Journal of Inflammation Research Dovepress

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# FSCN1 is a Potential Therapeutic Target for Atherosclerosis Revealed by Single-Cell and Bulk RNA Sequencing

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**Background:** Atherosclerosis (AS) is the major cause of cardiovascular disease. Using integrated single-cell and bulk RNA sequencing data of atherosclerosis, we aimed to investigate the cell phenotype, intercellular communication, and potential therapeutic target in AS.

**Methods:** Single-cell sequencing data from aortic arch of Apoe$^{ko}$ mice in normal diet (ND) and high fat diet (HFD) groups (obtained from GSE206239) were analyzed by Seurat, singleR, ReactomeGSA, and cellchat package. scRNA-seq dataset GSE159677 from the carotid artery of the patients with carotid endarterectomy were used to validate the distribution of fascin actin-bundling protein 1 (FSCN1) in cell populations. Bulk RNA sequencing data (GSE43292 and GSE28829) were used to analyzed the expression of FSCN1in AS. A cross-sectional clinical study was utilized to examine the association between FSCN1 and AS. Circulating concentrations of FSCN1 were measured using ELISA kits and assessed using logistic regression analysis and receiver operating characteristic (ROC) curves. Apoe$^{ko}$ mice fed with HFD and MAECs treated with oxidized low-density lipoprotein (ox-LDL) were established to detect the expression of FSCN1. Furthermore, we knocked down FSCN1 in MAECs to observe its influence on pyroptosis and migration.

**Results:** The HFD group had a significantly lower percentage of T cells, fibroblasts, and B cells and a significantly higher percentage of monocytes/macrophages cells. Strong interactions between endothelial cell (EC) and fibroblast in ND groups, while EC interactions with smooth muscle cells (SMC) and T cells were stronger in HFD groups. Semaphorin 7 (SEMA7) mediated signaling pathways were enriched in HFD groups and targeted EC driving by SMC. FSCN1was mainly expressed in EC and had a high expression in human AS samples. The cross-sectional study identified that high level of FSCN1 was associated with increased risk of AS. We also observed that high expression of FSCN1 in ox-LDL-induced MAECs and Apoe$^{ko}$ mice fed with HFD. Knockdown of FSCN1 reduced pyroptosis and increased the migration in MAECs.

**Conclusion:** Knockdown of FSCN1 in EC could alleviate the development and progression of AS. FSCN1 may be a potential prognostic biomarker and therapeutic target in AS.

**Keywords:** FSCN1, atherosclerosis, endothelial cells, pyroptosis, migration

## Introduction

Atherosclerosis (AS), the pathological basis of coronary heart disease (CHD), is associated with lipid accumulation and chronic inflammation of the arterial wall.[1,2] Different cell types exit in atherosclerotic lesions participating in the initiation, progression, and rupturing of AS.[3] The complex interactions among various cell types governs atherosclerosis progression and plaque stability involving endothelial cells (ECs), smooth muscle cells (SMCs), and macrophages.[4] The interaction of ECs and SMCs mediate SMC phenotype transdifferentiation to potentially guard vascular homeostasis.[5] Furthermore, Crosstalk between macrophages and SMCs play a significant role in determining atherosclerotic plaque stability.[6] Single-cell RNA sequencing (scRNA-seq) is a technology characterizing molecular heterogeneity at the single

Received: 26 July 2024
Accepted: 22 November 2024
Published: 25 November 2024

cell level which has ushered in a deeper understanding of the cell phenotype, differentiation trajectory, and intercellular communication in AS.[7]

Atherosclerotic cardiovascular diseases remain one of the main causes of death worldwide.[8] Although some traditional risk factors such as low-density lipoprotein cholesterol level, fasting glucose level, smoking, blood pressure, triglyceride level, and high-density lipoprotein cholesterol level have been identified as related to AS, there are currently no reliable risk prediction biomarkers or promising therapeutic targets for AS.

Fascin actin-bundling protein 1 (FSCN1), a member of the Fascin family of actin-binding proteins, has been associated with cell migration, motility, invasion, and metastasis.[9,10] Recent studies have shown that FSCN1 is recognized as a candidate biomarker for multiple cancer types and as a potential therapeutic target.[11] Further, published studies have shown that the expression of FSCN1 was increased in symptomatic compared to asymptomatic patients with carotid plaques.[12] However, the role of FSCN1 in AS and its underlying molecular mechanisms remain poorly understood.

To investigate the cell phenotype, intercellular communication, and potential therapeutic target in AS, we analyzed single-cell and bulk RNA sequencing data of atherosclerosis. To evaluate the relationship between FSCN1 and AS, we detected the expression of FSCN1 protein in CHD patients and examined the association between FSCN1 and AS. We also investigated the expression and molecular mechanism of FSCN1 on AS in vivo and in vitro.

# Methods

## Bioinformatics Analysis

We performed single cell RNA sequencing (scRNA-seq) data of the GSE206239 and GSE159677 datasets (http://www.ncbi.mlm.nih.gov/geo/). The Seurat R package (version 4.4.0) was used to process and analyze. We performed differential gene expression analysis of the GSE43292 (32 atheroma plaque and 32 macroscopically intact tissue) and GSE28829 (16 advanced atherosclerotic plaque and 13 early atherosclerotic plaque) microarray datasets in the GEO database. The GEO2R was used to process and analyze.

## Study Participants

The patients including 28 patients with a genetic diagnosis of CHD and 14 patients without CHD were enrolled from November 1, 2022, to March 30, 2023, in Affiliated hospitals of Jiangsu University. This study was performed with the Declaration of Helsinki and approved by Ethics Committee of the Affiliated hospitals of Jiangsu University (approval number: KY2021K1226). All participants signed consent forms. Patients with the following criteria were included in the study: patients aged ≥18 years and <90 years old; patients diagnosed with or without CHD according to the diagnostic criteria of the Guidelines.[13] Patients with the following criteria were excluded from the study: patients with malignant tumors, autoimmune diseases, severe hepatic and renal insufficiency or blood disorders; Vulnerable groups such as behavioral abnormalities and mental cognitive disorders; Baseline data are incomplete. The flow chart of this study is described (Figure 1).

## Data Collection and Biochemical Measurements

Demographic data, including age, sex, smoking and alcohol intake histories, hypertension history, and diabetes duration, was taken from medical records. In addition, Biochemical parameters, including fasting blood glucose (FBG), glycated hemoglobin (HbA1c), serum total cholesterol (TC), triglyceride (TG), high- density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), were also taken from medical records. To evaluate the concentrations of FSCN1, samples were isolated from human serum and analyzed using ELISA kits from Jiangsu Meimian Industrial Co., Ltd. All procedures were performed according to instructions from the manufacturers. Each sample was analyzed in duplicate.

