Supplemental Data:

Table 1: Adenylate kinase and hexokinase activity. All results are mean±SD and expressed as nmol/min/mg protein.

	Control (n=10)	**CK-M** (n=6)	**p-value**
adenylate kinase	556 ± 81	493 ± 99	=0.21
hexokinase	198 ± 66	194 ± 63	=0.89

Table 1 Method: Heart tissues were powdered in liquid N_2 with mortar and pestle and extracted with 150 mmol/L NaCl, 60 mmol/L Tris-HCl (pH 7.5), 5 mmol/L EDTA, 0.2% Triton X-100, 1 mmol/L PMSF, 10 mg/mL leupeptin, and 1 mg/mL aprotinin. Extracts were centrifuged (10 minutes, 10, 000g, 4°C), and adenylate kinase and hexokinase enzyme activities were measured with coupled enzyme assays with a Spectra max M2 spectrophotometer at 340 nm, as previously described (Dzeja PP et al, *Circ Res.*1999; 84:1137-1143).

Figure 1: Isolated myocardial mitochondrial function. Mitochondrial function did not differ between control (white bars, n=7) and CK-M overexpressing (gray bars, n=7) mice for State 4 (left panel) or State 3 (center panel) respiration or for that indexed through Complex IV(right panel).*p*=NS for all comparisons.

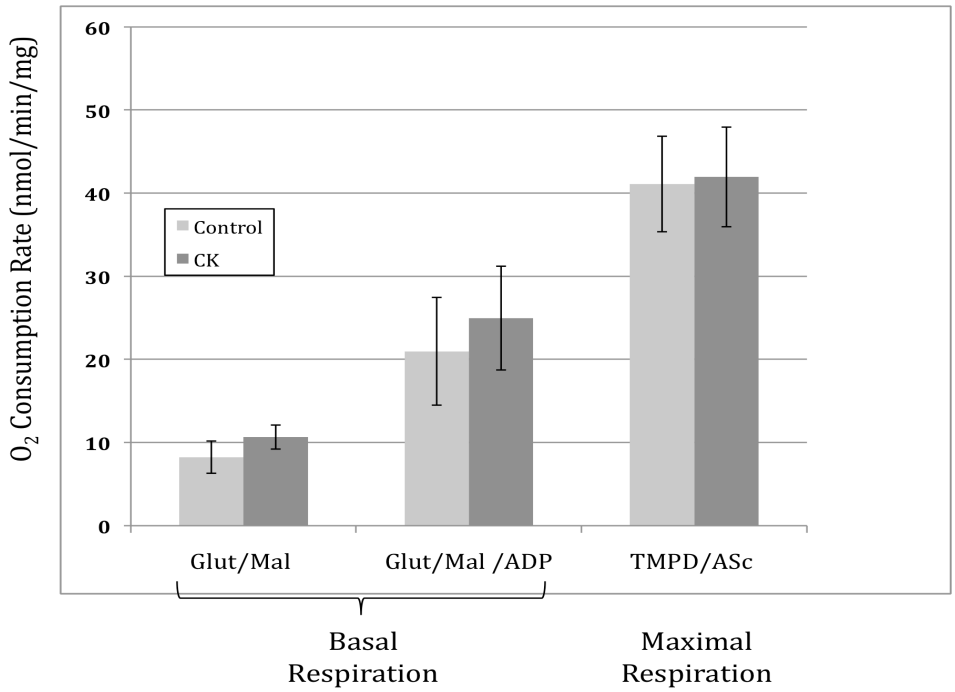


Figure 1 Methods:
Isolation of Mitochondria

Ventricles from 7 control mice and 7 CK overexpressors were each pooled into two groups for mitochondrial isolation. Tissues were minced into small pieces and rinsed with 10 ml of isolation buffer containing 20 mM HEPES pH 7.2, 200 mM mannitol, 50 mM sucrose, 1 mM EGTA, to remove residual blood. Each mince was homogenized in the same buffer (approx 25 mL) using a Potter-Elvehejm homogenizer with a Teflon pestle. The homogenates were

centrifuged at 700 x g for 10 minutes. to remove myofilaments. Mitochondria were isolated from the supernatants by centrifugation at 8000 x g for 10 minutes. The pellet, containing crude mitochondria, was washed twice by resuspension in isolation buffer and centrifugation as before. Mitochondria were stored in pellets, on ice, until use (< 1 hour).

