Supplemental Materials

Molecular Biology of the Cell

Finnigan et al.

SUPPORTING INFORMATION

for

Detection of protein-protein interactions at the septin collar in *Saccharomyces cerevisiae* using a tripartite split-GFP system

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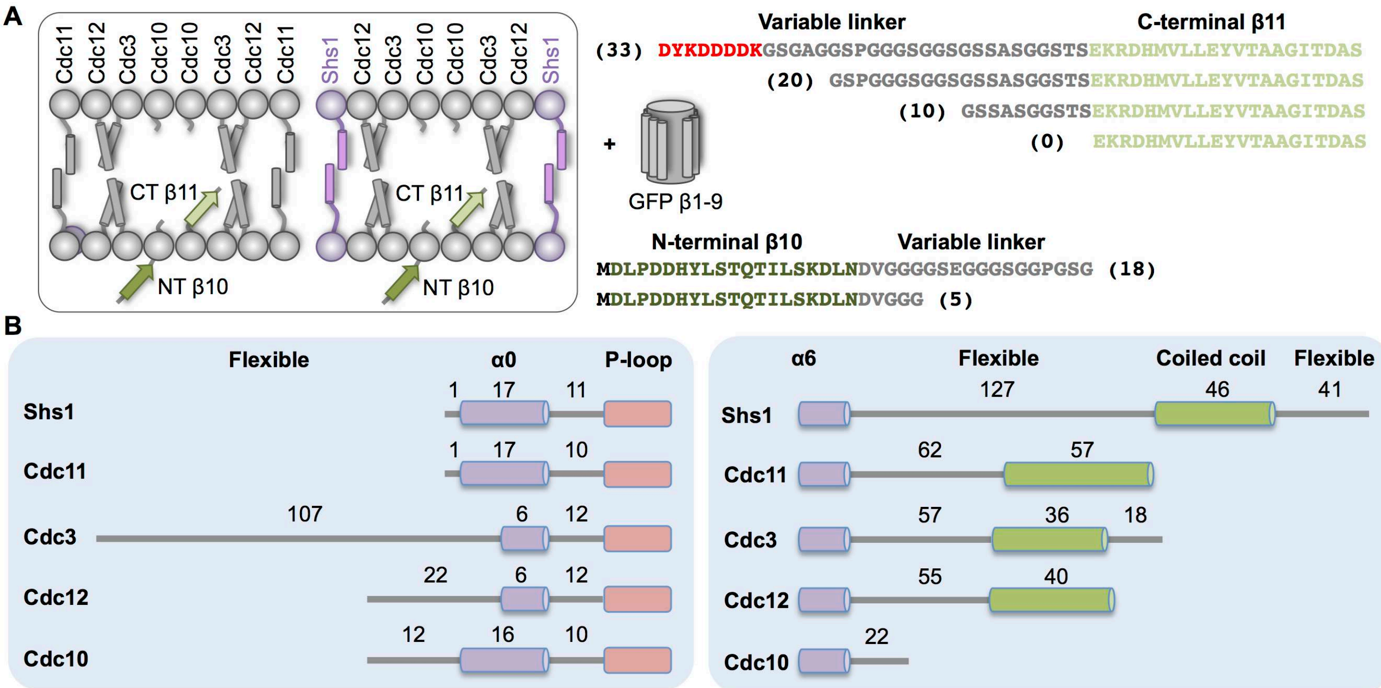

Figure S1. Tagging septin subunits for the tripartite split-GFP system. (A) *Left,* schematic diagram of the linear septin hetero-octamer, which can be capped with either of two alternative terminal subunits, Cdc11 or Shs1 (*purple*), and can polymerize end-to-end to form long laterally paired filaments. *Grey ball*, globular GTP-binding domain of each subunit; *cylinder,* C-terminal extension (CTE) with predicted α-helical and coiled coil-forming propensity (which only Cdc10 lacks); *dark green*, introduced N-terminal β10 tag; *light green*, introduced C-terminal β11 tag. When tagged versions of the same subunit are present in the diploid (*e.g.*, β10-Cdc10 and Cdc10-β11), no WT copy is present and 50% of the hetero-octamers will contain both tagged subunits. When two different tagged septins are combined (*e.g.*, β10-Cdc10 x Cdc3-β11), there is also a WT copy of each subunit present, which is not depicted in the diagram for clarity. In the latter case, in this representative subunit-subunit pairing (*e.g.*, β10-Cdc10 x Cdc3-β11), 7/16 (44%) of the hetero-octamers should contain both tagged subunits in a juxtaposed position. However, hetero-octamers are polymerized into filaments at the bud neck, greatly increasing the number of potential sites where juxtaposed tags will be present within any given septin collar, consequently the probability of detection (capture of the GFPβ1-9 barrel at multiple locations at the bud neck) is greatly enhanced. *Right,* C-terminal β11 tags were appended directly or with Ser- and Gly-rich linkers with the indicated lengths (10, 20 and 33 residues) and sequences indicated; N-terminal β10 tags were tethered, in most cases, by Gly-rich linkers of the lengths (5 and 18 residues) and sequences indicated. (B) Schematic representation of the naturally-occurring N-terminal (*left*) and C-terminal (*right*) extensions in the five yeast mitotic septins. Structure features follow the nomenclature of Sirajuddin *et al.* (2007).

