Supporting Information

Nowakowski et al. 10.1073/pnas.1220693110

SI Materials and Methods

Echocardiography. Echocardiography was carried out on mice 3–5 mo old. The animals were lightly anesthetized using 1% isoflurane. Images were collected with a GE Vivid 7 system using 13-MHz linear transducer (GE Healthcare). Left ventricular end-diastolic (LVEDD) and left-ventricular end-systolic (LVESD) dimensions were determined using M-mode measurements obtained by short-axis views at the midpapillary level. All data were averaged from at least three cardiac cycles. Fractional shortening was calculated from these data using the following formula: (LVEDD − LVESD)/LVEDD × 100. All echocardiography was carried out by a single reader who was blinded to the genotype of the animals.

Smooth Muscle Contractile Studies. All solutions and the methods for tissue preparation have been described in detail (1–4). Briefly, the mouse aorta was removed and placed into Ca^{2+}-free PSS solution [140 mM NaCl, 4.7 mM KCl, 1.2 mM $NaH_2PO_4{\cdot}7H_2O$, 2.0 mM 3-(N-morpholino)propanesulfonic acid buffer (MOPS), 0.02 mM EDTA, 1.2 mM $MgCl_2{\cdot}6H_2O$, 5.6 mM glucose, and 0.5 mM EGTA (pH 7.0)], cleared of connective tissue, and then cut into strips ∼400–500 μm long, 200 μm wide, and 50 μm thick and mounted between aluminum foil T-clips. Strips were then transferred to a mechanics workstation (Model 600; Aurora Scientific) and mounted between a force transducer (Akers AE 801, MEMSCAP) and servomotor (Aurora Scientific). The tissue was stretched to a level sufficient to just develop tension and then an additional 30%, defining the length for maximum force (Lo). Following stretching, tissue strips were skinned by transferring the preparation into a 1% Triton solution for 15–20 min. Strips were then returned to pCa 9 (negative logarithm of calcium ion concentration 9) and maximally activated with Ca^{2+} (pCa 4). The effects of 2-deoxy-ATP (dATP) were investigated in solutions containing either 5 mM ATP or 5 mM dATP.

Calcium solutions were prepared using a computer program designed to give a set of free ion concentrations that are adjusted for both temperature and ionic strength. The ionic strength for all solutions was 200 mM, and the experiments were carried out at a temperature of 22 °C. Solutions used were as follows: relaxing (pCa 9.0) containing (in mM) 25 N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 10 EGTA, 0.02 $CaCl_2$, 7.2 $MgCl_2$, 5.5 ATP dATP, 25 creatine phosphate, 56.5 potassium methanesulfonate (KMS), pH to 7.0 with 1 M KOH; and activating [5 mM ATP or 5 mM dATP (pCa 4) (in mM): 25 BES, 10 EGTA, 10.22 $CaCl_2$, 6.97 $MgCl_2$, 5.6 ATP/dATP, 25 creatine phosphate, 34.6 KMS, pH to 7.0 with 1 M KOH]. Creatine kinase was added before each experiment and all solutions contained 250 units/mL calmodulin.

Myosin Light Chain Phosphorylation. Myosin light chain LC_{20} phosphorylation was determined as described previously (1–4). Briefly, tissue strips were skinned and then placed in pCa 9 solution (5 mM MgATP or deoxy-MgATP) for 20 min. Strips were either kept in pCa 9 or transferred into pCa 4 solution (5 mM MgATP or deoxy-MgATP) for an additional 15 min. The tissue was then denatured in 15% (vol/vol) trichloroacetic acid in acetone for 30 min in liquid nitrogen. Following denaturation, strips were washed four times with acetone and air-dried. LC_{20} was solubilized in PAGE sample buffer containing 6 M urea, 20 mM glycine, 22 mM Tris·HCl (pH 8.6), and 1 mM EDTA. Glycerol (5% vol/vol) and bromophenol blue (0.1% wt/vol) were added to the samples before loading. The samples were resolved by a 19:1 (acrylamide:bisacrylamide) 10% polyacrylamide gel containing 40% (vol/vol) glycerol and 4 M urea and polymerized in a gel buffer containing 20 mM glycine and 22 mM Tris·HCl (pH 8.6). Resolved proteins were transferred to nitrocellulose membrane, and the phosphorylated and unphosphorylated LC_{20} were identified by immunoblotting using a LC_{20} mAb (Sigma). The percentage of LC_{20} phosphorylated was defined as the ratio between the LC_{20} band vs. the total LC_{20} (phosphorylated plus unphosphorylated forms) identified by the mAb.

