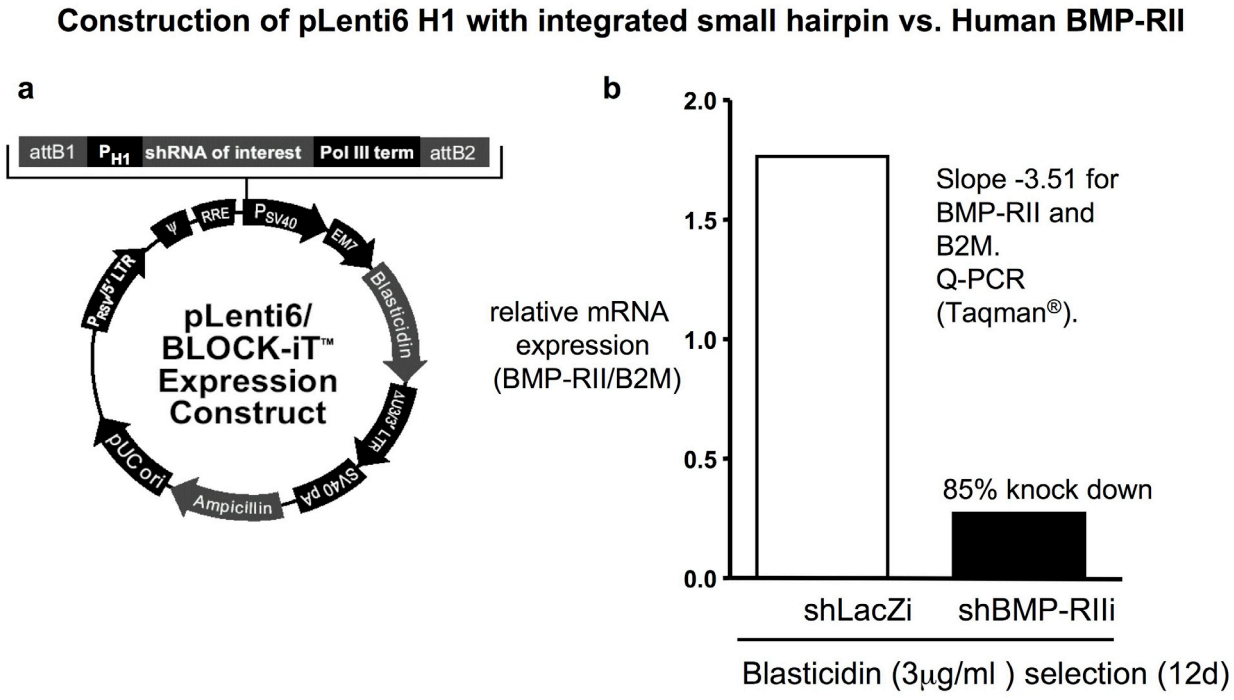
SUPPLEMENTARY METHODS

Mice Genotyping/RT-PCR Analysis. To detect the deletion of PPARγ exon 1 and exon 2, two primers (5'-primer: gtcacgttctgacaggactgtgtgac; 3'-primer: tatcactggagatctccgccaacagc) were designed to correspond to exon A1 and exon 4 of the PPARγ1 gene. RT-PCR could then be carried out to detect the full length 700bp transcript or the 300bp transcript containing the deletion as previously described (1). Total RNA was extracted from PASMC, aorta and lung with Trizol reagent (Invitrogen, Cat. # 15596-026). PASMC were obtained from pulmonary arteries of *SM22α Cre PPARγ $^{flox/flox}$* mice *(SMC PPARγ* -/-) and littermate control mice after 10 days in cell culture (5 mice per genotype and experiment). 3μg total RNA (1μg from aorta) was treated with DNase I for 30 min at 37ºC (Invitrogen, Cat# 18047019) followed by heating at 70°C for 15min (DNase I inactivation). After confirming appropriate RNA quality, RNA samples were subsequently reverse-transcribed (RT) by using the Superscript III reverse transcriptase kit (Invitrogen, Cat# 18080-044). 2μg RNA (0.5 from aorta) from each sample was incubated with 1ul Oligo dT at 70ºC for 10min and then put on ice. Samples were then incubated with 1xRT buffer, 1μl of 0.1M DTT, 1μl of 10μM dNTPs, and 1μl of Superscript III in a total 20μl volume at 50ºC for 1 hour. RT was stopped by heating samples at 70ºC for 15 min. After incubating the samples for 20 min. with 1μl of RNase H at 37ºC, the cDNA was subjected to the PCR reaction using Taq DNA polymerase kit (Invitrogen, Cat#10342-020). 5μl of RT product from each sample was used to mix with 3μl of 10 x buffer, 1μl of 50 mM $MgCl_2$, 0.5 μl of 10mM dNTPs, 1μl of each primer(20μM) and 1μl of Taq DNA polymerase in a total 30μl volume. Hot start PCR reaction was used at 94ºC for 3 min., followed by 35 cycles of 94ºC 30 sec, 65ºC 30 sec, 72ºC 1min. and then 72ºC 10min. incubation. PCR products were then run on a 1% agarose gel.

