Uncoupling of myofilament Ca^{2+}-sensitivity from troponin I phosphorylation can be reversed by Epigallocatechin-3-Gallate

Maria Papadaki, Petr G. Vikhorev, Steven B. Marston and Andrew E. Messer

National Heart and Lung Institute, Imperial College London, London, W12 0NN, UK

SUPPLEMENTARY MATERIAL

Supplement A

Supplementary Methods

Myofibril force assay methods

Measurement of contractility in phosphorylated and unphosphorylated mouse myofibrils

We used heart muscle from heterozygous *ACTC* E361G transgenic mice and non-transgenic (NTG: hybrid strain C57Bl/6xCBA/Ca) mice as the control (male and female, 28 weeks of age). Experiments and animal handling were done in accordance with College guidelines. Mice were killed by cervical dislocation as required by Schedule I of the UK Animals (Scientific Procedures) Act 1986.

Propranolol treatment

Mice were anesthetized with 5 % isoflurane (IsoFlo, Abbott Laboratories, UK) v/v in 100 % oxygen (0.5 mL/min), weighed and then transferred to a heated surgical table (VetTech, UK) where anaesthesia was maintained at 2.5 % isoflurane v/v in 100 % O_2 (0.5 mL/min) using a custom-made nose cone. A bolus of propranolol (8 mg/kg BW, Sigma, UK) was injected in to the subclavian vein. Mice were then kept in an anaesthetic induction chamber for 30 min with 1.5% isoflurane,

then the mice were sacrificed and the heart was removed and tissue samples (<5 mg) were dissected from the left ventricle, frozen and kept in liquid nitrogen until myofibrils were prepared. Control samples were obtained from anesthetized mice that did not receive propranolol.

Isolated myofibrils

Single myofibrils or thin bundles were prepared from one piece of frozen heart sample by permeabilisation and subsequent homogenization. Samples were immersed for 3 h in 2 mL of permeabilisation solution containing (mM): Tris 10 (pH 7.1), NaCl 132, KCl 5, $MgCl_2$ 1, EGTA 5, dithiothreitol (DTT) 5, NaN_3 10, 2,3-butanedione-monoxime (BDM) 20, and 1 % Triton X-100. All of our solutions contained the following protease inhibitors (μM): chymostatin 10, pepstatin 5, leupeptin 10, E-64 **(**trans-Epoxysuccinyl-L-leucylamido(4-guanidino)butane) 10**,** and PMSF (phenylmethlysufonyl fluoride) 200. Triton X-100 and BDM were then removed from the permeabilised samples using washing solution (like permeabilisation solution but without Triton X-100 and BDM), and finally the samples were homogenised for 15 s with Ultra-Turrax T10 blender (IKA Werke GmbH & Co., Staufen, Germany) to produce a suspension of myofibrils. The suspension was washed two times by centrifugation and suspension in the washing solution. A final pellet was dissolved in 300 μL of washing solution and kept on ice for use within 3 days.

Apparatus for measurement of myofibril contractility

Contraction and relaxation were initiated using a fast-solution change system and sensitive force transducer system [1,2], which was based on those described [3-6]. Briefly, our apparatus for measurement of force in single myofibrils was built around an inverted microscope (Eclipse Ti-U, Nikon UK Ltd., Surrey, UK) equipped with two micromanipulators (MP-285, Sutter Instruments, Novato, and Huxley-type micromanipulator) and a CCD camera (Rolera XR, Qimaging, Surrey, Canada). The myofibrils were manipulated by means of two fine glass microneedles mounted on the micromanipulators. One of them was a cantilever force sensor. Under illumination of a 5 mW HeNe laser, the shadow of the tip of the cantilever force sensor was projected on a photodiode position detector (Spot-2D, UDT Sensors, Hawthorne, CA). The extent of bending of the cantilever was proportional to the force on the cantilever, so the force produced by a myofibril was measured from the photodiode's current response. Each cantilever force sensor was calibrated by measuring its compliance using the needle of a micro-ammeter to apply known forces to the cantilever and observing the extent of bending; the range of measured cantilever compliances was 2-14 μm/μN.

Rapid activation and relaxation in myofibrils was achieved using an ultra-fast solution change system constructed from a double-barrelled micro-pipette mounted on a stepper motor that switched solutions in less than 10 ms. The micro-pipette was positioned perpendicular to the long axis of the myofibril. The relaxing and activating solutions were applied via adjacent barrels of the micro-pipette with flow being driven by gravity. Temperature was controlled to 17 °C The stepper motor controlled the position of the micro-pipette relative to the myofibril, thus enabling changes between the two solutions, each flowing in a laminar pattern.

