

Slt2 is required to activate ER-stress protective mechanisms through TORC1 inhibition and hexosamine pathway activation

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Table S1. *Saccharomyces cerevisiae* strains used in this study.

Strain	Genotype	Reference or source
BY4741 (wt)	<i>MATa ura3Δ his3-Δ1 leu-2Δ met15Δ</i>	Euroscarf
<i>slt2</i>	BY4741 <i>slt2::kanMX4</i>	Euroscarf
<i>Gfa1-TAP</i>	BY4741 <i>GFA1-TAP::HIS3MX6</i>	Horizon Discovery
<i>ire1</i>	BY4741 <i>ire1::kanMX4</i>	Euroscarf
<i>hac1</i>	BY4741 <i>hac1::kanMX4</i>	Euroscarf
<i>chs3</i>	BY4741 <i>chs3::kanMX4</i>	Euroscarf
<i>slt2 chs3</i>	BY4741 <i>slt2::kanMX4 chs3::hphMX4</i>	This study
<i>reg1</i>	BY4741 <i>reg1::kanMX4</i>	Euroscarf
<i>snf1</i>	BY4741 <i>snf1::kanMX4</i>	Euroscarf
<i>reg1 snf1</i>	BY4741 <i>reg1::kanMX4 snf1::natMX4</i>	[57]
<i>bck1</i>	BY4741 <i>bck1::kanMX4</i>	Euroscarf
<i>adc17</i>	BY4741 <i>adc17::kanMX4</i>	Euroscarf
<i>rpn4</i>	BY4741 <i>rpn4::kanMX4</i>	Euroscarf
<i>hsm3</i>	BY4741 <i>hsm3::kanMX4</i>	Euroscarf
<i>nas6</i>	BY4741 <i>nas6::kanMX4</i>	Euroscarf
<i>rpn14</i>	BY4741 <i>rpn14::kanMX4</i>	Euroscarf
<i>atg8</i>	BY4741 <i>atg8::kanMX4</i>	Euroscarf
<i>atg1</i>	BY4741 <i>atg1::kanMX4</i>	Euroscarf
<i>atg12</i>	BY4741 <i>atg12::kanMX4</i>	Euroscarf
<i>atg13</i>	BY4741 <i>atg13::kanMX4</i>	Euroscarf
<i>atg33</i>	BY4741 <i>atg33::kanMX4</i>	Euroscarf

Table S2. Oligonucleotides used in this study.

Name	Sequence	Used for
KAN-S2	GTCAAGGAGGGTATTCTGG	Verification integration
CHS3-S1	CATCAAGTACCGGTGTAAACCCAAATGCAACTCGTCGGAGCGTACGCTGCAGGTCGAC	Deletion of <i>CHS3</i> . Forward primer
CHS3-S2	GTA CTCAAACACTTGAATTGCAGTTACCCAAGATTGTGCCATCGATGAATTCGAGCTC	Deletion of <i>CHS3</i> . Reverse primer
CHS3-V1	TTGTAGTAAATCCTGCTAG	Verification deletion <i>CHS3</i>
Oligo-dT	TTTTTTTTTTTTTTTTTTT (A, C or G)	cDNA synthesis
GFA1-F1	GGTGTTTTGGCAGGTGAGTT	Quantification by qRT-PCR of <i>GFA1</i> mRNA. Forward primer
GFA1-R1	GGTTTGCAGGTCGATTGATT	Quantification by qRT-PCR of <i>GFA1</i> mRNA. Reverse primer
ACT1-F1	GGATCTTCTACTACATCAGC	Quantification by qRT-PCR of <i>ACT1</i> mRNA. Forward primer
ACT1-R1	CACATACCAGAACCGTTATC	Quantification by qRT-PCR of <i>ACT1</i> mRNA. Reverse primer

Table S3. Plasmids used in this study.

Plasmid	Description	Source or reference
pMCZ-Y	A high copy 2 μ <i>URA3</i> plasmid containing a <i>UPRE-lacZ</i> reporter (a gift from Prof Mori).	[43]
pAG32	The plasmid contains the <i>hph</i> gene from <i>Klebsiella pneumoniae</i> encoding hygromycin B phosphotransferase and confers resistance to the antibiotic hygromycin B.	[34]
pRS415-GFP-ATG8	Centromeric plasmid containing a fusion of GFP at the N-terminus of <i>ATG8</i> .	[80]
pFCM493	YEpl3 (<i>LEU2</i>) derivative containing the <i>GFAI</i> gene	[48]
pKK1	Centromeric plasmid with <i>LEU2</i> selection marker.	[46]
pMS109 (pHAC1)	Fragment containing the <i>HAC1</i> gene without introns inserted between the promoter and terminator of <i>PGK1</i> in pKK1.	[46]

