Supplemental Information

Supplement to: 'Macrophage SR-BI modulates autophagy via VPS34 complex and PPARα transcription of *Tfeb* in atherosclerosis'

Huan Tao[1], Patricia G. Yancey[1], John L. Blakemore[1], Youmin Zhang[1], Lei Ding[1], W. Gray Jerome[3], Jonathan D. Brown[1,] Kasey C. Vickers[1], MacRae F. Linton[1,2]

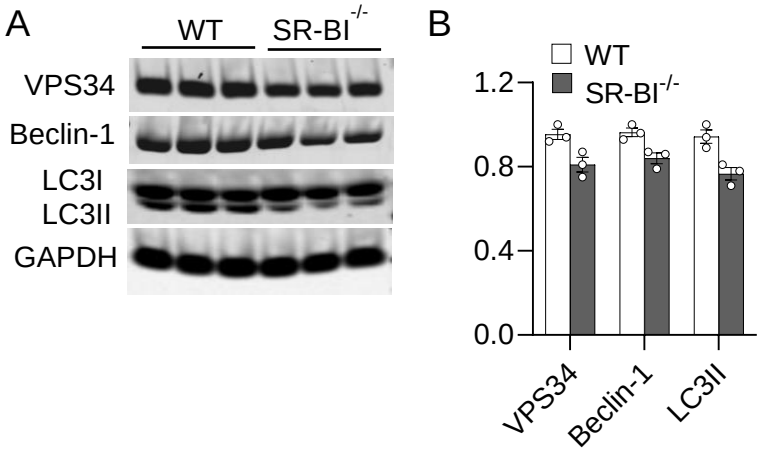
Vanderbilt University School of Medicine, Nashville, TN

1. Department of Medicine, Atherosclerosis Research Unit, Division of Cardiovascular Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232
2. Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232
3. Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

Address correspondence to:
MacRae F. Linton, MD
2220 Pierce Avenue, Nashville, TN
Department of Cardiovascular Medicine, Atherosclerosis Research Unit
Vanderbilt University Medical Center
Email: macrae.linton@vanderbilt.edu

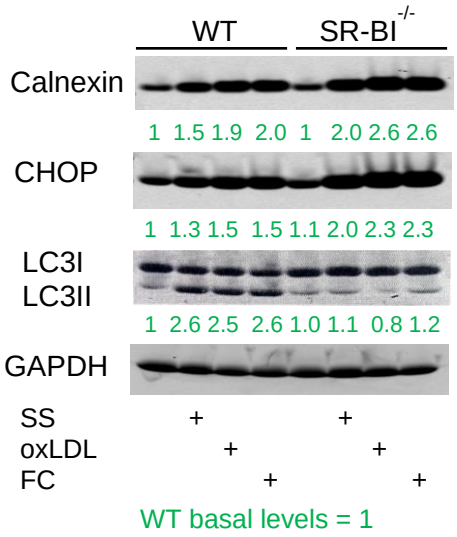
Running title: SR-BI regulates autophagy in atherosclerosis

Supplemental Figure 1



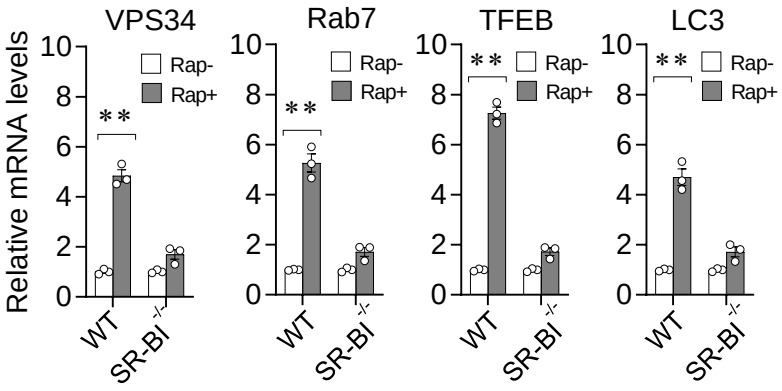
Supplemental Figure 1. Basal levels of autophagy proteins in WT and *Sr-b1*$^{-/-}$ control macrophages. A-B) As a control for ER stress induction, peritoneal macrophages were incubated for 24 h in DMEM containing 10% FBS. The protein levels of VPS34, Beclin-1, and LC3-II in cells from 3 experiments were detected by western blotting. B) The western blots were analyzed by dot quantitation and shown are the mean ± SEM for 3 experiments.

