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Supplementary Materials for

Myosin-binding protein C corrects an intrinsic inhomogeneity in cardiac excitation-contraction coupling

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This PDF file includes:

H2: Supplementary Materials

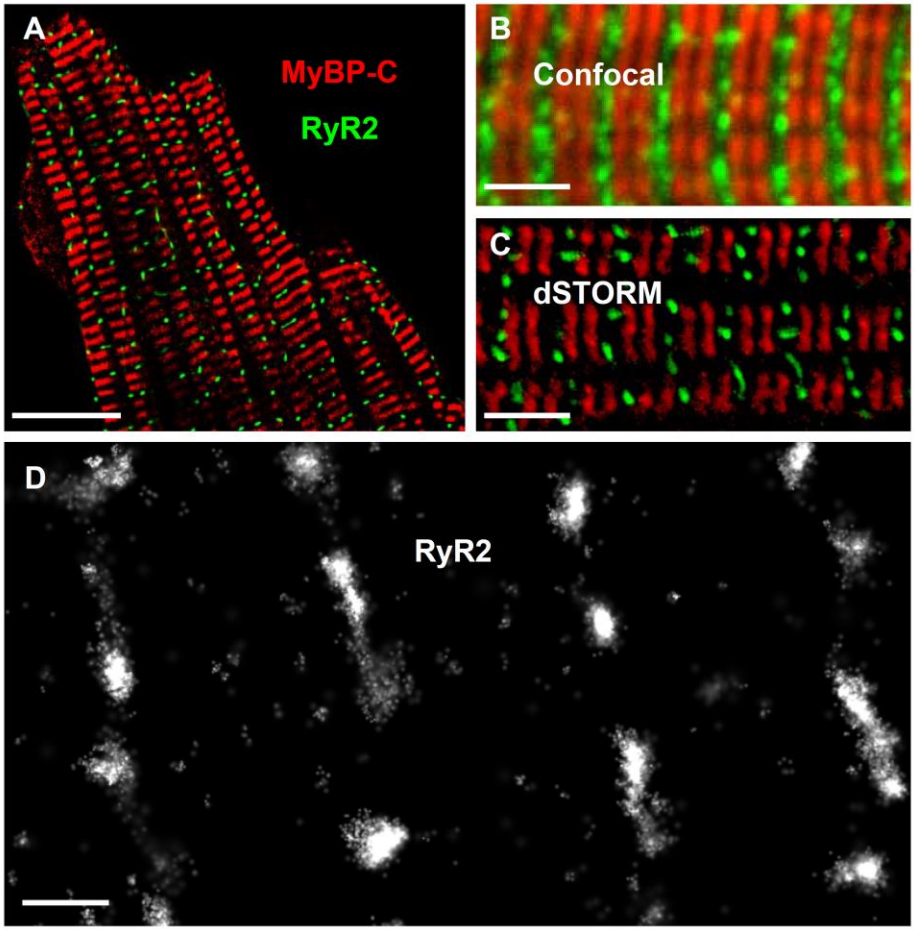

Fig. S1. Two-color dSTORM super-resolution imaging of MyBP-C and ryanodine receptors (RyR2). **(A)** Ventricular myocyte labeled with N-terminal MyBP-C antibody conjugated to AlexaFluor-647 (red) and RyR2 antibody conjugated to AlexaFluor-488 (green). Scale bar is 10 μm. **(B)** Comparison of confocal image (identical labeling as in **A**) with **(C)** dSTORM image of similar region in two different ventricular myocytes. Scale bars are 2μm. **(D)** Zoom in of dSTORM image of RyR2 antibody conjugated to AlexaFluor-647 to show punctate RyR2 clusters aligned at Z-lines at high resolution. Scale bar is 500 nm.

