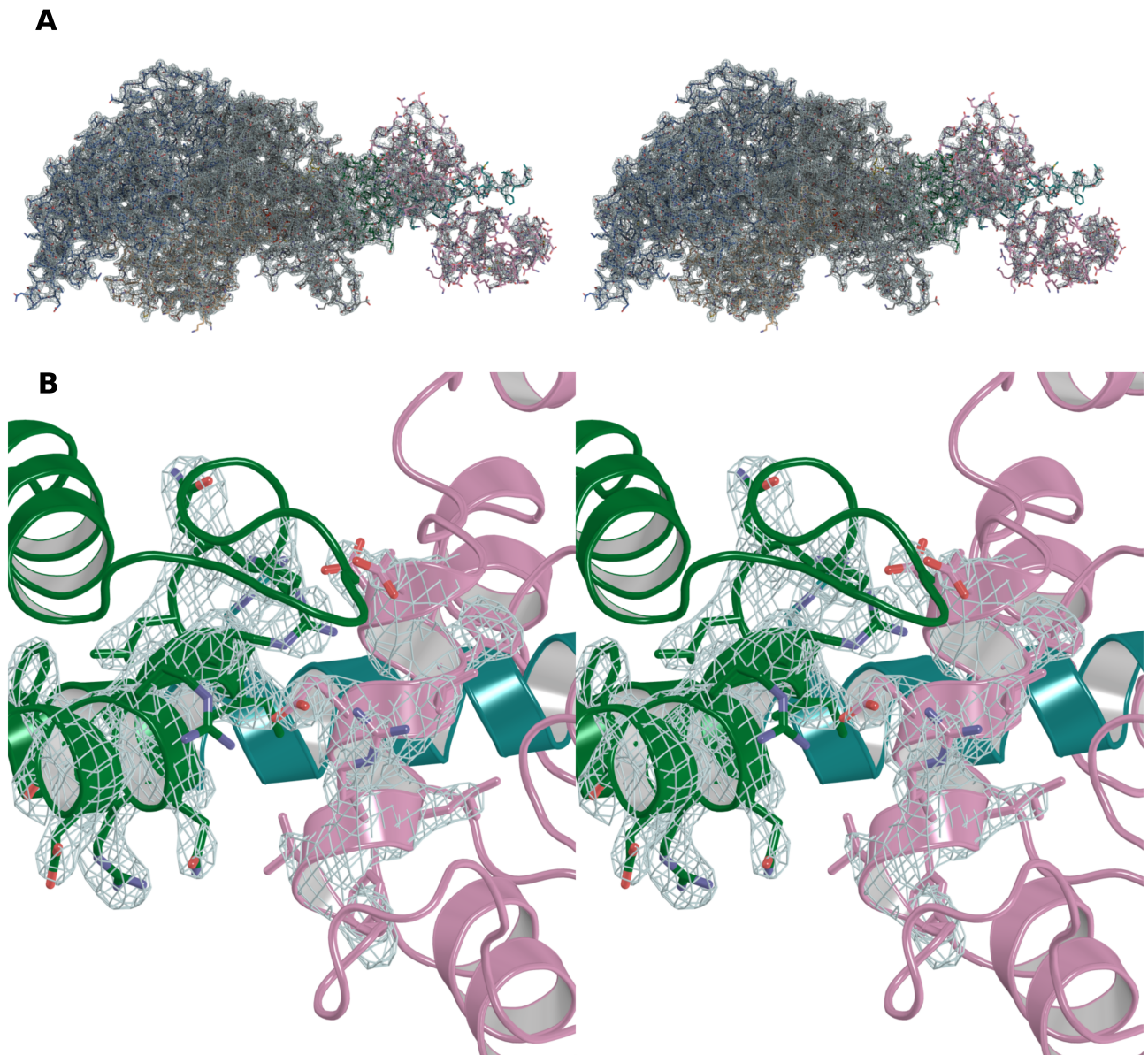


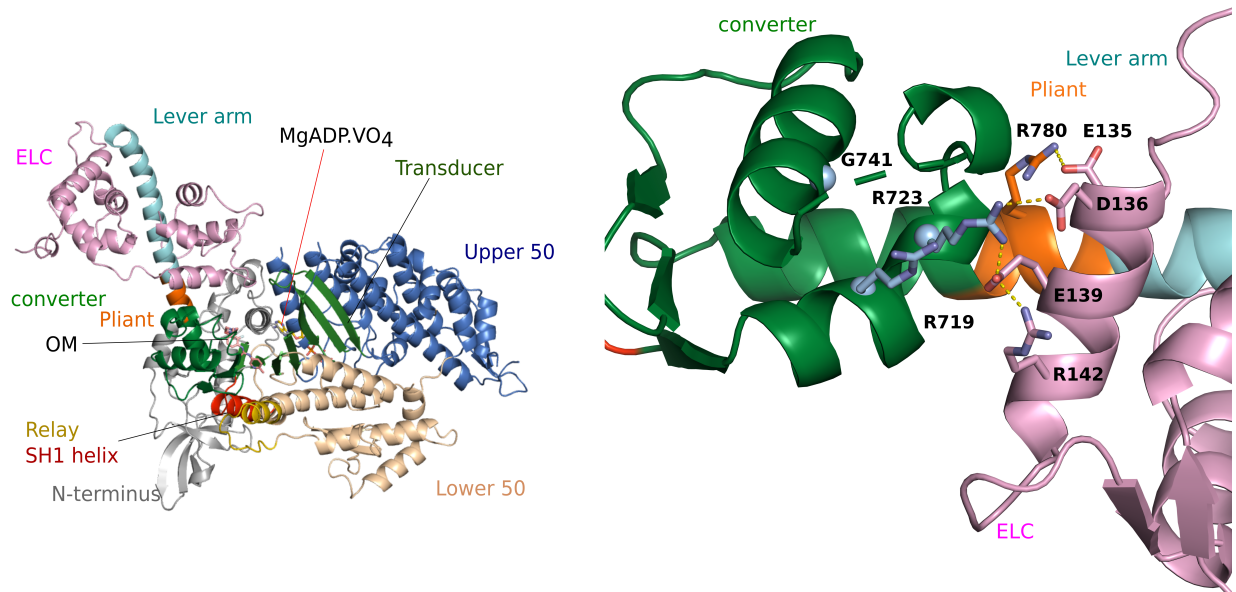
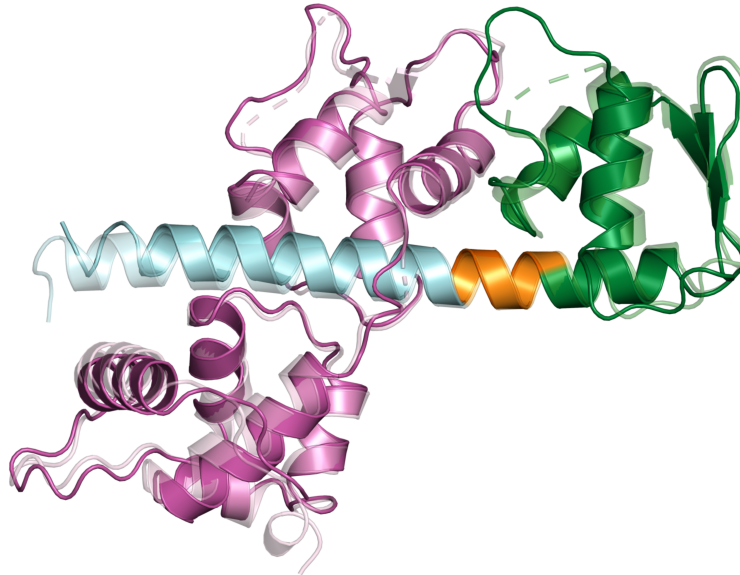
**Hypertrophic cardiomyopathy disease results from disparate impairments of
cardiac myosin function and auto-inhibition**

