Supplemental Materials

Molecular Biology of the Cell

Heasley et al.

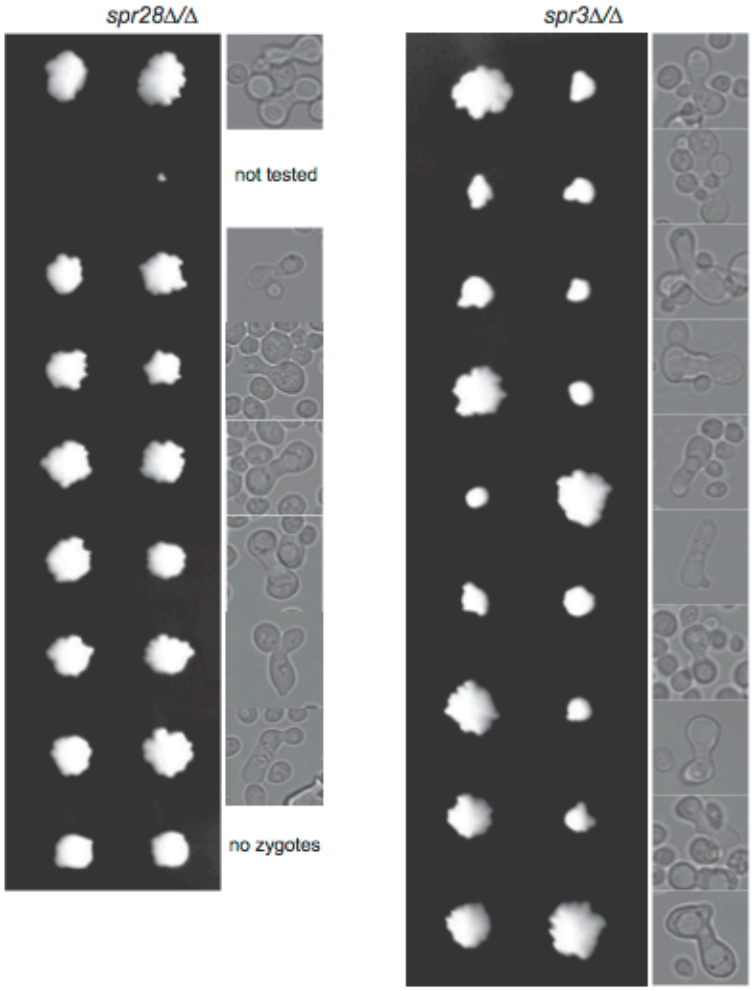

Figure S1. Non-sister dyads produced by septin-mutant cells. Utilizing a method to ensure ascus fidelity following enzymatic ascal wall removal (Davidow *et al.*, 1980), dyads produced by *spr3*Δ/Δ and *spr28*Δ/Δ diploids were dissected on YPD medium, allowed to grow into colonies at 22˚ for 4 days, and photographed. As *MAT* is centromere-linked and heterozygous in diploid cells, in NSDs the spores from a given dyad are usually able to mate with each other (Davidow *et al.*, 1980). Cells from dyad colonies were mixed together on YPD and and incubated overnight. From these "mating" mixes cells were resuspended in water and visualized microscopically to identify zygotes, indicative of mating; images of representative zygotes are shown. If no zygote could be found, the dyad was scored as a sister dyad. For each genotype, the proportion of NSDs (7 of 8 for *spr28*Δ/Δ, 9 of 9 for *spr3*Δ/Δ) was significantly different than random; $P < 0.05$ for two-tailed Chi squared tests compared to frequency expected from random segregation (50%). We note that the increased heterogeneity in colony shape/size in the *spr3*Δ/Δ mutants was largely due to an increase in the proportion of petites (i.e., cells lacking functional mitochondrial DNA). PSMs in LEP mutants (*ady3*Δ) capture mitochondria inefficiently, and perturbing septins (indirectly, via deletion of *GIP1*) makes this defect somewhat worse (Suda *et al.*, 2007). Thus, we speculate that the differences we observed in colony appearance reflect a similar problem in *spr3*Δ/Δ mutants.

