ADAMTS18 Deficiency Leads to Pulmonary Hypoplasia and Bronchial Microfibril Accumulation

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HIGHLIGHTS
ADAMTS18 serves as a morphogen in early lung development
ADAMTS18 deficiency increases lung susceptibility to injuries
ADAMTS18 affects airway branching by regulating bronchial microfibril abundance
ADAMTS18 Deficiency Leads to Pulmonary Hypoplasia and Bronchial Microfibril Accumulation

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SUMMARY

ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) are secreted metalloproteinases that play a major role in the assembly and degradation of the extracellular matrix (ECM). In this study, we show that ADAMTS18, produced by the epithelial cells of distal airways and mesenchymal cells in lung apex at early embryonic stages, serves as a morphogen in lung development. ADAMTS18 deficiency leads to reduced number and length of bronchi, tipped lung apexes, and dilated alveoli. These developmental defects worsen lipopolysaccharide-induced acute lung injury and bleomycin-induced lung fibrosis in adult Adamts18-deficient mice. ADAMTS18 deficiency also causes increased levels of fibrillin1 and fibrillin2, bronchial microfibril accumulation, decreased focal adhesion kinase signaling, and disruption of F-actin organization. Our findings indicate that ECM homeostasis mediated by ADAMTS18 is pivotal in airway branching morphogenesis.

INTRODUCTION

The lungs provide two vital physiological functions including passive gas exchange (alveolar respiration) and innate immune defense against microbial infections. Early lung development has a lifelong effect on respiratory health and disease (Stocks et al., 2013). Factors that adversely affect lung development may accelerate lung function decline and worsen respiratory morbidity in adulthood. Therefore, identification of key cellular and molecular mechanisms involved in early lung development is important for the development of novel strategies to prevent lung diseases.

ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) are a group of 19 secreted metalloproteinases with major roles in the assembly and degradation of the extracellular matrix (ECM). Previous studies have shown that some of these enzymes are produced by lung cells and are involved in lung pathophysiology. Among them, ADAMTS1 is secreted by developing lung epithelial cells at embryonic stages (Thai and Iruela-Arispe, 2002). Adams9 mRNAs are expressed in interstitial cells at E14.5 (Jungers et al., 2005). Adams10 mRNAs are present in the cells surrounding the bronchial tree and blood vessels at E14.5 to E17.5 (Somerville et al., 2004). ADAMTS1, 4, 9, 12, and 15 have been implicated in asthma (Di Valentin et al., 2009; Kurz et al., 2006; Paulissen et al., 2006).

In humans, ADAMTS18 mutations have been linked to tumorigenesis (Jin et al., 2007), developmental eye disorders (Aldahmesh et al., 2011, 2013; Peluso et al., 2013), reduced bone mineral density (Xiong et al., 2009), and decreased white matter integrity of the brain (Lopez et al., 2012). To further study the role of ADAMTS18 in vivo, we developed an Adamts18 knockout (Adamts18−−) mouse strain (Lu et al., 2017) and found that Adamts18−− mice exhibited severely dilated alveoli. This novel finding prompted further studies on the role of ADAMTS18 in lung pathophysiology.

In this study, we demonstrated that Adamts18 is spatiotemporally expressed in the branching epithelium of distal airways and mesenchymal cells in lung apex at early development stages. We also found that ADAMTS18 deficiency leads to reduced number and length of bronchi, tipped lung apex, dilated alveoli, and increased susceptibility to lung injuries. In addition, ADAMTS18 was found to affect bronchus branching partly by interacting with fibrillin1 (FBN1) and regulating the abundance of microfibrils.
RESULTS

Expression of Adamts18 in Mouse Lungs

To investigate the role of ADAMTS18 in lung development, Adamts18 mRNA distribution at different developmental stages was determined by in situ hybridization. In embryonic lungs (E9.5–E12.5), Adamts18 mRNAs were detected in the epithelium of branching tips and mesenchymal cells in lung apex. At pseudo-glandular stage (E12.5–E16.5), Adamts18 mRNAs were most abundant in distal epithelium. At saccular stage (E17.5–P4), the levels of Adamts18 mRNAs were very low in the epithelium of the distal part of bronchiole. At alveolarization stage (P5–P21), Adamts18 mRNAs were barely detectable in both airway and alveolar cells (Figure 1A). Determination of Adamts18 mRNA levels by quantitative RT-PCR (qRT-PCR) in lung tissues from E11.5 to 16-month-old mice demonstrated that Adamts18 is a phase-specific gene and is expressed only in the early embryonic stages (Figure 1B).

Decreased Number and Length of Bronchi due to ADAMTS18 Deficiency

At E14.5, the lung apexes of Adamts18⁻/⁻ mice were bilaterally tipped, whereas those of Adamts18⁺/⁺ mice had a smooth contour (Figure 2A). To characterize branching morphogenesis, E11.5 lungs of both Adamts18⁻/⁻ and Adamts18⁺/⁺ mice were in vitro cultured and examined every 24 h for 72 h (Figure 2B). No macroscopic differences between the two groups of lung explants were observed at the time of dissection. At 24 h of culture, Adamts18⁻/⁻ lungs exhibited an abnormal branching pattern at the distal part of the right lung. At 48 and 72 h of culture, the number of branches in Adamts18⁻/⁻ lungs was significantly decreased compared with that of Adamts18⁺/⁺ lungs (48 h, 35.5 ± 0.5 versus 25 ± 2.9, p = 0.031; 72 h, 56.5 ± 2.5 versus 41 ± 1.6, p = 0.007) (Figure 2C). In addition, the lengths of R5 and R2 secondary bronchi (Figure 2D) in cultured Adamts18⁻/⁻ lung explants were significantly shorter than those of Adamts18⁺/⁺ lung explants (R5, 7.07 ± 0.14 mm versus 4.41 ± 0.61 mm, p = 0.018; R2, 5.61 ± 0.06 mm versus 3.94 ±
Abnormal Lung Morphology in *Adamts18<sup>−/−</sup>* Mice

Adult *Adamts18<sup>−/−</sup>* lungs exhibited several morphologic features that are different from those of *Adamts18<sup>+/+</sup>* lungs, including tipped distal part, bulged center part in the ventral side of left lobes, and shorter axis length (*Figure 3A*). There was no significant difference in the ratio of lung weight to body weight between *Adamts18<sup>−/−</sup>* and *Adamts18<sup>+/+</sup>* mice (*Figure 3B*). Histological analyses of lungs showed linear atelectasis at postnatal day 1 and dilated alveoli with decreased number of radical alveolar counts.
(RACs) in Adamts18-/- mice after alveolar maturation (Figures 3C and 3D). Elastin and collagen are the two main ECM proteins during alveolar septation. With Hart’s staining, a thicker elastin layer was observed on the alveolar walls of 2-week-old Adamts18/C0/C0 mice compared with their Adamts18+/+ littermates (Figure 3E). Determination of mRNA levels by qRT-PCR of key proteins involved in elastic fiber synthesis, assembly, and degradation revealed that only the expression of Tropoelastin and elastin degradation protease Mmp2 was significantly increased in Adamts18-/- lungs; no significant change in the expression of...
other molecules was observed (Figure S1A). In addition, lung collagen of the two genotypes of mice showed no significant difference at mRNA levels by qRT-PCR (Figure S1B) and protein levels by Sirius red staining (Figure S1C).

**Increased Susceptibility to LPS-Induced Acute Lung Injury and Bleomycin-Induced Lung Fibrosis due to ADAMTS18 Deficiency**

There were no differences in basic lung functions between *Adamts18*+/− and *Adamts18*−/− mice (Table S1). However, *Adamts18*−/− mice (8 weeks old) showed more severe pathological injury (e.g., inflammation and bleeding) with a higher pathological score than *Adamts18*+/− littermates (8 weeks old) after intra-peritoneal injection of lipopolysaccharide (LPS) (Figures 4A and 4B). Bronchoalveolar lavage fluid (BALF) cell counts were significantly increased 24 h after LPS injection in both genotypes of mice compared with saline-injected control mice (Figure 4C). However, *Adamts18*−/− lungs had a higher percentage of polymorphonuclear neutrophil (PMN) in BALF than *Adamts18*+/− lungs (Figure 4D). *Adamts18*−/− lungs also showed a significant increase in CD11b+ neutrophil infiltration and interleukin (IL)-6 expression in injured lung tissues (Figures 4E and 4F). The release of neutrophil extracellular traps (NETs) was barely detectable in both genotypes of mice (Figure 4G).

