SUPPLEMENTAL MATERIAL

Supplemental Methods.

Bone marrow transplantation. At 8 weeks of age, female or male *Ldlr*$^{-/-}$ mice were lethally irradiated with 2 doses of 6.5 Gy from a cesium g source with a 4 h time interval. At 24 h after irradiation, mice were injected with 5-10*10^{6} bone marrow (BM) cells in DMEM containing 2% FBS from control, *Myl-Abc*^{dko}, *Nlrp3*$^{-/-}$, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice. All BM donors were littermate controls. Mice were allowed to recover for 5 weeks after BM transplantation before Western-type diet (WTD; Harlan Teklad TD88137) feeding for 4, 8, or 12 weeks, or chow diet feeding. After the recovery period, peripheral blood was collected and DNA isolated to determine the efficiency of BM reconstitution by quantification of wild-type *Ldlr* DNA compared with actin (for the *Myl-Abc*^{dko} and control BM transplantation) or wild-type *Nlrp3* or *Caspase1* DNA compared with actin (for transplantation with *Nlrp3*$^{-/-}$ or *Caspase1*$^{-/-}$*11*$^{-/-}$ BM). For all BM transplantation studies, reconstitution was >95%. For all studies, animals were randomized according to genotype or treatment.

Total Cholesterol and IL-18 Analysis in mouse plasma. Blood samples were collected from mice by tail bleeding into heparin-coated tubes. Plasma was separated by centrifugation, and assayed for total cholesterol using an enzymatic kit from Wako (Cholesterol E; 999-02601) and IL-18 using an ELISA from R&D systems (7625).

IL-18 and IL-1β Analysis in human plasma. Patients were subjected to a 12 hour fast before blood collection into heparin-coated tubes. Plasma was collected and assayed for IL-1β and IL-18 using ELISAs from R&D systems (HSBL00D and 7620, respectively). Since no data had been obtained on these parameters in humans carrying *ABCA1* mutations previously, we could not perform power calculations to determine sample size, and therefore included as many *ABCA1* mutations carriers and their age-and-gender-matched controls as possible, from the Academic Medical Center, Amsterdam, and the University of Pennsylvania.

Splenic homogenates, isolation of splenic CD11b$^+$, CD115$^+$, and Ly6G$^+$ cells, Caspase-1 cleavage, Caspase-11 cleavage, cholesterol accumulation, inflammasome priming, IL-1β and IL-18 secretion, ATP secretion. Spleens were mashed on a 40 μm filter and red blood cells were lysed (BD Pharm Lyse, BD Bioscience). CD11b$^+$, CD115$^+$, and Ly6G$^+$ cells were isolated using CD11b$^+$, CD115$^+$, or Ly6G$^+$ coated beads, respectively (Miltenyi Biotec). Caspase-1 and Caspase-11 cleavage were assessed by Western blot using primary antibodies from eBioscience (14-9832) and Sigma (C1354), respectively, and an anti-rat secondary antibody from Cell Signaling (7077). To assess free cholesterol and total cholesterol accumulation in Ly6G$^+$ neutrophils, lipids were extracted using the Folch method, and subsequently dissolved in isopropanol. Free cholesterol and total cholesterol were assessed using kits from Wako (Free Cholesterol E; 993-02501, and Cholesterol E; 999-02601, respectively). To assess inflammasome priming, RNA from splenic CD11b$^+$ or Ly6G$^+$ cells was isolated using a Qiagen RNeasy kit and cDNA was synthesized using kits from Thermo Scientific (Maxima First Strand cDNA synthesis kit; 1642). Primers were exactly similar to those reported previously.[1, 2] SYBR Green Master Mix was from Applied Biosystems (by ThermoScientific; 4385612) and qPCR was run on a StepOne Plus Real-time PCR Systems from Applied Biosystems. IL-1β and IL-18 secretion from splenic CD11b$^+$ cells were determined at 4 hours after collection using ELISAs from R&D Systems (MLB00C and 7625, respectively). ATP secretion from CD115$^+$ cells was determined at 2 hours after collection using a luciferase assay from Molecular Probes (A22066). Apyrase (5 units) treatments were used as a negative control. Apyrase was from Sigma Aldrich (A6535).

Thioglycollate-elicited macrophages. Mice were injected with thioglycollate (1 ml; 3% thioglycollate (BD; 211716) in PBS) i.p. and peritoneal macrophages were collected via PBS lavage at 4 days after the injection. Samples were analyzed for caspase-1 by Western blot or inflammasome priming was assessed after mRNA isolation, as described above. For analysis of IL-18 cleavage, we used a primary antibody from abcam (ab71495).

Bone marrow derived macrophages and inflammasome activation. Bone marrow was isolated by flushing bones with PBS. Cells were then cultured in DMEM supplemented with 10% FBS, 1% pen-strep, and 20% L929-cell conditioned medium for a period of 7 days, to obtain fully differentiated macrophages. To induce NLRP3 inflammasome activation, cells were stimulated with LPS (100 ng/ml; Sigma-Aldrich, L3024) for 4 h. Cells were washed and subsequently incubated with ATP (500 μM; Sigma-Aldrich, GE27-2056-01) for 2 h. IL-1β and IL-18 were measured in the medium using ELISA as described above, and cells were lysed to assess cell protein.

Microscopy. For imaging of cholesterol crystals, spleens were mashed and immediately transferred to DMEM containing 10% FBS and 1% pen-strep, which was kept at 37°C throughout the experiment. Cells were kept at 37°C while imaging cholesterol crystals. We added Cell Tracker (Life Technologies; C2110) to visualize the cells, and confocal microscopy was performed using a Leica SP2 AOBS confocal laser-scanning microscope, as described.[3] Cells were imaged using a Nikon Ti (Eclipse) inverted microscope with Ultraview Spinning Disc (CSU-X1) confocal scanner (Perkin Elmer). Images were captured with an Orca-ERCamera using Volocity (Perkin Elmer).

For imaging of free cholesterol accumulation in lysosomes, splenic $CD115^+$ and $CD11b^+$ cells were isolated as described above and allowed to adhere o/n in DMEM supplemented with 10% FBS, 1% pen-strep and 20% L929-cell conditioned medium. Cells were then stained with LysoTracker Red DND-99 (Invitrogen; L7528), 100 nM for 1 h at 37°C, fixed using 3% paraformaldehyde, and subsequently stained with filipin (Sigma; F-9765) at 0.05 mg/ml in 10% FBS for 2 h at room temperature. After 3 PBS washes, cells were mounted using ProLong Gold Antifade Mountant with DAPI (Thermofisher; P3693). Cells were imaged using a Leica DMI6000B microscope running Leica software.

For imaging of neutrophil extracellular traps, blood neutrophils were isolated using Ly6G coated beads (Miltenyi Biotec) according to the manufacturer's instructions. Cells were plated on Poly-L-Lysine coated plates and allowed to adhere for 30 min at 37°C in DMEM containing 10% FBS and 1% pen-strep. Cells

were washed using PBS and then incubated in HBSS for 4 h at 37°C with or without 200 ng/ml PMA (Sigma Aldrich; P1585). After another PBS wash, cells were fixed in 2% paraformaldehyde (15 min; RT). Cells were washed twice using PBS and incubated at 4°C o/n in PBS 1% BSA containing Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (1:250; Abcam; ab5103). Subsequently, cells were washed and incubated in PBS containing Anti-rabbit CF 647 (Sigma;SAB4600184) antibody (1:200) for 1 h at RT. Cells were mounted using ProLong Gold Antifade Mountant with DAPI (Thermofisher; P3693) and imaged using a Leica DMI6000B microscope running Leica software. The overlap of citrullinated histones and DAPI was quantified using Image-Pro Plus software (Media Cybernetics, USA) and expressed as % of total cells.

Flow cytometry. Blood samples were collected by tail bleeding into EDTA coated tubes and immediately put on ice. For analysis of blood leukocyte subsets, tubes were kept at 4°C for the whole procedure unless stated otherwise. Red blood cells (RBCs) were lysed (BD Pharm Lyse, BD Bioscience) and white blood cells were centrifuged, washed, and resuspended in HBSS (0.1% BSA, 5 mM EDTA). Cells were stained with a cocktail of antibodies against CD45-APC-Cy7, Ly6-C/G-PerCP-Cy5.5 (BD Pharmingen; 557659 and 561103, respectively), and CD115-APC (eBioscience; 17-1152). Monocytes were identified as $CD45^{hi}CD115^{hi}$ and further separated into Ly6-C^{hi} and Ly6-C^{lo} subsets, and neutrophils were identified as $CD45^{hi}CD115^{lo}$Ly6-C/G^{hi} (Gr-1).

