

RESEARCH | Article in Press

Targeting endothelial thioredoxin-interacting protein (TXNIP) attenuates pulmonary vascular remodeling

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Received: 19 May 2026 / Accepted: 16 September 2026
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Abstract

Background Pulmonary hypertension (PH) is a severe cardiopulmonary disease with high morbidity and mortality worldwide. Pulmonary vascular endothelial cell dysfunction is a core component of the etiology and progression of PH. Here we address the hypothesis that the thioredoxin-interacting protein (TXNIP), known to play a pivotal role in cellular growth, survival, and homeostasis in various diseases, plays a key role in the pathogenesis of pulmonary vascular remodeling, which characterizes PH.

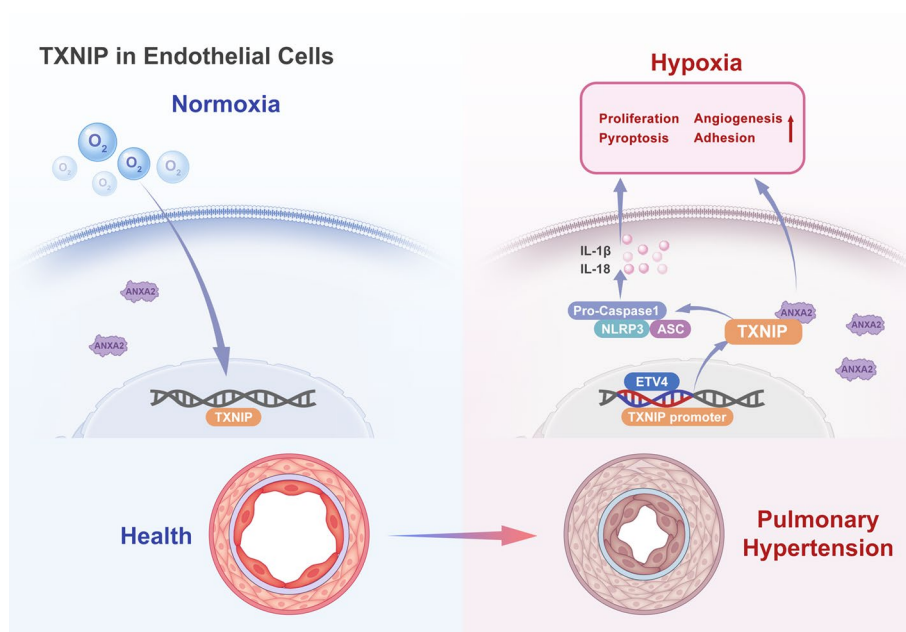
Methods Expression of TXNIP was measured in lung tissue from patients with PH and animal surrogates. Global and endothelial-specific TXNIP inhibition or overexpression were used to explore the roles of TXNIP in PH. Small hairpin RNA and plasmids were used to examine functional behavior in human pulmonary artery endothelial cells. Histological, transcriptomic, proteomic, and single-cell RNA sequencing (scRNA-seq) were used to investigate the mechanisms by which TXNIP contributes to the pathogenesis of PH.

Results Multiomics analyses revealed elevated TXNIP expression in the lung tissues of animal surrogates of PH and from human patients with PH. In the surrogates, global deletion of TXNIP attenuated the development of PH and pulmonary vascular remodeling. In vitro, TXNIP was upregulated in endothelial cells rather than smooth muscle cells and neutrophils. Furthermore, in mice, endothelial-specific inhibition of TXNIP attenuated the progression of pulmonary hypertension. Conversely, TXNIP overexpression accelerated disease progression. Mechanistically, TXNIP induced endothelial dysfunction by promoting cell proliferation, angiogenesis, and adhesion. E-twenty-six-specific sequence variant 4 (ETV4) induced TXNIP transcription and nuclear translocation. Subsequently, TXNIP bonded with annexin A2 (ANXA2) to promote endothelial dysfunction. Moreover, pharmacological inhibition of TXNIP attenuated pulmonary vascular remodeling and PH development.

Conclusions TXNIP plays a crucial role in endothelial homeostasis during the development of PH and is a promising therapeutic target for PH.

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Graphical abstract

Keywords Pulmonary hypertension · Thioredoxin-interacting protein · Endothelial dysfunction · Pulmonary vascular remodeling

Abbreviations

ANXA2	Annexin A2
α SMA	Alpha smooth muscle actin
CD31	Platelet endothelial cell adhesion molecule-1
EC	Endothelial cell
ETV4	E-twenty-six-specific sequence variant 4
hPAECs	Human pulmonary artery endothelial cells
hPASMCs	Human smooth muscle cells
mPAP	Mean pulmonary arterial pressure
PAT	Pulmonary artery acceleration time
PH	Pulmonary hypertension
RV/BW	Right ventricular/body weight
RVHI	Right ventricular hypertrophy index
RVSP	Right ventricular systolic pressure
RVWT	RV free wall thickness
SuHx	SU5416/hypoxia
TXNIP	Thioredoxin-interacting protein
TAPSE	Tricuspid annular plane systolic excursion

Introduction

Pulmonary hypertension (PH) is a severe cardiopulmonary disease caused by pulmonary vascular remodeling, resulting in progressive elevation of the mean pulmonary arterial pressure (mPAP), which in turn results in right heart failure, which may be fatal [1]. Despite numerous studies in past decades, the long-term prognosis of PH remains poor [2–4], reflecting a lack of understanding of its pathophysiology, which is a prerequisite for the identification of novel, relevant therapeutic targets.

Current clinical evaluation of PH relies on complementary noninvasive and invasive approaches. Transthoracic echocardiography is widely used for initial screening and assessment of right ventricular structure and function, whereas confirming right heart catheterization remains the reference standard for confirming diagnosis and defining pulmonary hemodynamics [5]. However, these approaches primarily reflect established physiological abnormalities and provide limited insight into the molecular processes driving pulmonary vascular remodeling. Therefore, there remains an unmet need for reliable biomarkers and molecular approaches capable of identifying disease-associated pathways and potential therapeutic targets. Recent advances in proteomic and multiomic technologies have facilitated the discovery of novel molecular signatures and regulatory network involved in cardiopulmonary vascular diseases, providing valuable strategies for elucidating PH pathogenesis [6].

Pulmonary vascular remodeling, considered a hallmark of PH, may result from a range of pathological changes, including pulmonary artery endothelial cell (EC) dysfunction, excessive proliferation of pulmonary smooth muscle cells, and inflammatory cellular infiltration [7]. Of these, EC dysfunction is recognized as an early and pivotal element of pulmonary vascular remodeling, with EC apoptosis in the early stages transiting to the emergence of hyperproliferative, apoptosis-resistant cells thought to play a critical role in the progression of PH [8–10]. These endothelial abnormalities in turn promote aberrant angiogenesis, inflammation, and metabolic dysregulation, all of which contribute to progressive elevation of the pulmonary vascular resistance. At the present time, the etiology of this EC dysfunction is poorly understood.

Our preliminary bioinformatic analysis identified thioredoxin-interacting protein (TXNIP) [11] as a key candidate molecule implicated in mediating the EC injury resulting in PH. TXNIP, also referred to as TBP2 or VDUP-1, is a member of the thioredoxin system and belongs to the α -arrestin protein family [12, 13]. As a central regulator of inflammation and oxidative stress, TXNIP plays a pivotal role in many cellular functions involving growth and survival, and has been implicated in the pathogenesis of various diseases [14–16]. In baseline, normoxia conditions, TXNIP forms an inactive complex with thioredoxin. Under conditions of oxidative stress, however, elevated reactive oxygen species trigger the dissociation of TXNIP from thioredoxin, thereby initiating the TXNIP signaling cascade. Expression of TXNIP in local endothelial cells has been shown to be increased in a number of cardiovascular and metabolic diseases [17–19]. Although a previous study reported elevated expression of TXNIP in the lung tissue of mice with PH [20], its precise biological function, and in particular, the mechanisms by which it may influence pulmonary vascular remodeling, remain elusive.

In this study, we have investigated, systematically and in detail, the role of TXNIP in promoting EC dysfunction during the progression of PH. We first assessed its clinical relevance by examining TXNIP expression in lung tissues from patients with PH and donor controls. To establish its functional significance, we employed animal surrogates with both global and endothelial cell-specific TXNIP knockdown and overexpression. We further elucidated the mechanisms by which TXNIP drives endothelial dysfunction and pulmonary vascular remodeling. Finally, we evaluated the potential of targeting TXNIP to attenuate pulmonary vascular remodeling in experimental PH. Collectively, these studies were designed to clarify the pathogenic role of TXNIP in PH and to determine whether or not TXNIP represents a viable target for future therapeutic intervention.

Methods

Human lung samples

Lung tissues were obtained from patients with pulmonary arterial hypertension (PAH) and matched healthy controls in the course of lung transplantation or lobectomy. Tissues were fixed immediately in 10% formaldehyde, and then embedded in paraffin. The clinical data pertaining to these patients may be seen in our previous paper and Supplementary Table S1.

Animal models

C57BL/6 mice and Sprague–Dawley rats were purchased from the Vital River Laboratory Animal Technology Company (Beijing, China). *TXNIP* knockout (*TXNIP*^{−/−}) mice (strain no. T012534) were purchased from Gem-Pharmatech Co., Ltd (Jiangsu, China). All animals were randomly divided into various groups for study. Animals were maintained in a specific pathogen-free facility (12-h light–dark cycle, humidity 60–70%, temperature 24–26 °C).

Adeno-associated virus serotype 2 (AAV2) knockdown (HBAAV2/VEC-TIE-Mir30-m-Txnip-ZsGreen) and AAV2 overexpression (HBAAV2/VEC-TIE-m-Txnip-3*flag-ZsGreen) vectors targeting *TXNIP* were constructed (Hanbio, Shanghai, China). In addition, $1.4\text{--}1.8 \times 10^{12}$ genomic titer vectors were prepared in sterile saline. Male mice aged 8 weeks were anesthetized and then infected with the AAV vector (50 μL with 10^{11} viral genome copies) by intratracheal injection as described before. Then, the mice were randomly divided into a control group and a SuHx group, and the model of PH generated as described above [36].

C57BL/6 mice (8 weeks old, body weight 22–25 g) were randomly separated into the indicated groups and treated with vehicle and TXNIP inhibitor (Selleck, E1272, TX, 40 mg/kg per dose). SRI-37330 was given every other day for 28 days. Control groups were injected with equal amounts of vehicle.

