SUPPLEMENTARY DATA

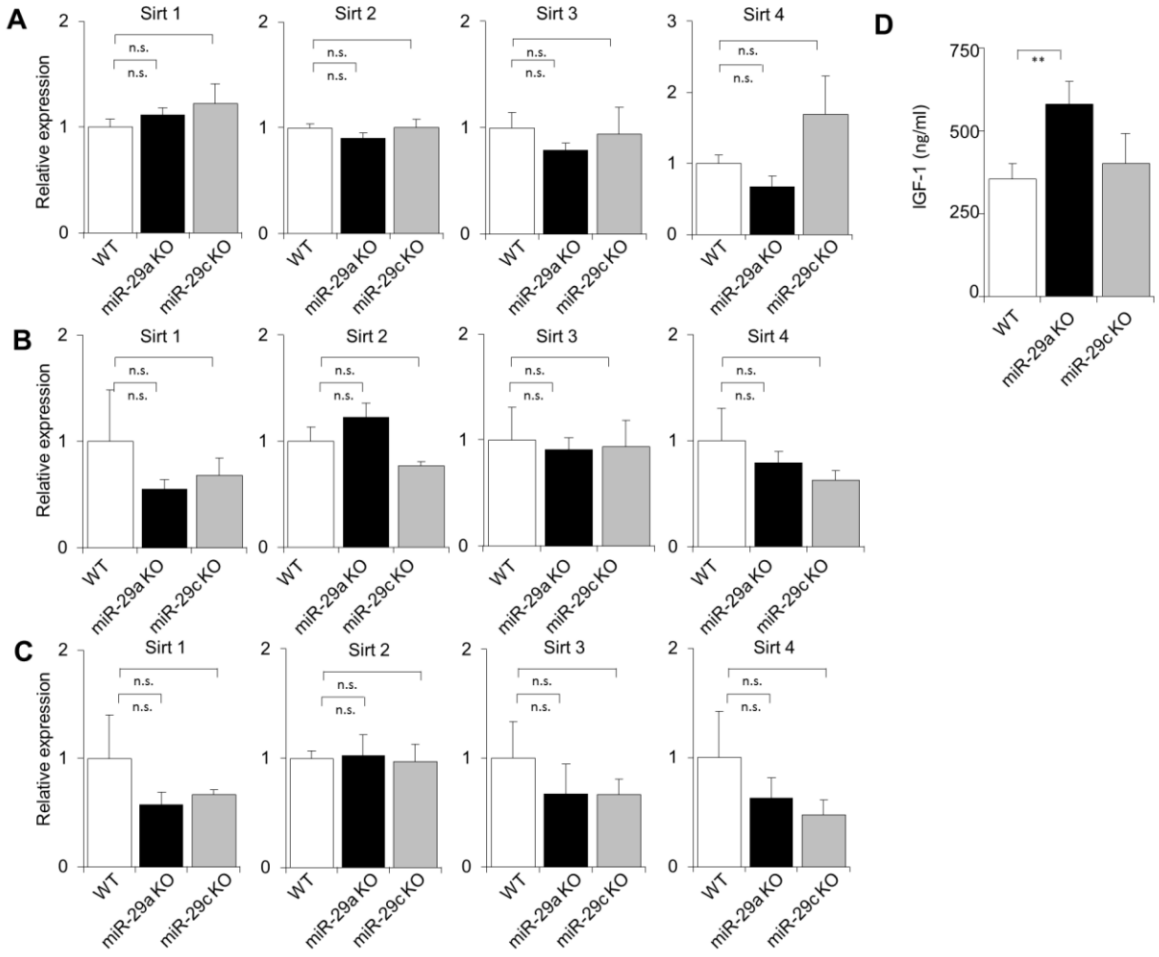
Supplementary experimental procedures

Generation of miR-29c floxed and knockout mice

A ~10kb KpnI/KpnI fragment containing the miR-29b-2/c cluster was amplified as fragments of ~2 kb and ~4 kb from isolated 129/Ola genomic DNA and subsequently cloned in a PGEM-T vector after A-tailing. A mutated loxP site (loxp*) was generated from oligos containing a SpeI site and inserted on a SphI site ~0.4 kb downstream of miR-29c. The targeting vector was subjected to subcloning of another loxp* site followed by the hygromycin B resistance gene, with a PGK promoter and flanked by two FRT sites in the NheI site 0.4 kb upstream of the miR-29b. The targeting construct was finalized by ligating all genomic fragments and subcloning the genetically altered KpnI fragment into a pUC18 vector complimented by the diphtheria toxin negative selection cassette (DTA). The vector was linearized with XmaI and purified prior to electroporation into E14 129/Ola ES cells. Hygromycin B-resistant clones were selected with 60μg/ml hygromycin and were screened by Southern blotting. Recombination was confirmed using ELT PCR (Roche) for the 5' arm (primers FW: gaaagtggtctgggctgtgt and RV: cccaagatgcatagtgtcg), 3' arm (primers FW: acagccaggagccataaaaa and RV: ccacacacgtctccatttct) and hygromycin region (primers FW: acagccaggagccataaaaa and RV: cccaagatgcatagtgtcg). Selected clones were confirmed by sequencing and were brought together with morula-aggregated embryos of Swiss mice for the generation of chimeras. The chimeras were then back-crossed with C57BL/6 mice for more than seven generations. Knockout mice were generated by intercrossing with EIIa-Cre mice[6], with the Cre then bred out of the strain. Genotyping of knockout mice was performed through PCR for the wildtype (422bp, FW: gctgccaaggttagagatca and RV: gaggagggtcagagtccaca) and knockout bands (490bp, FW: acagccaggagccataaaaa and RV: gaggagggtcagagtccaca).

