Supplemental Materials

Methods

Animal model

All procedures were approved by the Ethical Committee of Qilu Hospital of Shandong University (Approval No. DWLL-2018-018). A model of progressive atherosclerosis was generated in ApoE$^{-/-}$ mice (Vital River Laboratory Animal Technology Co., Beijing, China) by high-fat diet (HFD) feeding for different durations (8, 12, 18, 24 and 38 weeks) to investigate the dynamic changes in LAMP-2A expression. Immunofluorescence analysis was used to examine two stages of atherosclerosis ("early" versus "advanced" lesions, as defined by the duration of HFD feeding, 8 weeks and 24 weeks, respectively) [47, 48].

Macrophage-specific LAMP-2A (L2A)-deficient mice on a C57BL/6 background were generated using loxP insertion to selectively delete the exon region in the LAMP-2 gene that encodes the LAMP-2A variant (L2A$^{fl/fl}$ mice, Viewsolid Biotech, Beijing, China) and crossed with Cre-recombinase transgenic mice under the control of the Lysosomal M promoter (Jackson Laboratory). L2A$^{fl/fl}$ LysM-cre mice (L2A-mØKO mice) were further crossed with ApoE$^{-/-}$ mice (C57BL/6 background) to generate L2A$^{fl/fl}$/LysM-Cre/ApoE$^{-/-}$ mice (L2A-mØKO/ApoE$^{-/-}$mice). Eight-week-old L2A-mØKO/ApoE$^{-/-}$ mice and their L2A$^{fl/fl}$/LysM-Cre (-)/ApoE$^{-/-}$ littermates were fed a HFD (21% [wt/wt] fat, 1.0% cholesterol, 0.3% sodium cholate and 5% saccharose) for 16 weeks.

For *in vivo* LPS challenge, L2A-mØKO mice and their control littermates

were injected intraperitoneally with 1 mg/kg LPS (L2880, *Escherichia coli*, 055:B5, Sigma, USA). After 2 h, mice were euthanized and their serum was collected for subsequent experiments. As previous studies reported that estrogen may exert a protective effect on atherosclerosis [46], we used only male mice in this study to avoid potential conflicting results.

Animal randomization and determination of group size

A random number table was used for mouse randomization. Before grouping, all mice were first ordered by their weight from 1 to n and each of them was assigned one of the consecutive random numbers selected from the random number table. Then, all the selected random numbers were ranged from small to large and every consecutive 5 mice were chosen as one group. All data were collected and analyzed by two observers who were not aware of group assignment and treatment of mice. No samples or animals were excluded from data analysis. According to our study [49], after 16 weeks of high-fat feeding (HFD), the proportion of aortic atherosclerotic lesions in ApoE-/- mice was approximately 10%, and a deletion of macroautophagy-related genes (eg, Atg5 or p62) resulted in a double increase in aortic plaque area relative to their control groups [50-52]. Assuming type I error (α) and type II error (β) are 5% and 0.20, respectively, with a power >0.80, the sample size required is 4. By taking into account of accidental mouse death, we set n=5 in each mouse group of this study. Assuming type I error (α) and type II error (β) are 5% and 0.20, respectively, with a power >0.80, the sample size required is 4. By taking

account of accidental mouse death, we set n=5 in each mouse group of this study.

Human coronary artery sampling

Human coronary atherosclerotic plaques were obtained from autopsy specimens of 5 male body donors with coronary heart disease after sudden coronary death, and the bodies were provided by the Red Cross Society of Shandong Province, China. The human study protocol was approved by the Ethical Committee of Qilu Hospital of Shandong University (Approval No. KYLL-2016-333). The coronary arteries were embedded with optimal cutting temperature compound (OCT, Sakura, Japan) and cut into 7-μm-thick cross sections for histopathological staining.

Atherosclerotic lesion analysis

The atherosclerotic lesions in the aorta were quantified by *en face* analysis and aortic root cross-sectional measurement. For *en face* analysis, mouse aortas were placed in cold normal saline, cleaned of fatty tissue, and incised longitudinally at the thoracic aorta and aortic arch. The aortas were stained with 0.5% oil red O (ORO, Yiyuan Biotechnology, Guangzhou, China) for 30 min prior to visualization. Images were taken with a digital camera (70D, Canon, Japan). The ORO-stained lesions were quantified as a percentage of the total aortic area with SigmaScan Pro 5 (Systat Software, Inc.). For aortic root cross-sectioning, the heart with attached aortic roots was embedded in OCT, and serially sectioned at 7μm. Slides were fixed with 4% paraformaldehyde and

stained with ORO and hematoxylin-eosin (H&E; Solarbio, Beijing Solarbio Science & Technology, China) following the manufacturer's protocol. Measurement of the aortic atherosclerotic lesions was performed by investigators blind to mouse genotype.

Histopathological staining

For immunohistochemical (IHC) staining, frozen cross-sections were incubated with 3% H_2O_2 for 10 min at room temperature. Then, sections were blocked with 5% normal goat serum in PBS for 1 hour at room temperature and incubated with the corresponding primary antibodies at 4℃ overnight: rat monoclonal antibody against MOMA-2 (MCA519G; AbD, UK), rabbit polyclonal antibody against LAMP-2A (AMC2, Invitrogen, USA) and rabbit monoclonal antibody against α-smooth muscle actin (α-SMA, #19245, CST, USA). On the next day, sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (PV-9001, ZSJB-BIO, China) or goat anti-rat IgG (PV-9004, ZSJB-BIO, China) for 30 min at room temperature. The positive reactions of tissue sections were developed using an AEC Peroxidase Substrate Kit (#A2010, Solarbio, China) displayed as red color. Sections reacting with non-immune IgG as well as secondary antibodies were used as negative controls. The proportion of positive staining area of LAMP-2A was measured with the use of Image-Pro Plus software (Media Cybernetics, Inc., USA) by investigators blind to mouse genotype.

For immunofluorescent staining, frozen cross-sections were incubated with

3% H_2O_2 for 10 min and permeabilized with 0.4% Triton-X-100 in PBS for 15 min at room temperature. Then, sections were blocked with 5% normal donkey serum in PBS for 1 hour at room temperature and incubated with the above primary antibodies, as well as a mouse monoclonal antibody against CD68 (OM245833; OmnimABs, USA), rat monoclonal antibody against F4/80 (ab16911, Abcam, UK), rat monoclonal antibody against LAMP1(ab25245, Abcam, UK), and mouse monoclonal antibody against NLRP3 (AG-20B-0014; AdipoGen, USA). On the next day, sections were incubated with donkey anti-mouse IgG H&L (Alexa Fluor® 594) (ab150112, Abcam, UK), donkey anti-rat IgG H&L (Alexa Fluor® 594) (ab150156, Abcam, UK) or donkey anti-rabbit IgG H&L (Alexa Fluor® 488) (ab150061, Abcam, UK) for 1 h at room temperature. After washing, sections were stained with undiluted Fluoroshield Mounting Medium with DAPI (ab104139, Abcam, UK). Sections reacting with non-immune IgG as well as secondary antibodies were used as negative controls. An electric upright microscope (DS-Ri2, Nikon, Japan) was used for imaging.

