Supplemental experimental procedures

Strain construction and growth conditions

Cells were grown for microscopy in exponential phase at 25°C in YE5S rich liquid medium in 50 ml flasks in the dark to a density of 2 to 4 x 10^6/ml (measured by OD_{595}). To repress the expression of Cdc15p, *41xnmt1cdc15* cells were grown overnight in YE5S, washed twice with EMM5S medium and grown in EMM5S with 2.8 μM thiamine for 24 h before processed for microscopy. To inhibit the Bgs1p enzymatic activity cells were treated either with DMSO or 320 μM Aculeacin A with DMSO for 15 min at room temperature prior to imaging with the drug.

For some microscopy experiments cells were synchronized by growing exponential cultures with 20 mM hydroxyurea for 6 h at 25°C in EMM5S. Cells were then washed twice with EMM5S medium, and the cell cycle was resumed at 25°C in EMM5S supplemented with 2.8 μM thiamine for 2 h before imaging at 25°C.

Various domains of Cdc15p were tagged with mCherry at their N-termini and expressed in *S. pombe* under the control of the native promoters from the *leu1*$^+$ locus. The plasmids encoding the domains of Cdc15p were constructed by standard cloning procedures. Briefly to construct a pJK148 vector containing the promoter for *cdc15*$^+$(pJK148-P_{cdc15}), the 5' UTR (-433 to +3) plus start codon ATG of *cdc15*$^+$ was amplified by PCR from *S. pombe* genomic DNA and cloned into the pJK148 vector digested with *EcoR*I and *Pac*I. The cDNA encoding the mCherry was amplified from pKS391 (pFA6a-mCherry-natMX6) and cloned into pJK148-P_{cdc15} vector digested with *Pac*I and *Not*I. DNA fragments encoding the Cdc15p domains, FBD_{1-297}, $MD_{298-868}$ and $MDSH3_{298-927}$ were amplified from the *cdc15*$^+$cDNA by PCR and cloned into the pJK148-P_{cdc15}-mCherry vector digested with *Not*I and *Sac*II. Four repeats of TCC were added in the reverse primer for mCherry creating a linker of four glycines between mCherry and the domains of Cdc15p. Additional amino acids introduced by the sequence of *Not*I were removed by site directed mutagenesis. All plasmids were linearized by digestion with *Nde*I/*Nru*I before transforming into *S. pombe* cells.

Microscopy

Cells were centrifuged at 800 *g* for 1 min and washed twice into EMM5S media. Live-cell microscopy was performed at 23–25°C with cells on a thin pad of 25% gelatin in EMM5S medium supplemented with 0.1 mM *n*-propyl-gallate (Arasada and Pollard, 2011; Wu et al., 2008). Fluorescence images of live cells were acquired with an Olympus IX-71 microscope with

a 100×/NA 1.4 Plan Apo lens (Olympus) and a CSU-X1 (Andor Technology) confocal spinning disk confocal system equipped with an iXON-EMCCD camera (Andor Technology).

Image Analysis

S. pombe cells expressing a type II myosin regulatory light chain Rlc1p-tdTomato and spindle pole body protein Sad1p-GFP or mCherry tagged tubulin Atb2p were used to put cytokinetic events on an absolute time scale with time zero defined as the separation of the spindle pole bodies (Wu et al., 2003). Formation of cytokinesis nodes was determined by the appearance of Rlc1p-tdTomato fluorescence in puncta around the cell center in maximum intensity projected images. Condensation of nodes into a complete contractile ring was determined by comparing maximum intensity projected images with 3D reconstructions. The onset of ring constriction was determined by measuring the change the ring diameter over time. The circumference of the contractile ring was measured by tracking the position of the ring in each z-slice at every time point using an ImageJ plugin JFilament (Smith et al., 2010). The distance between the brightest pixels of GFP-Bgs1p in the median plane of the cell was used to calculate circumference of the cleavage furrow.

