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Supporting Material

The Upstream AMP-Activated Kinase Kinase Complex LKB1/Mo25/STRAD Uniquely Impacts Sarcomeric Contractile Function and Posttranslational Modification

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Table S1. Composition of Relaxing, Activating, and Preactivating Solutions						
Solution	**Na_2ATP**	**$MgCl_2$**	**EGTA**	**HDTA**	**Ca-EGTA**	**Kprop**
Relaxing	5.95	6.41	10	--	--	50.25
Preactivating	5.95	6.25	--	10	--	50.51
Activating	6.08	6.20	--	--	10	29.98

Table S1 Composition of Relaxing, Preactivating, and Activating Solutions (mM). *Ca-EGTA is made by mixing equimolar amounts of $CaCl_2$ and EGTA. In addition to the above constituents all solutions contained the following (mM): phosphocreatine 10, N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) 100, phenylmethylsulfonyl fluoride (PMSF) 0.1, dithiothreitol (DTT) 1, 50 U/ mL creatine phosphokinase, and protease inhibitor cocktail (Sigma) 4µLcocktail/ml solution. Free Mg^{2+} and Mg-ATP concentration were 1 and 5 mM, respectively. Relaxing and activating solutions were mixed to obtain the desired range of free [Ca^{2+}]. The pH is adjusted to 7.0 at 15.0 °C with KOH.*

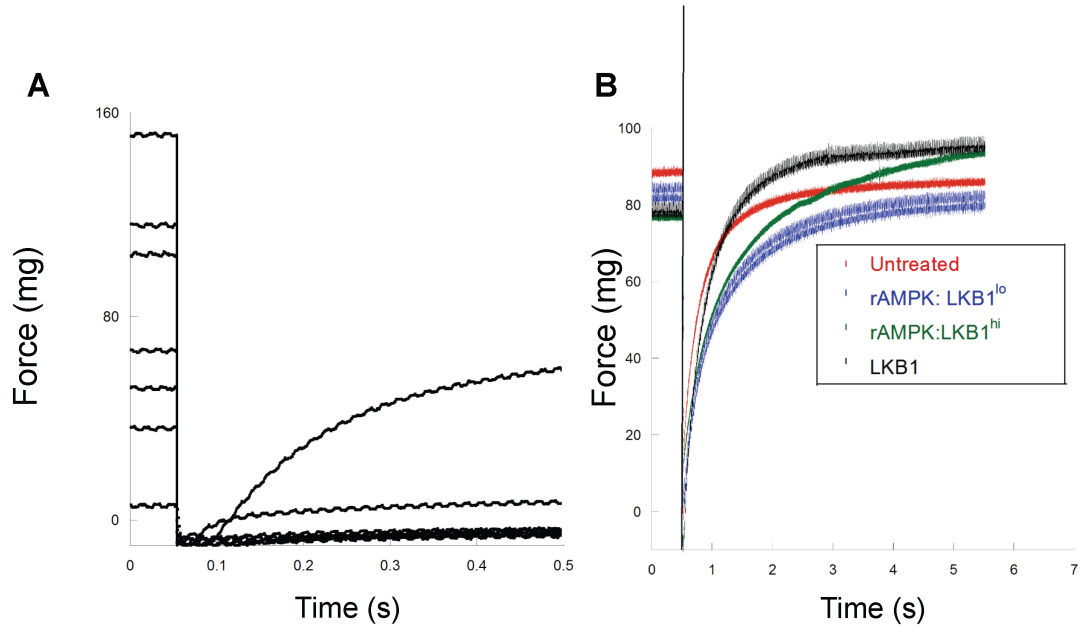

Figure S1: Mechanical Protocols Used for Determining Ca^{2+}-Sensitivity of Tension and the Rate Constant for Tension Redevelopment. A) Step release protocol used for the determination of Ca^{2+}-sensitivity of tension. *Fibers were allowed to reach steady state tension development, as depicted by the first force plateau in the figure. Once steady state was achieved, fibers were quickly released by 20% of total fiber length prior to fiber relaxation. This step release identified the zero force level. Therefore, the total tension was calculated by finding the difference between force at maximum steady state tension and zero force. Total force is then calculated for multiple Ca^{2+} concentrations. The amount of active tension generation per fiber was also calculated by subtracting passive tension (tension generated in a purely relaxing solution, at a given sarcomere length) from the total tension. Active tension was then plotted against corresponding Ca^{2+} concentrations to render the force- Ca^{2+} relationship.* ***B) Rate constant for tension redevelopment.*** *Raw trace of release-restretch protocol for each experimental group at approximately 30% of maximal tension generation. Following steady state tension generation, fibers were quickly released by 15% of total fiber length for 5ms. After the release, fibers were rapidly stretched (1ms) to 15% over original length to release all weakly bound cross bridges. Tension was then allowed to redevelop, and the rate constant for tension redevelopment ($\mathbf{k}_{tr}$) was calculated. Note the shallower slope in the rAMPK: $LKB1^{lo}$ group compared to untreated fibers.*

