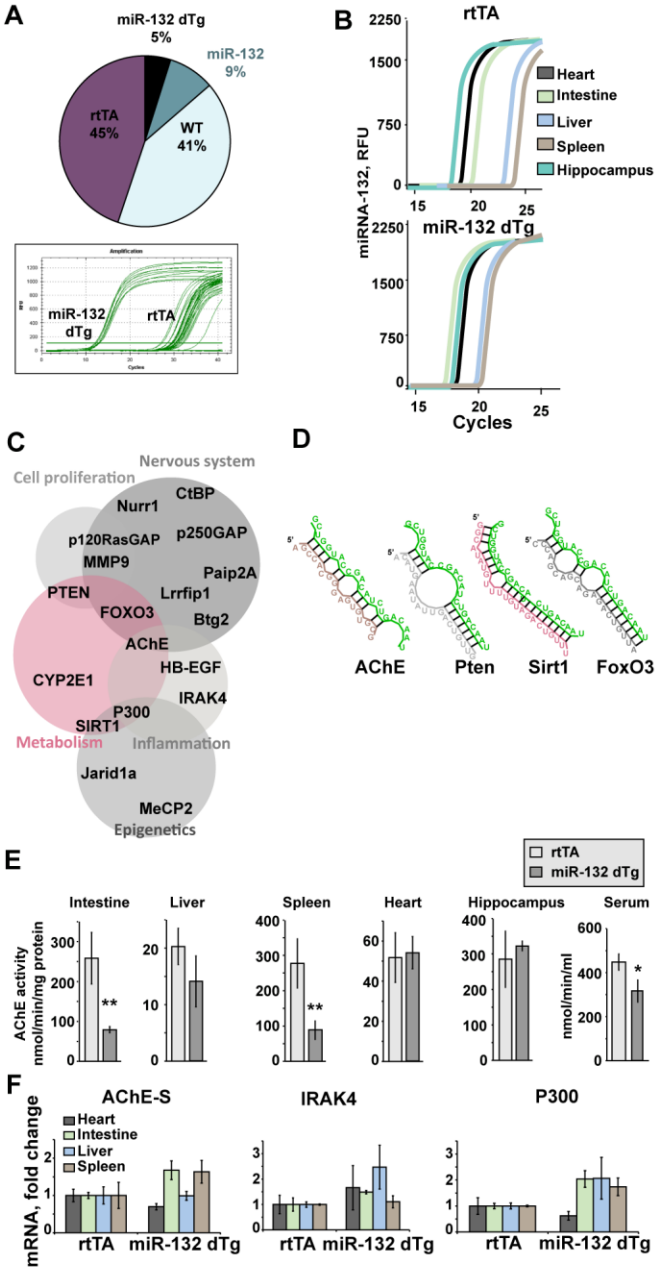
Supplementary material

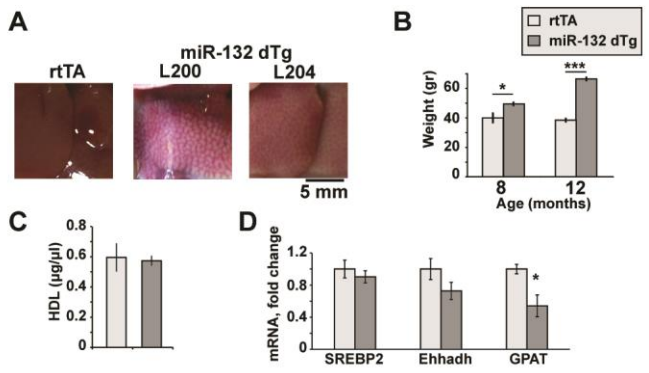
SUPPLEMENTARY FIGURES



Supplementary Figure 1: Characterization of miR-132 dTg mice.

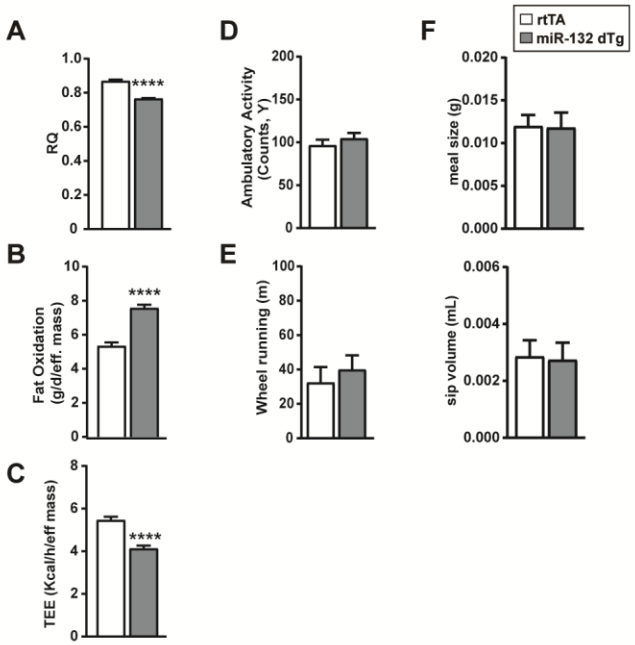
(A) Ratio of transgenic mice progeny, and representative nested real-time PCR genotyping results of doxycycline-inducible miR-132 dTg mice, compared to their rtTA littermate controls.

(B) Excess miR-132 levels observed in the heart, intestine, liver, spleen but not hippocampus of miR-132 dTg mice (n=12 for rtTA, n=5 for miR-132. $P<0.05$ in all cases). (C) Examples of validated miR-132 targets and their biological functions. (D) Predicted structures of the potential binding site sequences of miR-132 with the AChE, Pten, Sirt1, and FoxO3 transcripts. (E) Reduction of AChE activity in a number of miR-132 dTg tissues (n=4). (F) mRNA levels of the miR-132 targets AChE-S, IRAK4 and P300 are insignificantly changed in various tissues from miR-132 dTg mice (n=5 for rtTA, n=7 for miR-132, n.s. in all cases).



