Supplementary Information

Supplementary Figure Legends

Figure S1. Expression of miRNAs in mouse models, corticosterone levels, and genome browser data mining. Related to Figure 1

A Expression levels of miRNAs 379, 382, 541, 134, and 409-5p in livers of wt and GR dimerization-deficient (GR\textsuperscript{dim}) mice treated daily with dexamethasone (DEX, 1 mg/kg BW) or vehicle (2% EtOH in isotonic saline) (n=3-5) for 28 days. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): ***p<0.001 or **p<0.01

B Distribution plot of mean microarray signal intensity of hepatic miRNAs detected in the screening. Data are from 544 miRNAs detected in 32 biological replicates. Q1 and Q3 are the 1\textsuperscript{st} and 3\textsuperscript{rd} quartile, respectively.

C Correlation of hepatic mmu-miR-379 levels with serum corticosterone in wt, db/db, NZB, and NZO mice. Given are the correlation coefficients and the p-values.

D Hepatic miR-379 levels in wild-type mice treated with negative control (NC) or GR-specific miRNA rAAV (n=4). Bar graphs show mean ± SEM; t-test: ***p<0.001

E Quantitative PCR analysis of miR-379 expression levels in primary hepatocytes treated with 1 \textmu M dexamethasone or vehicle (2% EtOH in PBS) for 9 h. Bar graphs show mean ± SEM; t-test: *p<0.05

F Bioinformatic analysis using the UCSC Genome Browser (http://genome.ucsc.edu/) showing the putative miR-379 promoter, including glucocorticoid receptor (GR) binding sites derived from ChIP-Seq studies, DNase susceptible regions, and regions of histone trimethylation. Bar
graphs show mean ± SEM; t-test or ANOVA (with post-hoc test): ***p<0.001, ***p<0.01, or *p<0.05.
Figure S2. Metabolic phenotypes upon miR-379 inhibition and over-expression. Related to Figure 2

A Northern blot of miR-379 in C57Bl/6J mice treated with anti-miR-379 or scrambled control locked-nucleic acid (LNA) (n=5).

B Serum alanine aminotransferase (ALT) levels of same mice as in A.

C RT-qPCR levels of miR-379 in comparison to miRNAs 411, 382, and 541 from same animals as in A. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): ***p<0.001

D – F Liver glycogen (D), triglyceride (E), and cholesterol (F) of same animals as in A. Bar graphs show mean ± SEM.

G Blood glucose levels during a glucose tolerance test (GTT) of wt mice treated with PBS, scrambled or anti-miR-379 LNA. Bar graphs show mean ± SEM.

H Cholesterol profiles of FPLC-fractionated serum of same mice as in A under fed conditions. VLDL, IDL/LDL, and HDL peaks are indicated.

I HOMA-IR of same mice as in A. Bar graphs show mean ± SEM; t-test: **p<0.01

J Protein levels of total and phosphorylated FoxO1 (78-82 kDa) in mice upon treatment with anti-miR-379 or scrambled control LNA.

K RT-qPCR levels of Pepck1 in mice upon treatment with anti-miR-379 or scrambled control LNA. Bar graphs show mean ± SEM; t-test: ***p<0.001

L Blood glucose levels during a pyruvate tolerance test (PTT) of wt mice treated with scrambled or anti-miR-379 LNA (n=5). Line graphs show mean ± SEM; ANOVA (with post-hoc test): *p<0.05

M Fasting blood glucose of same mice as in A. Bar graphs show mean ± SEM.
N Northern blot of miR-379 in livers of wt mice treated with isotonic saline, rAAV negative control (NC) or rAAV for miR-379 overexpression (n=4).

O Serum ALT levels of same mice as in N.

P, Q Total serum cholesterol (P) and serum cholesterol profile (Q) derived from FPLC-analysis of same mice as in N. VLDL, IDL/LDL, and HDL peaks are indicated. Bar graphs show mean ± SEM.
Figure S3. Metabolic functional assays and miR-379 targets. Related to Figure 3

A Normalized oxygen consumption rate (OCR) of Hepa1-6 cells treated with anti-miR-379 or scrambled control tough decoy construct (n=8). Data generated using XF96 Extracellular Flux Analyser (Seahorse).

