Fang et al., http://www.jcb.org/cgi/content/full/jcb.201005134/DC1

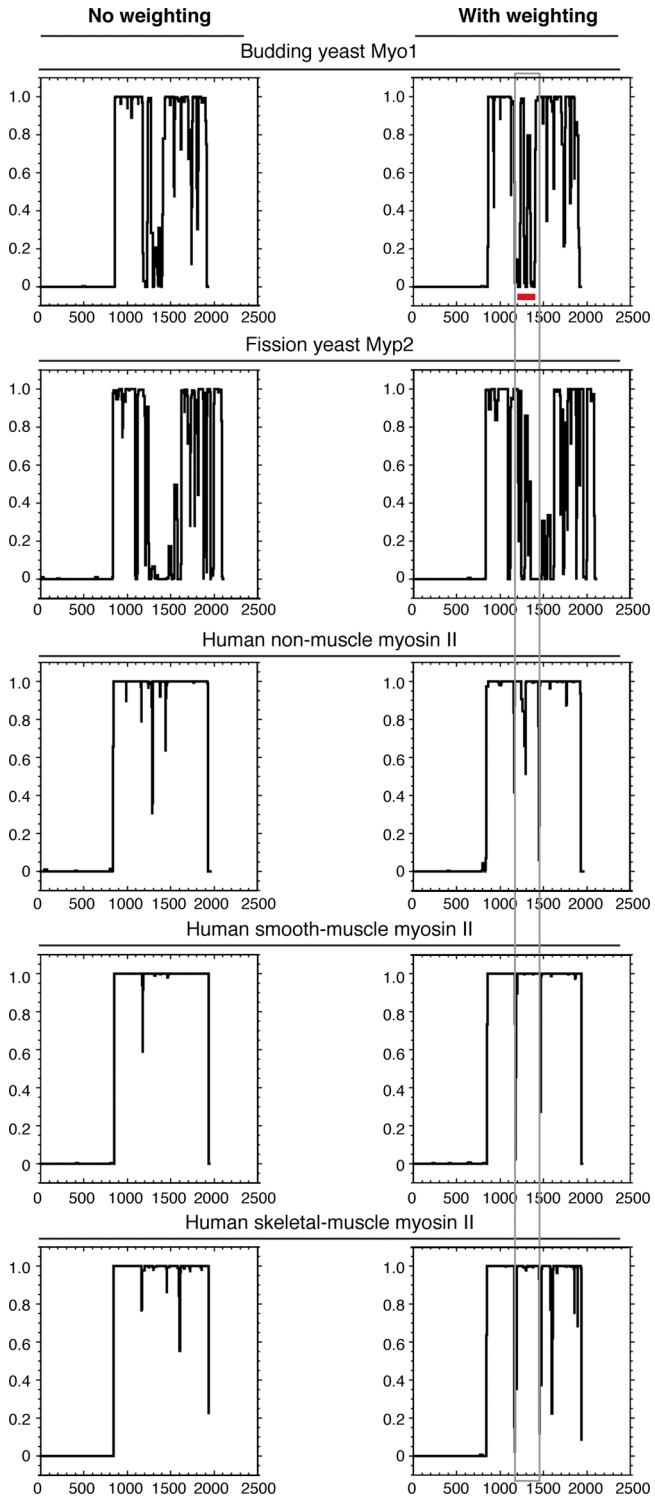


Figure S1. **Coiled-coil profiles of different myosin IIs.** The coiled-coil profiles of *S. cerevisiae* myosin II (Myo1), fission yeast myosin II Myp2 (NP_593816.1), human nonmuscle myosin II heavy chain A (NP_002464), human smooth muscle myosin II heavy chain 11 isoform SM2B (NP_001035202), and human skeletal muscle myosin II heavy chain 1 (NP_005954) were predicted by the Coils program (Lupas et al., 1991) using window width of 28 residues, with (right panels) and without (left panels) 2.5-fold weighting of positions a and d to identify false positive coiled-coil–forming probability caused by highly charged sequences. Red bar (top right panel), the proline-rich region of Myo1 tail; grey box (right panels), the putative "hinge" regions of all myosin IIs (to our knowledge, a hinge in the tail of a skeletal muscle myosin II has not been experimentally demonstrated).

