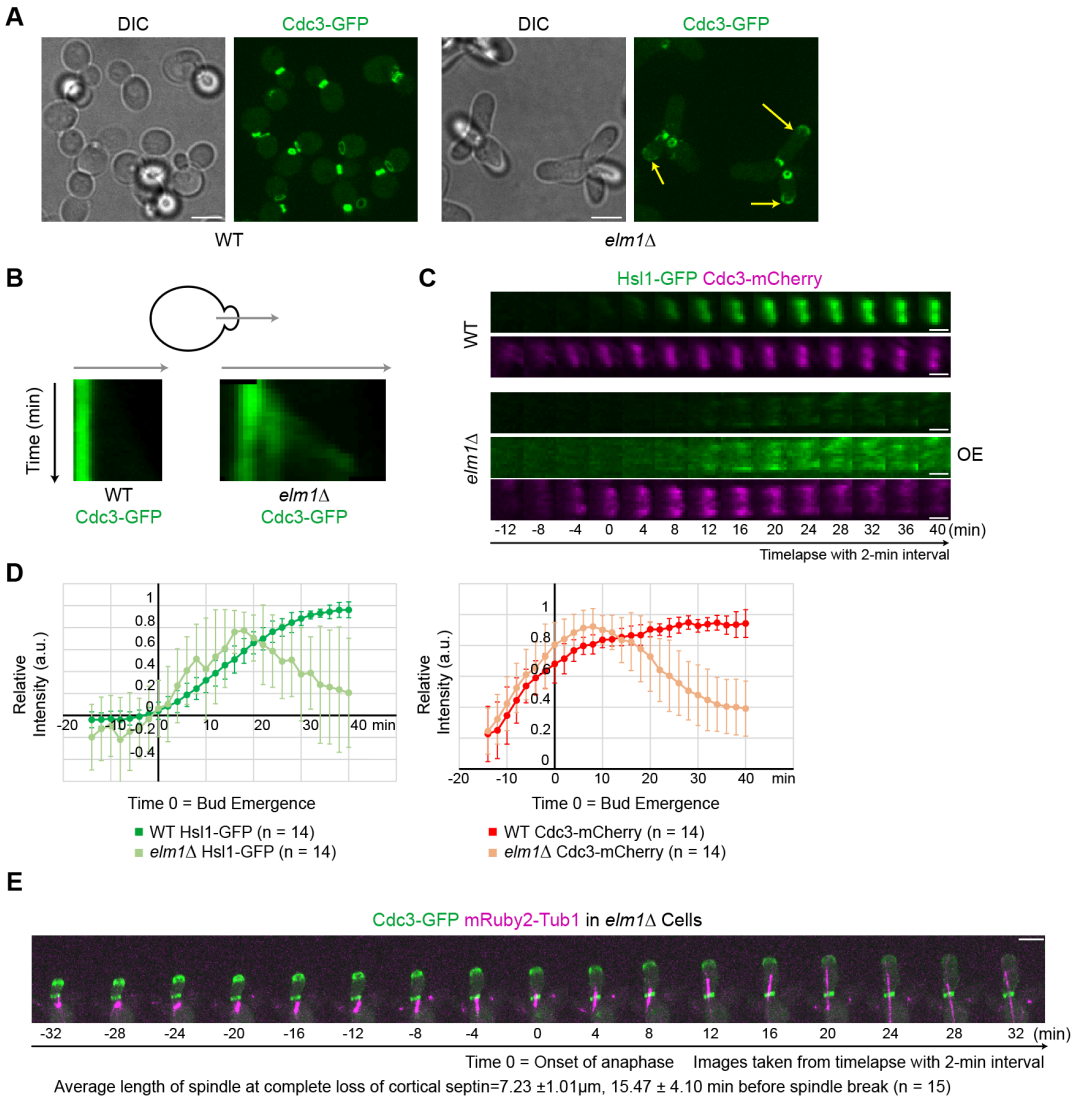

Figure S1. Deletion of *ELM1* causes destabilization of the septin hourglass preferentially at the daughter side from bud emergence to late anaphase. Related to Figure 1.

(A) Representative images of cell morphology (Differential interference contrast, DIC) and septin localization (Cdc3-GFP) for WT (YEF8102) and *elm1Δ* (YEF8393) cells. Yellow arrows indicate septins localized at the bud cortex. Scale bar = 5μm. (B) Kymographs of Cdc3-GFP in budding cells of indicated strains (WT: YEF7498, *elm1Δ*: YEF8087). Gray arrow in cartoon depiction shows the region of interest used to generate kymograph on the X-axis from the bud neck (green in cartoon) to the growing bud tip. Top of each kymograph is at bud emergence with time in minutes (min) on the Y-axis. (C) Montages show maximum-intensity projections of indicated fluorescent protein from 12 min before to 40 min after bud emergence with selected frames from time-lapse series taken with a 2-min interval for the representative WT (YEF8954) and *elm1Δ* (YEF8995) cells. OE = over-enhanced with higher brightness than above image to visualize the weak signal. Scale bar = 1 μm. (D) Quantification of cells in (S1C). Shown is cytoplasmic subtracted intensity of indicated fluorescent protein from the sum projection normalized to the maximum value measured in the given number cells for each strain. The mean is plotted with error bars being the standard deviation. (E) Montages show maximum-intensity projections of indicated fluorescent protein from 32 min before and after initiation of anaphase with selected frames from a time-lapse series taken with a 2-min interval for a representative *elm1Δ* cell (YEF8393). T = 0 is anaphase onset as seen by initiation of mitotic spindle elongation and penetration into the bud. Scale bar = 5 μm. The length of the mitotic spindle was measured at the time of loss of cortical Cdc3-GFP signal as well as the timing before spindle break and presented as the mean ± S.D. for the indicated number of cells. See also Video S1.

