Supplemental Figures and Legends:

A

Con BMP4 TGFβ1

Fibronectin — 220 kDa

β-Actin — 42 kDa

Fibronectin Rel β−Actin

B

☐ Control
☐ BMP4 (green)
☐ TGFβ1

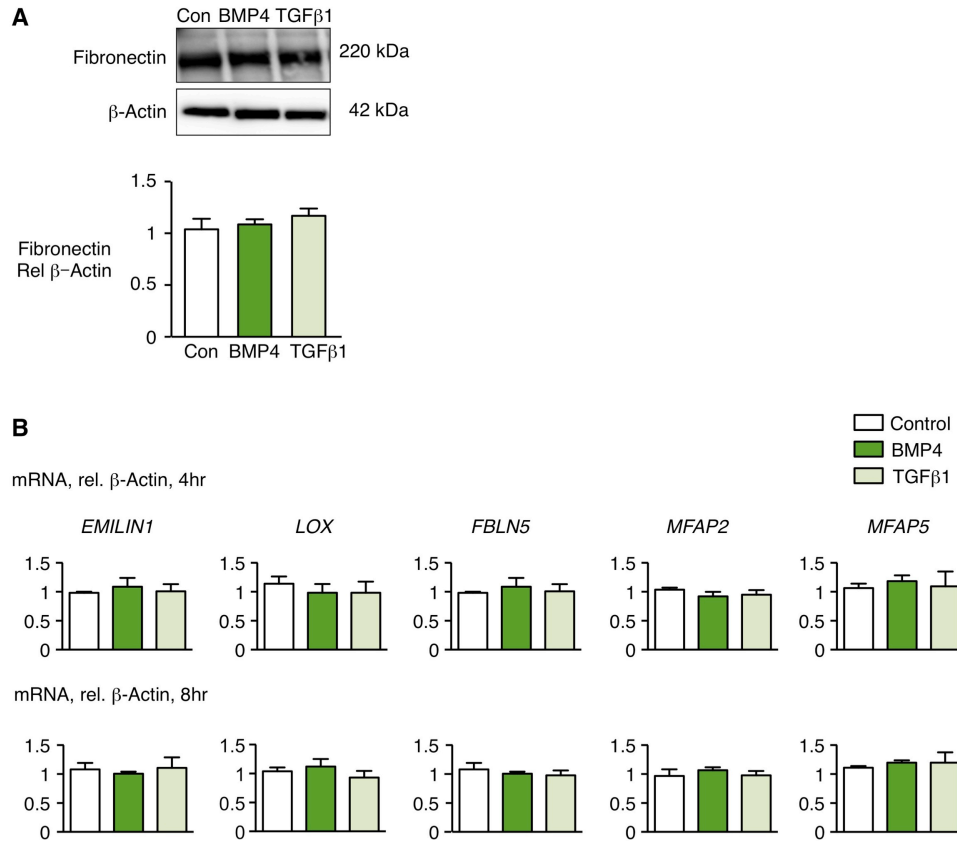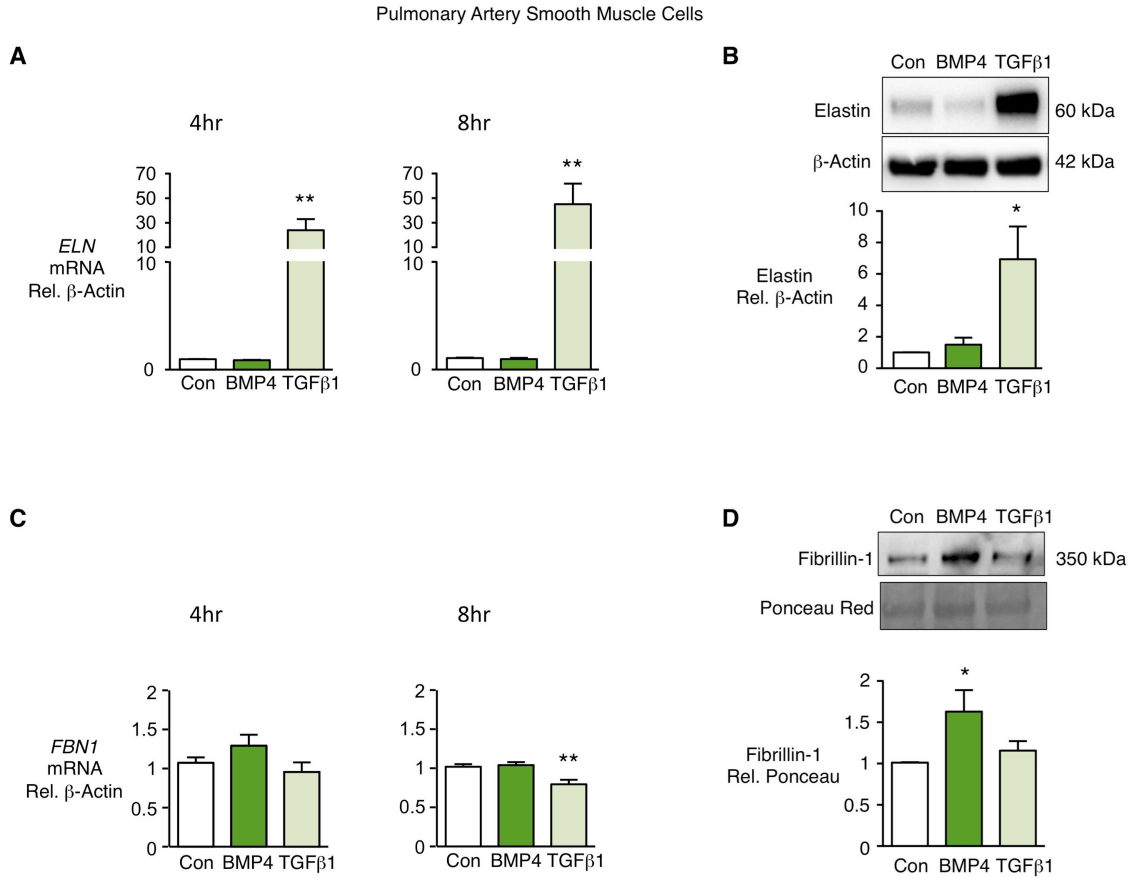mRNA, rel. β-Actin, 4hr