An 8-channel valve (HVXM 8-5, Hamilton, VWR, UK), controlled by a stepper motor, was used to perfuse one barrel of the micro-pipette with a range of different activating solutions. We applied 8 different solutions, ranging from low to high [Ca^{2+}]. Myofibrils were first activated at the highest concentration of Ca^{2+} and then solutions ranging from low to high [Ca^{2+}] were applied with contractions being initiated from 10^{-8} M Ca^{2+}. EGCG was added to all solutions at a concentration of 10 μM. The time lag in a change of solutions was less than 5 s.

Experimental solutions

Activating (3.98-0.79 μM Ca^{2+}) and relaxing (0.01 μM Ca^{2+}) solutions contained (mM): MOPS 10 (pH 7.0), MgATP 5, free Mg^{2+} 1, DTT 5, phosphocreatine 10, and creatine kinase (200 Units/mL). The Ca-EGTA:EGTA ratio was set to obtain 10 mM total EGTA and the desired free [Ca^{2+}]. Potassium propionate and sodium sulphate were added to adjust the ionic strength of the final solution to 200 mM.

(-)-Epigallocatechin gallate (EGCG, Sigma-Aldrich, UK) was dissolved in 10 mM HCl solution to a final concentration of 10 mM and stored at -20 °C for up to one week as a stock solution. EGCG from the stock solution was added to activating and relaxing solutions at a concentration of 10 μM. Myofibrils were incubated in EGCG containing relaxing solution for 15-30 min at 17 °C before measurements were started.

Experimental Protocol

A small droplet of myofibril solution was placed on the bottom of the glass chamber (temperature controlled to 17 °C). Then the chamber was filled with relaxing solution. The selected myofibril or thin bundle was positioned horizontally between the microneedles described above and viewed with the video camera mounted on the microscope. Sarcomere length (SL) was set to 2.17, while observing the striation pattern in the myofibril image using the fast Fourier transform analysis function in LabVIEW. The length and diameter of the myofibril was measured using ImageJ (Rasband, W.S., ImageJ, U.S. Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011). Cross-sectional area was calculated from the observed diameter assuming circular cross-section. Contraction and relaxation was initiated by the fast solution switch system described above. At each [Ca^{2+}] a number of activation-relaxation cycles (range 1-3) were performed by the myofibril and the average value of measured parameters for the set of cycles was reported. Each contraction lasted for 2-5 s.

The kinetic parameters, maximum force (F_{max}), rate of force development (k_{ACT}), duration (t_{LIN}) of slow relaxation phase and the rate of fast relaxation phase (k_{REL}) were determined from the tension traces. The slow relaxation phase was counted from the initiation of the micro-pipette movement. The beginning of the exponential phase of relaxation was considered as the end of slow relaxation phase.

Data Collection and Analysis

Apparatus control and data recording were done using a data acquisition device (NI USB-6251, National Instruments, Newbury, UK) and custom-written software in LabVIEW 2011 (National Instruments, Newbury, UK). The rate constants for the exponential force development upon activation (k_{ACT}) and fast phase of relaxation (k_{REL}) were evaluated by curve-fitting using Levenberg-Marquardt nonlinear least square algorithm in LabVIEW.

The maximum force was obtained by curve-fitting force development upon activation. The force data from the force-[Ca^{2+}] experiments were fitted to the Hill equation: $y=y_0+F_{max}[Ca^{2+}]^{n_H}/(EC_{50}{}^{n_H}+[Ca^{2+}]^{n_H})$ by adjusting the values of n_H and EC_{50}. Data were fitted and compared by an unpaired *t*-test using GraphPad Prism 6 (GraphPad software, San Diego, CA). Best fit parameters marked as ambiguous by GraphPad Prism were excluded from further analysis. In some cases the bottom plateau (y_0) was constrained to a constant value of 0.

The table (Table 3) reports the means and SE of EC_{50}, n_H, F_{max} and k_{ACT}, t_{LIN} and k_{REL} values at maximum activation, determined in 5-15 experiments with separate myofibrils.

Supplement B

Analysis of measurements of the Ca^{2+}-dependence of fraction motile and sliding speed (motile velocity) measured by *in vitro* motility assay, fitted to the 4-parameter Hill equation. The effects of TnI phosphorylation in the presence and absence of EMD57033, Bepridil and EGCG on EC_{50}, n_H, maximum fraction motile and maximum sliding speed are shown.

