SUPPLEMENTAL MATERIALS

Myeloid LXR deficiency induces inflammatory gene expression in foamy macrophages and accelerates atherosclerosis

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Running Title: Myeloid LXR on atherosclerosis

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Supplemental Methods

- Immunofluorescent staining and immunohistochemistry

For macrophage staining, paraffin-embedded sections were rehydrated, retrieved antigens and incubated with 1 % goat serum to remove nonspecific antibody binding. Sections were incubated with rat anti-Mac-2 (Galectin-3) (Cedarlane, cat No. CL8942AP, clone No. M3/38, RRID: AB_10060357, 1:10,000) overnight, and then with goat anti-rat IgG (H+L), CF 647 (Sigma-Aldrich, St. Louis, MO, cat No. SAB4600186, 1:200) for 30 minutes at room temperature, and mounted with ProLong Gold Antifade Mountant with DAPI reagent (Thermo Fisher Scientific). To detect proliferative macrophages, sections were stained with rabbit anti-Ki-67 (Vector Laboratories, Inc. Burlingame, CA, cat No. VP-K451, RRID: AB_2314701, 1:1,500) and rat anti-Mac-2, and then with Alexa Fluor (AF) 488-conjugated goat anti-rat IgG (Thermo Fisher Scientific, cat No. A-11006, RRID: AB_141373, 1:1,000) and AF647-conjugated goat anti-rabbit IgG (Thermo Fisher Scientific, cat No. A-21244, RRID: AB_2535812, 1:1,000). For IL-1β staining, sections were stained with rabbit anti-IL-1β (Novus Biologicals, Centennial, CO, cat No. NB600-633, RRID: AB_10001060, 1:100) and rat anti-Mac2 and with the same secondary antibodies as for Ki-67 staining. For neutrophil staining, sections were stained with rat anti-Ly6G (BioLegend, cat No. 127602, clone No. 1A8, RRID: AB_1089180, 1:200), and then with goat anti-rat IgG (H+L), CF 647. To detect NETs formation, sections were stained with biotinylated anti-myeloperoxidase (MPO) (R&D Systems, cat No. BAF3667, RRID: AB_2146326, 1:30) and anti-Histone H3 (citrulline R2 + R8 + R17) (Abcam, Cambridge, UK, cat No. ab5103, 1:300), and then with AF488-conjugated streptavidin (Thermo Fisher Scientific, cat No. S11223, 1:200) and goat anti-rabbit IgG (H+L), CF 647 (Sigma-Aldrich, St. Louis, MO, ca t No. SAB4600184, RRID: AB_2665437, 1:200). For 15-LOX staining, sections were stained with rabbit anti-15-LOX (Abcam, cat No. ab244205, 1:1,000) and rat anti-Mac-2.

For the staining of lipid peroxidation, paraffin-embedded sections were rehydrated, retrieved antigens and incubated with 1 % goat serum to remove nonspecific antibody bindings. Sections were incubated with anti-4-Hydroxynonenal (HNE) (Abcam, cat No. ab46545, RRID: AB_722490, 1:500) overnight, and the visualized with ImmPACT DAB substrate solution (Vector Laboratories, Inc. Burlingame, CA).

The stained samples were analyzed with DMI 6000B fluorescent microscopy (Leica Microsystems, Wetzlar, Germany), and the positive area was calculated with ImageJ software in a blind manner between genotypes. One or two sections were analyzed for the staining per each mice. The isotype control staining was shown in Supplemental Fig. 4 or reported previously[47, 48].

- Monocyte tracking analysis

Ldlr$^{-/-}$ mice transplanted with bone marrow cells from floxed control or *Myl-Lxr*^{dko}

mice were used after 11-weeks WTD feeding. To remove Gr-1^{lo} monocytes transiently, clodronate liposome solution (Liposoma BV, Amsterdam, Netherlands, 200 μL per 20 g of body weight) was injected intravenously into mice prior to 72 hours beads injection. Fluorescent-labeled latex beads (Fluoresbrite YG Microspheres 1.00 μm) (Polysciences, Inc., Warrington, PA, 1:4 in PBS, 200 μL per 20 g of body weight) were injected intravenously, and after 24 hours, the frequency of monocyte labeling was assessed with flowcytometry. Mice were sacrificed 5 days after beads injection. OCT-embedded frozen sections of heart were cut into 8 μm width, fixed with 4 % paraformaldehyde (PFA), and stained with rat anti-Mac-2. The number of beads in Mac-2-positive lesion were counted under fluorescent microscopy and normalized with the frequency of beads labeling in Gr-1^{hi} monocytes. For detection of blood monocytes, cells were stained with LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Thermo Fisher Scientific, 1:1,000), anti-CD16/32 Fc blocker, PE-Cy7-conjugated anti-CD11b, APC-conjugated anti-CD115, and BV421-conjugated anti-Gr-1. We confirmed that beads were selectively incorporated into Gr-1^{hi} monocytes. The percentage of beads-positive Gr-1^{hi} monocytes (Beads^{+}Gr-1^{hi} cells in CD115^{+}CD11b^{+}-gated cells) was analyzed with BD FACS Canto II (BD Biosciences, San Jose, CA) and calculated with FlowJo 10.4 software (Tree Star, Ashland, OR).

- Western blotting

The CD11b^{+} cells were isolated from splenocytes using magnetic beads (Miltenyi Biotec, Cologne, Germany), Western blot analysis was performed with rat anti-Caspase-1 (Thermo Fisher Scientific, cat No. 14-9832-82, clone No. 5B10, RRID: AB_2016691, 1:250) and visualized with an enhanced chemiluminescence detection system.

