

SUPPLEMENTAL MATERIAL AND METHODS

Title: Novel mouse model of coronary atherosclerosis with MI: insights into human CAD

Running title: Selective vulnerability of coronary atherogenesis

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SUPPLEMENTAL MATERIALS AND METHODS

Animals

C57BL/6J mice were purchased from HFK Bioscience (Beijing, China). *ApoE*^{-/-} mice that had been backcrossed to a C57BL/6J background for over 10 generations were purchased from the National Resource Center for Mutant Mice (T001458, Nanjing, China). Gene-targeting service was provided by Beijing Biocytogen (Beijing, China) to construct *ApoE*^{SA/SA} mouse model, in which an *Scarb1* knockdown cassette (marked as ‘S’ in the genotype) is driven by the endogenous *ApoE* promoter and an angiotensin II (Ang II) overproduction cassette (marked as ‘A’ in the genotype) is inducible upon doxycycline administration. Briefly, by using the CRISPR/Cas9 technique, the *ApoE* gene sequence of C57BL/6J mouse strain was replaced between exon 2 and exon 4 with a sequence that contains an expression cassette encoding PDZ domain of PDZK1 (that can downregulate SCARB1 expression²¹ at protein level), the *Scarb1* knockdown cassette encoding interference RNA for *Scarb1*, and an Ang II overproduction cassette followed by gaussia luciferase and Tet-on operon. Southern blotting was performed to verify the presence of correctly recombined allele in the targeted mice: a single 16.3 kb fragment by *Scal* indicated wildtype allele, a 10 kb fragment by *Scal* and a 7.5 kb fragment by *BamH I* indicated successfully targeted allele. Embryonic stem cells of C57BL/6J origin were used for gene targeting. Founder mice were established and crossed to C57BL/6J strain, the germline positive offsprings were inter-crossed to generate heterozygotes (*ApoE*^{SA/+}) and homozygotes (*ApoE*^{SA/SA}). Eight to 9 weeks old female (18-20g) and male mice (19-22g) were used in the study. *ApoE*^{SA/SA}, *ApoE*^{SA/+}, *ApoE*^{-/-} mice and wildtype mice were kept and bred in the same condition. Mouse grouping information was blind to investigators during the experimental operation and data collection.

All research mice used in this study were housed in standard individual ventilated cages with

no more than six mice per cage in a specific pathogen free animal facility at Fuwai Hospital. Normal laboratory diet (ND) and a Western diet (WD, 21% wt/wt fat and 0.2% wt/wt cholesterol) were purchased from HFK Bioscience (Beijing, China). Diet and water were available ad libitum. Cages and water bottles were changed every week. The facility was maintained at $22 \pm 2^{\circ}\text{C}$, with humidity of $55 \pm 15\%$, and kept in a 12-hour light-dark cycle. All animal experiments were approved by the Institutional Animal Care and Use Committee, the Experimental Animal Center, Fuwai Hospital, National Center for Cardiovascular Diseases, China. Welfare-related assessments, measurements and interventions were carried out before, during, and after the experiment. Animal studies are reported in compliance with the ARRIVE guidelines. The animals were randomly allocated to different experimental groups and the experimenters were blinded to animal genotype and grouping information. Sample size was determined based on previous studies in the field that used similar experimental designs.^{9, 28} The basic characteristics of animals used in this study are showed in Table S4.

Drug administration

To induce Ang II overproduction, *ApoE^{SA/SA}* mice were administered doxycycline (Dox, 1 mg/mL, A600889, Sangon Biotech, Shanghai, China) water that was prepared freshly, protected from light, and replaced every other day. Norepinephrine bitartrate monohydrate (HY-13715B, MCE, USA) at 100 ng/min and Ang II (HY-13948, MCE, USA) at 12.5 or 50 ng/min, was infused to mice for 4 weeks via osmotic minipump (Alzet Osmotic Pump, Model 2004). Losartan (HY-17512, MCE, USA) was administered at 30 mg/kg/day by gavage. Methotrexate (A600612, Sangon Biotech, Shanghai, China) was administered at 1 mg/kg by intraperitoneal injection on the first day of WD feeding, then once per week. Saline was used as vehicle control. Celecoxib (HY-14398, MCE, USA) was administered at 100 mg /kg/day by gavage for 3 days.

RNA isolation and quantitative real-time PCR

For RNA isolation, cell or tissue samples were homogenized in TRIzol (1 mL, 15596018CN, Thermo Fisher Scientific, USA). The solution was then mixed with chloroform (200 μ L, 67-66-3), and centrifuged (12,000 rpm; 15 min; 4°C). The aqueous phase was collected, mixed with the same volume of isopropanol (67-63-0), and centrifuged (12,000 rpm; 10 min; 4°C). After washing with ethanol (75% vol/vol, 64-17-5), the concentration of RNA was determined by an ultraviolet spectrophotometer (NanoDrop2000, Thermo Fisher, USA,). Chloroform, isopropanol and ethanol were purchased from Bei Jing Tong Guang Fine Chemicals Company (China). PrimeScriptTMRT Master Mix (RR036A, Takara, Shiga Prefecture, Japan) was used for cDNA preparation and quantitative real-time reverse transcription polymerase chain reaction (qPCR) was performed using TransStart Tip Green qPCR SuperMix (AQ142-21, TransGen Biotech, Beijing, China) and QuantStudio5 (Thermo Fisher, USA, A28139). The following condition was used: 95 °C for 5 min, followed by 40 cycles of amplification (95 °C for 20 s, then 60 °C for 15 s, and 72 °C for 30 s). The 2^{- $\Delta\Delta$ CT} method was used for data analysis. The primers used in this study are listed in Table S5.

Western blot analyses

For Western blot analyses, tissues were lysed in RIPA buffer containing protease inhibitors (P0038, Beyotime Biotechnology, Shanghai, China). After centrifugation (12000 rpm; 10 min), lysates were mixed with a loading buffer, then resolved by SDS-PAGE (10% wt/vol, BE6930-15, EASYBIO, Beijing, China), and transferred onto polyvinylidene fluoride membranes (ISEQ00010, Millipore, USA). Membranes were probed with the indicated antibodies overnight at 4°C, followed by incubation with secondary antibody for 2 hours at room temperature. Finally, membranes were incubated with ECL luminous liquid (32209, Thermo Fisher Scientific, Waltham, USA). Signals from immunoreactive bands were visualized using a FluorChem System (ProteinSimple, California, USA). For immunoblot analysis of plasma

lipoproteins in fractions resolved by size exclusion FPLC, the equivoluminal components were mixed with loading buffer and then fractionated with SDS-PAGE (10% wt/vol).

The following antibodies were used: rabbit anti-scavenger receptor B I antibody (1:1000; abcam, ab52629, Cambridge, UK), rabbit anti-apolipoprotein A-I antibody (1:1000; abcam, ab227455, Cambridge, UK), mouse anti-beta actin antibody (1:5000, ab8226, abcam, Cambridge, UK), HRP conjugated goat anti-rabbit secondary antibody (1:5000; G-21234, Thermo Fisher Scientific, Waltham, USA), and HRP conjugated goat anti-mouse IgG (1:5000, HS201, TansGen Biotech, Shanghai, China).

Gaussia luciferase assay

Gaussia luciferase was used to label Ang II by genetic manipulation, and was supported to systemic overproduce in *ApoE^{SA/SA}* mice administrated with doxycycline. For Gaussia luciferase assay, EDTA-anti-clotted blood was collected via tail bleeding. Mice were euthanized and perfused with PBS (20 mL). Heart (50 mg) and aorta (10 mg, including abdominal aorta) were collected in PBS (1 mL) and homogenized. Gaussia luciferase assay was performed by using Secrete-Pair™ Gaussian luciferase assay kit (LF031, GeneCopoeia, Guangzhou, China). Briefly, blood (2 µL) or tissue homogenate (5 µL) was pipetted into each well of a 96-well black plate in triplicate. Then assay working solution (50 µL) containing substrate (coelenterazine) was added to each sample, followed by incubation at room temperature for 1 minute, and then luminescence was measured by Infinite M200 Pro (Tecan, Männedorf, Switzerland) with 60 seconds of delay and 3 seconds of integration.

Blood pressure measurement

Systolic blood pressure was recorded by tail-cuff plethysmography using the MRBP system (IITC Life Science, CA, USA) in conscious animals at a specified time of the day. As reported previously,³² the mice were acclimated to the experimental environment by being restrained in

a plastic tube with a tail cuff for seven consecutive days. Every day the mice were restrained and placed in the chamber with temperature maintained at 34°C. After 10 min of the acclimation, three consecutive measurements were performed with successive 30 s cycles. The measurement was repeated if necessary to obtain the stable readings, the mean of at least 3 stable readings was used. The equipment is operated by two operators who were blinded to the intervention of the mice. Systolic blood pressure was analyzed by another independent investigator in a blinded manner.

Plasma lipid measurements

Blood samples were collected by retro-orbital venous plexus puncture after mice were anesthetized. Plasma concentrations of total cholesterol (CHOL), triglycerides (TG), ApoB containing lipoprotein and HDL-C were measured using commercial kits (BioSino, Beijing, China) and analyzed by Fuwai Hospital using UniCel DxC 800 Synchron (Backman, ME, USA). To quantify the ratio of unesterified cholesterol to total cholesterol, plasma unesterified cholesterol and total cholesterol were measured using commercial kits (E1006 and E1005, ApplyGen, Beijing, China). Optical density was measured by Infinite M200 Pro (Tecan, Swiss).

Myocardial injury marker detection

Mouse Creatine Kinase Myocardial Band (CKMB) ELISA kit (E-EL-M0355, Elabscience, Wuhan, China) and mouse cardiac Troponin I (cTnI) ELISA kit (E-EL-M1203, Elabscience, Wuhan, China) were used to assess myocardial injury in mice, according to the manufacturer's instructions.

Myocardial ischemia/reperfusion model

Myocardial ischemia-reperfusion (MI/R) injury was introduced in male mice by ligation of the left anterior descending (LAD) coronary artery for 30 min, followed by 24-hour reperfusion, as detailed in our previous publication.²⁸ Plasma was harvested 24 hours post MI/R.

Ttriphenyltetrazolium chloride staining

After euthanization, mouse heart and brain were dissected out, rinsed in PBS, frozen at -80°C for 30 min. The hearts were thereafter cut into 5 slices starting from the apex. Each slice was approximately 0.5 mm thick. The slices were incubated with 1% 2,3,5 triphenyltetrazolium chloride (T8877, TTC, Sigma, USA) in phosphate buffer (0.1 mol/L) at 37°C for 10 min in a dark room and then fixed in formalin (10% wt/vol) for 4 h. A stereomicroscope (Zeiss, Germany) was used to take images. The mouse brain was evenly cut into 6 cross-sectional slices. The slices were incubated with TTC (1% wt/vol) at 37°C for 30 min in a dark room and then fixed in formalin (10% wt/vol) for 1 day. A stereomicroscope (Zeiss, Germany) was used to take images. Viable tissue was stained red, whereas infarcted tissue remained unstained (white).

Myocardial histological analyses

Following anesthesia, mice were euthanized by exsanguination and hearts were collected and stored in sucrose solution (30% wt/vol) for 1 day. The top half of the heart (with aortic root) was embedded in OCT (4583, SAKURA, USA) and cross-sectioned ($8\text{ }\mu\text{m}$) from the mid-point to the sinus, at $200\text{ }\mu\text{m}$ intervals. Coronary atherosclerosis was assessed in 3 transverse sections, and the mean lesion area of the 3 sections was used to represent each mouse. Atherosclerotic lesions were stained with Oil Red O (O9755, Sigma, USA). Elastic fibers in coronary arteries were stained by Weigert (DC0066, Leagene, Beijing, China), which stains elastic fiber black, collagen-rich fibrotic tissue pink, and other brown. Cardiac fibrosis was identified by Sirius red staining (DC0041, Leagene, Beijing, China) (collagen fibers stain red and cardiac muscle fibers stain yellow). For immunofluorescence, slides were baked for 20 min, and then transferred to phosphate buffer to remove the OCT. Next, slides were fixed in alcohol (95% vol/vol) for 10 min. After rinsed with PBS, the slides were incubated with a goat serum (ZLI-9056, ZSGB-BIO, Beijing, China) with Triton X-100 (0.3% vol/wt) for 90 min. Following the

incubation with primary antibodies overnight at 4°C, samples were incubated with fluorescence-labeled secondary antibodies (1:200) for 3 h at 37 °C. Coverslips were mounted with a VectaShield medium containing DAPI to stain nuclei (ZLI-9556, ZSGB-BIO, Beijing, China). Slides incubated with IgG isotype control (3900, CST, USA) were used as negative controls. Images were taken by an Axiovert 200M microscope (Zeiss, Oberkochen, Germany) or by Panoramic SCAN (3DHISTECH, Budapest, Hungary).

Antibodies (and corresponding stained cells) used in this study are listed as below. Alexa Fluor 594 conjugated wheat germ agglutinin (Thermo Fisher Scientific, W11261, 1:50, Waltham, USA) was directly used for cardiomyocyte profile. Rabbit anti-CD31 antibody (1:50 endothelial cells, 77699T) was purchased from CST, USA. Rabbit anti-alpha smooth muscle actin antibody (ab5694, 1:800, smooth muscle cells), mouse anti-ICAM1 antibody (ab171123, 1:50), rabbit anti-CD41 antibody (1:50, platelets, ab134131), rat anti-CD3 antibody (1:50, T cells, ab11089), rat anti-Ly6G antibody (1:50, neutrophils, ab210204), rat anti-F4/80 antibody (1:50, macrophages, ab6640), rabbit anti-fibrinogen beta chain antibody (1:3500, blood clots, ab189490), mouse anti-myeloperoxidase (MPO) antibody (1:50, ab90810), and Alexa Fluor 594 conjugated donkey anti-rat secondary antibody (ab150156) were purchased from Abcam, Cambridge, UK. Rat anti-erythroid lineage (TER-119) antibody (sc-19592) was purchased from Santa Cruz, USA. Alexa Fluor 594 AffiniPure goat anti-rabbit IgG (33112ES60), Alexa Fluor 488 AffiniPure goat anti-rabbit (33106ES60), FITC-AffiniPure goat anti-rat IgG (33307ES60), Alexa Fluor 594 AffiniPure goat anti-mouse IgG (33212ES60), and FITC-AffiniPure goat anti-mouse IgG (33207ES60) were purchased from YEASEN, Shanghai, China.

