

Calvert et al., <http://www.jcb.org/cgi/content/full/jcb.201101055/DC1>

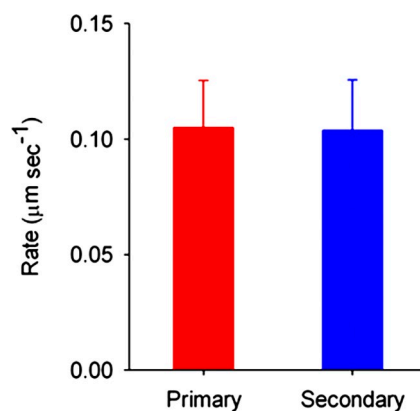


Figure S1. **Mean rates of constriction in primary and secondary hyphae.** The rate of constriction was calculated in cells with a mean circumference of 25 μm from both primary (red, $n = 10$) and secondary (blue, $n = 10$) hyphae. Error bars indicate the standard deviation from the mean.

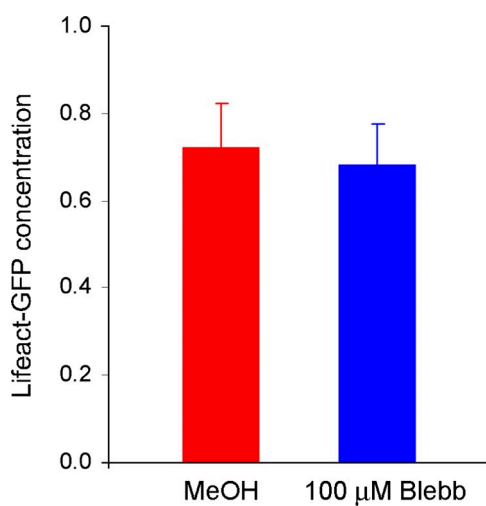


Figure S2. **Quantitation of the concentration of ring-associated Lifeact-GFP after treatment with low-dose blebbistatin.** Concentration of Lifeact-GFP in constricting rings in medium-sized cells (mean initial circumference = 24 μm) treated with MeOH alone (red, $n = 16$) or 100 μM blebbistatin (blue, $n = 15$), expressed in relative units of fluorescence intensity per confocal volume. Error bars indicate the standard deviation from the mean.

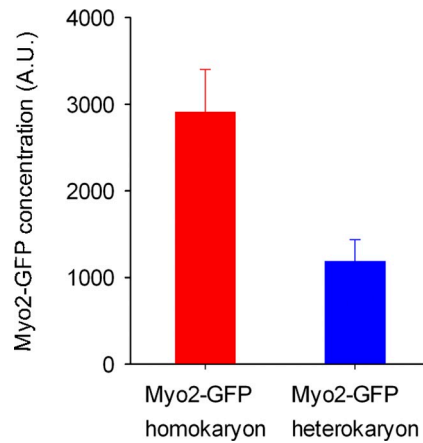


Figure S3. **Quantitation of the concentration of ring-associated Myo2-GFP in homokaryon and heterokaryon strains.** The Myo2-GFP intensity in medium-sized cells (mean circumference = 25 μm) of the Myo2-GFP homokaryon (red, $n = 10$) and heterokaryon (blue, $n = 14$) strains, expressed in arbitrary units of fluorescence intensity. Error bars indicate the standard deviation from the mean.

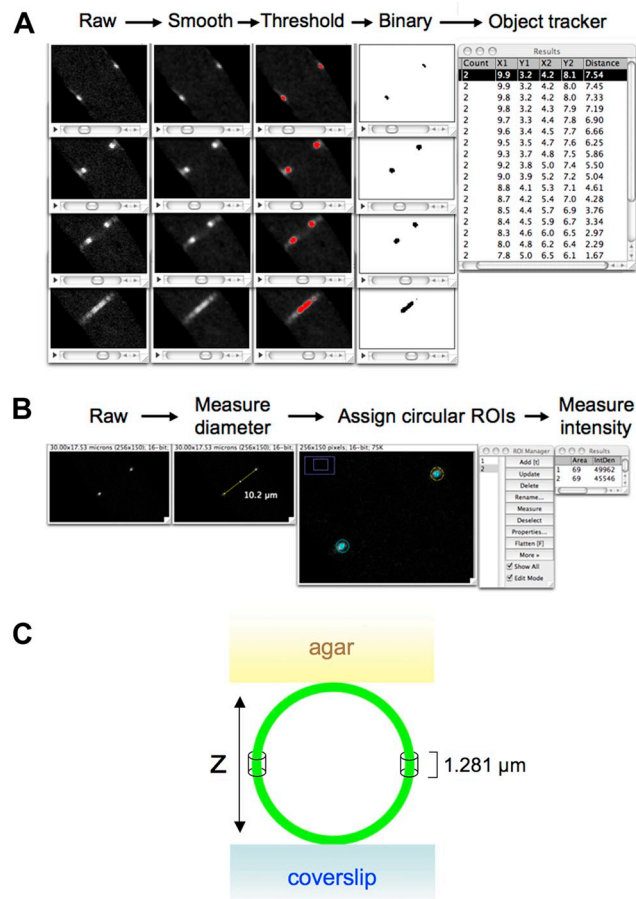
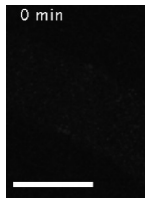
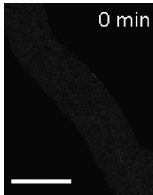


