Methods

Mice

Myeloid-specific *Cpt2* knockout mice were described earlier [1]. For the generation of a *Cpt1a*$^{fl/fl}$ mouse line, a *Cpt1a* conditional knockout ES cell clone (HEPD0727_3_A09) was purchased from EUCOMM (http://www.mousephenotype.org/). In brief, ES cells were injected into mouse blastocysts collected from C57BL/6N mice, which were then implanted into the uteri of pseudo pregnant surrogate mothers. Chimeric mice born to the foster mothers were bred with C57BL/6N mice to obtain germline offspring. These mice were supposed to contain two loxP sites floxing the Exon 4 of the *Cpt1a* gene, but our genotyping results indicated the presence of only the upstream (Intron 3) loxP, while the downstream (Intron 4) loxP was missing. The basis for this error is unknown but presumably was due either to an original ES cell genotyping error or because the ES cells shipped by the cell provider contained a mixed population. To remedy this genetic defect, we inserted a new loxP site into Intron 4 using the CRISPR/Cas9 method. Briefly, a CRISPR sgRNA (CCAGATGAGCCGTCTGCCCAGGG) designed to cut Intron 4 was made by *in vitro* transcription, and a single strand oligonucleotides (CAGTGACCAAACCCAGAGCTTCATGCCTGGCTTACCCAGATGAGCCGTCTGATAACTTCGTA TAGCATACATTATACGAAGTTATGGATCCCCCAGGGAAGGACATCCAAAGCCCCAACGAGC TGCTTTTATAAGCTTAGC) containing a loxP site flanked by short homologous arms was used as a donor for mediating the targeted loxP insertion. The sgRNA, *Casp9* mRNA, and donor oligos were co-microinjected, at the concentration of 20 ng/μl, 50 ng/μl, and 100 ng/μl, respectively, into zygotes generated by *in vitro* fertilization using sperm obtained from a male mouse containing the 5' *Cpt1a* loxP site and eggs collected from wildtype C57BL/6N mice. The injected embryos were cultured overnight in M16 medium and then implanted into the oviducts of pseudo pregnant foster mothers. Mice born to the foster mothers were genotyped by PCR and DNA sequenced to identify mice with the correct insertion of an Intron 4 loxP site within the same allele as the upstream loxP site. The founders were bred with C57BL/6N mice to expand the colony for subsequent studies. To generate myeloid-specific *Cpt1a* knockout mice, the *Cpt1a*$^{fl/fl}$ mice were crossed with C57BL/6 *Lyz2*-Cre transgenic mice [2] obtained from Jackson Laboratory. For atherosclerosis experiment, CPT2 M-KO mice were crossed with C57BL/6 ApoE knockout mice [3]. Male mice were fed a Western diet (42% calorie from fat; TD88137; Envigo) or a normal chow (NIH-31) for 17 (first cohort) or 20 weeks (second cohort) starting at the age of 8 weeks. All animal studies were done in accordance and approval of the National Heart, Lung, and Blood Institute (NHLBI) Animal Care and Use Committee.

Cells

Isolation of bone marrow-derived macrophages (BMDMs) was performed as previously described [1]. Differentiated macrophages were incubated for 48 h in either high 25 mM glucose medium (DMEM, penicillin-streptomycin and 10% FBS; described as 'Glu') or media containing 6 mM glucose supplemented with oleic acid (MEM (M4655; Sigma-Aldrich), penicillin-streptomycin, oleate-BSA (150 μM oleate conjugated with 23 μM BSA) and 0.5 mM carnitine (C0283; Sigma-Aldrich); described as 'Ole'). Stock solutions of 5 mM oleate-BSA were prepared by the conjugation of oleic acid (O1008; Sigma-Aldrich) and BSA (152401; MP Biomedicals). In some experiments, cells were incubated with oxLDL (770252-6; Kalen Biochemical), sulfosuccinimidyl oleate (SSO) (11211; Cayman), etomoxir (4539; Tocris Bioscience), recombinant murine IL-4 (214-14; Peprotech), palmitate-BSA, N-acetyl-L-Cysteine (NAC) (20261; Cayman), sodium acetate (S2889; Sigma-Aldrich), SR202 (21846; Cayman), or T0070907 (10026; Cayman) as described. For NLRP3 inflammasome activation, cells were pretreated with lipopolysaccharide (LPS) (L3012; Sigma-Aldrich). After 6 h, washed cells were stimulated with nigericin (tlrl-nig; InvivoGen) or ATP (tlrl-atpl; InvivoGen) for 1 h, or oleate-BSA or palmitate-BSA for 24 h. Stock solution of 5 mM palmitate-BSA was prepared by the conjugation of sodium palmitate (P9767; Sigma-Aldrich) and BSA.

Quantitative PCR

For mRNA quantification, RNA was isolated using RNeasy Mini Kit (74106; Qiagen) with (for tissues) or without (for BMDMs) TRIzol (15596026; Thermo Fischer) and cDNA was synthesized using iScript DNA Synthesis Kit (1708891; Bio-Rad) or SuperScript III First-Strand Synthesis SuperMix (18080400; ThermoFisher) or PrimeScript RT reagent Kit (RR037A; Takara). The cDNA was amplified by specific primers using FastStart Universal SYBR Green Master (Rox) (04913914001; Roche) and analyzed by a LightCycler 96 System (Roche). Expression levels were normalized to *Rplp0*. Primers were synthesized by Integrated DNA Technologies. The sequences are shown in Supplemental Table 1.

Immunoblotting

For CPT1a detection, a mitochondrial-enriched fraction was prepared as described previously [1]. Total protein extracts of BMDMs and aortic tissue were lysed in RIPA buffer (89901; Thermo Fischer) with cOmplete, Mini (11836170001; Roche) and PhosSTOP (04906837001; Roche). The samples were loaded into 4-20% or 7.5 % Mini-PROTEAN TGX Precast Protein Gel (4561096 or 4561026; Bio-Rad), and transferred to a Trans-Blot Turbo Midi Nitrocellulose Transfer Pack (1704158 or 1704159; Bio-Rad) by a Trans-Blot Turbo Transfer System (Bio-Rad). The blots were acquired and analyzed by an Odyssey CLx Imaging System (LI-COR). Blots were quantified with ImageJ software (NIH). For detection of IL-1β levels in the culture medium, protein was precipitated with methanol and chloroform. Details of primary antibodies are shown in Supplemental Table 2.

Metabolomics and ATP analysis

Cellular acylcarnitine levels were analyzed by the Metabolomics Core at the Sanford Burnham Prebys Medical Discovery Institute. ATP content was measured with Luminescent ATP Detection Assay Kit (ab113849; Abcam) and a 1450 MicroBeta TriLux Microplate Scintillation and Luminescence Counter (Perkin Elmar) and analyzed according to the manufacturer's recommendations.