Supplemental Figure 2



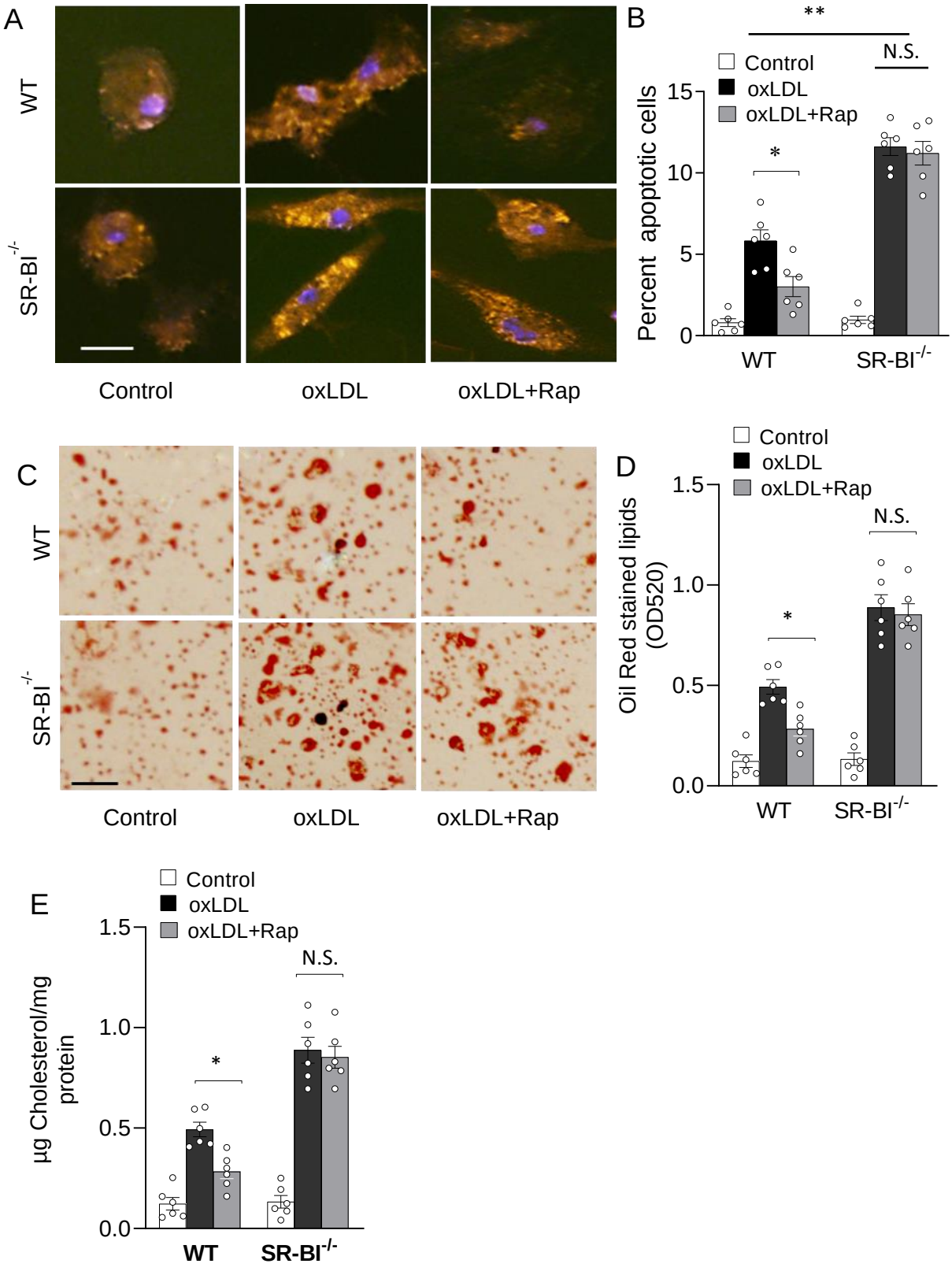
Supplemental Figure 2. Serum starvation (SS), oxidized LDL treatment, and free cholesterol (FC) enrichment promote an ER stress response in WT and *Sr-b1*$^{-/-}$ macrophages. WT or *Sr-b1*$^{-/-}$ macrophages were incubated for 24 h in DMEM containing 10%FBS (C) or in DMEM alone to subject the cells to serum starvation (SS). Alternatively, cells were incubated for 24 h with DMEM containing 100 µg/ml of oxidized LDL (oxLDL) or for FC enrichment, the cells were incubated for 24 h with 100 µg acetylated LDL/ml and 5 µg/ml of the ACAT inhibitor, Sandoz 58035 (FC). The protein levels of calnexin, CHOP, and LC3-II were then detected by western blotting and analyzed by dot quantitation. The blots are representative, and the numbers are the mean of three experiments where the values are normalized to basal WT levels.

Supplemental Figure 3



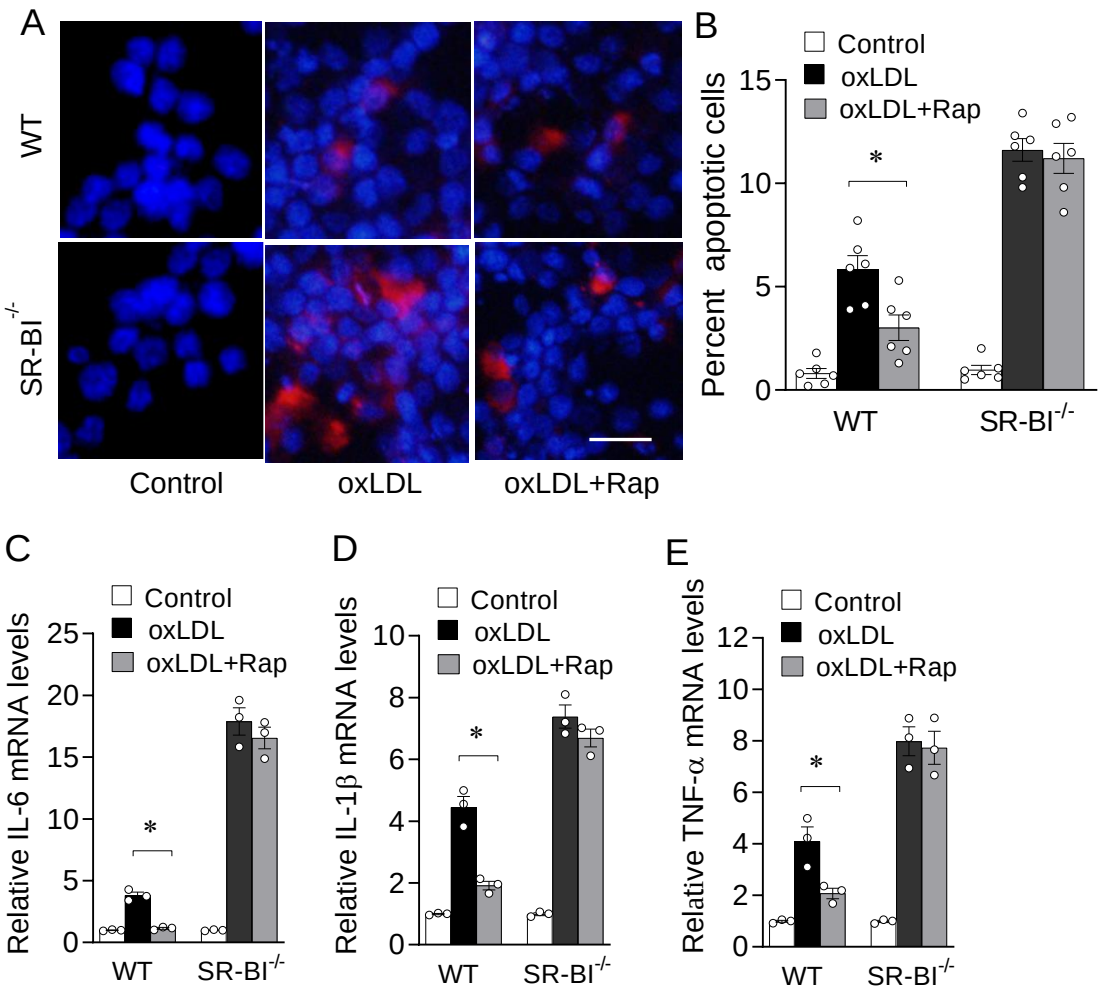
Supplemental Figure 3. The effects of rapamycin treatment on mRNA levels of autophagy genes in WT versus *Sr-b1*$^{-/-}$ cells. WT or *Sr-b1*$^{-/-}$ cells were incubated for 24 h in DMEM containing 10%FBS or 300 nM Rapamycin. Then, the mRNA levels of VPS34, Rab7, TFEB, and LC3 were measured by real time PCR. The data are expressed as mean ± SEM from three independent experiments. n = 3 per group, ** $p < 0.01$. One-way ANOVA with Bonferroni's post hoc test.

