SUPPLEMENTAL MATERIAL

Supplemental Methods

shRNAs

The BMPR2 (Mouse: TRCN0000022529, Human: TRCN0000000456), CTCF (TRCN0000014548), DNMT1 (TRCN0000021890), FOXK2 (TRCN0000016288), SIN3a (TRCN0000021777) and TET1 shRNAs (TRCN0000075024) were cloned in the pLKO.1 lentiviral expression vector and were obtained from Dharmacon.

Lentivirus preparation

The constructs and viral packaging plasmids (pSPAX2 and pMD2.G) were co-transfected into 293T cells using Lipofectamine 2000 (Invitrogen Life Technologies) per the manufacturer's recommendations. The media was removed 12 hours post-transfection and replace with fresh media. Virus particles were harvested 48 hours later. After infection with lentivirus shRNA particles, protein and RNA expression was determined by immunoblotting and RT-qPCR to validate all knockdown or overexpression in the present study.

siRNA experiments

hPASMCs and hPAECs were seeded at 250 000 cells/well in 6-well plates and maintained in a 37°C incubator with 5% CO2 for 24 hrs before transfection. For siRNA knockdown experiments, human SIN3a siRNA (AM16708; clone ID: 108731) and negative control siRNAs were purchased from Invitrogen. Lipofectamine RNAiMax was used to transfect 30 nM/well of siRNA following the manufacturer's instructions. SIN3a knockdown was confirmed by immunoblotting and RT-qPCR.

Cell proliferation

hPASMCs and hPAECs proliferation were measured by 5-Bromo-2'-deoxyuridine (BrdU) incorporation using the Cell Proliferation ELISA, BrdU (colorimetric) assay (Roche, Indianapolis, IN), according to the manufacturer's instructions.

Cell Migration

Migration of hPASMCs and hPAECs was assessed using a micro Boyden Chamber QCM™ 24- Well Colorimetric Cell Migration assay (ECDM 508, Millipore, Billerica, MA) according to the manufacturer's instructions. Migrated cells were quantified using a microplate reader at 560 nm by colorimetric assay. All experiments were performed in triplicate, and data are expressed as % migrated of control cells.

Reagents and inhibitors

Romidepsin (potent HDAC1 and HDAC2 inhibitor) and GSK126 (highly selective EZH2 methyltransferase inhibitor) were both purchased from SelleckChem. Concentrations and catalog numbers are provided in Table S1.

ChIP-qPCR

Cells were washed twice with ice-cold PBS after removing culture media, and chromatin was cross-linked immediately by adding 1% formaldehyde (Sigma-Aldrich, F8775) prepared in cell culture media for 10 minutes at room temperature with rocking. After cross-linking, formaldehyde solution was aspirated, and excessive formaldehyde was quenched by adding 1x glycine for 10 minutes at room temperature with rocking. Glycine was removed, cross-linked cells were washed once with ice-cold PBS to remove residual

glycine, and cells were collected using a sterile cell scraper. Cells were resuspended and washed by centrifugation in sterile ice-cold PBS containing 1x Halt protease inhibitor cocktail (Thermo Fischer).

To the cell pellet, cell lysis buffer containing protease inhibitor cocktail was added, followed by the addition of Nuclear Lysis Buffer and ChIP assay was carried out as recommended by the manufacturer using EZ-Magna ChIP™ Chromatin Immunoprecipitation Kit (Millipore, 17-10086). Briefly, soluble chromatin was prepared by centrifugation at 10,000g for 10 minutes at 4°C after sonication. About 50ul of chromatin was used per immunoprecipitation, and 5ul (10% of IP) of chromatin was saved as input material to determine relative enrichment in IP sample. 5ug of antibody was used per IP, and incubation was carried out overnight at 4oC in a rotating wheel platform. Antibodies used for ChIP are as follows: H3K27me3 (Abcam, ab6002), FOXK2 (Abcam, ab5298), CTCF (Cell Signaling, #3418). Non-specific IgG was incubated simultaneously to each reaction as control. After IP, the chromatin-antibody conjugates were washed sequentially with low salt buffer, high salt buffer, lithium chloride buffer, TE buffer by rotation at 4°C. Reverse cross-linking of Protein-DNA complexes was carried out in presence of Proteinase K at 62°C for 2 hours with shaking (1400rpm), followed by 95°C for 10 minutes. Previously saved 10% input material was also included during reverse cross-linking. Input DNA and immunoprecipitated DNA were precipitated overnight at -80°C, and DNA was resuspended in equal volumes of nuclease-free water. A 300bp region within BMPR2 promoter was amplified using the following primers: forward - GCAGCCTCTCACACCCACT and reverse – GGCAGCCCTAGTCGCATC. Relative enrichment of BMPR2 promoter sequence in input, ChIP, and IgG samples was quantified using Fast SYBR Green qPCR system (Applied Biosystems). DNA isolated from negative IgG control IP samples showed undetermined to negligible DNA amplification for BMPR2 promoter ensuring antibody specificity. Enrichment of DNA sequence (BMPR2 promoter) in IP samples

was then calculated as percentage of input (% input) within the sample. Fold change between groups was expressed as relative to control samples.

ChIP Sequencing and Data Analysis

For ChIP-seq experiments in hPASMC, the samples were prepared and processed as above using the EZ Magna ChIP™ Chromatin Immunoprecipitation Kit (Millipore) and a specific H3K27me3 antibody (Abcam, ab6002) overnight to pull down genomic DNAs. To ensure successful library preparation and sequencing, all samples received first undergo quality control assessment before sequencing. Chromatin DNA quality was assessed by (1) Nanodrop for preliminary quantitation, (2) Agarose Gel Electrophoresis to test RNA degradation and potential contamination (3) 2100 Bioanalyzer (Agilent Technologies). ChIP DNA samples were sequenced by Novogene (Sacramento, CA, USA).

Methyl-qPCR

Methylation-specific PCR (MS-qPCR or MSP) is one of the most commonly used methods for gene/sequence-specific detection of DNA methylation. Genomic DNA was extracted from both lung tissues and cells (hPASMCs and hPAECs) using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA samples were directly sent to the Epigentek company to perform all the MS-PCR experiments. Epigentek designed specific primers for methylation. Briefly, 100 ng of DNA was used to perform bisulfite conversion using the Methylamp DNA Bisulfite Conversion Kit (Epigentek Cat. # P-1001). The conversion efficiency of bisulfite-treated DNA was determined by real-time PCR using two primer pairs using control DNA. The first primer pair targets bisulfite-converted DNA (β-actin), and the second primer pairs are against unconverted DNA (GAPDH), for the same bisulfite-treated DNA sample. DNA was shown to be >99% converted.RT-qPCR was performed in duplicate using 1μl of

modified DNA, and gene-specific primers were designed for the mouse and rat promoter/exon 1 region of BMPR2 (Met: methylated target, Un: unmethylated target); Met-BMPR2-Forward: 5'-TTTAGTTACGAGGGAGCG-3'; Met-BMPR2-Reverse: 5'-TTATCCTACCCGAATTACAA-3'; Un-BMPR2-Forward:5'-TTTTTTAGTTATGAGGGAGTG-3; Un-BMPR2-Reverse: 5'-TATCCTACCCAAATTACAATAC-3'. The primer-bound sequences are the same for both mouse and rat. For Human samples, the Beta-actin housekeep gene was used as an internal control (indicating an input amount of bisulfite-converted DNA), and fully methylated HeLa DNA was used as a positive control. Gene-specific primers were designed against the promoter region of the human BMPR2 (NG_009363.1): Met-BMPR2-Forward: 5'-ATAGGGTCGGGAAGAGTCG-3'; Met-BMPR2-Reverse: 5'-GCCGCAACTACCAATCTACG-3'; Un-BMPR2-Forward:5'-AGGGTTGGGAAGAGTTGGAG-3; Un-BMPR2-Reverse: 5'-ATCACCACAACTACCAATCTACA-3'.

Data analysis was performed using qPCR Ct values to calculate the % methylation index of each target region: $\%\ \text{Methylation index (MI)} = \left[1 - \left(\frac{2^{(Ct\,M - Ct\,uM)}}{2^{(Ct\,M - Ct\,uM)} + 1}\right)\right] \times 100\%$. The Methylation Index measures the degree of methylation at a specific gene site. A higher MI indicates a higher degree of methylation. A relative comparison of the samples can be made based on the MI. The MI for each target gene for fully methylated DNA is defined as 100%. Data are shown as relative to control.

