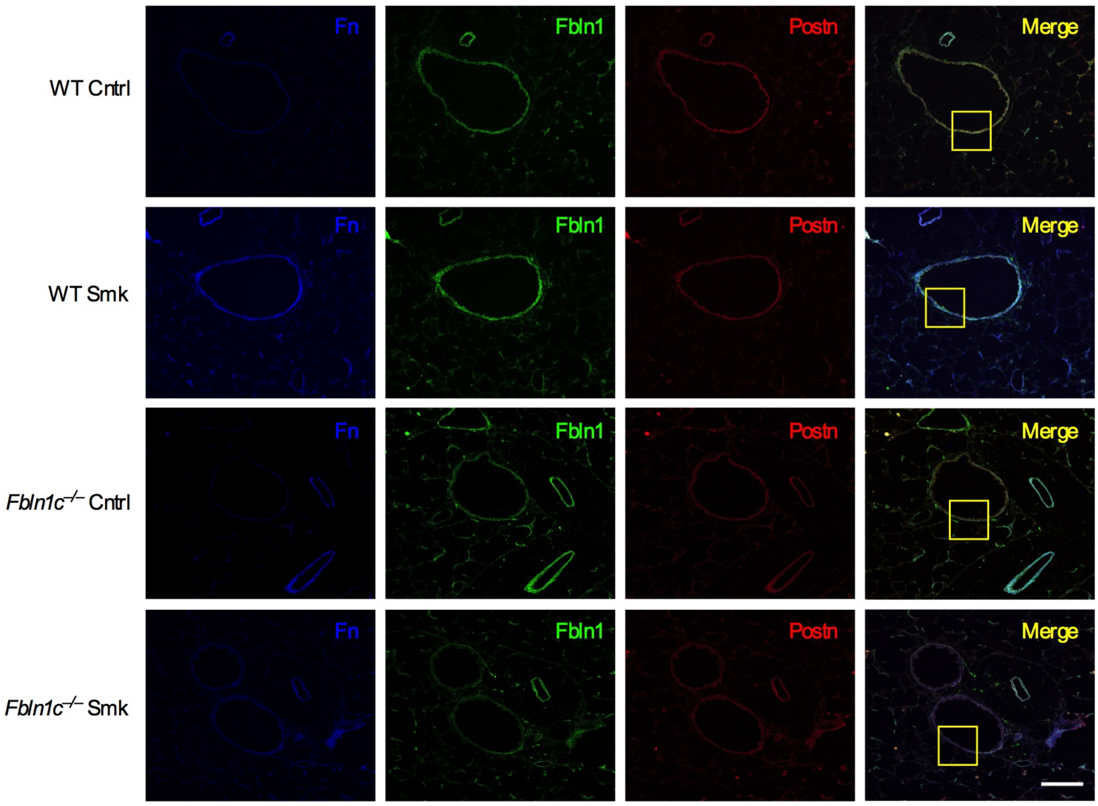
Supplementary Figure 6. Irrespective of the presence or absence of *Fbln1c* other ECM proteins are not altered in experimental COPD. WT and *Fbln1c* deficient ($^{-/-}$) mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. Versican (Vcan), hyaluronan and proteoglycan link protein (Hapln1), and extracellular matrix protein 1 (ECM1) proteins level in whole lungs by immunoblot (top), and fold change of protein densitometry normalized to β-actin (bottom), n=5-6. Results are mean ± SEM. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.



