Original Article



# TRIM59 attenuates ox-LDL-induced endothelial cell inflammation, apoptosis, and monocyte adhesion through AnxA2

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**Background:** Atherosclerosis (AS), a chronic inflammatory vascular disease, is a cause of heart attack and ischemic stroke. Tripartite motif-containing protein 59 (TRIM59), a member of the tripartite motif family, has been reported to be involved in inflammatory diseases. This study was to investigate the role of TRIM59 in oxidized low-density lipoprotein (ox-LDL)-induced endothelial cells and examine the mechanism of TRIM59.

**Methods:** To simulate a cellular model of AS *in vitro*, varying concentrations of ox-LDL (i.e., 20, 40, 60, 80, and 100 μg/mL) were used to treat the human umbilical vein endothelial cells (HUVECs) for 24 h. The messenger ribonucleic acid (RNA) and protein levels of TRIM59, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), and annexin 2 (AnxA2) were examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot. The transfection efficacy of overexpression (Ov)-TRIM59 and small-interfering RNA-AnxA2 was examined by RT-qPCR and western blot. Cell counting kit-8 assays, lactate dehydrogenase (LDH) assays, enzyme-linked immunosorbent assays, and terminal-deoxynucleotidyl transferase mediated nick end labeling staining were used to examine viability, LDH expression, inflammation, and apoptosis in HUVECs. The protein levels of B-cell lymphoma 2, Bcl-2-associated X (BAX), cleaved caspase3, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 were assessed by western blot. Additionally, the adhesion of THP-1 to ox-LDL-induced HUVECs was detected using monocyte adhesion assays and the binding of TRIM59 and AnxA2 was verified by co-immunoprecipitation.

**Results:** This study showed that TRIM59 expression was decreased in the ox-LDL-induced HUVECs while LOX-1 expression was increased. After transfection with Ov-TRIM59, TRIM59 in ox-LDL-induced HUVECs was increased, and TRIM59 overexpression alleviated the viability damage, inflammation, and apoptosis of the ox-LDL-induced HUVECs. In addition, THP-1 adhesion to the ox-LDL-induced HUVECs was also suppressed by TRIM59 overexpression. This study also showed that TRIM59 could bind to AnxA2 and promote AnxA2 expression in ox-LDL-stimulated HUVECs. Moreover, the rescue experiments revealed that TRIM59 suppressed the viability damage, inflammation, apoptosis, and monocyte adhesion of the ox-LDL-induced HUVECs via AnxA2.

**Conclusions:** TRIM59 protected against ox-LDL-induced AS by binding to AnxA2.

**Keywords:** Atherosclerosis (AS); TRIM59; AnxA2; inflammation; monocyte adhesion

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## Introduction

As a main potential pathology of cardiovascular disease, atherosclerosis (AS) is a major contributor to morbidity and mortality worldwide (1,2). AS, which often occurs in medium-and large-sized arteries that are composed of endothelial cells, vascular smooth muscle cells and other vascular cells, results from the retention of lipoproteins, such as low-density lipoprotein (LDL) in the intima of the arteries (3,4). AS is often considered as a chronic inflammatory disease, as inflammation plays a critical role in the whole process of atherosclerotic development (5,6). In addition, various risk factors have been reported to be associated with the advancement of AS, including hyperinsulinemia, insulin resistance, and adverse lipoprotein profile (7). In recent years, the potential of molecular-targeted therapy in the treatment of AS has been highlighted (8,9). Consequently, the identification of additional potential biomarkers involved in the progression of AS is of great significance.

The tripartite motif (TRIM) protein family, which comprises nearly 80 members, has an N-terminal region (10). A number of studies have shown that TRIM family proteins act as critical regulators in biological activities, including cell proliferation, cell-cycle progression, apoptosis, and natural viral immunity (11-13). Tripartite motif-containing protein 59 (TRIM59) is majorly localized in the cytoplasm and appears to possess an anti-inflammatory property (14). A previous study showed that the depletion of TRIM59 could exacerbate the endothelial inflammatory response (15). Additionally, TRIM59 was involved in cardiovascular disease and the level of TRIM59 was found to be significantly decreased during atherosclerosis progression (16). However, the functional effects of TRIM59 on the apoptosis and monocyte adhesion in AS haven't been investigated.

Annexin A2 (AnxA2), which is a member of the annexin family, is a 6-kDa calcium-dependent phospholipid binding protein that exists in a variety of eukaryotic cells (17). A previous study showed that AnxA2 participates in a wide range of cellular functions, such as vesicle transport, cell division, and cell growth (18). Li *et al.* conjectured that AnxA2 plays vital role in the advancement of AS (19). Interestingly, the Biogrid database (https://thebiogrid.org/) predicts that TRIM59 binds to AnxA2. Nevertheless, the relationship between TRIM59 and AnxA2 in AS remains obscure.

The present study was conducted to investigate the functional role of TRIM59 in ox-LDL-induced endothelial cell inflammation, apoptosis, and monocyte adhesion, and to discuss its relationship with AnxA2 in AS. The findings of this study may lead to the development of novel molecular-targeted therapies for AS. Additionally, this study was the first to explore the relationship between TRIM59 and AnxA2 in AS. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6044/rc).

### Highlight box

**Key Findings**

- TRIM59 might be a potential targeted-therapy for the treatment of atherosclerosis (AS).

**What is known and what is new?**

- TRIM59 overexpression alleviated the viability damage, inflammation and apoptosis of the ox-LDL-induced HUVECs.
- TRIM59 bound to AnxA2 and protected against ox-LDL-induced AS by binding to AnxA2.

**What is the implication, and what should change now?**

- Targeting of TRIM59 might be effective for the amelioration of AS.

## Methods

### *Cell culture and treatment*

Human umbilical vein endothelial cells (HUVECs) were provided by BeNa Culture Collection (cat. no. BNCC342247; Henan, China) and cultivated in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin at 37 ℃ with 5% carbon dioxide. To establish the AS model, varying concentrations of ox-LDL (20, 40, 60, 80, and 100 μg/mL) were used to treat the HUVECs for 24 h.

### *Cell transfection*

For the transfection, pc-DNA3.1 vectors containing the complete sequence of TRIM59 (Ov-TRIM59), the empty vector (Ov-NC), small-interfering ribonucleic acid targeting AnxA2 (siRNA-AnxA2-1/2), and the corresponding negative control (siRNA-NC) were constructed by GenePharma (Shanghai, China). The transfection of the above recombinants into the ox-LDL-induced HUVECs for 24 h at 37 ℃ was conducted using

Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Inc.). After transfection for 24 h, the cells were collected for the subsequent experiments.

### *Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)*

The RNA was extracted from the sample cells with TRIzol® reagent (Invitrogen) and reverse transcribed into complementary DNA using the QuantiTect Reverse Transcription kit (Qiagen GmbH) in accordance with the standard protocol. Subsequently, the RNA was amplified using the SYBR Green PCR Master Mix (Takara, Toyobo, Japan) on the 7500 Fast Real-Time PCR system. Finally, relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ (20) method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as an endogenous control. The following primer sequences were used: TRIM59 forward primer: 5'-CCCCCAAACCACGAGATCAA-3', reverse primer: 5'-CCAACATCACAGAGAGCCGT-3'; LOX-1 forward primer: 5'-TGCGACTCTAGGGGTCCTTT-3', reverse primer: 5'-TGCTGGATGAAGTCCTGAACAAT-3'; AnxA2 forward primer: 5'-GGTTGAACACATTGGCCT CAG-3', reverse primer: 5'-TGTTCAAAGCATCCCGC TCA-3' or GAPDH forward primer: 5'-TGTGGGCA TCAATGGATTTGG-3', reverse primer: 5'-ACACCATG TATTCCGGGTCAAT-3'.

