

# Rutaecarpine mediates phenotypic transition of vascular smooth muscle cells via ADAM17-TNF-NLRP3 signaling pathway

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## Research Article

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**Additional Declarations:** No competing interests reported.

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# Abstract

The Chinese herbal monomer rutaecarpine is extracted from *Evodia rutaecarpa*, which has been widely used for hundreds of years to treat hypertension. Rutaecarpine can inhibit pressure overload-induced vascular remodeling and oxidized low-density lipoprotein-induced phenotype transition in vascular smooth muscle cells (VSMCs). However, further studies are needed to elucidate the role of rutaecarpine in preventing VSMC phenotypic transition and its underlying mechanisms. This study aimed to explore the inhibitory effect of rutaecarpine on angiotensin (Ang) II-induced VSMC phenotypic transition and its mechanisms. In vitro experiments revealed that rutaecarpine inhibited Ang II-induced VSMC phenotypic transition. Further studies revealed that a disintegrin and metalloproteinase 17 (ADAM17) mediated Ang II-induced VSMC phenotypic transition through tumor necrosis factor (TNF)-NLRP3 signaling pathway. Moreover, treatment with rutaecarpine could inhibit the ADAM17-TNF-NLRP3 signaling pathway in VSMCs stimulated with Ang II. In conclusion, rutaecarpine prevents Ang II-induced VSMC phenotypic transition via the ADAM17-TNF-NLRP3 signaling pathway.

## 1. Introduction

According to the *Global Report on Hypertension 2025*, there are approximately 1.4 billion hypertensive patients aged 30–75 years worldwide (Farrar J and Frieden T. 2025). The most critical clinical outcome of hypertension is end-organ damage, such as heart failure and stroke. Even though the hypertensive patients have received the optimal therapies recommended by the hypertension guidelines, they still have a 50% greater risk than the subjects showing normotensive states (Struthers AD and George J. 2015), indicating the urgent need for add-on therapy to target hypertensive end-organ damage. Vascular remodeling is an active process of structural alteration of blood vessels and the pathological basis of hypertension, causing hypertensive end-organ damage, and it is associated with poor cardiovascular outcomes (Cameron AC et al. 2016). Vascular smooth muscle cells (VSMCs) are the major component of the vessel wall's middle layer and take part in vascular remodeling. In response to cytokine [e.g., angiotensin (Ang) II] stimulation or mechanical stress, VSMCs undergo phenotypic transition from the contractile state to the synthetic state, thereby contributing to vascular remodeling (Brown IAM et al. 2018). Concurrently, the increases in VSMC proliferation and migration, in the production and reorganization of the extracellular matrix, and in the expression of differentiation markers are observed (Owens GK et al. 2004; Alexander MR and Owens GK. 2012; Bennett MR et al. 2016; Xu Y et al. 2024). Therefore, inhibiting or reversing VSMC phenotypic transition prevents vascular remodeling and subsequent hypertensive end-organ damage.

Rutaecarpine is extracted from the Chinese herbal drug *Evodia rutaecarpa*, which has been adopted to treat hypertension for hundreds of years (Tian KM et al 2019). Our previous findings showed that rutaecarpine reduces blood pressure and inhibits vascular remodeling in spontaneously hypertensive and renal hypertensive rats (Zeng SY et al 2019; Qin XP et al 2007; Qin XP et al 2009). Furthermore, Wang ML et al (2019) reported that rutaecarpine inhibited the VSMC phenotypic transition induced by oxidized low-density lipoprotein in vitro. However, previous studies have not elucidated the beneficial

effect of rutaecarpine on cytokines (e.g., Ang II)-induced VSMC phenotypic transition. Therefore, this study aimed to examine the protective role of rutaecarpine in Ang II-induced VSMC phenotypic transition and its underlying mechanisms.

## 2. Materials and Methods

### 2.1 Ethical approval

All experimental procedures were approved by the Medical Ethics Committee of the Affiliated Guangdong Second Provincial General Hospital of Jinan University, China.

### 2.2 Reagents

Rutaecarpine (84-26-4) was provided by Chengdu Must Bio-Technology Co., Ltd (Sichuan, China). Antibodies against ADAM17 (ab39162; Abcam), actin alpha 2 (ACTA2; 14395-1-AP; Proteintech), myosin heavy chain 11 (MYH11; 21404-1-AP; Proteintech), NLRP3 (68102-1-Ig; Proteintech), and GAPDH (60004-1-Ig; Proteintech) were used. Fetal bovine serum (FBS, A115-500) and Dulbecco's modified Eagle medium (DMEM) were purchased from Nobimpex Biotechnology (Germany), and Ang II (4474-91-3) was provided by AbMole (Shanghai, China). Further, Lipofectamine 2000 was bought from Invitrogen (Carlsbad, USA).

### 2.3 Cell culture

The VSMC cell line A7R5 (research resource identifier, CSTR:19375.09.3101RAT GNR 7), derived from the thoracic artery of embryonic rats (unidentified sex), was obtained from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) in August 2023. This cell line has not been previously reported as misidentified or contaminated. The VSMCs were cultured in DMEM supplemented with 10% FBS under normal conditions (37°C, 5% carbon dioxide). The cell line was confirmed using short tandem repeat (STR) assays; the STR match was 85%, and the cell line tested negative for mycoplasma contamination. When the VSMCs reached approximately 80% confluence, cells were incubated for 24 h in DMEM containing 0.1% FBS before Ang II stimulation. VSMCs were pretreated with 10 µmol/L rutaecarpine for 30 minutes, as previously described (Wang ML et al. 2019).

### 2.4 RNA Interference

Small interfering RNA (siRNA) sequences against rat ADAM17 were synthesized by Shanghai Gene Pharma. The ADAM17 antisense sequence 5'-ACUUCACACUGUA CUCGCUTT-3' was used, and the scrambled sequence was adopted as the negative control. The VSMCs were transfected with 100 nmol/L siRNA or the negative control by using Lipofectamine 2000<sup>TM</sup> according to the manufacturer's instructions.

### 2.5 Cell viability assay

Cell viability was examined using the Cell Counting Kit-8 (Hangzhou Fude Biological Technology Co., Ltd, China) according to the manufacturer's instructions.

## **2.6 Western blotting**

Western blotting was done according to previously described standard procedures (Chen X et al. 2014).

