Supplemental Material

Expanded Materials & Methods

Human subjects. The demographic and clinical characteristics of the recruited subjects are presented in **Online Table I**. Consecutive consenting patients diagnosed with KD (American Heart Association criteria) [5] were randomly recruited to the study (Guangzhou Women and Children's Medical Center Human Investigation Committee No. 2017102710). KD subjects with underlying congenital heart disease, such as mitral valve prolapses, bicuspid aortic valve, and/or hemodynamically insignificant ventricular septal defects, were excluded. All recruited KD patients were treated with IVIG (2 g/kg) and aspirin (30–50 mg/kg/day) during the acute phase. In our cohort study (2017102710), a subset of KD patients with GCAA (n=12) were randomly enrolled in the clinical trial for simvastatin treatment (0.25-0.5 mg/kg/day). The same amount of age- and illness day- matched subjects with GCAA were treated with IVIG and aspirin as control. Details of KD patients with GCAA treated with or without statin are given in **Online Table II**. Age- and sex- matched healthy controls were children from annual outpatient visits. Healthy subjects with history of specific diseases, including primary coagulation disorder, severe infection, anemia, and/or thrombocytopenia; or with history of specific medication, such as anticoagulation, antiplatelet drugs, and glucocorticoid hormone, will be excluded (**Online Table III**). All the subjects are randomly recruited in our study. Written informed consent was obtained from the patients' guardians and adolescent or child assent was obtained. Coronary disease was determined using established echocardiography scores where coronary artery dimensions were described by Z-worst, the maximal Z-score (standard deviation units from the mean) of the internal diameter of the left anterior descending (LAD) or left main coronary artery (LMCA) normalized for body surface area. A normal coronary artery dimension was defined as a Z-worst < 2.5. Small aneurysm with a Z-worst ≥2.5 to <5; Medium aneurysm: ≥5 to <10, with absolute dimension <8 mm; Large or giant aneurysm: ≥10, or absolute dimension ≥8 mm [1, 5].

Platelet and PMP purification. Venous blood was drawn from healthy controls and KD patients. The platelets were prepared as previously described [18]. Briefly, platelet rich plasma

(PRP) was obtained by centrifugation at 250 g for 20 minutes. PRP was then treated with 100 nM Prostaglandin E1 (PGE1, Sigma) and passed through Acrodisc® WBC (White blood cell) syringe filter (Pall Inc, Part no AP-4851) for leukocyte removal. Briefly, 10 ml syringe barrel was attached to the filter inlet and mount the system over a 15 ml collection tube. Add the PRP to the syringe barrel and let it drain through the filter by gravity at a flow rate of 2.5-3.0 ml/min. After PRP passing through the device, wash it twice with 5 ml HBSS at a rate of approximately 3 drops per second. The suspension in collection tube was then centrifuged at 1000 g for 5 min. The sediment was resuspended to 10^8 platelets/ml in HEPES-Tyrode's buffer. Purity of platelet preparation was determined by flow cytometry (BD FACSCanto^{TM}) analysis using platelet markers (>99% CD41 positive). The RNA was extracted by addition of TRIzol (Invitrogen) and stored at -80℃ until analysis.

Platelet-derived microparticles (PMPs) were isolated from platelet-poor plasma, as previously described [24]. Briefly, PMPs were harvested by centrifugation at 20,000g for 90 minutes at 18°C, and were resuspended in HEPES-Tyrode's buffer. Purity of PMPs was determined by flow cytometry (BD FACSCanto^{TM}) analysis using platelet markers (>90% CD41 positive), and Nanosight NS300 (Malvern, UK) with approximately 100 to 400 nm in diameter. The supernatant fraction (plasma with neither platelets nor PMPs) was collected and stored at -80 ℃ until analysis.

Flow cytometry. Cell surface P-selectin (CD62P, Biolegend) expression in platelets was determined by flow cytometry (BD FACSCanto^{TM}) using PE anti-human CD62P (P-Selectin) antibody. Briefly, platelet suspensions (10^8 platelets/ml) were incubated with the fluorescent antibody at room temperature for 15 min, transferred to Eppendorf tubes and washed (2–3 times) with HEPES-Tyrode's buffer. Cell pellets were re-suspended in HEPES-Tyrode's buffer and analyzed by flow cytometry. Data were collected from 10,000 platelets on a BD FACSCanto^{TM} and analyzed using FlowJo-V10 software.

Enzyme-linked immunosorbent assay (ELISA). The level of PDGF-BB in the supernatant of VSMCs after incubation with platelets for 48 hours was determined by Human platelet derived growth factor BB, PDGF-BB ELISA Kit (CSB-E08923h, CUSABIO). The amount of platelet factor 4 (PF4), beta-thromboglobulin (β-TG), and tumor necrosis factor-alpha (TNF-α)

in human and murine plasma were determined by platelet factor 4, PF-4 ELISA Kit (CUSABIO, CSB-07884m; CSB-07882h), β-Thromboglobulin, β-TG ELISA Kit (CUSABIO, CSB-07888m; CSB-07886h), Tumor necrosis factor-alpha, TNF-α ELISA Kit (CUSABIO, CSB-E04741m, CSB-E04740h), respectively. The amount of CD62P in murine plasma was determined by P-Selectin (CD62P) ELISA Kit (CUSABIO, CSB-E04709m). Briefly, 100 μl of plasma was incubated with the capture and secondary antibodies according to the manufacturer's instructions. The intensity was measured at 450 nm (OD450) with reference measurement at 540 nm (OD540). The optical imperfections in the plate was corrected by subtracting the readings at 540 nm from the readings at 450 nm. A standard curve was plotted according to the manufacturer's instructions. The amount of protein of interest in each sample was calculated according to the standard curve.

Hematological analysis. Laboratory parameters, such as platelet counts, mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR) were analyzed using an automated hematology analyzer XS-800i (Sysmex, Japan).

miRNA sequencing and data analysis. Total RNA was extracted using a miRNeasy mini kit (QIAGEN) according to the manufacturer's protocols. The extracted RNA quality and quantity was determined using a Nano Drop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific) and purified by polyacrylamide gel electrophoresis (PAGE). Adenylated 3' adapters were then added to the 18~30 nt small RNAs, followed by the ligation of 5' adapters. The adapter-ligated products were subsequently transcribed into cDNA and amplified by RT-PCR. The PCR products with target fragments 115~140 bp were selected, purified, and further sequenced using the BGISEQ-500 platform (BGI-Shenzhen, China). After filtering the low-quality and contaminant tags, the remaining tags were mapped to the reference genome and other miRNA databases. The miRNA expression level including existing miRNA, known miRNA and novel miRNA, was calculated and normalized to transcripts per million (TPM). Heatmaps were drawn to assess for intersection and union DESs (digital gene expression profiles) for differentially expressed miRNA. The miRNAs with a fold change greater than 1.2 and P value higher than 0.05 were regarded as significantly differentially expressed miRNAs. Results from the sequencing was deposited in the NCBI database

(https://dataview.ncbi.nlm.nih.gov/object/PRJNA576349?reviewer=p6vck51k19t8fa37om7rh48ldo). Many of the top 11 induced miRNA likely have pleiotrophic effects on many components of the repair process including on VSMC, endothelial cells, myofibroblasts and white blood cells. In selecting which miRNA to further study, our inclusion criteria included 1) very high abundance in platelets, 2) not normally found in VSMC 3) can be horizontally transferred to VSMC and 4) has recognized significant effects on VSMC.

Reverse transcription quantitative PCR (RT-qPCR). MiRNA from platelets was purified using a miRNeasy mini kit (QIAGEN). For the quantification of pre-miR-223, total RNA was extracted from platelets using TRIzol reagent (Invitrogen). The extracted RNA was reverse transcribed using the PrimeScriptTM RT reagent Kit (Takara), and analyzed by qPCR using the Hairpin-it^{TM} miRNA qPCR Quantitation Kit (GenePharma), according to the manufactures' instructions. Values were normalized to small nuclear RNA U6 (RNU6), or human β-actin and expressed as $2^{-(Ct(target\ gene) - Ct(target\ gene))}$ for miRNA (miR-223-3p) and mRNA (pre-miR-223), respectively. For plasma samples, RNA was isolated using the miRNeasy Serum/Plasma kit (QIAGEN). The C. elegan cel-miR-39 (GenePharma) was used as the spike-in control. The primer sequences used in qPCR are listed in **Online Table V**.

Cell culture. Human coronary VSMCs were purchased from Cell Applications (San Diego, CA) at passage 3. Cells were cultured in smooth muscle cell media supplemented with 10% fetal bovine serum (FBS), smooth muscle cell growth supplement and 1% penicillin streptomycin (GIBCO). VSMCs were co-cultured with platelets from HC and KD patients with CAA or NCAA.

Immunofluorescence. Cells were fixed and stained according to the standard protocols. Briefly, isolated human platelets (10^8 platelets/ml in HEPES-Tyrode's buffer) were treated with CellTracker™ Green CMFDA (Life technologies) at a concentration of 1μM for 30 min in darkness. After washing with PBS for at least three times, fluorescent platelets were resuspended in HEPES-Tyrode's buffer. VSMCs were incubated with fluorescent platelets for 2, 4, 24 hours at 37℃ under 5% CO_2. Cells were washed at least 3 times, then fixed with 4% paraformaldehyde and incubated with Alexa Fluor 594-conjugated anti-ACTA2 (1:200, Abcam, catalog ab202510) for 2 hours at 37℃. Hoechst staining was performed to visualize

the nuclei. Images were taken with immunofluorescence confocal microscopy (×100 oil immersion lens, Leica SP8).

For tissue sections, the slides were stained with antibodies for PDGF receptor beta (PDGFRβ, 1:50, Santa Cruz Biotechnology, catalog sc-374573), CD45 (1:100, Abcam, catalog 23910), smoothelin (1:100, Santa cruz, catalog sc-376902), FITC anti-mouse CD41 (1:100, Biolegend, catalog 133903), MMP9 (1:100, Abcam, catalog 38898), Cleaved Caspase-3 (1:250, Cell signaling, catalog 9579S) overnight at 4℃ after 1 hour blocking in PBS containing 5% normal goat serum, 5% bovine serum albumin and 0.1% Triton X-100 at room temperature. After incubation with each of these primary antibodies, the slides were washed in PBS, and incubated with secondary antibody Alexa Fluor 488-conjugated IgG secondary antibody (ThermoFisher Scientific) for 60 min at room temperature. The slides were then incubated with Alexa Fluor 594-conjugated anti-ACTA2 (1:200, Abcam, catalog ab202510) for 2 hours at 37℃. DAPI staining was performed to visualize the nuclei (Vector Laboratories, Inc.). The Alexa Fluor 488 green fluorescent signal was obtained by excitation at 488 nm and detected within 510-560 nm emission range. The Alexa Fluor 594 red fluorescent signal was obtained by excitation at 561nm and detected within 590-650 nm emission range. Two slides, each with 3-6 sections were assessed for each group for immunofluorescent staining. Immunofluorescent images were captured using Leica SP8 confocal microscopy (Leica). The fluorescent intensity was measured by ImageJ in the region of interest in each section in a blinded manner, and the mean fluorescent intensity (MFI) was the average value of the fluorescent intensity in different sections of the same group.

