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ORIGINAL RESEARCH - CLINICAL

ABCA1-Super Enhancer RNA Promotes Cholesterol Efflux, Reduces Macrophage-Mediated Inflammation and Atherosclerosis

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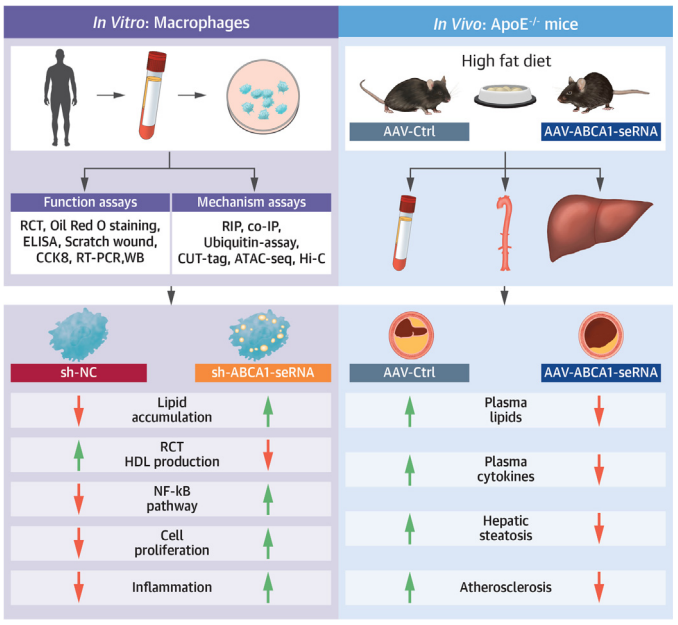
VISUAL ABSTRACT



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HIGHLIGHTS

- **ABCA1-seRNA was identified as a novel super-enhancer RNA.**
- **ABCA1-seRNA positively regulates ABCA1 expression by recruiting mediator (MED23) and transcriptional factor (RXRα and LXRα).**
- **ABCA1-seRNA negatively regulates NF-κB activation by ubiquitination degradation P65.**
- **ABCA1-seRNA involves in the constitute of chromatin loops and it also relies on the loop to produce effects on anti-atherosclerosis.**

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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SUMMARY

We describe a previously uncharacterized ATP-binding cassette A1 super enhancer RNA (ABCA1-seRNA)-mediated cholesterol efflux. In addition, it promoted macrophage inflammatory cytokine release, and was causally correlated with coronary artery disease severity. Mechanistically, ABCA1-seRNA upregulated cholesterol efflux by interacting with mediator complex subunit 23 and recruiting retinoid X receptor-alpha and liver X receptor-alpha to promote ABCA1 transcription in a *cis* manner. Meanwhile, ABCA1-seRNA induced P65 ubiquitination degradation, and thereby repressed the macrophage inflammatory response. Consistently, overexpression of ABCA1-seRNA in ApoE$^{-/-}$ mice decreased plasma lipids, cytokines, and atherosclerotic plaques. These findings indicate ABCA1-seRNA is a critical epigenetic regulator that maintains cholesterol homeostasis and modulates inflammation, thus promising a therapeutic target for atherosclerotic cardiovascular diseases. (JACC Basic Transl Sci. 2024;9:1388–1405)

ABBREVIATIONS AND ACRONYMS

ABCA1-seRNA = ATP-binding cassette A1 super enhancer RNA

ASCVD = atherosclerotic cardiovascular disease

ATAC = assays for transposase-accessible chromatin with high-throughput sequencing

BL-Hi-C = Bridge Linker High-through chromosome conformation capture

CAD = coronary artery disease

CUT&Tag = Cleavage Under Targets and Tag mentation

HDL = high-density lipoprotein

LXRa = liver X receptor-alpha

MED23 = Mediator complex subunit 23

RCT = cholesterol reversal transport

RXRa = retinoid X receptor-alpha

Atherosclerotic cardiovascular disease (ASCVD), the leading cause of mortality worldwide,[1] is a major economic and health care burden on society.[2] Therefore, elucidating the mechanisms that regulate the pathophysiology of atherosclerosis is crucial for developing novel therapeutic targets for ASCVD.[3] Atherosclerosis is a lipid-driven chronic inflammatory process initiated by the accumulation of low-density lipoproteins (LDLs), remnant lipoprotein particles, and macrophages in vessel walls.[3,4] Some guidelines have suggested that LDL-cholesterol (LDL-C) is an optimal therapeutic target for ASCVD.[5] However, ASCVD has a high incidence rate even among individuals with low LDL-C levels.[6] Thus, there is an urgent need to develop novel alternative therapies targeting non-LDL-C lipids and inflammation to mitigate residual ASCVD risk.[7]

As reported, cholesterol reversal transport (RCT) is the process of transporting excess cholesterol from peripheral tissue to liver tissue via high-density lipoprotein (HDL).[8] Functional HDL mediates cholesterol efflux, facilitates foam cell migration, and induces M2 macrophage polarization. Thus, the serum HDL concentration is a major contributor to the regression of ASCVD.[9] It is well-established that ABCA1 and ABCG1 synergistically participate in HDL synthesis, pre-b-HDL discoid particles were generated by ApoA1 and free cholesterol with ABCA1 mediated, free cholesterol uptake by these particles leads to their maturation to spherical HDL2, which subsequently serves as an acceptor of more cholesterol through ABCG1 transporter leading to maturation to HDL3 particles ultimately involved in RCT.[10,11] ABCA1, a key regulator of HDL biogenesis, attenuates cholesterol accumulation and residual inflammatory effect via macrophages. Patients with Tangier disease harboring loss-of-function mutations in ABCA1 are susceptible to coronary heart disease and are characterized by HDL deficiency and increased plasma levels of interleukin (IL)-1β and IL-18,[12] indicating that ABCA1 may be a promising target to preventing ASCVD.

Enhancers are classical *cis*-acting regulatory elements that recruit transcription factors (TFs) and collaborate with cofactors to exert their functions in tissue-specific and temporal-specific gene regulation.[13] In contrast to typical enhancers, super enhancers (SEs) are a cluster of enhancers that encompass a large open chromatin domain enriched with acetylated histone 3 lysine 27 (H3K27ac) and master TFs.[14] SE RNAs (seRNAs), a subclass of noncoding RNAs transcribed from active SEs,[15] play critical roles in the transcription of various genes[16] and the modulation of chromatin looping.[16] However, the physical and functional correlations between the dynamic transcriptional changes in seRNAs and the transcription of cholesterol homeostasis-related genes remain unclear.

To identify novel seRNAs involved in maintaining cholesterol homeostasis, we performed genomic screening that integrated the SE database,[17] long noncoding RNA (lncRNA) database,[18] and signals of cholesterol traits from genome-wide association studies (GWASs).[19] ABCA1-seRNA, an SE-derived lncRNA, initially garnered our interest and was further demonstrated to be a key epigenetic regulator that robustly promotes cholesterol efflux and attenuates macrophage inflammation and atherosclerotic lesion formation. Our findings revealed a novel regulatory role for an SE-derived lncRNA in the

fundamental aspects of atherosclerosis development and demonstrated a potential therapeutic target for ASCVD.

METHODS

The methods are extensively described in the Supplemental Appendix (Supplemental Tables 1 to 18).

STUDY SUBJECTS AND PERIPHERAL BLOOD MONONUCLEAR CELL ISOLATION. The peripheral blood samples and epidemiological data were collected from our research center in the Department of Clinical Chemistry, Tongji Hospital. All subjects were recruited from the Tongji Hospital in Wuhan, Hubei Province. A total of 1,854 patients were enrolled in the study. All participants signed informed consent documents and accepted a standard examination in the morning that included the following: a detailed past medical history inquiry, such as hypertension, type 2 diabetes, hyperlipidemia history of coronary artery disease (CAD), and other essential information; anthropometric parameters, for instance sex, age, heart rate, measurement of blood pressure, and drinking and smoking status; serum biochemistry index of glucose, total cholesterol, triglycerides, HDL-cholesterol (HDL-C) and LDL-C measured by the Roche modular DPP system, according to standard procedures. Peripheral blood mononuclear cells (PBMCs) were isolated from each patient's peripheral whole blood with Human Lymphocyte Separation Medium (TBD Science, LTS1077) according to the manufacturer's instructions. In brief, fresh anticoagulant blood was mixed with saline solution and then carefully added to the surface of lymphocyte separation medium. Next, we gradient centrifugated for 30 minutes at 300*g* and collected lymphocytes. Lymphocytes were washed and suspended with phosphate-buffered saline (PBS) for 5 minutes at 1,000 rpm. Finally, the obtained cell pellets were lymphocytes. The characteristics of the study subjects are listed in Supplemental Table 1.

