SUPPLEMENTAL MATERIAL

Supplemental Methods

Materials: siHif1α (sc-35562), siHif1β (sc-29734), siUSF1 (sc-36784), siUSF2 (sc-36785) as well as lentiviruses expressing shRNA against Clock (sc-35075-v) were from Santa Cruz Biotechnology. Antibodies against NPC1L1 (Novus, NB400-128), ABCA1 (Abcom, NB400-105), ABCG1 (Abcom, ab52617), HIF1α (sc-35562PR), HIF1β (sc-8077), USF1 (SC-36784PR), USF2 (SC-36785PR), and other antibodies (1) were purchased. [^{14}C]Cholesterol, [^{3}H]cholesterol, [^{3}H]oleic acid and [^{3}H]triolein were from NEN LifeScience Products. Alexa Flour 488 labeled AcLDL (L-23380) was from Invitrogen.

Animals and diets: All single knockout mice on C57Bl6J background were purchased from the Jackson Laboratory. Double knockout mice were generated at SUNY Downstate. *Clk*$^{\Delta 19/\Delta 19}$ mice are infertile. Therefore, *Clk*$^{\Delta 19/wt}$, *Clk*$^{\Delta 19/wt}$*Ldlr*$^{-/-}$, and *Clk*$^{\Delta 19/wt}$*Apoe*$^{-/-}$ mice were bred to obtained *Clk*$^{\Delta 19/\Delta 19}$, *Clk*$^{wt/wt}$, *Clk*$^{\Delta 19/\Delta 19}$*Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$, *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ mice. Male, 2-3 months old *Clk*$^{\Delta 19/\Delta 19}$ and *Clk*$^{wt/wt}$ siblings were placed on Paigen's atherogenic diet (TD88051; Harlan Teklad; Madison, WI) containing 1.25% cholesterol and 0.5% cholic acid for 8 weeks. Male (2-3 months) *Clk*$^{\Delta 19/\Delta 19}$*Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice were fed a western diet (0.15% cholesterol, 20% saturated fat; Research Diets) for 2 months for atherosclerosis studies. *Apoe*$^{-/-}$ and *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice were maintained on a regular chow diet from weaning until sacrifice or fed a western diet as indicated. Mice were housed in an air-conditioned room at 22°C±0.5°C with a 12-hour lighting schedule (700–1900 hours). Animal experiments were approved by the Animal Care and Use Committee of the SUNY Downstate Medical Center.

Macrophages: Bone marrow-derived macrophages were obtained from *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice (2;3). For gene expression studies, cells were placed in 10% DMEM plus 25% L-cell-conditioned medium for 1 week (2). Cells were then treated with or without oxLDL (200 μg/ml) as indicated for 8 hours. Total RNA was extracted and analyzed by real-time PCR (4). For cholesterol efflux assays, bone marrow derived macrophages were labeled with [^{3}H]cholesterol (5.0 μCi/ml) for 24 h, washed with PBS, incubated in DMEM containing 0.2% BSA for 1 h and then in the same media in the absence or presence of apoAI (15 μg/ml) or HDL (50 μg/ml) for 8 hours. Radioactivity in the medium and total cell-associated radioactivity was determined by scintillation counting. The assays were performed in quadruplicate and are presented as percent efflux (2).

The human monocytic cell line THP-1 (TIB-202) was maintained in RPMI 1640 media supplemented with 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 µg/ml) and 10% fetal bovine serum. To induce differentiation, cells (5×10^5 to 10^6 per ml) were seeded in serum-free medium with 200 nM phorbol myristic acid (cat#16561-29-8, Sigma) for 24 h. After incubation, nonattached cells were removed by aspiration, and the adherent cells were washed three times with RPMI 1640 and were transfected with indicated siRNAs as suggested by the manufacturer (Santa Cruz). Cells were then cultured for 48 h in RPMI containing 5% heat-inactivated fetal bovine serum. Human THP-1 macrophages were treated with siControl or siClock for 48 h and used for different experiments.

Quantification of plasma lipids, lipoproteins and apolipoproteins as well as tissue lipids: After a 4-h fast, blood was obtained from mice anesthetized with methoxyflurane (Pitman–Moore, Mundelein, IL), placed into pre-cooled tubes containing EDTA (final concentration 4 mM), and centrifuged (2500 × *g* for 20 min at 4°C) to obtain plasma. Total cholesterol (TC), triglycerides (TG), phospholipids (PL), and free cholesterol (FC) were assayed using commercial kits (5). Plasma high density lipoproteins (HDL) were isolated after the precipitation of apoB-lipoproteins with phosphotungstate/$MgCl_2$ reagent (HDL-cholesterol; Sigma). Total and HDL triglycerides and cholesterol were measured using commercial kits. Non-HDL apoB-lipoprotein triglyceride and cholesterol were determined by subtracting HDL lipid values from totals. Plasma levels of murine apoA-I, apoB and apoE were quantified by western blotting using polyclonal antibodies as previously reported (4).

In vivo absorption of lipids: Mice were injected intraperitoneally with 0.5 ml of Poloxamer P407 in PBS (1:6, v/v) and then gavaged with 50 µl of olive oil containing [^{3}H]cholesterol with cold cholesterol at 12:00. Blood was collected from the tail, and plasma was used for liquid scintillation counting (5).

Uptake and secretion of lipids by enterocytes: Enterocytes were isolated using EDTA (5-8) as described earlier. To study uptake, isolated enterocytes from *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice were incubated in triplicate with 1 µCi/ml of [^{3}H]cholesterol. At different times, enterocytes were centrifuged, washed and counted. To measure secretion, enterocytes were incubated in triplicate with 1 µCi/ml of [^{3}H]cholesterol for 1 h, centrifuged, washed and then incubated in fresh media for different time. Media were collected and counted as previously reported (1).

To study the distribution of cholesterol in chylomicrons and HDL, conditioned media was adjusted to a density of 1.10 g/ml by the addition of KBr and overlaid with different density solution as described earlier (9). After ultracentrifugation large chylomicrons (fraction 1), small chylomicrons (fraction 2), VLDL (fraction 3) and other lipoproteins were collected from the top of the gradient (9) and radioactivity was measured.

Evaluation of atherosclerosis: The proximal aorta was collected after saline perfusion through the left ventricle. Aortas were placed in 4% phosphate buffered formaldehyde at 4°C for 24 h. The aortic root and ascending aorta were sectioned at a thickness of 10 μm, and alternate sections were saved on slides and stained with Oil-Red-O for neutral lipids and hematoxylin. Ten cross sections/animal from the aortic root were evaluated for lesion areas as previously reported (10;11). Moreover, ten sequential sections (10 μm thick) of the brachiocephalic artery per animal were stained and mean areas of lipid staining were determined. Sections were also stained with trichrome (collagen), hematoxylin/eosin (cytoplasm and nucleus), and Chromaview kit (elastin). Necrotic areas with no collagen and eosin staining were measured. The percent collagen and necrotic area per total plaque area (n=20 plaques) were determined. To evaluate cellularity of the lesion, sections were immunostained for macrophages (AIA31240, Accurate Chemical and Scientific Corp) and smooth muscle cells (anti-SMC actin 1A4, Zymed). Primary antibodies were incubated at room temperature in 3% serum matched to the species of the secondary antibodies. Biotinylated secondary antibodies were incubated for 30 minutes followed by 45 minute horseradish peroxidase conjugated streptavidin and visualized with diaminobenzidine. Nuclei were counterstained with hemalaune. The mean area of staining per section per animal for 8 sections was determined for each animal. Positive macroscopic lesions, and the mean areas of lipid staining were quantified using Image Pro Plus software (10-12). For *en face* analysis, whole aortas were isolated, and the fatty streaks in the lumen were stained with Oil Red O, and the percentage staining was quantified for each animal (10-12).

