Molecular determinants of cardiac myocyte performance as conferred by isoform-specific TnI residues

by

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Supporting Material

Methods:

Ventricular myocyte isolation, gene transfer, and primary culture Adult rat ventricular myocyte isolation was performed as previously described [1, 2]. Briefly, adult female rats were anaesthetized by inhalation of isoflurane followed by i.p. injection of heparin (1500 U/kg) and Nembutal (162.5 U/kg). Following enzymatic digestion by retro-grade perfusion with collagenase and gentle trituration of the cardiac ventricles, cardiac myocytes were plated on laminin-coated glass coverslips ($2x10^4$myocytes/coverslip) and cultured in M199 media (Sigma, supplemented with 10 mmol/L glutathione, 26.2 mmol/L sodium bicarbonate, 0.02% bovine serum albumin, and 50 U/ml penicillin-streptomycin, with pH adjusted to 7.4, additionally insulin (5µg/ml), transferrin (5µg/ml) and selenite (5ng/ml) (ITS)were added (Sigma I1884)).One hour after plating, nonadherent cells were removed and recombinant adenovirus expressing cTnI-Flag isoforms were applied to the cells as previously described [3, 4]. Cells were cultured for three days to provide sufficient time for stoichiometric replacement of the troponin I isoforms.

Immunoblot detection Myocytes on day 3 after transduction were scraped off cover slips and placed in Laemmli sample buffer. Proteins were separated by SDS-PAGE and transferred to a PVDF membrane for immunodetection. After blocking in Li-Cor blocking buffer, membranes were probed with a pan troponin I antibody (MAB1691; 1:5000, Chemicon) or cTnI specific antibody (4C2; 1:4000, Advanced ImmunoChemical, Inc). Indirect immunodetection was carried out using a fluorescently labeled anti-mouse secondary antibody (Li-Cor, IRDye 680 conjugated

affinity purified; 1:10,000). Western blot analysis was accomplished using the infrared imaging system, Odyssey (Li-Cor, Inc.) and images analyzed using Odyssey software v3.0.

Indirect Immunofluorescence Myocytes day 3 after transduction were fixed in 4% paraformaldehyde and blocked in 20% goat serum. Fixed coverslips were stained with primary antibody M2 Flag (1:250, Sigma) and secondary antibody Alexafluor 488 conjugated goat anti-mouse (1:500, Molecular Probes). Immunofluorescence was visualized on a Zeiss Axioskop LSM 510 laser scanning confocal microscope. Images shown are a Multiple intensity project produced in Zen 2009, of a Z-stack through the entire cell.

Contractility measurements in single intact myocytes Sarcomere length dynamics and kinetics were measured as previously described [1, 2]. Briefly, cover slips containing single isolated myocytes, day three after isolation, were placed on an inverted microscope (Nikon, Eclipse TE2000) and electrically stimulated at 0.2 Hz in a 37°C media bath. Sarcomere length recordings were collected (1000Hz) using a CCD camera (MyoCam, IonOptix). Myocytes that did not follow the pacing protocol (0.2Hz) were excluded. Sarcomere length shortening and relaxation kinetics were calculated using IonOptix software. Myocytes were initially analyzed under baseline conditions in M199 pH 7.4 (no bicarbonate, no ITS) and then analyzed in M199 pH 6.2 (no bicarbonate, no ITS) for acidosis. Since control (non-transduced) and cTnI-FLAG transduced myocytes had no functional difference in sarcomere dynamics the data were combined into the control dataset.