Targeted bisulfite Sequencing

Genomic DNA was extracted from lung tissues from non-PAH controls or PAH patients using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. DNA samples were sent to Epigentek to proceed with bisulfite conversion, MS-PCR, sample library preparation, sample library quantification, Bioanalyzer QC, multiplexed NGS on an Illumina HiSeq 4000, and data analysis. Briefly, 100 ng of DNA was bisulfite-treated with the Methylamp DNA Modification Kit (EpiGentek,

Cat. # P-1001). The conversion efficiency of bisulfite-treated DNA was determined by real-time PCR using two primer pairs using control DNA. The first primer pair is against bisulfite-converted DNA (β-actin), and the second primer pairs are against unconverted DNA (GAPDH), for the same bisulfite-treated DNA sample. DNA was shown to be >99% converted. For MS-PCR, qPCR was performed in duplicate using 1µl of the modified DNA and gene-specific primers (Table 3) designed for modified DNA template. Three primer pairs were designed by Epigentek and used to selectively amplify the bisulfite converted DNA sequences in the following DNA regions: BMPR2 (Chr2: NC_000002.12: 202376007-20237606). Next, the PCR products were then purified and quantified. The sample library (15 nM) was verified on an Agilent Bioanalyzer and subjected to next-generation sequencing using an Illumina HiSeq 4000. Epigentek also performed the bioinformatic analysis.

Total RNA isolation, cDNA preparation, and quantitative RT-PCR analysis

For the mRNA expression analyses, total RNA was extracted using TRIzol™ (Invitrogen) and purified using RNeasy mini columns (Qiagen). The cDNA synthesis kit (Applied Biosystems, Foster City, CA) was used to generate cDNA according to the manufacturer's instructions. Quantitative RT-PCR was performed using the PerfeCTa SYBR™ Green FastMix kit (Quantabio) according to the manufacturer's instructions. Samples were analyzed using the ABI Prism 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Fold changes in gene expression were determined using the relative comparison method with normalization to a housekeeping gene as an internal control. The primer sequences are provided in Supplemental Table 3.

mRNA sequencing and transcriptome data analysis

Total RNA was extracted using TRIzol™ (Invitrogen) and purified using RNeasy mini columns (Qiagen), according to the manufacturer's recommendations. To ensure successful library preparation and

sequencing, all samples first undergo quality control assessment. RNA sample quality was assessed by NanoDrop and Agilent 2100 BioAnalyzer. In addition, RNA degradation and contamination were monitored on 1% agarose gels. Samples with RNA integrity number (RIN) above 7, OD260/280: 2, and OD260/230 $\geq$ 2 were used for RNA-seq. Eukaryotic mRNA sequencing (mRNA-Seq) utilizes the technology of next-generation sequencing (NGS) to reveal the expression profiles of mRNA. Illumina sequencing was carried out by Novogene (Sacramento, CA, USA) using a state-of-the-art Illumina NovaSeq 6000 platform, 250-300 bp insert cDNA library with paired-end 150 bp sequencing strategy.

Differential gene expression

Read counts obtained from Gene Expression Analysis were used for differential expression analysis. For the samples with biological triplicates, differential expression analysis of two conditions/groups was performed using the DESeq2 R package (Anders et al., 2010) to provide statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate.

Bioinformatics and data visualization

Data analysis and visualization were performed using Clustergrammer (Fernandez et al., 2017), which is freely available at http://amp.pharm.mssm.edu/clustergrammer/. Clustergrammer is a web-based tool for visualizing and analyzing high-dimensional data as interactive and shareable hierarchically clustered heatmaps. Clustergrammer enables intuitive exploration of high-dimensional data and has several optional biology-specific features. *Volcano plots.* Volcano plots are used to infer the overall distribution of differentially expressed genes. Log2-fold changes and statistical significance of each gene were calculated

by performing a differential gene expression analysis. Gene fold changes were transformed using log2 and displayed on the x-axis; P-values were corrected using the Benjamini-Hochberg method, transformed using –log10, and displayed on the y-axis. The p-value cutoff to display significant genes on the plot was setup at 0.05. The threshold of the absolute log2-Fold Changes to indicate differentially expressed genes was set up at 1.5. *Heatmap.* Prior to displaying the heatmap, the raw gene counts were normalized using the logCPM method, filtered by selecting the 2500 genes with most variable expression, and finally transformed using the Z-score method.

SDS-PAGE and Immunoblot analysis

Cell and tissue lysates were prepared using RIPA lysis buffer (Invitrogen) containing a Protease Inhibitor Cocktail (Roche) and a Phosphatase Inhibitor Cocktail (Sigma-Aldrich). After centrifugation for 20 min at 15000 × *g*, the protein concentrations were determined using a bicinchoninic acid (BCA) assay (Thermofisher Scientific). The proteins were then separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skim milk and hybridized overnight at 4°C with the primary antibodies listed in Table S1. The membranes were then incubated with the appropriate secondary HRP-conjugated antibody (Cell signaling), and the blots were developed using the ECL System (Thermo Scientific, Rockford, IL).

Immunostaining

Frozen lung sections (8 μm) were incubated in cold acetone for 20 min. Sections were then incubated with a blocking solution 10% normal goat serum for 1 hr at room temperature and incubated overnight at 4°C with a specific antibody against SIN3a (1:100) and alpha-SMA (1:250). Sections were washed three times with PBS and incubated with a secondary antibody coupled to Alexa Fluor®488 or Alexa Fluor®633

(1:200, Molecular probes) for 1h at 37C. Cover slides were mounted using a vectashield mounting medium (Vector Laboratories).

Hematoxylin & Eosin, Masson's trichrome staining and

Lung tissues were collected and inflated using a PBS/OCT solution (50:50), frozen in OCT, and kept at -80C until use. Lung sections were cut to 8 μm and stained with hematoxylin and eosin (Sigma-Aldrich) and Masson's trichrome (Sigma-Aldrich) according to the manufacturer's instructions. Sections were visualized using light microscopy. The medial thickness and collagen deposition were quantified using ImageJ software.

Wheat Germ Agglutinin (WGA) Immunostaining

RV sections were fixed were incubated in cold acetone for 20 min and then incubated with a blocking solution 10% normal goat serum for 1 hr at room temperature. RV sections were stained using a fluorescence-tagged wheat germ agglutinin antibody (WGA) (Invitrogen) overnight at 4°C. Sections were imaged using a Zeiss Observer Z.1 microscope (Carl Zeiss) at ×160 magnification. The outlines of cardiac myocytes were traced, and the cardiomyocyte area was analyzed using ImageJ software.

Hemodynamic Studies

Mice were anesthetized with (2-4%) isoflurane and intubated via tracheotomy. They were then mechanically ventilated using 1-2% isoflurane and oxygen. Once the pericardium was opened and the heart was fully accessible, an ultrasonic flow probe (flow probe 2.5S176; Transonic Systems Inc., Ithaca, NY) was inserted into the RV or PA to collect the right ventricular systolic pressure *(*RVSP) and PAP,

respectively. Hemodynamic data were recorded using a Scisense PV Control Unit (Scisense, Ontario, Canada).

Right Ventricular Weight Measurement

The heart was harvested and rinsed with cold PBS to remove the blood and any clots. Atria and connecting vessels were removed and discarded. Then, RV was separated from the left ventricle and weighed. The Fulton Index was calculated by the weight ratio of the RV weight to the LV plus septum weight to illustrate the RV hypertrophy.

Supplemental Tables

Supplemental Table 1. PAH patient pulmonary hemodynamics pre-lung transplant

	Gender	Diagnosis	Date of LTx	Age at LTx	Pre-LTx TPR (Wood units)	Pre-LTx mPAP (mmHg)	Pre-LTx CI (l/min/m2)
Patient 1	Woman	IPAH	05/19/2011	31	14.5	69	2.45
Patient 2	Man	IPAH	08/20/2010	49	13	43	3.23
Patient 3	Woman	IPAH	01/29/2011	59	9.4	53	2.85
Patient 4	Woman	IPAH	12/04/2011	31	14	61	1.6
Patient 5	Woman	IPAH	07/29/2011	18	26.4	100	1.7

Supplemental Table 2. Non-PAH control patient diagnosis at the time of surgery

	Gender	Diagnosis
Patient 1	Man	Squamous cell carcinoma
Patient 2	Man	Adenocarcinoma
Patient 3	Man	Adenocarcinoma
Patient 4	Man	Squamous cell carcinoma

Supplemental Table 3. Primer sequences for RT-qPCR analysis; clone ID; catalog numbers for shRNAs and siRNA (Open Biosystems); antibodies; source and concentration of chemical inhibitors.

