Online Supplemental Material

Materials and Methods

Primary Cardiomyocyte Cultures

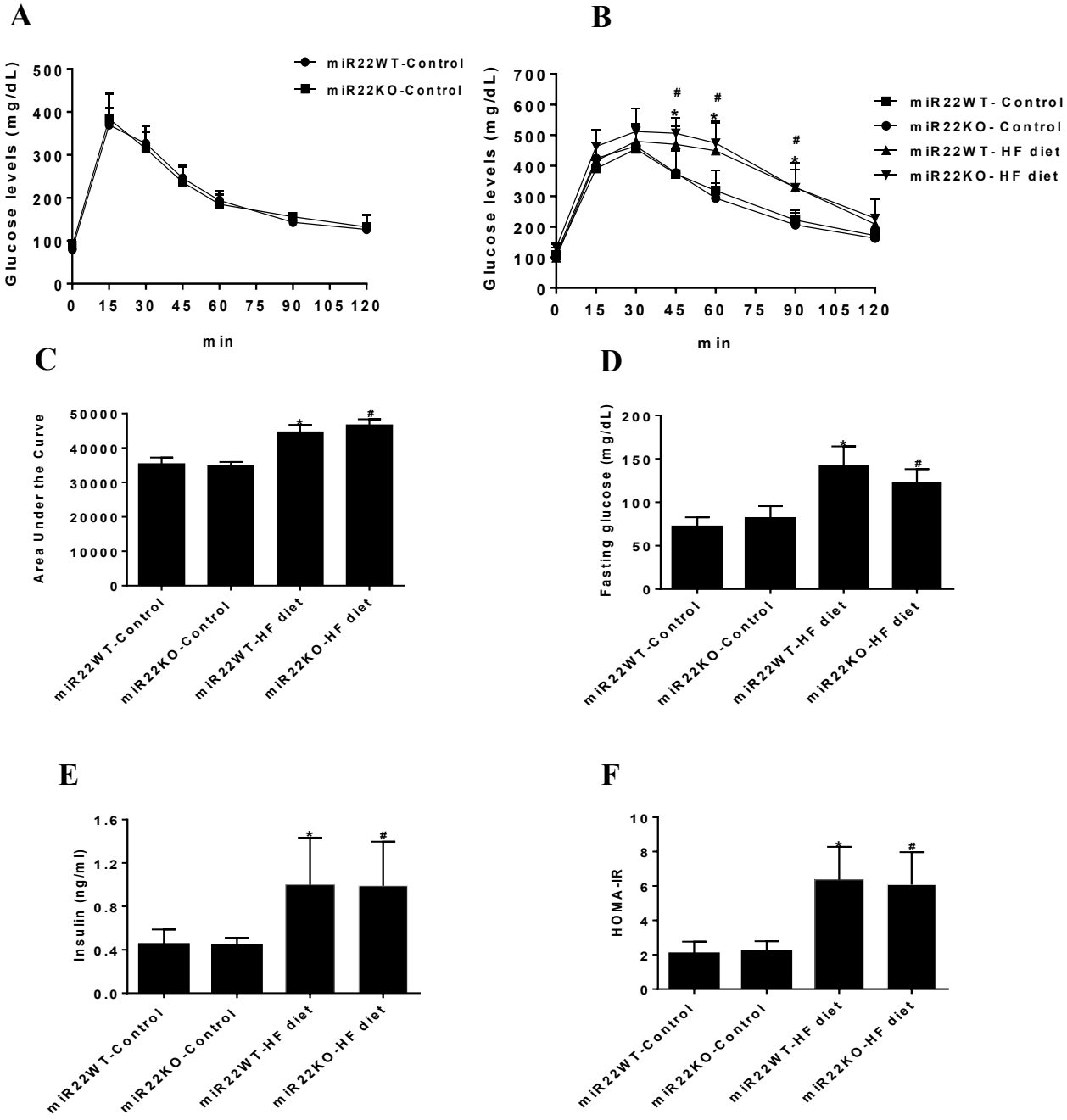
Neonatal rat cardiomyocyte cultures were prepared as previously described (Huang et al., Circ Res 2013;112:1234-43). Briefly, cardiomyocytes were isolated by enzymatic dissociation of one day old neonatal rat hearts using the Neonatal Cardiomyocyte Isolation kit (Cellutron, Life Technologies). Dissociated cells were plated for 2 hours to remove fibroblasts. The unattached cells were transfered to precoated plates containing medium supplemented with serum. Twenty-four hours after plating, cells were submitted to serum-free medium overnight. Cardiomyocyte cultures were transfected with 50 nM of miR-22 mimic duplex, miR-22 hairpin inhibitor and control oligonucleotide (Life Technologies) using Lipofectamine RNAiMAX transfection reagent. Six hours later, cells were treated with 50ng/ml of leptin (Sigma), which is a concentration sufficient to induce cardiomyocyte hypertrophy (Guedes et al., 2016). Cells were harvested 24 hours after leptin treatment for RNA isolation or 48 hours later for immunochemistry analyses.