We measured the local numbers of GFP or YFP molecules in the region of the cleavage furrow using sum images and a rectangular box (11 x 4 pixels). The fluorescence intensity was corrected for background fluorescence, acquisition photobleaching, camera noise and uneven illumination as follows. We measured the background fluorescence including both cytoplasmic fluorescence and fluorescence from the trans-Golgi vesicles underlying the contractile ring using a box wider by 2 pixels. The fluorescence intensity within the measuring area was corrected for background by subtracting the intensity of the surrounding area (Hoffman et al., 2001). The offset pixel intensity of the same exposure time was subtracted from the sum images to correct for camera noise. Uneven illumination was corrected from images of a thin film of Alexa 488 dye solution sealed under a coverslip on a glass slide. The numbers of molecules were calculated from the fluorescence intensities using a calibration curve constructed using seven mEGFP- or GFP or mYFP- or YFP tagged proteins (Fimbrin, Fim1p; Actin related protein 3, Arp3p; Actin related protein 2, Arp2p; Actin related protein C5, ArpC5p; Capping protein subunit, Acp2p; Type II Myosin, Myo2p and α-Actinin, Ain1p) (Wu et al., 2008; Wu and Pollard, 2005). Time courses of the average numbers of molecules were produced by aligning individual time courses to time zero, the separation of spindle pole bodies, and averaging the number of molecules at

each time point.

Subcellular Fractionation

Cell lysates were fractionated by sedimentation velocity ultracentrifugation in a sucrose gradient described for budding yeast (Antebi and Fink, 1992; Barrowman et al., 2010; Moseley et al., 2009). One liter of cells were grown at 25°C in nutrient rich media YE5S to an OD_{595} of 0.4. Cells were washed once with cold 10 mM sodium azide and converted to spheroplasts by gentle shaking at 120 rpm for 1.5 h at 32°C in 1.4 M sorbitol, 50 mM potassium phosphate pH 7.5, 10 mM sodium azide, 0.4% 2-mercaptoethanol and a mixture of 1 mg/ml Zymolase and lytic enzyme (Sigma Aldrich). Spheroplasts were pelleted through a sorbitol cushion (1.7 M sorbitol, 50 mM potassium phosphate pH 7.5) at 600 *g* for 10 min, resuspended in lysis buffer containing 20 mM HEPES pH 7.5, 12.5% sucrose, 1 mM EDTA and EDTA free complete protease inhibitor mix (Roche Diagnostics) and lysed by 8 passages through a 5/8 inch long 25-gauge needle. Centrifuging the lysate at 500 g for 5 min pelleted unbroken cells. One milliliter of cleared lysate was loaded on the top of a sucrose gradient consisting of eleven 1 ml steps (18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 60% sucrose) in 10 mM HEPES pH 7.5, 1 mM $MgCl_2$. Gradients were centrifuged at 38,000 rpm in an SW41 rotor at 4°C for 140 min. Fractions of 250 µl or 500 µl were carefully collected from the top of the gradient and analyzed by SDS-PAGE and immunoblotting. Proteins were transferred to PVDF membranes in transfer buffer (25 mM Tris-Base, 192 mM glycine, 20% methanol) at a constant voltage of 30 volts overnight at 4°C. Under these conditions, the vast majority of the protein transferred to the PVDF membranes. Blots were probed with anti-GFP polyclonal antibodies, anti-DsRed polyclonal antibodies (Clontech), anti-mCherry monoclonal antibody 1C51 (Abcam) and then with peroxidase-conjugated goat anti-rabbit IgG (Abcam). Blots were reacted with ECL reagent from ECL Plus detection kit (GE Healthcare Life Sciences, RPN2132) and exposed to Kodak BioMax MR film, which was developed, scanned and quantified using Image J. To quantify the intensity of the signal, we drew a rectangular area large enough to contain the brightest band of the target protein and this box was used to measure the total pixel intensity of the signal in every lane of the immunoblot. The intensity within the measuring area was corrected for background by subtracting the mean intensity of 7-10 different areas from random places on the exposed films.

