SUPPLEMENTAL MATERIAL

Intracellular AIBP regulates oxidized LDL-induced mitophagy in macrophages

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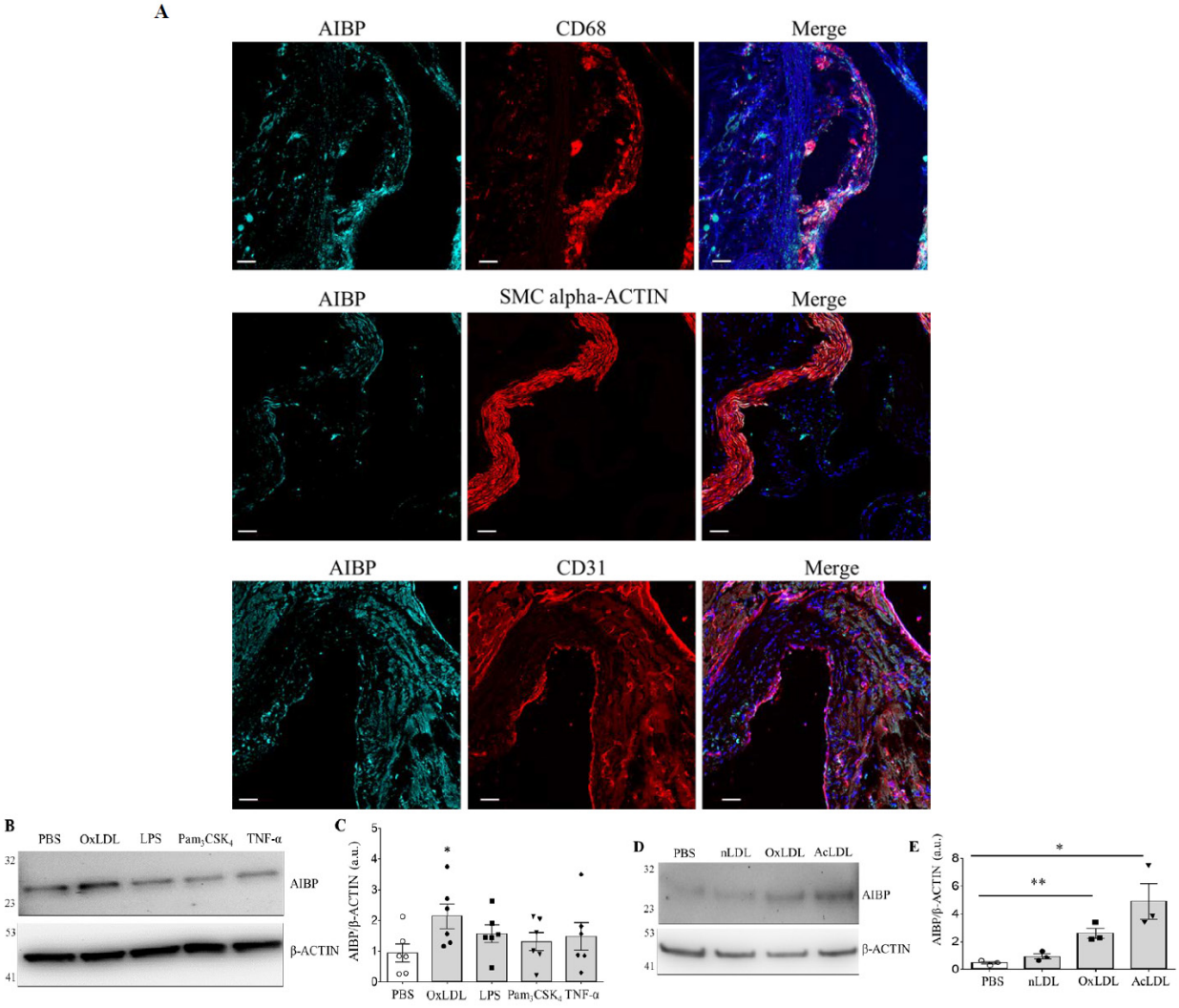
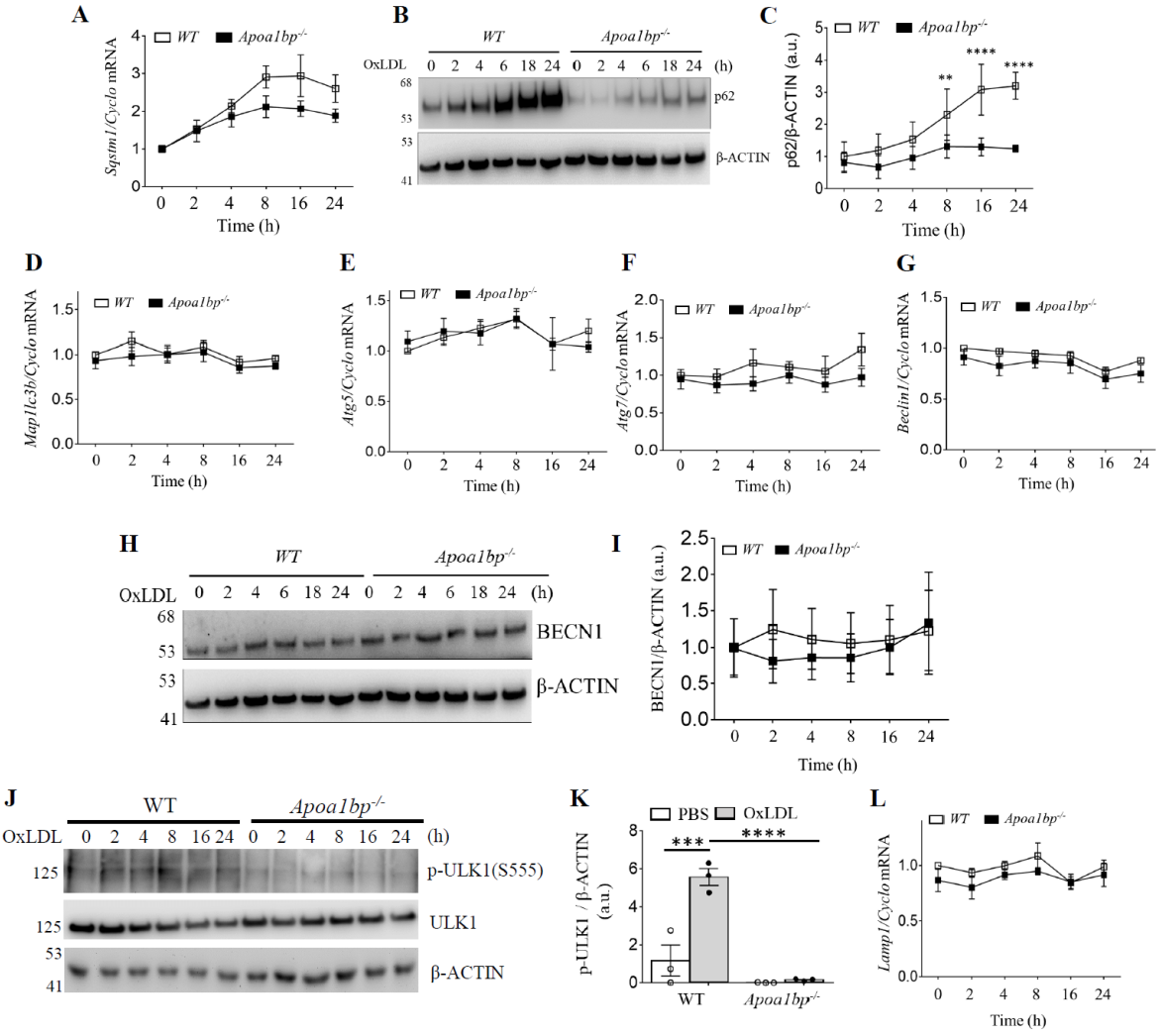
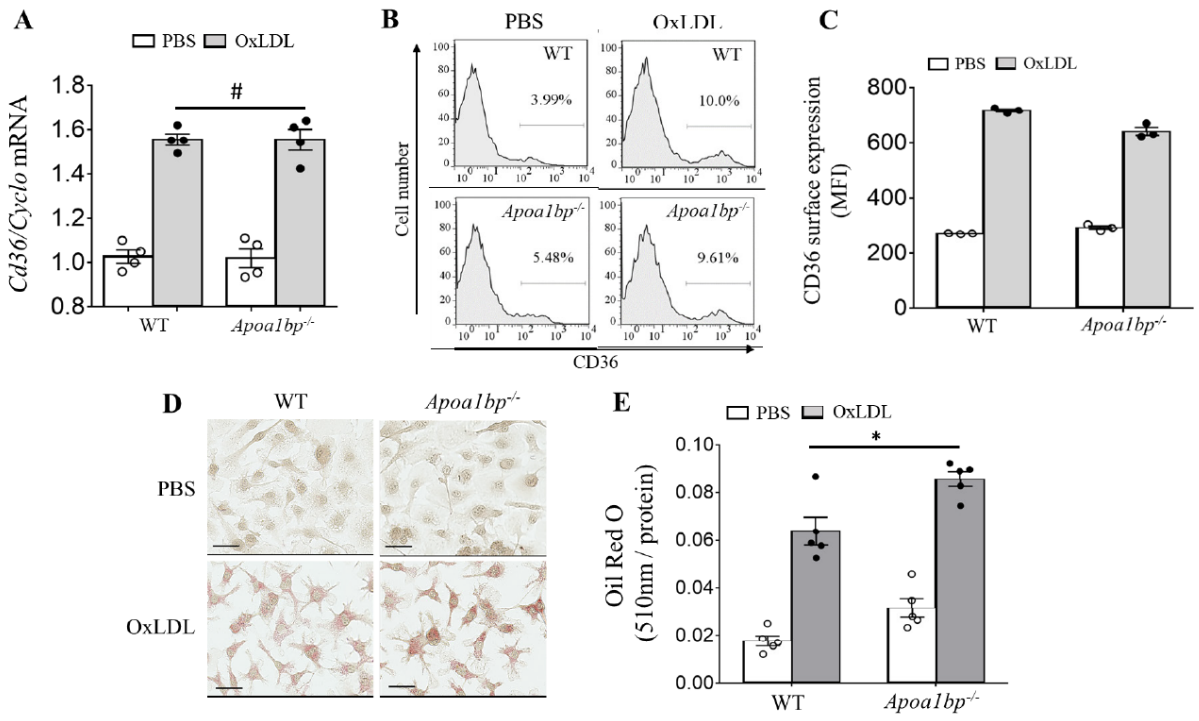