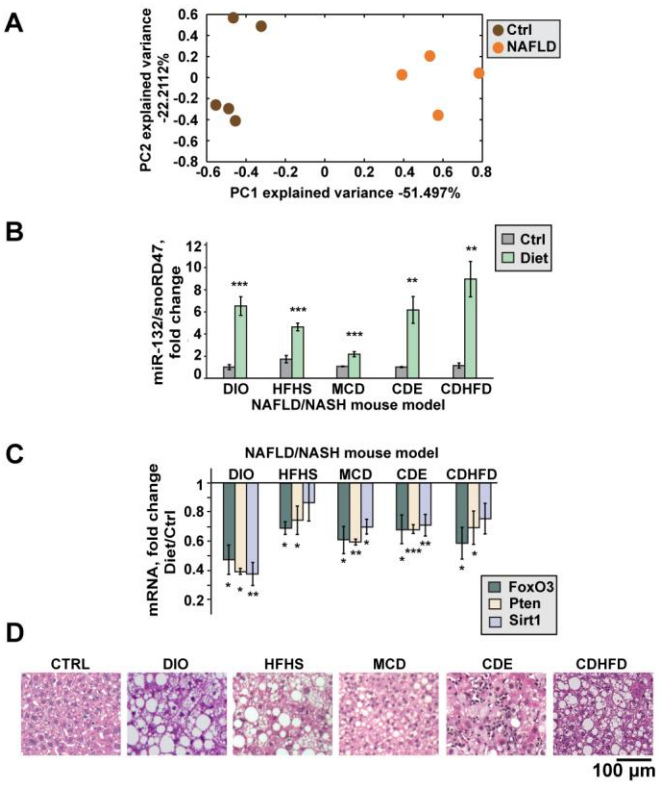
Supplementary Figure 2: miR-132 dTg mice phenotype.

(A) Representative image of fatty liver in two additional miR-132 dTg lines. (B) Body weight of 8 and 12-month old miR-132 dTg mice showing greater weight compared to their rtTA littermates, without doxycycline. (C) Unchanged HDL levels in serum of miR-132 dTg mice compared to littermates. (D) mRNA transcripts in miR-132 dTg mice compared to rtTA, normalized to multiple housekeeping genes and then to rtTA controls. Data were obtained by Fluidigm analysis and are representative of three experiments (n=3 per group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ determined by Student's t-test. Values are expressed as mean ±SEM).



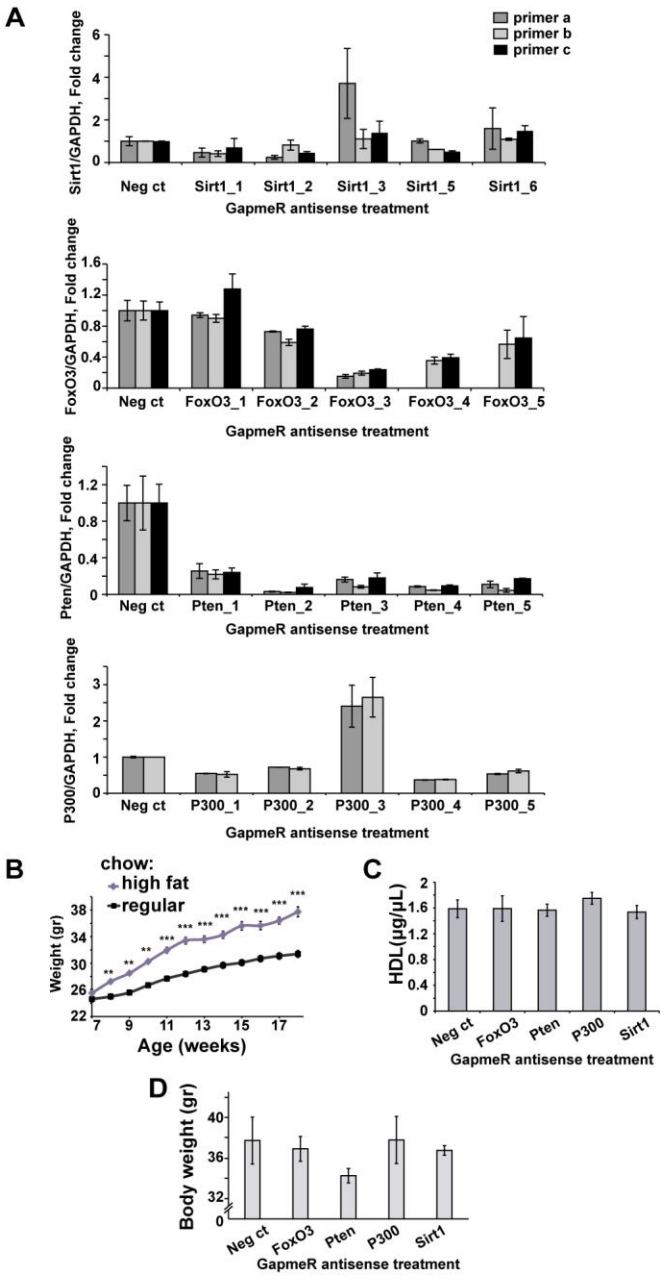
Supplementary Figure 3: miR-132 dTg mice show a lower metabolic rate.

Mice were monitored by the Promethion High-Definition Behavioral Phenotyping System over a 24 h period. MiR-132 dTg mice show a lower respiratory quotient (A), higher fat oxidation (B), and lower total energy expenditure (C) compared to their rtTA littermates. These changes are not related to their ambulatory activity (D) or wheel running patterns (E) which remained unchanged. The meal size and sip volume (F) did not differ between the groups. All parameters were normalized to effective mass (calculated by ANCOVA). Data are mean±SEM from 3-9 mice per group. *P<0.05, **P<0.01, ***P<0.001.



Supplementary Figure 4: Hepatic miR-132 is elevated while its targets are reduced in human NAFLD and in multiple NAFLD/NASH mouse models.