Supplemental Figures

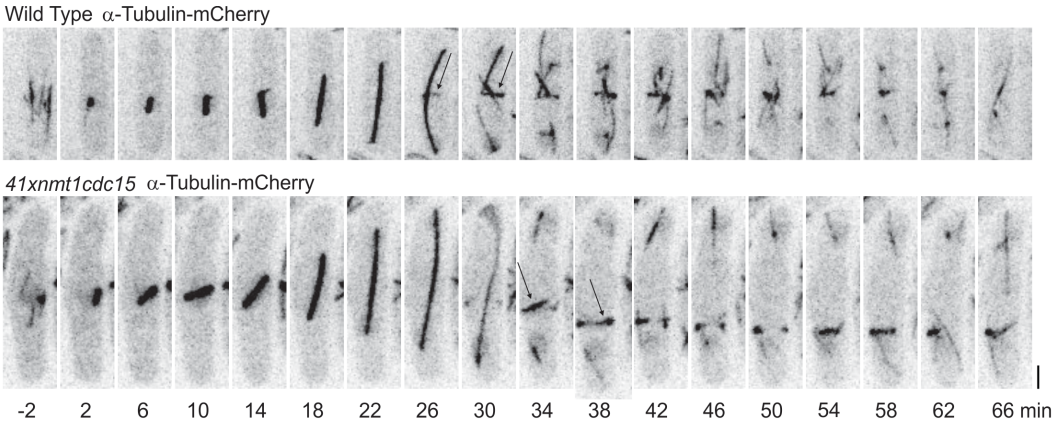
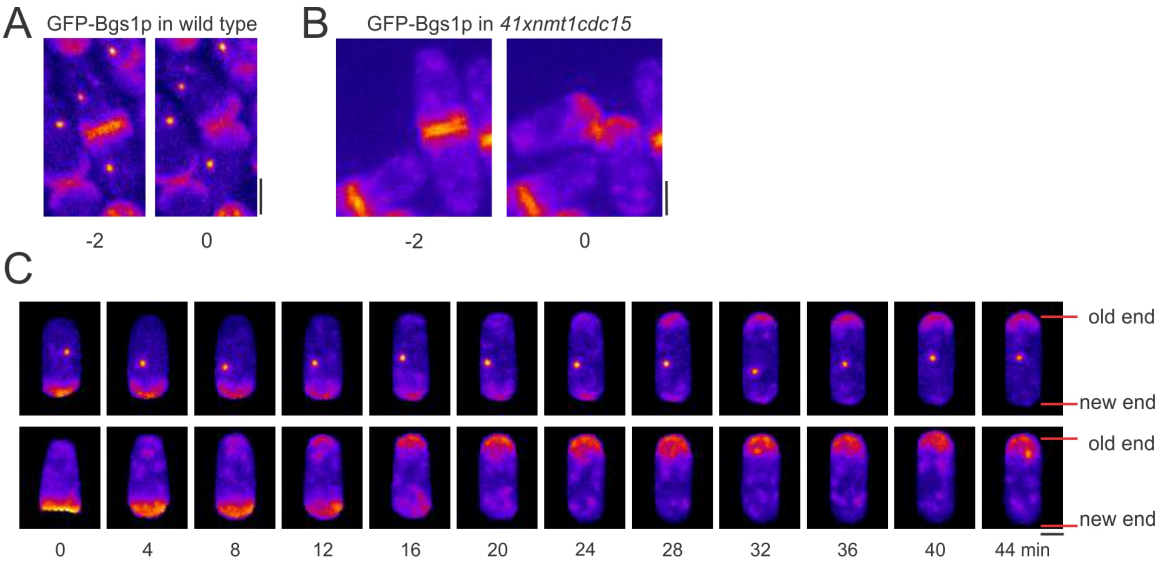


Figure S1, Related to Figure 2: Assembly of the post anaphase array of microtubules in *41xnmt1cdc15* cells depleted of Cdc15p.

Time series of negative contrast fluorescence micrographs (maximum intensity projections of 18 confocal z-sections at 4 min intervals) of mitotic cells expressing α-tubulin tagged with mCherry. (Upper panel) wild type cells and (lower panel) cells depleted of Cdc15p. Black arrows mark post anaphase arrays of microtubules. Scale bar 2 μm.



