

Supplemental Materials

Molecular Biology of the Cell

Wei et al.

Supplementary Material

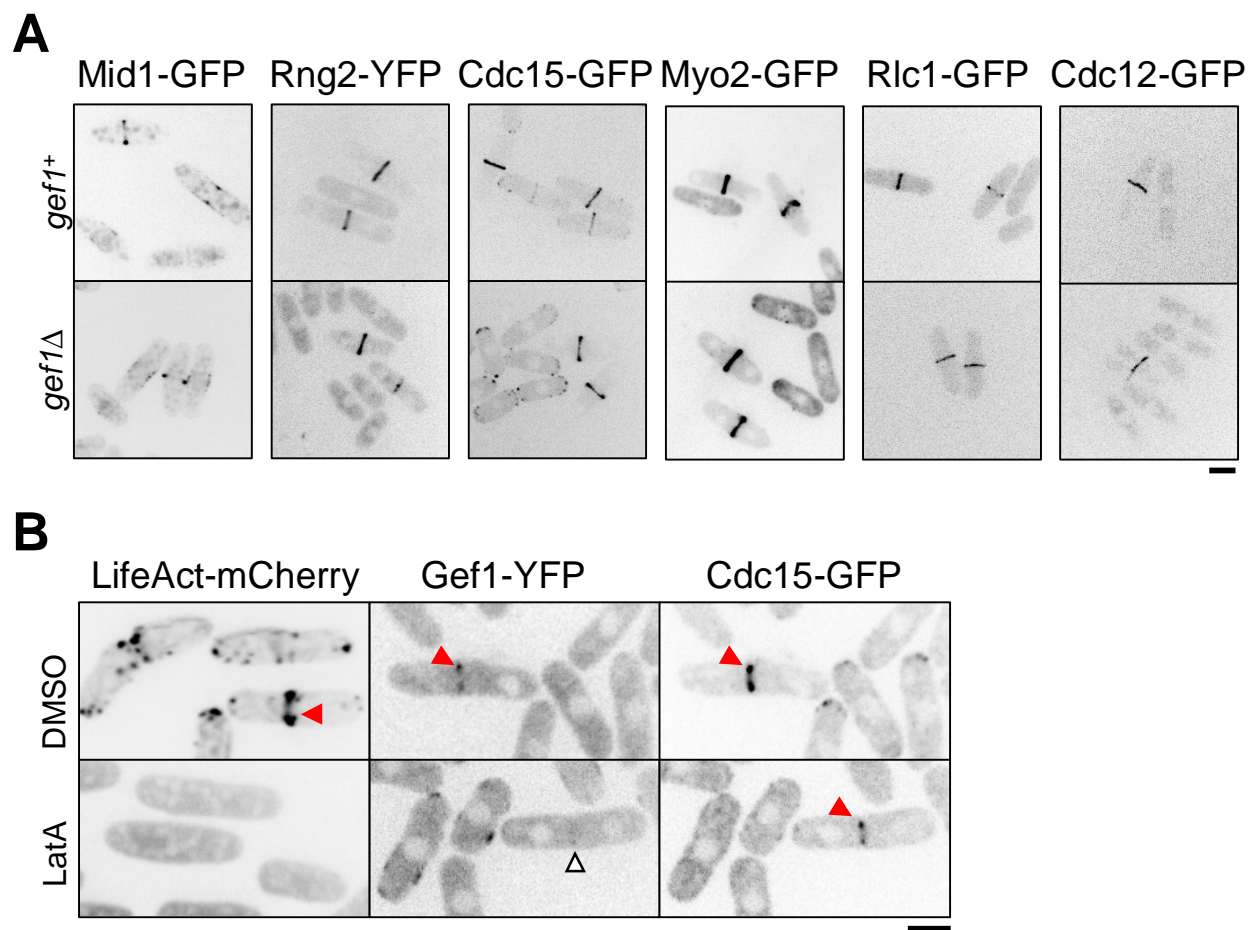


Figure S1: A. Gef1 is not required for recruitment of actomyosin ring assembly proteins. Localization of Mid1-GFP, Rng2-YFP, Cdc15-GFP, Myo2-GFP, Rlc1-GFP and Cdc12-GFP in *gef1*⁺ and *gef1*Δ cells were observed at the site of cell division. **B. Gef1 localization to the division site is actin dependent.** Cell expressing LifeAct-mCherry or Gef1-3xYFP and Cdc15-GFP treated with DMSO and 100 μM of Latrunculin A (LatA) for 30 mins. Red arrowheads indicate the presence and white arrowheads indicate absence of signals at cell division site. Bars, 5 μm.

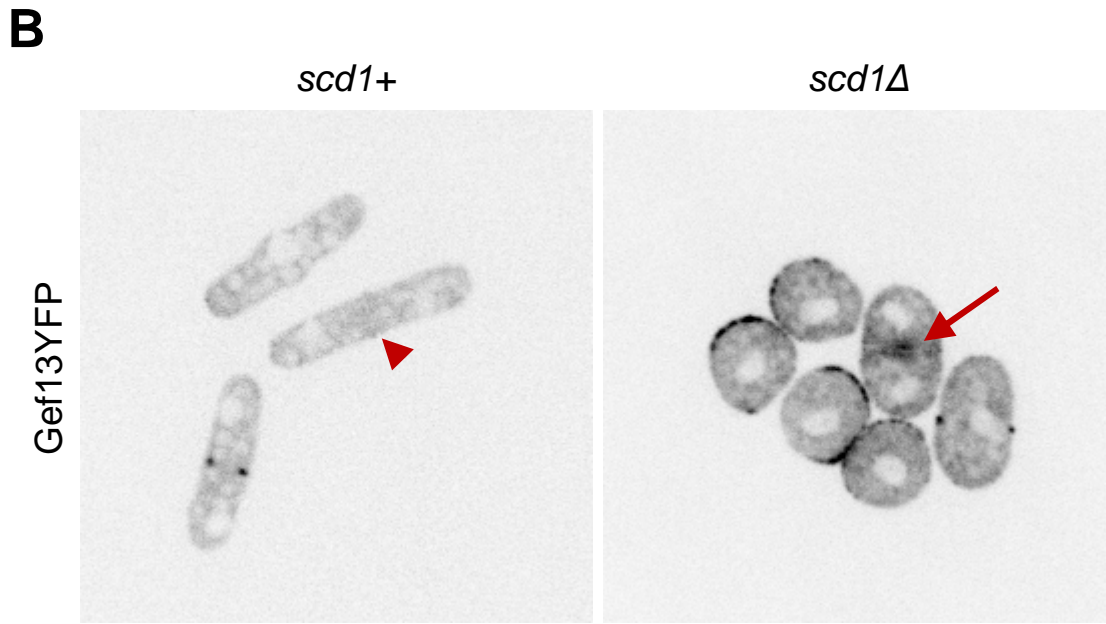
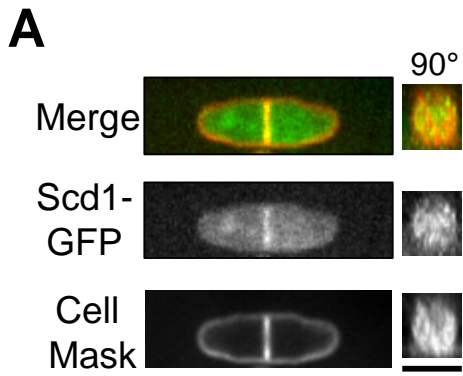


