Table S1. Strains used in this study.

Strains	Genotype	Source
LLY36	*MAT***a/α** *lys2Δ0/lys2Δ0 ura3Δ0/ura3Δ0 his3Δ200/his3Δ200 leu2Δ0/leu2Δ0*	Personal communication
RNT1	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0*	This study
rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 rnt1Δ::HIS3*	This study
HSL1-GFP	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HSL1-GFP::HIS3MX6*	Invitrogen
HSL1-GFP *rnt1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HSL1-GFP::HIS3MX6 rnt1Δ*::KanMX	This study
SWI4-GFP	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SWI4-GFP::HIS3MX6*	Invitrogen
SWI4-GFP *rnt1Δ*	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SWI4-GFP::HIS3MX6 rnt1Δ*::KanMX	This study
RNT1-GFP	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RNT1-GFP::HIS3MX6*	Invitrogen
P_{Gal1}-*BEM2*	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0* [pBG1805-BEM2][a]	This study
P_{Gal1}-*HSL1*	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0* [pEGH-HSL1][a]	This study
P_{Gal1}-*RLM1*	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0* [pBG1805-RLM1][a]	This study
P_{Gal1}-*SIC1*	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0* [pBG1805-SIC1][a]	This study
P_{Gal1}-*SLT2*	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0* [pBG1805-SLT2][a]	This study
P_{Gal1}-*SWI4*	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0* [pEGH-SWI4][a]	This study
bar1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 bar1Δ::KANMX*	This study
bar1Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 bar1Δ::KANMX rnt1Δ::HIS3*	This study
bck1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 bck1Δ::KANMX*	This study
bck1Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 bck1Δ::KANMX rnt1Δ::HIS3*	This study
hog1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 hog1Δ::KANMX*	This study
hog1Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 hog1Δ::KANMX rnt1Δ::HIS3*	This study
hsl1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 hsl1Δ::KANMX*	This study
hsl1Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 hsl1Δ::KANMX rnt1Δ::HIS3*	This study
mkk1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 mkk1Δ::KANMX*	This study
mkk1Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 mkk1Δ::KANMX rnt1Δ::HIS3*	This study
mkk2Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 mkk2Δ::KANMX*	This study
mkk2Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 mkk2Δ::KANMX rnt1Δ::HIS3*	This study
rlm1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 rlm1Δ::KANMX*	This study
rlm1Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 rlm1Δ::KANMX rnt1Δ::HIS3*	This study
slt2Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 slt2Δ::KANMX*	This study
slt2Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 slt2Δ::KANMX rnt1Δ::HIS3*	This study

swi4Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 swi4Δ::KANMX*	This study
swi4Δ rnt1Δ	*MAT***a** *lys2Δ0 ura3Δ0 his3Δ200 leu2Δ0 swi4Δ::KANMX rnt1Δ::HIS3*	This study
GFP	*rnt1Δ* [pRS315/GFP][b]	This study
GFP-RNT1	*rnt1Δ* [pRS315/GFP/RNT1][b]	This study
GFP-rnt1-D247R	*rnt1Δ* [pRS315/GFP/rnt1-D247/R][b]	This study
rnt1-ts	*rnt1Δ* [pRS315/rnt1-I338/T][b]	This study
rat1-1 xrn1Δ	*MAT***α** *ade3 his3 leu2 trp1 xrn1Δ::URA3 rat1-1*	(58)
xrn1Δ	*MAT***a** *ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 xrn1Δ::KANMX*	Open Biosystems

[a] Plasmids bought from Open Biosystems and transformed into the *RNT1* strain.

[b] Plasmids described previously in (57) and transformed into *rnt1Δ* strain.

Table S2. Primers used in this study.