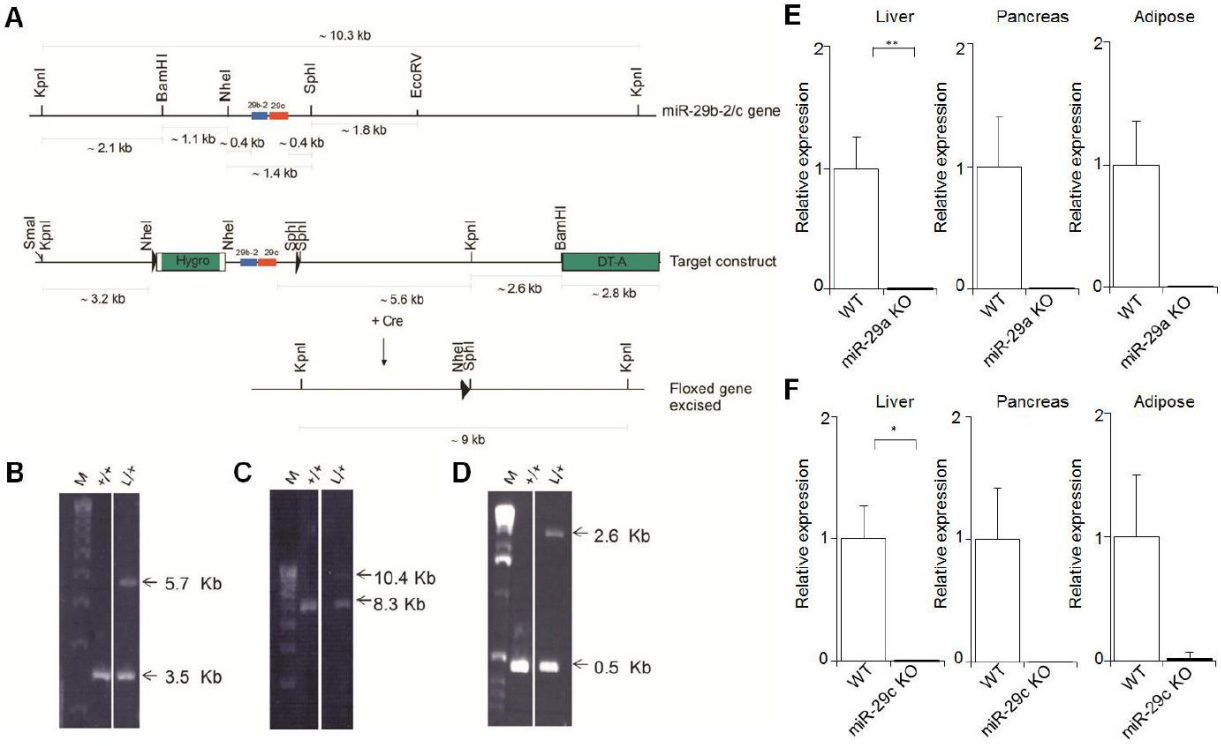
SUPPLEMENTARY DATA

Supplementary Figure S1. miR-29a regulates IGF-1 expression, but not that of SIRT1-4. (**A)** Whole pancreas, **(B)** liver and **(C)** white adipose tissue was taken from wildtype, *miR-29a*$^{-/-}$ mice and *miR-29c*$^{-/-}$ mice (n=5,4,3) and qPCR was performed for *Sirt1, Sirt2, Sirt3 and Sirt4* relative to *Rpl37a*. **(D)** Wildtype, *miR-29a*$^{-/-}$ and *miR-29c*$^{-/-}$ mice were fasted for six hours. Serum IGF-1 concentrations were measured on fasting serum (n=8,6,7). Median ± SEM, ** p<0.01.



