Materials and Methods

Animals

All animal procedures were conducted in compliance with protocols approved by the University of Maryland's Institutional Animal Care and Use Committee. All experimental mice were males on *C57Bl6/J* background (JAX: 000664). *Lyz2/LysM*$^{cre/cre}$ mice (JAX: 004781) were crossed separately with Becnflox/flox (JAX: 028794) and *Cd36*$^{flox/flox}$ (JAX: 032276) mice to generate *Becn1*$^{flox/flox}$*; Lyz2*$^{Cre/Cre}$ and *Cd36*$^{flox/flox}$*; Lyz2*$^{Cre/Cre}$ monocyte and microglia-specific knockout mice. All offspring were genotyped for the presence of both the floxed and Cre alleles following the genotyping guidelines provided by The Jackson Laboratory. All mice were maintained on sterilized bedding in a specific pathogen-free (SPF) facility with a 12-hour light/dark cycle.

Controlled Cortical Impact (CCI)

Traumatic brain injury (TBI) was induced in mice using a standard controlled cortical impact (CCI) device equipped with a microprocessor-regulated pneumatic impactor, as previously described[1,2]. Following anesthesia induction with 3% isoflurane, a 10-mm midline scalp incision was made to expose the skull. The skin and fascia were retracted, and a 4-mm craniotomy was performed over the central region of the left parietal bone. A moderate TBI was delivered using a 2.3-mm diameter impactor tip at a velocity of 4 m/s, deformation depth of 1.5 mm, and dwell time 100 ms. Sham-operated animals underwent the same surgical procedures, excluding the craniotomy and impact.

DESI-MSI

Flash frozen brain tissues from four CCI and three sham mice were sectioned at 20 µm thickness using a cryostat (HM 550; Thermo Scientific, Waltham, MA). Tissues were dried in a desiccator for 15 minutes at room temperature prior to analysis. DESI- MSI was conducted using a HDMS select series Cyclic IMS mass spectrometer (Waters Corporation, Wilmslow, UK) equipped with a DESI XS source and a heated transfer line. The capillary voltage for the DESI XS source was set to 0.75 kV, with a cone voltage of 60 V, a source temperature of 150 °C, and a heated transfer line temperature of 300 °C for more efficient ion transfer. Samples were run at 50 µm spatial resolution, maintaining a scan speed of 1 second per scan during DESI- MSI analysis in positive

ion mode. DESI flow was controlled by an Acquity Binary Solvent Manager (Waters Corporation, Milford, MA), maintaining a constant flow of 2 µL/min throughout the duration of the experiments. MSI data was first processed using High-Definition Imaging (HDI) software v1.6 (Waters Corporation, Milford, MA), where lipids were tentatively annotated based on accurate mass. Annotated data was then exported to SCiLS Lab v2025 (Bruker, https://www.bruker.com/) (Bruker, Billerica, Massachusetts) for co-registration and visualization.

Lipidomics

Lipid Extraction: Total lipid extracts from the lysosomes were prepared using methyl tert butyl ether (MTBE) lipid extraction protocol[3] with slight modifications as described previously[4,5]. Briefly, 400 µL of cold methanol and 10 µL of internal standard (EquiSPLASH) were added to each sample. The sample was incubated at 4°C, 650 rpm shaking for 15 min. Next, 500 µL of cold MTBE was added followed by incubation at 4°C for 1 h with 650 rpm shaking. Cold water (500 µL) was added slowly, and the resulting extract was maintained 4°C, 650 rpm shaking for 15 min. Phase separation was completed by centrifugation at 8,000 g for 8 min at 4 °C. The upper, organic phase was removed and set aside on ice. The bottom, aqueous phase was re-extracted with 200 µL of MTBE followed by 15 min incubation at 4 °C with 650 rpm shaking. Phase separation was completed by centrifugation at 8,000 g for 8 min at 4 °C. The upper, organic phase was removed and combined with a previous organic extract. The latter was dried under a steady stream of nitrogen at 30 °C. The recovered lipids were reconstituted in 100 µL of acetonitrile: isopropanol: water (1:2:1, v/v/v).