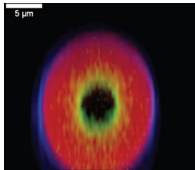
Figure S4. **Image analysis methodology.** (A) The duration and rate of contractility were determined by measuring the decrease in ring diameter during constriction for time-series acquired at a medial focal plane. The raw data were initially smoothed, then segmented by an intensity threshold, before using the Object Tracker plug-in in ImageJ. (B) The fluorescence intensity was measured in single images, acquired at a medial focal plane with a known confocal slice thickness, just after ring constriction had initiated. The diameter of the ring and intensity (integrated density) within two circular regions of interest (ROIs), drawn over the two intense spots of the ring, were measured using ImageJ. (C) The ring-associated protein concentration was determined as the mean of the two measurements described in B divided by the confocal volume (a cylinder equaling the product of the circular ROIs and the confocal slice thickness). Total protein in the ring was calculated by multiplying the concentration by the circumference of the ring.



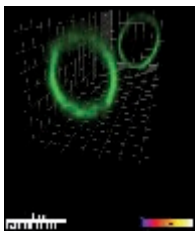
Video 1. **Myo2p-GFP localization during cortical ring formation and constriction.** Endogenously labeled Myo2-GFP was observed in wild-type *N. crassa* during vegetative growth by time-lapse (images acquired every 15 s for the duration of ring constriction) microscopy using a confocal laser scanning microscope (model TCS SP5; Leica). The time is indicated in minutes. Bar, 10 μ m.



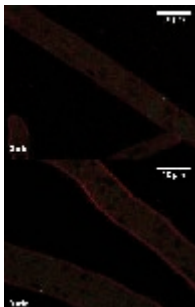
Video 2. **Lifeact-GFP localization during cortical ring formation and constriction.** The actin-binding probe Lifeact-GFP was transformed into wild-type *N. crassa* and observed during vegetative growth by time-lapse (images acquired every 15 s for the duration of ring constriction) microscopy using a confocal laser scanning microscope (model TCS SP5; Leica). The time is indicated in minutes. Bar, 10 μ m.



Video 3. **Membrane formation, cell wall deposition, and actomyosin ring constriction in *N. crassa*.** The process of septation was observed in wild-type *N. crassa* during vegetative growth using Lifeact-GFP (green), FM4-64 (red), and calcofluor (blue) to label actin, cell membrane, and cell wall, respectively. A 3D time-lapse was acquired (z-slices every 1 μ m) every 38 s for 5 min using a confocal laser scanning microscope (model TCS SP5; Leica) then rendered into the video shown using Imaris software (Bitplane). The time is indicated in minutes. Bar, 5 μ m.



Video 4. **Turnover of myosin II during ring constriction.** Fluorescence recovery after photobleaching (FRAP) of Myo2-GFP (green) in a hypha with two rings in close proximity, rendered into a 3D image. The ring in the foreground was selectively photobleached after the first image while the second ring in the background remained unbleached. A 3D time-lapse was acquired (z-slices every 0.8 μ m) every 20 s for 10 min using a confocal laser scanning microscope (model FV1000; Olympus) then rendered into the video shown using Imaris software (Bitplane). The time is indicated in minutes. Bar, 10 μ m.



Video 5. **Decreased ring-associated myosin II causes a decreased rate of ring constriction.** Endogenously labeled Myo2-GFP (green) and FM4-64 membrane dye (red) were observed in wild-type *N. crassa* after treatment with MeOH alone (top) or 100 μ M blebbistatin (bottom). The images were acquired every 30 s for up to 24 min by time-lapse microscopy using a confocal laser scanning microscope (model TCS SP5; Leica). The time is indicated in minutes. Bar, 10 μ m.