

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

**Isabel E. Sánchez-Adriá<sup>a</sup>, Gemma Sanmartín<sup>a</sup>, Jose A. Prieto<sup>a</sup>, Francisco Estruch<sup>b</sup>, and Francisca Randez-Gil<sup>a,\*</sup>**

<sup>a</sup> Department of Biotechnology, Instituto de Agroquímica y Tecnología de los Alimentos, Consejo Superior de Investigaciones Científicas, Avda. Agustín Escardino, 7. 46980-Paterna, Valencia, Spain

<sup>b</sup> Departament of Biochemistry and Molecular Biology, Universitat de València, Dr. Moliner 50, 46100-Burjassot, Spain

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

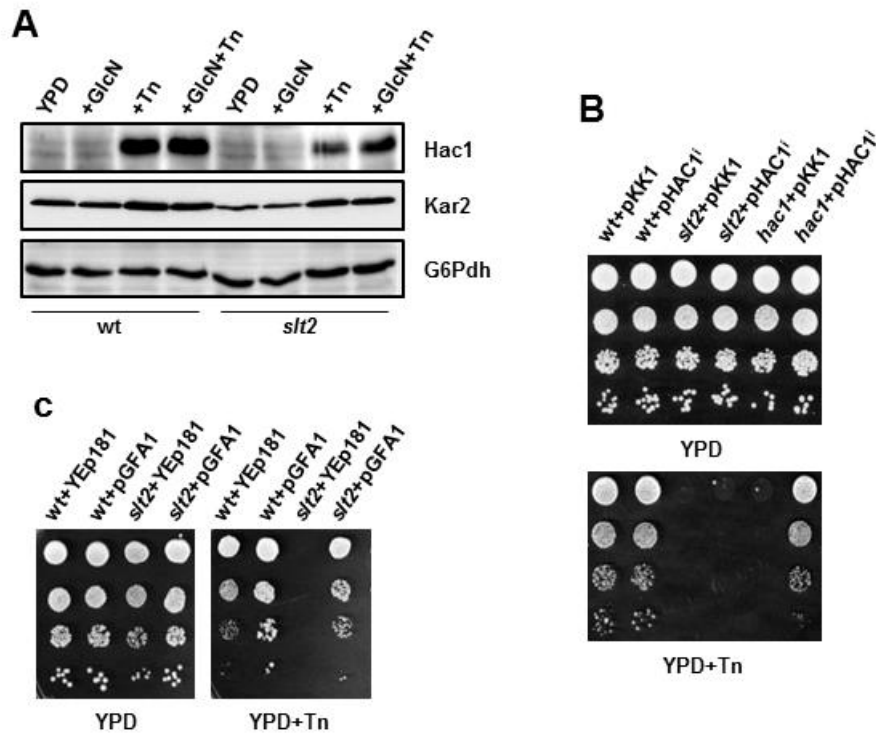
Strain	Genotype	Reference or source
BY4741 (wt)	<i>MATa ura3Δ his3-Δ1 leu-2Δ met15Δ</i>	Euroscarf
<i>slt2</i>	BY4741 <i>slt2::kanMX4</i>	Euroscarf
<i>Gfa1-TAP</i>	BY4741 <i>GFA1-TAP::HIS3MX6</i>	Horizon Discovery
<i>ire1</i>	BY4741 <i>ire1::kanMX4</i>	Euroscarf
<i>hac1</i>	BY4741 <i>hac1::kanMX4</i>	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.

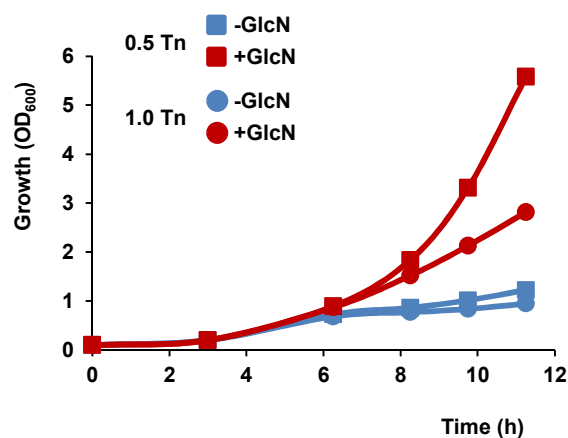
Name	Sequence	Used for
KAN-S2	GTCAAGGAGGGTATTCTGG	Verification integration
CHS3-S1	CATCAAGTACCGGTGTAAACCCAAATGCAACTCGTCGGAGCGTACGCTGCAGGTCGAC	Deletion of <i>CHS3</i> . Forward primer
CHS3-S2	GTACTCAAACACTTGAATTGCAGTTACCCAAGATTGTGCCATCGATGAATTCGAGCTC	Deletion of <i>CHS3</i> . Reverse primer
CHS3-V1	TTGTAGTAAATCCTGCTAG	Verification deletion <i>CHS3</i>
Oligo-dT	TTTTTTTTTTTTTTTTTT (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.

