Supplemental Materials

Title*: The role of lipoprotein lipase in macrophage polarization *in vitro* and *in vivo

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DOI: 10.1161/ATVBAHA.119.312389

Supplemental Methods

Animals: All procedures were approved by the Institutional Animal Care and Use Committee at New York University Langone Health. Mice were maintained in a temperature-controlled (25°C) facility with a 12 h-light/dark cycle and given free access to water and food, except when fasting blood was obtained. Mice were fed a laboratory standard rodent chow diet or a high fat diet (HFD) (60% fat kcal; Research Diet #D12492), as indicated. Euthanasia was done by intraperitoneal injection of ketamine 100 mg/kg and xylazine 10 mg/kg followed by cervical dislocation.

Blood Leukocytes: Tail blood was collected into EDTA-lined tubes and immediately placed on ice. All subsequent steps were performed on ice. Red blood cells were lysed, and white blood cells were centrifuged, washed, and resuspended in FACS buffer. Cells were stained with a cocktail of antibodies against CD45-Alexa Fluor 450 (Invitrogen), Ly6-C/G-PerCP-Cy5.5 (BD Pharmigen), and CD115- APC (eBioscience). Monocytes were identified as CD45hiCD115hi and further identified into $Ly6C^{hi}$ and $Ly6C^{lo}$; neutrophils were identified as CD45hiCD115loLy6-C/Ghi (Gr-1).

Hematopoietic Stem Cells: BM was harvested from femurs and tibiae by flushing with ice cold PBS. Spleens were minced into small pieces, gently rubbed against the mesh in a cell strainer and flushed with PBS. The cell suspension was centrifuged at 500 g for 5 mins. The supernatant was aspirated and the cells were resuspended in red blood cell lysis buffer for 5 mins. Lysis was stopped by adding an excess amount of flow buffer, and the cell suspension was once again centrifuged and aspirated. A single cell suspension was prepared by gently pipetting up and down with 200 µl of flow buffer. This was followed by incubation with a cocktail of antibodies against lineage-committed cells (B220, CD19, CD11b, CD3e, TER-119, CD2, CD8, CD4, Ly6-C/G: All FITC, eBioscience), Sca1-Pacific Blue, and ckit-APC Cy7). HSPCs were identified as lin-, Sac1+, and ckit+ (LSK), while the hematopoietic progenitor subsets were separated using antibodies to CD16/CD32 (FcγRII/III) and CD34. Common myeloid progenitors were identified as lin-, Sca1-, ckit+, CD34int, FcγRII/IIIint, and granulocyte-Mφ progenitors as lin-, Sca1-, ckit+, CD34int, FcγRII/IIIhi. BM Mφs were identified by negative gating against (B220, CD19, CD11b, CD3e, TER-119, CD2, CD8, CD4, Ly6-C/G: All FITC, eBioscience) and then CD115+, Gr1lo, F4/80+, CD169+, and either CD11c positive or negative. Flow cytometry was performed using an LSRII (for analysis) or FACS Aria (for sorting), with both machines running FACS DiVa software. All flow cytometry data were analyzed using FlowJo software (Tree Star Inc.).

Adipose Tissue Macrophage (ATM) Isolation: Freshly dissected adipose tissue was minced into fine pieces (<10 mg) and centrifuged to remove connective tissue debris. Minced tissues were digested by liberase TM (Roche) for 20 mins and centrifuged at 500 g for 5 mins. The pellets were collected as stromal vascular cells (SVCs), and buoyant cells were collected as adipocytes. The SVCs were resuspended in erythrocyte lysis buffer (BD Biosciences) and the erythrocyte-depleted SVCs were incubated with F4/80-APC, CD11b-PE, and CD11c-PETR for Mφ purification. ATM subpopulations were separated by F4/80+CD11b+ (FBs) and F4/80+CD11b+CD11c+ (FBCs); the gating strategy is shown in Supplemental Figure V.

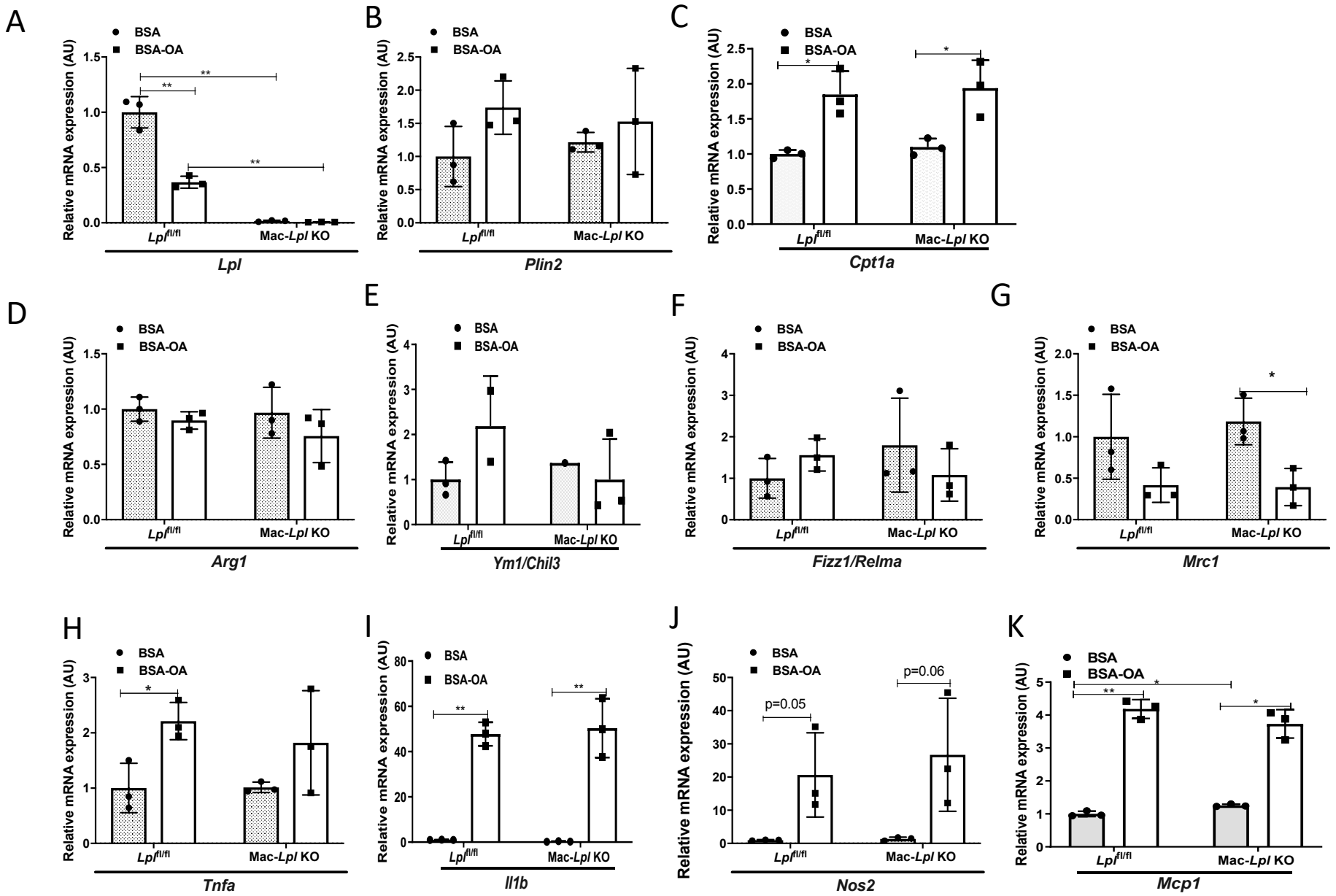
Supplemental Methods (Cont'd)

