Supplementary Materials and Methods

Human liver samples

Cohort 1. The study population included a group of 35 obese adult patients with body mass index (BMI) ≥ 35 kg/m^2 and a liver biopsy compatible with NAFLD. Participants were recruited from patients undergoing elective bariatric surgery at the University Hospital of Salamanca, Spain. As controls, we included 11 individuals with BMI < 35 kg/m^2 who underwent laparoscopic cholecystectomy for gallstones. Liver biopsies were taken under direct vision during surgery. Baseline characteristics of these groups and exclusion criteria were previously published (1) and are shown in Supplementary table 1.

Cohort 2. The study population included 21 biopsy-proven NAFLD patients (13 NAFL and 8 NASH), and 18 patients with histologically normal liver (NL), with a BMI < 30 kg/m2. All of them patients drank less than 20 g/day of alcohol, were not receiving any potentially hepatotoxic drug, had no analytical evidence of iron overload, and were seronegative for autoantibodies and for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus. All participants studied underwent a liver biopsy under direct vision during programmed cholecystectomy at the Santa Cristina University Hospital in Madrid, Spain. Baseline characteristics of this cohort are shown in Supplementary table 2.

Cohort 3. The study population included a group of 109 adult patients with body mass index (BMI) ≥ 25 kg/m2 and a liver biopsy compatible with NAFLD. All participants underwent a liver biopsy performed under sedation and local anaesthesia and guided by ultrasound using the Menghini technique at Hospital Clinico Universidad Católica de Chile. Baseline characteristics of this cohort are shown in Supplementary table 3. The inclusion criteria were persistent elevation of plasma levels of aminotransferases (aspartate aminotransferase and alanine aminotransferase), an imaging study consistent

with fatty liver and biopsy consistent with a diagnosis of NAFLD. Serum samples obtained from these patients were used for LPI serum analysis.

Histologic diagnosis of liver biopsies from NAFLD patients was classified into two groups: simple steatosis without hepatocellular ballooning nor lobular inflammation, also termed NAFL, and NASH. Minimal criteria for NASH included the combined presence of grade 1 steatosis, hepatocellular ballooning and lobular inflammation with or without fibrosis. It is worth to mention that control patients were non-diabetic, did not have features of metabolic syndrome and had a normal liver histology. The study was performed in agreement with the Declaration of Helsinki, and with local and national laws. The Human Ethics Committees of the three Hospitals approved the study procedures and all participants signed an informed written consent to undergo liver biopsy. Handling of liver biopsies of all the study patients was similar in the hospitals and 1.5 cm long liver tissue piece was immediately fixed and paraffin embedded for histological analysis, and the remaining liver tissue was snap-frozen and stored at -80ºC until use for experimental purposes. All biopsy samples were read by an expert pathologist in a blindly manner.

LPI serum analysis in humans

Metabolite extraction was accomplished by fractionating the samples into pools of species with similar physicochemical properties, using appropriate combinations of organic solvents. Briefly, proteins were precipitated from the defrosted serum samples by adding 4 volumes of cold methanol spiked with metabolites not detected in unspiked human serum extracts. After a brief vortex, samples were incubated overnight at -20 ˚C. Supernatants were collected after centrifugation at 18,000 x g for 15 minutes, dried and reconstituted in methanol before being transferred to vials for UHPLC-MS analysis.

Chromatographic separation and mass spectrometric detection conditions employed were previously described (2).

Data pre-processing was carried out using the TargetLynx application manager for MassLynx 4.1 software (Waters Corporation). The LC-MS features (as defined by retention time and mass-to-charge ratio pairs, Rt-m/z) included in this study were identified prior to the analysis. Data normalization was performed following the procedure previously described (3), which included an intra-batch (multiple internal standard response correction) and an inter-batch (variable specific inter-batch single point external calibration using repeat extracts of a commercial serum sample) normalization. Metabolomic data are represented as means ± standard deviation of the mean. Differences between groups were tested using Student's t-test. Significance was defined as $P<0.05$. All calculations were performed using statistical software package R v.3.1.1 (R Development Core Team, 2011; https://cran.r-project.org/).

Animals and diets

8 weeks old male C57BL/6J mice were kept under 12 hours light/dark cycle and had *ad libitum* access to standard diet, HFD (D12451 45% fat, Research Diets), vHFD (D12492 60% fat, Research Diets), MCD (A02082002BR, Research Diets) or CDHFD (D05010402; 45% fat, Research Diets) for the specified times. In the study with LPI (L7635, MERCK), mice fed a standard diet daily received intraperitoneal (i.p.) injections of 0.5mg/kg LPI for 7 days, using saline 10% (v/v) DMSO as the vehicle. Selected doses were chosen from previous studies (4). Food intake and body weight were measured daily following the acute i.p. injections or once a week during experimental phase in all the remaining experiments. Animal protocols were approved by the Committee at the University of Santiago de Compostela.

Generation of lentiviral particles

The specific shRNA sequences for knockdown of GPR55 or a control shRNA targeting Luciferase were designed using GPP Web Portal Tool (available at https://portals.broadinstitute.org/gpp/public/). The oligos targeting the transcripts of interest were synthesized and subcloned into pLKO.1 puro GFP vectors (Addgene). Lentivirus production were performed as described (5). Briefly, HEK293T cells were maintained in high-glucose DMEM supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin and streptomycin and plated at a density of 8×106 cells per 150 mm dish to be transfected 24 hours later with PEI, (Polyethylenimine; 408727, Sigma-Aldrich) and 20 μg of pLKO.shRNAs plasmids along with 10 μg of psPAX2 and pMD2.G packaging mix. 24 hours later medium was changed, and virus-containing supernatants were collected 48h and 72h post transfection. Lentiviral particles were concentrated using centrifugal filter units with 0.22μm pore size (UFC903024, Amicon). The target sequences of the shRNAs used in this study were:

shGPR55 #1: 5'-TCTGGTGAGGAACCGCTTTAT-3';

shGPR55 #2: 5'-GCGAAAGGGCAGGGAATTTAG-3',

shLuciferase: 5'-CCTAAGGTTAAGTCGCCCTCG-3'.

Tail vein injections for in vivo lentiviral gene silencing

Mice were held in a specific restrainer for intravenous injections, Tailveiner (TV-150, Bioseb). The injections into the tail veins were carried out using gauge needles. Mice were administered with 100 μl of lentiviral GPR55 shRNA ($1x10^9$ TU /mL) or control shRNA ($1x10^9$ TU /mL). In the study using mice treated with LPI, the lentivirus was injected 3 weeks before the administration of LPI for 7 days. In the study using mice fed

at vHFD diet for 10 weeks, the lentivirus was injected during the 6th week. In the study using mice fed at MCD diet, the lentivirus was injected 4 weeks before feeding them at MCD diet for another 4 weeks. In the study using the CCl_4 mice model, the lentivirus was injected during the 1st week.

Carbon tetrachloride (CCl_4) mice model

CCl_4 (06-3545, Strem Chemicals) was administered by i.p. injection at a dose of 0.6 ml/kg once a week for 6 weeks to induce liver fibrosis in mice (6). Corn oil was used as vehicle.

In vivo GS-0976 treatment

GS-0976 (HY-16901, MedChemExpress), an ACC inhibitor, was daily injected i.p. at a dose of 10 mg/kg for 7 days in mice (7). These animals were co-administered with LPI or vehicle for the same period of time.

Isolation and culture of primary hepatocytes, hepatic stellate cells (HSCs).