Atherosclerotic lesion measurement

Atherosclerosis was measured for *en face* preparations of the thoracic aorta and for cardiac

cross-sections by Oil Red O staining as described previously.³³ All images were collected by one operator who was blinded to the intervention grouping of the mice, by using a Zeiss microscope equipped with a ZEISS Axiocam 506 color camera (Zeiss, Oberkochen, Germany) and quantified using Image-Pro Plus 6.0 software (Media Cybernetics Inc., Rockville, MD, USA) by another independent investigator in a blind manner.

Myocardial microcirculatory blood perfusion

Blood flow in the heart was real-time imaged, based on laser speckle contrast analysis (LASCA) technology (PeriCam PSI system, Perimed, Stockholm, Sweden). Briefly, mice were placed on a heating pad under anesthetization with isoflurane (1.5% wt/vol, RWD, R510-22-10, Shenzhen, China) inhalation and ventilated to maintain breathing. The thoracic cavity was opened to expose the heart. Blood flow of the heart was measured immediately through 30 s laser scanning. Blood perfusion was expressed in arbitrary perfusion units (PU).

Echocardiography

Mouse echocardiography was performed under anesthetization with isoflurane (1.5% wt/vol) inhalation. The echocardiography images were obtained with a high-resolution Vevo 2100 imaging system (Fujifilm VisualSonics, Toronto, Canada). Left ventricle (LV) internal diameters and wall thicknesses were determined using parasternal short axis M-mode images at the level of the papillary muscle. Mean of three cardiac cycles was used to represent individual mice. Ejection fraction and fractional shortening were calculated using the Vevo2100 software. All ultrasonic images were collected by one operator who was blinded to the manipulation grouping of the mice. Ultrasound imaging was analyzed offline using Vevo Lab software by another independent investigator in a blind manner.

Cytokine array analysis

Plasma (undiluted) was probed for cytokine profiling using the RayBio Mouse Cytokine

Antibody Array kits according to the manufacturer's instructions (RayBiotech; Norcross, GA, USA). Quantification was done by Genetools from Syngene. Data were imported into an Excel spreadsheet, normalized against a control across membranes and final values were calculated.

Human tissue collection

The use of human tissue in the present study was approved by the Human Ethics Committee of Fuwai Hospital, Chinese Academy of Medical Sciences. Written informed consent was obtained from each patient. Human coronary arteries (HCAs) were obtained from 19 heart transplant (HTx) organ donors at Fuwai hospital. Nine of the patients had cardiomyopathy with normocholesterolemic and normotensive (Cluster 1) and the other 10 have hypercholesterolemic and hypertensive (Cluster 2). Human internal mammary arteries were obtained from 13 coronary artery bypass grafting (CABG) patients. These samples were sent to the laboratory within 3 hours of the operation.

The characteristics of these patients are described in Table S6. Each patient was fully evaluated by the ethics committee of heart transplantation that composed of cardiac surgeon, cardiologist, radiologist, and anesthetist before being enrolled on the transplant waiting list. Hearts and internal mammary arteries were stored in an ice-cold, sterile organ-protecting solution buffer. After arrival at the laboratory, a tertiary branch of the left anterior descending coronary artery (diameter range, 100 to 500 μm ; mean diameter, 300 μm) was isolated in a cold (4°C), oxygenated Krebs solution (118.3 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 25 mM NaHCO_3 , and 11.1 mM glucose).

Wire myography

Mouse artery wire myography: After mice were sacrificed by CO_2 overexposure, coronary and the second-order branches of femoral arteries were isolated for vessel motor reactivity studies. Briefly, after fatty connective tissues were removed, the artery was cut into several rings (~2.5

1 mm length). Each ring was bathed in Krebs solution that was continually aerated with a gas
2 mixture of O₂ (95%vol/vol) plus CO₂ (5%vol/vol). The rings were suspended in the chambers
3 of a multi myograph (620 M, Myo Technology A/S, Aarhus, Denmark) using two tungsten
4 wires. Krebs (5 mL) solution in the chamber was maintained at 37°C using a built-in heat-
5 exchange device. The pH values were between 7.3 and 7.5. A segment was stretched between
6 the wires under a determined previously optimal tension (~3 mN for femoral arteries, ~1.5
7 mN for coronary arteries). After 30 minutes equilibration, the arteries were contracted initially
8 with 60 mM KCl (62.70 mM NaCl, 60 mM KCl, 2.52 mM CaCl₂, 1.17 mM MgSO₄, 24.88 mM
9 NaHCO₃, 1.18 mM KH₂PO₄, 11.1 mM glucose, all these chemical reagents were purchased
10 from Sangon Biotech), followed by 3 washes with warmed Krebs solution. To determine
11 endothelium-dependent vasodilation, arteries were contracted with U46619 (HY-108566 ,
12 femoral arteries were contracted with 30 nM U46619, coronary arteries from wildtype mice
13 were contracted with 100 nM, coronary arteries from transgenic mice were contracted with 200
14 nM), followed by adding acetylcholine (HY-B0282, ACh, in a range of concentration from
15 3nM - 10 µM), or by adding isoproterenol (HY-B0468, ISO) at 3nM. Endothelium-independent
16 vasodilation was examined by determining vascular response to ISO (in a range of
17 concentration from 1nM - 10 µM) or sodium nitroprusside (HY-B0564, SNP, in a range of
18 concentration from 1nM - 10 µM)) or norepinephrine (HY-13715, NE, in a range of
19 concentration from 1nM - 10 µM) in U46619-precontracted vessels. The vessels were then
20 washed three times again, then Ang II (100 nM), L-NAME (100 µM; HY-18729A, eNOS
21 inhibitor), SC560 (5 µM; HY-59105, COX-1 inhibitor) or celecoxib (5 µM; COX-2 inhibitor)
22 was added before measuring acetylcholine-induced vasodilation again, or phentolamine (10
23 µM; HY-12717, α adrenergic receptor antagonists) or propranolol (10 µM; HY-B0573B, β 1
24 and β 2 adrenergic receptor antagonists) was added before measuring NE/ISO-induced vascular
25 activity again. The above reagents were purchased from MCE, USA.

Human artery wire myography: This method is similar to mouse artery wire myography. The endothelium-independent vasodilation was examined by determining vasodilation of SNP. The segment was stretched between the wires under 5 mN, a pre-determined optimal tension.

Ex vivo Langendorff perfusion

Mice were anesthetized by an intraperitoneal injection of 1% pentobarbital sodium (9 μ L/g body weight). The hearts were isolated and perfused 1 h in a modified Langendorff system under constant pressure (70 mmHg) with gassed (94% O₂ – 6% CO₂ vol/vol) Krebs-Henseleit buffer solution (118.30 mM NaCl, 4.70 mM KCl, 2.52 mM CaCl₂, 1.17 mM MgSO₄, 24.88 mM NaHCO₃, 1.18 mM KH₂PO₄, 11.10 mM glucose) at 37°C. PGE₂ and 6k-PGF_{1 α} in effluent of ex vivo perfused hearts were measured using a UPLC-MS/MS technique.

Proteomics analyses

The proteomic workflow started with sample collection and preparation (pretreatment, protein extraction/separation, protein digestion and purification), followed by LC-MS/MS analysis (fractionation, ionization, data acquisition, protein identification and label-free quantitative analysis), and then analyzed by database search. Sample preparation and LC-MS/MS analyses were done by the State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), and Beijing Institute of Lifeomics (Beijing 102206, China).

Vascular preparation for proteomics determination

Coronary and femoral arteries were isolated from *ApoE*^{SA/SA} mice administered ND, or WD and doxycycline for either 3 or 28 days. Arteries collected from 6 male mice were pooled. Each experimental group included 5 pooled samples. Freshly prepared arteries were transferred to lysis buffer (1% DOC, 10 mM TCEP, 40 mM CAA, 100 mM Tris, pH 8.5; Reagents were purchased from Sigma, USA) and then denaturized at 95°C for 5 min. After cooling, samples

were sonicated for 5 min and lysed on ice for 30 min. Lysates were centrifuged at 4°C and 15,000 rpm for 15 min, and the supernatants were taken. The protein concentration of supernatants was quantified by Nanodrop, and 100 µg of protein of each sample was used for FASP enzymatic digestion. Finally, the peptides were quantified and used for LC-MS/MS analysis.

Label-free LC-MS/MS was performed on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific) coupled online to a nanoflow LC system (EASY-nLC 1200, Thermo Fisher Scientific). Peptide mixture (2,000 ng) of each sample was resuspended in solvent A (0.1% vol/wt formic acid (FA) in HPLC grade water) and delivered in solvent A onto a 2 cm self-packed trap column (100-µm inner diameter, 3 µm resin, ReproSil-Pur C18-AQ, DrMaisch GmbH). After concentration and desalting, the peptides were transferred to a 30 cm column (150-µm-inner-diameter, 1.9 µm resin, ReproSil-Pur C18-AQ, DrMaisch GmbH) and separated in a 150 min nonlinear gradient from 5% to 32% vol/wt solvent B (0.1% FA in ACN) at a flow rate of 600 nL/min. The gradients were as follows: 0–10 min, 5–11 % solvent B; 10–110 min, 11–25 % solvent B; 110–140 min, 25–40 % solvent B; 140–141 min, 40–95% solvent B; and 141–150 min, 95% solvent B.

Peptides were ionized using 2.1 kV spray voltage and a capillary temperature of 320°C. Data acquisition was performed in OT–IT mode. A full MS scan (300 to 1,400 m/z) was performed at a resolution of 120,000, a maximum injection time of 50 ms, and an automatic gain control (AGC) target value of 2e5. Subsequently, an MS2 event with a duty cycle of 3 s was performed in top-speed mode. 2+ to 6+ precursors were fragmented by higher-energy collision dissociation (HCD) with 35% normalized collision energy. The MS2 spectra were read out in the ion trap in rapid mode with an AGC target value of 5,000 and a maximum-injection time of 35 ms. Dynamic exclusion was set to 25 s.

MS data interpretation by database search: The acquired LC-MS/MS data (Thermo .raw files) were subjected to database search using MaxQuant with an integrated Andromeda search engine (v.2.0.3.0). Tandem mass spectra were searched against the UniProt mouse protein database (downloaded from UniProt on 09/12/2021, <https://www.uniprot.org/>), concatenated with a reverse decoy database. The mass tolerance for precursor ions was set as 20 ppm in the First search and 5 ppm in the Main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethylation of cysteine (+57.021 Da) was set as a fixed modification, while oxidation on methionine (+15.995 Da), and acetylation to N-termini (+42.011 Da) were configured as variable modifications. Searches allowed for up to 2 missed K/R cleavages by trypsin (full specificity). The enzyme was trypsin, allowing for two missed cleavage sites. Precursor quantification based on intensity was used for label-free quantification (LFQ). The false discovery rate (FDR) for peptide-spectrum-matches (PSMs), protein group, and modification site was configured at 1%, and the minimum score for modified peptides was set >40.

Filtering of the identification results: The identification results from MaxQuant (proteinGroups.txt) were processed using Perseus (V.2.0.3.1). Potential contaminating proteins, proteins matching the reverse database, and proteins of “identified by site”, were removed from the protein list. Furthermore, proteins identified by only one unique peptide were filtered out. The original LFQ intensity values were log₂-transformed. Proteins with at least three valid values in at least one sample group were kept. Missing observations were replaced by random values that were imputed from a normal distribution. Differentially expressed proteins were identified using unpaired Student’s t-test. Proteins with an individual P-value <0.05 and |log₂ fold change|>1.5 were considered as significantly differential proteins. Median normalization (Z-Score) was performed to account for systematic differences in protein signal distribution. Based on the data processed by Perseus,

principal component analysis, volcano plot, Venn diagram and heatmap of differentially expressed proteins were created by using Bioladder (<https://www.bioladder.cn/>). Enrichment of functional pathways, including GO Biological Processes and KEGG Pathway, were analyzed in Metascape (<http://metascape.org/gp/>).

Inclusion and exclusion criteria

All surviving animals at the end of experiments were included. Some animals fed WD and administered doxycycline water that exhibited inactivity, hunched posture (Video S3) and hind-limb paralysis (Video S4) were humanely euthanized, and their tissues were collected for analysis. Animals that accidentally died during anesthesia or blood pressure measurements were excluded.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9 software (GraphPad Software Inc., San Diego, California, USA). Mean $\pm$ SEM was used for data presentations. When two groups were involved, Student's two-tailed unpaired *t*-test was used for comparisons of continuous variables if passed normality and equal variance tests, otherwise, Mann-Whitney test was used. Comparison of multiple groups were analyzed using one-way ANOVA with Tukey multiple comparisons test or two-way ANOVA (or two-way repeated-measures ANOVA) when there were 2 experimental factors, which were validated by normality and equal variance tests; their post hoc tests are indicated in figure legends. Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank (Mantel-Cox) test.

