SUPPLEMENTARY DATA

Supplementary Table S1. Composition of the diet

Ingredients	Normal chow diet (NCD, %)	High fat diet (HFD, %)
Casein	20	25
DL-methionine	0.3	
Cornstarch	15	
Sucrose	50	8.9
Cellulose	5	6.5
Corn oil	5	
Salt mix	3.5	
Vitamin mix	1	1.3
Choline bitartrate	0.2	0.2
Mineral mix		1.3
Lard		31.7
Soybean oil		3.2
Maltodextrin 10		16.2
Potassium citrate, 1 H20		2.1
L-cystine		0.3
Dicalcium phosphate		1.7
Calcium carbonate		0.7

All experimental diets were purchased from Research diet and stored in aliquots at -20°C.

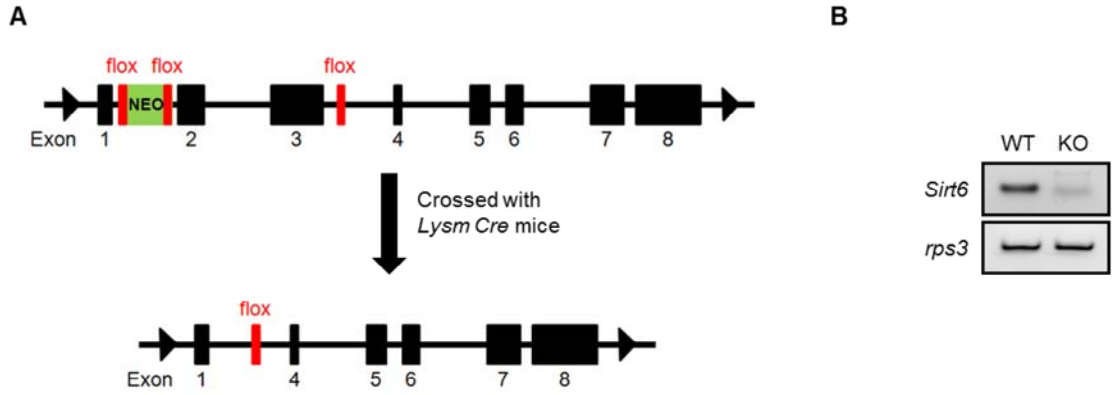
SUPPLEMENTARY DATA

Supplementary Table S2. Sequences and accession numbers for primers (forward, FOR; reverse, REV) for real-time RT-PCRand PCR analyses

Gene	Sequences for primers	Accession NO.
Sirt6	FOR: GACACAGAGACGGCTGGAAC REV: CAGACCCTCAAGCCATGTTT	NM_019812
Nos2	FOR: TTCTGTGCTGTCCCAGTGAG REV: TGAAGAAAACCCCTTGTGCT	NM_010927
Ifnb	FOR: CCCAGTGCTGGAGAAATTGT REV: CCCTATGGAGATGACGGAGA	NM_010510
Il1b	FOR: GGTCAAAGGTTTGGAAGCAG REV: TGTGAAATGCCACCTTTTGA	NM_008361
Tnfa	FOR: AGGGTCTGGGCCATAGAACT REV: CCACCACGCTCTTCTGTCTAC	NM_013693
Il6	FOR: ACCAGAGGAAATTTTCAATAGGC REV: TGATGCACTTGCAGAAAACA	NM_031168
F4/80	FOR: TTTCCTCGCCTGCTTCTTC REV: CCCCGTCTCTGTATTCAACC	NM_010130
Cd11b	FOR: AAGGATTCAGCAAGCCAGAA REV: TAGCAGGAAAGATGGGATGG	NM_008401
Cd11c	FOR: CACTCAGTGACTGCCCAAAA REV: CCTCAAGACAGGACATCGCT	NM_021334
Ccl2	FOR: ATTGGGATCATCTTGCTGGT REV: CCTGCTGTTCACAGTTGCC	NM_011333
Ccr2	FOR: AGCACATGTGGTGAATCCAA REV: TGCCATCATAAAGGAGCCA	NM_009915
Icam1	FOR: AACAGTTCACCTGCACGGAC REV: GTCACCGTTGTGATCCCTG	NM_010493
rps3	FOR: AATGAACCGAAGCACACCATAG REV: ATCAGAGAGTTGACCGCAGTTG	NM_012052