***In vivo* macrophage cholesterol efflux measurement** (2;13;14)**:** J774A.1 cells were grown in suspension in DMEM medium supplemented with 10% fetal bovine serum. Cells were radiolabeled with 5 μCi/mL ^{3}H-cholesterol and 50 μg/mL acetylated LDL for 48 hours. These labeled foam cells were washed twice, equilibrated in medium with 0.2% bovine serum albumin (BSA) for 8 hours, centrifuged, and resuspended in RPMI medium immediately before use. The

labeled foam cells were injected intraperitoneally into *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice and were caged individually with unlimited access to food and water. Blood was collected at 6, 12, 24, and 48 hours, and plasma samples were used for liquid scintillation counting. Feces were collected over 48 hours and stored at 4°C before extraction and counting. At 48 hours after injection, mice were exsanguinated and perfused with cold phosphate-buffered saline (PBS), and portions of the liver were removed and flash-frozen for lipid extraction and quantification of tracer counts.

Bone marrow-derived macrophages derived from *Clock*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice were loaded with cholesterol by incubation with acetylated LDL (50 μg protein/ml) and 5 μCi of [^{3}H]cholesterol for 24 hr. The labeled macrophages were injected intraperitoneally into WT mice. Plasma was collected at 6, 12, 24 and 48 hours and feces were collected at 48 hours to measure tracer counts. *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice were injected intraperitoneally with ^{3}H-cholesterol labeled J774 foam cells.

***Ex vivo* cholesterol efflux from macrophages:** Bone marrow-derived macrophages were cultured for 7 days, then labeled with [^{3}H]cholesterol carried by acetylated LDL as described above for J774A.1 cells. After labeling, cells were washed 3 times with PBS, then equilibrated with DMEM containing 0.2% BSA for 1 hour, and incubated with purified human HDL or apoA-I in 0.5 ml of DMEM containing 0.2% BSA. The medium was collected at 8 hours and centrifuged at 6000 x g for 10 min to remove cell debris. Radioactivity in the medium and total cell-associated radioactivity was determined by scintillation counting. Cholesterol efflux was expressed as the percentage of the radioactivity released from the cells into the medium relative to the total radioactivity in cells and medium as previously reported (3).

Alexa Fluor 488–AcLDL uptake: For acetylated low-density lipoprotein (AcLDL) uptake, bone marrow-derived macrophages from wildtype C57Bl6J mice were transfected with siClock or siControl for 48 hours and then incubated with Alexa Fluor 488–AcLDL (L-23380) (5 μg/mL) in serum-free DMEM media at 37°C for 3 hours. Bone marrow-derived macrophages derived from *Clock*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice were placed in 10% DMEM plus 25% L-cell-conditioned medium for 1 week, then cells were incubated with Alexa Fluor 488–AcLDL (5 μg/mL) in FBS free DMEM at 37°C for 3 hours. Cells were photographed using a BX-50-FLA fluorescence microscope (Olympus) at a magnification of ×40.

Measurement of lipid peroxides: Tissue samples were homogenized in 50 mM Tris-HCl buffer, pH 7.4, containing 1.15% KCl and centrifuged. The supernatants were used to measure lipid peroxides using TBARS assay kit (Cat#10009055, Cayman Chemical Company).

Determination of MTP Activity: After extensive washes with ice-cold PBS, 1-cm segments of proximal small intestine were homogenized with 1 ml of ice-cold 1 mM Tris-HCl (pH 7.6), 1 mM EGTA, and 1 mM $MgCl_2$ in a glass homogenizer. The homogenates were centrifuged (SW55 Ti rotor, 50,000 rpm, 10 °C, 1 h), and supernatants were used for an MTP assay (15) using a kit (Chylos, Inc.).

Immunoblot analysis: Proteins were separated under non-reducing conditions, transferred to nitrocellulose membranes, blocked for 2 h in 20 mM Tris, 137 mM NaCl, pH 7.5, containing 0.1% Tween 20 and 5% nonfat dry milk at room temperature. The blots were washed three times and incubated overnight at 4 °C in the same buffer containing 0.5% dry milk and a primary antibody (1:100-1:1000 dilution), washed, and then incubated with mouse horseradish peroxidase-conjugated secondary antibody (1:1000-1:4000) in 1.0% skim milk for 1 h at room temperature. Immune reactivity was detected by chemiluminescence as previously described (4).

Real-time PCR analysis: Total RNA was isolated using TRIzol™ (Invitrogen). Isolated total RNA from different tissues were reverse transcribed as previously described (1), and the resultant reaction mixtures were used for real-time PCR. 18S rRNA was used as the reference gene. The mRNA expression level of GAPDH was also measured as an internal control with GAPDH control Reagent (Applied Biosystems).

Luciferase reporter assays: Plasmids expressing luciferase under different ABCA1 promoter sequences (16) were used. For co-transfection studies FLAG-Clock plasmids (17;18), or siRNA, shRNA plasmids were utilized. Macrophages from wildtype C57Bl6J mice were seeded onto 48-well plates, transfected with 1.5 μg of a plasmid expressing firefly luciferase under the control of the ABCA1 promoter (16). Firefly and Renilla luciferase activities were determined using the Dual-Luciferase Assay System (Promega) was previously reported (5).

Chromatin immunoprecipitation (ChIP) assay: ChIP was used to study the binding of different transcription factors to the *ABCA1* promoter using goat polyclonal antibodies against HIF1α, HIF1β, USF1, and USF2 using kits as reported (5). DNA samples (n=3-4) recovered after

immunoprecipitation were subjected to PCR to detect coimmunoprecipitated DNA using the *ABCA1* promoter–specific primers for E-boxes (16) that flank the consensus HIF1α or Hif1β or USF1, USF2 binding sites in the human ABCA1 promoter. As negative controls, ChIP was performed in the absence of antibody or in the presence of rabbit IgG.

Bone marrow transplantation: Bone marrow cells were harvested from the tibias of donor mice (*Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ mice) as previously described (2). A total of 20 *Apoe*$^{-/-}$ mice (age 8 weeks) were lethally irradiated with 1000 rads (10Gy). Ten animals each were transplanted with bone marrow cells (5×10^6) derived from *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ or *Apoe*$^{-/-}$ mice via tail vein within 2 hours of irradiation. We monitored the process of cell replacement by polymerase chain reaction (PCR), using genomic DNA from mouse white blood cells as a template.