## **2.7 Real-Time Quantitative PCR**

Total RNA extraction and real-time quantitative PCR were performed as previously described. Primers used for interleukin (IL)-1 $\beta$ , IL-6, and TNF- $\alpha$  were as follows: IL-1 $\beta$ : 5-TCCTCTGTGACTCG TGGGAT-3' (forward primer), 5'-TCAGACAGCAC GAGGCATTT-3' (reverse primer); IL-6: 5'-TCCTACCCCAACTTCCAATGCTC-3' (forward primer), 5'-TTGGATGGT CTTGGTCCTTAGCC-3' (reverse primer); TNF - $\alpha$ : 5'-TGGCGTGTTTCATCCGTTCTC-3' (forward primer), 5'-CCCAGAGCCACAA TTCCCTT-3' (reverse primer); GAPDH: 5'-ATCAAGAAGGTGGTGAAGCA-3' (forward primer), 5'-AAGGTGGAAGAATGGGAGTTG-3' (reverse primer).

## **2.8 Statistical Analysis**

All experimental results are expressed as mean  $\pm$  standard deviation (SD). Statistical analyses were performed by an independent researcher using the unpaired t-test between two groups and one- or two-way ANOVA followed by Bonferroni's post hoc test among at least three groups, and statistical significance was set at  $P < 0.05$ .

# **3. Results**

## **3.1 Effect of rutaecarpine on Ang II-induced VSMC phenotypic transition.**

As shown in Figure 1, pretreatment with 10  $\mu$ mol/L rutaecarpine for 30 minutes inhibited Ang II-induced VSMC phenotypic transition, supported by reductions in cell viability and the protein levels of the contractile markers ACTA2 and MYH11. These findings indicated that treatment with rutaecarpine suppressed Ang II-induced VSMC phenotypic transition.

## **3.2 ADAM17 is required for Ang II-induced VSMC phenotypic transition.**

As shown in Figure 2a-d, ADAM17 protein level was elevated in contrast to those of VSMC contractile markers ACTA2 and MYH11 upon Ang II stimulation in cultured VSMCs; however, ADAM17 siRNA significantly decreased the protein levels of the contractile markers ACTA2 and MYH11 compared with those in the Ang II group. Furthermore, results from the VSMC viability assay using the Cell Counting Kit-8 showed that ADAM17 deletion prevented Ang II-induced VSMC proliferation (Figure 2e). Thus, ADAM17 mediated Ang II-induced VSMC phenotypic transition.

## **3.3 ADAM17-TNF axis promoted Ang II-induced VSMC phenotypic transition via NLRP3 inflammasome-induced inflammation.**

In vitro experiments demonstrated that transfection with ADAM17 siRNA resulted in a significant reduction in TNF- $\alpha$  mRNA levels in Ang II-stimulated VSMCs (Figure 3a). Notably, treatment with the TNF inhibitor etanercept also decreased ADAM17 protein level in VSMCs treated with Ang II (Figure 3b-c). Etanercept significantly suppressed Ang II-induced VSMC phenotypic transition, as indicated by significant increases in the contractile markers ACTA2 and MYH11 compared to levels in the Ang II group (Figure 3d-e). Moreover, the VSMC viability assay revealed that etanercept protected against Ang II-induced VSMC proliferation (Figure 3f). These findings indicated that ADAM17-TNF axis advanced Ang II-induced VSMC phenotypic transition.

VSMC phenotypic transition is often associated with the changes in inflammatory cytokines (Zhang F et al. 2021). As shown in Figure 4, ADAM17 inhibition markedly reduced NLRP3 protein level, as well as the mRNA levels of pro-inflammatory factors (e.g., IL-1 $\beta$  and IL-6) in Ang II-treated VSMCs. Furthermore, the TNF inhibitor etanercept prevented NLRP3-induced inflammation in Ang II-treated VSMCs, as indicated by reductions in the mRNA levels of the pro-inflammatory factors IL-1 $\beta$  and IL-6 compared with the Ang II group (Figure 5). Moreover, treatment with the NLRP3 inflammasome inhibitor MCC950 inhibited Ang II-induced VSMC phenotypic transition and inflammation, as evidenced by reduced cell viability, reduced protein levels of the contractile markers ACTA2 and MYH11, as well as reduced mRNA levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in VSMCs (Figure 6). Collectively, the ADAM17-TNF axis mediates Ang II-induced VSMC phenotypic transition through NLRP3 inflammasome-induced inflammation.

### **3.4 Rutaecarpine inhibited Ang II- induced VSMC phenotypic transition via the ADAM17-TNF axis and NLRP3 inflammasome-induced inflammation.**

Treatment with rutaecarpine suppressed Ang II-induced reduction of TNF- $\alpha$  mRNA level, ADAM17 protein level, as well as NLRP3 protein level (Figure 7, Figure 8a-b). As shown in Figure 8c-d, pretreatment with 10  $\mu$ mol/L rutaecarpine for 30 minutes reduced the mRNA levels of IL-6, IL-1 $\beta$  in Ang II-treated VSMCs. Thus, rutaecarpine prevented Ang II-induced VSMC phenotypic transition involving in the ADAM17- TNF axis and NLRP3 inflammasome-induced inflammation.

## **4. Discussion**

In this study, there are three interrelated discoveries: (1) rutaecarpine inhibited Ang II -induced VSMC phenotypic transition; (2) the ADAM17-TNF axis advanced Ang II -induced VSMC phenotypic transition through NLRP3 inflammasome-induced inflammation; and (3) treatment with rutaecarpine suppressed the ADAM17-TNF axis and NLRP3 inflammasome-induced inflammation.

A series of studies have examined the beneficial role of rutaecarpine in hypertension and subsequent vascular remodeling. Our earlier studies have verified the protective action of rutaecarpine against hypertension and subsequent vascular remodeling (Zeng SY et al. 2019; Qin XP et al. 2007; Qin XP et al. 2009). Moreover, we further revealed that treatment with rutaecarpine markedly suppressed Ang II-induced VSMC proliferation and hypoxia-induced proliferation of human pulmonary artery smooth muscle cells (Li YJ et al. 2014; Deng J et al. 2018). In the present study, in vitro data further confirmed

the protective action of rutaecarpine against VSMC phenotypic transition, providing additional experimental evidence for its use in the treatment of hypertension.

