

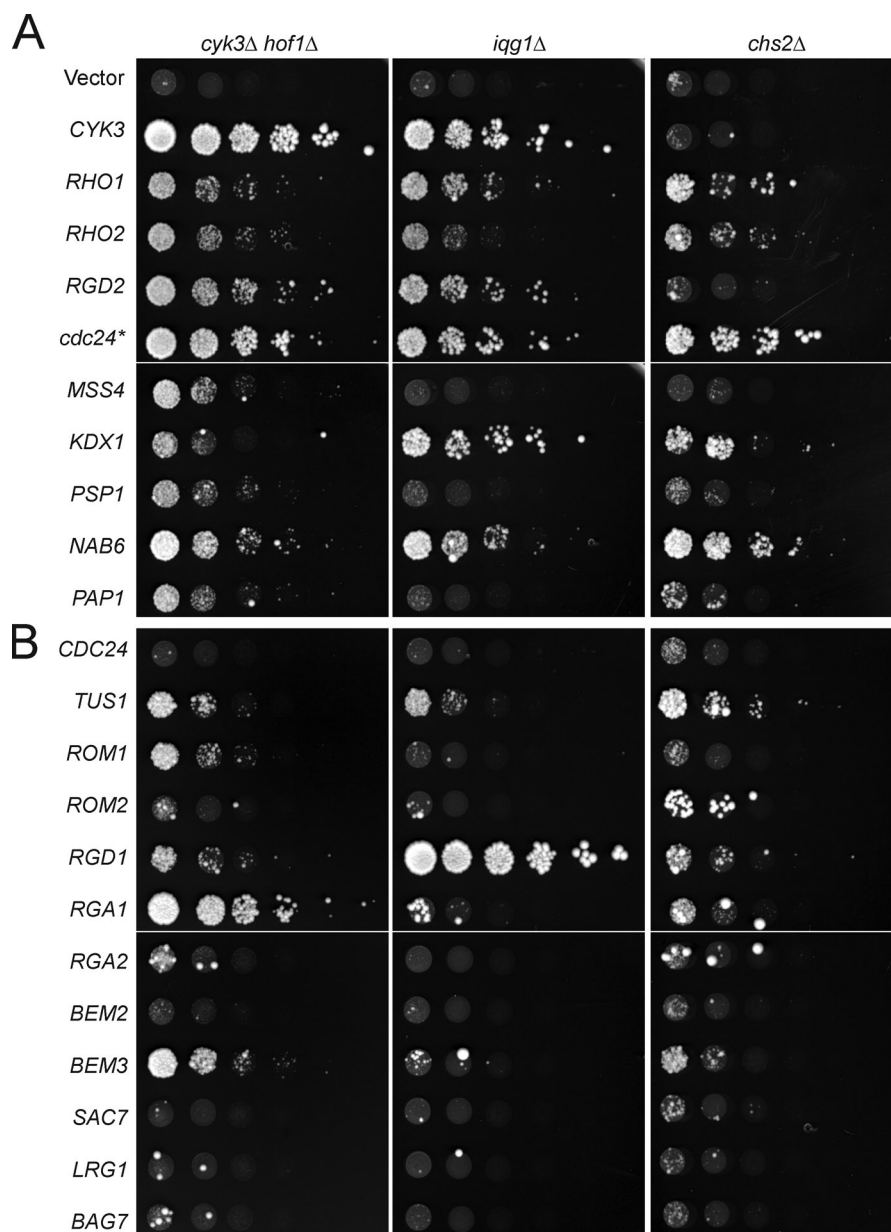
Onishi et al., <http://www.jcb.org/cgi/content/full/jcb.201302001/DC1>

Figure S1. ***iqg1Δ* suppression results and the suppression by some GEFs and GAPs.** This figure complements Fig. 2. (A) An expanded version of the data shown in Fig. 2 A with inclusion also of the *iqg1Δ* (strain RNY2242) suppression results. (B) Suppression of the three cytokinesis mutants by overexpression of certain GEFs and GAPs of Rho1 and Cdc42. This experiment was performed as described in Fig. 2 A; plasmids used are listed in Table 2.