Application	Gene symbol	Species	Forward primer (5′-3′)	Reverse primer (5′-3′)
RT-qPCR	BMPR2	Human	ATCCAGATTATTCTTCCTCCTC	TCACGATGCTGTCAGTATG
		Mouse	CGGTGGTGTTTGTGGTGGTT	TGAGCATTCGATGTCCAGCA
		Rat	CTTTGCCCTCCTGCTTCTTGG	CCAAGGTCTTGTTGATACGGGTC
	SIN3a	Human	TCAACCACCACCCCAACATC	CTGGCCGGAGTATGTGCTTG
		Mouse	GATTCCAGGGCCAACCAGAC	AGCCACCTGGGCATACACCT
		Rat	TTGGGAACGGCTCCATCTTT	GTCTGGTTGGCCCTGGAATC
	EZH2	Human	TCCCGCTGAGGATGTGGATA	GGGCACGAACTGTCACAAGG
		Mouse	AACAGTAGCAGACCCAGCAC	AGGAGCTGGACGTCTCATCT
		Rat	ACCCCAAACATCGATGGACC	TGGTCCACAAGGCTTGTTGT
	SUZ12	Human	TGCATTGCCCTTGGTGTACT	GCAAATCCAGGTTGGCGATG
		Mouse	GGAGACGCTGACTACAGAGC	CAGGTCGTCTCTGGCTTCTG
		Rat	TACTCGGCCTCCTCCTCTTC	GGAGGAAAAGCTCGTGGTCA
	DNMT1	Human	TAAGCACGGTCACCTGTGTC	CGATGAGGGCCTTTTCACCT
		Mouse	AGCCGCTCAAAGCAAAAGTG	TGGGGTTCATCCACAGCATC
		Rat	GCAAAGCAAGTGCAATCCCA	TCAAAGACGGACAGTGCCTC
	ELP3	Human	TACGGCGGCGCAGAAATGAG	GATATCCACCAGGCGGGGCT
		Mouse	ATCCCAATGCCCCTTGTCAG	TCTCCGTACCAACTCGACCT
		Rat	AGCTCTACCCTACCCTGGTG	TCCGAGCCACCAACTCAATC
	MBD4	Human	ACAACTGCTCACCAACCAGGA	GGTGACCGAGGAGGTGTCCA
		Mouse	AGTCCGTGGAGAGAGCCTAG	CAGTGGGCTCTTGGAGAAGG
		Rat	GCCTGTCTGAAGCTTCCTGT	TGGCAGGGGCTTAAGACATG
	TET1	Human	AGGACTCTGGGTTCTGAAAA	AGGACTCTGGGTTCTGAAAA
		Mouse	GAGCCTGTTCCTCGATGTGG	CAAACCCACCTGAGGCTGTT
		Rat	CCCCAGTCCCCTCCATCTA	TTTCCTGAGGGCTGATGCTG
	ANP	Mouse	GCTTCCAGGCCATATTGGAG	GGGGGCATGACCTCATCTT
		Rat	CCCGACCCACGCCAGCATGG	CAACTGCTTTCTGAAAGGGGT
	BNP	Mouse	CTGGGAAGTCCTAGCCAGTC	TTTTCTCTTATCAGCTCCAGCA
		Rat	ACAATCCACGATGCAGAAGCT	GGGCCTTGGTCCTTTGAGA
	β-MHC	Mouse	ACTGTCAACACTAAGAGGGTCA	TTGGATGATTTGATCTTCCAGGG
		Rat	ACAGAGGAAGACAGGAAGAACCTAC	CACAAGATCTACTCCTCATTCAG
	COLI	Mouse	CTGGCAAGAAGGGAGATGA	CACCATCCAAACCACTGAAA
		Rat	AATGGTGCTCCTGGTATTGC	GGTTCACCACTGTTGCCTTT
	COLIII	Mouse	GATGGAAACCCTGGATCAGA	GCACCAGGAGAACCATTTTC
		Rat	GAGATGTCTGGAAGCCAGAACCATG	ATCTCCCTTGGGGCCTTGAGGT
	CTGF	Mouse	GTCTGCGCCAAGCAGCTG	ACGTCCATGCTGCACAGGG
		Rat	CCCGTTAGCCTCGCCTTGG	GGTACACGGACCCACCGAA
	TGFβ	Mouse	CCTGCAAGACCATCGACATGGAG	GGTCGCGGGTGCTGTTGTA
		Rat	GGTCGCGGGTGCTGTTGTA	CCTGCAAGACCATCGACATGGAG
	TET2	Human	AACGGGCTTCCATTCTGGAG	AGCCTGATGGAACAGGACAG
	HDAC1	Human Mouse Rat	GCGATGTCCGTCTGCTGCTTAT	CTGAGGAGATGACCAAGTACCACA
	HDAC2	Human Mouse Rat	TCACAATCAAGGGCAACTGCAGTCT	ACAGGAGACTTGAGGGATATTGGTGC
	MECP2	Human	GCCTGGCTGTGACCAAGTAT	GCCTGGCTGTGACCAAGTAT
		Mouse	GGAAGGGACTGAAGACCTGC	TGGTGGTGATGATGGTGCTC
		Rat	AACCACTACAGCCTTCAGCC	CACGAATGATGGAACGTCGC

	CTCF	Human	GAATTGGTTCGGCATCGTCG	GTGTCCCTGCTGGCATAACT
		Mouse	GAATTGGTTCGGCATCGTCG	TGTTTGGGCTGGTTGGTTCT
		Rat	ATTGAACCTGAGCCAGAGCC	TGGCTGGTTCTGTTTGGGTT
	FOXK2	Human	GGACAGGGCAGTGAACGTTA	CTTGGGCAAGAACGGGGTAT
		Mouse	TATGTTGGCCACCCATGCAT	TTCTCTCACAGCTGCTGGTG
		Rat	TATGTTGGCCACCCATGCAT	TTCTCTCACAGCTGCTGGTG
	WHSC1L1	Human	AGTGACAGCTCCGTCTCCGC	GCCGCCGACAAAGAAATCTTGC
	WHSC1	Human	CAGCAGCCCCCTATTGCTGC	GCTGCATCTCCTGCAACGCA
	PHF8	Human	TGGGGACAGAACGCGGGTAG	TCCCACGGTCTCTGCGTTGT
	KDM6B	Human	CCCCACCCACTGTGGTCTGT	ATCACCCGTGTCTCCGCCTC
	JHDM1D	Human	CGAGGCAGGCAGACAGCAAA	TCAGGGACCTCCACCAATTCAGA
	SIN3b	Human	CACCCATGAGCGACAGATCC	GGGCTGCTGGTAGGTTTTGG
		Mouse	GTTGTCACGGATGGCTCCTG	CTCCTCCTCCTTGGCCTTCA
		Rat	TCCCAAGAACGGCAAGTTGA	CACCTGGCCTCTGTCCTCCT
	SAP45	Human Mouse Rat	AGGACGACGAGCGCAGCT	TGTTCAGTTTCCAGCTGGAGGAAGA
	SAP130	Human Mouse Rat	AATCCAGTGGCCATGGAAACCC	CTGCATGGGGTTCAGGTGGACT
	SAP180	Human Mouse Rat	TTCTTCAACAGTGGAAGTTCAGGATGA	GCTGGTCTAGTGTTTCACTTTCAGC
	RBBP4	Human Mouse Rat	GAAATCAAGATCAACCATGAAGGAGAAGT	GGTCATCTGAAGCACTAAGTAAGTGCC
	RBBP7	Human Mouse Rat	ACCTGGTTATGACCCATGCTCTTCA	GCTTTAGCATCCACAATTTTGCCTTC

shRNAs	Gene symbol	Clone ID	Catalog number
Mouse	BMPR2	TRCN0000022529	RMM3981-201753556
Human	BMPR2	TRCN0000000456	RHS3979-201732730
	CTCF	TRCN0000014548	RHS3979-201746126
	DNMT1	TRCN0000021890	RHS3979-201752917
	FOXK2	TRCN0000016288	RHS3979-201747866
	SIN3a	TRCN0000021777	RHS3979-201752804
	TET1	TRCN0000075024	RHS3979-201790100

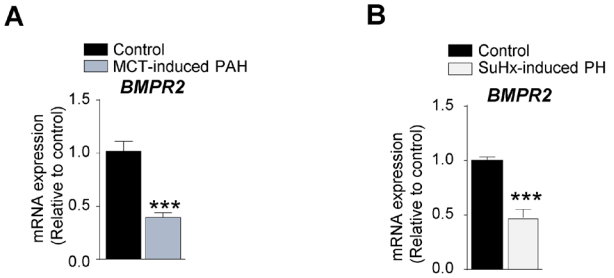
Overexpression	Gene symbol	Clone ID	Catalog number
	SIN3a	ccsbBroad304_11788	OHS6085-213584153
	EZH2	ccsbBroad304_00526	OHS6085-213573212
	BMPR2	Adenovirus	Gift from Dr. Akiko Hata

siRNA	Gene symbol	siRNA ID	Catalog number
	SIN3a	108731	AM16708

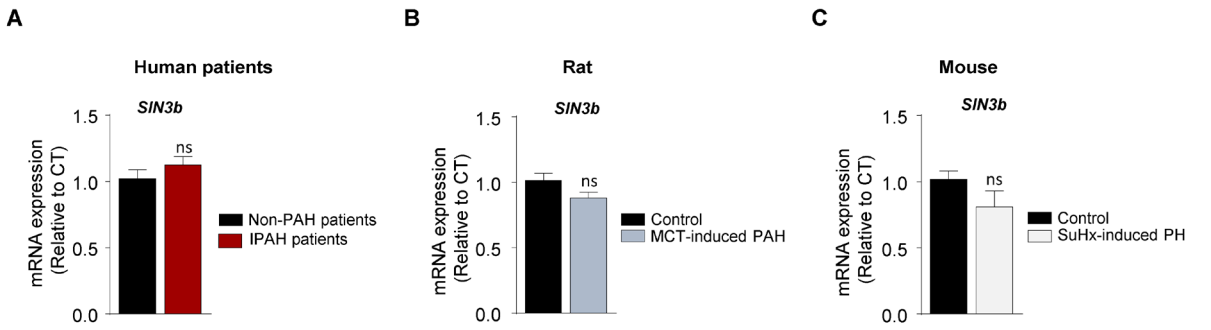
Immunoblotting	Protein symbol	Antibody source	Dilution
	SIN3a	Cell signaling	1:1000
	BMPR2	Cell signaling	1:1000
	Cyclin D1	BD Pharmingen	1:1000
	EZH2	Cell signaling	1:1000
	HDAC1	Cell signaling	1:1000