Seahorse analysis

The oxygen consumption rate (OCR) of cells was analyzed using a Seahorse XFe96 Analyzer (Agilent Technologies). For the mito stress test, cells were cultured in a 96-well Seahorse plate (75,000 cells/well) and incubated with the indicated medium. After 48 h, the medium was changed for 45 min to the assay medium (Agilent Seahorse XF Assay Medium (102365-100; Agilent Technologies) with 25 mM glucose). To assess the ability to oxidize exogenous fatty acids, cells were cultured in a 96-well Seahorse plate (75,000 cells/well) and incubated overnight with the substrate-limited medium (Agilent Seahorse XF Base Medium (102353-100; Agilent Technologies) with 0.5 mM glucose, 1 mM GlutaMAX (35050061; Thermo Fischer), 0.5 mM carnitine and 1% FBS). The next day, the medium was changed for 45 min to the FAO assay medium (111 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 2.0 mM $MgSO_4$, 1.2 mM Na_2HPO_4, 2.5 mM glucose, 0.5 mM carnitine and 5 mM HEPES) and palmitate-BSA (200 μM palmitate conjugated with 34 μM BSA) or BSA (34 μM) (102720-100; Agilent Technologies) were added and the OCR was analyzed. Results are shown as picomoles oxygen consumption per min per mg protein. Where appropriate, the cells were treated with the ATP synthase inhibitor oligomycin (1.0 μM; O4876; Sigma-Aldrich), the chemical uncoupler FCCP (1.5 μM; C2920; Sigma-Aldrich) and the electron transport inhibitor antimycin A (0.5 μM; A8674; Sigma-Aldrich). Basal OCR was assessed before the addition of any mitochondrial inhibitor and maximal OCR was assessed between the addition of FCCP and antimycin A.

Flow cytometry

Flow cytometry data was acquired and analyzed with a BD FACSCanto or FACSCanto II or BD LSR II or BD FACSymphony (all BD Biosciences). Mitochondrial ROS generation was evaluated using 5 μM MitoSOX (M36008; Thermo Fischer). For neutral lipid staining, cells were stained with 2 μM BODIPY

493/503 (D3922; Thermo Fisher) for 15 minutes. For the oxLDL uptake assay, 10 μg human DiI high oxidized low-density lipoprotein (DiI-oxLDL) (770262-9; Kalen Biomedical) was initially added to cells. After 3-4 h, cells were washed and analyzed. Cell surface CD36 expression was assessed using the specified antibody (see Supplemental Table 2).

Cellular lipid imaging and quantification

For neutral lipid imaging, cells were washed, fixed with 4% PFA in PBS and blocked with 1% BSA in PBS. After 30 minutes, cells were stained with 2 μM BODIPY 493/503 and 5 μM Draq5 (62251; Thermo Fisher), a nuclear marker, for an additional 30 minutes. The images were acquired with a Leica TCS SP5 confocal microscope (Leica). For cholesterol quantification, cells were washed, and the cholesterol was extracted with 200 μl of a chloroform: isopropanol: NP-40 (7: 11: 0.1) solution. After removing the organic solvent, dried lipids were dissolved with 200 μl of the Cholesterol Assay Buffer contained in the Cholesterol Fluorometric Assay Kit (10007460; Cayman Chemical) and measured by a SpectraMax Gemini EM Microplate Reader (Molecular Devices) and analyzed according to the manufacturer's recommendations. For free FAs quantification, cells were washed and the free FAs were extracted with 200 μl of 1% Triton X-100 in 100% chloroform. After transferring the organic phase, dried lipids were dissolved with 200 μl of PBS and analyzed using the Free Fatty Acid Fluorometric Assay Kit (700310; Cayman Chemical) along with a SpectraMax Gemini EM Microplate Reader and in accordance with the manufacturer's recommendations.

Small interfering RNA (siRNA)

To knockdown *Cpt1a* in BMDMs, siGENOME non-targeting (D-001210-01-05) (siCtrl) and mouse Cpt1a SMARTpool (M-042456-01-0005) (si*Cpt1a*) were transfected in combination with Lipofectamine 2000 Transfection Reagent (11668019; Thermo Fisher).

Cytokine quantification (ELISA)

IL-1β secretion in the culture media was quantified by enzyme-linked immunosorbent assay (ELISA) with specific antibodies, avidin-HRP (18-4100-51; Thermo Fischer), and TMB solution (00-4201-56; Thermo Fischer), and measured by a SpectraMax Plus 384 Microplate Reader (Molecular Devices). Details of the antibodies used are shown in Supplemental Table 2.

Cholesterol efflux

Cells were labelled with 5 μCi/ml of [1,2-3H]cholesterol (NET139250UC; Perkin Elmar). After 24 h, cells were washed and incubated with the Ole medium. After 24 h, cells were washed and incubated with or without 15 μg/ml of recombinant human apolipoprotein A-I (ApoA-I) (350-11; PeproTech) or 50 μg/ml of human high-density lipoprotein (HDL) (770300-4; Kalen Biomedical). After 24 h, the radioactivity of the culture medium and cell lysate was measured by a 1450 MicroBeta TriLux Microplate Scintillation and Luminescence Counter. The cholesterol efflux was calculated as the percentage of total cholesterol efflux into the medium after subtracting the no accepter control counts.

Atherosclerosis assessment

Mice were euthanized by CO_2 and perfused with phosphate-buffered saline (PBS). For *en face* analysis, thoraco-abdominal aortas were fixed by 4% paraformaldehyde/5% sucrose in PBS for 24 h and stored in PBS. Atherosclerosis was assessed by Oil red O staining (O1391; Sigma-Aldrich) and the images were acquired using a Leica MZ FLIII (Leica) and quantified with ImageJ software. For cross-sectional analysis, hearts were fixed with 4% PFA/5% sucrose in PBS for 24 h, cryoprotected by 10% sucrose in PBS followed by 20% sucrose in PBS over 48 h, and embedded in OTC compound (4583; Sakura Finetek). Atherosclerosis was assessed by Oil red O staining and the images were captured using NanoZoomer 2.0-RS (Hamamatsu) and quantified with ImageJ software. There were two cohorts of

animals, with the first cohort used for serum and morphometric analysis and the second cohort used for gene and protein expression analysis.

