Supplementary File

Materials and Methods

Animal models

The animal care and use committee of the University of Illinois at Chicago approved all animal protocols, which also conform to the "Guide for the Care and Use of Laboratory Animals" published by the United States National Institutes of Health, 8th edition, revised 2011. All animals used in this study were 3-5 months old. The mutant E180G α-tropomyosin (Tm180 linked to HCM) transgenic mouse model was produced in the FVB/NCrl strain as previously described (1) and the non-transgenic littermates were used as negative controls (Tm-WT). The Tm-WT (n=6) and Tm180 (n=9) of mixed sex were randomized into groups for the determination of tension-pCa relations in skinned fibers. The transgenic mice expressing cTnI-ND (n=8), an N-terminal 1-28 amino acid deletion, were heterozygotes as previously described (2) and the non-transgenic littermates were used as negative controls cTnI-WT (n=8).

Protein purification and reconstitution of troponin complexes

The mouse troponin subunits were all expressed in E. coli Rosetta 2 (DE3) cells from Millipore Sigma. The Myc-tagged mouse cardiac troponin T (cTnT) was purified as described previously (3). The mouse cardiac troponin C (cTnC), cTnI-WT, and cTnI-ND recombinant proteins were purified as previously described (2, 4). The mouse cardiac troponin complex was reconstituted from individual subunits as described (5, 6).

Ca^{2+}-dependent activation of force in skinned fibers and exchange of endogenous troponin with cTnI-ND troponin.

All reagents used are listed in the Supplementary Table S1. Measurement of steady-state isometric tension of skinned fiber bundles and cTn exchange were conducted as described with minor modifications (7-10). The FVB/NCrl mice (Tm-WT and Tm180) were heparinized (1,000 IU/kg) and anesthetized with a mixture of 200 mg/kg ketamine and 20 mg/kg xylazine, following the American Veterinary Medical Association Panel on Euthanasia Guidelines 2013. Following cardiectomy, we isolated left ventricular papillary muscles and dissected fiber bundles 4-8 mm long with a diameter of ~150-200 μm in high relax (HR) buffer (10 mM EGTA, 41.89 mM K-Prop, 100 mM BES, 6.75 mM MgCl2, 6.22 mM Na2ATP, 10 mM Na2CrP, 5 mM Na azide, pH 7.0 with inhibitors as described (10). The bundles were "skinned" by soaking for 30 min at room temperature in HR buffer containing 1% (v/v) Triton X-100. In fibers testing the effects of the presence of cTnI-ND, we exchanged the endogenous native cTn complex with exogenous Tn complex consisting of mouse cardiac cTnC, myc-tagged cTnT, as a marker, and mouse cTnI-WT or cTnI-ND. To exchange the cTn, we incubated the fibers with 26 μM of either cTnI-WT or cTnI-ND containing Tn complex in exchange buffer (200 mM KCl, 5 mM MgCl2, 5 mM EGTA, 1 mM DTT, 20 mM MOPS, 1:100 (v/v) protease inhibitor cocktail [Sigma Chemical Co. St Louis, Mo], pH 6.5) for 14.5-15.5 hours at 4°C. The next day the fibers were removed from the exchange solution and washed in HR buffer, after which the tension/pCa measurements were carried out. We mounted the fibers using cellulose-acetate glue between a force transducer and a micromanipulator; the sarcomere length (SL) was set to 2.2 μm using laser diffraction patterns and maintained throughout the experiment. After an initial stimulation to generate force at pCa 4.5, we return

the fibers to HR solution. Cross-sectional area was calculated from measures of the width and diameter along three points of the length of the fiber. The fibers were then sequentially incubated in solutions containing a range of pCa (-log[Ca^{2+}]) values prepared by mixing various ratios of the HR buffer and HR buffer containing 9.99 mM $CaCl_2$ (pCa 4.5). Force was measured in the pCa range of 8.0 to 4.5 and recorded on a chart recorder. All measurements were made at 21-22°C. The tension measurements were plotted as a function of pCa and fitted to a modified nonlinear least-squares regression analysis of the Hill equation using GraphPad Prism 8. The fitted curve yields pCa_{50} (pCa required to obtain half maximum tension) a measure of Ca^{2+} sensitivity, maximum tension, and the Hill coefficient (a measure of steepness related to cooperativity).

