

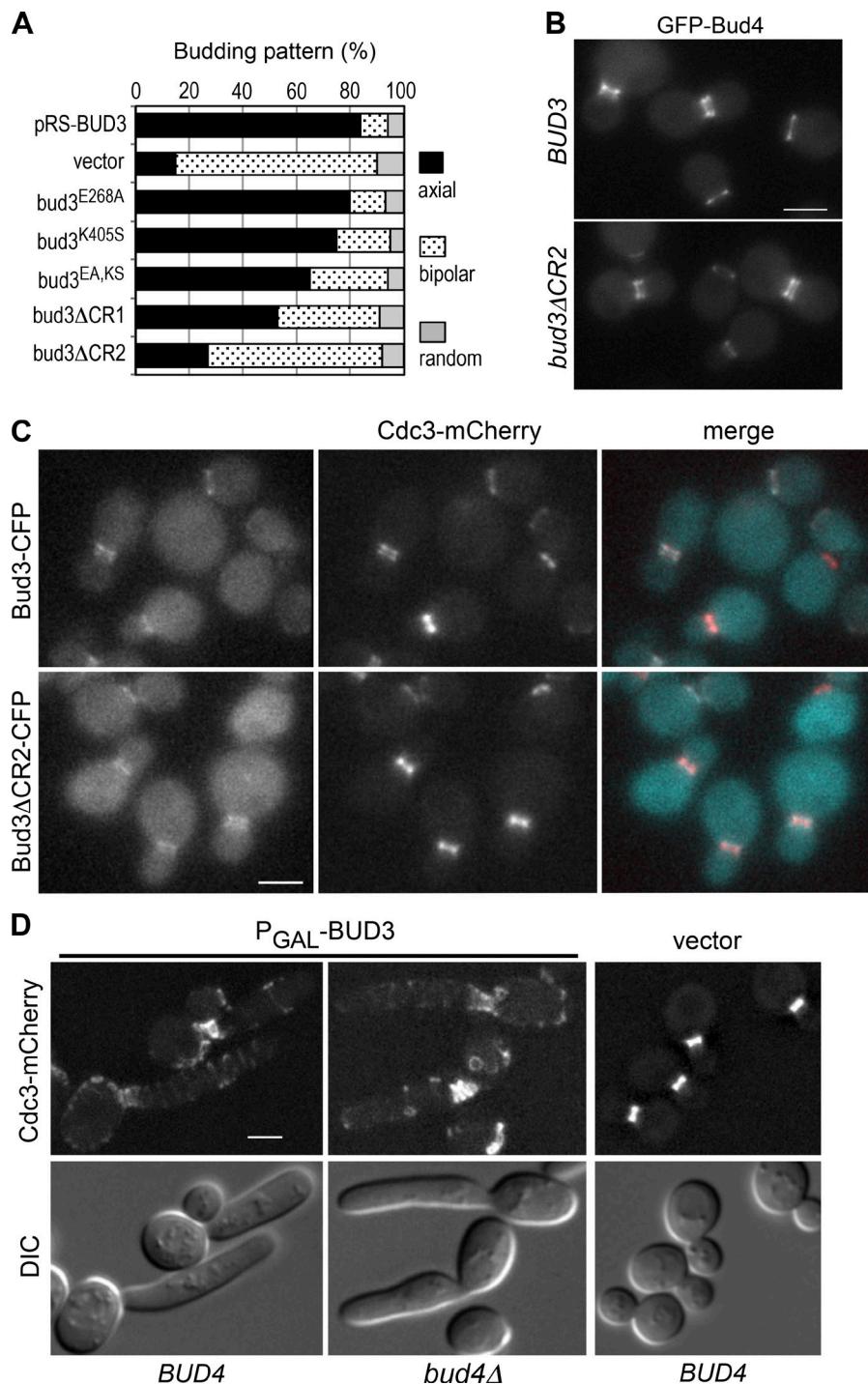
Kang et al., <http://www.jcb.org/cgi/content/full/jcb.201402040/DC1>

