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Supplementary Materials for

ACTRIIA-Fc rebalances activin/GDF versus BMP signaling in pulmonary hypertension

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Fig. S15. Treatment with ACTRIIA-Fc improves echocardiographic measures of RV function and PH after SU-Hx exposure.
Fig. S16. ACTRIIA-Fc increases apoptosis in small vessels of severe obliterative PH.
Fig. S17. ACTRIIA-Fc normalized *p*-SMAD2/3 expression in the severe obliterative SU-Hx rat model.
Fig. S18. ACTRIIA-Fc attenuated expression of Pai-1 (*Serpine1*), activin A (*Inhba*), E-selectin (*Sele*), and P-selectin (*Selp)* mRNA in the severe obliterative SU-Hx rat model.
Fig. S19. Treatment of rats with ACTRIIA-Fc in the severe obliterative SU-Hx model did not affect red cell mass.
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Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/12/543/eaaz5660/DC1)

Data file S1 (Micorosoft Excel format). Raw data for main figures.
Data file S2 (Micorosoft Excel format). Raw data for supplementary figures.

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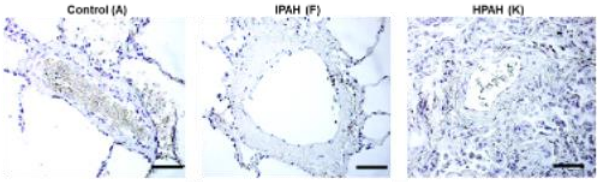
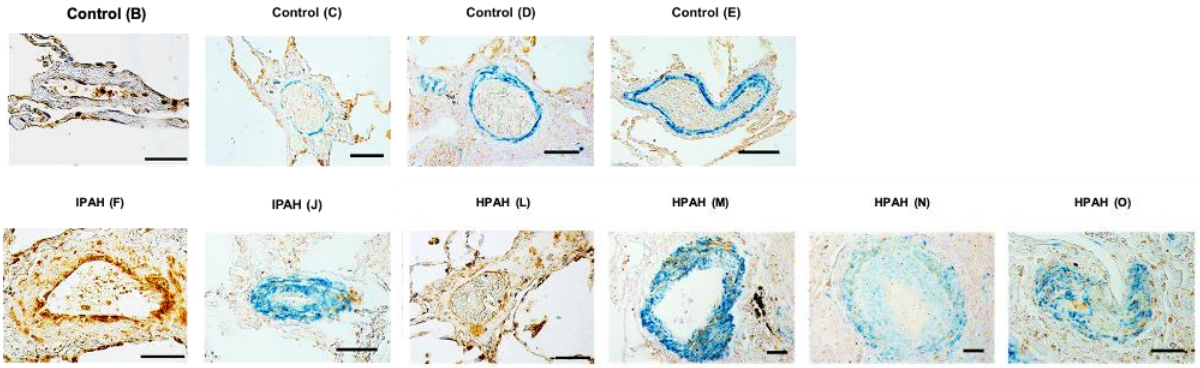


activin A



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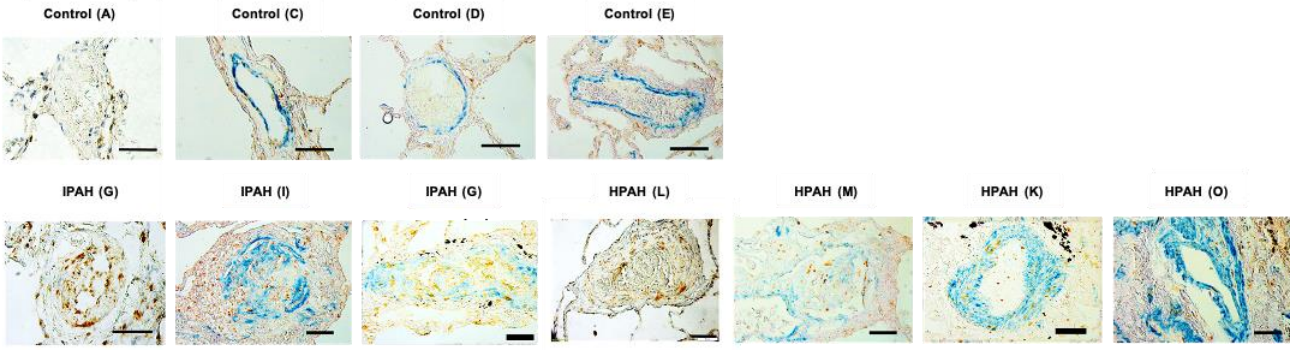

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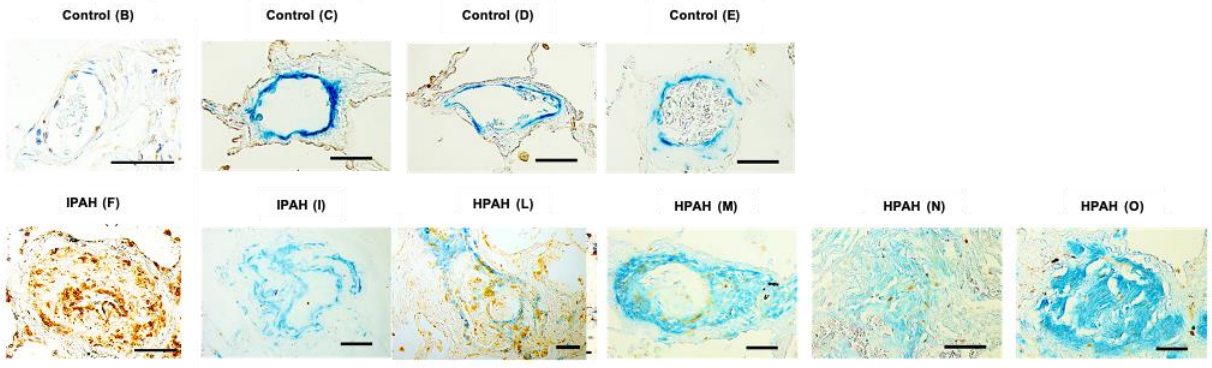

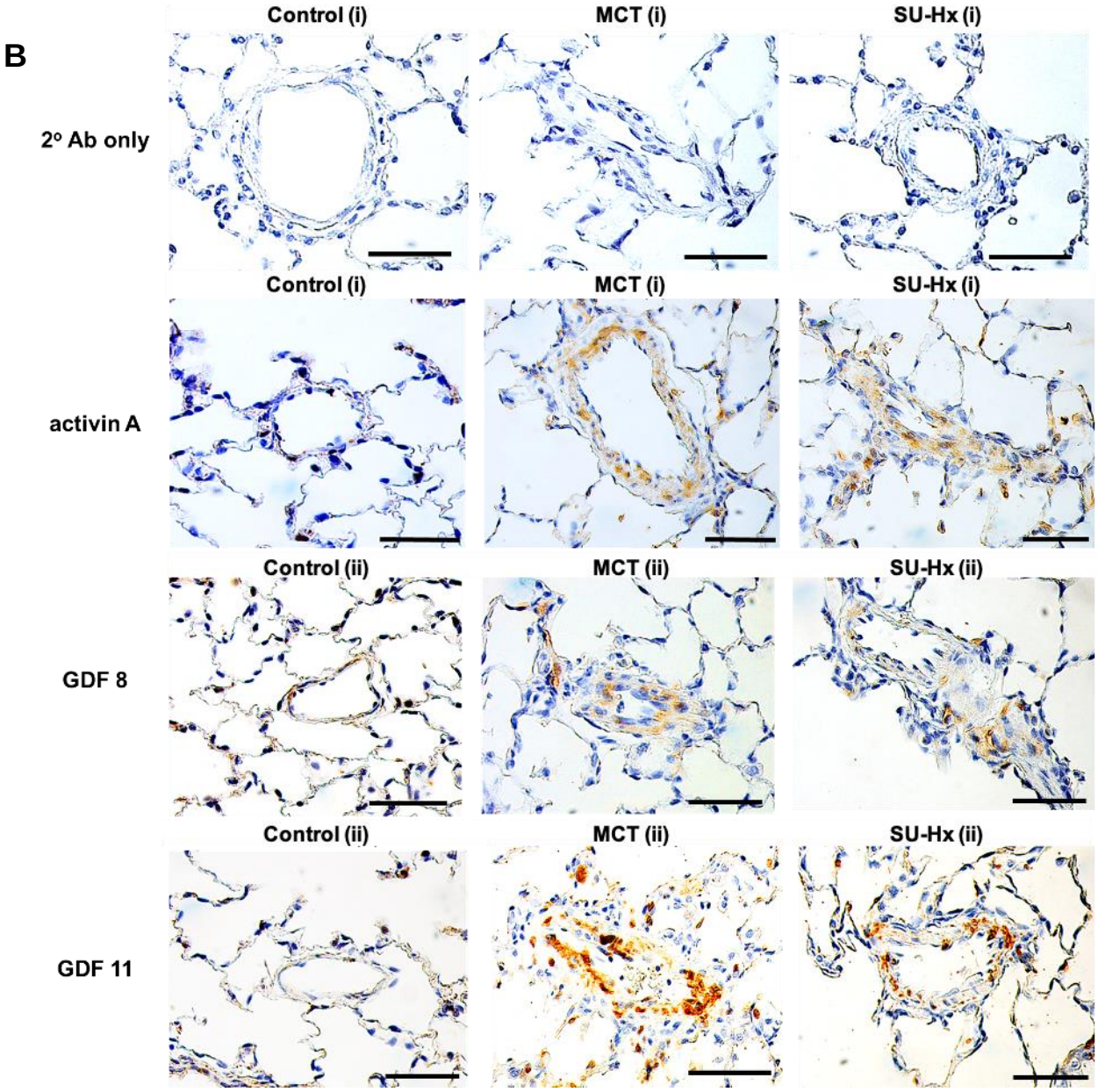

Fig. S1. Specificity of immunohistochemistry for activin A, GDF 8, and GDF 11 in human and rat lung tissue. Staining of paraffin embedded tissue sections with HRP-conjugated anti-goat IgG in normal and diseased **(A)** human and **(B)** rat tissues (top panels), in comparison to specific immunostaining for activin A, GDF8, and GDF11 seen with the addition of primary antibody in pulmonary vascular lesions of HPAH and IPAH human lung tissues, and MCT- and SU-Hx-treated rats. Samples are labeled (A-O) to represent distinct human explants (*n* = 15) or labeled (i-iii) to represent distinct rats (*n* = 3). Scale bars = 50 µm.

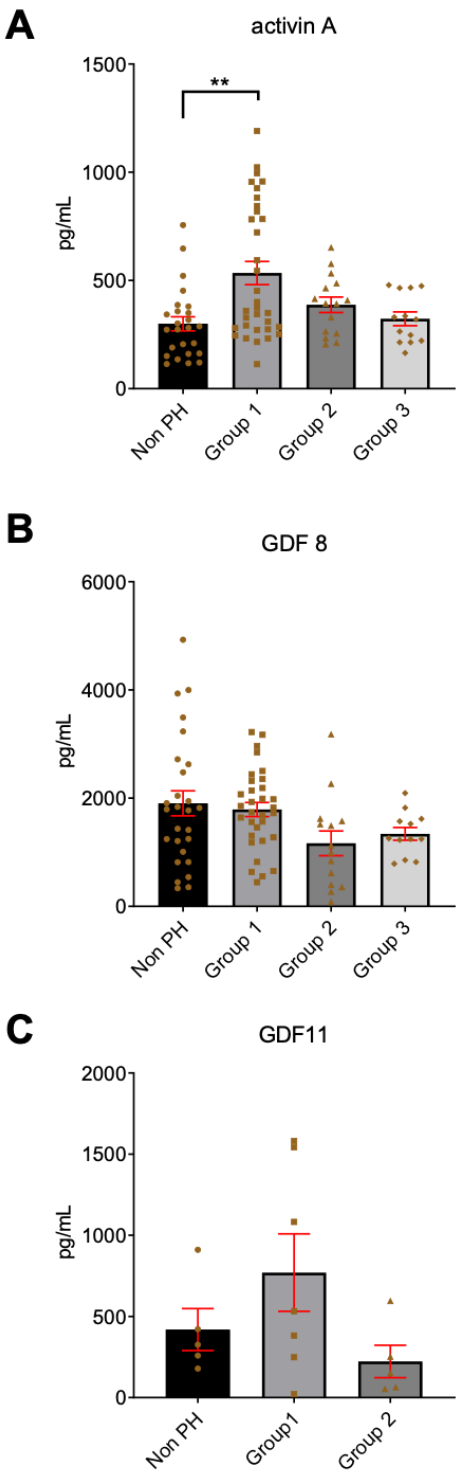

Fig. S2. Circulating activin A is elevated in WHO Group 1 PAH. Serum **(A)** activin A, **(B)** GDF8, and **(C)** GDF11 in samples obtained from a series of 135 patients including controls (no PH), Group 1 PAH, Group 2 PH, or Group 3 PH. Values are shown as mean ± SEM, *n* = 5-40 per group, ***P* < 0.01, one-way ANOVA with Dunnett's test for multiple comparisons.