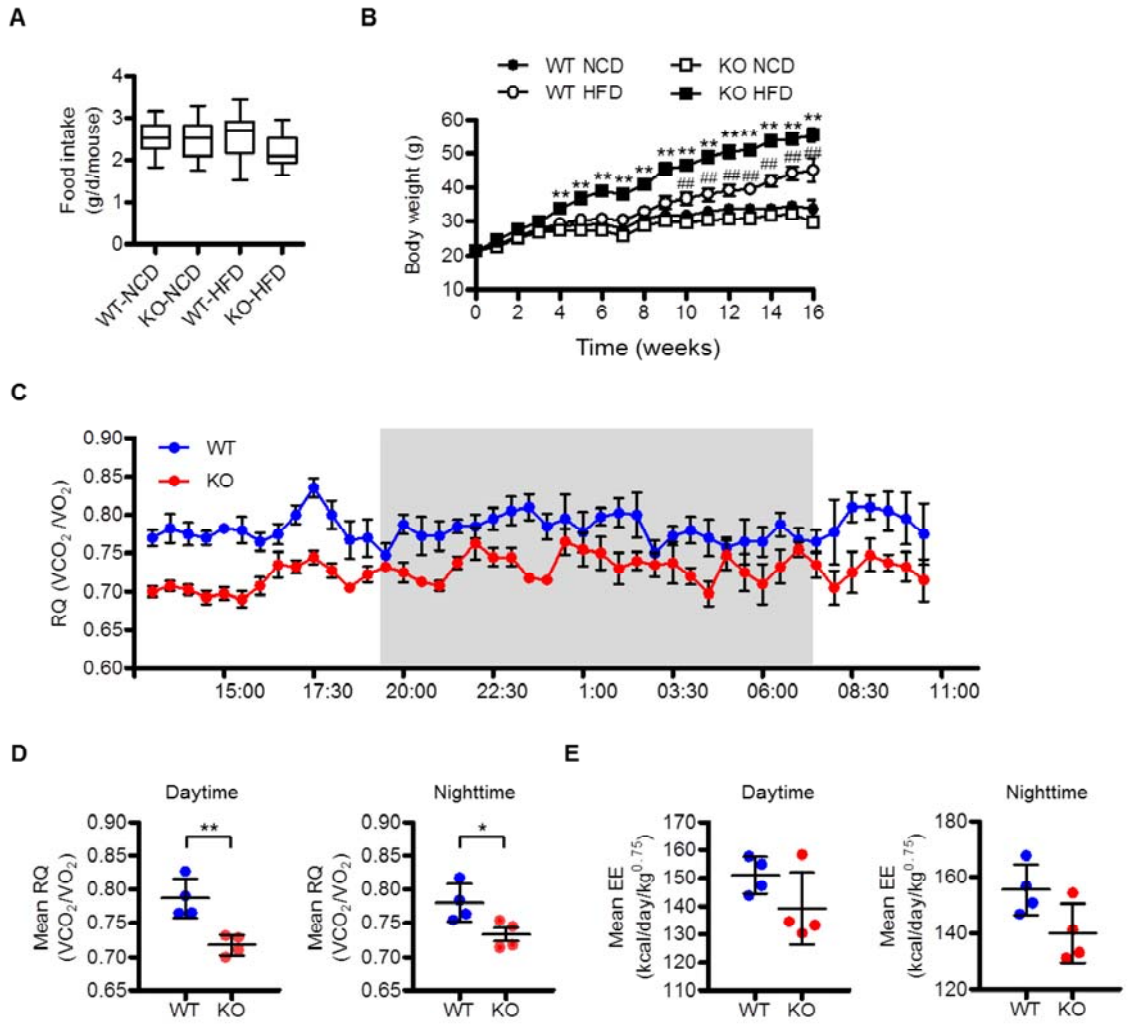
SUPPLEMENTARY DATA

Supplementary Figure S1. Generation of myeloid-specific Sirt6 knockout (mS6KO) mice. (A) Schematic diagram illustrating myeloid-specific deletion of Sirt6 using *LysM-Cre*. **(B)** RT-PCR confirmation of Sirt6 deletion in mS6KO mice.