Lentiviral small hairpin RNAi gene silencing (shRNAi against human BMP-RII): For longterm gene silencing of human BMP-RII, we constructed a pLentivirus 6 with an integrated small hairpin oligonucleotide (bottom 5' to 3': (DNA) - AAA AGC AGA TGG ACG CAT GGA ATA TTT CGA TAT TCC ATG CGT CCA TCT GC; top 5' to 3': (DNA) – AAA AGG ACA ATA TTA TGC TCG AAA GTT CGC TTT CGA GCA TAA TAT TGT CC) directed against the mRNA of human BMP-RII, using an inducible H1 RNAi entry vector kit (Invitrogen #K4920-00). Lentivirus was made and propagated with a lentiviral RNAi expression system kit (Invitrogen, #K4943-00), by transfecting 293FT cells with the shRNA-H1-TO-human BMP-RII pLenti6 construct using Lipofectamine 2000 (Invitrogen) and Vira Power Packaging Mix (Invitrogen, #K4944-00) according to the manufacturer's instructions. Virus-containing supernatants were harvested 48 and 72h posttransfection, ultra-filtered (Milipore, centricon Plus-70), and titrated on HT1080 cells (ATCC). A multiplicity of infection (MOI) of one was used for transfection of human PASMC (Cascade biologics, Portland, OR) with shBMP-RII pLenti6 (see Supplementary Figure 1 online) following the manufacturer's instructions: Cells were incubated with virus mix and polybrene (8μg/ml) for 6h, and then changed to full growth-medium. Forty-eight hours after the beginning of transfection, blasticidin (3μg/ml) was added to Medium 231 medium including 100U/ml Penicillin, 0.1mg/ml streptomycin and smooth muscle growth supplement (SMGS, Cascade Biologics, Portland, OR). A kill curve for blasticidin had been performed on HPASMC and revealed cell death in untransfected HPASMC by day 5 of blasticidin incubation (3μg/ml). By day 12 of blasticidin selection, we confirmed by q-PCR a 85% stable knock down of BMP-RII in shBMP-RIIi vs. shLacZi (control) transfected HPASMC.



Supplementary Figure 1 **a,** Construction of a pLentivirus 6 (pLenti 6) with integrated small hairpin oligonucleotide *vs.* human bone morphogenetic protein receptor II (shBMP-RIIi). For longterm gene silencing of human BMP-RII, we constructed a pLentivirus 6 with an integrated small hairpin oligonucleotide (for details see Supplementary Methods below) directed against the mRNA of human BMP-RII, using an inducible H1 RNAi entry vector kit and lentiviral RNAi expression system kit (Invitrogen #K4920-00, #K4943-00). **b,** Lentivirus was made and propagated in 293FT cells and human PASMC were transfected as described in the Supplementary Methods below. After 12 days blasticidin selection, we confirmed a 85% stable knock down of human BMP-RII vs. shLacZi control in human PASMC.

attB1, attB2: DNA recombination sequences that permit recombinational cloning of the gene of interest from a Gateway® entry clone (Invitrogen). **P_{H1}**, human H1 promotor: Expression of the shRNA of interest from pENTR™/H1/TO (or a suitable destination vector following LR

recombination) is controlled by the human H1 promoter. The endogenous human H1 promoter normally controls expression of H1 RNA, the RNA component of human RNase P involved in tRNA processing. This particular promoter to control vector-based expression of shRNA molecules in mammalian cells was choosen for the following reasons: 1.) The promoter is recognized by RNA Polymerase III and controls high-level, constitutive expression of shRNA. 2.) The promoter is active in most mammalian cell types. 3.) The promoter is a type III Pol III promoter in that all elements required to control expression of the shRNA are located upstream of the transcription start site. **Pol III term**, RNA polymerase III. **P_{SV40}**, SV40 early promoter and origin: Allows high-level expression of the selection marker and episomal replication in cells expressing the SV40 large T antigen. **EM7** promoter: synthetic prokaryotic promoter for expression of the selection marker in E. coli. **Blasticidin resistance gene**: permits selection of stably transduced mammalian cell lines. **ΔU3/HIV-1 truncated 3′ LTR**: Allows viral packaging but self-inactivates the 5′ LTR for biosafety purposes. The element also contains a polyadenylation signal for transcription termination and polyadenylation of mRNA in transduced cells. **SV40 pA**, SV40 polyadenylation signal. **Ampicillin** resistance gene (β-lactamase): allows selection of the plasmid in *E. coli*. **pUC ori**: permits high-copy replication and maintenance in *E. coli*. **P_{RSV}/5' LTR**, Rous Sarcoma Virus (RSV) enhancer/promoter: Allows Tat-independent production of viral mRNA. HIV-1 truncated 5′ LTR: Permits viral packaging and reverse transcription of the viral mRNA. Ψ, HIV-1 psi (ψ) packaging signal: Allows viral packaging. **RRE**, HIV-1 Rev response element (RRE): Permits Rev-dependent nuclear export of unspliced viral mRNA.

Small interfering (si) RNA gene silencing of human BMP-RII. To achieve effective gene knockdown, siRNA duplexes specific for BMP-RII (Dharmacon on-target plus; accession number NM_001204) were transfected into human PASMCs using Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol. Knockdown efficiency was evaluated 48

hours later by measuring protein levels in cell lysates via immunoblotting. Transfection of nontargeting siRNA duplexes (siCONTROL, Dharmacon, Inc) was performed simultaneously to serve as control in all experiments.