Several studies have focused on the mechanisms by which rutaecarpine modulates hypertensive vascular remodeling. Our previous studies have shown that calcitonin gene-related peptide and prolylcarboxypeptidase participate in hypertensive vascular remodeling (Zeng SY et al. 2019; Qin XP et al. 2007; Qin XP et al. 2009). Furthermore, Wang ML et al. reported that rutaecarpine prevented the up-regulation of connexin 43 levels through transient receptor potential vanilloid subtype 1/[Ca<sup>2+</sup>]<sub>i</sub>/calmodulin /NF-κB signaling, thereby preventing ox-LDL-induced VSMC dysfunction (Wang ML et al. 2019). Nevertheless, it does not fully elucidate the mechanisms by which rutaecarpine exerts its effects on hypertensive vascular remodeling.

ADAM17 has attracted more attention in hypertensive vascular remodeling. Vascular ADAM17 deficiency prevents vascular medial hypertrophy and perivascular fibrosis in Ang II-induced hypertensive rats, independent of alteration in blood pressure (Takayanagi T et al. 2016). In vitro experiments revealed that ADAM17 is required for Ang II-induced VSMC proliferation and hypertrophy (Ohtsu H et al. 2006; Zeng SY et al. 2018). In the present research, we further found that ADAM17 can promote Ang II-induced VSMC phenotypic transition, providing more details on its role in vascular remodeling.

As a cleaving enzyme, ADAM17 can cleave the 26 kDa pro-TNF-α into the soluble 17 kDa TNF-α, and it is necessary for Ang II-induced elevation of TNF-α level in VSMCs (Zeng SY. et al 2018; Black RA. 2002). In this research, we revealed that the TNF inhibitor etanercept inhibited Ang II-induced VSMC phenotypic transition and the elevation in ADAM17 level in VSMCs treated with Ang II. These findings indicate a positive feedback loop between ADAM17 and TNF. Simultaneously, we found that inhibition of both ADAM17 and TNF lessened inflammation through the NLRP3 inflammasome in Ang II-stimulated VSMCs. Disruption of NLRP3 prevents VSMC phenotypic transition in the aorta of spontaneously hypertensive rats, thus causing hypertensive vascular remodeling (Sun HJ et al. 2017). Furthermore, NLRP3 inflammasome mediates low-grade inflammation by caspase-1 -dependent release of the pro-inflammatory cytokine IL-1β during pressure overload (Higashikuni Y et al. 2023; Ertuglu LA et al. 2022). However, it is worth noting that our present study did not assess downstream markers of inflammasome activation (e.g., caspase-1 cleavage). Taken together, the ADAM17-TNF axis promotes Ang II-induced VSMC phenotypic transition through NLRP3 inflammasome-mediated inflammation.

In the present study, we revealed that rutaecarpine suppressed the ADAM17-TNF positive feedback loop and subsequent NLRP3 inflammasome-induced inflammation in VSMCs stimulated with Ang II. However, it does not examine whether rutaecarpine treatment prevents the ADAM17-TNF positive feedback loop and subsequent NLRP3 inflammasome-induced inflammation in the arteries of hypertensive models. Overall, inhibition of the ADAM17-TNF axis is associated with the beneficial action of rutaecarpine against VSMC phenotypic transition via NLRP3 inflammasome-induced inflammation.

## 5. Conclusions

In conclusion, rutaecarpine could prevent VSMC phenotypic transition, involving the ADAM17-TNF axis and subsequent NLRP3 inflammasome-induced inflammation. These findings will help us better understand the protective role of rutaecarpine in VSMC phenotypic transition and its underlying mechanisms, thereby providing more evidence for its use in preventing hypertensive vascular remodeling. However, this study examined only the inhibitory effect of rutaecarpine on the ADAM17-TNF axis and subsequent NLRP3 inflammasome-induced inflammation in VSMCs. Additional studies are needed to further confirm whether the positive feedback loop underlies the beneficial effect of rutaecarpine on the VSMC phenotypic transition.

## Declarations

### Author Contributions

Hai-Hong Tian: Conceptualization, Methodology, Investigation, Writing-original draft. [Ying Liu](#): Methodology, Investigation, Data curation, Visualization. Pei-Qi Shi: Formal analysis, Investigation, Validation. Yan, Yang: Investigation, Data curation. Yan-Fang Chen: Supervision, Funding acquisition. Chun Zhou: Supervision, Writing - Review & Editing. Si-Yu Zeng: Conceptualization, Supervision, Funding acquisition, Writing - Review & Editing.

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### Data Availability

No publicly available datasets were analyzed in this research. All data to support the findings of this research were generated from the experiments described in the manuscript and are available from the corresponding author upon reasonable request.

### Declarations

#### Clinical trial

Not applicable

#### Ethical approval

All experimental procedures were approved by the Medical Ethics Committee of the Affiliated Guangdong Second Provincial General Hospital of Jinan University, China.

