SUPPLEMENTAL MATERIALS

ADAM17 boosts cholesterol efflux and downstream effects of HDL on inflammatory pathways in macrophages

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Supplemental methods

Mice

Animals were kept under specific pathogen-free conditions with ad libitum access to food and water. Mice were fed a standard laboratory diet (Purina Mills diet 5053). Housing temperatures were kept within a range of 71-73°F (21.7-22.8°C). Water and cages were autoclaved, and cages were changed weekly. The health status of the mice was monitored using a dirty bedding sentinel program and no health status issues or changes in immune status were identified. It has been reported that male *APOA1^{Tg}* mice have higher plasma APOA1 levels than female mice.[1] Thus, to evaluate the effects on macrophage inflammatory properties by high levels of circulating HDL, we used male *APOA1^{Tg}* mice for all experiments. For consistency, we used male *Lyz2$^{Cre/Cre}$; Adam17$^{Wt/Wt}$* and *Lyz2$^{Cre/Cre}$*; *Adam17$^{fl/fl}$* mice for transplants and for experiments with bone marrow derived macrophages (BMDMs). Since we did not observe any differences between male and female mice in cell culture experiment with BMDMs from C57BL/6J mice, data from either male or female mice are shown, as described in the figure legends.

Isolation and culture of macrophages

BMDMs were obtained by isolating bone marrow cells from femurs and tibias of male and female mice and culturing the cells in RPMI1640 (Corning, Tewksbury, MA) supplemented with 30% L929-conditioned medium, 7% FBS (Thermo Fisher Scientific, Waltham, MA), and 1% penicillin-streptomycin (Thermo Fisher Scientific) in tissue culture plates (Thermo Fisher Scientific). Fresh medium was added on day 3 and day 5.

Quantitative PCR

Macrophages were washed twice with cold PBS and lysed in RNA lysis buffer (Zymo Research, Irvine, CA). RNA was isolated using RNA MiniPrep kits (Zymo Research), according to the manufacturer's protocol. cDNA was prepared using first strand synthesis kits (Thermo Fisher Scientific), and quantitative PCR (qPCR) was performed on an ABI 7900HT fast real-time PCR System (Applied Biosystems, CA) with SYBR reagents (Thermo Fisher Scientific). Assays were performed in 384-well plates with the following contents/well: 7.5 µl of PCR Master mix (Thermo Scientific, Cat # K0223), 4.3 µl of sterile water, 0.1 µl of each set of forward and reverse primers (0.13 µM final concentration of each) and 3 µl of DNA template, for a total reaction volume of 15 µl. Cycles included: one 2-min hold (50°C); a 10-min denaturation (95°C); and 40 cycles of denaturation (95°C for 30 s), annealing (60°C for 1 min), and extension (72°C for 30 s). The housekeeping gene *Rn18s* was used to account for variability in the initial quantities of mRNA.

HDL isolation from mouse plasma

HDL (density 1.125–1.21 g/ml) was isolated from EDTA plasma of *APOA1^{Tg}; Ldlr$^{-/-}$* mice by density gradient ultracentrifugation. Briefly, 335 µl of plasma was adjusted to a density of 1.21 g/ml by solid KBr, underlaid with 150 µl of KBr solution with a density of 1.21 g/ml, and spun at 511,260 x g at 5°C for 4.5 hr in a T120.1 rotor (Beckman Coulter, Pasadena, CA). The top 125 µl was collected, adjusted to a density of 1.063 g/ml with normal saline, and HDL was separated from more buoyant lipoproteins with another 2 hr spin at 511,260 x g in the T120.1 rotor. HDL was collected in 125 µl from the bottom of the tube and dialyzed against 20 mM potassium phosphate buffer with 100 µM DTPA, pH 7.4. HDL concentrations are given as total protein level.

Quantification of HDL particle size and concentration

HDL particle concentration (HDL-P) and size distribution were measured using calibrated ion mobility analysis (IMA).[2-4] This method yields a stoichiometry of APOA1 and sizes and relative

abundances of HDL subclasses that are in excellent agreement with those determined by non-denaturing gradient gel electrophoresis and analytical ultracentrifugation.[3, 5, 6] Particle concentration was calibrated using glucose oxidase and validated using external well-characterized plasma samples.

Effects of HDL on endothelial cells

Mouse microvascular endothelial cells were isolated by a magnetic bead positive selection method from hearts using an ICAM-2 antibody, as previously described.[7] In short, endothelial cells from C57BL/6J mouse hearts were placed on collagen pre-coated plates and cultured in endothelial cell medium at 37°C for 7–14 days to promote mouse heart endothelial cell outgrowth prior to passaging. The culture medium was replaced every three days, and cell cultures were subcultured at 90-95% confluence before studied between passages 2-3. For experiments, cells were treated with HDL (10-100 µg/ml) in the medium without growth factors for 18 hr, followed by stimulation with mouse recombinant TNFα (20 ng/ml, R&D Systems, Minneapolis, MN) for 6 hr. After extensive washing and addition of primary mouse bone marrow monocytes (monocyte enrichment kit, Stemcell Technologies, Vancouver, Canada) that had been labeled with calcein-acetoxymethyl ester (Sigma-Aldrich) for 30 min at 37°C, monocyte adhesion was estimated based on emitted fluorescence, as described previously.[8]

Cholesterol uptake using DiI-labeled AcLDL

For cholesterol uptake, BMDMs from C57BL/6J WT or ADAM17-deficient mice were cultured as described above in 24-well plates, and treated with 10 µg/ml DiI-labeled acetylated low-density lipoprotein (AcLDL) for 4 hr. The cells were then fixed for 10 min with 4% PFA and analyzed by fluorescence microscopy (Keyence All-in-One Fluorescence Microscope BZ-X800) at 20X magnification.

Lipogenesis assay

BMDMs from C57BL/6J WT or ADAM17-deficient mice were labeled with 0.5 µCi [^{3}H]sodium acetate per well (specific activity: >10 Ci/mmol; ViTrax; Placentia, CA) in the presence of 10 mM unlabeled sodium acetate for 18 hr. Cells were then washed twice with ice-cold PBS and scraped in 200 µl PBS for lipid extraction. Lipids were extracted according to a published method[9, 10] using 2:1 chloroform-methanol (v/v). Radioactivity in the chloroform phase containing total lipids was determined by mixing the samples with a scintillation cocktail (Insta-Fluor Plus, PerkinElmer Inc; USA) and using a TriCarb 2810 TR liquid scintillation analyzer.