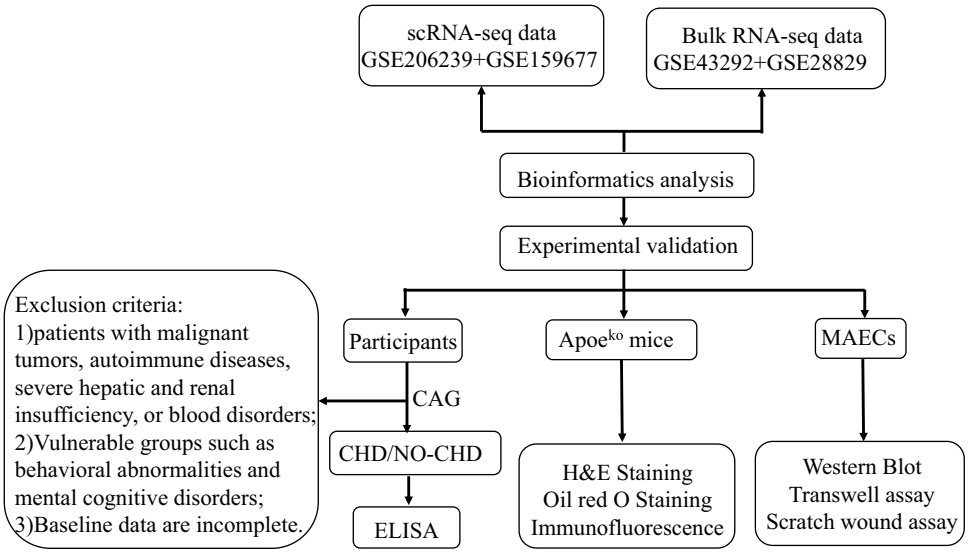


**Figure 1** Flow chart of the present study.

## Animal Experiments

Ethics approval for animal care and experimental procedures of this study was obtained from the Animal Care and Use Committee of Jiangsu University (approval ID: S CXK2013-0011). All animal experiments followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.5–23, revised 1996; NIH Bethesda, MD, USA). Wildtype C57BL/6 and ApoE knockout mice on a C57BL/6 background were purchased from the Jackson Laboratory (USA) and reared, housed, and maintained at the Experimental Animal Center of Jiangsu University. All mice were fed a high fat diet (HFD) (40 kcal.% fat, 20 kcal.% protein, 40 kcal.% carbohydrate and 1.25% cholesterol) for 24 weeks. At the end of the animal experiment, all mice were euthanized and the aortas were fixed in 4% paraformaldehyde.

## Cell Culture

Mouse aortic endothelial cells (MAECs) were cultured in DMEM supplemented with 10% FBS under a humidified 5% CO2 atmosphere at 37°C. Before all the experiments, Mouse aortic endothelial cells (MAECs) were pretreated 50 μg/mL oxidized low-density lipoprotein (ox-LDL) to simulate a high-fat environment.

## Oil Red O Staining

For en face lesion analysis of the aorta, the whole aorta was dissected and opened longitudinally. Then, the aortas were stained for 60 min in Oil Red O working solution (G1260, Solarbio, Beijing, China) and differentiated by 75% alcohol.

## H&E Staining

For H&E staining, Paraffin-embedded sections were dewaxed and hydrated to water. Then, the sections were stained with hematoxylin solution for 3 min and differentiated with differentiation solution for 3 min. Finally, Eosin Y Aqueous Solution was used to re-dyeing for 2 min and resinene was used to seal.

## Immunofluorescence

Paraffin-embedded sections were deparaffinized, rehydrated, antigen retrieval, and washed with PBS, followed blocked with 1% bovine serum albumin and 0.2% Triton X-100 for 1 h at 37°C. Fascin antibody (ab126772, Abcam, UK) and CD31 antibody (3528, cell signaling technology, USA) were diluted with 1:200 and incubated overnight at 4°C. Next, conjugated secondary antibodies (AB0152 and AB0141, abways, shanghai, China) were incubated with a 1:200 dilution for 1 h at 37°C in the dark. Images were captured using a fluorescence microscope.

## Western Blotting

MAECs were lysed in RIPA lysis buffer containing protease and phosphatase inhibitors. The proteins were separated by gel electrophoresis and then transferred onto PVDF membranes and incubated with primary antibodies at 4°C overnight. Membranes were incubated with primary antibodies overnight. Afterward, secondary antibodies were used to incubate at room temperature for 1 h and allow the detection of bands using ECL detection reagents (GE Healthcare).

## Scratch Wound Assay

MAECs were scratched by using a sterile micropipette tip after the cells had formed confluent monolayer. The cells were photographed at the beginning of the experiment and after 24 h. Randomly take 3 positions to compare the width of the scratches and the number of cells migrating into the scratches. ImageJ was used to measure the scratch area of cells and quantitative analysis.

## Transwell Assay

MAECs exponentially growing cells were implanted in a 24-well top chamber. DMEM containing 10% FBS was used as a chemical attractant in the lower chamber. The cells were treated with ox-LDL or siRNA and incubated for 24 h. Afterwards, the cells that did not tans-migrate through the pores were removed with a cotton swab and then the cells on the lower surface of the membrane were fixed with 4% PFA for 30 min and stained with 0.1% crystal violet for 20 min. The trans-migration of RAW 264.7 cells was counting the migrated cells in six randomly selected fields/well viewed and repeated three times.

## Statistical Analysis

Statistical analysis was performed with SPSS 25.0 (IBM Corp, Armonk, NY, USA) or GraphPad Prism 8. Quantitative values are expressed as the mean ± SD or median [interquartile range (IQR)]. After examining whether all variables were normally distributed by the Kolmogorov–Smirnov or Shapiro–Wilk test, statistical differences were determined by Mann Whitney test or Student's *t*-test for comparison between two groups and Kruskal–Wallis with Dunn's multiple comparisons test or one-way ANOVA followed by Bonferroni's multiple-comparison test for comparison among three or more groups. Categorical variables are presented as no. (%). Chi-square tests were performed to compare categorical variables. Logistic regression was used to evaluate possible factors affecting AS. The results were expressed using odds ratio (OR) and 95% confidence interval (CI). The clinical diagnostic performance of important variables in predicting FSCN1 was evaluated by plotting the receiver operating characteristic (ROC) curve, and the diagnostic cut-off value was determined by Youden index, and its specificity and sensitivity were calculated. $P<0.05$ was considered statistically significant.

# Results

## ScRNA-Seq Profiles Revealed Cell Types in Mouse Aortic Arch

ScRNA-seq dataset of aortic arch of $Apoe^{ko}$ mice (obtained from GSE206239) were used to compare the differences between normal diet (ND) and high fat diet (HFD) groups. A total of 8302 cells with 17,607 gene were identified after QC, which including 5454 cells from the ND group and 2848 from the HFD group. Cluster analysis was used for filtered cells, resulting in 16 distinct clusters (Figure 2A and B). By assessing the presence and abundance of canonical cell signature genes within each cluster, we identified 6 cell clusters. The clusters were identified to be monocytes/macrophages (Lgals3, Cd83 and Cd74), SMCs (Myh11, Tagln and Acta2), ECs (Aqp1, Cldn5 and Fabp4), T cells (Cd3g, Cd3d and Vps37b), B cells (Igkc, Cd79a and Cd79b), and fibroblasts (Col1a2, Col1a1 and Lum) (Figure 2C). We also calculated the percentage of 6 cell clusters for each sample group and found that HFD group had a significantly lower percentage of T cells, fibroblasts, and B cells and a significantly higher percentage of monocytes/macrophages (Figure 2D). Monocytes/macrophages, smooth muscle cells, and endothelial cells were identified as primary cell types. ReactomeGSA functional enrichment analysis suggested that these cell types mainly are involved in Sterols are 12-hydroxylated by CYP8B1, Biosynthesis of DPAn-3-derived protectins and resolvins, and NTF4 activates NTRK2 (TRKB) signaling (Figure 2E).