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS): Total lipid extracts were analyzed by liquid chromatography coupled to targeted tandem mass spectrometry (LC-MS/MS). The LC-MS/MS analyses were performed on an Ultimate 3000 Ultra High-Performance Liquid Chromatograph coupled to a Thermo TSQ Altis Tandem Quadrupole Mass Spectrometer (Thermo Scientific, San Jose, CA). LC-MS/MS methodology was adapted from the literature[6] and previous publications[5,7]. The separation was achieved using an ACQUITY Amide BEH column (1.7 µm; 2.1 x 100 mm) column (Waters, Milford, MA) maintained at 45 °C. Mobile phase compositions for solvents A and B consisted of ACN/H2O (95:5, v/v) and (50:50, v/v) respectively, with 10 mM ammonium acetate The gradient profile had a flow rate of 0.6 mL

min^{-1} and ramped from 0.1 to 20% B in 2 min, from 20 to 80% B in 3 min, dropped from 80 to 0.1% B in 0.1 min, and held 0.1% B for 2.9 min. Total chromatographic run time was 8.0 min. The injection volume was 2 µL. The auto-sampler was maintained at 7 °C. Electrospray ionization was achieved using either negative or positive mode. Mass spectrometry detection was done using selective reaction monitoring where predetermined precursor to product ion transitions were used. ESI source parameters were set as follows: voltage 3500 V in positive mode and −2500 V in negative mode, sheath gas (Arb) = 60, aux gas (Arb) = 15, sweep gas (Arb) = 1 and ion transfer tube temperature of 380 °C. Nitrogen was used as the nebulizer and argon as collision gas (1.5 mTor). The vaporizer temperature was set to 350 °C. LC-MS/MS data was acquired using Thermo's Xcalibur software and data processing was achieved using Xcalibur 4.2 and TraceFinder 5.1. Additional data analysis was done using Prism 6 (GraphPad, La Jolla, CA) and MetaboAnalyst[8]. Lipidomics data has been uploaded to Mendeley data repository. Additional data is available upon request.

BMDM isolation and culture and myelin phagocytosis

BMDMs were harvested from euthanized 12 – 14 weeks old female mice femurs and tibias under sterile conditions. Bones cleaned of muscle tissue and marrow were flushed using ice-cold PBS through a 23G needle into a collection tube. Cells were suspended by pipetting with a 1000 ul pipette, filtered through a 70 µm cell strainer to remove bone fragments and centrifuged at 400 × g for 5 minutes. Supernatant was discarded and pellet resuspended in RBC lysis buffer (Invitrogen, Cat# 00-4333-57) and left on ice for 10 minutes. Cells were centrifuged again (same speed and time) and the final white cells pellets resuspended and cultured in T75 flasks with BMDM media (RPMI containing 1% penicillin-streptomycin, 10% FBS, and 10% L929 conditioned media to provide M-CSF). Media was changed every 2-3 days. On day 7 cells were trypsinized and re-seeded on glass coverslips in 24 well plates at 2 ×105 overnight, then treated with 100ul/ml purified mouse myelin[9,10] 4 to 24 hours, as indicated. Cells were either fixated with 4% PFA for immunostaining or lysed with RIPA buffer for western blot[11,12].