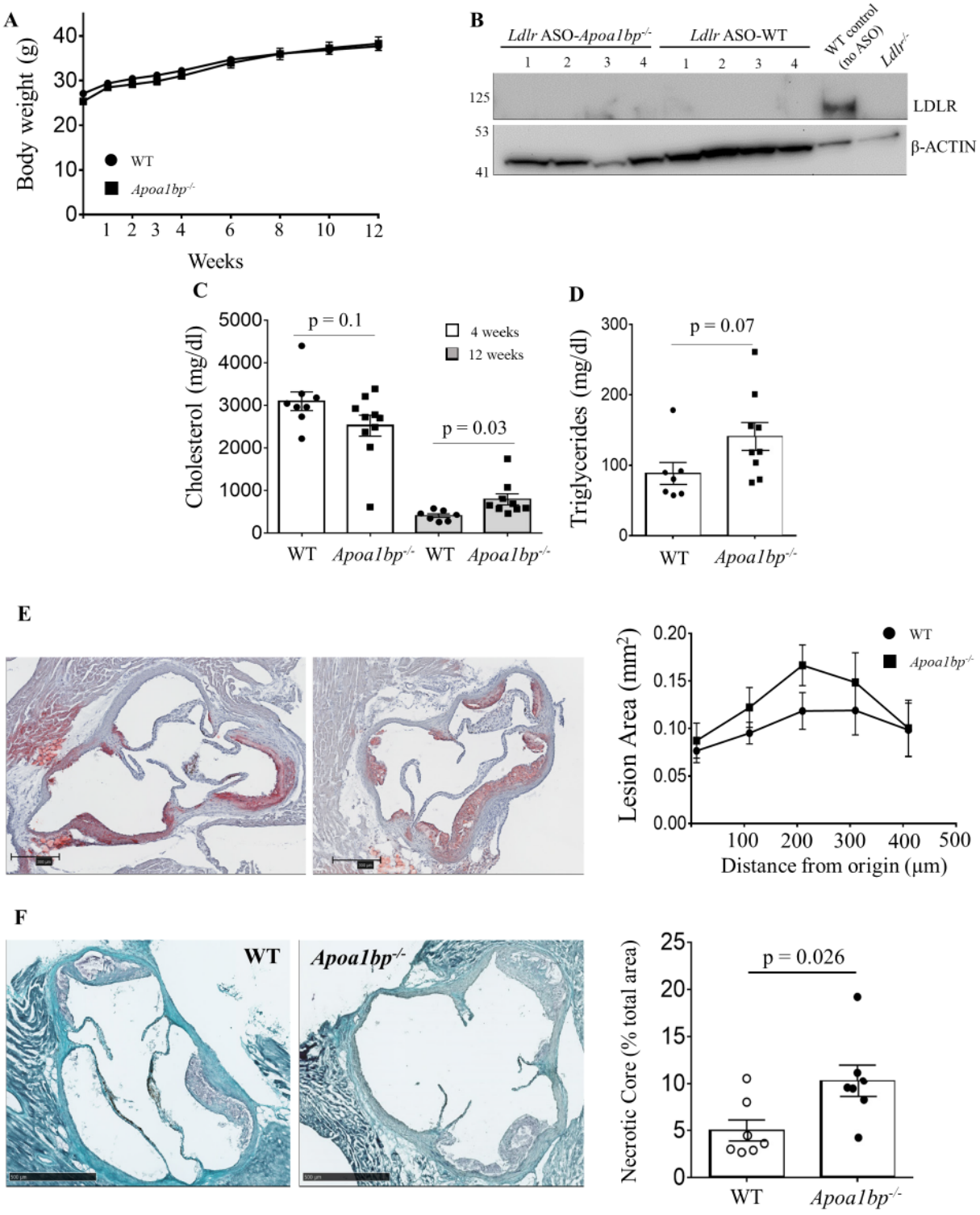
Supplemental Figure I. AIBP immunostaining colocalization with markers of macrophages, smooth muscle cells and endothelial cell in murine atherosclerotic lesion. (**A**) Sections from the aortic root of hypercholesterolemic WT mice were stained with the monoclonal anti-AIBP antibody BE-1 (cyan), CD68 (red), SMC alpha-ACTIN (red), or CD31 (red) antibodies. (**B-E**) BMDM isolated from WT mice were stimulated with 25 μg/ml OxLDL, 100 ng/ml LPS, 100 ng/ml Pam_3CSK_4, 20 ng/ml TNF-α, 25 μg/ml nLDL, or 25 μg/ml AcLDL for 24 h. Cell lysates were immunoblotted with polyclonal anti-AIBP and β-ACTIN antibodies. Mean±SEM; N=3-7. *, $p<0.05$; **, $p<0.005$. Scale bar,50 μm.