	DNMT1	Thermofisher	1:1000
	MECP2	Sigma Aldrich	1:1000
	pSMAD 1-5-9	Cell signaling	1:1000
	Tot-SMAD	Cell signaling	1:1000
	H3K27me3	Cell signaling	1:2000
	Histone H3	Cell signaling	1:2000
	TET1	Thermofisher	1:1000
	pENOS	Cell signaling	1:1000
	CTCF	Cell signaling	1:1000
	GAPDH	Sigma Aldrich	1:5000
Pharmacological agents	**Compounds**	**Concentration**	**Source**
	Romidepsin (HDAC inhibitor)	10 nmol	Selleckchem
	GSK126 (EZH2 inhibitor)	1 μM	MedChem Express
	Decitabine (DNMT1 inhibitor)	0.1 μM	MedChem Express

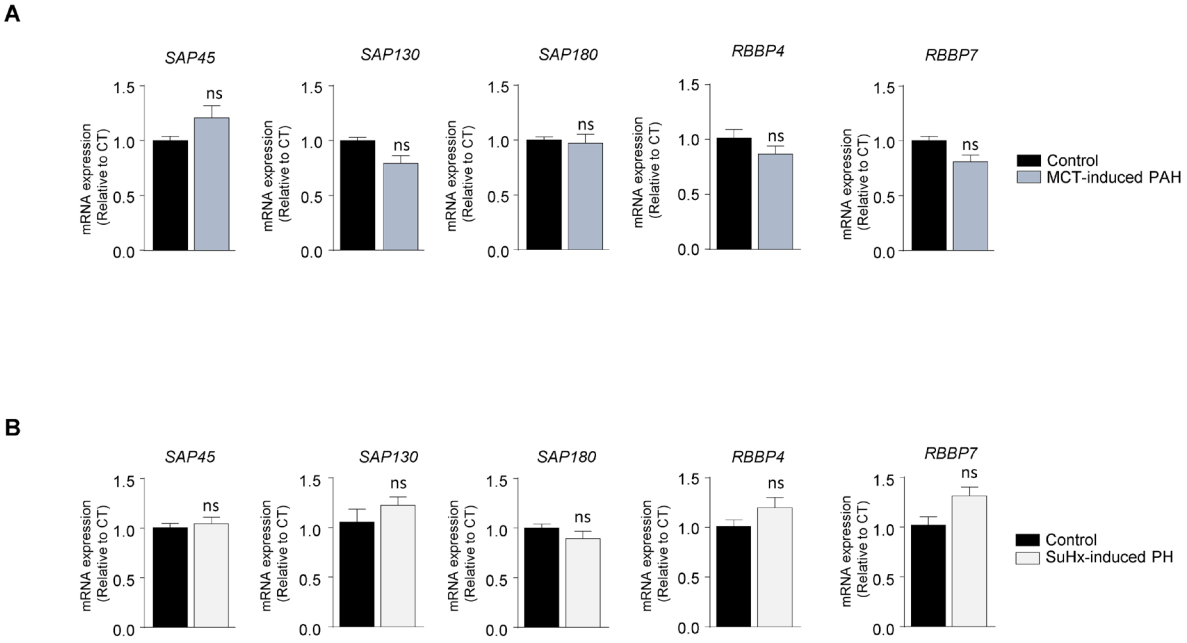
Supplemental Figures



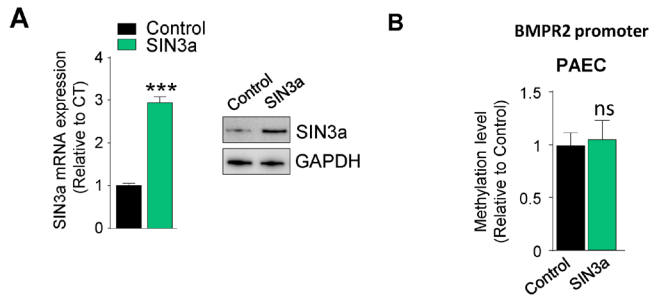
Supplemental Figure I. BMPR2 mRNA expression is downregulated in rodent models of PAH. A. BMPR2 mRNA expression was analyzed by RT-qPCR in lung tissue from controls and MCT-induced PAH rat model (n= 3). **B.** BMPR2 mRNA expression was analyzed by RT-qPCR in lung tissue from controls and SuHx-induced PAH mice model (n= 3). Data are presented as mean ±SEM; *** $P < 0.001$.



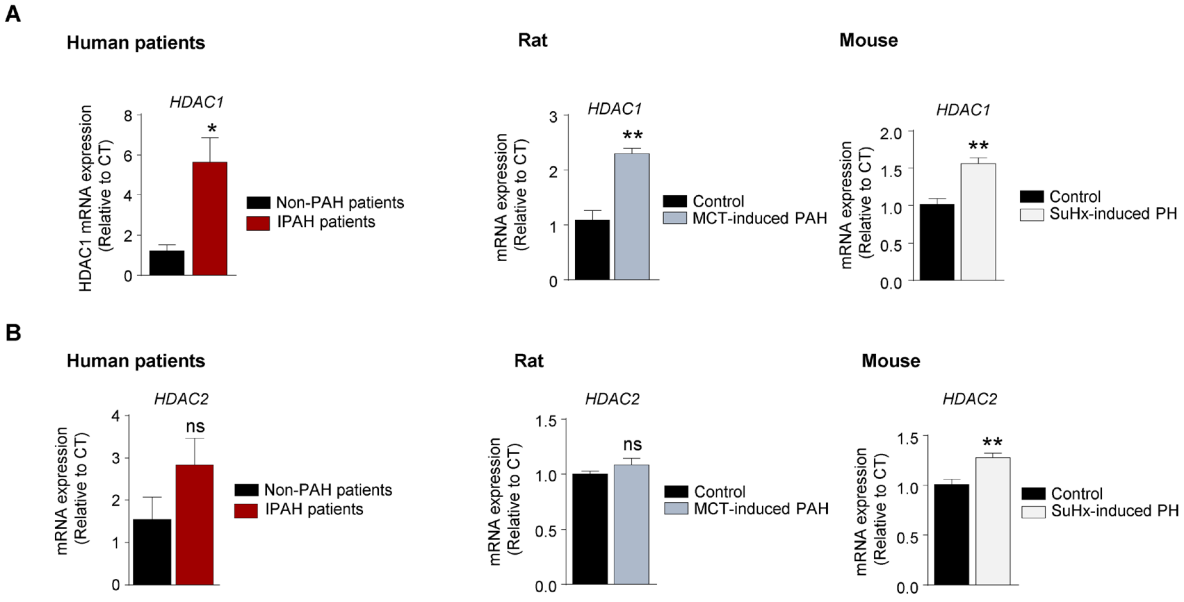
Supplemental Figure II. SIN3b mRNA expression is not altered in PAH. A-C. SIN3b mRNA expression was measured by RT-qPCR in lung tissues from non-PAH controls and patients with PAH (**A**), control sham and MCT-induced PAH rat model (**B**), control normoxia, and mouse SuHx-induced PAH model (**C**). Data are presented as mean ±SEM; ns=not significant.



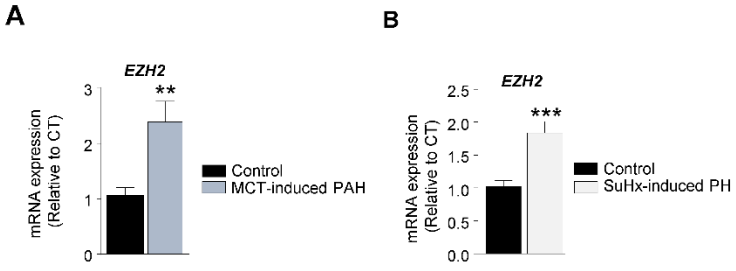
Supplemental Figure III. mRNA expression of the subunits of the SIN3 complex is not changed in animal models of PAH. A-B. SAP45, SAP130, SAP180, RBBP4, and RBBP7 mRNA expression was assessed by RT-qPCR in the MCT-induced PAH model (rat) and SuHx-induced PAH model (mouse). Data are presented as mean ±SEM; ns=not significant.



