SUPPLEMENTARY INFORMATION

Figure S1 (A) *UBP4* (*DOA4*) and *UBP5* mutants do not impair gene activation upon osmostress. Wild type and the indicated mutant strains were grown to mid-log phase in rich medium and then subjected to osmotic stress (0.4 M NaCl) for the indicated length of time. Total RNA was assayed by Northern blot for transcript levels of *STL1*, *CTT1* and *RDN18* (as a loading control). **(B)** *UBP3* mutant does not render osmo-sensitive cells. Wild type and the indicated mutant strains were spotted on YPD plates with or without 1 M NaCl or 2 M sorbitol. Growth was scored after 4 days.

Figure S2 Binding of the Hog1 SAPK to the stress-responsive promoters is not affected in a *ubp3* strain. Wild type (filled bars) and *ubp3* (open bars) strains carrying HA-tagged Hog1 were grown to mid-log phase and subjected to osmostress (0.4 M NaCl) for the indicated length of time. Proteins were immunoprecipitated with anti-HA monoclonal antibodies and binding to the promoter regions of *STL1* and *CTT1* loci was analysed. The real-time PCR results are shown as the fold induction of treated relative to non-treated (time zero) samples normalized to a telomere internal control. Data represent the mean and standard deviation of three independent experiments.

Figure S3 Ubp3^{S695A} associates with osmo-dependent genes in response to stress. Cells expressing HA-tagged Ubp3 (filled bars) or Ubp3^{S695A} (open bars) proteins were subjected to osmostress (0.4 M NaCl) for the indicated length of time. Proteins were immunoprecipitated with anti-HA monoclonal antibodies and binding to the promoter (left-hand panels) and ORF (right-hand panels) regions of *STL1* and *CTT1* loci was analysed. The real-time PCR results are shown as the fold induction of treated relative

to non-treated (time zero) samples normalized to a telomere internal control. Data are the mean and standard deviation of three independent experiments.

Figure S4 Protein levels of wild type and mutated versions of Ubp3 are expressed equally in yeast cells. Ubp3-HA, Ubp3-11m-HA, Ubp3-12m-HA, Ubp3^{S695A}-HA and Ubp3^{C496A} -tagged proteins were expressed in a low-copy vector in *ubp3* cells. Samples were taken at mid-log phase and fixed in TCA. Protein precipitates were treated with alkaline phosphatase and HA-tagged proteins were separated by SDS-PAGE and detected by immunoblotting using monoclonal anti-HA (upper panel). The membrane was stained with Naftol blue as a loading control (lower panel).

Figure S5 Cells lacking the phosphorylated Hog1 site of Ubp3 are not sensitive to 6-AU. Wild type and *ubp3* cells transformed with pRS416 (to yield a URA^{+} phenotype required for 6-AU sensitivity tests) and with the indicated plasmids were spotted onto yeast minimal plates lacking uracil and with or without 100 µg/ml 6-AU. The culture was scored after 3 days.

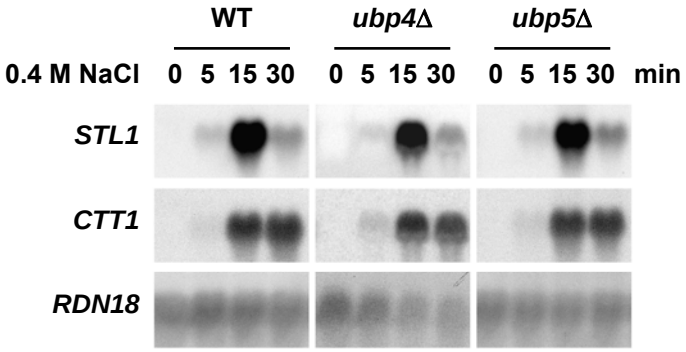
Figure S6 (A) Ubp3 is not required for *ADH1* mRNA production when expressed by the LexA-VP16 activator. *ubp3*Δ cells lacking endogenous *ADH1* and expressing an empty vector, a single copy of wild type Ubp3 or the unphosphorylatable mutant Ubp3^{S695A} were transformed with a vector carrying *ADH1* under the *LexA* promoter and with a plasmid containing the LexA binding domain fused to the VP16 transcriptional activator. Cells were treated with 0.4 M NaCl for the indicated length of time and transcript levels of *ADH1* and *RDN18* (as loading control) were measured. **(B)** The phosphorylation site of Ubp3 is required for proper RNA Pol II occupancy at *STL1*

