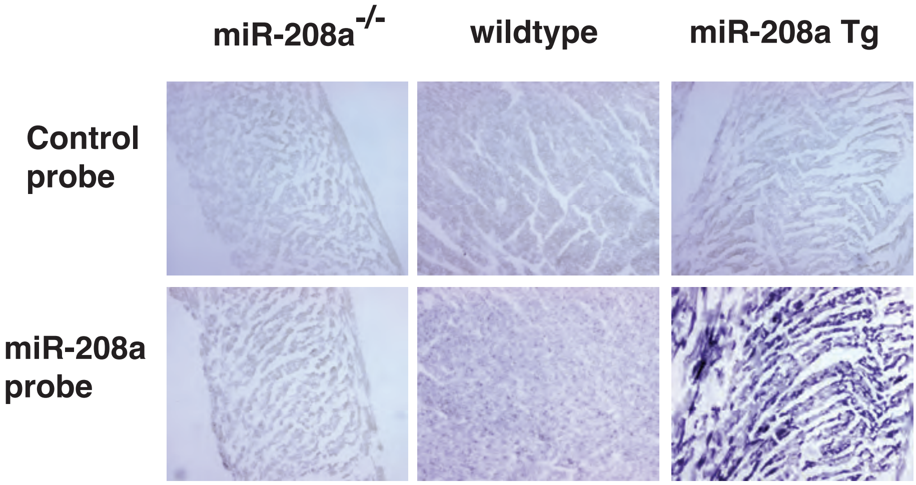
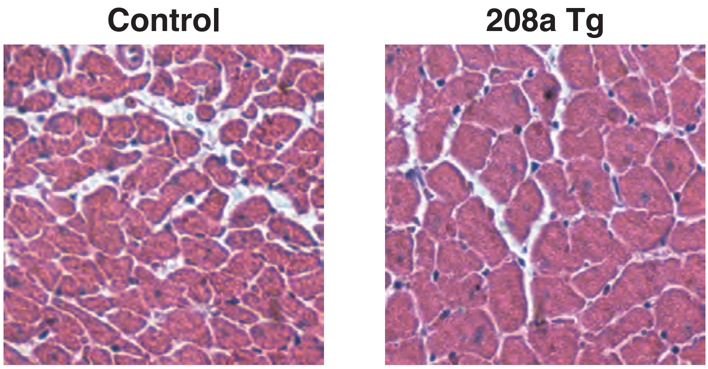
Callis et al, Supplemental Figure 1



Supplemental Figure 1

Representative images of adult miR-208a$^{-/-}$, wildtype, and miR-208a Tg mouse hearts hybridized with LNA miR-208a probes or scrambled control probes. miR-208 signal was completely absent from miR-208a$^{-/-}$ hearts and uniformly upregulated in miR-208a Tg hearts relative to wildtype hearts.

