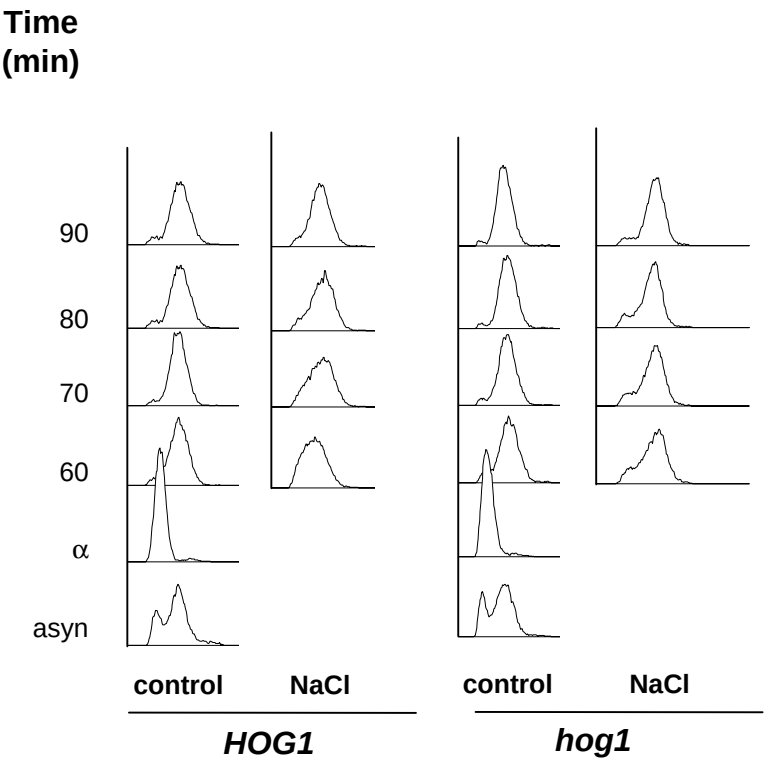
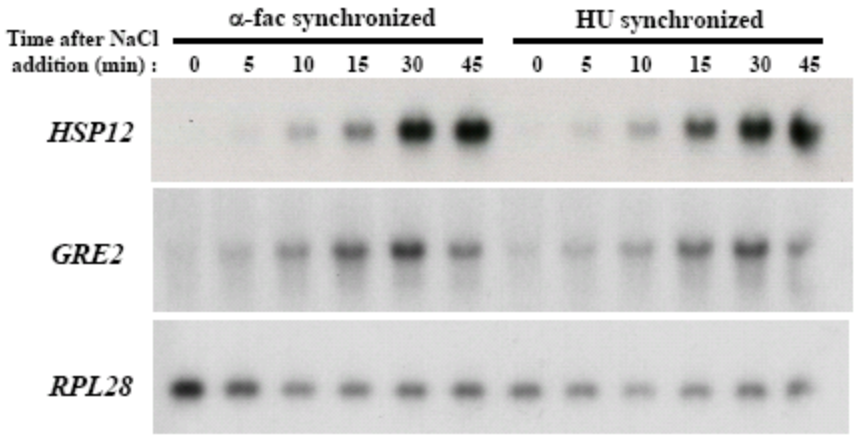


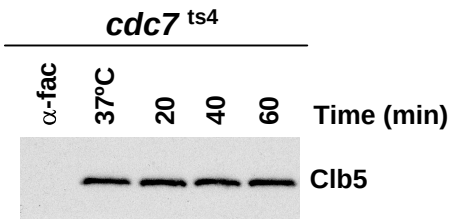
S1. Hog1 induces an S-phase delay. Exponentially growing wild type (*HOG1*) or *hog1* deficient cells (*hog1*) (asyn) were synchronized at G_1 phase with α-factor for 3 hours at 25ºC (α-fac), washed and released into fresh YPD. Cells were subjected to 0.4M NaCl 30 minutes after release (NaCl) or kept in YPD (control). Samples were taken at the indicated points after the treatment and assessed for total DNA content by FACS analysis.

Supp Fig2
Yaakov *et al*.