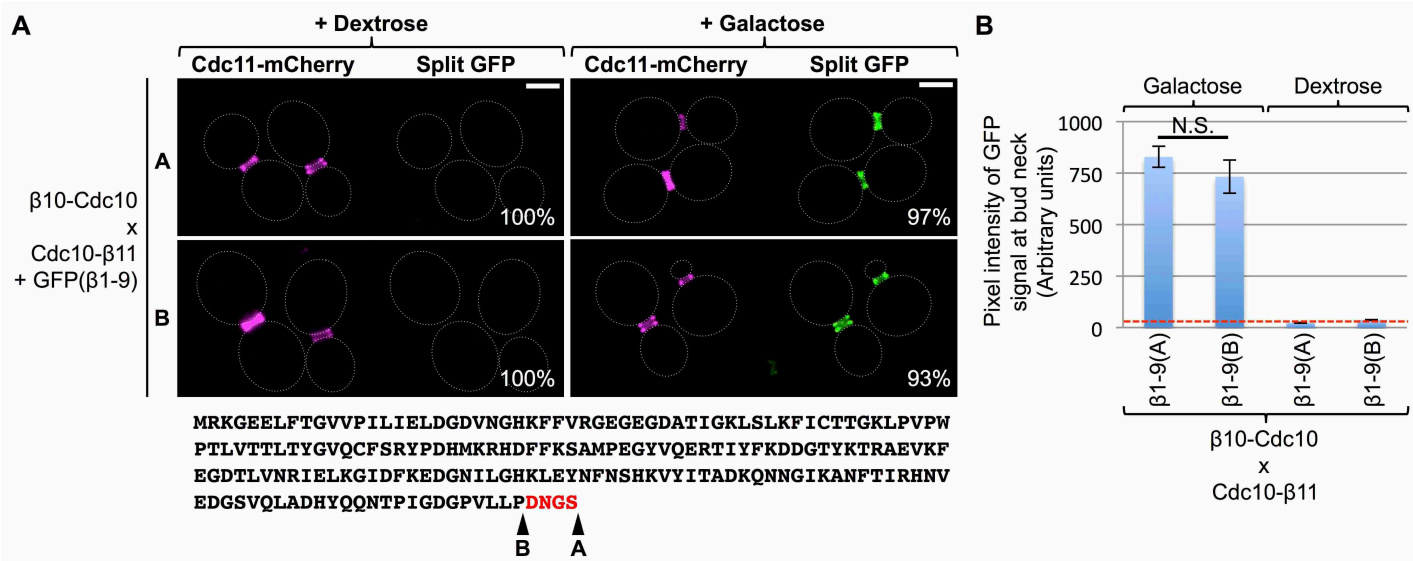

Figure S2. Both versions of the GFPβ1-9 barrel display robust GFP reconstitution *in vivo*. (A) In the initial report describing the tripartite split-GFP system (Cabantous et al., 2013), constructs of the GFPβ1-9 barrel that end at different positions after the 9th strand were used. Hence, we tested two such versions of GFPβ1-9 in yeast *in vivo*: A, which includes four additional residues (DNGS; *red*); and, B, ending after VLLP. Diploids (1 and 9, respectively) expressing β10-(linker)$_{32}$-Cdc10 and Cdc10-(linker)$_{33}$-β11, as well as Cdc11-mCherry, and carrying plasmids expressing either GFPβ1-9(A) (pGF-IVL794) or GFPβ1-9(B) (pGF-IVL795) from the inducible *GAL1/GAL10* promoter, were cultured overnight in 2% raffinose-0.2% sucrose medium selective for the presence of the plasmid, then back diluted into either the same medium containing 2% glucose (*left*) or 2% galactose (*right*), grown for 4.5 h, washed, and imaged by fluorescence microscopy. Glucose repression of either GFPβ1-9(A) or GFPβ1-9(B) expression prevented reconstitution of any GFP signal, whereas galactose induction of either GFPβ1-9(A) or GFPβ1-9(B) yielded equivalent, robust GFP fluorescence congruent with the bud neck marked with Cdc11-mCh. The percentage of the population (out of 50-100 representative budded cells) displaying the fluorescence pattern shown is indicated by the number in the lower right-hand corner of each panel. *Dotted white dotted line*, cell periphery; scale bar, 2 µm. (B) Quantification of the average pixel intensity of the GFP signal at the bud neck for the cultures shown in (A). *Dotted red line*, the average intrinsic background fluorescence of the bud neck in yeast cells lacking the components of the tripartite split-GFP system; *error bar*, S.E.M. There was no statistically significant difference (unpaired t-test, $p = 0.325$) between the average pixel intensity of the GFP signal at the bud neck when using either GFPβ1-9(A) or GFPβ1-9(B).