Mice were anesthetized with isoflurane (1.5% isoflurane in O_2), the abdomen was opened, and a catheter was inserted into the cava vein. Liver was perfused with buffer A [1X PBS, 5 mM EGTA] (37°C, oxygenated) and portal vein was cut. Subsequently, liver was perfused with buffer B [1X PBS, 1 mM $CaCl_2$, collagenase type I (Worthington)] (37°C, oxygenated). After the perfusion, liver was placed in a petri dish containing buffer C [1X PBS, 2 mM $CaCl_2$, 0.6% bovine serum albumin (BSA)] and disaggregated with forceps. Digested liver was filtered through sterile gauze. Then, mouse primary hepatocytes and HSC were isolated either from healthy control or mice fed during 4 weeks a 0.1%MCDD as previously described (6). Briefly, perfused livers were

centrifuged at 48g for 5 min. Supernatant was removed and hepatocytes present in pellet were resuspended in fresh

FBS 10%, Minimun Essential Medium (MEM; Gibco) containing penicillin (100 U/ml), streptomycin (100 U/ml), and glutamine (2 mM). HSC were further isolated from the supernatant by gradient centrifugation with Percoll Plus (GE Healthcare) and selective adherence.

Histological procedures

Frozen liver samples were cut in 8µm sections with a cryostat and stained in filtered Oil Red O for 10 min. After being washed in distilled water, sections were counterstained with Mayer´s hematoxylin for 3 min and mounted in aqueous mounting (glycerin jelly). Liver samples were fixed in 4% formaldehyde for 24 h and then dehydrated and embedded in paraffin. Sections of 4µm were cut with a microtome and stained using a standard Hematoxylin and Eosin alcoholic procedure according to the manufacturer´s instructions (BioOptica). Then, sections were rinsed with distilled water, dried at 37°C for 30 min and mounted with permanent (non-alcohol, non-xylene based) mounting media. For Sirius Red staining, samples fixated in paraffin were dewaxed, hydrated and stained in PicroSirius staining red for one hour. Then, samples were washed with distilled water, dehydrated in three changes of 100% ethanol and cleared in xylene and mounted in a resinous medium. For F4/80 inmunohistochemistry staining, samples fixated in paraffin were dewaxed, hydrated, pre-treated in PTLink TE buffer pH 9 and blocked with 3% peroxidase for 10 minutes. Then, sections were incubated with the primary antibody (ab111101, Abcam) at a concentration of 1:100 overnight and at room temperature, followed by an incubation with the secondary antibody (EnVision, DAKO) for 30 minutes at room temperature. After that, DAB developer was used for 1 minute and

sections were counterstained with Mayer´s hematoxylin for 10 min, dehydrated and mounted. In all the histological staining, up to 4 representative microphotographs of each animal at 20X or 40X were taken with a BX51 microscope equipped with a DP70 digital camera (Olympus). Lipids in Oil Red O- stained sections, collagen depositions in Sirius Red-stained sections and inflammatory infiltrates in F4/80-stained sections were quantified using FRIDA software (Framework for Image Dataset Analysis, the Johns Hopkins University).

Serum levels of metabolites

Mice were sacrificed 4 hours after the start of the light cycle. Whole trunk blood was collected and then spun for 15 min at 6000xg and 4°C. The supernatant was transferred to a new tube to obtain the serum. Serum cholesterol levels (1001093, Spinreact), triglycerides levels (1001310, Spinreact), free fatty acids levels (436-91995, 434-91795, WAKO), ALT activity (41283, Spinreact) and AST activity (41273, Spinreact) were measured by spectrophotometry in a ThermoScientific Multiskan GO spectrophotometer.

Triglycerides content in the liver

The extraction of hepatic triglycerides procedure was adapted from methods described previously (8). Briefly, 100 mg of tissue were homogenized in ice-cold chloroform-methanol (2:1, vol/vol) for 3 minutes, then extracted by shaking 3 hours at room temperature. For phase separation, distilled H_2O was added, samples were centrifuged, and the organic phase was collected. The organic solvent was evaporated to dryness under vacuum at room temperature. The content of triglycerides in each sample was then measured in duplicate by spectrophotometry (1001310, Spinreact).

Hydroxyproline assay

Hepatic collagen content was tested by measuring the levels of hydroxyproline in the liver with the Hydroxyproline Assay Kit (MAK008, MERCK) following the manufacturer´s instructions. Liver samples were hydrolyzed in concentrated hydrochloric acid at 120ºC for 3 hours, and evaporated to dryness under vacuum. We added chloramine T/oxidation buffer mixture and incubated it for 5 min at room temperature, and then DMAB reagent for 90 minutes at 60ºC. Absorbance was read at 560 nm and the amount of hydroxyproline calculated against the standard curve concentration.

Malonyl-CoA measurement in the liver

Hepatic malonyl-CoA levels were measured by Mouse Malonyl coenzyme A ELISA Kit (MBS705127, MyBioSource) according to manufacturer´s instructions. Briefly, 30 mg of liver were homogenized in PBS, and 0.5 mg of protein extract was used in each well for the sequential incubation steps in the provided 96-well plate. Absorbance was read at 450 nm and the amount malonyl-CoA calculated against the standard curve concentration.

Cell cultures

The THLE2 human hepatic cell line (American Type Culture Collection, ATCC) was grown in bronchial epithelial cell basal medium (BEBM; cc-3171, Lonza/Clonetics Corporation) supplemented with a growth factor BulleKit (cc-3170, Lonza/Clonetics Corporation), 70ng/mL phosphoethanolamine, 5 ng/mL epidermal growth factor, 10% (v/v) FBS and 1% (v/v) Glutamine-Penicillin-Streptomycin solution (MERCK). The HepG2 human hepatic cell line (European Collection of Animal Cell Cultures) was maintained as a monolayer culture using Minimum Essential Medium Eagle (EMEM; M2279, MERCK) supplemented with 10% (v/v) FBS, 1% (v/v) Glutamine-Penicillin-Streptomycin solution and 1% (v/v) Non-Essential Aminoacids as the growth medium.

The LX-2 human hepatic stellate cell line (SCC064, MERCK) was grown in Dulbecco's Modified Eagle's Medium (DMEM) high glucose (D5796, MERCK) supplemented with 2% (v/v) FBS and 1% (v/v) Glutamine-Penicillin-Streptomycin solution. All the cell lines were maintained at 37ºC in a humidified atmosphere containing 5% CO_2.

In vitro LPI treatment

$1.5x10^5$ THLE2 cells were seeded in 6-well plates and incubated with growth medium for 24 hours. Then, the medium was removed and replaced by fresh growth medium supplemented with LPI at 1µM or 10 µM in 1% (v/v) DMSO or with vehicle, PBS in 1% (v/v) DMSO. Selected doses were chosen from previous studies (9, 10). In the assays with HepG2, we seeded $4x10^5$ cells. Cells were exposed to LPI for 30 min, 1 hour or 6 hours and collected for mRNA and/or protein extraction. HepG2 cells were exposed to LPI for 24 hours and then the coverslips were placed in a 24-well with 300 µl of BEBM and incubated at 37ºC for 40 minutes with 2,4 µl of BODIPY 493/503 (green) to label lipid droplets (D3922, ThermoFisher). Coverslips were washed with PBS and fixed with 4% formaldehyde for 10 minutes. The coverslips were mounted in aqueous medium Fluoro-Gel (17985-10, Etectron Microscopy Sciences) with 4,6-diamino-2-phenylindole (DAPI) 1:1000 (blue). Representative confocal microphotographs were taken with a Leica A0B5-SP5 confocal microscope equipped with a high-grade color corrected plan apochromat lens for confocal scanning 63x/1.32 objective. Leica Confocal software was used for acquisition and analysis. Images are combinations of optical sections taken in the z axis at 0.5 mm intervals. To BODIPY 493/503 staining, HepG2 cells excited at 492 nm with the laser and fluorescence emission was acquired in a 414-479-nm band. To Oil Red O staining, HepG2 were washed with PBS, fixed with 4% formaldehyde and stained with Oil Red O to detect lipids. Representative microphotographs of each coverslip at 20X or

40X was taken with a BX51 microscope equipped with a DP70 digital camera and the lipids were quantified using FRIDA software. In the studies with human stellate cells, 3 x 10^5 LX-2 cells were seeded in 6-well plates, incubated with growth medium for 24 hours, treated with 1μM or 10 μM LPI or with the vehicle during the specified times and then and collected for mRNA and/or protein extraction.