open-reading frame in the *LexA-STL1* locus. *ubp3*Δ cells lacking endogenous *STL1* were transformed with a vector carrying *STL1* under the LexA promoter and with a plasmid containing the LexA binding domain fused to the VP16 transcriptional activator. This strain was transformed with an empty vector as a control, a wild type Ubp3 and the unphosphorylatable Ubp3^{S695A} proteins. Cells were subjected to osmostress (0.4 M NaCl, 10 minutes; open bars) or not (filled bars) and binding of Rpb1 to the *STL1* ORF region of the *LexA-STL1* construct was analysed by ChIP using anti-Rpb1 antibody (8WG16, Covance). The real-time PCR results are shown as the fold induction of treated relative to non-treated (time zero) samples normalized to a telomere internal control. Data are the mean and standard deviation of three independent experiments.

Figure S7 (A) Hog1 directly associates with Ubp3 *in vitro*. GST, GST-Ubp3 or His-Hog1 were expressed and purified from *E. coli*. GST proteins were incubated with His-Hog1 bound to beads and interacting proteins were probed using GST antibody (bound). Input represents 10% of total purified proteins. **(B)** *In vivo* binding of RNA Pol II and Ubp3 is not affected by osmostress or Hog1. Ubp3-HA-tagged protein was expressed from the wild type locus in wild type and *hog1* strains. Samples were taken before (–) or 10 min after (+) the addition of 0.4 M NaCl. The largest subunit of RNA Pol II (Rpb1) was immunoprecipitated with commercial anti-Rpb1 antibody (bottom) and the presence of Ubp3-HA was probed by immunoblotting using anti-HA antibodies (top). Total extract represents 50% of total input protein (middle).

Supplemental Figure 1
Solé *et al.*

A



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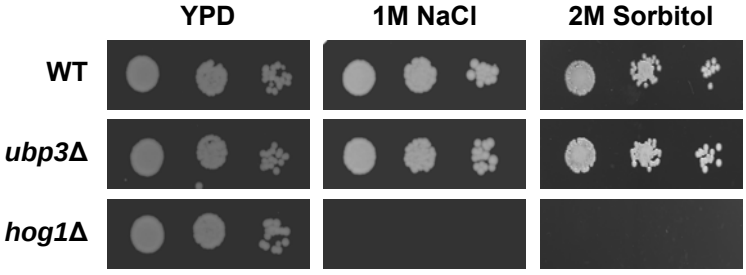
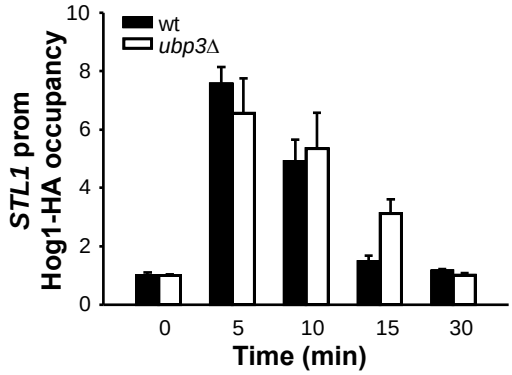
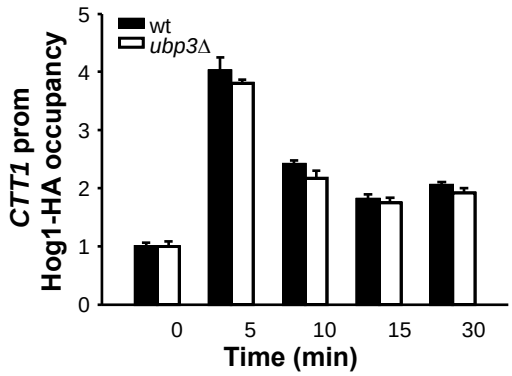
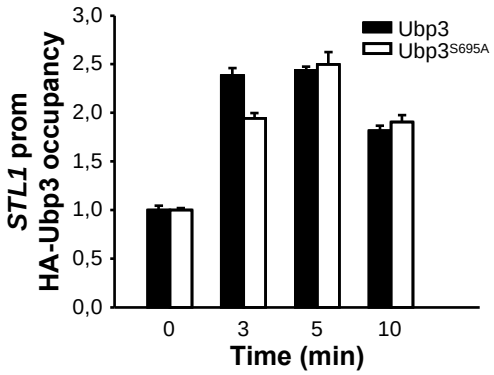