Figure S2 : (A) Scd1 localizes to the ingressing membrane during cytokinesis. Scd1-GFP expressing cells were stained with the membrane dye Cell Mask. 3D reconstructed cells division site is shown after 90° rotation. **(B) Scd1 is required for proper Gef1 localization at the division site and cell cortex.** Gef1-3xYFP expressing *scd1+* and *scd1Δ* cells were imaged. Red arrowhead shows loss of Gef1-3xYFP signal at the site of cell division post ring constriction. Red arrow shows Gef1-3xYFP at the division site post ring constriction. Gef1-3xYFP signal in non-dividing cells is more depolarized and randomly distributed at the cell cortex.

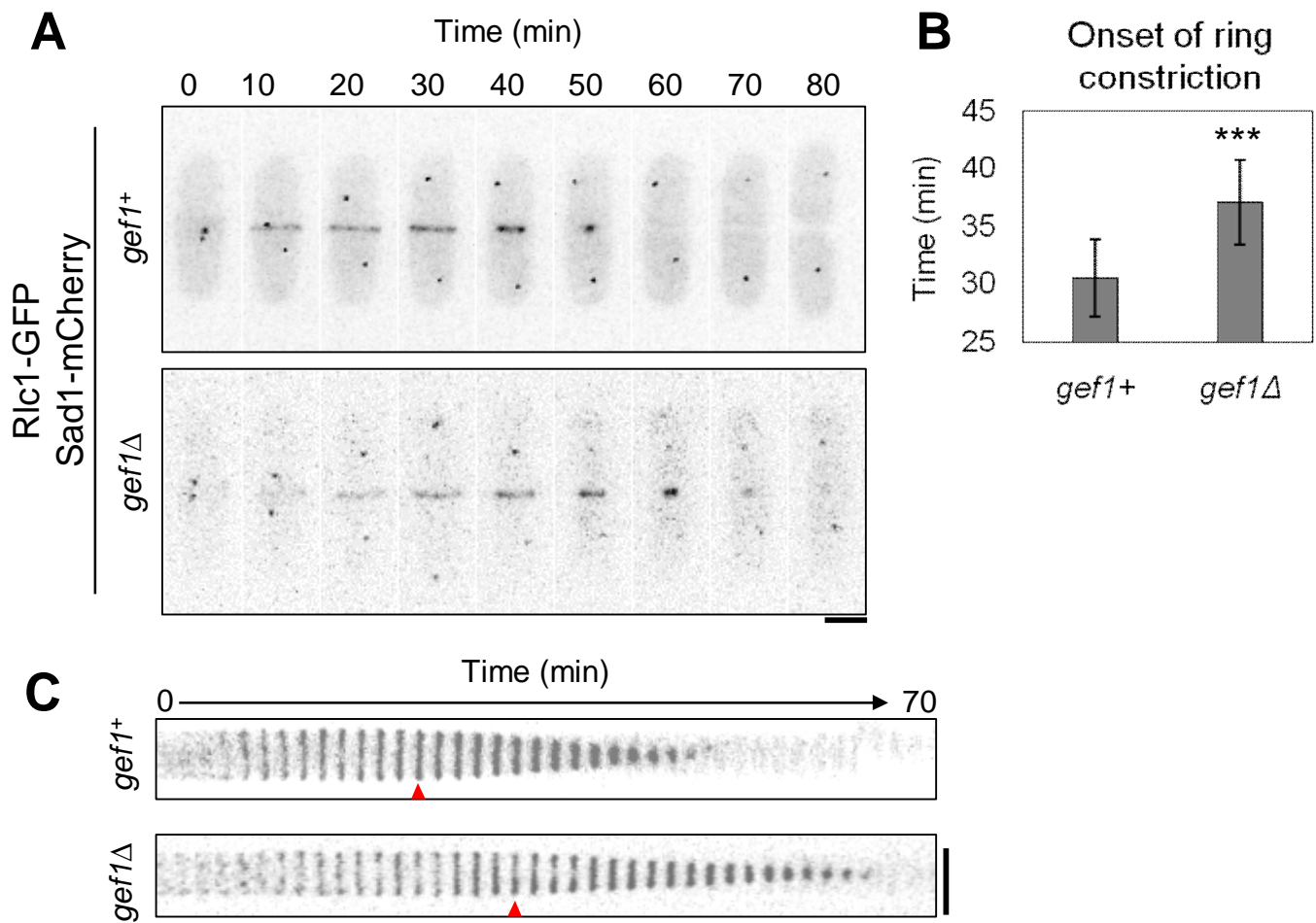


Figure S3: Gef1 is required for timely onset of actomyosin ring constriction. **A.** Actomyosin ring protein Rlc1-GFP and spindle pole body marker Sad1-mCherry observed over time in *gef1+* and *gef1Δ* cells at the site of cell division during cytokinesis. **B.** Quantification of onset of ring constriction in *gef1+* and *gef1Δ* cells. Student's t-test, *** $p < 0.001$. **C.** Kymograph of Rlc1-GFP at the actomyosin ring overtime since onset of spindle pole body separation. Scale bar, 5 μ m. m, minutes.