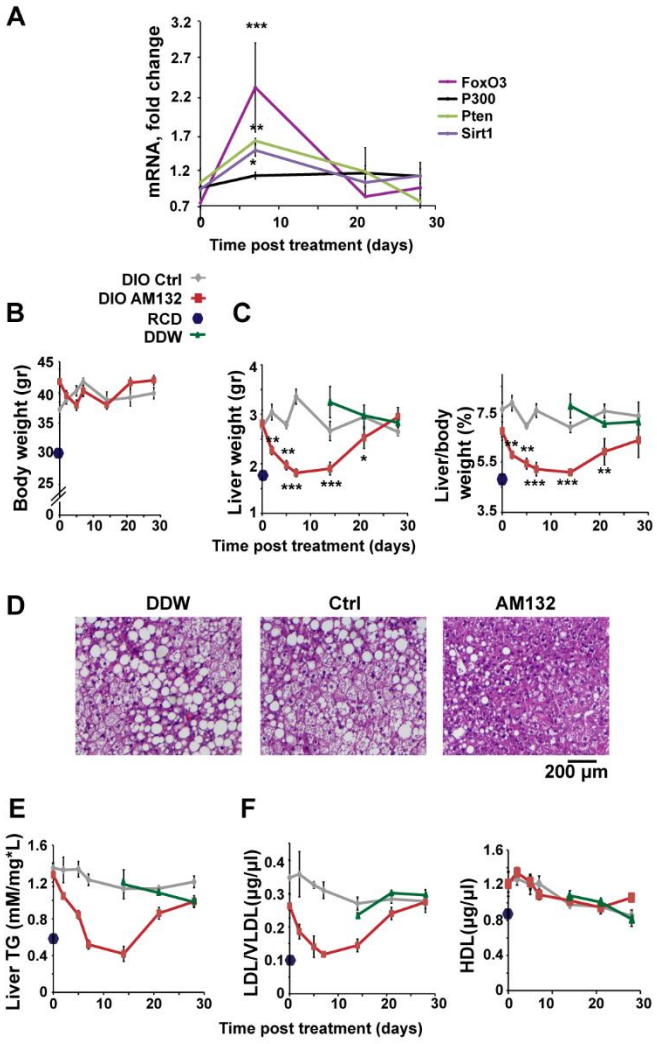
(A) PCA analysis of the expression levels of miR-132 and its targets in human patients diagnosed with NAFLD, correctly segregates diseased and healthy human tissue samples. (n=4-5). (B-D) Elevated levels of liver miR-132 and reduced levels of liver miR-132 target transcripts in 5 different NAFLD, NASH and MetS models, (DIO, HFHS, MCD, CDE, and CDHFD) compared to ctrl (n=4-5, each). Statistic info e.g. mean ±SEM (D) Representative Hematoxylin and Eosin stained liver sections from the above models.



Supplementary Figure 5: Individual miR-132 targets knockdown in DIO mice

(A) *In vitro* validation of knockdown efficiency of miR-132 target transcripts in the C2C12 mouse myoblast cell line: Cells were transfected with the five GapmeRs for each target, using HiPerfect transfection reagent (Qiagen), and RNA was extracted 48h post transfection. qRT-PCR quantification was performed using different primer pairs located outside of the GapmeR area.

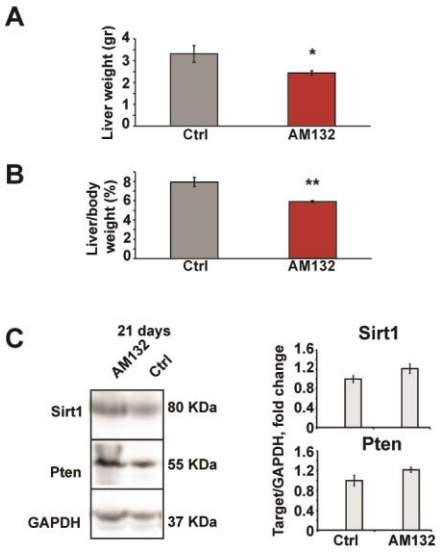
Knockdown is shown for Sirt1, FoxO3, Pten and P300. (B) Elevated body weights of DIO mice compared to regular chow diet (RCD; n=20). (C) Unaltered serum HDL in mice treated with target antisense GapmeRs (n=4). (D) Unaltered body weights of DIO mice treated with target antisense GapmeRs (n=4). Statistic info e.g. mean ±SEM.



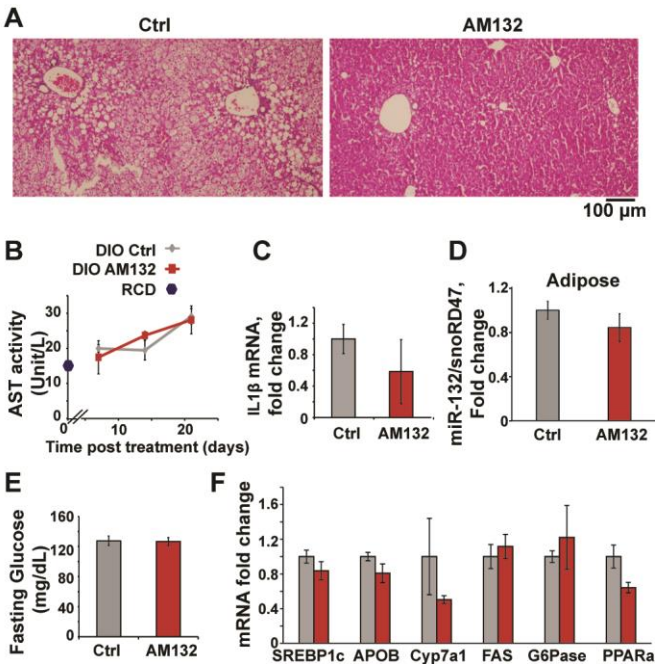
Supplementary Figure 6: Control antisense oligonucleotide treatment affects DIO mice similarly to DDW, unlike miR-132 suppression which reduces fat vacuoles, liver triglycerides and LDL/VLDL

(A) Levels of liver miR-132 target transcripts over time, in AM132 treated DIO mice, determined by Fluidigm qPCR and normalized to multiple housekeeping genes and then to control treatment (n=4). (B) Unchanged body weight of AM132 treated, DDW treated DIO mice or controls (n=4-9). (C) Reduced liver weight and liver/body weight of mice treated with AM132 but not DDW or AM608 which served as controls (n=3-9). (D) Hematoxylin and Eosin stained liver sections. Note reduced fat vacuoles in sections from AM132 treated mice. (E) Gradual and

transient reduction in liver triglycerides of mice treated with AM132 (n=3-9). (F) Reduced LDL/VLDL but not HDL levels in sera of mice treated with AM132 (n=3-9). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-way ANOVA). Bars show mean $\pm$SEM.