- Cell sorting

For sorting of lymphocytes and myeloid cells from spleen or blood, splenocytes or blood leukocytes were isolated after filtration with 40 μm nylon mesh and removal of red blood cells. Cells were stained with anti-CD16/32 Fc blocker (BioLegend, San Diego, CA, cat No. 101302, clone No. 93, RRID: AB_312801, 1:30), allophycocyanin (APC)-Cy7-conjugated anti-CD45 (BioLegend, cat No. 103116, clone No. 30-F11, RRID: AB_312981, 1:100), phycoerythrin (PE)-Cy7-conjugated anti-CD11b (BioLegend, cat No. 101216, clone No. M1/70, RRID: AB_312799, 1:200), Brilliant Violet (BV) 421-conjugated anti-Gr-1 (BioLegend, cat No. 108434, clone No. RB6-8C5, RRID: AB_2562219, 1:200), fluorescein isothiocyanate (FITC)-conjugated anti-B220 (BioLegend, cat No. 103206, clone No. RA3-6B2, RRID: AB_312991, 1:100), PerCP-Cy5.5-conjugated T cell receptor β (TCRβ) (BioLegend, cat No. 109228, clone No. H57-597, RRID: AB_1575173, 1:100) and APC-conjugated anti-F4/80 (BioLegend, cat No. 123116, clone No. BM8, RRID: AB_893481, 1:50 for spleen) or APC-conjugated anti-CD115 (BioLegend, cat No. 135532, clone No. AFS98, RRID: AB_2632740, 1:50 for

blood). Each of $CD45^+$-gated leukocytes such as T cells ($TCR\beta^+$ cells), B cells ($B220^+$ cells), neutrophils ($Gr\text{-}1^+CD11b^+$ cells), and monocyte in blood ($CD115^+$ cells) or macrophages in spleen ($F4/80^+$ cells) was sorted by FACSAria cell sorter (BD Biosciences).

For single cell RNA-sequencing, the digested aortic cells pooled from 10 mice for each group were incubated with LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit, anti-CD16/32 Fc blocker, Pacific Blue-conjugated anti-CD45 and propidium iodide (PI) (Supplemental Fig. 5A). Living $CD45^+$ cells ($LIVE/DEAD^-PI^-CD45^+$ cells) were sorted with FACSAria cell sorter.

For Sorting of foamy or nonfoamy macrophages, digested aortic cells isolated from *Ldlr*$^{-/-}$ male mice transplanted with bone marrow cells from floxed control or *Myl-Lxr*^{dko} on 20-weeks WTD feeding were used for the experiment. Cells were incubated with LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit, anti-CD16/32 Fc blocker, Pacific Blue-conjugated anti-CD45, PE-Cy7-conjugated anti-CD11b and APC-conjugated anti-CD64 (BioLegend, cat No. 139306, clone No. X54-5/7.1, RRID: AB_11219391, 1:50). Intracellular neutral lipid was stained with BODIPY 493/503 (4,4-Difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-*S*-indacene) (Thermo Fisher Scientific, 40 μM in PBS) for 30 minutes. Stained cells were subjected to FACSAria cell sorter, and separated to $CD45^+$-gated $CD64^+CD11b^+BODIPY^{hi}SSC^{hi}$ cells (foamy macrophages), $CD64^+CD11b^+BODIPY^{lo}SSC^{lo}$ cells (nonfoamy macrophages) and $CD64^-CD11b^+$ cells (mixed fraction of monocytes and neutrophils).

- Intracellular lipid staining in atherosclerotic plaque

For Oil red O staining, frozen sections were fixed with 4 % PFA for 15 minutes and stained with Oil red O reagent in isopropanol for 5 minutes. Sections were counterstained with Hematoxylin QS reagent (Vector Laboratories, Inc.) and mounted with VectaMount AQ Aqueous Mounting Medium (Vector Laboratories, Inc.). For BODIPY staining, frozen sections were fixed with 4 % PFA for 15 minutes and stained with rat anti-Mac-2 or rat anti-TREM-2 (Sigma-Aldrich, cat No. MABN755, clone No. 78, 1:100) overnight, and with goat anti-rat IgG (H+L), CF 647 for 30 minutes, and then incubated with BODIPY (40 nM in PBS) for 30 minutes.

Table S1 Mouse primer sequences for quantitative PCR analysis

Gene Symbol	Accession No.		Sequence [5' -> 3']	Amplicon Size (bp)
Nr1h3	NM_013839	Fw	AAACAGCTCCCTGGCTTCCT	102
		Rev	GTACCTCCGTGACGTCTCCA	
Nr1h2	NM_009473	Fw	AGATGGATGCCTTCATGCGG	133
		Rev	TCCGAATCTGCTCCTCAGAGA	
Alox15	NM_009660	Fw	GTGTCCCCCTGATGACTTGG	112
		Rev	CATTCCCACCACGTACCGAT	
Saa3	NM_011315	Fw	TTTCTCTTCCTGTTGTTCCCAGTC	120
		Rev	TCACAAGTATTTATTCAGCACATTG GGA	
Marco	NM_010766	Fw	CTGTCGCATGCTCGGTTAC	119
		Rev	TGCAGTCCCACAAACTGTTC	
Cxcl13	NM_018866	Fw	GGCCACGGTATTCTGGAAGC	75
		Rev	ACCGACAACAGTTGAAATCACTC	
Sectmla	NM_145373	Fw	GGCAGCAGTGGAGGTAACAT	99
		Rev	GGACAGGAGGCCTTAGGAGA	
Tst	NM_009437	Fw	ACAACCGGAGCCGGATATAG	105
		Rev	GGGCTCTTCTCGAAGCCATC	
Irg1	NM_008392	Fw	GCAACATGATGCTCAAGTCTG	96
		Rev	TGCTCCTCCGAATGATACCA	
Ccl22	NM_009137	Fw	TCTTGCTGTGGCAATTCAGA	92
		Rev	GAGGGTGACGGATGTAGTCC	
Trem2	NM_031254	Fw	ACAGCACCTCCAGGAATCAAG	82
		Rev	AACTTGCTCAGGAGAACGCA	
Apoe	NM_009696	Fw	TGCTGTTGGTCACATTGCTGA	186
		Rev	CTTGTGTGACTTGGGAGCTCTG	
Lpl	NM_008509	Fw	ATCGGGCCCAGCAACATTAT	118
		Rev	TGGACGTTGTCTAGGGGGTA	
Ctsl	NM_009984	Fw	TTAGTGCAGAGTGGCACCAG	175
		Rev	ATGTCACCGAAGGCGTTCAT	
Lipa	NM_021460	Fw	CCACTCCCGGAAGATGCAA	166
		Rev	GACCGAGTGTTCCTCACCAG	
Npc2	NM_023409	Fw	CCTATTCCTGAGCCTGACGG	134
		Rev	AGTTTCCATTCCACCACCAGT	
Ppib	NM_023409	Fw	GGAGATGGCACAGGAGGAA	76
		Rev	GCCCGTAGTGCTTCAGCTT	

