SUPPLEMENTAL MATERIALS

Aging-associated miR-217 aggravates atherosclerosis and promotes cardiovascular dysfunction

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Supplementary Figures

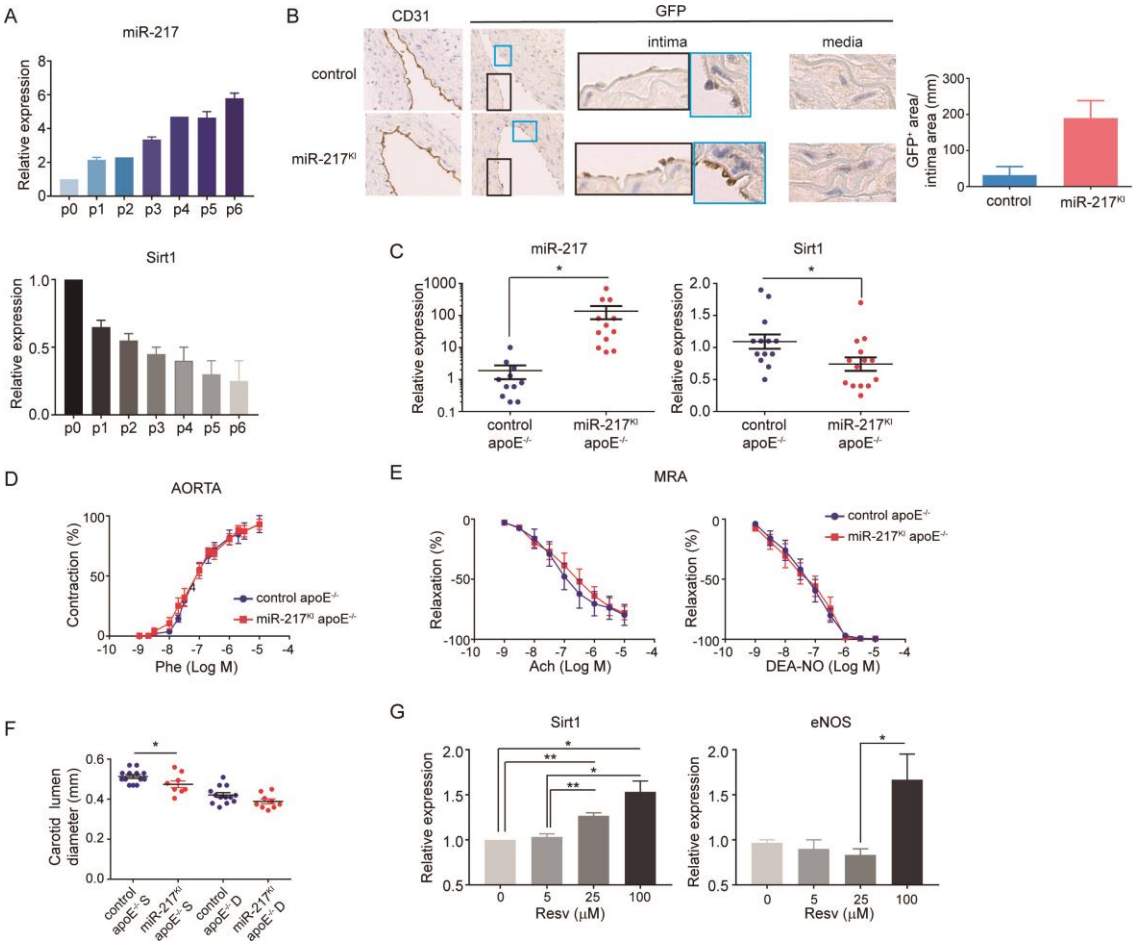

Figure SI. miR-217 expression alters vascular contractile function and promotes atherosclerosis development.