Histochemistry

To detect lesional macrophages, an anti-CD68 antibody (1:100 dilution) was used. The staining was visualized with VECTASTATIN ABC-AP Staining Kit (Alkaline Phosphatase, Mouse IgG) (AK-5002; Vector Laboratories) and VECTOR Red Alkaline Phosphatase (Red AP) Substrate Kit (SK-5100; Vector Laboratories). Collagen was detected using a Picrosirius Red Stain Kit (24901; Polysciences). These images were captured using NanoZoomer 2.0-RS. To detect smooth muscle cells, Cy3-conjugated anti-αSMA (1:500 dilution) was used. The images were acquired with a Leica TCS SP5 confocal microscope. Positive areas were quantified with ImageJ software. Details of the antibodies are shown in Supplemental Table 2. To detect reactive oxygen species (ROS), dihydroethidium (DHE) (12013; Cayman) was used. The frozen sections were stained with 20 μM DHE for 30 min. The images were acquired with a Leica TCS SP5 confocal microscope.

Plasma biochemistry

Plasma lipid contents (cholesterol, free FAs, and triglycerides) were measured with HDL and LDL/VLDL Quantitation Kit (MAK045-1KT; Sigma-Aldrich), Free Fatty Acid Quantitation Kit (MAK044-1KT; Sigma-Aldrich), and Serum Triglyceride Determination Kit (TR0100-1KT; Sigma-Aldrich), respectively.

Phenotyping

Mouse body weight was measured weekly. Body composition was analyzed with a minispec mq (Bruker). Blood glucose was determined using a Contour glucose meter (Bayer) and plasma insulin levels after overnight fasting were measured by mouse insulin ELISA kit (10-1249-01; Mercodia).

Statistical analysis

GraphPad Prism 7 (GraphPad) was used for statistical analysis. *P* values were calculated by unpaired two-tailed Student's *t*-test or one/two-way ANOVA with the Bonferroni correction as shown in figure legends. A p value less than 0.05 was considered significant.

Supplemental Fig. 1

(A) Transport of long-chain FAs into the mitochondrial matrix is achieved by CPT1 and CPT2. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; CACT; carnitine acylcarnitine translocase. Adapted from [1]. (B) Quantitative PCR analysis of mRNA of *Cpt1a* and *Cpt1b* in C57BL/6 wild type BMDMs (cells obtained from n = 3 mice). (C) Quantitative PCR analysis of *Cpt1a* mRNA in control and CPT1a M-KO BMDMs (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (D) Immunoblot analysis and the quantitative densitometry of CPT1a protein using mitochondrial lysate of control and CPT1a M-KO BMDMs (cells obtained from n = 2 mice per group). The mitochondrial protein HSP60 is shown as a loading control. (E) Quantitative PCR analysis of *Cpt1a* and *Cpt2* mRNA in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (F) acylcarnitines (AC) levels in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). (G) Basal and maximal oxygen consumption rate (OCR) in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group with each biological value representing a technical triplicate). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (H) Basal OCR in control and CPT1a M-KO BMDMs in the presence of BSA or palmitate-BSA (Palm-BSA) (cells obtained from n = 3 mice per group with each biological value representing a technical triplicate). Data are representative of two independent experiments. (I) Cellular ATP levels in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group with each biological value representing a technical duplicate). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (J) Representative and quantification of flow cytometry analyzing the level of MitoSOX fluorescence in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of three independent experiments. Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction or unpaired two-tailed Student's *t*-test (C, D, and F).

Supplemental Fig. 2

(A) Representative and quantification of flow cytometry analyzing the expression of CD36 in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (B) Quantitative PCR analysis of *Msr1* mRNA in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (C) Immunoblot analysis and the quantitative densitometry of SR-A protein in total lysate of control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). HSP90 is shown as a loading control. Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (D) Quantitative PCR analysis of *Olr1* mRNA in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h in response to LPS (100 ng/ml) for 6 h or 12 h. Data are representative of two independent experiments. (E) Total cellular free FAs content in control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation of media containing a FA substrate for 24 h, followed by media with (+) or without (−) oxLDL (50 μg/ml)

for 24 h (cells obtained from n = 3 mice per group with each biological value representing a technical triplicate). Data are representative of two independent experiments. (F) Representative and quantification of flow cytometry analyzing the oxLDL uptake in control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation of media containing a FA substrate with DMSO (DM) or SSO (100 μM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (G) Representative and quantification of flow cytometry analyzing the BODIPY 493/503 staining in control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation of media containing a FA substrate with DMSO (DM) or SSO (100 μM) for 24 h, followed by addition of oxLDL (50 μg/ml) for 24 h (cells obtained from n = 3 mice per group). Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction or two-way ANOVA with the Bonferroni correction (D).

Supplemental Fig. 3

(A) Quantitative PCR analysis of *Cd36* mRNA in C57BL/6 wild type BMDMs. Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) with or without etomoxir (3 μM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (B) Immunoblot analysis and the quantitative densitometry of CD36 protein in total lysate of C57BL/6 wild type BMDMs. Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) with or without etomoxir (3 μM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (C) Representative and quantification of flow cytometry analyzing the oxLDL uptake in C57BL/6 wild type BMDMs. Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) with or without etomoxir (3 μM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (D) Representative and quantification of flow cytometry analyzing the BODIPY 493/503 staining in C57BL/6 wild type BMDMs after incubation of media containing a FA substrate in the presence or absence of etomoxir (3 μM) for 24 h, followed by incubation with media with (+) or without (−) oxLDL (50 μg/ml) for 24 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (E) Quantitative PCR analysis of mRNA of *Cpt1a*, *Cpt2*, and *Cd36*, in siCtrl or *Cpt1a* siRNA (si*Cpt1a*)-transfected control and CPT2 M-KO BMDMs in the presence of a FA substrate for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (F) Immunoblot analysis and the quantitative densitometry of CD36 protein in siCtrl or si*Cpt1a*-transfected control and CPT2 M-KO BMDMs in the presence of a FA substrate for 48 h (cells obtained from n = 3 mice per group). (G) Representative and quantification of flow cytometry analyzing the BODIPY 493/503 staining in siCtrl or si*Cpt1a*-transfected control and CPT2 M-KO BMDMs after incubation of media containing a FA substrate for 24 h, followed by media with oxLDL (25 μg/ml) for 24 h (cells obtained from n = 3 mice per group). Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction. ns, not significant.

Supplemental Fig. 4

(A) Quantitative PCR analysis of mRNA of various M2 markers in control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation with (+) or without (−) IL-4 (10 ng/ml) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (B) Quantitative PCR analysis of mRNA of inflammatory M1 markers in control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation with or without IL-4 (10 ng/ml) for 24 h, followed by LPS (1 ng/ml) for 6 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (C) Immunoblot analysis and the quantitative densitometry of phosphorylated and total JNK in response to palmitate-BSA (500 μM; Palm) for 3 h in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (D) Quantitative PCR analysis

of mRNA levels for inflammatory (*Il6*, *Tnf*) and ER stress markers (*Hspa5*, *Xbp1*, *Atf4*, *Ddit3*) in response to palmitate-BSA (500 μM) for 3 h or 24 h in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction or two-way ANOVA with the Bonferroni correction (D).