1 SUPPLEMENTAL TABLE
2 Table S1. Plasma lipids in *ApoE*^{-/-} and *ApoE*^{SA/SA} mice.

Particle	Triglyceride								Total cholesterol							
Gender	Female				Male				Female				Male			
Diet	ND		WD - 5 weeks		ND		WD - 5 weeks		ND		WD -5 weeks		ND		WD - 5 weeks	
Genotype	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}
Concentration (mmom/L)	0.96	0.85	1.26	0.51	1.23	1.97	2.5	1.31	12.19	9.62	27.42	19.84	12.3	11.03	30.48	28.58
	0.86	1.75	1.31	0.77	0.9	0.8	2.97	0.94	9.16	15.09	29.47	15.39	10.18	9.37	27.08	26.31
	0.83	0.83	0.96	1.01	0.84	0.72	1.92	1.29	10.77	11.15	28.56	21.9	9.58	9.01	22.91	23.77
	0.84	0.78	0.78	1.15	0.72	1.16	1.75	1.69	11.17	9.99	23.64	22.7	8.15	11.04	24.09	27.96
	0.74	0.96	1.58	0.77	1.08	1.12	2.08	1.29	10.59	10.54	23.21	23.52	12.15	11.95	33.84	23.38
	1.6	0.81	1.64	0.69	1.39	0.78	2.32	0.82	11.13	10.73	19.35	27.26	9.22	8.47	26.3	29.37
	1.03	0.76	1.37	0.67	2.36	1.68	1.58	1.15	8.09	7.77	21.52	27.38	13.16	9.75	27.41	31.19
	0.8	0.92	1.6	1.12	1.89	0.8	1.82	1.21	7.3	9.31	21.02	27.29	8.5	9.07	30.94	27.64
	1.46	0.64	1.62	1.08	1.39	1.37	1.53	0.99	6.32	6.69	18.06	29.97	8.15	9.5	23.13	21.77
1.59	1.24	1.72	0.87	1.69	1.65	0.94	1.14	9.15	8.27	25.24	28.75	9.1	12.92	21.49	25.3	
Mean	1.071	0.954	1.384	0.864	1.349	1.205	1.941	1.183	9.587	9.916	23.75	24.4	10.05	10.21	26.77	26.53
Std. Deviation	0.3421	0.3215	0.3136	0.2176	0.5139	0.4459	0.5664	0.2422	1.903	2.298	3.887	4.561	1.844	1.45	4.015	2.963
Std. Error of Mean	0.1082	0.1017	0.09915	0.06882	0.1625	0.141	0.1791	0.07658	0.6017	0.7267	1.229	1.442	0.5831	0.4585	1.27	0.937
p value <i>ApoE</i> ^{-/-} vs. <i>ApoE</i> ^{SA/SA}	0.9883		5.38×10 ⁻⁴		0.5120		0.0021		0.7315		0.7353		0.8297		0.8809	
T tests	Kolmogorov-Smirnov test		Unpaired t test with Welch's correction													
Particle	ApoB containing lipoprotein								HDL-C							
Gender	Female				Male				Female				Male			
Diet	ND		WD - 5 weeks		ND		WD - 5 weeks		ND		WD - 5 weeks		ND		WD - 5 weeks	
Genotype	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{SA/SA}
Concentration (mmom/L)	9.8	6.71	23.98	22.85	9.32	8.57	32.22	36.29	2.32	3.48	5.76	9.31	2.88	2.85	4.46	11.62
	7.46	10.5	19.66	19.28	7.87	7.26	27.91	33.96	2.12	3.56	5.12	6.6	2.51	3.11	3.45	10.75
	9.34	7.6	19.78	28.59	6.8	7.14	23.73	31.3	2.36	4.01	4.87	10.68	2.77	2.89	4.52	7.43
	10.31	6.99	25.55	30.96	5.98	8.79	30.89	36.52	2.11	4.52	5.65	9.59	2.42	3.21	4.29	7.56
	8.62	7.36	34.48	30.9	8.75	9.97	39.93	32.2	2.43	3.61	5.17	10.76	2.59	3.26	5.81	7.14
	9.48	8.11	29.27	28.05	6.29	6.93	26.13	28.74	2	4.23	3.82	11.29	1.89	3.44	5.03	15.45
	6.52	7.3	29.04	27.68	11.76	6.73	28.61	30.57	1.86	3.15	4.1	9.6	1.41	2.71	4.31	7.85
	6.05	7.04	30.94	36.06	5.87	7.57	36.14	26.62	1.85	3.05	3.91	12.62	1.48	2.84	3.78	7.61
	4.79	5.03	26.25	32.2	5.84	8.84	26.06	18.97	1.34	3.02	3.45	9.84	1.62	2.86	1.59	5.72
8.9	5.47	33.95	30.41	9.24	11.23	22.41	24.61	1.47	2.38	3.58	11.33	1.43	3.02	5.75	8.05	
Mean	8.127	7.211	27.29	28.7	7.772	8.303	29.4	29.98	1.986	3.501	4.543	10.16	2.1	3.019	4.299	8.918
Std. Deviation	1.831	1.488	5.229	4.758	1.976	1.464	5.499	5.46	0.3652	0.6376	0.868	1.615	0.5918	0.2305	1.217	2.875
Std. Error of Mean	0.5791	0.4705	1.653	1.505	0.6247	0.4628	1.739	1.727	0.1155	0.2016	0.2745	0.5106	0.1871	0.07288	0.3848	0.9091
p value <i>ApoE</i> ^{-/-} vs. <i>ApoE</i> ^{SA/SA}	0.2361		0.5368		0.5041		0.8171		1.21×10 ⁻⁵		1.55 ×10 ⁻⁷		2.06×10 ⁻⁴		4.33×10 ⁻⁵	
T tests	Unpaired t test with Welch's correction												Mann Whitney test			

3 ND denotes normal diet, WD denotes western diet.

1 **Table S2. Profiling plasma lipids in mice under different treatments.**

Particle	Triglyceride							Total cholesterol						
Genotype	<i>ApoE</i> ^{-/-}	WT	<i>ApoE</i> ^{SA/+}	<i>ApoE</i> ^{SA/SA}				<i>ApoE</i> ^{-/-}	WT	<i>ApoE</i> ^{SA/+}	<i>ApoE</i> ^{SA/SA}			
Diet/treatment	ND+Dox	ND+Dox	ND+Dox	ND+Dox	WD	WD+Dox	ND	ND+Dox	ND+Dox	ND+Dox	ND+Dox	WD	WD+Dox	ND
Concentration (mmom/L)	1.46	0.77	0.99	0.62	0.69	1.23	0.81	7.56	2.16	4.67	16.06	27.26	29.18	10.73
	0.8	0.79	1.07	0.81	0.67	2.24	1.53	7.2	2.47	3.8	19.07	27.38	29.48	6.98
	1.03	0.78	0.82	1.3	1.68	3.19	0.76	8.2	2.5	4.38	23.72	27.87	28.68	7.77
	1.6	0.68	1.16	0.82	1.08	1.71	0.64	8.72	2.63	3.24	18.6	29.97	25.79	6.69
	1.59	0.76	0.79	0.72	0.87	1.87	0.92	8.75	2.44	3.54	24.9	28.75	30.23	9.31
	1.31	0.77	0.92	1.05	1.15	1.83		7.92	2.32	4.01	15.52	25.26	27.18	
	1.34	0.72	0.78	0.99	1.21	2.17		7.88	2.28	3.97	17.98	26.38	28.48	
	1.29	0.65	0.8	1.18	0.99	2.7		8.53	2.55	4.13	19.7	27.87	26.68	
					1.14	3.99						29.07	25.79	
Mean	1.303	0.74	0.9163	0.9363	1.053	2.248	0.932	8.095	2.419	3.968	19.44	27.66	28.27	8.296
Std. Deviation	0.2737	0.05127	0.1443	0.2342	0.2889	0.8342	0.3491	0.5586	0.1547	0.4523	3.337	1.377	1.86	1.699
Std. Error of Mean	0.09676	0.01813	0.05102	0.08278	0.09137	0.2638	0.1561	0.1975	0.05469	0.1599	1.18	0.4354	0.5881	0.7596
P value groups vs. <i>ApoE</i> ^{SA/SA} (WD+Dox)	2.76×10 ⁻⁴	1.81×10 ⁻⁸	3.81×10 ⁻⁷	5.38×10 ⁻⁷	1.16×10 ⁻⁶			1×10 ⁻¹⁵	1×10 ⁻¹⁵	1×10 ⁻¹⁵	1×10 ⁻¹⁵	0.9624		
p value Test	One-way ANOVA (Tukey's multiple comparisons test)							One-way ANOVA (Tukey's multiple comparisons test)						
Particle	ApoB containing lipoprotein							HDL						
Genotype	<i>ApoE</i> ^{-/-}	WT	<i>ApoE</i> ^{SA/+}	<i>ApoE</i> ^{SA/SA}				<i>ApoE</i> ^{-/-}	WT	<i>ApoE</i> ^{SA/+}	<i>ApoE</i> ^{SA/SA}			
Diet/treatment	ND+Dox	ND+Dox	ND+Dox	ND+Dox	WD	WD+Dox	ND	ND+Dox	ND+Dox	ND+Dox	ND+Dox	WD	WD+Dox	ND
Concentration (mmom/L)	4.79	0.58	1.52	8.45	28.05	31.2	8.11	1.85	1.63	3.47	3.72	11.29	8.62	4.23
	6.05	0.71	1.14	8.84	27.68	32.94	4.42	1.86	1.97	2.83	3.89	9.6	6.75	2.08
	6.52	0.54	1.31	11.4	35.58	29.82	7.3	2	1.69	3.38	4.38	9.12	5.32	3.15
	9.48	0.51	0.94	7.79	32.2	28.91	5.03	1.47	2.42	2.493	4.28	9.84	7.21	3.02
	8.9	0.37	1.11	9.51	30.41	34.04	7.04	1.34	1.33	2.68	4.82	11.33	8.87	3.05
	7.83	0.49	1.21	8.04	30.57	21.46		1.62	1.57	3.25	4.01	7.85	4.67	
	7.52	0.68	1.02	9.01	26.62	30.28		1.75	1.69	3.19	3.98	7.61	8.74	
	6.87	0.55	1.32	7.99	18.97	22.94		1.92	1.49	2.99	4.51	5.72	5.32	
					24.61	30.37						8.05	4.88	
Mean	7.245	0.5538	1.196	8.879	27.58	28.69	6.38	1.726	1.724	3.035	4.199	8.704	6.784	3.106
Std. Deviation	1.525	0.1076	0.1854	1.173	5.042	4.202	1.576	0.2306	0.3359	0.3469	0.3646	1.868	1.652	0.763
Std. Error of Mean	0.5391	0.03803	0.06555	0.4148	1.594	1.329	0.7049	0.08155	0.1188	0.1226	0.1289	0.5908	0.5225	0.3412
p value groups vs. <i>ApoE</i> ^{SA/SA} (WD+Dox)	1×10 ⁻¹⁵	1×10 ⁻¹⁵	1×10 ⁻¹⁵	1×10 ⁻¹⁵	0.96			3.82×10 ⁻¹¹	3.76×10 ⁻¹¹	1.42×10 ⁻⁷	2.25×10 ⁻⁴	5.44×10 ⁻³		
P value Test	One-way ANOVA (Tukey's multiple comparisons test)							One-way ANOVA (Tukey's multiple comparisons test)						

2 ND denotes normal diet, WD denotes western diet, Dox denotes doxycycline (administered in water).

3

1 **Table S3. Differentially expressed proteins in arteries of early and late stages of atherogenesis.**

Regulation	Group	Differentially expressed proteins (UniProt ID)
UP	CA-3d	P58022 , A2AUC9, O08663, O08734, O08999, O35345, O54931, O55135, O70373, O89017, P01592, P11930, P15327, P16294, P17918, P29268, P30416, P30681, P32233, P36552, P55937, P57784, Q8BWW3, P83917, P84084, P98078, Q05186, Q3ULW8, Q3UN02, Q3V1T4, Q4PZA2, Q4U4S6, Q60715, Q60716, Q60973, Q61210, Q61646, Q61704, Q61810, Q63961, Q64331, Q64339, Q64737, Q68FL6, Q6P5H2, Q6ZWM4, Q80XB4, Q80ZK0, Q810B6, Q8BKE6, Q8BMA6, Q8BXA1, Q8CFX1, Q8CG70, Q8CI32, Q8K297, Q8R050, Q8R0H9, Q8R2G6, Q8VCH8, Q8VDC0, Q8VHI3, Q8VI75, Q91VU0, Q91WQ3, Q920E5, Q921H9, Q921S7, Q921X9, Q9CR00, Q9CR42, Q9CWZ3, Q9CYA0, Q9D0W5, Q9D1Q4, Q9D404, Q9D662, Q9D8V0, Q9DC23, Q9ERG0, Q9JI33, Q9JJE7, Q9JLM8, Q9JLQ2, Q9JLR9, Q9QXS6, Q9QYS9, Q9R0E1, Q9R0E2, Q9Z0Y1, Q9Z1D1, Q9Z1T2, Q9Z2Z9
	CA-28d	A6X935, E9Q414, O08999, O35343, O35609, O35640, O54774, O55101, O55135, O55201, O70152, O70310, O70370, O70503, O88428, O88947, O89017, O89053, P06802, P10404, P10810, P10852, P11276, P16110, P16675, P17439, P17918, P18181, P19973, P20060, P20918, P21958, P22777, P22892, P27005, P27046, P29268, P29595, P29788, P30681, P32233, P33434, P35821, P36371, P41233, P41317, P43275, P49935, P50462, P50608, P58022, P58871, P61620, P62267, P62317, Q8BWW3, P62631, P62852, P62911, P70122, P70288, P97333, P97461, P97821, P98086, Q02105, Q03734, Q06890, Q07113, Q3U186, Q3ULF4, Q3UQ28, Q3V1T4, Q4LDD4, Q4U4S6, Q58A65, Q60648, Q60710, Q60715, Q60875, Q61093, Q61233, Q61543, Q61576, Q61702, Q61704, Q62009, Q62059, Q62189, Q64152, Q68FL6, Q6NZC7, Q6P542, Q6PGC1, Q6ZWM4, Q6ZWW3, Q7TSV4, Q7TT50, Q80SU7, Q80XB4, Q80YX1, Q810B6, Q8BHD7, Q8BKE6, Q8BMA6, Q8BVL3, Q8BWB1, Q8C0L8, Q8C2E7, Q8C4U3, Q8C8U0, Q8CFX1, Q8CG70, Q8CH18, Q8CI32, Q8CI59, Q8K1I3, Q8QZY9, Q8R054, Q8R0G9, Q8R105, Q8R2G6, Q8R3S6, Q8VCC9, Q8VCH8, Q8VDJ3, Q8VDT9, Q8VE37, Q8VEB4, Q8VHE0, Q91VH6, Q91VU0, Q91YJ2, Q91Z83, Q921M7, Q921S7, Q922Q4, Q99L45, Q99LD4, Q99LI8, Q99N93, Q99N94, Q99N96, Q9CQC6, Q9CQE1, Q9CQI6, Q9CQK7, Q9CR42, Q9D0Q7, Q9D0R8, Q9D1B9, Q9D1P0, Q9D236, Q9D404, Q9D662, Q9D7B7, Q9D7V9, Q9DBL7, Q9DCU6, Q9EPB4, Q9EPL9, Q9ERG0, Q9ESX5, Q9ET30, Q9ET66, Q9JIL4, Q9JIX8, Q9JJ18, Q9JKC8, Q9JLM8, Q9JM58, Q9QWK4, Q9QXS6, Q9QXZ0, Q9QYS9, Q9QZJ6, Q9R045, Q9R0E1, Q9R0E2, Q9WTX6, Q9WU07, Q9Z0J0, Q9Z0M5, Q9Z1D1, Q9Z2Z9 , A2AUC9, F8VPU2, O08663, O08692, O08734, O09012, O35345, O35386, O35405, O35744, O70165, P00848, P01027, P04186, P06797, P06800, P0DOV1, P0DOV2, P11031, P11103, P11835, P12265, P14106, P14434, P14483, P14576, P15379, P23780, P25911, P28650, P29351, P29416, P31532, P31725, P34928, P35285, P35288, P35831, P39039, P43883, P47713, P49182, P50114, P50428, P55065, P55096, P56387, P57784, P60603, P63280, P63328, P70297, P97298, P97863, P98063, Q01279, Q03350, Q04207, Q05186, Q07235, Q3TAS6, Q3TDQ1, Q3TWW8, Q3UHQ6, Q3UJB9, Q3UQN2, Q4VAA2, Q60716, Q60847, Q60973, Q61129, Q61210, Q61282, Q61398, Q61553, Q61733, Q640N1, Q64191, Q64331, Q64449, Q68FF6, Q6A068, Q6A0D4, Q6NVF9, Q6PAM0, Q6PDG5, Q6PDQ2, Q6PE01, Q6PHN9, Q6R891, Q6ZQM8, Q7TQ95, Q80V26, Q80VD1, Q80X95, Q80XL6, Q810A7, Q8BG07, Q8BGS2, Q8BLX7, Q8BTX9, Q8BXA1, Q8C052, Q8C0I1, Q8CBA2, Q8CCF0, Q8CEE7, Q8CG14, Q8JZU2, Q8K0L9, Q8K124, Q8K1E0, Q8K4Q0, Q8R050, Q8R0X7, Q8R395, Q8VBZ3, Q8VCG4, Q8VCI0, Q8VEA4, Q8VI36, Q91VS7, Q91WP0, Q91WQ3, Q91XV3, Q921X9, Q99K01, Q99LB6, Q99LE6, Q99LR1, Q99M71, Q9CQN7, Q9CR26, Q9CSU0, Q9CWZ3, Q9CXP8, Q9CY73, Q9CYD3, Q9D020, Q9D0A3, Q9D154, Q9D1G5, Q9D1I2, Q9D1I6, Q9D1J1, Q9D1M7, Q9D1N9, Q9D2V7, Q9D783, Q9D7N3, Q9D883, Q9D8V0, Q9D958, Q9DB27, Q9DBG7, Q9DC23, Q9EPM5, Q9ERR7, Q9ET22, Q9JHI0, Q9JIK5, Q9JLN9, Q9JLQ0, Q9JLQ2, Q9JLV6, Q9JM99, Q9QWR8, Q9QYF1, Q9QZ85, Q9R099, Q9R0G6, Q9R111, Q9R1R2, Q9R233, Q9WTQ8, Q9Z0G4, Q9Z1T2, Q9Z1W9, Q9Z2Q5
	FA-3d	O09118, P34928, P51910, P61939, P62855, Q9JIL4
	FA-28d	O09111, O88428, P14142, P34928, P55096, P82348, P98086, Q61210, Q62351, Q6PAV2, Q6PF93, Q7TSE6, Q80TL7, Q80X95, Q8BU30, Q8C3X8, Q8GUM2, Q91V01, Q91VK4, Q91YM4, Q9D289, Q9D6X6, Q9D8V0, Q9EQW7, Q9ER88, Q9ET22, Q9JLT4
Down	CA-3d	Q61982, Q9QZ19
	CA-28d	O54865, O70423, P02088, P09242, P10649, P31428, P37804, P68871, P97315, Q04447, Q61982, Q6PDN3, Q8K009, Q8VCT4, Q9CQ19, Q9D4J1 , P08226, P15089, P21844, P40936, Q6W8Q3, Q8BJD1, Q8BLI0, Q8BU85, Q91VC7, Q9CRC8, Q9JHR9, Q9R1T4
	FA-3d	P26262, P70297, P97298, Q65CL1, Q6X893, Q8VD62, Q8VHL1, Q91YD9, Q99KV1, Q9CQN3, Q9D1H6, Q9D404
	FA-28d	P26262, P58242 , P06683, P07759, P11859, P14094, P23953, P28665, P52430, Q00898, Q31125, Q3U5Q7, Q6SKR2, Q8BHC0, Q8BYN3, Q8VHC3, Q921J2, Q9D1H6, Q9D404, Q9R269, Q9Z1Q2