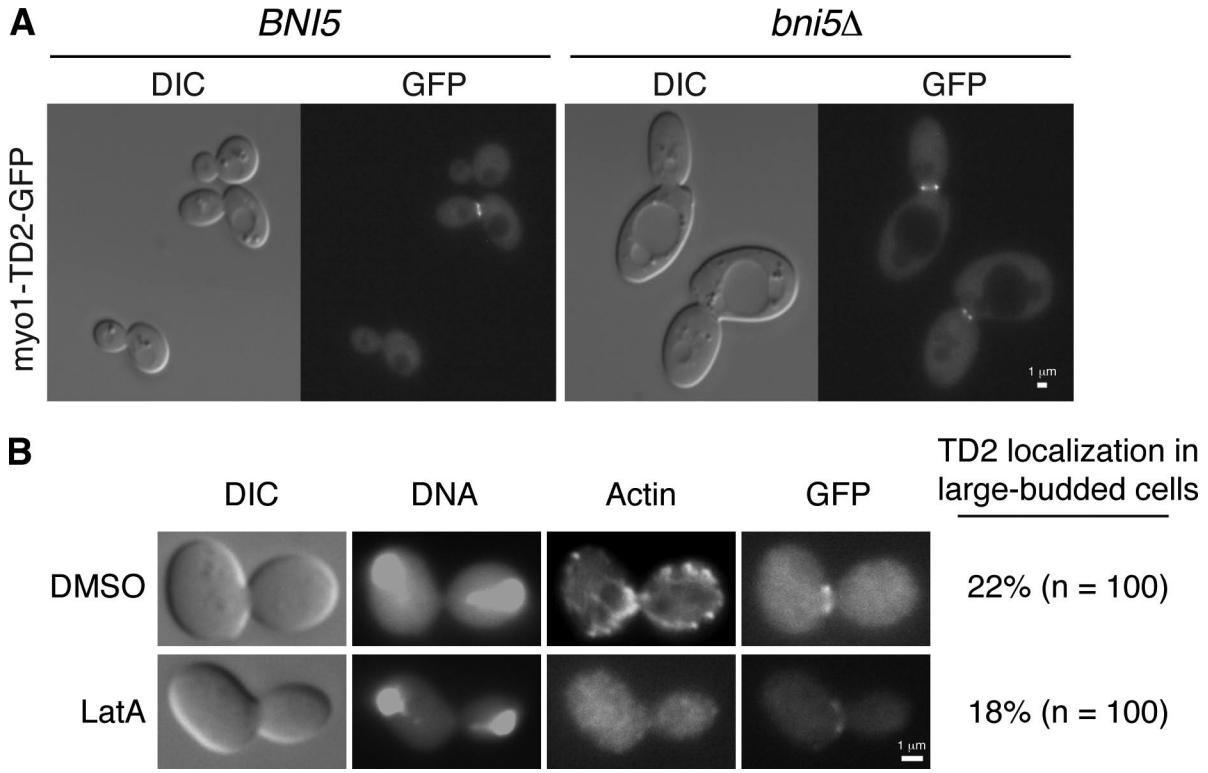


Figure S2. **Myo1-TD2-GFP localization in *bni5Δ* and LatA-treated cells.** (A) Localization of myo1-TD2-GFP in wild-type (*BNI5*) (YJL222A) and *bni5Δ* (XDY257) cells. Strains were grown in YPD at 23°C and observed by DIC and fluorescence microscopy. (B) Localization of myo1-TD2-GFP in LatA-treated cells. Three aliquots of the culture of strain YJL222A grown in YPD medium at 23°C were either untreated (not depicted) or treated with DMSO or 200 μM LatA for 30 min. Fixed cells were stained for DNA and F-actin, and then quantified for the TD2 localization.

