Supplementary Information

Cardiac ventricular myosin II and slow skeletal myosin II exhibit dissimilar chemo-mechanical properties despite bearing the same myosin heavy chain isoform.

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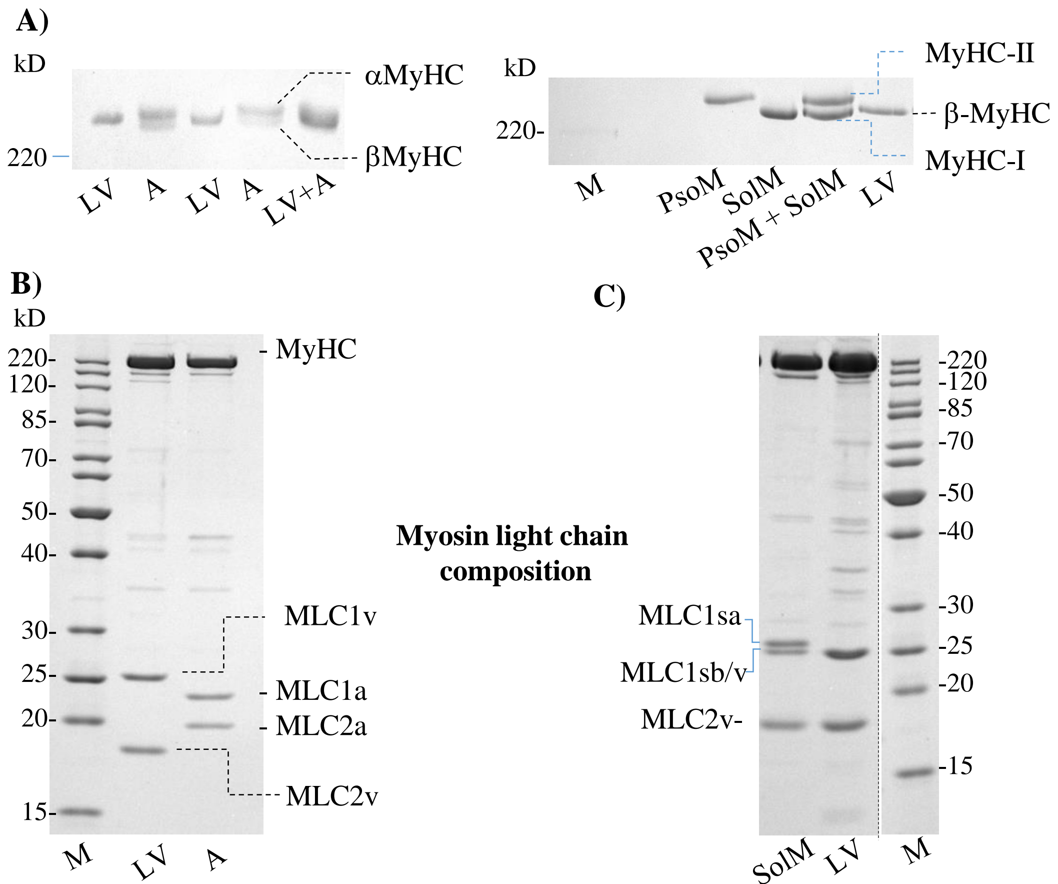

Figure S1. Myosin isoform gel for βM-II. Heavy and light chain composition among α- and β- myosin II as well as M. soleus myosin (SolM) and β- myosin compared. **A)** Left panel, myosin II isolated from rabbit left ventricle (LV) and atrium (A) tissue. 6.5 % acrylamid/bisacrylamide gel with 5 % glycerol was used to separate the α- and β- myosin heavy chain isoforms. The long gel (20 cm) was run for 18 hrs at room temperature and followed by coomassie staining. For lane 1 and 2 - 0.5 µg of protein sample, lane 3 and 4 – 0.375 µg sample, and for lane 5 – mixture of 0.5 µg each of atrial and ventricular myosin was loaded. While, two myosin heavy chain isoforms (α-MyHC and β-MyHC) as two distinct bands were detectible in atrium derived myosin, ventricular tissue showed only single band corresponding to β-MyHC. Note that below 5% contamination of α-MyHC in the ventricular tissue derived myosin is unlikely to be evident in the gel analysis. Right panel, the M. soleus and β- myosin are compared for the heavy chain isoforms. 8% Acrylamid/Bisacrylamid gel with 30% glycerol was used to separate the myosin heavy chain isoform. M. psoas M-II (PsoM) is also run alongside or as a mixture with SolM. Similar size bands for SolM and LV myosin heavy chains indicated as MyHC-I and β-MyHC, respectively, were observed, while MyHC-II is distinct from the two. B) The light chain compositions were analyzed on 12.5 % gel. Distinct bands corresponding to the essential and regulatory light chains typically associating with the α-MyHC and β-MyHC were observed. In each lane - 2 µg protein is loaded to check the light chain composition. C) Light chain composition of LV myosin and M. soleus myosin (SolM). 4 µg of proteins loaded in each lane. The dashed line indicates the trimmed area on the same gel to enclose the marker lane.

