SUPPLEMENTAL MATERIAL

Characterizing the Role of Glycogen Synthase Kinase (GSK)-3α/β in Macrophage Polarization and the Regulation of Pro-Atherogenic Pathways

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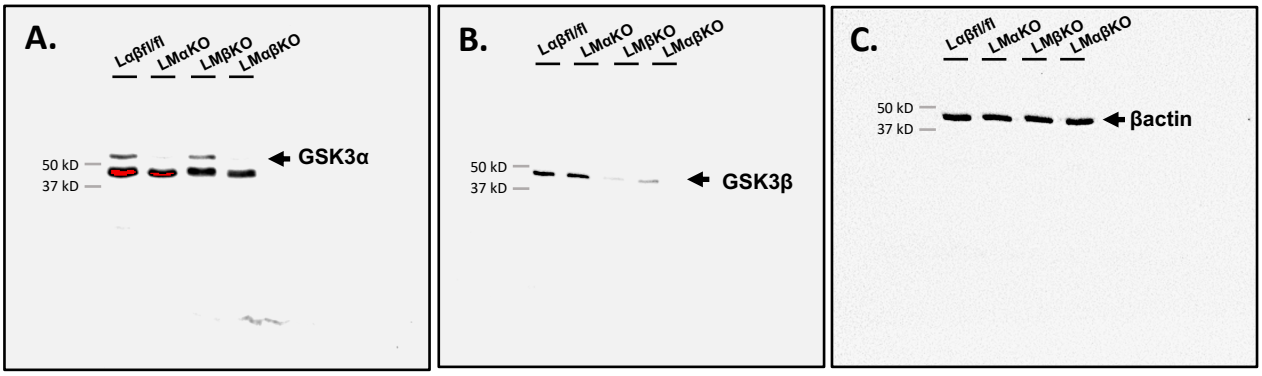
Supplementary Table I. RT-PCR primer sequences

Gene	Primer Sequence
β-actin	5'-GGC ACC ACA CCT TCT ACA ATG-3' 5'-GGG GTG TTG AAG GTC TCA AAC-3'
GSK3α	5' - GAG CGT TCC CAA GAA GTG G - 3'. 5' - GTG CCT GGT ATA CTA CTC CGA - 3'
GSK3β	5' - ATA AAG ATG GCA GCA AGG TAA CCA - 3' 5' - CTG ACT TCC TGT GGC CTG TCA - 3'
iNOS	5'-CAG CTG GGC TGT ACA AAC CTT-3' 5'-CAT TGG AAG TGA AGC GGT TCG-3'
Arg1	5'-ACC TGG CCT TTG TTG ATG TCC CTA-3' 5'-AGA GAT GCT TCC AAC TGC CAG ACT-3'
SR-A	5'-TGA ACG AGA GGA TGC TGA CTG-3' 5'-GGA GGG GCC ATT TTT AGT GC-3'
CD-36	5'-TCA TGC CAG TCG GAG ACA TGC TTA-3' 5'-AAC TGT CTG TAC ACA GTG GTG CCT-3'
ABCG1	5'-AGG TCT CAG CCT TCT AAA GTT CCT C-3' 5'-TCT CTC GAA GTG AAT GAA ATT TAT CG-3'
ABCA1	5'-GGT TTG GAG ATG GTT ATA CAA TAG TTG T-3' 5'-TTC CCG GAA ACG CAA GTC-3'
HO1	5'-CAG AAG AGG CTA AGA CCG CC-3' 5'-TCT GAC GAA GTG ACG CCA TC-3'
Nrf2	5'-CGA GAT ATA CGC AGG AGA GGT AAG A-3' 5'-GCT CGA CAA TGT TCT CCA GCT T-3'
Txnd1	5'-GGC TCA GAG GCT GTA TGG AG-3' 5'-TTC CAA TGG CCA AAA GAA AC-3'
CEBPB	5'-GCA CAA GGT GCT GGA GCT GAC GG-3' 5'-CTG CTT GAA CAA GTT CCG CAG GGT-3'
CREB	5′-ACC AGC AGA GTG GAG ATG CT-3′ 5′-ATG GCA ATG TAC TGC CCA CT-3′
SR-B1	5'-GGC TGC TGT TTG CTG CG-3' 5'-GCT GCT TGA TGA GGG AGG G-3'
LXR-α	5'-CCG ACA GAG CTT CGT CC- 3' 5'-CCC ACA GAC ACT GCA CAG- 3'
LCAT	5'- GCT GGC CTG GTA GAG GAG ATG –3' 5'- CCA AGG CTA TGC CCA ATG A -3'
FAS	5'-GCT GCG GAA ACT TCA GGA AAT-3' 5'-AGA GAC GTG TCA CTC CTG GAC TT-3'
HMG-CoA	5'-CTT GTG GAA TGC CTT GTG ATT G-3' 5'-AGC CGA AGC AGC ACA TGA T-3'
cMyc	5'-TGA CCT AAC TCG AGG AGG AGC TGG AAT C-3' 5'-AAG TTT GAG GCA GTT AAA ATT ATG GCT GAA GC-3'
S1PR1	5'-ACT TTG CGA GTG AGC TG-3' 5'-AGT GAG CCT TCA GTT ACA GC-3'
S1PR3	5'-TGG TGT GCG GCT GTC TAG TCA A-3' 5'-CAC AGC AAG CAG ACC TCC AGA-3'
MIF	5'-CAG TGG TGT CCG AGA AGT CAG-3' 5'-TAG GCG AAG GTG GAG TTG TT-3'

