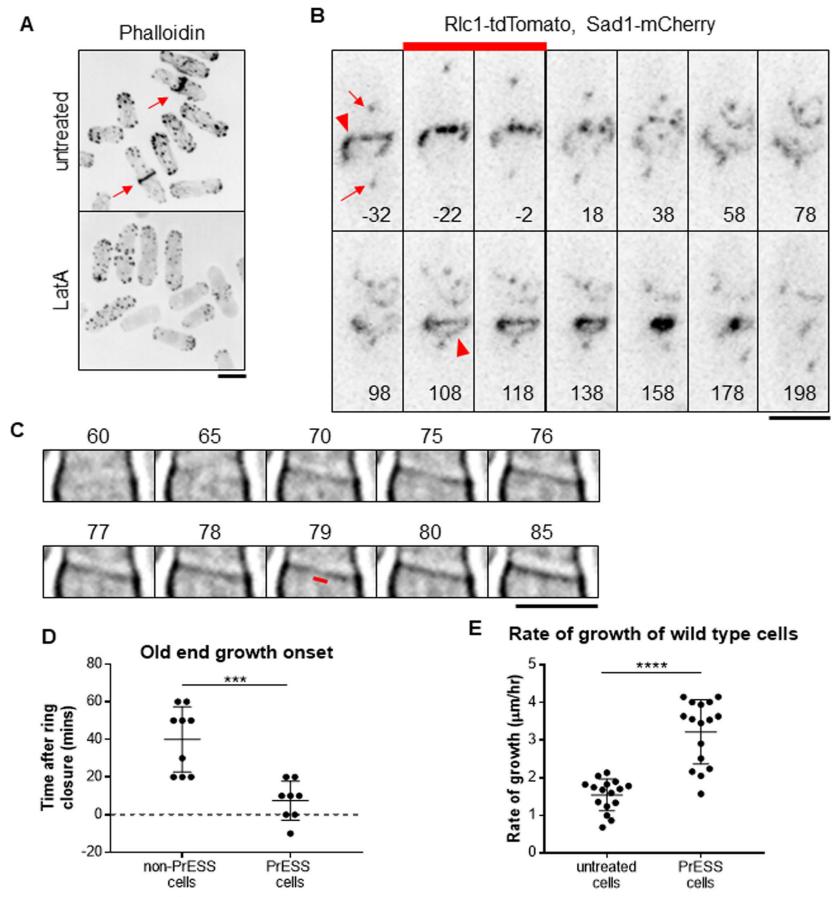


Fig. S1. A) Phalloidin staining of untreated cells and cells treated with 10 µM LatA for 30 minutes. Actomyosin rings are marked by red arrows. **B)** Rlc1-tdTomato (arrowhead) and Sad1-mCherry (arrows) mark the actomyosin ring and spindle pole bodies. Time 0 is ring closure. During a 30-minute 10 µM LatA treatment (**red bar**), the original actomyosin ring is disrupted. During recovery, a new ring is built, which undergoes constriction. **C)** In this sample cell, we determined that septum closure occurred at 80 minutes, because this is the first frame in which the gap that was still visible at 79 minutes (as shown by the red bar) was closed. **D)** Onset of growth in PrESS and non-PrESS cells after ring closure. **E)** Rate of old end growth of untreated and PrESS cells. ($p < 0.0001$; $n \geq 16$ cell ends. Statistical tests = Student t-test. Scale bars= 5µm.

A

PrESS phenotype frequency when LatA-treated at different cell cycle stages			
	G2	Mitosis	G1/S
% PrESS	0	100	0
n	17	10	7

B

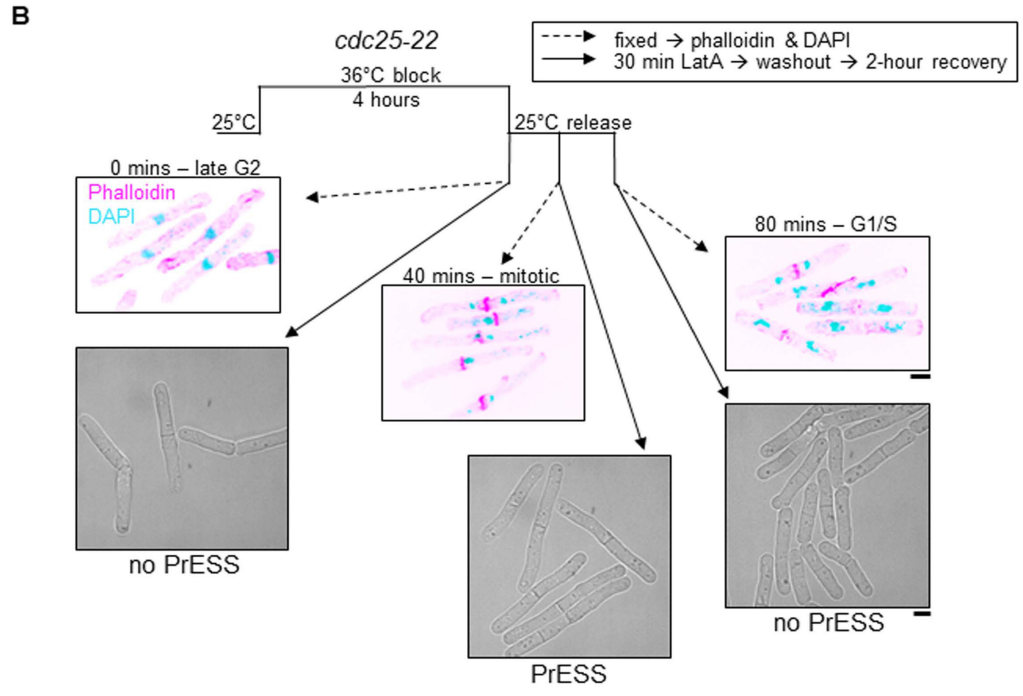


Fig. S2. A) Table showing the frequency of the PrESS phenotype when cells are treated in different cell cycle stages. **B)** *cdc25-22* cells were synchronized by cell cycle block and release. During the indicated cell cycle stages occurring 0, 40, and 80 minutes after release, a fraction of cells was fixed and stained with phalloidin (magenta) and DAPI (cyan), to mark the actomyosin ring and nucleus respectively, while the rest were treated for 30 minutes with LatA and then washed. Cells recovering from LatA treatment in G2 or G1/S does not yield PrESS cells while those undergoing mitosis exclusively yield PrESS cells. Scale bars, 5μm.