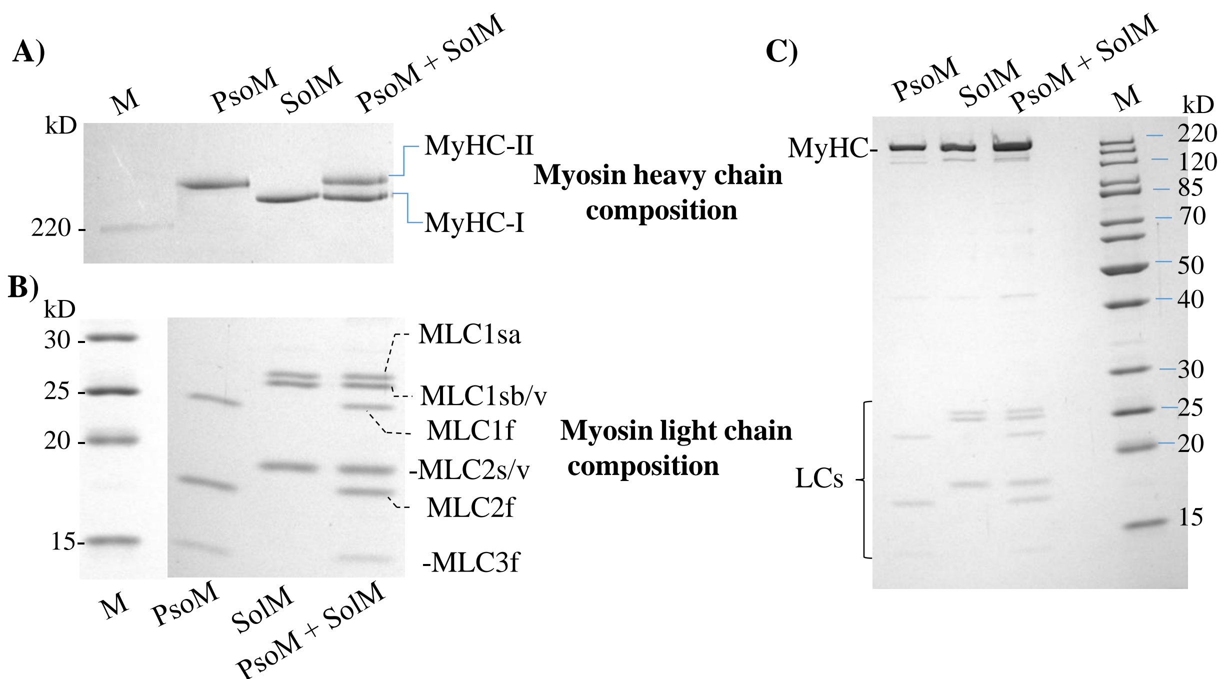

Figure S2. Myosin isoform gel for SolM-II. A) 8% acrylamid/bisacrylamide gel with 30 % glycerol was used to separate the myosin heavy chain isoform. The 20 cm custom-made gel was run for 25 hrs at 4°C. 0.5 µg of protein/lane was loaded. Lane 2 and 3 – isolated protein from two muscle sources M. psoas (PsoM) and M. soleus (SolM) . Lane 4- mixture of protein (0.5 µg each) used in lane 2 and 3 were loaded. Fast M. psoas myosin heavy chain (MyHC-II) is seen higher than slow M. soleus myosin (MyHC-I) with distinct banding. **B)** Same probes as in A) examined for corresponding light chains for the two myosin isoforms on 12.5 % SDS-PAGE gel. Distinct light chains, i.e., MLC1sa (27 kD), MLC1sb/v (24 kD), and MLC2s/v were seen to assemble with the SolMII, whereas PsoMII can be found in complex with MLC1f (20.7 kD), MLC3f (16.5 kD), and MLC2f. **C)** 12.5 % gel for the same sequence of myosins loaded showing both heavy and light chains. Note that the heavy chain isoforms cannot be distinguised in this gel. LCs- light chains, M- marker.