ETHICS STATEMENT. Human blood samples were collected from Tongji Hospital (Wuhan, China) between November 2019 and October 2021. All participants have written informed consents. This study was authorized by the Ethics Review Board of Tongji Hospital and Tongji Medical College and performed in accordance with the Declaration of Helsinki. The animal study was approved by the Committee on the Ethics of Animal Experiments of the Animal Research Committee of Tongji College and conducted strictly abiding by the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

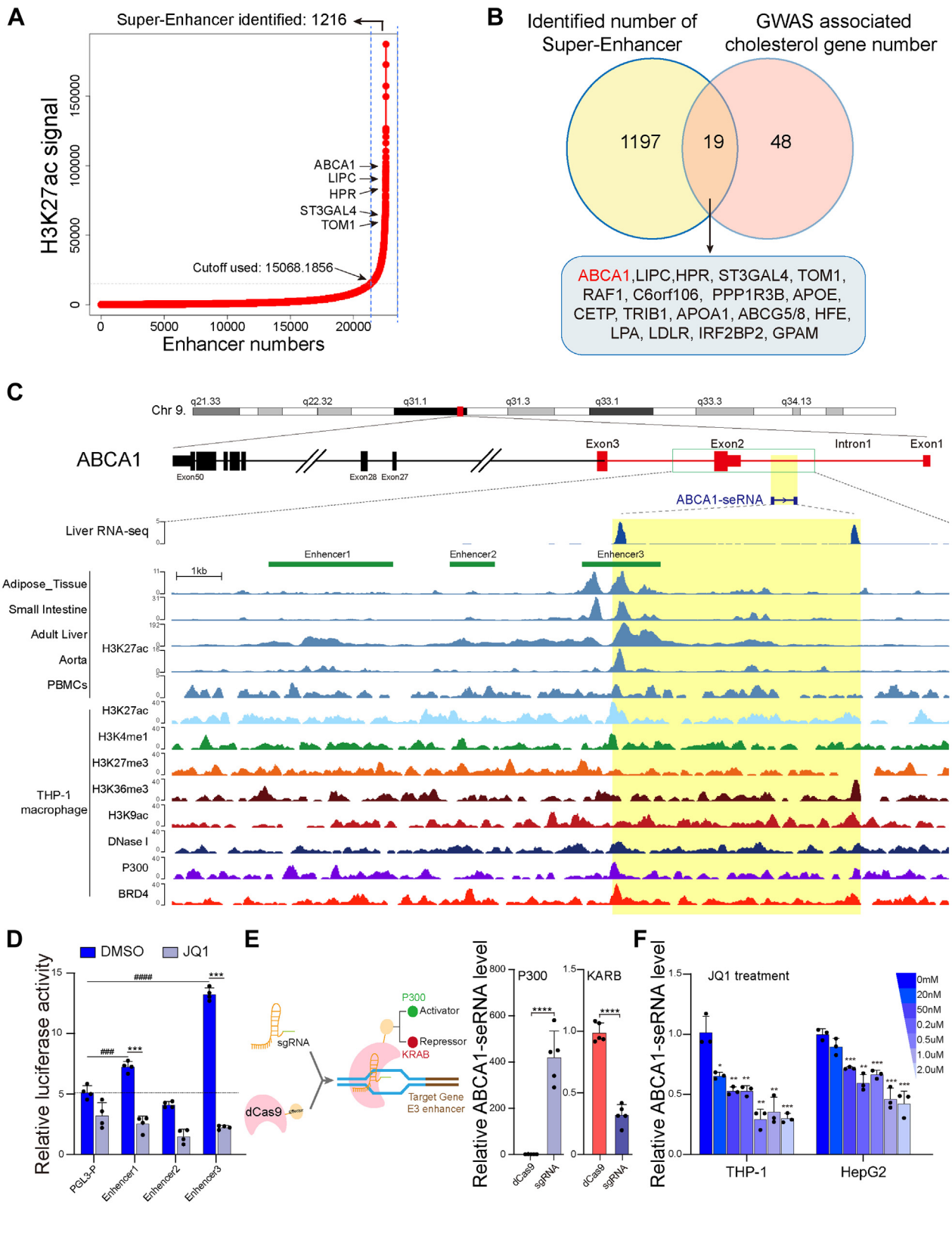
CELL CULTURE AND TRANSFECTION. Human aortic smooth muscle cells (HASMCs), human hepatocellular carcinoma cells (HepG2s), human umbilical vein endothelial cells (HUVECs), and human monocyte leukemia cells (THP-1) were obtained from ATCC. HASMCs and HepG2s were cultured in Dulbecco's modified Eagle's medium (Gibco, Life Technologies) with 10% fetal bovine serum (FBS) (Gibco, Life Technologies). HUVECs and THP-1 were cultured in RPMI 1640 Medium (HyClone) with 10% FBS (Gibco, Life Technologies). All cells were maintained in a humidified atmosphere incubator with 5% CO_2 at 37°C. A total of 50 nmol smart silence small interfering RNAs (siRNAs) or antisense oligonucleotides (ASOs) (Ribobio) against ABCA1-seRNA and negative control siRNAs (NC Ribobio) were transfected into HepG2 with lipofectamine2000 (Invitrogen) for 48 hours, and similar with transfect plasmids, according to the manufacturer's protocol. However, THP-1 cells transfect siRNAs with HiPerFect Transfection Reagent (QIAGEN) and plasmids were transfected with Attractene Transfection Reagent (QIAGEN) for 48 hours according to the manufacturer's protocol.

THP-1 cells were induced to be differentiated into macrophages, THP-1-derived macrophages (dmTHP-1), in vitro by treating phorbol 12-myristate 13-acetate (PMA) (MedChemExpress) with 100 nM final concentration for 48 hours. The details of the siRNA or ASO sequence are listed in Supplemental Table 2.

RNA SEQUENCING. RNA sequencing (RNA-seq) analysis was performed as previously. In brief, total RNA was extracted from indicated cells. The libraries were conducted by NEBNext UltraTM RNA Library Prep Kit (NEB #E7490), and then sequenced by Novaseq 6000. The reads were trimmed by Trimmomatic, aligned to human reference genome (hg38) by STAR. The read counts were produced with HTSeq-count. DESeq2 was used to analyze the differential expression genes according to reads per kilobase per million. The pathway analysis was performed by R or gene set enrichment analysis (GSEA) algorithm.[20] All sequence data have been uploaded to the GEO database.

BRIDGE LINKER-HI-C ASSAY. The Hi-C assay and library was conducted following the Bridge Linker-Hi-C (BL-Hi-C) protocol described with modifications, using MboI (NEB R0147) as the restriction enzyme. Briefly, 5×10^7 cells were washed twice with 1× PBS, then resuspended with a final concentration of 1% formaldehyde and cross-linked for 10 minutes with rotation at room temperature; a final concentration of 0.2 M glycine to quench the fixation for 5 minutes at

FIGURE 1 ABCA1-seRNA Is a Super-Enhancer-Derived Noncoding RNA



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room temperature. Cell pellets were resuspended by BL-Hi-C Lysis buffer (10 mM Tris, 10 mM NaCl, 0.2% NP40) containing 1× protease inhibitors for 10 minutes on ice. Nuclei were permeabilized with 0.5% sodium dodecyl sulfate on the thermomixer for 10 minutes at 62°C; a final concentration of 1% (v/v) Triton X-100 was added to quench the sodium dodecyl sulfate at 37°C. DNA was digested with NEBuffer 2 and 100 units of MboI at 37°C overnight on the thermomixer. The ends of restriction fragments were repaired for T4 DNA polymerase (Promeg(A) M4215) at 37°C for 50 minutes with agitation. Ten millimolar dATP solution (Thermo Fisher, 18252015) and Klenow Fragment (3′->5′ exo-) (NEB M0212) were added for A-tailing at 37°C for 50 minutes with agitation. For 1-step proximity ligation, we mixed 1,046 μL of ddH2O, 120 μL of 10 × T4 DNA ligase buffer, 4 μL of 200 ng/μL double-strand DNA Bridge linker, 12 μL of 10% (v/v) Triton X-100, 6 μL of 100 × bovine serum albumin, and 12 μL of T4 DNA ligase at 16°C overnight on a rotating wheel. After de-crosslinking at 65°C for 3 hours, ligated DNA was sheared to a length of approximately 300 to 500 base pair fragments and extracted with phenol/chloroform/isoamyl alcohol (25:24:1) and prepped for amplification Illumina sequencing library.

CLEAVAGE UNDER TARGETS AND TAG MENTATION. Cleavage Under Targets and Tag (CUT&Tag) assays were conducted with Hyperactive In Situ ChIP Library Prep Kit for Illumina (Vazyme Biotech, TD903) according to the manufacturer's protocol. Briefly, THP-1 macrophages were counted and harvested at room temperature. Then, cells were resuspended with 500 μL wash buffer and centrifuged for 3 minutes at 600*g*. Subsequently, cells were aliquoted 100 μL in prepared ConA beads and incubated on a rotating platform for 10 minutes at room temperature. Then, indicated primary antibodies and second antibodies were combined with cells. Hyperactive Tn5 transposon was applied to accurately cleavage DNA sequences near the target proteins. A total of 300 μL tagmentation buffer was added into product and incubated at 37°C for 1 hour to fragment the DNA sequences. At last, DNA was extracted for follow-up experiments. The details of antibodies are listed in Supplemental Table 10.

CUT&Tag-SEQ. Libraries for high-throughput sequence were amplified with Hyperactive In-Situ ChIP Library Prep Kit for Illumina (Vazyme Biotech, TD903) according to the protocol. A universal adapter i5 and a uniquely adapter i7 primer were from True-Prep Index Kit V4 for Illumina (Vazyme Biotech, TD501) with different barcodes for each sample. A thermocycler with a heated lid was used to amplify libraries under suitable conditions. Polymerase chain reaction (PCR) production purification was performed with 1.2 × volume of VAHTS DNA Clean Beads (Vazyme, N411-01) relative to PCR products according to the manufacturers. Finally, libraries were eluted with 22 μL sterilized ultrapure water and separated by magnetic stand. Library quality control was performed by a fragment analyzer and then sequenced in the Illumina NovaSeq 6000 platform. High-throughput sequencing service was provided by Jiayin Biotechnology Ltd.