Supplemental Tables

Table S1: Chemical composition (% weight) of different diets used in the study:

% by weight	**Chow**	**Western**	**Atherogenic**
Protein	23.9%	17.3%	20.5%
Carbohydrates	48.7%	48.5%	42.4%
Fat	5.0%	21.2%	37.1%
Cholesterol	0.02%	0.20%	1.25%
Cholic acid	-	-	0.5%
Source	LabDiet	Harland Tekland	Harland Tekland
Catalog #	5001	TD88137	TD88051

Table S2: Oligonucleotide sequences of primers used

RT-PCR primers	Forward (5′-3′)	Reverse (5′-3′)
mHIFα	CTGCGTGCATGTCTAATCTGTTC	GAGCGCGGAAAACTCTTGTT
mHif1β	CCCACCATTGCTTCTGGAAA	CGTCGCTTAATAGCCCTCTGTAC
mUSF1	GGCTTACTCAAGAGGTGGGAA	TGATTGGGCCCCCTTCTACT
mUSF2	ATGGAACCAGAACTCCTCGAGAT	CCTTCTCCGTTCGACTTCATTG
mCD36	TACCTGGGAGTTGGCGAGAA	GTTCCGATCACAGCCCATTC
mSR-A1	CAGTTCGACTGGTTGGTGGTAGT	ACTGGCCTTGGTGGAAGATCT
mABCA1	CCCAGAGCAAAAAGCGACTC	GGTCATCATCACTTTGGTCCTTG
mABCG1	ACAACTTCACAGAGGCCCAG	TTTCCCAGAGATCCCTTTCA
mSR-B1	ACGGCCAGAAGCCAGTAGTC	GACCTTTTGTCTGAACTCCCTGTAG
mNPC1L1	ATCCTCATCCTGGGCTTTGC	GCAAGGTGATCAGGAGGTTGA
mABCG5	GCAGGGACCAGTTCCAAGACT	ACGTCTCGCGCACAGTGA
mABCG8	AAAGTGAGGAGTGGACAGATGCT	TGCCTGTGATCACGTCGAGTAG
mABCG4	CATGAGGGAGCACTTGAACTACTG	CACCTGGAAGGGCACATCA
mLXRα	GCGTCCATTCAGAGCAAGTGT	TCACTCGTGGACATCCCAGAT
mLXRβ	GTCCCAGTTATGGCAGAGGA	GCCTTTTCCCCAAGATTCTC
mPPARγ	ATGCCAAAAATATCCCTGGTT	GGAGGCCAGCATGGTGTAGA
mPPARα	TCCGAGGGCTCTGTCATCA	TGACTGAGGAAGGGCTGGAA

mGAPDH	TGT GTC CGT CGT GGA TCT GA	CCT GCT TCA CCA CCT TCT TGA
mACAT1	TGGGTGCCACTTCGATGACT	TGAGTGCACACCCACCATTG
mMTP	CACACAACTGGCCTCTCATTAAAT	TGCCCCCATCAAGAAACACT
mACAT2	GGTGGAACTATGTGGCCAAGA	CCAGGATGAAGCAGGCATAGA
mSHP	TGGCCTCTACCCTCAAGAACA	TGATAGGGCGGAAGAAGAGATC
mPGC1α	TAGGCCCAGGTACGACAGC	GCTCTTTGCGGTATTCATCC
mPGC1β	CAAGCTCTGACGCTCTGAAGG	TTGGGGAGCAGGCTTTCAC
mRev-erbα	GGGCACAAGCAACATTACCA	CACGTCCCCACACACCTTAC
mRev-erbβ	TGGGACTTTTGAGGTTTTAATGG	GTGACAGTCCGTTCCTTTGC
mFXR	GATTTGGAATCGTACTCCCCATAC	GAAGCCCAGGTTGGAATAGTAAGA
mFoxO1	ACGAACTCGGAGGCTCCTTAG	GACTGGAGGTGGTCGAGTTGGACTG
mHNF4α	CAGGAGGAGCGTGAGGAAGA	CCACACATTGTCGGCTAAACC
mHNF1α	CCATTCTGAAAGAGCTGGAGAAC	GACTCCACCACGGCTTTCTG
mHNF1β	AAGGGCACCCCCATGAAG	CTCCCGTTGCTTTCTGACGTA
mCBP	GGATTCTGGAGAGATGTCGGAAT	CTCCATCGATCTCCTCAAAAGC
mP300	CAAAGGCTGCAAACGGAAA	AATGGCACCGGGCACTT
mNcoA-1	GGACTGGCTGGGAAGACCTAGTA	GCCACGAGTCATCACTTCTTGA
mTIF2	GCGTGCTAAGGAACTCTGGAA	AGTCTCGAGCAGACACAGGAACT
mRXR	TGGGCTTCGGGACTGGTA	TGCTGCTCACAGGGTTCATG
mSREBP2	GGTCCTCCATCAACGACAAAA	ACGCCAGACTTGTGCATCTTG
mGR	CCCCAGGTAAAGAGACAAACGA	TCTCCCGCCAAAGGAGAAA
mNF-κB	CTCTAGGTGCAATTCCGATGAGA	GGTGGTACATGACAGTGGATGCT
mZFN202	GAACTTCCGACGCTTTCGAT	GACAAAGCTCTCGAAGTCTGATGA
mRIP-140	GAACCTGGGCTTTTGAATGGA	CCATATCTGCGATGACCCACTT
mSP-3	GAAAAAGACTTCGGAGGGTAGCT	TGGTACCTCTCCCACCACCTT
mTRβ	TGGCAAATGCATGCGTAGA	CGTGTACCTCTGGCATACAGTAGTTC
mβ-actin	CTGGAGAAGAGCTATGAGCTGCCT	CTCCTGCTTGCTGATCCACATCTG
mTRF-2	GCGTGCTAAGGAACTCTGGAA	AGTCTCGAGCAGACACAGGAACT
mLDLR	AGGCTGTGGGCTCCATAGG	TGCGGTCCAGGGTCATCT
mSREBP1c	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
mLRH-1	CCTCCTGAGTCTCGCACAGG	AACTCCCGCTGATCGAACTG
mBMAL1	CAACCTTCCCGCAGCTAACA	TCCGCGATCATTCGACCTAT

