

Table S1. Comparison of the relevant clinical characteristics of the patients with pulmonary arterial hypertension undergoing lung transplantation and healthy lung donors.

	Healthy Controls (n = 5)	PAH (Group 1) (n = 4)	*P value
Ethnicity/Race	The Han Chinese		
Age (yr)	32.20±11.32	36.25±14.38	0.6496
Gender (male/female)	3/2	2/2	—
sPAP (mmHg)	N/A	87.00±27.93	—
mPAP (mmHg)	N/A	59.75±18.08	—
Treatment Status	N/A	triple combination therapy (ERA+PDE5i+PCA)	
Risk Stratification	N/A	high risk	
Underlying Diseases			
Hypertension	0	1	—
Tumor	0	0	—
Tuberculosis (past)	0	0	—
Depression	0	0	—

N/A, not available; PAH, pulmonary arterial hypertension; sPAP, systolic pulmonary artery pressure; mPAP, mean pulmonary artery pressure; *, difference between healthy controls and PAH group (Unpaired t test). Data are shown as the mean ± SD.

Table S2. Cluster cell and marker genes of each cell type.

Cluster	Cell type	Marker gene
0	B	Cd19, Ms4a1
1	Neutrophil	Fcgr3, Retnlg
2	Endothelial	Cldn5, Cdh5
3	AM	Ctsd, Lpl, Plet1, Chil3
4	T	Cd3d, Cd3e
5	cMonocyte	Ly6c2, Plac8
6	NKT	Cd3d, Cd3e, Nkg7
7	NK	Nkg7
8	Fibroblast	Colla1, Pdgfra, Colla2, Bmp4
9	ncMonocyte	Ly6c2-, Plac8+
10	DC	Batf3, Irf4
11	SMC	Des, Acta2, Tagln
12	IM	C1qb
13	proliferating AM	Pclaf, Mki67
14	AT1	Krt8, Hopx, Aqp5
15	AT2	Sftpa1, Muc1, Cxcl15, Sftpd
16	MAST	Ms4a2, Cpa3
17	Megakaryocytes	Ppbp, Itga2b

Table S3. Gene sequence primers

Gene	Primer
<i>TXNIP</i> (homo)	F-5'-ATATGGGTGTGTAGACTACTGGG-3' R-5'-GACATCCACCAGATCCACTACT-3'
<i>β-ACTIN</i> (homo)	F-5'-CTCACCATGGATGATGATTCGC-3' R-5'-CACATAGGAATCCTTCTGACCCA-3'
<i>Txnip</i> (mus)	F-5'-CGAGTCAAAGCCGTCAGGAT-3' R-5'-TTCATAGCGCAAGTAGTCCAAAGT-3'
<i>β-Actin</i> (mus)	F-5'-AATCGTGCGTGACATCAA-3' R-5'-GCTCGTTGCCAATAGTGA-3'

