Wloka et al., http://www.jcb.org/cgi/content/full/jcb.201208030/DC1

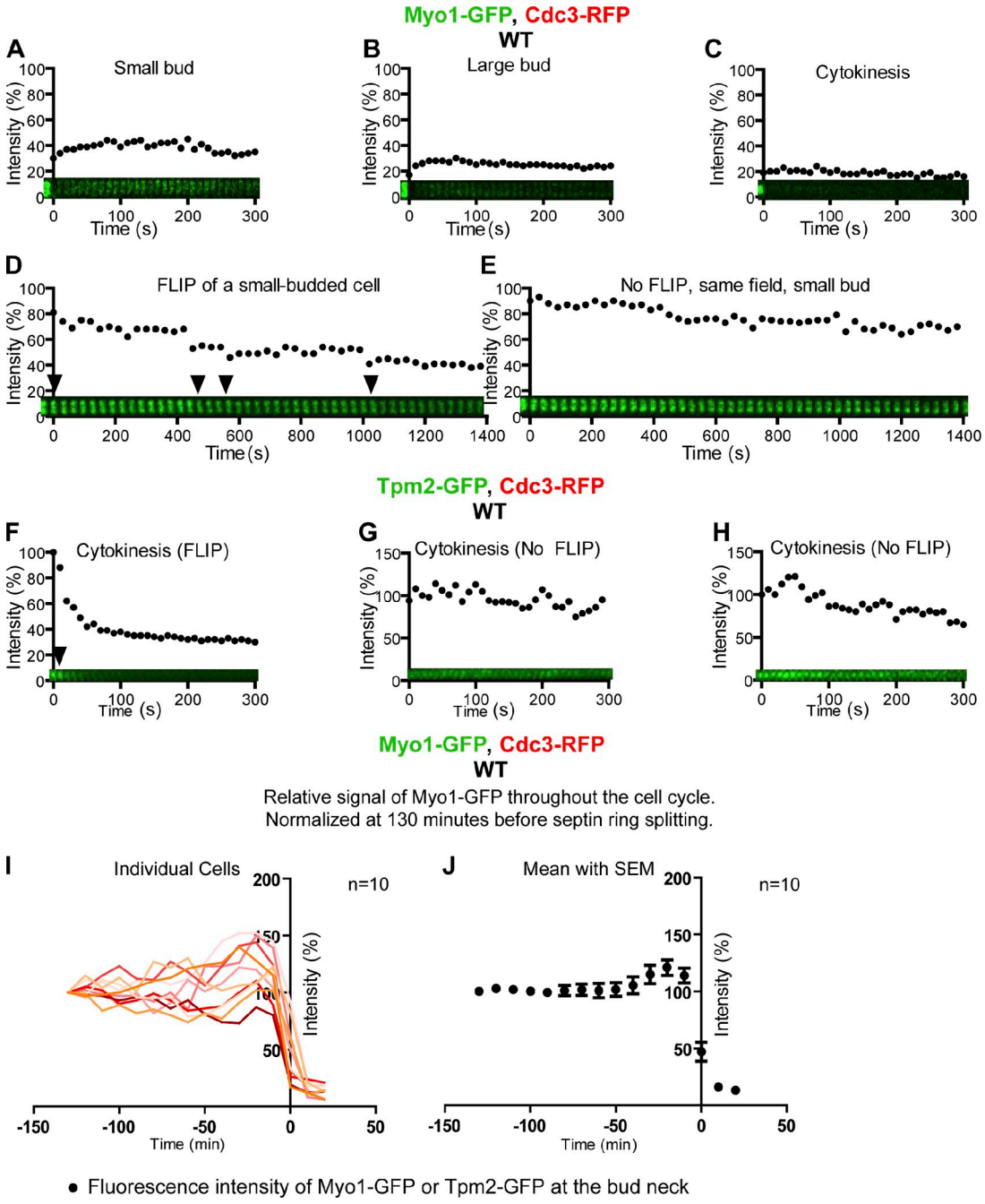


Figure S1. **Myo1 displays little cytosol–neck exchange, and its intensity at the bud neck remains relatively constant during the cell cycle.** (A–C) Full-ring bleaching of Myo1 at the division site in a small-budded cell (A), a large-budded cell (B), and a cell during cytokinesis (C). Strain XDY286 (*MYO1-GFP CDC3-RFP*) was grown in SC-Leu media to the exponential phase at 23°C, the full-ring of Myo1 at the division site was bleached in cells at different phases of the cell cycle, and fluorescence recovery was monitored over time (also see Video 1). (D and E). FLIP analysis of Myo1-GFP in a small-budded cell (D) and a comparable control cell (E) in the same imaged field. After sequential cytoplasmic bleaching, no significant decrease in the intensity of Myo1 at the bud neck was observed. Note that the intensity decreased to some extent immediately after the bleaching events (arrowheads), indicating that the scattering of the laser pulses may actually have bleached some of Myo1 at the neck region. The strain and growth condition were the same as described in A–C. (F–H) FLIP analysis of Tpm2-GFP in a cell undergoing cytokinesis (F) and two control cells (G and H) in the same imaged field. After a single cytoplasmic bleaching (arrowhead), the intensity of Tpm2-GFP at the bud neck decreased dramatically while displaying little or no change in the control cells. Note the difference in time scale (x axis) for the FLIP analysis of Myo1 and Tpm2. Strain YEF6197 (*TPM2-GFP CDC3-RFP*) was used, and the growth condition was the same as described in A–C. (I and J) The intensity of Myo1-GFP at the bud neck remains relatively constant during the cell division cycle. (I) The intensity of Myo1-GFP at the bud neck of individual cells of the strain XDY286 (*MYO1-GFP CDC3-RFP*) was followed by 3D spinning-disk microscopy from 130 min before the septin–hourglass splitting to the end of the AMR constriction, which roughly corresponds to the period from the small-budded stage to the end of cytokinesis. Time-lapse interval, 10 min (J). The mean and the SEM of the Myo1 intensity from I were calculated and plotted. WT, wild type.