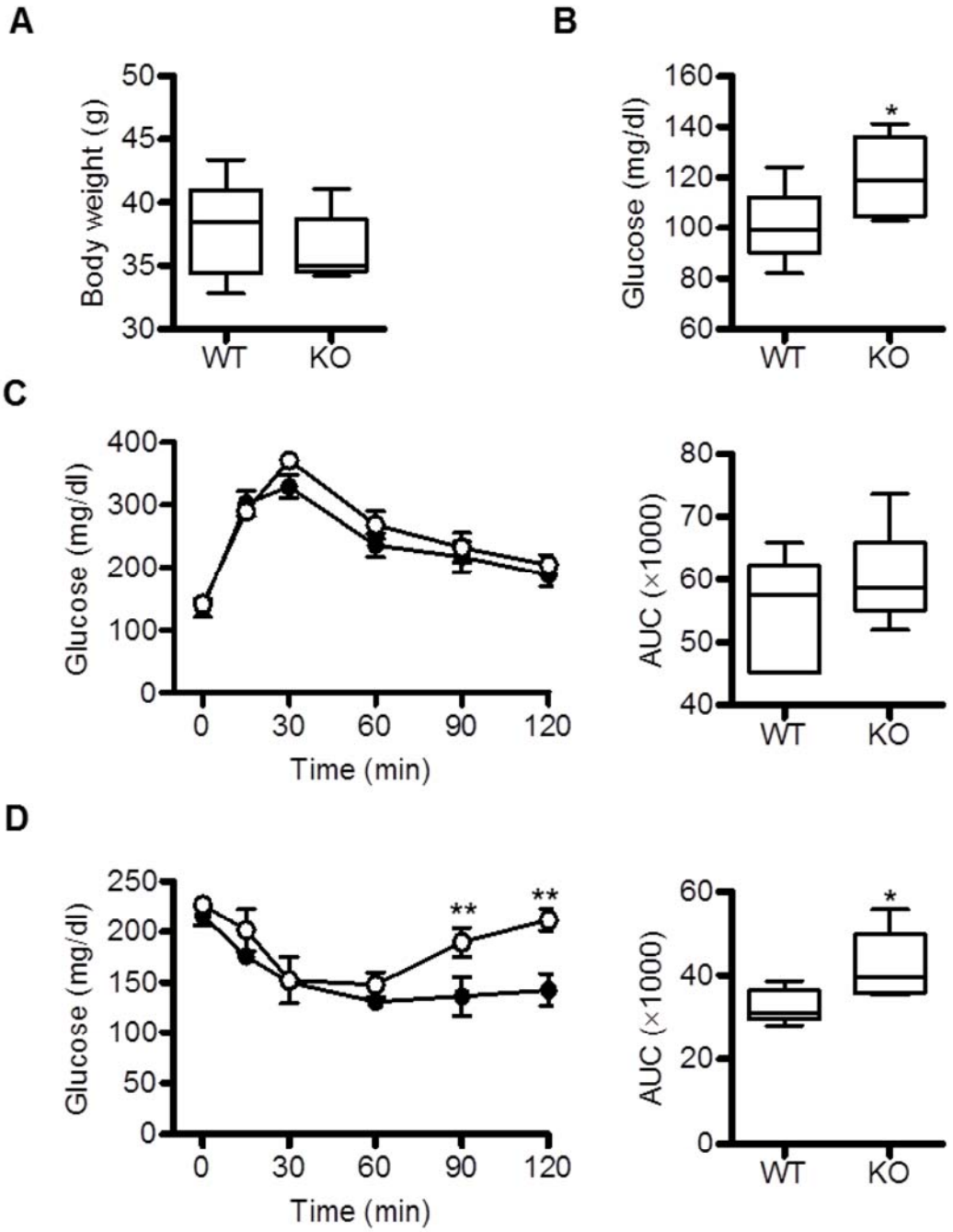
SUPPLEMENTARY DATA

Supplementary Figure S2. Metabolic phenotypes of mS6KO mice. Wild type and mS6KO mice were fed either a NCD or a HFD for 16 weeks. **(A, B)** Daily food intake and weekly body weight changes were measured (n=8). **(C-D)**The metabolic parameters were measured using an 8-chamber Oxymax system. Mice were acclimatized to cages for 24□h and data were collected for an additional 24□h (n=4). Values shown are mean±SD. $^{\#\#}p<0.01$ vs. NCD-fed WT mice; $^{*}p<0.05$ and $^{**}p<0.01$ vs. HFD-fedWT mice.