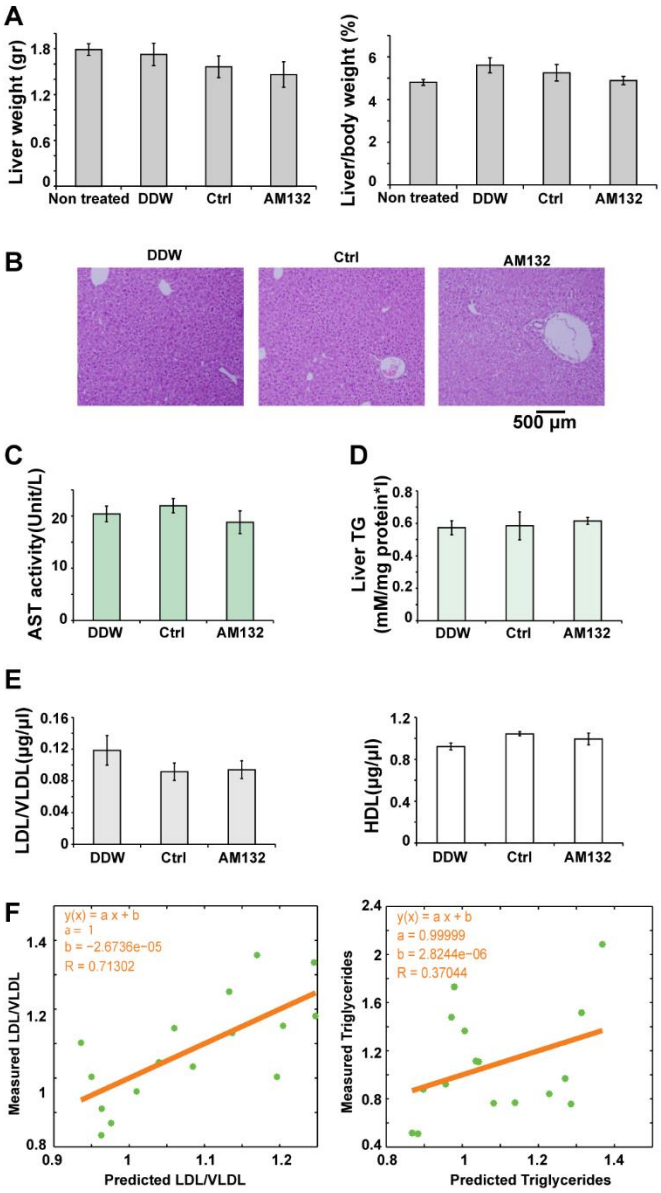


Supplementary Figure 7: Repeated injection of AM132 can extend the effect on the liver. (A) Reduced liver weight of AM132-treated DIO mice, 28 days post treatment, when injected every 14 days (n=6 for AM132, n=7 for controls) (B) Normalized liver/body weight of these mice. (C) Protein levels of Sirt1 and Pten, 21 days post-treatment. * $p<0.05$, ** $p<0.01$, AM132-treated mice compared to controls (Two-tailed Student's t-test). Bars show mean ±SEM.



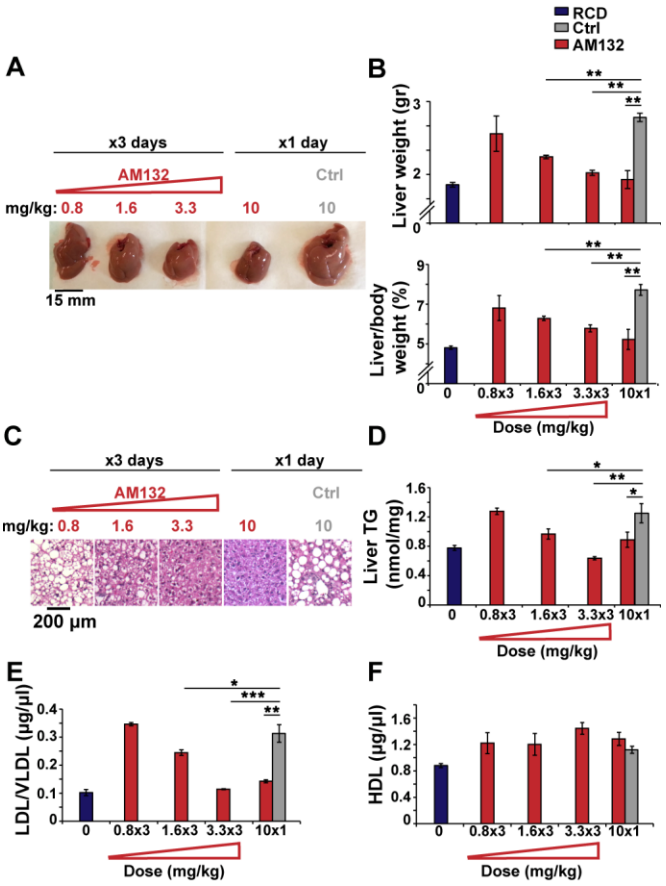
Supplementary Figure 8: MiR-132 suppression reduces fat vacuoles.

(A) Hematoxylin and Eosin stained liver sections of AM132 treated mice 7 days post treatment. Note zone 3 (pericentral zone) with reduction in macrovesicular and microvesicular steatosis. (B) Unchanged Aspartate Aminotransferase (AST) activity in sera of DIO mice treated with AM132 or controls (n=3-4). (C) qPCR-determined IL1β mRNA levels in livers of AM132-treated mice or controls. (D) Unchanged miR-132 levels in DIO mice adipose tissue 7 days post treatment (n=9). (E) Unchanged fasting glucose levels in DIO mice treated with AM132 or controls (n=8). (F) Additional metabolic transcripts in AM132-treated DIO mice compared to controls 7 days post-treatment (n=4). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (two-way ANOVA). Bars show mean +SEM.



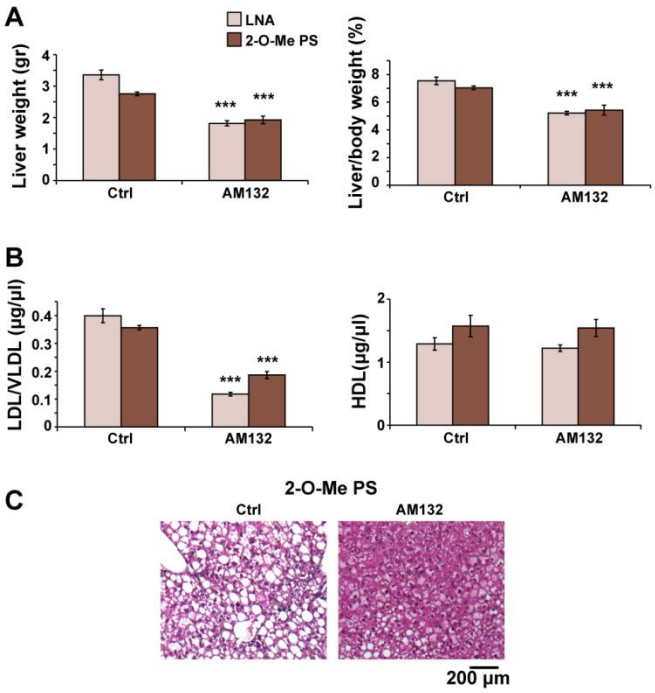
Supplementary Figure 9: Suppression of miR-132 in lean regular-chow fed mice, and PLS regression for LDL/VLDL and triglycerides

Lean RCD fed mice treated with AM132 show sustained liver weight and liver to body weight values (A), Hematoxylin and Eosin stained liver sections (B), AST activity (C), liver triglycerides (D), and serum LDL/VLDL and HDL (E) of these mice, n=5, one-way ANOVA, n.s. in all cases). (F) Correlation of predicted vs. measured LDL/VLDL and triglycerides for 16 mice in 4 different GapmeR injections. $p<0.05$ for LDL/VLDL, p= n.s. for triglycerides.



