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Vascular smooth muscle cell-derived SO₂ sulphenylated interferon regulatory factor 1 to inhibit VSMC senescence

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Background: Vascular smooth muscle cell (VSMC) senescence is a critical driver of vascular aging and various age-related cardiovascular diseases. Endogenous sulfur dioxide (SO₂), a newly identified key cardiovascular gaseous signaling mediator, accelerates collagen deposition and vascular remodeling in VSMCs when downregulated. However, its effects on VSMC senescence remain unclear.

Objective: This study focused on exploring the role of endogenous SO₂ in VSMC senescence and its associated molecular pathways.

Methods: Aged mice (24 months old), VSMC-specific aspartate aminotransferase 1 (AAT1) knockout (VSMC-AAT1-KO) mice, D-galactose (D-gal)-treated aorta rings and rat VSMC line A7r5 were used in the experiments. AAT1 expression was detected by Western blot and single-cell RNA sequencing. Senescence markers Tp53, p21^{Cip/Waf}, *interleukin 1β* (*IL-1β*) and *IL6* expression were detected by Western blot and real-time quantitative PCR. Senescence-associated β-galactosidase (SA-β-gal) activity was detected using SA-β-gal staining kit. Sulphenylation of interferon regulatory factor 1 (IRF1) was detected using a biotin switch assay. The plasmid for mutant IRF1 (mutation of cysteine 83 to serine, C83S) were constructed by site-directed mutagenesis.

Results: The expression of AAT1, a key enzyme for SO₂ production, was reduced in the aortic tissue of aged mice in comparison to young mice. VSMC-AAT1-KO mice exhibited elevated protein expression of senescence markers Tp53, p21^{Cip/Waf} and γ-H2AX in the aortic tissue. AAT1 knockdown in VSMCs elevated expression of Tp53, p21^{Cip/Waf}, *IL-1β* and *IL-6*, and enhanced SA-β-gal activity. While SO₂ donor supplementation rescued VSMC senescence caused by AAT1 knockdown and blocked aortic ring aging induced by D-gal. Mechanistically, SO₂ promoted IRF1 sulphenylation, inhibited IRF1 nuclear translocation, which in turn downregulated the expression of senescence markers and the activity of SA-β-gal. Furthermore, mutation of C83 in IRF1 abolished SO₂-mediated IRF1 sulphenylation and blocked the inhibitory effect of SO₂ on VSMC senescence.

Conclusion: Reduction of the endogenous SO₂/AAT1 pathway played a crucial role in driving VSMC senescence. Endogenous SO₂ counteracted VSMC senescence and vascular aging via the sulphenylation of IRF1 at C83.

KEYWORDS

endogenous SO₂, VSMC senescence, IRF1, sulphenylation, cysteine, post-translational modification

1 Introduction

Vascular aging, a core event in the human aging process, significantly contributes to cardiovascular disease progression (Lin et al., 2023). Vascular smooth muscle cells (VSMCs) (Lin et al., 2023) is a key component of blood vessels and their senescence drives vascular aging. Senescent VSMCs exhibit DNA damage, telomere damage and shortening, halted cell cycles, increased senescence-associated β -galactosidase (SA- β -gal) activity, and the senescence-associated secretory phenotype (SASP). These changes contribute to chronic vascular inflammation, vascular remodeling and impaired arterial function (Okuno et al., 2020; Zha et al., 2022). However, the endogenous regulatory mechanisms underlying VSMC senescence remain incompletely understood.

Endogenous sulfur dioxide (SO₂), a metabolite catalyzed by aspartate aminotransferase (AAT) using L-cysteine as the substrate, is a novel gaseous signaling molecule in cardiovascular system (Zhu et al., 2014; Zhou et al., 2020; Li et al., 2021). The deficiency of endogenous SO₂/AAT pathway aggravated age-related cardiovascular disease progression, such as hypertension, atherosclerosis, and calcification (Li et al., 2011; Tian et al., 2020; Huang et al., 2021; Huang et al., 2022). Surprisingly, SO₂ donor supplementation reduced the D-galactose (D-gal)-induced elevation of mean arterial pressure, ameliorated endothelial cell dysfunction, and lowered plasma angiotensin II concentrations in rats (Dai et al., 2018). These studies suggested that endogenous SO₂/AAT pathway deficiency might drive age-related disease progression. However, whether endogenous SO₂/AAT pathway inhibits vascular aging through VSMC senescence is unclear.

Interferon regulatory factor 1 (IRF1), a transcription factor of the IRF family, regulates cell cycle, proliferation, differentiation, and inflammation. Studies have shown that silencing IRF-1 significantly inhibited VSMC inflammatory responses, extracellular matrix remodeling, and migration (Du et al., 2019; Shen et al., 2020). IRF1 induced CDK inhibitor p21^{Cip/Waf} expression and led to G1 phase cell cycle arrest in coronary artery smooth muscle cells (Wessely et al., 2003). In addition, IRF1 promoted endothelial cell, fibroblast, and monocyte senescence (Upreti et al., 2010; Rossi et al., 2023). These studies suggested that targeting the IRF1 pathway could prevent cell senescence. However, it remains unclear whether endogenous SO₂-mediated anti-senescence effects are mediated through IRF1 inhibition.

Here, we demonstrated that downregulation of endogenous SO₂/AAT pathway promoted VSMC senescence during vascular aging. VSMC-derived SO₂ suppressed VSMC senescence via inhibiting IRF1 transcription activity. Mechanistically, SO₂-sulphenylated IRF1 at cysteine 83 suppressed IRF1 target gene expression and inhibited VSMC senescence phenotype. Our findings revealed VSMC-derived SO₂ acted as a novel protector against VSMC senescence via IRF1 sulphenylation.

2 Materials and methods

2.1 Materials

Sodium bisulfite (NaHSO₃, 243973-100G, Sigma) and sodium sulfite (Na₂SO₃, S4672-250G, Sigma) were freshly mixed in a 1:3 ratio (pH 7.4), which was used as the SO₂ donor. Tamoxifen (579002, Sigma, United States) was used to induce AAT1 knockout in the animal models. The primary reagents for cell culture included fetal bovine serum (FBS) (10099-141, Gibco, United States), penicillin-streptomycin solution (PS, 100 $\times$, 15140-122, ThermoScientific, United States), glutathione (LG, 100 $\times$, 25030081, Thermo Scientific, United States), and trypsin-containing EDTA (0.25%, 25200-056, ThermoScientific, United States). The primary antibodies used in this study were AAT1 (SAB2500473, Sigma, United States), β -actin (TA-09, Zsbio, China), Tp53 (2524, CST, United States), and p21^{Cip/Waf} (ab109199, Abcam, United States). Lentivirus-delivered Rat AAT1 shRNA (VB151210-10015, Cyagen, China) or scrambled shRNA (VB151214-10025, Cyagen, China) was used for the experiments. DAZ-2 (13382, Cayman, United States) and phosphine-biotin (13581, Cayman, United States) were used for the protein sulphenylation assays.

2.2 Cell culture and intervention

A7r5 (rat VSMCs) was purchased from the National Experimental Cell Resource Sharing Platform (1101RAT-PUMC000219, China) and cultured at 37°C in a 5% CO₂ incubator using Dulbecco's modified eagle's medium (DMEM) containing 10% FBS, 1% PS, and 1% LG.

At the appropriate cell density, A7r5 was infected with lentivirus containing AAT1 shRNA or scramble shRNA at a multiplicity of infection (MOI) of 10 in the presence of polybrene (1 μ g/mL). After 48 h, the culture medium was replaced with a puromycin (1 μ g/mL)-containing medium to establish a stably transfected cell pool. After 14 days of screening, stable cell lines were used for subsequent experiments.

Abbreviations: VSMC, vascular smooth muscle cell; SO₂, Sulfur dioxide; AAT, aspartate aminotransferase; IRF, interferon regulatory factor; SA- β -gal, senescence-associated β -galactosidase.

Stably transfected cell lines were divided into three groups: Scramble, AAT1 shRNA, and AAT1 shRNA + SO₂ groups. Cells stably transfected with scramble shRNA were used as controls. For the AAT1 shRNA + SO₂ group, cells were treated with 100 μ M SO₂ donor for 4 days. For the scrambled and AAT1 shRNA groups, the cells were treated with equal volumes of saline. The AAT1 shRNA sequences (5'-3') used in this study are as follows: TCGAATTGG AGCTGACTTCAGATACT. The scramble sequences (5'-3') used in this study are as follows: GCACTACCAGAGCTAACTCAGATA GTACT. All experiments were performed in duplicate and repeated independently three times (n = 3).

