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Supplemental Data

The Stress-Activated Hog1 Kinase Is a Selective Transcriptional Elongation Factor for Genes Responding to Osmotic Stress

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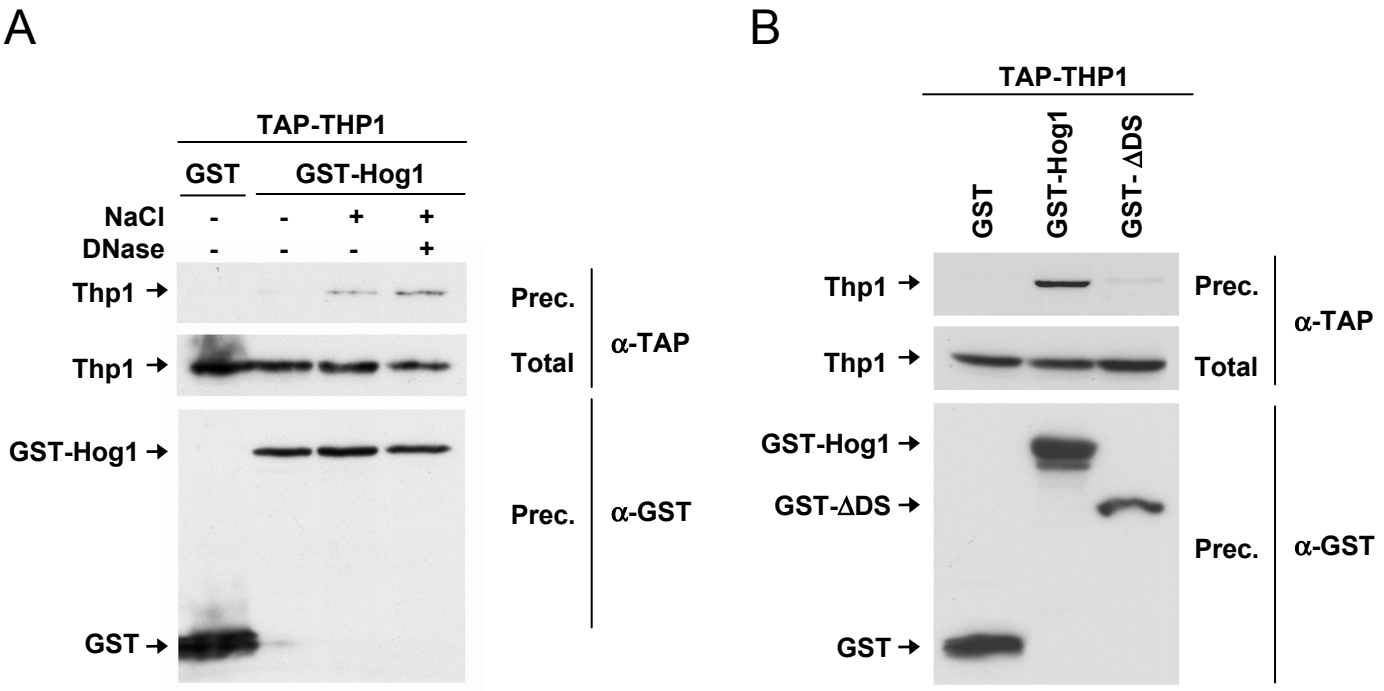

Figure S1. The interaction between Hog1 and the elongating polymerase complex is dependent on osmotic stress. (A) TAP-tagged Thp1 strain expressing GST or GST-Hog1 proteins was either treated to a brief osmotic shock (10 min, 0.4M NaCl) or untreated. GST proteins were pulled down by glutathione-Sepharose 4B in the presence of DNAse I (+) or not (-). The presence of TAP proteins was probed by immunoblotting using anti-TAP (upper panel). Total extract represents <20% of total input protein (middle panel). The amount of precipitated GST proteins was detected using anti-GST (lower panel). (B) Co-precipitation experiments were carried as in (A) with a full length Hog1 or a truncated Hog1 kinase that lacks the C-terminal region (residues 265-435). Western blots were performed as in (A).

