Garcia et al., http://www.jcb.org/cgi/content/full/jcb.201511029/DC1

Figure S1. **Sequence features of the sporulation-specific septins.** (A) Comparison of Spr3 to mitotic septins Cdc10 and Cdc12. (B) Comparison of Spr28 to mitotic septins Cdc11 and Shs1. Dashes, gaps introduced to maximize alignment of identical and similar residues. Colored residues, basic (red) and acidic (blue) side chains in helix α0. Boxes, the conserved G-1, G-2, and G-3 motifs diagnostic of GTP-binding proteins. Spr28 (and Shs1) lack a conserved Asp in G-2 important for stabilizing the binding of Mg^{2+}-GTP in septins and other G-proteins (Wittinghofer and Vetter, 2011). (*) Conserved Thr in Cdc10 and Cdc12 implicated by structural analysis (Sirajuddin et al., 2009) in their ability to hydrolyze bound GTP and the equivalent position in Cdc11, which is unable to hydrolyze bound GTP (Versele and Thorner, 2004).

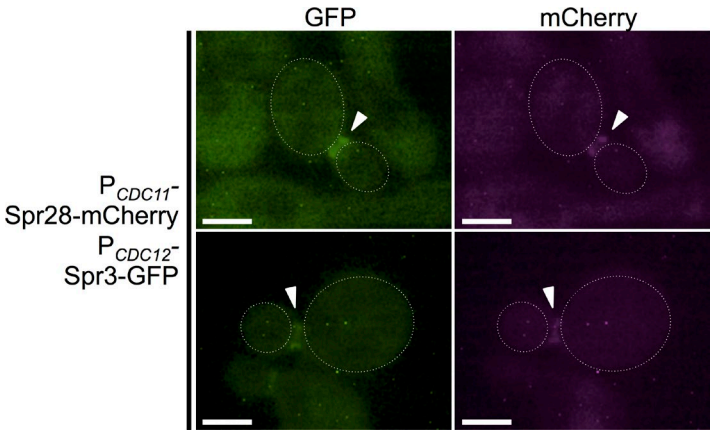

Figure S2. **Switching fluorescent tags on Spr3 and Spr28 does not alter their corecruitment to the bud neck in vegetative yeast cells.** A strain expressing all five mitotic septins and also coexpressing integrated Spr28-mCherry (under P_{CDC11} control) and integrated Spr3-GFP (under P_{CDC12} control) displayed a pattern of fluorescent signal both in the cytosol and at the bud neck in about the same proportion of the population as the same cells coexpressing integrated Spr28-GFP (under P_{CDC11} control) and integrated Spr3-mCherry (under P_{CDC12} control; see Fig. 6 B, right). Bar, 2 µm.

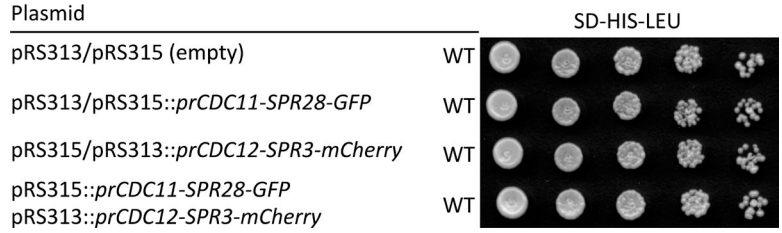

Figure S3. **Ectopic expression of *SPR3* and/or *SPR28* does not impede the growth of wild-type mitotic cells.** Strain BY4741 was transformed with an empty *HIS3*-marked *CEN* vector (pRS313) and an empty *LEU2*-marked *CEN* vector (pRS315) or derivatives of these plasmids expressing either Spr28-GFP or Spr3-mCherry, or both, as indicated, grown overnight in selective (-His-Leu) medium, serially diluted onto plates containing the same medium, and grown for 2 d at 30°C. WT, wild type.