Quantitative analysis of DNA by real-time PCR

Analysis of mitochondrial content in the heart and liver was performed using qPCR as previously described (Disatnik et al., J Exp Med. 2016;213:2655-2669). DNA isolation was performed using Proteinase K method. 0.625ng of DNA were used as templates for real-time PCR analysis. To detect nuclear DNA in the samples, we used GAPDH as a housekeeping gene. For assessment of mitochondrial DNA (mtDNA), we used NADH dehydrogenase 1 gene. PCR was performed using Sybr Select Master Mix (Applied Biosystems). Relative change in mtDNA content was calculated using the $2^{-\Delta\Delta CT}$ method.

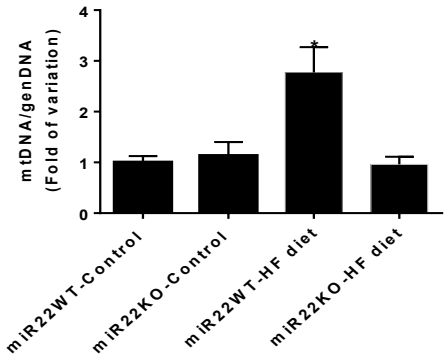
Supplemental Data

Supplemental Figure 1



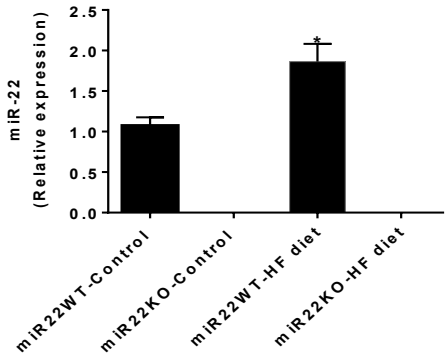
Supplemental Figure 1. (A) Intraperitoneal glucose tolerance test (iGTT) in miR-22WT and miR-22KO mice at 5 weeks of age fed control diet, before the treatment with different diets (n=8-10). (B) iGTT and; (C) area under the curve in miR22-WT and miR22-KO mice after 12 weeks of control diet and HF diet (n=6-7). (D) Fasting levels of glucose and; (E) insulin; (F) as well as HOMA-IR in miR22-WT and miR22-KO mice fed control diet and HF diet (n=5-7).

Supplemental Figure 2



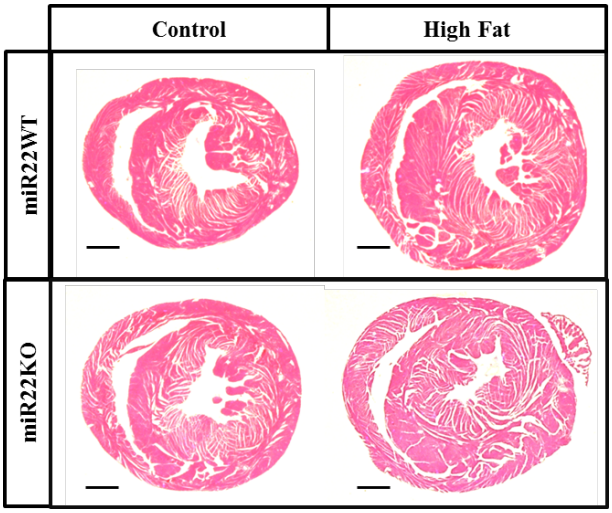
Supplemental Figure 2. Analysis of mitochondrial content in the liver of miR22-WT and miR22-KO mice fed control diet and HF diet, evaluated by qPCR (n=5-6). * vs miR22WT-control ($P<0.05$).