Primers	Sequences
RNT1del For	5’-GCGCATATAGAAGAGAGCAAAACTGTCCTATTTACAAGCT TTTCAAAACAAGATTGTACTGAGAGTGCAC
RNT1del Rev	5’-GCTAAAGAAAATCAATGCAAGTTCCATCATGGTTGTGTAA AAGGAACGTTCTGTGCGGTATTTCACACCG
HSL1 For 3’	5’-TATCCGAGGAATCTTCTCAA
HSL1 Rev 3’	5’-CGCTTCTTCAACTTCAACAT
SWI4 For 3’	5’-TTGGAATCGCCTTCTTCCCTTC
SWI4 Rev 3’	5’-GGTTGTGTCGACTTATGCGTTTGCCCTCAAATCC
ACT1 For	5’-ATGTTTAGAGGTTGCTGCTT
ACT1 Rev	5’-AGAGAGAAACCAGCGTAAAT
APC4 For 3’	5’-AAAGGAATCGTCAGTCAAGA
APC4 Rev 3’	5’-CCCTGATGTAAGCAGGAATA
HSL1 pe	5’-ATCTCTTCAGTAGTCTCTCTGG
SWI4 pe	5’-TAGATTGATTCAGCTTTTTCCTCC
RNT1-RT For	5’-TTAAGAAAGCTTGCCAAACCTGTCATTGAGG
RNT1-RT Rev	5’-CAACTATGGAATTAGGATCAACTGCAGTGGG
ACT1-RT For	5’-CAATGGATTCTGAGGTTGCTGCTTTG
ACT1-RT Rev	5’-CTTGGTCTACCGACGATAGATGGG
snR68-A-For	5’-AACTTGATGCTTCGATGGGG
snR68-A-Rev	5’-AAGTCGCGCCTGTATCCTTA
HSL1-RT-For	5’-TATCCGAGGAATCTTCTCAACTTGTGTTGC
HSL1-RT-Rev	5’-GTATGGGTTTGTGTATTTGATTCCATAGCGG
SWI4-RT-For	5’-CTGGCTACAATTGTCCAGGATGAGGAATC
SWI4-RT-Rev	5’-CAGTATTAGAAAATTTGGCGTCCGCTTTGG

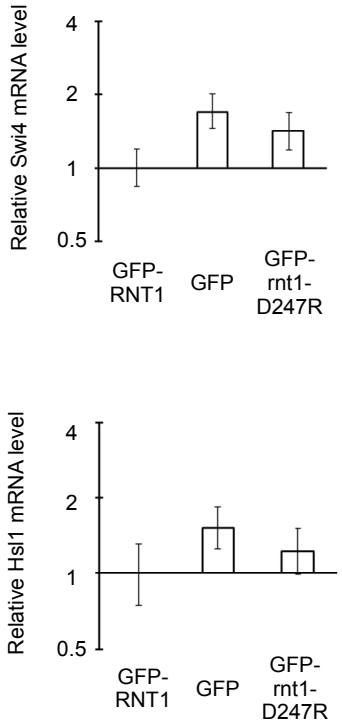

Figure S1. The loss of catalytic activity of Rnt1p causes the *in vivo* accumulation of Swi4 and Hsl1 mRNAs. RT-qPCR quantification of Swi4 and Hsl1 mRNAs from *rnt1*Δ cells expressing an empty vector (GFP) or a catalytic mutant of Rnt1p (GFP-rnt1-D247R) compared to GFP-RNT1 and normalized to Act1 mRNA.

