SUPPLEMENTARY METHODS

Allelic imbalance analysis in human biopsies

For homogenization of 5–10 mg frozen tissue and subsequent nucleic acid isolation, tubes with 1.4-mm ceramic beads (Precellys, Villeurbanne, France) and the AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) were used. Reverse transcription was performed using the Advantage RT-for-PCR Kit (Takara Clontech) with a random primer. Region of interest in the MBOAT7 transcript was amplified using the primers (TCCTTGTGTCTTTCGCTCC; TACACACGGTGACCTGTCA) with the following tag on each primer (ATG, CTG, GTG, ATC, CTC, GTC, AGT, CGT, TGT, CAG, TAG, GAG, (Metabion, Planegg/Steinkirchen). Cycling was done using the Bioline PCR mix (BIOLINE) in 25 µl reaction vol. at 94°C for 1min, 29 cycles at 60°C for 30 s, 72°C for 1 min, 94°C for 30 s, and a final elongation step at 72°C for 5 min. For library preparation and sequencing, Illumina technology was used. All sample-specifically tagged amplicons from a single sample were pooled together. For each pool an indexed sequencing library was prepared using Illumina TruSeq DNA PCR-free library preparation kit following the manufacturer's protocol with the exception that amplicons (around 500 ng) were directly introduced into end-repair skipping the fragmentation step. The libraries were quantified and quality checked using the Agilent Bioanalyzer 2100 and the Agilent DNA 7500 Kit (Agilent Technologies). Libraries were sequenced using the Illumina MiSeq (2 x 300 cycles, paired-end mode). Read information was extracted by MiSeq Control Software (MCS) v2.4.1.3 (Illumina). This includes demultiplexing of reads based on the Illumina indices and trimming of Illumina adapter sequences. Quality control of Sequence data was performed using FastQC. Demultiplexing of reads based on sample-specific PCR tags was done with an in-house script. Reads were filtered for the expected lengths and the correct primer pair

sequences. Only reads derived from molecules that were successfully sequenced in both forward and reverse direction were selected. Chimeras were excluded by filtering the read pairs for identical tags. Reads from each sample were allele-sorted and alleles counted.

Human Lipidomic Cohort

280 human liver samples were obtained from patients in Germany, in whom an intraoperative liver biopsy was indicated on clinical grounds such as during scheduled liver resection, exclusion of liver malignancy during major oncologic surgery or assessment of liver histology during bariatric surgery. Samples were snap frozen immediately in liquid nitrogen ensuring an ex vivo time of less than 40 seconds. Patients with evidence of viral hepatitis, hemochromatosis or alcohol consumption >20 g/day (women) and >30 g/day (men) were excluded. For all samples, *MBOAT7* rs641738C>T genotype as well as full phenotypic and histological information [1] generated by a single pathologist (C.R.) blinded to the lipidomic analysis was available (Table-1). Phenotypic groups (Table-1) were defined on the basis of clinical and histological parameters as follows [2]: Normal controls (BMI<30) and healthy obese (BMI≥30) patients showed histological fat content ≤5%, no histological inflammation, no ballooning and no fibrosis. NAFL was defined as histological fat contents above 5%, absence of lobular inflammation and ballooning, and with presence or absence of F1 fibrosis. Early NASH was defined as histological fat content above 5%, with presence of lobular inflammation, an NAS score below 5 and with presence or absence of F1 fibrosis. NASH was defined by an NAS score greater or equal to 5. The study protocol abides to the ethical guidelines of the 1975 Declaration of Helsinki and was approved

by the institutional review board (Universität Kiel, D425/07, A111/99), before study commencement.

Human NAFLD cohort

A total of 846 adult Caucasian NAFLD patients from tertiary referral centers in Austria (n=258), Germany (n=537) and Switzerland (n=51) who underwent percutaneous or surgical liver biopsy were included into this study (Supplementary-Table-1-2). NASH was defined by the NAFLD activity score (NAS). Presence of fibrosis was assessed histologically according to Kleiner classification [1] (stage-F0:no fibrosis; stage-F1:perisinusoidal fibrosis to portal/periportal fibrosis; stage-F2:perisinusoidal and portal/periportal fibrosis; stage-F3:bridging fibrosis). In all patients, infectious (e.g. viral hepatitis, HIV), immunological, drug-induced hepatic-steatosis (e.g. amiodarone, methotrexate, steroids, valproate, etc.) or hereditary causes (hereditary hemochromatosis, Wilson disease) of liver disease were excluded. Alcohol consumption was assessed by self-reporting; subjects with average alcohol consumption of more than 30 g/day (men) or 20 g/day (women) were excluded from this study. The liver biopsies were read by experienced pathologists in a blinded fashion. Portions of this NAFLD cohort have been described previously [3–5]. All patients gave their written consent. Subjects recruited for the genetic association study came from different sites, at which the study protocol was approved by the ethics committees of the participating institutions.

DNA preparation and genotyping of the human NAFLD cohort

Of all cases and controls, genomic DNA was extracted from peripheral blood samples according to standard procedures. Genomic DNA (1 µl) was amplified with the GenomiPhi (Amersham) whole-genome amplification kit. Genotyping of *MBOAT7* rs641738 (hcv8716820), was performed using the Taqman chemistry (Applied Biosystems, Foster City, CA, USA) on an automated platform with TECAN Freedom EVO and 384well TEMO liquid handling robots (TECAN, Männedorf, Switzerland) as described earlier [6,7]. Reactions were completed and read in a 7900 HT TaqMan sequence detector system (Applied Biosystems, Foster City, CA, USA). All process data were logged and administered with a database-driven LIMS [8].

Quantitative real time-PCR (qRT-PCR)

RNA was isolated using Nucleospin RNA II kit (Macherey-Nagel) and for cDNA synthesis IScript cDNA Synthesis Kit (Bio-Rad) was used. QRT-PCR was performed using SsoFast EvaGreen Supermix (Bio Rad), gene-specific primers (Supplementary table 10) and the Bio-Rad cycler system (Bio-Rad CFX 384 Real-time system). Calculation was based on the threshold cycle method (ΔΔCT) [9]. Expression levels were normalized to those of *B2m or 18s*.

Non parenchymal cell (NPC) isolation and flow cytometry

The liver-NPCs were isolated after perfusing the liver with ice-cold PBS. The tissue was minced and subjected to enzymatic digestion (crude collagenase at 2 mg/ml from Clostridium histolyticum and hyaluronidase at 0.5 mg/ml from bovine testis; Sigma Aldrich) in DMEM at 37°C for 30 minutes. Then, the cell suspension was passed through a strainer (100 µm) followed by centrifugation at 50 x g, to remove the

hepatocytes in the pellet. RBC lysis buffer (eBioscience) was used to perform RBC lysis, subsequently the cells were passed through a 40 µm strainer. Subsequently, the cells were washed in buffer containing 0.1% BSA and counted.

NPCs were subjected to flow cytometry analysis after staining with the respective antibodies. Within the $CD45^+$ population, macrophages were identified as $Ly6G^-/CD11b^+/F4/80^+$, monocytes as $Ly6G^-/CD11b^+/F4/80^-$ and neutrophils as $CD11b^+/Ly6G^+$. Within the $CD3^+$ population, $CD4^+$ and $CD8^+$ cells were identified. The cells were stained with anti-mouse antibodies, against CD3 (clone 145-2C11), CD4 (clone GK1.5), CD8 (clone 53-6.7), CD45 (clone 30-F11), CD11b (clone M1/70), F4/80 (clone BM8) and Ly6G (clone 1A8). Corresponding isotype control stainings were performed using the following antibodies: American hamster IgG for PeCy7, Rat IgG2b κ for APC, Rat IgG2b κ for PerCP, Rat IgG2a κ for PeCy7, Rat IgG2b κ for PerCP, Rat IgG2b κ for APC and Rat IgG2b κ control for Alexa488. Antibodies were purchased from BD Biosciences, eBioscience and Biolegend. Measurement was done using the FACS Canto II flow cytometer and the data were analyzed using FlowJo (Tree Star) software.

Histological analysis of mouse liver

PFA-fixed and paraffin-embedded liver sections were stained with hematoxylin/eosin (H&E). Briefly, sections were deparaffinized and rehydrated by subsequent submersions in the following solutions: xylol (twice) for 10 min each, 96% ethanol (twice), 70% ethanol, 40% ethanol and aqua dest for 1 min each. Sections were stained for 5 min in Hematoxylin solution (Thermo Fisher Scientific) and submerged in running tap water for 5 min. Thereafter, the slides were stained with Eosin-Y solution for 10 seconds and washed in tap water for 2 min. Finally, the slides were dehydrated by 1

min incubation in 40% ethanol, 70% ethanol, 96% ethanol (three times) and 100% ethanol (twice). Finally, slides were submerged in xylol (twice) for 2 min each and then mounted with Richard-Allan Scientific Cytoseal XYL (Thermo Fisher Scientific) mounting medium.