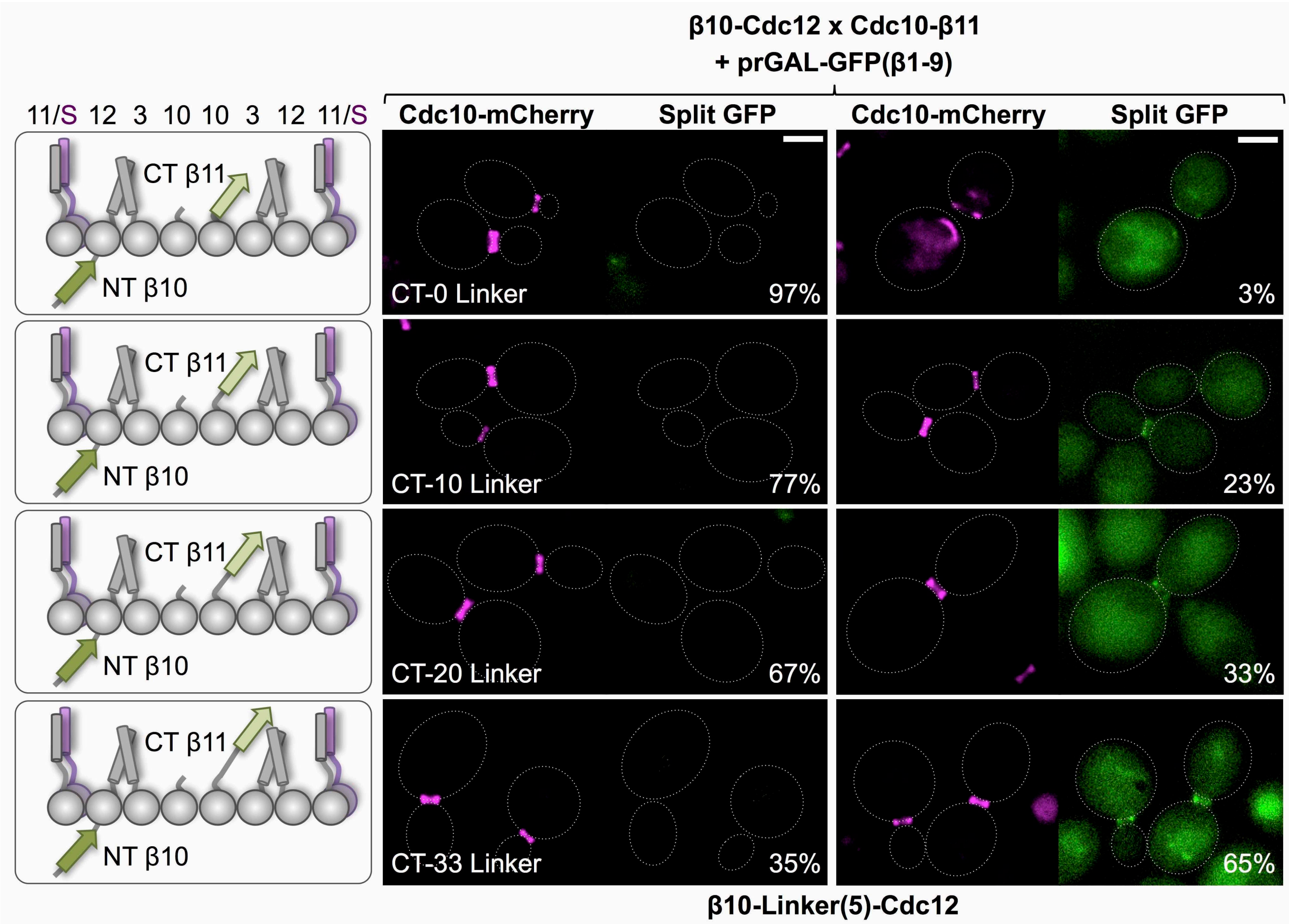

Figure S3. Interaction between Cdc10 and Cdc12 can be detected using the tripartite split-GFP method by extending the length of the linkers that tether the β10 and β11 tags. *Left*, schematic diagram of the septin hetero-octamer with the relative placement of the β10 and β11 tags on Cdc10 and Cdc12 (not precisely to scale). *Right*, Diploids (27, 13, 20, and 34) expressing β10-(linker)$_5$-Cdc12 (constant) with, respectively, Cdc10-β11, Cdc10-(linker)$_{10}$-β11, Cdc10-(linker)$_{20}$-β11, or Cdc10-(linker)$_{33}$-β11, as indicated, as well as Cdc10-mCh and a *CEN* plasmid expressing the GFPβ1-9 barrel from the *GAL1/GAL10* promoter (pGF-IVL794), were cultured, induce with galactose, harvested, washed and imaged by fluorescence microscopy as in Fig. S2A. The percentage of the population (out of 50-100 representative budded cells) displaying the fluorescence pattern shown is indicated by the number in the lower right-hand corner of each panel.

