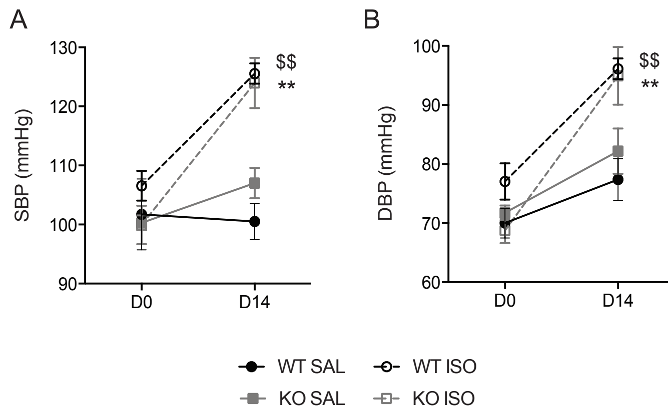
Figure S1



Supplemental Data

Surgeries

Transplantation. Brown adipose tissue transplantation was performed as described previously [1], using BAT removed from the intrascapular region of 12-week-old mice. After euthanasia of the donor mice by cervical dislocation, both lobes of BAT were removed. Twelve-week-old recipient mice were anesthetized by isoflurane inhalation. For each recipient mouse, the donor BAT of one mouse was transplanted into the visceral cavity. The transplant was carefully lodged deep between folds within the endogenous epididymal fat of the recipient.

Echocardiography

Transthoracic echocardiography was performed with a 13-MHz probe (Vivid 7, GE Medical Systems, Milwaukee, WI) in lightly sedated mice (50 mg/kg ketamine ip) at baseline (control), 14 days after Alzet pump implantation, or 8 weeks after MI, as previously described [2,3].

Measurements were made in M-mode by an observer blinded to the experimental group and taken in triplicate. Heart rate (HR), left ventricular end-diastolic internal diameter (LVEDD), LV end-systolic internal diameter (LVESD), end-diastolic posterior wall thickness (PWT) and interventricular septal thickness (IVS) were measured and fractional shortening of the LV (FS) calculated.

The LV mass index (LVMI) was calculated as 1.05 $[(AWT + PWT + LVEDD)^3 - LVEDD^3]$/body weight [4]. The thickness on radius ratio [5], H/R (defined as (PWT+IVS)/2 / LVEDD/2), an index of concentric hypertrophy, was calculated. In the MI model, the LV ejection fraction (LVEF) and fractional shortening (FS) was calculated

from 2D images as previously described [6].

Blood pressure

Systolic (SBP) and diastolic blood pressure (DBP) were measured non-invasively in awake mice by tail-cuff (MC4000MSP, Hatteras Instruments, Inc.) [7]. Mice were habituated to the blood pressure measurement device for 7 days. After this training period, the blood pressure was measured at baseline and Alzet pumps filled with saline or isoproterenol were implanted. Mice were trained every day during the infusion and a final blood pressure measurement was made after 14 days of isoproterenol or saline treatment.

Collection and analysis

Blood (200 µl) was collected by facial vein puncture in mice anesthetized using isoflurane in an EDTA tube. EDTA plasma was prepared by cold centrifugation at 2500 g for 15 min and stored at -70° C until use. Blood glucose was determined using a portable glucose meter (OneTouch Ultra Blue, Lifescan, Johnson & Johnson). Insulin levels were measured using the Ultra Sensitive Mouse Insulin Kit (Crystal Chem Inc.) according to the manufacturer instructions. Triglyceride levels were determined using a commercial Triglyceride Assay Kit (BioAssay Systems) according to the manufacturer instructions. All metabolic parameters were measured in mice fasted for 16 hours. Plasma levels of cTnI, Fabp3 and Myl3 were measured in EDTA plasma from mice treated for 3 days with saline or isoproterenol using the Muscle Injury Panel 3 mouse Kit (Meso Scale Diagnostics, Rockville, USA)

Glucose and insulin tolerance tests

For glucose tolerance tests (GTT), animals were fasted for 6 hours with free access to

drinking water. A baseline blood sample was collected from the tail of fully conscious mice, followed by an intraperitoneal injection of glucose (2.0 g/kg body weight), and blood was taken from the tail at 15, 30, 60, 90, and 120 minutes after injection. GTTs were performed at 12 weeks of age or 8 weeks after transplantation. For insulin tolerance tests (ITT) animals were fasted for 2 hours, and baseline blood samples were collected from the tail of fully conscious mice. Insulin (1 U/kg body weight) (Humulin; Eli Lilly) was administered by intraperitoneal injection, and blood samples were taken from the tail at 10, 15, 30, 45, and 60 minutes after injection. Glucose levels were determined from blood using a portable glucose meter (OneTouch Ultra Blue, Lifescan, Johnson & Johnson).