Quantitative Real Time-PCR Analyses: Total RNA was extracted from cells with silica-based membrane method by using PureLink RNA Mini kit (Ambion) for BMDMs or RNeasy Micro kit (Qiagen) for FACS-sorted cells. Complementary DNAs (cDNAs) were generated with a Verso cDNA synthesis kit (Thermo Fisher), and quantitative PCR was carried out with fluorescent double-stranded DNA dye (Fast SYBR Green Mastermix; Applied Biosystems). Data were normalized to *Rps3* with the ΔΔC(t) method and are presented as relative transcript levels. All primers are listed in Supplemental Table I.

Laser capture microdissection and quantitative Real Time PCR: Foam cells were then identified under a microscope and verified by positive CD68 staining. For each animal, CD68+ cells were captured from 50–60 frozen sections. After laser capture microdissection, RNA was isolated using the PicoPure Kit (Molecular Devices, Inc., Sunnyvale, CA), and quality and quantity were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA was converted to cDNA and amplified using the WT-Ovation Pico RNA Amplification Kit (NuGEN, San Carlos, CA).

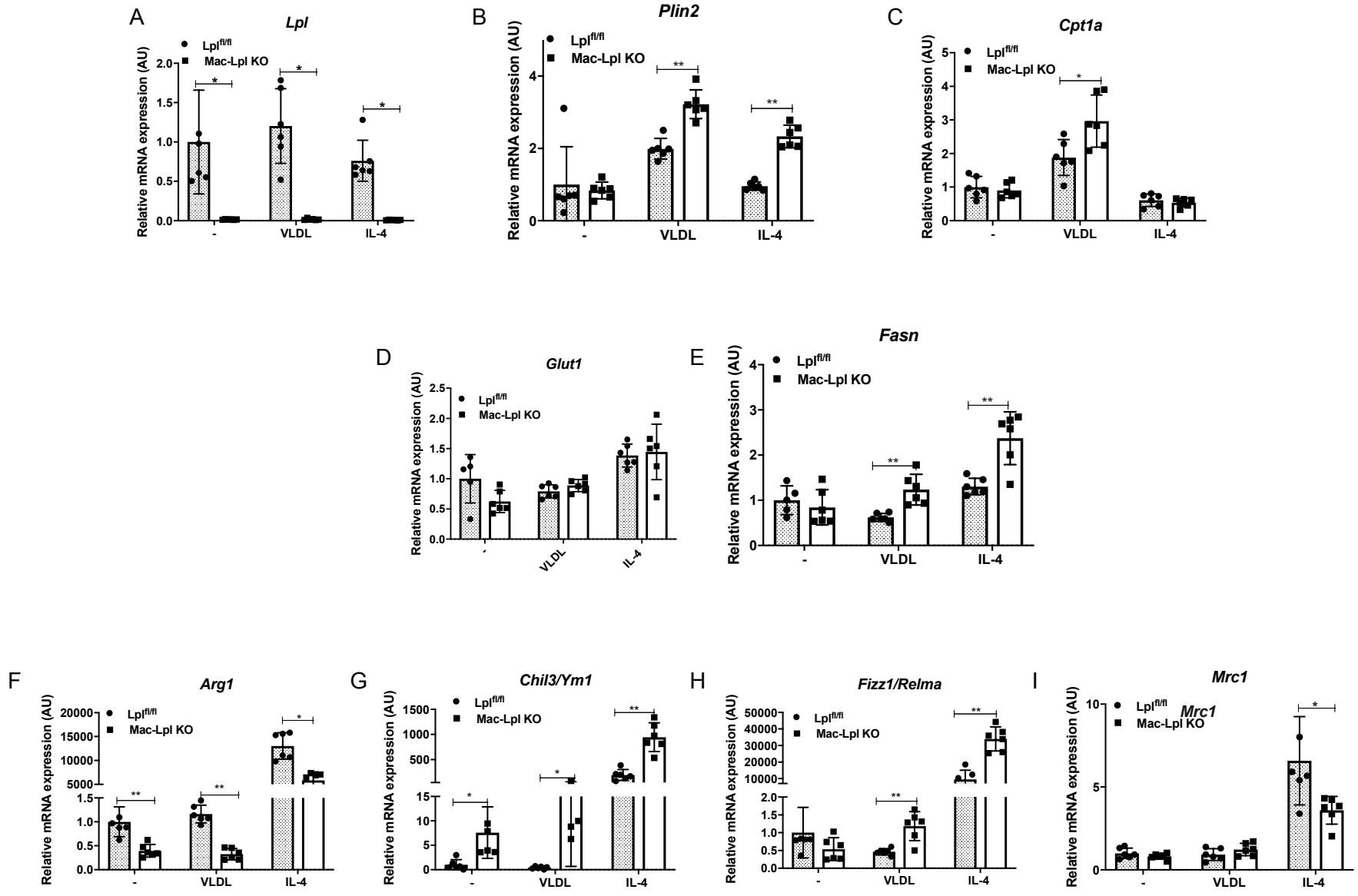
Gene	Forward (5’ - 3’)	Reverse (5’ - 3’)
Rps3	ATCAGAGAGTTGACCGCAGTTG	AATGAACCGAAGCACACCATAG
Lpl	AGGTGGACATCGGAGAACTG	TCCCTAGCACAGAAGATGACC3
Plin2	TCTGCGGCCATGACAAGTG	GCAGGCATAGGTATTGGCAAC
Cpt1a	TGCACTACGGAGTCCTGCAA	GGACAACCTCCATGGCTCAG
Glut1	TCGTAACGAGGAGAACCG	GGCCGTGTTGACGATA
Fasn	TCTTTCTAACAACCACCCTCTGG	CTTCACGACTCCATCACGAATG
Cd36	CCTTAAAGGAATCCCCGTGT	TGCATTTGCCAATGTCTAGC
Tnf	TGGAACTGGCAGAAGAGG	AGACAGAAGAGCGTGGTG
Il1b	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
Mcp1	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCCTTCTTG
Nos2	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGTTTCG
Arg1	TGGCTTGCGAGACGTAGAC	GCTCAGGTGAATCGGCCTTTT
Ym1	AGGCTTTGCGGTCCTGAT	CCAGCTGGTGAAGTAGCAGA
Fizz1	CCAATCCAGCTAACTATCCCTCC	AAGCCACAAGCACACCCAGT
Mrc1	TGATTACGAGCAGTGGAAGC	GTTCACCGTA-AGCCCAATTT
Il10	CTGGACAACATACTGCTAACCG	GGGCATCACTTCTACCAGGTAA