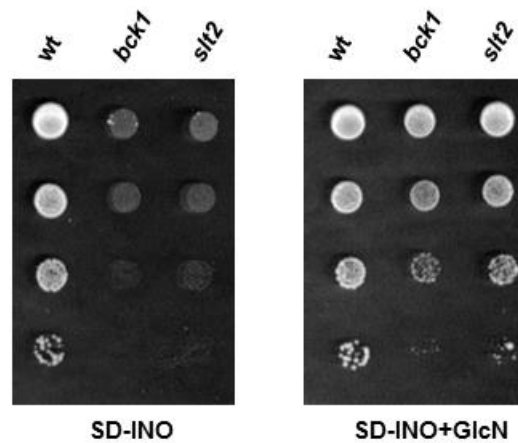
Plasmid	Description	Source or reference
pMCZ-Y	A high copy 2 $\mu$ <i>URA3</i> plasmid containing a <i>UPRE-lacZ</i> reporter (a gift from Prof Mori).	[43]
pAG32	The plasmid contains the <i>hph</i> gene from <i>Klebsiella pneumoniae</i> encoding hygromycin B phosphotransferase and confers resistance 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]



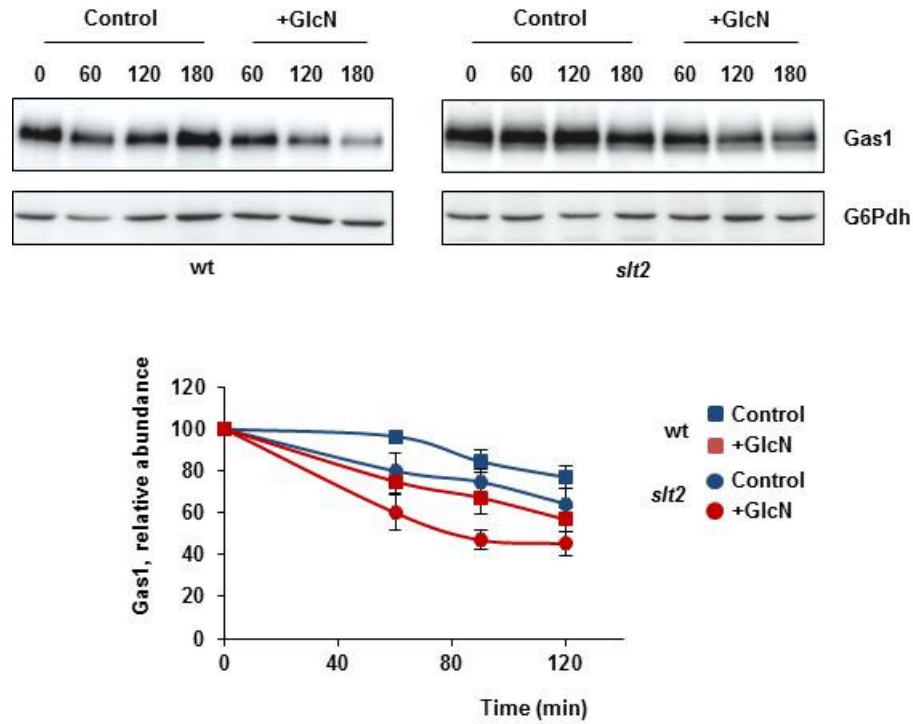
**Figure S1.** Overexpression of *GFA1* provides enhanced ER stress tolerance. (A) Protein extracts from wild-type (wt) and *slt2* mutant strains were analyzed by SDS-PAGE and Western blot by using Hac1 and Kar2 antibodies as described in the Materials and Methods section. YPD-grown cultures containing 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  $\mu$ 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 pHAC1<sup>i</sup> transformants of the BY4741 wild-type (wt), *slt2* and *hac1* mutant strains were spotted (2  $\mu$ l) onto YPD plates that lacked (YPD) or contained tunicamycin, Tn (0.5  $\mu$ g/ml; YPD+Tn) and were incubated at 30 °C for 2-4 days. The plasmid pHAC1<sup>i</sup> express the mature/spliced and active mRNA of *HAC1*. (C) Drop test of BY4741 wild-type (wt) and *slt2* mutant strains transformed with plasmids YEplac181 (empty plasmid; YEplac181) 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<sub>600</sub> = 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 µg/ml of tunicamycin (squares) or 1.0 µg/ml of the same drug (circles). Growth was followed by measuring the OD<sub>600</sub> at the indicated times. A representative experiment is shown.