Gene silencing and oleic acid exposure

THLE2, HepG2 and LX-2 cells were transfected with specific small-interference RNA (siRNA) to knock down the expression of GPR55 (siGENOME SMARTPool, M-005581-05, Dharmacon) or ACCα (siGENOME SMARTPool, L-004551-00-0005, Dharmacon). Control group was administered with a non-targeting siRNA (siGENOME Non-Targeting siRNA Pool, D-001206-13-05, Dhamacon). The transfection was performed using Dharmafect 1 reagent (T-2001-03, Dharmacon) for THLE2, TransIT-siQUEST reagent (MIR2115, Mirus) for HepG2 or Lipofectamine 2000 (11668-019, Invitrogen) for LX-2 following the protocol: 0.05 nmol of each siRNA diluted in 200μl of optiMEM (31985070, Life Technologies) was mixed with 6.5 μl of Dharmafect 1 (or 7.5 ul of TransIT-siQUEST for HepG2, or 6 μl of Lipofectamine 2000 for LX-2) diluted in 193.5 μl of optiMEM; the mixture was added into each well, resulting in a final volume of 1.5ml with growth medium. The medium was replaced with fresh medium after 8 hours, and cells were collected after a total of 48 hours to check the efficiency of silencing by Real Time PCR and/or Western blot. In the study with oleic acid, THLE2 cells with or without the silencing of GPR55 were exposed to FBS-free medium supplemented with 1mM of oleic acid (O1383, MERCK) bound to fatty acid free BSA (BSA-FAF-1U, Capricorn) 2:1 molar ratio for 24 hours to induce lipid accumulation. Controls were supplemented with fatty acid free BSA alone. In the study with LPI, cells with down-regulation of GPR55 or

ACCα were exposed to 10 μM LPI or the vehicle for 12 hours (in LX-2) or 24 hours (in THLE2 and HepG2). Cells were stained with Oil Red O to detect lipids.

De novo lipogenesis

$4x10^5$ HepG2 were seeded and incubated with growth medium. Cells were transfected with control or GPR55 specific siRNA for 6 hours and after that, maintained in fresh growth medium for 36 hours. Then HepG2 were treated with LPI or with vehicle. After 12 hours, the metabolic assays were performed on presence of LPI or vehicle. For analysis of *de novo* lipogenesis, cells were incubated overnight in new FBS-free medium supplemented with 0.5% (w/v) fatty acid free BSA and glucose to a final concentration of 22 mM (4 g/L) in the presence of vehicle or LPI. Then, cells were incubated for 4 hours with fresh medium supplemented with 100 nM insulin and 20 μM acetate containing 20 μCi/ml [^{3}H] -acetate (Perkin Elmer) in presence of vehicle or LPI (11). After the incubation, cells were harvested and washed four times with ice cold PBS (pH 7.4). Cells and medium were separated; lipids were extracted and separated by thin layer chromatography (12). Lipid classes were visualized by exposure to iodine vapour, the corresponding bands were scraped, and the label incorporated into lipids was determined by scintillation counting and expressed relative to the cell protein (13).

Fatty acid oxidation

The rate of fatty acid oxidation was determined by measuring the amount of $^{14}CO_2$ (complete oxidation) and the amount of ^{14}C labeled acid-soluble metabolites (incomplete oxidation) released, as described by others with minor modifications (13, 14). Briefly, cells were incubated for 4 hours with vehicle or LPI containing medium supplemented with 0.5% (w/v) fatty acid free BSA complexed with 0.2 mM palmitate containing 0.5

μCi/ml [1-^{14}C]-palmitate (Perkin Elmer). Medium was then collected in a tube containing Whatman filter paper soaked with 0.1 M NaOH in the cap and 500 μl of 3 M perchloric acid were added to release the CO_2, which was captured in the filter paper. The acidified medium was centrifuged at 21,000xg for 10 min to remove particulate matter. The radioactivity of CO_2 captured by the filter papers and the radioactivity in acid-soluble metabolites (the supernatants of the culture media) was measured by a scintillation counter and expressed relative to the cell protein (13).

Measurements of oxygen consumption rate

The respiration of HepG2 cells was measured at 37°C by high-resolution respirometry with the Seahorse Bioscience XFp Extracellular Flux Analyzer. For the measurement of the oxygen consumption rate (OCR), as the rate change of dissolved O2, HepG2 cells were seeded in a XFp cell culture microplate (103022-100, Seahorse Bioscience, Agilent Technologies), at 2.0 x 10^4 cells per well. After 6 hours of plating, growth medium was removed and replaced with pre-warmed assay medium, composed of Seahorse XF DMEM medium (103575-100, Seahorse Bioscience, Agilent Technologies) containing 1 mM sodium pyruvate, 2 mM L-glutamine and 10 mM glucose, and cultured at 37°C in room air. After equilibration in assay medium for 1 hour, three basal measurements of OCR were performed and then the LPI was injected. In the experiment with etomoxir (103260-100, Seahorse Bioscience, Agilent Technologies), it was added 15 min before the assay was initiated at a concentration of 4μM. The normalized data were expressed as a percentage of the baseline OCR, set to the rate just prior to the acute injection of LPI.

TGF-β1 treatment

LX-2 cells transfected with siRNA GPR55, siRNA ACCα or siRNA control were incubated with medium supplemented with recombinant human TGF-β1 (100-21, PreproTech) at a concentration of 8 ng/ml or the vehicle for 24 hours. After that, LX-2 cells were collected for mRNA extraction.

Cell proliferation

The *in vitro* viability test was performed with a (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) test (M2003, MERCK). Briefly, LX-2 cells were seeded at a density of 1 x 10^5 cells per well in 96-well plates. After 24 hours, cells downregulating GPR55, ACCα or control underwent the treatment of LPI for 12 hours. After the addition of MTT and solubilization of formazan, the cell viability in each well was measured in terms of optical density at a wavelength of 570 nm.

Real time PCR

RNA was extracted using Trizol reagent (15596018, Invitrogen) according to the manufacturer's instructions. 100 ng of total RNA were used for each RT reaction, and cDNA synthesis was performed using the M-MLV Reverse Transcriptase (28025013, ThermoFisher) and random primers. Negative control reactions, containing all reagents except the sample were used to ensure specificity of the PCR amplification. For real time PCR, we used a fluorescent temperature cycler (7500 Real Time PCR system, Applied Biosystems) following the manufacturer's instructions and SYBR green reagent (Precision®PLUS qPCR Master Mix with LOW ROX, PPLUS-LR, Primer-design). The PCR cycling conditions included an initial denaturation at 95ºC for 3 min followed by 40 cycles at 95°C for 5 seconds and 60°C for 32 seconds, with a holding stage of 95°C for

15 seconds, 60ºC for 1 min and 95ºC for 15 seconds. The oligonucleotide specific primers are shown in Supplementary table 4. All reactions were performed in duplicate. Expression levels were normalized to HPRT for each sample and expressed in relation (%) to the control group.

Western blot analysis

Western blots were performed as previously described (1, 15, 16). Briefly, total protein lysates from liver (20 µg) or cells (10 µg) were subjected to SDS-PAGE, electrotransferred onto polyvinylidene difluoride membranes and probed with the antibodies indicated in Supplementary table 5. For protein detection, horseradish peroxidase-conjugated secondary antibodies and chemiluminescence (Amersham Biosciences) were used. Membranes were exposed to radiograph film (Super RX Fuji Medical XRay Film, Fujifilm) and developed with developer and fixing liquids (AGFA) under appropriate dark room conditions. Protein expression was quantified by densitometric analysis with Image J software. Protein levels were normalized to GAPDH for each sample and expressed in relation to the control group.