Echocardiography

Echocardiography was performed using a high-resolution micro-ultrasound system (Vevo 2100, VisualSonics, Toronto, Ontario, Canada) to measure hemodynamic changes. Mice were anesthetized with isoflurane (3% for induction and 1.5% for maintenance), then placed on a warming plate in a supine position. Briefly, the right ventricular (RV) wall thickness (RVWT) during diastole was recorded by M-mode. The tricuspid annulus plane systolic excursion (TAPSE) was measured at the junction of the tricuspid valve plane and the RV free wall by M-mode from an apical four-chamber view. The pulmonary artery acceleration (PAT) and pulmonary valve peak velocity (PV-peak) were obtained from the short-axis section of parasternal aorta using the pulsed-wave doppler mode. All the measurements were obtained from more than three beats.

Hemodynamic and right ventricular hypertrophy assay

Right ventricular systolic pressure (RVSP) was frequently utilized as a surrogate measure for pulmonary arterial pressure. Mice were anesthetized with 2% tribromoethanol (50 mg/kg, intraperitoneal [i.p.]), then a 22-gauge needle inserted into the RV connected to a pressure transducer interfaced with the PowerLab system (AD Instruments, Sydney, Australia). The position of the needle was determined according to the waveform. Following the hemodynamic measurements, the mice were euthanized and the hearts and lungs collected following opening of the thorax. The RV free wall (RV) of the heart was dissected from the left ventricle and septum (LV + S), and these samples were weighed. The RV hypertrophy index (RVHI) was calculated using the formula: $\text{RV}/(\text{LV} + \text{S})$. The ratio of RV to body weight (BW) also served as an index of right ventricular hypertrophy.

Transcriptomic and proteomic data assay

Lung tissues were collected from mice exposed to SuHx for 1, 2, 3, and 4 weeks. Total RNA was extracted for transcriptomic analysis, while proteins were isolated separately for proteomic analysis. For transcriptomic analysis, the clustering of the index-coded samples was performed on a cBotCluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Novaseq platform and 150-bp paired-end reads were generated. For proteomic analysis, lung tissues were ground individually in liquid nitrogen and lysed with SDT. The raw files were searched and analyzed using the DIA-NN library search software, according to the protein database. Gene Ontology (GO) and InterPro (IPR) functional analyses were conducted using the interproscan program against the nonredundant protein database (including Pfam, PRINTS, ProDom, SMART, ProSite, and PANTHER), and the databases of Clusters of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to analyze protein families and pathways.

scRNA-seq and analysis

Lung tissues from experimental mice were dissociated and processed using the 10X Genomics Chromium Single Cell 3' v2 and V(D)J reagent kits (10X Genomics) following the manufacturer's protocol. Sequencing libraries were generated and sequenced on an Illumina HiSeq 3000 platform with 150-bp paired-end reads. Differentially expressed genes (DEGs) were identified for each cell cluster using the "FindAllMarkers" function, and clusters were annotated based on established marker genes. Transcription factor activity among different cell clusters was inferred using the R package decoupleR (version 2.9.7) and subsequently visualized as heat maps to illustrate differential activity across cell clusters.

Histopathology assays

The right lung tissues and hearts of the mice were inflated with 10% formaldehyde for more than 24 h then embedded in paraffin and sectioned into 4- μ m slices.

Pulmonary vascular remodeling was analyzed following hematoxylin and eosin (H&E) staining. Medial thickness percentage (MT%) was calculated using the formula: $(\text{circumference}_{\text{ext}}/\pi - \text{circumference}_{\text{int}}/\pi) / (\text{circumference}_{\text{ext}}/\pi) \times 100$. Vessels (≤ 100 μ m external diameter) were measured and divided into four groups: 0–25 μ m, 26–50 μ m, 51–75 μ m, and 76–100 μ m in diameter. Images were captured with a Nikon microscope system, and circumferences were measured using its image analysis software.

For immunohistochemistry staining, sections were subjected to deparaffinization and rehydration followed by antigen retrieval in citrate acid buffer (pH 6.0). The sections were then permeabilized with 0.3% Triton X-100, incubated with 3% hydrogen peroxide and blocked with 5% bovine serum albumin (BSA). The sections were then incubated with primary antibodies against TXNIP (ab188865, 1:800, Abcam) and ETV4 (ab189826, 1:4000, Abcam) and detected with horseradish peroxidase (HRP)-conjugated secondary antibodies and the 3,3'-diaminobenzidine (DAB) detection method.

For immunofluorescence staining, sections were incubated with primary antibody against TXNIP (ab188865, 1:200, Abcam), ETV4 (ab189826, 1:200, Abcam), CD31 (ab182981, 1:200, Abcam), and α -SMA (BM0002, 1:200, Boster) followed by Alexa Fluor 488 or 594 secondary antibody and counterstained with 4',6-diamidino-2-phenylindole (DAPI). Images were analyzed with ImageJ, where individual thresholds were established for channel-specific positivity assignment.

For wheat germ agglutinin (WGA) staining, slides were treated with 5-mM WGA dye (W11262, Invitrogen) and counterstained with DAPI. Positive staining was quantified in nine random microscopic fields per mouse using ImageJ, with cell counts standardized to the area of the field (per mm²).

Cell culture

Human pulmonary arterial endothelial cells (hPAECs; ScienCell, 3100) and human pulmonary arterial smooth muscle cells (hPASMCs; ScienCell, 3110) were cultured in culture medium (extracellular matrix medium [ECM]; ScienCell, 1001; SMC Medium, SMCM, 1101). Cells were used at passage 3–6 in all experiments. The 293T cells (Peking Union Medical College School of Basic Medicine Cell Bank) were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, 11965092, NY, USA). Human neutrophils were collected from healthy donors using Histopaque-1119 (Sigma-Aldrich, 11191, Germany) and Histopaque-1077 (Sigma-Aldrich, 0771, Germany). Cells were cultured in a humidified incubator with 5% CO₂ at 37 °C. For the hypoxia experiment, hPAECs and hPASMCs were placed in the hypoxia modular incubator chamber (3% O₂).

Small interfering RNAs and cell transfection

Small interfering RNAs (siRNAs) for TXNIP (si-*TXNIP*) and a negative control (NC) were purchased from GenePharma. The sequences are as follows:

si-*TXNIP*, sense: 5'-GGAGGUGUGUGAAGUUACUTT-3', antisense: 5'-AGUAACUUCACACACCUCCTT-3';

si-*ETV4*, sense: 5'-GCCUACGACUCAGAUGUCATT-3', antisense: 5'-UGACAUCUGAGUCGUAGGCTT-3';

NC, sense: 5'-UUCUCCGAACGUGUCACGUTT-3', antisense: 5'-ACGUGACACGUUCGGAGAATT-3'.

For hPAEC transfection with siRNA, cells at 50–70% confluency were transfected with si-*TXNIP* or NC using Lipofectamine RNAiMAX (Invitrogen, 13778150) according to the manufacturer's protocol. Then, 20 µl of mixture containing siRNA (20 pM for each) and transfection reagents (5 µl for each) were added to the cells for 6 h at 37 °C and then replaced with fresh culture medium. To overexpress TXNIP, ETV4, or ANXA2 in hPAECs, the plasmid of TXNIP (lone template: NM_006472.6), ETV4 (lone template: NM_001261439), or ANXA2 (lone template: NM_004039.2) was transferred into cells using Lipofectamine™ 3000 (Invitrogen, L3000015).

Tubule formation and cell adhesion assays

To evaluate the angiogenic ability of hPAECs, a tubule formation assay was performed as previously described[35]. Cells were seeded on top of BD Matrigel (BD Biosciences, 356234, NJ, USA). Tube formation was quantified by calculating the total tube length in six random fields after 24 h. Tracks of hPAECs organized into networks of cellular cords were counted in six random fields. Cellular adhesion capacity was assessed using fibronectin (30 µL of 20 µg/mL; Millipore, FC010, MA)-coated 96-well plates (10⁴ per well in 5% fetal bovine serum (FBS) and growth factor-free ECM). Data analysis was performed as published previously [35].

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from lung tissues and hPAECs using Trizol reagent (Invitrogen, 15596018CN). The concentration of RNA was measured using a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). The first-strand complementary DNA (cDNA) was synthesized using the Reverse Transcript Reagent Kit (abm, G592, Richmond, BC, Canada). qRT-PCR was performed using the BlasTaq™ 2X qPCR MasterMix kit (abm, G892, Richmond, BC, Canada) on an ABI 7500 Real-Time PCR System (Applied Biosystems, 4351106, Paisley, UK). Cycling conditions were 95 °C for 3 min, 95 °C for 15 s, and 60 °C for 1 min for 40 cycles. The relative expression of the target gene messenger RNA (mRNA) was determined using the 2^{-ΔΔC_t} method and normalized to the amount of endogenous β-ACTIN. The primer sequences are shown in Supplementary Table S3.

Western blotting

Proteins were extracted from lung tissues, hPAECs, hPASCs, and neutrophils in ice-cold radioimmunoprecipitation assay (RIPA) buffer supplemented with protease and phosphatase inhibitors (Roche, 04693116001 and 04906837001, Switzerland). The protein concentration was quantified using the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, A55860) following the manufacturer's protocol. Equal amounts of protein (30 µg) were loaded by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to NC membranes (Millipore, HATF00010, MA, USA). Membranes were blocked with a rapid blocking buffer (Epizyme Biotech, PS108P, China) for 20–30 min and then incubated with primary antibodies, including rabbit anti-TXNIP antibody (Abcam, ab188865, 1:1000), rabbit anti-pro Caspase-1 + p10 + p12 antibody (Abcam, ab179515, 1:1000), rabbit anti-IL-1 beta antibody (Abcam, ab283818, 1:1000), rabbit anti-TMS1/ASC antibody (Abcam, ab309497, 1:1000), rabbit anti-ETV4 antibody (Abcam, ab189826, 1:1000), rabbit anti-Histone H3 antibody (Abcam, ab1791, 1:1000); rabbit anti-GFP antibody (Abcam, ab290, 1:2000), rabbit anti-Flag antibody (Abcam, ab205606, 1:3000), mouse anti-beta actin antibody (Abcam, ab6276, 1:5000), rabbit anti-NLRP3 antibody (ThermoFisher, PA5-79740, 1:1000), and rabbit anti-IL18 antibody (Proteintech, 10663–1-AP, 1:1000) at 4 °C overnight. According to the origin of the primary antibody, membranes were incubated with fluorophore-conjugated secondary antibodies (LI-COR, 926–32211 and 926–68020, 1:5000) for 1 h at room temperature. Blots were developed using an Odyssey infrared imaging system (LI-COR Biosciences). Band intensities were quantified by Image Studio software (LI-COR Biosciences), comparing the target protein with the loading control.

