Supplemental Materials

Molecular Biology of the Cell

Miller et al.

Table S1. Yeast strains used in this study

Strain	Relevant Genotype[a]	Source/Comments
YEF473A	**a** *his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52*	Bi and Pringle, 1996
HPY1197	**a** *VC-CDC42-KAN*	Kang *et al.,* 2010
HPY1213	**a** *YFP^{C}-RSR1-TRP1*	Kang *et al.,* 2010
HPY1522	**a** *YFP^{C}-rsr1^{K16N}-TRP1*	Kang *et al.,* 2010
HPY1552	**a** *YFP^{C}-rsr1^{G12V}-TRP1*	Kang *et al.,* 2010
HPY3340	**a** *VC-CDC42-KAN BEM1-YFP^{N}-URA3*	This Study[b]
HPY3441	**a** *YFP^{C}-RSR1-TRP1 BEM1-YFP^{N}-URA3*	This Study[b]
HPY3442	**a** *YFP^{C}-rsr1^{K16N}-TRP1 BEM1-YFP^{N}-URA3*	This Study[b]
HPY3443	**a** *YFP^{C}-rsr1^{G12V}-TRP1 BEM1-YFP^{N}-URA3*	This Study[b]
HPY3444	**a** *VC-CDC42-KAN bem1$^{Δ281-345}$-YFP^{N}-URA3*	This Study[b]
HPY3445	**a** *YFP^{C}-RSR1-TRP1 bem1$^{Δ281-345}$-YFP^{N}-URA3*	This Study[b]
HPY3446	**a** *YFP^{C}-rsr1^{K16N}-TRP1 bem1$^{Δ281-345}$-YFP^{N}-URA3*	This Study[b]
HPY3447	**a** *YFP^{C}-rsr1^{G12V}-TRP1 bem1$^{Δ281-345}$-YFP^{N}-URA3*	This Study[b]
HPY3448	**a** *VC-CDC42-KAN bem1$^{Δ345-408}$-YFP^{N}-URA3*	This Study[b]
HPY3449	**a** *YFP^{C}-RSR1-TRP1 bem1$^{Δ345-408}$-YFP^{N}-URA3*	This Study[b]
HPY3450	**a** *YFP^{C}-rsr1^{K16N}-TRP1 bem1$^{Δ345-408}$-YFP^{N}-URA3*	This Study[b]
HPY3451	**a** *YFP^{C}-rsr1^{G12V}-TRP1 bem1$^{Δ345-408}$-YFP^{N}-URA3*	This Study[b]
HPY2671	α *WHI5-GFP-TRP1 PBD-tdTomato-URA3*	Lee *et al.,* 2015
HPY2669	α *rsr1Δ::URA3 WHI5-GFP-KAN PBD-tdTomato-URA3*	Lee *et al.,* 2015
HPY3296	**a** *BEM1-GFP-LEU2 WHI5-mCherry-hph*	This Study[c]
HPY3300	**a** *rsr1Δ::TRP1 BEM1-GFP-LEU2 WHI5-mCherry-hph*	This Study[c]
HPY3218	α *rsr1Δ::URA3 rsr1^{K16N}-TRP1 WHI5-GFP-KAN PBD-tdTomato-URA3*	This Study[d, e]
HPY3190	**a**/α *WHI5-GFP-KAN/ WHI5-GFP-KAN PBD-tdTomato-URA3/ PBD-tdTomato-URA3*	This Study[d]
HPY3259	**a**/α *rsr1Δ::URA3/rsr1Δ::URA3 rsr1^{K16N}-TRP1/rsr1^{K16N}-TRP1 WHI5-GFP-KAN/WHI5-GFP-KAN PBD-tdTomato-URA3/PBD-tdTomato-URA3*	This Study[d, e]