Macrophage culture and treatment

Primary peritoneal macrophages were obtained from L2A-mØKO mice and their control littermates as previously described[3]. Briefly, 1 mL of 3% sterile starch was intraperitoneally injected into each mouse, and 3 days later, mice were euthanized and sterilized in 75% ethanol for 10 s. The skin of the inferior ventral abdomen was incised to expose peritoneum and 15 mL of cold PBS was intraperitoneally injected. The cell suspension was aspirated from the peritoneal

cavity and centrifuged at 800 rpm at 4 °C for 5 min. The supernatant was removed and the cell pellet was resuspended with DMEM supplemented with 10% FCS (Gibco) and 1% antibiotics (Gibco). The cells were counted and incubated at 37 °C for 2 h. The non-adherent cells were removed using PBS and the adherent macrophages were incubated in complete medium. The macrophages were treated with the following reagents: LPS (L2880, *E. coli*, 055:B5, Sigma, USA), ATP (A7699, Sigma, USA) and cycloheximide (CHX, 2112, CST, USA).

Western blotting

Protein lysates were extracted from peritoneal macrophages on ice using RIPA lysis buffer (R0020, Solarbio, China) supplemented with complete protease inhibitor cocktail (P6730, Solarbio, China). Protein lysates of mouse whole aorta were extracted using protein extraction kit (SD-001/SN-002, Invent, USA). Samples were separated, transferred and incubated as previously described[45] with primary antibodies against the following: LAMP-2A (AMC2, Invitrogen, USA), LAMP2 (ab13524, Abcam, UK), LAMP1 (#9091, CST, USA), NLRP3 (ab263899, Abcam, UK), pro-Caspase-1 + p10 + p12 (ab179515, Abcam, UK), ASC (D2W8U, #67824, CST, USA), IL-1β (p31) (ab234437, Abcam, UK), IL-1β (p17) (#63124, CST, USA), JNK (#9252, CST, USA), p-JNK (#9251, CST, USA), p38 MAPK (#8690, CST, USA), p-p38 MAPK (#4511, CST, USA), p44/42 MAPK (Erk1/2) (#4695, CST, USA), p-p44/42 MAPK (Erk1/2) (#4370, CST, USA), NF-κB p65 (#8242, CST, USA), p-NF-κB p65 (Ser536) (#3033, CST, USA), IκBα

(#4812, CST, USA), p-IκBα (Ser32) (#2859, CST, USA), histone H3 (#4499, CST, USA), perilipin 2 (GP40, Progen Biotechnik, Germany), SQSTM1/p62 (#39749, CST, USA), LC3B (#43566, CST, USA), Atg7 (#8558, CST, USA), Atg5 (#12994, CST, USA), Beclin-1 (#3495, CST, USA), Cathepsin B (ab214428, Abcam, UK), Cathepsin D (ab75852, Abcam, UK), and β-actin (#4970, CST, USA). On the second day, the membranes were incubated with HRP-conjugated secondary antibodies (ZB-2301, ZB-2305, ZB-2307, ZSJB-BIO, China) or at room temperature for 1.5 h. After washing, signals were detected by adding immobilon ECL ultra western HRP substrate (WBULS0500, Millipore, USA) and imaged by using a luminescent image analyzer (Amersham Imager 600, GE, USA).

Production of polyclonal antibodies against LAMP-2B and LAMP-2C

Rabbit polyclonal antibodies against LAMP-2B and LAMP-2C were produced by AtaGenix Laboratories (Wuhan, China). Synthetic peptides consisting of the cytosolic tail of mouse LAMP-2B (aa 399 to 413, FISYMIGRRKSRTGY) or that of LAMP-2C (aa 401 to the C-terminus, YLIGRRKTYAGYQTL) were used to produce antibodies that recognize only LAMP-2B or LAMP-2C, respectively.

Enrichment of active Caspase-1 and IL-1β in the cellular supernatant

Cleaved Caspase-1 (p10 + p12) and IL-1β (p17) in the cellular supernatant were enriched and assessed by Western blotting. Briefly, after macrophages were treated with LPS/ATP, the cellular supernatant was mixed with 4 volumes of cold acetone and placed in a refrigerator at -80℃ overnight. The next day,

the mixture was centrifuged at 1,0000×g for 10 min. The supernatant was carefully removed, and the tube was opened for 30 min to allow the acetone to evaporate completely. To dissolve the pellets, 1× SDS loading buffer was added, and the mixture was boiled at 100°C for 10 min.

Transfections and adenovirus infection

Adenovirus containing plasmids expressing LAMP-2A or tandem fluorescent mRFP-GFP-LC3 were produced by BioSune Biotechnology (Shanghai, China) and HANBIO (HB-AP2100001, China), respectively. For transfection of mRFP-GFP-LC3 adenovirus, mouse peritoneal macrophages (5×10^5 cells/well) were seeded in 12-well plates. For overexpression of LAMP-2A, mouse peritoneal macrophages (5×10^5 cells/well) were seeded in 6-well plates. Transfection of primary peritoneal macrophages was conducted with a MOI=300 in the presence of 5 μg/mL polybrene for 12 h. After transfection for 12h, the supernatant was removed and the cells were cultured in fresh medium for another 48 h, according to the manufacturer's instructions.

Plasmid construction and transfection

Flag-tagged LAMP-2A (Flag-LAMP-2A), Flag-HSC70, Myc-tagged NLRP3 (Myc-NLRP3) or mutant Myc-NLRP3 (Myc-NLRP3 Mut), Myc-Caspase-1 and Myc-ASC plasmids were provided by BioSune Biotechnology (Shanghai, China). The plasmids were transiently transfected into HEK293T cells with Lipofectamine 2000 (#11668019, Thermo Fisher) according to the manufacturer's instructions.

ELISA

The concentrations of mouse IL-1β (#MHSLB00 and #MLB00C, R&D Systems,), IL-18 (#7625, R&D Systems) and TNF-α (#MHSTA50 and #MTA00B, R&D Systems) in the serum and cellular supernatants were measured using ELISA kits.

RNA quantitation

Total RNA was extracted from the primary macrophages of mice in the different groups using an RNAfast2000 Total RNA Extraction Kit (Fastagen, China) according to the manufacturer's instructions. cDNA was synthesized using a PrimeScript RT Reagent Kit (Vazyme Biotech, China). Synthesis was performed by subjecting each sample to 5 minutes at 25°C for annealing, 15 minutes at 55°C to allow for reverse transcription and 2 minutes at 85°C for reverse transcriptase inactivation. The obtained cDNA was subjected to Q-PCR using SYBR Green (Vazyme Biotech, China) for the relative quantification of IL-1β, IL-18 and TNF-α mRNA expression. The following PCR amplification condition was used: 2 minutes at 50°C for Uracil-N-Glycosylase incubation, 10 minutes at 95°C for polymerase activation and 40 consecutive cycles for amplification (15 seconds at 95°C for denaturation and 60 seconds at 58°C for annealment/extension). β-actin was used to normalize mRNA levels and the 2-ΔΔCt method used for comparisons. The reverse transcription primer sequences used for the target genes were shown in Online Table 1.

Immunofluorescence

Primary peritoneal macrophages were seeded in 12-well dishes containing a cover glass. Cells were washed in PBS and fixed with 4% PFA at room temperature for 20 min followed by permeabilization with 0.3% Triton X-100 in PBS for 5 min. The fixed cells were blocked with 5% normal goat or donkey serum in PBS for 1 hour at room temperature and incubated with the corresponding primary antibodies in PBS at 4℃ overnight: the formation of NLRP3/ASC specks was used to assess NLRP3 inflammasome activation by double immunofluorescence for NLRP3 (AG-20B-0014, AdipoGen, USA) and ASC (D2W8U, #67824, CST, USA). The colocalization of NLRP3 and LAMP-2A or HSC70 (ab51052, Abcam, UK) was also investigated using double immunofluorescence. Secondary donkey anti-mouse Alexa594 (ab150112, Abcam, UK) or donkey anti-rabbit Alexa488 (ab150061, Abcam, UK) antibodies were used. Slides reacting with non-immune IgG and secondary antibodies were used as negative controls. Slides were imaged using an upright electric fluorescence microscope (DS-Ri2, Nikon, Japan).