Sup Fig1

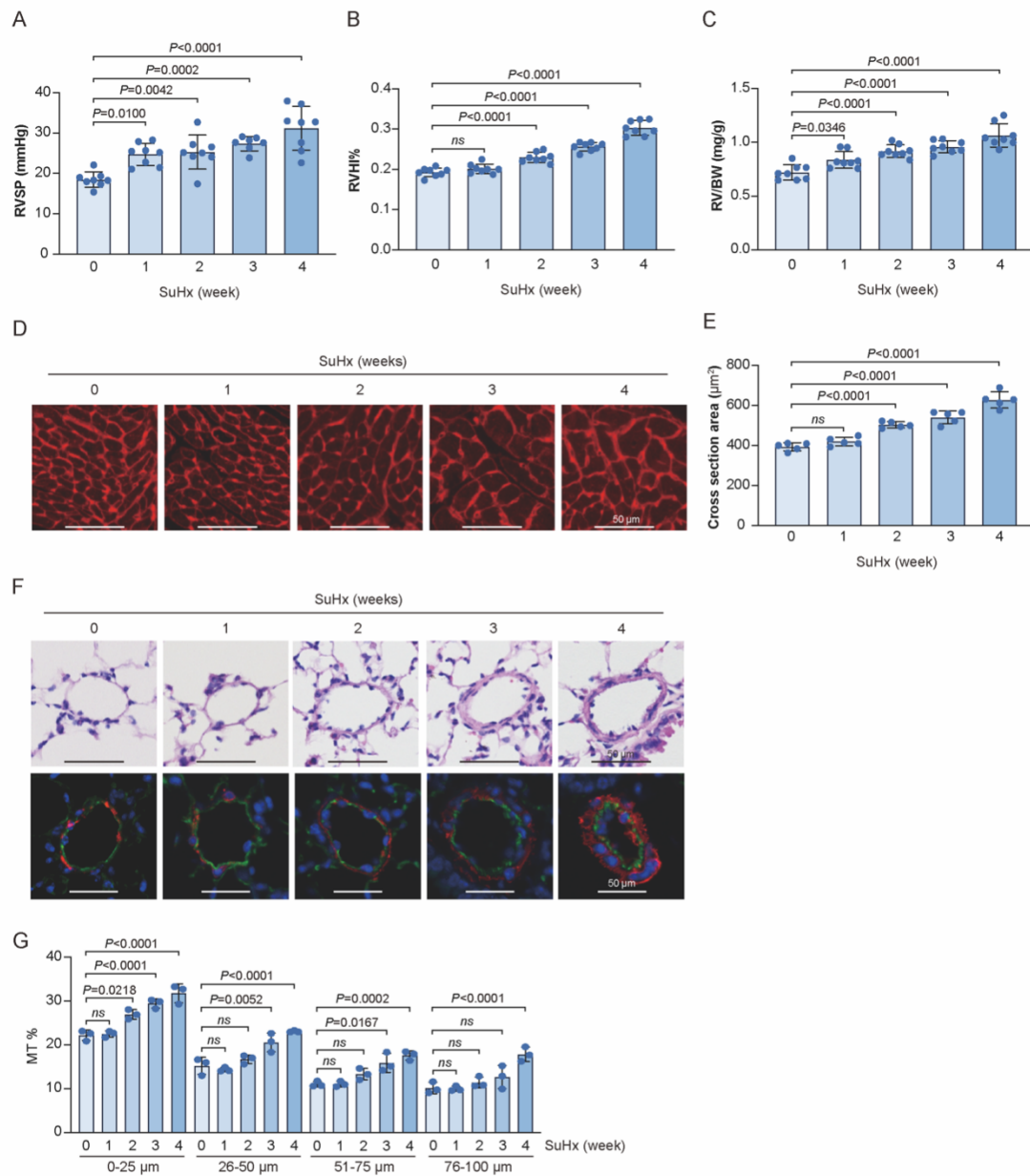


Figure S1 Time course of haemodynamic and histological changes in the SuHx murine PH surrogate. **A:** Right ventricular systolic pressure (RVSP) measured at indicated time points during PH progression. **B** and **C:** Changes in right ventricular hypertrophy index percentage (RVHI%) and the ratio of right ventricular to body weight (RV/BW). **D** and **E:** WGA staining and quantification analysis of cardiomyocyte hypertrophy in the RV (bar = 50 μm). **F:** Representative images of haematoxylin and eosin (H&E) and α-SMA (smooth muscle α-actin)/CD31 immunofluorescent (IF) staining in lung sections of the experimental mice (bar = 50

μm). **G:** Quantification of MT% of the pulmonary vasculature for vessels of 0-25 μm , 26-50 μm , 51-75 μm and 76-100 μm in diameter. n=3-5 per group: statistical analysis using one-way ANOVA.