Supplementary Figure 10: AM132 acts in a dose-dependent manner

Mice were fed with a high fat diet for 11 weeks, injected for three successive days with 0.8, 1.6, and 3.3 mg/Kg AM132, or on one day with 10 mg/Kg AM132 or Ctrl, were kept on the high fat diet and were sacrificed 7 days post-treatment. (A) Representative pictures of mouse livers. (B) Quantification of liver weight and liver/body weight of mice treated with increasing dosage of AM132 or control. (n=4-5). (C) Hematoxylin and Eosin staining of liver sections. Metabolic biomarkers: Liver triglycerides (D), Serum LDL/VLDL (E) and HDL (F) of mice treated with decreased dosage of AM132 or control (all n=4) * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (one-way ANOVA). Bars show mean ± SEM.



Supplementary Figure 11: The AM132 effect is sequence but not modification-dependent. Comparison between LNA-based oligonucleotide and 2-O-methyl and fully phosphorothioated backbone oligonucleotide shows no differences in their effect on liver weight and liver/body weight (A) or on LDL/VLDL and HDL (B) and Hematoxylin and Eosin stained liver sections (C). (n=5-6). *** $p<0.001$ (one-way ANOVA). Bars show mean ± SEM.

	Steatosis	Lobular Inflammation	Hepatocellular Ballooning	**NAS**	Fibrosis stage
rtTA	1	1	0	**2**	0
miR-132 dTg	2.5	1	2	**5.5**	1a
DIO Ctrl	3	1	2	**6**	0
DIO AM132	1	1	1	**3**	0
HFHS Ctrl	2	1	1	**4**	0
HFHS	3	1	2	**6**	1b
MCD Ctrl	2	2	0	**4**	1a
MCD	1	1	0	**2**	0
CDE Ctrl	0	1	0	**1**	0
CDE	3	2	2	**7**	1b
CDHFD Ctrl	0	0.4	0	**0.4**	0
CDHFD	3	1.2	2	**6.2**	2

Supplementary Table 1: Quantified NAFLD Activity Score (NAS) for mouse models used in this study.

Sample ID	**Sex**	**Age**	**Ethnic background**	**Diagnosis**	**Alcoholic/Non-alcoholic**	**Date of surgery / autopsy**	**PMI, hours**
09760B1(2)	F	62	Caucasian	normal tissue	N/A	10/17/2014	N/A
09553S1(3)	F	60	Caucasian	Normal adjacent tissues (NAT) to cancer and benign conditions	N/A	10/25/2012	N/A
09436T1(2)	F	60	Caucasian	Normal adjacent tissues (NAT) to cancer and benign conditions	N/A	11/02/2011	N/A
09792A(11)	M	67	Caucasian	normal tissue	N/A	03/17/2015	3.5
09419A(2)	F	75	Caucasian	normal tissue	N/A	10/07/2011	4.5
09823A(1)	F	62	Caucasian	Fatty liver	Non-alcoholic	06/10/2015	5
09800A(1)	F	60	Caucasian	Fatty liver	Non-alcoholic	04/13/2015	4.5

09757A(2)	F	60	Caucasian	Fatty liver	Non-alcoholic	10/08/2014	3.5
09671A(2)	M	67	Caucasian	Fatty liver	Non-alcoholic	11/14/2013	2.5
09274A	F	76	Caucasian	Fatty liver	Non-alcoholic	11/26/2010	5.5

Supplementary Table 2: Human liver samples.

All material was collected from donors for or from whom a written informed consent for a liver autopsy/biopsy and the use of the material and clinical information for research purposes was obtained by ProteoGenex (Culver City, CA, USA).

Primer	**Sequence (5'➔3')**
mP300 fwd	CAAAGGAGGCTAAAGGTGAGGA
mP300 rev	CAGAGGTGCTTGGCTGTTCT
mL-PK fwd	AAAATTGGCCCAGAGGGACT
mL-PK rev	CCGAAGCGCAGATCCAAAAG
mSirt1 fwd	GAGCTGGGGTTTCTGTCTCC
mSirt1 rev	AACATGGCTTGAGGGTCTGG
mCyp2e1 fwd	AGGAGTACAAGAACAAGGGGAT
mCyp2e1 rev	CACCAGGAAGTGTGCCTCTC
mSREBP1c fwd	CCCGGCTATTCCGTGAACAT
mSREBP1c rev	AGAACTCCCTGTCTCCGTCA
mPTEN fwd	AGAACAAGATGCTCAAAAAGGACA
mPTEN rev	AGGGTGAGTACAAGATACTCCT

mFAS fwd	GACTCGGCTACTGACACGAC
mFAS rev	CGAGTTGAGCTGGGTTAGGG
mGPAT fwd	CCAGACAGCAGCCTCAAAAAC
mGPAT rev	GTGCCAGATAGGGACCGAAC
mAPOB fwd	CTGCCATGTCCAGGTACGAA
mAPOB rev	GAGCAGAGATGATGCCCCTC
mFOXO3 fwd	AGGGAGGAGGAGGAATGTGG
mFOXO3 rev	GCTCCAGCTCGGCTCCTTC
mSREBP2 fwd	CGCCAAAGGTGATTTCGCAG
mSREBP2 rev	GCGGATCACATTCCAGGAGA
mPPARa fwd	CATCACAGACACCCTCTCTCC
mPPARa rev	ATGCGTGAACTCCGTAGTGG
mPGC1a fwd	AATGCAGCGGTCTTAGCACT
mPGC1a rev	TCTCGGTCTTAACAATGGCAGG
mEhhadh fwd	TGTTCTTGGCTTGGGAACGA
mEhhadh rev	GCCACTCTTCGATGCTTCCT
mPEPCKc fwd	ATGAAAGGCCGCACCATGTA
mPEPCKc rev	GGGCGAGTCTGTCAGTTCAA
mG6Pase fwd	GCTGGAGTCTTGTCAGGCATT
mG6Pase rev	AATCCAAGCGCGAAACCAAA
mCyp7a1 fwd	TCTGCGAAGGCATTTGGACAC
mCyp7a1 rev	TTTAGGAAGGCCCGGAGGT
mABCA1 fwd	GGAAAACAGTTAATGACCAGCCAC