Tissue Analysis

Mice were euthanized, and the LV was blotted, weighed, fixed, and embedded in paraffin. Serial heart sections from each group were analyzed. To assess the degree of LV fibrosis, 5 μM thick sections from the mid ventricular region were stained with PicroSirius red. The ratio of collagen deposition (indicated by red staining) to total myocardial area was outlined in 10 areas of interest and quantified by an automated analysis program (IP Lab Spectrum; Signal Analytics, Vienna, VA) as described [8].

Measurement of mRNA expression levels

Ventricular and BAT samples from mice treated for 3 days with isoproterenol or saline were frozen in liquid nitrogen and stored at –70°C until use. RNA was extracted using the Trizol (Ambion, Life Technologies, Austin, Texas, USA) method according to the manufacturer’s instructions. One μg of RNA was used in the Applied Bioscience Multiscribe Reverse Transcriptase cDNA Synthesis Kit (Applied Bioscience) for RT-PCR to produce cDNA using random hexameric primers. cDNA was subsequently used for

relative expression quantitation using the Applied Bioscience Taqman FAST Advanced Master Mix or the FAST SYBR Green I Master mix (Applied Biosciences) in a Lightcycler® 480 (Roche). To this end subunit specific primer-probe sets were purchased for UCP1, UCP2 and UCP3 (Mm00494069_m1, Mm00627599_m1, Mm00494077_m1, respectively, Life Technologies). To normalize the data in BAT and LV, the geometric mean of the 2 most stable housekeeping genes, 60S ribosomal protein L13 (*RPL13*) and beta-actin (*ACTB*), out of 5 tested genes, calculated with the geNorm™ Housekeeping Gene Selection Kit (PrimerDesign Ltd) was used as described [9]. In LV after MI 18S ribosomal protein (18S) was used as housekeeping gene. Each sample was measured in triplicate to determine the threshold cycle (Ct). For each sample, the normalization factor was calculated as the difference between the geometric mean Ct of the housekeeping genes of the sample and the mean Ct of the housekeeping genes of all samples. The level of target mRNA, relative to the mean of the reference housekeeping genes, was calculated by raising 2 to the power of [40 - (Ct of target - housekeeping gene normalization factor)]. Data ± SE are plotted as relative amount vs. WT Saline = 100%.

CCTGCTGCTCTCAAGGTTGTT	RPL13a F
TGGTTGTCACTGCCTGGTACTT	RPL13a R
GAAACTCTGCTTCGCTGCATT	HMBS F
TGCCCATCTTTCATCACTGTATG	HMBS R
TCTACCGTGAATCTTGGCTGTAAA	TBP F
TTCTCATGATGACTGCAGCAAA	TBP R
ATGCACGCAGAAAGAAATAGCAA'	B2M F
AGCTATCTAGGATATTTCCAATTTTTGAA	B2M R
GCTTCTAGGCGGACTGTTACTGA	ACTB F
GCCATGCCAATGTTGTCTCTTAT	ACTB R
CGGCTACCACATCCAAGGAA	18S F
GCTGGAATTACCGCGGCT	18S R

Immunoblotting

Protein samples from the LV of mice treated for 3 days with isoproterenol or saline were prepared by homogenization in extraction buffer (RIPA-buffer, Boston Bioproducts, MA, USA) in the presence of protease (Halt Protease Inhibitor Cocktail, Pierce Thermo Fisher Scientific, Rockford, IL) and phosphatase (Halt Phosphatase Inhibitor Cocktail, Pierce Thermo Fisher Scientific, Rockford, IL) inhibitors. Homogenates were centrifuged at 20000 g for 20 min at 4°C, and the resultant supernatant was stored at -70°C until use. Fifteen micrograms of protein was loaded in each lane of all Western blots. Antibodies included P-ERK1/2, ERK1/2, P-AKT and AKT (Cell Signaling Technologies). Detection and analysis was performed with the Odyssey system (Odyssey 2.1.12, Li-Cor Biosciences) using fluorophore-coupled secondary antibodies (goat anti-rabbit IRDye® 800CW, Li-Cor Biosciences). Results were normalized to total ERK1/2 and AKT, respectively, and expressed as a percentage of WT saline.