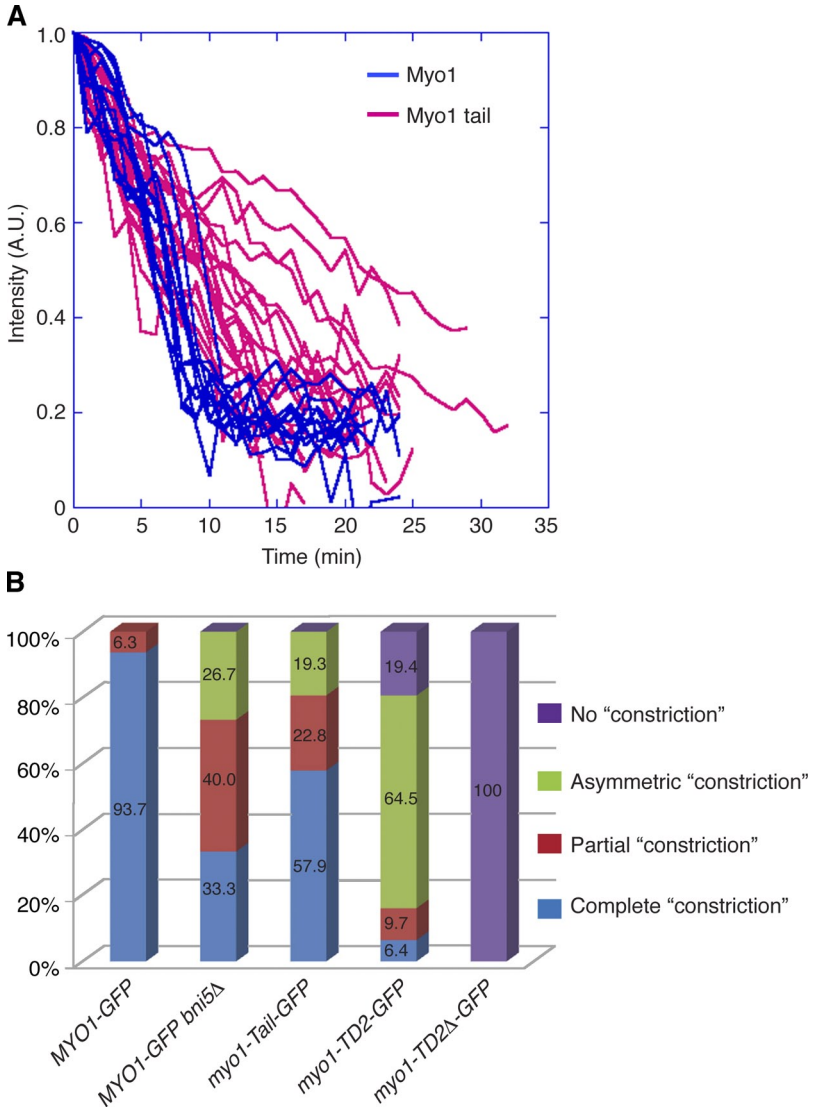
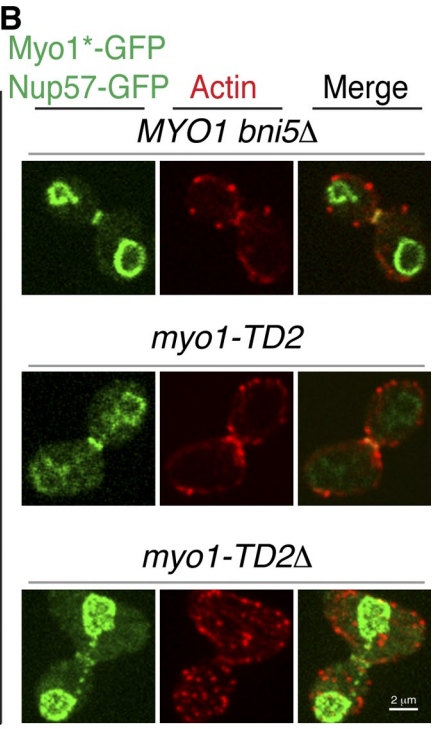