Supplemental Figure 4



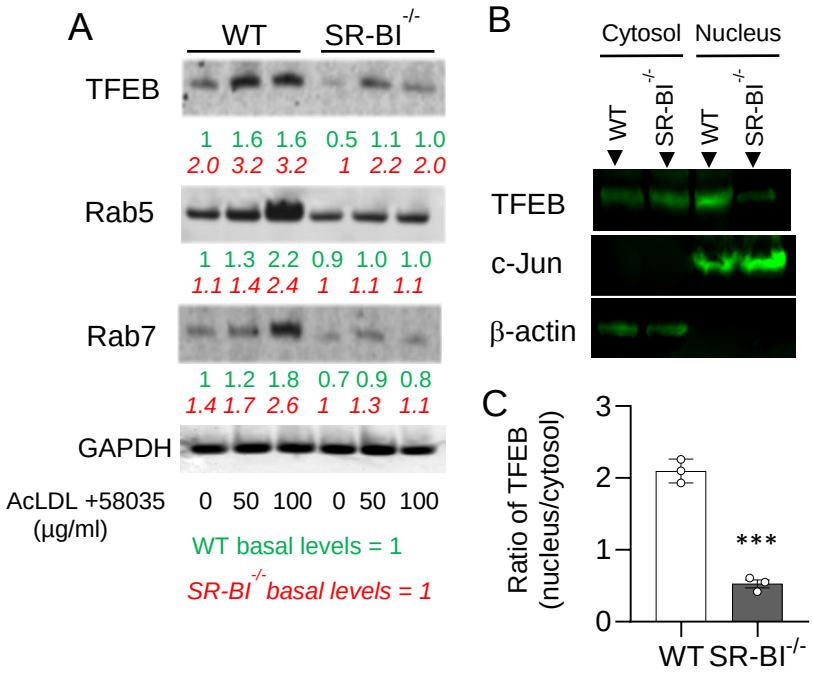
Supplemental Figure 4. Macrophage *Sr-b1* deficiency promotes lipid droplet deposition resulting from defective autophagy. A to E, WT and *Sr-b1*$^{-/-}$ macrophages were treated for 24 h with DMEM alone, oxidized LDL, or oxidized LDL with 300 nM rapamycin. A) The macrophages were then immunostained with anti-perilipin-2 antibody to detect lipid droplets (Yellow) and the nuclei were counterstained with Hoechst (blue). Bar = 20 μm. B) The number of lipid droplets was quantitated using ImageJ software. n = 6 per group from three independent experiments. Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, One-way ANOVA with Bonferroni's post hoc test. C) Representative images of the Oil-Red-O staining lipid droplets. Bar = 20 μm. D) The solubilized Oil-Red-O stained lipids were quantitated by spectrophotometer at 520 nm. n = 6 per group from three independent experiments. Data are presented as mean ± SEM. * $p < 0.05$, One-way ANOVA with Bonferroni's post hoc test. E) Cellular cholesterol levels were measured by enzymatic cholesterol assay. (B, D, and E). Data are presented as mean ± SEM, n = 6 per group from three independent experiments. * $p < 0.05$, One-way ANOVA with Bonferroni's post hoc test.

Supplemental Figure 5



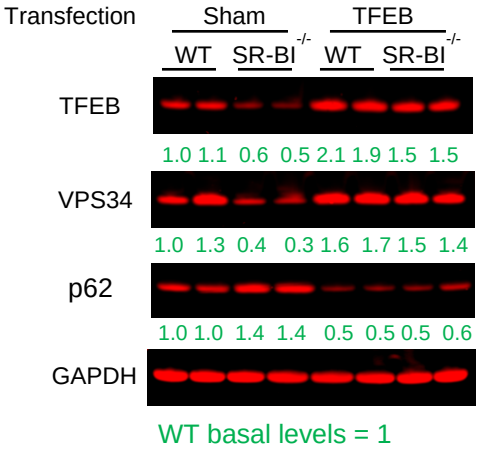
Supplemental Figure 5. Impaired autophagy in *Sr-b1*$^{-/-}$ macrophages causes increased cell death and inflammation. A-E, WT and *Sr-b1*$^{-/-}$ macrophages were treated for 24 h with DMEM alone, or 100 µg/ml of oxLDL with and without 300 nM Rapamycin. A-B) Cell apoptosis was detected by Cy3-labeled Annexin V (Red). Nuclei were counterstained with Hoechst (blue). Shown are representative images. Bar = 20 µm. (A) and the quantitation (B) of the number of apoptotic cells, n = 6 per group from 3 independent experiments. Data are presented as mean ± SEM. * $p < 0.05$. One-way ANOVA with Bonferroni's post hoc test. C-E) The mRNA levels of IL-6 (C), IL-1β (D), and TNF-α (E) were measured by real time qPCR. Data are expressed as mean ± SEM, n = 3 per group from three independent experiments, * $p < 0.05$. One-way ANOVA with Bonferroni's post hoc test.