B Abdominal white adipose tissue lipoprotein lipase (LPL) activity in C57Bl/6J mice treated with anti-miR-379 or scrambled control LNA (n=5). Bar graphs show mean ± SEM.

C Hepatic VLDL release in db/db mice treated with anti-miR-379 or scrambled control LNA (n=7). Times after tyloxapol injection are indicated. Data are mean ± SEM; ANOVA (with post-hoc test): *p<0.05.

D, E Organ distribution after 4 minutes (D) and serum VLDL ³H-triolein clearance (E) in C57Bl/6J mice treated with anti-miR-379 or scrambled control LNA (n=6-7). Mice were fasted for 6 hours and given an intravenous dosage of VLDL ³H-triolein. Data are mean ± SEM; t-test: **p<0.01

F Serum VLDL triglyceride levels in a lipid tolerance test in db/db mice treated with an anti-miR-379 or scrambled control Tough Decoy (TuD) construct delivered by rAAV (n=5-6). Mice were fasted for 16 hours and given a 100 µL oral fat load of olive oil spiked-in with ³H-triolein. Data are mean ± SEM; ANOVA (with post-hoc test): ***p<0.001, **p<0.01, or *p<0.05.

G VLDL specific activity of animals in F.

H Intestinal ³H-triolein radioactivity of animals in F.

I, J Vertebrate-conserved miR-379 binding sites in the coding sequences of the respective LSR (I) and LDLR (J) transcripts as predicted by RNA22.

K Quantitative densitometric analysis of Western blot in Figure 3C. Data are mean ± SEM; ANOVA (with post-hoc test): **p<0.01
L) Quantitative densitometric analysis of Western blot in Figure 3D. Data are mean ± SEM; ANOVA (with post-hoc test): *p<0.05
**Figure S4. Functional characterization of the miR-379-identified targets.** Related to Figure 3

**A** Schematic of miR-379 target identification and characterization. Web-based programs used include RNA22, DAVID, and MiRTiF.

**B - D** Western blot of liver extracts (B) from *wt* mice treated with control or LSR shRNA-containing adenovirus and with either anti-miR-379 or scrambled control LNA (n=7). Densitometric analysis of the Western blot for LDLR (C) and LSR (D) proteins. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): ***p<0.001

**E, F** Serum triglyceride (E) and cholesterol (F) profiles of FPLC-fractionated serum of same mice as in B-D. VLDL, IDL/LDL, and HDL peaks are indicated.

**G** Quantitative miR-379 PCR levels from livers of *wt* or *LDLRKO* mice treated with control or LSR shRNA-containing adenovirus and with anti-miR-379 or scrambled control LNA (n=7). Bar graphs show mean ± SEM; ANOVA (with post-hoc test): ***p<0.001

**H, I** Quantitative densitometric analysis of Western blot in Figure 3E for LDLR (H) and LSR (I) proteins. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): ***p<0.001 or **p<0.01

**J** Cholesterol profiles of FPLC-fractionated serum of *LDLRKO* mice treated with control or LSR shRNA-containing adenovirus and with either anti-miR-379 or scrambled control LNA (n=7). Same animals as in G. VLDL, IDL/LDL, and HDL peaks are indicated.
Figure S5. Other metabolic phenotypes affected by miR-379 inhibition in obese mice.

Related to Figure 4

A Expression levels of hepatic miR-379 in db/db mice treated with anti-miR-379 or scrambled LNA (n=7) as determined by qPCR. Bar graphs show mean ± SEM; t-test: ***p<0.001

B Fasting blood glucose levels of db/db mice treated with anti-miR-379 or scrambled control LNA (n=7 per group). Data show mean ± SEM; t-test: **p<0.01

C, D Total serum cholesterol (C) and serum cholesterol profiles (D) derived from FPLC-analysis of same mice as in A. VLDL, IDL/LDL, and HDL peaks are indicated.