Coimmunoprecipitation assay

Coimmunoprecipitation (Co-IP) was performed using Immunoprecipitation Kits (ab206996, Abcam, UK). Macrophages treated with or without LPS were used to detect the endogenous interaction between NLRP3 and HSC70 or LAMP-2A. These interactions were validated in HEK293T cells (ACS-4500, ATCC, USA) transfected with exogenous plasmids expressing Flag-LAMP-2A, Flag-HSC70, or Myc-NLRP3 or its mutants, Myc-Caspase-1 or Myc-ASC. For the analysis of

NLRP3 ubiquitination upon NLRP3 inflammasome activation in macrophages, the following primary antibodies were used for immunoblot analysis: Ubiquitin (#3933, CST, USA), K48-linkage Specific Polyubiquitin (#8081, CST, USA), K63-linkage Specific Polyubiquitin (#5621, CST, USA). The general Co-IP protocol consisted of sample preparation, antibody binding, and preparation of protein A/G beads, bead capture and elution. Briefly, cells were cold lysed using lysis buffer (nondenaturing) containing a protease inhibitor cocktail and mixed on a rotary mixer for 30 min at 4°C. After centrifugation at 10,000 ×g at 4°C for 10 min, the supernatants were incubated with primary antibodies overnight at 4°C on a rotary mixer. The same amount of normal rabbit IgG (#2729, CST, USA) was used as a control. The next day, the Protein A/G Sepharose was washed twice with wash buffer and suspended as a 50% slurry in wash buffer. Thirty microliters of protein A/G Sepharose beads were added to each tube and incubated for 2 h at 4°C. The protein A/G Sepharose was collected, washed 3 times with wash buffer and centrifuged at 4°C, with the supernatant aspirated between washes. Then, 40 μL of 2× SDS-PAGE loading buffer (P0015B, Beyotime, China) was added to the beads, which were boiled for five mins and analyzed by immunoblotting.

Assessment of lysosomal function

Measuring Physiological lysosomal pH Shifts

One day before the experiment, cells were seeded into a 6 well plate at a density of 1×10^6/well, After treatment with PBS, NH_4CL or CHIR99021 (CHIR)

for 1 h, cells were incubated with LysoSensorTM Yellow/Blue DND-160 (2 μM, L7545, Thermo Fisher) for 5 min. Cells were washed three times with 1X PBS. The microplate reader (Synergy HTX, BioTek, US) was set at excitation wavelength 360nm and emission wavelength 450nm for reading of LysoSensorTM Yellow/Blue DND-160 fluorescence. The average pH was calculated according to the fluorescence intensity ratio.

Measuring β-galactosidase Activity

The activity of β-galactosidase of lysosomes were measured using a micro β-galactosidase assay kit (BC2585, Solarbio, China). One day before the experiment, cells were seeded into a 6 well plate at a density of 1×10^6/well. After treatment with PBS, NH_4CL or CHIR99021 (CHIR) for 1 h, cells were collected into the centrifuge tubes. 1ml of extract per 5 million cells was added and ultrasonic processor was used to break up cells (Ice bath, 20% power, 3 s of ultrasound stimulation, 10 s interval, 30 repetitions). Then cells were centrifuged at 15000 g for 15 min and the supernatant were obtained for analysis. Sample, reagent 1, reagent 2 and reagent 3 were consecutively added into the 96-well plate. The absorbance of the samples was determined with a multi-mode microplate reader (Synergy HTX, BioTek, US) with a wavelength of 400 nm.

Measuring Active Lysosomal Cathepsin D

One day before the experiment, cells were seeded into a 12 well plate at a density of 5×10^5/well. After treatment with PBS, NH_4CL or CHIR99021 (CHIR)

for 1 h, cells were incubated with LysoTracker Red (1μM, CY-SC012, Cytoskeleton) for 15 minutes at 37°. After washing of cells three times with PBS, cells were stained with undiluted Fluoroshield Mounting Medium with DAPI (ab104139, Abcam, UK). An electric upright microscope (DS-Ri2, Nikon, Japan) was used for imaging.

Lysosome isolation

Lysosomes were isolated from primary peritoneal macrophages using a Lysosome Isolation Kit (#LYSISO1, Sigma, USA). The method consisted of two main parts: preparation of a crude lysosomal fraction (CLF) and further purification of the CLF. Briefly, approximately 2×10^8 cells were trypsinized, centrifuged, and resuspended in extraction buffer. The cells were broken in a 7-ml Dounce homogenizer and assessed using trypan blue staining solution to ascertain the degree of breakage. After the sample had been centrifuged at 1,000 ×g for 10 min, the supernatant was collected and centrifuged at 20,000 ×g for 20 min. The supernatant was removed to collect the pellet, which was suspended in a minimal volume of extraction buffer, and the resulting material was the CLF. For further purification of the CLF, it was diluted in a solution containing 19% OptiPrep density gradient medium, with the resulting sample referred to as the diluted OptiPrep fraction (DOF). Calcium chloride was added to a final concentration of 8 mM, and the DOF was incubated for 15 min to remove contaminant mitochondria and endoplasmic reticulum, after which the DOF was centrifuged at 5,000g, and the supernatant was collected. The

intactness of the lysosomes was assessed using the dye Neutral Red. All procedures were carried out according to the manufacturer's instructions.

Analysis of autophagic flux

Macroautophagy activity was analyzed in two ways: LC3B-II flux and mRFP-GFP-LC3 adenovirus transfection. For LC3B-II flux, macrophages were cultured in serum-rich (Rest) or serum-free DMEM media (activated, Act) for 12 h, and cultured for the final 4 h in the presence (+) or absence (−) of bafilomycin A1 (Baf A1, A8627, APExBIO, USA). Immunoblot analysis was used to detect autophagosome membrane–associated form (LC3B-II). LC3B-II flux was calculated as the ratio of treated lines to untreated lines after normalization to β-actin. For fluorescence microscopy analysis of autophagic flux, macrophages were transfected with adenovirus containing plasmids expressing tandem fluorescent mRFP-GFP-LC3 (HB-AP2100001, HANBIO, China) for 36 h, and cultured for the final 12 h in serum-rich (Rest) or serum-free DMEM media (activated, Act). Yellow (merge of GFP signal and RFP signal) particles represented early autophagosomes. Red fluorescent particles represent autolysosomes.

Statistical analyses

Statistical analysis was performed with SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). Continuous data with Gaussian distribution were expressed as mean and standard error of the mean (SEM). Continuous data with a non-Gaussian distribution were expressed as medians and quartiles. Categorical data were

expressed as number (%). Tests for assessing significance were detailed in each figure legend and precise p values of significant changes were indicated in each figure. The number of biological and technical replicates in each experimental group was indicated in each figure legend. Shapiro-Wilk test was first used to assess Gaussian distribution. As the sample size in the present study was relatively small (n=5), Mann-Whitney U test was used for between-group comparison, and Kruskal-Wallis test followed by Nemenyi post-hoc test used for multiple group comparisons. Multiple linear mixed effects modelling was used for two-group comparison of repeated measures with the treatment group variable being the fixed effect and the biological replicates the random effect. No experiment-wide/across-test multiple test correction was applied in this study. Difference was considered significant if $p<0.05$. Representative images reflecting the average results of each experiment were chosen in relevant figures of this study.

