Supplemental Materials

Molecular Biology of the Cell

Clayton et al.

SUPPLEMENTARY MATERIAL

Fission yeast tropomyosin specifies directed transport of myosin-V along actin cables

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Running head: Tropomyosin sorts myosin-V function.

Supplementary Movie Legends

Movie 1. Cdc8p-decorated actin filaments support the processive movement of single Myo52p-Qdot molecules.
Left: Failure of Myo52p-HMM-Qdots (green) to move processively on bare actin filaments (red) in the presence of 2 mM ATP. *Right*: In contrast, Myo52p-HMM-Qdots can move processively on Cdc8p-decorated actin filaments. Data were collected using TIRF microscopy with a capture rate of 30 frames/s and are played back at this speed. Scale bar: 2 μm.

Movie 2. Cdc8p-decorated actin filaments support the processive movement of single Myo52p-Cy3 molecules.
Cy3-labeled Myo52p-HMM (green) moving processively on actin-Cdc8 filaments (red) in the presence of 2 mM ATP. Data were collected using TIRF microscopy with a capture rate of 5 frames/s and are played back at this speed. Scale bar: 2 μm.

Movie 3. Processive movement of Myo52p molecules on bare actin and Cdc8p-decorated actin filaments at low concentrations of ATP.
Left: A Myo52p-HMM-Qdot (green) moving processively on bare actin filaments (red) in the presence of 10 μM ATP. *Right*: Myo52p-HMM-Qdots moving processively on Cdc8p-decorated actin filaments in 10 μM ATP. Data were collected using TIRF microscopy with a capture rate of 1 frame/s and are played back at 30 frames/s (sped-up 30x). Scale bar: 2 μm.

Movie 4. Cdc8p-decorated actin filaments support movement of Qdots by multiple Myo52p molecules.
Left: Failure of Qdots (green) bound by multiple Myo52p-HMM motors to move along bare actin filaments (red) in the presence of 2 mM ATP. *Right*: In contrast, Qdots bound by multiple Myo52p-HMM motors can move processively on Cdc8p-decorated actin filaments. Data were collected using TIRF microscopy with a capture rate of 30 frames/s and are played back at this speed. Scale bar: 2 μm.

Movie 5. The directed motility of Myo52p molecules in the cell does not rely on the C-terminal cargo-binding domain.
In vivo directed motility of Myo52pΔCBD-3xGFP particles (green) made from clusters of molecules lacking their C-terminal cargo-binding domains in the *myo52ΔCBD-3xGFP* strain. Data were collected using epi-fluorescence microscopy with a capture rate of 10 frames/s; played back at 100 frames/s (sped up 10x).

Supplementary Figures and Legends

Supplementary Figure S1.

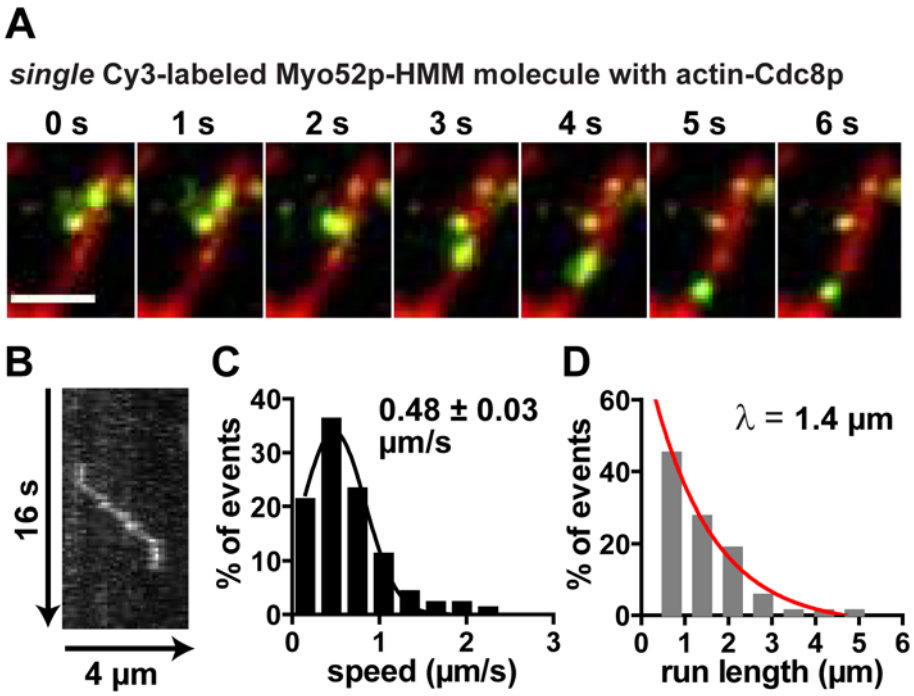

Figure S1. Cy3-labeled Myo52p molecules move processively along actin-Cdc8p filaments. Single Myo52p-HMM molecules were coupled to Cy3 in order to track their ability to move along Alexa 635-phalloidin-labeled actin filaments using TIRF microscopy. A) Movement of a representative Myo52p-Cy3 molecule (green) along a Cdc8p-decorated actin filament (red). Bar: 2 µm. Other representative events are presented in Supplementary Movie 2. B) Representative kymograph summarizing a typical Myo52p-Cy3 run along an actin-Cdc8p filament. C) Histogram summarizing the distribution of Myo52p-Cy3 speeds (n=100). D) Histogram of run lengths for Myo52p-Cy3 along Cdc8p-decorated actin tracks (n=114). The red curve shows the exponential fit ($y = Ae^{-x/\lambda}$) to determine the run length λ.

