Supplemental Figures and Tables

miR-7a regulates pancreatic β-cell function

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Figure S1
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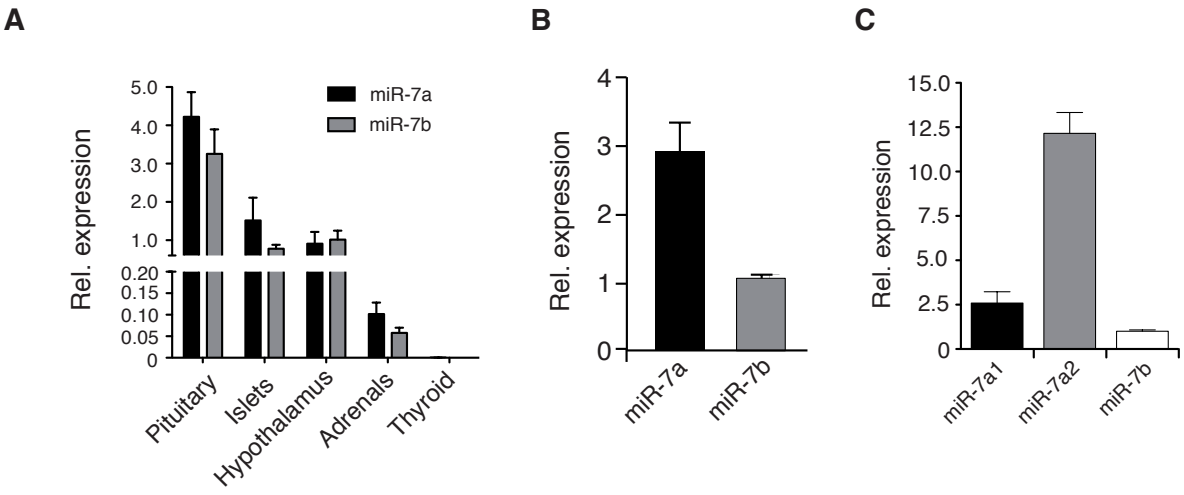

Figure S1 Expression of miR-7 in pancreatic islets (A) Expression of mature miR-7a and miR-7b levels in mouse neuroendocrine tissue measured by qPCR (n=3). (B) miR-7a and miR-7b in donor human pancreatic islet measured by qPCR. (C) Pre-miR-7 levels in mouse pancreatic islets at 15 weeks of age (n=4). Data are mean ± s.d.

Figure S2
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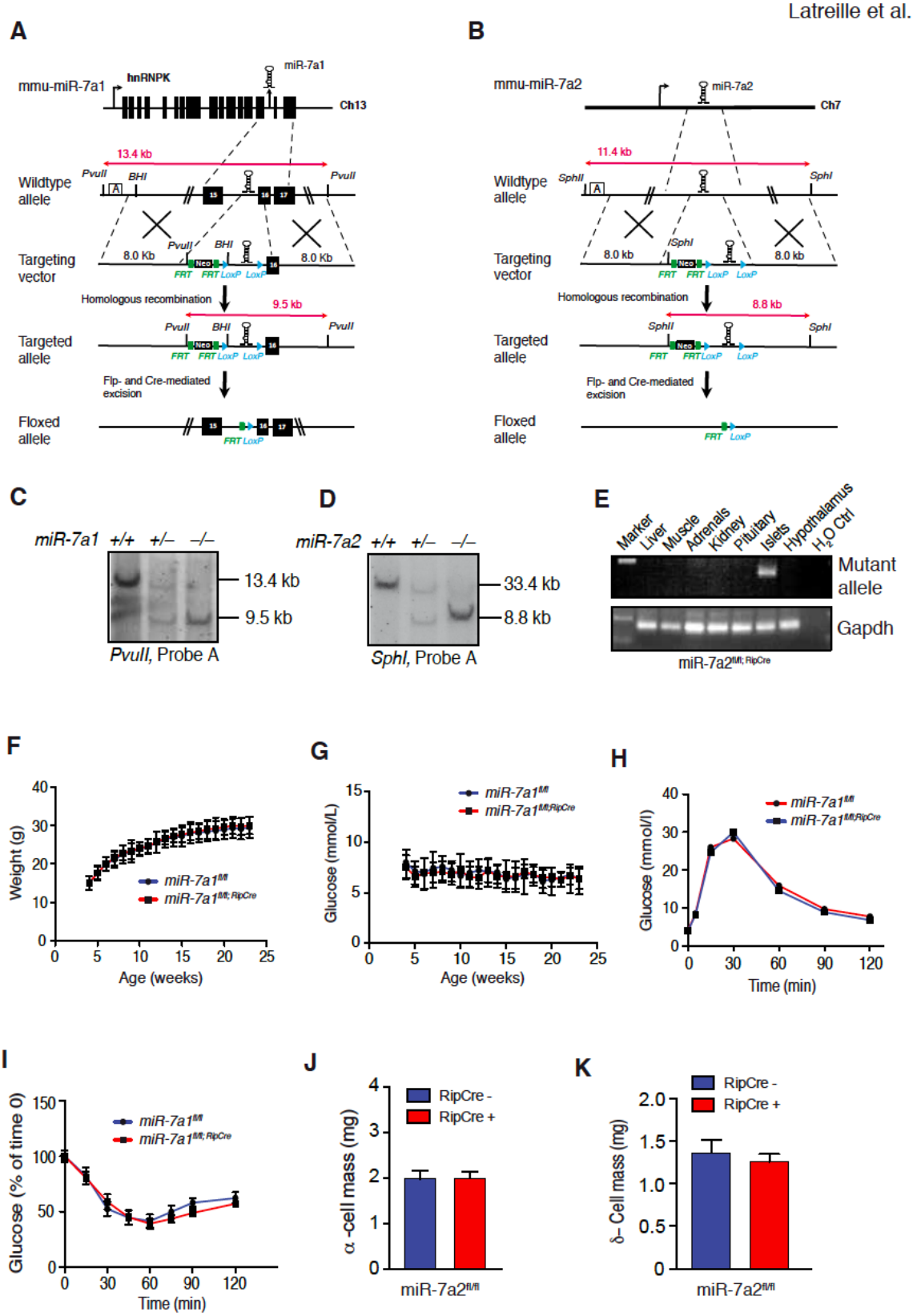