HPY3331	**a**/α *rsr1Δ::URA3/rsr1Δ::URA3 WHI5-GFP-KAN/WHI5-GFP-KAN PBD-tdTomato-URA3/PBD-tdTomato-URA3*	This Study[d]
DLY9875	α *PBD-tdTomato-URA3 BEM1-GFP-LEU2*	Daniel Lew
DLY13038	**a** *CDC24-GFP-TRP1 BEM1-tdTomato-HIS3*	Daniel Lew
HPY3349	**a**/α *CDC24-GFP-TRP1/CDC24-GFP-TRP1 BEM1-tdTomato-HIS3/BEM1-tdTomato-HIS3*	This Study
HPY3367	**a**/α *rsr1Δ::URA3/rsr1Δ::URA3 BEM1-tdTomato-HIS3/BEM1-tdTomato-HIS3 CDC24-GFP-LEU2/CDC24-GFP-LEU2*	This Study
HPY3370	**a**/α *rsr1Δ::URA3/rsr1Δ::URA3 $rsr1^{K16N}$-TRP1/$rsr1^{K16N}$-TRP1 BEM1-tdTomato-HIS3/ BEM1-tdTomato-HIS3 WHI5-GFP-KAN/WHI5-GFP-KAN*	This Study[d, e]
HPY3342	α *rsr1Δ::URA3 $rsr1^{K16N}$-TRP1 BEM1-GFP-LEU2 WHI5-mCherry-hph*	This Study[c, e]
HPY3426	**a** *GFP-SEC4-URA3 WHI5-mCherry-hph*	This Study[c]
HPY3427	α *rsr1Δ::TRP1 GFP-SEC4-URA3 WHI5-mCherry-hph*	This Study[e]
HPY3430	**a** *rsr1Δ::URA3 $rsr1^{K16N}$-TRP GFP-SEC4-URA3 WHI5-mCherry-hph*	This Study[c, e]
HPY3319	**a** *BEM1-tdTomato-HIS3 WHI5-GFP-TRP1*	This Study[d]
HPY3461	α *EXO70-tdTomato-KAN WHI5-GFP-TRP1*	This Study[d]
HPY3473	**a** *BEM1-YFP^N-URA3 EXO70-VC-KAN*	This Study[f]
HPY3483	**a** *BEM1-YFP^N-URA3 CDC24-VC-KAN*	This Study[f]
HPY3368	**a** *rsr1Δ::URA3 $rsr1^{K16N}$-TRP1 WHI5-GFP-KAN BEM1-tdTomato-HIS3*	This Study [d, e]
HPY3347	**a** *rsr1Δ::URA3 CDC24-GFP-TRP1 BEM1-tdTomato-HIS3*	This Study
HPY3380	**a**/α *BEM1-tdTomato-HIS3/ BEM1-tdTomato-HIS3 WHI5-GFP-TRP1/WHI5-GFP-TRP1*	This Study[d]
HPY3231	**a**/α *WHI5-mCherry-hph/WHI5-mCherry-hph CDC24-GFP-TRP1/CDC24-GFP-TRP1*	This Study[c]
HPY3467	**a**/α *rsr1Δ::URA3/rsr1Δ::URA3 BEM1-tdTomato-HIS3/BEM1-tdTomato-HIS3 WHI5-GFP-TRP1/ WHI5-GFP-TRP1*	This Study[d]
HPY3336	**a** *BEM1-tdTomato-HIS3*	This Study
HPY3480	**a**/α *YFP^C-$rsr1^{G12V}$-TRP1/YFP^C-$rsr1^{G12V}$-TRP1 BEM1-YFP^N-URA3/ BEM1-YFP^N-URA3*	This Study[b]
HPY3482	**a**/α *YFP^C-$rsr1^{K16N}$-TRP1/YFP^C-$rsr1^{K16N}$-TRP1 BEM1-YFP^N-URA3/ BEM1-YFP^N-URA3*	This Study[b]

[a] All strains are congenic to YEF473A. The original strains and plasmids expressing Gic2-PBD-tdTomato were previously described (Tong *et al*., 2007) (kind gifts from E. Bi, University of Pennsylvania). The original strains and plasmids expressing Bem1-GFP (Kozubowski *et al.,* 2008), Bem1-tdTomato (Howell *et*

al., 2012), and GFP-Sec4 (Chen *et al.*, 2012) were previously described (gifts from D. Lew, Duke University).
[b] pRS306 plasmid carrying BEM1-YFP^{N} or bem1$^{\Delta 281\text{-}345}$-YFP^{N} or bem1$^{\Delta 345\text{-}408}$-YFP^{N} was linearized with *Stu*I and integrated at the *ura3* locus.
[c] *WHI5-mCherry* was constructed by the C-terminal tagging method described in Longtine *et al*. (1998) using pBS35 (mCherry, hygromycin-B selection; a gift from Yeast Resource Center, University of Washington, Seattle, WA), replacing the endogenous *WHI5* (Miller *et al.,* 2017).
[d] *WHI5-GFP* was constructed by the C-terminal tagging method described in Longtine *et al*., (1998), using pFA6a-GFP-TRP1 or pFA6a-GFP-kanMX6 replacing the endogenous *WHI5* (Kang *et al*., 2014).
[e] rsr1^{K16N}-TRP1 contains a YFP tag at the N terminus (Park *et al.,* 2002). Since the GFP signal was much stronger than YFP, exposure time was set so that no YFP signal was detectable in images.
[f] *EXO70-VC* and *CDC24-VC* were constructed by the PCR-based C-terminal tagging method (Longtine *et al.,* 1998) using HP0038 pFA6a-VC-kanMX6 (Sung *et al.,* 2007) (a kind gift of W-K Huh, Seoul National University)