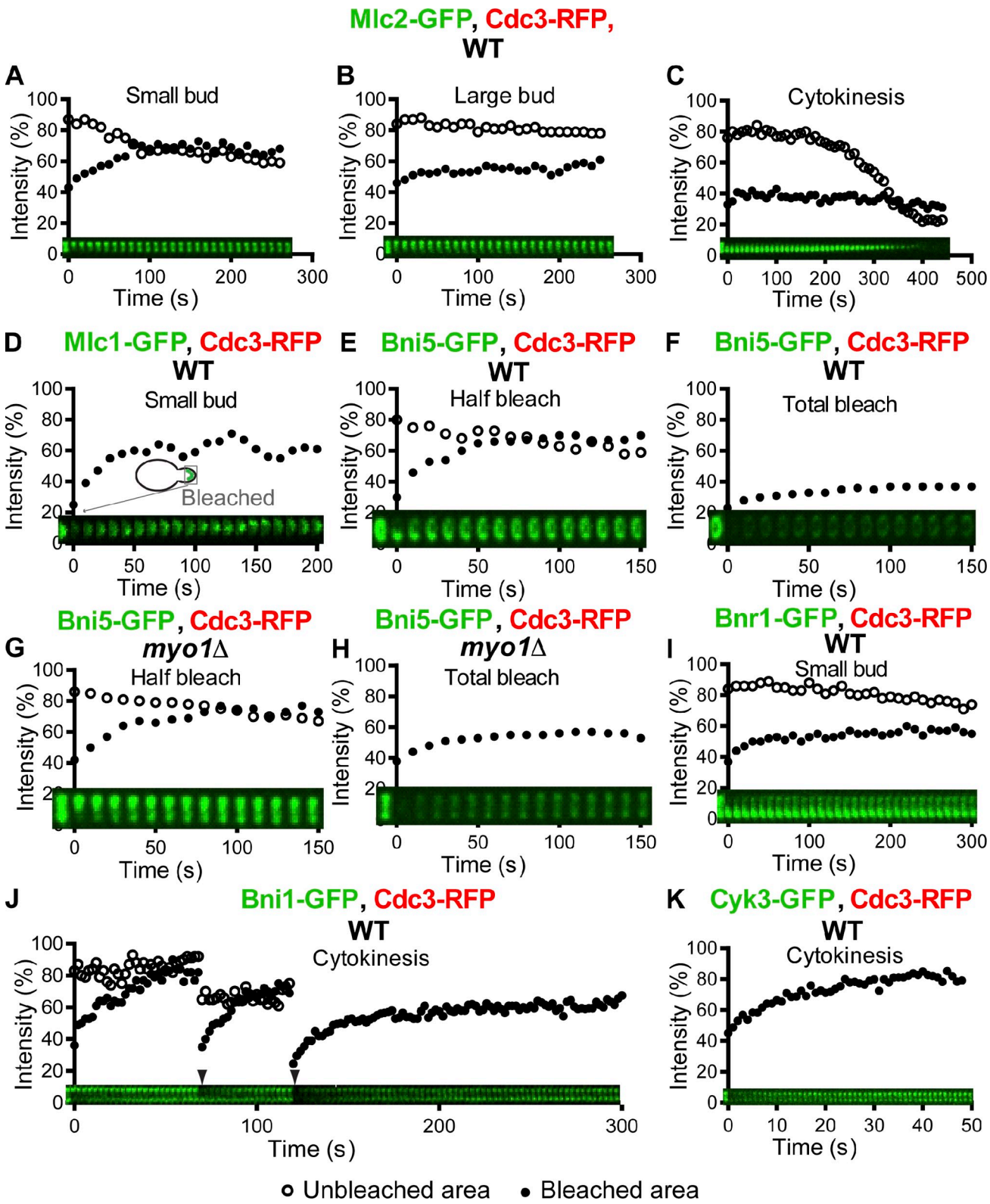


Figure S2. **Dynamics of Mlc2, Mlc1, Bni5, Bnr1, Bni1, and Cyk3 during the cell cycle.** (A–C) Mlc2 displays similar dynamics as Myo1 during the cell cycle. Mlc2-GFP in a small-budded cell (A), a large-budded cell (B), and a cell undergoing cytokinesis (C) of the strain YEF6069 (*MLC2-GFP CDC3-RFP*) was analyzed by FRAP. (D) Mlc1 at the bud cortex is dynamic during polarized bud growth. Strain: YEF6065 (*CDC3-RFP*, pUG34-MLC1). (E–H) Bni5 is mobile at the division site in the presence or absence of Myo1. Half-ring (E and G) and full-ring (F and H) bleaching of Bni5-GFP was performed on cells of strains YEF6899 (*BNI5-GFP CDC3-RFP*; wild type [WT]) and YEF6904 (*myo1Δ BNI5-GFP CDC3-RFP*; *myo1Δ*). (I) Bnr1 is relatively stable throughout its localization at the bud neck. Bnr1-GFP in small-budded and large-budded (unpublished data) cells of the strain YEF6135 (*CDC3-RFP*, pUG23-BNR1) was analyzed by FRAP. (J) Bni1 is highly dynamic during cytokinesis. Bni1-GFP in the strain YEF6134 (*CDC3-RFP*, YEp13-BNI1-GFP) was bleached sequentially (time 0 and arrowheads) during cytokinesis. (K) Cyk3 is dynamic during cytokinesis. Full-ring of Cyk3-GFP in strain YEF6130 (*CYK3-GFP CDC3-RFP*) was analyzed by FRAP. Note the differences in x-axis scaling.