Figure S2 Generation of conditional miR-7a1/2 null alleles and metabolic phenotyping. (**A-B**) Strategy used to generate miR-7a1 (A) and miR-7a2 (B) mutant mice by homologous recombination. miRNA sequences were flanked with loxP sites and recombination induced by breeding mice with RipCre transgenics. (**C**) Southern blotting of tail genomic DNA from wild type (+/+), heterozygote (+/-) and homozygote (-/-) miR-7a1 mutant mice crossed to DeleterCre mice. DNA was digested with PvuII and southern blotting performed with miR-7a1 Probe A (see scheme in A). Wild type allele: 13.4 kb; Mutant allele: 9.5 kb. (**D**) Southern blotting of miR-7a2 mutant as indicated in (C). Genomic DNA was digested with SphI and Southern blotting performed with miR-7a2 Probe A (see scheme in B). Wild type allele: 11.4 kb; Mutant allele: 8.8 kb. (**E**) Selective deletion of the miR-7a2 gene in islets of *miR-7a2*$^{fl/fl;RipCre}$ mice. Genomic DNA from indicated mouse tissues was used in PCR with miR-7a2 primers spanning LoxP sites (Top) and within Gapdh the locus as control (Bottom). (**F-K**) Metabolic characterization of miR-7a1 mutant mice. (**F**) Weight of *miR-7a1*$^{fl/fl;RipCre}$ and control mice (n=10-12). (**G**) *Ad libitum*-fed blood glucose levels in *miR-7a1*$^{fl/fl;RipCre}$ and control mice (n=10-12). (**H**) Intraperitoneal Glucose Tolerance Test in overnight fasted *miR-7a1*$^{fl/fl;RipCre}$ and control mice at 10 week of age (3g/kg)(n=10). (**I**) Intraperitoneal Insulin Tolerance Test in 6h fasted *miR-7a1*$^{fl/fl;RipCre}$ and control mice at 11 week of age (0.75U/kg)(n=10). Pancreatic α-cell (**J**) and δ-cell (**K**) masses of 25-week-old *miR-7a2*$^{fl/fl;RipCre}$ and control mice (n=5-6). Data shown are mean ± s. e. m.

Figure S3
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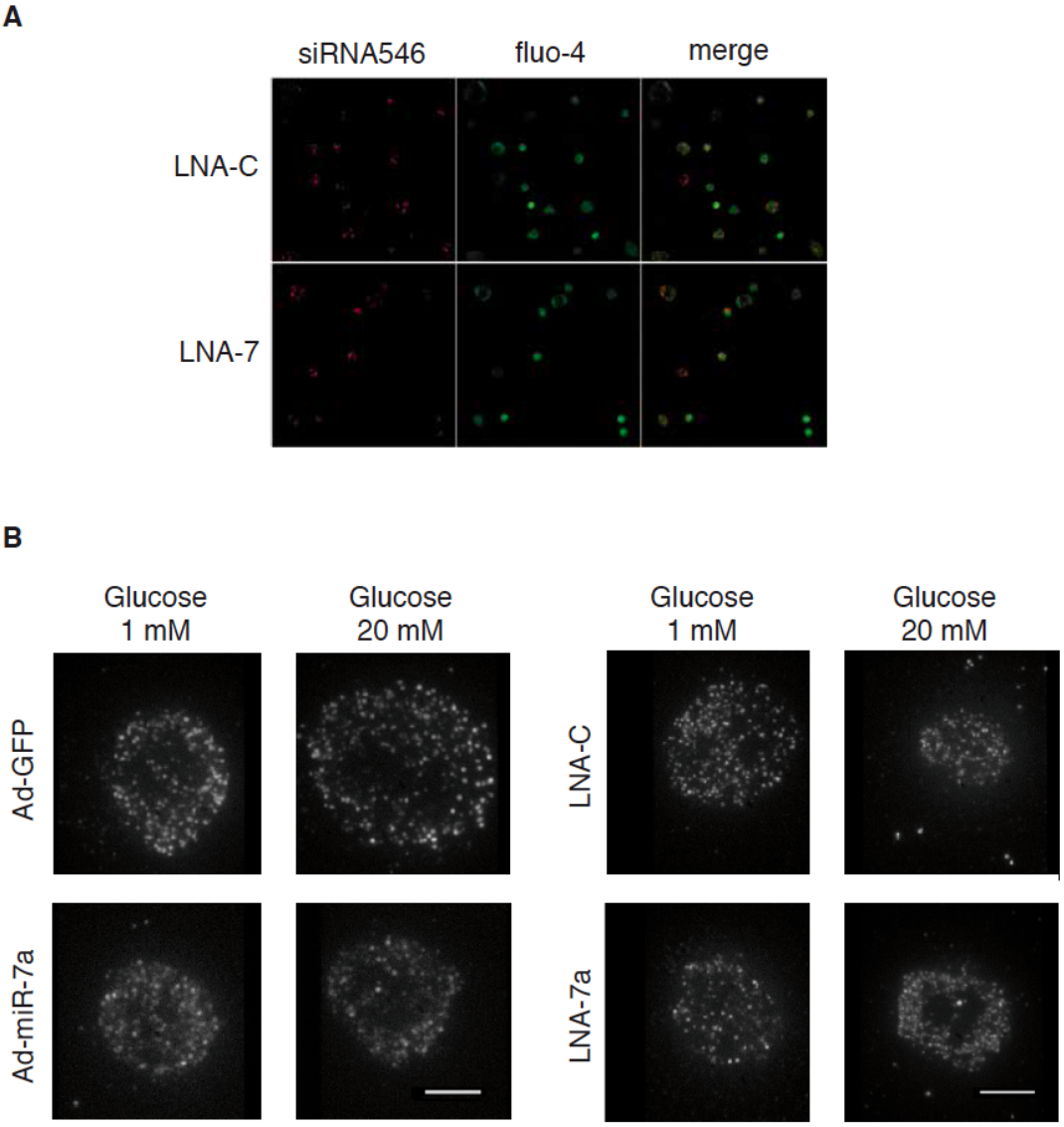

Figure S3 TIRF microscopy in primary-β-cells (**A**) Transfection efficiency of LNA-miR-7a inhibitors cotransfected with labeled-Alexa546 siRNAs in primary mouse β-cells. Fluo-4 shows the loading efficiency of the dye in calcium measurements.(**B**) TIRF microscopy in primary mouse β-cells. Docked granules visualized by TIRF microscopy and insulin immunocyto-chemistry in control cells (LNA-C or Ad-GFP) and after inhibition (LNA-7a) or overexpression (Ad-miR-7a) of miR-7a in primary β-cells incubated at 1 or 20 mM glucose for 15 min as indicated. Scale bar: 5 µm.

Figure S4
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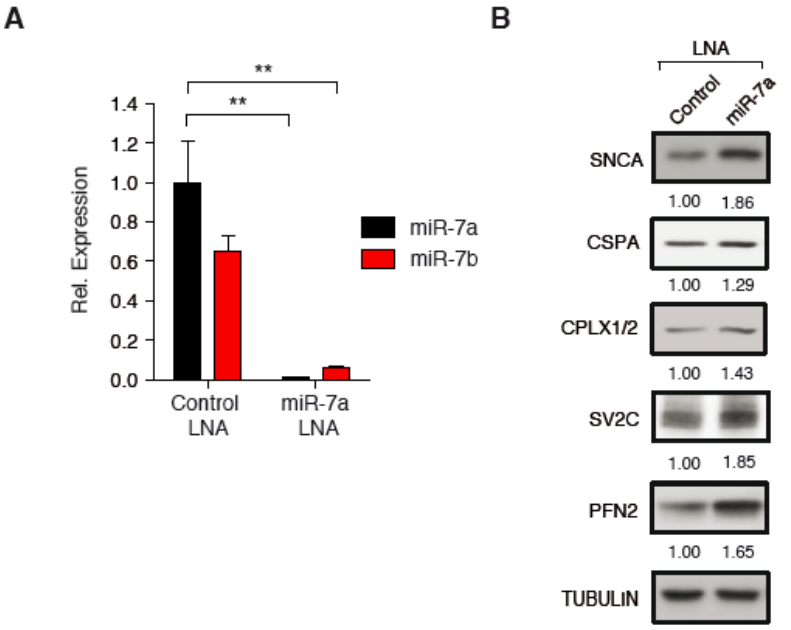

Figure S4 Depression of exocytotic targets by miR-7a inhibitors in MIN6 cells.
(A) Inhibition of miR-7 by miR-7a LNA inhibitors. MIN6 cells were transfected with Control or miR-7a LNA inhibitors (Exiqon) for 48h. Cells were subjected to RNA extraction and the levels of miR-7a and miR-7b were measured by qPCR (n=3). Data shown are mean ± s.d **: $P < 0.01$. (B) Immnublotting experiment of MIN6 cells treated with control (C-) or miR-7a LNA inhibitors for 48h. Shown are representative immunoblots of at least 3 independent transfections. Densitometric analysis were performed using the Image J program over TUBULIN levels.

