Krementsova et al., http://www.jcb.org/cgi/content/full/jcb.201106146/DC1

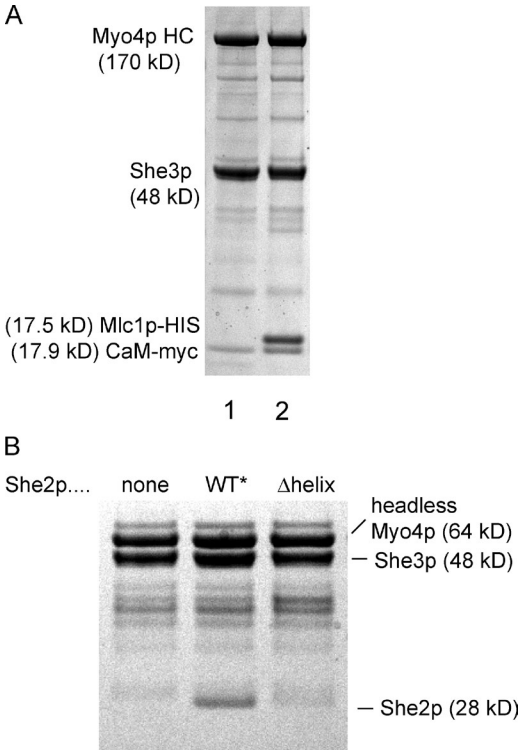


Figure S1. **Gels of expressed Myo4p–She3p and a FLAG resin pull-down assay.** (A) SDS gel of baculovirus-expressed and purified Myo4p–She3p expressed with yeast CaM alone (lane 1) or both yeast CaM and Mlc1p (lane 2). Mlc1p was His tagged, and CaM was Myc tagged (see Materials and methods). The protein used in this study was expressed with both Mlc1p and yeast CaM. Both proteins bind to the lever arm, with Mlc1p as the predominant light chain. Molecular masses of the proteins are indicated. HC, heavy chain. (B) A FLAG-tagged truncated Myo4p–She3p construct lacking the head was incubated with either WT* or the Δhelix-She2p construct. FLAG resin was used to isolate the complex, which was analyzed by SDS gels. Deletion of the helix abolished the ability of She2p to bind the Myo4p–She3p complex. Molecular masses of the proteins are indicated.

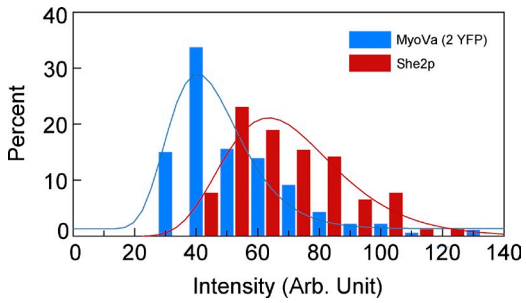


Figure S2. **Comparison of fluorescence intensity of She2p-YFP versus myosin Va–YFP.** The intensity of tetrameric She2p-YFP was compared with that of myosin Va (MyoVa)–YFP. Myosin Va is a known dimer and therefore contains exactly two YFP molecules. Myosin Va–YFP and She2p-YFP were adsorbed directly to a glass coverslip and imaged by TIRF microscopy. The histogram shows a comparison of intensities (peak intensity with background subtracted) for myosin Va–YFP (n = 189) and She2p-YFP (n = 169). As expected, the intensity distribution for each followed a log-normal distribution, shown as solid lines (Mutch et al., 2007). The distribution for She2p-YFP shifted right toward higher intensities, relative to myosin Va–YFP. The peak intensity of She2p-YFP was 63.5 ± 1.7 versus a peak intensity of 40.4 ± 1.3 for myosin Va, corresponding to ∼3.1 YFPs per She2p. This result indicates that we are visualizing single She2p tetramers and not larger aggregates. Arb. Unit, arbitrary unit.

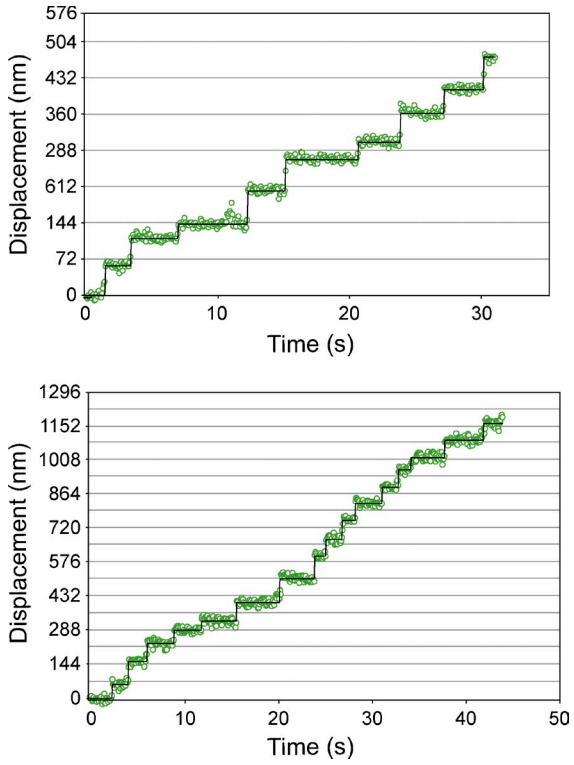
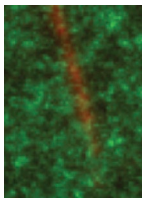


Figure S3. **Stepping pattern of Myo4p–She3p bound to She2p.** A single head of a Myo4p–She3p complex bound to She2p was labeled with a Qdot. Qdot displacement versus time is shown by open circles. Qdot positions were determined by the ImageJ plugin SpotTracker (Sage et al., 2005). Steps were identified using the Kerssemakers step-finding algorithm (solid lines; Kerssemakers et al., 2006). Each plot shows a single representative trace. A total of 15 traces from three experiments was analyzed.



Video 1. **A Myo4p–She3p-She2p motor complex moving processively on an actin filament.** Myo4p–She3p was mixed with She2p-YFP and visualized by TIRF microscopy. YFP particles (green) were observed to move processively along Alexa Fluor 594–labeled actin filaments (red) in 1 mM MgATP. The field of view is 5.3 × 7.4 µm. Data were collected at 10 frames per second and played back at 30 frames per second.

References

Mutch, S.A., B.S. Fujimoto, C.L. Kuyper, J.S. Kuo, S.M. Bajjalieh, and D.T. Chiu. 2007. Deconvolving single-molecule intensity distributions for quantitative microscopy measurements. *Biophys. J.* 92:2926–2943. http://dx.doi.org/10.1529/biophysj.106.101428