Supplemental materials and methods

Protein analysis
Protein was quantified with the BCA kit (Pierce Chemical Co.). 10% SDS-PAGE acrylamide gels were prepared as described, stained with Coomassie or transferred to nitrocellulose in the presence of 0.1% SDS and 20% methanol. Gels were probed with a 1:5,000 dilution of G2 goat anti-actin serum (Karpova et al., 1993) and an HRP-conjugated secondary antibody (Sigma-Aldrich). Densitometry was performed on scanned images with NIH Image.

Actin depolymerization assays were performed essentially as described previously (Wear et al., 2003). Actin was purified from porcine skeletal muscle and pyrene labeled. 50 μl aliquots of 5 μM actin, 20% pyrene labeled, were polymerized for >2 h at RT in the presence of 10 mM imidazole, pH 7.0, 50 mM KCl, 2 mM MgCl₂, and 1 mM EGTA. One reaction was added to 2.45 ml of 10 mM imidazole, pH 7.0, with or without 1 M sorbitol and/or 0.2 mM potassium phosphate. Pyrene fluorescence was detected and the fluorescence values were normalized to the appropriate buffer.

Myosin S1 preparation and use
Myosin S1 was prepared by an adaptation of the protocol described in Margossian and Lowey (1982). Myosin was extracted from fresh porcine skeletal muscle and stored as a 50% ammonium sulfate precipitate at 4°C. 5–10 mg of myosin was digested with 50 nM papain for 7 min at 25°C, and the digestion stopped by 1 mM iodoacetamide. The reaction was dialyzed into 50 mM Tris, pH 6.8, 2 mM MgCl₂, 2 mM DTT for 2 h, and centrifuged at 40,000 g for 30 min. The supernatant was collected and diluted 64-fold for labeling. Grids with actin patch samples were incubated with latrunculin A as described above, washed twice with KS, and incubated with the diluted S1 for 5 min before fixing and processing as described above.

References