## Supplemental Table S1. *S. pombe* strains used in this study

| Strain | Genotype | Source/Reference |
|--------|----------|------------------|
| JW36   | h¹ kanMX6-41nmt1-3HA-ain1 | Wu et al., 2001 |
| JW37   | h¹ kanMX6-81nmt1-3HA-ain1 | Wu et al., 2001 |
| JW47   | h¹ ain1-3HA-kanMX6 | Wu et al., 2001 |
| JW50   | h¹ kanMX6-41nmt1-ain1 | Wu et al., 2001 |
| JW81   | h⁰ ade6-M210 leu1-32 ura4-D18 | Wu et al., 2003 |
| JW84   | h¹ ain1-Δ1::kanMX6 ade6-216 ura4-D18 | Wu et al., 2001 |
| JW96   | h¹ ain1-13Myc-kanMX6 | Wu et al., 2001 |
| JW109  | h¹ fim1-GFP(S65T)-kanMX6 | Wu et al., 2001 |
| JW110  | h¹ kanMX6-3nmt1-fim1 | Wu et al., 2001 |
| JW113  | h¹ kanMX6-3nmt1-GFP(S65T)-fim1 | Wu et al., 2001 |
| JW114  | kanMX6-3nmt1-GFP(S65T)-fim1 | Wu et al., 2001 |
| JW139  | h¹ fim1-Δ1::kanMX6 ade6 leu1-32 ura4-D18 | Wu et al., 2001 |
| JW144  | ain1-Δ1::kanMX6 kanMX6-41nmt1-fim1 | Wu et al., 2001 |
| JW175  | h¹ kanMX6-3nmt1-ain1 ura4-D18 | Wu et al., 2001 |
| JW740  | h¹ ade6-M216 leu1-32 ura4-D18 | Lab collection |
| JW949  | h¹ rlc1-mYFP-kanMX6 ade6-M210 leu1-32 ura4-D18 | Wu et al., 2003 |
| JW1040 | h¹ fim1-mCFP-kanMX6 ade6-M210 leu1-32 ura4-D18 | This study |
| JW1056 | fim1-mYFP-kanMX6 sad1-CFP-kanMX6 ade6-M210 leu1-32 ura4-D18 | This study |
| JW1109 | h¹ kanMX6-Pmyo2-mEGFP-myo2 ade6-M210 leu1-32 ura4-D18 | Coffman et al., 2009 |
| JW1110 | h¹ kanMX6-Pmyo2-mYFP-myo2 ade6-M210 leu1-32 ura4-D18 | This study |
| JW1136 | kanMX6-Pmyo2-mEGFP-myo2 ain1-Δ1::kanMX6 ade6-M210 leu1-32 ura4-D18 | Coffman et al., 2009 |
| JW1201 | kanMX6-Pmyo2-mEGFP-myo2 ain1-Δ1::kanMX6 kanMX6-41nmt1-fim1 ade6-M210 leu1-32 ura4-D18 | This study |
| JW1328 | h¹ cdc15-140 leu1-32 | Fankhauser et al., 1995 |
| JW1341 | h¹ rlc1-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18 | Wu et al., 2011 |
| JW1349 | 41nmt1-GFP-CHD (rng2)-leu1⁺ rlc1-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18 | Vavylonis et al., 2008 |
| JW1439 | ain1-3GF-kanMX6 rlc1-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18 | Coffman et al., 2009 |
| JW1443 | ain1-3YFP-kanMX6 sad1-CFP-kanMX6 ade6-M210 leu1-32 ura4-D18 | Coffman et al., 2009 |
| JW1512 | 41nmt1-GFP-CHD(rng2)-leu1⁺ rlc1-tdTomato-natMX6 kanMX6-3nmt1-ain1 ade6-M210 ura4-D18 | This study |
| JW1516 | cdc12-tdTomato-kanMX6 kanMX6-Pcdc15-GFP(S65T)-cdc15 ade6-M210 leu1-32 ura4-D18 | Coffman et al., 2009 |
| JW1565 | ain1-Δ1::kanMX6 41nmt1-GFP-CHD (rng2)-leu1⁺ rlc1-tdTomato-natMX6 ade6-M210 ura4-D18 | This study |
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Wu P, Zhao R, Ye Y, Wu J-Q (2011). Roles of the DYRK kinase Pom2 in cytokinesis, mitochondrial morphology, and sporulation in fission yeast. PLoS ONE 6, e28000.
Table S2. Additional varied model parameters not shown in Figures 5-8 and S6-8.