Determination of Tn exchange efficiency and Western blot analysis

All reagents and antibodies used are listed in the Supplementary Table S1. The exchanged fibers were solubilized in 20 µL of sample buffer (8M Urea, 2M thiourea, 50mM Tris pH 6.8, 75mM DTT, 3% (w/v) SDS and 0.005% (w/w) bromophenol blue) to separate endogenous TnT from exogenous myc-tagged TnT. All 20 µL of the sample was loaded onto a 16 cm X 18 cm X 1 mm 8%T, 1.1%C bis SDS-PAGE resolving gel (11) and a 4%T, 3.4%C bis stacking gel run in an SE600 Hoefer gel box at 22 mA with constant cooling to 8°C. After the dye front reached the bottom, the gel was cut for the region of interest to fit into a BioRad criterion tank blotter and the proteins were transferred onto 0.2 µm PVDF membrane, and Western Blot analysis was performed as previously described (12) for all experiments. The primary antibody used to probe the membranes was a mouse monoclonal anti-TnT from ThermoFisher # MS-295-PO diluted 1:1000 in 1% BSA-TBS-T (bovine serum albumin- tris-buffered saline with 0.1% (v/v) Tween-20). The secondary antibody was a horse anti-mouse IgG HRP-linked from Cell Signaling # 7076S diluted 1:20000 in %5 non-fat dry milk in TBS-T. The membranes were incubated with SuperSignal West Femto maximum sensitivity substrate reagent from Thermo Scientific and imaged with BioRad's Chemidoc MP. Immunoblot images were analyzed with Image Lab v. 6.0.1 BioRad and GraphPad Prism v. 8.4.3.

Statistical analysis

The force-pCa measurements and Western blots were analyzed with GraphPad Prism v. 8.4.3. All comparisons were normally distributed (Shapiro-Wilk tested) and when equal variance (F-test) an unpaired t-test was used, however, if the variance was unequal an unpaired t-test with Welch's correction was used for binary comparisons between Tm180 vs Tm-WT or cTnI-WT vs cTnI-ND. A 2-way ANOVA with Tukey's multiple comparison testing was used to compare more than two groups with two independent variables as in the exchanged force-pCa measurements. Gaussian distribution was tested with Shapiro-Wilk and Spearman's test for heteroscedasticity. The mean ± SEM was reported for all measurements with a P-value significance set at 0.05.

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Supplementary Table S1. Antibodies and reagents used in study.

Antibodies/Reagents	**Catalog #**	**Supplier**
M-mAb anti-cardiac troponin T	MS-295-PO	Thermo Fisher Scientific
Horse anti-mouse IgG HRP-linked	7076S	Cell Signaling Technology
Urea	U20200	Research Products International
Thiourea	T7875	Sigma-Aldrich
Tris-Base	BP152	Thermo Fisher Scientific
DTT	DTT10	GoldBio
Bromophenol Blue	161-0404	BioRad
Immun-bolt PVDF membrane	162-0177	BioRad
Tween-20	BP377	Thermo Fisher Scientific
Non-Fat dry milk	232100	BD Difco
Super Signal West Femto	34096	Thermo Fisher Scientific
EGTA	E4378	Sigma-Aldrich
Propionic acid	P1386	Sigma-Aldrich
Potassium Hydroxide	P250	Thermo Fisher Scientific
BES	B9879	Sigma-Aldrich
MgCl2	M1028	Sigma-Aldrich
Na2ATP	A3377	Sigma-Aldrich
Disodium phosphocreatine	P7936	Sigma-Aldrich
Na azide	S2002	Sigma-Aldrich
MOPS	M5162	Sigma-Aldrich
Protease inhibitors	P8340	Sigma-Aldrich
Triton X-100	ADX100	Anatrace
CaCl2	21114	Honeywell Fluka

Abbreviations used: M-mAb, mouse monoclonal antibody.