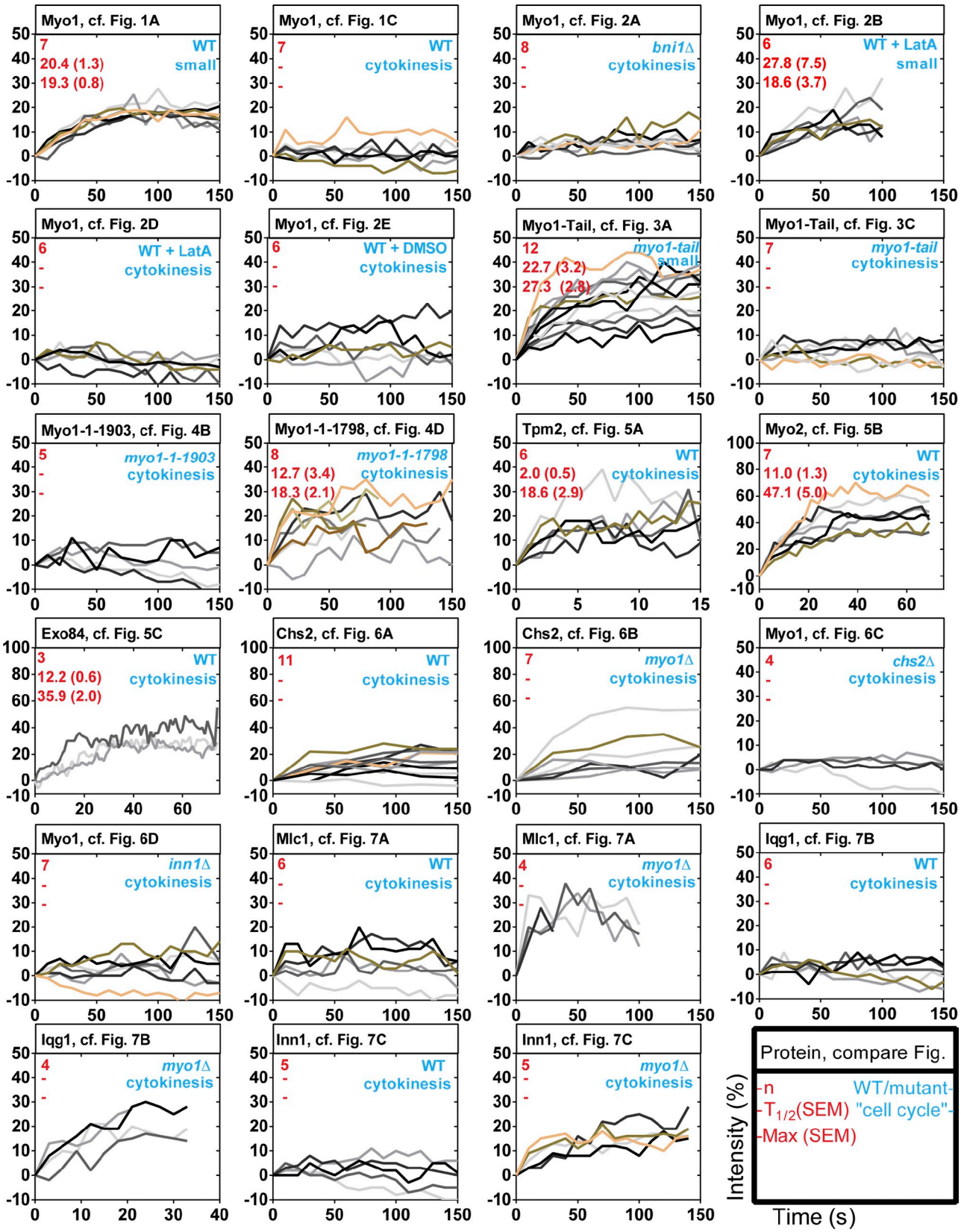


Figure S3. **Individual recovery curves and quantitative analyses for the indicated proteins.** The diagram at the bottom right denotes the information included in each FRAP plot. Red color indicates the number of cells analyzed (*n*), the time required for half of the maximal recovery ($t_{1/2}$ [±SEM]), the maximal recovery (Max [±SEM]), or no quantitative analysis because of little or no recovery or no need (−). Light blue indicates the genotypes of the strains (wild type [WT]/mutant) and the cell cycle stages (cell cycle). Only the traces of fluorescence intensities over the bleached areas are shown. For the purpose of comparison, the starting values on the y axis for all individual curves are set to 0 for all the FRAP plots. The relationships between the plots in this figure and those in the main figures are indicated at the top of each plot.