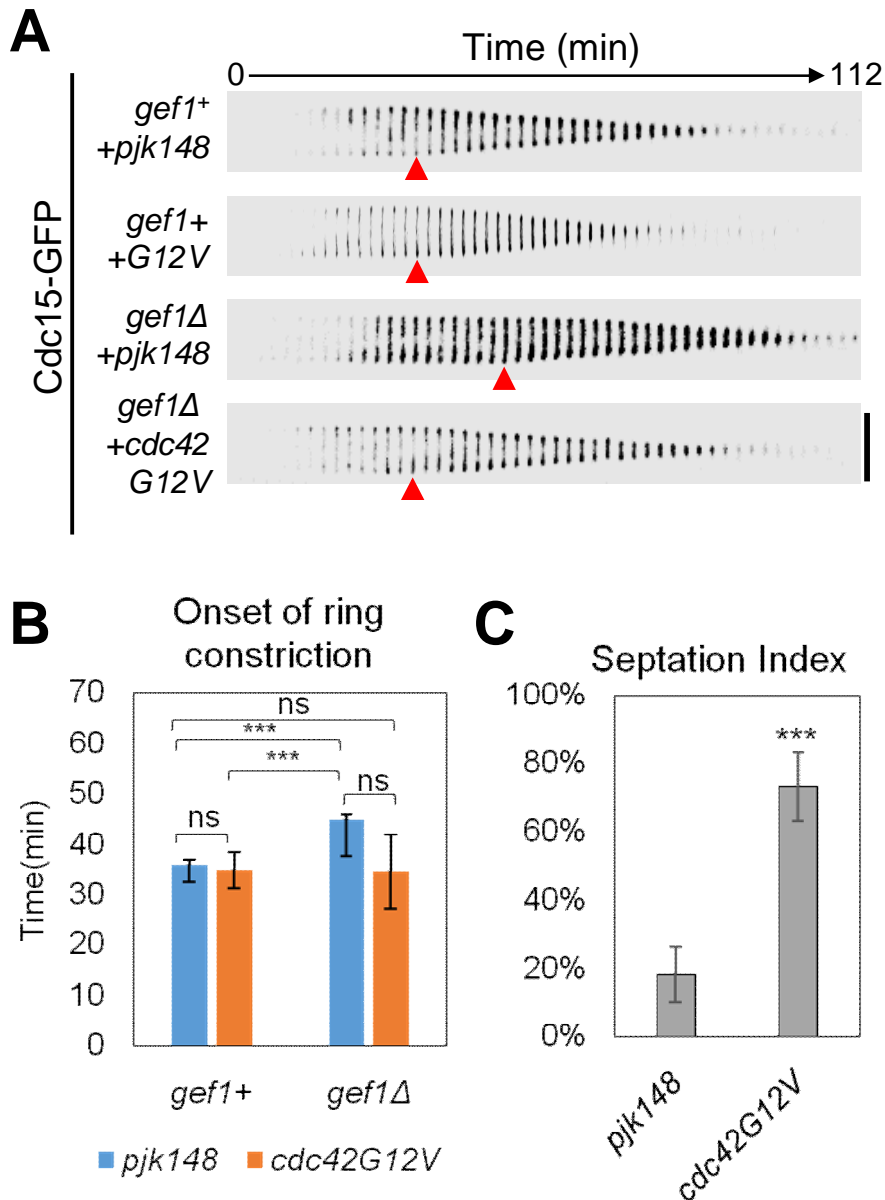


Figure S4: Constitutively active Cdc42 rescues delay in onset of ring constriction in *gef1Δ* cells. (A) Kymographs of cytokinetic ring represented by Cdc15-GFP in indicated cells.

Red arrowheads- onset of cytokinetic ring constriction. Bars, 5 μ m. (B) Quantification of onset of cytokinetic ring constriction as shown in A. $n > 16$ cells. (C) Septation index of control and Cdc41G12V overexpressing cells. $n > 16$. ***, $p < 0.001$. ns, not significant. Error bars, SD.

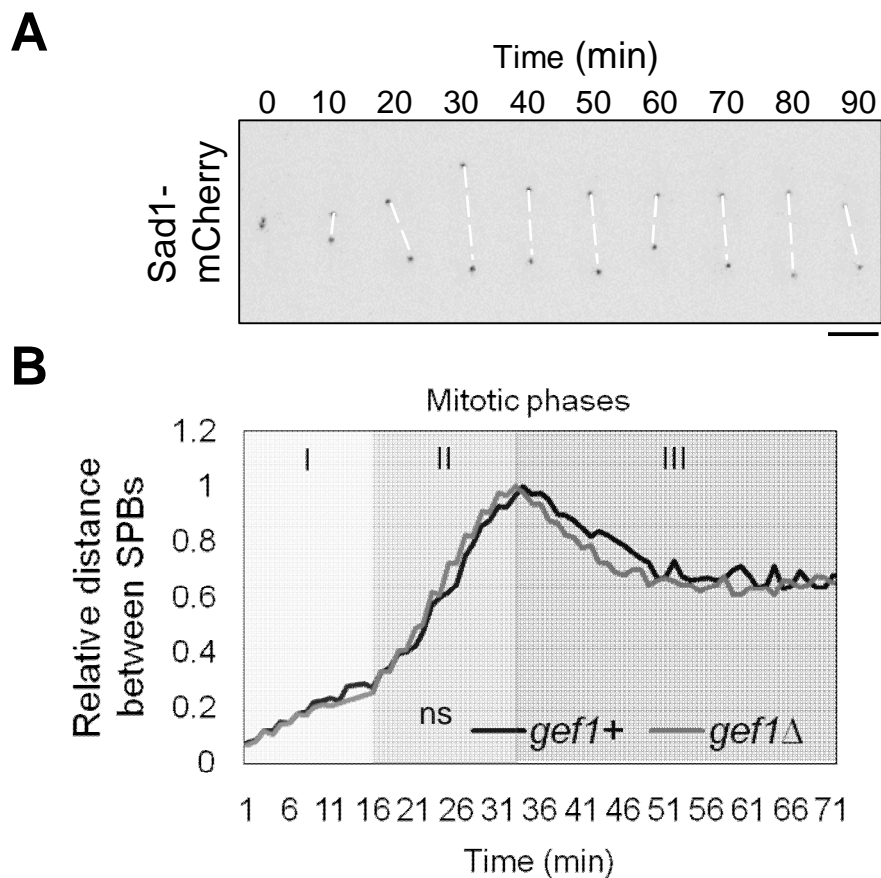


Figure S5: Gef1 does not affect mitotic events. **A.** SPB dynamics since onset of anaphase by Sad1-mCherry. White dashed lines indicate the distance between the two SPBs each time point. Bar, 5 μ m. **B.** Quantification of the distance between two spindle pole bodies over time in *gef1+* and *gef1* Δ cells. $n > 14$ cells. Three stages are defined by SPBs' distance: I, Anaphase A; II, Anaphase B; III, Telophase. ns, not significant. m, minutes.