Table S2. Plasmids used in this study

Plasmid	Description	Source
pHP2238	pRS426-BEM1-YFP^{N}, 2μ, *URA3*, carrying the complete ORF of *BEM1* with 217bp upstream and 268bp downstream.	This Study
pHP2239	pRS426-bem1$^{\Delta 1\text{-}147}$-YFP^{N}, 2μ, *URA3*, the same as pRS426-BEM1-YFP^{N} except carrying a deletion of aa1-147.	This Study
pHP2240	pRS426-bem1$^{\Delta 148\text{-}280}$-YFP^{N}, 2μ, *URA3*, the same as pRS426-BEM1-YFP^{N} except carrying a deletion of aa148-280.	This Study
pHP2247	pRS426-bem1^{K482A}-YFP^{N}, 2μ, *URA3*, the same as pRS426-BEM1-YFP^{N} except carrying the mutation K482A in the PB1 domain.	This Study
pHP2248	pRS426-bem1$^{\Delta 412\text{-}551}$-YFP^{N}, 2μ, *URA3*, the same as pRS426-BEM1-YFP^{N} except carrying a deletion of aa412-551.	This Study
pHP2253	pRS306-BEM1-YFP^{N}, integrative, *URA3*, carrying the complete ORF of *BEM1* with 217bp upstream and 268bp downstream.	This Study
pHP2254	pRS306-bem1$^{\Delta 281\text{-}345}$-YFP^{N}, integrative, *URA3*, the same as pRS306-BEM1-YFP^{N} except carrying a deletion of aa281-345.	This Study
pHP2255	pRS306-bem1$^{\Delta 345\text{-}408}$-YFP^{N}, integrative, *URA3*, the same as pRS306-BEM1-YFP^{N} except carrying a deletion of aa345-408.	This Study
pHP744	YEp13, 2μ, *LEU2*	Broach *et al*., 1979
PB268	YEp13-RSR1^{K16N}, 2μ, *LEU2*, carrying the complete ORF of *RSR1* with the K16N mutation	Ruggieri *et al.,* 1992
HP0038	pFA6a-VC-kanMX6	Sung *et al.,* 2007
pRS4	pGEX-RSR1	Park *et al*., 1997
pHP610	pTrcHisA-BEM1(aa44-551)	Park *et al*., 1997

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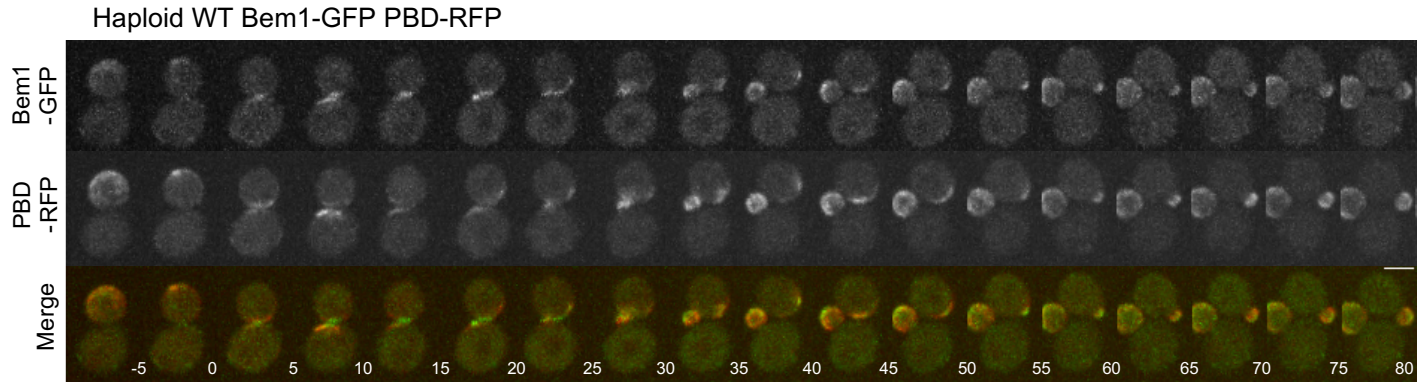

Fig. S1. Time-lapse images of Bem1-GFP and PBD-RFP in haploid WT cells at 30°C. Numbers indicate time (min) relative to the onset of cytokinesis (t=0). Bars, 3 μm.

