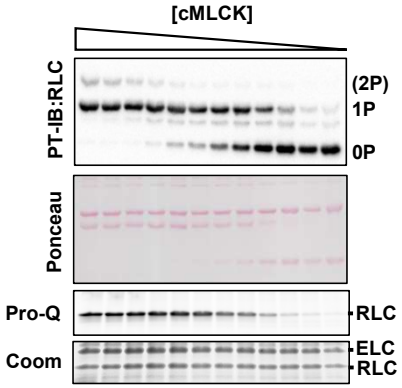
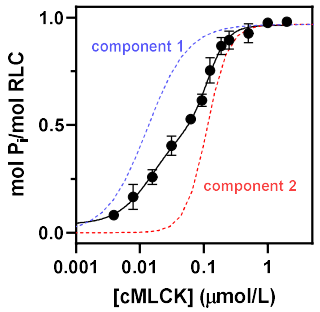
1. RLCs in intact myofibrils are phosphorylated with at least two different kinetics/EC_{50}

A

Cardiac Myofibrils



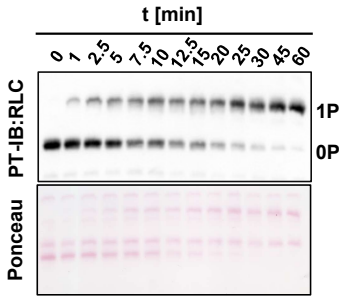


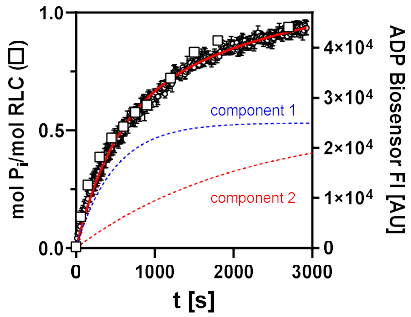
Biphasic preferred to monophasic model?

Yes

AIC: 75% probability

ESSF: p = 0.0349





Biphasic preferred to monophasic model?

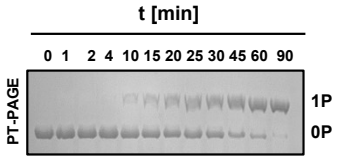
Yes

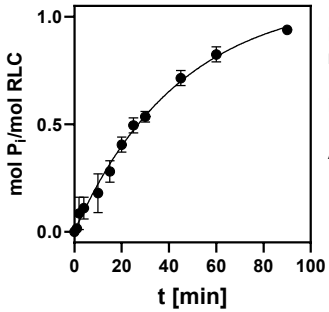
AIC: >99.99% probability

ESSF: p < 0.0001

B

Cardiac Regulatory Light Chain





Biphasic preferred to monophasic model?

