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## Supplementary Materials for

### **The hypertrophic cardiomyopathy mutations R403Q and R663H increase the number of myosin heads available to interact with actin**

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**Table S1. Summary of mechano-chemical properties of human  $\beta$ -cardiac R663H sS1 and R403Q sS1.**

HCM mutation	Intrinsic force mutant/WT ratio	Velocity mutant/WT ratio	ATPase mutant/WT ratio
R403Q	$0.8 \pm 0.1$	$1.2 \pm 0.1$	$1.2 \pm 0.1$
R663H	$1.1 \pm 0.1$	$1.02 \pm 0.02$	$0.9 \pm 0.1$

The ratios of mutant/WT for basic mechano-chemical properties of sS1 are presented. The data for human  $\beta$ -cardiac R403Q sS1 is taken from our published data (28).

**Table S2. Actin-activated ATPase activity values for human  $\beta$ -cardiac 2-hep HMM and 25-hep HMM.**

	2-hep HMM $k_{cat}$ ( $s^{-1}$ )	2-hep HMM $K_M$ ( $\mu M$ )	25-hep HMM $k_{cat}$ ( $s^{-1}$ )	25-hep HMM $K_M$ ( $\mu M$ )	25-hep HMM:2-hep HMM (%)
WT	$2.4 \pm 0.1$	$4 \pm 1$	$1.4 \pm 0.1$	$9 \pm 2$	$57 \pm 3$
R403Q	$2.7 \pm 0.2$	$20 \pm 3$	$2.2 \pm 0.2$	$27 \pm 6$	$80 \pm 8$
R663H	$2.8 \pm 0.2$	$6 \pm 1$	$1.9 \pm 0.1$	$6 \pm 2$	$67 \pm 3$

The values of  $k_{cat}$  and  $K_M$  were obtained from the fitting of data shown in Fig. 3A (R403Q) and 3B (R663H). WT values were obtained from our previous work (8).

**Table S3. Summary of mant-ATP single turnover data of myosin.**

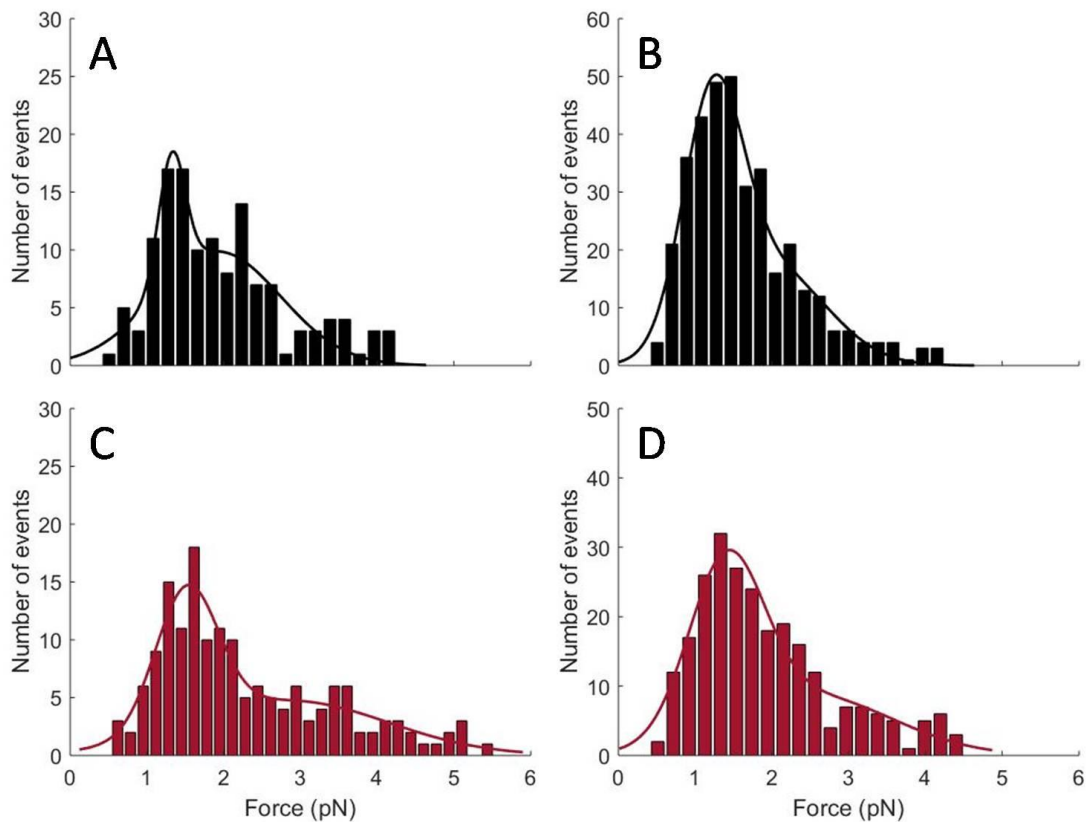
	SRX rate (1/s)	% of SRX	DRX rate (1/s)	% of DRX
WT	0.0038 ± 0.0003	45 ± 3	0.020 ± 0.003	55 ± 3
R403Q	0.0024 ± 0.0005	21 ± 6	0.016 ± 0.002	79 ± 6
R663H	0.0027 ± 0.0005	18 ± 3	0.027 ± 0.004	82 ± 3