| Parameter         | Successful range | Used value | Comments* |
|-------------------|------------------|------------|-----------|
| $l_x$ (nm)        | 0 - 80           | 50         | Effective crosslinker size. For values larger than 80 nm, actin filaments form thick bundles. |
| $r_{capt}$ (nm)   | 100 - 200        | 150        | Value representing the node size. For values less than ~ 100 nm, connections are rare and condensation is slower than experiments. Values larger than 200 nm lead to multiple connections and fast condensation. |
| $k_{bead-node}$ (pN/µm) | 1 - 3            | 2          | This empirical parameter was tuned to allow long-lived connections between nodes and filament beads, without simultaneously pinning the beads to the nodes. Values less than 1 pN/µm are too weak to keep actin filament beads near nodes. Values larger than 3 pN are too strong to allow passage of myosin nodes from bead to bead along growing actin filaments. |
| $t_{turn}$ (s)    | 15 - 30          | 20         | Lifetime of actin filaments. Values less than 15 s lead to very short actin filaments that promote clump formation. Values of order 40 s or larger generate very long filaments that crosslink into bundles, which run through nodes and promote node clump formation. |
| $F_{myo}$ (pN)    | 2 - 12           | 4          | Myosin pulling force. Value that reproduces observed node speeds. |
| $F_{rep}$ (pN)    | > 5              | 80         | Short-range node repulsive force. Forces less than 5 pN cannot counteract the forces due to node connections |

* Other parameters were fixed to those corresponding to wt cells.
VIDEO LEGENDS

**Video 1.** Deletion of ain1 induces clump formation during node condensation into the contractile ring. Rlc1-tdTomato node condensation in wt (left panel) and ain1Δ cells (right panel). Cells were grown and imaged at 25°C. A stack of 24 z sections spaced at 0.2 µm was taken every 30 s. The stack at each time point was projected into a 2D image using a maximum-intensity projection. Display rate, 8 projection frames/s. Related to Figure 1A.

**Video 2.** mEGFP-Myo2 nodes condense abnormally into clumps in ain1Δ 41nmt1-fim1 cells. Cells were grown in YE5S + 5 µg/ml thiamine for 22 h at 25°C. A stack of 21 z sections spaced at 0.2 µm was taken every minute. The stack at each time point was projected into a 2D image using a maximum-intensity projection. Display rate, 8 projection frames/s. Related to Figure 1A.

**Video 3.** Clumps capture nearby nodes using actin filaments in the absence of Ain1. rlc1-tdTomato 41nmt1-GFP-CHD ain1Δ cells were grown in EMM5S for 24 h at 25°C. Maximum projections of three z sections spaced at 0.2 µm taken every 3 s. Rlc1-tdTomato nodes (left), GFP-CHD marked actin filaments (middle), and merged images (Rlc1 in red and CHD in green) are shown. Display rate, 8 projection frames/s. Related to Figure 1G.

**Video 4.** Ain1 overexpression promotes the formation of stable linear structures during contractile-ring formation. 3nmt1-ain1 rlc1-tdTomato cells were grown in EMM5S (inducing condition) for 18 h at 25°C. A stack of 21 z sections spaced at 0.2 µm was taken every minute. The stack at each time point was projected into a 2D image using a maximum-intensity projection. Display rate, 8 projection frames/s. Related to Figure 2B.

**Video 5.** Fim1 overexpression prevents nodes from condensing into the contractile ring and nodes spread along the long axis of the cell over time. 3nmt1-fim1 rlc1-tdTomato cells were grown in EMM5S (inducing condition) for 22 h and imaged at 25°C. A stack of 21 z sections spaced at 0.2 µm was taken every minute. The stack at each time point was projected into a 2D image using a maximum-intensity projection. Display rate, 8 projection frames/s. Related to Supplemental Figure S4B.

**Video 6.** Actin network condenses into disorganized thick actin bundles instead of a contractile ring when Ain1 is overexpressed. Actin filaments were marked using GFP-CHD in wt (left panel) and 3nmt1-ain1 cells (right panel). Cells were grown in EMM5S (inducing condition) for 18 h at 25°C. A stack of 3 z sections spaced at 0.25 µm was taken every 10 s. The stack at each time point was projected into a 2D image using a maximum-intensity projection. Display rate, 8 projection frames/s. Related to Figure 3D.