EMILIN1   *LOX*   *FBLN5*   *MFAP2*   *MFAP5*

mRNA, rel. β-Actin, 8hr

Supplemental Figure I: TGFβ1 and BMP4 do not affect fibronectin protein levels, nor mRNA levels of elastic fiber-associated proteins emilin-1, lysyl oxidase, fibulin-5, MAGP1 and MAGP2

Pulmonary artery fibroblasts (PAF) from an unused donor control lung between passages 3-6 were cultured and stimulated with BMP4 (10ng/ml) or TGFβ1 (2ng/ml), as described in Figure 1. **(A)** Fibronectin protein levels, measured 48h after stimulation. **(B)** mRNA of emilin-1 (*EMILIN1*), lysyl oxidase (*LOX*), fibulin-5 (*FBLN5*), MAGP1 (*MFAP2*), MAGP2 (*MFAP5*) relative to β-actin were measured four and eight hours following stimulation. Bars represent Mean±SEM of n=3 independent experiments.

Pulmonary Artery Smooth Muscle Cells

A

4hr

ELN mRNA Rel. β-Actin

**

8hr

**

B

Con BMP4 TGFβ1

Elastin 60 kDa

β-Actin 42 kDa

Elastin Rel. β-Actin

*

C

4hr

FBN1 mRNA Rel. β-Actin

8hr

**

D

Con BMP4 TGFβ1

Fibrillin-1 350 kDa

Ponceau Red

Fibrillin-1 Rel. Ponceau

*

Supplemental Figure II: TGFβ1 increases elastin mRNA and protein and BMP4 increases extracellular fibrillin-1 in human pulmonary artery smooth muscle cells (SMC).