2 Filtering conditions for differentially expressed proteins is $|\log_2 \text{ fold change}| > 1.5$ and Student's T-test $p\text{-value} < 0.05$. Among the differentially expressed proteins, those in
3 red color passed FDR (Benjamini-Hochberg) correction.

1 **Table S4. Animal use in the study.**

Figures	Gender	Number	Genotype	Group
Figure1B	Female	6	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	6	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
Figure1D	Female	6	<i>ApoE</i> ^{-/-}	ND
	Female	6	<i>ApoE</i> ^{SA/SA}	ND
	Female	6	<i>ApoE</i> ^{-/-}	WD
	Female	6	<i>ApoE</i> ^{SA/SA}	WD
Figure1E, 1F	Female	10	<i>ApoE</i> ^{-/-}	ND
	Female	10	<i>ApoE</i> ^{SA/SA}	ND
	Female	10	<i>ApoE</i> ^{-/-}	WD
	Female	10	<i>ApoE</i> ^{SA/SA}	WD
	Male	10	<i>ApoE</i> ^{-/-}	ND
	Male	10	<i>ApoE</i> ^{SA/SA}	ND
	Male	10	<i>ApoE</i> ^{-/-}	WD
	Male	10	<i>ApoE</i> ^{SA/SA}	WD
Figure1G, 1H	Female	5	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	5	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
Figure 2A,2B,2D,2E	Female	10	<i>ApoE</i> ^{-/-}	Pre-WD
	Female	10	<i>ApoE</i> ^{SA/SA}	Pre-WD
	Female	14	<i>ApoE</i> ^{-/-}	Post-WD
	Female	14	<i>ApoE</i> ^{SA/SA}	Post-WD
Figure 2G,2H	Female	14	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	14	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
Figure 2J	Female	6	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	6	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
Figure 2L	Female	3	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	3	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
	Female	1	WT	WT
Figure 3A	Female	18	<i>ApoE</i> ^{SA/SA}	WD
	Female	27	<i>ApoE</i> ^{SA/SA}	WD+Dox
	Male	18	<i>ApoE</i> ^{SA/SA}	WD
	Male	27	<i>ApoE</i> ^{SA/SA}	WD+Dox

Figures	Gender	Number	Genotype	Group
Figure 3C,3D	Male	16	<i>ApoE</i> ^{SA/SA}	WD
	Male	9	<i>ApoE</i> ^{SA/SA}	WD+Dox
Figure 3F	Female	7	<i>ApoE</i> ^{SA/SA}	WD
	Female	13	<i>ApoE</i> ^{SA/SA}	WD+Dox
Figure 3G	Female	17	<i>ApoE</i> ^{SA/SA}	WD
	Female	17	<i>ApoE</i> ^{SA/SA}	WD+Dox
Figure 4A	Female	7	<i>ApoE</i> ^{SA/SA}	NE
	Female	7	<i>ApoE</i> ^{SA/SA}	Ang II-L
	Female	12	<i>ApoE</i> ^{SA/SA}	Ang II-H
	Female	12	<i>ApoE</i> ^{SA/SA}	Dox
	Female	7	<i>ApoE</i> ^{SA/SA}	Dox+Losartan
	Female	7	<i>ApoE</i> ^{SA/SA}	NE
Figure 4B	Female	7	<i>ApoE</i> ^{SA/SA}	Ang II-L
	Female	9	<i>ApoE</i> ^{SA/SA}	Ang II-H
	Female	9	<i>ApoE</i> ^{SA/SA}	Dox
	Female	7	<i>ApoE</i> ^{SA/SA}	Dox+Losartan
Figure 4D,4G	Female	10	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	10	WT	WT
	Female	8	<i>ApoE</i> ^{SA/+}	<i>ApoE</i> ^{SA/+}
	Female	10	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
Figure 4E,4F	Female	8	<i>ApoE</i> ^{-/-}	<i>ApoE</i> ^{-/-}
	Female	8	WT	WT
	Female	8	<i>ApoE</i> ^{SA/+}	<i>ApoE</i> ^{SA/+}
	Female	8	<i>ApoE</i> ^{SA/SA}	<i>ApoE</i> ^{SA/SA}
Figure 5, Figure S14	Male	5	<i>ApoE</i> ^{SA/SA}	ND
	Male	5	<i>ApoE</i> ^{SA/SA}	WD+Dox-3d
	Male	5	<i>ApoE</i> ^{SA/SA}	WD+Dox-28d
Figure 6A, 6B, 6C, 6D, 6E	Male	9	<i>ApoE</i> ^{-/-}	ND
	Male	9	<i>ApoE</i> ^{SA/SA}	ND
	Male	9	<i>ApoE</i> ^{-/-}	WD+Dox-3d
	Male	9	<i>ApoE</i> ^{SA/SA}	WD+Dox-3d

Figures	Gender	Number	Genotype	Group
Figure 7C	Male	6	WT	Veh.
	Male	6	WT	Ang II
Figure 7A	Male	6	WT	KCl
	Male	6	WT	U46619
	Male	6	WT	Ang II
	Male	8	WT	NE
	Male	6	WT	Ang II
Figure 7B	Male	7	WT	ACh
	Male	6	WT	ISO
	Male	6	WT	NE
	Male	6	WT	Vehicle
Figure 7D,7E,7F	Male	6	WT	Propranolol
	Male	6	WT	Phentolamine
	Male	6	WT	Veh
Figure 7G	Male	6	WT	L-NAME
	Male	6	WT	Veh
Figure 7H	Male	10	WT	Veh
	Male	8	WT	SC560
Figure 8A,8B	Male	5	<i>ApoE^{SA/SA}</i>	ND
	Male	5	<i>ApoE^{SA/SA}</i>	WD+Dox
Figure 8C	Male	12	<i>ApoE^{SA/SA}</i>	WD+Veh.
	Male	12	<i>ApoE^{SA/SA}</i>	WD+MTX
	Male	23	<i>ApoE^{SA/SA}</i>	WD+Dox+Veh.
	Male	23	<i>ApoE^{SA/SA}</i>	WD+Dox+MTX
Figure 8E	Male	8	<i>ApoE^{SA/SA}</i>	Veh.
	Male	8	<i>ApoE^{SA/SA}</i>	MTX
Figure 8F,8G	Male	5	<i>ApoE^{SA/SA}</i>	Veh.
	Male	5	<i>ApoE^{SA/SA}</i>	MTX
Figure 8I	Male	8	<i>ApoE^{SA/SA}</i>	Veh.
	Male	6	<i>ApoE^{SA/SA}</i>	MTX
Figure 8J	Male	8	<i>ApoE^{SA/SA}</i>	Veh.
	Male	6	<i>ApoE^{SA/SA}</i>	MTX
	Male	6	<i>ApoE^{SA/SA}</i>	MTX+Celecoxib

Figures	Gender	Number	Genotype	Group
Figure S1	Male	6	WT	WT
	Male	6	<i>ApoE^{SA/SA}</i>	<i>ApoE^{SA/SA}</i>
Figure S2	Male	6	WT	ND
	Male	6	<i>ApoE^{SA/SA}</i>	ND
	Male	6	WT	WD
	Male	6	<i>ApoE^{SA/SA}</i>	WD
	Female	6	<i>ApoE^{SA/SA}</i>	No doxycycline
Figure S3	Female	6	<i>ApoE^{SA/SA}</i>	3 days doxycycline
	Male	6	<i>ApoE^{SA/SA}</i>	No doxycycline
	Male	6	<i>ApoE^{SA/SA}</i>	3 days doxycycline
	Female	10	<i>ApoE^{-/-}</i>	Pre-WD
Figure S 4B, 4C,4D,4E	Female	10	<i>ApoE^{SA/SA}</i>	Pre-WD
	Female	14	<i>ApoE^{-/-}</i>	Post-WD
	Female	14	<i>ApoE^{SA/SA}</i>	Post-WD
	Female	14	<i>ApoE^{SA/SA}</i>	<i>ApoE^{SA/SA}</i>
Figure S5	Female	18	<i>ApoE^{-/-}</i>	<i>ApoE^{-/-}</i>
	Female	5	<i>ApoE^{SA/SA}</i>	<i>ApoE^{SA/SA}</i>
Figure S6	Female	5	<i>ApoE^{-/-}</i>	<i>ApoE^{-/-}</i>
	Female	8	<i>ApoE^{SA/SA}</i>	WD+Dox
Figure S7B	Male	45	<i>ApoE^{SA/SA}</i>	WD+Dox
Figure S7C	2W	6	<i>ApoE^{SA/SA}</i>	WD+Dox
	14W	6	<i>ApoE^{SA/SA}</i>	WD+Dox
	28W	13	<i>ApoE^{SA/SA}</i>	WD+Dox
	56W	12	<i>ApoE^{SA/SA}</i>	WD+Dox
Figure S8B	Male	12	<i>ApoE^{SA/SA}</i>	WD
	Male	60	<i>ApoE^{SA/SA}</i>	WD+Dox
	Female	14	<i>ApoE^{SA/SA}</i>	WD
	Female	30	<i>ApoE^{SA/SA}</i>	WD+Dox

Figures	Gender	Number	Genotype	Group
Figure S 8F,8G	Male	3	<i>ApoE^{SA/SA}</i>	ND
	Female	3	<i>ApoE^{SA/SA}</i>	ND
	Female	6	<i>ApoE^{SA/SA}</i>	WD+Dox-14D
	Female	6	<i>ApoE^{SA/SA}</i>	WD+Dox-21D
	Male	7	<i>ApoE^{SA/SA}</i>	WD+Dox-28D
	Female	6	<i>ApoE^{SA/SA}</i>	WD+Dox-28D
	Male	8	<i>ApoE^{SA/SA}</i>	WD+Dox-56D
	Female	4	<i>ApoE^{SA/SA}</i>	WD+Dox-56D
	Male	3	WT	MI/R
Figure S 9B,9C,9D,9E	Male	16	<i>ApoE^{SA/SA}</i>	WD
	Male	9	<i>ApoE^{SA/SA}</i>	WD+Dox
Figure S12	Female	8	<i>ApoE^{-/-}</i>	<i>ApoE^{-/-}</i>
	Female	8	<i>ApoE^{SA/SA}</i>	<i>ApoE^{SA/SA}</i>
Figure S16	Male	6	WT	WT+Vehicle
	Male	6	WT	WT+SC560
	Male	6	<i>CoxI^{-/-}</i>	<i>CoxI^{-/-}</i>
Figure S17	Male	3	WT	CA-Vehicle
	Male	3	WT	CA-Celecoxib
	Male	5	WT	FA-Vehicle
	Male	5	WT	FA-Celecoxib
Figure S18	Female	17	<i>ApoE^{SA/SA}</i>	Veh.
	Female	17	<i>ApoE^{SA/SA}</i>	MTX
	Male	11	<i>ApoE^{SA/SA}</i>	Veh.
	Male	10	<i>ApoE^{SA/SA}</i>	MTX
Figure S19A	Male	7	<i>ApoE^{SA/SA}</i>	WD+Veh.
	Male	7	<i>ApoE^{SA/SA}</i>	WD+MTX
	Male	7	<i>ApoE^{SA/SA}</i>	WD+Dox+Veh.
	Male	7	<i>ApoE^{SA/SA}</i>	WD+Dox+MTX
Figure S 19B,19C,19D,19E,19F,19G,19H	Male	5	<i>ApoE^{SA/SA}</i>	WD+Veh.
	Male	5	<i>ApoE^{SA/SA}</i>	WD+MTX
	Male	5	<i>ApoE^{SA/SA}</i>	WD+Dox+Veh.
	Male	5	<i>ApoE^{SA/SA}</i>	WD+Dox+MTX

1 **Table S5. Primer sequences for qPCR**

Gene	Genebank ID	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size(bp)
<i>ApoE*</i>	NM_009696.4	AGGTGACAGATCAGCTCGAGTG	TCCTTTACTTCCGTCATAGTGTC	181
<i>Scarb1*</i>	NM_016741.2	CTGGTGGACAAATGGAACGG	ACCTTCGTTGGGTGGGTAGAC	261
<i>Sdf1*</i>	NM_001012477	AATGGACGCCAAGGTCGTC	CCACTTTAATTCGGGTCAATG	235
<i>Gapdh*</i>	GU214026.1	ATCCTTCCGTGTTCTACCC	AAGGTGGAAGAGTGGGAGTTG	192
<i>Il-1β³⁴</i>	NM_008361.4	CTCCCCAGGGCATGTAAAG	ACCCTGAGCGACCTGTCTTG	101
<i>Il-6³⁴</i>	NM_031168.2	GCTACCAAACCTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA	77
<i>Collagen I³⁴</i>	NM_007742.4	GAGTACTGGATCGACCCTAACCA	GACGGCTGAGTAGGGAACACA	101
<i>Collagen III³⁴</i>	NM_009930.2	TCCCCTGGAATCTGTGAATC	TGAGTCGAATTGGGGAGAAT	63
AngII-Gaussian*		GAGATTCCTGGGTTCAAGGACTT	TTAGAAGGGGTGGATGTATACGC	267
<i>Scarb1</i> knockdown cassette*		GGCTAAGGACTTGTTCGGAAG	ATTTACACTCTCTGGGGTTGAAGG	196

2 *: designed by Primer Premier 5.0.