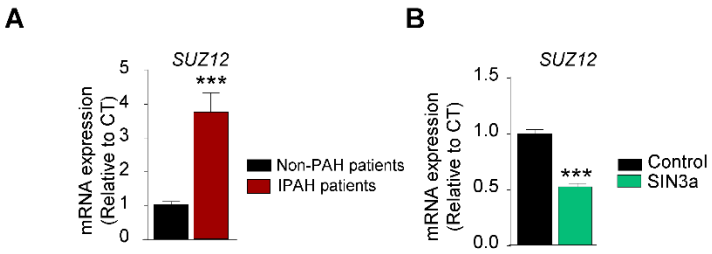
Supplemental Figure IV. Methylation level of the BMPR2 promoter in hPAECs. A. SIN3a mRNA (left) or protein (right) expression of the indicated proteins was analyzed by RT-qPCR and immunoblotting in hPAECs overexpressing either an empty vector (Vector) or SIN3a (SIN3a) lentivirus. **B**. Methylation level of the BMPR2 promoter region was analyzed by MS-PCR in hPAECs overexpressing either a control vector or SIN3a. Data are presented as mean ±SEM; ns = not significant, *** $P < 0.001$.



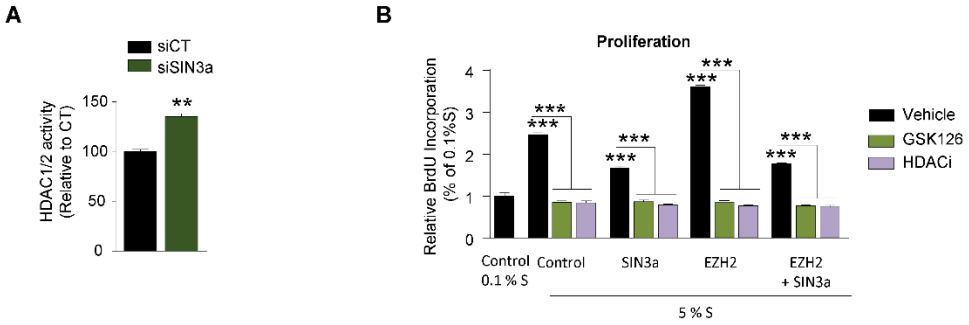
Supplemental Figure V. HDAC1 is upregulated in PAH. A. HDAC1 mRNA expression was measured by RT-qPCR in lung samples from PAH patients (human, left) panel, the MCT-induced PAH model (rat, middle panel) and SuHx-induced PH model (mouse, right panel)**. B.** HDAC2 mRNA expression was measured by RT-qPCR in lung tissues from non-PAH controls and patients with PAH (left panel), control sham and MCT-induced PAH in rats (middle panel), control normoxia and SuHx-induced PH model in mice (right panel). Data are presented as mean ± SEM; ns = not significant, * = $p<0.05$, ** = $p<0.01$.



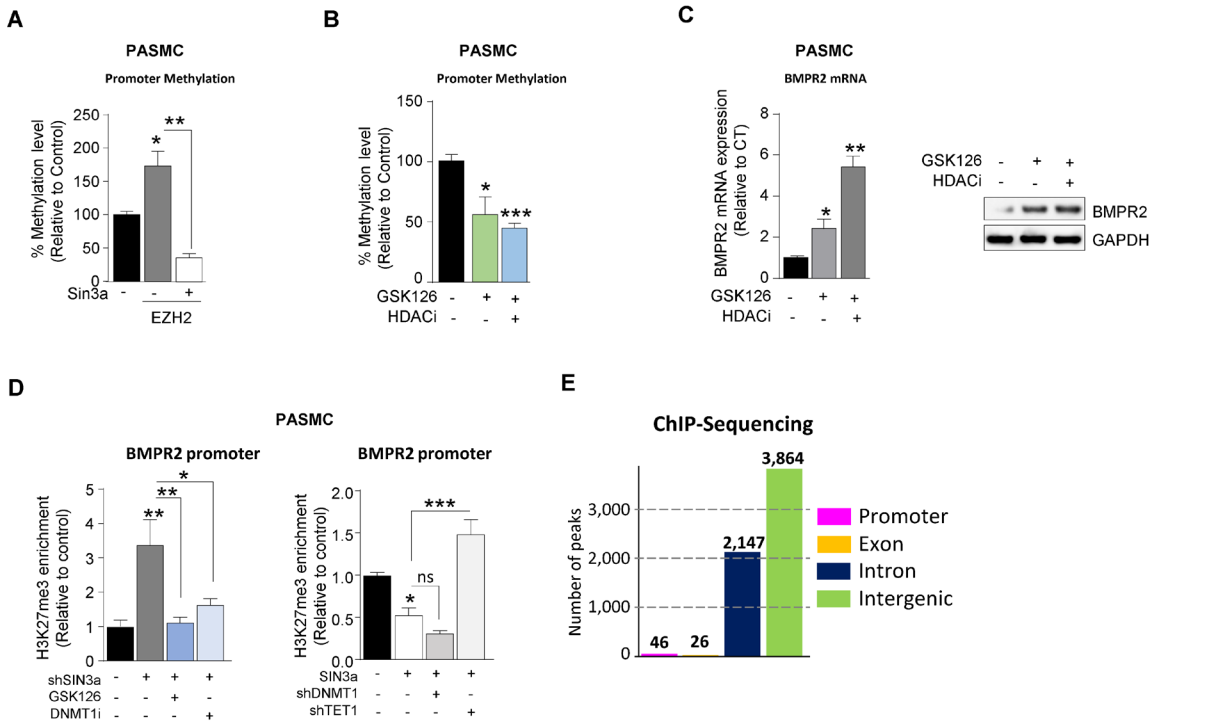
Supplemental Figure VI. Expression levels of the EZH2 transcripts are upregulated in PAH models. **A.** EZH2 mRNA expression was measured by RT-qPCR in lung samples from control sham and MCT-induced PAH model in rats. **B.** EZH2 mRNA expression was measured by RT-qPCR in lung samples from control normoxia and SuHx-induced PAH in mice. Data are presented as ±SEM; ** = $p<0.01$, *** $P < 0.001$.



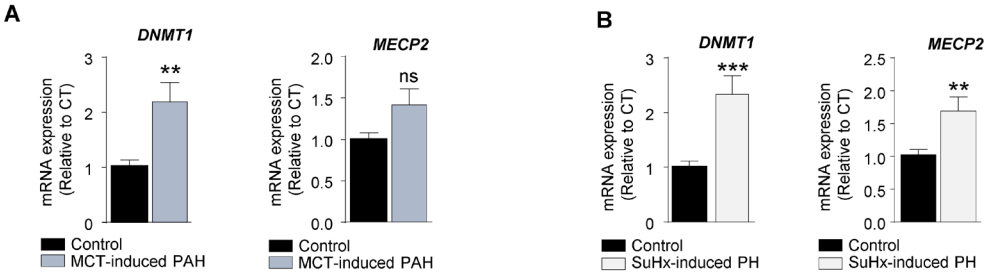
Supplemental Figure VII. SUZ12 is upregulated in lung samples from patients with PAH and down-regulated by SIN3a in PASMCs. A. SUZ12 mRNA was measured by RT-qPCR in lung tissues from non-PAH controls and iPAH patients. **B.** SUZ12 mRNA was measured by RT-qPCR in hPASMC overexpressing either SIN3a or an empty vector as control. Data are presented as mean ±SEM; *** $P < 0.001$.



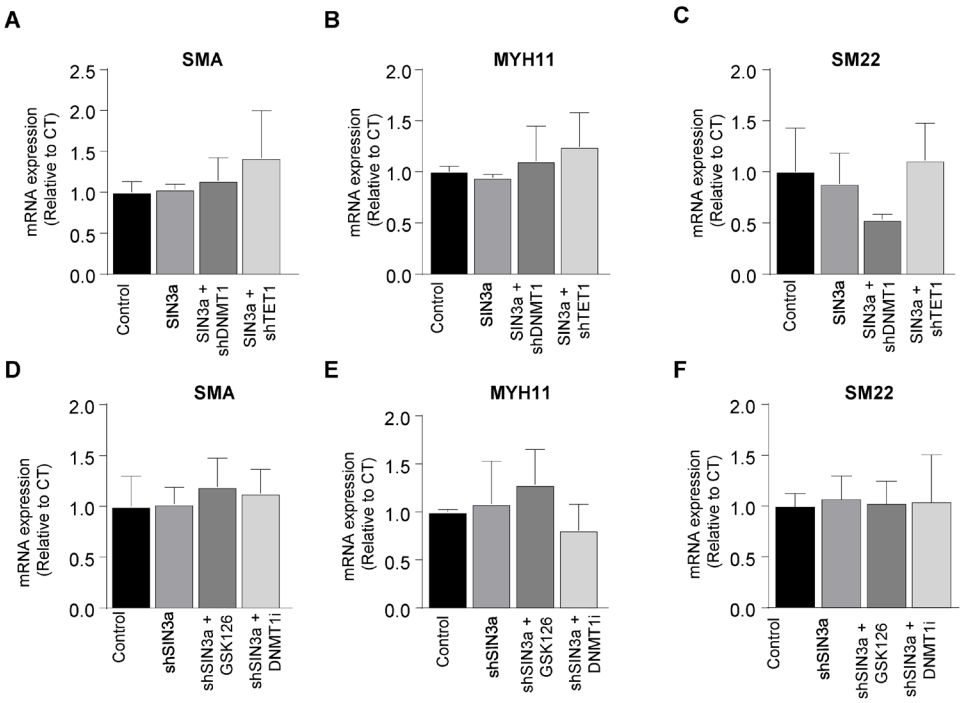
Supplemental Figure VIII. SIN3a regulates HDAC1/2 activity. A. hPASMC were transfected either with a specific siRNA against SIN3a (siSIN3a) or a non-silencing siRNA as control (siCT), and HDAC1/2 activity was determined after 72 hrs. **B.** Proliferation level was determined by BrdU in the indicated conditions after 72 hrs of treatment with the EZH2 inhibitor (GSK126, 1μM) or HDAC inhibitor (Romidepsin; HDACi; 5nM). Data are presented as mean ±SEM; ** = $p<0.01$, *** $P < 0.001$.