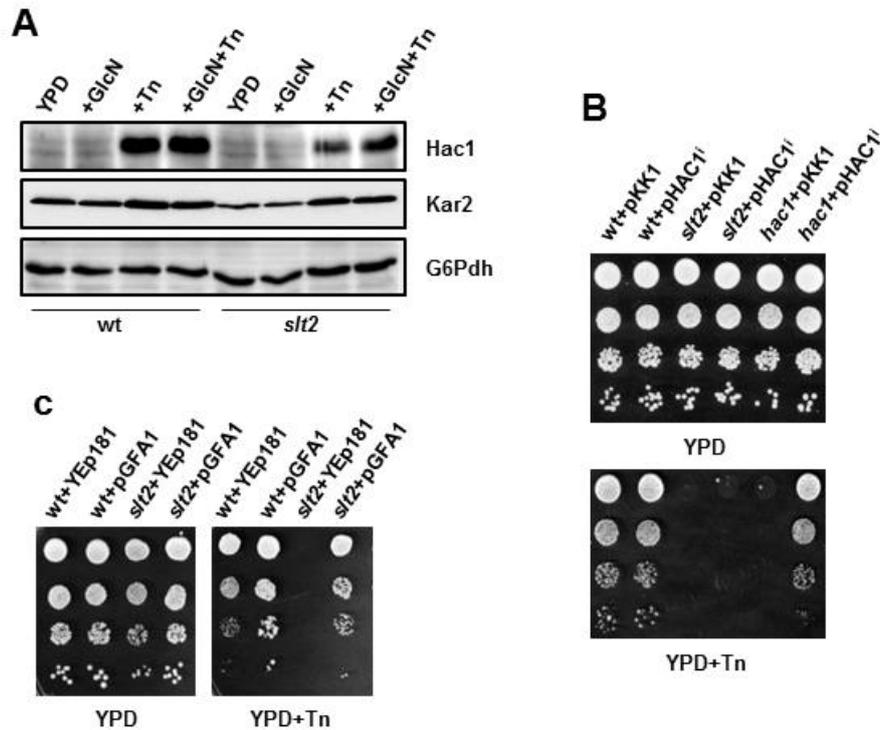


Figure S1. Overexpression of *GFA1* provides enhanced ER stress tolerance. (A) Protein extracts from wild-type (wt) and *slt2* mutant strains were analyzed by SDS-PAGE and Western blot by using Hac1 and Kar2 antibodies as described in the Materials and Methods section. YPD-grown cultures containing or lacking 11.5 mM glucosamine (GlcN) were grown until mid-log-phase ($OD_{600} \sim 0.5$) at 30 °C (control, YPD) and treated with 2 $\mu\text{g/ml}$ Tn for 90 min. The level of glucose-6-phosphate dehydrogenase (G6Pdh) was used as a loading control for crude extracts. (B) Serial dilutions ($1-10^{-3}$) of SCD-Ura-grown cultures ($OD_{600} \sim 0.8$) of pKK1 (control plasmid) and pHAC1¹ transformants of the BY4741 wild-type (wt), *slt2* and *hac1* mutant strains were spotted (2 μl) onto YPD plates that lacked (YPD) or contained tunicamycin, Tn (0.5 $\mu\text{g/ml}$; YPD+Tn) and were incubated at 30 °C for 2-4 days. The plasmid pHAC1¹ express the mature/spliced and active mRNA of *HAC1*. (C) Drop test of BY4741 wild-type (wt) and *slt2* mutant strains transformed with plasmids YEp181 (empty plasmid; YEp181) or pFCM493 (pGFA1). Cultures were diluted and spotted on YPD or tunicamycin-containing YPD (YPD+Tn) as described in (B). In all cases, representative experiments are shown.