2.3 Animal model construction

This study protocol received approval from the Animal Experimentation and Welfare Committee at Peking University First Hospital, with the approval number J2022051. All procedures involving animals adhered to the ethical guidelines established by the Peking University First Hospital for the management of animal research.

The 2- and 24-month-old C57BL/6J male mice used in this study were purchased from Sibeifu (Beijing, China), and the aortic tissues were collected to detect protein levels of Tp53, p21^{Cip/Waf} and AAT1. The 2-month-old mice were served as controls. VSMC-AAT1-KO mice were constructed using Cre/LoxP technology as described in previous study (Huang et al., 2021). Briefly, SM22 α -Cre transgenic (SM22 α -CreERT2⁺) mice and AAT1 flox/flox (AAT1 f/f) mice were purchased from Guangzhou Cyagen (Guangzhou, China). SM22 α -CreERT2⁺ and AAT1 f/f mice were hybridized to generate SM22 α -CreERT2⁺ AAT1 f/f mice. VSMC-AAT1-KO mice were obtained from 8-week-old male SM22 α -CreERT2⁺ AAT1 f/f mice by intraperitoneal injection of tamoxifen (2.5 mg/day, dissolved in ethanol/peanut oil [volume ratio 1:19]) for 5 consecutive days at 3-day intervals. Littermate AAT1 f/f mice were served as control. At the end, the aortic tissues of 5-month-old mice (AAT1 f/f and VSMC-AAT1-KO) were collected post euthanasia by excessive anesthesia. n = 6 independent biological samples.

2.4 Aortic ring assay

Eight-week-old C57BL/6J mice were selected for aortic ring culture experiments. The murine aorta was bluntly detached and placed in sterile phosphate-buffered saline (PBS), with the adventitia of the aorta and connective tissues stripped. The aorta was cut into 3-mm rings and transferred to complete DMEM containing 10% FBS, 1% PS, and 1% LG and cultured at 37°C in a 5% CO₂ incubator. The following day, the rings were divided into control, D-gal and D-gal + SO₂ groups. In the control group, the rings were cultured in complete DMEM. In the D-gal group, the rings were cultured in complete DMEM containing 7.5 g/L D-gal. In the D-gal + SO₂ group, the rings were cultured in DMEM containing 7.5 g/L D-gal, and 100 μ M SO₂ donor given twice daily. The medium was changed every 2 days. Aortic rings were collected after 7 days of induction for subsequent experiments. n = 6 independent biological samples.

2.5 SA- β -gal staining

Cells were fixed at room temperature for 15 min. After PBS washing, the working solution was applied and incubated overnight at 37°C without CO₂. The solution was then removed, followed by washing with PBS for three times. The cell observations were performed using a light microscope. A blue color indicates a positive signal. All experiments were performed in duplicate and repeated independently three times (n = 3).

2.6 Real-time quantitative PCR (RT-qPCR)

A7r5 cells were lysed to extract total RNA with TRIzol reagent. cDNA synthesis was performed using 2 μ g of total RNA and M-MLV reverse transcriptase. RT-qPCR was conducted using 2 $\times$ Go Taq qPCR Master Mix on a 7500 Fast Real-Time PCR System (Applied Biosystems), with β -actin serving as the control. The primers used in this study were as follows: Rat- β -actin-F 5'-ACC CGCGAGTACAACCTTCTT-3' and Rat- β -actin-R 5'-TATCGT CATCCATGGCGAACT-3'; Rat-IL1 β -F 5'-CACCTCTCAAGC AGAGCACAG-3' and Rat-IL1 β -R 5'-GGGTTCATGGTGAA GTCAAC-3'; Rat-IL6-F 5'-CCCACCCTCCAACAAAGATT-3' and Rat-IL6-R 5'-GCTCCAGAGCAGAATGAGCTA -3'. All experiments were performed in duplicate and repeated independently three times (n = 3).

2.7 Immunofluorescence

Aortic tissues were formalin-fixed, embedded in paraffin, 4 μ m sectioned, and dehydrated with xylene and graded ethanol concentrations. Antigen repair was performed using Tris-EDTA (pH 9.0) for 15 min. After permeabilization, the sections were incubated overnight with Tp53 (dilution 1:200), p21^{Cip/Waf} (dilution 1:200), γ -H2AX (dilution 1:200) antibodies. After incubation with the secondary antibody, the slices were sealed with a DAPI-containing sealer. Red (Tp53 and p21^{Cip/Waf}) or Green (γ -H2AX) fluorescence observed using Leica TCS SP8 MP FLIM indicated the expression of target protein. n = 6 independent biological samples.

Cells were fixed with 4% formaldehyde at room temperature for 15 min, permeabilized with pre-cooled methanol, and blocked with 5% bovine serum albumin. Subsequently, cells were incubated with primary antibody against IRF1 and Flag overnight at 4°C. After washing, cells were incubated with the corresponding secondary antibody and mounted with a DAPI-containing dye. Target protein expression was visualized as red fluorescence using a Leica TCS SP8 MP FLIM microscope. All experiments were performed in duplicate and repeated independently three times (n = 3).

2.8 Biotin switch assay for IRF1 sulphenylation

A7r5 cells were lysed in a non-denaturing lysis buffer (Appligen, Beijing, China) containing 5 mM DAZ-2 for 20 min. The supernatant was collected after centrifugation at 16,000 g for

4 min at 4°C and incubated with gentle shaking at 37°C for 2 h. DAZ-2-labeled lysates were reacted with 250 μ M phosphine-biotin (13581, Cayman, United States) at 37°C for 2 h. Neutravidin ultralink resin (53150, Thermo Fisher Scientific, United States) was added at a volume ratio of 1:10 and incubated overnight with shaking at 4°C. The suphenylated proteins enriched with resin were mixed with a 2 $\times$ non-denaturing sample buffer and boiled at 100°C for 10 min. The supernatant was collected by centrifugation at 5,000 g for 10 min and subjected to Western blot. All experiments were performed in duplicate and repeated independently three times ($n = 3$).

2.9 Plasmid transfection

Human IRF1 wild-type (WT) and C83S mutant plasmids were constructed (Transgene, China). When the A7r5 cells reached approximately 50%–60% confluence, cells were transfected with plasmids via Lipofectamine™ 3000 (L3000075, Thermo Fisher, United States), followed by medium replacement 6–8 h post-transfection.

2.10 Western blot

The protein expression of AAT1, GAPDH, Tp53, p21^{Cip/Waf}, and IRF1 was detected using Western blot. The mouse aortic tissue was homogenized in the lysis buffer. Cells were also lysed in lysis buffer for 30 min at 4°C. The total protein concentration was quantified using the BCA method. An equal amount of protein was taken for electrophoresis, and the membrane was subsequently blocked and incubated with primary antibody overnight at 4°C. Following incubation for 1 h with secondary antibodies at room temperature, the membrane was washed with PBST and processed on a FluorChem M MultiFluor System (Protein Simple, United States) with enhanced chemiluminescence solution (MA0186, Meilunbio, China). $n = 6$ independent biological samples in animal studies and $n = 3$ independent experiments performed in duplicate in cell studies.

2.11 Cell clustering of single-cell RNA sequencing (scRNA-seq) data

The original single-cell transcriptome data of aged aortic tissue used in this study is publicly available that can be found in the GEO database (GSE164585) (Xie et al., 2022). Aortas of 4-, 26-, and 86-week-old C57/BL6J mice were analyzed using single-cell RNA sequencing. Dimensional reduction was conducted using t-distributed stochastic neighbor embedding (UMAP), executed through the “RunUMAP” functions. Cluster identification was performed by the “FindClusters” function, resulting in the delineation of 16 distinct clusters from the comprehensive cell population. The “FindAllMarkers” function, employing the Wilcoxon rank-sum test, was used to identify signature genes for each cluster, enabling the assignment of cellular identities, which included various cell types such as B cells, fibroblasts, monocytes, and others.

2.12 Identification of differentially expressed genes (DEGs) and gene ontology (GO) analysis

DEGs from scRNA-seq were identified via “FindAllMarkers” or “FindMarkers” using the “Wilcox” test, yielding “p_val_adj” with Bonferroni correction and “avg_logFC.” Biomarkers were selected from DEGs with $p_val_adj \leq 0.05$ and $avg_logFC \geq 0.5$. GO enrichment analysis was conducted through Metascape and GO software. REVIGO summarized redundant GO terms based on semantic similarity, and non-redundant terms were visualized in Cytoscape, with nodes representing GO terms and 3% pairwise similarities as edges.