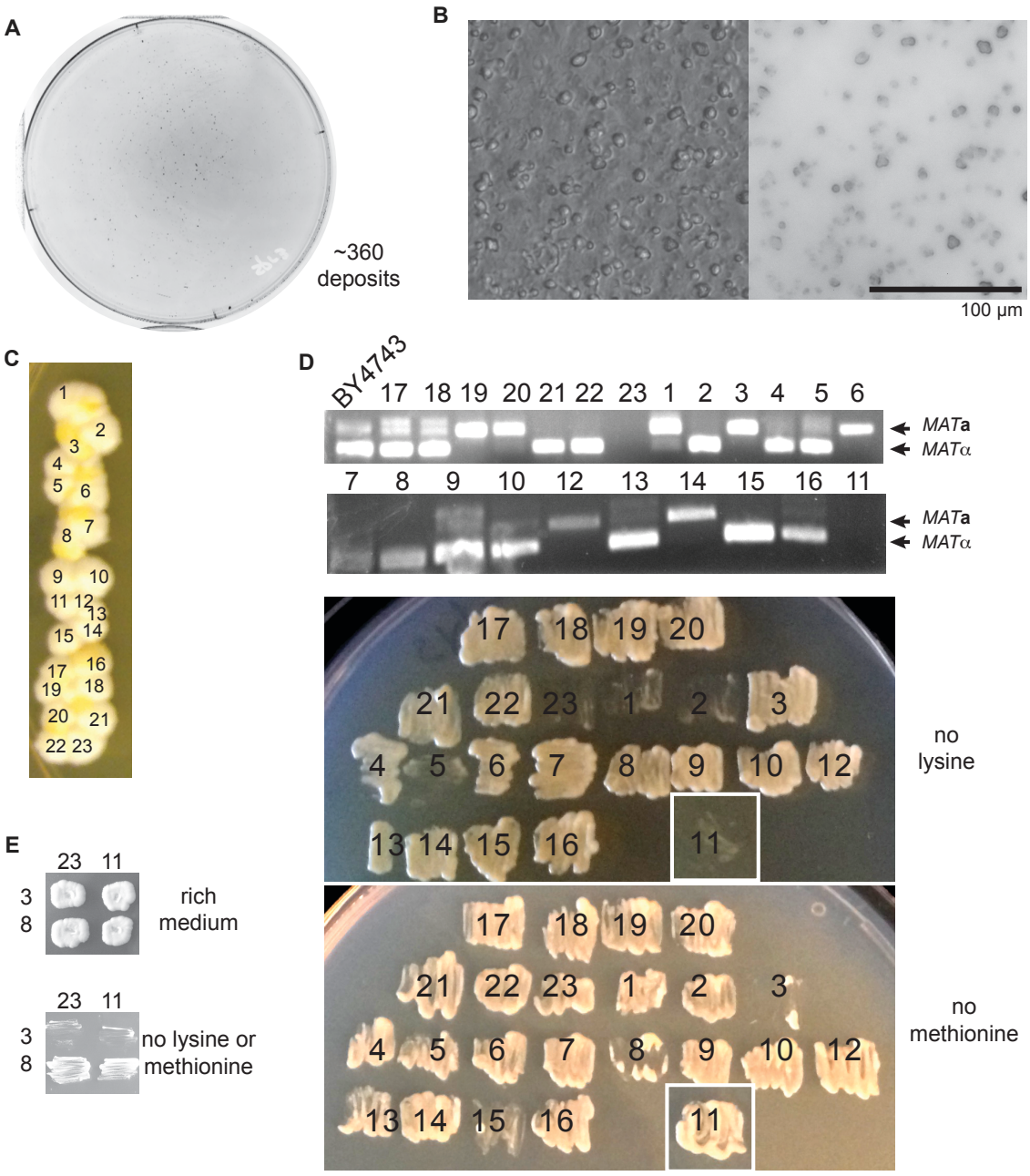

Figure S2. Cytological and genetic analysis of yeast cells in *Drosophila* feces. (A) Fluorescence scan of the lid of a petri dish in which adult *Drosophila* were fed a mixture of WT and *spr3*Δ/Δ sporulation cultures and allowed to defecate at will. Fecal deposits are visible as fluorescent speckles. (B) A single fecal deposit from the same lid as in (A) was examined by transmitted light and epifluorescence microscopy with a 40X objective and DAPI filter. (C) Resuspended fecal deposits from the lid in (A) were spread on a YPD plate and allowed to form colonies at 30˚. Yellow colonies are a non-yeast microbial contaminant. Numbers indicate the 23 distinct colonies analyzed in (D). (D). Top, multiplex colony PCR using *MAT* allele-specific primers (Huxley *et al.*, 1990) and the colonies in (C). The diploid WT strain BY4743 is included