Figure S5
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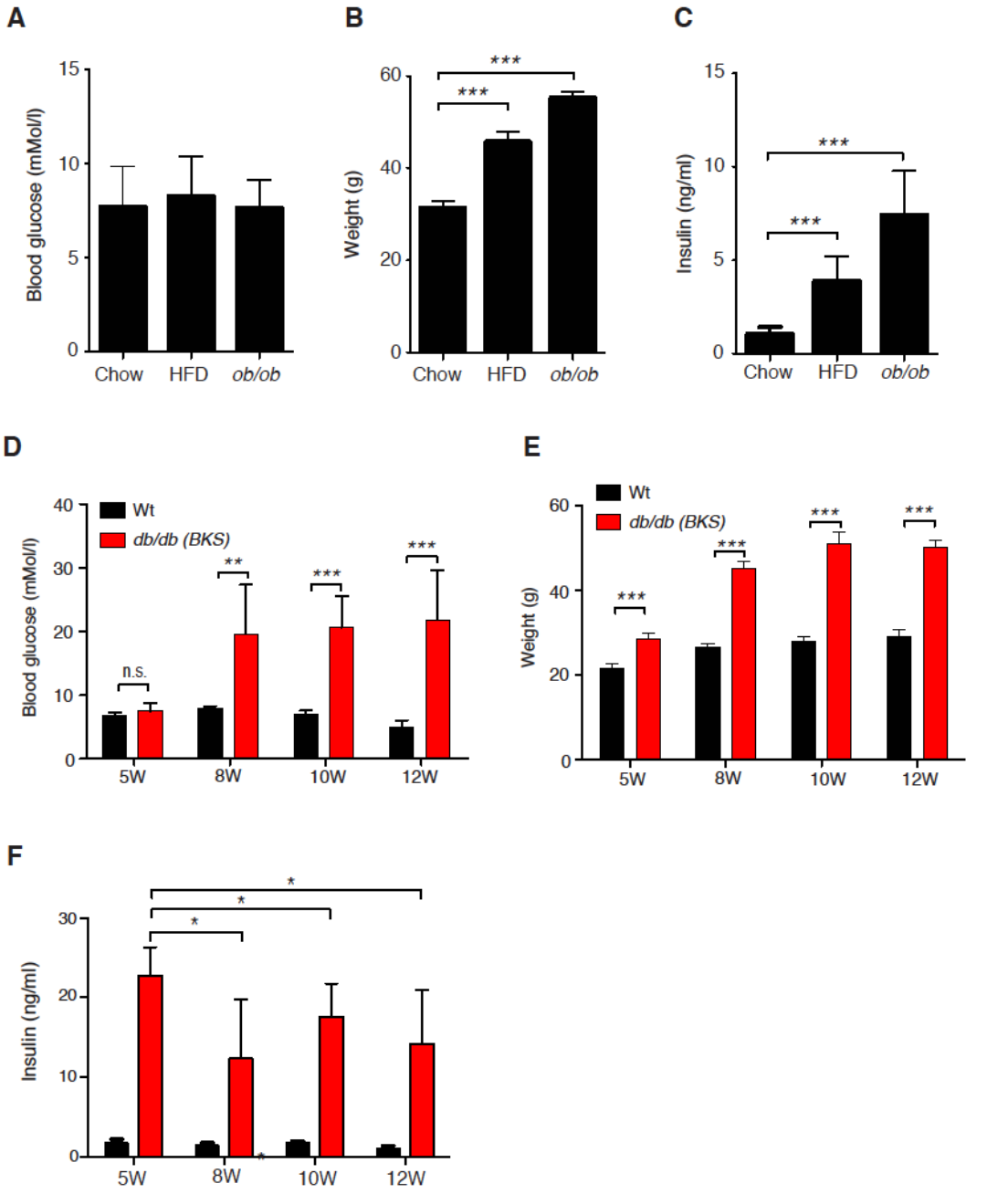

Figure S5 Metabolic profile of compensating and decompensating obese mice. (**A**) Blood glucose, (**B**) Weight and (**C**) Insulin levels in *Wt C57Bl/6* male mice maintained on chow or high fat diet (HFD) for 20 weeks, and 22-week-old *ob/ob (C57Bl/6)* males maintained on chow diet (n=4-5). (**D**) Blood glucose (**E**) weight and (**F**) insulin levels in Wt and *db/db* males mice (*BKS*) of indicated ages (n=5). Data shown are mean ± s.d. * P<0.05 , ** P <0.01, *** P <0.001.

Figure S6
Latreille et al.

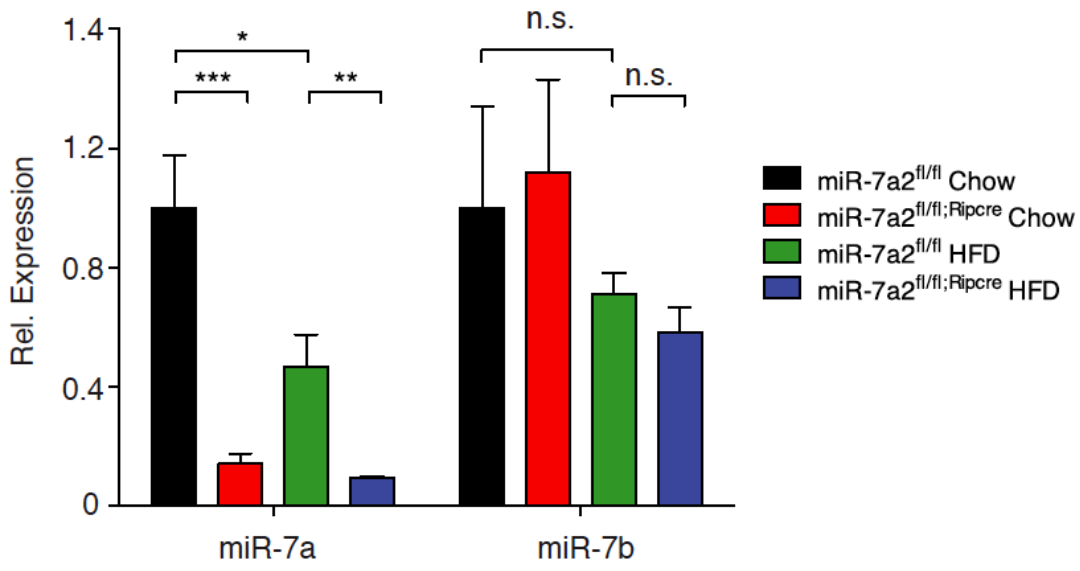

Figure S6. miR-7 levels in islet form *miR-7a2*$^{fl/fl;RipCre}$ mice fed a high fat diet.
Relative miR-7a and miR-7b expression in pancreatic islets of male *miR-7a2*$^{fl/fl}$ and *miR-7a2*$^{fl/fl;RipCre}$ maintained on chow or high fat diet (HFD) for 22 weeks determined by qPCR (n=4-8). Data shown are mean ± s.d. * P<0.05, ** P <0.01 and *** P <0.001.