### *Western blot*

The total proteins were extracted from the sample cells with radioimmunoprecipitation assay (RIPA) lysis buffer (NanJing SunShine Biotech Co., Ltd.), and the protein concentration was determined using a bicinchoninic acid (BCA)Assay kit (Beijing Dingguo Changsheng Biotechnology Co., Ltd.) in accordance with the standard protocol. After separation with sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10% gels), the proteins (60 μg/lane) were then transferred to the polyvinylidene fluoride membranes. Next, the membranes, which were impeded with 5% non-fat milk for 2 h at room temperature, were cultivated overnight with primary antibodies targeting TRIM59 (ab69639; 1:500; Abcam), LOX-1 (ab214427; 1:1,000; Abcam), Bcl-2 (ab32124; 1:1,000; Abcam), Bax (ab32503; 1:1,000; Abcam), Cleaved caspase3 (ab32042; 1:500; Abcam), intercellular adhesion molecule 1 (ICAM-1; ab282575; 1:1000; Abcam), vascular cell adhesion molecule 1 (VCAM-1; ab134047; 1:2,000; Abcam), AnxA2 (ab185957; 1:1,000; Abcam), or GAPDH (ab9485; 1:2,500; Abcam) at 4 ℃, and then cultivated with horseradish peroxidase–labeled anti-rabbit secondary antibody (ab6759; 1:5,000; Abcam) at room temperature for 2 h. The protein signals were visualized using enhanced chemiluminescence reagent (Thermo Fisher Scientific, Inc.) and ImageJ (Version 1.49; National Institutes of Health) was used in the densitometry analysis.

### *Cell counting kit-8 (CCK-8) assays*

The cell viability of the ox-LDL-induced HUVECs was assessed using CCK-8 assays. Initially, the ox-LDL-induced HUVECs were injected into 96-well plates and then cultivated for 24 h. Subsequently, 10 μL of CCK-8 reagent (Beyotime Institute of Biotechnology) was added to each well, and the cells were cultivated for a further 2 h. The optical density at 450 nm was determined by virtue of a microplate reader.

### *Measurement of lactate dehydrogenase (LDH)*

The level of LDH was examined using an LDH Assay Kit in accordance with the standard protocol. Nanjing Jiancheng Bioengineering institute was the supplier of the assay kit.

### *Enzyme-linked immunosorbent assay (ELISA)*

To examine the effects of TRIM59 overexpression on the inflammation of the ox-LDL-induced HUVECs, the releases of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) in the cell supernatants were measured using ELISA kits (Beyotime Institute of Biotechnology) in accordance with the standard protocol. Optical density was examined using a 450-nm microplate reader (Bio-Rad).

### *TUNEL*

The effects of TRIM59 overexpression on the apoptosis of the ox-LDL-induced HUVECs were evaluated using a terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) staining assay kit (Invitrogen) in strict accordance with the standard protocol. In brief, the ox-LDL-induced HUVECs were fixed with 4% paraformaldehyde for 15 min at room temperature and permeabilized with 0.25% Triton-X 100 for 15 min at room

temperature. Next, the phosphate buffered solution (PBS)–rinsed cells were cultivated with TUNEL reaction solution for 1 h in accordance with the standard protocol. The cell nuclei were stained by 4',6-diamidino-2-phenylindole for 5 min at room temperature. Finally, a florescent microscope was used to capture images of the apoptotic cells in 5 randomly selected fields.

### *Monocyte adhesion assays*

The HUVECs were cultivated in 24-well plates. In brief, 5 μM of 2',7'-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF AM) was used to label the THP-1 cells, which were diluted with RPMI 1640 medium at 37 ℃ for 30 min in the dark (21). The supernatants of the HUVEC culture medium were removed, and the labeled THP-1 cells were injected into 24-well plates at 37 ℃ for 1 h. PBS was used to rinse off any unadhered cells, and a fluorescent microscope was used to take photographs.

### *Co-immunoprecipitation (Co-IP)*

The total proteins that had been extracted from the sample cells with RIPA lysis buffer (NanJing SunShine Biotech Co., Ltd.) were quantified using a BCA Assay kit (Beijing Dingguo Changsheng Biotechnology Co., Ltd.) in accordance with the standard protocol. For the Co-IP, 500 μg of protein was cultivated with 1–2 μg of the antibodies specific to TRIM59 (ab178847; 1:40; Abcam) and AnxA2 (ab189473; 1:30; Abcam) overnight at 4 ℃. Next, 40 μL of protein A/G PLUS-Agarose beads (Invitrogen) was added, and the proteins were cultivated at room temperature for another 2 h. Next, the beads were rinsed with lysis buffer and centrifugated at 12,000 ×g for 2 min at 4 ℃. The re-suspension of the precipitated proteins was implemented in 2× SDS-PAGE loading buffer. Finally, the immuno-complexes were analyzed by western blot.

### *Statistical analysis*

All the experiments were independently repeated in triplicate. All the data are presented as the mean ± standard deviation (SD) and analyzed using GraphPad Prism 8.0 software (GraphPad software, Inc.). Differences between 2 groups were examined using an unpaired Student's *t*-test, and differences among 3 or more groups were examined using a 1-way analysis of variance with Tukey's post-hoc test. A P value <0.05 was considered statistically significant.

## Results

### *TRIM59 expression was downregulated in the ox-LDL-induced HUVECs*

Different concentrations of ox-LDL (i.e., 20, 40, 60, 80, and 100 μg/mL) were applied to treat the HUVECs, and RT-qPCR and western blot were used to estimate TRIM59. Compared to the 0-μg/mL group, the messenger RNA (mRNA) and protein expression levels of TRIM59 were significantly decreased by the ox-LDL treatment (*Figure 1A*). As *Figure 1B* shows, the ox-LDL treatment greatly enhanced the mRNA and protein expression levels of LOX-1 compared to the 0-μg/mL group.

### *TRIM59 overexpression alleviated the viability damage of the ox-LDL-induced HUVECs*

To induce TRIM59 expression, Ov-TRIM59 was transfected into the ox-LDL-induced HUVECs, and transfection efficacy was examined using RT-qPCR and western blot. Compared to the Ov-NC group, the mRNA and protein expression levels of TRIM59 in the ox-LDL-induced HUVECs were significantly increased after transfection with the Ov-TRIM59 plasmids (*Figure 2A*). The results of the CCK-8 assays revealed that ox-LDL induction significantly decreased the viability of the HUVECs (*Figure 2B*). However, the reduced viability of the HUVECs as a result of the ox-LDL stimulation was offset by the overexpression of TRIM59. In addition, the expression of LDH in the ox-LDL-induced HUVEC groups was increased following the transfection with Ov-TRIM59 in comparison to the ox-LDL + Ov-NC group (*Figure 2C*). Thus, TRIM59 overexpression relieved the viability damage of the ox-LDL-induced HUVECs.