Supplemental Figure 6



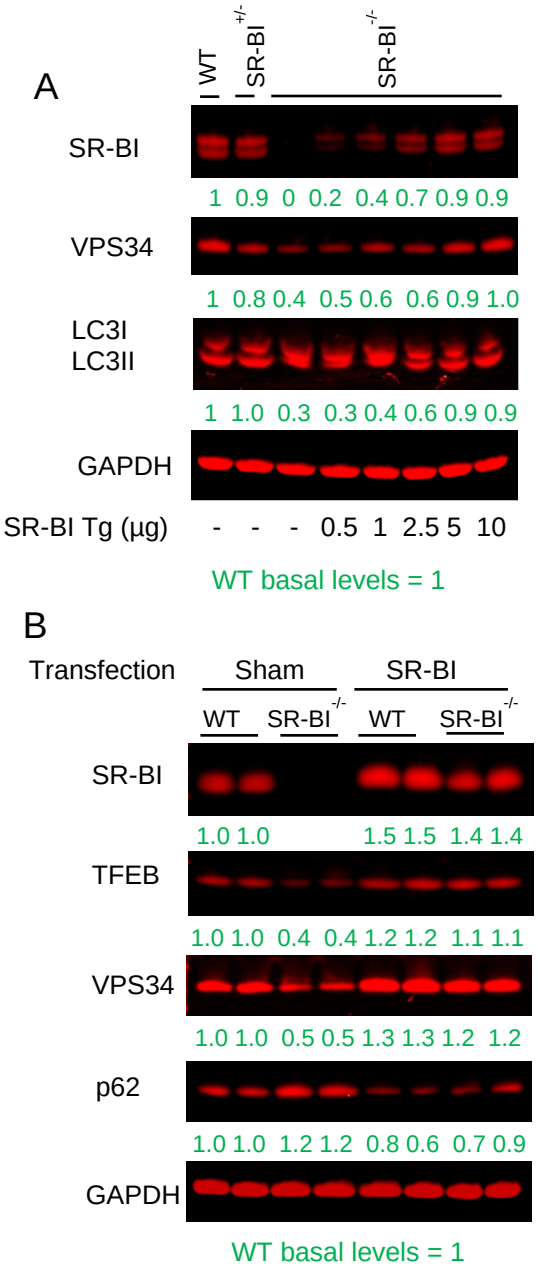
Supplemental Figure 6. Effects of *Sr-b1* expression on TFEB levels and localization in FC enriched macrophages. A) WT or *Sr-b1*$^{-/-}$ macrophages were incubated for 24 h in DMEM containing 10% FBS or containing 50 or 100 µg acetylated LDL/ml in the presence of the ACAT inhibitor, Sandoz 58035 (5 µg/ml). The levels of TFEB, Rab5, and Rab7 were analyzed by western blotting and dot quantitation. The blots are representative, and the numbers are the mean of three experiments, where the values are normalized to either basal WT (regular font) or basal *Sr-b1*$^{-/-}$ levels (italic font). B-C) WT or *Sr-b1*$^{-/-}$ macrophages were incubated for 24 h with 100 µg acetylated LDL/ml in the presence of the ACAT inhibitor, Sandoz 58035 (5 µg/ml). The cytoplasmic and nuclear levels of TFEB in response to FC enrichment were analyzed by western blotting and dot quantitation. B) The blots are representative, and C) the numbers are the mean of three experiments, where the values are normalized to either basal WT (green, regular font) or basal *Sr-b1*$^{-/-}$ levels (red, italic font). n = 3, *** $p < 0.001$, Unpaired Student t test.

Supplemental Figure 7



Supplemental Figure 7. The effect of forced expression of TFEB on autophagy activity in *Sr-b1*$^{-/-}$ macrophages. Thioglycollate-elicited WT and SR-B1$^{-/-}$ peritoneal macrophages were transfected with or without 5 µg plasmids per 5x10^{6} cells of pEGFP-N1-TFEB plasmid (Addgene #38119) with DharmaFECT reagent (Dharmacon) for 48 hours. After the treatment with 100 µg/ml acetylated LDL and 5 µg/ml of Sandoz 58035 for 24 hours, the whole cell lysates were prepared with RIPA buffer and protease inhibitor cocktail (Sigma). The expression levels of TFEB, VPS34, p62 and GAPDH were detected by Western blotting. The band density was quantitated using ImageJ software. The blots are representative, and the numbers are the mean of two experiments where the values are normalized to basal WT levels.

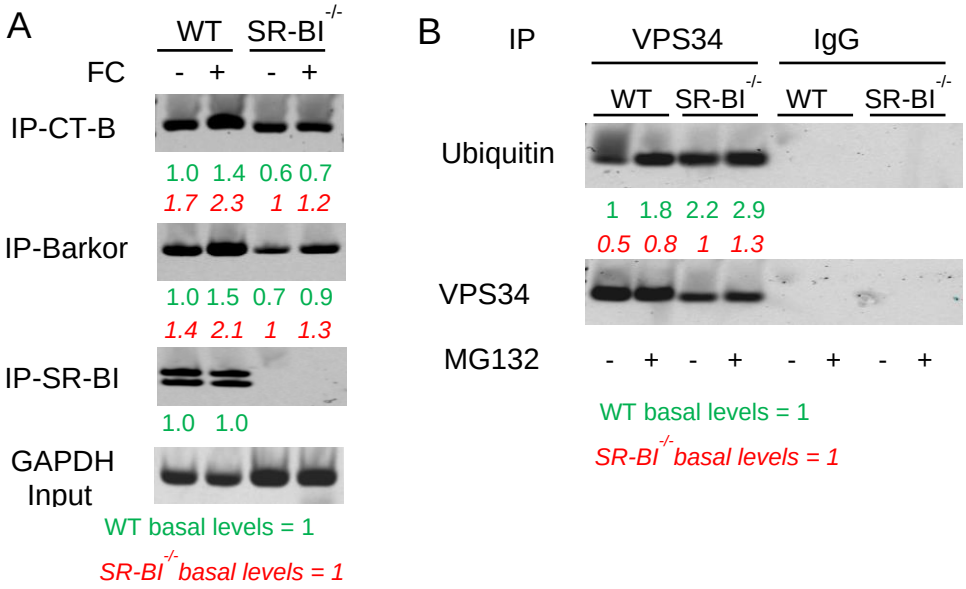
Supplemental Figure 8



Supplemental Figure 8. Forced expression of *Sr-b1* rescues autophagy activity in *Sr-b1*$^{-/-}$ macrophages. A) Thioglycollate-elicited peritoneal macrophages were isolated from WT, *Sr-b1*$^{+/-}$ and *Sr-b1*$^{-/-}$ mice. The *Sr-b1*$^{-/-}$ macrophages were transfected with a serial dose of pCMV6-SR-B1 plasmid (0 to 10 µg plasmids per 5x10^{6} cells). After the treatment with 100 µg/ml acetylated LDL and 5 µg/ml of Sandoz 58035 for 24 hours, whole cell lysates were prepared with RIPA buffer containing protease inhibitor cocktail. The protein levels of SR-B1, VPS34, LC3 and GAPDH were detected by Western blotting. The blots are representative, and the numbers are

