SUPPLEMENTAL FIGURE LEGENDS

Figure S1. ER stress restores normal septin morphology in *shs1*Δ cells (Related to Figure 1)

(A) Septin behavior in *shs1Δ* cells. Deletion of the septin subunit gene SHS1 causes gross alterations to septin morphology and localization under normal growth conditions. Septin was visualized by integration of CDC10-mCherry septin reporter at the genomic locus. Septin ring of *shs1Δ* cells were dispersed or localized away from the bud neck (white arrowhead). In addition to abnormal septin ring, many *shs1Δ* cells were elongated, similar to *cdc12-6* cells grown at non-permissive temperature (30^{o}C) (Figure 1C). Treatment of *shs1Δ* cells with Tm (1μg/ml, 3 hrs) to induce ER stress restored normal septin morphology and bud neck localization, also similar to the effects of ER stress on *cdc12-6* cells.

(B) Impaired growth of *shs1Δ* cells was rescued by ER stress. The growth of *shs1Δ* cells on YPD and YPD+Tm plates was compared to wild-type (WT) cells. Log phase cells spotted at five fold serial dilutions on YPD or YPD+Tm (0.4 μg/ml) plates were grown for 3 days. *shs1Δ* cell grew better than wild-type cell upon induction of ER stress with Tm, correlating with ER stress induced restoration of normal septin morphology and localization, and of normal cell shape to *shs1Δ* cells (shown in (A)).

(C) Inheritance of cER to *cdc12-6* daughter cells grown at non-permissive temperature (30^{o}C) is significantly reduced by ER stress induction with Tm (1μg/ml) for 3 hrs. These results are in good correlation with the restoration of normal septin morphology and localization, cell morphology, and growth of *cdc12-6* cells under ER stress (growth at 30^{o}C + Tm) as shown in Figures 1C & 1D. The ER in *cdc12-6* cells was visualized using Hmg1-GFP ER reporter.

Figure S2. Visualization of ER using HDEL-DsRed reporter, of vacuoles with

Vph1-mCherry, and of mitochondria using mito-dsRed, during ER stress. (Related to Figure 2)

(A) Quantitation of cells with cER in the daughter cell (bud) in WT and ER-stressed populations. For each class of cells (see Figure 2 for definition), at least 300 cells were counted to score the numbers containing cER in the daughter cell under normal conditions (grey bars) and in the presence of 1 μg/ml Tm for 3 hrs (black bars). The standard deviation was calculated from three independent experiments.

(B) Visualization of ER using the HDEL-DsRed ER reporter instead of Hmg1-GFP confirms delayed inheritance of cER in ER-stressed (1 μg/ml Tm for 3 hrs) cells. HDEL-DsRed alone, DIC alone, and a merged (HDEL-DsRed/DIC) image are shown.

(C)(D) Inheritance of vacuoles and mitochondria are not significantly affected by ER stress induction. Wild-type cells expressing a vacuolar marker Vph1-mCherry integrated at the genomic locus (Singh et al., 2008) (C), and a mitochondrial matrix marker pVT100-dsRedT1 (fusion of a mitochondrial targeting sequence and *dsRed-T1*) under the *ADH1* promoter (Meeusen et al., 2006, Suda et al., 2007) (D), were grown under non-stressed (YPD) conditions or in the presence of Tm (1 μg/ml) for 3 hrs, before visualization under the microscope.

Figure S3. *slt2Δ* cells are capable of induction of the Unfolded Protein Response (UPR) in response to ER stress and ER stress-induced phosphorylation of Slt2 occurs independent of the increase in total Slt2 protein level (Related to Figure 3)

(A) Tm treatment induced splicing of *HAC1* mRNA in both WT and *slt2Δ* cells at similar levels. Positions of unspliced and spliced *HAC1* mRNA on Northern blot are indicated.

(B) Northern blot of *SLT2* mRNA. Total RNA of WT, *hac1Δ*, and *rlm1Δ* cells were analyzed by Northern blot, probing with SLT2 coding sequence. The same blot was re-

probed with SCR1 as a loading control. Increase in Slt2 protein (see (C)) in response to ER stress was caused by increase in mRNA (lanes 1 & 2). *SLT2* mRNA level was also increased in the absence of the UPR transcription factor, Hac1 (lanes 3 & 4), consistent with the previous report (Chen et al., 2005). However, in *rlm1Δ cells*, *SLT2* mRNA level was minimum and did not increase after ER stress induction by Tm (lanes 5 & 6), suggesting that Rlm1, but not Hac1, is the transcription factor responsible for ER stress induced transcription of SLT2.