Flow cytometry

Mice were anesthetized using isoflurane and perfused transcardially with cold saline to remove circulating blood. Cortical brain tissue was collected and mechanically dissociated using a 70 µm

cell strainer. The resulting suspension was resuspended in RPMI-1640 medium. To facilitate enzymatic digestion, the suspension was treated with 10 U of papain, 10 mg/ml of DNase I, 1 mg/ml of collagenase-dispase (Roche, Cat# 10269638001), and 1 µl GolgiPlug containing brefeldin A, then incubated for 1 hour at 37°C on a shaker set to 200 rpm. Following digestion, leukocytes were isolated from brain tissue via a Percoll (GE Healthcare, Cat# GE17-5445-02) density gradient. Cells were first resuspended in 70% Percoll (GE Healthcare, Cat# GE17-5445-02)-HBSS (Thermo Fisher/Gibco, Cat# 14175095) and gently layered underneath a 30% Percoll (GE Healthcare, Cat# GE17-5445-02)-RPMI solution using a blunt-ended pipette tip (Sigma, Cat# CAD7942). This gradient was centrifuged at 400 × g for 20 minutes without braking. The myelin layer was carefully removed from the top of the 30% interface, and immune cells were collected from the 30%/70% interphase and washed in RPMI medium to create single-cell suspensions. For flow cytometry, these brain leukocytes were washed in FACS buffer (1× HBSS (Thermo Fisher/Gibco, Cat# 14175095) containing 5% FBS, 0.1% penicillin-streptomycin, and sodium azide) and incubated with Fc Block diluted 1:50 for 10 minutes on ice to prevent nonspecific binding. Cells were then stained with fluorophore-conjugated antibodies against surface markers. After staining, they were fixed and permeabilized using BD Fixation/Permeabilization solution (BD Biosciences, Cat# 554722) for 10 minutes, washed twice with BD Permeabilization/Wash Buffer (BD Biosciences, Cat# 554723), and incubated with intracellular antibody cocktails. Following a 30-minute incubation at 4°C, cells were washed again and resuspended in PBS for flow cytometry. Surface markers included CD45-eF450, CD11b-APCeF780 each used at 1:100 dilution. For viability staining, Zombie Aqua™ was prepared in DMSO and used at 1:50 dilution. Intracellular markers included TNF-PE-Cy7, 1:50, I-1β-PerCP-eF710, 1:100, LC3B-FITC 1:100, and SQSTM1/p62-AF647, 1:50.[13] Flow cytometric data were acquired using a 5-laser Cytek Aurora cytometer with SpectroFlo 3.30 software. Unmixing was done using single color controls (SCC). SCCs for dyes (BODIPY, FluoroMyelin, and Zombie) were made by cells and SCCs for antibodies made by compensation beads. Analysis was done with FCS Express™ 7.

CD11B+ cell isolation for single cell RNA sequencing and FACS

Cells were isolated using Adult Brain Dissociation Kit (Miltenyi Biotec, Cat# 130-107-677) (ABDK) following manufacturer's instructions, with modifications.[14,15] All procedures were

