Supporting Information

Greenberg et al. 10.1073/pnas.1009619107

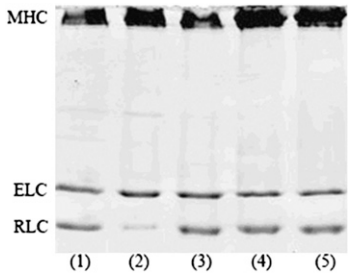

Fig. S1. Representative SDS/PAGE of native porcine myosin preparations. Triton/CDTA treatment yielded ~85% of RLC depletion. Depletion and exchange of the RLC were quantified using densitometry. Ten individual RLC-depleted myosin preparations demonstrated 86 ± 3% of RLC depletion (n = 10). Multiple RLC-reconstituted preparations were 95 ± 13% (n = 11), 97 ± 8% (n = 10), and 92 ± 9% (n = 9) reconstituted with human WT, N47K, and R58Q RLC, respectively. Shown are the following: 1, native porcine cardiac myosin; 2, RLC-depleted porcine cardiac myosin; 3, porcine cardiac myosin with exchanged human cardiac RLC (WT); 4, porcine cardiac myosin with exchanged human cardiac RLC bearing N47K; and 5, porcine cardiac myosin with exchanged human cardiac RLC bearing R58Q.

```
                    10        20        30        40        50        60
                     |         |         |         |         |         |
pig_cRLC     MSPKKAKKRADGANSNVFSMFEQTQIQEFKEAFTIMDQNRDGFIDKNDLRDTFAALGRVN
human_cRLC   MAPKKAKKRAGGANSNVFSMFEQTQIQEFKEAFTIMDQNRDGFIDKNDLRDTFAALGRVN

                    70        80        90       100       110       120
                     |         |         |         |         |         |
pig_cRLC     VKNEEIDEMIKEAPGPINFTVFLTMFGEKLKGADPEETILNAFKVFDPEGKGVLRADYVK
human_RLC    VKNEEIDEMIKEAPGPINFTVFLTMFGEKLKGADPEETILNAFKVFDPEGKGVLKADYVR

                   130       140       150       160
                     |         |         |         |
pig_cRLC     EMLTTQAERFSKEEIEQMFAAFPPDVTGNLDYKNLVHIITHGEEKD
human_RLC    EMLTTQAERFSKEEVDQMFAAFPPDVTGNLDYKNLVHIITHGEEKD
```

Fig. S2. Alignment of human (accession no. P10916) and porcine (accession no. Q8MHY0) cardiac RLC using MULTALIN. Human RLC was found to be 96% identical and 99% similar.

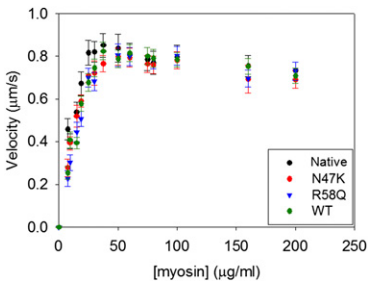

Fig. S3. The myosin duty cycle, the fraction of the myosin biochemical cycle spent attached to actin, was studied by examining the actin sliding velocity as a function of the concentration of myosin added to the flow cell. The unloaded in vitro motility assays were performed as previously described (1). Myosin (~200 μg) was precipitated in 10 mM DTT for 1 h on ice and collected by centrifugation at 16,000 × g for 30 min at 4 °C. The pellet was resuspended in 200 μL of myosin buffer (300 mM KCl, 25 mM imidazole, 1 mM EGTA, 4 mM $MgCl_2$, 10 mM DTT). Damaged rigor-like myosin heads were removed by mixing the myosin, 1 mM ATP, and 1.1 mM actin and centrifuging in an Airfuge for 30 min at 100,000 × g. Myosin was adsorbed to a nitrocellulose-coated coverslip surface by incubating 30 μL of myosin (100 μg/mL) in myosin buffer for 1 min, blocked by adding 30 μL of 0.5 mg/mL BSA (BSA) in myosin buffer, and washed with 60 μL actin buffer (25 mM KCl, 25 mM imidazole, 1 mM EGTA, 4 mM $MgCl_2$, 10 mM DTT). Then 30 μL of 1 μM unlabeled actin in actin buffer was added. After 2 min the flow cell was washed with 60 μL of actin buffer containing 1 mM ATP and then 120 μL of actin buffer without ATP. After incubation with 30 μL of 5 nM TRITC-labeled actin for 1 min, motility was initiated with motility buffer (actin buffer with the addition of 0.5% methyl cellulose, 1 mM ATP, 2 mM dextrose, 160 units glucose oxidase, and 2 μM catalase). The myosin concentration after centrifugation was determined using a Bradford assay (Bio-Rad). According to a detachment-limited model of actin motility (2), actin filaments in the motility assay will move at their maximal velocity if at least one myosin head interacts with the actin filament at any given time. A myosin with a higher duty cycle will require fewer myosin heads on the surface to move at a maximal velocity whereas a myosin with a lower duty cycle will require a greater concentration to move actin filaments at maximal velocity. As can be seen, there are no differences in either the maximal sliding velocity or the concentration of myosin necessary to reach maximal sliding velocity between any of the myosin isoforms examined (native, WT, N47K, and R58Q).