ASSAYS FOR TRANSPOSASE-ACCESSIBLE CHROMATIN WITH HIGH-THROUGHPUT SEQUENCING. The assays for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) were performed by Jiayin Biotechnology Ltd. and libraries were conducted using the protocols. Briefly, 4×10^5 THP-1 cells were collected, washed by cold 1 × PBS, and cell pellets were resuspended in 50 μL cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPAL CA-630) to generate the nuclei pellets. Next, the nuclei pellets were resuspended in the transposition reaction mix (25 μL T(D) 2.5 μL TDE1, 22.5 μL Nuclease Free H_2O) and incubated at 37°C for

FIGURE 1 Continued

(A) Distribution of H3K27ac chromatin immunoprecipitation sequencing (ChIP-seq) signals with respect to enhancers revealed 2 classes of enhancers. (B) Venn diagram showing the number of overlapping genes between the super enhancers and cholesterol genes as indicated. (C) Coordinate and enhancer mark modifications of ATP-binding cassette A1 super enhancer RNA (ABCA1-seRNA). Upper panel: Schematic of the human ABCA1 and ABCA1-seRNA gene loci on the Integrative Genomics Viewer (IGV). Middle panel: ChIP-seq profiles of H3K27ac at 3 enhancers (Enhancer1-Enhancer3) in different human tissues. Lower panel: Cleavage under targets and tag (CUT&Tag) sequencing profiles displaying different histones, DNase, P300, and BRD4 identifications at the ABCA1-seRNA gene locus. Yellow shade indicates amplified ABCA1-seRNA region. (D) Results of the luciferase reporter assay using the HEK293T cells after co-transfection with the pGL3-promoter reporter vector constructed with 3 indicated enhancers. PGL3-P was used as baseline control. $^{\#}P < 0.05$ vs PGL3-P, $^{*}P < 0.05$ vs dimethyl sulfoxide (DMSO). (E) Left: schematic diagram of clustered regularly interspaced palindrome repeat (CRISPR)-dead caspase 9 (dCas9) system. Right: Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analyses of ABCA1-seRNA expression after the fusion of the site-specific single-guide RNAs (sgRNAs) of the CRISPR-dCas9-p300 and dCas9-KRAB system with enhancer 3 region. (F) qRT-PCR analyses of ABCA1-seRNA expression after treated with different indicated concentrations of JQ1. $^{*}P < 0.05$ vs 0 mM JQ1. In bar plots, 1 dot represents data from 1 experiment. Results are expressed as mean ± SD derived from at least 3 independent experiments. For statistical analysis, the *P* values were calculated using a 2-tailed Student's *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

30 minutes. The DNA was purified by QIAGEN MinElute PCR Purification Kit followed by PCR amplification and library quality control. After that, the amplified libraries were sequenced by Illumina NovaSeq 6000.

The raw data were trimmed by Trimmomatic in which the main parameters were used the default reference values. Then, the trimmed data were aligned with Burrows-Wheeler-Aligner-Maximum Exact Match (BWA-MEM) to human reference genome (hg38) from University of California-Santa Cruz. The duplicate reads were removed using the markdup function of samtools. For peak calling, we used MACS2 to identify the peaks with the following parameters: -nomodel -f BAMPE -keep-dup 1 -q 0.05 -B -SPMR. For the motif analysis, we analyzed the peak regions to predict motifs by HOMER and matched them with existing motifs in major databases (eg, HOMER, JASPAR) to find corresponding TFs.

STATISTICAL ANALYSIS. The primary clinical features of the categorical variables were presented as counts and percentages, and the medians for the continuous variables were 25th-75th percentiles (Q1-Q3). Chi-square tests or Fisher exact tests were used to compare categorical variables, whereas the Wilcoxon rank-sum test was used to compare continuous variables.

Data are presented as the mean ± SEM from multiple individual human experiments or as the mean ± SD from at least triplicate measurements in a representative experiment, as stated in figure legends. Two-tailed Student's *t*-test or 1-way analysis of variance (ANOVA) with Bonferroni's post hoc test for multiple comparisons was applied to compare normally distributed data between groups. Not normally distributed data are presented as the median with 25th-75th percentiles (Q1-Q3) and compared using the Wilcoxon rank-sum (2 groups) or Kruskal-Wallis test (>2 groups). Spearman correlation assay was used to assess associations between gene expression and related human serum biochemical parameters, whereas categorical variables were compared using the chi-square test. The threshold was a 2-fold change with a false discovery rate (FDR) cutoff value of 0.05 for all sequencing data. All analyses were performed with SPSS 23.0 for Windows (Microsoft), and *P* value <0.05 was considered statistically significant.

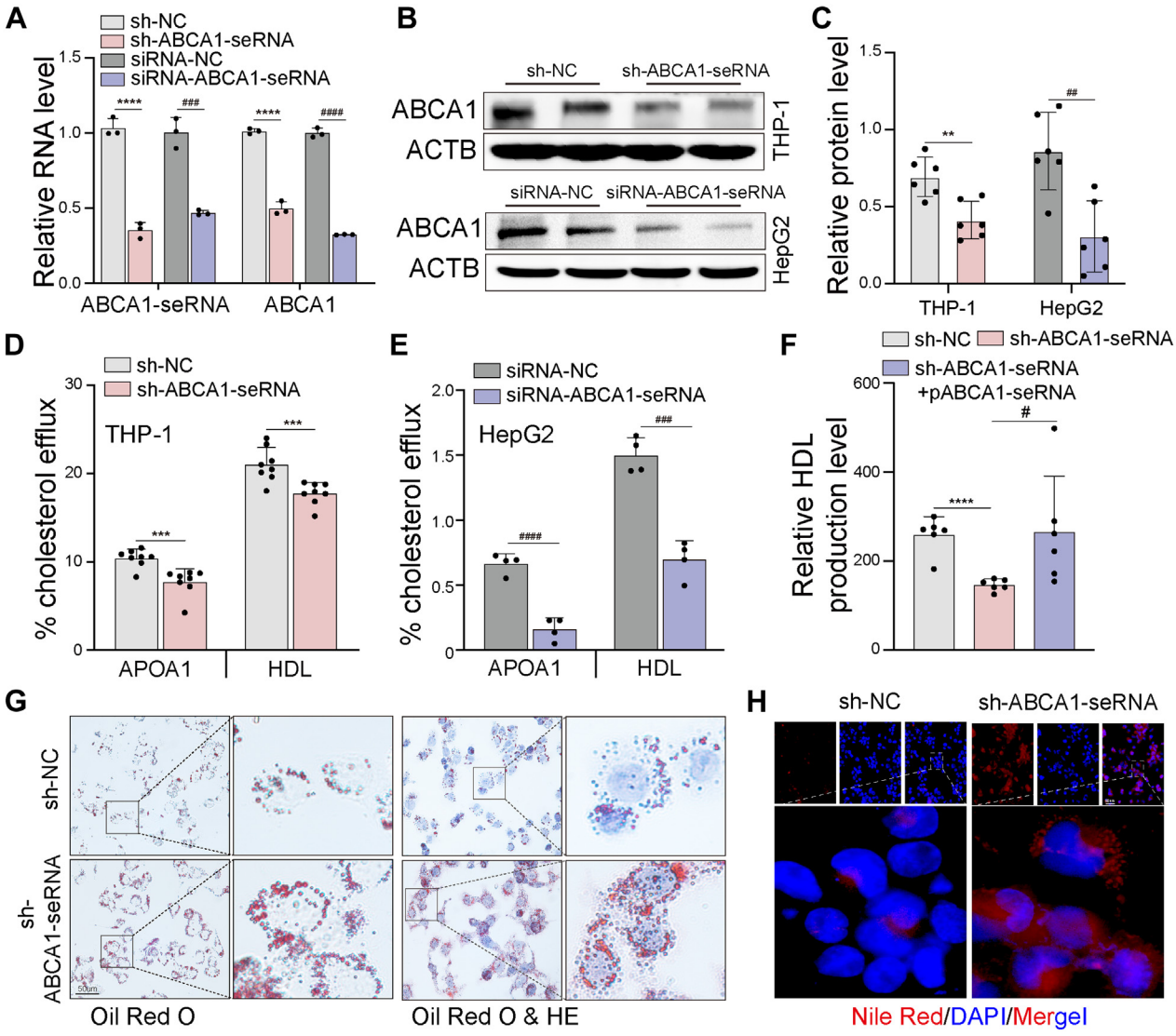
RESULTS

ABCA1-seRNA IS AN SE-DERIVED NONCODING RNA. To identify the key seRNAs involved in the regulation of cholesterol homeostasis, we initially performed integrated genomic screening and found that ABCA1 was associated with the highest levels of H3K27ac signal in the liver (**Figure 1A**, Supplemental Figure 1). Given that ABCA1 is a cholesterol homeostasis-related gene reported in GWAS (**Figure 1B**), we focused on this gene to examine SE-derived lncRNAs. An SE (SE_56105) that generates a long coding RNA (Supplemental Figure 2), named ABCA1-seRNA, was previously uncharacterized and garnered our interest. Similar to other lncRNAs, ABCA1-seRNA was transcribed with a poly A^+ tail (Supplemental Figures 3A and 3B) but without the coding capacity (Supplemental Figures 3C and 3D). RNAscope[21] analyses indicated that ABCA1-seRNA was detected in both the cytoplasmic and nuclear (Supplemental Figures 3E to 3G). RNA copy number analyses indicated that ABCA1-seRNA was present at approximately 10 copies per cell in THP-1 macrophages (Supplemental Figure 3H). Furthermore, with GW3965 stimulation, the expression was critically increased by ~5-fold (Supplemental Figure 3I).

One enhancer (Enhancer 3, named E3) was associated with significant enrichment of the H3K27ac signals (**Figure 1C**, middle). The luciferase assay results revealed that E3 was highly active (**Figure 1D**). In contrast, luciferase expression was significantly downregulated by the highly sensitive SE-associated gene transcriptional antagonist JQ1 (a BET bromodomain inhibitor of BRD4) (**Figure 1D**). Moreover, the transcriptional activity of ABCA1-seRNA was significantly induced in the presence of a transcriptional activation system (dCas9-p300) and repressed in the presence of a transcriptional repression system (dCas9-KRAB) with site-specific single-guide RNA specifically targeting the E3 region (**Figure 1E**). Reciprocally, ABCA1-seRNA expression was markedly repressed in the presence of JQ1 (**Figure 1F**).