Transmission electron microscopy (TEM). To detect the ultrastructural internalization of platelets into cells, transmission electron microscopy was performed as previously described [19]. Briefly, VSMCs were co-cultured with KD platelets for 4, 24, 48 hours, and then fixed with 2.5% glutaraldehyde for more than 2 hours. The fixed cells were then rinsed with 0.1 M sodium cacodylate buffer and post fixed with 1% osmium tetroxide for 1 hour at 4 ℃. Cells were washed 3 times in 0.1 M sodium cacodylate buffer and dehydrated in ethanol series. After embedded in Epon resin (EMbed-812 resin), stained with uranyl acetate and lead citrate, ultrathin sections (100 nm) were examined by the transmission electron microscope FEI

CM100 (Japan Electron Optics Laboratory). Images were recorded with an Advantage CCD camera using iTEM software (Olympus).

Transfection of VSMCs. VSMCs were plated at a density of 1×10^5 per well 24 h prior to transfection. Transient transfections were conducted for 24 hours with miR-223 mimic (agomiR-223), miR-223 inhibitor (antagomiR-223) or negative control (agomiR/antagomiR-NC) (GenePharma) at 100 nm by using lipofectamine RNAiMAX reagent (Invitrogen) according to the manufacturer's protocols. After replacing with fresh culture medium, VSMCs were then co-cultured with HC / KD (NCAA) platelets. The proteins of VSMCs were collected 48 hours after incubation, and RNA collected 24 hours after incubation. The sequences are listed in **Online Table VI**.

CCK-8 assay. Number of viable cells was analyzed using Cell Counting (CCK8) Kit-8 (Dojindo). VSMCs were cultured (10^4 per well) in 96-well plates overnight, followed by serum starvation for 24 hours. After incubation with platelets from HC and KD patients with NCAA for 48 hours, VSMCs were incubated with 10 μl WST-8 in fresh culture medium (100 μl) for 1 hour at 37 ℃. The absorbance of each sample was measured at 450 nm with a microplate reader (Thermo Scientific).

BrdU incorporation assay. VSMCs were treated as described above for 48 hours. Cell proliferation was also evaluated using Bromodeoxyuridine (BrdU) Cell Proliferation Assay Kit (Millipore) according to the manufacturer's protocol. Briefly, after 48 hours of incubation with platelets, cells were incubated with fresh culture medium containing BrdU for 2 hours. The cells were then fixed and incubated with peroxidase conjugated BrdU antibody for 1 hour. After washing, the cells were incubated with the peroxidase substrate and examined by a microplate reader (Thermo Scientific) at a wavelength of 450 nm (OD450) with reference measurement at 550 nm (OD550). Absorbance (OD450-OD550) values representing cell proliferation ability were compared between indicated groups.

Western blot analysis. Protein extracts of cells were separated by 12% SDS-PAGE gels and transferred to PVDF membranes. Primary antibodies for PCNA (Abcam, catalog ab29), Osteopontin (OPN, Abcam, catalog ab166709), α-smooth muscle actin (ACTA2, Abcam,

catalog ab119952), TAGLN (Abcam, catalog ab170902), smooth muscle calponin (CNN1, Abcam, catalog ab46794), and PDGFR beta (PDGFRβ, Abcam, catalog ab32570), were purchased from Abcam. Antibodies against α-tubulin (Abcam, catalog ab52866), α-Actinin/ACTN1 (Abcam, catalog ab18061) were used to demonstrate the amount of protein loaded in each sample. Protein bands were detected using Immobilon chemiluminescent substrate (Millipore). The pixel intensity of each band in the blot was measured using Image Lab software (USA). The band intensity of target protein (e.g. ACTA2, CNN1, TAGLN, PCNA, OPN, PDGFRβ) was divided by the band intensity of the loading control (α-tubulin).

Luciferase reporter assay. VSMCs were plated in 24 well plates and transfected with 0.5 μg psi-CHECK2-PDGFRβ 3'untranslated region (UTR) plasmid (Promega, C8021). Cells were (1) co-transfected with 50 nM miR-223 mimics (agomiR-223)/NC mimic (agomiR-NC); (2) incubated with platelets from HC and KD patients with NCAA; (3) co-transfected with 50 nM miR-223 inhibitor (antagomiR-223) followed by incubation with platelets from KD patients with NCAA, by using lipofectamine RNAiMAX reagent (Invitrogen). Dual luciferase activities (luminescence ratio of Firefly/Renilla) was measured using the Dual-Luciferase Reporter Assay System (Promega).

Ago2 Immunoprecipitation (Ago2-IP). VSMCs were co-cultured with platelets from KD patients with NCAA, or pre-treated with antagomiR-223 followed by incubation with platelets from KD patients with NCAA. Cells were detached from the culture dish using a cell scaper, and immunoprecipitated by protein G-agarose beads (MerckMillipore, catalog 17-701) conjugated with anti-Ago2 antibody (ab32381, Abcam) or isotypic IgG control, as previously described [25]. Ago2-associated RNA was reverse transcribed using the EasyScript First-Strand cDNA Synthesis SuperMix (Transgen) and analyzed by RT-qPCR using SYBR Green qPCR SuperMix (Invitrogen).

Droplet digital PCR (ddPCR). MiRNA from platelets was extracted and purified as described in the RT-qPCR. The extracted RNA was reverse transcribed using TaqMan miRNA Reverse Transcription kits and miRNA-specific RT-primers for U6 and miR-223-3p, respectively (Applied Biosystems, U6 catalog 001973; miR-223-3p catalog 002295). Synthesized cDNA in combination with Taqman™ MicroRNA Assays (Applied Biosystems, catalog 4427975)

were added in a 20 μl PCR reaction volume according to the manufacturer's protocol. The mixture was then mixed with 70 μl droplet generation oil for probes in a disposable droplet generator cartridge and applied to the QX200 droplet generator device for droplet generation (Bio-Rad). Each sample was then transferred into a 96-well PCR plate and PCR performed according to the manufacturer's protocol. At the end of the PCR reaction, the QX200 droplet reader (Bio-Rad) was used to count positive and negative droplets. The fraction of PCR-positive droplets enabled the target to be quantified according to a Poisson distribution. The resulting copies of miR-223-3p per microliter of reaction were normalized to copies of the reference (U6 per microliter of reaction).

LCWE preparation. L.casei (American Type Culture Collection, ATCC 11578) cell wall extract (LCWE) was prepared as previously described [20, 21]. In brief, L.casei were grown in Lactobacillus MRS broth (Difco) for 48 hours, harvested and washed with PBS. The harvested bacteria were lysed by two packed volume of 4% SDS/PBS overnight at 37℃, followed by 8 washes with PBS to remove the residual SDS. The cell wall fragment was then incubated sequentially with DNase, RNase, and trypsin to remove cytoplasmic materials. After sonication in a dry ice/ethanol bath for 2 hours at a pulse setting of 5.0 (10-s pulse/5-s pause), the cell wall fragments were centrifuged for 20 min at 12,000 rpm at 4 °C. The supernatant containing LCWE was harvested by centrifugation for a further 1 hour at 38,000 rpm at 4 °C. The concentration of the cell wall extract was quantified by phenol-sulfuric colorimetric determination of the total rhamnose content as described earlier [22].

Mice. The global miR-223 knockout (miR-223y/-) mice [23] (JAX stock #013198) and their WT littermates were purchased from Jackson Laboratory (Bar Harbor, USA) and backcrossed to C57BL/6 mice for ≥5 generations. The PF4-cre: mT/mG mice were created using C57BL/6-Tg(PF4-icre)Q3Rsko/J (stock No. 008535) and the mT/mG mice (B6.129(Cg)-Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J (stock No. 007676) [18]. The experiments were conducted under the authorization of Animal Care Committee in Guangzhou Medical University (2019-384). As male mice are prone for development of LCWE-induced abdominal aorta lesions, just male mice were included in the experiments [26]. All the animal procedures were performed by the same operator blinded to the mouse

genotype/treatment group, as well as the KD status for the in vivo animal model experiments. Images were assigned a numerical code to ensure that the selection of representative images is carried out in a blinded manner.

KD murine model. Male mice 4 weeks of age were injected intraperitoneally with 400 μg LCWE or PBS. Mice were sacrificed at two weeks post-injection and perfused with PBS containing heparin. Abdominal aorta were removed and embedded in optimum cutting temperature (OCT) compound for histological analysis. Blood samples (approximately 0.8 ml) were directly obtained from the right cardiac ventricle prior to perfusion. The PRP and platelet pellet were obtained as described in the human platelets. Purity of platelet preparation was determined by flow cytometry analysis using platelet markers (>99% CD41 positive).

One week after LCWE injection, miR-223 knockout (miR-223 KO) mice and wide type (WT) littermates were treated with PBS, agomiR-223 (GenePharma, 80mg/kg per mouse via intravenous injection), Atorvastatin calcium (Sigma-Aldrich, PHR1422, 10mg/kg/day per mouse by gavage) [27], Imatinib mesylate (MedchemExpress, #HY-50946, 50mg/kg/day per mouse by intraperitoneal injection) [28, 29], Rapamycin D (Sigma-Aldrich, V900930, 2mg/kg/day per mouse by intraperitoneal injection) [30, 31], respectively. For platelet infusion, washed platelets (0.25×10^9/200μl per mouse via intravenous injection) were suspended in HEPES-Tyrode's buffer and maintained at room temperature 30min prior to infusion [32]. After one week, the mice were sacrificed, and tissue sections were collected for use.