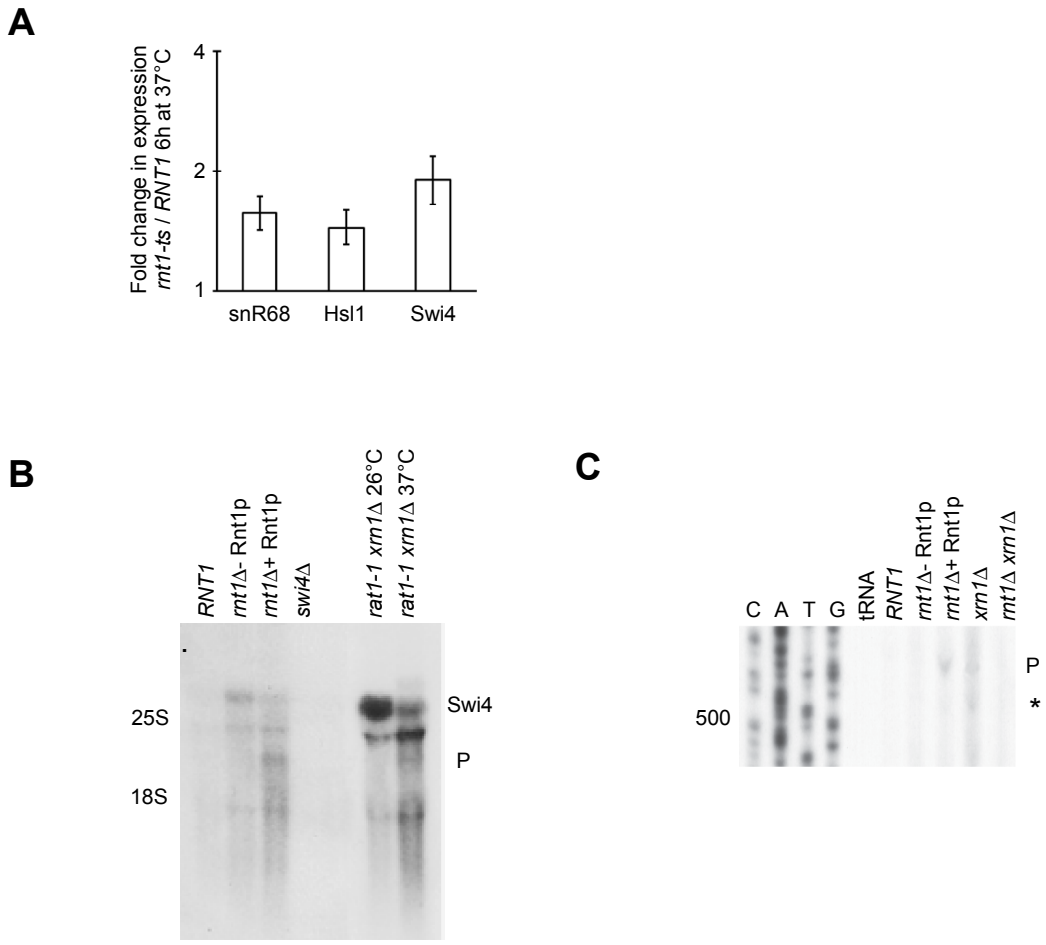

Figure S2. (A) The inactivation of a thermosensitive allele of *RNT1* causes the accumulation of Rnt1p substrates. Cells expressing *RNT1* or a thermosensitive allele of *RNT1* (*rnt1-ts*) were shifted from 26°C to 37°C for the indicated time. The relative levels of the known substrate snR68 (5' extension) and the mRNAs of Hsl1 and Swi4 in *RNT1* and *rnt1-ts* were quantified by qRT-PCR with respect to Act1 mRNA level and normalized to the expression before shift. The inactivation of 5'-3' exoribonucleases stabilizes Rnt1p's cleavage products. (B) Northern blot analysis of Swi4 mRNA from *rat1-1 xrn1*Δ cells grown at the permissive (26°C) or restrictive (37°C) temperature compared to Rnt1p total RNA *in vitro* cleavage reaction. Rnt1p cleavage product is labeled P. (C) Primer extension analysis of Hsl1 mRNA from *xrn1*Δ cells compared to Rnt1p total RNA *in vitro* cleavage reaction. The sequencing reaction of plasmid M13mp18 with Universal primer was loaded as a molecular weight marker. Sequencing reaction lanes and primer extension lanes from the gels are displayed at different contrast adjustments. The *in vitro* cleavage product of Rnt1p is labeled P. Asterisks denote RNA 5' ends that accumulate *in vivo* in x*rn1*Δ cells.

