Methods

Generation of MCJ-KO mice

MCJ-deficient mice have been described elsewhere[1].

The NIAAA model

The mouse model of chronic and binge ethanol feeding, also known as the NIAAA model, synergistically induces liver injury, inflammation and fatty liver, which mimics acute-on-chronic alcoholic liver injury in patients[2]. Wild-type (Wt) (n=14) and MCJ-KO (n=12) male mice aged 10-12 weeks with c57BL/6 background were initially fed the control Lieber-DeCarli diet (F1259, BIO-SERV) *ad libitum* for 5 days to acclimatize them to liquid diet and tube feeding. Afterwards, ethanol-ed groups were allowed free access to the ethanol Lieber-DeCarli diet (F1258, BIO-SERV) containing 5% (vol/vol) ethanol for 10 days, and control groups were pair-fed with the isocaloric control diet. At day 11, ethanol-fed and pair-fed mice were gavaged in the early morning with a single dose of ethanol (5g/kg body weight) or isocaloric maltose dextrin, respectively, and euthanized 9 h later (Supplemental Fig. 1). We set different experimental groups:

Group 1: 3-month-old male Wt (control fed n=5, ethanol fed=14) and MCJ-KO (control fed n=6, ethanol fed=12) mice were fed with the NIAAA model. At day 11, 5 h after the gavage, mice were orally administered with 100ul 0.6mg/gr body weight of FITC-Dextran and sacrificed 4 h after. Serum samples were directly assayed for the FITC-dextran measurement.

Group 2: 3-month-old male Wt (control fed n=5, ethanol fed n=5) and MCJ-KO (control fed n=5, ethanol fed n=8) mice were fed with the NIAAA model. At day 11, after an overnight fasting period and following the gavage, mice were subjected to an IPGTT. 2 h after, once the glucose tolerance test was finished, mice were sacrificed.

Group 3: 3-month-old male Wt (ethanol fed n=5) and MCJ-KO (ethanol fed n=5) mice were fed with the NIAAA model. At day 6 of the ethanol-diet, after an overnight fasting period, mice were subjected to an IPGTT, right after the oral gavage. At day 11, 9 h after the oral gavage, mice were sacrificed.

Group 4: 3-month-old male C57BL/6J wild-type mice followed the NIAAA model. After the initial acclimatization period, at day 5 of the ethanol Lieber-DeCarli diet, animals were subjected to an *in vivo* silencing RNA targeting *Mcj* (position 294-312) (n=7) or an unrelated siRNA control (n=7), receiving either 1.7 mg/Kg of specific *in vivo* siRNA (Custom Ambion, USA) or control siRNA (Sigma-Aldrich, USA) complexed with Invivofectamine ® 3.0 Reagent (Invitrogen, USA) following the manufacturer's instructions. At day 11, after an overnight fasting period and following the gavage, mice were subjected to an IPGTT. 2 h after, once the glucose tolerance test was finished, mice were sacrificed.

After the sacrifice, in all experimental groups, tissue samples were immediately frozen in liquid nitrogen for RNA and protein extraction, cryopreserved in optimal cutting temperature compound for Red Sudan staining, or fixed in 4% paraformaldehyde for immunohistochemical analysis.

The DUAL model

Tissue samples of mice that were fed with the DUAL model for 10, 23 and 52 weeks were kindly provided by Dr. FJ Cubero and Dr. Y Nevzorova[3].

Bisulfite pyrosequencing

DNA methylation patterns were analyzed by bisulfite pyrosequencing. Bisulfite modification of DNA was performed with the EZ DNA methylation-gold kit (Zymo Research) following the manufacturer's instructions. Primer sets for PCR amplification and sequencing were designed using the specific PyroMark assay design software

(version 2.0.01.15) (Supplemental Table 1). PCR amplification, pyrosequencing and quantification of methylation percentage were performed using PyroMark Q24 reagents, equipment, and software (Qiagen). The results of primer-1 CpG-2 (-333 bp from TSS), and primer-2 CpG-1 (+354 bp from TSS) are shown in the manuscript.

Histology

Formalin-fixed paraffin-embedded sections (5 µm thick) were deparaffinized with Histo-Clear I solution (Electron Microscopy Sciences, USA), hydrated with ethanol solutions and unmasked according to the primary antibody to be used and subjected to peroxide blocking (3% H_2O_2 in PBS, 10 min, RT). For staining with mouse-hosted antibodies in mouse tissues, samples were blocked with goat anti-mouse Fab fragment (Jackson Immunoresearch, USA) (1:10, 1h, RT) and then blocked with 2,5% normal goat serum (Vector, USA) (30 min, RT). Sections were incubated in a humid chamber with the primary antibody in antibody diluent with 0.02g/ml BSA followed by ImmPRESS goat anti-rat, anti-rabbit or anti-mouse (Vector, USA) HRP-conjugated secondary antibody incubation (30 min, RT). Colorimetric detection was confirmed with Vector VIP chromogen (Vector, USA) and sections were counterstained with Mayer´s hematoxylin. Samples were dehydrated in increasing concentrations of ethanol solutions until 100%, cleared in Histo-Clear I solution and mounted using DPX mounting medium. Images were taken with an upright light microscope, Axioimager D1 (Zeiss, Germany). Antibodies and conditions are described in Supplemental Table 2.

For the Red Sudan staining, O.C.T-included frozen liver samples were cut into 10 µm sections. Sections were washed in 60% isopropanol and then stained with fresh Sudan III (0.5% in isopropanol; Sigma Aldrich) solution for 30 min. Samples were then washed again in 60% isopropanol and then counterstained with Mayer's hematoxylin. The sections were then washed with distilled water and mounted in DPX mounting medium. Images were taken with an upright light microscope, Axioimager D1 (Zeiss, Germany).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed using the HRP-DAB Assay Kit (ab206386), as indicated by the manufacturer. Briefly, tissue sections embedded in paraffin were rehydrated with different alcohol concentrations until they finally reached TBS 1X. The samples were permeabilised with proteinase K and then the endogenous peroxidases were quenched with 3% H_2O_2 in methanol. Third, equilibration buffer was used before the labelling reaction with TdT enzyme plus TdT labelling reaction mix. After the reaction was stopped, the samples were blocked and the conjugate reactivated to finally develop the samples with the DAB solution. The tissue sections were counterstained with Methyl Green Counterstain solution. Then, the samples were fixed with a glass coverslip. Images were taken with an upright light microscope, Axioimager D1 (Zeiss, Germany).

Histopathological study

Samples of livers and colon were sectioned 4µm thick and stained with hematoxylin-eosin. Histopathological lesions were classified according to current histological scores[4,5]. All slides were analyzed by a pathologist in a blind manner regarding the genotype of the mice.