Effect of EMD on wild-type troponin

Fraction Motile	EC50, μM				Ratio	Hill co-efficient				Max fraction motile				EC50, μM				Ratio	Hill co-efficient				Max fraction motile				Ratio	Ratio
	P	SD	unP	SD	P/unP	P	SD	unP	SD	P	SD	unP	SD	P + EMD	SD	unP + EMD	SD	P+E/unP+E	P + EMD	SD	unP + EMD	SD	P + EMD	SD	unP +EMD	SD	P/P+EMD	unP/unP+EMD
Exp 1	0.12	0.01	0.06	0.01	2.00	2.34	0.75	1.97	0.33	0.82	0.04	0.83	0.04	0.03	0.003	0.03	0.002	1.07	1.16	0.14	1.19	0.13	0.87	0.01	0.86	0.02	3.44	1.84
Exp 2	0.15	0.04	0.11	0.02	1.44	1.72	0.52	1.19	0.21	0.81	0.02	0.81	0.02	0.08	0.017	0.07	0.011	1.01	1.16	0.29	1.09	0.18	0.82	0.04	0.81	0.02	2.05	1.44
Exp 3	0.13	0.01	0.08	0.01	1.71	2.28	0.29	1.88	0.21	0.78	0.04	0.80	0.02	0.07	0.002	0.06	0.004	1.12	1.23	0.05	1.37	0.11	0.84	0.03	0.85	0.01	1.87	1.23
Exp 4	0.23	0.01	0.12	0.02	1.93	1.98	0.08	0.98	0.15	0.78	0.03	0.74	0.02	0.05	0.002	0.04	0.003	1.11	1.47	0.14	1.72	0.69	0.77	0.06	0.76	0.05	4.91	2.83
Mean	0.16		0.09		1.77	2.08		1.51		0.80		0.80		0.06		0.05		1.08	1.26		1.34		0.83		0.82		3.07	1.83
Std Error	0.03		0.01		0.13	0.14		0.25		0.01		0.02		0.01		0.01		0.03	0.07		0.14		0.02		0.02		0.71	0.36

Sliding Speed	EC50, μM				Ratio	Hill co-efficient				ΔVmax, μm/sec				EC50, μM				Ratio	Hill co-efficient				ΔVmax, μm/sec				Ratio	Ratio
	P	SD	unP	SD	P/unP	P	SD	unP	SD	P	SD	unP	SD	P + EMD	SD	unP + EMD	SD	P+E/unP+E	P + EMD	SD	unP + EMD	SD	P + EMD	SD	unP +EMD	SD	P/P+EMD	unP/unP+EMD
Exp 1	0.08	0.01	0.04	0.005	2.08	5.11	2.21	1.94	0.57	1.23	0.02	1.30	0.05	0.03	0.01	0.03	0.01	1.06	0.93	0.32	1.00	0.39	1.70	0.12	1.69	0.12	1.38	1.30
Exp 2	0.09	0.01	0.04	0.003	2.06	3.02	1.28	1.60	0.22	1.38	0.07	1.33	0.03	0.03	0.01	0.03	0.01	1.14	0.82	0.20	0.96	0.28	1.58	0.09	1.51	0.08	1.14	1.14
Exp 3	0.09	0.02	0.05	0.005	1.74	4.50	2.13	1.65	0.30	1.26	0.08	1.38	0.04	0.03	0.01	0.03	0.01	0.96	0.89	0.24	0.90	0.21	1.59	0.09	1.63	0.09	1.26	1.18
Exp 4	0.29	0.07	0.18	0.074	1.58	1.03	0.22	0.50	0.10	1.73	0.35	1.19	0.13	0.05	0.03	0.05	0.03	1.11	0.82	0.35	0.88	0.43	1.32	0.15	1.33	0.16	0.76	1.12
Mean	0.14		0.08		1.87	3.42		1.42		1.40		1.30		0.04		0.04		1.07	0.87		0.94		1.55		1.54		1.14	1.18
Std Error	0.05		0.03		0.12	0.91		0.32		0.11		0.04		0.01		0.01		0.04	0.03		0.03		0.08		0.08		0.13	0.04

