Geymonat et al., http://www.jcb.org/cgi/content/full/jcb.201005070/DC1

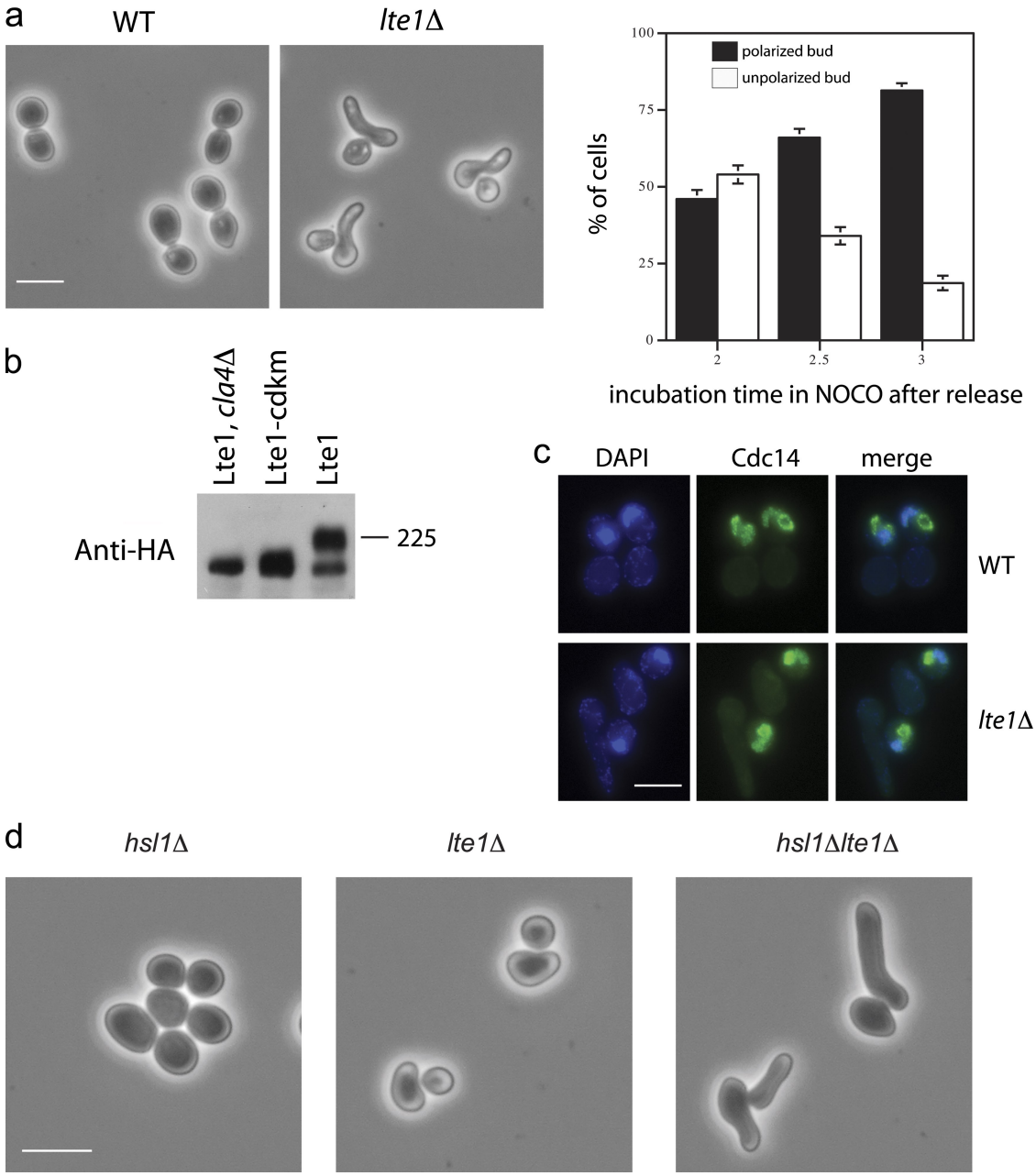


Figure S1. **Cell polarization in synchronized arrested cells does not depend on Cdc14 release.** (a) Wild-type (MGY302) and *lte1Δ* mutants (MGY296) were arrested with α-factor and released into nocodazole-containing medium. The degree of polarization was monitored for 3 h. (Left) Cell morphology after 3 h. (Right) Quantification of polarity ($n > 150$). Error bars show the standard deviation of three experiments. (b) Wild-type cells expressing Lte1-3HA (MGY205) or Lte-Cdk-3HA (MGY370) and *cla4Δ* mutants expressing Lte1-3HA (SY157) were arrested in metaphase with nocodazole. Lte1 was visualized by Western blotting. (c) GFP-Cdc14 was visualized in cells used in panel a after 3 h of arrest. Bar, 5 µm. (d) *hsl1Δ* (MGY321), *lte1Δ* (SY144), and *hsl1Δ lte1Δ* (MGY319) cells were treated with nocodazole for 2.5 h at 30°C.