For splenic homogenates, spleens were meshed onto a 40 μm filter and red blood cells were lysed (BD Pharm Lyse, BD Bioscience), and white blood cells were centrifuged, washed, and resuspended in HBSS (0.1% BSA, 5 mM EDTA). For further analysis, tubes were kept at 4°C for the whole procedure unless stated otherwise. To assess caspase-1 cleavage by caspase-1 FLICA staining, splenic cells were stained with antibodies against CD11b-APC (eBioscience; 17-0112) and F4/80-PECy7 (eBioscience; 25-4801), and subsequently incubated with caspase-1 FLICA probe (Immunochemistry; 98) for 1 h at 37°C, before analysis by flow cytometry. For lysosomal function studies, splenic cells were stained with antibodies against CD11b-APC (eBioscience; 17-0112) and F4/80-PB (eBioscience; 48-4801), and subsequently

incubated with LysoTracker Red DND-99 (Invitrogen; L7528), 100 nM for 1 h at 37°C, before analysis by flow cytometry. In other experiments, splenic cells were stained with antibodies against CD11b-APC (eBioscience; 17-0112) and F4/80-PECy7 (eBioscience; 25-4801), and subsequently incubated with LysoSensor Blue DND-167 (Invitrogen; L7533), 1 μM for 1 h at 37°C, before analysis by flow cytometry. For mitochondrial ROS studies, splenic cells were stained with antibodies against CD11b-APC (eBioscience; 17-0112) and F4/80-PB (eBioscience; 48-4801), and subsequently incubated with 5 μM MitoSOX red (Invitrogen; M36008) for 1 h at 37°C, before analysis by flow cytometry. For dead cell assays (necrosis/apoptosis), splenic cells were stained with a cocktail of antibodies against antibodies against CD11b-APC (eBioscience; 17-0112) and F4/80-PB (eBioscience; 48-4801), and subsequently cell death (necrosis/apoptosis) was assessed using the PI/Annexin V assay according to the manufacturer's instructions (Invitrogen; V13242), before analysis by flow cytometry. All samples were analyzed on an LSRII (BD Biosciences), running FACSDiVa software.

Reconstituted HDL injections. Reconstituted HDL (rHDL; CSL-111) was kindly provided by Dr. Samuel Wright. We injected control and *Myl-Abc*^{dko} BM transplanted *Ldlr*$^{-/-}$ mice that had been fed a WTD for 1 week with 120 mg/kg rHDL (i.v.). After one week, plasma IL-18 levels were determined as described above.

LPS mortality experiments. At 8 weeks of age, control, *Myl-Abc*^{dko}, *Nlrp3*$^{-/-}$, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, and *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice were injected with LPS (Invivogen; O111:B4) at a dose of 20 mg/kg (i.p.). Mice were fed a chow diet. Mortality was monitored every 6 h for a total duration of 48 h.

Aortic digestions. Aortas were isolated from control and *Myl-Abc*^{dko} BM transplanted *Ldlr*$^{-/-}$ mice after 8 weeks of WTD. Briefly, whole aortas were isolated, cut in small pieces and incubated for 1 h at 37°C with liberase TH (Sigma; libth-ro Roche), hyalorunidase, and DNase. Samples were centrifuged, CD11b^{+}

or $Ly6G^+$ fractions isolated, aortic cell homogenates lysed, and blotted for Caspase-1 and Caspase-11 as described above.

Atherosclerosis studies. After the indicated period of time on WTD, mice were sacrificed and the heart was isolated and fixed in phosphate-buffered formalin. Hearts were dehydrated and embedded in paraffin, and were cross-sectioned throughout the aortic root area. Haematoxylin-eosin staining was performed on the sections and the average from 6 sections for each animal was used to determine lesion size. Lesion size was quantified by morphometric analysis using Image-Pro Plus software (Media Cybernetics, USA).

Myeloperoxidase, Ly6G, Mac-2, and citrullinated histone staining on sections of the aortic root. Stainings were performed as described previously.[4] In brief, for antigen retrieval paraffin sections were incubated in Tris-Base EDTA at pH 9.0 (15-20 min; pressure cooker). Then sections were blocked in PBS containing 10% goat serum for 30 min at 4°C. Subsequently, sections were incubated o/n at 4°C with biotinylated myeloperoxidase (MPO) (1:30; R&D systems; BAF3667), Ly6G (1:200; BioLegend; 127602), or Mac-2 (1:10000; Cedarlane; CL8942). When citrullinated histones were stained, sections were concomitantly incubated with Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (1:300; Abcam; ab5103). For MPO staining, the sections were then incubated with Streptavidin Alexa Fluor 488 (1:200; Invitrogen/Life Technologies; S11223). Anti-rat CF 488A (1:200) was used as secondary antibody for Ly6G and Mac-2 staining. When citrullinated histones were stained, sections were concomitantly incubated with Alexa-647 (1:200; Invitrogen; A-21447). Sections were mounted using ProLong Gold Antifade Mountant with DAPI (Thermofisher; P3693) and imaged using a Leica DMI6000B microscope running Leica software. The MPO positive area was quantified using Image-Pro Plus software (Media Cybernetics, USA) and expressed as % of total lesion area. The overlap of MPO staining with citrullinated histone staining or Ly6G staining with citrullinated histone staining was assessed as neutrophil extracellular traps and quantified as % of total lesion area using Image-Pro Plus software (Media Cybernetics, USA).

Blinding procedures. Measurements of atherosclerotic lesion areas and composition were performed by an independent observer, blinded to the genotypes. Also investigators carrying out all other experiments and analysis of the data were blinded to the genotypes or treatment groups. Mouse and human samples were identified by numbers, not genotypes and the numbers and genotypes were only linked after completion of experiments and analysis.

Power calculations. For all animal studies, power calculations were performed based on atherosclerosis data reported by Duewell *et al*., indicating that NLRP3 inflammasome activation accelerated atherogenesis in *Ldlr*$^{-/-}$ mice.[5] Effect sizes were estimated at 65% and standard deviations at 38% of the highest value. Based on these calculations, a minimum of n=13 *Ldlr*$^{-/-}$ recipient mice per group were used for atherosclerosis experiments for ~80% chance to detect a difference where $p<0.05$. For further analyses, organs and plasma were obtained from mice used for atherosclerosis experiments. For mortality studies, power calculations were based on findings by Kayagaki *et al*. who showed that LPS-induced mortality is caspase-11 dependent.[6] We estimated based on these studies that at an LPS dose of 25% of the reported dose by Kayagaki *et al*., n=8 mice per group would be needed for ~80% chance to detect a 75% difference where $p<0.05$.

Supplemental Tables.

WTD (wks)	control	*Nlrp3*$^{-/-}$	*Myl-Abc*^{dko}	*Myl-Abc*^{dko}*Nlrp3*$^{-/-}$
12	1485±61	1349±46	623±23*	581±23*
8	1429±69	1342±43	543±23*	688±34*
WTD (wks)	**control**	***Casp1*$^{-/-}$*11*$^{-/-}$**	***Myl-Abc*^{dko}**	***Myl-Abc*^{dko}*Casp1*$^{-/-}$*11*$^{-/-}$**
12	1074±30	1070±26	603±18†	541±20†
8	1449±48	1338±37	656±33†	562±16†

Supplemental Table 1. Plasma cholesterol levels in *Ldlr*$^{-/-}$ mice used for atherosclerosis studies, arranged by bone marrow (BM) donor. *Ldlr*$^{-/-}$ mice were transplanted with BM from control, *Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, *Myl-Abc*^{dko}, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ BM at 8 weeks of age and fed WTD for 8 or 12 weeks (as indicated). Plasma cholesterol (mg/dL) was measured at the end of the study, when mice were sacrificed. *****P<0.001 compared to control and *Nlrp3*$^{-/-}$ BM donor mice; **†**P<0.001 compared to control and *Caspase1*$^{-/-}$*11*$^{-/-}$ BM donor mice. One-way ANOVA with a Bonferroni post-test was used to assess statistical significant differences between 4 groups.