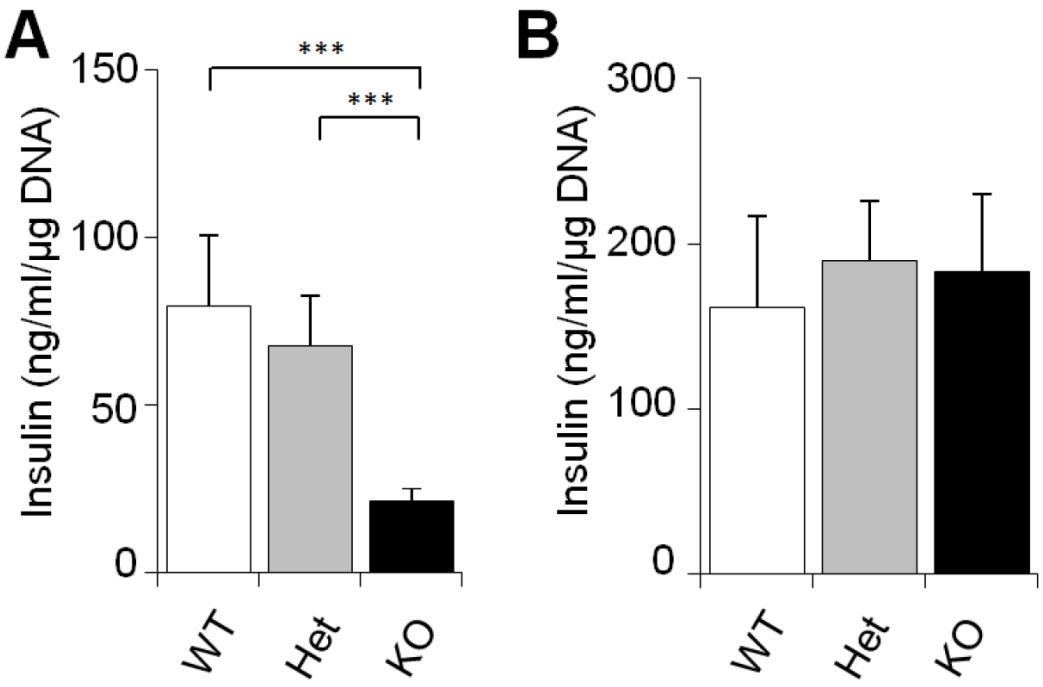
SUPPLEMENTARY DATA

Supplementary Figure S2. Generation of miR-29c floxed and knockout mice. (A) Schematic representation of the *miR-29b-2/c* cluster targeting construct. The wildtype gene is given in the top panel, the targeting construct in the middle panel and the somatic construct in the bottom panel. The precursors of miR-29b-2 and miR-29c are shown in blue and red boxes respectively, while the positive (Hygromycin B) and the negative (DT-A) cassettes are indicated as green boxes. The *frt* sites are depicted as white boxes, while the *loxP** sites as black arrows. Restriction sites used for the generation of the construct are shown. Positions of the probes used for screening of the correct clones via Southern Blotting are shown. **(B-D)** Homologous recombination of transfected ES cells by long PCR analysis for the **(B)** 5´arm, **(C)** 3´arm and **(D)** hygromycin cassette. Predicted sizes for the wildtype allele is 3.5kb, 8.3kb and 0.5kb, while predicted sizes for the floxed allele are 5.7kb, 10.4kb and 2.6kb, respectively. (**E)** Whole liver, pancreas, and white adipose tissue was taken from wildtype and *miR-29a*$^{-/-}$ mice (n=6,6) for qPCR of *miR-29a* relative to *Sno202*. **(F)** Whole liver, pancreas, and white adipose tissue was taken from wildtype and *miR-29c*$^{-/-}$ mice (n=6,5). qPCR was performed for *miR-29c* relative to *Sno202*. Median ± SEM, * p<0.05, ** p<0.01.



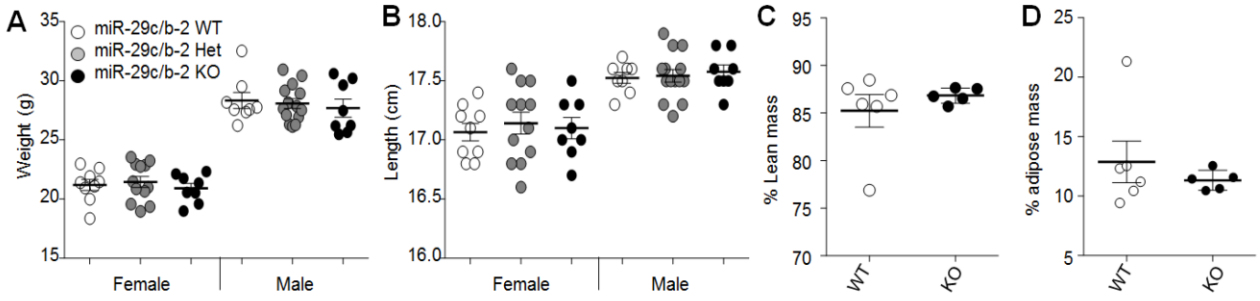
SUPPLEMENTARY DATA

Supplementary Figure S3. miR-29a is required for efficient insulin secretion *in vitro*. Insulin secretion from the islets of wildtype and *miR-29a*$^{-/-}$ mice after culturing in the presence of **(A)** 5mM and **(B)** 25mM glucose (n=4/group). Median ± SEM. *** p<0.001.