### *TRIM59 overexpression alleviated the inflammation and apoptosis of the ox-LDL-induced HUVECs*

The ELISA results showed that the levels of TNF-α, IL-6, and IL-8 were greatly increased in ox-LDL group compared to the control group, while TRIM59 overexpression produced the opposite effects in these inflammatory cytokines as evidenced by decreased expression levels of TNF-α, IL-6 and IL-8 in the ox-LDL + Ov-TRIM59 group (*Figure 3A*). Additionally, the apoptosis in ox-LDL + Ov-TRIM59 group was significantly decreased compared to the ox-LDL + Ov-NC group (*Figure 3B*). Further, ox-LDL induction decreased Bcl-2 levels and increased the Bax

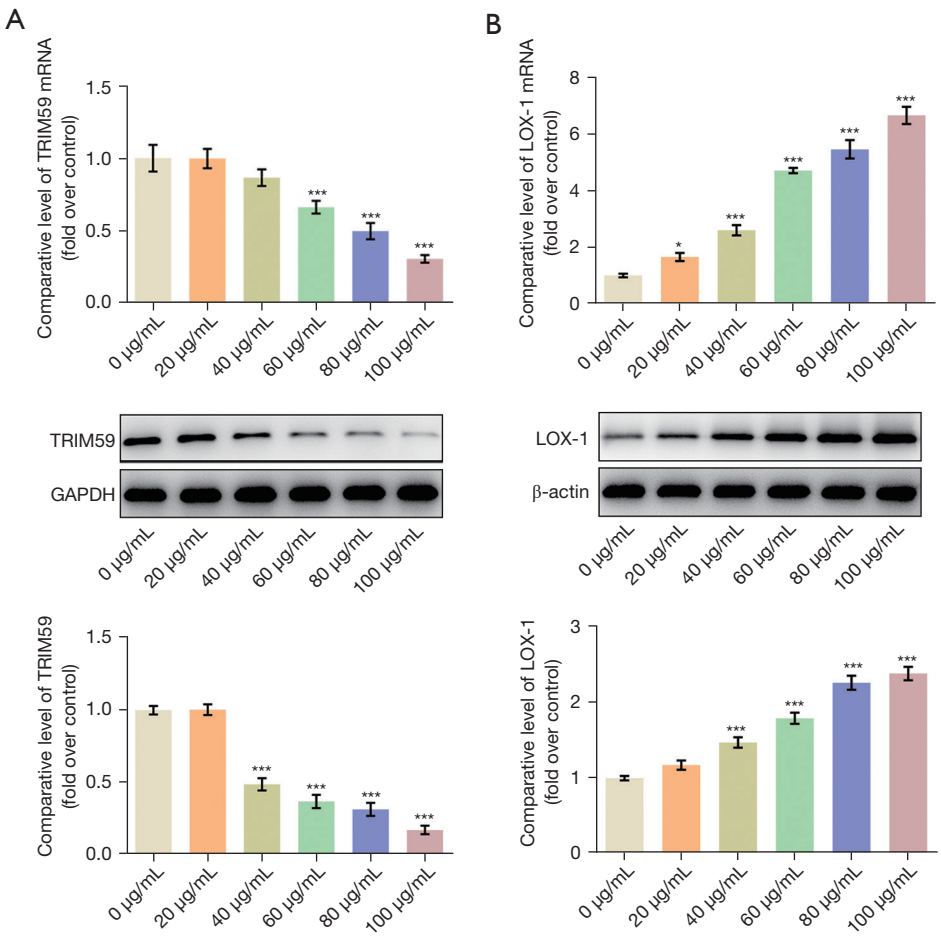


**Figure 1** TRIM59 expression was downregulated in the ox-LDL-induced HUVECs. (A) The mRNA and protein expression levels of TRIM59 in the ox-LDL-induced HUVECs were detected using RT-qPCR and western blot. (B) The mRNA and protein expression levels of LOX-1 in the ox-LDL-induced HUVECs were detected by RT-qPCR and western blot. *, P<0.05 and ***, P<0.001 *vs.* 0-μg/mL group. TRIM59, tripartite motif-containing protein 59; HUVECs, human umbilical vein endothelial cells; ox-LDL, oxidized low-density lipoprotein; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

and Cleaved caspase3 levels in ox-LDL group compared to the Control group, which were subsequently reversed after the overexpression of TRIM59 (*Figure 3C*). These results showed that TRIM59 overexpression alleviated the inflammation and apoptosis of the ox-LDL-induced HUVECs.

### *TRIM59 overexpression suppressed THP-1 adhesion to the ox-LDL-induced HUVECs*

Compared to the Control group, the THP-1 adhesion to the HUVECs was greatly increased in ox-LDL group, while TRIM59 overexpression markedly reversed THP-1 adhesion, which suggests that TRIM59 overexpression exerts protective effects on endothelial function disrupted by ox-LDL (*Figure 4A*). Additionally, western blots were employed to evaluate ICAM-1 and VCAM-1, and the results showed that ox-LDL stimulation significantly increased the levels of ICAM-1 and VCAM-1 in the HUVECs, which were then decreased after the transfection with Ov-TRIM59 plasmids (*Figure 4B*). Thus, TRIM59 overexpression suppressed THP-1 adhesion to the ox-LDL-induced HUVECs.

### *TRIM59 could bind to AnxA2 and promote AnxA2 expression in the HUVECs*

The expression of AnxA2 was examined by RT-qPCR

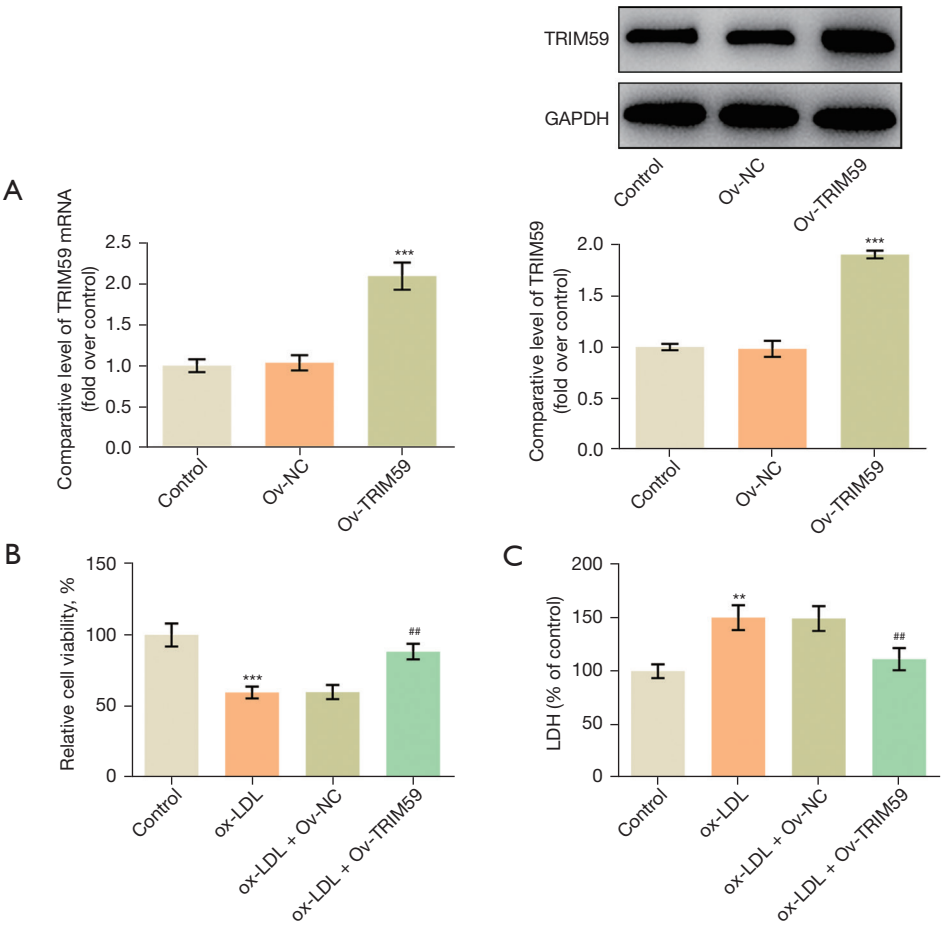


**Figure 2** TRIM59 overexpression alleviated the viability damage of the ox-LDL-induced HUVECs. (A) The transfection efficacy of Ov-TRIM59 was examined using RT-qPCR and western blot. ***, P<0.001 *vs.* Ov-NC. (B) The viability of the ox-LDL-induced HUVECs was detected by CCK-8 assays. ***, P<0.001 *vs.* Control, ##, P<0.01 *vs.* ox-LDL + Ov-NC group. (C) The levels of LDH were detected by LDH assays. **, P<0.01 *vs.* Control, ##, P<0.01 *vs.* ox-LDL + Ov-NC group. TRIM59, tripartite motif-containing protein 59; HUVECs, human umbilical vein endothelial cells; ox-LDL, oxidized low-density lipoprotein; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; Ov, overexpression; NC, negative control; LDH, lactate dehydrogenase.