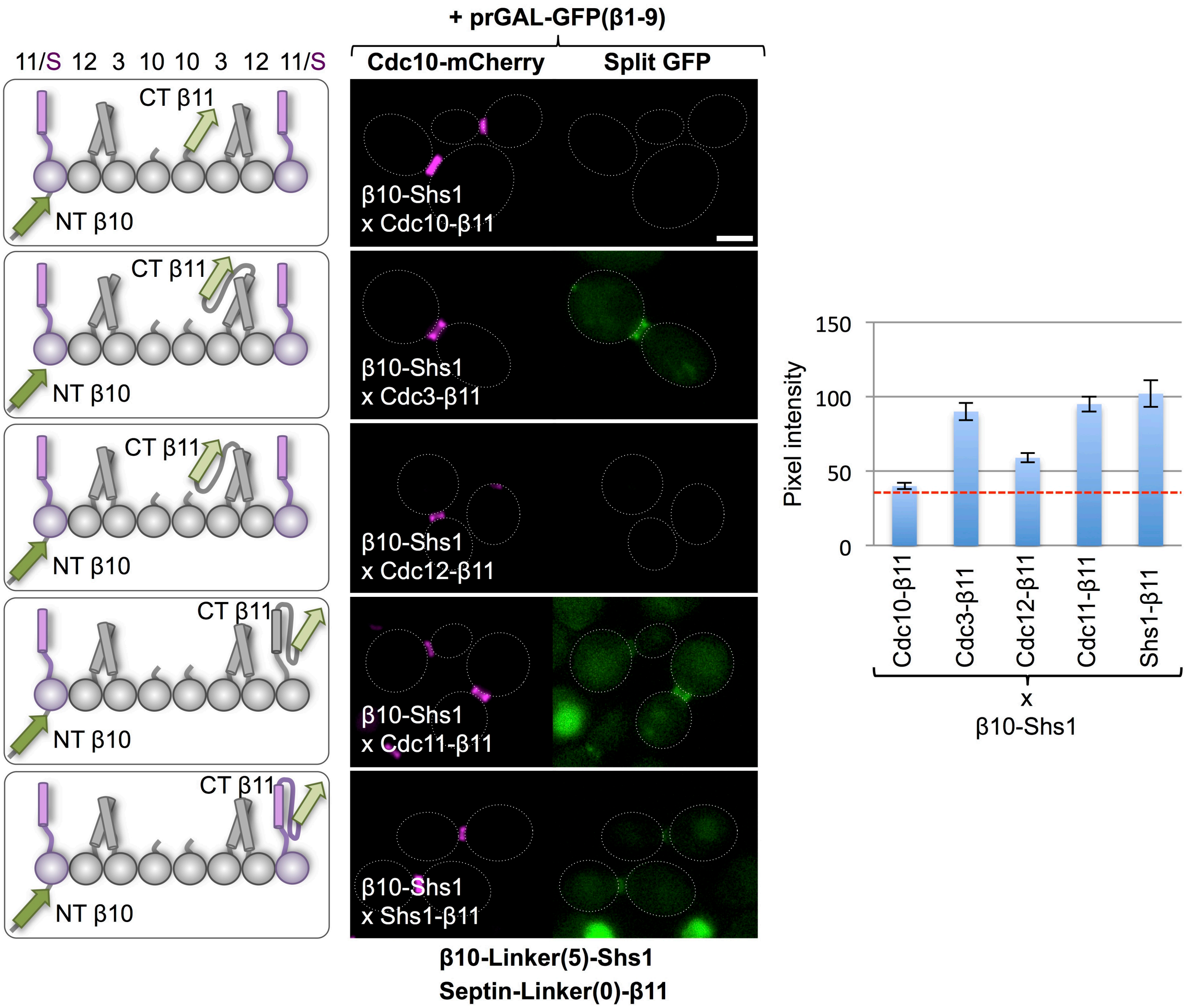

Figure S4. Interaction of the non-essential subunit Shs1 with the other mitotic septins assessed using the tripartite split-GFP method. (A) Diploids (108-112) expressing β10-(linker)$_5$-Shs1 and versions of all five mitotic septins to which the C-terminal β11 tag had been directly appended (i.e., no linker), and Cdc10-mCh (*left*), as well as the GFPβ1-9 barrel under the contorl of the *GAL1/GAL10* promoter on a *CEN* plasmid (pGF-IVL794) (*not shown*), were cultured, induced, harvested, washed and imaged by fluorescence microscopy as in Fig. S2A (*middle*). As always, the same exposure time was used for image capture and the images were scaled identically. *Dotted white lines*, cell periphery; scale bar, 2 μm. *Right, q*uantification using Image J of the average pixel intensity of the GFP signal at the bud neck (25-50 budded cells) for the cultures shown (*middle*). *Dotted red line*, the average intrinsic background fluorescence of the bud neck in yeast cells lacking the components of the tripartite split-GFP system; *error bar*, S.E.M

Table S1. Yeast strains used in Supplemental Figures 1-4.