Supplementary Figure S2.

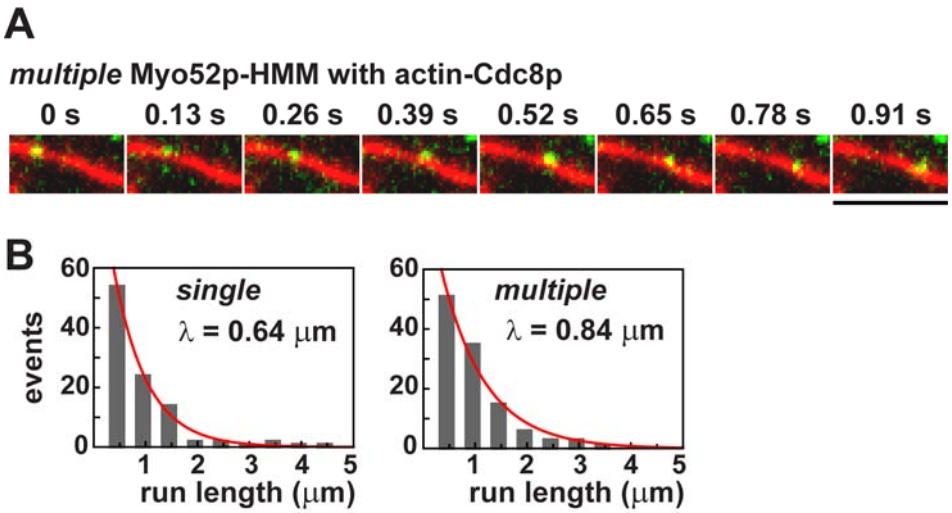

Figure S2. Cdc8p activates the motility of fluorescence Qdots coated with multiple Myo52p motors. Qdots were saturated with Myo52p-HMM molecules (yielding ~3-5 motors/Qdot) to track the ability of multiple Myo52p molecules to support movement along rhodamine phalloidin-labeled actin filaments using TIRF microscopy. Only actin filaments decorated with tropomyosin (Cdc8p) supported movement of these Myo52p-coated Qdots. A) Movement of a representative Myo52p-coated Qdot (green) along a Cdc8p-decorated actin filament (red). Bar: 4 μm. Other representative events are presented in Supplementary Movie 4. B) Histograms comparing run lengths of Qdots associated with single (n=103) versus multiple (n=117) Myo52p molecules along Cdc8p-decorated actin tracks. The red curves show exponential fits ($y = Ae^{-x/\lambda}$) to determine the run length λ.

Supplementary Figure S3.

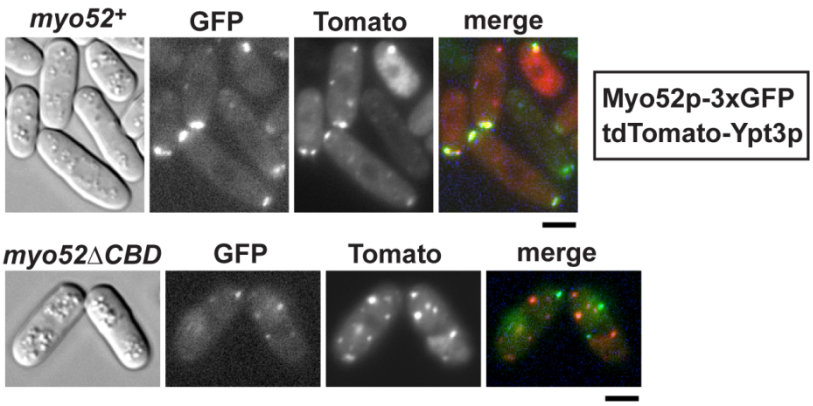

Figure S3. The cellular function of Myo52p relies on its C-terminal cargo-binding domain. Wild-type *myo52-3xGFP* and mutant *myo52ΔCBD-3xGFP* cells were grown at 25°C in YE5S rich media prior to microscopic imaging. Representative DIC and epi-fluorescence images showing the localization of Myo52p-3xGFP or Myo52pΔCBD-3xGFP (GFP) and a Myo52p cargo (GTPase Ypt3p, Tomato-Ypt3p) in wild-type and mutant cells. Unlike in wild-type cells, Ypt3p is not transported with Myo52p to sites of polarized growth in the absence of the cargo-binding domain. Bars: 4 μm.

Supplementary Figure S4.

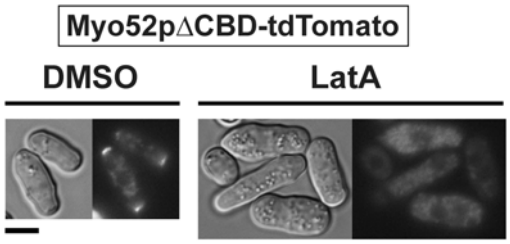

Figure S4. Polarized localization of truncated Myo52p lacking its cargo-binding domain relies on actin filaments.
myo52ΔCBD-tdTomato cells were grown at 25^{o}C in YE5S rich media prior to imaging. Representative DIC and fluorescence micrographs show the sub-cellular localization of Myo52pΔCBD-tdTomato upon treatment with DMSO (control, left) or 10 μM Latrunculin A (right). Bar: 4 μm.