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*Supplementary Information for*

# **Exosomes from short-time high-fat or high-sucrose fed mice induce hepatic steatosis through different pathways**

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## **This PDF file includes**

Supplementary Methods

Tables S1 to S13

Figures S1 to S7

## Supplementary Methods

### *Animal studies*

All *in vivo* studies were performed at the School of Medicine Animal Facilities (University of Barcelona). Procedures were conducted in accordance with principles of laboratory animal care (European and local government guidelines) and approved by the Animal Research Committee of the University of Barcelona (register number: 46/18). C57BL/6J male mice were used throughout the study. Mice were fed standard chow with either tap water or tap water supplemented with 50 % food-grade sucrose, or a high-fat diet (45 % calories from fat, *Open-Source Diets* #D12451) for the periods indicated below. Food and drink intake and animal weight were controlled weekly. Glucose tolerance test was performed by i.p. injection of 2 g/Kg glucose after 6 h fasting. Insulin sensibility was determined by i.p. injection of 0.5 U/Kg insulin after 6 h fasting. Pyruvate tolerance test was performed by i.p. injection of 1 g/Kg pyruvate after o/n fasting. Fructose tolerance test was performed by oral administration of 4g/Kg fructose after 6 h fasting. In all cases, tail blood glucose was measured at 0, 15, 30, 60 and 120 min using reactive strips in a *NovaPro* glucometer (1, 2). Circulating triglyceride levels were measured in tail blood with reactive strips using an *Accutrend* GCT glucometer after 6 h fasting and at 0 and 60 min during the fructose tolerance test. Plasma was obtained from 50 µl of tail blood centrifuged at 1,500 xg for 20 min (4°C) in microvette EDTA tubes (*Sarstedt*). Free fatty acids were measured from 5 µl plasma after 6 h fasting with NEFA HR(2) Assay Kit (*Wako chemicals*). β-hydroxybutyrate levels were measured from 30 µl plasma after o/n fasting with Amplite Colorimetric Beta-Hydroxybutyrate Ketone Body Assay Kit (*AAT Bioquest*). Alanine Aminotransferase activity was measured from 10 µl plasma after 6h fasting with ALT/SGPT Activity Assay Kit (*Biovision*). Insulin levels were determined from 5 µl plasma at the 0- and 15-min time points during the glucose tolerance test and at the 0- and 30-min points during the pyruvate tolerance test with Mouse Insulin ELISA Kit (*Alpha Diagnostic International*). The HOMA-IR index was calculated as  $(\text{Insulin}_{\text{mIU/L}} \times \text{Glucose}_{\text{mg/dL}}) / 405$ , with glucose in mg/dL and insulin in mIU/L. Fat distribution was analyzed at the Experimental Magnetic Resonance Imaging Unit of the IDIBAPS. Briefly, mice were anesthetized by isoflurane inhalation and placed in supine position on a methacrylate bed equipped with a temperature maintenance system using a hot water circuit. Imaging technique is standardized and lasts around 30 min per mouse, using BioSpec 70/30 horizontal animal scanner (3). Adipose areas were quantified with ImageJ FIJI. At the sacrifice, liver, eWAT, sWAT and gastrocnemius muscle were dissected, weighed and flash-frozen in liquid N<sub>2</sub> for posterior protein and RNA analyses. Tibia length was measured with a caliber. Hepatic free fatty acid and triglyceride contents were measured after extraction with 3 M KOH and 65% ethanol with the NEFA-HR(2) Kit and the GPO-POD Colorimetric Kit (*Spinreact*) respectively as described (1, 2).

### *Experimental models*

**DONORS:** 15-week-old male mice were fed standard chow (CT), standard chow with drinking water supplemented with 50% sucrose (SAC), or a high-fat diet (HFD) for 6 or 15 weeks. Four independent cohorts (n=6/group) were analyzed at the 6-weeks' time point and two more at the 15-weeks' time point (n=4/group). **INJECTED:** A first cohort of control male mice fed standard chow were injected i.v. through the tail vein with 100 µl PBS containing 200 µg exosomes isolated from plasma of CT (iCT), SAC (iSAC) or HFD (iHFD) mice and sacrificed 72h afterwards (acute treatment, n=4/group). A second cohort received a first i.v. injection of 100 µg exosomes and two more injections of 50 µg each, separated by 4 days (chronic treatment, n=4/group). Metabolic analyses were performed starting 15 days after the first injection. **EGFP:** For biodistribution studies, transgenic male mice overexpressing EGFP (*Jackson*) (4) were injected i.v. with 100 µl PBS containing 50 µg exosomes isolated from plasma of CT, HFD or SAC mice and transfected with 500 pmol siEGFP (*Thermo Fisher Scientific*). As a positive control, i.v. injection of 500 pmol siEGFP coupled to Invivofectamine 3.0 was performed in parallel (n=3/group). EGFP fluorescence was analyzed *in vivo* with an IVIS Imaging System and mice were sacrificed 72 h after injection for microscopy analysis of the tissues of interest in a TCS SPE confocal microscope. In addition, 100 mg of the indicated tissues were lysed in 500 µl RIPA lysis buffer and 100 µl were measured in a Tecan Fluorimeter with 480 nm excitation, 515 nm emission and a gain of 70.

### *Indirect calorimetry*

Gas exchange during the glucose and fructose tolerance tests was measured in a closed one-lane treadmill with the CaloSys apparatus TSE Systems. Each mouse was individually placed in the stationary treadmill chamber without inclination for 5 min to allow for gas equilibration. Chamber was then opened, mice were administered with the specified glucose or fructose doses through oral gavage and immediately replaced in the chamber.  $\text{VO}_2$  and  $\text{VCO}_2$  measurements were continued for the next 50 min. From these values, the respiratory exchange ratio was determined as  $\text{VCO}_2/\text{VO}_2$ , carbohydrate oxidation was determined as  $4.55 \times \text{VCO}_2 - 3.21 \text{VO}_2$ , and energy expenditure was determined as  $(\text{VO}_2 \times 3.94 + \text{VCO}_2 \times 1.11) / 1.44$  (5). For a set of experiments, mice were pretreated with an i.p injection of 10 mg/Kg FASN inhibitor C75 (*Sigma*) 1h before starting the test (6). At the end of each experiment, glucose and triglycerides were measured with reactive strips.

### *Exosome isolation, characterization, and transfection*

Exosomes were isolated from 500  $\mu\text{l}$  mouse plasma obtained by cardiac puncture at sacrifice. Briefly, plasma was diluted with an equal volume of PBS and samples were sequentially centrifuged at 2,000  $\times g$  for 30 min (4°C), 10,000  $\times g$  for 45 min (4°C), filtered through a 0.22  $\mu\text{m}$  syringe filter and ultracentrifuged o/n at 120,000  $\times g$  (4°C) using a S110AT Rotor in a Sorvall MX 150 ultracentrifuge. Pellets were resuspended in PBS and ultracentrifuged again at 120,000  $\times g$  for 3 h (4°C). The final pellets were resuspended in 100  $\mu\text{l}$  PBS.

Interstitial liver exosomes were isolated as previously described by us and others (2, 7). Briefly, CT, HFD and SAC mice were perfused through the heart at the left ventricle with 10 ml PBS supplemented with 0.5 mM EGTA at a rate of 2.5 ml/min for 4 min to remove blood from the tissues. EGTA was washed out with a second perfusion of 10 ml PBS supplemented with 0.5% BSA. Mice were finally perfused under the same settings with 10 ml 0.5 mg/ml collagenase IV (*Sigma-Aldrich*) in HBSS (*Sigma-Aldrich*) maintained at 37°C. Liver was dissected and finely minced with forceps to avoid cell rupture. Digestion was stopped by adding 8 ml PBS with BSA and tissue suspension was centrifuged at 100  $\times g$  for 5 min twice to precipitate undigested material. Cleared supernatants were further processed to isolate exosomes by following the protocol described for plasma samples. After the 2,000  $\times g$  and 10,000  $\times g$  centrifugations, supernatants were further cleaned by size exclusion chromatography using SmartSEC columns (*SBI Biosciences*). Samples were then filtered through a 0.22  $\mu\text{m}$  syringe filter and ultracentrifuged as described. The final pellets containing the liver interstitial exosomes were resuspended in 100  $\mu\text{l}$  PBS.

Total exosome protein was quantified by Bradford Assay (*Sigma-Aldrich*) and complementary analyses by measuring the acetylcholinesterase activity known to be associated with exosomes were also performed in 5  $\mu\text{l}$  of exosomes in suspension with Exocet Exosome Quantification Assay Kit (*System Biosciences*). Vesicle morphology was analyzed by negative staining in a Transmission Electron Microscope. Briefly, 30  $\mu\text{l}$  exosomes diluted 1/10 to 1/20 with PBS were allowed to dry on top of formvar carbon-coated grids for 25 min and contrasted with 2 % uranyl acetate for 2 min. Preparations were observed in a JEOL 1010 100 kV TEM. Particle number and diameter was determined by Nanoparticle Tracking Analysis with NanoSight LM10 equipment using different dilutions (1/10 to 1/50) and the following parameters: camera at 30 frames per second, camera level at 16, temperature between 21–25°C and video recording time 60 s. Nanosight Software analyzed raw data videos by triplicate. For western blotting of exosome membrane markers, 10  $\mu\text{l}$  exosomes were resolved by 12% SDS-PAGE and transferred to a NTC membrane (*Whatman*). Anti-CD63 (H-193) (sc-15363, *Santa Cruz Biotechnology*) and anti-CD9 (EPR2949) (ab92726, *Abcam*) were diluted 1/250 and 1/500 respectively in TBS (20mM Tris, 150mM NaCl, pH7.5) supplemented with 5% BSA and visualized by blotting with HRP-conjugated secondary anti-rabbit antibody (NA934V, *GE Healthcare Bio-Sciences*). Chemiluminescence was detected by using the ECL Plus Reagents (*GE Healthcare Bio-Sciences*), in a LAS4000 Lumi-Imager.

Exosomes (50  $\mu\text{g}$ ) were transfected with 500 pmol siEGFP by using Exo-Fect Exosome Transfection Reagent (*System Biosciences*) as described (1, 2).

### *Histochemistry and immunofluorescence*

Tissues were fixed with 4 % PFA o/n. Liver was equilibrated in 30 % sucrose and embedded in OCT, whereas eWAT was embedded in paraffin. 10 µm sections were obtained with a CM 1950 cryostat or a RM2135 microtome respectively and applied to poly-lysine coated slides. Oil Red O and Hematoxylin & Eosin stainings were performed by following the protocols at IHCWorld (<http://www.ihcworld.com>). Liver sections were mounted in Aquatex mounting media (Merck) and adipose sections were mounted in Pertex (HistoLab). Images were obtained with a BX41TF trinocular microscope. Adipocyte size and number were determined from the Hematoxylin & Eosin- stained sections using ImageJ FIJI. To study biodistribution of siEGFP-transfected exosomes and siEGFP-Invivofectamine complexes, liver and gastrocnemius muscle were embedded in OCT to obtain 10 µm sections, whereas eWAT was finely minced with scissors and applied to a slide. Nuclei were stained with 1:500 Hoestch in PBS for 3 min and samples were mounted with Dako fluorescence mounting medium. Images were collected using TCS SPE confocal microscope.

### *Western blotting*

Total protein was extracted using RIPA lysis buffer from frozen tissue samples after powdering with a ceramic mortar and pestle in liquid N<sub>2</sub>. Protease and phosphatase inhibitors were added fresh to the lysis buffer. 50 µg quantified by Bradford Assay (Sigma-Aldich) were resolved by 7.5 % Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad) and transferred to a NTC membrane (Whatman). After 5% BSA blocking, rabbit anti-ACACA (ab72046), anti-FASN (ab22759) (both Abcam) and anti-TUBBA (T6074) (Sigma) antibodies were diluted 1:1000 in 1% TBS-BSA and visualized by blotting with 1:5000 HRP-conjugated secondary anti-rabbit or anti-mouse antibody (both GE Healthcare). Chemiluminescence was detected with Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific) in a LAS4000 Lumi-Imager.

### *RNA isolation and gene and miRNA expression analysis*

Total RNA was extracted from frozen liver, eWAT and gastrocnemius muscle with miRNeasy Mini Kit (Qiagen), treated with DNase, and quantified by absorbance with Nanodrop. 250 ng RNA was used to analyze size and integrity in RNA TapeStation 4200. 150 ng good quality RNA samples (RIN >9) with a ratio 260/280=1.8-2 were used for microarray hybridization. Fragmentation and Biotin labelling of ss-cDNA was prepared according to Affymetrix WT PLUS Reagent Kit user guide, using an automated system (Biomek FX System). Next, ss-cDNA was hybridized for 17hr at 45°C on Clariom™ S HT, mouse array plate, using the automated GeneTitan System, which includes the hybridization oven, Fluidic Station and Scanner. We analyzed 3 tissues in 6 groups of mice (CT, HFD, SAC, iCT, iHFD and iSAC) for a total of n=18 types of samples, performing biological quadruplicates for a total of n=72 microarray hybridizations. Microarray hybridization data was analyzed with Transcriptome Analysis Console 4.0 (Applied Biosystems) using RMA analysis. PCAs, volcano plots, hierarchical clustering and pathway enrichment analysis were performed with the same software. Heatmaps of pathways identified by enrichment analysis of genes were created with [heatmapper.ca](http://heatmapper.ca). Venn's diagrams for comparing up lists of genes were created with Venny 2.1 (<https://bioinfogp.cnb.csic.es>).