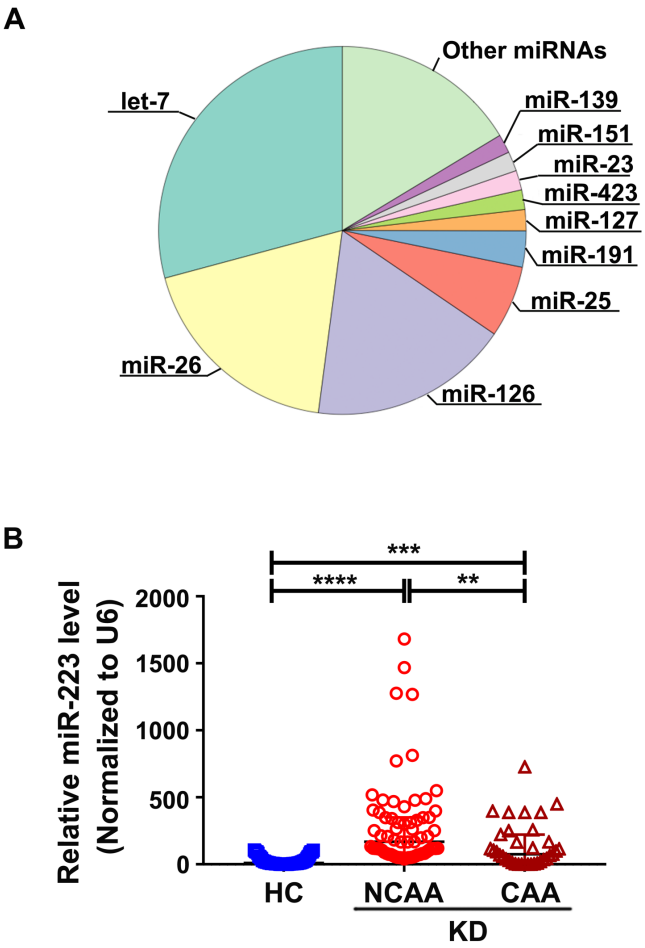
Fluorescent in situ hybridization (FISH). FISH was performed according to the manufacturers' protocol (BersinBio). Briefly, frozen sections were fixed with 4% paraformaldehyde, and treated with proteinase K at room temperature. After dehydration in ethanol for 5 min, the probes targeting miR-223-3p and PDGFRβ (BersinBio) was then added to the sections respectively. The nuclei were stained with DAPI. The LacZ was served as a negative control for PDGFRβ and miR-223-3p staining. The images were acquired with Leica SP8 (Leica) confocal microscopy.

Immunohistochemical staining. The abdominal aorta from PBS or LCWE-injected mice were fixed in 4% paraformaldehyde for 20 min and transferred to 30% sucrose in PBS before

embedding in OCT compound and frozen. The 5-μm frozen sections were applied with hematoxylin-eosin (H & E). Two slides, each with 3-6 sections were assessed for each mouse for immunohistochemical staining. Briefly, the sections were immersed in xylene and alcohol followed by staining with hematoxylin for 5 min, then stained with eosin for 2 min. All the images were obtained at the same light source. The areas circumscribed by the internal elastic lamina (IEL) and external elastic lamina (EEL) were measured by tracing along the respective vessel regions using ImageJ software in a blinded manner. The areas of thickened medial layer were calculated by the difference between EEL and IEL. The areas of thickened medial layer were compared between groups and subjected to statistical analysis for significance.

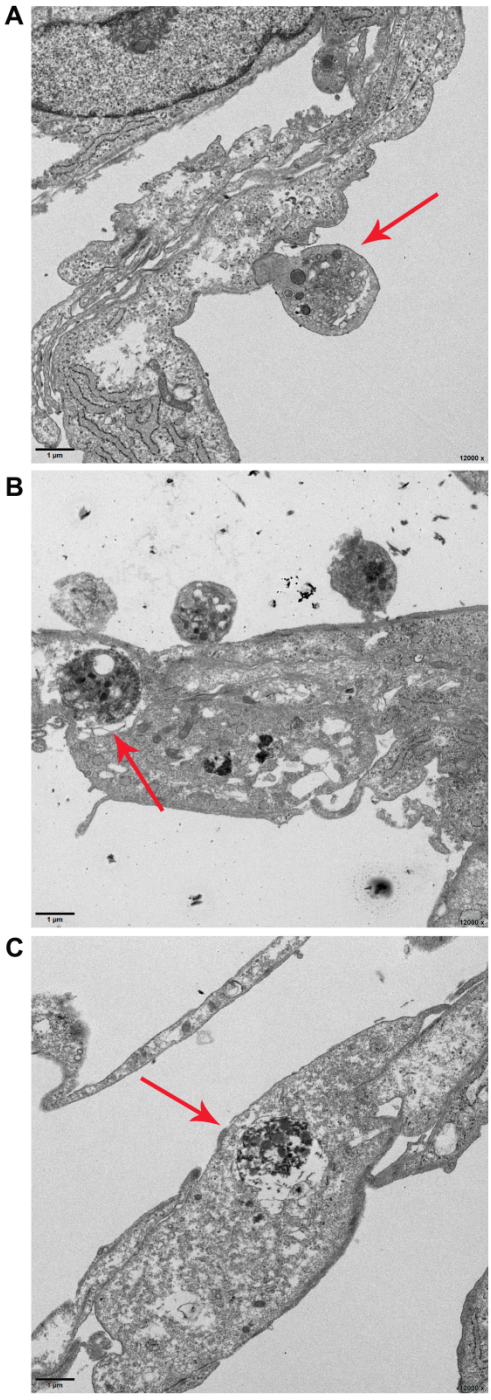
Online Figures

Online Figure I



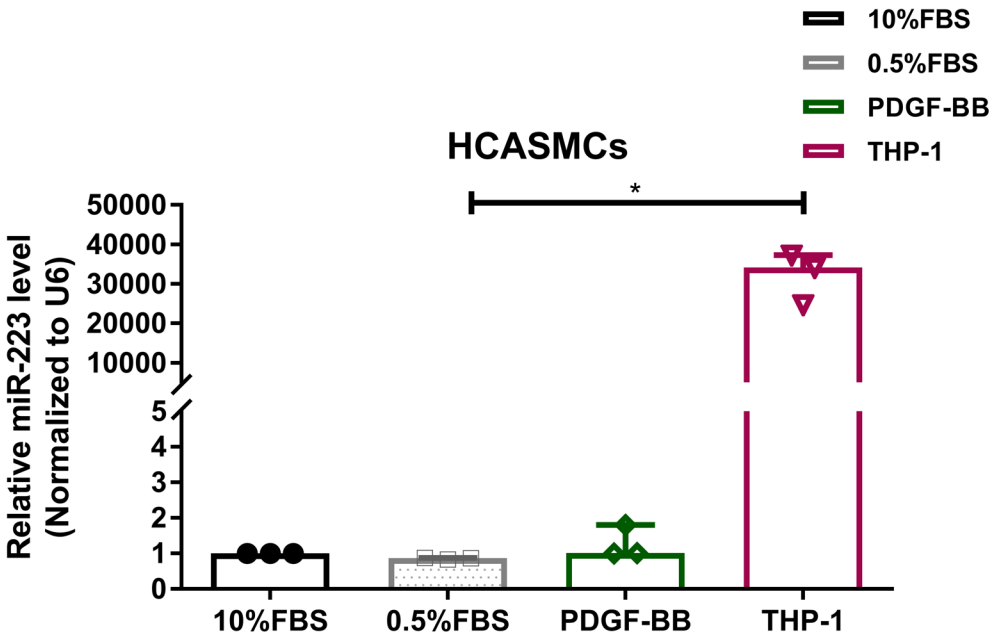
Online Figure I. Platelet miRNA profiling in platelets from kawasaki disease (KD) patients. (**A**) Distribution of the 10 most abundant miRNA families detected in platelets from KD patients. (**B**) Relative expression of miR-223 in platelets from healthy controls (HC) (n=67) and KD patients with non-coronary arterial aneurysm (NCAA) (n=67) or coronary arterial aneurysm (CAA) (n=35) were determined by quantitative Real-time PCR (RT-qPCR) (Kruskall-Wallis test and Dunn's multiple comparisons test). $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

Online Figure II



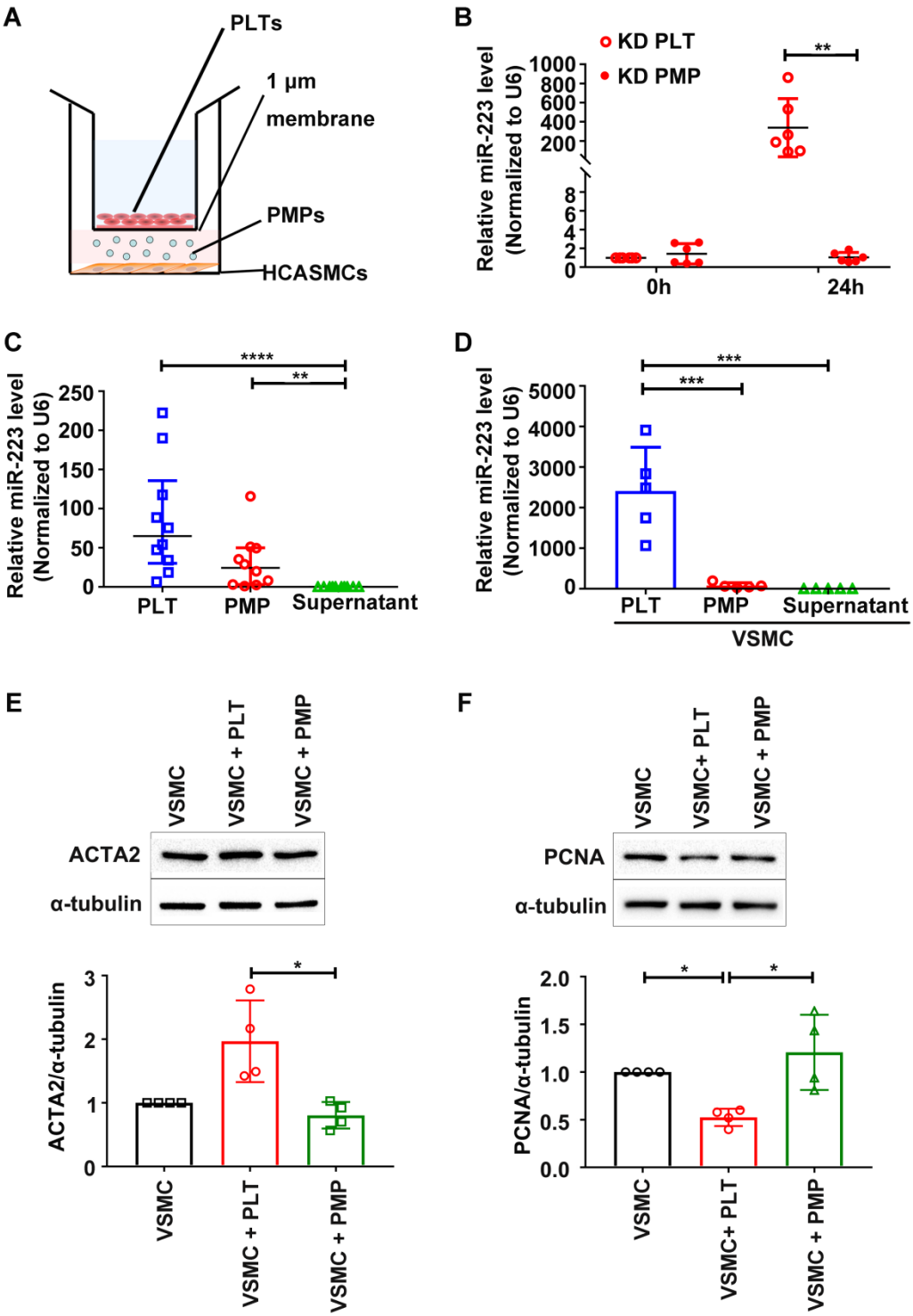
Online Figure II. Platelets were internalized by VSMCs. Lower magnification of each micrograph of KD platelets internalized into HCASMCs after 24 hour incubation was taken by transmission electron microscopy. Scale bar: 1 μm.