Figure S3. **Disassembly kinetics of Myo1 tail and the constriction behaviors of Myo1 and its fragments in wild-type and mutant cells.** (A) Disassembly defect of Myo1 tail during and after cytokinesis. Fluorescence intensities of Myo1-GFP (n = 12) and myo1-Tail-GFP (n = 19) rings were measured and plotted over time. Time 0 represents the beginning of ring constriction. (B) Quantitative analysis of the time-lapse data. The strain names and/or their genotypes are indicated in the Fig. 4 A legend as well as on the plot. Asymmetric constriction, Myo1 ring is not positioned at the center of the septin hourglass or rings; Partial constriction, the final diameter of Myo1 ring is larger than 0.5 µm; Complete constriction, the final diameter of Myo1 ring is smaller than or equal to 0.5 µm.

A

Strain (*MYO1*-GFP)	Actin ring in telophase cells (n = 100)
MYO1	98%
MYO1 bni5Δ	72%
myo1-Tail	92%
myo1-TD2	33%[a]
myo1-TD2Δ	42%[a]
myo1Δ	5%[a]



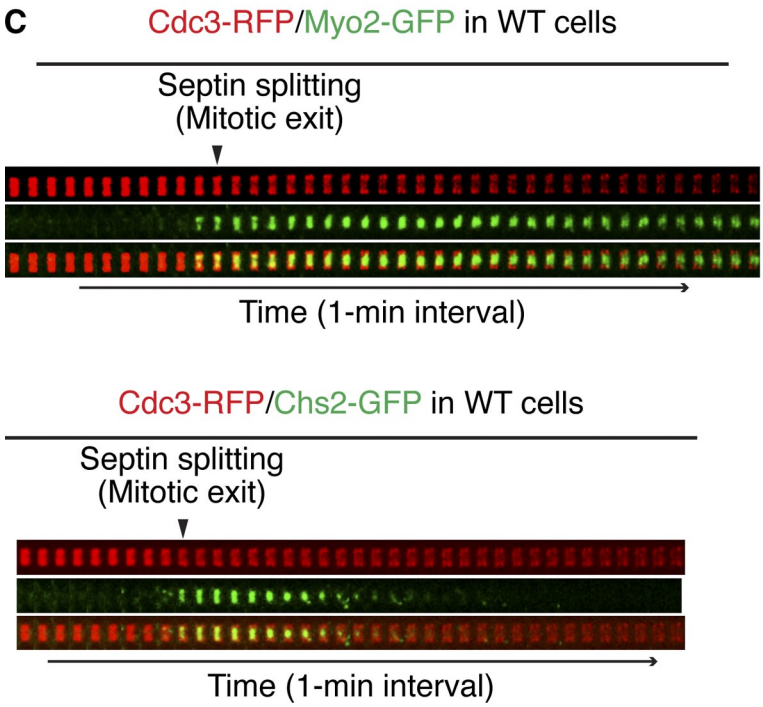
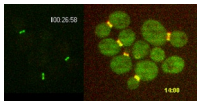
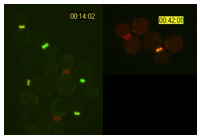