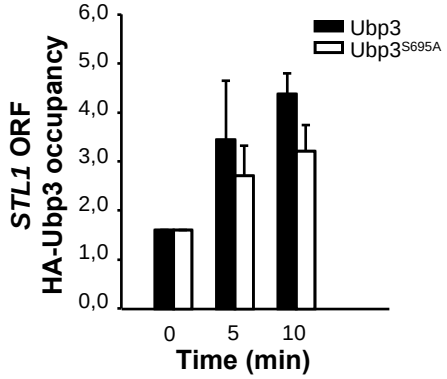
Supplemental Figure 2
Solé *et al.*

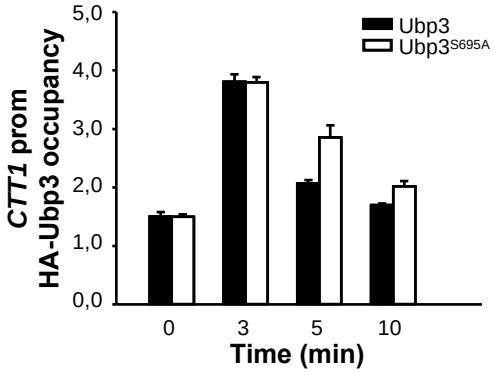


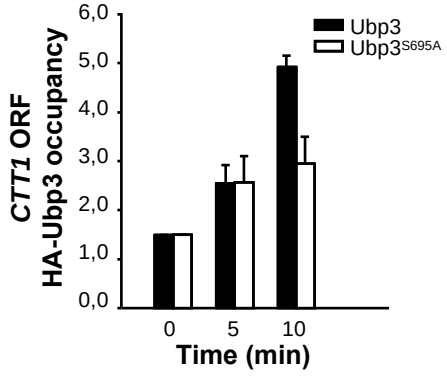


Supplemental Figure 3
Solé *et al.*

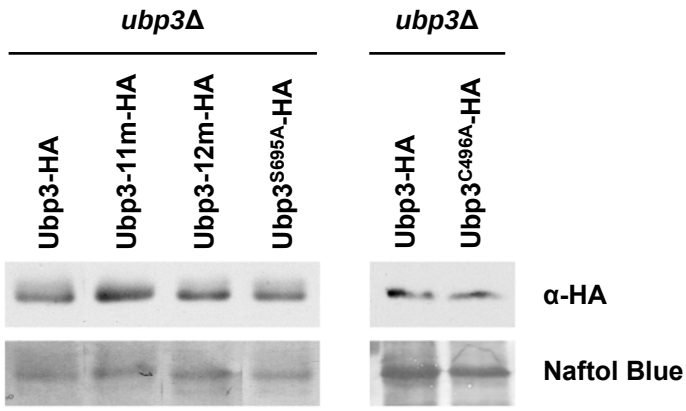




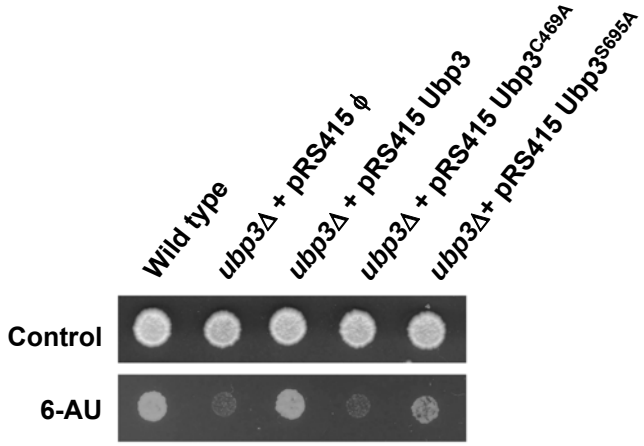




Supplemental Figure 4
Solé *et al.*

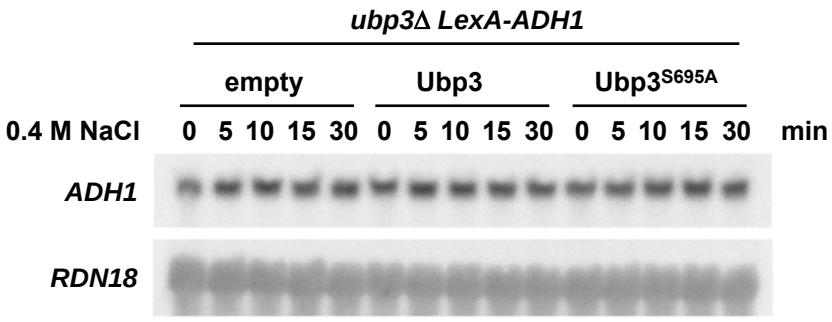


Supplemental Figure 5
Solé *et al.*



Supplemental Figure 6