Determination of ROS in liver sections

O.C.T- embedded 8 µm sections were incubated with MnTBAP 150 µM 1h at RT. The samples were then incubated with dihydroethidium (DHE) 5 µM for 30 min at 37 ºC and sections were mounted with Fluoromount-G (Southern Biotech, USA) containing 0.7 mg/l of DAPI to counterstain nuclei. Images were taken using an Axioimager D1 (Zeiss, Germany).

Liver transaminases activity, total bilirubin, prothrombin, and albumin levels determination in mouse serum

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum activity, together with total bilirubin, prothrombin and albumin concentration was determined by using the Selectra Junior Spinlab 100 automated analyzer (Vital Scientific, The Netherlands) according to the manufacturer's instructions. Standard controls were run before each determination.

Liver Triglyceride (TG) content measurement

30 mg of liver tissue were homogenized with PBS, and mixed gently with chloroform-methanol (2:1, V:V). Then, samples were centrifuged at 4200*g* at 4ºC for 10 min. The organic phase was evaporated. The pellet was then re-suspended with 1% Triton X-100 in ethanol and re-evaporated. Finally, the samples were re-suspended in 500 µl of PBS and aliquoted to analyze triglycerides using an automatized Selectra Junior Spinlab 100 analyzer (Vital Scientific, The Netherlands).

Fatty acid oxidation (FAO) rate determination in fresh tissue

Fatty Acid Oxidation (FAO) was measured by using a commercial Kit (Biomedical Research, USA). Briefly, 20 mg of liver homogenates in 750 µl of cell lysis solution 1x were used. Liver samples were centrifuged at 10000 rpm for 5 min. Protein determination was determined in supernatant fractions using a Bradford Assay. Protein samples were normalized and 15ug were loaded per well with the respective loading control and samples. Samples were incubated for 2 h at 37 ºC with 50 µl FAO assay solution. Reaction was stopped by adding 50ul of 3% Acetic Acid and colorimetric determination was finally determined at 492nm using a spectrophotometer.

Liver Succinate Dehydrogenase (SDH_2) activity quantification

The Succinate Dehydrogenase Activity Colorimetric Assay Kit (MAK197, Sigma-Aldrich) was used to measure the hepatic Succinate Dehydrogenase activity. 5mg of frozen tissue were manually homogenized in 50ul ice-cold SDH Assay Buffer (Sigma-Aldrich). Measurements (absorbance 600nm) were done every five minutes for 40 minutes.

Respiration studies in liver mitochondria

The respiration of liver mitochondria was measured at 37°C by high-resolution respirometry with the Seahorse Bioscience XF24 Extracellular Flux Analyzer. Liver mitochondria were isolated as recommended by Agilent Seahorse Application note. Glutamate (10mM) and Malate (10mM) were used as substrates to quantify State 1. Succinate (10 mM) and rotenone (2 µM) were used as substrates to quantify State 2. State 3 was initiated with ADP, State 4 induced with the addition of oligomycin (State 4o), and FCCP-induced maximal uncoupler-stimulated respiration (State 3u) were sequentially measured. The normalized data were expressed as pmol of O2 per minute or milli-pH units (mpH) per minute.

Measurement of hepatic Ethanol and Acetaldehyde content

250mg of liver tissue was used to measure either the hepatic Ethanol or Acetaldehyde content. Homogenized biological tissue was mixed with 500 µL of 1 M perchloric acid and mixed for 2 min and adjusted the pH to 7.0 - 8.0 using approximately 500 µL of 1 M KOH. Samples were stored on ice for 20 min to precipitate potassium perchlorate and allow separation of the fat (if present). Volumes of the samples were centrifuged at 13000*g*, 4ºC for 10 min and the clarified supernatant recovered for use in the assay.10 µL of standards, blanks, and samples were mixed with 200 µl of distilled water, 20 µl of buffer and 20 µl of NAD+, the absorbances of the initial solutions were read after a 2 min reaction. After that, an aliquot of 2 µl of either ADH or Al-DH (for hepatic ethanol and acetaldehyde, respectively) was added; the absorbance was registered after approx. 5 min. All measurements were analyzed on a costar 96-well clear flat-bottomed plate

(Corning, USA) in a Citation 3 (BioTek Instruments Inc., USA) with UV-Vis absorbance monochromators at 340 nm. The amount of NADH formed in this reaction is stoichiometric with twice the amount of ethanol, and stoichiometric with the amount of acetaldehyde.

Lipopolysaccharides (LPS) quantification in serum

Serum LPS levels were determined by ELISA using the Mouse Lipopolysaccharides (LPS) ELISA Kit (CSB-E13066m, Cusabio, USA) according to the manufacturer´s recommendations.

Lipopolysaccharides (LPS) content measurement in liver tissue

Mouse Lipopolysaccharides (LPS) ELISA Kit (CSB-E13066m, Cusabio, USA) was used to measure hepatic LPS content in 5mg of frozen livers manually homogenized in 200ul PBS 1x with protease inhibitors, stored overnight at -20ºC and centrifuged at 5,000*g* for 10 min at 4ºC. Supernatants were diluted 1:500 and 50ul were assayed. Optical density was determined using a SpectraMax M2 microplate reader (Molecular Devices, USA) set to 450nm (correction was set to 540nm and the reading was subtracted).

Isolation of mouse primary hepatocytes

Primary hepatocytes were obtained by *in situ* perfusion of the liver with collagenase Type I (Worthington, USA) digestion. Cell density was estimated in a Neubauer counting chamber and the desired density of mouse primary hepatocytes was seeded on collagen type I (Corning)-coated culture plates using the designated cell culture media and paced in an incubator at 37ªC, 5% CO2-95%air.

Primary mouse Kupffer cell (KC) isolation and culture

Following isolation of mouse hepatocytes by perfusion with Colagenase Type I, supernatants from the hepatocyte wash were joined together and centrifuged (1350g,

10', 4°C). The pellet was resuspended in 10mL preservation buffer and then loaded onto a 25/50% percoll PLUS (GE Healthcare, UK) gradient and again centrifuged (1350g, 30', 4°C) with minimum acceleration/deceleration. The non-parenchymal cells were collected with a pipette from the interface between the two density cushions of 25% and 50%. Collected cells were centrifuged again (1350g, 10', 4°C) and the resulting pellet was resuspended in DMEM (Gibco). Kupffer cells were removed from the media by selective adherence, by incubating the resuspended cells on uncoated plastic culture plates for 8 min at 37°C. Primary Kupffer cells were incubated in 10% DMEM medium supplemented with 1% PSA and 1% Glutamine at 37°C in a humidified atmosphere of 5% CO2-95% air.