Supplemental Figure 3



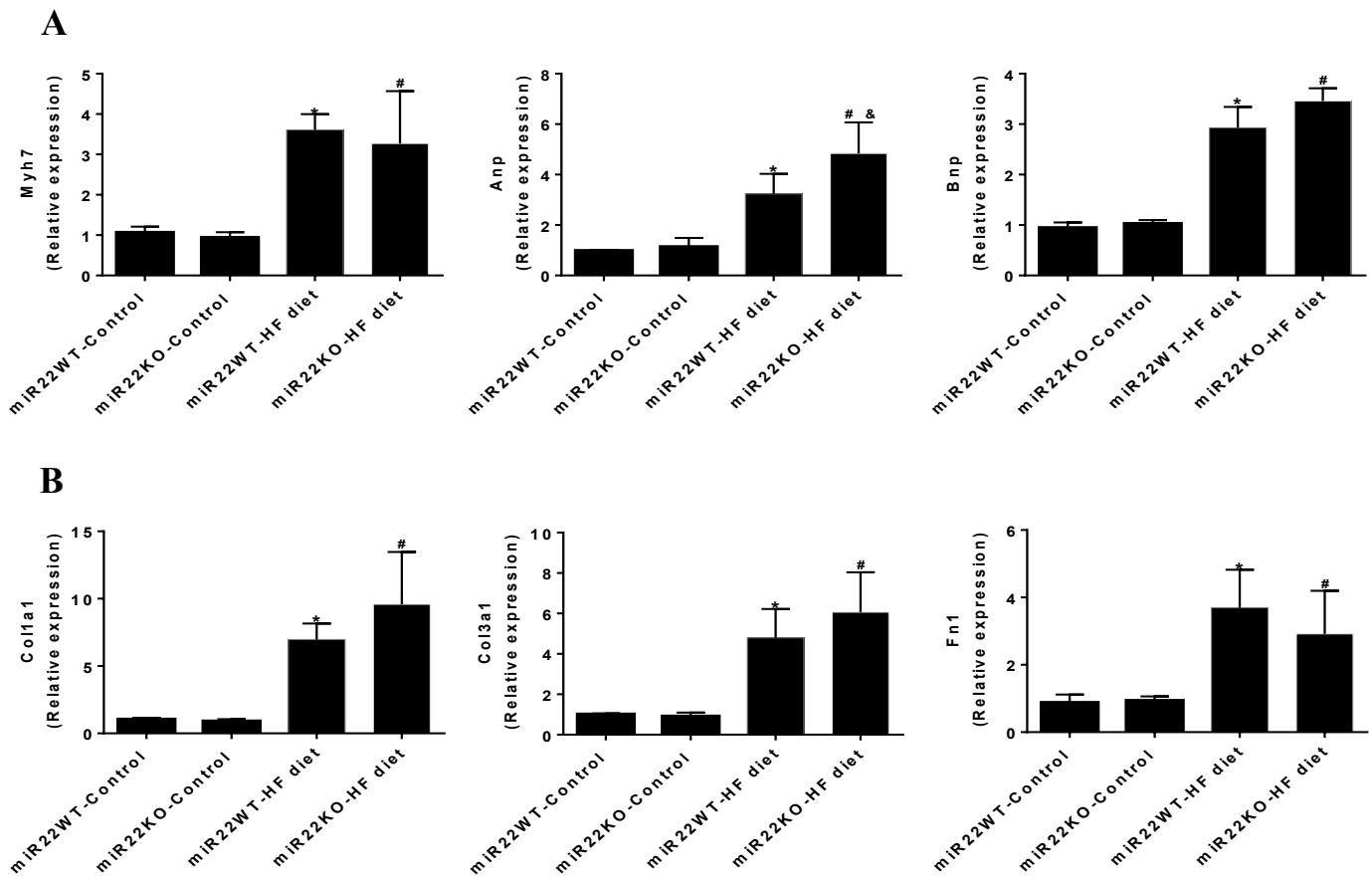
Supplemental Figure 3. miR-22 expression in the heart of miR22-WT and miR22-KO mice fed control diet and HF diet for 12 weeks, evaluated by qPCR (n=4-5). * vs miR22WT-control ($P<0.05$).