performed on ice/4 °C, as indicated; all plasticware was pre-coated with coating buffer (3% BSA in DPBS-CMF) to prevent cell adhesion. Anesthetized mice were transcardially perfused with 20 mL cold perfusion solution (30 mL DPBS-CMF with 2 U/mL heparin). Brains were dissected on ice and regions of interest micro-dissected into petri dishes containing 1 mL cold Enzyme Mix 1. 30 µL of Enzyme Mix 2 was added to each C Tube containing tissue, attach tubes to gentle MACS Octo Dissociator, and run the customized protocol.[15] After dissociation, cells were filtered through 70 µm mesh into 50 mL tubes pre-wet with 500 µL cold DPBS-CMF. C Tubes were rised with 9.5 mL DPBS-CMF and add to the filters then centrifuged at 300 × g for 8 min at 4 °C. Supernatant aspirated carefully. For debris removal, the pellet was gently resuspended in 1550 µL DPBS-CMF, mixed with 450 µL cold Debris Removal Solution, and overlaid with 2 × 1 mL DPBS-CMF. The mix was centrifuged at 3000 × g, 10 min, 4 °C then top two layers were discarded. The remnant was diluted to 5 mL with cold DPBS-CMF, inverted gently 5 times and centrifuged at 1000 × g for 10 min. Supernatant was aspirated and the pellet was resuspended in 90 µL MACS buffer [95 ml DPBS-CMF with 5 ml FBS (5% final) and 200 µL EDTA 0.5M (final 1mM)]. 10 µL CD11b microbeads was added and mixed by pipetting gently and left to incubate on ice for 15 min. Then 1 mL MACS buffer added and mixed by pipetting and centrifuged at 300 × g for 8 min at 4 °C. Meanwhile LS columns set on the magnetic stand and washed suing 2 ml MACS buffer. Also, 30 µm filters were pre-wet by using 1 ml MACS buffer. Once centrifuge was done, supernatant was aspirated, and the pellet was resuspended with 500 MACS buffer and loaded to LS the column and rinsed with 2 mL MACS buffer (washing column with 3 mL MACS buffer is recommended). CD11b+ cells were eluted by removing columns from the magnet and flushing with 2 × 5 mL MACS buffer. Eluates spinned at 300 × g for 8 min, aspirated carefully (pellet may be invisible). For downstream applications like scRNA-seq or FACS, pellet resuspended in 400 µL cold 1× PBS and original tube was rinsed with 800 µL PBS then combined and spinned again and finally resuspended in MACS buffer (for scRNAseq) or FACS buffer (for FACS with that was done using BD Aria sorter).

Single cell RNA sequencing and Seurat Analysis

Single cells in MASC buffer were subjected to encapsulation and library preparation that was carried out using the 10x Genomics Chromium v2 platform, following the manufacturer's protocols. Each sample was processed as an independent batch, with a capture target of at

least10,000 cells. Both cDNA and library amplifications were performed with 12 PCR cycles. Sequencing was conducted on the Illumina NovaSeq 6000 S2 flow cell (RRID:SCR_016387), according to the manufacturer's recommendations. An initial low-depth sequencing run (~1,000 reads per cell) was performed on an Illumina NextSeq instrument to validate input concentration and sample quality. Final sequencing was performed at approximately 100,000 reads per cell using the Illumina NovaSeq platform. Post-sequencing, library complexity and saturation were assessed using the Preseq package. Samples with saturation levels below 60% were sequenced further to ensure sufficient coverage, guided by Preseq projections. All samples underwent alignment and preprocessing using the Cell Ranger pipeline (version: cellranger-8.0.1, Transcriptome: GRCm39-2024-A). Analysis was done using Seurat (R package, v5.x). Quality control filtering was applied to retain cells that met the following criteria: detection of 200 to 10,000 genes per cell, total unique molecular identifier (UMI) counts between 1,000 and 15,000, and no more than 20% of reads aligning to mitochondrial genes resulting an average of 15,371 cells from sham samples and 12,493 cell from CCI samples. The majority of sn/scRNAseq analyses are comparisons between relatively stable cell populations, such as cells from organisms of different genotypes, ages or established disease states. Consistent with these dynamic changes, we detected significantly higher numbers of different mRNA transcripts in cells from injured as compared to sham mice (average read depth of 4,662/cell for sham, 10,289/cell for CCI; Supp. Figure S2A). To account for this, we performed stringent quality control assessments including number of RNA features, mitochondrial RNA content, normality of distribution[16]. The higher number of RNA features in TBI was justified by cell cycle analysis (Supp. Figure S2B, Figure 2F) indicating proliferation of monocytes after TBI. Individual samples as Seurat objects were integrated using IntegrateLayers() Seurat function. Differentially expressed (DE) genes were defined by FindAllMarkers() Seurat function that uses Wilcoxon rand sum test. Identity assignment to Seurat clusters done manually using canonical microglia and macrophages markers. These included canonical homeostatic markers (e.g. Tmem119)[17] which were used to identify homeostatic microglia clusters (Hom_MG_1-5). Additional clusters expressing high levels of homeostatic markers like Tmem119 but also positive for some activation markers (e.g. Lpl and Cybb) were designated as surveillance microglia (Surv_MG_1-5). Clusters with high expression of disease associated microglia (DAM) markers (e.g. Lyz2)[18] along with high microglial markers were identified as DAM_1-5. Clusters with high DAM