Cell Culture and Functional Assays: *Primary murine PASMC were isolated from* 5 male 14-15-week old mice of apoE deficient (apoE -/-) and C57Bl/6 mice, as well as *SM22α Cre PPARγ* $^{flox/flox}$ (SMC PPARγ -/-) mice and littermate control mice, using a modified protocol as previously described (2): The main extralobular pulmonary arteries were dissected, cleaned from adherent tissue, cut in small pieces and digested for 90 min. in dispersion media containing 0.53mg/ml elastase (Roche), 0.53mg/ml collagenase II (Worthington), 2 mg/ml albumin (Sigma), 0.2mg/ml soybean trypsin inhibitor (Worthington), 40μM $CaCl_2$, in HBSS buffer (Gibco). PASMCs were then cultured in DMEM (Gibco) containing FBS (20% for 3 days, then reduced to 10%), 2mM L-glutamine and 100U/ml Penicillin, 0.1mg/ml streptomycin. Passages 3-4 were used for further studies. Smooth muscle cell identity was verified by positive immunohistochemistry staining for SMα-actin (Sigma Aldrich, St. Louis, MO) (>95% of cells stained positive for SMα-actin). PASMC were grown to 70% confluence in DMEM and then cultured for 24h in starvation media (DMEM, 0.1% FBS, 2mM L-glutamine, 100U/ml Penicillin, 0.1mg/ml streptomycin).

Primary human PASMC were purchased from Cascade Biologics (Portland, OR) and maintained cell culture flasks (25-150cm^2) containing Medium 231, Smooth Muscle Growth Supplement (SMGS), 100U/ml penicillin G, 0.1mg/ml Streptomycin sulfate, and 0.25μg/ml Amphotericin B (PSA Solution) (all Cascade Biologics). Cells were received at passage 3 and used between passages 5 and 9. Moreover, normal PASMC were isolated from surgical resection specimens derived from patients undergoing lobectomy or pneumonectomy for suspected lung tumor (control). Only uninvolved tissue was used. PASMC were explanted peripheral pulmonary arteries (<1-2mm external diameters, as previously described (3, 4). Cells were maintained in 10% FBS/DMEM and used for experiments between passages 4 and 6.

Additional PASMC were obtained from a patient undergoing heart-lung transplantation for familial PAH and known to harbor a mutation in the BMP-RII receptor. The isolate used was obtained from a patient in which a premature stop codon is inserted in place of tryptophan at position of the amino acid sequence (W9X). The smooth muscle phenotype of isolated cells was confirmed by positive immunofluorescence with antibodies to anti-α-smooth muscle actin antibody (IA4) and anti–smooth muscle specific myosin (hsm-v), as described(6).

Cell Counts: PASMC were seeded at $2.5x10^4$ cells per well of a 24-well plate in 500µl of growth medium and allowed to adhere overnight. The medium was removed and the cells washed 3 times with PBS prior to the addition of starvation media (DMEM, 0.1% FBS, penicillin/streptomycin) and incubated at 37°C, 5% CO2 for 24h (murine PASMC) or 48h (human PASMC) prior to PDGF-BB stimulation for 0h and 72h (treatments and concentrations stated in the figure legends). Cells were washed twice with PBS and trypsinized in 150µl of Trypsin/EDTA for 7min., followed by the addition of 150µl trypsin neutralizer (all Cascade Biologics, Portland, OR). The cells were then resuspended and counted in a hemacytometer (3-6 wells per condition, 4 counts per well).

MTT Cell Proliferation Assay (ATCC, Manassas, VA): 3 x 10^3 HPASMC per well were seeded and allowed to adhere on a 96-well plate overnight. After removal of the medium, the cells were washed 3 times with PBS prior to the addition of Opti-MEM I (Gibco, Gaithersburg, MD) containing 0.1% FBS, penicillin/streptomycin, and incubated at 37°C, 5% CO2 for 48h prior to stimulation. The cells were stimulated with PDGF-BB (20ng/ml) for 72h and then incubated with the yellow MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) for 6h at 37°C, followed by the addition of detergent (room temperature, stored overnight in the dark). The absorbance was measured in a microtiter plate reader (Biorad, Hercules, CA) at 570nm the next day.

Western immunoblotting. *Preparation of total cell lysates.* PASMC were washed three times with ice-cold PBS. Cell lysates were prepared by adding boiling lysis buffer (10mM Tris HCl, 1% SDS, PMSF 0.2mM, protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO), phosphatase inhibitors cocktails #1 and #2) to the cells, scraping into a 1.5ml microcentrifuge tube and boiling for 10min. prior to centrifugation. The supernatants were transferred to fresh microcentrifuge tubes and stored – 80°C.