Robert-Paganin et al., 2018



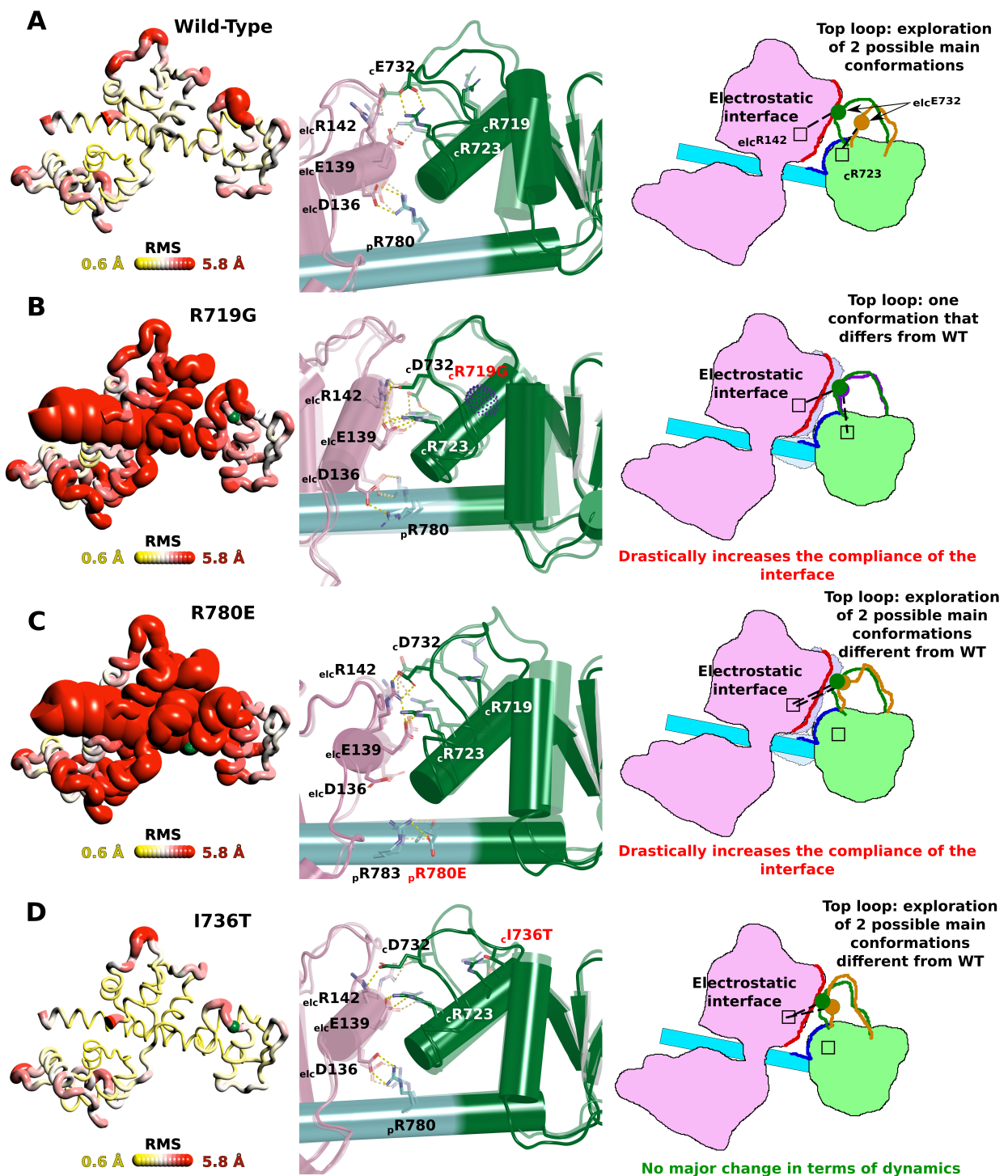
Supplementary Figure 1: Electron density map of the post-rigor S1 (PR-S1) crystal structure and the ELC/converter interface

(A) Stereo view of the electron density map of the crystal structure of the β -cardiac myosin S1 fragment in the post-rigor state (PR-S1). The electron density map in pale cyan corresponds to a 2Fo-Fc map with a contour of 1.0σ . The different subdomains of the model (stick representation) are colored: N-terminus (grey); U50 (marine blue); L50 (wheat); relay (yellow); SH1 helix (red); converter (green); lever arm (cyan); ELC (light pink). **(B)** Stereo view of the electron density map (2Fo-Fc; contour of 1.0σ) for the converter/ELC interface. Residues involved in the interface are well defined in electron density and the side chains can be clearly identified. The ELC backbone is clearly defined as well as the side chains of the E helix that interacts with the converter.