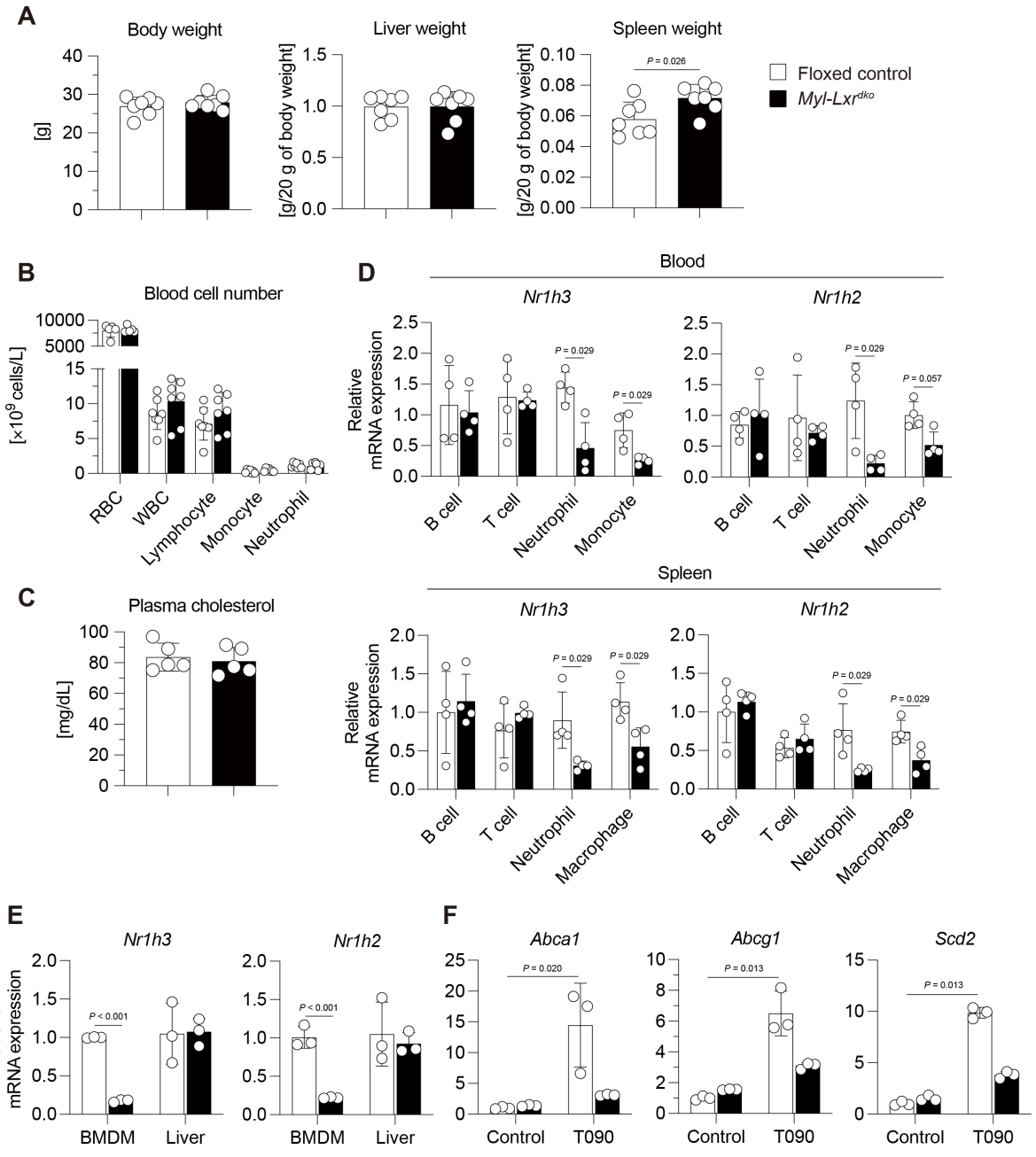

A
Body weight
Liver weight
Spleen weight
[g]
[g/20 g of body weight]
P = 0.026
Floxed control
Myl-Lxr^dko
B
Blood cell number
[×10^9 cells/L]
RBC
WBC
Lymphocyte
Monocyte
Neutrophil
C
Plasma cholesterol
[mg/dL]
D
Blood
Nr1h3
Nr1h2
Relative mRNA expression
B cell
T cell
Neutrophil
Monocyte
P = 0.029
P = 0.057
Spleen
Macrophage
E
Nr1h3
Nr1h2
P < 0.001
BMDM
Liver
F
Abca1
Abcg1
Scd2
P = 0.020
P = 0.013
Control
T090

Fig. S1 Myeloid cell-specific LXRα/β-deficient mice. Body weight, liver weight or spleen weight (**A**), blood cell counts (**B**) and plasma total cholesterol levels (**C**) in male floxed control or *Myl-Lxr*^{dko} mice on normal laboratory diet-fed condition. (n = 7 for each group). (**D**) Gene expression of *Nr1h3* or *Nr1h2* in lymphocyte (B cells or T cells) and myeloid cells (neutrophils, and monocyte or macrophages) sorted from whole blood or spleen. (n = 4 for each group). Statistical analysis was performed by Mann-Whitney U test. (**E**) Expression of *Nr1h3* or *Nr1h2* in bone marrow-derived macrophages (BMDMs) or whole liver. Statistical analysis was performed by unpaired Student *t*-tests. (**F**) Ligand-dependent LXR target gene expression in macrophages. BMDMs were treated with dimethyl sulfoxide (DMSO, control) or T0901317 (T090, 1 μM) for 16 hours (n = 4 for each group). The expression of *Abca1*, *Abcg1* and *Scd-2* were evaluated by qPCR. Statistical analysis was performed by Kruskal-Wallis test followed by Dunn's multiple comparisons. All data are shown as mean ± SD and white dots indicate individual values.

