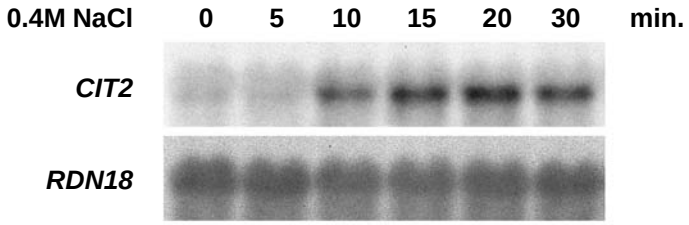
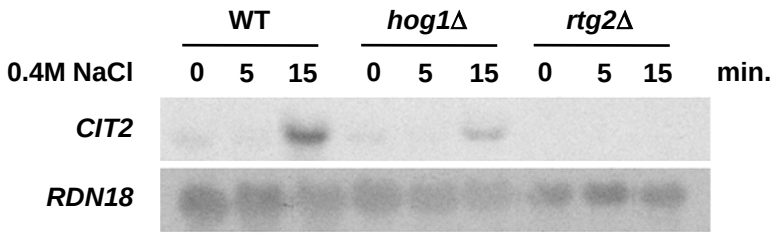


Figure S1. Activation on RTG pathway by osmostress is observed independently of the presence of excess glutamine. Wilde type strain was grown to mid-log phase in YPD medium and subjected or not to 0.4M NaCl for the indicated times. Total RNA was assayed by northern blot for transcript levels of *CIT2* and *RDN18* (as a loading control).

A



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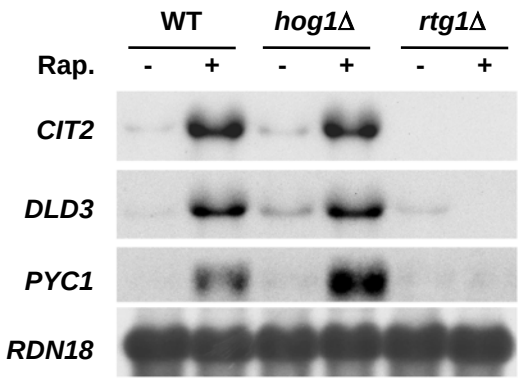

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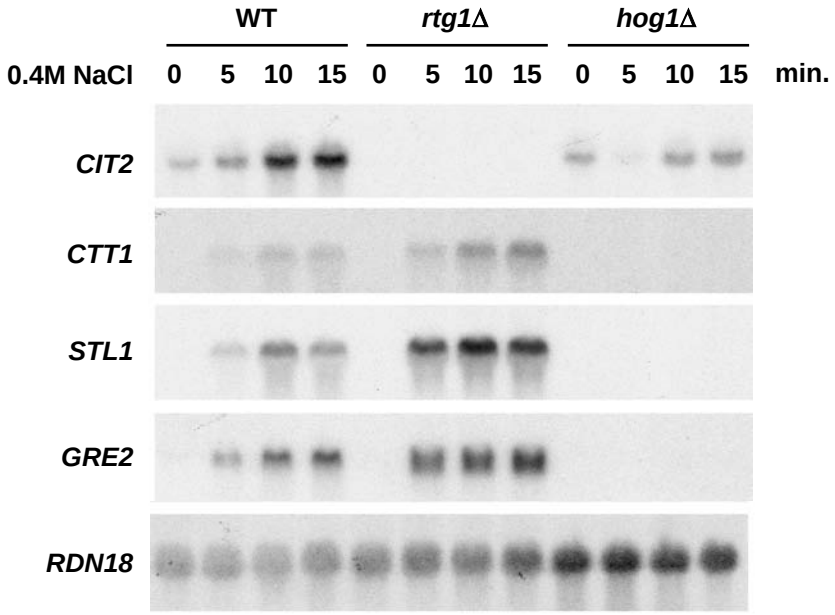
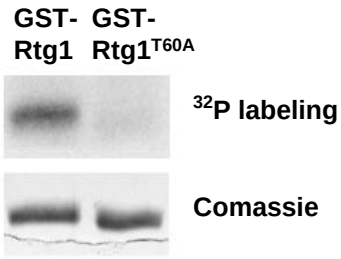