A**B**

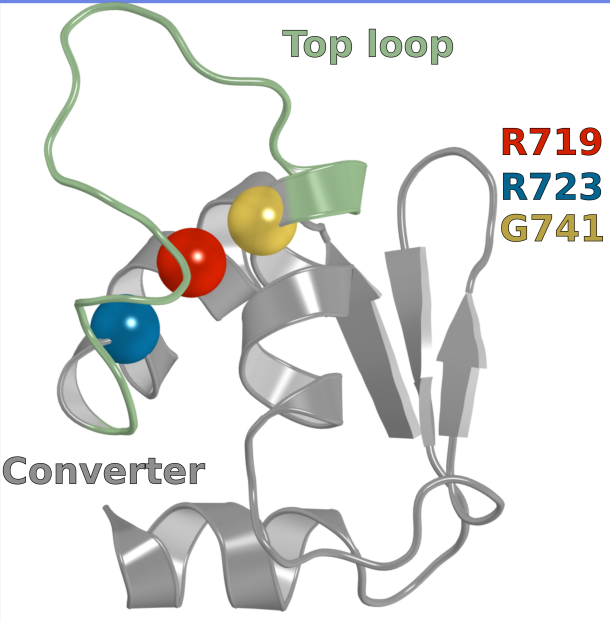
Supplementary Figure 2: The ELC/converter interface and the lever arm of the β -cardiac myosin are conserved in structure in the post-rigor (PR) and in the pre-powerstroke (PPS) states

(A) On the left, X-ray structure of β -cardiac myosin S1 bound to the activator omecamtiv mecarbil (OM) in the pre-powerstroke state (PDB code: 5N69). On the right, interface between the converter and the ELC as found in the PPS-state. (B) Cartoon representation of the lever arm region (converter, pliant, IQ motif and ELC) of β -cardiac myosin in the PR state (same color code as in Fig. 2A and 2B) superimposed with that found in the PPS state structure (translucid) (PDB code: 5N69). Both structures superimpose on this region with a root mean square deviation (RMSD) of 0.982 Å.



Supplementary Figure 3: Dynamics of the Converter/ELC interface

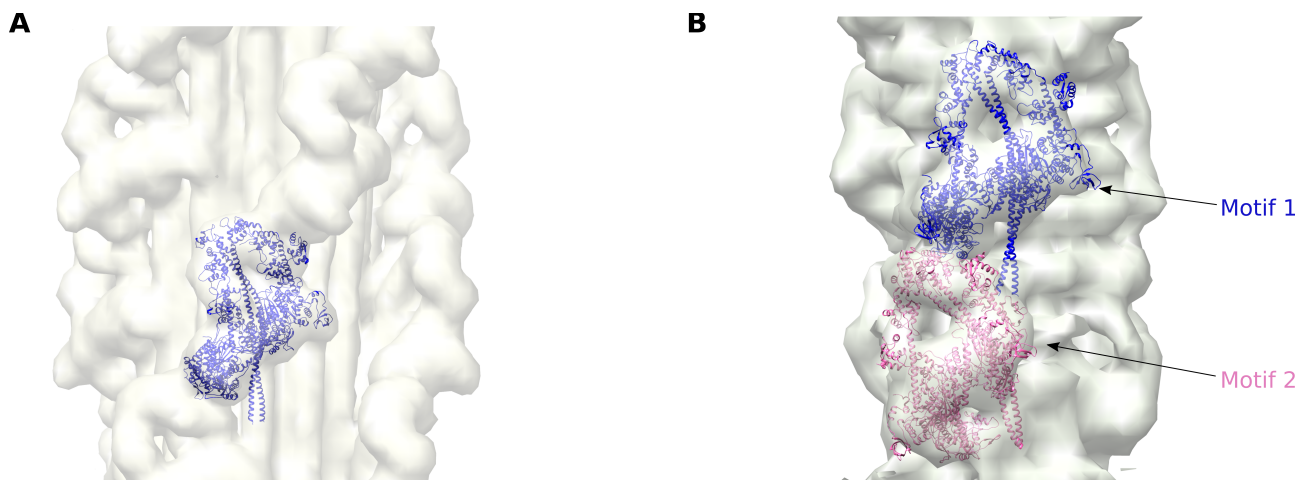
Schematic representation of the results from the molecular dynamics simulations for the wild-type (WT), two proof-of-concept mutations (R719A and R780E) as well as one HCM (I736T) mutant that have been analyzed in silico. On the left, a Putty representation is displayed for a β -cardiac myosin construct that includes the residues 701-806 and the ELC. Root mean square (RMS) fluctuations during 30 ns simulations are represented with a RMS scale ranging from 0.6 Å (in yellow) to 5.8 Å (in red). Structures represented here correspond to the most populated structural state. In each structure, the position of the residue mutated is labeled in red. On the center, a schematic representation of the region containing the converter, the essential light chain (ELC) and the lever arm is displayed. The different populations of the top loop allowed by the dynamics of this region are drawn and the nature of the interactions between the converter and the ELC is also represented. In each case a state is represented opaque and the others are in transparency in order to best compare the different populations. On the right, the interface between the ELC, the converter and the pliant region is represented. The different positions (positions are colored differently) schematized on the right are represented on the structure with the position of all key-residues that maintain the interface and its plasticity. On the center and on the left, the myosin subdomains are colored differently: the converter in green; the IQ region in cyan and the ELC in light pink. (A) WT, (B) R719G, (C) R780E, (D) I736T.