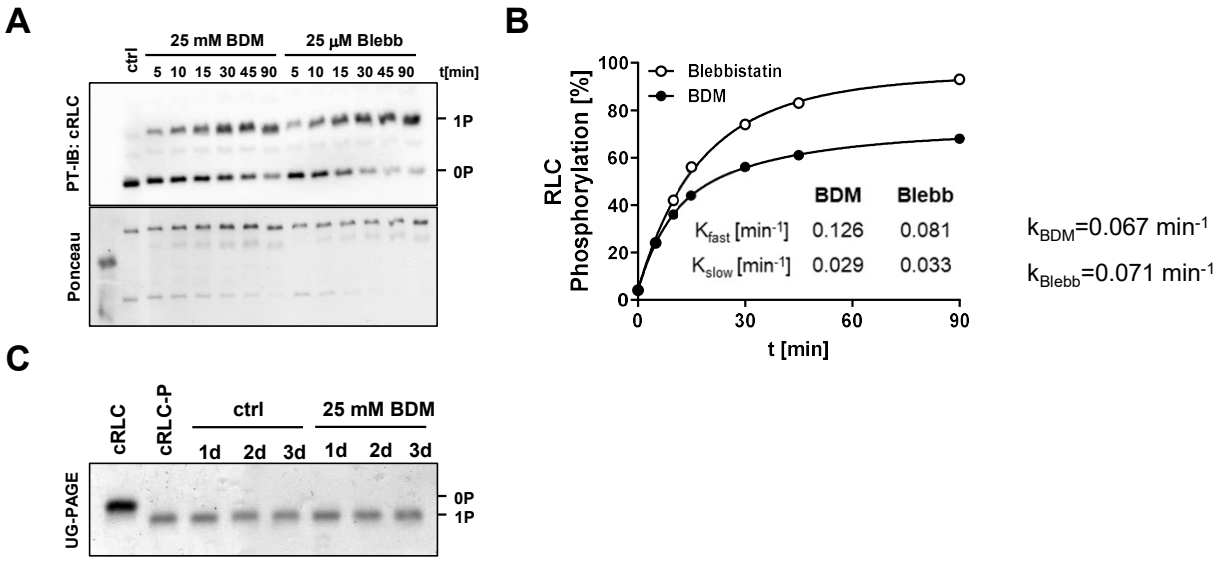
No

AIC: 95.55% probability for monophasic

C

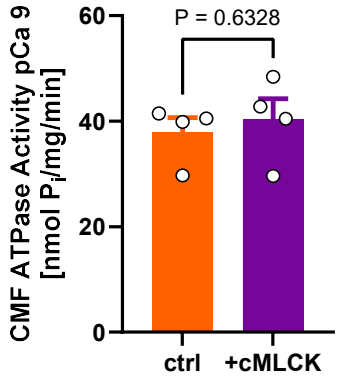
Synthetic Myosin Filaments

Supplementary Figure 1 – Optimization of conditions for myofibrillar kinase assay.