## Consent for publication

Not applicable

## Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

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## Figures

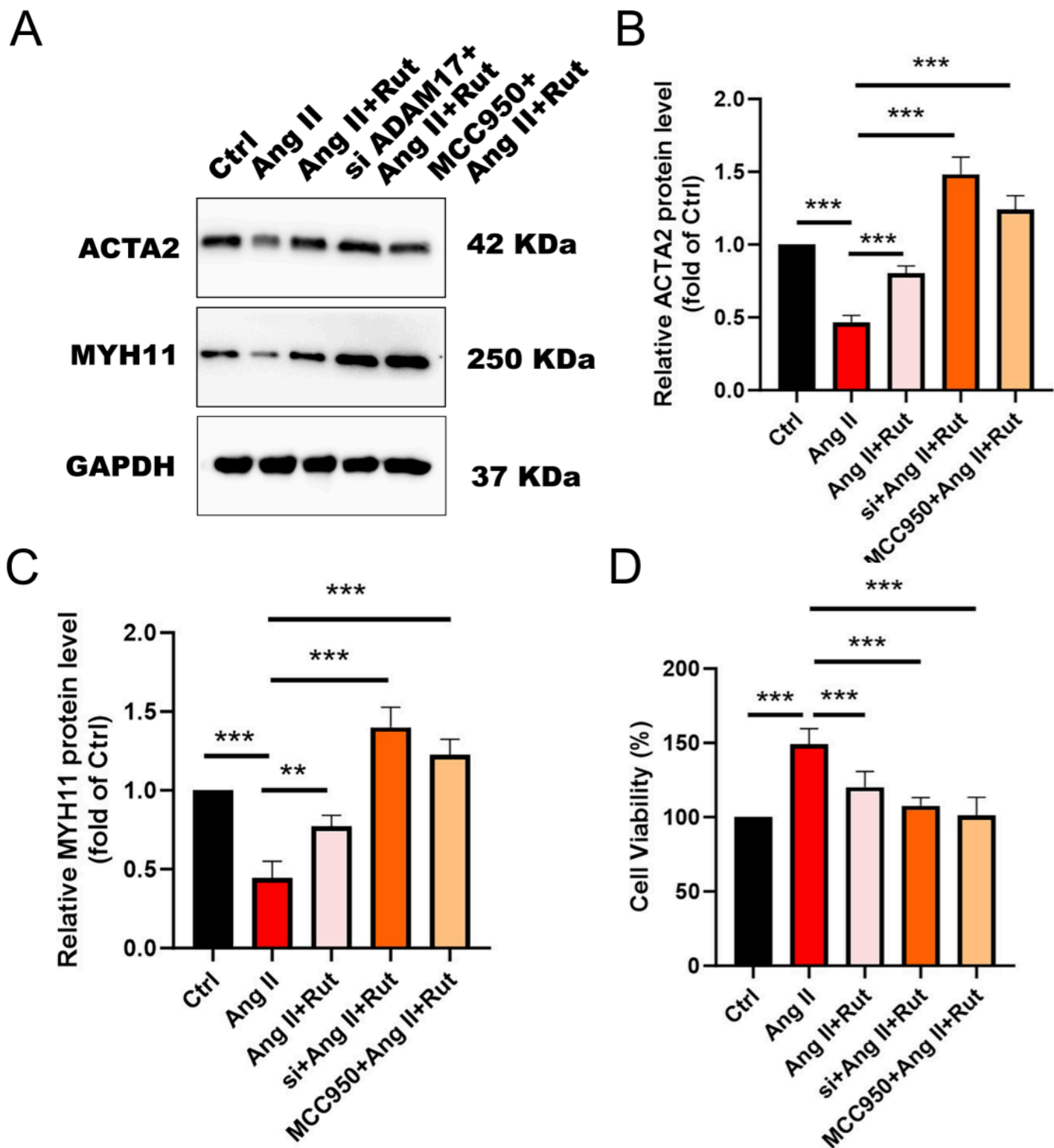


**Figure 1**

Rutaecarpine inhibited phenotypic transition in VSMCs treated with Ang II. After being pretreated with 10  $\mu$ mol/L rutaecarpine for 30 minutes, VSMCs were then stimulated with 100 nmol/L Ang II for 24 hours. A: Representative western blotting photograph; B-C: The protein levels of contractile markers ACTA2 (B), MYH11 (C); D: Cell viability.  $n = 4$  independent experiments. Ang II: angiotensin II. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

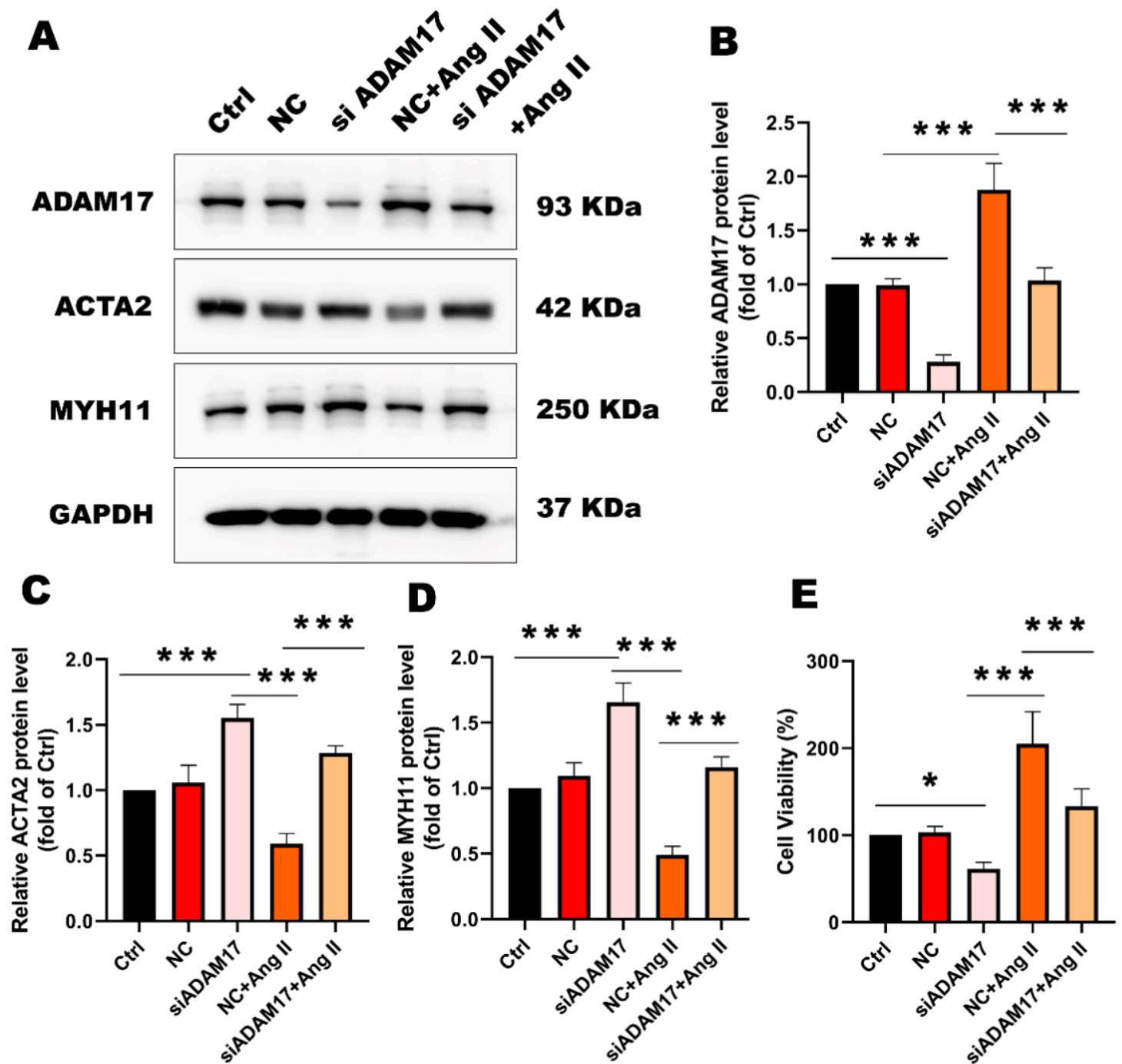


**Figure 2**

Genetic disruption of ADAM17 inhibited Ang II-induced VSMC phenotypic transition. After being transfected with 100 nmol/l ADAM17 siRNA for 30 hours, VSMCs were then treated with 100 nmol/l Ang II for 24 hours. A: Representative photograph of western blotting; B: ADAM17 protein level; C-D: The protein levels of contractile markers ACTA2 (C) and MYH11 (D); E: Cell viability.  $n = 4$  independent experiments. Ang II: angiotensin II; NC: negative control. \* $p < 0.05$ , \*\*\* $p < 0.001$ .