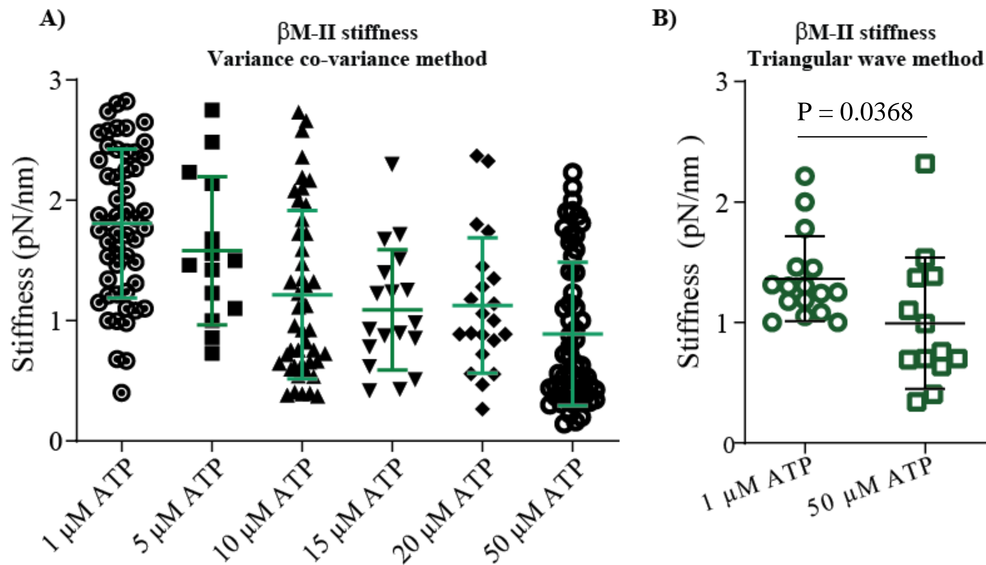

Figure S3. Stiffness of βM-II. **A)** Stiffness of individual βMII molecules measured at various ATP concentrations shown. Each data point indicates the average stiffness determined from 100s of AM binding events for individual myosin molecule. At 1 µM ATP, N = 59, n = 8211; 5 µM ATP, N = 18, n = 3219; 10 µM ATP, N = 48, n = 6263; 15 µM ATP, N = 18, n = 1647; at 20 µM ATP, N = 22, n = 1678; at 50 µM ATP, N = 63, n = 5371. Myosin Stiffness estimated using Variance-covariance method, P <0.0001 when 1 and 50 µM ATP were compared. **B)** Myosin stiffness measured at low (1 µM) and high (50 µM) ATP concentration using triangular wave method. 1 µM ATP; N= 17, n= 802, 50 µM ATP; N= 13, n = 301, N = number of molecules, n = number of events. Unpaired t-test used for statistical analysis.

