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Quercetin attenuates atherosclerosis via TNF-α/IRF-1-mediated suppression of the NLRP3/caspase-1 pyroptosis pathway

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Abstract

Despite the established anti-atherosclerotic properties of quercetin, the molecular mechanisms underlying its regulation of macrophage pyroptosis, a key driver of plaque instability, remain poorly understood. We investigated whether quercetin suppresses pyroptosis through coordinated inhibition of the tumor necrosis factor alpha/interferon regulatory factor-1 (TNF-α/IRF-1) axis and the NOD-like receptor family pyrin domain-containing 3 (NLRP3)/caspase-1 signaling pathway, two critical mediators of inflammasome activation. Atherosclerosis (AS) was induced in apolipoprotein E knockout mice fed a high-fat diet for 12 weeks, followed by oral administration of quercetin (50 mg/kg/day) or the caspase-1 inhibitor VX-765 (10 mg/kg/day) for 8 weeks. In parallel, THP-1–derived foam cells were treated with 80 μg/mL oxidized low-density lipoprotein (ox-LDL) for 48 h, quercetin (10 μM, administered 24 h after ox-LDL exposure), or VX-765 (10 μM, administered 24 h after ox-LDL exposure). Cardiac function, serum and cell supernatant cytokines (interleukin 1 beta [IL-1β], IL-18, TNF-α, and interferon beta [IFN-β]), and pyroptosis-related proteins (NLRP3, caspase-1, gasdermin D [GSDMD], IRF-1, and TNF-α) were evaluated using echocardiography, histopathology, enzyme-linked immunosorbent assays, immunofluorescence, and immunoblotting. Quercetin significantly improved cardiac function, reduced the aortic plaque area, and decreased serum IL-1β levels by 23.5% ($P < 0.01$) and TNF-α levels by 15.7% ($P < 0.05$). Mechanistically, quercetin inhibited IRF-1 nuclear translocation, suppressed transcriptional activation of caspase-1 and GSDMD, and inhibited NLRP3 inflammasome activation, thereby attenuating caspase-1–dependent GSDMD cleavage. These findings suggest that quercetin may represent a promising flavonoid-based therapeutic strategy for inflammation-driven AS.

Keywords: Quercetin; Atherosclerosis; Pyroptosis; TNF-α/IRF-1 axis; NLRP3 inflammasome.

Introduction

Cardiovascular diseases (CVDs), including heart failure, arrhythmias, hypertension, stroke, and myocardial infarction, remain the leading cause of morbidity and mortality worldwide [1]. Among CVDs, atherosclerosis (AS), a chronic inflammatory disorder characterized by lipid-rich plaque formation and arterial wall remodeling, serves as the primary pathological driver of fatal cardiovascular events, including coronary artery disease and ischemic stroke [2]. Despite the efficacy of lipid-lowering therapies, substantial residual inflammatory risk persists in patients with AS, as evidenced by elevated inflammatory biomarkers, such as high-sensitivity C-reactive protein (CRP), even after optimal low-density lipoprotein (LDL) reduction [3, 4]. This observation highlights the critical and independent role of inflammation in AS progression. Landmark clinical trials, such as the Canakinumab Anti-inflammatory Thrombosis Outcomes Study, have provided definitive proof-of-concept that selectively targeting inflammation, such as through interleukin-1 beta (IL-1β) inhibition with canakinumab, significantly reduces cardiovascular events [5]. However, the complexity of inflammatory signaling within atherosclerotic plaques and the need for therapies that selectively target pathological inflammation while preserving host immunity continue to present significant challenges for clinical translation [4, 6-8].

The progression of AS is driven by maladaptive crosstalk among endothelial cells, vascular smooth muscle cells, and macrophages. Chronic vascular inflammation plays a central role in this process by promoting monocyte recruitment, foam cell formation, and necrotic core expansion [9, 10]. Recent studies have identified macrophage pyroptosis, a lytic and pro-inflammatory form of cell death, as a pivotal mechanism contributing to plaque instability. Unlike apoptosis, pyroptosis is mediated by inflammasome activation (e.g., NOD-like receptor family pyrin domain-containing 3 [NLRP3] inflammasome), caspase-1/4/5/11–dependent cleavage of gasdermin D (GSDMD), and the subsequent release of IL-1β and IL-18, which further exacerbate immune cell infiltration and extracellular matrix degradation [11-14]. Although dysregulated pyroptosis has been implicated in AS progression, the upstream signaling pathways governing this process, particularly those amenable to pharmacological intervention, remain poorly understood.

Interferon regulatory factor 1 (IRF-1), a master transcriptional regulator of inflammatory signaling networks, is activated by STAT1-dependent pathways, including type I interferon signaling [15], and synergizes with IRF-8 to promote macrophage-mediated inflammation [16]. Critically, this tumor necrosis factor alpha (TNF-α)– and interferon-inducible factor has emerged as a key orchestrator of inflammatory responses during AS development. Mechanistically, IRF-1 directly binds to the promoter regions of caspase-1 and GSDMD, thereby enhancing their expression and promoting endothelial and macrophage pyroptosis [17,

18]. The non-canonical nuclear factor kappa B (NF-κB) pathway plays a pivotal role in mediating endothelial pyroptosis during atherogenesis. Specifically, this pathway activates NF-κB–inducing kinase, which facilitates RelB/p52 binding to the IRF-1 promoter, resulting in enhanced IRF-1 expression. Subsequently, IRF-1 directly binds to the promoter regions of caspase-1 and GSDMD, amplifying their transcription and triggering NLRP3 inflammasome–dependent pyroptosis [18]. Clinically, the pathogenic role of IRF-1 has also been demonstrated in acute coronary syndrome (ACS), where IRF-1 overexpression in plaque macrophages correlates with disease severity. Notably, IRF-1 exacerbates macrophage pyroptosis in ACS by suppressing the anti-pyroptotic circRNA_0029589 through METTL3-mediated m6A modification [19]. Collectively, these findings establish IRF-1 as a critical regulatory node linking NF-κB signaling and epigenetic regulation in pyroptosis-mediated plaque instability, emphasizing its therapeutic potential as both a transcriptional and post-transcriptional target. Beyond AS, IRF-1 has been shown to exacerbate tissue damage through pyroptosis in diverse inflammatory diseases, including diabetic nephropathy [20], Crohn's disease [21], multiple sclerosis [22], and diabetic retinopathy [23]. Its involvement in pyroptosis-associated tissue damage across these pathological conditions underscores the potential of IRF-1 as a broad therapeutic target in chronic inflammatory disorders.

Despite these advances, a critical knowledge gap remains. TNF-α is pathologically upregulated in atherosclerotic lesions [24, 25] and serves as a potent transcriptional activator of IRF-1 expression [26]; however, no natural compounds have yet been identified that effectively disrupt the TNF-α/IRF-1 axis to suppress pyroptosis in AS, thereby limiting translational applications.

Quercetin, a natural flavonoid abundant in fruits and vegetables, exhibits multifaceted anti-atherogenic properties through its antioxidant, anti-inflammatory, and plaque-stabilizing activities [27-30]. Emerging evidence also suggests a regulatory role for quercetin in pyroptosis. For instance, quercetin suppresses oxidized LDL (ox-LDL)–induced pyroptosis in THP-1 macrophages by inhibiting NLRP3 inflammasome assembly through nuclear factor erythroid 2-related factor 2–dependent pathways [31]. In apolipoprotein E knockout ($ApoE^{-/-}$) mice, quercetin attenuates AS progression by reducing macrophage pyroptosis and IL-1β secretion [32]. Furthermore, quercetin alleviates neurotoxicity and intestinal inflammation by targeting reactive oxygen species/ mitogen-activated protein kinase (MAPK)/NF-κB signaling [33, 34], suggesting broader anti-inflammatory effects mediated through multiple pathways. However, given the critical role of the TNF-α/IRF-1 axis in inflammasome activation and pyroptosis, whether quercetin exerts its anti-pyroptotic effects through this pathway remains unknown. This knowledge gap hinders the development of flavonoid-based therapeutic strategies targeting both NLRP3 and IRF-1 pathways.

In the present study, we investigated the protective effects of quercetin against macrophage pyroptosis in AS and elucidated its mechanism through dual inhibition of the TNF-α/IRF-1 axis and the NLRP3 inflammasome. By integrating both in vivo (ApoE$^{-/-}$ mice) and in vitro (ox-LDL–induced foam cells) models, we demonstrated that quercetin disrupts IRF-1 nuclear translocation, suppresses caspase-1/GSDMD activation, and attenuates vascular inflammation. The mechanistic scheme is illustrated in Figure 1.These findings not only broaden the current understanding of quercetin-mediated anti-inflammatory actions but also propose a novel therapeutic paradigm for AS by targeting pyroptosis through natural product–derived interventions.

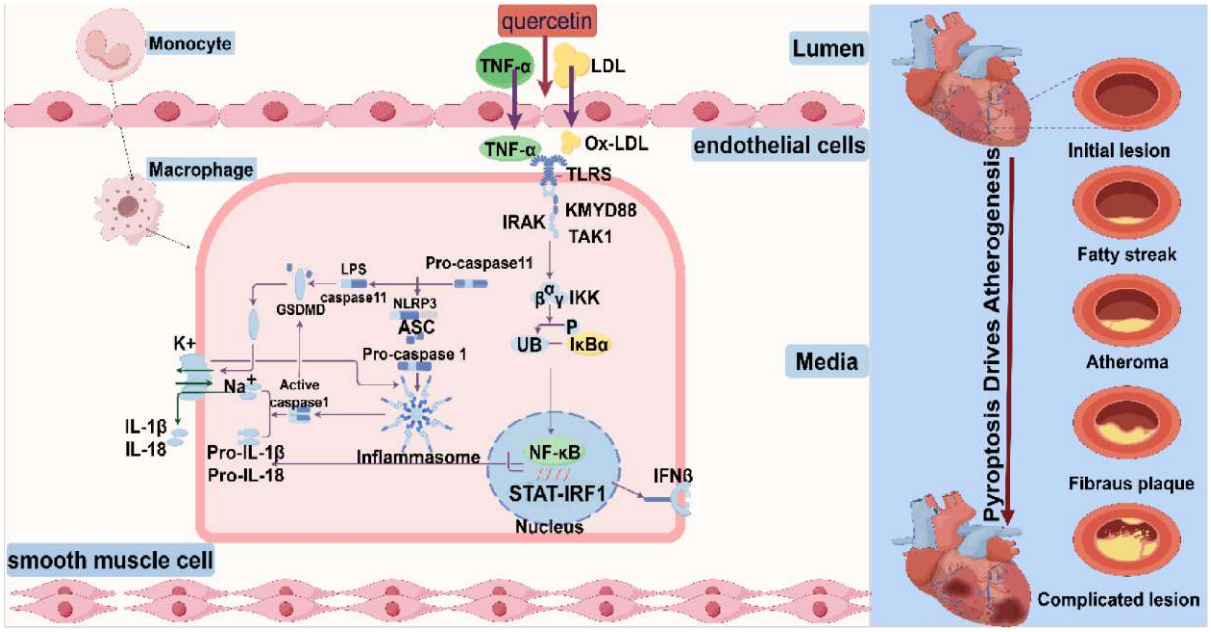


Fig. 1 Proposed mechanism of quercetin in suppressing ox-LDL-induced atherosclerosis

A high-fat diet increases LDL levels, which undergo oxidation within the vascular wall to generate ox-LDL. ox-LDL promotes macrophage activation and stimulates the secretion of TNF-α and IFN-β, which synergistically activate IRF-1. IRF-1 facilitates NLRP3 inflammasome assembly and promotes the transcription of caspase-1 and GSDMD. Activated caspase-1 subsequently cleaves GSDMD, triggering pyroptosis and inducing the release of IL-1β and IL-18, thereby exacerbating plaque instability. Quercetin exerts dual anti-pyroptotic effects through the (1) inhibition of TNF-α/IRF-1 signaling via blockade of IRF-1 nuclear translocation and (2) direct suppression of NLRP3 inflammasome activation. Collectively, these effects attenuate pyroptosis and promote atherosclerotic plaque stabilization.

Abbreviations: ox-LDL, low-density lipoprotein; NLRP3, NOD-like receptor family pyrin domain-containing 3; GSDMD, gasdermin D; IL, interleukin; TNF-α, tumor necrosis factor alpha; TLRS, toll-like receptors; NF-κb, nuclear factor kappa B; IRF-1, interferon regulatory factor-1; IFN-β, interferon beta; UB,; γ-IKK,.

Materials and methods

Animal experiments

Animals and diets

A total of 45 male ApoE$^{-/-}$ mice weighing (age: 5–6 weeks; weight: 17 ± 2 g; SCXK [Jing] 2021-0006) and 15 male C57BL/6 mice (age: 5–8 weeks; weight: 20 ± 3 g; SYXK [Xin] 2018-0003) were obtained from the Vital River Laboratory (Beijing) and Xinjiang Medical University Animal Center, respectively. After 1 week of acclimatization in specific pathogen-free facilities maintained at 22 ± 1 °C and 50–60% relative humidity, mice were assigned to the following groups: control group (C57BL/6) fed a standard chow diet (10% kcal from fat; D12450J, Research Diets) and an AS model group (ApoE$^{-/-}$) fed a high-fat diet (21% lard and 0.15% cholesterol; D12108C, Research Diets) for 12 weeks.