Effect of Bepridil on wild-type troponin

Fraction Motile	EC50, μM				Ratio	Hill co-efficient				Max fraction motile				EC50, μM				Ratio	Hill co-efficient				Max %				Ratio	Ratio
	P	SD	unP	SD	P/unP	P	SD	unP	SD	P	SD	unP	SD	P+Bepridil	SD	unP+Bepridil	SD	P+B/unP+B	P+Bepridil	SD	unP+Bepridil	SD	P+Bepridil	SD	unP+Bepridil	SD	P/P+Bep	unP/unP+Bep
Exp 1	0.11	0.002	0.06	0.00720	1.74	3.44	0.59	1.74	0.33	0.95	0.060	0.98	0.03	0.04	0.0011	0.04	0.001	1.06	1.87	0.11	1.69	0.31	0.99	0.03	1.00	0.01	2.58	1.84
Exp 2	0.14	0.029	0.08	0.00340	1.83	1.81	0.58	2.08	0.16	0.97	0.030	0.98	0.02	0.05	0.0015	0.04	0.001	1.04	1.77	0.12	2.37	0.13	0.97	0.03	0.95	0.02	3.06	1.02
Exp 3	0.14	0.016	0.08	0.00056	1.74	1.94	0.43	1.86	0.02	0.94	0.030	0.93	0.02	0.04	0.0041	0.04	0.001	1.00	1.92	0.37	1.86	0.12	0.97	0.01	0.96	0.02	3.19	1.01
Exp 4	0.13	0.006	0.07	0.01300	1.77	2.36	0.31	1.81	0.49	0.92	0.001	0.92	0.02	0.04	0.0003	0.04	0.001	1.05	1.71	0.02	1.73	0.09	0.93	0.02	0.94	0.01	2.92	1.38
Exp 5	0.12	0.002	0.07	0.00003	1.68	3.28	0.38	2.25	0.00	0.94	0.008	0.94	0.01	0.05	0.0005	0.05	0.003	1.01	2.23	0.04	2.32	0.24	0.96	0.02	0.96	0.01	2.35	1.47
Exp 6	0.11	0.017	0.07	0.00094	1.62	2.23	1.04	2.03	0.04	0.98	0.020	0.97	0.01	0.05	0.0007	0.05	0.003	1.01	2.31	0.08	2.22	0.28	0.96	0.02	0.98	0.01	2.44	0.97
Mean	0.13		0.07		1.73	2.51		1.96		0.95		0.95		0.05		0.04		1.03	1.97		2.03		0.96		0.96		2.76	1.28
Std Error	0.01		0.002		0.03	0.28		0.08		0.01		0.01		0.001		0.001		0.01	0.10		0.13		0.01		0.01		0.14	0.14

Sliding Speed	EC50, μM				Ratio	Hill co-efficient				ΔVmax, μm/sec				EC50, μM				Ratio	Hill co-efficient				ΔVmax, μm/sec				Ratio	Ratio
	P	SD	unP	SD	P/unP	P	SD	unP	SD	P	SD	unP	SD	P+Bepridil	SD	unP+Bepridil	SD	P+B/unP+B	P+Bepridil	SD	unP+Bepridil	SD	P+Bepridil	SD	unP+Bepridil	SD	P/P+Bep	unP/unP+Bep
Exp 1	0.12	0.001	0.09	0.022	1.34	2.91	0.18	1.17	0.34	2.29	0.010	2.41	0.18	0.06	0.013	0.05	0.0095	1.28	1.13	0.36	1.85	1.56	2.70	0.18	2.57	0.08	1.99	1.18
Exp 2	0.18	0.012	0.09	0.013	1.91	1.12	0.69	0.89	0.12	2.44	0.640	2.58	0.14	0.07	0.049	0.06	0.0150	1.13	1.32	0.52	1.17	0.35	2.95	0.87	2.59	0.25	2.48	1.21
Exp 3	0.10	0.007	0.06	0.003	1.71	1.95	0.27	1.40	0.08	1.73	0.060	1.78	0.03	0.03	0.002	0.04	0.0026	0.93	1.46	0.13	1.41	0.18	2.06	0.04	1.86	0.06	3.24	1.19
Exp 4	0.14	0.019	0.07	0.005	2.10	2.06	0.53	1.43	0.17	1.45	0.070	1.38	0.04	0.02	0.011	0.05	0.0110	0.49	0.91	0.39	0.93	0.26	1.76	0.15	1.79	0.11	5.91	1.21
Exp 5	0.12	0.005	0.09	0.031	1.33	2.40	0.34	1.14	0.44	1.84	0.050	1.98	0.27	0.05	0.001	0.05	0.0015	1.02	1.39	0.05	1.32	0.07	1.93	0.02	2.04	0.03	2.53	1.05
Exp 6	0.11	0.002	0.07	0.006	1.64	2.21	0.11	1.98	0.25	2.55	0.030	2.64	0.11	0.05	0.002	0.05	0.0001	1.01	1.96	0.13	1.90	0.01	2.67	0.05	2.61	0.00	2.40	1.05
Mean	0.13		0.08		1.67	2.11		1.34		2.05		2.13		0.05		0.05		0.98	1.36		1.43		2.35		2.24		3.09	1.15
Std Error	0.01		0.01		0.13	0.24		0.15		0.18		0.20		0.01		0.004		0.11	0.14		0.16		0.20		0.16		0.59	0.03