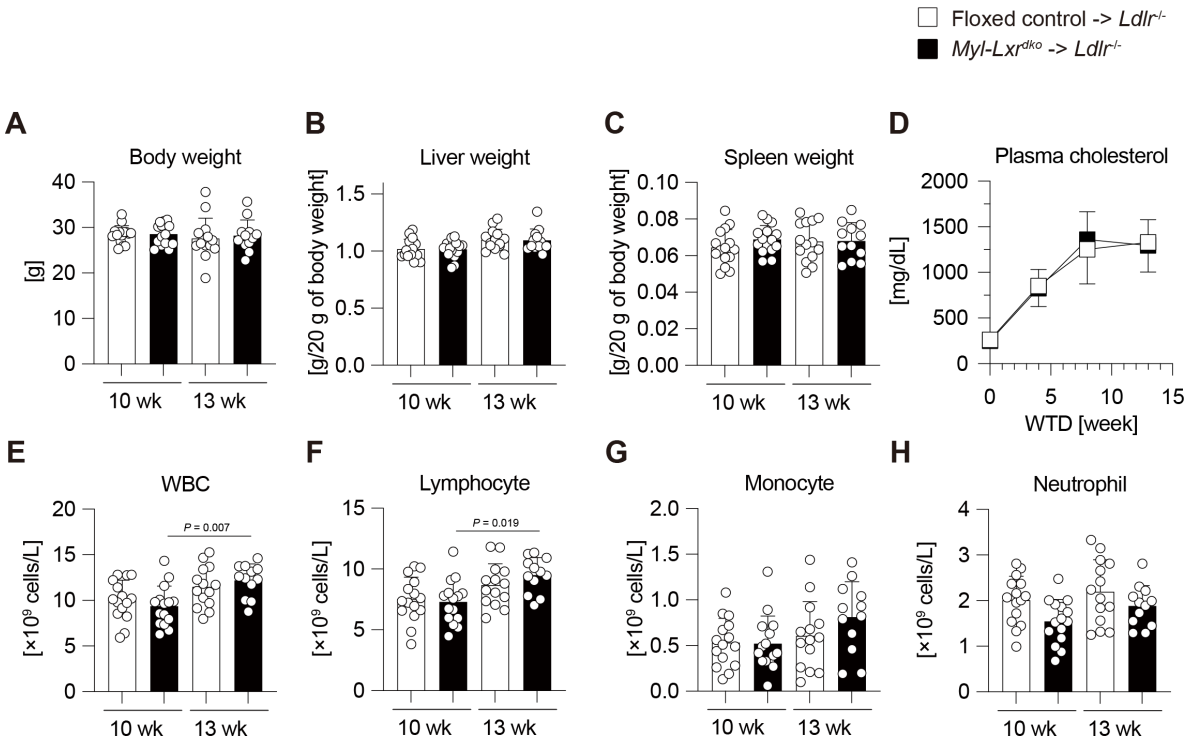


Fig. S2 Tissue weight and blood white blood cell contents in atherosclerosis mice. *Ldlr*$^{-/-}$ mice were transplanted with bone marrow cells from floxed control or *Myl-Lxr*^{dko} mice after WTD feeding for 10 or 13 weeks (n = 13-15 for each group). (**A**) Body weight, (**B**) liver weight and (**C**) spleen weight per 20 g of body weight. (**D**) Plasma total cholesterol levels during WTD feeding. (**E**-**H**) The number of total white blood cells (WBC), lymphocytes, monocytes and neutrophils were measured with complete blood cell tests. Statistical analysis was performed by Kruskal-Wallis test followed by Dunn's multiple comparisons. All data are shown as mean ± SD and white dots indicate individual values.

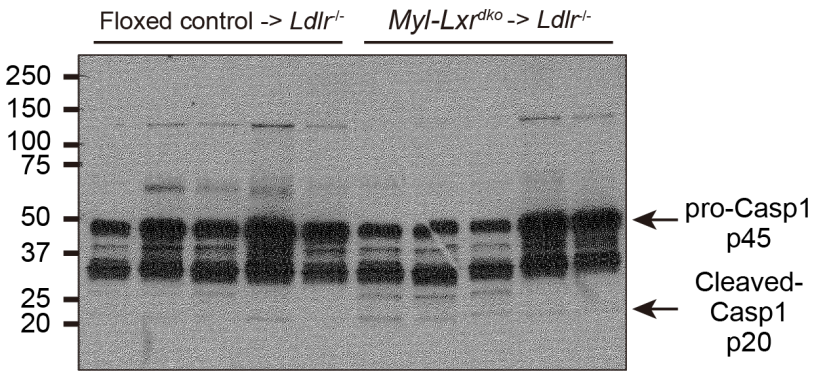


Fig. S3 Inflammasome activation in splenic CD11b^{+} cells. The CD11b^{+} cells were isolated from spleen of *Ldlr*$^{-/-}$ mice transplanted with bone marrow cells from floxed control or *Myl-Lxr*^{dko} mice after WTD feeding for 10 weeks (n = 5 for each group). The levels of cleaved form of Caspase-1 were assessed by western blotting using anti-caspase-1 antibody.

