Geymonat et al., http://www.jcb.org/cgi/content/full/jcb.200905114/DC1

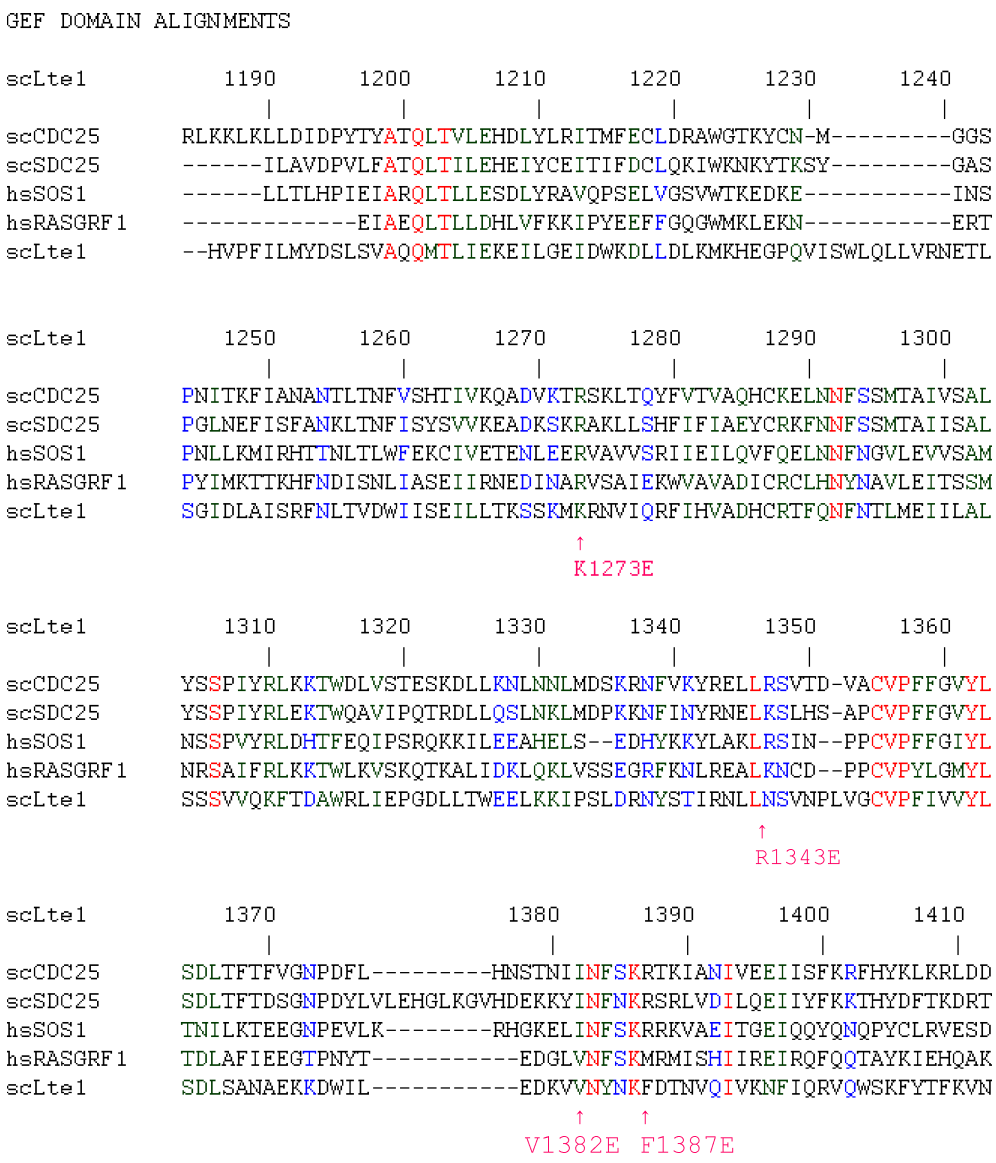


Figure S1. **Protein sequence alignment between the GEF domains of *S. cerevisiae* Cdc25 and Sdc25, hsSOS1, hsRASGRF1, and the putative GEF domain of scLte1.** Alignments were made using Clustal W (http://npsa-pbil.ibcp.fr; Chenna et al., 2003). Upward arrows indicate point mutations made for this study. Red indicates identity, green indicates strong similarity, and blue indicates weak similarity.