Online Figure III



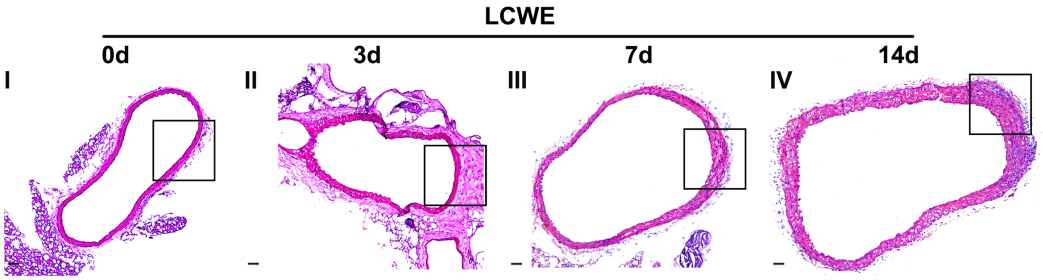
Online Figure III. VSMCs generate only negligible amount of endogenous miR-223 under different condition. The expression of miR-223 in VSMCs cultured in complete medium (10% FBS) (n=3), VSMCs cultured in serum-free condition (0.5% FBS) (n=3), VSMCs stimulated by platelet-derived growth factor-BB (PDGF-BB) (n=3) were determined. The human monocyte line THP-1 cells, which have high level of endogenous miR-223 (n=3) were used as positive control (Kruskall-Wallis test and Dunn's multiple comparisons test). $^{*}P<0.05$.

Online Figure IV



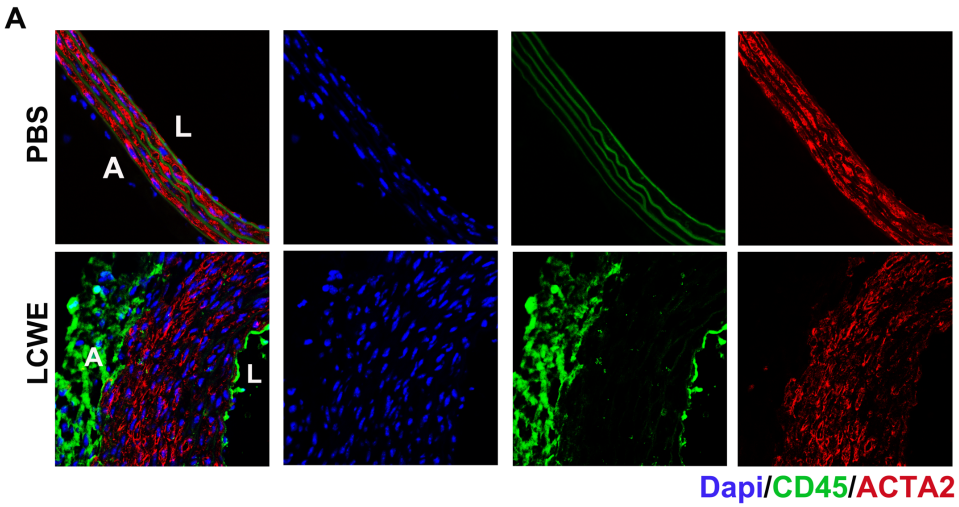
Online Figure IV. An increased miR-233 level in VSMC co-cultured with KD platelets was primarily due to platelet mediated miR-223 transfer. (**A**) A transwell chamber was used to allow KD platelet-derived microparticle (PMPs) pass through, but not platelets. (**B**) Level of miR-223 in VSMC incubated with KD platelets (PLTs) with or without a transwell chamber after 24 hours (KD PMP: n=4, KD PLT: n=4, RM Two-way ANOVA and Sidak's multiple comparisons test). (**C**) Level of miR-223 in KD platelets (PLT: n=10), KD PMPs (PMP: n=10), and KD plasma with neither platelets nor PMPs (Supernatant: n=10) from the same volume of blood (Kruskall-Wallis test and Dunn's multiple comparisons test). (**D**) Level of miR-223 in VSMCs after 24 hour incubation with PLT (n=5), PMP (n=5), and Supernatant (n=5) (One-way ANOVA and Tukey's multiple comparisons test). The protein expression and quantification of ACTA2 (**E**), and PCNA (**F**) in VSMCs, or VSMCs co-cultured with PLT, PMP (n=4, Kruskall-Wallis test and Dunn's multiple comparisons test). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

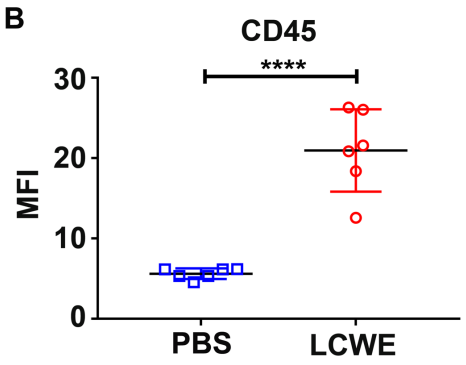
Online Figure V



Online Figure V. Development of abdominal aorta lesions in lactobacillus casei cell wall extract (LCWE)-induced KD murine model. The abdominal aorta tissues were collected from day 0 to day 14 after LCWE-injection into WT littermates. Hematoxylin and eosin (H & E) -statined cross sections for each whole vessel from LCWE-injected WT littermates were shown. Photomicrographs from I-IV show the process of LCWE-induced abdominal aorta lesions (n=30). Scale bar: 50μm.

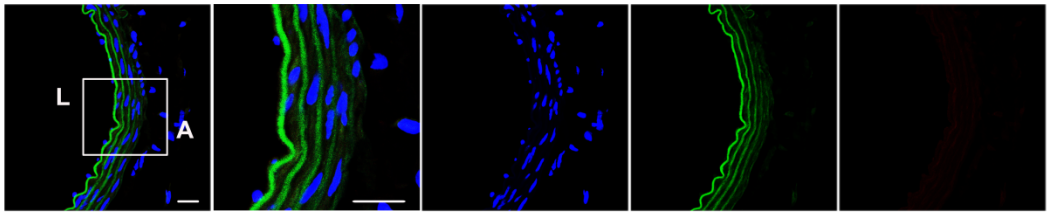
Online Figure VI





Online Figure VI. Significant inflammatory cells were infiltrated in adventitia at three days after LCWE administration. (**A**) Corresponding immunofluorescence of CD45 and ACTA2 in abdominal aorta sections from PBS (n=6) and LCWE-injected WT littermates (n=6) at three days after LCWE administration. CD45 stained as green, ACTA2 as Red, and nuclei visualized with DAPI (blue). Scale bar: 20 μm. (**B**) Quantification of CD45 expression in the injured arteries (Unpaired t test). Abbreviations: PBS, PBS-injected WT littermates; LCWE, LCWE-injected WT littermates; MFI, mean fluorescence intensity; A, adventitia; L, lumen. $^{****}P<0.0001$.

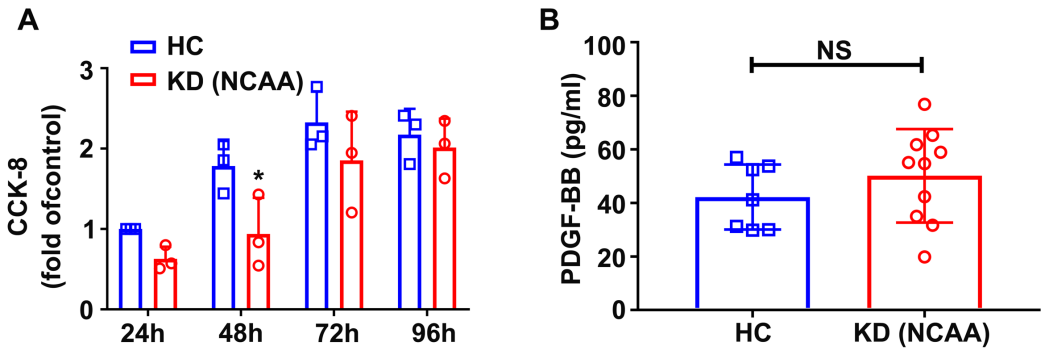
Online Figure VII



Dapi/LacZ/LacZ

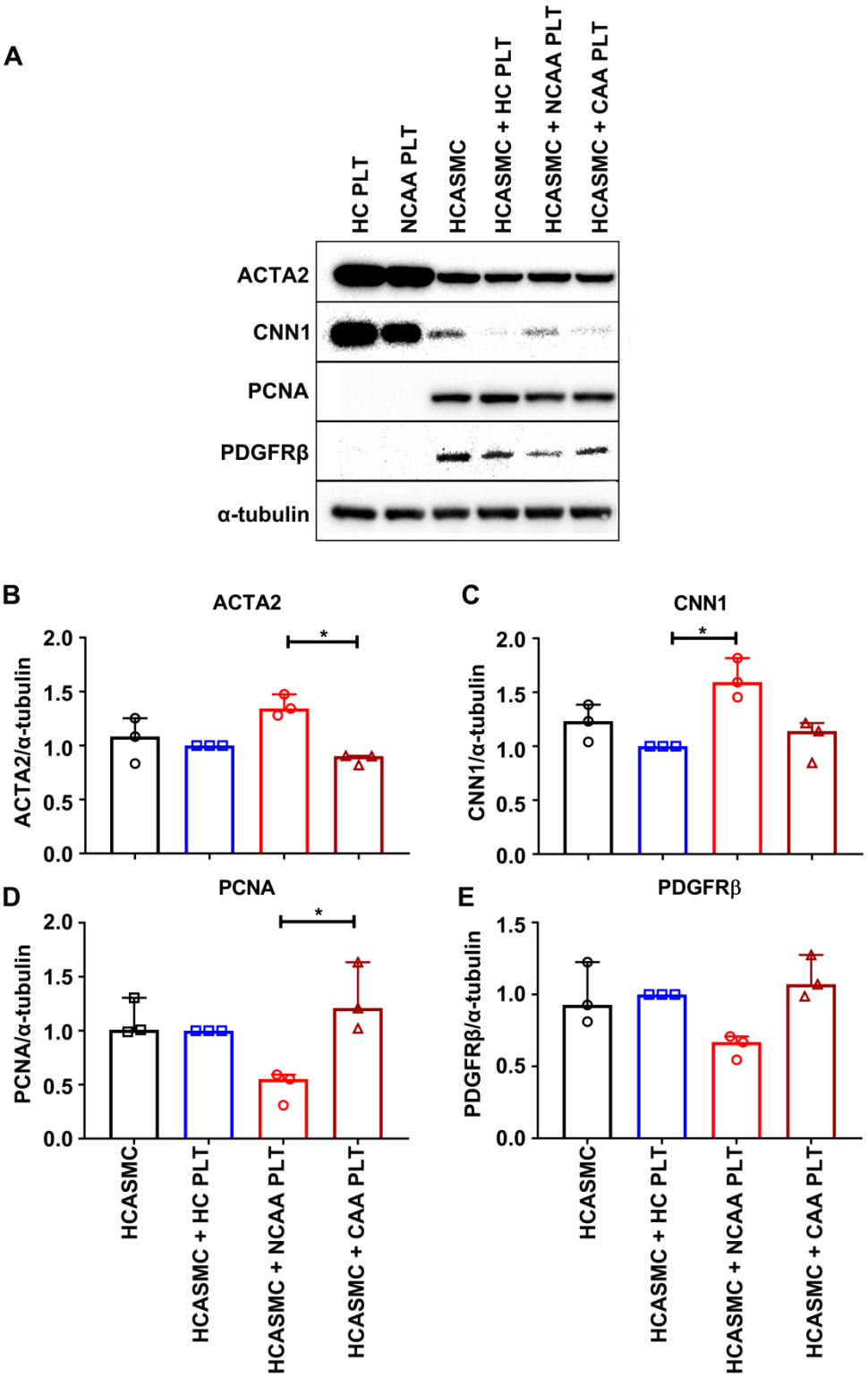
Online Figure VII. The negative control for fluorescent in situ hybridization (FISH) was shown in sections from LCWE-injected mice. The LacZ was used as the negative control for PDGFRβ (green) and miR-223 (red) staining. The nuclei were stained with DAPI (blue). Scale bar: 20 μm.