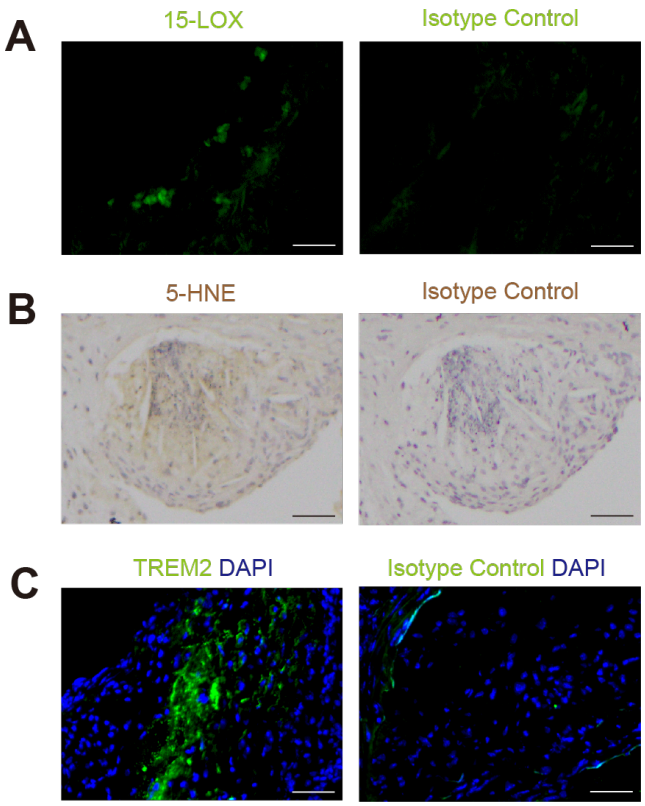
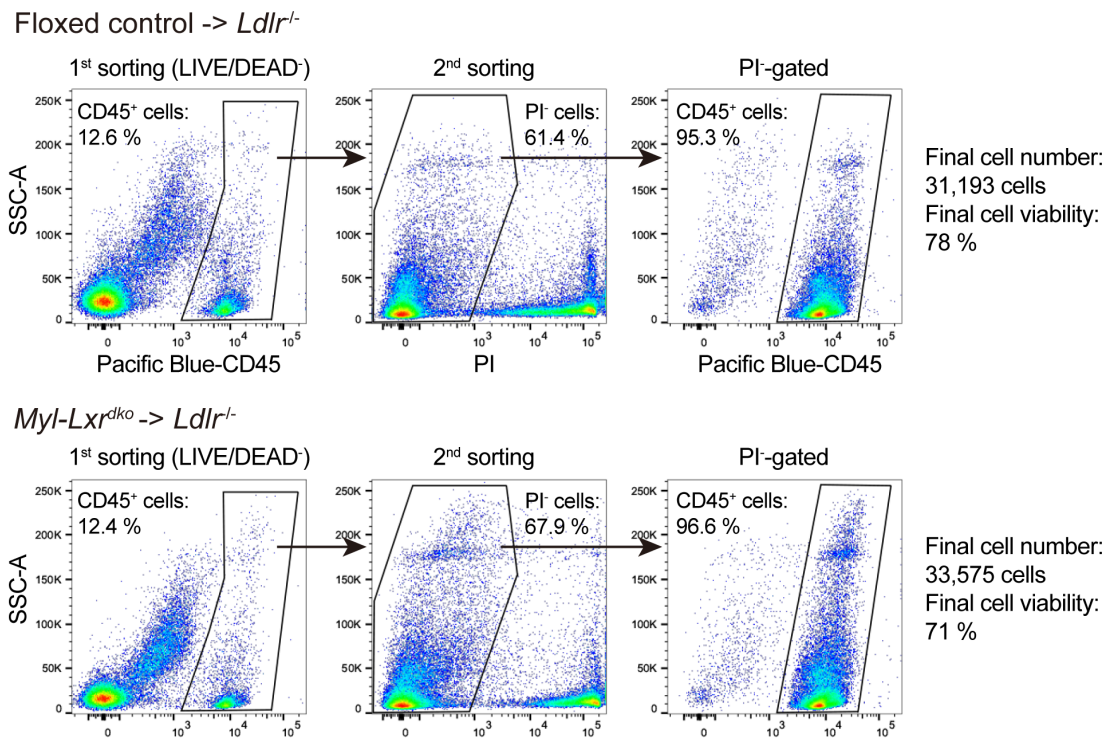


Fig. S4 Isotype control staining for 15-LOX, 4-HNE and TREM2. (**A**) Paraffin section of aortic root was stained with anti-15-LOX antibody or isotype control antibody (anti-rabbit IgG), and anti-5-HNE antibody or isotype control antibody (anti-rabbit IgG) (**B**). (**C**) Frozen section was stained with anti-TREM2 antibody or isotype control antibody (anti-rat IgG).

A



B

Group	Min # of Cells	Max # of UMI	Min # of Genes	Max # of Genes	Max % of Mitochondrial Reads	Total # of Genes	Total # of Cells
Floxed Control -> *Ldlr*$^{-/-}$	10	25,000	200	4,000	10	14,169	3,932
Myl-Lxr^{dko} -> *Ldlr*$^{-/-}$	10	25,000	200	4,000	10	14,006	2,907

Fig. S5 Sorting of aortic CD45^{+} cells for scRNA-seq. (**A**) The digested aortic cells pooled from 10 mice for each group were stained with LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit, Pacific Blue-conjugated anti-CD45, and then LIVE/DEAD^{-}PI^{-}CD45^{+} cells were sorted with FACSAria cell sorter (1st sorting). For the second sorting, cells were propidium iodide (PI) and living CD45^{+} cells (PI^{-}CD45^{+} cells) were separated again with cell sorter. After cell sorting, 31,193 cells from floxed control-BMT *Ldlr*$^{-/-}$ group (final cell viability: 78 %) or 33,575 cells from *Myl-Lxr*^{dko}-BMT *Ldlr*$^{-/-}$ mice group (final cell viability: 78 %) were subjected to Chromium library preparation system, respectively. (**B**) The detailed filtering procedure of scRNA analysis.