Preparation of subcellular fractions (nuclear matrix, nuclear extract, cytoplasmic extract) were performed using a modified low-salt-high-salt-protocol as previously described (7, 8). HPASMC were washed and then scraped in ice-cold PBS. After centrifugation (1850g, 10min., 4°C), the cell pellet was washed again in PBS. After spin down, the cells were then lysed by resupension in hypotonic buffer (HEPES 10mM, $MgCl_2$ x 6 H_2O 1.5mM, KCl 19mM, PMSF 0.2mM, DTT 0.5mM), cell swelling on ice for 20min., followed by 15 strokes with a Dounce homogenizer (B pestle). Nuclei were then pelleted at 3300g for 15min., 4°C. *For cytoplasmic extract preparation*, 0.11 vol. of 10X cytoplasmatic extract buffer (HEPES 300mM, $MgCl_2$ x 6 H_2O 30mM, NaCl 1.4M) was added to the supernatant. After high speed centrifugation at 20,000g for 30min. at 4°C, the supernatant was designated as cytoplasmic extract and stored at – 80°C. *For nuclear extract preparation*,1/2 packed nuclear volume of high salt buffer (HEPES 20mM, $MgCl_2$ x 6 H_2O1.5mM, NaCl 800mM, glycerol 25%, EDTA-Na 0.2mM, PMSF 0.2mM, DTT 0.5mM) was added dropwise to the nuclear pellet, vortexed for 40min. at 4°C., then centrifuged at 20,000g for 30min. at 4°C. The resulting supernatant was designated as nuclear extract and stored at – 80°C. *For nuclear matrix extract preparation*, 1/2 packed nuclear volume (pnv) of high salt buffer was added. The pellet was boiled for 10min, and the nuclear matrix fraction was extracted by vortexing in 2x packed nuclear volume (pnv) SDS buffer for 60min. at 20°C. After centrifugation at 20,000g for 30min., 4°C, the salt resistant supernatant was designated as

nuclear matrix fraction and stored at – 80°C. All buffers contained protease and phosphatase inhibitors (Sigma-Aldrich, phosphatase inhibitor cocktails #1 and #2).

Protein concentration was determined by the Lowry protein assay (Biorad, Hercules, CA). Equal amounts of protein were loaded onto each lane of a 4-12% Bis-Tris gel and subjected to electrophoresis under reducing conditions. After blotting, PVDF-membranes (Invitrogen, Carlsbad, CA) were blocked for 1h (milkpowder 5% in TBS/tween 0.1-0.2%) and incubated with rabbit polyclonal antibodies raised against ERK 1/2 (Cell Signaling, Danvers, MA), , pSmad 1/5/8 or total Smad1 (both Cell Signaling, Danvers, MA), or mouse monoclonal antibodies against phosphoERK 1/2 (Cell Signaling), PPARγ (Santa Cruz, Santa Cruz, CA), apolipoprotein E (Abcam, Cambridge, MA) or BMP-RII (BD Biosciences Pharmingen, San Jose, CA). Binding of secondary HRP-antibodies were visualized by ECL or ECL plus chemiluminescent (Amersham, Princeton, NJ). Normalization for total cell protein was performed by re-probing the membrane with a mouse monoclonal antibody against α-tubulin (Sigma-Aldrich, St. Louis, MO). Normalization for total protein in cell fractions was achieved by correcting for Ponceau S stain.

PPARγ-DNA-Binding Assay. A multiplex transcription factor (TF) assay (Marligen Biosciences, Ijamsville, MD) was performed as previously described (9, 10). Briefly, multiple biotin labeled TF DNA probes (DNA multiplex probe mix) are mixed with one nuclear extract protein sample to allow transcription factor-DNA binding. In the following digestion step, DNA sequences that are not bound (and "protected") by specific transcription factors are destroyed by proprietary reagents. A mix of colored beads (1000 of each color per specific transcription factor) with attached DNA-oligonucleotides complimentary to a sequence in the specific TF DNA probes is added to the tube. Then, DNA binding sites hybridize to their respective beads (PPARγ core binding site: 5'-TGACCTTTGACC-3') and the entire sample is measured in a Luminex-100 instrumentation (Luminex, Houston, TX) that reads at least 100 signals per

