Supplemental Methods

Mouse model Generation and analysis of transgenic mice expressing a histidine-modified cardiac troponin I (cTnI A164H) with FLAG epitope was previously described (Figure 1a)[15]. The procedures used in this study are in agreement with the guidelines of the University of Michigan and approved by the University of Michigan Committee on the Use and Care of Animals. The University of Michigan is accredited by the American Association of Accreditation of Laboratory Animal Health Care, and the animal care use program conforms to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23). Aged mice were susceptible to complications during Millar catheterization resulting in significant loss of animals during the protocol. Three mice died (2 nontransgenic, 1 transgenic) during catheter placement and three had irreversible ventricular arrhythmias (1 nontransgenic, 2 transgenic) that prevented proper data acquisition either during baseline measurements or during the hypoxic challenge. Mice used in this study were exclusively from line 892, which was the highest expressing Tg mouse line for this model.

Conductance micromanometry Measurements of in vivo cardiovascular hemodynamics were obtained using conductance micromanometry as previously performed by this laboratory[15]. Mice were anesthetized and intubated via a tracheal cannulation and ventilated via a pressure controlled ventilator with 1% isoflurane. A 1.0 French Millar pressure-volume catheter (PVR-1045; Millar Instruments Inc., Houston, Texas, USA) was inserted into the right carotid artery and advanced into the left ventricle. Pressure-volume loops were collected on line at 1 kHz. Data were analyzed with PVAN 3.2

software (Millar Instruments Inc.). Inferior vena cava occlusions were also performed to obtain the end systolic and end diastolic pressure-volume relationships. After obtaining baseline hemodynamics (ventilated with 100% O_2), mice were exposed to an acute hypoxic challenge (12% O_2 balanced with nitrogen). Data were acquired until systolic failure which was defined as the point when LV pressure reached 65% of initial peak systolic pressure. At this point, mice were recovered using 100% O_2 in order to obtain calibration data.

Echocardiography Anesthesia was induced with 3% isoflurane and then maintained at 1% for the duration of the procedure. Transthoracic echocardiography was performed in the supine or left lateral position. Two-dimensional, M-mode, Doppler and tissue Doppler echocardiographic images were recorded using a Visual Sonics' Vevo 770 high resolution *in vivo* micro-imaging system. 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. Fractional shortening and ejection fraction were calculated from the M-mode parasternal short axis view. 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_a) and late (A_a) diastolic tissue velocities of the lateral annulus.

Adult mouse myocyte isolation and analysis Adult mouse cardiac myocytes were isolated from 3-6 month old mice as described previously [19]. Between 5 x 105 and 1 x 106 rod-shaped cells were obtained from a single mouse heart and subjected to sarcomere shortening and Ca2+-transient analysis by loading with Fura 2AM (2 μM) as previously

described[20]. An in vitro calibration was performed to convert the fluorescence ratio to [Ca2+] as described[21]. To determine the Ca2+ content of the sarcoplasmic reticulum, electrical pacing was discontinued and caffeine (20 mM) was rapidly applied using a capillary tube perfusion system (Warner Instruments, SF-77B Perfusion Fast-Step).