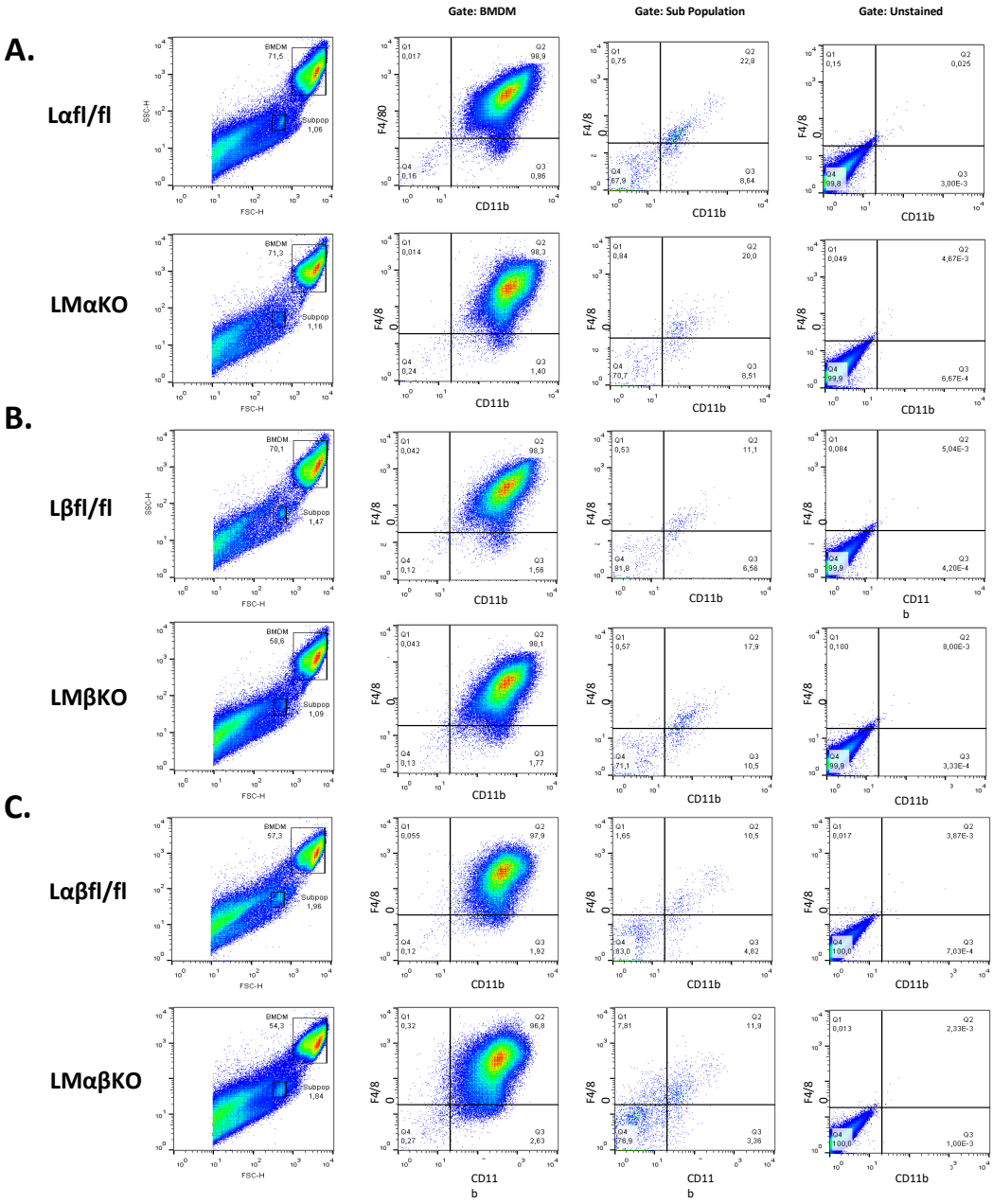
Supplementary Table II. Complete blood cell count