Data Analysis and Statistics

Data are expressed as mean ± standard error mean (SEM). Statistical significance was determined by two-tailed Student's t-test when two groups were compared or ANOVA and post hoc two-tailed Bonferroni test when more than two groups were compared. $P < 0.05$ was considered significant for all the analysis. Test were performed with Prism Software Version 6.0 (GraphPad).

Supplementary Tables

Supplementary table 1. Characteristics of obese patients and non-obese controls used for GPR55 mRNA expression (cohort 1). Variables are presented as mean (standard deviation) or absolute frequency (percentage) are compared by means of Student´s T test or χ^2 test. NAFLD: non-alcoholic fatty liver disease. BMI: body mass index. AST: aspartate aminotransferase. ALT: alanine aminotransferase. NAS: NAFLD Activity Score. NAFLD (NAS score < 4; n=8) and NASH (NAS score > 4; n=27).

Variable	Obese patients with NAFLD (n=35)	Controls (n=11)	P value
Age (years)	43.7 (11.4)	50.4 (17.5)	0.140
Female: male ratio	29:9	7:4	0.451
Hypertension (n)	15 (39.5)	2 (18.2)	0.287
Diabetes mellitus (n)	8 (21.1)	0 (0)	0.172
BMI (kg/ m^2)	49.2 (6.9)	27.2 (4.24)	<0.001*
Fasting blood sugar (mg/dl)	104.2 (34.4)	93.67 (14.3)	0.376
AST (IU/l)	24.8 (13.4)	21.0 (3.8)	0.137
ALT (IU/l)	30.8 (17.6)	28.4 (12.6)	0.703
Bilirubin (mg/dl)	0.43 (0.2)	0.49 (0.27)	0.383
Total cholesterol (mg/dl)	199.1 (33.6)	207.0 (37.3)	0.563
Triglycerides (mg/dl)	129.9 (50.0)	97.6 (38.3)	0.096
LDL-cholesterol (mg/dl)	118.7 (35.6)	124.8 (36.0)	0.668
HDL-cholesterol (mg/dl)	49.0 (13.2)	62.7 (18.5)	0.020*
NAS score	4.9 (1.5)	0	<0.001*
Steatosis	2.1 (0.9)	0	<0.001*
Lobular inflammation	1.6 (0.8)	0	<0.001*
Hepatocyte ballooning	1.4 (0.6)	0	<0.001*

Supplementary table 2. Characteristics of non-obese patients and non-obese controls used for GPR55 mRNA expression (cohort 2). Data are shown as mean ± standard deviation or as number of cases (%). NL, normal liver; NAFLD, non-alcoholic fatty liver disease; NS, not significant; HOMA, homeostatic model assessment; ALT, alanine aminotransferase; AST, aspartate aminotransferase; G-GT, gamma-glutamyltransferase.

Variable	NL (n=18)	NAFL (n=21)	P value
Age (years)	51.6 ± 13.5	51.1 ± 14.2	NS
Body mass index (kg/m^2)	25.16 ± 2.5	27.03 ± 2.8	0.071
Glucose (mg/dL)	99.5 ± 33.5	90.8 ± 10.5	NS
Insulin levels (□U/L)	7.5 ± 3.9	9.3 ± 5.9	NS
HOMA score	1.9 ± 1.7	2.1 ± 1.4	NS
Triglycerides (mg/dl)	135.5 ± 62.5	142.3 ± 79.3	0.003*
Total cholesterol	193.7 ± 85.8	204.6 ± 98.4	NS
ALT (IU/L)	17.3 ± 6.3	37.7 ± 31.2	< 0.001*
AST (IU/L)	17.5 ± 3.9	25.5 ± 14.1	0.025*
G-GT (IU/L)	34.2 ± 36.6	57.9 ± 51.7	0.049*
Alkaline phosphatase (IU/L)	68.1 ± 18.7	64.8 ± 17.7	NS
Steatosis			
Grade 0	18 (100%)	0	
Grade 1	0	14 (66.7%)	
Grade 2	0	5 (23.8%)	
Grade 3	0	2 (9.5%)	
Inflammation			
Grade 0	18 (100%)	13 (61.9%)	
Grade 1		5 (23.8%)	
Grade 2		2 (9.5%)	
Grade 3		1 (4.8%)	
Hepatocyte ballooning			
Grade 0	18 (100%)	13 (61.9%)	
Grade 1		5 (23.8%)	
Grade 2		2 (9.5%)	
Grade 3		1 (4.8%)	
Fibrosis			
Stage 0	18 (100%)	13 (61.9%)	
Stage 1		6 (28.6)	
Stage 2		2 (9.5%)	

Supplementary table 3. Characteristics of patients used for LPI serum measurement (cohort 3). Data are shown as mean ± standard deviation. NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; ALT, alanine aminotransferase; AST, aspartate aminotransferase; G-GT, gamma-glutamyltransferase.

Variable	NAFL	NASH
N (female)	31 (16)	78 (53)
Age (years)	47.1 ± 12.27	48.18 ± 12.84
Body mass index (kg/m^2)	31.66 ± 4.22	31.63 ± 4.54
N diabetics (female)	9 (4)	17 (12)
ALT (IU/l)	50.15 ± 47.21	96.53 ± 69.24
AST (IU/l)	30.19 ± 17.80	66.67 ± 49.60
G-GT (IU/l)	59.31 ± 70.87	74.04 ± 70.43
Total cholesterol (mg/dl)	206.96 ± 50.54	190.60 ± 42.42
Triglycerides (mg/dl)	163.7 ± 71.02	160 ± 64.10
Glycemia (mg/dl)	100.07 ± 25.71	101.85 ± 22.38
N (Steatosis Grade 1)	16	16
N (Steatosis Grade 2)	9	27
N (Steatosis Grade 3)	4	24
N (Inflammation Grade 0)	19	3
N (Inflammation Grade 1)	9	29
N (Inflammation Grade 2)	1	28
N (Inflammation Grade 3)	0	7
N (Ballooning Grade 0)	23	3
N (Ballooning Grade 1)	5	36
N (Ballooning Grade 2)	1	28

Supplementary table 4. Primers and probes used for gene amplification.

Name	Primer sequence 5′→ 3′
Human ACCα	FW ACCACCAATGCCAAAGTAGC RV CTGCAGGTTCTCAATGCAAA
Human ACTA2	FW TTCAATGTCCCAGCCATGTA RV GAAGGAATAGCCACGCTCAG
Human Collagen 1α1	FW GCTCGTGGAAATGATGGTGC RV ACCCTGGGGACCTTCAGAG
Human Collagen 1α2	FW GCAGGAGGTTTCGGCTAAGT RV CAGACTCCTTGTGTCGCAGA
Human GPR55	FW AAGAACCCACAGACCAGGTG RV CTCTGCCCAAGACACTCTCC
Human HPRT	FW ACCCCACGAAGTGTTGGATA RV AAGCAGATGGCCACAGAACT
Human TGFβ1	FW GGACCAGTGGGGAACACTAC RV AGAGTCCCTGCATCTCAGAGT
Mus musculus Collagen 1α1	FW CCTAATGCTGCCTTTTCTGC RV ATGTCCCAGCAGGATTTGAG
Mus musculus Collagen 1α2	FW CCGTGCTTCTCAGAACATCA RV CTTGCCCCATTCATTTGTCT
Mus musculus GPR55	FW AGTCCATATCCCCACCTTCC RV AGGGAGAGCACCAGCAGTAA
Mus musculus HPRT	FW AAGCTTGCTGGTGAAAAGGA RV TTGCGCTCATCTTAGGCTTT
Mus musculus IL-6	FW AGTTGCCTTCTTGGGACTGA RV TCCACGATTTCCCAGAGAAC
Mus musculus αSMA	FW CTGACAGAGGCACCACTGAA RV CATCTCCAGAGTCCAGCACA
Mus musculus SREBP1c	FW GCGGCTGTTGTCTACCATAAG RV TGTTGCCATGGAGATAGCATCTC
Mus musculus TGFβ1	FW TTGCTTCAGCTCCACAGAGA RV TGGTTGTAGAGGGCAAGGAC
Mus musculus TNFα	FW AGCCCCCAGTCTGTATCCTT RV CTCCCTTTGCAGAACTCAGG

Supplementary table 5. Antibodies used for western blot.