ABCA1-seRNA IS A KEY REGULATOR OF ABCA1 TRANSCRIPTION AND CHOLESTEROL EFFLUX. Some lncRNAs regulate the expression of adjacent genes.[22] Hence, we hypothesized that *cis*-acting ABCA1-seRNA may impact the expression of ABCA1 and consequently regulate cholesterol efflux. Lentivirus-mediated RNA interference (RNAi) (Supplemental Figure 4A) was used to efficiently knock down ABCA1-seRNA (**Figure 2A**, Supplemental Figure 4B). ABCA1 was markedly downregulated following ABCA1-seRNA knockdown (**Figures 2A to 2C**). Accordingly, cholesterol efflux was downregulated with ABCA1-seRNA knockdown (**Figures 2D and 2E**). Similar results were confirmed using ASOs, the use of which involved a complementary acute loss-of-function approach targeting ABCA1-seRNA

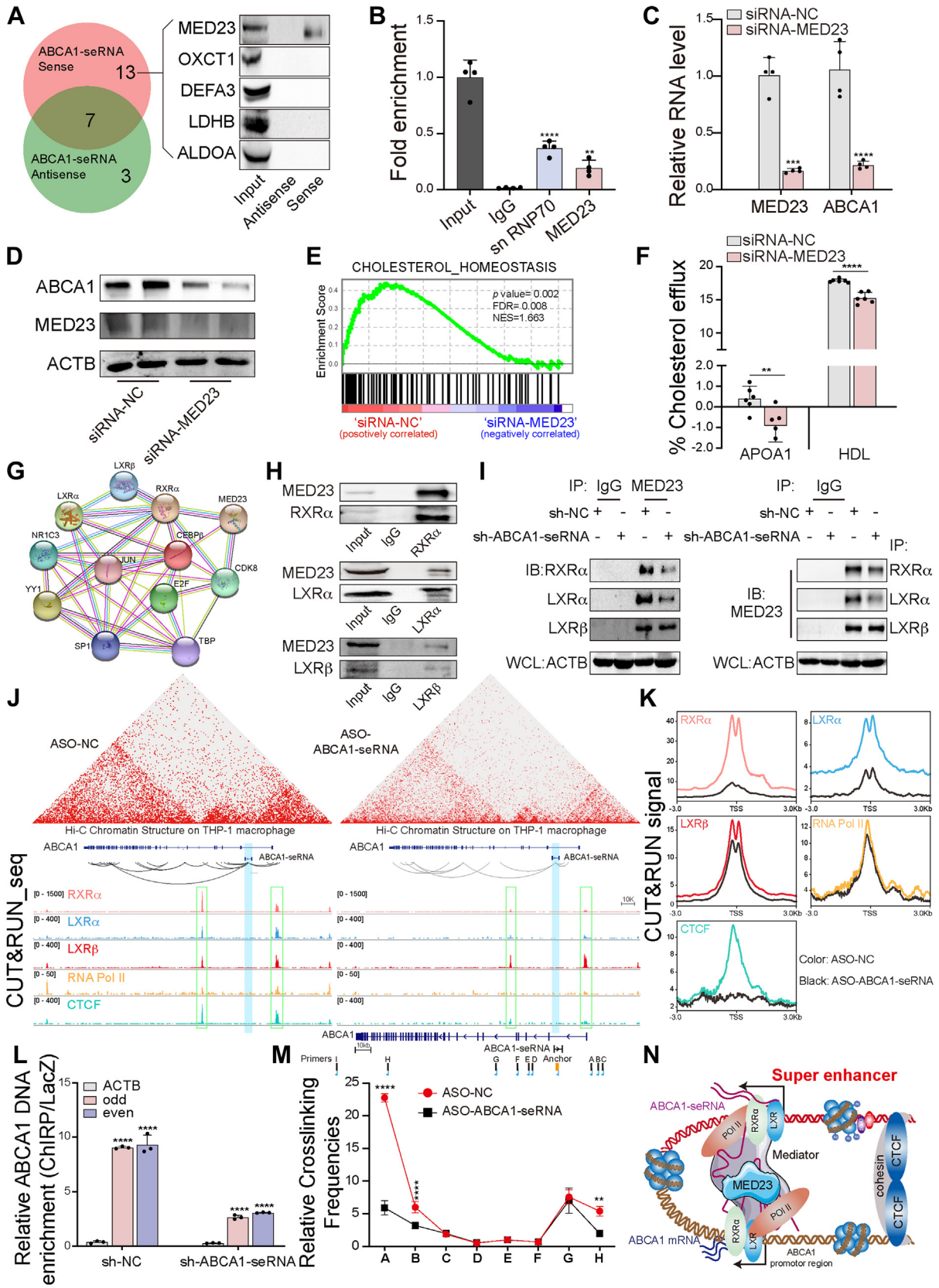
FIGURE 2 ABCA1-seRNA Modulates ABCA1 Expression and Affects Cholesterol Efflux



(A) qRT-PCR analyses of ABCA-seRNA and ABCA1 expression. Stable knockdown of ABCA1-seRNA using a short hairpin RNA (shRNA) and psi-LVRU6GP plasmid (sh-ABCA1-seRNA) or negative control (sh-NC). Approximately 70% decrease in ABCA1-seRNA levels cause at least 50% decrease in ABCA1 mRNA in THP-1 macrophages. Silencing, using custom smart silence small interfering RNA (siRNA) of ABCA1-seRNA (siRNA-ABCA1-seRNA) or negative control (siRNA-NC) in HepG2 cells, of ABCA1-seRNA significantly attenuates the expression of ABCA1 mRNA. (B and C) Western blot and quantitative analyses of ABCA1 protein level at indicated groups. (D and E) Cholesterol efflux was significantly downregulated with ABCA1-seRNA knockdown. (A to E) $^{*}P < 0.05$ vs sh-NC; $^{\#}P < 0.05$ vs siRNA-NC. (F) sh-ABCA1-seRNA significantly lowered high-density lipoprotein (HDL) production compared with sh-N (C) while ABCA1-seRNA overexpression rescued sh-ABCA1-seRNA-mediated reduction of HDL production. ABCA-seRNA was overexpressed by pcDNA3.1(+) vector, named pABCA-seRNA. $^{*}P < 0.05$ vs sh-NC; $^{\#}P < 0.05$ sh-ABCA1-seRNA+pABCA-seRNA vs sh-ABCA1-seRNA. (G) Hematoxylin-eosin (HE) and Oil Red O staining of lipid droplets at indicated THP-1 macrophages. (H) Nile red staining to observe lipid droplet accumulation. Lipid droplet accumulation in ABCA1-seRNA-knockdown THP-1 macrophages was upregulated more than that in the control. Cells were stained with Nile red to observe lipid droplet distribution (red). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). In bar plots, 1 dot represents data from 1 experiment. Results are expressed as mean ± SD derived from at least 3 independent experiments. For statistical analysis, the *P* values were calculated using a 2-tailed Student's *t*-test. compared with the control group. $^{\#/*}P < 0.05$, $^{\#\#/**}P < 0.01$, $^{\#\#\#/***}P < 0.001$. n.s., not significant; other abbreviations as in Figure 1.

expression (Supplemental Figures 4D to 4F). Conversely, overexpression of ABCA1-seRNA upregulated ABCA1 expression, resulting in a significant increase in cholesterol efflux (Supplemental Figures 4G to 4K). ABCA1-seRNA knockdown significantly decreased HDL production in THP-1 macrophages, whereas overexpression of ABCA1-seRNA rescued sh-ABCA1-seRNA-mediated reduction of HDL

FIGURE 3 ABCA1-seRNA Interacts With MED23 and Recruits RXRα and LXRα to Mediate ABCA1 Transcription



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production (**Figure 2F**); direct overexpression of ABCA1-seRNA also increased HDL in trend (Supplemental Figure 4L). We performed Oil Red O staining and found that lipid droplets were enriched in THP-1 cells with ABCA1-seRNA knockdown (**Figure 2G**). These results were further confirmed by Nile red staining (**Figure 2H**).

ABCA1-seRNA INTERACTS WITH MEDIATOR COMPLEX SUBUNIT 23 AND RECRUITS RXRα AND LXRα TO MEDIATE ABCA1 TRANSCRIPTION. To explore the molecular mechanism underlying the ABCA1-seRNA-mediated regulation of ABCA1 transcription, mass spectrometry was used to identify the proteins that bind to the transcript (**Figure 3A**, left). Mediator complex subunit 23 (MED23), has been identified as a candidate protein. The interaction between MED23 and ABCA1-seRNA was confirmed using western blotting and RNA immunoprecipitation (RIP) assays (**Figure 3A**, Supplemental Figures 5A and 5B). The knockdown of MED23 abolished the expression of ABCA1 (**Figures 3C and 3D**), indicating that MED23 is essential for ABCA1 transcription. Consistent with this, the cholesterol metabolism pathway (**Figure 3E**, Supplemental Figures 6A and 6B) and cholesterol efflux (**Figure 3F**, Supplemental Figure 5C) were significantly downregulated in macrophages with MED23 knockdown.

MED23 serves as a transcriptional coactivator[23] and regulates gene transcription by recruiting other TFs.[24] Protein-protein interaction network analyses indicated potential interactions among MED23, RXRα, and LXRβ (**Figure 3G**). Hence, we hypothesized that MED23 binds and interacts with these TFs to enhance the expression of ABCA1. Co-immunoprecipitation experiments further confirmed the interactions between MED23 and TFs (**Figure 3H**). ABCA1-seRNA knockdown significantly decreased the binding of MED23 to RXRα and LXRα but did not affect its binding to LXRβ (**Figure 3I**). Conversely, overexpression of ABCA1-seRNA promoted MED23-mediated recruitment of these TFs (Supplemental Figure 6C).

