## **Supporting Information**

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## SI Text

Rigor in frog intact fibers was induced by means of metabolic poisons, as previously described (1). The SL was adjusted before the rigor induction. Second-harmonic generation (SHG) measurements in rigor were performed at room temperature.

Fig. S1 shows SHG polarization anisotropy (SPA) results from rigor obtained in intact frog fibers (gray circles). The data show a large dispersion compared to skinned rigor data from rabbit muscle (red circles). This dispersion is most likely due to the complexity of rigor preparation in intact fibers.

The dispersion was somewhat reduced when rigor was induced following frog fiber demembranation obtained as follows. After SPA measurements, the intact fiber was skinned for 20 min at room temperature in frog-rigor solution [EDTA 34 mM, N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES)

 Piazzesi G, et al. (2007) Skeletal muscle performance determined by modulation of number of myosin motors rather than motor force or stroke size. Cell 131:784–795. 100 mM, pH 7.1] with added 2,3-Butanedione monoxime (BDM) (20 mM) and Triton X-100 (0.5%). The SL was adjusted in relaxing solution. Rigor was induced in the presence of BDM (20 mM), incubating the fiber for 10 min at 10 °C in prerigor solution (Na<sub>2</sub>ATP 0.5 mM, MgCl<sub>2</sub> 3.0 mM, EGTA 10 mM, TES 100 mM, pH 7.1, BDM 20 mM), and then in frog-rigor solution at 1 °C. This preparation allowed us to perform SPA measurements on the same fiber in rigor at different SL (Fig. S1, blue symbols). The data confirm the same linear decrease of  $\gamma$  with SL, as that found in skinned rigor fibers from rabbit. Moreover, in frog fibers at an SL between 2.0 and 2.2  $\mu$ m, we found the following: in intact fibers  $\gamma_{\rm rig}=0.73\pm0.05~(n=2);$  in skinned fibers  $\gamma_{\rm rig}=0.69\pm0.04~(n=2).$  These data are in good agreement with measurements on skinned rabbit fibers at full overlap.

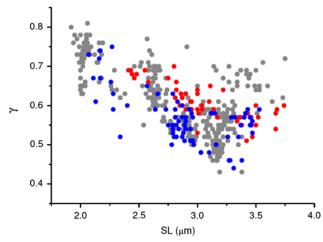


Fig. S1. Measured γ versus SL in rigor in 10 intact frog fibers (gray circles), in four skinned frog fibers (blue circles), and three skinned rabbit fibers (red circles).