Measurement of Mitochondrial Respiration

Final preparations were resuspended 20 mM HEPES, 137 mM KCl, 2.5 mM $MgCl_2$, 2 mM KH_2PO_4, 0.5 mM EGTA, 0.2% (w/v) BSA, pH 7.3 and were aliquoted into 96 wells (5 µg protein/well) of a polyethyleneimine-coated XF96 cell culture microplate (Seahorse Bioscience). The plate was centrifuged at 3000 x g for 14 minutes at 4°C in an A-4-62 rotor. Plates, containing 200µL of assay buffer per well, were used immediately. The cell culture microplate was incubated at 37°C and loaded into the Seahorse XF96 extracellular flux analyzer following the manufacturer's instructions. All experiments were performed at 37°C. Glutamate/Malate (5 mM each) were used as substrates to measure basal respiration. ADP-stimulated respiration was measured by the inclusion of 1mM ADP. Respiration measured, using N,N,N',N'-Tetramethyl-p-Phenylenediamine (TMPD) and Ascorbate as substrates, is a function of Complex IV activity, and approximates maximal respiration. Oxygen consumption data were acquired over 2 minutes (15 data points) from a mimimum of 7 technical replicates. The oxygen consumption rates were determined by using a compartment model-based 'deconvolution' algorithm which compensated for oxygen diffusion phenomena occurring around the entrapped volume, and for the response time of the probe. Data were analyzed using the two-tailed Student's T-test.

Figure 2: Mitochondrial anatomy

Electron micrographs for control (A), CK-M overexpressor (B), control TAC (C), d.) CK-M overexpressor TAC hearts. There is a bar in the lower right portion of each figure to indicate magnifcation.