**Video 7.** Simulations in Figure 5G. Video shows node condensation into clumps, ring and meshwork for three values of crosslinking parameter $\alpha$ (1 s/frame, 500 s total; top to bottom: $\alpha = 0, 0.7, 1$).

**Video 8.** Increasing Myo2 concentration partially rescues the defects in contractile-ring formation when Ain1 is overexpressed. 41nmt1-ain1 rlc1-tdTomato (left panel) and 2x myo2 41nmt1-ain1 rlc1-tdTomato cells (right panel) were grown in EMM5S (inducing condition) for 24 h at 25°C. Stacks of 21 z sections spaced at 0.2 µm were taken every minute. The stack at each time point was projected into a 2D image using a maximum-intensity projection. Display rate, 8 projection frames/s. Related to Figure 8C.
Supplemental Figure S1: Abnormal node condensation in ain1 and fim1 mutants is not due to defects in initial node distribution. (A and B) Initial node distribution over the long cell axis has no defects in ain1 and fim1 mutants. (A) The regions in rectangles were used for measuring node intensities. (B) Gaussian fits of the mean intensity measured for each strain. n, R², and FWHM (mean ± SD) are indicated. Cells were grown in repressing condition (YES5S +thiamin). (C and D) Times are in min. (C) Mid1 forms clumps in ain1Δ as indicated by red arrow. Times are relative. (D) Deletion of both ain1 and fim1 results in clump formation during node condensation. Localization of Rlc1-tdTomato in wt and ain1Δ fim1Δ cells after growing spores for 24 h in YES5S. Clump formation is indicated by red arrow. (E-G) Localization of Ain1 and Fim1 to the division site during node condensation. (E) Cells expressing either Sad1-CFP Ain1-3YFP or Sad1-CFP Fim1-mYFP were imaged and distances between SPBs were used to determine the timing for Ain1/Fim1 localization. Green and gray diamonds represent whether Ain1 or Fim1 are detectable or not at a meshwork/the contractile ring, respectively; the red solid and dashed lines mark the mean and SDs of SPB distances when mYFP-Myo2 nodes begin to condense in a parallel experiment. (F and G) Example of Ain1 or Fim1 localization in wt and mutant cells. (H) Fim1 protein level in 41nmt1-GFP-fim1 cells after induction for 25 h. (I) Radial projections of Myo2 clump formation from linear structures during late stage of node condensation in ain1Δ. Red and blue arrowheads indicate linear and clump structures, respectively. Time in min. (J and K) 2D porosity (J) and largest gap (K) visualized by mEGFP-Myo2 during node condensation in ain1Δ. Bars, 2 μm.
Supplemental Figure S2: The SIN pathway is critical for contractile-ring assembly from the clumps in ain1Δ but is dispensable for recruiting Ain1 to the division site; Ain1 and Cdc15 are independent of each other for localization. Times, in min, are relative. (A, C, and D) Cells were grown in YE5S at 36°C for 2 h before imaging at 36°C. (A) The SIN pathway is required for ring assembly after clump formation. Time courses of Rlc1-tdTomato localization in ain1Δ and ain1Δ with SIN mutants cdc11-123 and cdc7-24 at 36°C. Red arrows indicate clump formation. Note that clumps disappeared over time and no ring formed in the double mutants. (B) ain1Δ is synthetic sick/lethal with SIN and cdc15 mutants. Serial dilutions on EMM5S + Phloxin B were grown at 30°C for 2 days before scanning. (C) The SIN pathway is not required for Ain1 division-site localization. Time courses of Rlc1 and Ain1 localization in wt and cdc7-24 mutant at 36°C. Rlc1-tdTomato is used as a control to observe ring collapse (white arrows) when the SIN pathway is inactivated. (D) Ain1 does not depend on Cdc15 for division-site localization. In cdc15-140, Ain1-mEGFP localizes as a ring before collapsing (white arrows) a few min after its formation. (E and F) Cdc15 localizes normally at the contractile ring in ain1Δ. (E) Time courses of GFP-Cdc15 localization in wt and ain1Δ. Top: maximum-intensity projection; bottom: radial projection. (F) GFP-Cdc15 fluorescence intensity at the division site in the boxed region as indicated in E. Mean and SD are plotted. Bars, 2 μm.
Supplemental Figure S3: Ain1 protein levels, dynamics, and distribution in cells overexpressing Ain1. (A and B) Ain1 protein levels revealed by western blotting. Actin was used as a loading control. (A) Only GFP-Ain1 full length band is observed. Strains were grown for indicated time in inducing condition. (B) Ain1 protein levels increase as a function of nmt1 promoter strengths and the inducing time in EMM5S medium. Signals were quantified and the ratios to wt are indicated. Note that no degradation bands are observed. (C) Ain1 is dynamic in the contractile ring regardless of its cellular concentrations in FRAP assays. Fluorescence recovery curves (mean ± SEM) after photobleaching with half time (mean ± SD) for four strains are shown. (D and E) Rlc1 node distribution along the long cell axis has no defects in cells overexpressing Ain1. (D) The regions in rectangles on the micrographs were used for measuring node intensities. (E) Gaussian fits of the mean intensity measured for each strain as color coded to the micrographs. n, R², and FWHM (mean ± SD) are indicated. Bars, 2 µm.