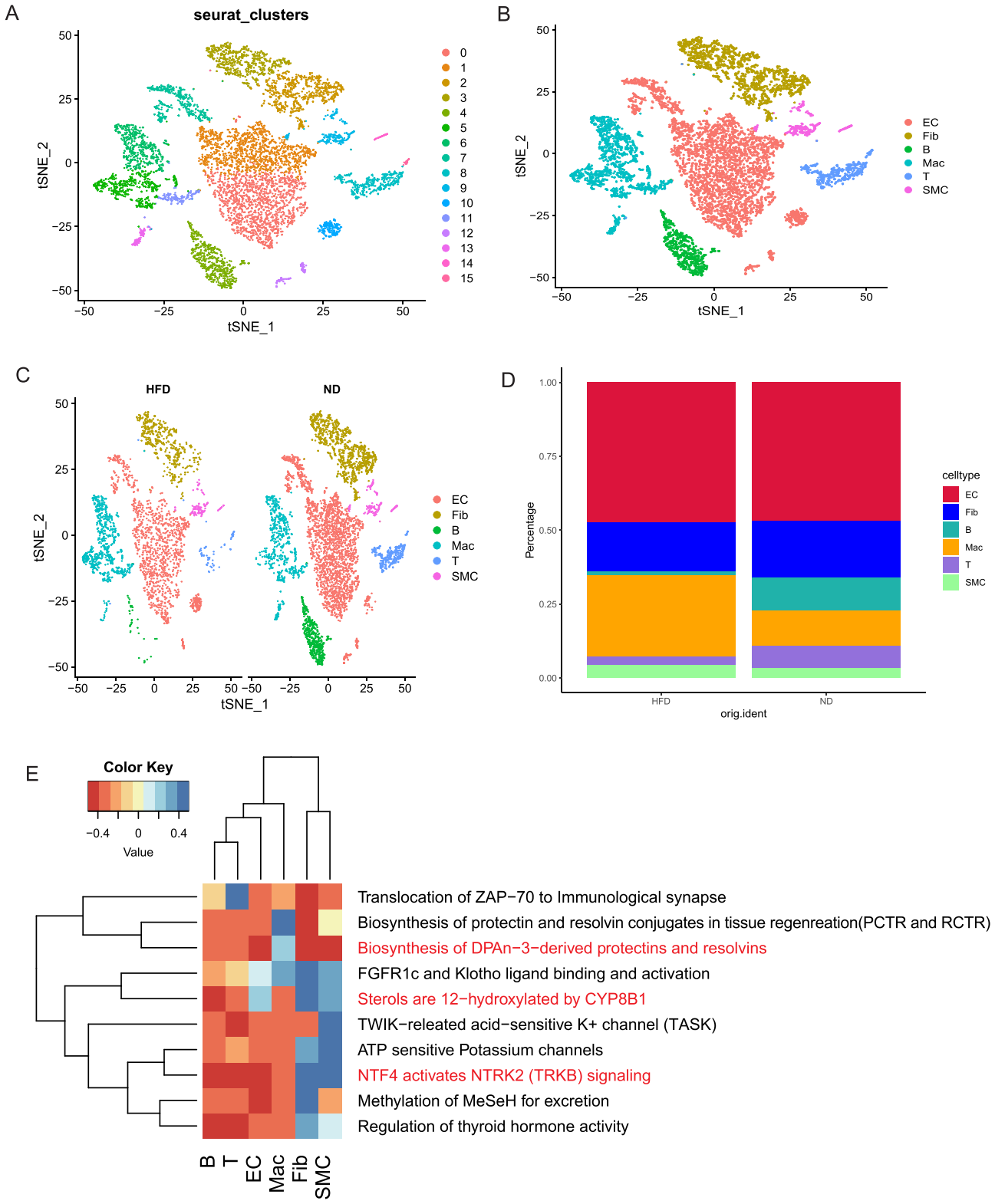


**Figure 2** Overview of cell populations was profiled. (**A**) t-distributed stochastic neighbor embedding (t-SNE) represents the aligned gene expression data in ND and HFD groups. (**B** and **C**) t-SNE shows the identified major aortic cell types in ND and HFD groups. Monocytes/macrophages: Mac; smooth muscle cells: SMC; endothelial cell: EC; T cells: T; B cells: B; fibroblasts: Fib. (**D**) The proportions of various cell types in ND and HFD groups. (**E**) Functional enrichment analysis for the identified hub cell types using "ReactomeGSA" package.

## Cell–cell Communication Network in Mouse Atherosclerosis

To assess the communication and interactions between various cell phenotypes in mouse atherosclerosis, we dissected intercellular communication using the R package "CellChat". We observed the number of cell–cell communications and the interaction weight among 6 cell phenotypes. In particular, the results showed strong interactions between EC and fibroblast in ND groups, while EC interactions with SMC and T cells were stronger in HFD groups (Figure 3A and B). Furthermore, we investigated the outgoing communication patterns of cell populations in ND and HFD groups. Fibroblast mediated the highest outgoing communication in ND and HFD groups (Figure 3C). Differential signaling pathway enrichment analysis among cell populations revealed Galectin- and CD22 -mediated signaling pathways enriched in ND groups, whereas Semaphorin 7 (SEMA7)- and osteopontin (SPP1)-mediated signaling pathways were enriched in HFD groups (Figure 3D). Signaling involving SEMA7 specifically targeted EC and was mostly driven by SMC (Figure 3E). This is consistent with previous studies implicating these signals in vascular inflammation and atherosclerosis.[14] Furthermore, we identified the significant signaling pathways from EC or SMC to other cell types (Figure 3F and G).