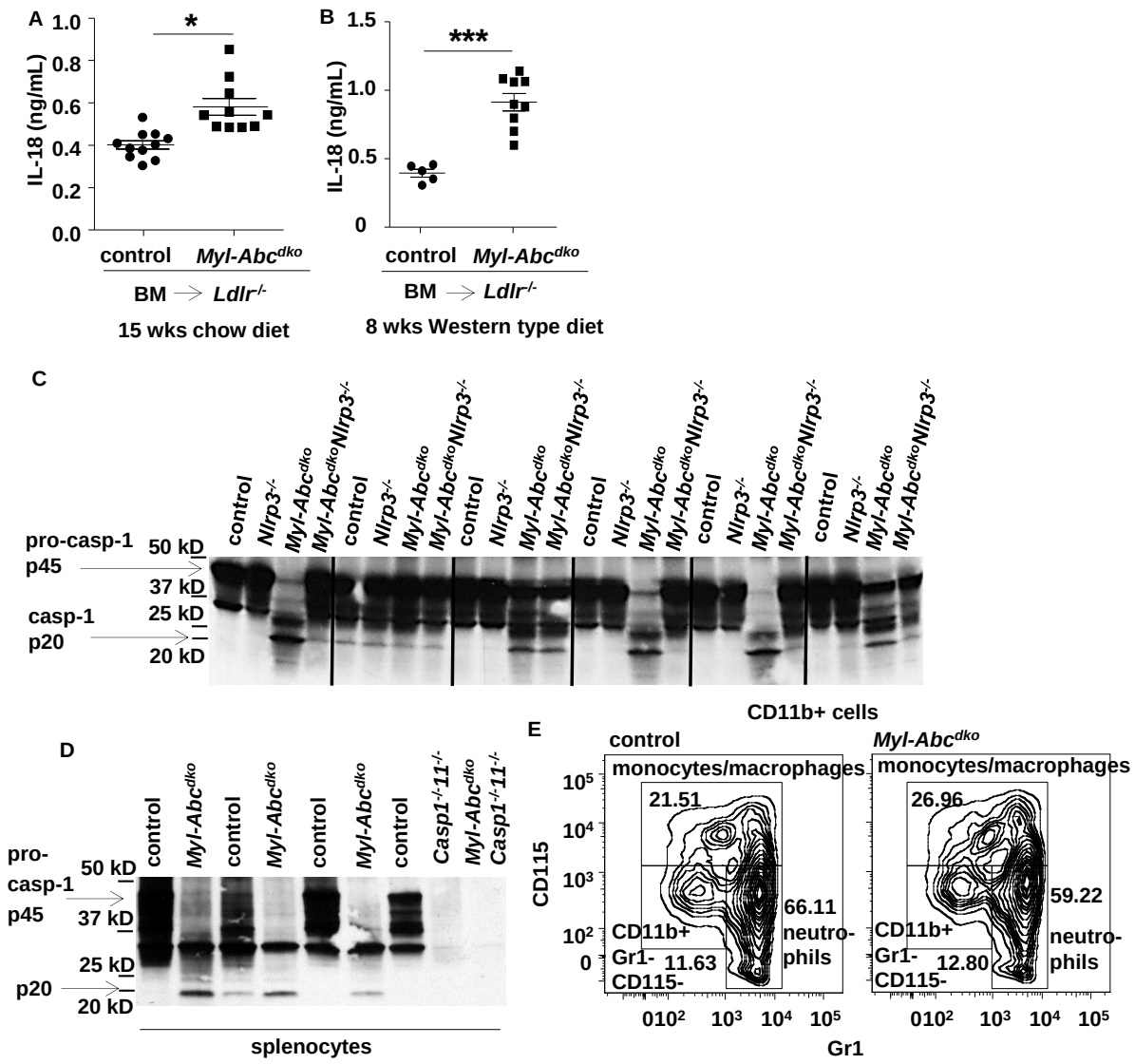
Outcome	Group (n)	Overall type III test of grouping factor	Estimated marginal means (original scale)	[95%-confidence interval] (original scale)	Pairwise comparison (Bonferroni corrected)
IL-1β	controls (13)	<0.001	0.0256	[0.0000 ; 0.0595]	1-2: n.s. 1-3: p<0.001 2-3: p<0.001
	ABCA1 heterozygous mutation carriers (29)		0.0267	[0.0022 ; 0.0518]	
	TD patients (7)		0.1673	[0.1163 ; 0.2206]	
IL-18	controls (17)	<0.001	286.2	[237.527 ; 344.909]	1-2: n.s. 1-3: p<0.001 2-3: p<0.001
	ABCA1 heterozygous mutation carriers (29)		283.6	[239.838 ; 335.338]	
	TD patients (7)		930.5	[695.424 ; 1244.90]	

Supplemental Table 2. Tangier Disease patients, homozygous for *ABCA1* loss-of-function mutations, show signs of inflammasome activation. IL-1β and IL-18 levels were measured in plasma of Tangier Disease (TD) patients carrying a homozygous loss-of-function mutation for *ABCA1*, and gender and age matched heterozygous *ABCA1* mutation carriers and controls. Some of the heterozygous *ABCA1* mutation carriers were family members of TD patients.

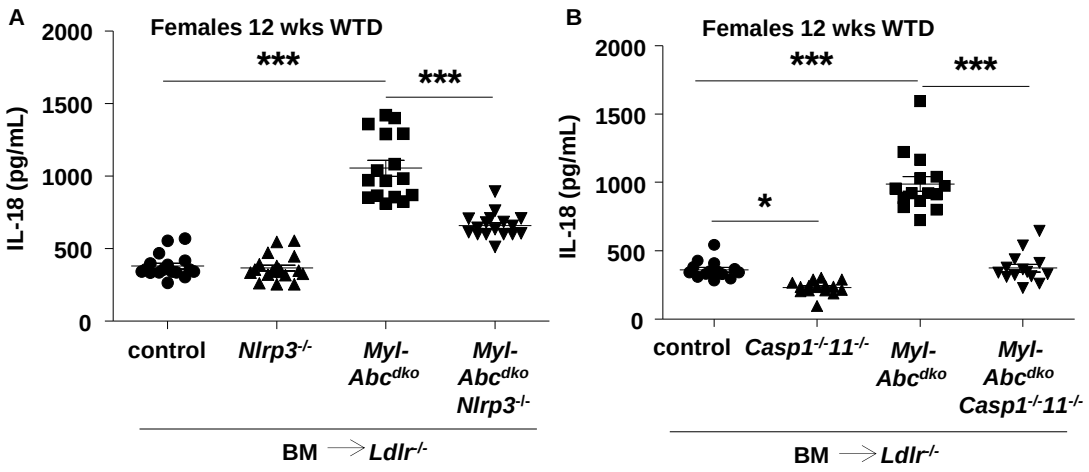
To define differences between: (1) age-and-gender-matched controls, (2) *ABCA1* heterozygous mutation carriers, and (3) Tangier Disease patients, mixed effects models with random intercepts were used taking into account data-clustering due to family members. Restricted maximum likelihood estimation and type 3 generalized score statistics were used for testing significance of the factor 'group' and data were (log-)transformed to meet model assumptions of normality. Significance levels for pairwise comparisons of estimated marginal means were Bonferroni-corrected to account for multiple testing.

For each outcome, presented are the overall p-value of the effect of group, the estimated marginal means (back-transformed to the original scale) with their 95%-confidence intervals based on these models. Significance levels for the pairwise comparisons of these estimated marginal means are given in the last column and are Bonferroni-corrected to account for multiple testing. All analyses were performed using SPSS software, version 23.0.0.3.

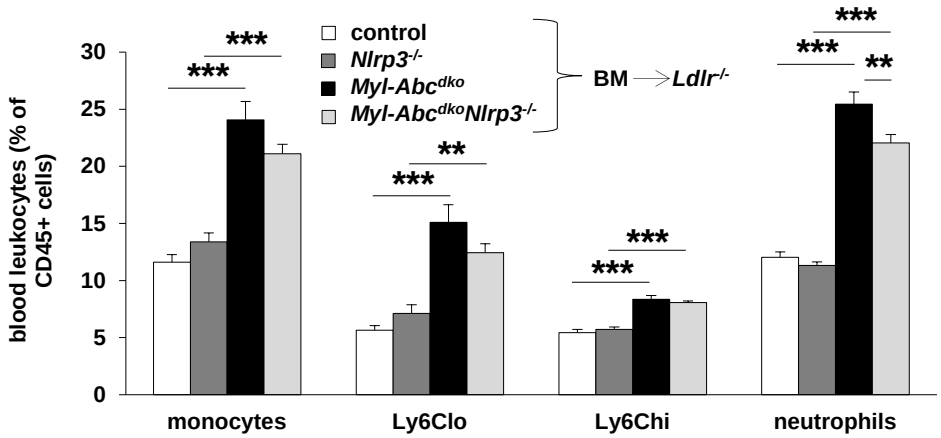
Supplemental Figures.



