

Transection of the Cervical Sympathetic Trunk Ameliorates Pulmonary Arterial Hypertension probably by Suppressing Transcriptome Profile and calcium signaling in rat model

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Article

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Abstract

Background

Pulmonary arterial hypertension (PAH) is mostly induced by excessive proliferation of pulmonary arterial smooth muscle cells (PASMCs) with undefined mechanism. This study investigated the function and mechanism of transection of the cervical sympathetic trunk (TCST) on the progression of monocrotaline (MCT)-induced PAH in rats and explored the underlying mechanism.

Methods

A rat model of PAH was established by MCT injection. We evaluated the restoration of pulmonary architecture and hemodynamic parameters following TCST. Transcriptome sequencing (RNA-seq) was performed to identify key regulatory genes and signaling pathways that are particularly correlated with PASMC proliferation. The identified genes and pathways were further validated in the PAH model.

Results

TCST significantly attenuated pulmonary vascular remodeling and improved hemodynamic condition, as evidenced by reduced mean pulmonary arterial pressure (mPAP), right ventricular systolic pressure (RVSP), and right ventricular hypertrophy index (RVHI) compared to the PAH+sham group. RNA-seq analysis revealed that TCST suppressed calcium signaling and cell proliferation pathways, with downregulated DEGs enriched in immune responses, inflammatory responses, and the calcium signaling pathway. Differential expression analysis showed downregulation of the calcium pathway genes *Adra1d* and *Tacr1*, as well as their transcriptional regulator *Foxa3*. Further qPCR experiments confirmed decreased levels of *Adra1d*, *Tacr1*, *Plcb1*, and *Foxa3*. ELISA confirmed the inhibited expression of inositol trisphosphate (IP3), which directly regulate intracellular calcium concentration.

Conclusions

We explored the mechanism of TCST in ameliorating PAH by the suppression of the *Foxa3/Adra1d-Tacr1* axis involved in calcium signaling, revealing a novel link between PAH and calcium-driven vascular remodeling, and a mechanistic basis for TCST as a potential therapeutic strategy for PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive and heterogeneous disorder characterized by a sustained elevation in mean pulmonary arterial pressure (mPAP) > 20 mmHg at rest, arising predominantly from excessive pulmonary vasoconstriction and maladaptive structural remodeling of the pulmonary vasculature (1, 2). This hemodynamic abnormality arises predominantly from excessive

pulmonary vasoconstriction and maladaptive structural remodeling of the pulmonary vasculature. This pathological process leads to increased pulmonary vascular resistance (PVR), right ventricular (RV) afterload, and ultimately RV failure and death (3, 4). Current pharmacological management primarily targets three classical pathways: the prostacyclin, endothelin-1 and nitric oxide systems (5). These therapies aim to modulate pulmonary vascular tone and induce vasodilation, thereby reducing right ventricular afterload and improving cardiopulmonary hemodynamics (6). While they significantly enhance patients' quality of life and exercise capacity, their long-term survival benefits remain limited, underscoring the urgent need for novel therapeutic strategies (7).

However, research indicates that beyond conventional pathways, several abnormal signaling pathways play crucial roles in the pathogenesis of PAH. Aberrant proliferation of pulmonary arterial smooth muscle cells (PASMCs) is a hallmark of pulmonary vascular remodeling, driven by dysregulated signaling cascades including MAPK (8), PI3K/Akt (9), and calcium-dependent pathways (10). Concurrently, inflammation has emerged as a critical contributor to PAH pathogenesis, characterized by perivascular infiltration of macrophages, T lymphocytes, and mast cells, as well as elevated levels of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α (11, 12). These inflammatory processes interact closely with PASMC proliferation, as cytokines can directly stimulate smooth muscle cell growth and migration, while activated immune cells release growth factors that exacerbate vascular remodeling (13, 14). Notably, accumulating evidence suggests that targeting either PASMC proliferation or inflammation—or ideally both—may offer therapeutic benefit in PAH (8, 12). Consequently, a deeper investigation into the underlying pathogenic mechanisms of PAH is of critical importance, as it holds significant scientific and clinical importance for identifying novel therapeutic targets and developing effective intervention strategies.

Pulmonary vessels receive innervation from sympathetic, parasympathetic and sensory nerves. Among these, sympathetic nerves mediate vasoconstriction and elevate vascular resistance primarily via α -adrenergic receptors (15). Accumulating evidence over the past two decades has consistently demonstrated that PAH is frequently associated with overactivation of the sympathetic nervous system (SNS) (16–19). Elevated SNS activity in PAH patients correlates with poor prognosis (16, 20), and may even serve as an independent predictor of clinical worsening (18, 21). Interventions that mitigate sympathetic overexcitation, such as beta-blockers, can reduce cardiac load, lower blood pressure and decrease myocardial oxygen consumption, thereby conferring cardioprotective effects (22, 23). Given the recognized role of SNS activation in PAH, various strategies to attenuate this activation have been explored (15), including renal denervation (24, 25) and pulmonary artery denervation (PADN) (26–28). Although some experimental and clinical studies suggest that these approaches can reduce mPAP and slow PAH progression, their precise mechanisms remain incompletely elucidated. Moreover, radiofrequency ablation carries risks of vascular stenosis or unintended damage to parasympathetic nerves, which may compromise their safety profile (29). Therefore, there is a pressing need to develop more precise and safer methods for sympathetic intervention.

Stellate ganglion block (SGB) is a technique that modulates sympathetic nerve activity, potentially improving immune function, correcting neuroendocrine imbalances and alleviating systemic sympathetic overexcitation. In recent years, SGB has demonstrated efficacy in treating various conditions (30–32). Transection of the cervical sympathetic trunk (TCST) is considered an appropriate animal model to simulate the long-term effects of SGB (33). Preliminary studies indicate that TCST can ameliorate PAH symptoms in rats (29); however, the specific molecular mechanisms underlying this improvement remain unclear. Given that sympathetic activation not only promotes PASMC proliferation but also drives pulmonary inflammation through the release of norepinephrine and neuropeptides (34, 35), we hypothesized that TCST may exert its anti-PAH effects by suppressing both PASMC proliferation and inflammatory responses.

In this study, we established a monocrotaline (MCT)-induced rat model of PAH to systematically evaluate the effects of TCST on pulmonary arterial pressure, right ventricular function and lung tissue pathology. Furthermore, we employed whole transcriptome sequencing to analyze differentially expressed genes and their associated signaling pathways in lung tissue following TCST intervention. Our aim was to elucidate the potential molecular mechanisms by which TCST alleviates PAH, with a particular focus on its regulatory effects on both PASMC proliferation and inflammatory responses, thereby providing a theoretical foundation for developing novel PAH treatment strategies based on neuroimmunomodulation.

2. Materials and Methods

Animals

Adult male Sprague-Dawley rats (weight 180–220 g, age 6–8 weeks) were obtained from Hubei BioNT Animal Center. All animals were housed under specific pathogen-free conditions with a 12 h light/dark cycle, controlled temperature (22–24°C), and humidity (50–60%), with free access to standard chow and water. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Hubei BioNT Experimental Center (Approval No. BNB-2025-023) and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

MCT-induced PAH Rat Model and TCST Surgery

Following a 7-day acclimation period, rats were randomly assigned to four groups (n = 8 per group): (1) Normal Control (NC) group: received a subcutaneous injection of an equal volume of phosphate-buffered saline (PBS); (2) PAH group: received a single subcutaneous injection of monocrotaline (MCT, 60 mg/kg, C101555-50, ALADDIN, China); (3) PAH+sham group: received MCT injection followed by sham surgery, which involved exposure of the cervical sympathetic trunk without ligation or transection; (4) PAH+TCST group: received MCT injection followed by transection of the cervical sympathetic trunk (TCST).

For TCST surgery, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (40 mg/kg). A midline cervical incision was made, and the left cervical sympathetic trunk was carefully

isolated under a stereomicroscope. In the PAH+TCST group, the sympathetic trunk was ligated with 6 – 0 silk suture and transected distally to the ligation. Successful TCST was confirmed by the onset of ipsilateral Horner's syndrome (ptosis, miosis, and enophthalmos) 24 h post-surgery. All animals were sacrificed for tissue collection 28 days after MCT injection.

Right Ventricular Systolic Pressure (RVSP) Measurement

Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and placed in a supine position. A polyethylene catheter (PE-50) filled with heparinized saline was inserted into the right jugular vein and carefully advanced into the right ventricle. RVSP was recorded using a pressure transducer connected to a data acquisition system.