1 **Table S6. Basic characteristics of heart transplant and CABG patients**

Variables	Heart transplant patients		CABG patients
	Cluster 1 (n=9)	Cluster 2 (n=10)	(n=13)
Age, years	41.56±16.3	51.9±11.32	65.9±4.3
Male, n	8	10	13
Chest pain, n	1	3	4
NYHA-FC I / II / III / IV, n	0/1/5/3	0/0/5/5	1/8/4/0
LVEF, %	28.7±5.4	32.1±9.6	56.8±8.8
Medical history			
Smoking, n	1	3	9
Hypertension, n	0	10	13
Hyperlipidemia, n	0	10	13
Type II diabetes mellitus, n	0	3	7
Medications			
Diuretics, n	3	7	11
Antidiabetic agents, n	1	5	2
Antihypertensive agents, n	3	6	8
Lipid-lowering agents, n	0	3	7
Antithrombotic agent or anticoagulant, n	1	5	4

2 CABG, coronary artery bypass grafting; NYHA-FC, New York Heart Association functional classification; LVEF, left ventricular ejection fraction; Cluster 1, patients with
3 heart failure that had not been diagnosed with hypertension and hyperlipidemia; Cluster 2, patients with heart failure that had been diagnosed with both hypertension and
4 hyperlipidemia.

SUPPLEMENTAL FIGURES AND FIGURE LEGENDS

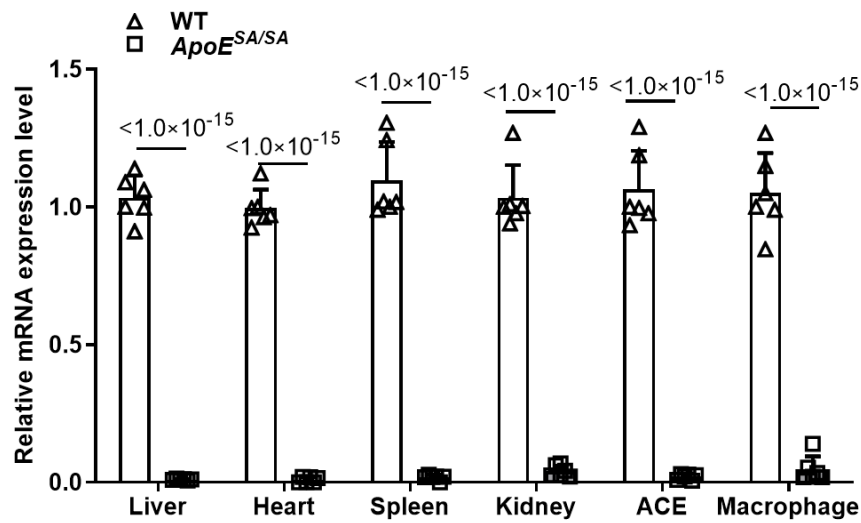
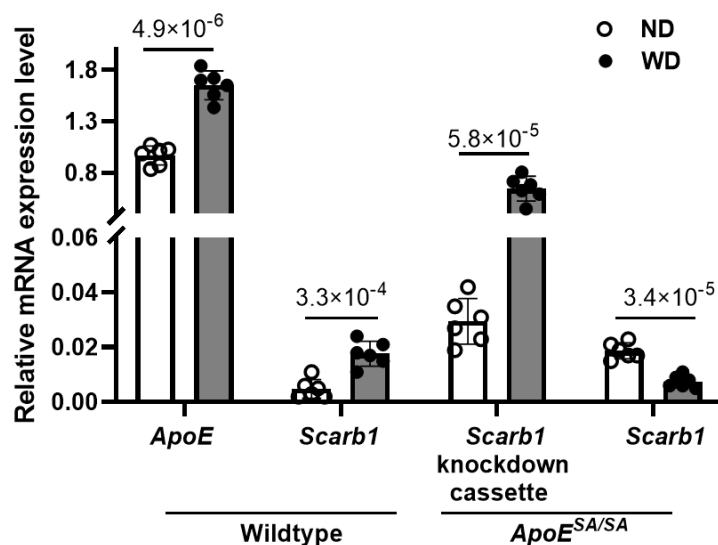


Figure S1. *ApoE* transcription detection in mice.

Tissue abundance of *ApoE* mRNA detected with qPCR, n=6 per group. Eight-week-old wildtype (WT) or *ApoE*^{SA/SA} mice fed normal diet. Student's *t*-test was used. AEC: aortic endothelial cell.

1



2

3 **Figure S2. WD feeding increased *ApoE* transcription, *ApoE* promoter-driven *Scarb1***
 4 **knockdown cassette transcription and the *Scarb1* interference therein.**

5 Wildtype or *ApoE*^{SA/SA} mice were fed WD for 28 days, liver were collected for transcription
 6 detection by RT-PCR. Transcription of *ApoE* and *Scarb1* in wildtype mice and transcription of
 7 *Scarb1* knockdown cassette and *Scarb1* in *ApoE*^{SA/SA} mice were determined. ND: normal
 8 laboratory diet. n=6 males. Student's *t*-test was used for statistical analysis. Eight-week-old
 9 male mice were used.

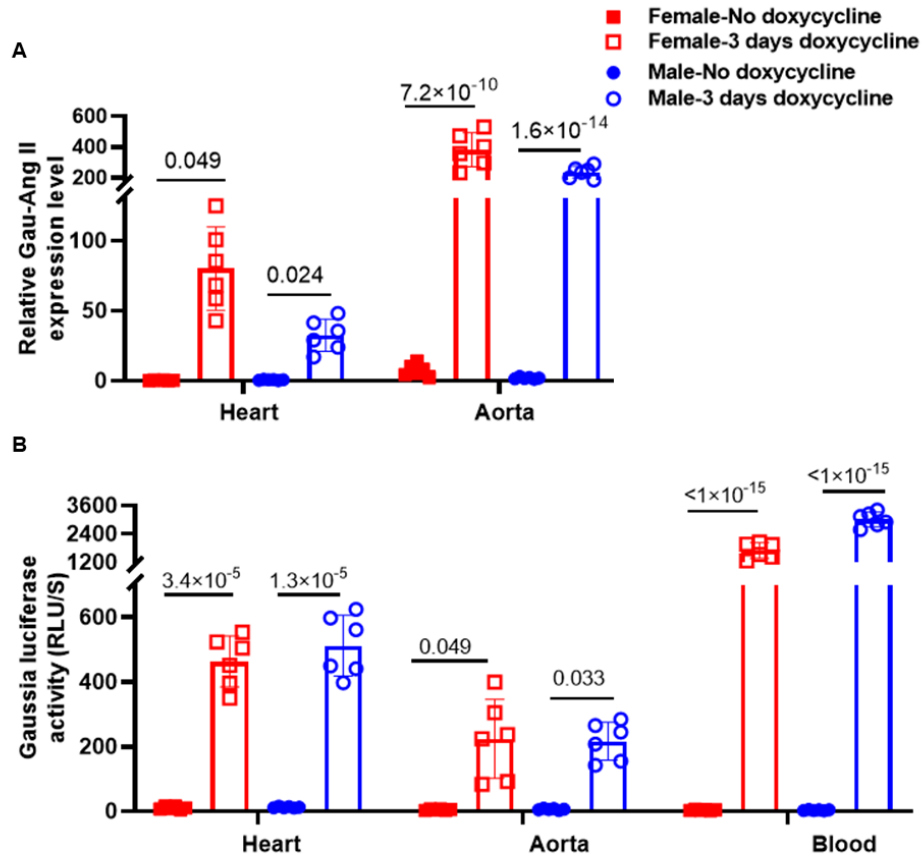


Figure S3. Tissue expression of AngII and its reporter Gaussian luciferase.

Eight-week-old *ApoE^{SA/SA}* mice were fed WD and administered doxycycline in drinking water for 3 days. Different tissues were collected for Ang II-Gaussian detection. A: Ang II-Gaussian transcription detection; B: Gaussian luciferase activity measurement. n=6. Student's *t*-test was used for statistical analysis (A, B).

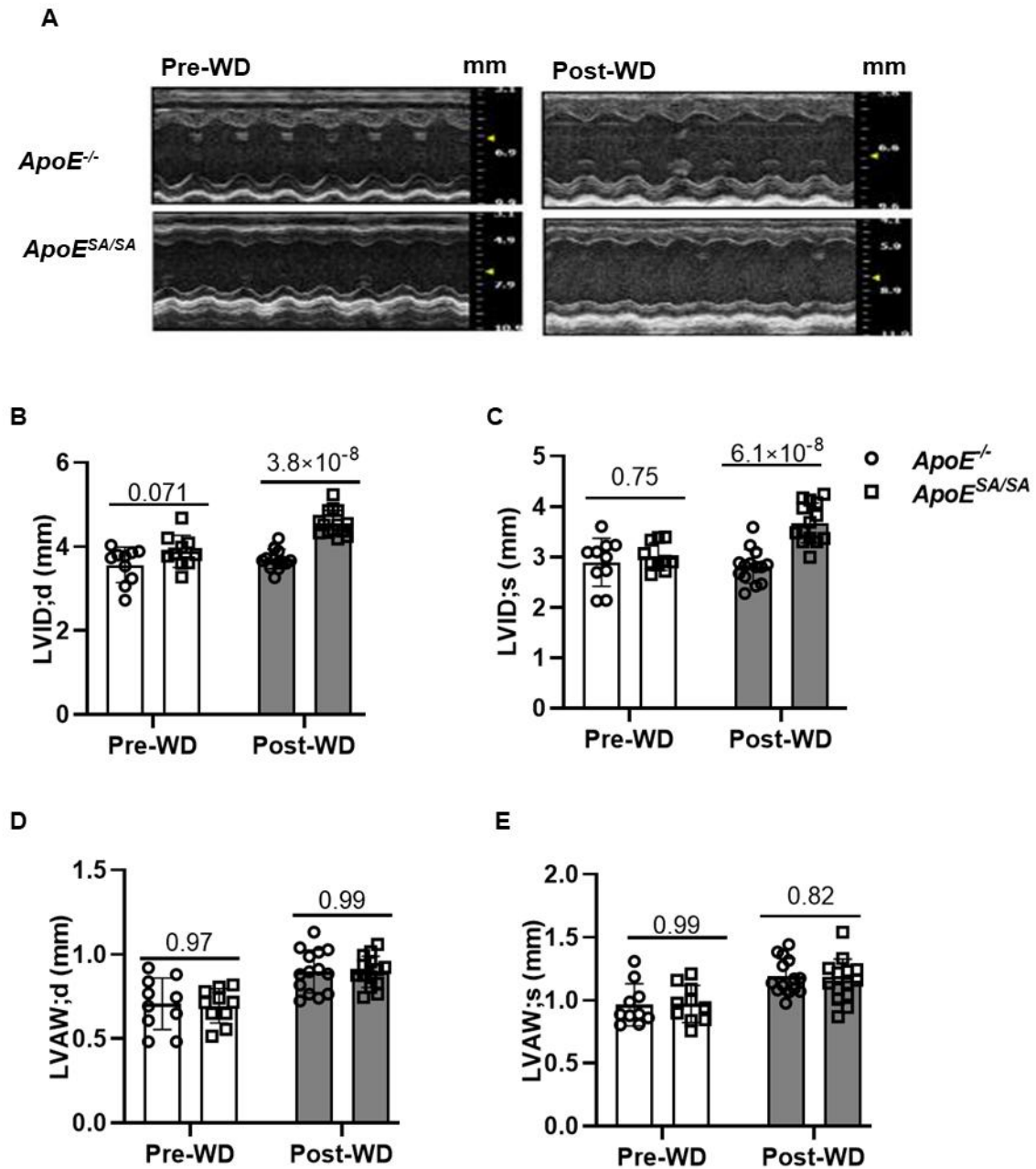


Figure S4. Assessment of cardiac function by echocardiography.

Eight-9 week-old female mice were fed WD for 16 weeks. A: Representative M-mode short-axis views of LV. B-E: Echocardiographic parameters measured before initiation of WD feeding and 16 weeks after. LV: left ventricle; ID: internal diameter; d: diastole; s: systole; AW: anterior wall. Pre-WD group n=10, Post-WD group n=14. Two-way ANOVA with Šídák's multiple comparisons test was used for statistical analysis.