For Picro Sirius red staining, the slides were deparaffinized and stained in hematoxylin for 8 min. The slides were then washed in tap water and stained in Picro Sirius red solution for 1 h. Next, the slides were shortly washed in 30% acetic acid and treated with 96% ethanol (twice) for 4 min each, followed by isopropanol for 4 min. Finally, slides were submerged in xylol solution and then mounted with mounting medium.

For Oil red O staining of the liver, cryo-sections were used. First, the sections were fixed in 10% formaldehyde for 30 min and then washed in PBS. The slides were then incubated in Oil red O solution for 10 min. Then, the slides were washed in 60% isopropanol and followed by PBS. Then, the slides were stained in Mayer's Hematoxylin for 30 sec and washing in tap water. The slides were mounted with VectaMount (TM) Mounting Medium. Images were taken using the Axio Observer.Z1 microscope (Carl Zeiss). An analyser slider with lambda-plate (Zeiss) for circular polarized light was used for imaging of Picro Sirius red-stained slides. ImageJ software was used for quantification of Picro Sirius red staining.

Genomic DNA isolation and detection of Mboat7 exon 5 deletion

To detect the *Mboat7* exon 5 deletion, genomic DNA was isolated from primary mouse hepatocytes using QIAamp DNA micro kit (Qiagen). PCR was performed using DreamTaq Green PCR master mix (Thermo Fisher Scientific) with *Mboat7* exon 5 flanking primers 5'CAS-F1 (AAGGCGCATAACGATACCAC) and 3'LOXP-R1 (ACTGATGGCGAGCTCAGACC). After amplification, the PCR product was run on a

2% agarose (Serva) gel together with 50 bp and 100 bp ladder (Generuler DNA ladders, Thermo Fisher Scientific). The gel was imaged on Peqlab "Quantum-ST4-1100".

Hepatic hydroxyproline quantification

Hydroxyproline (HYP) concentration was determined biochemically using a Hydroxyproline Assay Kit (Sigma-Aldrich, Darmstadt, Germany), according to the manufacturer's protocol. Briefly, liver tissue (10-20 mg) was hydrolyzed in 600 µl of 6N HCl at 110^0 C for 16 h and then cleared from insoluble by passing through a 0.2 µm nylon filter. Standard concentrations of all-trans HYP were processed in the same way and used to establish a calibration curve. 50 µl aliquots of hydrolysates or standards were dried under a nitrogen stream and the residues were solubilized in chloramine T for 25 min at RT. This was followed by incubation with freshly prepared Erlich's reagent for 20 min at 65^0 C. Finally, HYP concentration was determined by the reaction of oxidized hydroxyproline with 4-(dimethylamino) benzaldehyde, which results in a colorimetric (560 nm) product proportional to the hydroxyproline present. OD values were plotted on a calibration curve to calculate the hepatic HYP concentrations.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity assays

ALT and AST activity assays (Sigma-Aldrich) were performed in serum samples according to kit protocol.

Multi-spot immunoassay

Multi-spot assay was performed using Mouse proinflammatory panel 1 kit (Meso Scale Discovery) to measure IFNγ, IL1β, IL2, IL4, IL5, IL6, KC/GRO, IL10, IL12p70 and

TNFα protein levels. Tissue homogenates were used to perform the assay according to kit protocol.

ANGPTL3 ELISA

ANGPTL3 ELISA (ThermoFisher Scientific) was performed in serum samples according to kit protocol.

Protein quantification

Whole liver lysates were prepared by disrupting the tissue in RIPA buffer supplemented with cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail and PhosSTOP phosphatase inhibitor cocktail (Sigma Aldrich). Protein concentration was measured using BCA protein assay kit (ThermoFisher Scientific). TIMP1 (Abcam), eIF2α (Cell Signaling), peIF2α (Ser51, Cell signaling), CHOP (Santa Cruz), ATF4 (Cell Signaling) and vinculin (Cell Signaling) antibodies were used for immunoblotting. Imaging was performed using the UV Imager FusionFX7 with the Fusion software. Vinculin was used for normalization. Analysis was performed using ImageJ software

Primary hepatocyte isolation

Primary hepatocyte isolation was performed according to a previously published protocol [10]. Briefly, 8-week old mice were anesthetized and the liver was perfused via the inferior vena cava and portal vein using a pump which allows perfusion at a flow rate of 5 ml/min, while the buffers are maintained at 37° C using a water bath. First, the liver was perfused with HBSS solution (containing 0.5 mM EDTA) for 7 minutes, followed by DMEM (ThermoFisher Scientific) containing 1 mg/ml Collagenase type 1 (ThermoFisher Scientific) for 10 minutes. The digested liver was excised and shaken gently in DMEM during which the hepatocytes dissociate. The isolated

hepatocytes were passed through a 100 µm cell strainer and washed twice at 4° C. Cells were resuspended in DMEM/F-12 medium (ThermoFisher Scientific) supplemented with 10% FBS, 1% Penicillin/Streptomycin and L-glutamine. Cells were seeded in 10% collagen (from calfskin, Sigma Aldrich) coated cell culture plates. For Oil Red O staining of hepatocytes, cells were fixed in 10% formaldehyde. Next cells were then washed in water, 60% isopropanol and allowed to dry. Cells were incubated with freshly prepared Oil Red O solution for 1 h at room temperature. Finally, the cells were washed in tap water three times and imaged.

Mouse and Human lipidomic analysis

Overview of lipidomic analysis

Approximately 25 mg of liver tissue was homogenized in 300 µL of neat isopropanol and the protein concentration was determined by BCA assay (Thermo Fisher Scientific, Rockford IL). Lipids were extracted from aliquots containing an equivalent of 50 µg of total protein and quantified by shotgun lipidomics as described [11,12]; For more detail see below. For oxylipin and free fatty acid measurement, approximately 30 mg of liver tissue was used for sample preparation using LC/ESI-MS/MS at Lipidomix GmbH (Berlin, Germany) as described in a separate section below. The oxylipins and fatty acids were normalized to total protein concentration.

Lipid extraction from mouse and human liver tissues

Approximately 25 mg of liver tissue was homogenized in 300 µL of neat isopropanol and the protein concentration was determined by BCA assay (Thermo Fisher Scientific, Rockford IL). Aliquots containing an equivalent of 50 µg of total protein were used for lipid extraction [11,12]. After evaporation of the organic phase, lipid extracts were

reconstituted in 400 µL of 2:1 MeOH / $CHCl_3$ for mouse samples and in 600 µl 2:1 MeOH/CHCl3 for human samples and stored at -20° C. 10 µl of liver lipid extract was diluted with 90 µL spray solution (4:2:1 IPA/MeOH/$CHCl_3$ + 7.5 mM ammonium formate) for mass spectrometric analysis.

Human lipidomic data processing

The lipidomics data generated from the human liver samples were quality-checked according to the following steps. First, selected samples were chosen for the pilot run. The goal of the selection was to find the extreme of results (i.e. Extremely fatty and non-fatty samples).

Sample where ranked from fatty to non-fatty samples, in order for the lipid profile of these samples to be used to distinguish between fatty and non-fatty conditions. To consider all the features and not just the fatty feature, a regression analysis was performed, specifically gradient boosted tree as these are well suited for ranking models, the initial data was numerically formatted by covering categorical data into values, e.g. male, female to 1,0 and by removing incomplete entries, additionally a feature was added based on the concurrence of multiple factors, e.g. fatty, inflamed and fibrosis. Later for the full cohort run, 36 samples from the pilot were pooled together in order to create a single quality control (QC) sample. A QS sample was added to each batch to control for inter-batch variation and for the instrument stability. Variation of the measurements across all the batches is considered acceptable (e.g. QC standard deviation was 15% for neutral lipids, and 24% for phospholipids). Each sample was measured twice, and technical replicates were retrieved by splitting a sample lipid extract in running solution into two separate wells. The noise in spectra was reduced based on a repetition rate filtering performed with the PeakStrainer. Lipid

identification was performed with the LipidXplorer v. 1.2.4. The quantification of lipid species, based on known concentrations of internal standards, was performed using in-house scripts. Lipid species for which the internal standard signal was detected but the signal for the species itself was not detected were set to zero. All internal standards were detected in all the samples. The obtained lipidomics dataset underwent two further filters. First, for any given lipid species, when the variation between technical replicates was below 40% the respective mean was taken, otherwise the value was set to NaN. To avoid loss of measurements close to the detection limit, one exception to this first filter has been applied: If a lipid species detected in only one of the technical replicates has a measured value smaller or equal to twice the minimum detected for the respective lipid species (i.e. value ≤ 2*min(species)), this non-zero value has been rescued. Second, a lipid species was set to zero in all samples of one patient group (normal control, healthy obese, NAFL, NASH) if present in less than 15% of the samples of this group.