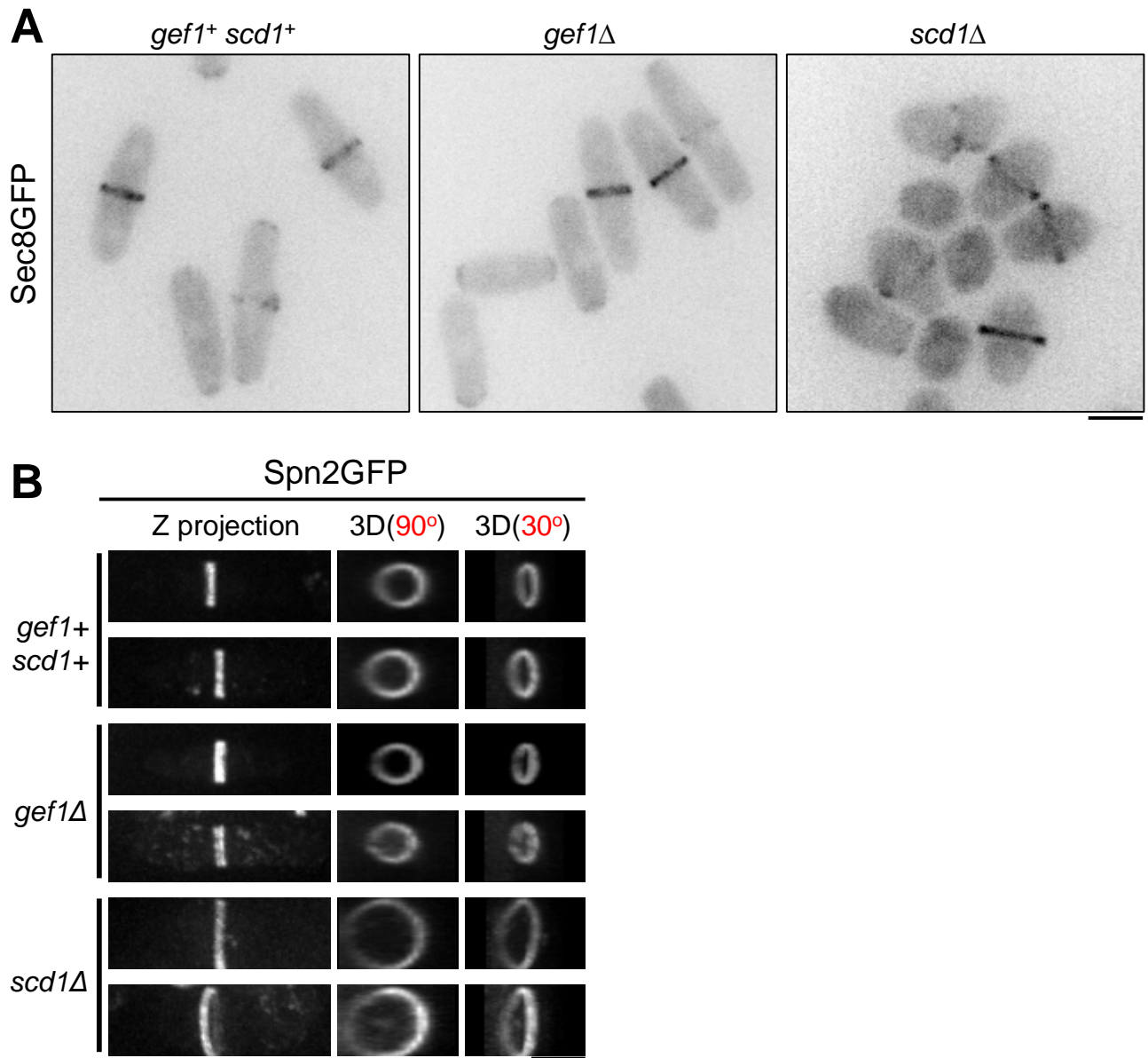


Figure S6. Gef1 and Scd1 do not affect the localization of the Exocyst complex or the septin ring during cytokinesis. (A) Cells as indicated expressing the exocyst protein Sec8-GFP were imaged. (B) Indicated cells expressing the septin ring protein Spn2-GFP were analyzed during cytokinesis. 3D reconstructed rings rotated at 90° and 30° are shown. Bars, 5 μ m