R719W -Intrinsic force decreases 15-30 % -Actin and regulated thin filament gliding velocities increases 15-20 % - # mobile filaments decreases 2-5 % - Velocity under load similar to WT	 Top loop R719 R723 G741 Converter
R723G -Intrinsic force decreases 15-30 % -Actin and regulated thin filament gliding velocities increases 15-20 % - # mobile filaments decreases 2-5 % - Velocity under load similar to WT	G741R -Intrinsic force similar to WT -Actin and regulated thin filament gliding velocities similar to WT - # Mobile filaments similar to WT - Velocity under load similar to WT

in vitro results from Kawana et al., 2017

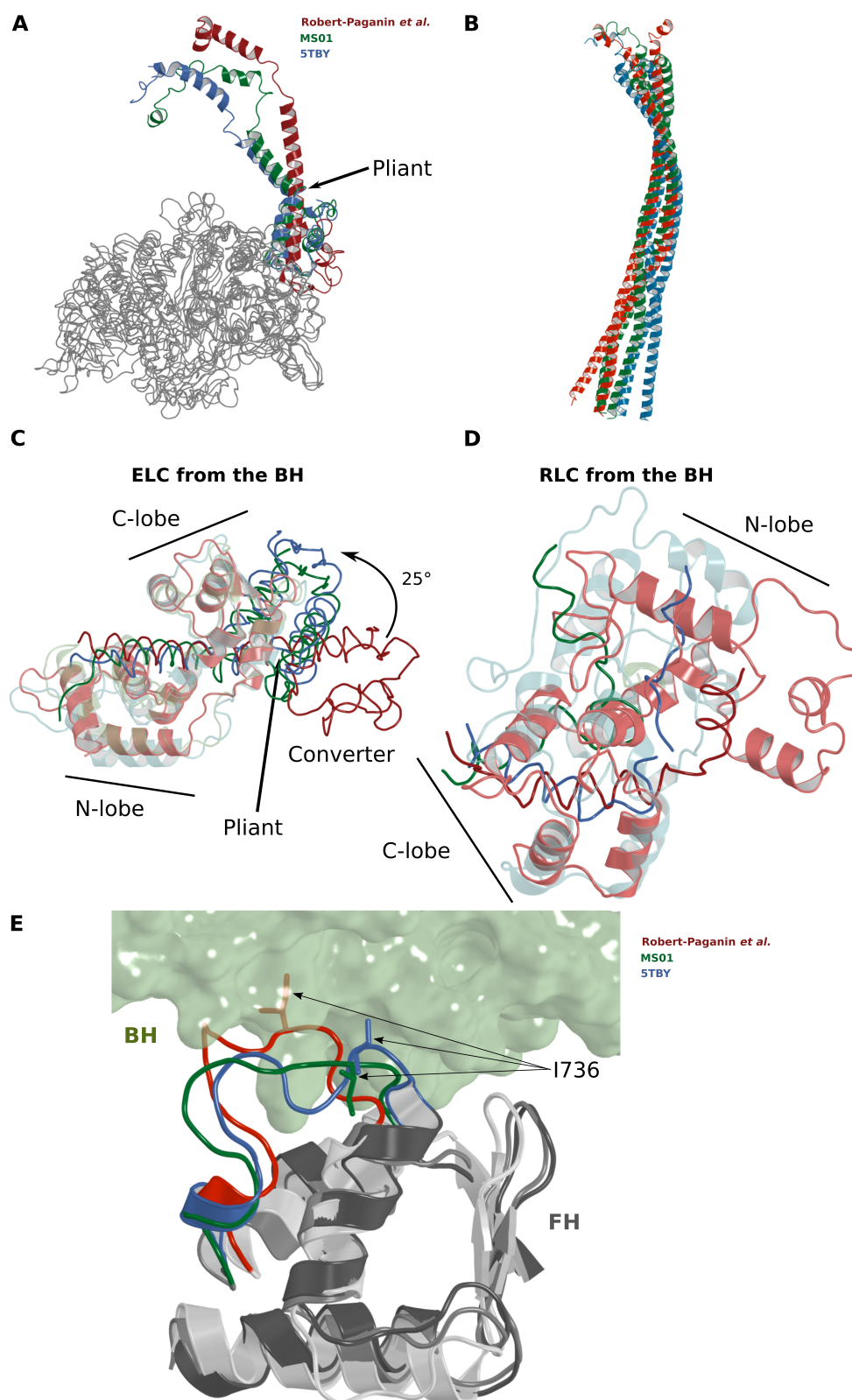
Supplementary Figure 4: Consequences of HCM mutations in the converter on the biomechanical properties of β -cardiac myosin as reported from the Kawana et al. studies performed in the Spudich laboratory.