Figure S7
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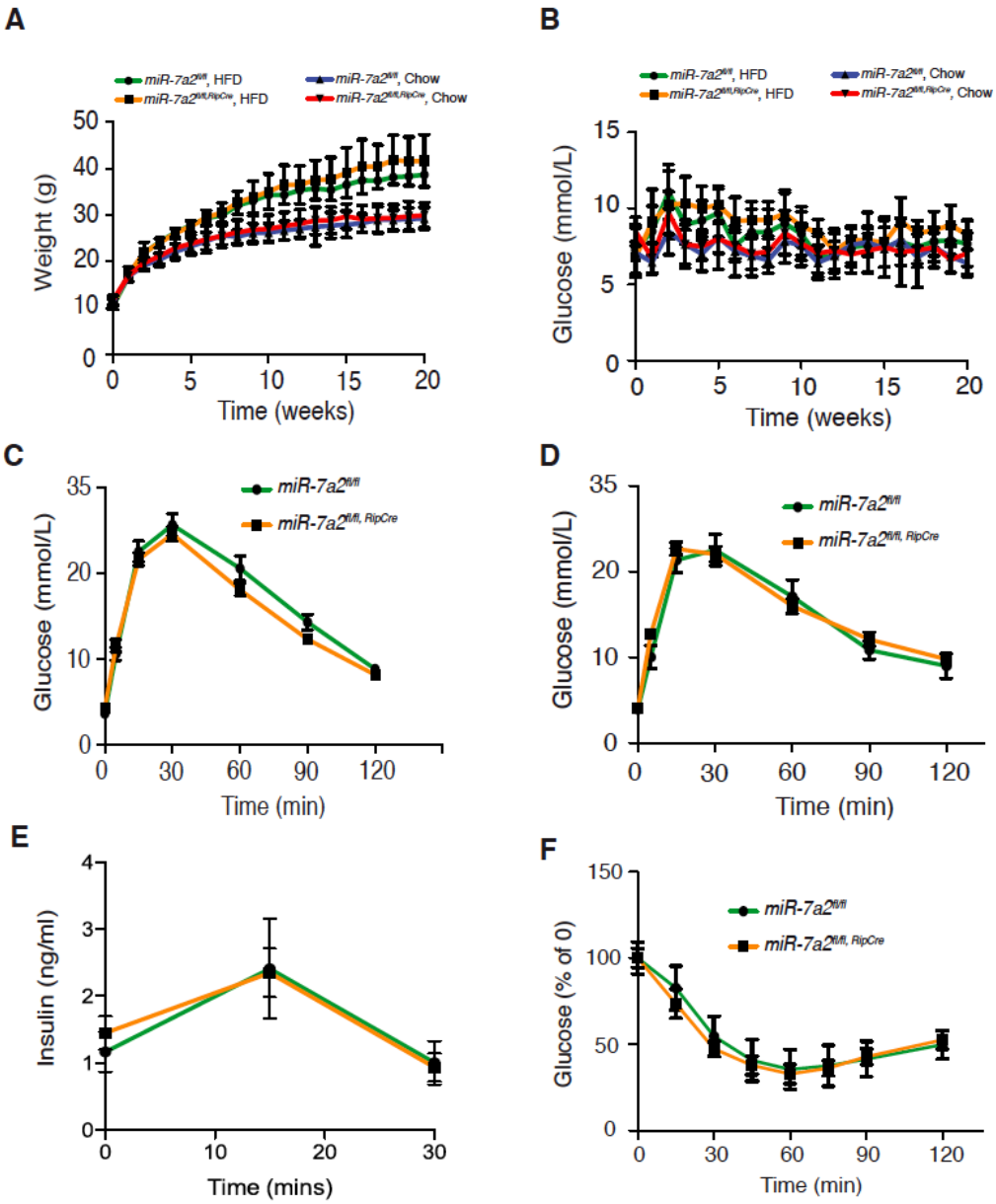

Figure S7 Metabolic characterization of obese *miR-7a2*$^{fl/fl;RipCre}$ mice fed a High Fat Diet (HFD). Body weight (**A**) and Ad libitum-fed blood glucose levels (**B**) of *miR-7a2*$^{fl/fl;RipCre}$ and control mice maintained HFD (n=11-14). Intraperitoneal Glucose Tolerance Test (IPGTT; 1.5g/kg) in overnight fasted *miR-7a2*$^{fl/fl;RipCre}$ and control mice after 12 (**C**) and 20 (**D**) weeks of HFD feeding (n=6-11). (**E**) In vivo insulin excursion in overnight fasted *miR-7a2*$^{fl/fl;RipCre}$ and controls after 15 weeks of HFD feeding (3g/kg; n=7-11). (**F**) Intraperitoneal Insulin Tolerance Test (IPITT; 0.75U/kg) in 6h fasted *miR-7a2*$^{fl/fl;RipCre}$ and control mice after 15 weeks on HFD (n=6-12). Data shown are mean ± s.e.m. except for panels A and B where s.d. is shown.

Figure S8
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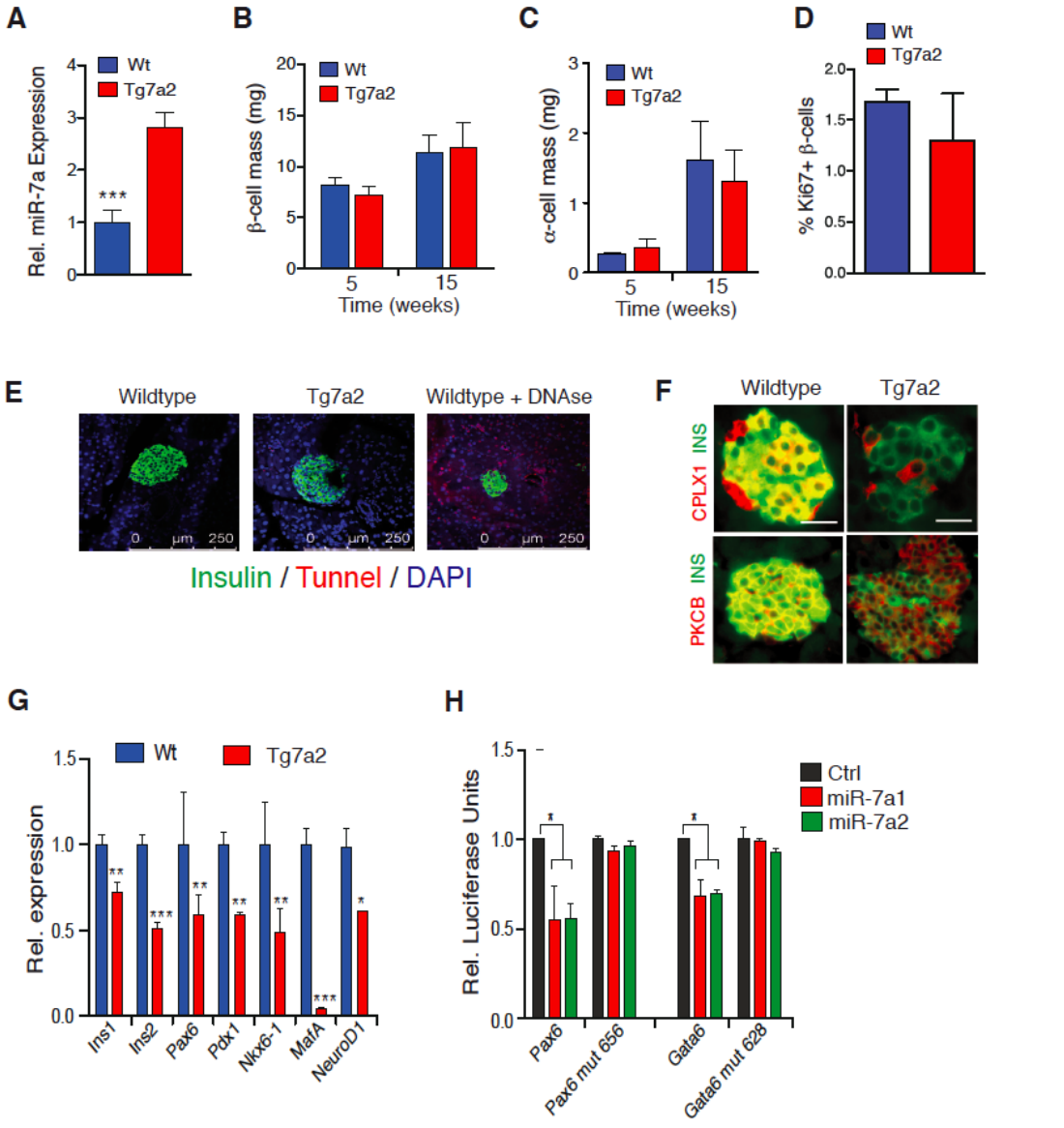