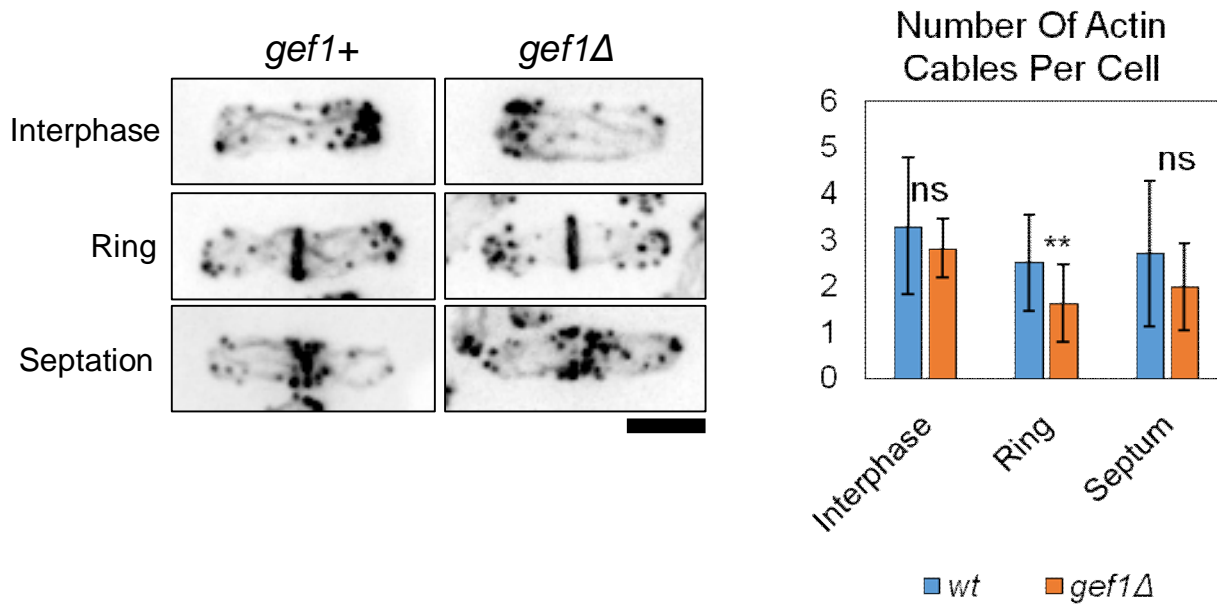


Figure S7: Gef1 is required for non-medial actin cables during cytokinesis.

Quantification of actin cables observed in Alexa-fluor phalloidin stained *gef1+* and *gef1Δ* cells during interphase, ring phase of cytokinesis and during the septum phase. $n > 10$. **, $p = 0.004$. ns, not significant. Error bars, SD.

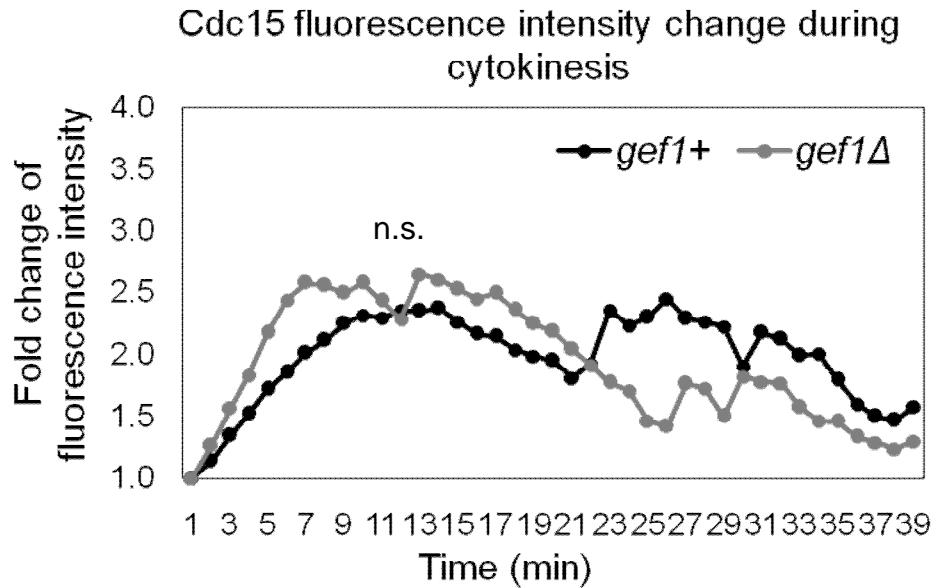


Figure S8: Gef1 does not affect the recruitment of the actomyosin ring protein Cdc15 during ring maturation. Quantification of fold increase in Cdc15-GFP levels at the cell division site in *gef1+* and *gef1Δ* cells over time. Time 0 is onset of maturation phase as determined by the first appearance of a fully assembled Cdc15-GFP ring. Student's t-test; ns, not significant. min, minutes.