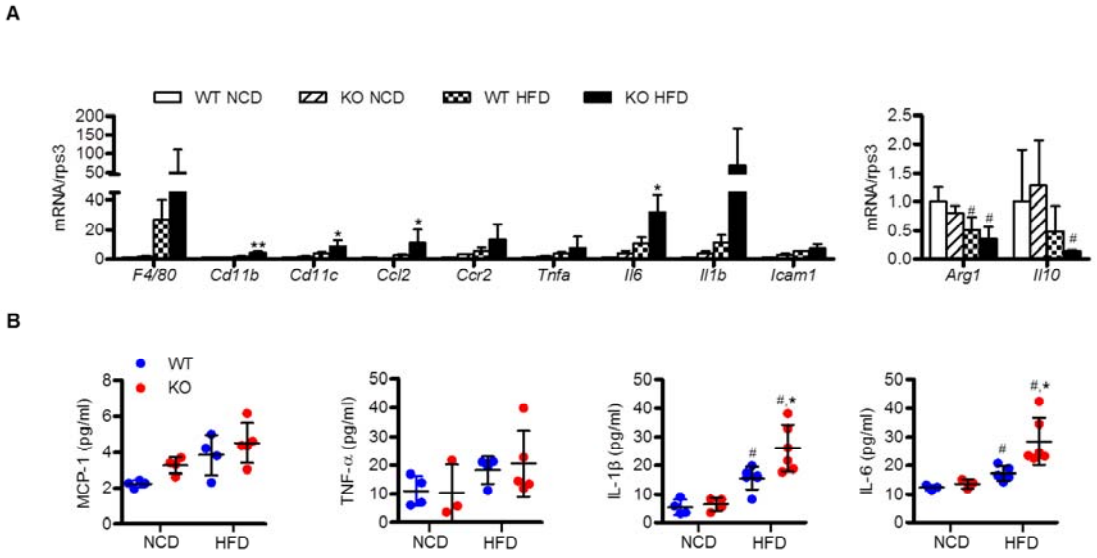
SUPPLEMENTARY DATA

Supplementary Figure S3. Whole body glucose tolerance in WT and mS6KO mice with similar body weight. Wild type and mS6KO mice were fed HFD for 8 weeks and mice with similar body weight were chosen (n=6). Body weight **(A)**, fasting plasma glucose levels **(B)**, plasma glucose levels during glucose tolerance test **(C)** and insulin tolerance test **(D)**. Areas under the curve were compared. Values shown are mean±SD. $^{*}p<0.05$and $^{**}p<0.01$vs. HFD-fedWT mice.