Figure S8 Dedifferentiation of β-cells in Tg7a2 mice (**A**) Relative miR-7a expression in islets of Tg7a2 mice at 5 week of age. β-cell (**B**) and α-cell (**C**) mass of 5 and 15-week-old Tg7a2 and control mice (n=5-6). (**D**) Percentage of Ki67-positive β-cell nuclei of of Tg7a2 and control mice (n=3). (**E**) Representative images of 5-week-old Tg7a2 and control pancreata processed in Tunne (Red) and insulin (green) staining. Treatment with DNAseI serves as a positive control. (**F**) Co-immunostaining for insulin (green) and CPLX1/2 or PKCB (red) in Tg7a2 and control mice at 5 weeks of age. Note that residual CPLX1/2 positive cells are negative for insulin and thus unlikely overexpressing miR-7a. Scale bar 50 μm. (**G**) Relative expression of indicated genes in Tg7a2 islets at 10 weeks of age (n=5). (**H**) Relative luciferase activity of Pax6 and Gata6 wildtype and mutant 3'-UTRs in HEK 293 cells cotransfected with pcDNA3 (Ctrl), pcDNA3-miR-7a1 or pcDNA-miR-7a2 performed in triplicate (n=3-4). Data show are mean ± s.d. except B and C where mean ± s.e.m. *: P <0.05, **: P<0.01, ***: P<0.001.

Figure S9
Latreille et al.

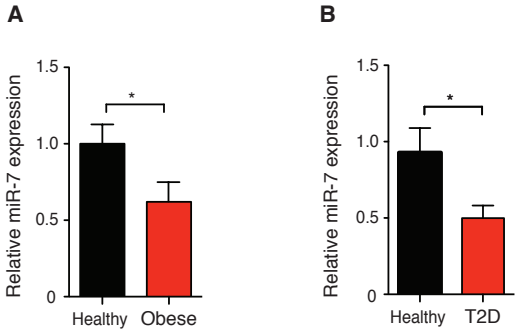

Figure S9 Levels of miR-7a in obese and diabetic (T2D) human islets. (**A**) miR-7a levels in islets of healthy (BMI<28) and obese / non-diabetic (BMI>28) humans (n=5-8). (**B**) miR-7a levels in human healthy and T2D islets determined by qPCR (n=9-10). Data are mean ± s.e.m. *: $p < 0.05$

Supplemental Table 1
Human obese patients characteristics

Sample	Age (years)	BMI (kg/m^2)	HbA1c (mmol/mol)
Healthy	47.6 ± 8.3	24.7 ± 2.2	5.6 ± 0.3
Obese	51.8 ± 12.0 $^{n.s.}$	33.1 ± 2.5 ***	5.9 ± 0.2 *

Data shown are mean ± s.d. * P <0.05, *** P <0.001, n.s. non significant

Supplemental Table 2
Human T2D patients characteristics

Sample	Gender (M:F)	Age (years)	BMI (kg/m^2)	HbA1c (mmol/mol)
Control	(5:5)	56.9 ± 7.9	26.7 ± 3.3	5.5 ± 0.2
T2D	(5:4)	54.2 ± 8.3 $^{n.s.}$	28.4 ± 4.3 $^{n.s.}$	6.6 ± 0.5

Data shown are mean ± s.d. * P <0.05, *** P <0.001, n.s. non significant

Supplemental Table 3
Primer sequences used genotyping of miR-7 mutant mice* #

Sequence	Forward strand (5'-3')	Reverse strand (5'-3')
RIP2 promoter	TCGCTCATGCAACGACACCAAG	AACAATGACCTGGAAGATAGTCAGGG
Pre-miR7a2	CCC**GGTACC**GAAGGTGGCTAGCTGTACCAC	CCC**AAGCTT**CTCCCCCCGCCCCACACAGCTGC
Tg7a2 Genotyping	CATGTGGCAGACTGGGACTTGTTGTTG	CTATAAAGCTAGTGGGGATTCAGTAAC
miR-7a1 16kb locus	CTACACTGGCTACTGGGCCCTGTGCGCCGCCGTCAACCGCGCGCTCGACCGCTCTCCTGAGTAGGACAAATC	GAAAAGACCACGTCTTATCACAAAACTAGTGTTATAGTAGGGCTAGTAGTTCACAGCTTGTCTGTAAGCGGATG
miR-7a2 16kb locus	GGACCTTTATTGCGCCACCCTTTCTCAAACTGGGATGATTATCGCAATCGGCTCTCCTGAGTAGGACAAATC	CTTTAGTTAAAATAGGAATTCCCACCTACTGAATTTACCTGTTTGTTTACTCACAGCTTGTCTGTAAGCGGATG
miR-7a1 Genotyping	TACAGGAACACAGGACCAGATACAGAACG	TTTAGAAGTTAGATTCCCCAAGTGACGGAAACAC
miR-7a2 Genotyping	CGCTCTTGGAGCTGCCAGGATATTG	CAGGTGGACAGCTCATCTCTCTATATCTTCTTCG
Rip-Cre Genotyping	CCTGTTTTGCACGTTCACCG	ATGCTTCTGTCCGTTTGCCG

