Supplemental Materials and Methods

Subtotal nephrectomy: 5/6 nephrectomies (5/6Nx) were performed under isoflurane anesthesia. Briefly, a left-flank incision exposed the left kidney, which was decapsulated to avoid ureter and adrenal damage, and the upper and lower poles were resected. The entire right kidney was decapsulated and removed via right-flank incision. Animals were maintained on normal chow diet throughout the entire study. At time of sacrifice, blood was collected with 10% volume sodium citrate and centrifuged at >10,000 rpm for 10 min to collect plasma. After PBS perfusion, tissues were dissected and snap-frozen in liquid nitrogen for long-term storage at -80°C. Hearts and ascending aorta were embedded in OCT and snap-frozen for sectioning. Endothelium of the descending aorta was lysed by flushing the aorta with Qiazol, as previously described[1].

HDL Isolation: The study was approved by Vanderbilt University Institutional Review Board and written informed consent was obtained for each donor. Whole blood was collected by venipuncture into EDTA tubes from healthy donors. The whole blood was centrifuged for 15 minutes at 4°C to separate the plasma from cells. The HDL was isolated from fresh plasma by density gradient ultracentrifugation with potassium bromide adjustment to obtain a density range of 1.063-1.21 g/mL[2]. HDL utilized for the *in vivo* studies was from a single donor or a pool of two donors so that the mice within a cohort all received the same source of HDL. The LNAs were complexed to HDL by overnight incubation at 37°C.

Histology: For cross-sectional analyses of the atherosclerotic lesions, OCT embedded hearts and ascending aortae were analyzed in consecutive 5μm sections (Leica CM3050s cryostat). To quantify lesion neutral lipid content, the sectioned aortas were stained with Oil-Red-O and presented as total lesion area (μm^2), as previously described[3]. Macrophage positive areas of the cross-sectioned lesions were assessed by immunohistochemistry for CD68 (1:200, BioRAD, Hercules, CA) and MOMA-2 (Serotec, Raleigh, NC). Cryosections for MOMA-2 staining were fixed in acetone prior to staining, as previously described[4, 5]. To assess the collagen content,

proximal aorta cross-sections were stained with Masson's trichrome and aniline blue, as previously described[6]. The collagen positive area is expressed as a ratio of aniline blue-to-Oil red O staining.[6] To assess necrotic area in atherosclerotic lesions, proximal aorta cross-sections were stained with hematoxylin and eosin (H&E). Both stained lesion and acellular/anuclear areas in intimal lesions were quantified as total atherosclerotic lesions. Necrotic areas were calculated from the ratio of acellular/anuclear areas to total atherosclerotic lesion, as described previously[5, 7]. Lesion images were collected using a Nikon ECLIPSE E400 system and quantification of lesion staining was completed with Image J software by a technician blinded to the group assignments.

Physiological Analyses: Cholesterol Assay: Plasma cholesterol levels were quantified by colorimetric assays for total cholesterol, as per manufacturer's instructions (Wako). Assay microplates were read at 500nm wavelength on a Synergy Mx plate reader and total cholesterol levels were calculated using a standard curve. Blood urea nitrogen (BUN): Serum BUN levels were determined using a commercially available kit (BioAssay Systems, Hayward, CA) following the manufacturer's instructions, as previously described[8]. Blood pressure: Systolic blood pressure (SBP) levels were measured by tail-cuff plethysmography (BP-2000 Blood Pressure Analysis System, Visitech Systems) in trained conscious mice, as previously described[5, 6, 9]**.**

Total RNA Sequencing: High-throughput total RNA (mRNA) sequencing: Total RNA sequencing libraries were generated from 10 ng of total RNA isolated from aortic endothelium using Ovation RNA-Seq kits for model organisms (Nugen). Briefly, total RNA was DNase I digested and cDNA was synthesized by RT-PCR and fragmented using Covaris S2 system to produce fragments of approximately 250 bp (sweeping frequency, 10% duty cycle, intensity of 5, 100 cycles/burst, 5 cycles of 60 s each). Blunt ends were generated by end-repair reaction followed by ligation of unique barcode adapters for each sample. Next, rRNA were removed by

insert-dependent adaptor cleavage (InDA-C) procedure and the final library was amplified by
54 PCR (20 cycles). The library quantity and quality were analyzed by Qubit (Invitrogen) and
Bioanalyzer (Agilent), respectively. Paired-end sequencing (PE75) was performed on the
56 Illumina HiSeq3000 platform. Sequencing data analysis: Reads were trimmed to remove
adapter sequences using Cutadapt v1.16[10] and aligned to the GENCODE GRCm38.p5 genome
58 using STAR v2.5.3a[11]. GENCODE vM12 gene annotations were provided to STAR to improve
the accuracy of mapping. Quality control on both raw reads and adaptor-trimmed reads was
60 performed using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc). featureCounts
v1.15.2[12] was used to count the number of mapped reads to each gene. Significantly differential
62 expressed genes with p-value < 0.05 and absolute fold change > 1.5 were detected by DESeq2
(v1.18.1)[13]. Pathway analysis was completed using MetaCore software (GeneGo).
64