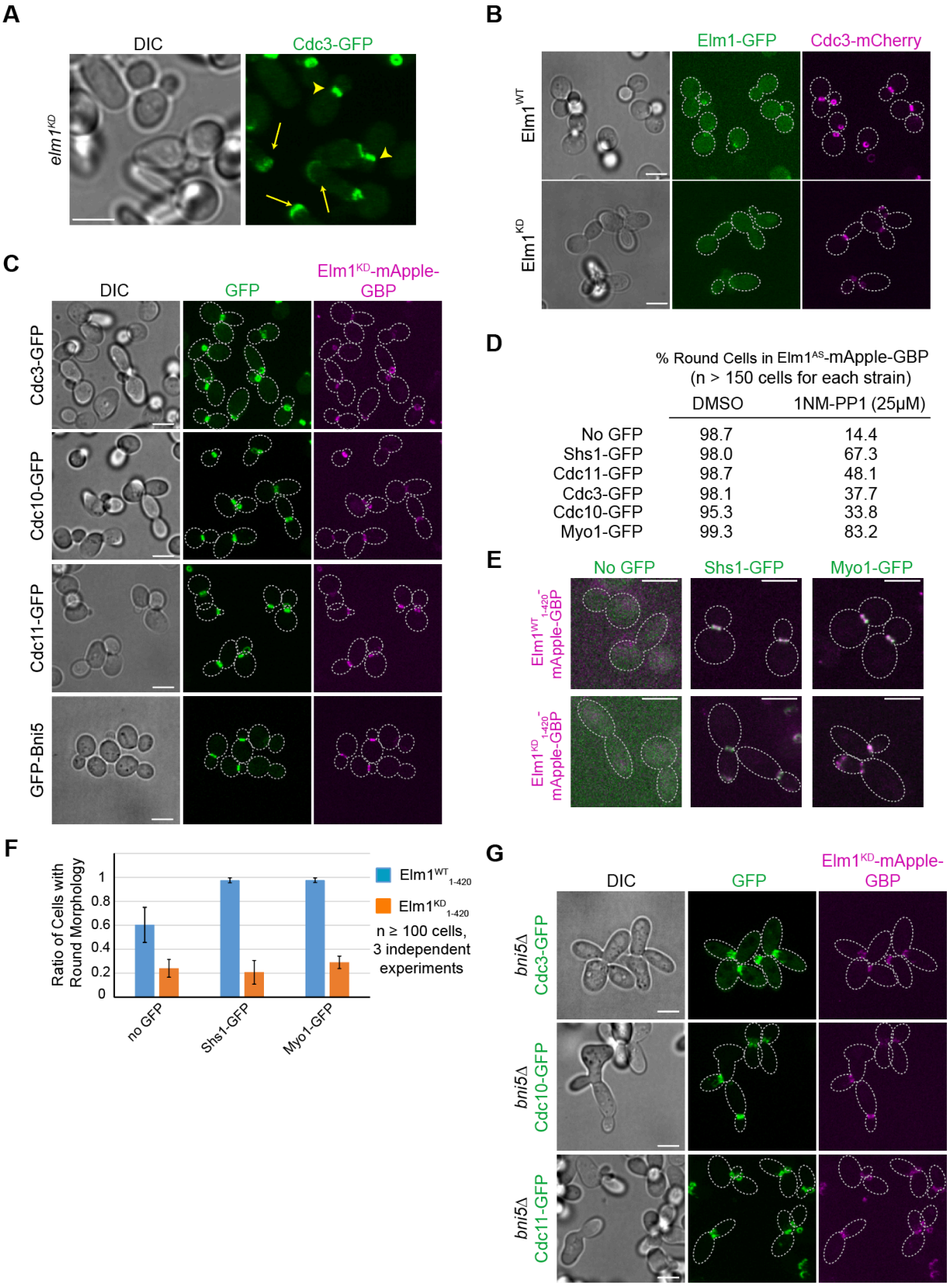
A
DIC
Cdc3-GFP
elm1KD
B
Elm1-GFP
Cdc3-mCherry
Elm1WT
Elm1KD
C
DIC
GFP
Elm1KD-mApple-GBP
Cdc3-GFP
Cdc10-GFP
Cdc11-GFP
GFP-Bni5
D
% Round Cells in Elm1AS-mApple-GBP
(n > 150 cells for each strain)
DMSO
1NM-PP1 (25μM)
No GFP 98.7 14.4
Shs1-GFP 98.0 67.3
Cdc11-GFP 98.7 48.1
Cdc3-GFP 98.1 37.7
Cdc10-GFP 95.3 33.8
Myo1-GFP 99.3 83.2
E
No GFP
Shs1-GFP
Myo1-GFP
Elm1WT1-420-mApple-GBP
Elm1KD1-420-mApple-GBP
F
Ratio of Cells with Round Morphology
1
0.8
0.6
0.4
0.2
0
no GFP
Shs1-GFP
Myo1-GFP
Elm1WT1-420
Elm1KD1-420
n ≥ 100 cells,
3 independent
experiments
G
DIC
GFP
Elm1KD-mApple-GBP
bni5Δ Cdc3-GFP
bni5Δ Cdc10-GFP
bni5Δ Cdc11-GFP

Figure S2. Tethering Elm1 to the bud neck largely bypasses the requirement for kinase activity. Related to Figure 2.

(A) Representative images of cell morphology (DIC) and septin localization (Cdc3-GFP) for *elm1^{KD}* cells (YEF9271). Yellow arrows indicate septins localized at the bud cortex in elongated cells, yellow arrowheads indicate septins with normal bud-neck localization in round cells. Scale bar = 5µm. (B) Representative images of cell morphology (DIC) and the localization of Elm1, Elm1^{KD}, and septin (as indicated by Elm1-GFP/Elm1^{KD}-GFP in green and Cdc3-mCherry in magenta, respectively) for WT (YEF8299) and *elm1^{KD}* (YEF9287) cells. Dotted white line is cell periphery. Scale bar = 5µm. (C) Representative images of indicated strains with Elm1^{KD}-mApple-GBP tethered to various GFP-tagged septins. Strains used are as follows from top to bottom: YEF9342 (*CDC3-GFP*), YEF9972 (*CDC10-GFP*), YEF9782 (*CDC11-GFP*), YEF10349 *(GFP*-BNI5) Images are maximum projections. Dotted line is cell periphery. Scale bar = 5 µm. See also Figure 2. Quantified in Figure 2B. (D) Quantification of the ratio of round cells in strains with Elm1^{AS}-mApple-GBP and indicated GFP-tagged protein for a minimum of 150 cells for each strain treated with either DMSO (control) or 25µM 1NM-PP1 (inhibitor). Strains used are as follows from top to bottom: YEF9460 (no GFP), YEF10107 (*SHS1-GFP*), YEF10106 (*CDC11-GFP*), YEF9501 (*CDC3-GFP*), YEF10105 (*CDC10-GFP*), and YEF9503 (*MYO1-GFP*). (E) Representative images of indicated strains with WT or kinase-dead Elm1$_{1-420}$-mApple-GBP tethered to various GFP-tagged proteins. Strains used are as follows: YEF9589 (*ELM1$_{1-420}$-mApple-GBP* no GFP), YEF10001 (*ELM1$_{1-420}$-mApple-GBP SHS1-GFP*), YEF10003 (*ELM1$_{1-420}$-mApple-GBP MYO1-GFP*), YEF9884 (*elm1$^{KD}{}_{1-420}$-mApple-GBP* no GFP), YEF9938 (*elm1$^{KD}{}_{1-420}$-mApple-GBP SHS1-GFP*), and YEF9940 (*elm1$^{KD}{}_{1-420}$-mApple-GBP MYO1-GFP*). Images are merged color maximum projections. Dotted line is cell periphery. Scale bars = 5 µm. (F) Quantification of the ratio of round cells in strains used in (S2E). Plotted is the average of 3 independent experiments of n > 100 cells. Error bars are standard deviation. (G) Representative images of indicated *bni5Δ* strains with Elm1^{KD}-mApple-GBP tethered to various GFP-tagged septins. Strains used are as follows from top to bottom: YEF10089 (*CDC3-GFP*), YEF9973 (*CDC10-GFP*), and YEF9644 (*CDC11-GFP*). Images are maximum projections. Dotted line is cell periphery. Scale bar = 5 µm. See also Figure 2. Quantified in Figure 2D.