Dual-luciferase assay

To investigate the regulatory role of the ETV4 gene on the TXNIP gene promoter, a dual-luciferase assay was performed. In brief, the TXNIP gene promoter region (500–2000 bp upstream of the transcription start site) was cloned into a fluorescent reporter plasmid (pGL3-Basic). HEK293T cells were cotransfected with pGL3-TXNIP-promoter plasmid and an ETV4 overexpression plasmid for 48 h. Firefly and Renilla luciferase were determined using the Dual-Luciferase Reporter Assay System.

Immunoprecipitation–mass spectrometry (IP–MS)

TXNIP antibody was added to hPAECs lysates for immunoprecipitation (IP), with rabbit immunoglobulin G (IgG) used as a negative control. The immunoprecipitated proteins were subsequently subjected to liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis performed by PTM Bio Co., Ltd. Candidate substrate proteins interacting with TXNIP were identified based on protein scores and molecular weights from the mass spectrometry data.

Immunoprecipitation (IP)

hPAECs: cells were lysed in IP lysis buffer containing a protease inhibitor cocktail, and total protein lysates were quantified. Immunoprecipitation was performed using TXNIP (Proteintech, 18243–1-AP) primary antibodies conjugated to Protein G PLUS–Agarose beads (Santa Cruz, SC-2003). After incubation, the antibody–protein–bead complexes were washed three times with IP lysis buffer, and bound proteins were eluted with SDS sample loading buffer. The immunoprecipitated proteins were analyzed by western blotting.

The 293 T cells: the HEK293T cell line was cultured in DMEM and transfected with GFP-ANXA2 and Flag-TXNIP cloned into the PcDNA3.1(+) karyotic expression vector. After 48 h, cells were harvested and IP RIPA cell lysis buffer added. Cell lysates were incubated with anti-GFP monoclonal antibody (Proteintech, 66002–1-Ig), or anti-Flag monoclonal antibody (Sigma, F1804) at 4 °C overnight. The lysates were then incubated with Protein A and Protein G agarose (Santa Cruz, SC-2003). Immune complexes were washed and resolved

by SDS–polyacrylamide gel electrophoresis (PAGE), and immunoblotting was performed with the following primary antibodies: anti-GFP monoclonal antibody (ab290, 1:2000, Abcam); anti-Flag monoclonal antibody (Abcam, ab205606, 1:3000); anti-rabbit IgG (H + L) peroxidase labeled antibody (KPL, 1:10,000, 5220–0336); anti-mouse IgG (H + L) peroxidase labeled antibody (KPL, 1:10,000, 5220–0341); IPKine™ HRP, mouse anti-rabbit IgG light chain specific antibody (LCS) (Abbkine, A25022, 1:5000), and IPKine HRP AffiniPure goat anti-mouse IgG light chain specific antibody (Abbkine, A25012, 1:5000).

Statistical analysis

All statistical analyses were performed with GraphPad Prism 10.0 (GraphPad software, San Diego, CA, USA). Data from groups are expressed as the mean $\pm$ standard deviation (SD). The unpaired, two-tailed Student's *t*-test was performed to compare the differences between two groups. Multiple group comparisons were conducted using one-way or two-way analysis of variance (ANOVA) with Bonferroni's post hoc test. A *p*-value < 0.05 was considered to be statistically significant.

Results

TXNIP expression is elevated in PH lung tissues

To investigate molecular events underlying the progression of PH, we established a time-course SuHx-PH murine surrogate (Fig. 1A). In line with the hemodynamic abnormalities and pulmonary vascular remodeling characteristic of PH in human subjects, the animals exhibited progressive increases in pulmonary vascular wall thickness, right ventricular systolic pressure (RVSP), right ventricular hypertrophy index (RVHI), right ventricular/body weight (RV/BW), and right-sided cardiomyocyte hypertrophy between consecutive pairs of timepoints (Fig. 1B; Supplementary Fig. S1). We then performed RNA-seq and proteomic profiling of lung tissues across these timepoints. A total of 19 genes were persistently upregulated after SuHx exposure, among which TXNIP drew our particular attention as a consistently elevated candidate (Fig. 1C–E). Given the important role of TXNIP in cellular homeostasis and endothelial function, its persistent upregulation pointed to a possible central role in PH progression, prompting further investigation.

Next, we measured the expression of TXNIP in the lung tissues from patients with pulmonary arterial hypertension (PAH) and healthy donors. Immunohistochemical staining and western blotting revealed that global TXNIP expression was significantly elevated in the lungs of the patients with PAH compared with healthy donors (Fig. 1F–G). In addition, analysis of expression data from GEO datasets GSE210248 [21] revealed elevated TXNIP mRNA expression in pulmonary artery tissues from patients with PH compared with donor controls (Supplementary Fig. S2A,B). Furthermore, we also observed significant upregulation of TXNIP expression in the lung tissue of the murine surrogate of PH and the MCT-induced surrogate of PH in rats (Fig. 1H–J; Supplementary Fig. S3). Collectively, TXNIP expression was then upregulated in the course of development of PH in both human patients and the animal surrogates.

TXNIP deletion attenuates SuHx-induced PH in mice

To investigate the role of TXNIP in PH, we generated *TXNIP* knockout (*TXNIP-KO*) mice (Supplementary Fig. S4A,B). *TXNIP-KO* mice and wild type (WT) mice were exposed to SuHx for 4 weeks as before (Fig. 2A). Western blotting analysis confirmed the absence of TXNIP protein in the lung tissues of the *TXNIP-KO* mice (Supplementary Fig. S4C). In the animals exposed to SuHx, the increase in the mean right ventricular systolic pressure (RVSP) measured in the wild-type animals was significantly reduced in the *TXNIP-KO* animals to such a degree as to be no longer significantly different to that in the control groups (Fig. 2B,C). The same findings pertained

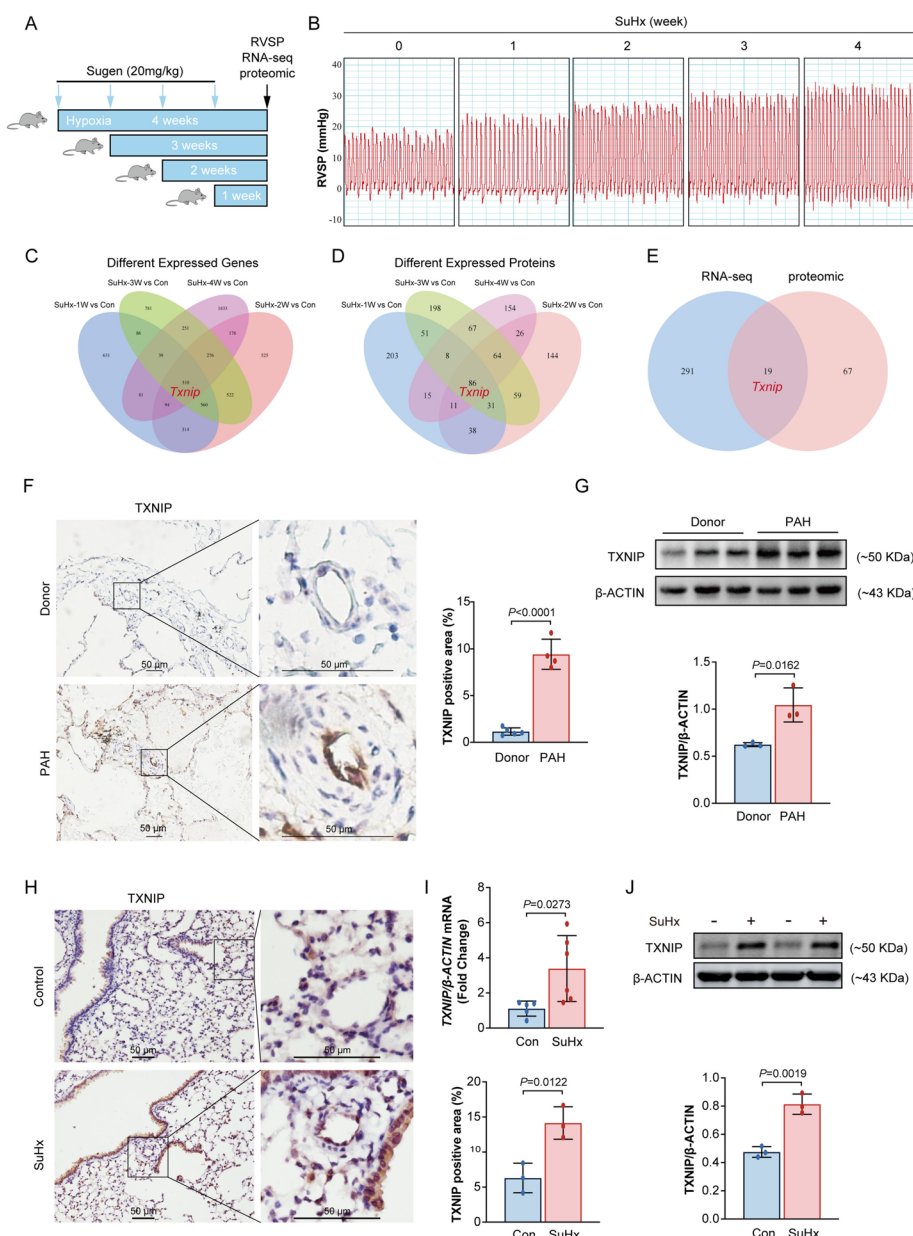
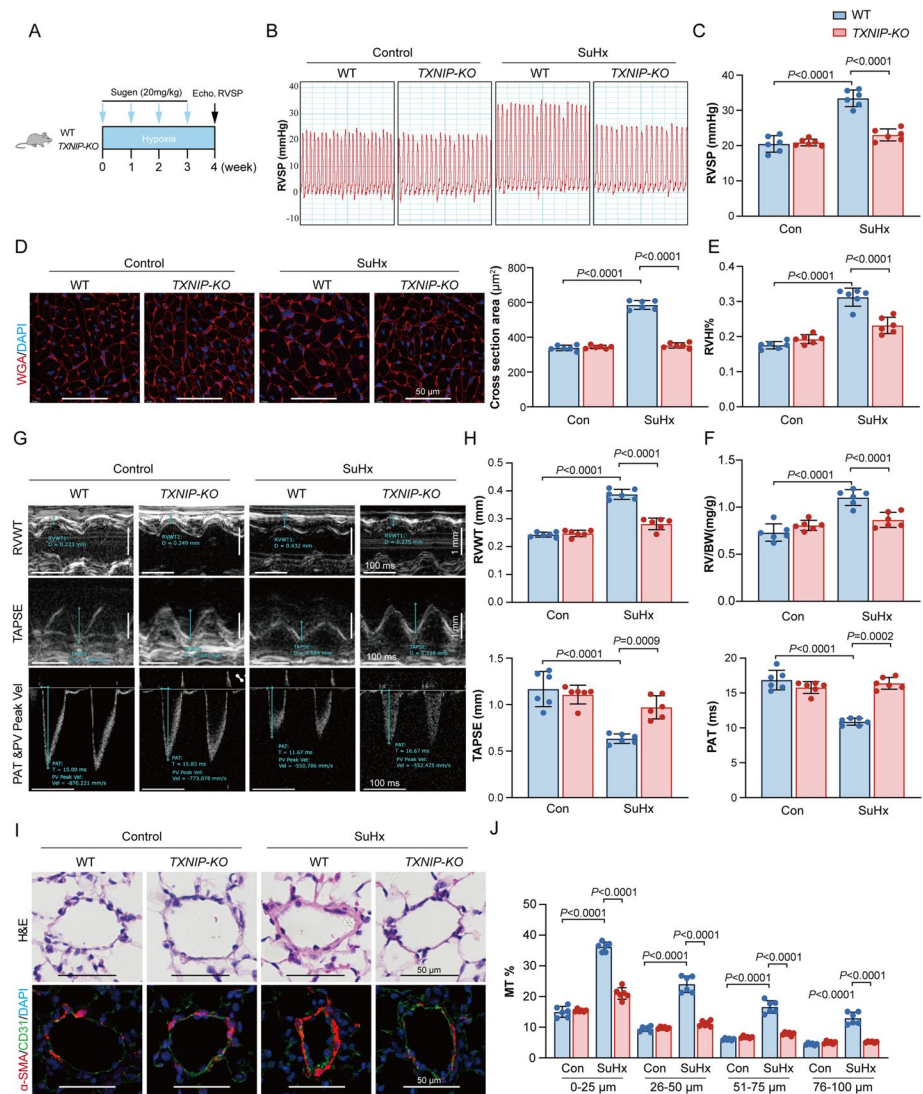