Strain	**Genotype**	**Reference**
BY4741	*MAT**a** leu2Δ ura3Δ met15Δ his3Δ*	(Brachmann et al., 1998)
BY4742	*MAT**α** leu2Δ ura3Δ met15Δ his3Δ*	(Brachmann et al., 1998)
GFY-1794	BY4742; *cdc10Δ::GFPβ10::Linker(32)::CDC10::ADH1(t)::Hyg*^{R} *cdc11Δ::CDC11::mCherry::SpHIS5*	This study
GFY-1570	BY4741; *cdc10Δ::CDC10::Linker(33)::GFPβ11::ADH1(t)::Nat*^{R}	This study
GFY-1803	BY4742; *cdc12Δ::GFPβ10::Linker(5)::CDC12::ADH1(t)::Hyg*^{R} *cdc10Δ::CDC10::mCherry::SpHIS5*	This study
GFY-1851	BY4741; *cdc10Δ::CDC10::GFPβ11::ADH1(t)::Hyg*^{R}	This study
GFY-1852	BY4741; *cdc10Δ::CDC10::Linker(10)::GFPβ11::ADH1(t)::Hyg*^{R}	This study
GFY-1853	BY4741; *cdc10Δ::CDC10::Linker(20)::GFPβ11::ADH1(t)::Hyg*^{R}	This study
GFY-1807	BY4742; *shs1Δ::GFPβ10::Linker(5)::SHS1::ADH1(t)::Hyg*^{R} *cdc10Δ::CDC10::mCherry::SpHIS5*	This study
GFY-1845	BY4741; *cdc3Δ::CDC3::GFPβ11::ADH1(t)::Hyg*^{R}	This study
GFY-1848	BY4741; *cdc12Δ::CDC12::GFPβ11::ADH1(t)::Hyg*^{R}	This study
GFY-1842	BY4741; *cdc11Δ::CDC11::GFPβ11::ADH1(t)::Hyg*^{R}	This study
GFY-1839	BY4741; *shs1Δ::SHS1::GFPβ11::ADH1(t)::Hyg*^{R}	This study

Table S2. Plasmids used in Supplemental Figures 1-4.

pRS315	CEN; *LEU2 AMP*	(Sikorski and Hieter, 1989)
pGF-IVL794	pRS315; *prGAL1/10::GFPβ1-9(A)::ADH1(t)::Kan*^{R}	This study
pGF-IVL795	pRS315; *prGAL1/10::GFPβ1-9(B)::ADH1(t)::Kan*^{R}	This study

Table S3. Diploid strains used in this study.

Diploid Number	Haploid strains crossed (+ plamids)	Notes[2]
1[1]	GFY-1794 x GFY-1570 (+pGF-IVL794)	Negative Controls
2	GFY-1794 x BY4741 (+pGF-IVL794)	Negative Controls
3	GFY-59 x GFY-1570 (+pGF-IVL794)	Negative Controls
4	GFY-59 x BY4741 (+pGF-IVL794)	Negative Controls
5	GFY-59 x BY4741 (+pRS315 empty)	Negative Controls
6	GFY-1794 x GFY-1570 (+pRS315 empty)	Negative Controls
7	GFY-59 x GFY-1570 (+pRS315 empty)	Negative Controls
8	GFY-1794 x BY4741 (+pRS315 empty)	Negative Controls
9	GFY-1794 x GFY-1570 (+pGF-IVL795)	Negative Controls
164	GFY-1793 x GFY-1979 (+pGF-IVL794)	Neg. Controls (Pgk1)
165	GFY-1796 x GFY-1979 (+pGF-IVL794)	Neg. Controls (Pgk1)
166	GFY-1795 x GFY-1979 (+pGF-IVL794)	Neg. Controls (Pgk1)
167	GFY-1797 x GFY-1979 (+pGF-IVL794)	Neg. Controls (Pgk1)
168	GFY-1806 x GFY-1979 (+pGF-IVL794)	Neg. Controls (Pgk1)
170	GFY-1793 x GFY-1983 (+pGF-IVL794)	Neg. Controls (Hsp82)
171	GFY-1796 x GFY-1983 (+pGF-IVL794)	Neg. Controls (Hsp82)
172	GFY-1795 x GFY-1983 (+pGF-IVL794)	Neg. Controls (Hsp82)
173	GFY-1797 x GFY-1983 (+pGF-IVL794)	Neg. Controls (Hsp82)
174	GFY-1806 x GFY-1983 (+pGF-IVL794)	Neg. Controls (Hsp82)