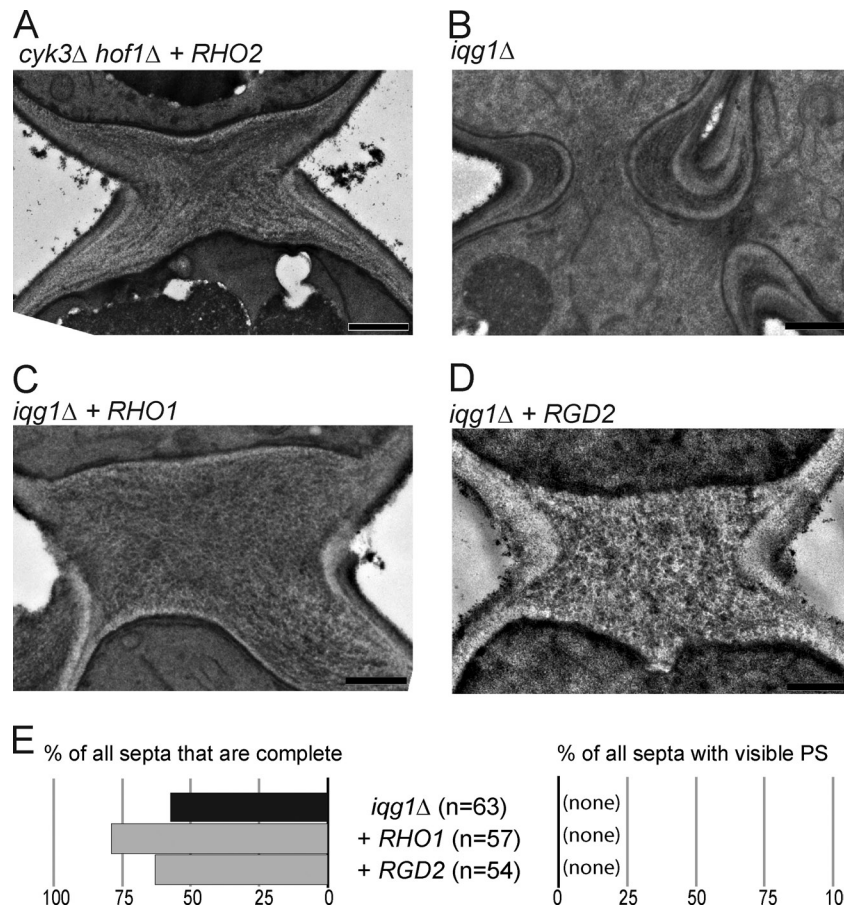


Figure S2. **EM images of *cyk3Δ hof1Δ* and *iqg1Δ* cells expressing some of the suppressors.** This figure complements Fig. 3. (A) Activation of SS formation without restoration of PS formation in a *cyk3Δ hof1Δ* mutant by overexpression of *RHO2* (strain MOY681). See Fig. 3 E for quantification. (B–D) Activation of SS formation in an *iqg1Δ* mutant by overexpression of *RHO1* or *RGD2* (strains KO969, KO1372, and MOY355). Bar, 0.5 μ m. (E) *iqg1Δ* cells from B–D with visible septal structures were scored for successful bud-neck closure (left) or the presence of PS-like structures (right).

