Supplemental Figure Legends

Supplemental Figure 1 (Related to Figure 1).
Relative mRNA expression levels for representative unsaturated fatty acid related genes determined by microarray analysis of RNA from thioglycollate-elicited macrophages treated with KLA for 0, 1, 6, 24 hours. .

Supplemental Figure 2 (Related to Figure 2).
UCSC genome browser images illustrating normalized tag counts for LXR at target loci, in thioglycollate-elicited macrophages treated with KLA for indicated time points.

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A. Venn diagram of overlap between LXR and SREBP1 ChIP-Seq peaks in thioglycollate-elicited macrophages.
B. Functional annotations of genes vicinal to genomic regions cobound by LXR and SREBP1.
C. Distribution of H4K5Ac and GRO-Seq tag densities, in the vicinity of genomic regions cobound by LXR and SREBP1, in thioglycollate-elicited macrophages treated with KLA for indicated times.
D. Western blot of nuclear content of SREBP1 in thioglycollate-elicited macrophages treated with either KLA for 0, 1, 3, 6, 20 hours, or GW3965 for 20 hours, as indicated. Control nuclear extracts from transfected with control or *Srebf1* siRNA thioglycollate-elicited macrophages are provided as indicated.

Supplemental Figure 4 (Related to Figure 5).
A. UCSC genome browser images illustrating relative expression levels for representative unsaturated fatty acid related genes WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with KLA for 24 hours.
B. UCSC genome browser images illustrating relative expression levels for representative unsaturated fatty acid related genes in control or *Srebf1* siRNA transfected thioglycollate-elicited macrophages treated with KLA for 24 hours.
C. Scatter plot depicting the relationship between fold change of KLA repressed-induced genes, comparing RNA-seq from KLA-treated thioglycollate-elicited macrophages, transfected with control or *Srebf1* siRNA. Gray dots represent all uniquely expressed genes. Red dots represent all KLA repressed-induced genes.
D. Hierarchical clustering and heatmap of the fold change in expression levels of KLA

repressed-induced genes, comparing RNA-seq from KLA-treated thioglycollate-elicited macrophages, transfected with control or *Srebf1* siRNA. (FDR < 0.01, RPKM > 0.5).

E. Relative mRNA expression of *Scd2* in WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with 100ng/ml Pam3CSK4 for the indicated times.

F. Relative mRNA expression of *Scd2* in WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with 20ng/ml PolyI:C for the indicated times.

Values are expressed as mean ± SEM. *p<0.05, **p<0.01.

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A. UCSC genome browser images illustrating relative expression levels for representative inflammatory genes (KLA induced-repressed) comparing RNA-seq from KLA-treated thioglycollate-elicited macrophages, transfected with control or *Srebf1* siRNA.

B. Relative levels of GRO-Seq signal at loci of KLA induced-repressed genes in thioglycollate-elicited macrophages treated with KLA for indicated times. Boxes encompass the 25th to 75th % changes. Whiskers extend to 10th and 90th percentiles. The median normalized tag distribution is indicated by the central horizontal bar.

C. Distribution of p65 tag densities at loci of KLA induced-repressed genes WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with KLA for 24 hours.

D. Distribution of p65 tag densities at loci of KLA induced-repressed genes in *Srebf1*$^{-/-}$ 1.5-fold > WT bone marrow-derived macrophages with KLA for 24 hours.

E. Relative mRNA expression of inflammatory genes in WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with 100ng/ml Pam3CSK4 for the indicated times.

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Values are expressed as mean ± SEM. *p<0.05, **p<0.01.

Supplemental Figure 6 (Related to Figure 7).

A. Lipidomics analysis of unsaturated fatty acid (9Z-PO) levels in WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with KLA for the indicated times.

B. Relative mRNA expression of *Cxcl9* in control or *Srebf1* siRNA transfected thioglycollate-elicited macrophages treated with KLA for 24 hours, with or without supplementation with the indicated exogenous fatty acids (20μM) at 12h post KLA treatment. Values are expressed as mean ± SEM. *p<0.05.