Online Figure VIII



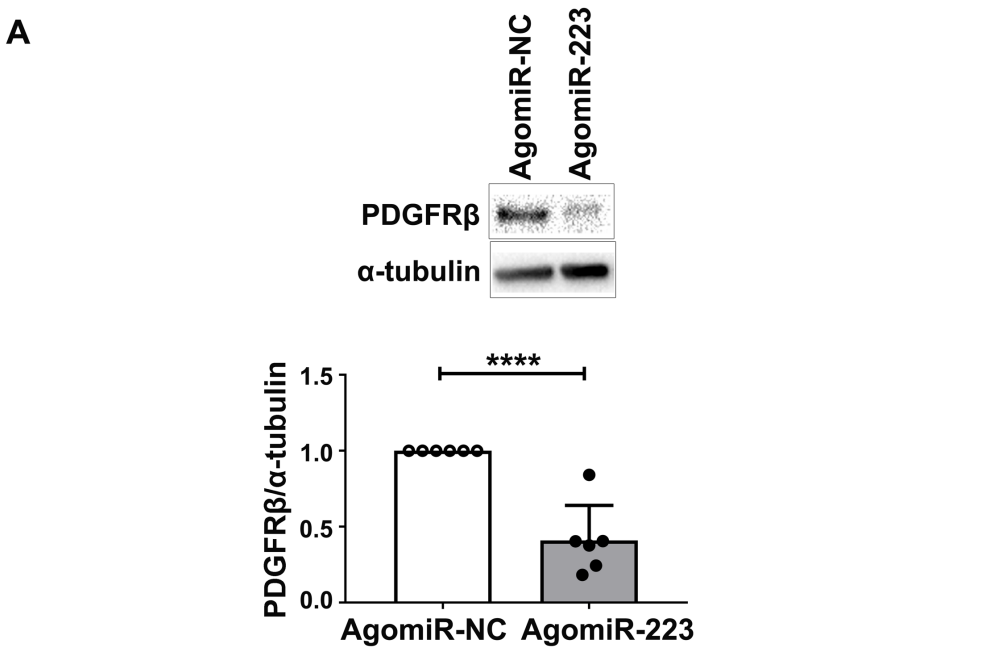
Online Figure VIII. KD platelets induced reduction of VSMC proliferation, which was not due to changes in PDGF-BB level. (**A**) VSMCs were co-cultured with platelets from HC and KD patients with NCAA, and cell proliferation was assessed by CCK-8 assays after 24, 48, 72, 96 hours of incubation (n=3, RM Two-way ANOVA and Sidak's multiple comparisons test). (**B**) The level of PDGF-BB in the supernatant of VSMCs after incubation with platelets from HC (n=7) and KD patients with NCAA (n=10) for 48 hours were determined (Unpaired t test). Abbreviations: HC, VSMCs co-cultured with platelets from HC; KD (NCAA), VSMCs co-cultured with platelets from KD patients with NCAA; NS, not significant. $^{*}P<0.05$.

Online Figure IX



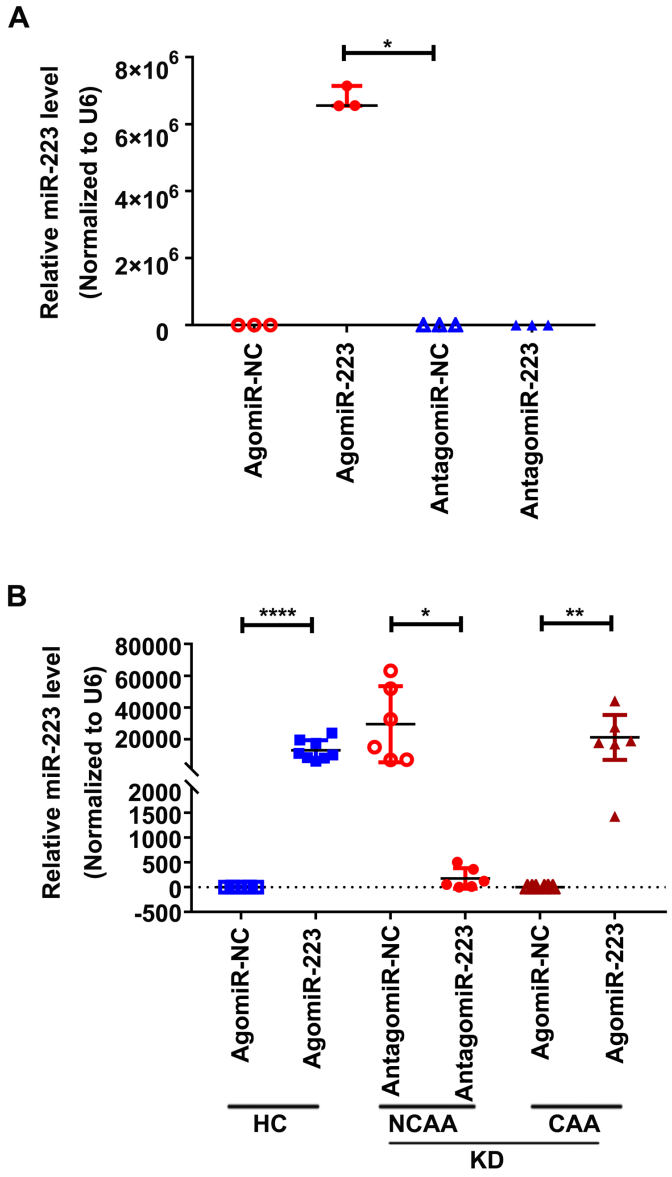
Online Figure IX. The protein expression of VSMC differentiation markers (ACTA2, CNN1 and de-differentiation markers (PCNA, PDGFRβ) were determined in HC platelets (HC PLT: n=3), NCAA platelets (NCAA PLT: n=3), VSMCs (HCASMC, n=3), and VSMCs co-cultured with platelets from HC (HCASMC + HC PLT: n=3), KD patients with NCAA (HCASMC + NCAA PLT: n=3), and CAA (HCASMC + CAA PLT: n=3), respectively. (**A**) The representative blots and (**B**) quantification of each protein were shown (Kruskall-Wallis test and Dunn's multiple comparisons test). $^{*}P<0.05$.

Online Figure X



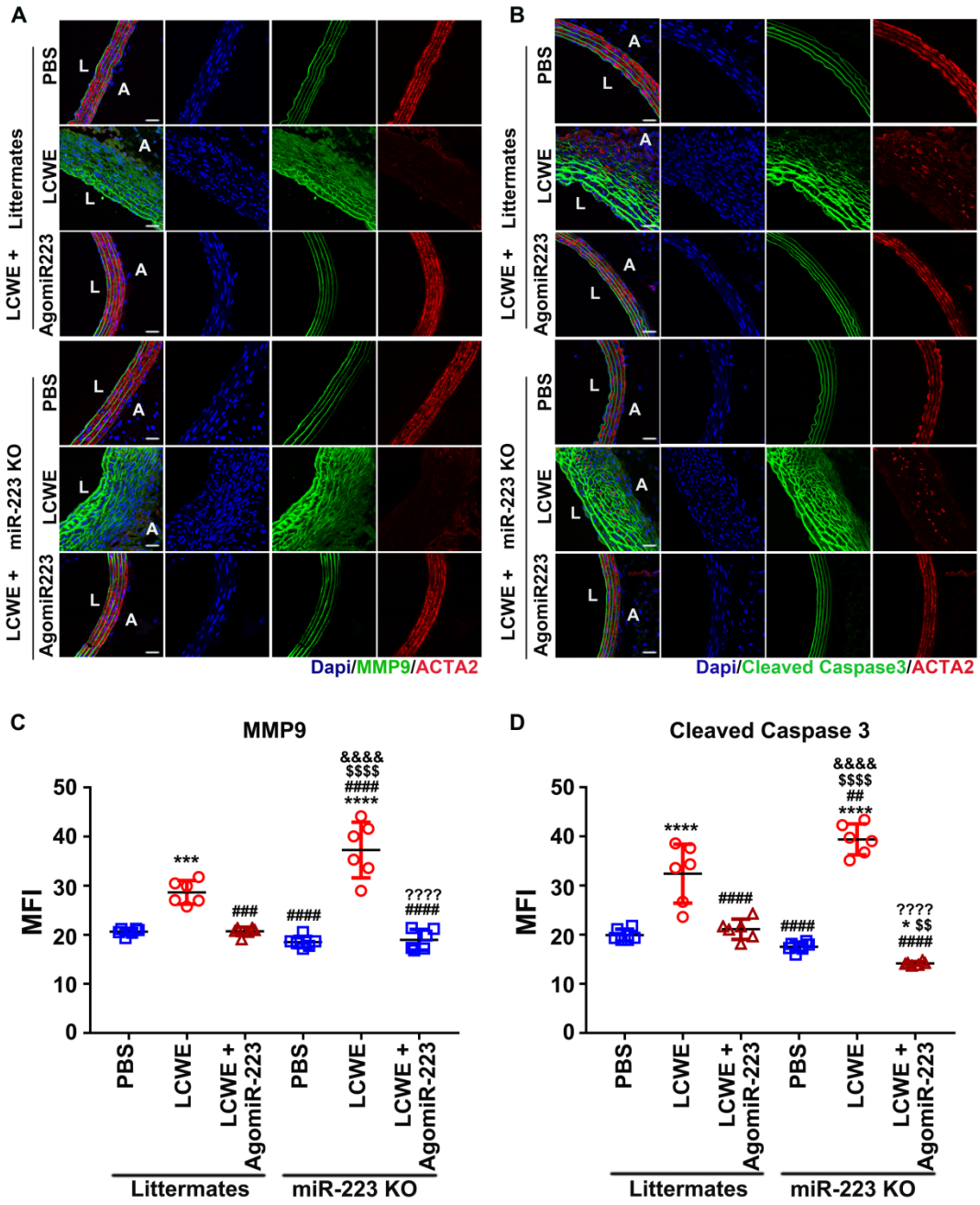
Online Figure X. MiR-223 directly target PDGFRβ. (**A**) The protein expression of PDGFRβ in VSMCs transfected with miR-223 mimic (agomiR-223) and NC mimic (agomiR-NC) was determined (n=6, Unpaired t test). (**B**) Schematic representation of PDGFRβ 3'UTRs showing putative miRNA target site. $^{****}P<0.0001$.