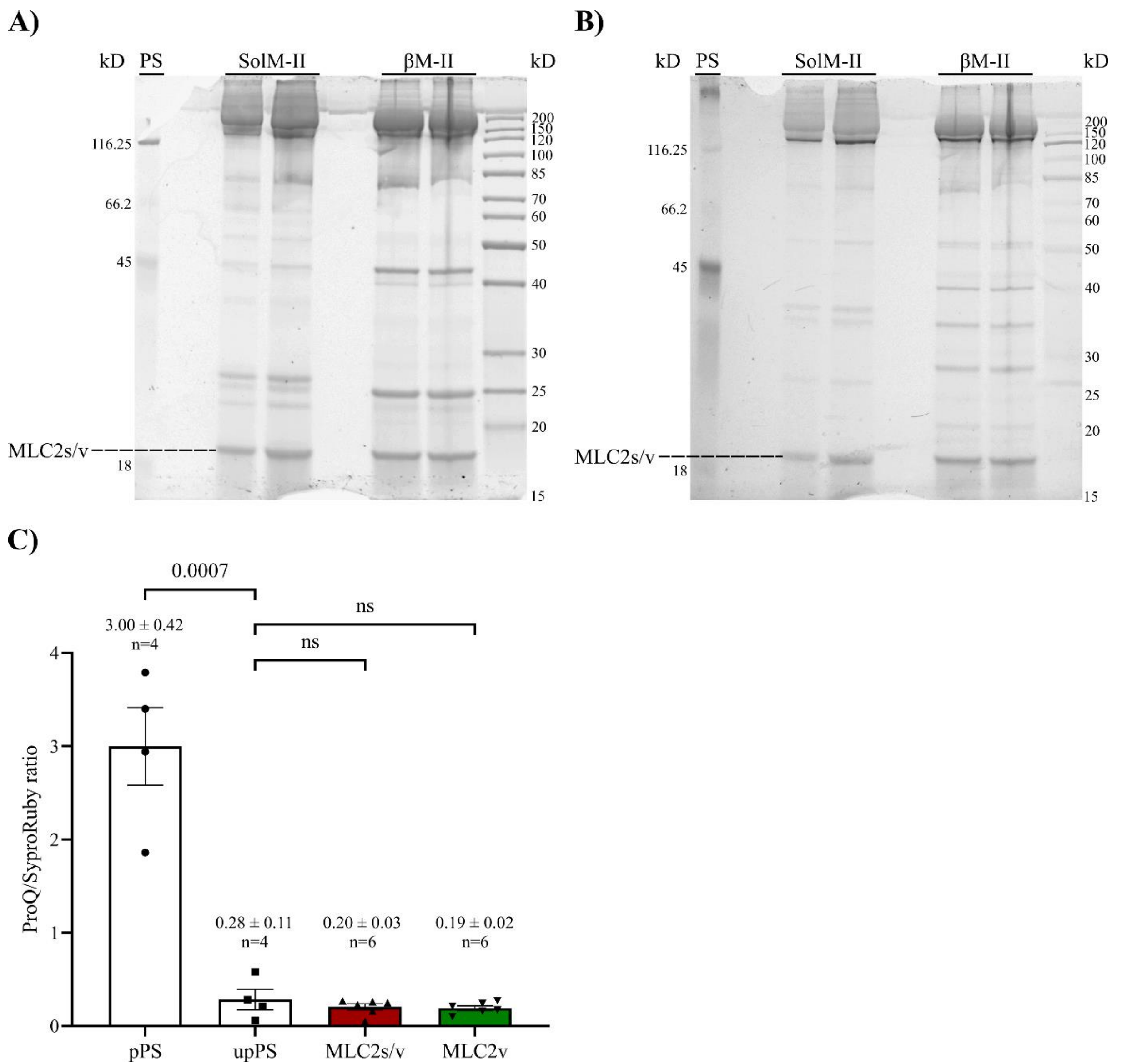

Figure S4. **Myosin regulatory light chain phosphorylation.**

Representative images displaying **(A)** SYPRO® Ruby and, **(B)** Pro-Q® stained pictures of the same gel to quantify the phosphorylation levels of MLC2v/s for SolM-II and βM-II. Two lanes each for SolM-II and βM-II myosin are shown. The densitometric analysis was performed on the indicated MLC2s/v bands for each lane. PS= PeppermintStick^{TM} **C)** Bar graph with individual data points showing Pro-Q®/Sypro®Ruby (D/S) ratios for the controls and MLC2s/v. MLC2s/v and MLC2v indicate the light chain assembled with SolM-II and βM-II, respectively. pPS and upPS indicate phosphorylated (45 kDa) and unphosphorylated (116.25 kDa) protein standard bands. No significant difference in the phosphorylation levels of MLC2s/v was observed between SolM-II and βM-II. The error bars are standard deviations (SD). Tested for significance with an unpaired t test. ns – non-significant, n= number of analyzed lanes.