Supplemental Fig. 5

(A) IL-1β secretion in response to nigericin (10 μM; Ni) or ATP (5 mM) for 1 h in LPS (200 ng/ml, 6 h)-primed control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (B) Immunoblot analysis of proteins of IL-1β (p17) in response to nigericin (10 μM; Ni) or ATP (5 mM) for 1 h in LPS (200 ng/ml, 6 h)-primed control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (C) IL-1β secretion in response to a 24 h incubation with oleate-BSA (500 μM; Ole) or palmitate-BSA (500 μM; Palm) in LPS (200 ng/ml, 6 h)-primed control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction.

Supplemental Fig. 6

(A) Quantitative PCR analysis of *Cd36* mRNA in control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without NAC (1 mM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (B) Immunoblot analysis and the quantitative densitometry of CD36 protein in total lysate of control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without NAC (1 mM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (C) Representative and quantification of flow cytometry analyzing the oxLDL uptake in control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without NAC (1 mM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (D) Representative and quantification of flow cytometry analyzing the BODIPY 493/503 staining in control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without NAC (1 mM) for 24 h, followed by addition of oxLDL (25 μg/ml) for 24 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (E) Cellular ATP levels in control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without acetate (1 mM) for 48 h (cells obtained from n = 3 mice per group). (F) Immunoblot analysis and the quantitative densitometry of CD36 protein in total lysate of control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without acetate (1 mM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (G) Representative and quantification of flow cytometry analyzing the oxLDL uptake in control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without acetate (1 mM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (H) Representative and quantification of flow cytometry analyzing the BODIPY 493/503 staining in control and CPT1a M-KO BMDMs after incubation of media containing a FA substrate with or without acetate (1 mM) for 24 h, followed by addition of oxLDL (25 μg/ml) for 24 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction.

Supplemental Fig. 7

(A) Quantitative PCR analysis of *Abca1* and *Abcg1* mRNA in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of three independent experiments. (B) Immunoblot analysis and the quantitative densitometry of ABCA1 and ABCG1 proteins in total lysate of control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (C) Apolipoprotein A-I (ApoA-I; an acceptor for ABCA1) or high-density lipoprotein (HDL; an acceptor for ABCG1)-dependent cholesterol efflux in control, CPT1a M-KO or CPT2 M-KO BMDMs in the presence of a FA substrate for 48 h (cells obtained from n = 2-3 mice per group). Data are representative of two independent experiments (ApoA-I). (D) Immunoblot analysis and the quantitative densitometry of CD36 protein in total lysate of control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation of media containing a FA substrate with or without SR202 (50 μM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (E) Immunoblot analysis and the quantitative densitometry of CD36 protein in total lysate of control, CPT1a M-KO or CPT2 M-KO BMDMs after incubation of media containing a FA substrate with or without T0070907 (2 μM) for 48 h (cells obtained from n = 3 mice per group). Data are representative of two independent experiments. (F) Quantitative PCR analysis of *Pparg* mRNA in control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. (G) Immunoblot analysis and the quantitative densitometry of PPARγ protein in total lysate of control, CPT1a M-KO or CPT2 M-KO BMDMs (cells obtained from n = 3 mice per group). Analysis was performed in standard high glucose media (Glu) or in the presence of a FA substrate (Ole) for 48 h. Data are representative of two independent experiments. Results represent mean ± s.d. **P* <.05, by one-way ANOVA with the Bonferroni correction or unpaired two-tailed Student's *t*-test (C).