Online Figure XI



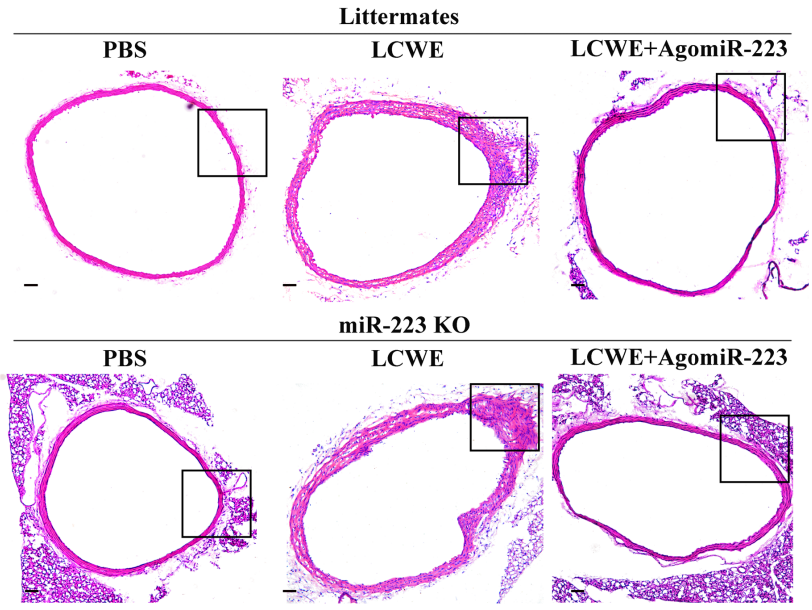
Online Figure XI. The intracellular level of miR-223 in VSMCs after transfection. (**A**) VSMCs were transfected with miR-223 mimic (agomiR-223) or inhibitor (antagomiR-223) for 48 hours. The level of miR-223 in VSMCs was detected by RT-qPCR (n=3, Kruskall-Wallis test and Dunn's multiple comparisons test). (**B**) VSMCs were pre-treated with agomiR-223 or antagomiR-223, followed by incubation with platelets from HC and KD patients with NCAA or CAA. The intracellular level of miR-223 in VSMCs was determined (HC: n=8, NCAA: n=6, CAA: n=6, Unpaired t test for each group). Abbreviations: HC, VSMCs co-cultured with platelets from HC; KD (NCAA), VSMCs co-cultured with platelets from KD patients with NCAA; CAA, VSMCs co-cultured with platelets from KD patients with CAA. $^{*}P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$.

Online Figure XII



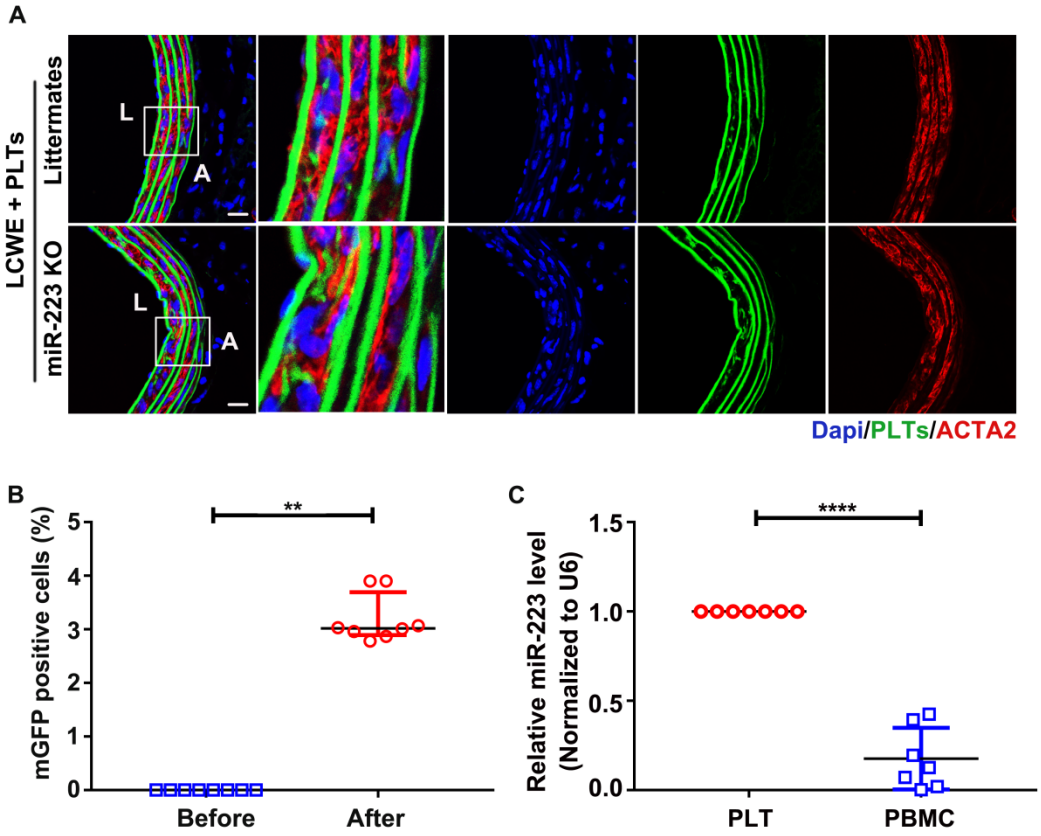
Online Figure XII. **MMP9 and cleaved caspase-3 were significantly increased in the injured arteries of LCWE-injected mice**. Representative immunofluorescent images of MMP9 (**A**) and cleaved caspase-3 (**B**) in sections from PBS-littermates: n=7, LCWE-littermates: n=24, LCWE-littermates + agomiR-223: n=7, PBS-miR-223 KO: n=7, LCWE-miR-223 KO: n=13, and LCWE-miR-223 KO + agomiR-223: n=7. PBS injected mice were used as vehicle control. MMP9 and cleaved caspase-3 were stained as green, ACTA2 as red, and nuclei visualized with DAPI. Scale bar: 20 μm. Quantification of MMP9 (**C**) and cleaved caspase-3 (**D**) expression in the injured arteries (One-way ANOVA and Tukey's multiple comparisons test). Abbreviations: Littermates, WT littermates; miR-223 KO, miR-223 KO mice; PBS, PBS-injected mice; LCWE, LCWE-injected mice; LCWE+AgomiR-223, LCWE-injected mice followed by administration with agomiR-223. $^{*}P<0.05$, $^{***}P<0.001$, $^{****}P<0.0001$ versus PBS-Littermates; $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, $^{\#\#\#\#}P<0.0001$ versus LCWE-Littermates; $^{\$\$}P<0.01$, $^{\$\$\$\$}P<0.0001$ versus LCWE-Littermates + AgomiR-223; $^{\&\&\&\&}P<0.0001$ versus PBS-miR-223 KO; $^{????}P<0.0001$ versus LCWE-miR-223 KO.

Online Figure XIII



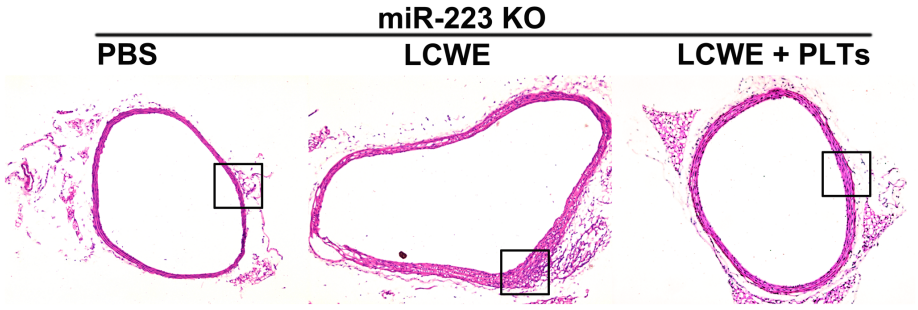
Online Figure XIII. Deficiency of miR-223 aggravates LCWE-induced abdominal aorta lesions, which was resolved by administration with agomiR-223. H & E-stained cross sections for each whole vessels from PBS or LCWE-injected mice. Scale bar: 50μm. Abbreviations: Littermates, WT littermates; miR-223 KO, miR-223 KO mice; PBS, PBS-injected mice; LCWE, LCWE-injected mice; LCWE+AgomiR-223, LCWE-injected mice followed by administration with agomiR-223.

Online Figure XIV



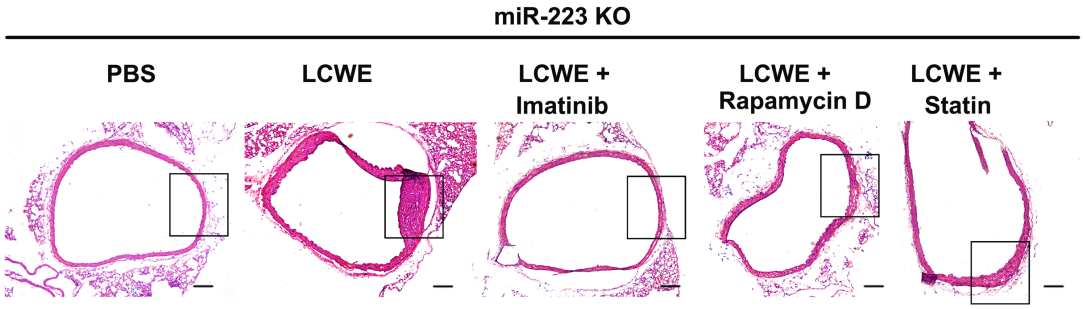
Online Figure XIV. Evaluation of efficacy of platelet infusion in KD murine model. One week after LCWE injection, washed platelets (0.25×10^9 per mouse) isolated from PF4-cre: mT/mG mice were intravenously injected, the abdominal aorta tissues were collected after one week. (**A**) Representative immunofluorescent images of GFP-labeled platelets and ACTA2 in sections from LCWE-injected miR-223 KO mice. ACTA2 stained as Red, and nuclei visualized with DAPI. Scale bar: 20 μm. (**B**) Percentage of mGFP positive cells in circulation of mice before and after platelet infusion was measured (n=8, Wilcoxon matched-pairs signed rank test). (**C**) Relative levels of miR-223 in platelets (PLTs) and peripheral blood mononuclear cells (PBMCs) isolated from the same volume of blood (n=7, Unpaired t test). Abbreviations: miR-223 KO, miR-223 KO mice; LCWE, LCWE-injected mice; LCWE + PLTs, LCWE-injected mice followed by infusion with PLTs; Before, before platelet infusion; After, after platelet infusion. $^{**}P<0.01$, $^{****}P<0.0001$.

