Calmodulin bound to the first IQ motif is responsible for calcium-dependent regulation of myosin 5a

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Supplemental Figure Legends

Figure S1. SDS-PAGE (4-20%) of the purified myosin 5a constructs. M5aΔT (lane 1), M5aΔT-IQ26 (lane 2), M5aHMM (lane 3), MD-IQ1-6 (lane 4), MD-IQ1/2 (lane 5), MD-IQ1 (lane 6), and MD (lane 7). Arrowheads indicate the CaM co-purified with myosin 5a heavy chain.

Figure S2. Inhibition of the ATPase activities of M5aΔT and M5aΔT-IQ26 by GST-GTD. M5aΔT-IQ26 was created by substituting IQ2 (aa. 788 - 812) of M5aΔT with IQ6 (aa. 884 -908). The ATPase activities were measured in the presence of 100 mM NaCl in EGTA (open triangles) and pCa4 conditions (closed triangles). The K_d of GST-GTD to M5aΔT (**A**) and M5aΔT-IQ26 (**B**), obtained by a quadratic fit, were 41.8 nM and 116.7 nM respectively.

Figure S3. Ca^{2+} induces the dissociation of CaM from IQ2 but not from IQ1 and IQ6. (**A**) MD-IQ1, MD-IQ1/2, or MD-IQ1/6 was mixed with F-actin in EGTA or pCa4 conditions in the absence of ATP and subject to ultracentrifuge to precipitate F-actin. The F-actin co-precipitated proteins were separated by SDS-PAGE (4-20%) and visualized by Commassie Brilliant Blue staining. (**B**) The amounts of myosin 5a heavy chain and co-precipitated CaM were measured with NIH Image J. The ratio of CaM cosedimentated with myosin 5a heavy chain in pCa4 conditions versus that in EGTA conditions was scored and presented. Data are the average of two independent assays.

Figure S4. Effects of ionic strength on the inhibition of M5aHMM by GST-GTD. (**A**) Inhibition of M5aHMM ATPase activity by GST-GTD at various concentrations of NaCl in EGTA conditions. The K_d of GST-GTD to M5aHMM were obtained by a quadratic fit (in 50 mM and 100 mM NaCl conditions) or hyperbolic fit (in 150 mM, 200 mM, and 250 mM NaCl conditions). (**B**) Semilog plot of apparent K_d versus the concentrations of NaCl.

Figure S5. SDS-PAGE (4-20%) of the purified MD-IQ3 (lane 1) and MD-IQ5 (lane 2). MD-IQ3 or MD-IQ5 was coexpressed with CaM and purified by Anti-FLAG affinity chromatography. Note: CaM associates with MD-IQ3 but not with MD-IQ5.

Figure S6. SDS-PAGE (4-20%) of the purified MD-IQ1, MD-IQ1-short, and CaM variants. MD-IQ1 or MD-IQ1-short was coexpressed with CaM-WT or CaM-C and purified by Anti-FLAG affinity chromatography. Lane 1, MD-IQ1 coexpressed with CaM-C; lane 2, MD-IQ1 coexpressed with CaM-WT, lane 3, MD-IQ1-short (aa. 1 – 783) coexpressed with CaM-C; lane 4, CaM-WT; lane 5, CaM-C; lane 6, CaM-N.