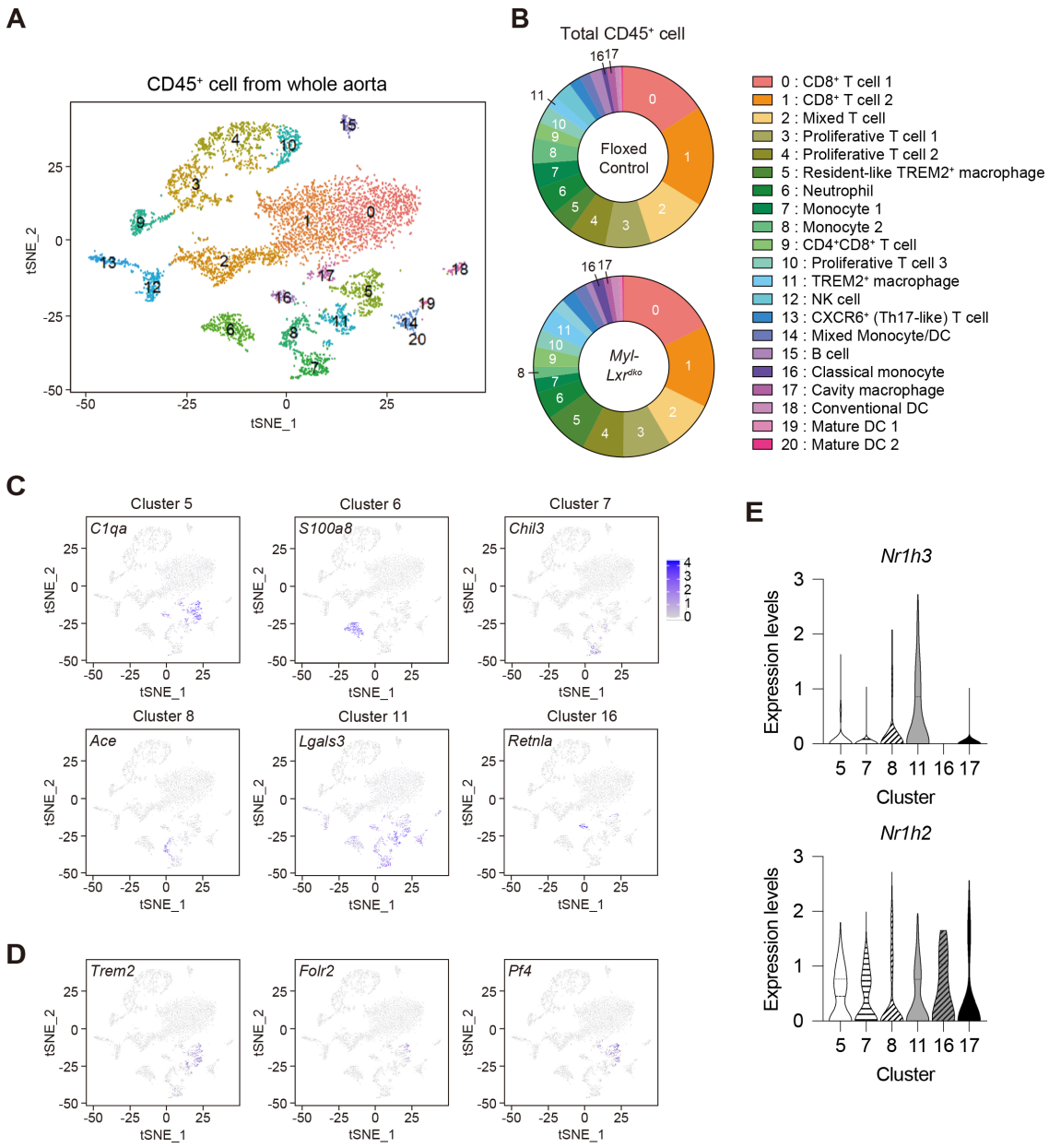
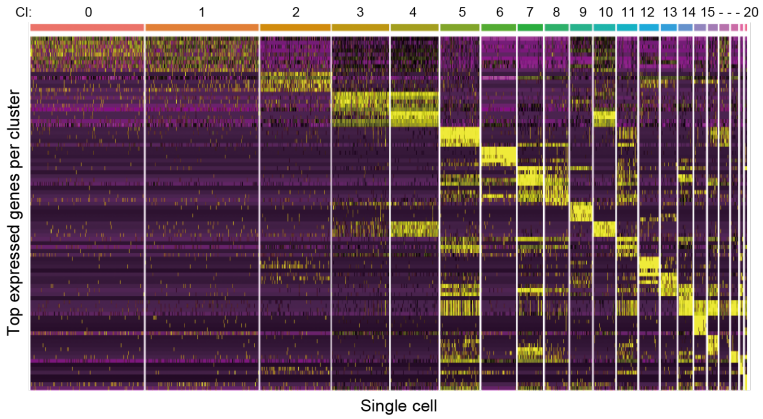


Fig. S6 Overall population of immune cells in atherosclerotic plaques. (**A**) Representative t-distributed stochastic neighbor embedding (tSNE) plot of CD45$^+$ cells isolated from whole aorta by scRNA-seq analysis. (**B**) Percentage of each cluster (Cl.) in total CD45$^+$ cells in floxed control- or *Myl-Lxr*^{dko}-BMT *Ldlr*$^{-/-}$ mice. Each percentage were normalized with total cell number. (**C**) The representative tSNE plots for myeloid cell cluster-specific gene expressions of *C1qa* (cluster 5), *S100a8* (cluster 6), *Chil3* (cluster 7), *Ace* (cluster 8), *Lgal3* (cluster 11) and *Retnla* (cluster 16). (**D**) The representative tSNE plots for macrophage markers, *Trem2*, *Folr2* and *Pf4*. (**E**) Expression of *Nr1h3* (LXRα) and *Nr1h2* (LXRβ) in myeloid cell clusters in floxed control-BMT *Ldlr*$^{-/-}$ mice.

A



B

Cluster ID	Top 10 Differentially Expressed Gene Markers	Cell Type
0	*Ccr9, P3h3, Dntt, Slamf1, Sgk1, Pou3f1, Id3, Cd8a, Mafb, Terf2ip*	$CD8^+$ T cell 1
1	*Trbc2, Dntt, Cd8a, Tubb2b, Sh2d1a, Trbc1, Ccr9, H2-Q2, Ctsl, Kcnq1ot1*	$CD8^+$ T cell 2
2	*Tmsb10, Itm2a, Cd2, Cd5, Dusp10, Vps37b, Nr4a3, Crem, Ccnd2, Hivep3*	Mixed T cell
3	*Mcm3, Dut, Pclaf, Mcm6, Anp32b, Hmgn2, Ran, Stmn1, Ranbp1, Rrm2*	Proliferative T cell 1
4	*Birc5, Top2a, Pclaf, Tuba1b, Stmn1, Hmgb2, Hmgn2, Ube2c, Rrm2, H2afx*	Proliferative T cell 2
5	*C1qb, C1qc, C1qa, Ms4a7, Selenop, Apoe, Pf4, H2-Ab1, H2-Eb1, H2-Aa*	Resident-like $TREM2^+$ macrophage
6	*Retnlg, Csf3r, Mmp9, Il1b, S100a8, S100a9, Slpi, Msrb1, S100a11, Cxcl2*	Neutrophil
7	*Ms4a4c, Ly6c2, Ifitm6, Chil3, Fn1, Ms4a6c, Plac8, Ifitm3, Lyz2, S100a4*	Monocyte 1
8	*Ace, Cybb, Ifitm3, Lst1, Gngt2, Eno3, Pou2f2, Ear2, Hes1, Polr2l*	Monocyte 2
9	*Gm4632, Tcrg-C2, Myl10, Tcrg-C1, Il2ra, Cdk6, Plac8, Cd3g, Hes1, Olfr1369-ps1*	$CD4^+CD8^+$ T cell
10	*Cenpf, Ube2c, Cenpa, Cdc20, Birc5, Ccnb2, Cdca8, Prc1, Hmgb2, Top2a*	Proliferative T cell 3
11	*Lgals3, Ctsb, Plin2, Psap, Lgmn, Rnh1, Apoe, Fabp5, Spp1, Ccl2*	$TREM2^+$ macrophage
12	*Nkg7, Gzmb, Klrd1, Ccl5, Ms4a4b, AW112010, Ctsw, Ctla2a, H2-Q7, Hcst*	NK cell
13	*Pxdc1, Il7r, Cxcr6, H2-Q7, S100a4, Itgb7, S100a10, Tmem176b, Tmem176a, Ctla2a*	$CXCR6^+$ (Th17-like) T cell
14	*Cd209a, Napsa, H2-DMb1, H2-DMa, Gm2a, Cd74, S100a4, H2-Ab1, H2-Eb1, H2-Aa*	Mixed monocyte/DC
15	*Ms4a1, Iglc3, Fcmr, Iglc2, Iglc1, Ebf1, Cd79a, Igkc, Cd79b, Ighm*	B cell
16	*Fcrls, Fn1, Ccl9, Pltp, Ccr2, Ccl6, Retnla, H2-Ab1, Lyz1, Lpl*	Classical monocyte
17	*C1qb, C1qc, Apoe, C1qa, Selenop, F13a1, Ninj1, Pf4, Serpinb6a, Blvrb*	Cavity macrophage
18	*Naaa, Irf8, Plbd1, H2-DMb1, Cst3, H2-Ab1, H2-Eb1, Ckb, H2-Aa, Cd74*	Conventional DC
19	*Fscn1, Anxa3, Ccr7, Basp1, Bcl2a1b, H2-Eb1, Cd74, H2-Aa, Cst3, Fabp5*	Mature DC1
20	*Iglc3, Siglech, Cox6a2, Rnase6, Ly6c2, Ly6a, Irf8, Tcf4, Bst2, Ctsl*	Mature DC2