For exosomal miRNA analysis, total RNA was extracted from 25 µl isolated plasma exosomes or liver interstitial exosomes with miRNeasy Mini Kit (Qiagen). Exosomes used for RNA isolation were pretreated with 0.1 µg/µl RNase A for 40 min at 37°C to eliminate contamination from cellular rupture, particularly in the case of interstitial exosomes. RNase digestion was stopped by addition of RNasin (Promega Corporation). An equal volume of each RNA sample was retrotranscribed with mircury LNA™ Universal RT microRNA PCR (Qiagen). Profiling of 375 miRNAs was performed by Real time RT-PCR using predesigned panels with LNA specific primers (Qiagen) in a 7900HT Fast Thermocycler (Applied Biosystems). Differential expression was determined with GenEx Software v.6 (Exiqon) after normalization to the global mean Ct of the plate.

### Clinical cohort

All participants gave written informed consent, and the studies were approved by the Research and Ethics Committees of the Hospital Clinic, University of Barcelona (register 2011/6945). A total of 40 serum samples from well-characterized male individuals with different degrees of glucose tolerance according to American Diabetes Association established criteria (8) were selected from among those stored in the Hospital Clínic-IDIBAPS Biobank: 13 control subjects, 10 prediabetic individuals with isolated impaired fasting glucose, 9 prediabetic subjects with isolated impaired glucose tolerance, and 8 newly diagnosed T2D patients. Circulating miRNAs were extracted from 200 µl serum with the miRNeasy kit and analyzed by real-time RT-PCR using 384-well panels with primers specific for the 176 miRNAs most commonly found in plasma/serum (*Qiagen*), in a Roche LC480 II thermocycler. Differential expression was analyzed with GenEx software (*Exiqon*) by normalizing to the mean Ct of the whole plate. Results obtained by analyzing the samples according to glycemic state were published in (9). For the current study, subjects were distributed according to the fatty liver index, calculated as described (9, 10), and circulating *miR-22-3p* values: control group (FLI<60), hepatic steatosis with high *miR-22-3p* (High22) and hepatic steatosis with low *miR-22-3p* (Low22). Additional information regarding inclusion criteria and biochemical parameter measurement can be found in (9). Heatmap of correlation coefficients was created with [heatmapper.ca](http://heatmapper.ca). Receiver Operating Characteristic curves were plotted using ROC Station and the area under the curve was calculated to determine discrimination accuracy.

### Statistics

Differences between groups were determined by either t-test analysis when only two groups were compared or by One-way ANOVA with t-test analysis for the pair wise comparison of 3 or more groups with different number of values. The Shapiro Wilk test p-value was performed to ascertain normality of the samples. Symbols indicate significance with respect to each control group, unless otherwise indicated.

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**Table S1.** Modified pathways identified by enrichment analysis of differentially expressed genes in liver of HFD or SAC mice as compared with CT mice.

LIVER HFD vs CT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Cholesterol biosynthesis	0	-	13	<i>Sqle,Idi1</i>	0.0000
Cholesterol metabolism	1	<i>Fads2</i>	17	<i>Hmgcr,Mvk</i>	0.0000
PPAR signaling pathway	4	<i>Fabp4,Cpt1a</i>	7	<i>Acsl3,Fabp5</i>	0.0000
Oxidation by CYP450	3	<i>Cyp21a1</i>	4	<i>Cyp51,Cyp7a1</i>	0.0001
Statin pathway	2	<i>Apoc2,Apoa4</i>	2	<i>Cyp7a1,Hmgcr</i>	0.0024
Retinol metabolism	3	<i>Abcg8,Abcg5</i>	2	<i>Retsat,Crabp2</i>	0.0052
$\omega$ 3/ $\omega$ 6 fatty acid synthesis	1	<i>Fads2</i>	2	<i>Pla2g5,Acsl3</i>	0.0123
Eicosanoid metabolism	0	-	3	<i>Cyp2c37</i>	0.0123
Fatty acid beta-oxidation	3	<i>Fabp2,Fabp4</i>	1	<i>Acsl3</i>	0.0134
Type II interferon signaling	2	<i>Tap1,Cxcl9</i>	2	<i>Socs3,Icam1</i>	0.0165
Fatty acid biosynthesis	0	-	3	<i>Acsl3,Acacb</i>	0.0251
Endochondral ossification	3	<i>Igf2,Frzb</i>	2	<i>Hmgcs1,Sox9</i>	0.0342
p53 signaling	3	<i>Cdkn1a,Thbs1</i>	2	<i>Gadd45g,Sesn1</i>	0.0480
LIVER SAC vs CT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
$\omega$ 9 fatty acid synthesis	6	<i>Fasn,Elovl5</i>	0	-	0.0000
Fatty acid biosynthesis	7	<i>Acsl5,Acaca</i>	0	-	0.0000
Glycolysis	7	<i>Eno3,Slc2a5</i>	1	<i>Slc2a1</i>	0.0000
Pentose phosphate pathway	4	<i>G6pdx,Rpia</i>	0	-	0.0000
Cholesterol metabolism	9	<i>Hmgcr,Fads1</i>	0	-	0.0002
Eicosanoid metabolism	1	<i>Cyp2c38</i>	4	<i>Cyp2c44</i>	0.0002
Complement activation	0	-	5	<i>C8a,C2</i>	0.0002
Glycolysis & gluconeogenesis	7	<i>Gpi1,Pklr</i>	1	<i>Slc2a1</i>	0.0003
Striated muscle contraction	6	<i>Myl1,Acta1</i>	1	<i>Neb</i>	0.0008
mRNA processing	1	<i>Oas1a</i>	4	<i>Eif4e,Elavl3</i>	0.0014
Cholesterol biosynthesis	4	<i>Fdps,Mvd</i>	0	-	0.00143
Elongation of VLCFA	4	<i>Elovl5,Fads1</i>	0	-	0.0018
PPAR signaling pathway	9	<i>Acaa1b,Acsl5</i>	0	-	0.0021
$\omega$ 3/ $\omega$ 6 fatty acid synthesis	4	<i>Fads2,Acot2</i>	0	-	0.0023
Oxidation by CYPP450	3	<i>Cyp8b1</i>	3	<i>Cyp26a1</i>	0.0024
Statin pathway	4	<i>Apoc2,Apoa4</i>	0	-	0.0044
Steroid biosynthesis	1	<i>Cyp17a1</i>	2	<i>Hsd3b4,F13b</i>	0.0091
Ala and asp metabolism	1	<i>Pcx</i>	2	<i>Ass1,Agxt</i>	0.0137
Oxidative stress	5	<i>G6pdx,Slc6a9</i>	3	<i>Gsta1,Idh2</i>	0.0144
TCA cycle	3	<i>Pdk4,Pdk1</i>	1	<i>Idh2</i>	0.0213
Leptin-insulin signaling	1	<i>Lepr</i>	2	<i>Socs3,Socs2</i>	0.0228
Glutathione metabolism	3	<i>Gstt1,G6pdx</i>	0	-	0.0264
Urea cycle	0	-	3	<i>Ass1,Oat</i>	0.0303
Fatty acid beta-oxidation	4	<i>Acsl5,Acss2</i>	0	-	0.0318
Triacylglyceride synthesis	2	<i>Gpam,Mogat1</i>	1	<i>Agpat5</i>	0.0436

**Table S2.** Modified pathways identified by enrichment analysis of differentially expressed genes in eWAT of HFD or SAC mice as compared with CT mice.

eWAT HFD vs CT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
B cell receptor signaling	57	<i>Grb2,Arpc2</i>	9	<i>Foxo1,Cr2</i>	0.0000
Complement and coagulation	7	<i>C1qa,C3ar1</i>	28	<i>Fgb,C9</i>	0.0000
Focal adhesion	45	<i>Col4a1,Rac2</i>	24	<i>Met,Egfr</i>	0.0000
Oxidative stress pathway	8	<i>Cd44,Hpgds</i>	30	<i>Ptgs1,Sod1</i>	0.0000
Cell cycle	30	<i>E2f2,Plk1</i>	6	<i>Hdac2,Cdh1</i>	0.0000
Chemokine signaling	50	<i>Cx3cr1,Arrb2</i>	13	<i>Pik3r1,Gnb5</i>	0.0000
TCA cycle	0	-	17	<i>Pdha1,Pdhh</i>	0.0000
T cell receptor signaling	44	<i>Map4k1,Was</i>	3	<i>Tuba4a,PPP3cb</i>	0.0000
Electron transport chain	2	<i>Atpif1,Ucp2</i>	35	<i>Cox6c,Atp5</i>	0.0000
Ribosomal proteins	2	<i>Rps6ka1</i>	1	<i>Rpl36</i>	0.0001
Mapk signaling pathway	28	<i>Stmn1,Ptpn7</i>	27	<i>Akt2,Ngf</i>	0.0001
Adipogenesis genes	18	<i>Ddit3,Cebpb</i>	27	<i>Retn,Adipoq</i>	0.0001
PPAR signaling pathway	9	<i>Fabp7,Cpt1b</i>	22	<i>Pparg,Apoa1</i>	0.0001
p53 signaling	21	<i>Chek1,Bax</i>	6	<i>Mdm4,Perp</i>	0.0002
Eicosanoid synthesis	3	<i>Tbxas1,Ltc4s</i>	8	<i>Ptgs2,Dpep1</i>	0.0003
Glucuronidation	0	-	10	<i>Ugp2,Pgm3</i>	0.0004
Apoptosis	21	<i>Casp9,Bak1</i>	8	<i>Birc2,Irf6</i>	0.0006
Glycolysis	3	<i>Tpi1,Hk3</i>	14	<i>Slc2a4,Slc2a5</i>	0.0006
Macrophage markers	5	<i>Cd83,Cd68</i>	2	<i>F3,Cd163</i>	0.0008
Estrogen metabolism	0	-	8	<i>Ugt1a2,Gstm1</i>	0.00114
Fatty acid biosynthesis	0	-	11	<i>Fasn,Acaca</i>	0.0014
Insulin signaling	23	<i>Pik3cd,Arf6</i>	25	<i>Irs1,Irs2</i>	0.0019
Statin pathway	4	<i>Pltp,Ldlr</i>	6	<i>Apoc3,Abca1</i>	0.0023
Glycogen metabolism	3	<i>Gys2,PPP2r5b</i>	11	<i>Pygm,Gys1</i>	0.0040
Fatty acid beta-oxidation	4	<i>Tpi1,Cpt1a</i>	9	<i>Acadm,Pnpla2</i>	0.0171
Toll-like receptor signaling	24	<i>Tlr1,Tlr9</i>	5	<i>Pik3r1,Irf3</i>	0.0212
IL-6 signaling pathway	20	<i>Il6,Fes</i>	9	<i>Gab1,Ncoa1</i>	0.0222
Prostaglandin synthesis	7	<i>Anxa5,Anxa1</i>	4	<i>Ptgis,Hpgd</i>	0.0394
eWAT SAC vs CT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Ribosomal proteins	0	-	40	<i>Rps18,Rpl3</i>	0.0000
Cholesterol biosynthesis	10	<i>Fdps,Sqle</i>	1	<i>Fdft1</i>	0.0000
Cholesterol metabolism	14	<i>Dhcr7,Sc5d</i>	3	<i>Hmgcs2,Fdft1</i>	0.0000
Fatty acid beta-oxidation	5	<i>Acss2,Cpt1a</i>	4	<i>Acadl,Crat</i>	0.0014
Fatty acid biosynthesis	3	<i>Acss2,Acly</i>	3	<i>Echdc3,Ech1</i>	0.0063
Oxidative stress	3	<i>G6pdx,Pgd</i>	12	<i>Ggt1,Sod1</i>	0.0065
LCFA $\beta$ -oxidation	2	<i>Cpt1a,AcsL3</i>	3	<i>Acadl,Acadm</i>	0.0067
Complement activation	1	<i>Masp1</i>	4	<i>C2,C3,C9</i>	0.0089
Glycolysis	5	<i>Pklr,Aldoa</i>	3	<i>Slc2a3,Pdp1</i>	0.0091
$\omega$ 9 fatty acid synthesis	4	<i>AcsL3,Elovl6</i>	0	-	0.0212
p53 signaling	6	<i>Cdkn1a,Casp9</i>	5	<i>Rrm2b,Sesn1</i>	0.0217
Retinol metabolism	2	<i>Rara,Scarb1</i>	5	<i>Aldh1a3,Rbp1</i>	0.0329
Oxidation by CYP450	4	<i>Cyb5b,Cyb5r1</i>	3	<i>Cyp1b1,Cyp2e1</i>	0.0372
PPAR signaling pathway	6	<i>Scd2,AcsL3</i>	6	<i>Acsbg1,Acadl</i>	0.0379



**Table S3.** Modified pathways identified by enrichment analysis of differentially expressed genes in gastrocnemius muscle of HFD or SAC mice as compared with CT mice.