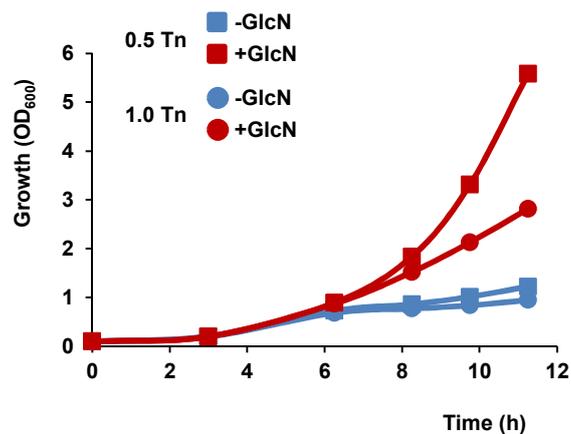


Figure S2. Glucosamine supplementation stimulates the growth of *Saccharomyces cerevisiae* in the presence of the ER-stress inducer tunicamycin. Overnight YPD-grown cultures of the BY4741 wild-type strain were refreshed in YPD ($OD_{600} = 0.1$) lacking (-GlcN; blue lines and symbols) or containing 11.5 mM glucosamine (+GlcN; red lines and symbols) and grown at 30°C for 3 h. Cultures were split in two and incubated at 30 °C in the presence of 0.5 $\mu\text{g/ml}$ of tunicamycin (squares) or 1.0 $\mu\text{g/ml}$ of the same drug (circles). Growth was followed by measuring the OD_{600} at the indicated times. A representative experiment is shown.

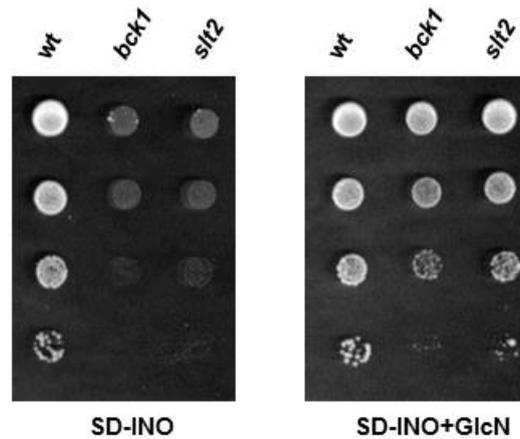


Figure S3. Glucosamine addition partially overcomes the inositol requirement of CWI pathway mutants. YPD-grown cultures ($OD_{600} \sim 0.8$) of the BY4741 wild-type (wt), *bck1*, and *slt2* mutant strains were centrifuged and the pellets were washed with water, resuspended in SD-INO medium at the same OD_{600} , and serial dilutions ($1-10^{-3}$) were spotted ($2 \mu\text{l}$) onto SD-INO plates lacking (SD-INO) or containing 11.5 mM glucosamine (SD-INO+GlcN). Plates were incubated at 30 °C and inspected for growth after 2-4 days. A representative experiment is shown.