Supplemental Figure 4



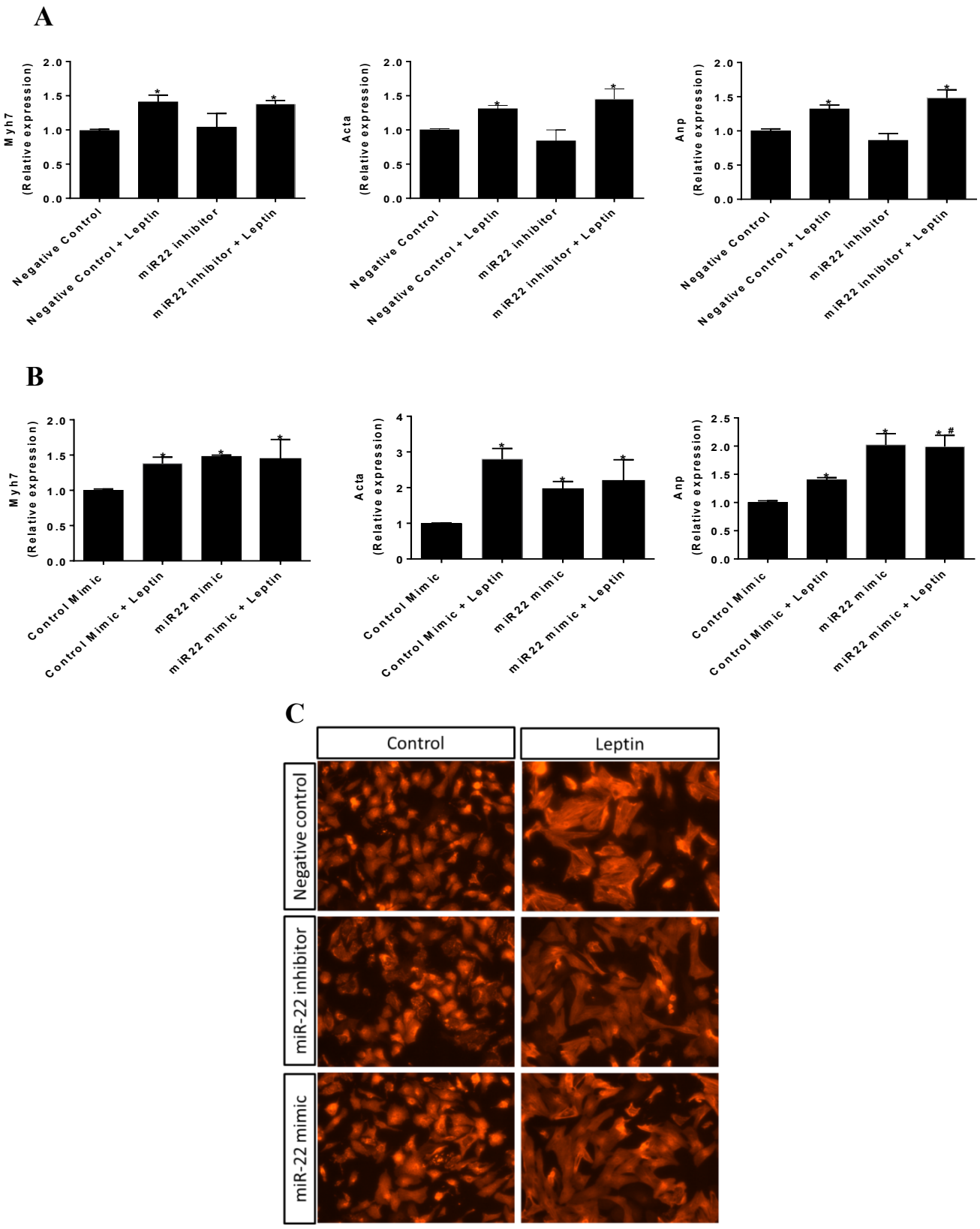
Supplemental Figure 4. Analysis of heart morphology in miR22-WT and miR22-KO mice fed control diet and HF diet for 12 weeks. Heart transverse sections were stained with Hematoxylin and eosin. Bars, 1 mm.