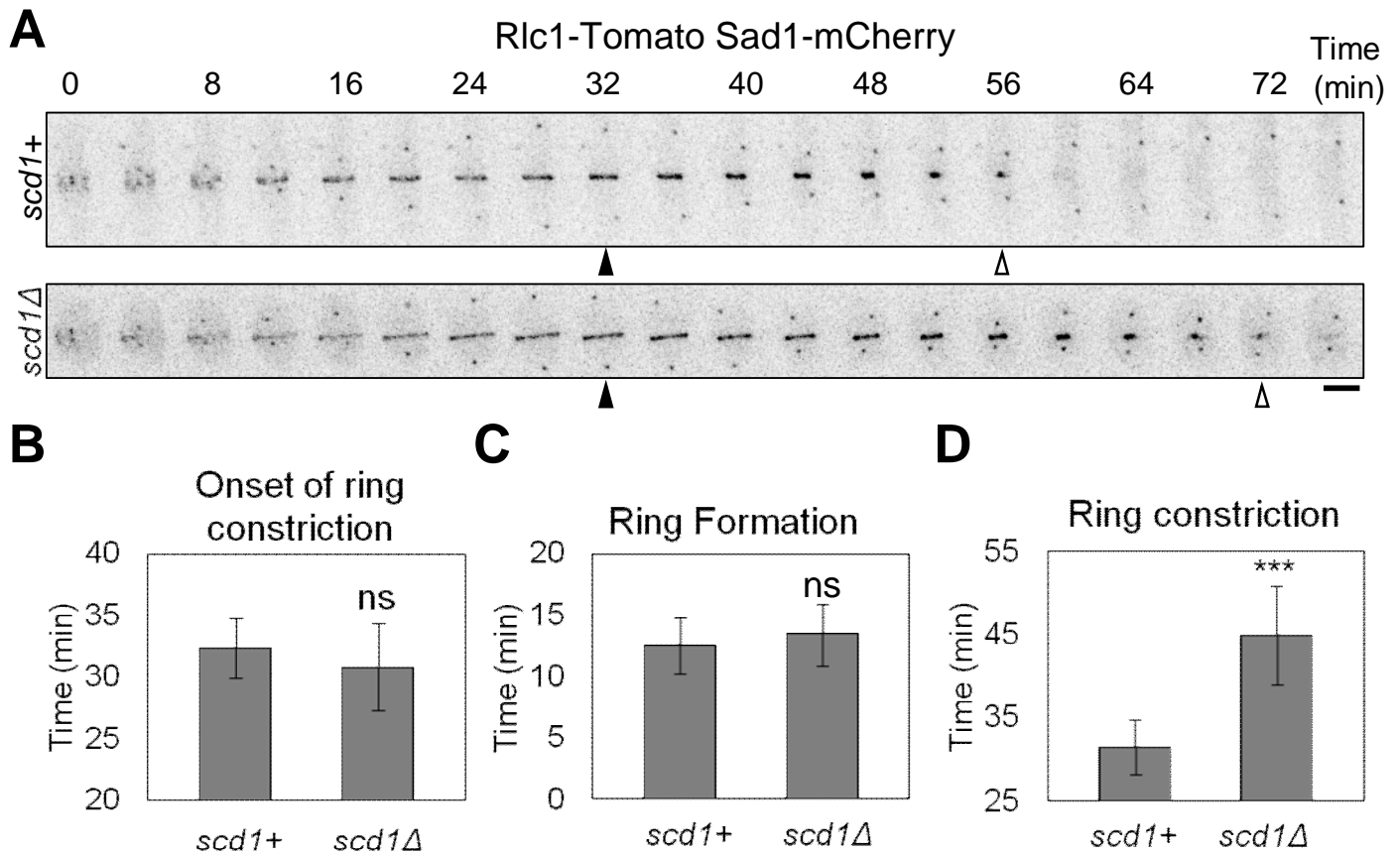


Figure S9: Scd1 is not required for timely onset of actomyosin ring constriction. (A)

Actomyosin ring protein Rlc1-GFP and spindle pole body marker Sad1-mCherry observed over time in *scd1+* and *scd1Δ* cells at the site of cell division during cytokinesis. Bar, 5 μ m. **(B)** Quantification of onset of ring constriction in *scd1+* and *scd1Δ* cells. **(C)** Quantification of actomyosin ring assembly in *scd1+* and *scd1Δ* cells. ns, not significant. **(D)** Quantification of duration of actomyosin ring constriction in *scd1+* and *scd1Δ* cells. ***, $p < 0.0001$. min, minutes.

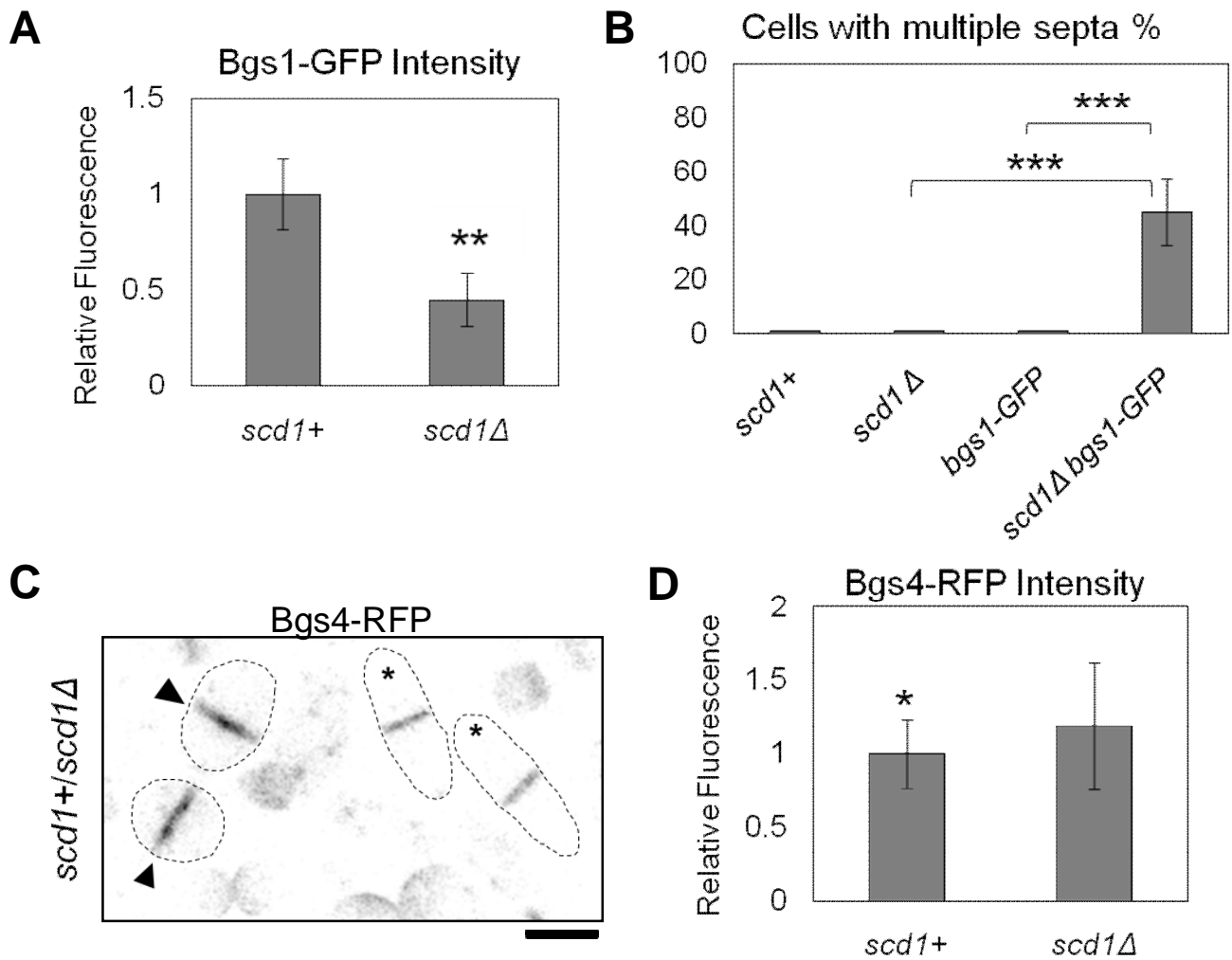


Figure S10. Scd1 is required for normal septum formation. (A) Quantification of Bgs1-GFP levels at the cell division site in *scd1+* and *scd1Δ* cells as shown in Figure 8A. $n>23$ **, $p=0.0014$. (B) Quantification of multi-septated cells over total septated cells in indicated cells as shown in Figure 8B. $n>286$. ***, $p=0.0001$. (C) Cells expressing Bgs4-RFP in *scd1+* and *scd1Δ* cells analyzed in the same field. *scd1+* cells are depicted by asterisks and *scd1Δ* cells are depicted by arrowheads. Bars, 5 μ m (D) Quantification of Bgs4-RFP levels at the cell division site in the indicated cells as shown in C. $n>21$. **, $p=0.04$. Error bars, SD.