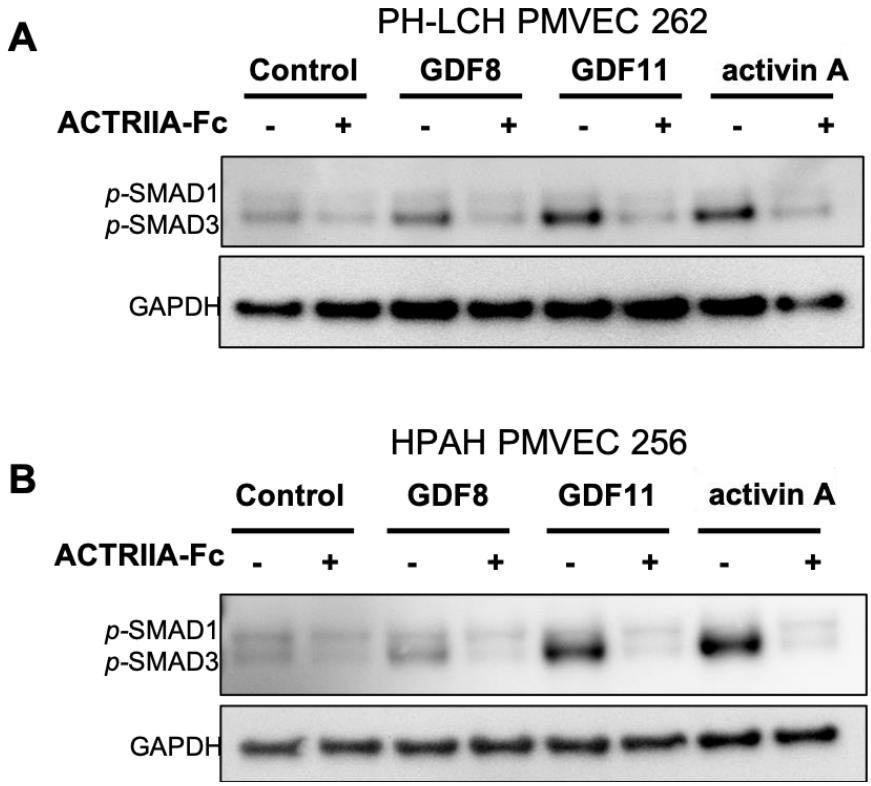

Fig. S3. ACTRIIA-Fc modulates GDF8-, GDF11-, activin A–, and activin B–mediated signaling in microvascular endothelial and PASMCs isolated from PH donors. (A) Western blot analysis of phosphorylation of SMAD1 and SMAD3 in human PMVECs isolated from a donor with Group 5 PH due to Langerhans Cell Histiocytosis (PH-LCH, donor 262) treated with GDF8 (50 ng/mL), GDF11 (50 ng/mL), activin A (50 ng/mL), and activin B (50 ng/mL) for 30 min with or without treatment with ACTRIIA-Fc (2500 ng/mL). **(B)** Western blot analysis of phosphorylation of SMAD1 and SMAD3 in human PMVECs isolated from a donor with *BMPR2*-mutation positive HPAH (donor 256) treated with GDF8 (50 ng/mL), GDF11 (50 ng/mL), activin A (50 ng/mL), and activin B (50 ng/mL) for 30 min with or without treatment with ACTRIIA-Fc (2500 ng/mL). GAPDH serves as loading control. These results are representative of 4 independent experiments performed with 4 distinct cell donors.

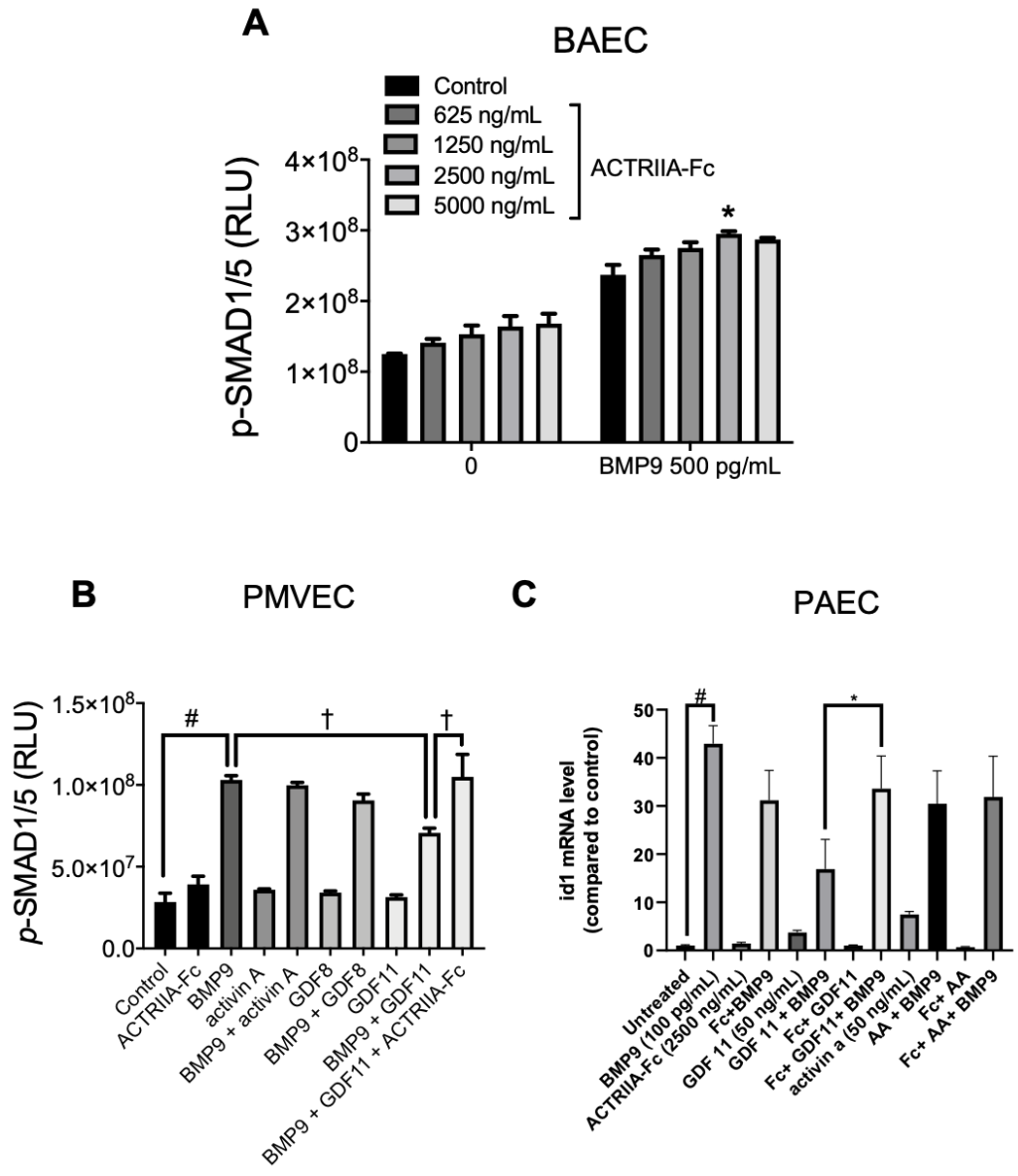

Fig. S4. ACTRIIA-Fc enhances BMP9-induced signaling and blocks inhibition of BMP9 signaling by GDF11 in multiple endothelial cell lineages. (A) The activation of SMAD1/5 was analyzed in bovine aortic endothelial cells (BAECs) treated with or without BMP9 (500 pg/mL) with varying concentrations of ACTRIIA-Fc by In-Cell Western. **(B)** The activation of SMAD1/5 was analyzed by In-Cell Western in PMVECs co-treated with GDF11 (50 ng/mL) and BMP9 (500 pg/mL) in comparison to treatment with BMP9, with or without ACTRIIA-Fc (2500 ng/mL). Data from (A-B) represent mean ± SEM. **P* < 0.05 ***P* < 0.01, †*P* < 0.001, #*P* < 0.0001 as indicated, one-way ANOVA with Sidak's or Tukey's test for multiple comparisons (*n* = 3-6). **(C)** Expression of *Id1* mRNA in human pulmonary aortic endothelial cells (PAECs) co-treated with GDF8, GDF11, and activin A (50 ng/mL each) or treated with BMP9 (500 pg/mL), with or without ACTRIIA-Fc (2500 ng/mL). Values are shown as mean ± SEM, n=3-4 per group. *p<0.05, **p<0.01, †p<0.001, #p<0.0001 in comparison to without ACTRIIA-Fc treatment, two-way ANOVA with Dunnett's multiple comparisons test. These data are representative of 4 independent experiments performed using 3 distinct PMVEC or PAEC donors.

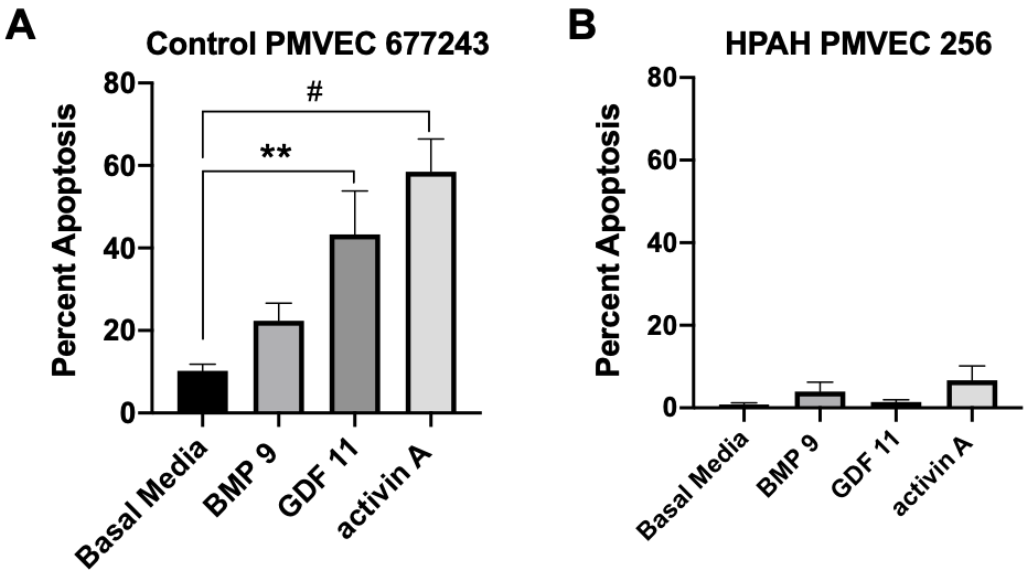

Fig. S5. Differential basal and ligand-modulated apoptosis in PMVECs isolated from healthy and PH donors. (**A**) Apoptosis analyzed by TUNEL and DAPI staining in control PMVECs (donor 677243) and (**B**) HPAH HPMVECs (donor 256) cultured in basal media (0.5% FBS) with or without the addition of BMP9 (1 ng/mL), GDF11 (100 ng/mL), or activin A (100 ng/mL) for 48 hours in chamber slides. The basal frequency of apoptotic cells was higher in control than HPAH-derived PMVECs (p=0.003). Data represent mean ± SEM. $^{*}P < 0.05$ $^{**}P < 0.01$, $^{\dagger}P < 0.001$, $^{\#}P < 0.0001$ as indicated, one-way ANOVA with Sidak's or Tukey's test for multiple comparisons (n=3-6). These data are representative of 3 independent experiments performed with 3 distinct control and PAH-derived PMVEC isolates.