Mean Pulmonary Arterial Pressure (mPAP) Measurement

Following RVSP measurement, a left thoracotomy was performed to expose the heart and pulmonary artery. A PE-50 catheter was inserted into the main pulmonary artery through the right ventricular outflow tract, and mPAP was recorded using the same pressure transducer and data acquisition system.

Right Ventricular Hypertrophy Index (RVHI) Measurement

After hemodynamic measurements, hearts were excised via thoracotomy and washed with cold PBS. The right ventricle (RV) was carefully separated from the left ventricle plus septum (LV + SP). After blotting dry with filter paper, the RV and LV + SP tissues were weighed separately. RVHI was calculated using the following formula: $RVHI = [RV / (LV + SP)] \times 100\%$.

Euthanasia and Anesthesia

For euthanasia, rats were intraperitoneally injected with 2.5% tribromoethanol at a dose of 50 μ L/g body weight. For anesthesia during experimental procedures, rats were initially induced with 3–5% isoflurane at an oxygen flow rate of 1 L/min, and subsequently maintained with 1–2% isoflurane at an oxygen flow rate of 1 L/min.

Tissue Processing

Following animal euthanasia, intact pathological tissues were collected and immediately fixed in 4% paraformaldehyde (PFA) for 48 h at 4°C. After fixation, tissues were dehydrated through a graded ethanol series (70%, 80%, 95%, and 100%), cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at 4 μ m thickness using a microtome (KD-2258, KEDI, China). Sections were mounted on glass slides and dried at 65°C for 2 h.

Hematoxylin and Eosin (H&E) Staining

Paraffin-embedded sections were deparaffinized in xylene (two changes, 20 min each) and rehydrated through a graded ethanol series (100%, 95%, 80%, 70%, and distilled water, 5 min each). Sections were

stained with hematoxylin for 6 min, rinsed in distilled water, differentiated in 0.3% hydrochloric acid alcohol for 10 s, and blued in PBS for 5 min. Subsequently, sections were stained with eosin for 5 min, dehydrated through graded ethanol, cleared in xylene, and mounted with neutral resin. Images were captured using a whole-slide scanner (3DHISTECH, Hungary). Pulmonary vascular remodeling was assessed by measuring the medial wall thickness of small pulmonary arteries (50–100 µm in diameter) using ImageJ software.

RNA Extraction and Sequencing

Total RNA was isolated using TRIZOL reagent (15596-018, Ambion, USA) according to the manufacturer's protocol. RNA quality and concentration were assessed using an Ultrafine spectrophotometer (N50 Touch, IMPLIN, Germany), and RNA integrity was confirmed by 1.0% agarose gel electrophoresis. For each sample, 1 µg of total RNA was treated with RQ1 DNase (M6101, Promega, USA) to eliminate residual genomic DNA.

RNA sequencing was conducted by Wuhan Ruixing Biotech Co., Ltd (<http://www.rxbio.cc>). For each sample, 1 µg of total RNA was treated with RQ1 DNase (M6101, Promega, USA) to remove residual DNA. Strand-specific RNA-seq libraries were constructed using the VAHTS® Universal V8 RNA-seq Library Prep Kit for Illumina (NR605, Vazyme, China). mRNA was enriched with VAHTS mRNA Capture Beads (N401, Vazyme, China), followed by fragmentation and synthesis of double-stranded cDNA. After end repair, A-tailing, and ligation to VAHTS RNA Multiplex Oligos Set 1 for Illumina (N323, Vazyme, China), the ligated products were amplified, purified, quantified and stored at – 80°C until sequencing. The use of dUTP marking during second-strand synthesis enabled strand-specific sequencing.

Libraries were sequenced on the Illumina NovaSeq Xplus platform following the manufacturer's instructions, generating 150 bp paired-end reads.

RNA-seq Data Processing and Alignment

Raw reads containing more than two undefined bases (N) were discarded. Adapter sequences and low-quality bases were trimmed using FASTX-Toolkit (v0.0.13). Reads shorter than 16 nt after trimming were also removed. The resulting clean reads were aligned to the gencode_GRCh38_v45 reference genome using HISAT2(36) with an allowance of four mismatches. Uniquely mapped reads were used to quantify gene expression levels, reported as FPKM (fragments per kilobase of transcript per million fragments mapped) (37).

Differential Gene Expression Analysis

We used uniquely mapped reads for raw read count quantification at the gene level by HTSeq (version 2.0.2). Raw counts, but not FPKM values, were used for DESeq2 (38) analysis, whereas FPKM was used only for visualization of gene expression. Benjamini-Hochberg (BH) false discovery rate (FDR) correction with adjusted *P*-value (FDR) < 0.05, combined with $|\log_2 \text{fold change}| > 1$ as the cut-off for Differentially expressed genes (DEGs) identification.

Functional enrichment analysis

To sort out functional categories of DEGs, Gene Ontology (GO) functional enrichment and KEGG pathways analyses were performed using the KOBAS 2.0 server (39). Enrichment was defined using a hypergeometric test with Benjamini-Hochberg false discovery rate (FDR) correction ($FDR < 0.05$).

Transcription Factor Prediction

Transcription factors (TFs) with binding sites in the promoter regions of *Adra1d* and *Tacr1* were predicted using the Gene Transcription Regulation Database (GTRD). The promoter region (-2000 bp to +200 bp relative to the transcription start site) for each gene was queried in the GTRD to compile a list of all TFs with ChIP-seq-supported binding evidence. To focus on biologically relevant regulators, this list of potential TFs was then cross-referenced with the differentially expressed genes (DEGs) identified from our RNA-seq data. TFs present in both the GTRD-predicted binding list and the DEGs were considered high-confidence candidate regulators(olp-TFs).

Co expression analysis

Co-expression analysis was conducted between olp-TFs and the target genes *Adra1d* and *Tacr1*. The Pearson correlation coefficient was calculated for each TF-target pair, and pairs satisfying $|r| \geq 0.9$ with a p -value < 0.05 were considered statistically significant and selected for further analysis.

Quantitative Real-Time PCR (qPCR)

RNA Extraction and Quality Control: Total RNA was extracted from lung tissues using TRIzol reagent according to the manufacturer's instructions. RNA concentration and purity were measured spectrophotometrically (NanoDrop). RNA integrity was confirmed by agarose gel electrophoresis. **cDNA Synthesis:** 1 µg of total RNA was reverse-transcribed into first-strand cDNA using a PrimeScript™ RT Reagent Kit with gDNA Eraser, following the supplier's protocol. **qPCR Amplification:** qPCR reactions were performed in triplicate using SYBR Green Premix on a StepOnePlus Real-Time PCR System. The reaction mix (20 µL) contained 10 µL SYBR Green Mix, 0.4 µM of each primer, 2 µL cDNA template, and RNase-free water. **Cycling conditions:** 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. **Melt-curve analysis** verified amplification specificity. **Data Analysis:** Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method (40), with β -actin as the endogenous control. The primer sequences used in the study are shown in Table 1.

Table 1
Primer sequences for RT-qPCR experiment.

Foxa3-RAT-F:	ATGTCCATCGCAGCATCCG
Foxa3-RAT-R:	CATACCGTGAAGGCAGTTAGAC
Plcb1-F:	GCCGTGTAATTCAGTGTTCAA
Plcb1-R:	ACAGTGTACAAGGGCAACATT
Tacr1-F:	GTGACATCCGTGAACATCCTC
Tacr1-R:	TCAACAAGGCTGGCAGAAGT
Adra1d-F:	GGGAACATACAGTTAGGAGTGT
Adra1d-R:	GAGGAAGTCAATGGACCTACC

ELISA

Sample Preparation: Lung tissue homogenates were prepared in cold PBS, centrifuged (10,000 g, 15 min, 4°C), and supernatants were collected. Protein concentration was determined using a BCA Protein Assay Kit. Assay Procedure: IP3 levels were quantified using a commercial ELISA kit according to the manufacturer's instructions. Briefly, 50 µL of sample or standard was added to pre-coated wells, followed by 50 µL of detection antibody. After 1 h incubation at 37°C, wells were washed, and 100 µL of HRP-conjugated secondary antibody was added for 30 min. Following another wash, 90 µL of TMB substrate was added and incubated for 15 min in the dark. The reaction was stopped with 50 µL stop solution. Measurement and Calculation: Absorbance was measured at 450 nm using a microplate reader. IP3 concentrations were interpolated from the standard curve and normalized to total protein content.