Fig. 1 TXNIP expression is elevated in PH lung tissues. **A–E** Establishment of the SuHx-induced murine surrogate of PH and the timepoint sampling strategy ($n = 5$). **A** Schematic diagram of the murine surrogate. **B** Right ventricular systolic pressure (RVSP) measured at indicated timepoints during PH progression. **C** Venn diagram showing the overlap of differentially expressed genes (DEGs) from lung tissue transcriptomic profiling at multiple timepoints ($|\log_2FC| \geq 0.3$, false discovery rate [FDR] < 0.05). **D** Venn diagram illustrating the overlap of differentially expressed proteins (DEPs) identified by proteomic analysis across the different timepoints ($|\log_2FC| \geq 0.3$, FDR < 0.05). **E** Integrated Venn diagram depicting the intersection between transcriptomic DEGs and proteomic DEPs, revealing commonly dysregulated molecules during PH development. **F** Immunohistochemical staining and quantitative analysis of TXNIP protein expression in lung sections of healthy donors and patients with PAH ($n = 4-5$; bar = 50 μ m; statistical analysis by one-way ANOVA). **G** Western blotting and quantitative analysis of TXNIP protein expression in lung tissues of healthy donors and patients with PAH ($n = 3$, statistical analysis by one-way ANOVA). **H–J** Expression of TXNIP in murine lung tissues under control and SuHx-induced conditions. **H** Immunohistochemical staining and quantification of TXNIP protein ($n = 3$ per group; bar = 50 μ m). **I** Representative qRT-PCR of TXNIP mRNA expression ($n = 5-6$). **J** Representative western blotting of TXNIP protein expression ($n = 3$). Statistical analysis was carried out using an unpaired t -test

Fig. 2 TXNIP deletion attenuates SuHx-induced PH in mice. **A** Schematic diagram of establishment of experimental murine model. **B–J** WT or *TXNIP*^{-/-} mice were maintained for 4 weeks in SuHx or control conditions ($n = 6$ per group). **B** and **C** Final right ventricular systolic pressures (RVSP). **D** WGA staining and quantitative analysis of cardiomyocyte hypertrophy in the right ventricles of the experimental animals (bar = 50 μ m). **E** and **F** Changes in the right ventricular hypertrophy index (%) (RVHI%) and the ratio of right ventricular to body weight. **G** and **H** Representative echocardiographic images and quantification of RV free wall thickness (RVWT), tricuspid annular plane systolic excursion (TAPSE), and pulmonary artery acceleration time (PAT). **I** Representative images of hematoxylin and eosin (H&E) and α -SMA (smooth muscle α -actin)/CD31 immunofluorescence (IF) staining in lung sections of the experimental mice (bar = 50 μ m). **J** Quantification of medial thickness (MT%) for vessels 0–25 μ m, 26–50 μ m, 51–75 μ m, and 76–100 μ m in diameter. Statistical analysis was performed using two-way ANOVA followed by Bonferroni correction for six pairwise comparisons among the four experimental groups



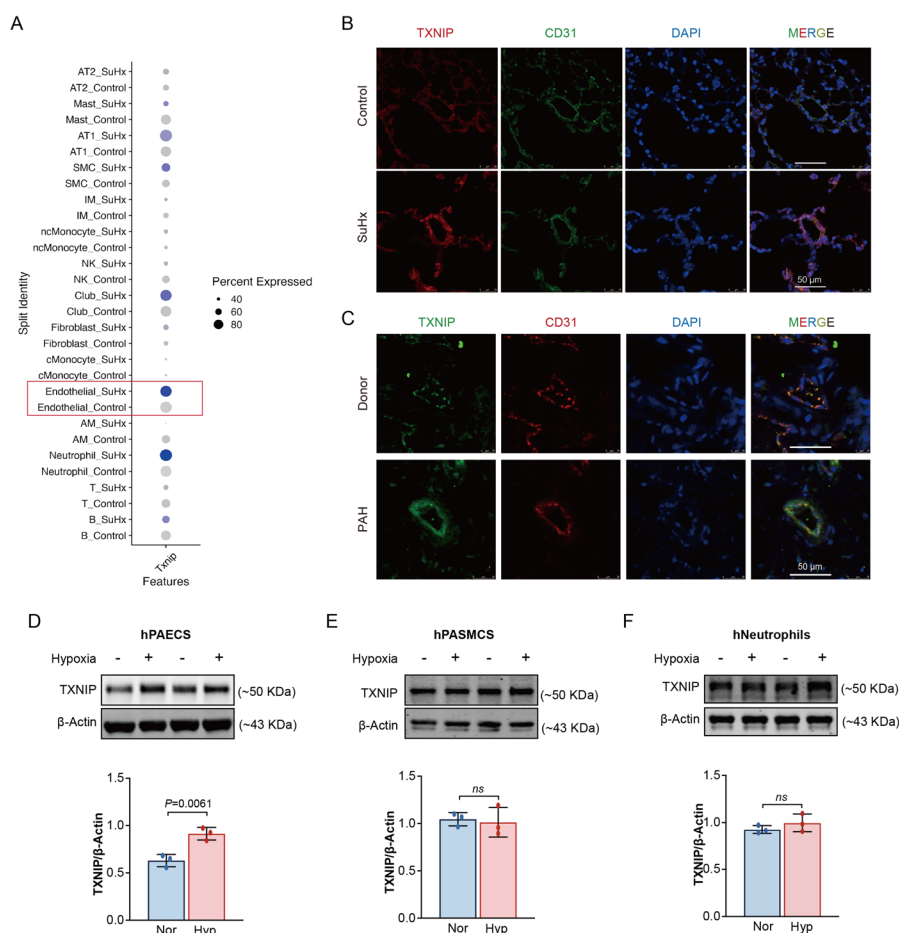
with the mean cross-sectional area of the right heart, the right ventricular hypertrophy index (RVHI%) and the right ventricular/body weight ratio (RV/BW) (Fig. 2D–F). Similarly, echocardiographic measurements showed that the increase in the mean RV free wall thickness (RVWT) and the reduction of the mean pulmonary artery acceleration time (PAT) and the tricuspid annular plane systolic excursion (TAPSE) observed in the WT mice were abolished in the *TXNIP*-KO mice (Fig. 2G,H). H&E and immunofluorescence staining also showed significantly attenuated pulmonary vascular thickening in *TXNIP*-KO mice compared with the WT mice (Fig. 2I,J). These findings clearly support the hypothesis that TXNIP plays a pivotal role in the development of PH.

TXNIP in pulmonary artery endothelial cells plays an important role in vascular remodeling

To investigate the cellular expression of TXNIP, we analyzed scRNA-seq data from the murine surrogate of PH. We found that TXNIP was enriched principally in endothelial cells, smooth muscle cells, and neutrophils, and that its expression was specifically elevated in endothelial cells in association with SuHx (Fig. 3A). Furthermore, the GEO datasets showed a significant increase in TXNIP mRNA expression specifically within endothelial cells from patients with PH relative to donor controls (Supplementary Fig. S2C,D). In addition, we observed elevated TXNIP expression in the lung vascular intima of animal surrogates of PH and from human patients suffering

Fig. 3 TXNIP is predominantly elevated in endothelial cells in PH.

A Dot plot showing the relative expression of TXNIP across a range of cellular phenotypes of murine models. **B** IF staining of TXNIP in the lung tissues from control and SuHx-treated mice (bar = 50 μ m). **C** IF staining of TXNIP in the lung tissues from healthy donors and patients with PAH (bar = 50 μ m). **D–F** Western blotting and quantification analysis of TXNIP expression in human cells under either normoxic or hypoxic conditions ($n = 3$, statistical analysis by unpaired t -test). **D** Human pulmonary artery endothelial cells (hPAECs). **E** Human pulmonary smooth muscle cells (hPASCs). **F** Human neutrophils (hNeutrophils)



from PAH. We confirmed the colocalization of TXNIP⁺ cells and endothelial cells (CD31⁺ cells; Fig. 3B,C). In the present study, TXNIP expression was significantly upregulated in the hPAECs compared with hPASCs and neutrophils, so we focused on the role of TXNIP in ECs (Fig. 3D–F).