Hematological Parameters	Units	Lαβfl/fl	LMαKO	LMβKO	LMαβKO	Normal Range
Leukocytes:						
White Blood cells, WBC	K/µL	2.55 ± 0.26	2.42 ± 0.12	2.56 ± 0.41	3.09 ± 0.90	1.8-10.7
Neutrophils, NE	K/µL	0.66 ± 0.09	0.60 ± 0.02	0.52 ± 0.08	0.86 ± 0.31	0.1-2.4
Lymphocytes, LY	K/µL	1.75 ± 0.13	1.68 ± 0.09	1.89 ± 0.32	1.89 ± 0.43	0.9-9.3
Monocytes, MO	K/µL	0.07 ± 0.02	0.11 ± 0.02	0.07 ± 0.03	0.15 ± 0.05	0.0-0.4
Eosinophils, EO	K/µL	0.06 ± 0.04	0.02 ± 0.01	0.06 ± 0.03	0.14 ± 0.07	0.0-0.2
Basophils, BA	K/µL	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.04	0.0-0.2
Erythrocytes:						
Red Blood Cells, RBC	M/µL	7.94 ± 0.33	8.03 ± 0.11	8.44 ± 0.03	7.69 ± 0.23	6.36-9.42
Hematocrit, HCT	%	38.46 ± 0.92	38.40 ± 0.29	37.42 ± 0.55	36.60 ± 0.88	35.1-45.4
Mean Corpuscular Volume, MCV	fL	48.62 ± 1.38	47.85 ± 0.68	44.36 ± 0.66	47.65 ± 0.33	45.4-60.3
Mean Corpuscular Hemoglobin, MCH	pg	11.40 ± 1.18	8.93 ± 0.74	11.86 ± 0.13	12.83 ± 0.26	14.1-19.3
MCH Concentration, MCHC	g/dL	23.46 ± 2.44	18.70 ± 1.74	26.74 ± 0.37	26.93 ± 0.41	30.2-34.2
Red Cell Distribution Width, RDW	%	17.34 ± 0.50	16.65 ± 0.27	17.16 ± 0.43	16.40 ± 0.11	12.4-27.0
Thrombocytes:						
Platelets, PLT	K/µL	722.00 ± 93.01	801.00 ± 59.34	768.60 ± 67.71	705.00 ± 146.19	592.0-2972.0
Mean Platelet Volume, MPV	fL	4.82 ± 0.19	4.60 ± 0.12	4.50 ± 0.11	4.80 ± 0.16	5.0-20.0