Supplemental Figure 5



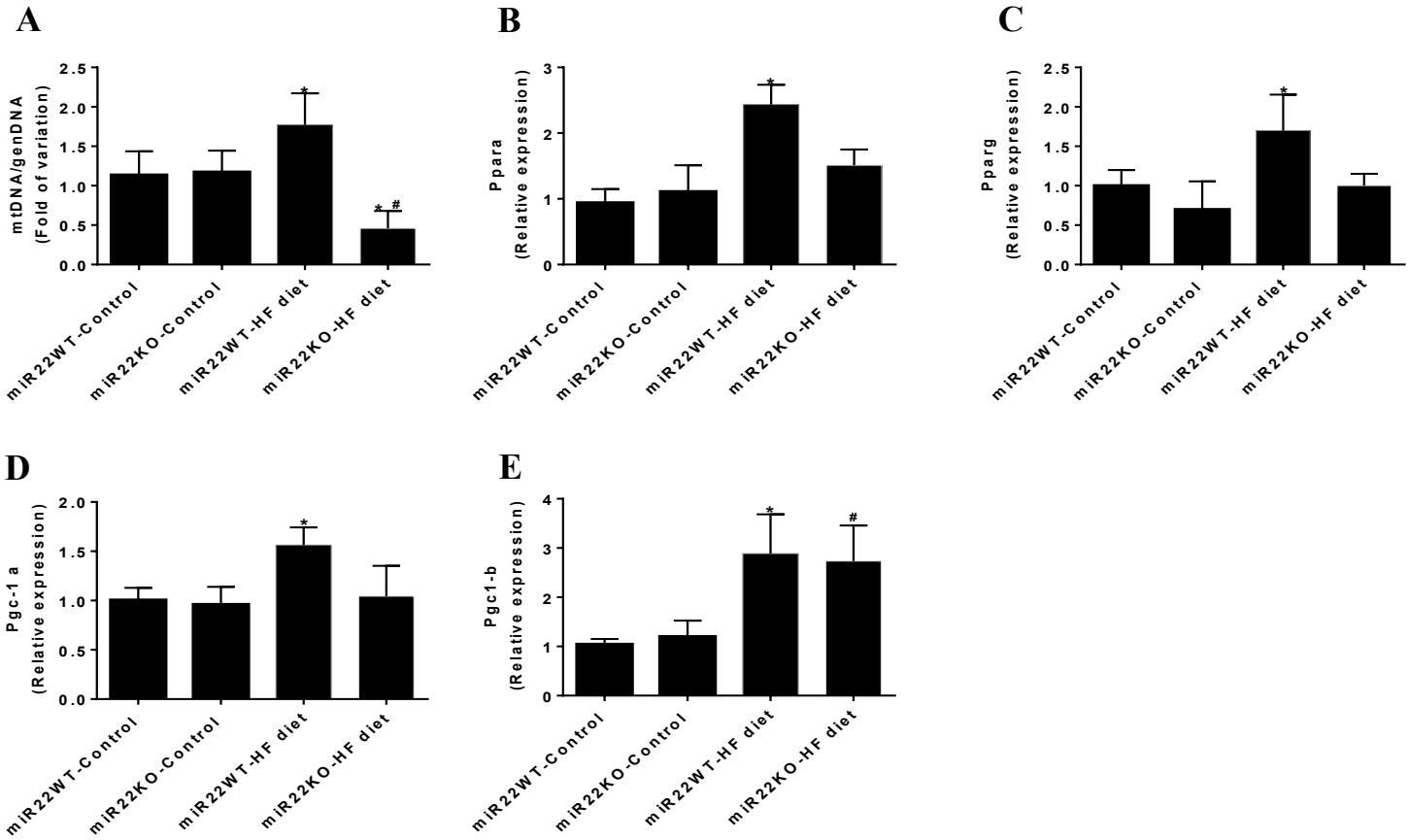
Supplemental Figure 5. (A) Analysis of mRNA levels of cardiac hypertrophy markers (Myh7, Anp and Bnp) and (B) fibrosis markers (Col1a, Col3a and Fn1) in the hearts of miR22-WT and miR22-KO mice fed control diet and HF diet, evaluated by qPCR (n=4-5).