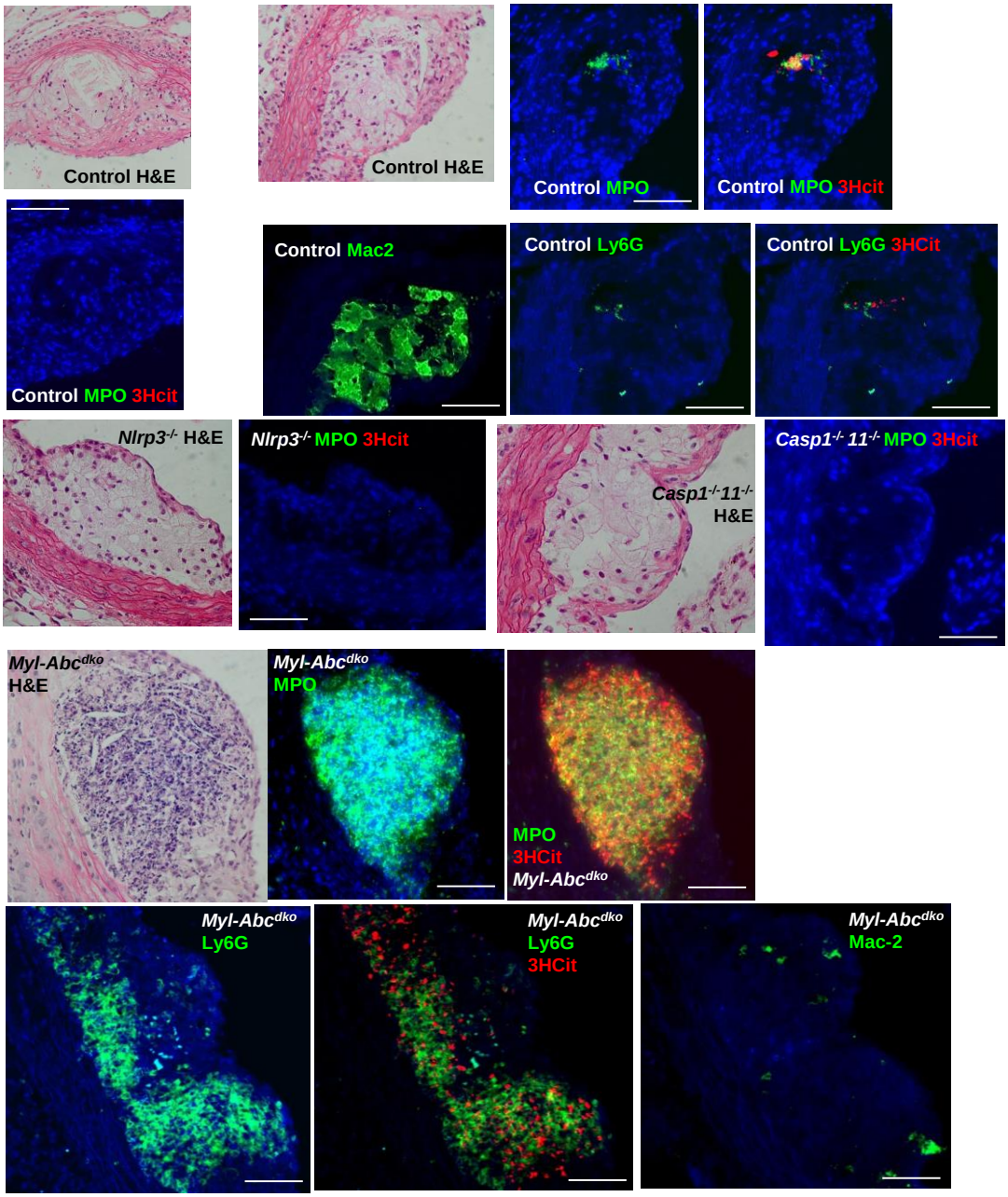
Supplementary Figure 1. Effects of myeloid *Abca1/g1* deficiency on inflammasome activation in *Ldlr*$^{-/-}$ mice. *Ldlr*$^{-/-}$ mice were transplanted with bone marrow (BM) from control, *Myl-Abc*^{dko} , *Nlrp3*$^{-/-}$, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice, as indicated, and fed chow diet for 20 weeks or Western-type diet (WTD) for 8 weeks. The genotypes of the BM donors are indicated. (**A**-**B**) Plasma IL-18 levels in mice fed chow (**A**) or Western type diet (**B**). n=5-11 mice per group; each datapoint represents one mouse. **P*<0.05, ****P*<0.001 by t-test. (**C**) Myeloid *Abca1/g1* deficiency enhances Caspase-1 cleavage in splenocytes of mice fed WTD with reversal by *Nlrp3* deficiency. Complete blot of data shown in Figure 1C. (**D**) Myeloid *Abca1/g1* deficiency enhances Caspase-1 cleavage in splenocytes of mice fed WTD; specificity of Caspase-1 antibody is shown. (**E**) Splenic CD11b^{+} cells from mice fed WTD were isolated using CD11b^{+} beads and subsequently stained for CD115 and Gr1. The CD11b^{+} cell populations (as indicated) were identified using flow cytometry.



Supplementary Figure 2. Effects of inflammasome components on plasma IL-18 levels in mice fed WTD for 12 weeks. *Ldlr*$^{-/-}$ mice were transplanted with BM from control, *Myl-Abc*^{dko}, *Nlrp3*$^{-/-}$, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice, as indicated, and fed WTD for 12 weeks. (**A-B**) Plasma IL-18 levels (n=11-20; each datapoint represents one mouse). *P<0.05, ***P<0.001 by one-way ANOVA.