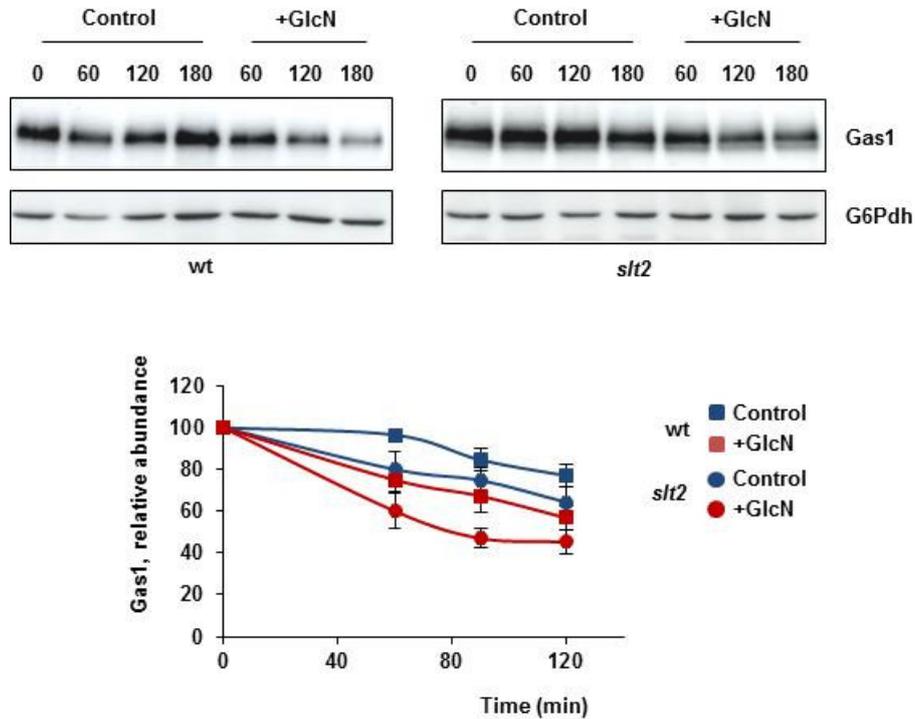


Figure S4. Glucosamine reduces Gas1 cellular content. Cells of the wild-type BY4741 (wt) and *slt2* mutant strains were grown to the mid-logarithmic phase ($OD_{600} \sim 0.5$) in YPD medium at 30 °C. An aliquot was withdrawn for the analysis (time 0) and the rest of the culture was treated with 11.5 mM glucosamine (GlcN) for the indicated times. Protein extracts from untreated (time 0; control) and GlcN-treated samples were separated by SDS-PAGE, and Gas1 abundance was analyzed by Western blot and immunodetection by using a rabbit polyclonal anti-Gas1 antibody as described in the Materials and Methods section. The level of glucose-6-phosphate dehydrogenase (G6Pdh) was used as a loading control for crude extracts. The graph shows the abundance of Gas1 in each time point relative to that of the corresponding control (time 0) for untreated and GlcN-treated samples of each strain analyzed. Data are the mean (\pm SD) of three independent biological replicates.

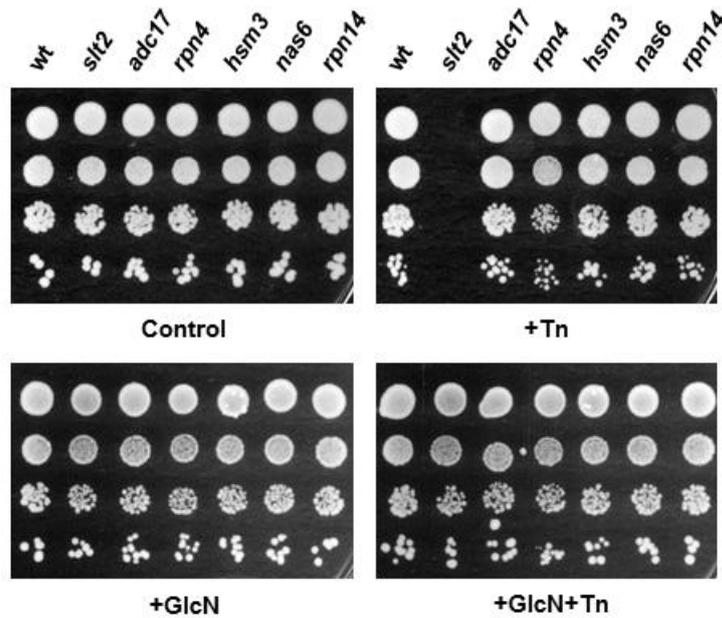


Figure S5. Knock-out of RACs does not cause increased ER-stress sensitivity. Cultures of BY4741 wild-type (wt), *slt2* and stress-inducible RAC (regulatory particle assembly-chaperone) deletion strains lacking *ADC7*, *RPN4*, *HSM3*, *NAS6* or *RPN14* were diluted ($1-10^{-3}$) and spotted ($2 \mu\text{l}$) on plates containing or lacking 11.5 mM glucosamine (GlcN) and/or 0.5 $\mu\text{g/ml}$ tunicamycin (Tn). Plates were incubated at 30 °C and inspected for growth after 2-4 days. A representative experiment is shown.

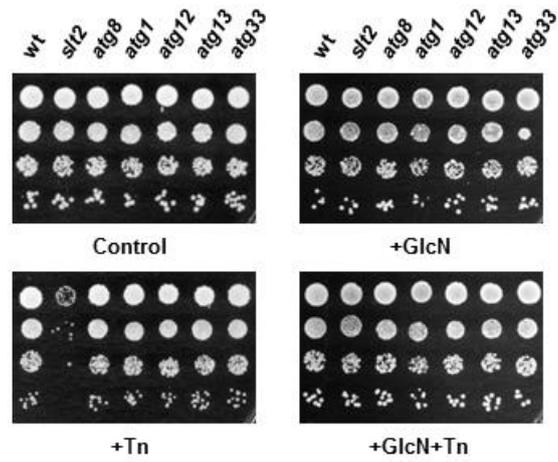


Figure S6. *ATG* genes essential for bulk autophagy are dispensable for ER stress tolerance. Drop test of BY4741 wild-type (wt) and *slt2*, *atg8*, *atg1*, *atg12*, *atg13* and *atg33* mutant strains was carried out on plates lacking or containing 11.5 mM glucosamine (GlcN) and/or 0.5 µg/ml tunicamycin (Tn).