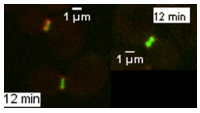
Figure S4. **Actin ring assembly in different yeast mutants and localization of Myo2 and Chs2 during the cell cycle.** (A) Actin ring assembly in *bni5Δ* and *myo1* mutants. Cells of the strains XDY41 (*MYO1-GFP*), XDY254 (*MYO1-GFP bni5Δ*), YJL335A (*myo1-Tail-GFP*), YJL222A (*myo1-TD2-GFP*), YJL488A (*myo1-TD2Δ-GFP*), and YEF1804 (*myo1Δ*) were grown in YPD at 23°C and then fixed and stained for F-actin and DNA. Cells with segregated DNA positioned at the extreme ends of the mother–daughter axis (in late anaphase or telophase) were scored for actin ring assembly. "a" indicates a range of disorganized actin structures at the bud neck, from very smooth and bright rings to very disorganized and faint ring-like structures. (B) Cells of the strains YEF6316 (*bni5Δ MYO1-GFP NUP57-GFP*; top row), YEF6310 (*myo1-TD2-GFP NUP57-GFP*; middle row), and YEF6308 (*myo1-TD2Δ-GFP NUP57-GFP*; bottom row) were grown in SC-Ura media at 23°C, fixed, and stained for actin, and then imaged by spinning-disk confocal microscopy along Z-axis at a 0.1-μm increment (71 Z-sections were taken for all three strains). For clear visualization of the actin ring, images of a single focal plane near the middle of the cell were presented. Myo1*-GFP indicates GFP-tagged Myo1 or its fragments. (C) Timing of Myo2 and Chs2 localization with respect to septin-hourglass splitting during the cell cycle. Cells of YEF5986 (*MYO2-GFP CDC3-mCherry*) and YEF5874 (*CHS2-GFP CDC3-mCherry*) were imaged by 3D dual-color time-lapse microscopy at 23°C with a 1-min interval. Montage images of the representative time-lapse data are shown here.



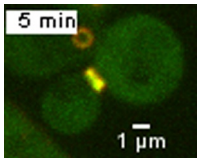
Video 1. **Localization of Bni5 versus Myo1 and myo1-mTD1 during the cell cycle.** (Left) Bni5 disappears from the bud neck at the onset of cytokinesis, whereas full-length Myo1 stays until the completion of cytokinesis. Strain: YEF6321 (**a** *MYO1-GFP BNI5-mCherry*). Green, Myo1-GFP; red, Bni5-RFP. 2-min time-lapse interval is shown. (Right) Strain: YEF6326 (**a** *myo1-mTD1-GFP BNI5-mCherry*). Green, myo1-mTD1-GFP; red, Bni5-RFP. 2-min time-lapse interval is shown.



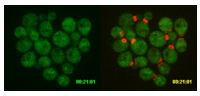
Video 2. **Full-length Myo1 disappears from the bud neck at the onset of cytokinesis in *mlc1-11* and *iqg1Δ* cells.** (Left) Strain: YEF6179 (**a** *mlc1-11 MYO1-GFP CDC3-mCherry:LEU2*). Green, Myo1-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. (Right) Strain: YEF6325 (**a** *iqg1Δ CDC3-mCherry:LEU2*, pRS316-MYO1-C-GFP). Green, Myo1-GFP; red, Cdc3-RFP. 1.5-min time-lapse interval is shown.



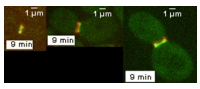
Video 3. **Constriction patterns of the rings formed by full-length Myo1 versus its tail.** (Left) Symmetric and complete constriction of Myo1-GFP ring in wild-type cells. Strain: XDY286 (**a** *MYO1-GFP CDC3-mCherry:LEU2*). Green, Myo1-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. (Right) Symmetric and partial constriction of myo1-Tail-GFP ring and the "cutting" of the myo1-Tail-GFP ring presumably by the forming PS. Strain: XDY288 (**a** *myo1-Tail-GFP CDC3-mCherry:LEU2*). Green, myo1-Tail-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown.