mCLCOK	CACCGACAAAGATCCCTACTGAT	TGAGACATCGCTGGCTGTGT
mIL-2	CTCCTTGTCAACAGCGCACCCA	TCAATTCTGTGGCCTGCTTGGGC
mIL-12A	GCTGCTGAAATCTTCTCACCGTGCA	TGACACGCTGGACCGGCAC
mIL-12B	CACCTGCCCAACTGCCGAGG	TAGCTCCCTGGCTCTGCGGG
mIL-17A	GGAAGCTCAGTGCCGCCACC	CGGACAATGGAGGCCACGCA
mG-CSF	CAGCGCTCTGCCACCATCCC	TGCAGGGCCTGGCTAGAGCA
mGS-CSF	TCTCAGCACCCACCCGCTCA	GCCCCGTAGACCCTGCTCGA
hCLOCK	AAAAAACCCCATAGTGCCTTGA	CTGACACCCACATGCTGTTAAAA
hABCA1	TGGTCTCCAAGCAGAGTGTG	GAGCAGCAGCTCCCAAATAC
hABCG1	ACAACTTCACAGAGGCCCAG	TTTCCCAGAGATCCCTTTCA
hSBR1	CTGTGGGTGAGATCATGTGG	GCCAGAAGTCAACCTTGCTC
hHlF1α	CCACAGCTGACCAGTTATGATTG	GAGTAATTCTTCACCCTGGAGTAGGT
hHIF1β	CTGTCAGATATGGTACCCACCTGTA	GCCATGCGTAAGATGGTTAGCT
hUSF1	AACCACCGCTTGTCTGAAGAAC	GTTGTCGAAGCACGTCATTGTC
hUSF2	AGGCCAGTTCTACGTCATGATGA	GATCGTCCTCTGTGTTCCTGTCT
hβ-actin	CACACTGTGCCCATCTACGA	TTCTCTACGGAGGAGCTGGA
Chip assay primers	Forward (5′-3′)	Reverse (5′-3′)
mABCA1	GGACCCTAAGACACCTGCTG	TCCCGGCCTCTGTTTATGTA
Genotyping primers		
$mClock^{\Delta 19/\Delta 19}$	AGCACCTTCCTTTGCAGTTCG	TGTGCTCAGACAGAATAAGTA
$mClock^{wt/wt}$	GGT CAA GGG CTA CAG GTA	TGG GGT AAA AAG ACC TCT TGC C
$mApoe^{-/-}$	ACTACTACACAGGATGCCTAGCC	AGGGTGAAAGAGCTGGACACTC
$mApoe^{wt/wt}$	TGT GAC TTG GGA GCT CTG CAG C	GCC TAG CCG AGG GAG AGC CG
$mLdlr^{-/-}$	AAT CCA TCT TGT TCA ATG GCC GAT C	CCA TAT GCA TCC CCA GTC TT
$mLdlr^{wt/wt}$	GCG ATG GAT ACA CTC ACT GC	CCA TAT GCA TCC CCA GTC TT

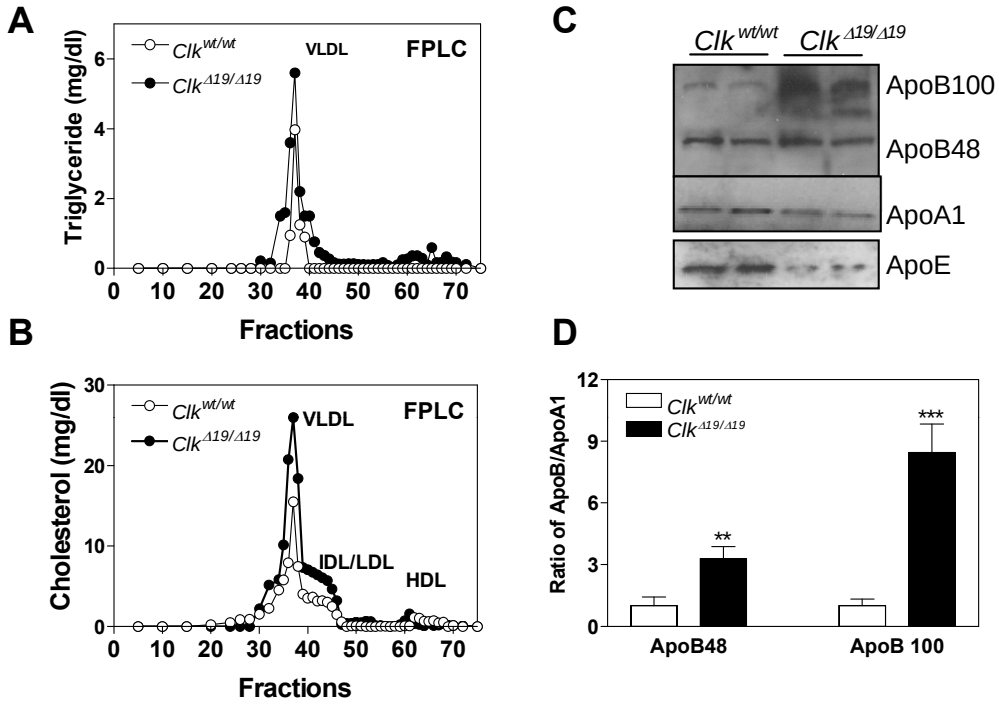

Fig S1: Changes in Plasma and hepatic lipids in *Clk*$^{\Delta 19/\Delta 19}$ mice fed an atherogentic diet.

10 weeks old male *Clk*$^{\Delta 19/\Delta 19}$ mice and wild type littemates were fed an atherogentic diet for 2 months. Plasma and liver were collected at 12:00. Plasma was subjected to FPLC and total triglyceride (A) and cholesterol (B) were measured in different fractions. Plasma was fractionated on SDS-PAGE and subjected to western blotting using anti-ApoB, anti-ApoA1 or anti-Apoe antibodies (C). Bands correspondig to ApoB100 and ApoB48, and ApoA1 were quantified and ApoB/ApoA1 ratios were plotted (D). Each group represents mean±SD, n=6-9. ** $P < 0.01$; *** $P < 0.001$, compared to *Clk*$^{wt/wt}$ mice.