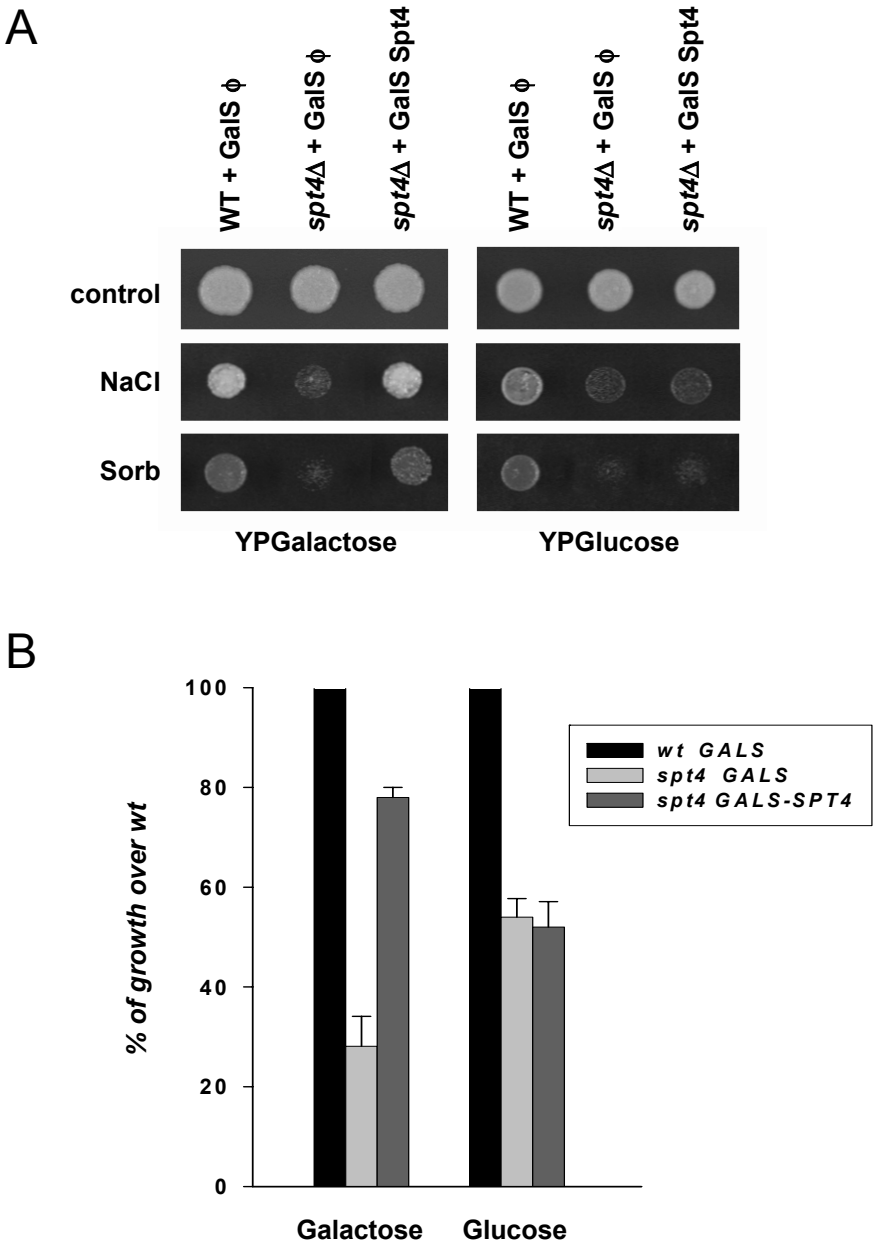

Figure S2. Spt4 is required for efficient cell survival upon stress. (A) Wild type and *spt4* mutant strains were transformed with a vector expressing *SPT4* under the control of a *GAL1* promoter and spotted on YP galactose plates with 0.8 M NaCl or 2M Sorbitol and YP glucose plates with 1.2M NaCl or 2.2M Sorbitol. Growth was scored after 4 days. (B) Cells as in (A) were grown in liquid media in the presence of galactose and then shifted to galactose o glucose in the presence of 0.8 M NaCl. Growth was monitored after 16h and data is given as % of growth over wild type (mean of four independent measurements).