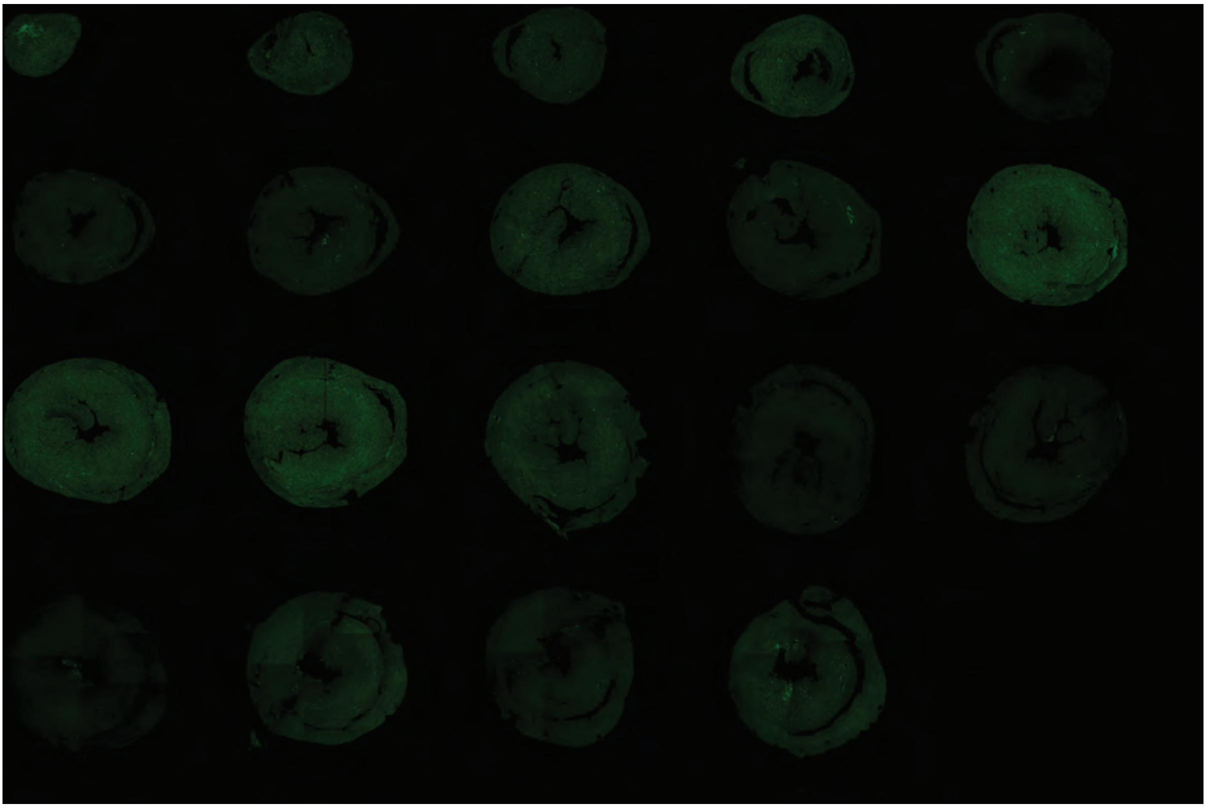
Callis et al, Supplemental Figure 2



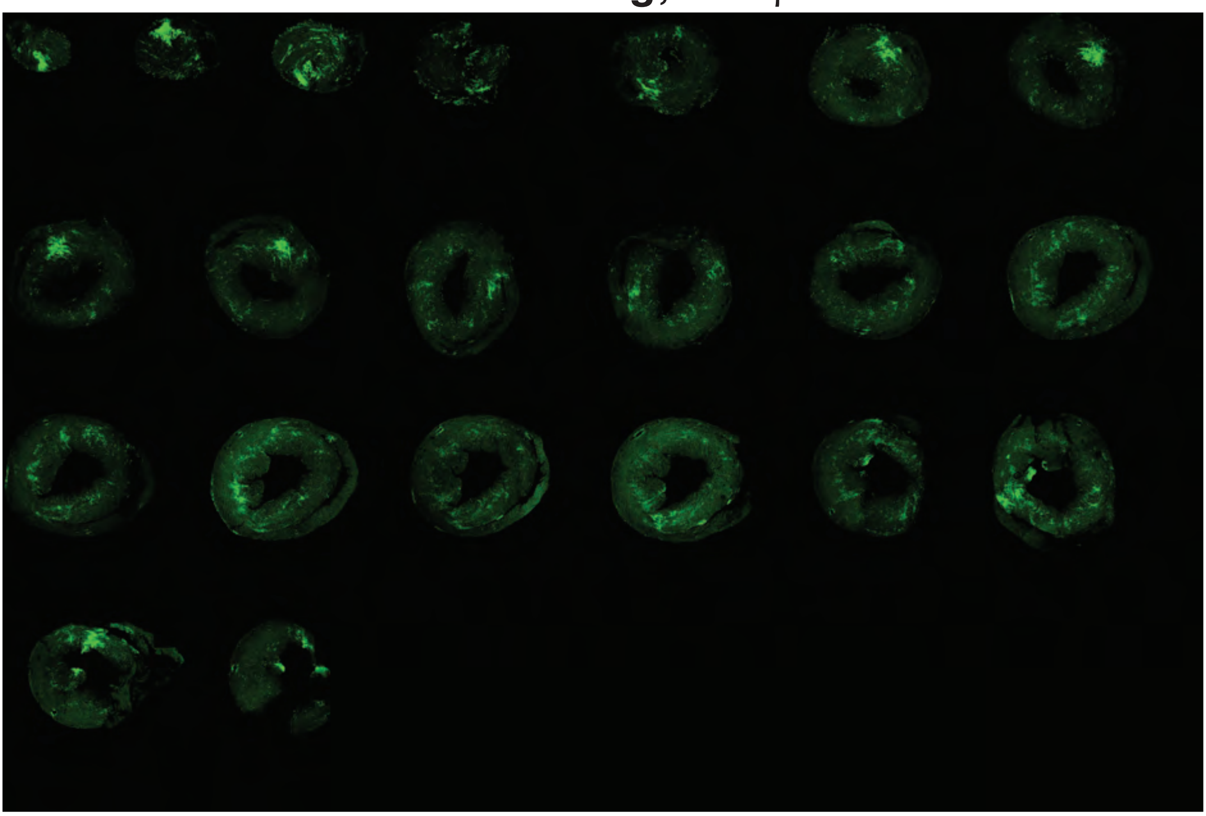
Supplemental Figure 2
Microscopic view of H&E stained histological sections from control and miR-208a Tg hearts.