Isolated Perfused Mouse Heart Preparation and ^{31}P NMR Spectroscopy. Myocardial energetics and left ventricular (LV) function were measured in Langendorff-isolated heart preparations combined with ^{31}P NMR spectroscopy as described previously (5–7). In brief, excised mouse hearts were perfused at a constant pressure of 80 mmHg with a modified Krebs–Henseleit buffer consisting of (in mmol/L): 118 NaCl, 25 $NaHCO_3$, 5.3 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 0.5 EDTA, 5.5 glucose, and 0.5 pyruvate, equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). Temperature was maintained at 37.5 °C throughout the protocol. After 20 min of equilibration, baseline function was monitored for 10 min at a fixed end diastolic pressure of 8–10 mmHg by way of a water-filled balloon inserted into the left ventricle (LV). After baseline, dobutamine (DOB) was infused at 5% of the coronary flow at a final concentration of 300 nM for 20 min. Dynamic changes in high energy phosphate content [phosphocreatine (PCr), ATP, and inorganic phosphate (Pi)] were monitored using ^{31}P NMR spectroscopy simultaneously with continuous recording of LV function via a data acquisition system (PowerLab; ADInstruments) during the entire perfusion protocol.

The ^{31}P NMR spectra were acquired on a 14T magnet interfaced with an Avance III console and TopSpin V2.1 software (Bruker Biospin). Spectra were obtained by averaging 120 free induction decays (FIDs) over a time period of 5 min at a pulse angle of 60°, acquisition time of 0.4 s, and recycle time of 2.14 s Frequency-domain NMR spectra were obtained by Fourier transformation of the FIDs and analyzed using 20-Hz exponential multiplication and zero- and first-order phase corrections. The average of γ- and β-ATP peak areas obtained at baseline were set to 100% and used as the reference value for all peaks in the ^{31}P NMR spectra. Intracellular pH was determined by comparison of the chemical shift of inorganic phosphorous and PCr.

Adult Mouse Cell Isolation and Contractile Assessment. Adult mouse cardiomyocytes were isolated by enzymatic digestion from 3- to 5-mo-old mice as described previously (8) in accordance with Alliance for Cellular Signaling protocol. Briefly, the hearts were rapidly excised and cannulated through the aorta and perfused with a calcium-free buffer to arrest the heart. Myocytes were dissociated via a collagenase-based solution. The ventricles were then removed, minced, and placed in fresh digestion buffer. The digestion reaction was stopped, and cells were reintroduced to calcium by multiple resuspensions in solutions with increasing calcium concentrations. Finally, cells were resuspended in 37 °C standard media (DMEM) supplemented with penicillin G (100 U/mL) and streptomycin (100 μg/mL).

Contractile assessment of cells was carried out on the same day as isolation. Cells were treated with Fura-2–acetoxymethyl ester (1 μL/mL media) and ultimately resuspended in Tyrode's buffer [in mmol/L: 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5.4 KCl, 140 NaCl, 10 Hepes, 0.33 NaH_2PO_4, 5 glucose (pH 7.4)] and plated on 25-mm^2 glass coverslips. Cell shortening and relengthening was recorded and measured using IonOptix SarcLen system video microscopy. A