Sup Fig2

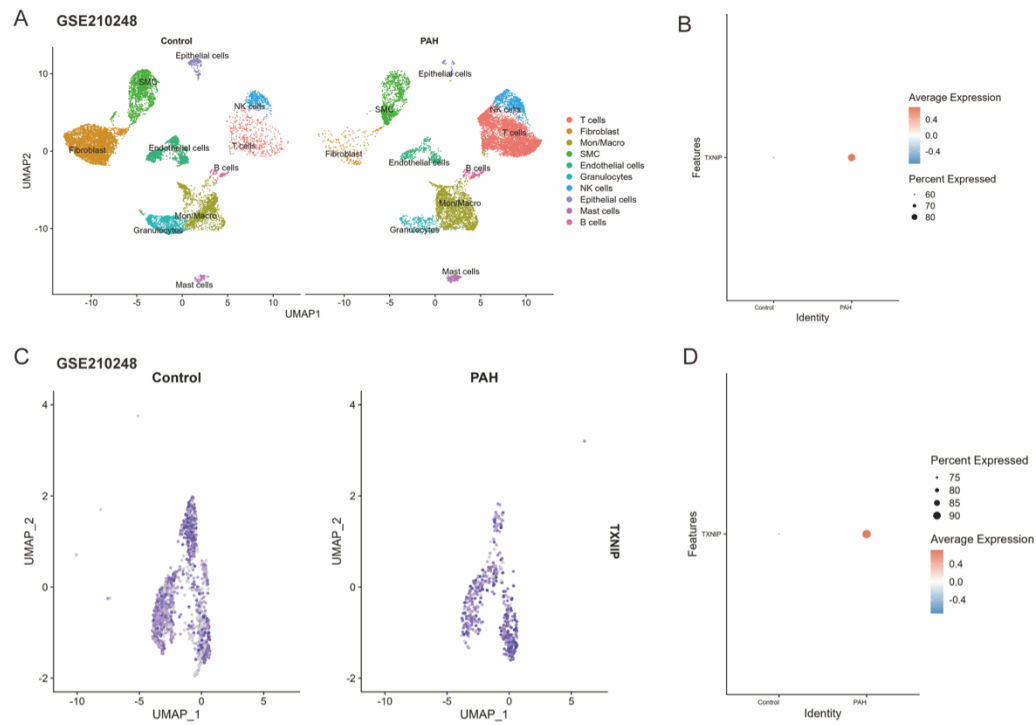


Figure S2 Elevation of TXNIP expression in GEO datasets GSE210248. A: UMAP visualization showed the presence of 10 distinct lung cell types, highlighting the diversity of cell populations within the tissue. **B:** Dot plot showing the degree of expression of TXNIP in the tissue. **C:** UMAP visualization showing TXNIP expression in endothelial cells. **D:** Dot plot showing the degree of expression of TXNIP in endothelial cells.