Gas HFD vs CT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Electron transport chain	35	<i>Cox6c, Atp5f1</i>	0	-	0.0000
Fatty acid beta-oxidation	16	<i>Cpt2, Acads</i>	2	<i>Lipf, Acsl3</i>	0.0000
Cholesterol metabolism	3	<i>Acat1, Acsl1</i>	18	<i>Srebf1, Abca1</i>	0.0000
PPAR signaling pathway	10	<i>Cd36, Ppara</i>	14	<i>Adipoq, Plin1</i>	0.0000
Fatty acid biosynthesis	6	<i>Acsl1, Acaa2</i>	6	<i>Fasn, Acaca</i>	0.0000
Oxidative phosphorylation	19	<i>Ndufb9, Ndufs1</i>	0	-	0.0000
LCFA beta-oxidation	9	<i>Acadl, Acadm</i>	1	<i>Acsl3</i>	0.0000
$\omega$ 9 fatty acid synthesis	2	<i>Acsl1, Acot2</i>	7	<i>Fads1, Fads2</i>	0.0000
Complement and coagulation	1	<i>Masp1</i>	16	<i>C1qa, Kng1</i>	0.0000
Statin pathway	1	<i>Lpl</i>	7	<i>Ldlr, Abca1</i>	0.0001
Glucuronidation	0	-	7	<i>Ugt1a5, Ugt2b1</i>	0.0001
Synthesis of ketone bodies	3	<i>Acat1, Oxct1</i>	1	<i>Hmgcs2</i>	0.0001
Estrogen metabolism	0	-	6	<i>Ugt1a10, Cyp1a</i>	0.0002
TCA cycle	8	<i>Pdk4, Sdhb</i>	1	<i>Pcx</i>	0.0004
$\omega$ 3/ $\omega$ 6 fatty acid synthesis	2	<i>Acsl1, Acot2</i>	4	<i>Elovl5, Acsl3</i>	0.0013
Glycolysis & gluconeogenesis	4	<i>Dld, Ldhd</i>	7	<i>Pck1, Aldob</i>	0.0013
NRs in lipid metabolism	1	<i>Ppara</i>	7	<i>Abca1, Vdr</i>	0.0016
SREBF and cholesterol	1	<i>Ppara</i>	4	<i>Srebf1, Srebf2</i>	0.0021
Gas SAC vs CT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Electron transport chain	16	<i>Cox6b1, Uqcrl10</i>	0	-	0.0000
Apoptosis	3	<i>Bad, Casp3</i>	8	<i>Irf1, Tnf</i>	0.0010
Oxidative phosphorylation	9	<i>Atp5g1, Ndufb2</i>	0	-	0.0011
Complement and coagulation	0	-	9	<i>Tfpi, C3</i>	0.0020
Cholesterol metabolism	0	-	7	<i>Hmgcr, Fads2</i>	0.0180
B cell receptor signaling	0	-	14	<i>Cd22, Vav2</i>	0.0183
Type II interferon signaling	0	-	5	<i>Irf4, Irf8</i>	0.0186
Glycerolipids	1	<i>Crls1</i>	3	<i>Dgat1, Pnpla2</i>	0.0195
Omega-9 fatty acid synthesis	0	-	3	<i>Elovl5, Fads2</i>	0.0241
TNF-alpha NF-kB signaling	2	<i>Casp3, Pebp1</i>	13	<i>Nfkb1a, Nfkb2</i>	0.0312
Elongation of VLCFAs	0	-	3	<i>Elovl5, Fads1</i>	0.0346
Fatty acid $\omega$ -oxidation	0	-	2	<i>Cyp1a2, Cyp2e1</i>	0.0379
IL-5 signaling pathway	0	-	7	<i>Prkcb, Itgb2</i>	0.0386
$\Omega$ 3/ $\omega$ 6 fatty acid synthesis	0	-	3	<i>Fads2, Fads1</i>	0.0406
Complement activation	0	-	3	<i>C3, C6, C4b</i>	0.0406
Toll-like receptor signaling	3	<i>Irf7, Tlr8</i>	6	<i>Cd40, Nfkb2</i>	0.0461
NRs in lipid metabolism	0	-	4	<i>Vdr, Nr1h4</i>	0.0470



**Table S4.** Modified pathways identified by enrichment analysis of differentially expressed genes in liver of mice injected with plasma exosomes from HFD or SAC mice as compared with mice injected with plasma exosomes of CT mice.

LIVER iHFD vs iCT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
GPCRs, other	0	-	1	<i>Olfr48</i>	0.0018
mRNA processing	6	<i>Bicc1,Dhx8</i>	5	<i>Mettl3,Snrpd3</i>	0.0232
FAS pathway	2	<i>Mapkapk2</i>	3	<i>Casp8,Fas</i>	0.0247
Inflammatory response	2	<i>Fn1,Tnfrsf1b</i>	2	<i>Lamc2,Il5ra</i>	0.0413
LIVER iSAC vs iCT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Glycolysis & gluconeogenesis	5	<i>Slc2a5,G6pc</i>	1	<i>Eno3</i>	0.0322
Glycolysis	4	<i>Hk1,Pdk2</i>	1	<i>Eno3</i>	0.0346
IL-5 signaling pathway	5	<i>Foxo3,Itgb2</i>	2	<i>Il5ra,Sdcbp</i>	0.0479
GPCRs, other	4	<i>Cysltr1,Olfr24</i>	0	-	0.0496

**Table S5.** Modified pathways identified by enrichment analysis of differentially expressed genes in eWAT of mice injected with plasma exosomes from HFD or SAC mice as compared with mice injected with plasma exosomes of CT mice.

eWAT iHFD vs iCT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
mRNA processing	5	<i>Prpf4, Col4a3</i>	7	<i>Rngtt, Snrpb2</i>	0.0002
Eicosanoid synthesis	5	<i>Ptgds, Ggt1</i>	1	<i>Pnpla8</i>	0.0008
Oxylipins	6	<i>Alox8, Alox12</i>	0	-	0.0019
Prostaglandin synthesis	1	<i>Ptgds</i>	5	<i>Anxa5, Anxa1</i>	0.0120
Elongation of VLCFAs	4	<i>Fads2, Elovl4</i>	0	-	0.0158
$\omega$ 3/ $\omega$ 6 fatty acid synthesis	4	<i>Fads1, Acox3</i>	0	-	0.01965
Pluripotency	6	<i>Tcf7, Nr6a</i>	3	<i>P4ha1, Nr0b1</i>	0.0206
Focal adhesion	11	<i>Mylk, Araf</i>	9	<i>Col4a1, Fyn</i>	0.0309
T cell receptor signaling	1	<i>Plcg1</i>	2	<i>Cd247, Fyn</i>	0.0490
eWAT iSAC vs iCT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Ala and asp metabolism	2	<i>Asl, Agxt</i>	0	-	0.0436

**Table S6.** Modified pathways identified by enrichment analysis of differentially expressed genes in gastrocnemius muscle of mice injected with plasma exosomes from HFD or SAC mice as compared with mice injected with plasma exosomes of CT mice.

Gas iHFD vs iCT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
mRNA processing	3	<i>Zbp1, Col4a3</i>	2	<i>Cpsf1, Snrpa1</i>	0.0089
Nucleotide metabolism	2	<i>Mthfd2, Polb</i>	1	<i>Polg</i>	0.0179
Blood clotting cascade	0	-	3	<i>Serpinf2, Plg</i>	0.0206
Statin pathway	0	-	3	<i>Apoc3, Apoa1</i>	0.0206
G1 to S cell cycle control	3	<i>Cdkn2d, Atm</i>	2	<i>Cdk6, Rpa3</i>	0.0373
Gas iSAC vs iCT					
Pathway	Up	Representative Up genes	Down	Representative Down genes	p-value
Striated muscle contraction	6	<i>Mybpc1, Myh7</i>	0	-	0.0017
mRNA processing	3	<i>Adar, Col4a3</i>	1	<i>Dazl</i>	0.0033
B cell receptor signaling	7	<i>Dok1, Nfatc2</i>	4	<i>Ptpn18, Ccne1</i>	0.0055
Inflammatory response	4	<i>Col1a1, Col1a2</i>	0	-	0.0102
Fatty acid omega-oxidation	0	-	2	<i>Cyp1a1, Cyp2e1</i>	0.0157
Focal adhesion	10	<i>Thbs4, Itga9</i>	1	<i>Pgf</i>	0.0427

**Table S7.** miRNAs differentially expressed in exosomes isolated from plasma of HFD as compared with CT mice (n=3/group).

(PCT) vs (PHFD)	Fold change	Difference (A-B log scale)	P-Value
<i>miR-122-5p</i>	-8.11	-3.02	<b>0.0321</b>
<i>miR-101b-3p</i>	-4.19	-2.07	<b>0.0438</b>
<i>miR-378a-3p</i>	-1.88	-0.91	<b>0.0033</b>
<i>miR-19b-3p</i>	-1.77	-0.82	<b>0.0482</b>
<i>miR-107-3p</i>	-1.74	-0.80	<b>0.0122</b>
<i>miR-223-3p</i>	-1.56	-0.64	<b>0.0092</b>
<i>miR-23b-3p</i>	1.47	0.55	<b>0.0495</b>
<i>RNU1A1</i>	1.91	0.93	<b>0.0228</b>
<i>RNU5G</i>	2.11	1.08	<b>0.0198</b>
<i>miR-130b-3p</i>	2.84	1.50	<b>0.0403</b>
<i>miR-199a-5p</i>	2.95	1.56	<b>0.0050</b>
<i>miR-200c-3p</i>	1.66	0.73	0.0548
<i>miR-7a-5p</i>	2.66	1.41	0.0584
<i>miR-24-3p</i>	1.47	0.56	0.0647
<i>miR-100-5p</i>	1.86	0.90	0.0695
<i>miR-151-5p</i>	2.60	1.38	0.0728
<i>miR-296-5p</i>	-1.83	-0.87	0.0881
<i>miR-375-3p</i>	2.17	1.12	0.1076
<i>rno-miR-223-3p</i>	-1.60	-0.68	0.1102
<i>miR-339-5p</i>	1.51	0.59	0.1169
<i>miR-18a-5p</i>	-2.84	-1.51	0.1173
<i>rno-miR-143-3p</i>	-2.01	-1.01	0.1237
<i>miR-219a-5p</i>	-1.71	-0.78	0.1419
<i>miR-484</i>	-2.16	-1.11	0.1420
<i>miR-297a-5p</i>	1.73	0.79	0.1438
<i>miR-365-3p</i>	-3.71	-1.89	0.1513
<i>miR-10b-5p</i>	1.95	0.96	0.1562
<i>miR-30e-5p</i>	1.86	0.89	0.1663
<i>rno-miR-214-3p</i>	1.98	0.99	0.1683
<i>miR-106b-5p</i>	-1.54	-0.62	0.1746
<i>miR-652-3p</i>	-2.09	-1.07	0.1811
<i>miR-15a-5p</i>	1.47	0.56	0.1928
<i>miR-210-3p</i>	-1.68	-0.75	0.2044
<i>miR-142-5p</i>	1.72	0.78	0.2046
<i>miR-148a-3p</i>	-2.47	-1.31	0.2085
<i>miR-324-5p</i>	2.00	1.00	0.2127
<i>let-7a-5p</i>	1.57	0.65	0.2132
<i>miR-350-3p</i>	-2.37	-1.25	0.2226
<i>miR-142-3p</i>	1.71	0.77	0.2247
<i>miR-328-3p</i>	1.38	0.46	0.2296
<i>miR-451a</i>	-1.75	-0.81	0.2332
<i>miR-500-3p</i>	2.63	1.40	0.2419
<i>miR-140-5p</i>	1.50	0.58	0.2440
<i>miR-16-5p</i>	-1.23	-0.29	0.2444
<i>miR-425-5p</i>	1.45	0.54	0.2562
<i>miR-467a-5p</i>	1.42	0.50	0.2662
<i>miR-19a-3p</i>	-3.11	-1.64	0.2711

<i>miR-532-5p</i>	-2.01	-1.01	0.2713
<i>miR-151-3p</i>	2.47	1.30	0.2727
<i>let-7g-5p</i>	-1.27	-0.35	0.2775
<i>miR-21a-5p</i>	-1.20	-0.26	0.2783
<i>let-7d-5p</i>	2.02	1.02	0.2840
<i>miR-34a-5p</i>	1.69	0.76	0.2930
<i>miR-30c-5p</i>	1.47	0.55	0.2951
<i>let-7c-5p</i>	1.24	0.31	0.2954
<i>miR-146a-5p</i>	1.40	0.48	0.3004
<i>let-7f-5p</i>	1.46	0.54	0.3070
<i>miR-423-5p</i>	1.64	0.71	0.3178
<i>miR-101a-3p</i>	-1.50	-0.59	0.3203
<i>miR-150-5p</i>	1.21	0.28	0.3285
<i>let-7e-5p</i>	2.66	1.41	0.3305
<i>miR-212-3p</i>	1.82	0.87	0.3615
<i>miR-29b-3p</i>	-1.09	-0.13	0.3697
<i>miR-322-5p</i>	-1.30	-0.38	0.3756
<i>miR-497-5p</i>	-1.19	-0.25	0.3864
<i>miR-145a-5p</i>	1.53	0.62	0.4015
<i>miR-574-3p</i>	1.60	0.68	0.4056
<i>miR-3107-5p</i>	-1.24	-0.31	0.4103
<i>U6 snRNA</i>	2.69	1.43	0.4148
<i>rno-miR-338-3p</i>	-1.58	-0.66	0.4273
<i>miR-361-5p</i>	-1.39	-0.47	0.4311
<i>let-7b-5p</i>	-1.30	-0.38	0.4331
<i>miR-195a-5p</i>	1.52	0.61	0.4493
<i>miR-25-3p</i>	-1.38	-0.47	0.4546
<i>miR-30d-5p</i>	-1.49	-0.58	0.4680
<i>miR-30b-5p</i>	1.25	0.33	0.4804
<i>miR-191-5p</i>	-1.26	-0.34	0.4842
<i>miR-193b-3p</i>	1.56	0.64	0.4880
<i>miR-152-3p</i>	-1.66	-0.73	0.5147
<i>miR-125b-5p</i>	1.21	0.28	0.5305
<i>miR-22-3p</i>	-1.18	-0.24	0.5314
<i>miR-103-3p</i>	1.44	0.53	0.5351
<i>miR-326-3p</i>	-1.15	-0.20	0.5365
<i>miR-29c-3p</i>	-1.19	-0.25	0.5579
<i>miR-20a-5p</i>	-1.28	-0.35	0.5625
<i>miR-194-5p</i>	-1.41	-0.49	0.5645
<i>miR-130a-3p</i>	-1.21	-0.27	0.5716
<i>miR-99a-5p</i>	1.36	0.44	0.5727
<i>miR-320-3p</i>	1.33	0.41	0.5772
<i>miR-192-5p</i>	-1.31	-0.39	0.5967
<i>miR-222-3p</i>	-1.64	-0.71	0.5992
<i>miR-23a-3p</i>	-1.11	-0.15	0.6212
<i>miR-125a-5p</i>	-1.21	-0.27	0.6507
<i>miR-188-5p</i>	-1.36	-0.45	0.6563
<i>miR-143-3p</i>	-1.22	-0.29	0.6641
<i>miR-214-3p</i>	1.13	0.18	0.7077
<i>miR-301a-3p</i>	1.19	0.25	0.7254
<i>miR-27b-3p</i>	-1.20	-0.27	0.7297