and western blot, and the results showed that the mRNA and protein expression levels of AnxA2 were significantly decreased in ox-LDL group compared to the control group (*Figure 5A*). The Biogrid database (https://thebiogrid.org/) predicts that TRIM59 binds to AnxA2. To verify this finding, Co-IP assays were performed. As *Figure 5B,5C* showed, TRIM59 expression was enriched in antibodies targeting AnxA2. The western blot results showed that the level of AnxA2 was decreased in ox-LDL group compared to the control group, while TRIM59 overexpression produced the opposite effects as evidenced by the increased AnxA2 expression in the ox-LDL + Ov-TRIM59 group compared to the ox-LDL + Ov-NC group (*Figure 5D*). Collectively, the above results revealed that TRIM59 could bind to AnxA2 and promote AnxA2 expression in the HUVECs.

### *TRIM59 overexpression alleviated the viability damage of the ox-LDL-induced HUVECs via AnxA2*

To knockdown AnxA2 expression, siRNA specific to AnxA2 was transfected into the ox-LDL-induced HUVECs, and the transfection efficacy was examined using RT-qPCR and western blot. Compared to the siRNA-NC group,

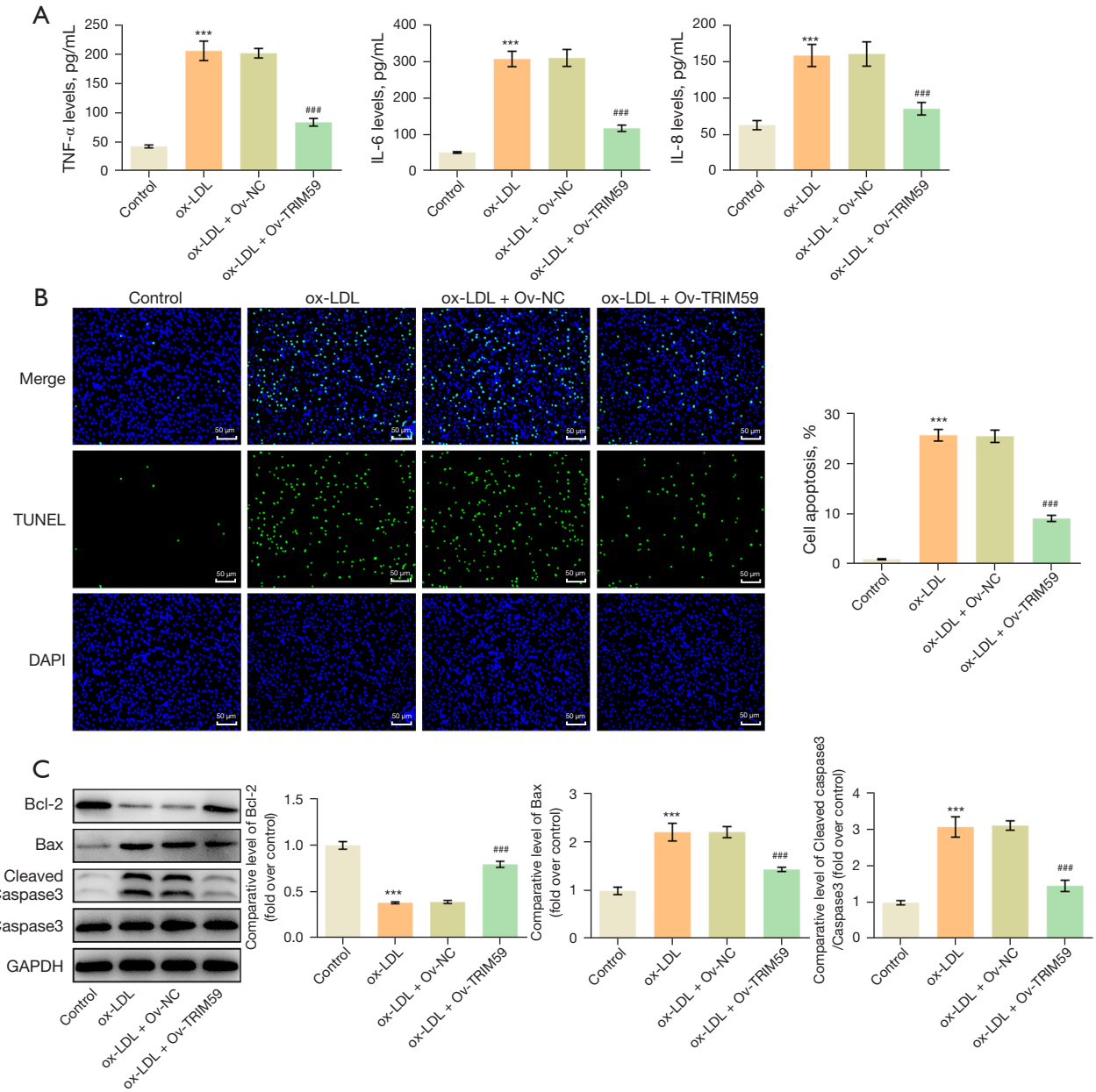


**Figure 3** TRIM59 overexpression alleviated the inflammation and apoptosis of the ox-LDL-induced HUVECs. (A) The levels of TNF-α, IL-6, and IL-8 in the ox-LDL-induced HUVECs were detected using ELISAs. (B) The apoptosis of the ox-LDL-induced HUVECs was detected using TUNEL. DAPI was used for staining. (C) The expression levels of Bcl-2, Bax, and cleaved caspase3 in the ox-LDL-induced HUVECs were detected using western blot. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL + Ov-NC group. TRIM59, tripartite motif-containing protein 59; HUVECs, human umbilical vein endothelial cells; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; ox-LDL, oxidized low-density lipoprotein; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; Ov, overexpression; NC, negative control; ELISA, enzyme-linked immunosorbent assay; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling; DAPI, 4',6-diamidino-2-phenylindole.

