García-Martínez *et al.*,

SUPPLEMENTARY INFORMATION

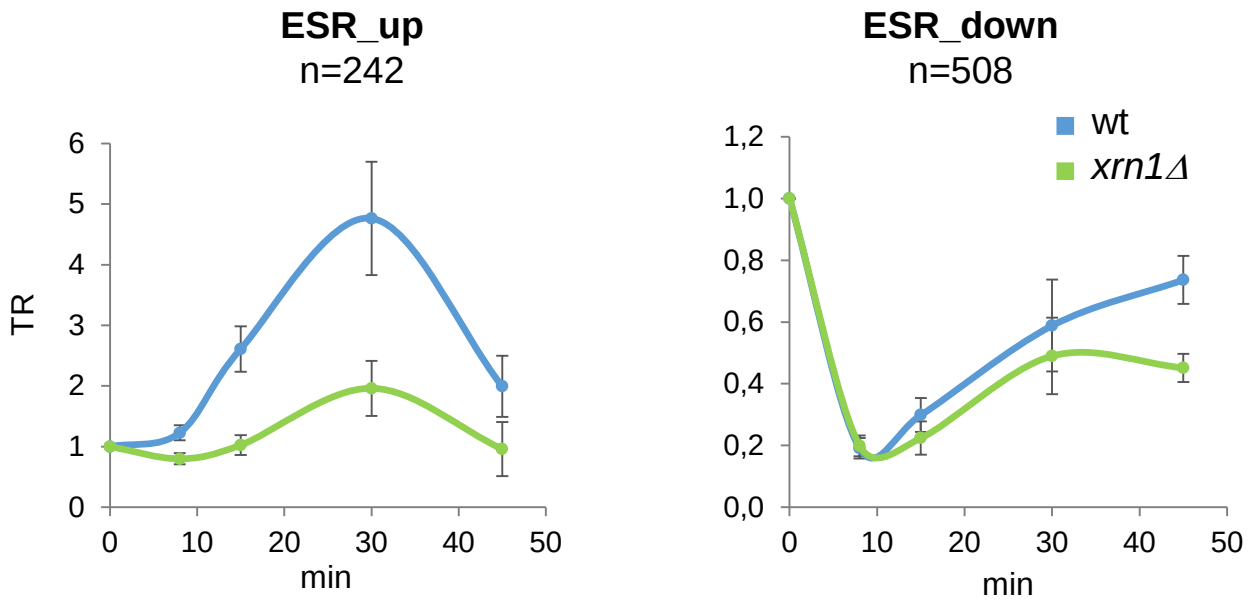
Table S1. Yeast strains used in this study.

Strain	Relevant Genotype	Source
BY4741	MATa *his3Δ1 leu2Δ met15Δ ura3Δ*	Euroscarf
xrn1Δ	BY4741 *xrn1Δ::KanMX4*	Euroscarf
Y836	BY4741 *xrn1Δ::(Ca)ura3Δ XRN1-WT*	A gift from M. Choder[1]
Xrn1-wt-FLAG	BY4741 *xrn1Δ::(Ca)ura3Δ::XRN1-FLAG*	A gift from M. Choder[1]
LMY5.2 (HA-TFIIS)	BY4741 *TRP1::pADH::3HA::TFIIS*	(33)
HA-TFIIS *xrn1Δ*	BY4741 *TRP1::pADH::3HA::TFIIS xrn1Δ::KanMX4*	(55)
SPT5-myc	BY4741 *SPT5-myc::HPH*	A gift from S. Chavez[2]
SPT5-myc *xrn1Δ*	BY4741 *SPT5-myc::HPH xrn1Δ:: KanMX4*	A gift from S. Chavez[2]

[1] Technion-Israel Institute of Technology, Israel
[2] Instituto de Biomedicina de Sevilla, Universidad de Sevilla, Spain

Table S2. List of primers used in this study.