(A) qPCR analysis of miR-217 and Sirt1 expression in primary mouse lung endothelial cells (MLEC) along serial passages in *in vitro* cell culture. Statistical significance versus passage 0 (p0) expression is shown. (B) Immunohistochemical detection of GFP expression in $CD31^{+}$ aortic endothelial cells of VE-Cad CreERT2 control mice and miR-217-overexpressing miR-217KI mice after tamoxifen (TMX) injection. Representative images of the aortic intima

and media are shown. Quatification of GFP^+ area in the aorta intima (right). (C) qPCR analysis of aortic miR-217 and Sirt1 expression in control $apoE^{-/-}$ and endothelium-specific miR-217-overexpressing $miR\text{-}217^{KI}$ $apoE^{-/-}$ post TMX injection fed with HFD for 9 weeks. Each dot represents an individual mouse. (D) Phenylephrine-induced contraction of aortas from male control $apoE^{-/-}$ and $miR\text{-}217^{KI}$ $apoE^{-/-}$ mice fed with HFD for 12 weeks. (E) Acetylcholine-induced relaxation (left) and dose-response relaxation to an exogenous NO source by DEA-NO (right) of mesenteric resistance arteries from male control $apoE^{-/-}$ and $miR\text{-}217^{KI}$ $apoE^{-/-}$ mice fed with HFD for 12 weeks. (F) Systolic (S) and diastolic (D) left carotid lumen diameter assessed by echocardiography in control $apoE^{-/-}$ and $miR\text{-}217^{KI}$ $apoE^{-/-}$ mice fed with HFD for 12 weeks. $p<0.05$ unpaired t test. (G) Resveratrol induces Sirt1 and eNOS expression in endothelial cells. qPCR analysis of Sirt1 and Nos3 (eNOS) in HUVECs after 3 days in culture in the presence of different concentrations of resveratrol.