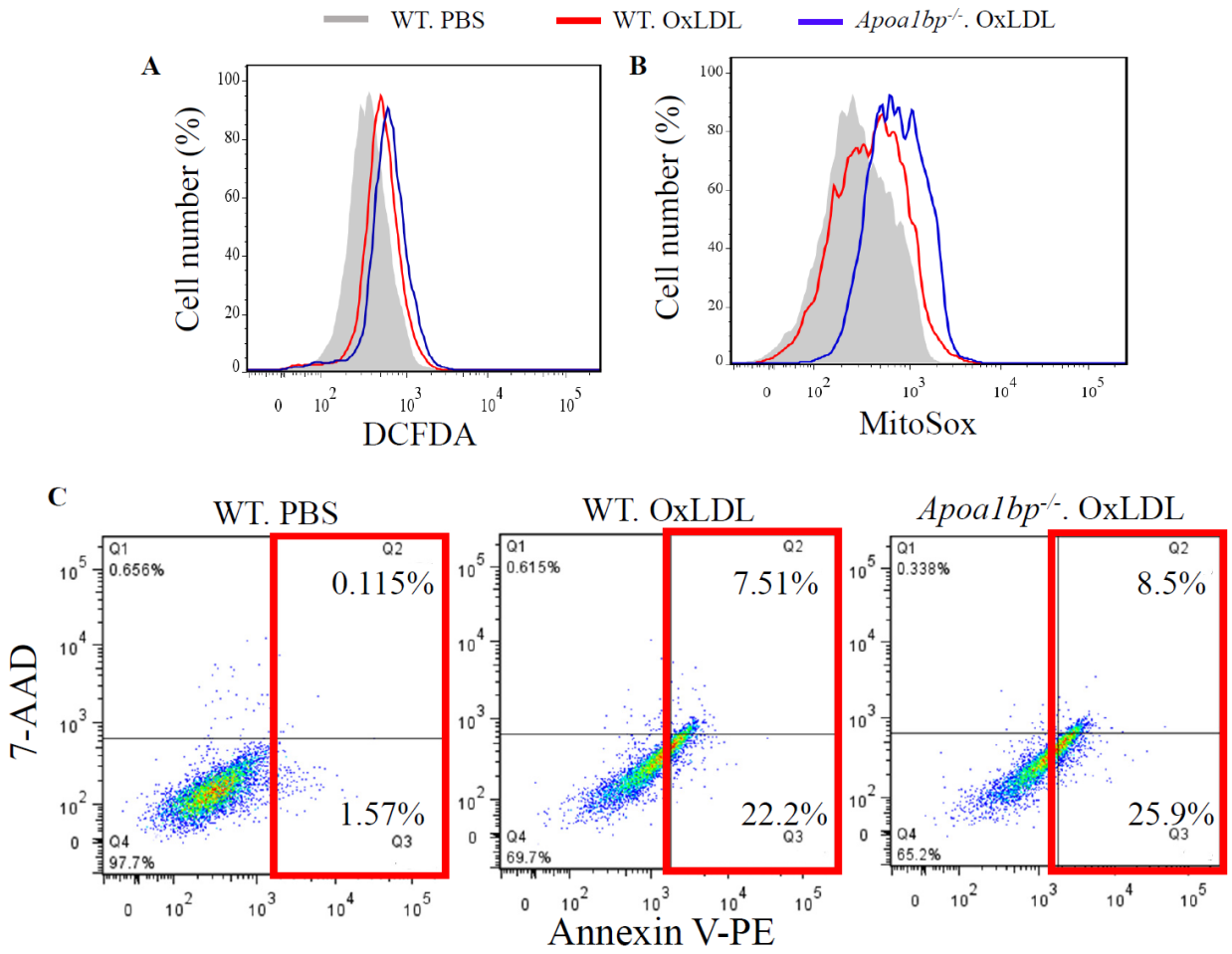
Supplemental Figure II. Expression of genes involved in autophagy in in WT and *Apoa1bp*$^{-/-}$ macrophages. BMDM isolated from *WT* and *Apoa1bp*$^{-/-}$ mice were stimulated with 25 μg/ml OxLDL for indicated time. (**A**) mRNA expression of *Sqstm1* was measured by qPCR. (**B-C**) SQSTM1/p62 expression was measured by immunoblotting and band intensities were quantified. (**D-G**) mRNA expression of *Map1lc3b*, *Atg5*, *Atg7*, and *Beclin1* were measured by qPCR. (**H-I**) Expression of Beclin1 was measured by immunoblotting and band intensities were quantified. (**J-K**) Expression of total ULK1 and phosphorylation of ULK1 was measured by immunoblotting and band intensities at 24 h were quantified. (**L**) mRNA expression of *Lamp1* was measured by qPCR. Mean±SEM; N=3-7. **, p<0.005; ***, p<0.0005; ****, p<0.0001.