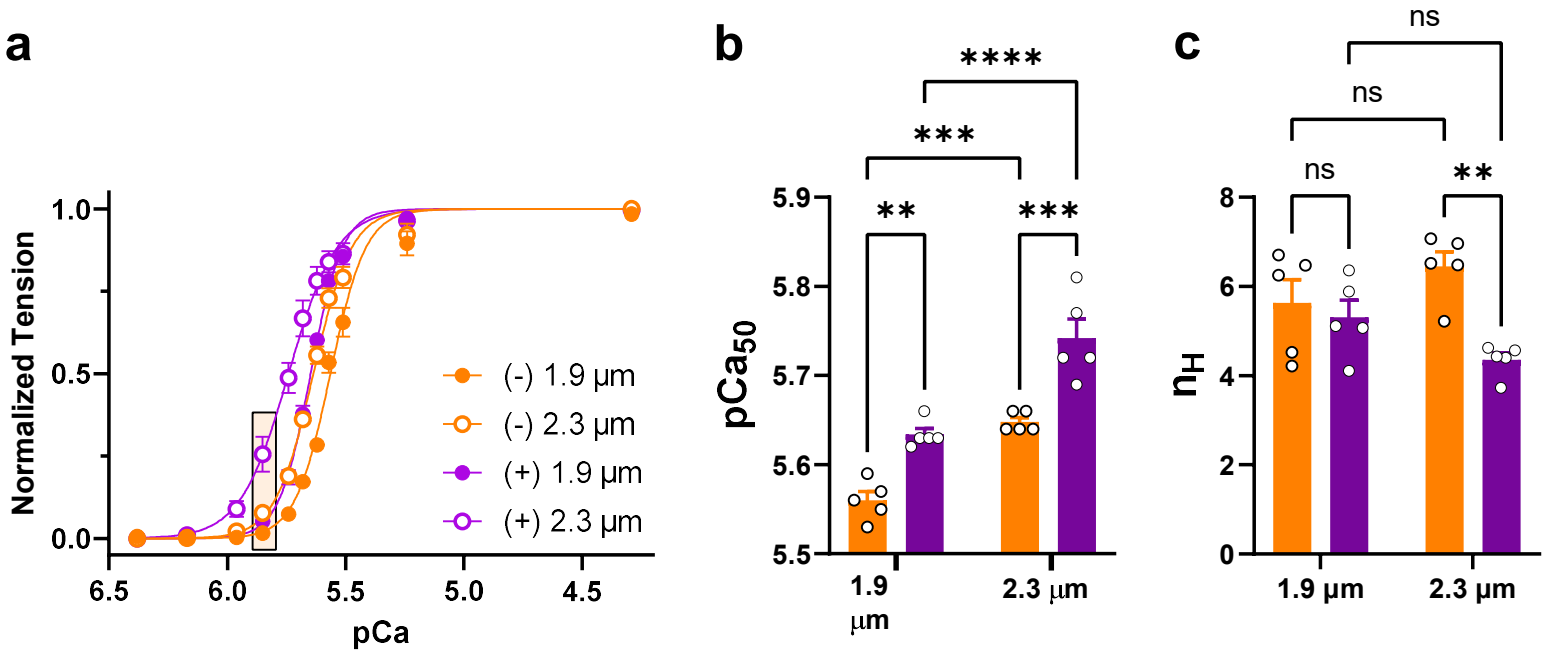


Supplementary Figure 2 – Effect of RLC phos on CMF ATPase in relaxing conditions

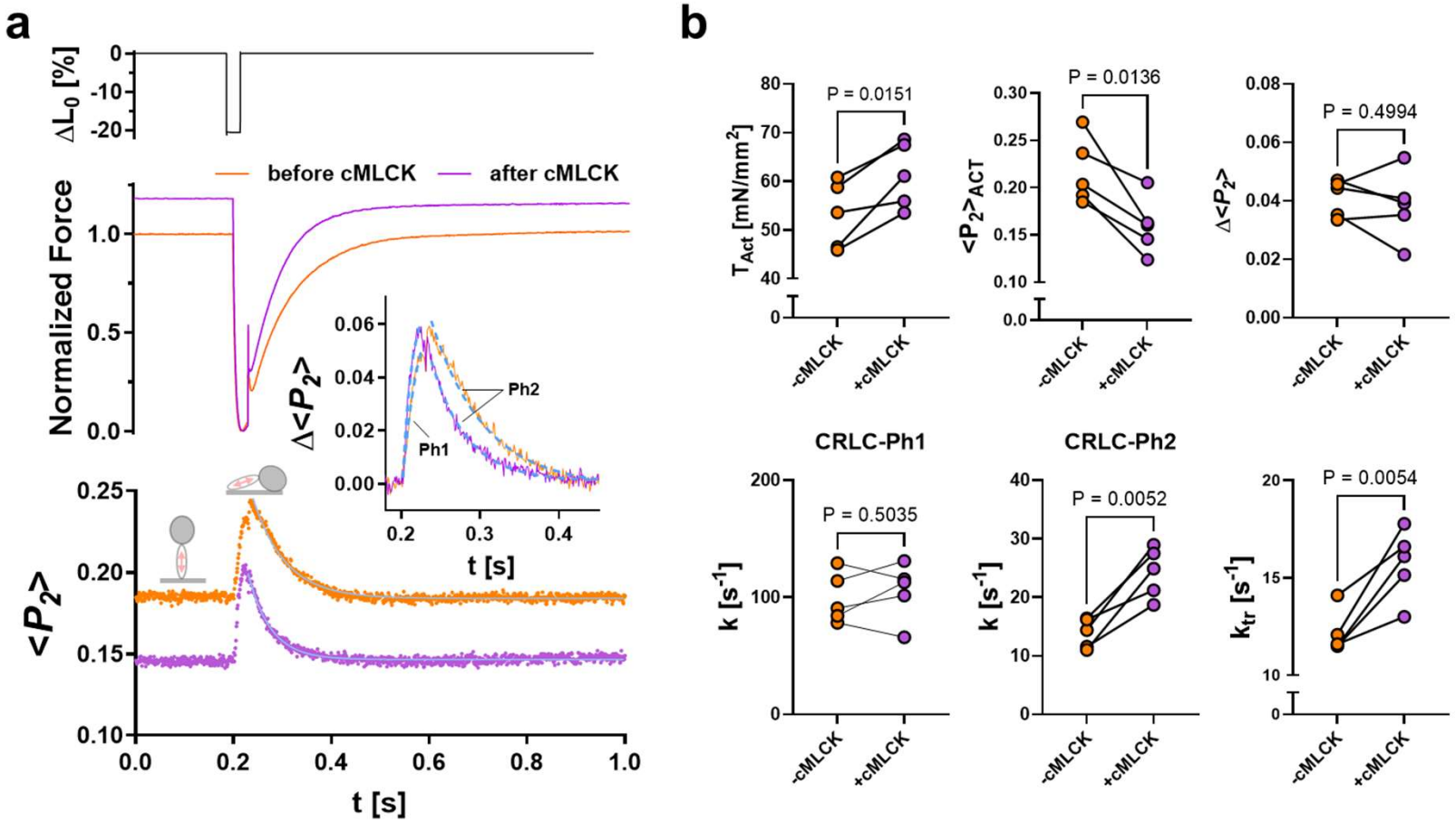
File: '170628_CMF_ATPase pCa 9'