Statistical analysis

All statistical analyses were performed using R software (v4.0.5). Data are presented as mean ± standard deviation (SD) from at least three independent experiments. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Comparisons between two groups were performed using Student's t-test. Pearson correlation analysis was used for co-expression analysis. A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1 TCST Alleviates Pulmonary Arterial Hypertension in MCT-Induced PAH Rat

To investigate whether TCST mitigates PAH pathology, we established a PAH model in rats using MCT and performed TCST. Successful TCST was confirmed by the onset of Horner's syndrome on the

ipsilateral side, presenting as enophthalmos, miosis and ptosis (Fig. 1A). Compared with the negative control (NC) group, rats in the PAH and PAH+sham groups exhibited significant alveolar structural damage, marked thickening of the pulmonary interstitium and small pulmonary arterial walls, luminal stenosis, disrupted endothelial cell continuity and medial smooth muscle cell proliferation and hypertrophy with disorganized arrangement. Most alveolar structures were effaced. In contrast, the PAH+TCST group showed notable improvements in these pathological features, including relatively preserved alveolar architecture and slight alveolar dilation in some regions (Fig. 1B). Hemodynamically, mPAP (Fig. 1C) and right ventricular systolic pressure (RVSP) (Fig. 1D) were significantly elevated in the PAH and PAH+sham groups compared to the NC group. However, both mPAP (Fig. 1C) and RVSP (Fig. 1D) were significantly reduced in the PAH+TCST group, and the extent of right ventricular hypertrophy was also ameliorated (Fig. 1E). These findings collectively demonstrate that TCST effectively alleviates the symptoms of PAH in the rat model.

(A) Schematic diagram of an experimental workflow involving different treatment groups for PAH in mice. Normal Control (NC) mice received PBS; PAH was induced by MCT injection; PAH + Sham mice underwent sham surgery following MCT induction; and PAH + PSCT mice underwent transection of the cervical sympathetic trunk after MCT induction. (B) Representative H&E-stained lung sections from each experimental group, Scale bar = 50 μ m.; (C) Mean pulmonary arterial pressure (mPAP) measured 28 days post-MCT injection. (D) Right ventricular systolic pressure (RVSP) measured 28 days post-MCT injection.; (E) Evaluation of the right ventricular hypertrophy index (RVHI) in MCT-induced PAH rats. Data are presented as mean $\pm$ SD (n = 8 per group). ** p < 0.01, *** p < 0.001, **** p < 0.001 (one-way ANOVA followed by Tukey's post hoc test).

3.2 TCST Modulates Gene Expression to Attenuate PAH Through Inhibition of Inflammation and Calcium-Driven Proliferation

To elucidate the molecular mechanisms underlying the therapeutic effect of TCST, we performed RNA-seq on lung tissues from the PAH+sham and PAH+TCST groups. Principal component analysis revealed that TCST induced substantial gene expression profile changes between these two groups (Fig. 2A). Comparative analysis identified 500 differentially expressed genes (DEGs) in the PAH+TCST group vs. the PAH+sham group, including 61 up-regulated and 439 down-regulated genes (Fig. 2B). To further investigate the functional roles of the DEGs, GO and KEGG enrichment analyses were performed. For the up-regulated DEGs, GO analysis revealed enrichment in molecular functions such as protein binding, RNA polymerase II cis-regulatory region sequence-specific DNA binding, as well as biological processes including inflammatory response, immune response, apoptotic response, and positive regulation of apoptosis. KEGG pathway analysis showed that up-regulated genes were enriched in the cytokine-cytokine receptor interaction signaling pathway (Fig. 2C). Regarding the down-regulated DEGs, GO enrichment analysis identified molecular functions such as dynein chain binding, calcium ion binding, calcium-dependent protein binding, ATP binding, ATP hydrolysis activity, and growth factor activity. Biological processes included adaptive immune response, sperm axoneme assembly, sperm motility,

cilium movement, positive regulation of calcium ion concentration, and positive regulation of cell proliferation. KEGG pathway analysis further revealed that down-regulated genes were enriched in pathways such as the complement cascade, cytokine-cytokine receptor interaction, primary immunodeficiency, calcium signaling pathway, PPAR signaling pathway, AMPK signaling pathway, and PI3K-Akt signaling pathway (Fig. 2D). Collectively, these transcriptomic data suggest that TCST exerts its therapeutic effects in PAH by modulating multiple biological processes, with a particular emphasis on suppressing calcium signaling and cell proliferation pathways, while also influencing inflammatory and immune-related pathways. These findings provide a molecular framework for further investigating the key regulators and downstream targets underlying TCST-mediated amelioration of PAH.

(A) Principal component analysis (PCA) of transcriptomic profiles after normalization of gene expression levels. The confidence ellipse is shown for each experimental group (n = 3 per group); (B) Volcano plot displaying the distribution of differentially expressed genes (DEGs) between PAH+TCST and PAH+sham groups; (C) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (41–43) of up-regulated DEGs; (D) GO and KEGG pathway enrichment analysis (41–43) of down-regulated DEGs.

3.3 TCST Targets the *Foxa3*/*Adra1d*-*Tacr1* Axis to Attenuate Calcium Signaling

To identify key genes involved in calcium signaling underlying the therapeutic effects of TCST, we integrated calcium-related gene sets from both GO and KEGG enrichment analyses. A total of eight genes known to promote calcium influx were downregulated following TCST: *Cacna1b*, *Adra1d*, *Tacr1*, *P2rx6*, *Ccr10*, *Cd24*, *Cxcl13*, and *Fpr2* (Fig. 3A). Among these, *Adra1d* (adrenergic receptor alpha 1D) and *Tacr1* (tachykinin receptor 1) were of particular interest, as they were consistently enriched in both GO and KEGG pathways associated with downregulated genes (Fig. 3B). Additionally, the expression of *Plcb1*, which encodes phospholipase C (PLC)—a key enzyme that promotes downstream calcium mobilization—was also significantly suppressed following TCST (Fig. 3C).

To explore the upstream regulators of *Adra1d* and *Tacr1*, we screened for transcription factors with predicted binding activity to their promoter regions using the GTRD database. Intersecting these candidates with the downregulated DEGs identified from our RNA-seq data revealed *Foxa3* as a common transcription factor potentially regulating both *Adra1d* and *Tacr1* (Fig. 3D-E). To further validate this regulatory relationship, we used the JASPAR database to obtain the DNA-binding motif of FOXA3 and applied the FIMO tool to predict putative binding sites within the promoter regions of *Adra1d* and *Tacr1*. Multiple potential binding sites for this motif (GTAAACA) were identified in the promoters of both genes (Fig. 3F). Co-expression analysis further substantiated a strong positive correlation between *Foxa3* expression with both *Adra1d* ($r = 0.96$, $p = 0.002$) and *Tacr1* ($r = 0.95$, $p = 0.004$) (Fig. 3G). Collectively, these findings suggest that TCST may suppress the transcription factor FOXA3, leading to down-regulation of its target genes *Adra1d* and *Tacr1*, ultimately resulting in attenuated intracellular calcium

signaling. This FOXA3-mediated attenuation of the calcium pathway represents a plausible mechanism through which TCST alleviates PAH.

(A) Heatmap showing expression levels of eight calcium influx-related genes (*Cacna1b*, *Adra1d*, *Tacr1*, *P2rx6*, *Ccr10*, *Cd24*, *Cxcl13*, and *Fpr2*) that were downregulated following TCST. (B) Bar plot showing expression levels of *Adra1d* and *Tacr1* across groups; (C) Bar plot showing expression levels of *Plcb1* across groups; (D) Venn diagram illustrating the overlap between predicted transcription factors for *Adra1d* and *Tacr1* promoters using the GTRD database and down-regulated DEGs; (E) Bar plot showing expression of *Foxa3* across groups; (F) Schematic representation of predicted FOXA3 binding sites within the promoter regions of *Adra1d* and *Tacr1*, identified using the JASPAR and FIMO databases. (G) Scatter plots showing co-expression correlation between *Foxa3* and the target genes *Adra1d* and *Tacr1*. Data are presented as mean $\pm$ SD (n = 3 per group for RNA-seq). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.4 Verification of TCST's Regulatory Effect on Key Molecules in the FOXA3/Calcium Signaling Pathway

To further validate the molecular mechanisms suggested by the transcriptomic analysis, we verified the key molecules at the transcriptional and protein levels using qPCR and ELISA techniques. qPCR results showed that compared to the PAH+sham group, the mRNA expression levels of *Foxa3* and its downstream target genes *Adra1d* and *Tacr1* in the lung tissues of the PAH+TCST group were significantly downregulated (Fig. 4A-B). Concurrently, the expression levels of the PLC-encoding genes *Plcb1*, were also significantly reduced (Fig. 4C). These findings are highly consistent with the RNA-seq result, confirming at the molecular level that TCST can inhibit the expression of *Foxa3* and its regulated calcium signaling pathway-related genes.