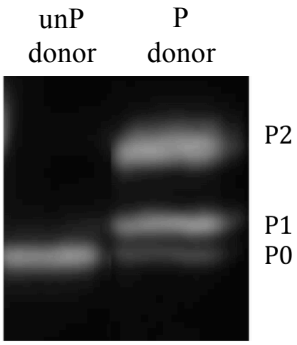
Effect of EGCG on wild-type troponin

Fraction Motile	EC50, µM				Ratio	Hill co-efficient				Max fraction motile					EC50, µM				Ratio	Hill co-efficient				Max fraction motile				Ratio	Ratio
	P	SD	unP	SD	P/unP	P	SD	unP	SD	P	SD	unP	SD		P + EGCG	SD	unP + EGCG	SD	P+E/unP+E	P + EGCG	SD	unP + EGCG	SD	P + EGCG	SD	unP + EGCG	SD	P+EGCG/P	unP+EGCG/unP
Exp 1	0.18	0.055				1.30	0.44			0.77	0.03				0.27	0.0660				1.51	0.530			0.60	0.02			1.51	
Exp 2	0.15	0.020	0.06	0.009	2.42	2.59	0.88	1.72	0.42	0.78	0.01	0.85	0.04		0.18	0.0120	0.14	0.02	1.33	3.24	0.380	3.27	1.36	0.86	0.04	0.83	0.03	1.24	2.25
Exp 3	0.27	0.026	0.11	0.063	2.53	1.39	0.16	0.75	0.36	0.71	0.04	0.66	0.02		0.34	0.0540	0.21	0.04	1.61	1.65	0.470	1.53	0.31	0.66	0.02	0.64	0.01	1.24	1.95
Exp 4	0.08	0.013	0.04	0.045	1.91	0.98	0.24	2.06	1.08	0.92	0.03	0.93	0.02		0.35	0.0350	0.20	0.03	1.70	2.39	0.540	1.39	0.17	0.65	0.01	0.73	0.02	4.58	5.15
Exp 5	0.13	0.017	0.06	0.003	2.15	1.49	0.26	1.87	0.13	0.95	0.01	0.96	0.02		0.22	0.0120	0.14	0.02	1.54	1.60	0.099	1.31	0.17	0.86	0.04	0.92	0.03	1.69	2.36
Exp 6	0.07	0.010	0.03	0.007	2.04	1.82	0.40	1.01	0.28	0.96	0.02	0.96	0.02		0.20	0.0350	0.08	0.01	2.50	1.49	0.290	1.53	0.19	0.85	0.03	0.92	0.02	2.77	2.26
Exp 7	0.12	0.001	0.05	0.001	2.39	1.68	0.03	1.61	0.03	0.97	0.01	0.96	0.01		0.24	0.0003	0.14	0.02	1.72	1.87	0.003	1.68	0.35	0.92	0.04	0.94	0.02	2.05	2.84
Mean	0.14		0.06		2.24	1.61		1.50		0.87		0.89			0.26		0.15		1.73	1.96		1.79		0.77		0.83		2.15	2.80
Std Error	0.03		0.01		0.10	0.19		0.21		0.04		0.05			0.02		0.02		0.16	0.24		0.30		0.05		0.05		0.45	0.48