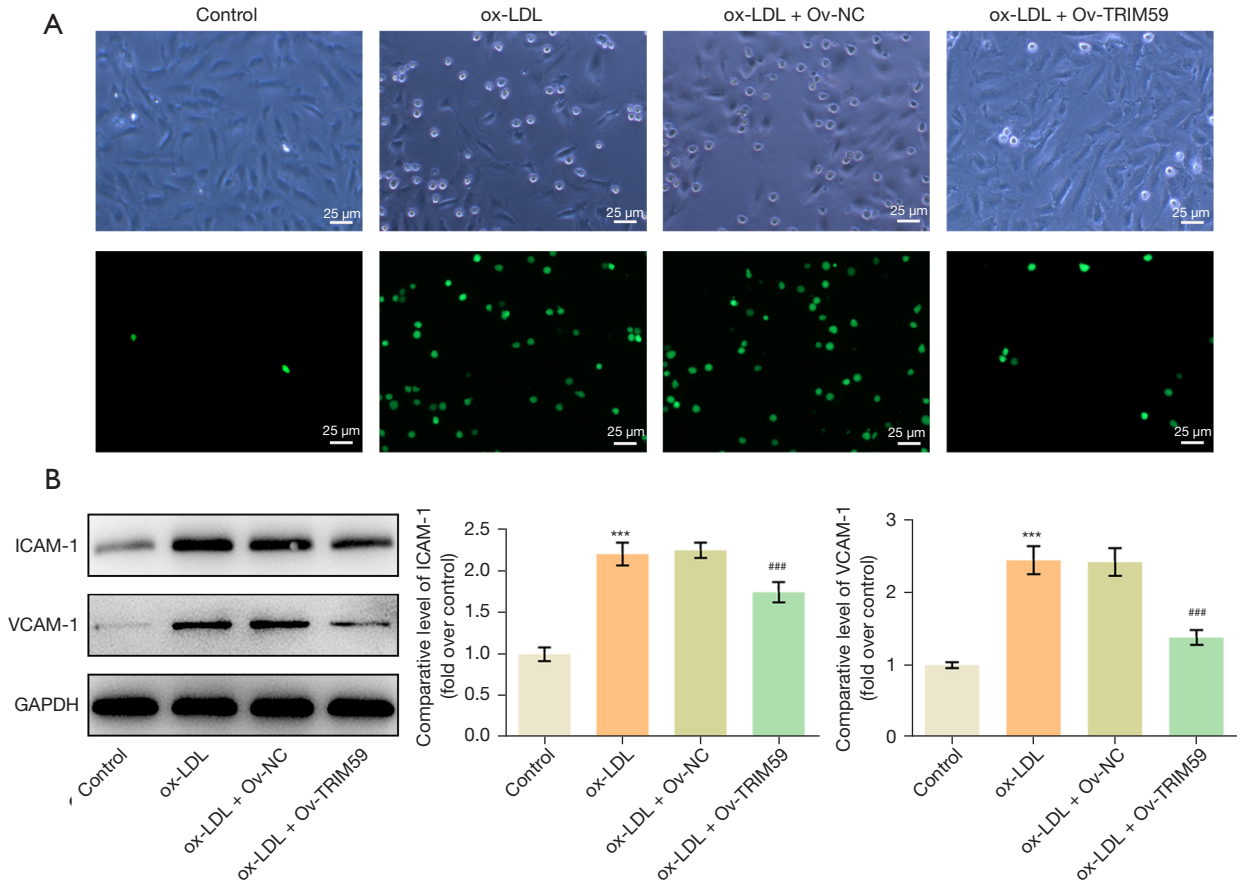


**Figure 4** TRIM59 overexpression suppressed THP-1 adhesion to the ox-LDL-induced HUVECs. (A) The THP-1 adhesion to the ox-LDL-induced HUVECs was detected using monocyte adhesion assays. 2',7'-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF AM) is used for staining. (B) The expression levels of ICAM-1 and VCAM-1 were detected by western blot. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL + Ov-NC group. TRIM59, tripartite motif-containing protein 59; ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells; Ov, overexpression; NC, negative control.

AnxA2 expression was greatly decreased in siRNA-AnxA2-1 or siRNA-AnxA2-2 group (*Figure 6A*). It was noted that AnxA2 was more lowly expressed in the siRNA-AnxA2-1 group than the siRNA-AnxA2-2 group; thus, siRNA-AnxA2-1 was subsequently employed in the following experiments. Compared to the ox-LDL group, the decreased viability of the HUVECs because of the ox-LDL induction was revived in ox-LDL + Ov-TRIM59 group, which was then reversed by AnxA2 silencing (*Figure 6B*). Additionally, AnxA2 depletion partially enhanced the level of LDH in the ox-LDL-induced HUVECs with TRIM59 overexpression compared to that in the ox-LDL + Ov-TRIM59 + siRNA-NC group (*Figure 6C*). To summarize, TRIM59 overexpression alleviated the viability damage of the ox-LDL-induced HUVECs via AnxA2.

### *TRIM59 overexpression alleviated the inflammation and apoptosis of the ox-LDL-induced HUVECs via AnxA2*

The ELISA results revealed that TRIM59 overexpression decreased the levels of TNF-α, IL-6, and IL-8 in the ox-LDL-induced HUVEC group compared to those in the ox-LDL group, while AnxA2 exhibited the opposite effects on these inflammatory cytokines as shown by the elevated levels of TNF-α, IL-6, and IL-8 in the ox-LDL + Ov-TRIM59 + siRNA-AnxA2 group (*Figure 7A*). As *Figure 7B* shows, TRIM59 overexpression suppressed the apoptosis of the ox-LDL-induced HUVEC group compared to the ox-LDL group. However, the reduced apoptosis in the ox-LDL-induced HUVEC group transfected with Ov-TRIM59 was promoted after depleting AnxA2 expression compared to the ox-LDL + Ov-TRIM59 + siRNA-NC