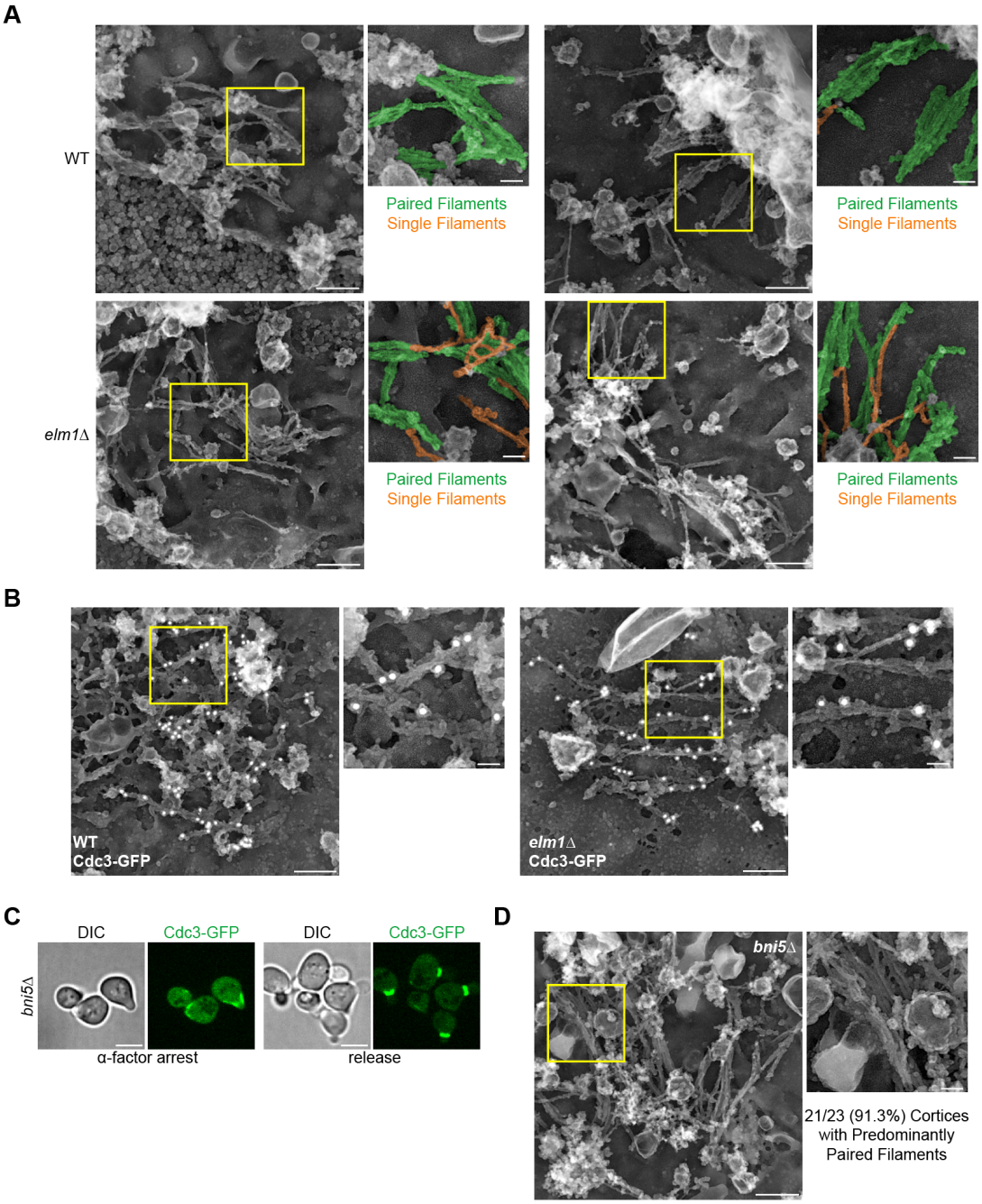

Figure S3. Deletion of *ELM1*, but not *BNI5*, causes defects in paired filament formation in the septin hourglass. Related to Figure 3.

(A) Representative PREM images of indicated strains (WT: YEF9327, *elm1Δ*: YEF9334) after α-factor arrest/release. Inset to the right is of area in yellow square. Scale bar in large image = 200nm, inset = 50nm. See also Figure 3. (B) Representative PREM images of indicated strains (WT: YEF9333, *elm1Δ*: YEF9431) after α-factor arrest/release and immunogold-labeling of Cdc3-GFP using 18nm gold-conjugated secondary antibody (white dots). Inset to the right is of area in yellow square. Scale bar in large image = 200nm, inset = 50nm. (C) Representative images of *bni5Δ* cells (YEF9767) at 2.5 hours in the presence of α-factor and at 1.5 hour after the release from α-factor. Scale bar = 5μm (D) Representative PREM images of *bni5Δ* strain (YEF9619) after α-factor arrest/release. Inset to the right is of area in yellow square. Scale bar in large image = 200nm, inset = 50nm. Quantification presented is number of cortices examined to contain only paired filaments in observed structures as in Figure 3C.

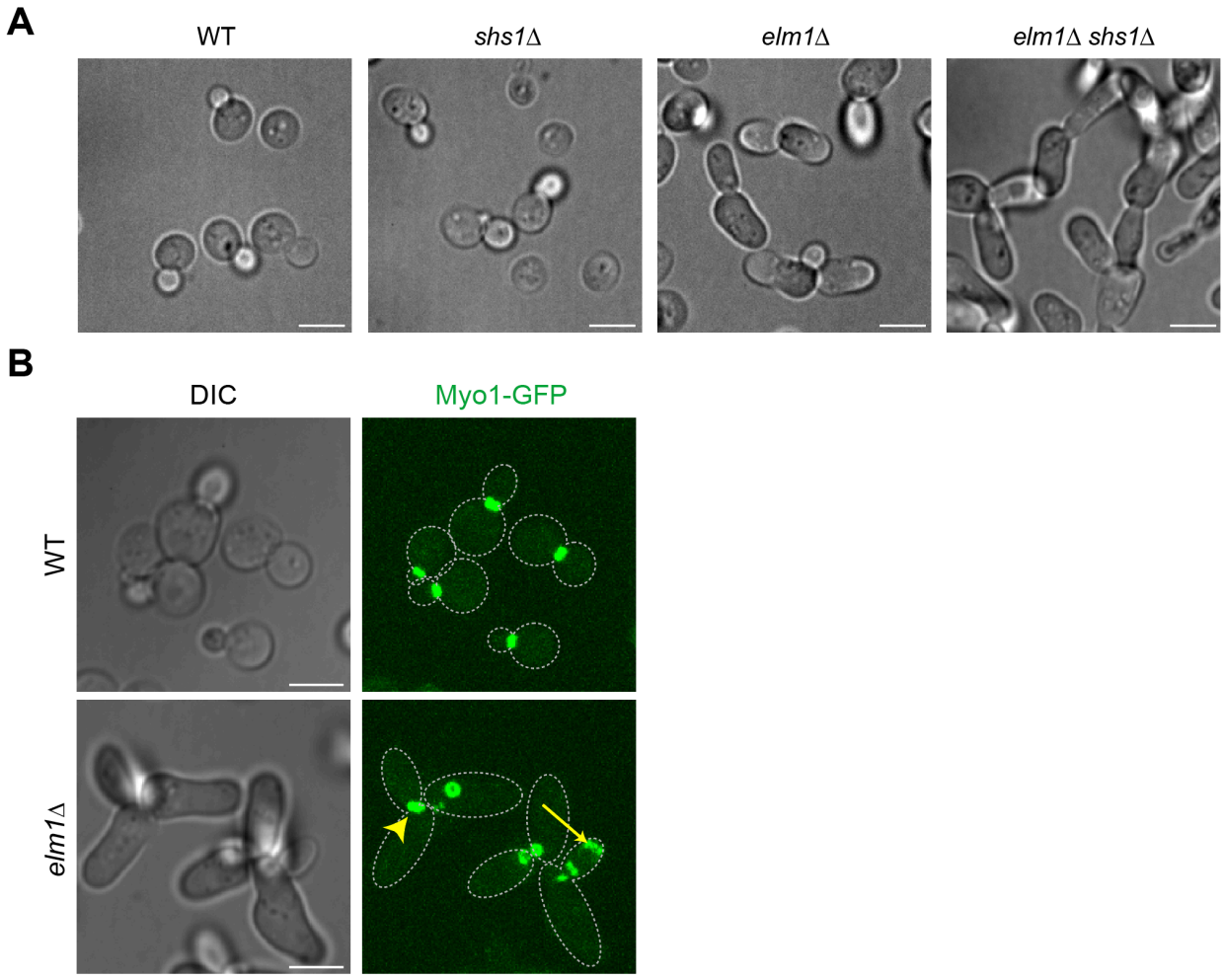

Figure S4. Elm1 and Shs1 cooperatively control cytokinesis presumably through their distinct roles in septin hourglass assembly. Related to Figure 4.

(A) Representative images of cell morphology (DIC) for WT (YEF8118), *shs1Δ* (YEF8244), *elm1Δ* (YEF8195), and *elm1Δ shs1Δ* (YEF8246) cells. Scale bar = 5µm. (B) Representative images of cell morphology (DIC) and Myo1-GFP in WT (YEF8367) and *elm1Δ* (YEF8381) cells. Yellow arrow indicates cortical Myo1 signal and yellow arrowhead indicates Myo1 returned to the bud neck during cytokinesis. Scale bar = 5µm.