2.13 Transcription factor prediction

Transcription factor prediction was performed using CheA3 online tools (<https://maayanlab.cloud/chea3/>).

2.14 Statistical analysis

Statistical analysis was performed with SPSS (version 17.0; IBM, United States) and GraphPad Prism 8.0 (GraphPad Software Inc.), and results were represented as mean $\pm$ standard deviation (SD). When the variance was uniform and satisfied the normal distribution, an independent sample t-test was used to compare the two groups. One-way ANOVA followed by Bonferroni post-doc analysis was used to compare the difference among multiple groups. Statistical significance was defined as $p < 0.05$.

3 Results

3.1 Endogenous SO₂/AAT1 pathway was downregulated during vascular aging

We initially measured the expression of senescence markers and SO₂-generating enzymes in the aortic tissues of young (2-month-old) and aged (24-month-old) mice. The results showed that, compared to young (2-month-old) mice, senescence markers Tp53 and p21^{Cip/Waf} were increased in the aortic tissues of aged (24-month-old) mice. However, the protein expression of AAT1, a key enzyme in SO₂ generation, showed a significantly reduced expression in the aortic tissues of aged (24-month-old) mice (Figure 1A).

To identify the cell populations responsible for reduced AAT1 expression in aortic tissue during aging process, we performed dimensionality reduction and clustering analysis of single-cell transcriptomic data from 4-week-old, 26-week-old, and 86-week-old mice. As a result, we finally grouped cells into 16 distinct clusters, comprising dendritic cells (DC), fibroblasts, B cells, CD8⁺ T cells, CD4⁺ T cells, lymphatic endothelial cells, macroECs, mastocytes, mesothelial cells, microECs, monocytes, neuron cells, neutrophils, pericytes, plasmocytes, and VSMCs (Figure 1B). We next performed gene expression mapping, which revealed that AAT1, the

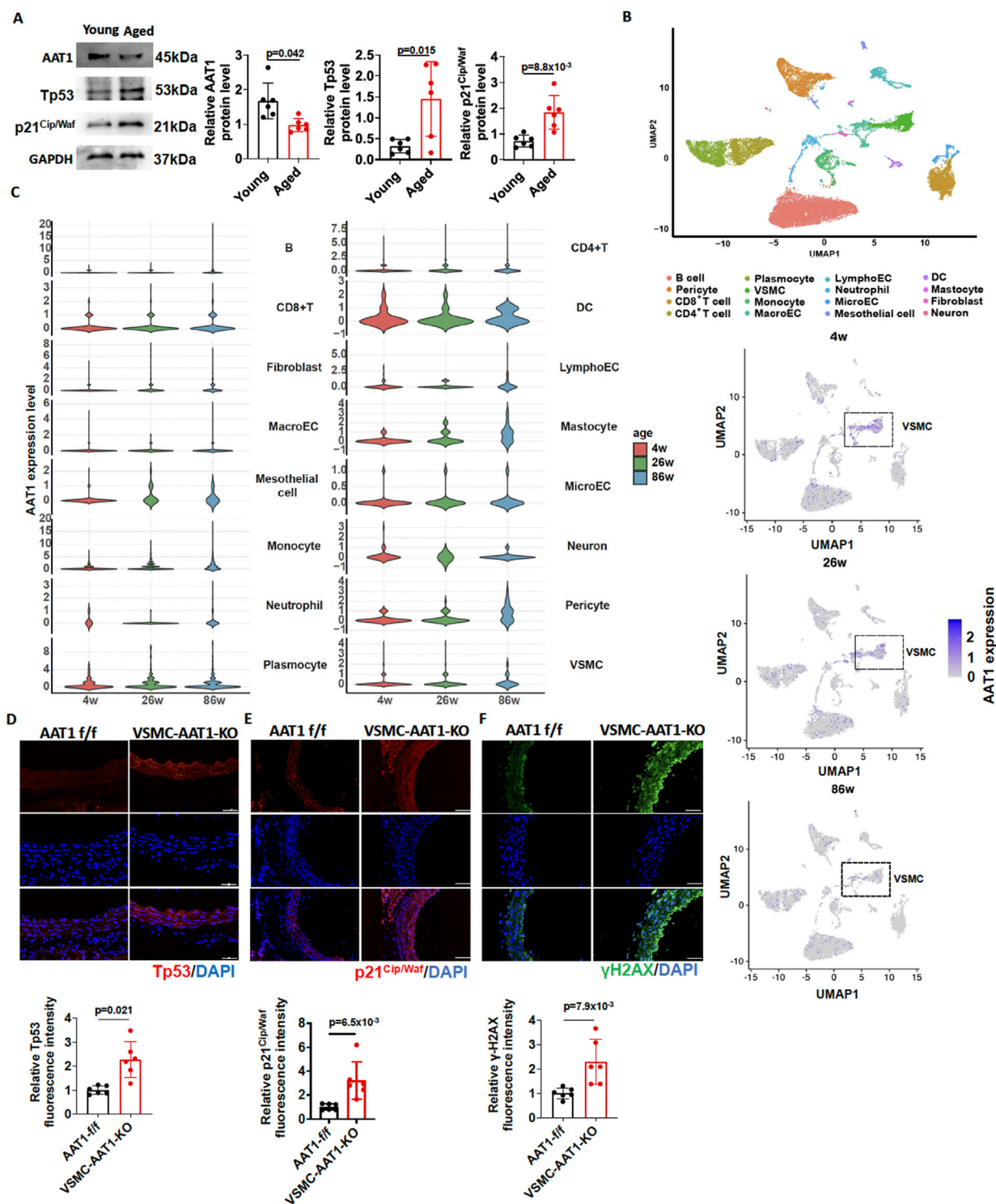


FIGURE 1

Downregulation of the endogenous SO_2 /AAT1 pathway during vascular aging. (A) Western blot analysis was used to detect AAT1, Tp53, and p21^{Cip/Waf} protein expression in the aortic tissues of young (2 months) and aged (24 months) mice ($n = 6$ independent biological samples). Two-tailed unpaired student's t-test was used. (B) UMAP plot of 16 segregated cell clusters in the aortic tissues of 4-, 26-, and 86-week mice. (C) Violin plots showing AAT1 expression levels across 16 cell clusters (upper panel). Each circle represents AAT1 gene expression level in one VSMC (lower panel). (D) Detection of Tp53 expression in the aortic tissues of 5-month-old VSMC-AAT1-KO and control AAT1 f/f mice using immunofluorescence ($n = 6$ independent biological samples), scale bar: 100 μm . Two-tailed unpaired student's t-test was used. (E) Detection of p21^{Cip/Waf} expression in the aortic tissues of 5-month-old VSMC-AAT1-KO and control AAT1 f/f mice using immunofluorescence ($n = 6$ independent biological samples), scale bar: 100 μm . Two-tailed unpaired student's t-test was used. (F) Detection of γ -H2AX expression in the aortic tissues of 5-month-old VSMC-AAT1-KO and control AAT1 f/f mice using immunofluorescence ($n = 6$ independent biological samples), scale bar: 50 μm . Two-tailed unpaired student's t-test was used. Data are expressed as mean $\pm$ SD.