Primer	Sequence	Use
STL1TATA-1	5´-TTTTGTCCCCTTCAACGCTG-3´	QPCR of *STL1* promoter
STL1-2	5´-TATGAGTGTGACTACTCCTG-3´	QPCR of *STL1* promoter
3-STL1-5b	5´-CCGGAAGAAGTTTGGAGGAA-3´	QPCR of *STL1* ORF
3-STL1-3c	5´-GGGCAAATGGTTAGCAACTC-3´	QPCR of *STL1* ORF
GRE3-7	5´-GGGGGCCTATCAAGTAAATT-3´	QPCR of *GRE3* promoter
5-GRE3-2	5´-GAAGGATTGCACTGACAACT-3´	QPCR of *GRE3* promoter
GRE3-1	5´-CAAAACCATCCAGGCAGTAC-3´	QPCR of *GRE3* ORF
GRE3-2	5´-CTCTCTGAGTTGCCCATCTA-3´	QPCR of *GRE3* ORF
GAL1-ORF3 F	5'-ATCCGGCATCGAACGGTTA-3'	QPCR of *GAL1* ORF
GAL1-ORF3 R	5'-TCAAGGATTGTGCGACATCG-3'	QPCR of *GAL1* ORF
M1	5´-TCGTTCCAATTTACGCTGGTT-3´	QPCR of *ACT1* ORF
M2	5´-CGGCCAAATCGATTCTCAA-3´	QPCR of *ACT1* ORF
18S-F	5'-CATGGCCGTTCTTAGTTG GT-3'	QPCR of rRNA 18S
18S-R	5'-ATTGCCTCAAACTTCCATCG-3'	QPCR of rRNA 18S
M11	5´-ACTTCCAGGTGGTAACAACGAA -3´	QPCR of *RPL30* ORF
M12	5´-AAATAGAGACAACACCGACTCTGAATAA -3´	QPCR of *RPL30* ORF



Supplementary Figure S1. The absence of Xrn1 reduces the transcriptional ESR during osmotic stress. Genomic data were obtained for wt and *xrn1Δ* treated with 0.6 M KCl for 0, 8, 15, 30 and 45 min as indicated in Figure 1. Graphical representation of the TR medians and standard deviation for ESR genes (as defined in (59)) upregulated (242 genes, left panel) and ESR downregulated (508 genes, right panel) in response to osmotic stress. All data were normalized to an arbitrary value of 1 at t=0.