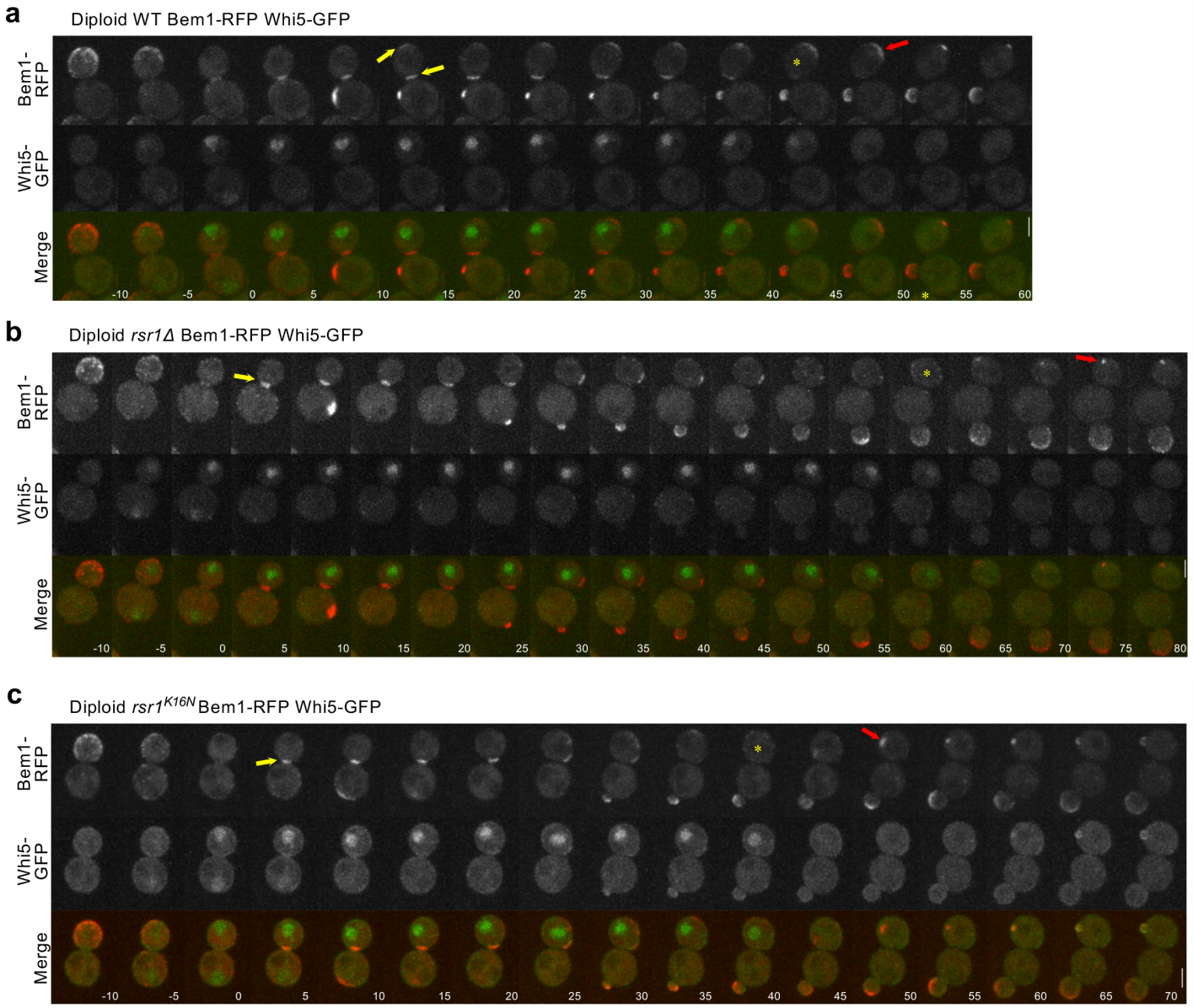

Fig. S2. Localization of Bem1-RFP and Whi5-GFP in diploid WT and *rsr1* mutant cells. Time-lapse images of Bem1-RFP and Whi5-GFP in (a) *WT*, (b) *rsr1*Δ, and (c) *rsr1*^{K16N} cells at 22°C. Numbers indicate time (min) relative to the onset of cytokinesis (t=0). Asterisks mark the T_1-T_2 transition in daughter cells. Yellow and red arrows mark localized Bem1-RFP signal during T_1 and at the incipient bud site during T_2, respectively. Bars, 3 μm.