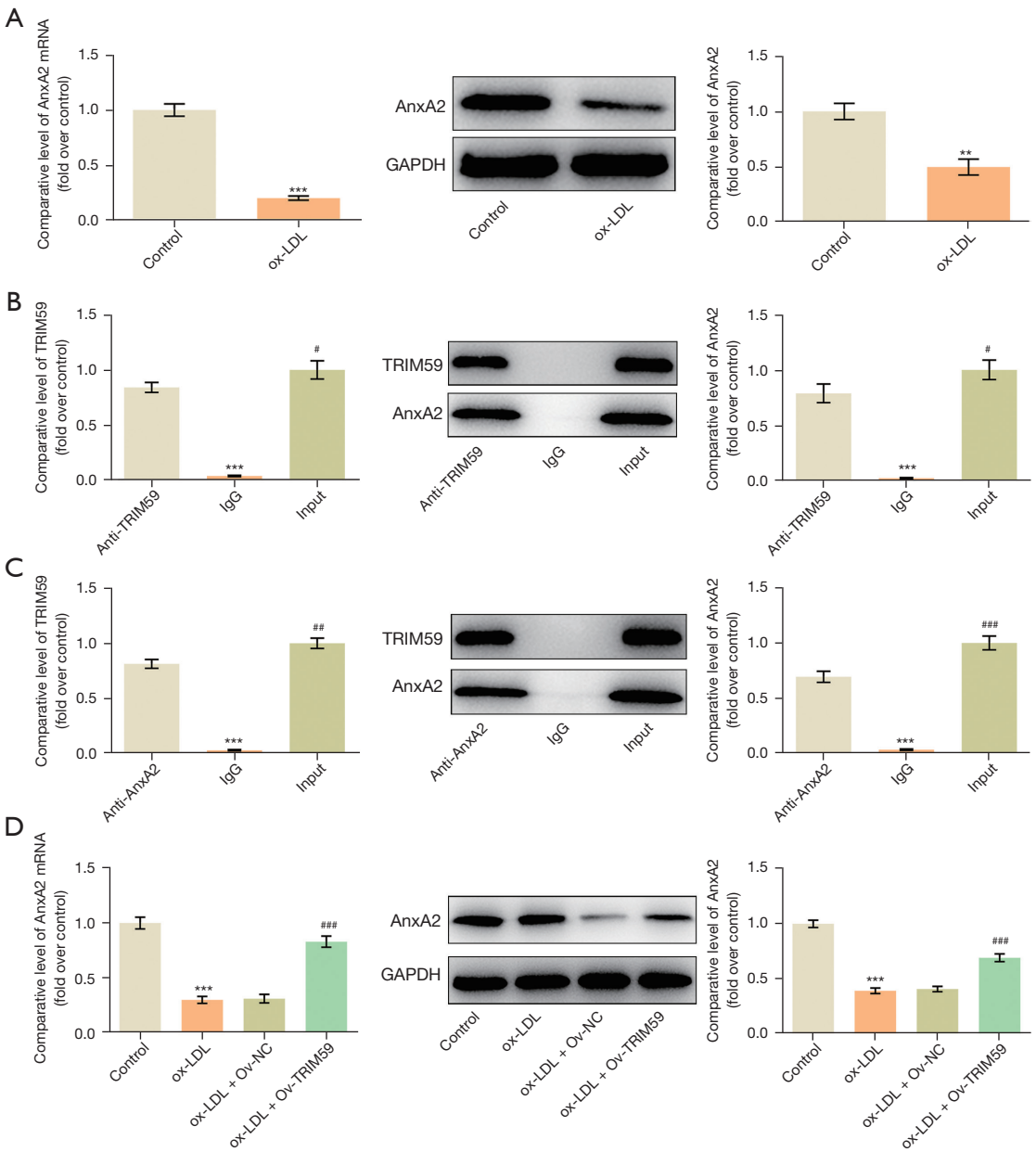


**Figure 5** TRIM59 could bind to AnxA2 and promote AnxA2 expression in the HUVECs. (A) The mRNA and protein expression levels of AnxA2 in the ox-LDL-induced HUVECs were detected using RT-qPCR and western blot. **, P<0.01 and ***, P<0.001 *vs.* Control. (B,C) The binding of TRIM59 and AnxA2 was detected using Co-IP. ***, P<0.001 *vs.* Anti-TRIM59 group *vs.* Anti-AnxA2 group, #, P<0.05, ##, P<0.01 and ###, P<0.001 *vs.* IgG group. (D) The mRNA and protein expression levels of AnxA2 in the TRIM59-overexpressed ox-LDL-induced HUVECs were detected by RT-qPCR and western blot. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL + Ov-NC group. TRIM59, tripartite motif-containing protein 59; HUVECs, human umbilical vein endothelial cells; ox-LDL, oxidized low-density lipoprotein; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; Ov, overexpression; NC, negative control; AnxA2, annexin 2; Co-IP, co-immunoprecipitation.

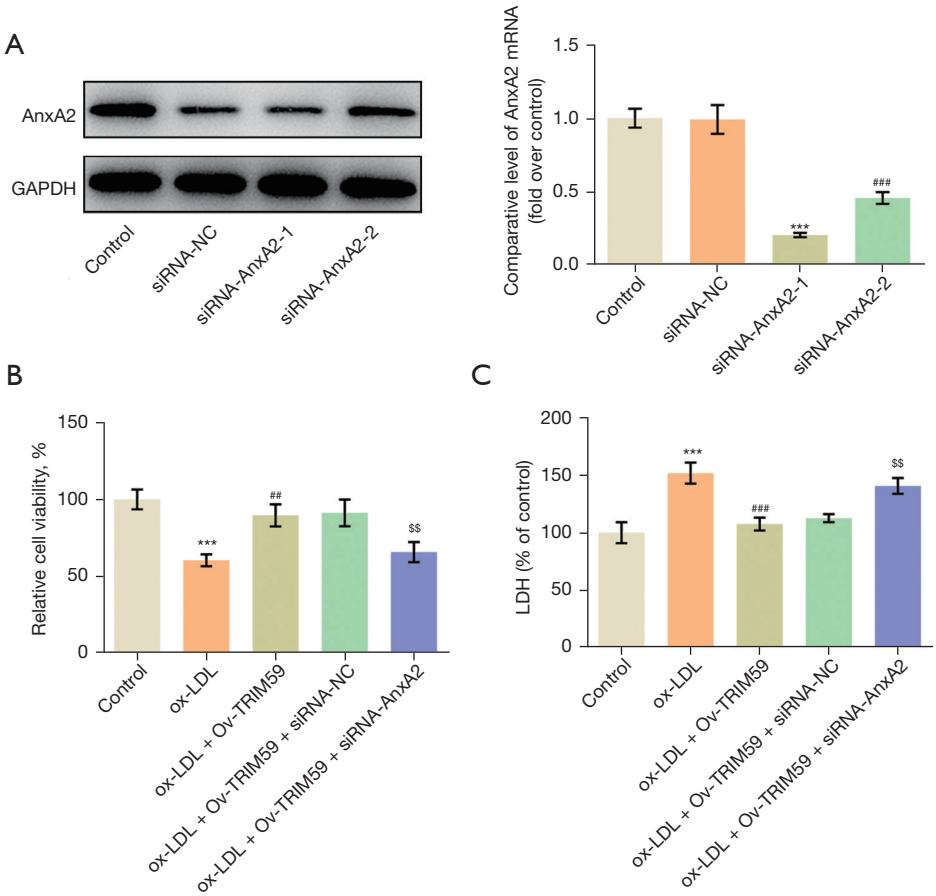


**Figure 6** TRIM59 overexpression alleviated the viability damage of the ox-LDL-induced HUVECs via AnxA2. (A) The transfection efficacy of siRNA-AnxA2 was detected using RT-qPCR and western blot. ***, P<0.001 *vs.* siRNA-NC, ###, P<0.001 *vs.* siRNA-NC. (B) The viability of the transfected ox-LDL-induced HUVECs was detected using CCK-8 assay. (C) The levels of LDH in transfected ox-LDL-induced HUVECs was detected using LDH assays. ***, P<0.001 *vs.* Control, ##, P<0.01 and ###, P<0.001 *vs.* ox-LDL group, $$, P<0.01 *vs.* ox-LDL + Ov-TRIM59 + siRNA-NC group. TRIM59, tripartite motif-containing protein 59; ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells; LDH, lactate dehydrogenase; Ov, overexpression; NC, negative control; AnxA2, annexin 2; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; CCK-8, cell counting kit-8.

group. Additionally, TRIM59 overexpression increased Bcl-2 expression but decreased the levels of Bax and Cleaved caspase3 compared to the ox-LDL group, which were then reversed by AnxA2 deficiency (*Figure 7C*). Thus, TRIM59 overexpression alleviated the inflammation and apoptosis of the ox-LDL-induced HUVECs via AnxA2.

### *TRIM59 overexpression suppressed THP-1 adhesion to the ox-LDL-induced HUVECs via AnxA2*

The results showed that the THP-1 adhesion to HUVECs was greatly reduced in ox-LDL + Ov-TRIM59 group compared to the ox-LDL group, while AnxA2 knockdown exhibited promotive effects as shown by the increased THP-1 adhesion in the ox-LDL + Ov-TRIM59 + siRNA-AnxA2 group (*Figure 8A*). Further, the increased levels of ICAM-1 and VCAM-1 in the HUVECs due to ox-LDL induction were significantly decreased in ox-LDL + Ov-TRIM59 group compared to the ox-LDL group, which were subsequently enhanced by AnxA2 depletion (*Figure 8B*). Thus, TRIM59 overexpression suppressed THP-1 adhesion to the ox-LDL-induced HUVECs via AnxA2.