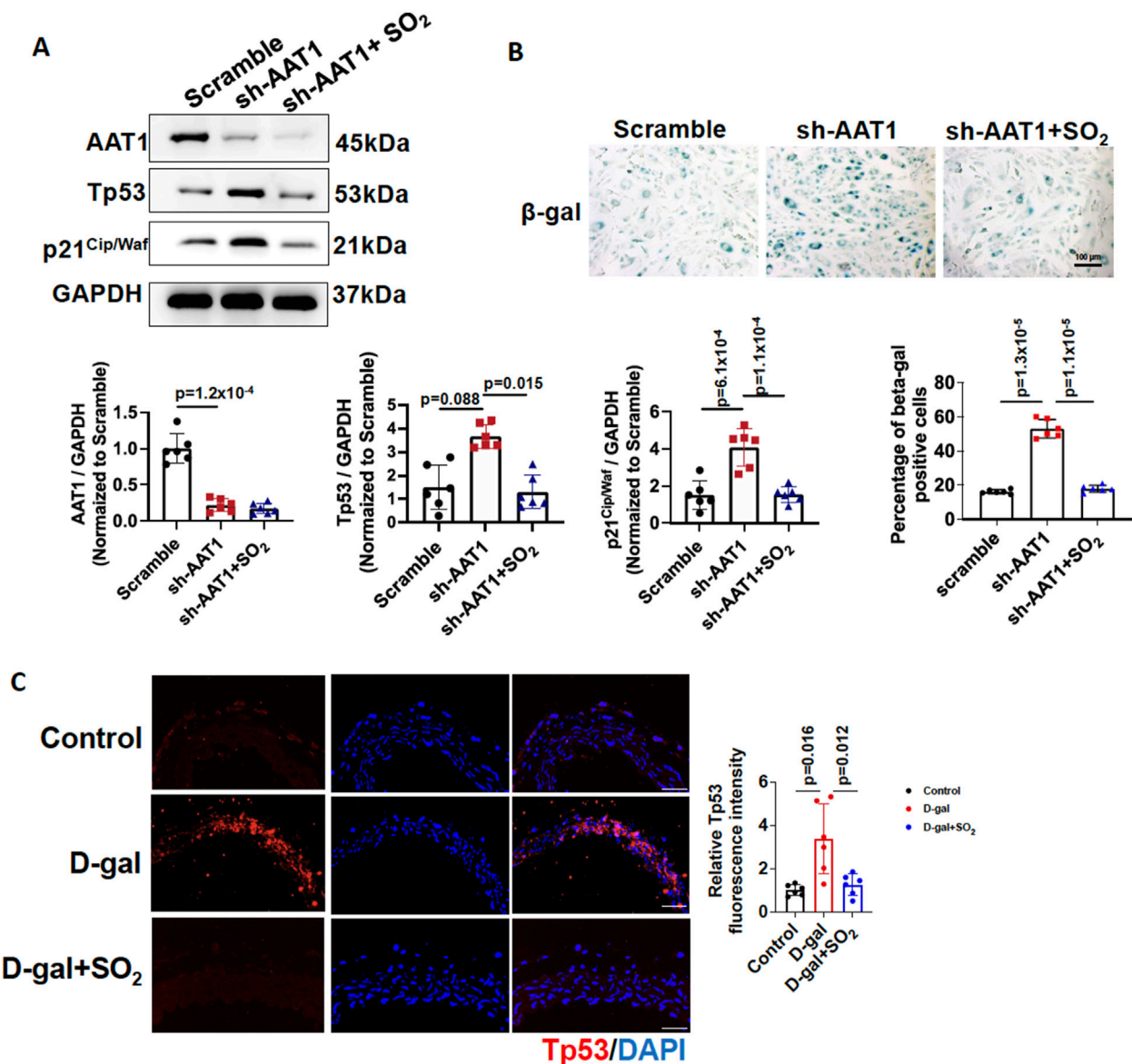


FIGURE 2 Endogenous SO₂/AAT1 deficiency contributed to VSMC senescence. **(A)** Western blot analysis was used to detect AAT1, Tp53, and p21^{Cip/Waf} protein levels in VSMCs transfected with scrambled or AAT1 shRNA (n = 3 independent experiments performed in duplicate). One-way ANOVA followed by Bonferroni post-doc analysis was used. **(B)** SA-β-gal activity in AAT1-knockdown VSMCs (n = 3 independent experiments performed in duplicate). Scale bar: 100 μm. One-way ANOVA followed by Bonferroni post-doc analysis was used. **(C)** Immunofluorescence analysis of Tp53 protein expression in D-gal-induced aortic rings with or without SO₂ donor (n = 6 independent biological samples), scale bar: 100 μm. One-way ANOVA followed by Bonferroni post-doc analysis was used. Data are expressed as mean ± SD.

SO₂-generating enzyme, was mainly reduced in VSMC from aged (86-week-old) mice (Figure 1C). Moreover, AAT1 expression was upregulated in mastocytes and pericytes from aged (86-week-old) mice (Figure 1C).

Therefore, we detected the expression of senescence markers Tp53, p21^{Cip/Waf} and γ-H2AX in the aortic tissues of 5-month-old AAT1 *f/f* and VSMC-AAT1-KO mice. As expected, the expression of Tp53, p21^{Cip/Waf}, and γ-H2AX was elevated in the aorta of 5-month-old VSMC-AAT1-KO mice compared to 5-month-old AAT1 *f/f* mice (Figures 1D–F), suggesting that the downregulation of endogenous SO₂/AAT1 pathway might be associated with vascular aging.

3.2 Endogenous SO₂/AAT1 deficiency contributed to VSMC senescence

To confirm the importance of endogenous SO₂/AAT1 pathway in VSMC senescence, we generated VSMCs with stable knockdown of AAT1 by transfecting AAT1 shRNA, and cells stably transfected with Scramble shRNA were used as controls. AAT1-knockdown VSMCs were then treated with or without SO₂ donor. As expected, senescence markers Tp53 and p21^{Cip/Waf} protein levels increased in AAT1-knockdown VSMCs, which were rescued by SO₂ donor supplementation (Figures 2A, B). Moreover, SA-β-gal activity increased in AAT1-knockdown VSMCs, which was rescued by

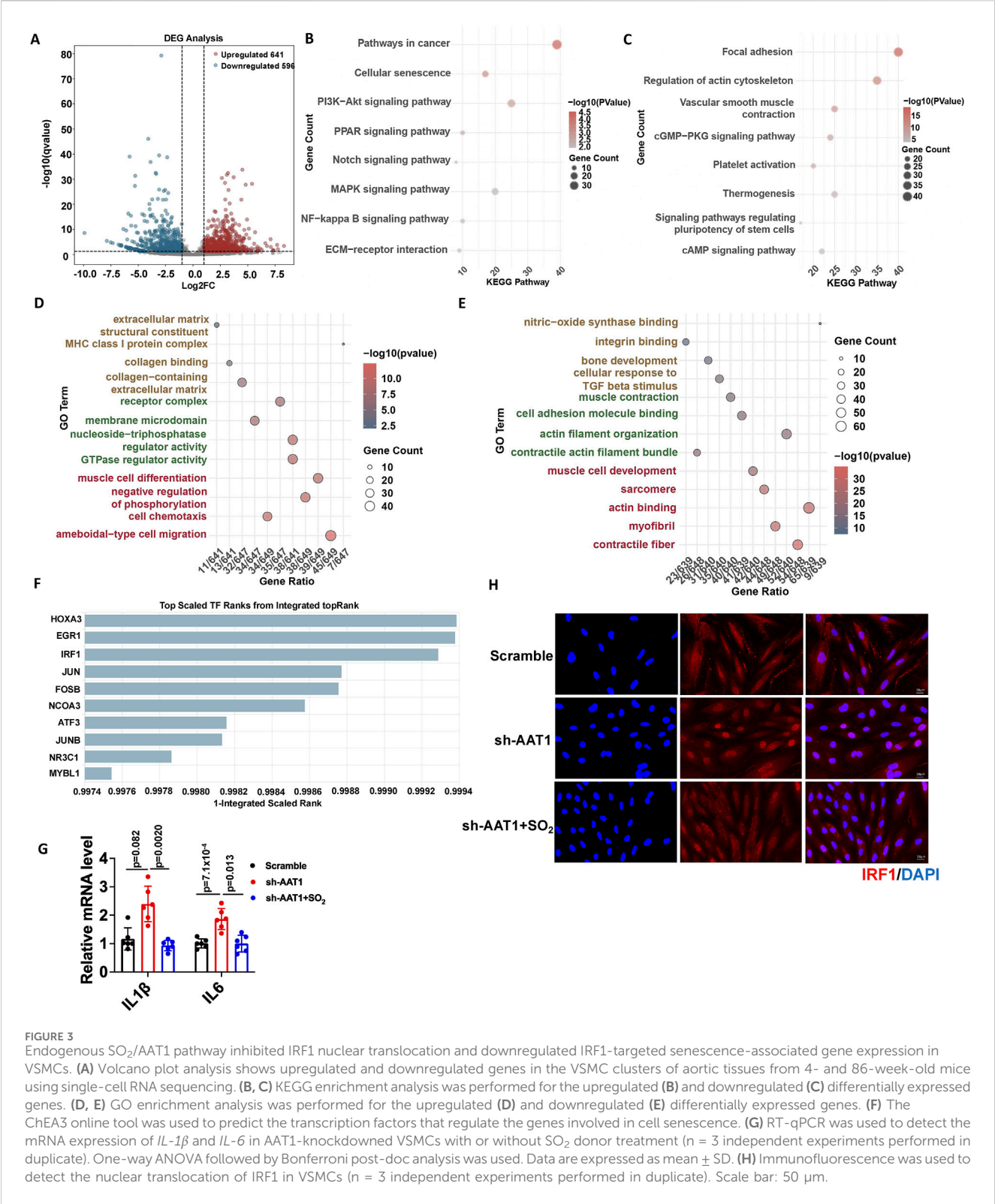


FIGURE 3 Endogenous SO₂/AAT1 pathway inhibited IRF1 nuclear translocation and downregulated IRF1-targeted senescence-associated gene expression in VSMCs. (A) Volcano plot analysis shows upregulated and downregulated genes in the VSMC clusters of aortic tissues from 4- and 86-week-old mice using single-cell RNA sequencing. (B, C) KEGG enrichment analysis was performed for the upregulated (B) and downregulated (C) differentially expressed genes. (D, E) GO enrichment analysis was performed for the upregulated (D) and downregulated (E) differentially expressed genes. (F) The ChEA3 online tool was used to predict the transcription factors that regulate the genes involved in cell senescence. (G) RT-qPCR was used to detect the mRNA expression of *IL-1β* and *IL-6* in AAT1-knockdown VSMCs with or without SO₂ donor treatment (n = 3 independent experiments performed in duplicate). One-way ANOVA followed by Bonferroni post-doc analysis was used. Data are expressed as mean ± SD. (H) Immunofluorescence was used to detect the nuclear translocation of IRF1 in VSMCs (n = 3 independent experiments performed in duplicate). Scale bar: 50 μm.