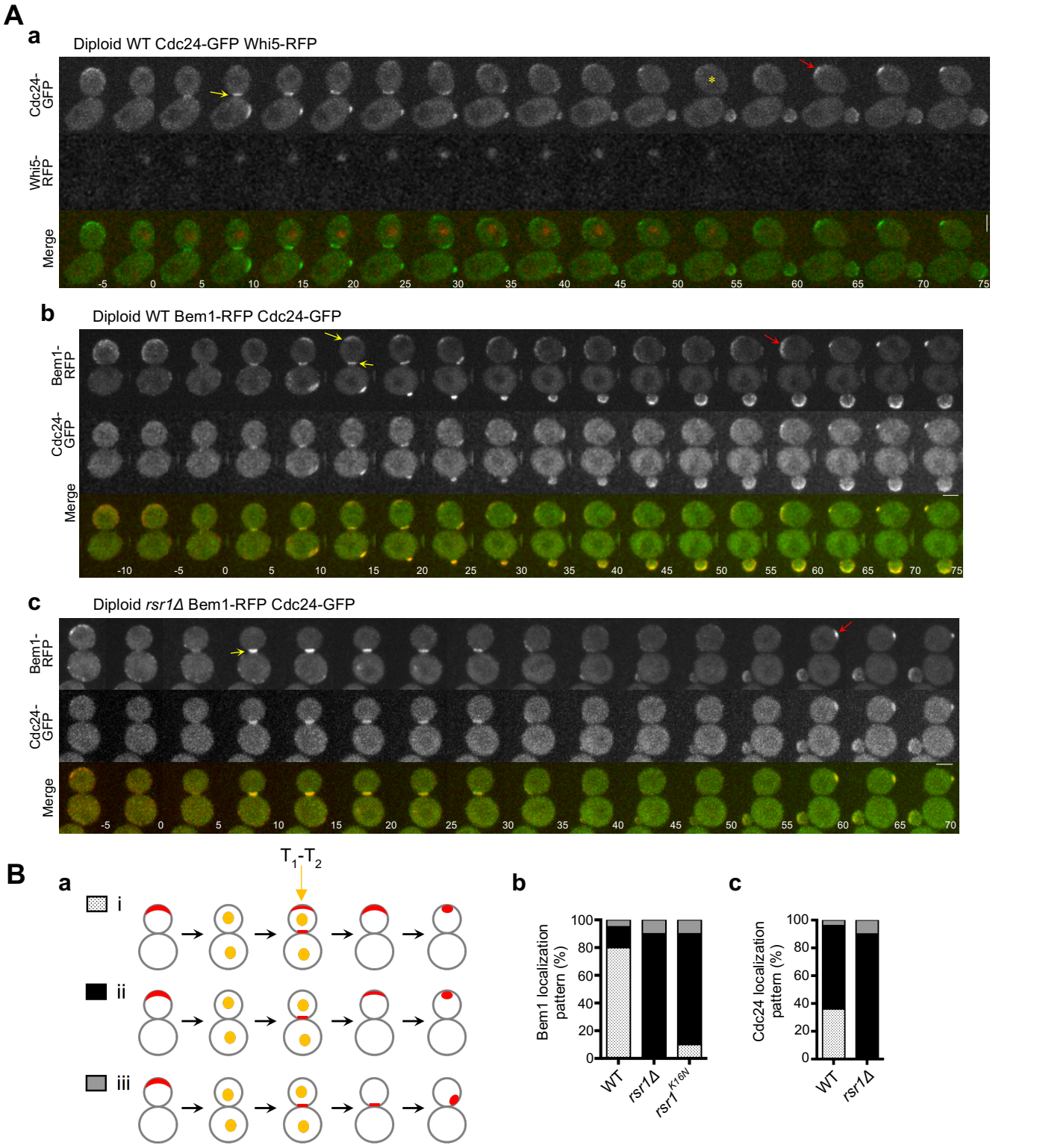

Fig. S3. Localization of Bem1-RFP and Cdc24-GFP Bem1 in diploid WT and *rsr1* cells. (A) Time-lapse images of (a) Cdc24-GFP and Whi5-RFP in WT cells; and Bem1-RFP and Cdc24-GFP in (b) WT and (c) *rsr1*Δ cells at 22°C. Numbers indicate time (min) relative to the onset of cytokinesis (t=0). An asterisk in (a) marks the T_1-T_2 transition in the daughter cell. Yellow and red arrows mark localized Cdc24 or Bem1 signals during T_1 and at the incipient bud site during T_2, respectively (a-c). Bars, 3 μm. (B) Localization pattern (%) of Bem1 (b) or Cdc24 (c), marked in red in (a), during T_1 and T_2 (Whi5 in yellow) in daughter cells is summarized from time-lapse images of WT (n=45), *rsr1*^{K16N} (n=10), and *rsr1*Δ cells (n=20).