Figure S1. Characterization of *bud3* DH domain mutants and *BUD3* overexpression. (A) Budding pattern of a *bud3Δ* mutant carrying the *WT* or each *bud3* mutant on a multicopy plasmid. Cells were grown overnight in SC-Leu. *bud3^{EA, KS}* denotes the *bud3^{E268A, K405S}* mutation. See also Fig. 1 A. (B) Localization of GFP-Bud4 in *WT* (top) and in *bud3ΔCR2* (bottom) cells. (C) Colocalization of Cdc3-mCherry with Bud3-CFP (top) or Bud3^{ΔCR2}-CFP (bottom). Bud3-CFP (cyan) and Cdc3-mCherry (red) in merged images. (D) Localization of Cdc3-mCherry in *BUD4* or *bud4Δ* cells overexpressing *BUD3* from the *GAL1* promoter on a plasmid and in *BUD4* cells carrying a vector control. Cells were grown in SRaf-Ura and then shifted to SGal-Ura for 6 h at 30°C. Images on the top were deconvolved. DIC, differential interference contrast. Bars, 3 μm.

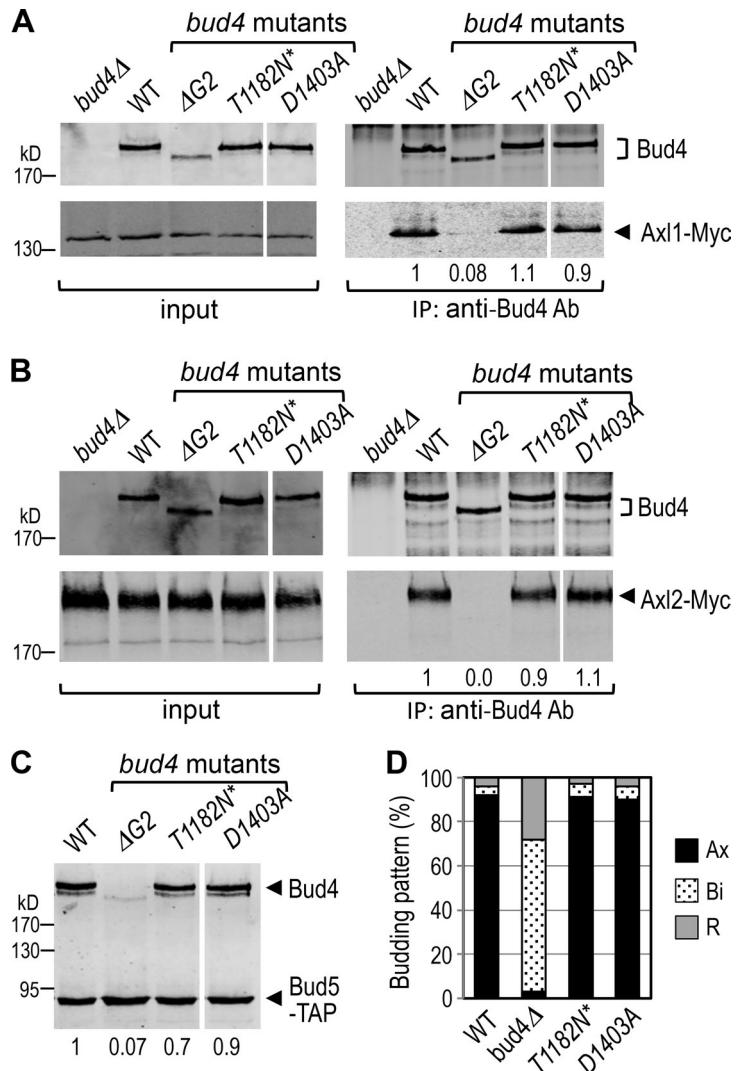


Figure S2. The GDP-locked (or nucleotide free) form of Bud4 is not defective in the axial landmark assembly and bud site selection. (A and B) Coimmunoprecipitation of Axl1-Myc and Axl2-Myc with Bud4. Extracts prepared from the *AXL1-Myc* (A) and *AXL2-Myc* (B) strains, each of which expresses either WT or a *bud4* mutant—*bud4^Δ*, *bud4^{ΔG2}*, *bud4^{T1182N, K1183S}* ($T1182N^*$), or *bud4^{D1403A}*—were subjected to immunoprecipitation using anti-Bud4 