Further, to investigate the role of endothelial TXNIP in the pathogenesis of PH, we generated mice with TXNIP knockdown confined to the endothelial cells by intratracheal administration of adeno-associated virus-mediated TXNIP shRNA (AAV-shTXNIP) or vector control (AAV-shNC), the success of which was verified by staining (Fig. 4A–D). Both AAV-shTXNIP- and AAV-shNC-transfected mice were exposed to SuHx for 4 weeks. In response to SuHx, AAV-shTXNIP mice exhibited a significantly mitigated PH phenotype compared with the AAV-shNC mice, as reflected by the significantly reduced mean RVSP, RVHI, and RVWT and the significantly elevated mean TAPSE and PAT (Fig. 4E–I). Histological staining revealed that vascular thickening and right heart hypertrophy were increased in AAV-shNC mice, and that both of these phenomena were essentially abolished in the AAV-shTXNIP mice (Fig. 4J–L).

Conversely, we again employed adeno-associated virus to establish mice overexpressing TXNIP in endothelial cells (AAV-oeTXNIP; Supplementary Fig. S5A). We first confirmed that TXNIP was overexpressed in the lung endothelial cells of the experimental mice (Supplementary Fig. S5B–D). Compared with control mice (AAV-oeNC), the mean RVSP, RVHI, and RVWT were all significantly elevated in the AAV-oeTXNIP mice at baseline (labeled Normoxia in Supplementary Fig. S5), and these differences were further accentuated in the animals exposed to SuHx. Echocardiographic analysis showed that TXNIP overexpression in ECs further aggravated the abnormalities of TAPSE and PAT, effects that were again further accentuated in the animals exposed to SuHx

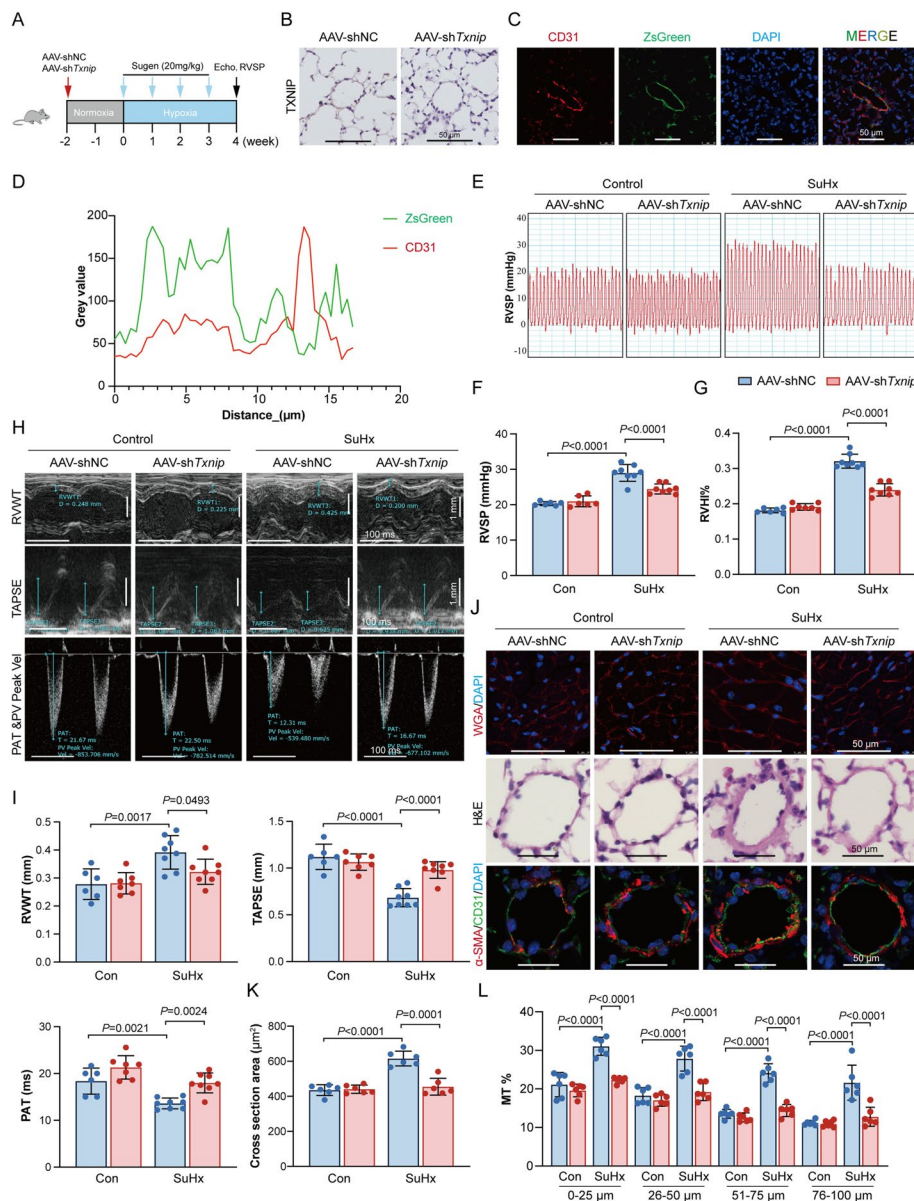


Fig. 4 TXNIP inhibition in pulmonary artery endothelial cells alleviates SuHx-induced vascular remodeling. **A** Schematic diagram of the establishment of the experimental murine surrogate. AAV-shNC or AAV-shTxnip mice were maintained for 4 weeks in SuHx or control conditions ($n = 6-8$ per group). **B** Immunohistochemistry (IHC) staining of TXNIP in the lung sections of experimental mice (bar = 50 μm). **C** and **D** Representative images and quantification of ZsGreen and CD31 IF staining within the lung sections of experimental mice (bar = 50 μm). **E** and **F** Changes in right ventricular systolic pressure (RVSP). **G** Changes in right ventricular hypertrophy index (%) (RVHI%). **H** and **I** Representative echocardiographic images and quantification of right ventricular free 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 IF staining of lung sections in the experimental mice (bar = 50 μm). **K** Quantification of the cardiomyocyte cross-sectional area in the right ventricle. **L** Quantification of MT% of the pulmonary vasculature for vessels 0–25 μm , 26–50 μm , 51–75 μm , and 76–100 μm in diameter. Statistical analysis was performed using two-way ANOVA followed by Bonferroni correction for six pairwise comparisons among the four experimental groups

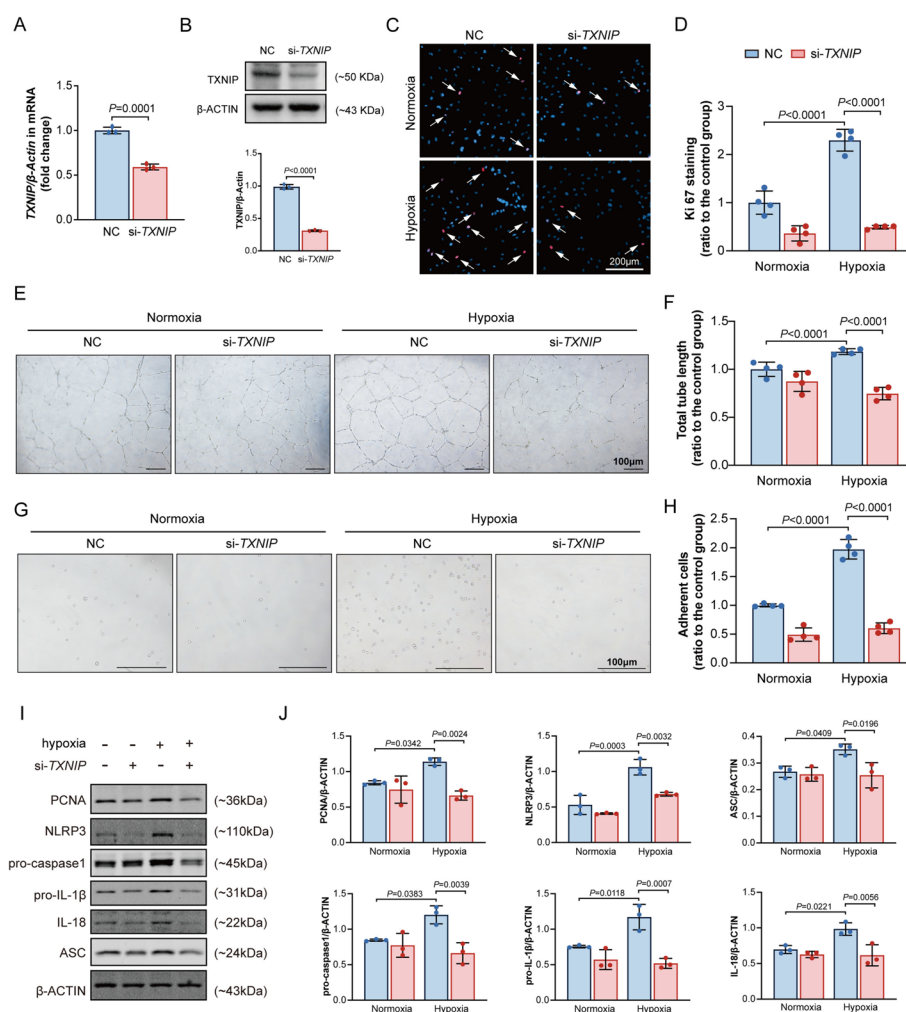
(Supplementary Fig. S5E–K). Finally, pulmonary vascular remodeling induced by SuHx was significantly exaggerated in the AAV-oe*TXNIP* mice compared with the control groups (Supplementary Fig. S5K,L). These findings clearly further support the hypothesis that *TXNIP* expressed in pulmonary artery endothelial cells is a key promoter of the pathogenesis of PH.

TXNIP promotes endothelial cell dysfunction

Since endothelial dysfunction is an important feature of vascular remodeling, we were interested to investigate whether *TXNIP* may influence other physiological functions of hPAECs. *TXNIP* knockdown using small interfering RNA significantly attenuated the mean hypoxia-induced proliferation of hPAECs (Fig. 5A–D). *TXNIP* knockdown also significantly reduced the mean expression of PCNA by hPAECs (Fig. 5I,J). Furthermore, *TXNIP* knockdown significantly suppressed the enhanced angiogenesis and adhesion of hPAECs maintained in conditions of hypoxia (Fig. 5E–H). Finally, inhibition of *TXNIP* expression significantly alleviated hypoxia-induced activation of the NLRP3 inflammasome pathway in hPAECs (Fig. 5I,J).

Additionally, to confirm the effect of *TXNIP* on endothelial dysfunction, we overexpressed *TXNIP* in hPAECs using *TXNIP*-encoding plasmids. *TXNIP* overexpression significantly enhanced hPAEC tubular vessel formation and adhesion (Supplementary Fig. S6A–C). These data are clearly consistent with the hypothesis that *TXNIP* promotes key aspects of endothelial cellular dysfunction implicated in PH pathogenesis, including proliferation, angiogenesis, and adhesion.