Supplemental References

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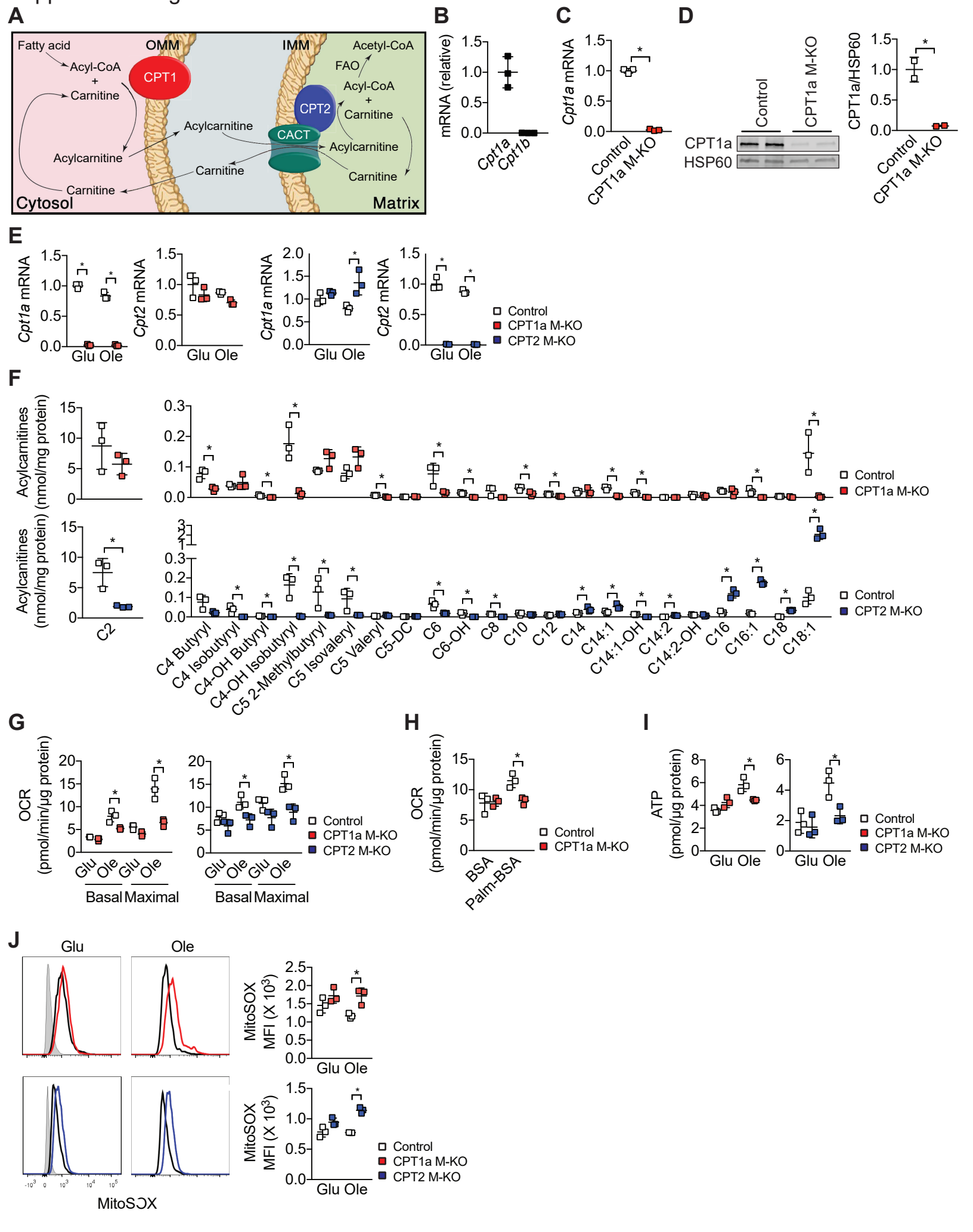
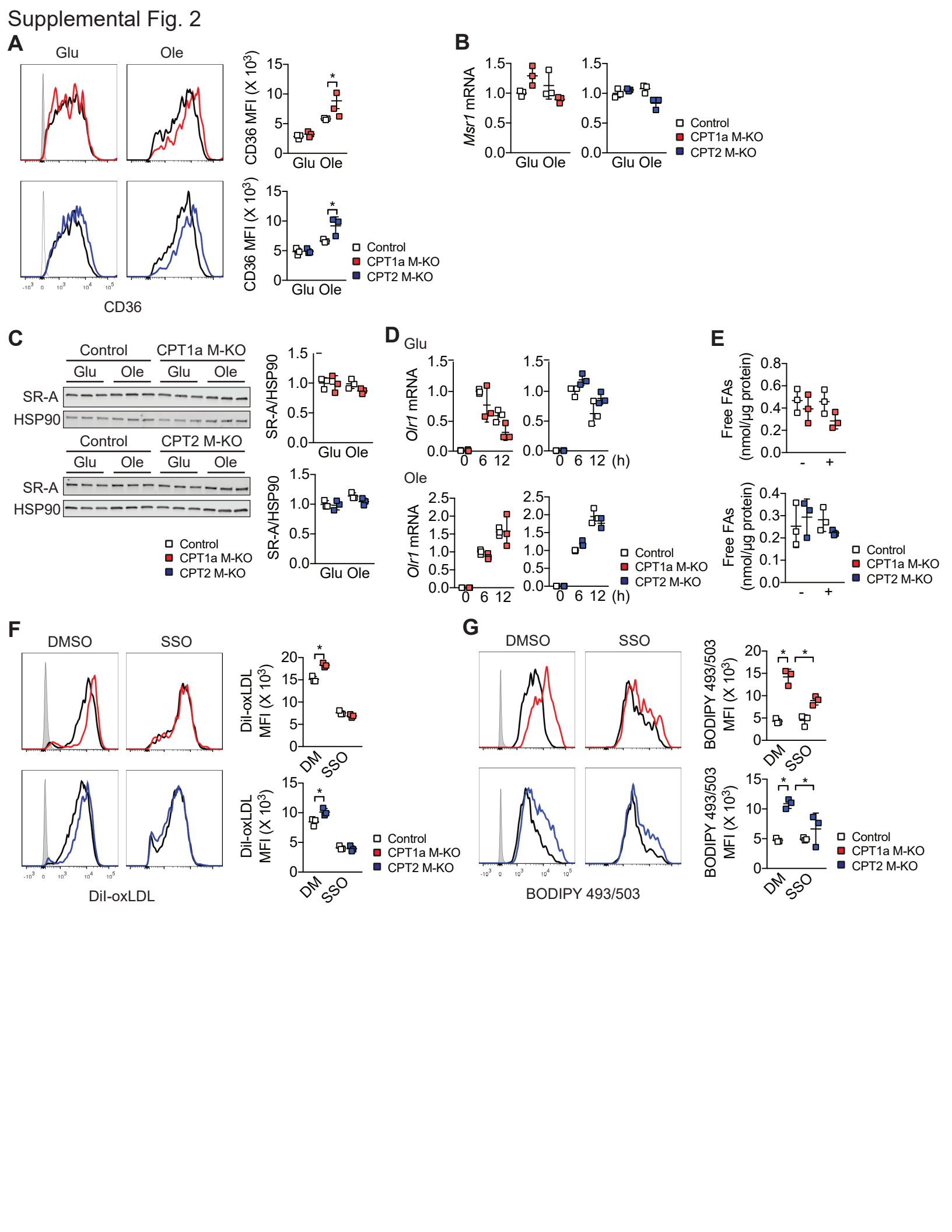
Supplemental Fig. 1