markers and lower expression of microglial markers were identified as brain associated macrophages (BAM_1-7). BAM identity was further confirmed based on expression of infiltration (e.g. Ccr2) or macrophage differentiation (e.g. Pf4) markers[19]. Additional minor clusters included immune cells such as dendritic cells, neutrophils, NK and T cells, as well as astrocytes and mature and immature oligodendrocytes (Supp. Figure 2C).

Western blot analysis

Cell lysates were obtained by directly lysing BMDMs cultured in 24-well plates using RIPA buffer. Proteins from cellular lysates were separated on 4–20% gradient SDS-PAGE gels, followed by transfer onto PVDF membranes using a semi-dry blotting apparatus (Bio-Rad). The membranes were first blocked with 5% non-fat milk in TBST buffer (Tris-buffered saline containing 0.05% Tween 20), then incubated overnight at 4°C with primary antibodies diluted in 1% BSA in TBST (β-Actin/ACTB,1:10,000; SQSTM1/p62,1:1000; LC3,1:1000). The next day, membranes were treated with HRP-conjugated secondary antibodies for 1 hour at room temperature in blocking solution. Protein signals were detected using either SuperSignal West Dura or SuperSignal West Pico chemiluminescent substrates and visualized with the Chemi-Doc imaging system (Bio-Rad, Universal Hood II). Band intensities were quantified using Image Lab software (Bio-Rad) and normalized to loading controls.

Immunofluorescence staining

Mice were anesthetized using isoflurane and perfused transcardially with cold saline, followed by 4% paraformaldehyde (PFA, pH 7.4). Dissected brains were post-fixed in 4% PFA and cryoprotected in 30% sucrose before being sectioned into 20-µm-thick frozen slices, following previously established procedures[1]. For each immunofluorescence experiment, four coronal sections spaced 1–1.2 mm apart across the lesion site were collected per animal. Tissue sections were blocked with 5% goat serum in 1× phosphate-buffered saline (PBS) containing 0.025% Triton X100. For lysosomal labeling, sections were first treated with 0.1 M glycine in PBS for 30 minutes, followed by permeabilization with 0.2% saponin in PBS for another 30 minutes. They were then blocked in PBS containing 0.04% saponin, 5% goat or donkey serum, and 0.05% BSA. Sections were incubated overnight at 4°C with primary antibodies, followed by a 2-hour incubation at room temperature with appropriate secondary antibodies in blocking solution.

DAPI was used to counterstain nuclei. BODIPY staining was done as a separate step right after permeabilization. BODIPY stock was made by reconstitution of the BODIPY™ 493/503 (Thermo Fisher, Cat# D3922) in DMSO (1 mg/ml). Stock was diluted 1:5000 in PBS and used applied on the sections for 1 hour in dark. Then washed three times with PBS and blocked.

Epifluorescence image acquisition and quantification

Fluorescence images were captured using a Nikon Eclipse Ti-E/Ni-E microscope and analyzed with Nikon Elements software (version 4.12.01). Nikon 20X/0.75 Plan-Apochromat Lambda DIC objective lens (cellular neutral lipid and lipid droplet accumulation) or 60X /1.40 Plan-Apochromat Lambda DIC objective lens (BODIPY engulfment inside lysosome). Emission wavelengths used for detection included 460 nm for DAPI, 535 nm for BODIPY and Alexa Fluor 488, 620 nm for Alexa Fluor 546 and Alexa Fluor 568, 670 nm for Alexa Fluor 633, and 756 nm for Alexa Fluor 750. Z-stacks were captured at 1 m intervals and processed with the NIS-Elements Extended Depth of Focus (EDF) algorithm to reconstruct a single in-focus composite image. Consistent exposure times were maintained across all tissue sections within each experimental group. Quantification was carried out using Elements software. Nuclei were identified using the Spot Detection algorithm, while immunofluorescent marker-positive cells were detected using the Detect Regional Maxima algorithm in combination with global thresholding, as previously described2. The number of marker-positive cells was normalized to the total number of nuclei per image.