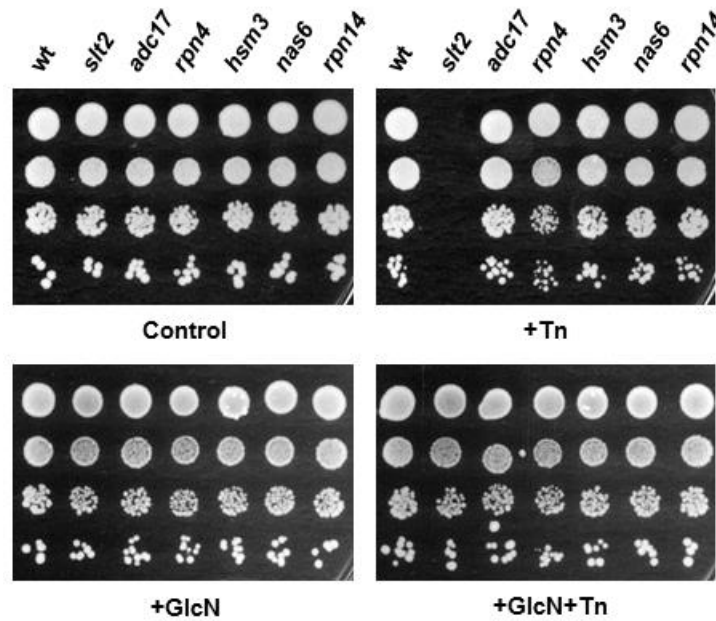


**Figure S3.** Glucosamine addition partially overcomes the inositol requirement of CWI pathway mutants. YPD-grown cultures ( $OD_{600} \sim 0.8$ ) of the BY4741 wild-type (wt), *bck1*, and *slt2* mutant strains were centrifuged and the pellets were washed with water, resuspended in SD-INO medium at 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.

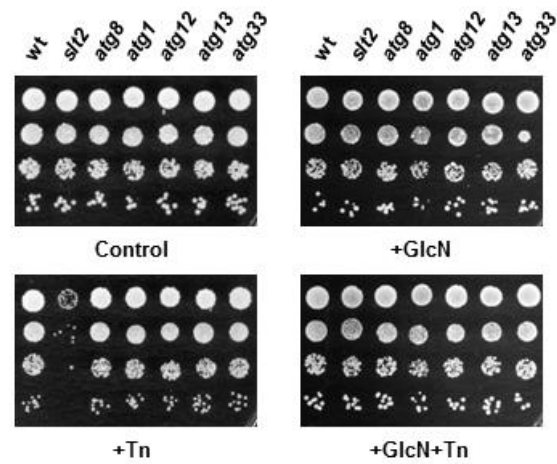


**Figure S4.** Glucosamine reduces Gas1 cellular content. Cells of the wild-type BY4741 (wt) and *slt2* mutant strains were grown to the mid-logarithmic phase ( $OD_{600} \sim 0.5$ ) in YPD medium at 30 °C. An aliquot was withdrawn for the analysis (time 0) and the rest of the culture was treated with 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  $\mu$ l) on plates containing or lacking 11.5 mM glucosamine (GlcN) and/or 0.5  $\mu$ 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  $\mu$ g/ml tunicamycin (Tn).