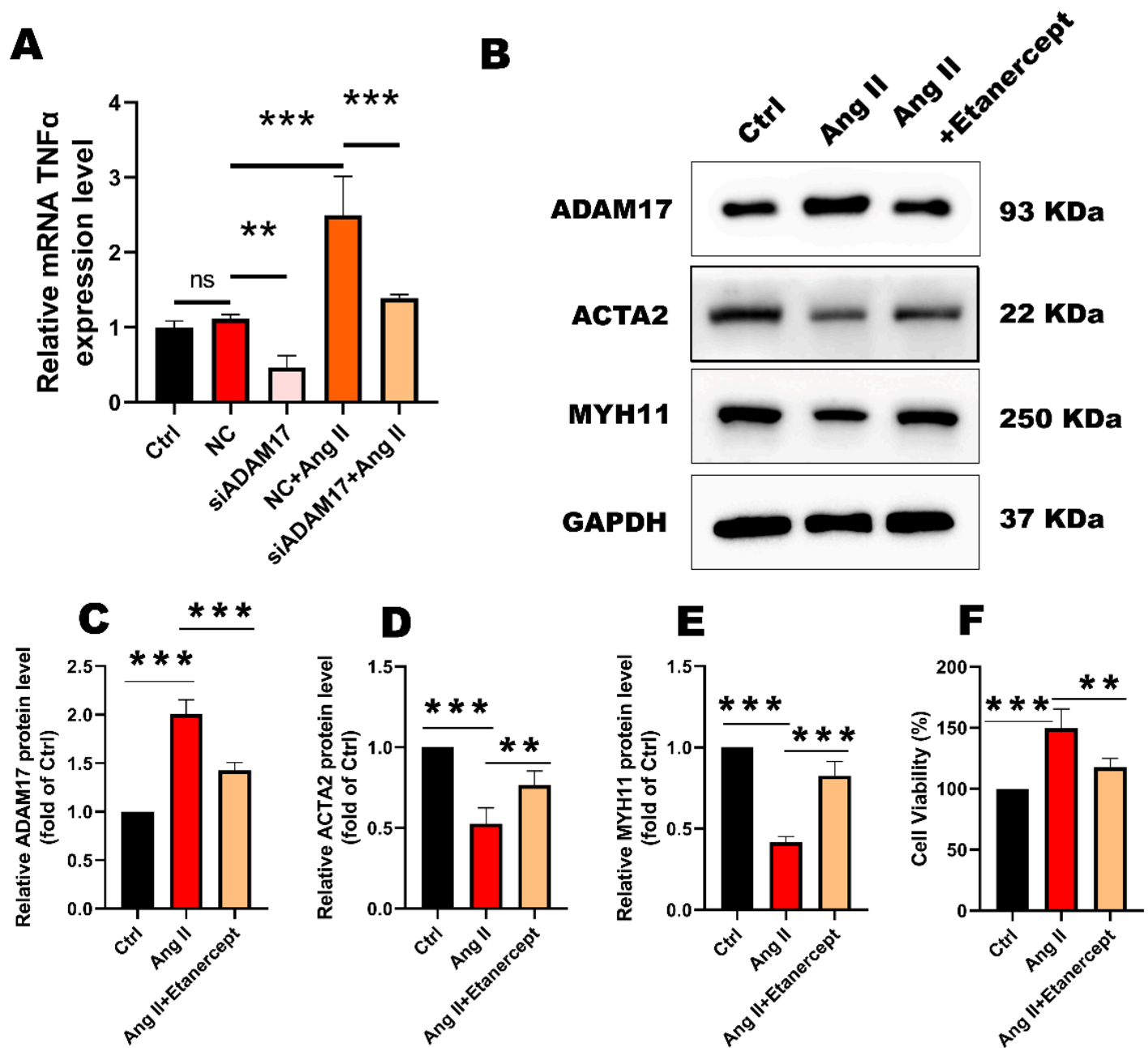


**Figure 3**

ADAM17-TNF axis mediated Ang II-induced VSMC phenotype transition. A: After being transfected with 100 nmol/L ADAM17 siRNA for 30 hours, VSMCs were then treated with 100 nmol/L Ang II for 24 hours. TNF- $\alpha$  mRNA level (A),  $n = 4$  independent experiments. B-F: After being pretreated with 20ng/ml TNF inhibitor etanercept for 30 minutes, VSMCs were then treated with 100 nmol/L Ang II for 24 hours. B: Representative photograph of western blotting; C: ADAM17 protein level; D-E: The protein levels of contractile markers ACTA2 (D) and MYH11 (E); F: Cell viability.  $n = 4$  independent experiments. Ang II: angiotensin II; NC: negative control. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