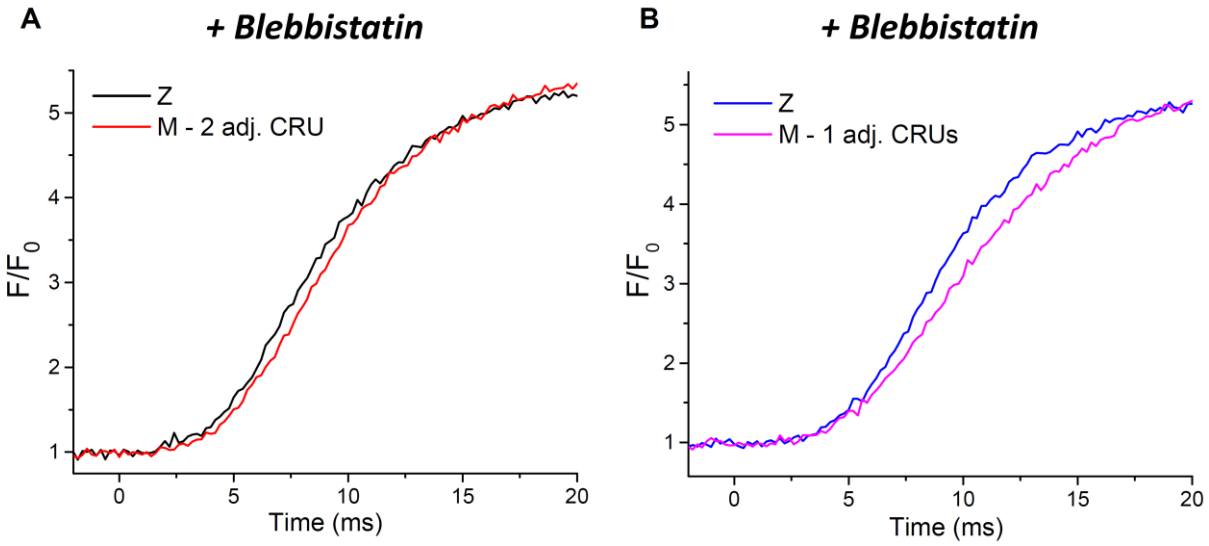

Fig. S2. Treatment with the contractile inhibitor blebbistatin does not obscure sarcomeric calcium gradients. **(A and B)** Rising phase of $[Ca^{2+}]_i$ transient at Z-line and adjacent M-lines when flanked by 2 firing CRUs **(A)** or only 1 firing CRU **(B)** in the presence of 5μM blebbistatin to block cardiac contraction.

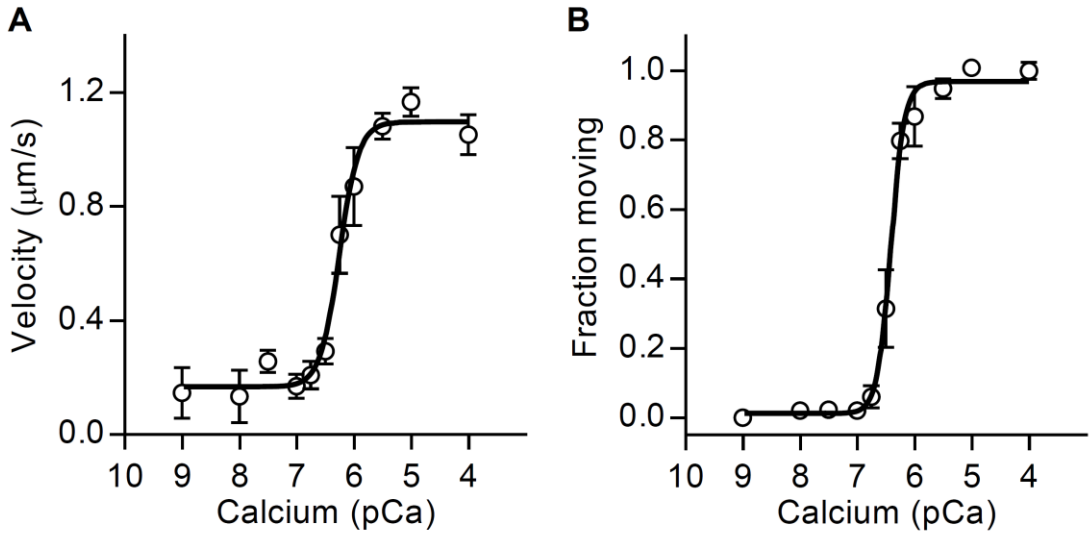

Fig. S3. Calcium dependent regulation of native thin filament motion. **(A)** Velocity ± SEM- and **(B)** fraction of thin filaments moving ± SEM-pCa plots for native thin filaments on a bed of monomeric myosin in an *in vitro* motility assay. Data fitted with sigmoidal-dose response curves with similar pCa_{50} values (6.3 ± 0.04 and 6.4 ± 0.02 respectively).