*Adamts18*−/− mice also exhibited a higher mortality rate than *Adamts18*+/− mice (Figure 5A) and more severe lung inflammation and fibrosis after intratracheal injection of bleomycin (Figures 5B–5D). To determine whether LPS or bleomycin induced *Adamts18* expression, *Adamts18* mRNAs levels in the lungs of LPS- or bleomycin-treated *Adamts18*+/− mice were measured by qRT-PCR. Results showed that LPS or bleomycin treatment did not result in increased transcription of *Adamts18* mRNAs in the lungs of these mice at various time points after the treatment (Figures 4H and 5E).

**Branching-Related Signaling Molecules in *Adamts18*−/− Lungs**

Airway branching is controlled by growth factors and matrix proteins in the epithelium and mesenchyme (Stocks et al., 2013). To determine whether the aberrant bronchus structure in *Adamts18*−/− lungs is related to altered expressions of these factors, mRNA levels of several critical signaling transducers were determined at E14.5. The mRNA levels of *Fgf10*, *Wnt2*, and *Bmp4* in lung tissues were similar between the two genotypes of mice. However, mRNA levels of *Fgfr2* and *Shh* were significantly increased in *Adamts18*−/− lungs compared with *Adamts18*+/− lungs (Figure S2A). *Hhip* and *Ptch1* genes are direct targets of SHH signaling (Kugler et al., 2015), and *Ext1* has been shown to control SHH-FGF10 signaling (He et al., 2017). Results showed that mRNA levels of *Hhip*, *Ptch1*, and *Ext1* were comparable between *Adamts18*−/− and *Adamts18*+/− lungs (Figure S2B). Immunohistochemistry (IHC) analysis of the distribution of *FGF10* and *FGFR2* in E14.5 lungs also showed no significant difference between the two genotypes of mice (Figure S2C).

**Embryonic Lung Proteomes**

To investigate the role of ADAMTS18 in embryonic bronchus branching, proteins in E14.5 lungs from *Adamts18*+/− and *Adamts18*−/− mice were analyzed by label-free mass spectrometry. A total of 5,797 proteins were identified (data not show). The abundance of 203 lung proteins (3.5%) was significantly different between *Adamts18*+/− and *Adamts18*−/− lungs. Gene ontology term and pathway analyses of significantly changed proteins by Metascape revealed enrichment of proteins of several pathways related to ribosome, supramolecular fiber organization, and protein folding (Figures S3A and S3B). Forty-three proteins were enriched in the category of supramolecular fiber organization, suggesting disarrangements in actin fiber and ECM (Table S2). Among them, the abundance of two major components of microfibrils, fibrillin1 (FBN1) and FBN2, was increased in *Adamts18*−/− lungs.

**Increased Levels of FBN1 and FBN2 and Accumulation of Microfibrils in *Adamts18*−/− Bronchi**

Precise spatiotemporal regulation of ECM proteins is essential for lung development (Zhou et al., 2018). Western blotting confirmed that the levels of FBN1 and FBN2 were significantly higher in *Adamts18*−/− mice than in *Adamts18*+/− mice (Figure 6A). FBN1 and FBN2 proteins were barely detectable in the distal airway of E14.5 *Adamts18*+/− mice by immunofluorescence staining. However, deposition of FBN1 and FBN2 was seen around the distal airway epithelium of E14.5 *Adamts18*−/− lungs (Figure 6B). These two fibrillin proteins were also found in the proximal airway, but there was no significant difference in their abundance in *Adamts18*+/− and *Adamts18*−/− lungs (Figure S4). Transmission electron microscopic images
showed that Adamts18−−/− lungs had a thicker layer of microfibrils in the basement membrane surrounding epithelial tubes (Figure 6C). Results of qRT-PCR analysis showed that Fbn2 mRNA levels were 1.4-fold higher in Adamts18−−/− lungs than in Adamts18+/+ lungs, whereas Fbn1 mRNA levels in Adamts18−−/− lungs

Figure 4. Increased Susceptibility of Adamts18−−/− Mice to Lipopolysaccharide (LPS)-Induced Acute Lung Injury

(A) Representative images of H&E-stained lung sections of saline-treated (left panels) or LPS-treated (right panels) mice. Scale bar, 50 μm.

(B) Pathological grade of lung injury (n = 5/group).

(C) Total number of cells in bronchoalveolar lavage fluid (BALF) collected 24 h after LPS treatment. Results are expressed as mean ± SD (n = 3/group, *p < 0.05, Student’s t test).

(D) Diff-quick staining of BALF cells for quantification of neutrophils (% polymorphonuclear neutrophil). N, neutrophil; E, eosinophil; M, monocyte. Scale bar, 50 μm. Results are expressed as mean ± SD (n = 3/group, *p < 0.05, Student’s t test).

(E) Immunostaining of CD11b+ neutrophils in lung sections of LPS-treated mice. Quantification of CD11b+ cells in each microscopic field was performed with ImageJ. Each lung section was analyzed for 5 fields. Results are expressed as mean ± SD (N = 5/group, ***p < 0.001, Student’s t test). Scale bar, 50 μm.

(F) IL-6 expression in lung tissues of saline- or LPS-treated mice was analyzed by ELISA. Results are expressed as mean ± SD (n = 5/group, *p < 0.05, Student’s t test).

(G) Confocal microscopy of Cit-H3+MPO+ neutrophil extracellular traps (NETs) in mouse lung sections. Scale bar, 100 μm.

(H) Relative mRNA levels of Adamts18 at different time points of LPS-treated Adamts18+/+ mice determined by quantitative real-time RT-PCR (n = 3/time point). The quantity of Adamts18 mRNA was normalized to that of the housekeeping gene Gapdh using the ΔΔCt method. Data are expressed as mean ± SEM. See also Table S4.
were comparable to those in Adamts18+/+ lungs (Figure 6D). These observations suggest that the increase in FBN1 protein level in Adamts18/C0/C0 lungs was not due to an increase in Fbn1 mRNA transcription.

Interaction between ADAMTS18 and FBN Proteins
To investigate whether ADAMTS18 binds to fibrillins, HEK293T cells transiently transfected with Adamts18-myc-ddk were seeded on mouse dermal fibroblast cells (DFCs), which provided FBN1 and FBN2 proteins in vitro (Figure 6E). ADAMTS18 was found to co-localize with both exogenous FBN1 and FBN2 proteins. Co-immunoprecipitation (co-IP) was then performed to confirm the interaction between ADAMTS18 and fibrillins. Cell lysates of co-cultures of Adamts18-myc-ddk transfected 293T cells and DFCs were incubated overnight with anti-DDK (FLAG tag) agarose beads. A 180-kDa band of ADAMTS18-MYC-DDK was observed by western blotting, and no band in the sample of untransfected cells was seen. FBN1 was pulled down by ADAMTS18 and detected with anti-FBN1-C-terminal antibody, whereas there was no co-IP of FBN2 with ADAMTS18 (Figure 6F). This result suggests that ADAMTS18 binds to FBN1.

Recovery of Adamts18−/− Lung Morphogenesis by Inhibiting FBN Expression
To investigate whether decreasing the abundance of FBN1 or FBN2 in mutant lungs could rescue their branching defects, Fbn1 or Fbn2 antisense (AS)-phosphorothioated oligodeoxynucleotides (ODNs) were
Figure 6. Interactions between ADAMTS18 and Fibrillins

(A) Western blotting results of FBN1 and FBN2 in E14.5 lungs of Admats18+/+ and Admats18+/− mice. The relative quantity of FBN1 and FBN2 proteins is normalized to that of GAPDH and expressed as mean ± SD (n = 4).