C

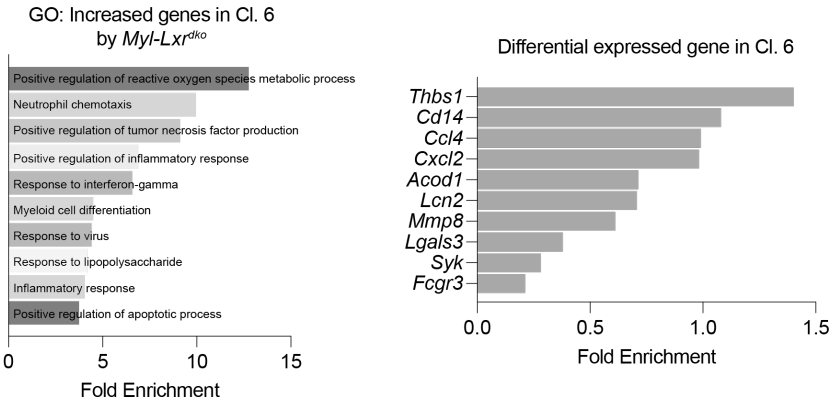


Fig. S7 Differential gene expression of $CD45^+$ leukocytes in atherosclerotic plaques. (**A**) The heatmap of top expressed genes per each cluster. (**B**) The list of top 10 differentially expressed gene markers per each cluster. (**C**) GO analysis in increased genes by myeloid LXRα/β deficiency (> 1.2-fold, Bonferroni-adjusted *P* value < 0.05) and differential expressed genes in cluster 6.

