Supplemental Material

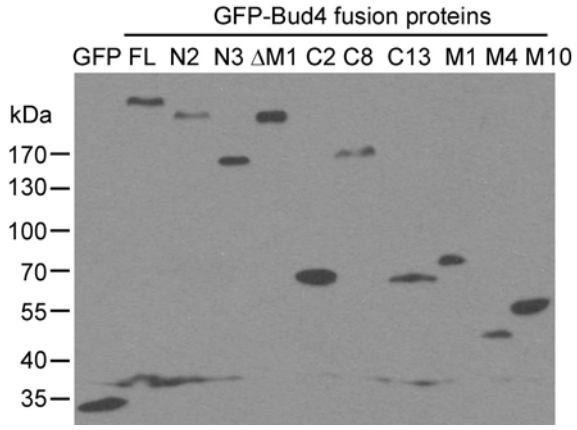

FIG S1 Expression of Bud4 segments in yeast cells.

Cells of strain YEF473A carrying pBG2 (GFP, vector) or pBG2-BUD4 segments were grown in SC-Ura medium. Cell lysates prepared from the yeast strains were separated by 7.5% SDS-PAGE and immunoblotted with an anti-GFP antibody. Molecular weight of GFP-fusion proteins: Bud4-FL (full-length, a.a. 1-1447, 186.6 kDa), Bud4-N2 (a.a. 1-1066, 144.7 kDa), Bud4-N3 (a.a. 1-622, 95.8 kDa), Bud4-ΔM1 (a.a. 1-622 plus 881-1447, 158.2 kDa), Bud4-C2 (a.a. 1067-1447, 69.3 kDa), Bud4-C8 (a.a. 623-1447, 118.1 kDa), Bud4-C13 (a.a. 1082-1447, 67.7 kDa), Bud4-M1 (a.a. 623-880, 55.8 kDa), Bud4-M4 (a.a. 623-774, 44.1 kDa), and Bud4-M10 (a.a. 623-804, 47.4 kDa). GFP (238 a.a. plus linker, 29.6 kDa). Note: The GFP-Bud4-M1 segment reproducibly migrated slower than predicted.

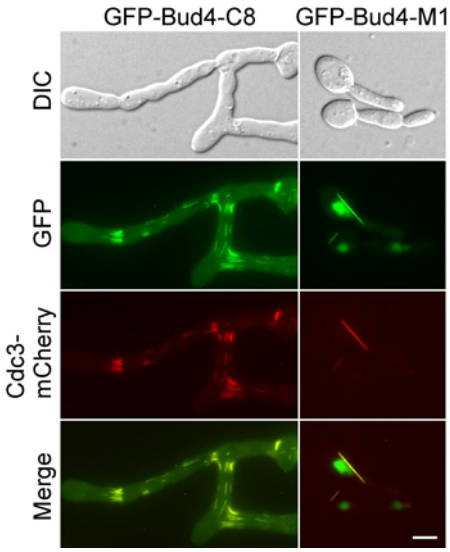

FIG S2 Co-localization of overexpressed GFP-Bud4-C8 and GFP-Bud4-M1 with the disorganized septins.

Cells of strain JGY2923 (*bud4*Δ *CDC3-mCherry:LEU2*) carrying plasmid pGGFP316-BUD4-C8 and pGGFP316-BUD4-M1 were grown on SRG-Ura plate at 30℃ for 20 hr. Images were taken by two-color fluorescence microscopy. Bar, 5 μm.

TABLE S1 Yeast strains used in this study

Strain	Genotype	Source
JMY314.1-4b	α *leu2-3,112 ura3-1 his3-11,15 trp1-1 ade2-1 can1-100* (W303 background)	1
YEF473A	a *his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52*	2
YEF473B	α *his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52*	2
YEF1238	As YEF473A except *gin4Δ::TRP1*	E. Bi
YEF1342	As YEF473A except *cla4Δ::HIS3*	E. Bi
YEF2218	As YEF473B except *cdc12-6*	E. Bi
YEF3570	As YEF473A except *bud3Δ::HIS3*	J. R. Pringle
YEF3572	As YEF473A except *bud4Δ::HIS3*	J. R. Pringle
YEF4603	As YEF473B except *shs1Δ::kanMX*	E. Bi
JGY781	As YEF473B except *bud3Δ::HIS3 bud4Δ::HIS3*	This study
JGY2482	As JMY314.1-4b except *ade3Δ::kanMX*	This study
JGY2737	As YEF473B except *bud4Δ::TRP1 shs1Δ::kanMX*	This study
JGY2903	As JGY2482 except *iqg1Δ::His3MX6*	This study
JGY2923	As YEF473A except *bud4Δ::HIS3 CDC3-mCherry:LEU2*	This study
JGY2966	As YEF473A except *bud4Δ::HIS3 CDC3-mCherry:LEU2 SPC42-mCherry:TRP1*	This study
JGY3035	As YEF473B except *bud3Δ::HIS3 shs1Δ::kanMX*	Segregants from the cross between YEF3570 and YEF4603.
JGY3040	As YEF473B except *bud3Δ::HIS3 cdc12-6*	Segregants from the cross between YEF3570 and YEF2218.
JGY3042	As YEF473B except *bud4Δ::HIS3 cdc12-6*	Segregants from the cross between YEF3572 and YEF2218.
pJ69-4A	a *his3-Δ200 leu2-3,112 trp1-901 ura3-52 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	3
pJ69-4α	α *his3-Δ200 leu2-3,112 trp1-901 ura3-52 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	3

REFERENCE

1. **Ko N, Nishihama R, Tully GH, Ostapenko D, Solomon MJ, Morgan DO, Pringle JR.** 2007. Identification of yeast IQGAP (Iqg1p) as an anaphase-promoting-complex substrate and its role in actomyosin-ring-independent cytokinesis. Mol. Biol. Cell **18:**5139-5153.

2. **Bi E, Pringle JR.** 1996. *ZDS1* and *ZDS2*, genes whose products may regulate Cdc42p in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**5264-5675.

3. **James P, Halladay J, Craig EA.** 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics **144:**1425-1436.