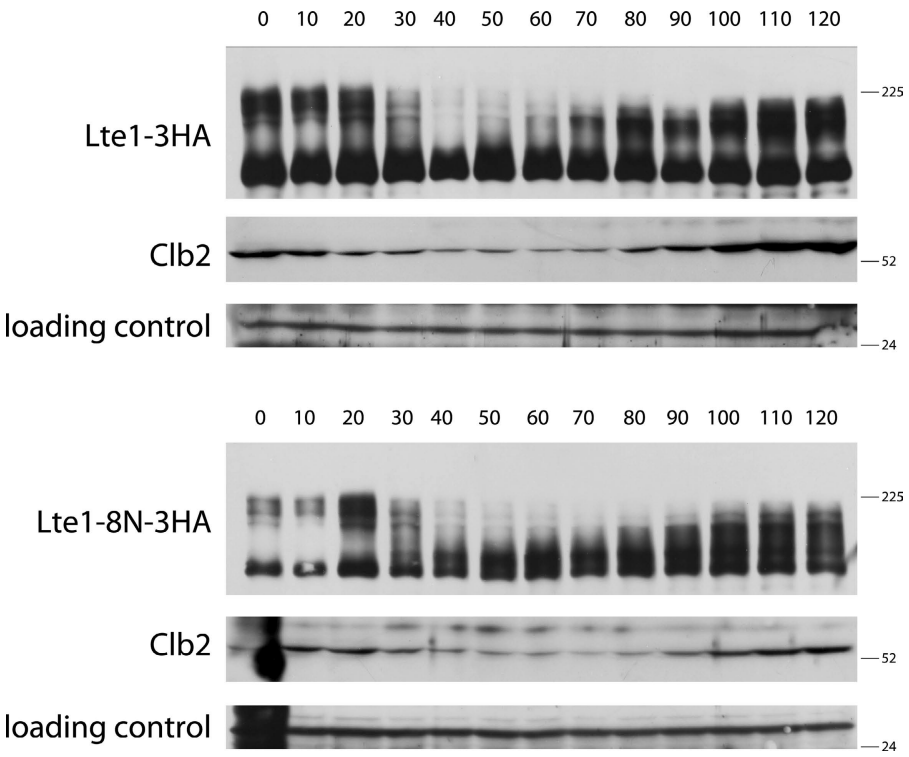


Figure S2. **Cell cycle phosphorylation patterns of wild-type Lte1 and Lte1-8N.** *dbf2-2* mutants expressing Lte1-3HA (MGY583; top panels) or Lte1-8N-3HA (MGY584; bottom panels) were arrested at 37°C for 2 h then released at 23°C. Samples were collected at 10-min intervals. Lte1-3HA and Clb2 were visualized by Western blotting. An unspecific 26-kD protein revealed by the anti-Clb2 antibody served as a loading control.