as a positive control for both alleles. Below, the same colonies were patched on solid synthetic medium lacking the indicated nutrient and incubated overnight at 30˚. The patch representing colony #11 was on a different part of the same plates and is shown as an inset for the sake of image space. (E) To determine the mating types of colonies from (C) for which PCR failed in (D), cells from the indicated colonies were mixed together on rich medium overnight at 30˚C and then patched from the mix to medium lacking lysine and methionine, to select for diploids resulting from successful mating.

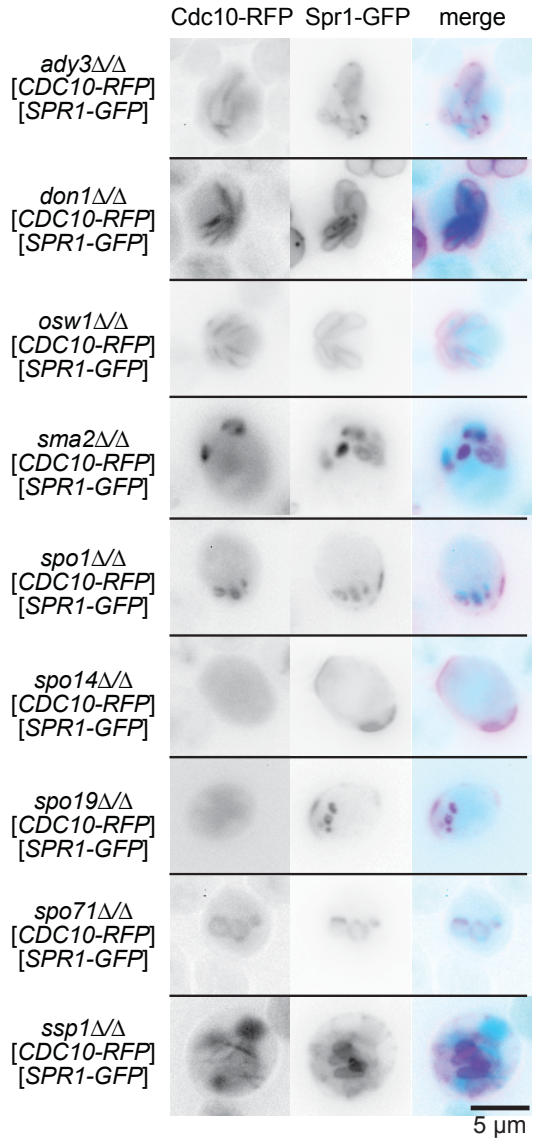

Figure S3. Septin localization and lack of functional rescue by septin overexpression in various non-septin-mutant backgrounds. Cdc10-RFP (pZL04) and Spr1-GFP (pSpr1-GFP) were co-expressed from plasmids in strains homozygous for deletion alleles of the indicated genes. Cells were sporulated as in Figure 1A and imaged with Texas Red and GFP filter cubes. Images of representative cells were false-colored and overlaid.