Sliding Speed	EC50, µM				Ratio	Hill co-efficient				ΔVmax, µm/sec					EC50, µM				Ratio	Hill co-efficient				ΔVmax, µm/sec				Ratio	Ratio
	P	SD	unP	SD	P/unP	P	SD	unP	SD	P	SD	unP	SD		P + EGCG	SD	unP + EGCG	SD	P+E/unP+E	P + EGCG	SD	unP + EGCG	SD	P + EGCG	SD	unP + EGCG	SD	P+EGCG/P	unP+EGCG/unP
Exp 1	0.14	0.0370				1.31	0.38			1.51	0.19				0.27	0.0520				1.42	0.380			0.84	0.090			1.96	
Exp 2	0.12	0.0130	0.05	0.020	2.21	2.72	0.59	1.76	0.56	1.43	0.12	1.45	0.27		0.15	0.0064	0.11	0.003	1.35	1.94	0.140	1.87	0.09	1.35	0.030	1.34	0.02	1.28	2.09
Exp 3	0.13	0.0280	0.04	0.006	3.00	1.52	0.40	1.91	0.51	1.45	0.15	1.25	0.08		0.24	0.0570	0.12	0.009	2.09	2.13	0.690	1.92	0.26	1.27	0.150	1.23	0.05	1.90	2.74
Exp 4	0.11	0.0430	0.04	0.014	2.53	0.86	0.31	1.26	0.79	2.09	0.22	1.85	0.10		0.57	0.1600	0.14	0.056	3.98	0.84	0.190	0.75	0.23	1.22	0.160	1.19	0.14	5.06	3.21
Exp 5	0.11	0.0028	0.06	0.014	1.70	1.17	0.03	1.07	0.01	2.01	0.02	2.04	0.18		0.32	0.0170	0.08	0.006	4.09	0.89	0.210	1.38	0.14	1.50	0.160	1.64	0.05	2.96	1.23
Exp 6	0.07	0.0004	0.05	0.004	1.29	2.80	0.03	2.38	0.29	2.67	0.01	2.74	0.11		0.24	0.0003	0.09	0.019	2.84	1.63	0.002	1.69	0.57	1.84	0.001	1.95	0.21	3.52	1.59
Exp 7	0.11	0.0110	0.06	0.001	1.94	1.30	0.16	1.41	0.05	2.18	0.10	2.18	0.02		0.27	0.0500	0.09	0.009	3.12	1.11	0.190	1.63	0.26	1.63	0.120	1.74	0.09	2.52	1.57
Mean	0.11		0.05		2.11	1.67		1.63		1.91		1.92			0.30		0.10		2.91	1.42		1.54		1.38		1.52		2.74	2.07
Std Error	0.01		0.003		0.25	0.29		0.20		0.18		0.22			0.05		0.01		0.44	0.19		0.18		0.12		0.13		0.48	0.31

Supplement C

Phosphate affinity SDS-PAGE measurements of TnI phosphorylation levels in the troponin used in these studies



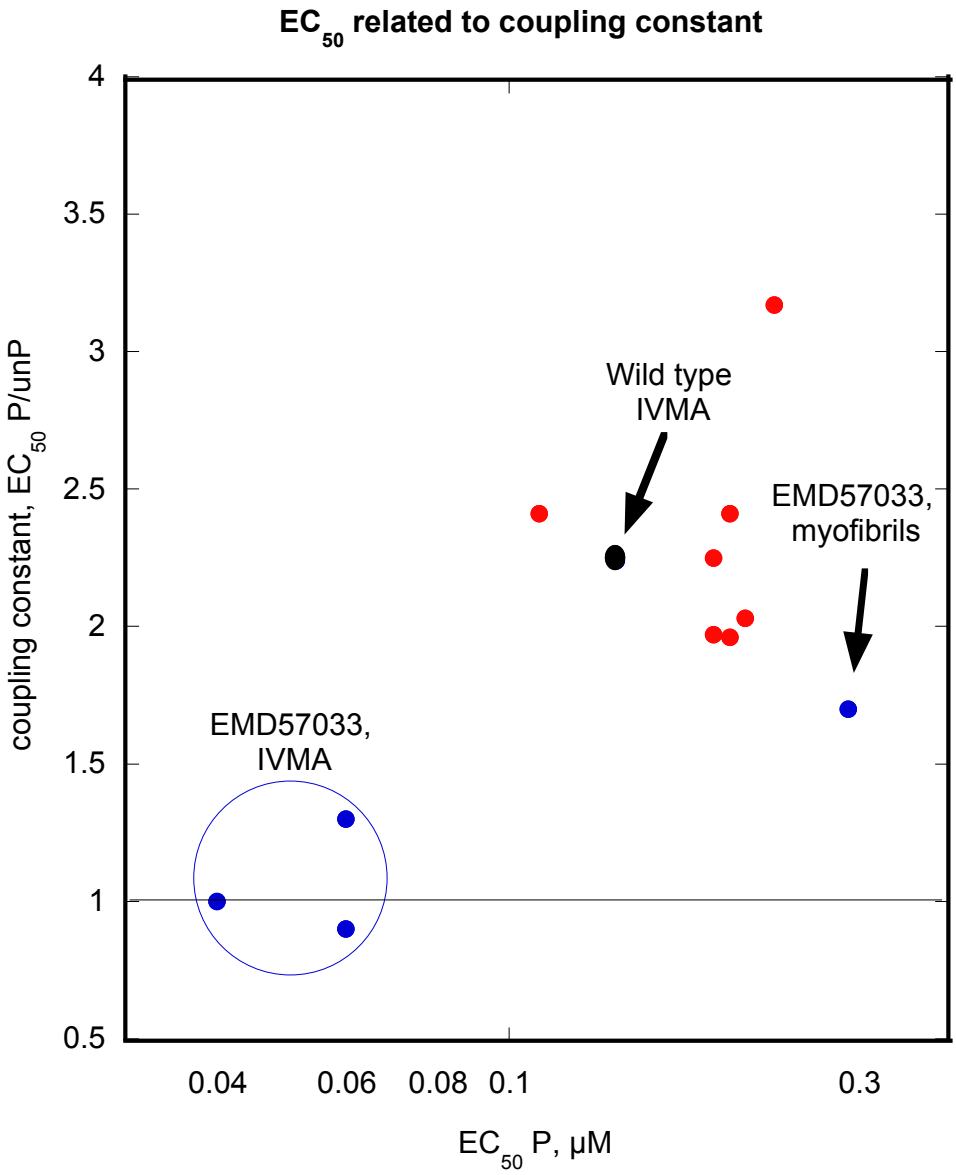
Human donor example: NJ (kindly provided by Cris Dos Remedios)