Callis et al, Supplemental Figure 3

Control; YFP-βMHC



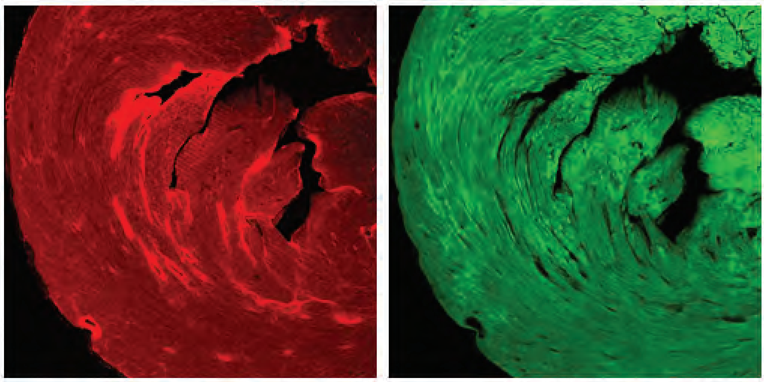
miR-208a Tg; YFP-βMHC



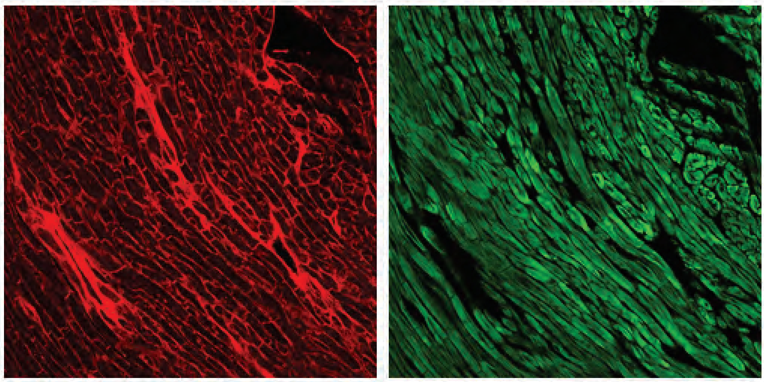
Supplemental Figure 3
Confocal microscropy for YFP detection on serial coronal sections from control; YFP-βMHC and miR-208a Tg; YFP-βMHC hearts (from left to right, top to bottom: apex to the top of the ventricles).