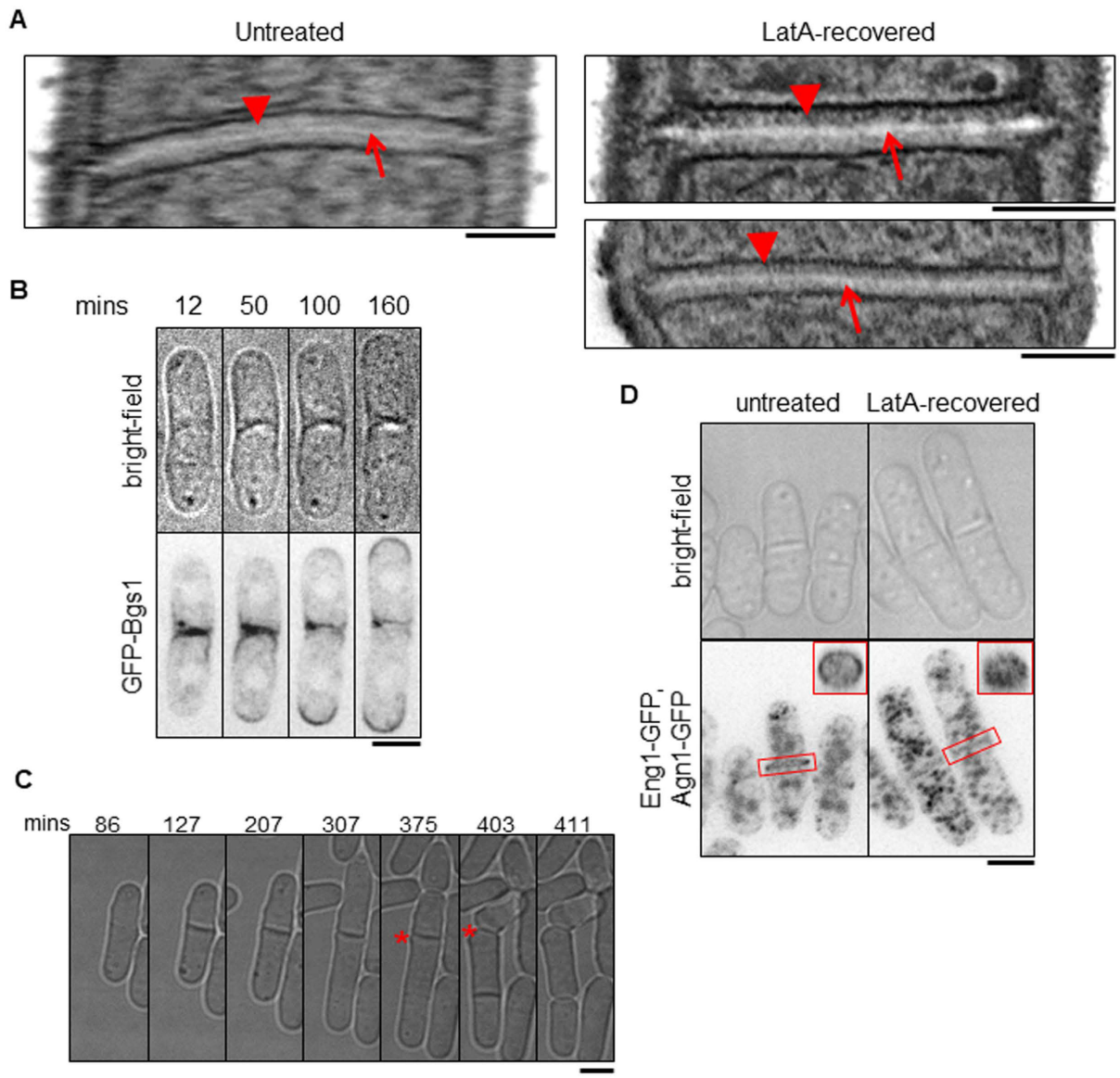


Fig. S3. A) Electron micrographs of wild-type septa; untreated (left) and LatA-treated after 2.5-hour recovery (right). Arrows indicate primary septum, while arrowheads indicate secondary septum. Scale bars, 500nm. **B)** Montage of a time lapse of GFP-Bgs1 localization in a PrESS cell. Minutes are since LatA washout. Scale bar, 5μm **C)** The original septum of a PrESS cell sometimes separates (asterisk) albeit after a delay. Minutes are since LatA washout. **D)** Septum-digesting enzymes, Eng1-GFP and Agn1-GFP, in control cells show cortical distribution at the septum, effectuating a ring, while in PrESS cells their localization appears as a disc all over the septum barrier. The LatA-treated cells shown were imaged 3 hours after LatA washout. 3D-reconstructed membrane barriers are shown in the insets. Scale bars, 5μm.

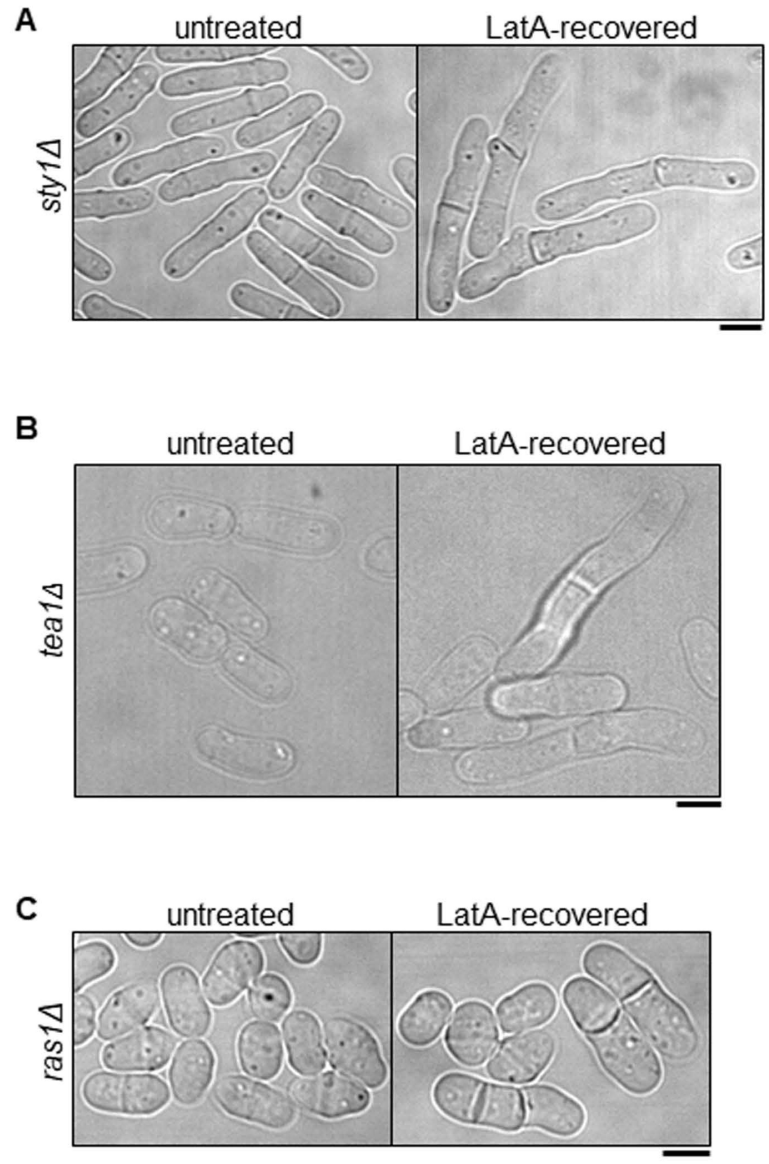


Fig. S4. The PrESS phenotype was observed in mutants of the MAP kinase *sty1Δ* **(A)**, *tea1Δ* **(B)** and GTPase *ras1Δ* **(C)**. Scale bars, 5µm.