Annotation of lipid classes and species

The measured glycerolipids are triacylglycerols (TG) and diacylglycerols (DG); the glycerophospholipids and lyso-glycerophospholipids are phosphatidic acids (PA), phosphatidylinositols (PI), phosphatidylserines (PS), phosphatidylglycerols (PG), phosphatidylethanolamines (PE), phosphatidylcholines (PC), ether phosphatidylethanolamines (PE *O*-), ether phosphatidylcholines (PC *O*-), lyso-phosphatidic acids (LPA), lyso-phosphatidylinositols (LPI), lyso-phosphatidylcholines (LPC) and lyso-phosphatidylethanolamines (LPE); the sphingolipids are ceramides (Cer) and sphingomyelins (SM); the sterols are cholesterol (Chol) and cholesterol ester (CE). Glycero- and glycerophospholipid species were annotated as previously

described [13] using the total number of carbon atoms: total number of double bonds in both fatty acid / fatty alcohol moieties. Sphingolipid species were annotated by the total number of carbon atoms: double bonds: hydroxyl groups at the ceramide backbone.

Common chemicals and lipid standards

All solvents were of LC-MS or better grade. Synthetic lipid standards were purchased from Avanti Polar Lipids (Alabaster AL). Stocks of internal standards were stored in sealed glass ampoules at -20° C until they were used for preparing the internal standard mix in 10:3 methyl-*tert*-butyl ether (MTBE) / methanol (MeOH). 700 µL of the internal standard mix for mouse samples contained: 1778 pmol of D_7-cholesterol; 2080 pmol of D_7- CE 16:0; 1000 pmol of D_5-TG 50:0; 465 pmol of D_5-DG 34:0; 1321 pmol of PC 25:0; 386 pmol of LPC 13:0; 590 pmol of PE 25:0; 85 pmol of LPE 13:0; 240 pmol of PI 25:0, 164 pmol of PG 25:0; 73 pmol of Cer 30:1:2; 185 pmol of PA 25:0; 91 pmol of LPA 13:0; 271 pmol of SM 30:1:2; 32 pmol of LPI 13:0, 160 pmol of PS 25:0, 59 pmol of LPS 13:0 and 75 pmol of LPG 13:0.

700 µL of the internal standard mix for human samples contained: 1778 pmol of D_7-cholesterol; 2215 pmol of D_7- CE 16:0; 1041 pmol of D_5-TG 50:0; 595 pmol of D_7-DG 33:1; 1376 pmol of PC 25:0; 386 pmol of LPC 13:0; 589 pmol of PE 25:0; 85 pmol of LPE 13:0; 480 pmol of PI 25:0, 140 pmol of PG 25:0; 73 pmol of Cer 30:1:2; 127 pmol of D_7-PA 33:1; 78 pmol of U-^{13}C LPA 16:0; 271 pmol of d18-SM 30:1:1; 64 pmol of LPI 13:0, 137 pmol of PS 25:0, 74 pmol of LPS 13:0; 75 pmol of LPG 13:0 and 137 pmol of CL 56:4.

Lipids quantification by shotgun mass spectrometry

The mass spectrometric analysis was performed on a Q Exactive instrument (Thermo Fischer Scientific, Bremen, Germany) equipped with a robotic nanoflow ion source TriVersa NanoMate (Advion BioSciences, Ithaca NY) using nanoelectrospray chips with a spraying nozzle diameter of 4.1 µm. The ion source was controlled by the Chipsoft 8.3.1 software (Advion BioSciences). Ionization voltage was + 0.96 kV in positive and − 0.96 kV in negative mode; backpressure was 1.25 psi in both modes [14]. The temperature of the ion transfer capillary was 200° C; *S*-lens RF level was 50%. Each sample was analyzed for 11 min. FT MS spectra were acquired within the range of *m/z* 400 – 1000 from 0 min to 0.2 min in positive and within the range of *m/z* 350 – 1000 from 6.2 min to 6.4 min in negative mode at the mass resolution of $R_{m/z\ 200}$=140000; automated gain control (AGC) of 3×10^6 and with the maximal injection time of 3000 ms. *t*-SIM in positive (1.7 to 6 min) and negative (6.4 to 11 min) mode was acquired with $R_{m/z\ 200}$=140000; automated gain control of 5×10^4; maximum injection time of 650 ms; isolation window of 20 Th and scan range of *m/z* 400 to 1000 in positive and *m/z* 350 to 1000 in negative mode, respectively. The inclusion list of masses targeted in *t*-SIM analyses started at *m/z* 355 in negative and *m/z* 405 in positive ion mode and other masses were computed by adding 10 Th increment (*i.e. m/z* 355, 365, 375) up to *m/z* 1005.

Free cholesterol was quantified by parallel reaction monitoring FT MS/MS within the time range of 0.2 to 1.7 min. For FT MS/MS the number of micro scans was set to 1; precursor isolation window: 0.8 Da, normalized collision energy (nCE):12.5%; AGC: 5×10^4 and maximum injection time: 3000 ms. Spectra were pre-processed using repetition rate filtering software PeakStrainer [15] and stitched together by an in-house

developed script [16]. Lipids were identified by LipidXplorer software [17]. Molecular Fragmentation Query Language (MFQL) queries were compiled for PC, PC *O*-, LPC, LPC *O*-, PE, PE *O*-, LPE, PI, LPI, PG, LPG, PA, LPA, PS, LPS, SM, TG, DG, Cer, Chol and CE lipid classes. Lipids were identified by matching of accurately determined intact masses (mass accuracy better than 5 ppm) and quantified by comparing isotopically corrected abundances of their molecular ions with abundances of internal standards of the same lipid class.

Oxylipin measurement

Sample preparation

Lipidomix GmbH, Berlin performed the oxylipin measurement. Approximately 30 mg of liver tissue was homogenized and spiked with an internal standard consisting of 15-HETE-d8, 14,15-DHET-D11, 14,15-EET-d8, LTB4-d4, 20-HETE-d6, PGE2-d2 (10 ng each; Cayman Chemical, Ann Arbor, USA); this was followed by the addition of 500 µL of methanol and 300 µL of 10 M sodium hydroxide solution and was shook vigorously for 30 min at 60° C for alkaline hydrolysis. The samples were brought to pH 6 with 500 µL of 1 M sodium acetate buffer and acetic acid for hydrolyzed samples. Then, the samples were centrifuged and the supernatant was added to Bond Elute Certify II columns (Agilent Technologies, Santa Clara, USA) for solid-phase extraction; the columns were preconditioned with 3 ml methanol, followed by 3 ml of 0.1 mol/L phosphate buffer containing 5 % methanol (pH 6). The columns were washed with 3 mL methanol/H2O (50/50, vol/vol). For elution, 2 ml of n-hexane: ethyl acetate 25:75 with 1 % acetic acid was used. An SPE Vacuum Manifold was used for extraction. A heating block at 40° C was used to evaporate the eluate under a stream of nitrogen to

obtain solid residues. This was dissolved in 70 µl of acetonitrile/water 50:50 and transferred to an HPLC autosampler vial.

LC/ESI-MS/MS

The residues were analyzed using an Agilent 1290 HPLC system with a multi-sampler, binary pump, column thermostat (with a Zorbax Eclipse plus C-18, 2.1 x 150 mm), 1.8 µm column using a solvent system of aqueous acetic acid (0.05%) and acetonitrile / methanol (50:50). The elution gradient was started with 5 % organic phase, which was increased within 0.5 min to 56, 5.5 min to 61%, 18.5 min to 87%, 18.6 min to 98% and held there for 6.5 min. The flow rate was 0.3 ml/min and the injection volume were 15 µL. The HPLC was coupled with an Agilent 6490 Triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, USA) with electrospray ionization source. The following source parameters were applied: drying gas: 115° C / 16 L / min, sheath gas: 390° C / 12 L /min, capillary voltage: 4300 V, nebulizer pressure: 35 psi and nozzle voltage: 1950 V. The analysis was performed with multiple reaction monitoring in negative mode. Further details are given below.