Supplemental Table I. List of primers used for quantitative PCR



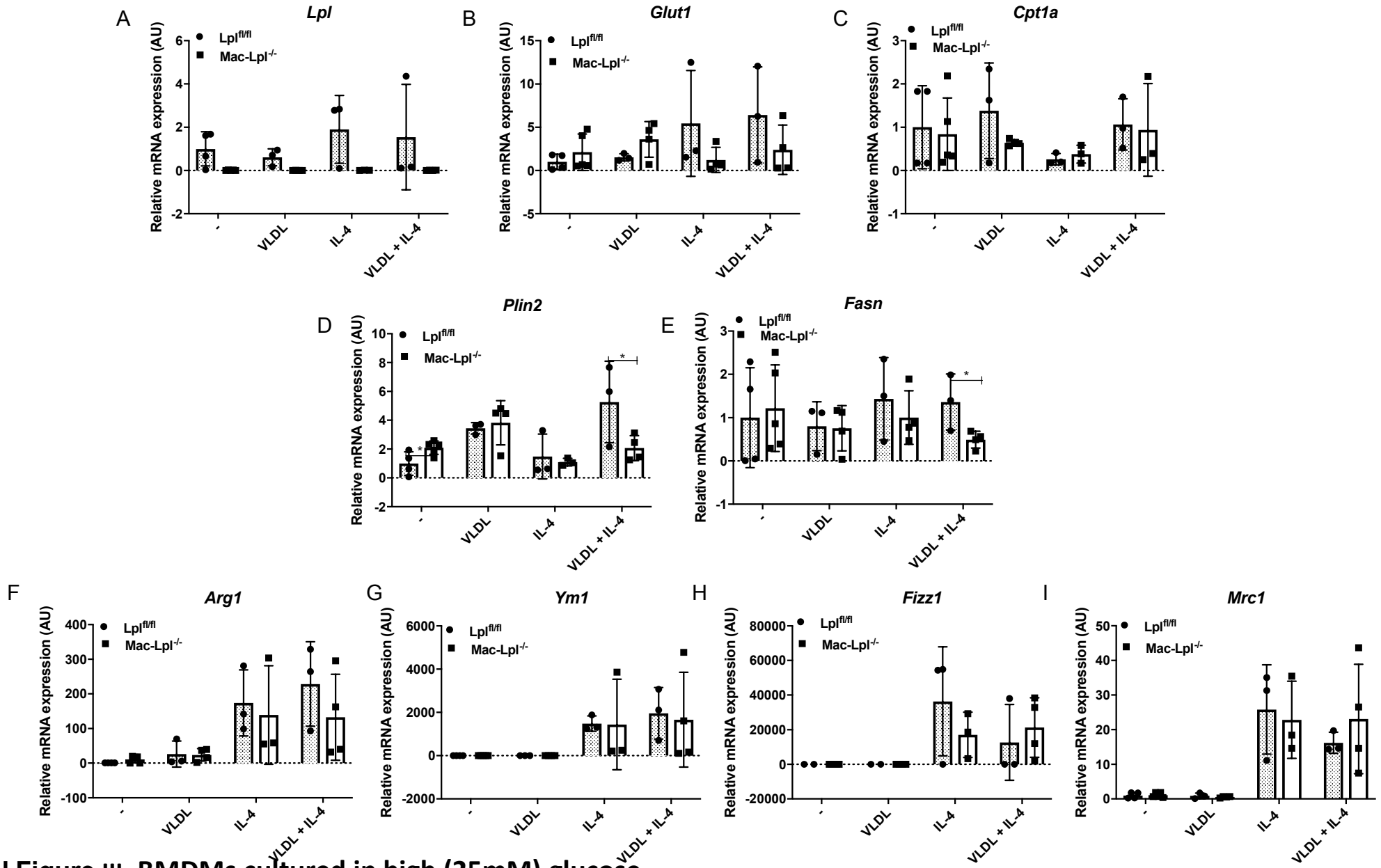
Supplemental Figure I. *Lpl* deficiency does not affect alternative activation of macrophages upon oleic acid treatment *in vitro*

BMDMs from *Lpl*$^{fl/fl}$ and Mac-*Lpl*$^{-/-}$ mice were used to measure metabolism- related and M1/M2-related gene expression in the presence of oleic acid (OA). (A) The gene expression of *Lpl* in BMDMs. (B) The expression of a lipid droplet-related protein, *Plin2*, gene in BMDMs. (C) The expression of a fatty acid oxidation-related gene, *Cpt1a* in BMDMs. (D-G) gene expression of anti-inflammatory genes (*Arg1*, *Ym1*, *Fizz1*, *Mrc1*) in OA treated BMDMs. (H-K) The expression of inflammatory genes (*Tnfa*, *I1b*, *Nos2*, *Mcp1*) in OA treated BMDMs. N=3/group.*p<0.05, **p<0.01. Results are represented mean ± SD and compared using Two-way ANOVA, Sidak's multiple comparison.