(B) Representative immunohistochemical images of FBN1 and FBN2 in E14.5 lung sections. * denote distal airways, and white arrows indicate FBN1 or FBN2 distribution around distal airways. White dotted curves mark visceral pleura. Scale bar, 100 μm.

(C) Transmission electron microscopic images of E14.5 lungs. 1, 2 and 3, 4 are selected fields of the basement membrane of Admats18+/+ and Admats18+/− lungs. The lengths of red arrows indicate the thickness of the microfibril layer outside the lamina dense.

(D) Relative mRNA levels of Fbn1 and Fbn2 determined by real-time RT-PCR. The levels of Fbn1 and Fbn2 mRNAs are normalized to those of the housekeeping gene Gapdh using the ΔΔCt method. Data are expressed as mean ± SEM (n = 7).

(E) Colocalization of ADAMTS18 and fibrillins. Admats18-myc-ddk transiently transfected HEK293T cells were co-cultured with or without mouse dermal fibroblasts (DFCs) and stained with DAPI (blue), anti-FBN1 (red), anti-FBN2 (green), and antibodies against DDK (green) or MYC (red) to label ADAMTS18. Merged yellow sites showed co-localization of ADAMTS18 and FBN1 or FBN2. Scale bar, 100 μm.

(F) Western blotting results of co-IP. *p < 0.05, **p < 0.01. These experiments were repeated independently at least three times.

See also Figures S3 and S4, Tables S3 and S4.
added to lung explant cultures (Figure 7). For lung explants of Adamts18+/+ mice, Fbn1 ASODN treatment had little effect on airway branching; however, Fbn2 ASODN induced dysmorphogenesis of the lung explants, including reduced number of branches and decreased length of secondary bronchi, as previously reported (Yang et al., 1999). For lung explants of Adamts18+/−/+ mice, Fbn1 ASODN treatment partially restored the length of secondary bronchi resulting in a profound increase in the length of R5 secondary bronchi. Treatment with Fbn2 ASODN resulted in increased number of branches and lengths of R5, L3, and L1 secondary bronchi.

**Altered Cytoskeleton Signaling in Adamts18−/− Lungs**

In addition to fibrillins, other ECM proteins involved in branching were also examined (Table S3). Some of these ECM proteins, such as Col1a2, Col3a1, Lama1, Lama3, Lamb1, Lamc1, dystroglycan, nidogen1, and Ctgf, showed significant difference in mRNA or protein levels between the two genotypes of mice.

ECM provides mechanical strength to the epithelium and induces new branches in the lungs. The mechanical signal is transduced in part by the focal adhesion kinase (FAK) in lung epithelial cells (Gjorevski and Nelson, 2010). By IHC analyses, total FAK levels in Adamts18+/+ and Adamts18−/− lungs were found to be similar, but the levels of Tyr297-phosphorylated FAK were lower in lung epithelial cells of Adamts18−/− mice (Figure 8A). The RhoA GTPase, which regulates cell cytoskeleton arrangement in conjunction with FAK (Provenzano and Keely, 2011), also showed a lower activation level in Adamts18−/− lungs (Figure 8B). In proteomic study, 43 proteins involved in supramolecular fiber organization were found to be differentially expressed, most of which were involved in F-actin filament assembly (Table S2). The level
of the cytoskeleton protein Tmsb10, which binds and stabilizes G-actin (Fanni et al., 2011), was found to be significantly decreased (0.3-fold) in Adamts18−/− lungs. This result was confirmed by IHC (Figure 8C). Phallopidin staining showed less F-actin distribution on the apical surface of the epithelial cells facing the lumen in Adamts18−/− lungs (Figure 8D). These data indicate that ADAMTS18 deficiency caused F-actin disorganization, which may result in reduced cell mobility.

In vitro transwell assays showed a significant decrease in the migration of E14.5 Adamts18−/− mouse embryonic fibroblasts (MEFs) compared with Adamts18+/+ lung MEFs (Figure 8E).

DISCUSSION
ADAMTS18 is a poorly characterized member of the ADAMTS family of metalloproteinases. Previously, Ataca et al. created Adamts18−/− C57Bl6/Ola mice in which exons 8–9 of the Adamts18 gene was deleted and found that these mice have a higher percentage of adjacent bronchioles and larger airspaces with thinner walls (Ataca et al., 2016). Recently, Rudge et al. generated another strain of Adamts18−/− mice (VG12442) by deleting a 3,616-bp fragment encompassing the region between the ATG codon in exon 1
and the end of exon 3 (Rutledge et al., 2019). They found that these Adamts18−/− mice have shorter primary branches but maintain the ability to form secondary lateral branches in E12.5 lungs. These findings indicate that ADAMTS18 is crucial for early lung development. However, the mechanisms by which ADAMTS18 affects lung morphogenesis and the effect of ADAMTS18 deficiency on lung function remain largely unknown. In this study, we performed experiments using another Adamts18−/− mouse strain with the C57BL6/126Sv background. In this mouse strain, exons 5–6 of the Adamts18 gene are deleted (Lu et al., 2017). These Adamts18−/− mice exhibit reduced numbers and lengths of bronchi, tipped lung apexes, and dilated alveoli. These developmental defects worsen LPS-induced acute lung injury and bleomycin-induced lung fibrosis in adult Adamts18−/− mice. By examining the bronchial ECM of these mice, we revealed a novel function of ADAMTS18 in modulating fibrillin microfibril formation.

Mouse microfibrils are mainly composed of FBN1 and FBN2. Our results showed that Adamts18−/− lungs had more microfibrils in the basement membrane surrounding the distal airway at E14.5 (Figures 6A–6C). The accumulation of microfibrils in the bronchial wall of Adamts18−/− mice was mainly due to increased FBN2 expression (Figures 6A and 6B). Inhibition of FBN1 or FBN2 expression by ASODNs revealed that FBN2 plays a more important role than FBN1 in early bronchial development (Figure 7). Similar to our findings, Hubmacher et al. found that Adams2 deletion results in bronchial fibrillin microfibril accumulation due to increased FBN2 deposition on the bronchial wall (Hubmacher et al., 2015). Adams2 deletion increases bronchial FBN2 expression only at protein level, and Adams2 is shown to bind directly to FBN2. However, our data showed that FBN2 levels were increased at both protein and mRNA levels in the lungs of Adamts18−/− mice (Figures 6A, 6B, and 6D), suggesting that the increased FBN2 expression is due to enhanced Fbn2 mRNA transcription. It is possible that ADAMTS18 directly processes certain ECMs of microfibril networks, such as FBN1, thus altering tissue stiffness and mechano-signaling and resulting in secondary transcription of other ECM protein genes. In addition to altered Fbn2 mRNA levels, we have previously observed increased laminin transcription in Adamts18−/− adipose tissue and embryonic brains affecting early adipocyte differentiation and neurite formation (Zhu et al., 2018, 2019). This possibility was further indicated by the finding that ADAMTS18 regulates mammary stem cell niche by cleaving fibronectin. This action may lead to changes in the abundance of collagen I, collagen IV, laminin, and collagen XVIII (Ataca et al., 2020).

In this study, we found that ADAMTS18 co-localizes with both FBN1 and FBN2 in the ECM of cultured fibroblasts (Figure 6E). Co-IP results showed that FBN1, but not FBN2, was pulled down by ADAMTS18 (Figure 6F). These data suggest that ADAMTS18 binds to FBN1. Similar to our findings, previous studies showed that some ADAMTS and ADAMTSL proteases can bind to FBN1 or FBN2 or both. Among them, ADAMTSL10 has two FBN1 binding sites and binds to both the N (exons 8–11) and C termini of FBN1 (Hubmacher and Apte, 2011; Kutz et al., 2011). ADAMTS6 has been shown to bind to an N-terminal region of FBN1 (exons 8–11) (Cain et al., 2016). ADAMTS17 binds to both FBN1 and FBN2 (Hubmacher et al., 2017). ADAMTSL2, ADAMTSL4, and ADAMTSL6 are known to bind FBN1 (Gabriel et al., 2012; Le Goff et al., 2011; Tsutsui et al., 2010). ADAMTSL5 has been shown to bind both FBN1 and FBN2 (Bader et al., 2012). We speculate that ADAMTS18 forms a complex with FBN1 and regulates the activity of FBN2 in the fibrillin microfibril scaffold. Because of technical difficulties (Mead and Apte, 2018), we have yet to purify full-length ADAMTS18 proteins for further affinity analysis.