E Serum corticosterone levels measured by ELISA of same mice as in A.

F Quantitative PCR analyses of lipogenic genes in livers of mice in A: Pparg ( Peroxisome proliferator-activated receptor gamma), Acc ( Acetyl-CoA carboxylase), Fasn ( Fatty acid synthase), Scd1 ( Stearoyl-CoA desaturase 1), Srebp ( Sterol regulatory element-binding protein), and Lipin. Data show mean ± SEM; t-test: *p<0.05

G Liver sections stained with Oil Red O and hematoxylin from representative animals in A.

H Quantitative densitometric analysis of Western blot in Figure 4D. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): *p<0.05

I Expression levels of hepatic miR-379 in NZO mice treated with anti-miR-379 or scrambled LNA (n=7) as determined by qPCR. Bar graphs show mean ± SEM; t-test: ***p<0.001

J, K Serum triglycerides (J) and serum cholesterol profile (K) derived from FPLC-analysis of same mice in I. Bar graphs show mean ± SEM; t-test: **p<0.01

L Quantitative densitometric analysis of Western blot in Figure 4F. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): **p<0.01
Figure S6. Phenotypes of mice upon miR-379 inhibition using rAAV-delivered tough decoy.

Related to Figure 4

A Expression levels of miR-379 from livers of db/db mice treated with anti-miR-379 or scrambled control Tough Decoy (TuD) construct delivered by rAAV (n=8) as determined by qPCR. Bar graphs show mean ± SEM; t-test: **p<0.01

B, C Serum triglycerides (B) and cholesterol (C) of same mice as in A. Bar graphs show mean ± SEM; t-test: *p<0.05

D Serum cholesterol profile as determined by FPLC-analysis of same mice as in A. VLDL, IDL/LDL, and HDL peaks are indicated.

E Quantitative PCR analysis of miR-379 from livers of C57Bl/6J mice fed with low-fat (10%) or high-fat (60%) diet for 12 weeks and treated with either anti-miR-379 or scrambled control Tough Decoy (TuD) construct delivered by rAAV (n=7). Bar graphs show mean ± SEM; ANOVA (with post-hoc test): *p<0.05

F – K Body weight (F), serum cholesterol (G), blood glucose (H), serum triglyceride (I) & cholesterol profiles of FPLC-fractionated serum (J) and serum corticosterone levels (K) of same mice as in E. Bar graphs show mean ± SEM; ANOVA (with post-hoc test): ***p<0.001 or *p<0.05; n.s.: not significant
Supplementary Table Legends

**Suppl. Table 1:** Demographic and serum profiles of obese cases (n=64) and healthy controls (n=10) recruited in the study.

**Suppl. Table 2:** Nucleotide sequences of LNAs, tough decoy (TuD) inserts, dual-luciferase vector inserts, miRNA mimics, and ChIP-qPCR primers.
Figure S1

A

miR-379

miR-382

miR-541

miR-134

miR-409-5p

Vehicle

DEX

Vehicle

DEX

Vehicle

DEX

Vehicle

DEX

Vehicle

DEX

B

N=544

miR-122

miR-379

C

Corr. coef. = 0.908

Corr. coef. = 0.775

p = 0.00180

p = 0.00238

D

Relative miR Levels

(miR-379/snoRNA-202)

rAAV-miR-NC

rAAV-miR-GR

Vehicle

DEX

Vehicle

DEX

Vehicle

DEX

E

Relative miR Levels

(miR/snoRNA-202)

Vehicle

DEX

Vehicle

DEX

Vehicle

DEX

F

cnr14 ( 32.31)