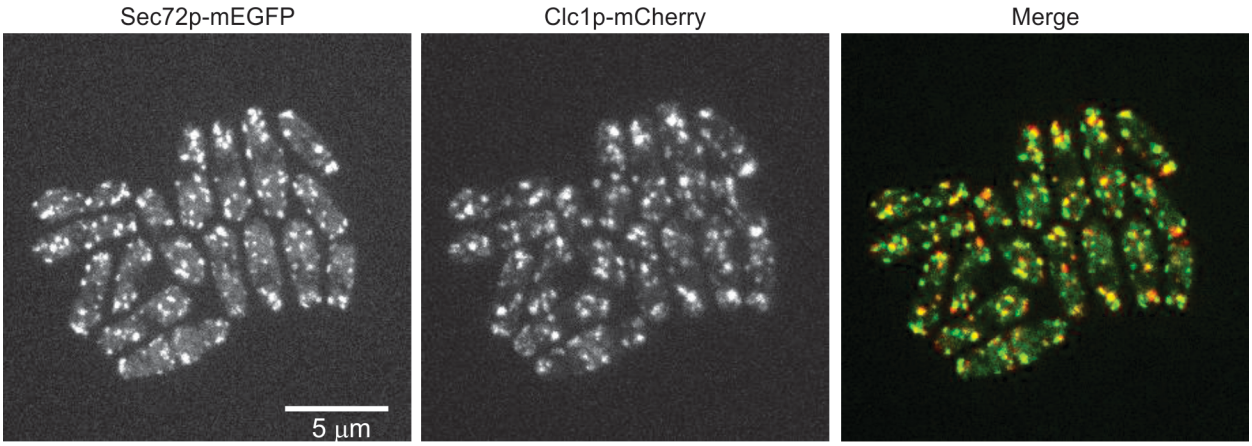
Supplemental Figure S2, Related to Figure 4: Recruitment of Bgs1p-GFP to the cell tips in interphase.

(A and B) Fluorescence micrographs (maximum intensity projections of 20 confocal z-sections, $\Delta z = 0.36$ µm) of cells before cell separation (t = -2 min) and at the time of cell separation (t = 0 min).

