SUPPORTING INFORMATION

Regulatory Light Chain Mutants Linked to Heart Disease Modify the Cardiac Myosin Lever-Arm

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Construction of the microfluidic device. Figure 2 shows the channel pattern drawn in Adobe Photoshop at 1200 dpi resolution with each square pixel ~21 μm on a side. The pattern was printed onto Toner Transfer Paper (Pulsar, Crawford FL) using a Samsung Laserjet printer (model ML-3471ND) at 1200 dpi resolution. Toner was then transferred using pressure and heat to a brass substrate cut from shim stock (0.032 inch thick, Amazon). Brass etching was performed with a 20% solution (% w/v) ammonium persulfate (APS, Sigma). Regions protected by the toner are not etched. We etched to depths of 20-40 μm using the total substrate weight to monitor progress. The depth was occasionally measured experimentally using the narrow depth of focus for the TIRF objective as described previously (*1*).

Horizontal tapered stripes are 250-1000 μm wide to allow adequate flow of solution around differently sized fiber bundles. The smallest fibers were mounted at the narrow end. Tapered stripe depth alternated between 20 μm (depicted in the figure) and 40 μm to accommodate the 50-100 μm diameter fibers. The pattern long dimension is ~1.5 cm and it fits on a 22 x 30 mm #1 glass coverslip (~0.15 mm thick).

Figure S1 shows the elements needed for construction of the 3-dimensional PDMS substrate in the microfluidic device shown in **Figure 2**. The brass master was placed in a petri dish and 6, 1.5 cm long, 0.8 mm diameter wires were epoxied to the raised portions of the brass corresponding to the ends of the etched channels where fluid will enter and exit the microfluidic device. The wires are supported by the petri dish edge until the epoxy hardens. 1 mm thick spacers not show are placed on the outer edges of the brass master and in the foreground and background to support the hovering glass spacer. Unpolymerized polydimethylsiloxane (PDMS) was poured into a perti dish and over the brass master. The dish was transferred to a hot plate and heated to 80 ^{o}C for 2 hours to polymerize the PDMS as described previously (*2*). After heating the perti dish and wires were removed from the PDMS and the substrate was cut to fit on top of a glass coverslip. The PDMS forms a spontaneous water tight seal with the glass. Pipet tips (20 μL) filled with solution are inserted into holes on one side of the substrate left by the wires. Suction is applied to the other side using another pipet tip to draw the solution through the channel. After testing for good flow the PDMS substrate is removed from the coverslip and washed with water then ethanol and pumped down in a vacuum chamber to remove liquid. The PDMS substrate is placed channel side up into a clean petri dish until needed.

SHG from muscle fibers. **Figure S2** top shows the SHG and fast Fourier transform (FFT) amplitude images for active isometric and relaxed permeabilized papillary fibers. The FFT amplitude meridionals are plotted at the bottom of **Figure S2** where spatial frequencies from active and relaxed fibers are compared. The FFT amplitude spatial frequencies are calibrated using an image generated with a 1-dimensional pure sinusoidal intensity oscillation. The simulated intensity also has random disordering added to resemble frequencies in the real muscle fiber. The SHG images imply relaxed and active fibers have a similar average sarcomere spacing of 2.33 μm. This is normal for resting or isometric cardiac muscle.

Scatter plots comparing WT and mutant species. Dipole moment spherical polar coordinates (β,α) for fibers in rigor (blue solid square), isometric contraction (red solid square), and relaxation (open blue square). Coordinates are defined relative to a lab frame z-axis parallel to the fiber symmetry axis, x-axis in the plane of the coverslip, and y-axis normal to the coverslip plane pointing into the aqueous medium. Arrows originate on various high free-energy active coordinates in sub-states A-E as indicated and terminate with pointy end on minimum free-energy rigor A-state cross-bridges. Blue or green arrows designate a positive or negative projection scalar (eq. 3) where same color single molecules reside in the same half-sarcomere. The blue and green arrows arranged like the hands of a clock are the average orientation of the blue or green arrows connecting coordinates. They are referred to in the text as $\overrightarrow{\Delta g}(\pm)$ and have amplitudes proportional to the average free energy separating the isometric active cross-bridge from the minimum free-energy rigor A-state cross-bridge computed using eq. 2.