Layered H3K4Me1

Layered H3K4Me3

Layered H3K27Ac

DNase Clusters

ENCODE

Digital

DNase

Hypersensitivity

Clusters

http://genome.ucsc.edu/
**Figure S2**

A) Scrambled Control vs. Anti-miR-379 LNA

B) Serum ALT, U/L

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Scrambled Control</th>
<th>Anti-miR-379 LNA</th>
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<tbody>
<tr>
<td>17 – 77</td>
<td>37.06 ± 11.08</td>
<td>27.57 ± 8.96</td>
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</tbody>
</table>

Note: Data are mean ± SEM; p = 0.76

C) Relative miR Levels

D) Liver Glycerogen (μmol/g Liver)

E) Liver TG (μmol/g Liver)

F) Liver Cholesterol (μmol/g Liver)

G) Glucose, mM

H) Cholesterol, μg/fraction

I) HOMA-IR

J) p-FoxO1 (S256)

K) Relative mRNA Levels

L) Blood Glucose, mM

M) Blood Glucose, mM

N) PBS vs. rAAV-miR-NC vs. rAAV-miR-379 OE

O) Serum ALT, U/L

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>rAAV-miR-NC</th>
<th>rAAV-miR-379 OE</th>
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<tr>
<td>17 – 77</td>
<td>20.31 ± 4.00</td>
<td>19.21 ± 11.36</td>
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Note: Data are mean ± SEM; p = 0.673