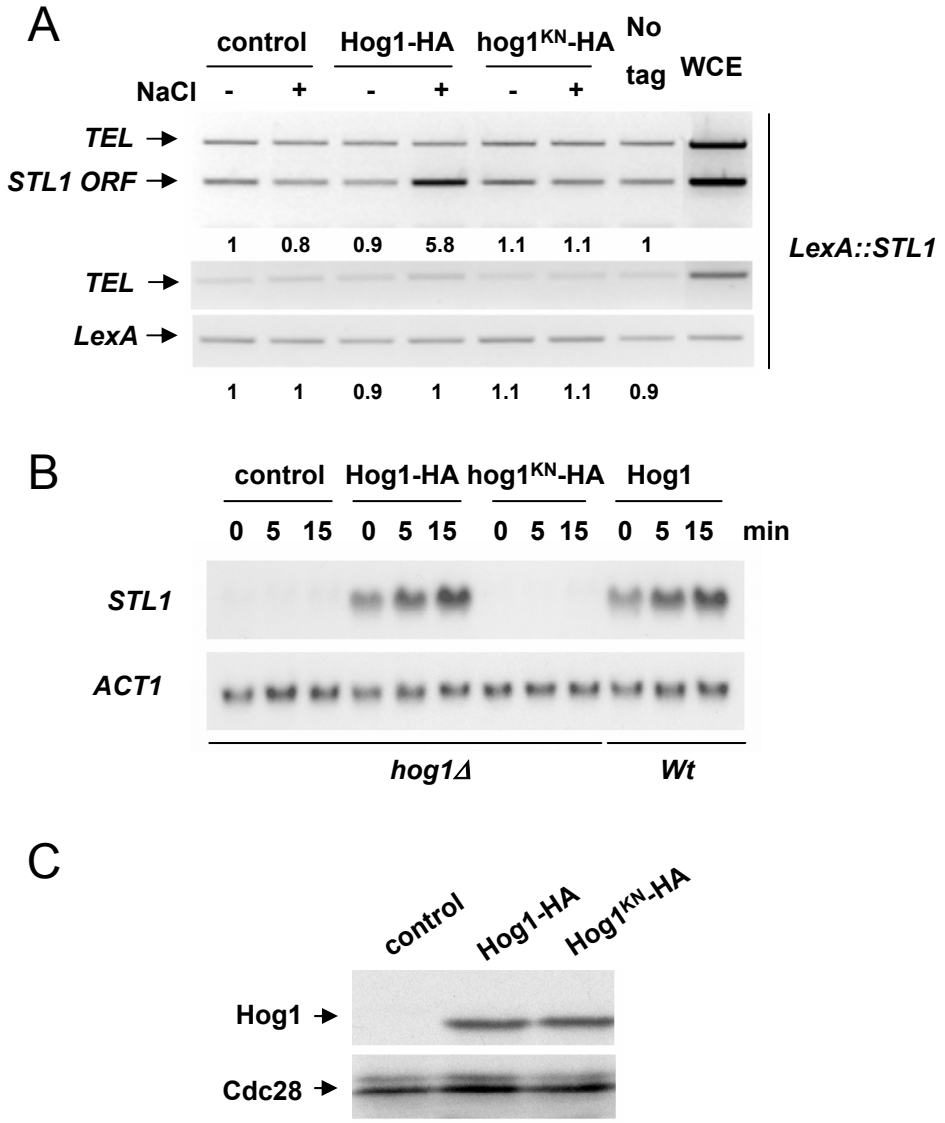

Figure S3. Hog1 catalytic activity is required for the role of Hog1 in elongation. (**A)** Catalytic activity is required for Hog1 recruitment to *STL1*. Binding of wild type Hog1 (Hog1-HA) or the catalytically inactive hog1 (hog1^{KN}-HA) to the *LexA* promoter or *STL1* ORF regions of the *LexA-STL1* construct was analysed by ChIP. *hog1* cells (also *stl1Δ*) containing the *LexA-STL1* construct and the LexA-Vp16 were transformed with empty control plasmid or plasmids containing HA tag Hog1 or the catalytically inactive Hog1 and ChIPs were preformed before or after (+) osmostress. BY4741 strain was used as a control (no tag). Quantification is depicted as fold binding over *TEL1*.
(**B)** Accumulation of *STL1* mRNA depends on Hog1 catalytic activity. Cells as in A or wild type cells carrying the *LexA-STL1* construct together with the LexA-VP16 or empty plasmid (control) were subjected to osmostress and mRNA analysed by northern blot. **(C)** the catalytic inactive hog1^{KN} is expressed similarly to wild type Hog1. Yeast extracts containing an empty vector or vectors expressing HA-tagged wild type Hog1 or catalytically inactive hog1^{KN} were probed using anti-HA and anti-Cdc28 (pSTAIRE) antibodies (for normalization).