SO₂ donor supplementation. These findings suggested that AAT1 knockdown led to VSMC senescence.

To determine the protective effects of endogenous SO₂ on vascular aging, aortic rings treated with DMEM medium containing 7.5 g/L D-gal were used as a vascular aging model and incubated with or without an SO₂ donor. Aortic rings treated with DMEM medium were used as controls. Compared to the control group, D-gal significantly upregulated Tp53 expression in aortic rings, and supplementation with the SO₂ donor reduced Tp53 protein expression (Figure 2C). These results suggested that sufficient SO₂ protected against vascular aging.

3.3 Endogenous SO₂/AAT1 pathway inhibited IRF1 nuclear translocation and downregulated IRF1-targeted senescence-associated gene expression

Next, we explored the potential mechanisms underlying the inhibitory effect of the endogenous SO₂/AAT1 pathway on VSMC senescence. First, we analyzed the DEGs in VSMC clusters of aortic tissue from 4- and 86-week-old mice using single-cell RNA sequencing (4-week-old mice as controls), which revealed 641 upregulated and 596 downregulated genes (Figure 3A). KEGG enrichment analyses of these DEGs showed that the upregulated pathways were mainly related to cell senescence (Figure 3B). GO enrichment analyses of these DEGs showed that the upregulated pathways were mainly related to MHC class I complex (Figure 3D), which is also a hallmark of aging (Suryadevara et al., 2024). Whereas the downregulated pathways in KEGG enrichment analyses were mainly related to VSMC contraction and extracellular matrix remodeling (Figure 3C). GO enrichment analyses of these DEGs showed that the downregulated pathways were mainly related to muscle contraction, which is also a marker of vascular aging (Figure 3E).

We then used the CheA3 online tool to predict the potential transcription factors that regulate genes involved in cell senescence and found that IRF1 might play an important role in VSMC senescence (Figure 3F). IRF1 target genes include *IL-1β* and *IL-6*, which are SASP factors. The mRNA levels of *IL-1β* and *IL-6* increased in the AAT1-knockdown VSMCs compared with the scramble group but reduced after SO₂ donor supplementation (Figure 3G). Furthermore, IRF1 nuclear translocation increased in AAT1-knockdown VSMCs, which reduced after SO₂ donor supplementation (Figure 3H). These results suggested that the endogenous SO₂/AAT1 pathway inhibited IRF1 nuclear translocation and downregulated IRF1-targeted senescence-associated gene expression in VSMCs.

3.4 Endogenous SO₂ inhibited VSMC senescence by sulphenylating IRF1 at cysteine 83 (C83)

Various kinds of protein modification were involved in aging-related vascular diseases (Zu et al., 2010; Zou et al., 2018; Zhao et al., 2021; Zou et al., 2023). Previous studies have shown that SO₂ regulates protein function via sulphenylation (Huang et al., 2021). Moreover, cysteine 83 (C83) in IRF1 mediates its affinity with DNA (Antonczyk et al., 2019; Jefferies, 2019). Therefore, we hypothesized that SO₂ could inhibit IRF1 transcription activity and its downstream inflammation-mediated VSMC senescence by sulphenylating IRF1 at C83. First, we constructed Flag-IRF1-WT (Flag-tagged wild-type IRF1) and Flag-IRF1-C83S (mutation at cysteine 83 by serine) plasmids and detected the IRF1 sulphenylation. Cells transfected with the corresponding plasmids treated without SO₂ as controls. The results showed that the SO₂ donor could sulphenylate IRF1 in VSMCs transfected with the Flag-IRF1-WT plasmid. However, in VSMCs transfected with the Flag-IRF1-C83S plasmid, supplementation with SO₂ failed to increase the sulphenylated IRF1 levels (Figure 4A).

These results indicated that SO₂ sulphenylated IRF1 at C83 in VSMCs.

Meanwhile, in VSMCs transfected with Flag-IRF1-WT plasmid, AAT1 knockdown enhanced IRF1 nuclear translocation compared to the scramble group (control), which could be attenuated by the SO₂ donor supplementation (Figure 4B). However, in VSMCs transfected with Flag-IRF1-C83S plasmid, neither AAT1 knockdown nor SO₂ donor supplementation affected IRF1 nuclear translocation (Figure 4B). These results revealed that sulphenylation of IRF1 at C83 is essential for SO₂-inhibited IRF1 nuclear translocation (Figure 4B).

Furthermore, we examined whether C83 was a target of SO₂ for inhibiting the senescence phenotype of VSMCs. In VSMCs transfected with Flag-IRF1-WT plasmid, AAT1 knockdown increased *IL-1β* and *IL-6* mRNA levels (Figure 4C), elevated Tp53 and p21^{Cip1/Waf} protein levels (Figure 4D), and enhanced SA-β-gal activity (Figure 4E) compared to the scramble group, while SO₂ donor supplementation downregulated the levels of IRF1 target genes, SASP factors and SA-β-gal activity compared to AAT1-knockdown group. However, in VSMCs transfected with Flag-IRF1-C83S plasmid, neither AAT1 knockdown nor SO₂ donor supplementation affected the VSMC senescence phenotype (Figures 4C–E). These results further confirmed that endogenous SO₂ could inhibit VSMC senescence phenotype through IRF1 sulphenylation at C83.

4 Discussion

Vascular aging is the pathological basis of various age-related cardiovascular diseases and can trigger or aggravate disease progression (Ungvari et al., 2020; Abdellatif et al., 2023). VSMC senescence is a major contributor to vascular aging (Cui et al., 2021). However, the mechanisms underlying VSMC senescence remain unclear. Here, we demonstrated that the endogenous SO₂/AAT1 pathway was downregulated in the aortic tissues of aged mice. Insufficient endogenous SO₂/AAT1 pathway led to VSMC senescence, whereas SO₂ supplementation rescued the AAT1-knockdown-induced VSMC senescent phenotype. Our study further revealed that endogenous SO₂ attenuated VSMC senescence by sulphenylating IRF1 at C83 and inhibiting its nuclear translocation and downstream SASP gene transcription. These findings provided new insights into the mechanism of VSMC senescence and revealed the role of SO₂/AAT1 as endogenous regulators of vascular aging.