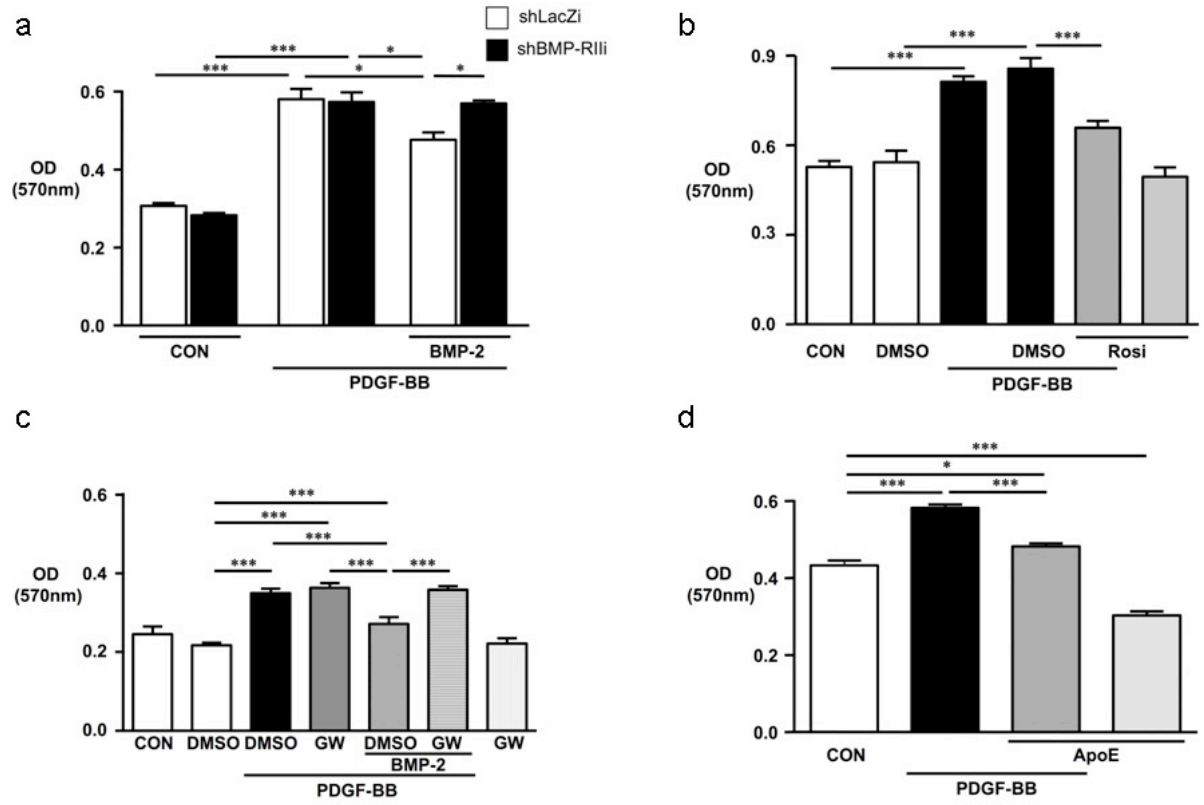
colored bead type. Nuclear extract samples were run in triplicate so that 300 different data points are collected for each DNA binding site.

Immunohistochemistry/Confocal Microscopy. Serum starved Human PASMC were seeded on 4-chamber slides (250μl), stimulated with BMP-2 (10ng/ml), washed and fixated with paraformaldehyde 4% at room temperature, incubated in blocking buffer (5% goat serum, 0.02% BSA, Triton X-100 0.1% in TBS) for 30min., and then incubated with primary polyclonal antibody (rabbit anti human) against phosphoERK1/2 (Cell Signalling, Danvers, MA) overnight at 4°C. The fixed cells were then carefully washed three times, and incubated with secondary antibody (goat anti rabbit, Alexa 488, Molecular Probes/Invitrogen, Carlsbad, CA), and again washed three times. For mounting, antifade-DAPI (Component A, slow fade-antifade kit S-24635, Mol. Probes/Invitrogen) was given on the cover slips, and slides were sealed with colorless nail polish. Images were acquired on a Zeiss LSM 510 two-photon confocal laser scanning microscope. Confocal micrographs were processed with Openlab 3.1.4 and Volocity 3.0 software (Improvision, Coventry, UK).

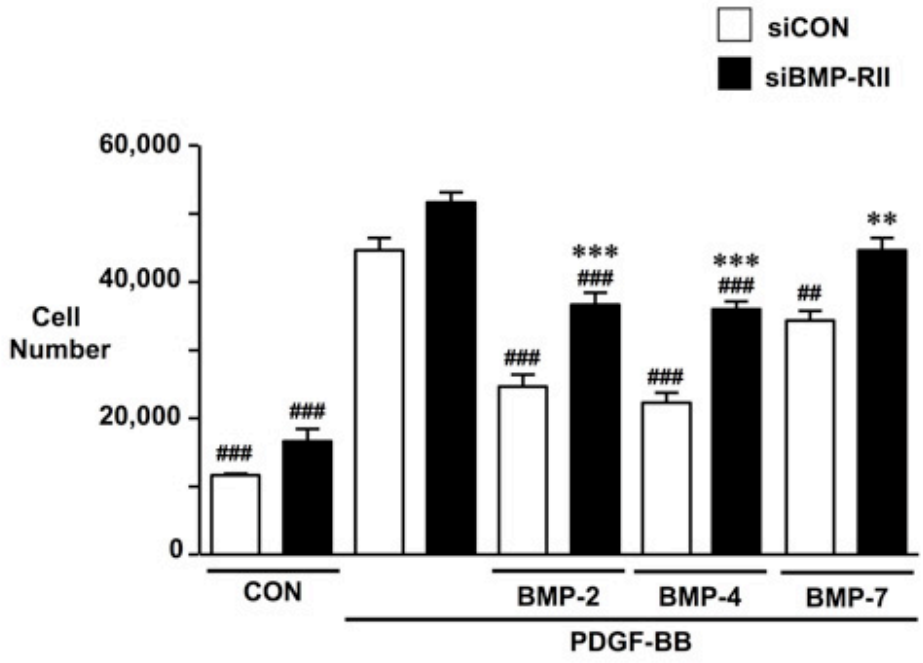
Hemodynamic Measurements in Mice. RV catheterisation: A 1.4 F catheter (Millar Instruments, Houston, Texas) was inserted into the right jugular vein and then placed into the RV free cavity, as previously described (11). Using the PowerLab/4SP recording unit (AD Instruments, Colorado Springs, CO), 3-5 tracings at different time points were averaged to determine the RVSP, maximal rate of pressure development (dp/dt max.; RV systolic function) and maximal rate of pressure decay (dp/dt min.; RV diastolic function). *Systemic blood pressure measurements (tail cuff method):* At least 5 recordings per mouse were averaged to determine systolic BP, MAP and diastolic BP. *Echocardiography:* Fractional shortening (FS) and heart rate (HR) were determined in M-mode. Ejection fraction (EF) and cardiac output (CO) were estimated using the Teichholz formula (12).