Strain	Genotype	Source
YEF473A	**a** *his3 leu2 lys2 trp1 ura3*	[1]
JGY1326 (YEF4162)	As YEF473A except *CDC3-mCherry-spHIS5*	[2]
YEF5804	As YEF473A except *CDC3-mCherry-LEU2*	[3]
YEF5995	As YEF473A except *CDC3-GFP-LEU2*	[4]
YEF7498	As YEF473A except *CDC3-GFP-LEU2 NUP57-mCherry-URA3*	This study[a]
YEF8087	As YEF473A except *elm1Δ::KanMX6 CDC3-GFP-LEU2 NUP57-mCherry-URA3*	This study[b]
YEF8102	As YEF473A except *CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-URA3*	This study[c]
YEF8118	As YEF473A except *CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HIS3*	This study[d]
YEF8123	As YEF473A except *elm1Δ::URA3-KanMX6*	This study[e]
YEF8132	As YEF473A except *pHis3-mRuby2-TUB1-URA3*	This study[c]
YEF8195	As YEF473A except *elm1Δ::URA3-KanMX6 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HIS3*	This study[f]
YEF8219	As YEF473A except *CHS2-GFP-HIS3 pHis3-mRuby2-TUB1-URA3*	This study[g]
YEF8244	As YEF473A except *shs1Δ::TRP1 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HIS3*	This study[h]
YEF8246	As YEF473A except *shs1Δ::TRP1 elm1Δ::URA3-KanMX6 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HIS3*	This study[h]
YEF8247	As YEF473A except *elm1Δ::KanMX6 CHS2-GFP-HIS3MX6 pHis3-mRuby2-TUB1-URA3*	This study[b]
YEF8250	As YEF473A except *ELM1-GFP-SpHIS5 pHis3-mRuby2-TUB1-URA3*	This study[i]
YEF8299	As YEF473A except *ELM1-GFP-CaURA3 CDC3-mCherry-spHIS5*	This study[j]
YEF8367	As YEF473A except *MYO1-GFP-HIS3 pHis3-mRuby2-TUB1-URA3*	This study[k]
YEF8381	As YEF473A except *elm1Δ::KanMX6 MYO1-GFP-HIS3 pHis3-mRuby2-TUB1-URA3*	This study[b]
YEF8390	As YEF473A except *pHis3-mRuby2-TUB1-HPH*	This study[l]
YEF8393	As YEF473A except *elm1Δ::KanMX6 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-URA3*	This study[b]
YEF8502	As YEF473A except *shs1Δ::TRP1 MYO1-GFP-HIS3 pHis3-mRuby2-TUB1-URA3*	This study[h]
YEF8503	As YEF473A except *shs1Δ::TRP1 elm1Δ::KanMX6 MYO1-GFP-HIS3 pHis3-mRuby2-TUB1-URA3*	This study[h]
YEF8817	As YEF473A except *BNI4-GFP-NatMX6 CDC3-mCherry-LEU2*	This study[m]
YEF8818	As YEF473A except *KCC4-GFP-NatMX6 CDC3-mCherry-LEU2*	This study[n]
YEF8911	As YEF473A except *shs1Δ::TRP1 CHS2-GFP-HIS3MX6 pHis3-mRuby2-TUB1-URA3*	This study[h]
YEF8912	As YEF473A except *shs1Δ::TRP1 elm1Δ::KanMX6 CHS2-GFP-HIS3MX6 pHis3-mRuby2-TUB1-URA3*	This study[h]
YEF8914	As YEF473A except *elm1Δ::KanMX6 BNI4-GFP-NatMX6 CDC3-mCherry-LEU2*	This study[b]
YEF8915	As YEF473A except *elm1Δ::KanMX6 KCC4-GFP-NatMX6 CDC3-mCherry-LEU2*	This study[b]
YEF8954	As YEF473A except *HSL1-GFP-NatMX6 CDC3-mCherry-LEU2*	This study[o]
YEF8995	As YEF473A except *elm1Δ::KanMX6 HSL1-GFP-NatMX6 CDC3-mCherry-LEU2*	This study[b]
YEF9180	As YEF473A except *CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH*	This study[l]
YEF9206	As YEF473A except $ELM1^{KD(K117R)}$	This study[p]
YEF9214	As YEF473A except *elm1Δ::URA3-KanMX6 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH*	This study[f]
YEF9271	As YEF473A except $ELM1^{KD(K117R)}$ *CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH*	This study[p]
YEF9273	As YEF473A except $ELM1^{KD(K117R)}$*-GFP-SpHIS5*	This study[q]

YEF9287	As YEF473A except *ELM1$^{KD(K117R)}$-GFP-SpHIS5 CDC3-mCherry-LEU2*	This study[r]
YEF9305	As YEF473A except *ELM1-GFP-CaURA3 CDC3-mCherry-LEU2*	This study[s]
YEF9327	As YEF473A except *bar1Δ::HIS3MX6*	This study[t]
YEF9330	As YEF473A except *ELM1$^{AS(T200A)}$*	This study[u]
YEF9333	As YEF473A except *bar1Δ::HIS3MX6 CDC3-GFP-LEU2*	This study[v]
YEF9334	As YEF473A except *bar1Δ::HIS3MX6 elm1Δ::KanMX6*	This study[b]
YEF9335	As YEF473A except *ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[w]
YEF9341	As YEF473A except *bar1Δ::HIS3MX6 elm1Δ::KanMX6 CDC3-GFP-LEU2*	This study[b]
YEF9342	As YEF473A except *CDC3-GFP-LEU2 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[v]
YEF9362	As YEF473A except *SHS1-GFP-HIS3 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[x]
YEF9370	As YEF473A except *MYO1-GFP-HIS3 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[k]
YEF9431	As YEF473A except *bar1Δ::TRP1*	This study[y]
YEF9460	As YEF473A except *ELM1$^{AS(T200A)}$-mApple-GBP-CaURA3*	This study[z]
YEF9486	As YEF473A except *ELM1$_{1-420}$-GFP-SpHIS5*	This study[aa]
YEF9501	As YEF473A except *CDC3-GFP-LEU2 ELM1$^{AS(T200A)}$-mApple-GBP-CaURA3*	This study[v]
YEF9503	As YEF473A except *MYO1-GFP-HIS3 ELM1$^{AS(T200A)}$-mApple-GBP-CaURA3*	This study[k]
YEF9594	As YEF473A except *bni5Δ::KanMX6 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[ab]
YEF9598	As YEF473A except *ELM1$_{1-420}$-mApple-GBP-CaURA3*	This study[ac]
YEF9619	As YEF473A except *bar1Δ::TRP1 bni5Δ::HIS3*	This study[ad]
YEF9644	As YEF473A except *bni5Δ::KanMX6 CDC11-GFP-HIS3 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[ae]
YEF9645	As YEF473A except *bni5Δ::KanMX6 SHS1-GFP-HIS3 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[x]
YEF9646	As YEF473A except *bni5Δ::KanMX6 MYO1-GFP-HIS3 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[k]
YEF9767	As YEF473A except *bar1Δ::TRP1 bni5Δ::HIS3 CDC3-GFP-LEU2*	This study[v]
YEF9782	As YEF473A except *CDC11-GFP-HIS3 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[ae]
YEF9843	As YEF473A except *ELM1$^{KD(K117R)}$$_{1-420}$-GFP-SpHIS5*	This study[af]
YEF9884	As YEF473A except *ELM1$^{KD(K117R)}$$_{1-420}$-mApple-GBP-CaURA3*	This study[ag]
YEF9935	As YEF473A except *elm1Δ::KanMX6 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH*	This study[b]
YEF9938	As YEF473A except *SHS1-GFP-HIS3 ELM1$^{KD(K117R)}$$_{1-420}$-mApple-GBP-CaURA3*	This study[y]
YEF9940	As YEF473A except *MYO1-GFP-HIS3 ELM1$^{KD(K117R)}$$_{1-420}$-mApple-GBP-CaURA3*	This study[k]
YEF9972	As YEF473A except *CDC10-GFP-SpHIS5 ELM1$^{KD(K1179R)}$-mApple-GBP-CaURA3*	This study[ah]
YEF9973	As YEF473A except *bni5Δ::KanMX6 CDC10-GFP-SpHIS5 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[ah]
YEF10001	As YEF473A except *ELM1$_{1-420}$-mApple-GBP-CaURA3 SHS1-GFP-HIS3*	This study[x]
YEF10003	As YEF473A except *ELM1$_{1-420}$-mApple-GBP-CaURA3 MYO1-GFP-HIS3*	This study[k]
YEF10089	As YEF473A except *bni5Δ::KanMX6 CDC3-GFP-LEU2 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[v]
YEF10105	As YEF473A except *CDC10-GFP-SpHIS5 ELM1$^{AS(T200A)}$-mApple-GBP-CaURA3*	This study[ah]
YEF10106	As YEF473A except *CDC11-GFP-HIS3 ELM1$^{AS(T200A)}$-mApple-GBP-CaURA3*	This study[ae]
YEF10107	As YEF473A except *SHS1-GFP-HIS3 ELM1$^{AS(T200A)}$-mApple-GBP-CaURA3*	This study[x]
YEF10243	As YEF473A except *bni5NΔ::URA3-KanMX6*	This study[ai]
YEF10263	As YEF473A except *CDC10-mApple-GBP-SpHIS5*	This study[aj]
YEF10276	As YEF473A except *yoEGFP-BNI5*	This study[ak]