Callis et al, Supplemental Figure 4

A



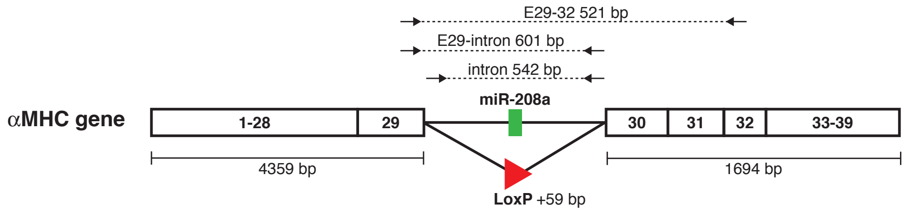
B



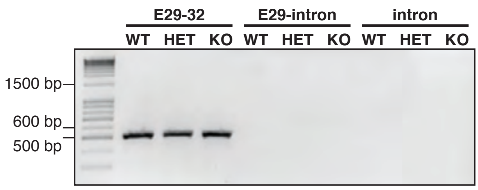
Supplemental Figure 4
(**A**) Representative confocal fluorescent image of coronal section from an adult YFP-βMHC; miR-208a Tg heart treated with PTU for 6 weeks. PTU resulted in uniform YFP-βMHC (green) expression throughout the myocardium, consistent with inhibition of thyroid hormone signaling. Wheat germ agglutinin-TRITC staining in red. (**B**) PTU-treated YFP-βMHC; miR-208a Tg heart imaged with a 20x objective for YFP-βMHC (green) expression and wheat germ agglutinin-TRITC staining (red).

Callis et al, Supplemental Figure 5

A



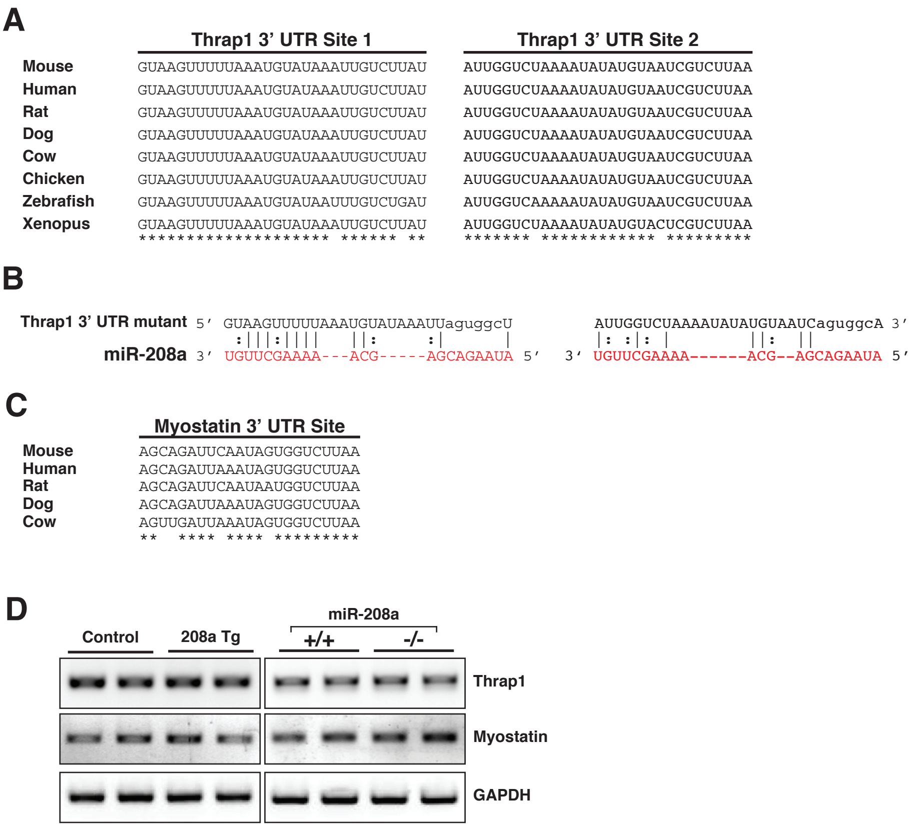
B



Supplemental Figure 5

Splicing of αMHC transcript containing mutant miR-208a allele is undisturbed. (**A**) Diagram of αMHC gene showing the proper splicing pattern of the intron that encodes the miR-208a allele or a mutant allele that carries a loxP site instead. Location of primers and regions amplified marked by arrows and dashed lines. (**B**) Results of PCR analysis using genomic DNA from wildtype (WT), miR-208+/- (Het) or miR-208-/- (KO) animals and the primer sets as marked.