Figure S2. RTG-dependent gene expression in osmostress is controlled by Hog1. **(A)** The integrity of the RTG pathway is required for gene expression upon osmostress. Wild-type and *RTG2* mutant cells were grown in MD-Gln to logarithmic phase and subjected to osmotic shock (0.4M NaCl) for the indicated times. Total RNA was assayed by northern blot analysis for *CIT2* and *RDN18* as a loading control. **(B)** Hog1 is not required for induction of RTG-dependent genes under rapamycin treatment. The indicated strains were grown as in (A) and treated or not with 1μg/ml of rapamycin (Rap) for 15 minutes. Total RNA was assayed as in (A). **(C)** Rtg1 transcription factor is required specifically for induction of RTG-dependent genes in osmostress. The indicated strains were grown and treated as in (A).

A



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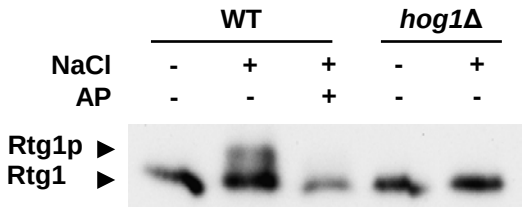

C

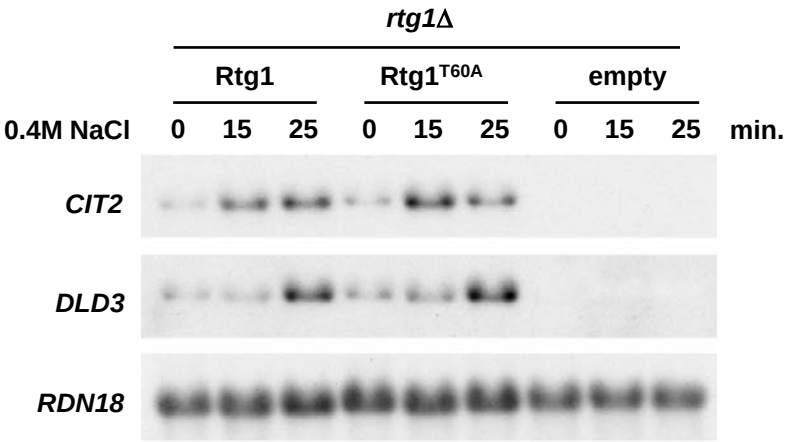

Figure S3. Phosphorylation of Rtg1 by Hog1 does not alter RTG-mediated gene expression. **(A)** Hog1 phosphorylates *in vitro* Rtg1 at Thr60. Full length Rtg1 and Rtg1^{T60A} were purified from *E. coli* and tested for their ability to be phosphorylated by an *in vitro*-activated Hog1. Phosphorylated proteins were resolved by SDS-PAGE and detected by autoradiography (upper panel). GST-tagged Rtg1 proteins were detected by comassie staining (lower panel). **(B)** Rtg1 is phosphorylated upon osmostress in a Hog1-dependent manner. Wild-type and *hog1*Δ cells expressing Rtg1-HA were grown to 0.6-1 OD_{660}. Samples were taken before (-) or 5 min after (+) the addition of NaCl to a final concentration of 0.4M, and the extracts were treated (+) or not (-) with 10U of alkaline phosphatase (AP). Rtg1-HA was detected by immunoblotting using anti-HA monoclonal antibody. **(C)** Rtg1 phosphorylation by Hog1 is not essential for activation of gene expression upon osmostress. The *rtg1*Δ strain carrying plasmids expressing wild-type Rtg1 or the indicated mutants were grown to mid-log phase and subjected to osmotic stress (0.4M NaCl) for the indicated times. Total RNA was assayed by northern blot for transcript levels of *CIT2*, *DLD3* and *RDN18* (as a loading control).