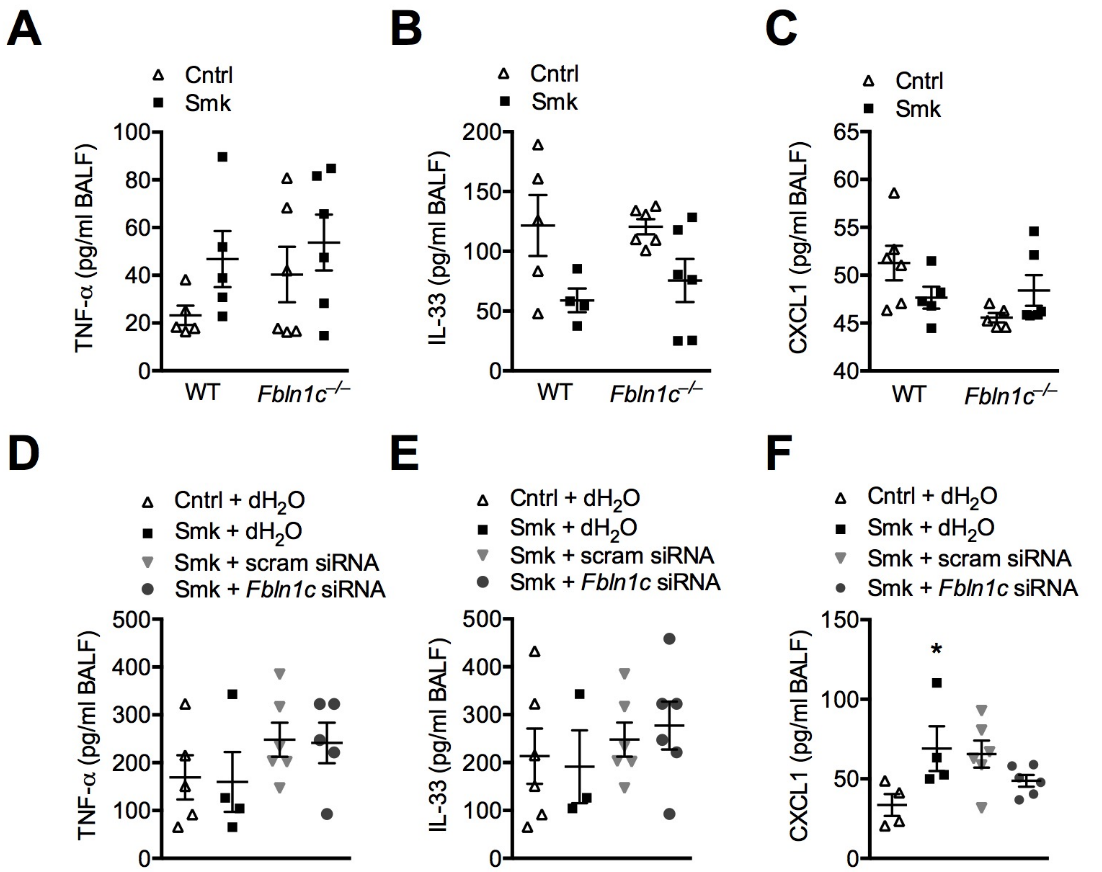
Supplementary Figure 7. Tenascin-c (Tnc), type I collagen-α1 (Col1a1), and periostin (Postn) co-localize around mouse small airways in experimental COPD. Wild type (WT) and *Fbln1c* deficient ($^{-/-}$) mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. Co-localization of Tnc (blue), Col1a1 (green) and Postn (red) around mouse small airways (scale bar, 50 µm, area magnified in Figure 4E is boxed). n=3.