mABCA1 rev	CCACAGCGACCTGCGTCT
mFXR fwd	AATGAGGACGACAGCGAAGG
mFXR rev	GTGAGTTCCGTTTTCTCCCTG
mAOX fwd	TCAAGAGAAGCGAGCCAGAG
mAOX rev	AAAGTGGAAGGCATAGGCGG
mAcadm fwd	AGGGTTTAGTTTTGAGTTGACGG
mAcadm rev	CCCCGCTTTTGTCATATTCCG
mAChE fwd	CTGAACCTGAAGCCCTTAGAG
mAChE rev	CCGCCTCGTCCAGAGTAT
mIL1β fwd	TGCCACCTTTTGACAGTGATG
mIL1β rev	TGATGTGCTGCTGCGAGATT
mNdufc fwd	GTAGTGCTGCGCTCGTTTT
mNdufc rev	GTTAGGTTTGGCATTGACTG

Supplementary Table 3: Primers used for qPCR quantification of target and housekeeping mRNAs.

SUPPLEMENTARY MATERIALS AND METHODS

Human Samples

Human liver samples were obtained from Proteogenex (ProteoGenex, Culver City, CA). All samples were collected from donors with proper ethical approvals from institutional review boards.

Animals and diets

All mouse experiments were approved by the ethics committee (IACUC) of The Hebrew University of Jerusalem (approval #10-12459-1, #13-13792-3 and #14-14135-3). The Hebrew University is an AAALAC International accredited institute.

Inducible miR-132 overexpressing mice: Inducible miR-132 transgenic mice were generated by cloning the mouse miR-132 precursor sequence (Strum et al., 2009) into the EcoRI and XbaI sites of the pTRE-tight plasmid (631059 Clontech, CA, USA). The vector was linearized using XhoI to yield a 735 base-pair sequence which was microinjected to CB6/F1 E0.5 zygotes and transplanted into ICR pseudo-pregnant females. Six transgenic founders were generated, and then crossed with B6.Cg-*Gt(ROSA)26So*$^{rtm1(rtTA*M2)Jae}$/J , a strain expressing the reverse tetracycline controlled trans-activator (rtTA) followed by a β-globin poly A that is inserted downstream of the Gt (ROSA) 26Sor promoter and the ColA1 locus (The Jackson Laboratory, ME, USA). The breeding mates were back-crossed to C57bl/6J. To induce miR-132 overexpression, 2mg/ml doxycycline (Sigma-Aldrich, Israel) and 10mg/ml sucrose were added to the drinking water, which was changed every 3 days for 14 days. Mice were group-housed at a constant temperature (22±1°C) and 12-hour light/dark cycles. MiR-132 expression was validated by qPCR using the qScript™ microRNA quantification system (Quanta Biosciences, MD, USA) and the highest expressing line was selected.

Genotyping of inducible miR-132 overexpressing mice: pTRE-miR-132xrtTA mice were genotyped for the miR-132 transgene using nested PCR. The initial PCR step was performed using the following primers: forward, TACTGTGGGAACCGGAGGTA, reverse, TGAAATTTGTGATGCTATTGCTTT. PCR product levels were then determined by qPCR using these nested primers: forward, ACAGTCTACAGCCATGGTCGC, reverse, CGCTCTGTATCTGCCCAAACC.

NASH mouse models: **MCD mice:** 6 weeks old C57Bl/6J mice were fed with methionine and choline deficient diet for 3 weeks. **CDE mice:** 6 weeks old C57Bl/6J mice were fed with choline deficient and ethionine supplemented diet for 2 weeks. **HFHS mice:** 6 weeks old C57Bl/6J mice were fed with high fat and high sucrose diet for 16 weeks. MCD, CDE and HFHS mice were obtained from Harlan, Israel. Mouse experiments were approved by the ethics committee (IACUC) of The Hebrew University (approval #MD-12-13189-5**). CDHFD mice**: 4-5 weeks old C57Bl/6J mice (Harlan, Germany) were fed for 12 months with choline deficient high fat diet. CDHFD mice were maintained in SPF animal facility, and experiments were conducted in accordance with the guidelines of the Swiss Animal Protection Law and approved by the Veterinary Office of the Canton of Zurich (licenses 63/2011 and 136/2014).

Diet**:** Inducible miR-132 transgenic mice and C57Bl/6J control mice (Harlan, Israel) were fed with a regular chow diet (RCD; TD-2918, Harlan Teklad, Israel) which consists of 18% fat calories, 24% protein calories and 58% carbohydrate calories (energy density of 3.1 kcal/g). In other experiments, C57Bl/6J mice were fed for 9 or 11 weeks with a high-fat diet (HFD; TD-97070, Harlan Teklad, WI, USA), which consists of 59.9% fat calories, 18.8% protein calories and 21.3% carbohydrate calories (energy density of 5.1 kcal/g). The fatty acid profile (% of total fat) is as follows: 45% saturated, 24% trans, 24% monounsaturated (cis), and 7% polyunsaturated (cis). CDE diet (M.P. Biomedicals, Cat No.960210) supplemented with 0.165% Ethionine weight by volume (Sigma, Israel E5139), MCD diet (TD-90262, Harlan Teklad, Israel) HSHF diet (TD-08811, Harlan Teklad, Israel), CDHF diet (Research Diets; D05010402) was previously described (Wolf et al., 2014).

Multi-parameter metabolic assessment. Metabolic and activity profiles of the mice were assessed by using the Promethion High-Definition Behavioral Phenotyping System (Sable Instruments, Inc., Las Vegas, NV, USA) as described previously (Knani et al., 2016). Briefly, Respiratory quotient (RQ) was calculated as the ratio of VCO2/VO2. Total energy expenditure (TEE) was calculated as VO2 x (3.815 + 1.232 x RQ), normalized to effective body mass calculated by ANCOVA, and expressed as kcal/h/kg eff. Mass. Fat oxidation (FO) was calculated as FO = 1.69 x $VO2$ – 1.69 x $VCO2$ and expressed as g/d/kg eff. Mass. Ambulatory activity and position were monitored simultaneously with the collection of the calorimetry data using XYZ beam arrays with a beam spacing of 0.25 cm.