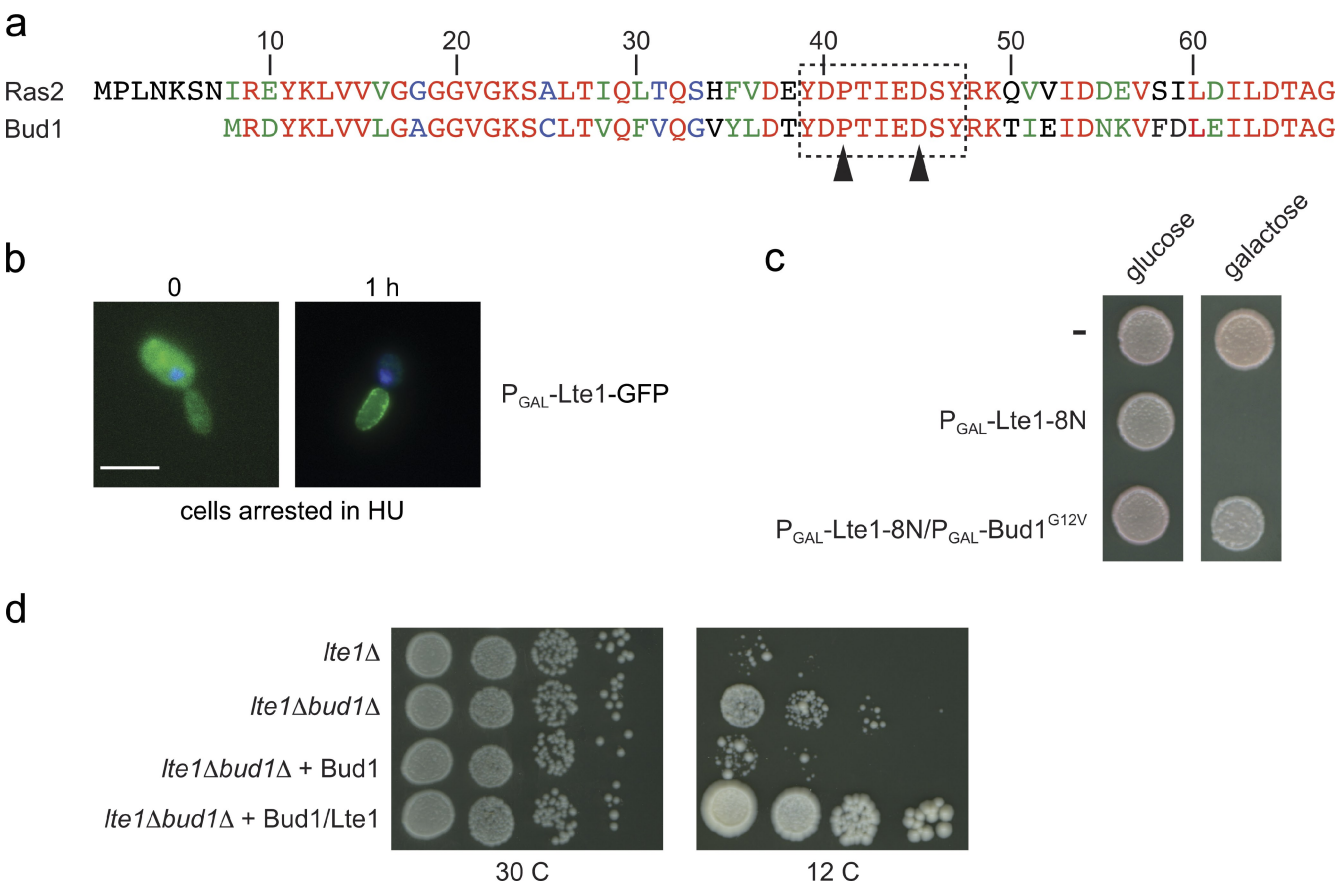


Figure S3. **Bud1 interacts genetically with Lte1.** (a) Alignment of the N-terminal region of Ras2 and Bud1. The dotted line identifies the effector domain and arrows indicate mutations that in Ras2 impair Ras–Lte1 interaction (Seshan and Amon, 2005). (b) Cells expressing Lte1-GFP under *GAL1* promoter control (SY148) were cultivated in YP-sucrose and arrested in HU for 2 h. Galactose was then added and Lte1-GFP was analyzed after 1 h. (c) 5×10^3 cells expressing Lte1-8N-GFP (MGY449) alone or in combination with $Bud1^{Val12}$ (MGY555) under *GAL1* promoter control were spotted on YP-glucose or YP-galactose and incubated for 3 d at 30°C. (d) *lte1Δ* (SY144), *lte1Δ bud1Δ* (MGY516), *lte1Δ bud1Δ* transformed with a plasmid expressing Bud1 (MGY530) alone or in combination with a plasmid expressing Lte1 (MGY533) were spotted on YPD plates and incubated at 30°C for 2 d or at 12°C for 20 d.