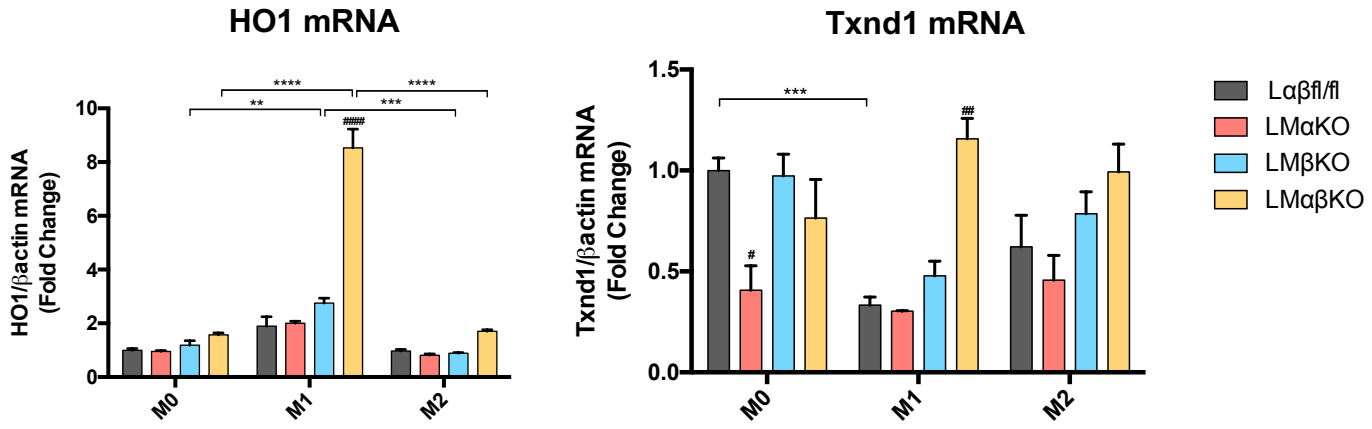
n=4-5 per group



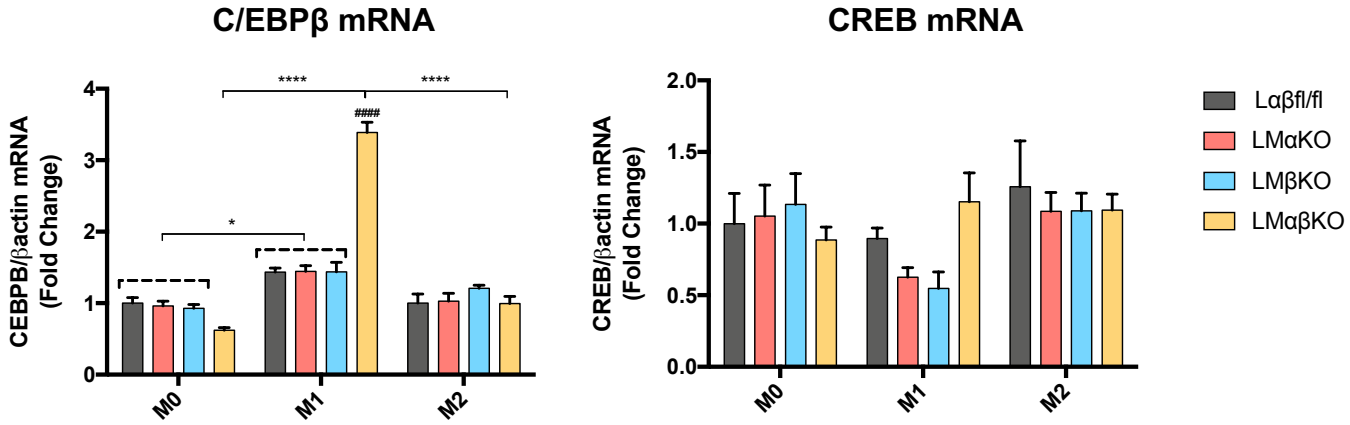
Supplementary Figure I. Uncropped Images of western blot analysis of BMDM.