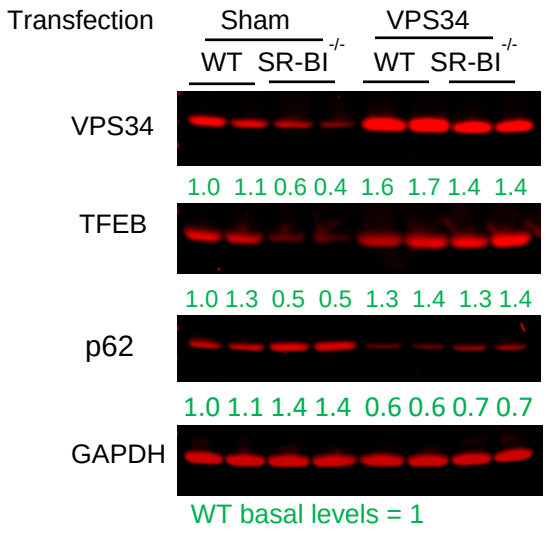
the mean of two experiments where the values are normalized to basal WT levels. B) The WT and *Sr-b1*$^{-/-}$ peritoneal macrophages were treated with 100 µg/ml acetylated LDL and 5 µg/ml Sandoz 58035 for 24 hours, and then transfected with or without 5 µg plasmids per 5x10^{6} cells of pCMV-SR-B1 (Sino Biological, Cat#, MG50317-NY) with DharmaFECT reagent (Dharmacon) for 48 hours. The whole cell lysates were prepared with RIPA buffer containing protease inhibitor cocktail. The expression levels of SR-B1, TFEB, VPS34, p62 and GAPDH were detected by Western blotting. The blots are representative, and the numbers are the mean of two experiments where the values are normalized to either basal WT levels.

Supplemental Figure 9



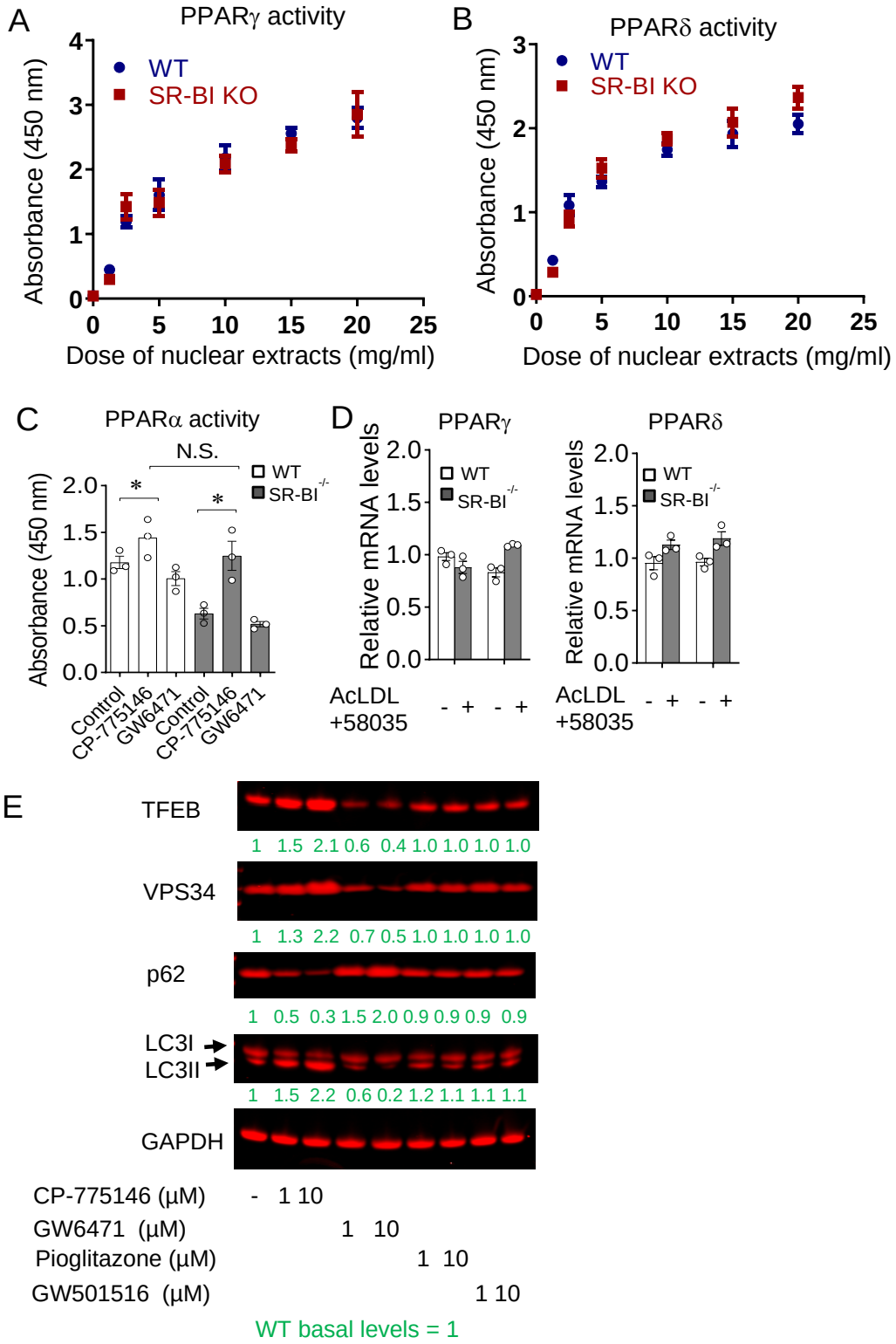
Supplemental Figure 9. Effects of SR-BI expression on Barkor and cholesterol domain content of intracellular membranes and on VPS34 ubiquitination. A) WT or SR-BI$^{-/-}$ macrophages were incubated for 24h in DMEM containing 10% FBS or containing 100 µg acetylated LDL/ml in the presence of the ACAT inhibitor, Sandoz 58035 (5 µg/ml). The cells were incubated with cholera toxin B (CT-B), which interacts with lipid rafts, and, after crosslinking, the plasma membranes were separated from intracellular membranes by ultracentrifugal plasma membrane isolation. The cholesterol domains were immunoprecipitated with anti-CT-B antibody and protein A/G magnetic beads. Barkor and SR-BI were then detected by western blotting. The blots are representative, and the numbers are the mean of two independent experiments, where the values are normalized to either basal WT (green, regular font) or basal SR-BI$^{-/-}$ levels (red, italic font). B) WT or SR-BI$^{-/-}$ macrophages were incubated for 24h in DMEM containing 100 µg acetylated LDL/ml and the ACAT inhibitor, Sandoz 58035 (5 µg/ml), alone or in the presence of the proteasomal degradation inhibitor MG132. Cell lysates were cross-linked and immunoprecipitated with anti-VPS34 antibody or IgG and protein A/G magnetic beads. Ubiquitinated VPS34 was then detected in immunoprecipitated proteins by western blotting. The blots are representative, and the numbers are the mean of two independent experiments, where the values are normalized to either basal WT (green, regular font) or basal SR-BI$^{-/-}$ levels (red, italic font).