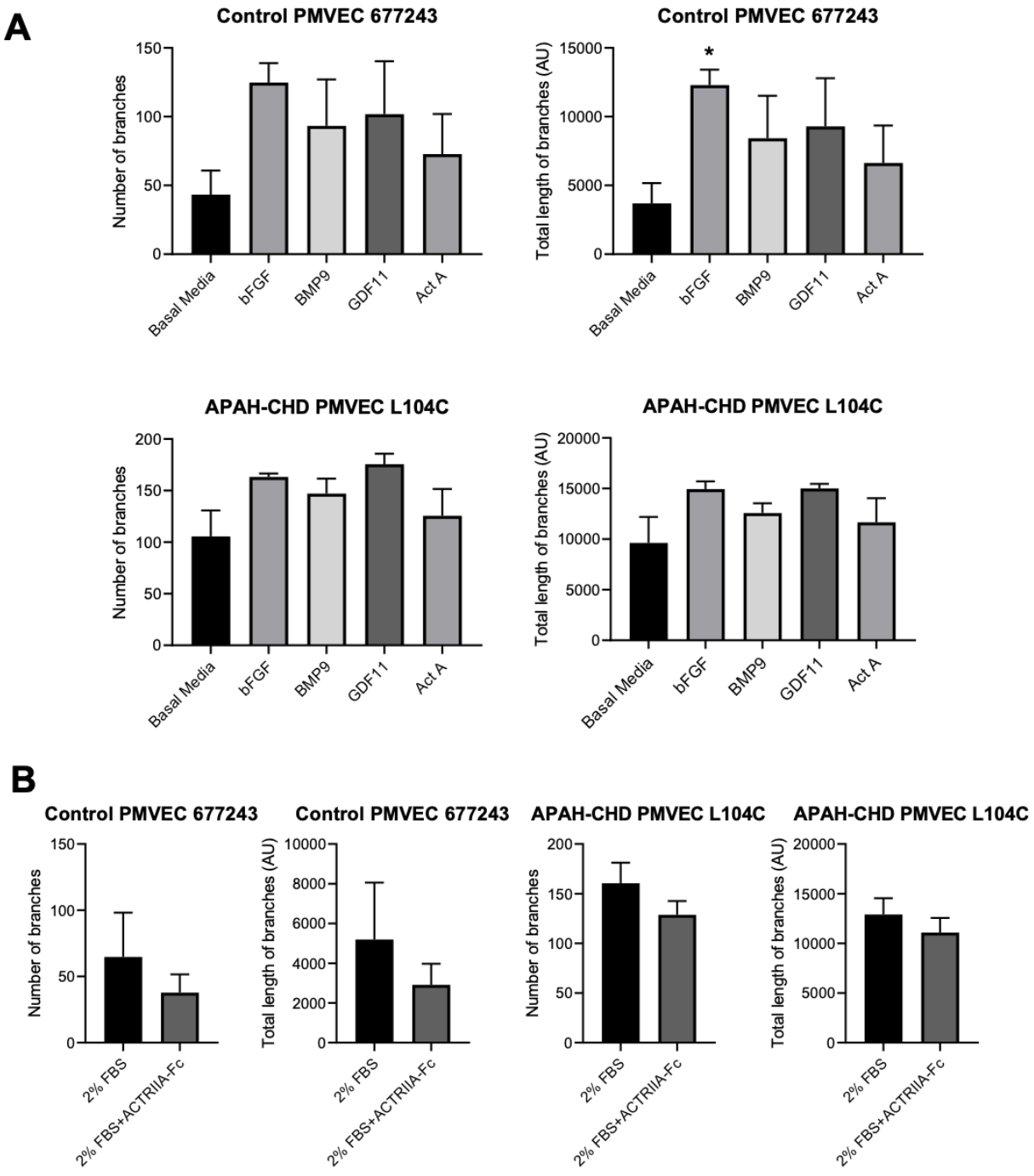

Fig. S6. No significant impact of ACTRIIA-Fc or its ligands on endothelial tube formation in PMVECs from control and PAH donors. (A) Control and PAH-derived PMVECs were plated in Geltrex matrix-coated wells and treated with VEGF (10 ng/mL), bFGF (10 ng/mL), BMP9 (1 ng/mL), GDF11 (100 ng/L), GDF8 (100 ng/mL), activin A (100 ng/mL), or activin B (100 ng/mL) in basal media (0.5% FBS) and numbers of branches and branch lengths quantified at 14 h using the ImageJ Angiogenesis Analyzer suite. **(B)** Control and PAH-derived PMVECs were treated with 2% FBS in basal media with or without the addition of ACTRIIA-Fc (2500 ng/mL), and branches and branch lengths quantified at 14 h. Data represent mean ± SEM, n=4. **P* < 0.05 as indicated vs. basal media, Brown-Forsythe and Welch ANOVA. These results are representative of two independent experiments using two distinct control or PAH donor-derived cells each.

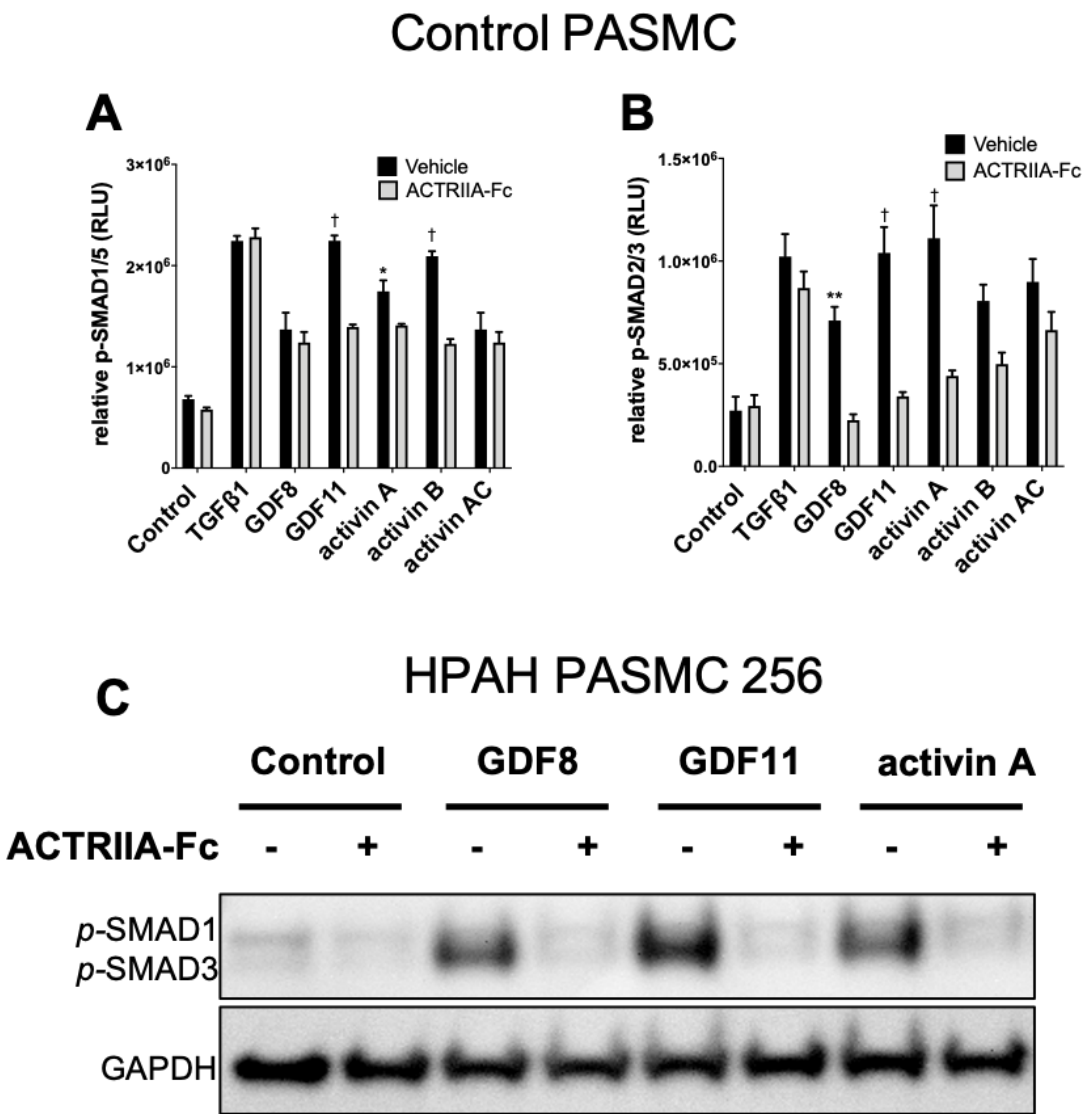

Fig. S7. Impact of ACTRIIA-Fc on ligand-mediated SMAD signaling in control and PAH-derived PASMCs. (**A**) Activation of SMAD1/5 in human PASMCs exposed to GDF11 (100 ng/mL), activin A (100 ng/mL), activin B (100 ng/mL), TGFβ1 (1.25 ng/mL), GDF8 (100 ng/mL), and activin AC (100 ng/mL) in the presence or absence of ACTRIIA-Fc (2500 ng/mL) analyzed by In-Cell Western. (**B**) Activation of SMAD2/3 in human PASMCs under the same conditions as in (A). For (A-B), all values are shown as mean ± SEM, n=3-8 per group. **P* < 0.05, ***P* < 0.01, †*P* < 0.001 in comparison with ACTRIIA-Fc treatment, one-way ANOVA with Sidak's test for multiple comparisons. (**C**) Western blot showing activation of SMAD1 and SMAD3 in HPAH-derived PASMCs (donor 256) treated with GDF8 (50 ng/mL), GDF11 (50 ng/mL), and activin A (50 ng/mL) with or without ACTRIIA-Fc (2500 ng/mL). These results are representative of 3 independent experiments performed with 3 distinct control or PAH donor derived PASMC isolates.