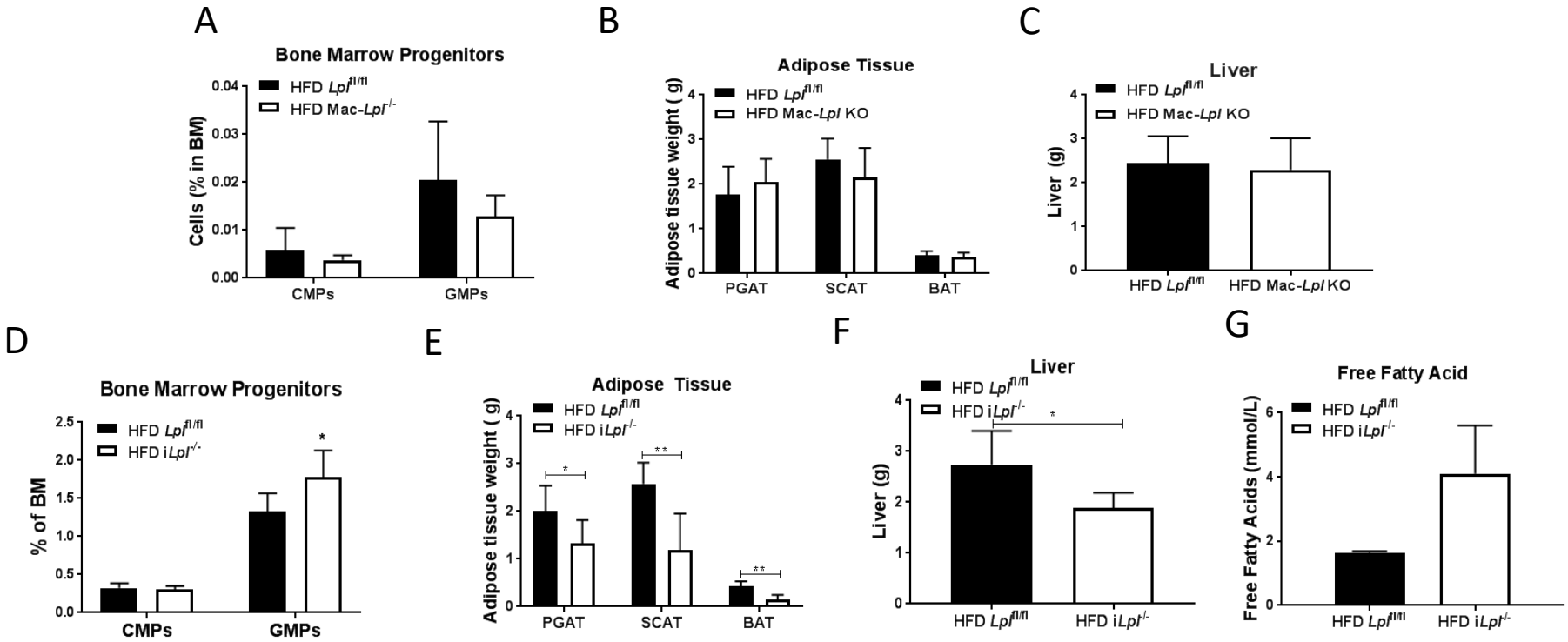
Supplemental Figure II. BMDMs culture in normal glucose (5mM)

BMDMs from *Lpl*$^{fl/fl}$ and Mac-*Lpl*$^{-/-}$ mice were used to measure metabolism- related and M1/M2-related gene expression in the presence of human VLDL (100ug/mL), IL-4 (20ng/mL) and normal glucose (5mM). (A) The gene expression of *Lpl* in BMDMs. (B) The expression of a lipid droplet-related protein, *Plin2*, gene in BMDMs. (C) The expression of a fatty acid oxidation-related gene, *Cpt1a* in BMDMs. (D) The expression of a glucose transporter, *Glut1* (*Slc2a1*), gene in BMDMs. (E)The expression of fatty acid synthase gene in BMDMs. (F-I) The expression of canonical M2 (anti-inflammatory) genes in BMDMs. N=6/group. *p<0.05, **p<0.01. Results are represented mean ± SD and compared using Two-way ANOVA, Sidak's multiple comparison.



