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Supplementary Materials for

Biophysical properties of human β-cardiac myosin with converter mutations that cause hypertrophic cardiomyopathy

Masataka Kawana, Saswata S. Sarkar, Shirley Sutton, Kathleen M. Ruppel, James A. Spudich

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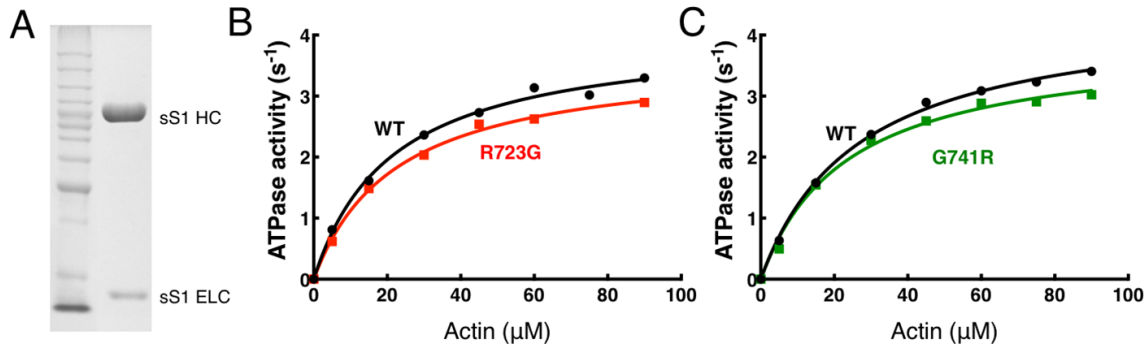

fig. S1. sS1 purification and ATPase measurements. (**A**) Typical column fraction of purified sS1 myosin construct, with top band showing the heavy chain, and bottom band showing the ELC. (**B**) Representative actin-activated sS1 ATPase curve for WT vs R723G human β-cardiac sS1. (**C**) Representative actin-activated sS1 ATPase curve for WT vs G741R human β-cardiac sS1.

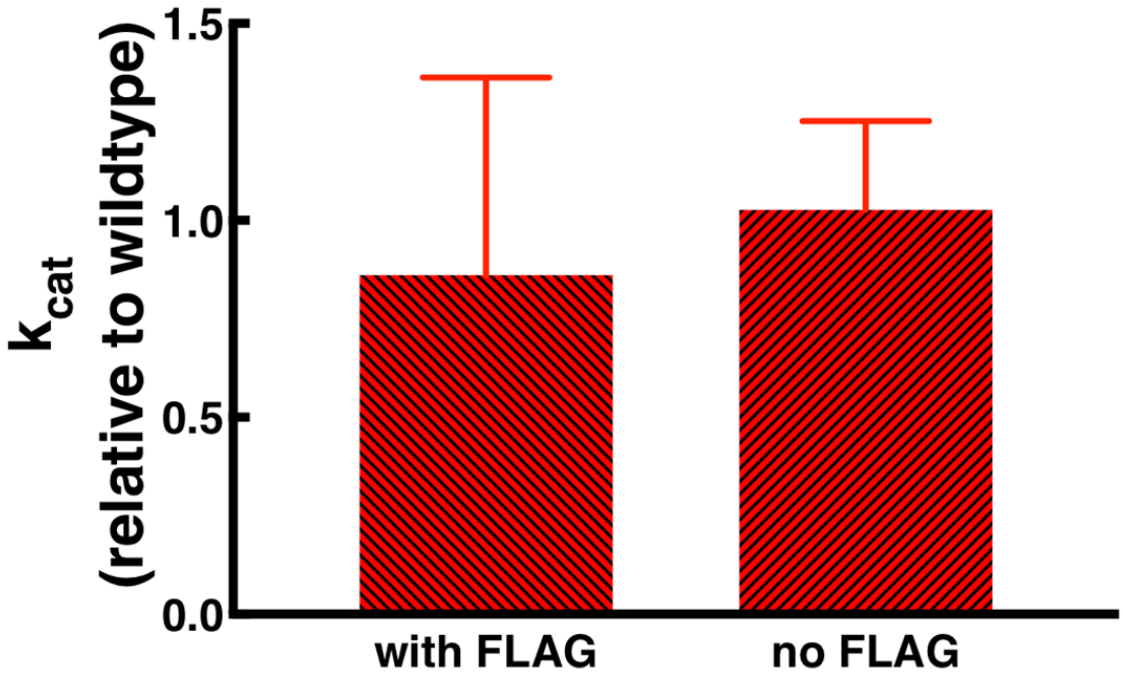

fig. S2. Comparison of k_{cat} (relative to WT) for R723G human β-cardiac sS1 with and without a FLAG tag on the ELC. Data with the FLAG tag is the average of 2 independent experiments. Data without the FLAG tag is the average of 5 independent experiments.