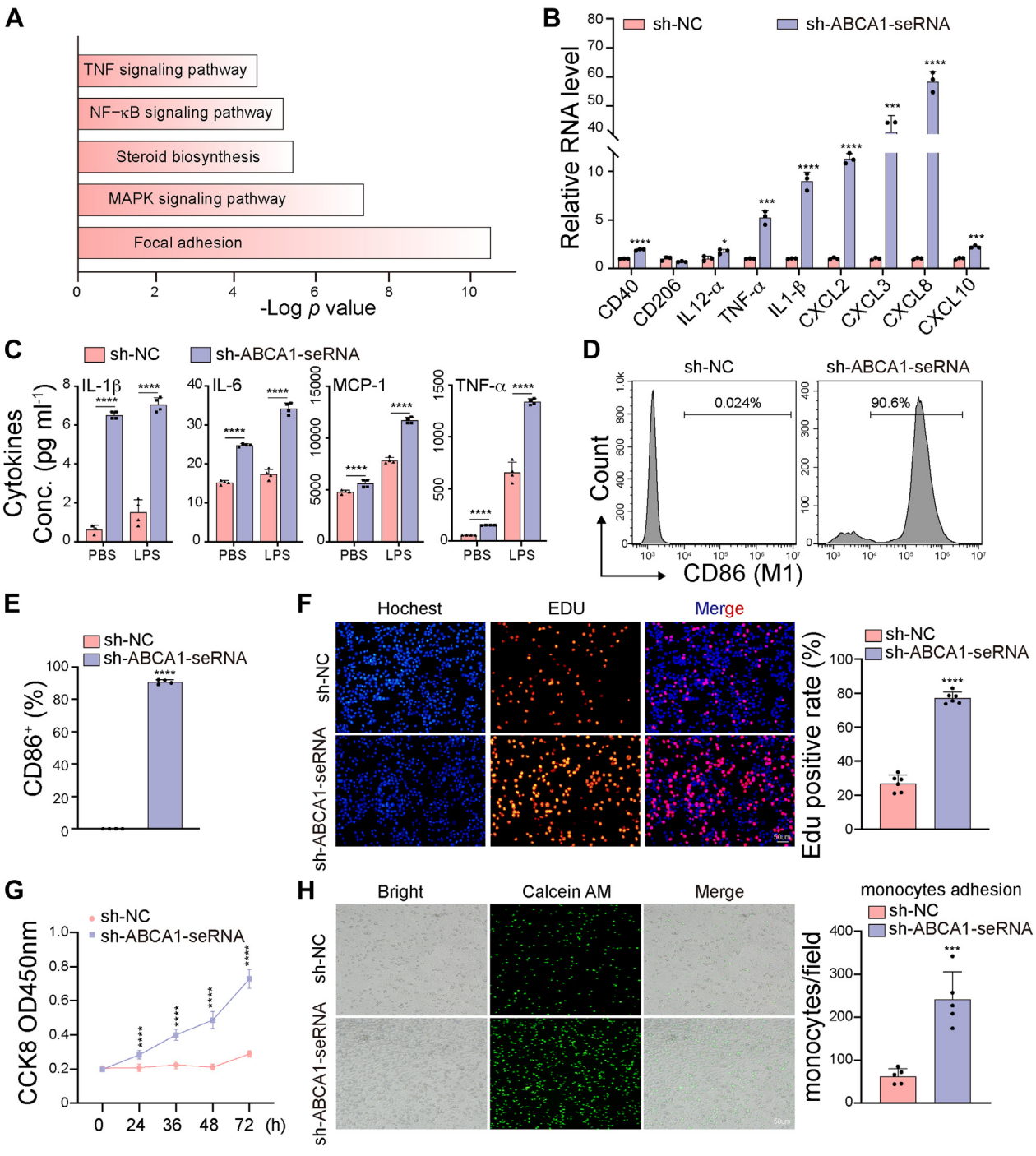
Subsequently, we performed a series of CUT&RUN assays and observed that the knockdown of ABCA1-seRNA significantly decreased the levels of TFs, RNA Pol II, and CTCF occupancy at the ABCA1 gene locus. Notably, knockdown of ABCA1-seRNA almost abolished peak enrichment of RXRα and LXRα around the transcription start site (TSS), indicating that ABCA1-seRNA is crucial and selectively enhances RXRα- and LXRα-regulated gene expression, including that of ABCA1 (**Figures 3J and 3K**).

To investigate whether ABCA1-seRNA was recruited to the ABCA1 gene locus, we conducted chromatin isolation by RNA purification quantitative PCR (**Figure 3L**). We found that ABCA1-seRNA could bind directly to the ABCA1 gene locus, and the corresponding levels were significantly attenuated by ABCA1-seRNA interference. To further determine the potential role of ABCA1-seRNA at the ABCA1 gene locus, we performed a BL-Hi-C[25] and 3C[26] assay to assess the interactions between ABCA1-seRNA and

FIGURE 3 Continued

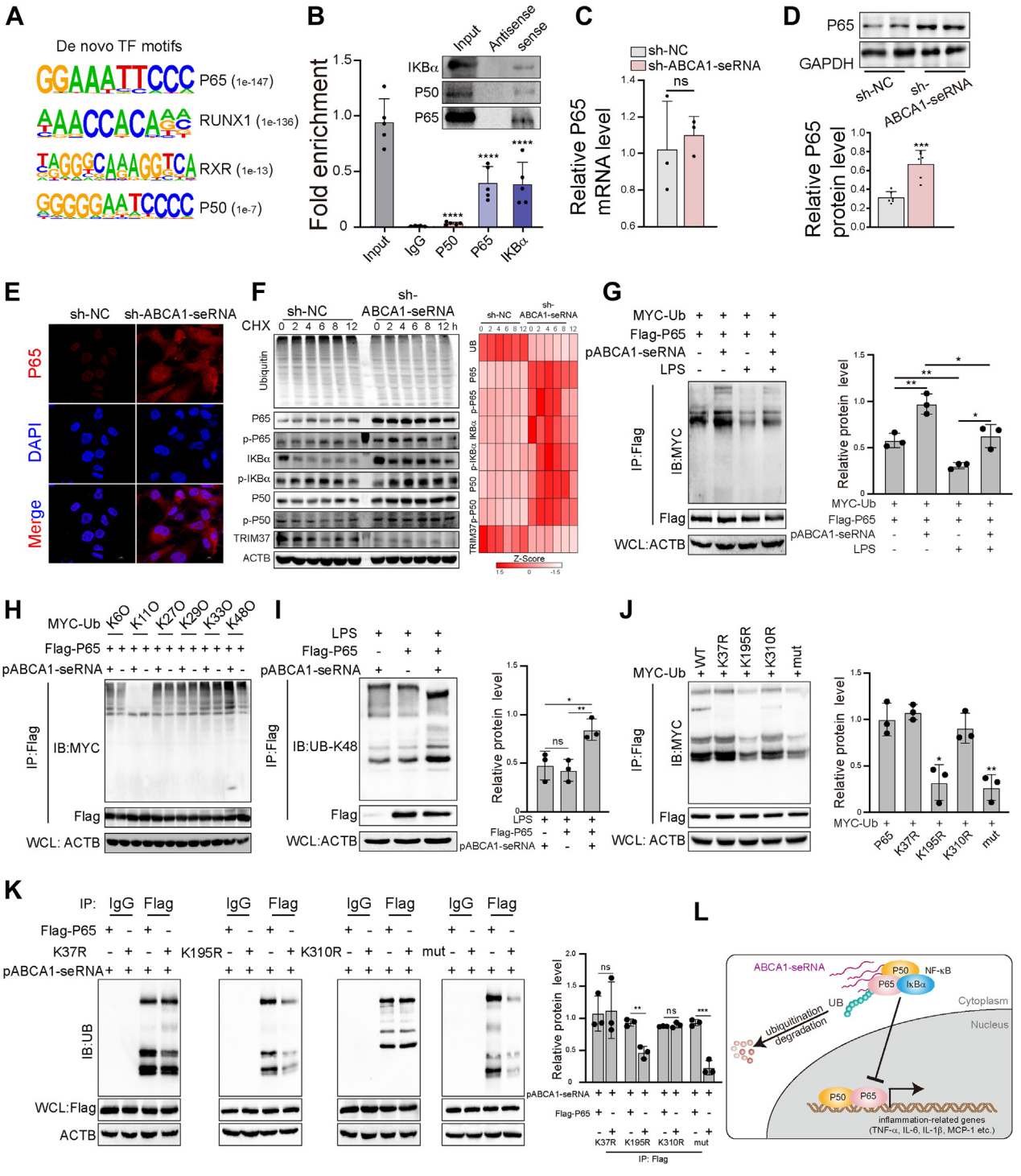
(A) Venn diagram demonstrated the number of proteins separately binding with ABCA1-seRNA-sense and ABCA1-seRNA-antisense strands (left). Western blotting analyses revealed that only mediator subunit complex 23 (MED23) specifically binds to ABCA1-seRNA (right). (B) RNA immunoprecipitation (RIP) and qRT-PCR analyses ABCA1-seRNA expression. *$P < 0.05$ vs immunoglobulin (Ig)G. (C and D) qRT-PCR and western blot analyses of MED23 and ABCA1 expression at indicated groups. (E) Gene set enrichment analysis (GSEA) data demonstrated that the cholesterol homeostasis pathway was enriched in macrophages lacking MED23 mRNA. The enrichment plot shows the enrichment scores of the cholesterol homeostasis pathway-associated genes. Datasets were compared by analysis of variance (ANOVA) and filtered using a 2-fold change in expression and false discovery rate (FDR) of 0.05. (F) Cholesterol efflux in siRNA-MED23 transfected macrophages was significantly downregulated when compared with that in siRNA-NC group. (C and F) *$P < 0.05$ vs siRNA-NC. (G) Protein-protein interaction (PPI) network predicted the interaction between transcription factors (TFs) and MED23. In the plot, nodes are denoted as proteins, and lines are used to represent their interactions. (H) Immunoprecipitation (IP) and western blotting analyses of binding of MED23 with retinoid X receptor-alpha (RXRα), liver X receptor-alpha (LXRα), and LXRβ proteins. (I) Western blot showing the results of Co-IP assays in THP-1 cells at indicated groups. (J) Bridge Linker High-through chromosome conformation capture (BL-Hi-C) annotation revealed interaction between ABCA1 and ABCA1-seRNA. Upper: Heatmap representing chromatin conformation around the ABCA1 locus measured by BL-Hi-C; Middle: Virtual Hi-C line plots showing cross-linking contact frequency at each indicated genomic position; Bottom: CUT&RUN-seq profiles of TFs, RNPII, and CTCF at ABCA1-seRNA and ABCA1 locus. (K) Transcription factors CUT&RUN-seq signals at the ABCA1-seRNA and ABCA1 locus transcriptional start site (TSS) (spanning −3.0 kb to +3.0 kb). (L) Chromatin isolation by RNA purification (ChIRP) analyses of the binding of ABCA1-seRNA to the ABCA1 gene promoter locus. Results are expressed as fold enrichment of ACTB or ABCA1 DNA sequence in ABCA1-seRNA compared with lacZ RNA ChIRP. *$P < 0.05$ vs ACTB. (M) Upper panel: Schematic graph shows the ABCA1 and ABCA1-seRNA gene loci. Lower panel: The relative cross-linking frequency between the anchor region and distal fragments (fragments A to H) was determined by chromosome conformation capture (3C) -qPCR and normalized to the control region (fragment I) to detect the effect of ABCA1-seRNA on chromatin interactions in the genome. *$P < 0.05$ vs ASO-NC. (N) Model for the super enhancer-derived noncoding RNA ABCA1-seRNA-mediated regulation of ABCA1 transcription. Right: The transcriptional factors, RXRα and LXRα, associate with RNA Pol II, and bind to the promoter region of ABCA1-seRNA to upregulate its expression. Left: ABCA1-seRNA recruits the transcriptional machinery (MED23, RXRα, LXRα, and RNA Pol II) to enhance the expression of ABCA1. In bar plots, 1 dot represents data from 1 experiment. Results are expressed as mean ± SD derived from at least 3 independent experiments. For statistical analysis, the *P* values were calculated using a 2-tailed Student's *t*-test. **$P < 0.01$, ***$P < 0.001$. Abbreviations as in **Figures 1 and 2**.