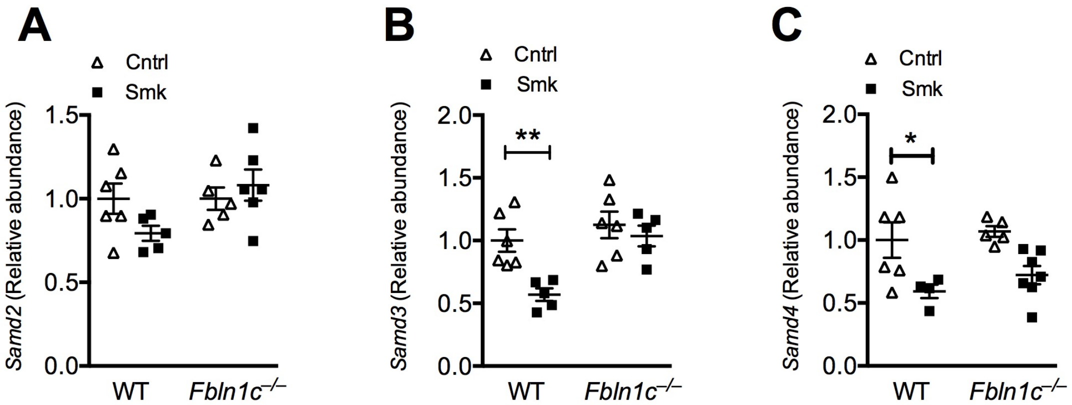
Supplementary Figure 8. Tenascin-c (Tnc), fibulin-1 (Fbln1), and periostin (Postn) co-localize around mouse mall airways in experimental COPD. WT and *Fbln1c* deficient ($^{-/-}$) mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. Co-localization of Tnc (blue), Fbln1 (green) and Postn (red) around mouse small airways (scale bar, 50 μm, area magnified in Figure 4F is boxed). n=3.



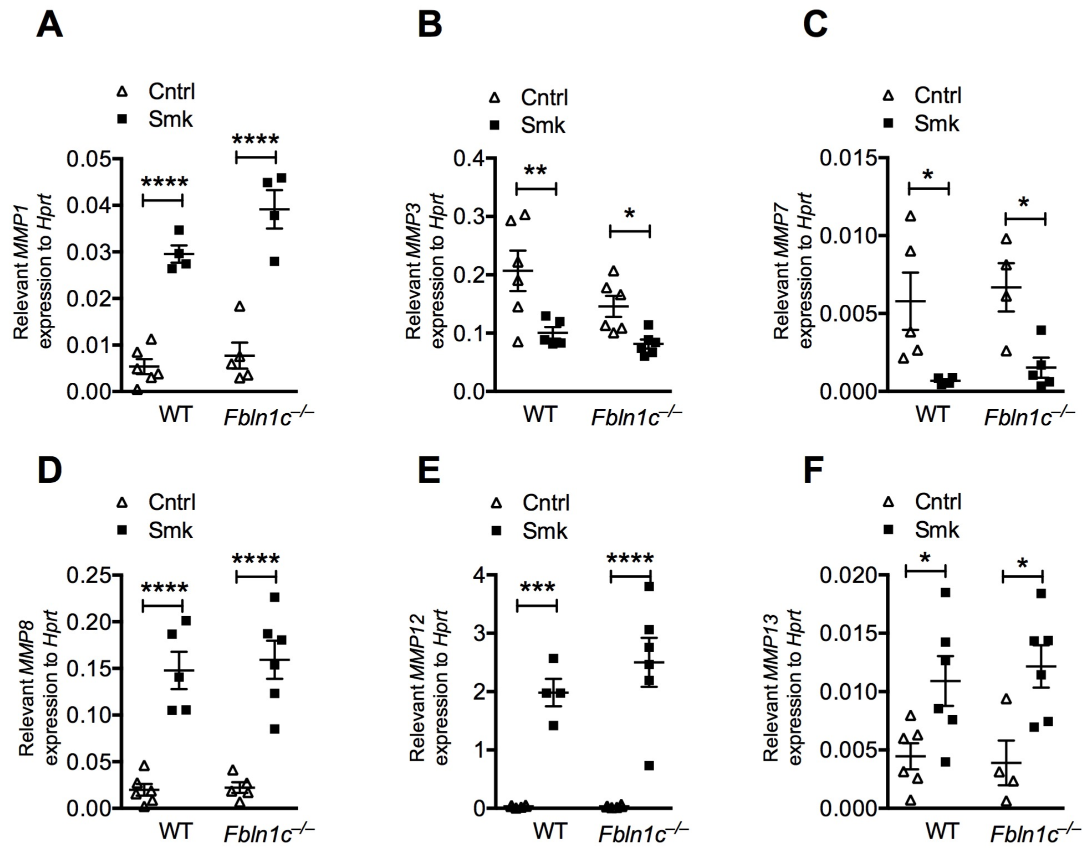
Supplementary Figure 9. Fibronectin (Fn), fibulin-1 (Fbln1), and periostin (Postn) co-localized around mouse small airways in experimental COPD. WT and *Fbln1c* deficient ($^{-/-}$) mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. Co-localization of Fn (blue), Postn (red) and Fbln1 (green) around mouse small airways (scale bar, 50 µm, area magnified in Figure 4E is boxed). n=3.