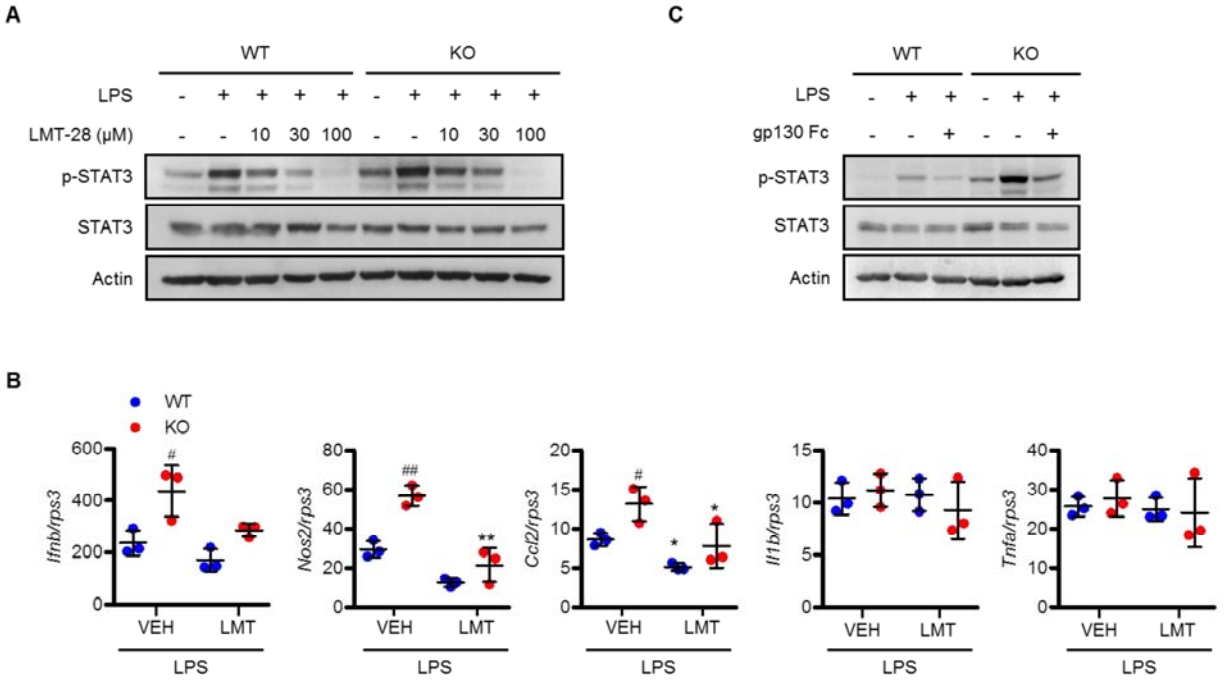
SUPPLEMENTARY DATA

Supplementary Figure S4. Effects of myeloid Sirt6 deletion on hepatic and systemic inflammation. **(A)** After 16 weeks on either a NCD or a HFD, the liver expression levels of macrophage infiltration-related genes were determined by real-time RT-PCR (n=4-8). **(B)** The plasma levels of MCP-1,TNF-α, IL-1β,andIL-6were determined by ELISA(n=4-6). Values shown are mean±SD. #p<0.05vs. NCD-fed mice; $^{*}p$<0.05 and $^{**}p$<0.01 vs. HFD-fedWT mice.