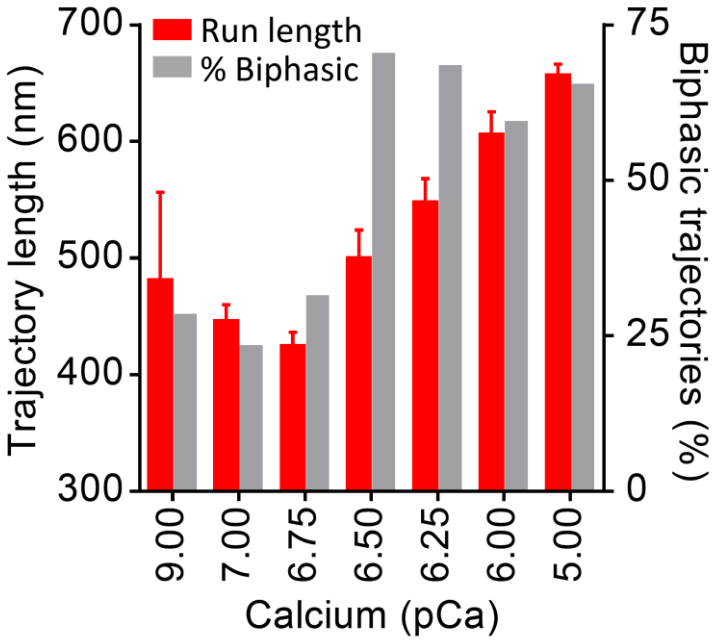

Fig. S4. Trajectory length ± SEM- and percent trajectories with biphasic velocities-pCa histograms for thin filaments on λ-phosphatase treated thick filaments containing dephosphorylated MyBP-C.