## Discussion

As a chronic low-grade inflammatory disease, AS affects

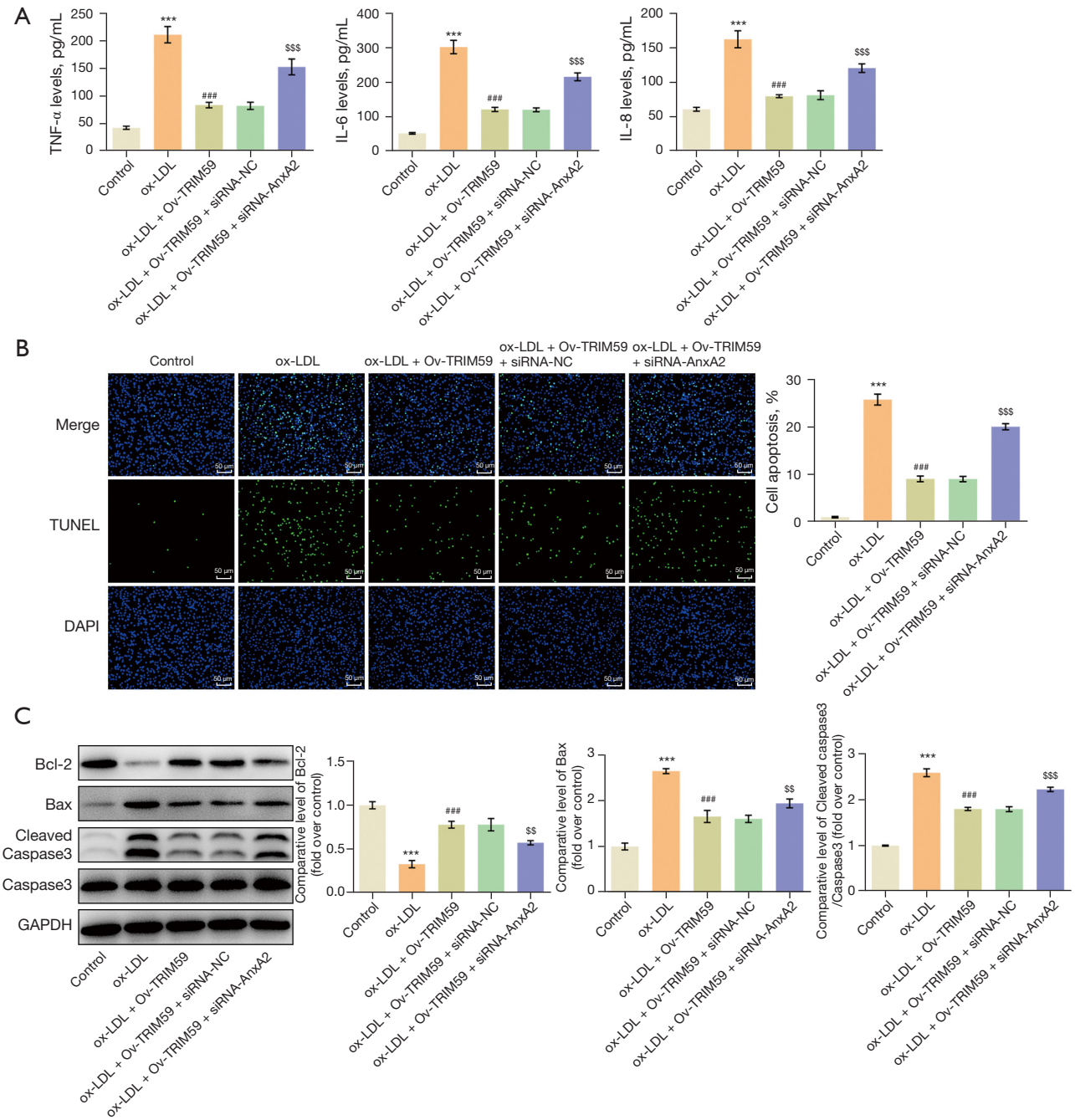


**Figure 7** TRIM59 overexpression alleviated the inflammation and apoptosis of the ox-LDL-induced HUVECs via AnxA2. (A) The levels of TNF-α, IL-6, and IL-8 in transfected ox-LDL-induced HUVECs were detected using ELISA. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL group, $$$, P<0.01 *vs.* ox-LDL + Ov-TRIM59 + siRNA-NC. (B) The apoptosis of the transfected ox-LDL-induced HUVECs was detected using TUNEL. DAPI was used for staining. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL group, $$$, P<0.01 *vs.* ox-LDL + Ov-TRIM59 + siRNA-NC. (C) The expression levels of Bcl-2, Bax, and cleaved caspase3 in the transfected ox-LDL-induced HUVECs were detected using western blot. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL group, $$, P<0.01 and $$$, P<0.01 *vs.* ox-LDL + Ov-TRIM59 + siRNA-NC. TRIM59, tripartite motif-containing protein 59; ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells; Ov, overexpression; NC, negative control; ELISA, enzyme-linked immunosorbent assay; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling; DAPI, 4',6-diamidino-2-phenylindole.