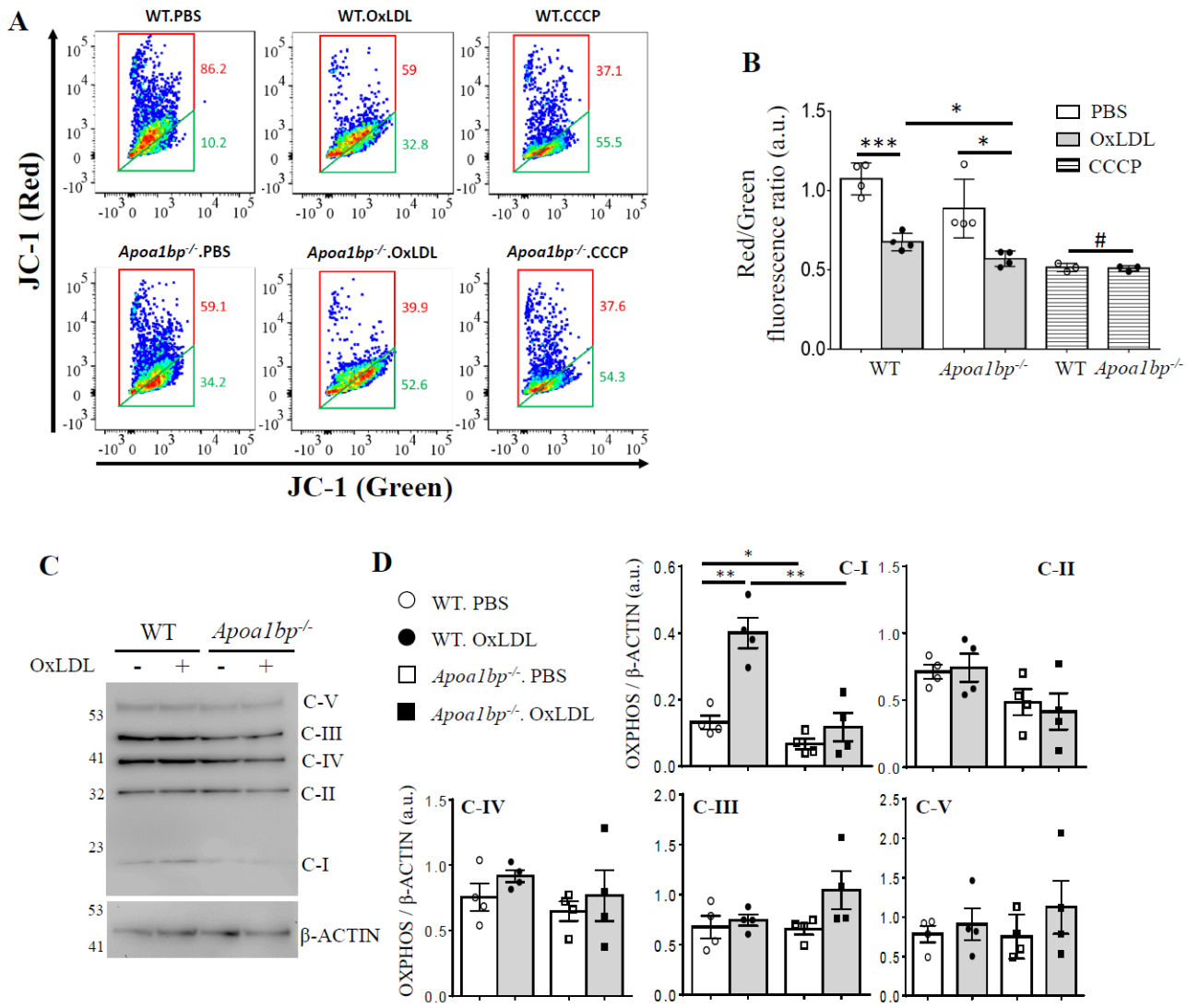
Supplemental Figure III. CD36 expression and lipid accumulation in WT and *Apoa1bp*$^{-/-}$ macrophage in response to OxLDL. BMDM isolated from *WT* and *Apoa1bp*$^{-/-}$ mice were stimulated with 25 μg/ml OxLDL for 24 h. (**A**) mRNA expression of *Cd36* was measured by qPCR. (**B-C**) CD36 expression on cell surface was measured by flow cytometry and MFI was quantified. (**D-E**) BMDM were stained with Oil Red O and for quantification cells were extracted with isopropanol and optical density at 510nm was measured. Mean±SEM; N=3-5. #, p>0.05. Scale bar, 25 μm