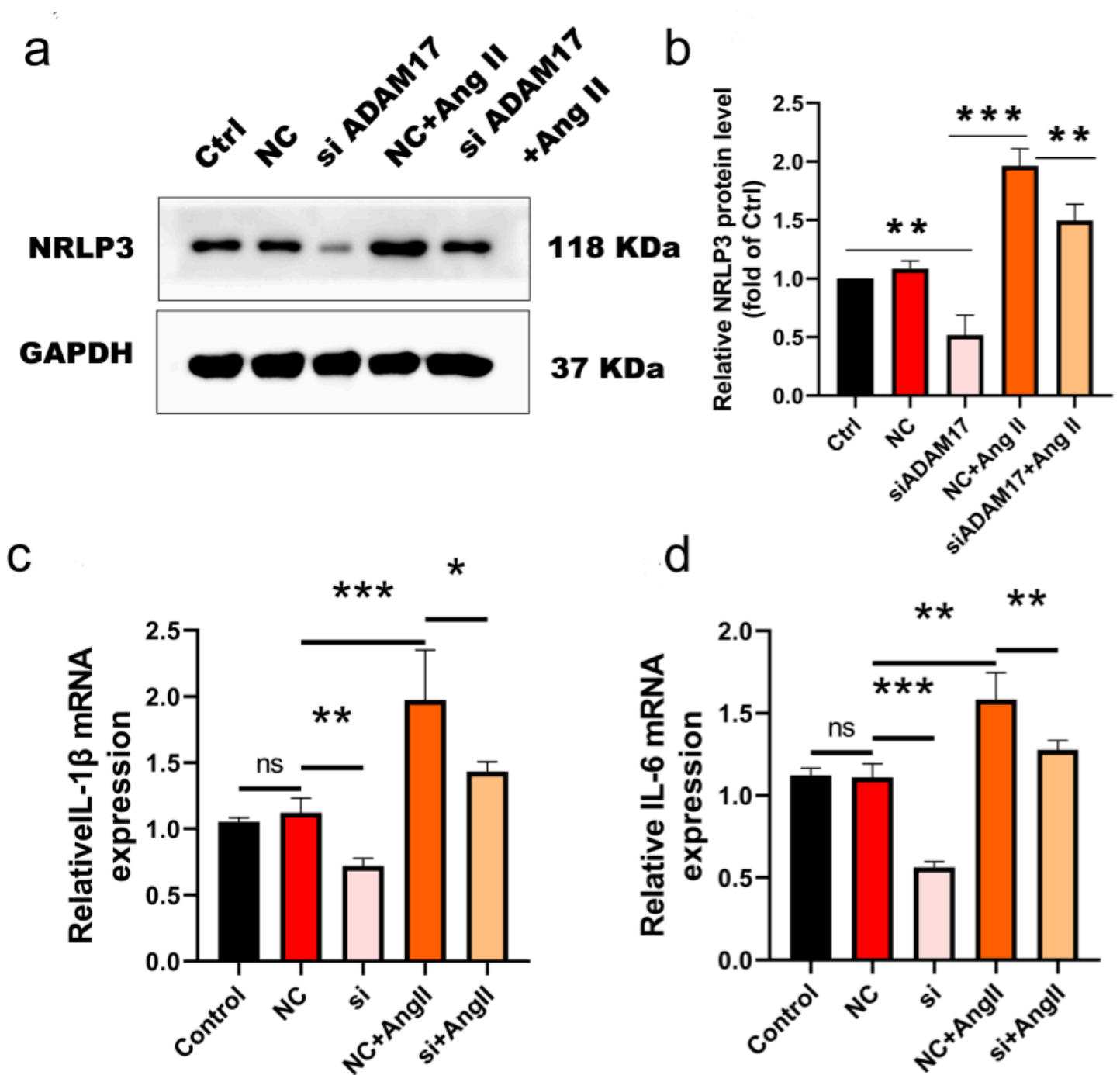


**Figure 4**

ADAM17 siRNA alleviated NLRP3 inflammasome-induced inflammation in Ang II-stimulated VSMCs. After being transfected with 100 nmol/L ADAM17 siRNA for 30 hours, VSMCs were then treated with 100 nmol/L Ang II for 24 hours. A: Representative photograph of western blotting; B: NLRP3 protein level; C-D: The mRNA levels of IL-6 (C) and IL-1β (D).  $n = 4$  independent experiments. Ang II: angiotensin II; NC: negative control. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