Fibrillin microfibrils represent pivotal ECM signaling platforms integrating the functions of transforming growth factor β, bone morphogenetic protein (BMP), and mechano-signaling (Ramirez and Sakai, 2010). ECM mechanical properties are affected by elastic fibers, fibrillar collagens, glycosaminoglycans, and related proteoglycans. Fibrillar collagens provide tissue stiffness and strength, whereas microfibril-containing elastic fibers are associated with extensibility and resilience (Humphrey et al., 2014). Thus, increased microfibril composition in ECM results in a compliant matrix. Surrounding cells sense the mechanics of ECM through integrins, focal adhesions proteins, and actomyosin cytoskeleton. It has been demonstrated that the phosphorylation level of FAK increases in response to changes in the stiffness of ECM (Du et al., 2016), and FAK signaling is suppressed in compliant ECM (Humphrey et al., 2014). Therefore, ADAMTS18 deficiency increases the levels of fibrillin and tissue compliance, resulting in down regulation of FAK signaling.

Epithelial-mesenchymal transition (EMT) also plays key roles in lung development. BMP, WNT, and FGF signaling induce EMT during branching morphogenesis (Nieto et al., 2016). We found that mRNA levels...
of Bmp4, Wnt2, and Fgf10 in lung tissues of Adamts18+/+ and Adamts18−/− mice were similar (Figure S2) and that those of Fgfr2 were significantly increased in Adamts18−/− lungs compared with Adamts18+/+ lungs (Figure S2). These pathways can activate one or more EMT-driving transcription factors such as Snail1 and Snail2 (Nieto et al., 2016). However, Snail1 and Snail2 mRNA levels showed no difference in Adamts18+/+ and Adamts18−/− lung tissues (Figure S5A). The hallmark of EMT is loss of epithelial cell-cell adhesion molecule E-cadherin and/or concomitant expression of mesenchymal markers such as N-cadherin, vimentin, and alpha-smooth muscle actin (Nieto et al., 2016). The expression levels of E-cadherin, N-cadherin, and Vimentin also showed no difference in lung tissues of Adamts18+/+ and Adamts18−/− mice (Figure S5B). Reorganization of the actin cytoskeleton and activation of the RhoA GTPase equip epithelial cells with the mesenchymal traits of migration. Although major EMT biomarkers detected were not changed in Adamts18−/− lungs, Adamts18−/− distal epithelial cells showed fewer F-actin bundles and reduced activation of RhoA GTPase during branching morphogenesis, suggesting diminished migratory property of terminal epithelial cells and EMT involvement.

The lungs of Adamts18−/− mice exhibited several structural defects, including linear atelectasis and dilated alveoli with decreased number of RACs (Figure 3D). As Adamts18 mRNA is not expressed at the alveolization stage, these lung defects are likely the secondary effect of bronchodyplasia. We observed a thicker elastin layer on alveolar walls of Adamts18−/− lungs than those of Adamts18+/+ lungs (Figures 3E and S1). Normally, elastin is distributed in alveolar tips and guides the formation of alveoli (Zhou et al., 2018). It is likely that excessive fibrillin accumulation in mice with ADAMTS18 deficiency promotes elastic fiber synthesis, leading to increased elastin production and ectopic elastin deposition on alveolar walls.

In the study of LPS-induced acute lung injury and bleomycin-induced lung fibrosis, adult Adamts18−/− mice demonstrated a high susceptibility to lung inflammation and fibrosis (Figures 4 and 5). LPS treatment may induce production and release of proinflammatory cytokines IL-1, tumor necrosis factor-α, IL-6, and chemokines (IL-8 and macrophage inflammatory protein-2), leading to recruitment of neutrophils and acute lung injury (Moreland et al., 2002). Although release of NETs was not observed in both Adamts18+/+ and Adamts18−/− mice 24 h after LPS injection, increased capillary permeability, interstitial edema, and more serious tissue damages were clearly observed in Adamts18−/− lungs. These symptoms may be due to elevated levels of the proinflammatory cytokine IL-6 and infiltration of CD11b+ neutrophils.

Taken together, results of this study indicate that ADAMTS18 is secreted by bronchial epithelial cells and binds to FBN1. In vivo, ADAMTS18 deficiency causes increased levels of FBN1 and FBN2 and accumulation of microfibrils in bronchi. Accumulation of microfibrils causes a weakened FAK signaling and abnormal F-actin organizations. In vitro, ADAMTS18 deficiency causes a reduction in the migration of embryonic fibroblasts.

**Limitations of the Study**

In the present study, we demonstrated that ADAMTS18 regulates early lung development in a microfibril-dependent manner by binding to fibrillin1 protein. However, the binding site of ADAMTS18 on fibrillin1 and how it affects fibrillin abundance remain to be investigated.

**Resource Availability**

**Lead Contact**

Further information and requests for resources and reagents will be fulfilled by the Lead Contact, Wei Zhang (wzhang@sat.ecnu.edu.cn).

**Materials Availability**

This study did not generate new unique reagents.

**Data and Code Availability**

The raw data of this article are available from the leading contact upon request.

**METHODS**

All methods can be found in the accompanying Transparent Methods supplemental file.
**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101472.

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**AUTHOR CONTRIBUTIONS**

T.L., S.D., and W.Z. conceived the study and designed the experiments. T.L., X.L., and C.W. performed experiments and analyzed data. S.Y., Q.Z., and T.Z. genotyped mice and maintained mouse colonies. R.Z., Y.-H.P., T.M.W., Z.C., and B.-S.D. provided valuable advice. T.L., S.D., and W.Z. conceived the study and designed the experiments. T.L., X.L., and C.W. performed experiments and analyzed data. S.Y., Q.Z., and T.Z. genotyped mice and maintained mouse colonies.

**DECLARATION OF INTERESTS**

The authors declare that they have no conflict of interest.

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Supplemental Information

ADAMTS18 Deficiency Leads to Pulmonary Hypoplasia and Bronchial Microfibril Accumulation

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Figure S1. Effect of *Adams18* on elastin and collagen in the lungs of two-week-old mice, related to Figure 3 and Table S5.  

A) qRT-PCR results of key proteins involved in elastic fiber synthesis, assembly, and degradation, including *Tropoelastin (Eln)*, *Lox*, *Fibrillin 1 (Fbn1)*, *Fibrillin 2 (Fbn2)*, *Fibulin 4 (Fbln4)*, *Fibulin 5 (Fbln5)*, *Tgfβ1*, *Ltbp1*, *Integrin α5 (Itga5)*, *Integrin β3 (Itga3)*, *Mmp2*, and *Mmp12*.  

B) qRT-PCR results of *Col1a1*, *Col3a1*, and *Col4a1*.  

C) Lung collagen revealed by Sirius red staining. Scale bar, 50 μm. The relative quantity of target mRNA was normalized to that of the housekeeping gene *Gapdh* using the ΔΔCt method. Data are expressed as mean ± s.d. Statistical significance: *P < 0.05; **P < 0.01
Figure S2. FGF10, Wnt2, Bmp4 and Shh signaling activity in the lungs of Adamts18^{+/+} and Adamts18^{-/-} mice, related to Figure 2, Table S4 and S5. A-B) Relative mRNA levels of Fgf10, Fgfr2, Wnt2, Bmp4, Shh, Hhip, Ptc1, and Ext1
determined by qRT-PCR. The relative quantity of target mRNA was normalized to that of the housekeeping gene Gapdh using the \( \Delta\Delta C_t \) method. Data are expressed as mean ± SEM. C) Representative immunostaining images of Fgf10 and Fgfr2 in E14.5 lungs from Adamts18\(^{+/+}\) and Adamts18\(^{-/-}\) mice. Scale bar, 25 \( \mu \)m.