To functionally confirm the altered activity of the calcium signaling pathway, we measured the level of the critical intracellular second messenger, inositol trisphosphate (IP3). IP3 is produced by activated PLC and is a key molecule mediating calcium ion release from the endoplasmic reticulum. ELISA detection revealed that the IP3 content in the lung tissues of the PAH+TCST group was significantly decreased compared to the PAH+sham group (Fig. 4D). This result directly indicates that following TCST intervention, the activity of the PLC-dependent calcium signaling pathway in lung tissue is suppressed, leading to a diminished intracellular calcium mobilization capacity.

(A-C) Bar plot showing qPCR validation of *Foxa3*, *Adra1d*, *Tacr1* and *Plcb1* expression across groups; (D) Bar plot showing ELISA measurement of IP3 concentration across groups. Data are presented as mean $\pm$ SD (n = 6 per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

This study systematically evaluated the therapeutic potential of TCST and investigated its underlying molecular mechanisms in an MCT-induced rat model of PAH. TCST significantly attenuated pulmonary vascular remodeling, reduced mPAP and RVSP, and ameliorated right ventricular hypertrophy. RNA-seq

analysis revealed that TCST induced broad alterations in the lung transcriptome, with downregulated DEGs enriched in immune responses, inflammatory responses, cell proliferation and the calcium signaling pathway. Further investigation indicated that TCST suppresses the *Foxa3/Adra1d-Tacr1* axis, ultimately resulting in inhibition of the calcium signaling pathway and attenuation of PASMC proliferation. Given the established role of calcium signaling in integrating both proliferative and inflammatory signals(44), we speculate that suppression of the *Foxa3/Adra1d-Tacr1* axis and downstream calcium signaling may contribute to reduced PASMC proliferation and, potentially, attenuated pulmonary inflammation, thereby collectively ameliorating PAH. The proposed molecular mechanism underlying the therapeutic effect of TCST in PAH is summarized schematically in Fig. 5.

From a neuro-regulatory standpoint, pulmonary vascular innervation derives from branches of the vagus nerve and the cervicothoracic sympathetic trunk, comprising adrenergic, cholinergic and sensory fibers(45). PAH is often accompanied by sympathetic overactivity, evidenced by increased muscle sympathetic nerve activity and reduced heart rate variability, the degree of which correlates with disease severity(16, 18). This provides a rationale for employing sympathetic blockade strategies in PAH treatment. Compared to renal denervation and PADN, TCST offers the potential advantage of selectively interrupting sympathetic signaling while potentially sparing parasympathetic nerves and minimizing vascular structural damage (29). Furthermore, by targeting the cervical sympathetic trunk, TCST can mimic the long-term effects of SGB and may avoid adverse effects associated with β -blockers, such as negative inotropy (33, 46), thereby enabling a more precise intervention targeting core pathological processes in PAH.

Our transcriptomic analysis provides a comprehensive overview of the systemic regulatory mechanisms engaged by TCST. The enrichment of downregulated genes in immune-inflammatory, cell proliferation and calcium signaling pathways suggests that TCST exerts its therapeutic effects by targeting interconnected pathological processes. Aberrant PASMC proliferation is a cornerstone of vascular remodeling in PAH, intimately linked to elevated intracellular calcium concentration (3, 8, 47, 48). Similarly, chronic inflammation characterized by perivascular inflammatory cell infiltration and elevated pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α —is increasingly recognized as a critical driver of PAH pathogenesis (11, 12). Importantly, calcium signaling serves as a central node integrating these two processes: elevated cytosolic Ca²⁺ not only directly promotes PASMC proliferation but also activates pro-inflammatory transcription factors including NF- κ B and NFAT, thereby amplifying inflammatory responses (49). In our study, TCST significantly downregulated multiple calcium signaling-related genes (e.g., *Adra1d*, *Tacr1*, *Plcb1*) and reduced IP3 levels, while KEGG pathway analysis revealed downregulation of inflammation-related pathways such as cytokine-cytokine receptor interaction and the complement cascade (Fig. 2D). These findings suggest that TCST may concurrently attenuate both PASMC proliferation and pulmonary inflammation through suppression of calcium signaling.

Delving deeper into the specific regulatory nodes of the calcium signaling pathway, we identified *Adra1d* and *Tacr1* as key elements. ADRA1D (α 1D-adrenergic receptor) and TACR1 (tachykinin receptor 1) are G protein-coupled receptor (GPCR) that activate phospholipase C (PLC), generating IP₃ and DAG to mediate

intracellular Ca^{2+} release(50, 51). Norepinephrine (NE) binding to ADRA1D initiates sympathetic signal transduction (52, 53), and in PAH models, has been shown to mediate PASMC proliferation via the calcium-dependent pathways (54). In our study, TCST intervention significantly downregulated both *Adra1d* and *Tacr1*, indicating that TCST can molecularly dampen sympathetic-driven pulmonary vascular calcium signaling. Subsequent bioinformatics analysis and co-expression validation pinpointed the transcription factor *Foxa3* as an upstream regulator of this process. The strong positive correlation between *Foxa3* expression and both *Adra1d* and *Tacr1*, coupled with its predicted binding activity, suggests a novel regulatory axis: TCST $\rightarrow$ $\downarrow$ Foxa3 $\rightarrow$ $\downarrow$ (*Adra1d*, *Tacr1*) $\rightarrow$ Inhibition of Calcium Signaling. Given the established role of calcium signaling in both PASMC proliferation and inflammatory activation, this axis may contribute to dual anti-proliferative and anti-inflammatory effects, thereby ameliorating pulmonary vascular remodeling in PAH.

Nevertheless, this study has limitations. First, the proposed TCST–Foxa3–calcium signaling pathway axis is primarily founded on bioinformatic correlations and *in vitro* expression validation. Direct transcriptional regulation of *Adra1d* and *Tacr1* by *Foxa3* requires further causal validation through experiments such as chromatin immunoprecipitation (ChIP) and dual-luciferase reporter assays. Second, while our focus was on PASMC proliferation, the potential effects of TCST on pulmonary arterial endothelial cell function, endothelial-mesenchymal transition (EndMT) and the systemic neuroendocrine system warrant further investigation. Third, although our transcriptomic data suggest an anti-inflammatory effect of TCST, direct evidence such as measurement of cytokine levels (e.g., IL-1 β , IL-6, TNF- α) or inflammatory cell infiltration in lung tissues would strengthen this conclusion. Finally, translating these findings from animal models to clinical application presents challenges, including optimizing SGB parameters to achieve sustained and precise sympathetic inhibition in patients.

Conclusion

In summary, this study demonstrates that TCST effectively alleviates MCT-induced PAH in rats by suppressing the *Foxa3/Adra1d-Tacr1* axis and downstream calcium signaling. Transcriptomic analysis revealed that TCST downregulated genes involved in calcium signaling, cell proliferation, and inflammatory pathways. Given that calcium signaling integrates both proliferative and inflammatory signals, we propose that TCST exerts dual anti-proliferative and anti-inflammatory effects through this pathway. These findings provide a mechanistic basis for developing neuromodulatory strategies for PAH. These findings not only deepen our understanding of sympathetic nervous system involvement in PAH pathogenesis but also provide a significant theoretical foundation and identify potential targets for developing combination treatment strategies for PAH based on neuromodulation.