Online Table I

The reverse transcription primer sequences used for the target genes

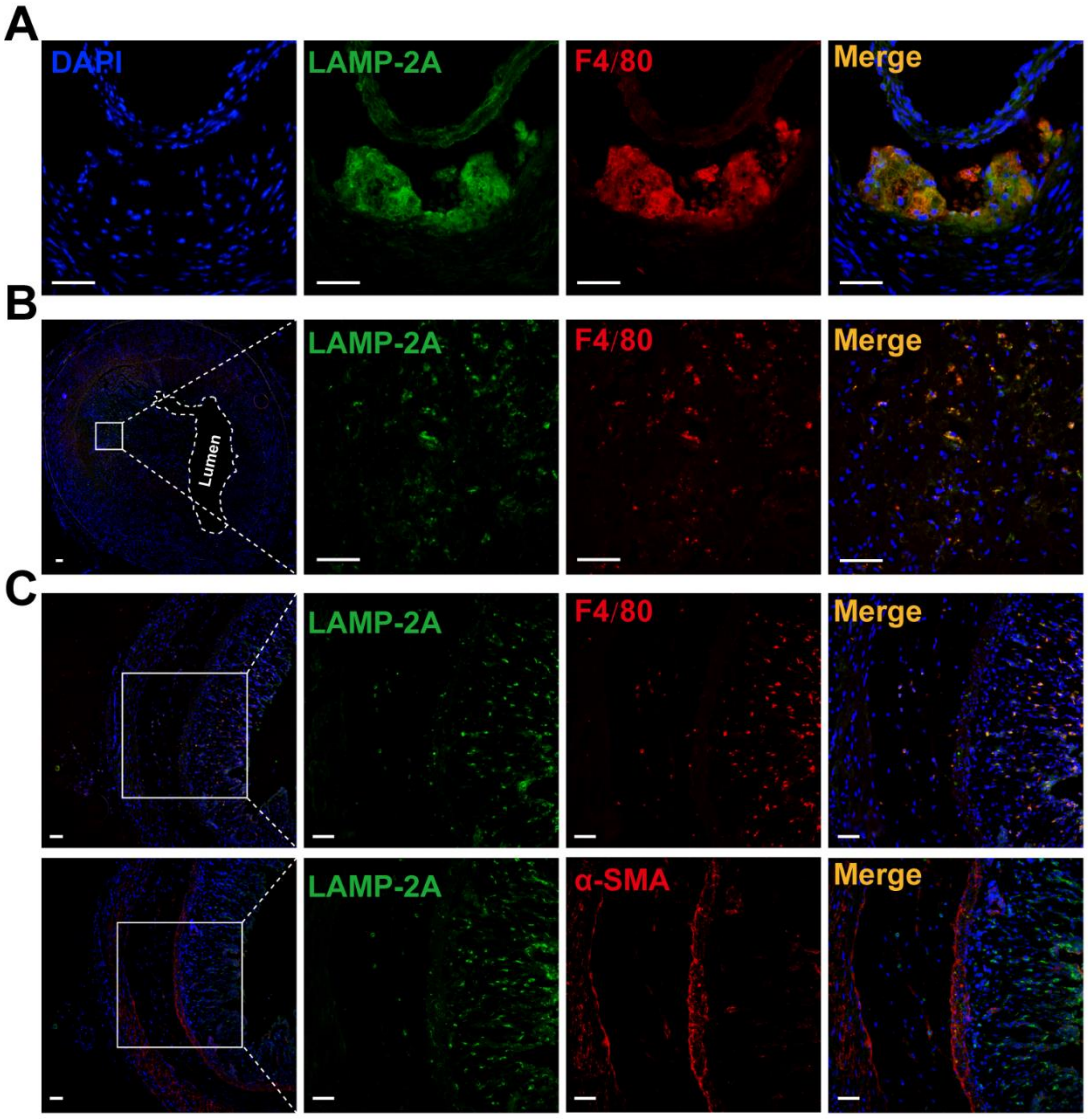
Genes	Primer type	Sequence 5'-3'
IL-1β	Forward	ACCTTCCAGGATGAGGACATGA
	Reverse	AACGTCACACACCAGCAGGTTA
IL-18	Forward	CGGCCAAAGTTGTCTGATTCC
	Reverse	ACTCTTGCGTCAACTTCAAGG
TNF-α	Forward	GCCACCACGCTCTTCTGTCT
	Reverse	TGAGGGTCTGGGCCATAGAAC
NLRP3	Forward	TGAGGGTCTGGGCCATAGAAC
	Reverse	CTGCGTGTAGCGACTGTTGAG
β-actin	Forward	TGTTACCAACTGGGACGACA
	Reverse	CTGGGTCATCTTTTCACGGT

Online Table II

Metabolic profile in control ApoE$^{-/-}$ mice and L2A-møKO/ApoE$^{-/-}$ Mice

Characteristics	**ApoE$^{-/-}$** (n=7)	**L2A-møKO/ApoE$^{-/-}$** (n=7)	**p values**
BW (g)	27.30 (26.80, 28.10)	25.60 (24.10, 26.60)	0.053
Glucose (mmol/L)	8.00 (7.20, 9.00)	7.50 (7.00, 9.10)	0.710
TC (mmol/L)	35.11 (31.05, 48.97)	36.50 (28.02, 42.71)	0.535
TG (mmol/L)	2.29 (1.81, 2.91)	2.31 (1.68, 2.65)	0.710
HDL-C (mmol/L)	1.40 (1.28, 1.75)	1.55 (1.36, 1.73)	0.805
LDL-C (mmol/L)	13.87 (13.57, 22.65)	18.47 (11.77, 18.83)	0.710

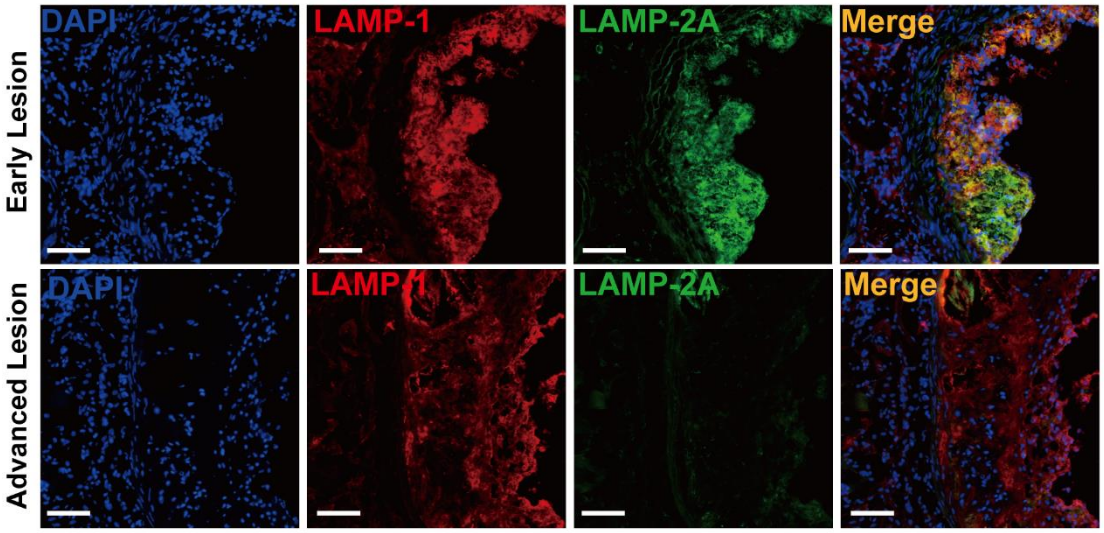
BW, body weight; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. n=7 per group. Mann-Whitney Test was used for statistical comparisons. Data were presented as medians and quartiles (25th and 75th percentiles). There was no statistically significant difference in these parameters between two groups of mice.



Online Figure I. Detection of CMA marker LAMP-2A *in vivo*.