Protein target	Manufacturer (catalog number)	Species reactivity	Dilution
Acetyl-Coa Carboxylase, ACC	Abcam (Ab45174)	Rabbit monoclonal	1:1000
Phospho- acetyl-Coa Carboxylase (Ser 79), pACC	Cell Signaling (3661)	Rabbit polyclonal	1:1000
Phospho-AMPKα (Thr172)	Cell Signaling (2535)	Rabbit monoclonal	1:1000
Carnitine Palmitoyltransferase 1A, CPT1A	Abcam (Ab128568)	Mouse monoclonal	1:1000
Cleaved caspase 3	Cell Signaling (9664)	Rabbit monoclonal	1:1000
Cleaved caspase 7	Cell Signaling (9491)	Rabbit polyclonal	1:1000
Phospho- EIF2α (Ser 52)	Santa Cruz Biotechnology (Sc-101670)	Rabbit polyclonal	1:1000
Fatty Acid Synthase, FAS	Abcam (Ab128870)	Rabbit monoclonal	1:1000
Glyceraldehyde 3- phosphate dehydrogenase, GAPDH	Merck (CB1001)	Mouse monoclonal	1:5000
GPR55	Abcam (Ab203663)	Rabbit polyclonal	1:1000
Phospho- IRE1 (Ser 724)	Novus Biologicals (NB100-2323)	Rabbit polyclonal	1:1000
Lipoprotein lipase, LPL	Santa Cruz Biotechnology (Sc-32885)	Rabbit polyclonal	1:1000
Phospho- PERK (Thr 981)	Santa Cruz Biotechnology (Sc-32577)	Rabbit polyclonal	1:1000
Sterol Regulatory Element-Binding Transcription Factor 1, SREBP1	Abcam (ab3259)	Mouse monoclonal	1:1000
α-Tubulin	Merck (T5168)	Mouse monoclonal	1:5000
X-box binding protein 1 spliced isoform, XBP1s	Santa Cruz Biotechnology (Sc-7160)	Rabbit polyclonal	1:1000

Supplementary Figures

Supplementary Figure 1. Correlation between NAS score and GPR55 in the liver of non-obese patients with NAFLD (cohort 2). (Pearson correlation test). HPRT was used to normalize mRNA levels. (n= 39)

Supplementary Figure 2. Serum levels of different species of LPI are elevated in obese patients with NAFLD as scores of steatosis, inflammation and ballooning increase (cohort 3). (A) Serum levels of the LPI (16:0), LPI (18:1) and LPI (18:1) isomer in obese patients with NAFLD (cohort 3: n=31 with fatty liver without NASH; n=78 with NASH) according to steatosis, inflammation and ballooning scores. (B) Serum levels of the different species of LPI in obese patients with NAFLD, comparing diabetic (n=26) and non-diabetic patients (n=83). Data are presented as mean ± standard error mean (SEM). Statistical differences are denoted by *P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 3. GPR55 regulates lipid accumulation in HepG2 human hepatocytes through phosho-ACC. (A) GPR55 mRNA in HepG2 cells treated with different doses of LPI (n=4-6). (B) Protein levels of markers of lipid metabolism in cells following the administration with 10 µM LPI for 6 hours (n=4). Dual channel fluorescent microphotographs and Oil Red O staining of HepG2 cells showing staining of lipids (BODIPY 493/503, green) and nuclei (DAPI, blue) after the administration of LPI for 24 hours (n=6-8). Right graphs show total lipid content normalized to total number of nuclei per field. (C) Protein levels of apoptosis markers in HepG2 cells treated with 10µM LPI for 6 hours (n=4). (D) mRNA levels of GPR55 in HepG2 cells transfected with siRNA GPR55 or siRNA control for 48 hours (n=4). Oil Red O staining of HepG2 cells after silencing GPR55, treated with vehicle or LPI. Lipids were quantified and normalized to total number of nuclei per field (n=6). Protein levels of pAMPKα, pACC and ACC were measured (n=4-6). (E) Oil Red O staining of HepG2 cells after silencing ACCα, treated with vehicle or LPI (n=6). GAPDH and HPRT were used to normalize protein and mRNA levels. Dividing lines indicate splicing in the same gel. Data are presented as mean ± standard error mean (SEM). Statistical differences are denoted by *P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 4. GPR55 controls lipogenesis and β-oxidation in hepatocytes. *De novo* triglyceride, diacylglicerol and fatty acid lipogenesis (A) (n=5), and palmitate oxidation (B) (n=5) in HepG2 cells treated with vehicle or LPI. (C) Oxygen consumption rate (OCR) in HepG2 cells treated with vehicle or LPI (n=3), and treated with etomoxir +LPI (n=3). Data are presented as mean ± standard error mean (SEM). Statistical differences are denoted by *P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 5. LPI upregulates hepatic GPR55 and induces liver steatosis in mice. C57BL/6J mice fed a standard diet were randomly treated with either vehicle or LPI (0.5mg/kg) for 7 days (n=7). (A) Body weight change. (B) Food intake. (C) Liver mass and serum levels of AST, ALT, triglycerides, non-esterified fatty acids (NEFAs) and cholesterol. (D) Hematoxylin and Eosin staining (upper panel) and Oil Red O staining (lower panel) of liver sections. Lipids were quantified in Oil Red O stained sections. Hepatic triglycerides content was also directly measured. (E) Hepatic mRNA levels of GPR55. (F) Levels of proteins involved in lipid metabolism. HPRT and GAPDH were used to normalize mRNA and protein levels. Dividing lines indicate splicing in the same gel. Data are presented as mean ± standard error mean (SEM). Statistical differences are denoted by *P<0.05, ***P<0.001.

Supplementary Figure 6. The downregulation of hepatic GPR55 ameliorates non-alcoholic steatohepatitis (NASH) induced by methionine-choline-deficient diet (MCD) in mice. C57BL/6J mice randomly received a tail vein injection (TVI) with lentiviral vectors encoding GPR55 shRNA or control shRNA and 4 weeks later were fed a MCD diet for 4 weeks. (A) GPR55 mRNA levels in the liver. (B) Liver mass and serum levels of triglycerides, non-esterified fatty acids (NEFAs) and cholesterol. Levels of liver proteins involved in (C) lipid metabolism and (D) ER stress markers. (E) Hepatic gene expression of inflammatory markers. (F) Liver protein levels of cleaved isoforms of apoptotic markers caspase 3 and caspase 7. HPRT and GAPDH were used to normalize mRNA and protein levels. Dividing lines indicate splicing in the same gel. Data are presented as mean ± standard error mean (SEM) (n=7). Statistical differences are denoted by *P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 7. (A) mRNA levels of GPR55 in LX-2 cells transfected with siRNA GPR55 or siRNA control for 48 hours (n=6 per group). (B) mRNA levels of ACC in LX-2 cells transfected with siRNA ACCα or siRNA control for 48 hours (n=6 per group). (C) Gene expression of hepatic stellate cell (HSCs) markers and GPR55 following the administration of TGFβ1 for 24 hours in LX2 cells (n=8). (D) mRNA levels of HSC activation markers in LX-2 cells transfected with siRNA GPR55 or siRNA control and treated with TGFβ1 or vehicle for 24 hours (n=6-8). (E) TGFβ1 mRNA levels in LX-2 cells after 12 hours of treatment with 10μM LPI (n=8). HPRT was used to normalize mRNA levels. Data are presented as mean ± standard error mean (SEM). Statistical differences are denoted by **P<0.01, ***P<0.001