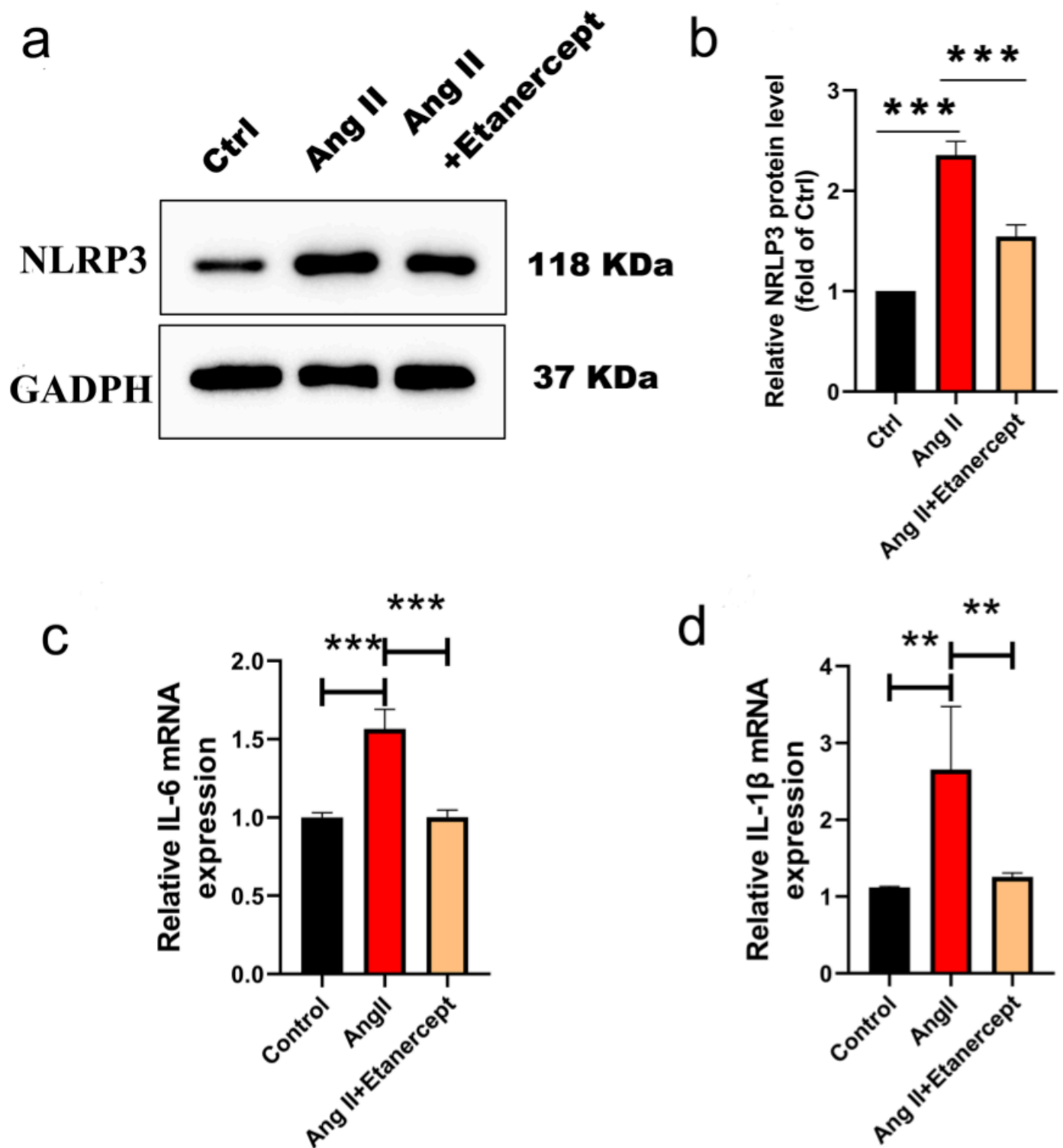


**Figure 5**

The TNF inhibitor etanercept protected against NLRP3 inflammasome- induced inflammation in Ang II-stimulated VSMCs. After pretreatment with 20 ng/ml etanercept for 30 minutes, VSMCs were treated with 100 nmol/L Ang II for 24 hours. A: Representative photograph of western blotting; B: NLRP3 protein level; C-D: The mRNA levels of IL-6 (C), IL-1 $\beta$  (D).  $n = 4$  independent experiments. Ang II: angiotensin II. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

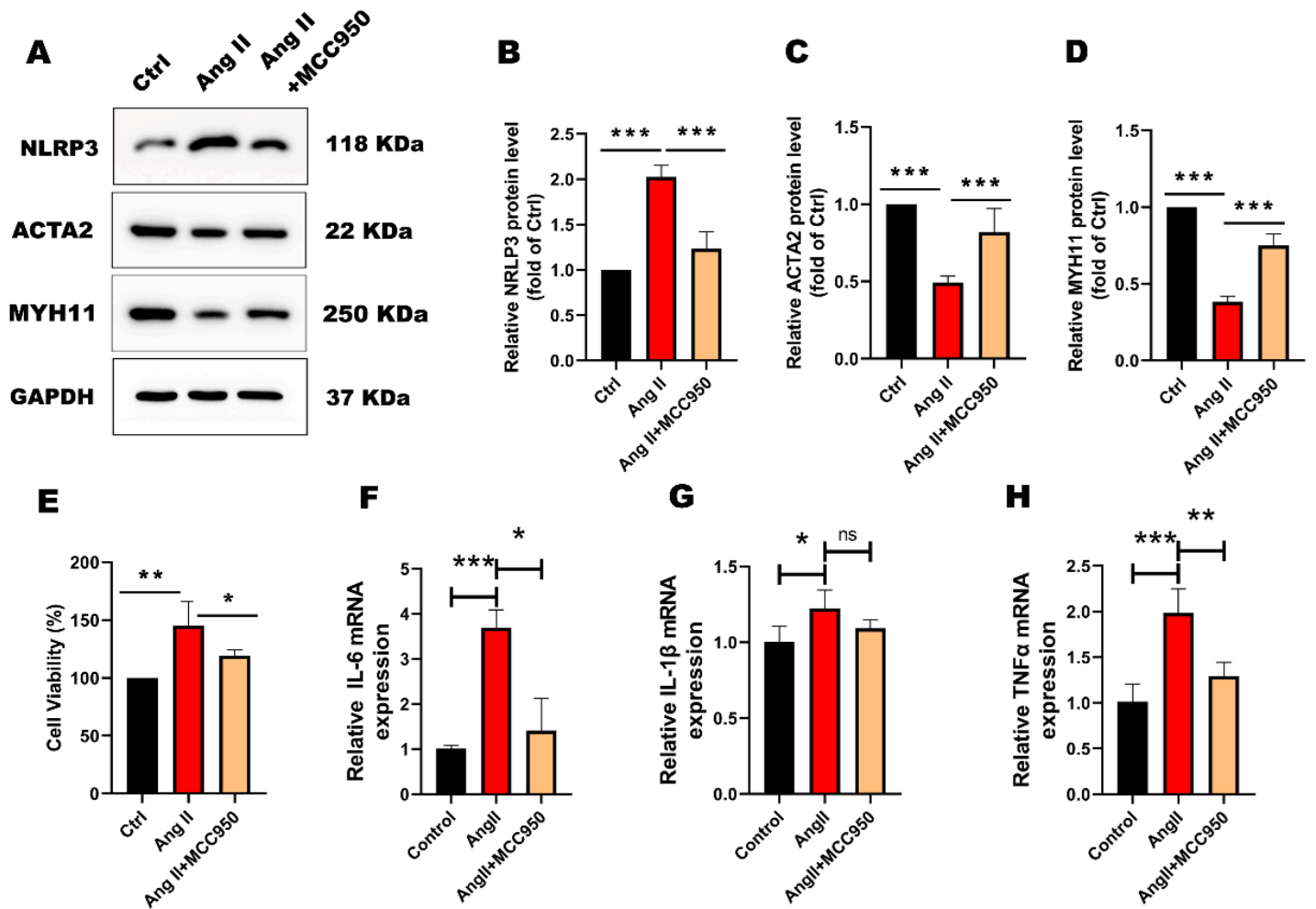


**Figure 6**

Treatment with NLRP3 inflammasome inhibitor MCC950 suppressed Ang II-induced phenotypic transition and inflammation in VSMCs. After pretreatment with 25  $\mu\text{mol/L}$  MCC950 for 30 minutes, VSMCs were treated with 100 nmol/L Ang II for 24 hours. A: Representative photograph of western blotting; B: NLRP3 protein level; C-D: The protein levels of contractile markers ACTA2 (C) and MYH11 (D); E: Cell viability; F-H: The mRNA levels of IL-6 (F), IL-1 $\beta$  (G), and TNF- $\alpha$  (F).  $n = 4$  independent experiments. Ang II: angiotensin II. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