## Expression of FSCN1 in as from GEO Database

Fascin actin-bundling protein 1 (FSCN1), a member of the Fascin family of actin-binding proteins, was upregulated in symptomatic patients with carotid plaques.[9] To investigate the expression pattern of FSCN1 among the different cell populations in atherosclerosis, we found that FSCN1 was mainly expressed in EC (Figure 4A and B). Furthermore, we analyzed the scRNA-seq dataset GSE159677 from the carotid artery of the patients with carotid endarterectomy. This is consistent with the scRNA-seq dataset GSE206239 implicating that FSCN1was mainly expressed in EC (Figure 4C–E). Next, we performed an analysis of bulk RNA-seq data (GSE43292) to further identify the Expression differences of FSCN1 in human AS and normal samples. The results indicated that the FSCN1 gene expressed higher in AS than normal samples (Figure 4F). Similarly, the high expression of FSCN1 in advanced atherosclerotic plaque from human carotid compared with

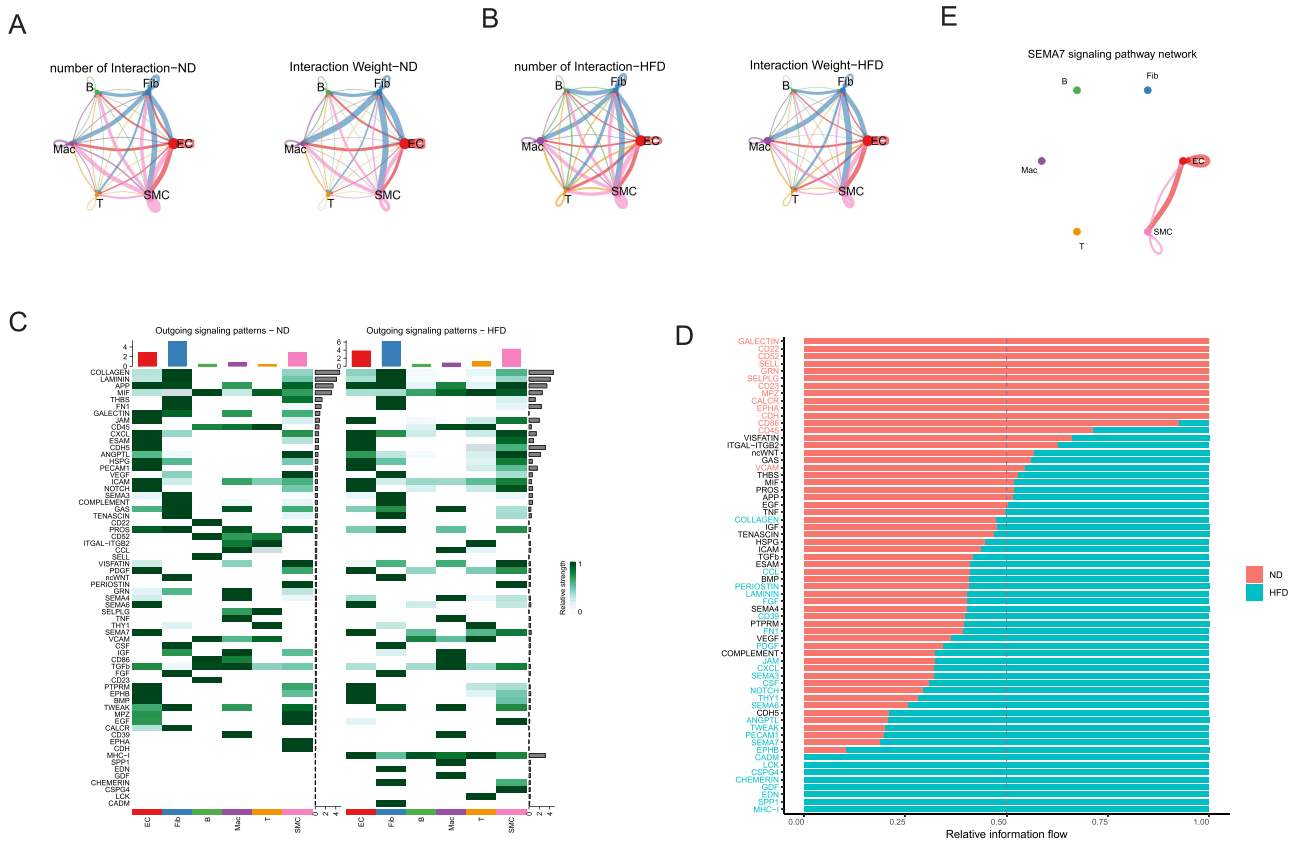


**Figure 3** Contiune.

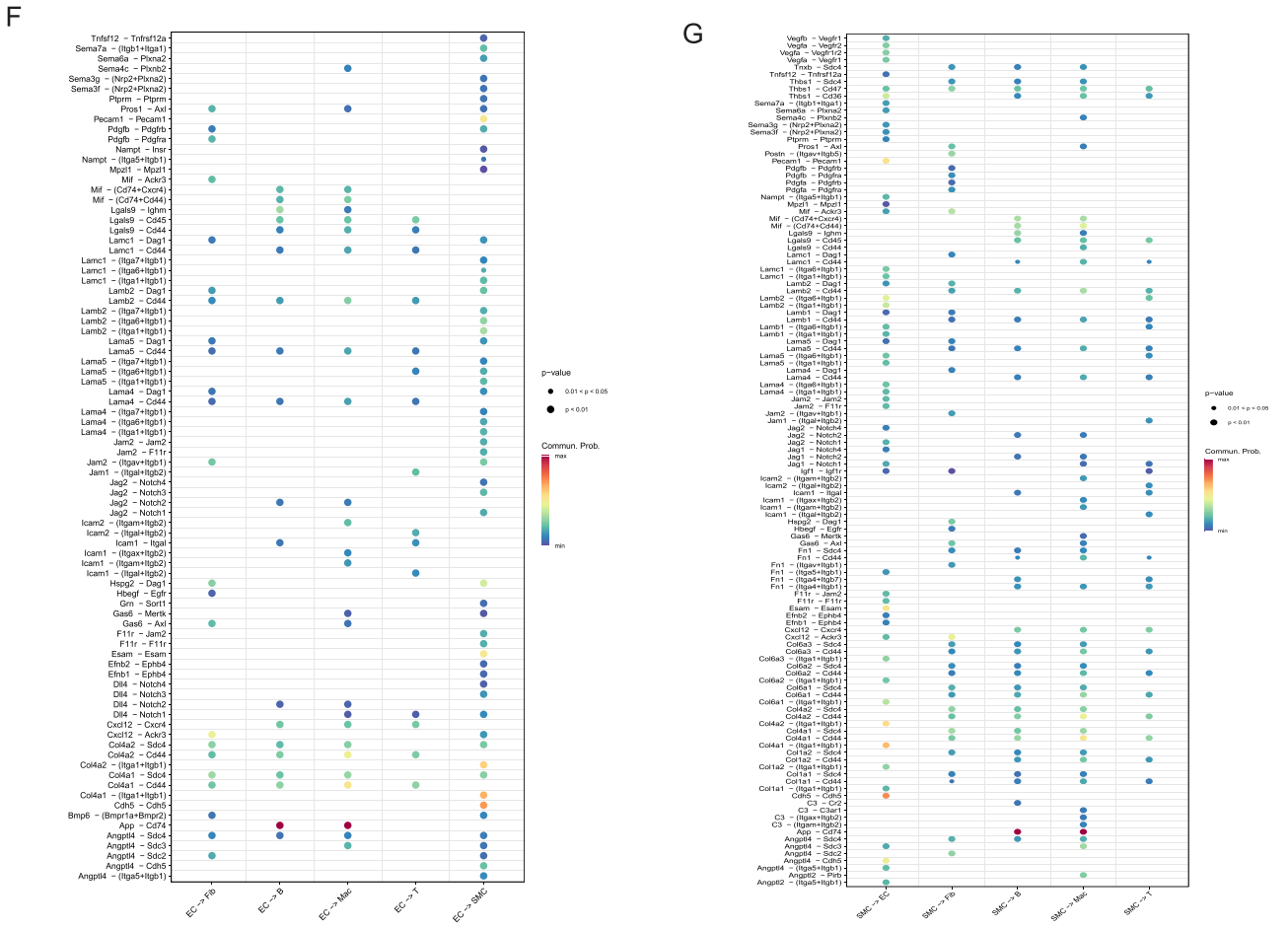


**Figure 3** Global cell–cell communication signals participated in atherosclerosis. (**A** and **B**) The line width represents the interaction number and interaction weight among hub cell types in ND and HFD groups. (**C**) Significant signaling of hub cell types in outgoing communication patterns in ND and HFD groups. (**D**) Bar plot illustrating significant signaling pathways in ND and HFD groups. (**E**) Circle plot depicting sources and targets for SEMA7 signaling. (**F** and **G**) Summary dot plot of ligand-receptor interactions.