FIGURE 4 Knockdown of ABCA1-seRNA Activates NF-κB Inflammatory Signaling and Promotes Polarization and Migration of M1 Macrophages



(A) Kyoto Encyclopedia of Genes and Genomes analyses of RNA-sequencing data revealed the enrichment of the NF-κB signaling pathway in THP-1 macrophages with ABCA1-seRNA knockdown. (B) qRT-PCR analyses of NF-κB signaling pathway-related genes regulated by ABCA1-seRNA in THP-1 cells. (C) Levels of human inflammatory cytokines interleukin (IL)-1β, IL-6, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor (TNF)-α in the supernatant of THP-1 macrophages at indicated groups treated with phosphate-buffered saline (PBS) or lipopolysaccharide (LPS) (10 ng/mL) were quantified using enzyme-linked immunosorbent assay (ELISA). (D) and (E) Flow cytometry analyses of M1 polarization surface marker CD86+ at indicated groups. (F) Results of the 5-ethynyl-2′-deoxyuridine (EdU) assay indicated that ABCA1-seRNA knockdown markedly increased the percentage of EdU-positive macrophages. (G) Cell Counting Kit 8 (CCK8) assay results revealed that ABCA1-seRNA knockdown significantly increased macrophage proliferation. (H) ABCA1-seRNA knockdown increased the ability of THP-1 macrophages to adhere to human umbilical vein endothelial cells (HUVECs) upon stimulation with TNF for 4 h. (B) through (H) *$P < 0.05$ vs sh-NC. In bar plots, 1 dot represents data from 1 experiment. Results are expressed as mean ± SD derived from at least 3 independent experiments. For statistical analysis, the P values were calculated using a 2-tailed Student's t-test. *$P < 0.05$, ***$P < 0.001$. Abbreviations as in Figures 1 to 3.

FIGURE 5 ABCA1-seRNA Knockdown Dysregulates Inflammation by Modulating P65 Expression



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the ABCA1 gene locus (Figures 3J and 3M). Results indicate that knocking down ABCA1-seRNA impaired the interactions, along with downregulation of ABCA1. Collectively, these results suggest that ABCA1-seRNA mediates transcription of ABCA1 by participating in chromatin looping (Figure 3N).

ABCA1-seRNA DEFICIENCY ACTIVATES INFLAMMATORY SIGNALING AND FACILITATES POLARIZATION, PROLIFERATION, AND MIGRATION OF M1 MACROPHAGES. To explore the potential effect of ABCA1-seRNA beyond the ABCA1 locus, the global gene profile in macrophages under ABCA1-seRNA knockdown was subjected to unbiased pathway analyses (Supplemental Figure 7). Furthermore, the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses revealed significant activation of the NF-κB signaling pathway, which may mediate the inflammatory response (Figures 4A and 4B). Consistent with the findings of this analysis, the secretion of inflammatory cytokines was significantly increased with ABCA1-seRNA or ABCA1 knockdown (Figure 4C, Supplemental Figures 8C and 8D); however, it was decreased in the ABCA1-seRNA-overexpressing group (Supplemental Figure 8B). Interestingly, the inflammatory cytokine (Mcp-1, Tnf-α, Il-6, and Il-1β) levels were restored on overexpression of ABCA1-seRNA in THP-1 cells with ABCA1 knockdown (Supplemental Figure 8D).

The 2 major functional states of macrophages, M1 and M2 polarization, represent the proinflammatory and anti-inflammatory states, respectively. Macrophage transition between the M1 and M2 states promotes the progression of cardiovascular diseases.[27] To further explore the ability of ABCA1-seRNA to modulate macrophage function, flow cytometry was performed to analyze the ratio of $CD86^{+}/CD206^{+}$ cells (macrophage M1/M2 polarization surface marker). ABCA1-seRNA deletion significantly increased the proportion of cells with M1 polarization (Figures 4D and 4E). Inversely, the proportion of cells with M2 polarization was decreased (Supplemental Figures 8E and 8F). In addition, ABCA1-seRNA deficiency facilitated macrophage proliferation (Figures 4F and 4G) and migration (Supplemental Figures 8G and 8H), as well as adherence of THP-1 monocytes to HUVECs (Figure 4H).

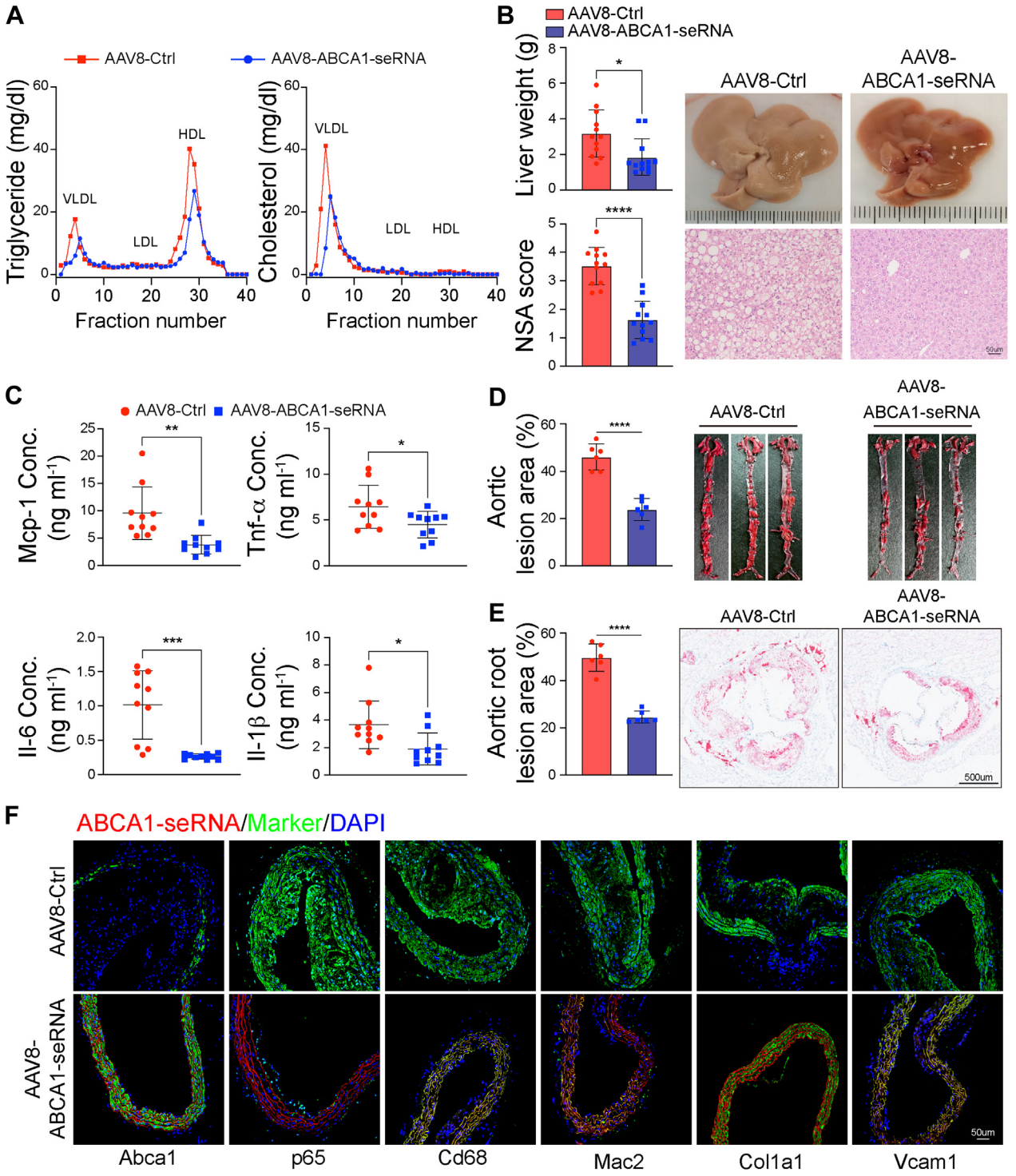
ABCA1-seRNA SUPPRESSES NF-κB ACTIVITY BY PROMOTING P65 UBIQUITINATION. Next, the mechanism of action of ABCA1-seRNA beyond the ABCA1 locus was examined. Consistent with the previous results, the motif of P65, a key regulator in the NF-κB signaling pathway was significantly enriched (Figure 5A). As expected, we verified the specific interactions between ABCA1-seRNA and NF-κB subunits P65/P50/IκBα using RIP and pull-down assays (Figure 5B, Supplemental Figure 9M). Next, our promoter luciferase transcriptional reporter assays indicated that ABCA1-seRNA predominantly regulated the NF-κB activity (Supplemental Figure 9A).