antibodies. The *bud4^{ΔG2}* mutant carries a deletion of the G2–G4 boxes (Kang et al., 2012). The *bud4^{T1182N, K1183S}* (which carries a mutation analogous to *ras^{S17N}*) and *bud4^{D1403A}* (which is analogous to *ras^{D119N}*) are expected to express the GDP-locked (or nucleotide free) Bud4. Input and immunoprecipitated proteins were analyzed by immunoblotting with anti-Bud4 antibodies (top) and the anti-Myc antibody (bottom). Numbers indicate relative amounts of Axl1-Myc (A) or Axl2-Myc (B) recovered with each WT or mutant Bud4 protein, normalized to the recovery with the WT Bud4. The *bud4^{D1403A}* mutant was analyzed on the same gel, but the lane was separated by another sample, and thus dividing lines were added in A–C. (C) Copurification of WT or mutant Bud4 with Bud5-TAP. Extracts prepared from the *BUD5-TAP* strains, each of which carries either WT or a mutant *BUD4* allele, were subjected to IgG-agarose pull-down assays. Bud4 and Bud5-TAP were detected with anti-Bud4 antibodies. Numbers indicate normalized WT and mutant Bud4 proteins copurified with Bud5-TAP. (D) Budding pattern (percentage) of haploid WT (HPY210) and *bud4* mutants. Cells were grown at 30°C in YPD. IP, immunoprecipitation.

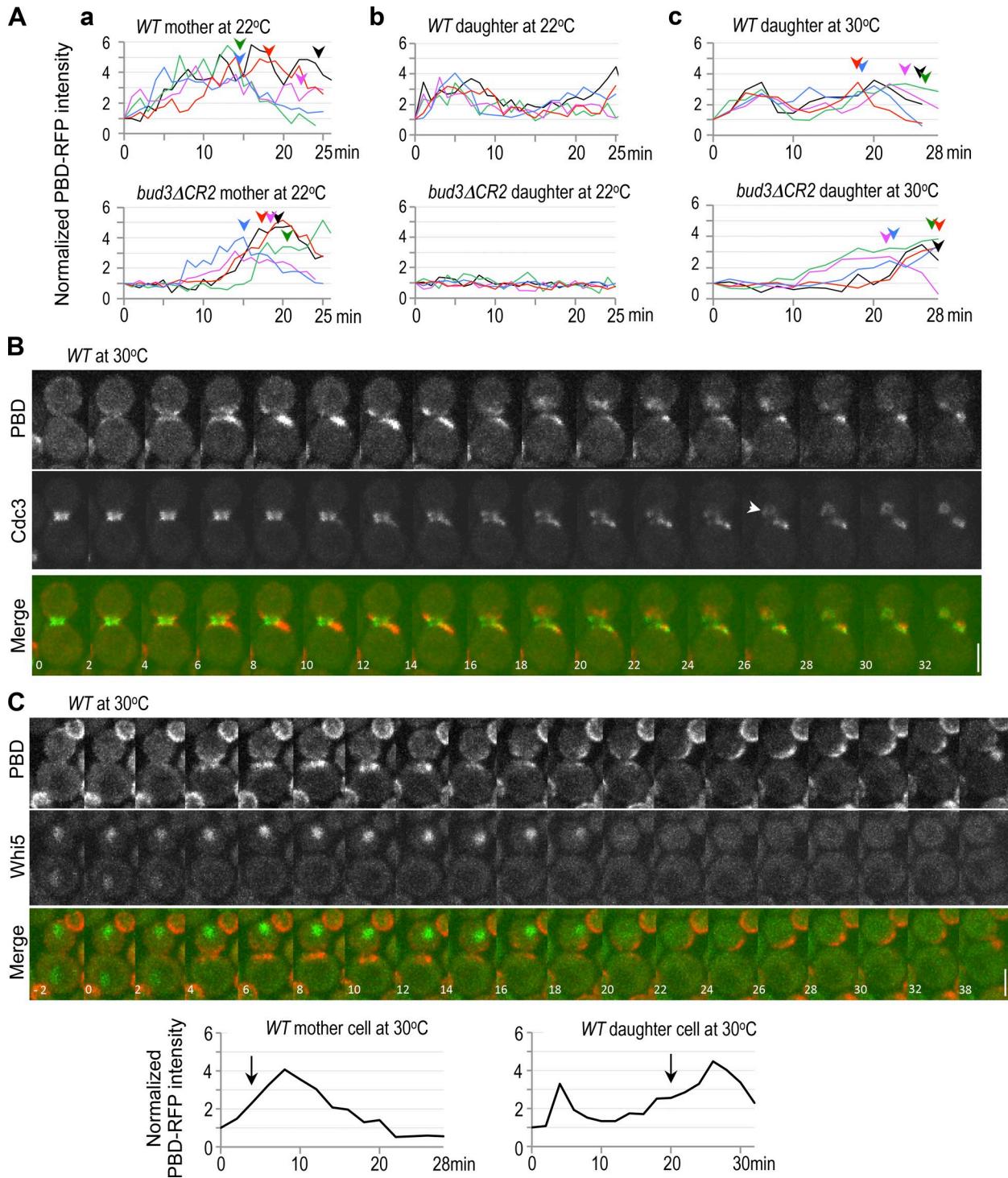
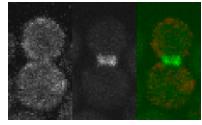
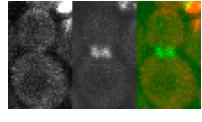