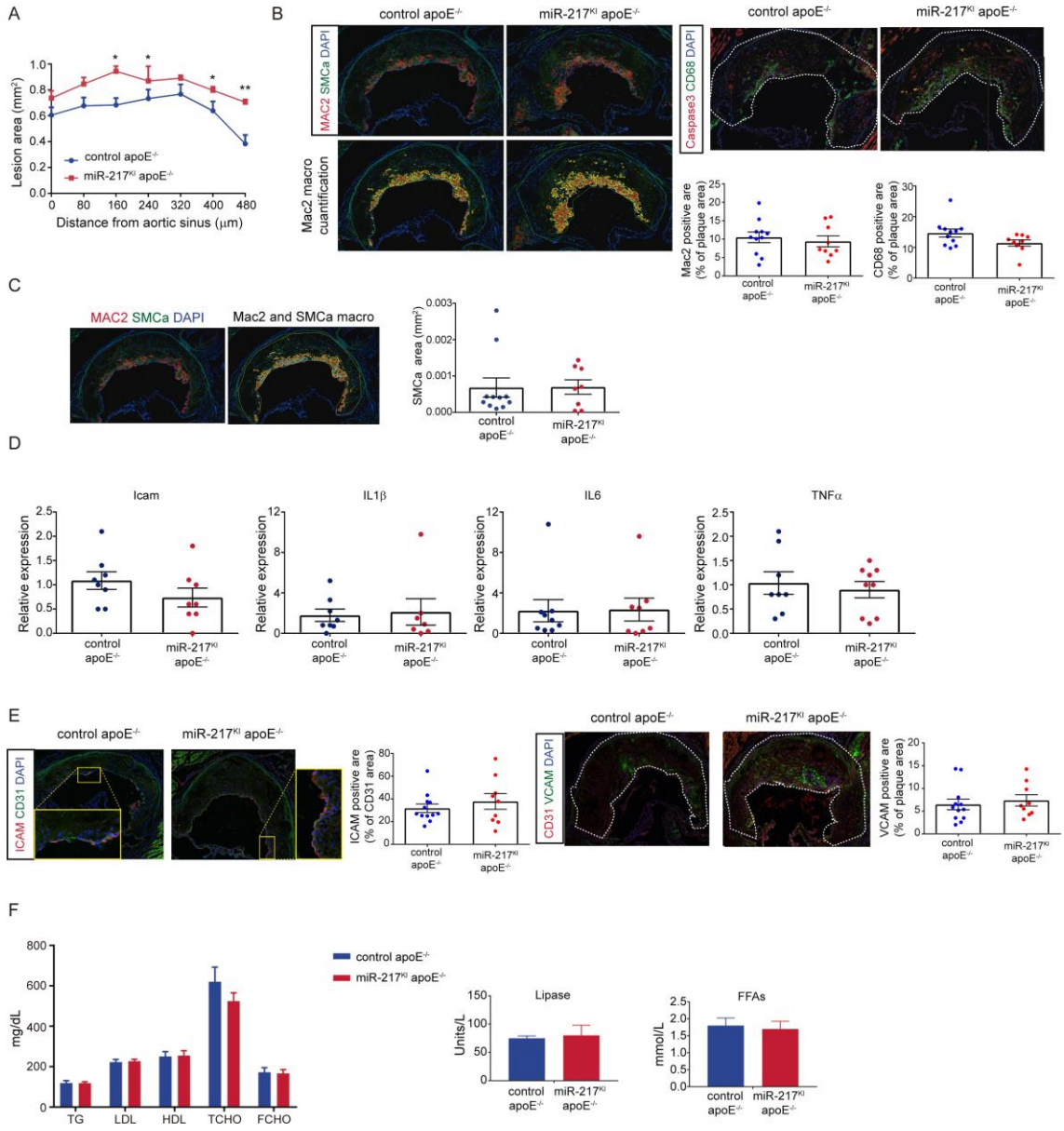

Figure SII. Endothelial miR-217 expression enhances atherosclerosis development.

(A) Quantification of atherosclerotic lesion area (mm^2) by oil red O staining in aortic root heart cryosections in control $apoE^{-/-}$ and $miR\text{-}217^{KI}$ $apoE^{-/-}$ mice fed with HFD for 7 weeks. Each point represents the mean lesion area determined at a specific distance from the aortic sinus* $p<0.05$, ** $p<0.01$ unpaired t test. (B-C) Automatic quantification of confocal microscopy Mac2, CD68 (B) and SMCa (C)

immunofluorescence within atheroma plaque with an Image J automatic threshold analysis of immunofluorescence images (macro). Representative image examples are shown. (D) qPCR analysis of inflammatory an endothelial cell activation molecules in RNAs extracted from the aorta of control apoE$^{-/-}$ and miR-217^{KI} apoE$^{-/-}$ HFD fed mice. (E) Icam1 and Vcam1 immunofluorescence stainings in atheroma plaques of control apoE-/- and miR-217KI apoE-/- HFD fed mice. (F) Blood lipid analysis content in control apoE$^{-/-}$ (n=5) and miR-217^{KI} apoE$^{-/-}$ (n=5) HFD fed mice. Triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TCHO), free cholesterol (FCHO) and free fatty acids (FFAs).