Experimental procedures for supplemental information

Reagents

Kdo2-Lipid A and desmosterol were purchased from Avanti Polar Lipids and processed as described (http://www.lipidmaps.org/protocols/). GW3965 and Ethyl palmitate (purity >95%) were purchased from Sigma. Pam3CSK4 and Poly(I:C) were purchased from Invivogen. The unsaturated omega fatty acids 9Z-palmitoleic acid, EPA and DHA were purchased from Cayman Chemicals. LPS from E. coli serotype O55:B5 (phenol extracted and then chromatographically purified by gel filtration) was purchased from Sigma. Highly purified EPA ethyl ester (purity >99%) was obtained from Mochida pharmaceutical, Co., Ltd. (Tokyo, Japan).

Isolation and culture of human monocyte-derived macrophages (HMDMs)

Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque 1077 (M&P Biomedical) washed and residual red blood cells lysed using molecular grade water. Monocytes were isolated from purified PBMCs using the Human Pan monocyte isolation kit (Miltenyi) and OctoMACS separator per manufacturer instructions. Purified monocytes were counted and plated in Complete Macrophage Media (Macrophage-Serum Free Media (M-SFM) (Gibco), 1x Penicillin/Streptomycin (PS), Nutridoma-SP (Roche), 1x Fungizone (Gibco), M-CSF 100ng/ml (Peprotech)) and allowed to differentiate for one week with media changed every 3 days. Macrophages were treated with 100ng/ml KLA for indicated time points and RNA harvested using Quick RNA Mini Kit (Zymo).

RNA and qPCR analysis

Total RNA was isolated from cells and purified using RNeasy columns and RNase free DNase digestion according to the manufacturer's instructions (QIAGEN). For quantitative PCR analysis, either 1nM of cDNA or ChIP DNA was used for real-time PCR with gene- or locus-specific primers. Quantitative PCR was performed on an Applied Biosystems StepOne Plus™ system using SYBR GreenER mastermix (Invitrogen) and the following conditions: 10 min at 50ºC and 10min at 95ºC, followed by 40 cycles of 15 s at 95ºC and 30 secs at 60ºC. Primer sequences for RT-qPCR analysis are indicated in supplementary.

siRNA experiments

Thioglycollate-elicited peritoneal macrophages were plated at 2.0x10e6 cells per well of a 6-well plate in growth medium. Cells were transfected with non-specific control

siRNA or SMARTpool siRNAs (Dharmacon) using Deliver X transfection reagent (Panomics) according to the manufacturer's instructions. For siRNA replicate experiments, the SMARTpool was deconvoluted and different individual target specific siRNAs were utilized. At 20 hours post transfection, cells were switched stabilization media containing RPMI-1640 and 0.5% FCS. At 40 hours post transfection, cells were treated with vehicle (PBS) or 100ng/ml KLA for indicating times. For the fatty acid rescue experiments, cells were treated with 100ng/ml KLA for 12 hours, then supplemented for an additional 12 hours with either vehicle or 20μM indicated fatty acids complexed with FA-free low-endotoxin BSA (Sigma, final FA:BSA molar ratio was 5:1).