Endogenous SO₂ exerts various cellular functions, including anti-inflammatory, anti-oxidative, anti-DNA damage and anti-apoptotic effects. Macrophage M1 polarization plays an important role in inflammation-related diseases. Macrophage-derived SO₂ inhibits macrophage M1 polarization and the secretion of inflammatory cytokines (Zhu et al., 2020; Zhang et al., 2025). Mast cell-derived SO₂ is responsible for inhibiting IgE-mediated and hypoxia-driven mast cell degranulation (Zhang et al., 2021; Song et al., 2024). Cardiomyocyte senescence is a key risk factor for the progression of cardiovascular diseases. Cardiomyocyte-derived SO₂ deficiency leads to cardiomyocyte senescence, while SO₂ donor supplementation can restore heart function and inhibit heart aging. SO₂ sulphenylated STAT3 at C259 to inhibit its nuclear translocation and DNA binding

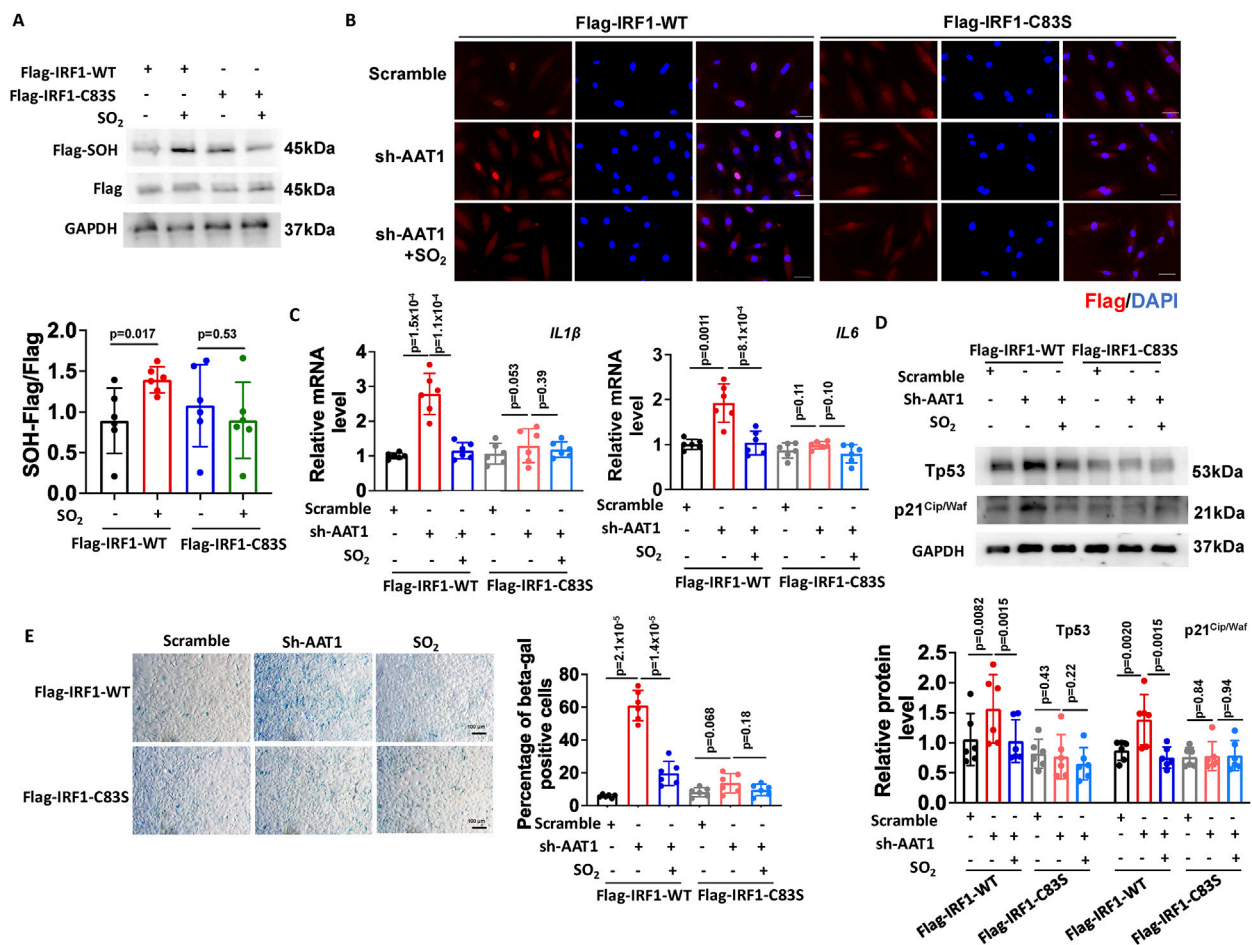


FIGURE 4

Endogenous SO₂ inhibited VSMC senescence by sulphenylating IRF1 at cysteine 83. (A) Sulphenylation of IRF1 in VSMCs transfected with IRF1-WT or IRF1-C83S plasmids was detected by a biotin switch assay ($n = 3$ independent experiments performed in duplicate). One-way ANOVA followed by Bonferroni post-doc analysis was used. (B) Immunofluorescence was used to detect the nuclear translocation of Flag-IRF1-WT and Flag-IRF1-C83S in VSMCs ($n = 3$ independent experiments performed in duplicate). Scale bar: 50 μ m. (C) The mRNA expression of *IL-1 β* and *IL-6* was determined by RT-qPCR ($n = 3$ independent experiments performed in duplicate). One-way ANOVA followed by Bonferroni post-doc analysis was used. (D) Protein expression of Tp53 and p21^{Cip/Waf} was determined using Western blotting ($n = 3$ independent experiments performed in duplicate). One-way ANOVA followed by Bonferroni post-doc analysis was used. (E) SA- β -gal staining was used to detect SA- β -gal activity ($n = 3$ independent experiments performed in duplicate). Scale bar: 100 μ m. One-way ANOVA followed by Bonferroni post-doc analysis was used. Data are expressed as mean $\pm$ SD.

ability, thereby inhibiting DNA damage and reducing cardiomyocyte senescence (Zhang et al., 2024). Our results suggested that VSMC-derived SO₂ could attenuated VSMC senescence and vascular aging. These studies suggested that endogenous SO₂ is an important molecule in maintaining body homeostasis.

Atherosclerosis and hypertension are typical age-related diseases, and VSMC senescence promotes their progression (Bos et al., 2012). SO₂ supplementation could reduce the area of atherosclerotic plaques in the aorta and coronary arteries of atherosclerosis rats (Li et al., 2011; Tian et al., 2020). In addition, SO₂ alleviated D-gal-induced hypertension and maintained vascular homeostasis by inhibiting the angiotensin II/angiotensin II type 1 receptor pathway (Dai et al., 2018). These studies suggested that SO₂ was involved in the regulation of age-related vascular disease. However, the relationship between endogenous SO₂ and vascular aging, especially VSMC senescence, remains unclear. In this study, we found that SO₂/AAT1 pathway was downregulated in the aortic tissues of aged mice. VSMC cluster were identified as the main

contributor of AAT1 downregulation in aortic tissue of aged mice by scRNA-seq analysis. However, according to dimensionality reduction and clustering analysis and AAT1 expression mapping, there were still immune cells-derived AAT1 upregulated. Due to data distribution dispersion, the expression of AAT1 in B cell, CD4⁺T cell, CD8⁺T cell and DC seemed to be upregulated in the aortic tissues of aged mice (86w group), but there was no significant difference. Moreover, the expression of AAT1 in mastocytes was upregulated in the aortic tissues of aged mice (86w group). Given that mastocytes is important immune cells in mediating vascular inflammation, upregulation of AAT1 in mastocytes might act as a compensatory role to antagonize vascular aging-related and inflammation. As expected, VSMC-AAT1 KO increased the level of Tp53, p21^{Cip/Waf} and γ -H2AX and promoted VSMC senescence in aortic tissues. In addition, AAT1 knockdown in VSMCs promoted VSMC senescence with the increased Tp53/p21^{Cip/Waf} protein levels, SA- β -gal activity, and SASP factors *IL-1 β* and *IL-6* mRNA levels. SO₂ supplementation inhibits AAT1 knockdown-induced VSMC senescence. Furthermore,

SO₂ supplementation inhibited D-gal-induced Tp53 expression in the aortic rings. These findings suggested that downregulation of the endogenous SO₂/AAT1 pathway was an important mechanism for VSMC senescence. Supplement of SO₂ might be a potential strategy to inhibited VSMC senescence and aging related vascular disease, such as atherosclerosis and hypertension.

However, the mechanisms by which endogenous SO₂ inhibits VSMC senescence remain unclear. Single-cell transcriptome analysis of aortic tissues of 4 and 86-week mice indicated that IRF1 might play an important role in VSMC senescence. Previous studies have shown that IRF1 plays an important role in innate and acquired immune responses (Zeng et al., 2024). In addition, IRF1 is involved in the regulation of cell cycle, cell migration, apoptosis, differentiation, and inflammation. Silencing IRF-1 can significantly inhibit the VSMC inflammatory response and extracellular matrix remodeling, proliferation, and migration (Du et al., 2019; Shen et al., 2020). Additionally, IRF-1 activation is a risk factor for atherosclerosis (Du et al., 2019; Shen et al., 2020; Fan et al., 2023; Qian et al., 2024). IRF-1 causes growth arrest in coronary artery smooth muscle cells mainly by arresting the coronary artery smooth muscle cell G1 cell cycle and inducing the upregulation of the CDK inhibitor p21^{Cip/Waf} (Wessely et al., 2003). In addition, IRF1 activation promotes endothelial cells, fibroblast, and monocyte senescence (Upreti et al., 2010; Rossi et al., 2023). These studies suggest that inhibiting IRF1 signaling may prevent cellular senescence. In this study, we demonstrated that endogenous SO₂ inhibited IRF1 transcription activity in a redox modification dependent manner. In detail, AAT1 knockdown increased IRF1-WT nuclear translocation but failed to promote nuclear translocation of IRF1 with C83S mutation, suggesting that sulphenylation of IRF1 at C83 was responsible for the inhibitory effect of SO₂ on IRF1 nuclear translocation. Meanwhile, endogenous SO₂ reduced the expression of IRF1-targeted senescence-associated factors, including *IL-1β*, *IL-6*, and p21^{Cip/Waf}, indicating that the endogenous SO₂/AAT1 pathway inhibited VSMC senescence in association with IRF1 activation.