References

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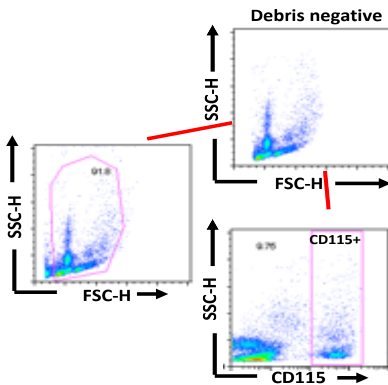
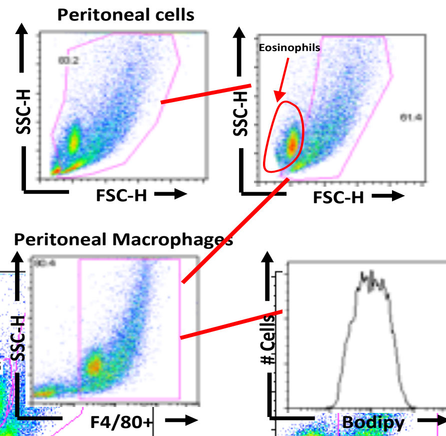
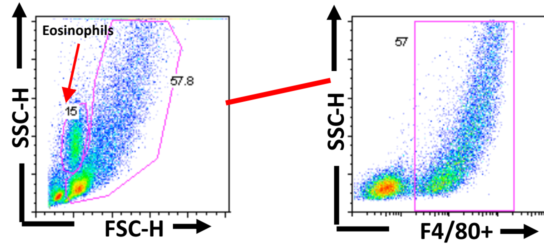
A Gating strategy for CD115+ monocytes

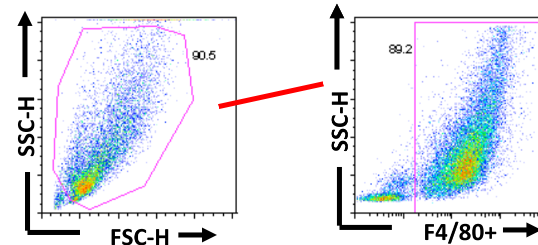


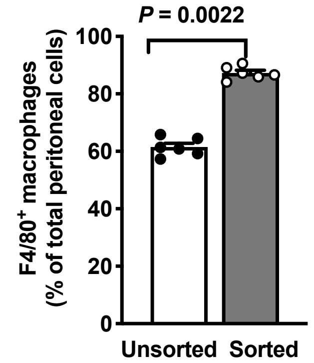
B Gating strategy for BODIPY



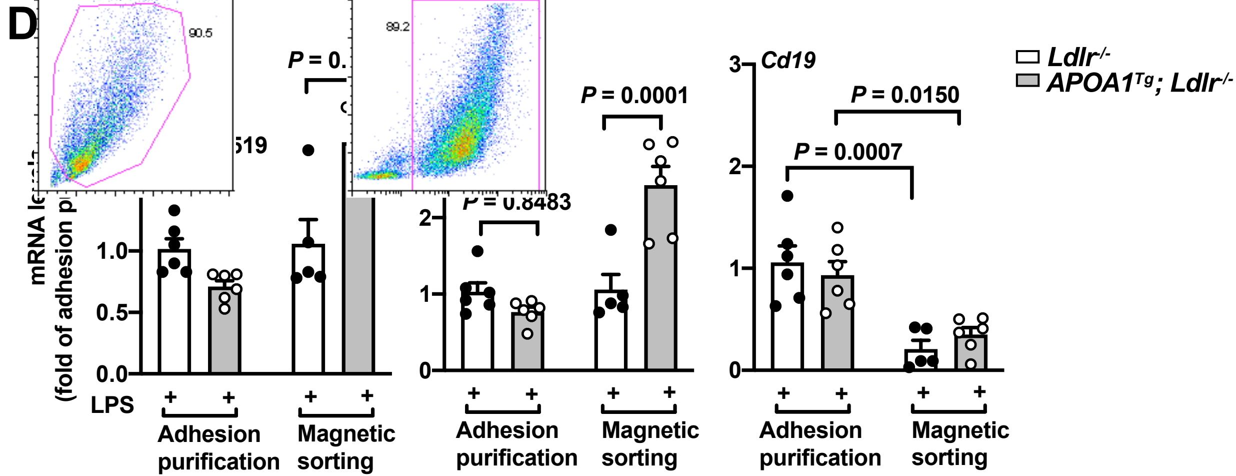
C Unsorted peritoneal cells — Sorted peritoneal cells