(C) ER stress induced phosphorylation of Slt2 and increased overall levels of Slt2 protein. WT and *rlm1Δ* cells were treated with Tm (1 μg/ml) for 2 hrs before preparation of total cell extract for western blot analyses. Phosphorylated Slt2 (phospho-Slt2) was visualized by probing with anti-phospho Slt2 specific antibody, while total Slt2 was analyzed with anti-Slt2 antibody. Pgk1 level examined by anti-Pgk1 antibody was used as a loading control. In addition to phosphorylation, total level of Slt2 increased upon ER stress induction. RLM1 codes for a MADS-box transcription factor similar to serum response factor-like protein and is thought to be a target for Slt2 MAP Kinase (Levin, 2005). The increase in total Slt2 protein level was diminished in *rlm1Δ* cells, suggesting that Rlm1 is responsible for increase in Slt2 protein level (compare WT in lanes 1 & 2 with *rlm1Δ* cells, lanes 3 & 4). Note that phosphorylation of Slt2 increased even in *rlm1Δ* cells upon ER stress (lane 4, phospho-Slt2), revealing that ER stress induces both phosphorylation and increase in overall protein level of Slt2 and that phosphorylation does not require increase in Slt2 protein level.

Figure S4. Kinases upstream of Slt2 are essential for ER stress surveillance (ERSU) (Related to Figure 4)

pkc1Δ (A) and *bck1Δ* (B) cells carrying ER reporter (Hmg1-GFP) and septin reporter (Cdc10-mCherry) integrated at the corresponding genomic loci were analyzed for ER behavior and septin ring morphology by fluorescence microscopy. Under non-stressed conditions (YPD), ER distribution and septin ring localization (at the bud neck) are

normal. Following ER stress (by Tm 1 μg/ml, 3 hrs), normal septin ring morphology and normal ER behavior persisted (Examples of daughter cells with cER are shown with white arrows), indicating that septin and cER in these knockout cells are unaffected by ER stress. Similar results were seen with *slt2Δ* cells in response to ER stress (Figure 4). *pkc1Δ* cells were grown in YPD in the presence of 1M sorbitol. 1M sorbitol does not influence ERSU phenotypes (see Figures 5A vs 5E). *bck1Δ* cells were grown in YPD.

Figure S5. Involvement of Wsc1 in ERSU is distinct from its functioning in previously reported signaling pathways (Related to Figure 5)

(A) In the absence of *WSC1*, cER enters daughter cell following ER stress induction. *wsc1Δ* cells carrying Hmg1-GFP reporter were grown in YPD or under ER stress inducing condition (YPD + Tm 1 μg/ml for 3 hrs). A white arrowhead shows a daughter cell with cER.

(B) Neither *ROM1* nor *ROM2* is not involved in the ERSU pathway. Either *rom1Δ* or *rom2Δ* cells can support growth on a YPD plate with Tm. Five-fold serial dilutions of the indicated mutant cells were grown on medium with and without Tm (0.4 μg/ml).

(C) Both *rom1Δ* and *rom2Δ* cells can phosphorylate Slt2 upon ER stress at a level similar to ER stressed WT cells. Samples were collected and analyzed by western blot for Slt2 phosphorylation (top), total Slt2 (middle) and Pgk1 (loading control, bottom) as described in Figure S3C.

(D) The UPR pathway was not activated during a secretory block induced in *sec1-1* cells. UPR activation was monitored by splicing of *HAC1* mRNA. Upon tunicamycin (+Tm) treatment, northern analysis showed appearance of spliced form of *HAC1* mRNA (lane 2). In contrast, the lack of *HAC1* splicing during the secretory block induced in *sec1-1* cells grown at 37°C indicates that ER stress was not induced (lanes 7-10). Shifting temperature to 37°C in WT cells did not induce *HAC1* mRNA splicing (lanes 3-6).