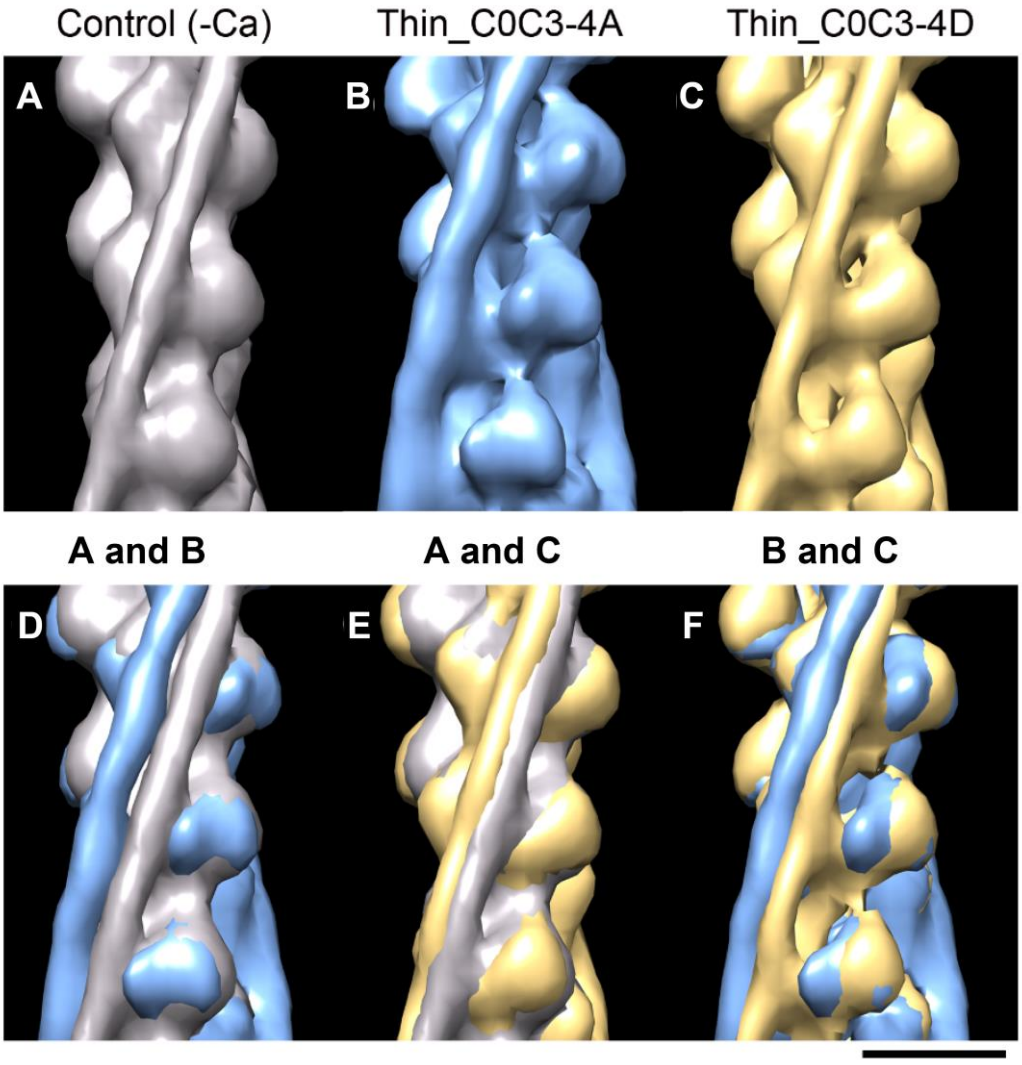

Fig. S5. 3D reconstructions of low Ca^{2+} native thin filaments decorated with C0C3 (actin:C0C3 = 1:3) in which the 4 phosphorylatable serines have been replaced with alanines (fully dephosphorylated, C0C3-4A) or with aspartic acid residues (phosphomimetic, C0C3-4D). **(A)** Undecorated (control) filament (cf. Fig. 3F). **(B)** Decorated with C0C3-4A, showing shift of Tm from "blocked" to "closed" position (Fig. 3H). **(C)** Decorated with C0C3-4D, showing smaller Tm movement towards the "closed" position (Fig. 3I). **(D-F)** Superpositions of the reconstructions in A-C highlighting the greater movement of Tm with C0C3-4A than with C0C3-4D (Fig. 3H, I). Filaments oriented with pointed end up. Scale bar = 5 nm.

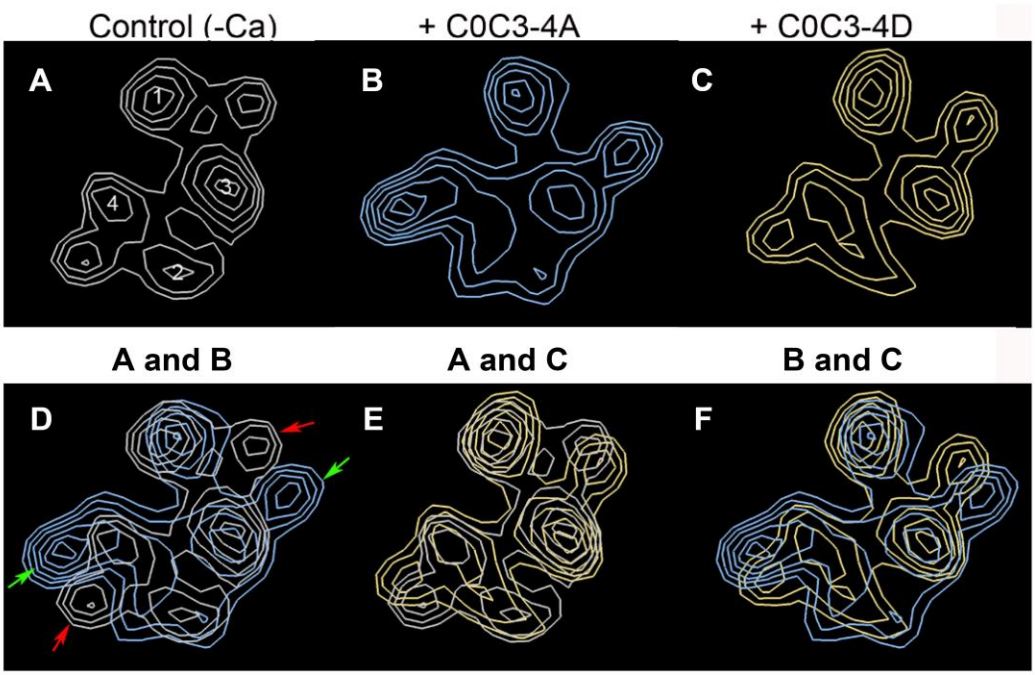

Fig. S6. Transverse sections of the respective low-Ca^{2+} reconstructions in Extended Data Fig. 5, in which native thin filaments have been decorated with C0C3-4A and C0C3-4D. **(A)** Control filament showing positioning of Tm near the junction of actin subdomain 1 (SD1) and SD3 ("blocked") position. **(B)** Decorated with C0C3-4A showing shift of Tm on to SD3 ("closed" position). **(C)** Decorated with C0C3-4D, showing smaller Tm movement towards the "closed" position. **(D-F)** Superpositions of the reconstructions in A-C highlighting the greater movement of Tm with C0C3-4A than with C0C3-4D. Arrows represent the "blocked" (red) and "closed" (green) positions of Tm.

