Supplemental Data

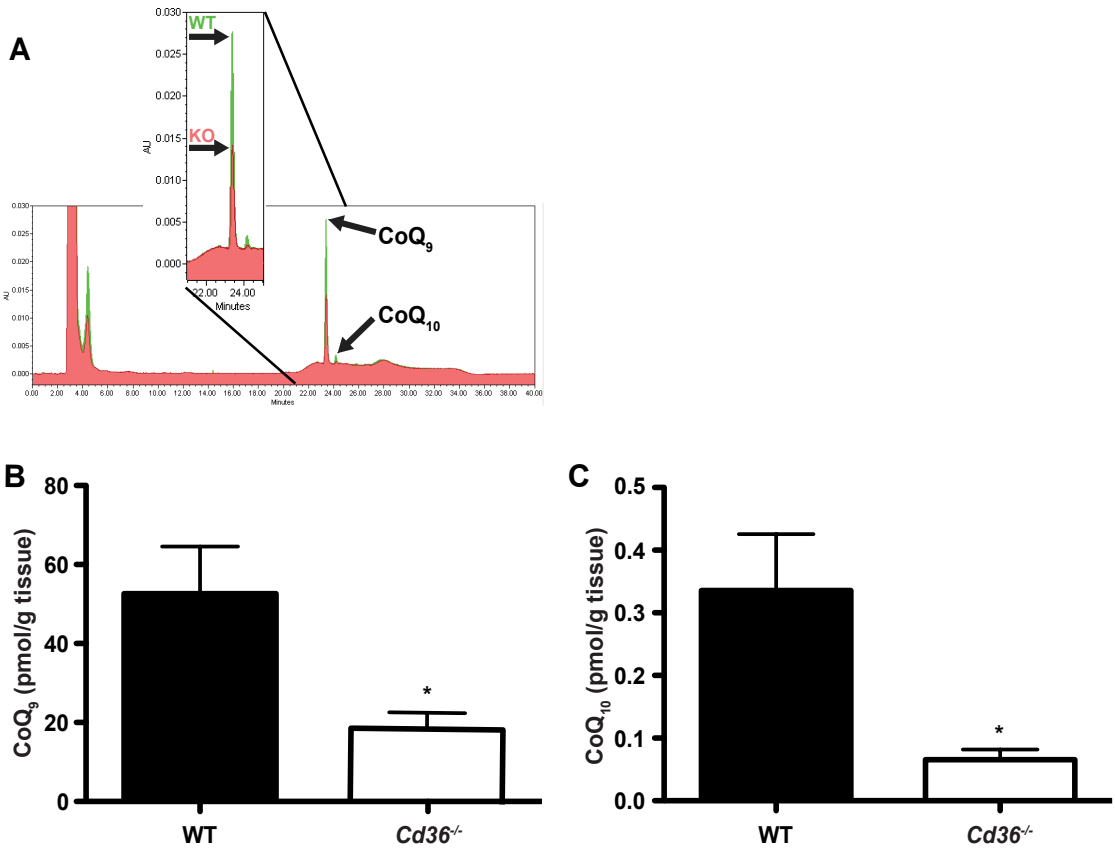

Figure S1, related to Figure 1. CoQ deficiency in *Cd36*$^{-/-}$ BAT.

(A) Representative HPLC chromatograms from WT (green) and *Cd36*$^{-/-}$ (pink) BAT are displayed. The CoQ_9 and CoQ_{10} peaks overlay is shown in inset. CoQ_9 (B) and CoQ_{10} (C) levels were measured in WT and *Cd36*$^{-/-}$ BAT by mass spectrometry (n=3). *p<0.05. Error bars, SEM.