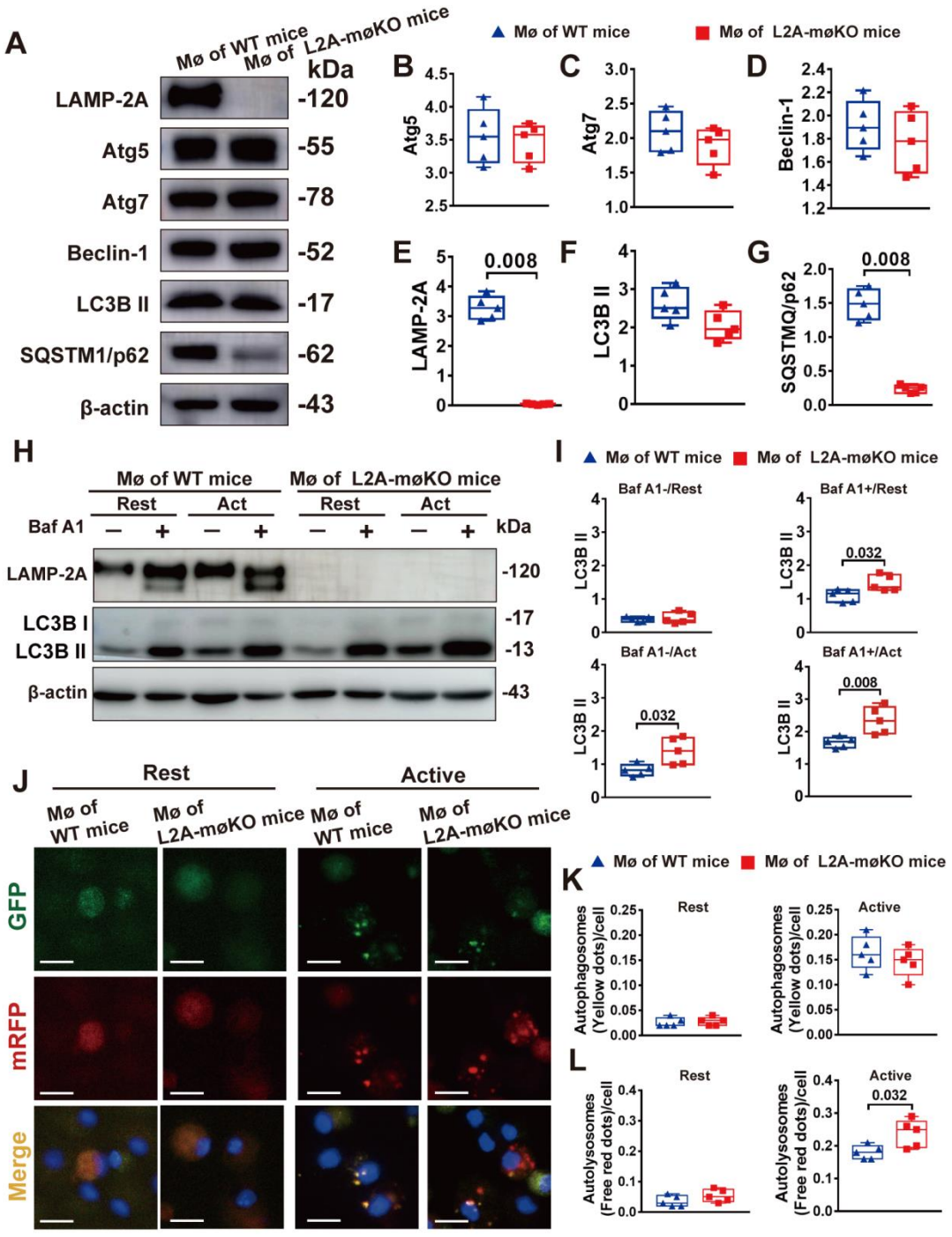
(**A**) Representative immunofluorescence analysis to detect colocalization (yellow particles) of LAMP-2A (green particles) and F4/80 (red particles) in frozen sections from the aortic root of ApoE$^{-/-}$ mice (n=5, male) fed a HFD for 12 weeks. Scale bar=100μm. (**B**) Representative immunofluorescence analysis to detect colocalization (yellow particles) of LAMP-2A (green particles) and F4/80 (red particles) in frozen sections from human coronary atherosclerotic plaques. Scale bar=100μm. (**C**) Representative immunofluorescence analysis

to detect colocalization (yellow particles) of LAMP-2A (green particles), F4/80 (red particles) and α-SMA (red particles) in frozen sections from the colitis tissues of mice (n=5, male). Scale bar=100μm.



Online Figure II. Alterations of LAMP-1 and LAMP-2A in progressive atherosclerosis

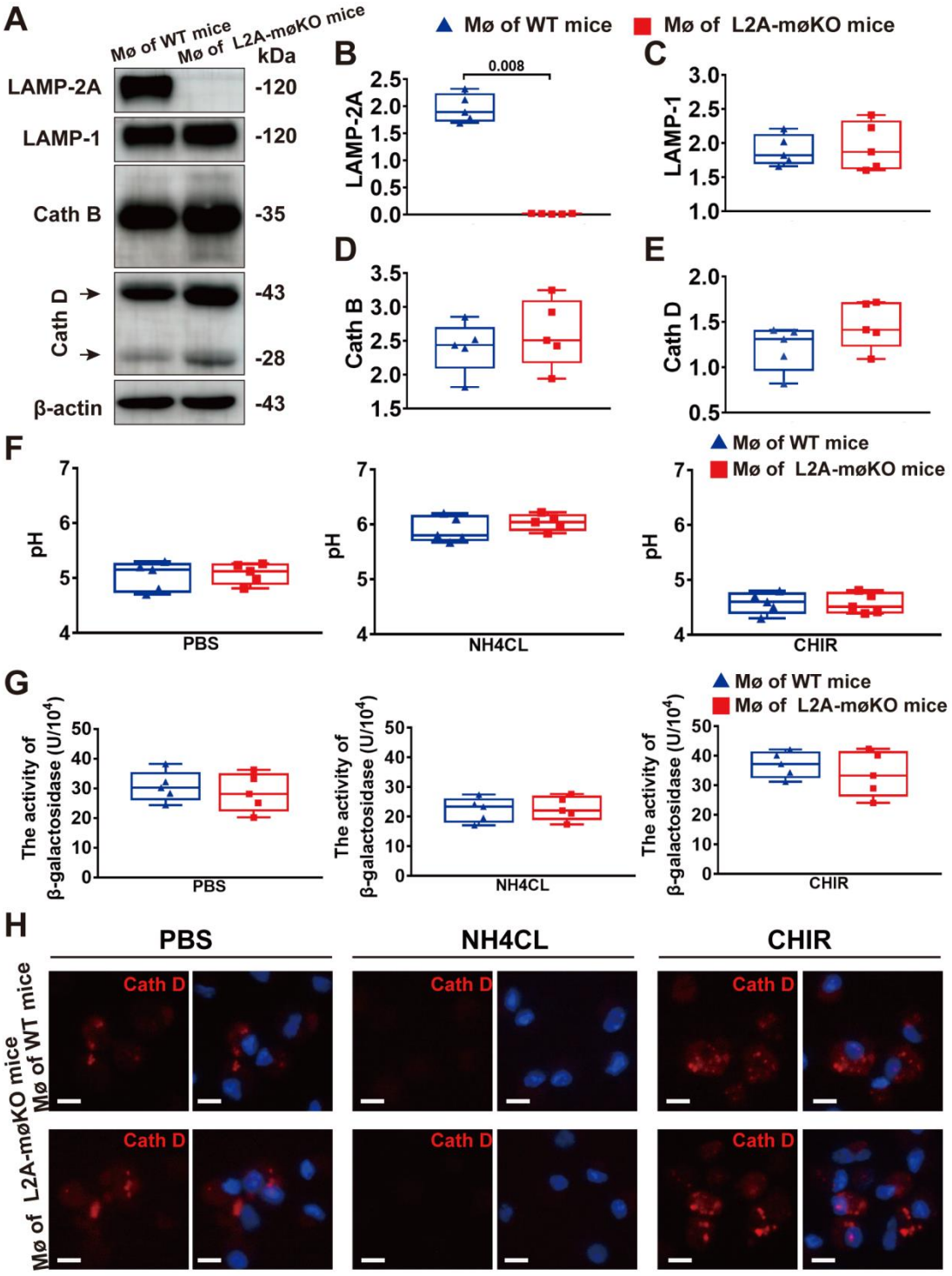
Representative double immunofluorescence images for LAMP-1 (red particles) and LAMP-2A (green particles) in “early lesions” (fed a HFD for 8 weeks) and “advanced lesions” (fed a HFD for 24 weeks). n = 5 in each group. Scale bar=100μm.



