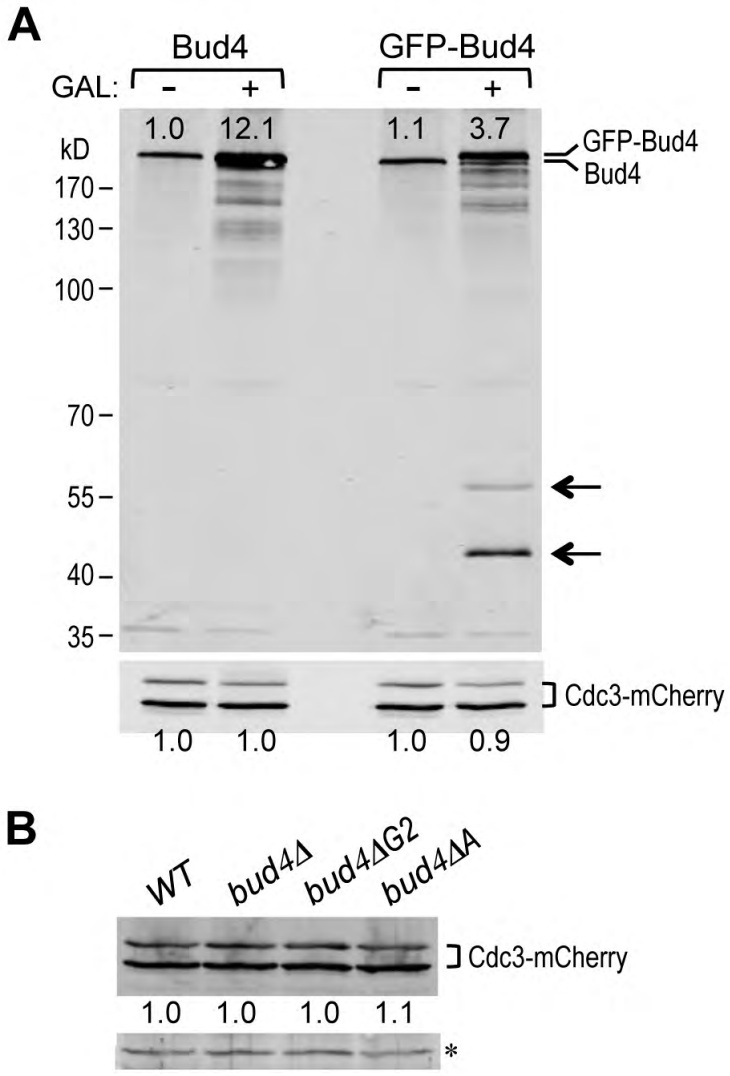

Fig. S1. A. The level of Bud4 and GFP-Bud4 expressed from the *GAL1* promoter. The *BUD4 CDC3-mCherry* strain (HPY2008) carrying pRD53-BUD4 or pRD53-GFP-BUD4 was grown in SC-URA media containing 2% raffinose (-), and then galactose was added (+; final 2%) for 6 hrs. Bud4 and GFP-Bud4 were detected by immnuoblotting using polyclonal anti-Bud4 antibodies (upper panel); and Cdc3-mCherry was detected with anti-DsRED antibodies (lower panel). Numbers represent the relative protein levels normalized against Bud4 or Cdc3 shown in the first lane. The Bud4 and GFP-Bud4 levels were elevated about 12-fold and 3.3-fold, respectively, after induction with galactose, while the Cdc3-mCherry levels remained about the same. The lower level of GFP-Bud4 compared to Bud4 upon Gal induction might be partly due to degradation of GFP-Bud4 (see bands marked with arrows). **B.** The Cdc3-mCherry level in wild type and *bud4* mutants. Cdc3-mCherry was detected with anti-DsRED antibodies (upper panel), and Bem1 was detected with anti-Bem1 antibodies as a loading control (lower panel; marked with *). Numbers represent the relative Cdc3-mCherry protein levels normalized against that in the wild-type strain.