To further decipher the effects of ABCA1-seRNA on P65 in vivo, we analyzed the expression of P65. The expression of P65 was markedly altered at the protein level but not at the mRNA level (Figures 5C to 5E, Supplemental Figures 9B to 9D). As expected, we found that MG132 increased P65 protein expression when ABCA1-seRNA was overexpressed (Supplemental Figure 9E). In addition, overexpression of ABCA1-seRNA sharply increased

FIGURE 5 Continued

(A) Enrichment of the 4 identified de novo TF sequence motifs in differentially accessible regions in ABCA1-seRNA knockdown THP-1 macrophages. (B) Right: Western blot analyses revealed that P65/P50/IκBα interacted with ABCA1-seRNA. Left: RIP and qRT-PCR analyses also demonstrated this interaction. The presence of ABCA1-seRNA in the complexes was detected by qRT-PCR. The expression levels of target proteins were normalized to those of ACTB. $*P < 0.05$ vs IgG. (C) qRT-PCR analyses revealed that the P65 mRNA levels were not affected by ABCA1-seRNA. (D) Western blot analyses revealed that the P65 protein levels were markedly upregulated in ABCA1-seRNA knockdown THP-1 macrophages. For C to D, $*P < 0.05$ vs sh-NC. (E) Immunofluorescence staining of P65 (red) in sh-ABCA1-seRNA and sh-NC THP-1 macrophages. (F) Western blot analyses revealed that ABCA1-seRNA knockdown decreased the polyubiquitination level and increased the total and phosphorylated levels of NF-κB in THP-1 macrophages at indicated groups. (G) Western blot analyses of P65 ubiquitination in HEK293T cells at indicated groups. (H) Western blot analyses of P65 ubiquitination in HEK293T cells transfected with different types of MYC-tagged ubiquitin (K6O, K11O, K27O, K29O, K33O, and K48O) plasmids. In the K6O mutant, all lysine residues that were ubiquitinated were mutant (D) except for the K6 residue. (I) Western blot analyses of K48-linked ubiquitination of P65. (J) Western blot analyses of immunoprecipitated samples obtained from the lysates of HEK293T cells transfected with MYC-tagged ubiquitin and flag-tagged P65 or different types of P65 mutants (K37R, K195R, K310R, and mut). In the K37R mutant, only K37 residue was mutated to arginine. In mut, 3 lysine residues at 37, 195, and 310 sites all were mutated to arginine. (K) Western blot showing the ubiquitination levels of P65 in HEK293T cells at indicated groups. For G to K, ABCA1-seRNA was overexpressed by pcDNA3.1(+) vector, named pABCA1-seRNA. pcDNA3.1(+) empty vector was used as the negative control, named pCtrl. (L) Schematic showing the mechanism of ABCA1-seRNA involvement in the process of ubiquitination-mediated degradation of P65 and trans-regulation inflammatory genes expression. ABCA1-seRNA interacts with P65 and promotes its ubiquitination and subsequent degradation, which suppressed the nuclear translocation of P65 and its ability to modulate the expression of inflammatory genes. In bar plots, 1 dot represents data from 1 experiment. Results are expressed as mean ± SD derived from at least 3 independent experiments. For statistical analysis, the *P* values were calculated using a 2-tailed Student's *t*-test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. n.s. = not significant; other abbreviations as in Figures 1 to 4.

FIGURE 6 Therapeutic Potential of Targeting the ABCA1-seRNA



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total protein ubiquitin levels (Supplemental Figures 9F and 9H). Hence, we speculated that ABCA1-seRNA promoted P65 degradation at least partly via the ubiquitin-proteasome pathway. In contrast, ABCA1-seRNA ablation suppressed polyubiquitination, and P65/P50/IκBα protein expression was also increased along with phosphorylation of P65/P50/IκBα (**Figure 5F**, Supplemental Figure 9H).

To substantiate these observations, we performed a co-immunoprecipitation assay and found endogenous P65/P50/IκBα and ubiquitin could reciprocally co-immunoprecipitate (Supplemental Figures 9I to 9K). Furthermore, overexpression of ABCA1-seRNA promoted P65 polyubiquitination with or without lipopolysaccharide (LPS) stimulation (**Figure 5G**). Notably, K48-linked ubiquitination of P65 was significantly affected by ABCA1-seRNA overexpression in response to LPS stimulation (**Figures 5H and 5I**). To determine the target lysine residue for ubiquitination in P65, the lysine residues of P65 were replaced with arginine, and the mutant plasmids were co-transfected with ABCA1-seRNA and PGL3-NF-κB in HEK293T cells. The results revealed that ubiquitination of P65 at 6 sites (K37, K79, K93, K122, K123, and K195) did not ablate the NF-κB activation in response to ABCA1-seRNA (Supplemental Figure 9L), which means these may be the key sites that interact with ubiquitin. Additional co-immunoprecipitation experiments confirmed that Lys-195 residue of P65 was the key site of the polyubiquitination in response to ABCA1-seRNA (**Figures 5J and 5K**). These results demonstrated the presence of the ABCA1-seRNA-P65-NF-κB axis, which is functionally involved in the inflammatory response (**Figure 5L**).

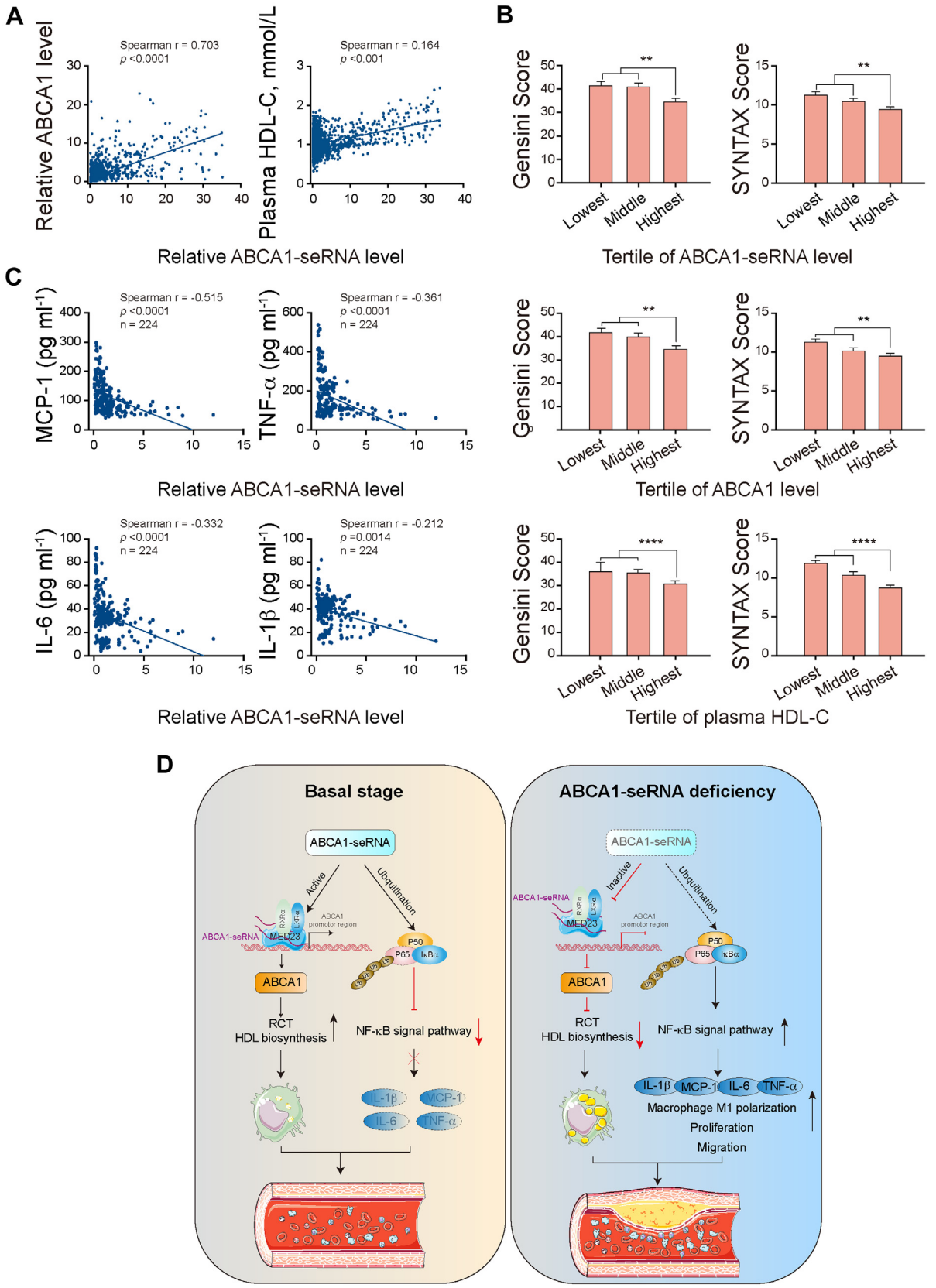
THERAPEUTIC EFFECTS OF ABCA1-seRNA ON LIPIDS, INFLAMMATION, AND AORTIC PLAQUE FORMATION. To further determine the role of ABCA1-seRNA in the lipid metabolic profile and atherosclerosis lesion formation, we used adeno-associated virus 8 (AAV8)-based vectors to deliver human ABCA1-seRNA in the C57BL/6C and ApoE$^{-/-}$ models. Intriguingly, ABCA1-seRNA overexpression significantly decreased levels of serum cholesterol and triglycerides in C57BL/6 mice (**Figure 6A**). Moreover, gross and histological examination of the livers derived from C57BL/6 mice with ABCA1-seRNA overexpression showed changes consistent with lipid alleviation (**Figure 6B**). In the same way, in the ApoE$^{-/-}$ mice, the levels of serum cytokines (Mcp-1, Tnf-α, Il-6, and Il-1β) and circulating lipids (total cholesterol and LDL-C) were significantly lowered in the AAV8-ABCA1-seRNA treatment group (**Figure 6C**, Supplemental Figure 10). In addition, en face analyses of the atherosclerotic plaque area of the aorta tree and atherosclerotic burden of the aortic root revealed that ABCA1-seRNA protected against atherosclerosis (**Figures 6D and 6E**). Moreover, compared with AAV8-Ctrl, AAV8-ABCA1-seRNA exhibited lower immune cell infiltration (Cd68, Mac2, and Vcam1) and fibrosis (Col1a1) (**Figure 6F**). These results further demonstrate that exogenous overexpression of ABCA1-seRNA improved the lipid profile, alleviated inflammation, and thereby attenuated atherosclerotic lesion formation in mice.