Online Figure III. Effect of LAMP-2A deficiency on macroautophagy.

(A) Representative immunoblot images and (**B-G**) quantitative analysis of the indicated macroautophagy-related protein expression in primary peritoneal macrophages from wild-type (WT, C57 background, male) and macrophage-specific L2A-knockout (L2A-mØKO, C57 background, male) mice. Five

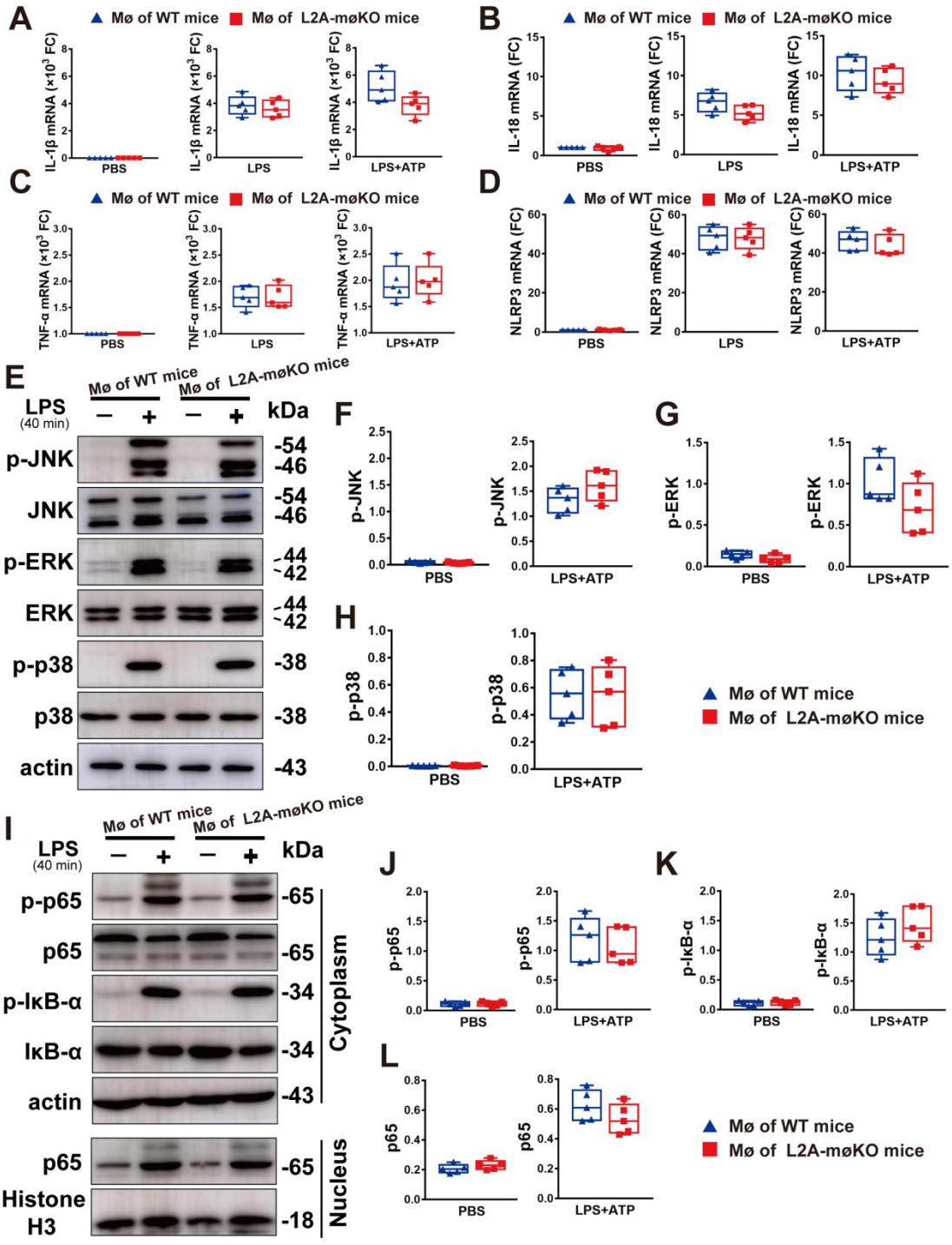
independent experiments were performed. Statistical analysis was performed using Mann-Whitney Test. Data were presented as medians and quartiles. Atg5, autophagy protein 5; Atg7, autophagy protein 7; LC3B, microtubule-associated proteins 1A/1B light chain 3B. (**H**) Representative immunoblot images and **(I)** quantitative analysis to detect the autophagy-related protein LC3 in its cytosolic form (LC3-I) and autophagosome membrane–associated form (LC3-II) in primary peritoneal macrophages from wild-type (WT, C57 background, male) and macrophage-specific L2A-knockout (L2A-mØKO, C57 background, male) mice. Macrophages were cultured in serum-rich (Rest) or serum-free DMEM media (activated, Act) for 12 h, and cultured for the final 4 h in the presence (+) or absence (−) of bafilomycin A1 (Baf A1). Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between WT and L2A-møKO groups. Statistical analysis was conducted using Mann-Whitney Test. **(J)** Representative images and **(K-L)** statistical analysis of autophagic flux in primary peritoneal macrophages from wild-type (WT, C57 background) and macrophage-specific L2A-knockout (L2A-mØKO, C57 background) mice by using mRFP-GFP-LC3 adenovirus transfection. Five independent experiments were performed. Data were presented as medians and quartiles. Statistical analysis was made using Mann-Whitney Test. Comparison was made only between WT and L2A-møKO groups. Scale bar=10μm.



Online Figure IV. Effect of LAMP-2A deficiency on lysosomal function.

(A) Representative western blot images and (**B-E**) quantitative analysis of protein expression of LAMP-2A, LAMP-1, Cathepsin B (Cath B) and Cathepsin D (Cath D) in primary peritoneal macrophages from wild-type (WT, C57

background, male) and macrophage-specific L2A-knockout (L2A-mØKO, C57 background, male) mice. Five independent experiments were performed. Data were presented as medians and quartiles. Statistical analysis was conducted using Mann-Whitney Test. (**F**) LysoSensor pH quantitation to detect the pH values of lysosomes in primary peritoneal macrophages from WT and L2A-mØKO mice (male). Macrophages were treated with PBS, NH4CL and CHIR99021 (CHIR) for 1 h, respectively. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between WT and L2A-møKO groups Statistical analysis was performed using Mann-Whitney Test. (**G**) Quantitation of the activity of β-galactosidase in primary peritoneal macrophages from WT and L2A-mØKO mice. Macrophages were treated with PBS, NH4CL and CHIR99021 (CHIR) for 1 h, respectively. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between WT and L2A-møKO groups. Statistical analysis was done using Mann-Whitney Test. (**H**) Representative immunofluorescence images of SiR-Lysosome assay for measuring active lysosomal cathepsin D in primary peritoneal macrophages from WT and L2A-mØKO mice. Macrophages were treated with PBS, NH4CL and CHIR99021 (CHIR) for 1 h, respectively. Five independent experiments were performed. Scale bar=10μm.