ChIP

For p65, H4K5ac, H3K27ac, RNA polymerase II, PU.1 and H3K4me2 ChIP, 2x10e7 Thioglycollate-elicited peritoneal macrophages or bone marrow-derived macrophages were used as described previously (Spann et al., 2012). Briefly, for p65 cells were crosslinked in 2mM Disuccinimmidyl Glutarate (Pierce) and 1% formaldehyde. For H4K5ac, H3K27ac, RNA polymerase II, PU.1 and H3K4me2 cells were crosslinked in 1% formaldehyde. Cell were collected and resuspended in swelling buffer (10mM HEPES/KOH pH7.9, 85mM KCl, 1mM EDTA, 0.5% IGEPAL CA-630) with protease inhibitors for 5min. Cells were spun down and resuspended in 500ul lysis buffer (50mM Tris/HCL pH7.4, 1% SDS, 0.5% Empigen BB, 10mM DETA) with protease inhibitors, and chromatin was sheared by sonication. Lysate was diluted with 750ul dilution buffer (20mM Tris/HCl, 100mM NaCl, 0.5% TritonX-100, 2mM EDTA), 1% taken as input DNA, and immunoprecipitation was carried out with Dynabeads protein G coated with specific antibody for overnight. The beads were washed two times each with wash buffer I (20mM Tris/HCl, 150mM NaCl, 0.1% SDS, 1% Triton X-100, 2mM EDTA), wash buffer II (10mM Tris/HCl, 250mM LiCl, 1% IGEPAL CA-630, 0.7% Na-deoxycholate, 1mM EDTA), TE plus 0.2% triton X-100 and TE plus 50mM NaCl and eluted with elution buffer (TE, 2% SDS). DNA was reverse-crosslinked and purified using ChIP DNA Clean & Concentrator (Zymo Research) according to the manufacturer's instructions.

For SREBP1 and LXR ChIP, primary macrophages were used as previously described (Spann et al., 2012). Cells were crosslinked in 2mM Disuccinimmidyl Glutarate (Pierce) and 1% formaldehyde. Cell were collected and resuspended in swelling buffer (10mM HEPES/KOH pH7.9, 85mM KCl, 1mM EDTA, 0.5% IGEPAL CA-630) with protease inhibitors for 5min. Cells were spun down and resuspended for

lysis in 1ml RIPA buffer (10 mM Tris/HCl pH7.6, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 0.1% Na-Deoxycholate, 1% Triton X-100, 1x protease inhibitor cocktail (Roche), 1 mM PMSF) and chromatin was sheared by sonication. Samples were recovered and spun down at 13,000 rpm, 4°C for 10 minutes. 1% supernatant was taken as input DNA and immunoprecipitation was carried out with Dynabeads protein G coated with specific antibody for overnight. .

Beads were washed three times with each buffer by rotating in 1 ml buffer at 4°C for 5 minutes: RIPA buffer (10 mM Tris/HCl pH7.6, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 0.1%Na-Deoxycholate, 1% Triton X-100, 1xprotease inhibitor cocktail (Roche), 1 mM PMSF), LiCl buffer (0.25 M LiCl, 1% NP40, 1%NaDOC), TE plus 0.2% triton X-100 and TE plus 50mM NaCl. Immunoprecipitated chromatin was eluted twice at 37°C for 1h with 100 µl elution buffer (TE, 1% SDS, 250 mM NaCl, 0.5mg/ml RNaseA) followed by proteinase K treatment. DNA was isolated using the ChIP DNA Clean & Concentrator (Zymo Research) according to the manufacturer's instructions.

Antibodies against p65 (sc-372X), SREBP (Santa Cruz Biotechnology: sc-8984X, sc-13551X, sc367X), LXR (Santa Cruz Biotechnology: sc-1000X, Abcam: ab-41902, Active Motif: 61177), H4K5ac (Millipore, 07-327), H3K27ac (Abcam: ab4729), RNA polymerase II (Santa Cruz Biotechnology: sc-899X, Abcam: ab26721, PU.1 (Santa Cruz Biotechnology: sc-352X) and H3K4me2 (Millipore, cat# 07030) were purchased as indicated.