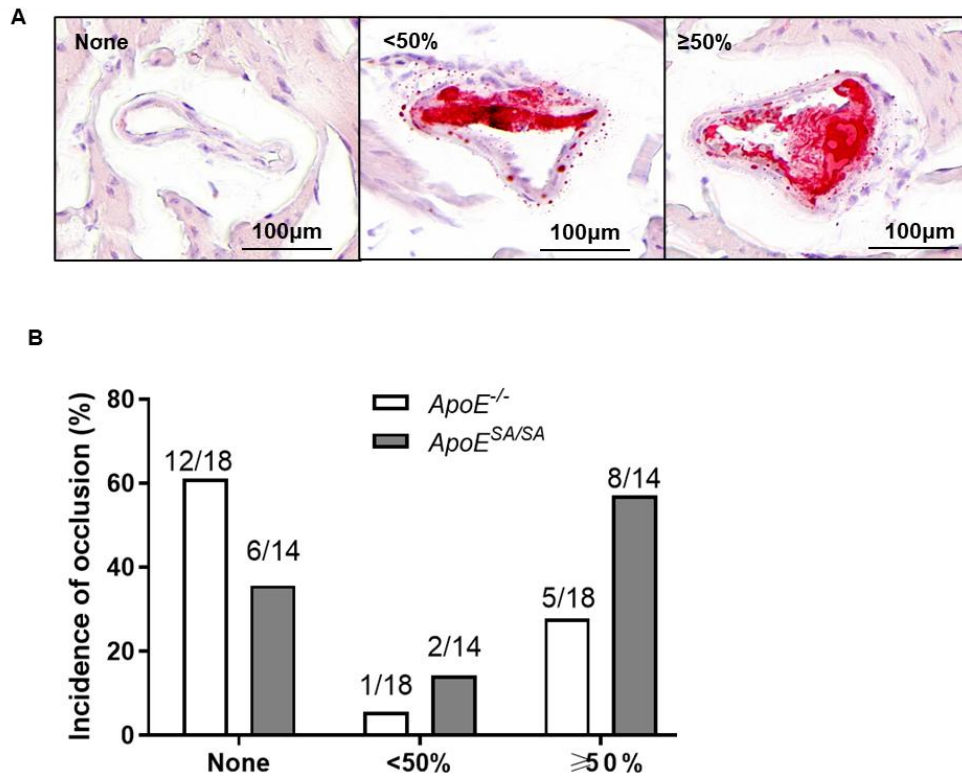


Figure S5. Coronary artery plaque analysis.

ApoE^{SA/SA} or *ApoE*^{-/-} mice (female, 8-9 weeks old) were fed WD for 16 weeks, heart was collected for pathological analysis, n=14 *ApoE*^{SA/SA} mice, n=18 *ApoE*^{-/-} mice. Cardiac sections were taken at three levels of 200 μm interval for coronary artery staining. A: Representative Oil Red O-stained coronary arteries with different degrees of occlusion, namely, no occlusion (left), <50% occlusion (middle), ≥50% occlusion (right). B: The incidence of mice showing coronary occlusion of different extent. The three cardiac cross sections of each animal were scanned entirely, and, mice showing one or more ≥50% occluded arteries, one or more <50% occluded arteries, and no occluded arteries, were counted and presented as percentage of total animal number of each group (incidence of occlusion, with absolute number ratio shown above each column). Mice with vessels of both ≥50% and <50% occlusions are counted for both categories.

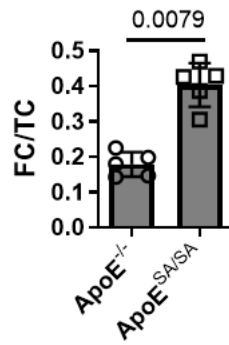


Figure S6. Ratio of free cholesterol to total cholesterol in mouse plasma

Eight-9 week-old female mice were fed WD for 16 weeks. Ratios of free cholesterol (FC) to total cholesterol (TC) was determined. n=5, Student's t test.

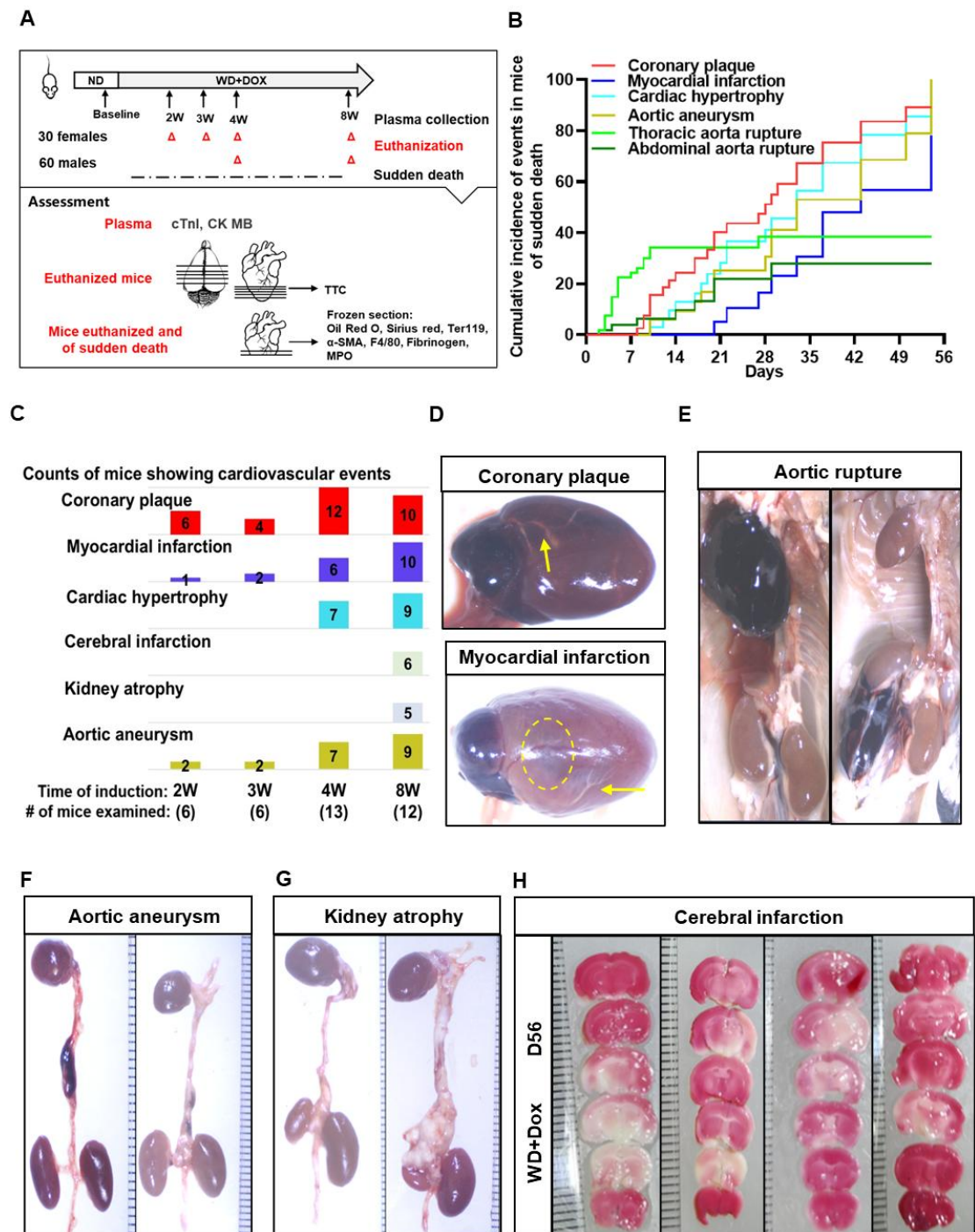


Figure S7. Cardiovascular events following hypercholesterolemia and hypertension induction in *ApoE^{SA/SA}* mice.

A: Flowchart of study design. Eight to nine weeks old *ApoE^{SA/SA}* mice (60 males and 30 females) were initiated with western diet (WD) and doxycycline (Dox) water to induce hypercholesterolemia and hypertension for up to eight weeks. The condition of the mice were

1 monitored every day, necropsies were performed on mice found dead, and their cardiovascular
2 abnormalities were recorded. Some female mice were euthanized at 2, 3, 4, and 8 weeks of
3 induction, some male mice were euthanized at 4 and 8 weeks of induction, and their gross
4 pathology was examined. Plasma and hearts were collected for myocardial injury biomarker
5 analysis or histology examination. B: Cumulative incidence of cardiovascular pathologic
6 events based on autopsy analysis (45 males and 8 females). Mice with severe coronary plaques,
7 visible to naked eyes, were counted; MI was defined by the presence of pale-colored
8 myocardium or ventricular wall thinning (more transparent); cardiac hypertrophy was defined
9 by heart weight greater than 190 mg (normal heart weight ranged 100-150 mg); abdominal
10 aortic aneurysm was defined by expansion of aortic diameter by 50% or above compared to
11 normal diameter; thoracic or abdominal aorta rupture was adjudged by existence of blood clot
12 in the thoracic or abdominal cavity. C: Counts of cardiovascular events in euthanized mice.
13 Mice were sacrificed at 2, 3, 4, and 8 weeks (W) of double-hit induction and numbers of mice
14 showing specific cardiovascular phenotype are presented. Numbers of mice examined at each
15 timepoint are shown in parathesis at the bottom. D: Representative images of hearts with
16 coronary plaques (yellow arrows) and infarct myocardium (yellow dotted circle). E-G:
17 Representative images of aortic rupture (E), aortic aneurysm (F) and kidney atrophy (G). H:
18 TTC staining of brain tissue showed stroke in mice euthanized at 56 days of WD + Dox
19 induction.

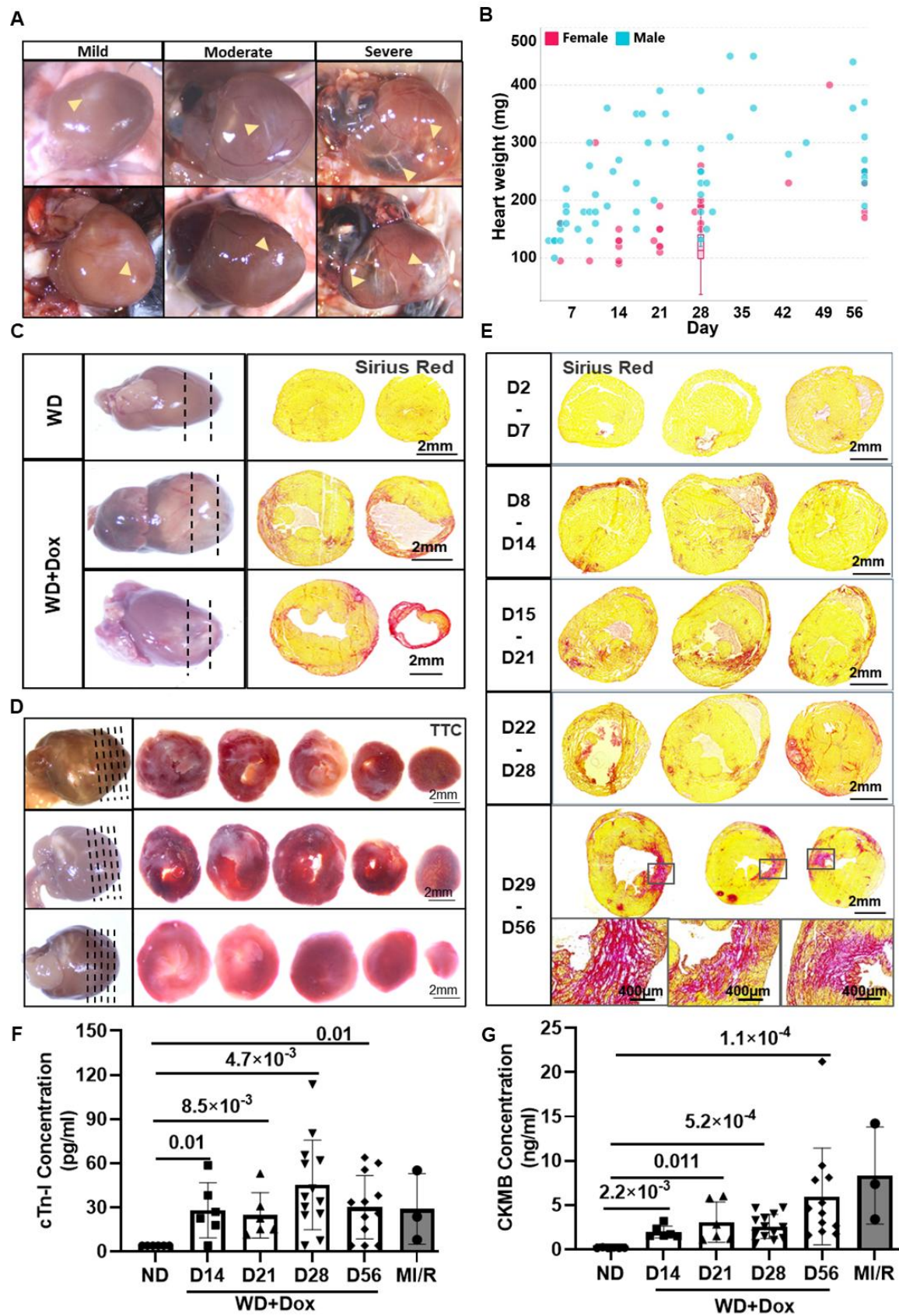


Figure S8. Spontaneous myocardial infarction in *ApoE^{ES/SA}* mice following western diet feeding and hypertension induction

A total of 90 mice (60 males and 30 females) were examined, with the study design detailed in Figure S7. A: Representative images of the hearts with myocardial infarction. B: Individual heart weight is shown in a scatter diagram relative to time of autopsy or euthanization. For reference, 12 males and 14 females were initiated with WD only, and their heart weight was examined on Day 28 and shown in box diagram. C: Representative images of cardiac sections stained with Sirius Red (tissue section is illustrated to the left). Hearts from mice treated with WD only were used as control. Ventricular thinning of the infarct region was observed in some mice treated with WD + Dox. D: Myocardium infarct region (pale) stained by TTC for euthanized mice. E: Representative images of Sirius Red-stained cardiac sections at different stages of double-hit induction. Collagen fibers stain red, and cardiac muscle fibers stain yellow. Note that cardiac fibrosis worsened over time. Insets show regions under high magnification. F-G: Plasma levels of cTnI (F) and CK-MB (G). Plasma was collected at days 14, 21, 28, and 56 of double induction. Plasma from mice that underwent 30-minute ischemia followed by 24-hour reperfusion (MI/R) was used as control. n=6 (3 females and 3 males) in ND group, n=6 females in D14 group, n=6 females in D21 group, n=13 (6 females and 7 males) in D28 group, n= 12 (4 females and 8 males) in D56 group, n=3 males in MI/R group. Student's t-test (B, F and G). ND: normal diet. D: day, MI/R: Myocardial Infarction/Reperfusion.