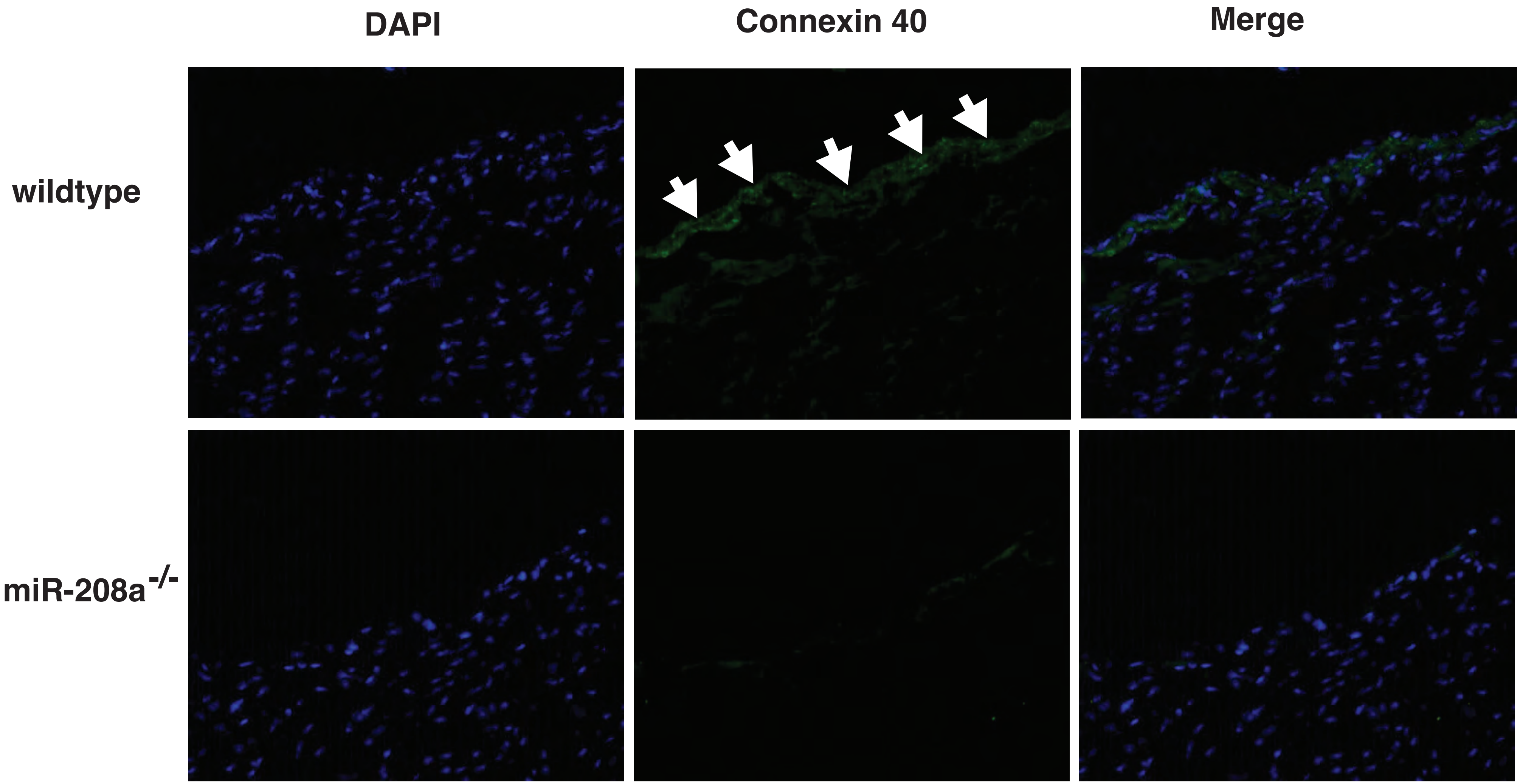
Callis et al, Supplemental Figure 6



Supplemental Figure 6

(A) Target sites for miR-208a in the 3' UTR of Thrap1 show a high level of cross-species sequence conservation. (B) Alignment of miR-208a with mutated Thrap1 target sites. Lower case lettering indicates mutant nucleotides. (C) Target site for miR-208a in the 3' UTR of myostatin shows a high level of cross species sequence conservation. (D) RT-PCR analysis for Thrap1 and myostatin transcript levels in hearts from 4 month-old miR-208a Tg versus control animals and miR-208a-/- versus wild type (+/+) animals. GAPDH serves as loading control.

Callis et al, Supplemental Figure 7



Supplemental Figure 7 Immunohistochemistry of connexin 40 in ventricle septums of 3 month old wildtype and miR-208a knockout hearts. DAPI labels nuclei. Arrowheads indicate the expression of connexin 40 in the heart of wildtype mouse.

Callis et al, Supplemental Figure 8

A

```
                  GATA4 Target Site
             ______________________
miR-208a  3' TGTTCGAAAAACGAGCAGAATA 5'
               |  | ||    |:||||||
Mouse     5' CAACCCGTTAACATTGTCTTAA 3'
Human        AAACCTGTTAACATTGTCTTAA
Rat          CAACCCGTTAACATTGTCTTAA
Dog          AAACCTGTTAACATTGTCTTAA
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Supplemental Figure 8

(A) Alignment of miR-208a with conserved target site in the 3' UTR of GATA4.

Supplemental Table 1

Echocardiography of dimensions and function of 7 month-old miR-208a transgenic mice

	Control n = 5			miR-208a Tg n = 5		