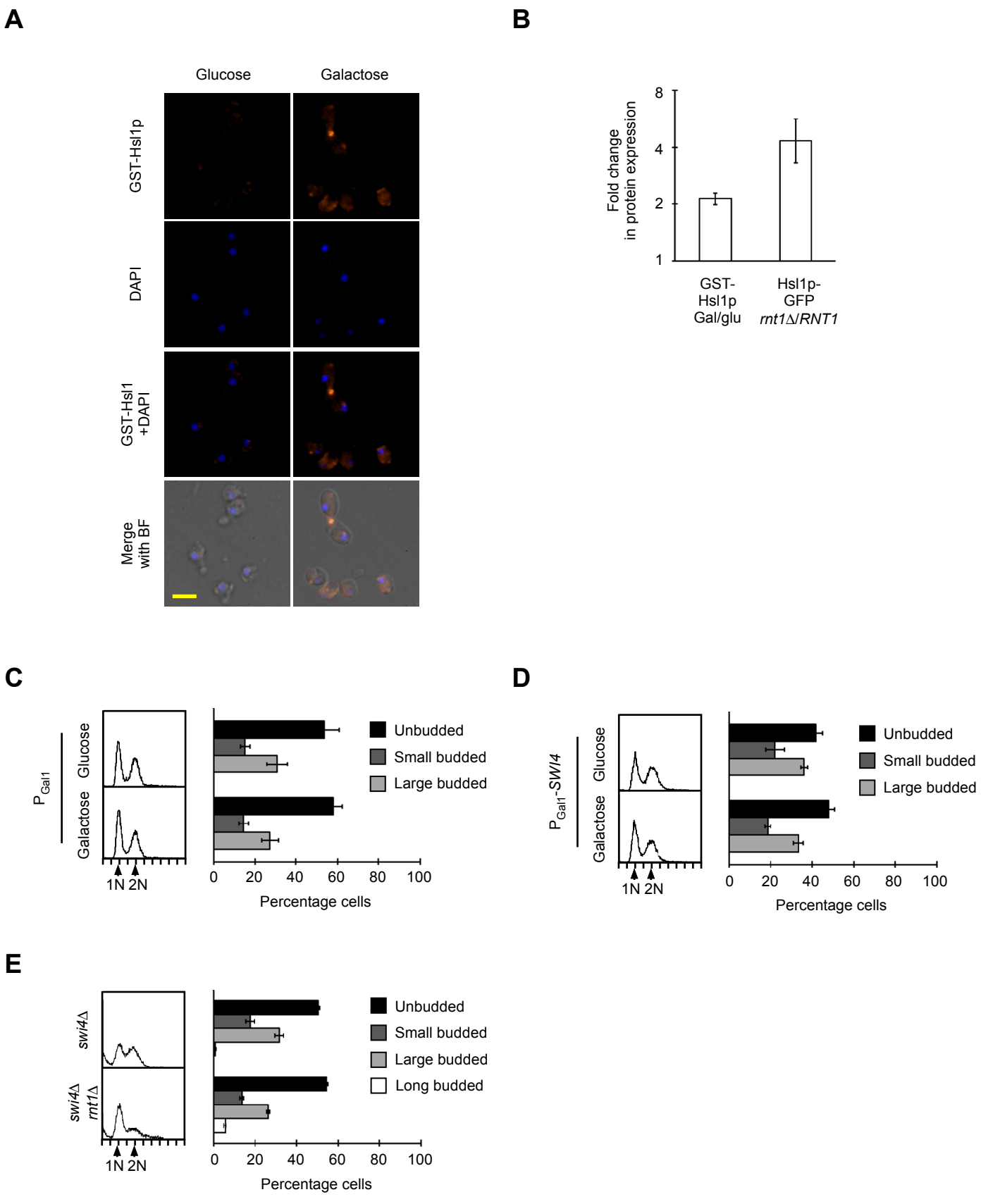

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Figure S3

Figure S3. (A) Hsl1p expressed from the Gal promoter was observed by immunofluorescence using antibodies against a GST tag fused in N-terminal in cells grown in glucose or after induction for 6 hours in galactose. The scale bar represents 5 μm. (B) The signal above background was measured by immunofluorescence for cells treated as in (A) and compared to the change in expression of Hsl1p-GFP in absence of *RNT1* as described in Figure 2. (C) *RNT1* cells expressing an empty vector were grown in glucose or galactose for 6 hours and analyzed using flow cytometry or light microscopy. The left panel shows DNA content analysis by flow cytometry with 1N and 2N peaks indicated. The bar graph on the right shows the distribution of cells according to the bud size and morphology. The data presented is an average of three independent experiments with error bars corresponding to SD. (D) *RNT1* cells expressing *SWI4* under the control of an inducible galactose promoter were grown under repressive (glucose) or inducible conditions (2% galactose for 6 hours) and analyzed as in C. (E) The effect of the deletion of *SWI4* in presence or absence of *RNT1* was assessed as in C for cells grown in YEPD.