Compound name	Precursor ion (m/z)	Product ion (m/z)	Collision energy	Ret time (min)
17,18-DiHETE	335.2	87	20	10.246
17,18-DiHETE	335.2	247.2	17	10.246
LTB4-D4	339.2	197.1	16	10.346
14,15-DiHETE	335.2	127	18	10.646
14,15-DiHETE	335.2	207.1	18	10.646
11,12-DiHETE	335.2	167.1	16	10.836
11,12-DiHETE	335.2	225.1	16	10.836
8,9-DiHETE	335.2	127.1	21	11.225
8,9-DiHETE	335.2	185.2	16	11.225
12,13-DiHOME	313.2	129.1	22	11.375
12,13-DiHOME	313.2	183.2	20	11.375
9,10-DiHOME	313.2	171.2	24	11.755
9,10-DiHOME	313.2	201.1	21	11.755
5,6-DiHETE	335.2	145	16	12.065
14,15-DHET-D11	348.3	207.1	20	12.175
14,15-DHET	337.2	129.1	22	12.305
14,15-DHET	337.2	207.2	17	12.305
19,20-DiHDPA	361.2	87	19	12.505
19,20-DiHDPA	361.2	273.3	15	12.505
16,17-DiHDPA	361.2	189.1	18	12.845
16,17-DiHDPA	361.2	233.2	15	12.845
11,12-DHET	337.2	167.2	18	12.945
11,12-DHET	337.2	197.2	18	12.945
19-HEPE	317.2	229.2	14	12.975
19-HEPE	317.2	255.2	12	12.975
13,14-DiHDPA	361.2	149.1	18	13.025
13,14-DiHDPA	361.2	193.2	16	13.025
20-HEPE	317.2	243.2	14	13.045
20-HEPE	317.2	287.2	14	13.045
18-HEPE	317.2	215.2	13	13.375
18-HEPE	317.2	259.2	9	13.375
8,9-DHET	337.2	127	22	13.525
8,9-DHET	337.2	185.2	16	13.525
15-HEPE	317.2	175.1	13	13.805
15-HEPE	317.2	219.2	11	13.805
19-HETE	319.2	231.2	18	13.985

Compound name	Precursor ion (m/z)	Product ion (m/z)	Collision energy	Ret time (min)
19-HETE	319.2	275.2	16	13.985
8-HEPE	317.2	155.1	13	14.025
8-HEPE	317.2	161.1	17	14.025
7,8-DiHDPA	361.2	113.1	18	14.075
7,8-DiHDPA	361.2	127.1	16	14.075
20-HETE-d6	325.2	295.2	18	14.125
20-HETE	319.2	245.2	16	14.175
20-HETE	319.2	289.2	19	14.175
12-HEPE	317.2	135.1	13	14.205
12-HEPE	317.2	179.1	13	14.205
9-HEPE	317.2	149.1	13	14.335
9-HEPE	317.2	167.1	13	14.335
5,6-DHET	337.2	145.1	16	14.335
18-HETE	319.2	217.2	16	14.405
18-HETE	319.2	261.2	16	14.405
17-HETE	319.2	203.2	16	14.465
17-HETE	319.2	247.2	14	14.465
5-HEPE	317.2	115.1	11	14.655
5-HEPE	317.2	201.1	13	14.655
16-HETE	319.2	233.2	14	14.725
16-HETE	319.2	257.2	14	14.725
21-HDHA	343.2	255.2	14	14.97
21-HDHA	343.2	299.2	11	14.97
22-HDHA	343.2	269.2	13	15.01
22-HDHA	343.2	313.2	12	15.01
13-HODE	295.2	195.2	18	15.06
17,18-EEQ	317.2	215.1	12	15.21
17,18-EEQ	317.2	259.2	10	15.21
20-HDHA	343.2	227.2	15	15.26
20-HDHA	343.2	241.2	11	15.26
15-HETE-d8	327.2	226.2	11	15.26
15-HETE	319.2	121.1	16	15.46
15-HETE	319.2	219.2	11	15.46
16-HDHA	343.2	233.2	11	15.56
17-HDHA	343.2	201.1	13	15.59
17-HDHA	343.2	245.2	10	15.59

Compound name	Precursor ion (m/z)	Product ion (m/z)	Collision energy	Ret time (min)
14,15-EEQ	317.2	207.1	11	15.76
14,15-EEQ	317.2	219.1	9	15.76
13-HDHA	343.2	193.2	12	15.76
13-HDHA	343.2	221.2	10	15.76
11-HETE	319.2	149.1	22	15.83
11-HETE	319.2	167.1	14	15.83
14-HDHA	343.2	161.1	13	15.86
14-HDHA	343.2	205.1	11	15.86
11,12-EEQ	317.2	167.1	11	15.86
11,12-EEQ	317.2	179.1	9	15.86
10-HDHA	343.2	153.1	14	15.91
10-HDHA	343.2	181.1	11	15.91
8,9-EEQ	317.2	127.1	13	15.93
8,9-EEQ	317.2	155.1	9	15.93
8-HETE	319.2	127.1	22	16.12
8-HETE	319.2	155.1	12	16.12
5,6-EEQ	317.2	189.1	11	16.13
12-HETE	319.2	135.1	14	16.14
12-HETE	319.2	179.2	13	16.14
11-HDHA	343.2	121.1	13	16.14
11-HDHA	343.2	149.1	12	16.14
7-HDHA	343.2	113.1	18	16.31
7-HDHA	343.2	141.1	11	16.31
9-HETE	319.2	123.1	15	16.44
9-HETE	319.2	151.1	13	16.44
8-HDHA	343.2	109	12	16.54
8-HDHA	343.2	189.1	11	16.54
5-HETE	319.2	115.1	14	16.74
5-HETE	319.2	191.2	14	16.74
19,20-EDP	343.2	241.2	11	17.006
19,20-EDP	343.2	285.2	7	17.006
12,13-EpOME	295.3	183	18	17.106
12,13-EpOME	295.3	195.2	16	17.106
4-HDHA	343.2	101	13	17.206
4-HDHA	343.2	133.1	14	17.206
9,10-EpOME	295.3	171.2	16	17.236

Compound name	Precursor ion (m/z)	Product ion (m/z)	Collision energy	Ret time (min)
9,10-EpOME	295.3	183	22	17.236
14,15-EET	319.2	203.2	14	17.256
14,15-EET	319.2	219.2	9	17.256
16,17-EDP	343.2	201.1	10	17.506
16,17-EDP	343.2	233.2	9	17.506
13,14-EDP	343.2	193.1	9	17.606
13,14-EDP	343.2	234.2	7	17.606
10,11-EDP	343.2	153.1	11	17.706
10,11-EDP	343.2	190.1	7	17.706
8,9-EET-d11	330.3	268.2	12	17.826
11,12-EET	319.2	167.1	14	17.886
11,12-EET	319.2	179.1	12	17.886
7,8-EDP	343.2	109.1	16	18.006
7,8-EDP	343.2	189.2	10	18.006
5,6-EET	319.2	191.2	9	18.006
8,9-EET	319.2	127.1	15	18.056
8,9-EET	319.2	155.1	11	18.056

Free fatty acid measurement

Free fatty acid measurement was performed by Lipidomix GmbH, Berlin. 30 mg of liver tissue was homogenized in citrate buffer and extracted following Folch's procedure. Extracts were evaporated under nitrogen and dissolved in 100 µL ethanol. An aliquot was diluted 1:10 with isopropanol containing internal standards (C15:0, C21:0 1000 ng/mL, C20:4-d8, C18:2-d4 100 ng/mL, C20:5-d5, C22:6-d5 20 ng/mL Cayman Chemical, Ann Arbor MI). HPLC-measurement was performed using an Agilent 1290 HPLC system with a binary pump, autosampler, and column thermostat equipped with a Phenomenex Kinetex-C18 column 2.6 µm, 2.1 x 150 mm column (Phenomenex, Aschaffenburg, DE), using a solvent system of acetic acid (0.05%) and acetonitrile. All solvents and buffers used were in LC-MS-grade (VWR, Germany). The solvent

gradient started at 70 % acetonitrile and was increased to 98 % within 10 min and hold until 14 min with a flow rate of 0.4 mL/min and 5 µL injection volume. The HPLC was coupled with an Agilent 6470 triple quad mass spectrometer with electrospray ionization source operated in negative selected ion mode (parameters are given below).

Compound	Internal standard	Mass	Fragmentor
C 12:0		199	140
C 14:1		225	140
C 14:0		227	140
C 15:0	x	241	140
C 16:1		253	140
C 16:0		255	140
C 18:3		277	140
C 18:2		279	140
C 18:1		281	140
C 18:2-d4	x	283	140
C 18:2-d4	x	283	140
C 18:0		283	140
C 20:5		301	140
C 20:4		303	140
C 20:3		305	140
C 20:2		307	140
C 20:1		309	140
C 20:5-d5	x	306	140
C 20:0		311	140
C 20:4-d8	x	311	140
C 21:0	x	325	140
C 22:6		327	140
C 22:5		329	140
C 22:6-d5	x	332	140
C 22:0		339	140
C 22:1		337	140
C 24:0		367	140
C 24:1		365	140

Ion source parameters

Gas Temp	210° C
Gas Flow	7 L/min
Nebulizer press	45 psi

Sheath Gas Temp	350° C
Sheath Gas Flow	11 L/min
Capillary voltage	4000 V
Nozzle voltage	1500 V

The oxylipins and fatty acids were normalized to the total protein concentration. The data was Log_2 transformed and the Shapiro test was applied to check for normal distribution. When normality was passed, Welch's t-test was performed and when it did not pass normality Wilcoxon test was performed. Benjamini-Hochberg correction was used to further adjust the P values. The analysis was performed using R 3.6.0.

