

1 **Supplementary Information**

2 **Fibulin-1c regulates transforming growth factor- $\beta$**   
3 **activation in pulmonary tissue fibrosis**

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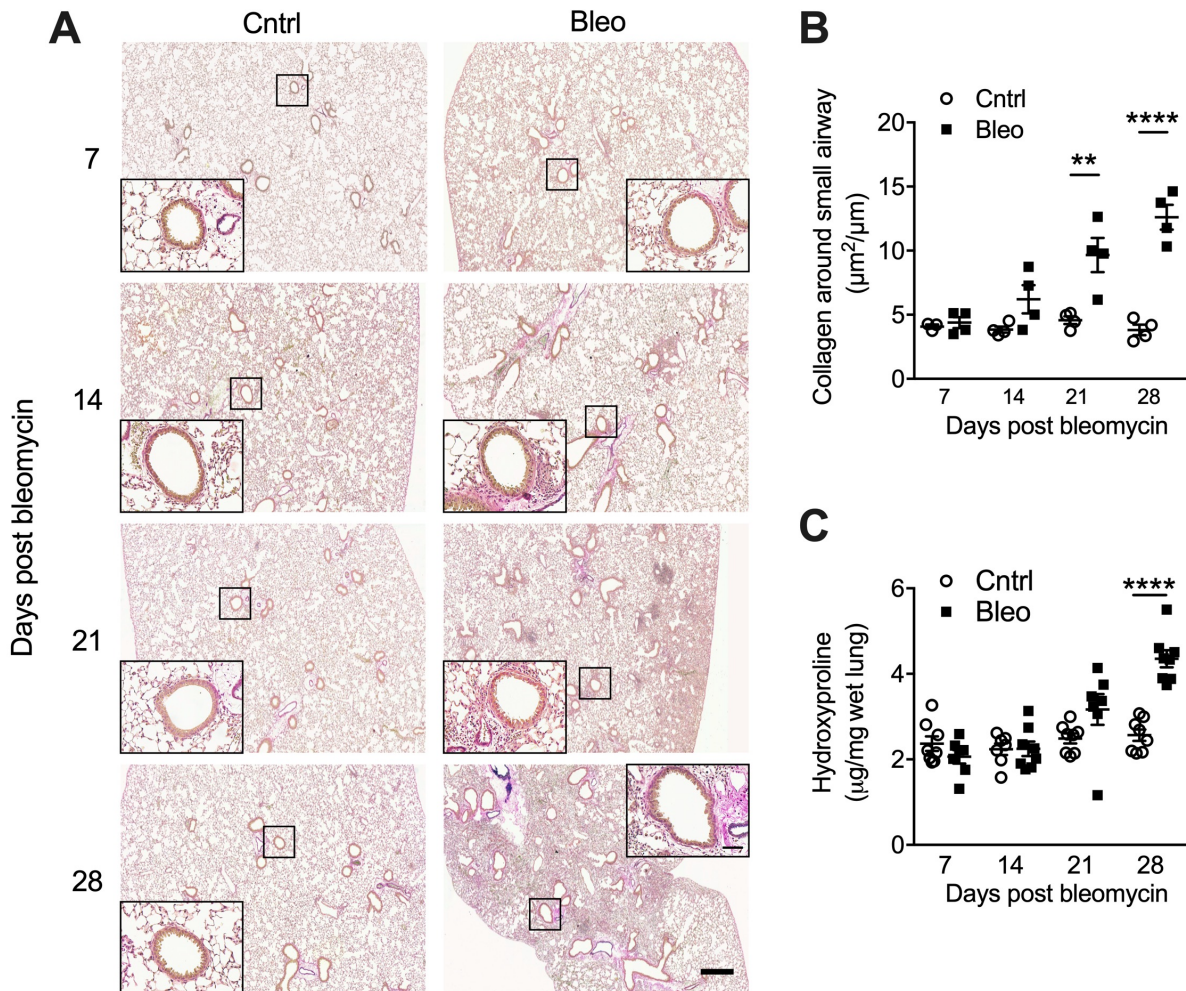