Cloning: pAdtrack-CMV-FAM220A Vector: cDNA was reverse transcribed from total RNA
66 isolated from (human) HEK293 cells using high-capacity cDNA RT kit (Life Technologies) with
oligo dT primers. The coding region of *FAM220A* was amplified from cDNA using Phusion High-
68 Fidelity DNA polymerase (New England BioLabs) with the following primers that added BglII and
Xhol restriction enzyme sites: forward primer 5'-
70 AGATCTCACCATGAGGGACAGAAGAGGGCCCC-3', reverse primer 5'-
CTCGAGGAACCTAAGGGCTGCACAGTTTG-3'. The amplified product (883 bp) was separated
72 using an agarose gel and purified with a Zymoclean gel DNA recovery kit (Zymo Research). The
amplified *FAM220A* coding region was cloned into a Zero Blunt Topo PCR Cloning Vector and
74 transformed into E. coli (Invitrogen). The *FAM220A* coding region was digested out of the Topo
vector with BglII and Xhol (New England BioLabs) and gel-purified as previous described. The
76 purified fragment was ligated into a pAdtrack-CMV[143] vector using T4 ligase (Promega). The
pAdtrack-CMV-FAM220A vector was confirmed by PCR, restriction enzyme digestion, and
78 Sanger sequencing. All vector purifications were performed with either Wizard Plus SV

Minipreps DNA purification system (Promega) or PureLink HiPure plasmid midiprep kit (Invitrogen). Luciferase constructs: The full length Human *FAM220A* 3'UTR was cloned down-stream of Firefly luciferase in the pEZX-MT06 gene reporter construct which also contains a transfection control *Renilla* luciferase gene (Genecopeia). Site-directed mutagenesis was performed using custom designed mutagenesis primers (Agilent Quikchange Primer Design tool) to generate a 2 base pair deletion within the putative miR-92a-3p target sites harbored in the human *FAM220A* 3' UTR using the Quikchange II site-directed mutagenesis kit (Agilent), as per manufacturer's instructions. The mutation was confirmed by Sanger sequencing. The putative targets sites for miR-92a-3p in the mouse *Fam220a* 3' UTR and miR-489-3p in the *Tgfb2* 3' UTR were also cloned separately into pEZX-MT01 reporter constructs down-stream of Firefly luciferase. Briefly, oligonucleotides of the 35-40 bp sequence including the putative target site and surrounding sequence were phosphorylated with T4 polynucleotide kinase (New England Biolabs) at 37°C for 30 minutes followed phenol chloroform purification. The phosphorylated oligonucleotides were denaturation and slowly cooled to anneal the oligonucleotides. The annealed oligonucleotides were ligated into XhoI and XbaI digested pEZX-MT01 vector (Genecopeia) with T4 DNA ligase (Promega) at RT for 1 hr followed by transformation into *E. coli* (Invitrogen). The pEZX-MT01 vectors containing miRNA target sites were confirmed by PCR, restriction enzyme digestion, and Sanger sequencing. All vector purifications performed with either Wizard Plus SV Minipreps DNA purification system (Promega) or PureLink HiPure plasmid midiprep kit (Invitrogen).

Gene reporter (luciferase) assays: HEK293 cells were dually transfected with gene reporter luciferase constructs (described above) in the absence and presence of 50nM miR-92a-3p or miR-489-3p mimetics using Dharmafect Duo transfection reagent (Dharmacon). To quantify Firefly and *Renilla* luciferase activity 48 h post-transfection, luc-pair duo-luciferase assay kit 2.0 (Genecopeia) was used, according to manufacturer's instructions. Firefly luciferase activity were

normalized to transfection control *Renilla* luciferase activity, and ratios were reported as fold changes to the control cells that received no mimetics.