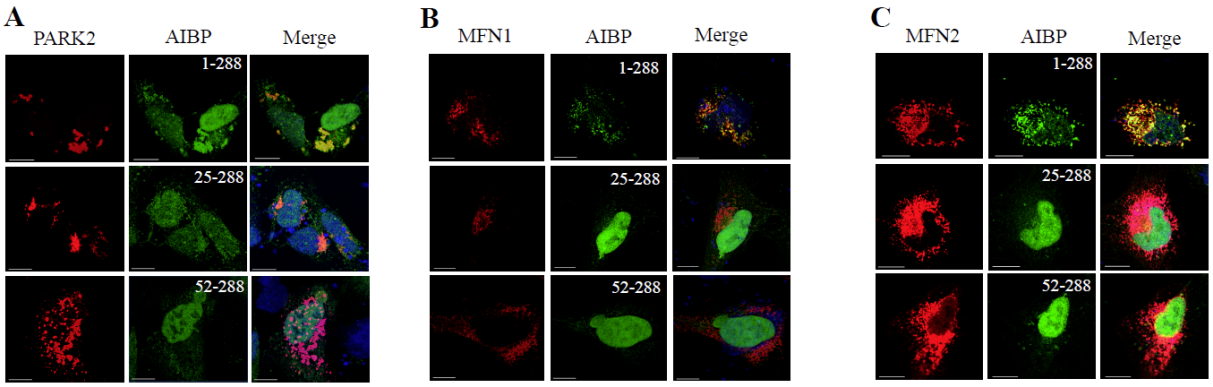
Supplemental Figure IV. Weight, plasma lipids and atherosclerosis in WT and *Apoa1bp*$^{-/-}$ mice injected with *Ldlr* ASO and fed a Western diet. (**A**) Weight gain. (**B**) Liver expression of the LDLR protein. (**C**) Total cholesterol levels at 4 and 12 weeks after the start of ASO/diet intervention. (**D**) Triglyceride levels at 12 weeks. (**E**) Aortic root atherosclerotic lesions representative Oil Red O images and size as a function of the distance from the first leaflet appearance. (**F**) Representative images of aortic root stained with Gomori's trichrome and necrotic core areas as the percentage of total lesion area. Mean±SEM; N=7-10. Scale bars, 300µm (E) and 500µm (F).