Supplemental Figure 6



Supplemental Figure 6. (A) Analysis of the role of miR-22 in leptin-induced cardiomyocyte hypertrophy. For loss of function of miR-22, cardiomyocytes were treated with leptin (50ng/ml) combined with negative control (50nM) or miR-22 inhibitor (50nM) for 24 hours. (B) For gain of function of miR-22, cardiomyocytes were treated with leptin (50ng/ml) combined with control mimic (50nM) or miR-22 mimic (50nM) for 24 hours. Analysis of gene expression of Myh7, Acta and Anp was evaluated by qPCR (n=3). * vs respective controls ($P<0.05$); # vs control mimic + leptin ($P<0.05$). (C) Some cardiomyocytes cultures were treated for 48 hours to perform immunochemistry for α-actinin.

Supplemental Figure 7



Supplemental Figure 7. (A) Analysis of mitochondrial content in the heart of miR22-WT and miR22-KO mice fed control diet and HF diet, evaluated by qPCR (n=5-6). (B-E) Analysis of gene expression of Ppara, Pparg, Pgc-1α and Pgc-1β in the heart of miR22-WT and miR22-KO mice fed control diet and HF diet (n=5-6). * vs miR22WT-control ($P<0.05$); # vs miR22KO-control ($P<0.05$).

Supplemental Table 1

	miR22WT-Control (n=7)	miR22KO-Control (n=7)	miR22WT-HF diet (n=7)	miR22KO-HF diet (n=7)
Body weight (g)	27.33±3.44	25.20±1.92	39.25±2.49	39.61±5.19
Liver (g)	1.03±0.16	1.11±0.16	1.16±0.11 *	1.13±0.12 #
Epididymal fat (g)	0.39±0.10	0.46±0.26	2.35±0.27 *	1.68±0.37 #&
Retroperitoneal fat (g)	0.33±0.19	0.27±0.09	0.75±0.12 *	0.55±0.18 #
Lungs (mg)	196.27±39.13	195.03±28.27	207.32±37.59	200.50±37.10
Heart (mg)	111.23±17.89	119.38±8.71	161.48±18.55 *	157.92±15.22 #

Supplemental Table 1. Morphometric parameters of miR22-WT and miR22-KO mice fed control diet and HF diet for 12 weeks. * miR22WT-control ($P<0.05$); # vs miR22KO-control ($P<0.05$); & vs miR22WT-HF diet ($P<0.05$).

Supplemental Table 2

	miR22WT-Control (n=5)	miR22KO-Control (n=5)	miR22WT-HF diet (n=7)	miR22KO-HF diet (n=7)
IVS;d (mm)	0.52±0.11	0.55±0.07	0.58±0.07	0.64±0.08
IVS;s (mm)	0.68±0.10	0.75±0.09	0.67±0.06	0.71±0.19
LVID;d (mm)	2.37±0.69	2.73±0.20	2.37±0.54	2.77±0.39
LVID;s (mm)	1.02±0.30	1.31±0.33	1.19±0.56	1.44±0.43
LVPW;d (mm)	0.65±0.02	0.64±0.04	0.78±0.05 *	0.83±0.10 #
LVPW;s (mm)	0.76±0.15	0.80±0.18	0.82±0.15	0.97±0.17
EF (%)	87.62±4.46	83.83±8.71	83.08±11.02	78.94±10.49
FS (%)	55.90±7.73	52.17±9.97	51.45±10.97	48.31±10.17
LV mass (mg)	36.74±20.88	46.24±6.98	38.22±14.94	59.19±16.80
LV mass (Corrected, mg)	29.39±16.71	36.99±5.59	30.57±11.95	47.35±13.44
LV vol;d (ul)	21.95±14.39	28.10±5.06	21.17±12.87	29.64±10.77
LV vol;s (ul)	2.54±1.94	4.69±3.31	2.59±2.31	4.61±2.17
Heart rate (bpm)	664.26±33.55	654.30±23.54	664.22±78.54	634.08±73.30

Supplemental Table 2. Echocardiographic analyses of miR22-WT and miR22-KO mice fed control diet and HF diet for 12 weeks. End-diastolic interventricular septum (IVS;d), end-systolic interventricular septum (IVS;s), end-diastolic left ventricular (LV) internal diameter (LVID;d), end-systolic LV internal diameter (LVID;s), LV end-diastolic posterior wall thickness (LVPW;d); LV end-systolic posterior wall thickness (LVPW;s); ejection fraction (EF), fractional shortening (FS), LV end-diastolic volume (LV vol;d), LV end-systolic volume (LV vol;s). * miR22WT-control ($P<0.05$); # vs miR22KO-control ($P<0.05$).