Isolation of liver infiltrating immune cells for flow cytometry

80mg of fresh liver tissue were digested with Type I collagenase (LS004196, Worthington Biochemical Corp., USA) (2mg/ml in HBSS), at 37ºC, 200rpm for 40 min. Samples were filtered through a 70uM strainer (22363548, Fisher Scientific, Spain) and washed with PBS-2%FBS at 500*g* for 5 min. Then, a 33,75% Percoll™ PLUS (17-5445-01, GE Healthcare, USA)) gradient at 693*g*, for 12 min 20ºC was used to separate the hepatocytes and stellate cells from the immune cells, that led in the bottom. The pellet was resuspended in 1mL red blood cell lysis solution (A10492-01, Gibco, USA) and incubated for 4 min to eliminate erythrocytes. The reaction was stopped using PBS (14190-094, Gibco, USA). Another washing step was performed, and cells were resuspended in 200ul PBS, ready for staining.

Isolation of pancreas infiltrating immune cells for flow cytometry

Pancreas were digested with Type V collagenase (17104-019, Gibco, USA) as previously described[6]. Samples were then filtered through a 70uM strainer (22363548, Fisher Scientific, Spain) and washed twice with ice cold HBSS at 300xG for 3 min. Cells were resuspended in 200ul PBS, ready for staining.

Flow cytometry

After isolation, cells were stained with live/dead fixable green (1:1000 in PBS, Invitrogen, USA, L23101) at 4ºC for 30 min. A wash step was performed at 600*g* 5 min before adding 1:200 of Fc Blocker (Biolegend, USA, 101319) for 5 min. Then, 1:200 of primary antibodies was added in staining buffer (00-4222-26, Thermofisher, USA) and incubated for 30 min at 4 ºC. Another washing step was performed, and cells were resuspended in 200 μL of staining buffer for acquisition in FACSymphony (BD, USA) and results were analyzed by flowjo (BD, USA). Antibodies used for staining: CD45-BV480 (BD, 566073), CD3-BUV737 (BD, 612803), CD4-BUV395 (BD, 563790), CD8-BUV563 (BD, 748535), CD19-APC (Biolegend, 115512), GR1-BV711 (Biolegend, 108443), NK1.1-BV605 (Biolegend, 108753), CD11B-BUV805 (BD,741934), F4/80-APC-fire750 (Biolegend, 123151) and CD11C-PE (Biolegend, 117307).

Quantification of hepatic GSSG and GSH levels

Liver extracts were analyzed with an Acquity UPLC system (Waters, USA) coupled to a Time of Flight mass spectrometer (ToF MS, SYNAPT G2, Waters, USA). A 2.1 x 100 mm, 1.7 mm BEH amide column (Waters, USA), stabilized at 40ºc, was used to separate the analytes before entering the MS. Solvent A (aqueous phase) consisted of 99.5% water, 0.5% formic acid, and 20mM ammonium formate, while solvent B (organic phase) consisted of 29.5% water, 70% MeCN, 0.5% formic acid and 1mM ammonium formate. The extracted ion trace was obtained for GSH (m/Z= 308.0916) and GSSG (m/z= 613.1598) and subsequently smoothed (2 points, 2 iterations) and integrated with QuanLynx software (Waters, USA).

Gut metagenomic analysis

Faecal DNA extraction

Genomic DNA was extracted from 180 mg approximately of faecal samples using the Qiagen Fast DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer instructions. Briefly, an initial step of bead beating was included to enhance homogenization, and the lysis temperature was increased up to 95 ºC to recover DNA from bacteria that are difficult to lyse. DNA concentration was measured with a NanoDrop-1000 spectrophotometer (NanoDrop Technologies, USA) and DNA samples were stored at -20 ºC until further analysis.

16S data analysis methods

The amplicon sequencing protocol targets a fusion fragment containing the V3 and V4 regions (about 459bp) of the 16S genes with the primers designed surrounding conserved regions. The full-length primer sequences, using standard IUPAC nucleotide nomenclature, to follow the protocol targeting this fusion region are:

16S Amplicon PCR Forward Primer
5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG

16S Amplicon PCR Reverse Primer
5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

DNA amplicon libraries were generated following Illumina Inc.'s recommendations. The amplification reactions consisted of:

	Volume
Microbial DNA (5 ng/µl)	2.5 µl
Amplicon PCR Forward Primer 1 µM	5 µl
Amplicon PCR Reverse Primer 1 µM	5 µl
2x KAPA HiFi HotStart ReadyMix (KK2602)	12.5 µl
Total	**25 µl**

PCR cycling was programed with an initial denaturation at 95ºC for 3 min, followed by 25 cycles of annealing (95ºC - 30 seconds, 55ºC - 30 seconds, 72ºC - 30 seconds) and an extension at 72ºC for 5 min.

Then, Illumina Inc.'s sequencing adaptors and dual-index barcodes (Nextera XT index kit v2, FC-131-2001) were added to each amplicon (see Illumina Inc.'s Protocol for details) and, after PCR purification, libraries were normalized and pooled prior to sequencing. The pool containing indexed amplicons was loaded onto the MiSeq reagent cartridge v3 (MS-102-3003), spiked with 25% PhiX control to improve base calling during sequencing, as recommended by Illumina for amplicon sequencing. Sequencing was conducted using a paired end, 2x300pb cycle run on an Illumina MiSeq sequencing system.

Sequencing was done by FISABIO Sequencing Core Facility, who also performed the quality assessment, using *prinseq-lite*[7] with the following parameters (min_length: 50, trim_qual_right: 30, trim_qual_type: mean, trim_qual_window: 20), and the sequence joining, with *FLASH* software[8] using default parameters.

Microbiome sequences bioinformatics analysis

Joined reads were uploaded to QIIME2 software (v2019.7)[9], specifying the type parameter (SampleData[SequencesWithQuality]) and QIIME2 format option for FASTQ data input (SingleEndFastqManifestPhred33). Samples were then clustered *de novo* into Operational Taxonomic Units (OTUs), using the 97% similarity threshold using dada2 plugin[10]. The resulting OTU table was then rarefied to 45,000 reads per sample, when no increase in diversity was obtained from including more reads. Rarefied table was aligned with mafft plugin[11] and the OTUs phylogenetic tree was then obtained using fasttree plugin[12]. Several alpha and beta diversity indexes were computed with diversity plugin and exported for posterior analysis. Finally, OTUs were annotated with GreenGenes 13_8 database and the resulting table was exported for posterior analysis.