Declarations

Data availability

Our RNA-seq data have been deposited in the NCBI Gene Expression Omnibus (GEO) under the accession number GSE328273. The dataset is currently private and will be publicly released on April 30, 2026. To allow access during the peer-review process, we provide the following reviewer token: qdslycmqtxwhbyb. Reviewers can access the data via the temporary link below: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE328273>

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

1. Mocumbi, A. et al. Pulmonary hypertension. *Nat. reviews Disease primers*. **10**, 1. 10.1038/s41572-023-00486-7((2024).
2. Humbert, M. et al. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J* ;**61**. 10.1183/13993003.00879-2022(2023).
3. Adu-Amankwaah, J. et al. Pulmonary Hypertension: Molecular Mechanisms and Clinical Studies. *MedComm*.6:e70134. (2025). 10.1002/mco2.70134
4. Johnson, S. et al. Pulmonary Hypertension: A Contemporary Review. *American journal of respiratory and critical care medicine*.208:528 – 48. (2023). 10.1164/rccm.202302-0327SO(
5. Maron, B. A. et al. Pulmonary Arterial Hypertension: Diagnosis, Treatment, and Novel Advances. *Am. J. Respir. Crit Care Med*. **203**, 1472–1487. 10.1164/rccm.202012-4317SO((2021).
6. Westerhof, B. E., Saouti, N., van der Laarse, W. J., Westerhof, N. & Vonk Noordegraaf, A. Treatment strategies for the right heart in pulmonary hypertension. *Cardiovascular. Res*. **113**, 1465–1473. 10.1093/cvr/cvx148 (2017).
7. Sahay, S. et al. Contemporary Treatment of Pulmonary Arterial Hypertension: A U.S. Perspective. *Am. J. Respir. Crit Care Med*. **210**, 581–592. 10.1164/rccm.202405-0914SO((2024).
8. Adu-Amankwaah, J. et al. Signaling pathways and targeted therapy for pulmonary hypertension. *Signal. Transduct. Target. therapy*. **10**, 207. 10.1038/s41392-025-02287-8((2025).
9. Zhao, M., Chen, N., Li, X., Lin, L. & Chen, X. MiR-19a modulates hypoxia-mediated cell proliferation and migration via repressing PTEN in human pulmonary arterial smooth muscle. *Life Sci*. **239**, 116928. 10.1016/j.lfs.2019.116928 (2019).
10. Balistrieri, A., Makino, A. & Yuan, J. X. Pathophysiology and pathogenic mechanisms of pulmonary hypertension: role of membrane receptors, ion channels, and Ca(2+) signaling. *Physiol Rev* ;**103**:1827–1897. 10.1152/physrev.00030.2021(2023).

11. Hu, Y., Chi, L., Kuebler, W. M. & Goldenberg, N. M. Perivascular Inflammation in Pulmonary Arterial Hypertension. *Cells*. **9**. (2020). 10.3390/cells9112338
12. Zhang, Y. et al. Immunotherapy for Pulmonary Arterial Hypertension: From the Pathogenesis to Clinical Management. *Int. J. Mol. Sci.* **25** 10.3390/ijms25158427 (2024).
13. Si, M., Hu, Y., Jin, Z., Mao, Y. & Kang, L. NF- κ B and pulmonary hypertension: Advances in mechanistic research and therapeutic applications. *Am. heart J. plus: Cardiol. Res. Pract.* **60**, 100669. 10.1016/j.ahjo.2025.100669 (2025).
14. Landy, C. et al. Resolution of inflammation in pulmonary arterial hypertension, the influence of sex- An IUPHAR immunopharmacology committee review. *Br. J. Pharmacol.* **182**, 5706–5733. 10.1111/bph.70207 (2025).
15. Vaillancourt, M. et al. Autonomic nervous system involvement in pulmonary arterial hypertension. *Respir. Res.* **18**, 201. 10.1186/s12931-017-0679-6 (2017).
16. Velez-Roa, S. et al. Increased sympathetic nerve activity in pulmonary artery hypertension. *Circulation* **110**, 1308–1312. 10.1161/01.Cir.0000140724.90898.D3 (2004).
17. Naeije, R., Richter, M. J. & Rubin, L. J. The physiological basis of pulmonary arterial hypertension. *Eur Respir J* ;**59**. 10.1183/13993003.02334-2021(2022).
18. Ciarka, A., Doan, V., Velez-Roa, S., Naeije, R. & van de Borne, P. Prognostic significance of sympathetic nervous system activation in pulmonary arterial hypertension. *Am. J. Respir. Crit Care Med.* **181**, 1269–1275. 10.1164/rccm.200912-1856OC((2010).
19. Zeder, K., Siew, E. D., Kovacs, G., Brittain, E. L. & Maron, B. A. Pulmonary hypertension and chronic kidney disease: prevalence, pathophysiology and outcomes. *Nature reviews Nephrology* **20**:742–754. 10.1038/s41581-024-00857-7(2024).
20. Hemnes, A. R. & Brittain, E. L. Autonomic Nervous System in Pulmonary Arterial Hypertension: Time to Rest and Digest. *Circulation* **137**, 925–927. 10.1161/circulationaha.117.032355 (2018).
21. Wensel, R. et al. Impaired cardiac autonomic control relates to disease severity in pulmonary hypertension. *Eur. Respir J.* **34**, 895–901. 10.1183/09031936.00145708 (2009).
22. Provencher, S. et al. Deleterious effects of beta-blockers on exercise capacity and hemodynamics in patients with portopulmonary hypertension. *Gastroenterology* **130**:120–126. 10.1053/j.gastro.2005.10.013(2006).
23. de Man, F. S. et al. Bisoprolol delays progression towards right heart failure in experimental pulmonary hypertension. *Circ. Heart. Fail.* **5**, 97–105. 10.1161/circheartfailure.111.964494 (2012).
24. Qingyan, Z. et al. Beneficial Effects of Renal Denervation on Pulmonary Vascular Remodeling in Experimental Pulmonary Artery Hypertension. *Revista espanola de cardiologia (English ed.)*. **68**:562 – 70. 10.1016/j.rec.2014.11.022(2015).
25. Liu, Q. et al. Effects of renal denervation on monocrotaline induced pulmonary remodeling. *Oncotarget* **8**, 46846–46855. 10.18632/oncotarget.15154 (2017).

26. Chen, S. L. et al. Pulmonary artery denervation to treat pulmonary arterial hypertension: the single-center, prospective, first-in-man PADN-1 study (first-in-man pulmonary artery denervation for treatment of pulmonary artery hypertension). *J Am. Coll. Cardiol* ;**62**:1092–1100. 10.1016/j.jacc.2013.05.075(2013).
27. Chen, S. L. et al. Percutaneous pulmonary artery denervation completely abolishes experimental pulmonary arterial hypertension in vivo. *EuroIntervention: J. EuroPCR Collab. Working Group. Interventional Cardiol. Eur. Soc. Cardiol.* **9**, 269–276. 10.4244/eijv9i2a43 (2013).
28. Chen, S. L. et al. Hemodynamic, functional, and clinical responses to pulmonary artery denervation in patients with pulmonary arterial hypertension of different causes: phase II results from the Pulmonary Artery Denervation-1 study. *Circ. Cardiovasc. Interv.* **8**, e002837. 10.1161/circinterventions.115.002837 (2015).
29. Zhao, Y. et al. Transection of the cervical sympathetic trunk inhibits the progression of pulmonary arterial hypertension via ERK-1/2 Signalling. *Respiratory research* **20**:121. 10.1186/s12931-019-1090-2(2019).
30. Shin, J. H., Kim, D. W., Yang, J. Y. & Lee, W. I. Efficacy of stellate ganglion block in cholinergic urticaria with acquired generalized hypohidrosis. *Korean J. Pain.* **25**, 278–280. 10.3344/kjp.2012.25.4.278 (2012).
31. Chouairi, F. et al. A Multicenter Study of Stellate Ganglion Block as a Temporizing Treatment for Refractory Ventricular Arrhythmias. *JACC Clin. electrophysiology* **10**:750–758. 10.1016/j.jacep.2023.12.012(2024).
32. Fudim, M., Boortz-Marx, R., Patel, C. B., Sun, A. Y. & Piccini, J. P. Autonomic Modulation for the Treatment of Ventricular Arrhythmias: Therapeutic Use of Percutaneous Stellate Ganglion Blocks. *J. Cardiovasc. Electrophys.* **28**, 446–449. 10.1111/jce.13152 (2017).
33. Long, J. et al. Effects of transection of cervical sympathetic trunk on cognitive function of traumatic brain injury rats. *Neuropsychiatr. Dis. Treat.* **15**, 1121–1131. 10.2147/ndt.S199450 (2019).
34. Li, W. et al. A brain-to-lung signal from GABAergic neurons to ADRB2(+) interstitial macrophages promotes pulmonary inflammatory responses. *Immunity*.58:2069-85.e9. 10.1016/j.immuni.2025.05.005(2025).
35. Liu, R. et al. Norepinephrine stimulation of alpha1D-adrenoceptor promotes proliferation of pulmonary artery smooth muscle cells via ERK-1/2 signaling. *Int. J. Biochem. Cell. Biol.* **88**, 100–112. 10.1016/j.biocel.2017.05.001 (2017).
36. Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Nature methods*.12:357 – 60. (2015).
37. Trapnell, C. et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* **28**, 511–515 (2010).
38. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 1–21 (2014).