(E) Imposition of a secretory block in *S. cerevisiae* is known to repress transcription of the mRNA transcript coding for the ribosomal protein *RPL32* through the arrest of secretion response (Carr et al., 1999). After shifting both WT and *sec1-1* cells to the non-permissive temperature 37°C for the indicated amount of time, total RNA samples from each time point were analyzed by northern blot for the level of *RPL32* mRNA and *SCR1* RNA as a loading control. A dramatic loss of *RPL32* RNA confirmed that a secretory block was induced upon shifting *sec1-1* cells to 37°C.

(F) The *sec1-1* mutation induces a secretory block, but no ER stress**.** Septin ring was monitored in *sec1-1* cells genomically expressing Cdc10-GFP at permissive (24°C), semi-permissive (30°C), and non-permissive temperature (37°C). A *sec1-1*-induced secretory block affected the size of the daughter cell, but the septin ring targeted to its normal position at the bud neck.

Figure S6. Inheritance of the stressed cER into the daughter has deleterious consequences to the cell (Related to Figure 6 and 7)

(A) The effect of Latrunculin B (LatB) on ER and septin distribution was visualized with the ER Hmg1-GFP reporter and the septin reporter Shs1-GFP, respectively. GFP/DIC merged images shown Figure 7A were separated to GFP or DIC alone. Both wild-type (left) or *slt2*Δ cells (right) were treated with 1 μg/ml Tm & 400 μM LatB for 2 hrs.

(B) Quantitation of Lat B effect on cER in the daughter cell. For each of the three classes of cells, the number of wild-type and *slt2*Δ cells containing cER in the daughter cell was counted (n=300) during growth under normal conditions (dark gray bars) or in the presence of 1 μg/ml Tm (2 hrs) (light gray bars) or 1 μg/ml Tm & 400 μM LatB (2 hrs) (black bars). The average of three independent experiments is depicted; error bars represent standard deviation (SD). cER inheritance in Tm-treated WT cells was not significantly affected by inclusion of LatB (WT, compare Tm to LatB+Tm).

(C) Prevention of cER inheritance by *MYO4* gene deletion (Estrada et al., 2003), instead of LatB, rescues *slt2*Δ cell growth on Tm plates (0.1 μg/ml Tm + 1M sorbitol). *myo4*Δ *slt2*Δ cells (two different isolates) were able to grow better than *slt2*Δ cells or *myo4*Δ cells on Tm plates at the specific concentration range of Tm (0.1 μg/ml). Deletion of *MYO4* in *slt2*Δ cells mimicked the rescue effect of LatB seen for *slt2*Δ cells grown with Tm (Figure 7B). Curiously, however, we found that neither *myo4*Δ*slt2*Δ nor *slt2*Δ cells were able to grow at higher concentration of Tm (0.2μg/ml + 1M sorbitol).

(D) cER in *myo4*Δ*slt2*Δ cells carrying Hmg1-GFP under no stress and ER stressed conditions (+Tm, 1 μg/ml for 3 hrs). Examples of daughter cells with little cER are indicated by white arrowheads. Under normal growth conditions, most *myo4*Δ*slt2*Δ cells did not exhibit cER inheritance defects. This was surprising since *MYO4* was reported to impair cER inheritance under normal growth conditions (Estrada et al., JCB *163*: 1255-1266 2003). Further, since *slt2*Δ cells are not defective for cER inheritance under normal growth (Figure 4C & 4D), we anticipated cER inheritance defect in *myo4*Δ*slt2*Δ under the normal growth. Nevertheless, we did observe cER inheritance delay to many *myo4*Δ*slt2*Δ daughter cells upon ER stress induction. Taken together, our result showing the rescued growth of *myo4*Δ*slt2*Δ cells under low concentration of Tm is consistent with the minor delay of cER inheritance seen in *myo4*Δ*slt2*Δ cells.

(E) *act1-1* temperature sensitive mutant grew better than wild-type cells in the presence of Tm (+Tm 0. 4 μg/ml) at permissive (24 °C). At semi-permissive temperature (30°C), *act1-1* grew significantly slower than wild-type cell under no ER stress. In the presence of Tm, however, *act1-1* cells grew slightly better than wild-type cells. Similarly, at permissive temperature (24 °C), while *act1-1* and WT cells grew similarly under no ER stress, ER stress caused *act1-1* cell to grow better than WT. (*act1-1* is known to display mild actin defect even at permissive temperature). Together, these results are consistent with the idea that mild actin defect helps cER inheritance delay upon induction of ER stress, allowing *act1-1* to grow better than WT cells.