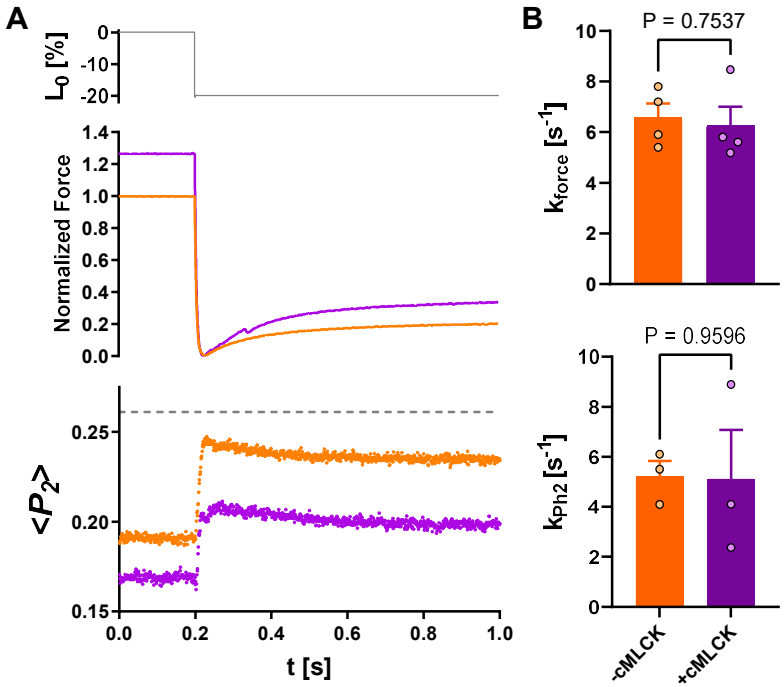
2. Myosin RLC phosphorylation increases force production at sup-optimal calcium concentrations only at long sarcomere length