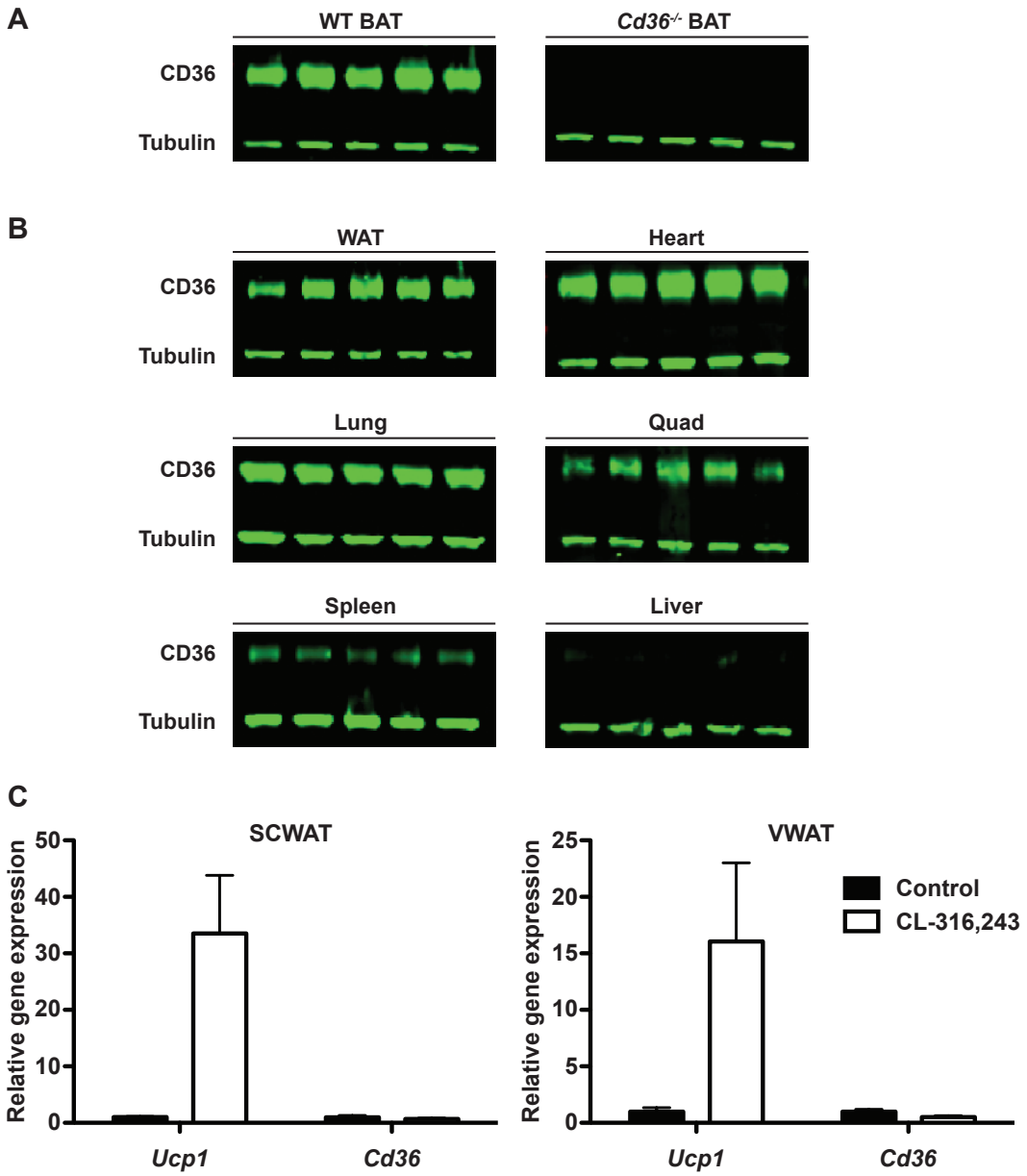

Figure S2, related to Figure 2. Analysis of CD36 expression in various tissues.

Western blot for CD36 and tubulin in WT and *Cd36*$^{-/-}$ BAT (A) and WT WAT, heart, lung, quadriceps muscle, spleen, and liver (B) (n=5). (C) Gene expression in subcutaneous WAT (SCWAT) and visceral WAT (VWAT) after daily treatment with CL-316,243 for 7 days. Error bars, SEM.