YEF10278	As YEF473A except *bni5NΔ::URA3-KanMX6 ELM1$^{KD(K117R)}$*	This study[al]
YEF10279	As YEF473A except *elm1Δ::KanMX6 CDC10-mApple-GBP-SpHIS5*	This study[b]
YEF10294	As YEF473A except *CDC10-mApple-GBP-SpHIS5, pGFP316-CDC10*	This study[am]
YEF10295	As YEF473A except *elm1Δ::KanMX6 CDC10-mApple-GBP-SpHIS5, pGFP316-CDC10*	This study[am]
YEF10302	As YEF473A except *hof1Δ::TRP1 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH*	This study[an]
YEF10311	As YEF473A except *CDC10-mApple-SpHIS5*	This study[ao]
YEF10333	As YEF473A except *yoEGFP-BNI5 ELM1$^{KD(K117R)}$*	This study[ap]
YEF10334	As YEF473A except *hof1Δ::TRP1 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH, pRS316-HOF1*	This study[aq]
YEF10349	As YEF473A except *yoEGFP-BNI5 ELM1$^{KD(K117R)}$-mApple-GBP-CaURA3*	This study[z]
YEF10352	As YEF473A except *elm1Δ::KanMX6 CDC10-mApple-SpHIS5*	This study[b]
YEF10356	As YEF473A except *elm1Δ::KanMX6 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH, pRS316-HOF1*	This study[aq]
YEF10357	As YEF473A except *CDC10-mApple-SpHIS5, pGFP316-CDC10*	This study[am]
YEF10358	As YEF473A except *elm1Δ::KanMX6 CDC10-mApple-SpHIS5, pGFP316-CDC10*	This study[am]
YEF10364	As YEF473A except *elm1Δ::KanMX6 hof1Δ::TRP1 CDC3-GFP-LEU2 pHis3-mRuby2-TUB1-HPH, pRS316-HOF1*	This study[b]

Table S1. Strains used in this study. Related to STAR METHODS.

[a] A DNA fragment carrying *NUP57-mCherry-URA3* was amplified by PCR using the plasmid pFA6a-mCherry-URA3 (a gift from C. Burd at Yale University) as the template DNA and the pair of primers Nup57-mCherry-Ura3-F2 and Nup57-mCherry-Ura3-R1, and then transformed into YEF5995 to generate YEF7498.

[b] A DNA fragment carrying *elm1Δ::KanMX6* was amplified by PCR using the chromosomal DNA from YEF7515 (our lab stock) as the template DNA and the pair of primers Elm1-F-check and Elm1-R-check, and then transformed into YEF7498, YEF8219, YEF8367, YEF8102, YEF8817, YEF8818, YEF8954, YEF9327, YEF9333, YEF9336, YEF9734, YEF9180, YEF10263, YEF10311, and YEF10334 to generate YEF8087, 8247, YEF8381, YEF8393, YEF8914, YEF8915, YEF8995, YEF9334, YEF9341, YEF9369, YEF9748, YEF9935, YEF10279, YEF10352, and YEF10364 respectively.

[c] BsaBI-digested plasmid pHIS3p:mRuby2-Tub1+3'UTR::URA3 (integrated at the *TUB1* locus) [5] was transformed into YEF5995 and YEF473A to generate YEF8102 and YEF8132, respectively.

[d] XbaI-digested plasmid pHIS3p:mRuby2-Tub1+3'UTR::HIS3 (integrated at the *TUB1* locus) [5] was transformed into YEF5995 to generate YEF8118.

[e] A DNA fragment carrying *elm1Δ::URA3-KanMX6* was amplified by PCR using the plasmid pFA6a-URA3-KanMX6 (a gift from John Pringle) as the template DNA and the pair of primers Elm1-F1 and Elm1-R1, and then transformed into YEF473A to generate YEF8123.

[f] A DNA fragment carrying *elm1Δ::URA3-KanMX6* was amplified by PCR using the chromosomal DNA from YEF8123 as the template DNA and the pair of primers Elm1-F-check and Elm1-R-check, and then transformed into YEF8118 and YEF9180 to generate YEF8195 and YEF9214, respectively.

[g] A DNA fragment carrying CHS2-GFP-HIS3 was amplified by PCR using the chromosomal DNA from YEF5762 (our lab stock) as the template DNA and the pair of primers Chs2-Ftag-check and Chs2-R-check, and then transformed into YEF8132 to generate YEF8219.

[h] A DNA fragment carrying *shs1Δ::TRP1* was amplified by PCR using the chromosomal DNA from YEF7506 (our lab stock) as the template DNA and the pair of primers Shs1-F-check and Shs1-R-check, and then transformed into YEF8118, YEF8195, YEF8367, YEF8381, YEF8219, and YEF8247 to generate YEF8244, YEF8246, YEF8502, YEF8503, YEF8911, and YEF8912, respectively.

[i] A DNA fragment carrying Elm1-GFP-SpHIS5 was amplified by PCR using pFA6a-link-yEGFP-SpHis5 [6] as the template and the pair of primers Elm1-F5 and Elm1-R3, and then transformed into YEF8132 to generate YEF8250.

[j] A DNA fragment carrying Elm1-GFP-CaURA3 was amplified by PCR using pFA6a-link-yEGFP-CaURA3 [6] as the template and the pair of primers Elm1-F5 and Elm1-R3, and then transformed into JGY1326 (YEF4162) to generate YEF8299.