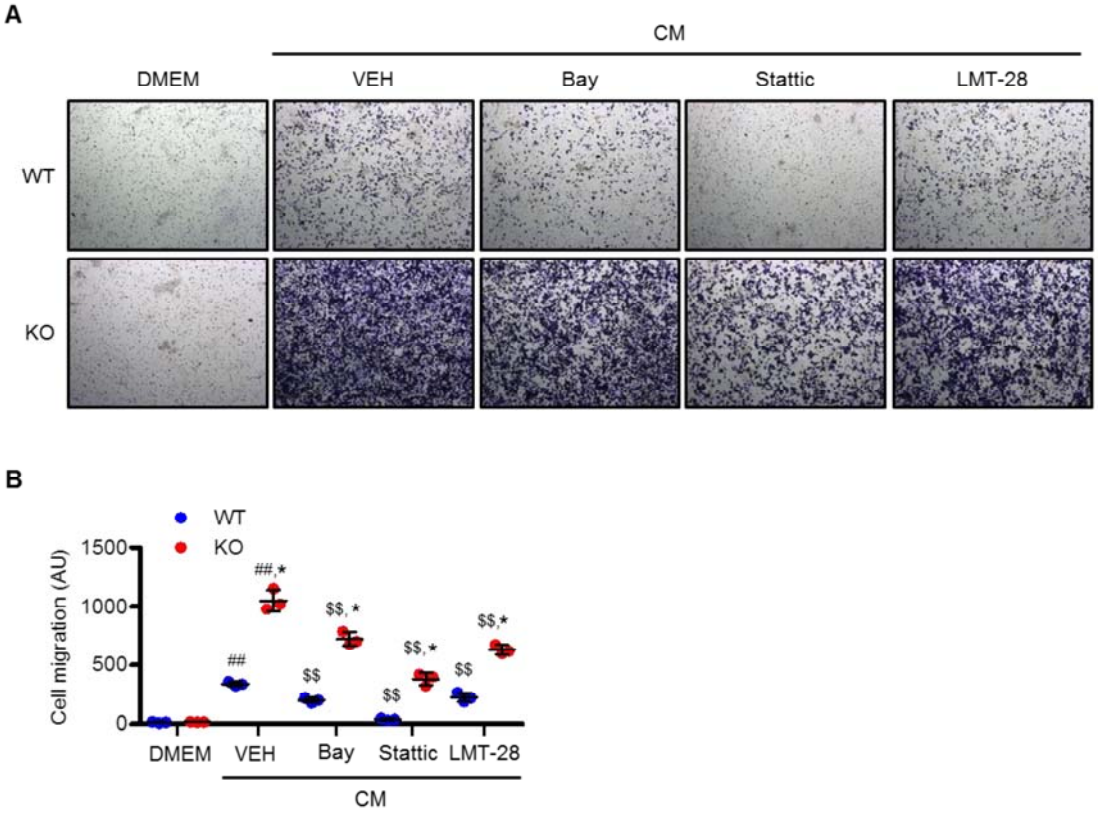
SUPPLEMENTARY DATA

Supplementary Figure S5. Regulation of M1 polarization by IL-6 receptor antagonist. (A-B) BMMs from WT or mS6KO mice were treated with LMT-28 at the indicated concentrations. STAT3 activation and M1 marker expression were analyzed by western blotting and real-time RT-PCR, respectively. Values shown are mean±SD(n=3). $^{\#}p<0.05$ and $^{\#\#}p<0.01$ vs. WT VEH+LPS; $^{*}p<0.05$ and $^{**}p<0.01$ vs. VEH+LPS.**(C)** BMMs were preincubated with soluble gp130 (sGP130, 100 ng/ml) for 1 h and then treated with 10 ng/ml LPS for 2 h. The levels of STAT3 activation were assessed.