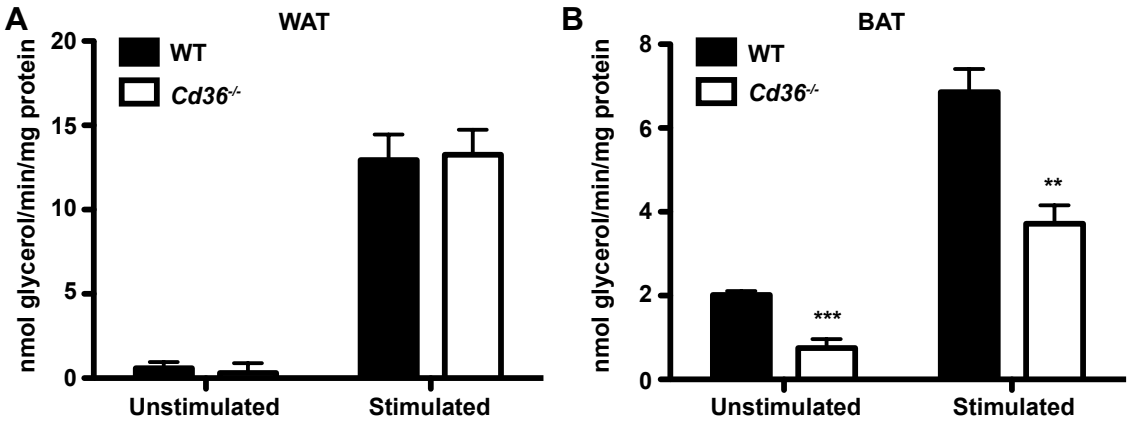

Figure S3, related to Figure 2. Impaired lipolysis in *Cd36*$^{-/-}$ BAT

Lipolysis rate (nmol of glycerol released/minute/mg protein of tissue lysate) in unstimulated or IBMX/forskolin-stimulated WT and *Cd36*$^{-/-}$ WAT (A) and BAT (B) chunks (n=3). **p<0.005; ***p<0.0005. Error bars, SEM.

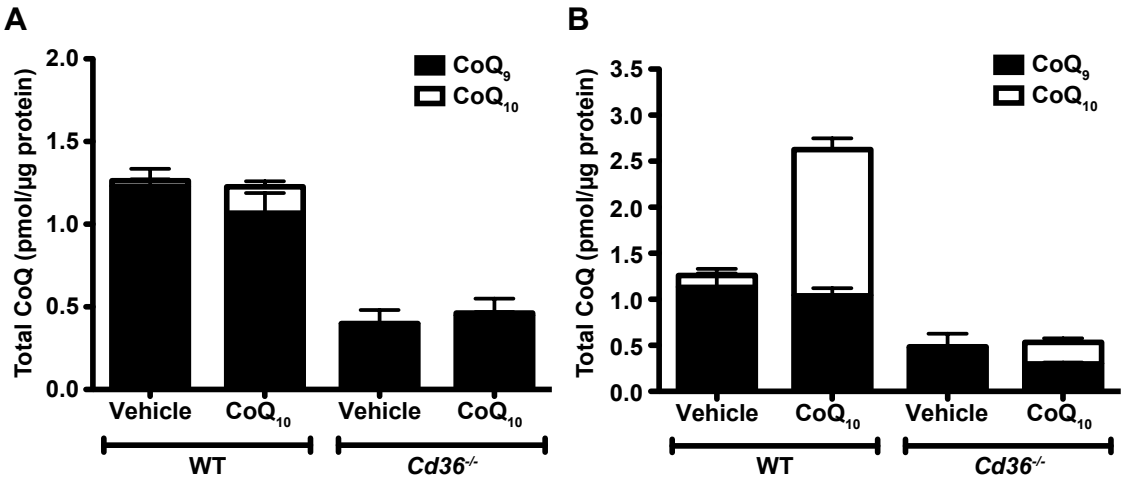

Figure S4, related to Figure 1 and Discussion. Administration of CoQ does not increase total CoQ levels in *Cd36*$^{-/-}$ BAT

(A) WT and *Cd36*$^{-/-}$ mice were injected intraperitoneally with either vehicle (Intralipid) or CoQ_{10} (n=8-9). (B) WT and *Cd36*$^{-/-}$ mice were injected intraperitoneally with either vehicle or a highly concentrated commercial solution of CoQ_{10} (n=3). After 24 h tissues were isolated and CoQ levels were measured by HPLC and normalized to µg of protein.