Supplementary Figure 10. Cytokine and chemokine changes in the airways in BALF in experimental COPD. WT and *Fbln1c* deficient ($^{-/-}$) mice were exposed to cigarette smoke (CS) for eight-weeks to induce experimental COPD, controls were exposed to normal air. (**A**) TNF-α, (**B**) IL-33, and (**C**) CXCL1 proteins in bronchoalveaolar lavage fluid (BALF) were measured by ELISA. WT mice were exposed to CS for eight-weeks to induce experimental COPD and were treated with *Fbln1c* or scrambled siRNA from weeks 6-8 of eight-weeks of CS exposure. (**D**) TNF-α, (**E**) IL-33 and (**F**) CXCL1 protein in BALF were measured by ELISA. n=5-6. Results are mean ± SEM. *P<0.05 compared to air-exposed WT controls. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.



Supplementary Figure 11. Absence of *Fbln1c* prevents reductions in *Smad3* and *Smad4* mRNA in the lungs in experimental COPD. WT and *Fbln* deficient ($^{-/-}$) mice were exposed to cigarette smoke (CS) for eight-weeks to induce experimental COPD, controls were exposed to normal air. (**A**) *Smad2*, (**B**) *Smad3*, and (**C**) *Smad4* mRNA levels in whole lungs determined by qPCR, n=5-6. Results are mean ± SEM. *P<0.05, **P<0.01 compared to WT controls. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.



Supplementary Figure 12. MMP mRNA levels in the lungs in experimental COPD. WT and *Fbln* deficient ($^{-/-}$) mice were exposed to cigarette smoke (CS) for eight-weeks to induce experimental COPD, controls were exposed to normal air. (**A**) *MMP1*, (**B**) *MMP3*, (**C**) *MMP7*, (**D**) *MMP8*, (**E**) *MMP12*, and (**F**) *MMP13* mRNA levels in whole lungs determined by qPCR, n=5-6. Results are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to normal air-exposed WT or *Fbln1c*$^{-/-}$controls. Statistical differences were determined with one-way ANOVA followed by Bonferroni post-test.

Supplementary Table 1. Extracellular matrix (ECM) protein gene expression in whole lungs in experimental COPD. WT mice were exposed to cigarette smoke for eight-weeks to induce experimental COPD, controls were exposed to normal air. ECM protein gene expression in the lungs was assessed by PCR array. Red = up-regulated >2-fold, blue = down-regulated >2-fold.