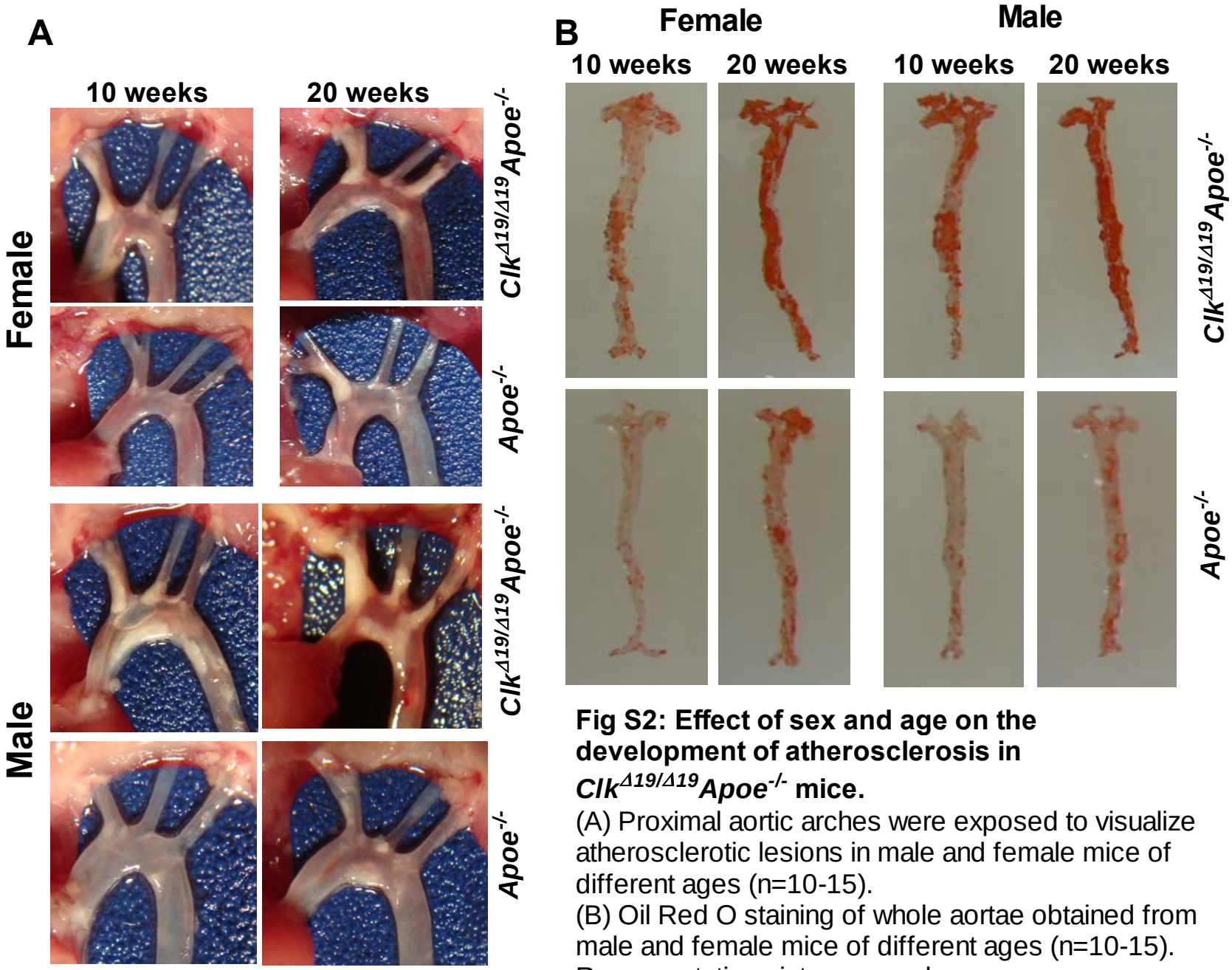

Fig S2: Effect of sex and age on the development of atherosclerosis in *Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$* mice.

(A) Proximal aortic arches were exposed to visualize atherosclerotic lesions in male and female mice of different ages (n=10-15).

(B) Oil Red O staining of whole aortae obtained from male and female mice of different ages (n=10-15). Representative pictures are shown.

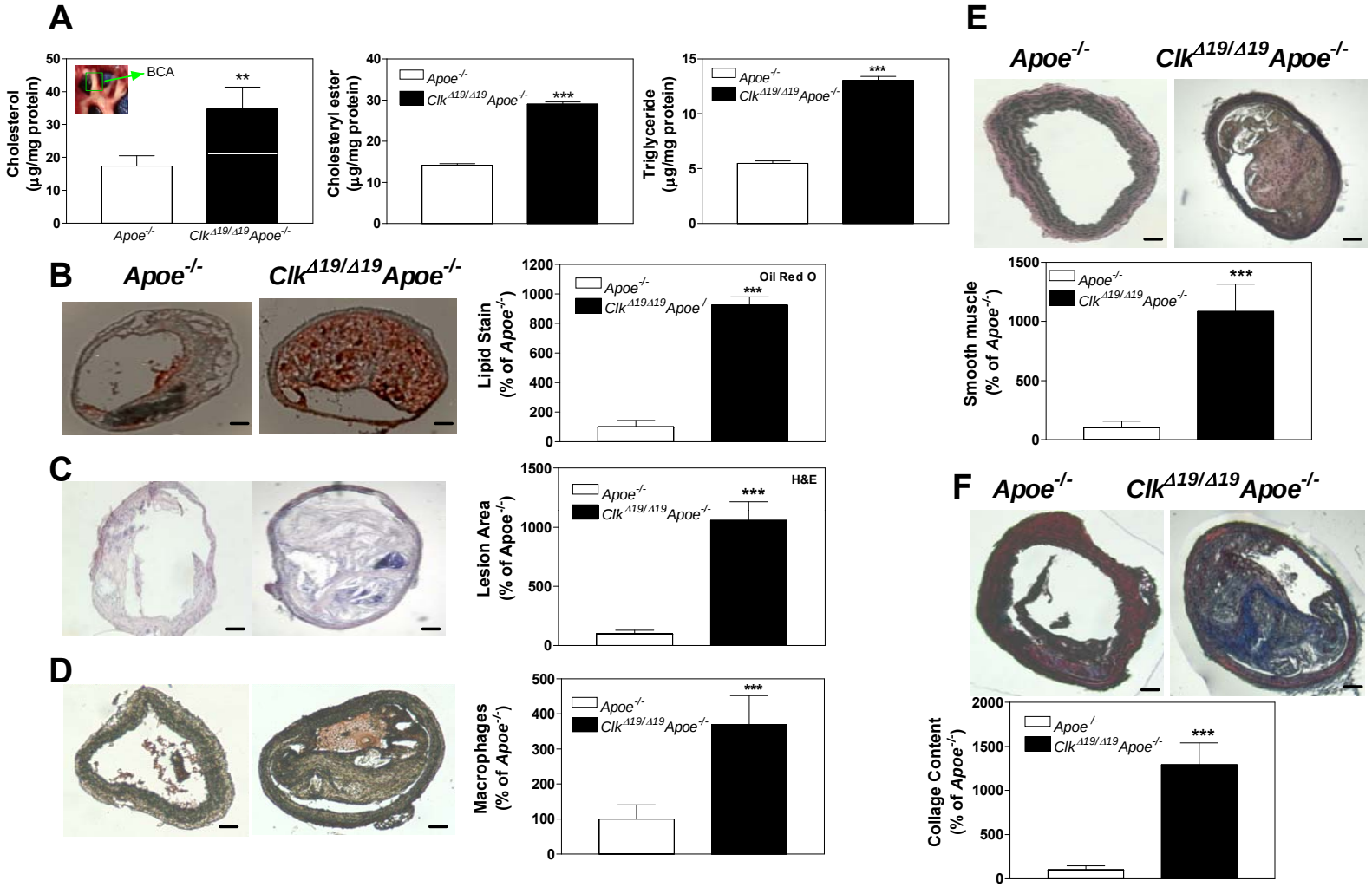

Fig S3: Atherosclerotic lesions in the brachiocephalic artery (BCA) of *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice. (A) BCA was collected as marked in the inset of Panel A, lipids were extracted, and cholesterol, cholesterol esters and triglycerides were measured. (B) Serial sections of BCA were stained with Oil Red O and lesion areas were quantified. (C) Hematoxylin and eosin stained lesions and their quantifications. (D) Sections were stained to determine macrophages and quantified. (E) Sections were stained to quantify smooth muscle cells. (F) Collagen content was quantified after Trichrome staining. Each group represents mean±SD, n=10-15. ** P < 0.01; *** P < 0.001,Scale bars: 40 µM .