Note: Data are mean ± SEM; p = 0.76
Figure S3

**A** BSA + DMSO
- Scrambled TuD Control
- TuD-Anti-miR-379
- Mock Transfection

**B** LPL Activity (nmol/mg/min)
- Scrambled Control
- Anti-miR-379 LNA

**C** Serum TG, mM
- Scrambled Control
- Anti-miR-379 LNA

**D** Time [min]
- Scrambled Control
- Anti-miR-379 LNA

**E** [3H]-TG, μmol
- Scrambled Control
- Anti-miR-379 LNA

**F** Serum VLDL TG, mM
- Scrambled Control
- Anti-miR-379 LNA

**G** LSR Coding Sequence
- Human
- Mouse
- Cow
- Rat
- Dog
- Zebrafish

**H** LDLR Coding Sequence
- Human
- Mouse
- Zebrafish

**I** LDLR Specific Activity, [3H]-TG AUC/(mM*4h)/(mM*4h)
- TuD-Scrambled
- TuD-Anti-miR-379

**J** Relative Signal Intensity, AU
- LDLR or LSR/VCP
- Scrambled Control
- Anti-miR-379 LNA

**K** VLDL Specific Activity, [3H]-TG, μM
- TuD-Scrambled
- TuD-Anti-miR-379

**L** Tissue clearance (absolute), nmol/h [3H]-TG
- Liver
- BAT
- pgWAT
- GCM
Figure S4

A. miR-379 Predicted Targets

B. miR-379 targets in the liver (n=51)

C. Functional Annotation Clustering

D. miR-379 KD/OE Phenotypes

E. miR-379 KD/OE Phenotypes

F. miR-379 KD/OE Phenotypes

G. miR-379 KD/OE Phenotypes

H. miR-379 KD/OE Phenotypes

I. miR-379 KD/OE Phenotypes

J. miR-379 KD/OE Phenotypes
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-T2D, Obese</th>
<th>Diabetic-Obese</th>
<th>Controls</th>
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<tr>
<td>Age at surgery</td>
<td>46 ± 9</td>
<td>49 ± 10</td>
<td>54 ± 5</td>
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<td>Gender (Male/Female)*</td>
<td>11/26</td>
<td>10/17</td>
<td>5/5</td>
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<tr>
<td>Body weight, kg</td>
<td>131.6 ± 21.6z</td>
<td>130.5 ± 19.4z</td>
<td>69.9 ± 8.3</td>
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<td>BMI, kg/m²</td>
<td>45.1 ± 6.1z</td>
<td>45.2 ± 6.5z</td>
<td>23.8 ± 0.8</td>
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<tr>
<td>Systolic BP, mm Hg</td>
<td>141.9 ± 13.2z</td>
<td>143.1 ± 15.1z</td>
<td>120.4 ± 2.1</td>
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<td>Diastolic BP, mm Hg</td>
<td>90.4 ± 8.2z</td>
<td>92.1 ± 9.8z</td>
<td>80.9 ± 1.5</td>
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<td>Fasting Plasma Glucose, mM</td>
<td>4.9 ± 0.5z</td>
<td>6.5 ± 1.5z</td>
<td>5.2 ± 0.1</td>
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<td>Fasting Plasma Insulin, mU/L</td>
<td>10.8 ± 7.5z</td>
<td>22.8 ± 8.2z</td>
<td>12.1 ± 1.2</td>
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<tr>
<td>HOMA-IR</td>
<td>2.4 ± 1.8z</td>
<td>6.6 ± 2.8z</td>
<td>0.4 ± 0.0</td>
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<td>Total Cholesterol, mM</td>
<td>5.1 ± 1.3</td>
<td>5.5 ± 1.2</td>
<td>4.8 ± 0.1</td>
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<td>LDL Cholesterol, mM</td>
<td>3.1 ± 1.1</td>
<td>3.5 ± 1.2</td>
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<td>HDL Cholesterol, mM</td>
<td>1.1 ± 0.2z</td>
<td>1.2 ± 0.2z</td>
<td>1.4 ± 0.1</td>
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<td>Free Fatty Acids, mM</td>
<td>0.6 ± 0.5z</td>
<td>0.8 ± 0.6z</td>
<td>0.2 ± 0.0</td>
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<td>Leptin, ng/mL</td>
<td>46.5 ± 20.9z</td>
<td>42.3 ± 22.5z</td>
<td>9.0 ± 1.3</td>
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<td>Medications</td>
<td>None</td>
<td>Metformin alone or in combination with Liraglutide or Pioglitazone</td>
<td>None</td>
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* Counts
** Sig. vs Control
z Sig. Non-T2D, obese vs Diabetic-obese

Data are Mean ± SD
### Supplemental Table 2

<table>
<thead>
<tr>
<th><strong>LNA Oligonucleotides</strong></th>
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<tr>
<td>Anti-miR-379</td>
<td>GTTCCATAGTCTACC</td>
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<td>LNA Scrambled</td>
<td>ACGTCTATACGCCCA</td>
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<th><strong>Tough Decoy Inserts</strong></th>
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<td>TuD-Anti-miR-379</td>
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<td><strong>Fwd</strong></td>
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<td><strong>Rev</strong></td>
<td>GATCTGACGGGCTAGGATCATCTTTTGTGAGCTACTAGATTGGAAGTGGCTAGGGTTGATTTCTTGAGCAGGATCTTACAGATTTGGAAGTGGCTAGGGTTGATTTCTTG</td>
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<td>TuD-Scrambled</td>
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<td>GATCTGACGGGCTAGGATCATCTTTTGTGAGCTACTAGATTGGAAGTGGCTAGGGTTGATTTCTTGAGCAGGATCTTACAGATTTGGAAGTGGCTAGGGTTGATTTCTTG</td>
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*Note: Sequences in italics are the SalI and BglII overhangs; Underlined sequences are the miR-379 binding sites or the scrambled sequence.*

<table>
<thead>
<tr>
<th><strong>psiCHECK™-2 Vector Inserts</strong></th>
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<td><strong>LSR_MBS</strong></td>
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</table>

*Note: Sequences in italics are the XhoI and NotI overhangs; Underlined sequences are the miR-379 binding sites or the mutated sequence; MBS – MicroRNA Binding Site*

<table>
<thead>
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<th><strong>MicroRNA Mimics</strong></th>
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<tr>
<td>mmu-miR-379</td>
<td>UGGUAAGAUAUUGAAACGUAG (MIMAT0000743)</td>
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<tr>
<td>cel-miR-293b</td>
<td>UUGUACUAACAAAAAGUACUG (MIMAT0000295)</td>
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<table>
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<tr>
<th><strong>GR ChIP qPCR Primers</strong></th>
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