# SUPPLEMENTAL INFORMATION

## Supplemental Figures

**Figure S1. AntimiR-208a Treatment Effect on Cardiac Function, Lipid Accumulation, Food Intake, Activity and Cardiac Gene Expression, Related to Figure 1.**

(A) Heart weight from antimiR-208a and control antimiR treated mice on NC or HF for 6 weeks, n=5-13

(B) Fractional shortening for antimiR-208a and control antimiR treated mice on NC for 6 weeks, n=5

(C) Heart rate in beats per minute for antimiR-208a and control antimiR treated mice on NC for 6 weeks, n=5

(D) Pictures of visceral WAT and liver from antimiR-208a and control antimiR treated mice on NC for 6 weeks

(E) Food consumption n=11

(F) Physical activity, average beam breaks in the X,Y and Z axis over a 12 hour light/dark cycle n=11

(G) Cardiac Med13 mRNA levels from antimiR-208a or control antimiR treated mice on NC. n=5

(H) Altered cardiac metabolic gene expression from antimiR-208a or control antimiR treated mice on NC. n=5

Data is represented as mean ± SEM $*p<0.05$

**Figure S2. Characterization of αMHC-Med13 TG Mice, Related to Figure 2.**

(A) Multi-tissue analysis comparing Med13 mRNA levels from αMHC-Med13 TG mice (Line 1) compared to WT mice by quantitative RT-PCR.

(B) Representative immunoblot of Med13 from WT and αMHC-Med13 TG hearts. Anti-tubulin was used as loading control.

(C) H&E stain from αMHC-Med13 TG and WT mouse hearts.

(D) BNP and ANF levels in αMHC-Med13 TG and WT mice on NC

(E) Nose-to-anus length for 12 week old αMHC-Med13 TG and WT mice on NC.

(F) Male 6 week old αMHC-Med13 TG (Line 2, over-expressing Med13 3-fold in the heart)) and WT mice growth curves and percent change in body weight on HF, n=9-14

(G) Female 6 week old αMHC-Med13 TG mice (Line 1) and WT growth curves (left) and percent change in bodyweight on HF and NC for 16 weeks, n=7 or NC, n=3

(H) Body composition analysis of αMHC-Med13 TG (Line 1) and WT mice at 7 and 12 months of age. n=3-5

(I) Oil red O stained hearts and liver from high fat treated αMHC-Med13 TG (Line 1) and WT mice. Scale=40μM

Data are represented as mean ± SEM. * $p<0.05$ αMHC-Med13 TG vs WT

**S3. Core Body Temperature is Comparable in αMHC-Med13 TG and WT mice, Related to Figure 3.**

Core body temperature of αMHC-Med13 TG (Line 1) and WT mice measured at room temperature. (n=5)

**S4. Analysis of heart, WAT, BAT and liver microarray data comparing αMHC-Med13 TG and WT mice, Related to Figure 4.**

Tissue and RNA was isolated from 4 week old mice and analyzed by microarray. Genes that were altered greater than two-fold up or down in the array were further analyzed using Panther software and are displayed as the fraction of changes in biological processes.

**Figure S5. Characterization of Med13$^{fl/fl}$; αMHC-Cre cKO, Related to Figure 6.**

(A) Schematic diagram of putative Med13 protein domains, the arrow represents the end of the Med13 resulting from targeted gene deletion. Arms of homology flanking exons 7 and 8 were used to insert loxP sites, a neomycin cassette and DTA for homologous recombination. Cre-mediated recombination of the Med13 cKO removes exons 7 and 8.

(B) PCR from fl/+, +/- and +/+ Med13 genotypes.

(C) Med13$^{fl/fl}$; αMHC-Cre conditional knockout offspring chart

(D) Expression of Med13 mRNA in heart, n=3.

(E) H&E stain for 6 week old Med13$^{fl/fl}$; αMHC-Cre and Med13$^{fl/fl}$ hearts.

(F) Representative traces of an echocardiograph from $Med13^{fl/fl}$; $\alpha$MHC-Cre and $Med13^{fl/fl}$.

(G) Cardiac fractional shortening from $Med13^{fl/fl}$; $\alpha$MHC-Cre and $Med13^{fl/fl}$ mice. (n=5-7)

(H) Heart rate from $Med13^{fl/fl}$; $\alpha$MHC-Cre and $Med13^{fl/fl}$ mice. (n=5-7)

(I-J) Diastolic and systolic internal ventricular diameter from $Med13^{fl/fl}$; $\alpha$MHC-Cre and $Med13^{fl/fl}$ mice. (n=5-7)

(K) $Med13^{fl/fl}$ and $Med13^{fl/fl}$; $\alpha$MHC-cre cKO images displaying excessive WAT and fatty liver in mice after 12 weeks on HF diet.