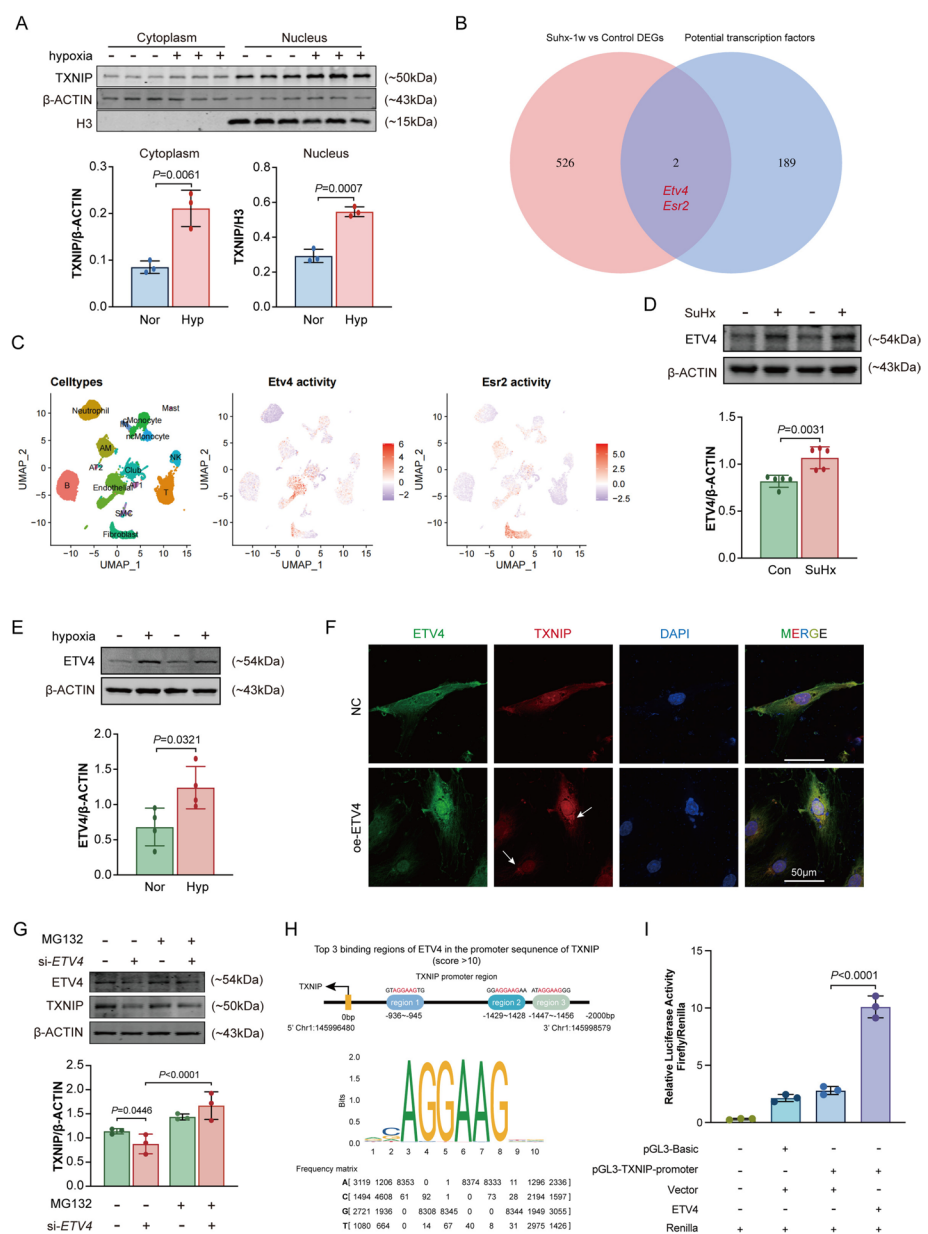
Fig. 5 *TXNIP* promotes endothelial cell dysfunction. **A** and **B** hPAECs were transfected with NC or si-*TXNIP* ($n = 3$ per group, unpaired t -test was used). **A** qRT-PCR analysis of *TXNIP* in hPAECs. **B** Western blotting analysis of *TXNIP* in hPAECs. **C–J** hPAECs were transfected with NC or si-*TXNIP* and then exposed either to normoxia or hypoxia. ($n = 3–4$ per group). **C** and **D** IF staining and quantification of Ki-67 in hPAECs (bar = 200 μ m). **E** and **F** Representative images and quantification analysis of angiogenesis by hPAECs (bar = 100 μ m). **G** and **H** Representative images and quantification analysis of hPAEC adhesion (bar = 100 μ m). **I** and **J** Representative western blot of protein expression and quantification analysis. Two-way ANOVA was performed, followed by Bonferroni correction for six pairwise comparisons among the four experimental groups



ETV4 promotes TXNIP transcription in PH

Furthermore, to explore the mechanism of TXNIP upregulation by hPAECs under conditions of hypoxia, we measured the expression and subcellular location of TXNIP in hPAECs cultured in conditions of normoxia or hypoxia. Compared with cells maintained in normoxia conditions, hypoxia markedly increased TXNIP expression in the cell nuclei (Fig. 6A). Given that hypoxia increased both the expression and nuclear localization of TXNIP, we next focused on elucidating the mechanism driving its upregulation. By overlapping 528 DEGs identified in lung tissues after 1 week of SuHx exposure and 191 putative TXNIP transcription factors predicted by the UCSC database, we found two differentially expressed transcription factors (Fig. 6B). To generate an overview of the role of ETV4 and ESR2 in lung tissue, we used the “decoupleR” package to analyze its activity in all cell types. ETV4 activity was predominantly enriched in endothelial cells, while ESR2 activity was predominantly enriched in fibroblast cells (Fig. 6C). Transcription factor activity was analyzed using the “decoupleR” package, with results shown in a uniform manifold approximation and projection (UMAP) plot. D Western blotting and quantification analysis of ETV4 expression in murine lung tissue under control and SuHx-induced conditions ($n = 5$, unpaired t -test). E Western blotting and quantification analysis of ETV4 expression in hPAECs under either normoxic or hypoxic conditions ($n = 4$, unpaired t -test). F IF staining of TXNIP and ETV4 in hPAECs transfected with NC or oe-ETV4. G Western blotting and quantification analysis of TXNIP expression in hPAECs transfected with si-ETV4 or vector control (NC) and then treated with DMSO or MG132 ($n = 3$, two-way ANOVA). H Putative ETV4-binding sites identified in the promoter region of the TXNIP gene. I Dual-luciferase reporter assays showing that ETV4 binds to the TXNIP promoter ($n = 3$ per group, one-way ANOVA)

Fig. 6 ETV4 regulates TXNIP transcription in PH. **A** Western blotting and quantification analysis of TXNIP expression in nuclear and cytoplasmic expression of hPAECs ($n = 3$, unpaired t -test). **B** Integrated Venn diagram depicting the candidate transcription factors regulating TXNIP after 1 week of SuHx exposure. **C** Transcription factor activity was analyzed using the “decoupleR” package, with results shown in a uniform manifold approximation and projection (UMAP) plot. **D** Western blotting and quantification analysis of ETV4 expression in murine lung tissue under control and SuHx-induced conditions ($n = 5$, unpaired t -test). **E** Western blotting and quantification analysis of ETV4 expression in hPAECs under either normoxic or hypoxic conditions ($n = 4$, unpaired t -test). **F** IF staining of TXNIP and ETV4 in hPAECs transfected with NC or oe-ETV4. **G** Western blotting and quantification analysis of TXNIP expression in hPAECs transfected with si-ETV4 or vector control (NC) and then treated with DMSO or MG132 ($n = 3$, two-way ANOVA). **H** Putative ETV4-binding sites identified in the promoter region of the TXNIP gene. **I** Dual-luciferase reporter assays showing that ETV4 binds to the TXNIP promoter ($n = 3$ per group, one-way ANOVA)



The polyoma enhancer activator protein (PEA3) belongs to E26 transformation-specific (ETS) variant transcription factor family, including ETV1, ETV4, and ETV5. ETV4 activates gene expression by recognizing specific DNA sequences with a 5'-GGAA/T-3' core in the promoter [22]. ETV4 is a transcription factor, the expression of which may be modulated by signaling pathways triggered by hypoxia [23]. Under hypoxic stress, reductions in ETV4 activity or expression have been associated with protective effects against hypoxia-induced cell death in certain cellular contexts [24]. Consequently, we were interested in investigating whether or not ETV4 modulates TXNIP expression.

ETV4 expression was elevated in the lung tissue of mice exposed to SuHx and hypoxia and in hPAECs exposed to hypoxia in vitro (Fig. 6D,E; Supplementary Fig. S7A). Overexpression of ETV4 by plasmid transfection induced TXNIP upregulation and facilitated its translocation from the nucleus to the cytoplasm of the hPAECs (Fig. 6F; Supplementary Fig. S7B). Furthermore, ETV4 inhibition significantly abrogated the expression of TXNIP in hPAECs (Fig. 6G). To elucidate how ETV4 transcriptionally regulates TXNIP expression, we used the JASPAR database to identify three putative ETV4 binding sites within the promoter regions of TXNIP (Fig. 6H). A dual-luciferase reporter gene was further used to verify ETV4 binding to the TXNIP promoter (Fig. 6I). Inhibition of ETV4 expression further significantly alleviated tube formation and adhesion of hPAECs maintained in conditions of hypoxia (Supplementary Fig. S8A–C). Taken together, these data clearly support the hypothesis that ETV4 binding to the TXNIP promoter drives TXNIP transcription in endothelial cells.