[k] A DNA fragment carrying Myo1-GFP-HIS3 was amplified by PCR using the chromosomal DNA from YEF5291 (our lab stock) as the template DNA and the pair of primers Myo1-Ftag-check and Myo1-R-check, and then transformed into YEF8132, YEF9335, YEF9460, YEF9594, YEF9884, and YEF9598 to generate YEF8367, YEF9370, YEF9503, YEF9646, YEF9940, YEF10003, respectively.
[l] BsaBI-digested plasmid pHis3:mRuby2-Tub1+3'UTR::HPH [5] was transformed into YEF473A and YEF5995 to generate strains YEF8390 and YEF9180, respectively.
[m] A DNA fragment carrying Bni4-GFP-NatMX6 was amplified by PCR using pFA6a-link-yEGFP-NatMX6 (See plasmid generation methods) as the template and the pair of primers Bni4-F5 and Bni4-R3, and then transformed into YEF5804 to generate YEF8817.
[n] A DNA fragment carrying Kcc4-GFP-NatMX6 was amplified by PCR using pFA6a-link-yEGFP-NatMX6 (See plasmid generation methods) as the template and the pair of primers Kcc4-F5 and Kcc4-R3, and then transformed into YEF5804 to generate YEF8818.
[o] A DNA fragment carrying Hsl1-GFP-NatMX6 was amplified by PCR using pFA6a-link-yEGFP-NatMX6 (See plasmid generation methods) as the template and the pair of primers Hsl1-F5 and Hsl1-R3, and then transformed into YEF5804 to generate YEF8954.
[p] To generate endogenous *ELM1* with the kinase dead mutation K117R, two DNA fragments were generated by PCR using the chromosomal DNA from YEF473A as the template DNA: fragment 1 used Elm1-F-check and Elm1-K117R-R, fragment 2 used Elm1-K117R-F and Elm1-R-check. These two fragments have 20 bases of homology between them via the K117R primers to site directed mutagenize the K amino acid to R. A second amplification by PCR was done using the above two fragments as the template DNA (after gel extraction and purification) and the pair of primers Elm1-F-check and Elm1-R-check to combine the two fragments. This long fragment was then transformed into YEF8123 and YEF9214 and selected for the loss of the URA3-KanMX6 cassette to generate YEF9206 and YEF9271, respectively.
[q] A DNA fragment carrying Elm1-GFP-SpHIS5 was amplified by PCR using the chromosomal DNA from YEF8250 as the template DNA and the pair of primers Elm1-Ftag-check and Elm1-R-check, and then transformed into YEF9206 to generate YEF9273.
[r] BglII-digested YIp128-CDC3-mCherry (integrated at the *CDC3* locus) [7] was transformed into YEF9273 to generate YEF9287.
[s] A DNA fragment carrying Elm1-GFP-CaURA3 was amplified by PCR using the chromosomal DNA from YEF8299 as the template DNA and the pair of primers Elm1-Ftag-check and Elm1-R-check, and then transformed into YEF 5804 to generate YEF9305.
[t] A DNA fragment carrying *bar1Δ::HIS3MX6* was amplified by PCR using the plasmid pFA6a-HIS3MX6 [8] as the template DNA and the pair of primers Bar1-F1 and Bar1-R1, and then transformed into YEF473A to generate YEF9327.
[u] A DNA fragment carrying *ELM* with the T200A mutation making the kinase sensitive to the bulky analog 1NM-PP1 was amplified by PCR using the chromosomal DNA from SA2 (YEF8090 in our stock, a gift from Douglass Kellogg) as the template DNA and the pair of primers Elm1-F-check and Elm1-R-check, and then transformed into YEF8123 and selected for the loss of the URA3-KanMX6 cassette to generate YEF9330.
[v] BglII-digested YIp128-CDC3-GFP (integrated at the *CDC3* locus) [9] was transformed into YEF9327, YEF9335, YEF9460, YEF9619, and YEF9594 to generated strains YEF9333, YEF9342, and YEF9501, YEF9767, and YEF10089, respectively.
[w] A DNA fragment carrying Elm1-mApple-GBP-CaURA3 was amplified by PCR using the plasmid pFA6a-link-mApple-GBP-CaURA3 (see plasmid generation methods) as the template DNA and the pair of primers Elm1-F5 and Elm1-R3, and then transformed into YEF9206 to generate YEF9335.
[x] A DNA fragment carrying Shs1-GFP-HIS3 was amplified by PCR using the chromosomal DNA from YEF6651 (our lab stock) as the template DNA and the pair of primers Shs1-Ftag-check and Shs1-R-check, and then transformed into YEF9335, YEF9594, YEF9884, YEF9598, and YEF9460 to generate YEF9362, YEF9645, YEF9938, YEF10001, and YEF10107, respectively.
[y] A DNA fragment carrying *bar1Δ::TRP1* was amplified by PCR using the plasmid pFA6a-TRP1 [8] as the template DNA and the pair of primers Bar1-F1 and Bar1-R1, and then transformed into YEF473A to generate YEF9431.
[z] A DNA fragment carrying Elm1-mApple-GBP-CaURA3 was amplified by PCR using the chromosomal DNA from YEF9335 as the template DNA and the pair of primers Elm1-Ftag-check and Elm1-R-check, and then transformed into YEF9330 and YEF10333 to generate YEF9460 and YEF10349, respectively.
[aa] To generate the first 420 amino acids of ELM1 tagged with GFP at the endogenous *ELM1* locus, two DNA fragments were generated by PCR: fragment 1 used the chromosomal DNA from YEF473A with the pair of primers Elm1-F-check and Elm1-420-R, fragment 2 used the chromosomal DNA from YEF8250 with the pair of primers

Elm1-420-F5 and Elm1-R-check. These two fragments have 40 bases of homology between them via the F5 region present in the R primer of fragment 1 and the F primer of fragment 2. A second amplification by PCR was done using the above two fragments as the template DNA (after gel extraction and purification) and the pair of primers Elm1-F-check and Elm1-R-check to combine the two fragments. This long fragment was then transformed into YEF8123 and selected for the loss of the URA3-KanMX6 cassette to generate YEF9486.

[ab] A DNA fragment containing *bni5Δ::KanMX6* was amplified by PCR using the chromosomal DNA from YEF7253 (our lab stock) as the template DNA and the pair of primers Bni5-244bp US-Start and Bni5-381bp-DS-Stop, and then transformed into YEF9335 to generate YEF9594.

[ac] To generate the first 420 amino acids of ELM1 tagged with mApple-GBP at the endogenous *ELM1* locus, two DNA fragments were generated by PCR: fragment 1 used the chromosomal DNA from YEF473A with the pair of primers Elm1-F-check and Elm1-420-R, fragment 2 used the chromosomal DNA from YEF9335 with the pair of primers Elm1-420-F5 and Elm1-R-check. These two fragments have 40 bases of homology between them via the F5 region present in the R primer of fragment 1 and the F primer of fragment 2. A second amplification by PCR was done using the above two fragments as the template DNA (after gel extraction and purification) and the pair of primers Elm1-F-check and Elm1-R-check to combine the two fragments. This long fragment was then transformed into YEF9486 and selected for the loss of the GFP-HIS cassette to generate YEF9598.

[ad] A DNA fragment containing *bni5Δ::HIS3MX6* was amplified by PCR using the chromosomal DNA from YEF6316 (our lab stock) as the template DNA and the pair of primers Bni5-244bp-US-Start and Bni5-381bp-DS-Stop, and then transformed into YEF9431 to generate YEF9619.

[ae] A DNA fragment carrying Cdc11-GFP-HIS3 was amplified by PCR using the chromosomal DNA from YEF6652 (our lab stock) as the template DNA and the pair of primers Cdc11-Ftag-check and Cdc11-R-check, and then transformed into YEF9594, YEF9335, and YEF9460 to generate YEF9644, YEF9782, and YEF10106, respectively.

[af] To generate the first 420 amino acids of ELM1^{KD} tagged with GFP at the endogenous *ELM1* locus, two DNA fragments were generated by PCR: fragment 1 used the chromosomal DNA from YEF9206 with the pair of primers Elm1-F-check and Elm1-420-R, fragment 2 used the chromosomal DNA from YEF8250 with the pair of primers Elm1-420-F5 and Elm1-R-check. These two fragments have 40 bases of homology between them via the F5 region present in the R primer of fragment 1 and the F primer of fragment 2. A second amplification by PCR was done using the above two fragments as the template DNA (after gel extraction and purification) and the pair of primers Elm1-F-check and Elm1-R-check to combine the two fragments. This long fragment was then transformed into YEF8123 and selected for the loss of the URA3-KanMX6 cassette to generate YEF9843.

[ag] To generate the first 420 amino acids of ELM1^{KD} tagged with mApple-GBP at the endogenous *ELM1* locus, two DNA fragments were generated by PCR: fragment 1 used the chromosomal DNA from YEF9206 with the pair of primers Elm1-F-check and Elm1-420-R, fragment 2 used the chromosomal DNA from YEF9335 with the pair of primers Elm1-420-F5 and Elm1-R-check. These two fragments have 40 bases of homology between them via the F5 region present in the R primer of fragment 1 and the F primer of fragment 2. A second amplification by PCR was done using the above two fragments as the template DNA (after gel extraction and purification) and the pair of primers Elm1-F-check and Elm1-R-check to combine the two fragments. This long fragment was then transformed into YEF9843 and selected for the loss of the GFP-HIS cassette to generate YEF9884.

[ah] A DNA fragment carrying Cdc10-GFP-SpHIS5 was amplified by PCR using the chromosomal DNA from YEF9420 as the template and the pair of primers Cdc10-Ftag-check and Cdc10-R-check, and then transformed into YEF9335, YEF9594, and YEF9960 to generate YEF9972, YEF9973, and YEF10105, respectively.

[ai] A DNA fragment carrying *bni5NΔ::URA3-KanMX6* that would insert *URA3-KanMX6* cassette just downstream of the start codon of *BNI5* was amplified by PCR using the plasmid pFA6a-URA3-KanMX6 (a gift from John Pringle) as the template DNA and the pair of primers Bni5-F1 and Bni5N-term-R1, and then transformed into YEF473A to generate YEF10243.