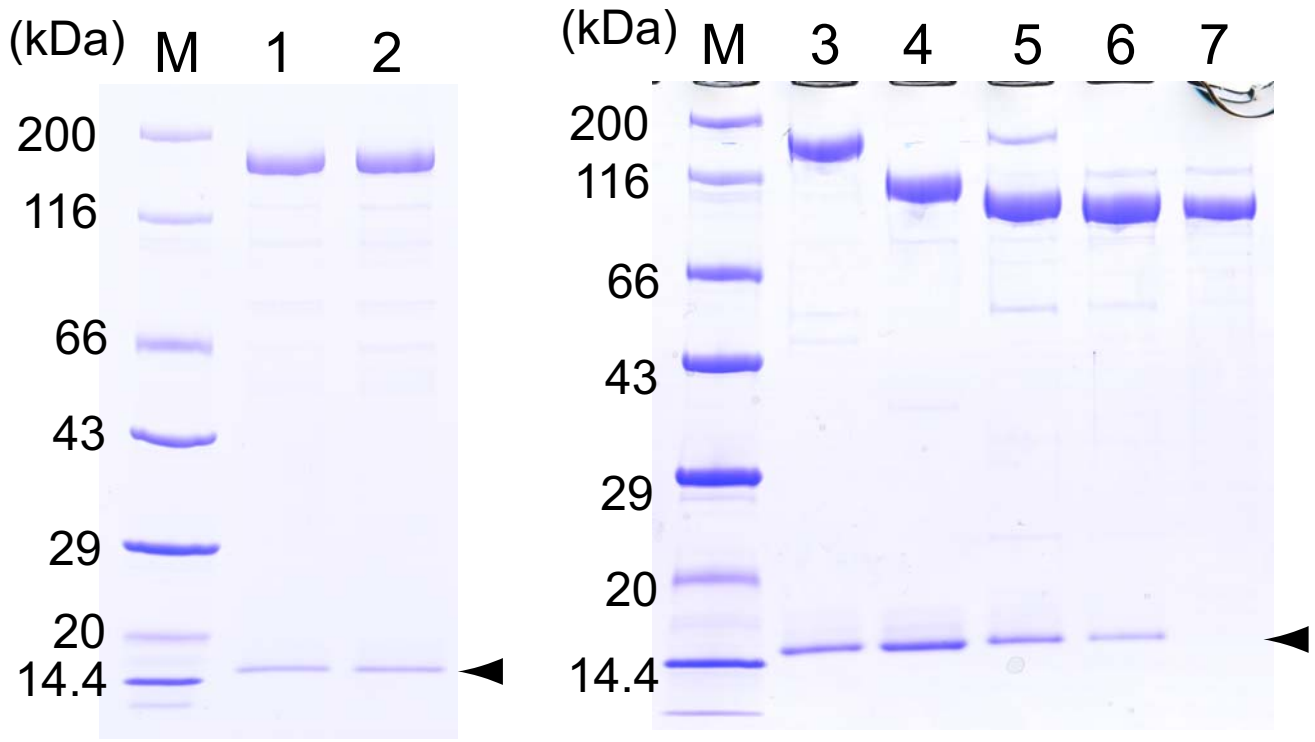

Figure S1 (Lu et al.)