<i>miR-205-5p</i>	-1.11	-0.15	0.7385
<i>miR-423-3p</i>	-1.05	-0.07	0.7524
<i>miR-93-5p</i>	1.12	0.17	0.7534
<i>miR-1a-3p</i>	-1.14	-0.19	0.7576
<i>miR-203-3p</i>	-1.16	-0.22	0.7924
<i>miR-200b-3p</i>	-1.19	-0.25	0.7927
<i>miR-139-5p</i>	1.07	0.10	0.8047
<i>miR-141-3p</i>	1.10	0.14	0.8063
<i>miR-148b-3p</i>	-1.16	-0.21	0.8101
<i>miR-362-3p</i>	-1.15	-0.21	0.8365
<i>miR-199a-3p</i>	1.06	0.08	0.8532
<i>miR-27a-3p</i>	1.05	0.08	0.8595
<i>miR-17-5p</i>	1.05	0.08	0.8665
<i>let-7i-5p</i>	1.04	0.06	0.8854
<i>miR-33-5p</i>	1.06	0.08	0.8855
<i>miR-132-3p</i>	-1.09	-0.13	0.9113
<i>rno-miR-345-5p</i>	1.09	0.13	0.9155
<i>miR-126a-3p</i>	1.02	0.03	0.9225
<i>miR-133b-3p</i>	-1.03	-0.04	0.9488
<i>miR-29a-3p</i>	1.00	0.01	0.9491
<i>miR-133a-3p</i>	-1.03	-0.04	0.9550
<i>miR-15b-5p</i>	1.02	0.03	0.9623
<i>miR-144-3p</i>	-1.01	-0.01	0.9873
<i>miR-342-3p</i>	-1.00	0.00	1.0000
<i>miR-185-5p</i>	1.00	0.00	1.0000

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**Table S8.** miRNAs differentially expressed in exosomes isolated from plasma of SAC as compared with CT mice (n=3/group).

(PCT) vs (PHFD)	Fold change	Difference (A-B log scale)	P-Value
<i>miR-200b-3p</i>	-3.69	-1.88	<b>0.0306</b>
<i>miR-122-5p</i>	-2.86	-1.52	<b>0.0298</b>
<i>miR-322-5p</i>	-2.39	-1.25	<b>0.0177</b>
<i>miR-223-3p</i>	-1.42	-0.50	<b>0.0208</b>
<i>miR-29b-3p</i>	1.42	0.51	<b>0.0242</b>
<i>miR-23b-3p</i>	1.50	0.58	<b>0.0464</b>
<i>miR-301a-3p</i>	1.66	0.73	<b>0.0414</b>
<i>miR-100-5p</i>	2.09	1.06	<b>0.0191</b>
<i>miR-339-5p</i>	2.19	1.13	<b>0.0118</b>
<i>miR-142-5p</i>	2.21	1.14	<b>0.0145</b>
<i>miR-151-3p</i>	2.87	1.52	<b>0.0381</b>
<i>miR-199a-5p</i>	3.39	1.76	<b>0.0333</b>
<i>miR-467a-5p</i>	4.04	2.01	<b>0.0076</b>
<i>miR-101a-3p</i>	-2.86	-1.51	0.0542
<i>miR-130a-3p</i>	1.55	0.63	0.0604
<i>let-7c-5p</i>	1.55	0.63	0.0652
<i>rno-miR-223-3p</i>	-1.60	-0.68	0.0653
<i>miR-194-5p</i>	-2.91	-1.54	0.0675
<i>miR-151-5p</i>	1.99	0.99	0.0710
<i>miR-423-5p</i>	2.23	1.15	0.0740
<i>miR-7a-5p</i>	1.84	0.88	0.0759
<i>miR-425-5p</i>	1.68	0.75	0.0782
<i>miR-30b-5p</i>	2.50	1.32	0.0808
<i>miR-141-3p</i>	-1.68	-0.75	0.0887
<i>miR-143-3p</i>	-2.14	-1.10	0.0942
<i>miR-3107-5p</i>	-1.77	-0.83	0.1082
<i>miR-203-3p</i>	-2.92	-1.55	0.1141
<i>let-7a-5p</i>	1.81	0.85	0.1207
<i>miR-152-3p</i>	-1.82	-0.86	0.1317
<i>miR-326-3p</i>	1.59	0.67	0.1383
<i>miR-17-5p</i>	-1.48	-0.57	0.1397
<i>miR-212-3p</i>	2.62	1.39	0.1521
<i>miR-365-3p</i>	-2.04	-1.03	0.1531
<i>miR-423-3p</i>	-1.40	-0.49	0.1716
<i>miR-324-5p</i>	1.92	0.94	0.1738
<i>miR-30c-5p</i>	1.75	0.81	0.1830
<i>rno-miR-143-3p</i>	-1.90	-0.93	0.1853
<i>miR-142-3p</i>	1.82	0.86	0.1870
<i>miR-19b-3p</i>	-1.24	-0.31	0.1887
<i>miR-16-5p</i>	-1.63	-0.70	0.1898
<i>miR-205-5p</i>	-1.78	-0.83	0.1957
<i>miR-139-5p</i>	1.35	0.43	0.1979
<i>miR-574-3p</i>	-2.00	-1.00	0.2016
<i>miR-106b-5p</i>	-1.50	-0.59	0.2035
<i>miR-133a-3p</i>	1.75	0.81	0.2203
<i>miR-107-3p</i>	-2.10	-1.07	0.2242
<i>miR-532-5p</i>	2.05	1.03	0.2274



<i>miR-101b-3p</i>	-1.67	-0.74	0.2287
<i>let-7g-5p</i>	1.36	0.44	0.2302
<i>miR-210-3p</i>	-1.76	-0.81	0.2427
<i>let-7d-5p</i>	1.80	0.85	0.2539
<i>miR-296-5p</i>	1.45	0.53	0.2618
<i>miR-19a-3p</i>	-3.37	-1.75	0.2678
<i>miR-103-3p</i>	2.31	1.21	0.2961
<i>miR-24-3p</i>	1.22	0.29	0.2968
<i>miR-146a-5p</i>	1.33	0.41	0.3008
<i>miR-200c-3p</i>	2.24	1.16	0.3051
<i>miR-27b-3p</i>	-1.54	-0.62	0.3103
<i>let-7e-5p</i>	2.86	1.52	0.3179
<i>miR-25-3p</i>	-1.69	-0.75	0.3251
<i>miR-29c-3p</i>	-1.22	-0.29	0.3352
<i>U6 snRNA</i>	3.44	1.78	0.3394
<i>miR-350-3p</i>	-1.48	-0.57	0.3482
<i>miR-93-5p</i>	-1.28	-0.35	0.3592
<i>miR-29a-3p</i>	1.10	0.14	0.3668
<i>miR-27a-3p</i>	1.73	0.79	0.3777
<i>miR-378a-3p</i>	1.06	0.09	0.3928
<i>miR-193b-3p</i>	1.77	0.82	0.3956
<i>miR-185-5p</i>	1.68	0.75	0.3977
<i>miR-22-3p</i>	-1.29	-0.36	0.4071
<i>miR-484</i>	-1.39	-0.48	0.4173
<i>miR-500-3p</i>	1.79	0.84	0.4247
<i>miR-23a-3p</i>	-1.18	-0.24	0.4324
<i>miR-20a-5p</i>	-1.36	-0.45	0.4490
<i>miR-30e-5p</i>	1.36	0.45	0.4587
<i>RNU5G</i>	1.33	0.41	0.4641
<i>miR-195a-5p</i>	1.43	0.52	0.4789
<i>miR-34a-5p</i>	-1.21	-0.27	0.4906
<i>miR-126a-3p</i>	-1.12	-0.17	0.4908
<i>miR-10b-5p</i>	1.49	0.57	0.4945
<i>miR-132-3p</i>	1.37	0.46	0.4960
<i>miR-214-3p</i>	-1.17	-0.22	0.5013
<i>miR-297a-5p</i>	1.21	0.27	0.5038
<i>miR-150-5p</i>	1.15	0.20	0.5262
<i>miR-320-3p</i>	1.48	0.56	0.5620
<i>rno-miR-214-3p</i>	1.28	0.35	0.5625
<i>miR-30d-5p</i>	-1.33	-0.41	0.5748
<i>miR-148a-3p</i>	1.28	0.35	0.5822
<i>let-7f-5p</i>	1.31	0.39	0.5900
<i>miR-451a</i>	-1.31	-0.39	0.5949
<i>miR-497-5p</i>	-1.22	-0.29	0.5992
<i>miR-99a-5p</i>	-1.27	-0.34	0.6243
<i>rno-miR-338-3p</i>	1.19	0.25	0.6254
<i>miR-18a-5p</i>	-1.42	-0.50	0.6293
<i>miR-133b-3p</i>	1.19	0.25	0.6881
<i>miR-199a-3p</i>	-1.23	-0.30	0.6928
<i>miR-361-5p</i>	1.13	0.18	0.7168
<i>miR-1a-3p</i>	1.15	0.20	0.7208

<i>miR-192-5p</i>	-1.08	-0.11	0.7397
<i>miR-130b-3p</i>	1.20	0.26	0.7462
<i>miR-362-3p</i>	-1.31	-0.39	0.7472
<i>miR-328-3p</i>	1.06	0.09	0.7487
<i>miR-140-5p</i>	-1.17	-0.23	0.7728
<i>miR-652-3p</i>	-1.29	-0.36	0.7788
<i>miR-144-3p</i>	-1.10	-0.14	0.7895
<i>miR-188-5p</i>	1.16	0.22	0.7967
<i>miR-145a-5p</i>	1.08	0.11	0.8013
<i>rno-miR-345-5p</i>	-1.20	-0.26	0.8144
<i>miR-33-5p</i>	-1.08	-0.11	0.8264
<i>miR-342-3p</i>	-1.07	-0.10	0.8451
<i>miR-148b-3p</i>	1.07	0.10	0.8503
<i>miR-222-3p</i>	1.17	0.23	0.8644
<i>let-7b-5p</i>	1.03	0.05	0.8957
<i>miR-21a-5p</i>	-1.02	-0.03	0.9012
<i>miR-15a-5p</i>	1.03	0.05	0.9080
<i>miR-219a-5p</i>	1.04	0.05	0.9110
<i>miR-125b-5p</i>	1.03	0.04	0.9216
<i>miR-125a-5p</i>	1.02	0.03	0.9639
<i>let-7i-5p</i>	1.01	0.01	0.9745
<i>miR-191-5p</i>	1.01	0.01	0.9863
<i>RNU1A1</i>	1.01	0.01	0.9877
<i>miR-15b-5p</i>	-1.01	-0.01	0.9881
<i>miR-375-3p</i>	1.00	0.01	0.9895

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**Table S9.** miRNAs differentially expressed in exosomes isolated from liver as compared with liver tissue in CT mice (exosomes n=4, tissue n=3).

(Exosomes) vs (Tissue)	Fold change	Difference (A-B log scale)	P-Value
<i>RNU1A1</i>	-25.94	-4.70	0.0182
<i>miR-144-3p</i>	-15.55	-3.96	0.0010
<i>miR-339-5p</i>	-12.50	-3.64	0.0007
<i>RNU5G</i>	-12.48	-3.64	0.0104
<i>miR-125a-5p</i>	-9.45	-3.24	0.0008
<i>miR-212-3p</i>	-9.45	-3.24	0.0188
<i>U6 snRNA</i>	-9.29	-3.22	0.0007
<i>miR-200c-3p</i>	-9.29	-3.22	0.0019
<i>miR-331-3p</i>	-8.82	-3.14	0.0058
<i>miR-107-3p</i>	-7.61	-2.93	0.0022
<i>miR-3107-5p</i>	-7.04	-2.82	0.0013
<i>rno-miR-146b-5p</i>	-6.96	-2.80	0.0272
<i>miR-484</i>	-6.36	-2.67	0.0080
<i>miR-451a</i>	-6.33	-2.66	0.0008
<i>miR-141-3p</i>	-5.59	-2.48	0.0142
<i>miR-139-5p</i>	-5.57	-2.48	0.0038
<i>miR-149-5p</i>	-5.12	-2.36	0.0277
<i>miR-379-5p</i>	-4.79	-2.26	0.0140
<i>miR-26a-5p</i>	-4.76	-2.25	0.0244
<i>rno-miR-223-3p</i>	-4.40	-2.14	0.0023
<i>miR-142-3p</i>	-4.39	-2.14	0.0022
<i>miR-31-5p</i>	-4.36	-2.12	0.0013
<i>let-7e-5p</i>	-4.31	-2.11	0.0022
<i>miR-30b-5p</i>	-4.20	-2.07	0.0018
<i>rno-miR-200b-3p</i>	-3.91	-1.97	0.0364
<i>miR-28a-5p</i>	-3.80	-1.93	0.0004
<i>let-7a-5p</i>	-3.80	-1.92	0.0028
<i>miR-551b-3p</i>	-3.73	-1.90	0.0429
<i>let-7f-5p</i>	-3.71	-1.89	0.0038
<i>miR-374b-5p</i>	-3.64	-1.86	0.0173
<i>miR-30c-5p</i>	-3.55	-1.83	0.0022
<i>miR-103-3p</i>	-3.39	-1.76	0.0026
<i>miR-150-5p</i>	-3.25	-1.70	0.0052
<i>rno-miR-450a-5p</i>	-3.05	-1.61	0.0101
<i>miR-99b-5p</i>	-3.04	-1.60	0.0011
<i>let-7c-5p</i>	-2.90	-1.54	0.0050
<i>miR-223-3p</i>	-2.90	-1.53	1E-04
<i>let-7d-5p</i>	-2.83	-1.50	0.0049
<i>miR-214-3p</i>	-2.64	-1.40	0.0286
<i>miR-145a-5p</i>	-2.53	-1.34	0.0180
<i>miR-98-5p</i>	-2.49	-1.31	0.0096
<i>let-7g-5p</i>	-2.47	-1.31	0.0030
<i>miR-203-3p</i>	-2.32	-1.21	0.0033
<i>miR-429-3p</i>	-2.25	-1.17	0.0219
<i>miR-142-5p</i>	-2.25	-1.17	0.0221
<i>miR-181a-5p</i>	-2.19	-1.13	0.0081
<i>miR-342-3p</i>	-2.19	-1.13	0.0051