40× objective lens (Olympus uWD 40) and 25× intermediate lenses were used for video microscopy, and all measurements were performed on a heated stage set to 37 °C. Cells were not measured if they did not respond to field stimulation of 1:1 and/or if their resting sarcomere lengths were below 1.70 μm. Field stimulation was performed with a 4-ms square suprathreshold (10 V) pulse at 1, 3, and 5 Hz through parallel platinum electrodes. Cell shortening was recorded by illuminating the myocytes with red transmitted light (>600 nm). Calcium transients were measured in Fura-2–loaded myocytes using IonOptix equipment as described previously (9). Briefly, calcium transients were recorded by measuring Fura-2 fluorescence (IonOptix spectrophotometer with stepper switch) passed through a 510-nm emission filter to a photomultiplier tube using the interpolated pseudoratiometric method with a 380-nm excitation during and a 360-nm excitation at the beginning and the end of 20 second recording events. The cell and sarcomere length measurements were recorded simultaneously with Fura-2 fluorescence by computer acquisition and were subsequently analyzed using the proprietary IonOptix software. Experiments were performed by four different experimentalists and were analyzed in duplicate by two different analysts. No differences were observed between mice, experimentalists, or analysts.

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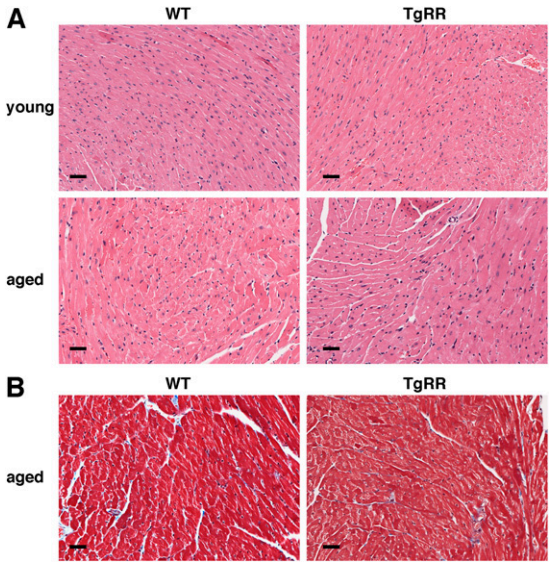

Fig. S1. Histological assessment of WT hearts and hearts from transgenic mice that overexpress ribonucleotide reductase (TgRR). Cardiac muscle morphology in TgRR and WT control mice. Tissues from young (3-mo-old) or aged (12-mo-old) mice were stained with H&E (*A*) or with Masson's trichrome (*B*). (Scale bar: 25 μm.)