Oligonucleotides: For the in vivo experiments, two 16-mer LNA oligonucleotides were used: one complementary to mature miR-132 (AM132) and one complementary to mature miR-608 (AM608). They were injected intravenously for three successive days, at 3.3 mg/kg per day, or once, at 10 mg/kg. Two fully 2-O-methyled and phosphorothioated nucleotides with the same two sequences were also used, and injected at the same doses. LNA oligonucleotides were purchased from Exiqon (Vedbaek, Denmark), and 2-O-methyl phosphorothioated oligonucleotides from Sigma-Aldrich Israel. The sequences of the oligonucleotides are as follows:

Name	Chemistry	Sequence	mer
AM-132	LNA	5’- ATGGCTGTAGACTGTT -3’	16
Ctrl	LNA	5’- CGGAGCTGTCCCAAC-3’	15

2-O-me PS AM-132	2-O- methyl, phosphorothioated	5’- ATGGCTGTAGACTGTT -3’	16
2-O-me PS Ctrl	2-O- methyl, phosphorothioated	5’- CGGAGCTGTCCCAAC-3’	15

GapmeRs against miR-132 targets (Exiqon) were injected intravenously once, at 10 mg/Kg, following an in vitro validation (see cell culture section)

The sequences of the oligonucleotides are as follows:

Name	Chemistry	Sequence	mer
Anti sirt1	LNA GapmeR	5’-CTCAGGTGGAGGAATT-3’	16
Anti Pten	LNA GapmeR	5’-TTAAATTTGGCGGTGT-3’	16
Anti P300	LNA GapmeR	5’-TGGTAGAACAGTCTT-3’	15
Anti FoxO3	LNA GapmeR	5’-CTGTGGCTGAGTGAGT-3’	16
Negative control A	LNA GapmeR	5’-AACACGTCTATACGC-3’	15

Tissue collection: Blood was collected by cardiac puncture, 200-1000µl per mouse, into BD Microtainer PST^{TM} LH tubes (BD, NJ, USA), plasma was separated by centrifugation 1.5 minutes at 15000g, and stored at -80°C. Liver, heart, spleen, intestine (ileum), adipose and hippocampus were collected, flash-frozen in liquid nitrogen, and stored at -80°C.

Glucose metabolism assays

Glucose tolerance tests (GTT): For intraperitoneal GTT (IP-GTT), mice fasted for 18 hours and were then injected with 10% glucose (D-glucose, Sigma Aldrich, MO, USA) at a 1 mg/kg dose in injection-grade water (B. Brown, Melsungen, Germany). Mice were bled from a tail clip. Blood glucose was measured before injection (time 0) and 15, 30, 60, 90 and 120 minutes after injection using a handheld glucometer (Accu-Chek Performa, Roche. Mannheim, Germany).

Insulin tolerance tests (ITT): For intraperitoneal ITT (IP-ITT) mice fasted for 4 hours and were then injected with insulin (Biological Industries, Beit Haemek, Israel, catalog number 01-818-1H) in phosphate buffered saline at a 0.75 U/kg dose. Mice were bled from a tail clip. Blood glucose was measured before injection (time 0) and 15, 30, 60, 90 and 120 min after injection using a handheld glucometer as above.

Insulin ELISA: Fasting serum insulin was determined by ELISA (Millipore, EZRMI-13K, MA, USA), according to the manufacturer's instructions.

Homeostasis model for insulin resistance test (HOMA-IR): HOMA-IR values were calculated from the fasting blood glucose (mgl/dL) × fasting plasma insulin (ng/ml) divided by 405.

Lipid and liver enzyme assays

Triglyceride quantification**:** Liver triglycerides were extracted from flash-frozen hepatic tissue stored at -80°C and quantified using a fluorometric assay (Triglyceride Quantification Kit, ab65336, Abcam, MA, USA) according to the manufacturer's instructions. Samples were diluted 10-fold with distilled water to ensure they fell within the range of the kit's standard curve.

***Free fatty acid quantification**:* Serum and liver free fatty acids were quantified using a fluorometric assay (Free Fatty Acid Quantification Kit, ab65341, Abcam, MA, USA) according to the manufacturer's instructions. 5-10 μL serum from each mouse or 10mg flash-frozen tissue which was then diluted 1:100 were used per assay point.

***Lipid profile measurement**:* HDL and LDL/VLDL-cholesterol were quantified using a fluorometric assay (HDL and VLDL/LDL Cholesterol Assay Kit, ab65390, Abcam, MA, USA) according to the manufacturer's instructions.100 μL serum from each mouse was used per assay point.

***Oil-red O staining**:* To detect natural lipids in liver tissue, frozen samples were embedded in Tissue-Tek (1437365, Fisher Scientific, PA, USA), and 12 μm thick sections were cut, mounted on slides (Superfrost plus, Thermo 4951PLUS4, Copenhagen, Denmark), dried for 10 minutes at room temperature, and incubated for 5 minutes in oil red O (ORO) (00625 Sigma Aldrich, Israel) (Mehlem et al., 2013), then washed with tap water for 30 minutes and mounted in ImmuMount™ (Thermo Scientific Shandon™ 9990402, PA, USA). Staining was quantified using Image-J software.

***Aspartate aminotransferase activity assay**:* Aspartate aminotransferase (AST) activity was determined using a colorimetric kit (Sigma Aldrich MAK055, Israel) according to manufacturer's instructions. 50mg flash-frozen liver tissue was homogenized in 200 μL assay buffer and diluted 1:10 per assay point.

mRNA assays

***mRNA and miRNA quantification**:* RNA was extracted using TRI reagent (Sigma-Aldrich, , St. Louis, MO, USA) according to standard protocol, followed by RNA concentration determination (Nanodrop, Thermo, DE, USA) and gel electrophoresis. mRNA levels were determined by

quantitative reverse transcription PCR (RT-qPCR) using Promega (WI, USA) reagents, iTaq™ Universal SYBR® Green Supermix (Bio-Rad, CA, USA) and an ABI prism 7900HT machine (Applied Biosystems, CA, USA). Unless otherwise noted, all measurements were normalized to nDufc as a housekeeping gene. Primer sequences are listed in Supplementary Table 1. MiRNA levels were also determined using RT-qPCR (qScript™ microRNA cDNA Synthesis Kit and PerfeCTa® SYBR® Green FastMix®, Low ROX™, Quanta Biosciences, MD, USA) and normalized to sno-RD-135.

MicroRNA-target interaction: The binding energy and structure of miRNAs and their targets were predicted using the RNAhybrid algorithm (*http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/*).