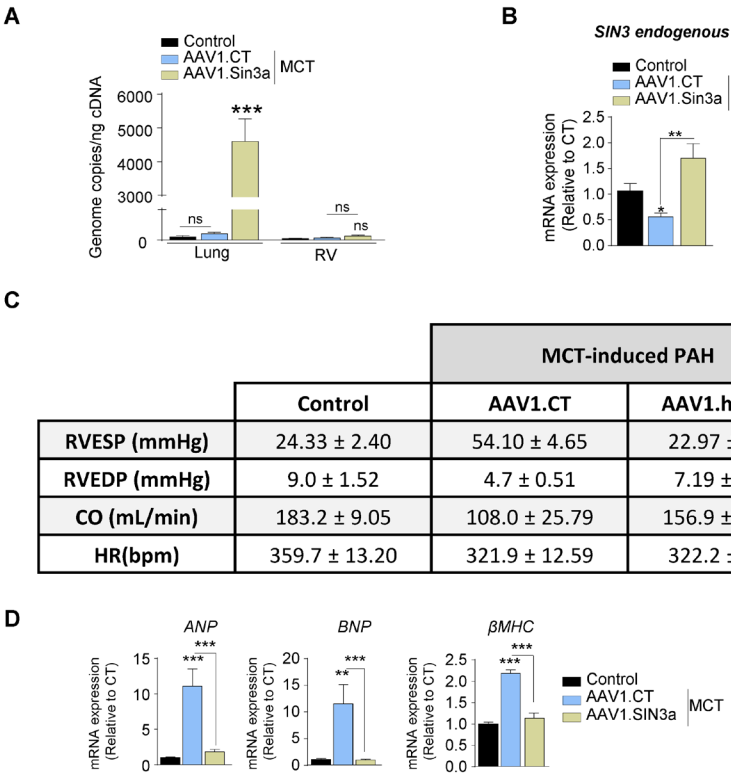
Supplemental Figure IX. SIN3a regulates DNA methylation and H3K27me3 level in the BMPR2 promoter region. A. The DNA methylation level of the BMPR2 promoter region was analyzed by MS-PCR in hPASMC overexpressing either EZH2 alone or in combination with SIN3a. **B.** The methylation level of the BMPR2 promoter region was analyzed by MS-PCR in hPASMC treated with Romidepsin alone (HDACi) or in combination with EZH2 inhibitor (GSK126). **C.** BMPR2 mRNA (left) and protein expression (right) were analyzed by RT-qPCR and western blot in hPASMC treated with GSK126 alone or in combination with HDACi for 48 hrs. **D.** Left panel: ChIP-qPCR analysis of H3K27me3 at the BMPR2 promoter in hPASMC overexpressing a non-silencing shRNA or a specific shRNA against SIN3a (shSIN3a). The cells were treated either with an inhibitor of EZH2 (GSK126) or with an inhibitor of DNMT1 (DNMT1i; Decitabine) for 72 hrs. Promoter occupancy levels are expressed as the fold change relative to the control cells infected with a non-silencing shRNA. Right panel: ChIP-qPCR analysis of H3K27me3 at the BMPR2 promoter in hPASMC overexpressing SIN3a alone or in combination with a specific shRNA against TET1 (shTET1) or DNMT1 (shDNMT1). Promoter occupancy levels are expressed as the fold change relative to the control cells infected with an empty vector. **E.** Peak distribution across promoters, exons, intra- and inter-genic regions after H3K27me3 ChIP-sequencing in hPASMC overexpressing SIN3a. Data are presented as mean ±SEM; ns=not significant, * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.



Supplemental Figure X. DNMT1 and MECP2 are upregulated in rodent models of PAH. A. DNMT1 (left panel) and MECP2 (right panel) mRNA expression was analyzed by RT-qPCR in lung tissue from control and monocrotaline-induced PAH rat model (n= 3). **B.** DNMT1 (left panel) and MECP2 (right panel) mRNA expression was analyzed by RT-qPCR in lung tissue from control and SuHx-induced PAH mice model (n= 3). Data are presented as mean ±SEM; ns=not significant, ** = $p<0.01$, *** $P < 0.001$.

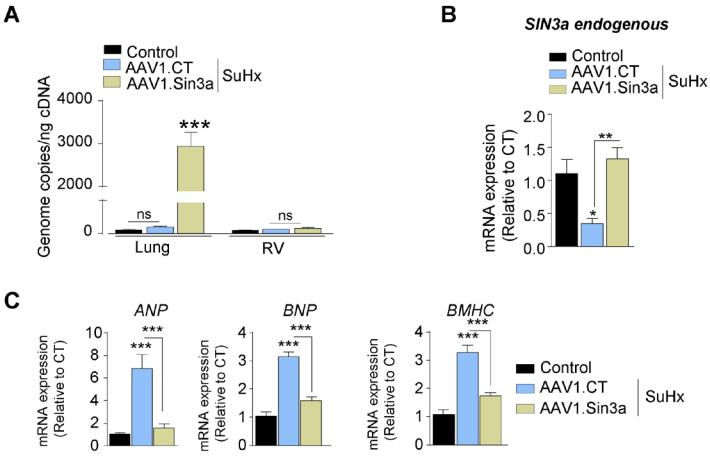


Supplemental Figure XI. SIN3a overexpression does not affect the SMC phenotype *in vitro*. A-C. hPASMC were infected with a lentivirus encoding SIN3a alone or in combination with a specific shRNA against DNMT1 (shDNMT1) or TET1 (shTET1). The expression level of several SMC markers, including α smooth muscle actin (α-SMA) (**A**), Myosin Heavy Chain 11 (Myh11) (**B**), and Smooth Muscle Protein 22-Alpha (SM-22) (**C**) was measured in the indicated conditions by RT-qPCR. **D-F.** PASMCs were infected with a specific shRNA against SIN3a for 72 hours and treated either with GSK126 or Decitabine (DNMT1i) for 48 hrs. The mRNA levels of α-SMA (**D**), Myh11 (**E**), SM-22 (**F**) was determined by RT-qPCR.