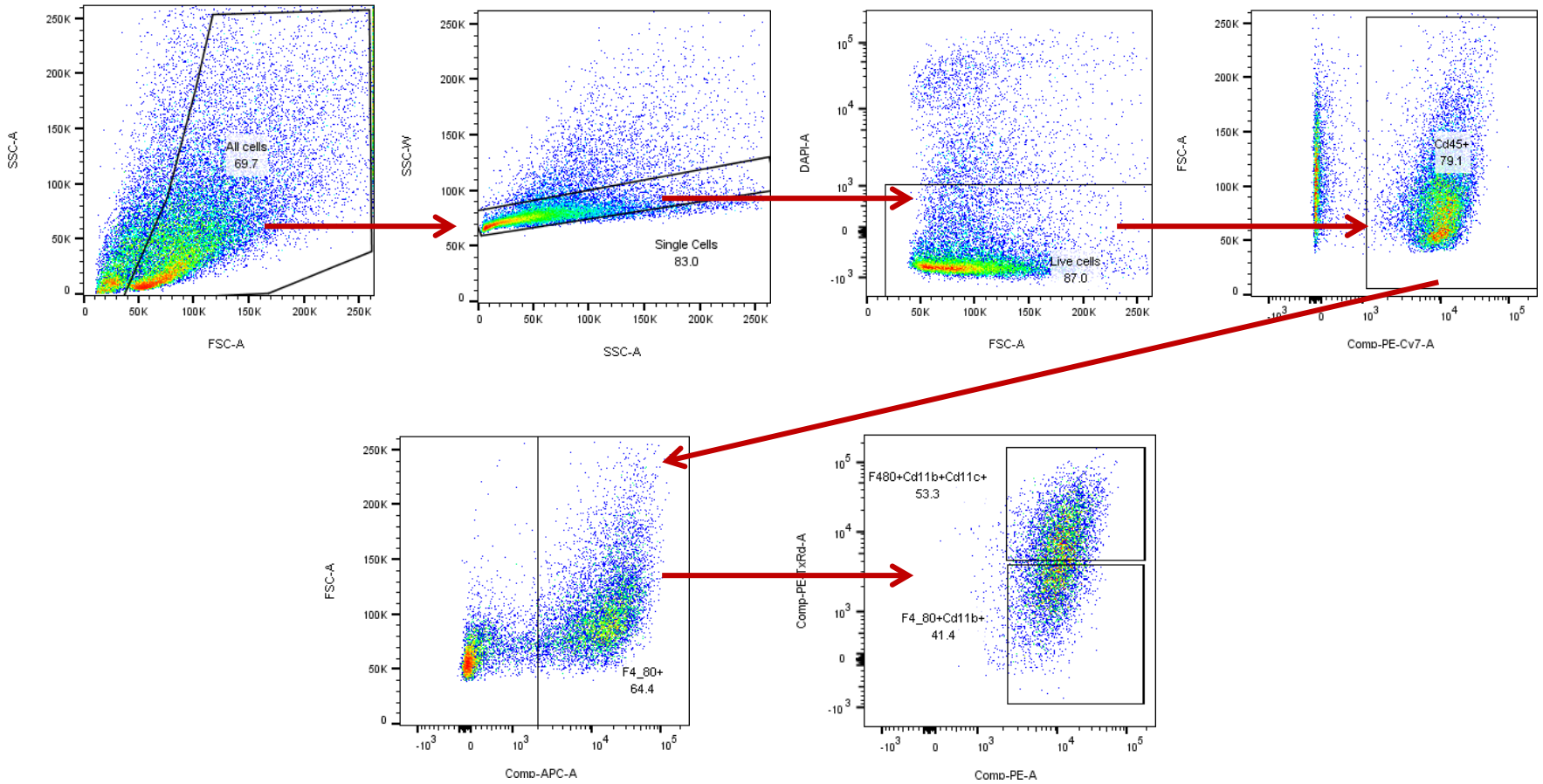
Supplemental Figure III. BMDMs cultured in high (25mM) glucose

BMDMs from *Lpl*$^{fl/fl}$ and Mac-*Lpl*$^{-/-}$ mice were used to measure metabolism- related and M1/M2-related gene expression in the presence of human VLDL (100ug/mL), IL-4 (20ng/mL) and high glucose (25mM). (A) The gene expression of *Lpl* in BMDMs. (B) The expression of a glucose transporter, *Glut1* (*Slc2a1*), gene in BMDMs. (C) The expression of a fatty acid oxidation-related gene, *Cpt1a* in BMDMs. (D) The expression of a lipid droplet-related protein, *Plin2*, gene in BMDMs. (E)The expression of fatty acid synthase gene in BMDMs. (F-I) The expression of canonical M2 (anti-inflammatory) genes in BMDMs. N=3-4/group. *p<0.05, **p<0.01. Results are represented mean ± SD and compared using Two-way ANOVA, Sidak's multiple comparison.

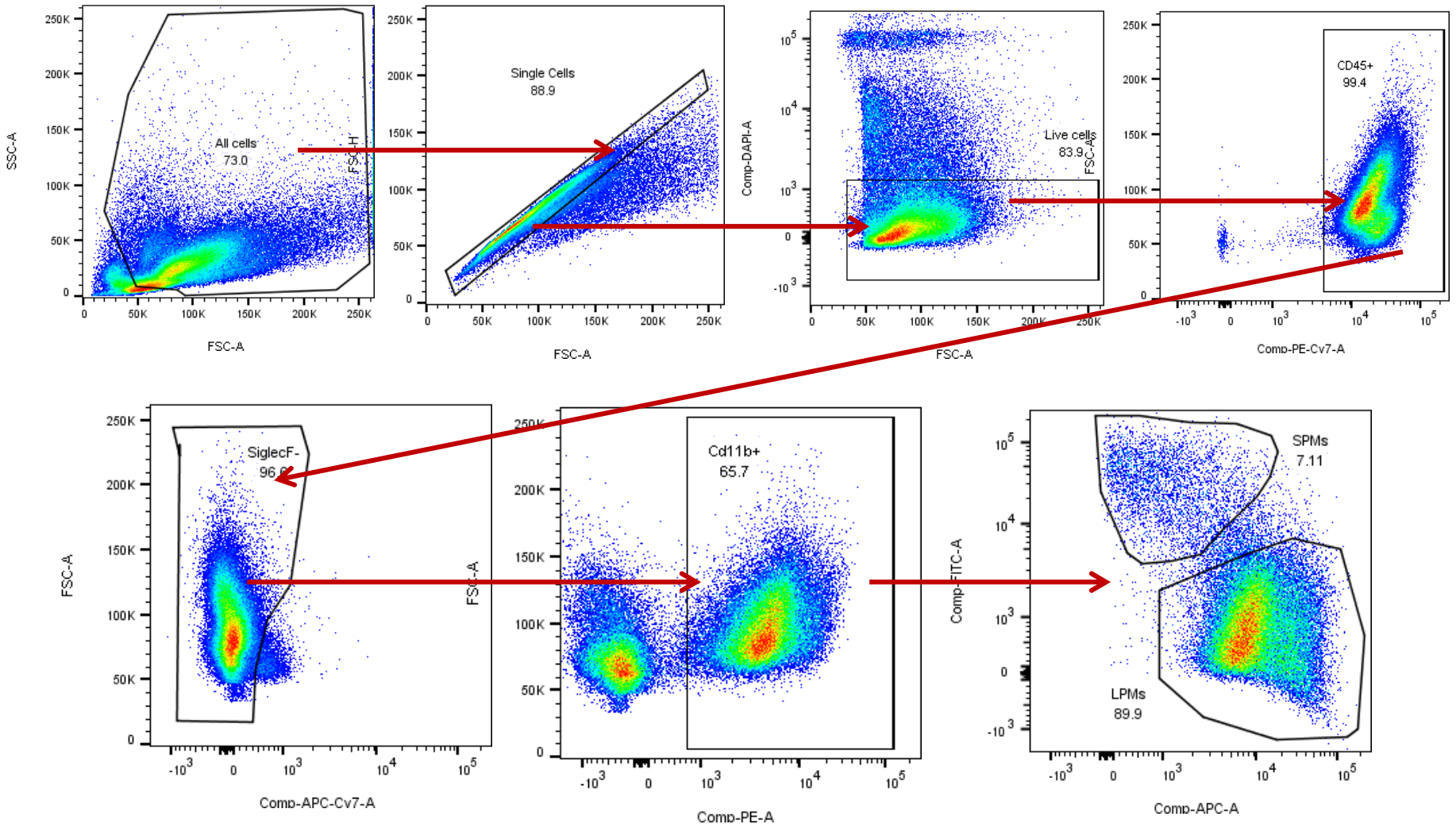


Supplemental Figure IV. Bone marrow progenitors and weight of tissue in Mac-*Lpl*$^{-/-}$and i*Lpl*$^{-/-}$ mice fed with a HFD