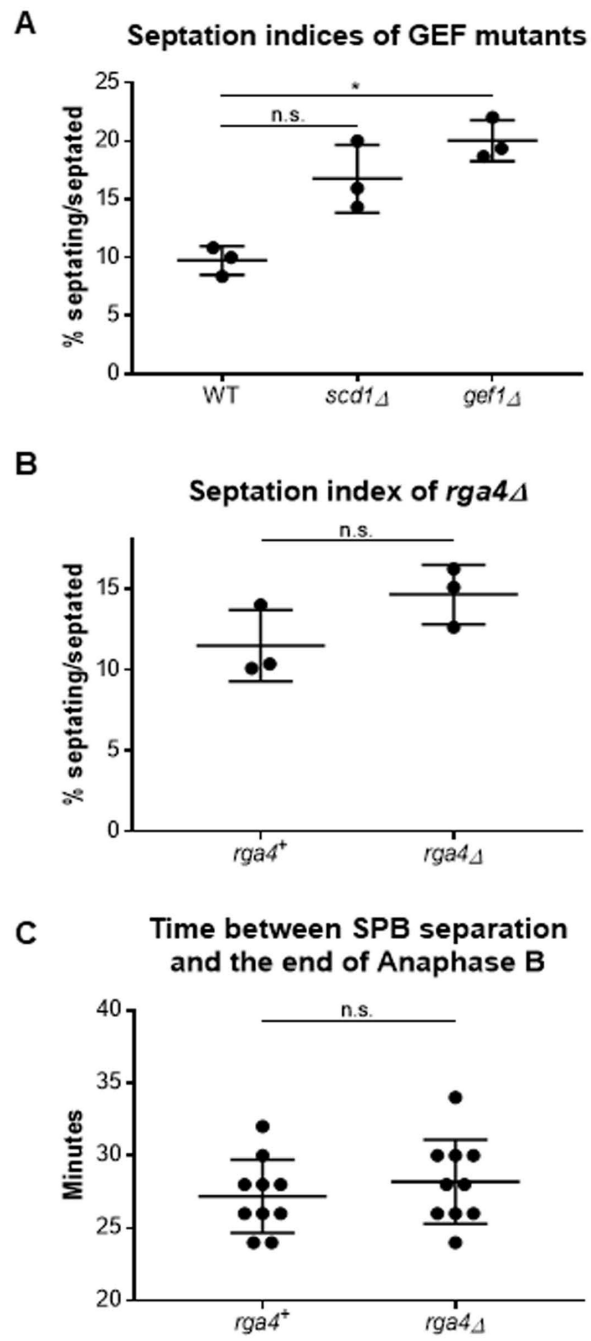


Fig. S5. A) Septation indices of the Cdc42 GEF mutants *scd1*Δ and *gef1*Δ. *gef1*Δ shows significantly more septated cells than the wild type (p = 0.0396), while *scd1*Δ does not (p = 0.1017). **B)** Septation index of the Cdc42 GAP mutant *rga4*Δ, which was similar to wild type (p = 0.1277). **C)** Quantification of the time between spindle pole body separation (completion of spindle formation) and the end of Anaphase B. We find no significant difference between *rga4*$^{+}$ and *rga4*Δ cells. Statistical tests used for A is Ordinary one-way ANOVA with Tukey's multiple comparisons and for B and C is Student's t-test.

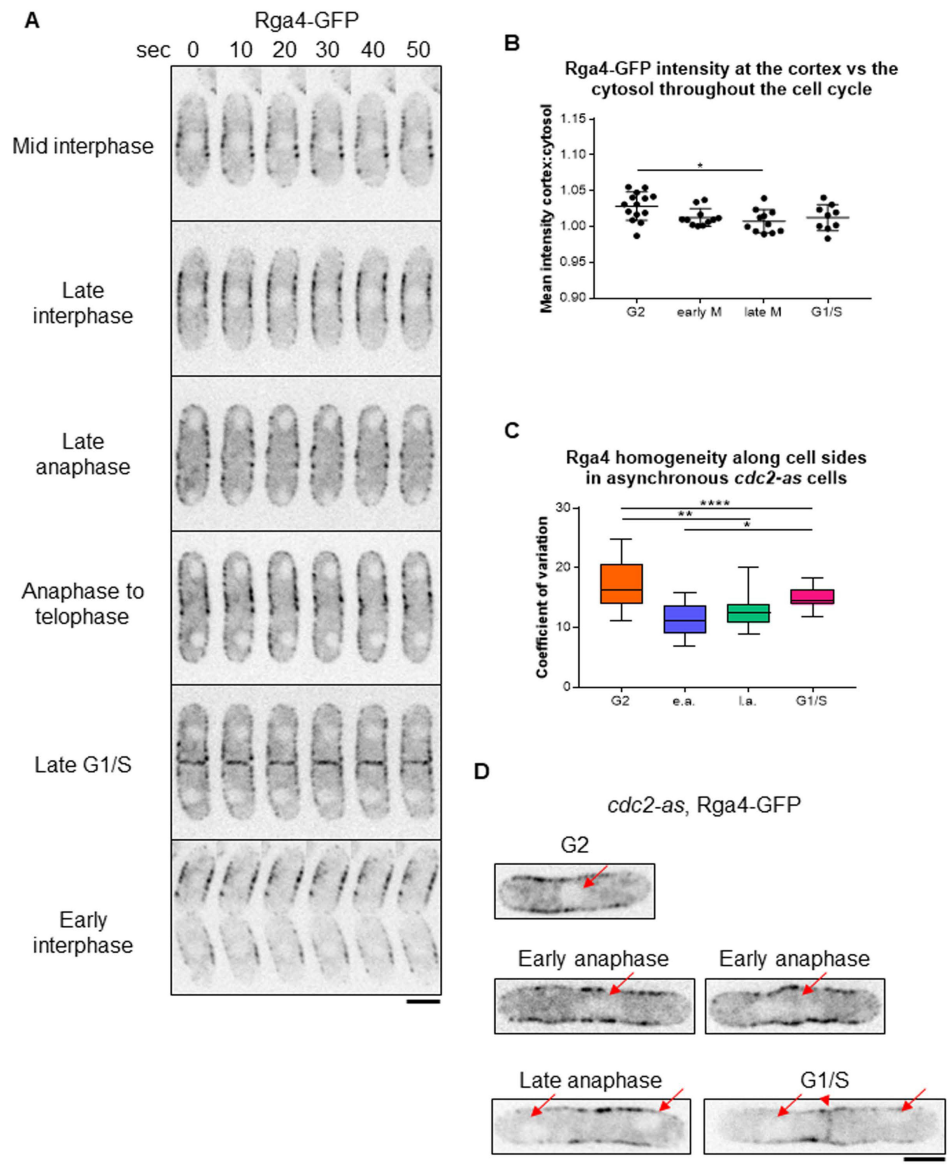


Fig. S6. A) Montages of the same cells in Figure 6B. Images are from the first minute of the timelapse with an interval of 10 seconds. **B)** Quantification of Rga4-GFP intensity throughout the cell cycle. The values are ratios of the intensity at the cortex to the intensity in the cytosol. The intensity of Rga4-GFP is lower in the cytoplasm during G2 than late mitosis ($p = 0.0189$). **C)** Quantification of the homogeneity of Rga4-GFP in asynchronous *cdc2-as* cells without inhibitor treatment. e.a. = early anaphase; l.a. = late anaphase. Ordinary one-way ANOVA with Tukey's multiple comparisons. **D)** Sample Rga4-GFP expressing *cdc2-as* cells demonstrating how cells in different cell cycle stages were selected. Arrows show a single nucleus in G2, an elongated nucleus in early anaphase, a binuclear cell with no signal at the division site for late anaphase, and a binuclear cell with signal at the division site (arrowhead) for post-anaphase cells. Scale bars, 5μm.