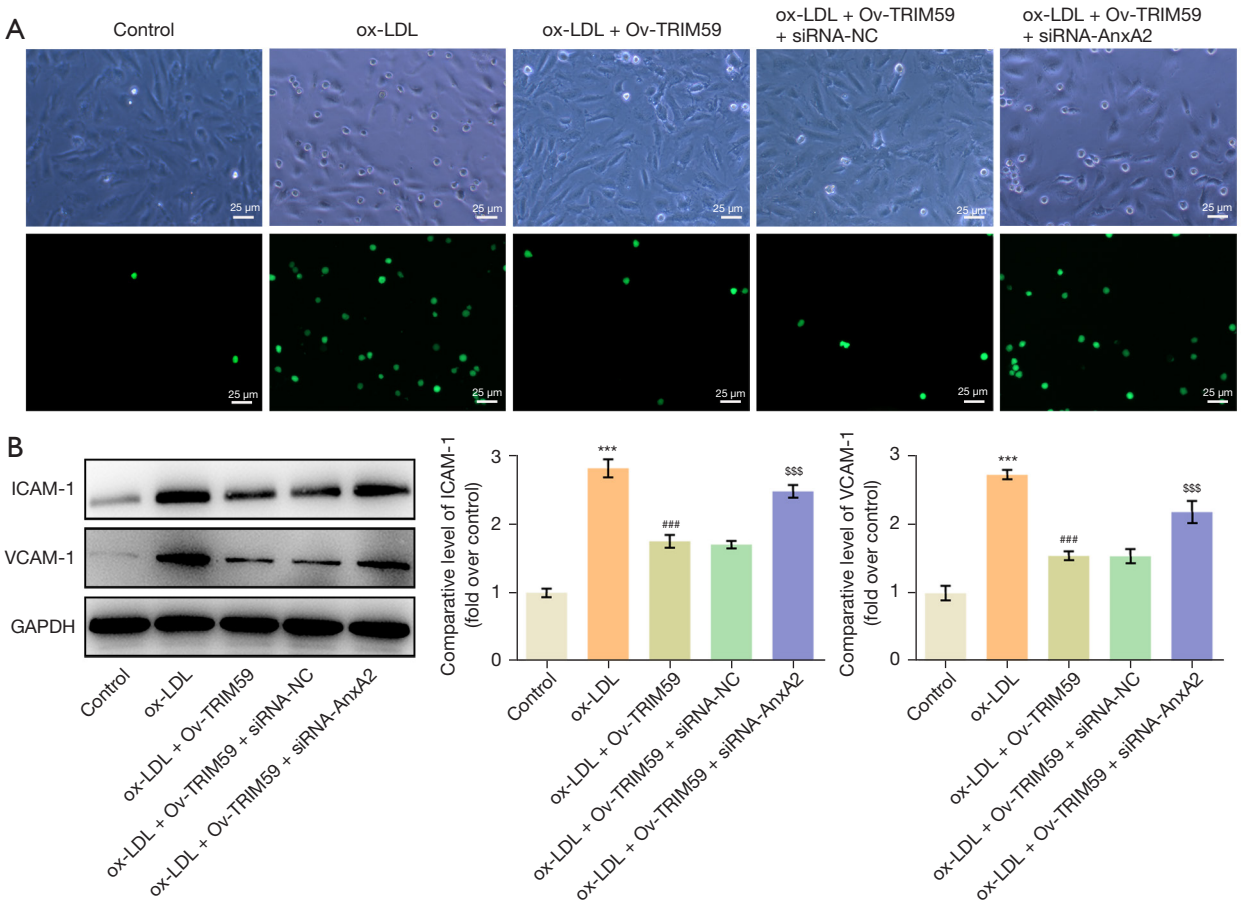


**Figure 8** TRIM59 overexpression suppressed THP-1 adhesion to the ox-LDL-induced HUVECs via AnxA2. (A) The THP-1 adhesion to the ox-LDL-induced HUVECs was detected using monocyte adhesion assays. 2',7'-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF AM) is used for staining. (B) The expression levels of ICAM-1 and VCAM-1 in the transfected ox-LDL-induced HUVECs were detected using western blot. ***, P<0.001 *vs.* Control, ###, P<0.001 *vs.* ox-LDL group, $$$, P<0.01 *vs.* ox-LDL + Ov-TRIM59 + siRNA-NC. TRIM59, tripartite motif-containing protein 59; ox-LDL, oxidized low-density lipoprotein; HUVECs, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; Ov, overexpression; NC, negative control; AnxA2, annexin 2.

large and medium-sized arteries and is thought to be a predominant underlying contributor to cardiovascular disease (22). Ox-LDL is a critical player in AS, and ox-LDL is often used to establish AS models *in vitro* (23). In this study, varying concentrations of ox-LDL (20, 40, 60, 80, and 100 μg/mL) were administered to HUVECs for 24 h. LOX-1, a type II membrane glycoprotein receptor, has been shown to be a crucial effector of AS (24). In this study, it was found that ox-LDL induction significantly elevated the expression of LOX-1 in the HUVECs in a concentration-dependent manner. TRIM59, a TRIM family member, contains a really interesting new gene (RING)-finger domain, 1 zinc-binding motif, and an associated coiled-coil region (25). This study also showed that the mRNA and protein expression levels of TRIM59 in the HUVECs were reduced by ox-LDL induction. To induce TRIM59 overexpression, Ov-TRIM59 was transfected into the ox-LDL-induced HUVECs. The transfection efficacy was examined using RT-qPCR and western blot, and the results showed that the mRNA and protein expression levels of TRIM59 were greatly increased. A previous study showed that endothelial injury in the atheroprone arteries is a predominant initiating factor in the advancement of AS (26). Besides, Chen and co-workers have put forward that the depletion of TRIM59 could aggravate endothelial injury induced by LPS (15). In the present study, TRIM59

overexpression was shown to revive the viability of the ox-LDL-induced HUVECs. Additionally, the increased LDH level in the HUVECs due to ox-LDL induction was reduced after TRIM59 overexpression was induced.

Decreased bioavailability of nitric oxide (NO) arising downstream of endothelial oxidative stress can cause endothelial dysfunction (27). Previous studies have reported that the inhibition of inflammation, apoptosis and ferroptosis can attenuate endothelial dysfunction (28,29). Research has shown that inflammation plays a crucial role in the initiation and the progression of atherosclerotic plaque (6). Additionally, low-grade, chronic inflammation of the arterial wall is a typical characteristic of AS (30). An increasing number of studies have shown that targeting inflammation is a novel therapeutic approach in the treatment of AS (31,32). Notably, TRIM59 has been shown to possess an anti-inflammatory property. For example, the inflammatory response resulting from myocardial ischemia reperfusion injury was attenuated after TRIM59 overexpression was induced (33). Additionally, An *et al.* showed that TRIM59 might be a potential therapeutic target for the inflammatory disease AS (16). TNF-α, IL-6, and IL-8 are pro-inflammatory cytokines (34). The present study showed that the elevated expression levels of TNF-α, IL-6 and IL-8 in the HUVECs due to ox-LDL induction were greatly reduced by TRIM59 overexpression. Ox-LDL–mediated endothelium apoptosis is an important cause of AS (35). In this study, TRIM59 overexpression was shown to decrease the apoptosis level of the ox-LDL-induced HUVECs. Bcl-2 is an anti-apoptotic protein, and Bax and cleaved caspase-3 are proapoptotic proteins (36). In this study, TRIM59 upregulation increased Bcl-2 levels but decreased the levels of Bax and cleaved caspase-3 in the ox-LDL-induced HUVECs.

The initiation of early AS induced by ox-LDL is also accompanied by the recruitment of monocytes by endothelial cell-derived ligands, mainly including VCAM-1 and ICAM-1, which subsequently enter the subendothelial space and transform into foam cells that develop into atherosclerotic plaques (21,37). Thus, it is of great significance to protect the function of endothelial cells from exogenous factors in anti-AS (38,39). The present study showed that TRIM59 overexpression inhibited THP-1 adhesion to HUVECs. Further, the increased levels of ICAM-1 and VCAM-1 in the HUVECs resulting from ox-LDL induction later decreased after TRIM59 overexpression was induced.

The Biogrid database predicts that TRIM59 binds to AnxA2. Moreover, a previous study also showed that TRIM59 binds to AnxA2 and TRIM59 overexpression promotes the expression of AnxA2 (40). Interestingly, Li *et al.* showed that AnxA2 was involved in the progression of AS (19). The RT-qPCR and western blot results revealed that AnxA2 expression was decreased in the ox-LDL-induced HUVECs. Co-IP assays confirmed that TRIM59 could bind to AnxA2 in the ox-LDL-induced HUVECs. Moreover, TRIM59 overexpression was shown to enhance the expression of AnxA2 in the ox-LDL-induced HUVECs. To knockdown AnxA2 expression, siRNA-AnxA2 was transfected into the ox-LDL-induced HUVECs and the expression of AnxA2 was found to be significantly decreased by the siRNA-AnxA2. In many cases, AnxA2 participates in various developmental processes through gene regulation. For example, hsa_circ_0003928 interference attenuated apoptosis and inflammation in high glucose-induced renal tubular cells via AnxA2 (41). Bao *et al.* showed that miR-101 overexpression regulated the viability of gastric cancer cells by targeting AnxA2 (42). Further, AnxA2 silencing was reported to decrease monocyte adhesion in AS (43). The results of the rescue experiments showed that the inhibitory effects of TRIM59 overexpression on ox-LDL-induced endothelial cell inflammation, apoptosis, and monocyte adhesion were reversed by AnxA2 silencing, which suggests that TRIM59 helps to protect against AS via AnxA2.

## Conclusions

To summarize, this study examined the effects of TRIM59 on the viability, inflammation, apoptosis, and THP-1 adhesion of the ox-LDL-induced HUVECs, and showed that TRIM59 could bind to AnxA2. This was the first study to reveal the mechanism by which TRIM59 suppressed the development of AS. In the future, this conclusion will be further validated in clinical and *in vivo* samples.

### *Limitations*

There are some limitations of our study. Firstly, this study didn't explore any signaling pathways to further support our findings. Secondly, the methods that used to measure cell apoptosis and inflammation are not rich enough.

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## Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6044/rc

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*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6044/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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