To validate the establishment of the AS model, serum lipid profiles, including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), and LDL cholesterol (LDL-C), were analyzed in randomly selected mice (n = 3 per group) after 12 weeks of dietary intervention using an automated biochemistry analyzer (Hitachi 7020). In addition, hematoxylin and eosin (H&E) staining (Biosharp; BL-700B) of the aortic arch section was performed to histologically assess atherosclerotic plaque formation.

Treatment groups

ApoE$^{-/-}$ mice (n = 36) were randomized into three groups (n = 12/group):

1. Model group: Oral gavage of distilled water (0.2 mL/10 g body weight/day).
2. Quercetin group: Quercetin treatment at 50 mg/kg/day (Sigma-Aldrich, Q4951). The dose was calculated using human-to-mouse conversion (9.1 times the typical adult clinical dose).
3. VX-765 group: Caspase-1 inhibitor treatment with VX-765 (ABMole, M3532) at 10 mg/kg/day.

Treatments were administered once daily for 8 weeks, with weekly dose adjustments based on weekly body weight measurements.

Tissue collection and analysis

Mice were anesthetized with 2% isoflurane (RWD Life Science) and euthanized via cervical dislocation. Blood samples were collected via cardiac puncture and centrifuged at 3,000 × g at 4 °C for 10 min to isolate serum. Aortic tissues were dissected and processed as follows:

- Aortic arch/thoracic aorta: Fixed in 4% paraformaldehyde (Beijing Lanjieke, BL539A) for 24 h.

- Abdominal aorta: Snap-frozen in liquid nitrogen for subsequent protein analysis.

Histopathological staining

En face Oil Red O staining

Excised aortas were longitudinally opened, rinsed in saline, fixed in 4% paraformaldehyde for 10 min at room temperature, and stained with 0.3% Oil Red O (Sigma #1320-06-5) for 10 min. Following destaining in 75% ethanol, tissues were mounted on moistened black paper for imaging. The percentage of the aortic arch plaque area (%) was calculated using ImageJ software (National Institutes of Health) as follows:

Plaque area (%) = (Oil Red O-positive area / total aortic area) × 100.

H&E and Masson's trichrome staining

Paraffin-embedded sections (thickness: 5 μm) were stained with H&E (Biosharp; BL-700B) or Masson's trichrome kits (Solarbio; G1340).

Immunohistochemistry

Tissue sections were subjected to antigen retrieval using 10 mM citrate buffer (pH 6.0) at 95 °C for 20 min, followed by blocking of endogenous peroxidase activity using 3% H_2O_2. Sections were incubated overnight at 4 °C with the following primary antibodies: NLRP3 (Abcam, ab270449; 1:200), caspase-1 (Abcam, ab138483; 1:200), and GSDMD (Abcam, ab219800; 1:200). Horseradish peroxidase (HRP)–conjugated secondary antibodies (ZSGB-BIO, PV6001; 1:500) and DAB substrate (Abcam, ab64238) were applied for visualization. Images were captured using a Nikon Eclipse E100 microscope.

Western blotting

Frozen aortic tissues were homogenized in RIPA lysis buffer (Solarbio; R0010) supplemented with a protease inhibitor cocktail. Protein concentrations were determined using a bicinchoninic acid assay kit (Solarbio; PC0020). Equal amounts of protein (25 μg/lane) were separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels (Solarbio; P1200) and transferred onto polyvinylidene fluoride membranes (Millipore). The membranes were incubated with the following primary antibodies: NLRP3 (1:1000; Abcam; Ab270449), caspase-1 (1:1000; Abcam; Ab138483), GSDMD (1:1000; Abcam; Ab219800), TNF-α (CST, #11948; 1:1000), IRF-1 (CST, #8478; 1:1000), and Glyceraldehyde-3-phosphate dehydrogenase

(GAPDH; Proteintech, 60004-1-Ig; 1:5000). Subsequently, membranes were incubated with HRP-conjugated anti-rabbit/mouse IgG secondary antibodies (ZSGB-BIO; 1:5000). Protein bands were visualized using enhanced chemiluminescence reagent (Beijing Lanjieke; BL523A) and quantified using Image Lab software, version 6.1 (Bio-Rad).

Serum analysis

Lipid profiling

TC, TG, HDL-C, and LDL-C levels were measured enzymatically using an automated biochemical analyzer (Hitachi 7020).

Cytokine quantification

Serum cytokine levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Mouse cytokines included IL-1β (Shanghai Youxuan; YX-E28912), IL-18 (Shanghai Youxuan; YX-E21218), TNF-α (Shanghai Youxuan; YX-E20220), and IFN-β (Shanghai Youxuan; YX-E20334). Human cytokines included IL-1β (Shanghai Youxuan; YX-E10083) and IL-18 (Shanghai Youxuan; YX-E10092).

Cell experiments

THP-1 macrophage differentiation

THP-1 monocytes (Procel; CL-0233) were cultured in RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco). Differentiation into macrophages was induced by treatment with 100 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma; P1585) for 48 h and was confirmed by CD68 immunofluorescence staining (Abcam; ab213363).

Foam cell induction

Macrophages were incubated with 80 μg/mL ox-LDL (Guangzhou Yiyuan; YB-002) for 48 h to induce foam cell formation. Lipid accumulation was confirmed by Oil Red O staining.

Treatment groups

Cells were assigned to the following treatment groups:

- Control group: RPMI-1640 medium alone.

- ox-LDL group: treatment with 80 μg/mL ox-LDL for 48 h.
- ox-LDL + Quercetin group: Treatment with 10 μM quercetin administered 24 h after ox-LDL exposure (Supplementary Fig. S1).
- ox-LDL + VX-765: Treatment with 10 μM VX-765 (ABMole; M3532) administered 24 h after ox-LDL exposure (Supplementary Fig. S2).

Cell viability and death assays

Cell viability was assessed using a Cell Counting Kit-8 (CCK-8) assay (Biosharp; BA00208) at 12, 24, and 48 h following treatment. Pyroptosis was quantified using Hoechst 33342/propidium iodide (PI) dual staining with a commercially available kit (Solarbio; CA1120).

Statistical analysis

Data are expressed as mean ± standard deviation. Data normality was assessed using the Shapiro–Wilk test. Comparisons among multiple groups were performed using one-way analysis of variance with Tukey's post hoc test (GraphPad Prism 8). Nonparametric data were analyzed using the Kruskal–Wallis test. A P-value < 0.05 was considered statistically significant.

Results

Systemic manifestations of the AS model

ApoE$^{-/-}$ mice fed a high-fat diet for 12 weeks exhibited significant phenotypic alterations. Body weight increased from 15 ± 2 g to 35 ± 3 g, and fur texture became oily and glossy (Fig. 2B). The survival rate was 85%, with partial mortality attributed to feed intolerance. These findings confirmed successful induction of metabolic disturbances.

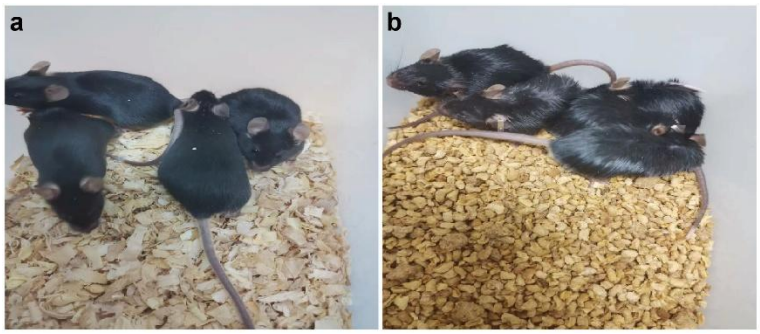


Fig. 2 Phenotypic characterization of ApoE$^{-/-}$ mice after a 12-week high-fat diet intervention

(A) Control group (standard chow diet). (B) Model group (high-fat diet). Representative image showing oily/glistening fur texture (yellow arrow) and increased adiposity.

Validation of the atherogenic phenotype was further confirmed through biochemical and histological analyses conducted at week 12. Dyslipidemia was evident, with serum TC and LDL-C levels increasing by 8.9-fold and 49.2-fold, respectively ($P < 0.01$ vs. control), whereas HDL-C levels decreased by 19.9% ($P < 0.05$; Fig. 3A). Histological examination using HE staining revealed foam cell–rich atheromatous lesions within the aortic walls of the model group, whereas no such lesions were observed in the control group (Figs. 3B and 3C).

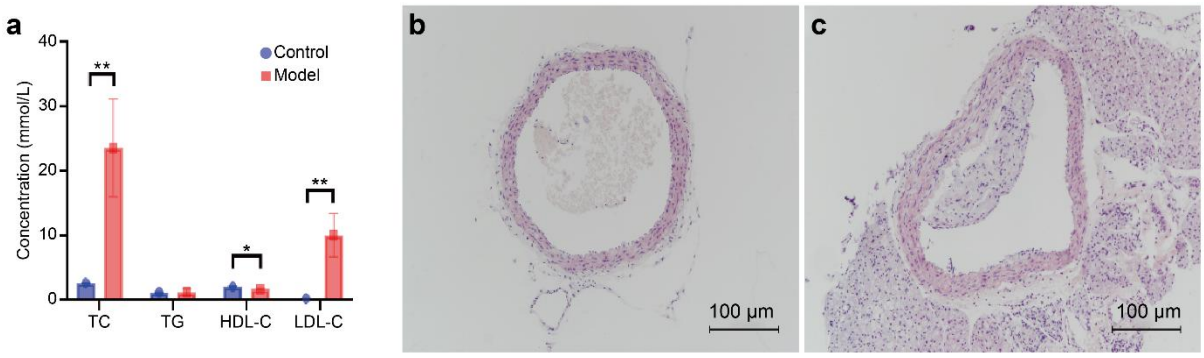


Fig. 3 Validation of the atherogenic phenotype: Dyslipidemia and aortic plaque formation in ApoE$^{-/-}$ mice following a 12-week high-fat diet intervention

(A) Serum lipid profiles showing total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels (n = 3/group). (B) Representative image of normal aortic architecture in the control group (hematoxylin and eosin [H&E] staining, ×100 magnification). (C) Representative image of atheromatous plaques with foam cell infiltration in the model group (H&E staining, ×100 magnification).

Quercetin improves lipid metabolism and cardiac function in AS mice

To evaluate the systemic effects of quercetin on AS progression, we first analyzed serum lipid profiles and cardiac function in ApoE$^{-/-}$ mice following 8 weeks of treatment. Compared with the control group, the model group exhibited marked dyslipidemia characterized by elevated levels of TC ($P < 0.01$), TGs ($P < 0.05$), and LDL-C ($P < 0.01$), accompanied by decreased HDL-C levels ($P < 0.01$) (Fig. 4). Notably, quercetin treatment attenuated these lipid abnormalities, resulting in reductions in TC, TG, and LDL-C levels; however, these changes did not reach statistical significance ($P > 0.05$).

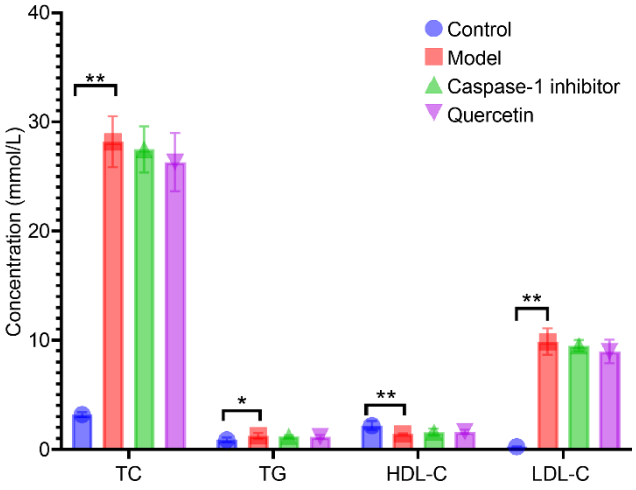


Fig. 4 Quercetin modulates serum lipid profiles in atherosclerotic ApoE$^{-/-}$ mice

Abbreviations: TC, total cholesterol; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

To further evaluate the impact of AS on cardiac performance, echocardiographic assessment demonstrated impaired cardiac function in the model group, including increased left ventricular internal diameter at end-systole ($P < 0.05$) and left ventricular end-systolic volume ($P < 0.05$), together with reduced ejection fraction ($P < 0.01$) and fractional shortening ($P < 0.01$). Importantly, quercetin treatment partially restored cardiac function, as evidenced by improved left ventricular posterior wall thickness at end-systole ($P < 0.05$) (Figs. 5 and 6). These findings suggest that quercetin ameliorates AS-associated cardiac dysfunction, potentially through mechanisms distinct from those of caspase-1 inhibition.