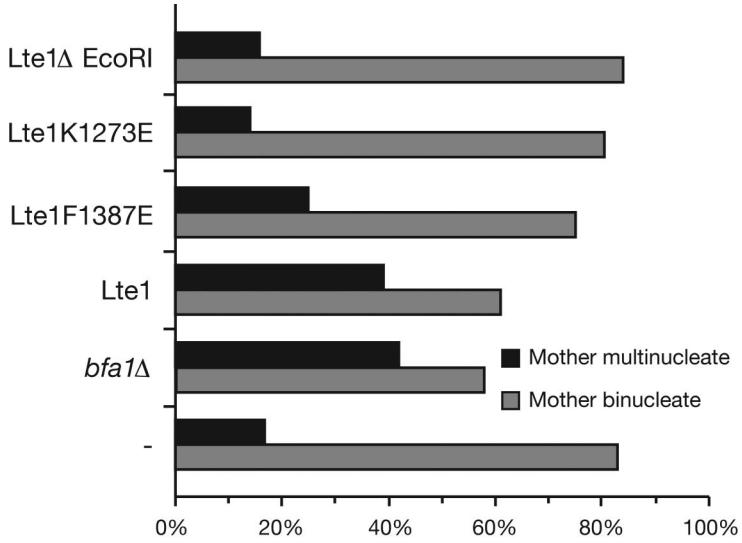
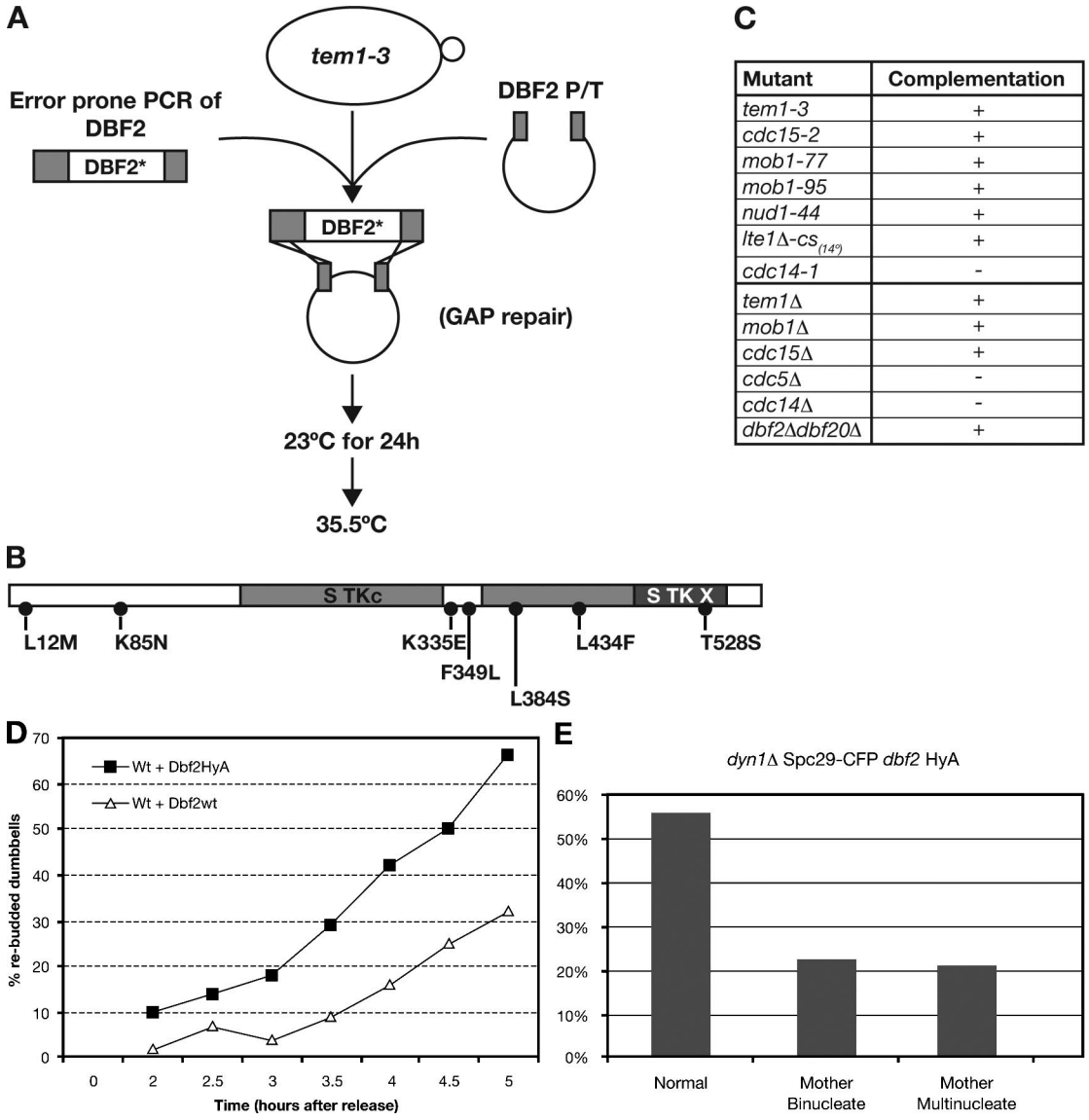