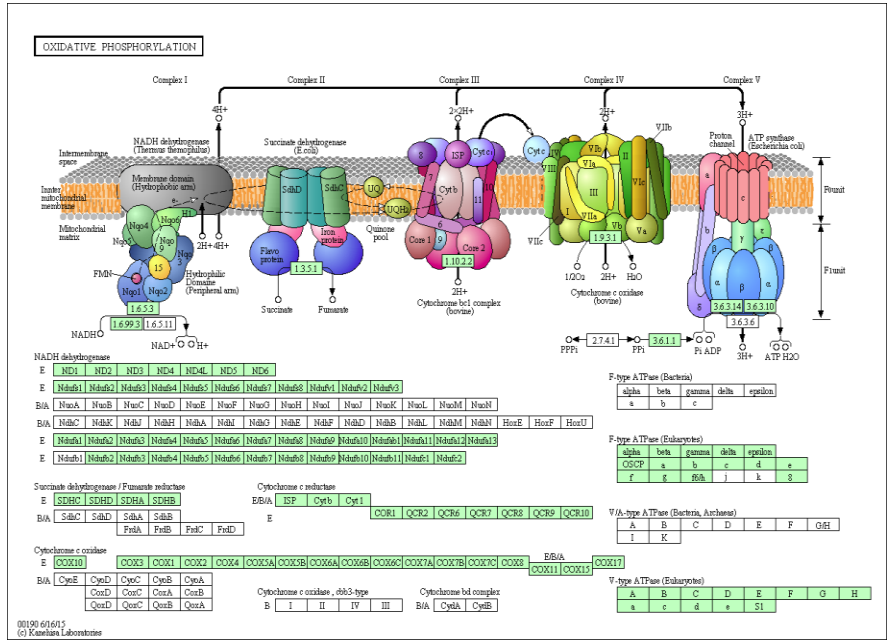
(A) Bone marrow progenitors in *Mac-Lpl*$^{-/-}$ (B) Adipose tissue weight (g) in *Mac-Lpl*$^{-/-}$ (C) Liver weight (g) in *Mac-Lpl*$^{-/-}$ (D) Bone marrow progenitors in *iLpl*$^{-/-}$ (B) Adipose tissue weight (g) in *iLpl*$^{-/-}$ (C) Liver weight (g) in *iLpl*$^{-/-}$ (G) Free fatty acids (mmol/L) in *Mac-Lpl*$^{-/-}$ *and iLpl*$^{-/-}$ mice. N=4-7/group. *p<0.05, **p<0.01. Results are represented mean ± SD and compared using Two-way ANOVA, Sidak's multiple comparison.