Figure S1

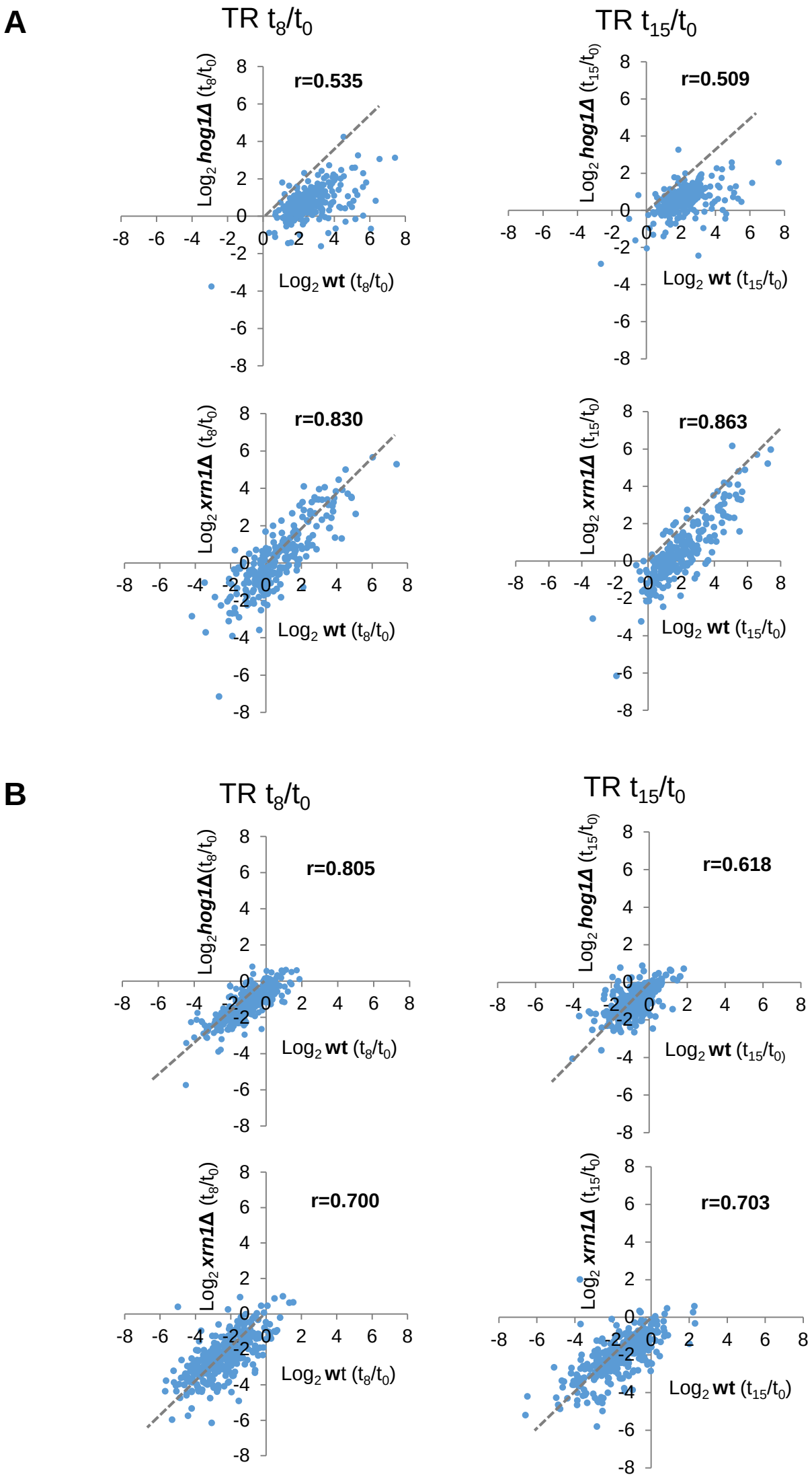
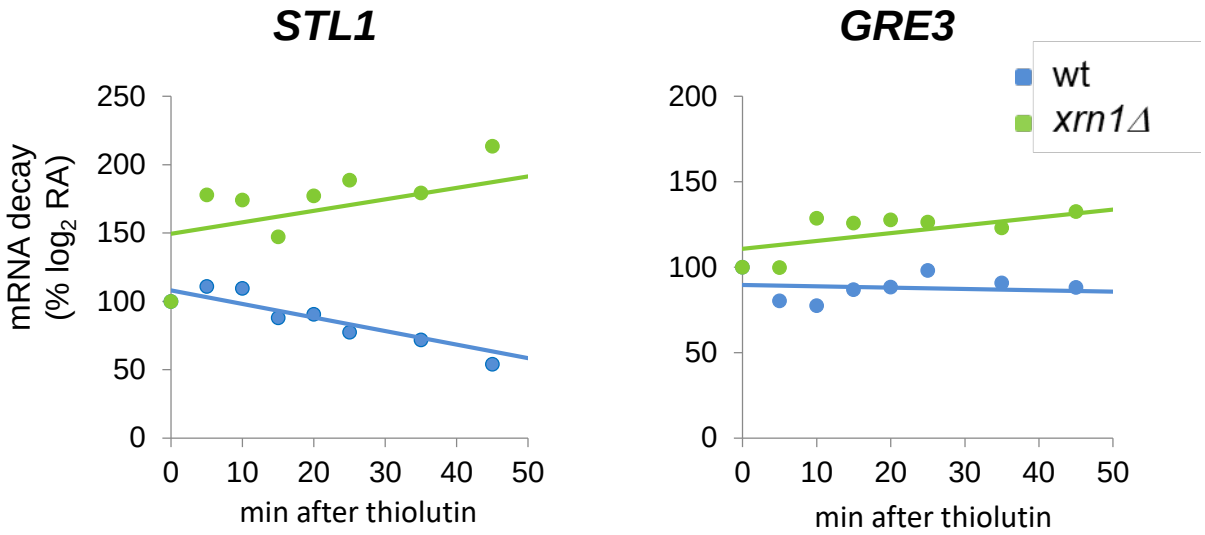

A
TR t_8/t_0
TR t_{15}/t_0
r=0.535
r=0.509
r=0.830
r=0.863
Log2 hog1Δ (t_8/t_0)
Log2 hog1Δ (t_{15}/t_0)
Log2 xrn1Δ (t_8/t_0)
Log2 xrn1Δ (t_{15}/t_0)
Log2 wt (t_8/t_0)
Log2 wt (t_{15}/t_0)
B
TR t_8/t_0
TR t_{15}/t_0
r=0.805
r=0.618
r=0.700
r=0.703
Log2hog1Δ(t_8/t_0)
Log2 hog1Δ (t_{15}/t_0)
Log2 xrn1Δ (t_8/t_0)
Log2 xrn1Δ (t_{15}/t_0)
Log2 wt (t_8/t_0)
Log2 wt (t_{15}/t_0)