Table S1. Yeast strains used in this study

STRAIN	RELEVANT GENOTYPE	SOURCE/REFERENCE
BY4741	*MAT**a** his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	(Brachmann *et al.*, 1998)
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	(Brachmann *et al.*, 1998)
BY4743	*MAT**a**/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/MET15 lys2Δ0/LYS2*	(Brachmann *et al.*, 1998)
YMVB20	BY4743 *cdc12Δ::kanMX4/+*	(Winzeler *et al.*, 1999)
YMVB33[a]	*MAT**a** his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 cdc3Δ::kanMX4* [*CDC3 URA3*]	(McMurray *et al.*, 2011)
YMVB61[a]	BY4741 *cdc12Δ::kanMX4* [*CDC12 URA3*]	(McMurray *et al.*, 2011)
JTY4338[a]	BY4742 *cdc10Δ::kanMX4* [*CDC10 URA3*]	(McMurray *et al.*, 2011)
MJY201[a]	BY4741 *cdc11Δ::HIS3MX6* [*CDC11 URA3*]	(McMurray *et al.*, 2011)
JRY8012	BY4741 *pdr5Δ::kanMX snq1Δ::kanMX yor1Δ::kanMX*	(Jeong *et al.*, 2007)
MMY0019[b]	JRY8012 *HTB2-tdTomato::HIS3MX*	This work
FY2839	*MAT**a** his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 MKT1(G30) RME1 TAO3(Q1493)*	Fred Winston, Harvard Medical School
FY2742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 MKT1(G30) RME1 TAO3(Q1493)*	Fred Winston, Harvard Medical School
MMY0150[c]	FY2742 *spr3Δ::kanMX4*	This work
MMY0151	BY4742 *spr3Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0220[c]	BY4742 *spr3Δ::kanMX4*	This work
MMY0152	BY4741 *spr28Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0153	BY4742 *spr28Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0155[c]	FY2742 *spr28Δ::kanMX4*	This work
MMY0184	BY4741 *spr3Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0185[c]	FY2839 *spr3Δ::kanMX4*	This work
MMY0186[c]	FY2839 *spr28Δ::kanMX4*	This work
MMY0187	BY4743 *sma1Δ::kanMX4/sma1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0233	BY4741 *sma1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0188	BY4743 *ssp1Δ::kanMX4/ssp1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0189	BY4743 *sma2Δ::kanMX4/sma2Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0192	BY4743 *ssp1Δ::kanMX4/ssp1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0193	BY4743 *osw1Δ::kanMX4/osw1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0195	BY4743 *don1Δ::kanMX4/don1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0196	BY4743 *spo71Δ::kanMX4/spo71Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0197	BY4743 *spo1Δ::kanMX4/spo1Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0198	BY4743 *spo19Δ::kanMX4/spo19Δ::kanMX4*	(Winzeler *et al.*, 1999)
MMY0199	BY4743 *spo14Δ::kanMX4/spo14Δ::kanMX4*	(Winzeler *et al.*, 1999)

MMY0200[d]	BY4743 *spr3Δ::kanMX4/spr3Δ::kanMX4 HTB2-tdTomato::HIS3MX/HTB2*	This work
MMY0238[d]	BY4743 *spr28Δ::kanMX4/spr28Δ::kanMX4 HTB2-tdTomato::HIS3MX/HTB2*	This work
MMY0236[d]	BY4743 *HTB2-tdTomato::HIS3MX/HTB2*	This work
MMY0201	BY4743 *CDC3/cdc3Δ::kanMX*	(Winzeler *et al.*, 1999)
MMY0202	BY4743 *SHS1/shs1Δ::kanMX*	(Winzeler *et al.*, 1999)
MMY0203[e]	BY4743 *ADY3-GFP::URA3*	This work
MMY0204[e]	BY4743 *spr3Δ::kanMX/spr3Δ::kanMX ADY3-GFP::URA3*	This work
MMY0232[f]	*MAT***a**/α *spr3Δ::kanMX/spr3Δ::kanMX sma1Δ::kanMX/sma1Δ::kanMX* [pLA10]	This work
792-10-1[g]	*MAT***a** *ade2-1 can1-100 trp1-1 ura3-1 his3-11,15 LEU2:: P_{URA3}–tetR-GFP CEN4::tetOx448::URA3*	(Jin *et al.*, 2012)
yJM808[h]	*MAT***a** *can1-100 trp1-1 ura3-1 his3-11,15 LEU2:: P_{URA3}–tetR-GFP CEN4::tetOx448::URA3 SPC110-DsRed::HIS3*	Jeff Moore, University of Colorado Anschutz Medical Campus
MMY0234	yJM808 x MMY0220	This work