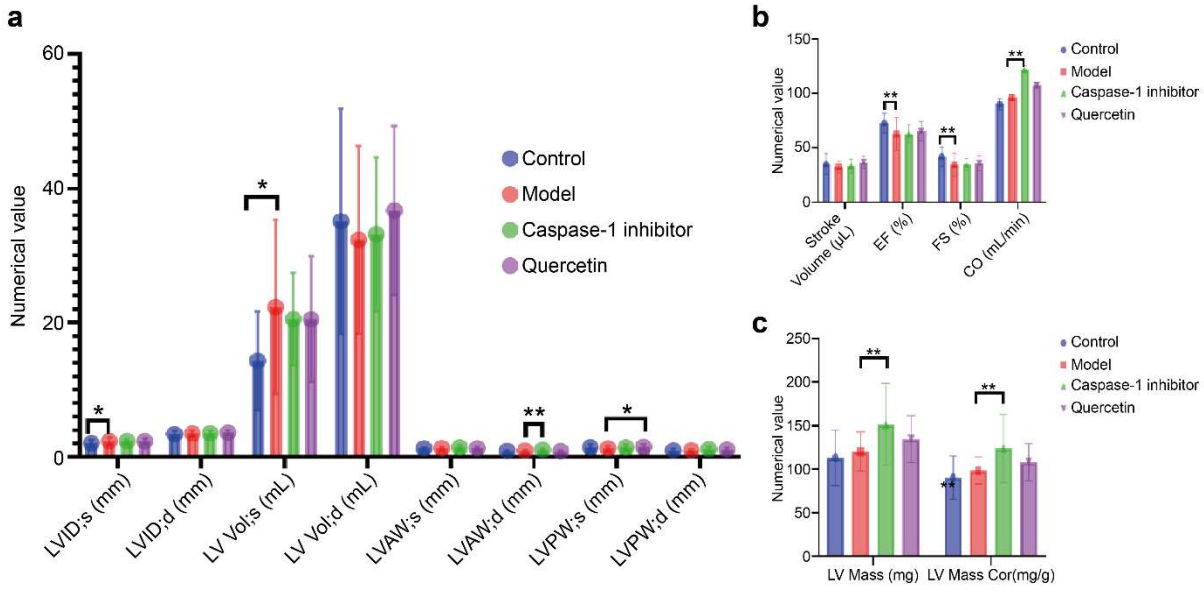


Fig. 5 Quercetin improves cardiac structure, function, and mass parameters in atherosclerotic mice

(A) Cardiac structural parameters. (B) Cardiac functional parameters. (C) Cardiac mass parameters. Abbreviations: LVID; left ventricular internal diameter; LV Vol; left ventricular volume; EF; ejection fraction; FS; fractional shortening; CO; cardiac output; LV Mass Cor; left ventricular mass corrected for body weight; LVAW; left ventricular anterior wall; LVPW; left ventricular posterior wall.

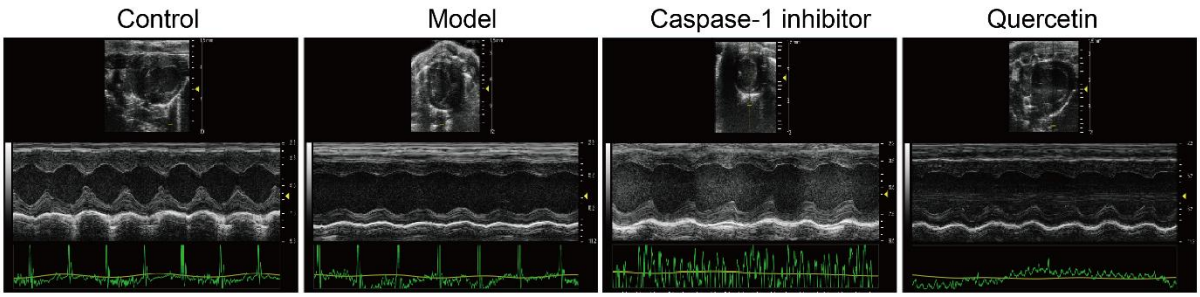


Fig. 6 Comparison of cardiac function among the experimental groups

Quercetin attenuates atherosclerotic plaque formation and inflammation

Oil Red O staining of aortic arches demonstrated significant differences in lipid deposition. The model group exhibited markedly increased lipid plaque area ($P < 0.01$), whereas quercetin treatment significantly reduced lipid accumulation ($P < 0.05$). These quantitative findings further support the plaque-stabilizing effects of quercetin (Representative images shown in Fig. 7).

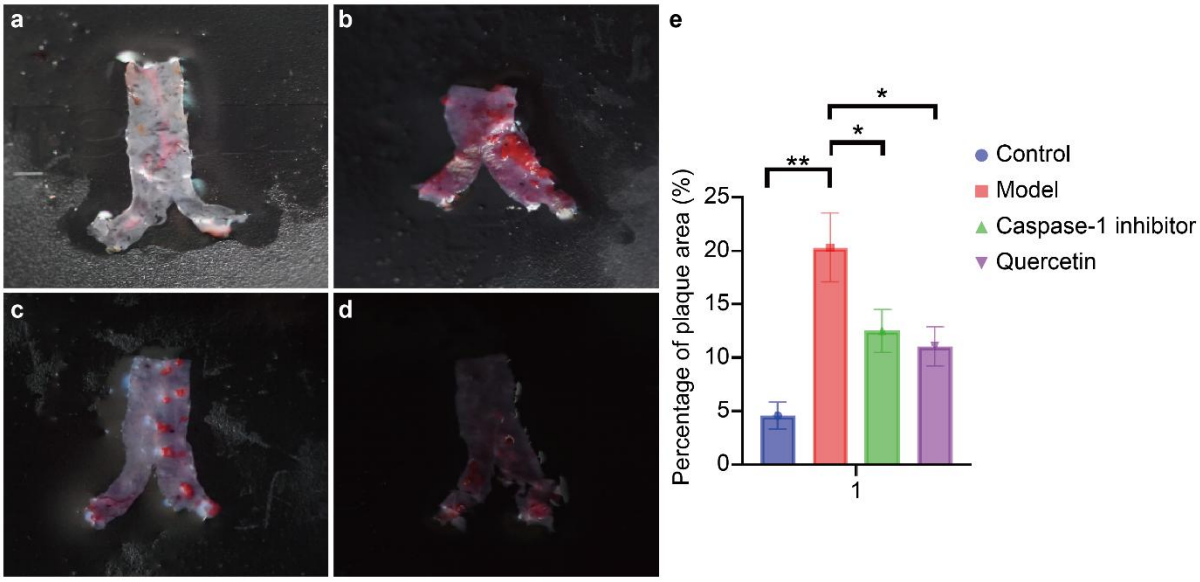


Fig. 7 Quercetin attenuates lipid deposition in the aortic arch in atherosclerotic mice

(A) Control group. (B) Model group. (C) Caspase-1 inhibitor group. (D) Quercetin group. (E) Quantified plaque area (%).

Histopathological analysis using H&E and Masson's trichrome staining demonstrated extensive lipid deposition and disrupted collagen architecture within aortic plaques in the model group. By contrast, quercetin-treated mice exhibited a significant reduction in the plaque area and improved collagen fiber organization (Fig. 8), indicating enhanced plaque stability.

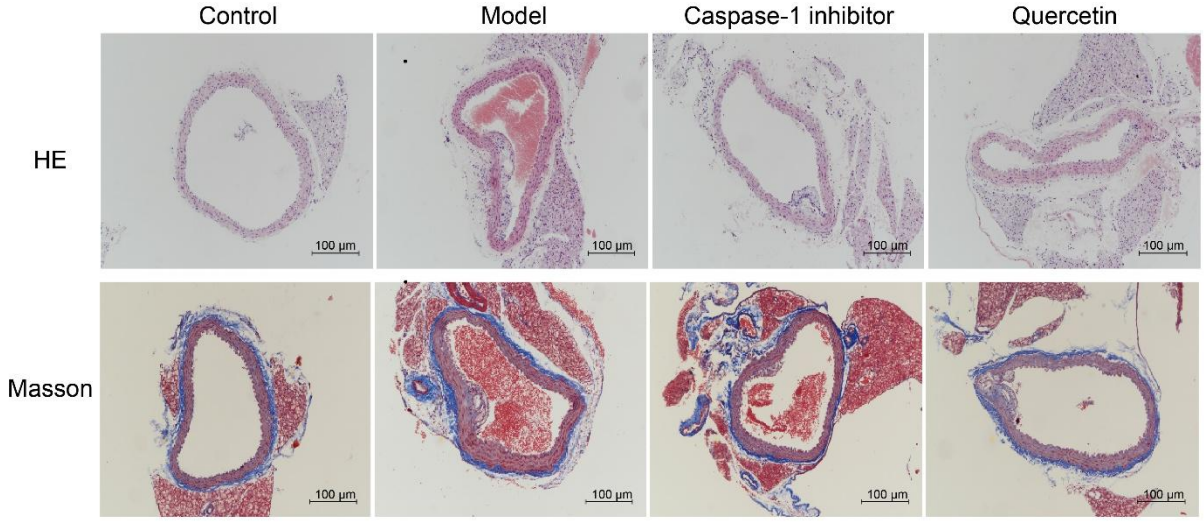


Fig. 8 Representative images of hematoxylin and eosin (H&E) and Masson's trichrome staining (×100 magnification)

To further validate these histopathological findings, serum levels of pyroptosis-related cytokines were measured. Compared with the control group, the model group exhibited elevated IL-1β (14.18 ± 4.32 pg/mL vs. 4.13 ± 0.98 pg/mL, $P < 0.01$) and IL-18 levels (41.18 ± 6.14 pg/mL vs. 20.31 ± 2.85 pg/mL, $P < 0.01$). Quercetin treatment significantly attenuated these increases, reducing IL-1β levels by 23.5% ($P < 0.05$) and IL-18 levels by 29.8% ($P < 0.01$) (Fig. 9).

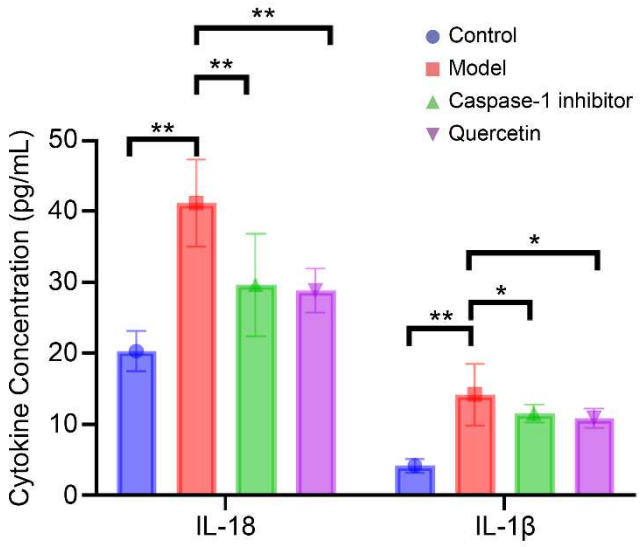


Fig. 9 Quercetin suppresses the production of serum pyroptosis-related cytokines in atherosclerotic ApoE$^{-/-}$ mice

Abbreviations: IL, interleukin

Furthermore, quercetin treatment alleviated systemic inflammation by modulating key leukocyte populations. The total leukocyte count was significantly elevated in the model group (1.43 ± 0.87 ×10⁹/L vs. 0.59 ± 0.28 ×10⁹/L; $P < 0.05$ vs. control) and was reduced following quercetin treatment (0.86 ± 0.34 ×10⁹/L; $P < 0.05$ vs. model), corresponding to a 39.9% reduction. The neutrophil ratio, a key indicator of systemic inflammatory burden, was markedly increased in atherosclerotic mice (66.02 ± 4.19% vs 26.76 ± 3.49% in controls; $P < 0.01$) but was significantly attenuated following quercetin treatment (43.75 ± 6.72%; $P < 0.05$ vs. model). In addition, monocyte counts, which contribute to macrophage infiltration within plaques, were significantly reduced by quercetin treatment (0.06 ± 0.02 ×10⁹/L vs. 0.11 ± 0.07 ×10⁹/L in the model group; $P < 0.05$). Collectively, these coordinated improvements in leukocyte parameters suggest that quercetin attenuates systemic inflammation and promotes stabilization of atherosclerotic plaques (Fig. 10).