early atherosclerotic plaque by analyzing the bulk RNA-seq dataset GSE28829 (Figure 4G). To investigate the physically binding protein that interact with FSCN1, STRING (https://string-preview.org/) was used to make PPI network diagram. The results indicated that the FSCN1 may interact with AKT1, RAB35, or FLNA (Figure 4H).

## High Serum Level of FSCN1 Was Positively Correlated with Increased Risk of CHD

Coronary heart disease (CHD), which is closely associated with atherosclerosis, is a major cause of death in developed countries.[8] To validate the results of our Bioinformatic analyses, we included 42 individuals, including 14(33.3%) individuals of a control group without CHD, and 28 (66.7%) individuals of CHD patients. The characteristics of study groups are shown in Table 1. Furthermore, an ELISA kit was used to detect the serum level of FSCN1 in CHD patients. The ELISA results demonstrate a significant increase in the concentration of FSCN1 in patients with CHD compared with NO-CHD (13.3±0.6 ng/mL vs 18.8±0.5 ng/mL, $P< 0.0001$; Figure 4I).

In addition, in order to better explore the relationship between FSCN1 and CHD, some factors that might affect CHD were used to perform Logistic regression analysis. As shown in Table 2, FSCN1 was an independent risk factor for CHD ($P= 0.002$, odds ratios (OR) = 2.37, 95% CI = 1.36–4.08). Also, after adjusting for confounding factors such as sex, age, diabetes, and hypertension, high levels of FSCN1 were still associated with an increased risk of CHD ($P= 0.006$, OR = 2.82, 95% CI = 1.35–5.91). ROC curve was used to analyze the diagnostic performance of serum FSCN1 in AS. The AUC of serum FSCN1 for AS was 0.9388 (95% CI: 0.8695 to 1.000, $P< 0.001$). The optimal cut-off value of serum