<i>miR-423-3p</i>	-2.19	-1.13	0.0138
<i>miR-125b-5p</i>	-2.00	-1.00	0.0039
<i>miR-23b-3p</i>	-1.82	-0.87	0.0017
<i>miR-192-5p</i>	-1.56	-0.65	0.0382
<i>miR-425-5p</i>	-1.22	-0.29	0.0453
<i>miR-15b-5p</i>	1.54	0.62	0.0057
<i>miR-100-5p</i>	1.55	0.63	0.0040
<i>miR-143-3p</i>	1.65	0.72	0.0084
<i>miR-27a-3p</i>	1.81	0.85	0.0293
<i>miR-378a-3p</i>	2.12	1.08	0.0011
<i>miR-199a-3p</i>	2.17	1.12	0.0014
<i>miR-30a-5p</i>	2.21	1.14	0.0288
<i>miR-29a-3p</i>	2.35	1.24	0.0258
<i>miR-365-3p</i>	2.38	1.25	0.0499
<i>miR-185-5p</i>	2.43	1.28	0.0257
<i>rno-miR-338-3p</i>	2.43	1.28	0.0479
<i>miR-382-5p</i>	2.87	1.52	0.0407
<i>miR-15a-5p</i>	2.94	1.56	0.0008
<i>miR-322-5p</i>	3.01	1.59	0.0025
<i>miR-423-5p</i>	3.01	1.59	0.0021
<i>miR-146a-5p</i>	3.08	1.62	0.0118
<i>miR-23a-3p</i>	3.28	1.71	0.0002
<i>miR-101a-3p</i>	3.50	1.81	0.0004
<i>miR-676-3p</i>	3.72	1.90	0.0020
<i>miR-27b-3p</i>	3.79	1.92	0.0002
<i>miR-132-3p</i>	3.81	1.93	0.0009
<i>miR-532-5p</i>	4.03	2.01	0.0039
<i>let-7a-1-3p</i>	4.03	2.01	0.0081
<i>miR-122-5p</i>	4.11	2.04	0.0017
<i>miR-362-3p</i>	4.43	2.15	0.0428
<i>miR-25-3p</i>	4.52	2.18	0.0002
<i>miR-10b-5p</i>	4.74	2.24	0.0014
<i>miR-324-3p</i>	4.79	2.26	7E-05
<i>miR-21a-5p</i>	4.81	2.27	3E-05
<i>miR-101b-3p</i>	5.14	2.36	3E-06
<i>miR-320-3p</i>	5.45	2.45	0.0020
<i>miR-201-5p</i>	5.61	2.49	0.0156
<i>miR-34a-5p</i>	5.74	2.52	0.0004
<i>miR-222-3p</i>	6.18	2.63	0.0001
<i>miR-497-5p</i>	6.22	2.64	0.0013
<i>miR-335-5p</i>	6.28	2.65	4E-05
<i>miR-361-5p</i>	7.95	2.99	0.0036
<i>miR-130a-3p</i>	8.64	3.11	7E-05
<i>miR-22-3p</i>	9.48	3.24	5E-06
<i>miR-210-3p</i>	9.73	3.28	0.0044
<i>miR-152-3p</i>	10.18	3.35	1E-06
<i>miR-221-3p</i>	11.37	3.51	8E-05
<i>miR-148b-3p</i>	15.37	3.94	4E-05
<i>miR-148a-3p</i>	18.53	4.21	8E-08
<i>miR-592-5p</i>	22.22	4.47	0.0063
<i>miR-7a-5p</i>	2.73	1.45	0.0508

<i>let-7i-5p</i>	1.96	0.97	0.0508
<i>miR-218-5p</i>	-2.50	-1.32	0.0509
<i>miR-18a-5p</i>	-2.91	-1.54	0.0528
<i>miR-106b-5p</i>	-2.16	-1.11	0.0536
<i>miR-18a-3p</i>	3.18	1.67	0.0537
<i>miR-187-3p</i>	-2.20	-1.14	0.0552
<i>miR-26b-5p</i>	-2.62	-1.39	0.0606
<i>miR-99a-5p</i>	1.45	0.53	0.0634
<i>miR-29c-3p</i>	3.03	1.60	0.0706
<i>rno-miR-143-3p</i>	1.39	0.48	0.0710
<i>miR-455-5p</i>	-2.19	-1.13	0.0720
<i>rno-miR-200c-3p</i>	-3.92	-1.97	0.0729
<i>miR-350-3p</i>	-3.89	-1.96	0.0765
<i>miR-351-5p</i>	-5.98	-2.58	0.0786
<i>miR-194-5p</i>	1.44	0.52	0.0810
<i>miR-190a-5p</i>	-2.77	-1.47	0.0894
<i>miR-376c-3p</i>	-3.50	-1.81	0.0975
<i>miR-151-3p</i>	1.88	0.91	0.0983
<i>miR-200a-3p</i>	-1.94	-0.95	0.1090
<i>miR-375-3p</i>	-2.55	-1.35	0.1138
<i>miR-328-3p</i>	4.59	2.20	0.1160
<i>miR-191-5p</i>	-1.58	-0.66	0.1174
<i>miR-193a-3p</i>	2.53	1.34	0.1217
<i>miR-501-3p</i>	2.22	1.15	0.1219
<i>miR-30e-5p</i>	1.80	0.85	0.1219
<i>miR-296-5p</i>	-3.60	-1.85	0.1239
<i>miR-183-5p</i>	-2.42	-1.27	0.1427
<i>miR-154-5p</i>	1.37	0.46	0.1455
<i>miR-19a-3p</i>	1.88	0.91	0.1484
<i>miR-219a-5p</i>	2.54	1.35	0.1542
<i>miR-20a-3p</i>	-3.64	-1.86	0.1556
<i>miR-30d-5p</i>	-1.22	-0.29	0.1557
<i>miR-345-5p</i>	-1.97	-0.97	0.1684
<i>miR-93-5p</i>	-1.35	-0.43	0.1696
<i>miR-874-3p</i>	1.52	0.60	0.1723
<i>miR-128-3p</i>	-3.64	-1.86	0.1758
<i>miR-130b-3p</i>	1.96	0.97	0.1843
<i>miR-188-5p</i>	2.22	1.15	0.1955
<i>miR-434-3p</i>	-2.06	-1.04	0.1986
<i>miR-181b-5p</i>	-1.58	-0.66	0.2052
<i>miR-450a-5p</i>	-1.36	-0.44	0.2214
<i>let-7b-5p</i>	-1.61	-0.69	0.2232
<i>miR-137-3p</i>	1.61	0.68	0.2386
<i>miR-1a-3p</i>	3.00	1.59	0.2395
<i>miR-133b-3p</i>	2.43	1.28	0.2595
<i>rno-miR-551b-3p</i>	-1.67	-0.74	0.2654
<i>miR-151-5p</i>	-1.36	-0.45	0.2749
<i>miR-32-5p</i>	2.23	1.16	0.2765
<i>miR-195a-5p</i>	-1.22	-0.28	0.2767
<i>miR-16-5p</i>	-1.56	-0.64	0.2817
<i>miR-200b-3p</i>	-1.24	-0.31	0.2932

<i>rno-miR-214-3p</i>	-1.79	-0.84	0.2984
<i>miR-17-5p</i>	-1.88	-0.91	0.2985
<i>miR-199a-5p</i>	-1.41	-0.50	0.3277
<i>miR-338-3p</i>	1.67	0.74	0.3282
<i>rno-miR-136-5p</i>	2.50	1.32	0.3319
<i>miR-30c-2-3p</i>	-2.19	-1.13	0.3362
<i>miR-421-3p</i>	2.05	1.04	0.3371
<i>miR-182-5p</i>	-1.75	-0.81	0.3438
<i>miR-301a-3p</i>	-1.73	-0.79	0.3547
<i>miR-339-3p</i>	-1.69	-0.76	0.3907
<i>miR-106a-5p</i>	-1.57	-0.65	0.3914
<i>miR-140-5p</i>	1.19	0.25	0.4202
<i>miR-33-5p</i>	-1.71	-0.78	0.4227
<i>rno-miR-340-3p</i>	-1.67	-0.74	0.4578
<i>miR-652-3p</i>	-1.25	-0.32	0.4674
<i>miR-126a-3p</i>	-1.19	-0.26	0.5131
<i>miR-324-5p</i>	1.50	0.59	0.5169
<i>rno-miR-345-5p</i>	1.26	0.34	0.5537
<i>miR-96-5p</i>	1.20	0.27	0.5918
<i>miR-133a-3p</i>	1.60	0.68	0.6104
<i>miR-503-5p</i>	1.28	0.35	0.6187
<i>miR-500-3p</i>	-1.15	-0.20	0.6836
<i>miR-19b-3p</i>	1.17	0.23	0.7439
<i>miR-136-5p</i>	1.21	0.28	0.7631
<i>miR-411-5p</i>	-1.26	-0.33	0.7859
<i>miR-24-3p</i>	1.06	0.08	0.7967
<i>miR-193b-3p</i>	1.12	0.17	0.8004
<i>miR-574-3p</i>	-1.07	-0.09	0.8069
<i>miR-467a-5p</i>	-1.20	-0.26	0.8104
<i>miR-217-5p</i>	-1.08	-0.12	0.8254
<i>miR-326-3p</i>	1.12	0.17	0.8634
<i>miR-802-5p</i>	-1.04	-0.06	0.8906
<i>miR-139-3p</i>	1.09	0.13	0.9098
<i>miR-20a-5p</i>	1.03	0.05	0.9148
<i>miR-297a-5p</i>	-1.05	-0.07	0.9228
<i>miR-340-5p</i>	1.04	0.06	0.9460
<i>miR-29b-3p</i>	1.06	0.08	0.9479

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**Table S10.** miRNAs differentially expressed in exosomes isolated from liver of HFD as compared with CT mice (n=4/group).

(LCT) vs (LHFD)	Fold change	Difference (A-B log scale)	P-Value
<i>miR-34a-5p</i>	-2.73	-1.45	<b>0.0000</b>
<i>miR-497-5p</i>	-1.88	-0.91	<b>0.0381</b>
<i>miR-676-3p</i>	-1.67	-0.74	<b>0.0380</b>
<i>miR-152-3p</i>	-1.52	-0.60	<b>0.0317</b>
<i>miR-15b-5p</i>	-1.49	-0.57	<b>0.0104</b>
<i>miR-148a-3p</i>	-1.47	-0.56	<b>0.0239</b>
<i>miR-22-3p</i>	-1.37	-0.46	<b>0.0457</b>
<i>miR-378a-3p</i>	-1.36	-0.44	<b>0.0008</b>
<i>miR-23b-3p</i>	1.31	0.38	<b>0.0195</b>
<i>miR-93-5p</i>	1.60	0.68	<b>0.0456</b>
<i>miR-143-3p</i>	1.66	0.73	<b>0.0059</b>
<i>miR-30d-5p</i>	1.70	0.76	<b>0.0098</b>
<i>miR-99b-5p</i>	1.74	0.80	<b>0.0486</b>
<i>miR-30a-5p</i>	1.78	0.84	<b>0.0484</b>
<i>miR-181a-5p</i>	1.94	0.95	<b>0.0107</b>
<i>miR-200b-3p</i>	1.95	0.96	<b>0.0250</b>
<i>miR-150-5p</i>	3.16	1.66	<b>0.0454</b>
<i>miR-450a-5p</i>	3.35	1.74	<b>0.0137</b>
<i>rno-miR-143-3p</i>	1.50	0.58	0.0533
<i>let-7g-5p</i>	1.49	0.57	0.0563
<i>rno-miR-223-3p</i>	1.62	0.70	0.0637
<i>miR-3107-5p</i>	2.15	1.11	0.0638
<i>miR-425-5p</i>	1.36	0.44	0.0707
<i>miR-15a-5p</i>	-1.43	-0.51	0.0715
<i>miR-142-5p</i>	1.75	0.81	0.0725
<i>miR-101b-3p</i>	-1.23	-0.30	0.0734
<i>miR-210-3p</i>	-1.41	-0.49	0.0817
<i>U6 snRNA</i>	-1.98	-0.99	0.0821
<i>miR-30c-5p</i>	1.57	0.65	0.0827
<i>miR-126a-3p</i>	1.61	0.69	0.0863
<i>miR-125b-5p</i>	1.32	0.40	0.0882
<i>rno-miR-338-3p</i>	1.81	0.86	0.0917
<i>miR-142-3p</i>	1.61	0.69	0.0923
<i>miR-199a-5p</i>	1.58	0.66	0.1033
<i>miR-30e-5p</i>	1.71	0.78	0.1088
<i>miR-193a-3p</i>	3.04	1.60	0.1124
<i>miR-146a-5p</i>	1.61	0.69	0.1157
<i>miR-503-5p</i>	2.38	1.25	0.1212
<i>miR-361-5p</i>	-1.38	-0.46	0.1214
<i>miR-122-5p</i>	1.47	0.56	0.1241
<i>miR-322-5p</i>	-1.36	-0.44	0.1393
<i>miR-148b-3p</i>	-1.66	-0.73	0.1418
<i>miR-99a-5p</i>	-1.31	-0.39	0.1435
<i>miR-194-5p</i>	-1.24	-0.31	0.1657
<i>miR-190a-5p</i>	-1.62	-0.70	0.1770
<i>miR-423-5p</i>	-1.26	-0.34	0.1786
<i>miR-342-3p</i>	1.53	0.61	0.1809