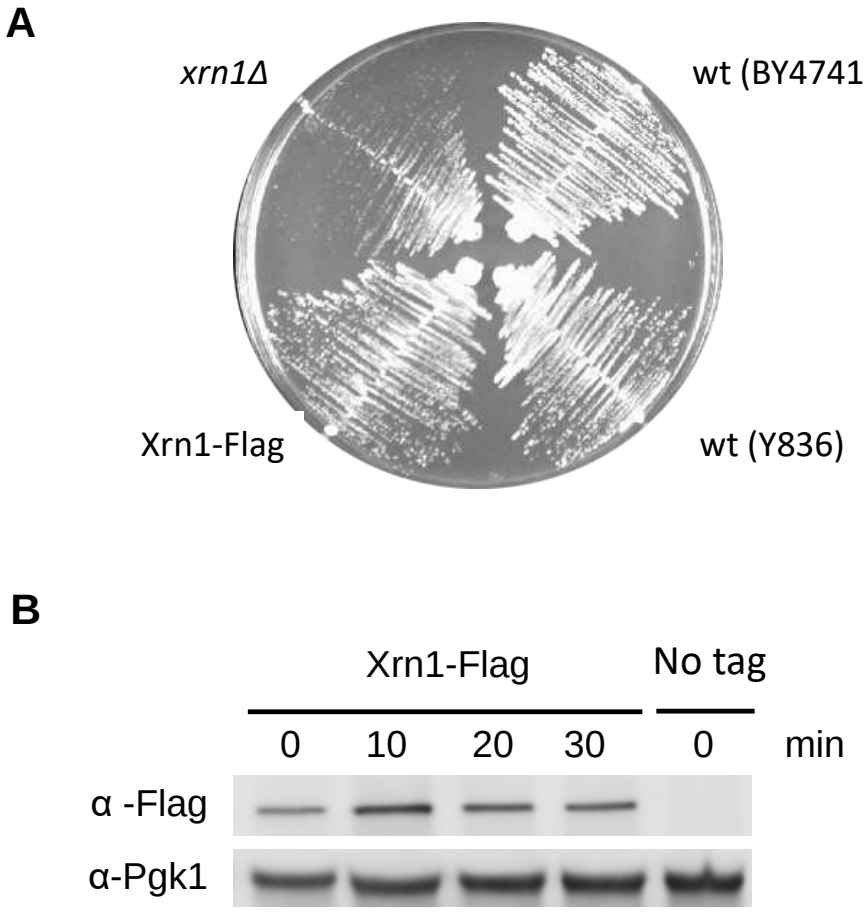
Figure S2

Supplementary Figure S2. Comparison of the transcriptional response to osmotic stress of *hog1* and *xrn1Δ* mutants *vs.* wt. (A, B) Genomic TR data obtained with *hog1* (8) (upper panels) and *xrn1Δ* (this work) (lower panels) mutants at 8 min (left panels) and 15 min (right panels) of mild osmotic stress was compared to the data obtained with the wild type in each experiment. The ratios of TR at t=8 or 15 min vs. t=0 (plotted in $\log_2$ scale) for each mutant were represented respect the same ratio for the wild type for the common set of upregulated genes (A) and downregulated genes (B) in both experiments ((8) and this work). The Pearson correlation coefficient (r) for each comparison is given.



Supplementary Figure S3. **Estimation of *STL1 and GRE3* mRNA stability upon osmotic stress in wt and *xrn1Δ* strains.** Cells were treated with 0.6 M KCl for 30 min and then 5 µg/mL of thiolutin was added to stop transcription and samples were taken at different times. Samples were analysed by RT-qPCR using specific primers for *STL1* and *GRE3* (see Supplementary Table S2) and normalized using the 18S RNA. Data are presented as the percentage of the $\log_2$ of the mRNA and it is represented respect to the initial (100%) over time. One representative experiment is shown, similar to the one in Figure 4C.

Figure S3



Supplementary Figure S4. **Functionality of Xrn1-Flag.** (A) Growth on YPD plate of *xrn1Δ* mutant and its corresponding wt strain (BY4741) and of Xrn1-Flag version and its corresponding wt strain (Y836) (see strains in Supplementary Table S1). The reduced growth of the *xrn1Δ* mutant strain but not of the Xrn1-Flag strain can be observed. (B) Expression of Xrn1-Flag protein under osmotic stress conditions analysed by western blot using anti-Flag antibodies. Anti-Pgk1 antibody was used as loading control. The samples were taken at different times of 0.6 M KCl treatment.

Figure S4