Supplemental Figure V. Representative histogram and dot plots to illustrate quantitative results shown in Figure 4. Cellular(**A**) and mitochondrial (**B**) ROS. (**C**) Apoptotic (annexin V) and necrotic (7AAD) cells.



Supplemental Figure VI. AIBP regulates mitochondrial function in macrophage. (**A and B**) Mitochondrial membrane potential was measured using JC-1 dye in a flow cytometry assay, and red/green fluorescence intensity ratio was quantified as described in Methods. Ten μM CCCP, an uncoupling agent, was used as a positive control for membrane depolarization. (**C and D**) Expression of OXPHOS complexes I-V was measured by immunoblot. Mean±SEM; N=3-4. *, p<0.05; **, p<0.005; ***, p<0.0005; ****, p<0.0001; #, p>0.05.



Supplemental Figure VII. AIBP associates with PARK2, MFN1 and MFN2. HepG2 cells were co-transfected with flag-tagged AIBPs (1-289 aa, 25-289 aa, or 52-289 aa) together with (**A**) PARK2-myc, (**B**) MFN1-myc, or (**C**) MFN2-myc. Cells were stained with anti-flag and anti-myc antibodies, and DAPI. Scale bar: 10 μm.

MAJOR RESOURCES TABLE

Animals

Species (Mouse)	Vendor or Source	Background strain	Sex
C57BL/6J	The Jackson Laboratory; Stock No: 000664	C57BL/6J	Male
Apoa1bp$^{-/-}$	Developed and bred at UCSD	C57BL/6J	Male

Cultured cells

Name	Vendor or Source	Catalog No
BMDM	Isolated from C57BL/6J and *Apoa1bp*$^{-/-}$ mice	N/A
HEK293	ATCC	CRL-1573
HepG2	ATCC	HB-8065
Murine myeloma	ATCC	CRL-1580
L929 fibroblasts	ATCC	CCL-1
Platinum-E Retroviral packaging cell line	Cell BioLabs	RV-101

Antibodies

Target antigen	Vendor or Source	Catalog No	Working concentration
LC3	Cell Signaling Technology	3868	1:1000 (WB)
VDAC	Cell Signaling Technology	4661	1:1000 (WB)
SIRT3	Cell Signaling Technology	5490	1:1000 (WB) 1:100 (IF)
Phospho-ULK1 (Ser555)	Cell Signaling Technology	5869	1:1000 (WB)
goat anti-rabbit HRP	Cell Signaling Technology	7074	1:3000 (WB)
goat anti-mouse HRP	Cell Signaling Technology	7076	1:3000 (WB)
SQSTM1/p62	Abcam	Ab56416	0.3 µg/ml (WB) 10 µg/ml (IF)
LDLR	Proteintech	10785-1-AP	0.6 µg/ml (WB)
CD68	BioLegend	137001	5 µg/ml (IF)
CD36	BioLegend	102612	2 µg/ml (FC)
CD31	BD Bioscience	562939	2 µg/ml (IF)
CD16/CD32	BD Bioscience	553141	10 µg/ml (FC)
myc	Santa Cruz Biotechnology	sc-40	0.2 µg/ml (WB) 10 µg/ml (IF) 2 µg/ml (IP)
ha	Santa Cruz Biotechnology	sc-805	0.2 µg/ml (WB)
Ub-HRP	Santa Cruz Biotechnology	sc-8017	0.2 µg/ml (WB)
ULK1	Santa Cruz Biotechnology	sc-390904	0.2 µg/ml (WB)
Beclin 1	Santa Cruz Biotechnology	sc-11427	0.2 µg/ml (WB)
Parkin	Santa Cruz Biotechnology	sc-32282	0.2 µg/ml (WB) 2 µg/ml (IP)
flag	Sigma	F3165	0.2 µg/ml (WB) 10 µg/ml (IF) 2 µg/ml (IP)
OXPHOS	Invitrogen	45-7999	1:5000 (WB)

Alpha-Smooth muscle actin eFluor-570	Invitrogen	41-9760-82	2 µg/ml (IF)
Alexa Fluor-568 conjugated goat anti-rat IgG antibody	Invitrogen	A-11077	5 µg/ml (IF)
Alexa Fluor-568 conjugated goat anti-mouse IgG antibody	Invitrogen	A-11031	5 µg/ml (IF)
Alexa Fluor-647 conjugated goat anti-mouse IgG antibody	Invitrogen	A-21237	5 µg/ml (IF)
Alexa Fluor-488 conjugated goat anti-rat IgG antibody	Invitrogen	A-11006	5 µg/ml (IF)
Alexa Fluor-488 conjugated goat anti-rabbit IgG antibody	Invitrogen	A-11034	5 µg/ml (IF)
Mouse monoclonal AIBP (BE-1)	Generated in house	N/A	10 µg/ml (IF)
Rabbit polyclonal AIBP	Generated in house	N/A	1:5000 (WB)