39. Xie, C. et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res.* **39**, W316–W22 (2011).
40. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego Calif).* **25**, 402–408. 10.1006/meth.2001.1262 (2001).
41. Besal, R., Adamič, P., Beović, B. & Papst, L. Systemic Antimicrobial Treatment of Chronic Osteomyelitis in Adults: A Narrative Review. *Antibiotics (Basel, Switzerland)*. **12**. (2023). 10.3390/antibiotics12060944
42. Kanehisa, M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* **28**, 1947–1951. 10.1002/pro.3715 (2019).
43. Kanehisa, M. & Goto, S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30. 10.1093/nar/28.1.27 (2000).
44. Giorgi, C., Danese, A., Missiroli, S., Patergnani, S. & Pinton, P. Calcium Dynamics as a Machine for Decoding Signals. *Trends Cell. Biol.* **28**, 258–273. 10.1016/j.tcb.2018.01.002 (2018).
45. Townsley, M. I. Structure and composition of pulmonary arteries, capillaries, and veins. *Compr. Physiol.* **2**, 675–709. 10.1002/cphy.c100081 (2012).
46. Wang, J. et al. Carotid Baroreceptor Stimulation Improves Pulmonary Arterial Remodeling and Right Ventricular Dysfunction in Pulmonary Arterial Hypertension. *JACC. Basic to translational science*. **9**:475 – 92. 10.1016/j.jacbts.2024.01.012(2024).
47. Schermuly, R. T., Ghofrani, H. A., Wilkins, M. R. & Grimminger, F. Mechanisms of disease: pulmonary arterial hypertension. *Nat. Rev. Cardiol.* **8**, 443–455. 10.1038/nrcardio.2011.87 (2011).
48. Xiao, L. & Tong, X. [Advances in molecular mechanism of vascular remodeling in pulmonary arterial hypertension]. *Zhejiang da xue xue bao. Yi xue ban = Journal of Zhejiang University. Medical sciences*. **48**:102 – 10. 10.3785/j.issn.1008-9292.2019.02.15(2019).
49. Song, S. et al. STIM2 (Stromal Interaction Molecule 2)-Mediated Increase in Resting Cytosolic Free Ca(2+) Concentration Stimulates PASMC Proliferation in Pulmonary Arterial Hypertension. *Hypertension (Dallas, Tex.:)*. **71**:518 – 29.).71:518 – 29. (1979). 10.1161/hypertensionaha.117.10503(2018).
50. Kraboth, Z. et al. Involvement of the Catecholamine Pathway in Glioblastoma Development. *Cells*. **10**. (2021). 10.3390/cells10030549
51. Hu, G. et al. IGFs Potentiate TAC3-induced SLα Expression via Upregulation of TACR3 Expression in Grass Carp Pituitary Cells. *Cells* **8**. 10.3390/cells8080887 (2019).
52. Filippi, S. et al. alpha(1D)-adrenoceptors cause endothelium-dependent vasodilatation in the rat mesenteric vascular bed. *J. Pharmacol. Exp. Ther.* **296**, 869–875 (2001).
53. Vinci, M. C., Bellik, L., Filippi, S., Ledda, F. & Parenti, A. Trophic effects induced by alpha1D-adrenoceptors on endothelial cells are potentiated by hypoxia. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H2140–H2147. 10.1152/ajpheart.00390.2007 (2007).

54. Luo, Q. et al. alpha1A-adrenoceptor is involved in norepinephrine-induced proliferation of pulmonary artery smooth muscle cells via CaMKII signaling. *J. Cell. Biochem.* **120**, 9345–9355. 10.1002/jcb.28210 (2019).

Figures

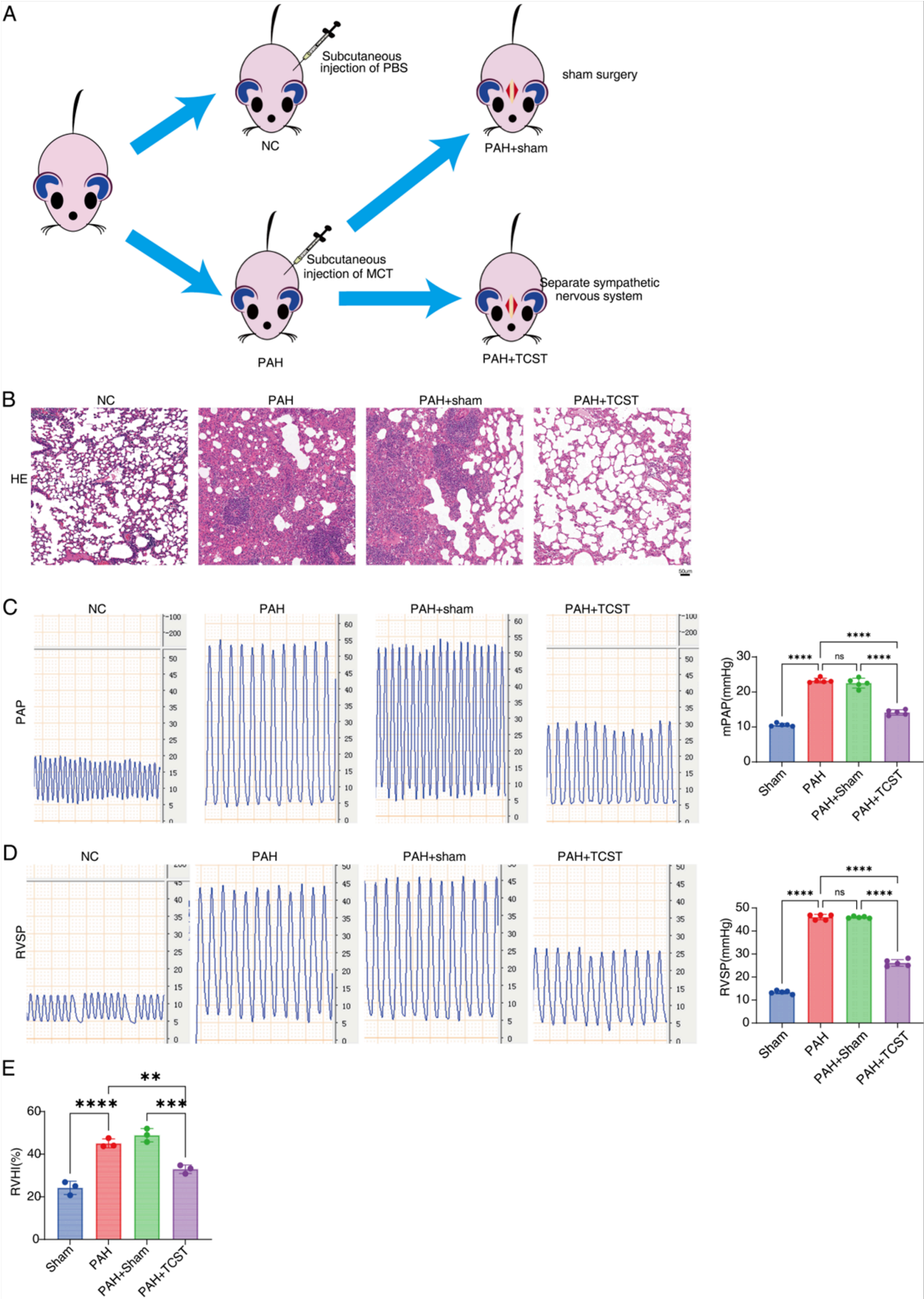


Figure 1

TCST ameliorates pulmonary pathology and hemodynamics in MCT-induced PAH rats

(A) Schematic diagram of an experimental workflow involving different treatment groups for PAH in mice. Normal Control (NC) mice received PBS; PAH was induced by MCT injection; PAH + Sham mice underwent sham surgery following MCT induction; and PAH + PSCT mice underwent transection of the cervical sympathetic trunk after MCT induction. (B) Representative H&E-stained lung sections from each experimental group, Scale bar = 50 μ m.; (C) Mean pulmonary arterial pressure (mPAP) measured 28 days post-MCT injection. (D) Right ventricular systolic pressure (RVSP) measured 28 days post-MCT injection.; (E) Evaluation of the right ventricular hypertrophy index (RVHI) in MCT-induced PAH rats. Data are presented as mean $\pm$ SD (n = 8 per group). ** p < 0.01, *** p < 0.001, **** p < 0.001 (one-way ANOVA followed by Tukey's post hoc test).