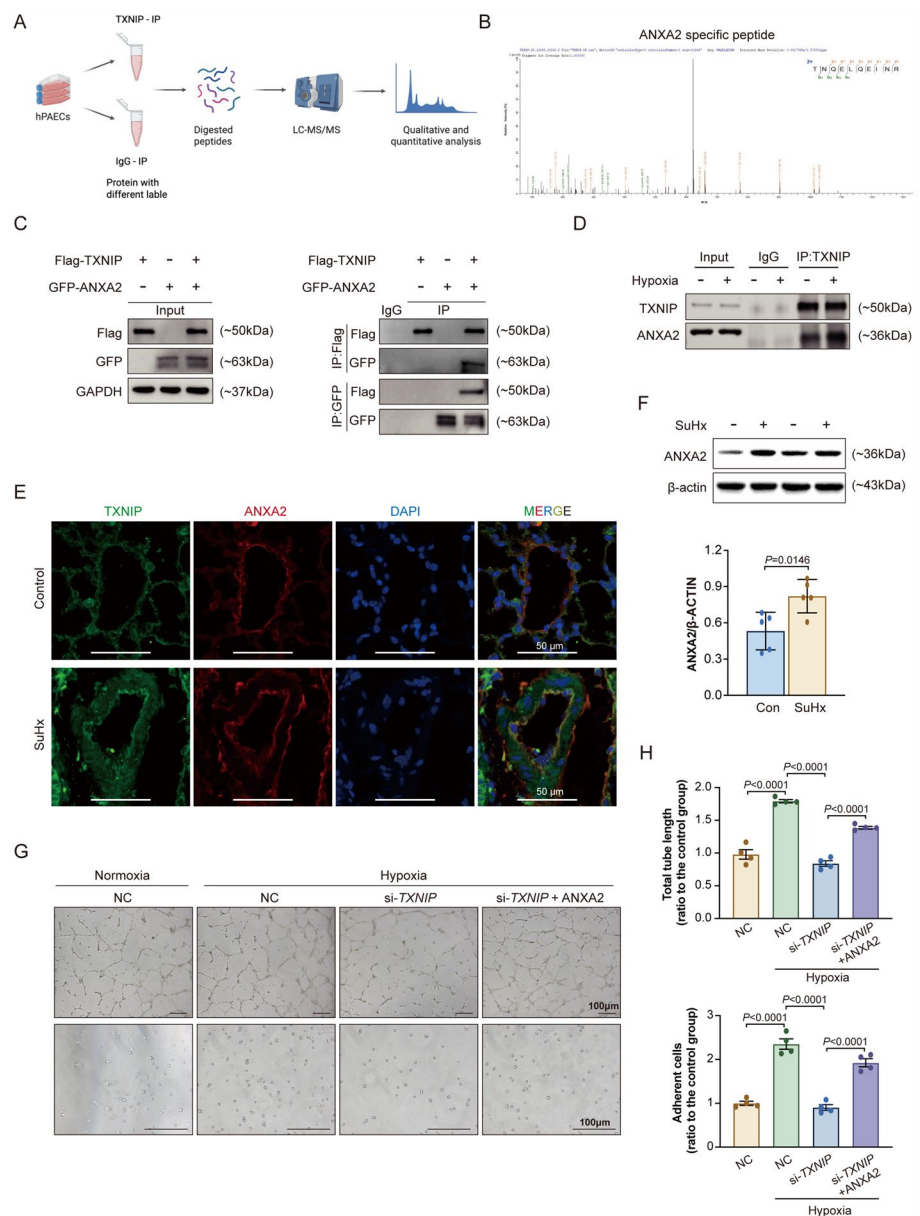
ANXA2 is a binding partner of TXNIP and promotes endothelial dysfunction

In response to stress, TXNIP translocates from the cell nucleus to the cytoplasm and influences cellular apoptosis [16]. Using western blotting, we found that TXNIP was induced to translocate from the nucleus to the cytoplasm of hPAECs under conditions of hypoxia. Immunoprecipitation mass spectrometry revealed a total of 68 proteins that interact specifically with TXNIP in hPAECs (Fig. 7A). Of these, ANXA2 drew our attention because it has been reported to play a critical role in the regulation of PH and vascular remodeling [25, 26]. Representative MS spectra of ANXA2 are shown in Fig. 7B. To determine whether or not TXNIP binds to ANXA2, we incubated HEK293T cells or a lysate of hPAECs with TXNIP antibody (Fig. 7C,D). These findings were further confirmed by immunofluorescence staining in which the ANXA2 immunoreactivities were colocalized with TXNIP in the lung tissues of SuHx-PH mice (Fig. 7E). TXNIP interacted with ANXA2 following IP–MS assays. In addition, ANXA2 expression was increased in the lung tissue of the mice exposed to SuHx (Fig. 7F). Moreover, overexpression of ANXA2 abrogated the restoration of angiogenesis and adhesion induced by TXNIP knockdown when the cells were cultured in hypoxia conditions (Fig. 7G,H). These observations clearly support the hypothesis that TXNIP mediates endothelial dysfunction at least partly by interacting with ANXA2 and facilitating its activation.

Pharmacological inhibition of TXNIP alleviates SuHx-induced PH development in mice

To further investigate whether TXNIP could be a therapeutic target for the prophylaxis and treatment of PH, we treated animals in our SuHx surrogate of PH intraperitoneally with the specific TXNIP inhibitor SRI-37330 for 4 weeks (Fig. 8A). The mean expression of TXNIP was significantly reduced in the lung tissue of the SRI-37330-treated mice (Fig. 8B,C). Furthermore, the elevation of the RVSP, and markers of right heart hypertrophy (RVHI, RV/BW, and cross-sectional area of cardiomyocytes), as well as the pulmonary vascular remodeling induced by SuHx, markedly attenuated following SRI-37330 treatment (Fig. 8D,E,H,I). SRI-37330 treatment also improved echocardiographic parameters RVWT, TAPSE, and PAT (Fig. 8F,G). In addition, SRI-37330 also attenuated hypoxia-induced endothelial dysfunction in vitro in this surrogate (Supplementary Fig. S9). These data are fully compatible with the hypothesis that SRI-37330 alleviates the progression of PH and that pharmacological inhibition of TXNIP may limit the development of experimental PH.

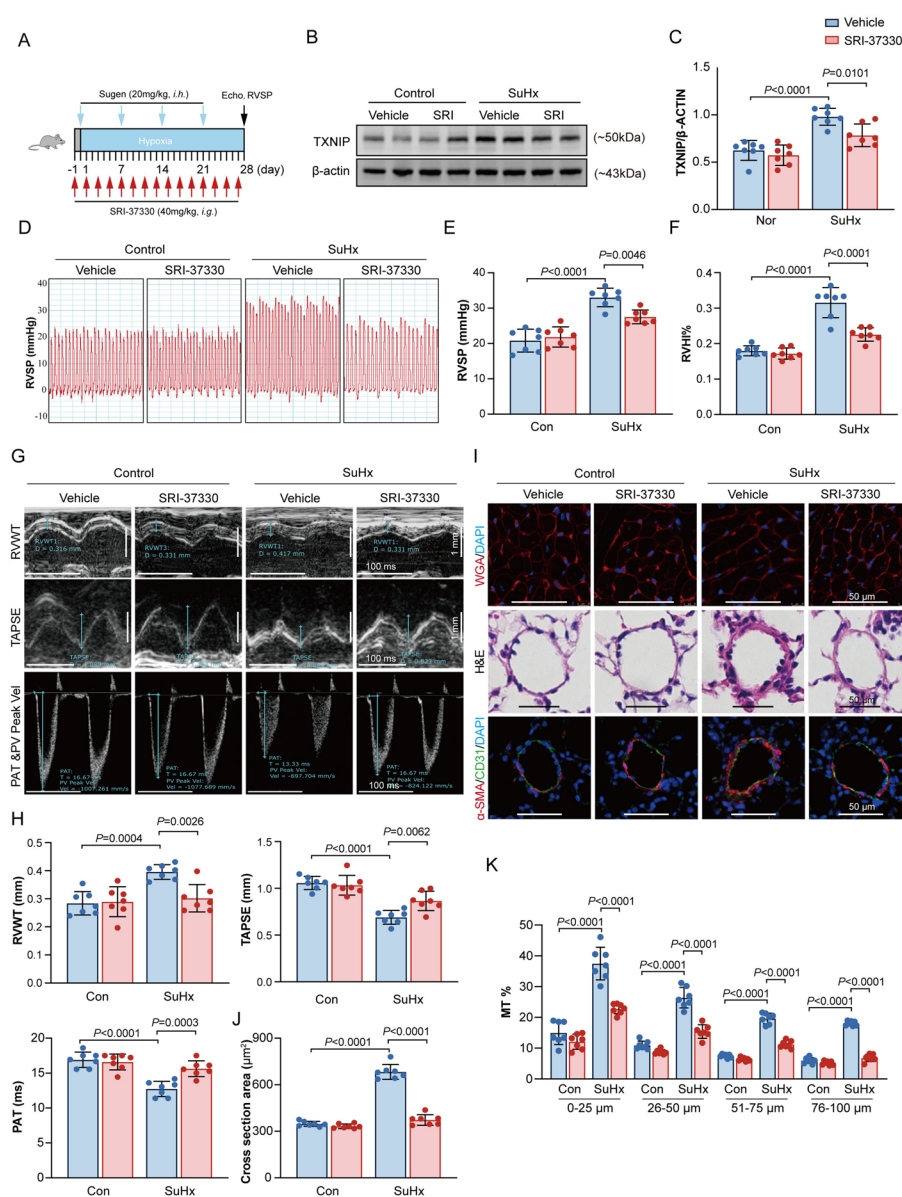
Fig. 7 ANXA2 is a binding partner of TXNIP and promotes endothelial dysfunction. **A** Schematic representation of quantitative proteomic screen to identify proteins binding to TXNIP. **B** Mass spectrographic analysis showing that ANXA2 is one of the proteins that interact with TXNIP. **C** Lysate analysis of HEK293T cells transfected with Flag-TXNIP and GFP-ANXA2. Lysates were immunoprecipitated with anti-Flag or anti-GFP. TXNIP and ANXA2 were detected by western blotting, with IgG employed as a negative control. **D** hPAEC lysates were immunoprecipitated with anti-IgG or anti-TXNIP antibody. **E** IF staining of TXNIP and ANXA2 in murine lung tissues under control and SuHx-induced conditions. **F** Western blotting and quantification analysis of ANXA2 expression in murine lung tissue under control and SuHx-induced conditions ($n = 5$, unpaired t -test). **G** and **H** Representative images and quantification analysis of adhesion and angiogenesis of hPAECs exposed to hypoxia following si-TXNIP transfection in the presence or absence of ANXA2 ($n = 4$ per group, one-way ANOVA)



Discussion

Pulmonary vascular remodeling is a critical pathogenic feature driving the development and progression of PH, a widespread disease with high morbidity and mortality that currently lacks any effective treatment. In this study, we have clearly demonstrated that TXNIP expression is elevated both in patients with PH and our murine surrogate. Mice in which TXNIP was deleted or inhibited were resistant to SuHx-mediated pulmonary vascular remodeling, with its associated hemodynamic abnormalities and right ventricular hypertrophy. Notably, endothelial TXNIP knockdown attenuated, whereas endothelial TXNIP overexpression aggravated SuHx-induced PH. Elevated TXNIP expression was also associated with the enhanced vascular tubular remodeling, cellular adhesion, and activation of the NLRP3 signaling cascade in endothelial cells. These data clearly support the hypothesis that TXNIP exacerbates the endothelial dysfunction that results in PH. Mechanistically, we have shown that ETV4 promotes TXNIP transcription and nuclear translocation, as a result of which TXNIP induces endothelial

Fig. 8 Pharmacological inhibition of TXNIP alleviates SuHx-induced PH in mice. **A** Schematic diagram of establishment of the experimental murine model. SRI-37330 or vehicle control was administered intraperitoneally to C57BL/6 mice under control and SuHx-induced conditions ($n = 7$ per group). **B** and **C** Western blotting and quantification analysis of TXNIP expression in the lungs of experimental mice. **D** and **E** Changes in RVSP. **F** Changes in RVHI. **G** and **H** Representative echocardiographic images and quantification of RVWT, TAPSE, and PAT. **I** WGA staining of right ventricular sections and H&E and α -SMA/CD31 IF staining of lung sections in the experimental mice (bar = 50 μ m). **J** Quantification of cardiomyocyte right ventricular cross-sectional area. **K** Quantification of MT% of the pulmonary vasculature of the experimental mice. Two-way ANOVA was performed, followed by Bonferroni correction for six pairwise comparisons among the four experimental groups



dysfunction through its interaction with ANXA2. These findings clearly identify TXNIP as a mediator and a promising therapeutic target in the pathogenesis of PH.