<i>miR-151-5p</i>	1.36	0.45	0.1860
<i>miR-185-5p</i>	-1.38	-0.47	0.1950
<i>miR-223-3p</i>	1.28	0.35	0.2017
<i>miR-21a-5p</i>	1.24	0.32	0.2065
<i>miR-532-5p</i>	-1.34	-0.42	0.2066
<i>let-7c-5p</i>	1.33	0.41	0.2101
<i>let-7f-5p</i>	1.31	0.39	0.2153
<i>miR-130b-3p</i>	-1.70	-0.77	0.2216
<i>miR-140-5p</i>	1.23	0.30	0.2269
<i>miR-17-5p</i>	1.85	0.89	0.2310
<i>miR-28a-5p</i>	1.42	0.50	0.2409
<i>miR-106b-5p</i>	1.54	0.62	0.2410
<i>miR-141-3p</i>	-2.22	-1.15	0.2474
<i>miR-423-3p</i>	-12.76	-3.67	0.2580
<i>miR-362-3p</i>	1.82	0.86	0.2625
<i>miR-429-3p</i>	1.27	0.34	0.2707
<i>miR-20a-5p</i>	1.34	0.42	0.2756
<i>miR-29b-3p</i>	2.34	1.23	0.2791
<i>let-7e-5p</i>	1.35	0.44	0.2857
<i>miR-144-3p</i>	1.89	0.92	0.2867
<i>miR-139-5p</i>	1.49	0.57	0.3012
<i>miR-31-5p</i>	1.42	0.51	0.3082
<i>miR-345-5p</i>	-1.58	-0.66	0.3115
<i>let-7a-5p</i>	1.29	0.37	0.3149
<i>miR-501-3p</i>	1.45	0.54	0.3175
<i>miR-222-3p</i>	-1.12	-0.16	0.3200
<i>miR-219a-5p</i>	1.85	0.89	0.3247
<i>miR-382-5p</i>	1.60	0.68	0.3318
<i>miR-335-5p</i>	-1.42	-0.50	0.3343
<i>miR-324-3p</i>	1.21	0.27	0.3538
<i>miR-10b-5p</i>	-1.27	-0.35	0.3688
<i>miR-191-5p</i>	1.26	0.34	0.3716
<i>miR-467a-5p</i>	2.03	1.02	0.3820
<i>miR-133a-3p</i>	-2.18	-1.12	0.3850
<i>let-7d-5p</i>	1.26	0.33	0.3895
<i>miR-16-5p</i>	1.35	0.43	0.4120
<i>miR-26b-5p</i>	1.47	0.56	0.4137
<i>miR-30b-5p</i>	1.24	0.31	0.4218
<i>miR-103-3p</i>	1.22	0.28	0.4349
<i>miR-137-3p</i>	1.35	0.43	0.4414
<i>miR-201-5p</i>	1.41	0.49	0.4451
<i>miR-100-5p</i>	1.12	0.16	0.4560
<i>miR-375-3p</i>	-1.49	-0.58	0.4589
<i>let-7a-1-3p</i>	1.29	0.37	0.4641
<i>miR-101a-3p</i>	-1.10	-0.14	0.4824
<i>miR-133b-3p</i>	-1.56	-0.64	0.4934
<i>miR-874-3p</i>	-1.25	-0.32	0.4967
<i>miR-199a-3p</i>	1.12	0.16	0.4977
<i>miR-324-5p</i>	-1.41	-0.50	0.5024
<i>miR-365-3p</i>	-1.23	-0.30	0.5063
<i>miR-203-3p</i>	1.14	0.18	0.5156

<i>miR-188-5p</i>	-1.42	-0.51	0.5174
<i>miR-26a-5p</i>	1.28	0.36	0.5393
<i>miR-98-5p</i>	1.31	0.39	0.5411
<i>miR-154-5p</i>	1.41	0.49	0.5550
<i>RNU1A1</i>	-1.59	-0.67	0.5889
<i>miR-339-5p</i>	-1.32	-0.40	0.5913
<i>miR-297a-5p</i>	-1.30	-0.38	0.5975
<i>miR-500-3p</i>	1.24	0.31	0.6027
<i>rno-miR-214-3p</i>	-1.30	-0.37	0.6076
<i>miR-214-3p</i>	-1.14	-0.19	0.6296
<i>miR-30c-2-3p</i>	-1.35	-0.43	0.6366
<i>miR-19b-3p</i>	1.28	0.35	0.6401
<i>miR-27a-3p</i>	1.08	0.11	0.6627
<i>miR-350-3p</i>	1.26	0.34	0.6761
<i>miR-29a-3p</i>	-1.11	-0.15	0.6786
<i>miR-32-5p</i>	-1.32	-0.40	0.6787
<i>miR-132-3p</i>	-1.05	-0.07	0.6829
<i>miR-200a-3p</i>	1.19	0.25	0.6876
<i>miR-421-3p</i>	1.33	0.41	0.6978
<i>miR-33-5p</i>	1.36	0.44	0.7090
<i>miR-326-3p</i>	1.26	0.34	0.7154
<i>miR-7a-5p</i>	1.14	0.19	0.7164
<i>miR-434-3p</i>	1.22	0.29	0.7178
<i>miR-25-3p</i>	-1.09	-0.12	0.7198
<i>miR-340-5p</i>	-1.21	-0.27	0.7258
<i>miR-455-5p</i>	-1.11	-0.15	0.7278
<i>miR-107-3p</i>	-1.12	-0.17	0.7289
<i>miR-221-3p</i>	1.06	0.08	0.7570
<i>miR-18a-3p</i>	-1.14	-0.19	0.7643
<i>rno-miR-345-5p</i>	-1.14	-0.19	0.7645
<i>miR-484</i>	1.12	0.17	0.7662
<i>miR-18a-5p</i>	-1.15	-0.21	0.7695
<i>let-7b-5p</i>	1.10	0.13	0.7772
<i>miR-592-5p</i>	-1.06	-0.08	0.7816
<i>miR-151-3p</i>	1.05	0.08	0.7868
<i>miR-192-5p</i>	1.05	0.06	0.7965
<i>miR-181b-5p</i>	1.14	0.19	0.8011
<i>RNU5G</i>	1.17	0.23	0.8053
<i>miR-802-5p</i>	-1.06	-0.08	0.8109
<i>miR-130a-3p</i>	-1.05	-0.06	0.8218
<i>miR-331-3p</i>	-1.15	-0.20	0.8222
<i>miR-23a-3p</i>	1.04	0.06	0.8241
<i>miR-24-3p</i>	1.05	0.07	0.8474
<i>miR-27b-3p</i>	-1.02	-0.03	0.8632
<i>miR-320-3p</i>	-1.05	-0.06	0.8673
<i>miR-338-3p</i>	-1.09	-0.12	0.8731
<i>miR-301a-3p</i>	1.08	0.11	0.8846
<i>miR-1a-3p</i>	-1.06	-0.08	0.9300
<i>miR-652-3p</i>	-1.03	-0.04	0.9313
<i>miR-125a-5p</i>	-1.04	-0.05	0.9389
<i>let-7i-5p</i>	1.02	0.02	0.9456

<i>miR-328-3p</i>	1.05	0.07	0.9472
<i>miR-193b-3p</i>	-1.02	-0.03	0.9662
<i>miR-195a-5p</i>	-1.01	-0.01	0.9742
<i>miR-187-3p</i>	1.01	0.02	0.9743
<i>miR-145a-5p</i>	1.01	0.01	0.9750
<i>miR-29c-3p</i>	-1.01	-0.02	0.9800
<i>miR-19a-3p</i>	-1.01	-0.01	0.9882
<i>miR-574-3p</i>	1.00	0.01	0.9893
<i>miR-200c-3p</i>	-1.00	0.00	1.0000

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**Table S11.** miRNAs differentially expressed in exosomes isolated from liver of SAC as compared with CT mice (n=4/group).

<b>(LCT) vs (LSAC)</b>	<b>Fold change</b>	<b>Difference (A-B log scale)</b>	<b>P-Value</b>
<i>miR-190a-5p</i>	-2.93	-1.55	<b>0.0332</b>
<i>miR-214-3p</i>	-2.39	-1.26	<b>0.0379</b>
<i>miR-335-5p</i>	-2.20	-1.14	<b>0.0134</b>
<i>miR-34a-5p</i>	-2.18	-1.13	<b>0.0031</b>
<i>miR-223-3p</i>	-1.62	-0.69	<b>0.0192</b>
<i>miR-143-3p</i>	1.55	0.63	<b>0.0097</b>
<i>miR-27b-3p</i>	1.78	0.83	<b>0.0209</b>
<i>miR-324-3p</i>	1.86	0.90	<b>0.0232</b>
<i>miR-122-5p</i>	1.93	0.94	<b>0.0355</b>
<i>miR-221-3p</i>	1.94	0.95	<b>0.0437</b>
<i>miR-592-5p</i>	2.04	1.03	<b>0.0435</b>
<i>miR-22-3p</i>	2.09	1.07	<b>0.0026</b>
<i>miR-193a-3p</i>	4.66	2.22	<b>0.0137</b>
<i>rno-miR-223-3p</i>	-2.18	-1.12	0.0514
<i>miR-101b-3p</i>	1.34	0.42	0.0577
<i>miR-29a-3p</i>	1.92	0.94	0.0601
<i>miR-425-5p</i>	1.57	0.65	0.0648
<i>miR-30e-5p</i>	2.19	1.13	0.0665
<i>miR-31-5p</i>	-1.72	-0.78	0.0679
<i>miR-210-3p</i>	1.54	0.62	0.0686
<i>miR-200a-3p</i>	-2.09	-1.06	0.0697
<i>miR-365-3p</i>	1.96	0.97	0.0698
<i>rno-miR-338-3p</i>	2.24	1.16	0.0736
<i>miR-30b-5p</i>	-1.67	-0.74	0.0747
<i>miR-501-3p</i>	2.31	1.21	0.0752
<i>miR-133a-3p</i>	-2.88	-1.53	0.0779
<i>let-7f-5p</i>	-1.48	-0.57	0.0789
<i>miR-200c-3p</i>	-2.49	-1.31	0.0801
<i>rno-miR-143-3p</i>	1.35	0.43	0.0811
<i>miR-154-5p</i>	-1.74	-0.80	0.0821
<i>miR-142-3p</i>	-1.66	-0.73	0.0847
<i>miR-148b-3p</i>	1.65	0.72	0.0905
<i>miR-141-3p</i>	-2.48	-1.31	0.0928
<i>miR-362-3p</i>	2.71	1.44	0.0969
<i>let-7e-5p</i>	-1.66	-0.73	0.1032
<i>miR-107-3p</i>	-1.86	-0.90	0.1088
<i>miR-27a-3p</i>	1.43	0.52	0.1192
<i>miR-455-5p</i>	-1.62	-0.69	0.1271
<i>miR-99a-5p</i>	1.37	0.45	0.1292
<i>let-7d-5p</i>	-1.45	-0.54	0.1339
<i>miR-10b-5p</i>	2.25	1.17	0.1369
<i>miR-500-3p</i>	1.69	0.75	0.1375
<i>miR-125b-5p</i>	1.35	0.43	0.1447
<i>miR-18a-3p</i>	2.18	1.12	0.1498
<i>miR-193b-3p</i>	1.73	0.79	0.1537
<i>miR-15a-5p</i>	1.30	0.38	0.1607
<i>miR-28a-5p</i>	-1.56	-0.65	0.1623