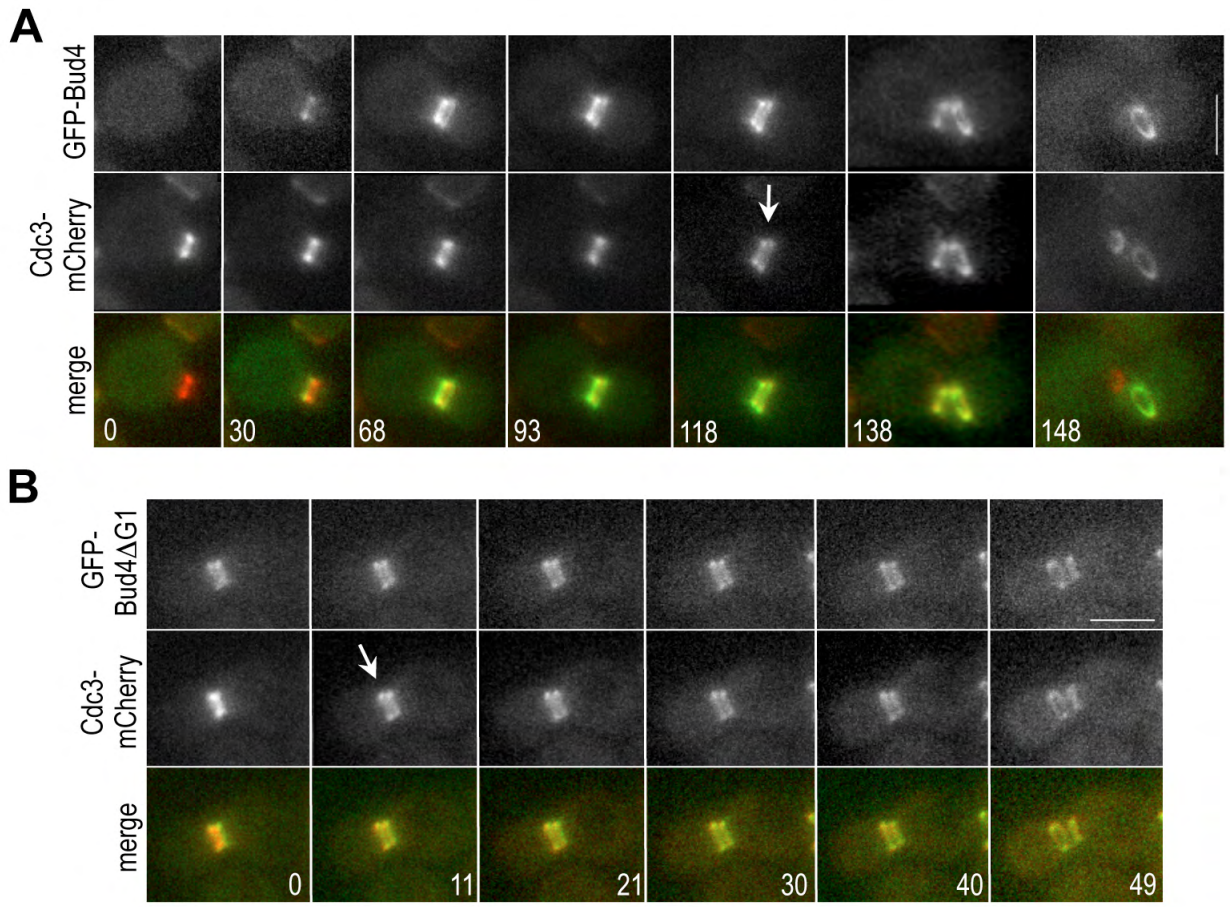

Fig. S2. Time-lapse images of cells expressing Cdc3-mCherry and (**A**) GFP-Bud4 (HPY2111) or (**B**) GFP-Bud4ΔG1 (HPY2176). Images were captured at 23°C, and relative time (in min) after the first image is shown. Arrows depict splitting of the septin ring at the beginning of cytokinesis. Note: GFP-Bud4 appears to form a double ring at the mother-bud neck before septin ring splitting. Scale bar: 3μm.