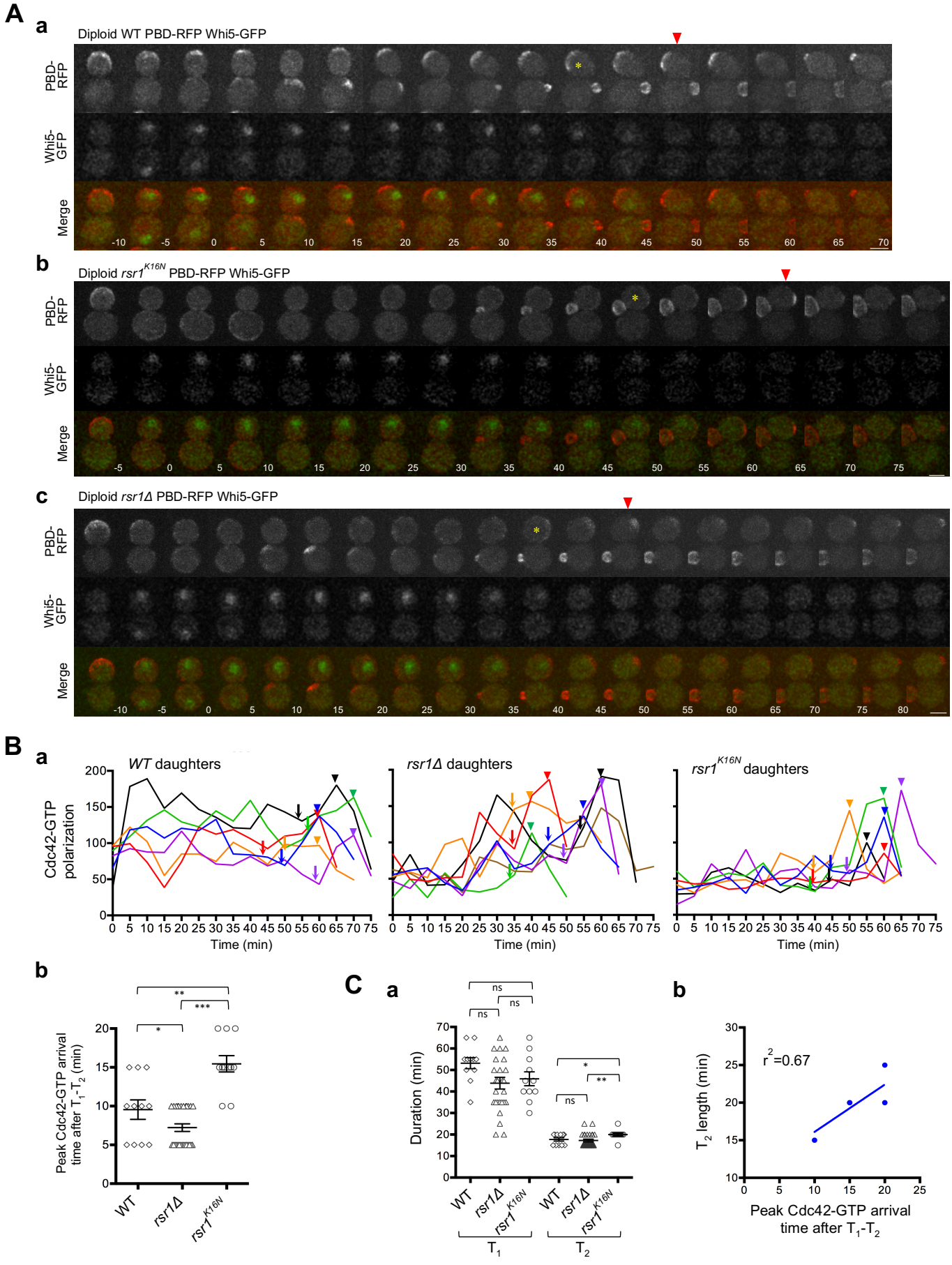

Fig. S4. The second phase of Cdc42 polarization is delayed in diploid cells expressing GDP-locked Rsr1.
(A) Time-lapse images of PBD-RFP and Whi5-GFP in (a) WT, (b) *rsr1*^{K16N}, and (c) *rsr1*Δ cells at 22°C.

Numbers indicate time (min) relative to the onset of cytokinesis (t=0). Asterisks mark the T_1-T_2 transition in daughter cells. Bars, 3 μm. (B) (a) Representative graphs showing Cdc42-GTP polarization over time (min) from the onset of cytokinesis (t=0) in daughter cells. Each colored line shows Cdc42 polarization in a single daughter cell. The T_1-T_2 transition point and when Cdc42-GTP level peaked during T_2 in each cell are marked with an arrow and an arrowhead, respectively, in the same color. (b) Quantification of the time (min) from T_1-T_2 transition until Cdc42-GTP reached a maximum level during T_2 in WT (n=11), *rsr1*Δ (n=27), and $rsr1^{K16N}$ (n=11) daughter cells. Values are shown for each individual cell. (C) (a) Length of T_1 and T_2 (min) in each daughter cell (WT, n =11; *rsr1*Δ, n =27; and $rsr1^{K16N}$, n =11). (b) Correlation analysis of T_2 length and peak Cdc42-GTP arrival time after the T_1-T_2 transition in $rsr1^{K16N}$ cells (n=11).