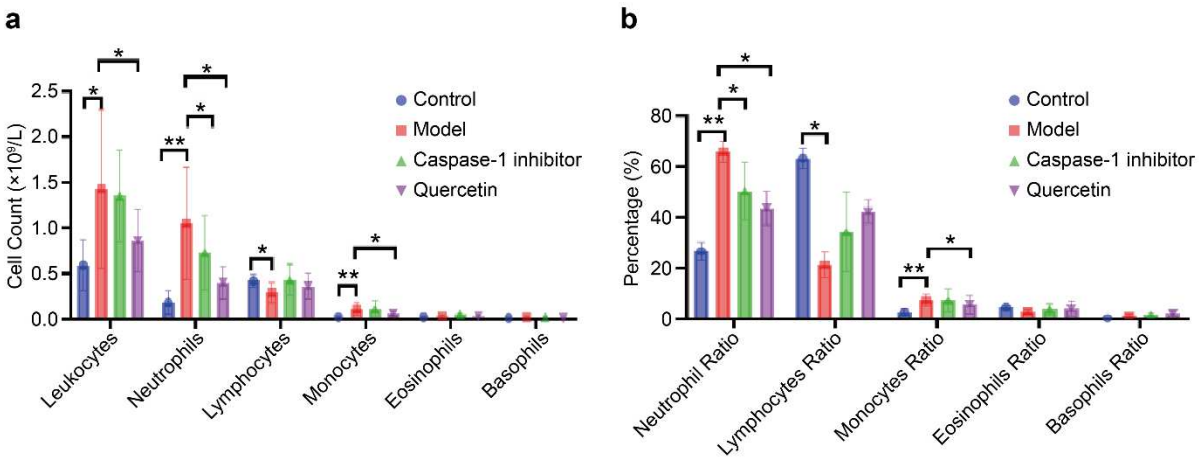


Fig. 10 Quercetin treatment attenuates systemic inflammatory burden in atherosclerotic ApoE$^{-/-}$ mice

(A) Leukocyte counts. (B) Leukocyte ratios.

Quercetin inhibits pyroptosis via the TNF-α/IRF-1 and NLRP3/caspase-1 pathways

To elucidate the molecular mechanisms underlying the anti-pyroptotic effects of quercetin, we systematically examined key components of the TNF-α/IRF-1 axis and NLRP3/caspase-1 signaling pathway. First, immunohistochemical analyses revealed significant upregulation of NLRP3, caspase-1, and GSDMD expression in aortic plaques of the model group ($P < 0.0001$ vs. control), consistent with the activation of pyroptosis signaling. Importantly, quercetin treatment markedly reduced the expression of these proteins to levels comparable to those observed following caspase-1 inhibition (Figs. 11 and 12), suggesting broad regulatory effects on pyroptosis-associated markers.

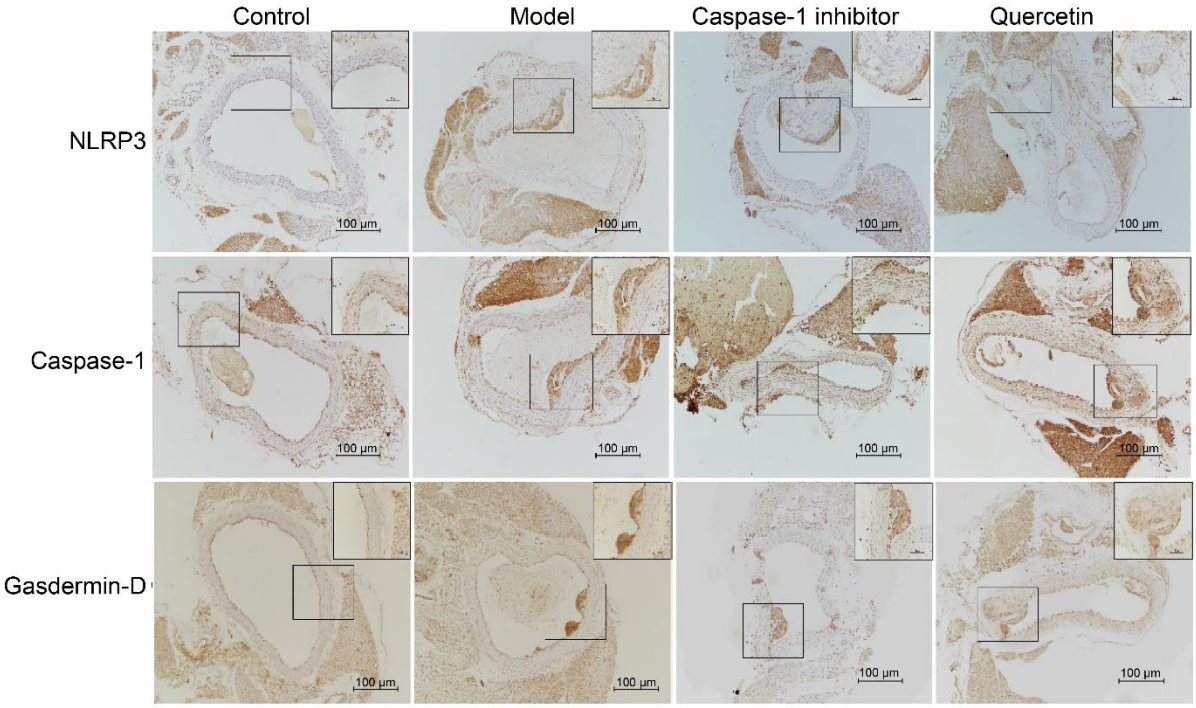


Fig. 11 Quercetin attenuates pyroptosis-related protein expression in atherosclerotic arteries

Immunohistochemical staining of NLRP3, caspase-1, and GSDMD (scale bar: 100 μm).

Abbreviations: NLRP3, NOD-like receptor family pyrin domain-containing 3; GSDMD, gasdermin D.

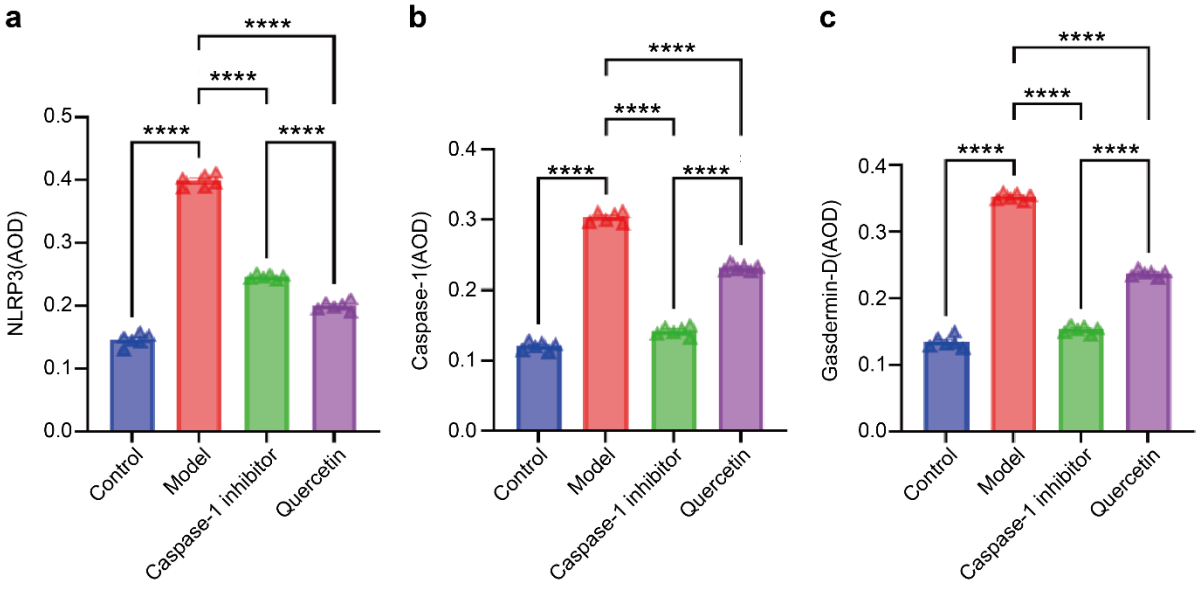


Fig. 12 Quercetin suppresses pyroptosis-related protein activation in atherosclerotic plaques

(A) Quantification of NOD-like receptor family pyrin domain-containing 3 (NLRP3) expression. (B) Quantification of caspase-1 expression. (C) Quantification of gasdermin-D expression.

To further investigate upstream mechanisms contributing to IRF-1 activation, we quantified systemic inflammatory cytokine levels in mouse serum. ELISA analysis demonstrated significantly higher TNF-α (112.86 ± 6.37 pg/mL vs. 82.37 ± 5.49 pg/mL, $P < 0.01$) and IFN-β levels (20.64 ± 1.21 pg/mL vs. 17.32 ± 0.86 pg/mL, $P < 0.01$) in the model group than in the control group. Quercetin treatment significantly reduced TNF-α (95.04 ± 3.34 pg/mL; 15.7% reduction, $P < 0.05$) and IFN-β levels (18.22 ± 1.15 pg/mL; 11.7% reduction, $P < 0.05$), suggesting an association between cytokine suppression and the anti-pyroptotic effects of quercetin (Fig. 13).

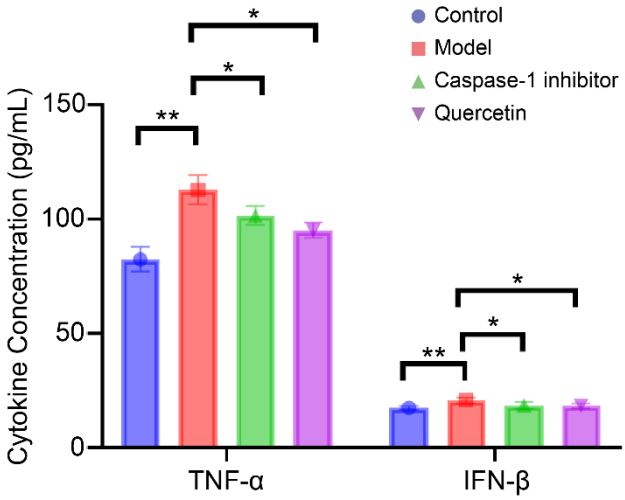


Fig. 13 Quercetin attenuates serum tumor necrosis factor alpha (TNF-α) and interferon beta (IFN-β) levels in atherosclerotic ApoE$^{-/-}$ mice

To mechanistically establish the relationship between cytokine signaling and pyroptosis activation, Western blot analysis was performed. The results demonstrated that quercetin attenuated TNF-α/IFN-β–driven IRF-1 nuclear translocation ($P < 0.01$ vs. model group), thereby suppressing transcriptional activation of caspase-1 and GSDMD (Fig. 14). Simultaneously, quercetin directly inhibited NLRP3 inflammasome assembly, resulting in reduced caspase-1-dependent GSDMD cleavage ($P < 0.01$). These dual inhibitory effects, targeting both upstream cytokine signaling (TNF-α/IFN-β/IRF-1) and downstream inflammasome activation (NLRP3/caspase-1), highlight the unique ability of quercetin to disrupt pyroptosis at transcriptional and post-translational levels.

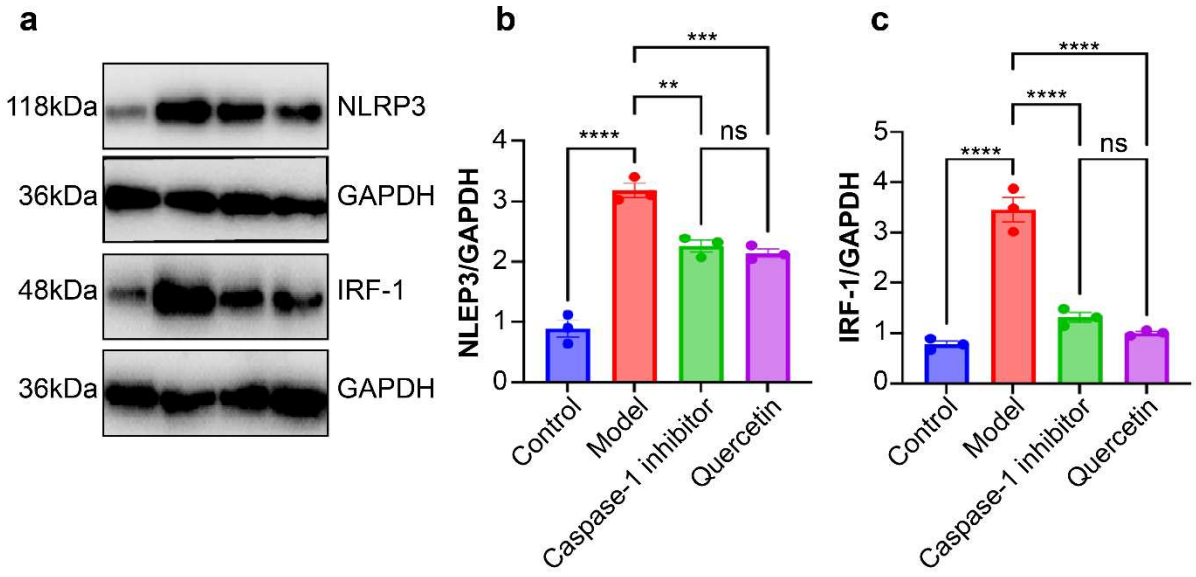


Fig. 14 Quercetin inhibits pyroptosis through dual suppression of IRF-1 nuclear translocation and NLRP3 inflammasome activation

(A) Representative Western blot images of IRF-1 and NLRP3 protein expression, with GAPDH as the loading control. (B) Quantitative analysis of NLRP3 expression normalized to GAPDH. (C) Quantitative analysis of IRF-1 expression normalized to GAPDH.