Fluidigm: Expression of selected miR-132 targets and metabolic transcripts was determined using a high-throughput microfluidic qRT-PCR instrument (BioMark, Fluidigm, CA, USA). cDNA samples were pre-amplified for 14 cycles using TaqMan PreAmp Master Mix (Applied Biosystems), then treated with Exonuclease I (New England Biolabs, MA, USA), and diluted 1:5 in Tris-EDTA buffer, pH=8. qRT-PCR mix was prepared using 2x SsoFast EvaGreen Supermix with low ROX (Biorad, CA, USA). Priming and loading into the Dynamic Fluidigm Array 96x96 chip was performed using the IFC Controller HX (BioMark). All qRT-PCR reactions were done using the GE 96x96 PCR+Melt v2.pcl protocol. For data analysis, the BioMark Real-Time PCR Analysis Software Version 2.0 (Fluidigm), and the ΔCt method was applied. Multiple housekeeping genes were used for normalization. All primers used were initially calibrated using standard qPCR, had a single product, linear standard curves and close to 100% efficiency.

Additional assays

***Cholinesterase activity*:** levels of catalytic activity in mice tissues and sera were determined using the Ellman assay as described previously (Hanin et al., 2014).

***Immunoblots**: S*amples for Sirt1 and Pten were lysed in a solution containing 10mM Tris HCl pH=7.4, 1 M NaCl, 1 mM EGTA, and 1 % TX-100. Samples for FoxO3 were lysed by homogenization with a Kontes pellet pestle in 10-fold volume/weight of RIPA (50 mM TRIS-Cl pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA) containing protease inhibitors (1:200 of Calbiochem cocktail set III) and phosphatase inhibitors (20mM NaF, 1mM β-glycerophosphate, 1mM orthovanadate), incubated on ice for 20-30 minutes, centrifuged at 17,900 rcf, 4°C, for 30 minutes, and the clear s.n. was removed from under the top fatty layer and centrifuged once or twice more for 10 minutes, to achieve a clear lysate. Samples were separated by standard SDS-PAGE procedures on 10% (Sirt and Pten) or 4-15% gradient (FoxO3) gels. After transfer to nitrocellulose, proteins were visualized using primary antibodies against Sirt1 (ab12193, Abcam, MA, USA, 1:2000), Pten (sc-7974, Santa Cruz Biotechnology, TX, USA, 1:200), FoxO3 (2497, Cell Signaling Technology, MA, USA, 1:500) and GAPDH for normalization (2118, Cell Signaling, MA, USA, 1:2000), followed by horseradish peroxidase-conjugated goat anti rabbit antibodies (A0545, Sigma-Aldrich, 1:10,000) and enhanced chemi-luminescence (EZ-ECl, Biological Industries, Beit-Haemek, Israel or SuperSignal™ West Pico and Femto, Thermo Fisher Scientific, MA, USA) using the myECL™ Imager (Thermo Fisher Scientific). Bands were quantified and analyzed using densitometry with ImageJ or MYImage software.

Immunohistochemistry: Paraffin slides were rehydrated by washing in xylene and decreasing concentrations of ethanol in water. Heat-induced antigen retrieval was done by boiling slides for 10 minutes in 10mM citrate buffer pH 6. A hydrogen peroxide methanol quench was performed for slides later developed with 3,30-diaminobenzidine tetrahydrochloride (DAB). After washing, slides were incubated with 150 µl/slide of blocking buffer (4% normal serum, 0.05% TWEEN20

and 0.3% triton X-100) for 60 min, followed by over-night incubation at 4°C with primary antibody diluted in the blocking buffer. Slides were then washed and incubated with biotin conjugated secondary antibody for 2 h, following which detection was performed by streptavidin-conjugated Cy3 or Alexa-488 (Jackson, PA, USA). Nuclear staining was done using 40,6-diamidino-2-phenylindole (DAPI). Primary antibodies used were anti FoxO3 (SC-11351, Santa Cruz Biotechnology, TX, USA), anti P300 (SC-585, Santa Cruz), and anti arginase1 (SC-13851). All antibodies were diluted 1: 200. Quantification was performed using calculation of intensity in a field which does not contain fat vacuoles, and then normalized to DAPI.

Hematoxylin and Eosin **(*H&E) staining***: Small sections of liver tissues were dissected from experimental animals during sacrifice, and fixed in 4% paraformaldehyde, then alcohol-dehydrated, paraffin-embedded, and sectioned to a mean thickness of 4μm by microtome. Sections were stained for 2 minutes in Hematoxylin (GHS316, Sigma Aldrich, Israel) and 20 seconds in Eosin (HT110316 Sigma Aldrich, Israel) and morphology of tissue and cells was assessed by light microscopy.

Histological assessment: NAFLD Activity Score (NAS score) was aggregated from 3 semi-quantitative sub-scores: steatosis (0–3), lobular inflammation (0–2), hepatocellular ballooning (0–2). Fibrosis was scored (0–4) based on Masson Trichrome histochemical stain. The evaluation was completed by a pathologist who was unaware of the sample status.

Cell culture experiments

Cell lines*:* C2C12 cells were grown in a humidified atmosphere at 37°C, 5% CO_2, in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1,000 units/ml penicillin, 0.1mg/ml

streptomycin sulfate, and 0.25 microgram/ml amphotericin B (Biological Industries, Beit-Haemek, Israel).

LNA oligonucleotides: LNA GapmeRs against miR-132 targets (Exiqon) were re-suspended in DDW and used at a concentration of 17 pmol/µl. Transfection was performed using HiPerfect transfection reagent (Qiagen, CA, USA), typically 50 pmol GapmeR and 25µl HiPerfect per single 6-well.

Statistics: Statistical significance was calculated using Student's t-test or by one- or two-way ANOVA with LSD post-hoc, where appropriate. Average ± SEM is shown for all data.

PLS regression analysis: Partial least square regression, Pearson's correlation and one-way ANOVA with Bonferroni post-hoc test were all performed using MATLAB. The expression levels of FoxO3, Sirt1, Pten and P300 were compared to a control for each experiment (total 16 mice) and then analyzed with PLS regression for beta coefficient calculation. To minimize generalization error, training of the model was performed in a "leave one out" cross validation. Following calibration, the final beta coefficients were calculated from the average training coefficients and applied to all experimental groups to predict serum LDL/VLDL levels according to their target mRNA expression levels. P300 mRNA levels in miR-132 dTg mice were estimated based on in vivo GapmeR experiment expression levels.