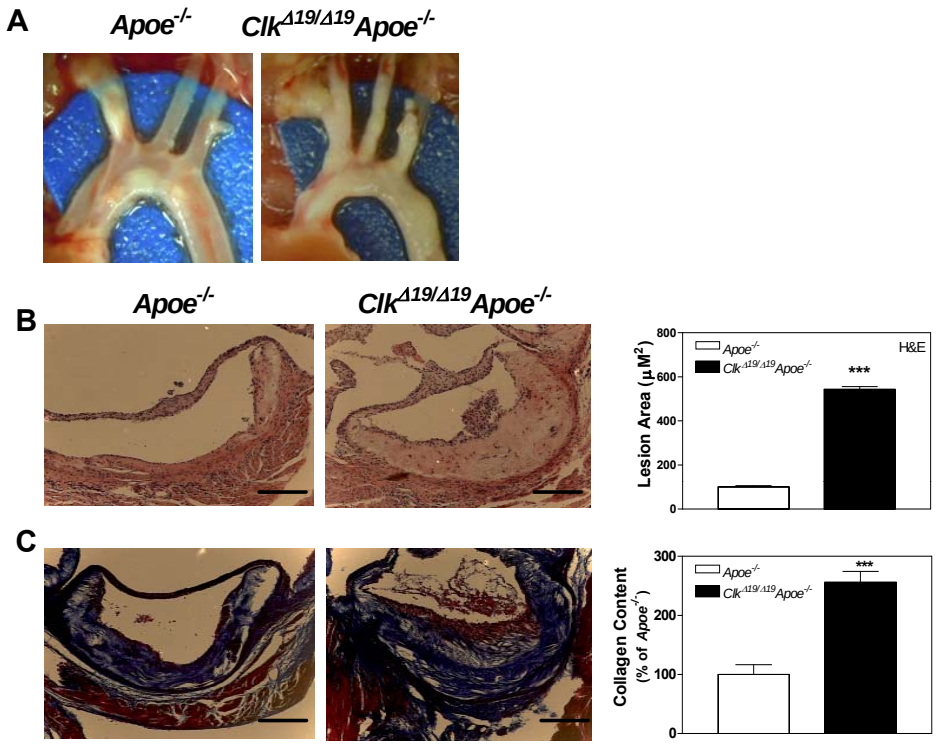

Fig S4: Effect of a western diet on atherosclerosis in *Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$* mice.
Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$ mice and *Apoe$^{-/-}$* mice (male, 8-12 weeks old, n=12) were fed a western diet for one month and used for analysis. (A) Proximal aortic arches were exposed to visualize atherosclerotic lesions in male (n=10-15). (B) Aortic sections were stained with hematoxylin and eosin and lesion areas were quantified. (C) Sections were stained with Trichrome to measure collagen content.. Each group represents mean±SD, n=12. *** $P < 0.001$, scale bars: 100 μM.

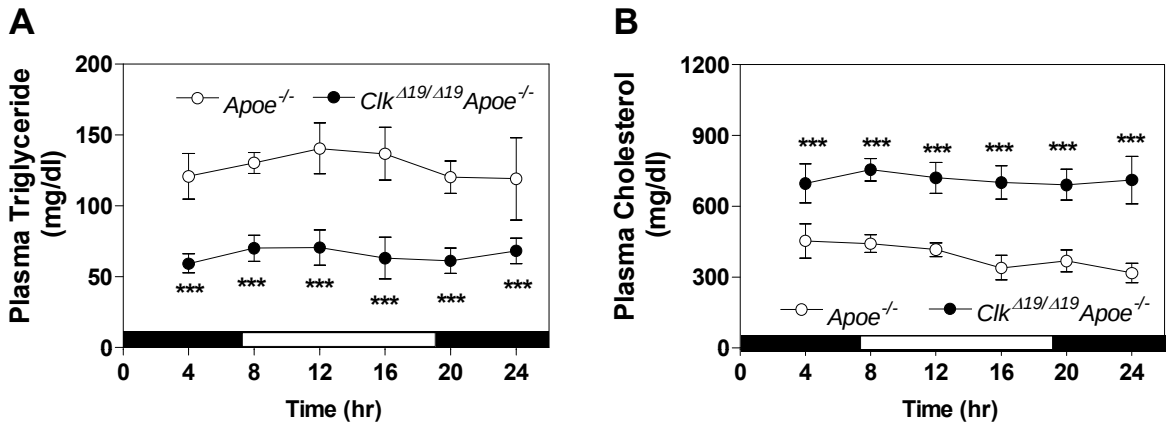

Fig S5: Diurnal rhythm of total plasma triglyceride and cholesterol in *Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$* mice.

Plasma were collected from *Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$* mice and *Apoe$^{-/-}$* mice (male,10 weeks old) at different time of a day, mice were kept in a 12 h light/dark cycle with free access to chow and water. Total Plasma triglycerides (A) and cholesterol (B) were measured. Each group represents mean±SD, n=9, ***P <0.001.

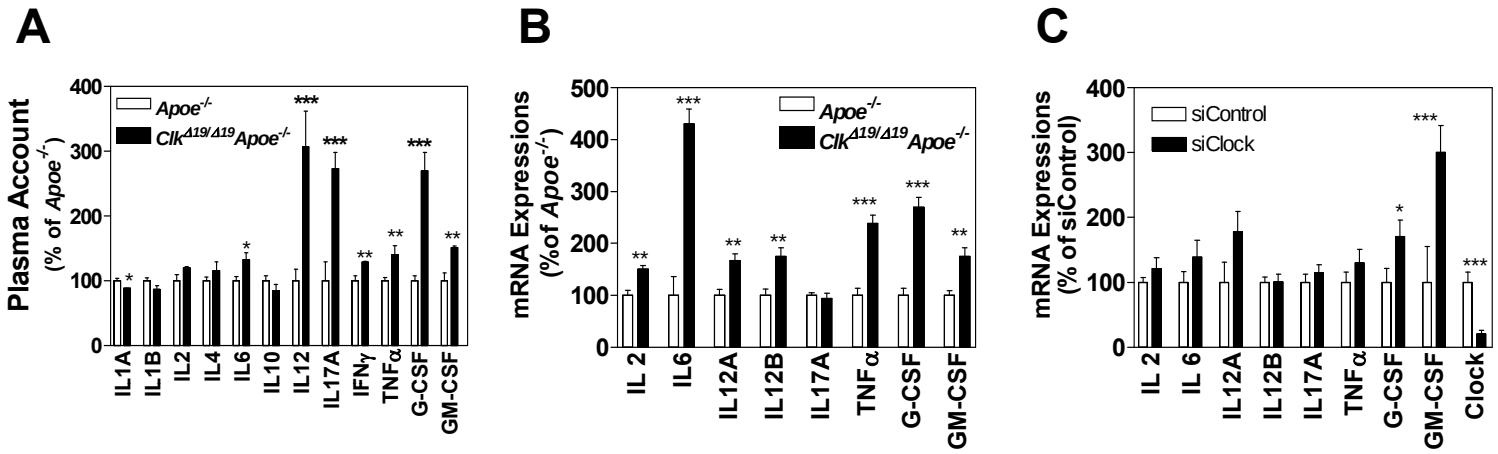

Fig S6: Increased inflammatory response in Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$ mice.

(A) Plasma cytokines were measured using inflammatory kit (mouse inflammatory Cytokines #MEM-004A, SABioscience). (B) mRNA expression of inflammatory marker genes in bone marrow macrophages of male mice (8 months old) was measured by RT-PCR. (C) Isolated bone marrow macrophages from wild type C57Bl/6J mice were treated with siControl or siClock. After 48 h, RNA was isolated to quantify mRNA levels of Clock, and inflammatory maker genes. Each group represents mean±SD, n=6-9.* $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$.