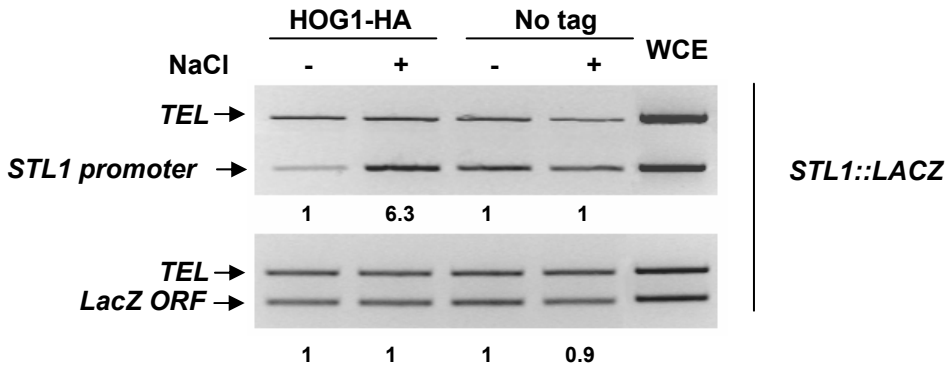

Figure S4. Hog1 is recruited to the promoter of *STL1::LacZ* but not to the LacZ ORF in response to osmostress. Binding of Hog1-HA to the *STL1* promoter or *LacZ* ORF regions of the *STL1::LacZ* construct was analysed by ChIP in wild type cells (*stl1Δ*) subjected (+) or not (-) to osmostress. BY4741 was used as a control (No tag). Quantification is depicted as fold binding over *TEL1* in absence (-) or presence of stress (+).

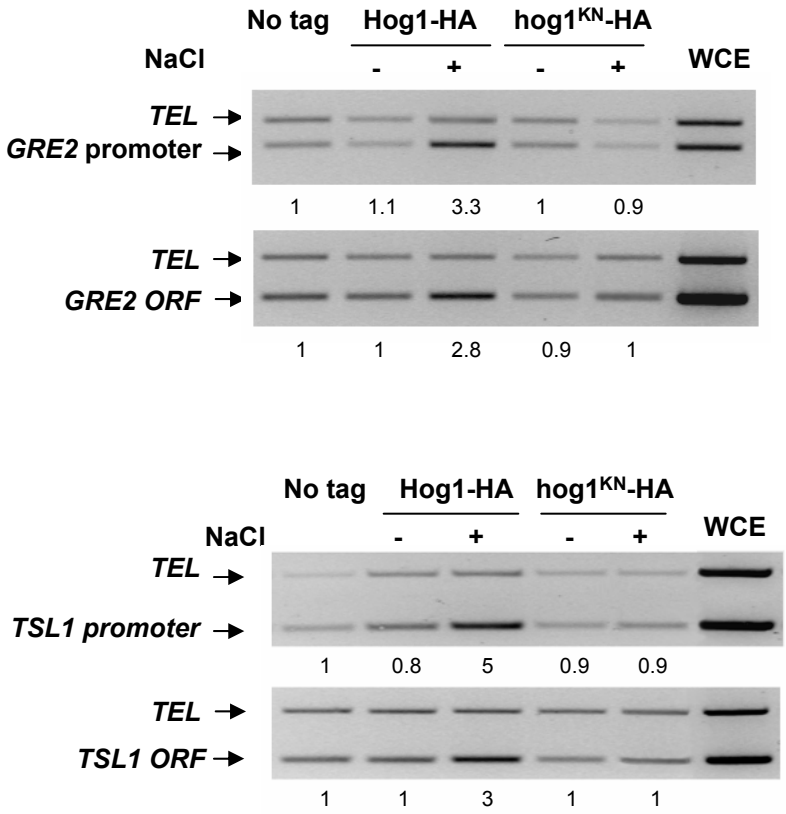

Figure S5. The catalytically inactive Hog1 (*hog1^{KN}*) does not bind to the ORF or promoters of osmoresponsive genes. Binding of wild type Hog1 (Hog1-HA) or the catalytically inactive hog1 (hog1^{KN}-HA) to the *GRE2 and TSL1* promoter and ORF regions was analysed by ChIP. *hog1* cells (also *stl1Δ*) were transformed with empty control plasmid or plasmids containing HA tag Hog1 or the catalytically inactive hog1^{KN} and ChIPs were preformed before or after (+) osmostress. BY4741 strain was used as a control (no tag). Quantification is depicted as fold binding over *TEL1*.