Supporting Information

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SI Text

Materials. Restriction enzymes and modifying enzymes were purchased from New England Biolabs unless indicated otherwise. Pfu Ultra High-Fidelity DNA polymerase was purchased from Stratagene. Oligonucleotides were synthesized by Invitrogen. Vector plasmid pFastBacHT and pET30c were purchased from Invitrogen and Novagen, respectively. Anti-FLAG M2 affinity gel, FLAG peptide, phosphoenol pyruvate, 2,4-dinitrophenyl-hydrazine, pyruvate kinase, and L-Glutathione were from Sigma. Nickel-nitrilotriacetic acid (Ni-NTA)-agarose and Glutathione Sepharose 4B were purchased from Qiagen and GE Healthcare, respectively. Actin was prepared from rabbit skeletal muscle according to Spudich and Watt (1). Recombinant calmodulin was expressed in *Escherichia coli* and purified as described (2).

Glutar-Aldehyde Cross-Linking. DM7AHMM (0.7 mg/mL) or DM7AHMMLZ (0.7 mg/mL) was incubated with 0.025% glutar-aldehyde, 100 mM NaCl, 20 mM Hepes·KOH (pH 7.5), and 0.05 mM EGTA for 15 min at 25 °C. The reactions were terminated by addition of 70 mM Tris·HCl (pH 6.8) and 1% 2-mercapto-ethanol. The cross-linking products were analyzed by SDS/PAGE according to ref. 3.

Pull-Down Assay. DM7AHMM was incubated in buffer containing 20 mM Mops (pH 7.0), 50 mM KCl, 1 mM $MgCl_2$, and 0.1 mM DTT in the presence of 1 mM EGTA-$CaCl_2$ buffer system at 25 °C for 10 min, then was incubated with 20–50 μL of anti-FLAG antibody resin for 90 min at 4 °C. The beads were washed extensively, and proteins were eluted in Laemmli sample buffer and resolved by SDS/PAGE. Densitometric analysis was performed with National Institutes of Health ImageJ version 1.38 software.

1. Spudich JA, Watt S (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J Biol Chem* 246:4866–4871.
2. Ikebe M, et al. (1998) A hinge at the central helix of the regulatory light chain of myosin is critical for phosphorylation-dependent regulation of smooth muscle myosin motor activity. *J Biol Chem* 273:17702–17707.
3. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.

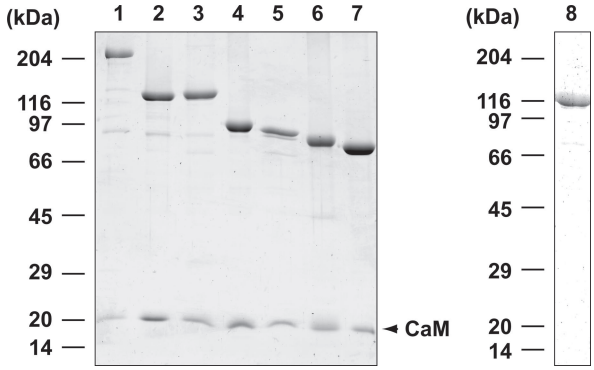

Fig. S1. SDS/PAGE (4–20% gradient gel) of the purified DM7A constructs. The heavy chains were coexpressed with calmodulin (CaM). Lane 1, DM7AFull; lane 2, DM7AHMM; lane 3, DM7AHMMLZ; lane 4, DM7AIQ5; lane 5, DM7AIQ5ΔSAH; lane 6, DM7AIQ2; lane 7, DM7AIQ1; lane 8, DM7Atail. Molecular mass (kDa) is indicated at left.

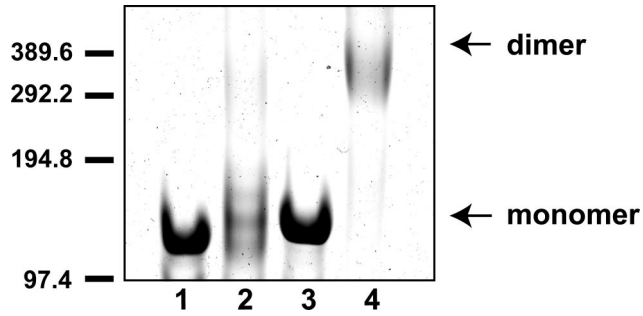

Fig. S2. Cross-linking of DM7AHMM. Cross-linking of DM7AHMM and DM7AHMMLZ was done as described in *SI Text*. The samples before and after cross-linking were analyzed by SDS/PAGE. Molecular mass (kDa) is indicated at left. Lane 1, DM7AHMM; lane 2, DM7AHMM + cross-linker; lane 3, DM7AHMMLZ; lane 4, DM7AHMMLZ + cross-linker.