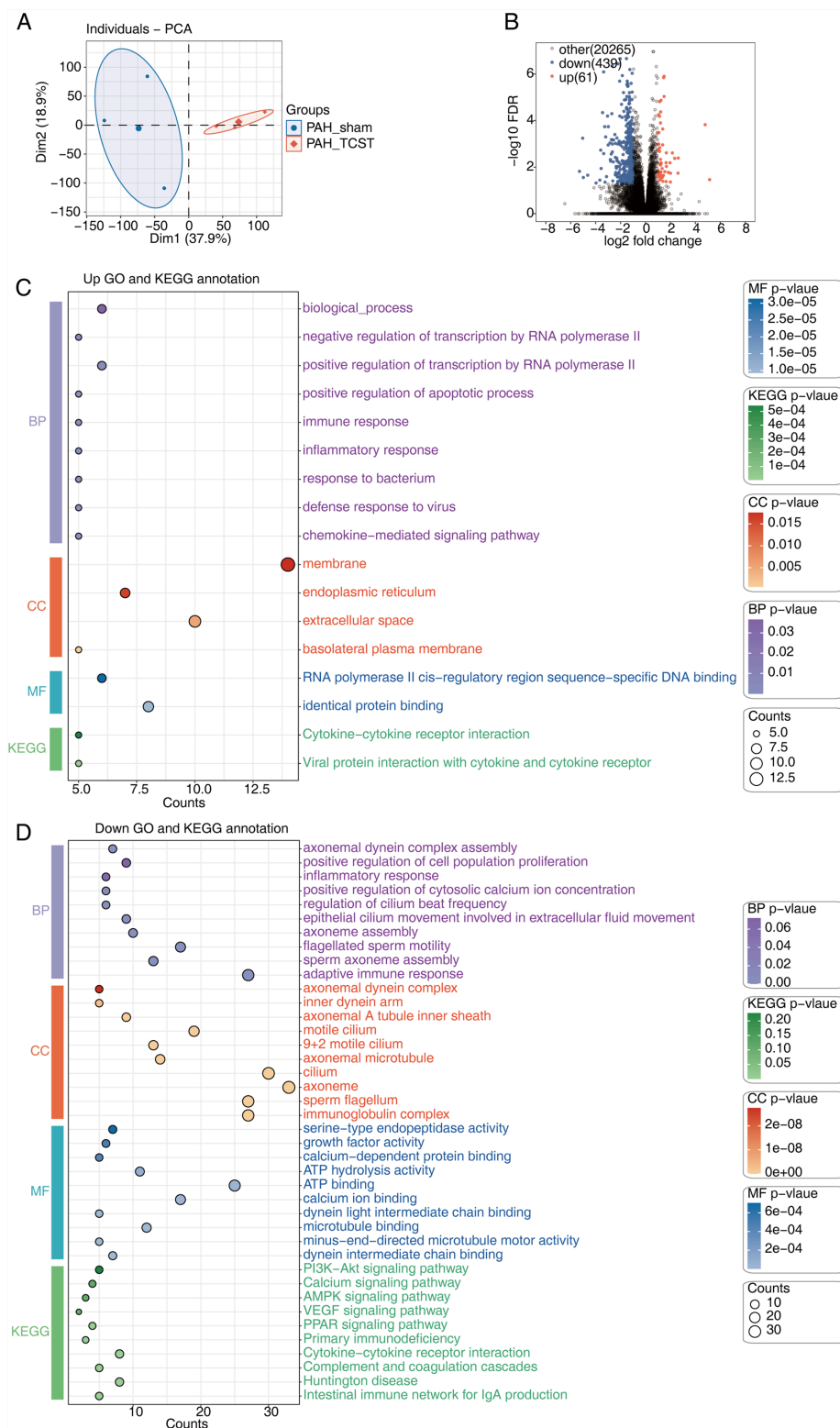


Figure 2

TCST modulates the lung transcriptome in PAH rats

(A) Principal component analysis (PCA) of transcriptomic profiles after normalization of gene expression levels. The confidence ellipse is shown for each experimental group (n = 3 per group) (B) Volcano plot displaying the distribution of differentially expressed genes (DEGs) between PAH+TCST and PAH+sham

groups; (C) Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (41-43) of up-regulated DEGs (D) GO and KEGG pathway enrichment analysis (41-43) of down-regulated DEGs.

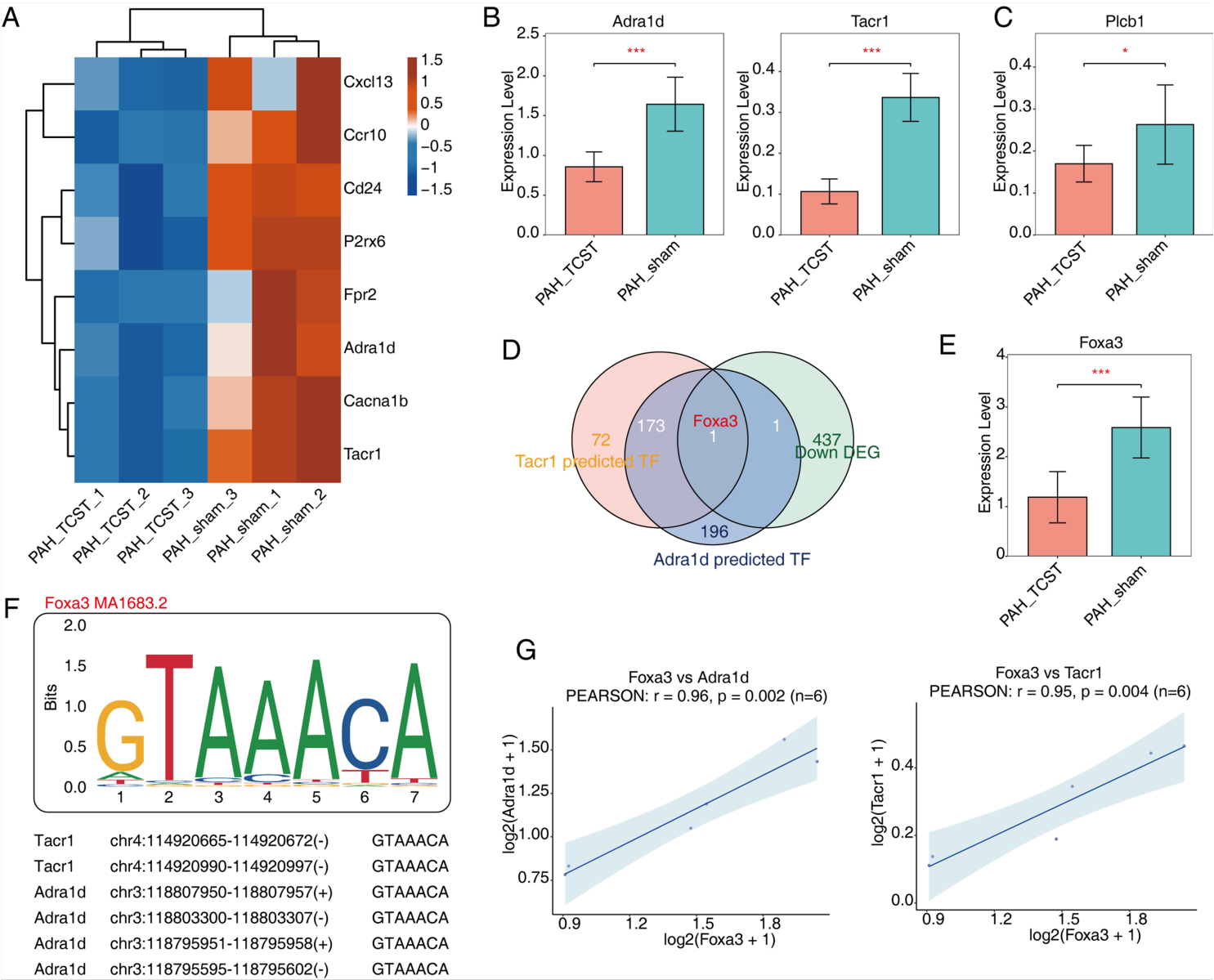


Figure 3

TCST regulates the calcium signaling pathway by suppressing Foxa3 and its target genes Adra1d/Tacr1

(A) Heatmap showing expression levels of eight calcium influx-related genes (*Cacna1b*, *Adra1d*, *Tacr1*, *P2rx6*, *Ccr10*, *Cd24*, *Cxcl13*, and *Fpr2*) that were downregulated following TCST. (B) Bar plot showing expression levels of *Adra1d* and *Tacr1* across groups (C) Bar plot showing expression levels of *Plcb1* across groups (D) Venn diagram illustrating the overlap between predicted transcription factors for *Adra1d* and *Tacr1* promoters using the GTRD database and down-regulated DEGs (E) Bar plot showing expression of *Foxa3* across groups (F) Schematic representation of predicted FOXA3 binding sites

within the promoter regions of *Adra1d* and *Tacr1*, identified using the JASPAR and FIMO databases. (G) Scatter plots showing co-expression correlation between *Foxa3* and the target genes *Adra1d* and *Tacr1*. Data are presented as mean $\pm$ SD ($n = 3$ per group for RNA-seq). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