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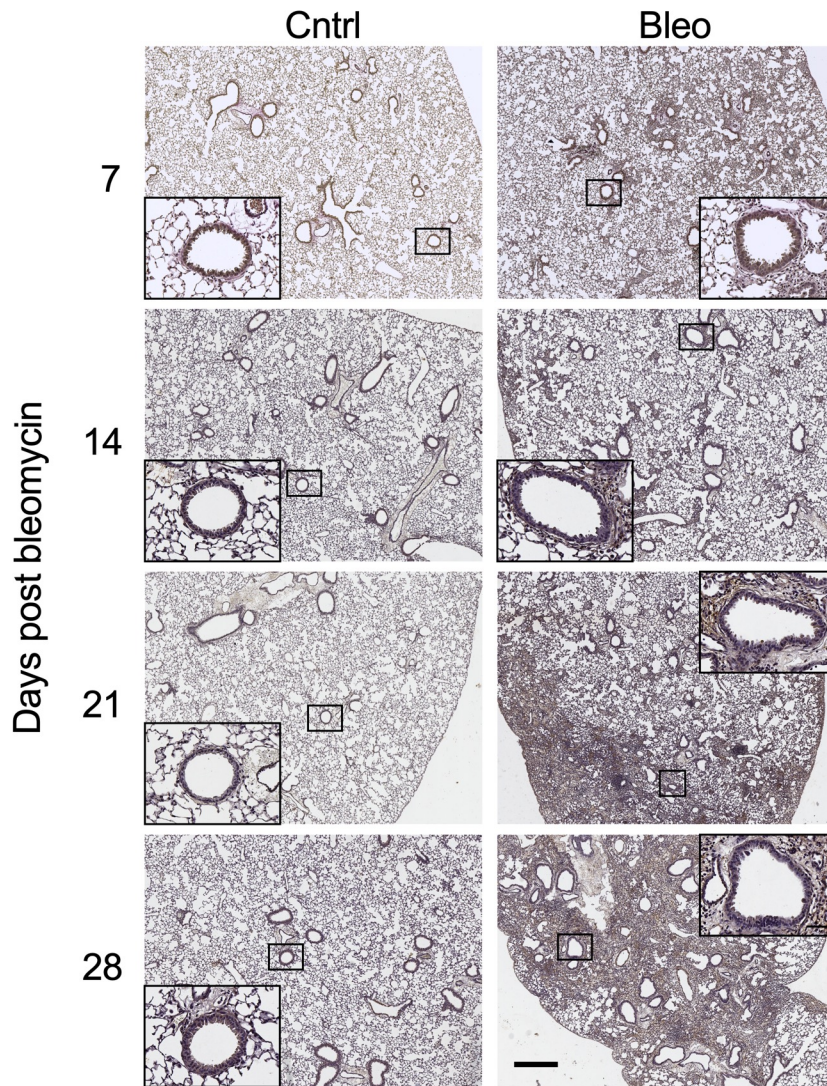
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29 **Supplementary Figures and Figure legends**