1. Greenberg MJ, et al. (2009) Regulatory light chain mutations associated with cardiomyopathy affect myosin mechanics and kinetics. *J Mol Cell Cardiol* 46:108–115.
2. Siemankowski RF, Wiseman MO, White HD (1985) ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc Natl Acad Sci USA* 82:658–662.

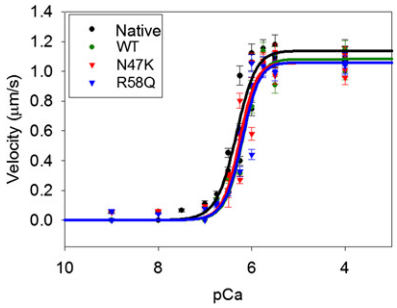

Fig. S4. The regulation of thin filament sliding by troponin and tropomyosin was also studied with the in vitro motility assay. The procedure for regulated motility assays was similar to that for the unregulated motility assays (*Materials and Methods*), except 150 nM tropomyosin and troponin in actin buffer was added to the flow cell after the TRITC phalloidin-labeled actin incubation and incubated for 10 min. A total of 30 μL of appropriate pCa motility buffer was added with the inclusion of 75 nM troponin and 75 nM tropomyosin. The pCa_{50} and the Hill coefficients, *H*, were determined by fitting the equation $V = \frac{V_{max}[Ca^{2+}]^H}{pCa_{50}^H + [Ca^{2+}]^H}$ to the data of velocity versus calcium where V_{max} is the maximal velocity and *H* is the Hill coefficient. As can be seen from the data, the relationship between calcium concentration and velocity is unchanged by the mutations. There is no difference in the maximal sliding velocity of regulated thin filaments when compared with the WT [V_{max}(WT) = 1.08 ± 0.05 μm/s, V_{max}(native) = 1.14 ± 0.04 μm/s, *P* = 0.441; V_{max}(N47K) = 1.06 ± 0.05 μm/s, *P* = 0.747; V_{max}(R58Q) = 1.06 ± 0.05 μm/s, *P* = 0.774]. Furthermore, there are no differences in the cooperativity of thin filament activation between any of the myosins examined when compared with the WT [*H*(WT) = 34 ± 7, *H*(native) = 30 ± 5, *P* = 0.652; *H*(N47K) = 35 ± 7, *P* = 0.911; *H*(R58Q) = 34 ± 7, *P* = 0.958]. Finally, there is no significant difference in calcium sensitivity as indicated by the pCa_{50}, the amount of calcium necessary for half-maximal activation [pCa_{50}(WT) = 6.19 ± 0.05, pCa_{50}(native) = 6.33 ± 0.04, *P* = 0.04 (which is not significant when corrected for multiple comparisons using the Holm *t* test); pCa_{50}(N47K) = 6.26 ± 0.04, *P* = 0.312; pCa_{50}(R58Q) = 6.21 ± 0.05, *P* = 0.833]. Thus there are no differences between the WT and either R58Q or N47K in regulated motility assays.