Supplemental Fig. 2



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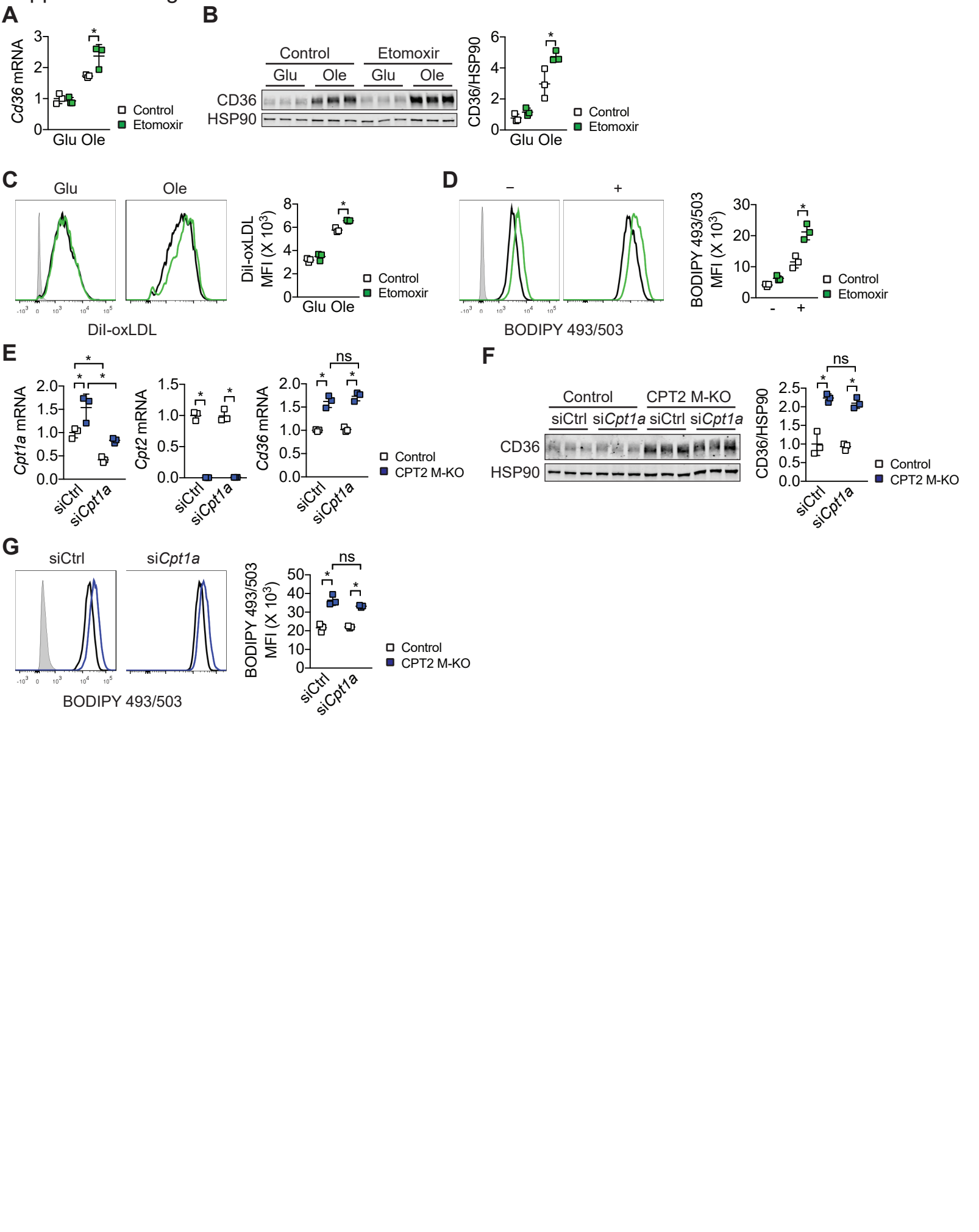
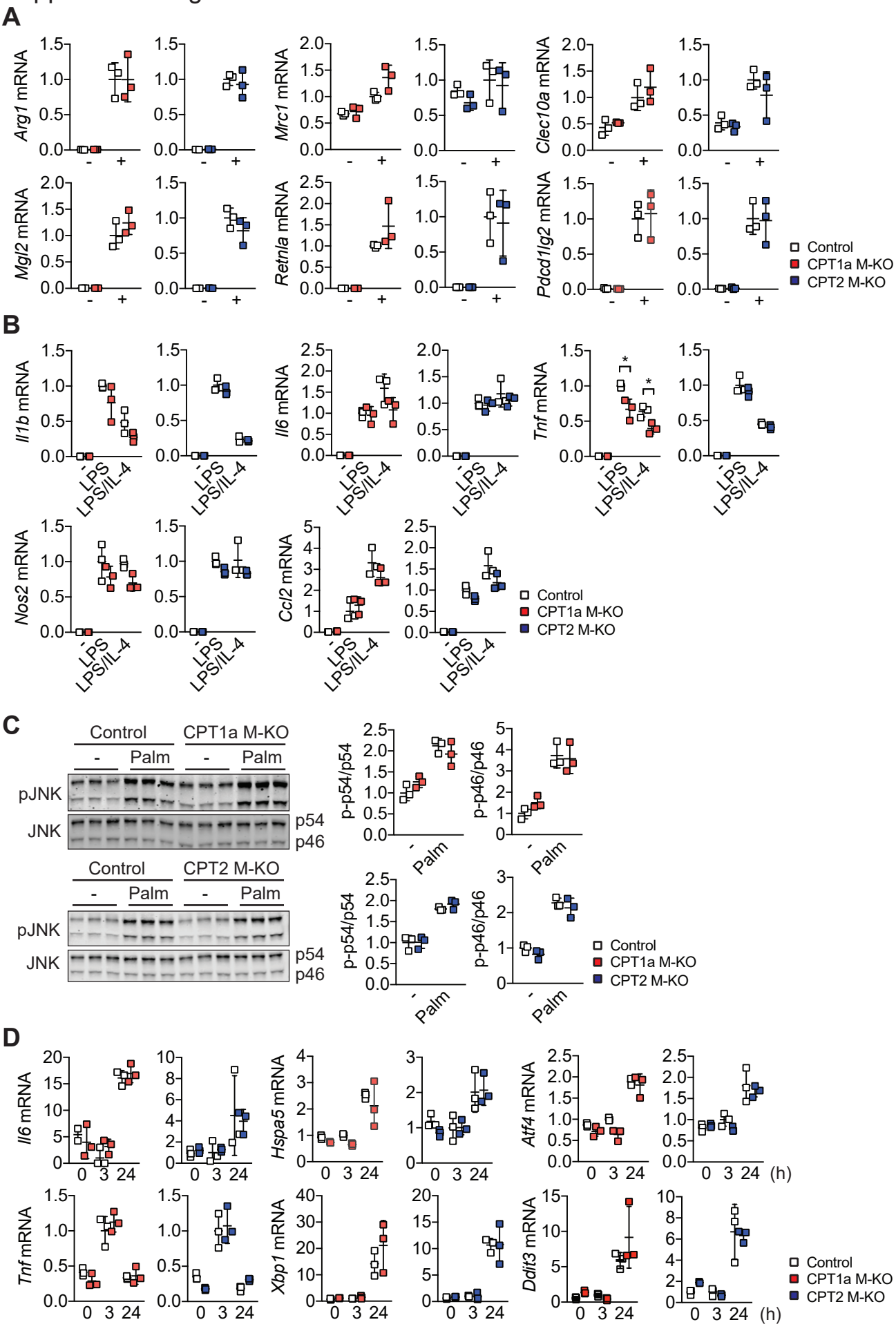
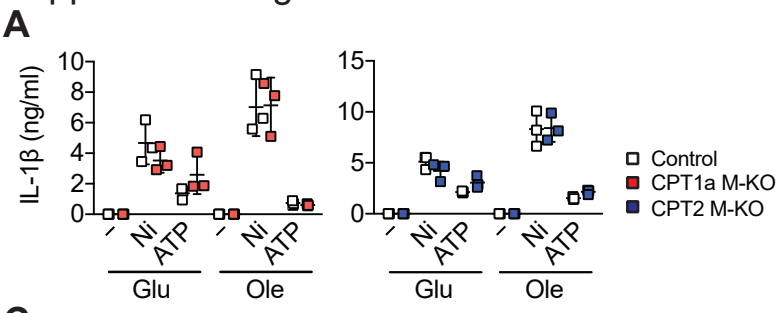
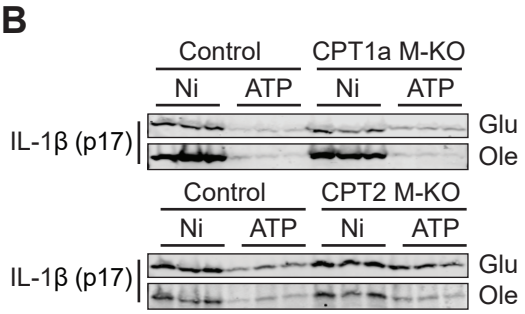