Abbreviations: IRF-1; interferon regulatory factor-1; NLRP3, NOD-like receptor family pyrin domain-containing 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Notably, although the caspase-1 inhibitor VX-765 also reduced TNF-α and IFN-β levels, its effects were less pronounced than those observed with quercetin treatment (Fig. 13). This finding suggests that the anti-inflammatory effects of quercetin may extend beyond direct caspase-1 inhibition and potentially involve transcriptional modulation of IRF-1 signaling and suppression of pro-inflammatory cytokine production.

Induction of monocyte differentiation and establishment of the foam cell model

The human monocytic cell line THP-1 was utilized in this study. Cell viability at varying cell densities and time points was assessed using the CCK-8 assay. The results demonstrated that cells cultured at a density of 2×10^6 cells/mL exhibited overcrowding under microscopic observation. Comparative analysis of post-induction morphology between this concentration and 1×10^6 cells/mL, together with evidence from previous studies, identified 1×10^6 cells/mL as the optimal density for subsequent experiments (Fig. 15A). Accordingly, 1×10^6 cells/mL was selected as the standard culture density.

To establish a macrophage model, induction conditions were optimized by comparing morphological changes observed at 24 h and 48 h under different serum concentrations and PMA doses. Serum-free medium supplemented with 100 ng/mL PMA for 24 h was identified as the optimal induction condition. Prior to induction, THP-1 cells displayed a round, suspended morphology; however, after induction, cells differentiated into adherent macrophages exhibiting irregular morphologies, including discoid and spindle-like shapes (Fig. 15B–C). Successful differentiation was further confirmed by immunofluorescence staining, which demonstrated positive green fluorescence for the macrophage surface marker CD68 (Fig. 15D–F).

Using this model, foam cells were generated by stimulating differentiated macrophages with 80 μg/mL ox-LDL for 48 h. Oil Red O staining revealed abundant intracellular red-stained lipid droplets in ox-LDL–treated cells (Fig. 15H), whereas untreated macrophages showed minimal lipid accumulation (Fig. 15G), confirming successful establishment of the foam cell model.

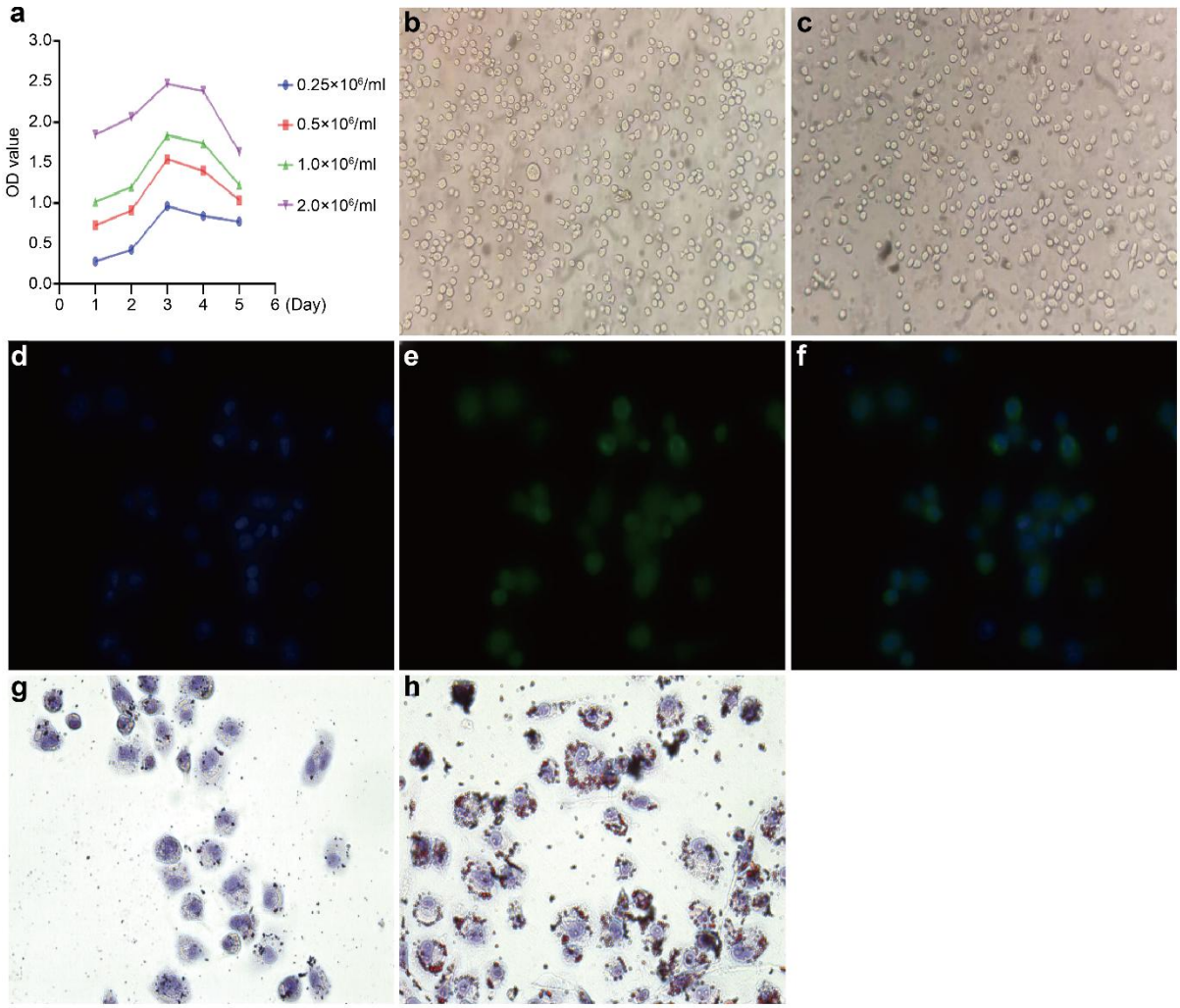


Fig. 15 Optimization of THP-1 monocyte culture, PMA-induced macrophage differentiation, and ox-LDL–induced foam cell formation

(A) Growth curve of THP-1 monocytes determined by the CCK-8 assay. (B) Untreated THP-1 monocytes exhibiting suspended morphology (×200 magnification). (C) Macrophages differentiated with 100 ng/mL PMA under serum-free conditions for 24 h, showing adherent and irregular morphology (×200 magnification). (D–F) Immunofluorescence validation of macrophage differentiation: (D) DAPI nuclear staining (blue), (E) CD68 surface marker staining (green), and (F) merged image confirming CD68-positive macrophages (×400 magnification). (G, H) Oil Red O staining: (G) Control macrophages without detectable lipid droplets and (H) foam cells induced by 80 μg/mL ox-LDL for 48 h, showing intracellular red-stained lipid droplets (×400 magnification).

Abbreviations: PMA, phorbol 12-myristate 13-acetate; CCK-8, Cell Counting Kit-8; DAPI, 4',6-diamidino-2-phenylindole; ox-LDL, oxidized low-density lipoprotein; OD, optical density.

Quercetin attenuates pyroptosis in foam cells via dual pathway inhibition

To validate the translational relevance of the in vivo findings, we replicated AS-associated pyroptosis in ox-LDL–treated THP-1-derived foam cells. First, pyroptotic cell death was initially evaluated using Hoechst 33342/PI dual staining. Compared with the control group, ox-LDL exposure treatment increased the proportion of PI-positive cells, indicating membrane rupture and pyroptotic cell death (Fig. 16). Notably, quercetin treatment significantly reduced the number of PI-positive cells, mirroring the protective effects of the caspase-1 inhibitor VX-765 (Fig. 17). These findings confirm the ability of quercetin to suppress ox-LDL–induced pyroptosis in human-derived foam cells.

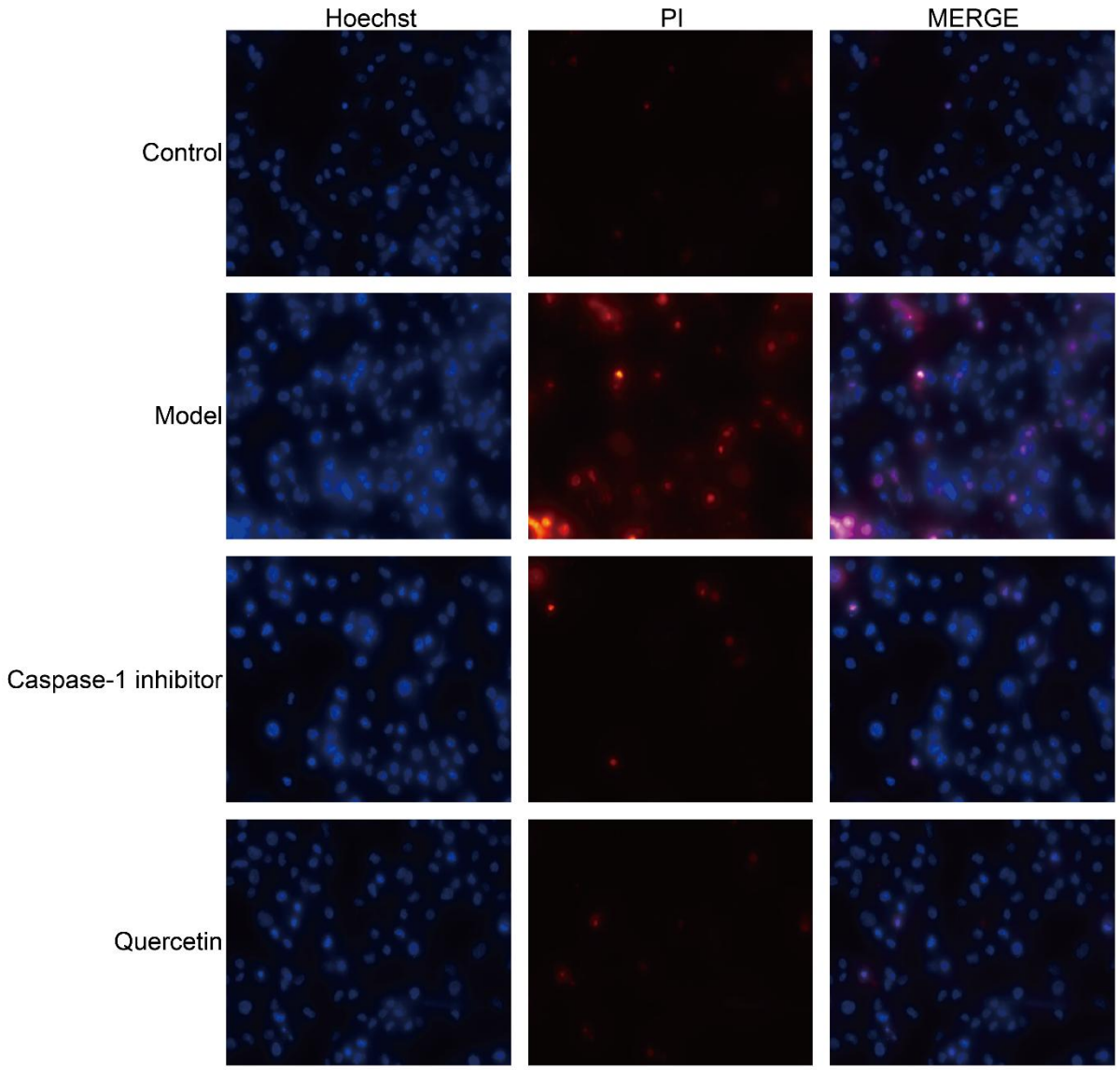


Fig. 16 Quercetin attenuates pyroptotic cell death in ox-LDL (oxidized low-density lipoprotein)–treated foam cells (Hoechst/propidium iodide (PI) staining; scale bar: 50 μm)

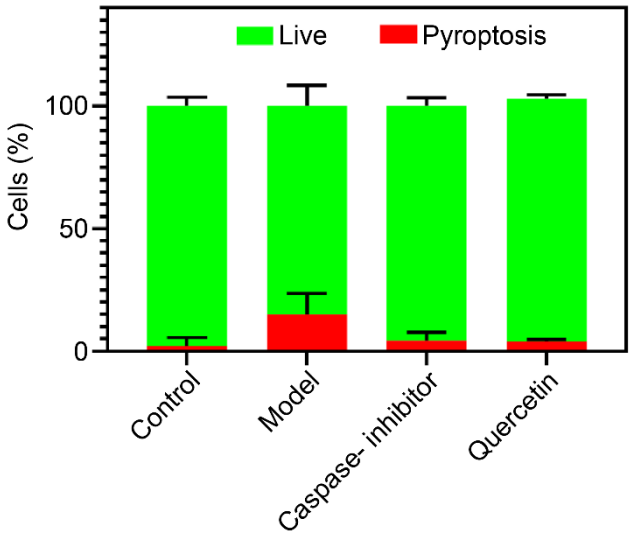


Fig. 17 Quercetin and caspase-1 inhibitor suppresses pyroptosis (quantification of propidium iodide (PI)–positive cells)

To further investigate the molecular mechanisms involved, we analyzed TNF-α and IRF-1 expression by immunofluorescence staining. ox-LDL stimulation significantly upregulated the expression of both TNF-α ($P < 0.001$ vs. control) and nuclear IRF-1 ($P < 0.0001$ vs. control), consistent with activation of pyroptotic signaling pathways. Quercetin treatment significantly reduced TNF-α expression ($P < 0.001$) and inhibited IRF-1 nuclear translocation ($P < 0.0001$), suggesting disruption of the TNF-α/IRF-1 signaling axis (Figs. 18–20).