RNA sequencing

Library preparation

Total RNA with an integrity number between 8-9 was used. mRNA was isolated from 1 µg of total RNA by poly-dT enrichment using the NEBNext Poly(A) mRNA Magnetic Isolation module, according to the manufacturer's instructions. The final elution was done in 15 µl of 2x first-strand cDNA synthesis buffer (NEBnext, NEB). After chemical fragmentation, by incubating the samples for 15 min at 94° C, the samples were subjected to the workflow for strand-specific RNA-Seq library preparation (Ultra Directional RNA Library Prep, NEB). For ligation, custom adaptors were used (Adaptor-Oligo 1: 5'-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT-3', Adaptor-Oligo 2: 5'-P-GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT CAC-3'). After ligation, adapters were depleted using an XP bead purification system (Beckman Coulter) by adding beads in a ratio of 1:1, followed by an index PCR (15 cycles) using Illumina compatible index primer. After double XP bead purification (with beads added in a ratio 1:1), libraries were quantified on a Fragment Analyser run with an NGS Assay

Kit (Agilent) and loaded on a Nextseq500 flow cell with 75 cycles by pooling the samples in respect of their molarity aiming at 30 million reads per sample.

Data processing and analysis of RNA sequencing data

Reads were trimmed with flexbar v3.5.0 [18] for adapter contamination. The reads were then aligned to the mouse genome reference GRCm38 with the STAR v2.6.1d [19] and the per-sample 2-pass mapping strategy processing all reads in both passes as described in the documentation. Read counts were summarized to Gencode gene models vM21 [20] with featureCounts v1.6.1 [21] counting primary alignments only. EdgeR (v3.16.5) was used to detect differentially expressed genes with maximal FDR of 0.05 and minimal absolute logFC of 0.5. We discarded genes for which fewer than three samples had counts per million value above 1, calculated normalization factors and robustly estimated the dispersion. Gene Trail 2 1.6 was used to check the enrichment pathways of the deregulated genes.

To perform gene set enrichment analysis (GSEA), differential gene expression data (containing all expressed genes) were ranked using the -log10 transform of the p-value and then signed as positive or negative based on the direction of fold change. Then, the GSEA software (Broad Institute) was used to perform the GSEA pre-ranked analysis (1000 permutations, minimum term size of 15 and maximum term size of 500) [22,23]. As input, the annotated gene sets from Molecular Signatures Database (MSigDB) were used, specifically the GO_Extra_Celluar_Matrix gene set and the Hallmark (v6.2) gene sets [24–26]. Heat maps were generated using the web-based tool Morpheus (https://software.broadinstitute.org/morpheus).

Statistical analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Inc., La Jolla, CA, USA) software. For the comparison of quantitative measurements, the Mann-Whitney U test or Unpaired t test was used. Data are expressed as mean ± standard error of the mean (SEM).

Oxylipins and free fatty acids were normalized to total protein concentration and analysed using R 3.6.0. Data was Log_2 transformed and Shapiro test was applied to check for normal distribution. When normality was passed, Welch's t-test was performed and if not, then Wilcoxon test was performed. Benjamini-Hochberg correction was used to further adjust the P values.

Genetic analyses were calculated using an additive model. Differences between the groups were compared by logistic regression analysis adjusted for sex, age, BMI and presence of T2DM (Supplementary Table 1).

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SUPPLEMENTARY TABLES

Supplementary Table 1: Patient samples used for genetic analysis (NAFLD Cohort)

Demographic, clinical and histological characteristics of the patient samples for the analysis of the phenotypic impact of MBOAT7 rs641738 genotype on NAFLD phenotype. The data for the two patient strata based on BMI are provided. Quantitative parameters are provided as median with interquartile range in brackets.

	BMI ≤ 35	**BMI > 35**
n	361	485
Sex (% males)	69.3%	32.4%
Age	54 [43-62]	43 [34-51]
BMI	28.0 [25.7-30.4]	48.7 [43.6-53.5]
Diabetes Type 2	32.1%	36.5%
Liver fat (percent)	25 [10-50]	30 [10-60]
NAS fat	1 [1-2]	1 [1-2]
NAS ballooning	0 [0-1]	0 [0-1]
NAS inflammation	0 [0-0]	0 [0-1]
NAS score	2 [1-3]	2 [1-3]
Fibrosis stage 0 (n)	225	295
Fibrosis stage 1-3 (n)	109	179
Fibrosis stage 4 (n)	27	11
ALT (IU/L)	39 [23-70]	33 [22-49]
AST (IU/L)	32 [24-51]	26 [21-36]
GGT (IU/L)	62 [32-133]	36 [24-51]
rs641738 (CC/CT/TT)	110/178/73	136/243/106
rs641738 MAF (T)	0.45	0.47

Supplementary Table 2: Patient samples used for genetic analysis (NAFLD Cohort)

Additional demographic, clinical and histological characteristics of the patient samples for the analysis of the phenotypic impact of MBOAT7 rs641738 genotype on NAFLD phenotype. Values are given as mean with standard error in parentheses. *ANOVA; **Pearson Chi-Square; *** Test of deviation from Hardy-Weinberg-equilibrium

Total (n=846)	***MBOAT7* rs641738 (CC)**	***MBOAT7* rs641738 (CT)**	***MBOAT7* rs641738 (TT)**	**P value (CC) vs (CT) vs (TT)**
n	246	421	179	0.964***
Sex (% females)	49%	53%	53%	0.602**
Age	46.07 (0.82)	47.80 (0.66)	47.21 (0.94)	0.260*
BMI	40.18 (0.83)	39.94 (0.59)	40.72 (0.89)	0.780*
Diabetes Type 2	30%	37%	36%	0.145**
Liver fat (percent)	32.41 (1.65)	34.41 (1.28)	36.01 (2.11)	0.371*
NAS fat	1.55 (0.05)	1.61 (0.04)	1.63 (0.06)	0.526*
NAS ballooning	0.26 (0.03)	0.37 (0.03)	0.34 (0.04)	0.063*
NAS inflammation	0.31 (0.04)	0.30 (0.03)	0.32 (0.04)	0.897*
NAS score	2.10 (0.09)	2.27 (0.07)	2.28 (0.11)	0.288*
Fibrosis stage	0.57 (0.06)	0.72 (0.05)	0.78 (0.09)	0.095*
Fibrosis stage BMI ≤ 35 (n=361, 43%)	0.60 (0.11)	0.84 (0.09)	1.14 (0.17)	0.020*
Fibrosis stage BMI > 35 (n=485, 57%)	0.54 (0.08)	0.63 (0.06)	0.54 (0.08)	0.578*
ALT (IU/L)	44.81 (2.55)	43.01 (1.96)	39.96 (3.24)	0,487*
AST (IU/L)	32.45 (1.31)	37.75 (2.98)	31.71 (1.75)	0.207*
GGT (IU/L)	65.53 (6.99)	77.86 (10.81)	44.41 (3.47)	0.094*

Supplementary Tables 3: High fat choline deficient methionine low diet (HFCDD) composition

High fat choline deficient methionin low diet (A06071302, Research Diet)		
Class description	**Ingredients**	**Grams**
Protein	Glutamic Acid, L	38.20 g
Protein	Proline, L	17.80 g
Protein	Leucine, L	15.80 g
Protein	Lysine, L, HCl	13.20 g
Protein	Aspartic Acid, L	12.10 g
Protein	Serine, L	10.00 g
Protein	Valine, L	9.30 g
Protein	Tyrosine, L	9.20 g
Protein	Phenylalanine, L	8.40 g
Protein	Isoleucine, L	7.60 g
Protein	Threonine, L	7.20 g
Protein	Arginine, L	6.00 g
Protein	Alanine, L	5.10 g
Protein	Histidine, L, HCl, H2O	4.60 g
Protein	Cystine, L	4.20 g
Protein	Glycine	3.00 g
Protein	Tryptophan, L	2.10 g
Protein	Methionine, L	0.80 g
Carbohydrate	Lodex 10	130.10 g
Carbohydrate	Sucrose, Fine Granulated	72.80 g
Fiber	Solka Floc, FCC200	50.00 g
Fat	Lard	245.00 g
Fat	Soybean Oil, USP	25.00 g
Mineral	Calcium Phosphate, Dibasic,Calcium Carbonate, Sodium Chloride, Magnesium Sulfate, Heptahydrate, Magnesium Oxide, Ferric Citrate, Manganese Carbonate Hydrate, Zinc Carbonate, Chromium Potassium Sulfate, Copper Carbonate, Ammonium Molybdate Tetrahydrate, Sodium Fluoride, Sodium Selenite, Potassium Iodate. (Ref. S10026B rResearch Diet)	50.00 g
Mineral	Sodium Bicarbonate	7.50 g

Vitamin	Vitamin E Acetate, Niacin, Biotin, Pantothenic Acid, Vitamin D3, Vitamin B12, Mannitol, Vitamin A Acetate, Pyridoxine HCl, Riboflavin, Thiamine HCl, Folic Acid, Menadione Sodium Bisulfite. (Ref. V10001C Research diet)	1.00 g
Dye	Dye, Blue FD&C #1, Alum. Lake 35-42%	0.03 g
Dye	Dye, Red FD&C #40, Alum. Lake 35-42%	0.03 g
	Total:	756.05 g
	Caloric information of physiological fuel values	
	Protein	18%Kcal
	Fat	62%Kcal
	Carbohydrate	21%Kcal
	Energy density	5.21Kcal/g

Supplementary Table 4: Differential mRNA expression in 10-week old Mboat7$^{\Delta hep}$ mice livers

The table provides a list of differentially expressed transcripts between Mboat7$^{\Delta hep}$ and Mboat7^{WT} mice using a false discovery rate (FDR) of 0.05. The table content is ordered by FDR in decreasing order. logFC: log fold change.