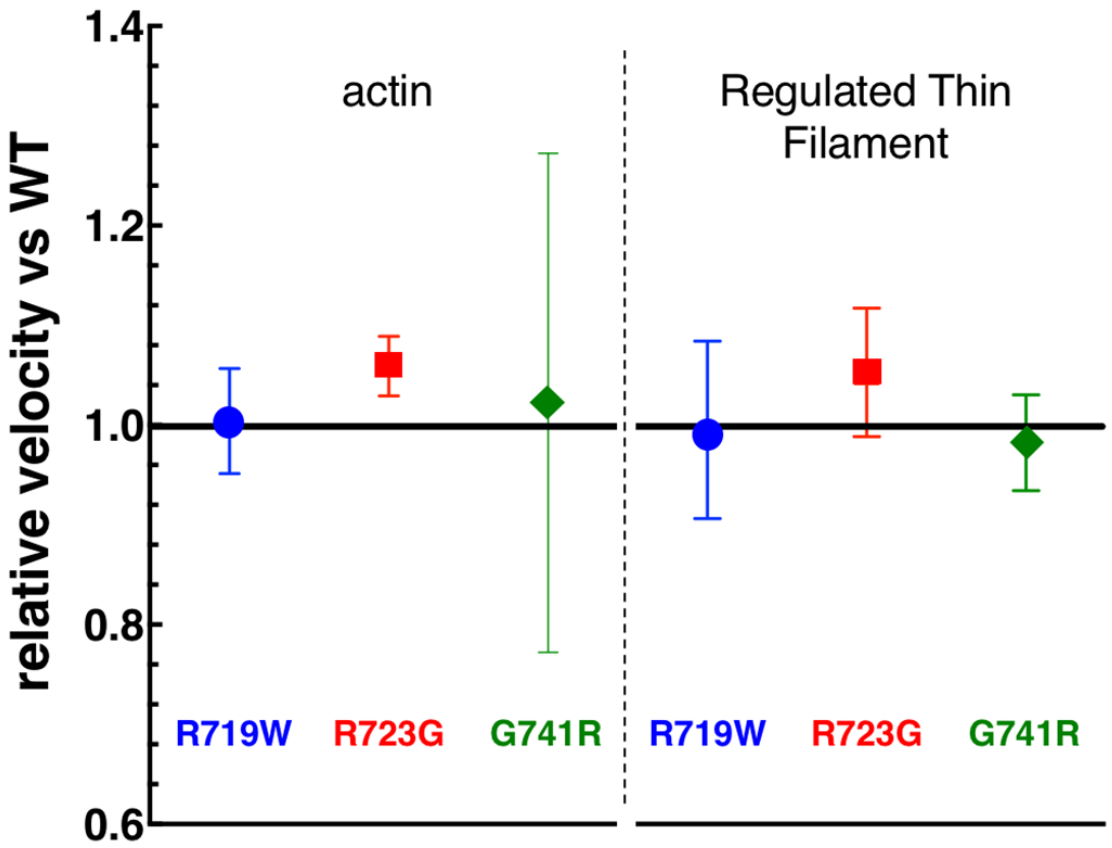

fig. S3. Unloaded MVEL measurements for actin and RTF. The MVEL velocities relative to WT are shown for gliding actin filaments (left) and RTFs (right, in 10^{-5} M Ca^{2+}) driven by the three mutant human β-cardiac sS1 proteins. Each mutant protein was normalized against its matching WT protein prepared and assayed on the same days. Each data point is a mean of the relative velocity (Error bars represent ± 95% confidence interval).