Online Figure XV



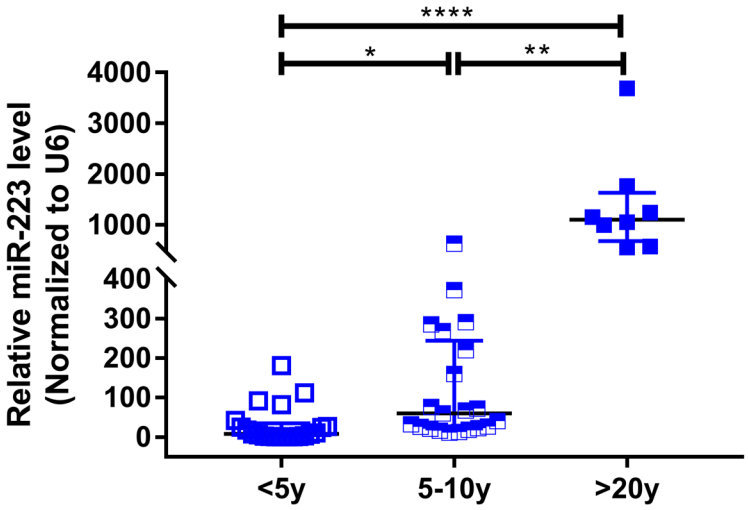
Online Figure XV. The LCWE-induced medial thickening was significantly attenuated by the adoptive platelet transfer. H & E -stained cross sections for each whole vessels from LCWE injected miR-223 KO mice after the adoptive platelet transfer. Scale bar: 50μm. Abbreviations: miR-223 KO, miR-223 KO mice; LCWE, LCWE-injected mice; LCWE + PLTs, LCWE-injected mice followed by infusion with PLTs.

Online Figure XVI



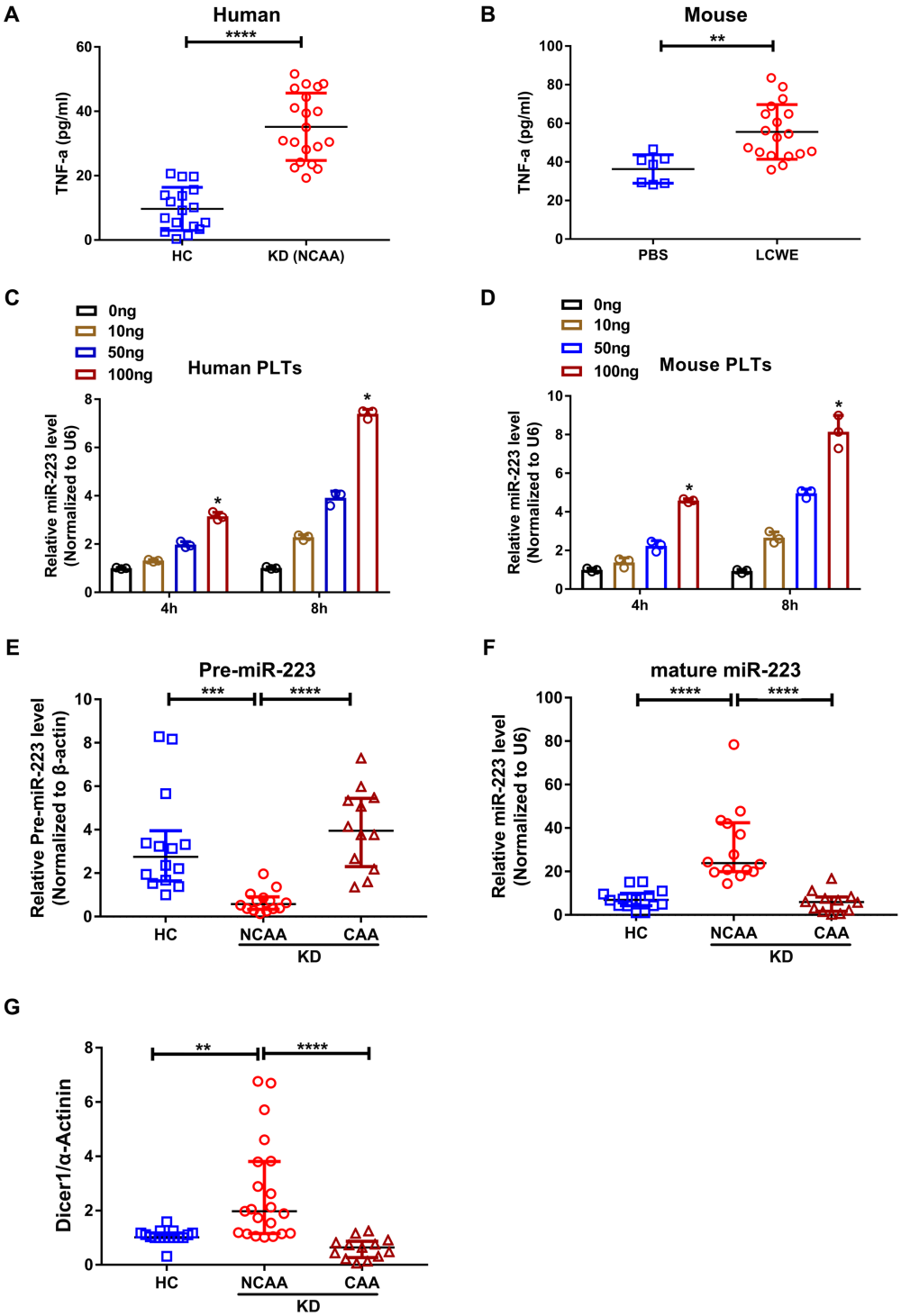
Online Figure XVI. Targeting miR-223-PDGFRβ axis attenuated LCWE-induced abdominal aorta lesions in KD mice. Representative H & E -stained cross sections for each whole vessels from PBS-injected (n=6), LCWE injected (n=7), LCWE injected miR-223 KO mice followed by administration with Imatinib mesylate (Imatinib) (n=7), Rapamycin D (n=7), Atorvastatin calcium (Statin) (n=7). Abbreviations: miR-223 KO, miR-223 KO mice; PBS, PBS-injected mice; LCWE, LCWE-injected mice; LCWE + Imatinib, LCWE-injected mice followed by administration with Imatinib; LCWE + Rapamycin D, LCWE-injected mice followed by administration with Rapamycin D; LCWE + Statin, LCWE-injected mice followed by administration with Statin. Scale bar: 50μm.

Online Figure XVII



Online Figure XVII. Level of platelet miR-223 increases with age. The levels of miR-223 in platelets from HC less than 5 years of age (<5y: n=21), between 5 and 10 years of age (5-10y: n=21), above 20 years of age (>20y: n=8) were detected by RT-qPCR (Kruskall-Wallis test and Dunn's multiple comparisons test). $^{*}P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$.

Online Figure XVIII



Online Figure XVIII. Platelets from KD patients with NCAA and CAA exhibit differential expression of miR-223. (**A**) Levels of TNF-alpha (TNF-α) in plasma from HC (n=17) and KD patients (n=20) and (**B**) plasma from PBS- (n=7) and LCWE-injected mice (n=18) were determined (Unpaired t test). Levels of miR-223 in (**C**) human (human PLTs) and (**D**) murine platelets (murine PLTs) were measured after incubation with increasing concentration of TNF-α for 4, 8 hours, and normalized to U6 (n=3, Kruskall-Wallis test and Dunn's multiple comparisons test). Levels of (**E**) pre-miR-223 and (**F**) miR-223 were measured in platelets from HC (n=14), NCAA (n=14) and CAA (n=12). Pre-miR-223 was normalized to β-actin, and miR-223 was normalized to U6 (Kruskall-Wallis test and Dunn's multiple comparisons test). (**G**) The expression of Dicer1 was determined in platelets from HC (n=16), NCAA (n=21) and CAA (n=13) (Kruskall-Wallis test and Dunn's multiple comparisons test). Abbreviations: PBS, PBS-injected mice; LCWE, LCWE-injected mice. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

Online Tables

Online Table I. Characteristics of HC and KD patients with CAA or NCAA.

		KD		
	HC	**NCAA**	**CAA**	
Variables	n =96	n = 155	n = 63	*P*-value
Ethnicity (n)	Han (96)	Han (155)	Han (63)	NA
Age, months, (IQR)	24 (12-36)	24 (12-36)	25 (12-48)	0.2654
Male, n (%)	54 (56)	97 (63)	47 (75)	0.0630
Coronary artery status, n (%)				
SCAA	NA	0	24 (38)	NA
MCAA	NA	0	18 (29)	NA
GCAA	NA	0	21 (33)	NA
Normal	NA	155 (100)	0	NA
Z-worst, median (range)	< 2.5	< 2.5	5.2 (3.5 - 7.06)	NA
Laboratory Data, median (IQR)				
PLT (10^9/L)	321 (266-399)	392 (316-498)****	461 (334-666)****	4.86E-10
MPV (fL)	9.5 (8.9-10.2)	9.7 (9.0-10.5)	9.5 (8.9-10.4)	0.3112
PDW (%)	9.6 (8.8-10.5)	9.8 (9.1-10.9)	9.5 (8.9-10.1)	0.0700
P-LCR (%)	19.7(15.1-23.9)	21.0 (16.1-25.1)	19.0 (15.3-22.8)	0.1518
WBC (10^9/L)	8.9 (6.8-11.3)	10.5 (7.5-13.9)***	9.4 (7.6-13.5)*	0.0005
RBC (10^{12}/L)	4.6 (4.3-4.9)	4.3 (3.9-4.7) ****	4.2 (3.9-4.6) ****	4.48E-09
HGB (g/L)	122 (117-128)	112 (100-124) ****	108 (99-118) ****	4.63E-13
hs-CRP (mg/L)	0.5 (0.5-1.1)	19.8 (0.8-87.1)****	12.9 (0.5-64.8)****	0.0000
ESR (mm/h)	NA	31 (17-45)	29.5 (15.2-51)	0.9281

Online Table I. Characteristics of HC and KD patients with NCAA or CAA. *P*-value was calculated by Kruskal-Wallis test followed by Dunn's multiple comparisons test for continuous variables. Mann-Whitney test was used to analyze difference between NCAA and CAA group. Abbreviations: HC, healthy control; KD, Kawasaki disease; IQR, interquartile range; CAA, coronary artery aneurysm; NCAA, non-coronary artery aneurysm; SCAA, small coronary artery aneurysm; MCAA, medium coronary artery aneurysm; GCAA, giant coronary artery aneurysm; PLT, platelet; MPV, mean platelet volume; PDW, platelet distribution width; P-LCR, platelet large cell rate; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; hs-CRP: high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate. NA, not applicable, $^{*}P<0.05$, $^{***}P<0.001$, $^{****}P<0.0001$ versus HC group.