Supplemental Table 4
Quantitative PCR primers sequences

Mouse Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
pre-miR-7a1	GCCTGTAGAAAATGTAGAAGAGAC	TATGGCAGACTGTGATTTGTTG
pre-miR-7a2	AAGTCAGGGGAGCAGGCAC	TGGCAGACTGGGACTTGTTGT
pre-miR-7b	GAGAGAGAGAGAAGCACTTGAGGG	GAGGCTGGCTGTGACTTGTTGT
Ins1	CAGAGAGGAGGTACTTTGGACTATAAA	GCCATGTTGAAACAATGACCT
Ins2	GAAGTGGAGGACCCACAAGT	GCTGGTGCAGCACTGATCTA
Pax6	ACTTCAGTACCAGGGCAACC	GAACTGATGGAGTTGGTGTTCTC
Pdx1	TTCCCGAATGGAACCGAGC	GTAGGCAGTACGGGTCCTCT
Nkx6-1	TCAGTCAAGGTCTGGTTCC	CGATTTGTGCTTTTTCAGCA
Mafa	AGGAGGAGGTCATCCGACTG	CTTCTCGCTCTCCAGAATGTG
Neurod1	GACCCAGAAACTGTCTAAAATAGAGACA	AAGGAGACCAGATCAGGGCTTT
Glut2	TCTTCACGGCTGTCTCTGTG	AATCATCCCGGTTAGGAACA
Sncα	AAGAAGAGTCTGTTCGCTGGA	AAAGATGTATTTTTGCTCCACACTT
Cspα	CTGCTGTGGGAAATGCAAG	GCTGTATGACGATCGGTGTG
Cplx1	CCATGGAGTTCGTGATGAAA	CCAAGCATCTTCCCCATGT
Pkcβ	GAAACTCGAACGCAAGGAGA	ACCGGTCGAAGTTTTCAGC
Pfn2	CCGGACAAAGAGTCAAGGTGGGG	GACCAAGACTCTCCCGGCCCT
Wipf2	ACCACTGTCCGCTCCTTCT	TCTGGAGCAGGGAAGTCCT
Phactr1	TCAGACATTATGGATGGACCAG	GGATCTTGGGACAGGAGGAC
Zdhhc9	ACACTCTTCTTTGCCTTCGAGT	ATAGCGGCAAACACAGGAAT
Snap25	GCTCCTCCACTCTTGCTACC	CAGCAAGTCAGTGGTGCTTC
Sox9	GAGCCGGATCTGAAGAGGGA	GCTTGACGTGTGGCTTGTTC
36B4	GCCGTGATGCCCAGGGAAGACA	CATCTGCTTGGAGCCCACGTTG

Supplemental Table 5
Mouse 3'-UTR primer sequences [#]

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Pax6 3'-UTR	CCC**GTCGAC**AGAGAGAAG GAGAGAGCGTGTGATCGA GAG	CCC**GTCGAC**ATTATAGAAATC ATTCTGAGGATTTCTAGG
Gata6 3'-UTR	CCC**GCTAGC**GCTGGTGCTA CCAAGAGGCAAGGAGGGC	CCC**CTCGAG**CACTGTACAGAAC ACAAAACAATACC
Sncα 3'-UTR	CCC**GCTAGC**GAATGTCATT GCACCCA	CCC**CTCGAG**TTATTTTATTTTC CACC
Sncα-V5-UTR	CCC**GTTTAAAC**GAATGTC ATTGCACCCA	CCC**GTTTAAAC**TTATTTTATT TTCCACC
Cspα 3-UTR	CCC**GCTAGC**AGATCAGTTT CAAAGTGATAATAG	CCC**CTCGAG**AGGATTTCTTTGT GTGTAGTCCTG
Cplx1 3-UTR	CCC**GCTAGC**TGATATTGGG TCAGCACCAGGAGC	CCC**CTCGAG**CACGGTGTGAAAT CACTGTTTATTG
Pkcβ 3-UTR	CCC**GTCGAC**ACAAAGTCAG CTCCTGGCCACAGGGGTCG	CCC**GTCGAC**GGGGCAATGCTCT TATTCTAAACTAAACAG
Pfn2 3-UTR	CCC**GCTAGC**CTGCTAGGCA GACTGTTAAG	CCC**CTCGAG**CAGCCAAGTCCAC AATAATGC
Basp1 3-UTR	CCC**GCTAGC**TGTCTCTATC CTATACTAACTTGTTTCAA ATTGG	CCC**CTCGAG**CCATTTTTCCAAT GGGTGGTGTG
Wipf2 3-UTR	CCC**GCTAGC**GTCCCTAGCT ACATGGTCAGG	CCC**CTCGAG**CAGTTACTATCAG TGGCAAAC
Zdhhc9 3-UTR	CCC**GCTAGC**GACTAATCAG TACTTCCATTAAGCC	CCC**CTCGAG**AAGTCGTGGTTTG CACCCCTCCCTG
Pax6 Mut (656-662)	TACAATTTGTTTTATGTCA AAATGTAAGTATTTGTAG TAACTAGAAGTCCTCAGAA TGATTTCTATAATCTCGAC	GTCGAGATTATAGAAATCATTC TGAGGACTTCTAGTTACTACAA ATACTTACATTTTGACATAAAA CAAATTGTA
Gata6 Mut (628-634)	CACTTAAAAAAATTATTGT GTACATTTACTAAAAATAT CTTGTTTGCTACCCTGTTT ACAAATTCCAAGTGACCT	AGGTCACTTGGAATTTGTAAAC AGGGTAGCAAACAAGATATTT TTAGTAAATGTACACAATAAT TTTTTTAAGTG
Sncα Mut (120-126)	ACCTTTTCTCAAAGCTGTA CAGTGTGTTTCAAAGTAGT AAGTCAGCAGTGATCGGCG TC	GACGCCGATCACTGCTGACTTA CTACTTTGAAACACACTGTACA GCTTTGAGAAAAGGT
Cspα Mut (2610-2616)	GTTTCTGTTTGTGCCCAGT GATGTCTTTCCTTGTAGTA AGTTTGACTGTATTTTAAG ATTACTTTAGATGATTCT	AGAATCATCTAAAGTAATCTT AAAATACAGTCAAACTTACTAC AAGGAAAGACATCACTGGGCAC AAACAGAAAC
Pkcβ Mut (6152-6158)	TCAGGCCGAATGTCTGTTA CCAATTCCAGTAGTAAGGG ATTCATGGTGCCT	AGGCACCATGAATCCTTTACTA CTGGAATTGGTAACAGACATTC GGCCTGA

Pfn2 Mut (483-489)	GGAGCATTTCCTTGGCTCA TCTAGTAGTAAGTTTGTAG CGCATGGTTGGGAGGAAA	TTTCCTCCCAACCATGCGCTAC AAACTTACTACTAGATGAGCCA AGGAAATGCTCC

Supplemental Table 6: siRNAs sequences

Gene	Forward strand (5'-3')	Reverse strand (5'-3')
Sncα	GCCUGAAGCCUAAGAAUGUUU	ACAUUCUUAGGCUUCAGGCUU

*Underline: Minimal Vector binding sites (GeneBridges).

Bold: Restriction site introduced in primer (overhang) and used for cloning DNA fragment in target vector.