Figure S3. Related to Figure 6. A) Gene ontology (GO) term and pathway analysis. B) Protein-protein interactome network analysis of differentially expressed proteins by Metascape.
Figure S4. Immunohistochemical localization of fibrillin1 (FBN1) and FBN2 around E14.5 large airways, related to Figure 6 and Table S4. B: bronchi. Scale bar = 200 μm.

Figure S5. Epithelial–mesenchymal transition (EMT) signaling in the lungs of
Adams18\(^{+/+}\) and Adams18\(^{-/-}\) mice, related to Figure S3 and Table S5. A-B).

Relative mRNA levels of Snail 1, Snail 2 (A), E-cadherin (Cdh1), N-cadherin (Cdh2), and Vimentin (Vim) (B) Determination of mRNA levels by qRT-PCR. The relative quantity of target mRNA was normalized to that of the housekeeping gene Gapdh using the \(\Delta\Delta Ct\) method. Data are expressed as mean ± SEM.
## Supplemental Tables

### Table S1. Lung functions of 12-week-old mice, related to Figure 3.

|                | Male        | Female       | P value | Male        | Female       | P value |
|----------------|-------------|--------------|---------|-------------|--------------|---------|
|                | Adamts18+/+ | Adamts18−/−  | P value | Adamts18+/+ | Adamts18−/−  | P value |
| Ti (s)         | 0.23 ± 0.03 | 0.23 ± 0.08  | 0.95    | 0.33 ± 0.09 | 0.31 ± 0.11  | 0.94    |
| Te (s)         | 0.12 ± 0.01 | 0.12 ± 0.02  | 0.73    | 0.12 ± 0.01 | 0.15 ± 0.04  | 0.59    |
| f/min          | 172.16 ± 22.60 | 184.98 ± 57.09 | 0.62 | 136.67 ± 22.87 | 143.62 ± 53.72 | 0.78      |
| Phigh (cmH₂O)  | 25.89 ± 0.34 | 25.81 ± 6.15 | 0.75    | 26.91 ± 0.63 | 27.26 ± 0.53  | 0.33    |
| Pmean (cmH₂O)  | 6.20 ± 0.08  | 6.15±0.13    | 0.45    | 6.42 ± 0.15  | 6.50 ± 0.15   | 0.42    |
| TVb (μL)       | 1.4 ± 0.2   | 1.6 ± 0.2    | 0.31    | 1.1 ± 0.2   | 1.3 ± 0.2     | 0.18    |
| MVb (mL)       | 0.24 ± 0.05 | 0.30 ± 0.13  | 0.37    | 0.15 ± 0.04 | 0.19 ± 0.10   | 0.42    |
| PIF/PEF (mL/s) | 0.0111 ±    | 0.0135 ±    | 0.43    | 0.0078 ±    | 0.0102 ±     | 0.11    |
| (mL)           | 0.0021      | 0.0067       |         | 0.0012      | 0.0032       |         |

Ti: Time of inspiration; Te: time of expiration; f/min: frequency per minute; Phigh: airway pressure high; Pmean: airway pressure mean; TVb: tidal volume; MVb: minute volume; PIF/PEF: peak inspiratory/expiratory flow.
Table S2. 43 proteins involved in supramolecular fiber organization, related to Figure 8.

| NO. | Genes  | Protein Description                                      | UniProtIds | Fold (5 Adams18/5 Adams18+/+) | P Value  | AVG Ratio |
|-----|--------|----------------------------------------------------------|------------|-------------------------------|----------|-----------|
| 1   | Tmsb10 | Thymosin beta-10 60S ribosomal protein L13a              | Q6ZWY8     | 0.303                         | 0.029    | 1.723     |
| 2   | Rpl13a | Thymosin beta-10 60S ribosomal protein L13a              | P19253     | 0.691                         | <0.001   | 0.533     |
| 3   | Fbn2   | Fibrillin-2                                              | Q61555     | 1.401                         | <0.001   | 0.486     |
| 4   | Fbn1   | Fibrillin-1                                              | Q61554     | 1.295                         | 0.001    | 0.373     |
| 5   | Pfdn1  | Prefoldin 1                                              | Q9CQF7     | 0.779                         | 0.046    | 0.360     |
| 6   | Col1a2 | Collagen alpha-2(I) chain                                | Q01149     | 1.283                         | 0.037    | 0.360     |
| 7   | Col3a1 | Collagen alpha-1(III) chain                              | P08121     | 1.270                         | 0.018    | 0.345     |
| 8   | Tpm3   | Tropomyosin alpha-3 chain                                | D3Z6I8;    | 0.824                         | <0.001   | 0.279     |
| 9   | Apoe   | Apolipoprotein E                                         | P08226     | 0.825                         | 0.021    | 0.278     |
| 10  | Dpysl3 | Dihydropyrimidinase-related protein 3                    | E9PWE8     | 1.195                         | 0.049    | 0.257     |
| 11  | Apoa1  | Apolipoprotein A-I                                       | Q00623     | 0.837                         | 0.009    | 0.257     |
| 12  | Arpc3  | Actin-related protein 2/3 complex subunit 3              | Q9JM76     | 0.863                         | 0.013    | 0.213     |
| 13  | Clu    | Clusterin                                                | Q06890     | 1.150                         | 0.024    | 0.202     |
| 14  | Rpl4   | 39S ribosomal protein L41, mitochondrial                 | Q9CQN7     | 1.128                         | <0.001   | 0.174     |
| 15  | Lars   | Leucine-tRNA ligase, cytoplasmic ADP-riboseylation factor-like protein 2 | Q8BMJ2     | 0.890                         | 0.046    | 0.168     |
| 16  | Arl2   | Alpha-2-antiplasmin                                       | Q9D0J4     | 0.891                         | 0.027    | 0.167     |
| 17  | Hist1h1b| Histone H1.5                                              | P43276     | 0.894                         | 0.001    | 0.162     |
| 18  | Serpinf2| Alpha-2-antiplasmin                                       | Q61247     | 0.909                         | 0.034    | 0.138     |
| 19  | Vim    | Vimentin                                                 | P20152     | 0.916                         | 0.017    | 0.127     |
| 20  | Dynclh1| Cytoplasmic dynein 1, heavy chain 1                       | Q9JHU4     | 0.918                         | 0.022    | 0.123     |
| 21  | Apoa4  | Apolipoprotein A-IV                                       | P06728     | 0.924                         | 0.029    | 0.114     |
| 22  | Psmc4  | 26S proteasome regulatory subunit 6B                      | P54775     | 0.925                         | 0.019    | 0.112     |
| 23  | Hsp90ab1| Heat shock protein HSP 90-beta                           | P11499     | 0.932                         | 0.024    | 0.102     |
| 24  | Sptan1 | Spectrin alpha chain, non-erythrocytic 1                  | P16546     | 0.933                         | 0.002    | 0.100     |
| 25  | Rdx    | Radixin                                                  | P26043     | 0.934                         | 0.012    | 0.099     |
| 26  | Krt8   | Keratin, type II                                          | P11679     | 0.938                         | 0.048    | 0.092     |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 27 | Hsp90b1 | Endoplasmic | P08113 | 0.940 | 0.001 | 0.089 |
| 28 | Add1 | Alpha-adducin | Q9QYC0 | 1.057 | 0.014 | 0.080 |
| 29 | Serpinh1 | Serpin H1 | P19324 | 0.952 | 0.001 | 0.071 |
| 30 | Actn4 | Alpha-actinin-4 | P57780 | 0.953 | 0.014 | 0.069 |
| 31 | Ran | GTP-binding nuclear protein Ran | P62827 | 0.959 | 0.020 | 0.060 |
| 32 | Farp1 | pleckstrin domain-containing protein | F8VPU2 | 0.959 | 0.027 | 0.060 |
| 33 | Myh10 | Myosin-10 | Q3UH59 | 0.964 | 0.017 | 0.053 |
| 34 | Atp2a2 | Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 | O55143 | 1.035 | 0.044 | 0.050 |
| 35 | Rock2 | Rho-associated protein kinase 2 | A0A1Y7V MN0 | 1.034 | 0.040 | 0.048 |
| 36 | Acta2 | Actin, aortic smooth muscle | P62737 | 0.968 | 0.008 | 0.047 |
| 37 | Mapk1 | Mitogen-activated protein kinase 1 | P63085 | 0.972 | 0.044 | 0.041 |
| 38 | Hspa8 | Heat shock cognate 71 kDa protein | P63017 | 0.981 | 0.016 | 0.028 |
| 39 | Kif2c | Kinesin-like protein KIF2C | Q922S8 | 0.984 | 0.038 | 0.023 |
| 40 | Hist1h1d | Histone H1.3 | P43277 | 0.984 | 0.001 | 0.023 |
| 41 | Map1b | Microtubule-associated protein 1B | P14873 | 1.016 | 0.013 | 0.023 |
| 42 | Sec24b | Sec24-related gene family, member B (S. cerevisiae) | Q80ZX0 | 0.986 | 0.05 | 0.020 |
| 43 | Arhgef2 | Rho guanine nucleotide exchange factor 2 | H3BJ40 | 0.99 | 0.044 | 0.014 |