WB, Westernblot; IF, Immunofluorscence; FC, Flow cytometry; IP, Immunoprecipitation

Oligonucleotides

Gene	Sequence	Vendor or Source
Cyclophilin	Forward : 5'TGGAGAGCACCAAGACAGACA3' Reverse : 5'TGCCGGAGTCGACAATGAT3'	This paper
Atg5	Forward : 5'CTCCTCAGAGAAGTCTGTCCTT3' Reverse : 5'GGTTTCCAGCATTGGCTCTAT3'	This paper
Atg7	Forward : 5'TCCTGAGAGCATCCCTCTAAT3' Reverse : 5'GGCTCGACACAGATCATCATAG3'	This paper
Beclin1	Forward : 5'CAGGAACTCACAGCTCCATTAC3' Reverse : 5'CCATCCTGGCGAGTTTCAATA3'	This paper
Map1lc3b	Forward : 5'GCTTGCAGCTCAATGCTAAC3' Reverse : 5'TCTCTCTCACTCTCGTACACTT3'	This paper
Sqstm1	Forward : 5'AACAGATGGAGTCGGGAAAC3' Reverse : 5'AGACTGGAGTTCACCTGTAGA3'	This paper
Lamp1	Forward : GACCCTGAAAGTGGAGAACAA Reverse : GGGCATCAGGAAGAGTCATATT	This paper
Cd36	Forward : 5'TGGAGCAACTGGTGGATGGTT3' Reverse : 5'TTTTCTACGTGGCCCGGTTC3'	This paper

Other

Description	**Vendor and Sources**	**Catalog No**
Western diet	Envigo	TD.96121
Ldlr ASO	Ionis Pharmaceuticals, Inc	ION 713852
Total cholesterol quantification kit	Biovision	K603
Triglycerides quantification kit	EnzyChrom	ETGA-200
Oil red O	Sigma	O0625
Hematoxylin	Vector Laboratories	H-3404
Protein A resin	Genesee Scientific	20-528
Protein G resin	Genesee Scientific	20-537

Protease Inhibitor Cocktail	MCE	HY-K0010
Formaldehyde	ThermoFisher Scientific	BP531-500
Vector Red substrate	Vector	SK-5100
Simpo-Mount	IHC World	EO3-18
DMEM	Cellgro	10-013-CV
FBS	Omega Scientific	FB-01
Gentamicin	Omega Scientific	GT-10
Human LDL	Alfa Aesar	BT-603
AcLDL	Lee Biosolutions	360-28-10
Concentrator	Millipore	UFC810024
DCF-DA	Invitrogen	D399
MitoSox Red	Invitrogen	M36008
Bafilomycin A1	Calbiochem	196000
JC-1 assay kit	ThermoFisher Scientific	M34152
CCCP	Sigma-Aldrich	C2759
GenJet In Vitro DNA transfection reagent	SignaGen Laboratories	SL100488
Nu-PAGE Bis-Tris gels	Invitrogen	NP0321BOX NP0322BOX NP0323BOX
TBS	Apex Bioresearch Products	18-236B
DPBS	Corning	20-030-CV
2-Propanol	Fisher Scientific	A416500
Nucleospin RNA columns	Clontech	740984
EcoDry	Clontech	639549
KAPA SYBR FAST qPCR kit	KAPA Biosystems	KK4602
Prolong Gold AntiFade reagent with DAPI	Cell Signaling Technology	8961
Annexin V-FITC apoptosis detection kit	eBioscience	BMS500FI
Puromycin	InvivoGen	ant-pr-1
Blaticidin	InvivoGen	ant-bl-05
Hexadimenthrine bromide (polybrene)	Sigma	H9268
Mitochondria isolation kit	ThermoFisher Scientific	89874
pCHAC-mito-mKeima	Addgene	
In Situ Cell Death Detection Kit, TMR red	Sigma-Aldrich	12156792910
pCHAC-mt-mKeima	Addgene	72342
ImagePro	Media Cybernetics	software
Image J	NIH	software
GraphPad Prism	GraphPad Software	software