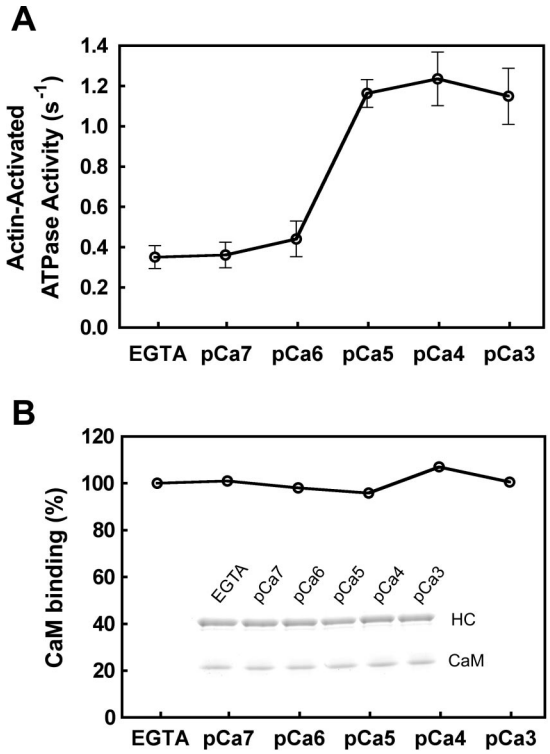

Fig. S3. pCa dependence of the tail-induced inhibition of the actin-activated ATPase activity of DM7AHMM (*A*) and dissociation of calmodulin light chain (*B*). The assay conditions are the same as Fig. 2, except for the use of 0.7 μM DM7Atail. Values are mean with SE from 4 independent experiments. Dissociation of calmodulin light chain from DM7AHMM was determined by pull-down assay as described in *SI Text*.

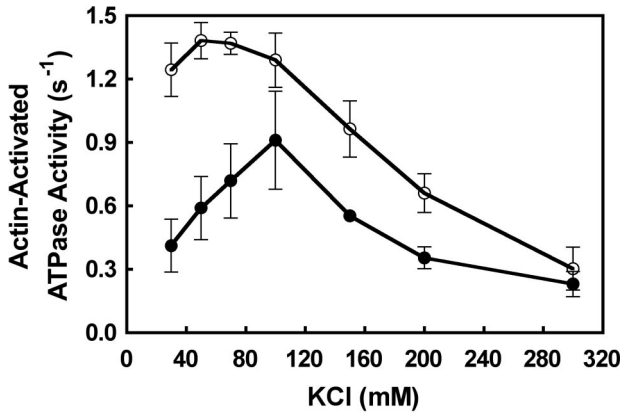

Fig. S4. Effect of ionic strength on the tail-induced inhibition of the actin-activated ATPase activity of DM7AHMM under EGTA condition. ○, absence of exogenous DM7Atail; ●, presence of 0.5 μM exogenous DM7Atail. Assay conditions are as described in *Materials and Methods*.

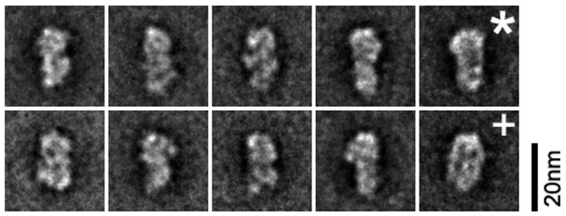

Fig. S5. Averaged images of DM7AFull in EGTA. Montage of some averaged images taken form DM7AFull molecules in 50 mM NaAcetate solution. Note that most averages showed closely packed structure (consisting of 20-30 images in each), thus it was difficult to identify the tail and the head domains separately. Asterisk and cross marked averages were selected and presented in Fig. 6F based on the most separation between the domains. Scale bar indicates 20 nm.