Sup Fig3

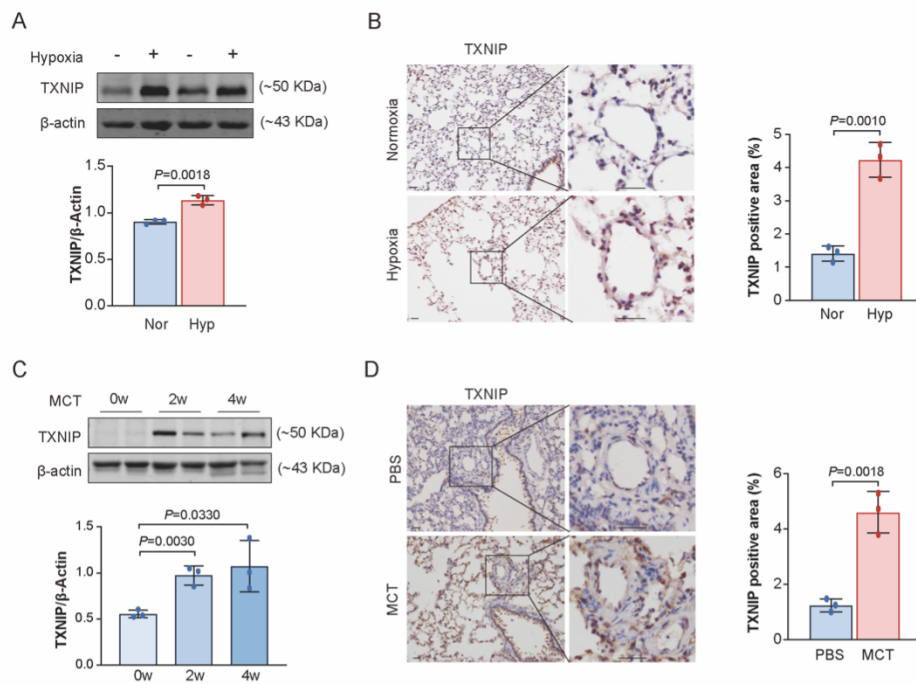


Figure S3 Elevation of TXNIP expression in the lungs of murine and rat surrogates of PH. **A:** Representative Western blot and quantification of TXNIP expression in the lungs of mice with chronic hypoxia-induced PH (n=3 per group). **B:** IHC and quantification analysis of TXNIP expression in sections of lung tissues of mice with chronic hypoxia-induced PH (n=3 per group). **C:** Representative Western blot and quantification analysis of TXNIP expression in the lungs of rats with MCT-induced PH (n=3 per group). **D:** immunohistochemical staining and quantification analysis of TXNIP expression in sections of lung tissues of rats with MCT-induced PH (n=3 per group): statistical analysis by unpaired t-test.

Sup Fig4

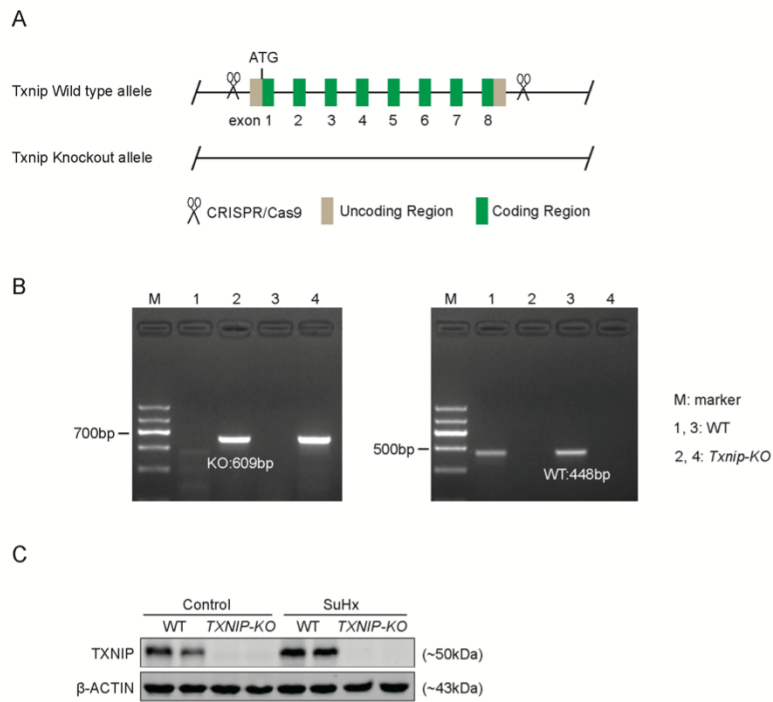


Figure S4 Construction and verification of TXNIP knockout (TXNIP-KO) mice.

A: Schematic of the strategy used to generate TXNIP-KO mice. TXNIP-KO mice were constructed by deleting exons 1-8 of the gene using the CRISPR/Cas9 system.

B: PCR genotyping of TXNIP-KO mice and their wild-type (WT) littermates. **C:** Western blotting analysis of TXNIP protein expression in the lungs of WT and TXNIP-KO mice with and without experimental PH.