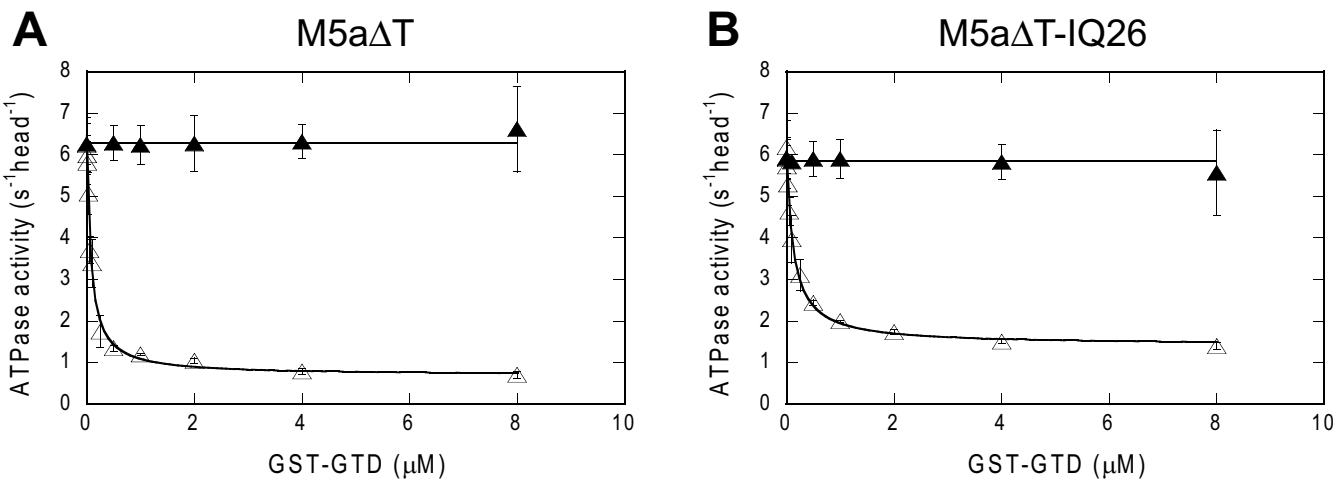

Figure S2 (Lu et al.)

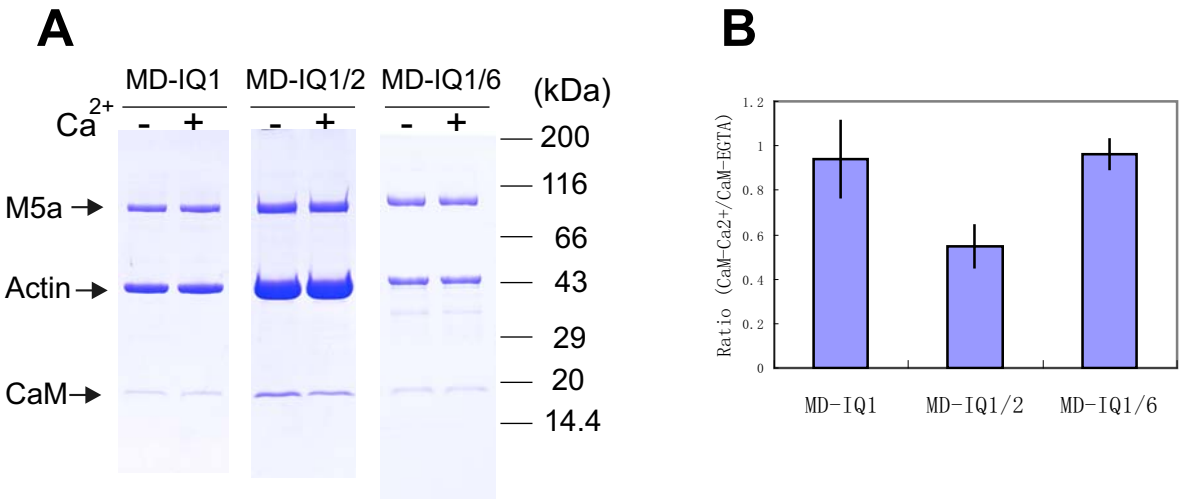

Figure S3 (Lu et al.)