[a] To make heterozygous BY4743-background diploid strains, this haploid was mated with an appropriate partner and loss of the *URA3*-marked plasmid was selected using medium with 5-fluoro-orotic acid (Boeke *et al.*, 1984).
[b] JRY8012 was transformed with an integrating PCR product, amplified from template plasmid pKT146, that contained a *tdTomato::his5MX* cassette and flanking homology targeting the end of the *HTB2* ORF.
[c] The parent strain was transformed with a PCR product, amplified from genomic DNA of the appropriate strain from the deletion collection (Winzeler *et al.*, 1999), that contained a *kanMX6* cassette and flanking homology targeting the entire *SPR3* ORF.
[d] The *HTB2-tdTomato::his5MX* allele was amplified from genomic DNA of strain MMY0019 using primers flanking the *HTB2* locus, and transformed into an appropriate diploid strain.
[e] pAM34-2 was linearized with *Eco*RI and integrated at the *ADY3* locus.
[f] MMY0233 was mated to MMY0220, the heterozygous diploid was sporulated, and *sma1Δ spr3Δ* spores with complementary mating types were mated together. The resulting diploid was transformed with pLA10.
[g] The *tetO* array is integrated ~2 kb from *CEN4* in this W303-background strain.
[h] 792-10-1 was backcrossed six times with a strain of the YEF473 strain background harboring *SPC110-DsRed::HIS3*.

Table S2. Plasmids used in this work

PLASMID	RELEVANT PROPERTIES	SOURCE/REFERENCE
pLA10	*CEN URA3 CDC10-GFP*	(Cid *et al.*, 1998)
pZL04	*CEN HIS3 CDC10-mCherry*	Jeremy Thorner, Univ. California at Berkeley
pMVB66	*CEN URA3 CDC12*	Jeremy Thorner, Univ. California at Berkeley
pMVB100	*CEN URA3 CDC3*	(Versele *et al.*, 2004)
pML113	*CEN LEU2 his3MX CDC12-eCitrine*	(Nagaraj *et al.*, 2008)
pSPR1-GFP (B1913)	*CEN URA3 SPR1-GFP*	JoAnne Engebrecht, Univ. California at Davis
pRS316	*CEN URA3*	(Sikorski and Hieter, 1989)
pKT146	*tdTomato::his5MX*	(Sheff and Thorn, 2004)
YEpP_{GAL}–*GST-His6-URA3*	2 μm *URA3* P_{GAL}–*GST-His6-URA3*	(Sopko *et al.*, 2006)
YEpP_{GAL}–*GST-His6-URA3*	2 μm *URA3* P_{GAL}–*GST-His6-URA3*	(Sopko *et al.*, 2006)
YEpP_{GAL}–*GST-His6-SPR3*	2 μm *URA3* P_{GAL}–*GST-His6-SPR3*	(Sopko *et al.*, 2006)
YEpP_{GAL}–*GST-His6-SSP1*	2 μm *URA3* P_{GAL}–*GST-His6-SSP1*	(Sopko *et al.*, 2006)
pAM34-2	*URA3 GFP-ADY3*	(Moreno-Borchart *et al.*, 2001)

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