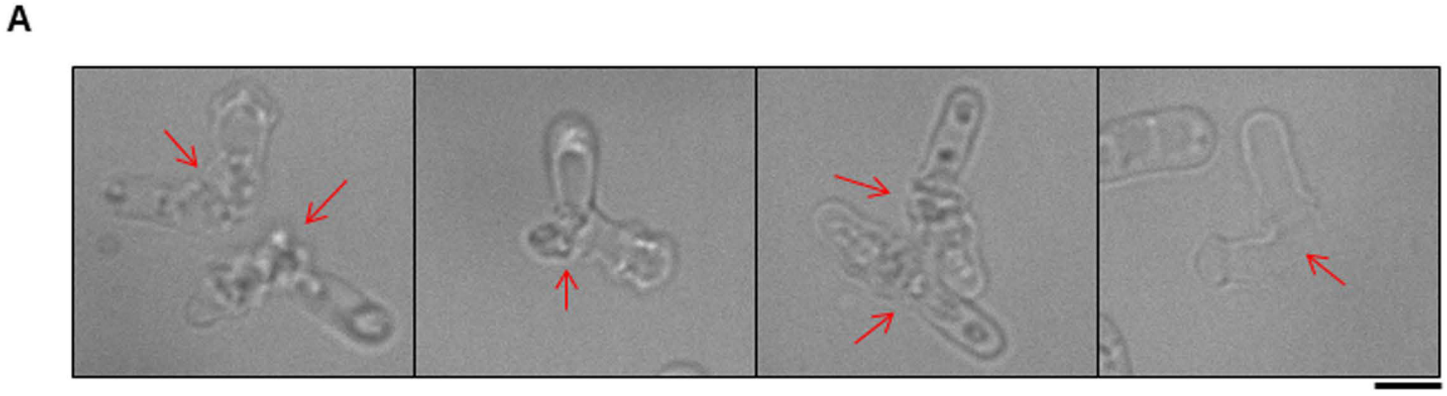


Fig. S7. A) Examples of lysed cells (arrows) in wild type cells expressing *nak1-mor2* (-thiamine). Cells mostly lyse at the division site. **B)** *gef1*Δ and *rga6*Δ cells under conditions where they repress *nak1-mor2* or express *nak1-mor2*. Lysed cells are shown with arrows. Scale bars, 5µm.