Human pulmonary artery smooth muscle cells (SMC) isolated from unused donor lungs and used between passage 3-6 were stimulated with BMP4 (10ng/ml) or TGFβ1 (2ng/ml) or vehicle (Con). **(A, C)** Fold change in mRNA of *ELN* and *FBN1* was measured four and eight hours after stimulation. **(B, D)** Representative immunoblots above and densitometry below for elastin in cell lysates and fibrillin-1 in conditioned media in 48h after stimulation. Beta-actin and Ponceau staining were used as loading controls for cell lysates and conditioned media respectively. Values are normalized to Con. Bars represent Mean±SEM of n=3-5 independent experiments, *p<0.05, **p<0.01 vs. Con, by one-way ANOVA and post-hoc Bonferroni test.
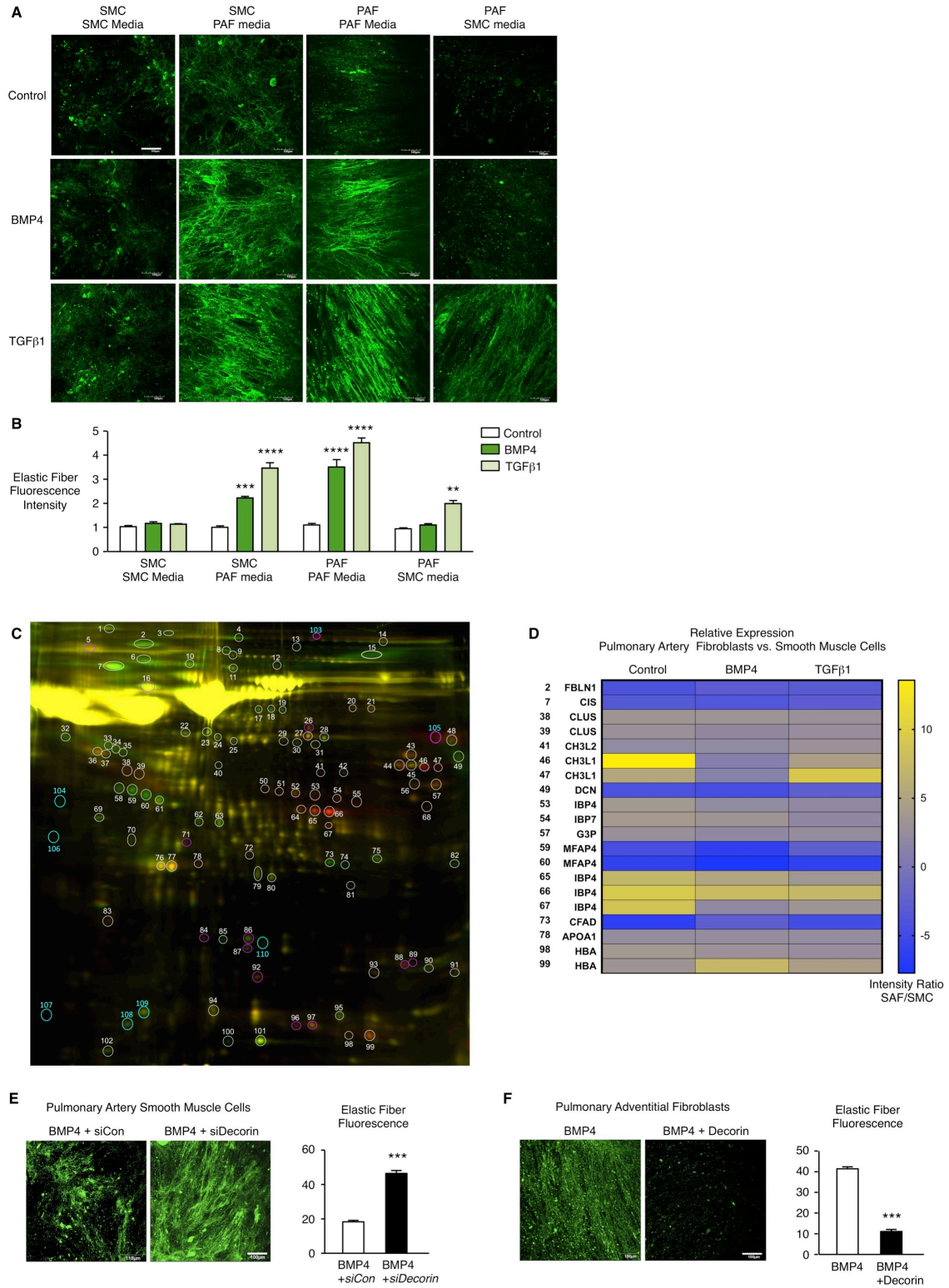
Supplemental Figure III: PAF show reduced fiber formation following exposure to SMC media, while SMC show improved fiber formation following exposure to PAF media.
(Legend on the following page)

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*(Figure on the previous page)*

(A) Human PAF and SMC from unused donor lungs were cultured with BMP4 (10ng/ml) or TGFβ1 (2ng/ml) for 24 hours and the conditioned media was added from PAF to SMC and from SMC to PAF respectively for six days. Scale bar=100 μm. **(B)** Elastin positive fibers were visualized and quantified as previously described in figure 2. Bars represent Mean ±SEM of n=3. **p<0.01, ***p<0.001, ****p<0.0001 vs. Donor, unstimulated, by two-way ANOVA and post-hoc Bonferroni test.