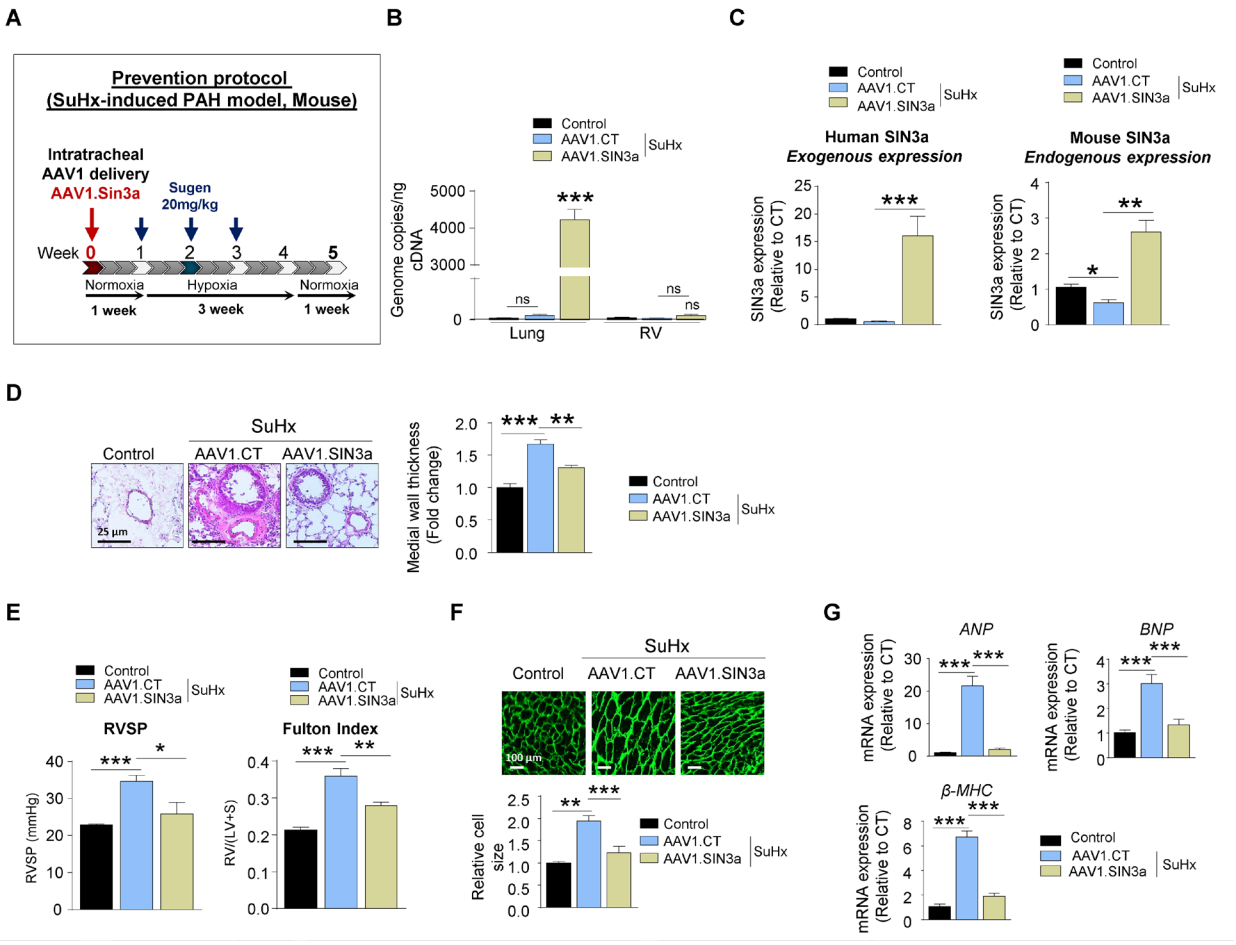


		MCT-induced PAH	
	Control	**AAV1.CT**	**AAV1.hSIN3A**
RVESP (mmHg)	24.33 ± 2.40	54.10 ± 4.65	22.97 ± 2.06
RVEDP (mmHg)	9.0 ± 1.52	4.7 ± 0.51	7.19 ± 1.93
CO (mL/min)	183.2 ± 9.05	108.0 ± 25.79	156.9 ± 19.05
HR(bpm)	359.7 ± 13.20	321.9 ± 12.59	322.2 ± 9.82

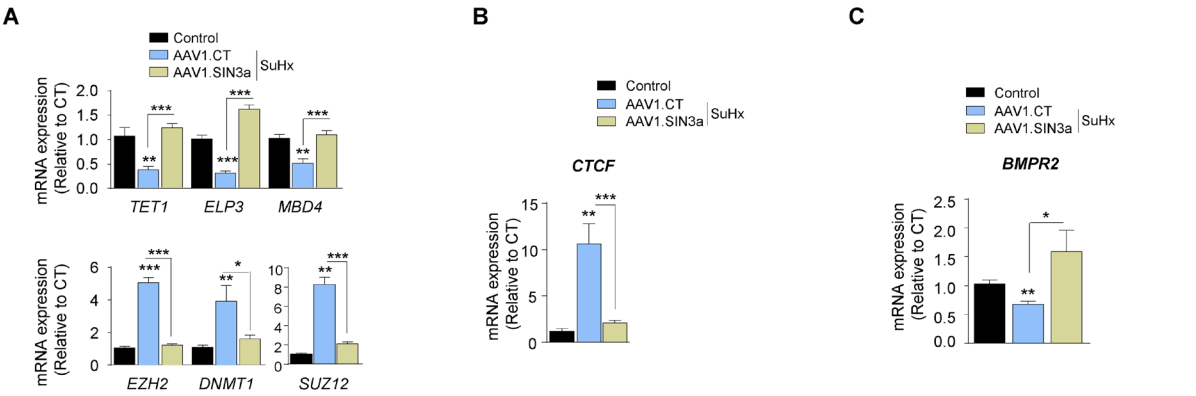
Supplemental Figure XII. Therapeutic efficacy of AAV1.hSIN3a gene therapy in MCT-induced rat model of PAH. A. Viral genome copies were assessed in the rat lungs and the RV to determine the efficiency of the IT delivery of AAV1.hSIN3a gene transfer in the MCT-induced PAH model. **B.** Endogenous SIN3a mRNA level was assessed in lung tissues by RT-qPCR in the MCT-induced PAH model. **C.** Cardiac hemodynamics were measured invasively in Control and MCT-PAH rats treated with aerosolized AAV1.CT, or AAV1.hSIN3a. RVESP: Right Ventricular End-Systolic Pressure; RVEDP: Right Ventricular End-Diastolic Pressure; CO: Cardiac Output; HR: Heart rate. **D.** Cardiac hypertrophy–related transcripts expression levels (ANP, BNP, β-MHC) in control and MCT-induced PAH rats treated either with AAV1.CT or AAV1.hSIN3a. Data are presented as mean ±SEM; ns=not significant, * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.



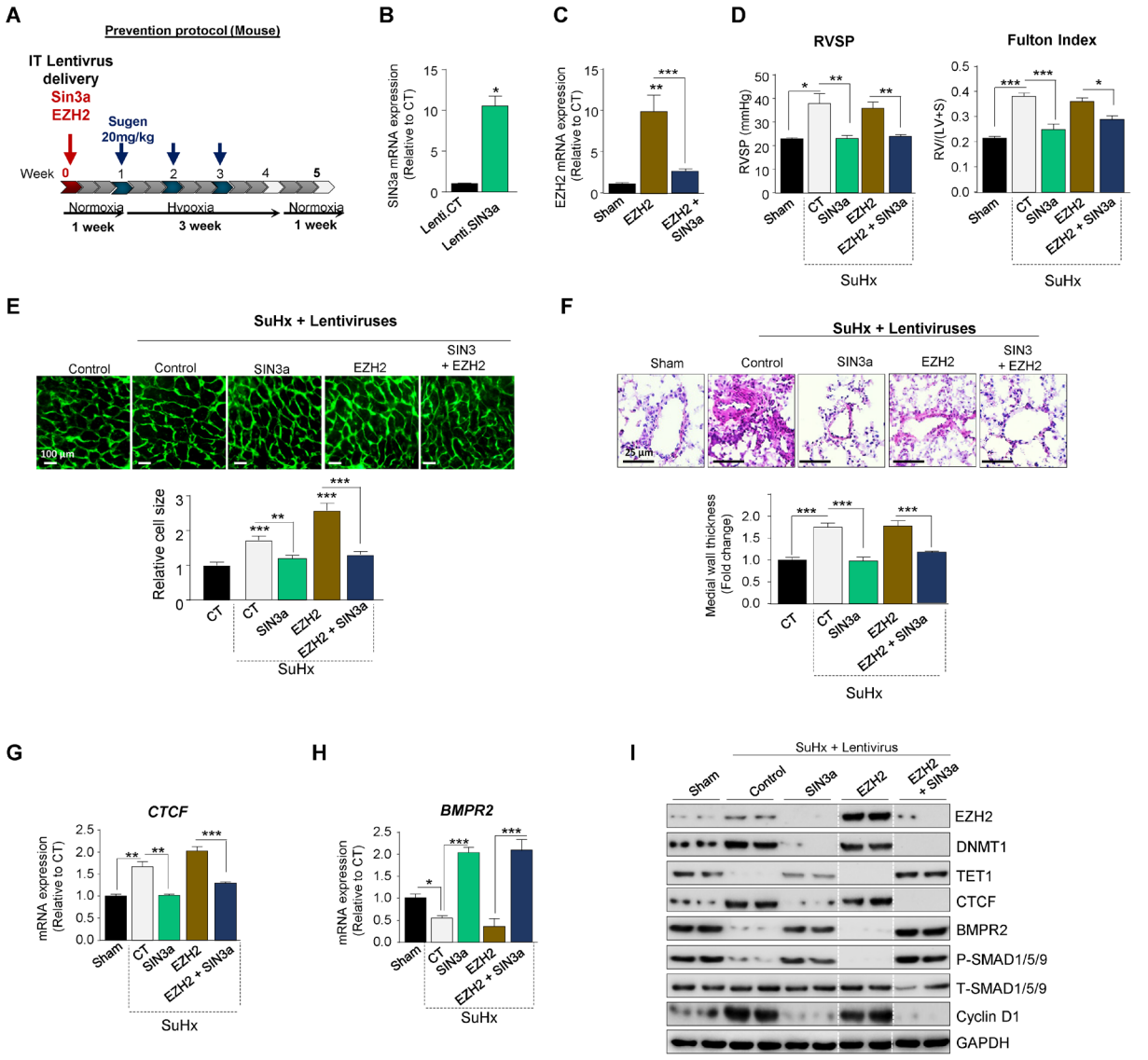
Supplemental Figure XIII. Therapeutic efficacy of AAV1.hSIN3a gene therapy in the SuHx-induced PAH model in mice. A. Viral genome copies were measured in the mice lungs and RV to determine the efficiency of the IT delivery of AAV1.hSIN3a gene transfer in the SuHx-induced PAH model. **B.** Endogenous SIN3a mRNA level was assessed in lung tissues by RT-qPCR in the SuHx-induced PAH model. **C.** Cardiac hypertrophy–related transcripts expression levels (ANP, BNP, β-MHC) were measured by RT-qPCR in control and SuHx-induced PAH mice treated either with AAV1.CT or AAV1.hSIN3a. Data are presented as mean ±SEM; ns=not significant, * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.