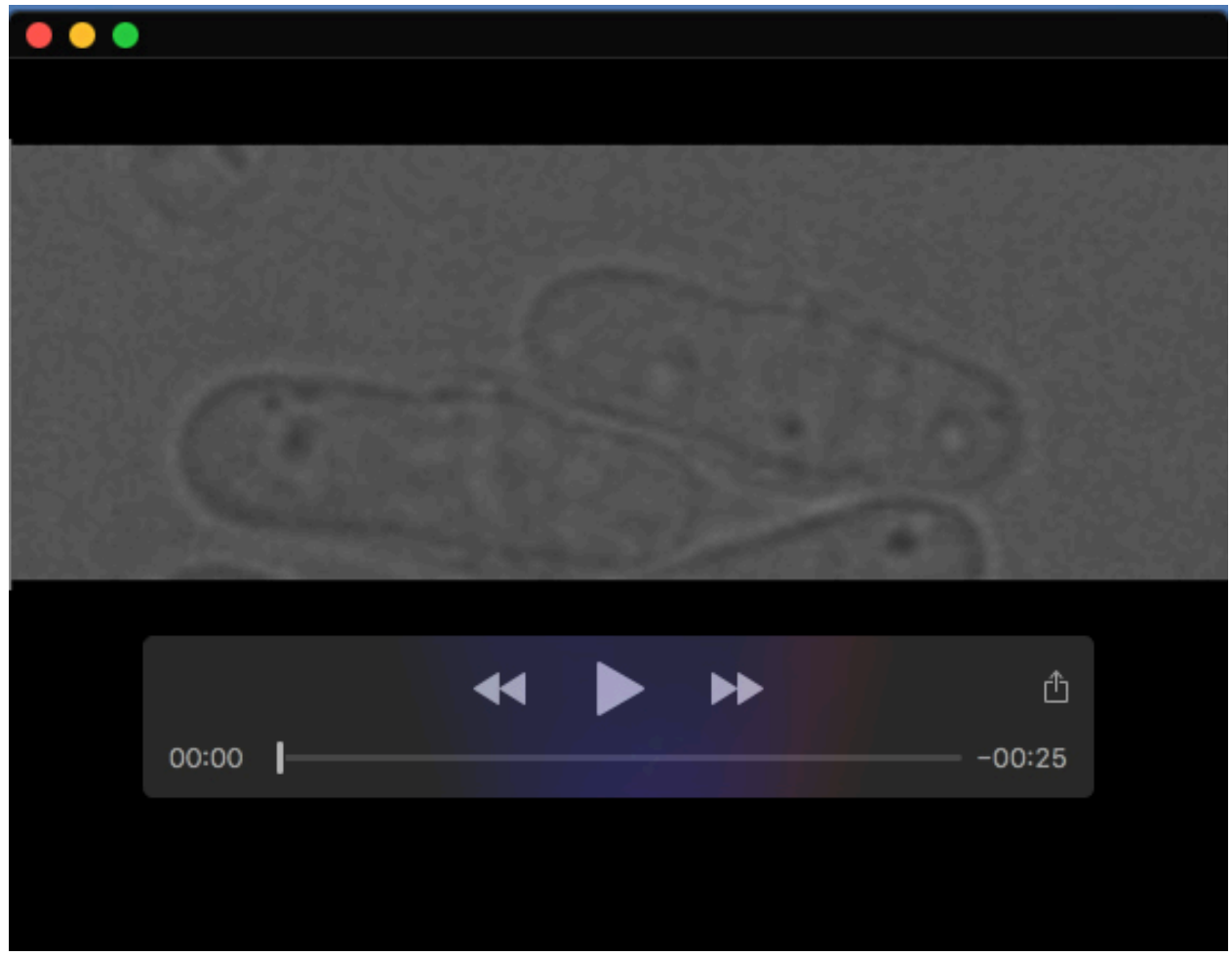
Table S1. Strains list

Strain	Genotype	Source
PN567	*h+ ade6-704 leu1-32 ura4-d18*	Paul Nurse
FV467	*h+ tea1∆::ura4+ ura4-D18 ade6-M210*	(Verde et al., 1995)
FV513	*h- rga4Δ::ura4+ ade6-704 leu1-32 ura4-d18*	(Das et al., 2007)
FV784	*h+ rga4-gfp::KanMX ade6-704 ura4-D18 leu1-32*	(Das et al., 2007)
JM1745	*h- sty1Δ:ura4+ leu1-32 ura4-D18*	(Millar et al., 1995)
JX125	*h90 Δscd1::ura4+ ade6 leu1-32 ura4-d18 h210*	(Hirota et al., 2003)
JZ521	*h90 ras1Δ::ura4+ ade6-M210 leu1-32 ura4-D18*	(Hakuno et al., 1996)
PPG2601	*h+ Δgef1::ura4+ ura4-D18 leu1-32*	(Coll et al., 2003)
PPG3750	*h- bgs1Δ::ura4 Pbgs1::GFP-bgs1:leu1 leu1-32 ura4-D18 his3-D1 h-*	(Cortes et al., 2002)
PPG5668	*h+ scd2-GFP-kanMX6+ leu1-32 ura4-d18*	(Rincon et al., 2007)
WF206	*h- cdc25-22 ade6-704 leu1-32 ura4-D18*	(Russell and Nurse, 1986)
YSM740	*h+ myo52-tdTomato-NATrMX ade6-M216 leu1-32 ura4-D18*	(Martin et al., 2007)
YSM947	*h+ scd1-3GFP-kanMX+ ade6-m216 leu1-32 ura4-d18*	(Bendezu and Martin, 2013)
YMD317	*CRIB-GFP-ura4+ rlc1-Tomato-NATr sad1-mCherry:kanMX ade6-M21X leu1-32 ura4-D18 his7+*	Lab stock
YMD546	*bgs1Δ::ura4 Pbgs1::GFP-bgs1:leu1+ Rlc1-tdTomato-NATr Sad1-mCherry:kanMX leu1-32 ura4-D18*	(Wei et al., 2016)
YMD772	*rga4-GFP-KanMX rlc1-tdTomato-NATr sad1-mCherry:kanR ade6-M216 leu1-32 ura4-D18 his7+*	Lab stock
YMD824	*eng1-GFP-KanMX agn1-GFP-KanMX leu1-32 ura4-D18*	This study
YMD1200	*h- [pREP41X-Nak1-Mor2] ade6-704 leu1-32 ura4-d18*	This study

YMD1253	*[pREP41X] CRIB-GFP-ura4+ ade6-704 leu1-32 ura4-d18*	This study
YMD1255	*[pREP41X-Nak1-Mor2] CRIB-GFP-ura4+ ade6-704 leu1-32 ura4-d18*	This study
YMD1509	*h90 cdc2-as-bsd rga4-GFP::KanMX leu1-32 ura4-D18 ade6-M216*	This study
YMD1512	*h- rga4Δ::ura4+ [pREP41X-Nak1-Mor2] ade6-704 leu1-32 ura4-d18*	This study
YMD1735	*h- rga6Δ::ura4+ [pREP41X-Nak1-Mor2] ade6-704 leu1-32 ura4-d18*	This study
YMD1737	*h- gef1Δ::ura4+ [pREP41X-Nak1-Mor2] ade6-704 leu1-32 ura4-d18*	This study
YMD1741	*h- rga6Δ::ura4+ [pREP41X] ade6-704 leu1-32 ura4-d18*	This study
YMD1743	*h- gef1Δ::ura4+ [pREP41X] ade6-704 leu1-32 ura4-d18*	This study
YMD1831	*h- rga4Δ::ura4+ [pREP41X-Nak1-Mor2] CRIB-GFP-ura4+ ade6-704 leu1-32 ura4-d18*	This study
YMD1835	*h- rga4Δ::ura4+ [pREP41X] CRIB-GFP-ura4+ ade6-704 leu1-32 ura4-d18*	This study

Table S2. Candidate screen of mutants to identify changes in the PrESS phenotype. All mutants listed here showed the PrESS phenotype during LatA recovery unless marked with an asterisk.

Protein	**Mutant**	**Role**
Cdc16	*cdc16-116******	SIN inhibitor
Scd1	*scd1Δ*	Cdc42 GEF
Gef1	*gef1Δ*	Cdc42 GEF
Gef1	*gef1S112A*	Cdc42 GEF
Rga4	*rga4Δ*	Cdc42 GAP
Rga6	*rga6Δ*	Cdc42 GAP
Scd2	*scd2Δ*	Scaffold for Scd1
Rdi1	*rdi1Δ*	Cdc42 GDI
Ras1	*ras1Δ*	RAS GTPase
Tea1	*tea1Δ*	Polarity marker
Tea4	*tea4Δ*	Polarity marker
For3	*for3Δ*	Actin regulator
Myo1	*myo1Δ*	Type I myosin
Sec8	*sec8-1*	Exocyst component
Exo70	*exo70Δ*	Exocyst component
Sty1	*sty1Δ*	MAP kinase
Orb2 / Shk1 / Pak1	*orb2-34*	PAK kinase
Plo1	*plo1.as8*	Polo kinase
Cdc10	*cdc10-129*	Cell cycle regulator



Movie 1. PrESS Cells. Cells treated with 10µm LatA for 30 minutes and washed and allowed to recover. Cells initiate growth at the ends before the septum matures and fail to separate.

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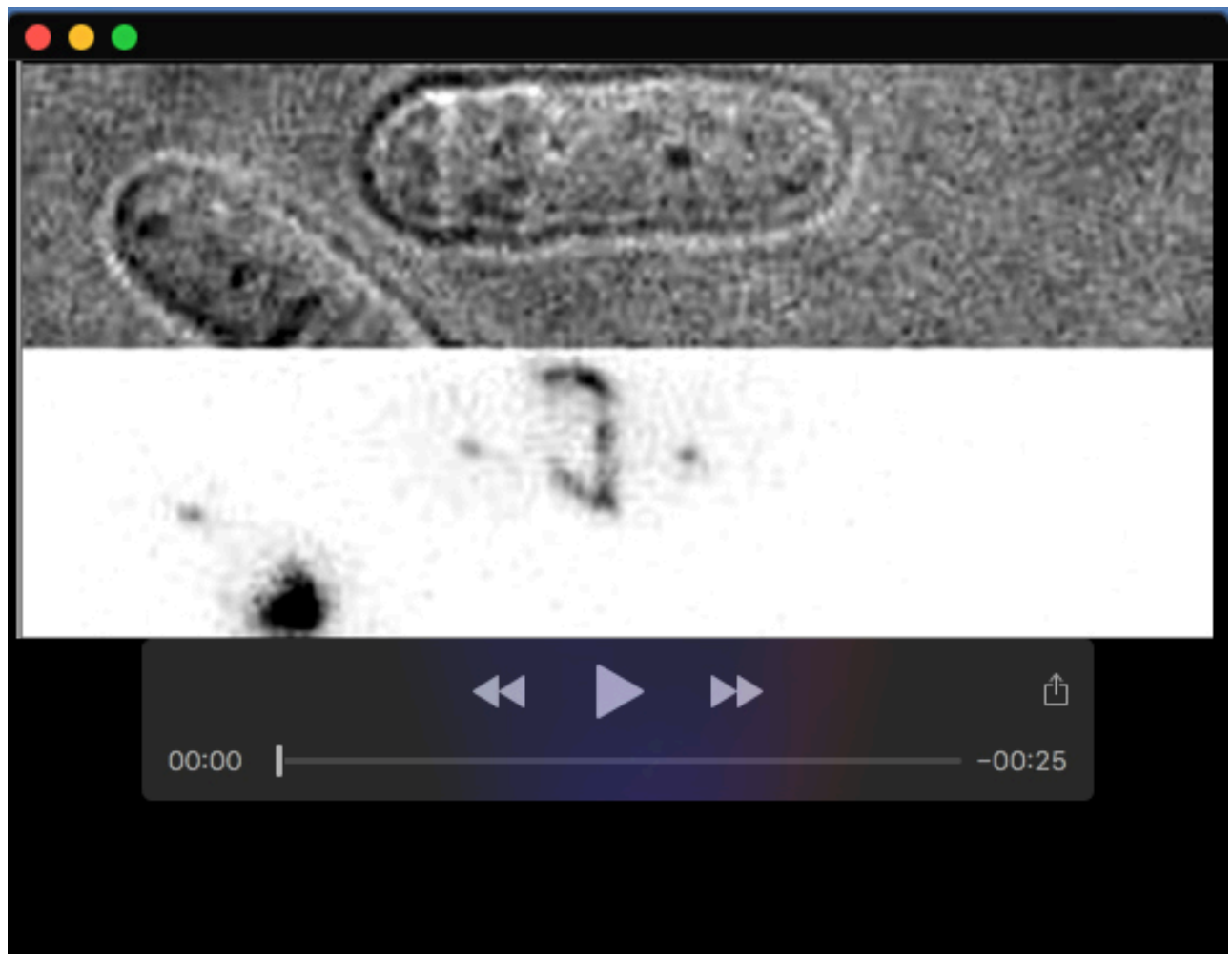