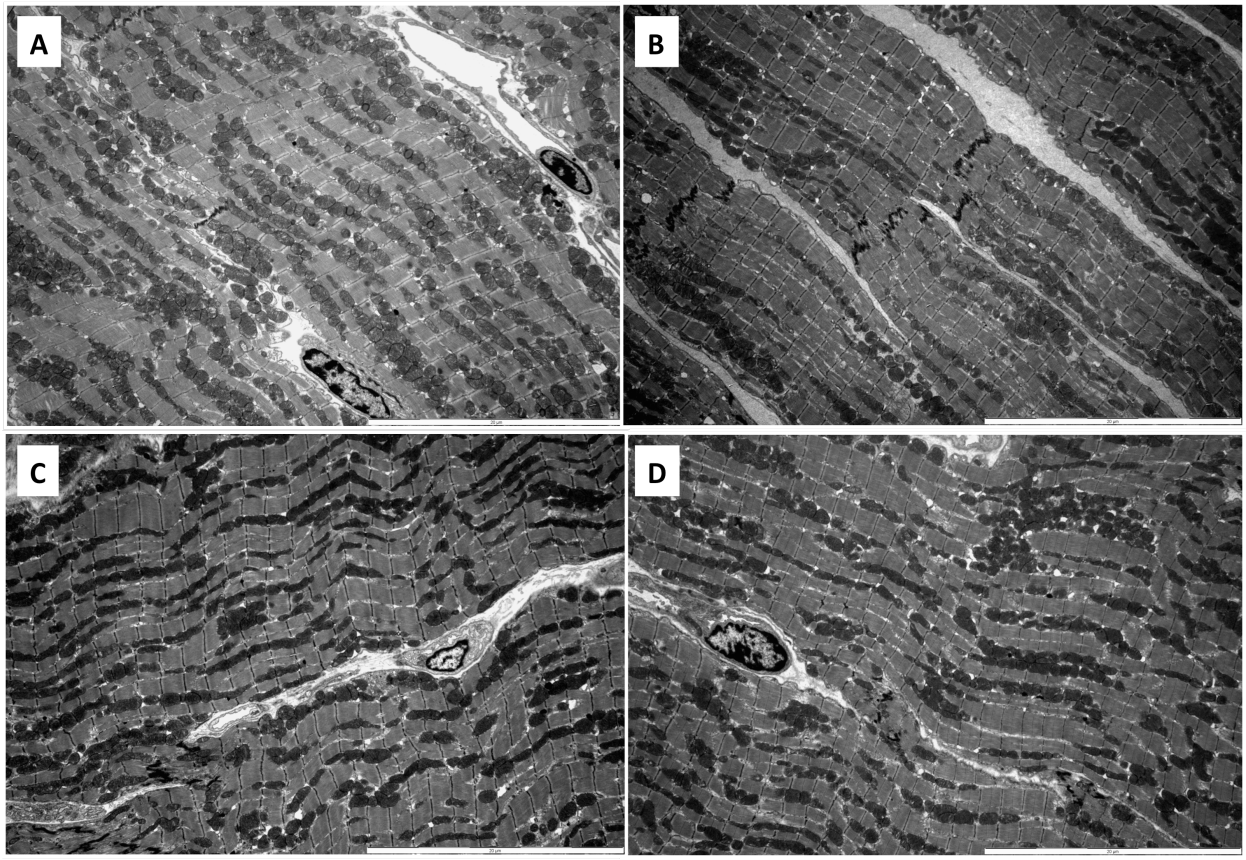


***Figure 2 Methods*:** For transmission electron microscopy (TEM), heart tissues were chemically fixed in 3.0% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.3) for 24 hours at 4ºC, rinsed in 0.1M sodium phosphate buffer, and postfixed in 1% osmium tetroxide in the same buffer for 1 hour at room temperature. The tissues were dehydrated in a graded series of ethanol and embedded in epoxy resin (EPON 812). Ultrathin sections (~100nm) were cut with a diamond knife (DIATOME), placed on 200 mesh copper grids, and stained with saturated uranyl acetate (10 min) followed by lead citrate (2 min). The sections were examined and digitally photographed with a transmission electron microscope (CM12 TEM;Philips) operating at 60 keV with magnification at x2650.

Figure 3: Contractile effects of CK-M overexpression before and during dobutamine stimulation in sham hearts. In vivo MRI assessment before (A-F) and during dobutamine (G-L) of myocardial EF (%, panels A,G), SV (μl, panels B,H), CO (ml/min, panels C,I), ESV (μl, panels D,J), EDV (μl, panels E,K) and LV mass (mg, panels F,L) in control (gray bars) and CK-M (black bars).*p*=NS for all comparisons.