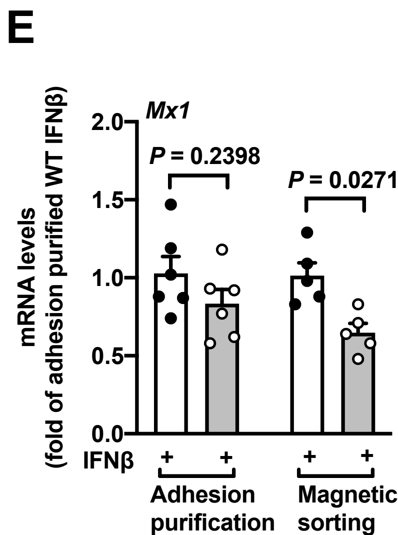




D



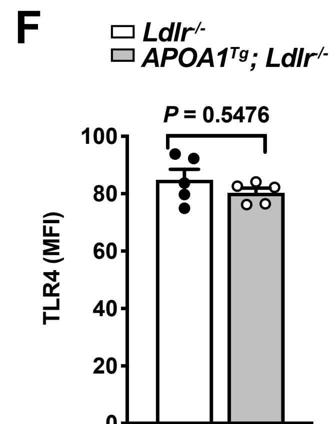
E



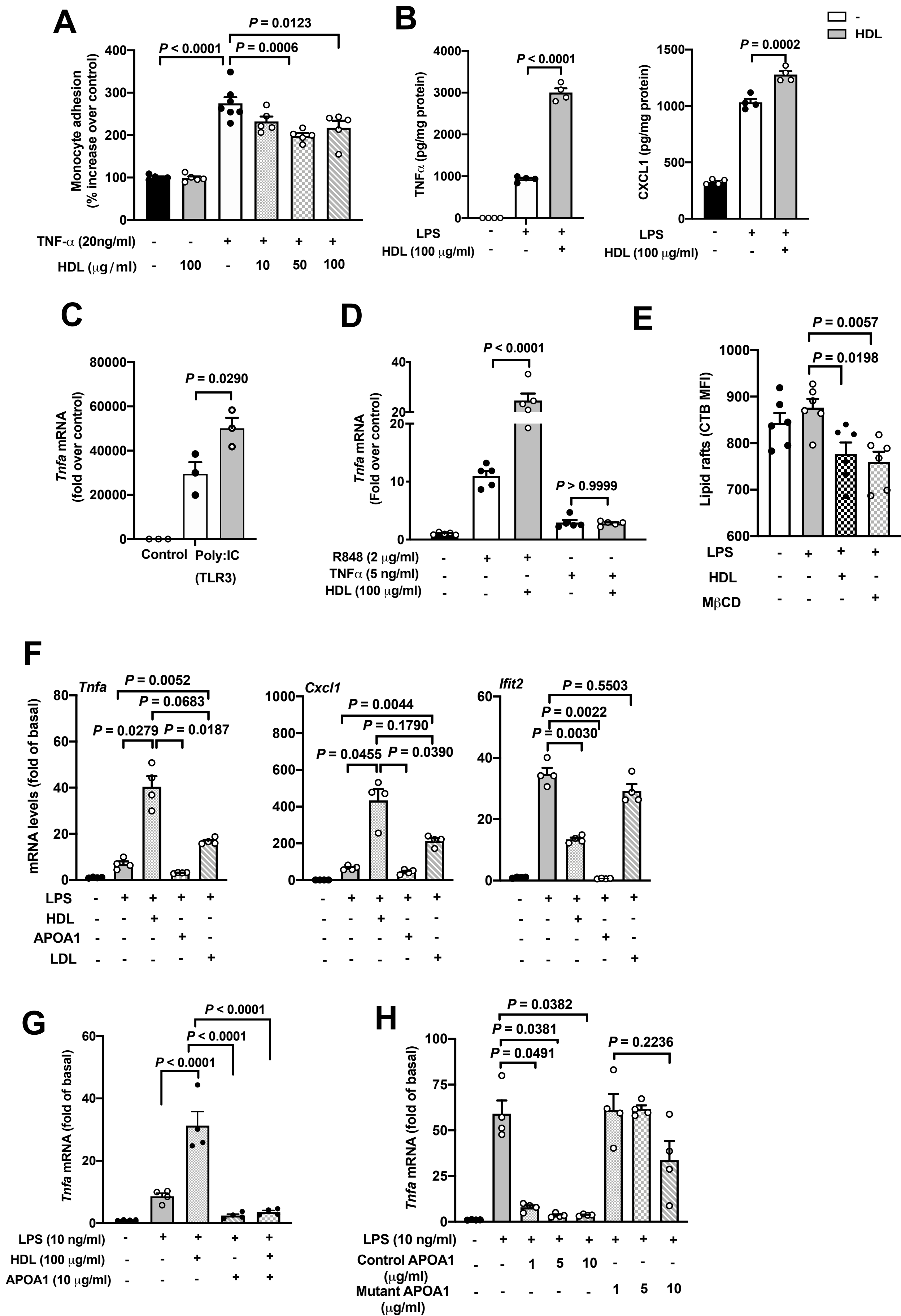
Ifit2

P > 0.9999 — P = 0.0450

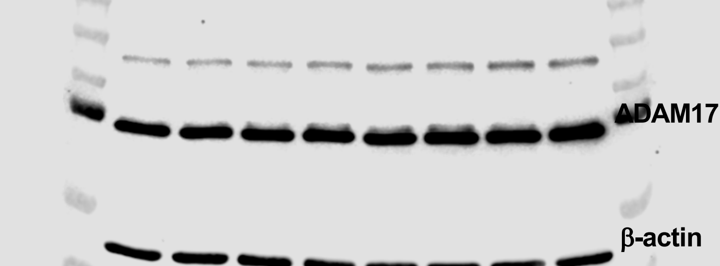
F

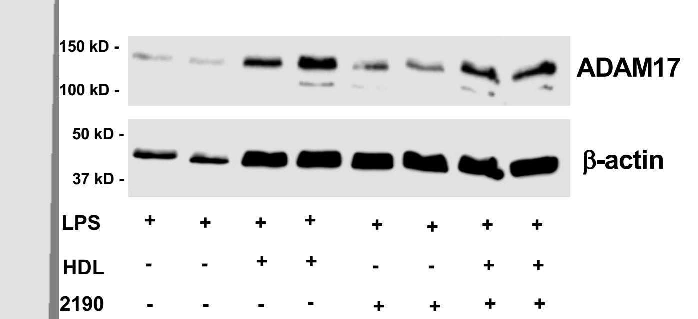


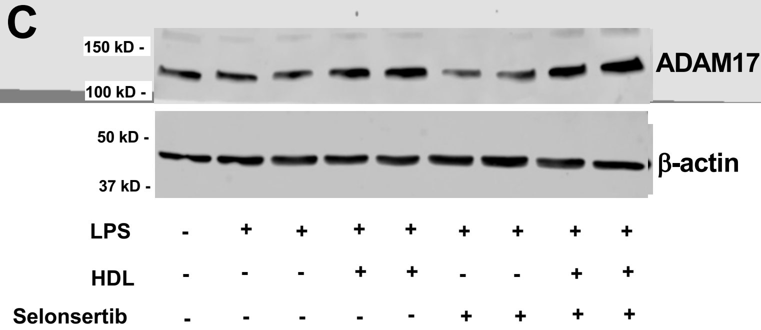
Supplemental Figure I. A. Gating strategy for CD115. **B.** Gating strategy for BODIPY in thioglycolate-elicited peritoneal macrophages. **C.** Gating strategy for F4/80+ macrophages from thioglycollate-elicited peritoneal cells before and after sorting using a macrophage negative selection kit. Cell population purity is shown on the right. **D.** Peritoneal cells from male *Ldlr*$^{-/-}$ and *APOA1*^{Tg}; *Ldlr*$^{-/-}$ mice were collected 4 days after thioglycolate injection. Macrophages were purified from other cell types either by 1 hr adhesion or by using a macrophage negative selection kit followed by 1 hr adhesion before stimulation with LPS (10 ng/ml) or IFNβ (1 ng/ml) for 4 hr. At the end of treatment, the effect on gene expression of *Tnfa, Cxcl1* and *Cd19* in LPS-stimulated cells was measured by qPCR (n=5-6). **E.** Gene expression of *Ifit2* and *Mx1* in IFNβ-stimulated cells was determined by qPCR (n=5-6). **F.** Peritoneal cells from male *Ldlr*$^{-/-}$ and *APOA1*^{Tg}; *Ldlr*$^{-/-}$ mice were collected 4 days after thioglycolate injection. Macrophages were isolated from other cell types using a macrophage isolation kit and cell surface expression of TLR4 was determined by flow cytometry. Data are shown as mean ± SEM. *P* values were determined by unpaired two-tailed Mann-Whitney test (C, F) and by two-way ANOVA followed by Sidak's multiple comparison tests (D, E).

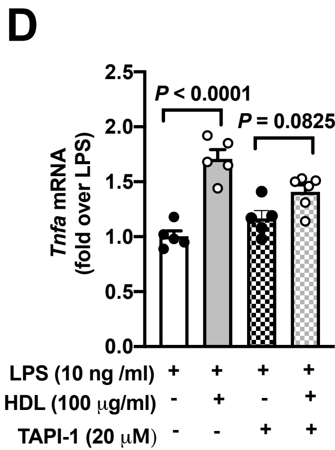

A
B
C
D
E
F
G
H
-
HDL
P < 0.0001
P = 0.0006
P = 0.0123
Monocyte adhesion (% increase over control)
TNF-α (20ng/ml)
HDL (μg/ml)
TNFα (pg/mg protein)
CXCL1 (pg/mg protein)
LPS
HDL (100 μg/ml)
P = 0.0002
P = 0.0290
Tnfa mRNA (fold over control)
Control
Poly:IC (TLR3)
P > 0.9999
R848 (2 μg/ml)
TNFα (5 ng/ml)
P = 0.0057
P = 0.0198
Lipid rafts (CTB MFI)
MβCD
Tnfa
Cxcl1
Ifit2
P = 0.0052
P = 0.0683
P = 0.0279
P = 0.0187
P = 0.0044
P = 0.1790
P = 0.0455
P = 0.0390
P = 0.5503
P = 0.0022
P = 0.0030
mRNA levels (fold of basal)
APOA1
LDL
Tnfa mRNA (fold of basal)
LPS (10 ng/ml)
APOA1 (10 μg/ml)
P = 0.0382
P = 0.0381
P = 0.0491
P = 0.2236
Control APOA1 (μg/ml)
Mutant APOA1 (μg/ml)