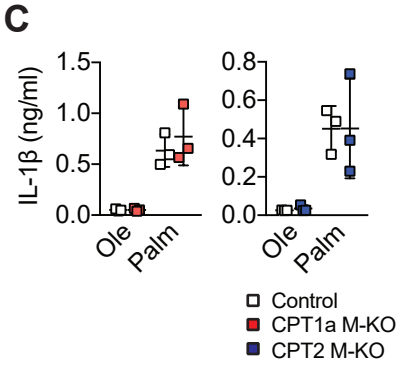
Supplemental Fig. 4



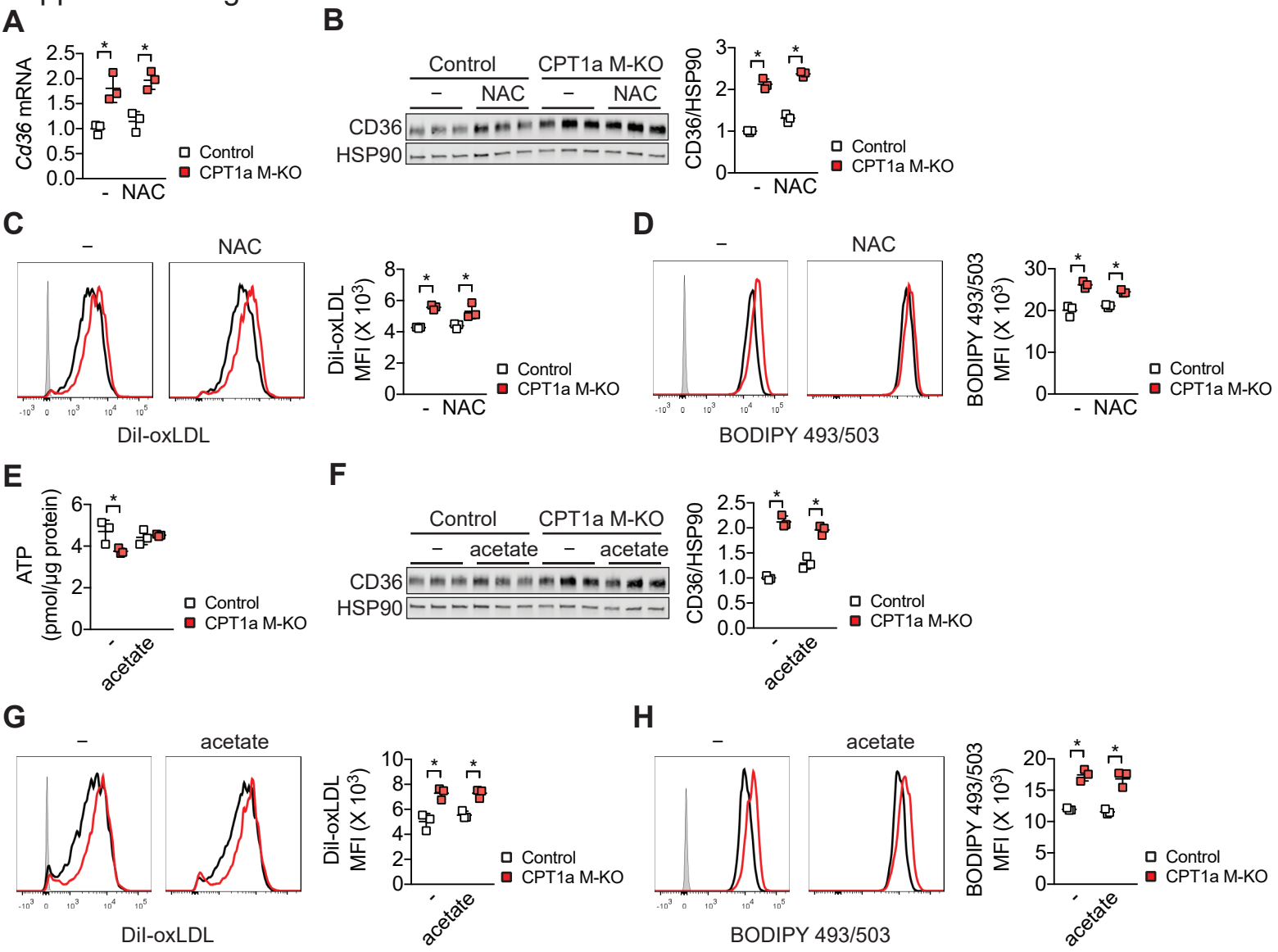
Supplemental Fig. 5



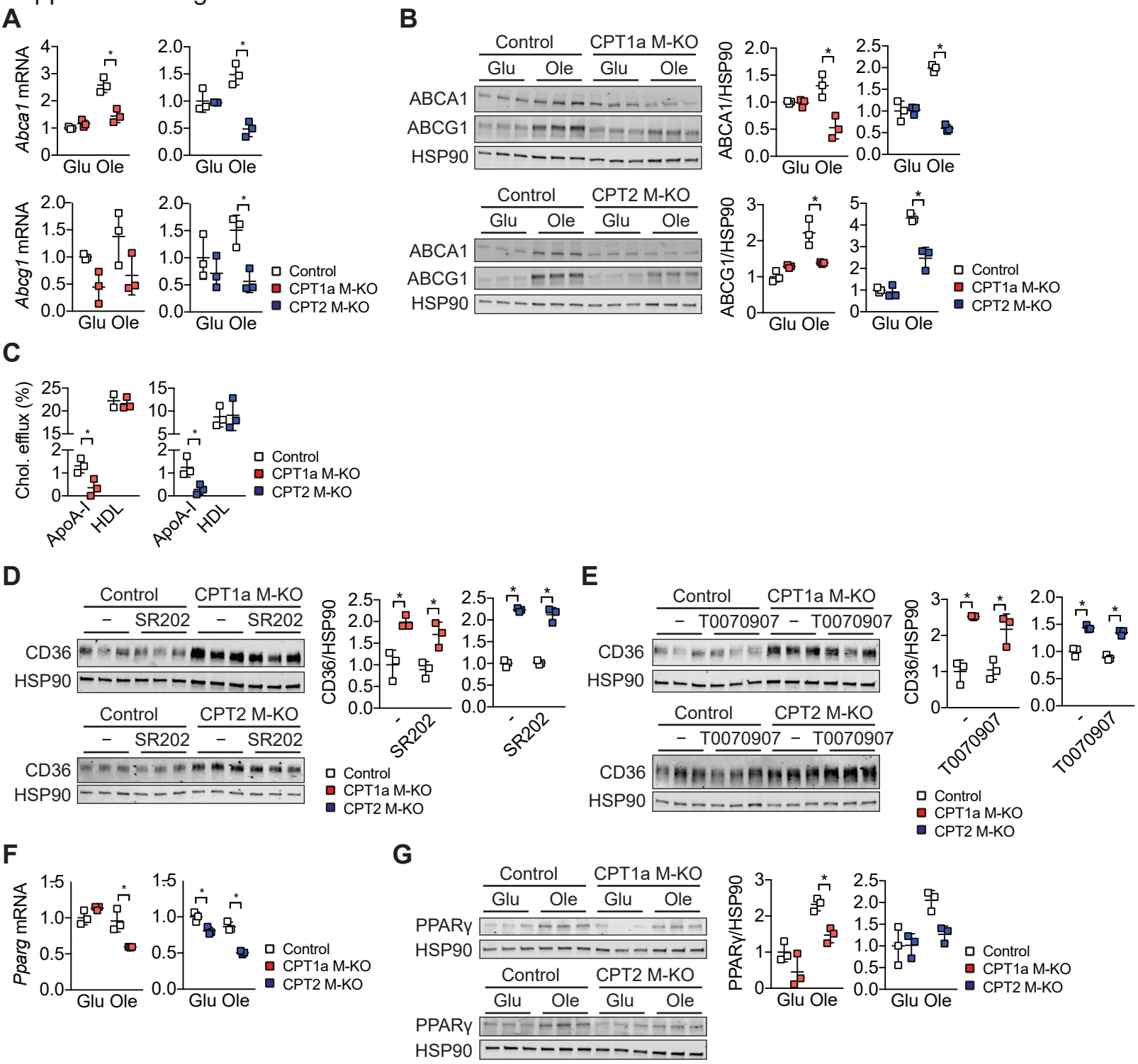




Supplemental Fig. 6



Supplemental Fig. 7



Supplemental Table 1. Primers for quantitative PCR (all mouse).

Gene	**Forward**	**Reverse**
Rplp0	AGATTCGGGATATGCTGTTGG	AAAGCCTGGAAGAAGGAGGTC
Cpt1a	CTATGCGCTACTCGCTGAAGG	GGCTTTCGACCCGAGAAGA
Cpt1b	GCACACCAGGCAGTAGCTTT	CAGGAGTTGATTCCAGACAGGTA
Cpt2	CAGACAGTGGCTACCTATGAATCCT	TGGTCAGCTGGCCATGGTATTTGGA
Cd36	TCCTCTGACATTTGCAGGTCTATC	AAAGGCATTGGCTGGAAGAA
Msr1	GCACAATCTGTGATGATCGCT	CCCAGCATCTTCTGAATGTGAA
Olr1	CAAGATGAAGCCTGCGAATGA	ACCTGGCGTAATTGTGTCCAC
Ppard	AGCACATCTACAACGCCTACCTGA	TCGATGTCGTGGATGACAAAGGGT
Pparg	AGGCCGAGAAGGAGAAGCTGTTG	TGGCCACCTCTTTGCTCTGCTC
Ppargc1a	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
Ppargc1b	TCCTGTAAAAGCCCGGAGTAT	GCTCTGGTAGGGGCAGTGA
Nr1h3	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT
Fabp4	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Mrc1	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
Clec10a	TGCAACAGCTGAGGAAGGACTTGA	AACCAATAGCAGCTGCCTTCATGC
Mgl2	GCATGAAGGCAGCTGCTATTGGTT	TAGGCCCATCCAGCTAAGCACATT
Il1b	AAATACCTGTGGCCTTGGGC	CTTGGGATCCACACTCTCCAG
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Tnf	ACGGCATGGATCTCAAAGAC	GTGGGTGAGGAGCACGTAGT
Nos2	GAGGCCCAGGAGGAGAGAGATCCG	TCCATGCAGACAACCTTGGTGTTG