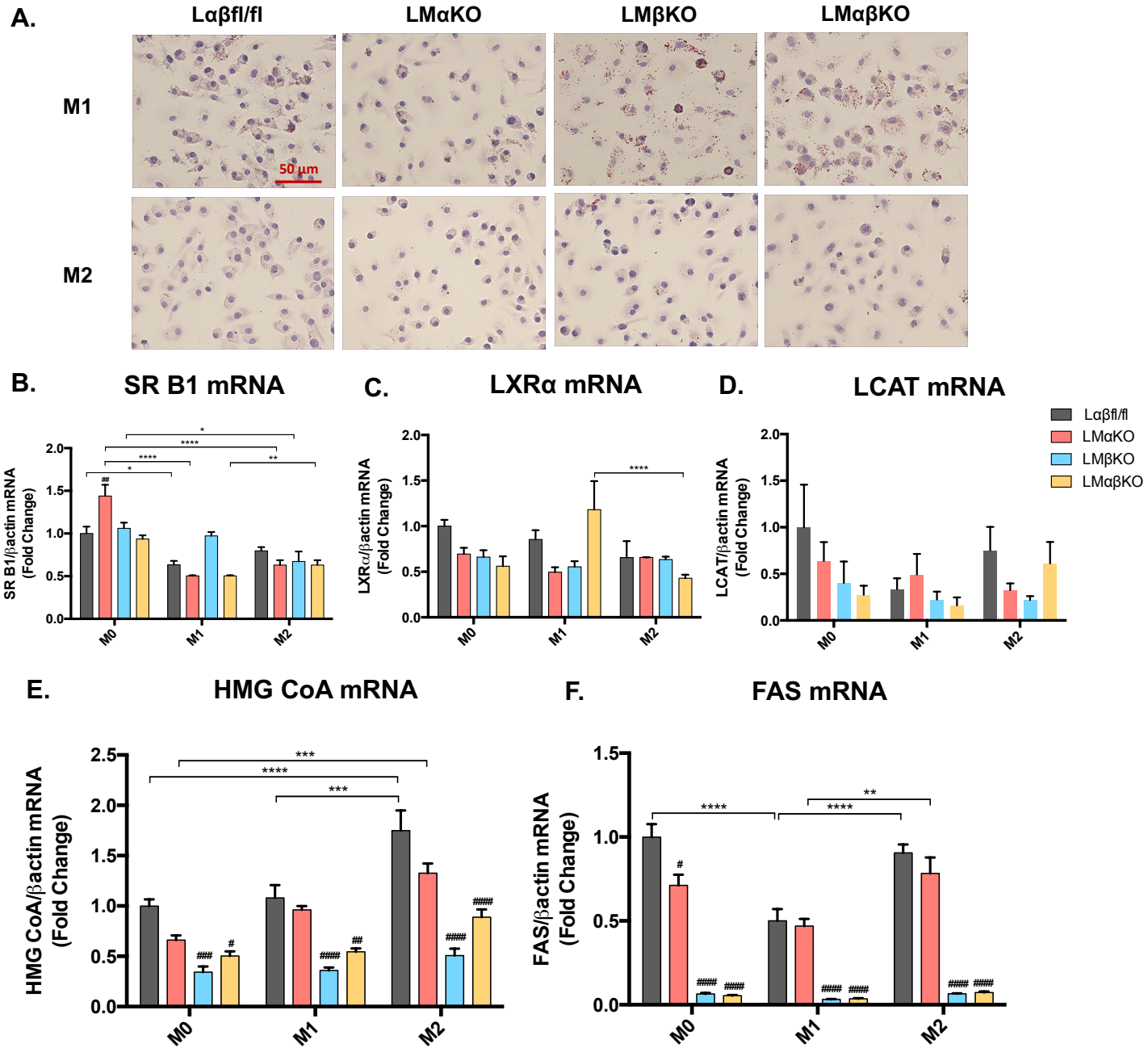
Supplementary Figure II. Effect of GSK3α and/or GSK3β deficiency on macrophage differentiation. To determine the bone marrow progenitor cells differentiation into macrophages, cells were labelled with antibodies against the macrophage-specific surface markers, CD11b and F4/80. Cells from each experimental group were examined on a BD FACS calibur flow cytometer. Representative contour diagram of CD11b/F4/8 of BMDM from **A.** GSK3α deficient mice**, B.** GSK3β deficient mice and **C.** GSK3α and GSK3β deficient mice in compare to their respective control mice.



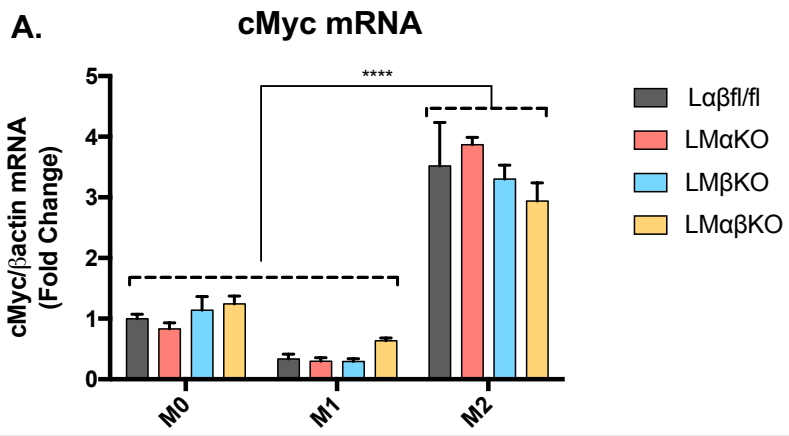
Supplementary Figure III. Mox macrophage polarization. Polarization efficiency were examined by quantify the transcription expression of the gene associated with Mox macrophage polarization by using RT-PCR. **A.** HO1 **B.** Txnd1. Data are normalized to the βactin reference gene. Results are reported as the fold change relative to control M0. n=3-4; mean ± SEM; * is the comparison between UT, LPS(M1), and IL-4(M2) treatments; # is the comparison between control and KOs; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