Table S1. Yeast strains used in this study

Strain		Relevant Genotype	Source/Comments
YEF473	**a**/α	*his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 trp1-Δ63/ trp1-Δ63 ura3-52/ura3-52*	(Bi and Pringle, 1996)
HPY210	**a**		(Singh et al., 2008)
HPY1023	**a**	*bud4Δ::LEU2*	(Kang et al., 2012)
HPY1027	**a**	*bud4ΔG2-TRP1::bud4Δ::LEU2*	(Kang et al., 2012)
HPY1032	**a**	*BUD4-TRP1::bud4Δ::LEU2*	This study
HPY1034	**a**	*GFP-BUD4-TRP1::bud4Δ::LEU2*	(Kang et al., 2012)
HPY2000	**a**	*bud4Δ::kanMX4*	This study
HPY2008	**a**	*CDC3-mCherry-LEU2*	This study
HPY2053	**a**	*GFP-TUB1-URA3::ura3Δ CDC3-mCherry-LEU2*	This study
HPY2088	**a**	*GFP-TUB1-URA3::ura3Δ CDC3-mCherry-LEU2 bud4Δ::kanMX4*	This study
HPY2099	**a**	*GFP-TUB1-URA3::ura3Δ CDC3-mCherry-LEU2 bud4ΔG2-TRP1::bud4Δ::kanMX4*	This study
HPY2111	**a**	*GFP-BUD4-TRP1::bud4Δ::LEU2 CDC3-mCherry-LEU2*	This study
HPY2132	**a**	*GFP-bud4ΔN8-TRP1::bud4Δ::LEU2*	This study
HPY2138	**a**	*bud4ΔN5-TRP1::bud4Δ::LEU2*	This study
HPY2139	**a**	*bud4ΔA-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2 GFP-TUB1-URA3*	This study
HPY2141	**a**	*bud4ΔA-TRP1::bud4Δ::LEU2*	This study
HPY2152	**a**	*GFP-bud4ΔA-TRP1::bud4Δ::LEU2 CDC3-mCherry-LEU2*	This study
HPY2156	**a**	*bud4ΔN6-TRP1::bud4Δ::LEU2*	This study
HPY2157	**a**	*bud4ΔN8-TRP1::bud4Δ::LEU2*	This study
HPY2158	**a**	*GFP-bud4ΔN6-TRP1::bud4Δ::LEU2*	This study
HPY2159	**a**	*GFP-bud4ΔN5-TRP1::bud4Δ::LEU2*	This study
HPY2173	**a**	*bud4ΔG1-TRP1::bud4Δ::LEU2*	(Kang et al., 2012)
HPY2176	**a**	*GFP-bud4ΔG1-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2180	**a**	*bud4ΔB-TRP1::bud4Δ::LEU2*	This study
HPY2181	**a**	*bud4ΔC-TRP1::bud4Δ::LEU2*	This study
HPY2202	**a**	*GFP-bud4ΔA-TRP1::bud4Δ::LEU2 BUD3-mCherry- kanMX4*	This study

HPY2207	**a**	*bud4ΔD-TRP1::bud4Δ::LEU2*	This study
HPY2217	**a**	*GFP-BUD4-TRP1::bud4Δ::LEU2 BUD3-mCherry- kanMX4*	(Kang et al., 2012)
HPY2249	**a**	*GFP-bud4ΔG2-TRP1::bud4Δ::LEU2 BUD3-mCherry- kanMX4*	This study
HPY2218	**a**	*GFP-bud4ΔE-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2219	**a**	*GFP-bud4ΔD-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2220	**a**	*bud4ΔN7-TRP1::bud4Δ::LEU2*	This study
HPY2225	**a**	*bud4ΔE-TRP1::bud4Δ::LEU2*	This study
HPY2226	**a**	*GFP-bud4ΔN7-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2229	**a**	*bud4ΔF-TRP1::bud4Δ::LEU2*	This study
HPY2231	**a**	*GFP-bud4ΔB-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2232	**a**	*GFP-bud4ΔC-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2265	**a**	*GFP-bud4ΔG2-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2314	**a**	*GFP-bud4ΔF-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2323	**a**	*GFP-bud4ΔI-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY2324	**a**	*GFP-bud4ΔH-TRP1::bud4Δ::kanMX4 CDC3-mCherry-LEU2*	This study
HPY16*	**a**	*ura3-52 trp1-Δ63 leu2 his3-Δ1 pep4-3*	(Park et al., 1993)
HPY1511*	**a**	*BUD3-Myc$_{13}$-kanMX6*	(Kang et al., 2012)
HPY1514*	**a**	*BUD3-Myc$_{13}$-kanMX6 bud4Δ::LEU2*	(Kang et al., 2012)
HPY2254*	**a**	*bud4ΔA-TRP1::bud4Δ::LEU2 BUD3-Myc$_{13}$-kanMX6*	This study
HPY2255*	**a**	*bud4ΔE-TRP1::bud4Δ::LEU2 BUD3-Myc$_{13}$-kanMX6*	This study
HPY1991*	**a**	*bud4ΔG2-TRP1::bud4Δ::LEU2 BUD3-Myc$_{13}$-kanMX6*	This study