Western Blotting: Total protein was isolated from scraped cells using RIPA buffer containing Halt phosphatase and protease inhibitors (Thermofisher). After 1 h of rotation at 4°C, cell debris were removed by centrifugation and protein concentrations were quantified by Pierce BCA assays (Thermofisher). Total protein lysates were analyzed by gel electrophoresis on NuPAGE 4-12% Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membranes using the iblot system (Invitrogen). Membranes were blocked in 5% milk TBS + 0.1% tween (TBS-T) for 30 min followed by incubation with mouse anti-STAT3 (1:5,000, BD Biosciences), rabbit anti-STAT3 pY705 (1:2,000, diluted in TBS-T + 5% BSA, Cell Signaling), or mouse anti-GAPDH (1:10,000, Sigma) antibodies diluted in 5% milk TBS-T overnight at 4°C. Horseradish peroxidase (HRP) conjugated anti-mouse (1:15,000, Promega) and HRP-anti-rabbit (1:20,000, Promega) secondary antibodies were diluted in 5% milk TBS-T and incubated for 1 h at RT. Membranes were developed using Western lightening plus (Perkin Elmer) or Amersham ECL prime (GE) reagents and exposed to x-ray film (Phenix). Western blot band density was calculated using Image J software.

Supplemental References

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Supplemental Figure Legends

Supplemental Figure 1: Isolation of mouse aortic endothelium for RNA analyses. (**A**) Illustration of method used to shear the aorta endothelial cells for total RNA isolation. (**B**) Real-time PCR quantification of the endothelial cell marker *Pecam1* and smooth muscle cell marker *Acta2* in the endothelium, remaining aortae, and whole aortae. N=5-6.

Supplemental Figure 2: LNA-92a inhibits endothelial miR-92a-3p levels. Real-time PCR quantification of miR-92a-3p for aorta endothelium of *Apoe*$^{-/-}$ mice treated with HDL, LNA-92a, and HDL complexed with LNA-92a. N=3. One-way ANOVA with Bonferroni α=0.016.

Supplemental Figure 3: LNA inhibition of miR-92a-3p and miR-489-3p levels in remaining aortae and liver. Real-time PCR quantification of miR-92a-3p for (**A**) remaining aorta after shearing/isolation of endothelium and (**B**) liver. N=4. (**C**) Real-time PCR quantification of miR-489-3p for remaining aorta after shearing/isolation of endothelium. N=4. One-way ANOVA with Bonferroni α=0.01.

Supplemental Figure 4: Histology for atherosclerotic lesion collagen content. Histological images for aortic cross-sections for collagen content stained with Masson's trichrome stain. Scale bar represents 100µm.

Supplemental Figure 5: Dual inhibition of miR-92a-3p and miR-489-3p failed to alter total macrophage content. (**A**) Immunohistochemical images of aortic cross-sections for macrophage marker MOMA-2. (**B**) Quantification of inflammatory cell content by MOMA-2 staining normalized by total lesion area (Oil-Red-O, ORO) for *Apoe*$^{-/-}$;5/6Nx treated mice. One-way ANOVA. N=5-13. Scale bar represents 500µm.

Supplemental Figure 6: Systolic blood pressure and blood urea nitrogen levels. (**A**) SBP and (**B**) BUN of *Apoe*$^{-/-}$;5/6nx cohort 8 weeks after surgery and 1 week post-injection. One-way ANOVA with Bonferroni α=0.025. N=4-6.

Supplemental Figure 7: Plasma total cholesterol levels. Locked-nucleic acid (LNA) dual inhibition of miR-92a-3p and miR-489-3p. Quantification of plasma total cholesterol of *Apoe*$^{-/-}$;5/6Nx mice. One-way ANOVA with Bonferroni correction α=0.008. N=13-20.

Supplemental Figure 8: Differential gene expression for locked nucleic acid (LNA) miRNA inhibition in Apoe-/-;5/6Nx mice. MA plot of the log10 DESeq2 normalized mean expression and the log2 fold change of aortic endothelial gene expression for (**A**) HDL, (**B**) HDL+LNA-92a, and (**C**) HDL+LNA-489 compared to saline control with significantly altered genes determined by DESEQ2 analysis highlighted in red. Fold change ≥ 1.5 indicated by the green lines. N=4. Venn diagrams of (**D**) significantly up-regulated genes and (**E**) significantly down-regulated genes.

Supplemental Figure 9: miR-92a-3p regulation of FAM220A and STAT3 activation in human endothelial cells. Real-time PCR quantification of (**A**) miR-92a-3p and (**B**) miR-489-3p suppression post transfection of LNAs in HCAEC. N=9. (**C**) Real-time PCR quantification of *FAM220A* in HCAEC transfected with multiple concentrations of LNA-92a. N=9. (**D**) Real-time PCR quantification of miR-92a-3p overexpression in HCAEC transfected with miR-92a-3p mimic and siRNA *FAM220A*. N=8-12. Quantification of western blots for (**E**) P-STAT3 and (**F**) total STAT3 to GAPDH for miR-92a-3p overexpression in HCEAC. N=8. Mann-Whitney non-parametric tests were used for comparisons between two groups. One-way ANOVA with Bonferroni α=0.025 were used for two comparisons and Bonferroni α=0.017 were used for three comparisons.