Data analysis

OTU table was then clustered into both phylum and genus levels, using the R package *phyloseq*. Genus-clustered dataset was then transformed using the center log-ratio approach, in order to assess for the compositional nature of microbiome data[13] using *ALDEx2* R package[14]. Differential abundance significance between EtOH mice groups was assessed following the ALDEx2 pipeline, with default parameters, using Student's t-test approach. Significance results were then corrected for multiple testing using Benjamini-Hochberg approach (False Discovery Rate). Significance was established at 10% FDR threshold. Both statistical analyses and data visualization was done in R v3.6 (R Development Core Team; http://cran.r-project.org).

Analysis of intestinal permeability using FITC-Dextran

Mice where administered 100ul of FITC-dextran 0.6mg/g of animal by oral gavage and after 4h, mice were sacrificed, and blood was collected in order to get the serum. Serum samples were diluted 1:1 in dPBS and added into a 96-well microplate, where the FITC concentration was determined using a SpectraMax M2 microplate (excitation of 485nm and emission wavelength of 528nm). Serum from mice not administered with FITC-dextran was used to determine the background.

Obtention of Human pancreatic islets

Human islets were either obtained from The Cell Isolation and Transplantation Center (Department of Surgery, Geneva; Switzerland) or purchased from Tebu-Bio (Barcelona, Spain) from deceased individuals with informed consents obtained from their families. The donors did not have a previous history of glucose intolerance. The use of human islets was performed in compliance with the Declaration of Helsinki, ICH/Good Clinical Practice.To recover after arrival, human islet preparations were washed, handpicked, and subsequently cultured for 2 days in CMRL-1066 (ThermoFisher

Scientific) containing 5.6 mM glucose, and supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 100 µg/ml gentamycin (Sigma-Aldrich, Madrid).

Intra peritoneal glucose tolerance test (IPGTT)

Animal followed an overnight fasting period before starting the IPGTT. The glucose solution was freshly prepared: 1 g of glucose (Sigma, USA) was resuspended in 5mL of sterile PBS and filtered through 0.22um Millipore filter. Mice were then weighed, and the tip of the tail vein was nicked using a pair of sterile scissors. After a baseline glucose measurement, glucose was injected into the intraperitoneal cavity using an insulin syringe, 2g/kg body weight. At 15, 30, 60 and 120 min blood glucose was measured by gently massaging a small drop of blood onto the glucometer strip. Blood glucose was measured using an automatic glucometer (Accu-Chek Aviva, Roche, USA).

Insulin in serum

Blood insulin levels were determined by ELISA using Mouse Insulin ELISA (Mercodia AB, Sweden, Ref.10-1247-01) according to the manufacturer´s recommendations.

Pancreatic Islets of Langerhans isolation, cell isolation and culture

For the *in vitro* studies, islets of Langerhans were isolated as previously described[15]. Briefly, after pancreas digestion with Collagenase V (C9263, Sigma, USA) in a stationary bath at 37ºC, islets were separated by centrifugation and hand-picked under a microscope. Once isolated, islets were cultured in RPMI-1640 supplemented with 10% FBS, 2 mM L-glutamine, 3 mM glucose, 1% sodium pyruvate, 1% HEPES 1M and 1% penicillin/streptomycin (Invitrogen-Thermo Fisher Scientific, Spain), allowing them to recover.

Pancreatic Islets ATP determination

Total ATP content was determined as previously described[16]. Briefly, pancreatic islets total ATP was first extracted. Islets were washed twice in cold Krebs Ringer Bicarbonate

Buffer (KRB) supplemented with 5.6 mmol/L glucose and 3% bovine serum albumin (BSA) (Sigma-Aldrich, USA) and centrifuged for 1 min at 100x *g*. Then, supernatant was removed, 600 µL of somatic cell ATP releasing reagent (Merck Life Science, Spain) was added to the islet pellets and samples were sonicated on ice for 1 min (Branson 450 Digital Sonifier, Marshall Scientific, USA). Afterwards, 400 µL of the ATP releasing reagent was added to the samples and they were centrifuged for 15 min at 1400x *g* (4ºC). Finally, 800 µL of supernatant was collected and stored at -80ºC for further measurement of total ATP. Finally, the total ATP content was quantified using the ATP Assay kit (Abcam, UK) following manufacturer´s instructions. Fluorescence (Ex/Em= 535/587/nm) was read using a Varioskan Flash microplate reader (Thermo Scientific, Finland). ATP values were normalized to DNA content from each batch.

Respiration studies in pancreatic islets

Respiration studies in pancreatic islets were performed as previously described in (Ref) using islet-capture plates of the XF24 extracellular flux analyzer (Agilent, Seahorse Bioscience). Briefly, freshly isolated 30 size-matched islets were handpicked into individual wells and incubated 6h with XF DMEM assay media with 11mM glucose and 1% FBS. With the help of a dissecting microscope all the islets were placed in the depression at the bottom of the well and on the top, the prewet islet capture screens. Glucose (20mM), Oligomycin (5uM), FCCP (1uM) and Rot/AA (5uM) were used for the respiration studies. The normalized data were expressed as pmol of O2 per minute or milli-pH units (mpH) per minute, per ug protein.

Static Insulin Measurements

Insulin measurements were performed as previously described[17]. Briefly, to measure glucose-stimulated insulin secretion (GSIS), the fresh collagenase-isolated islets were incubated for 1 h at 37 °C in fresh KRB supplemented with 5.6 mmol/L glucose and 3%

BSA (Sigma-Aldrich, USA). The medium was continuously bubbled with a mixture of 95% O_2:5% $CO_2$to obtain a final pH of 7.4. The medium was then replaced, and the islets were incubated in groups of 5 in 1 mL of KRB supplemented with 1% BSA and glucose at various concentrations (2.75, 5.5, 11.1, 16.7 and 22.2 mmol/L) for an additional 60 min at 37 ºC. Then, the supernatant was collected and stored at −80 °C for the subsequent insulin measurements. Insulin was assayed by ELISA using the kit from Mercodia per the manufacturer's instructions and Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, USA) was used to measure islet DNA. Insulin values were normalized to DNA content from each batch. Standard curves and experimental points were performed in triplicate.