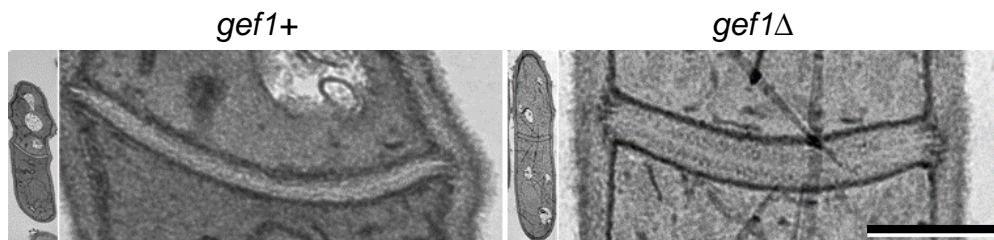


Figure S11. Loss of *gef1* does not show septum morphology defects but shows thicker septum. Electron microscopy images of *gef1*⁺ and *gef1* Δ cells. Bar, 1 μ m.

SUPPLEMENTARY MOVIE LEGENDS

Movie S1: **Spatiotemporal localization of CRIB-3XGFP during cytokinesis.** Left panel shows CRIB-3XGFP. Right panel shows cytokinetic ring marker Rlc1-Tomato and SPB marker Sad1-mCherry. Frame interval is 2 minute. Frame rate is 2 fps.

Movie S2: **Spatiotemporal localization of CRIB-3XGFP in *gef1Δ* cells during cytokinesis.** Left panel shows CRIB-3XGFP. Right panel shows cytokinetic ring marker Rlc1-Tomato and SPB marker Sad1-mCherry. Frame interval is 2 minute. Frame rate is 2 fps.

Movie S3: **Spatiotemporal localization of CRIB-3XGFP in *scd1Δ* cells during cytokinesis.** Left panel shows CRIB-3XGFP. Right panel shows cytokinetic ring marker Rlc1-Tomato and SPB marker Sad1-mCherry. Frame interval is 2 minute. Frame rate is 2 fps.

Movie S4: **Loss of Gef1 delays onset of ring constriction.** Cdc15-GFP shows the cytokinetic ring in the cell division site in upper panel. Sad1-mCherry as SPB marker is shown in the bottom panel. Left panel shows a *gef1⁺* cell, right panel shows a *gef1Δ* cell. Both *gef1⁺* and *gef1Δ* movies start from initial SPB separation. Frame interval is 2 minute. Frame rate is 2 fps.

Movie S5: **Bgs1 recruitment to the division site is delayed in *gef1Δ* cells.** Bgs1-GFP at the cell division site is shown in upper panel. Bottom panel shows cytokinetic ring marker Rlc1-Tomato and SPB marker Sad1-mCherry. Left panel shows a *gef1⁺* cell, right panel is a *gef1Δ* cell. Both *gef1⁺* and *gef1Δ* movies start playing from separation of SPBs. Sad1-mCherry as SPB marker was used. Frame interval is 2 minute. Frame rate is 2 fps.

Movie S6: **Loss of Scd1 delays ring constriction but not onset of ring constriction.** Rlc1-Tomato shows the cytokinetic ring in the cell division site. Sad1-mCherry represents SPBs separation. Left panels show bright field images. Right panels shows both Rlc1-Tomato and Sad1-mCherry. Upper panel shows a *scd1⁺* cell, bottom panel shows a *scd1Δ* cell. Both *scd1⁺*

and *scd1Δ* movies start playing from separation of SPBs. Frame interval is 2 minute. Frame rate is 2 fps.

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

Strain	Genotype	Source
PN972	<i>h⁻</i>	<i>P. Nurse</i>
PPG5660	<i>h⁺ cdc42-1625-kanMX leu1-32 ura4-D18</i>	(<i>Martin et al., 2007</i>)
YMD208	<i>cdc15-Tomato-NAT^r gef1-3xYFP-kanMX6 ade6-M216/704 his7⁺</i>	<i>This study</i>
YMD373	<i>cdc15-Tomato-NAT^r scd1-3xGFP-kanMX ad6-m216 leu1-32 ura4-d18 his7⁺</i>	<i>This study</i>
MBY6843	<i>h⁺ LifeAct-mCherry: leu⁺ ade6-M216 leu1-32 ura4-D18</i>	(<i>Huang et al., 2012</i>)
YMD317	<i>CRIB-GFP-ura4⁺ rlc1-Tomato-NAT^r sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 his7⁺</i>	<i>This study</i>
YMD488	<i>Δgef1::ura4⁺ CRIB-GFP-ura4⁺,rlc1-Tomato-NAT^r sad1-mCherry:kanMX ura4-D18 leu1-32 his7⁺</i>	<i>This study</i>
YMD530	<i>scd1::ura4⁺ CRIB-GFP-ura4⁺ rlc1-Tomato-NAT^r sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 his7⁺</i>	<i>This study</i>
YMD133	<i>cdc15-GFP: kanMX6 sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18</i>	<i>This study</i>
YMD131	<i>Δgef1::ura4⁺ cdc15-GFP: kanMX6 sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18</i>	<i>This study</i>
YMD542	<i>Δgef1::ura4⁺ bgs1D::ura4 Pbgs1::GFP-bgs1:leu1⁺ Rlc1-Tomato-NAT^r Sad1-mCherry:kanMX leu1-32 ura4-D18</i>	<i>This study</i>
YMD546	<i>bgs1D::ura4 Pbgs1::GFP-bgs1:leu1⁺ Rlc1-Tomato-NAT^r Sad1-mCherry:kanMX leu1-32 ura4-D18</i>	<i>This study</i>
YMD498	<i>cdc15-GFP: kanMX6 sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 pj148-nmt41x-leu1⁺</i>	<i>This study</i>
YMD473	<i>Δgef1::ura4⁺ cdc15-GFP: kanMX6 sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 pj148-nmt41x-leu1⁺</i>	<i>This study</i>
YMD474	<i>Δgef1::ura4⁺ cdc15-GFP: kanMX6 sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 pj148-nmt41x-cdc42G12V-leu1⁺</i>	<i>This study</i>
PPG2601	<i>h⁺ Δgef1::ura4⁺ ura4-D18 leu1-32</i>	(<i>Coll et al., 2003</i>)
KLG2955	<i>h⁻ mid1Δ::ura4⁺</i>	<i>K. Gould</i>
YMD377	<i>Δgef1::ura4⁺ mid1Δ::ura4⁺ ura4-D18 leu1-32</i>	<i>This study</i>
KGY3019	<i>h⁺ cdc15-GFP: kanMX6 ade6-M210 leu1-32 ura4-D18</i>	(<i>Carnahan and Gould, 2003</i>)
YMD61	<i>Δgef1::ura4⁺ cdc15-GFP: kanMX6 ade6-M210 leu1-32 ura4-D18</i>	<i>This study</i>