Lung tissue preparation. After abdominal aortic dissection, lungs were perfused in vivo by injecting 5ml normal saline into the beating RV. Lungs were tracheally injected with 10% formalin, fixed overnight, and then embedded either in paraffin for standard histology (Hematoxylin & Eosin, Elastic van Gieson, Movat pentachrome). Prior to fixation, a subset of left lungs were infused with barium-gelatin via the central PA (13) to label peripheral pulmonary arteries for morphometric analysis. The barium was infused by hand with similar endpoints of pre-capillary filling of all small vessels at alveolar duct and wall level. The total number of peripheral arteries was calculated as a ratio of number of arteries per surface area (5 random fields per slide) and per 100 alveoli in each field (200x magnification). Muscularized and non-muscularized peripheral (alveolar wall) pulmonary arteries were counted at 400x magnification in 5 random fields per lung. (200x magnfication = 1 field)

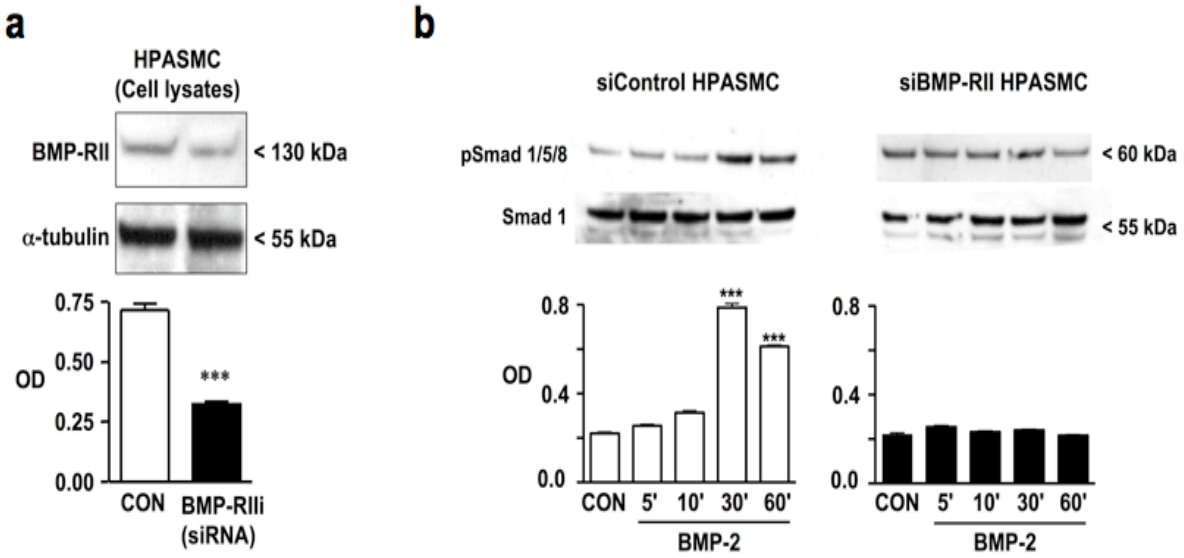
SUPPLEMENTARY DATA



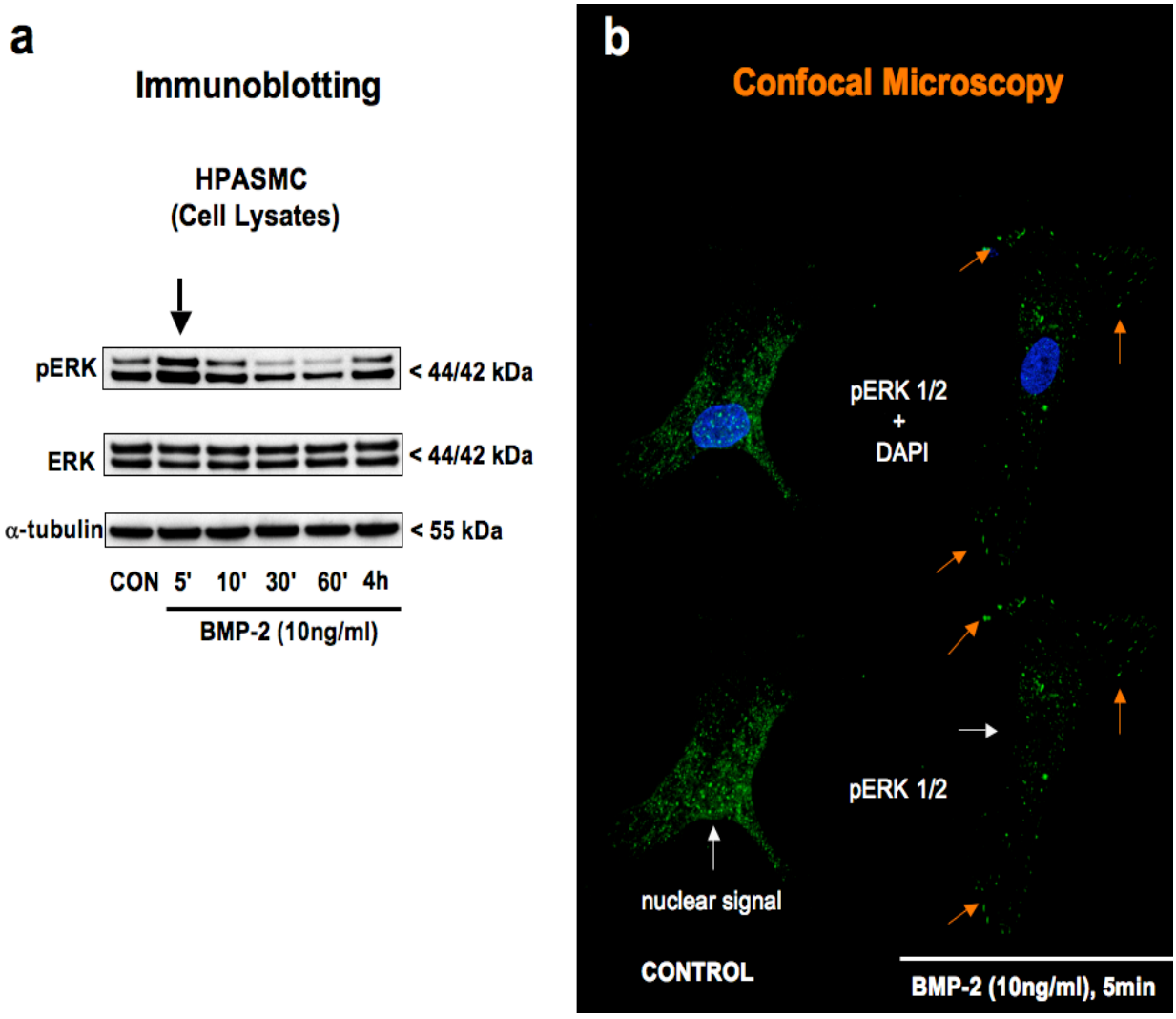
Supplementary Figure 2 BMP-2 (**a,c**), the PPARγ agonist rosiglitazone (**b**), and apolipoprotein E (**d**) inhibit proliferation of human pulmonary artery smooth muscle cells (PASMC) induced by PDGF-BB (20ng/ml). **a**, Recombinant BMP-2 inhibits proliferation in LacZi control but not in shBMR2i PASMC in which BMP-receptor II expression is suppressed by shRNAi pLenti 6. **b,** Rosiglitazone (1μM) blocks human PASMC proliferation. The expansion of the y axis reflects higher baseline OD values. **c**, The inhibitory effect of BMP-2 on human PASMC proliferation is lost in the presence of the