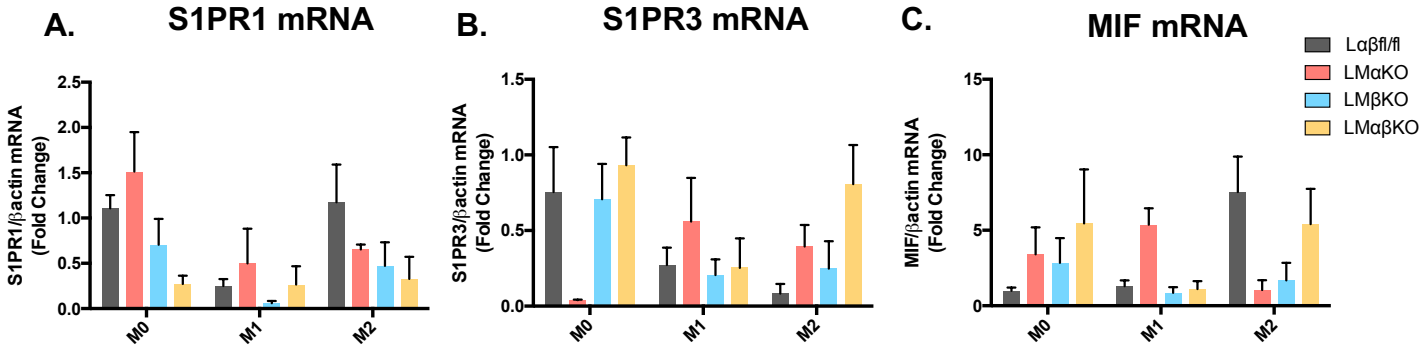
Supplementary Figure IV. Effect of GSK3α and/or GSK3β deficiency on transcription factors expression involved in inflammation. Expression of transcription factors were examined by quantify gene expression using RT-PCR. **A.** C/EBPβ **B.** CREB. Data are normalized to the βactin reference gene. Results are reported as the fold change relative to control M0. n=3-4; mean ± SEM; * is the comparison between UT, LPS(M1), and IL-4(M2) treatments; *p<0.05, ****p<0.0001.



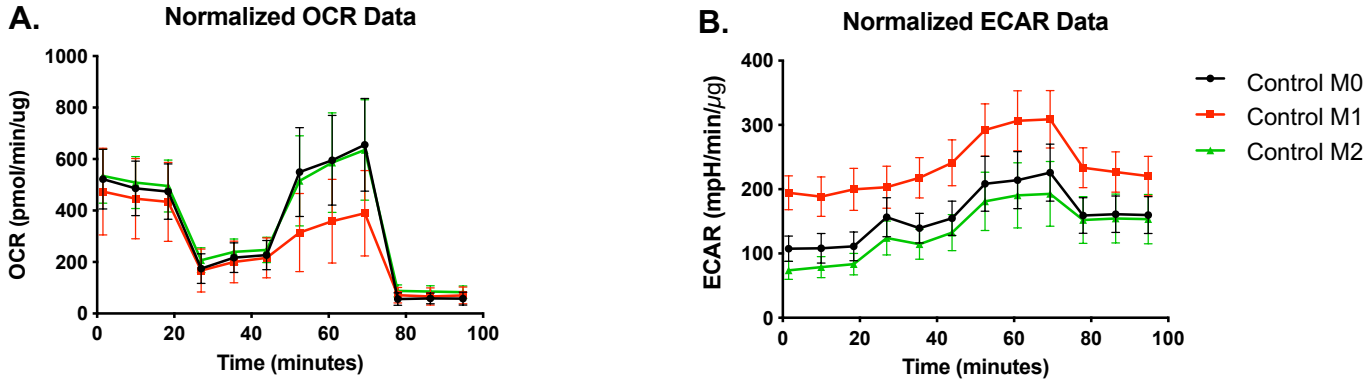
Supplementary Figure V. Effect of GSK3α and/or GSK3β deficiency on lipid accumulation and metabolism. Lipid accumulation of these macrophages were determined by Oil Red O staining. **A.** Oil red O staining of GSK3α and/or GSK3β deficient macrophages. Transcription expression of the gene associated with lipid accumulation and metabolism were quantified **B.** SR B1 **C.** LXRα **D.** LCAT **E.** HMG CoA **F.** FAS. Results are reported as the fold change relative to control UT. n=3-4; mean ± SEM; * is the comparison between UT, LPS(M1), and IL-4(M2) treatments; # is the comparison between control and KOs; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