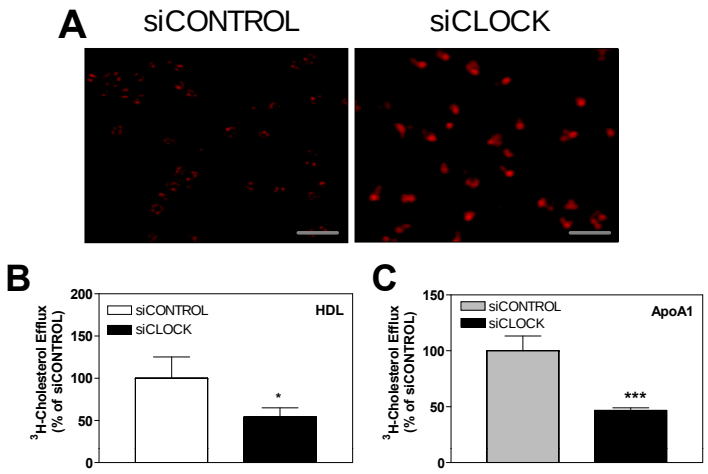

Fig S7: Clock knockdown increases uptake of modified lipoproteins and reduces cholesterol efflux in human THP-1 macrophages :

(A) After 48 h, they were incubated with DiI-labeled AcLDL (5 μg/ml) in the presence or absence of cold AcLDL (200 μg/ml) for 6 h and then visualized under a fluorescent microscope, Scale bars: 50 μM.

(B-C) For efflux studies, cells were labeled with cholesterol by incubating cells with [^{3}H]cholesterol and acetylated LDL. After labeling, cells were washed 3 times with PBS, then equilibrated with DMEM, 0.2% BSA for 1 hour, and incubated with 10 μg/ml purified human HDL (B) or apoA-I (C) in 0.5 ml of DMEM containing 0.2% BSA. The medium was collected at 8 hours and centrifuged (6000 x g, 10 minutes) to remove cell debris. Radioactivity in the medium and total cell-associated radioactivity was determined by scintillation counting. Mean± SD, n=6. * $P < 0.05$, *** $P < 0.001$.

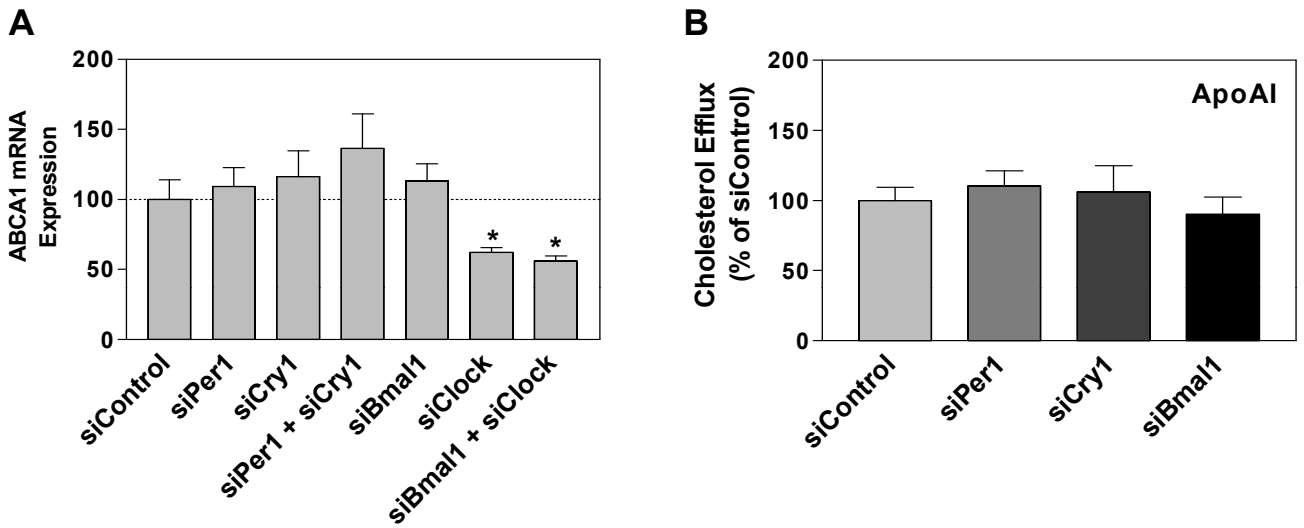

Fig S8: Effect of knockdown of different circadian clock genes on ABCA1 expression and cholesterol efflux.

(A) Wild type bone marrow macrophages were transfected with different indicated siRNAs. After 48 h, ABCA1 mRNA levels were quantified in these cells.

(B) Wildtype bone marrow macrophages were transfected with siRNAs against few clock genes and then used to measure cholesterol efflux to ApoAI.

Each group represents mean±SD, n=6, * $P < 0.05$.

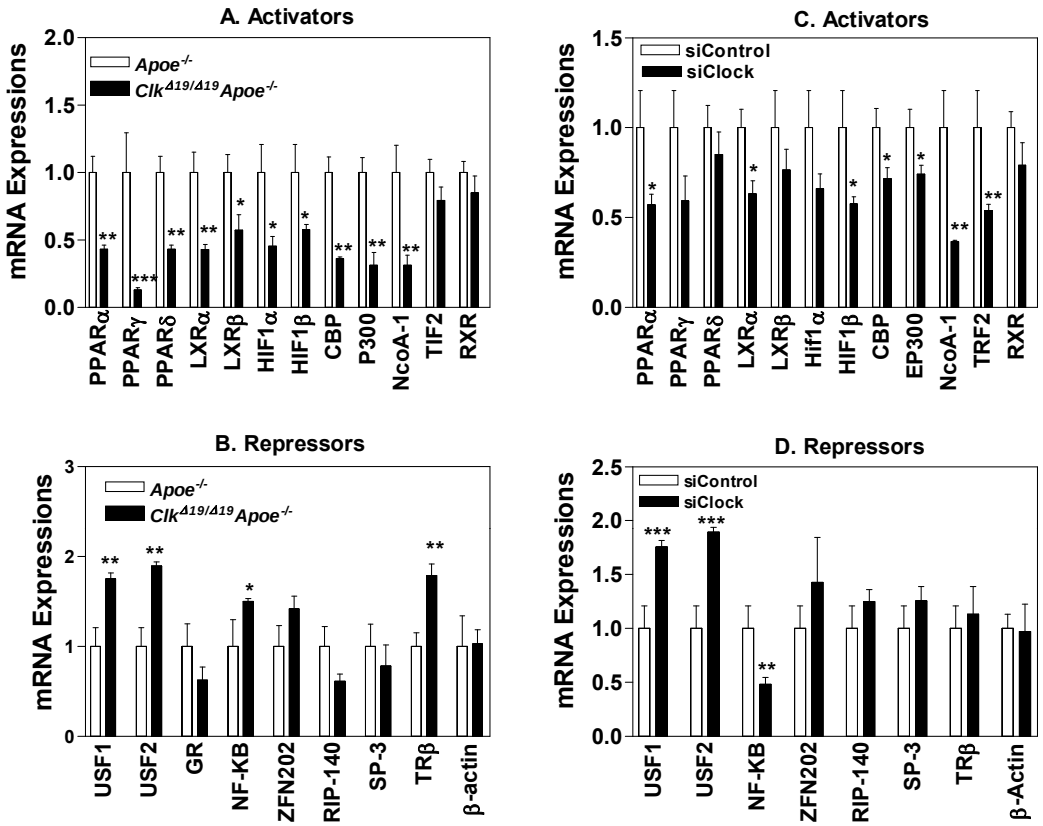

Fig S9: Gene expression of different transcription factors in bone marrow macrophages of *Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$* mice.

(A-B) Bone marrow macrophages isolated from *Clk$^{\Delta 19/\Delta 19}$Apoe$^{-/-}$* and *Apoe$^{-/-}$* mice were used to measure mRNA levels of different transcription factors that are known to regulate ABCA1.