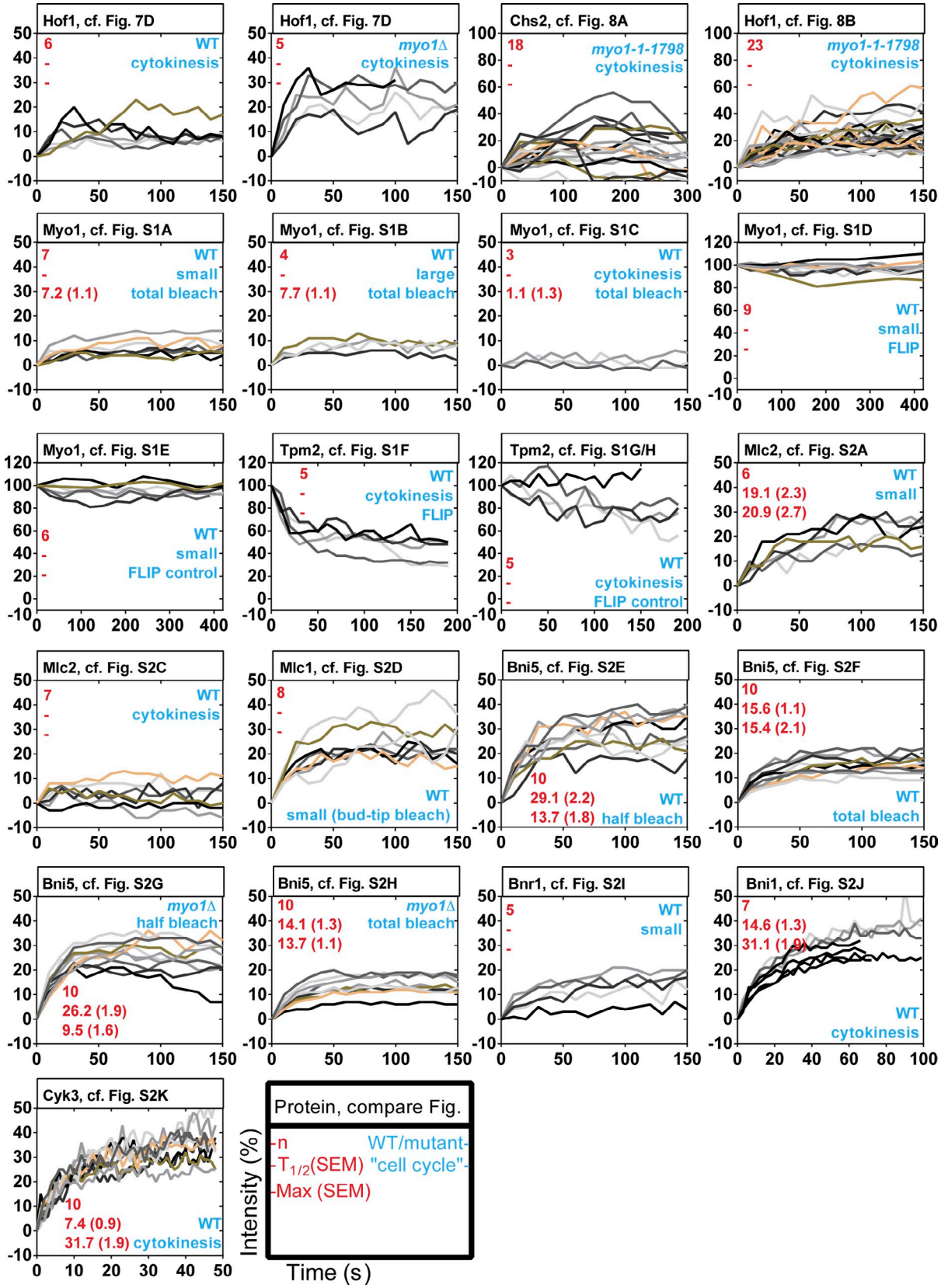
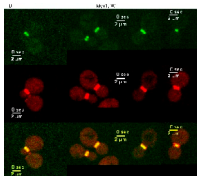
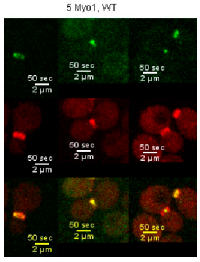


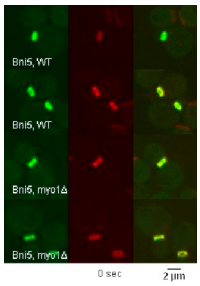
Figure S4. **Individual curves and quantitative analyses for the indicated proteins.** The diagram at the bottom right denotes the information included in each FRAP or FLIP plot. Red color indicates the number of cells analyzed (*n*), the time required for half of the maximal recovery ($t_{1/2}$ [±SEM]), the maximal recovery (Max [±SEM]), or no quantitative analysis because of little or no recovery or no need (−). Light blue indicates the genotypes of the strains (wild type [WT]/mutant) and the cell cycle stages (cell cycle). Only the traces of fluorescence intensities over the bleached areas in the FRAP experiments or the bud–neck areas in the FLIP experiments are shown. For the purpose of comparison, the starting values on the y axis for all individual curves are set to 0 for all the FRAP plots and to 100 for all the FLIP plots. The relationships between the plots in this figure and those in the main figures are indicated at the top of each plot.