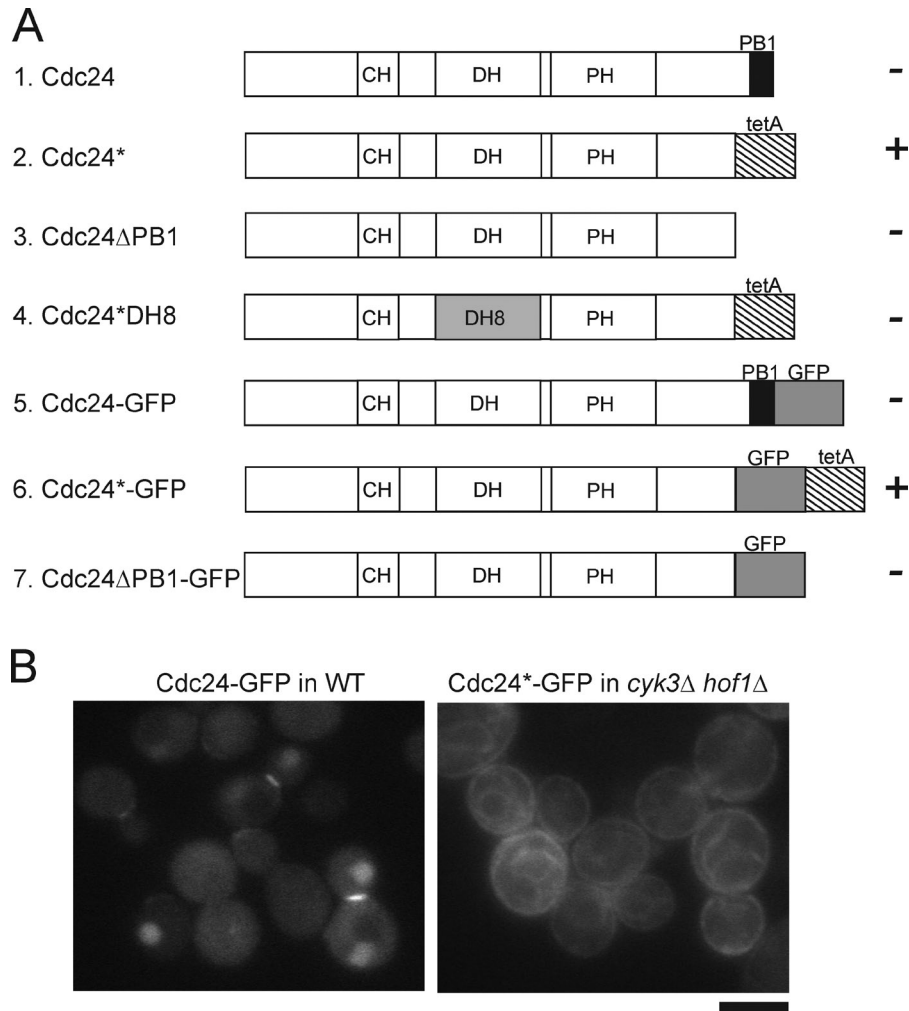


Figure S3. Evidence that suppression of *cyk3* Δ *hof1* Δ by *cdc24 reflects inhibition rather than activation of bud-neck Cdc42 activity.** This figure complements Fig. 2 and Fig. S1 A. (A) Abilities of various constructs to suppress *cyk3* Δ *hof1* Δ (strain MOY68) when expressed from high-copy plasmids (Table 2). (1 and 5) Wild-type *CDC24* showed no detectable suppression (Fig. S1 B) whether or not it was fused to *GFP* sequences. (2) The *cdc24** plasmid isolated in the suppressor screen contained a fragment of *CDC24* ending at position 2,340 (and thus lacking the sequence encoding the C-terminal PB1 domain) and fused in frame to a fragment of the tetracycline resistance gene *tetA* (encoding a transmembrane fragment) in the YEpl3 vector. (6) Suppression ability was retained when *GFP* sequences were inserted between the *CDC24* and *tetA* sequences. (3 and 7) *CDC24* truncated at the same point, but not fused to *tetA*, showed no detectable suppression whether or not it was fused to *GFP* sequences. (4) A plasmid encoding Cdc24* with N452G and E453G mutations (DH8; Mionnet et al., 2008) showed no detectable suppression, indicating that Cdc42 binding is required for suppression. CH, calponin-homology domain; DH, Dbl homology RhoGEF domain; PH, pleckstrin homology domain; PB1, Phox and Bem1 domain. (B) Failure of Cdc24* to accumulate at the division site. (left) Wild-type (WT; YEF473A) cells expressing Cdc24-GFP showed nuclear and bud-neck localization of full-length Cdc24. (right) *cyk3* Δ *hof1* Δ cells (MOY68) expressing Cdc24*-GFP showed only disseminated plasma and intracellular membrane localization of Cdc24*. Growth was on SC-Leu (left) or SC-Leu + 5-FOA (right) medium. Bar, 5 μ m.