Figure S7. ER stress induces increased cell death (Related to Figure 7)

(A) The vital dye FUN-1 stains metabolically active yeast WT cells on cylindrical intravacuolar red rod-like structures (CIVSs) (Essary and Marshall, 2009**),** while cells rendered metabolically inactive by treatment with 10% bleach display diffuse green and red staining. (a) DIC, (b) red channel, (c) green channel, and (d) merge of red and green channels of normal (upper panels) or metabolically inactive (lower panels) cells are shown.

(B) & (C) Propidium iodide (PI) staining of untreated and Tm treated (1 μg/ml for 4 hrs) wild-type (WT) or *slt2*Δ cells to enumerate dead cells. The fluorescent dye PI is membrane impermeable and thus excluded from viable cells, staining only dead cells. Following ER stress, dead cells (stained with PI) are noted in both cell populations, but are much more abundant in *slt2*Δ cells. The population of PI-stained WT cells consisted of small daughter cells attached to the mother cell. In contrast, both mother and daughter of *slt2*Δ cells were PI-stained. Furthermore, simultaneous treatment of cells with 400 μM LatB and 1 μg/ml Tm for 4 hrs reduced the number of PI stained cells in both cell types. These results are consistent with the growth rescue of stressed *slt2*Δ cells by LatB (shown in Figure 7B). Strikingly, the population of PI-stained *slt2Δ* cells in the presence LatB was similar to ER stressed wild-type cells. Quantitation of PI stained cells (n= 100) is shown in (C).

(D) & (E) ER stress induction in *ero1-1* cells increases the number of cells stained with PI. When grown at permissive temperature (24 $^{\circ}$C), essentially no *ero1-1* cells were stained by PI. Temperature shift to the non-permissive temperature (37°C, 3 hrs) resulted in a reproducible fraction of *ero1-1* stained by PI, although this fraction was smaller than for cells induced for ER stress by Tm treatment. Quantitation of stained cells is shown in (D). At least, 100 cells were counted for each data set, and standard deviation was calculated from three repeats of experiments.

SUPPLEMENTAL TABLE

Supplemental Table 1 A list of candidate genes examined (Related to Figure 3 and 4)

A list of candidate genes was chosen based on their localization to the ER, and their functions (or presumed functions) associated with ER.

Candidate Genes tested
1. SLT2
2. IRE1
3. AUX1
4. MYO4
5. SHE3
6. ICE2
7. YOP1
8. RTN1
9. RTN2
10.SBH1
11. SBH2
12. SCS2
13. SEC3
14. SEC8
15. YPT11
16. OSH1
17 OSH2
18. HSP104

Supplemental Table 2 Strains used in this study (Related to all the Figures)

Strain number	Relevant genotype	Source
MNY1031	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, his3-11,15, bar1::LEU2, CDC10-GFP::KanMX*	This study
MNY1032	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, his3-11,15, bar1::LEU2, SHS1-GFP::KanMX*	This study
MNY1033	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, his3-11,15, bar1::LEU2, CDC11-GFP::KanMX*	This study
MNY1034	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, ero1-1::HIS3, bar1::LEU2*	This study
MNY1035	*MATa, cdc12-6, his3, leu2, lys2, trp1, ura3, ade2*	Y. Barral
MNY1036	*MATa, cdc12-6, his3, leu2, lys2, trp1, ura3, ade2, CDC10-mCherry::KanMX*	This study
MNY1037	*MATa, leu2-3,112, trp1-1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3*	This study
MNY1038	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11, bar1Δ::LEU2*	This study
MNY1039	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11, bar1 Δ::LEU2, ire1Δ::KanMX*	This study
MNY1040	*MATa, leu2-3,112, trp1-1::CDC10-mCherry:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3*	This study
MNY1041	*MATa, leu2-3,112, trp1-1::CDC10-mCherry:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3, ire1 Δ::KanMX*	This study
MNY1042	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, his3-11,bar1Δ::LEU2, slt2Δ::KanMX*	This study
MNY1043	*MATa, leu2-3,112, trp1-1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3, slt2 Δ::KanMX*	This study