Online Table II: Characteristics of KD patients treated with or without Statin.

	Statin + group	Statin - group	
Variables	n=12	n=12	*P*-value
Ethnicity (n)	Han (12)	Han (12)	NA
Age, months, (IQR)	48 (36-69)	53 (19-105)	0.5992
Male, n (%)	12 (100%)	12 (100%)	1.0000
Illness month, median (IQR)	12 (1-40)	4 (1-37)	0.8867
Statin dose:			
0.25mg/kg/day	9 (75)	NA	NA
0.5mg/kg/day	3 (25)	NA	NA
Z-worst, median (range)	8.04 (6.83-9.50)	6.87 (5.96-8.68)	0.4047
IVIG resistant, n (%)	1 (8.33%)	1 (8.33%)	1.0000
Laboratory Data, median (IQR)			
PLT (10^9/L)	348 (280-454)	371 (297-407)	0.7051
MPV (fL)	9.6 (9.1-10)	9.3 (8.9-9.7)	0.5223
PDW (%)	9.8 (9.2-11.7)	9.5 (8.9-10.5)	0.5336
P-LCR (%)	21.4 (17-25.1)	18.7 (15.8-22.7)	0.6411
WBC (10^9/L)	9.9 (7.3-11.6)	9.4 (6.3-10.9)	0.9887
RBC (10^{12}/L)	4.5 (4.3-4.7)	4.6 (4.3-5.1)	0.4185
HGB (g/L)	117 (113-122)	116 (96-124)	0.3437
hs-CRP (mg/L)	2.2 (0.5-25.2)	3 (0.5-29.6)	0.9702

Online Table II. Characteristics of KD (GCAA) patients treated with or without Statin. *P*-value was calculated by Mann-Whitney test or Unpaired t test for continuous variables. Illness month was calculated since first calendar day of fever. Abbreviations: KD, Kawasaki Disease; GCAA, giant coronary artery aneurysm; Simvastatin: Statin; Statin +, GCAA patients treated with statin; Statin -, GCAA patients treated without statin; IQR, interquartile range; IVIG, intravenous immunoglobulin; PLT, platelet; MPV, mean platelet volume; PDW, platelet distribution width; P-LCR, platelet large cell rate; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; hs-CRP: high-sensitivity C-reactive protein; NA, not applicable.

Online Table III. Characteristics of healthy controls with age between 5 and 10 or greater than 20.

	< 5y	**5-10 y**	**> 20y**	
Variables	n=21	n=21	n=8	*P*-value
Ethnicity (n)	Han (21)	Han (21)	Han (8)	NA
Age, years, (IQR)	1 (0.5-2)	8 (6.5-9)****	27 (24.5-30.5)$^{****\#}$	7.93E-10
Male, n (%)	12 (57.1)	11 (52.3)	4 (50)	0.9244
Laboratory Data, median (IQR)				
PLT (10^9/L)	336 (294-404)	336 (277-373)	329 (322-337)	0.6928
WBC (10^9/L)	8.3 (8.0-10.4)	7.1 (6.2-8.1)**	7.3 (5.3-8.5)*	0.8761
RBC (10^{12}/L)	4.6 (4.4-4.7)	4.5 (4.4-4.8)	4.8 (4.5-5.5)	0.2322
HGB (g/L)	126 (118-131)	128 (122-137)	141 (127-159)$^{**\#}$	0.0467

Online Table III. Characteristics of healthy subjects with age between 5 and 10 or greater than 20. *P*-value was calculated by One-way ANOVA or Kruskal-Wallis test for continuous variables. Abbreviations: IQR, interquartile range; PLT, platelet; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin. NA, not applicable. $^{*}P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$ versus <5y group; $^{\#}P<0.05$ versus 5-10y group.

Online Table IV. Measurement of platelet activation in HC and KD patients with NCAA.

	HC	KD (NCAA)	
Variables, median (IQR)	(n=32)	(n=32)	*P*-value
CD62P (%)	38.4 (18.7-58.8)	59.2 (45.3-73.7)***	0.0006
PF4 (ng/ml)	203.6 (116.9-277.2)	262.3 (212.1-324.6) **	0.0038
β-TG (ng/ml)	12.74 (7.95-19.33)	22.58 (18.25-29.16) ****	2.19E-05
CD42b/GPIb alpha (μg/ml)	3.11 (2.12-4.17)	6.38 (5.47-7.80) ****	8.43E-10

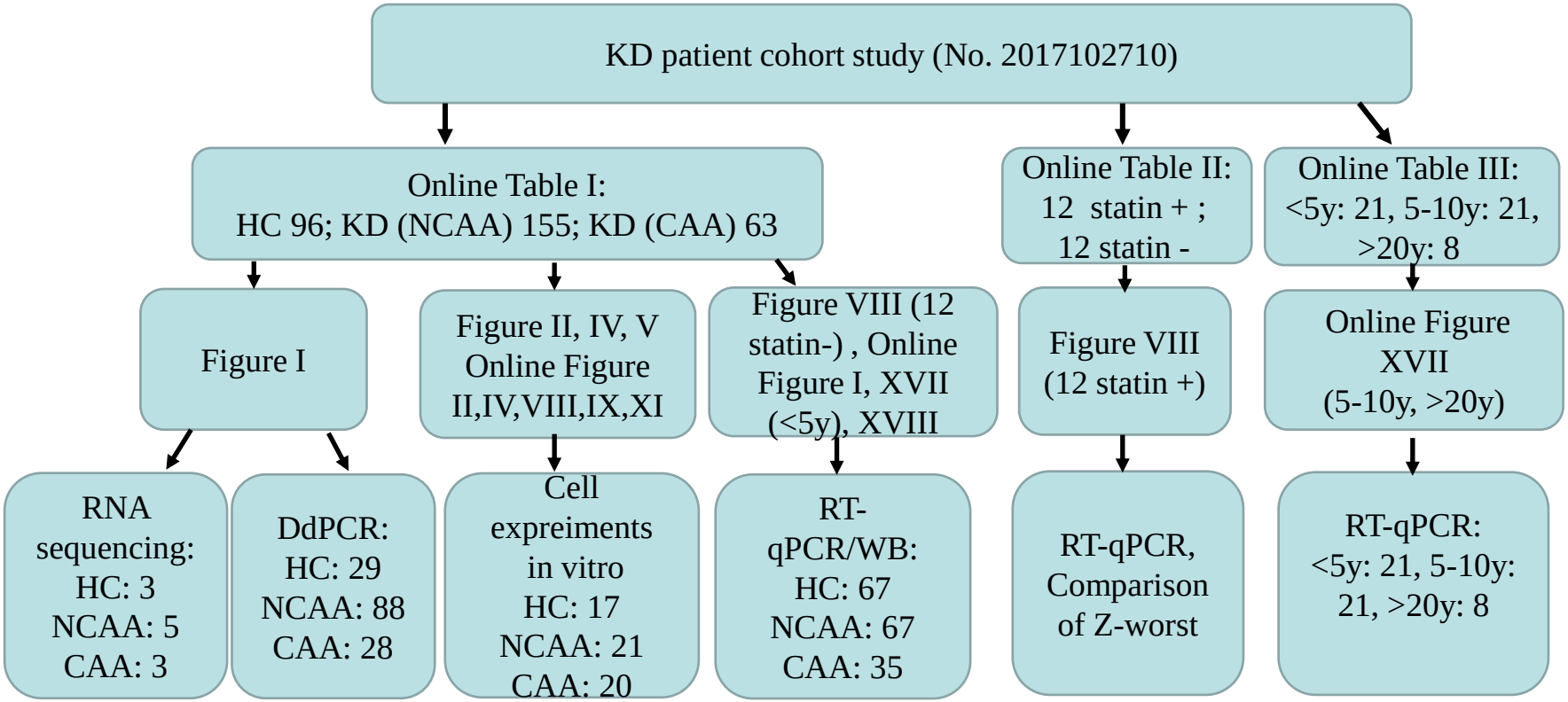
Online Table IV. Measurements of platelet activation in HC and KD patients with NCAA. *P*-value was calculated by Mann-Whitney test or Unpaired t test. CD62P was measured by flow cytometry, the amount of β-TG, PF4, GPIb in human plasma were determined by ELISA kit. Abbreviations: HC: healthy control; KD: Kawasaki Disease; NCAA, non-coronary artery aneurysm; CD62P: P-selectin; PF4: platelet factor 4; β-TG: beta-thromboglobulin. $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ versus HC group.

Online Table V. Primers for RT-qPCR.

Primer	Forward Sequence	Reverse Sequence
hsa-miR-223	GTTGCTCCTGTCAGTTTGTCAAA	TATGGTTGTTCACGACTCCTTCAC
mmu-miR-223	GTTGCTCCTGTCAGTTTGTCAAA	TATGGTTGTTCACGACTCCTTCAC
cel-miR-39-3p	CGTCGATCACCGGGTGTAAA	TATGGTTGTTCTGCTCTCTGTCTC
Pre-miR-223	GCAGTGCCACGCTCCGTGTA	TGCCGCACTTGGGGTATTTGACA
snU6	ATTGGAACGATACAGAGAAGATT	GGAACGCTTCACGAATTTG
β-actin	CGGGAAATCGTGCGTGACAT	AAGGAAGGCTGGAAGAGTGC

Online Table VI. The sequences for miR-223 mimic (agomiR-223) or inhibitor (antagomiR-223).

Gene	Sense (5'-3')	Anti-sense (5'-3')
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
hsa-miR-223 mimic (agomiR-223)	UGUCAGUUUUGUCAAAUACCCA	GGGUAUUUGACAAACUGACAUU
hsa-miR-223 inhibitor (antagomiR-223)	UGGGGUAUUUGACAAACUGACA	
cel-miR-39-3p mimic	UCACCGGGUGUAAAUCAGCUUG	AGCUGAUUUACACCCGGUGAUU


KD patient cohort study (No. 2017102710)
Online Table I:
HC 96; KD (NCAA) 155; KD (CAA) 63
Online Table II:
12 statin + ;
12 statin -
Online Table III:
<5y: 21, 5-10y: 21,
>20y: 8
Figure I
Figure II, IV, V
Online Figure
II,IV,VIII,IX,XI
Figure VIII (12
statin-) , Online
Figure I, XVII
(<5y), XVIII
Figure VIII
(12 statin +)
Online Figure
XVII
(5-10y, >20y)
RNA
sequencing:
HC: 3
NCAA: 5
CAA: 3
DdPCR:
HC: 29
NCAA: 88
CAA: 28
Cell
expreiments
in vitro
HC: 17
NCAA: 21
CAA: 20
RT-
qPCR/WB:
HC: 67
NCAA: 67
CAA: 35
RT-qPCR,
Comparison
of Z-worst
RT-qPCR:
<5y: 21, 5-10y:
21, >20y: 8