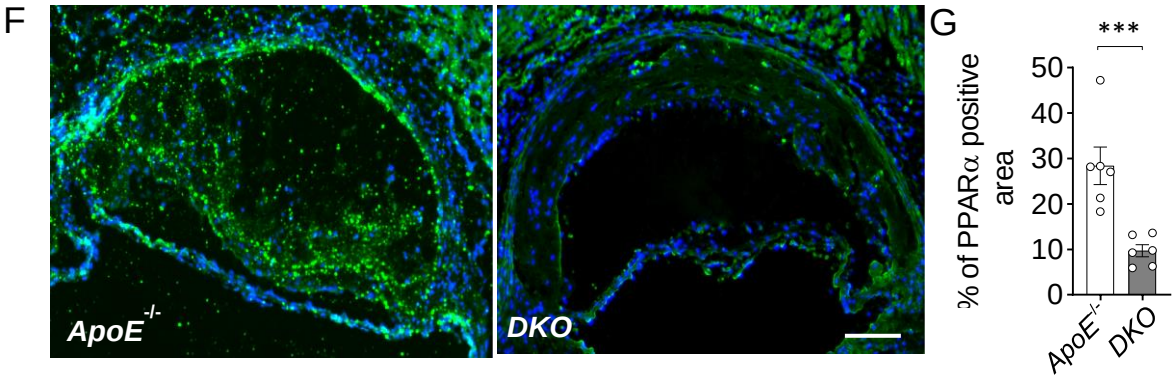
Supplemental Figure 10



Supplemental Figure 10. Forced expression of VPS34 rescues autophagy activity in SR-BI$^{-/-}$ macrophages. WT and SR-BI$^{-/-}$ peritoneal macrophages were transfected with or without 5 µg plasmids per 5x10^{6} cells of pcDNA4-VPS34 (Addgene #24398) with DharmaFECT reagent (Dharmacon) for 48 hours. Cells were then incubated with 100 µg/ml acetylated LDL and 5 µg/ml of Sandoz 58035 for 24 hours. The whole cell lysates were prepared with RIPA buffer containing protease inhibitor cocktail. The expression levels of VPS34, TFEB, p62 and GAPDH were detected by Western blotting. The blots are representative, and the numbers are the mean of two independent experiments, where the values are normalized to basal WT levels.

Supplemental Figure 11





Supplemental Figure 11. SR-BI deficiency causes attenuated PPARα, not PPARγ/δ activity in macrophage foam cells. A-B) PPARγ/δ activity was measured using serial doses of nuclear extracts from WT and SR-BI knockout macrophages treated with 100 µg/ml acetylated LDL and 5 µg/ml Sandoz 58035 for 24 hours. The data are expressed as mean ± SEM from three independent experiments. C) PPARα activity was measured in FC enriched WT and SR-BI$^{-/-}$ macrophages treated with vehicle alone or with CP-775146 or GW6471. The data are expressed as mean ± SEM from three independent experiments. n = 3 per group, $* p < 0.05$, One-way ANOVA with Bonferroni's post hoc test. D) The gene expression of PPARγ/δ was measured by real time PCR from WT and SR-BI$^{-/-}$ macrophages treated either with 10% FBS or 100 µg/ml acetylated LDL and 5 µg/ml Sandoz 58035 for 24 hours. The data are expressed as mean ± SEM from three independent experiments. E) The expression of TFEB, VPS34, p62, LC3I/II, and GAPDH were detected by Western blot in WT macrophages treated with or without different doses of CP-775146, GW6471, Pioglitazone, and GW501516. The blots are representative, and the numbers are the mean of two independent experiments. F-G) Hematopoietic SR-BI deletion leads to attenuated PPARα expression in atherosclerotic lesions. *ApoE*$^{-/-}$ mice were transplanted with *ApoE*$^{-/-}$ or *ApoE*$^{-/-}$*SR-BI*$^{-/-}$ (*DKO*) bone marrow and fed with Western diet for 8 weeks. F) Proximal atherosclerotic aortic sections were stained with anti-PPARα primary antibody and Alexa 488-labeled secondary antibody (Green). Nuclei were counterstained with Hoechst (Blue). Scale Bar = 50 µm. G) PPARα positive area and total lesion area were quantified by ImageJ software. The data are expressed as mean ± SEM, n = 6 biologically independent mice per group, Mann-Whitney test.

Supplemental Methods

Cell Culture, Autophagy Induction, siRNA, and Transfections

Peritoneal macrophages were isolated from mice reconstituted with WT, *Sr-b1*$^{-/-}$, *Apoe*$^{-/-}$, and double *Sr-b1*$^{-/-}$*Apoe*$^{-/-}$ (DKO) bone marrow (BM), as previously described. To stimulate autophagy, an ER stress response was induced in macrophages by starvation, FC enrichment, or oxidized LDL treatment. Control macrophages were incubated for 24 h with DMEM containing 10% FBS. Macrophages were subjected to starvation by incubation for 24 h in DMEM alone (no serum). For FC enrichment, macrophages were incubated for 24 h with 100 µg acetylated LDL/ml in the presence of the ACAT inhibitor, Sandoz 58035(5 µg/ml). Macrophages were incubated for 24 h with 100 µg oxidized LDL/ml (Alfa Aesar). To knock down *Tfeb* gene expression, 25 nM siRNA of mouse *Tfeb* (Ambion) was transfected into J774A1 cells (ATCC) with DharmaFECT reagent (Dharmacon) for 24 hours. For transient transfections, Lipofectamine LTX and Plus reagent (Invitrogen) or jetPEI-Macrophage DNA Transfection Reagent (Polyplus-transfection) with pcCMV6-SR-BI (Origene), pcDNA4-VPS34 (Addgene #24398) (1) or pEGFP-N1-TFEB (Addgene #38119) (2) or scramble control plasmids were applied to macrophages for 48 h.