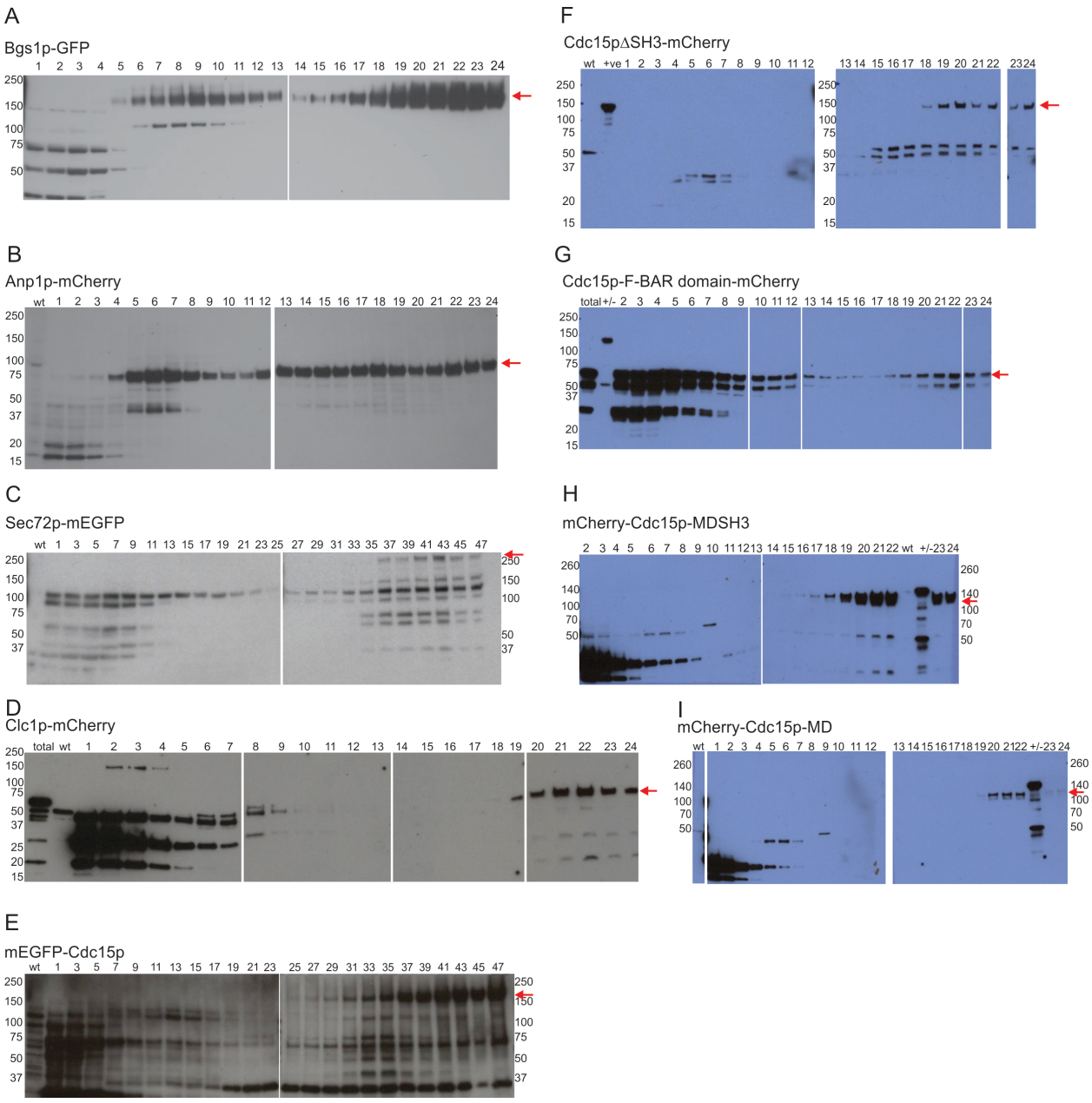
(A) Wild type cells expressing GFP-Bgs1p and Sad1p-GFP.

(B) *41xnmt1cdc15 cells* depleted of Cdc15p expressing GFP-Bgs1p and Sad1p-RFP.

(C) Time series of fluorescence micrographs (maximum intensity projected of 20 confocal z-slices, $\Delta z = 0.36$ µm) of (upper panel) wild type cells expressing GFP-Bgs1p and Sad1p-GFP and (lower panel) cells depleted of Cdc15p expressing GFP-Bgs1p and Sad1p-RFP showing the recruitment of GFP-Bgs1p to the old end after the separation of the two daughter cells. Time zero is the first time point after cell separation. Old and new ends are labeled. Scale bar, 2 µm.