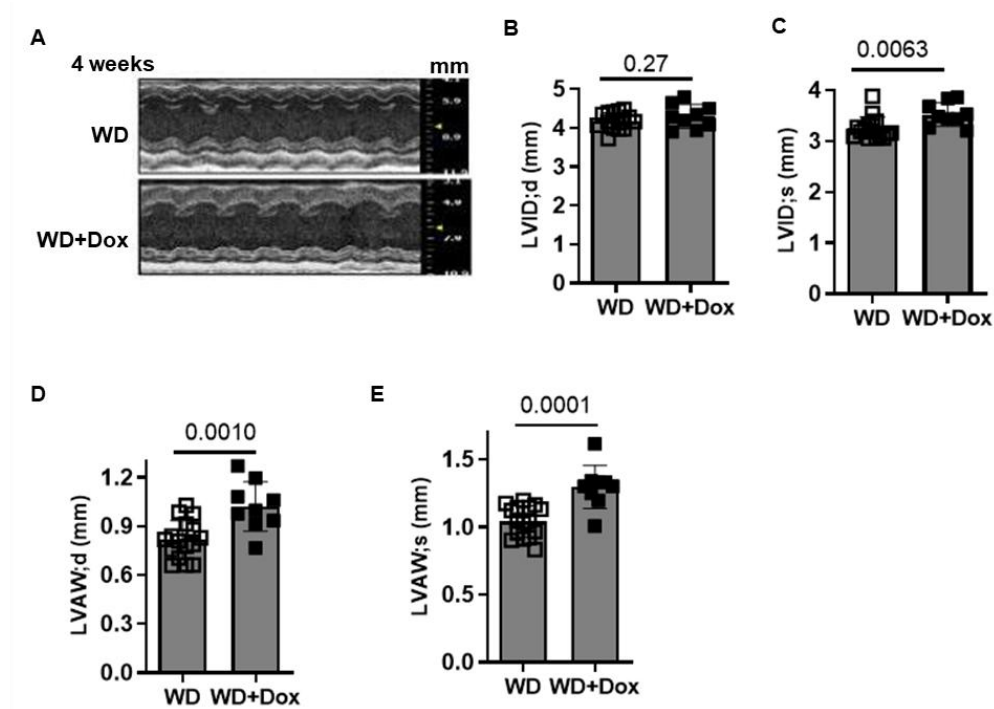


Figure S9. Echocardiography assessment of *ApoE*^{SA/SA} mice

Eight-week-old male *ApoE*^{SA/SA} mice were fed WD alone (WD group) or fed WD and administered doxycycline in drinking water (WD+Dox group), for 4 weeks. A: Representative M-mode short-axis views of the left ventricle. B-E: Echocardiographic parameters. WD+Dox group: n=9, WD group n=16. Student's *t*-test was used for statistical analysis.

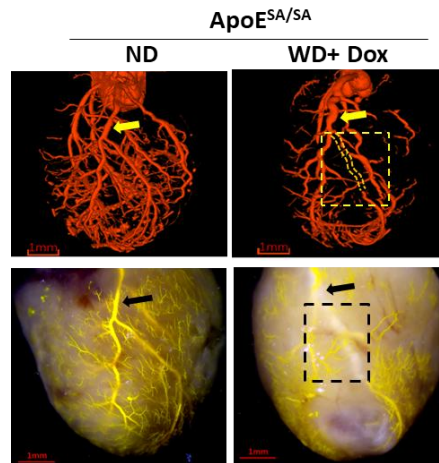


Figure S10. Micro-CT imaging of mouse hearts

Coronary vascular imaging by micro-CT (upper panels) and stereomicroscope (lower panels) for the Microfil-prefilled hearts from *ApoE^{SA/SA}* mice that were treated for four weeks with ND (left panels) or with WD plus Dox (right panels). The boxed areas show an occlusive coronary artery with atherosclerotic lesions visible under the stereomicroscope but missing in the micro-CT image (demarked by the two dotted lines). Arrows indicate the left anterior descending artery.

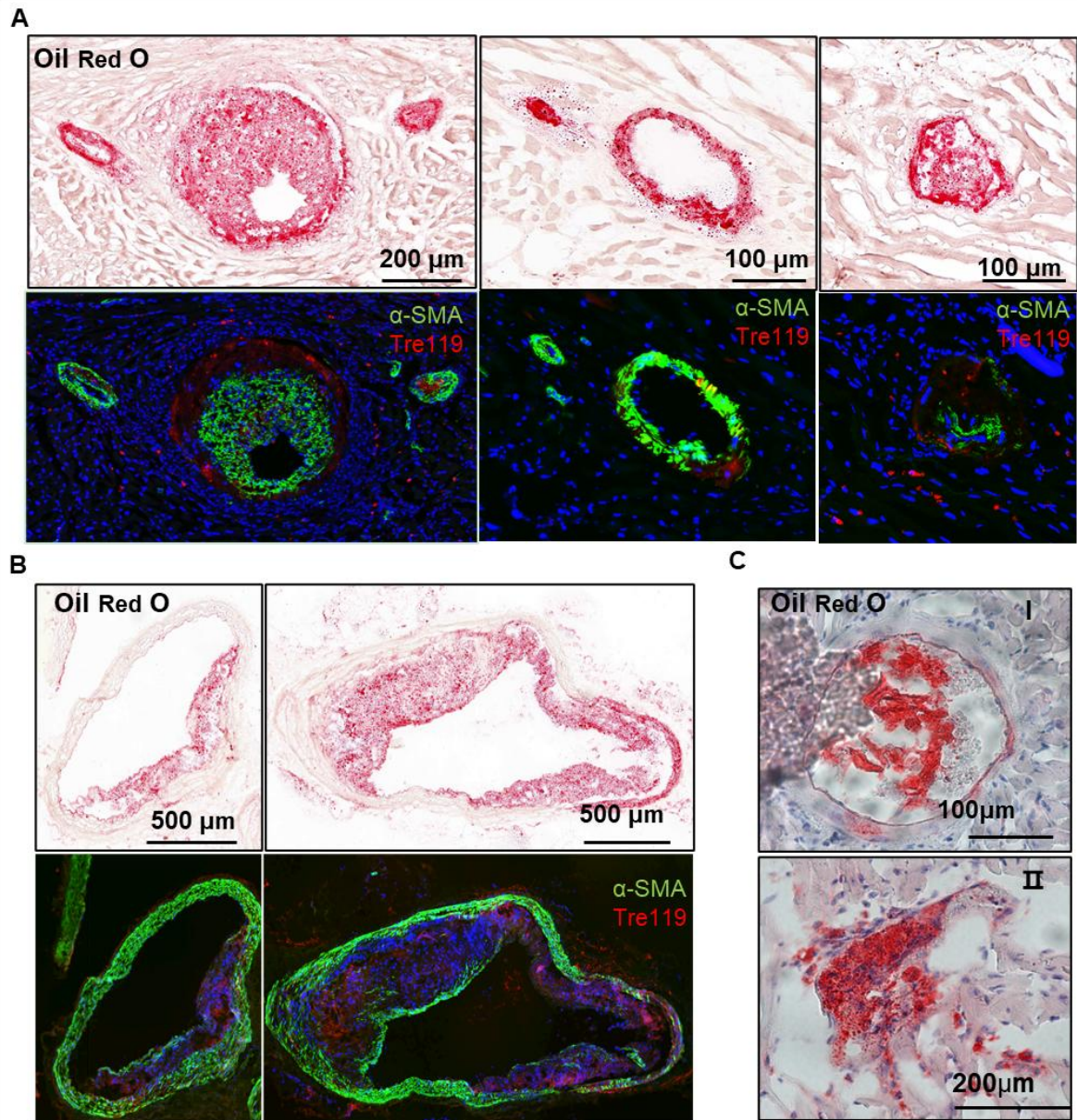


Figure S11. Histology staining of atherosclerotic lesions and plaque rupture

Representative images of coronary atherosclerotic lesions (A) and carotid atherosclerotic lesions (B) stained with Oil Red O (upper panel) and with antibodies for smooth muscle cells (α -SMA+) and red blood cells (Tre119+) (lower panel). C: Representative images showing distal blockade likely resulted from plaque rupture.

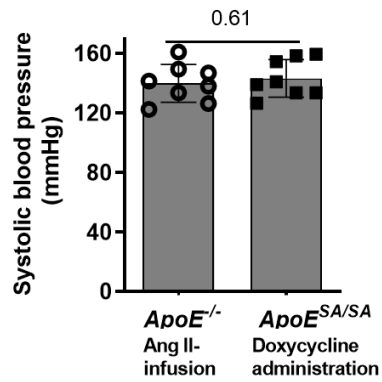


Figure S12. Assessment of systolic blood pressure.

Eight-week-old female *ApoE*^{-/-} mice were infused with Ang II (50 ng/min) for 4 weeks. Eight-week-old female *ApoE*^{SA/SA} mice were administered doxycycline for 4 weeks. Blood pressure was measured at 4 weeks of treatment. n=8, Student's *t*-test.

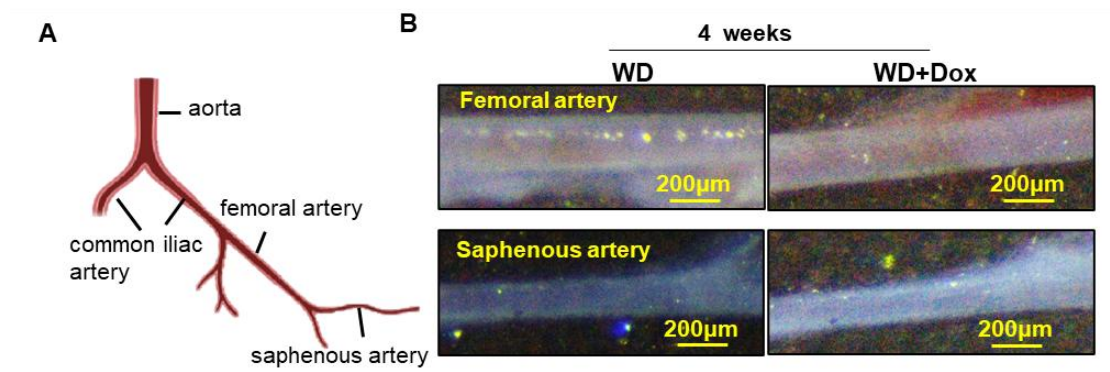


Figure S13. Gross images of femoral arteries in *ApoE^{SA/SA}* mice

Eight-week-old male *ApoE^{SA/SA}* mice were fed a Western diet (WD group) or fed WD and administered doxycycline (WD+Dox group). A: Illustration of arterial anatomy in the hind limb. B: Representative images of hind limb arteries. Femoral artery with about 240-290 µm vessel diameter was used in this study. The vessel diameter of the saphenous artery was about 140-200µm.

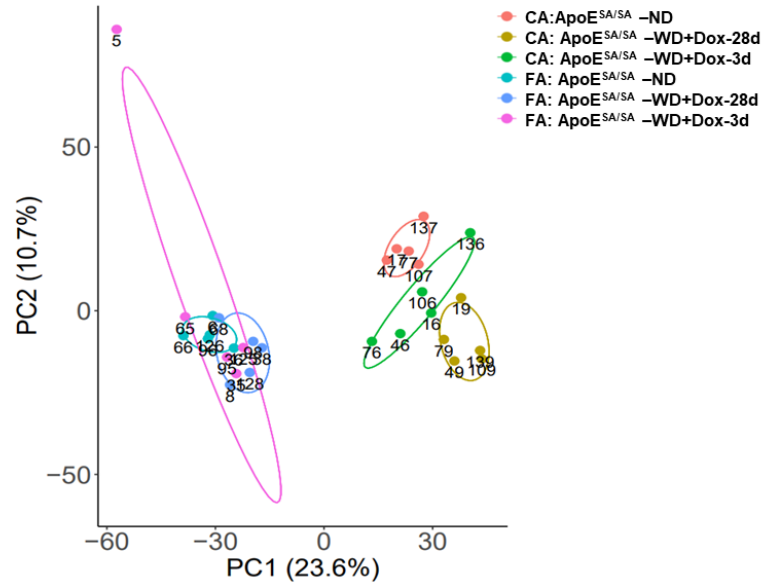
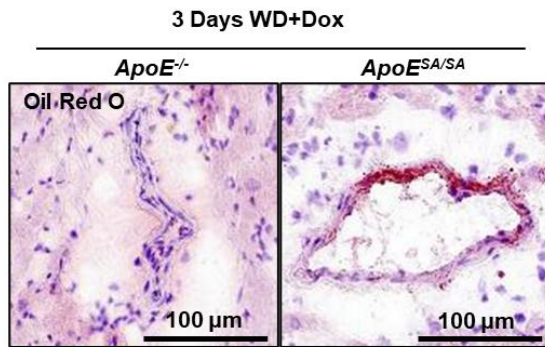


Figure S14. Principal Component Analysis (PCA) of proteomic data

PCA two-dimensional scatter plot represents the differential protein expression patterns of coronary and femoral arteries isolated from male *ApoE^{SA/SA}* mice. PCA analysis of whole samples dataset. One outlier in FA vessels from mice with 3 days WD plus doxycycline was removed in further analysis because it had too many missing values. Splashes represent different samples. Numbers represent sample ID. CA: coronary artery; FA: femoral artery; ND: normal diet. n=5 male mice.

A



B

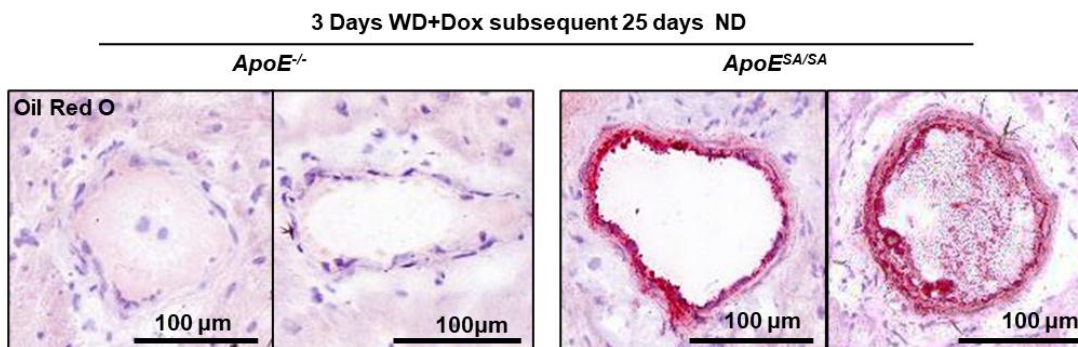


Figure S15. Early coronary lesions in *ApoE*^{SA/SA} mice.