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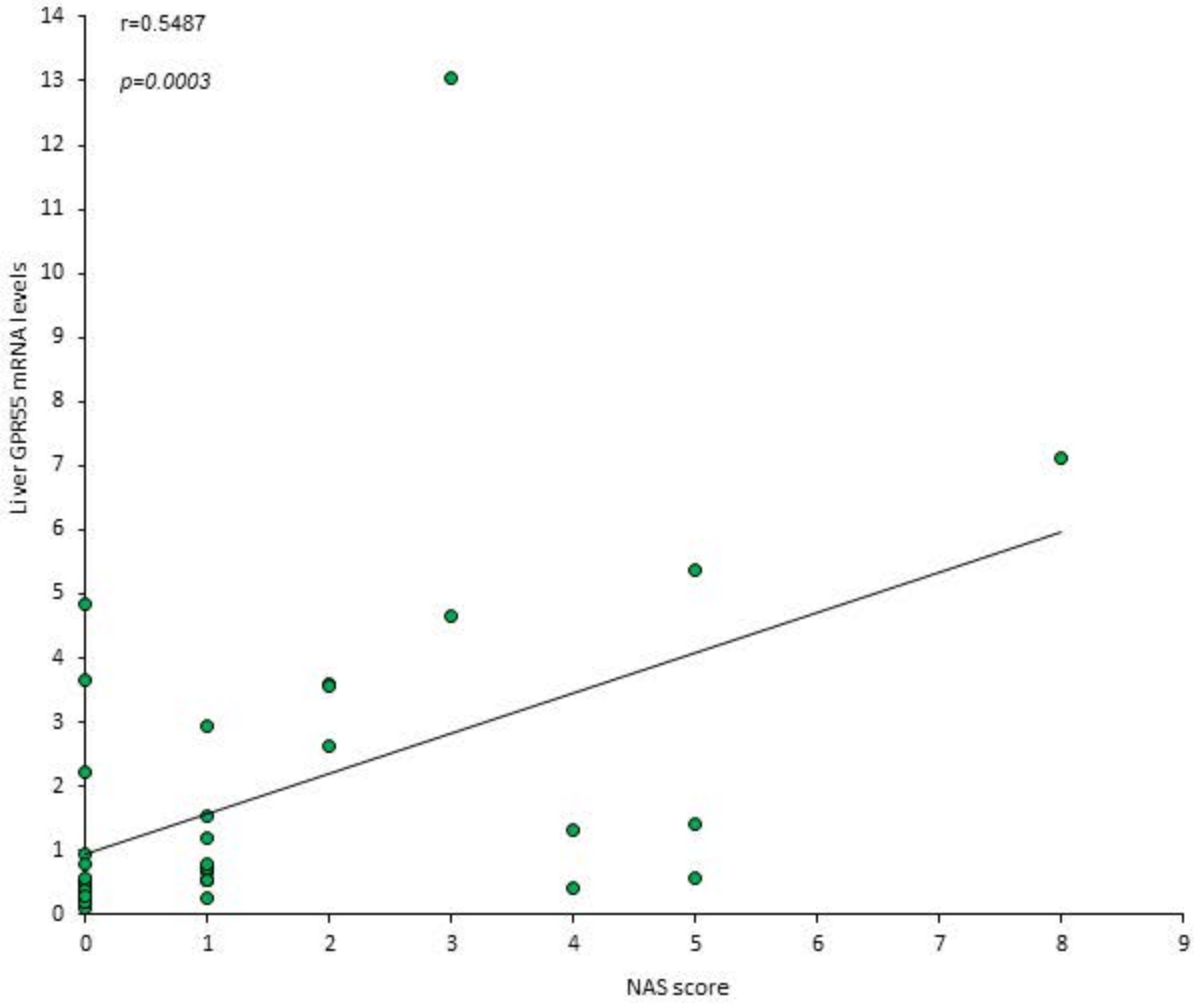
Supplementary figure 1



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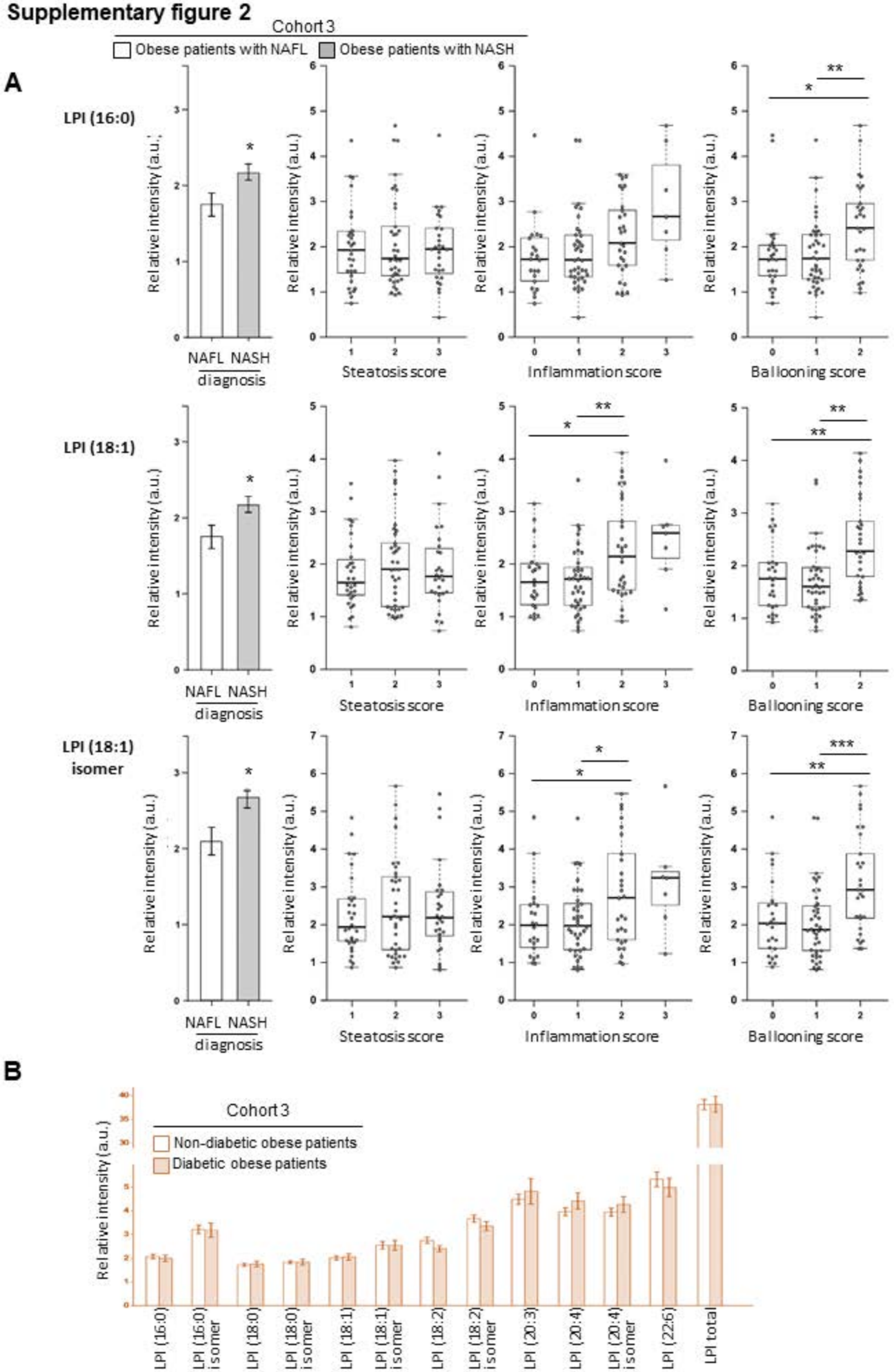