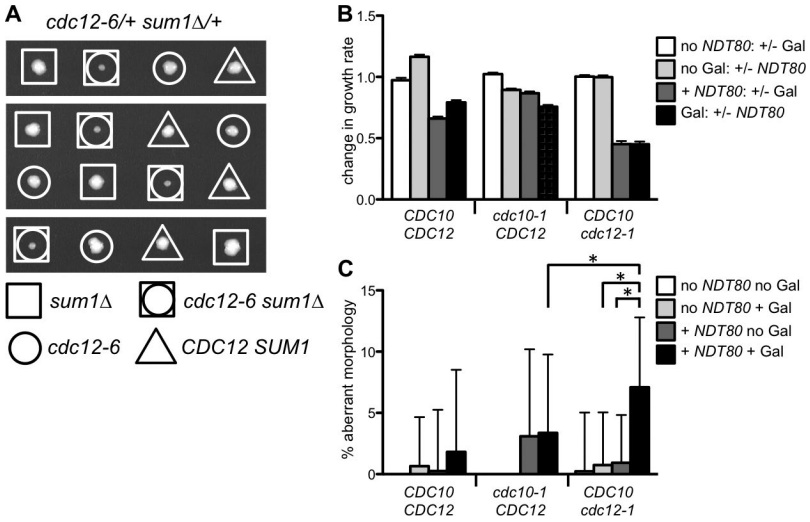

Figure S4. **Ectopic expression of sporulation-specific septins in mitotic cells exacerbates the growth defects caused by mutations in mitosis-specific septins.** (A) A *cdc12-6* derivative of wild-type strain BY4741 was crossed with a *sum1Δ* derivative of wild-type strain BY4742, and the four meiotic progeny from individual tetrads (rows) were separated on rich (YPD) solid medium and incubated for 3 d at 26°C. Symbols indicate the spore genotypes as surmised from marker phenotypes. Sum1 is a component of a repressive complex that prevents *SPR3* transcription during vegetative growth (McCord et al., 2003) and, in a high-throughput screen for genetic interactions (Costanzo et al., 2010), a *sum1Δ* mutation exhibited a more-than-additive synthetic defect in colony growth rate at 30°C when combined with cells carrying the septin alleles *cdc11-2^{ts}*, *cdc11-5^{ts}*, and *cdc12-1^{ts}*. Also, it has been reported that ectopic expression of Spr3 at the permissive temperature in either *cdc12-6^{ts}* or *cdc11-6^{ts}* cells interferes with mitotic septin function, as judged by an increased frequency of cells with an elongated morphology and/or a defect in cytokinesis (Fares et al., 1996). Finally, we introduced a *sum1Δ* mutation into a strain carrying the *cdc12(T48N)* allele, which has only a very mild effect on Cdc12 function (Versele and Thorner, 2004), and found that the resulting double mutants exhibited a subpopulation of elongated cells that was absent in cultures of either single mutant (not depicted). (B) Eight replicate cultures of BY4741 and each of the derivatives of the indicated genotypes were grown in liquid rich raffinose medium (YPRaf) at 24.5°C and $A_{630\,nm}$ was measured at 10-min intervals over the course of 15 h, from which growth rate was calculated, in the presence (+) or absence (– or no) of an integrated copy of the *NDT80* transcriptional activator expressed under the control of the galactose (Gal)-inducible *GAL1/10* promoter, with (+) or without (– or no) Gal added to final concentration of 0.05%. Ndt80 is a meiosis-specific transcriptional activator that competes with the Sum1 repressor by binding to distinct, but overlapping, sites in the *SPR3* promoter (Pierce et al., 2003) and is sufficient to drive *SPR3* expression in mitotically dividing cells (Chu and Herskowitz, 1998). We used only 0.05% Gal because high-level Ndt80 expression is severely detrimental to mitotic proliferation, due apparently to misregulation of multiple B-type cyclin genes (Chu and Herskowitz, 1998), and, as shown in B, at the low level of *NDT80* expression elicited by 0.05%, there was only a slight effect on the doubling time of wild-type cells. Note that Spr3 production driven by this low level of ectopic Ndt80 was sufficient to deleteriously affect the growth of cells carrying a mutation in Cdc12, the septin subunit replaced by Spr3, but not in a different septin subunit (Cdc10). Bars, ratio of the mean growth rate to the mean growth rate for the no NDT80 control; error bars, standard deviation of each mean. (C) Cultures from B were examined under the microscope and the percentage of cells with a markedly elongated morphology were scored. Error bars are the standard error of the proportion. *, $P < 0.05$ according to one-tailed Fisher's exact test; only the most informative such comparisons are indicated. Approximately 200 cells were examined for each sample.