Movie 2. Time-lapse movie of a cell in mitosis undergoing LatA treatment, then washout after 30 mins. This cells recovers and initiates growth (asterisk) soon after ring closure. Upper panel is brightfield imaging, lower panel is Rlc1-TdTomato to mark the actomyosin ring and Sad1-mCherry to mark the spindle pole bodies.

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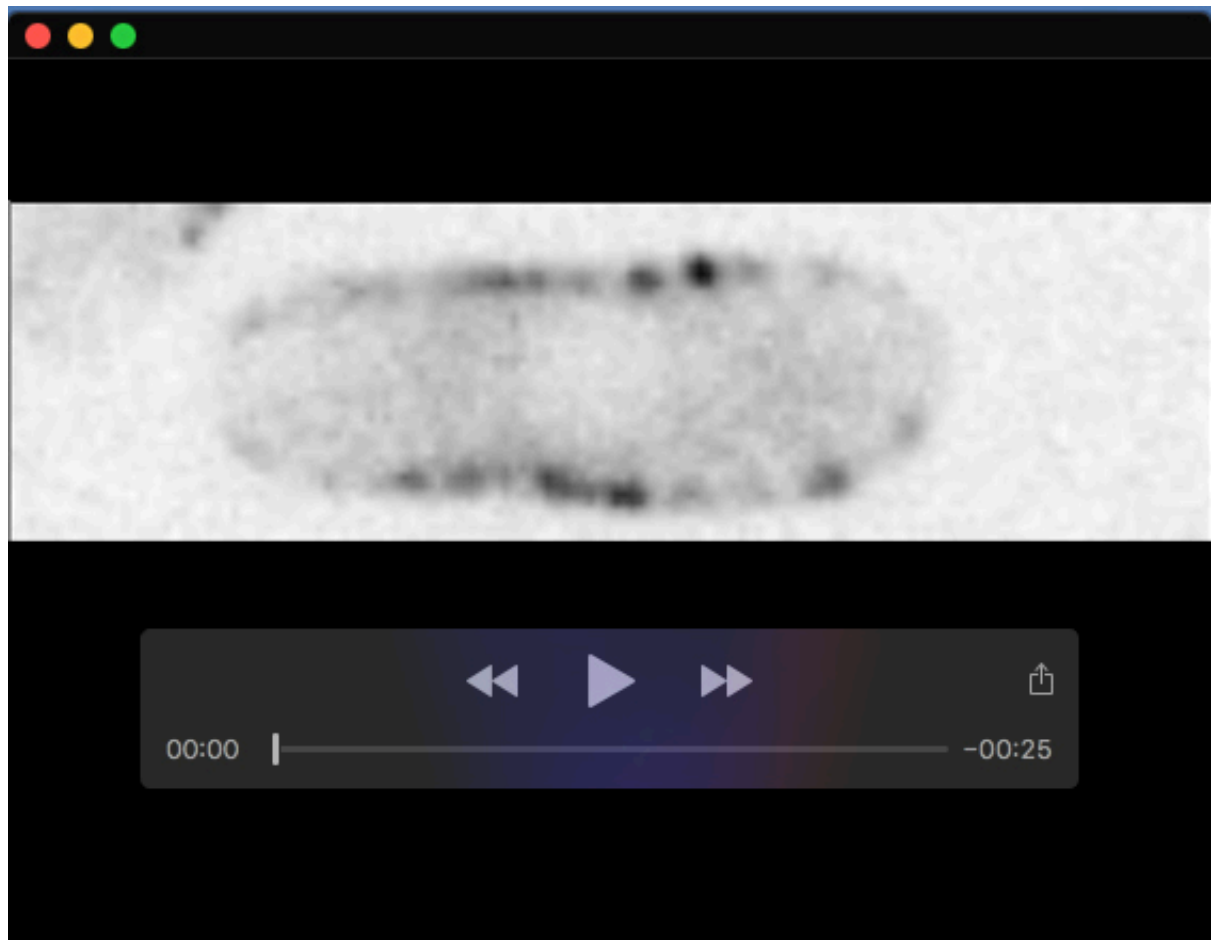

Movie 3. Rga4-GFP dynamics in mid-G2 phase. *rga4-GFP*-expressing cells were imaged every 10 secs for 5 min to obtain Rga4-GFP dynamics in different cell cycle stages.

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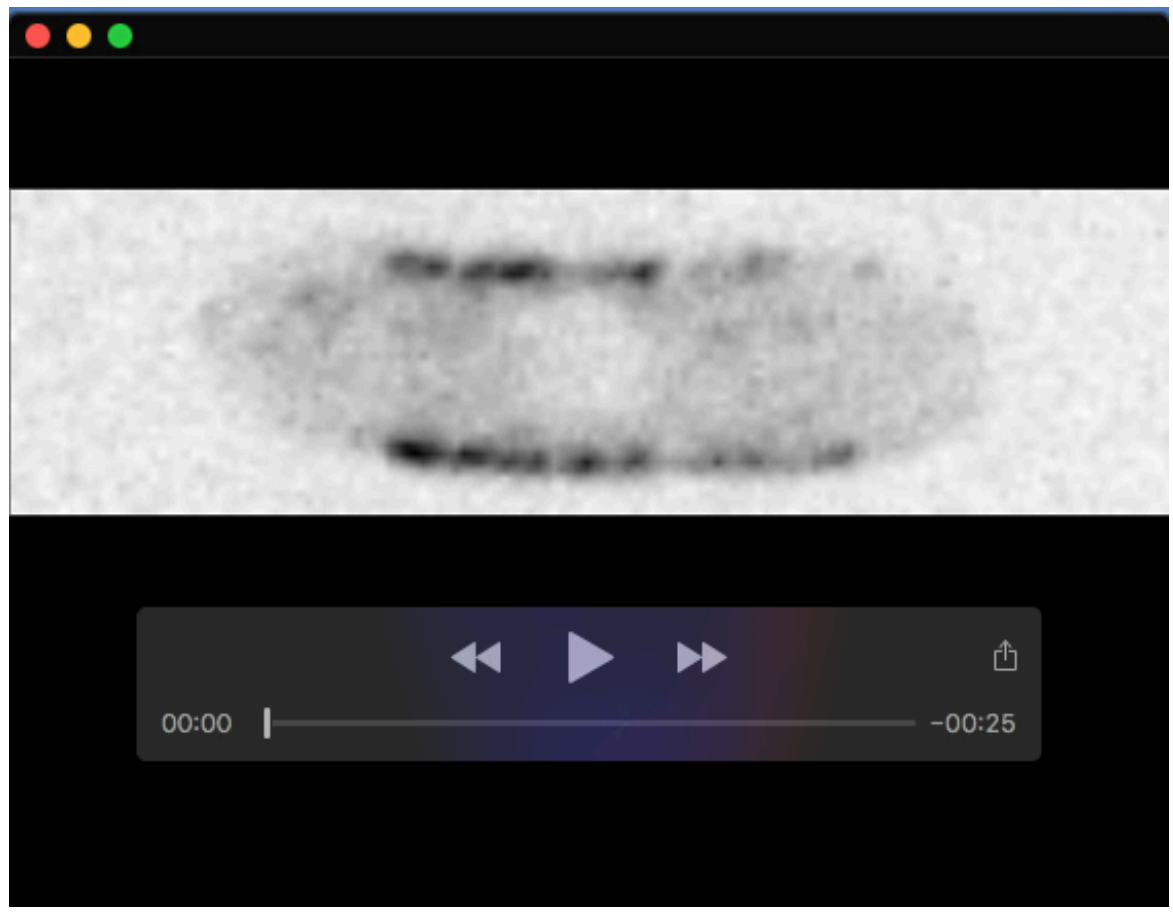