<i>let-7g-5p</i>	-1.30	-0.38	0.1624
<i>miR-3107-5p</i>	1.94	0.96	0.1648
<i>miR-152-3p</i>	-1.17	-0.23	0.1662
<i>miR-181b-5p</i>	-1.65	-0.72	0.1669
<i>miR-802-5p</i>	1.58	0.66	0.1802
<i>miR-106b-5p</i>	-1.53	-0.61	0.1814
<i>miR-350-3p</i>	-2.23	-1.16	0.1975
<i>miR-15b-5p</i>	1.44	0.53	0.2021
<i>miR-24-3p</i>	1.30	0.38	0.2071
<i>miR-93-5p</i>	1.34	0.42	0.2113
<i>miR-133b-3p</i>	-1.79	-0.84	0.2130
<i>miR-130b-3p</i>	-1.67	-0.74	0.2134
<i>rno-miR-345-5p</i>	1.62	0.69	0.2172
<i>miR-125a-5p</i>	-1.76	-0.82	0.2310
<i>miR-297a-5p</i>	-2.50	-1.32	0.2360
<i>miR-29c-3p</i>	1.86	0.90	0.2403
<i>miR-26a-5p</i>	-1.63	-0.70	0.2418
<i>miR-301a-3p</i>	-1.60	-0.68	0.2544
<i>miR-188-5p</i>	-2.01	-1.01	0.2548
<i>miR-450a-5p</i>	1.25	0.32	0.2641
<i>miR-99b-5p</i>	-1.54	-0.63	0.2713
<i>miR-148a-3p</i>	1.42	0.51	0.2796
<i>miR-7a-5p</i>	1.49	0.57	0.2803
<i>miR-16-5p</i>	1.50	0.59	0.2915
<i>miR-423-5p</i>	1.22	0.29	0.2955
<i>miR-219a-5p</i>	1.82	0.86	0.3051
<i>miR-132-3p</i>	1.33	0.41	0.3152
<i>miR-434-3p</i>	1.43	0.51	0.3266
<i>miR-676-3p</i>	1.47	0.55	0.3280
<i>miR-222-3p</i>	1.40	0.48	0.3333
<i>miR-574-3p</i>	-1.30	-0.37	0.3344
<i>miR-199a-5p</i>	-1.42	-0.50	0.3364
<i>miR-324-5p</i>	-1.71	-0.78	0.3404
<i>miR-339-5p</i>	-1.34	-0.42	0.3489
<i>let-7a-5p</i>	-1.29	-0.37	0.3514
<i>miR-30c-5p</i>	-1.26	-0.33	0.3857
<i>U6 snRNA</i>	-1.55	-0.63	0.3863
<i>miR-142-5p</i>	1.23	0.30	0.3865
<i>miR-467a-5p</i>	-1.84	-0.88	0.3919
<i>miR-21a-5p</i>	1.21	0.27	0.4030
<i>miR-429-3p</i>	-1.52	-0.61	0.4173
<i>miR-33-5p</i>	1.63	0.71	0.4282
<i>miR-194-5p</i>	1.14	0.19	0.4325
<i>miR-151-5p</i>	-1.19	-0.25	0.4470
<i>miR-30a-5p</i>	1.38	0.47	0.4504
<i>miR-19a-3p</i>	1.33	0.41	0.4862
<i>miR-185-5p</i>	1.20	0.27	0.5064
<i>miR-98-5p</i>	-1.25	-0.32	0.5112
<i>miR-137-3p</i>	1.31	0.38	0.5155
<i>miR-103-3p</i>	-1.17	-0.23	0.5310
<i>miR-497-5p</i>	-1.20	-0.26	0.5325

<i>miR-421-3p</i>	1.50	0.58	0.5389
<i>miR-144-3p</i>	1.37	0.45	0.5525
<i>let-7c-5p</i>	-1.14	-0.18	0.5529
<i>miR-191-5p</i>	-1.15	-0.20	0.5625
<i>miR-342-3p</i>	-1.14	-0.18	0.5647
<i>miR-503-5p</i>	1.34	0.42	0.5695
<i>miR-140-5p</i>	-1.19	-0.25	0.5788
<i>RNU5G</i>	-1.44	-0.52	0.6036
<i>miR-328-3p</i>	1.50	0.59	0.6064
<i>miR-101a-3p</i>	1.11	0.15	0.6323
<i>miR-19b-3p</i>	1.23	0.30	0.6357
<i>miR-23a-3p</i>	-1.07	-0.10	0.6381
<i>miR-199a-3p</i>	1.07	0.09	0.6516
<i>miR-200b-3p</i>	1.11	0.16	0.6662
<i>miR-25-3p</i>	1.14	0.18	0.6798
<i>let-7a-1-3p</i>	1.18	0.23	0.6823
<i>miR-375-3p</i>	-1.15	-0.20	0.6839
<i>miR-331-3p</i>	-1.22	-0.29	0.6871
<i>miR-29b-3p</i>	1.32	0.40	0.6919
<i>miR-361-5p</i>	1.08	0.11	0.6968
<i>miR-203-3p</i>	-1.07	-0.10	0.7237
<i>miR-423-3p</i>	-1.15	-0.20	0.7256
<i>miR-30c-2-3p</i>	-1.19	-0.25	0.7331
<i>let-7b-5p</i>	1.16	0.21	0.7345
<i>miR-23b-3p</i>	1.03	0.05	0.7354
<i>miR-652-3p</i>	-1.09	-0.12	0.7438
<i>miR-322-5p</i>	1.07	0.10	0.7556
<i>miR-146a-5p</i>	-1.09	-0.13	0.7614
<i>miR-181a-5p</i>	1.12	0.16	0.7757
<i>miR-345-5p</i>	1.12	0.16	0.7834
<i>miR-17-5p</i>	1.14	0.19	0.7853
<i>miR-378a-3p</i>	-1.04	-0.05	0.7872
<i>miR-192-5p</i>	-1.05	-0.07	0.7874
<i>miR-30d-5p</i>	-1.05	-0.07	0.7910
<i>miR-320-3p</i>	-1.08	-0.11	0.7968
<i>miR-20a-5p</i>	1.07	0.10	0.8074
<i>miR-126a-3p</i>	-1.07	-0.10	0.8078
<i>miR-18a-5p</i>	1.09	0.12	0.8180
<i>miR-187-3p</i>	1.12	0.16	0.8227
<i>let-7i-5p</i>	-1.06	-0.08	0.8318
<i>miR-874-3p</i>	1.11	0.15	0.8332
<i>miR-26b-5p</i>	-1.09	-0.12	0.8393
<i>miR-130a-3p</i>	-1.06	-0.09	0.8465
<i>miR-151-3p</i>	1.07	0.09	0.8753
<i>miR-100-5p</i>	1.02	0.03	0.8799
<i>miR-201-5p</i>	-1.06	-0.08	0.8811
<i>miR-32-5p</i>	-1.10	-0.14	0.8846
<i>miR-340-5p</i>	1.08	0.12	0.8872
<i>miR-145a-5p</i>	-1.03	-0.04	0.9184
<i>miR-195a-5p</i>	-1.02	-0.02	0.9353
<i>miR-326-3p</i>	1.03	0.04	0.9616

<i>rno-miR-214-3p</i>	1.02	0.03	0.9657
<i>RNU1A1</i>	-1.04	-0.05	0.9677
<i>miR-150-5p</i>	-1.01	-0.02	0.9726
<i>miR-382-5p</i>	1.01	0.02	0.9753
<i>miR-1a-3p</i>	-1.02	-0.02	0.9759
<i>miR-484</i>	1.02	0.03	0.9767
<i>miR-338-3p</i>	1.01	0.01	0.9814
<i>miR-139-5p</i>	1.01	0.01	0.9893
<i>miR-532-5p</i>	-1.00	0.00	1.0000

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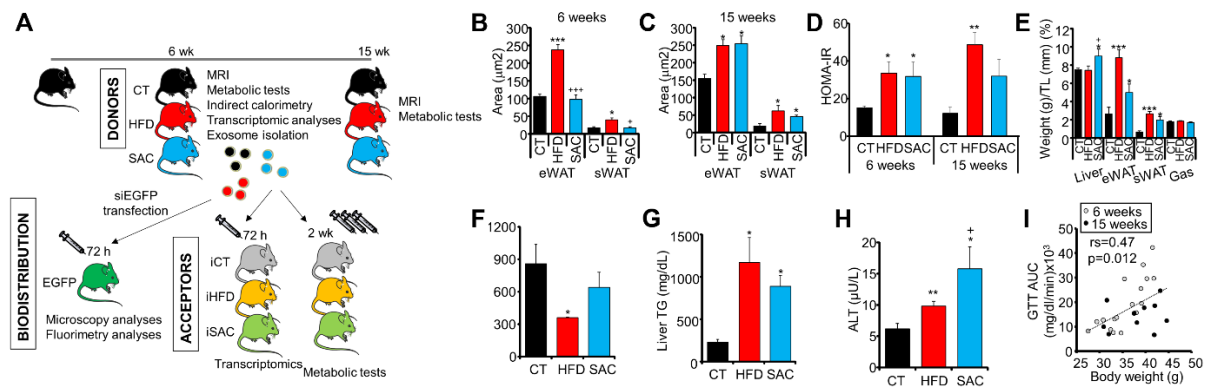


**Table S12.** Correlation coefficients between clinical parameters and selected circulating miRNAs in a human cohort. Boldface indicates  $p < 0.05$  ( $n = 40$ ). WHR= waist/hip ratio; FPI= fasting plasma insulin; FPG= fasting plasma glucose; G120= glucose at the 2h point during an oral glucose tolerance test.

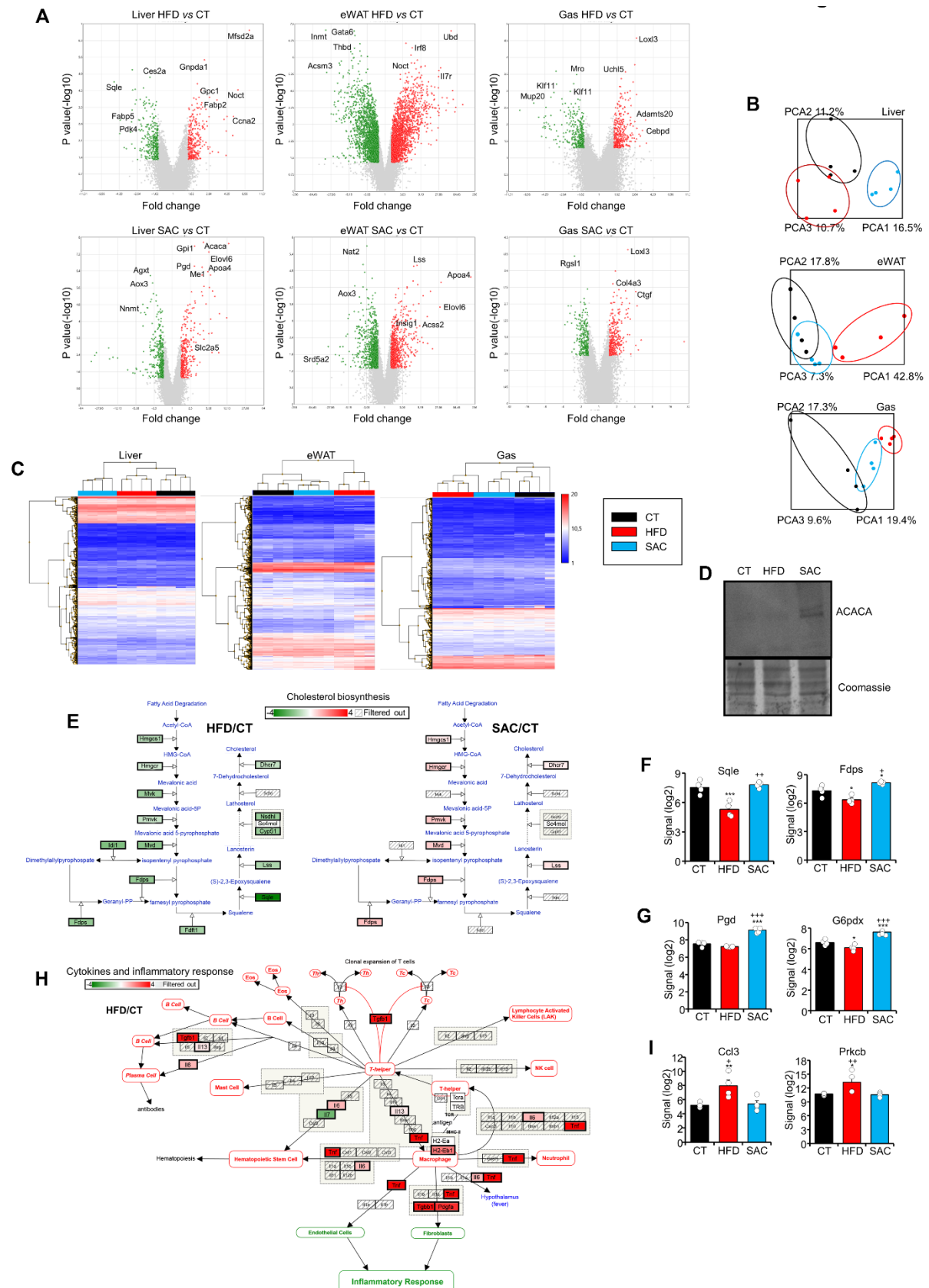
	<i>miR-122-5p</i>	<i>miR-378-3p</i>	<i>miR-19b-3p</i>	<i>miR-101-3p</i>	<i>miR-107-3p</i>	<i>miR-223-3p</i>	<i>miR-22-3p</i>
<b>BMI</b>	-0.130	0.218	-0.059	0.002	-0.175	-0.202	<b>0.351</b>
<b>WHR</b>	0.065	0.301	0.087	-0.046	-0.184	0.002	<b>0.405</b>
<b>FLI</b>	0.170	0.230	-0.034	-0.111	-0.289	-0.127	<b>0.409</b>
<b>TG (mmol/L)</b>	<b>0.340</b>	0.090	0.032	-0.197	-0.217	-0.026	<b>0.322</b>
<b>CHOL (mmol/L)</b>	-0.082	<b>-0.368</b>	0.043	-0.009	0.113	-0.006	0.170
<b>LDL (mmol/L)</b>	-0.113	-0.248	0.036	0.118	0.027	-0.030	0.232
<b>HDL (mmol/L)</b>	-0.277	<b>-0.406</b>	-0.076	0.020	<b>0.403</b>	0.112	-0.285
<b>FPI (mIU/L)</b>	0.152	<b>0.397</b>	-0.209	0.107	-0.118	-0.301	<b>0.436</b>
<b>FPG (mg/dl)</b>	-0.058	0.057	0.099	-0.179	0.010	<b>-0.413</b>	<b>0.462</b>
<b>G120 (mg/dl)</b>	0.116	0.069	0.078	0.058	-0.177	-0.116	<b>0.403</b>
<b>HOMA-IR</b>	0.101	<b>0.356</b>	-0.171	0.008	-0.090	<b>-0.387</b>	<b>0.494</b>