Supplementary Figure 3. Effects of inflammasome components on blood leukocytes in control or *Myl-Abc^{dko}* BM transplanted *Ldlr$^{-/-}$* mice. *Ldlr$^{-/-}$* mice were transplanted with bone marrow (BM) from control, *Myl-Abc^{dko}*, *Nlrp3$^{-/-}$*, or *Myl-Abc^{dko}Nlrp3$^{-/-}$* mice, as indicated, and fed WTD for 4-8 weeks. Blood leukocyte levels were assessed by flow cytometry. n=15 mice per group. ***P*<0.01, ****P*<0.001 by one-way ANOVA.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Nlrp3-/- MPO 3Hcit
Casp1-/-11-/- H&E
Casp1-/- 11-/- MPO 3Hcit
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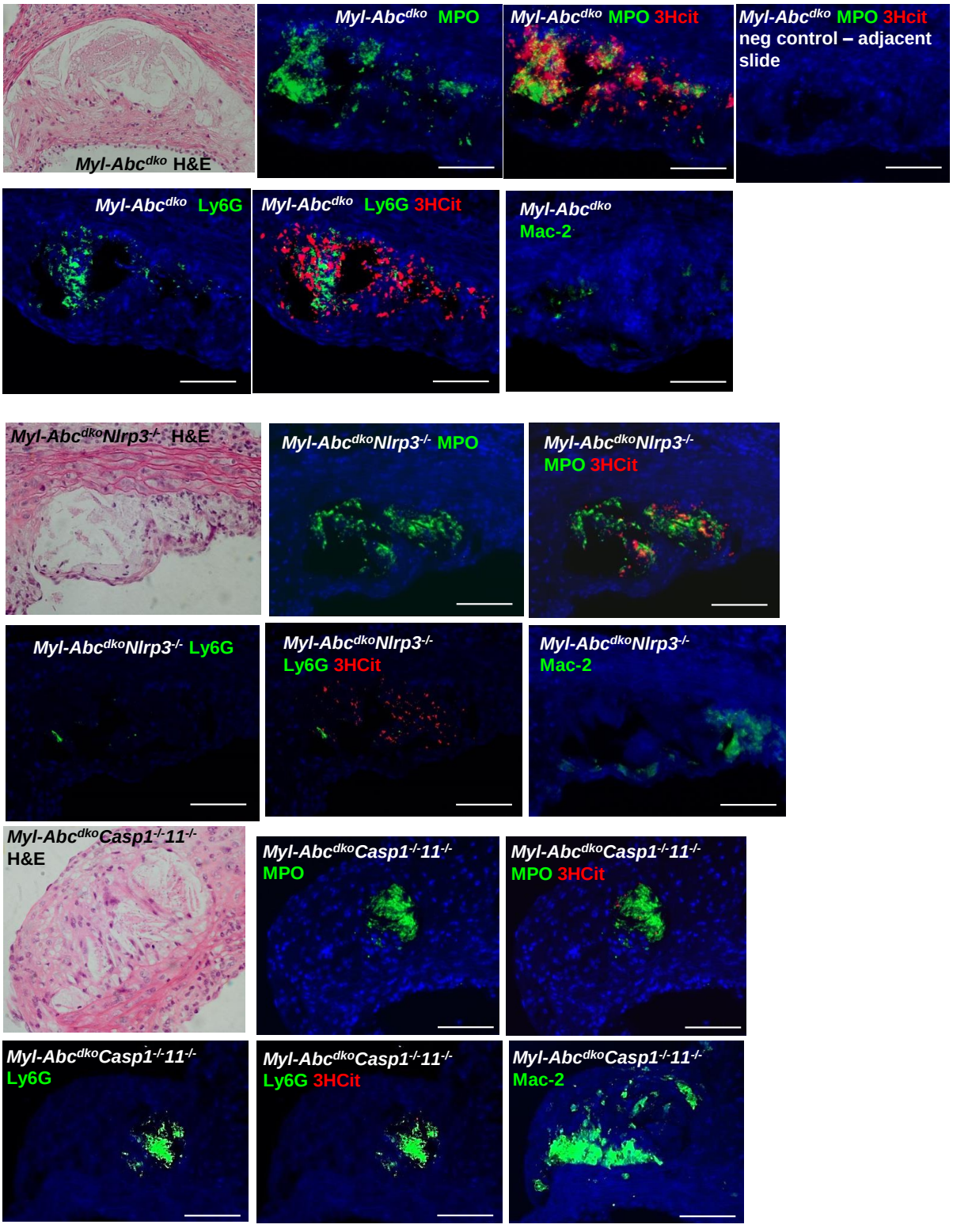
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Myl-Abc^dko MPO 3Hcit
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Myl-Abc^dko Ly6G 3HCit
Myl-Abc^dko
Mac-2
Myl-Abc^dkoNlrp3^-/- H&E
Myl-Abc^dkoNlrp3^-/- MPO
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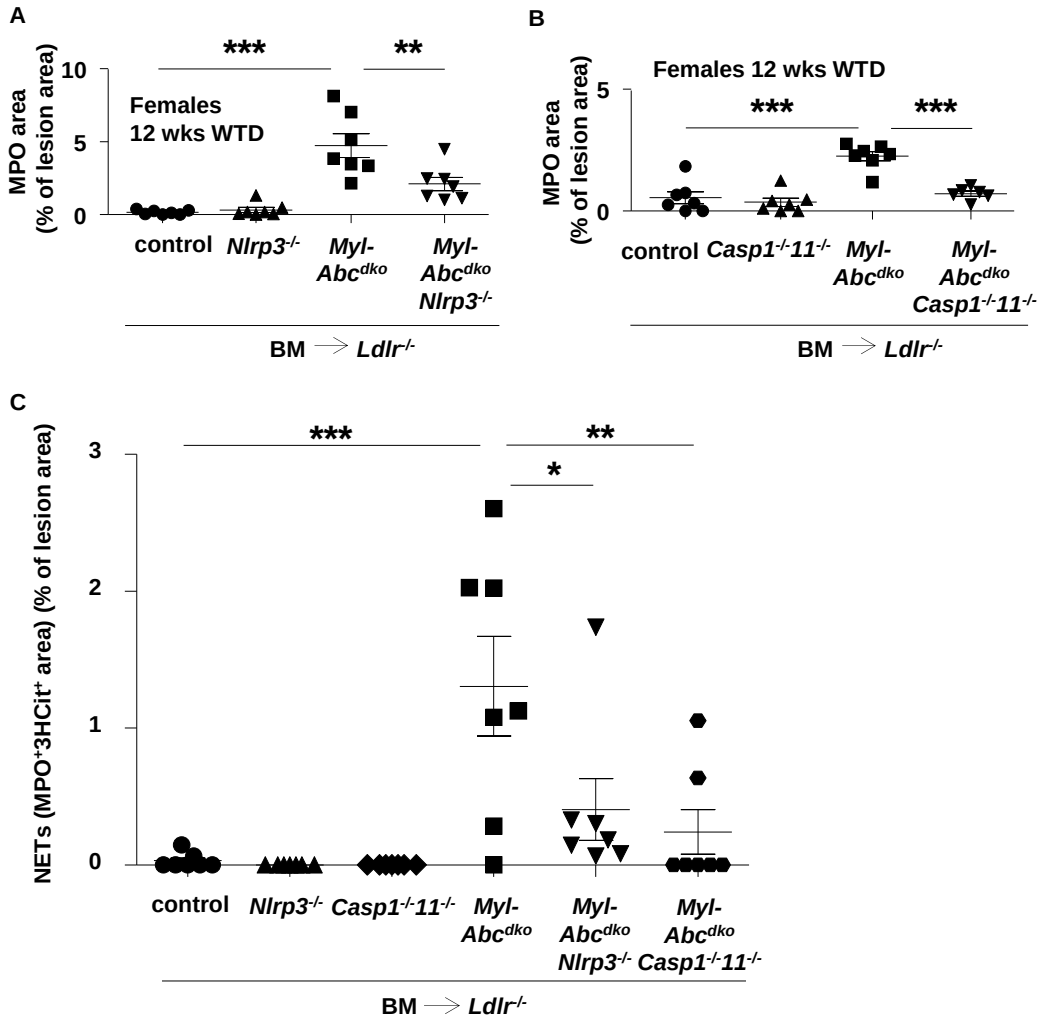
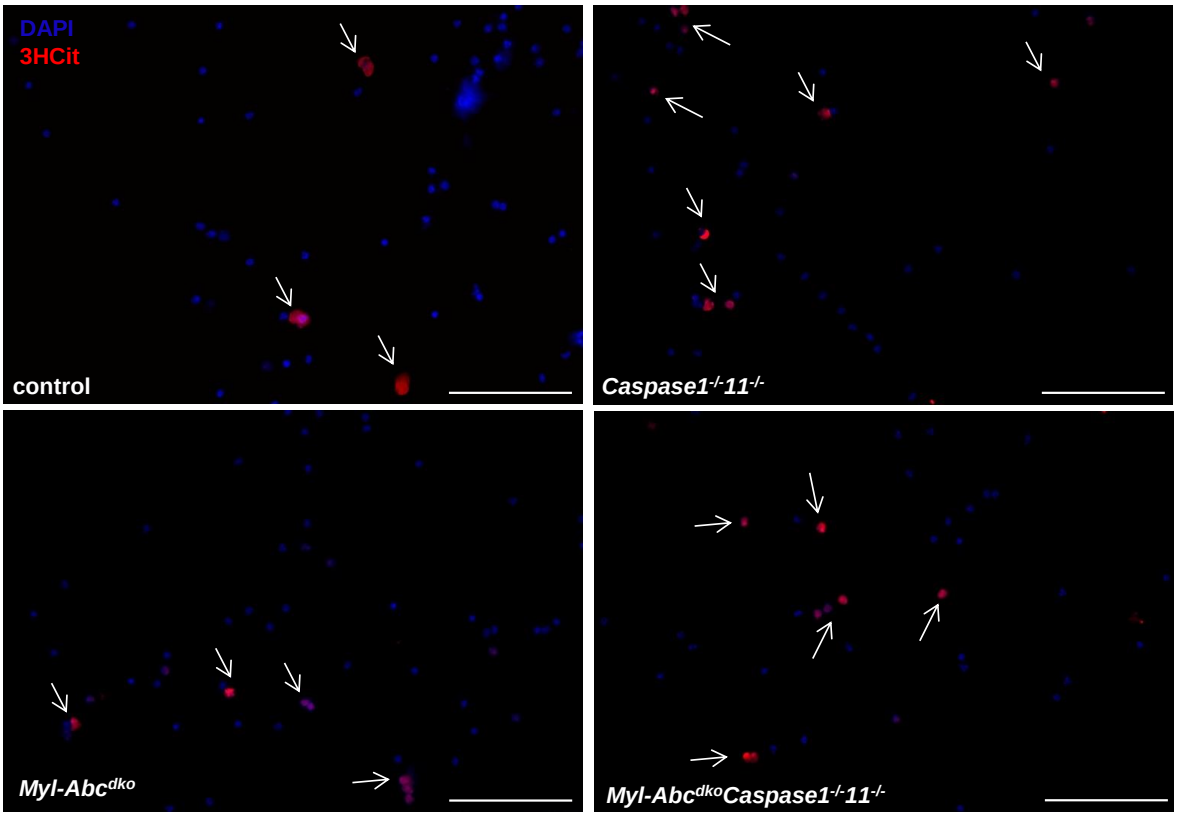
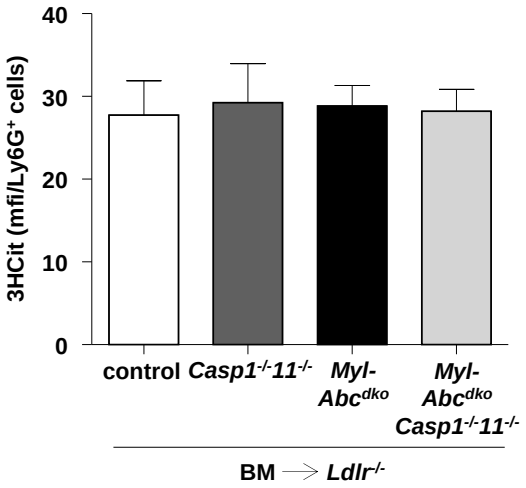
Myl-Abc^dkoCasp1^-/-11^-/-
Mac-2

Supplementary Figure 4. Effects of inflammasome components on neutrophil accumulation and neutrophil extracellular trap formation in atherosclerotic lesions. *Ldlr*$^{-/-}$ mice were transplanted with BM from control, *Myl-Abc*^{dko}, *Nlrp3*$^{-/-}$, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice, as indicated, and fed WTD for 8 weeks. Mice were sacrificed, hearts isolated, and atherosclerotic lesions were stained with haematoxylin and eosin (H&E) or an antibody to myeloperoxidase (MPO) in the absence or presence of an antibody to citrullinated histones 2, 8, and 17 (3HCit). To measure neutrophil extracellular traps (NETs), we quantified the overlap of MPO and 3HCit or Ly6G and 3HCit. From each group, one or two representative examples are shown for consecutive sections of H&E staining, MPO staining and combined MPO and 3Hcit staining. The specificity of the MPO and 3Hcit antibodies is also shown. Scale bars represent 100 μm.