Troponin Sample	Phosphorylated mols Pi/mol TnI	Unphosphorylated mols Pi/mol TnI
Human donor	1.42±0.20	0.20±0.20
Human K280N	1.57±0.105	0.96
Human K36Q	1.31±0.01	0.09±0.004
Human G159D	1.47	0.20

Supplement D

Relationship between the coupling constant and EC_{50}

Black, wild-type; blue, +EMD57033; red, HCM and DCM mutant +EGCG



Supplement E

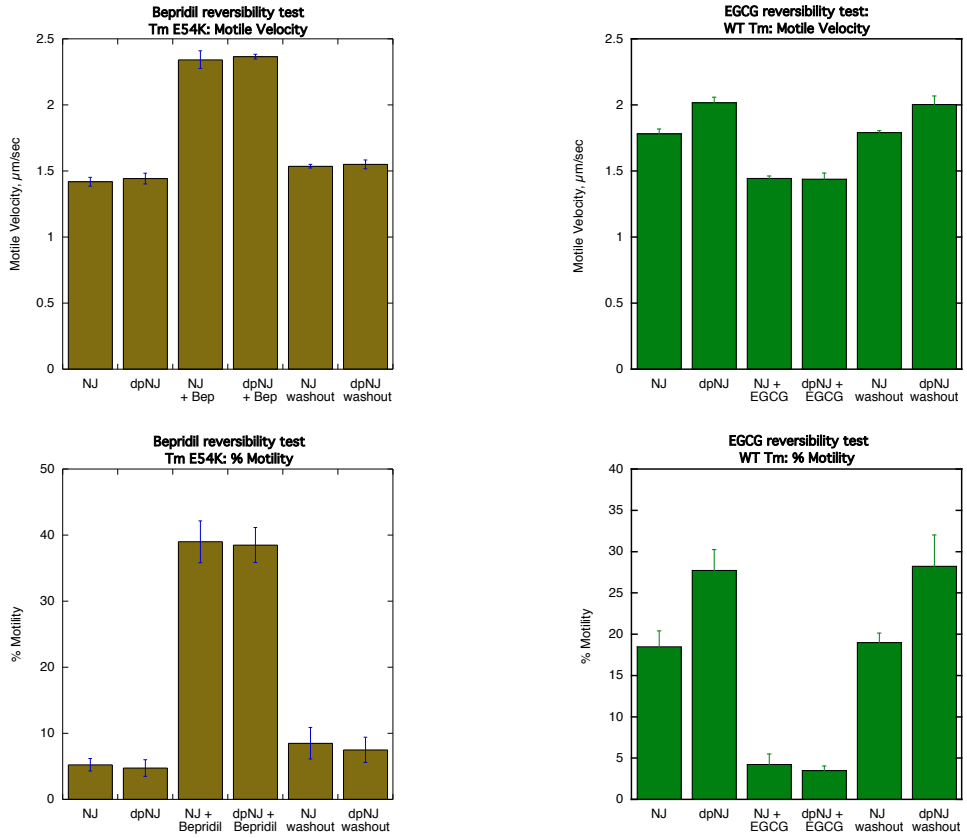
Effects of TnI phosphorylation and EGCG treatment on Ca^{2+}sensitivity analysed by two-way ANOVA.