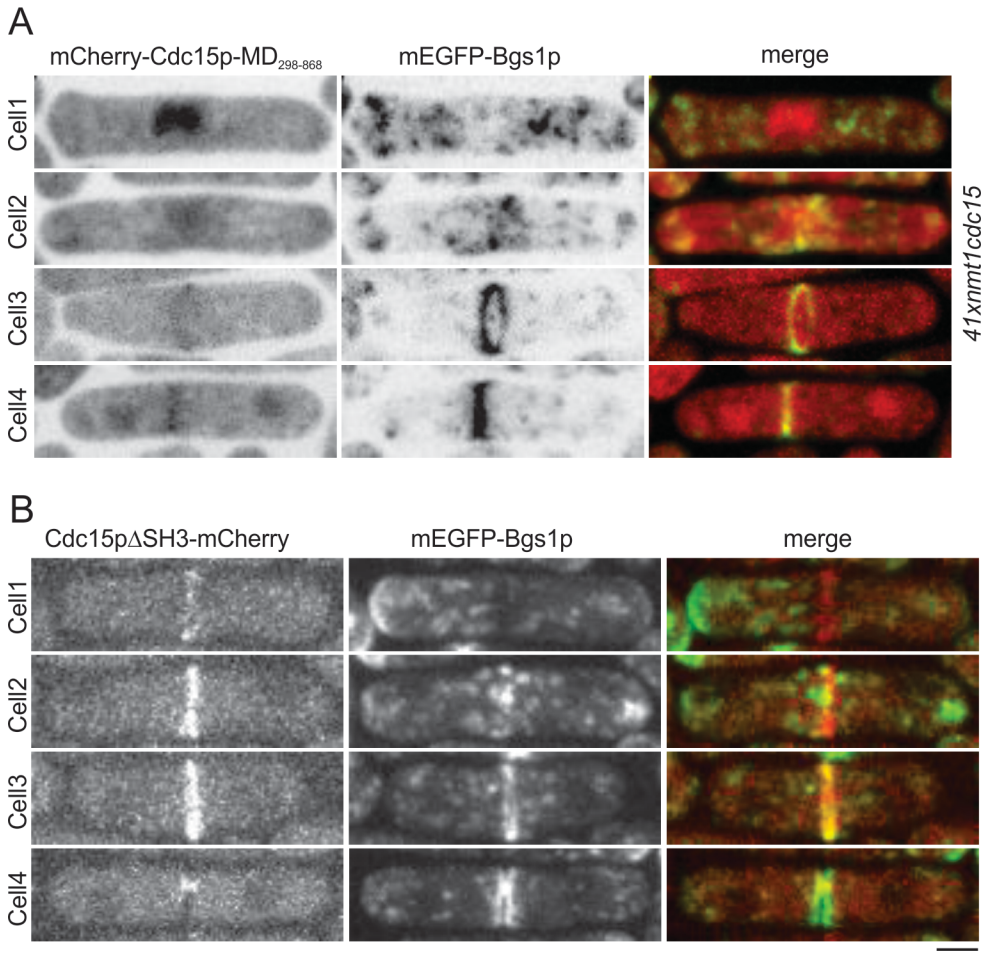


Supplemental Figure S3, Related to Figure 5: Localization of clathrin light chain Clc1p-mCherry and late-Golgi compartment protein Sec72p-mEGFP by fluorescence microscopy of live cells. Each panel is a maximum intensity projection of a stack of 20 spinning disk confocal sections taken at 0.36-μm intervals: (left) mEGFP-Sec72p, (middle) Clc1-mCherry and (right) merge of the images from the two channels. Scale Bar, 5 μm.



Supplemental Figure S4 related to Figure 5. Subcellular distribution of GFP-Bgs1p, cis-Golgi marker Anp1p, trans-Golgi marker Sec72p, clathrin light chain Clc1p, F-BAR domain containing protein Cdc15p and Cdc15p domains analyzed by sedimentation velocity on 16-60% sucrose step gradients. Samples of lysates were layered on to top of the sucrose gradient and centrifuged at 100,000g for 140 min. Fractions were collected manually from the top and analyzed by SDS-PAGE and immunoblotting with antibodies against GFP or mCherry. Red arrows indicate bands that were quantified.

(A-F) Total cell lysates from wild type cells expressing full length (A) GFP-Bgs1p, (B) Anp1p-mCherry, (C) Sec72p-mEGFP, (D) Clc1p-mCherry (E) mEGFP-Cdc15p and (F) mEGFP-Cdc15pΔSH3 (Cdc15p lacking the SH3 domain).
(G-I) Total cell lysates from Cdc15p-depleted *41xnmt1cdc15* cells expressing (G) mCherry-Cdc15p-$FBD_{1\text{-}297}$ (F-BAR domain), (H) mCherry-Cdc15p-$MDSH3_{298\text{-}927}$ (Cdc15p lacking the F-BAR domain) and (I) mCherry-Cdc15p-$MD_{298\text{-}868}$ (middle region between the F-BAR domain and the SH3 domain) under the control of the *cdc15*$^{+}$ promoter in the *leu1*$^{+}$ locus.