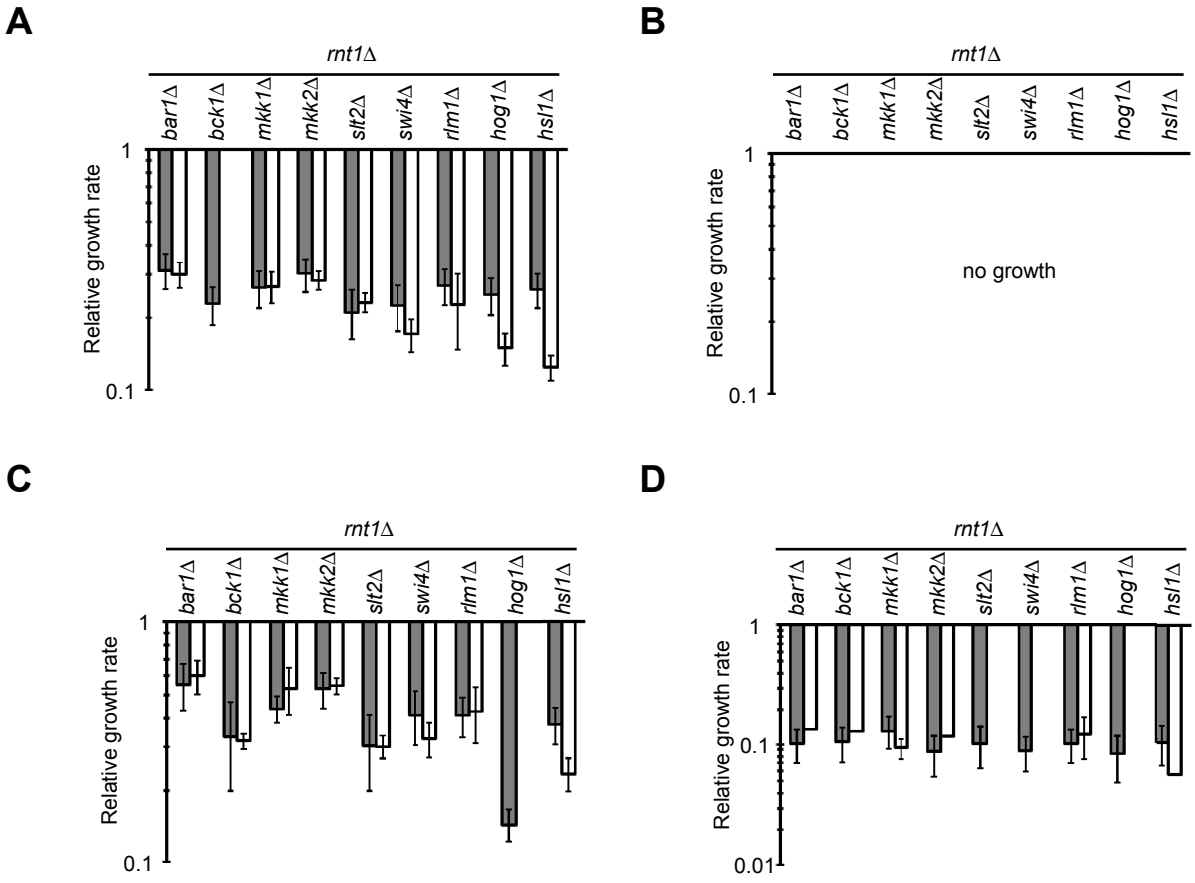

Figure S4. *RNT1* genetically interacts with cell wall stress response factors. *rnt1*Δ cells carrying deletion in 8 genes implicated in hyper and hypo-osmotic stress response or an unrelated gene (*BAR1*) were generated and assayed for growth in liquid media containing or not 1 M sorbitol at 26 or 37°C. The relative growth rates of each single mutants and double mutants were calculated with respect to the wild-type for each growth condition. The expected relative growth rate of the double mutants calculated from the product of the relative growth rates of the two single mutants are reported in the gray bars. The white bars are the actual double mutants relative growth rates. (A) YEPD 26°C. (B) YEPD 37°C. (C) YEPD + 1 M sorbitol 26°C. (D) YEPD + 1 M sorbitol 37°C.