3. RLC phosphorylation increases rate of force redevelopment and rate of myosin activation after slack-restretch

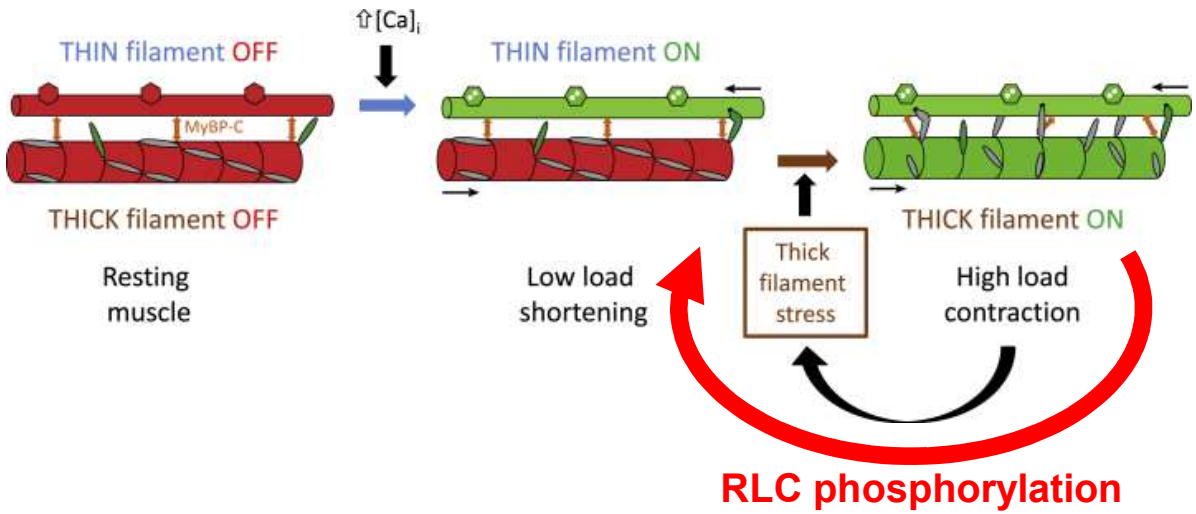


4. RLC phosphorylation does <u>NOT</u> increases rate of force redevelopment and rate of myosin activation after length step (slack)