Supplemental Methods

Generation of miR-7a-1 and miR-7a-2 knockout mice

A 16 kb fragment encompassing mmu-miR-7a-1 (BAC 454-E16) and mmu-miR-7a-2 (BAC 520-A12) was recombined into a minimal vector using homology arms-containing primers according to the protocol supplied by GeneBridges (http://www.genebridges.com - Primer listed in Supplemental Table 1). The miR-7a-1 targeting vector was engineered by introducing a *loxP*-containing neo^r cassette 22 bp downstream of the miRNA precursor sequence. Upon bacterial Cre expression, the neo^r cassette was removed and a second Frt/loxP/neo^r cassette was introduced 252 bp upstream of the gene (Figure S2A). The miR-7a-2 targeting vector was designed by introducing loxP sites 410 bp upstream and 323 bp downstream of the gene, respectively (Figure S2B). Upon linearization, targeting vectors were electroporated in C5BL/6N embryonic stem cells, clones were selected with G418 (Invitrogen) and verified by Southern blotting. Properly targeted clones were microinjected in BALB/c blastocytes and subsequently transferred into pseudopregnant females to generate chimeric offspring. Chimeras were bred with C57BL/6 female mice to produce heterozygotes, which were crossed with Flipase mice to delete the neo^r cassette. Mice were then bred with RipCre (1) or DeleterCre (B6.C-Tg(CMV-cre)1Cgn/j – Jackson Laboratory) transgenic mice for deletion of miR-7a1 and miR-7a2 selectively in pancreatic β-cells or in all tissues of mice, respectively. Genotyping of miR-7a1 and miR-7a2 knockout mice was performed by PCR on DNA isolated from 2 weeks old mice with primers listed in Supplemental Table 1. All procedures were carried out in accordance with institutional policies following approval from the Animal Ethical Committee.

Generation of miR-7a2 transgenic mice

Transgenic mice expressing miR-7a-2 under the regulation of the Rat Insulin Promoter (RIP) was created by subcloning a 948 bp fragment of the rat insulin II promoter in pCRII (Invitrogen) by TOPO cloning generating pCRII-RIP. A 141 bp fragment encompassing the genomic murine pre-miR-7a-2 sequence was inserted at KpnI and HindII sites of pCRII-RIP generating pCRII-RIP-miR-7a-2. A 1.2 kb DNA fragment generated upon digestion of pCRII0-RIP-miR-7a-2 with NsiI

and containing the pRIP-miR-7a2 transgene was microinjected into male pronuclei of *C57BL/6N* zygotes to generate Tg7a2 transgenic mice. Three transgenic (F40, F42, F45) founder lines, designated as B6N-Tg(Rip-7a2), were characterized. All lines displayed similar expression levels of miR-7a and metabolic phenotypes. Data presented here were obtained with F42. All mice were maintained on a pure C57BL/6N background. Genotyping of transgenic and RIP-miR-7 mice was performed by PCR with primers listed in Table S1.

Southern Blotting

Tail biopsies were processed in Sarkosyl-based buffer supplemented with 50mg/ml Proteinase K at 56°C. Clarified supernatants were extracted once with Phenol/Chloroform/Isoamyl alcohol (Sigma) before precipitation with 2 volumes of ethanol. Genomic was digested at 37°C for 15 hours, loaded on 0.8% Tris-Acetate-EDTA agarose gels and transferred to nylon blotting membrane (BioRad). Membranes were UV-cross-linked and incubated at 85°C for 1h. ^{32P}Radialoabeled PCR probes were prepared by Prime-It Random Primer Labeling kit (Agilent Technologies) and purified by G50 gel filtration (GE Healthcare). Membrane were incubated with probes for 16hrs at 60°C, washed and exposed to screens.

Secretion studies

Islet secretion studies were performed on size-matched islets following collagenase digestion and overnight culture in RPMI medium 1640, 5.5 mM glucose supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin). Islet were incubated in Dulbecco's PBS-Hepes-BSA buffer 1 mM glucose for 1 h before being transferred to Dulbecco's buffer containing 3.3 mM and 16.7 mM glucose solutions for 1 hour for static incubations. MIN6 secretion assays were performed 24 h following transfections. Briefly, cells were incubated for 16h in DMEM medium containing 1 g/l glucose, Glutamax, 2% FBS, and 5.5 µM 2-mercaptoethanol. Media were changed to Dulbecco's PBS-Hepes-BSA buffer 2 mM glucose for 1 hour before being transferred to Dulbecco's buffer containing either 1 mM or 25 mM glucose. Supernatants were and islets or MIN6 cells were processed by acid-ethanol

precipitation overnight at -20°C. Insulin levels were measured by ELISA. Values are normalized to total insulin content.

Insulin measurements

For measuring mouse serum insulin levels, blood was collected from the tail vein in heparinized capillaries, the serum was separated by centrifugation and insulin measured by ELISA (Chrystal Chem). For determining total pancreatic insulin contents, hormones were extracted by acid-ethanol extraction and concentrations were determined by ELISA.

Cell Culture, transfection and viral infections

MIN6 cells were maintained in DMEM medium as described previously (2). HEK293 cells were cultivated in DMEM media containing 4.5 g/l glucose and 10% FBS under 5% CO_2 atmosphere at 37 °C. All transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufactures instructions. Concentrations of 50 nM were used for siRNA transfection (Sigma-Supplemental Table 6). Single-strand antisense control or miR-7a LNA inhibitors (Exiqon) were transfected at a final concentration of 20 nM. All cells were harvested 48hrs post transfection. MIN6 adenoviral infections were performed at a MOI of 250 for 48 h in DMEM medium containing 4.5 g/l glucose, Glutamax, 2% FBS, and 5.5 μM 2-mercaptoethanol in triplicates.

Luciferase assays

HEK293 cells were cultured in 24-well plates and transfected with 100 ng of pmirGLO reporters together with 400ng of pCDNA-miR-7a-1 or -2. Cells were harvested and assayed 48h after transfection using the Dual-Luciferase Reporter Assay System (Promega). Results were normalized to the Renilla luciferase control and expressed relative to the average value of the controls.

Immunoblotting

Whole cell protein extracts were obtained by lysing cells in PBS containing 150 mM NaCl, 1% Triton X-100 supplemented with Complete, EDTA-free Protease inhibitors (Roche) and Halt Phosphatase inhibitors (Pierce). Laemmli buffer was

added to samples, separated by SDS-PAGE, transferred by electroblotting and membranes blocked in 10% fat free milk/ TBS-0.1% Tween 20 (Sigma). Membranes were incubated with appropriate antibodies overnight at 4°C. Membranes were exposed to secondary antibodies for 1h at room temperature. Immunoblotting protein levels were quantified by densitometry using NIH ImageJ software (http://rsbweb.nih.gov/ij/download).

Plasmids and adenovirus

Mouse miR-7a-1 and miR-7a-2 -expressing vectors were generated by PCR amplification of 450 bp and 250 bp spanning genomic sequences of pre-miR-7a-1 and pre-miR-7a-2, respectively. Fragments were cloned at XhoI sites of pcDNA3 and pAD5 for adenovirus production (Viraquest). All miR-7 adenoviruses expressed GFP from an independent promoter. Expression was confirmed by Taqman qPCR. V5-Tagged *Snca* was generated by cloning respective coding sequences into pCDNA3.1/V5-His-TOPO (Invitrogen) using the following primer: *Snca* For 5'-ATGGATGTGTTCATGAAAGGAC-3'; *Snca* Rev 5-GGCTTCAGGCTCATAGTCTTGGTAGC; Mouse 3'-UTR sequences of putative miR-7 target genes were PCR-amplified from MIN6 cells or pancreatic islets with specific primers (Supplemental Table 5) and cloned in the pmirGLO. DNA fragments containing at least 150bp spanning miR-7 binding sites were cloned, otherwise full-length 3'-UTRs were amplified. Mutagenesis of the miR-7 seed motif (TCTTCCN to TAGTAAG) in the 3'-UTR of selected targets was done using QuickChange Lightning kit (Agilent Technologies). All constructs and derived mutants were confirmed by DNA sequencing.