Sup Fig5

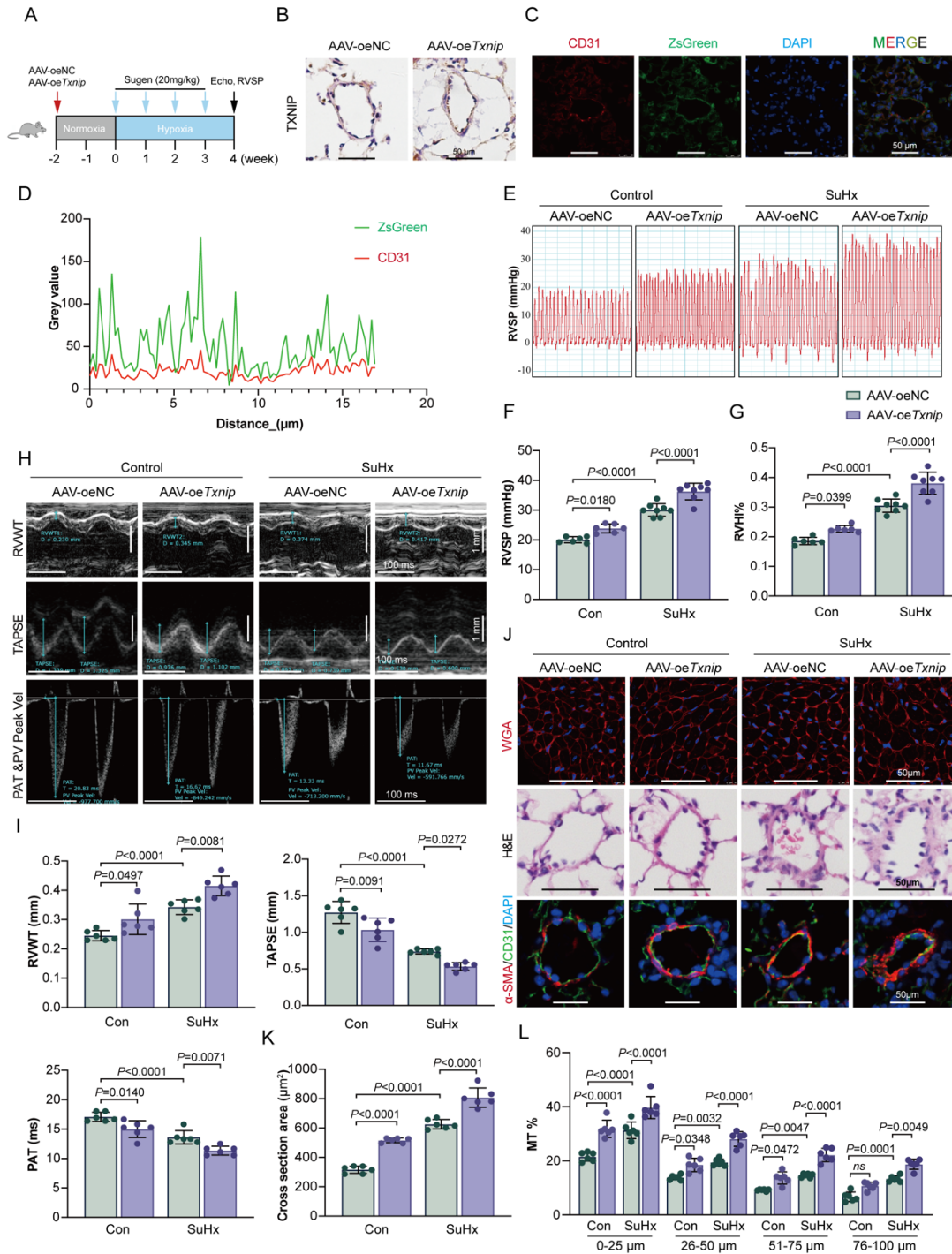


Figure S5 TXNIP overexpression in pulmonary artery endothelial cells aggravates SuHx-induced vascular remodelling. **A:** Schematic diagram of the experimental murine model. Groups of AAV-Vec-oeNC and AAV-Vec-oe*TXNIP* infected mice were exposed to hypoxia and weekly SuHx injections (SuHx); control