Figure S2. **Assay for the bypass of the SPoC by overexpression of different alleles of *LTE1*.** *dyn1Δ SPC29-CFP* (MGY396), *dyn1Δ bfa1Δ SPC29-CFP* (MGY406), and MGY396 transformed with plasmid expressing Lte1 or Lte1-F1387E, -K1273E, or -ΔEcoRI under control of the *GAL1-10* promoter were cultivated in 2% YEP-galactose overnight at 14°C. Bi- and multinucleate cells were counted and represented as a percentage of each other ($n > 100$).



C

Mutant	Complementation
tem1-3	+
cdc15-2	+
mob1-77	+
mob1-95	+
nud1-44	+
lte1Δ-cs$_{(14^{o})}$	+
cdc14-1	-
tem1Δ	+
mob1Δ	+
cdc15Δ	+
cdc5Δ	-
cdc14Δ	-
dbf2Δdbf20Δ	+

Figure S3. **Isolation and characterization of the *dbf2* HyA allele.** (A) The *dbf2* HyA allele was generated using an error-prone PCR whose products were cotransformed into a *tem1-3* strain with a linearized plasmid containing the promoter and the terminator of *DBF2*, allowing gap repair of the construct to occur by homologous recombination. (B) The HyA allele contains seven substitutive mutations: L12M, K85N, K335E, F349L, L384S, L434F, and T528S. (C) Ability of *dbf2* HyA allele to complement different MEN mutants. (D) The *dbf2* HyA allele bypasses the spindle assembly checkpoint in wild-type (Wt) cells as determined by scoring cell rebudding after α-factor arrest and release into 15 μg/ml of nocodazole-containing medium. (E) The *dbf2* HyA allele bypasses the SPoC, as determined by the accumulation of multinucleate cells in a *dyn1Δ* strain cultivated overnight at 14°C (n > 100).

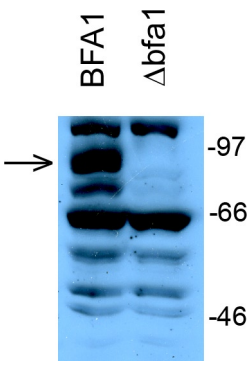
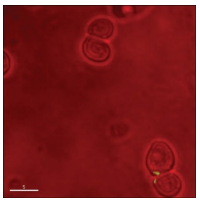
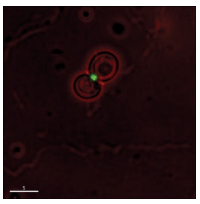


Figure S4. **Specificity assay for the purified rabbit anti-Bfa1 antibody.** A Western blot of soluble protein extracts from *S. cerevisiae* 15D and MGY389 *bfa1Δ* was probed using the rabbit anti-Bfa1 antibody. The arrow indicates the major position of an 85-kD protein that is absent in extracts from the *bfa1Δ* mutant. Molecular mass is indicated in kilodaltons.



Video 1. **The polarity cap behavior in wild-type cells.** Polarity cap localization in wild-type LTE1 cultivated at 30°C was monitored by fluorescence microscopy of Spa2- and Kel1-GFP in *S. cerevisiae* MGY308. The video is shown at 1.5 frames/s. Bar, 5 μm.



Video 2. **How polarity cap behavior is affected in an *lte1Δ* mutant.** Polarity cap localization in *lte1Δ* mutants at 30°C was monitored by fluorescence microscopy of Spa2- and Kel1-GFP in *S. cerevisiae* MGY309. The video is shown at 1.5 frames/s. Bar, 5 μm.

Reference

Chenna, R., H. Sugawara, T. Koike, R. Lopez, T.J. Gibson, D.G. Higgins, and J.D. Thompson. 2003. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res.* 31:3497–3500. doi:10.1093/nar/gkg500