Supplemental figures

Figure S1.

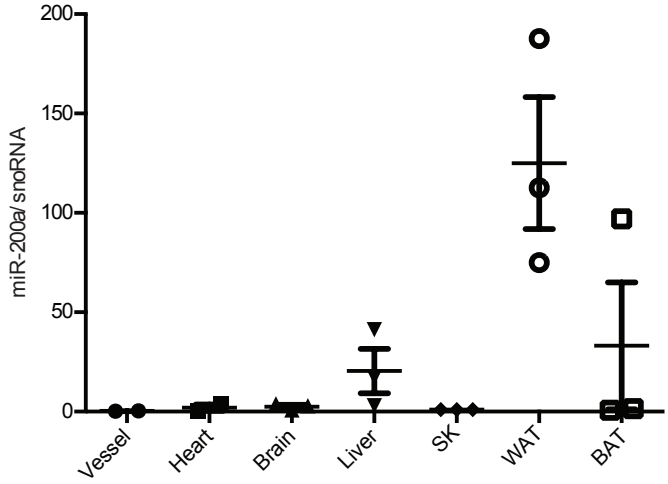

Figure. S1: Tissue expression pattern of miR-200a. Indicated issues were collected from C57/BL6 mice and mature miR-200a levels were measured by qRT-PCR. SK: skeletal muscle; WAT: white adipose tissue; BAT: brown adipose tissue. snoRNA 202 was used as the internal control. n=3. Data is represented as mean ± SEM.

Figure S2.

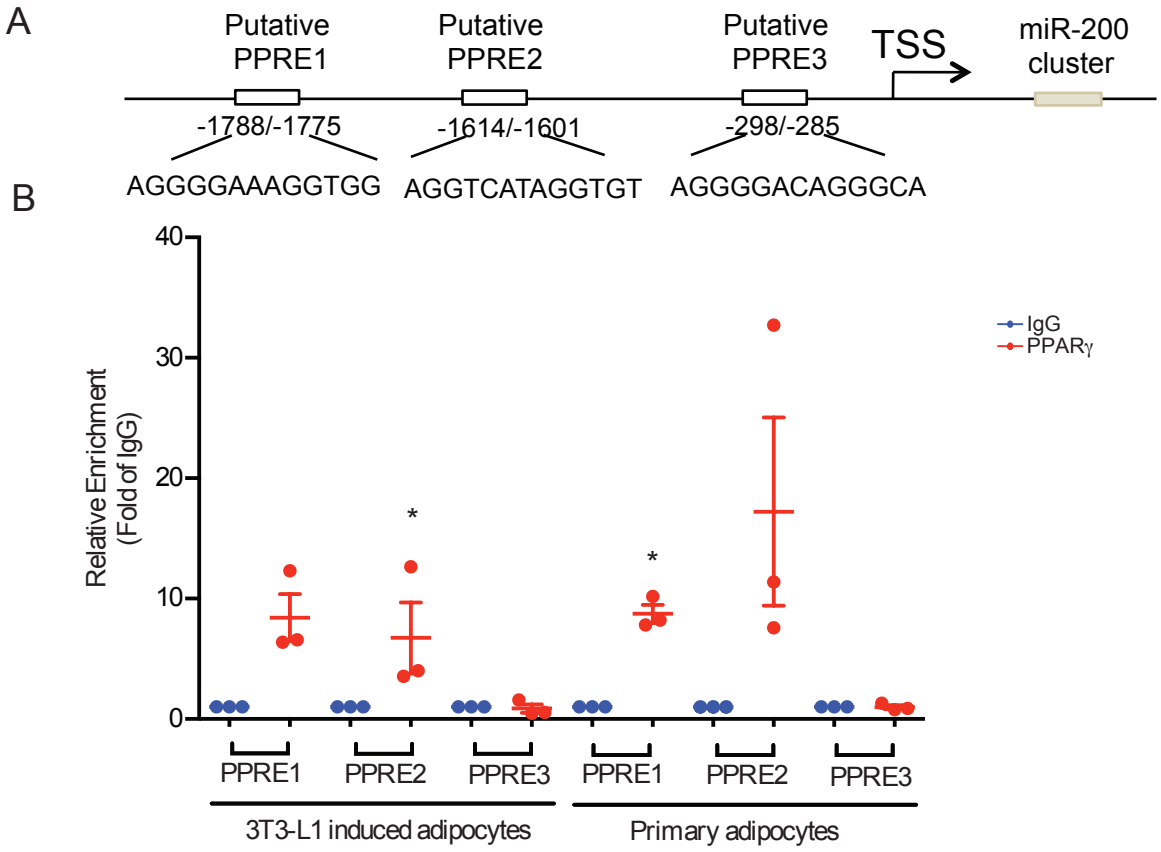

Figure. S2: PPARγ binds to the promoter of miR-200 cluster. (A) Bioinformatics revealed three putative conserved PPARγ responsive elements (PPREs) upstream of the transcription start site (TSS) of the miR-200 cluster. (B) 3T3-L1 induced adipocytes and primary adipocytes from C57/BL6 mice were cross-linked and immunoprecipitated with anti-PPARγ antibody or IgG. The immunoprecipitates were amplified with PCR using primers flanking the putative PPREs. Experiment was replicated three times. Data are represented as mean ± SEM; *, $p < 0.05$ according to 2-tailed Student's t-test.