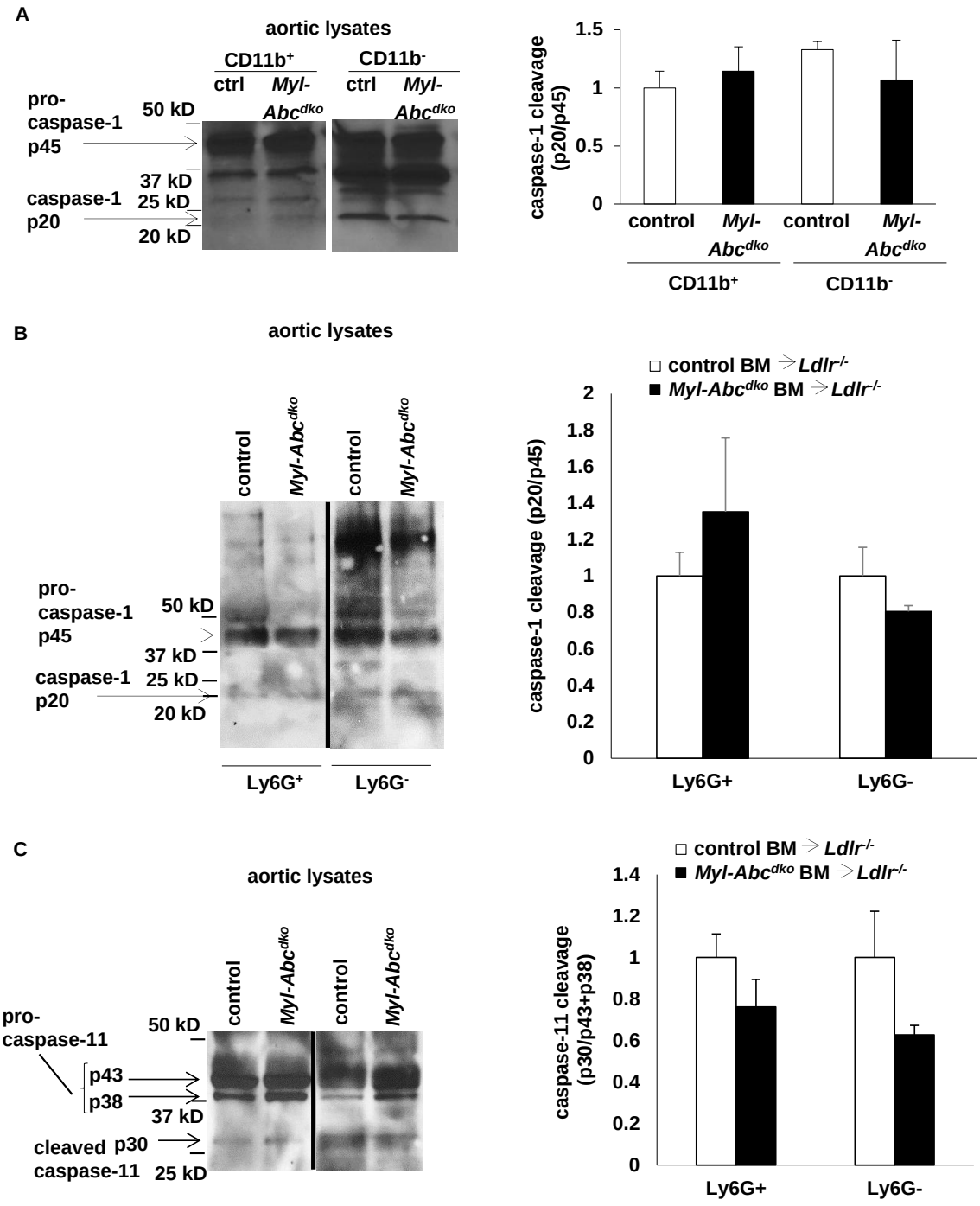


Supplementary Figure 5. Inflammasome activation promotes neutrophil accumulation and neutrophil extracellular trap formation in atherosclerotic lesions of myeloid *Abca1/g1* deficient mice in mice fed WTD for 12 weeks. *Ldlr*$^{-/-}$ mice were transplanted with BM from control, *Myl-Abc*^{dko}, *Nlrp3*$^{-/-}$, *Myl-Abc*^{dko}*Nlrp3*$^{-/-}$, *Caspase1*$^{-/-}$*11*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice, as indicated, and fed WTD for 12 weeks. In atherosclerotic lesions, neutrophil activation was assessed using MPO staining (**A** and **B**), and NETs were assessed by staining for MPO and citrullinated histones 2, 8, and 17 (3HCit) and quantifying the overlap of MPO and 3HCit (**C**). n=6 mice per group. Each datapoint represents one mouse. *P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA with Bonferroni post-test.