animals were not (Con). **B:** Immunohistochemical staining of TXNIP in the lung sections of the experimental mice (bar=50 μ m). **C and D:** Representative images and quantification of ZsGreen and CD31 IF staining within the lung sections of experimental mice (bar=50 μ m). **E and F:** Comparison of the mean right ventricular systolic pressures (RVSP) in the 4 groups. **G:** comparison of the mean right ventricular hypertrophy indices (RVHI) in the 4 groups. **H and I:** Representative echocardiographic images and quantification of right ventricular wall thickness (RVWT), tricuspid annular plane systolic excursion (TAPSE) and pulmonary artery acceleration time (PAT). **J:** WGA staining of right ventricular sections and H&E and α -SMA /CD31 immunofluorescent staining of lung sections in the experimental mice (bar=50 μ m). **K:** Quantification of cardiomyocyte cross-sectional area in the right ventricle. **L:** Quantification of MT% of the pulmonary vasculature of the experimental mice. Statistical analysis was by two-way ANOVA followed by Bonferroni correction for six pairwise comparisons among the four experimental groups.

Sup Fig6

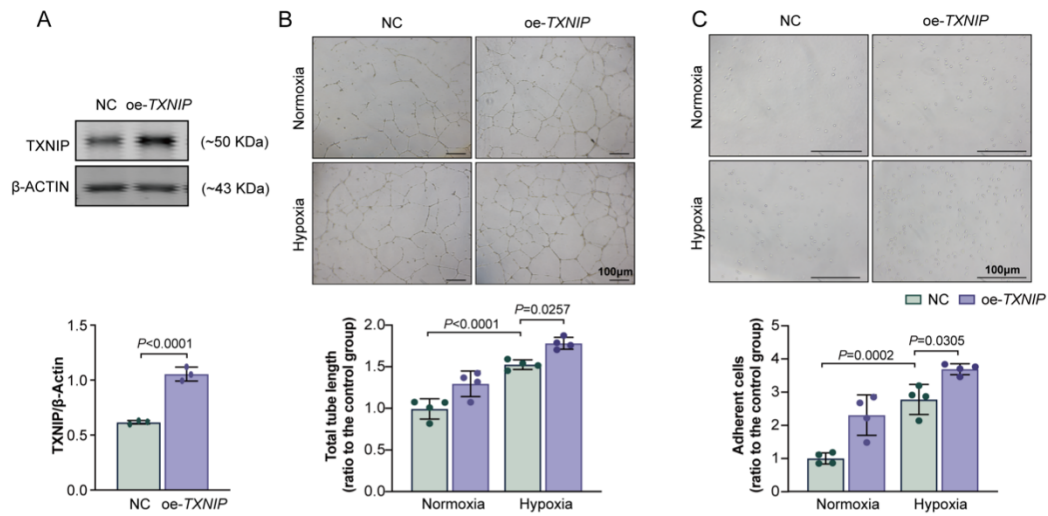


Figure S6 TXNIP promotes endothelial dysfunction. **A:** Western blotting analysis of TXNIP in hPAECs transfected with NC or oe-TXNIP (n=3 per group, unpaired t-test was used). **B** and **C:** hPAECs were transfected with NC or oe-TXNIP and then exposed either to normoxia or to hypoxia. (n=4 per group). **B:** Representative images and quantification analysis of hPAEC angiogenesis (bar=100 μm). **C:** Representative images and quantification analysis of hPAEC adhesion (bar=100 μm). Statistical analysis was by two-way ANOVA followed by Bonferroni correction for six pairwise comparisons among the four experimental groups.