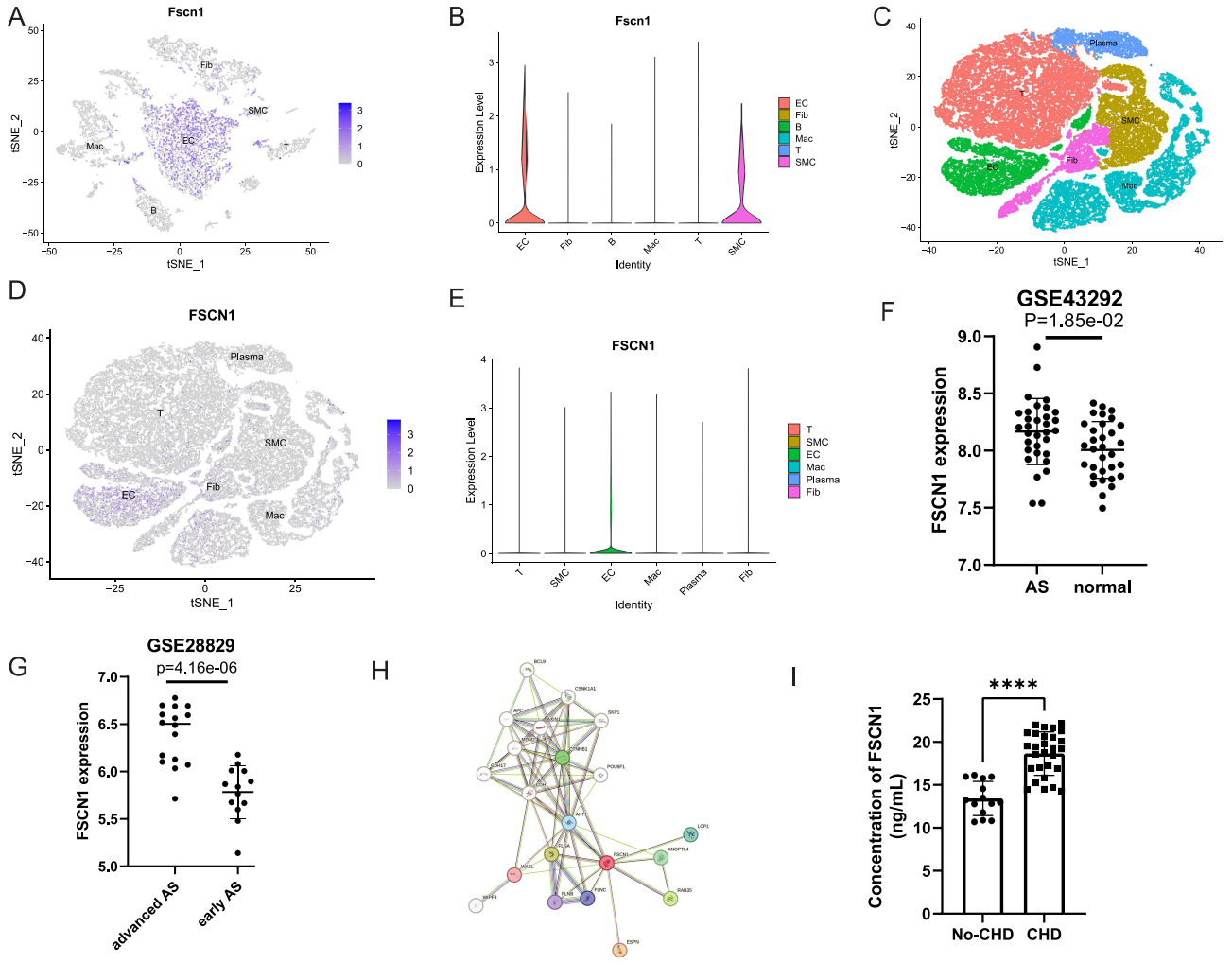


**Figure 4** Expression of FSCN1 in atherosclerosis. (**A**) Gene expression patterns projected onto t-SNE plots of FSCN1 (scale: log-transformed gene expression). (**B**) Gene expression patterns projected onto Violin plots of FSCN1 (scale: log-transformed gene expression). (**C**) t-SNE plot of aggregate cells from the carotid artery of the patients with carotid endarterectomy with colors denoting different cell clusters in scRNA-seq data (GSE159677). (**D**) Gene expression patterns projected onto t-SNE plots of FSCN1 (scale: log-transformed gene expression). (**E**) Gene expression patterns projected onto Violin plots of FSCN1 (scale: log-transformed gene expression). (**F**) Relative FSCN1 expression in atheroma plaque vs Macroscopically intact tissue in BulkRNA-seq data (GSE43292). (**G**) Relative FSCN1 expression in advanced atherosclerotic plaque vs early atherosclerotic plaque in RNA-seq data (GSE28829). (**H**) FSCN1-binding proteins obtained by the STRING tool. (**I**) ELISA results showing concentrations of FSCN1 in serum from CHD and NO-CHD patients. ****P<0.0001, compared with the NO-CHD group.

FSCN1 was 16.42 ng/mL for distinguishing AS with high sensitivity (78.57%) and specificity (100%). Taken together, these data demonstrated that high serum level of FSCN1 was positively correlated with increased risk of atherosclerotic CHD.

## FSCN1 was Upregulated During as in vivo and in vitro

To explore the change of FSCN1 in AS, we established Apoe$^{ko}$ mice fed with an HFD for 24 weeks to establish the AS model. As shown in Figure 5A, Oil Red O staining showed increased lipid accumulation in the whole aorta of Apoe$^{ko}$ mice compared with Apoe$^{WT}$ mice. Co-immunostaining of FSCN1 and ECs marker CD31 showed that FSCN1 is expressed on ECs at the root of the aorta and increased in Apoe$^{ko}$ mice compared with Apoe$^{WT}$ mice (Figure 5B and C). Furthermore, we constructed experiments on MAECs in vitro. It's observed that the FSCN1 expression was elevated in MAECs treated with ox-LDL, which is consistent with the mouse model observations (Figure 5D and E).

**Table 1** Basal Characteristics in NO-CHD and CHD Patients

| Characteristic | Total (n=42) | NO-CHD (n=14) | CHD (n=28) | *P* Value |
|---|---|---|---|---|
| Age (years) | 66.0±5.2 | 72.5±2.5 | 59.5±8.5 | 0.207 |
| Male sex, no. (%) | 25(59.5) | 10(71.4) | 15(53.6) | 0.266 |
| BMI, kg/m$^2$ | 26.3±1.5 | 24.9±2.1 | 27.7±2.2 | 0.116 |
| SBP, mmHg | 172.5±11.1 | 170.0±10.0 | 175.0±25.0 | 0.988 |
| DBP, mmHg | 90.0±10.8 | 75.0±5.0 | 105.0±15.0 | 0.991 |
| Current smoker, no. (%) | 13 (31.0) | 6(42.9) | 7(25.9) | 0.453 |
| Alcohol user, no. (%) | 10 (23.8) | 5(35.7) | 5(19.2) | 0.444 |
| Hypertension, no. (%) | 23 (54.8) | 6(42.9) | 17(60.7) | 0.273 |
| Diabetes, no. (%) | 14 (33.3) | 3(21.4) | 11(39.3) | 0.418 |
| FPG, mmol/L | 7.1±0.7 | 6.1±1.0 | 8.2±0.4 | 0.210 |
| HbA1c (%) | 7.3±1.1 | 5.4±0.4 | 9.3±0.1 | 0.139 |
| Total cholesterol, mmol/L | 4.7±0.5 | 4.8±0.9 | 4.7±0.9 | 0.626 |
| Triglycerides, mmol/L | 2.0±0.5 | 1.4±0.9 | 2.5±0.5 | 0.431 |
| HDL cholesterol, mmol/L | 1.4±0.2 | 1.7±0.1 | 1.2±0.1 | 0.133 |
| LDL cholesterol, mmol/L | 2.6±0.4 | 2.4±0.7 | 2.8±0.5 | 0.269 |
| CAC score | 55.5±18.8 | 39.0±39.0 | 72.0±8.0 | 0.345 |
| FSCN1, ng/mL | 17.0±3.6 | 13.3±0.6 | 18.8±0.5 | <0.001 |

**Notes**: Values are expressed as mean ± SD or number (%). Statistical significance was assessed using two-tailed t-tests.
**Abbreviations**: CHD, coronary heart disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, Hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

**Table 2** OR of CHD for FSCN1 Estimated by Logistic Regression

| Characteristic | Model 1 OR (95% CI) | *P* Value | Model 2 OR (95% CI) | *P* Value |
|---|---|---|---|---|
| FSCN1 | 2.37 (1.36–4.08) | 0.002 | 2.82 (1.35–5.91) | 0.006 |

**Notes**: Model 1: unadjusted. Model 2: adjusted for sex, age, diabetes, and hypertension.
**Abbreviations**: OR, odds ratios; CHD, coronary heart disease.

## Effects of FSCN1 Knockdown on MAECs Pyroptosis and Migration

To investigate the effect of FSCN1 on ECs, we knocked down FSCN1 by infecting siRNA to select the one with the strongest knockdown efficiency evident of which Western blotting confirmed a ~75% fall in at the protein level and ensure that siFSCN1 infection had lower FSCN1 expression (Figure 6A and B). It was demonstrated that pyroptosis is involved in atherosclerosis and plays an important role in atherosclerotic lesion progression and plaque stability.[15] To study whether FSCN1 plays a role in ECs pyroptosis, the expression levels of proteins involved in pyroptosis were detected. As shown in Figure 6C–G, the expression levels of pyroptosis markers, including NLRP3, GSDMD, Caspase-1, and IL-1β, were significantly elevated in ox-LDL-treated MAECs. While ox-LDL activation increased the expression of pyroptosis-related protein, this effect was reversed by si-FSCN1 treatment. FSCN1, a member of the Fascin family of actin-binding proteins, has been associated with cell migration.[11] To clarify the effect of FSCN1 knockdown on ECs migration capacity, transwell and scratch wound assays were used. The results showed that FSCN1 knockdown significantly increased MAECs migration (Figure 6H–K).