Supplementary Table 5: Differential mRNA expression in Mboat7$^{\Delta hep}$ mice livers after 6 weeks of HFCDD feeding

The table provides a list of differentially expressed transcripts between Mboat7$^{\Delta hep}$ and Mboat7^{WT} mice using a false discovery rate (FDR) of 0.05. The table content is ordered by FDR in decreasing order. logFC: log fold change.

Supplementary Table 6: Hepatic oxylipin measurements on a chow diet

Hepatic-oxylipin concentrations in Mboat7^{WT} and Mboat7$^{\Delta hep}$ mice fed a chow diet for 10 weeks. Abbreviations are provided at the end of the table. The data were analyzed using R and the oxylipins were normalized to the total protein concentration. The data was Log2 transformed and Shapiro test was applied to check for normal distribution. When normality was passed, then Welch's t-test was performed and when it did not pass normality then Wilcoxon test was performed. The Benjamini-Hochberg correction was used to further adjust the P values [27].

Oxylipin species	Mboat7^{WT} (mean in ng/g total protein)	Standard deviation	Mboat7$^{\Delta hep}$ (mean in ng/g total protein)	Standard deviation	Adjusted p value
13-HODE	16858	2465	15628	1988	0.6299
9.10-EpOME	30422	9979	27444	8028	0.814
12.13-EpOME	42766	10442	43268	10666	0.9835
9.10-DiHOME	277	46	349	94	0.3796
12.13-DiHOME	394	42	510	111	0.2304
5.6-EET	14717	1794	14708	2567	0.9835
8.9-EET	6577	715	6596	1056	0.9921
11.12-EET	6515	1489	8141	2761	0.5411
14.15-EET	11137	2417	13908	5183	0.5796
5.6-DHET	375	61	378	48	0.9835
8.9-DHET	607	102	634	133	0.9327
11.12-DHET	427	121	511	222	0.7943
14.15-DHET	519	192	720	384	0.6095
5.6-EEQ	4858	1748	3749	1614	0.5411
8.9-EEQ	149	51	129	44	0.7279
11.12-EEQ	128	30	121	28	0.8831
14.15-EEQ	272	52	279	83	0.9835
17.18-EEQ	611	194	645	207	0.9307
5.6-DiHETE	55	10	56	7	0.8953
8.9-DiHETE	6	1	6	1	0.8421
11.12-DiHETE	7	2	8	2	0.6437
14.15-DiHETE	15	5	19	7	0.5464
17.18-DiHETE	42	14	52	9	0.4833
7.8-EDP	3751	576	4224	649	0.5363
10.11-EDP	4276	641	5337	639	0.2216
13.14-EDP	2591	439	3695	854	0.2216
16.17-EDP	5372	1263	8372	3694	0.3033
19.20-EDP	35323	4403	43605	11644	0.4833
7.8-DiHDPA	222	43	305	67	0.2304

Oxylipin species	Mboat7^{WT} (mean in ng/g total protein)	Standard deviation	Mboat7$^{\Delta hep}$ (mean in ng/g total protein)	Standard deviation	Adjusted p value
10.11-DiHDPA	107	19	163	34	0.1903
13.14-DiHDPA	168	46	270	112	0.2424
16.17-DiHDPA	353	138	601	334	0.3237
19.20-DiHDPA	407	82	547	127	0.2424
5-HETE	3761	243	3652	480	0.8221
8-HETE	1513	107	1543	272	0.9835
9-HETE	2451	319	2518	488	0.9835
11-HETE	3530	443	3551	601	0.9921
12-HETE	5489	3445	5679	3020	0.9737
15-HETE	5164	816	5095	862	0.9835
16-HETE	554	99	554	134	0.9835
17-HETE	142	37	174	49	0.5464
18-HETE	238	53	277	92	0.7279
19-HETE	302	56	310	91	0.9921
20-HETE	1063	134	1009	237	0.8007
LTB4	22	5	28	5	0.3033
12-HpETE	628	109	537	162	0.5411
5-HEPE	173	43	192	40	0.6555
8-HEPE	37	7	33	4	0.6008
9-HEPE	33	4	31	8	0.6437
12-HEPE	281	227	285	106	0.8421
15-HEPE	54	6	48	11	0.5604
18-HEPE	99	20	87	21	0.5604
19-HEPE	273	36	351	64	0.2304
20-HEPE	742	43	970	205	0.2304
4-HDHA	2209	134	2392	347	0.6095
7-HDHA	994	63	1307	185	0.1903
8-HDHA	1095	87	1416	245	0.2216
10-HDHA	777	61	970	188	0.2458
11-HDHA	1012	100	1289	228	0.2304
13-HDHA	1193	98	1480	258	0.2424
14-HDHA	711	95	894	184	0.2424
16-HDHA	1173	133	1420	243	0.2642
17-HDHA	1411	189	1690	297	0.3033
20-HDHA	3089	336	3690	611	0.2642
21-HDHA	23608	2486	25907	6604	0.8032
22-HDHA	2566	727	2135	744	0.5604
NPD x	11	1	12	2	0.5382

Oxylipin abbreviations: 13-HODE, 13-Hydroxyoctadecadienoic acid; 9.10-EpOME, 9(10)-Epoxy-12Z-octadecenoic acid; 12.13-EpOME, 12,13-epoxy-9(Z)-octadecenoic acid; 9.10-DiHOME, 9,10-dihydroxy-12Z-octadecenoic acid; 12.13-DiHOME, 12,13-dihydroxy-9Z-octadecenoic acid; 5.6-EET, 5,6-epoxy-8Z,11Z,14Z-eicosatrienoic acid; 8.9-EET, 8,9-epoxy-5Z,11Z,14Z-eicosatrienoic acid; 11.12-EET, 11,(12)-epoxy-5Z,8Z,14Z-eicosatrienoic acid; 14.15-EET, 14(15)-epoxy-5Z,8Z,11Z-eicosatrienoic acid; 5.6-DHET, 5,6-Dihydroxy-8,11,14-icosatrienoic acid; 8.9-DHET, 8,9-dihydroxy-5Z,11Z,14Z-icosatrienoic acid; 11.12-DHET, 11,12-dihydroxy-5Z,8Z,14Z-eicosatrienoic acid; 14.15-DHET, 14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid; 5.6-EEQ, 5,6-epoxy-8*Z*,11*Z*,14*Z*,17*Z*-eicosatetraenoic acid; 8.9-EEQ, 8,9-epoxy-5*Z*,11*Z*,14*Z*,17*Z*-eicosatetraenoic acid; 11.12-EEQ, 11,12-epoxy-5*Z*,8*Z*,14*Z*,17*Z*-eicosatetraenoic acid; 14.15-EEQ, 14,15-epoxy-5*Z*,8*Z*,11*Z*,17*Z*-eicosatetraenoic acid; 17.18-EEQ, 17,18-epoxy-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; 5.6-DiHETE, 5,6-dihydroxy-8Z,11Z,14Z,17Z-eicosatetraenoic acid; 8.9-DiHETE, 8,9-dihydroxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid; 11.12-DiHETE, 11,12-dihydroxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid; 14.15-DiHETE, 14,15-dihydroxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid; 17.18-DiHETE, 17,18-dihydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; 7.8-EDP, 7,8-epoxy-4*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosapentaenoic acid; 10.11-EDP, 10,11-epoxy-4*Z*,7*Z*,13*Z*,16*Z*,19*Z*-docosapentaenoic acid; 13.14-EDP, 13,14-epoxy-4*Z*,7*Z*,10*Z*,16*Z*,19*Z*-docosapentaenoic acid; 16.17-EDP, 16,17-epoxy-4*Z*,7*Z*,10*Z*,13*Z*,19*Z*-docosapentaenoic acid; 19.20-EDP, 19,20-epoxy-4*Z*, 7*Z*,10*Z*,13*Z*,16*Z*-docosapentaenoic acid; 7.8-DiHDPA, 7,8-dihydroxydocosa-4Z,10Z,13Z,16Z,19Z-pentaenoic acid; 10.11-DiHDPA, 10,11-dihydroxy-4Z,7Z,13Z,16Z,19Z-docosapentaenoic acid; 13.14-DiHDPA, 13,14-dihydroxy-4Z,7Z,10Z,16Z,19Z-docosapentaenoic acid; 16.17-DiHDPA, 16,17-dihydroxy-4Z,7Z,10Z,13Z,19Z-docosapentaenoic acid; 19.20-DiHDPA, 19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid; 5-HETE, 5-hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid; 8-HETE, 8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid; 9-HETE, 9-hydroxy-5*Z*,7*E*,11*Z*,14*Z*-eicosatetraenoic acid; 11-HETE, 11-hydroxy-5*Z*,8*Z*,11*E*,14*Z*-eicosatetraenoic acid; 12-HETE, 12-hydroxy-5,8,10,14-eicosatetraenoic acid; 15-HETE, 15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid; 16-HETE, 16-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; 17-HETE, 17-hydroxy-5Z,8Z,11Z,14Z-