272	GFY-1793 x GFY-2035 (+pGF-IVL794)	Neg. Controls (Cdc19)
273	GFY-1796 x GFY-2035 (+pGF-IVL794)	Neg. Controls (Cdc19)
274	GFY-1795 x GFY-2035 (+pGF-IVL794)	Neg. Controls (Cdc19)
275	GFY-1797 x GFY-2035 (+pGF-IVL794)	Neg. Controls (Cdc19)
276	GFY-1806 x GFY-2035 (+pGF-IVL794)	Neg. Controls (Cdc19)
277	GFY-1793 x GFY-2036 (+pGF-IVL794)	Neg. Controls (Gpp1)
278	GFY-1796 x GFY-2036 (+pGF-IVL794)	Neg. Controls (Gpp1)
279	GFY-1795 x GFY-2036 (+pGF-IVL794)	Neg. Controls (Gpp1)
280	GFY-1797 x GFY-2036 (+pGF-IVL794)	Neg. Controls (Gpp1)
281	GFY-1806 x GFY-2036 (+pGF-IVL794)	Neg. Controls (Gpp1)
282	GFY-1793 x GFY-2043 (+pGF-IVL794)	Neg. Controls (Tpi1)
283	GFY-1796 x GFY-2043 (+pGF-IVL794)	Neg. Controls (Tpi1)
284	GFY-1795 x GFY-2043 (+pGF-IVL794)	Neg. Controls (Tpi1)
285	GFY-1797 x GFY-2043 (+pGF-IVL794)	Neg. Controls (Tpi1)
286	GFY-1806 x GFY-2043 (+pGF-IVL794)	Neg. Controls (Tpi1)
25	GFY-1801 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
24	GFY-1798 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
27	GFY-1803 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
26	GFY-1804 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
28	GFY-1807 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
39	GFY-1796 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)

38	GFY-1793 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
41	GFY-1797 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
40	GFY-1795 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
42	GFY-1806 x GFY-1851 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
11	GFY-1801 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
10	GFY-1798 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
13	GFY-1803 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
12	GFY-1804 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
14	GFY-1807 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
46	GFY-1796 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
45	GFY-1793 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
48	GFY-1797 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
47	GFY-1795 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
49	GFY-1806 x GFY-1852 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
18	GFY-1801 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
17	GFY-1798 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
20	GFY-1803 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
19	GFY-1804 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
21	GFY-1807 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
53	GFY-1796 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
52	GFY-1793 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)

55	GFY-1797 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
54	GFY-1795 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
56	GFY-1806 x GFY-1853 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
32	GFY-1801 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
31	GFY-1798 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
34	GFY-1803 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
33	GFY-1804 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
35	GFY-1807 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
60	GFY-1796 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
59	GFY-1793 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
62	GFY-1797 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
61	GFY-1795 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
63	GFY-1806 x GFY-1570 (+pGF-IVL794)	Septin x Septin (Cdc10-β11)
67	GFY-1798 x GFY-1851 (+pGF-IVL794)	Septin x Septin (β10-Cdc3)
66	GFY-1798 x GFY-1845 (+pGF-IVL794)	Septin x Septin (β10-Cdc3)
69	GFY-1798 x GFY-1848 (+pGF-IVL794)	Septin x Septin (β10-Cdc3)
68	GFY-1798 x GFY-1842 (+pGF-IVL794)	Septin x Septin (β10-Cdc3)
70	GFY-1798 x GFY-1839 (+pGF-IVL794)	Septin x Septin (β10-Cdc3)
74	GFY-1801 x GFY-1851 (+pGF-IVL794)	Septin x Septin (β10-Cdc10)
73	GFY-1801 x GFY-1845 (+pGF-IVL794)	Septin x Septin (β10-Cdc10)
76	GFY-1801 x GFY-1848 (+pGF-IVL794)	Septin x Septin (β10-Cdc10)

75	GFY-1801 x GFY-1842 (+pGF-IVL794)	Septin x Septin (β10-Cdc10)
77	GFY-1801 x GFY-1839 (+pGF-IVL794)	Septin x Septin (β10-Cdc10)
81	GFY-1804 x GFY-1851 (+pGF-IVL794)	Septin x Septin (β10-Cdc11)
80	GFY-1804 x GFY-1845 (+pGF-IVL794)	Septin x Septin (β10-Cdc11)
83	GFY-1804 x GFY-1848 (+pGF-IVL794)	Septin x Septin (β10-Cdc11)
82	GFY-1804 x GFY-1842 (+pGF-IVL794)	Septin x Septin (β10-Cdc11)
84	GFY-1804 x GFY-1839 (+pGF-IVL794)	Septin x Septin (β10-Cdc11)
109	GFY-1807 x GFY-1851 (+pGF-IVL794)	Septin x Septin (β10-Shs1)
108	GFY-1807 x GFY-1845 (+pGF-IVL794)	Septin x Septin (β10-Shs1)
111	GFY-1807 x GFY-1848 (+pGF-IVL794)	Septin x Septin (β10-Shs1)
110	GFY-1807 x GFY-1842 (+pGF-IVL794)	Septin x Septin (β10-Shs1)
112	GFY-1807 x GFY-1839 (+pGF-IVL794)	Septin x Septin (β10-Shs1)
102	GFY-1803 x GFY-1851 (+pGF-IVL794)	Septin x Septin (β10-Cdc12)
101	GFY-1803 x GFY-1845 (+pGF-IVL794)	Septin x Septin (β10-Cdc12)
104	GFY-1803 x GFY-1848 (+pGF-IVL794)	Septin x Septin (β10-Cdc12)
103	GFY-1803 x GFY-1842 (+pGF-IVL794)	Septin x Septin (β10-Cdc12)
105	GFY-1803 x GFY-1839 (+pGF-IVL794)	Septin x Septin (β10-Cdc12)
151	GFY-1809 x GFY-1570 (+pGF-IVL794)	Septin x β10-Bni5
150	GFY-1809 x GFY-1572 (+pGF-IVL794)	Septin x β10-Bni5
153	GFY-1809 x GFY-1571 (+pGF-IVL794)	Septin x β10-Bni5
152	GFY-1809 x GFY-1573 (+pGF-IVL794)	Septin x β10-Bni5