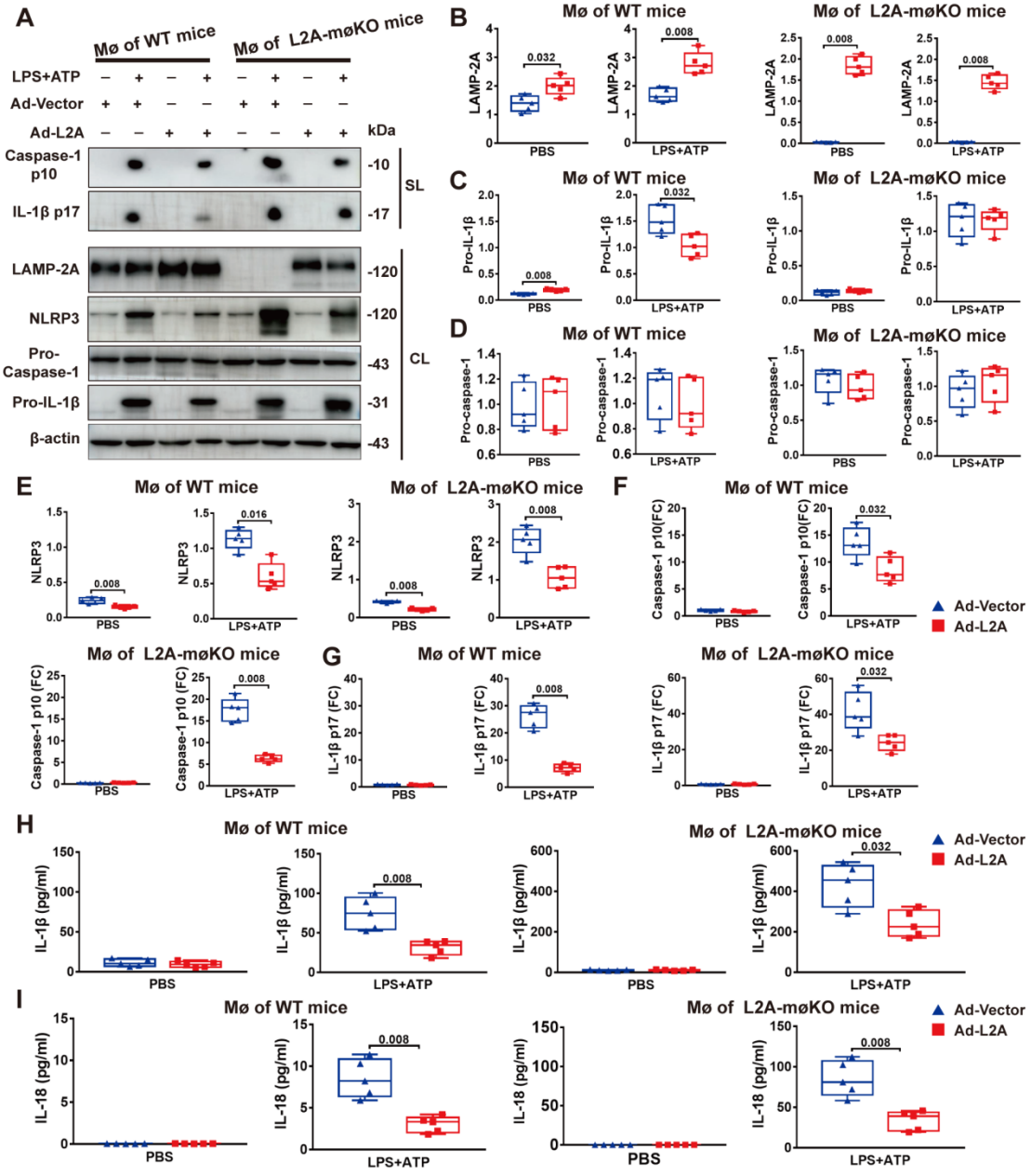


Online Figure V Effect of LAMP-2A deficiency on transcription of IL-1β, IL-18, TNF-α and NLRP3

(**A-C**) RT-PCR analysis of IL-1β, IL-18 and TNF-α mRNA expression in mouse peritoneal macrophages from wild-type (WT) and macrophage-specific L2A-knockout (L2A-mØKO) mice. Macrophages were primed with LPS (100 ng/ml)

for 8 h, followed by stimulation with ATP (5 mM) for 30 min. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between WT and L2A-møKO groups. Statistical analysis was made using Mann-Whitney Test. (**D**) RT-PCR analysis of NLRP3 mRNA expression in mouse peritoneal macrophages from wild-type and macrophage-specific L2A-knockout (L2A-mØKO) mice. Macrophages were primed with LPS (100 ng/ml) for 8 h, followed by stimulation with ATP (5 mM) for 30 min. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between WT and L2A-møKO groups. Statistical analysis was carried out using Mann-Whitney Test. (**E-H**) Representative immunoblot images and quantitative analysis of protein expression of MAPK pathways in mouse peritoneal macrophages from wild-type and macrophage-specific L2A-knockout (L2A-mØKO) mice. Macrophages were treated with LPS (100 ng/ml) for 40 min. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between WT and L2A-møKO groups. Statistical analysis was conducted using Mann-Whitney Test. (**I-L**) Representative immunoblot images and quantitative analysis of protein expression of IκBa/NF-κB pathways from mouse peritoneal macrophages of wild-type and macrophage-specific L2A-knockout (L2A-mØKO) mice. Macrophages were treated with LPS (100 ng/ml) for 40 min. Five independent experiments were performed. Data were presented as medians and quartiles.

Comparison was made only between WT and L2A-møKO groups. Statistical analysis was done using Mann-Whitney Test. There was no significant difference in these parameters between two groups of mice.



Online Figure VI Gain-of Function experiments investigating the effect of LAMP2A rescue on NLRP3 inflammasome activation

(**A**) Representative western blot images and **(B-G)** quantitative analysis of protein expression in cellular supernatants (SNs) and cell lysates (CLs) of mouse peritoneal macrophages from WT and L2A-mØKO mice infected with adenovirus vector or adenovirus expressing LAMP-2A (L2A). Macrophages were stimulated with LPS (100 ng/ml) for 8 h with or without subsequent

stimulation with ATP (5 mM) for 30 min. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between Ad-Vector and Ad-L2A groups. Statistical analysis was performed using Mann-Whitney Test. **(H-I)** ELISA was used to detect IL-1β and IL-18 levels in the supernatants of mouse peritoneal macrophages from WT and L2A-mØKO mice. Macrophages were infected with adenovirus vector or adenovirus expressing LAMP-2A, and stimulated with LPS (100 ng/ml) for 8 h with or without subsequent stimulation with ATP (5 mM) for 30 min. Five independent experiments were performed. Data were presented as medians and quartiles. Comparison was made only between Ad-Vector and Ad-L2A groups. Statistical analysis was made using Mann-Whitney Test.