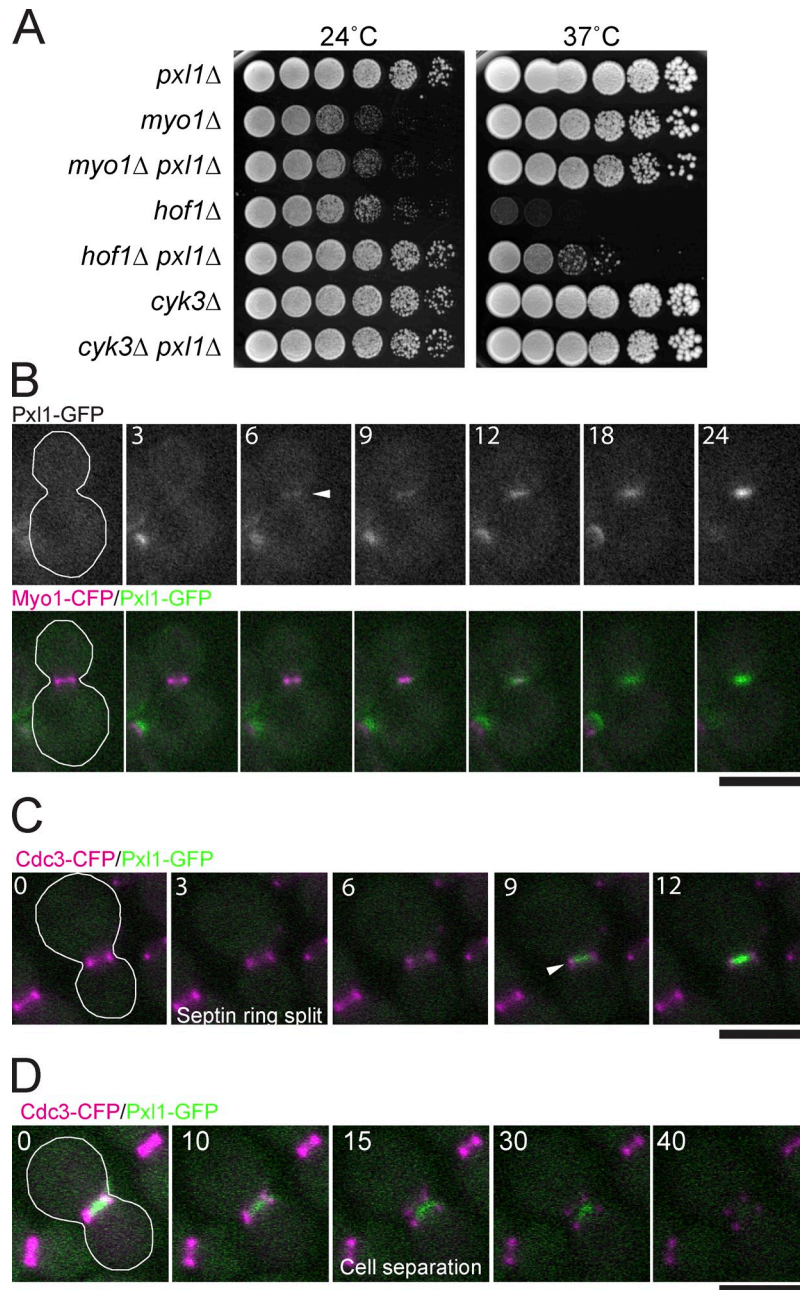


Figure S4. **Possible role of the Paxillin homologue Pxl1 in regulating SS formation.** The yeast paxillin homologue Pxl1 was originally identified as an allele-specific dosage suppressor of *cdc42* point mutations (Gao et al., 2004). Pxl1 appears from both genetic and biochemical evidence to be a negative regulator of Rho1 and localizes to sites of polarized growth, including the bud neck (Gao et al., 2004; Mackin et al., 2004), but whether it has a role in cytokinesis is unknown. (A–D) Deletion of *PXL1* restored the growth defects *cyk3Δ hof1Δ* (Fig. 2 B) and of *hof1Δ* (A), possibly through activation of SS formation, and that Pxl1-GFP localizes to the division site at the timing and pattern consistent with SS formation (B–D). Unlike its homologue in *S. pombe* (Ge and Balasubramanian, 2008), *pxl1Δ* cells did not show any synthetic growth defect with *myo1Δ* (A), defects in AMR integrity (not depicted), or localization to the division site during AMR constriction (B), indicating that the roles of Pxl1 in cytokinesis slightly differ between the organisms, despite the similar molecular function in inhibiting Rho1 (Pinar et al., 2008). (A) Genetic interaction between *pxl1Δ* and *hof1Δ* but not *myo1Δ* or *cyk3Δ*. Strains RNY469 (*myo1Δ* [*URA3 MYO1*]) and MOY428 (*myo1Δ pxl1Δ* [*URA3 MYO1*]) were streaked on an SC + 5-FOA plate to eliminate the *URA3 MYO1* plasmids, spotted