[aj] A DNA fragment carrying Cdc10-mApple-GBP-SpHIS5 was amplified by PCR using the plasmid pFA6a-link-mApple-GBP-SpHIS5 (see plasmid generation methods) as the template DNA and the pair of primers Cdc10-F5 and Cdc10-R3, then transformed into YEF473A to generate YEF10263.

[ak] A DNA fragment carrying yoEGFP with flanking sequences to integrate at the N-terminus of *BNI5* was amplified by PCR using plasmid YIp128-proACT1-yEGFP-TPM1-tADH1 (our lab stock) as the template DNA and pair of primers Bni5NGFP-F and Bni5NGFP-R, then transformed into YEF10243 and selected for the loss of the *URA3-KanMX6* cassette to generate YEF10276.

[al] A DNA fragment carrying *bni5NΔ::URA3-KanMX6* that would insert *URA3-KanMX6* cassette just downstream of the start codon of *BNI5* was amplified by PCR using the chromosomal DNA from YEF10243 as the template DNA and the pair of primers Bni5-244bp US-Start and Bni5Nterm-Rtag-check, and then transformed into YEF9206 to generate YEF10278.

[am] Plasmid pGFP316-CDC10 [10] (CEN, URA3, Cdc10-GFP) was transformed into strains YEF10263, YEF10279, YEF10311, and YEF10352 to generate strains YEF10294, YEF10295, YEF10357, and YEF10358, respectively.
[an] A DNA fragment carrying *hof1Δ::TRP1* was amplified by PCR using the chromosomal DNA from YEF4460 (our lab stock) as the template DNA and the pair of primers HOF1-400up-from-Start and HOF1-350down-from-Stop, and then transformed into YEF9180 to generate YEF10302.
[ao] A DNA fragment carrying Cdc10-mApple-SpHIS5 was amplified by PCR using the plasmid pFA6a-link-mApple-SpHIS5 [6] as the template DNA and the pair of primers Cdc10-F5 and Cdc10-R3, then transformed into YEF473A to generate YEF10311.
[ap] A DNA fragment carrying yoEGFP with flanking sequences to integrate at the N-terminus of *BNI5* was amplified by PCR using the chromosomal DNA from YEF10276 as the template DNA and pair of primers Bni5-244bp US-Start and Bni5Nterm-Rtag-check, then transformed into YEF10278 and selected for the loss of the *URA3-KanMX6* cassette to generate YEF10333.
[aq] Plasmid pRS316-HOF1 [11], (CEN, URA3, *HOF1*) was transformed into strains YEF10302 and YEF9935 to generate strains YEF10334 and YEF10356, respectively.

Oligonucleotides	**Function**	**Identifier**
Nup57-mCherry-Ura3-F2 : GAAAGATGCTGCAATTGTAAAAAAATATAAAAATAAAACGC GGATCCCCGGGTTAATTAA	For the construction of *NUP57-mCherry-URA3*	N/A
Nup57-mCherry-Ura3-R1: CGATCTTTATACAATTCAGTCATTGATTTAAGTAACCTGAGA ATTCGAGCTCGTTTAAAC	For the construction of *NUP57-mCherry-URA3*	N/A
Elm1-F-check: GAGGAACTTACTTGATCCTTCTTGAAG	For the amplification of *elm1ΔKanMX6, $ELM1^{KD}$, $ELM1^{AS}$,* and $ELM1_{1-420}$	P1139
Elm1-R-check: GATTTCGCGACACAGTGG	For the amplification of *elm1ΔKanMX6, ELM1-GFP-SpHIS5, ELM1-GFP-CaURA3, ELM1-mApple-GBP-CaURA3, $ELM1^{KD}$, $ELM1^{AS}$,* and $ELM1_{1-420}$	P1140
Elm1-F1: TTTTTTGAACGCCAGGTTAACAATAATTACTTAGCATGAACG GATCCCCGGGTTAATTAA	For the construction of *elm1Δ::URA3-KanMX6*	P1125
Elm1-R1: CAGCTAACCCAATCCGACAGATATCATCCTGTAGTTTCATGA ATTCGAGCTCGTTTAAAC	For the construction of *elm1Δ::URA3-KanMX6*	P1126
Chs2-Ftag-check: GAATTGTGATGATTTGGATGC	For the amplification of *CHS2-GFP-HIS3*	F123
Chs2-R-check: TCAAAAGCTCTTGATGCCCA	For the amplification of *CHS2-GFP-HIS3*	R093
Shs1-F-check: ACCACCTTTTTCCATACGA	For the amplification of *shs1Δ::TRP1*	Y178
Shs1-R-check: GTTACGGGAAATCATGATAG	For the amplification of *shs1Δ::TRP1* and *SHS1-GFP-HIS3*	P768
ELM1-F5: TGTAAAAACGTATCTGAACTTTGCAGATAATGGTCAAATAG GTGACGGTGCTGGTTTA	For the construction of *ELM1-GFPSpHIS5*, *ELM1-GFP-CaURA3, and ELM1-mApple-GBP-CaURA3.*	P1129
Elm1-R3: CAGCTAACCCAATCCGACAGATATCATCCTGTAGTTTCATTC GATGAATTCGAGCTCG	For the construction of *ELM1-GFPSpHIS5*, *ELM1-GFP-CaURA3, and ELM1-mApple-GBP-CaURA3.*	P1130
Myo1-159bp-US-Stop: CTAGCGAATAAAAATAGAAGCGA	For the amplification of *MYO1-GFP-HIS3*	N/A
Myo1-249bp-DS-Stop: GATACGGGGTGAAAGAGTT	For the amplification of *MYO1-GFP-HIS3*	N/A
Bni4-F5: GGAAGTACACGATGATTCGCGATGTTACACACATTTTTATGG TGACGGTGCTGGTTTA	For the construction of *BNI4-GFP-NatMX6*	P1151
Bni4-R3: GTATGATTTGATTCATTTCCATTTCTCCCAGTTTTCTGCTTCG ATGAATTCGAGCTCG	For the construction of *BNI4-GFP-NatMX6*	P1152
Kcc4-F5: AATCCAAATTATTTTACAAAAAGAAGGTGTTTTGGACAAAG GTGACGGTGCTGGTTTA	For the construction of *KCC4-GFP-NatMX6*	P1147
Kcc4-R3: CGTATTGTCCATTTGGGGATCGATTATCCCTCCCTTTTTTTCG ATGAATTCGAGCTCG	For the construction of *KCC4-GFP-NatMX6*	P1148
Hsl1-F5: TGATGATGTGGAGAGAGTAATTCGAAATGCCGGACGTTCAG GTGACGGTGCTGGTTTA	For the construction of *HSL1-GFP-NatMX6*	P1159