A

CLUSTAL 0(1.2.0) multiple aliqnment of human Caspase-1

```
ASC   MADKVLKEKRKLFIRSMGEGTINGLLDELLQTRVLNKEEMEKVKRENATVM
KFERQ ------------------------------------------------------------

ASC   DKTRALIDSVIPKGA----------------------------------------------
KFERQ ------------------------------------------------------------

ASC   ----------PEHKTSDSTFLVFMSHGIREGICGKKHSEQVPDILQLNAIFNMKK
KFERQ ------------------------------------------------------------

ASC   AHIEKDFIAFCSSTPDNVSWRHPTMGSVFIGRLIEHMQEYACSCDVEEIFRK
KFERQ ------------------------------------------------------------

ASC   VRFSFEQPDGRAQMPTTERVTLTRCFYLFPGH
KFERQ ----------------------------------
```

CLUSTAL 0(1.2.0) multiple aliqnment of mouse Caspase-1

```
ASC   MADKILRAKRKQFINSVSIGTINGLLDELLEKRVLNQEEMDKIKLANITAMDKA
KFERQ ------------------------------------------------------------

ASC   RDLCDHVSKKGPQASQ--------------------------------------------
KFERQ ------------------------------------------------------------
                      182   186
ASC   TEFQHLSPRVGAQVDLREMKLLLEDLGYTVKVKENLTALEMVKE--------------
KFERQ ----------------------KFERQ-------------------------------

ASC   ------------------------SWRHPVRGSLFIESLIKHMKEYAWSCDLEDIFRKVRFSF
KFERQ ------------------------------------------------------------
          379 383
ASC   EQPEFRLQMPTADRVTLTKRFYLFPGH
KFERQ ------KFERQ-----------------------------------
```

B

CLUSTAL 0(1.2.0) multiple aliqnment of human ASC

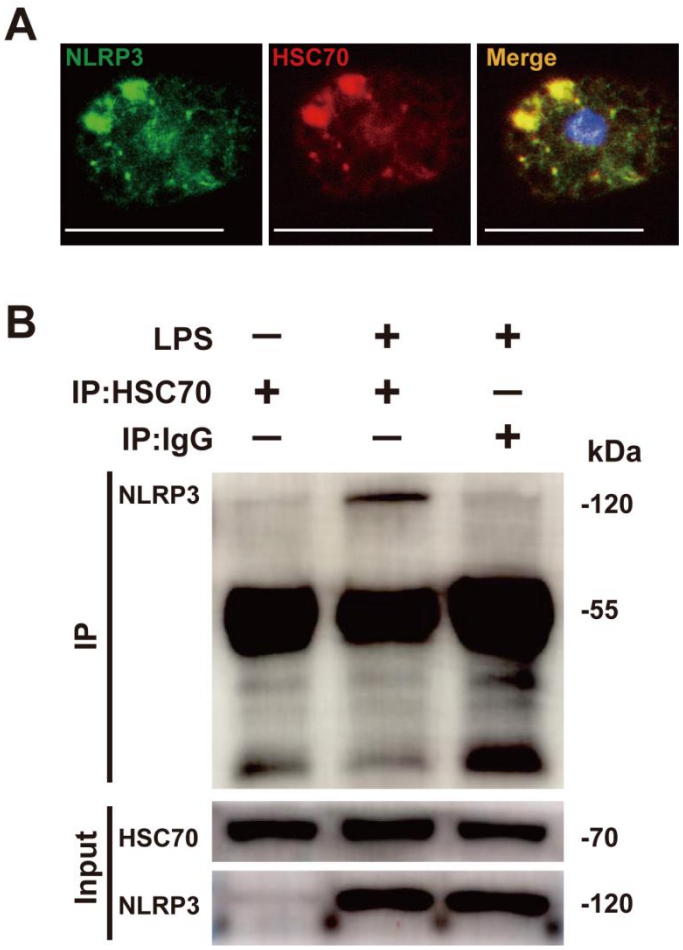
```
ASC   MGRARDAILDALENLTAEELKKFKLKLLSVPLREGYGRIPRGALLSMDALDLT
KFERQ ------------------------------------------------------------

ASC   DKLVSFYLETYGAELTANVLRDMGLQEMAGQLQAATHQGSGAAPAGIQAPP
KFERQ ------------------------------------------------------------

ASC   QSAAKPGLHFIDQHRAALIARVTNVEWLLDALYGKVLTDEQYQAVRAEPTNP
KFERQ ------------------------------------------------------------

ASC   SKMRKLFSFTPAWNWTCKDLLLQALRESQSYLVEDLERS
KFERQ ----------------------------------------
```

CLUSTAL 0(1.2.0) multiple aliqnment of mouse ASC

```
ASC   MGRARDAILDALENLSGDELKKFKMKLLTVQLREGYGRIPRGALLQMDAIDL
KFERQ ------------------------------------------------------------

ASC   TDKLVSYYLESYGLELTMTVLRDMGLQELAEQLQTTKEESGAVAAAASVPAQ
KFERQ ------------------------------------------------------------

ASC   STARTGHFVDQHRQALIARVTEVDGVLDALHGSVLTEGQYQAVRAETTSQD
KFERQ ------------------------------------------------------------

ASC   KMRKLFSFVPSWNLTCKDSLLQALKEIHPYLVMDLEQS
KFERQ ------------------------------------
```

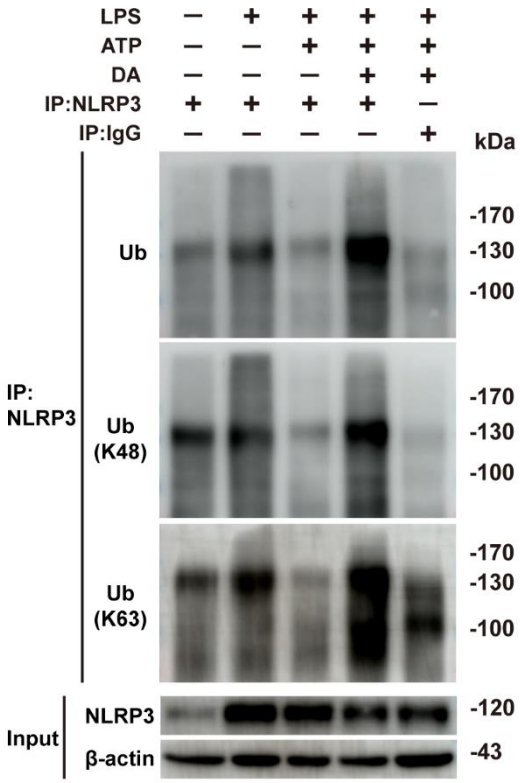
Online Figure VII. Alignment of the amino acid sequences of mouse and human Caspase-1 and ASC with the KFERQ motif

(A) The amino acid sequence of human and mouse Caspase-1. In the KFERQ motifs, Q (in red) and the other four amino acids (in purple) were marked. (B)The amino acid sequence of human and mice ASC.



Online Figure VIII. NLRP3 interacts with HSC70

(A) Colocalization (yellow particles) of NLRP3 (green particles) with HSC70 (red particles) in peritoneal macrophages treated with LPS and ATP. Bar=20μm. (**B**) Co-IP of endogenous NLRP3 with HSC70 from LPS-primed mouse peritoneal macrophages. Five independent experiments were performed. Scale bar=10μm.



Online Figure IX. NLRP3 protein undergoes de-ubiquitination during NLRP3 inflammasome activation

Immunoblot analysis of lysates from WT peritoneal macrophages treated with LPS, ATP or dopamine (DA), followed by IP with anti-NLRP3, probed with anti-Ub, anti-K48-Ub or anti-K63-Ub. DA, a compound proved to promote NLRP3 ubiquitination, was used as a positive control. Five independent experiments were performed.