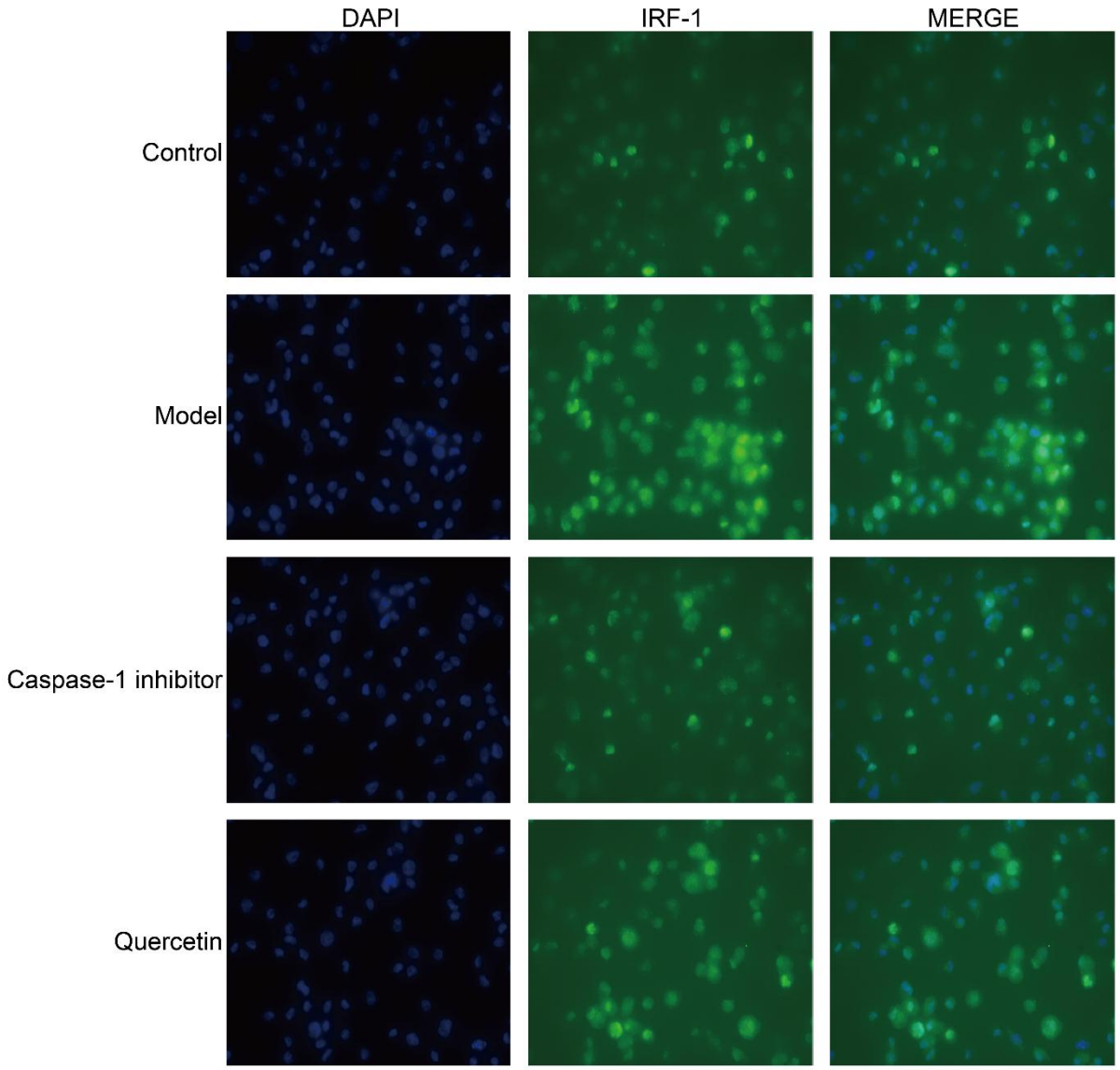


Fig. 18 Quercetin attenuates interferon regulatory factor-1 (IRF-1) activation in foam cells (immunofluorescence staining of IRF-1; scale bar: 50 μm)

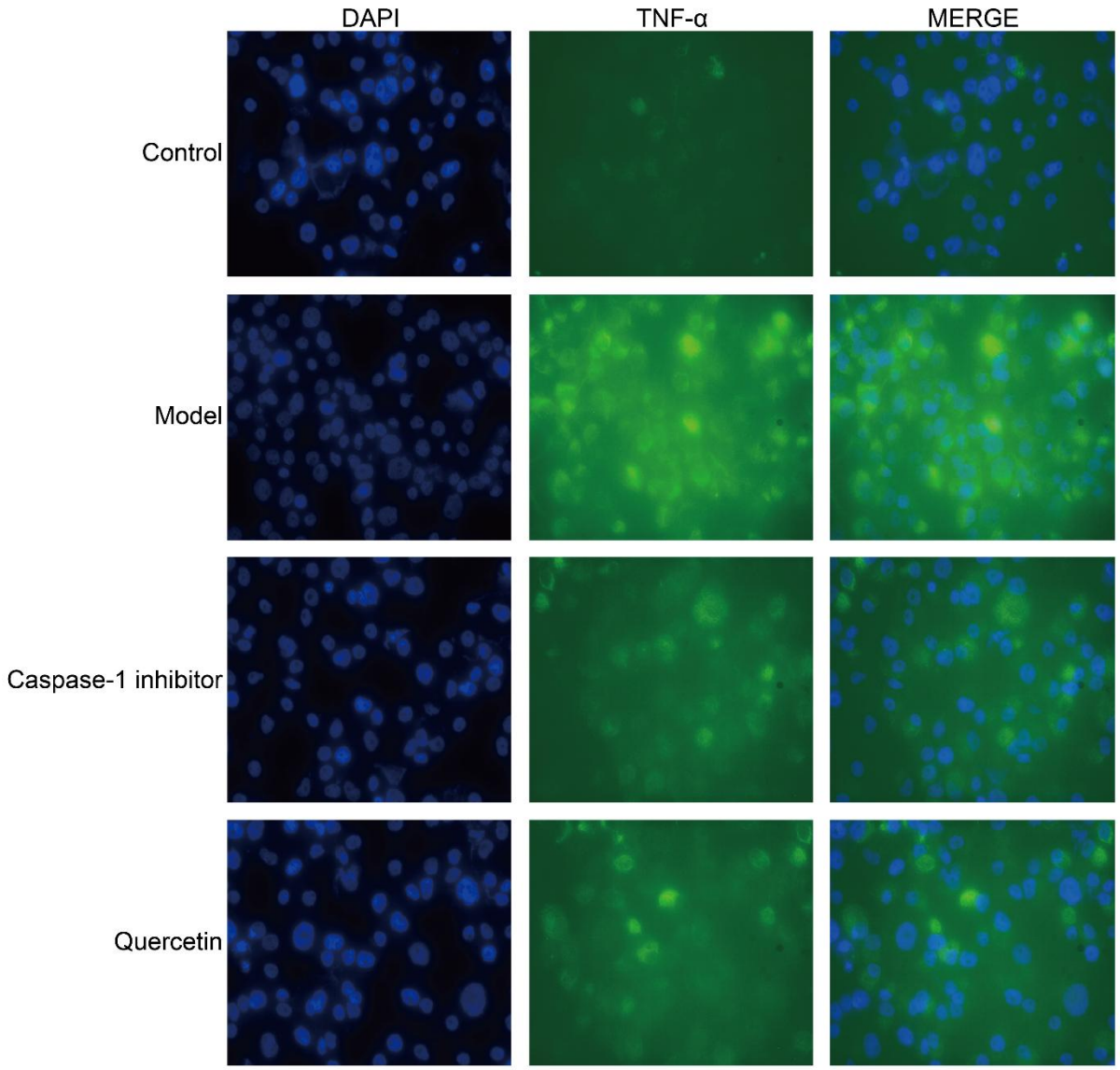


Fig. 19 Quercetin attenuates tumor necrosis factor alpha (TNF-α) activation in foam cells (immunofluorescence staining of TNF-α; scale bar: 50 μm)

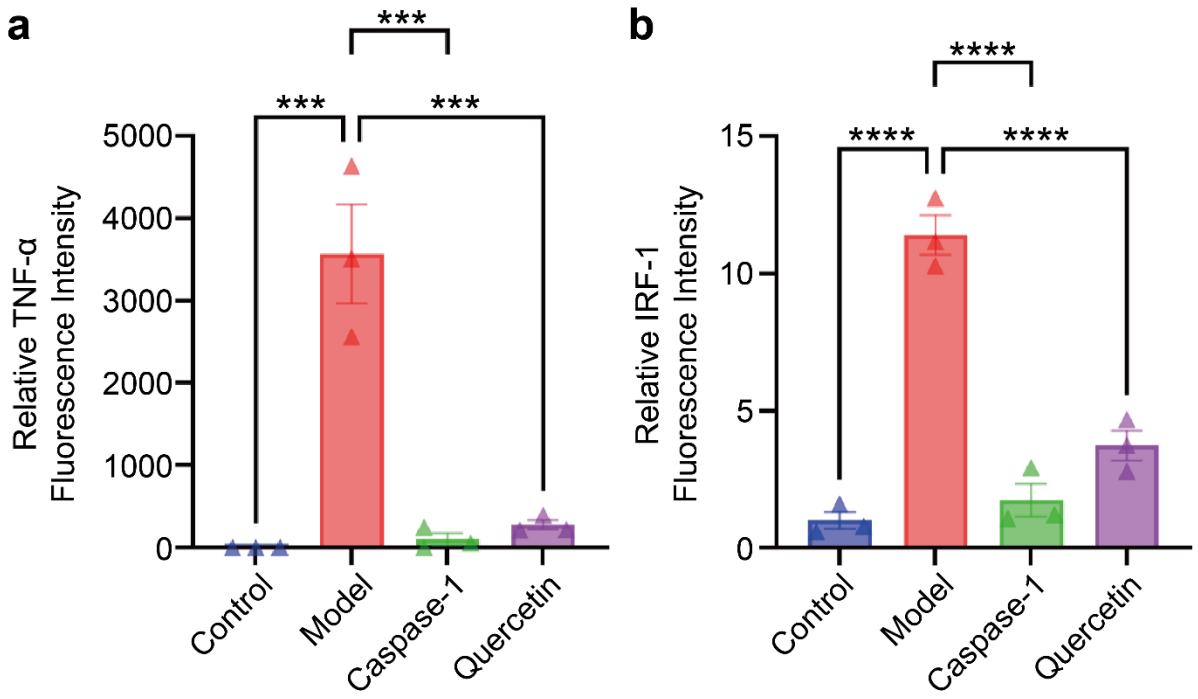


Fig. 20 Quercetin downregulates TNF-α/IRF-1 expression in foam cells

(A) Relative fluorescence intensity of TNF-α. (B) Relative fluorescence intensity of IRF-1.

Abbreviations: TNF-α, tumor necrosis factor alpha; IRF-1, interferon regulatory factor-1.

Consistent with animal experimental findings, quercetin significantly suppressed ox-LDL–induced NLRP3 expression ($P < 0.01$), caspase-1 activation ($P < 0.0001$), GSDMD cleavage—a key factor in pyroptosis ($P < 0.0001$)—and pro-inflammatory cytokine release, including IL-1β (38% reduction) and IL-18 (36% reduction; $P < 0.01$) (Figs. 21–25), validating the potential of quercetin for dual-pathway targeting.