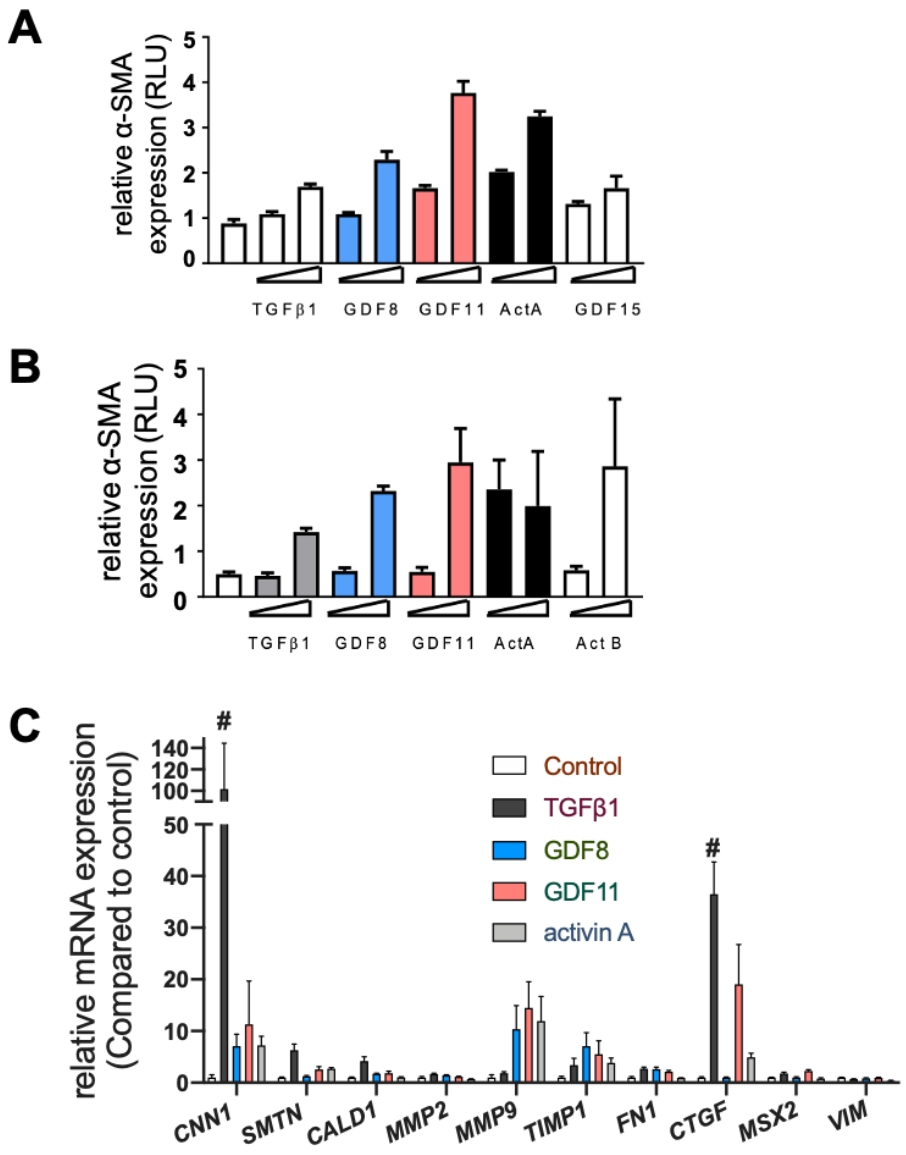

Fig. S8. Modulation of smooth muscle phenotypic genes by BMP, GDF, activin, and TGFβ ligands in human PASMCs. (A-B) The expression of α-SMA was assayed by In-Cell Western in PASMCs following 72 h treatment with varying concentrations of TGFβ1 (0.25 and 2.5 ng/mL), GDF8 (25 and 100 ng/mL), GDF11 (25 and 100 ng/mL), activin A (25 and 100 ng/mL), activin B (25 and 100 ng/mL) and GDF15 (25 and 100 ng/mL). The ability of GDF8, GDF11, and activin A to induce the expression of α-SMA was consistent across multiple isolates of human PASMCs obtained from distinct donors. **(C)** Expression of smooth muscle phenotypic genes known to be regulated by TGFβ1 in PASMCs following exposure to GDF8, GDF11 and activin A. For **A-C**, all values are shown as mean ± SEM, n=3-8 per group. **P* < 0.05, ***P* < 0.01, †*P* < 0.001, $^{\#}$*P* <0.001 in comparison with control, one-way ANOVA with Sidak's test for multiple comparisons.

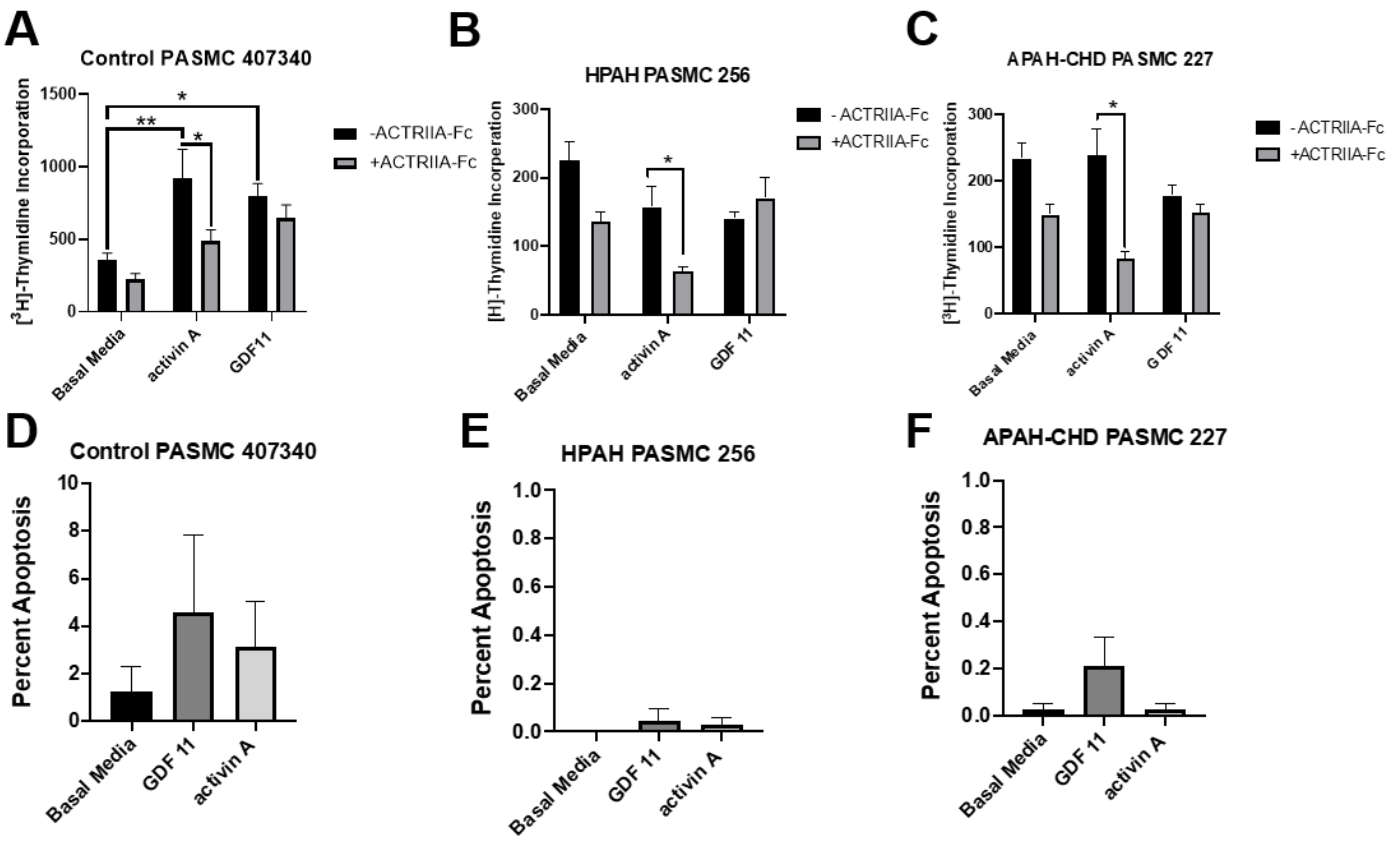

Fig. S9. Impact of ACTRIIA-Fc on proliferation and apoptosis in PASMCs isolated from both control and PH donors. (**A**) Control PASMCs (donor 407340), (**B**) HPAH PASMCs (donor 256), and (**C**) APAH-CHD PASMCs (isolated from donor 227) were deprived of serum for 24 h and treated with GDF11 (100 ng/mL), activin A (100 ng/mL), or complete media with or without ACTRIIA-Fc (2500 ng/mL) for 48 h, and proliferation measured by ^{3}H-thymidine incorporation during the last 6 h of culture. Data represent mean ± SEM. **P* < 0.05 ***P* < 0.01, †*P* < 0.001, $^{\#}$*P* < 0.0001 as indicated, one-way ANOVA with Sidak's or Tukey's test for multiple comparisons (n = 3-6). (**D**) Control PASMCs (donor 407340), (**E**) HPAH PASMCs (donor 256), and (**F**) APAH-CHD PASMCs (donor 227) were cultured in basal media with or without exogenous GDF11 (100 ng/mL) or activin A (100 ng/mL) for 48 h in chamber slides and apoptotic cells were measured by TUNEL and DAPI staining. Data represent mean ± SEM (n = 3-6). These data are representative of 3 independent experiments performed with 3 distinct control or PAH-derived PASMC isolates.

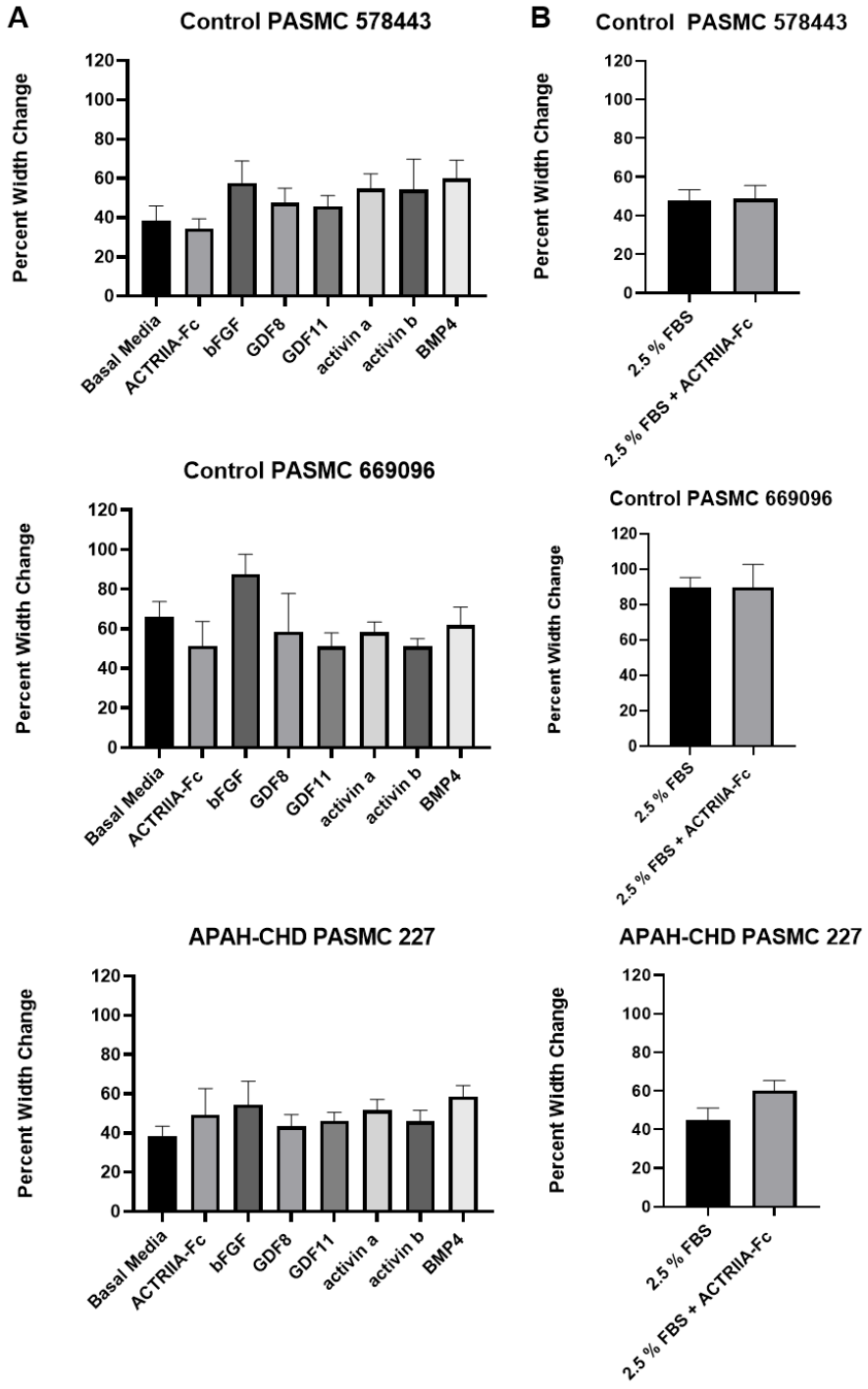

Fig. S10. Impact of ACTRIIA-Fc on cell migration in cultured PASMCs from healthy and PH donors. Cells were grown to confluency and treated with (**A**) ACTRIIA-Fc (2500 ng/mL), bFGF (10 ng/mL), GDF 8 (100 ng/mL), GDF 11 (100 ng/L), activin A (100 ng/mL), activin B (100 ng/mL), or BMP4 (25 ng/mL) in 0.25% FBS basal media, or (**B**) treated with 2.5% FBS basal media with and without the addition ACTRIIA-Fc (2500 ng/mL). Following scratch injury, the monolayer was photographed and width measured at multiple sites at 0 and 48 h for each condition. (n=10 replicate measurements for each condition). These data are representative of 2 independent experiments performed with 2 distinct control or PAH-derived PASMC isolates.