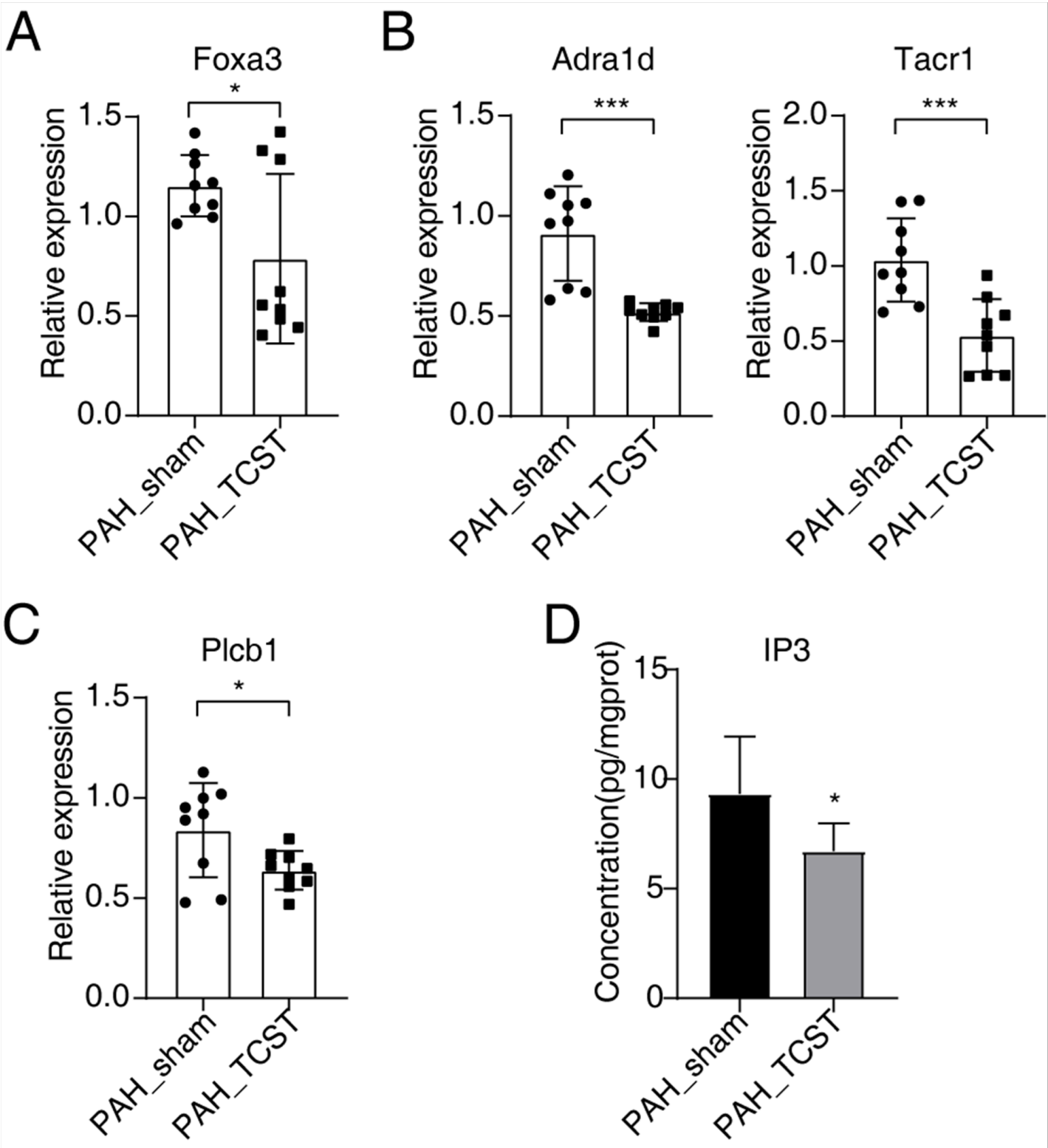


Figure 4

TCST suppresses the *Foxa3*-driven calcium signaling pathway in PAH rats

(A-C) Bar plot showing qPCR validation of *Foxa3*, *Adra1d*, *Tacr1* and *Plcb1* expression across groups (D) Bar plot showing ELISA measurement of IP3 concentration across groups. Data are presented as mean $\pm$ SD (n = 6 per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

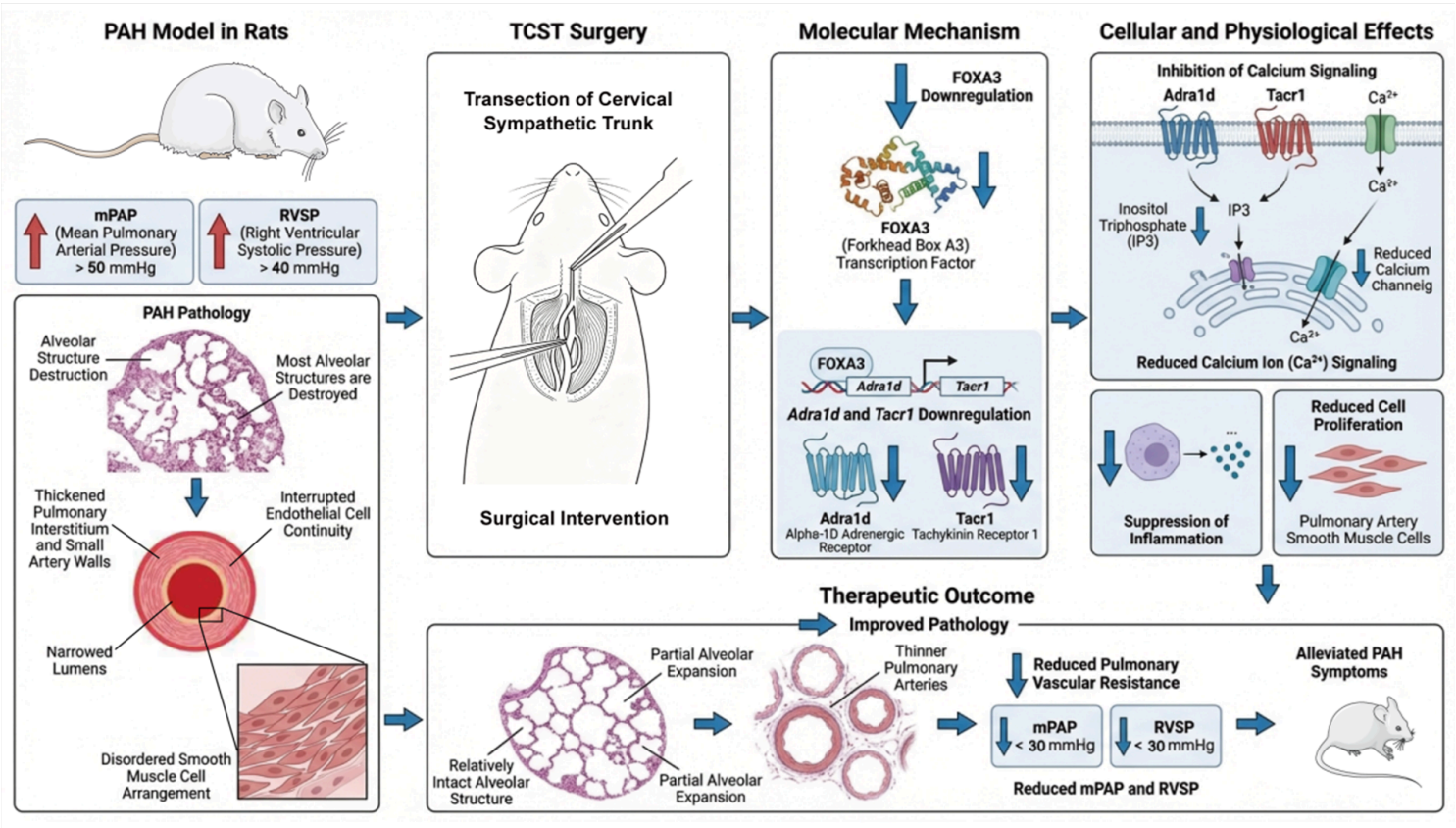


Figure 5

Schematic diagram of the proposed mechanism