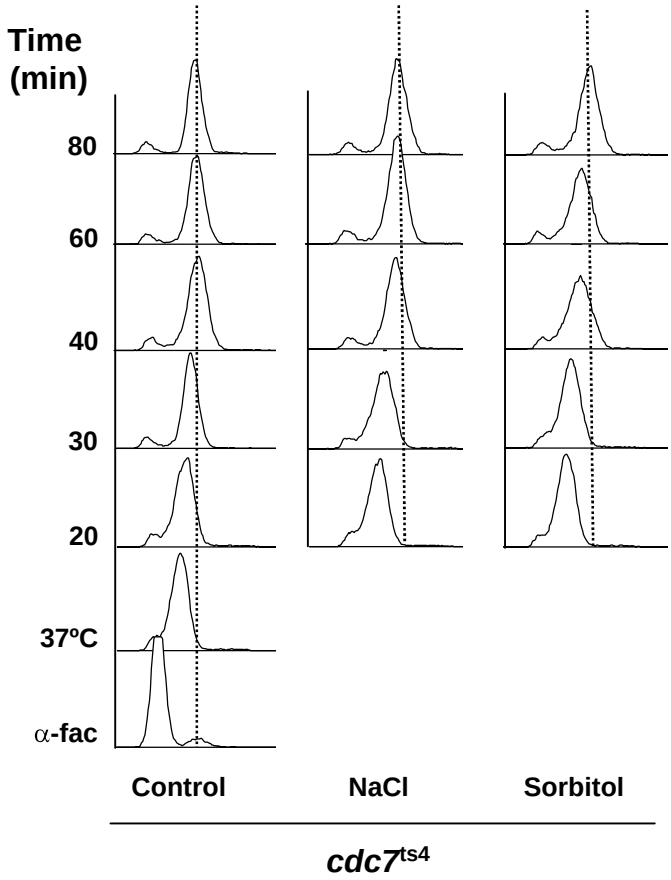
S2. Hog1 target genes are equally induced in S-phase arrested cells. Exponential cultures of the BY4741strain were synchronized with either α-factor for 3 hours or with HU for 2 hours and stressed with 0.4M NaCl. Samples were taken at the indicated times, RNA was extracted and Northerblot analysis was performed with probes for the *HSP12*, *GRE2* and *RPL28* (as a loading control) mRNA species.

Supp Fig3
Yaakov *et al*.



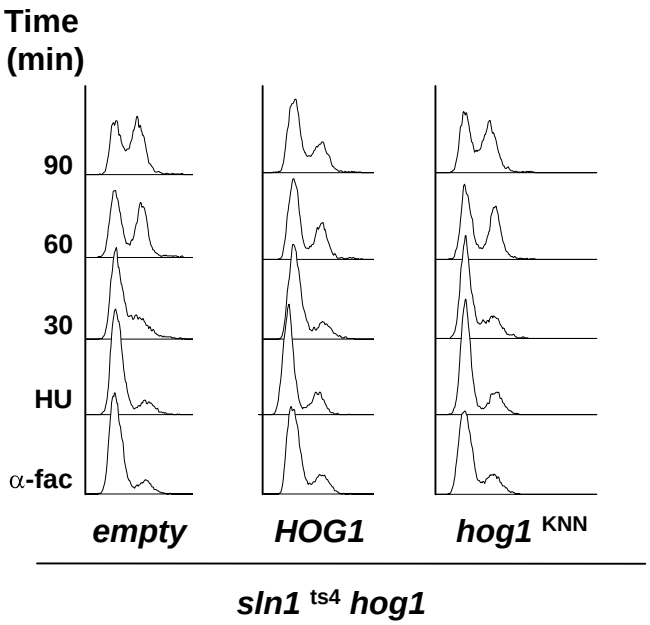
S3. *cdc7^{ts4}* cells display high levels of Clb5 at non-permissive temperature. *cdc7*^{ts4} cells that contained TAP-tagged Clb5 at its endogenous locus were synchronized with α-factor for 3 hours, washed and released into fresh medium at 37ºC. After 1 hour incubation at 37ºC, cells were released at 25ºC to let the cells progress through the cell cycle. Samples were analyzed by SDS-PAGE, blotted and probed with α-TAP antibodies to detect Clb5.

Supp Fig4
Yaakov *et al*.



S4. Cells delay S phase when osmostressed with Sorbitol or NaCl. An exponential culture of *cdc7*^{ts4} cells grown at 25ºC was incubated with α-factor for 3 hours (α-fac), washed and released into S-phase at 37ºC for 1 hour (37ºC) to synchronize the cells in S-phase. The culture was then shifted back to 25ºC and left untreated (control) or subjected to 0.4 M NaCl (NaCl) or 0.8 M Sorbitol (Sorbitol).

Supp Fig5
Yaakov *et al*.



S5. Hog1 induced S-phase arrest depends on its catalytic activity. A *sln1*^{ts4} *hog1* strain carrying an empty centromeric plasmid (empty) or bearing either the wild type *HOG1* (*HOG1*) or a catalytically inactive form of *hog1* (*hog1*^{KNN}) were grown at the permissive temperature of 25ºC, synchronized with α-fac for 3hours, washed and released into HU containing medium for 1h to synchronize the cells in S-phase. Upon release from HU, the cells were shifted to 37ºC to activate the HOG pathway. Samples were taken and processed by FACS at the given times .