irreversible PPARγ antagonist GW9662 (1μM). **d**, recombinant apolipoprotein E (apoE 10μM) inhibits human PASMC proliferation. Starvation, stimulation with PDGF-BB and MTT assay as described under methods. Bars represent mean±SEM (n = 4-8; n = 12-14 for PDGF-BB in Fig. 2b and 2d). ∗ $p < 0.05$; ∗∗∗ $p < 0.001$.



Supplementary Figure 3 Antiproliferative effects of BMP-2, BMP-4, and BMP-7 in sicontrol and siBMP-RII (knock down) human PASMC. To achieve effective gene knockdown, siRNA duplexes specific for BMP-RII were transfected into human PASMCs using Lipofectamine 2000 (Invitrogen). Knock-down efficiency was evaluated 48 hours later by measuring protein levels in cell lysates by western immunoblotting. Transfection of nontargeting siRNA duplexes (siCONTROL, Dharmacon, Inc) was performed simultaneously to serve as a control in all experiments. PASMC were seeded at $2.5x10^4$ cells per well of a 24-well plate in 500µl of growth medium and allowed to adhere overnight. The cells were washed with PBS prior to the addition of starvation media (0.1% FBS) and incubated for 48h, and then stimulated with PDGF-BB (20ng/ml) for 72h. BMP-2, BMP-4 and BMP-7 (all 10ng/ml) were added to quiescent cells 30min. prior to PDGF-BB stimulation. Cell numbers in controls at time points 0h and 72h were not significantly different. Bars represent mean±SEM (n = 3). ANOVA with Bonferroni's multiple comparison test. ## $p < 0.01$; ### $p < 0.001$ denote comparisons with PDGF-BB stimulation of either siCON or siBMP-RII cells, in the absence of BMPs. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ denote comparisons between siCON and siBMP-RII for each ligand (BMP-2, BMP-4, BMP-7).