		CoQ_9 (pmol/µg protein or µl serum)	CoQ_{10} (pmol/µg protein or µl serum)	
Tissue	**Genotype**	**Mean ± SEM**	**Mean ± SEM**	**N**
BAT	WT	2.2389 ± 0.2530	0.2093 ± 0.0339	6
	Cd36$^{-/-}$	0.7645 ± 0.1341 ***	0.1011 ± 0.0174 ***	6
Liver	WT	0.6134 ± 0.1284	0.0033 ± 0.0003	5
	Cd36$^{-/-}$	0.9015 ± 0.1173 **	0.0078 ± 0.0019	4
Heart	WT	0.6692 ± 0.0526	0.1128 ± 0.0131	4
	Cd36$^{-/-}$	0.6251 ± 0.0726	0.0972 ± 0.0137	4
Soleus	WT	179.6024 ± 10.6717	16.9808 ± 2.6677	3
	Cd36$^{-/-}$	171.1216 ± 1.5504	16.0791 ± 1.0224	3
Gastroc	WT	421.2820 ± 47.1222	35.9012 ± 8.0316	3
	Cd36$^{-/-}$	472.4202 ± 42.0899	49.2020 ± 3.7380	3
Quad	WT	0.2260 ± 0.0377	0.0242 ± 0.0049	3
	Cd36$^{-/-}$	0.2208 ± 0.0447	0.0231 ± 0.0046	3
Adr. Gland	WT	1.0063 ± 0.0374	0.4630 ± 0.0561	6
	Cd36$^{-/-}$	0.9329 ± 0.1009	0.3564 ± 0.0532	6
Brain	WT	0.1708 ± 0.0105	0.0890 ± 0.0053	6
	Cd36$^{-/-}$	0.1847 ± 0.0187	0.0928 ± 0.0094	6
Serum	WT	0.0124 ± 0.0009	0.0063 ± 0.0015	8
	Cd36$^{-/-}$	0.0114 ± 0.0013	0.0081 ± 0.0006	7

Table S1, related to Figure 1. CoQ levels in WT and *Cd36*$^{-/-}$ tissues

CoQ_9 and CoQ_{10} levels in BAT, liver, heart, soleus, gastrocnemius, quadriceps, adrenal gland, brain, and serum from WT and *Cd36*$^{-/-}$ mice were measured by HPLC and normalized to µg of protein (n=3-6).

Tables S2 and S3, related to Figure 2. Loss of CD36 alters gene expression in BAT

Genome-wide expression analysis was performed on RNA from WT and *Cd36*$^{-/-}$ BAT (n=3 per group). Using a 2-fold change and a $p \leq 0.05$ threshold, 55 genes were identified to be downregulated (Table S2) and 279 genes were found to be upregulated (Table S3) in *Cd36*$^{-/-}$ BAT compared to WT BAT. The *Cd36*$^{-/-}$ versus WT fold change (KO vs. WT.FC) represents the average of samples analyzed in triplicates.

Supplemental Experimental Procedures

Primary Mature Brown Adipocyte Isolation

Interscapular BAT was dissected and chopped with scissors after complete removal of the surrounding WAT. The small pieces were digested in 2mg/ml type I collagenase (Life Technologies) for 40 min at 37°C with gentle shaking. The cell suspension was filtered through a 100μm nylon mesh and centrifuged at 200g for 10 min. For the fatty acid uptake and fatty acid oxidation assays, the top layer containing the mature adipocytes was washed twice in Krebs-Henseleit Buffer containing 1% BSA and 18mM glucose. Cells were then washed in BSA-free Krebs-Henseleit Buffer with low glucose (5mM) and treated as described below. For CoQ uptake assays, the top layer containing the mature adipocytes was washed twice in DMEM and treated as described below.

Antibodies

Anti-mouse CD36 (goat polyclonal) and anti-mouse UCP1 (rabbit polyclonal) antibodies were purchased from R&D Systems and Sigma, respectively. The β-tubulin (E7) (mouse monoclonal) antibody developed by M. Klymkowsky was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242. Secondary antibodies were purchased from Licor.

Cholesterol Analysis

Cholesterol was extracted using the Folch method (Chen and Resh, 2002; Folch et al., 1957). For tissues, 100mg tissue was homogenized in 5ml 2:1 chloroform:methanol and

incubated at room temperature for 20 min with agitation. Samples were centrifuged for 5 min at 2000rpm and 1ml 0.9% NaCl was added, vortexed briefly, and centrifuged again. The top layer was removed and discarded and the bottom layer was washed again with 1ml 0.9% NaCl, vortexed, and centrifuged. The bottom layer was transferred to a glass tube and allowed to dry under a stream of nitrogen for 1 h. The dried film was resuspended in 1ml isopropanol. Cholesterol levels were measured using 1µl of extracted cholesterol or serum with the Liquicolor kit according to manufacturer's recommendations (Stanbio Laboratory #1010-430).