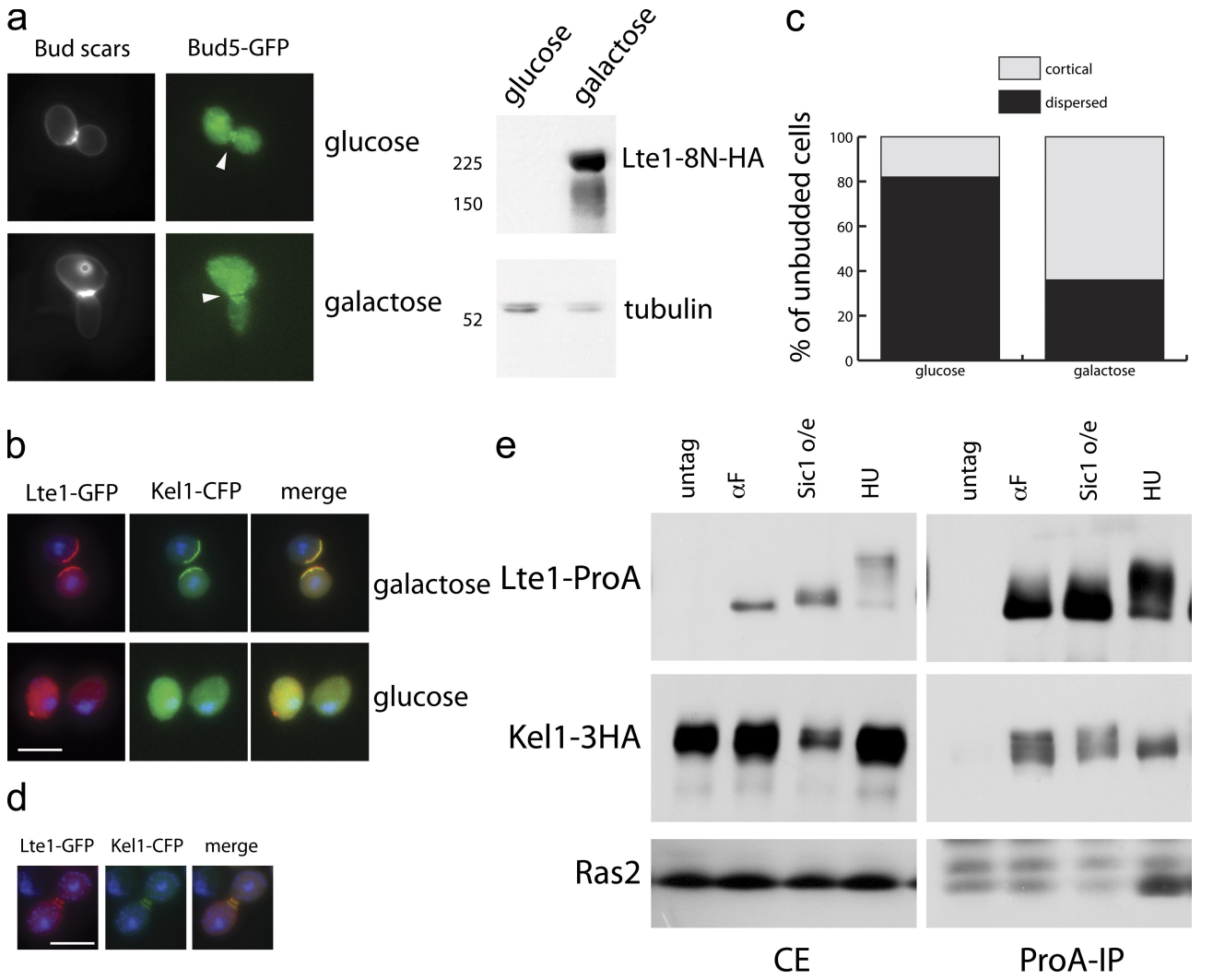
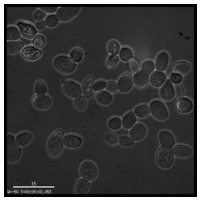
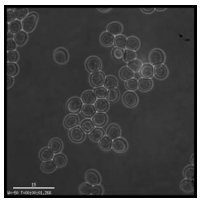


Figure S4. **Lte1-Kel1 localization and interaction during cell cycle arrest points.** (a) Cells expressing Bud5-GFP and Lte1-8N-HA under the control of the *GAL1* promoter (MGY589) were cultivated in glucose or galactose. Bud scars and Bud5-GFP were observed. Arrows indicate Bud5 signal at the bud neck. Levels of Lte1-8N were also analyzed by Western blotting in the two conditions. (b and c) Cells expressing Lte1-GFP from a *MET3* promoter, $CDC42^{G12V}$ from a *GAL1* promoter, and Kel1-CFP (MGY574) were cultivated in minimal medium without methionine and 2% sucrose. The culture was then split and 2% glucose or galactose was added for an additional 2 h. Cells were fixed and unbudded cells were counted for cortical or dispersed signals of Lte1 and Kel1 ($n > 150$). (d) Example of double ring staining of Lte1 and Kel1 at the neck in cells described in b. (e) Cells expressing Lte1-ProA and Kel1-3HA were arrested in α-factor, HU, or by Sic1 overexpression. Protein A–tagged proteins were immunoprecipitated and associated Kel1-3HA or Ras2 was detected by Western blotting.



Video 1. ***lte1Δ* cells expressing nondegradable Clb2 under *GAL1* promoter control (MGY218) were cultivated in YP plus 2% sucrose.** Cells were then transferred to an agar plug containing 2% galactose and observed under the microscope in a 30°C chamber for 12–14 h. Images were taken every 10 min.



Video 2. **Wild-type cells expressing nondegradable Clb2 under *GAL1* promoter control (MGY232) were cultivated in YP plus 2% sucrose.** Cells were then transferred to an agar plug containing 2% galactose and observed under the microscope in a 30°C chamber for 12–14 h. Images were taken every 10 min.

Reference

Seshan, A., and A. Amon. 2005. Ras and the Rho effector Cla4 collaborate to target and anchor Lte1 at the bud cortex. *Cell Cycle.* 4:940–946.