Aging is a key independent risk factor of various vascular diseases, for which the post-translational modification-dependent mechanisms remain largely unknown. Previous studies have shown that SO₂ exerts its cardiovascular protective effect through the sulphenylation of target proteins (Smad3, AAT, NF-κB p65, and CypD) at cysteine residues (Chen et al., 2017; Song et al., 2020; Huang et al., 2021; Lv et al., 2021). IRF1 is a DNA-binding transcription factor that interacts with DNA through its C83 residue (Antonczyk et al., 2019; Jefferies, 2019). Therefore, we hypothesized that SO₂ might sulphenylate IRF1 at C83 to inhibit VSMC senescence. Our study confirmed that SO₂ sulphenylates IRF1 at C83 in VSMCs. Notably, in IRF1-C83S plasmid-transfected VSMCs, neither AAT1 knockdown nor SO₂ supplementation affected the expression of IRF1-targeted senescence-associated factors or the VSMC senescence phenotype, confirming that SO₂ inhibits VSMC senescence by sulphenylating IRF1 at C83. Considering the important regulatory role of IRF1 in the immune system (Chavez et al., 2025; Rahlf and Tarakanova, 2025) and AAT1 upregulation in mastocytes, SO₂-mediated IRF1 sulphenylation might play a critical regulatory role in regulating mastocytes-mediated vascular inflammation, as well as modulating the pathogenesis of atherosclerosis (Lavillegrand et al., 2024), pulmonary arterial hypertension (Zhang et al., 2021), and allergic diseases (Galli and Tsai, 2012), which require further study. However, this research still has limitations. Identification of

IRF1 sulphenylation using liquid chromatography coupled to tandem mass spectrometry merits study. Furthermore, the usage of a IRF1-C83S knock-in mouse line to investigate the role of IRF1 sulphenylation in VSMC senescence and vascular aging will be more conclusive.

This study demonstrated that downregulation of the endogenous SO₂/AAT1 pathway significantly contributed to VSMC senescence. Endogenous SO₂ mitigated VSMC senescence and vascular aging by sulphenylating IRF1 at C83. These findings provided new insights into the mechanisms underlying VSMC senescence and therapeutic strategies for VSMC senescence and vascular aging. Regulating IRF1 sulphenylation by supplementation of SO₂ donors or AAT1 activators would be a promising approach to the development of new antagonistic therapies against aging and aging-related cardiovascular diseases.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The animal study was approved by the Animal Experimentation and Welfare Committee at Peking University First Hospital. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

BQ: Conceptualization, Formal Analysis, Investigation, Writing—original draft, Writing—review and editing, Methodology. SZ: Conceptualization, Investigation, Methodology, Writing—original draft. SG: Software, Investigation, Writing—original draft. ZY: Software, Methodology, Writing—original draft. DW: Investigation, Supervision, Writing—original draft. KL: Investigation, Methodology, Writing—original draft. XY: Investigation, Methodology, Writing—original draft. CT: Conceptualization, Supervision, Writing—original draft. JD: Funding acquisition, Writing—review and editing, Supervision, Writing—original draft. HJ: Funding acquisition, Supervision, Writing—review and editing, Conceptualization, Writing—original draft. YH: Funding acquisition, Supervision, Writing—review and editing, Conceptualization, Writing—original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Abdellatif, M., Rainer, P. P., Sedej, S., and Kroemer, G. (2023). Hallmarks of cardiovascular ageing. *Nat. Rev. Cardiol.* 20 (11), 754–777. doi:10.1038/s41569-023-00881-3
- Antonczyk, A., Krist, B., Sajek, M., Michalska, A., Piaszyk-Borychowska, A., Plens-Galaska, M., et al. (2019). Direct inhibition of IRF-dependent transcriptional regulatory mechanisms associated with disease. *Front. Immunol.* 10, 1176. doi:10.3389/fimmu.2019.01176
- Bos, D., van der Rijk, M. J., Geeraedts, T. E., Hofman, A., Krestin, G. P., Witteman, J. C., et al. (2012). Intracranial carotid artery atherosclerosis: prevalence and risk factors in the general population. *Stroke* 43 (7), 1878–1884. doi:10.1161/strokeaha.111.648667
- Chavez, C., Lin, K., Malveaux, A., Gorin, A., Brizuela, S., Cheng, Q. J., et al. (2025). IRF1 cooperates with ISGF3 or GAF to form innate immune *de novo* enhancers in macrophages. *Sci. Signal* 18 (868), eado8860. doi:10.1126/scisignal.ado8860
- Chen, S., Huang, Y., Liu, Z., Yu, W., Zhang, H., Li, K., et al. (2017). Sulphur dioxide suppresses inflammatory response by sulphenylating NF- κ B p65 at Cys(38) in a rat model of acute lung injury. *Clin. Sci. (Lond)* 131 (21), 2655–2670. doi:10.1042/cs20170274
- Cui, X. Y., Zhan, J. K., and Liu, Y. S. (2021). Roles and functions of antisense lncRNA in vascular aging. *Ageing Res. Rev.* 72, 101480. doi:10.1016/j.arr.2021.101480
- Dai, J., Liu, R., Zhao, J., and Zhang, A. (2018). Sulfur dioxide improves endothelial dysfunction by downregulating the angiotensin II/AT(1)R pathway in D-galactose-induced aging rats. *J. Renin Angiotensin Aldosterone Syst.* 19 (2), 1470320318778898. doi:10.1177/1470320318778898
- Du, M., Wang, X., Mao, X., Yang, L., Huang, K., Zhang, F., et al. (2019). Absence of interferon regulatory factor 1 protects against atherosclerosis in apolipoprotein E-deficient mice. *Theranostics* 9 (16), 4688–4703. doi:10.7150/thno.36862
- Fan, X., Li, Q., Wang, Y., Zhang, D. M., Zhou, J., Chen, Q., et al. (2023). Non-canonical NF- κ B contributes to endothelial pyroptosis and atherogenesis dependent on IRF-1. *Transl. Res.* 255, 1–13. doi:10.1016/j.tlrs.2022.11.001
- Galli, S. J., and Tsai, M. (2012). IgE and mast cells in allergic disease. *Nat. Med.* 18 (5), 693–704. doi:10.1038/nm.2755
- Huang, Y., Li, Z., Zhang, H., Tang, H., Zhang, H., Wang, C., et al. (2021). Endogenous SO(2)-dependent Smad3 redox modification controls vascular remodeling. *Redox Biol.* 41, 101898. doi:10.1016/j.redox.2021.101898
- Huang, Y., Zhang, H., Lv, B., Tang, C., Du, J., and Jin, H. (2022). Sulfur dioxide: endogenous generation, biological effects, detection, and therapeutic potential. *Antioxid. Redox Signal* 36 (4–6), 256–274. doi:10.1089/ars.2021.0213
- Jefferies, C. A. (2019). Regulating IRFs in IFN driven disease. *Front. Immunol.* 10, 325. doi:10.3389/fimmu.2019.00325
- Lavillegrand, J. R., Al-Rifai, R., Thietart, S., Guyon, T., Vandestienne, M., Cohen, R., et al. (2024). Alternating high-fat diet enhances atherosclerosis by neutrophil reprogramming. *Nature* 634 (8033), 447–456. doi:10.1038/s41586-024-07693-6
- Li, W., Tang, C., Jin, H., and Du, J. (2011). Regulatory effects of sulfur dioxide on the development of atherosclerotic lesions and vascular hydrogen sulfide in atherosclerotic rats. *Atherosclerosis* 215 (2), 323–330. doi:10.1016/j.atherosclerosis.2010.12.037
- Li, Y., Feng, Y., Ye, X., Peng, H., Du, J., Yao, X., et al. (2021). Endogenous SO(2) controls cell apoptosis: the state-of-the-art. *Front. Cell Dev. Biol.* 9, 729728. doi:10.3389/fcell.2021.729728
- Lin, M. J., Hu, S. L., Tian, Y., Zhang, J., Liang, N., Sun, R., et al. (2023). Targeting vascular smooth muscle cell senescence: a novel strategy for vascular diseases. *J. Cardiovasc Transl. Res.* 16 (5), 1010–1020. doi:10.1007/s12265-023-10377-7
- Lv, B., Peng, H., Qiu, B., Zhang, L., Ge, M., Bu, D., et al. (2021). Sulphenylation of CypD at cysteine 104: a novel mechanism by which SO(2) inhibits cardiomyocyte apoptosis. *Front. Cell Dev. Biol.* 9, 784799. doi:10.3389/fcell.2021.784799
- Okuno, K., Cicalese, S., Elliott, K. J., Kawai, T., Hashimoto, T., and Eguchi, S. (2020). Targeting molecular mechanism of vascular smooth muscle senescence induced by angiotensin II, A potential therapy via senolytics and senomorphics. *Int. J. Mol. Sci.* 21 (18), 6579. doi:10.3390/ijms21186579
- Qian, Z., Shaofang, F., Chen, C., Chunhua, S., Nan, W., and Chao, L. (2024). IL-33 suppresses the progression of atherosclerosis via the ERK1/2-IRF1-VCAM-1 pathway. *Cardiovasc Drugs Ther.* 38 (3), 569–580. doi:10.1007/s10557-023-07523-3
- Rahlf, C. R., and Tarakanova, V. L. (2025). Role of Interferon Regulatory Factor 1 in acute and chronic virus infections. *Virology* 603, 110386. doi:10.1016/j.virol.2024.110386
- Rossi, M., Anerillas, C., Idda, M. L., Munk, R., Shin, C. H., Donega, S., et al. (2023). Pleiotropic effects of BAFF on the senescence-associated secretome and growth arrest. *Elife* 12, e84238. doi:10.7554/eLife.84238
- Shen, Y., Sun, Z., Mao, S., Zhang, Y., Jiang, W., and Wang, H. (2020). IRF-1 contributes to the pathological phenotype of VSMCs during atherogenesis by increasing CCL19 transcription. *Aging (Albany NY)* 13 (1), 933–943. doi:10.18632/aging.202204
- Song, J., Zheng, J., Li, Z., Fu, L., Yang, J., Li, K., et al. (2024). Sulfur dioxide inhibits mast cell degranulation by sulphenylation of galectin-9 at cysteine 74. *Front. Immunol.* 15, 1369326. doi:10.3389/fimmu.2024.1369326
- Song, Y., Peng, H., Bu, D., Ding, X., Yang, F., Zhu, Z., et al. (2020). Negative auto-regulation of sulfur dioxide generation in vascular endothelial cells: AAT1 S-sulphenylation. *Biochem. Biophys. Res. Commun.* S0006-291, 231–237. doi:10.1016/j.bbrc.2020.02.040
- Suryadevara, V., Hudgins, A. D., Rajesh, A., Pappalardo, A., Karpova, A., Dey, A. K., et al. (2024). SenNet recommendations for detecting senescent cells in different tissues. *Nat. Rev. Mol. Cell Biol.* 25 (12), 1001–1023. doi:10.1038/s41580-024-00738-8
- Tian, X., Zhang, Q., Huang, Y., Chen, S., Tang, C., Sun, Y., et al. (2020). Endothelin-1 downregulates sulfur dioxide/aspartate aminotransferase pathway via reactive oxygen species to promote the proliferation and migration of vascular smooth muscle cells. *Oxid. Med. Cell Longev.* 2020, 9367673. doi:10.1155/2020/9367673
- Ungvari, Z., Tarantini, S., Sorond, F., Merkely, B., and Csizsar, A. (2020). Mechanisms of vascular aging, A geroscience perspective: JACC focus seminar. *J. Am. Coll. Cardiol.* 75 (8), 931–941. doi:10.1016/j.jacc.2019.11.061
- Upreti, M., Koonce, N. A., Hennings, L., Chambers, T. C., and Griffin, R. J. (2010). Pegylated IFN- α sensitizes melanoma cells to chemotherapy and causes premature senescence in endothelial cells by IRF-1 mediated signaling. *Cell Death Dis.* 1 (8), e67. doi:10.1038/cddis.2010.43
- Wessely, R., Hengst, L., Jaschke, B., Wegener, F., Richter, T., Lupetti, R., et al. (2003). A central role of interferon regulatory factor-1 for the limitation of neointimal hyperplasia. *Hum. Mol. Genet.* 12 (2), 177–187. doi:10.1093/hmg/ddg018
- Xie, W., Ke, Y., You, Q., Li, J., Chen, L., Li, D., et al. (2022). Single-cell RNA sequencing and assay for transposase-accessible chromatin using sequencing reveals cellular and molecular dynamics of aortic aging in mice. *Arterioscler. Thromb. Vasc. Biol.* 42 (2), 156–171. doi:10.1161/atvbaha.121.316883