RNA-Sequencing Library Preparation

Sequencing libraries were prepared from polyA enriched mRNA, either as previously described (Kaikkonen, et al. 2013), or as follows. Poly A enriched mRNA was fragmented, in 2x Superscript III first-strand buffer with 10mM DTT (Invitrogen), by incubation at 94°C for 9 minutes, then immediately chilled on ice before the next step. The 10 µL of fragmented mRNA, 0.5 µL of Random primer (Invitrogen), 0.5 µL of Oligo dT primer (Invitrogen), 0.5 µL of SUPERase-In (Ambion), 1 µL of dNTPs (10 mM) and 1 µL of DTT (10 mM) were heated at 50°C for three minutes. At the end of incubation, 5.8 µL of water, 1 µL of DTT (100 mM), 0.1 µL Actinomycin D (2 µg/µL), 0.2 µL of 1% Tween-20 (Sigma) and 0.2 µL of Superscript III (Invitrogen) were added and incubated in a PCR machine using the following conditions: 25°C for 10 minutes, 50°C for 50 minutes, and a 4°C hold. The product was then purified with RNAClean XP beads according to manufacture's instruction and eluted with 10 µL nuclease-free water. The RNA/cDNA double-stranded hybrid was then added to 1.5 µL of Blue Buffer (Enzymatics), 1.1 µL of dUTP mix (10 mM dATP, dCTP, dGTP and 20 mM

dUTP), 0.2 µL of RNAse H (5 U/µL), 1.05 µL of water, 1 µL of DNA polymerase I (Enzymatics) and 0.15 µL of 1% Tween-20. The mixture was incubated at 16°C for 1 hour. The resulting dUTP-marked dsDNA was purified using 28 µL of Sera-Mag Speedbeads (Thermo Fisher Scientific), diluted with 20% PEG8000, 2.5M NaCl to final of 13% PEG, eluted with 40 µL EB buffer (10 mM Tris-Cl, pH 8.5) and frozen -80°C. The purified dsDNA (40 µL) underwent end repair by blunting, A-tailing and adapter ligation as previously described (Heinz, S. et al. Molecular Cell 38, 576-589 (2010)) using barcoded adapters (NextFlex, Bioo Scientific). Libraries were PCR-amplified for 9-14 cycles, size selected by gel extraction, quantified Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and sequenced on either a Genome Analyzer II (Illumina) or Hi-Seq 2000 (Illumina) for 51 cycles.

LC/MS Analysis of Fatty Acids

Lipids were extracted by bringing up cells in 1 mL of PBS, followed by the addition of 1 mL MeOH and 2 mL $CHCL_3$, doped with internal standard (20 pmol C17:1 heptadecanoic acid (C17:1 FFA)). The mixture was vortexed and centrifuged at 2200 g for 6 min to separate aqueous (top) and organic (bottom) phase. The organic phase containing the extracted lipids was dried under N_2, re-solubilized in 50 uL MeOH and subjected to LC/MS analysis. A fraction (~10 uL) was injected onto a Thermo TSQ Quantiva LC/MS. LC separation was achieved using a Gemini 5U C18 column (Phenomenex). The LC solvents were as follows: buffer A, 95:5 H_2O/MeOH + 0.1% NH_4OH; buffer B, 60:35:5 iPrOH/MeOH/H_2O + 0.1% NH_40H. A typical LC run was 15 min and consisted the following steps: 0.1 ml/min 100% buffer A for 2 min, 0.5 ml/min linear gradient to 100% buffer B over 8 minutes, 0.5 ml/min 100% buffer B for 2 min, and equilibration with 0.5 ml/min 100% buffer A for 3 min. MS analyses were performed using an electrospray ionization (ESI) in negative ion mode. Data was collected using an m/z of 150-500, and DHA, EPA, and 9z-palmitoleic acid were filtered using the following mass ranges m/z 327-327.5, 301-301.5, and 253-253.5, respectively. These fatty acids were quantified by measuring their area under the peak and normalizing to an internal standard.

Protein analysis

For the SREBP activation assay, nuclear and cytoplasmic extracts were prepared from thioglycollate-elicited peritoneal macrophages treated with siRNA, GW3965, or KLA as indicated in figure legend. Protein extract were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) according to manufacturer

instructions. Anti-mouse SREBP1 antibodies are generous gifts from Dr. Timothy Osborne, Director of Integrative Metabolism Program, Sanford Burnham Prebys Medical Discovery Institute, Orlando, FL.