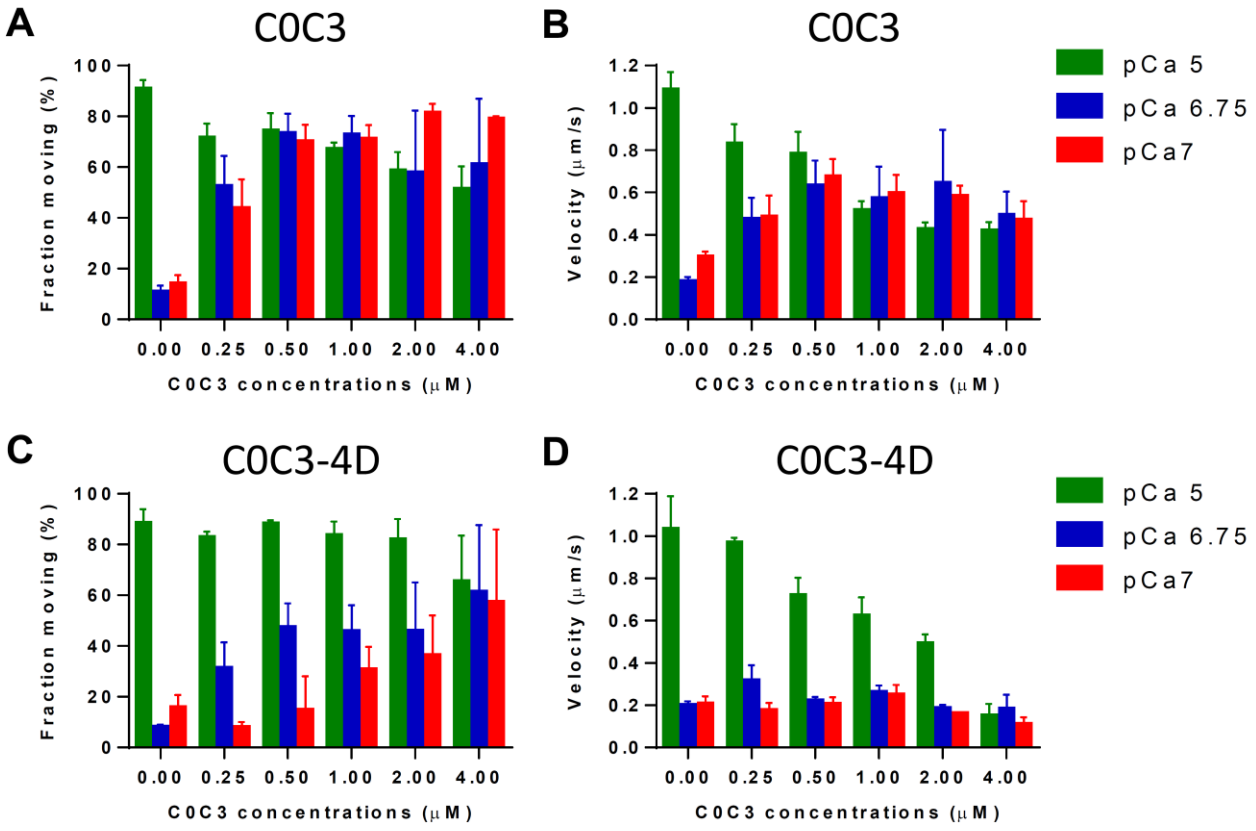

Fig. S7. Phosphorylation of MyBP-C N-terminal domains modulates their ability to activate native thin filament motion in the *in vitro* motility assay on a bed of monomeric myosin. **(A and B)** Concentration dependent effect of (dephosphorylated) C0C3 N-terminal fragments on the fraction of native thin filaments moving and their sliding velocities. Mean ± SEM (N=4). **(A)** Addition of C0C3 initiates filament motion at low calcium levels pCa 6.75 (blue) and 7 (red). **(B)** Although C0C3 initiates sliding at low calcium levels its presence in the assay inhibits maximal velocities when the system is fully calcium activated (pCa 5, green). **(C and D)** As in **A** and **B** upon addition of C0C3 with the four phosphorylatable serines within the M-domain (Fig 1B) replaced by phosphomimetic aspartic acid residues (C0C3-4D) to mimic phosphorylation. Mean ± SEM (N=4). **(C)** Addition of C0C3-4D is still capable of initiating thin filament sliding but to a lesser degree than C0C3. **(D)** Despite C0C3-4D activating thin filament motion, the thin filaments slide at slow velocities, which is indicative of myosin only having partial access to the thin filament as the thin filament is not completely activated at the lower calcium concentrations.