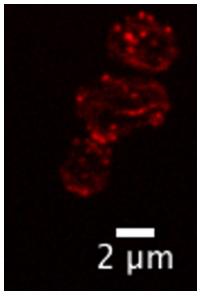
Video 4. **Disappearance of the myo1-TD2Δ-GFP ring from the bud neck before the onset of cytokinesis.** Strain: XDY289 (**a** *myo1-TD2Δ-GFP CDC3-mCherry:LEU2*). Green, myo1-TD2Δ-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown.



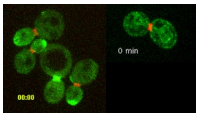
Video 5. **myo1-TD2Δ-GFP completely fails to localize to the bud neck in *bni5Δ* cells.** Strain: YEF6323 (**a** *bni5Δ myo1-TD2Δ-GFP CDC3-mCherry:LEU2*). Green, myo1-TD2Δ-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. Left, GFP channel; right, merged channels.



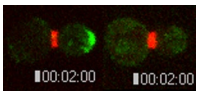
Video 6. **Localization of Myo1 in *bni5Δ* cells versus localization of myo1-TD2.** (Left) Asymmetric and complete constriction of Myo1-GFP ring in *bni5Δ* cells. Strain: XDY287 (**a** *bni5Δ MYO1-GFP CDC3-mCherry:LEU2*). Green, Myo1-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. (Middle) Symmetric and complete constriction of myo1-TD2-GFP ring. Strain: XDY290 (**a** *myo1-TD2-GFP CDC3-mCherry:LEU2*). Green, myo1-TD2-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. (Right) Asymmetric constriction of myo1-TD2-GFP ring. Strain and imaging conditions are the same as those for the middle video.



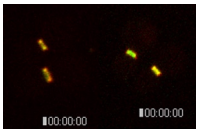
Video 7. **3D construction of the actin ring in a *myo1-Tail* cell.** Strain: YEF6309 (**a** *myo1-Tail-GFP NUP57-GFP*). Red, F-actin. 46 Z-sections with a 0.1-μm increment were used for the 3D reconstruction.



Video 8. **Localization of Myo1 versus Myo2 and Chs2 during the cell cycle.** (Left) Myo1-RFP and Myo2-GFP localization during the cell cycle. Strain: YKT662 (**a** *MYO2-GFP*, pRS316-MYO1-mCherry). Green, Myo2-GFP; red, Myo1-RFP. 1-min time-lapse interval is shown. (Right) Myo1-RFP and Chs2-GFP localization during the cell cycle. Strain: YEF5762 (**a** *CHS2-GFP*, pRS316-MYO1-mCherry). Green, Chs2-GFP; red, Myo1-RFP. 1-min time-lapse interval is shown.



Video 9. **Localization of Myo2 and Chs2 versus Cdc3 during the cell cycle.** (Left) Myo2-GFP and Cdc3-RFP localization during the cell cycle. Strain: YEF5986 (**a** *MYO2-GFP CDC3-mCherry*). Green, Myo2-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. (Right) Chs2-GFP and Cdc3-RFP localization during the cell cycle. Strain: YEF5874 (**a** *CHS2-GFP CDC3-mCherry*). Green, Chs2-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown.



Video 10. **Constriction patterns of Myo1 ring in the presence of full-length *IQG1* versus *iqg1(Δ2-411).*** (Left) Normal constriction of Myo1-GFP ring in the presence of full-length *IQG1*. Strain: RNY2596 (**a** *iqg1Δ MYO1-GFP CDC3-mCherry*, pRS315-IQG1-GST). Green, Myo1-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown. (Right) Abnormal constriction of Myo1-GFP ring in the presence of *iqg1(Δ2-411)* (cell 1). Strain: RNY2597 (**a** *iqg1Δ MYO1-GFP CDC3-mCherry*, pRS315-IQG1(Δ2-411)-GST). Green, Myo1-GFP; red, Cdc3-RFP. 1-min time-lapse interval is shown.

Reference

Lupas, A., M. Van Dyke, and J. Stock. 1991. Predicting coiled coils from protein sequences. *Science.* 252:1162–1164. doi:10.1126/science.252.5009.1162