Sup Fig7

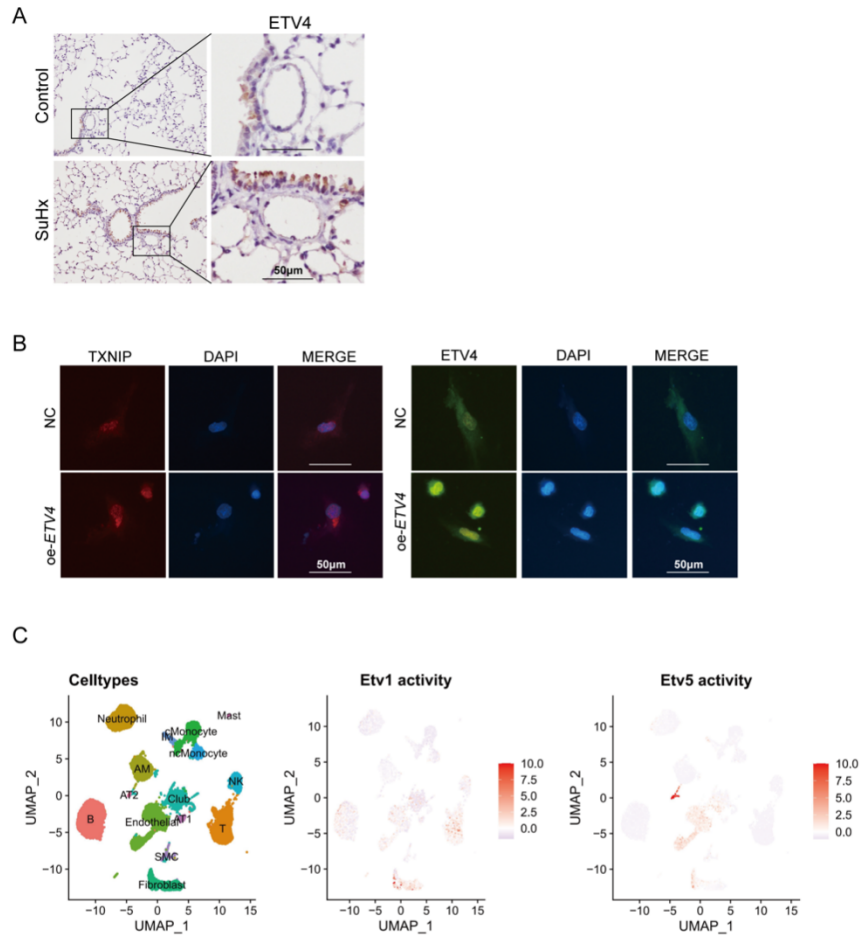


Figure S7 ETV4 expression was elevated in the lung tissue of the SuHx-PH mice and promoted TXNIP transcription in hPAECs. A: Immunohistochemical staining and quantification of TXNIP protein in murine lung tissue under control and SuHx-induced conditions. **B:** Immunofluorescence staining of TXNIP or ETV4 in hPAECs with transfected with oe-ETV4 or vector control (NC). **C:** Transcription factor activity was analysed using the ‘decoupleR’ package, with results shown in a UMAP plot.