ABCA1-seRNA EXPRESSION IN HUMAN MACROPHAGES IS NEGATIVELY CORRELATED WITH INFLAMMATORY CYTOKINES AND THE SEVERITY OF CAD. To determine the clinical relevance of ABCA1-seRNA in CAD, a cross-sectional population study was performed with 1,854 patients. The clinical characteristics of the patients are presented in Supplemental Table 1. ABCA1-seRNA expression in PBMCs was positively correlated with ABCA1 expression and HDL-C concentration (**Figure 7A**). ABCA1-seRNA levels were inversely correlated with the Gensini and SYNTAX scores (**Figure 7B**). In addition, levels of ABCA1-seRNA were inversely correlated with the concentrations of inflammatory cytokines, including MCP-1, tumor

FIGURE 6 Continued

(A) Fast performance liquid chromatography (FPLC) analyses triglyceride and total cholesterol fractions from C57BL/6 mice fed a western diet for 16 weeks between 2 groups detected by routine enzymatic assays. Comparison of the total serum cholesterol, triglyceride, HDL-C, and LDL-C levels in male C57BL/6 mice fed a western diet for 16 weeks between 2 groups. (B) Left: Analyses and quantification liver weights and NAS score of male C57BL/6 mice fed a western diet for 16 weeks and then transduced with control (AAV8-Ctrl, n = 12) or vectors targeting ABCA1-seRNA (AAV8-ABCA1-seRNA, n = 12). Right: Representative (12 images per group) gross appearance of the livers derived from AAV8-Ctrl and AAV8-ABCA1-seRNA mice after 16 weeks on a western diet. (C) Secreted mouse inflammatory cytokines Mcp-1, Tnf-α, Il-6, and Il-1β in the serum were quantified by ELISA after fed with high-fat diet (HFD) for 24 weeks (n = 10 per group). (D) Representative images of the aorta tree stained with Oil Red O in ApoE$^{-/-}$ mice after being fed with western diet for 24 weeks (n = 6 per group). The bar chart (left) shows the quantification statistics of the atherosclerotic plaque lesion area (right) (n = 6 per group). (E) Oil Red O staining of frozen sections of the aortic root in ApoE$^{-/-}$ mice after being fed with western diet for 24 weeks (n = 10 per group). For (B) through (E), 1 dot represents data from 1 mouse, results are expressed as mean ± SEM (C) or mean ± SD (B, D, and E), for statistical analysis, the *P* values were calculated using a 2-tailed Student's *t*-test. *$P < 0.05$ vs AAV8-Ctrl. (F) Representative images of immunofluorescence staining for inflammatory factors (green) and ABCA1-seRNA (red) in aortic vascular tissues of ApoE$^{-/-}$ mice after injection with adeno-associated virus 8 (AAV8) and fed with western diet for 24 weeks. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NAS = nonalcoholic fatty liver disease activity score; other abbreviations as in **Figures 1 to 4**.

FIGURE 7 ABCA1-seRNA Expression Is Negatively Correlated With Inflammatory Cytokines and the Severity of CAD



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necrosis factor-α, IL-6, and IL-1β (Figure 7C). Subsequently, the correlation between the dosage of ABCA1-seRNA vascular lesions, and the severity of CAD was examined.

DISCUSSION

This study demonstrated that ABCA1-seRNA functions as the key epigenetic regulator and coordinates genetic transcriptional regulation to activate ABCA1 and repress the NF-κB signaling pathway. Mechanistically, ABCA1-seRNA is an SE-derived noncoding RNA that initially interacts with MED23 to recruit RXRα and LXRα and forms an RNA-protein complex, leading to upregulation of the expression of nearby ABCA1 and other RXRα- and LXRα-dependent genes. In contrast, ABCA1-seRNA suppressed the NF-κB signaling pathway by interacting with P65 and mediating its ubiquitination. Thus, ABCA1-seRNA promotes cholesterol efflux, reduces macrophage-mediated inflammation and atherosclerosis, and contributes to severity of coronary heart disease (Figure 7D). This study revealed novel and intricate layers of regulatory mechanisms involving seRNAs, which play a role in modulating cholesterol metabolism and macrophage inflammation. Furthermore, this research enhanced our comprehension of the existing mechanisms underlying seRNA activity.

Given that ABCA1-seRNA interacts with RXRα/LXRα and is essential for RXR/LXR-mediated gene expression, we speculated that ABCA1-seRNA might be capable of modulating the cognate gene by functioning as a *trans*-acting TF or cofactor. In addition, an unbiased approach was used to identify P65 as a key TF in macrophage inflammation. These data demonstrate that cholesterol-activator ABCA1-seRNA acts as a potent macular decoy P65 ubiquitination site that restricts inflammation. Our study indicates that seRNAs participate in transcriptional regulation through both *cis*- and *trans*-acting mechanisms.

Atherosclerosis does not only involve dysfunctional lipid storage but can also be characterized as an inflammatory disorder.[28] Recent clinical trials have demonstrated that targeting inflammatory mediators (especially IL-1β) can reduce cardiovascular events, indicating that anti-inflammatory interventions in atherosclerosis are clinically efficacious.[29] NF-κB signaling is a central responder and regulator of inflammatory factors linked to atherosclerosis.[30] This may represent the mechanism underlying the correlation of ABCA1-seRNA with the severity and risk of CAD.

Different from antibody-based PCSK9 inhibitors and/or siRNA-based inhibitor therapies, our data suggest that ABCA1-seRNA expression is responsive to anti-atherogenic action and improves lipid level. This hints that ABCA1-seRNA mimics can be used as a therapeutic strategy. This treatment model will have several potential advantages: 1) based on key target sequences synthesizes active lncRNA mimics and achieved tissue-specific distribution according to organ-targeting labeled peptides; 2) because of resistance to DNase and RNase, it has well stability in vivo; 3) GalNAc-tag helps to enrich ABCA1-seRNA mimics in the liver. Moreover, natural agonist of ABCA1-seRNA could be used in clinical drug development and may have low potential for organ toxicity.

STUDY LIMITATIONS. First, although exogenous ABCA1-seRNA could effectively attenuate atherosclerosis and lipid metabolic profiles, we did not find any new functional sequences homologous to ABCA1-seRNA in the mouse genome. Second our study also indicates that P65 degradation not only depends on ubiquitination but may also involve other mechanisms, such as macroautophagy and caspases. Furthermore, many regulating factors are affected by ABCA1-seRNA such as interaction protein RUNX1. Hence, future studies are warranted to explore the potential mechanisms of ABCA1-seRNA including *trans*-regulation, in depth.

FIGURE 7 Continued

(A) Scatter plots showing a positive correlation between ABCA1-seRNA, ABCA1 mRNA expression levels, and plasma levels of HDL-C in peripheral blood mononuclear cells (PBMCs). RNA expression levels were measured using qRT-PCR. Relative expression levels were calculated using the $2^{\Delta\Delta Ct}$ method. (B) The correlation between tertiles of ABCA1-seRNA, ABCA1 mRNA, and plasma HDL-C levels with Gensini or SYNTAX score. Data are mean ± SEM and were compared by 1-way ANOVA followed by Bonferroni's multiple comparison test. (C) Serum levels of MCP-1, TNF-α, IL-6, and IL-1β in each group were quantified using an enzyme-linked immunosorbent assay (ELISA). The participants were randomly selected from the included population (n = 224). (D) Summary of the mechanism of ABCA1-seRNA. A model illustrating the potential regulatory roles of ABCA1-seRNA in modulating cholesterol homeostasis and vascular inflammation in atherosclerosis progress. ABCA1-seRNA deficiency activates the NF-κB signaling pathway by inhibiting the polyubiquitination-mediated degradation of P65. In addition, ABCA1-seRNA deficiency attenuates the ability of cells to reverse cholesterol transport and increases intracellular lipid droplet accumulation. These changes can cause arterial vascular disease. Hence, targeting ABCA1-seRNA is a promising approach for preventing atherosclerosis and coronary artery disease (CAD). $^{**}P < 0.01$, $^{***}P < 0.001$.

CONCLUSIONS

In summary, we have identified ABCA1-seRNA as a novel SE-derived RNA associated with ABCA1. ABCA1-seRNA affected cholesterol efflux via *cis*-regulation ABCA1 expression. In addition, ABCA1-seRNA interacts with P65 and mediates its ubiquitination, resulting in the suppression of the downstream signaling cascades of NF-κB and macrophage inflammatory responses. Therefore, ABCA1-seRNA was identified as a novel epigenetic regulator in the modulation of cholesterol efflux and macrophage inflammation. Hence, it can be considered a potential locus-specific target for epigenetic therapy aimed at the prevention and treatment of CAD.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study revealed ABCA1-seRNA as an epigenetic factor and therapeutic target of atherosclerosis. Population study finding ABCA1-seRNA is significantly negatively associated with the risk of CAD. Animal experiments finding exogenous ABCA1-seRNA has significant therapeutic effect on atherosclerosis APOE$^{-/-}$ mice.

TRANSLATIONAL OUTLOOK 1: The current findings suggest that ABCA1-seRNA maintained cholesterol homeostasis via *cis*-regulation ABCA1 expression. In addition, it interacts with P65 and mediates its ubiquitination, resulting in the suppression of the downstream signaling cascades of NF-κB and inflammatory responses in macrophages.

TRANSLATIONAL OUTLOOK 2: Understanding the role of super-enhancer RNA on atherosclerosis may uncover the epigenetic modifications in ASCVD and provides a potential therapeutic option.

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APPENDIX For an expanded Methods section and supplemental figures, and tables, please see the online version of this paper.