



Supplemental Figure 2. Phosphorylation of purified LC₂₀ and whole myosin by ZIPK-L93G.

(A), Purified recombinant ZIPK-L93G (0.16 μ M) was incubated with purified LC₂₀ (1 μ M) and either ATP or 6-Phe-ATP (0.2 mM) as substrate. Reactions were terminated at the indicated time, and then LC₂₀ phosphorylation status was analyzed by Phos-tag SDS-PAGE (10 ng LC₂₀/lane) with subsequent detection of unphosphorylated, monophosphorylated (-1P) and diphosphorylated (-2P) forms by western blotting. **(B)**, Experiments were repeated with purified whole myosin as substrate at the same LC₂₀ concentration (1 μ M). **(C)**, Coomassie brilliant blue (CBB)-stained SDS-PAGE (12.5% acrylamide) of purified LC₂₀ and myosin substrates used for the phosphorylation assays. The results are representative of 3 independent experiments.