# Discussion

Atherosclerosis (AS) is a chronic inflammatory disease characterized by lipid accumulation of the arterial wall.[16,17] Moreover, valid prognostic biomarkers and putative therapeutic strategies targeting atherosclerosis can alter patient outcomes and may open the way for atherosclerosis protection.[18,19] However, at present, there are not enough studies of markers and treatment targets on the prognosis of AS. It is known that GEO is a public microarray dataset that can mine

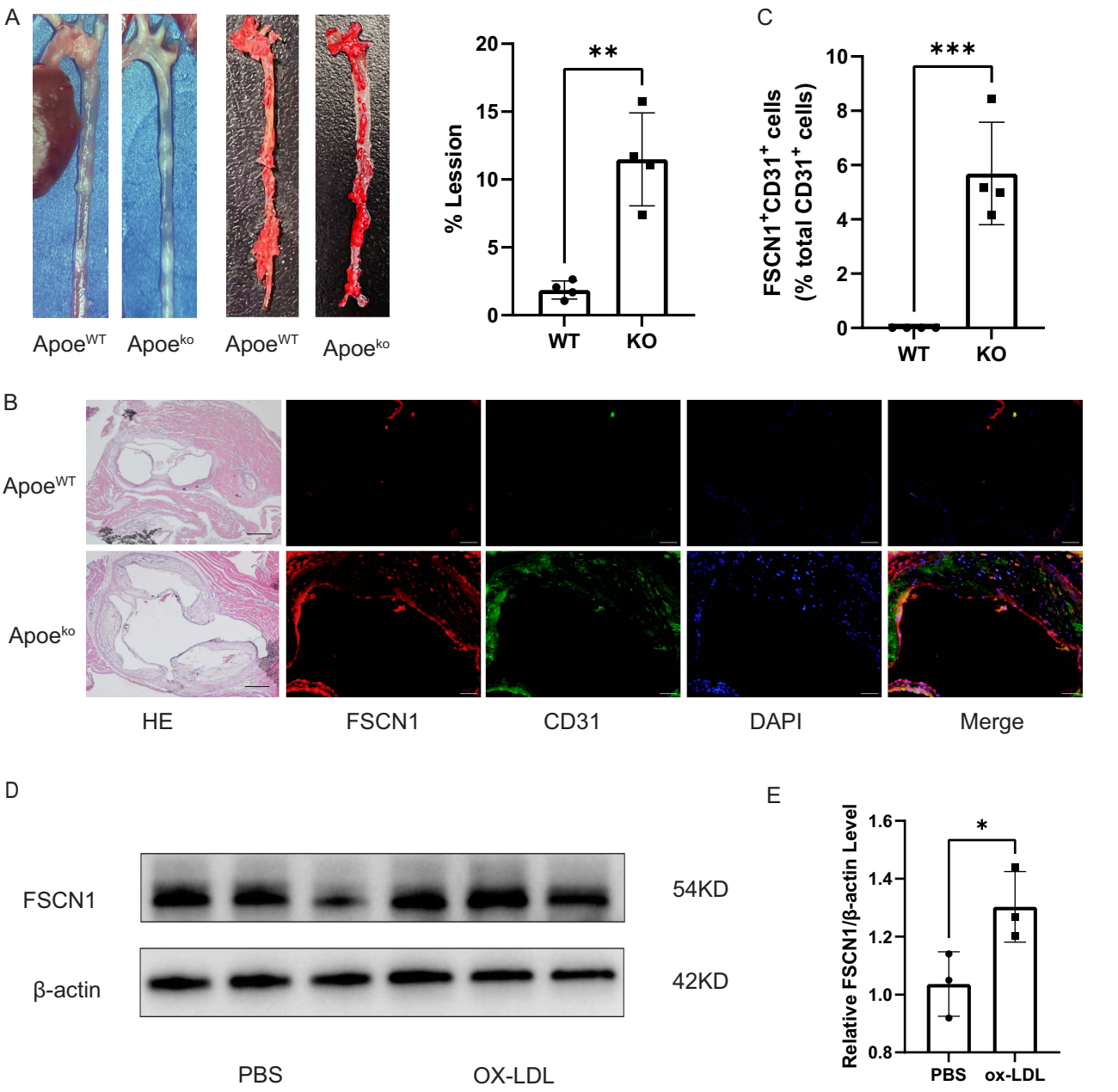


**Figure 5** The expression of FSCN1 during AS in vivo and in vitro. (**A**) The representative images of Oil red O-staining of en face aorta. **P<0.01, compared with Apoe$^{ko}$ group. (**B**) Representative images of aortic root sections stained with H&E staining and FSCN1 expression in aortic macrophages of Apoe$^{ko}$ hyperlipidemic and Apoe$^{WT}$ mice by co-staining with ECs marker CD31. Scale bars are 200 μm (H&E staining) and 50μm (Immunofluorescence). (**C**) quantitative data for FSCN1$^{+}$CD31$^{+}$cells. ***P<0.001, compared with Apoe$^{ko}$ group. (**D** and **E**) MAECs treated with ox-LDL or PBS for 24 h. The protein levels were determined by western analyses. *P<0.05, compared with the PBS group.

consolidate and integrate multifaceted data to discover new targets for disease. GSE206239, a single-cell RNA-seq for the aortic arch of Apoe$^{ko}$ mice, was firstly identified a high-resolution characterization of monocytes/macrophages and strong interactions between EC and SMCs in HFD groups. Importantly, ECs expressed a higher level of FSCN1. Furthermore, GSE43292 (32 atheroma plaque and 32 macroscopically intact tissue) and GSE28829 (16 advanced atherosclerotic plaque and 13 early atherosclerotic plaque) microarray datasets were used to verify. Based on the results of the Bioinformatics analysis, we suspected that FSCN1 in ECs may be a new diagnostic marker and therapeutic target for atherosclerosis.

