Supplementary information, Data S1

Materials and Methods

Northern blot analysis

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA quality was assessed by 1% agarose gel electrophoresis in the presence of ethidium bromide. Northern blot analysis was performed as described using 20 μg total RNA from each sample. Probes were synthesized in Invitrogen Biotechnology Co, Ltd (Beijing, China) as following: miR-23a, 5'-GGAAATCCCTGGCAATGTGAT-3'; miR-23b, 5'-GGTAATCCCTGGCAATGTGAT-3'; miR-27a, 5'-GCGGAACTTAGCCACTGTGAA -3'; miR-27b, 5'-GCAGAACTTAGCCACTGTGAA-3'. A U6 probe, 5'-CGAATTTGCGT GTCATCCTTGCG-3', was served as a loading control. Probes were labeled with ^{32}P γ-ATP using T4 polynucleotide kinase (New England Biolabs, Inc., Ipswich, MA, USA).

Generation of adenovirus expressing the antisense miR-27b

Six tandems of sequences complementary to mouse miR-27b were ligated into pAd-track-CMV cut by XhoI and XbaI, and then recombined with pAd-easy vector in BJ5183 competent cells to get the pAd-anti-miR-27b vector. The linearized vector was transfected into 293A cells to generate the adenovirus expressing the antisense miR-27b (Ad-anti-miR-27b).

Generation of miR-24-1 and miR-27b expression plasmids

miR-24-1 was amplified from mouse genomic DNA using the following primer pairs: 5'-TTTGGATTCGTTCTTTGTCCCTGCTGGGCGTGGT-3' and 5'-TTTGTCGACCAA CACTGAAGCCACACCTCACAAT-3'. miR-27b was amplified using primers 5'-TTT GGATTCTCACATTGCCAGGGATTACCAC-3' and 5'-TTTGTCGACTCAGCACGCTG TTTGCACTCTT-3'. The PCR products were then cut with BamHI and SalI, and ligated into

pIRES2 to generate the miR-24-1 and miR-27b expression plasmids pIRES2-miR-24 and pIRES2-miR-27b.

Luciferase assay

For promoter assay, a 2941-bp genomic fragment upstream of the transcriptional start site of miR-27b precursor (a region between (-2863) ~ (+78) bp), was amplified from mouse genomic DNA using the following primer pairs: 5'-TTTACGCGTGTTGCTGGGATTTGAACTCTGGACC-3' and 5'-ACTACTCGAGG AGCCTACTTGCCGTGCGAGCCTAC-3'. The PCR product was cloned into pGL3-basic (Promega, Madison, WI, USA) to generate pGL3-27b. The construct (pGL3-27b-mut) containing miR-27b promoter without Smad-binding site (CAGACAT) was also constructed.

For target assay, we performed luciferase reporter experiments in the HEK293 cells. A 230 bp 3'-UTR fragment of PPAR-γ was amplified using primers 5'-TTAGATCTAGGACTTGTATTAGCAGGAA-3' and 5'-TTACGCGTAATTTTATAATGT GGTAATT-3', and cloned into pGL3-CM luciferase reporter vector (Ambion, Inc.). The construct (Luc-PPAR-γ mutant) containing a mutated PPAR-γ-UTR (ACUGUGA was mutated to AGAAUUC) was also constructed. For luciferase assay, reporter plasmids were co-transfected using lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). The phRG-TK vector (Promega, Madison, WI, USA) that expresses a synthetic renilla luciferase was used for normalizing transfection efficiency. After transfection, luciferase activities were measured with a dual luciferase reporter assay kit (invitrogen E1910) using LB 960 Centro XS3 luminometer (Berthold Technologies, GmbH & Co. KG, Germany).

Isolation and cell culture of primary cardiomyocytes

Hearts from 1-2 days old rats were aseptically removed. Their ventricles were dissected, minced and trypsinized overnight at 4 °C. The next day, cells were dissociated with collagenase and pre-plated for 2 h at 37 °C. The non-adherent cardiomyocytes were removed

and plated in 24-well plates in DMEM/F-12 medium containing 10% FBS and 0.1 mM bromodeoxyuridine (Sigma). 1×10^5 cells/cm^2 were seeded in 24-well plate for further experiments. This procedure yielded cultures with 90 ~ 95% cardiomyocytes, as assessed by microscopic observation of cell beating.