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 31 **Supplementary Figure 1. Excess collagen deposition in whole lungs and around**  
 32 **the airways in bleomycin-induced experimental pulmonary fibrosis.** A single  
 33 bleomycin challenge induced pulmonary fibrosis in WT mice. Controls received PBS.  
 34 **(A)** A time-course of lung sections stained with Verhoeff's-Van Gieson stain. Scale  
 35 bar=500 µm; inserts show expanded images of indicated regions, scale bar=50 µm.  
 36 Images are representative of n=24-40 airways from n=4-8 mice per group. **(B)** A time-  
 37 course of quantification of collagen area around small airways with normalization to  
 38 the perimeter of the basement membrane (Pbm) (n=7-8). **(C)** Total collagen levels  
 39 were assessed by measuring hydroxyproline in whole lung tissues (n=8). Statistical

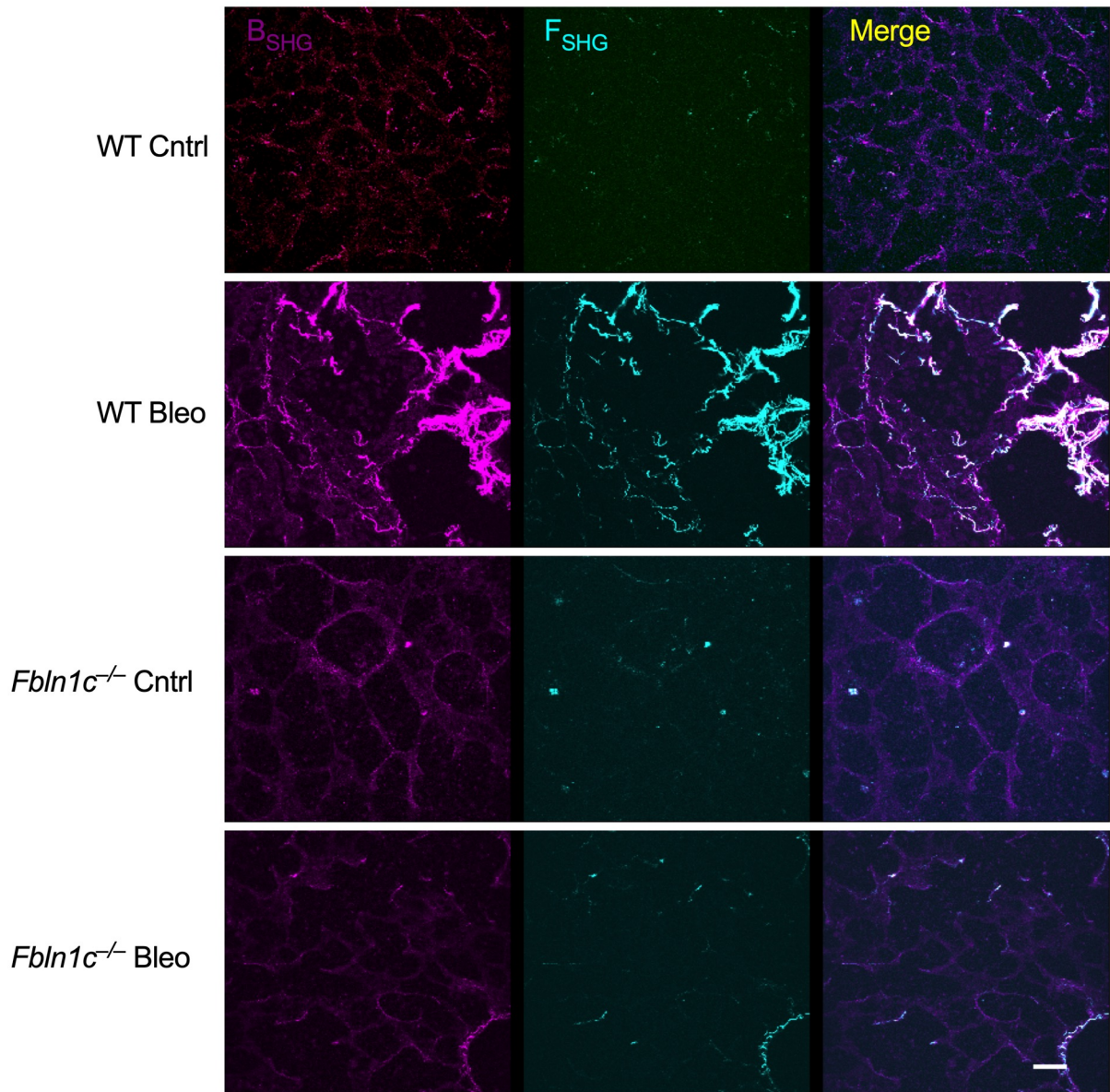
40 differences were determined with two-tailed student t-test. \*\*P<0.01, \*\*\*\*P<0.0001  
41 compared to PBS-challenged mouse controls.



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43 **Supplementary Figure 2. Fbln1c is increased around the airways in bleomycin-**  
 44 **induced experimental pulmonary fibrosis.** A single bleomycin challenge was used  
 45 to induce pulmonary fibrosis in WT mice. Controls received PBS. A time-course of lung  
 46 sections were assessed for Fbln1 protein levels around small airways using  
 47 immunohistochemistry. Scale bar=500  $\mu$ m; inserts show expanded images of indicated  
 48 regions, scale bar=50  $\mu$ m. Images are representative of n=24-40 airways from n=4-8  
 49 mice per group.





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51 **Supplementary Figure 3. Bleomycin challenge of *Fbln1c*<sup>-/-</sup> mice does not**

52 **increase collagen fibers around the airways.** A single bleomycin challenge was