Hsl1-R3: CAAATTATTGTTGTATAATTATATAACATCTATATAGAATTC GATGAATTCGAGCTCG	For the construction of *HSL1-GFP-NatMX6*	P1160
Elm1-K117R-R: CAAGGTTTTTTTGGTATAATCCTGACAGCAACAACCTTGCCT A	For the construction of *ELM1$^{KD(K117R)}$*	P1165
Elm1-K117R-F: TAGGCAAGGTTGTTGCTGTCAGGATTATACCAAAAAAACCT TG	For the construction of *ELM1$^{KD(K117R)}$*	P1164
Elm1-Ftag-check: CCTAAAGAGAACGGGAACAGAAC	For the amplification of *ELM1-GFPSpHIS5*, *ELM1-GFP-CaURA3, and ELM1-mApple-GBP-CaURA3.*	P1119
Bni5-F1: TGGTGATGCTATGTTAGTGTGAAATAGAACAACAGAAACGC GGATCCCCGGGTTAATTAA	For the construction of *bni5NΔ::URA3-KanMX6*	P1067
Bni5-Nterm R1: GAGAAAGCCTCTTCTTTATCTTGTCCTGGTCCAAGCCCATGA ATTCGAGCTCGTTTAAAC	For the construction of *bni5NΔ::URA3-KanMX6*	P1194
Bni5-Nterm GFP-F: TGGTGATGCTATGTTAGTGTGAAATAGAACAACAGAAACGA TGTCTAAAGGTGAAGAATT	For the construction of *yoEGFP-BNI5*	P1196
Bni5-Nterm GFP-R: GAGAAAGCCTCTTCTTTATCTTGTCCTGGTCCAAGCCCATAC CACCTGTTCCTCCGCTAC	For the construction of *yoEGFP-BNI5*	P1197
Bni5-Nterm-R Tag check: TGGGAATATGGACCTGTGCG	For the amplification of *bni5NΔ::URA3-KanMX6* and *yoEGFP-BNI5*	P1195
Bni5-244bp-US-Start: CGGATCTTTGGCAAATGTATG	For the amplification of *bni5Δ::HIS3*, *bni5Δ::KanMX6*, *bni5NΔ::URA3-KanMX6,* and *yoEGFP-BNI5*	N/A
Bni5-381bp-DS-Stop: ACAAAGTTAGCAGGGTTATCGC	For the amplification of *bni5Δ::HIS3 and bni5Δ::KanMX6*	N/A
Bar1-F1: ATCATACCAAAATAAAAAGAGTGTCTAGAAGGGTCATATAC GGATCCCCGGGTTAATTAA	For the construction of *bar1Δ::HIS3* and *bar1Δ::TRP1*	P1170
Bar1-R1: TGATATTTATATGCTATAAAGAAATTGTACTCCAGATTTCGA ATTCGAGCTCGTTTAAAC	For the construction of *bar1Δ::HIS3* and *bar1Δ::TRP1*	P1171
Shs1-Ftag-check: GAAACCGTTCCATATGTCTTG	For the amplification of *SHS1-GFP-HIS3*	P1117
Elm1-420-R: TAAACCAGCACCGTCACCAATTTGACTGTGATTCCTAGAATC TATGG	For the construction of *ELM1$_{1-420}$*	P1184
Elm1-420-F5: GCGTGACCAGCCCATAGATTCTAGGAATCACAGTCAAATTG GTGACGGTGCTGGTTTA	For the construction of *ELM1$_{1-420}$*	P1191
Cdc11-Ftag-check: GTCATCGTCCACCACAACAAG	For the amplification of *CDC11-GFP-HIS3*	P1121
Cdc11-R-check: CGATAATGACGATCCACACAAG	For the amplification of *CDC11-GFP-HIS3*	P1122
Cdc10-F5: TCGTTCCTCAGCTCATATGTCTAGCAACGCCATTCAACGTGG TGACGGTGCTGGTTTA	For the construction of *CDC10-mApple-*SpHIS5 and *CDC10-mApple-GBP-*SpHIS5	P786
Cdc10-R3 AATAACATAAGATATATAATCACCACCATTCTTATGAGATTC GATGAATTCGAGCTCG	For the construction of *CDC10-mApple-*SpHIS5 and *CDC10-mApple-GBP-*SpHIS5	P787

Cdc10-Ftag-check: GCAGTGGTTGGTTCTGAG	For the amplification of *CDC10-GFP*-SpHIS5	P1135
Cdc10-R-check: CGCATTATGTCATTATGTAAAACC	For the amplification of *CDC10-GFP*-SpHIS5	P1136
HOF1-400up-from-Start: ACTGCAAGCAACAAGGAGTTCTCC	For the amplification of *hof1Δ::TRP1*	P205
HOF1-350down-from-Stop: TTCGTAACAAGTGACTCTAATGATA	For the amplification of *hof1Δ::TRP1*	P486
pR32-pmeI: GTTTAAACAATACGACTCACTATAGGGAG	For the construction of pFA6a-linker-yoEGFP-natMX6	N/A
pR29-bglII: CACATACGATTTAGGTGACAC	For the construction of pFA6a-linker-yoEGFP-natMX6	N/A
pRS315-F-PacI: GATAAAAACATAGAAAGG	For the construction of pFA6a-linker-yomApple-GBP-CaURA3 and pFA6a-linker-yomApple-GBP-SpHIS5	F045
GBP-R-AscI-term: GTCATGGCGCGCCTTAATGGTGGTGATGGTG	For the construction of pFA6a-linker-yomApple-GBP-CaURA3 and pFA6a-linker-yomApple-GBP-SpHIS5	R108
Elm1-F-BamHI FL: GGTTCCGCGTGGATCCATGTCACCTCGACAGCTTATAC	For the construction of pGEX-4T1-$Elm1_{FL}$ and pGEX-4T1-$Elm1_{1-420}$	P1003
Elm1-R-XhoI FL: GATGCGGCCGCTCGAGCTATATTTGACCATTATCTGC	For the construction of pGEX-4T1-$Elm1_{FL}$ and pGEX-4T1-$Elm1_{421-640}$	P1004
Elm1-F-BamHI 421-640: GGTTCCGCGTGGATCCTCATCGTCCAGTGTGAACCCCG	For the construction of pGEX-4T1-$Elm1_{421-640}$	P1005
Elm1-R-XhoI 1-420: GATGCGGCCGCTCGAGCTAAATTTGACTGTGATTCC	For the construction of pGEX-4T1-$Elm1_{1-420}$	P1006
Bni5-F-SspI: TGTACTTCCAATCCAATATTATGGGCTTGGACCAGGACAAG	For the construction of pET-His6-Sumo-Bni5	P1065
Bni5-R-Bam HI: GACGGCGCTCGAATTCGGATCCTCATTTAGTTCCAATCCAA	For the construction of pET-His6-Sumo-Bni5	P1066

Table S2. Oligonucleotides used in this study. Related to STAR METHODS.

Supplemental References:

S1. Bi, E., and Pringle, J.R. (1996). *ZDS1* and *ZDS2*, genes whose products may regulate Cdc42p in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *16*, 5264-5275.

S2. Iwase, M., Luo, J., Nagaraj, S., Longtine, M., Kim, H.B., Haarer, B.K., Caruso, C., Tong, Z., Pringle, J.R., and Bi, E. (2006). Role of a cdc42p effector pathway in recruitment of the yeast septins to the presumptive bud site. Mol. Biol. Cell *17*, 1110-1125.

S3. Wloka, C., Vallen, E.A., Thé, L., Fang, X., Oh, Y., and Bi, E. (2013). Immobile myosin-II plays a scaffolding role during cytokinesis in budding yeast. J. Cell Biol. *200*, 271-286.

S4. Ong, K., Wloka, C., Okada, S., Svitkina, T., and Bi, E. (2014). Architecture and dynamic remodelling of the septin cytoskeleton during the cell cycle. Nat. Commun. *5*, 5698.

S5. Markus, S.M., Omer, S., Baranowski, K., and Lee, W.L. (2015). Improved plasmids for fluorescent protein tagging of microtubules in *Saccharomyces cerevisiae*. Traffic *16*, 773-786.

S6. Lee, S., Lim, W.A., and Thorn, K.S. (2013). Improved blue, green, and red fluorescent protein tagging vectors for *S. cerevisiae*. PLoS One *8*, e67902.

S7. Gao, X.D., Sperber, L.M., Kane, S.A., Tong, Z., Hin Yan Tong, A., Boone, C., and Bi, E. (2007). Sequential and distinct roles of the cadherin domain-containing protein Axl2p in cell polarization in yeast cell cycle. Mol. Biol. Cell *18*, 2542-2560.

S8. Longtine, M.S., McKenzie, A., III,, DeMarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast *14*, 953-961.

S9. Caviston, J.P., Longtine, M., Pringle, J.R., and Bi, E. (2003). The role of Cdc42p GTPase-activating proteins in assembly of the septin ring in yeast. Mol. Biol. Cell *14*, 4051-4066.

S10. Iwase, M., and Toh-e, A. (2001). Nis1 encoded by YNL078W: a new neck protein of Saccharomyces cerevisiae. Genes Genet. Syst. *76*, 335-343.

S11. Vallen, E.A., Caviston, J., and Bi, E. (2000). Roles of Hof1p, Bni1p, Bnr1p, and Myo1p in cytokinesis in *Saccharomyces cerevisiae*. Mol. Biol. Cell *11*, 593-611.