Solé *et al.*

A



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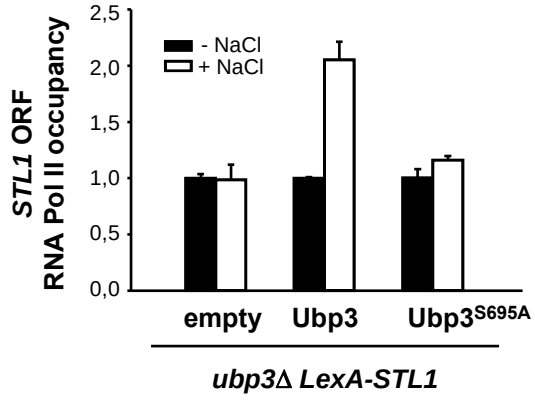

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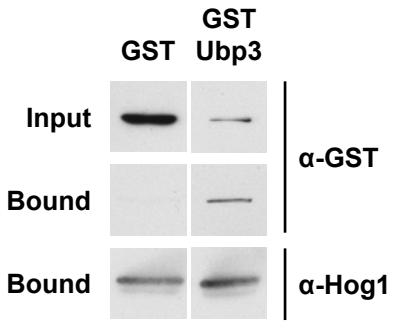
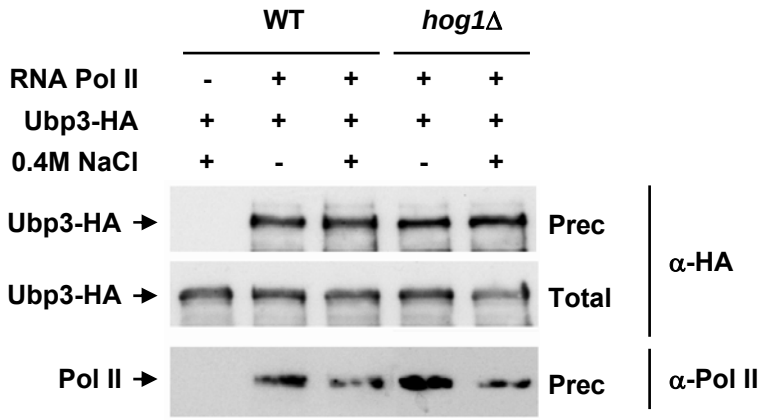

GST
GST Ubp3
Input
Bound
α-GST
Bound
α-Hog1

B


WT
hog1Δ
RNA Pol II - + + + +
Ubp3-HA + + + + +
0.4M NaCl + - + - +
Ubp3-HA
Prec
Ubp3-HA
Total
α-HA
Pol II
Prec
α-Pol II