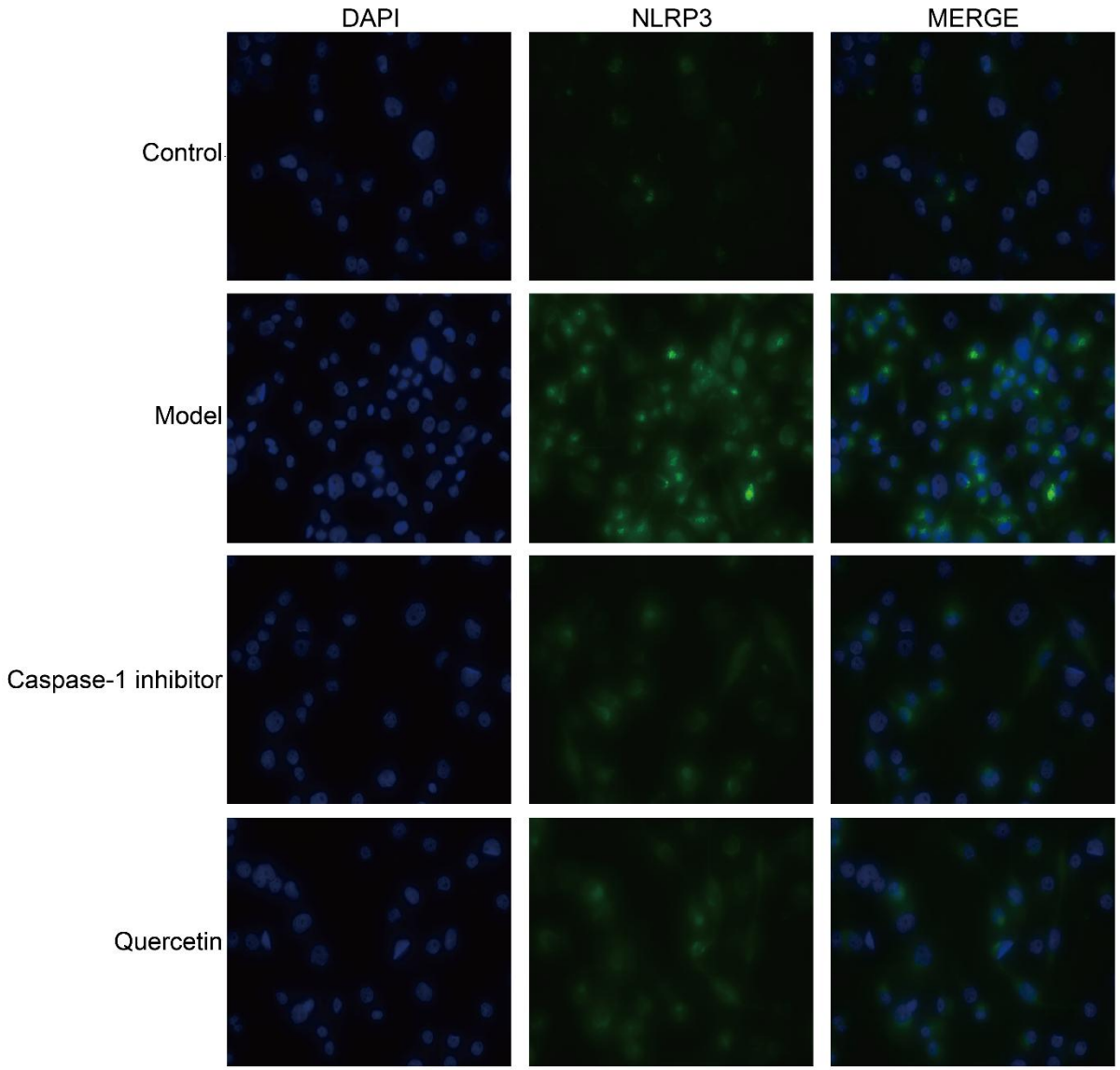


Fig. 21 Quercetin attenuates NOD-like receptor family pyrin domain-containing 3 (NLRP3) activation in foam cells (immunofluorescence staining of NLRP3; scale bar: 50 μm)

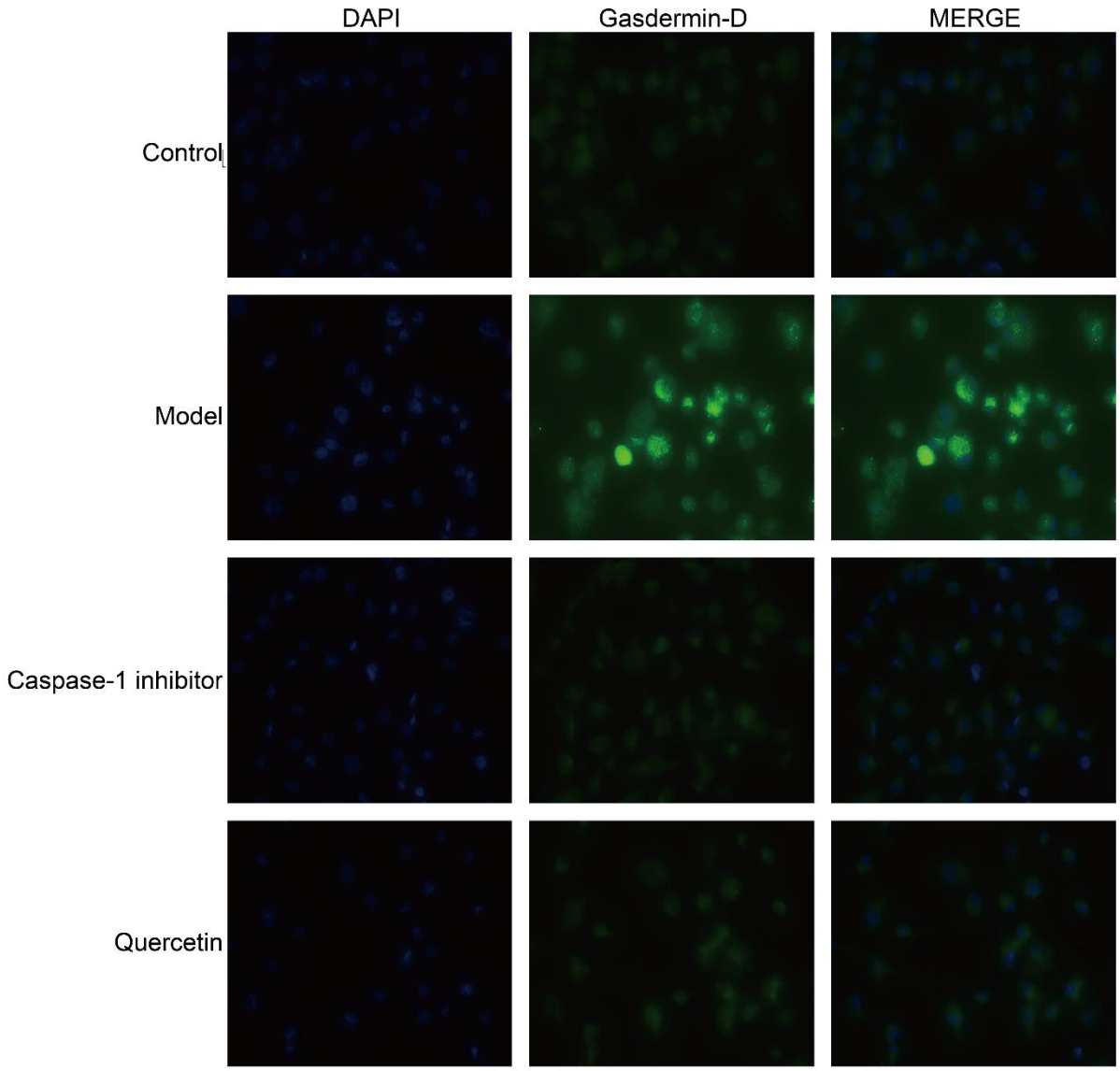


Fig. 22 Quercetin attenuates caspase-1 activation in foam cells (immunofluorescence staining of caspase-1; scale bar: 50 μm)

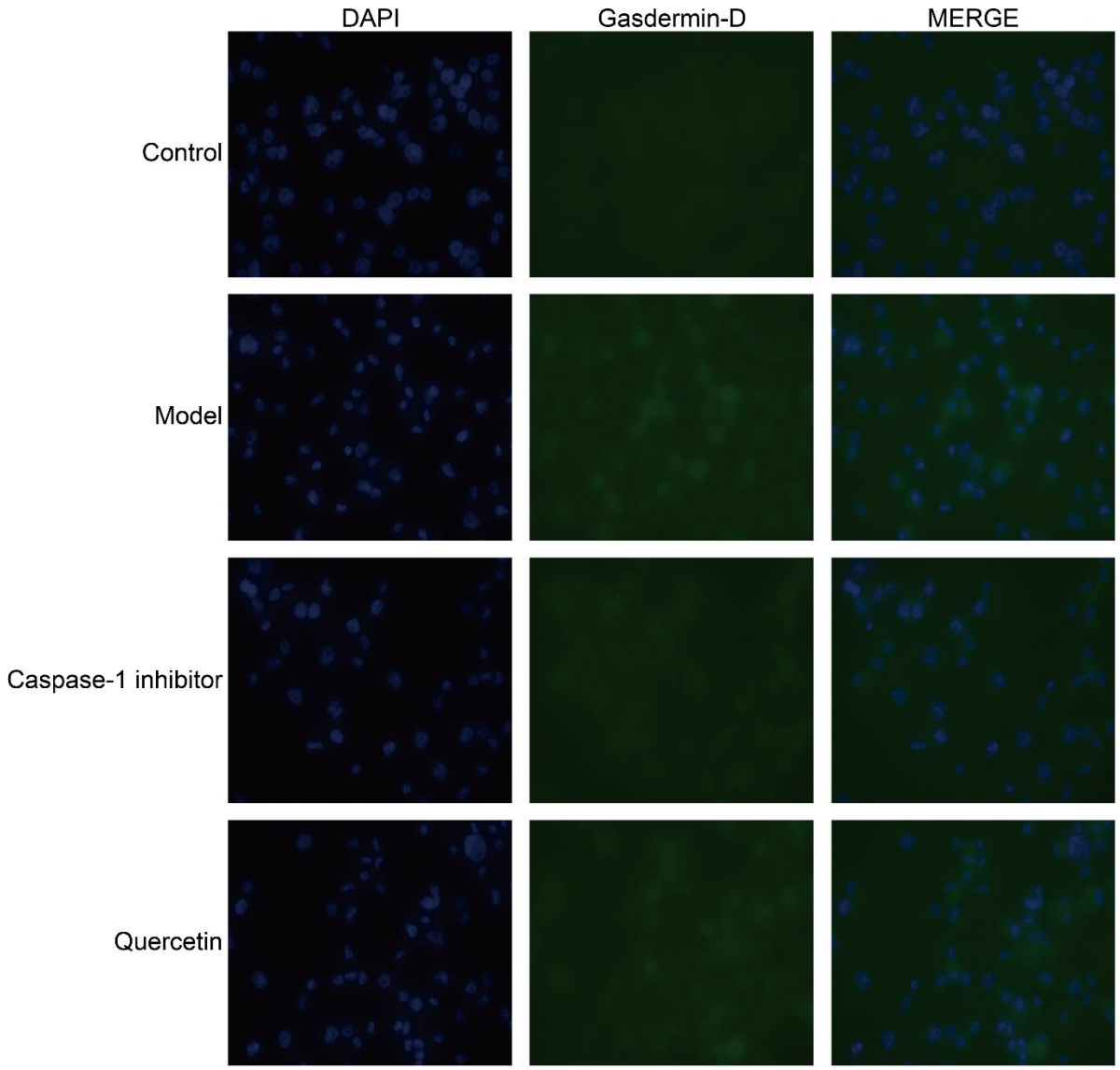


Fig. 23 Quercetin attenuates gasdermin-D activation in foam cells (immunofluorescence staining of gasdermin-D; scale bar: 50 μm)

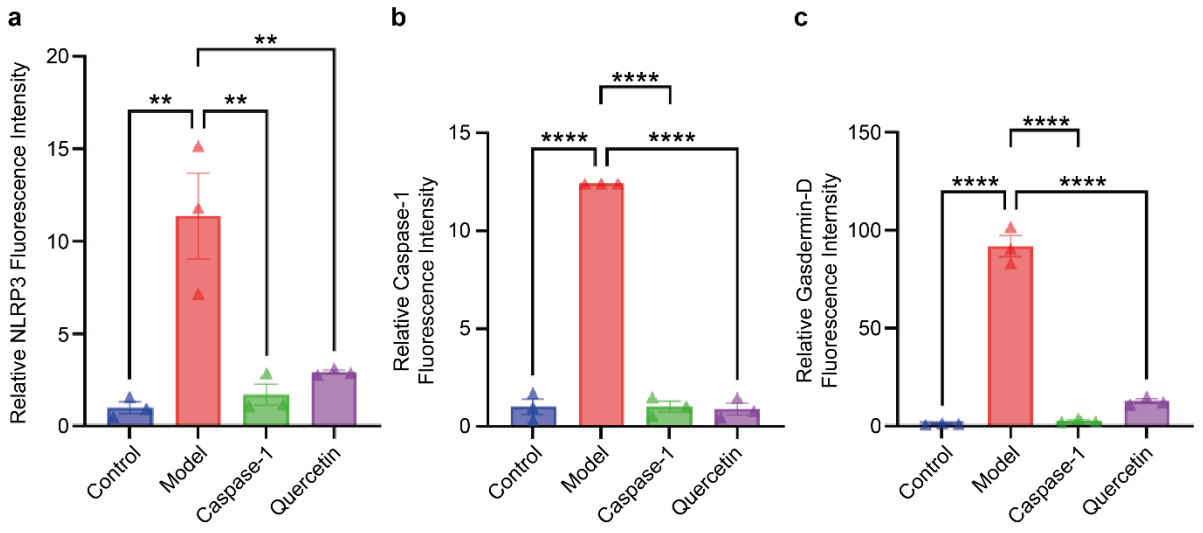


Fig. 24 Quercetin downregulates NOD-like receptor family pyrin domain-containing 3 (NLRP3)/caspase-1/gasdermin-D expression in foam cells

(A) Relative fluorescence intensity of NLRP3. (B) Relative fluorescence intensity of ascpase-1. (C) Relative fluorescence intensity of gasdermin-D