Gene		4wk (Smk vs Cntrl)	8wk (Smk vs Cntrl)
Symbol	Description	Fold change	Fold change
Adamts1	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	-1.3824	-1.894
Adamts2	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 2	-2.4338	-2.2306
Adamts5	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 5	-1.2494	-1.9284
Adamts8	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 8	-1.4552	-1.2856
Cd44	CD44 antigen	-1.0397	1.5153
Cdh1	Cadherin 1	-1.4562	-1.4503
Cdh2	Cadherin 2	-1.182	-1.2332
Cdh3	Cadherin 3	-6.4183	-1.7248
Cdh4	Cadherin 4	1.3926	-1.01
Cntn1	Contactin 1	-1.0426	-1.3008
Col1a1	Collagen, type I, alpha 1	-2.5039	-1.3513
Col2a1	Collagen, type II, alpha 1	-3.6558	-1.2171
Col3a1	Collagen, type III, alpha 1	-1.6739	-1.9391
Col4a1	Collagen, type IV, alpha 1	-1.618	-2.1146
Col4a2	Collagen, type IV, alpha 2	-1.7401	-1.6238
Col4a3	Collagen, type IV, alpha 3	-1.034	-1.2972
Col5a1	Collagen, type V, alpha 1	-1.2076	-2.5305
Col6a1	Collagen, type VI, alpha 1	-1.7114	-1.5642
Ctgf	Connective tissue growth factor	1.2199	-1.05
Ctnna1	Catenin (cadherin associated protein), alpha 1	-1.096	-1.4403
Ctnna2	Catenin (cadherin associated protein), alpha 2	-2.5144	-1.3504
Ctnnb1	Catenin (cadherin associated protein), beta 1	-1.0586	-1.2366
Ecm1	Extracellular matrix protein 1	-1.2279	1.0641
Emilin1	Elastin microfibril interfacer 1	-1.0601	-1.1411
Entpd1	Ectonucleoside triphosphate diphosphohydrolase 1	-1.3033	-1.6261
Fbln1	Fibulin 1	-1.173	-1.3485
Fn1	Fibronectin 1	1.5239	1.3118
Hapln1	Hyaluronan and proteoglycan link protein 1	1.3945	-2.8194
Hc	Hemolytic complement	-1.7804	-2.0454
Icam1	Intercellular adhesion molecule 1	1.5229	1.3137
Itga2	Integrin alpha 2	-1.4144	-1.7476
Itga3	Integrin alpha 3	-2.1919	-1.6904
Itga4	Integrin alpha 4	-2.0437	-1.357
Itga5	Integrin alpha 5 (fibronectin receptor alpha)	1.1915	-1.1522
Itgae	Integrin alpha E, epithelial-associated	-2.221	-1.1797
Itgal	Integrin alpha L	-2.5144	-1.3589
Itgam	Integrin alpha M	-1.0304	2.1623
Itgav	Integrin alpha V	-1.8637	-1.1978
Itgax	Integrin alpha X	1.0724	2.3369
Itgb1	Integrin beta 1 (fibronectin receptor beta)	-1.1617	-1.4463
Itgb2	Integrin beta 2	1.5624	2.3418
Itgb3	Integrin beta 3	-1.6315	-1.7332
Itgb4	Integrin beta 4	-1.4076	-1.533
Lama1	Laminin, alpha 1	1.5624	1.7131
Lama2	Laminin, alpha 2	-1.3863	1.0752
Lama3	Laminin, alpha 3	-1.0513	-1.5151
Lamb2	Laminin, beta 2	-1.8741	-1.3208
Lamb3	Laminin, beta 3	-1.3969	1.2645
Lamc1	Laminin, gamma 1	1.0881	-1.6081
Mmp10	Matrix metallopeptidase 10	-8.096	5.0616
Mmp11	Matrix metallopeptidase 11	-2.4321	-1.2679
Mmp12	Matrix metallopeptidase 12	6.3107	12.3514
Mmp13	Matrix metallopeptidase 13	-1.1465	3.4982
Mmp14	Matrix metallopeptidase 14 (membrane-inserted)	-2.7706	-1.4166
Mmp15	Matrix metallopeptidase 15	-1.1836	1.2231
Mmp1a	Matrix metallopeptidase 1a (interstitial collagenase)	-1.1552	1.0985
Mmp2	Matrix metallopeptidase 2	-1.8279	-1.0558
Mmp3	Matrix metallopeptidase 3	-4.2257	-3.2839
Mmp7	Matrix metallopeptidase 7	-5.5067	1.5611
Mmp8	Matrix metallopeptidase 8	2.4654	2.6273
Mmp9	Matrix metallopeptidase 9	-1.5683	-1.0863
Ncam1	Neural cell adhesion molecule 1	-1.8963	-2.1015
Ncam2	Neural cell adhesion molecule 2	-5.3044	-1.5491
Pecam1	Platelet/endothelial cell adhesion molecule 1	-1.3824	-1.7501
Postn	Periostin, osteoblast specific factor	1.028	-1.7893
Sele	Selectin, endothelial cell	-2.7419	-1.4353
Sell	Selectin, lymphocyte	-1.565	-1.2145
Selp	Selectin, platelet	1.753	-1.1618
Sgce	Sarcoglycan, epsilon	1.7062	-1.6104
Sparc	Secreted acidic cysteine rich glycoprotein	1.111	-1.5729
Spock1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan 1	-6.4183	-1.4009
Spp1	Secreted phosphoprotein 1	3.5945	6.8905