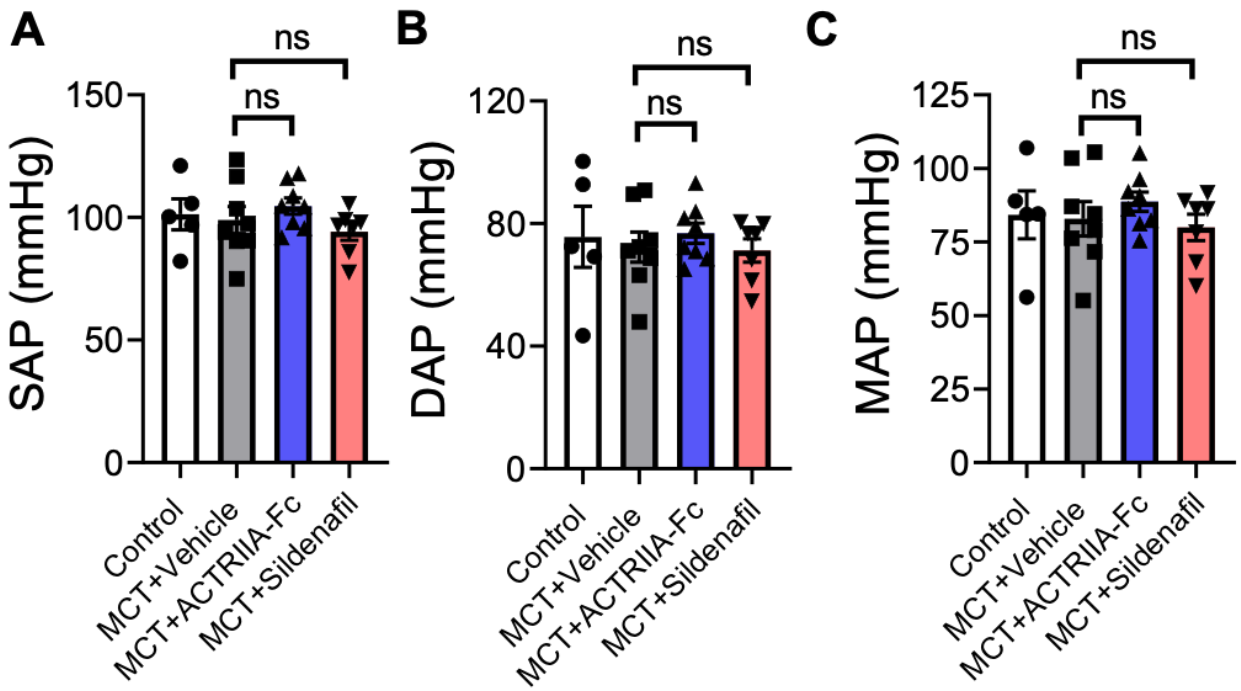

Fig. S11. Treatment with ACTRIIA-Fc or sildenafil does not alter systemic arterial blood pressure in MCT-treated rats. (**A**) Systolic arterial pressure (SAP), (**B**) diastolic arterial pressure (DAP), and (**C**) mean arterial pressure (MAP) in rats given MCT (40 mg/kg s.c.) followed by 4 weeks of treatment with ACTRIIA-Fc (15 mg/kg, i.p. twice weekly), sildenafil (30 mg/kg, p.o. BID), or vehicle (10 mM TBS). Values are shown as mean ± SEM, n=5-8 per group. (NS=non-significant, one-way ANOVA with Dunnett's test for multiple comparisons)

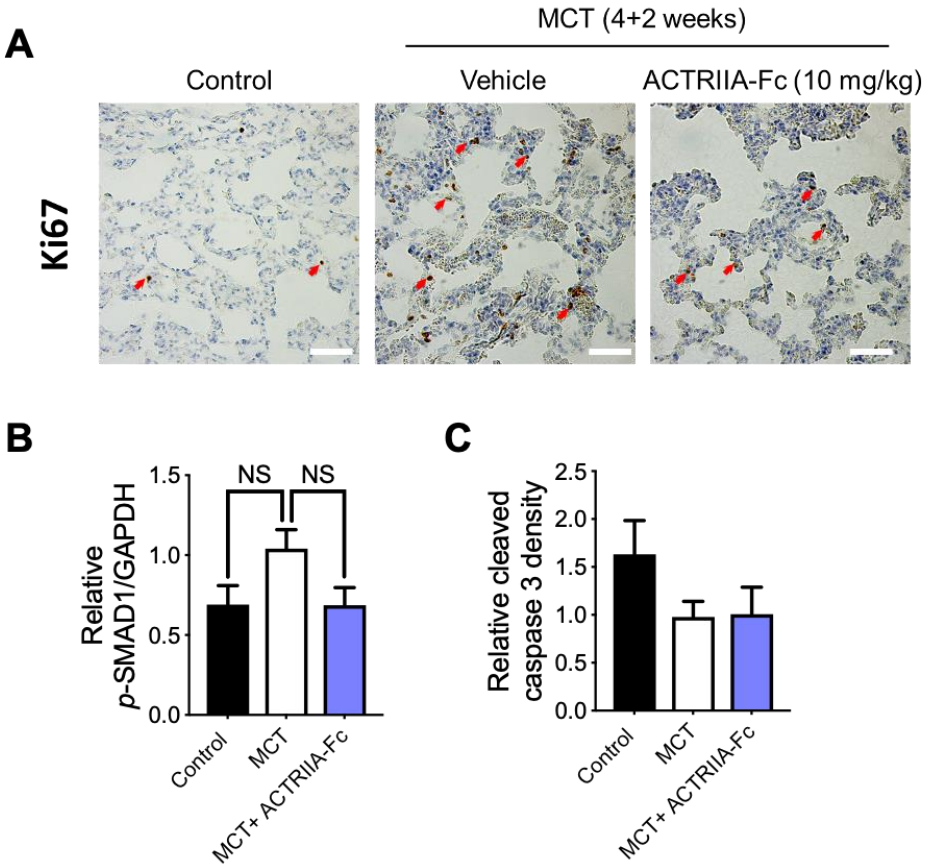

Fig. S12. Effects of ACTRIIA-Fc on cell proliferation and signaling in experimental PH. (**A**) Representative images of Ki67-positive cells (brown, red arrows) in rat lung tissue from rats exposed to MCT for 4 weeks followed by treatment with vehicle or ACTRIIA-Fc (10 mg/kg twice weekly s.c.) for 2 weeks as compared to normoxic control rats. Scale bars = 50 µm (**B**) Lung protein extracts obtained from rats in (A) analyzed for phosphorylated SMAD1/5 by densitometric analysis of Western blot. (**C**) Expression of cleaved caspase 3 in lung protein extracts obtained from rats in (A) analyzed as in (B). Values are shown as mean ± SEM, n=5 per group. (NS = non-significant, one-way ANOVA with Dunnett's test for multiple comparisons)

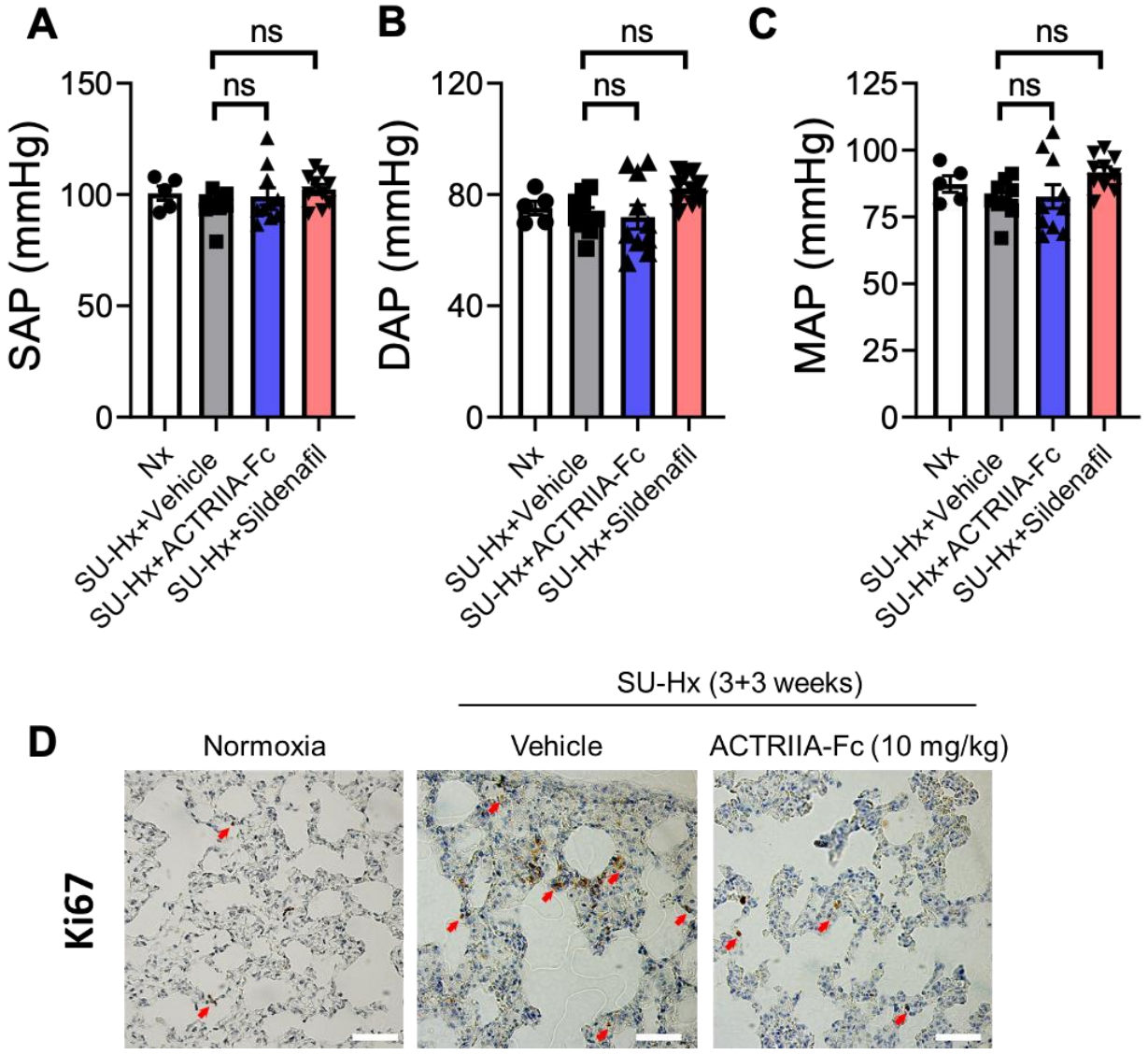

Fig. S13. Treatment with ACTRIIA-Fc or sildenafil does not alter systemic arterial blood pressure in the SU-Hx rat model. (**A**) Systolic arterial pressure (SAP), (**B**) diastolic arterial pressure (DAP), and (**C**) mean arterial pressure (MAP) in rats treated with SU-Hx (200 mg/kg s.c., FIO_2=0.10 for four weeks) with prophylactic treatment with ACTRIIA-Fc (10 mg/kg, i.p. twice weekly), sildenafil (60 mg/kg, p.o. BID), or vehicle (10 mM TBS) for 4 weeks. Values are shown as mean ± SEM, n=5-10 per group. (NS=not significant, one-way ANOVA with Dunnett's test for multiple comparisons). (**D**) In the SU-Hx rescue treatment model, representative images of Ki67-positive cells (brown, red arrows) in rat lungs from rats exposed to SU-Hx for 3 weeks followed by vehicle or ACTRIIA-Fc (10 mg/kg twice weekly i.p.) for 3 weeks, as compared to normoxic control rats. Scale bars = 50 µm.