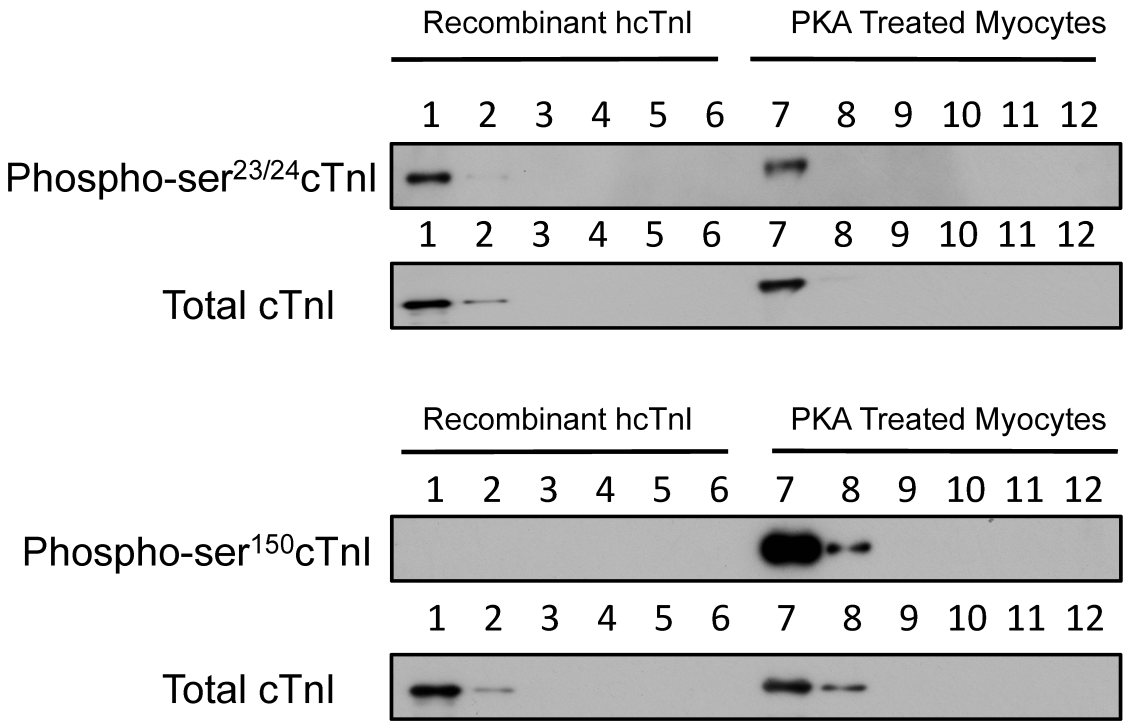

Figure S2: Relative Promiscuity of Phospho-Specific Antibodies. *Human recombinant troponin I (hcTnI), expressed to not have post-transalational modifications, and Protein Kinase A (PKA) treated myocytes were diluted and probed using phospho-specific and pan-TnI antibodes. In lane 1, hcTnI was loaded to the same extent as used in figs. 5,6,7. Lanes 2-6 are 10 fold serial dilutions of lane 1 hcTnI. In lane 7, a PKA treated myocyte sample was loaded to the same extent as in fig. 5,6,7. Lanes 8-12 contain 10 fold serial dilutions of lane 7 PKA treated myocytes. As seen in the top blot, there was signifigant reactivity of the phospho-ser$^{23/24}$cTnI antibody with both unphosphoryalted hcTnI and with PKA treated control. However, when using the phospho-ser^{150}cTnI antibody, there is no cross reactivity of the phospho-antibody with unphosphoryalted hcTnI.*