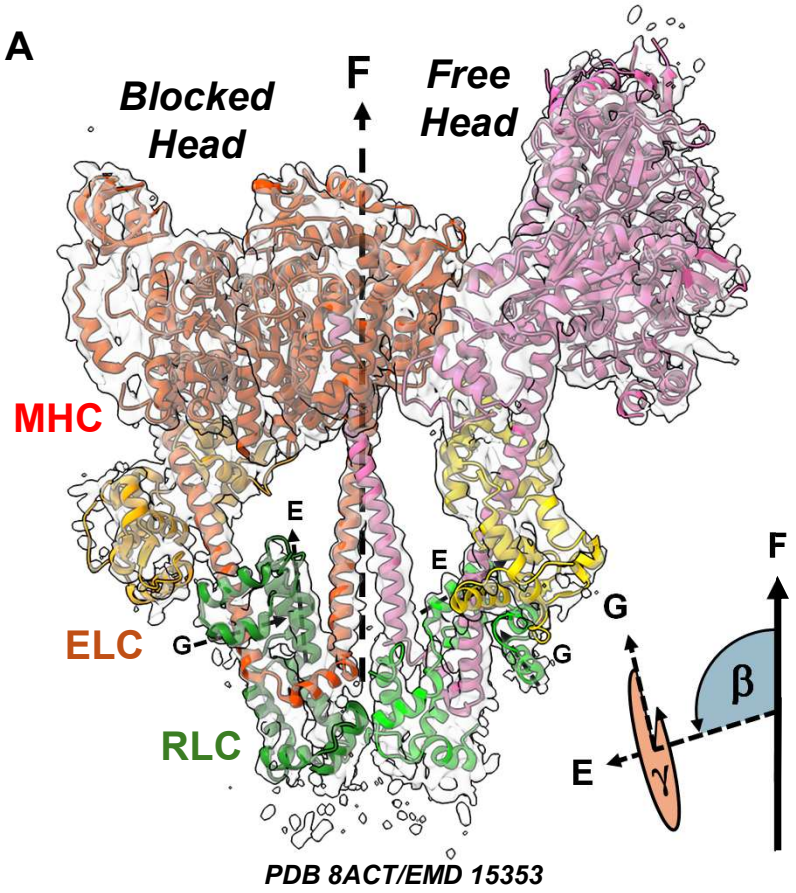


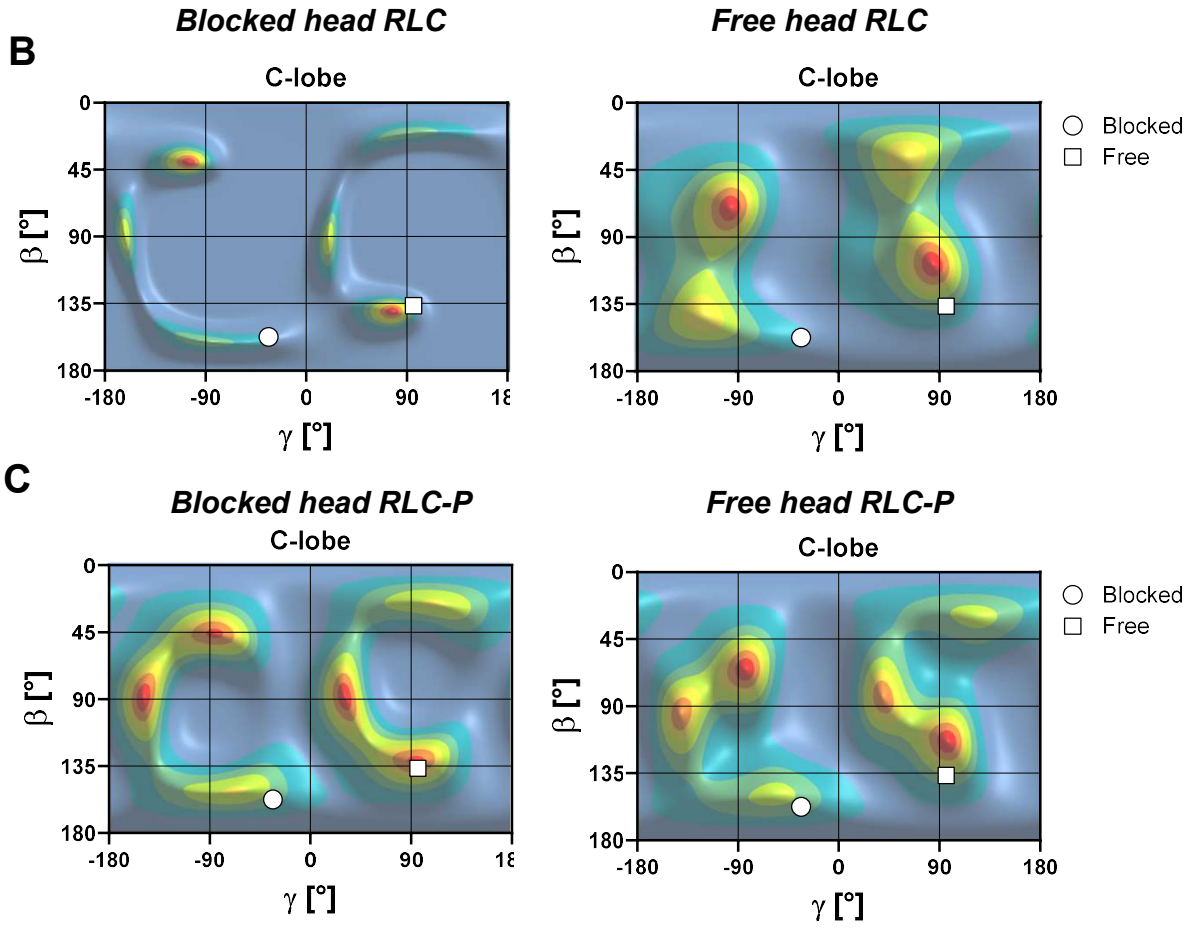
Hypothesis:

RLC phosphorylation increases the 'gain' of the thick filament mechano-sensing

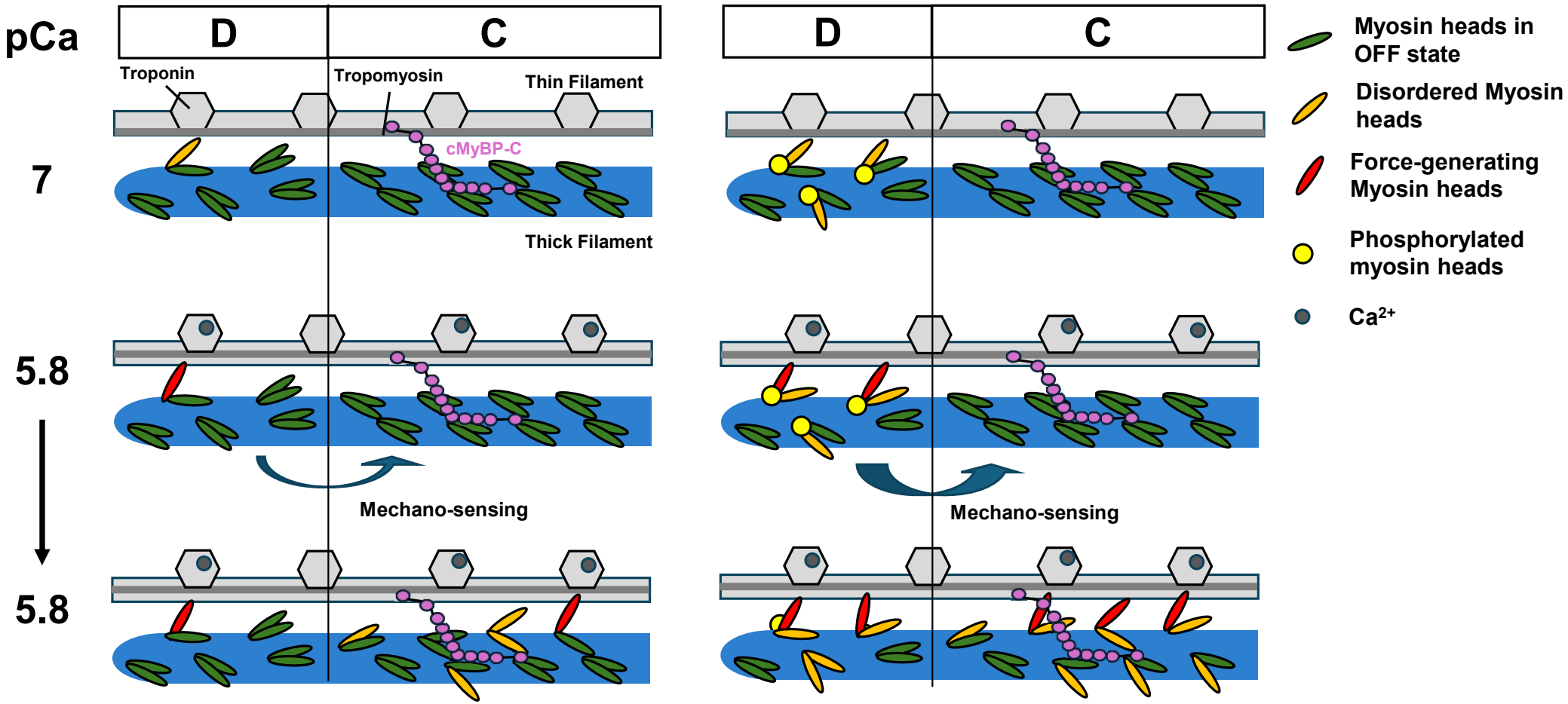


5. Hypothesis 1: RLC phosphorylation activates the 'blocked head'

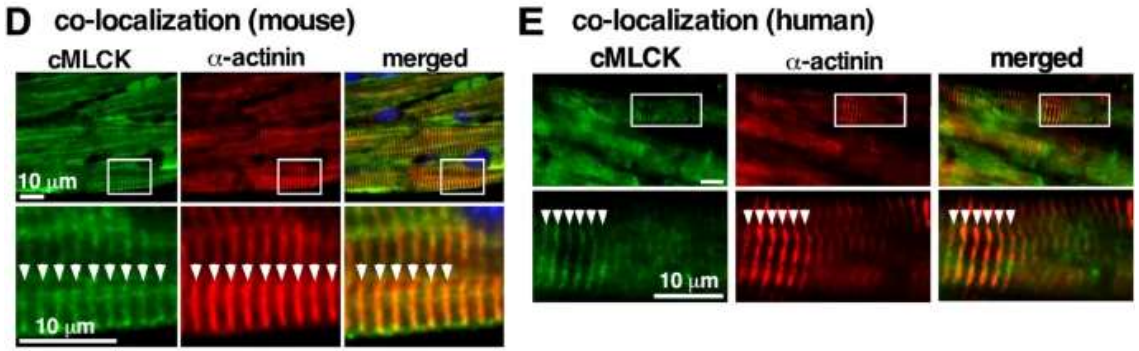




Hypothesis 2: Myosin heads/RLCs in the D-zone are preferentially phosphorylated by cMLCK
Phosphorylated 'sentient' heads in the D-zone facilitate force-dependent activation of thick filament