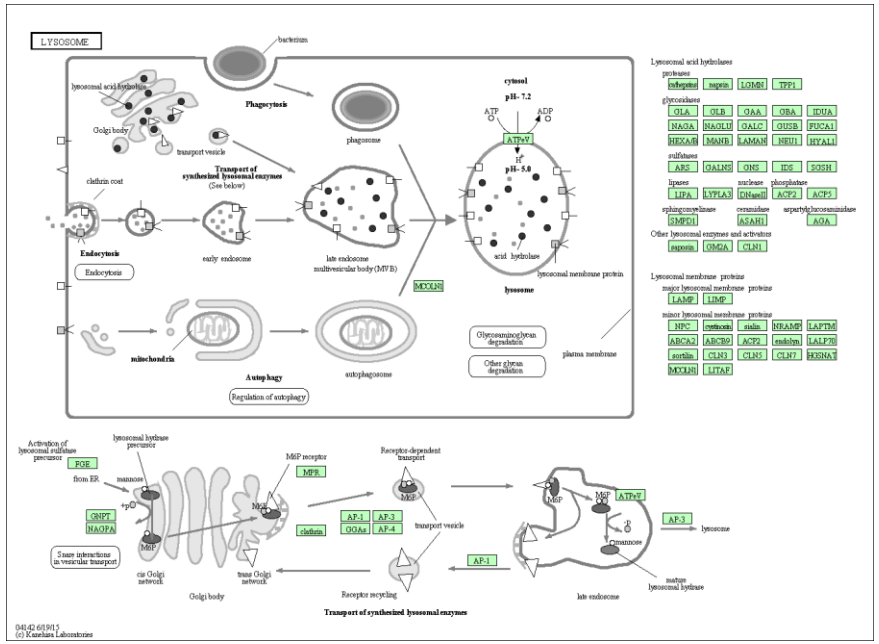


Supplemental Figure V. Adipose tissue macrophages (ATM) gating strategy

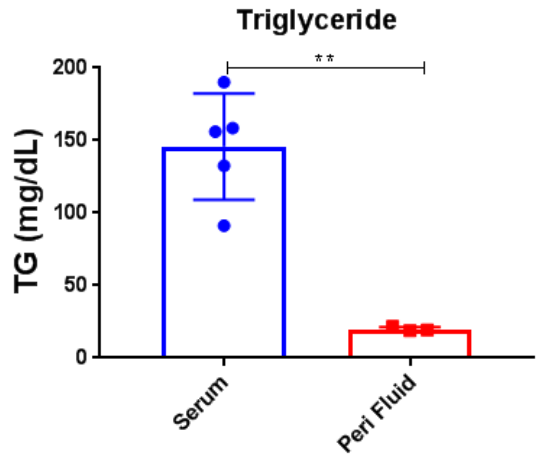


Supplemental Figure VI. Peritoneal macrophages gating strategy

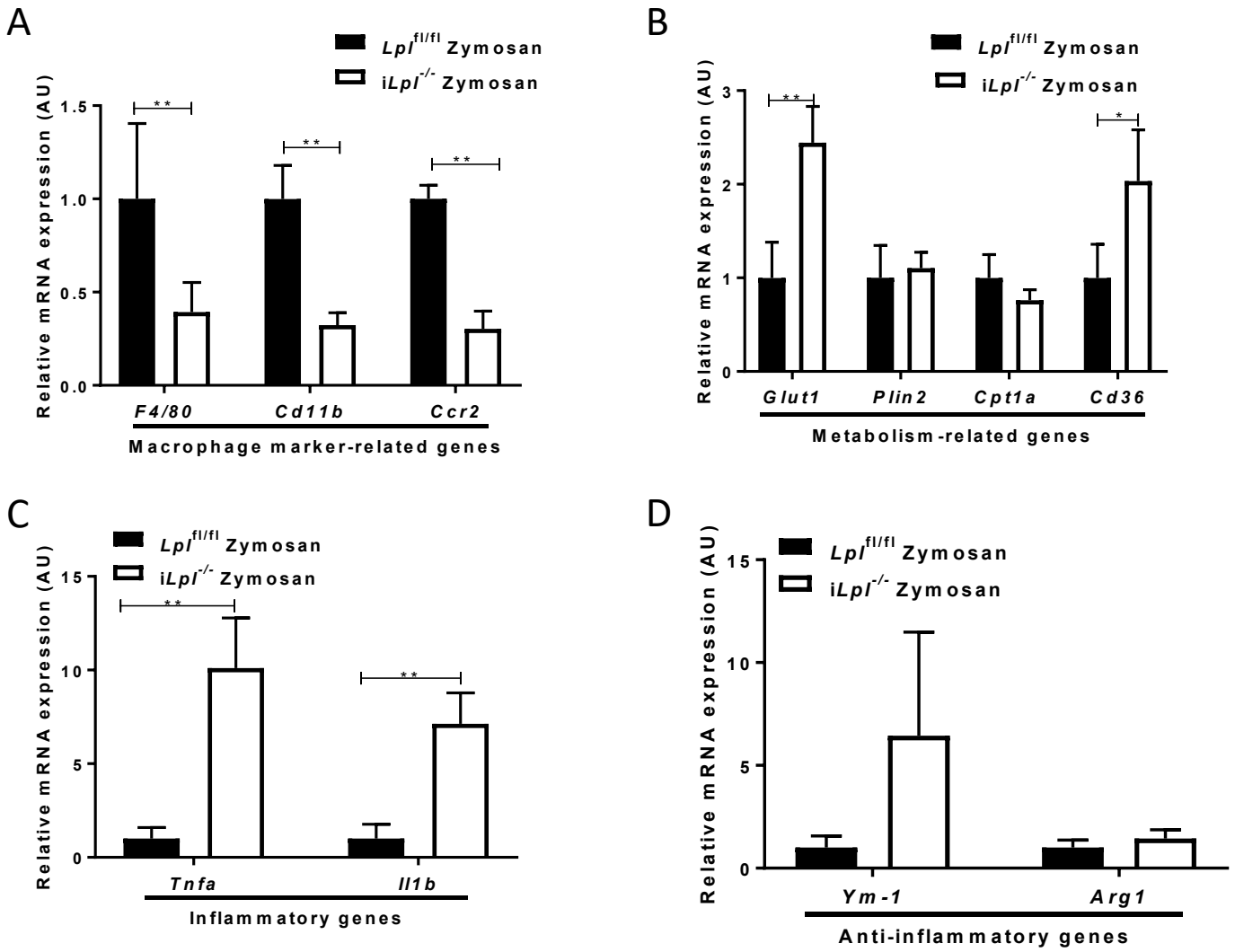




Supplemental Figure VII. KEGG Mapper Analysis- Oxidative Phosphorylation and Lysosome Pathway (Genes cut off q-value <0.05)