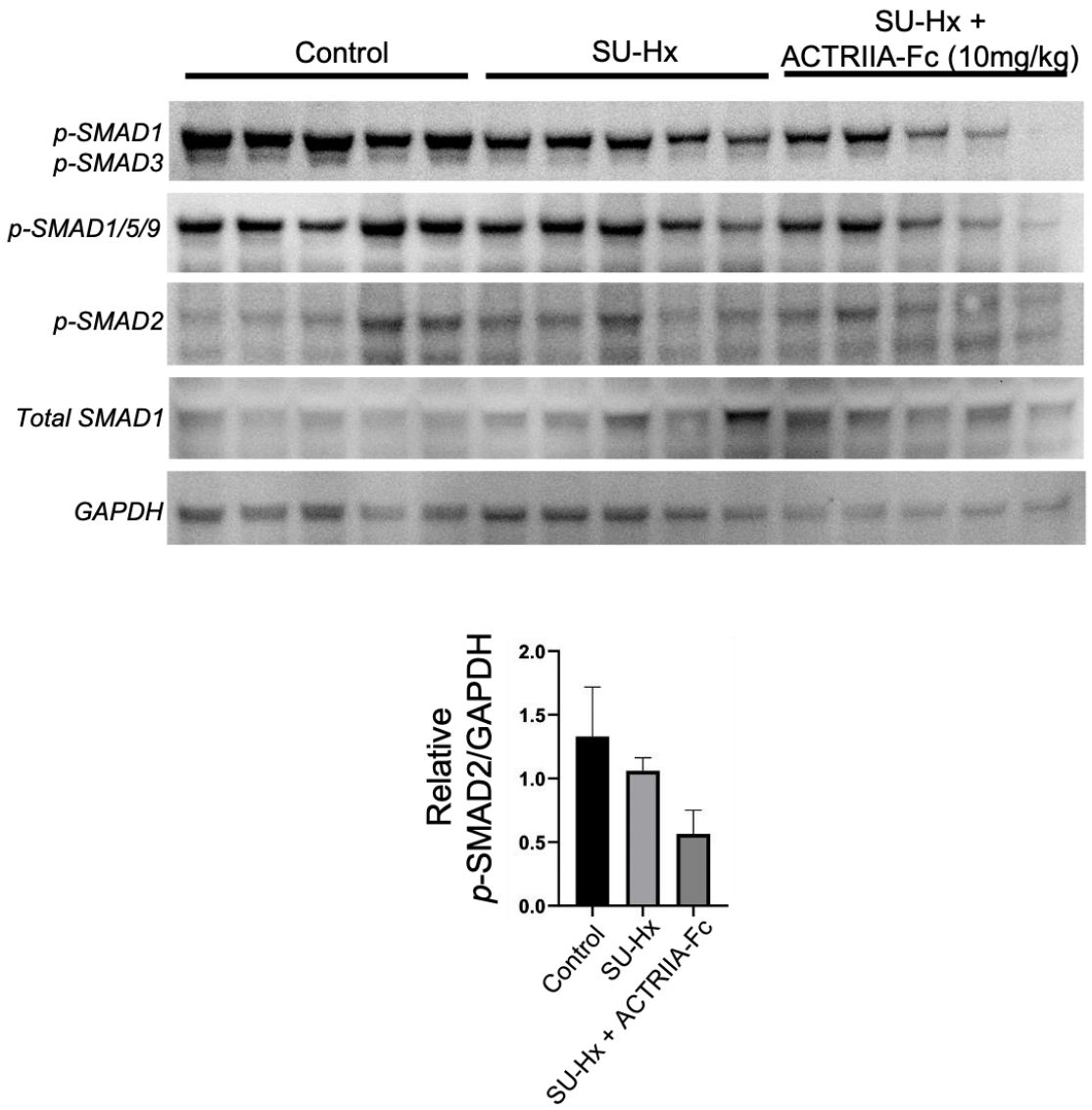

Fig. S14. Western blot analysis of SMAD signaling in the SU-Hx rescue model. Whole lung extracts from rats exposed to SU-Hx for 3 weeks followed by 3 weeks of treatment with vehicle or ACTRIIA-Fc were analyzed by Western blot for *p*-SMAD1/5/9, *p*-SMAD1/3, *p*-SMAD2, total SMAD1 and GAPDH, and subjected to densitometric analysis and normalized to GAPDH.

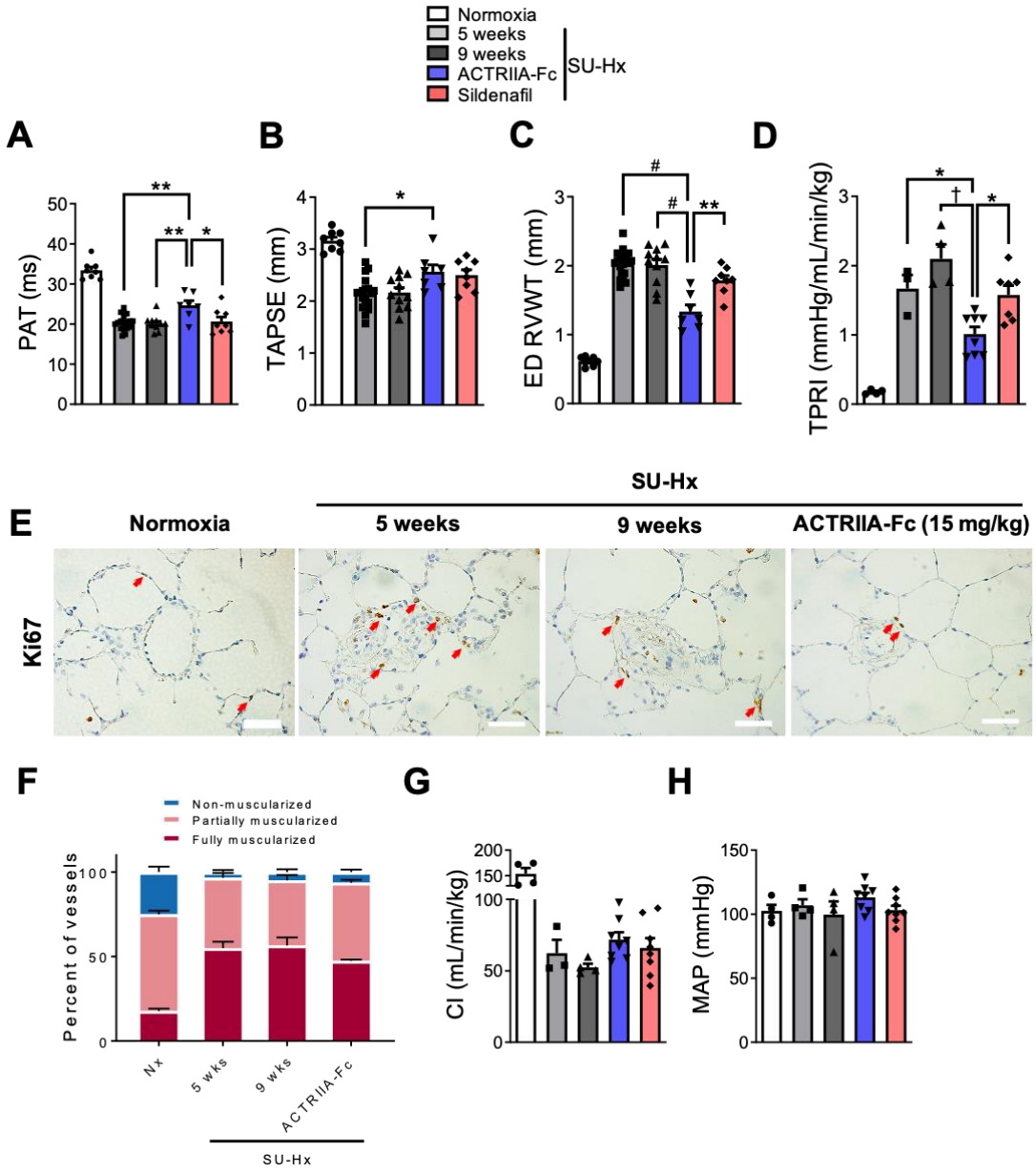

Fig. S15. Treatment with ACTRIIA-Fc improves echocardiographic measures of RV function and PH after SU-Hx exposure. (A) Ultrasound measurement of pulmonary arterial acceleration time (PAT), **(B)** RV function by tricuspid annular plane systolic excursion (TAPSE), **(C)** RV hypertrophy measured by end-diastolic right ventricular wall thickness (ED RVWT), and **(D)** total peripheral resistance index (TPRI) in ACTRIIA-Fc-treated, sildenafil-treated, or untreated animals at weeks 5 and 9 following SU-Hx. Values are shown as mean ± SEM, n=3-19 per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{\dagger}P < 0.001$, $^{\#}P < 0.0001$ one-way ANOVA with Tukey's test for multiple comparisons. **(E)** Representative images of Ki67-positive cells (brown, red arrows) in lung tissue from SU-Hx rats treated with or without ACTRIIA-Fc. Scale bars = 50 μm. **(F)** The percentage of muscularized small vessels (<50 μm diameter) analyzed by histomorphometry as in (E), n = 3-5 per group (30-50 vessels counted per sample). Values are shown as mean ± SEM, two-way ANOVA with Dunnett's test for multiple comparisons. **(G)** Cardiac index (CI) and **(H)** systemic mean arterial pressure (MAP) in SU-Hx rats treated as in (A). For **G-H**, values are shown as mean ± SEM, n=3-8 per group. One-way ANOVA with Tukey's post-test.