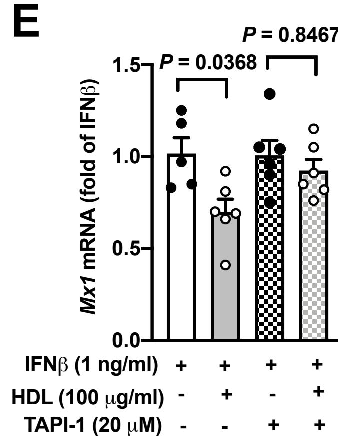
Supplemental Figure II. A. Mouse heart microvascular endothelial cells were pre-treated with HDL at different concentrations for 18 hr. The cells were then stimulated with TNFα (20 ng/ml) for an additional 6 hr. Primary mouse bone marrow monocytes were added to the monolayer of TNFα-stimulated endothelial cells to determine the effect of HDL on adhesion of monocytes to the endothelium. **B**. BMDMs from female C57BL/6J mice were primed with HDL (100 µg/ml) for 18 hr, washed and stimulated with LPS (10 ng/ml) for 4 hr. At the end of the treatment, conditioned media were used to analyze soluble TNFα and CXCL1 (n=4). **C**. Effect of HDL on poly:IC (10 µg/ml)-induced gene expression of *Tnfa*. **D.** BMDMs from female C57BL/6J mice were pre-treated with HDL (100 µg/ml for 18 hr) and the cells were then stimulated with LPS (10 ng/ml), R848 (2 µg/ml), or TNFα (5 ng/ml) in the absence of HDL. At the end of the 4 hr stimulation, the cells were used to analyze gene expression of *Tnfa*. **E.** BMDMs from male C57BL/6J mice were treated with HDL (100 µg/ml, 18 hr) or methyl β-cyclodextrin (MβCD, 10 µM, 1 hr), washed and stimulated with LPS (10 ng/ml) for 4 hr. At the end of the treatment, the cells were stained for lipid raft with cholera toxin B (CTB) and mean fluorescent intensity (MFI) was determined by flow cytometry (n=6; experiment performed twice with similar results). **F**. BMDMs from male C57BL/6J mice were primed with either HDL (100 µg/ml), human APOA1 (50 µg/ml) or LDL (100 µg/ml) for 18 hr, washed and stimulated with LPS (10 ng/ml) for 4 hr. At the end of the treatment, cells were used to analyze gene expression *Tnfa*, *Cxcl1* and *Ifit2* (n=4; experiment performed twice with similar results). **G**. BMDMs from male C57BL/6J mice were pre-treated with HDL (100 µg/ml) and APOA1 (10 µg/ml) alone or in combination for 18 hr, washed and stimulated with LPS (10 ng/ml) for 4 hr. At the end of the treatment, cells were used to analyze gene expression *Tnfa*, (n=4). **H.** BMDMs from male C57BL/6J mice were primed with either control wildtype APOA1 or an APOA1 carboxyl-terminal deletion mutant at indicated concentrations for 18 hr, washed and stimulated with LPS (10 ng/ml) for 4 hr. At the end of the treatment, cells were used to analyze gene expression *Tnfa*, (n=4). Data are shown as mean ± SEM. *P* values were determined by Brown-Forsythe and Welch ANOVA with Dunnett's multiple comparison tests (A, B – TNFα, E, F), one-way ANOVA followed by Tukey's multiple comparison tests (C, D, B – CXCL1, G) or by two-way ANOVA followed by Sidak's multiple comparison tests (H). Unless otherwise stated, data are representative of at least three independent experiments performed in replicates.

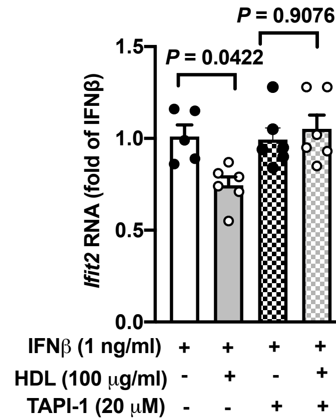

ADAM17
β-actin

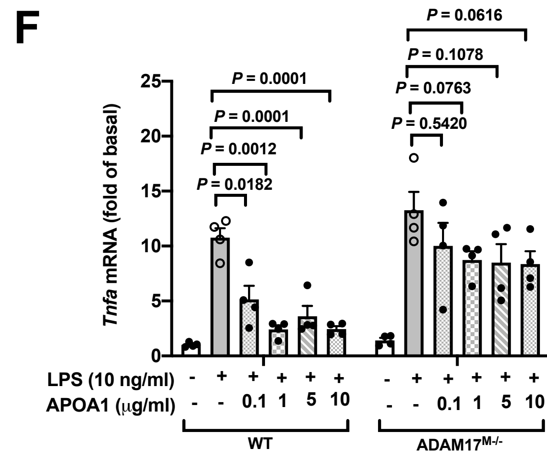

150 kD -
100 kD -
ADAM17
50 kD -
37 kD -
β-actin
LPS + + + + + + + +
HDL - - + + - - + +
2190 - - - - + + + +


C
150 kD -
100 kD -
ADAM17
50 kD -
37 kD -
β-actin
LPS - + + + + + + + +
HDL - - - + + - - + +
Selonsertib - - - - - + + + +