Table S1

cTnC: cTnI WT				cTnC:sTnI				cTnC: cTnI QAEH HSD				cTnC:cTnI QAEH HSP			
	pK_a^{int} ($\varepsilon_{in}=4$)	$\Delta pK_a^{charges}$ ($\varepsilon_{eff}=40$)	pK_a^{p}		pK_a^{int} ($\varepsilon_{in}=4$)	$\Delta pK_a^{charges}$ ($\varepsilon_{eff}=40$)	pK_a^{p}		pK_a^{int} ($\varepsilon_{in}=4$)	$\Delta pK_a^{charges}$ ($\varepsilon_{eff}=40$)	pK_a^{p}		pK_a^{int} ($\varepsilon_{in}=4$)	$\Delta pK_a^{charges}$ ($\varepsilon_{eff}=40$)	pK_a^{p}
cTnC				**cTnC**				**cTnC**				**cTnC**			
GLU-14	4.04	-0.27	3.78	**GLU-14**	4.06	-0.12	3.93	**GLU-14**	4.40	-0.43	3.97	**GLU-14**	4.01	-0.33	3.68
GLU-15	3.99	**0.05**	4.04	**GLU-15**	4.41	**-0.82**	3.59	**GLU-15**	3.81	**0.02**	3.83	**GLU-15**	4.33	**-1.26**	3.07
LYS-17	9.69	1.07	10.76	**LYS-17**	9.85	1.08	10.93	**LYS-17**	9.77	1.24	11.01	**LYS-17**	9.69	1.09	10.78
GLU-19	4.83	**-0.61**	4.22	**GLU-19**	5.69	**-1.41**	4.28	**GLU-19**	4.17	**-0.39**	3.78	**GLU-19**	5.15	**-1.22**	3.94
LYS-21	8.89	1.41	10.30	**LYS-21**	9.63	1.05	10.68	**LYS-21**	9.61	0.85	10.46	**LYS-21**	9.63	1.17	10.80
ASP-25	4.90	-0.38	4.52	**ASP-25**	4.07	-0.23	3.84	**ASP-25**	4.55	-0.87	3.68	**ASP-25**	4.83	-0.82	4.01
GLU-32	4.84	0.08	4.92	**GLU-32**	4.13	0.24	4.37	**GLU-32**	5.14	-1.03	4.11	**GLU-32**	4.88	-0.49	4.39
ASP-33	3.69	0.02	3.72	**ASP-33**	3.34	0.12	3.46	**ASP-33**	2.98	-0.13	2.86	**ASP-33**	3.76	-0.19	3.56
LYS-39	9.75	**1.12**	10.88	**LYS-39**	9.97	**0.24**	10.20	**LYS-39**	9.39	**1.19**	10.59	**LYS-39**	8.98	**1.26**	10.24
GLU-40	4.60	**-0.91**	3.69	**GLU-40**	5.88	**-1.22**	4.66	**GLU-40**	5.26	**-1.13**	4.13	**GLU-40**	5.73	**-1.59**	4.14
LYS-43	9.45	**0.36**	9.81	**LYS-43**	9.86	**1.05**	10.91	**LYS-43**	7.72	**1.82**	9.54	**LYS-43**	8.51	**1.54**	10.05
cTnI WT				**sTnI**				**cTnI QAEH**				**cTnI QAEH**			
ASP-153	3.88	-0.18	3.69	**ASP-121**	3.89	-1.42	2.47	**ASP-153**	3.73	-0.52	3.21	**ASP-153**	3.28	-1.13	2.15
				ARG-125	11.52	0.04	11.56	**ARG-157**	11.58	0.16	11.75	**ARG-157**	11.61	0.05	11.66
ARG-163	11.01	0.17	11.18	**LYS-131**	10.32	0.19	10.51	**ARG-163**	11.34	0.18	11.52	**ARG-163**	11.91	-0.01	11.89
				HIS-132	3.89	**0.91**	4.81	**HIS-164**	5.93	**-0.35**	5.58	**HIS-164**	4.76	**0.30**	5.05
LYS-165	10.13	0.43	10.56	**LYS-133**	9.79	1.22	11.01	**LYS-165**	10.22	0.04	10.25	**LYS-165**	9.77	0.33	10.10
GLU-166	4.70	-0.45	4.25												
ASP-169	5.03	-1.18	3.85	**ASP-137**	3.74	-0.09	3.65	**ASP-169**	4.47	-0.46	4.01	**ASP-169**	4.45	-0.70	3.76
ARG-171	11.90	0.00	11.91	**ARG-139**	11.14	0.14	11.28	**ARG-171**	11.97	-0.05	11.92	**ARG-171**	11.71	0.09	11.80
HIS-173	6.56	0.60	7.16												

Table S1: pK_a analysis of cTnC and TnI. Predicted pK_a measurements for all ionizable groups highlighting regions of difference between cTnI and sTnI (bold). Averaged pK_a for three independent frames for three simulations for all groups.

Supporting References

1. Herron, T.J., et al., *Calcium-independent negative inotropy by beta-myosin heavy chain gene transfer in cardiac myocytes.* Circ Res, 2007. **100**(8): p. 1182-90.
2. Davis, J., et al., *Thin filament disinhibition by restrictive cardiomyopathy mutant R193H troponin I induces Ca2+-independent mechanical tone and acute myocyte remodeling.* Circ Res, 2007. **100**(10): p. 1494-502.
3. Palpant, N.J., et al., *pH-responsive titratable inotropic performance of histidine-modified cardiac troponin I.* Biophys J, 2012. **102**(7): p. 1570-9.
4. Davis, J., et al., *Allele and species dependent contractile defects by restrictive and hypertrophic cardiomyopathy-linked troponin I mutants.* J Mol Cell Cardiol, 2008. **44**(5): p. 891-904.