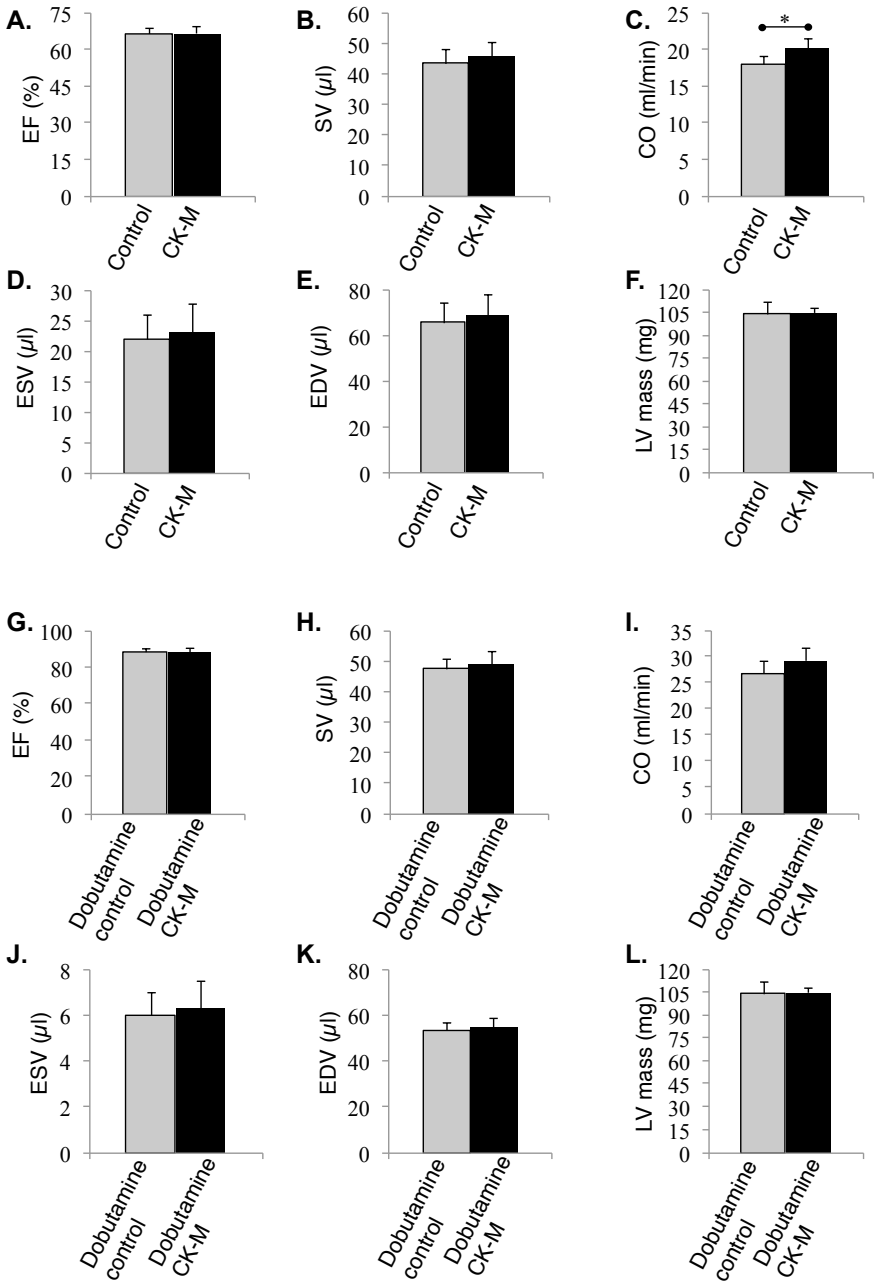
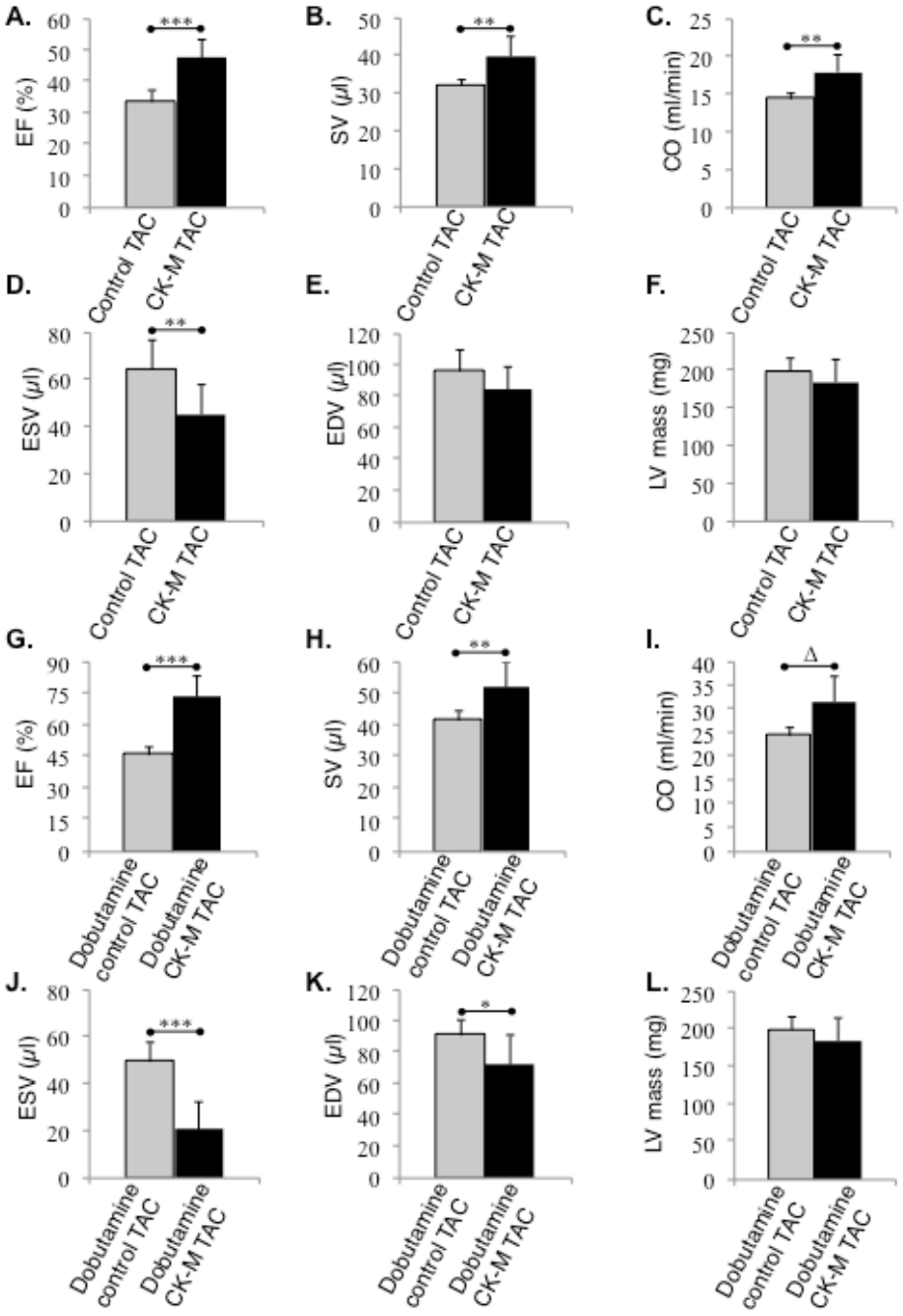


Figure 4: Contractile effects of CK-M overexpression before and during dobutamine stimulation in TAC hearts. In vivo MRI assessment before (A-F) and during dobutamine (G-L) of myocardial EF (%, panels A,G), SV (μl, panels B,H), CO (ml/min, panels C,I), ESV (μl, panels D,J), EDV (μl, panels E,K) and LV mass (mg, panels F,L) in control (gray bars) and CK-M (black bars). *p<0.05, **p<0.005, Δp<0.006, ***p<0.0001



Figures 3 and 4 Methods: Animals were studied with MRI before and after administration of 1.5μg/g body weight dobutamine ip. The inotropic effect of dobutamine was observed after a mean interval of 14±2 minutes and persisted for 80±10 minutes in preliminary studies in five animals. MRI data acquisition in the Control and CK-M study animals reported above in Figures 3 and 4 was completed within 40±5 minutes and thus all within the interval of increased stress induced by dobutamine.

Figure 5: The expression of the mitochondrial isoform of creatine kinase is not increased when the cytosolic muscle isoform (CK-M) transgene is turned on in CK-M transgenic mice.