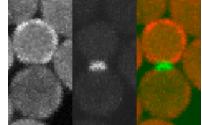
Figure S3. Quantification of PBD-RFP and localization of PBD-RFP and Whi5-GFP. (A) PBD-RFP intensity is compared in mother cells (a) and daughter cells (b) of WT and *bud3ΔCR2* at 22°C (including those shown in Fig. 5 C) and daughter cells (c) of WT and *bud3ΔCR2* at 30°C. PBD-RFP intensity was normalized to intensity at the onset of cytokinesis ($t = 0$). Each colored arrowhead marks the time point when new septin clouds were first visible (or estimated) in each mother cell at 22°C (a) or in each daughter cell at 30°C (c) for each corresponding colored line. (B) Time-lapse imaging of PBD-RFP and Cdc3-GFP in WT cells at 30°C. Arrowheads mark the first appearance of a new septin in a daughter cell. Numbers indicate time (in minutes) from the onset of cytokinesis ($t = 0$). Quantification of PBD-RFP intensity in the daughter cell is shown in A (c, purple line on the top). (C) Time-lapse imaging of PBD-RFP and Whi5-GFP in a haploid WT cell at 30°C. (bottom) PBD-RFP intensity at each time point is normalized to intensity at $t = 0$. Arrows mark time points when Whi5-GFP completely exited from the nucleus. Bars, 3 μ m.



Video 1. **Localization of Cdc42-GTP in haploid WT yeast cells.** Gic2-PBD-tdTomato (left), Cdc3-GFP (middle), and merge (right) are shown from time-lapse images of haploid WT cells. Images were captured every minute using a spinning-disk confocal microscope (UltraVIEW Vox CSU-X1 system; PerkinElmer) at 22°C. The video shows frames for 25 min starting from the onset of cytokinesis. Display rate is 8 frames/s. Selected images are shown in Fig. 5 A.



Video 2. **Localization of Cdc42-GTP in the *bud3ΔCR2* mutant cells.** Gic2-PBD-tdTomato (left), Cdc3-GFP (middle), and merge (right) are shown from time-lapse images of haploid *bud3ΔCR2* cells. Images were captured every minute using a spinning-disk confocal microscope (UltraVIEW Vox CSU-X1 system; PerkinElmer) at 22°C. The video shows frames for 25 min starting from the onset of cytokinesis. Display rate is 8 frames/s. Selected images are shown in Fig. 5 B.



Video 3. **Localization of Cdc42-GTP in the *cdc24-4* mutant cells.** Gic2-PBD-tdTomato (left), Cdc3-GFP (middle), and merge (right) are shown from time-lapse images of *cdc24-4* cells. Images were captured at 2-min intervals using a spinning-disk confocal microscope (UltraVIEW Vox CSU-X1 system; PerkinElmer) at 37°C. The video shows frames for 1 h and 26 min, starting from 2 h and 12 min after shifting to 37°C. Display rate is 8 frames/s. Selected images are shown in Fig. 5 D.