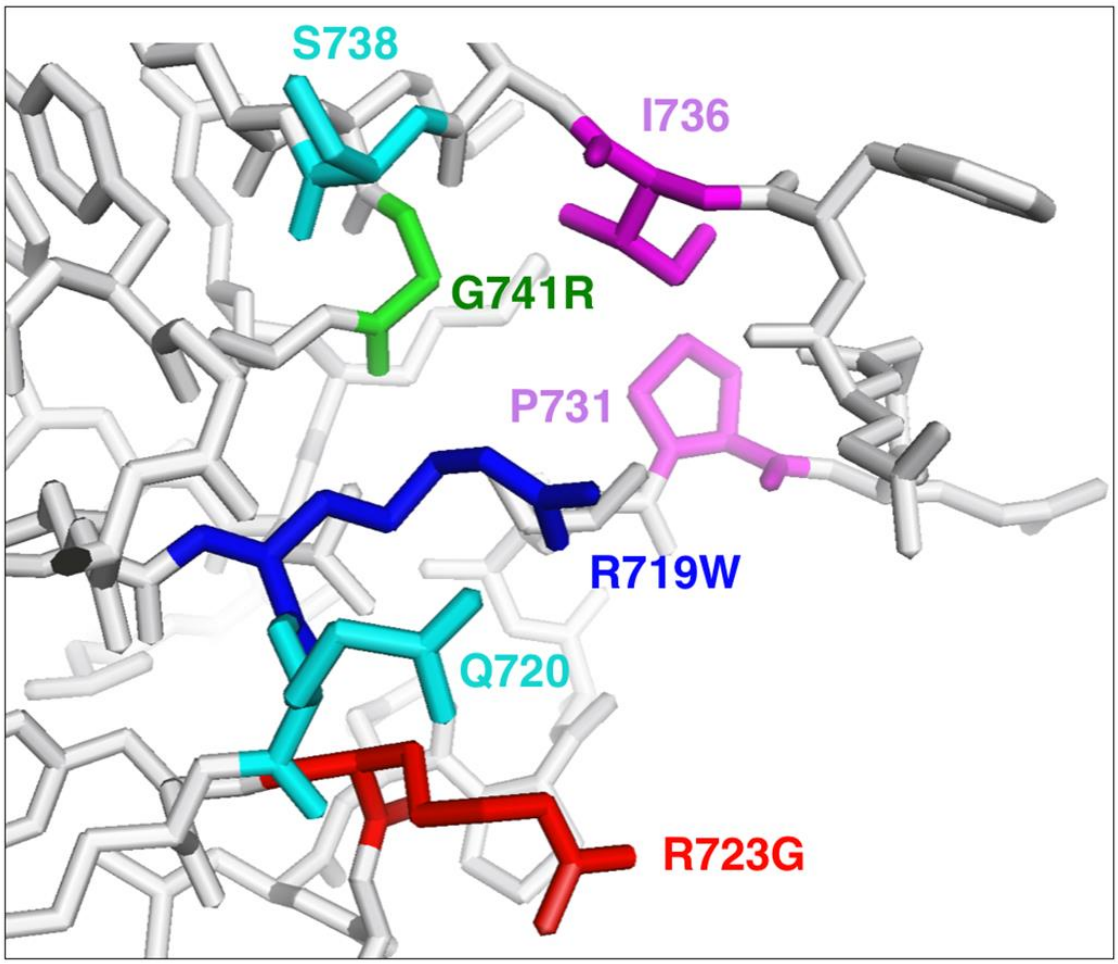

fig. S4. Structure of the part of the converter domain showing G741R (green), R719W (blue), and R723G (red), neighboring residues whose conflict with the G741R conversion (residues I736 and P731; magenta) and whose interactions with R719 may be lost by the R719W conversion.

Supplementary tables

table S1. Summary of k_{cat} and K_m values from actin-activated ATPase assays.

Mean +/- SEM	WT	R719W	WT	R723G	WT	G741R
k_{cat} (s^{-1})	5.7 +/- 0.4	5.4 +/- 0.4	3.3 +/- 0.2	3.2 +/- 0.2	5.1 +/- 0.3	4.6 +/- 0.3
K_m (μM)	34 +/- 5	34 +/- 4	23 +/- 2	30 +/- 4	26 +/- 3	23 +/- 3

Relative Change (95% CI)	R719W	R723G	G741R
k_{cat}	1.0 (0.8 - 1.1)	1.0 (0.8 - 1.2)	0.9 (0.8 - 1.0)
K_m	1.1 (0.8 - 1.4)	1.3 (1.0 - 1.7)	1.0 (0.6 - 1.3)

Upper table: Actual values from multiple paired preparations of wild type and mutant proteins as indicated. For the R719W mutant, 8 independent protein preparations with corresponding wild type controls were done, and 16 ATPase assays were performed. For R723G, 3 protein preps and 8 ATPase assays were performed. For G741R, 4 protein preps and 11 ATPase assays were performed. The k_{cat} and K_m values are expressed in mean +/- SEM. We saw variability in protein activity, with the k_{cat} of wild type ranging from 3 to 6 s^{-1}, and this variability was manifest over the course of several months. For example, the ATPase activity of the wild type protein was similar when R719W and G741R were studied, while it was lower during the time that R723G was studied later in the year). We made every effort to standardize the material and protein purification processes. Using combinations of reagents and fresh and frozen protein, we determined that the source of the variability was within the C2C12 cells themselves, rather than in the adenovirus or purification or assay reagents. We do not fully understand the reason for the variability at this time (thawing a new aliquot of cells did not alter our ATPase activities, and there was no change in media or fetal bovine serum lot), but have found that performing parallel preps of WT and mutant proteins has resulted in consistent **relative** changes, despite alterations in the actual values, as shown in the lower panel of the table.

Lower table: There were no relative differences in the k_{cat} and K_m values between matching wild type and mutant proteins, shown as relative changes with 95% confidence intervals.