RNA isolation and cDNA quantification

RNA was isolated from MIN6 cells, pancreatic islets or tissues using Trizol reagent (Invitrogen) according to the manufacturer's protocol. RNA was subjected to DNaseI treatment with the DNA-free kit (Invitrogen), based on manufacturer's recommendations. RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed by Roche 384 real time PCR machine and using gene specific primers (Supplemental Table 4) and Light Cycler® 480 SYBR Green Master

(Roche). Results were normalized to 36B4 mRNA. miR-7 levels were measured using the TaqMan microRNA Assays for miR-7a and 7b (Applied Biosystems) and the results were normalized to miR-16 levels. Levels of *pre-miR-7* were determined using primers listed in Supplemental Table 4 and as described in (3). For determination of miR-7 levels in T2D islets, the geometric mean of RNU44 and RNU48 was used for normalization according to GeNORM method (4).

Islets dispersion

Mouse or human pancreatic islets were incubated in Ca^{2+} and Mg^{2+} free PBS for 9 min at 37^{o}C and centrifuged for 5 min at 200g. Supernatant was removed and islet cells resuspended in RPMI 1640, 11 mM glucose supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin and seeded in 96-weel plate coated with 804G (bladder carcinoma) conditioned medium (5). The 18h later, dispersed islet cells were infected or transfected with DNA plasmids or LNA inhibitors using Lipo2000 as indicated above.

Estimation of number of docked granules

Isolated β-cells were transfected with a marker of transfection siRNA negative Control Alexa 546 in combination with either LNA-C or LNA-7 or infected with Ad-GFP (Control) or Ad-miR-7a (which also expresses GFP). The number of docked granules was estimated using an Olympus IX-81 total internal reflection fluorescence (TIRF) microscope equipped with a 150x/1.45 Apo lens (Olympus, UK). Following a 15min incubation at 1 or 20 mM glucose, cells were permeabilized using Triton X100 (0.1%), fixed in paraformaldehyde (4%) and immunostained with an antibody against insulin (p/n I8510 from Sigma) overnight at 4^{o}C and subsequently washed 3 times in PBS. Insulin immunoreactivity was visualized using an Alexa Fluor 488 (p/n 11073 from Invitrogen; for cells transfected with LNA or Alexa Fluor 555 (p/n A21435; for cells infected with the adenoviruses) goat anti-guinea-pig secondary antibodies. Granule number was determined using Metamorph software and normalized to the area of the footprint (vesicles/mm^2).

Computational Analysis

Raw data (CEL files) from the Affymetrix scanner were loaded into R using the R software (www.R-project.org) with the BioConductor affy package [Gentleman et al., Genome Biology 2004]. Probe intensities were corrected for optical noise, adjusted for non-specific binding and quantile normalized with the gcRMA algorithm (6). Per gene log2 fold-change were obtained through the following procedure. Probe sets for which more than half of the probes (i.e. 6 probes) mapped ambiguously (more than 1 match) to the genome were discarded, as were probe sets that mapped to multiple genes. We then collected all remaining probe sets matching a given gene, and averaged their log2 fold changes to obtain an expression change per gene. For the MIN6 over-expression data, we considered all genes for which at least one probeset was called present in either the GFP expression control or both the miR-7a and miR-7b overexpression experiments as expressed. Only these genes were retained for further analyses. In the *miR-7a2*$^{fl/fl;RipCre}$ mutant islet analysis, we considered all genes for which at least one probe set was called present in either the wildtype or the knockout as expressed. We investigated the effect of miR-7 over-expression on miR-7 target genes. We collected all genes that carried a canonical miR-7 binding site in the 3'-UTR, defined as a heptamer complementary to positions 2 to 8 of the miRNA, or to positions 2 to 7 with a 'U' at position 1 (7). 3'-UTR sequences were downloaded from the RefSeq database (NCBI) on January 18th 2011. From the genes "with ElMMo site", we also selected those containing one of the 100 top-scoring ElMMo-predicted sites. ElMMo is an algorithm that estimates the probability that a miRNA-complementary site is under evolutionary selective pressure (8). In addition, we defined a third group of genes with "no site" that did not carry a heptameric seed match to miR-7. For each of these groups of genes, we plotted the cumulative density function of log2-fold change as well as the 95% confidence interval on the mean log2-fold change. Finally, we tested for the tendency of genes "with site" or "with ElMMo site" to be repressed compared to genes with "no site" using Wilcoxon's rank sum test and reported the resulting p-values in the figure legend.

Gene Ontology (GO) enrichment analysis

To characterize the function of genes under direct and indirect control by miR-7 in MIN6 cells, we focused on genes up- and down-regulated by 30% upon infection by Ad-miR-7a2 compared to controls Ad-GFP. This represents a cut-off of log2 (1.3) = 0.38 on log2-fold changes. We further examined the subset of down-regulated genes with a match to miR-7 in the 3'-UTR to capture the possible function of miR-7 direct targets. Similarly, we determined genes up- and down-regulated in *miR-7a2*$^{fl/fl;RipCre}$ pancreatic islets compared to *miR-7a2*$^{fl/fl}$ controls. Changes in gene expression were smaller in the mutant islets experiment compared to Ad-miR-7a2 MIN6 cells, with a standard deviation of 0.39 on log2-fold changes in islets compared to 0.48 in MIN6. This represents a ratio of 0.81. We therefore set a cut-off of 0.81 x log2 (1.3) = 0.30 on log2-fold changes in the KO islets experiment and focused on genes regulated by 2^0.30=24%. We further examined the subset of genes up-regulated in *miR-7a2*$^{fl/fl;RipCre}$ islets that carried a seed match to miR-7 in their 3'-UTR. Finally, we sought to increase the specificity of the analysis by examining genes both down-regulated in the MIN6 and up-regulated in *miR-7a2*$^{fl/fl;RipCre}$ mutant islets, as well as genes both up-regulated in the MIN6 and down-regulated in the mutant islets. This leads to a total of 9 "study sets" in the terminology used by the Ontologizer.
GO annotation in mouse focuses primarily on Mouse Genome Informatics (MGI) genes. We therefore converted RefSeq mRNA IDs to MGI IDs using the annotation provided by the RefSeq database. We then performed GO enrichment analysis using the parent-child union method implemented in the command line version of the Ontologizer (9) and applying the Westfall-Young single step correction for multiple testing. For the MIN6 study sets, we defined the population (or background) set as all genes called expressed on the MIN6 microarrays. Background genes sets for mutant islets study sets were similarly defined as genes called expressed on the KO microarrays. Finally, genes expressed both in MIN6 and mutant *miR-7a2* islets defined the population set for study sets at the intersection of MIN6 and *miR-7a2*$^{fl/fl;RipCre}$ islets. Finally, we visualized all GO terms enriched with a P-value smaller than 0.05 in at least one gene set on a

heatmap by re-ordering GO terms by hierarchical clustering on the Euclidean space of -log10 P-values using Ward linkage.

Supplementary Reference

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