Table S1. Yeast strains used in this study

| Strain | Relevant genotype | Source |
|------------------------|---|---------------------------|
| BY4741 ^a | <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i> | GE Healthcare |
| DDY1301 ^a | <i>CDC42-LEU2 his3Δ200 leu2-3, 112 lys2-801 ura3-52</i> | Kozminski et al., 2000 |
| DDY1304 ^a | <i>cdc42-101-LEU2</i> | Kozminski et al., 2000 |
| DDY1327 ^a | <i>cdc42-118-LEU2</i> | Kozminski et al., 2000 |
| DDY1336 ^a | <i>cdc42-123-LEU2</i> | Kozminski et al., 2000 |
| EGY48 | <i>his3 trp1 ura3 LexA_{opplx67}-LEU2</i> | Gyuris et al., 1993 |
| HPY2623 | <i>his3 trp1 ura3 LexA_{opplx67}-LEU2 rsr1Δ::URA3</i> | This study |
| HPY210 ^b | <i>his3Δ200 leu2Δ1 lys2-801 trp1Δ63 ura3-52</i> | Singh et al., 2008 |
| IH2608 ^c | <i>leu2 trp1 ura3 his4 bud3::TRP1</i> | Herskowitz Lab collection |
| HPY16 ^d | <i>ura3-52 trp1Δ63 leu2 his3Δ1 pep4-3</i> | Park et al., 1993 |
| HPY906 ^a | <i>AXL1-TAP-HIS3</i> | GE Healthcare |
| HPY2128 ^a | <i>rho2Δ::kanMX4</i> | GE Healthcare |
| HPY2129 ^a | <i>rho4Δ::kanMX4</i> | GE Healthcare |
| HPY657 ^a | <i>rho5Δ::kanMX4</i> | GE Healthcare |
| HPY1023 ^b | <i>bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1028 ^b | <i>bud4^{T1182N, K1183S}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1031 ^b | <i>bud4^{D1403A}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1446 ^a | <i>BUD5-TAP-HIS3</i> | GE Healthcare |
| HPY1447 ^a | <i>AXL1-TAP-HIS3 bud3Δ::URA3</i> | Kang et al., 2012 |
| HPY1458 ^a | <i>BUD5-TAP-HIS3 bud3Δ::URA3</i> | Kang et al., 2012 |
| HPY1461 ^a | <i>AXL1-TAP-HIS3 bud3ΔCR2-LEU2:bud3Δ::URA3</i> | This study |
| HPY1462 ^b | <i>BUD3-LEU2:bud3Δ::URA3 GFP-BUD4-TRP1:bud4Δ::kanMX4</i> | This study |
| HPY1463 ^b | <i>bud3ΔCR2-LEU2:bud3Δ::URA3 GFP-BUD4-TRP1:bud4Δ::kanMX4</i> | This study |
| HPY1488 ^a | <i>BUD5-TAP-HIS3 bud3ΔCR2-LEU2:bud3Δ::URA3</i> | This study |
| HPY1493 ^a | <i>BUD5-TAP-HIS3 bud3Δ::URA3 AXL1-Myc₁₃-kanMX6</i> | Kang et al., 2012 |
| HPY1495 ^a | <i>BUD5-TAP-HIS3 bud3ΔCR2-LEU2:bud3Δ::URA3 AXL1-Myc₁₃-kanMX6</i> | This study |
| HPY1496 ^a | <i>BUD5-TAP-HIS3 AXL1-Myc₁₃-kanMX6</i> | Kang et al., 2012 |
| HPY1503 ^d | <i>AXL2-Myc₁₃-kanMX6 bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1514 ^d | <i>BUD3-Myc₁₃-kanMX6 bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1516 ^d | <i>bud3ΔCR2-LEU2-Myc₁₃-kanMX6:bud3Δ::URA3</i> | This study |