eicosatetraenoic acid; 18-HETE, 18-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; 19-HETE, 19-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; 20-HETE, 20-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid; LTB4, Leukotriene B4; 12-HpETE, 12-hydroperoxy-5Z,8Z,10E,14Z-eicosatetraenoic acid; 5-HEPE, 5-hydroxy-6E,8Z,11Z,14Z,17Z-eicosapentaenoic acid; 8-HEPE, 8-hydroxy-5Z,9E,11Z,14Z,17Z-eicosapentaenoic acid; 9-HEPE, 9-hydroxy-5Z,7E,11Z,14Z,17Z-eicosapentaenoic acid; 12-HEPE, 12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid; 15-HEPE, 15-hydroxy-5Z,8Z,11Z,13E,17Z-eicosapentaenoic acid; 18-HEPE, 18-hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid; 19-HEPE, 19-hydroxy-5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid; 20-HEPE, 20-hydroxy-5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid; 4-HDHA, 4-hydroxy-5E,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid; 7-HDHA, 7-hydroxy-4Z,8E,10Z,13Z,16Z,19Z-docosahexaenoic acid; 8-HDHA, 8-hydroxy-4Z,6E,10Z,13Z,16Z,19Z-docosahexaenoic acid; 10-HDHA, 10-hydroxy-4Z,7Z,11E,13Z,16Z,19Z-docosahexaenoic acid; 11-HDHA, 11-hydroxy-4Z,7Z,9E,13Z,16Z,19Z-docosahexaenoic acid; 13-HDHA, 13-hydroxy-4Z,7Z,10Z,14E,16Z,19Z-docosahexaenoic acid; 14-HDHA, 14-hydroxy-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid; 16-HDHA, 16-hydroxy-4Z,7Z,10Z,13Z,17E,19Z-docosahexaenoic acid; 17-HDHA, 17-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid; 20-HDHA, 20-hydroxy-4Z,7Z,10Z,13Z,16Z,18E-docosahexaenoic acid; 21-HDHA, 21-hydroxy-4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid; 22-HDHA, 22-hydroxy-4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid; NPDx- nonenzymatic isomer of NPD1 (Neuroprotectin D1).

Supplementary Tables 7: Hepatic oxylipin measurements on HFCDD diet

Hepatic-oxylipin concentrations in Mboat7^{WT} and Mboat7$^{\Delta hep}$ mice fed a HFCDD for 6 weeks. Abbreviations are provided at the end of Supplementary Table 5. The data was analyzed using R and the oxylipins were normalized to the total protein concentration. The data was Log2 transformed and the Shapiro test was applied to check for normal distribution. When normality was passed, then Welch's t-test was performed and when it did not pass normality then Wilcoxon test was performed. Benjamini-Hochberg correction was used to further adjust the P values [27].

Oxylipin	Mboat7^{WT} (mean, ng/g total protein)	Standard deviation	Mboat7$^{\Delta hep}$ (mean, ng/g total protein)	Standard deviation	Adjusted P value (Benjamini Hochberg correction)
13-HODE	115863	36385	127094	31710	0,7629
9,10-EpOME	60123	9653	49937	9594	0,1716
12,13-EpOME	93909	17602	76086	11161	0,1716
9,10-DiHOME	2246	570	2123	771	0,8325
12,13-DiHOME	3236	897	3086	924	0,8559
5,6-EET	56924	10699	41281	4987	0,0718
8,9-EET	16263	2954	11865	1823	0,0718
11,12-EET	11287	2107	8489	1197	0,0878
14,15-EET	16684	3778	10263	2761	0,0705
5,6-DHET	1459	352	1164	200	0,2717
8,9-DHET	941	286	758	157	0,4570
11,12-DHET	848	250	578	111	0,1438
14,15-DHET	883	224	630	179	0,1263
5,6-EEQ	17238	4951	12654	2703	0,1716
8,9-EEQ	487	127	332	83	0,0895
11,12-EEQ	337	83	248	63	0,1263
14,15-EEQ	417	126	361	100	0,6098
17,18-EEQ	899	295	631	203	0,1806
5,6-DiHETE	192	41	134	31	0,0895
8,9-DiHETE	12	4	10	1	0,3276
11,12-DiHETE	14	5	10	3	0,3070
14,15-DiHETE	42	12	32	10	0,2968
17,18-DiHETE	120	28	97	17	0,3032
7,8-EDP	11750	2451	9141	1740	0,1440
10,11-EDP	10322	2207	7659	1559	0,1098
13,14-EDP	5051	1021	3720	780	0,0895
16,17-EDP	7034	1524	5034	935	0,0895

Oxylipin	Mboat7^{WT} (mean, ng/g total protein)	Standard deviation	Mboat7$^{\Delta hep}$ (mean, ng/g total protein)	Standard deviation	Adjusted P value (Benjamini Hochberg correction)
19,20-EDP	70103	18273	42796	9738	0,0718
7,8-DiHDPA	391	106	432	74	0,6098
10,11-DiHDPA	265	81	229	37	0,7042
13,14-DiHDPA	438	140	335	69	0,3276
16,17-DiHDPA	808	208	572	111	0,1263
19,20-DiHDPA	2124	607	1631	323	0,2968
5-HETE	17664	2078	12780	2372	0,0892
8-HETE	3219	659	2739	372	0,3531
9-HETE	8787	2387	8195	1492	0,8559
11-HETE	12236	3452	11464	2055	0,8597
12-HETE	12918	3742	12753	4744	0,8962
15-HETE	20869	5005	19495	3016	0,8559
16-HETE	2005	579	1684	255	0,5797
17-HETE	559	153	310	112	0,0718
18-HETE	626	137	433	97	0,0878
19-HETE	340	58	303	37	0,4072
20-HETE	1629	392	1513	535	0,7738
LTB4	245	99	250	52	0,8559
12-HpETE	1141	208	842	181	0,0895
5-HEPE	2403	207	1393	367	0,0085
8-HEPE	99	32	86	19	0,7904
9-HEPE	142	56	144	41	0,8597
12-HEPE	792	674	876	631	0,8559
15-HEPE	282	99	291	76	0,8559
18-HEPE	444	148	460	106	0,8559
19-HEPE	770	166	542	150	0,1098
20-HEPE	1860	436	1251	427	0,1263
4-HDHA	6005	1116	5205	895	0,3646
7-HDHA	2858	673	2631	392	0,8438
8-HDHA	3433	770	3258	446	0,8597
10-HDHA	2877	662	2675	359	0,8559
11-HDHA	4219	1067	3989	517	0,8597
13-HDHA	4556	1112	4548	610	0,8962
14-HDHA	3164	815	3113	537	0,9726
16-HDHA	4532	1133	4567	591	0,7920
17-HDHA	5881	1442	6102	837	0,9497
20-HDHA	12648	3014	12625	1772	0,8984
21-HDHA	72722	21907	47874	14808	0,1263
22-HDHA	1864	586	1422	442	0,3276
NPD x	78	49	87	22	0,6098

Supplementary Table 8: Patient samples used for lipidomic analysis (Lipidomic Cohort)

Overview of the patients in the lipidomic cohort. Patients are grouped by MBOAT7 rs641738 genotype and histology using the NAS score. Values are given as counts with percentage and as mean with standard error in parentheses. *ANOVA; **Pearson Chi-Square; *** Test of deviation from Hardy-Weinberg-equilibrium.