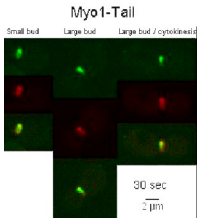
Video 1. **Full-ring bleaching of Myo1-GFP during the cell cycle.** Related to Fig. S1. Strain: XDY286 (*MYO1-GFP CDC3-RFP*). (left column) A small-budded cell; (middle two columns) large-budded cells; and (right column) a cell during cytokinesis. A 10-s time-lapse interval is shown. Green, Myo1-GFP; red, Cdc3-RFP.



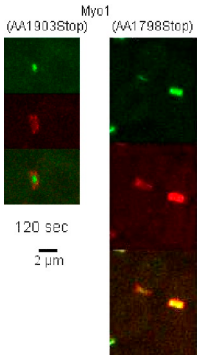
Video 2. **Half-ring bleaching of Myo1-GFP during the cell cycle.** Related to Fig. 1. Strain: XDY286 (*MYO1-GFP CDC3-RFP*). (left column) A small-budded cell; (middle column) a large-budded cell; and (right column) a cell undergoing cytokinesis. A 10-s time-lapse interval is shown. Green, Myo1-GFP; red, Cdc3-RFP.



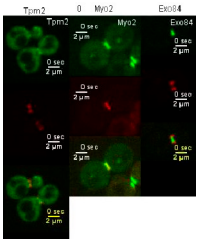
Video 3. **Half-ring and full-ring bleaching of Bni5-GFP in wild-type and *myo1Δ* cells before the onset of cytokinesis and the half-ring bleaching of Myo1-GFP in *bni1Δ* and LatA-treated cells.** (left, related to Fig. S2) Half-ring and full-ring bleaching of Bni5-GFP in wild-type and *myo1Δ* cells. Strains: YEF6899 (*BNI5-GFP CDC3-RFP*; wild type [WT]) and YEF6904 (*myo1Δ BNI5-GFP CDC3-RFP*; *myo1Δ*). A 10-s time-lapse interval is shown. Green, Bni5-GFP; red, Cdc3-RFP. (right, related to Fig. 2) Half-ring bleaching of Myo1-GFP in *bni1Δ* and LatA-treated cells. (left column) A cell undergoing cytokinesis of the strain YEF6116 (*bni1Δ MYO1-GFP CDC3-RFP*); (middle and right columns) a small-budded cell (middle) and a cell undergoing cytokinesis (right) of the LatA-treated strain XDY286 (*MYO1-GFP CDC3-RFP*). A 10-s time-lapse interval is shown. Green, Myo1-GFP; red, Cdc3-RFP.



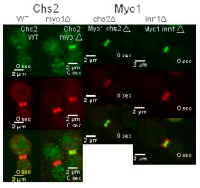
Video 4. **Half-ring bleaching of Myo1-Tail-GFP during the cell cycle.** Related to Fig. 3. Strain: XDY288 (*myo1-Tail-GFP CDC3-RFP*). (left column) A small-budded cell; (middle column) a large-budded cell; and (right column) a cell undergoing cytokinesis. A 10-s time-lapse interval is shown. Green, Myo1-Tail-GFP; red, Cdc3-RFP.



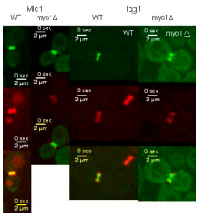
Video 5. **Half-ring bleaching of truncated Myo1-GFP proteins during cytokinesis.** Related to Fig. 4. (left column) Strain: YEF6617 (*myo1(AA1903Stop)-GFP CDC3-RFP*). (right column) A strain YEF6616 (*GFP-myo1(AA1798Stop) CDC3-RFP*). A 10-s time-lapse interval is shown. Green, truncated Myo1-GFP; red, Cdc3-RFP.