**AVG:** Absolute value of Log2
Table S3. Quantification of important ECM proteins involved in branching morphogenesis in E14.5 lungs, related to Figure 6.

| UniProtIds | Gene | Protein Description | mRNA H* (3 Adams18<sup>−/−</sup>/2 Adams18<sup>+/+</sup>) | J* (3 Adams18<sup>−/−</sup>/3 Adams18<sup>+/+</sup>) | Protein MS (5 Adams18<sup>−/−</sup>/5 Adams18<sup>+/+</sup>) | Subcellular Location |
|------------|------|---------------------|--------------------------|--------------------------|--------------------------|---------------------|
| P11087     | Col1a1 | Collagen alpha-1(I) chain | 1.01                               | 1.07                               | 1.36                               | Extracellular matrix |
| Q01149     | Col1a2 | Collagen alpha-2(I) chain | 0.96                               | 1.14                               | 1.28                               | Extracellular matrix |
| P08121     | Col3a1 | Collagen alpha-1(III) chain | 1.01                               | 1.10                               | 1.27                               | Extracellular matrix |
| P11276     | Fn1   | Fibronectin          | 1.22                               | 1.23                               | 0.93                               | Extracellular matrix |
| P19137     | Lama1  | Laminin subunit alpha-1 | 1.25 (p = 0.022)                  | 1.36                               | 0.82                               | Basement membrane   |
| Q61789     | Lama3  | Laminin subunit alpha-3 | 1.60 (p = 0.014)                  | 1.64 (p = 0.016)                  | 0.75                               | Basement membrane   |
| Q61001     | Lama5  | Laminin subunit alpha-5 | 1.17                               | 1.53                               | 0.89                               | Basement membrane   |
| P02469     | Lamb1  | Laminin subunit beta-1 | 1.29 (p = 0.008)                  | 1.29 (p = 0.015)                  | 0.97                               | Basement membrane   |
| P02468     | Lamc1  | Laminin subunit gamma-1 | 1.32 (p = 0.042)                  | 1.26                               | 0.98                               | Basement membrane   |
| Q3V3R4     | Itga1  | Integrin alpha-1     | 0.92                               | 1.28                               | 1.09                               | Membrane            |
| Q62469     | Itga2  | Integrin alpha-2     | 1.15                               | 1.19                               | 1.06                               | Membrane            |
| Accession | Gene Symbol | Description                          | Fold Change 1 | Fold Change 2 | Mean Change | Location         |
|-----------|-------------|--------------------------------------|---------------|---------------|-------------|-----------------|
| Q62470    | Itga3       | Integrin alpha-3                     | 1.12          | 1.24          | 0.99        | Membrane        |
| P43406    | Itgav       | Integrin alpha-v                     | -             | 0.98          | 0.99        | Membrane        |
| Q61739    | Itga6       | Integrin alpha-6                     | 0.95          | 1.37          | 1.11        | Membrane        |
| P09055    | Itgb1       | Integrin beta-1                      | -             | 0.94          | 0.99        | Membrane        |
| A2A863    | Itgb4       | Integrin beta-4                      | 0.81          | 1.77          | -           | Membrane        |
| P18828    | Sdc1        | Syndecan-1                           | 1.21          | -             | -           | Extracellular matrix |
| O35988    | Sdc4        | Syndecan-4                           | 1.10          | -             | -           | Extracellular matrix |
| Q62165    | Dag1        | Dystroglycan                          | 1.10          | 1.28* (P = 0.050) | 1.3        | Basement membrane |
| P10493    | Nid1        | Nidogen-1                            | 1.18          | 1.27 (P = 0.007) | 0.99        | Basement membrane |
| P33434    | Mmp2        | 72 kDa type IV collagenase           | 0.78          | -             | 0.97        | Extracellular matrix |
| P29268    | Ctgf        | CCN family member 2                  | 0.92          | 1.51 (P = 0.036) | -           | Extracellular matrix |
| P04202    | Tgfb1       | Transforming growth factor beta-1 proprotein | 0.84          | 1.02          | -           | Extracellular matrix |

*H and J represent mice from two different littermates.

“-”: mean data unavailable because of limited quantity of samples in real-time qPCR experiments or undetectable in MS analysis.
Table S4. Antibodies used in this study, related to Figure 4, 6, 8, S2, and S4.