The mean SRX and DRX rates and the mean percentages of myosin heads in SRX and DRX are reported with SEM for human  $\beta$ -cardiac WT, R663H and R403Q 25-hep HMM. The p values for WT and R403Q are 0.06 and 0.23 for SRX and DRX rates, respectively. The p values for WT and R663H are 0.10 and 0.15 for SRX and DRX rates, respectively.

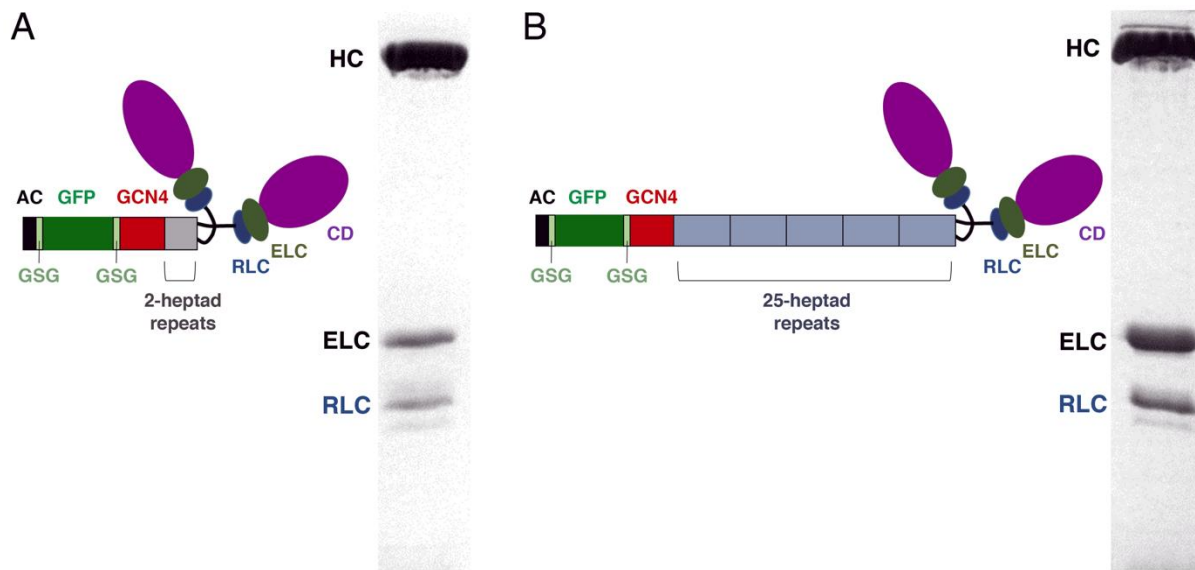
**Table S4. Summary of mant-ATP single turnover data of myosin and MyBP-C.**

	SRX rate (1/s)	% of SRX	DRX rate (1/s)	% of DRX
WT + C0-C7	0.0054 ± 0.0004	81 ± 7	0.040 ± 0.007	19 ± 7
R403Q + C0-C7	0.0035 ± 0.0005	29 ± 4	0.018 ± 0.001	71 ± 4
R663H + C0-C7	0.0038 ± 0.0007	26 ± 3	0.025 ± 0.002	74 ± 3