Movie 4. Rga4-GFP dynamics in late-G2 phase. *rga4-GFP*-expressing cells were imaged every 10 secs for 5 min to obtain Rga4-GFP dynamics in different cell cycle stages.

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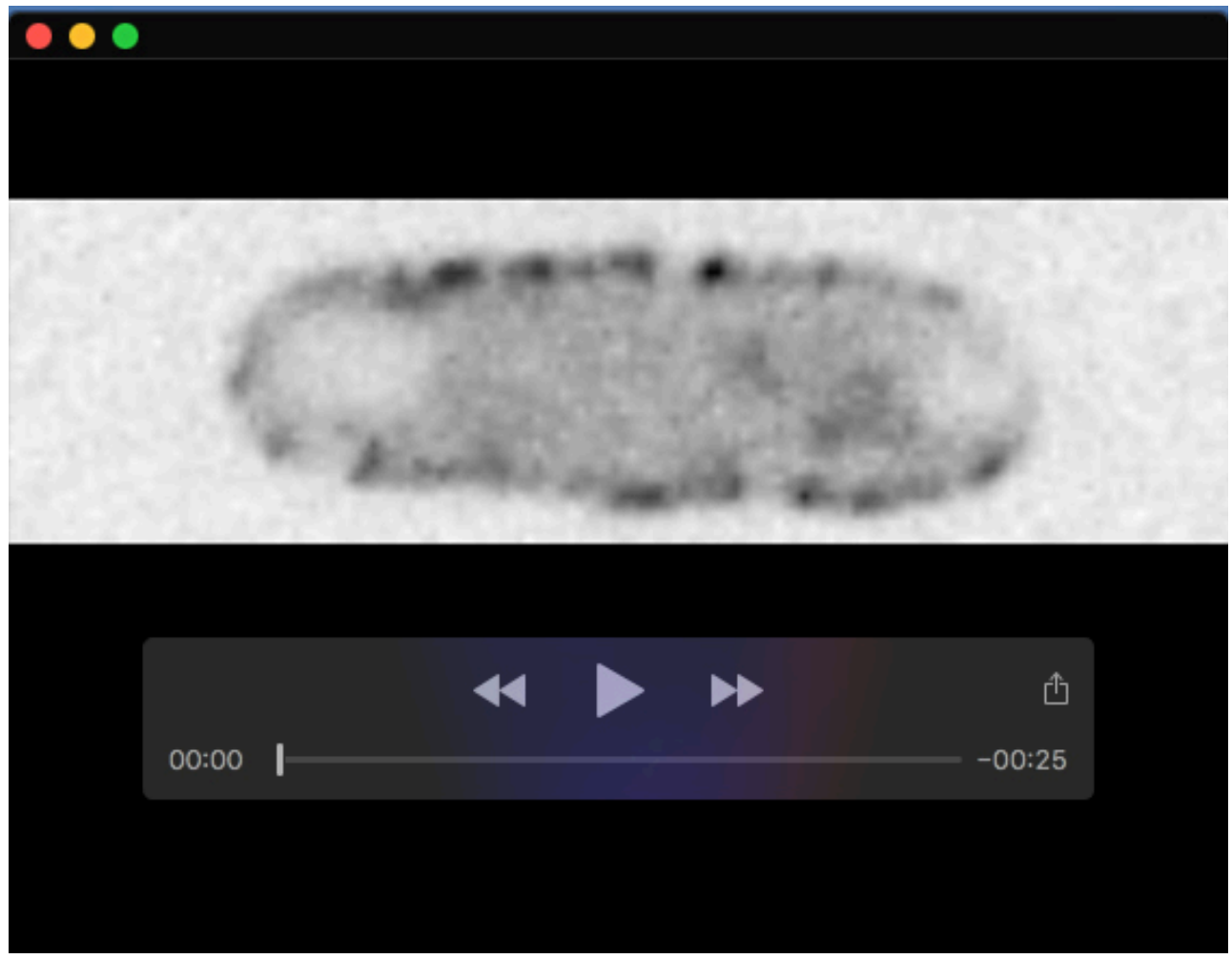
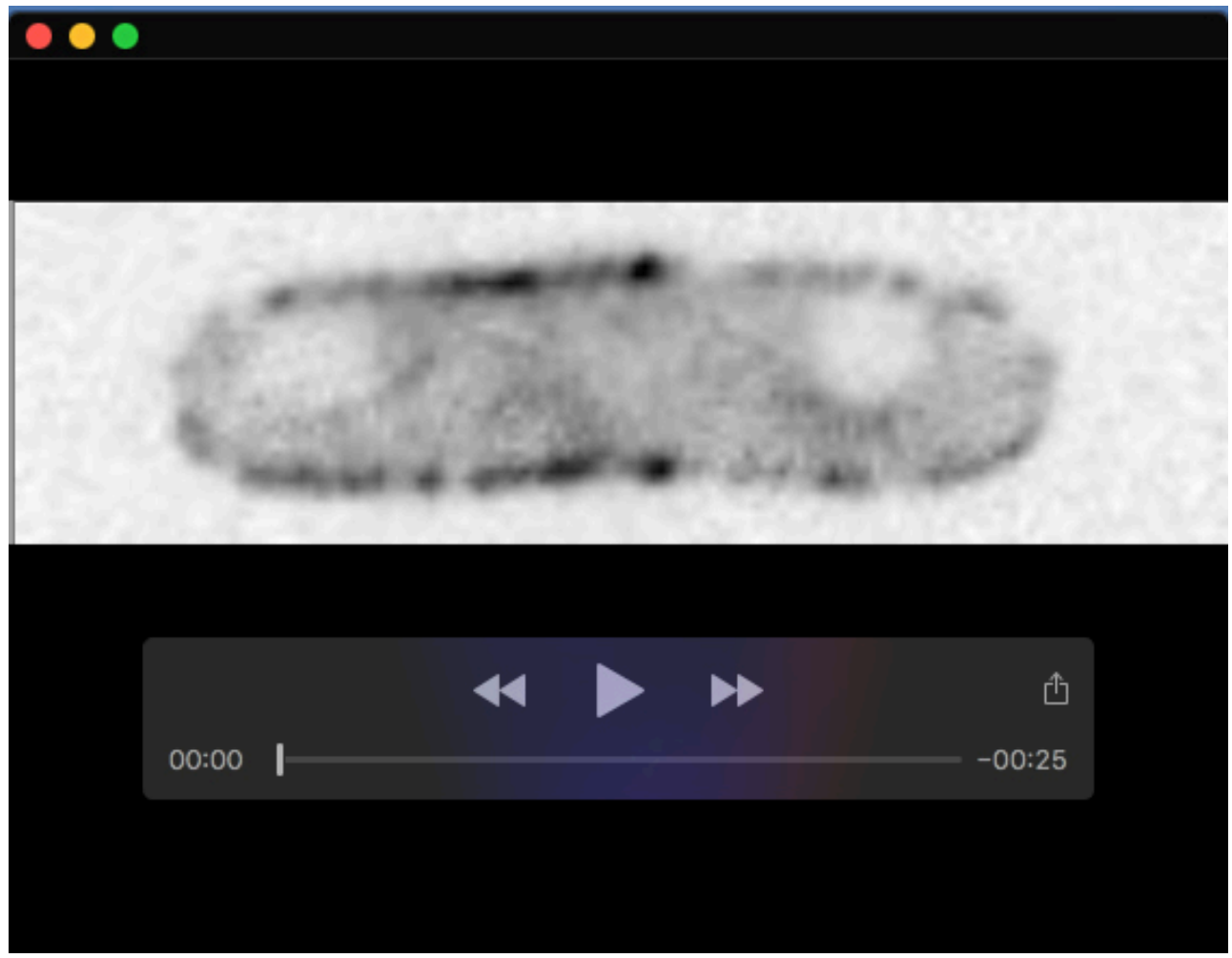
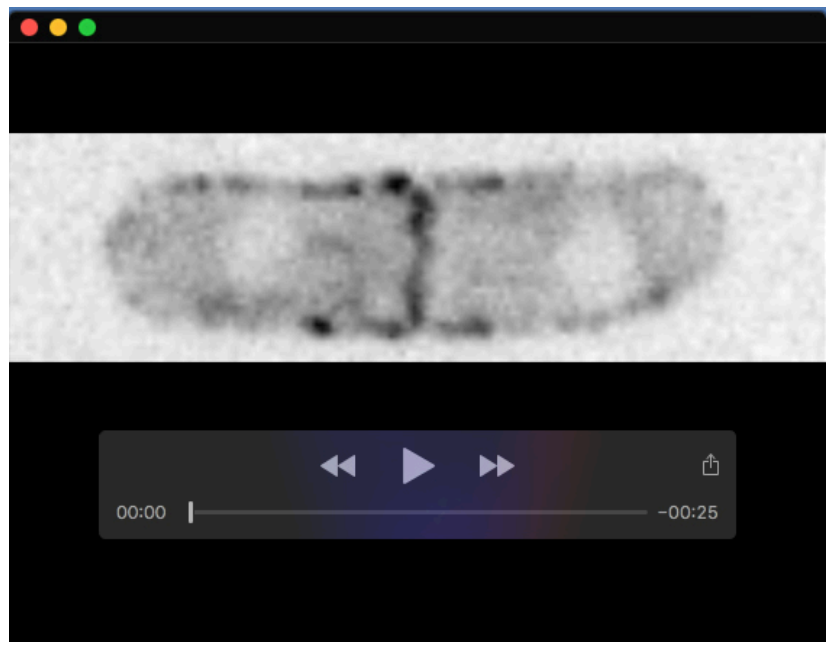