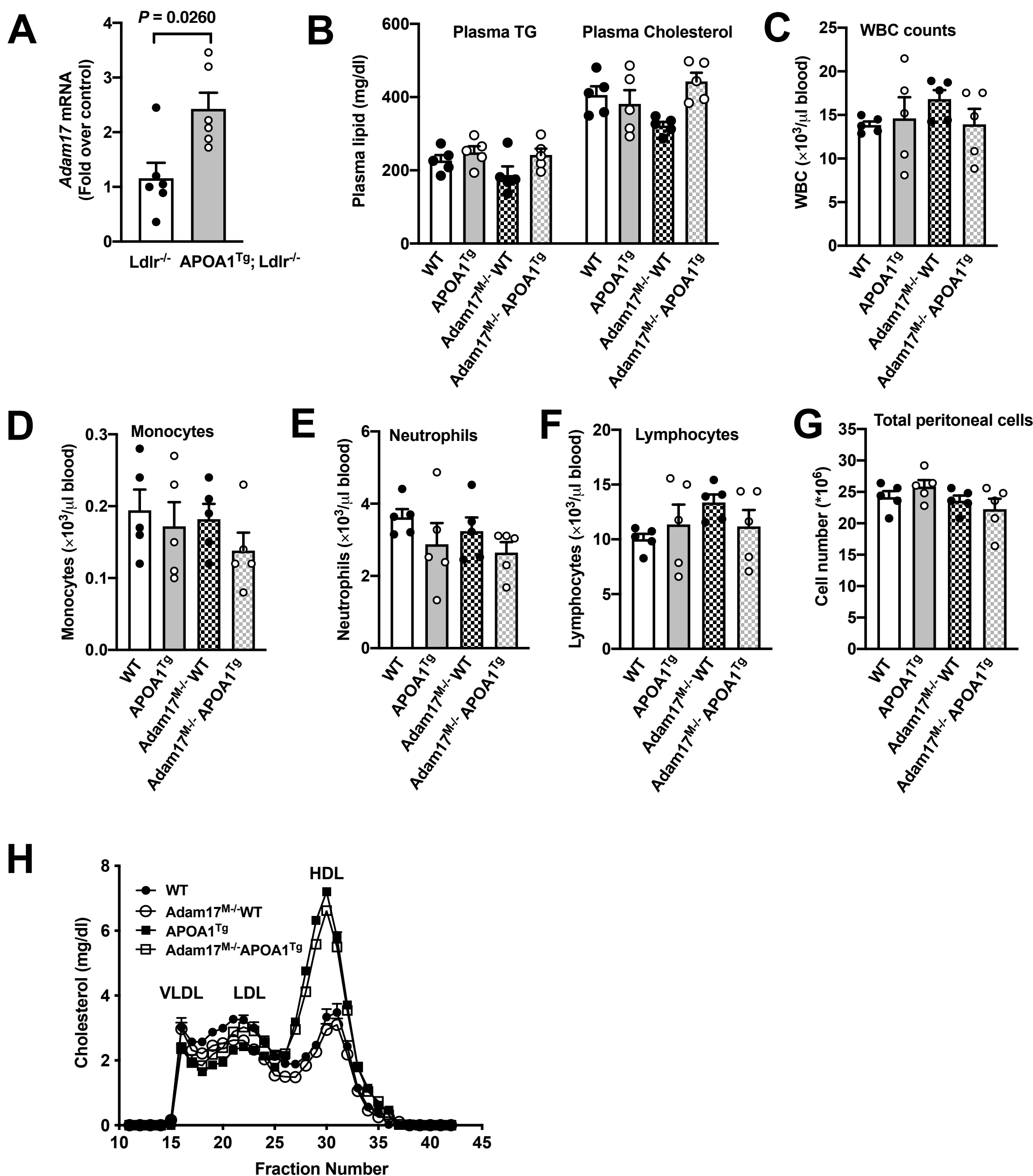

D
P < 0.0001
P = 0.0825
Tnfa mRNA (fold over LPS)
LPS (10 ng /ml) + + + +
HDL (100 μg/ml) - + - +
TAPI-1 (20 μM) - - + +


E
P = 0.0368
P = 0.8467
Mx1 mRNA (fold of IFNβ)
IFNβ (1 ng/ml) + + + +
HDL (100 μg/ml) - + - +
TAPI-1 (20 μM) - - + +

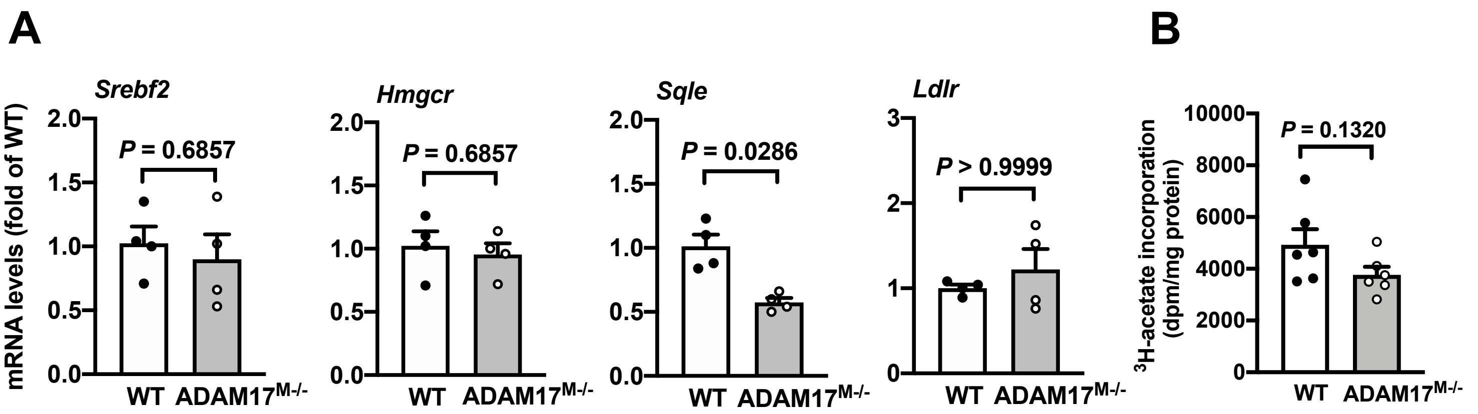

P = 0.0422
P = 0.9076
Ifit2 RNA (fold of IFNβ)
IFNβ (1 ng/ml) + + + +
HDL (100 μg/ml) - + - +
TAPI-1 (20 μM) - - + +

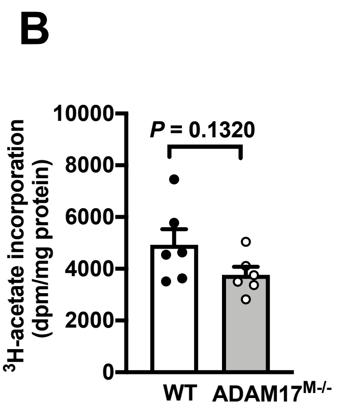

F
P = 0.0001
P = 0.0001
P = 0.0012
P = 0.0182
P = 0.0616
P = 0.1078
P = 0.0763
P = 0.5420
Tnfa mRNA (fold of basal)
LPS (10 ng/ml) - + + + + + - + + + + +
APOA1 (μg/ml) - - 0.1 1 5 10 - - 0.1 1 5 10
WT
ADAM17M-/-