[a] All strains except that those marked with asterisk (*) are congenic to YEF473 except as indicated. Strains marked with * are congenic to HPY16 except as indicated.

Table S2. Plasmids used in this study

Plasmid	Description[1]	Source/Comments
pRD53	*URA3* (CEN), *GAL1* promoter	R. Deshaies (California Institute of Technology)
pAFS125	pRS306-GFP-TUB1	(Straight et al., 1997)
pBS34	pFA6a-mCherry-kanMX6	YRC, Univ. of Washington, Seattle
pFJ115	pRS413-P_{ADH2}-HTB2-mCherry	(Egriboz et al., 2011)
pLP17	LEU2 (CEN), CDC12-GFP	(Lippincott and Li, 1998)
	YIplac128-CDC3-mCherry (LEU2)	(Tong et al., 2007)
pHP1392	pRS304*(= pRS304 lacking the *Not*I site)-*BUD4*	(Kang et al., 2012)
pHP1400	pRS304*-*Not*I-*BUD4*	(Kang et al., 2012)
pHP1851	pRS304*-*Not*I-*bud4ΔG1* (Δ aa 1175 - 1182)	(Kang et al., 2012)
pHP1395	pRS304*-*bud4ΔG2 (*Δ aa 1344 - 1404)	(Kang et al., 2012)
pHP1401	pRS304*-*GFP-BUD4*	(Kang et al., 2012)
pHP1854	pRS304*-*GFP-bud4ΔG1 (*Δ aa 1175 – 1182)	(Kang *et al.,* 2012)
pHP1403	pRS304*-*GFP-bud4ΔG2 (*Δ aa 1344 - 1404)	(Kang et al., 2012)
pHP1430	pRD53-BUD4	This study
pHP1819	pRD53*(= pRD53 lacking the *Not*I site)-GFP-BUD4	This study [3]
pHP1800	pRS304*-*Not*I-*bud4ΔN5(*Δ aa 2 – 510)	This study [2]
pHP1480	pRS304*-*Not*I-*bud4ΔN6(*Δ aa 2 – 883)	This study [2]
pHP1887	pRS304*-*Not*I-*bud4ΔN7(*Δ aa 2 – 1000)	This study [2]
pHP1481	pRS304*-*Not*I-*bud4ΔN8 (*Δ aa 2 – 1081)	This study [2]
pHP1845	pRS304*-*GFP-bud4ΔN5*(Δ aa 2 – 510)	This study [3]
pHP1843	pRS304*-*GFP-bud4ΔN6 (*Δ aa 2 – 883)	This study [3]
pHP1889	pRS304*-*GFP-bud4ΔN7 (*Δ aa 2 – 1000)	This study [3]