BW (g)	28.7	±	1.58	33.7	±	2.97
LV mass (mg)	104	±	4.10	169	±	10.1***
LV mass/BW (mg/g)	3.67	±	0.24	5.60	±	0.22**
HR (bpm)	666	±	15.1	672	±	18.7
IVSTD (mm)	0.89	±	0.03	1.16	±	0.10**
IVSTS (mm)	1.42	±	0.08	1.79	±	0.10**
PWTD (mm)	0.89	±	0.02	1.17	±	0.09**
PWTS (mm)	1.39	±	0.06	1.55	±	0.11
LVEDD (mm)	3.2	±	0.04	3.4	±	0.11*
LVESD (mm)	1.62	±	0.04	1.9	±	0.10**
FS%	49.5	±	0.71	41.495	±	0.33***

Transthoracic echocardiography on unanesthetized mice. Data are mean ± SEM. BW, body weight; LV, left ventricular; HR, heart rate; IVSTD, interventricular septal thickness in diastole; IVSTS, interventricular septal thickness in systole; PWTD, posterior wall thickness in diastole; PWTS, posterior wall thickness in systole; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension. LV mass index was calculated as (external LV diameter in diastole^3 – LV end-diastolic dimension^3) x 1.055. Fractional shortening (FS) was calculated as (LV end-diastolic dimension – LV end-systolic dimension)/LV end-diastolic. *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$.

Supplemental Table 2

Summary of 1-month and 6-month Surface ECG Findings

	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTc (ms)
1-month					
Control (n=6)	453 ± 26	34 ± 1	9 ± 1	51 ± 1	44 ± 1
208a Tg (n=7)	405 ± 27	49 ± 3**	11 ± 1	53 ± 1	43 ± 2
6-month					
Control (n=6)	425 ± 25	40 ± 2	10 ± 1	54 ± 2	45 ± 2
208a Tg (n=7)	436 ± 15	51 ± 3*	11 ± 1	59 ± 2	50 ± 2

HR, heart rate; bpm, beats per minute; ms, milliseconds; *, $P < 0.05$; **, $P < 0.001$.