Bone Marrow Transplantation and Atherosclerosis Analyses

Female *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice were lethally irradiated and transplanted with 5 × 10^6 BM cells from SR-BI$^{+/+}$ *Apoe*$^{-/-}$ and *Sr-b1*$^{-/-}$*Apoe*$^{-/-}$ mice or BM from WT, *Sr-b1*$^{-/-}$, *Apoe*$^{-/-}$, and DKO mice, respectively. After 4 weeks, the mice were placed on a Western-type diet for 8 weeks or 16 weeks. The extent of atherosclerosis was examined both in Oil-Red-O-stained cross-sections of the proximal aorta and by *en face* analysis using the KS300 imaging system (Kontron Elektronik GmbH) (3). Immunofluorescence staining was used to examine the autophagosome levels in atherosclerotic lesions (3). Briefly, 5 µm cross-sections of the proximal aorta were fixed in cold acetone (Sigma), blocked in Background Buster (Innovex), incubated with indicated primary antibodies (Beclin-1 and LC3II) at 4ºC for overnight, and then with fluorescently labeled secondary antibodies at 37C for 1 hour, the nucleus was counter stained with Hoechst, the images were processed with fluorescence microscope (Olympus IX81) and SlideBook 6 (Intelligent-Image) software and quantified using ImageJ software.

Plasma Membrane and Lysosome Preparation

Plasma membranes were extracted using a plasma membrane extraction kit (BioVision). Lysosomes were prepared from macrophage lysates by density gradient ultracentrifugation as described using the Lysosome Isolation Kit (Sigma) (4).

Immunoprecipitation and Western Blotting

Whole cell lysates were prepared with either RIPA or Pierce IP Lysis Buffer plus 0.5% protease inhibitor mixture (Sigma) alone or with the cross-linking reagent DPS (dithiobis succinimidylpropionate, Pierce) (5). For immunoprecipitation experiments, cross-linked lysates were immunoprecipitated with 10 µg of polyclonal antibody against mouse SR-BI (Novus); then 25 µL of magnetic beads (Invitrogen) was added, and the mixture was incubated for 1h at 4°C

with rotation. The magnetic beads were then collected, washed three times, and SDS-PAGE sample buffer containing either DTT (100 mM) or β-mercaptoethanol (1%) was added to the beads. After incubation at 70°C for 5 min, magnetic field was applied to the Magnetic Separation Rack (New England), and the supernatant was used for detecting mouse VPS34, Beclin-1, Rab7, LC3 or SR-BI by western blotting. Briefly, 30-60 μg of protein was resolved by NuPAGE Bis-Tris electrophoresis (Invitrogen), and transferred onto nitrocellulose membranes (Amersham Bioscience). Membranes were probed with primary rabbit antibodies specific for SR-BI (Novus, NB400-131), LC3B (Cell signaling, 3868S), p62 (Abcam, ab91526), VPS34 (Abcam, ab124905), Beclin-1(Novus, NBP1-00085), Rab7 (Cell signaling, 9367S), Lamp-1 (Millipore, AB2971), TFEB (Novus, NBP1-67872), Perilipin-2 (Novus, NB110-40877), Barkor/ATG14L (Novus, NBP2-43644), Bif-1 (Cell signaling, 4467S), ATG5 (Novus, NBP2-24389), Ubiquitin (Abcam, ab137031), CT-B (List Biological Laboratories, #703), c-Jun (Abcam, ab40766), PPARα (Abcam, ab227074), β actin (Sigma, ABT264) and GAPDH (Sigma, G9545), as well as fluorescently tagged IRDye 680/800 (LI-COR) secondary antibodies (926-68072/926-68073/926-32210/926-32214). Proteins were visualized and quantified by Odyssey 3.0 Quantification software (LI-COR). In experiments where Pierce Lysis Buffer was used with DPS as the cross-linking reagent and/or β-mercaptoethanol as the reducing reagent, the majority of SR-BI migrated as oligomers. SR-BI oligomer bands were confirmed by the absence of bands in *Sr-b1*$^{-/-}$ lysates or in anti-IgG control immunoprecipitates.

Oil-Red-O Staining of Foam Cells, Immunofluorescence Staining of Lipid Droplets, and Measurement of Cellular Cholesterol

Oil Red O staining was used to measure macrophage foam cell formation as previously reported (6). Briefly, the cultured cells were fixed with 10% formalin, stained with fresh 60% Oil Red O (Sigma) solution, and robustly washed with water, then images were captured by light microscopy. For quantification, cells were dried, extracted with 100% isopropanol, the solubilized stained lipids were quantified by spectrophotometer at 520 nm. The number of cytoplasmic lipid droplets was quantitated by immunofluorescence staining of perilipin-2. Briefly, the cells were cultured in the chambers, treated as indicated, then fixed with fresh 4% paraformaldehyde (Sigma) in PBS, permeabilized with 0.2% Triton X-100, blocked with 2% BSA in PBS, then incubated with anti-perilipin-2 antibody (Novus) at 4ºC for overnight, and Alexa 658 secondary antibody at room temperature for 1 hour, the nucleus was counter stained with Hoechst (Sigma), the images were processed using a fluorescence microscope (Olympus IX81) and SlideBook 6 (Intelligent-Image) software. Cellular cholesterol was measured by enzymatic cholesterol assay as described (7).

Electron, Fluorescence, and Confocal Microscopic Analyses

Electron microscopy was used to visualize autophagosome formation in macrophages. Briefly, a monolayer of cells was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7), then scraped and postfixed in 2% osmium tetroxide and 0.1M cacodylate buffer, dehydrated with a series of ethanol; and gradually infiltrated with Epon resin. Thin sections were stained with uranyl acetate and lead citrate. Transmission EM was performed using a FEI Tecnai T-12 operated at 100 kiloelectron volts to ultrastructurally analyze cell autophagosome content.

Images were processed with Metamorph (Universal Imaging Co.) and Photoshop (Adobe) software. Macrophage autophagosome levels were also detected by immunofluorescence staining using anti-LC3II primary and fluorescence labeled secondary antibodies. The intracellular locations and expression levels of SR-BI, Barkor/ATG14L, and cholesterol domain marker cholera toxin B subunit (CT-B) were detected by immunofluorescence staining and confocal microscopy (LSM 880 and Zen 2 software) using primary and relative Alex488, 568 or 647 donkey anti-rabbit/goat/mouse secondary antibodies.