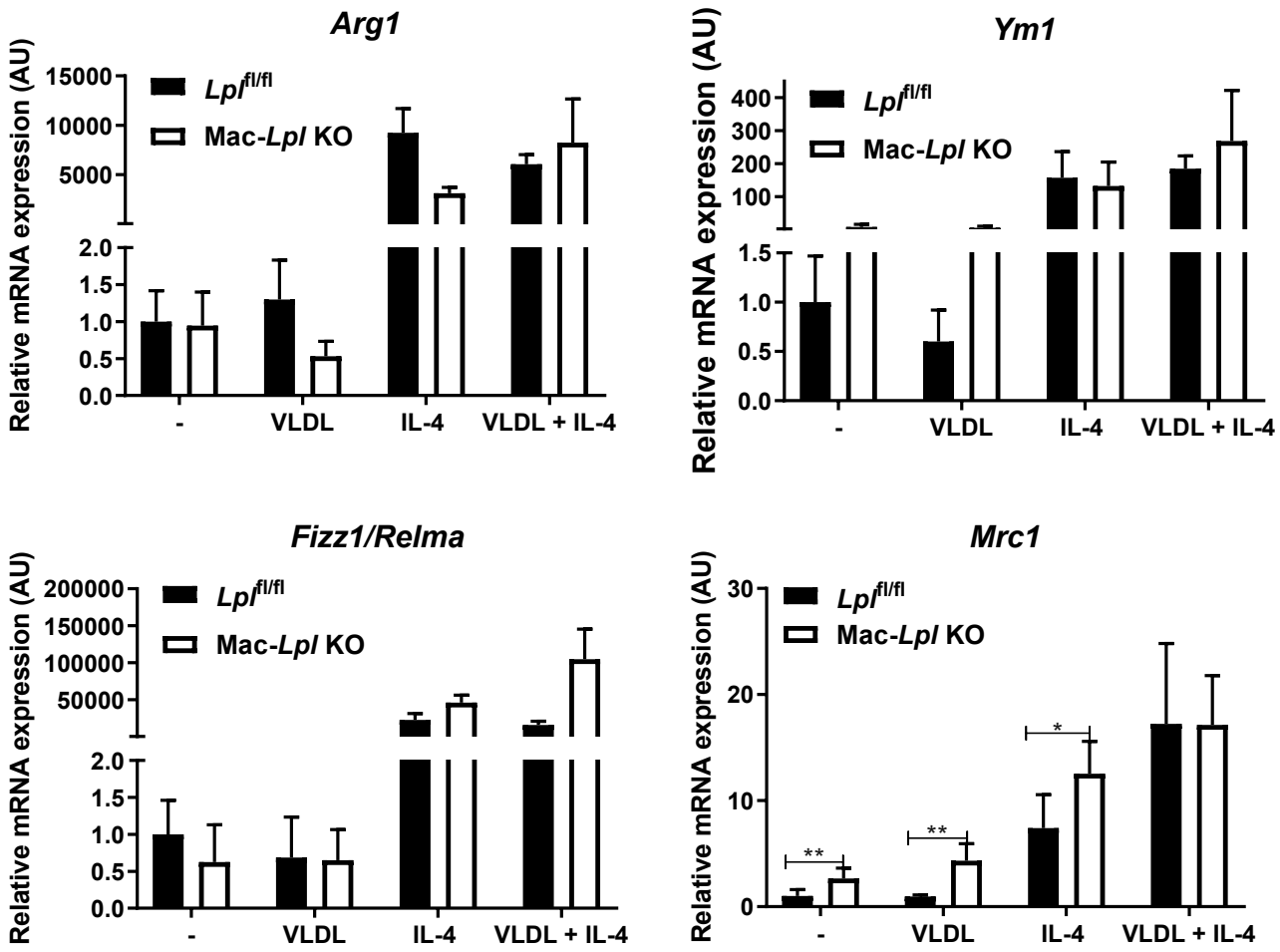


Supplemental Figure VIII. TG levels in plasma versus TG levels in peritoneal fluid



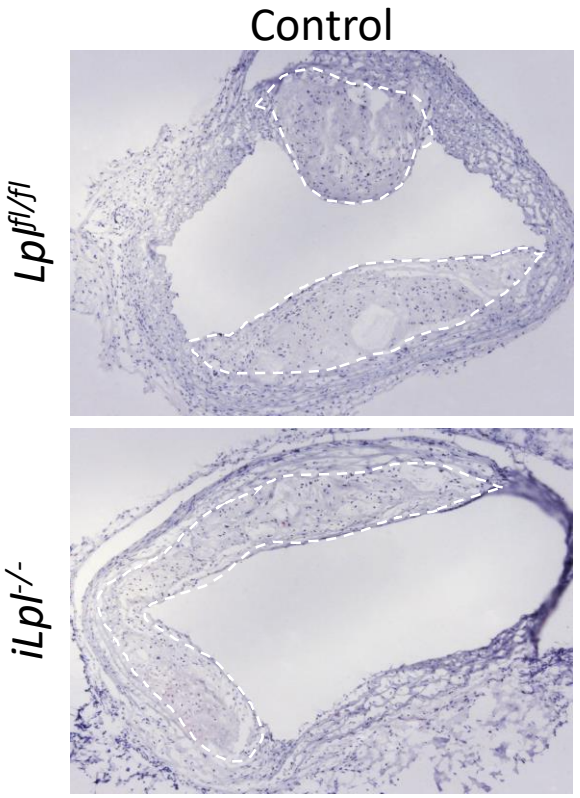
Supplemental Figure IX. Inflammatory and anti-inflammatory gene expression in total peritoneal macrophages upon zymosan stimuli

Peritoneal Macrophages from *Lpl*$^{fl/fl}$ and i*Lpl*$^{-/-}$ mice were used to measure metabolism- related and M1/M2-related gene expression. (A) Expression of macrophage marker related genes (F4/80, Cd11b, Ccr2). (B) The expression of metabolism-related genes (*Glut1, Plin2, Cpt1a, Cd36*). (C) The expression of inflammatory genes (*Tnfa, Il1b*).(D) The expression of anti-inflammatory genes (*Ym-1, Arg1*). N=4/group.*p<0.05, **p<0.01. Results are represented mean ± SD and compared using Two-way ANOVA, Sidak's multiple comparison.



Supplemental Figure X. BMDM culture in low (1mM) glucose

Side-by-side comparison of anti-inflammatory genes from Figure 1 and 2. N=6/group.*p<0.05, **p<0.01. Results are represented mean ± SD and compared using Two-way ANOVA, Sidak's multiple comparison.



Supplemental Figure XI. Control staining for CD68 and CD206

Negative controls were performed using an isotype control (Rat IgG2a) instead of the primary antibody.

Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Mouse	The Jackson Laboratory and Goldberg Laboratory	C57BL/6J	Males and Females

Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent - Male	*LysM*Cre	The Jackson Laboratory	C57BL/6J	
Parent - Male	Tg^{CreER} transgenic	The Jackson Laboratory	C57BL/6J	
Parent - Female	$Lpl^{fl/fl}$	Goldberg Laboratory	C57BL/6J	PMID: 15028738

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
CD68	Bio-Rad	MCA1957	5ug/mL
CD206/Mannose Receptor	Bio-Rad	MCA2235	5ug/mL
Rat IgG2a	Abcam	ab18450	5ug/mL
BV220 (CD45R)-FITC	eBioscience	11-0452082	2.5ug/mL
CD19-FITC	eBioscience	11-0193-82	2.5ug/mL
CD11b-FITC	eBioscience	11-0112-82	2.5ug/mL
TER-119-FITC	eBioscience	11-5921-82	2.5ug/mL
CD2 FITC	eBioscience	11-0021-82	2.5ug/mL
CD4-FITC	eBioscience	11-0041-82	2.5ug/mL
CD8a-FITC	eBioscience	11-0081-82	2.5ug/mL
Ly-6G(GR-1)-FITC	eBioscience	11-0031-82	2.5ug/mL
CD16/CD32-PE	eBioscience	12-0161-82	2.5ug/mL
Ly6A/E (Sca-1)-PE/Cy7	BioLegend	108114	2.5ug/mL
CD117 (c-kit)-APC/Cy7	BioLegend	105826	2.5ug/mL
CD34-Percp/Cy5.5	BioLegend	128608	2.5ug/mL
CD11b-PE	Life Technologies	RM2804	5ug/mL
F4/80-APC	AbD Serotec	NC9384293 (MCA497APC)	50ug/mL
CD11c-PETR	Life Technologies	MCD11C17	5ug/mL
CD45-PECy7	eBioscience	25-0451-82	2.5ug/mL

Mouse Fc Block	BD Bioscience	BDB553142	2.5ug/mL
CD115-PE	eBioscience	12-1152-82	2.5ug/mL
Ly6-6G/Ly-6C (Gr-1)-APC	BioLegend	108412	2.5ug/mL

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)
Primary Bone Marrow Cells	Not Applicable	F and M