MNY1044	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, his3-11,,bar1Δ::LEU2, CDC10-GFP::KanMX, slt2 Δ::NatMX*	This study
MNY1045	*MATa, leu2-3,112, trp1-1::CDC10-mCherry:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3, slt2Δ::KanMX*	This study
MNY1046	*MATa, leu2-3,112, trp1-1::CDC10-mCherry:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3, wsc1Δ::KanMX*	This study
MNY1047	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0::wsc1Δ:URA3, wsc2Δ::KanMX*	This study
MNY1048	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0::wsc1Δ:URA3, wsc3Δ::KanMX*	This study
MNY1049	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0::wsc1Δ:URA3, wsc4Δ::KanMX*	This study
MNY1050	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, rlm1Δ::KanMX*	Yeast KO collection
MNY1051	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, ire1Δ::KanMX*	Yeast KO collection
MNY1052	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, slt2Δ::KanMX*	Yeast KO collection
MNY1053	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, wsc1Δ::KanMX*	Yeast KO collection
MNY1054	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, wsc2Δ::KanMX*	Yeast KO collection
MNY1055	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, wsc3Δ::KanMX*	Yeast KO collection
MNY1056	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, wsc4Δ::KanMX*	Yeast KO collection
MNY1057	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, mid2Δ::KanMX*	Yeast KO collection
MNY1058	*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, mtl1Δ::KanMX*	Yeast KO collection
MNY1059	*MATa /MATα, his3Δ1/his2Δ1, leu2Δ0/leu2Δ0, LYS2/lys2Δ0, met15Δ0/MET15, ura3Δ0/ura3Δ0*	(Kim et al., 2008)
MNY1060	*MATa /MATα, trp1-1/trp1-1, leu2-3,112/leu2-3,112, ura3-52/ura3-52, his4/his4, bck1Δ::G418/bck1Δ::G418*	(Kim et al., 2008)
MNY1061	*MATa /MATα, his3Δ1/his2Δ1, leu2Δ0/leu2Δ0,LYS2/lys2Δ0, met15Δ0/MET15,ura3Δ0/ura3Δ, mkk1Δ::G418/mkk1Δ::G418 mkk2Δ::G418/mkk2Δ::G418*	(Kim et al., 2008)
MNY1062	*MATa, leu2-3,112, ura3-52, trp1-1, his4, can1r*	(Watanabe et al., 1994)
MNY1063	*MATa, leu2-3,112, ura3-52, trp1-1, his4, can1r, pkclΔ::LEU2*	(Watanabe et al., 1994)
MNY1064	*MATa*, *ura3-52*	P. Novick

MNY1065	*MATa, ura3-52, sec1-1*	P. Novick
MNY1066	*MATα, ura3-52, leu2-3,112, his3Δ200, trp1Δ901, lys2-801, suc2Δ9, WSC1-GFP::HIS3*	(Piao et al., 2007)
MNY1067	*MATα, ura3-52, leu2-3,112, his3Δ200, trp1Δ901, lys2-801, suc2Δ9, WSC1^{AAA}-GFP::HIS3*	(Piao et al., 2007)
MNY1068	*MATa*, *leu2-3,112, trp1-1, can1-100, ura3-1, his3-11,15, bar1Δ::LEU2, HDEL-DsRed::URA3*	This study
MNY1069	*MATa* his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *VPH1*-mcherry:: KanMx	(Dokudovskaya S, unpublished)
MNY1070	*MATα, leu2-3,112, trp1-1:: shs1Δ::TRP1:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15*	(MN Simon)
MNY1071	*MATα, leu2-3,112, trp1-1:: shs1Δ::TRP1:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15, , CDC10-mCherry::KanMX*	This study
MNY1072	*MATa, leu2-3,112, ura3-52, trp1-1, his4, can1r, , CDC10-mCherry::KanMX*	This study
MNY1073	*MATa, leu2-3,112, ura3-52, trp1-1, his4, can1r, pkclΔ::LEU2, CDC10-mCherry::KanMX*	This study
MNY1074	*MATa, leu2-3,112, trp1-1::CDC10-mCherry:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3, bck1 Δ::KanMX*	This study
MNY1075	*MATa, leu2-3,112, trp1-1::CDC10-mCherry:TRP1, can1-100, ura3-1::HMG1-GFP:URA3, ade2-1, his3-11,15::UPRE-lacZ:HIS3, ptc1 Δ::KanMX*	This study

