Supplementary Methods

Tnl substitutions and viral vector construction. We used a pGEM-3Z vector containing cTnl or ssTnl, and the QuikChange mutagenesis kit (Stratagene) to generate substitutions in cTnl and ssTnl. The primers used for mutagenesis of cTnl to cTnIA164H removed an Xma1 site and were 5’-ggcactagtggaccgggcacaaggaatctttggacctg-3’ (sense) and 5’-caggtccaggattcctttgtgcgggtccagtagtggcc-3’ (antisense). Primers were then extended using Pfu DNA polymerase and methylated parental DNA was subsequently digested with Dpn I. DNA was transformed into competent bacterial cells. Mutated DNA with appropriate restriction enzyme sites was sequenced prior to ligation of mutant cTnl and ssTnl cDNA into Ad5 viral shuttle vectors. All DNA sequences were verified by overlapping sequence runs. We produced and purified recombinant vectors as described previously1.

Adult rat cardiac myocyte isolation and adenoviral gene transfer. We isolated adult rat cardiac myocytes by enzymatic digestion and plated them on laminin-coated coverslips in Dulbecco’s modified Eagle’s medium (DMEM) containing 5% fetal bovine serum, 50 units/ml penicillin, and 50 g/ml streptomycin (P/S) as described previously2. After 2 h, we replaced media with adenovirus diluted in serum-free DMEM + P/S. Serum-free media was added after an hour incubation with adenovirus, and media was changed the day after adding virus and then every other day.
Transgenic mice. We mutagenized rat cTnI A164H using a Site-Directed Mutagenesis Kit (Stratagene) and verified the constructs by DNA sequencing. Constructs were microinjected into C57BL/6xSJL F₂ fertilized eggs and implanted in pseudopregnant females. Transgenes in mouse genomic DNA were detected by PCR using the following pairs: 5’-AGACAGATCCCTCCTATCTC-3’ in the Myh6 promoter and 5’-GTGATGTTCTTGGTGACTTTT-3’ complimentary to the rat cTnI. Transgenic founders were backcrossed to C57BL/6 mice. For all experiments described, we studied F1-F3 male and female progeny from a cross of SJL and C57BL/6 strains and used ntg littermates as controls. Previous studies showed that a flag epitope in cTnI had no detectable effects on hemodynamic function in transgenic mice³. All animal care and experimental procedures were approved by the University of Michigan Committee on Use and Care of Animals.

Isometric tension measurements in permeabilized myocytes. We isolated cardiac myocytes by mechanical disruption and permeabilized them in 0.2% Triton X-100. We measured Ca²⁺-activated tension in single myocytes attached to a force transducer via glass micropipets at pH 7.0 and pH 6.2 as described⁴. Tension-pCa (-log [Ca²⁺]) relationships were constructed by expressing tension at various submaximal Ca²⁺ concentrations as a fraction of tension at maximal activation (pCa = 4.0). The Ca²⁺ concentration required for half maximal force development was expressed as pCa₅₀.

Adult mouse myocyte isolation, morphometric analysis, sarcomere shortening and Ca²⁺ transients. For morphometric analysis, we plated cells on laminin-coated
coverslips in the presence and absence of 10 mM 2,3-butanedionemonoxime (BDM). Microscopic images were recorded and analyzed for cell area using morphometrics software (Metamorph). We measured dynamic sarcomere shortening and relengthening in intact myocytes using a high intensity video-based detection system (Ionoptix, Milton, MA) at 37 °C as described by us previously⁵,⁶. For baseline recordings, myocytes were incubated in media 199 supplemented with P/S, 10 mM HEPES, 0.2 mg/ml bovine serum albumin and 10nM glutathione. Recordings were also performed after exposing myocytes to an ischemia mimetic solution (pH 6.0), as described⁷. We measured Ca²⁺ transients in myocytes loaded with a fluorescence indicator (5- M fura2-AM) and paced at 0.2 Hz as described by us previously⁸.

³¹P NMR spectroscopy and energetic enzyme activities. FIDs (n=250, 60° pulse, recycle time 2.0 s) were Fourier transformed, phased, baseline corrected, and line broadened by 20 Hz. The resonance areas and chemical shifts were quantified using the MacNuts – Utility transform software (Acorn NMR Inc., Livermore, CA). For enzyme activity measurements, we homogenized 5 to 10 mg of ventricular tissue for 10 s in K-phosphate buffer (0.1 M) containing 1 mM EDTA and 1 mM β–mercaptoethanol, pH 7.4 (final concentration of 5 mg wet weight/ml). Triton X-100 was added to the homogenate at a final concentration of 0.1% for analysis of activities of creatine kinase (CK), the major glycolytic enzymes phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and lactate dehydrogenase (LDH), and the mitochondrial marker citrate synthase (CS), using standard spectrophotometric-based assays⁹. We measured cytochrome C oxidase using an assay kit from Sigma as described¹⁰.
**Echocardiography.** We measured systolic and diastolic dimensions and wall thickness in M-mode in the parasternal short axis view at the level of the papillary muscles\textsuperscript{11,12}. Fractional shortening and ejection fraction were calculated from the M-mode parasternal short axis view\textsuperscript{12}. We assessed diastolic function by conventional pulsed-wave spectral Doppler analysis of mitral valve inflow patterns (early [E] and late [A] filling waves). Doppler tissue imaging (DTI) was used to measure the early (E\textsubscript{a}) and late (A\textsubscript{a}) diastolic tissue velocities of the mitral annulus in the apical 4-chamber view.