Total protein isolation from liver tissue and Western blotting

Approximately, 50 mg of liver tissue were homogenized using a Precellys-24 apparatus (Bertin Technologuies, France). The lysis buffer (NaH_2PO_4 1.6 mM, Na_2HPO_4 8.4 mM, 0.1% Triton X-100, NaCl 0.1 M, 0.1% SDS, 0.5% sodium azide) used to homogenize liver tissue and the extracts of primary hepatocytes were supplemented with a protease and phosphatase inhibitor cocktail (Roche, USA). Total protein lysates were centrifuged (13000 rpm, 30 min, 4 ºC) and total protein content in the supernatant was quantified with the Bradford protein assay (BioRad, USA). Protein extracts from each sample were boiled at 95ºC for 10 min in SDS-PAGE sample buffer (50 mM Tris-HCl pH 6.8, 500 mM β-mercaptoethanol, 50% glycerol, 10% SDS, bromophenol blue). 7-20µg of protein from each sample were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in 8-15% acrylamide gels, using a Mini-PROTEAN Electrophoresis System (BioRad, USA). Protein gels were transferred onto nicrocellulose blotting membranes (GE Healthcare, USA). Membranes were blocked with 5% non-fat milk in Tris-buffered saline (TBS) PH 8 containing 0.1% Tween-20 (Sigma Aldrich, USA) (TBST-0.1%) for 60 min at RT, washed 3 times with TBST and incubated overnight at 4ºC with commercial antibody (1:1000), and incubated with

secondary antibody conjugated with horseradish-peroxidase (HRP) 1-2h RT (1:5000, anti-rabbit-IgG-HRP-linked and anti-mouse IgG-HRP-linked, Cell Signalling Tech, USA). Inmunoreactive proteins were detected by using Western Lightning Enhanced Chemiluminescence reagent (ECL, PerkinElmer, USA) and exposed to Super RX-N X-ray films (Fuji, Japan) in a Curix 60 Developer (AGFA, Belgium). Antibodies and conditions are described in Supplemental Table 3.

Total RNA isolation and quantitative real-time polymerase chain reaction

Total RNA was isolated from tissue using the Trizol reagent (Invitrogen)/ Chloroform (Sigma-Aldrich, USA). After analyzing its integrity and concentration, 2 µM of total RNA was treated with DNAse I (Invitrogen, USA) and cDNA was synthesized using M-MLV Reverse Transcriptase (Invitrogen, USA) in the presence of random primers, RNAase OUT and ribonucleotides. The resulting CDNA was diluted 1/10. Quantitative real-time (RT-PCR) was performed using SYBR ® Select Master Mix (Applied Biosystems, USA) using a Viia 7 Real-Time PCR System (Applied Biosystems, USA). Primers were designed using Primer-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Sigma Aldrich. The calculations were based on the differential of ΔCt cycles (expression) between the housekeeping (ARP) and the target gene. Primers sequences are described in Supplemental Table 4.

Proteomics

In solution digestion:

Protein was extracted in a sample containing 7M urea, 2M Thiourea, 4% CHAPS and 5mM DTT, then digested following the filter-aided FASP protocol described by Wisniewski et al[18] with minor modifications. Trypsin was added to a trypsin:protein ratio of 1:20, and the mixture was incubated overnight at 37ºC, dried out in a RVC2 25

speedvac concentrator (Christ, Germany), and resuspended in 0.1% FA. Peptides were desalted and resuspended in 0.1% FA using C18 stage tips (Millipore,USA).

Mass spectrometry analysis

Samples were analyzed in a timsTOF Pro with PASEF (Bruker Daltonics, USA) coupled online to a Evosep ONE liquid chromatograph (Evosep Biosystems, Denmark). 200ng were directly loaded onto the Evosep ONE and resolved using the 30 samples-per-day method.

Protein identification and quantification was carried out using PEAKS X software (Bioinformatics Solutions Inc., Canada). Searches were carried out against a database consisting of Mus musculus entries (Uniprot/Swissprot), with precursor and fragment tolerances of 20 ppm and 0.05 Da respectively. Only proteins identified with at least two peptides at FDR<1% were considered for further analysis. Protein abundances inferred from PEAKS were loaded onto Perseus platform[19], log2 transformed and imputated. A t-test was used to address significant differences in protein abundances within each sample group under analysis.

Functional enrichment proteomic analysis

To elucidate the possible molecular mechanism involved in *siMcj*, we required the use of bioinformatics tools that helped us to understand the whole protein interactions, pathways, and upstream regulators, through the use of Ingenuity Pathway Analysis (IPA, QIAGEN, USA). The program assesses the protein network using t-test and ratios between groups. IPA studies the protein enrichment using Fisher´s exact *p* value that measures overlap of observed and predicted regulated gene sets. Using *Z* score, IPA is able to predict the upstream regulators and its expectable functions.

SIRT1 activity analysis

Hepatic SIRT1 activity was measured using a commercial Kit (ab156065, Abcam, UK). Briefly, 20mg of liver tissue were used to extract the nuclei as shown in Papageorgiou et al[20]. Protein concentration was determined using a Bradford Assay and the purity of the fractions was confirmed by Wb. Nuclei were normalized to 60ug in 60ul, and samples were assayed in duplicates, 25ul per well, enzyme sample and the background control. The fluorometric determination was done using the SpectraMax M2 microplate reader, with excitation at 340-360nm and emission at 440-460nm.

NAD^+/NADH determination in liver tissue

Hepatic NAD^+/NADH levels were measured using a commercial Kit (ab65348, Abcam, UK). Briefly, 10mg of liver homogenates in 300ul of Extraction buffer were used. Samples were centrifuged at top speed for 5 min at 4ºC and the supernatant was assayed. Protein concentration was determined in supernatant fractions using a Bradford Assay. Protein samples were normalized to 200ug in 100ul; 50ul were kept on ice while 50ul were warmed for 30 min to degrade NAD^+. Samples were incubated for 5 min with the Reaction Mix and after adding the NADH Developer, the colorimetric determination was finally done at 450nm using the SpectraMax M2 microplate reader**.**

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Prism 9 (GraphPad Software, version 9.2.0, USA) was used to perform statistical analyses. A one-way analysis of variance (ANOVA) followed by Tukey (comparing all pairs of columns) was used for three or more groups, while Student's t-test was used for 2 groups. For human samples the Mann-Whitney U test was used for 2 groups. Grubbs' test was performed to determine the significant outliers. A $p < 0.05$ was considered statistically significant. Statistical parameters are reported in the figure legends.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1 (a) Schematic overview of the NIAAA model in Wt and MCJ-KO mice. (b) Relative Mcj expression in 23 and 52 weeks of DUAL Model. Values are represented as mean ± SEM. Student's t-test was used to compare two groups. *p<0.05 versus Wt.