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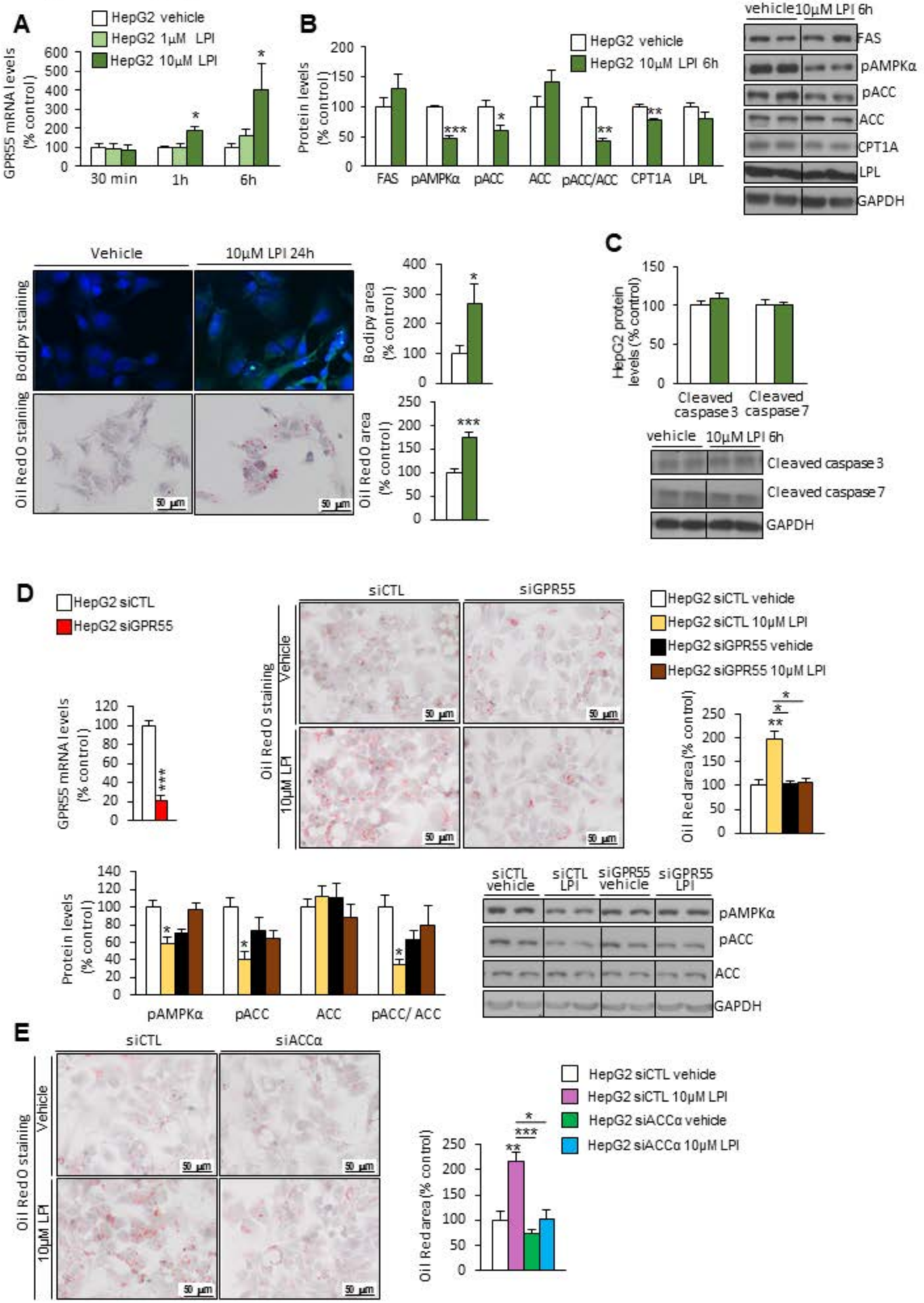

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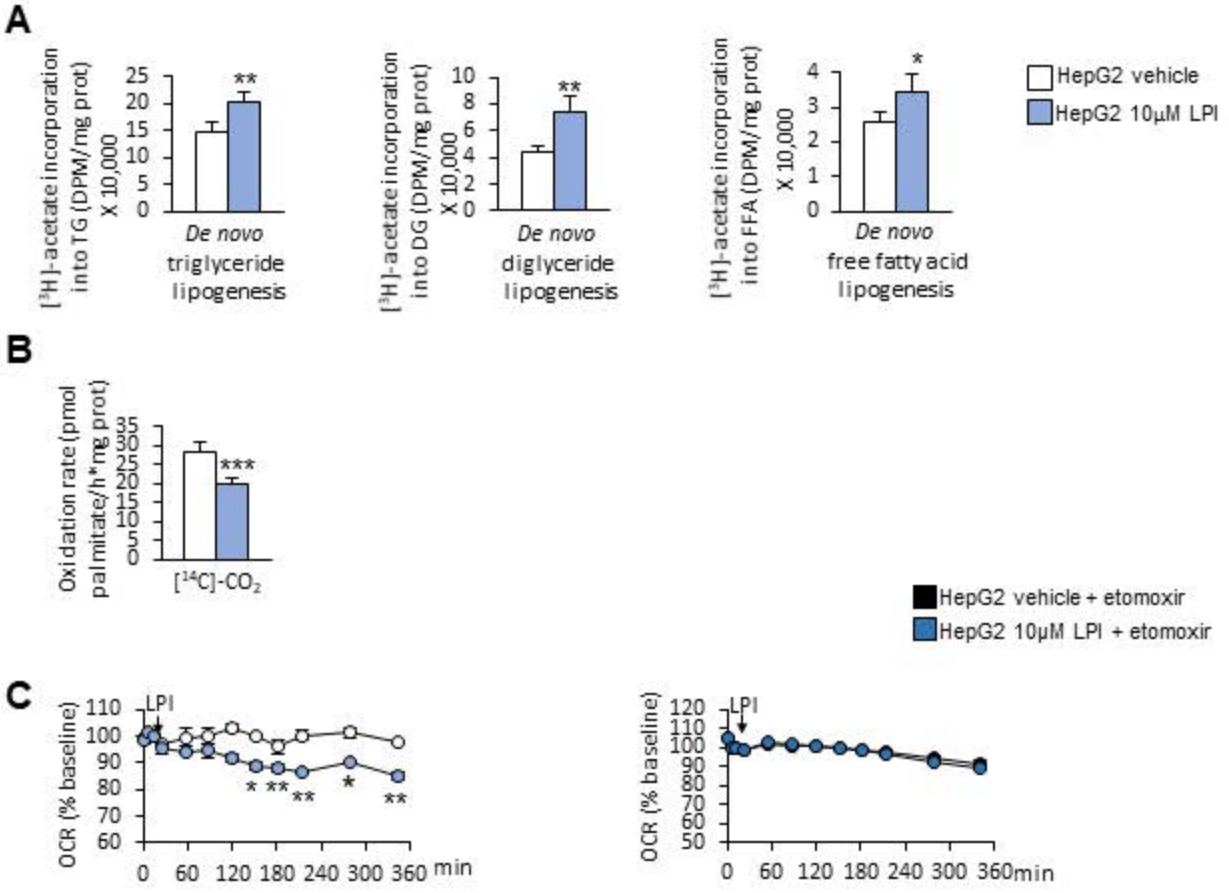