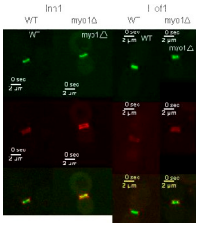
Video 6. **Full-ring bleaching of Tpm2-GFP, Myo2-GFP, and Exo84-GFP during cytokinesis.** Related to Fig. 5. (left column) strain YEF6197 (*TPM2-GFP CDC3-RFP*). A 1-s time-lapse interval is shown. Green, Tpm2-GFP; red, Cdc3-RFP. (middle column) Strain: YEF6001 (*MYO2-GFP*, pRS316-MYO1-mCherry). A 3-s time-lapse interval is shown. Green, Myo2-GFP; red, Myo1-RFP. (right column) Strain: YEF5862 (*EXO84-GFP CDC3-RFP*). A 1-s time-lapse interval is shown. Green, Exo84-GFP; red, Cdc3-RFP.



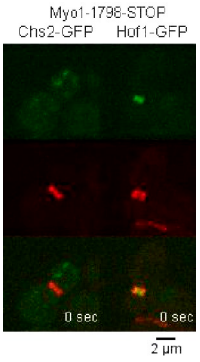
Video 7. **Half-ring bleaching of Chs2-GFP in wild-type and *myo1Δ* cells and of Myo1-GFP in *chs2Δ* and *inn1Δ* cells.** Related to Fig. 6. (first and second columns, counting from the left) Strains: YEF5874 (*CHS2-GFP CDC3-RFP*; first column) and YEF6336 (*myo1Δ CHS2-GFP CDC3-RFP*; second column). A 30-s time-lapse interval is shown. Green, Chs2-GFP; red, Cdc3-RFP. (third and fourth columns) Strains: YEF6273 (*chs2Δ MYO1-GFP CDC3-RFP*; third column) and YEF6230 (*inn1Δ MYO1-GFP CDC3-RFP*; fourth column). A 10-s time-lapse interval is shown. Green, Myo1-GFP; red, Cdc3-RFP. WT, wild type.



Video 8. **Half-ring and/or full-ring bleaching of Mlc1-GFP and Iqg1-GFP in wild-type and *myo1Δ* cells during cytokinesis.** Related to Fig. 7. (first and second columns, counting from the left) Strains: YEF6065 (*CDC3-RFP*, pUG34-MLC1; first column) and YEF6351 (*myo1Δ CDC3-RFP*, pUG34-MLC1; second column). Note that YEF6065 carries an *ade2* mutation, which presumably accounts for the red appearance of the vacuoles. A 10-s time-lapse interval is shown. Green, Mlc1-GFP; red, Cdc3-RFP. (third and fourth columns) Strains: YEF6140 (*CDC3-RFP*, pUG35-IQG1; third column) and YEF6356 (*myo1Δ CDC3-RFP*, pUG35-IQG1 fourth column). 10- and 3-s time-lapse intervals are shown for YEF6140 and YEF6356, respectively. Green, Iqg1-GFP; red, Cdc3-RFP. WT, wild type.



Video 9. **Half-ring and full-ring bleaching of Inn1-GFP and Hof1-GFP in wild-type and *myo1Δ* cells during cytokinesis.** Related to Fig. 7.(first and second columns, counting from the left) Strains: YEF6138 (*INN1-GFP CDC3-RFP*; first column) and YEF6357 (*myo1Δ INN1-GFP CDC3-RFP*; second column). (third and fourth columns) Strains: YEF6131 (*HOF1-GFP CDC3-RFP*; third column) and YEF6358 (*myo1Δ HOF1-GFP CDC3-RFP*; fourth column). A 10-s time-lapse interval is shown for all strains. Green, Inn1-GFP or Hof1-GFP; red, Cdc3-RFP.



Video 10. **Half-ring bleaching of Chs2-GFP and Hof1-GFP in *myo1-(AA1798Stop)* cells during cytokinesis.** Related to Fig. 8. (left column) Strain: YEF6771 (*myo1-(AA1798Stop) CHS2-GFP CDC3-RFP*). (right column) Strain: YEF6769 (*myo1-(AA1798Stop) HOF1-GFP CDC3-RFP*). 30- and 10-s time-lapse intervals are shown for YEF6771 and YEF6769, respectively. Green, Chs2-GFP or Hof1-GFP; red, Cdc3-RFP.