Figure S3.

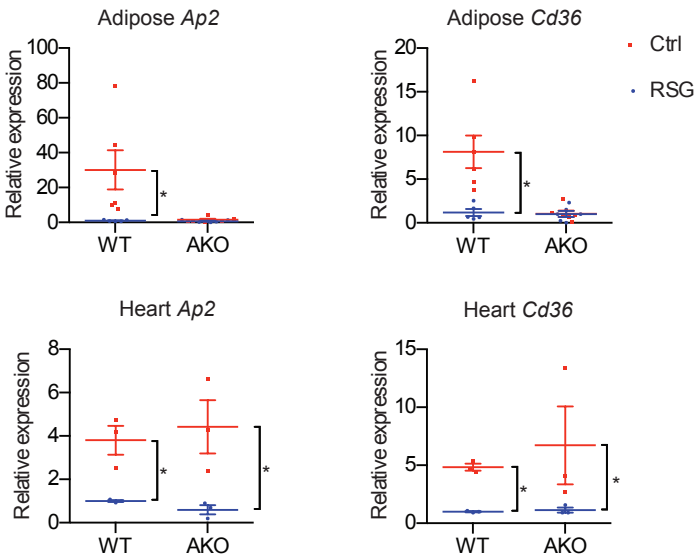

Figure. S3: The expression of PPARγ target genes in adipose and cardiac tissue. qRT-PCR detection of PPARγ target genes in adipose (A, B) and cardiac tissue (C, D) after 4 week RSG treatment of WT and adipose tissue-specific knockout (AKO) mice. n=3-5 mice per group. Data are represented as mean ± SEM; *, $p < 0.05$ according to one way ANOVA.

Supplemental Methods

ChIP assay

3T3-L1 induced adipocytes or primary adipocytes were cross-linked with 1% formaldehyde and quenched with 50 mM glycine-PBS before lysis and sonication. Sheared chromatin was immunoprecipitated with anti-PPARγ antibody (or control IgG) and protein A Dynabeads. The eluted immunoprecipitates were digested with proteinase K, DNA was extracted and underwent qPCR with primers specific for PPRE.

Supplemental Tables

Table S1. Antibodies used in this study.

Antibodies	Source	Catalogue Number
PPARγ	Santa cruz	sc-7196
GAPDH	Santa cruz	sc-32233
TSC1	Cell Signaling	4906
mTOR	Cell Signaling	2983
Phospho-mTOR (Ser2448)	Cell Signaling	5536

Table S1. Primers used in this study.

Name	Orientation	Sequence
Luciferase reporter cloning		
TSC1 3'UTR	Forward	GATGGTCAATCAGTGTTAACTTGC
TSC1 3'UTR	Reverse	GTTCACAGTTAAACACAAGCAACTG
ChIP Assay		
PPRE1	Forward	GGTTGAACAAAAACCAGGGT
PPRE1	Reverse	GGTCTGGTGCATATACACAA
PPRE2	Forward	AGTTATCCTTGTGGCTTCAG
PPRE2	Reverse	GGATCAGGCTCATCATTAGA
PPRE3	Forward	CACAGACACAAATACTGAGG
PPRE3	Reverse	GAGAACACCATTATGCCTTG
qRT-PCR		
pri-miR-200a	Forward	GTTCATGGCATCAGGTTTCC
pri-miR-200a	Reverse	GGGTCACCTTTGAACATCGT
AP2	Forward	GATGCCTTTGTGGGAACCT
AP2	Reverse	CTGTCGTCTGCGGTGATTT
CD36	Forward	ATGGGCTGTGATCGGAACTG
CD36	Reverse	TTTGCCACGTCATCTGGGTTT-
ANP	Forward	GATAGATGAAGGCAGGAAGCCGC

ANP	Reverse	AGGATTGGAGCCCAGAGTGGACTAGG
BNP	Forward	TGTTTCTGCTTTTCCTTTATCTGTC
BNP	Reverse	CTCCGACTTTTCTCTTATCAGCTC
GAPDH	Forward	CTCAAGATTGTCAGCAATGCATCC
GAPDH	Reverse	CCAGTGGATGCAGGGATGATGTTC