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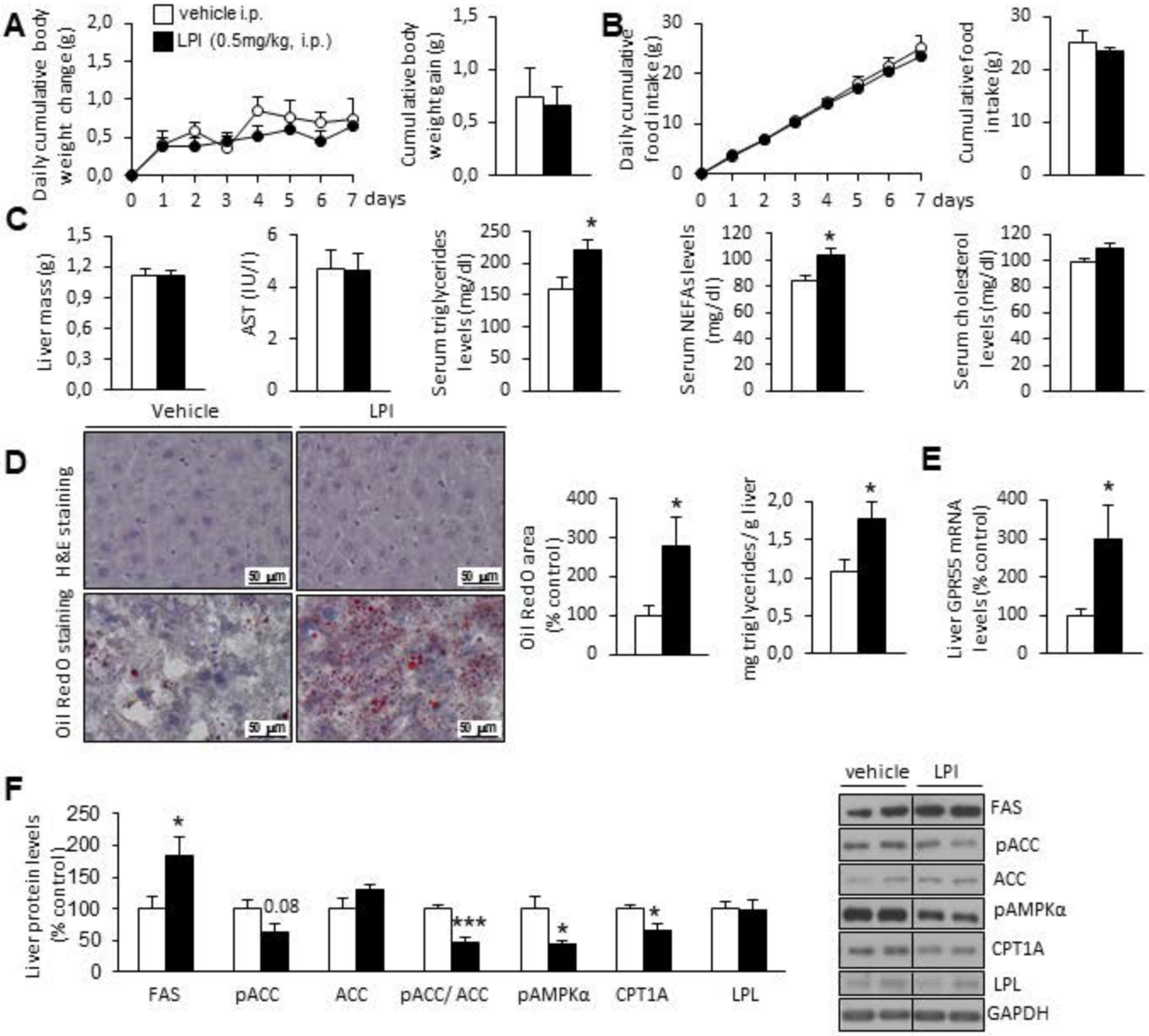

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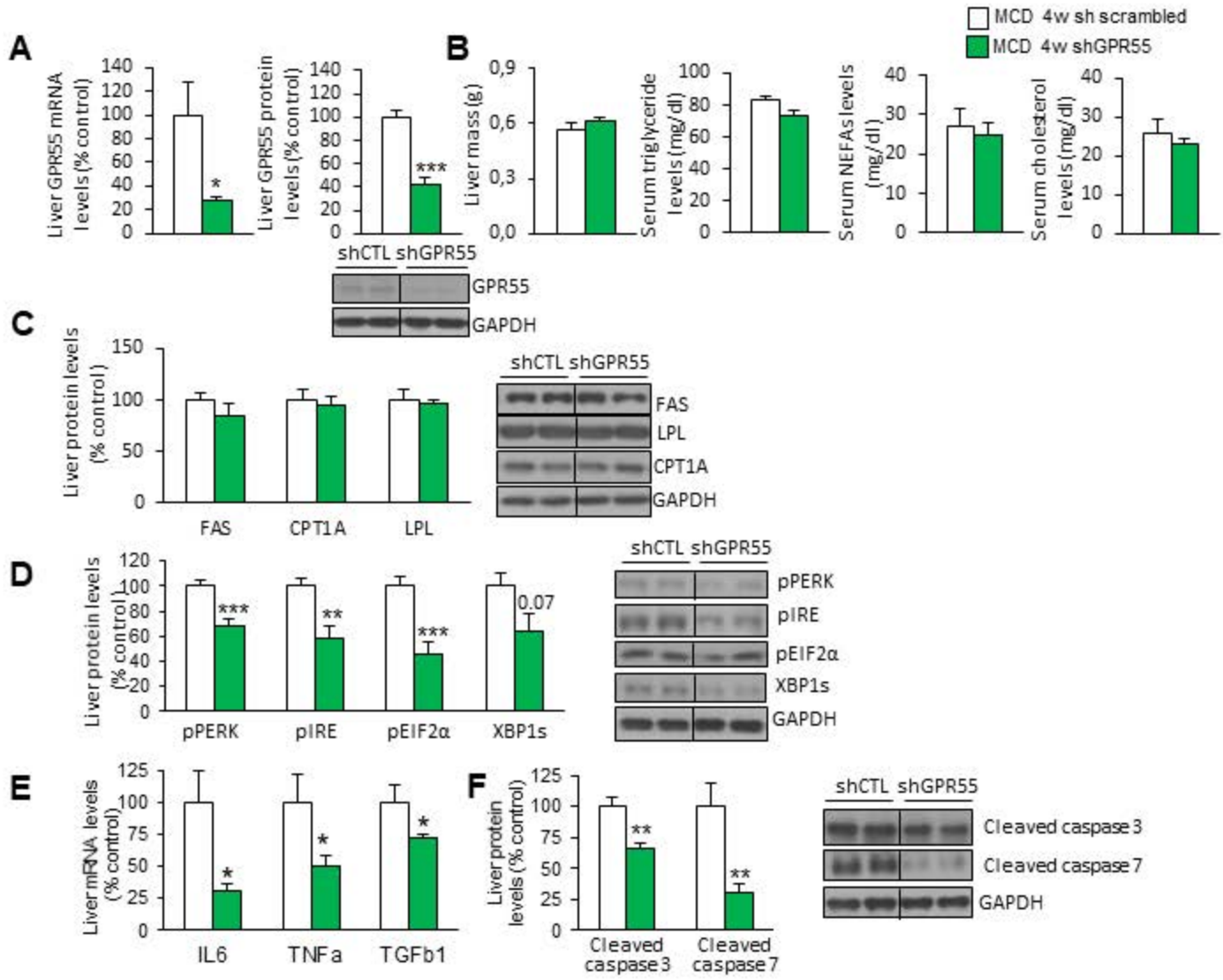

Supplementary figure 7