Syt1	Synaptotagmin I	-1.536	1.6662
Tgfbi	Transforming growth factor, beta induced	1.1589	1.5687
Thbs1	Thrombospondin 1	-1.7742	-1.9084
Thbs2	Thrombospondin 2	-1.762	-1.6834
Thbs3	Thrombospondin 3	-1.5159	-1.4176
Timp1	Tissue inhibitor of metalloproteinase 1	-1.1869	2.012
Timp2	Tissue inhibitor of metalloproteinase 2	1.0843	-1.4829
Timp3	Tissue inhibitor of metalloproteinase 3	-1.518	-1.7881
Tnc	Tenascin C	-1.8496	1.0214
Vcam1	Vascular cell adhesion molecule 1	-1.2747	-1.2349
Vcan	Versican	-1.9335	-1.6148
Vtn	Vitronectin	-1.6832	-2.1308

Supplementary Table 2. Human subject characteristics.

	Healthy	COPD	smoker	p-value
Number	8	9	5	NA
Sex (% female)	50%	55%	80%	P=0.6
Mean Age (SD)	60 (8)	65 (15)	55 (11)	P=0.71
Mean FEV_1 (SD)	104.3 (13.62)	46 (10.55)	92.3 (9.5)	P < 0.001
Cigarette (Pack/year; SD)	0	53 (34.8)	32 (9.4)	P=0.05
Years abstinent (SD)	0	9.6 (1.2)	0	NA
ICS (present treated)	0	100%	0	NA

FEV_1 refers to the forced expiratory volume in 1s expressed as a percentage of the predicated value.
The statistical analysis used for this table is ANOVA for multiple groups.
NA = Not applicable.

Supplementary Table 3. Primers used for qPCR analysis.

Gene	Forward primer 5' to 3'	Reverse primer 5' to 3'
Col1a1	CTTCACCTACAGCACCCTTGTG	TGACTGTCTTGCCCCAAGTTC
Fbln1c	AGAACTATCGCCGCTCCGCA	CCACCGCTGGCACTTGGATG
Fbln1d	GCTATGAGGACGGCATGACT	GGAAACTACGCCTCCCAACA
Smad2	AATACGGTAGATCAGTGGGACA	CAGTTTTCGATTGCCTTGAGC
Smad3	GTTCTCCAAACCTCTCCCCG	TGTGAGGCGTGGAATGTCTC
Smad4	AGCCGTCCTTACCCACTGAA	GGTGGTAGTGCTGTTATGATGGT
MMP1	GTCTTCTGGCACACGCTTTT	GGGCAGCAACAATAAACAA
MMP3	ACATGGAGACTTTGTCCCTTTTG	TTGGCTGAGTGGTAGAGTCCC
MMP7	TTTGCTGCCACCCATGAAT	ACATCACAGTACCGGGAACAGA
MMP8	GATTCAGAAGAAACGTGGACTCAA	CATCAAGGCACCAGGATCAGT
MMP12	GCTTGAGTTTTGATGGTGTCAC	GAAGTAATGTTGGTGGCTGGGA
MMP13	CCTTCTGGTCTTCTGGCACAC	GGCTGGGTCACACTTCTCTGG
HPRT	AGGCCAGACTTTGTTGGATTTGAA	CAACTTGCGCTCATCTTAGGCTTT