**Supplementary Materials**

**Movie S1.** Time-lapse movie (1140 s total) of 0.3 M actin filament (including 20% Alexa Fluor 488-labeled skeletal muscle actin and 80% non-labeled wild-type -actin) polymerization and depolymerization (related to Fig. 4A). Actin filaments were tethered on a glass surface with 50 nM NEM-myosin. Depolymerization was induced by loading TIRF buffer (10 mM imidazole, 50 mM KCl, 1 mM MgCl2, 1 mM EGTA, 100 mM DTT, 0.2 mM ATP, 0.02 mM CaCl2, pH 7.0) to remove any free actin monomer (indicated by "Buffer").

**Movie S2.** Time-lapse movie (1140 s total) of 0.3 M actin filament (including 20% Alexa Fluor 488-labeled skeletal muscle actin and 80% non-labeled mutant -actin) polymerization and depolymerization (related to Fig. 4B). Actin filaments were tethered on a glass surface with 50 nM NEM-myosin. Depolymerization was induced by loading TIRF buffer (10 mM imidazole, 50 mM KCl, 1 mM MgCl2, 1 mM EGTA, 100 mM DTT, 0.2 mM ATP, 0.02 mM CaCl2, pH 7.0) to remove any free actin monomer (indicated by "Buffer").