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains, media, and growth conditions

Deletion and epitope tagged strains were constructed using a one-step PCR-mediated technique (Longtine et al., 1998b). Plasmids p2188 (pRS315[*MPK1-3xHA*]), p2190(pRS315[*mpk1*(*T190A Y192F*)*-3xHA*]) and p2193 (pRS315[*mpk1*(*K54R*)*-3xHA]*) were transformed into MNY1045. ER localization was monitored using the previously-characterized ER membrane reporter plasmid, pRH 475, and luminal reporter plasmid, pRH 1827 (Du et al., 2001; Hampton et al., 1996). pRH 475 expressed Hmg1-GFP, the transmembrane NH2-terminal domain (1-702) of the ER resident protein HMG-CoA reductase isozyme I fused to GFP and was expressed from the strong, constitutive TDH3

promoter. pRH 1827 contained an HDEL-DsRed fusion protein bearing the signal sequence and an HDEL ER retrieval signal from *KAR2*.

All cells were grown in rich complete medium (YPD) except strains transformed with p2188, p2190 and p2193, which were grown in synthetic complete –LEU medium. Strains were grown at 30°C and examined during log phase, unless otherwise noted. For synchronized experiments, cells were treated with 50 ng/ml α factor (stored as a 1 mg/ml stock in PBS at –20°C) for 2.5 hours, washed twice with an equal volume of fresh medium, diluted to an OD of 0.25, and then allowed to recover for 15 minutes before treatment with Tm. For Slt2 immunoblot experiments, cells were grown to an OD_{600} of 1, then diluted to an OD of 0.25 before treatment with Tm or CFW.

Stock and final concentrations of drugs used in this study were as follows: Tunicamycin (Tm) (Calbiochem), final 1 μg/ml or as indicated; DTT (Fisher), final 2 mM or 4 mM, as indicated; Latrunculin B (LatB) (Calbiochem), final 400 μM; calcofluor white (CFW) (Sigma), final 10 μg/ml; and caspofungin (CP) (a generous gift from Merck), final 10 ng/ml. For plates, final concentrations were 0.2 μg/ml or 0.4 μg/ml Tm, as indicated, and 6 μM LatB.

Cell extracts, Northern blotting and immunoblotting

For Northern blotting, ten μg of total RNA were loaded on a 1.5% agarose gel with 6.7% formaldehyde, and transferred to zeta probe membrane (BioRad) in 10x SSC by capillary action overnight. Following UV-crosslinking, membranes were probed with a radio labeled DNA probe.

For Western blot analysis, protein was extracted as previously described (Bicknell et al., 2007), and 20 μg of protein were loaded on an 8% SDS-PAGE gel, and transferred to nitrocellulose. Primary antibodies used were anti-phospho p44/p42 MAP Kinase (Slt2) antibody (Cell Signaling) at a 1:1000 dilution overnight and anti-PGK (phosphoglycerate kinase) antibody (Molecular Probes) at a 1:10,000 dilution for one hour, and anti-Slt2 antibody (Santa Cruz Biotechnology) at a 1:1000 dilution for overnight at 4 ^{o}C. Anti- Secondary antibodies were HRP-conjugated donkey anti-rabbit antibody at 1:10,000 (GE Healthcare) for phosphor Slt2, and goat anti-mouse 1:10,000

(BioRad) for PGK. Membranes were developed with ECL Plus Western blotting detection reagent (GE Healthcare) and imaged using a Typhoon phosphorimager (GE Healthcare).

Microscopy

Tagged septin subunits and Hmg1-GFP were imaged in live log phase cells. To quantitate ER inheritance, 300 budded cells were counted, divided into 3 classes as described in Results, and scored for the presence or absence of cER in the bud. FUN1 staining was performed using FUN1 dye (Molecular Probes) at a final concentration of 10 μM for 1 ml of $3x10^6$ cells/ml for 30 minutes at 30°C in the dark. Propidium iodide (PI) staining was performed using a 1 mg/ml stock solution at a final concentration of 3μg/ml. Cells were imaged immediately after staining. No washing step was included.

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