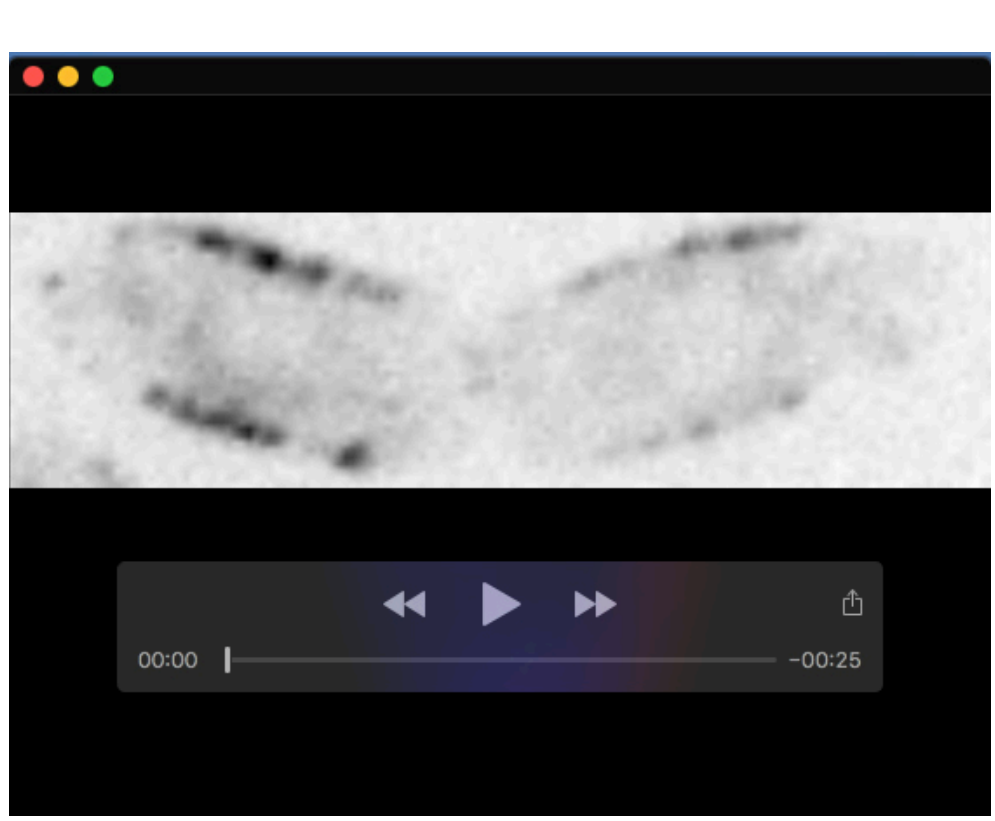
Movie 5. Rga4-GFP dynamics in late anaphase. *rga4-GFP*-expressing cells were imaged every 10 secs for 5 min to obtain Rga4-GFP dynamics in different cell cycle stages.



Movie 6. Rga4-GFP dynamics in telophase. *rga4*-GFP-expressing cells were imaged every 10 secs for 5 min to obtain Rga4-GFP dynamics in different cell cycle stages.



Movie 7. Rga4-GFP dynamics in G1/S phase. *rga4*-GFP-expressing cells were imaged every 10 secs for 5 min to obtain Rga4-GFP dynamics in different cell cycle stages.



Movie 8. Rga4-GFP dynamics in early-G2 phase. *rga4*-GFP-expressing cells were imaged every 10 secs for 5 min to obtain Rga4-GFP dynamics in different cell cycle stages.

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