It is widely recognized that endothelial dysfunction, abnormal proliferation of smooth muscle cells and fibroblasts, and inflammatory cellular infiltration are critical requisites for pulmonary vascular remodeling during PH development [27–31]. Endothelial cells participate in the development of vasoconstriction and vascular remodeling, which results from inappropriate release of diverse growth factors and agonists by the damaged endothelium [32–34], but the extent of this endothelial cell dysfunction and the key elements of this dysfunction that result in PH remain unknown. In our previous study we showed that the abnormal expression and function of homeostatic agents, including ENO1 and Cullin 5, in endothelial cells govern pulmonary vascular remodeling in PH [35, 36]. In the present study we have further shown that pulmonary endothelial cells are a key source of TXNIP production, which we have further implicated in the pathogenesis of PH, and that targeting endothelial TXNIP could alleviate pulmonary vascular remodeling. Suppression of TXNIP may represent a key prophylactic and therapeutic strategy for PH.

In line with our new findings, it is worthy to note that TXNIP has been shown to facilitate inflammation and metabolic reprogramming in various diseases. Under conditions of stress, TXNIP translocates to the cytoplasm, and inhibits the activity of thioredoxin-1 [37, 38]. TXNIP is an essential promoter in myocardial ischemia/reperfusion injury, since overexpression of TXNIP destroyed autophagosome clearance by increasing the concentration of reactive oxygen species, resulting in NLRP3 activation and ultimately the exacerbation of cardiomyocyte death [39, 40]. In addition, the TXNIP–NLRP3 axis has been implicated in cellular PANoptosis in the pathogenesis of diabetic retinopathy [41] and was reported to aggravate airways inflammation by increasing NLRP3 inflammasome protein expression in mice [42]. In addition to its reported contribution to oxidative stress, inflammation, and vascular dysfunction, TXNIP can support VEGF/VEGFR2 signaling and endothelial angiogenic responses, including proliferation and tube formation [11]. Conversely, under diabetic or metabolic stress, excessive TXNIP expression may impair VEGF responsiveness and ischemia-induced angiogenesis, whereas TXNIP reduction has been reported to restore vascular repair capacity [43, 44]. Thus, the vascular consequences of TXNIP inhibition may depend on the cell type and disease milieu, and our data specifically support the pathogenic role of endothelial TXNIP activation during pulmonary vascular remodeling in PH.

Although some previous studies have reported elevated TXNIP expression in the lungs in animal surrogates of PH, its effects on the vascular endothelium contributing to the pathogenesis of PH have, prior to the present study, received little attention [20, 45]. Inhibition of TXNIP rescued the activation of the NLRP3 inflammasome signaling pathway in endothelial cells maintained in conditions of hypoxia. Importantly, TXNIP upregulation was observed not only in the lung tissues from patients with PAH but also in the experimental chronic hypoxia-induced PH models, suggesting that TXNIP activation is associated with pulmonary vascular remodeling rather than being solely a consequence of advanced human disease. Nevertheless, our human cohort was relatively limited in size and consisted exclusively of patients with PAH, a subtype characterized by prominent pulmonary vascular remodeling and endothelial dysfunction. Therefore, further studies involving larger cohorts and diverse forms of PH are required to determine the broader clinical relevance of TXNIP dysregulation. Given that, suppression of TXNIP may serve as a PH mediator and a therapeutic target of PH, but the underlying mechanism needs to be investigated.

Notwithstanding our present findings, emerging studies have revealed that TXNIP may also exert its detrimental effects on endothelial cells by alternative mechanisms independent of oxidative stress. Recently, TXNIP was shown to interact with TAK1, thereby suppressing its activation and downstream JNK signaling, which ultimately restored the expression of nitric oxide synthase and NO production while inhibiting the release of proinflammatory mediators in endothelial cells [46]. In addition, other studies have revealed that transcription factors such as KLF11 and FOXO1 regulate TXNIP expression and activation, suggesting that TXNIP activity is tightly controlled at multiple regulatory levels under pathological conditions [47, 48]. Based on scRNA-seq analysis in our study, we speculated that ETV4 might act as a transcriptional activator of TXNIP. In line with this, our experimental data confirmed that ETV4 enhanced TXNIP transcription and cytoplasmic translocation. ETV4 functions as a transcriptional regulatory factor and promotes cellular proliferation and migration in many malignancies [49–52]. ETV4 plays multiple roles in endothelial cells, particularly in regulating angiogenic signaling and endothelial cell behavior in response to the angiopoietin-1 and Tie-2 receptor pathways. In endothelial cells, ETV4 is part of a network of ETS family transcriptional regulatory factors that coordinates survival, migration, and tube-like differentiation of endothelial cells, contributing to angiogenesis [53]. In addition, studies suggest that ETV4 is a co-activator of HIF-1-dependent transcription [24, 54]. Comparative analysis within the ETV transcription factor family (ETV1, ETV4, and ETV5) indicated that ETV4, but not ETV1 or ETV5 (Supplementary Fig. S7C), exhibited significantly enriched transcriptional activity in endothelial cells, highlighting its unique regulatory role in TXNIP expression in PH. In this study, we have demonstrated data clearly consistent with the hypothesis that ETV4 participates in the progression of PH by regulating TXNIP activation.

In the present study we have employed immunoprecipitation–mass spectrometric analysis to identify ANXA2 as a potential downstream effector of TXNIP. ANXA2, a member of the conserved annexin family, is known to function primarily in its phosphorylated form at N-terminal residues Tyr23, Ser11, and Ser25. Previous studies

have also reported that ANXA2 contributes to the development of PH by regulating vascular remodeling [25]. We speculate that TXNIP may influence endothelial function at least in part through modulation of ANXA2 activity. Although the precise mechanisms remain to be fully elucidated, our data suggest that ANXA2 may antagonize the beneficial effects of TXNIP knockdown, underscoring the functional relevance of the TXNIP–ANXA2 interaction. Collectively, these findings suggest the presence of a previously unrecognized ETV4–TXNIP–ANXA2 signaling axis that contributes to endothelial dysfunction in PH, offering new mechanistic insights and potential therapeutic targets for pulmonary vascular remodeling.

Currently, PH treatments approved for therapy for PH comprise drugs that enhance the nitric oxide–cyclic guanosine monophosphate biological pathway, prostacyclin pathway agonists, and endothelin pathway antagonists. Although these approaches have improved survival, they are inadequate to inhibit or rescue vascular remodeling [2]. In the present study we have clearly demonstrated the potential of SRI-37330, a TXNIP-selective inhibitor, to attenuate pulmonary vascular remodeling. A previous study also showed that SRI-37330 inhibits the activity of the TXNIP promoter and endogenous TXNIP mRNA expression in INS-1 cells [55]. It is likely, therefore, that drugs such as SRI-37330 and other agents targeting TXNIP will protect patients from the endothelial cell dysfunction resulting in the vascular remodeling, which results in PH.

In summary, our findings establish a previously unrecognized ETV4–TXNIP–ANXA2 axis as a critical regulator of PH pathogenesis and highlight TXNIP as a promising therapeutic target for arresting or reversing pulmonary vascular remodeling.

Supplementary Information The online version contains Supplementary Material available at <https://doi.org/10.1186/s11658-026-01033-4>.

Acknowledgments We are grateful to Professor Huaping Dai (China-Japan Friendship Hospital) for valuable insights.

Author contributions Zhenqiang Gao: investigation, methodology, visualization, and writing—original draft. Yu Guo: formal analysis, investigation, and methodology. Muzhi Zhang: formal analysis, methodology, and data curation. Huangyu Long: software, formal analysis, data curation, and visualization. Yang Liu: software, methodology, and data curation. Jialu Lv: software. Ruoyang Zhang: methodology. Lin Liu: data curation. Mengyu Xu: software. Ye Cui: supervision. Yufeng Jia: conceptualization. Qihong Zhang: writing—review. Lei Wang: conceptualization and writing—review and editing. Chris J. Corrigan: writing—review and editing. Ying Sun: conceptualization, supervision, project administration, funding acquisition, and writing—review and editing. Jie Liu: conceptualization, supervision, and project administration. Wei Wang: conceptualization, supervision, project administration, and funding acquisition.

Funding This work was supported by the National Natural Science Foundation of China (82090013, 82241011, 82470105, and 82270055), the Beijing Natural Science Foundation (7242010 and 7264236), and the Beijing Nova Program (20240484639).

Data availability The data analyzed in this article will be shared upon reasonable request from the corresponding authors. Sequencing data generated in the present study have been deposited at the Genome Sequence Archive (<https://ngdc.cnbc.ac.cn/gsa>) and are publicly available on the date of publication. The accession nos. of scRNA-seq, proteomic, and RNA-seq data are CRA020769, OMIX008029, and CRA020767, respectively.

Declarations

Ethics approval and consent to participate The human samples were approved by the Ethics Committee of the Capital Medical University (approval no. Z2021SY025) and were conducted in accordance with the latest version of the Declaration of Helsinki of 1975. The animal studies were approved by the Institutional Animal Care and Use Committee of Capital Medical University (approval no.: AEEI-2024-252) performed in accordance with the guidelines of the International Council for Laboratory Animal Science (ICLAS) to ensure ethical compliance.

Consent for publication All authors approved the final manuscript to be published.

Competing interests The authors declare no competing interests.

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