Confocal image acquisition and 3D rendering

Confocal fluorescence images were acquired with the Nikon CSU-W1 microscope equipped with 405, 488, 561, and 647 lasers, using a 60x (1.49 NA) TIRF oil-immersion objective and Nikon Elements software. For three-dimensional rendering, confocal z stacks were taken with a 60x objective equipped with a 1.5x magnifying lens, to achieve a magnification of 102x and a z-step size of 0.2um. Images were denoised and deconvolved using automatic deconvolution algorithms in Nikon Elements and were reconstructed in three dimensions in Imaris Bitplane software Individual microglial surface of fluorescence images were reconstructed using the ‘surface’ feature and default parameters to create a volumetric boundary of the cell. Following all the lysosomal signal outside of the reconstructed surfaces were masked to ‘0’, retaining only

lysosomal signal inside the reconstructed surfaces. After masking, both lysosomal surfaces and BODIPY-positive signals were reconstructed using the 'surface' feature and 'machine learning' algorithm parameters. The two surfaces (BODIPY-positive and lysosomes) were merged to create the final rendering. Representative images were deconvolved using TRUESHARP online deconvolution (Abberior). All analysis were performed on raw images.

Lysosomal enzyme assay

The enzymatic activities of lysosomal proteins cathepsin D (CTSD) and alpha-N-acetylglucosaminidase (NAGLU) were measured using respective fluorometric assay kits following the manufacturers' protocols. Enzyme activity levels in lysosomal fractions and total cell or tissue lysates were quantified based on changes in absorbance or fluorescence per microgram of protein. To assess cytosolic enzyme activity, values were calculated as a proportion of the total enzyme activity in the whole cell or tissue lysate, normalized per microgram of total protein.

Preparation of lysosome enriched fraction

Lysosomal fractions were isolated from cortical tissue of both sham and injured mice using previously established protocols1 using the Lysosomal Enrichment Kit for Tissue and Cultured Cells (Thermo Fisher, Cat# 89839) following the manufacturer's recommended procedure.

Lysosomal proteomics

We collected lysosomal samples and stored them at -80 ºC until assay. Cell lysis and protein digestion were performed similar as previously described[20,21]. samples were lysed in a lysis buffer containing 5% sodium dodecyl sulfate (Sigma, Cat# L4509), 50 mM triethylammonium bicarbonate (1 M, pH 8.0). Proteins were extracted and digested using S-trap micro columns (ProtiFi, NY). The eluted peptides from S-trap column were dried, and peptide concentration was determined using Pierce Quantitative Colorimetric Peptide Assay kit (Thermo Fisher, Cat# 23275), after reconstituted in 0.1% formic acid. LC-MS/MS-based proteomic analysis was conducted on a nanoACQUITY Ultra-Performance Liquid Chromatography system (Waters Corporation, Milford, MA USA) coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific, San Jose, CA USA) similar to our previous work[20,22,23]. Peptide