Supplemental Figure 2 (a) Liver immunohistochemical staining for H&E, Cleaved Caspase 3 and TUNEL in ethanol fed Wt and MCJ-KO mice. **(b)** Heatmap representing the differential hepatic expression of mRNA levels from genes involved in Apoptosis in Wt and MCJ-KO mice after NIAAA model (*Trail*= TNF-related apoptosis-inducing ligand; *Bax*= Bcl-2-associated X protein). **(c)** Total bilirubin levels in serum. **(d)** Liver immunohistochemical staining for Sudan Red, a marker for hepatic steatosis, F4/80, macrophage infiltration, and Sirius Red, fibrosis (upper panel) and quantification of F4/80 (bottom panel) in ethanol fed Wt and MCJ-KO mice. **(e)** Heatmap showing the differential hepatic expression of mRNA levels from genes involved in Lipid Metabolism in Wt and MCJ-KO mice after NIAAA model (*Fatp2*= Fatty acid transport protein 2; *Cpt1a*= Carnitine Palmitoyltransferase 1A; *Ppara*= Peroxisome proliferator-activated receptor alpha; *Acc*= Acetyl-CoA carboxylase; *FasN*= Fatty acid synthase; *Chrebp*= Carbohydrate-responsive element-binding protein; *Pparg*= Peroxisome proliferator-activated receptor gamma; *Glut2*= Glucose transporter 2). **(f)** Quantification of hepatic total CD45+ cells and a further characterization of different CD45+ populations using FACS in ethanol fed Wt and MCJ-KO mice. **(g)** Heatmap showing the differential hepatic expression of mRNA levels from genes involved in inflammation in Wt and MCJ-KO mice after NIAAA model (*Tnf*= Tumor necrosis factor; *Il-1b*= Interleukin 1 beta; *Cxcl1*= C-X-C Motif Chemokine Ligand 1; *Ccl2*= C-C Motif Chemokine Ligand 2; *Ccl5*= C-C Motif Chemokine Ligand 5; *Ccr2*= C-C Motif Chemokine Receptor 2; *Ccr5*= C-C Motif Chemokine Receptor 5; *Il-10*= Interleukin-10; *Ho-1*= Heme oxygenase-1). **(h)** Hepatic ethanol (left panel) and acetaldehyde (right panel) quantification in Control and NIAAA fed Wt and MCJ-KO mice. **(h)** Hepatic reduced to oxidized glutathione ratio in ethanol fed Wt

versus MCJ-KO mice. Values are represented as mean ± SEM. Student's t-test was used to compare two groups. *p<0.05, **p<0.01 and ***p<0.001 versus Wt.

Supplemental Figure 3 (a) Blood glucose levels following the acute ethanol administration**. (b)** Relative *MCJ* mRNA expression in human ~~and mice~~ pancreatic islets. **(c)** Relative *Mcj* mRNA expression in mice pancreatic islets with control and NIAAA diet. **(d)** Curves showing the blood glucose levels (left panel) and the resulting area under curve (right panel) during the IPGTT in Wt and MCJ-KO mice at the 6th day of the NIAAA model. **(e)** Number of pancreatic islets in Wt and MCJ-KO mice, control and ethanol fed. **(f)** Pancreatic immunohistochemical staining of Cleaved caspase 3 and iNOS in Control fed Wt and MCJ-KO mice. **(g)** Immunohistochemical staining of pancreatic acinar cells of Cleaved caspase 3 in Control and Ethanol-fed Wt and MCJ-KO mice. Values are represented as mean ± SEM. Student's t-test was used to compare two groups and one-way ANOVA followed by Sidak post-test was used to compare between multiple groups. *p<0.05, **p<0.01 and ***p< 0.001 versus Wt.

Supplemental Figure 4 (a) Schematic overview of the NIAAA model in siCtrl and siMCJ mice. **(b)** MCJ levels by western blotting (upper panel) and densitometric quantification (bottom panel) in siCtrl and siMCJ liver extracts after the NIAAA model. ß-actin was used as a loading control. **(c)** Relative *Mcj* mRNA expression in hepatocytes and Kupffer cells from ethanol fed Wt and LSS-MCJ mice. MCJ Values are represented as mean ± SEM. Student's t-test was used to compare two groups and one-way ANOVA followed by Sidak post-test was used to compare between multiple groups. **p<0.01 and ***p< 0.001 versus Wt.

Supplemental Figure 5 (a) Liver immunohistochemical staining for H&E, Cleaved Caspase 3, TUNEL and PCNA in ethanol fed siCtrl and LSS-MCJ mice. **(b)** Apoptotic Bcl2 and Bax and regenerative PCNA protein levels by western blotting (upper panel) and densitometric quantification (bottom panel) in siCtrl and siMCJ liver extracts after the NIAAA model. ß-actin was used as a loading control. **(c)** Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), Bilirubin, Prothrombin and Albumin levels in ethanol fed siCtrl and

LSS-MCJ mice. **(d)** Liver immunohistochemical staining for Sudan Red, F4/80 and Sirius Red in ethanol fed siCtrl and LSS-MCJ mice. **(e)** Hepatic ethanol (left panel) and acetaldehyde (right panel) quantification in NIAAA fed siCtrl and LSS-MCJ mice. **(f)** Heatmap showing the differential hepatic expression of mRNA levels from genes involved in Ethanol metabolism in siCtrl and LSS-MCJ mice after NIAAA model (*Adh1*= Alcohol dehydrogenase 1; *Aldh2*= Aldehyde dehydrogenase 2; *Cyp2e1*= Cytochrome P450 Family 2 Subfamily E Member 1). **(g)** Hepatic reduced to oxidized glutathione ratio in ethanol fed Wt versus LSS-MCJ mice. Values are represented as mean ± SEM. Student's t-test was used to compare two groups. *p<0.05 and **p<0.01 versus Wt.

Supplemental Figure 6 Relative *Mcj* mRNA levels in gut **(a)** and pancreatic ß islets **(b)** on control and ethanol-fed Wt and ethanol-fed LSS-MCJ mice. Values are represented as mean ± SEM. Student's t-test was used to compare two groups.

Supplemental Table 1. Primer sequences used for pyrosequencing analysis.

Primers	Forward (5’-3’)	Reverse (5’-3’)	Sequencing (5’-3’
Dnajc15-1	TAGTTGGGAAGGTTAAGTAAGGAAGTT	ATTTATTTATTTATACCACCCTCCACTA	GTTTTGTTTTTATTTTTAGGTATTA
Dnajc15-2	GGAGTTTTTTTTGGGGAAAAGTAG	AAATACTAAAAATCCCAACTATTCTCAC	GGGGAAAAGTAGTTAAGAATTA

Supplemental Table 2. Optimal conditions, concentration, reference and supplier for each antibody analyzed by Immunohistochemical staining.