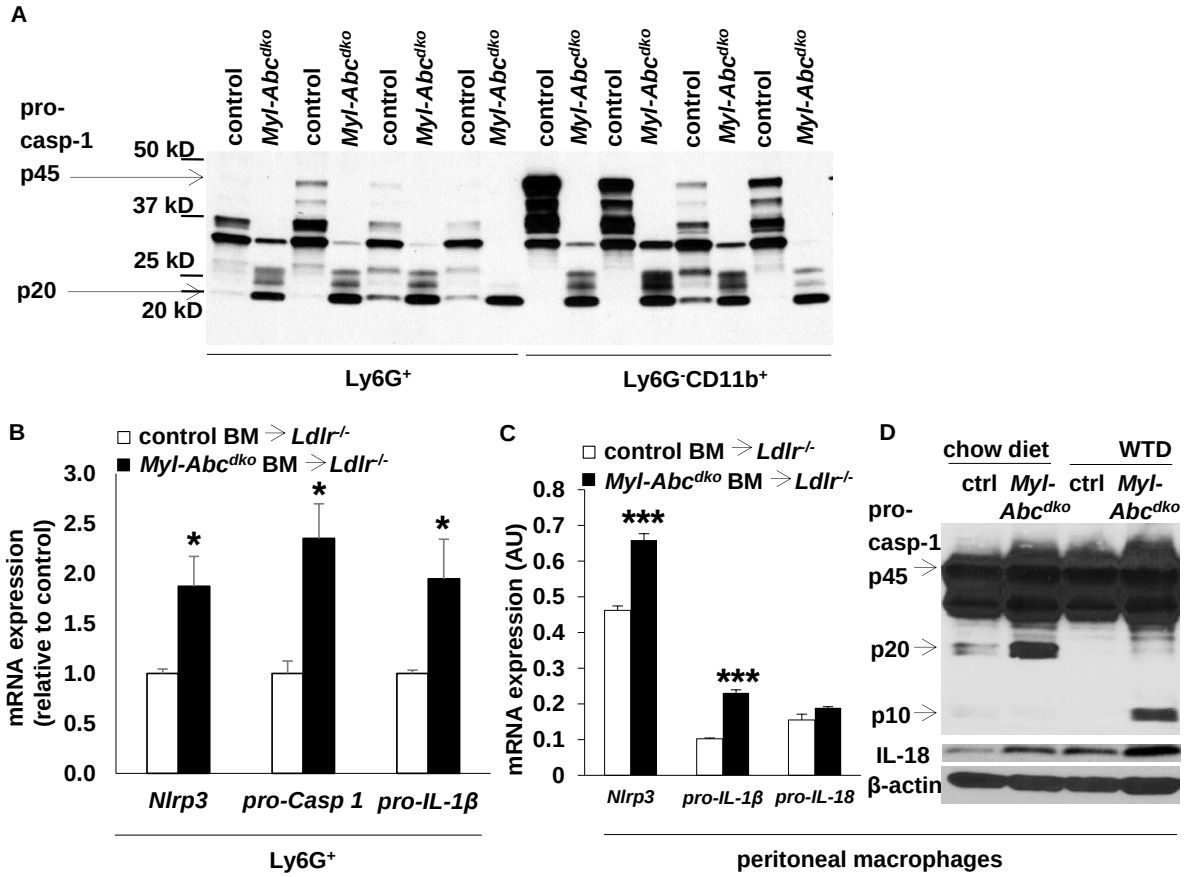




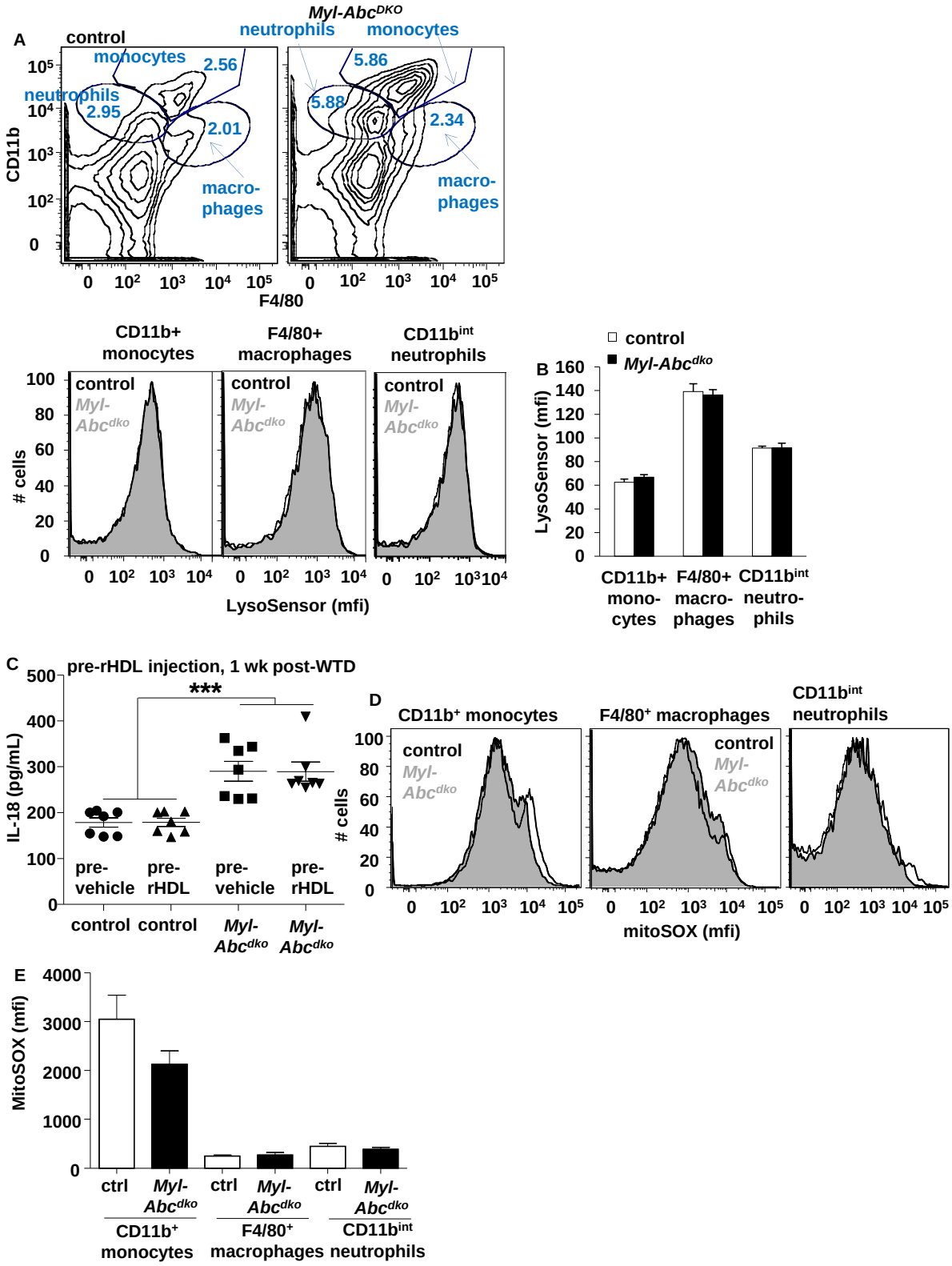
Supplementary Figure 6. Myeloid *Abca1/g1* deficiency or *Caspase1/11* deficiency did not affect citrullinated histones staining in cultured blood neutrophils of mice fed WTD for 8 weeks. *Ldlr*$^{-/-}$ mice were transplanted with BM from control, *Myl-Abc*^{dko}, *Caspase1*$^{-/-}$*11*$^{-/-}$, or *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice, as indicated, and fed WTD for 8 weeks. Blood neutrophils were isolated using Ly6G^{+} beads, allowed to adhere for 30 min at 37°C, and subsequenty incubated in HBSS for 4 h. Cells were stained for citrullinated histones 2, 8, and 17 (3HCit) and the mean fluorescent intensity of 3HCit per cell was quantified. n=4 of 3 mice pooled per genotype. Scale bars represent 100 µm.

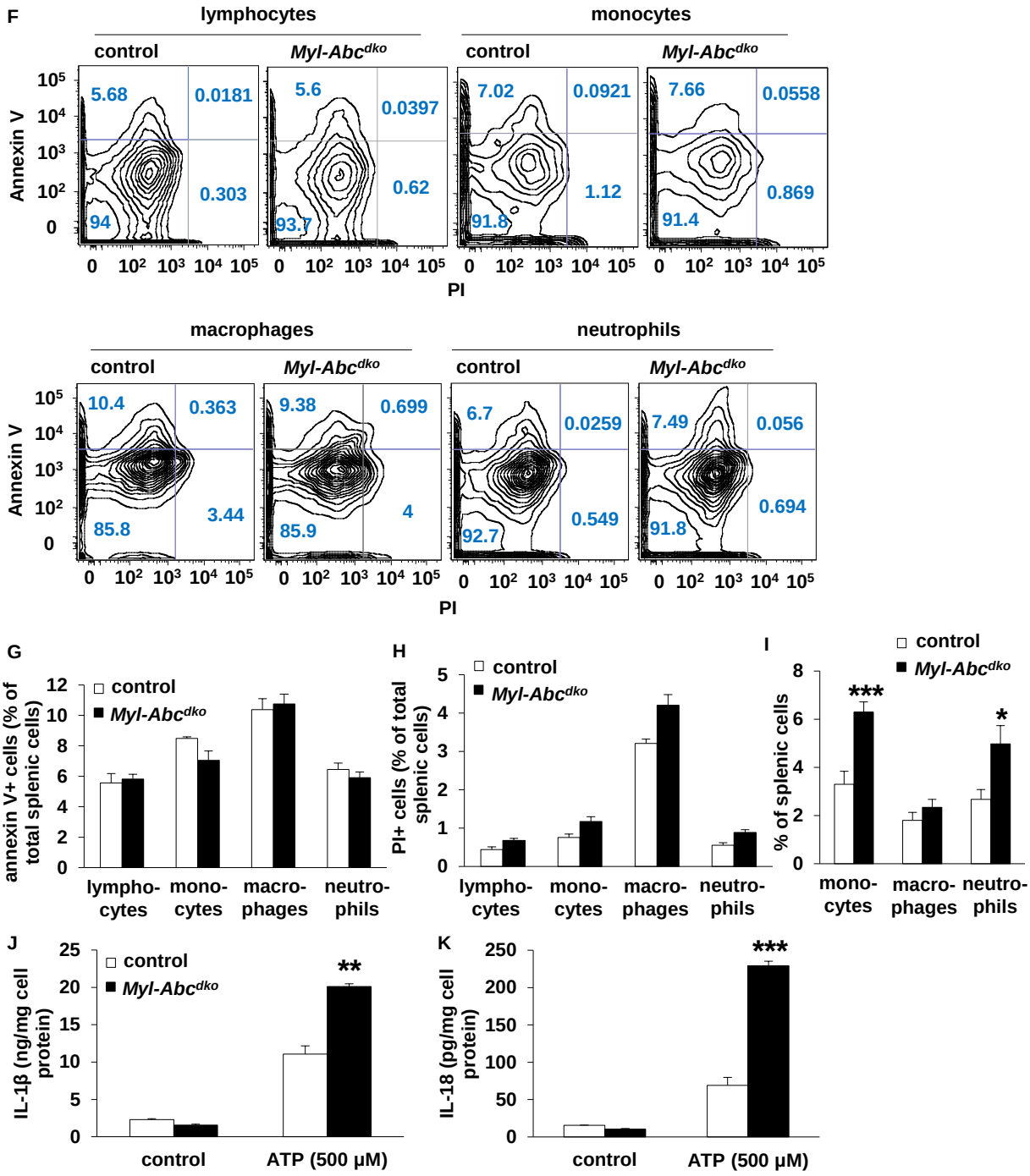


Supplementary Figure 7. Myeloid *Abca1/g1* deficiency does not affect Caspase-1 or Caspase-11 cleavage in CD11b$^+$ cells or Ly6G$^+$ neutrophils of atherosclerotic lesions. *Ldlr*$^{-/-}$ mice were transplanted with BM from control or *Myl-Abc*^{dko} mice, as indicated, and fed WTD for 8 weeks. Aortas were isolated, subjected to liberase digestion, CD11b$^+$ and CD11b$^-$ fractions were isolated (**A**) or Ly6G$^+$ and Ly6G$^-$ fractions were isolated (**B**), and Caspase-1 cleavage (**A-B**) or Caspase-11 cleavage (**C**) was assessed using Western blot (n=3 per group of 2 aortas pooled), and quantified.