Total (n=280)	**MBOAT7 rs641738 (CC), n=94**	**MBOAT7 rs641738 (CT), n=136**	**MBOAT7 rs641738 (TT), n=50**	**P value (CC) vs (CT) vs (TT)**
NAFLD n=214 (n, % genotype)	71 (33%)	105 (49%)	38 (18%)	.939***
NAFL n=135 (63%)	41 (30%)	71 (53%)	23 (17%)	.410***
early NASH n=49 (23%)	22 (45%)	17 (35%)	10 (20%)	.067***
NASH n=30 (14%)	8 (27%)	17 (56%)	5 (17%)	.428***
Age	43.5 (1.7)	44.6 (1.4)	43.8 (1.9)	.862*
BMI	50.1 (1.1)	47.9 (1.2)	48.3 (1.7)	.415*
sex % female	73%	63%	68%	.395**
Diabetes %	78%	64%	64%	.137**
ALT (IU/L)	40.1 (3.8)	35.7 (2.2)	36.7 (5.3)	.575*
AST (IU/L)	30.0 (1.8)	32.3 (1.7)	31.2 (4.0)	.707*
GGT (IU/L)	46.9 (5.9)	45.1 (3.4)	39.9 (4.5)	.678*
AP (U/I)	83.4 (4.0)	76.7 (2.1)	79.6 (6.2)	.336*
NAS score	2.39 (0.19)	2.30 (0.16)	2.66 (0.26)	.511* | .115**
NAS Fat	1.62 (0.10)	1.64 (0.08)	1.89 (0.15)	.204* | .488**
Fat (area in %)	35.4 (3.0)	35.3 (2.6)	47.0 (4.5)	**.049***

Total (n=280)	**MBOAT7 rs641738 (CC), n=94**	**MBOAT7 rs641738 (CT), n=136**	**MBOAT7 rs641738 (TT), n=50**	**P value (CC) vs (CT) vs (TT)**
NAS Ballooning	0.25 (0.06)	0.30 (0.05)	0.29 (0.08)	.862* | .919**
NAS Inflammation	0.52 (0.08)	0.37 (0.06)	0.47 (0.11)	.309* | .856**
Fibrosis stage	0.49 (0.08)	0.53 (0.08)	0.53 (0.11)	.947* | .657**
NAFLD with Fibrosis vs. without fibrosis (n, %) – NAS Inflammation (0)	11/30 (27%)	21/50 (30%)	10/13 (43%)	.355**
NAFLD with Fibrosis vs. without fibrosis (n, %) – NAS Inflammation (≥1)	20/10 (67%)	20/14 (59%)	7/8 (47%)	.434**
non-NALFD n=66 (n, % genotype)	23 (35%)	31 (47%)	12 (18%)	.784***
normal controls (NC) n=30	10 (33%)	14 (47%)	6 (20%)	.785***
healthy obese (HO) n=36	13 (36%)	17 (47%)	6 (17%)	.912***
Fat (area in %)	2.2 (0.5)	2.0 (0.4)	1.8 (0.6)	.870*
Age	50.5 (3.8)	56.1 (2.9)	54.8 (5.1)	.495*
BMI	37.7 (3.0)	36.8 (2.3)	36.3 (3.9)	.952*
sex (% female)	91%	74%	67%	.165**
Diabetes %	5%	12%	45%	**.010****
ALT (IU/L)	20.3 (1.9)	24.3 (2.1)	25.9 (3.7)	.284*
AST (IU/L)	23.0 (1.4)	29.9 (6.1)	26.8 (3.1)	.567*
GGT (IU/L)	55.1 (20.2)	51.7 (18.0)	33.1 (6.3)	.773*
AP (U/I)	88.0 (7.1)	80.4 (8.3)	86.2 (8.3)	.761*

Supplementary Table 9: Patient samples used for lipidomic analysis (Lipidomic Cohort)

Overview of the patients in the lipidomic cohort. Patients are grouped by histology using the NAS score as the main classification criterion. Values are given as counts and percentage for categorical parameters and as mean with standard error (SE) for continuous parameters. N/A: data not available.

Lipidomics cohort (n=280)			Groups				
			NC	HO	NAFL	early NASH	NASH
Count	N		30 (11%)	36 (13%)	135 (48%)	49 (18%)	30 (11%)
Age	Mean		69,03	41,28	44,61	43,14	43,23
	SE		1,68	1,83	1,23	2,05	1,89
BMI	Mean		23,89	47,9	46,85	50,11	55,06
	SE		0,62	1,16	0,98	1,41	1,46
Diabetes	NO		18 (60%)	29 (81%)	93 (69%)	31 (63%)	15 (50%)
	YES		5 (17%)	4 (11%)	33 (24%)	17 (35%)	13 (43%)
	N/A		7 (23%)	3 (8%)	9 (7%)	1 (2%)	2 (7%)
ALT (IU/L)	Mean		24,4	22,14	30,52	40,73	64,74
	SE		2,06	1,83	1,9	3,42	7,43
Fat (area in %)	Mean		1,48	2,46	27,16	41,20	77,33
	SE		0,45	0,36	1,85	3,14	1,59
NAS_score	0		30 (100%)	36 (100%)	0	0	0
	1		0	0	91 (67%)	0	0
	2		0	0	26 (19%)	17 (35%)	0
	3		0	0	15 (11%)	13 (27%)	0
	4		0	0	3 (2%)	19 (39%)	0
	5		0	0	0	0	19 (63%)
	6		0	0	0	0	9 (30%)
	7		0	0	0	0	1 (3%)
	8		0	0	0	0	1 (3%)
NAS_FAT	0		30 (100%)	36 (100%)	0	0	0
	1		0	0	100 (74%)	19 (39%)	0
	2		0	0	22 (16%)	22 (45%)	1 (3%)
	3		0	0	13 (10%)	8 (16%)	29 (97%)
NAS_BALLOONING	0		30 (100%)	36 (100%)	118 (87%)	40 (82%)	2 (7%)
	1		0	0	17 (13%)	9 (18%)	22 (73%)
	2		0	0	0	0	6 (20%)
NAS_INFLAMMATION	0		30 (100%)	36 (100%)	135 (100%)	0	0
	1		0	0	0	46 (94%)	22 (73%)
	2		0	0	0	2 (4%)	5 (17%)
	3		0	0	0	1 (2%)	3 (10%)
Activity score	0	none	28 (100%)	35 (100%)	118 (87%)	0	0

(Balloning+Inflammation)	1	mild	0	0	17 (13%)	37 (76%)	0
	2	moderate	0	0	0	11 (22%)	18 (60%)
	≥3	severe	0	0	0	1 (2%)	12 (40%)
Fibrosis stage	0		28 (100%)	35 (100%)	93 (69%)	25 (51%)	7 (23%)
	1		0	0	42 (31%)	17 (35%)	18 (60%)
	2		0	0	0	3 (6%)	0
	3		0	0	0	4 (8%)	5 (17%)
Fibrosis	NO		28 (100%)	35 (100%)	93 (69%)	25 (51%)	7 (23%)
	YES		0	0	42 (31%)	24 (49%)	23 (77%)

Supplementary Table 10: Sequences of gene-specific mouse primers used in qRT-PCR assays.

Gene name	Forward (5' to 3')	Reverse (5' to 3')
Mboat7	TACCGCACCTACCTGGATTG	AGAAGACCGGGATCATGTAGAA
B2m	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC
Col1a2	AAGGGTGCTACTGGACTCCC	TTGTTACCGGATTCTCCTTTGG
Col3a1	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Col5a2	TTGGAAACCTTCTCCATGTCAGA	TCCCCAGTGGGTGTTATAGGA
Tgfb2	CTTCGACGTGACAGACGCT	GCAGGGGCAGTGTAAACTTATT
Tnfa	AGCCCCCAGTCTGTATCCTTCT	AAGCCCATTTGAGTCCTTGATG
Il1b	ATCCCAAGCAATACCCAAAG	GTGCTGATGTACCAGTTGGG
Il6	CCTTCCTACCCCAATTTCCAAT	AACGCACTAGGTTTGCCGAGTA
Ifng	CTGGAGGAACTGGCAAAAGG	CTGGACCTGTGGGTTGTTGA
Scd1	GCGATACACTCTGGTGCTCA	CCCAGGGAAACCAGGATATT
Elovl3	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA
Elovl5	ATGGAACATTTCGATGCGTCA	GTCCCAGCCATACAATGAGTAAG
Acsf3	AGGAGTGGAAGTACGCATCAT	AACCCTGGAGTCACCTTTGTC
Decr2	GATTGTGAACATTACTGCCACCC	TCGCGTCATAGCATCCACAG
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
Acaa1a	ACGCATCGCCCAATTTCTGA	CCAGACAGGGACATGGACTC
Acox1	TCGAAGCCAGCGTTACGAG	GGTCTGCGATGCCAAATTCC
Ehhadh	ATGGCTGAGTATCTGAGGCTG	GGTCCAAACTAGCTTTCTGGAG
Fabp2	GTGGAAAGTAGACCGGAACGA	CCATCCTGTGTGATTGTCAGTT
Cyb5r3	TCGCCCGTCTGGTTCATCTA	GCCTCAGAGGGTACTTGATGTC
Angptl3	TCTACTGTGATACCCAATCAGGC	CATGTTTCGTTGAAGTCCTGTGA
18s	GTTCCGACCATAAACGATGCC	TGGTGGTGCCCTTCCGTCAAT
Acta2	GGACGTACAACTGGTATTGTGC	TCGGCAGTAGTCACGAAGGA

Primer sequences for detecting ER stress was derived from Oslowski et. al [28]

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