Antibodies	*Dilution*	*Incubation solution*	*Unmasking*	*Source*	*Identifier*
Anti-4 Hydroxynonenal antibody (4-HNE)	1:100	PBS-azide (0.01%)-BSA (2%)	none	Abcam	ab46545
Cleaved Caspase-3 (Liver)	1:50	PBS-azide (0.01%)-BSA (2%)	EDTA 5M, pH 8, 97ºC	Cell Signaling	9661
F4/80	1:50	PBS-azide (0.01%)-BSA (2%)	Proteinase K, 15' at RT	Serotec	MCA497-BB
ZO-1	1:500	PBS-azide (0.01%)-BSA (2%)	Proteinase K, Tris-EDTA, pH 8, 30'	Thermo	40-2300
Cleaved Caspase-3 (Pancreas)	1:400	PBS-Tween (0.2%)- BSA (2%)	Heat-induced antigen retrieval in citrate buffer (pH=6)	Cell Signaling	9661
iNOS (Pancreas)	1:100	PBS-Tween (0.2%)- BSA (2%)	Heat-induced antigen retrieval in citrate buffer (pH=6)	Thermo	PA1-036
Insulin (Pancreas)	1:400	PBS-Tween (0.2%)- BSA (2%)	Heat-induced antigen retrieval in citrate buffer (pH=6)	Cell Signaling	C27C9
Insulin (Pancreas)	1:500	PBS-Tween (0.2%)- BSA (2%)	Heat-induced antigen retrieval in citrate buffer (pH=6)	Sigma	I2018
PCNA	1:400	PBS-azide (0.01%)-BSA (2%)	citrates	Santa Cruz	sc-25280
MCJ	1:50	PBS-azide (0.01%)-BSA (2%)	citrates	Bio Mosaics	B0027R
Anti-Rabbit, Alexa Fluor 568	1:300	PBS-Tween (0.2%)- BSA (2%)	-	Thermo	A11011
Anti-Rabbit, Cy3	1:200	PBS-Tween (0.2%)- BSA (2%)	-	Jackson	711-165-152
Anti-Mouse, Alexa Fluor 488	1:300	PBS-Tween (0.2%)- BSA (2%)	-	Invitrogen	A11001

Anti-Rabbit-biotinylated, HRP-linked antibody	1:300	PBS-Tween (0.2%)- BSA (2%)	-	Jackson	111-035-003

Supplemental Table 3. Optimal conditions, concentration, reference and supplier for each antibody analyzed by Western Blot.

Antibodies	***Dilution***	***Incubation Solution***	***Source***	***Identifier***
MCJ	1:1000	TBS-Tween(0.1%)-milk (5%)	BioMosaics	B0027R
Bcl2	1:1000	TBS-Tween(0.1%)-milk (5%)	Abcam	Ab7973-1
Bax	1:1000	TBS-Tween(0.1%)-milk (5%)	Cell Signaling	2772S
BclxL	1:1000	TBS-Tween(0.1%)-milk (5%)	Santa Cruz	sc-7195
PCNA	1:500	TBS-Tween(0.1%)-milk (5%)	Santa Cruz Biotechnology	Sc-25280
Phospho-mTORC1(Ser2481)	1:1000	TBS-Tween(0.1%)-BSA (3%)	Cell Signaling	2974
mTORC1	1:1000	TBS-Tween(0.1%)-milk (5%)	Cell Signaling	2972
Phospho-AMPK (Thr172)	1:1000	TBS-Tween(0.1%)-BSA (3%)	Cell Signaling	2531
Phospho-S6 (Ser235/236)	1:1000	TBS-Tween(0.1%)-BSA (3%)	Cell Signaling	4857
β-Actin	1:5000	TBS-Tween(0.1%)-milk (5%)	Sigma	a5441
Anti-rabbit IgG, HRP-linked antibody	1:5000	TBS-Tween(0.1%)-milk (5%)	Cell Signaling	7074
Anti-mouse IgG, HRP-linked antibody	1:5000	TBS-Tween(0.1%)-milk (5%)	Cell Signaling	7076

Supplemental Table 4. Primer sequences used for RT-qPCR analysis.

Gene name	*Symbol*	*Species*	*Sequence primer*
Methylation Controled J-protein	***Mcj***	Mouse	FW 5'- ACGCCGACATCGACCACACAG -3'
			RV 5'- AATCTTCCTTGCTGTTGCVGTG -3'
Toll-like receptor 2	***Tlr2***	Mouse	FW 5'- GCATCCGAATTGCATCACCG-3'
			RV 5'- GAGCCAAAGAGCTCGTAGCA -3'
Toll-like receptor 4	***Tlr4***	Mouse	FW 5'- CCCACTGCTTCAGGCTACAA -3'
			RV 5'- GACCCTGACTGGCACTAACC -3'
Toll-like receptor 5	***Tlr5***	Mouse	FW 5'-AATCCCGCTTGGGAGAACAA-3'
			RV 5'-CAGGGGAACCAGGTATGCAG-3'
Activator Protein-1	***Ap-1***	Mouse	FW5'- GCACATCACCACTACACCGA -3'
			RV 5'- GGGAAGCGTGTTCTGGCTAT -3'
Hepcidin antimicrobial peptide	***Hamp***	Mouse	FW 5'- AGGGCAGACATTGCGATACC -3'
			RV 5'- GCAACAGATACCACACTGGGA -3'
TNF-related apoptosis-inducing ligand	***Trail***	Mouse	FW 5'- CCAACGAGATGAAGCAGC -3'
			RV 5'- CCATCAGTGGAGTCCCAG -3'
Bcl2 Associated X, Apoptosis Regulator	***Bax***	Mouse	FW 5'- GATCAGCTCGGGCACTTTAG-3'
			RV 5'- TGCAGAGGATGATTGCTGAC -3'
B-cell lymphoma 2	***Bcl2***	Mouse	FW 5'- GACCACAGGTGGCACAGGGC -3'
			RV 5'- ATGCTGGAGATGCGGACGCG-3'
Bcl2-like 1	***Bcl-xL***	Mouse	FW 5'-GGCCTTTTTCTCCTTTGGCG -3'
			RV 5'-GATCCACAAAAGTGTCCCAGC -3'
Fatty acid transport protein 2	***Fatp2***	Mouse	FW 5'- CCGCAGAAACCAAATGACCG-3'
			RV 5'- TGCCTTCAGTGGATGCGTAG-3'
Carnitine Palmitoyltransferase 1A	***Cpt1a***	Mouse	FW 5'- GACTCCGCTCGCTCATTCC -3'
			RV 5'- GAGATCGATGCCATCAGGGG -3
Peroxisome proliferator-activated receptor alpha	***Ppara***	Mouse	FW 5'- GAGGGTTGAGCTCAGTCAGG -3'
			RV 5'- GGTCACCTACGAGTGGCATT -3'
Acyl-CoA Dehydrogenase Long Chain	***Acadl***	Mouse	FW 5'- GTCCGATTGCCAGCTAATGC -3'
			RV 5'- CACAGGCAGAAATCGCCAAC -3
Acetyl-CoA carboxylase	***Acc***	Mouse	FW 5'- GCCTCAGGAGGATTTGCTGT-3'
			RV 5'- AGGATCTACCCAGGCCCACAT -3'
Fatty Acid synthase	***FasN***	Mouse	FW 5'- GGCCCCTCTGTTAATTGGCT -3'
			RV 5'- GGATCTCAGGGTTGGGGTTG -3'
Sterol regulatory element-binding protein 1	***Srebp1***	Mouse	FW 5'- GAGGCCAAGCTTTGGACCTGG -3'
			R 5'- CCTGCCTTCAGGCTTCTCAGG -3
Carbohydrate-responsive element-binding protein	***Chrebp***	Mouse	FW 5'- GCAAAAACTGTCTGCAAGGGT -3'
			RVV 5'- GGCAGCTCTGAGTCCCATAc-3