**Table S13.** Main clinical features and *miR-22-3p* values of the study population.

	<b>FLI&lt;60 (n=13)</b>	<b>FLI&gt;60 (n=27)</b>	<b>p</b>
<b>AGE (yr)</b>	55.38 ± 1.60	52.75 ± 0.90	0.132
<b>FLI</b>	44.55 ± 3.66	83.58 ± 2.21	<b>0.000</b>
<b>BMI (kg/m<sup>2</sup>)</b>	27.10 ± 0.61	31.05 ± 0.58	<b>0.000</b>
<b>Cholesterol (mmol/L)</b>	4.96 ± 0.27	5.04 ± 0.15	0.772
<b>HDL (mmol/L)</b>	1.17 ± 0.09	1.04 ± 0.05	0.164
<b>LDL (mmol/L)</b>	2.77 ± 0.19	2.77 ± 0.11	0.993
<b>TG (mmol/L)</b>	1.31 ± 0.18	2.34 ± 0.26	<b>0.013</b>
<b>Insulin (mIU/L)</b>	11.35 ± 1.78	14.88 ± 1.41	0.161
<b>Glucose (mg/dL)</b>	95.53 ± 3.50	99.51 ± 2.91	0.423
<b>HOMA-IR</b>	2.72 ± 0.45	3.75 ± 0.44	0.161
<b><i>miR-22-3p</i> (dCt)</b>	1.07 ± 0.08	1.38 ± 0.10	0.050

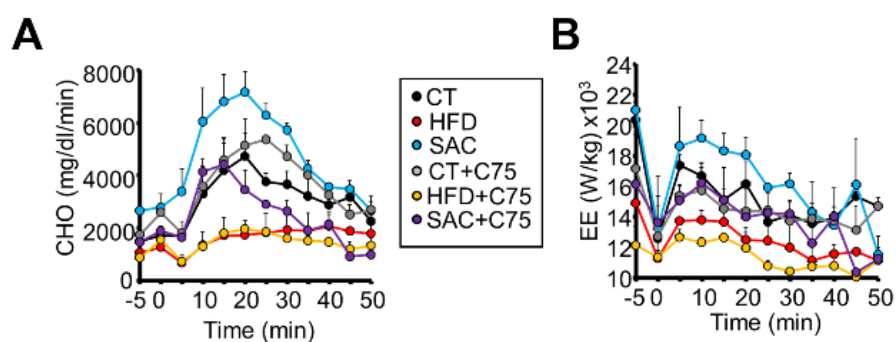


**Figure S1.** High-fat and high-sucrose diets lead to a convergent obese phenotype over time. **(A)** Scheme of animal experiments. **(B, C)** Quantification of fat distribution of mice fed either standard chow (CT), a high-fat diet (HFD) or standard chow with drinking water supplemented with 50% sucrose (SAC) after 6 **(B)** and 15 weeks **(C)** of diet, according to the MRI scans shown in Figure 1E. **(D)** HOMA-IR of the same mice as calculated from the values of glucose and insulin shown in Figure 1. **(E)** Percentage of tissue weight relative to tibia length at the time of sacrifice of CT, HFD and SAC mice after 6 weeks. **(F)** Adipocyte number in the eWAT of the same mice as determined from the IHC shown in figure 1O. **(G, H)** Liver TG content **(G)** and plasma ALT activity of the same mice **(H)**. **(I)** Correlation of body weight with the glucose AUC of the GTTs performed at 6 weeks (white circles) and 15 weeks (black circles).  $n=3/\text{group}$  (B, C, F),  $n=6/\text{group}$  (D, E, G, H),  $n=12/\text{group}$  (6 weeks) and  $n=3/\text{group}$  (15 weeks) (I). \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.005$  with respect to CT group. + $p<0.05$ , ++ $p<0.01$ , +++ $p<0.005$  with respect to HFD group.



**Figure S2.** HFD and SAC induce distinct gene expression profiles in eWAT and liver. **(A)** Volcano plots of HFD/CT (upper panels) and SAC/CT (lower panels) liver, eWAT and gastrocnemius muscle. **(B)** PCA of liver (upper panel), eWAT (middle panel) and muscle (lower panel). **(C)** Heatmaps showing hierarchical clustering of differentially expressed genes comparing CT, HFD and SAC groups. **(D)** Key DNL regulator ACACA is increased in SAC liver. **(E, F)** The cholesterol biosynthesis pathway is

decreased in HFD liver and increased in SAC liver. (G) Genes of the pentose phosphate pathway are increased in liver of SAC mice. (H, I) The cytokine and inflammatory response pathway is increased in HFD eWAT. n=4/group (A-I). \*p<0.05, \*\*p<0.01, \*\*\*p<0.005 with respect to CT group. \*p<0.05, \*\*p<0.01, \*\*\*p<0.005 with respect to HFD group.

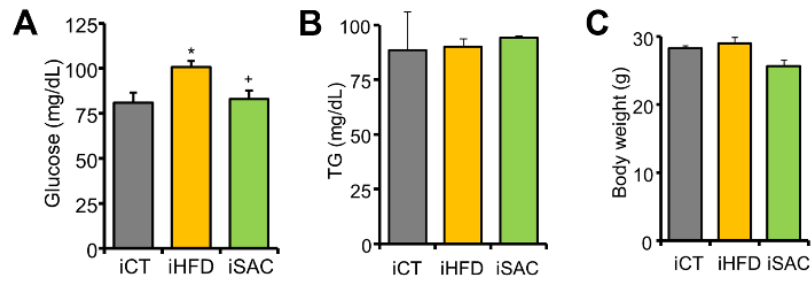


**Figure S3.** SAC mice rely on DNL to maintain glucose homeostasis. **(A, B)** Oral administration of glucose increases carbohydrate oxidation **(A)** and energy expenditure **(B)** in SAC mice to levels above CT mice, as calculated by  $VO_2$  and  $VCO_2$  values measured by indirect calorimetry, and FASN inhibitor C75 pretreatment blunts the increase.  $n=3/\text{group}$ .

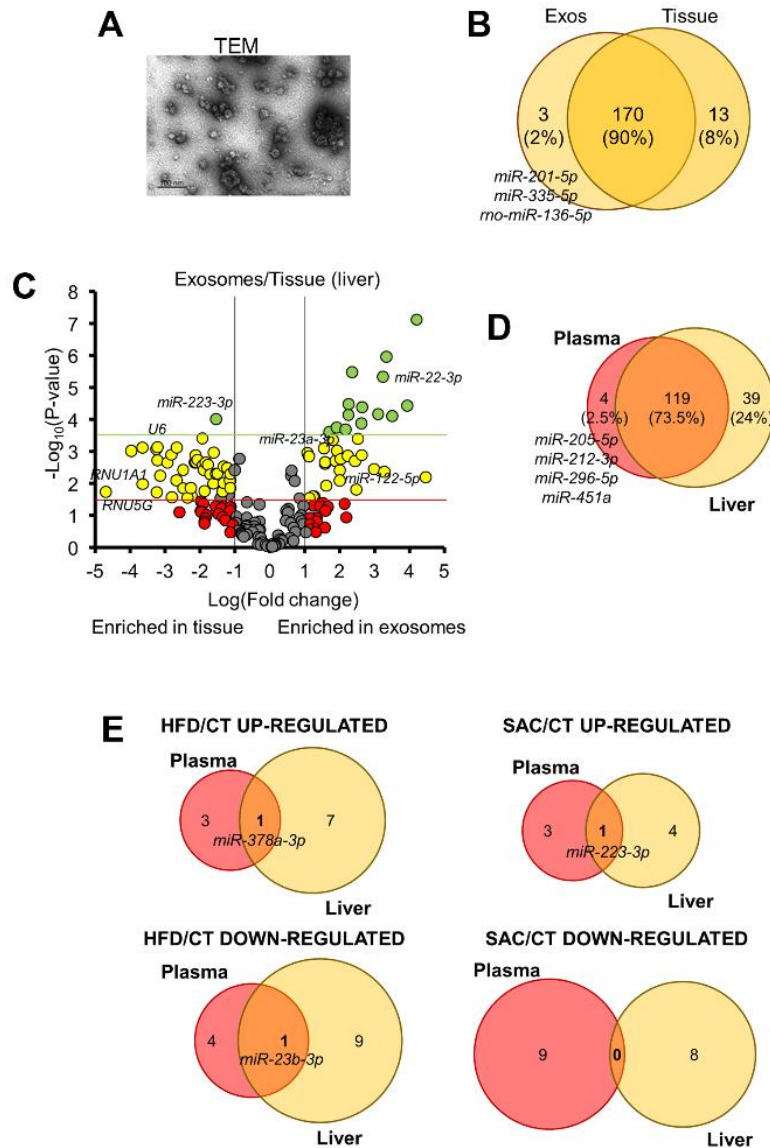




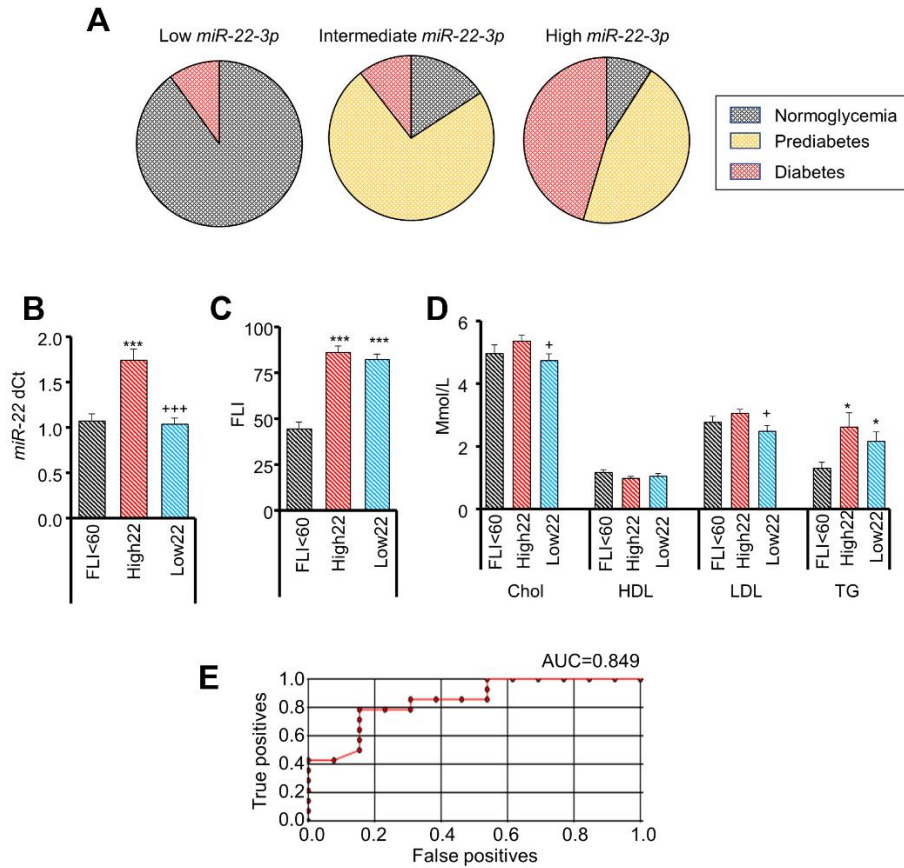
Global distribution of fluorescence was determined by IVISS scanning. (C) Volcano plots of liver, eWAT and gastrocnemius muscle of mice injected with HFD plasma exosomes (iHFD) (upper panels) or SAC exosomes (iSAC) (lower panels) as compared to those injected with CT exosomes (iCT). (D) PCA of liver (upper panel), eWAT (middle panel) and muscle (lower panel). (E) Heatmaps showing hierarchical clustering of differentially expressed genes comparing iCT, iHFD and iSAC groups. (F, G) Prostaglandin genes are decreased (F) and eicosanoid synthesis is increased in eWAT of iHFD mice (G). n=3/group (A, B), n=4/group (C-G). \*p<0.05, \*\*p<0.01, \*\*\*p<0.005 with respect to CT group. +p<0.05, ++p<0.01, +++p<0.005 with respect to HFD group.



**Figure S5.** Injection of HFD and SAC plasma exosomes reproduce the metabolic phenotype observed in donor mice. (A-C) Chronic treatment of control mice with exosomes isolated from plasma of CT, HFD and SAC mice show increased fasting plasma glucose in iHFD mice (A) with no changes in circulating triglyceride (B) or body weight (C) for any group.  $n=4/\text{group}$ . \* $p<0.05$  with respect to iCT group. <sup>+</sup> $p<0.05$  with respect to iHFD group.



**Figure S6.** The miRNA profile differs between HFD and SAC plasma and liver exosomes. (A) Liver exosomes are characterized by transmission electron microscopy. (B) Comparison of the detected miRNAs in control liver exosomes and tissue. (C) Volcano plot of liver exosomes and liver tissue shows enrichment of snRNAs in tissue as compared to exosome samples. (D) Comparison of the miRNAs detected by RT-PCR in plasma and tissue exosomes of CT mice. (E) Comparison of the upregulated and downregulated miRNAs in plasma and liver exosomes of HFD (left panels) and SAC mice (right panels).  $n=4/\text{group}$  (A-E).



**Figure S7.** Circulating *miR*-22-3p can be used as a biomarker for MAFLD stratification. (A) Subjects with increasing levels of circulating *miR*-22-3p (low < 1.0 < intermediate < 1.4 < high, relative values) display a more unfavorable clinical diagnosis. (B) Subjects with a low fatty liver index (FLI<60) show low levels of circulating *miR*-22-3p, whereas subjects with FLI>60 can be separated into two subgroups with either high (High22) or low (Low22) circulating *miR*-22-3p levels. (C, D) Individuals in the High22 group show equivalent FLI (C) but a worse lipid profile (D) than those in the Low22 group. (E) ROC analysis of circulating *miR*-22-3p as a parameter to discern HOMA-IR>2.7 in patients with FLI>60. n=40 (A-D), n=27 (E). \*p<0.05, \*\*\*p<0.005 with respect to FLI<60 group. \*p<0.05, \*\*\*p<0.005, with respect to High22 group.