**Isolated heart model (Langendorff).** Hearts were retrograde-perfused with a standard Krebs-Henseleit buffer (KHB: 118 mM NaCl, 25 mM NaHCO\textsubscript{3}, 15 mM glucose, 0.5 mM EDTA, 47 mM KCl, 1.2 mM MgSO\textsubscript{4}, 1.2 mM NaH\textsubscript{2}PO\textsubscript{4}, 2.5 mM CaCl\textsubscript{2}) equilibrated with 95% O\textsubscript{2} and 5% CO\textsubscript{2} (pH 7.4) at a constant pressure of 80 mmHg, and paced at 7 Hz. We monitored left ventricular pressure by means of a water-filled polyvinyl chloride balloon connected to a pressure transducer\textsuperscript{13,14}. Left ventricular pressure measurements and electrocardiograms were collected on-line at a sample rate of 500 s. We performed data analysis using AIDInstruments software (Chart for Windows, version 4.2.3).

**Left coronary artery ligation model.** We sedated mice with intraperitoneal sodium pentobarbital (45 mg/kg), intubated them orally, and ventilated them via a pressure-controlled ventilator with 1% isoflurane in 100% oxygen at a peak inspiratory pressure of 15 cm H\textsubscript{2}O and a respiratory rate of 60 breaths/min. With the aid of a dissecting
microscope, we exposed the heart via a left thoracotomy, and tied a 7-0 silk suture around the proximal portion of the left coronary artery (LCA) 1-2 mm from the left atrium. We filled the chest with warm sterile saline to evacuate air and closed the incision in layers using 5-0 silk suture. Sham-operated mice underwent thoracotomy without LCA ligation.

**Implantation of ECG radiotelemetry devices and in vivo ischemia-reperfusion.** We anesthetized animals with 0.75-1.5% inhaled isoflurane in 100% oxygen. With the aid of a dissecting stereomicroscope, we made a midline skin incision 2-3 cm long from the pelvis to the xiphoid process. We placed the ECG transmitter (TA10ETA-F20, Data Sciences International, St. Paul, MN) in the abdominal cavity with the two electrical leads passed through the abdominal wall and secured in the standard subcutaneous lead II positions. The appropriate radio signal confirmed functional implant. The peritoneal cavity was flooded with sterile saline to assure adequate hydration, and the transmitter rotated into position with the securing suture rib facing the wound edge. Six to eight interrupted non-absorbable suture (4-0 or 5-0) ties were used to secure the transmitter to the peritoneum. We closed the skin with staples and topical antiseptic applied. The animals were recovered for one week following implantation of the ECG telemetry device. To obtain baseline heart rates, we sampled ECGs for 10 s every 15 min and heart rates were averaged over a period of 48 h. We performed the ischemia-reperfusion protocol via a left thoracotomy in ventilated mice as described above for the permanent LCA ligation experiments. We placed the 7-0 silk suture under the LCA, lay a short segment of PE-10 tubing on the myocardium over the LCA, and tied the suture
on the tubing. We confirmed occlusion by visual inspection of myocardial pallor distal to
the suture and by ST-segment elevation on the ECG. After 30 min of ischemia,
reperfusion was achieved by gently removing the PE-10 occluder. The animals were
monitored for 24 h with continuous ECG recording. We sacrificed the mice for area at
risk (AAR) and infarct size (IS) measurement by retrograde perfusion of 1% Evans blue
dye followed by a 15 min incubation of sectioned tissue in 1% TTC at 37 ºC as
described\textsuperscript{13,15}. Images were acquired and analyzed using Image-Pro Express (version
5.0) software.

**Human myocyte isolation and gene transfer.** Myocytes were made calcium tolerant
over 1 h, resuspended in MEM media containing 5% FBS, and then allowed to attach
on laminin-coated coverslips (40,000 rod-shaped cells per coverslip) for 2 h. We
replaced media with serum-free media containing recombinant adenovirus for 1 h as
described earlier \textsuperscript{2}. We changed media daily and performed contractile function
measurements with field stimulation over a range of pacing frequencies including 0.2,
0.5, 1 and 2 Hz on day 4 post gene transfer. Myocyte contractile function was
comparable on days 1-4 after isolation in non-transduced cells. These studies were
conducted under Institutional Review Board approval # 2002-0413. Myocytes used in
gene transfer experiments were isolated from the hearts of 3 patients (41-66 years of
age) with diverse heart failure etiologies – one long-standing ischemic cardiomyopathy,
one long-standing non-ischemic cardiomyopathy, and one 3 months following acute
myocardial infarction and left ventricular assist device implantation. All patients were on
beta-blockers and angiotensin converting enzyme inhibitors, and one was receiving intravenous milrinone at the time of heart transplantation.

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