separation was effected on a nanoACQUITY Ultra-Performance Liquid Chromatography (UPLC) analytical column (BEH130 C18, 1.7 µm, 75 µm x 200 mm; Waters Corporation, Milford, MA, USA) using a 185-min linear gradient with 3-40% acetonitrile and 0.1% formic acid. Mass spectrometry conditions were as follows: Full MS scan resolution of 240,000, precursor ions fragmentation by high-energy collisional dissociation of 35%, and a maximum cycle time of 3 seconds. The Pierce HeLa Protein Digest Standard was injected between runs as an instrument quality control to monitor system performance. The resulting mass spectra were processed using Thermo Proteome Discoverer (PD, version 2.5.0.400, Thermo Fisher Scientific) and searched against a UniProt mouse (Mus musculus) reference proteome (release 2022.06, 17180 entries) using Sequest HT algorithm. Search parameters include carbamidomethylation of cysteines (+57.021 Da) as a static modification, methionine oxidation (+15.995 Da) as a dynamic modification, precursor mass tolerance of 20 ppm, fragment mass tolerance of 0.5 Da, and trypsin as a digestion enzyme. Tryptic missed cleavages were restricted to a maximum of two, with peptide lengths set between 6 and 144 residues. For protein quantification, the Minora feature detector, integrated in the PD, was used as described previously[24]. To ensure high data quality, proteins were further filtered to a 1% false discovery rate (FDR) threshold, calculated with the Percolator algorithm. Next, protein abundance values exported from PD were post-processed using Perseus software. Proteins with missing values were excluded to improve data quality. The quantitative protein data were log2 transformed and further normalized using median centering. Differentially expressed proteins (DEPs) were identified using a two-tailed Student's t-test (adjusted p-value < 0.05). Ingenuity Pathway Analysis (IPA) software (Qiagen, Germantown, MD) was used to identify dysregulated pathways and biological processes. Metabolanalyst (version 5.0) was used to generate PCA plots and heatmaps. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the project accession number PXD067157.

Quantitative real time PCR

Total RNA was extracted from cortex using TRIzol reagent and RNeasy Mini Kit (QIAGEN) following the manufacturer's protocol. The RNA was reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Synthesis Kit according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was carried out using the TaqMan

Universal Master Mix II. For each reaction, 2× Master Mix, 1 µl of cDNA (equivalent to 50 ng of input RNA), and the appropriate TaqMan Gene Expression Assay were combined in a final volume of 20 µl. All reactions were performed in duplicate. TaqMan Gene Expression Assays (primers) targeting mouse transcripts were utilized (detailed informations of each listed in the resources table). PCR amplification and data collection were conducted on a 7900HT Fast Real-Time PCR System (Applied Biosystems) using the manufacturer's software. The thermal cycling conditions included an initial hold at 50 °C for 2 minutes and 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds and annealing/extension at 60 °C for 1 minute. Gene expression levels were normalized to Gapdh (Applied Biosystems, Assay ID: Mm99999915_g1), and relative expression was calculated accordingly.

List of qPCR primers used (all from Applied Biosystems):

- Gapdh, Assay ID: Mm99999915_g1
- Npc1, Assay ID: Mm00435300_m1
- Npc2, Assay ID: Mm00499230_m1
- Scd1, Assay ID: Mm00772290_m1
- Abca1, Assay ID: Mm00442646_m1
- Elovl5, Assay ID: Mm00506717_m1
- Srebf1, Assay ID: Mm00550338_m1
- Srebf2, Assay ID: Mm01306292_m1
- Cebpa, Assay ID: Mm00514283_s1
- Tfeb, Assay ID: Mm00448968_m1
- Nr1h3, Assay ID: Mm00443451_m1
- Grn, Assay ID: Mm00433848_m1
- Lipa, Assay ID: Mm00498820_m1

- MafB, Assay ID: Mm00627481_s1
- Cd36, Assay ID: Mm00432403_m1
- Fasn, Assay ID: Mm00662319_m1
- Hmgcr, Assay ID: Mm01282499_m1
- Acat1, Assay ID: Mm00507463_m1
- Anxa5, Assay ID: Mm01293059_m1
- Nceh1, Assay ID: Mm00626772_m1
- Pparg, Assay ID: Mm01184322_m1
- Plin2, Assay ID: Mm00475794_m1
- Nlrp3, Assay ID: Mm00840904_m1
- Nfkb1, Assay ID: Mm00476361_m1
- Apoe, Assay ID: Mm01307193_g1

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