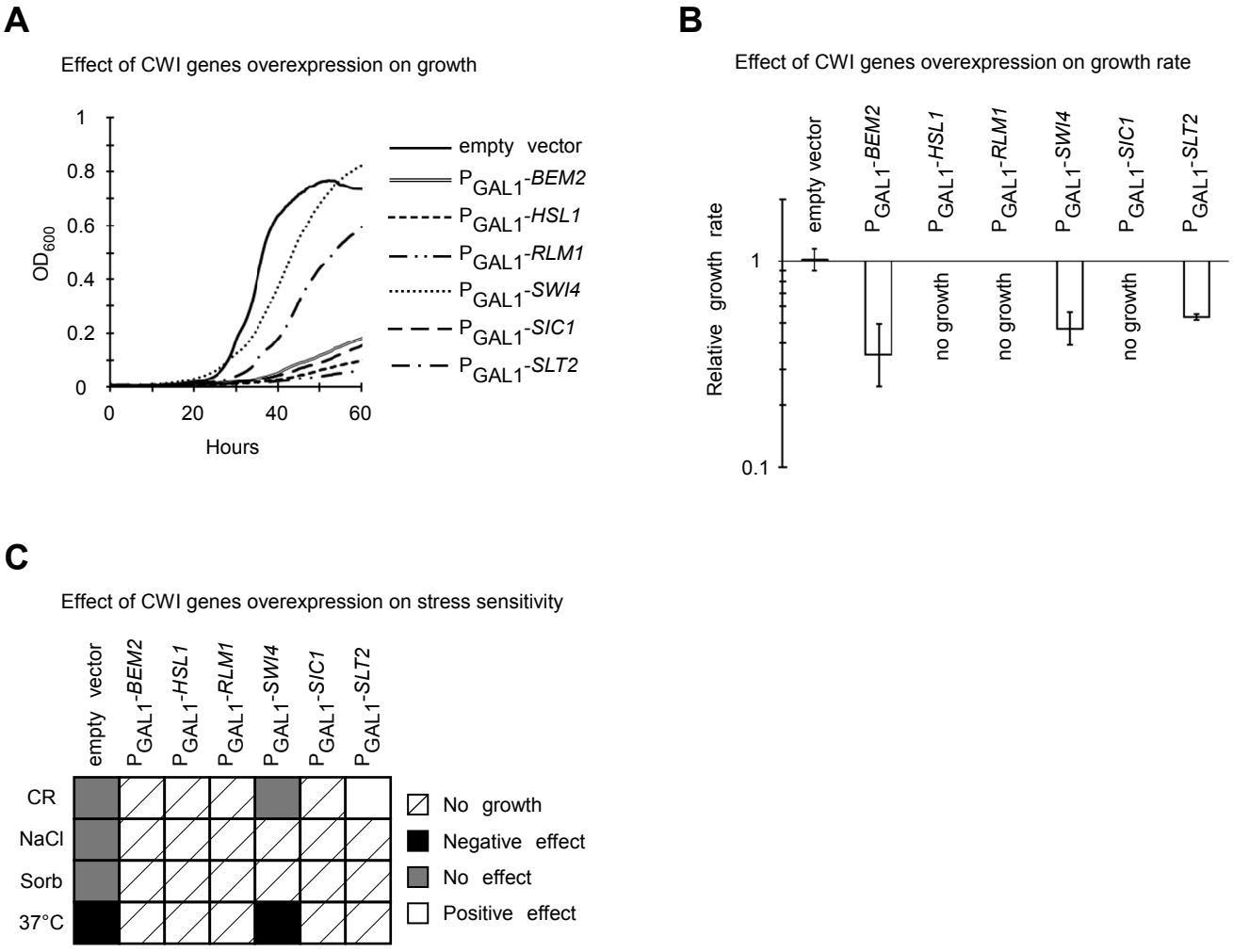

Figure 5. Overexpression of cell wall stress genes regulated by Rnt1p causes sensitivity to stress. Wild type cells transformed with plasmids expressing *BEM2*, *HSL1*, *RLM1*, *SWI4*, *SIC1* or *SLT2* under the control of a galactose inducible promoter were grown in synthetic media in presence of dextrose or galactose. (A) Growth curves under inducing conditions (galactose). (B) The effect of the overexpression on doubling time was calculated as relative growth rates for cells grown in galactose with respect to dextrose. Some growth rates that could not be calculated due to lack of growth in overexpression condition. (C) The effect of the overexpression of cell wall stress genes on stress response was assayed by comparing the growth in the presence of stress in inducing (galactose) and repressing (dextrose) conditions.

SUPPLEMENTARY METHODS

Immunofluorescence

GST-Hsl1p fusion protein was detected by immunofluorescence on yeast cells prepared as described in (20) using a rabbit anti-GST (Bethyl Laboratories, Montgomery, TX) at a dilution of 1:100 and a Texas-Red-X conjugated goat anti-rabbit antibody (Invitrogen Canada, Burlington, ON, Canada) at a dilution of 1:1000. Nuclei were stained with the DNA dye DAPI. Pictures were acquired on a Zeiss Axio Observer microscope (Carl Zeiss Canada) with a 100x/1.46 oil objective and analyzed using the Columbus software (Perkin Elmer) after image background correction.

SUPPLEMENTARY REFERENCES

57. Catala, M., Tremblay, M., Samson, E., Conconi, A., and Abou Elela, S. (2008) Deletion of Rnt1p alters the proportion of open versus closed rRNA gene repeats in yeast. *Mol. Cell. Biol.*, **28**, 619-629.

58. Johnson, A. (1997) Rat1p and Xrn1p are functionally interchangeable exoribonucleases that are restricted to and required in the nucleus and cytoplasm, respectively. *Mol. Cell. Biol*., **17**, 6122-6130.