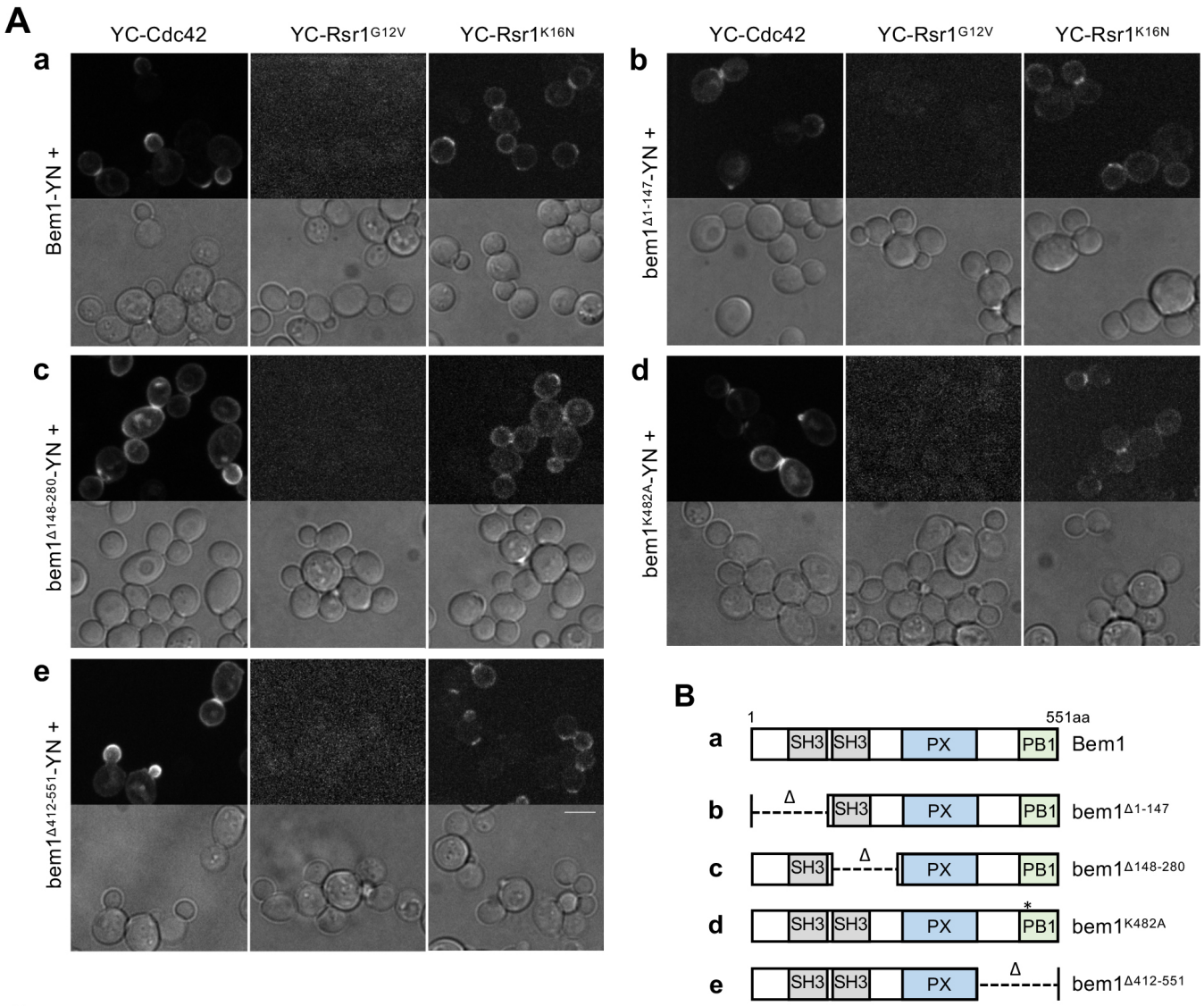

Fig. S5. Additional BiFC assays. (A) BiFC assays in haploid cells expressing YC-Cdc42, YC-Rsr1^{G12V}, or YC-Rsr1^{K16N} and carrying each multicopy plasmid for expression of WT or mutant Bem1 fused to YN, as marked. (B) Diagram of WT and mutant Bem1 proteins. Regions deleted are marked with delta (Δ). An asterisk (*) marks the K482A mutation. Bars, 5μm.