Supplemental Figure S4: Fim1 overexpression prevents nodes from condensing into the contractile ring. (A) Strong Fim1 overexpression leads to severe cytokinesis defects in both 3nmt1-fim1 and 3nmt1-fim1 ain1Δ cells. Indicated strains were grown in EMM5S for 24 h. (B-D) Fim1 overexpression affects nodes condensation. Times in min. Cells were grown in inducing condition for 22 h. (B) Time course of node movements in 3nmt1-fim1 (also see Video 5). (C) Linear structures but not the contractile ring form transiently (red arrowheads) in 3nmt1-fim1 cells. Images are radial projections. (D) Dynamics of the actin network in 3nmt1-fim1 GFP-CHD rlc1-tdTomato cells. Note that actin filaments/bundles did not form a more focused network at the division site. Bars, 2 μm.
**Supplemental Figure S5:** Crosslinker overexpression or depletion has no significant effects on F-actin concentrations in the contractile ring/meshwork. (A) Representative F-actin staining using Alexa Fluor 488 phalloidin in indicated strains. Settings of gray levels are indicated on the top. Cells were grown in EMM5S (inducing condition). Bars, 2 μm. (B) Quantification of total F-actin in the cell (top graph) or in the ring/meshwork (bottom graph). Horizontal bars indicate mean ± SD. (C) Representative F-actin staining using Alexa Fluor 568 phalloidin in indicated strains. Cells of wt and 41nmt1-fim1 ain1Δ were grown for 22 h in YE5S + thiamine (repressing condition) and then mixed before fixation. Mutant cells were identified using mEGFP-Myo2 signal. (D) Quantification of F-actin ring/meshwork in indicated strains. Horizontal bars indicate mean ± SD. Bars, 2 μm.
Supplemental Figure S6. **Additional model description and simulation results.** (A) Schematic of actin filament (green) polymerization out of nodes (red). The equilibrium length of the spring connecting the node and first filament bead, \( l_0(t) \), increases with time due to the polymerization of the filament by formins at the node. The actin filament polymerizes at an angle \( \varphi_{\text{pol}} \). A restoring torque proportional to \( \varphi_{\text{pol}} - \varphi_1 \) pushes the filament towards the preferred angle. (B-D) Additional quantitative comparisons of simulation results as in Figure 5G to experiments. (B) 2D porosity vs. time for different values of parameter \( \alpha \), with other parameters kept constant. Porosity was calculated similarly to the experiments (see Materials and methods) using a grid box size of 0.2 \( \mu \)m and counting empty boxes. As time progresses the total width of the grid was considered to be equal to two standard deviations of \( y \) node coordinates. The total length of the grid was kept constant and equal to the cell circumference. Failure of condensation into rings in the absence or over-expression of crosslinkers is indicated by large porosity at long times, similarly to experiments (Figures 1E and Supplemental Figure S1J). Error bars are SEM (n = 10 simulations). (C) Node displacements are biased toward large angles when \( \alpha \) is decreased. Nodes whose initial distance from the center was larger than 0.9 \( \mu \)m were tracked in the simulations for the first 150 s. We measured the angle between a line joining the initial node position to the final node position and the axis parallel to the long axis of the cell. The displacement was the Euclidean distance between initial and final points. We grouped displacement measurements into three bins: 0-30, 30-60, and 60-90 degrees. Nodes move at larger angles in the absence of crosslinking, similar to Figure 1H. (D) Probability of node velocities for different values of parameter \( \alpha \) shows similar trend to Figure 4, E and F. Node velocities were measured over a 5 s interval during the first 200 s after onset of condensation. Only velocities larger than 10 nm/s are shown.
Laporte et al., Figure S7
**Supplemental Figure S7**: Dependence of model results on parameter values. Snapshots and graphs (calculated at 500 s, mean of 10 simulations) showing the effect of changes in selected model parameters, with all other parameters unchanged compared to Figure 5G, $\alpha = 0.7$. Error bars: SEM. (A) Effect of changing polymerization stall force $F_{\text{stall}}^\text{pol}$ (see Materials and methods). In the presence of crosslinking, force-induced reduction of the polymerization rate causes the nodes to align in small disconnected clumps. (B) Effect of changing parameter $\zeta_{\text{rot}}$ that describes resistance to rotation of polymerization direction. For small $\zeta_{\text{rot}}$, filaments rotate and align within a single bundle. (C) Effect of changing model parameter $\mu$ that describes the reduction of myosin pulling force per filament by nodes inside actin bundles. For large $\mu$ (small reduction), myosin pulling forces inside bundles become too strong to maintain ring integrity. We used $\mu = 0.3$ in the main text. (D) Effect of changing the initial band width. Wide initial bands ($w > 5 \ \mu$m) fail to form narrow smooth rings.
A  \textit{cdc25-22}
\[\alpha = 0.7 \quad \alpha = 0.8 \quad \alpha = 0.9 \quad \alpha = 1.0\]