Primer Sequences

mFADS2s	TCCTGTCCCACATCATCGTCATGG
mFADS2as	GCTTGGGCCTGAGAGGTAGCGA
mCXCL9s	TCTGCCATGAAGTCCGCTGTTCT
mCXCL9as	GTGGATCGTGCCTCGGCTGGT
mNos2s	AGCCTTGCATCCTCATTGG
mNos2as	CACTCTCTTGCGGACCATCT
mElovl5s	TGAACTTCGGAAGCCGCTCGT
mElovl5as	GCACCAGTTCGAAGAGCACCGA
mFads1s	TGGTGCCCTTCATCCTCTGT
mFads1as	GGTGCCCAAAGTCATGCTGTA
mSCD2s	CCCCCTCCGCTTGGCTAGGG
mSCD2as	AGCTTGCAGCCACCGGTGTC
mCxcl1s	AGACTCCAGCCACACTCCAA
mCscl1as	TGACAGCGCAGCTCATTGG
mIL1a s	TTGGTTAAATGACCTGCAACA
mIL1a as	GAGCGCTCACGAACAGTTG
m36b4s	AGGGCGACCTGGAAGTCC
m36b4as	CCCACAATGAAGCATTTTGGA
mIFNb1s	CAGCTCCAAGAAAGGACGAAC
mIFNb1as	GGCAGTGTAACTCTTCTGCAT
mIFNa4s	TGATGAGCTACTACTGGTCAGC
mIFNa4as	GATCTCTTAGCACAAGGATGGC

References

Kaikkonen, M.U., Spann, N.J., Heinz, S., Romanoski, C.E., Allison, K.A., Stender, J.D., Chun, H.B., Tough, D.F., Prinjha, R.K., Benner, C.*, et al.* (2013). Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol Cell *51*, 310-325.

Spann, N.J., Garmire, L.X., McDonald, J.G., Myers, D.S., Milne, S.B., Shibata, N., Reichart, D., Fox, J.N., Shaked, I., Heudobler, D.*, et al.* (2012). Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. Cell *151*, 138-152.

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Supplemental Figure 2 (Related to Figure 2).
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B. UCSC genome browser images illustrating relative expression levels for representative unsaturated fatty acid related genes in control or *Srebf1* siRNA transfected thioglycollate-elicited macrophages treated with KLA for 24 hours.
C. Scatter plot depicting the relationship between fold change of KLA repressed-induced genes, comparing RNA-seq from KLA-treated thioglycollate-elicited macrophages, transfected with control or *Srebf1* siRNA. Gray dots represent all uniquely expressed genes. Red dots represent all KLA repressed-induced genes.
D. Hierarchical clustering and heatmap of the fold change in expression levels of KLA

repressed-induced genes, comparing RNA-seq from KLA-treated thioglycollate-elicited macrophages, transfected with control or *Srebf1* siRNA. (FDR < 0.01, RPKM > 0.5).

E. Relative mRNA expression of *Scd2* in WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with 100ng/ml Pam3CSK4 for the indicated times.

F. Relative mRNA expression of *Scd2* in WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with 20ng/ml PolyI:C for the indicated times.

Values are expressed as mean ± SEM. *p<0.05, **p<0.01.

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B. Relative levels of GRO-Seq signal at loci of KLA induced-repressed genes in thioglycollate-elicited macrophages treated with KLA for indicated times. Boxes encompass the 25th to 75th % changes. Whiskers extend to 10th and 90th percentiles. The median normalized tag distribution is indicated by the central horizontal bar.

C. Distribution of p65 tag densities at loci of KLA induced-repressed genes WT and *Srebf1*$^{-/-}$ bone marrow-derived macrophages treated with KLA for 24 hours.

D. Distribution of p65 tag densities at loci of KLA induced-repressed genes in *Srebf1*$^{-/-}$ 1.5-fold > WT bone marrow-derived macrophages with KLA for 24 hours.