pHP1834	pRS304*-*GFP-bud4ΔN8* (Δ aa 2 – 1081)	This study [3]
pHP1835	pRS316*-*Not*I-*bud4ΔA* (Δ aa 1069 – 1163)	This study [4]
pHP1836	pRS304*-*GFP-bud4ΔA* (Δaa1069 – 1163)	This study [4]
pHP1837	pRS304*-*Not*I-*bud4ΔA* (Δaa1069 – 1163)	This study [4]
pHP1864	pRS304*-*GFP-bud4ΔB* (Δ aa 805 – 997)	This study [5]
pHP1866	pRS304*-*Not*I-*bud4ΔB* (Δ aa 805 – 997)	This study [5]
pHP1865	pRS304*-*GFP-bud4ΔC* (Δ aa 989 – 1447)	This study [6]
pHP1867	pRS304*-*Not*I-*bud4ΔC* (Δ aa 989 – 1447)	This study [6]
pHP1881	pRS304*-*Not*I-*bud4ΔD* (Δ aa 530 – 811)	This study [7]
pHP1886	pRS304*-*GFP-bud4ΔD* (Δ aa 530 – 811)	This study [3]
pHP1882	pRS316*-*Not*I-*bud4ΔE* (Δaa1306 - 1328)	This study [8]
pHP1885	pRS304*-*GFP-bud4ΔE* (Δ aa 1306 - 1328)	This study [8]
pHP1888	pRS304*-*Not*I-*bud4ΔE* (Δaa 1306 - 1328)	This study [8]
pHP1893	pRS304*-*Not*I-*bud4ΔF* (Δ aa 530 – 997)	This study [9]
pHP1909	pRS304*-*GFP-bud4ΔF* (Δ aa 530 – 997)	This study [3]
pHP1914	pRS304*-*GFP-bud4ΔH* (aa 511 - 988)	This study [10]
pHP1913	pRS304*-*GFP-bud4ΔI* (aa 511 - 804)	This study [11]

[1]Residues deleted in each *bud4* allele are indicated after 'Δaa' in each plasmid whereas the residues remaining in *bud4ΔH* and *bud4ΔI* are indicated after 'aa'.

[2] The *N*–terminal deletion mutations of *bud4* were generated by PCR. First, separate PCR reactions were carried out using pHP1392 as a template and primers oBUD412 (5'-CCGCGGATCCAAGCTTCCCAC CCTTACC-3') and oBUD431 (5'- GACACGCGGCCGCAGTGAAATTGGTGTCGAC-3') for *Not*I-*bud4ΔN5*; and primers oBUD412 and oBUD432 (5'- GTACCGCGGCCGCATAAGAAAGCAACC TATTG-3') for *Not*I-*bud4ΔN6*; and primers oBUD412 and oBUD455 (5'-CACACGCGGCCGCGTC GTGAAAGCTGGAAACAAAC -3') for *Not*I-*bud4ΔN7* and primers oBUD412 and oBUD433 (5'- G

AGAGGCGGCCGCACGCTTGCATTATATGGAAC-3') for *Not*I-*bud4ΔN8*. The PCR products were digested with *Not*I and *BamH*I, and then ligated with pHP1400 digested with *Not*I and *BamH*I.

[3] The 720-bp *Not*I fragment encoding GFP$^{S65T,V163A,\ S175G}$ from pRS304-GFP-RSR1 (Park et al., 2002) was cloned into the *Not*I site for GFP-tagging.

[4] The *bud4ΔA* mutation was generated by PCR using pHP1400 as a template and primers oBUD452 (5'-CACCTCCGGAATTGGTCGAAATAGTAGAC-3') and oBUD412. The PCR product was digested with *BspE*I and *BamH*I, and then cloned into the same sites of pRS316*(= pRS316 lacking the *Not*I site) -*Not*I-BUD4, yielding pHP1835. The 2.87-kb *Sal*I-*BamH*I fragment of pHP1403 was then replaced with the 2.77-kb *Sal*I-*BamH*I fragment of pHP1835, yielding pHP1836. The 720-bp *Not*I fragment encoding GFP from pHP1836 was removed, yielding pHP1837.