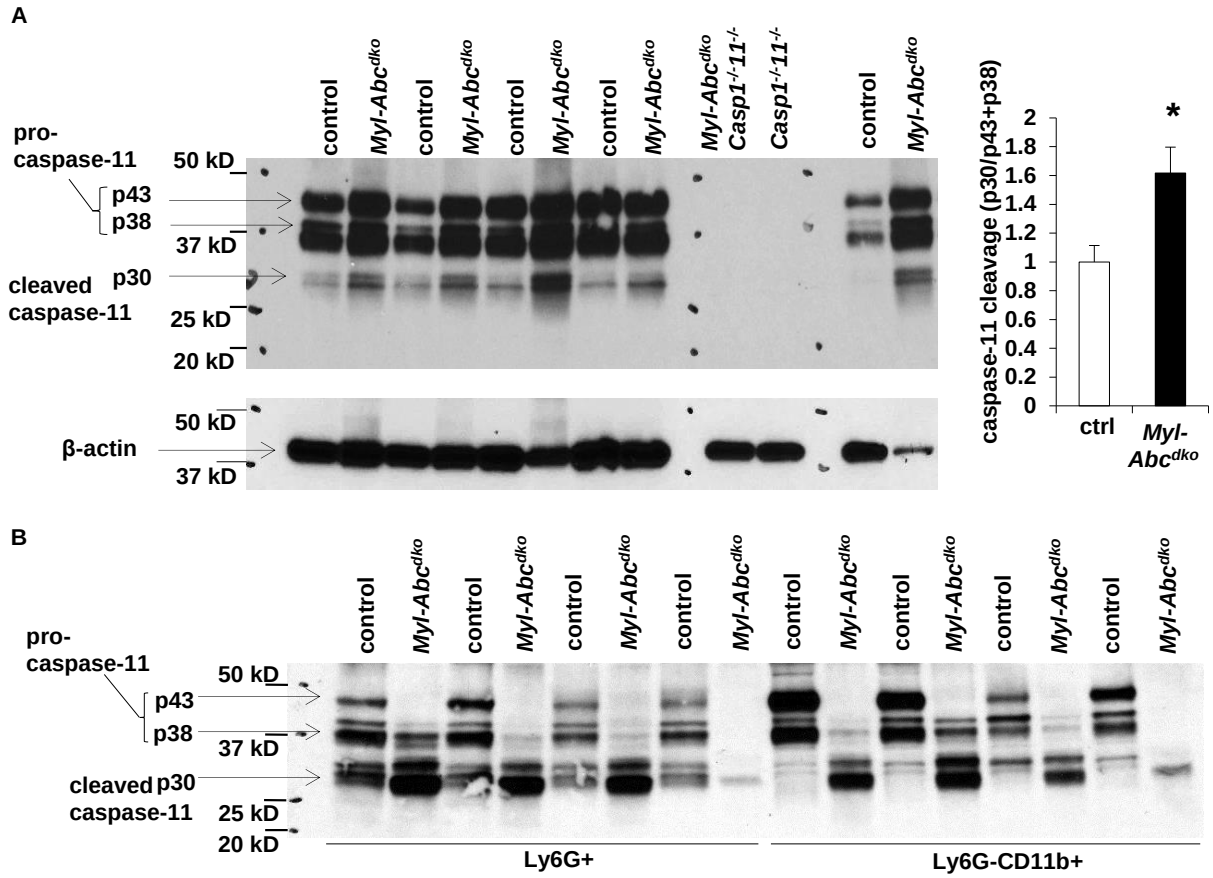


Supplementary Figure 8. Effects of myeloid *Abca1/g1* deficiency on inflammasome activation in *Ldlr*$^{-/-}$ mice. *Ldlr*$^{-/-}$ mice were transplanted with bone marrow (BM) from control or *Myl-Abc*^{dko} mice, as indicated, and fed Western-type diet (WTD) for 8 weeks. The genotypes of the BM donors are indicated. (**A**) Myeloid *Abca1/g1* deficiency enhances caspase-1 cleavage in Ly6G^{+} and Ly6G^{-}CD11b^{+} splenocytes of mice fed WTD. Complete blot of data shown in Figure 1I. (**B-C**) Myeloid *Abca1/g1* deficiency enhances inflammasome priming in splenic Ly6G^{+} neutrophils and thioglycollate-elicited macrophages from WTD fed *Ldlr*$^{-/-}$ mice. For (**C-D**), Mice were injected with thioglycollate, macrophages isolated, and (**C**) RNA extracted to determine inflammasome priming. n=4. ***P*<0.05, ****P*<0.001 by t-test. (**D**) Myeloid *Abca1/g1* deficiency enhances caspase-1 cleavage and IL-18 cleavage in thioglycollate-elicited macrophages from chow and WTD fed *Ldlr*$^{-/-}$ mice. Representative examples of n=4 replicates are shown.


A
control
Myl-Abc^DKO
neutrophils
monocytes
monocytes
2.56
neutrophils
2.95
2.01
macro-
phages
5.86
5.88
2.34
macro-
phages
CD11b
F4/80
CD11b+
monocytes
F4/80+
macrophages
CD11b^int
neutrophils
control
Myl-
Abc^dko
cells
LysoSensor (mfi)
B
control
Myl-Abc^dko
LysoSensor (mfi)
CD11b+
mono-
cytes
F4/80+
macro-
phages
CD11b^int
neutro-
phils
C
pre-rHDL injection, 1 wk post-WTD
***
IL-18 (pg/mL)
pre-
vehicle
pre-
rHDL
pre-
vehicle
pre-
rHDL
control
control
Myl-
Abc^dko
Myl-
Abc^dko
D
CD11b+ monocytes
F4/80+ macrophages
CD11b^int
neutrophils
control
Myl-
Abc^dko
cells
mitoSOX (mfi)
E
MitoSOX (mfi)
ctrl
Myl-
Abc^dko
CD11b+
monocytes
F4/80+
macrophages
CD11b^int
neutrophils


F
lymphocytes
monocytes
control
Myl-Abcdko
control
Myl-Abcdko
Annexin V
5.68
0.0181
0.303
94
5.6
0.0397
0.62
93.7
7.02
0.0921
1.12
91.8
7.66
0.0558
0.869
91.4
PI
macrophages
neutrophils
control
Myl-Abcdko
control
Myl-Abcdko
Annexin V
10.4
0.363
3.44
85.8
9.38
0.699
4
85.9
6.7
0.0259
0.549
92.7
7.49
0.056
0.694
91.8
PI
G
annexin V+ cells (% of total splenic cells)
control
Myl-Abcdko
lympho-cytes
mono-cytes
macro-phages
neutro-phils
H
PI+ cells (% of total splenic cells)
control
Myl-Abcdko
lympho-cytes
mono-cytes
macro-phages
neutro-phils
I
control
Myl-Abcdko
% of splenic cells
***
*
mono-cytes
macro-phages
neutro-phils
J
IL-1β (ng/mg cell protein)
control
Myl-Abcdko
**
control
ATP (500 μM)
K
IL-18 (pg/mg cell protein)
***
control
ATP (500 μM)

Supplementary Figure 9. Mechanisms of inflammasome activation in myeloid *Abca1/g1* deficiency. *Ldlr*$^{-/-}$ mice were transplanted with BM from control or *Myl-Abc*^{dko} mice, and fed WTD for 4 weeks unless indicated otherwise. The genotypes of the BM donors are indicated. (**A-B**) CD11b^{+} monocytes, CD11b^{int} neutrophils, and F4/80^{+} macrophages were gated as shown. Lysosomal pH was assessed by Lysosensor staining in splenic CD11b^{+} monocytes, F4/80^{+} macrophages, and CD11b^{int} neutrophils. Representative examples are shown (**A**), and quantified (**B**). n=6 mice per group. (**C**) *Ldlr*$^{-/-}$ mice were transplanted with BM from control or *Myl-Abc*^{dko} mice, fed WTD for 1 week, and plasma IL-18 levels were measured. n=14 mice per control or *Myl-Abc*^{dko} group. ***P<0.001 by t-test (each datapoint represents one mouse) These results are pre-vehicle and pre-reconstituted HDL (rHDL) injection. Mice with similar plasma IL-18 levels were randomly subdivided in 4 groups for vehicle and rHDL injection and these groups are indicated. (**D-E**) mitoROS production as assessed by mitoSOX staining in splenic CD11b^{+} monocytes, F4/80^{+} macrophages, and CD11b^{int} neutrophils gated as shown in (**A**). Representative examples are shown (**D**), and quantified (**E**). (**F-H**) splenocytes were stained with CD11b^{+} and F4/80^{+} antibodies as indicated in (**A**) and subsequently apoptotic cells were stained using Annexin V and necrotic cells using PI. Representative examples are shown (**F**) and quantified (**G-H**). n=6 mice per group. (**I**) Quantification of splenic CD11b^{+} monocytes, CD11b^{int} neutrophils, and F4/80^{+} macrophages, as gated in (**A**). n=6 mice per group. *P<0.05, ***P<0.001, by t-test. (**J**-**K**) Myeloid *Abca1/g1* deficiency enhanced IL-1β (**J**) and IL-18 (**K**) secretion in bone marrow derived macrophages first stimulated with LPS (100 ng/ml; 4 h) and subsequently with ATP (500 μM; 2 h). IL-1β and IL-18 secretion were assessed using ELISA and corrected for cell protein. **P<0.01, ***P<0.001, by t-test.



Supplementary Figure 10. Myeloid *Abca1/g1* deficiency activates the non-canonical inflammasome. (**A**) Control, *Myl-Abc*^{dko}, *Caspase1*$^{-/-}$*11*$^{-/-}$, and *Myl-Abc*^{dko}*Caspase1*$^{-/-}$*11*$^{-/-}$ mice were fed a chow diet for 20 weeks. Caspase-11 cleavage was assess in splenocytes by Western blot. Representative images are shown as well as specificity of the antibody. Caspase-11 cleavage is quantified. n=5 mice per group. **P*<0.05 by t-test. (**B**) *Ldlr*$^{-/-}$ mice were transplanted with BM from control or *Myl-Abc*^{dko} mice, and fed WTD for 8 weeks. Myeloid *Abca1/g1* deficiency enhances Caspase-11 cleavage in Ly6G^{+} and Ly6G^{-}CD11b^{+} splenocytes. Complete blot from data shown in Figure 6C.

Supplemental References

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