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B. Relative mRNA expression of *Cxcl9* in control or *Srebf1* siRNA transfected thioglycollate-elicited macrophages treated with KLA for 24 hours, with or without supplementation with the indicated exogenous fatty acids (20μM) at 12h post KLA treatment. Values are expressed as mean ± SEM. *p<0.05.

Experimental procedures for supplemental information

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Total RNA was isolated from cells and purified using RNeasy columns and RNase free DNase digestion according to the manufacturer's instructions (QIAGEN). For quantitative PCR analysis, either 1nM of cDNA or ChIP DNA was used for real-time PCR with gene- or locus-specific primers. Quantitative PCR was performed on an Applied Biosystems StepOne Plus™ system using SYBR GreenER mastermix (Invitrogen) and the following conditions: 10 min at 50ºC and 10min at 95ºC, followed by 40 cycles of 15 s at 95ºC and 30 secs at 60ºC. Primer sequences for RT-qPCR analysis are indicated in Primer Sequences section.

siRNA experiments

Thioglycollate-elicited peritoneal macrophages were plated at 2.0x10e6 cells per well of a 6-well plate in growth medium. Cells were transfected with non-specific control

siRNA or SMARTpool siRNAs (Dharmacon) using Deliver X transfection reagent (Panomics) according to the manufacturer's instructions. For siRNA replicate experiments, the SMARTpool was deconvoluted and different individual target specific siRNAs were utilized. At 20 hours post transfection, cells were switched stabilization media containing RPMI-1640 and 0.5% FCS. At 40 hours post transfection, cells were treated with vehicle (PBS) or 100ng/ml KLA for indicating times. For the fatty acid rescue experiments, cells were treated with 100ng/ml KLA for 12 hours, then supplemented for an additional 12 hours with either vehicle or 20μM indicated fatty acids complexed with FA-free low-endotoxin BSA (Sigma, final FA:BSA molar ratio was 5:1).

ChIP

For p65, H4K5ac, H3K27ac, RNA polymerase II, PU.1 and H3K4me2 ChIP, 2x10e7 Thioglycollate-elicited peritoneal macrophages or bone marrow-derived macrophages were used as described previously (Spann et al., 2012). Briefly, for p65 cells were crosslinked in 2mM Disuccinimmidyl Glutarate (Pierce) and 1% formaldehyde. For H4K5ac, H3K27ac, RNA polymerase II, PU.1 and H3K4me2 cells were crosslinked in 1% formaldehyde. Cell were collected and resuspended in swelling buffer (10mM HEPES/KOH pH7.9, 85mM KCl, 1mM EDTA, 0.5% IGEPAL CA-630) with protease inhibitors for 5min. Cells were spun down and resuspended in 500ul lysis buffer (50mM Tris/HCL pH7.4, 1% SDS, 0.5% Empigen BB, 10mM DETA) with protease inhibitors, and chromatin was sheared by sonication. Lysate was diluted with 750ul dilution buffer (20mM Tris/HCl, 100mM NaCl, 0.5% TritonX-100, 2mM EDTA), 1% taken as input DNA, and immunoprecipitation was carried out with Dynabeads protein G coated with specific antibody for overnight. The beads were washed two times each with wash buffer I (20mM Tris/HCl, 150mM NaCl, 0.1% SDS, 1% Triton X-100, 2mM EDTA), wash buffer II (10mM Tris/HCl, 250mM LiCl, 1% IGEPAL CA-630, 0.7% Na-deoxycholate, 1mM EDTA), TE plus 0.2% triton X-100 and TE plus 50mM NaCl and eluted with elution buffer (TE, 2% SDS). DNA was reverse-crosslinked and purified using ChIP DNA Clean & Concentrator (Zymo Research) according to the manufacturer's instructions.