[5] The *GFP-bud4ΔB* mutation was generated by two-step PCR strategy. First, separate PCR reactions were carried out using pHP1392 as a template and primers oBUD431 and oBUD448 (5'-GGTTCGCGC GCAATTTCAGCTTCGTCCTGGG -3'); and primers oBUD451 (5'-CGCGAGCGCGCCCTTTCAAG GTCGTGAAAGCTGGAAAC-3') and oBUD412. The PCR products were ligated together after *BssH*II digestion, and then used as template in the second PCR with primers oBUD431 and oBUD412. The resulting product was digested with *Sal*I and *BamH*I, and then replaced with the 3.05-kb *Sal*I-*BamH*I fragment of pHP1401, yielding pHP1864. The GFP moiety was then removed from pHP1864 by *Not*I digestion and self-ligation, yielding pHP1866.

[6] The *GFP-bud4ΔC* mutation was generated by two-step PCR strategy. First, separate PCR reactions were carried out using pHP1392 as a template and primers oBUD431 and oBUD450 (5'-GGTTGGCG CGCTATACTACGAACTGGAGTGG-3'); and primers oBUD454 (5'-GCAGGCGCGCGAAGTATA AATTTGAAAAGAAGTATGTACAG-3') and oBUD412. The PCR products were ligated together after *BssH*II digestion, and then processed for the second PCR with primers oBUD431 and oBUD412, and the resulting product was replaced with the 3.05-kb *Sal*I-*BamH*I fragment of pHP1401 as described above [5], yielding pHP1865. The GFP moiety was then removed from pHP1865 as described above [5], yielding pHP1867.

[7] The *bud4ΔD* mutation was generated by two-step PCR strategy. First, separate PCR reactions were carried out using pHP1343 as a template and primers oBUD419 (5'- GCCTGGGCGGCCGCCACGA CGCAGAGAGTACCGTAG-3') and oBUD446 (5'-GAGTCGCGCGCTTTTGCCAATGGTTCATTC ACTTCAG-3'); and primers oBUD449 (5'- GGAGGGCGCGCGCGGGTGATATCACTTTTAATAGG GG-3') and oBUD412. The PCR products were ligated together after *BssH*II digestion, and then used as

template in the second PCR with primers oBUD419 and oBUD412. The PCR product was digested with *Sal*I and *BamH*I, and then cloned into the same sites of pHP1400, yielding pHP1881.

[8] The *bud4ΔE* mutation was generated by two-step PCR strategy. First, separate PCR reactions were carried out using pHP1343 as a template and primers oBUD47 (5'-CCGCCACCTTCCATAAGAAAG C-3') and oBUD456 (5'-GGTGGGCGCGCTTTATAGATATTTTGTTGCAACTTGTATTTGG-3'); and primers oBUD457 (5'-TCGGTGCGCGCGGCTCGCAACTTTCTGGTTATC-3') and oBUD412. The PCR products were ligated together after *BssH*II digestion, and then used as template in the second PCR with primers oBUD47 and oBUD412. The PCR product was digested with *BspE*I and *BamH*I, and then cloned into the same sites of pRS316*-*Not*I-BUD4, yielding pHP1882. The 2.87-kb *Sal*I-*BamH*I fragment of pHP1403 was then replaced with the 2.94-kb *Sal*I-*BamH*I fragment of pHP1882, yielding pHP1885. The GFP moiety was removed from pHP1885 as described above [5], yielding pHP1888.

[9] The *bud4ΔF* mutation was generated by combining *bud4ΔB* and *bud4ΔD*. The larger DNA fragment from pHP1866 digested with *Not*I and *BssH*II was ligated with the 1.6-kb DNA fragment from pHP1881 digested with the same enzymes, yielding pHP1893.

[10] The *GFP-bud4ΔH* mutation was generated by PCR using pHP1867 as a template and primers oBUD431 and oBUD412. The PCR product was digested with *Sal*I and *BamH*I, and then replaced with the 3.05-kb *Sal*I-*BamH*I fragment of pHP1845.

[11] The *GFP-bud4ΔI* mutation was generated by two-step PCR strategy. First, separate PCR reactions were carried out using pHP1867 as a template and primers oBUD431 and oBUD448; and primers oBUD454 and oBUD412. The PCR products were ligated together after *BssH*II digestion, and then used as template in the second PCR with primers oBUD431 and oBUD412. The resulting product was digested with *Sal*I and *BamH*I, and then replaced with the 3.05-kb *Sal*I-*BamH*I fragment of pHP1845.