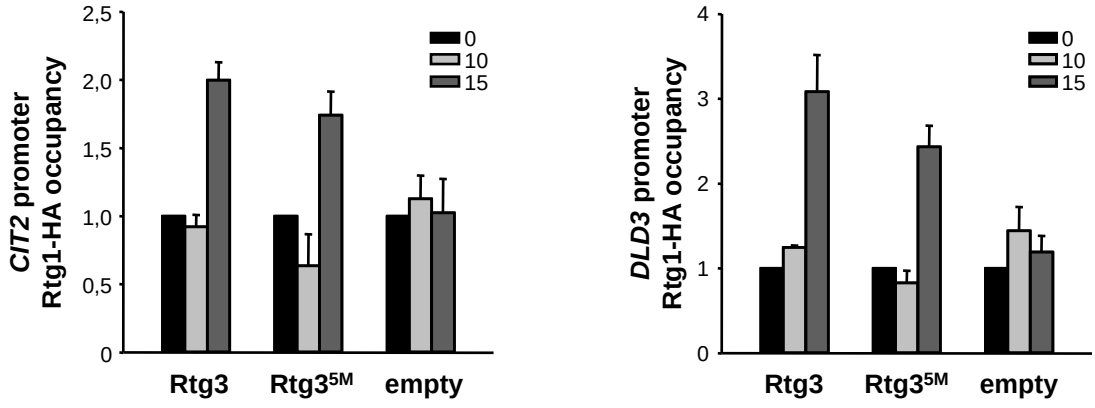

Figure S4. Hog1 phosphorylation sites in Rtg3 are not essential for Rtg1/3 chromatin binding. *rtg3*Δ strain with HA-tagged Rtg1 was transformed with empty plasmid or plasmids expressing wild-type Rtg3 or the Rtg3 non-phosphorylatable mutant ($Rtg3^{5M}$), grown to mid-log phase and subjected to osmotic stress (0.4M NaCl) for the indicated length of time. Tagged Rtg1 was immunoprecipitated with anti-HA monoclonal antibody and binding to the promoter regions of *CIT2* and *DLD3* loci was analysed by PCR. Results are shown as the fold induction of treated relative to non-treated (time zero) samples normalized to *TEL2*. Data are the mean and standard deviation of three independent experiments.

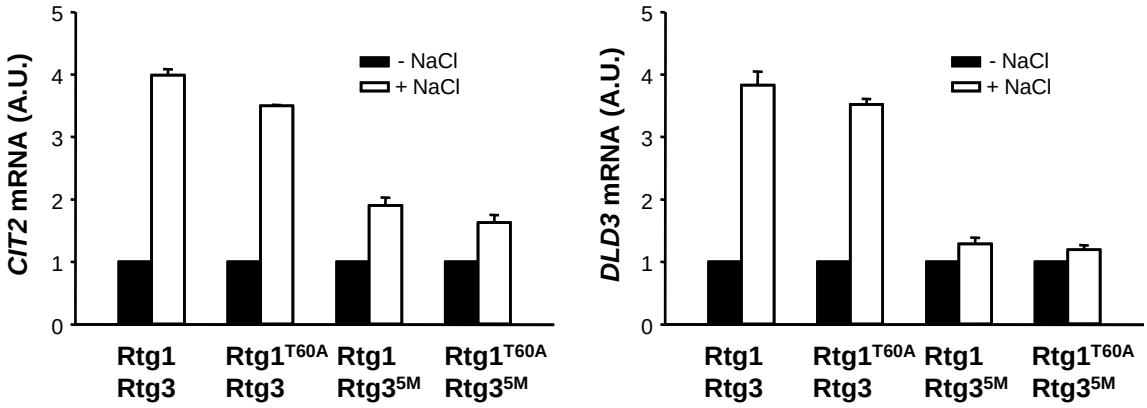

Figure S5. Rtg1 and Rtg3 phosphorylations by Hog1 are not synergic for activation of gene expression upon osmostress. The *rtg1*Δ*rtg3*Δ strain carrying monocopy plasmids expressing wild-type Rtg1, Rtg3 or the indicated mutants were grown to mid-log phase and subjected (+) or not (-) to osmostress (0.4M NaCl, 20 minutes). Total RNA was assayed by northern blot for transcript levels of *CIT2*, *DLD3* and *RDN18* (as a loading control). Quantification of northern blot is shown as the fold induction of treated against the untreated cultures. Data are the mean and standard deviation of three independent experiments.