Real-time RT- PCR

Reverse transcription PCR was performed using the mRNA selective PCR kit (DRR025A, TaKaRa, Dalian, China). Briefly, 2 μg total RNA was reversely transcribed by AMV reverse transcriptase XL. Real-time PCR was performed using the LightCycler system (Roche Ltd, Switzerland) with the FastStart DNA Master SYBR Green. Experiments were repeated at least three times. The following primers were used:

GAPDH

Sense: 5’-TGCCCAGAACATCATCCCT-3’

Antisense: 5’-GGTCCTCAGTGTAGCCCAAG-3’

ANF

Sense: 5’-GCCGGTAGAAGATGAGGTCA-3’

Antisense: 5’-GGGCTCCAATCCTGTCAATC-3’

SKA

Sense: 5’-GGCTCCCAGCACCATGAAGA-3’

Antisense: 5’-CAGCACGATTGTCGATTGTCG-3’

β-MHC

Sense: 5’-GTGAAGGGCATGAGGAAGAGT-3’

Antisense: 5’-AGGCCTTCACCTTCAGCTGC-3’

U6

Sense: 5'- TCGCTTCGGCAGCACATATACTA -3'

Antisense: 5'- TAGTATATGTGCTGCCGAAGCGA -3'

Pre-miR-27b

Sense: 5’-GTGCAGAGCTTAGCTGATTGG -3’

Antisense: 5’-CACTGTGAACAAAGCGGAAAC -3’

pri-miR-23b

Sense: 5’-GAAGGACAGCAGGCTGCACTGC-3’

Antisense: 5’-AAATCAGCATGCCAGGAACCCAAGC-3’

pri-miR-24-1

Sense: 5’-ATTTGCAGTCCAGGTCTCCATG-3’

Antisense: 5’-CTGAGCCAGTGTGTGAAATGAGAAC-3’

pre-miR-23b

Sense: 5’-TGGTGCTGCCCTCACCTGCTCT-3’

Antisense: 5’- GGTTCTGCAGGAGCAGCCAAGG-3’

pre-miR-24-1

Sense: 5’- GCCCAGCTGTGATTGGACCCGC -3’

Antisense: 5’- TGCTCAAGGGCTCGACTCCTGT-3’

miRNA real-time PCR

PCR was performed with miR-specific primers from the TaqMan miR assays (Applied Biosystems) in the the LightCycler system (Roche Ltd, Switzerland) according to the manufacturer’s protocol. Three biological replicates were used for analysis and all reactions were run in triplicates.

Cell immunostaining

Immunostaining was carried out mainly as described. Briefly, cardiomyocytes were fixed with 4% formaldehyde for 30 min at 4 °C and then treated with 0.5% Triton-X 100 in PBS for 5 min at room temperature. After that, cells were incubated with primary antibody against α-Actinin (Sigma) and Ki67 (Cell Signal Technology, Inc.) at 4 °C overnight, followed by

incubation with fluorescence-conjugated secondary antibody (1:100, Zhongshan, Beijing, China). The slides were stained with DAPI to visualize the nuclei. Photo capture was performed using a Nikon laser microscope (Eclipse E600, Nikon Instruments Inc, Japan). For each sample, more than five fields covering the whole slide were picked and positive cells and total cells were counted.

BrdU labeling

For BrdU labeling, mice at P5 were injected with BrdU (100 mg/g body weight). Ventricles were isolated 2 h later. BrdU-positive nuclei were detected using a monoclonal anti-BrdU antibody (Santa Cruz), followed by horseradish peroxidase (HRP)-conjugated secondary antibody. Sections were counterstained with hematoxylin. The proliferative index of cardiomyocytes was determined as the number of BrdU-positive nuclei reported to the total number of nuclei within a section. Proliferative index was determined for 3 mice per genotype.

Histology and immunohistology

Heart tissues were fixed in 4% PFA at 4 °C overnight, embedded in paraffin and sectioned at 5 μm. Sections were stained with hematoxylin and eosin (H&E), Masson trichrome (to examine interstitial fibrosis), laminin antibody (Boster Biological Technology) as described [6].

Generation of Ad-si-PPAR-γ

Oligonucleotides encoding shRNA against PPAR-γ mRNA were as following (bold sequence represented the shRNA target sequence): Si-PPAR-γ-s: 5’-GATCCCC**GTTTGAGTTTGCTGTGAAG**TTCAAGAGA**CTTCACAGCAAACTCA AA**CTTTTTGGAAG -3’; Si-PPAR-γ-a: 5’-TCGACTTCCAAAAA**GTTTGAGTTTGCTG TGAAG**TCTCTTGAA**CTTCACAGCAAAC**TCAAACGGG-3’. Oligonucleotides encoding

both strands of the targeting sequence were annealed and inserted into pSuperior.puro vector which contains H1 promoter. The cassette containing the H1 promoter plus the shRNA was transferred to adenoviral vector to generate the Ad-si-PPAR-γ.

Cloning and expression of PPAR-γ

PPAR-γ DNA was PCR-amplified from cDNA fragments of mouse hearts and inserted into the adenoviral vectors. The primers were as following:

PPAR-γ sense: 5'-TTTGTCGACATGACCATGGTTGACACAGA-3'

PPAR-γ antisense: 5'-TTTTCTAGACTAGTACAAGTCCTTGTAGA-3'

miR-27b antagomir

Chemically modified antagomir complementary to miR-27b were used to inhibit its expression. The antagomir sequence is 5'-GCAGAACUUAGCCACUGUGAA-3'. All of the bases were 2'-OMe modified. Antagomir oligonucleotides were synthesized and purified with high-performance liquid chromatography by GenePharma Co., Ltd.