154	GFY-1809 x GFY-1567 (+pGF-IVL794)	Septin x β10-Bni5
155	GFY-1809 x GFY-1735 (+pGF-IVL794)	Bni5-β11 x β10-Bni5
127	GFY-1796 x GFY-1735 (+pGF-IVL794)	Septin x Bni5-β11
120	GFY-1793 x GFY-1735 (+pGF-IVL794)	Septin x Bni5-β11
141	GFY-1797 x GFY-1735 (+pGF-IVL794)	Septin x Bni5-β11
134	GFY-1795 x GFY-1735 (+pGF-IVL794)	Septin x Bni5-β11
148	GFY-1806 x GFY-1735 (+pGF-IVL794)	Septin x Bni5-β11
158	GFY-1899 x GFY-1570 (+pGF-IVL794)	Septin x β10-Nis1
157	GFY-1899 x GFY-1572 (+pGF-IVL794)	Septin x β10-Nis1
160	GFY-1899 x GFY-1571 (+pGF-IVL794)	Septin x β10-Nis1
159	GFY-1899 x GFY-1573 (+pGF-IVL794)	Septin x β10-Nis1
161	GFY-1899 x GFY-1567 (+pGF-IVL794)	Septin x β10-Nis1
163	GFY-1899 x GFY-1854 (+pGF-IVL794)	Nis1-β11 x β10-Nis1
128	GFY-1796 x GFY-1854 (+pGF-IVL794)	Septin x Nis1-β11
121	GFY-1793 x GFY-1854 (+pGF-IVL794)	Septin x Nis1-β11
142	GFY-1797 x GFY-1854 (+pGF-IVL794)	Septin x Nis1-β11
135	GFY-1795 x GFY-1854 (+pGF-IVL794)	Septin x Nis1-β11
149	GFY-1806 x GFY-1854 (+pGF-IVL794)	Septin x Nis1-β11
177	GFY-1992 x GFY-1570 (+pGF-IVL794)	Septin x Hsl1(WT)
176	GFY-1992 x GFY-1572 (+pGF-IVL794)	Septin x Hsl1(WT)
179	GFY-1992 x GFY-1571 (+pGF-IVL794)	Septin x Hsl1(WT)

178	GFY-1992 x GFY-1573 (+pGF-IVL794)	Septin x Hsl1(WT)
180	GFY-1992 x GFY-1567 (+pGF-IVL794)	Septin x Hsl1(WT)
182	GFY-1995 x GFY-1570 (+pGF-IVL794)	Septin x Hsl1(611-950)
181	GFY-1995 x GFY-1572 (+pGF-IVL794)	Septin x Hsl1(611-950)
184	GFY-1995 x GFY-1571 (+pGF-IVL794)	Septin x Hsl1(611-950)
183	GFY-1995 x GFY-1573 (+pGF-IVL794)	Septin x Hsl1(611-950)
185	GFY-1995 x GFY-1567 (+pGF-IVL794)	Septin x Hsl1(611-950)
187	GFY-1997 x GFY-1570 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
186	GFY-1997 x GFY-1572 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
189	GFY-1997 x GFY-1571 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
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216	GFY-1997 x GFY-1846 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
217	GFY-1997 x GFY-1852 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
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219	GFY-1997 x GFY-1849 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
220	GFY-1997 x GFY-1840 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
221	GFY-1997 x GFY-1847 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
222	GFY-1997 x GFY-1853 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
223	GFY-1997 x GFY-1844 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
224	GFY-1997 x GFY-2044 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)

225	GFY-1997 x GFY-1841 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
262	GFY-1997 x GFY-1845 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
263	GFY-1997 x GFY-1851 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
264	GFY-1997 x GFY-1842 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
265	GFY-1997 x GFY-1848 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
266	GFY-1997 x GFY-1839 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
206	GFY-1996 x GFY-1846 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
207	GFY-1996 x GFY-1852 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
208	GFY-1996 x GFY-1843 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
209	GFY-1996 x GFY-1849 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
210	GFY-1996 x GFY-1840 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
211	GFY-1996 x GFY-1847 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
212	GFY-1996 x GFY-1853 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
213	GFY-1996 x GFY-1844 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
214	GFY-1996 x GFY-2044 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
215	GFY-1996 x GFY-1841 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
252	GFY-1996 x GFY-1572 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
253	GFY-1996 x GFY-1570 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
254	GFY-1996 x GFY-1573 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
255	GFY-1996 x GFY-1571 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
256	GFY-1996 x GFY-1567 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)