For SREBP1 and LXR ChIP, primary macrophages were used as previously described (Spann et al., 2012). Cells were crosslinked in 2mM Disuccinimmidyl Glutarate (Pierce) and 1% formaldehyde. Cell were collected and resuspended in swelling buffer (10mM HEPES/KOH pH7.9, 85mM KCl, 1mM EDTA, 0.5% IGEPAL CA-630) with protease inhibitors for 5min. Cells were spun down and resuspended for

lysis in 1ml RIPA buffer (10 mM Tris/HCl pH7.6, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 0.1% Na-Deoxycholate, 1% Triton X-100, 1x protease inhibitor cocktail (Roche), 1 mM PMSF) and chromatin was sheared by sonication. Samples were recovered and spun down at 13,000 rpm, 4°C for 10 minutes. 1% supernatant was taken as input DNA and immunoprecipitation was carried out with Dynabeads protein G coated with specific antibody for overnight. .

Beads were washed three times with each buffer by rotating in 1 ml buffer at 4°C for 5 minutes: RIPA buffer (10 mM Tris/HCl pH7.6, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 0.1%Na-Deoxycholate, 1% Triton X-100, 1xprotease inhibitor cocktail (Roche), 1 mM PMSF), LiCl buffer (0.25 M LiCl, 1% NP40, 1%NaDOC), TE plus 0.2% triton X-100 and TE plus 50mM NaCl. Immunoprecipitated chromatin was eluted twice at 37°C for 1h with 100 µl elution buffer (TE, 1% SDS, 250 mM NaCl, 0.5mg/ml RNaseA) followed by proteinase K treatment. DNA was isolated using the ChIP DNA Clean & Concentrator (Zymo Research) according to the manufacturer's instructions.

Antibodies against p65 (sc-372X), SREBP (Santa Cruz Biotechnology: sc-8984X, sc-13551X, sc367X), LXR (Santa Cruz Biotechnology: sc-1000X, Abcam: ab-41902, Active Motif: 61177), H4K5ac (Millipore, 07-327), H3K27ac (Abcam: ab4729), RNA polymerase II (Santa Cruz Biotechnology: sc-899X, Abcam: ab26721, PU.1 (Santa Cruz Biotechnology: sc-352X) and H3K4me2 (Millipore, cat# 07030) were purchased as indicated.

RNA-Sequencing Library Preparation

Sequencing libraries were prepared from polyA enriched mRNA, either as previously described (Kaikkonen, et al. 2013), or as follows. Poly A enriched mRNA was fragmented, in 2x Superscript III first-strand buffer with 10mM DTT (Invitrogen), by incubation at 94°C for 9 minutes, then immediately chilled on ice before the next step. The 10 µL of fragmented mRNA, 0.5 µL of Random primer (Invitrogen), 0.5 µL of Oligo dT primer (Invitrogen), 0.5 µL of SUPERase-In (Ambion), 1 µL of dNTPs (10 mM) and 1 µL of DTT (10 mM) were heated at 50°C for three minutes. At the end of incubation, 5.8 µL of water, 1 µL of DTT (100 mM), 0.1 µL Actinomycin D (2 µg/µL), 0.2 µL of 1% Tween-20 (Sigma) and 0.2 µL of Superscript III (Invitrogen) were added and incubated in a PCR machine using the following conditions: 25°C for 10 minutes, 50°C for 50 minutes, and a 4°C hold. The product was then purified with RNAClean XP beads according to manufacture's instruction and eluted with 10 µL nuclease-free water. The RNA/cDNA double-stranded hybrid was then added to 1.5 µL of Blue Buffer (Enzymatics), 1.1 µL of dUTP mix (10 mM dATP, dCTP, dGTP and 20 mM

dUTP), 0.2 µL of RNAse H (5 U/µL), 1.05 µL of water, 1 µL of DNA polymerase I (Enzymatics) and 0.15 µL of 1% Tween-20. The mixture was incubated at 16°C for 1 hour. The resulting dUTP-marked dsDNA was purified using 28 µL of Sera-Mag Speedbeads (Thermo Fisher Scientific), diluted with 20% PEG8000, 2.5M NaCl to final of 13% PEG, eluted with 40 µL EB buffer (10 mM Tris-Cl, pH 8.5) and frozen -80°C. The purified dsDNA (40 µL) underwent end repair by blunting, A-tailing and adapter ligation as previously described (Heinz, S. et al. Molecular Cell 38, 576-589 (2010)) using barcoded adapters (NextFlex, Bioo Scientific). Libraries were PCR-amplified for 9-14 cycles, size selected by gel extraction, quantified Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and sequenced on either a Genome Analyzer II (Illumina) or Hi-Seq 2000 (Illumina) for 51 cycles.