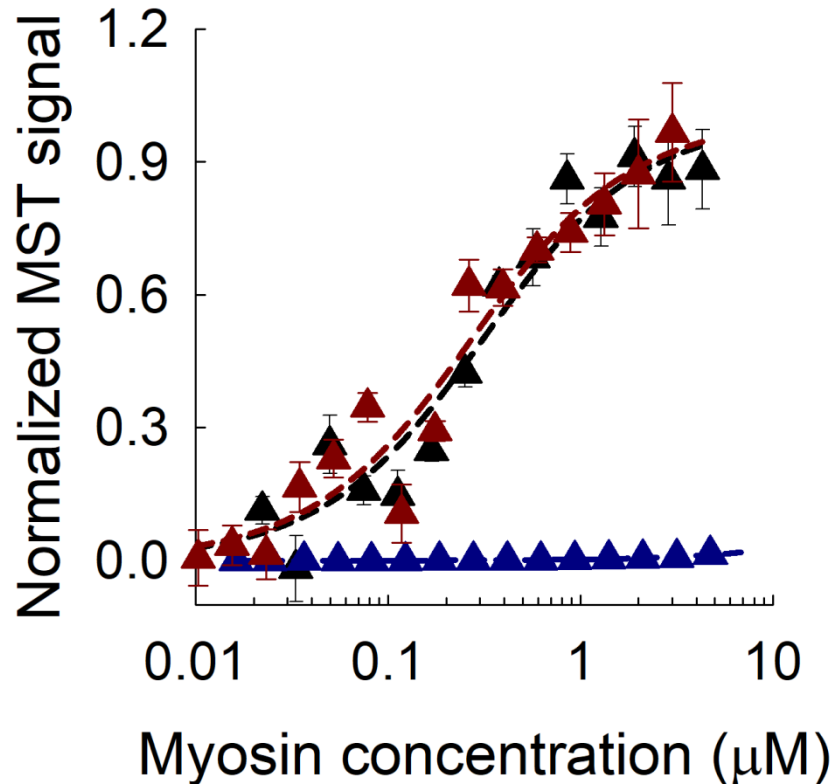
The mean SRX and DRX rates and the mean percentages of myosin heads in SRX and DRX are reported with SEM for human  $\beta$ -cardiac WT, R663H and R403Q 25-hep HMM with 10  $\mu$ M C0-C7. The p values for WT and R403Q are 0.01 and 0.05 for SRX and DRX rates, respectively. The p values for WT and R663H are 0.10 and 0.13 for SRX and DRX rates, respectively.



**Fig. S1. Intrinsic force measurements of WT and R663H human  $\beta$ -cardiac sS1.** (A-D) The data from Fig. 1B represented as force histograms. (A) Force histogram of a representative molecule of WT human  $\beta$ -cardiac sS1. (B) Cumulative distribution of four molecules of WT (number of events = 362). (C) Force histogram of a representative molecule of R663H human  $\beta$ -cardiac sS1. (D) Cumulative distribution of four molecules of R663H (number of events = 304). Each of the distributions was fitted to a double-Gaussian function and the fittings are shown.



**Fig. S2. SDS-PAGE gel images and schematic structures of human  $\beta$ -cardiac 2-hep HMM and 25-hep HMM.** Each construct has 2 heavy chains, 2 ELCs and 2 RLCs. (A) Human  $\beta$ -cardiac 2-hep HMM has 2-heptad repeats of S2 whereas (B) 25-hep HMM has 25-heptad repeats. The C-terminus of each construct has 1 leucine zipper (GCN4) followed by an e-GFP and an 8-residue (RGSIDTWV) PDZ-binding peptide (AC). There are flexible (GSG) linkers between GCN4 and GFP, and between GFP and AC. The gels were 15% acrylamide.



**Fig. S3. Binding data of human  $\beta$ -cardiac 25-hep HMM with the C0-C7 fragment of MyBP-C.** Representative Microscale Thermophoresis (MST) binding curves of human  $\beta$ -cardiac 25-hep HMM with the C0-C7 fragment of MyBP-C for WT (black), R663H (dark red) and R403Q (blue) are shown. Experiments were performed by mixing 50 nM labeled C0-C7 with different concentrations of 25-hep HMM. In this particular dataset, the  $K_d$  values obtained from data fitting are 280 nM and 250 nM for WT and R663H, respectively. For R403Q, 25-hep HMM showed no binding to C0-C7 up to 5  $\mu$ M 25-hep HMM. Data points represent the mean at each C0-C7 concentration with the SEM shown. The data are the average of 3 experiments from a single set of protein preparations.