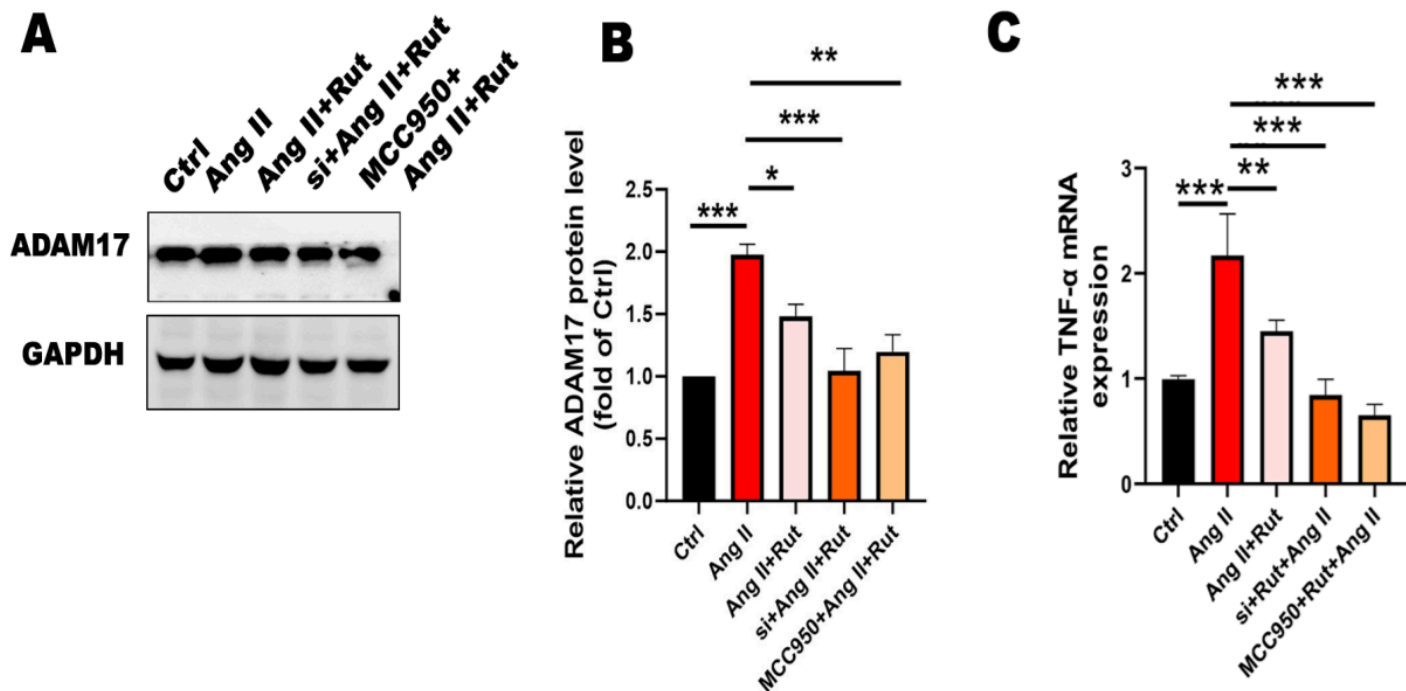


Figure 7

Rutaecarpine inhibited the ADAM17-TNF axis in VSMCs treated with Ang II. After pretreatment with 10  $\mu\text{mol/L}$  rutaecarpine for 30 minutes, VSMCs were treated with 100 nmol/L Ang II for 24 hours. A: Representative photograph of western blot; B: ADAM17 protein level; C: TNF- $\alpha$  mRNA level.  $n = 4$  independent experiments. Ang II: angiotensin II. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

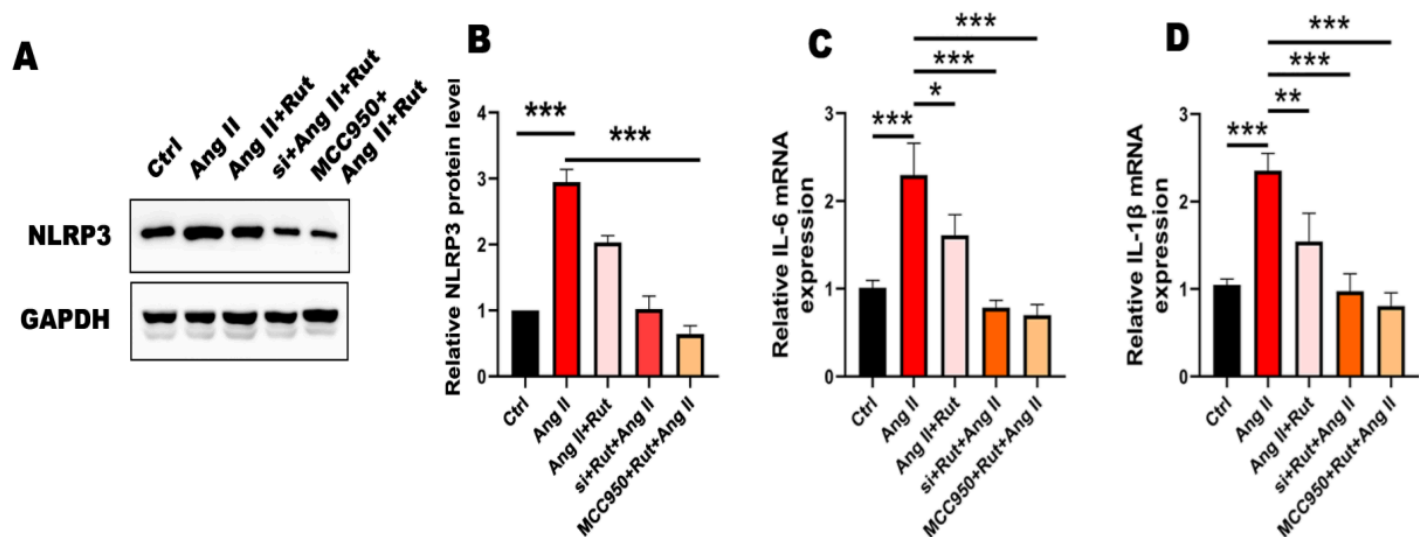


Figure 8

Rutaecarpine inhibited NLRP3 inflammasome-induced inflammation in Ang II-treated VSMCs. After being pretreated with 10  $\mu\text{mol/L}$  rutaecarpine for 30 minutes, VSMCs were then stimulated with 100 nmol/L

Ang II for 24 hours. A: NLRP3 protein level; B: IL-6 mRNA level; C: IL-1 $\beta$  mRNA level.  $n = 4$  independent experiments. Ang II: angiotensin II. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

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