Summary of the consequences of the mutations R719W, R723G and G741R compared to WT for the biomechanical properties of the cardiac myosin motor⁸³. On the top, right, the three mutations are located (colored spheres) on a cartoon representation of the β -cardiac myosin converter.



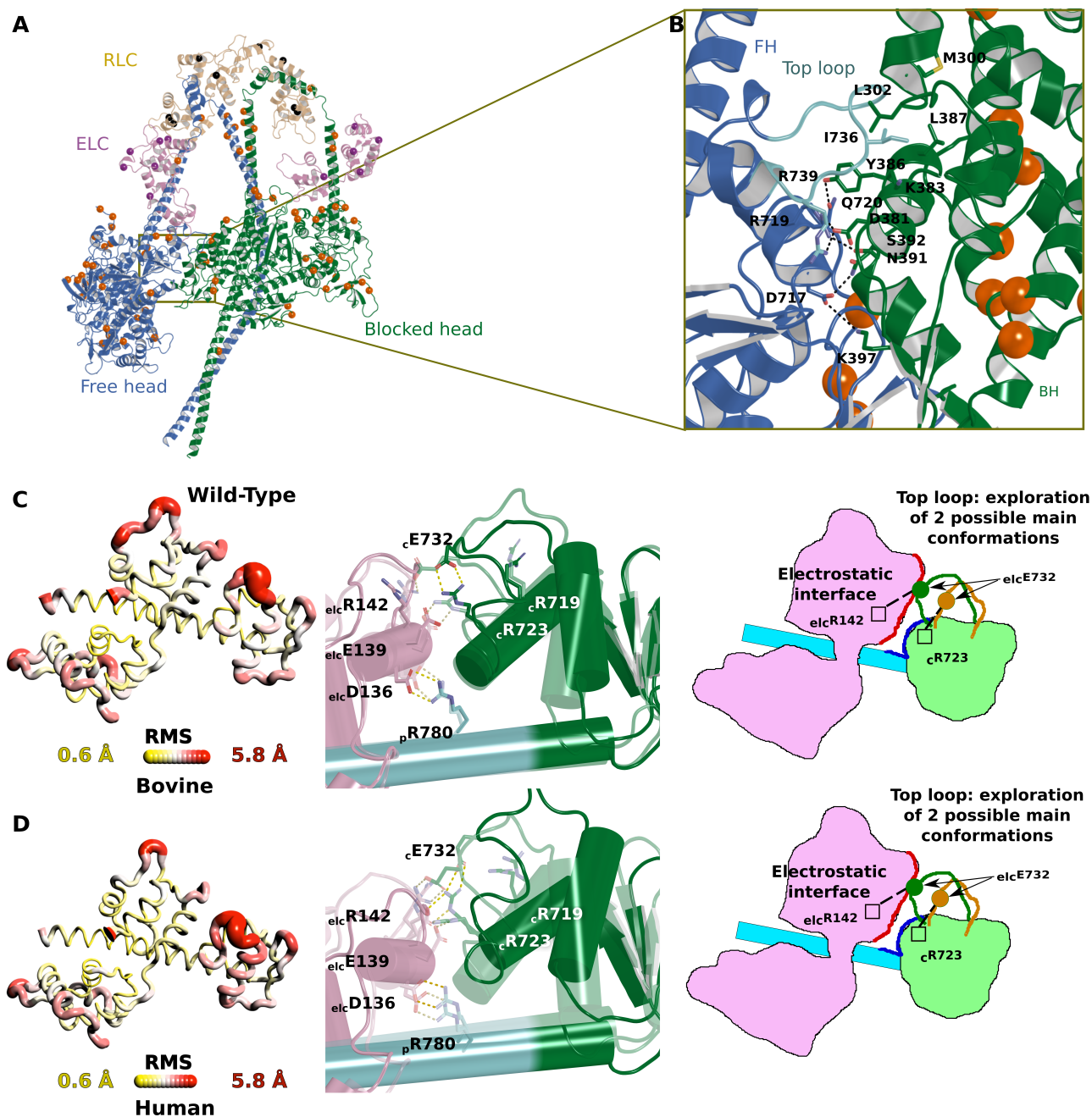
Supplementary Figure 5: Sequestered state model and its fit in the electron density map