Supplementary Figure 4. Knock-down of human BMP-RII prevents BMP-2 mediated phosphoSmad 1/5/8 (pSmad 1/5/8) signalling in human PASMC. **a**, Knock-down of human BMP-RII protein expression by small interfering RNA in human PASMC. To achieve effective gene knockdown, siRNA duplexes specific for BMP-RII were transfected into human PASMCs using Lipofectamine 2000. Nontargeting siRNA duplexes were used for control transfections (see Supplementary Methods). **b**, BMP-2 (10ng/ml) stimulation for 30min. induces Smad1/5/8 phosphorylation in siControl but not in siBMP-RII (knock-down) human PASMC. Of note, Smad 1/5/8 phosphorylation occurs after BMP-2 mediated activation of PPARγ (see Figure 2d, main manuscript). Cell culture, total cell lysate preparation and Western immunoblotting are described in the (Supplementary) Methods section. Arbitrary OD values (densitometry): Bars represent mean±SEM (n = 3). Unpaired two-tailed t-test (**a**) and ANOVA with Bonferroni's multiple comparison test (**b**). *** $p < 0.001$ versus control.



Supplementary Figure 5. BMP-2 induces rapid extra-nuclear ERK1/2 phosphorylation that is accompanied by a strong signal at the cyoplasmic membrane. **a**, Western immunoblotting of total cell lysates: pERK1/2, total ERK1/2, and α-tubulin (2nd loading control). Human PASMC were stimulated with BMP-2 (10ng/ml) for 5-60min., and 4h (n=2). Cell culture and preparation of total cell lysates (which include the cytoplasmic membrane fraction) as described in the Methods section. **b**, Immunohistochemistry/Confocal microscopy. DAPI = nuclear DNA stain, bright green = pERK1/2 stain (see Supplementary Methods section). BMP-2 stimulation (5min.) impairs the nuclear signal of pERK (white arrows). This is accompanied by strong pERK 1/2 staining at the cytoplasmic membrane (orange arrows; see also Figure 2b, main manuscript).

References (Supplement)

1. Hevener, A.L., He, W., Barak, Y., Le, J., Bandyopadhyay, G., Olson, P., Wilkes, J., Evans, R.M., and Olefsky, J. 2003. Muscle-specific Pparg deletion causes insulin resistance. *Nat Med* 9:1491-1497.
2. Fouty, B.W., Grimison, B., Fagan, K.A., Le Cras, T.D., Harral, J.W., Hoedt-Miller, M., Sclafani, R.A., and Rodman, D.M. 2001. p27(Kip1) is important in modulating pulmonary artery smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 25:652-658.
3. Yang, X., Long, L., Southwood, M., Rudarakanchana, N., Upton, P.D., Jeffery, T.K., Atkinson, C., Chen, H., Trembath, R.C., and Morrell, N.W. 2005. Dysfunctional Smad signaling contributes to abnormal smooth muscle cell proliferation in familial pulmonary arterial hypertension. *Circ Res* 96:1053-1063.
4. Wharton, J., Davie, N., Upton, P.D., Yacoub, M.H., Polak, J.M., and Morrell, N.W. 2000. Prostacyclin analogues differentially inhibit growth of distal and proximal human pulmonary artery smooth muscle cells. *Circulation* 102:3130-3136.
5. Morrell, N.W., Yang, X., Upton, P.D., Jourdan, K.B., Morgan, N., Sheares, K.K., and Trembath, R.C. 2001. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta(1) and bone morphogenetic proteins. *Circulation* 104:790-795.
6. Morrell, N.W., Upton, P.D., Kotecha, S., Huntley, A., Yacoub, M.H., Polak, J.M., and Wharton, J. 1999. Angiotensin II activates MAPK and stimulates growth of human pulmonary artery smooth muscle via AT1 receptors. *Am J Physiol* 277:L440-448.
7. Dignam, J.D., Lebovitz, R.M., and Roeder, R.G. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 11:1475-1489.
8. Mitani, Y., Zaidi, S.H., Dufourcq, P., Thompson, K., and Rabinovitch, M. 2000. Nitric oxide reduces vascular smooth muscle cell elastase activity through cGMP-mediated suppression of ERK phosphorylation and AML1B nuclear partitioning. *Faseb J* 14:805-814.
9. Hodge, D.R., Peng, B., Pompeia, C., Thomas, S., Cho, E., Clausen, P.A., Marquez, V.E., and Farrar, W.L. 2005. Epigenetic silencing of manganese superoxide dismutase (SOD-2) in KAS 6/1 human multiple myeloma cells increases cell proliferation. *Cancer Biol Ther* 4:585-592.
10. Shurin, G.V., Ferris, R.L., Tourkova, I.L., Perez, L., Lokshin, A., Balkir, L., Collins, B., Chatta, G.S., and Shurin, M.R. 2005. Loss of new chemokine CXCL14 in tumor tissue is associated with low infiltration by dendritic cells (DC), while restoration of human CXCL14 expression in tumor cells causes attraction of DC both in vitro and in vivo. *J Immunol* 174:5490-5498.
11. Hansmann, G., Wagner, R.A., Schellong, S., Perez, V.A., Urashima, T., Wang, L., Sheikh, A.Y., Suen, R.S., Stewart, D.J., and Rabinovitch, M. 2007. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. *Circulation* 115:1275-1284.
12. Teichholz, L.E., Kreulen, T., Herman, M.V., and Gorlin, R. 1976. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. *Am J Cardiol* 37:7-11.
13. Ye, C.L., and Rabinovitch, M. 1991. Inhibition of elastolysis by SC-37698 reduces development and progression of monocrotaline pulmonary hypertension. *Am J Physiol* 261:H1255-1267.