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- Zeng, C., Zhu, X., Li, H., Huang, Z., and Chen, M. (2024). The role of interferon regulatory factors in liver diseases. *Int. J. Mol. Sci.* 25 (13), 6874. doi:10.3390/ijms25136874
- Zha, Y., Zhuang, W., Yang, Y., Zhou, Y., Li, H., and Liang, J. (2022). Senescence in vascular smooth muscle cells and atherosclerosis. *Front. Cardiovasc Med.* 9, 910580. doi:10.3389/fcvm.2022.910580
- Zhang, H., Lv, B., Liu, K., Du, J., Jin, H., and Huang, Y. (2025). Sulfur dioxide controls M1 macrophage polarization by sulphenylation of prolyl hydroxylase 2 at cysteine 260. *Free Radic. Biol. Med.* 230, 33–47. doi:10.1016/j.freeradbiomed.2025.01.054
- Zhang, L., Jin, H., Song, Y., Chen, S. Y., Wang, Y., Sun, Y., et al. (2021). Endogenous sulfur dioxide is a novel inhibitor of hypoxia-induced mast cell degranulation. *J. Adv. Res.* 29, 55–65. doi:10.1016/j.jare.2020.08.017
- Zhang, S., Qiu, B., Lv, B., Yang, G., Tao, Y., Hu, Y., et al. (2024). Endogenous sulfur dioxide deficiency as a driver of cardiomyocyte senescence through abolishing sulphenylation of STAT3 at cysteine 259. *Redox Biol.* 71, 103124. doi:10.1016/j.redox.2024.103124
- Zhao, W., Zhang, X., and Rong, J. (2021). SUMOylation as a therapeutic target for myocardial infarction. *Front. Cardiovasc Med.* 8, 701583. doi:10.3389/fcvm.2021.701583
- Zhou, D., Zhang, Y., Du, J., Jin, H., Tang, C., and Huang, Y. (2020). Sulfur dioxide: an endogenous protector against myocardial injury. *J. Cardiovasc Pharmacol.* 76 (4), 389–396. doi:10.1097/fjc.0000000000000882
- Zhu, M., Du, J., Liu, A. D., Holmberg, L., Tang, C., and Jin, H. (2014). Effect of endogenous sulfur dioxide in regulating cardiovascular oxidative stress. *Histol. Histopathol.* 29 (9), 1107–1111. doi:10.14670/hh-29.1107
- Zhu, Z., Zhang, L., Chen, Q., Li, K., Yu, X., Tang, C., et al. (2020). Macrophage-derived sulfur dioxide is a novel inflammation regulator. *Biochem. Biophys. Res. Commun.* 524 (4), 916–922. doi:10.1016/j.bbrc.2020.02.013
- Zou, L., Yang, Y., Wang, Z., Fu, X., He, X., Song, J., et al. (2023). Lysine malonylation and its links to metabolism and diseases. *Aging Dis.* 14 (1), 84–98. doi:10.14336/ad.2022.0711
- Zou, R., Shi, W., Tao, J., Li, H., Lin, X., Yang, S., et al. (2018). SIRT5 and post-translational protein modifications: a potential therapeutic target for myocardial ischemia-reperfusion injury with regard to mitochondrial dynamics and oxidative metabolism. *Eur. J. Pharmacol.* 818, 410–418. doi:10.1016/j.ejphar.2017.11.005
- Zu, Y., Liu, L., Lee, M. Y., Xu, C., Liang, Y., Man, R. Y., et al. (2010). SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells. *Circ. Res.* 106 (8), 1384–1393. doi:10.1161/circresaha.109.215483