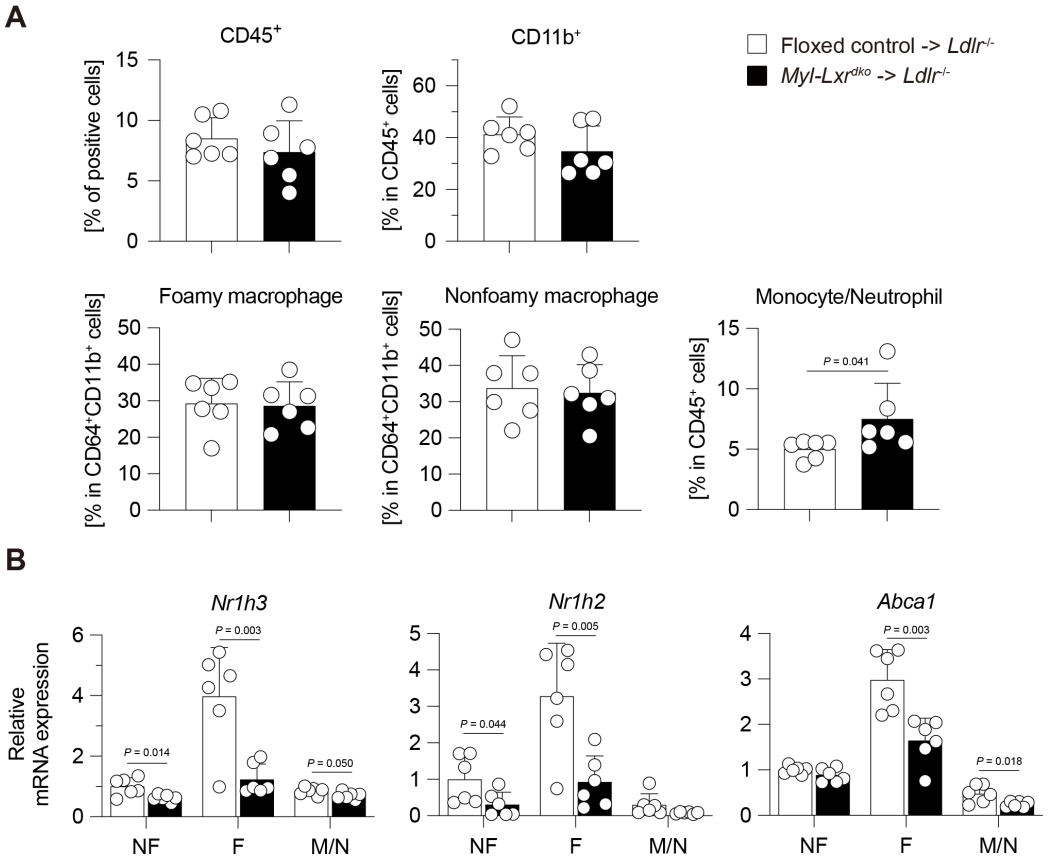


Fig. S8 Sorting of foamy macrophages, nonfoamy macrophages and monocyte/neutrophils from digested aortic cells. (**A**) The population of total CD45$^+$ cells, CD45$^+$-gated CD11b$^+$ cells, foamy macrophages (CD45$^+$-gated CD64$^+$CD11b$^+$BODIPY^{hi}SSC^{hi} cells), nonfoamy macrophages (CD45$^+$-gated CD64$^+$CD11b$^+$BODIPY^{lo}SSC^{lo} cells) and monocyte/neutrophils (CD45$^+$-gated CD64$^+$CD11b$^+$ cells) in *Ldlr*$^{-/-}$ mice transplanted with bone marrow cells isolated from floxed control- or *Myl-Lxr*^{dko}-BMT *Ldlr*$^{-/-}$ mice after WTD feeding for 20 weeks (n = 6 for each group). The digested aortic cells were stained with Pacific Blue-conjugated anti-CD45, PE-Cy7-conjugated anti-CD11b, APC-conjugated anti-CD64 and BODIPY. (**B**) Expression of *Nr1h3*, *Nr1h2* and *Abca1* in sorted foamy macrophages, nonfoamy macrophages and mixed cells of monocytes and neutrophils. (n = 6 for each group) All data are shown as mean ± SD and white dots indicate individual values. Statistical analysis was performed by Mann-Whitney U test.

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mus Musculus *Nr1h3*$^{floxed/floxed}$*Nr1h2*$^{floxed/floxed}$ (*Lxr*$\alpha^{fl/fl}$*Lxr*$\beta^{fl/fl}$)	Jan-Åke Gustafsson Laboratory, Karolinska Institute	C57BL/6J	M	
Mus Musculus *Nr1h3*$^{floxed/floxed}$*Nr1h2*$^{floxed/floxed}$-*LysM*-Cre (*Lxr*$\alpha^{fl/fl}$*Lxr*$\beta^{fl/fl}$-*LysM*-Cre)	Alan R. Tall Laboratory, Columbia University Irving Medical Center	C57BL/6J	M	
Mus Musculus B6.129S7-*Ldlr*^{tm1Her}/J	The Jackson Laboratory, stock No: 002207	C57BL/6J	M	

Genetically Modified Animals

	Species	Vendor or Source	Background Strain
Parent - Male	Mus Musculus B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J	The Jackson Laboratory, Stock No: 004781	C57BL/6J
Parent - Female	Mus Musculus *Nr1h3*$^{floxed/floxed}$*Nr1h2*$^{floxed/floxed}$ (*Lxr*$\alpha^{fl/fl}$*Lxr*$\beta^{fl/fl}$)	Jan-Åke Gustafsson Laboratory, Karolinska Institute	C57BL/6J

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
Mac-2	Cedarlane	CL8942AP	1:10,000	RRID: AB_10060357
Ki-67	Vector Laboratories, Inc.	VP-K451	1:1,500	RRID: AB_2314701
IL-1β	Novus Biologicals	NB600-633	1:100	RRID: AB_10001060
Ly6G	BioLegend	127602	2.5 μg/mL (1:200)	RRID: AB_1089180

MPO	R&D Systems	BAF3667	6.7 μg/mL (1:30)	RRID: AB_2146326
Histone H3 (citrulline R2 + R8 + R17)	Abcam	ab5103	1:300	
15-LOX	Abcam	ab244205	1:1,000	
4-HNE	Abcam	ab46545	1:500	RRID: AB_722490
TREM-2	Sigma-Aldrich	MABN755	1:100	
Caspase-1	Thermo Fisher Scientific	14-9832-82	1:250	RRID: AB_2016691
CD16/32 Fc blocker	BioLegend	101302	16.7 μg/mL (1:30)	RRID: AB_312801
APC-Cy7-conjugated anti-CD45	BioLegend	103116	2 μg/mL (1:100)	RRID: AB_312981
PE-Cy7-conjugated anti-CD11b	BioLegend	101216	1 μg/mL (1:200)	RRID: AB_312799
BV421-conjugated anti-Gr-1	BioLegend	108434	1:200	RRID: AB_2562219
FITC-conjugated anti-B220	BioLegend	103206	5 μg/mL (1:100)	RRID: AB_312991
PerCP-Cy5.5-conjugated TCRβ	BioLegend	109228	2 μg/mL (1:100)	RRID: AB_1575173
APC-conjugated anti-F4/80	BioLegend	123116	4 μg/mL (1:50)	RRID: AB_89348
APC-conjugated anti-CD115	BioLegend	135532	4 μg/mL (1:50)	RRID: AB_2632740
APC-conjugated anti-CD64	BioLegend	139306	4 μg/mL (1:50)	RRID: AB_11219391
rat IgG (H+L), CF 647	Sigma-Aldrich	SAB4600186	10 μg/mL (1:200)	
Alexa Fluor (AF) 488-conjugated goat anti-rat IgG	Thermo Fisher Scientific	A-11006	2 μg/mL (1:1,000)	RRID: AB_141373
AF647-conjugated goat	Thermo Fisher	A-21244	2 μg/mL (1:1,000)	RRID: AB_2535812

anti-rabbit IgG	Scientific			
rabbit IgG (H+L), CF 647	Sigma-Aldrich	SAB4600184	10 μg/mL (1:200)	RRID: AB_2665437

Other

Description	Source / Repository	Persistent ID / URL
Western-type diet	Harlan Teklad	TD88137
Cholesterol E	FUJIFILM Wako Pure Chemical Corporation	
IL-18 ELISA	MBL	
T0901317	Cayman Chemical Company	
TRIzol Reagent	Thermo Fisher Scientific	
RNeasy Micro Kit	Qiagen	
First Strand cDNA Synthesis Kit for RT-qPCR	Thermo Fisher Scientific	
Fast Power SYBR Green PCR Master Mix reagent	Thermo Fisher Scientific	
ProLong Gold Antifade Mountant with DAPI reagent	Thermo Fisher Scientific	
AF488-conjugated streptavidin	Thermo Fisher Scientific	S11223
Clodronate liposome solution	Liposoma BV	
Fluoresbrite YG Microspheres 1.00 mm	Polysciences, Inc.	
SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing	TaKaRa Bio. Inc.	
Nextera XT DNA Library Preparation Kit	Illumina	
BODIPY 493/503	Thermo Fisher Scientific	
Chromium library preparation system	10x Genomics	