Mutation	EC_{50} of thin filaments containing phosphorylated troponin µM ± SEM	EC_{50} of thin filaments containing unphosphorylated troponin µM ± SEM	EC_{50} of thin filaments treated with EGCG containing phosphorylated troponin µM ± SEM	EC_{50} of thin filaments treated with EGCG containing unphosphorylated troponin µM ± SEM
WT	0.14 ± 0.03(6)	0.059 ± 0.011(6)††	0.25 ± 0.03(6)**	0.15 ± 0.02(6)**††
DCM				
TPM1 E54K	0.11 ± 0.013(5)	0.11 ± 0.02(5)	0.23 ± 0.03(5)**	0.071 ± 0.005(5)††
TPM1 E40K	0.17 ± 0.05(3)	0.16 ± 0.05(3)	0.26 ± 0.04(3)**	0.058± 0.02(3)**††
TNNC1 G159D	0.092 ± 0.004(5)	0.095 ± 0.005(5)	0.19 ± 0.03(5)**	0.088 ± 0.005(5)††
TNNI3 K36Q	0.077 ± 0.006(3)	0.07 ± 0.011(3)	0.18 ± 0.03(3)**	0.088 ± 0.01(3)††
ACTC E361G	0.087 ± 0.002(5)	0.080 ± 0.002(5)	0.20 ± 0.02(5)**	0.081 ± 0.002(5)††
HCM				
TPM1 E180G	0.086 ± 0.013(3)	0.087 ± 0.011(3)	0.12 ± 0.02(3)	0.043 ± 0.0011(3)†
TNNT2 K280N	0.11 ± 0.012(3)	0.097 ± 0.004(3)	0.23 ± 0.04(3)**	0.11 ± 0.005(3)††
ACTC E99K	0.074 ± 0.005(5)	0.074 ± 0.005(5)	0.21 ± 0.03(5)**	0.10 ± 0.007(5)††

** $p<0.01$; for presence and absence of EGCG, † $p<0.05$, †† $p<0.01$; unphosphorylated compared with phosphorylated using paired two-way ANOVA. EC_{50} values rounded to 2 significant figures. In brackets is the number of experiments.

Supplement F

Reversibility of the effects of Bepridil and EGCG



To demonstrate reversibility, Bepridil or EGCG was incubated with thin filaments at 0.037μM Ca^{2+} in the normal way and then washed out after the filaments were added to the heavy meromyosin coated surface. Measurements were taken of the same motility chamber before and after the washing out. It is clear that the effects of both Bepridil and EGCG are fully reversible.

1. Vikhorev PG, Song W, Wilkinson R, Copeland O, Messer AE, Ferenczi MA, Marston SB. The Dilated Cardiomyopathy-Causing Mutation ACTC E361G in Cardiac Muscle Myofibrils Specifically Abolishes Modulation of Ca2+ Regulation by Phosphorylation of Troponin I. *Biophys J* 2014;**107**:2369-2380.
2. Song W, Vikhorev PG, Kashyap MN, Rowlands C, Ferenczi MA, Woledge RC, MacLeod K, Marston S, Curtin NA. Mechanical and energetic properties of papillary muscle from ACTC E99K transgenic mouse models of hypertrophic cardiomyopathy. *Am J Pysiol Heart Circ Physiol* 2013;**304**:H1513-H1524.
3. Ayittey PN, Walker JS, Rice JJ, de Tombe PP. Glass microneedles for force measurements: a finite-element analysis model. *Pflugers Arch* 2009;**457**:1415-1422.
4. de Tombe PP, Belus A, Piroddi N, Scellini B, Walker JS, Martin AF, Tesi C, Poggesi C. Myofilament calcium sensitivity does not affect cross-bridge activation-relaxation kinetics. *Am J Physiol Regul Integr Comp Physiol* 2006;**292**:R1129-R1136.
5. Tesi C, Piroddi N, Colomo F, Poggesi C. Relaxation kinetics following sudden Ca(2+) reduction in single myofibrils from skeletal muscle. *Biophys J* 2002;**83**:2142-2151.
6. Stehle R, Krüger M, Pfitzer G. Force kinetics and individual sarcomere dynamics in cardiac myofibrils after rapid ca(2+) changes. *Biophys J* 2002;**83**:2152-2161.