as in Fig. 2 A on YPD plates along with strains of the other indicated genotypes (MOY404, RNY2299, MOY445, MOY585, and MOY429), and incubated at the indicated temperature for 3 d. (B–D) Localization of Pxl1-GFP during cytokinesis. Strains MOY526 (*MYO1-CFP PXL1-GFP*; B) and MOY403 (*PXL1-GFP*; C and D) carrying plasmid YCp111-CDC3-CFP were cultured at 24°C to exponential phase in SC medium and observed by fluorescence microscopy. (B and C) Cells early in cytokinesis. Arrowheads show Pxl1-GFP at the division site. (D) A cell late in cytokinesis and cell separation. Times (minutes) from the start of observation in each series are indicated. Lines show cell outlines. Bars, 5 μm.

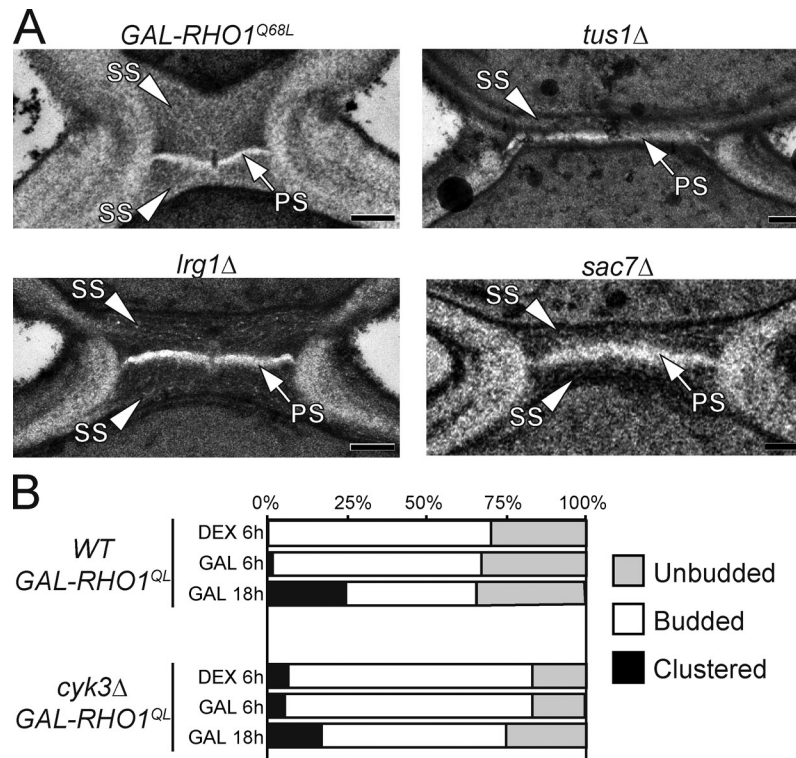


Figure S5. **Supporting evidence of cytokinesis defects caused by ectopically activated Rho1.** This figure complements Fig. 4. (A) Septum morphologies of a *GAL-RHO1^{Q68L}* strain (KN143) cultured in YMP (1% raffinose + 2% galactose) for 12 h and of *tus1Δ* (RNY879), *lrg1Δ* (MOY407), and *sac7Δ* (MOY405) mutants. Bars, 0.2 μ m. (B) Effect of constitutively active Rho1 on the cell cycle. *GAL-RHO1^{Q68L}* (KN143) and *cyk3Δ GAL-RHO1^{Q68L}* (MOY691) cells were cultured in YMP with either 2% dextrose (DEX) or 1% raffinose + 2% galactose (GAL) for the indicated times. More than 200 cells were counted per sample. WT, wild type.

References

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