(A) Fit of our model in the electron density of tarantula muscle filament (EMD code: EMD-1950). **(B)** Reconstitution of the cardiac filament and sequestered state. Fit of our model in the human cardiac muscle filament electron density map obtained from negative stained electron microscopy (EM) data (EMDB code EMD-2240). Since the geometry in cardiac filament is not purely helical⁹⁸, two motifs have been fitted in the filament, each motif is slightly rotated compared to the other.



Supplementary Figure 6: Comparison of sequestered state models

Comparison of the sequestered model we propose (red) with the two previously released models: MS01 (green) from Jim Spudich's lab³⁷ and 5TBY (blue) from Raul Padron's lab⁸. All the models have been superimposed on the N-ter region (1-171) of the blocked head (BH) of our model. **(A)** Comparison of the converter/lever arm region that is displayed in cartoon models, **(B)** Comparison of the S2 region. **(C)** Comparison of the converter/ELC region. **(D)** Comparison of the RLC region, RLC are aligned on the C-lobe. **(E)** Comparison of the interface between the FH-converter (cartoon) and the BH (transparent surface) in the model we present here (converter in white, top loop in red), the MS01 model (converter in light grey, top loop in green), and the 5TBY model (converter in dark grey, top loop in blue). For each structure, the side chain of Ile 736 is represented, showing that only in our model is this residue modeled in the interface between both heads.



Supplementary Figure 7: Comparison of the human and of bovine β -cardiac myosins

Cartoon representation of the minimized model of the sequestered state of human β -cardiac myosin. Polymorphism are represented as balls colored in orange, purple and black for the heavy chain, the essential light chain (ELC) and the regulatory light chain (RLC) respectively. **(B)** Region of interaction between the blocked head (BH) and the free head (FH), the top loop is colored in cyan. The interfaces are identical in the human and bovine models. **(C)** and **(D)** represent results from molecular dynamics on the lever arm of the bovine β -cardiac myosin **(C)** and of the human β -cardiac myosin **(D)**. On the left, a putty representation is displayed for a β -cardiac myosin construct that includes the residues 701-806 and the ELC. RMS fluctuations during 30 ns simulations are represented with a RMS scale ranging from 0.6 Å (in yellow) to 5.8 Å (in red). Structures represented here correspond to the most populated structural states. On the center, the interface between the ELC, the converter and the pliant region is represented. Different conformations are colored differently on the structure with the position of all key-residues that maintain the interface and its plasticity. A state is represented opaque and the others are in transparency in order to best compare the different populations. On the right, a schematic representation of the region containing the converter, the ELC and the lever arm is displayed. The different populations of the top loop allowed by the dynamics of this region are drawn and the nature of the interactions between the converter and the ELC is also represented. On the center and on the left, the myosin subdomains are colored differently: the converter in green; the IQ region in cyan and the ELC in light pink.

Residue directly involved in IHM interactions; Indirect effect on IHM interactions; **early onset HCM**

Mutations for which no effect on PPS is predicted => no effect on sequestered state									
Powerstroke altered	A426T	(transition slower after Pi release)					A430E		
Actin surface	V406M	V411I	I524L	K542R	G571R	H576R	D587V	V606M	
L50 near connectors	A583V								
Strut	N602S	E603K							

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