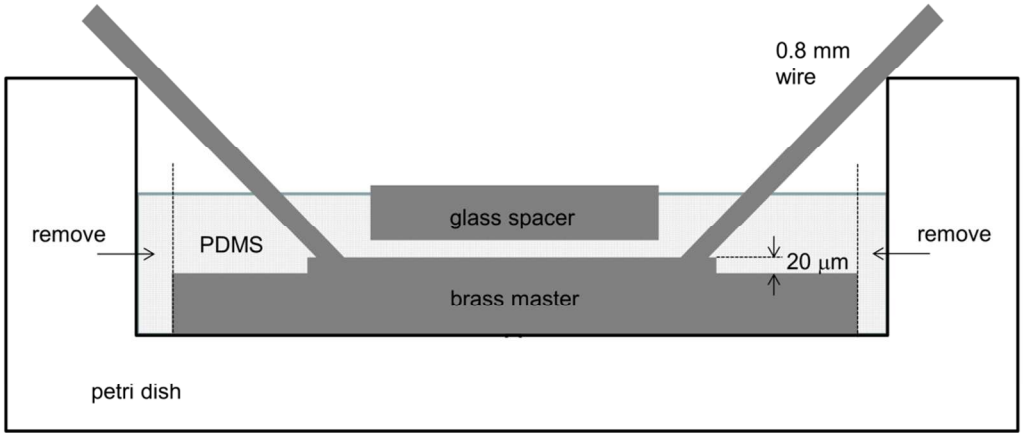
0.8 mm
wire
glass spacer
remove
PDMS
20 μm
remove
brass master
petri dish

Figure S1

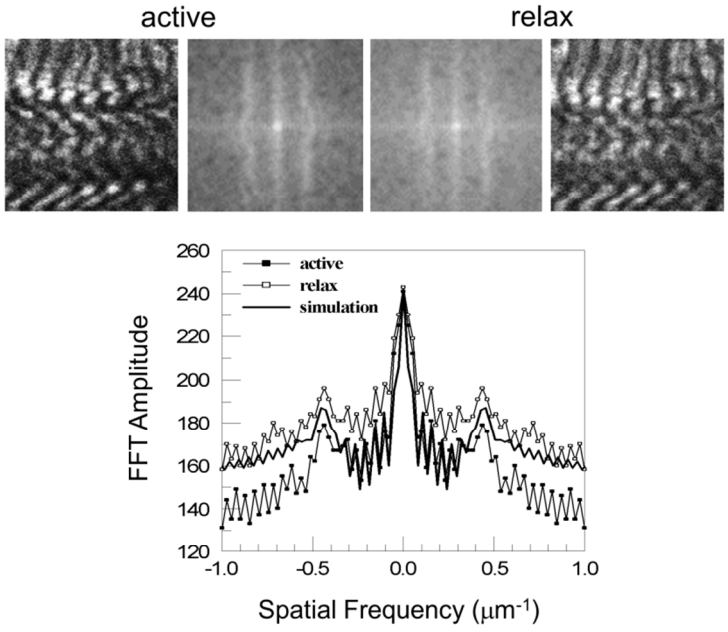
active
relax
active
relax
simulation
FFT Amplitude
260
240
220
200
180
160
140
120
-1.0
-0.5
0.0
0.5
1.0
Spatial Frequency (μm-1)