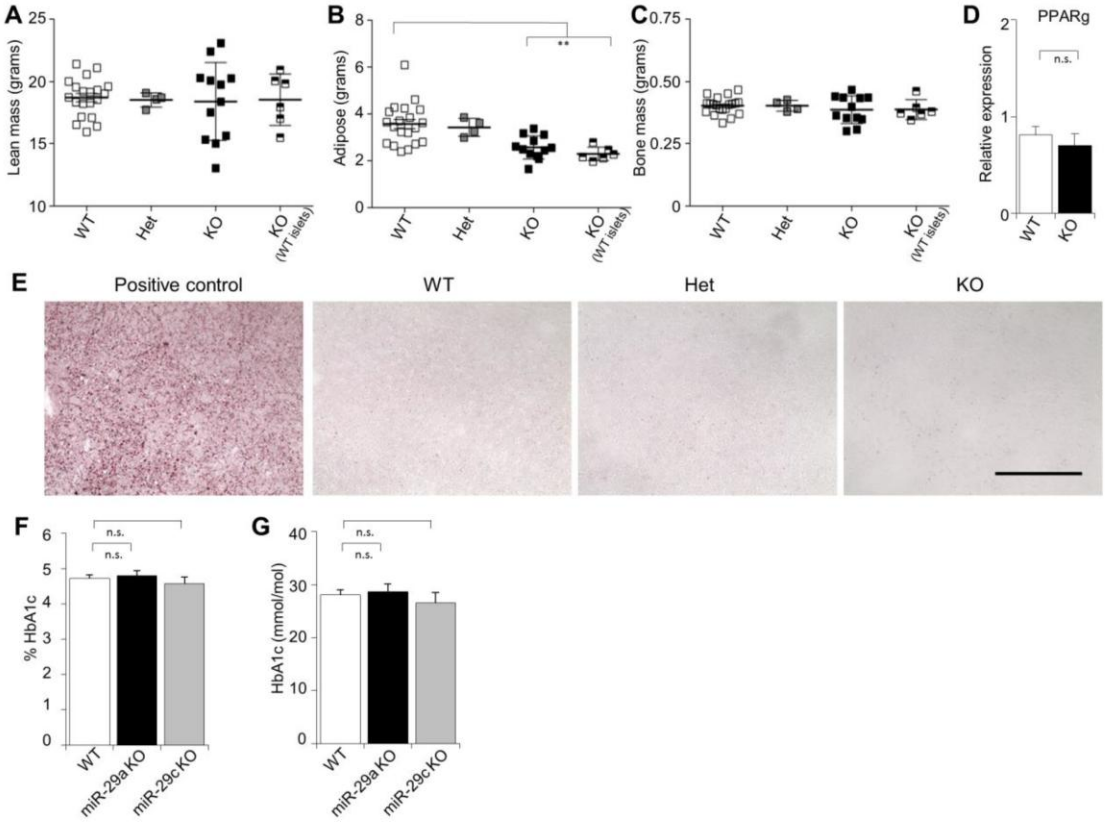
SUPPLEMENTARY DATA

Supplementary Figure S4. miR-29c is not required for adipose expansion. (A) Weights and (**B)** head-tail lengths of wildtype, *miR-29c*$^{+/-}$ and *miR-29c*$^{-/-}$ mice at 12 weeks of age, separated by sex (weights, n= 9,12,8 for female and n= 8,14,8 for male; length, n= 9,12,8 for female and n=8,14,8 for male). **(C)** Lean body mass and adipose tissue mass for wildtype and *miR-29c*$^{-/-}$ mice at 12 weeks of age, separated by sex (n= 5,5). Median ± SEM.