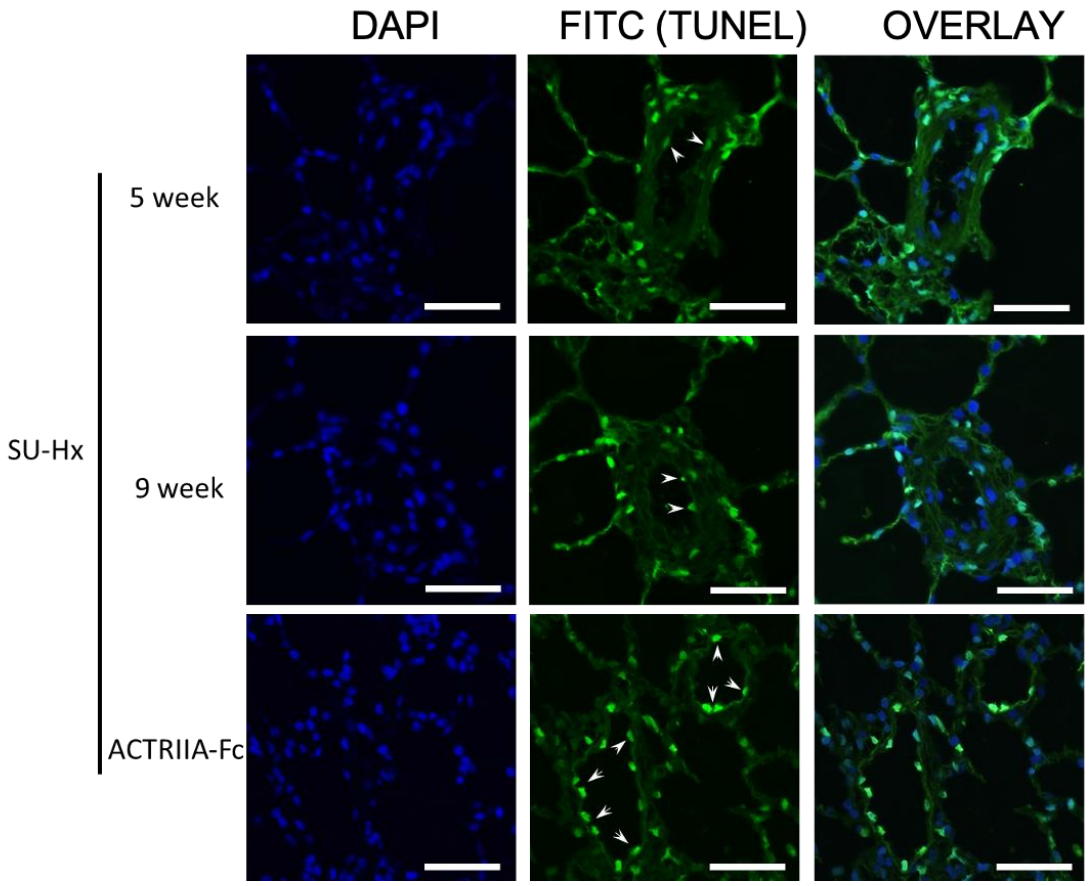

Fig. S16. ACTRIIA-Fc increases apoptosis in small vessels of severe obliterative PH. TUNEL-positive (green, white arrows) apoptotic cells in the walls of small vessels (<50 µm diameter) in lung tissues obtained from rats following 3 weeks of SU-Hx + 2 weeks or 6 weeks of normoxia (labeled 5 week and 9 week respectively) in paraffin-fixed sections counterstained with DAPI nuclear stain (blue), compared with treatment with ACTRIIA-Fc for 4 weeks following 3 weeks of SU-Hx + 2 weeks of normoxia (labeled ACTRIIA-Fc). Images shown are representative of 15-30 random fields analyzed from n=4-6 distinct animals in each treatment group, scale bars = 50 µm.

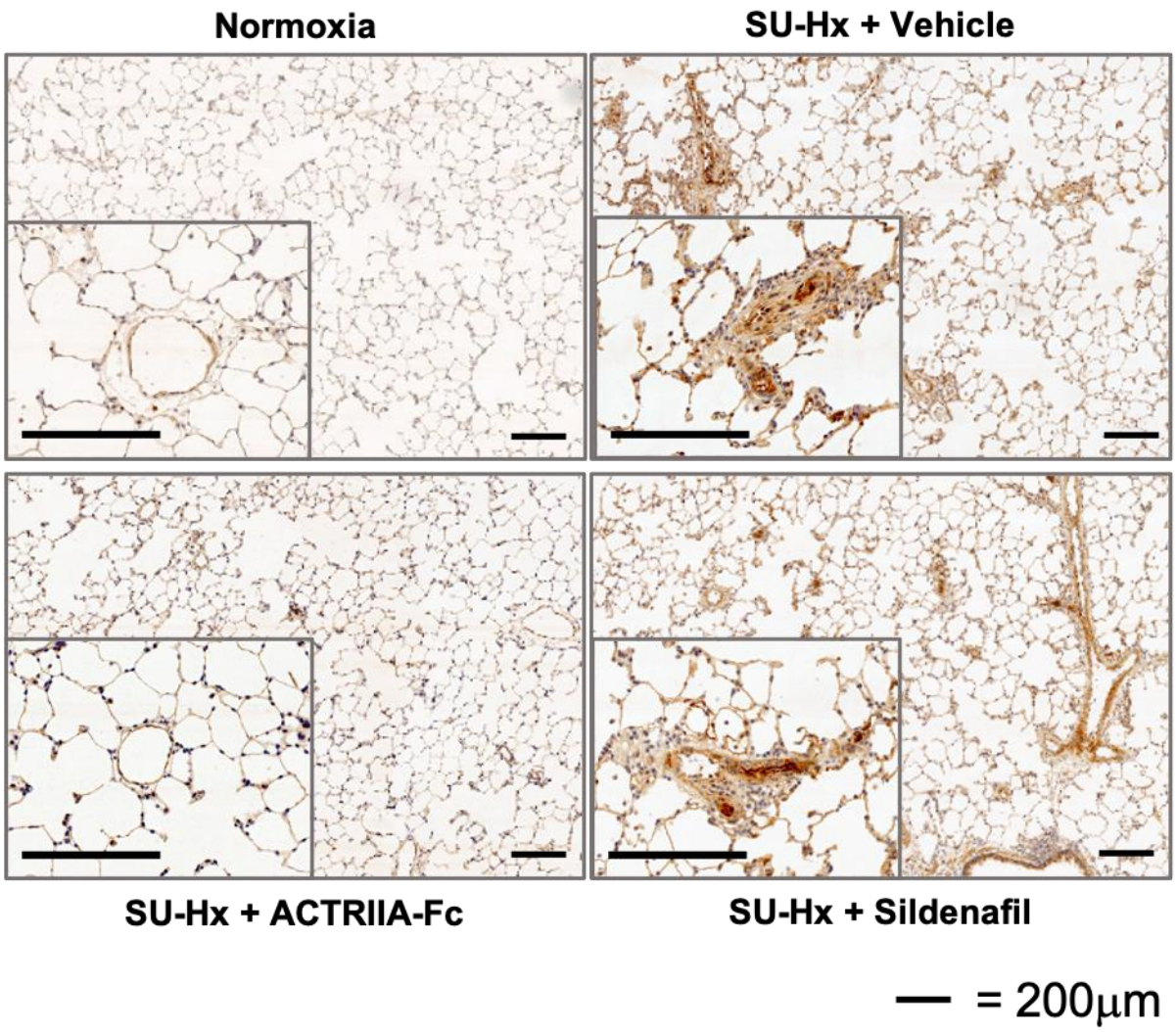

Fig. S17. ACTRIIA-Fc normalized *p*-SMAD2/3 expression in the severe obliterative SU-Hx rat model. Immunohistochemical staining for *p*-SMAD2/3 was performed on paraffin-embedded lung sections from SU-Hx exposed rats (3 weeks, followed by 2 weeks of normoxia, followed by 4 weeks of treatment with vehicle, sildenafil, or ACTRIIA-Fc) and normoxic controls. HRP-conjugated secondary antibody (DAB; brown) and nuclei counterstain with hematoxylin. Representative images are shown for each treatment group. Scale bars = 200 μm.

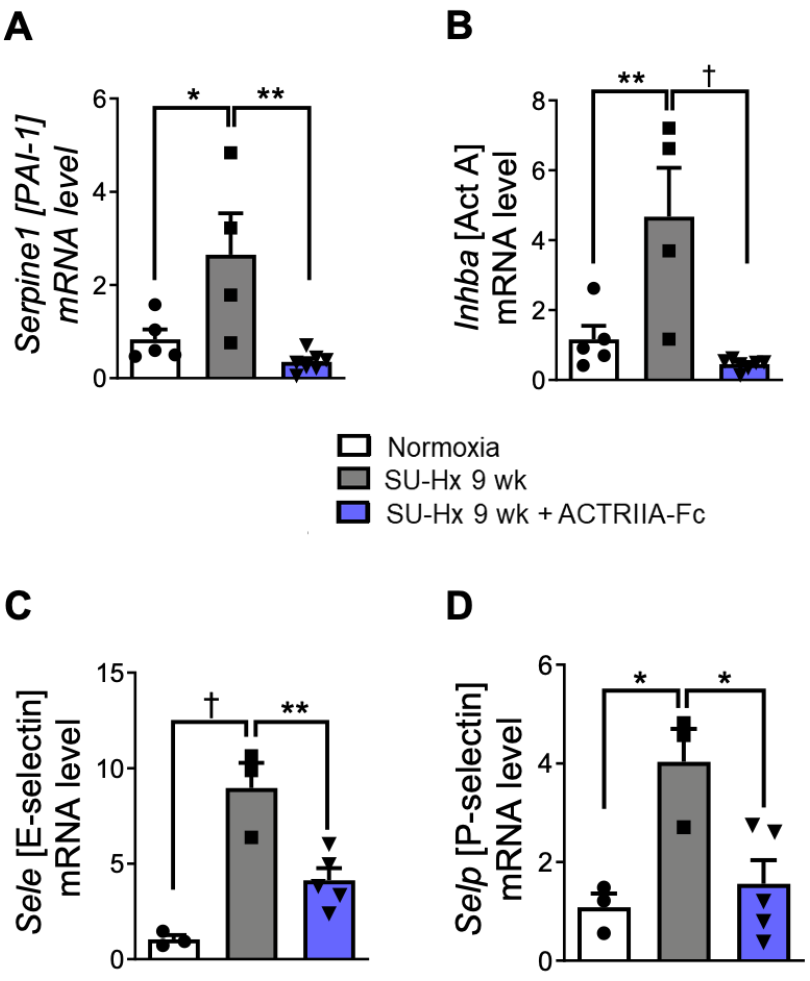

Fig. S18. ACTRIIA-Fc attenuated expression of Pai-1 (*Serpine1*), activin A (*Inhba*), E-selectin (*Sele*), and P-selectin (*Selp)* mRNA in the severe obliterative SU-Hx rat model. **(A)** *Serpine1* and **(B)** *Inhba* mRNA expression in rats with severe PH, following 3 weeks of SU-Hx and 6 weeks of normoxia, with or without delayed treatment with ACTRIIA-Fc. (**C**) *Sele* and **(D)** *Selp* mRNA expression in SU-Hx rats with or without delayed ACTRIIA-Fc treatment. Values are shown as mean ± SEM, n = 3-7 per group. *$P < 0.05$, **$P < 0.01$, $^{\dagger}P <$ 0.001 in comparison with SU-Hx 9 wk, one-way ANOVA with Dunnett's test for multiple comparisons.