Figure S2

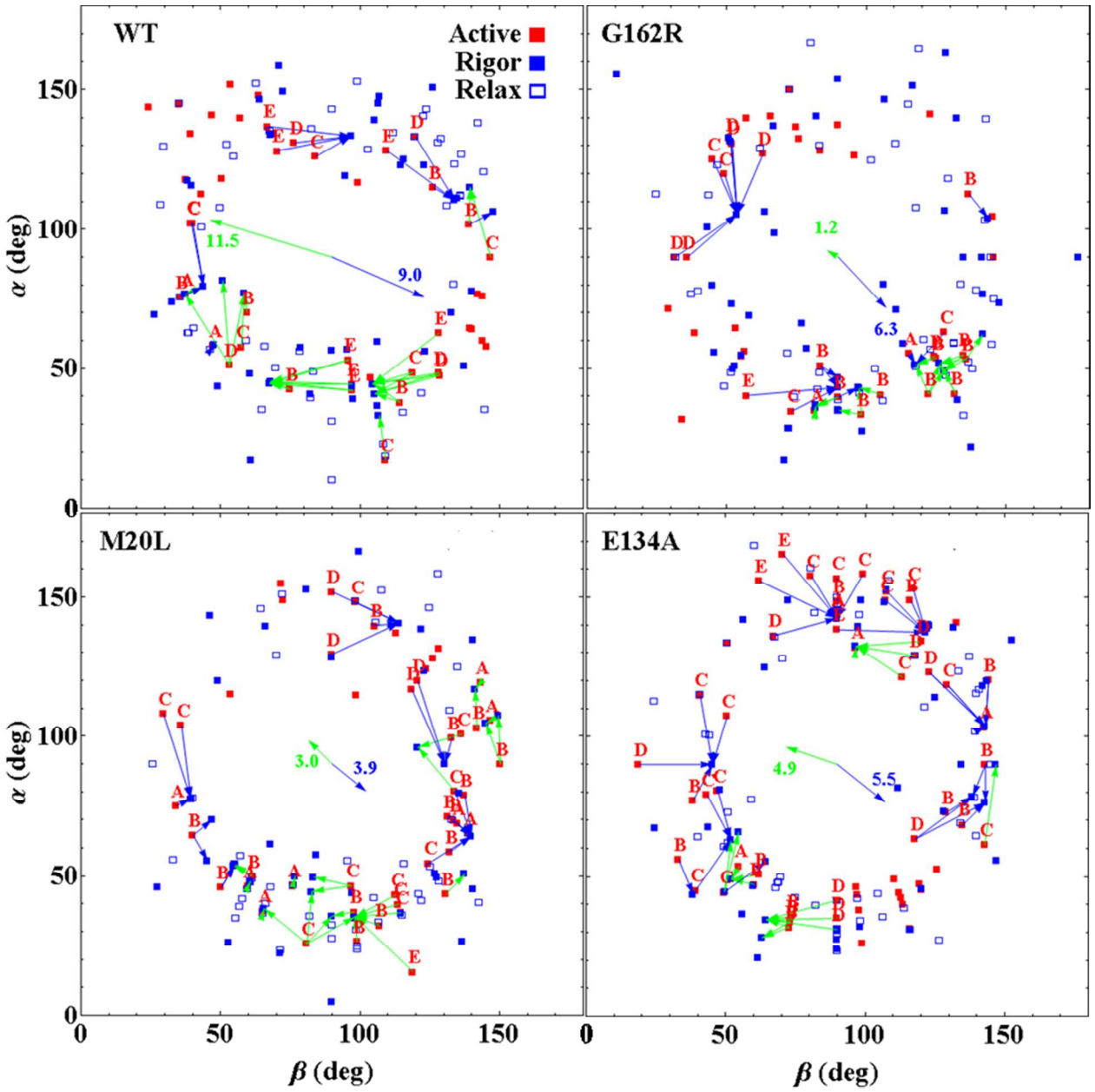
WT
Active
Rigor
Relax
G162R
M20L
E134A
α (deg)
β (deg)
0
50
100
150
11.5
9.0
1.2
6.3
3.0
3.9
4.9
5.5

Figure S3

References

1. Burghardt, T. P. (2012) Evanescent field shapes excitation profile under axial epi-illumination, *J.Biomed.Optics 17*, 066021.
2. Jo, B. H., Van Lerberghe, M., Motsegood, K. M., and Beebe, D. J. (2000) Three-Dimensional Micro-Channel Fabrication in Polydimethylsiloxane (PDMS) Elastomer, *J.Microelectriomechanical systems 9*, 76-81.