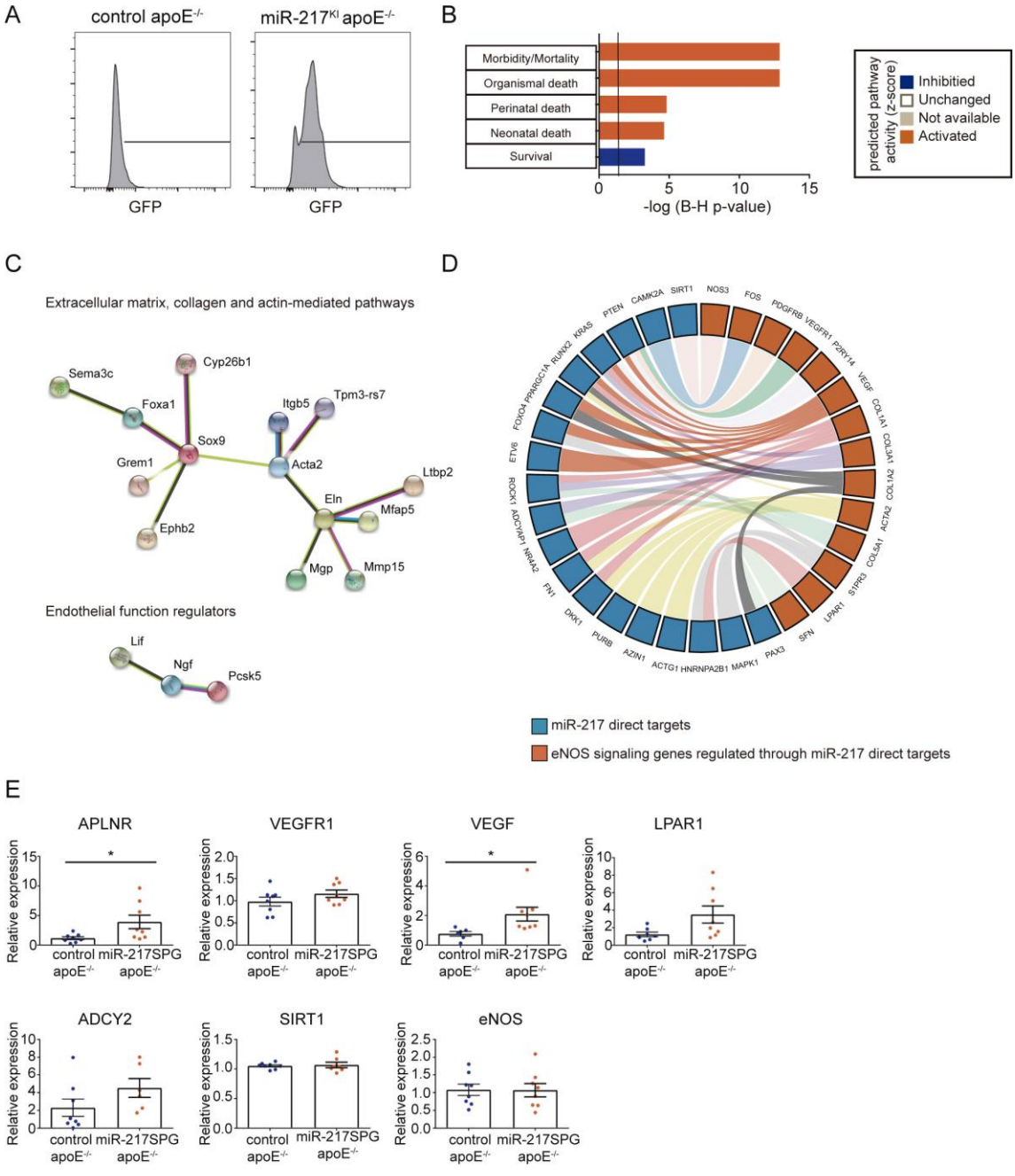

Figure SIII. miR-217 expression disrupts endothelial function.

(A) GFP expression analyzed by flow cytometry in MLECS isolated from control apoE$^{-/-}$ and miR-217^{KI} apoE$^{-/-}$ mice after tamoxifen administration. (B-D) RNA-Seq analysis in MLECs from miR-217KI apoE-/- or control apoE-/- mice (B) "Organism Survival" IPA pathway analysis of RNA-Seq gene expression

quantification in MLECs from miR-217KI apoE$^{-/-}$ or control apoE$^{-/-}$ mice. P-values were corrected for multiple testing using the Benjamini-Hochberg (B-H) false discovery rate. Pathway activity prediction is shown with a color code (blue: inhibited, orange: activated). (C-D) Analysis of downregulated miR-217 direct targets in miR-217KI apoE-/- MLECs. (C) String gene networks of downregulated genes within miR-217 direct targets, as predicted by TargetScan. (D) Circos plot showing eNOS signaling genes (in orange) downregulated by one or several miR-217 direct target genes (in blue). (E) qPCR analysis of the expression of eNOS signaling genes in control apoE-/- and miR-217-Sponge (miR-217SPG) apoE-/- transduced MLECs. * $p<0.05$ by unpaired t test.