(C) Human PAF and SMC from unused donor lungs were cultured with BMP4 (10ng/ml) or TGFβ1 (2ng/ml) for 24 as described in Figure 2 and Supplemental Figure II. A minimum of 0.3 mg of total protein was recovered and sent to Applied Biomics (Hayward, CA) for 2D DIGE analysis. The gel was run as follows: Cy2: Internal Standard (equally mixed samples); Cy3: SMC; Cy5: PAF. The figure shows an overlap of the 3 dyes. The data was analyzed using ImageQuant software and DeCyder 2D software to find significantly changed spots (analysis performed by Applied Biomics). Following this step, the images were analyzed and 20 spots were picked for mass-spectrometry identification. **(D)** Heat map of the 20 selected spots following mass-spectrometry, showing the fold-increase between treatments.

(E) SMC of donor controls were stimulated with BMP4 and transfected with siRNA oligonucleotides targeting decorin (DCN) *(siDecorin)* or with non-targeting siRNA (*siControl*) as described in Figure 2. Decorin inhibition increased BMP4-related elastic fiber formation. Elastic fibers were visualized by indirect immunofluorescence of elastin and quantified by Image J software. Scale bar=100μm. Bars represent Mean±SEM of n=3 independent experiments, ***p<0.001 vs. non-targeting siRNA (*siControl*) by two-way ANOVA and post-hoc Bonferroni test.

(F) PAF of donor controls were treated with BMP4 (10ng/mL) alone or with BMP4 (10ng/mL) plus decorin peptide (100μM, ab71694, Abcam) every other day for seven days as described in Figure 2. Addition of decorin peptide improved SMC-dependent elastic fiber formation. Scale bar=100μm. Bars represent Mean±SEM of n=3 independent experiments, ***p<0.001 vs. BMP4 plus decorin peptide, by two-way ANOVA and post-hoc Bonferroni test.
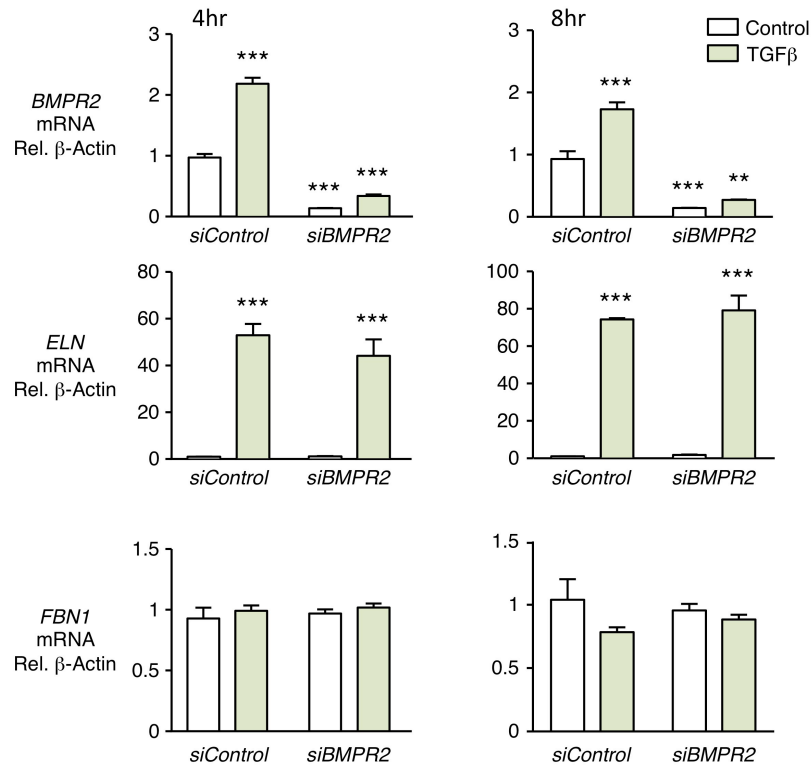
Supplemental Figure IV: TGFβ1 increases elastin mRNA despite a loss of BMPR2.