Supplemental Figure S5, Related to Figure 5: Localization of mCherry-Cdc15p domains. Fluorescence micrographs (maximum intensity projections of 20 confocal z-slices, $\Delta z = 0.36$ µm) of *41xnmt1cdc15* cells depleted of Cdc15p expressing mCherry-tagged Cdc15p constructs and GFP-Bgs1p. (First column) mCherry-Cdc15p construct, (second column) GFP-Bgs1p and (third column) a merge of the two fluorescence images: red, mCherry-Cdc15p-construct and green, GFP-Bgs1p. Scale bars 2 µm.

(A) Cdc15p middle domain mCherry-Cdc15p-$MD_{298\text{-}868}$. Columns 1 and 2 are reversed contrast. Top to bottom: Cell 1, interphase with mCherry-Cdc15p-$MD_{298\text{-}868}$ in the nucleus and mEGFP-Bgs1p in trans-Golgi vesicles; Cell 2, Anaphase B with mCherry-Cdc15p-$MD_{298\text{-}868}$ at the cell center and mEGFP-Bgs1p accumulated at the cell equator; Cell 3, end of Anaphase B with mCherry-Cdc15p-$MD_{298\text{-}868}$ in the contractile ring and mEGFP-Bgs1p concentrated in the cleavage furrow; Cell 4, final stages of cytokinesis.

(B) Cdc15p lacking the SH3 domain, Cdc15pΔSH3-mCherry. Top to bottom: Cell 1, metaphase or anaphase A with Cdc15pΔSH3-mCherry is in the nodes or a nascent ring and mEGFP-Bgs1p is at the cell tips; Cell 2, anaphase B with Cdc15pΔSH3-mCherry in the contractile ring and mEGFP-Bgs1p accumulating at the center; Cell 3, end of anaphase B with Cdc15pΔSH3-mCherry in the contractile ring and mEGFP-Bgs1p in the cleavage furrow; Cell 4, final stages of cytokinesis with Cdc15pΔSH3-mCherry in the remnant of the contractile ring and mEGFP-Bgs1p in the cleavage furrow.

Supplemental movies

Movie S1, Time-lapse movie of fluorescence micrographs of a *41xnmt1cdc15* cell depleted of Cdc15p expressing Rlc1p-tdTomato at 25°C, Related to Figure 1. Images are maximum intensity projections (ImageJ) of Z-series of confocal images acquired at 2 min intervals. Dimensions: 30.7 X 15.3 μm. Frame rate: 7 frames/s.

Movie S2, Time-lapse movie of fluorescence micrographs of a *41xnmt1cdc15* cell depleted of Cdc15p expressing Rlc1p-tdTomato at 25°C, Related to Figure 1. Images are maximum intensity projections (ImageJ) of Z-series of confocal images acquired at 2 s time intervals. Dimensions: 6.7 X 16 μm. Frame rate: 100 frames/s.

Movie S3, Time-lapse movies of fluorescence micrographs of contractile rings in wild type and mutant cells expressing Myp2p-YFP, Related to Figure 6. Images are optical sections in the plane of the contractile rings reconstructed from stacks of 18 z–sections acquired at 3 min intervals at 25°C. Top to bottom: wild type cell, cell depleted of Cdc15p, *cdc15*Δ*SH3* cell and Δ*clp1* cell. Dimensions: 6.13 X 21.33 μm. Frame rate: 7 frames/s.

Movie S4, Time-lapse movie of fluorescence micrographs of a contractile ring in a cell depleted of Cdc15p and expressing Rlc1p-tdTomato, Related to Figure 6. Images are optical sections in the plane of the contractile rings reconstructed at 3 min intervals at 25°C from stacks of 21 z-sections. Dimensions: 7.2 X 7.7 μm. Frame rate: 7 frames/s.

Movie S5, Time-lapse movie of fluorescence micrographs of Δ*myp2* cells expressing Rlc1p-GFP at 25°C, Related to Figure 7. Images are maximum intensity projections (ImageJ) of Z-series of 18 confocal z-sections at 2 min intervals at 25°C. Dimensions: 30.4 X 28 μm. Frame rate: 7 frames/s.