Metabolic Rates

VO_2 consumption, VCO_2, and heat production were recorded from individually caged mice treated with PBS or CL-316,243 (1mg/kg; intraperitoneal injection) in the comprehensive lab animal monitoring system (Columbus Instruments) as previously described (Wu et al., 2006). Values from periods of negligible locomotive activity (less than 20 UV beam breaks) were averaged during the three hours following the intraperitoneal injection. Values were normalized to body weight and corrected to the effective body mass value. Heat production was indirectly calculated, based on the observed respiratory exchange ratios.

Triacylglycerol Staining and Immunohistochemistry

BAT was fixed in 4% paraformaldehyde (PFA) and incubated overnight in cryopreservation medium containing 60% Superblock (10% FBS, 1% BSA, 0.05% Saponin, 0.25% normal donkey serum in HBSS), 30% sucrose, and 10% Optimum

Cutting Temperature Medium (Tissue-Tek OCT Compound, Sakura Finetek). 20μm cryosections were obtained using a Leica CM3050S cryostat and stored at -80°C until use**.** Intracellular TAG accumulation was determined by staining sections with BODIPY 493/503 (Invitrogen, #D3922). Z-stack imaging was performed on a Zeiss 510 UV/Vis Meta Laser Scanning Confocal Microscope. TAG storage size and percent of cellular volume were estimated in the 3D images reconstructed using the Imaris 7.2 package (Bitplane). For immunohistochemistry, sections were allowed to air dry and then post-fixed for 10 min in 4% PFA. Sections were rinsed in PBS, blocked for 1 h at room temperature in 3% normal goat serum (NGS), and then incubated in primary antibody overnight at 4°C in 3% NGS. The next day, sections were rinsed 3 times in PBS and incubated in secondary antibody at room temperature for 1 h in 3% NGS. Sections were then rinsed 3 times in PBS, stained with DAPI for 5 min, rinsed 3 times in PBS, and then visualized using a Zeiss 510 UV/Vis Meta Laser Scanning Confocal Microscope.

Western Blotting

Lysates from BAT were obtained by homogenizing tissue in RIPA buffer with protease inhibitors, centrifuging, and storing the supernatant at -80°C until use. 30μg of protein was incubated with SDS-disruption buffer, boiled for 5 min, briefly chilled and centrifuged, and then loaded onto a 4-20% SDS acrylamide gel. Gels were transferred to membranes using a wet transfer protocol for 1 h at 90V on ice. Membranes were blocked in 5% non-fat milk/1x TBST for 1 h at room temperature and incubated in primary antibody/1x TBST overnight at 4°C. Membranes were washed 3 times in 1x TBST, incubated in secondary antibody/1x TBST (Licor) for 1 h at room temperature, and

washed 3 times in 1x TBST. Immunoblotting was detected using the Licor imaging system.

CL-316,243 Treatment and Gene Expression

Mice were injected subcutaneously with either CL-316,243 (1μg/g body weight) or PBS every day for 7 days as previously described Guerra (Guerra et al., 1998). Subcutaneous and visceral WAT depots were collected and total RNA from tissues was extracted using the Aurum Total RNA Fatty and Fibrous Tissue Kit according to the manufacturer's protocol (Bio-Rad). 1μg of RNA was used to generate cDNA using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific #K1642) according to the manufacturer's protocol. RT-qPCR was performed using the TaqMan Universal Master Mix II protocol (Applied Biosystems #4440040). Primer-probe qPCR assays used in RT-qPCR were obtained from IDT.

Generation of HEK293 Cell Line Stably Overexpressing CD36

The pDNR-Lib vector containing human CD36 cDNA was purchased from Open Biosystems. To generate the CD36 expression construct for stable transfection, the human CD36 cDNA was cloned into the pCDNA3.1- vector as an EcoRI-HindIII fragment. HEK293 cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomyocin. Cells were seeded onto 6-well plates and transfected using the Fugene 6 protocol (Roche). In each transfection, 1.8μg of either pCDNA3.1 or pCDNA3.1-CD36 was mixed with 5.4ul of Fugene 6 and up to 90μl serum-free DMEM. The reaction was incubated for 30 min at room temperature, during

which time cells were washed with PBS and 1ml of DMEM with FBS and P/S was added to each well. Each reaction was then added to the appropriate well and allowed to incubate for 48 hours. Selection of stably transfected cells was carried out by treatment of transfected and non-transfected cells using 50ug/ml G418 until non-transfected cells died (approximately 9 days).