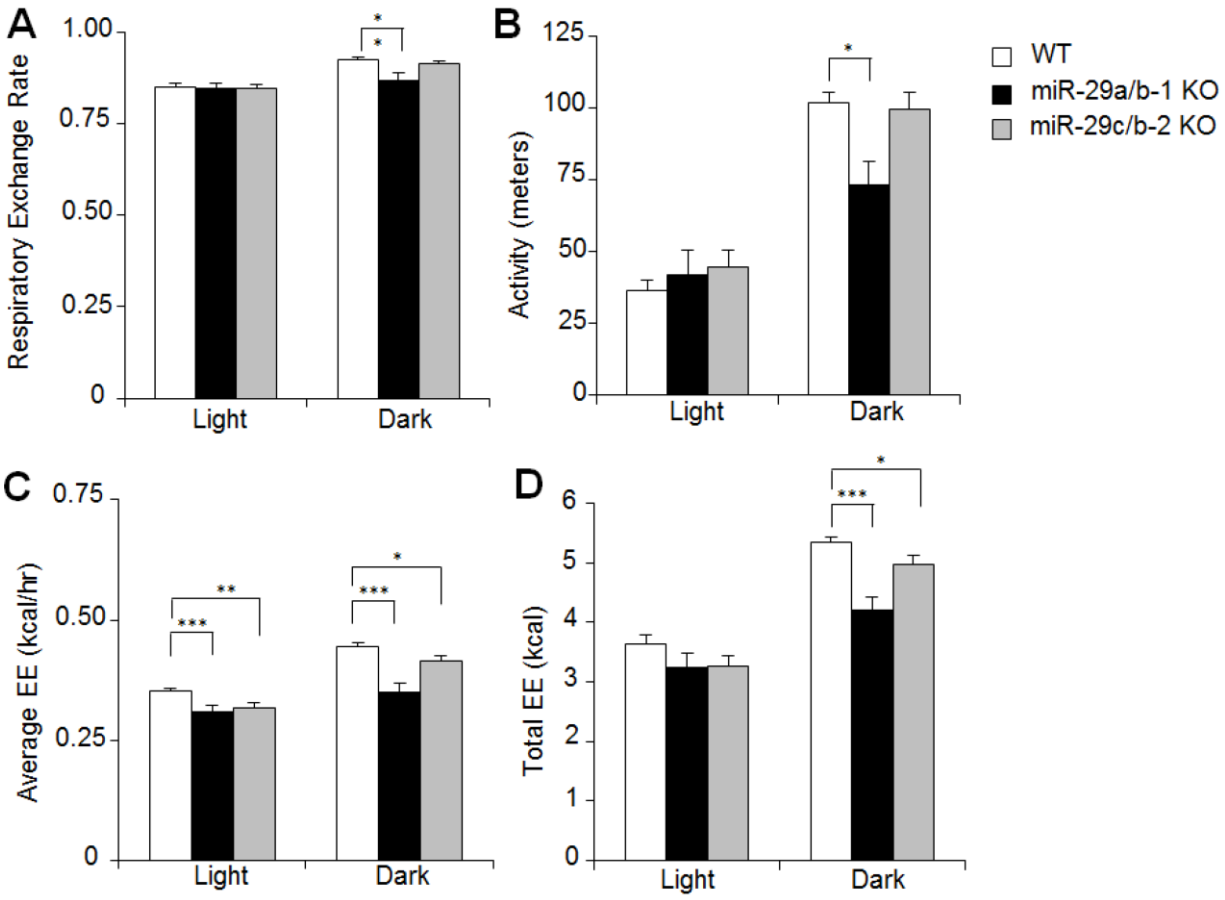
SUPPLEMENTARY DATA

Supplementary Figure S5. miR-29a-deficient mice have reduced adipose mass without lipid accumulation in the liver. Wildtype, *miR-29a*$^{+/-}$ and *miR-29a*$^{-/-}$ mice were measured at 10 weeks of age for the absolute mass of **(A)** lean tissue, (**B)** adipose tissue and **(C)** bone mass (n= 20,3,12,5). **(D)** cDNA was produced from the white adipose tissue of wildtype and *miR-29a*$^{-/-}$ mice and qPCR was performed for *Pparg* relative to *Rpl37a* (n=6,7). **(E)** The liver of *LepRDb/Db* mice (positive control for fatty liver), wildtype, *miR-29a*$^{+/-}$ and *miR-29a*$^{-/-}$ mice at 10 weeks of age was stained with Oil Red. Scale bar indicates 200µm. **(F)** Wildtype, *miR-29a*$^{+/-}$ and *miR-29a*$^{-/-}$ mice were measured at 10 weeks of age for HbA1c, expressed as %HbA1c or **(G)** mmol/mol (n=9,3,5). Median ± SEM. ** p<0.01.



SUPPLEMENTARY DATA

Supplementary Figure S6. Deficiency in miR-29 family members does not increase energy expenditure. Wildtype, *miR-29a*$^{-/-}$ and *miR-29c*$^{-/-}$ mice were measured at 10 weeks of age for **(A)** Respiratory Exchange Rate (RER), **(B)** activity (meters walked in cage), **(C)** average energy expenditure (EE), and **(D)** total EE (n= 8,4,8). Median ± SEM. * p<0.05, ** p<0.01, *** p<0.001.



SUPPLEMENTARY DATA

Supplementary Figure S7. miR-29-deficient mice have poorer insulin secretion after feeding on high fat diets. Wildtype, *miR-29a*$^{-/-}$ mice and *miR-29c*$^{-/-}$ mice on a high fat diet were fasted for six hours and challenged with exogenous insulin, prior to measurement of **(A)** blood glucose levels (n=5,5,4) and **(B)** serum insulin levels (n=5,5,4). Median ± SEM, * $p<0.05$, ** $p<0.001$.