VPS34 Kinase Activity Assay

Class III PI3K ELISA Kit (Echelon) was used to measure endogenously or exogenously expressed VPS34 kinase activity from macrophages or aortic root tissue following the manufacturer's instructions. Briefly, whole cell or tissue lysates were prepared with IP Lysis Buffer (Pierce) plus protease inhibitor and kinase inhibitor cocktail (Sigma), 500 μg of the lysates was immunoprecipitated with anti-VPS34 antibody. Then 20 μL of kinase reaction buffer (10 mM Tris, pH 8, 100 mM NaCl, 1 mM EDTA and 10 mM $MnCl_2$), 4 μL of 500 μM phosphatidylinositol (PI) substrate plus 1 μL of 1.25 mM ATP was added to the precipitated beads and incubated at 37 °C for 1 h. The reaction was quenched with 5 μL of 100 mM EDTA, diluted with 130 μL ddH_2O and 40 μL PI(3)P detection Buffer (provided by the Kit). Then the quenched reaction and PI(3)P detector protein were added together to the PI(3)P-coated microplate for competitive binding to the PI(3)P detector protein. The amount of PI(3)P detector protein bound to the plate was determined through colorimetric detection of absorbance at 450 nm.

Aortic Root RNA Isolation and Real-Time RT-PCR

Total RNA was isolated and purified using Aurum Total RNA kit (Bio-Rad) according to the manufacturer's protocol. Complementary DNA was synthesized with iScript reverse transcriptase (Bio-Rad). Relative quantitation of the target mRNA was performed using specific primers, SYBR probe (Bio-Rad), and iTaqDNA polymerase (Bio-Rad) on IQ5 thermocylcer (Bio-Rad) and normalized with 18S, as described earlier (3).

Supplemental table: the gene name and primer sequences applied for real time PCR.

Gene name	Forward 5' to 3'	Reverse 5' to 3'
Ulk1 (ATG1)	CCTGAAGGAACTAAAGCACGA	CTGCTGTAGGAAAAGCCTGA
Atg5 (ATG5)	CAGGGGTGTGCCTTCATATT	GCTTTTGCCAAGAGTCAGCTA
Becn1 (Beclin-1)	CTGTGCATTCCTCACACAGC	CCCAGCCAGGATGATGTCTA
Atg7(ATG7)	CTCCTTCTGGAGCAGTCAGC	CGAAGGTCAGGAGCAGAAAC
Map1lc3b (LC3b)	CCGGATGATCTTGACCAACT	GACCAGCACCCCAGTAAGAT
Rab5a (Rab5a)	ATTGGGGCTGCCTTTCTAAC	AGGACTTGCTTGCCTTTGAA
Rab7a (Rab7a)	GGCCTTCTACAGAGGTGCAG	TCTTTGTGGCCACTTGTCTG
*Rab9*a (Rab9a)	TGGCAGGAAAATCGTCTCTT	TGTCCCAAATCTGCATGGTA
Tfeb (TFEB)	GGGCTACATCAACCCTGAGA	CTTTCTTCTGCCGCTCCTT
Vps34 (VPS34)	CTAACGTGGAGGCAGATGGT	AATCTGTCCAGCCAATCCAC
Ppara (PPARα)	GCCTGTCTGTCGGGATGT	GGCTTCGTGGATTCTCTTG
Pparg (PPAPγ)	CAGGCTTGCTGAACGTGAAG	GGAGCACCTTGGCGAACA
Ppard (PPARδ)	ACAGTGACCTGGCGCTCTTC	TGGTGTCCTGGATGGCTTCT

Gel shift assay

In *Silico* methodology was applied to examine the potential existence of TFEB binding sites in the VPS34 gene promoter and 5' untranslated region (5' UTR) (8). The predicted and mutant DNA and TFEB consensus (5'-GTAGGCCACGTG ACCGGG-3') oligonucleotides were labeled with fluorescent IRDy700 dye (Invitrogene) and annealed into dsDNA. Then, 1 µL of 25 nM of a variety of TFEB consensus and VPS34 WT and mutant dsDNA was incubated with 5 µg TFEB protein (Origene) in each reaction for 20 min at room temperature using EMSA Buffer Kit (LI-COR) and separated by 5% TBE polyacrylamide gel electrophoresis following the company's instructions. Images were captured by Odyssey scanner (Li-COR).

Supplemental References

1. Sun, Q., Fan, W., Chen, K., Ding, X., Chen, S., and Zhong, Q. 2008. Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. *Proc Natl Acad Sci U S A* 105:19211-19216.
2. Roczniak-Ferguson, A., Petit, C.S., Froehlich, F., Qian, S., Ky, J., Angarola, B., Walther, T.C., and Ferguson, S.M. 2012. The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci Signal* 5:ra42.
3. Tao, H., Yancey, P.G., Babaev, V.R., Blakemore, J.L., Zhang, Y., Ding, L., Fazio, S., and Linton, M.F. 2015. Macrophage SR-BI mediates efferocytosis via Src/PI3K/Rac1 signaling and reduces atherosclerotic lesion necrosis. *J Lipid Res* 56:1449-1460.
4. Graham, J., Ford, T., and Rickwood, D. 1994. The preparation of subcellular organelles from mouse liver in self-generated gradients of iodixanol. *Anal Biochem* 220:367-373.
5. Staros, J.V. 1982. N-hydroxysulfosuccinimide active esters: bis(N-hydroxysulfosuccinimide) esters of two dicarboxylic acids are hydrophilic, membrane-impermeant, protein cross-linkers. *Biochemistry* 21:3950-3955.
6. Tao, H., Aakula, S., Abumrad, N.N., and Hajri, T. 2010. Peroxisome proliferator-activated receptor-gamma regulates the expression and function of very-low-density lipoprotein receptor. *Am J Physiol Endocrinol Metab* 298:E68-79.
7. Robinet, P., Wang, Z., Hazen, S.L., and Smith, J.D. 2010. A simple and sensitive enzymatic method for cholesterol quantification in macrophages and foam cells. *J Lipid Res* 51:3364-3369.
8. Oshchepkov, D.Y., and Levitsky, V.G. 2011. In silico prediction of transcriptional factor-binding sites. *Methods Mol Biol* 760:251-267.

Graphical Abstract