LC/MS Analysis of Fatty Acids

Lipids were extracted by bringing up cells in 1 mL of PBS, followed by the addition of 1 mL MeOH and 2 mL $CHCL_3$, doped with internal standard (20 pmol C17:1 heptadecanoic acid (C17:1 FFA)). The mixture was vortexed and centrifuged at 2200 g for 6 min to separate aqueous (top) and organic (bottom) phase. The organic phase containing the extracted lipids was dried under N_2, re-solubilized in 50 uL MeOH and subjected to LC/MS analysis. A fraction (~10 uL) was injected onto a Thermo TSQ Quantiva LC/MS. LC separation was achieved using a Gemini 5U C18 column (Phenomenex). The LC solvents were as follows: buffer A, 95:5 H_2O/MeOH + 0.1% NH_4OH; buffer B, 60:35:5 iPrOH/MeOH/H_2O + 0.1% NH_40H. A typical LC run was 15 min and consisted the following steps: 0.1 ml/min 100% buffer A for 2 min, 0.5 ml/min linear gradient to 100% buffer B over 8 minutes, 0.5 ml/min 100% buffer B for 2 min, and equilibration with 0.5 ml/min 100% buffer A for 3 min. MS analyses were performed using an electrospray ionization (ESI) in negative ion mode. Data was collected using an m/z of 150-500, and DHA, EPA, and 9z-palmitoleic acid were filtered using the following mass ranges m/z 327-327.5, 301-301.5, and 253-253.5, respectively. These fatty acids were quantified by measuring their area under the peak and normalizing to an internal standard.

Protein analysis

For the SREBP activation assay, nuclear and cytoplasmic extracts were prepared from thioglycollate-elicited peritoneal macrophages treated with siRNA, GW3965, or KLA as indicated in figure legend. Protein extract were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) according to manufacturer

instructions. Anti-mouse SREBP1 antibodies are generous gifts from Dr. Timothy Osborne, Director of Integrative Metabolism Program, Sanford Burnham Prebys Medical Discovery Institute, Orlando, FL.

Primer Sequences

mFADS2s	TCCTGTCCCACATCATCGTCATGG
mFADS2as	GCTTGGGCCTGAGAGGTAGCGA
mCXCL9s	TCTGCCATGAAGTCCGCTGTTCT
mCXCL9as	GTGGATCGTGCCTCGGCTGGT
mNos2s	AGCCTTGCATCCTCATTGG
mNos2as	CACTCTCTTGCGGACCATCT
mElovl5s	TGAACTTCGGAAGCCGCTCGT
mElovl5as	GCACCAGTTCGAAGAGCACCGA
mFads1s	TGGTGCCCTTCATCCTCTGT
mFads1as	GGTGCCCAAAGTCATGCTGTA
mSCD2s	CCCCCTCCGCTTGGCTAGGG
mSCD2as	AGCTTGCAGCCACCGGTGTC
mCxcl1s	AGACTCCAGCCACACTCCAA
mCscl1as	TGACAGCGCAGCTCATTGG
mIL1a s	TTGGTTAAATGACCTGCAACA
mIL1a as	GAGCGCTCACGAACAGTTG
m36b4s	AGGGCGACCTGGAAGTCC
m36b4as	CCCACAATGAAGCATTTTGGA
mIFNb1s	CAGCTCCAAGAAAGGACGAAC
mIFNb1as	GGCAGTGTAACTCTTCTGCAT
mIFNa4s	TGATGAGCTACTACTGGTCAGC
mIFNa4as	GATCTCTTAGCACAAGGATGGC

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