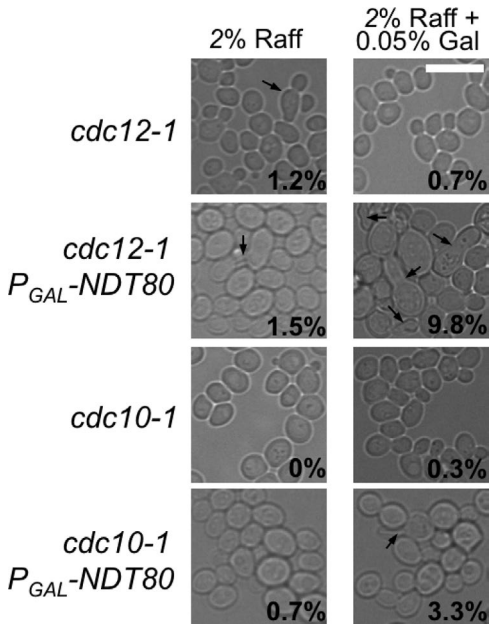

Figure S5. **Increased frequency of aberrant cellular morphology in mitotic *cdc12-1* mutants upon low-level ectopic expression of Ndt80.** A subset of the same strains (relevant genotypes are indicated) as in Fig. S4 C were spotted on solid YPRaf medium with or without 0.05% Gal as indicated and incubated for 3 d at 22°C before being scraped from the plate and resuspended in water. Cell morphology was assessed by bright-field microscopy using a 60× objective. Representative cells are shown. Arrows indicate aberrantly shaped cells. Inset numbers, values calculated from examination of at least 230 cells per sample. Bar, 10 µm.

Table S1. **Sequence relationships among *S. cerevisiae* septins**

Query	Cdc3 (520)[a]	Cdc10 (322)	Cdc11 (415)	Cdc12 (407)	Shs1 (551)	Spr3 (512)	Spr28 (423)
Cdc3 (520)	— I: 100% S: 100% M: 520	2.0e–50 I: 38% S: 58% M: 234	8.6e–48 I: 38% S: 63% M: 224	1.4e–57 I: 31% S: 52% M: 352	1.7e–41 I: 34% S: 57% M: 214	1.4e–40 I: 29% S: 51% M: 333	7.7e–34 I: 29% S: 50% M: 341
Cdc10 (322)	1.8e–49 I: 39% S: 60% M: 235	— I: 100% S: 100% M: 322	3.1e–52 I: 41% S: 61% M: 283	2.1e–55 I: 37% S: 56% M: 300	2.3e–34 I: 31% S: 53% M: 228	2.2e–42 I: 37% S: 55% M: 291	3.8e–34 I: 30% S: 54% M: 235
Cdc11 (415)	1.8e–47 I: 31% S: 54% M: 310	7.8e–56 I: 39% S: 58% M: 283	— I: 100% S: 100% M: 415	1.8e–47 I: 32% S: 51% M: 393	8.7e–62 I: 36% S: 55% M: 343	4.2e–41 I: 35% S: 57% M: 286	2.3e–51 I: 34% S: 54% M: 326
Cdc12 (407)	1.6e–54 I: 33% S: 55% M: 341	1.2e–50 I: 40% S: 58% M: 301	3.5e–44 I: 38% S: 58% M: 299	— I: 100% S: 100% M: 407	5.3e–38 I: 32% S: 54% M: 227	1.3e–53 I: 40% S: 63% M: 295	3.6e–32 I: 34% S: 54% M: 246
Shs1 (551)	2.1e–33 I: 36% S: 61% M: 214	1.5e–33 I: 31% S: 54% M: 228	3.8e–65 I: 40% S: 59% M: 286	6.8e–38 I: 32% S: 53% M: 227	— I: 100% S: 100% M: 551	9.8e–26 I: 29% S: 52% M: 269	8.0e–25 I: 33% S: 55% M: 221
Spr3 (512)	6.0e–49 I: 27% S: 46% M: 454	3.5e–44 I: 36% S: 54% M: 291	4.2e–41 I: 35% S: 57% M: 286	1.3e–60 I: 33% S: 54% M: 402	3.4e–25 I: 25% S: 44% M: 376	— I: 100% S: 100% M: 512	1.2e–25 I: 28% S: 51% M: 245
Spr28 (423)	1.2e–33 I: 32% S: 54% M: 255	3.4e–35 I: 31% S: 51% M: 235	2.6e–54 I: 37% S: 58% M: 245	1.3e–35 I: 32% S: 50% M: 246	6.7e–25 I: 33% S: 55% M: 221	6.6e–21 I: 34% S: 56% M: 149	— I: 100% S: 100% M: 423

Exponential is the E value derived using the WU-BLAST algorithm at the Saccharomyces Genome Database (http://www.yeastgenome.org/); the lower the number, the less likely the observed similarity is due to random chance and, thus, the more significant the match. I (percent amino acid sequence identity) and S (percent amino acid sequence similarity) over the indicated match length (M, in residues) derived from pairwise comparisons performed using the WU-BLAST algorithm available at the Saccharomyces Genome Database.
[a]Value in parentheses represents the number of residues.

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