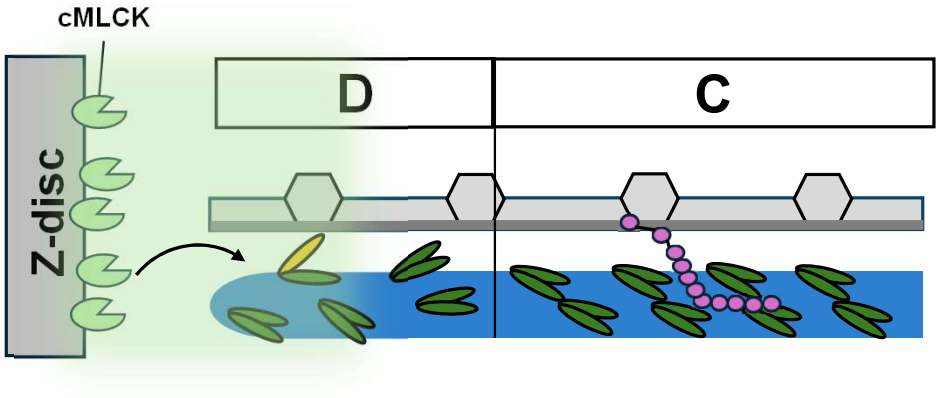


Additional data: cMLCK localizes to Z-disc or Z-disc adjacent regions of sarcomere



Ca et al., 2019, Scientific Reports

→ If cMLCK mainly localizes to Z-disc, D-zone heads are much closer and therefore more likely to get phosphorylated in vivo????



FiberSim Modelling

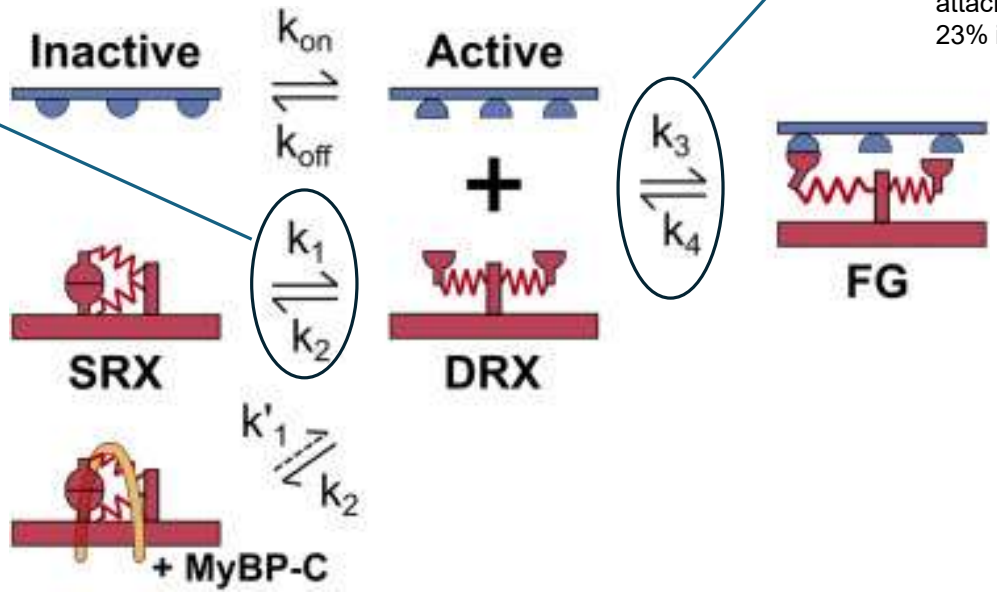
Stelzer et al., 2006, Circ Res
krel (=k4?) is about 30% slower after RLC phos; Kdf (=k3?) is about 2.5 times faster

Pulcastro et al., 2016, ABB
RLC phos slowed MgATP binding rate ~50% (=reduced k4 by 50%)

Campbell et al. 2018, Biophys J
→ Best fit assumes 50% increase in k1, no effect on k3 or k4
→ k1 = 6.17 s-1 before and 9.13 s-1 after phosphorylation

Sheikh et al., 2012, JCI
2.6-fold increase in the rate of crossbridge attachment (=k3?)
23% increase in crossbridge stiffness

Park-Holohan et al. 2021, PNAS
→ RLC phos does not change stress-dependent activstion of thick filamtn
→ = no change in k1 or k2?



<P_2> recovery during ktr protocol is twice as fast after RLC phos
(could indicate that k3 is twice as fast)