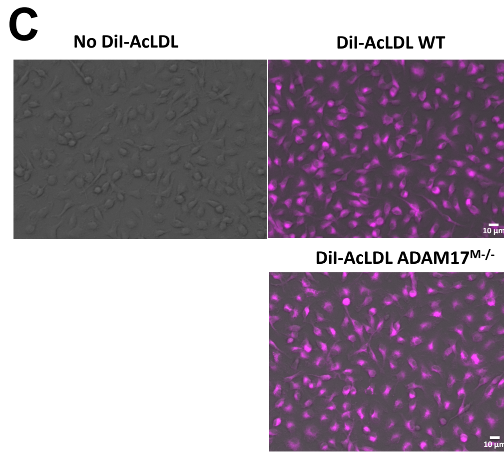
Supplemental Figure III. **A.** BMDMs were treated with either HDL (100 μg/ml, 18 hr) or methyl β-cyclodextrin (MβCD, 10 μM, 1 hr) and then washed and stimulated with LPS (10 ng/ml) for 4 hours. Protein expression of ADAM17 was analyzed at the end of the treatment by immunoblot. **B.** BMDMs were pre-treated with a p38MAPK inhibitor (SB202190, 10 μM) before HDL (100 μg/ml for 18 hr) treatment, washed, and then the cells were stimulated with LPS (10 ng/ml) for 4 hr in the presence of SB202190. At the end of the stimulation, protein expression of ADAM17 was analyzed by immunoblot. **C.** BMDMs were pre-treated with an ASK1 inhibitor (Selonsertib, 10 μM) before treatment with HDL (100 μg/ml for 18 hr), washed, and then stimulated with LPS (10 ng/ml) for 4 hr in the presence of Selonsertib. At the end of the stimulation, protein expression of ADAM17 was analyzed by immunoblot. **D.** BMDMs were pre-treated with TAPI-1 (20 μM, 48 hr) before HDL (100 μg/ml for 18 hr) treatment, and then stimulated with LPS (10 ng/ml) for 4 hr in the absence of HDL but in the presence of TAPI-1. At the end of the stimulation, the cells were used to analyze gene expression of *Tnfa* (n=5; experiment performed twice with similar results). **E.** BMDMs were pre-treated with TAPI-1 (20 μM, 48 hr) before HDL (100 μg/ml for 18 hr) treatment, and then stimulated with IFNβ (1 ng/ml) for 4 hr in the absence of HDL but in the presence of TAPI-1. At the end of the stimulation, the cells were used to analyze gene expression of *Mx1* and *Ifit2* (n=5-6). **F**. BMDMs from WT and ADAM17-deficient mice were pre-treated with APOA1 at indicated concentrations for 18 hr, washed and stimulated with LPS (10 ng/ml) for 4 hr. At the end of the treatment, the cells were used to analyze gene expression of *Tnfa*, (n=4). Data are shown as mean ± SEM. *P* values were determined by one-way ANOVA followed by Tukey's multiple comparison tests (D, E, F). Unless otherwise stated, data are representative of at least three independent experiments in BMDMs from male mice.

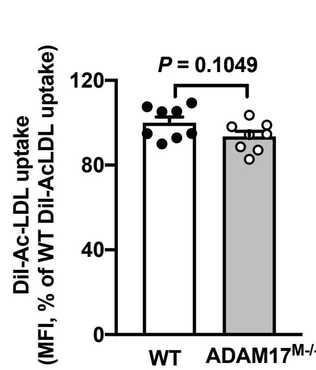


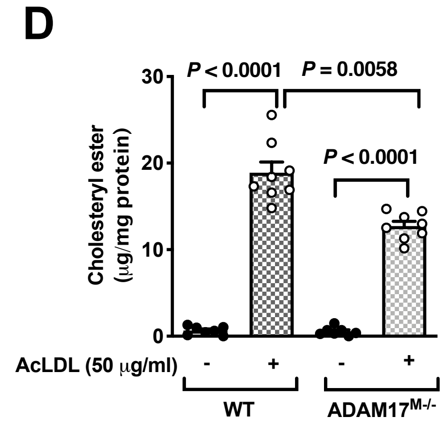
Supplemental Figure IV. **A.** Resident peritoneal cells from male *Ldlr*$^{-/-}$ and *APOA1*^{Tg}*; Ldlr*$^{-/-}$ mice were adhesion purified for 1 hr and then analyzed for the gene expression of *Adam17* (n=5). **B.** Bone marrow was harvested from myeloid cell-targeted ADAM17-deficient mice or C57BL/6J WT controls, erythrocytes were removed, and the cells were transplanted into lethally irradiated (10 Gy) male *Ldlr*$^{-/-}$ and *APOA1*^{Tg}*; Ldlr*$^{-/-}$ recipients (n=5). After 8 weeks of recovery, plasma cholesterol and triglycerides were measured using colorimetric assays. **C-F.** Blood was collected from the retro-orbital plexus under isoflurane sedation with EDTA as an anticoagulant. White blood cell (WBC) (**C**), total blood monocytes (**D**), neutrophils (**E**), and lymphocytes (**F**) were determined using a Hemavet automated counter. **G.** Thioglycolate-elicited peritoneal cells collected by peritoneal lavage were counted manually using a hemocytometer. **H.** Cholesterol lipoprotein profiles. Three plasma samples were analyzed per group. Plasma was pooled from a total of 5 animals per group. Data are shown as mean ± SEM. *P*-values were determined by unpaired two-tailed Mann-Whitney test (A) and one-way ANOVA (B-G).

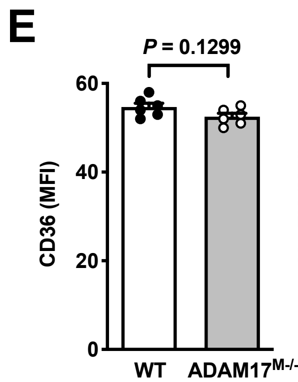

A
Srebf2
Hmgcr
Sqle
Ldlr
mRNA levels (fold of WT)
P = 0.6857
P = 0.6857
P = 0.0286
P > 0.9999
WT ADAM17M-/-