Ccl2	ATTGGGATCATCTTGCTGGT	CCTGCTGTTCACAGTTGCC
Hspa5	ACTTGGGGACCACCTATTCCT	ATCGCCAATCAGACGCTCC
Xbp1 (spliced form)	GAGTCCGCAGCAGGTG	AGGCTTGGTGTATACATGG
Atf4	GGGTTCTGTCTTCCACTCCA	AAGCAGCAGAGTCAGGCTTTC
Ddit3	CTGCCTTTCACCTTGGAGAC	CGTTTCCTGGGGATGAGATA
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Retnla	CCAATCCAGCTAACTATCCCTCC	ACCCAGTAGCAGTCATCCCA
Pdcd1lg2	CTGCCGATACTGAACCTGAGC	GCGGTCAAAATCGCACTCC
Abca1	CGTTTCCGGGAAGTGTCCTA	GCTAGAGATGACAAGGAGGATGGA
Abcg1	CTTCCCAGCTCCAGAAGGAC	CCTACTCTGTACCCGAGGGG

Supplemental Table 2. Antibodies.

Antibodies	Source	Identifier
Immunoblotting		
Anti-CPT1A antibody [EPR12740(B)]	Abcam	ab176320
HSP 60 Antibody (N-20)	Santa Cruz	sc-1052
CD36/SR-B3 Antibody	Novus	NB400-144
Purified Mouse Anti-Hsp90	BD Biosciences	610419
Anti-SRA1 antibody	Abcam	ab183725
Phospho-SAPK/JNK (Thr183/tyr185) (81E11)	Cell Signaling	4668
SAPK/JNK (56G8) Rabbit mAb	Cell Signaling	9258
Anti-IL1 beta antibody	Abcam	ab9722
Anti-ABCA1 antibody [AB.H10]	Abcam	ab18180
ABCG1 Antibody	Novus	NB400-132SS
PPARγ Antibody (E-8)	Santa Cruz	sc-7273
Flow cytometry		
APC anti-mouse CD36 Antibody	BioLegend	102611
ELISA		
IL-1 beta Monoclonal Antibody (B122)	Thermo Fisher	14-7012-85
IL-1 beta Polyclonal Antibody, Biotin	Thermo Fisher	13-7112-85
Immunohistochemistry		
Anti-CD68 antibody [KP1]	Abcam	ab955
Anti-Actin, α-Smooth Muscle - Cy3 antibody	Sigma	C6198