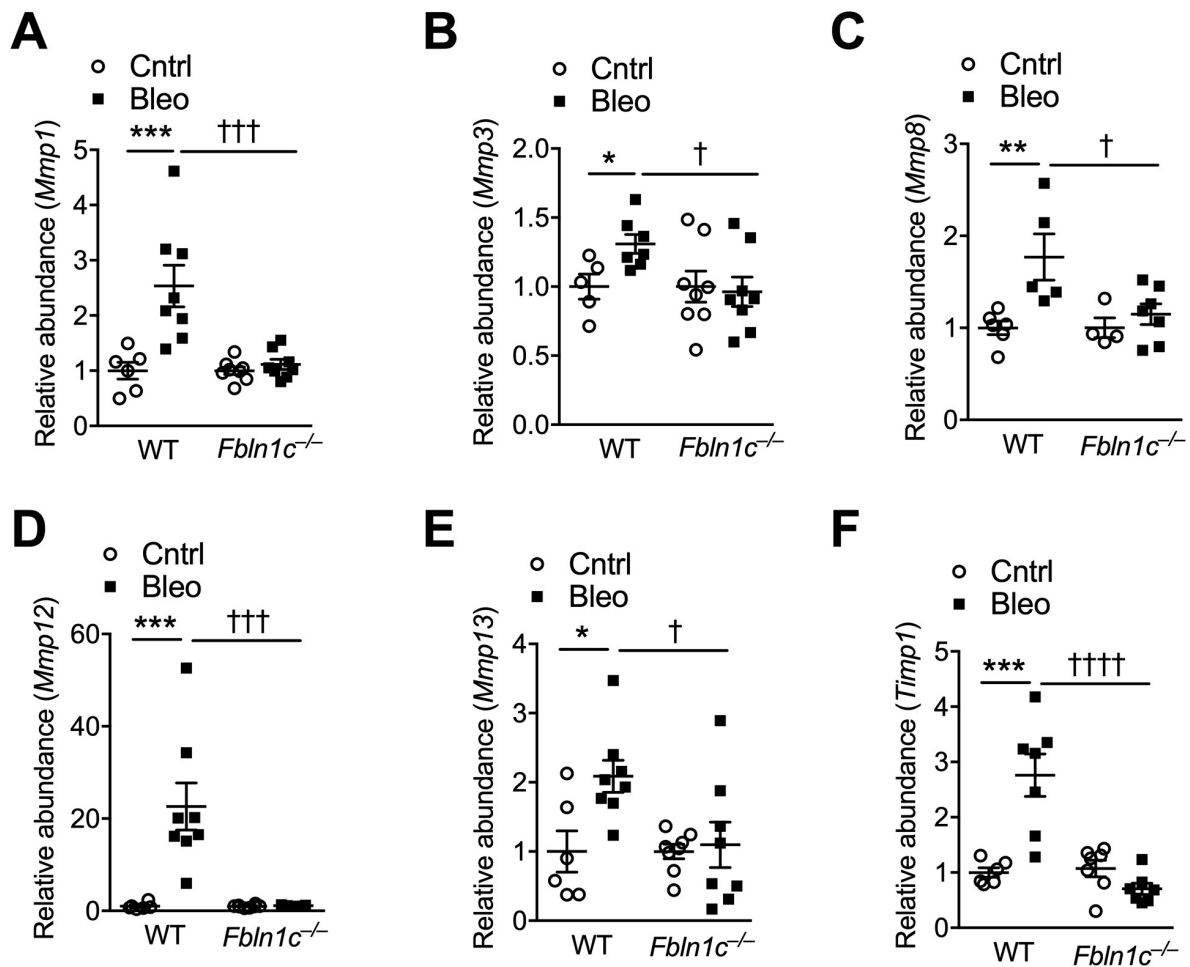
53 used to induce pulmonary fibrosis in WT and *Fbln1c*<sup>-/-</sup> mice. Controls received PBS.