Supplemental Figure XIV. Preventive intratracheal delivery of AAV1.hSIN3a decreased vascular and RV remodeling in the SuHx-induced PAH in mice. **A.** Schematic of the experimental design to assess the preventive efficacy of AAV1.hSIN3a gene therapy in the SuHx-induced PAH in mice. **B.** Viral genome copies were assessed in the mice lungs and RV to determine the efficiency of the IT delivery of AAV1.hSIN3a gene transfer. **C.** Exogenous (left) and endogenous (right) SIN3a mRNA levels were assessed in lung tissues by RT-qPCR. **D.** Representative hematoxylin and eosin-stained lung sections of the indicated mice (left panel). The graph represents the quantification of the medial thickness (right panel). **E.** RVSP (left) and Fulton index (right) were determined in control and SuHx-induced PAH mice treated either with AAV1.CT or AAV1.hSIN3a. **F.** RV sections were stained with fluorescence-tagged wheat germ agglutinin to measure RV cardiomyocyte cross-sectional area. **G.** Cardiac hypertrophy–related transcripts expression levels (ANP, BNP, β-MHC) were measured by RT-qPCR. Data are presented as mean ±SEM; ns=not significant, * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.

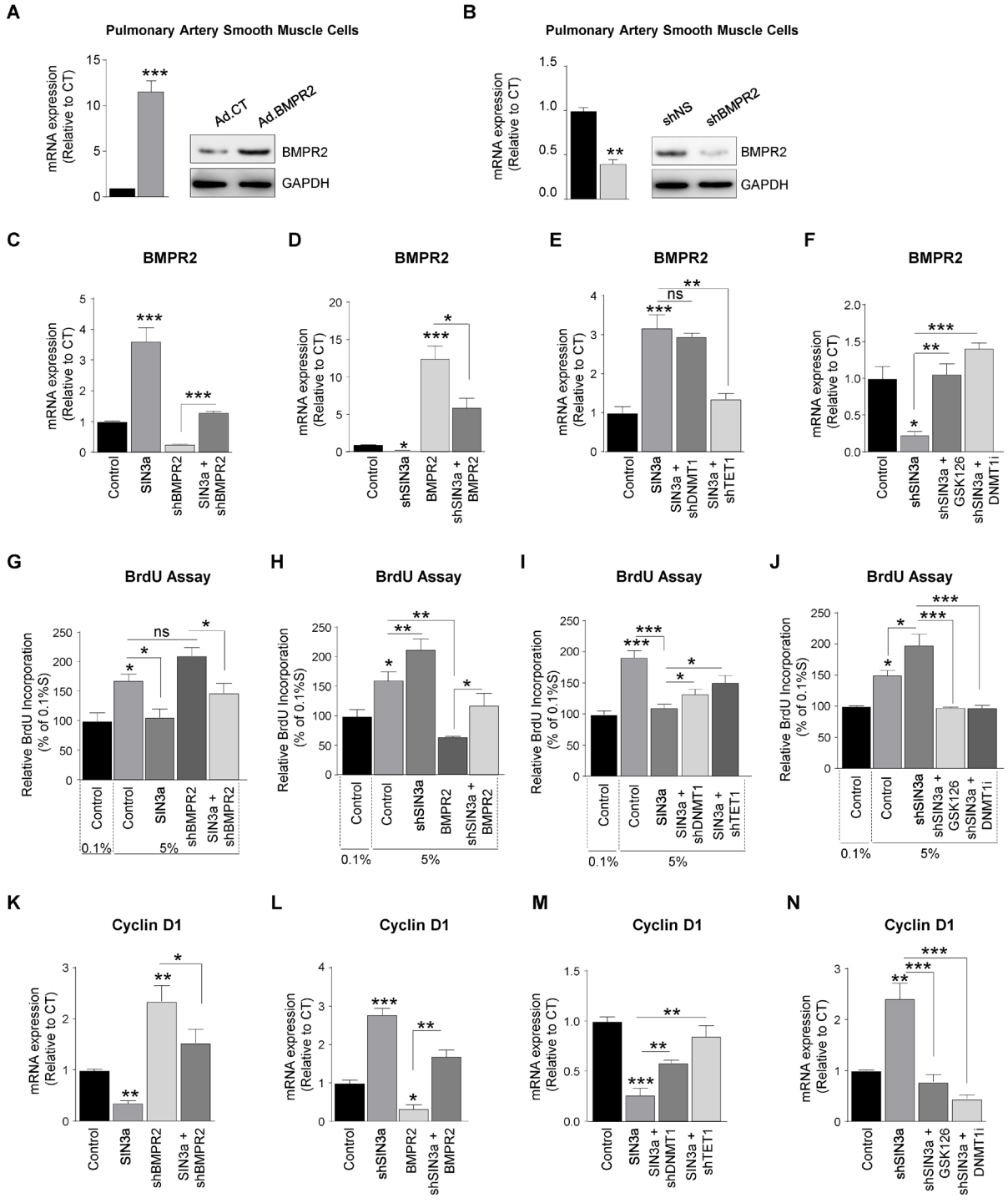


Supplemental Figure XV. Preventive intratracheal delivery of AAV1.hSIN3a regulates BMPR2 expression by modulating the expression of critical epigenetic regulators. A. Expression of the transcripts TET1, ELP3, MBD4, DNMT1, EZH2, SUZ12 was measured by RT-qPCR in lungs from control and SuHx-induced PAH mice treated either with AAV1.CT or AAV1.hSIN3a. **B.** Expression of the transcripts CTCF was measured by RT-qPCR. **C.** BMPR2 mRNA expression was assessed by RT-qPCR in lung samples from control and SuHx-induced PAH mice treated either with AAV1.CT or AAV1.hSIN3a. Data are presented as mean ±SEM; * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.



Supplemental Figure XVI. SIN3a lentivirus-mediated gene transfer reversed the effects of EZH2, prevented SuHx-induced PAH, and restored BMPR2 expression. A. Schematic of the experimental design to assess the therapeutic efficacy of SIN3a lentivirus-mediated gene transfer using the SuHx-induced PAH mouse model. **B-C.** mRNA expression of SIN3a and EZH2 was assessed by RT-qPCR in the indicated conditions. **D.** RVSP (left) and Fulton's index (right) were determined in normoxia (Control, CT) and SuHx-induced PAH mice treated either with Lenti.CT, Lenti.SIN3a or Lenti.EZH2 alone, or in combination with Lenti.SIN3a. **E.** RV sections were stained with fluorescence-tagged wheat germ agglutinin to measure RV cardiomyocyte cross-sectional area in the indicated mice. **F.** Representative hematoxylin and eosin-stained lung sections of the indicated mice. The graph represents the quantification of the medial thickness. (lower panel) **G.** CTCF mRNA levels were assessed by RT-qPCR in the indicated conditions. **H.** BMPR2 mRNA level was measured by RT-qPCR in normoxia (Control, CT) and SuHx-

induced PAH mice treated either with Lenti.CT, Lenti.SIN3a or Lenti.EZH2 alone, or in combination with Lenti.SIN3a. **I.** Lung homogenates were analyzed by Western blot for the indicated proteins. Representative immunoblots, and the respective densitometric quantitation for EZH2, BMPR2, DNMT1, TET1, CTCF, pSMAD1/5/9, and Cyclin D1. Protein expression was normalized to Total-SMAD and GAPDH. Data are presented as mean ±SEM; * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.



Supplemental Figure XVII. SIN3a regulates hPASMC proliferation in a BMPR2-dependent manner. A. BMPR2 mRNA (left) or protein (right) expression was analyzed by RT-qPCR and immunoblotting in hPASMC infected with an adenovirus encoding BMPR2 or an empty vector as control. **B.** BMPR2 mRNA (left) or protein (right) expression in hPASMC expressing a non-silencing (shNS) or the designated shRNA against BMPR2 (shBMPR2) was analyzed by RT-qPCR and immunoblotting. **C-**

F. BMPR2 mRNA was analyzed by RT-qPCR in the indicated conditions in hPASMC. **G-J.** Proliferation level was measured by BrdU assay in hPASMC in the indicated conditions after serum stimulation (0.1% or 5% FBS) for 72 hrs. **K-N.** Cyclin D1 mRNA was analyzed by RT-qPCR in the indicated conditions in hPASMC. Data are presented as mean ±SEM; ns=not significant, * = $p<0.05$, ** = $p<0.01$, *** $P < 0.001$.