| Name                     | Catalog number | Company                | Application (dilution) |
|--------------------------|----------------|------------------------|------------------------|
| Anti-MYC                 | TA150121       | Origene                | ICC (1:100)            |
| Anti-Fibrillin1          | Ab53076        | Abcam                  | ICC (1:100)            |
| Anti-DDK                 | TA150078       | Origene                | WB (1:5000)            |
| Anti-FBN1-C terminal     | LS-C358981     | LifeSpan Bioscience    | WB (1:400)             |
| Anti-FBN2                | Sc-393968      | Santa Cruz             | WB (1:200)             |
| Anti-FBN2                | 20252-1-AP     | Proteintech            | IHC (1:200)            |
| Anti-FAK                 | AF6397         | Affinity               | IHC (1:100)            |
| Anti-PFAK(Tyr397)        | AF3398         | Affinity               | IHC (1:100)            |
| Anti-TMSB10              | TA351779       | Origene                | IHC (1:50)             |
| Anti-Fgf10               | GTX55619       | Genetex                | IHC (1:100)            |
| Anti-Fgfr2               | 23328          | CST                    | IHC (1:200)            |
| Anti-CD11b               | Ab133357       | Abcam                  | IHC (1:2000)           |
| Anti-MPO                 | AF3667         | R&D system             | IHC (1:40)             |
| Anti-Histone H3          | NB100-57135    | Novus                  | IHC (1:200)            |
| CY™3 Affinity Goat IgG  | 112-165-003    | Jackson Immuno         | IHC/ICC (1:200)        |
| CY™3 Affinity Goat IgG  | 111-165-003    | Jackson Immuno         | IHC/ICC (1:200)        |
| Anti-Rat IgG             |                | Research               |                        |
| Alexa Fluor 488 Donkey IgG | 705-545-147  | Jackson Immuno         | IHC (1:200)            |
| Anti-Rabbit IgG          |                | Research               |                        |
| Alexa Fluor 647 Donkey IgG | 711-605-152 | Jackson Immuno         | IHC (1:200)            |
| Anti-Goat IgG            |                | Research               |                        |
| Gene     | Forward (5’ to 3’) | Reverse (5’ to 3’) |
|----------|-------------------|-------------------|
| Gapdh    | GTGGAGTCATACCTGGGAAACTATGGAGTG | AAATGGAAGGTCGTGAGTGTG |
| Adams18  | CCTCAAGTTGTCGTCCTCCATCA | GCTGAAGAAATCCACCGCAAGA |
| Fbn1     | GCCAGAAAAGGTATATCCAGC | ACACACTCTCCTCGGT |
| Fbn2     | GTGAAACCACACAGAATGTTA | GAAAGACGCGCATATCAGT |
| Eln      | CTCTGCTCTAGTCGTAAGA | CCACACACCAGGAAATGC |
| Lox      | TCTTCTGCTGCTGACAAACAA | GAGAACAGGTTCGGAACAG |
| Fbln4    | CTCTGGGCTTTTCTGCTTGT | GCCATCTGTCATCTCGGT |
| Fbln5    | GGCTCATACTCTTCGTGCT | GATGGTGAATGGCTGGTCT |
| Tgb1     | CTCCGCTGCTCGCTAGTGC | GCCCTTTGATGGACAGATCG |
| Ltb1     | GGTTATTTGCATCTTCCGCT | GAAATTTGAGGACAGACTG |
| Itgav    | GCCACAAAGACCCGTTAGGA | ACCAGACACCGGAGAAGT |
| Itgb3    | AGGGCAGTCTCTCTTGGTGT | CTTTGCTGCTGCTGCTT |
| Mmp2     | GACATCGGGAAGCAGCAAG | TGTGAACACATCCATCTGTG |
| Mmp12    | CACAGGAGAAATCTGAGTAC | AGGCAAGAAGGGAGACAG |
| Col1a1   | CGTGTACTGAGGATCTCCAGT | CAGAGAAGACAGGCTCAG |
| Col3a1   | CCACAGAAGATACGGACATC | TCCAGAGACAGGACTT |
| Col4a1   | AAATGGTACCTGGATCGTCAA | TCTGTGCAACTCCACGT |
| Fgfr10   | TCCGCTGACAGTCTCTCGGAGA | CACCGGCAAGGAGGGTT |
| Fgfr2    | CGCCAGCTCCTGCTGCTGCTT | AGGCTGGCTCCTCTGGT |
| Wnt2     | CCTGAGAAGAGCGTCAAC | GCCACTGCAACAGACT |
| Bmp4     | AGCCCGCTCTCAGCAGA | AAGGCTCAGAGAAGGTCAG |
| Shh      | CTCCGGAAGTCAAAGGAAACTCAC | GCCACTGTCATCACGAGAT |
| Hhip     | GGACCTCTATTGGAGTTGCAA | CGGGTCTGCGTGTGATG |
| Ptc1     | CTACAGGCAATAGGAAAGCAAGA | GAGAGGCTGAAGTGGGG |
| Ext1     | GTTGCCATTGTGAGGTTGCAC | TCTGTGCAACTGAGAGT |
| Col1a2   | CGATGTTGAACTCTGGGCTGAG | GGCAGCGGAGAGTTTT |
| Fn1      | AAGAGAAAGACAGAGCAATAAGA | TGGAGAGCATAGCAGACTT |
| Lama1    | GGTAGTATGACTCCTCATCAAAC | CAAGGCTGCAAGGAGAT |
| Lama3    | CTCAATGACCTCAGTCGAGA | TCTAAGACAGGATGG |
| Lama5    | TTGAGAAGATAGCAGATGTG | CGAAGATGAGGTAAGGAGA |
| Lamb1    | TCTGCTGAGTGTCACTGTA | GACACTGAGAAGGAGATG |
| Lamc1    | TGGCGGCAATGTGCTCACT | TGGCACTGTCACGAGCAT |
| Sdc1     | CTGGAGAAACAGCTTTCATC | ACAGCTCGGTATGTT |
| Sdc4     | ACCTCTGGAAGGCAGAATCT | GGCACGAAGGGCTCAG |
| Dag1     | GAGTGTAGCCTTCAGCAGT | CGAAGAAGAGGATGGT |
| Ctgf     | AAGGACGCCAGCAGCAGT | AGTGGTTCGCTGATG |
| Nid1     | AGAGCAACGGAGCCTTACAACAT | CCGGTAGCAGGACCTCCAT |
| Itga1    | CGGCTTCAGTGTCTATTTC | AGTACAGGCACCTGCCC |
| Itga2    | CGCAGAGAAGAGCTTACAT | CTCGGGATGTTTCCAC |
| Itga3    | AGAGACACATTGCCACAGA | CGCAGAGTTAAGGAGATG |

**Table S5.** Primers for quantitative real-time RT-PCR (qRT-PCR), related to Figure 1, 4, 5, 6, S1, S2, and S5.
| Gene  | Primer 1                        | Primer 2                        |
|-------|--------------------------------|--------------------------------|
| Itga6 | TCTCGTTCTTCGTTCCAGGTT          | GCAGCAGCGGTGACATCTA            |
| Itgb1 | TGGTCAGCAACGCATATCTG           | GTTACATTCCTCCAGCCAATCA         |
| Itgb4 | GACCAATGGCGAGATCACAG           | TCCACGAGCACCTTCTTCATA          |
| Snail1| GTCTGCAACGACCTGTGGAAA          | GGTCAGCAAAAGCACGGTTG           |
| Snail2| TCATCCTTGGGGCGTGTAAG           | GATGGCATGGGGGTCTGAAA           |
| Cdh1  | CAGCCGTTCTTTTGAGGGATT          | TGACGATGGTGAGCGGATG            |
| Cdh2  | ACAGGCAGCTTTACCGAAG            | CTTGAATCTGCTGGCTCGC            |
| Vim   | TTCTCTGGCACGTCTTGACC           | GCTTGGAACGTCCACATCG            |
**Transparent Methods**

**Reagents**
All reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise indicated. Primary antibodies used in this study are listed in Table S4.

**Animals**
*Adamts18* knockout (*Adamts18*−/−) and wildtype (*Adamts18*+/+) mice with the C57BL/6/129Sv background were generated and genotyped as previously described (Lu et al., 2017). Animals were maintained on a 12-h light/dark schedule (lights on at 06:00) in a specific pathogen-free facility. All procedures for animal experiments were approved by the Institutional Animal Care and Use Committee of East China Normal University (ECNU).

**RNA in situ hybridization**
RNA in situ hybridization (ISH) was performed as described previously (Zhu et al., 2019). Briefly, mouse lungs were fixed in 10% neutral buffered formalin for 24 h at room temperature (RT) and paraffin-embedded following standard methods. ISH was performed on 5-µm-thick sections using the RNAscope 2.5 HD Reagent Kit-RED (Advanced Cell Diagnostics, Hayward, CA). Specific probes were used to detect target mRNAs as described (Zhu et al., 2019).

**Quantitative Real-Time RT-PCR Analysis**
Quantitative real-time RT-PCR (qRT-PCR) was performed using the Step One Plus real-time PCR system (ThermoFisher, Carlsbad, CA) with SuperReal PreMix Plus (SYBR Green; TIANGEN). Primers used are listed in Table S5. The relative quantity of target mRNA was determined using the ΔΔCt method, with Gapdh as the reference gene. All reactions were performed in triplicates.

**Explant cultures**
Lung explant cultures were performed as previously described (Moral and Warburton, 2009). Briefly, E11.5 lungs were cultured on Nucleopore polycarbonate track-etch membranes (WHA-110414, Whatman) at 37°C in a 5% CO2 incubator for 72 h. For the rescue experiments, sense and antisense-phosphorothioated oligodeoxynucleotides (SODN and ASODN) were synthesized and added to the culture medium at the concentration of 0.5 µM for *Fbn1* or 1 µM for *Fbn2* (Kanwar et al., 1998; Yang et al., 1999). Sequences of the oligonucleotides are as follows: *Fbn1* sense ODN: 5’-GCCAGCGCGACCTCCAGCAGCCCTCCTCGCCGCAT-3’, *Fbn1* antisense ODN: 5’-ATGCGGCGAGGAGGGCTGCTGGAGGTCGCGCTGGC-3’, *Fbn2* sense ODN: 5’-CTCGGAGTATTTGCTGCTGCTGCGCTGGC-3’, *Fbn2* antisense ODN: 5’-GTCCGCAGGGCAGGACAGCAGGAAATACTCCGAG-3’. The culture medium was refreshed every 24 h. Images of explants were taken using an Mshot microscope with MS60 camera (Guangzhou, China). The number of branches was counted manually, and the length of each airway was calculated by the software Image Pro
Plus 6.0 (IPP, Media Cybernetics, Inc., Silver Spring, MD, USA).