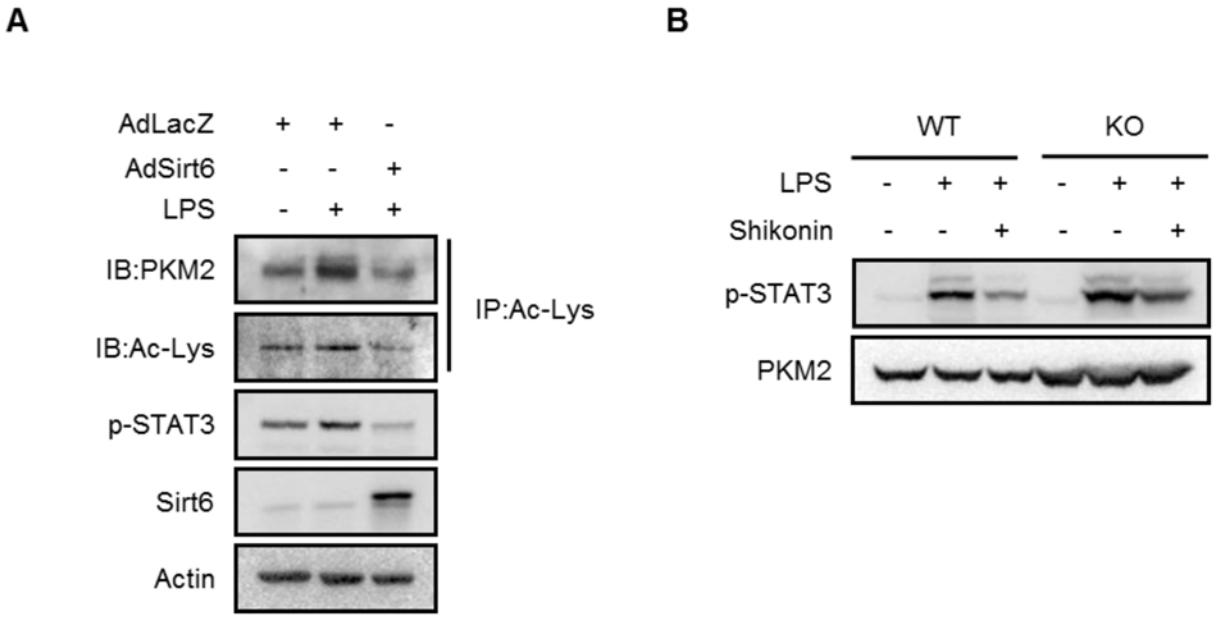
SUPPLEMENTARY DATA

Supplementary Figure S6. Suppression of cell migration by NF-κB-IL-6-STAT3 inhibitors. BMMs isolated from WT or mS6KO mice were treated with 20 μM Bay11-7802, 5 μM Stattic, or 30 μM LMT-28 for 3 h, after which migration toward adipocyte-conditioned medium (CM) was assessed. **(A)** Representative microphotographs of the migration assay. Cells were stainedwith crystal violet. **(B)** Quantification of the cells that migrated to the bottom of the insert. Results are expressed as arbitrary units (AU). Values shown are mean±SD(n=3). $^{\#\#}p<0.01$ vs.DMEM; $^{*}p<0.05$ vs. WT; $^{\$\$}p<0.01$ vs.CM+VEH.VEH, vehicle; Bay, Bay11-7802.



SUPPLEMENTARY DATA

Supplementary Figure S7. Suppression of PKM2 acetylation by Sirt6 overexpression in macrophages. (A) Intraperitoneal macrophages from WT mice were transduced with AdSirt6 or AdLacZ and then treated with LPS (10 ng/ml) for 2 h. Immunoprecipitation and immunoblotting for PKM2deacetylation were performed. **(B)** Macrophages were pretreated with 5 μM shikoninfor 1 h and then treated with 10 ng/ml LPS for 2 h. The levels of STAT3 activation were assessed.



SUPPLEMENTARY DATA

Supplementary Figure S8. The effect of re-expression of Sirt6 on the M1-related genes in Sirt6KO BMMs. BMMs isolated from WT or mS6KO mice were transduced with either AdLacZ or AdSirt6 and then treated with 10 ng/ml LPS for the indicated time periods. **(A)**Western blot analysis of intracellular signaling pathways.**(B)**Real-time RT-PCR analysis of M1 marker expression.Values shown are mean±SD(n=3). $^{\#}p<0.05$vs.WT+AdLacZ; $^{*}p<0.05$ vs. KO+AdLacZ.