Data are represented as mean ± SEM. * $p<0.05$

## Experimental Procedures

### Western Blot Analysis

Hearts were isolated, homogenized then and analyzed by Western blot using anti-Thrap1 (Med13) A301-278A-1 (Bethyl Laboratories) at a dilution of 1:1,000. β-tubulin antibody (Sigma) at a 1:2000 dilution was used as a protein loading control. Antibody signals were detected by ECL (Thermo Scientific).

### Telemetry

Male αMHC-Med13 TG and WT mice were implanted with TA10TA-F20 temperature transmitters (Data Sciences International) in the peritoneal cavity under general anesthesia. Mice were individually housed at 22C and allowed to recover for one week. Then, data were collected and analyzed using ART2.1 software (Data Sciences International).

### Generation of Med13 conditional knockout mice

Med13 cKO mice were generated using the pGKneo-F2L2dta vector which contains a neomycin resistance cassette for positive selection flanked by FRT sites for removal following germline transmission. Exons 7 and 8 in Med13 were flanked by LoxP sites for Cre-mediated excision (Figure S4). Mouse embryonic stem (ES) cells derived from 129SV mice were screened by PCR using primers designed to regions outside the arms of homology and within the targeting vector to identify positive clones. Positive PCR products were sequenced to confirm

insertion of the LoxP sites. Three targeted ES cell clones were selected for injection into blastocysts by the UT Southwestern Transgenic Core Facility. High percentage chimera male mice were bred to C57Bl6 females for germline transmission. Primers used for genotyping were: Med13 forward 5' CTTTTCATGTTCTGTGTATGTATCC 3', Med13 KO reverse 5' GGAAAACAGGGATAGGTACATATCCA 3' and Med13 conditional reverse 5' ACTCCTCGAATCTTTGCTC 3'. Heterozygous mice were then bred to transgenic mice expressing FlpE recombinase to remove the neomycin cassette. The $Med13^{fl/+}$ mice were then bred to αMHC-Cre transgenic mice and intercrossed to establish homozygous $Med13^{fl/fl}$; αMHC-Cre mice. Studies were done using Cre-negative, $Med13^{fl/fl}$ littermates as controls.

**Histology**

Hearts, WAT, BAT and liver were isolated and fixed in 4% paraformaldehyde (PFA) and processed for H&E staining. For Oil-red-O staining of heart and liver, tissues were fixed in 4% PFA overnight, incubated in 12% sucrose for 12 hours then in 18% sucrose overnight before being cryoembedded and sectioned by the UT Southwestern Histology Core. WAT cell size was analyzed using ImageJ software (http://rsb.info.nih.gov/ij/) (NIH). Measurements were done on at least 3 sections per tissue with 10 independent fields taken per section at 40X magnification.

**RT-PCR**

Sequence of primers for Sybergreen RT-PCR were generated using mouse primer depot software *(*mouseprimerdepot.nci.nih.gov)

**Med13**

Right primer sequence: 5'-ATCCATCAAGTGCCTGCTTC-3'

Left primer sequence: 5'-GTGCGGACTGAGGATCAACT-3'

**Thrsp**

Right primer sequence: 5'-TCGGGGTCTTCATCAGTCTT-3'

Left primer sequence: 5'-GCGGAAATACCAGGAAATGA-3'

**Elovl6**

Right primer sequence: 5'-AACTTGGCTCGCTTGTTCAT-3'

Left primer sequence: 5'-CCAATGGATGCAGGAAAACT-3'

**Apoc1**

Right primer sequence: 5'-AAATGCCTCTGAGAACCAGG-3'

Left primer sequence: 5'-GGAGTTTGGGAACACTTTGG-3'

**Cyp2a5**

Right primer sequence: 5'-GGCAGGTGTTTCATCACAGA-3'

Left primer sequence: 5'-CACTGCTTCGAATGATGCTG-3'

**Gpd1**

Right primer sequence: CTTGGTGTTGTCACCGAAGC

Left primer sequence:  CAAGAGGTGGACACAGTGGA

**Eno1**

Right primer sequence: AAAGATCTCTCTGGCGTGGA

Left primer sequence:  CTTAACGCTCTCCTCGGTGT