(C-D) Macrophages from wildtype C57Bl6J mice were treated with siControl or siClock for 48 h. mRNA levels of different transcription factors that are known to regulate ABCA1 were quantified. Mean±SD, n=6, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

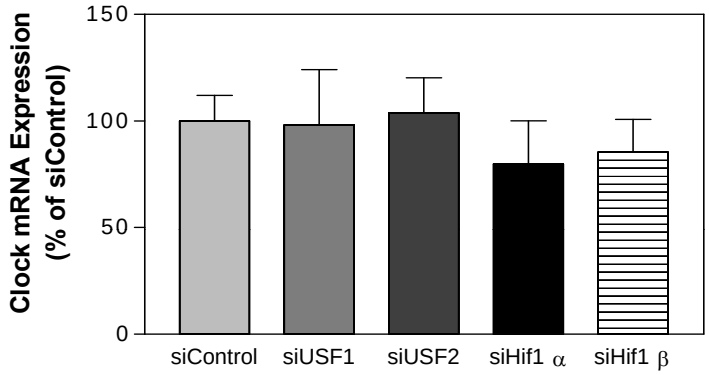

Fig S10: Clock is not regulated by USF1, USF2, HIF1α and HIF1β.
Wildtype macrophages were transfected with indicated siRNAs and changes in Clock mRNA were measured. Mean±SD, n=6.

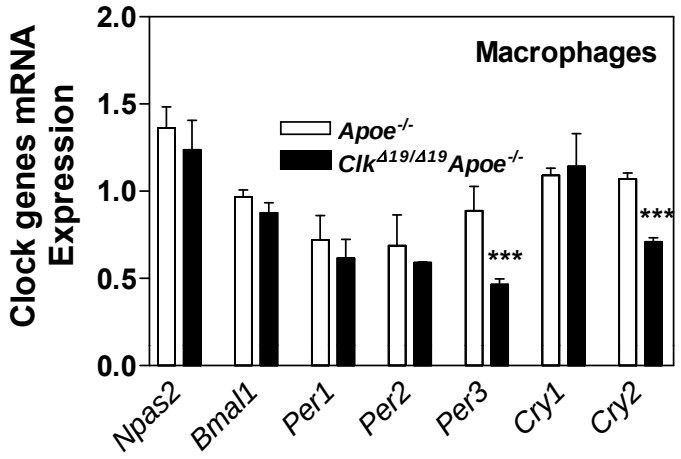

Fig S11: Expression of Clock genes in bone marrow macrophages of *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ mice.

Bone marrow macrophages isolated from *Clk*$^{\Delta 19/\Delta 19}$*Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ mice were used to measure different Clock genes mRNA levels. Mean±SD, n=9, *** P < 0.001.

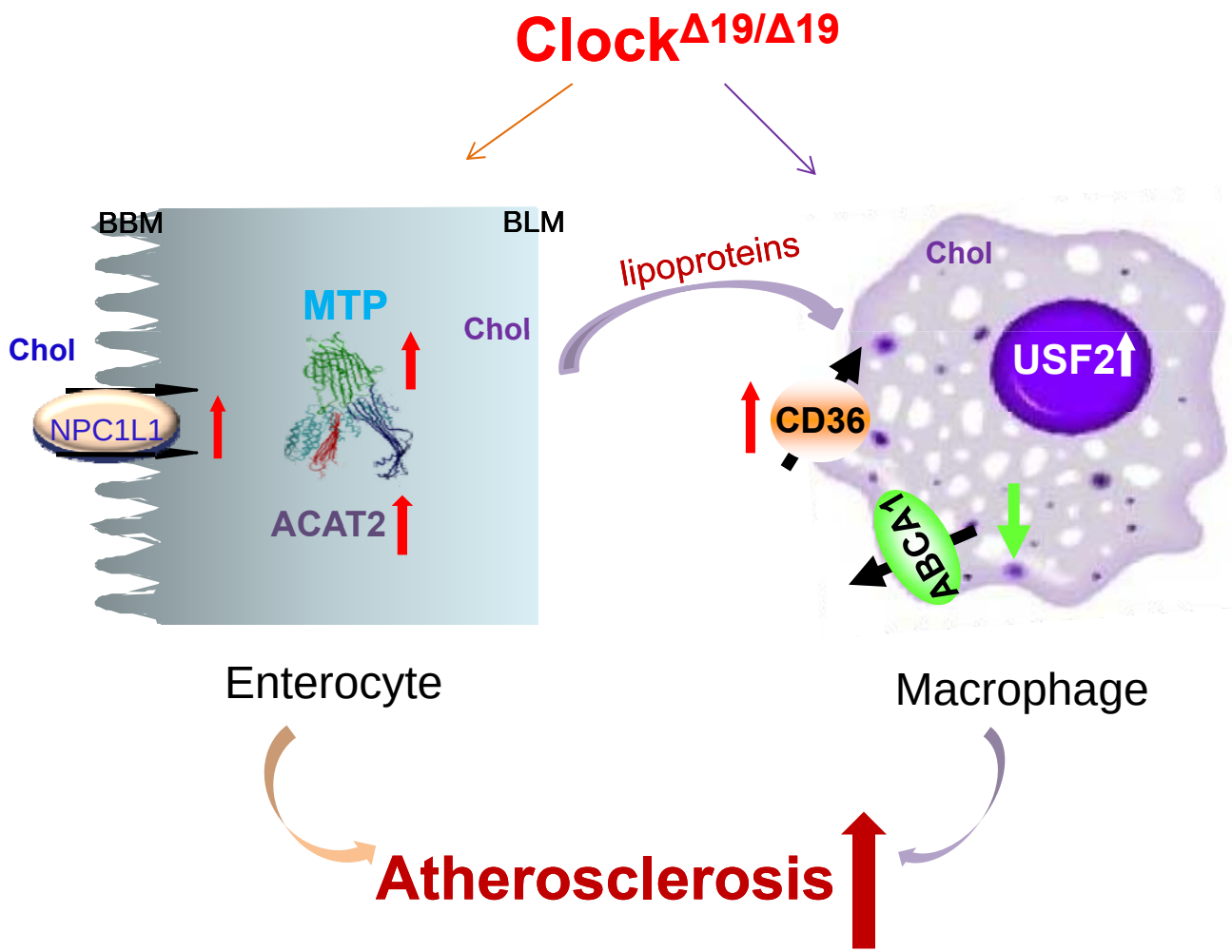

Fig S12: A schematic diagram explaining the regulation of different pathways and molecules modulated by Clock in enterocytes and macrophages. Data presented in this manuscript show that Clock regulates cholesterol metabolism in enterocytes and macrophages. Expression of a Clock mutant protein increases expression of NPC1L1, ACAT2 and MTP in enterocytes leading to enhanced uptake and secretion of cholesterol with chylomicrons. In the presence of the Clock mutant protein, macrophages take up more modified lipoproteins due to increased expression of scavenger receptors such as CD36. Further, these macrophages are defective in cholesterol efflux due to reduced expression of ABCA1. Molecular studies suggest that reduced expression of ABCA1 might be secondary to increased expression and binding of the USF2 repressor to the ABCA1 promoter. BBM, brush border membrane; BLM, basolateral membrane; Chol, cholesterol

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