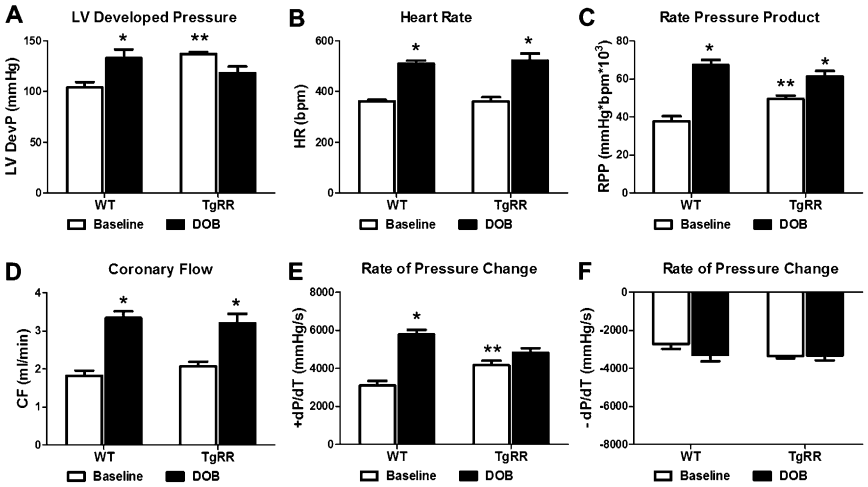

Fig. S2. Response of LV function during acute physiological demand. (*A–C*) LV developed pressure (LVDevP), heart rate (HR), and rate–pressure product (RPP), the product of LVDevP and HR, measured in isolated hearts perfused at baseline (BL) and at the end of DOB infusion. (*D*) Coronary flow, estimated by collecting the perfusate effluent over a 2-min period, in Langendorff heart preparations during normal workload and at the end of DOB infusion. (*E* and *F*) Rate of pressure change calculated by the first derivative of the LV pressure wave (dP/dt) in Langendorff heart preparations at baseline and at the end of DOB infusion. The positive maximum (+dP/dt) is an index of the rate of LV pressure development. The negative maximum (−dP/dt) is an index of the rate of ventricular relaxation. **P* < 0.05 vs. respective WT BL; ***P* < 0.05 vs. WT (*n* = 5 each group).

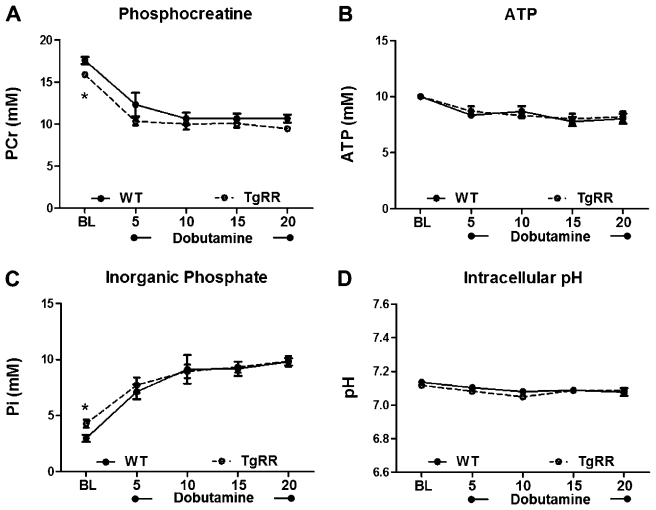

Fig. S3. Changes of high energy phosphate content during DOB challenge. (*A–C*) PCr, ATP, and Pi content measured by ^{31}P NMR spectroscopy in isolated perfused hearts at baseline (BL) and during 300 nM DOB infusion. **P* < 0.05 vs. WT at baseline (*n* = 5 each group). (*D*) Intracellular pH determined by the relative chemical shift between Pi and PCr at BL and during DOB infusion (*n* = 5 each group).

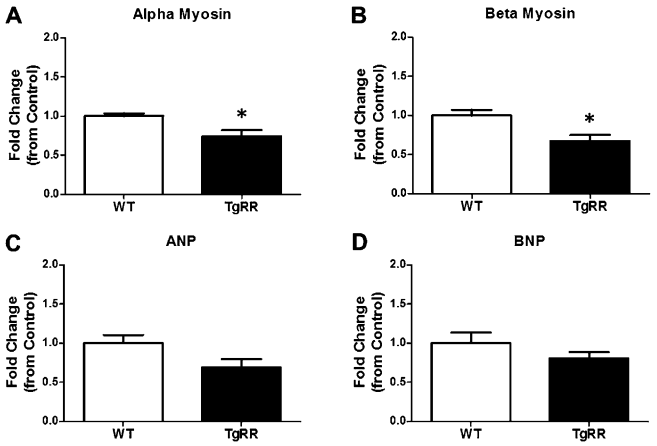

Fig. S4. Expression of myosin isoforms and hypertrophy genes. (*A*) α-Myosin heavy chain (*myh6*) gene expression. (*B*) β-Myosin heavy chain (*myh7*) gene expression. (*C*) Atrial natriuretic peptide (*anp*) gene expression. (*D*) Brain natriuretic peptide (*bnp*) gene expression. Specific genes were normalized to 18S ribosomal RNA and reported as fold change over control (WT) ± SEM. *$P < 0.05$ vs. WT ($n = 5$ each group).