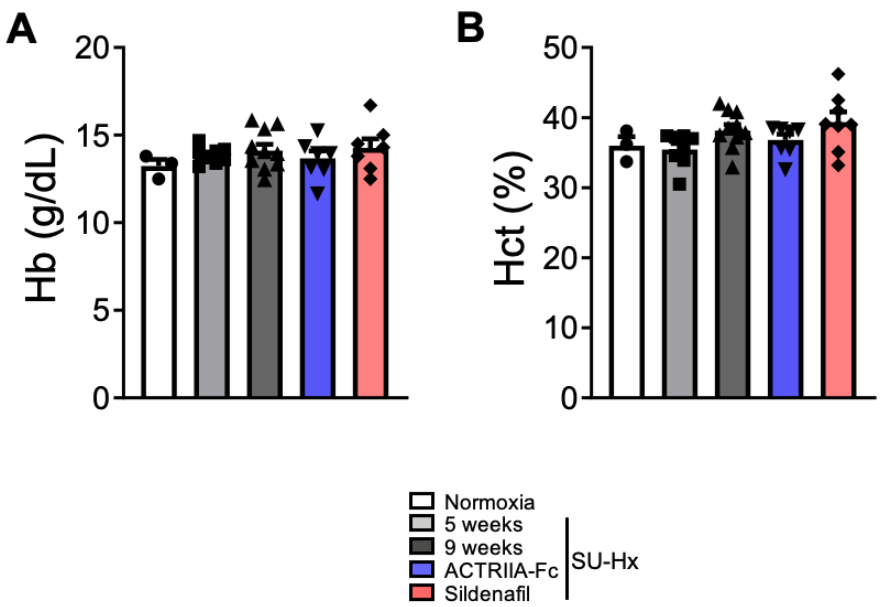

Fig. S19. Treatment of rats with ACTRIIA-Fc in the severe obliterative SU-Hx model did not affect red cell mass. (A) Hemoglobin (Hb) and **(B)** hematocrit (Hct) in SU-Hx-exposed rats treated with or without ACTRIIA-Fc at 5 and 9 weeks following SU-Hx. Values are shown as mean ± SEM, *n* = 3-9 per group. One-way ANOVA with Tukey's test for multiple comparisons.

Table S1. Donor characteristics of serum samples used in the present study.

	Non-PH	WHO Group 1	WHO Group 2	WHO Group 3
Total sample size, n	29	34	15	13
Gender, female subjects, n (%)	21 (72.4)	25 (73.5)	11 (73.3)	8 (61.5)
BMI mean ± SEM	27.1 ± 1.4	30.2 ± 1.8	34.7 ± 2.3	28.8 ± 2.2
Age years ± SEM	53.0 ± 2.8	52.5 ± 2.5	65.8 ± 3.1	64.3 ± 4.3
PAH etiology				
IPAH, n (%)	0 (0)	10 (29.4)	0 (0)	0 (0)
HPAH, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
APAH-CTD, n (%)	0 (0)	6 (17.6)	0 (0)	0 (0)
APAH-STIM, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
APAH-CHD, n (%)	0 (0)	1 (2.9)	0 (0)	0 (0)
APAH-HIV, n (%)	0 (0)	2 (5.9)	0 (0)	0 (0)
APAH-PoPH, n (%)	0 (0)	13 (38.2)	0 (0)	0 (0)
PVOD, n (%)	0 (0)	2 (5.9)	0 (0)	0 (0)
NYHA functional class, n (%)				
NYHA, all, n	27	28	13	12
Class I, n (%)	5 (17.2)	3 (8.8)	0 (0)	2 (15.4)
Class II, n (%)	10 (34.5)	10 (29.4)	8 (53.3)	4 (30.8)
Class III, n (%)	12 (41.4)	14 (41.2)	4 (26.7)	6 (46.2)
Class IV, n (%)	0 (0)	1 (2.9)	1 (6.7)	0 (0)
RHC measurements, mean ± SEM				
RAP (mmHg) ± SEM, n	4.80 ± 0.68, 25	9.63 ± 0.95, 32	13.29 ± 2.05, 14	7.85 ± 1.13, 13
mPAP (mmHg) ± SEM, n	19.00 ± 0.77, 28	50.53 ± 2.32, 34	42.80 ± 2.90, 15	33.23 ± 2.41, 13
PCWP (mmHg) ± SEM, n	10.07 ± 0.87, 29	11.03 ± 0.80, 34	24.47 ± 1.01, 15	11.77 ± 1.35, 13
CO (L/min) ± SEM, n	6.55 ± 0.56, 28	5.31 ± 0.36, 33	5.66 ± 0.44, 15	5.22 ± 0.53, 13
CI (L/min/m2) ± SEM, n	3.58 ± 0.26, 28	2.76 ± 0.18, 33	2.69 ± 0.17, 15	2.90 ± 0.25, 13
PVR (Wood units) ± SEM, n	1.38 ± 0.23, 13	8.45 ± 0.98, 25	3.48 ± 0.46, 15	4.87 ± 0.92, 13
PAH therapy, n (%)				
Prostacyclin analogue, n (%)	0 (0)	6 (17.6)	0 (0)	0 (0)
Endothelin antagonist, n (%)	0 (0)	6 (17.6)	2 (13.3)	2 (15.4)
PDE-5 inhibitor, n (%)	1 (3.4)	15 (44.1)	4 (26.7)	3 (23.1)

Table S2. Total number of rats and mice included in the present study.

	Experimental group	Starting number of animals	Hemodynamic data excluded from analysis due to premature mortality or low heart rate (<350 bpm) during right ventricular catheterization	Final number included in hemodynamic analysis
MCT prophylaxis	Control	5	0	5
	MCT+Vehicle	8	2	6
	MCT+ACTRIIA-Fc	9	2	7
	MCT+Sildenafil	7	1	6
MCT rescue	Control	6	0	6
	MCT+Vehicle	10	0	10
	MCT+ ACTRIIA-Fc 1 mg/kg	10	1	9
	MCT+ ACTRIIA-Fc 3 mg/kg	10	0	10
	MCT+ ACTRIIA-Fc 10 mg/kg	10	1	9
SU-Hx rat prophylaxis	Control (normoxia)	5	0	5
	SU-Hx+Vehicle	10	1	9
	SU-Hx+ACTRIIA-Fc	10	0	10
	SU-Hx+Sildenafil	10	0	10
SU-Hx rat rescue (3+3)	Control (normoxia)	5	0	5
	SU-Hx+Vehicle	8	0	8
	SU-Hx+ ACTRIIA-Fc 1 mg/kg	7	0	7
	SU-Hx+ ACTRIIA-Fc 3 mg/kg	7	0	7
	SU-Hx+ ACTRIIA-Fc 10 mg/kg	8	0	8
SU-Hx rat rescue (3+2+4)	Control (normoxia)	5	0	5
	SU-Hx+Vehicle	5	1	4
	SU-Hx+ACTRIIA-Fc	8	0	8
	SU-Hx+Sildenafil	7	0	7

Table S3. Antibodies used in the present study.

Antibody	Supplier	Catalog number	Dilution (IHC)	Dilution (ICW)	Dilution (IF)	Dilution (WB)
p-SMAD1/3/5	Abcam	ab52903				1:2000
p-SMAD1/5	Cell Signaling	9516		1:400		1:1000
p-SMAD2	Cell Signaling	8828		1:250		1:1000
p-SMAD2/3	Abcam	ab63399	1:100			
Total SMAD1	Cell Signaling	6944				1:1000
GAPDH	Thermo Fisher	MA5-15738				1:5000
vWF	Dako	A0082			1:400	
smooth muscle α-actin	Sigma	A2547	1:100	1:200	1:100	
Calponin	Abcam	ab46794		1:200		
Ki67	Cell Signaling	9129	1:200			
Activin A	Thermo Fisher	PA5-47004	10 ug/ml			1:2000
GDF8	R&D	AF788 (for IHC and WB)	10 ug/ml			1:2000
GDF11	R&D	AF1958 (for IHC)	15 ug/ml			1:2000
GDF11	R&D	MAB19581 (for WB)				1:2000
Cleaved caspase-3	Cell Signaling	9664				1:1000
Rabbit anti-goat IgG, HRP	Thermo Fisher	81-1620	1:100			1:10,000
Anti-mouse IgG, HRP	Cell Signaling	7076S	1:100	1:1000		1:10,000
Anti-rabbit IgG, HRP	Cell Signaling	7074S	1:100	1:1000		1:10,000
Goat Anti-rabbit IgG Alexa Flour 488	Abcam	ab150077			1:100	
Goat Anti-rabbit IgG Alexa Flour 555	Abcam	Ab150078			1:100	

Table S4. Sequences of primers used in the present study.

Species	Gene of interest	Forward	Reverse
Rat	*Pai-1*	CTTTATCCTGGGTCTCCCTG	TGATGCCTCCCTGACATACA
	Bmpr2	AATAATCTGGGTAAGGCC	GCAGAACGAACGCAACCTATCA
	Id1	TGGACGAACAGCAGGTGAACG	GCACTGATCTCGCCGTTCAGG
	Beta-actin	TGTCACCAACTGGGACGATA	ACCCTCATAGATGGGCACAG
Human	*αSMA*	GTGTTGCCCCTGAAGAGCAT	GCTGGGACATTGAAAGTCTCA
	Caldesmon	TGGAGGTGAATGCCCAGAAC	GAAGGCGTTTTTGGCGTCTTT
	Smoothelin	CCCTGGCATCCAAGCGTTT	CTCCACATCGTTCATGGACTC
	Calponin	CTGTCAGCCGAGGTTAAGAAC	GAGGCCGTCCATGAAGTTGTT
	MMP2	TACAGGATCATTGGCTACACACC	GGTCACATCGCTCCAGACT
	MMP9	TGTACCGCTATGGTTACACTCG	GGCAGGGACAGTTGCTTCT
	TIMP1	CTTCTGCAATTCCGACCTCGT	ACGCTGGTATAAGGTGGTCTG
	FN1	CGGTGGCTGTCAGTCAAAG	AAACCTCGGCTTCCTCCATAA
	18S	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT
	CTGF	TAGCAAGAGCTGGGTGTGTG	TTCACTTGCCACAAGCTGTC
	MSX2	GGGTCTAAAGCGGAAGTCACT	GATGGCGACCACTTTCTTGTT
	VIM	AGTCCACTGAGTACCGGAGAC	CATTTCACGCATCTGGCGTTC

Table S5. Donor characteristics of primary tissues used in the study.

PH donors	227	246	256	262	L104C
Diagnosis	Group 1.4.4 APAH-CHD	Group 1.1 IPAH	Group 1.2.1 HPAH-BMPR2	Group 5.2 PH-LCH	Group 1.4.4 APAH-CHD
Cell cultures	PASMC	N/A	PASMC; PMVEC	PASMC; PMVEC	PMVEC
Lung lysates	Yes	Yes	Yes	Yes	No
Age	29	66	66	45	48
Gender	F	F	M	F	F
Smoking	N	N	Remote	Y	N
BMI	20.9	23	24.4	18.8	21.1
mean PAP	69	48	69	74	56
PVR (WU)	7.2	8.8	3.66	21.2	8.6
Cardiac Index	6.19	2.55	5.8	1.9	3.3
FEV1	1.7	1.07	1.98	1.75	2.44
FEV1 (%)	53	45	51	56	81
FVC	2.54	2.01	3.42	2.65	3.55
FVC (%)	69	66	68	69	95
FEV1/FVC	0.67	0.53	0.58	0.66	0.69
TLC (%)	75	111	94	77	101
DLCO (%)	61	50	56	8	89

PMVEC control donors	547317	560578	677243
Age	57	39	7
Gender	M	F	F
Smoking	N	N	N

PASMC control donors	369143	407340	559495	578443	658401	669096
Age	43	56	64	51	52	51
Gender	M	F	M	F	F	M
Smoking	N	N	Y	Y	Y	N

PAH tissue sections	Diagnosis	Label
TB121567	Unused donor control	A
Control 1	Unused donor control	B
TB121584	Tumor adjacent normal lung	C
TB121545	Tumor adjacent normal lung	D
TB121658	Tumor adjacent normal lung	E
5401054	Group 1.1 IPAH	F
5031201	Group 1.1 IPAH	G
S951786	Group 1.1 IPAH	H
S001859	Group 1.1 IPAH	I
095559	Group 1.1 IPAH	J
5971353	Group 1.2.1 HPAH-BMPR2	K
5021254	Group 1.2.1 HPAH-BMPR2	L
S03406	Group 1.2.1 HPAH-BMPR2	M
S031201	Group 1.2.1 HPAH-BMPR2	N
S9850	Group 1.2.1 HPAH-BMPR2	O