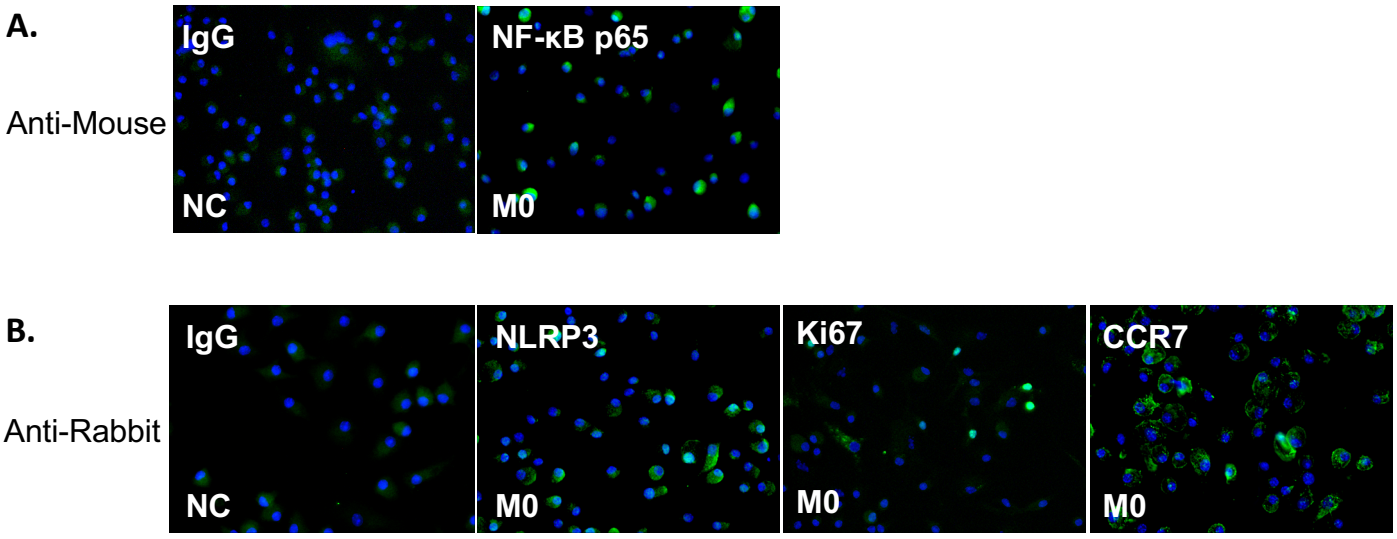
Supplementary Figure VI. Effect of GSK3α and/or GSK3β deficiency on proliferation. Transcription expression of the gene associated with proliferation were quantified **A.** cMyc. Results are reported as the fold change relative to control UT. n=3-4; mean ± SEM; * is the comparison between UT, LPS(M1), and IL-4(M2) treatments. ****p<0.0001.



Supplementary Figure VII. Effect of GSK3α and/or GSK3β deficiency on migration. Transcription expression of the gene associated with migration were quantified **A.** S1PR1 **B.** S1PR3 **C.** MIF. Results are reported as the fold change relative to control UT. n=3-4; mean ± SEM; * is the comparison between UT, LPS(M1), and IL-4(M2) treatments; # is the comparison between control and KOs; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplementary Figure VIII. Metabolism in M1 and M2 polarized BMDM. Metabolic activity of macrophages were determined by using seahorse extracellular flux analysis. **A.** OXPHOS was measure by analysing OCR (normalized to protein content) and **B**. Glycolysis was measure by analysing ECAR (normalized to protein content) in control BMDM. n=4; mean ± SEM; * is the comparison between control and KOs; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Supplementary Figure IX. Negative Controls for immunofluorescent staining. BMDMs were stained with pre-immune IgG antibodies to control for non-specific binding. Representative images of control IgG immunofluorescent staining and specific secondary antibody staining for **A.** anti-mouse, **B.** anti-rabbit . DAPI nuclear counterstaining is shown in blue. NC, negative control.