Human pulmonary artery fibroblasts (PAF) isolated from unused donor lungs and used between passage 3-6 were transfected with non-targeting siRNA *(siControl)* or with siRNA targeting BMPR2 (*siBMPR2)*. After recover for 24 hours, the cells were starved overnight, then stimulated with vehicle (Control) or TGFβ1 (2ng/ml). Fold-change in mRNA of *ELN* and *FBN1* (relative to siControl, unstimulated), was measured four and eight hours after stimulation. Bars represent Mean±SEM of n=3, **p<0.01, ***p<0.001 vs. Con, by one-way ANOVA and post-hoc Bonferroni test.
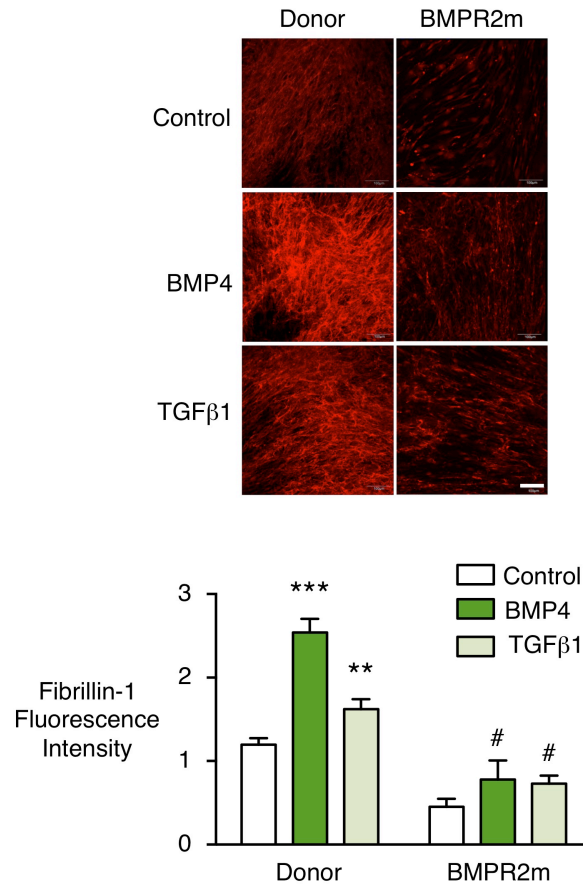
Supplemental Figure V: BMPR2m PAF show reduced fibrillin-1 in response to BMP4 and TGFβ1

Human PAF from unused donor lungs and from lungs of HPAH patients with a *BMPR2* mutation (BMPR2m) were cultured with BMP4 (10ng/ml) or TGFβ1 (2ng/ml) for 6 days. Fibrillin-1-positive fibers were visualized and quantified as described in Figure 2. Scale bar=100 μm. Bars represent Mean ±SEM of n=3. **p<0.01, ***p<0.001 vs. Donor, unstimulated; #p<0.05, BMPR2m vs. Donor, same treatment by two-way ANOVA and post-hoc Bonferroni test.
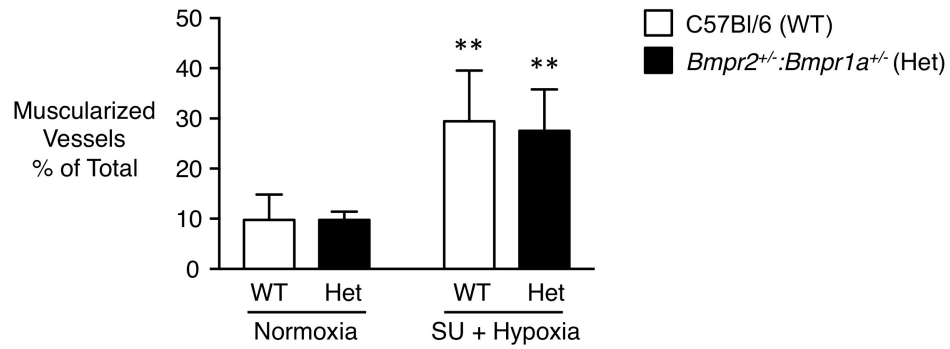
Supplemental Figure VI: Muscularization of distal pulmonary arteries

Bmpr2/1a mice (Het) and littermates controls (WT) were exposed to room air (normoxia) or 10% O_2 (hypoxia) with s.c injection of the VEGF receptor blocker Sugen 5416 (SU) once weekly for three weeks, as described in "Methods". Bars represent Mean±SEM of n=5-7 mice for normoxia and n=6-7 mice for SU + hypoxia.