**Lung cast**
Mice were euthanized by CO₂ asphyxiation. The trachea was exposed just below the larynx, and a catheter was inserted and securely tied with braided silk surgical suture. The lungs were inflated with casting agent (90 ml ethyl acetate, 10 g polyvinyl chloride, 2.7 ml dibutyl phthalate, and appropriate amount of oil paints), separated from trachea, and transferred to a 60°C oven. After the casting agent was solidified, the lungs were immersed in 50% HCl to remove tissues (only airways left). Airways were imaged using an Mshot microscope.

**Histology, immunohistochemistry, and immunofluorescence**
Lung tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. After dewaxing and rehydration, hematoxylin and eosin (HE) staining or Hart’s staining was performed on 5-μm sections to determine radial alveolar counts (RAC) and elastin distribution. Immunohistochemical staining of sections was performed using anti-CD11b, anti-histone H3, anti-MPO, anti-FBN1, anti-FBN2, anti-PFAK (Tyr397), anti-FAK, anti-TMSB10, anti-FGF10, and anti-FGFR2 antibodies. For immunofluorescence examination, 48 h after transfection, the cells were fixed with pre-cooled ethanol for 20 min. The samples were incubated with anti-ddk (or anti-myc), anti-fibrillin1, and anti-fibrillin2 antibodies overnight at 4°C, followed by incubation with the secondary antibody. Samples were counterstained with DAPI (MP, Carlsbad, CA) and imaged with a Leica SP8 confocal microscope (Leica Microsystems, Wetzlar, Germany).

FITC-phalloidin staining
E14.5 lung tissues were obtained, embedded in optimal cutting temperature (OCT) compound, and cut into 10-μm thick sections with a Leica CM3050 S cryostat microtome (Leica Biosystem, Wetzlar, Germany). The sections were fixed for 15 min with 4% formaldehyde in PBS and permeabilized with 0.5% triton X-100 in PBS for 20 min, followed by incubation with 0.1 mg/ml FITC-phalloidin diluted in PBS containing 1% BSA at 37°C for 2 h and counterstaining with DAPI. After mounting on slides, the sections were examined with a Leica SP8 confocal microscope.

**Lung function examination**
Mice were anesthetized with a mixture of urethane (14% m/v), alpha-chloralose (0.7% m/v), and sodium tetraborate decahydrate (0.7% m/v) in saline and connected to a computer-controlled ventilator via the tracheal cannula. After normal respiratory movements were recorded, mice were mechanically ventilated with room air at 110 breaths per min with the expiration/inspiration ratio of 20:10. Pulmonary function tests were performed using the AniRes2005 Lung Function System (Bestlab Technology Co., Ltd).

**LPS-induced acute lung injury**
Lipopolysaccharide (LPS) was diluted to a final concentration of 1 mg/ml in normal saline. Anesthetized mice were injected intraperitoneally (I.P.) with LPS at 10 mg/kg. Lung tissues were collected at different time points (0 h, 3 h, 6 h, 12 h, and 24 h) after LPS injection. Pathological grade of lung injury was defined as follows: Grade 0, normal alveoli, alveolar septum, and bronchi; Grade 1, partial alveolar septal congestion; Grade 2, moderate alveolar septal congestion and intra-alveolar hemorrhage; Grade 3, severe congestion and bleeding in alveolar septum and alveoli. Frozen lung tissues (around 30 mg) were homogenized to measure IL-6 using a commercially available ELISA kits (LYBD Bio-Technique Co, Ltd, Beijing, China), and data were normalized to protein concentration measured by the bicinchoninic acid (BCA) method.

**Bronchoalveolar lavage**

Mice were sacrificed by CO₂ asphyxiation and bronchoalveolar lavaged 3 times, each with 0.8 ml of PBS via a 22G catheter. The bronchoalveolar lavage fluid (BALF) was centrifuged at 400 xg for 7 min at 4°C, and the pellet was resuspended in 20 μl PBS containing 1% BSA. The resuspended cells were placed on a slide, dried, stained with Diff-Quick (Solarbio Life Science, Beijing, China), and counted under a microscope at 100x magnification.

**Bleomycin-induced lung fibrosis**

Mice were anesthetized and intratracheally instilled with 100 μl of saline or bleomycin sulfate dissolved in saline (1 mg/kg body weight), followed with 300 μl of air to ensure delivery to the distal airways. Mouse mortality was monitored for 25 days after bleomycin injection. To assess lung fibrosis, mice were sacrificed, and lung tissues were examined microscopically at 100 x magnification. The severity of fibrosis was determined according to the method of Ashcroft et al (Ashcroft et al., 1988) and scored as follows: grade 0, normal lung; grade 1, minimal fibrous thickening of alveolar or bronchiolar walls; grade 3, moderate thickening of walls without obvious damage to lung architecture; grade 5, increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses; grade 7, severe distortion of structure and large fibrous areas; grade 8, total fibrous obliteration.

**Proteomic analysis of embryonic lungs**

Whole lung tissues from E14.5 embryos were digested with sequencing grade trypsin (Promega) and fractionated with high PH reversed phase chromatography. The data-independent acquisition (DIA) analysis was performed on an Orbitrap Fusion LUMOS mass spectrometer (Thermo Fisher Scientific) connected to an Easy-nLC 1200 via an Easy Spray (Thermo Fisher Scientific). The DIA raw files were analyzed in Spectronaut X (Biognosys, Schlieren, Switzerland). Pathway enrichment analysis was performed with MetaScape (http://metascape.org/).
Transmission electron microscopy (TEM)
Distal parts of E14.5 lungs were fixed with 2.5% glutaraldehyde at 4°C, followed by fixation with osmium tetroxide, dehydration in alcohol, embedding in plastic, ultra-thin sectioning, flotation of the sections on aqueous medium, and staining with uranyl acetate and lead acetate. Images were taken with a Tecnai G2 Spirit BioTWIN transmission electron microscope (FEI, Hillsboro, Oregon).

ADAMTS18 and fibrillin1 (FBN1) expression
The plasmid for expression of Myc-DDK tagged mouse full-length Adamts18 (pCMV6-Adamts18, the ORF clone with sequence NM_172466 in pCMV6-Entry) was purchased from Origene (Rockville, MD). Plasmid DNA was introduced into HEK293T cells by transfection using Lipofectamine 2000 (Invitrogen/Life technologies, Carlsbad, CA).

Co-Immunoprecipitation (IP)
Mouse dermal fibroblast cells (DFCs) and HEK293T cells were co-cultured and transiently transfected with pCMV6-Adamts18 or empty vector. At 48 h, the cells were washed once with PBS and lysed in NP-40 buffer (50 mM Tris-base pH 7.5, 150 mM NaCl, 1% NP-40, and protease inhibitors). Cell lysates were incubated with anti-DDK agarose (TA150037, Origene, Rockville, MD) at 4°C overnight and washed with 1% NP40 washing buffer. The proteins eluted from the agarose beads were resolved by SDS-PAGE and analyzed by Western blotting with anti-fibrillin 1 C-terminal, anti-fibrillin 2, and anti-DDK (DYKDDDDK) primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibody. The immunoreactive bands were visualized with enhanced chemiluminescence (ECL) Western blot kit (Millipore, Boston, MA).

RhoA Activity
RhoA activity was detected using the Rho Activation Assay Biochem Kit (Cytoskeleton, Inc, Dencer, CO). Briefly, E15.5 lung tissues were isolated and homogenized in the lysis buffer. The cell lysate thus obtained was adjusted to 1 mg/ml of protein and then incubated with rhotekin-RBD beads at 4°C for 1 h. RhoA was then eluted from the rhotekin-RBD beads and analyzed by Western blotting with anti-RhoA antibody included in the kit.

Statistics
Data were analyzed by Student’s t test or Log-rank (Mantel-Cox) test using the software package Prism version 7 (GraphPad, La Jolla, CA, USA). Data are shown as mean ± SEM or mean ± SD. A p value < 0.05 was considered statistically significant.

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