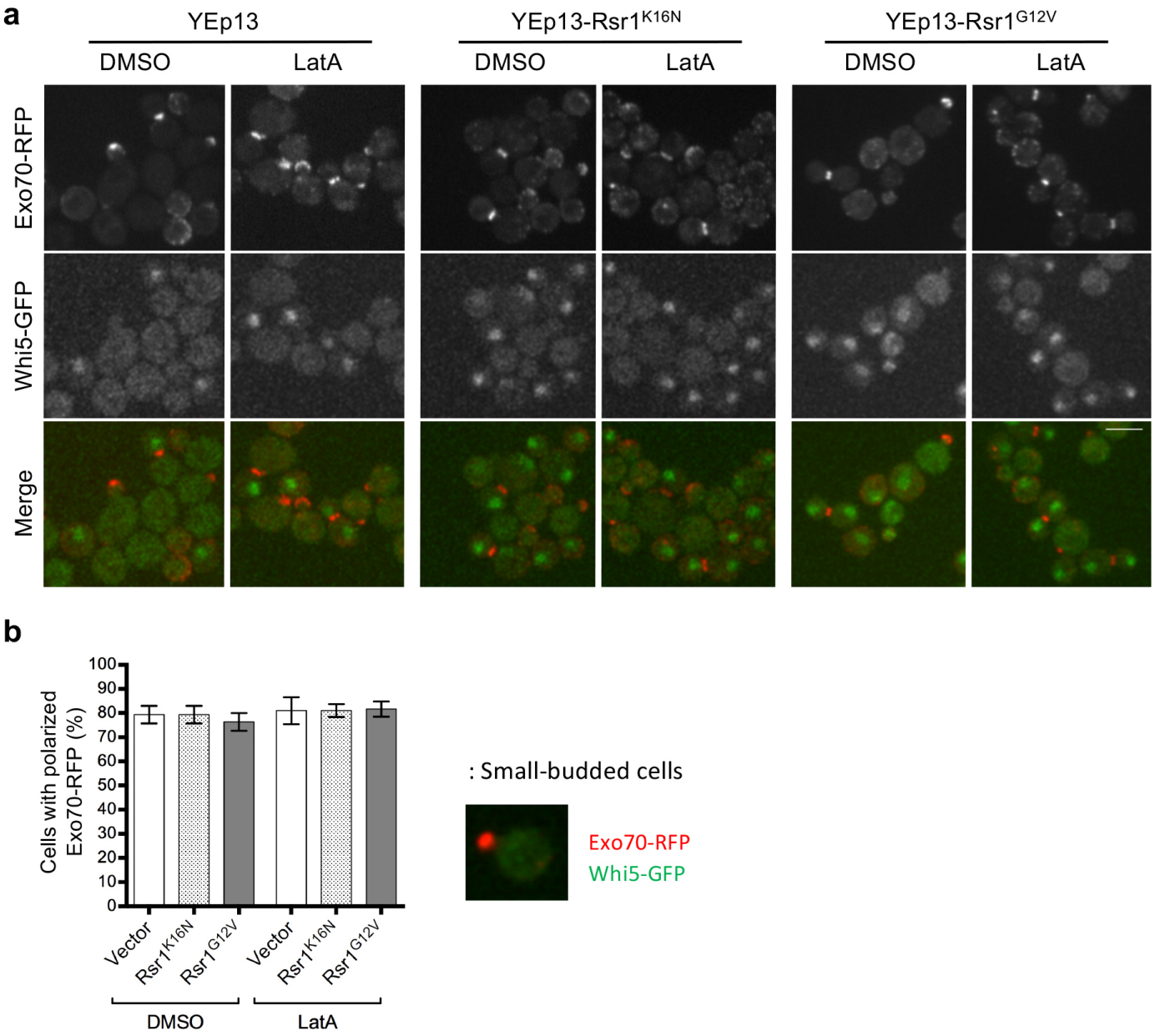

Fig. S6. Exo70 polarization in haploid cells treated with LatA or DMSO. (a) Images of LatA or DMSO-treated WT haploid cells expressing Exo70-RFP and Whi5-GFP and carrying each plasmid as marked. Bar 5μm. (b) The percentage of small-budded cells with polarized Exo70-RFP (n=100-270 for each sample per experiment). An example image of a small-budded cell expressing Exo70-RFP and Whi5-GFP is shown.

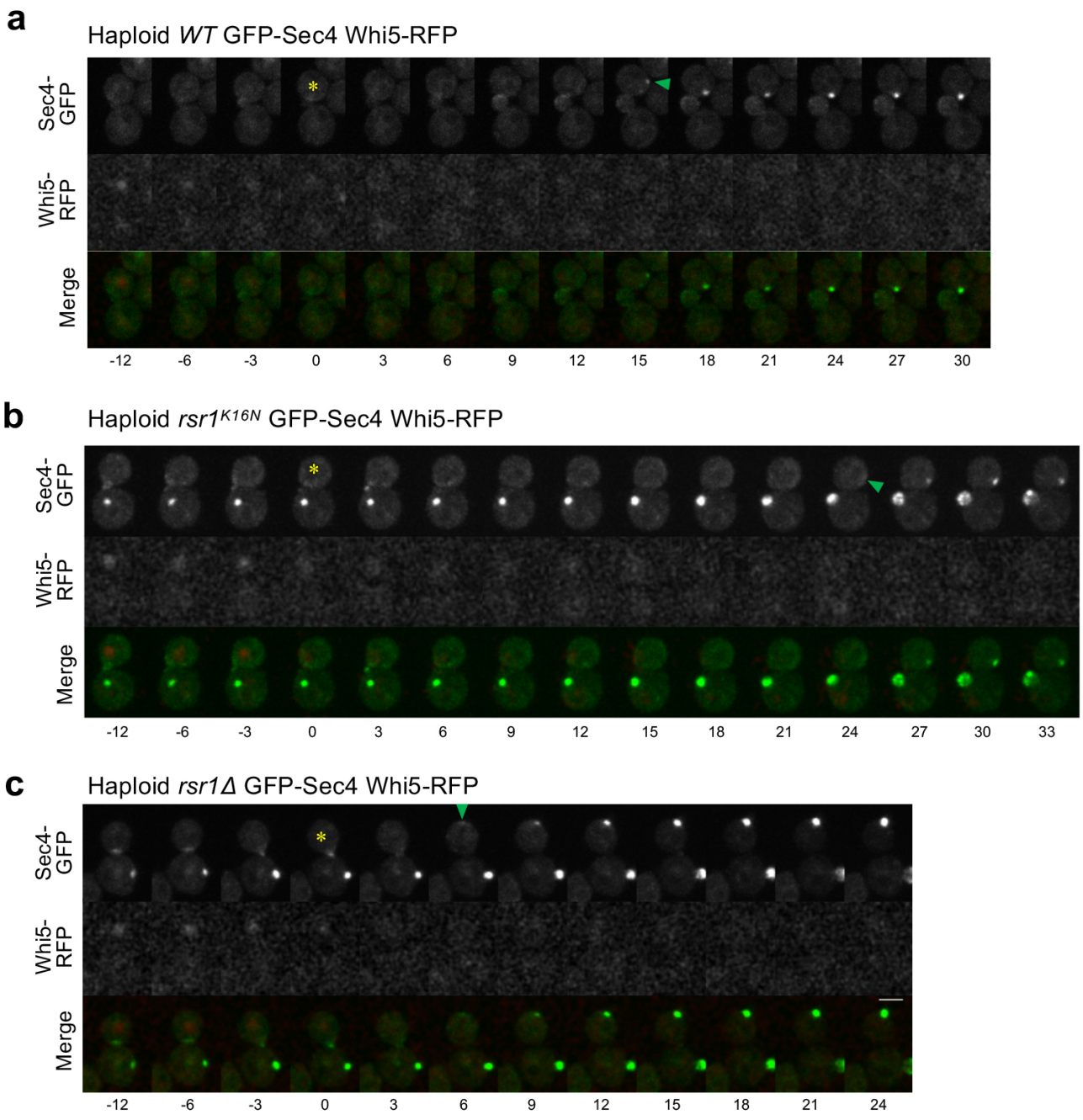

Fig. S7. Sec4 polarization in haploid cells. Time-lapse images of GFP-Sec4 and Whi5-RFP in (a) WT, (b) *rsr1*^{K16N}, and (c) *rsr1*Δ cells at 30°C. Numbers indicate time (min) relative to the T_1-T_2 transition (marked with an asterisk; t=0) in daughter cells. Green arrowheads point the first appearance of polarized GFP-Sec4. Bars, 3 μm.