Microarray Analysis

Genome-wide DNA microarray study was conducted as previously described (Vivar et al., 2010). RNA was quantitated and evaluated by capillary electrophoresis in the Experion system (Bio-Rad). cRNA samples were biotinylated and hybridized to a WG-6 BeadChip array (Illumina) consisting of 45,000 transcripts of the mouse genome by the UCSF genomic core facility. The differential expression analysis was performed using the Linear Models for Microarray Data package (Limma, available at www.bioconductor.org). Microarray dataset is available in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE64060.

Stereology and Electron Microscopy

Brown adipose tissue from WT and *Cd36*$^{-/-}$ mice were fixed overnight in 0.15M cacodylate buffer, 2% paraformaldehyde, and 2% glutaraldehyde. Tissues were then postfixed in 2% osmium tetroxide in 0.1M cacodylate buffer, dehydrated in ethanol series, embedded in propylene oxide and EPON. Thin sections were stained with 5% uranyl acetate and observed under a transmission electron microscope. Stereological

analysis was performed as previously described (Weibel et al., 1966) using the software program Image J (NIH) to place the grids.

Superoxide/H_2O_2 production

Rates of superoxide/H_2O_2 were measured as the rate of H_2O_2 production, as the two superoxide molecules are dismutated by exogenous and endogenous superoxide dismutases. H_2O_2 was detected using 50μM amplex red in the presence of 5 U/ml horseradish peroxidase and 25 U/ml superoxide dismutase in a Varian Cary Eclipse spectrofluorometer ($\lambda_{excitation}$ = 560nm, $\lambda_{emission}$ = 590nm) with constant stirring. Brown adipose mitochondria from WT and *Cd36*$^{-/-}$ mice was isolated according to standard procedures (Cannon and Nedergaard, 2008). Briefly, tissues were place in ice-cold 0.25M sucrose solution, minced on a pre-chilled cutting board and homogenized in 0.25M sucrose with 0.3% bovine serum albumin fatty acid free (BSA-FFA) (up to ten strokes). Mitochondria were resuspended in KHE [120mM KCl, 5mM HEPES, 1mM EGTA, pH 7.4] buffer and kept on ice until use. Protein was measured by Bradford (Bio-Rad). Mitochondria (0.05 mg protein/ml) were incubated for 5 min at 37°C under non-phosphorylative conditions (1μg/ml oligomycin) in KHE buffer supplemented with 5mM KH_2PO_4, 1mM $MgCl_2$, 0.3% BSA-FFA and 1mM GDP. 20mM glycerol 3-phosphate was added and the rates monitored for 5 min. The rates were calibrated with known amounts of H_2O_2 in the presence of glycerol 3-phosphate (Orr et al., 2012).

Coenzyme Q Measurement by Mass Spectrometry

For targeted lipid and lipophilic metabolite measurements by mass spectrometry, tissues were weighed and dounce homogenized in 6ml of 1:1 v/v hexane:ethyl acetate and 2ml of PBS containing 10nmol CoQ4 as an internal standard. The mixture was vortexed and then centrifuged (2000g for 10 min). The organic layer was removed, evaporated under a stream of nitrogen and resolubilized in 120µl of chloroform. Metabolite separation by liquid chromatography for lipophilic metabolites was achieved using a Gemini reverse-phase C5 column from Phenomonex. An aliquot of the extract (10µl) was injected for analysis with an Agilent G6430 QQQ instrument. For LC separation of lipid metabolites, mobile phase A consisted of 95:5 water:methanol and mobile phase B consisted of 60:35:5 isopropanol:methanol:water. Formic acid (0.1 %) was included to assist in ion formation in positive ionization mode. The flow rate for each run started at 0.1 ml/min with 100% A. At 5 min, the solvent was immediately changed to 60% B with a flow rate of 0.4 ml/min and increased 2 linearly to 100% B over 15 min. This was followed by an isocratic gradient of 100% B for 8 min at 0.5 ml/min before equilibrating for 3 min at 0% B at 0.5 ml/min. Metabolites were detected using single reaction monitoring. Metabolites were quantified by integrating the area under the curve and normalizing to the internal standard and tissue weight.

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