Oil red O staining of coronary arteries from eight-week-old male *ApoE*^{-/-} and *ApoE*^{SA/SA} mice that were fed WD and administered doxycycline for 3 days (A) and with an additional 25 days of normal diet feeding (B). Three independent experiments were performed and results were consistent, with representative images shown.

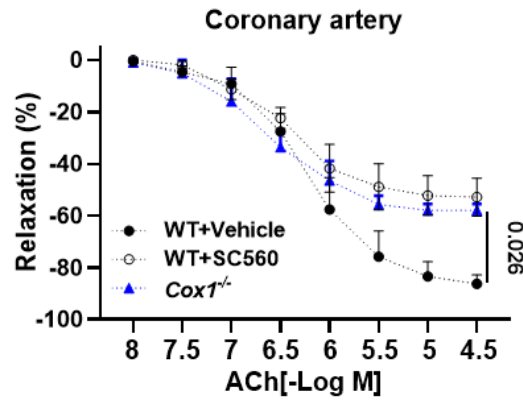


Figure S16. Prostaglandin synthesis blockade impaired coronary vasodilation response.

Coronary arteries were isolated from male wildtype (WT) or *Cox1*^{-/-} mice and relaxation response to ACh was determined. Relaxation was expressed as percentage of the contraction response to U46619. WT coronary arteries were also incubated with 5 μ M of SC560, a cyclooxygenase inhibitor that suppresses prostaglandin biosynthesis. Values are mean $\pm$ SEM. n=6 vessels from 6 mice. Cumulative concentration-response curves were analyzed by comparing area-under-curve with one-way ANOVA with Tukey multiple comparison test.

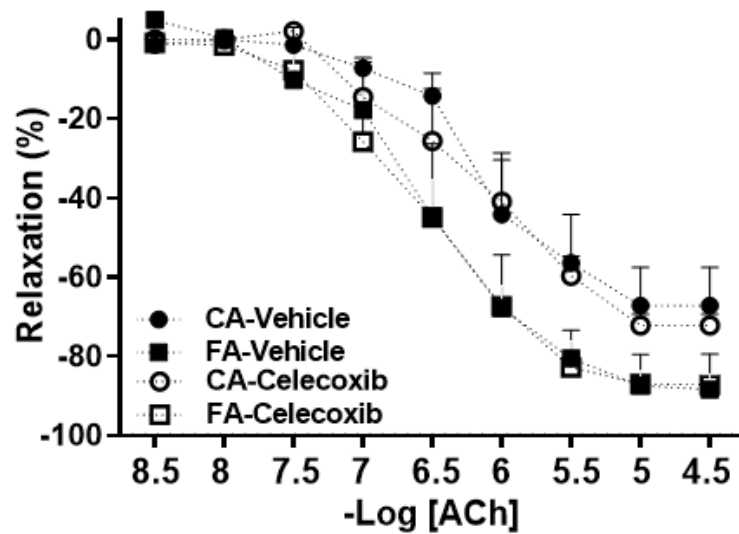


Figure S17. Inhibition of COX-2 did not suppress vasodilation.

Coronary arteries (CA) and femoral arteries (FA) were isolated from eight-week-old male wildtype mice and relaxation response to ACh was determined. Relaxation was expressed as percentage of the contraction response to U46619. The arteries were also incubated with celecoxib (5 μ M), a cyclooxygenase-2 inhibitor. Values are mean $\pm$ SEM. CA groups: n=3 vessels from 3 mice. FA groups: n=5 vessels from 5 mice. Cumulative concentration-response curves were analyzed by comparing area-under-curve with one-way ANOVA with Tukey multiple comparison test.

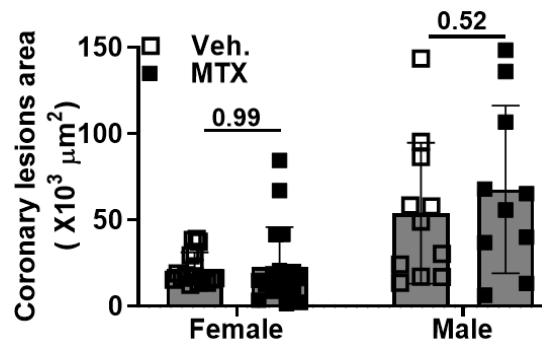


Figure S18. Coronary atherosclerotic lesion size in *ApoE^{SΔ/SΔ}* mice administered with MTX.

Eight weeks old *ApoE^{SΔ/SΔ}* mice were fed WD and administered doxycycline in drinking water, and were injected intraperitoneally with methotrexate (MTX, 1 mg/kg weekly) or vehicle. Coronary atherosclerotic lesions were quantified in surviving mice following 28 days administration. n=17, 17, 11, 10 mice for the groups labeled from left to right. Statistical analysis was performed by two-way ANOVA with Šídák's multiple comparison test.

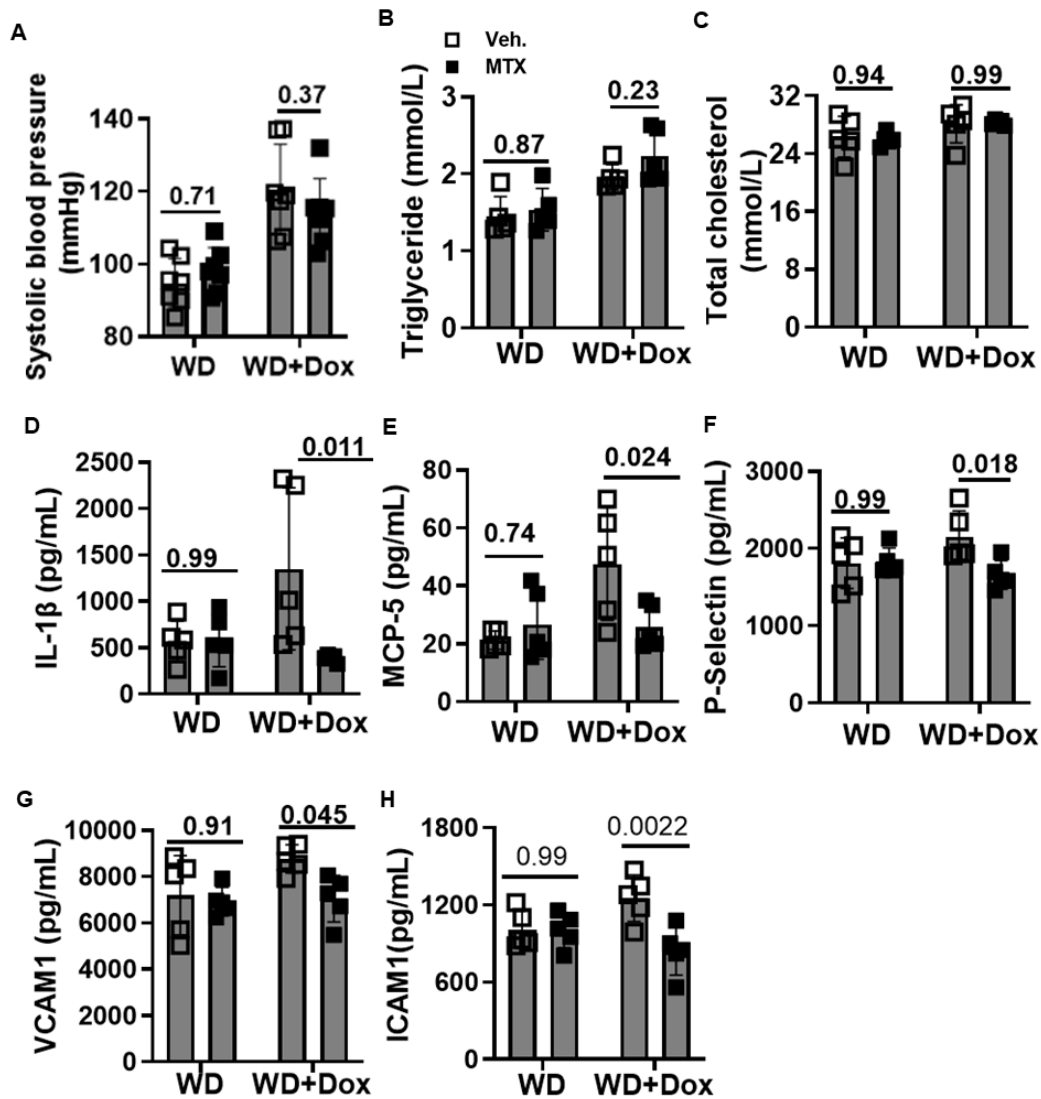


Figure S19. Blood pressure, plasma lipids and cytokines in *ApoE^{SA/SA}* mice administered with MTX.

Male *ApoE^{SA/SA}* mice were fed WD alone or fed WD and administered doxycycline (WD+Dox), and then randomized to receive methotrexate (MTX, 1 mg/kg weekly, intraperitoneal injection) or saline (Veh.). A: Systolic blood pressure was measured, n=7. Plasma concentrations of triglycerides (B) and total cholesterol (C) were measured, n=5. D-H: Plasma concentrations of IL-1 β , MPC-5, P-selectin, VCAM1 and ICAM1. n=5. Student's *t*-test was used to compare the two groups connected by line.

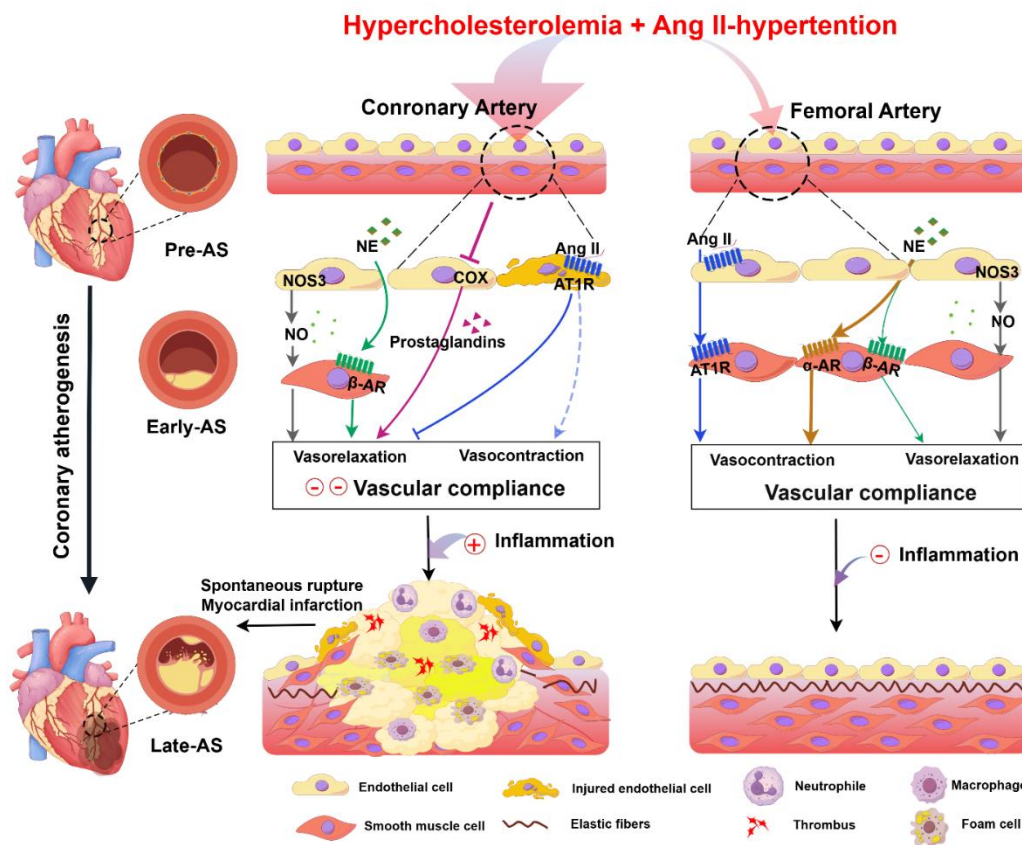


Figure S20. Selective susceptibility of coronary atherosclerosis to hypercholesterolemia and Ang II-induced hypertension underscores a distinctive coronary vasomotor reactivity.

Hypercholesterolemia and hypertension are two major risk factors for atherosclerotic cardiovascular disease. Genes for *ApoE* and *Scarb1* (the receptor for HDL) are associated with hypercholesterolemia in human genome-wide association study. When exposed to an excess risk of hypercholesterolemia, as shown here, upon deficiency of both *ApoE* and *Scarb1*, coronary artery develops mild atherosclerosis (AS). Impressively, combination of hypercholesterolemia and Ang II-induced hypertension exerts a strong synergistic effect on coronary atherogenesis compared to femoral atherogenesis, exhibiting cardinal features of human CAD that include endothelial erosion, myeloid cell infiltration, spontaneous plaque rupture, resulting in myocardial infarction, heart failure, and cardiovascular death with male gender predilection. Ang II, via its receptor AT1, confers a coronary risk beyond its hypertensive action, which is in contrast to a lack of alike severe coronary atherosclerosis in

1 norepinephrine (NE)-induced hypertensive mice. As a mechanistic basis, coronary
 2 endothelium-dependent vasodilation is mediated by both nitric oxide (NO) and vasodilating
 3 prostaglandins (PGs, particularly, PGI₂), as compared to an NO dominated physiology of
 4 femoral arteries. Vasomotor reaction of coronary arteries, relative to femoral arteries, is
 5 particularly vulnerable to Ang II exposure and to prostaglandin inhibition. This distinctive
 6 pathophysiology of coronary arteries may render an increased hemodynamic stress on the
 7 vessel wall due to lacking vascular motion compliance in the presence of hypercholesterolemia
 8 and hypertension. The curved anatomy of coronary arterial tree in a beating heart, might further
 9 predispose coronary arteries to atherosclerosis. In contrast to the impairment of coronary
 10 vasorelaxation by Ang II, NE dilates coronary arteries via β -adrenergic receptors (AR),
 11 increasing coronary vascular compliance and imposing a constraint on coronary atherogenesis.
 12 NE constricts peripheral arteries via α -AR increasing systemic blood pressure and may thus
 13 promote atherogenesis as a general hemodynamic stressor.
 14 Solid arrow denotes 'positive action; dotted arrow denotes null or weak effect; $\dashv$ denotes
 15 'inhibition'; $\oplus$ and $\ominus$ denote 'increase' and 'decrease', respectively; arrows thickness
 16 indicates relative (arbitrary) extent of action. The schematic diagram was drawn by Figdraw.