54 Collagen fibers were imaged by second harmonic generation (SHG) microscopy.

55 Collagen backward signal ( $B_{SHG}$ ) is violet, and collagen forward signal ( $F_{SHG}$ ) is cyan,

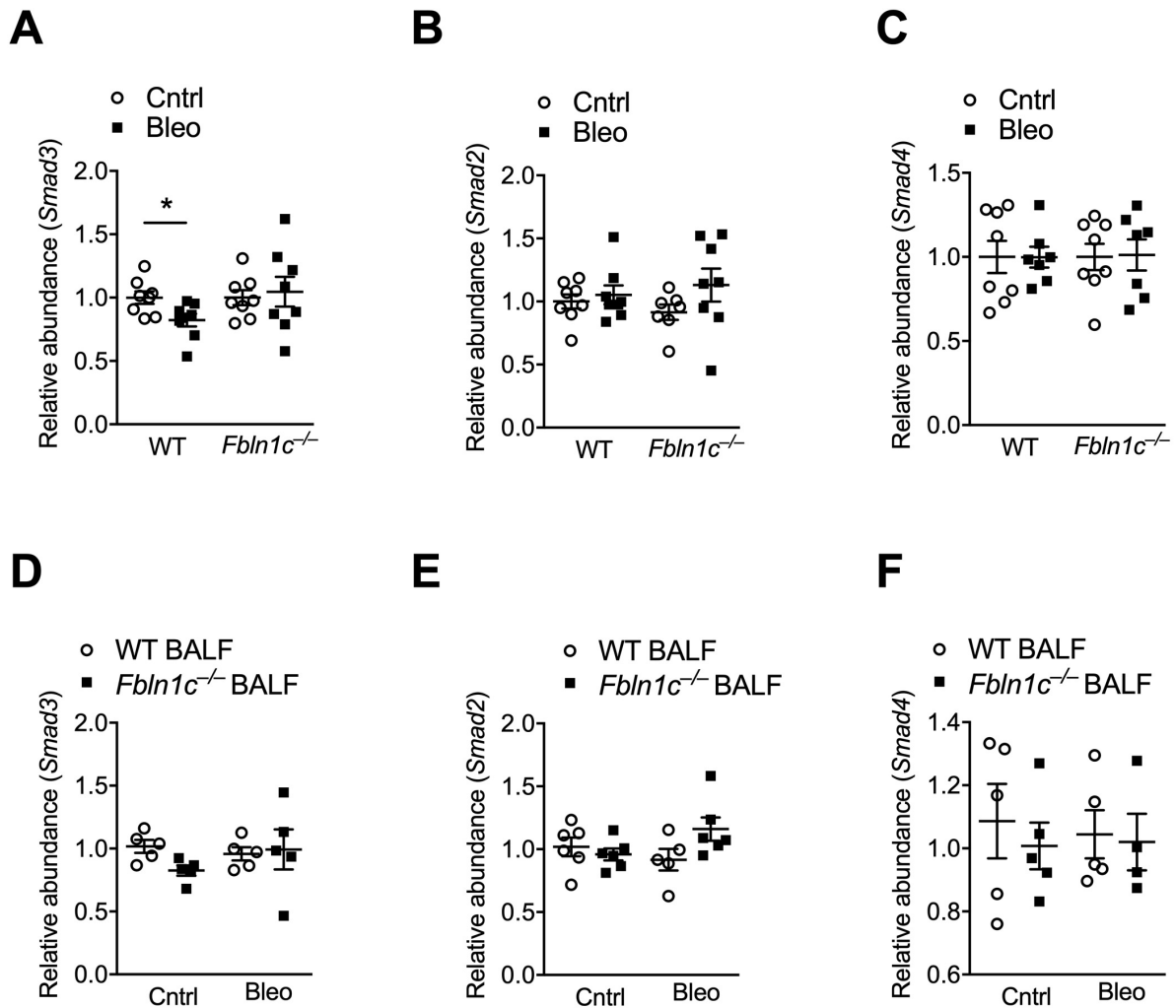
56 scale bar=100  $\mu$ m. Images are representative of n=40 airways from n=4 mice per

57 group.



58

59 **Supplementary Figure 4. Bleomycin challenge of *Fbln1c*<sup>-/-</sup> mice does not**  
 60 **increase the mRNA levels of *Mmps* or *Timp1* in whole lung tissues.** A single  
 61 bleomycin challenge was used to induce pulmonary fibrosis in WT and *Fbln1c*<sup>-/-</sup> mice.  
 62 Controls received PBS. **(A) *Mmp1*, (B) *Mmp3*, (C), *Mmp8*, (D) *Mmp12*, (E) *Mmp13* and**  
 63 **(F) *Timp1*** mRNA levels in lungs determined using qRT-PCR (n=6-8). Statistical  
 64 differences were determined with one-way ANOVA followed by Bonferroni post-test.  
 65 \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared to PBS-challenged WT mice. †P<0.05,  
 66 †††P<0.001, ††††P<0.0001 compared to bleomycin-challenged WT mice.



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68 **Supplementary Figure 5. TGF- $\beta$  challenge of *Fbln1c*<sup>-/-</sup> fibroblasts does not affect**

69 ***Smad3* mRNA levels, and bronchoalveolar lavage fluid (BALF) from *Fbln1c*<sup>-/-</sup>**

70 **mice reduces *Smad* gene levels in fibroblast from WT mice. Primary fibroblasts**

71 **were isolated from the lungs of WT and *Fbln1c*<sup>-/-</sup> mice and stimulated with TGF- $\beta$  or**

72 **media control for 24 h. (A) *Smad3*, (B) *Smad2* and (C) *Smad4* mRNA levels in**

73 **fibroblast lysates determined by qRT-PCR (n=6 of each genotype). Primary mouse**

74 **lung fibroblasts from WT mice were incubated with bronchoalveolar lavage fluid (BALF,**

75 **20 $\mu$ l each mouse from WT and *Fbln1c*<sup>-/-</sup> mice after 28 days bleomycin challenge and**

76 **controls for 6 hours. (D) *Smad3*, (E) *Smad2*, (F) and *Smad4* mRNA levels in fibroblast**



- 77 lysates determined by qRT-PCR. Statistical differences were determined with one-way
- 78 ANOVA followed by Bonferroni post-test. \*P<0.05 compared to media control.