B  \[\varepsilon = 0.03 \quad \varepsilon = 0.07\]

C  \[\varepsilon = 0.03 \quad \varepsilon = 0.07\]

Laporte et al., Figure S8
**Supplemental Figure S8:** Simulations showing node condensation in cdc25-22 cells and the effect of reduction of actin filament turnover by crosslinkers. (A) Simulation snapshots of ring formation in cdc25-22 cells. Initially 100 nodes are distributed according to Gaussian distribution (width = 4σ = 6.4 µm). Formation of the formation of meshwork of intersecting bundles/double rings was observed with increasing α (Ojic et al., 2011). (B and C) Simulation snapshots and results of a model that includes reduction of actin filament turnover by crosslinkers. To simulate this effect we assumed that for each filament $t_{\text{turn}} = t_{\text{turn}}^0 (1 + \varepsilon \cdot N_x / N_L)$, where $t_{\text{turn}}^0 = 20s$, $N_x$ is the total number of crosslinker connections, $N_L$ is the total number of beads of the filament, and $\varepsilon$ is a parameter that measures the strength of this effect. Thus, as filaments form bundles, their turnover time increases depending on the number of crosslinkers along the filament. In (B) we simultaneously changed the polymerization rate: $v_{\text{pol}} = v_{\text{pol}}^0 t_{\text{turn}}^0 / <t_{\text{turn}}>$, where $<t_{\text{turn}}>$ is the average turnover time of all filaments and $v_{\text{pol}}^0 = 0.1 \mu m/s$. Thus, the total F-actin remains constant during the late stages of condensation, as observed in experiments (see Figure 5). Left graph shows how $<t_{\text{turn}}>$ changes during condensation for given values of parameter $\varepsilon$. For $\varepsilon = 0.03$, it increases by about 40%, similar to Figure 3, F and G. Right graph shows band width at 500s for different values of parameter $\varepsilon$. Increasing $\varepsilon$ does not compromise the ability to form rings. (C) Same as B, but polymerization rate $v_{\text{pol}}$ is kept constant. Large misplaced bundles form, leading to aberrant accumulation of F-actin and failure of condensation. For large values of $\varepsilon$, bands fail to condense. Error bars: SEM, (n = 10).
**Supplemental Figure S9:** Measurements of myosin Myo2 level and the importance of the spectrin repeats in Ain1 function. (A-C) Myosin II level in nodes, but not node numbers, are doubled in the 2x myo2 strain. (A) Micrographs of mYFP-myo2 and 2x mYFP-myo2 cells. Both strains were imaged with the same setting and the images were adjusted with the same gray level. Note that cytoplasmic background is higher in 2x mYFP-myo2 strain. (B) mYFP-Myo2 fluorescence intensity at the division site in the green boxed region as indicated in A. Mean and SD are plotted. (C) The number of nodes is similar in mYFP-myo2 and 2x mYFP-myo2 strain. (D) The spectrin repeats of Ain1 are critical for its function. Time courses (in min) of wt and ain1::0SR cells during node condensation. Note that nodes condense into clumps in the ain1::0SR cell. Bars, 2 μm.