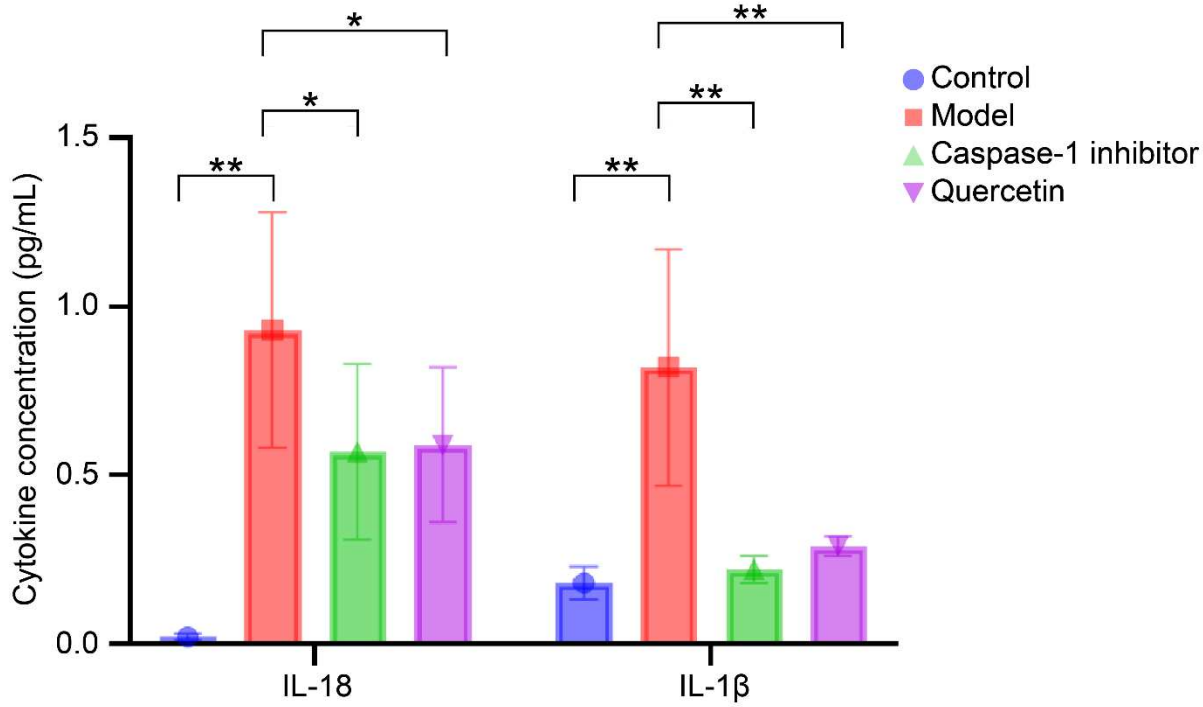


Fig. 25 Quercetin suppresses ox-LDL–induced IL-1β and IL-18 release in foam cells

Abbreviations: IL, interleukin; ox-LDL, oxidized low-density lipoprotein

Collectively, these in vitro findings are consistent with in vivo observations and further suggest the anti-pyroptotic effects quercetin are associated with the inhibition of TNF-α/IRF-1 signaling and NLRP3

inflammasome activation. By suppressing both upstream transcriptional regulators (IRF-1) and downstream pyroptotic pathways (NLRP3/caspase-1), quercetin may represent a potential therapeutic strategy for mitigating inflammation-associated pyroptosis in AS.

Discussion

Quercetin, a dietary flavonoid abundant in fruits and vegetables, has emerged as a multifunctional therapeutic agent against AS, with documented anti-inflammatory, antioxidant, and lipid-modulating properties [35, 36]. Previous studies have demonstrated that quercetin promotes cholesterol efflux [37] and activates extracellular signal-regulated kinase (ERK)–mediated autophagy [38-40]. In the present study, we further demonstrate that quercetin exerts anti-atherosclerotic effects through dual modulation of the TNF-α/IRF-1 and NLRP3/caspase-1 signaling pathways, thereby suppressing pyroptosis, a lytic inflammatory form of programmed cell death closely associated with plaque instability. These findings provide additional insight into the mechanisms through which natural compounds regulate inflammasome-driven vascular pathology.

TNF-α/IRF-1 signaling as a novel pyroptotic switch

During AS progression, TNF-α contributes to endothelial dysfunction and monocyte recruitment, while IRF-1 acts as a transcriptional regulator that promotes the expression of pyroptosis-associated effectors, including caspase-1 and GSDMD [17, 18]. Our findings suggest that quercetin modulates this axis through two complementary mechanisms. First, quercetin inhibits IRF-1 nuclear translocation. By attenuating TNF-α/IFN-β signaling, quercetin prevents IRF-1 from binding to caspase-1 and GSDMD promoter regions, effectively silencing their transcription. This observation is consistent with findings in diabetic vasculopathy, in which IRF-1 deficiency mitigates endothelial pyroptosis [41]. Second, quercetin suppresses NLRP3 inflammasome activation. The present findings suggest that quercetin inhibits NLRP3-ASC oligomerization, independent of IRF-1, thereby reducing caspase-1 activation and IL-1β/IL-18 maturation. Similar mechanisms have been reported in studies demonstrating quercetin-mediated inhibition of caspase-11-dependent noncanonical inflammasome activation during sepsis [42].

Clinical implications and regulatory complexity of IRF-1

Although IRF-1 suppression has been shown to attenuate endothelial pyroptosis [18], its role in antiviral immunity [43] highlights the importance of selective pharmacological modulation rather than complete

inhibition. In this context, quercetin may represent a potentially safer alternative to broad-spectrum TNF-α inhibition (e.g., biologics such as infliximab) because it appears to attenuate pathological IRF-1 hyperactivity while preserving its physiological functions. Moreover, our observation that quercetin partially normalized neutrophil-to-monocyte ratios, a biomarker of systemic inflammation, suggests its potential role in restoring immune homeostasis during AS progression. Additionally, the interaction of IRF-1 with STAT1 and NF-κB signaling pathways in alveolar epithelial cells [44] further underscores the complexity of IRF-1–mediated regulatory networks and suggests that quercetin may exert broader regulatory effects on inflammatory signaling pathways.

This dual-pathway inhibition highlights the superior efficacy of quercetin over caspase-1 inhibition (e.g., VX-765). Bioinformatics analysis using the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP; https://www.tcmsp-e.com/load_intro.php?id=43) suggests that quercetin interacts with 154 distinct molecular targets involved in inflammatory regulation, including transcriptional controllers (NF-κB p65, STAT1, and activator protein-1 [AP-1]), kinase signaling hubs (p38 MAPK, JAK2, and IKK-β), and inflammatory cytokines and chemokines (TNF-α, IL-1β, and IL-6).

Through interactions with multiple inflammatory pathways, quercetin may disrupt the TNF-α→NF-κB→IL-1β/IL-6 amplification axis (evident in TCMSP IDs: TNF/IL-1β/IL6), indicating its potent suppression ability of both local plaque-induced inflammation (via NF-κB/AP-1 inhibition) and systemic inflammation (via CRP/leukocyte normalization). Unlike single-target therapeutic approaches, the broader molecular profile of quercetin may contribute to enhanced plaque stabilization through coordinated regulation of transcriptional reprogramming (NF-κB/STAT1 suppression), signal transduction inhibition (MAPK/JAK pathway inhibition), and inflammatory mediator neutralization (TNF-α/IL-1β reduction).

Dietary quercetin: a holistic approach for inflammation management

Although pharmacological formulations of quercetin are limited by low bioavailability, its natural dietary sources may offer a pragmatic alternative. Quercetin predominantly exists in plants as glycosides (e.g., rutin and isoquercetin), which are hydrolyzed into bioactive aglycones in the gastrointestinal tract, thereby improving absorption efficiency [45, 46, 47]. For instance, onions—one of the richest dietary sources of quercetin—contain up to 300 mg/kg of quercetin glycosides, which are metabolized and subsequently exert systemic anti-inflammatory effects [48, 49]. Epidemiological studies have demonstrated that regular consumption of quercetin-rich foods (e.g., apples, berries, and capers) is associated with reduced cardiovascular risk and

improved endothelial function [50]. Furthermore, synergistic interactions between quercetin and other polyphenols present in whole foods, such as anthocyanins in berries and catechins in tea, may amplify its anti-inflammatory effects through multi-target modulation of NF-κB, NLRP3, and IRF-1 pathways [51, 52]

Promoting dietary patterns enriched with quercetin-containing foods may not only provide benefits in AS but also confer broader protection against chronic inflammatory conditions such as diabetes, neurodegenerative diseases, and inflammatory bowel disease [53-55]. This concept is consistent with the Mediterranean diet model, in which high polyphenol intake has been linked to longevity and reduced age-related morbidity [48, 56]. Therefore, encouraging dietary diversification through increased consumption of quercetin-rich plant-based foods represents a cost-effective and low-risk strategy that complements pharmacological interventions, particularly in populations with limited access to advanced therapies.

Translational challenges

Despite these promising findings, key issues remain. First, quercetin exhibits relatively low oral bioavailability [45], which may limit its therapeutic efficacy in clinical applications. Strategies, including nanoformulation development or structural modification (e.g., Ck-02 and Cf-02), may improve the efficacy of quercetin [45, 57-61]. Second, combining quercetin with statins or PCSK9 inhibitors may enhance anti-atherogenic effects. Moreover, previous studies demonstrating IL-33-mediated suppression of the IRF-1/vascular cell adhesion molecule-1 pathway through ERK1/2 signaling [62] suggest potential opportunities for synergistic intervention strategies involving flavonoid-based therapies.

Study limitations and future perspectives

Although this study provides insights into the novel dual-pathway mechanism by which quercetin attenuates pyroptosis in AS, several limitations should be acknowledged. First, the reliance on pharmacological inhibitors, such as VX-765, rather than genetic approaches, such as IRF-1 or NLRP3 knockout models, limits definitive assessment of causal relationships underlying the observed effects. Second, pharmacokinetic parameters, including tissue distribution and metabolite profiles of quercetin in atherosclerotic lesions, were not characterized due to technical constraints in tracer labeling and mass spectrometry analyses. Third, our findings were not validated in advanced human disease models, such as induced pluripotent stem cell–derived macrophages from patients with AS, which may more accurately recapitulate human pathophysiology.

These limitations provide direction for future investigations. First, mechanistic studies using myeloid-specific IRF-1/NLRP3 double-knockout mouse models may further clarify pathway hierarchy and potential compensatory interactions. Second, translational optimization strategies, including the development of nanoformulated quercetin systems, such as lipid–polymer hybrid nanoparticles, may enhance bioavailability and lesion-targeted delivery, guided by positron emission tomography/magnetic resonance multimodal imaging. Third, the development of patient-derived three-dimensional vascular organoid models may help assess interindividual variability and identify predictive biomarkers associated with quercetin responsiveness.

Importantly, these limitations do not diminish the core contribution of this study but rather identify opportunities for further investigation. Addressing these knowledge gaps may facilitate the development of flavonoid-based precision therapies for inflammation-associated CVDs.

Conclusions

In summary, this study demonstrates a novel anti-atherosclerotic mechanism of quercetin involving coordinated inhibition of the TNF-α/IRF-1 transcriptional axis and NLRP3 inflammasome assembly, resulting in suppression of pyroptosis and vascular inflammation. These findings s extend current understanding of conventional flavonoid-mediated cardioprotective mechanisms by suggesting that quercetin may influence both upstream regulatory pathways (IRF-1) and downstream effector pathways (NLRP3/caspase-1) involved in the pyroptotic cascade.

These findings further support the potential of quercetin as a dietary adjunct or therapeutic candidate for inflammation-driven CVDs. Future studies should focus on improving quercetin delivery systems, such as nanoencapsulation, and validating its efficacy in human studies, particularly in high-risk populations with persistent inflammatory burden despite lipid-lowering therapy. In addition, investigating potential interactions between quercetin and emerging anti-inflammatory biologics [63] may provide further insights into combinatorial therapeutic regimens aimed at improving plaque stability while minimizing systemic toxicity.

Statements & Declarations

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Author contributions

Dandan Wu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft. Yinxue Bai performed the experiments, prepared figures and/or tables, and approved the final draft. Qianqian Guo performed the experiments, prepared figures and/or tables, and approved the final draft. Shan Ren performed the experiments, prepared figures and/or tables, and approved the final draft. Xiaxia Zhang performed the experiments, prepared figures and/or tables, and approved the final draft. Huan He analyzed the data, prepared figures and/or tables, and approved the final draft. Yajie Zhang analyzed the data, prepared figures and/or tables, and approved the final draft. Dongqing An conceived and designed the experiments, authored and reviewed drafts of the article. All authors read and approved the final manuscript.

Data availability statement

All raw data and code generated or analyzed in this study are available from the corresponding author upon reasonable request.

Ethical approval: This study received ethical approval from the Ethics Committee of Xinjiang Medical University.

Consent to participate: Not applicable.

Consent to publish: Not applicable.

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