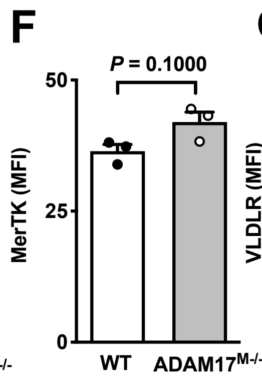

B
3H-acetate incorporation (dpm/mg protein)
P = 0.1320
WT ADAM17M-/-

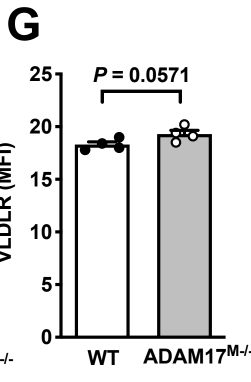

C
No DiI-AcLDL
DiI-AcLDL WT
DiI-AcLDL ADAM17M-/-

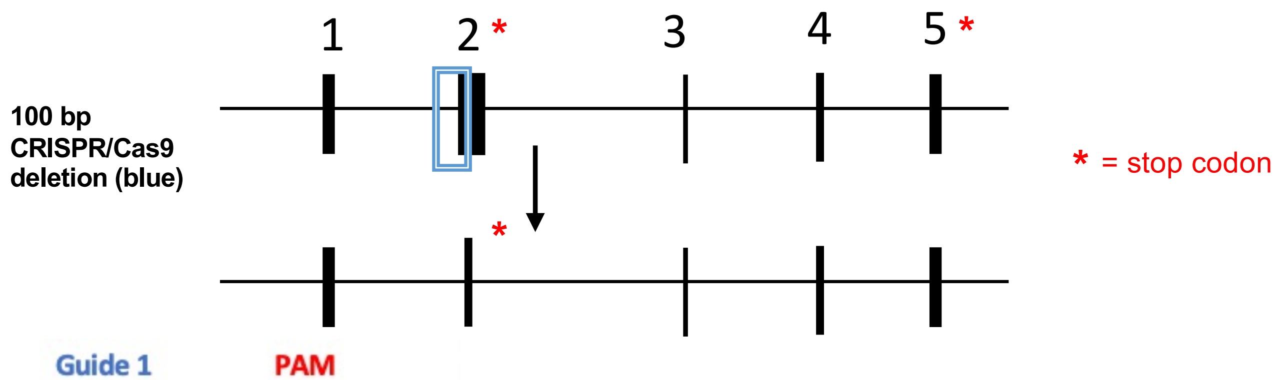

DiI-Ac-LDL uptake (MFI, % of WT DiI-AcLDL uptake)
P = 0.1049
WT ADAM17M-/-


D
Cholesteryl ester (µg/mg protein)
P < 0.0001
P = 0.0058
P < 0.0001
AcLDL (50 µg/ml)
WT
ADAM17M-/-


E
CD36 (MFI)
P = 0.1299
WT ADAM17M-/-


F
MerTK (MFI)
P = 0.1000
WT ADAM17M-/-


G
VLDLR (MFI)
P = 0.0571
WT ADAM17M-/-


H
Designing of guide sequence for CRISPR/Cas9 gene editing of TREM2
100 bp CRISPR/Cas9 deletion (blue)
* = stop codon
Guide 1
PAM
5'-TCAACACCACGGTGCTGCAGGGCATGGCCGGCCAGTCCTTGAGGGTGTCATGTACTTATGACGCCTTGAAGCACTGGGGAGACGCAAGGCCTGGTGTCGGCAGCT-3'
3'-AGTTGTGGTGCCACGACGTCCCGTACCGGCCGGTCAGGAAC TCCCACAGTACATGAATACTGCGGAACTTCGTGACCCCC TCTGCG TTCCGGACCACAGCCGTCGA-5'
PAM
Guide 2

Supplemental Figure V. **A.** C57BL/6J WT or ADAM17-deficient BMDMs were used to analyze gene expression of *Srebf2*, *Sqle, Hmgcr,* and *Ldlr* (n=4). **B.** BMDMs from C56BL/6 mice were incubated in the presence of ^{3}H-acetate, and ^{3}H-acetate incorporation into cellular lipids was analyzed as a measure of lipogenesis. **C.** C57BL/6J WT or ADAM17-deficient BMDMs were treated with DiI-labeled AcLDL (10 µg/ml). After 4 hr, lipid uptake was visualized with fluorescence microscopy (DiI in red, nuclei stained with DAPI in blue), and quantified. The results are shown as mean fluorescence intensity; percentage of BMDMs from WT mice (n=8). **D.** BMDMs from C57BL/6J mice were loaded with cholesterol using acetylated LDL (AcLDL; 50 µg/ml, 48 hr) and the cellular content of cholesteryl esters were analyzed. **E-G.** Effect of HDL (100 µg/ml) on cell surface expression of ADAM17 targets by flow cytometry in WT and ADAM17-deficient BMDMs. **E.** CD36, **F.** MerTK, and **G.** VLDL receptor. **H.** Location of CRISPR/Cas9 target sequences. Data are shown as mean ± SEM. *P*-values were determined by one-way ANOVA followed by Tukey's multiple comparison tests (D) and unpaired two-tailed Mann-Whitney test (A, C, E-G). Data are representative of at least three independent experiments in BMDMs from male mice.

Supplemental Table I: List of primers

Gene	Forward sequence	Reverse sequence
Abca1	CAGCTTCCATCCTCCTTGTC	CCACATCCACAACTGTCTGG
Abcg1	GGGATCTCTGGGAAATTCAACAGTG	GTGAGCAGAGCTTCTGGTAGCAAAC
Adam17	GGCAGAATATAACGTAGAGCCACT (Exon 4)	CTTCAGACTTATACACCAGC (Exon 5)
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Cd19	GGAGGCAATGTTGTGCTGC	ACAATCACTAGCAAGATGCCC
Cxcl1	TGGCTGGGATTCACCTCAAG	CCGTTACTTGGGGACACCTT
Hmgcr	GACTGTGGTTTGTGAAGCCG	GTTGTAGCCGCCTATCGTCC
Ifit2	CTGGGGAAACTATGCTTGGGT	ACTCTCTCGTTTTGGTTCTTGG
Ifit3	AGTGAGGTCAACCGGGAATCT	TCTAGGTGCTTTATGTAGGCCA
Il1b	GGGCTGCTTCCAAACCTTTG	TGATACTGCCTGCCTGAAGCTC
Irf7	TCCAGTTGATCCGCATAAGGT	CTTCCCTATTTTCCGTGGCTG
Ldlr	GGTATGAGGTTCCTGTCCAT	GGTATAGCCATCCTGGCTTC
Mx1	AAACCTGATCCGACTTCACTTCC	TGATCGTCTTCAAGGTTTCCTTGT
Rn18S	CATTAAATCAGTTATGGTTCCTTTGG	CCCGTCGGCATGTATTAGCT
Scrab1	TCTGGCGCTTTTTC- TATCGT	ACGGCCCATACCTCTAGCTT
Sqle	CTGGGCCTTGGAGATACAGT	TGCTTTCCGGAGACTCATGA
Srebf2	TAACCCCTTGACTTCCTTGC	CACACCATTTACCAGCCACA
Tnfa	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
C57BL/6J	The Jackson Laboratory Cat# 000664	C57BL/6J	Female/Male	https://www.jax.org/strain/000664
C57BL/6-Tg(*APOA1*)^{1Rub}/J	The Jackson Laboratory Cat# 001927	C57BL/6J	Male	https://www.jax.org/strain/001927
B6.129S7- *Ldlr*^{tm1Her}/J	The Jackson Laboratory Cat# 002207	C57BL/6J	Male	https://www.jax.org/strain/002207
B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J	The Jackson Laboratory Cat # 004781	C57BL/6J	Male	https://www.jax.org/strain/004781
Lyz2$^{Cre/Cre}$; *Adam1*$^{fl/fl}$	This study	C57BL/6J	Male	

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	C57BL/6J	The Jackson Laboratory Cat# 000664	C57BL/6J		https://www.jax.org/strain/000664
Parent - Female	C57BL/6J	The Jackson Laboratory Cat# 000664	C57BL/6J		https://www.jax.org/strain/000664
Parent - Male	B6.129S7-*Ldlr*^{tm1Her}/J	The Jackson Laboratory Cat# 002207	C57BL/6J	These mice were crossed to generate APOA1^{Tg} Ldlr$^{-/-}$ and Ldlr$^{-/-}$ littermates	https://www.jax.org/strain/002207
Parent - Female	C57BL/6-Tg(*APOA1*)^{1Rub}/J	The Jackson Laboratory Cat# 001927	C57BL/6J		https://www.jax.org/strain/001927
Parent - Male	*Adam17*$^{flox/flox}$	Elaine W. Raines, Department of Pathology, University of Washington, Seattle, WA (PMCID: PMC3622831)	C57BL/6J	These mice were crossed to generate Lyz2$^{Cre/Cre}$; *Adam17*$^{Wt/Wt}$ and Lyz2$^{Cre/Cre}$; *Adam17*$^{fl/fl}$ littermates	
Parent - Female	B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J	The Jackson Laboratory Cat # 004781	C57BL/6J		https://www.jax.org/strain/004781