PN527	<i>h⁻ ade6-M210 leu1-32 his3-D1 ura4-D18</i>	P. Nurse
FV242+	<i>h⁹⁰ leu1 ura4-d18 ade6 h210 scd1::ura4</i>	(Li et al., 2000)
PPG3750	<i>h⁻ bgs1D::ura4 Pbgs1::GFP-bgs1:leu1 leu1-32 ura4-D18 hys3-D1</i>	(Cortes et al., 2002)
YMD501	<i>h⁹⁰ scd1::ura4 bgs1D::ura4 Pbgs1::GFP-bgs1:leu1⁺ leu1-32 ura4-D18 hys3-D1</i>	This study
PPG4608	<i>h⁻ bgs4::ura4 Pbgs4::GFP-bgs4:leu1 leu1-32 ura4-D18 hys3-D1</i>	(Cortes et al., 2005)
YMD504	<i>h⁹⁰ scd1::ura4⁺ bgs4::ura4 Pbgs4::GFP-bgs4:leu1 leu1-32 ura4-D18 hys3-D1</i>	This study
YMD64	<i>Δgef1::ura4⁺ mid1-GFP ura4-D18 leu1-32</i>	This study
YDM403	<i>h⁻ mid1-GFP:kanMX6 ade6-M210 leu1-32 ura4-D18</i>	(Bahler et al., 1998)
YMD76	<i>Δgef1::ura4⁺ KanMX::YFP-rng2 ade6-M21X ura4-D18 leu1-32</i>	This study
JW937	<i>h⁻ kanMX6-Prng2-mYFP-rng2 ade6-M210 leu1-32 ura4-D18</i>	(Wu et al., 2003)
KG Y3019	<i>cdc15-GFP::kanMX, ade6-m210 ura4-d18 leu1-32</i>	(Carnahan and Gould, 2003)
YMD183	<i>Δgef1::ura4⁺ kanMX-GFP-myo2 ade6-M210 ura4-D18 leu1-32</i>	This study
JW766	<i>kanMX-GFP-myo2 ade6-M210 leu1-32 ura4-D18</i>	(Wu et al., 2003)
YMD156	<i>Δgef1::ura4⁺ rlc1-GFP:ura4⁺ ade6-M210 ura4-D18 leu1-32</i>	This study
KG Y1278	<i>h⁺ rlc1-GFP:ura4⁺ ade6-M210 leu1-32 ura4-D18</i>	(Naqvi et al., 2000)
YMD204	<i>Δgef1::ura4⁺ rlc1-GFP:ura4⁺ sad1-mCherry:kanMX ade6-M210 ura4-D18 leu1-32</i>	This study
YMD165	<i>rlc1-GFP:ura4⁺ sad1-mCherry:kanMX ade6-M210 ura4-D18 leu1-32</i>	This study
YMD275	<i>bgs4::ura4 Pbgs4::RFP-bgs4:leu1 cdc11-GFP:ura4⁺ ade6-M210 leu1-32 ura4-D18</i>	This study
YMD288	<i>Δgef1::ura4⁺ bgs4::ura4 Pbgs4::GFP-bgs4:leu1 rlc1-GFP:ura4⁺ ade6-M210 leu1-32 ura4-D18</i>	This study
YMD 457	<i>Δgef1::ura4⁺ bgs1D::ura4 Pbgs1::GFP-bgs1:leu1 leu1-32 ura4-D18 hys3-D1</i>	This study
YMD590	<i>myo52GFP sad1-mCherry:kanMX ade6-M210 ura4-D18 leu1-32</i>	This study
YMD594	<i>Δgef1::ura4⁺ myo52GFP sad1-mCherry:kanMX ade6-M210 ura4-D18 leu1-32</i>	This study
YMD 600	<i>bgs4::ura4 Pbgs4::GFP-bgs4:leu1 leu1-32 ura4-D18 hys3-</i>	This study

	<i>D1 cdc11-GFP:kanR ade6-M21x</i>	
YMD617	<i>Δgef1::ura4⁺ bgs4::ura4 P bgs4::GFP-bgs4:leu1 leu1-32 ura4-D18 hys3-D1 cdc11-GFP:kanR ade6-M21x</i>	<i>This study</i>
FV1405	<i>scd1::ura4⁺ Gef13yfp</i>	<i>This study</i>
YMD566	<i>Pjk148cdc42g12v cdc15gfp sad1-mCherry:kanMX ade6-M210 ura4-D18 leu1-32</i>	<i>This study</i>
YMD606	<i>Pjk148 ade6-M210 leu1-32 his3-D1 ura4-D18</i>	<i>This study</i>
YMD610	<i>Pjk148-nmt41-cdc42G12V ade6-M210 leu1-32 his3-D1 ura4-D18</i>	<i>This study</i>
YSM1720	<i>sec8-GFP-ura4⁺</i>	<i>Sophie Martin</i>
YMD84	<i>Δgef1::ura4⁺ sec8-GFP-ura4⁺</i>	<i>This study</i>
YMD508	<i>scd1::ura4⁺ sec8-GFP-ura4⁺</i>	<i>This study</i>
Spn2GFP	<i>Spn2-GFP::KanR Leu 1-32, ura4-D18, ade 6-m210 h-</i>	<i>(An et al., 2004)</i>
YMD613	<i>Δgef1::ura4⁺ Spn2-GFP::KanR Leu 1-32, ura4-D18, ade6-m210</i>	<i>This study</i>
YMD615	<i>scd1::ura4⁺ Spn2-GFP::KanR Leu 1-32, ura4-D18, ade6-m210</i>	<i>This study</i>
YMD527	<i>rlc1-tomato-NATr ade6-M21X leu1-32 his7+ sad1-mCherry:kanMX ura4-D18</i>	<i>This study</i>
YMD625	<i>scd1::ura4⁺ rlc1-tomato-NATr ade6-M216 leu1-32 ura4-D18 his7+ sad1mcherry</i>	<i>This study</i>

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