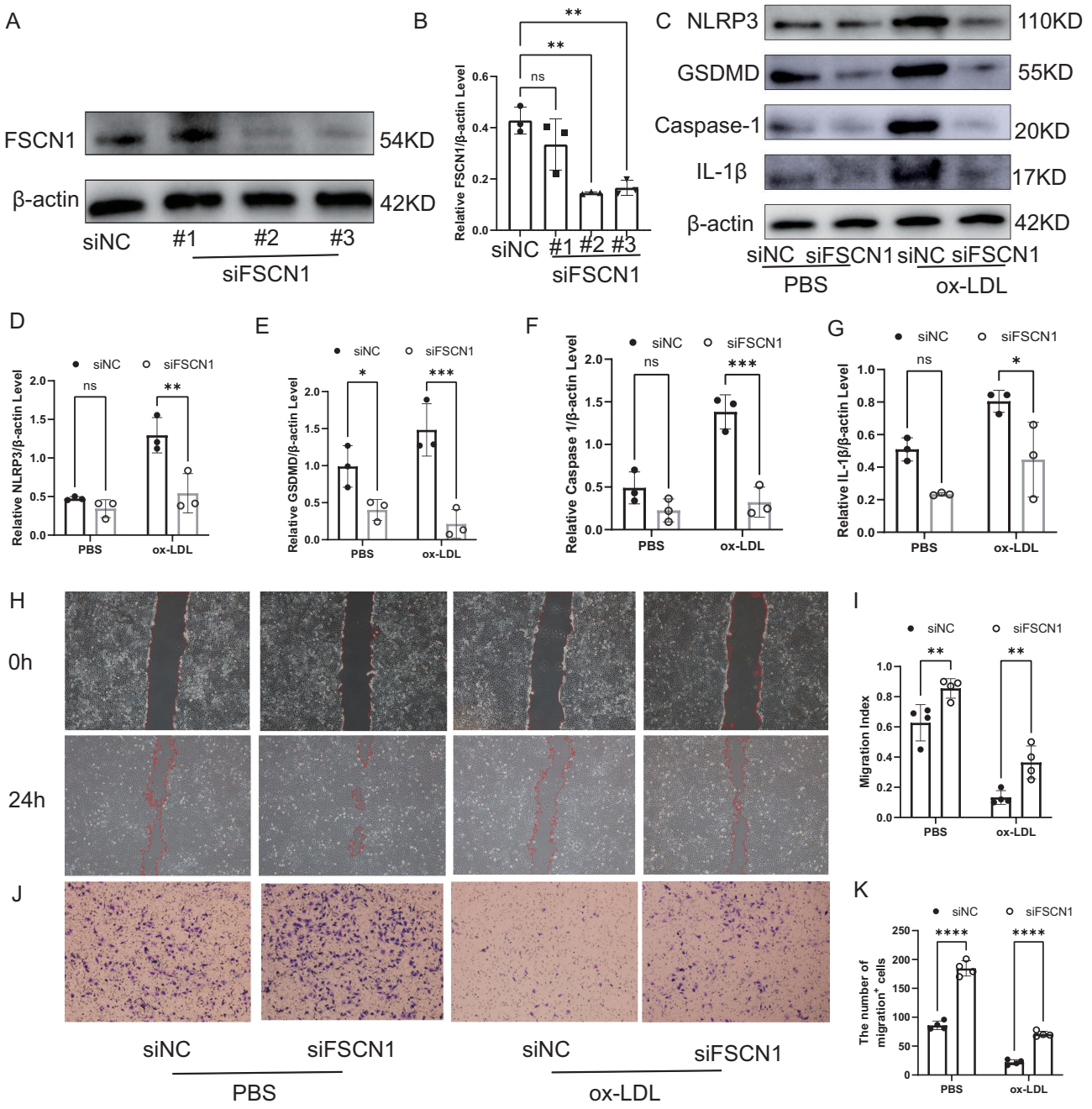


**Figure 6** Effect of FSCN1 on MAECs pyroptosis and migration. (**A**) Western blot was used to detect the knockdown efficiency of FSCN1 with three unique siRNAs. (**B**) The relative expression of the target protein was measured by Image J software. **P<0.01, compared with the siNC group. (**C**) The pyroptosis-related protein expression levels were measured by Western blotting analysis. (**D**) NLRP3; (**E**) GSDMD; (**F**) caspase-1; (**G**) IL-1β. β-actin levels were used as an internal control. The histogram reports mean ± SEM of protein band density from three experiments (normalized by comparison with β-actin). (**H** and **I**) Scratch wound assay was used to evaluate the cell migration capacity. (**J** and **K**) Representative images of transwell migration assay. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, compared with the siNC group.

In the present study, we revealed that the serum level of FSCN1 in CHD patients was significantly higher than that in the NO-CHD group. However, the correlation between the serum level of FSCN1 and lipid indexes (TC and LDL-C) had no statistical significance. Though our results had no statistical significance, its p-value was close to 0.05. If we expanded the sample size, the results might be more convincing. It is necessary to further explore the correlation between FSCN1 and lipid indexes by expanding the number of patients included in the study. Based on these observations, we established the AS model by Apoe$^{ko}$ mice and found that FSCN1 was highly expressed in ECs in atherosclerotic plaque. In addition,

FSCN1 was also upregulated in vitro MAECs cells treated with ox-LDL. To sum up, FSCN1 might be a biomarker in AS. However, the mechanism of FSCN1 in AS needs to be further explored.

FSCN1, a marker protein for mature dendritic cells (DCs),[20] plays a crucial role in DC maturation and migration.[21,22] Additionally, FSCN1 has originally been found to be involved in many different cancers and improved the motility of tumor cells to promote invasion and metastasis.[23–25] Previous studies demonstrated that vulnerable lesions in human carotid endarterectomy specimens were characterized by increased numbers of FSCN1-expressing mature DCs.[26] Here, we found that FSCN1 expression is a marker for the progression of AS. Our results are consistent with the previous study.[26]

It was reported that macrophage pyroptosis is implicated in atherosclerosis.[15,27] Pyroptosis, a novel proinflammatory-regulated cell death,[28] is the cleavage of GSDMD by inflammatory protease caspase-1 after NLRP3 inflammasome activation with subsequent pyroptotic cell death and cytokine IL-1β and IL-18 release.[29] FSCN1 was regulated by NLRP3 induced-IL-1β in tubular epithelial cells, indicating that FSCN1 might be involved in pyroptosis.[30] We demonstrated that FSCN1 knockdown attenuated pyroptosis in MAECs treated with ox-LDL. It was known that endothelial cell death including pyroptosis induces plaque instability and are closely associated with the progression of atherosclerosis.[31] Thus, our results provided evidence that FSCN1 in ECs could aggravate atherosclerotic plaque instability.

Following endothelial death, the proliferate and migration of the neighboring mature endothelial cells to heal the wound is the way to alleviate atherosclerosis.[32] FSCN1 was identified as an actin-bundling protein-regulating cell adhesion and migration in mature DCs, CAL-27, and SCC-25 cell lines.[9,33–35] Our study found that FSCN1 knockdown promotes MAECs migration. It was suggested that FSCN1 may have a regulatory effect on the accumulation of MAECs in plaques. However, the mechanism of FSCN1 in suppressing EC migration needs to be further explored.

The limitations of this study are as follows: 1) This study is a single center study with a relatively small sample size, which can easily lead to bias in the research data, further validation requires a large sample size multicenter study. (2) The specific mechanism by which FSCN1 regulates pyroptosis and migration in MAECs needs to be further explored.

## Conclusion

In summary, we have demonstrated that FSCN1 expression is upregulated during atherosclerosis, and FSCN1 knockdown in EC could attenuate the development and progression of atherosclerosis. The proatherogenic effects of FSCN1 in EC might involve acceleration of pyroptosis, and inhibition of migration. Therefore, FSCN1 may be a target for ameliorating atherosclerosis in the future.

## Data Sharing Statement

All data used to support the findings of this study are available from the corresponding author upon request.

## Ethics Approval and Informed Consent

This study was performed with the Declaration of Helsinki and approved by Ethics Committee of the Affiliated hospitals of Jiangsu University (approval number: KY2021K1226). Informed consent was obtained from all individual participants included in the study. Ethics approval for animal care and experimental procedures of this study was obtained from the Animal Care and Use Committee of Jiangsu University (approval ID: SCXK2013-0011). All animal experiments followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.5–23, revised 1996; NIH Bethesda, MD, USA).

## Author Contributions

Lili Zhang designed the study. Han Jiang contributed to the animal and clinical data. Lihua Li assisted with getting mice tissues. Yongjiang Qian performed the data analysis. Zhen Sun wrote the paper. Zhongqun Wang supervised the study. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This work was supported by the National Natural Science Foundation of China (82370457); Jiangsu Provincial Key Research and Development Program (BE2022780); Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX23_3754).

## Disclosure

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

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