DOI [to be added]

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
p-p38MAPK	Cell signaling	Cat # 9211	Not available 1:1000 from stock		
p38MAPK	Cell signaling	Cat # 9212	Not available 1:2000 from stock		
STAT1	Cell signaling	Cat # 9172	Not available 1:2000 from stock		
p-STAT1	Cell signaling	Cat # 9167	Not available 1:1000 from stock		
ADAM17	Cell signaling	Cat # 3976	Not available 1:1000 from stock		
β-actin	Sigma	Cat # A1978	Not available 1:10000 from stock		
Anti-rabbit IgG HRP-linked antibody	Cell signaling	Cat # 7074	Not available 1:3000 from stock		
Anti-mouse IgG HRP-linked antibody	GE Healthcare	Cat # NA931	Not available 1:10000 from stock		
CD16/CD32	eBioscience	Cat # 14-0161	1:4000 from stock		
PE-labeled Trem2	Invitrogen	Cat # MA5-28225	1:200 from stock		
PE-Cy7-labeled CD36 clone HM36	Biolegend	Cat # 102615	1:200 from stock		
APC-labeled MerTK clone DS5MMER	eBioscience	Cat # 17-5751-80	1:200 from stock		
FITC-labeled VLDL receptor	Abcam	Cat # ab75591	1:200 from stock		
PE-Cy7-labeled F4/80 clone BM8	eBioscience	Cat # 25-4801-82	1:2000 from stock		
V500-labeled CD11b clone M1/70	BD Horizon	Cat # 562127	1:1000 from stock		
Viability dye	eBioscience	Cat # 65-0863	1:2 from stock		
PE-labeled CD115 clone AFS98	eBioscience	Cat # 12-1152-83	1:1000 from stock		
PE/Cy7-labeled TLR4-clone SA15-21	Biolegend	Cat # 145408	1:200 from stock		

DOI [to be added]

Mouse TNFα antibody	R&D Systems	Cat # AF-410-SP	10 μg/ml		
Normal goat IgG control antibody	R&D Systems	Cat # AB-108-C	10 μg/ml		

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
Primers for Quantitative PCR	see Supplemental Table I	This study	
pSpCas9(BB)-2A-Puro (PX459) V2.0 plasmid	Addgene	Cat # 62988	
Single guide RNA (sgRNA) oligonucleotides for Trem2	(sgRNA1; CTGCAGCACCGTGGTGTTGA and sgRNA2;AAGGCCTGGTGTCGGCAGCT)	This study	

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Bone marrow-derived macrophages	Derived from mouse strains described above	Female/Male
Peritoneal macrophages	Derived from mouse strains described above	Male
Bone marrow-derived monocytes	Derived from C57BL/6J mice described above	Male
Mouse heart endothelial cells	Derived from *Ldlr*$^{-/-}$ mice described above	Male
L cells (mouse fibroblasts derived from subcutaneous connective tissue; areolar and adipose)	ATCC® CRL2648™	Male

Software and algorithms

Name	Vendor or Source	link
GraphPad Prism v8.4.3.	GraphPad Software	https://www.graphpad.com/scientificsoftware/ prism/

Other

Name	Vendor or Source	Catalog #
Lipopolysaccharide, ultrapure	List Biological Laboratories	Cat# NC9633766
Recombinant mouse IFNβ	R&D systems	Cat# 8234-MB
Thioglycollate	BD	Cat# BD 292788
Selonsertib	Selleckchem	Cat# S8292
Methyl-β-cyclodextrin	Sigma	Cat# C4555
Human acetylated LDL	Kalen Biomedical	Cat# 770201
Cyclic AMP	Sigma-Aldrich	Cat# A6885
Acyl-coenzyme A:cholesterol acyltransferase inhibitor (Sandoz 58-035)	Sigma-Aldrich	Cat# S9318
BODIPY 493/503	Invitrogen	Cat# D3922
Quick-RNA Miniprep	Zymo Research	Cat# R1055
BCA protein assay kit	Pierce	Cat# 23225
Supersignal West Pico Chemiluminescent substrate	Pierce	Cat# 34578
Cholesterol E (total cholesterol assay)	Wako Diagnostics	Cat# 999-02601
Triglyceride Reagent	Sigma-Aldrich	Cat # T2449
Hemavet MULTI-TROL Controls Mouse	Drew Scientific Inc	Cat # 600065
Macrophage Isolation Kit (Peritoneum), mouse	Miltenyi Biotec	Cat # 130-110-434

DOI [to be added]

Name	Vendor or Source	Catalog #
Recombinant mouse TNFα (aa 80-235)	R&D Systems	Cat # 410-MT
p38 MAPK inhibitor (SB202190)	Santa Cruz Biotechnology, Inc	Cat # sc-202334
ADAM17 inhibitor (TAPI-1)	Tocris	Cat # 6162
RPMI 1640	Corning	Cat # 10-040-CV
Protease Inhibitor Cocktail	Sigma-Aldrich	Cat # P8340
GM6001	Sigma-Aldrich	Cat # M5939
Fetal bovine serum	ThermoFisher Scientific	Cat # 26140079
Fast SYBR Green Master Mix	ThermoFisher Scientific	Cat # K0223
TNFα mouse ELISA Kit	ThermoFisher Scientific	Cat # 88-7324-86
CCL2 mouse ELISA Kit	ThermoFisher Scientific	Cat # 88-7391-88
Mouse CXCL1/KC DuoSet ELISA	R&D Systems	Cat # DY453-05
Peptide-N-glycosidase F	New England BioLabs	Cat # P0704S
Lipofectamine 3000 Reagent	ThermoFisher Scientific	Cat # L3000001
Maxima First Strand cDNA synthesis kit for RT-qPCR	ThermoFisher Scientific	Cat# K1642
Filipin complex from *Streptomyces filipinensis*	Sigma	Cat # F9765
Human APOA1	Academy Bio-medical Co.	Cat # 11P-UP205

DOI [to be added]

Sodium acetate, [^{3}H]	ViTrax	Cat # VT211
Sodium acetate trihydrate	VWR International J.T. Baker	Cat # 3462-05
Insta-Fluor Plus Scintillation Cocktail	PerkinElmer Inc	Cat # 6013167
Chloroform ACS reagent grade	ThermoFisher Scientific	Cat # RSOC00204C
Methanol	Sigma	Cat # 179337
Glycine	VWR International J.T. Baker	Cat # JT0582-1

DOI [to be added]