257	GFY-1996 x GFY-1845 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
258	GFY-1996 x GFY-1851 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
259	GFY-1996 x GFY-1842 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
260	GFY-1996 x GFY-1848 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
261	GFY-1996 x GFY-1839 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
226	GFY-1998 x GFY-1846 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
227	GFY-1998 x GFY-1852 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
228	GFY-1998 x GFY-1843 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
229	GFY-1998 x GFY-1849 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
230	GFY-1998 x GFY-1840 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
231	GFY-1998 x GFY-1847 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
232	GFY-1998 x GFY-1853 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
233	GFY-1998 x GFY-1844 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
234	GFY-1998 x GFY-2044 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
235	GFY-1998 x GFY-1841 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
247	GFY-1998 x GFY-1845 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
248	GFY-1998 x GFY-1851 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
249	GFY-1998 x GFY-1842 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
250	GFY-1998 x GFY-1848 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
251	GFY-1998 x GFY-1839 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)

267	GFY-1998 x GFY-1572 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
268	GFY-1998 x GFY-1570 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
269	GFY-1998 x GFY-1573 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
270	GFY-1998 x GFY-1571 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
271	GFY-1998 x GFY-1567 (+pGF-IVL794)	Septin x Hsl1(611-950; 1245-1518)
287[3]	GFY-1796 (+pGF-IVL1084) x BY4741 (+pGF-IVL1005)	Septins x Bud4(623-774)
288	GFY-1793 (+pGF-IVL1084) x BY4741 (+pGF-IVL1005)	Septins x Bud4(623-774)
289	GFY-1795 (+pGF-IVL1084) x BY4741 (+pGF-IVL1005)	Septins x Bud4(623-774)
290	GFY-1797 (+pGF-IVL1084) x BY4741 (+pGF-IVL1005)	Septins x Bud4(623-774)
291	GFY-1806 (+pGF-IVL1084) x BY4741 (+pGF-IVL1005)	Septins x Bud4(623-774)
292	GFY-1796 (+pGF-IVL1082) x BY4741 (+pGF-IVL1005)	Septins x Hof1(293-355)
293	GFY-1793 (+pGF-IVL1082) x BY4741 (+pGF-IVL1005)	Septins x Hof1(293-355)
294	GFY-1795 (+pGF-IVL1082) x BY4741 (+pGF-IVL1005)	Septins x Hof1(293-355)
295	GFY-1797 (+pGF-IVL1082) x BY4741 (+pGF-IVL1005)	Septins x Hof1(293-355)

296	GFY-1806 (+pGF-IVL1082) x BY4741 (+pGF-IVL1005)	Septins x Hof1(293-355)

[1]For all diploids, unless otherwise noted, the plasmid expressing GFPβ1-9 was transformed into the *MAT**a*** strain prior to mating and diploid selection. Additionally, all haploid strains were selected on medium containing 5-FOA to counter-select for any covering vectors expressing WT copies of the septin gene(s). Unless otherwise noted, yeast were mated on YPD for 24 hours at 30°C and replica-plated to SD-HIS-LEU, grown for 2 days, and transferred to a second SD-HIS-LEU plate and grown for 1-2 additional days.

[2]For clarity, the general category and description of each small grouping of diploid strains is given. In general, one protein fusion is held constant and compared against the remaining set of five tagged septins. The portion held constant in each grouping is briefly described (e.g. Cdc10-β11).

[3]Diploids strains expressing fragments of either Bud4 or Hof1 (expressed from plasmids) used a modified diploid selection procedure. Prior to transformation of the GFPβ1-9 plasmid or Bud4/Hof1-expressing plasmids, the septin covering vector was counter-selected on 5-FOA medium twice. Following mating, diploids were selected on SD-URA-LEU medium twice.

Table S4. Protein abundance for split GFP negative control proteins present in the cytosol

Protein	Description	Molecules / cell	Reference
Pgk1	3-phosphoglycerate kinase	314,000	(Ghaemmaghami et al., 2003)
		15,100	(Chong et al., 2015)
		561,265	(Kulak et al., 2014)
		42,840	(Newman et al., 2006)
Hsp82	Hsp90 protein chaperone	445,000	(Ghaemmaghami et al., 2003)
		4,167	(Chong et al., 2015)
		10,044	(Kulak et al., 2014)
		6,395	(Newman et al., 2006)
Gpp1	Glycerol-1-phosphatase	193,000	(Ghaemmaghami et al., 2003)
		14,626	(Chong et al., 2015)
		235,727	(Kulak et al., 2014)
		9,107	(Newman et al., 2006)
Tpi1	Triose phosphate isomerase	207,000	(Ghaemmaghami et al., 2003)
		5,770	(Chong et al., 2015)
		395,237	(Kulak et al., 2014)
		4,768	(Newman et al., 2006)
Cdc19	Pyruvate kinase	291,000	(Ghaemmaghami et al., 2003)
		404,162	(Kulak et al., 2014)
		11,387	(Newman et al., 2006)
Cdc11	Septin subunit	9280	(Ghaemmaghami et al., 2003)
		236	(Chong et al., 2015)
		3947	(Kulak et al., 2014)
		359	(Newman et al., 2006)

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