| HPY1687 ^d | <i>AXL1-Myc₁₃-kanMX6 bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1715 ^d | <i>AXL2-Myc₁₃-kanMX6 bud4ΔG2-TRP1:bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1716 ^d | <i>AXL2-Myc₁₃-kanMX6 bud4ΔG2-TRP1:bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1717 ^d | <i>AXL2-Myc₁₃-kanMX6 bud4^{T1182N, K1183S}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1719 ^d | <i>AXL2-Myc₁₃-kanMX6 bud4^{D1403A}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1720 ^d | <i>AXL1-Myc₁₃-kanMX6 BUD4-TRP1:bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1721 ^d | <i>AXL1-Myc₁₃-kanMX6 bud4ΔG2-TRP1:bud4Δ::LEU2</i> | Kang et al., 2012 |
| HPY1722 ^d | <i>AXL1-Myc₁₃-kanMX6 bud4^{T1182N, K1183S}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1724 ^d | <i>AXL1-Myc₁₃-kanMX6 bud4^{D1403A}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1725 ^d | <i>BUD5-TAP-HIS3 BUD4-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1726 ^d | <i>BUD5-TAP-HIS3 bud4ΔG2-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1727 ^d | <i>BUD5-TAP-HIS3 bud4^{T1182N, K1183S}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1729 ^d | <i>BUD5-TAP-HIS3 bud4^{D1403A}-TRP1:bud4Δ::LEU2</i> | This study |
| HPY1901 ^c | <i>BUD3-LEU2:bud3::TRP1</i> | This study |
| HPY1902 ^c | <i>bud3ΔCR1-LEU2:bud3::TRP1</i> | This study |
| HPY1903 ^c | <i>bud3ΔCR2-LEU2:bud3::TRP1</i> | This study |
| HPY2008 ^b | <i>CDC3-mCherry-LEU2</i> | Kang et al., 2012 |
| HPY2151 ^b | <i>bud4Δ::kanMX4 CDC3-mCherry-LEU2</i> | This study |
| HPY2316 ^d | <i>BUD3-Myc₁₃-kanMX6</i> | This study |
| HPY2446 ^d | <i>bud3Δ::HIS3</i> | This study |
| YEF6699-1 ^b | <i>GIC2-PBD^{W23A}-tdTomato-URA3 CDC3-GFP-LEU2</i> | Okada et al., 2013 |
| HPY2542 ^b | <i>bud3ΔCR2-LEU2:bud3Δ::URA3 GIC2-PBD^{W23A}-tdTomato-URA3, CDC3-GFP-LEU2</i> | This study |
| HPY2543 ^b | <i>kanMX6-P_{GAL1}-GFP-BUD3 GIC2-PBD^{W23A}-tdTomato-URA3</i> | This study |
| HPY2555 ^b | <i>TRP1-P_{GAL1}-GFP-bud3ΔCR2 GIC2-PBD^{W23A}-tdTomato-URA3</i> | This study |
| HPY2564 ^a | <i>CDC42-LEU2 AXL1-TAP-HIS3</i> | This study |
| HPY2565 ^a | <i>cdc42-101-LEU2 AXL1-TAP-HIS3</i> | This study |
| HPY2566 ^b | <i>BUD3-CFP-TRP1-LEU2:bud3Δ::URA3 CDC3-mCherry-LEU2</i> | This study |
| HPY2567 ^b | <i>bud3ΔCR2-CFP-TRP1-LEU2:bud3Δ::URA3 CDC3-mCherry-LEU2</i> | This study |
| HPY2579 ^e | <i>cdc24-4 GIC2-PBD^{W23A}-tdTomato-LEU2 ura3 leu2-3, 112 his3</i> | This study |
| HPY2586 ^b | <i>GIC2-PBD^{W23A}-tdTomato-URA3 WHI5-GFP-TRP</i> | This study |