Peroxisome proliferator-activated receptor gamma	***Pparg***	Mouse	FW 5'- GAATGCGAGTGGTCTTCCAT -3'
			RV 5'- TGCACTGCCTATGAGCACTT -3'
Tumor Necrosis Factor	***Tnf***	Mouse	FW 5'-AGCCCACGTCGTAGCAAACCAC-3'
			RV 5'-ATCGGCTGGCACCACTAGTTGGT-3'
Interleukin 1 beta	***Il-1β***	Mouse	FW 5'- ACACTCCTTAGTCCTCGGCCA -3'
			RV 5'- CCATCAGAGGCAAGGAGGAA -3
C-X-C Motif Chemokine Ligand 1	***Cxcl1***	Mouse	FW 5'- GGTGTCCCCAAGTAACGGAG -3'
			RV 5'- TTGTCAGAAGCCAGCGTTCA-3
C-C Motif Chemokine Ligand 2	***Ccl2***	Mouse	FW 5'- GACCCCAAGAAGGAATGGGT -3'
			RV 5'- ACCTTAGGGCAGATGCAGTT -3'
C-C Motif Chemokine Ligand 5	***Ccl5***	Mouse	FW 5'- TCGAGTGACAAACACGACTGC -3'
			RV 5'- GCTGCTTTGCCTACCTCTCC -3'
C-C Motif Chemokine Receptor 2	***Ccr2***	Mouse	FW 5'- ATCCACGGCATACTATCAACAT-3'
			RV 5'- CAAGGCTCACCATCATCGTAG -3
C-C Motif Chemokine Receptor 5	***Ccr5***	Mouse	FW 5'- GTGTGGAAAATGAGGACTGCAT-3'
			RV 5'- GTCAGAACGGTCAACTTTGGG-3
Interleukin 10	***Il-10***	Mouse	FW 5'- GGTTGCCAAGCCTTATCGGA -3'
			RV 5'- AATCTTCCTTGCTGTTGCCGTC -3'
Heme oxygenase 1	***Ho-1***	Mouse	FW 5'- AAGCTTTTGGGGTCCCTAGC -3'
			RV 5'-ACAGCTGCTTTTACAGGCCA-3'
Alcohol dehydrogenase 1	***Adh1***	Mouse	FW 5'- GTGGCCAAAATCGATGGAGC -3'
			RV 5'- GCAGAGCCATAGCCAGTTGA -3
Aldehyde dehydrogenase 2	***Aldh2***	Mouse	FW 5'- GCTGGGCTGACAAGTACCAT -3'
			RV 5'- CAGGCTCATGGCGGGTATAG -3
Cytochrome P450 Family 2 Subfamily E Member 1	***Cyp2e1***	Mouse	FW 5'-TCACTGGACATCAACTGCCC-3'
			RV 5'-ACATGGGTTCTTGGCTGTGT-3'
Sirtuin 1	***Sirt1***	Mouse	FW 5'- GATTGGCACCGATCCTCGAA -3'
			RV 5'-ACAATCTGCCACAGCGTCAT -3
Transcription factor A, mitochondrial	***Tfam***	Mouse	FW 5'- CTGCCTTCCTCTAGCCCGGG-3'
			RV 5'-GTAACAGCAGACAACTTGTG-3
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	***Pgcta***	Mouse	FW 5'-AGACAGGTGCCTTCAGTTCAC-3'
			RV 5'-ACCAGAGCAGCACACTCTATG-3'
Mitofusin1	***Mfn1***	Mouse	FW 5'-GGGTGCTGGGTTGCAGTATT-3'
			RV 5'-CCACTTGGTTGCCTGTACCA-3
OPA1, Mitochondrial dynamin like GTPase	***Opa1***	Mouse	FW 5'- TCTGAGGCCCTTCTCTTGTT-3'
			RV 5'-GTCTGACACCTTCCTGTAATGC-3
Mitochondrial fission1 protein	***Fis1***	Mouse	FW 5'- CCTGATTGATAAGGCCATGAA-3'
			RV 5'-ACAGCCAGTCCAATGAGTCC-3'
Mitochondrial fission factor	***Mff***	Mouse	FW 5'-TCAAAGCGAAGAGAGCCGAG-3'
			RV 5'- GTCCATTTTGGCGAACAGCA-3
Parkin	***Prkn***	Mouse	FW 5'- GAAGCAGCCAGAGGTCCAGTTA-3'
			RV 5'-TGAACTCGGAGCTTTCCAGGC-3
PTEN induced kinase1	***Pink1***	Mouse	FW 5'-GTGGGACTCAGATGGCTGTC-3'

			RV 5’-ACTGGAGCTGTTGAAAGGCAG-3’
Dynamin like 1	***Dnm1l***	Mouse	FW 5’-CTGGATCACGGGACAAGG-3’
			RV 5’-GTTGCCTGTTGTTGGTTCCT-3’
Sequestosome1	***Sqstm1***	Mouse	FW 5’- GGTGCACCCCAATGTGATCT-3’
			RV 5’-TGGGCACACACTGCACTTAT-3
Glyceraldehyde-3-Phosphate Dehydrogenase	***GAPDH***	Mouse/ Human	FW 5’- TTGATGGCAACAATCTCCAC -3’
			RV 5’- CGTCCCGTAGACAAAATGG -3
ARP	***ARP***	Mouse/ Human	FW 5’- CGACCTGGAAGTCCAACTAC -3’
			RV 5’- ATCTGCTGCATCTGCTTG -3
9S	***9S***	Mouse/ Human	FW 5’- GACTCCGGAACAAACGTGAGG -3’
			RV 5’- CTTCATCTTGCCCTCGTCCA-3’
Methylation Controled J-protein	***MCJ***	Human	FW 5’- CCTCGCCCAACAGTCATCAA -3’
			RV 5’- GGGACGGACTATGCTGACAC -3

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