Sup Fig8

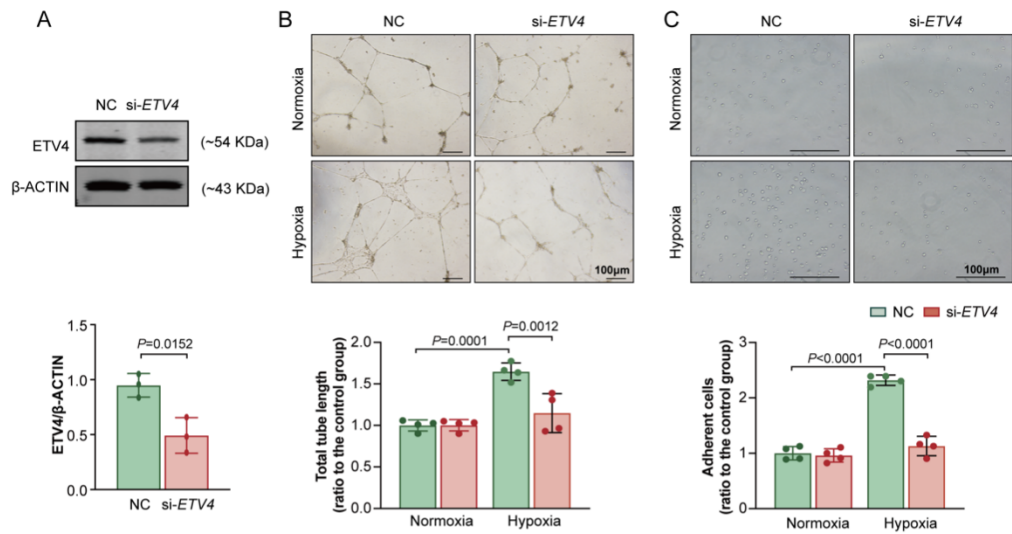


Figure S8 ETV4 knockdown alleviated hypoxia-induced endothelial dysfunction.

A: Western blotting analysis of ETV4 in hPAECs transfected with si-ETV4 or a control vector (NC) (n=3 per group, statistical analysis by unpaired t-test). **B and C:** hPAECs were transfected with si-ETV4 or a control vector (NC) and then exposed to either normoxia or hypoxia. (n=4 per group). **B:** Representative images and quantification analysis of hPAEC angiogenesis (bar=100 μm). **C:** Representative images and quantification analysis of hPAEC adhesion (bar=100 μm). Statistical analysis was by two-way ANOVA followed by Bonferroni correction for six pairwise comparisons among the four experimental groups.

Sup Fig9

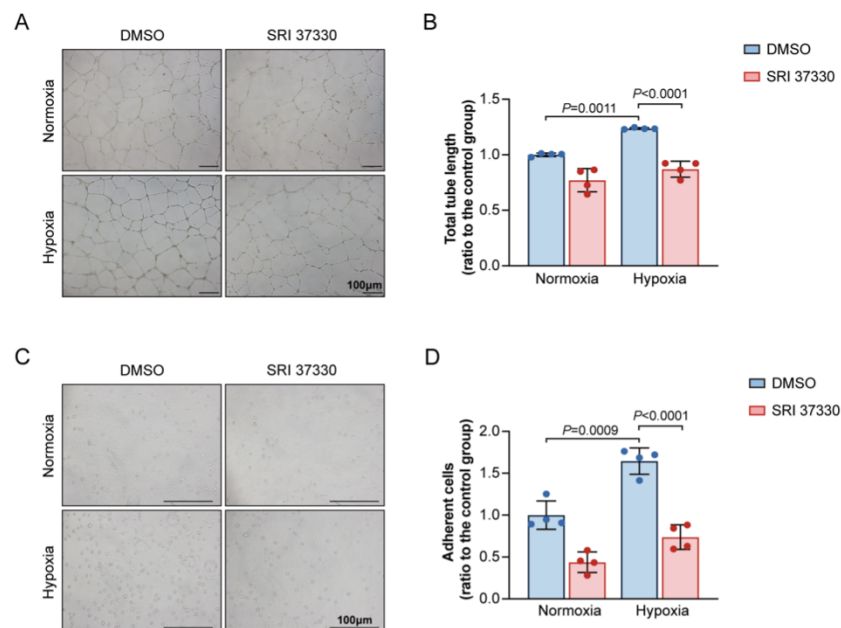


Figure S9 SRI-37330 attenuated hypoxia-induced endothelial dysfunction. A-D: hPAECs were treated with DMSO or SRI-37330 and then exposed either to normoxia or hypoxia. (n=4 per group). **A-B:** Representative images and quantification analysis of angiogenesis by hPAECs (bar=100 μ m). **C-D:** Representative images and quantification analysis of hPAEC adhesion (bar=100 μ m). Statistical analysis was by two-way ANOVA followed by Bonferroni correction for six pairwise comparisons among the four experimental groups.