^aStrains in an S288C background.^bStrains that are congenic to HPY210.^cStrains that are isogenic to 486 (Chant et al., 1995).^dStrains that are isogenic to HPY16 (Park et al., 1993) except as indicated.^eDerived from Y147 (Bender and Pringle, 1989).

Table S2. Plasmids used in this study

| Plasmid name | Description | Source |
|---------------------------------------|--|---------------------------|
| pRS425 | 2 μ , <i>LEU2</i> | Christianson et al., 1992 |
| pRS426 | 2 μ , <i>URA3</i> | Christianson et al., 1992 |
| pRS425-BUD3 | <i>BUD3</i> , 2 μ , <i>LEU2</i> | This study |
| pRD53 | <i>P_{GAL1}-BUD3</i> , CEN, <i>URA3</i> | Peter et al., 1996 |
| pRD53-BUD3 | <i>P_{GAL1}-BUD3</i> , CEN, <i>URA3</i> | This study |
| pRS4 | <i>pGEX-RSR1</i> | Holden et al., 1991 |
| pRS304-bud4 ^{T1182N, K1183S} | <i>bud4^{T1182N, K1183S}</i> , integrative, <i>TRP1</i> | This study |
| pRS304-bud4 ^{D1403A} | <i>bud4^{D1403A}</i> , integrative, <i>TRP1</i> | This study |
| pRS425-bud3 ^{E268A} | <i>bud3^{E268A}</i> , 2 μ , <i>LEU2</i> | This study |
| pRS425-bud3 ^{K405S} | <i>bud3^{K405S}</i> , 2 μ , <i>LEU2</i> | This study |
| pRS425-bud3 ^{EA, KS} | <i>bud3^{E268A, K405S}</i> , 2 μ , <i>LEU2</i> | This study |
| pRS425-bud3 Δ CR1 | <i>bud3^{A268-271}</i> , 2 μ , <i>LEU2</i> | This study |
| pRS425-bud3 Δ CR2 | <i>bud3^{A325-326, D328Q}</i> , 2 μ , <i>LEU2</i> | This study |
| pRS305-BUD3 | <i>BUD3</i> , integrative, <i>LEU2</i> | This study |
| pRS305-bud3 Δ CR1 | <i>bud3^{A268-271}</i> , integrative, <i>LEU2</i> | This study |
| pRS305-bud3 Δ CR2 | <i>bud3^{A325-326, D328Q}</i> , integrative, <i>LEU2</i> | This study |
| pRS426-BUD3 | <i>BUD3</i> , <i>URA3</i> , 2 μ | This study |
| pRS426-bud3 Δ CR2 | <i>bud3^{A325-326, D328Q}</i> , <i>URA3</i> , 2 μ | This study |
| pDLB2091 | <i>pGEX-KG-CDC42</i> | Gladfelter et al., 2002 |
| pEGKT-CDC42 | <i>P_{GAL1}-GST-CDC42</i> , <i>URA3</i> , 2 μ | Gao et al., 2007 |
| pEGKT-CDC42 ^{T17N} | <i>P_{GAL1}-GST-CDC42^{T17N}</i> , <i>URA3</i> , 2 μ | Gao et al., 2007 |
| pEGKT-CDC42 ^{Q61L} | <i>P_{GAL1}-GST-CDC42^{Q61L}</i> , <i>URA3</i> , 2 μ | Gao et al., 2007 |
| pEGKT-CDC42 ^{D57Y} | <i>P_{GAL1}-GST-CDC42^{D57Y}</i> , <i>URA3</i> , 2 μ | Gao et al., 2007 |
| pEG202 | <i>P_{ADH1}-LexA</i> , 2 μ , <i>HIS3</i> | Gyuris et al., 1993 |
| pEG202-Bud3(m) | <i>bud3(aa 1-656)</i> , pEG202 | This study |
| pJG4-5 | <i>P_{GAL1}-B42AD</i> , 2 μ , <i>TRP1</i> | Gyuris et al., 1993 |
| pJG4-5-cdc42 ^{C188S} | <i>cdc42^{C188S}</i> , pJG4-5 | Butty et al., 2002 |
| pJG4-5-CDC42 ^{G12V, C188S} | <i>cdc42^{G12V, C188S}</i> , pJG4-5 | Butty et al., 2002 |
| pJG4-5-CDC42 ^{D118A, C188S} | <i>cdc42^{D118A, C188S}</i> , pJG4-5 | This study |
| pJG4-5-CDC42 ^{G15A, C188S} | <i>cdc42^{G15A, C188S}</i> , pJG4-5 | This study |
| pMAL-c2 | MBP fusion vector | New England Biolabs, Inc. |
| pMAL-c2-Bud3(s) | MBP-Bud3(aa 211-498) | This study |
| pMAL-c2-bud3 Δ CR2(s) | MBP-Bud3 ^{A325-326, D328Q} (aa 211-498) | This study |
| Yiplac211-PBD-RFP | <i>GIC2-PBD^{W23A}-tdTomato</i> , integrative, <i>URA3</i> | Okada et al., 2013 |
| Yiplac128-PBD-RFP | <i>GIC2-PBD^{W23A}-tdTomato</i> , integrative, <i>LEU2</i> | This study |
| Yiplac128-CDC3-GFP | <i>CDC3-GFP</i> , integrative, <i>LEU2</i> | Tong et al., 2007 |
| Yiplac128-CDC3-mCherry | <i>CDC3-mCherry</i> , integrative, <i>LEU2</i> | Tong et al., 2007 |
| pRS316-CDC3-GFP | <i>CDC3-GFP</i> , CEN, <i>URA3</i> | M.S. Longtine |
| pFA6a-GFP-TRP1 | <i>GFP^{S65T}</i> , <i>TRP1</i> | Longtine et al., 1998 |