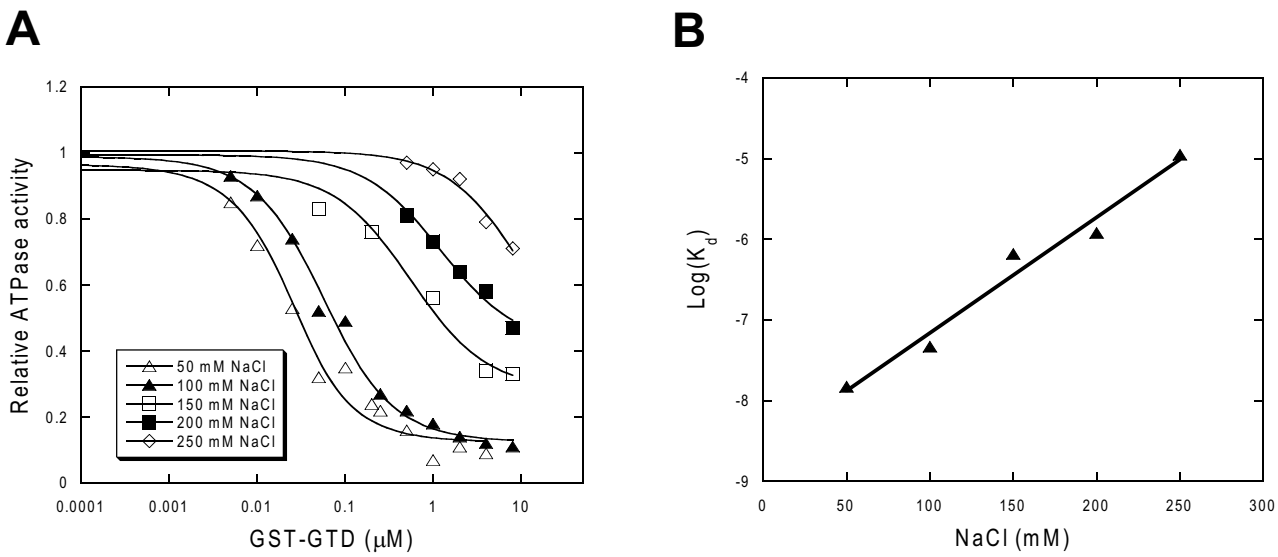

Figure S4 (Lu et al.)

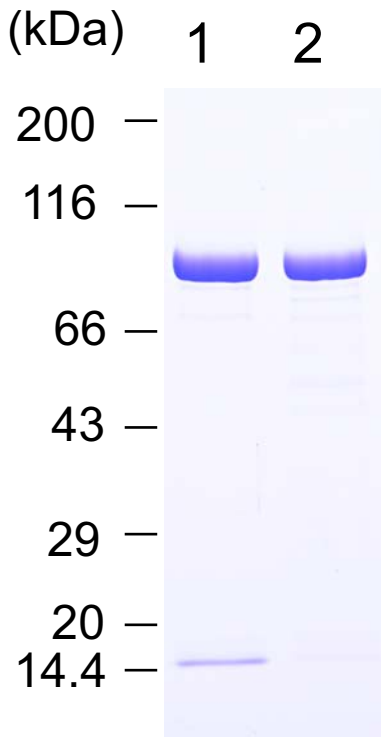

Figure S5 (Lu et al.)

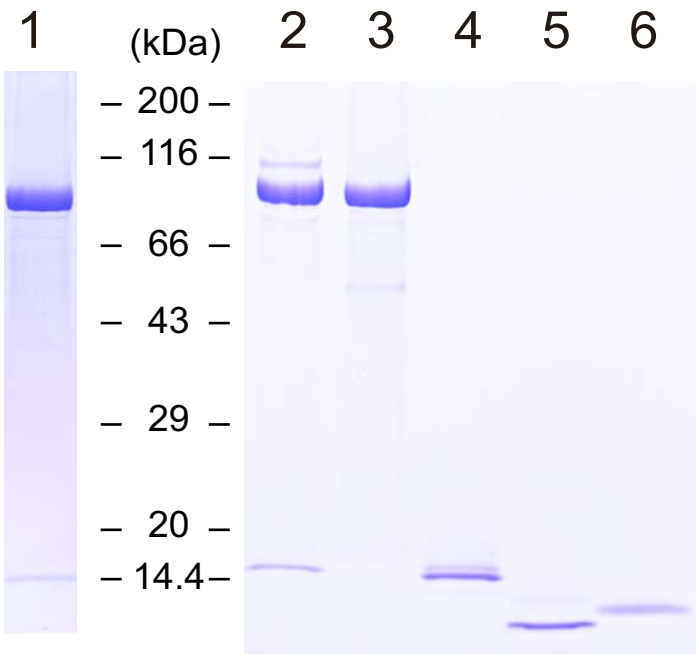

Figure S6 (Lu et al.)