Fig S1 | Expression of osmo-responsive genes remains mostly unchanged upon other environmental stresses. Total osmo-responsive genes (662) were compared to available expression arrays with different environmental stresses (Gasch et al., 2000) such as (A) heat stress (15 minutes at 37ºC), (B) oxidative stress (320 mM H₂O₂ for 30 minutes), (C) DTT (250 mM for 60 minutes) and (D) aminoacid starvation (30 minutes). Genes were determined to be induced (FC>2), repressed (FC<-2) or unchanged if fold change remained within the induced/repressed threshold upon stress. These genes are shown in red, green and grey respectively together with the percentage from the total genes considered.
Fig S2 | Hog1-dependent genes have higher expression than Hog1-independent genes upon osmostress. Comparison of average fold change upon stress of Hog1-dependent (black) and independent (white).
Fig S3 | MA Plots representing RNA Pol II in a subset of 100 genes (blue) whose expression is downregulated upon more than two fold upon osmostress and a group of 100 genes (yellow) whose fold change upon stress remained unchanged (FC 1 to 1.1) Left panels show wild type RNA Pol II recruitment while right panels represent Hog1-dependent recruitment of RNA Pol II.
**Fig S4** | **A.** Classification of Hog1 recruited genes or regions depending on their annotation in the SGD as RNA Pol II, RNA Pol III or Long Terminal Repeats (LTR). **B.** MA Plots representing Hog1 binding at the different sets of genes. A group of 100 Hog1-dependent genes are shown in green while 100 Hog1-independent genes are shown in red. In blue and yellow, sets of 100 genes that are downregulated and whose expression remains constant respectively as in Figure S3.
Fig S5 | Binding profile of Hog1 (A) and RNA Pol II (B) to CTT1 osmo-responsive gene. Binding of Hog1 and RNA Pol II (Rpb1) validated by chromatin immunoprecipitation as in Figure 3D at the indicated regions of CTT1 and times upon osmostress (0.4 M NaCl).
**Fig S6** | Genome-wide distribution of Hog1. Venn diagram showing the number of genes in which the ORF and/or promoter (500 bp upstream of ATG) regions were occupied by Hog1 (z-score > 4).
**Fig S7** | Association of RNA PolIII to tRNAs upon stress depends on HOG1. Association of specific subunit of RNA Pol III (Rpc82-myc) was assessed by ChIP upon osmostress (0.4M NaCl) at the indicated times and tRNA loci. Conventional PCR was performed and results are shown as fold induction of treated versus non-treated (time 0) and normalized against internal loading control (*TEL1*).
**Fig S8** | Kinetics of dose response (0.1M, 0.2M and 0.4M NaCl) expression of different osmostress genes (*STL1*, *CTT1* and *ALD3*). Total RNA was extracted at the indicated times and concentrations. Expression of osmo-responsive genes was probed and normalized with *ENO1* as loading control.
Fig S9 | Nucleosome occupancy maps determined by MNase-Seq genome-wide (A) and for Hog1-dependent genes (described in material and methods) in a wild type and hog1 strains (B). Coverage values of each position in the genome has been normalized, converted to reads per million (rpm) and represented in logarithmic scale. Genes (rows) are aligned by their TSS and sorted to maximize the correlation in 0 to 100 bp region. Blue, black, yellow and red indicate a low, medium, and high read density.