Table S3. Oligonucleotides used in this study

| Name | Sequence (5' → 3') |
|--------------|--|
| oBUD31 | GTGCGGATCCACCATCGAACTAAATAATAGG |
| oBUD32 | GTGGGAATTCAAATCCACTACATCATACACAC |
| oBUD33 | GTCAGGCCATTGCAACCGCTTATTACAACATACAAAC |
| oBUD34 | GTTTGTAGTTGATAACCGCTTGCAAATGGCCTTGAC |
| oBUD35 | GGACCTCAGGAAAACACTGAGTTAAAGTTAATTAGAGAGATTGTGAAACTCG |
| oBUD36 | CGACTTCCACAATCTCTATAATTAACTTTAACTCAGTAGTTCCTGAGGTCC |
| oBUD39 | CTGCGGATCCATGGAGAAAGACCTGTCGCTC |
| oBUD311 | GGAGAGCTGCAGGTTGCAAATGGCCTTGACAAG |
| oBUD312 | GGTGTGCTGCAGACAATTTCATTCAATTGGTAGAATTTC |
| oBUD313 | GGCGGCCTGCAGACAATTGATTGACTTCATC |
| oBUD314 | CCACCTCTGCAGTCGCTAAAGACAGCAATCCC |
| oBUD315 | CCAGGAAGCTTCATCATTCAAATCCACTACATC |
| oBUD332 | GCGCGGAATTCACTGGAGAAAGACCTGTCGTC |
| oBUD333 | GCGCGCTCGAGCTGGATGGTGTATGTTAG |
| oBUD47 | CCGCCACCTTCATAAGAAAGC |
| oBUD410 | AGAGTTTTTGGAAAAGAATTGCTATAAATTGAAAAGAAGTATG |
| oBUD411 | CTTCTTTCAAATTATACGAATTCTTCCAAAAAAACTCTTAC |
| oBUD412 | CCGGCGATCCAAGCTTCCCACCCCTTAC |
| oBUD413 | GCGGAATGCTCTAATGAGGAAAAAGCGCTTGTACAATAAGCTCCAGGAAGTTGTTG |
| oBUD414 | CAACAATTCTGGAGCTTATTGACCAAGCGCTTTTCCTCATTAGAGCATTCCGC |
| oPEG202UP | GGCATCTATTGAAGTAATAATAGCGCATG |
| oPEG202DOWN | GTGAAATGGGGAGCGATTGCGAGCATTTG |
| oCDC42G15A-1 | TTTGTGCGTATGGCTCTGGAAAACGTCCTCTAATC |
| oCDC42G15A-2 | GATTAGAAGGCACGTTTCGCGACAGCACCATACCGACAAC |
| oWHI51 | CGAACCGGAGCCCGACTCGGATACCGAAGTGGAGACGTCTGGATCCCCGGTTAATTAA |
| oWHI52 | CTAACTCGAGATTGCGGAGAAAAACTGTAACCACAGAATTGAGCTCGTTAAC |
| oWHI53 | CGGCTGCGAAACACTGATGC |
| oWHI54 | GCAGAGTACGCACTAGTGAC |

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