Supplementary Figure.

Figure S1. Effect of 14 days of isoproterenol infusion on systolic and diastolic blood pressure in wild-type (WT) and UCP1-deficient (UCP1$^{-/-}$) mice. Isoproterenol increased both systolic and diastolic blood pressure in both strains, however there was no difference in response between WT and UCP1$^{-/-}$ mice. **Panel A**: Systolic blood pressure. **Panel B**: Diastolic blood pressure. (WT SAL N=5, WT ISO N=5, KO SAL N=5, KO ISO N=5) **: p<0.01 vs. sham (saline infusion) of the same strain at 14 days, $$: p<0.001 vs. baseline (D0) in the same strain. WT: wild-type, KO: UCP1 $^{-/-}$ mice, D0; baseline, D14: 14 days of isoproterenol, SAL: saline infusion, ISO: isoproterenol infusion.

Supplementary Table S1. Echocardiographic measurements and post-mortem analysis of LV mass in female WT and UCP1$^{-/-}$ mice at baseline and after 14 days infusion with saline or isoproterenol.

	DAY	WT SAL N=15	WT ISO N=17	KO SAL N=14	KO ISO N=12
FS (%)	0	57±1	58±0.5	59±0.3	58±1
	14	57±1	58±1	59±1	61±1$^{\$}$
PWT (mm)	0	0.8±0.01	0.8±0.01	0.8±0.01	0.8±0.01
	14	0.8±0.02	0.9±0.02$^{\$*}$	0.8±0.01	1.0±0.02$^{\$*\#}$
IVS (mm)	0	0.7±0.01	0.7±0.01	0.7±0.01	0.7±0.01
	14	0.7±0.02	0.9±0.02$^{\$*}$	0.7±0.01	0.9±0.02$^{\$*\#}$
LVEDD (mm)	0	3.3±0.01	3.3±0.02	3.3±0.02	3.3±0.03
	14	3.2±0.04	3.1±0.05$^{\$}$	3.2±0.04	3.1±0.05$^{\$*}$
LVESD (mm)	0	1.4±0.02	1.4±0.02	1.3±0.01	1.4±0.03
	14	1.4±0.04	1.3±0.05	1.3±0.03	1.2±0.03$^{\$*\#}$
HR (bpm)	0	566±8	558±12	532±10	533±12
	14	550±8	644±7$^{\$*}$	517±12	572±14$^{*\#}$
LVMI (mg/g)	0	3.9±0.1	3.7±0.1	3.7±0.1	3.8±0.1
	14	3.5±0.1$^{\$}$	3.9±0.1$^{\$*}$	3.5±0.1	4.6±0.2$^{\$*\#}$
H/R	0	0.45±0.01	0.46±0.01	0.45±0.01	0.46±0.01
	14	0.46±0.01	0.56±0.02$^{\$*}$	0.47±0.01	0.62±0.02$^{\$*\#}$
LV/BW (mg/g)	14	3.2±0.1	3.6±0.1*	3.3±0.1	4.0±0.2$^{*\#}$

WT; wild-type, KO; UCP1-/-, SAL; saline, ISO; isoproterenol, FS; fractional shortening, PWT; end-diastolic posterior wall thickness, IVS; end-diastolic interventricular septal thickness, LVEDD; left ventricular end-diastolic internal diameter, LVESD; LV end-systolic internal diameter, HR; Heart rate, LVMI; LV mass index (echocardiography), H/R; thickness on radius ratio, BW; body weight. $: p<0.05 vs. same treatment and same group on day 0, *: p<0.05 vs. saline treatment on same day, #:p<0.05 vs. same treatment on same day in WT ISO

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