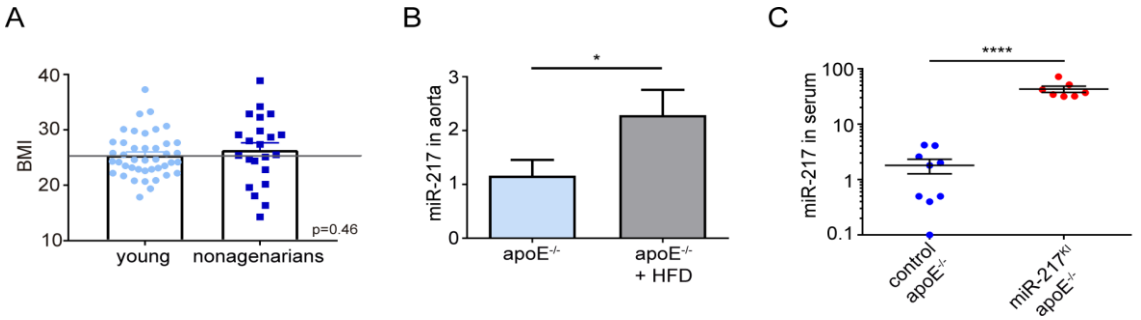

Figure SIV. Atherosclerosis is associated with increased endothelial expression of miR-217 and its release to serum.

(A) BMI of individuals young healthy controls (< 50 years old) and nonagenarian women is shown. Grey line indicates overweight (BMI≥ 25). Unpaired T test p value 0.46. (B) Aortic miR-217 expression is increased in mice with atherosclerosis. Real time RT-PCR of miR-217 from the aortas of age-matched apoE$^{-/-}$ mice fed standard chow (n=11) and apoE$^{-/-}$ mice fed a high-fat diet for 12 weeks to promote atherosclerosis (n=9). (C) Mice overexpressing miR-217 in the endothelium have elevated levels of miR-217 in serum. Quantitative RT-PCR analysis of serum miR-217 in control apoE$^{-/-}$ mice (miR-217$^{+/+}$ VECad CreERT2$^{TG/+}$ apoE$^{-/-}$) and miR-217^{KI} apoE$^{-/-}$ mice (miR-217$^{KI/+}$ VECad CreERT2$^{TG/+}$ apoE$^{-/-}$). * p<0.05, **** p<0.0001, unpaired t test.

Table SI. RNA-Seq transcriptome analysis on endothelial cells from miR-217KI apoE$^{-/-}$ and control apoE$^{-/-}$ mice. Excell spreadsheet.

Video SI: Endothelial miR-217 expression promotes left ventricular systolic dysfunction.

Left ventricle B-mode large axis echocardiography in control apoE-/- (left) and miR-217KI apoE-/- mice after 10 weeks on HFD.

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
ApoE-/-	Charles River	C57BL/6J	Male and Female	JAX mice stock 002052
VE-Cadherin Cdh5-CreERT2	Provided by. Dr. Ralf H. Adams (Max Planck Institute, Münster, Germany)	C57BL/6J	Male and Female	NA
Rosa26 miR-217 knock-in	Generated by V.G. Yebenes [15]	C57BL/6J	Male and Female	NA

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
GFP	Acris Antibodies	R1091P	IHC (1,7 μg/ml))	NA	https://m1.acris-antibodies.com/pdf/R1091P.pdf
CD31	Abcam	ab28364	IHC (1/80 dilution IF (1/200 dilution)	NA	https://www.abcam.com/cd31-antibody-ab28364.html
SMA-1 biotin	ThermoFisher Scientific	MS-113-B0	IF (0,2 μg/ml)	NA	https://assets.fishersci.com/TFS-Assets/APD/Specification-Sheets/D11617~.pdf
Mac-2	Cedarlane	CL8942AP	IF (5 μg/ml)	NA	https://www.cedarlanelabs.com/products/detail/cl8942le
CD68	Serotec	MCA1957	IF (2 μg/ml)	NA	https://www.bio-rad-antibodies.com/monoclonal/mouse-cd68-antibody-fa-11-mca1957.html?f=purified
VCAM-1	Abcam	ab134047	IF (1/200 dilution)	NA	https://www.abcam.com/vcam1-antibody-epr5047-ab134047.htm
Icam-1 biotin	BD Pharmingen	553251	IF (10 μg/ml)	NA	https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/mouse/biotin-hamster-anti-mouse-cd54-3e2/p/553251
CD31	Millipore	MAB1398Z	IF (1/100 dilution	NA	https://www.merckmillipore.com/ES/es/product/Anti-PECAM-1-Antibody-clone-2H8-Azide-Free,MM_NF-MAB1398Z
active caspase-3	R&D systems	AF835	IF (2 μg/ml)	NA	https://resources.rndsystems.com/pdfs/datasheets/af835.pdf

DOI [to be added]