Table S1
List of single non-essential gene deletion mutants that grew invasively in a genome-wide screen

Name	ORF	Agar Penetration-No Salt	Agar Penetration-0.5 M KCl	Salt Sensitivity	Protein Product
*HOG1**	YLR113W	-	+++	+	HOG pathway MAPK
*PBS2**	YJL128C	-	+++	+	HOG pathway MAPKK
*MNN11**	YJL183W	-	++	-	Subunit of a Golgi mannosyltransferase complex
PUN1	YLR414C	+	+	-	Putative protein of unknown function
SIN4	YNL236W	++	++	+	Subunit of the RNA polymerase II mediator complex
SNF4	YGL115W	++	++	+	Activating gamma subunit of the AMP-activated Snf1p kinase complex
TPS2	YDR074W	+	++	-	Phosphatase subunit of trehalose-6-phosphate synthase/phosphatase complex
KEX2	YNL238W	+	+	-	Subtilisin-like protease (proprotein convertase)
SPE1	YKL184W	++	-	+	Ornithine decarboxylase
ASC1	YMR116C	+	-	-	G-protein beta subunit and GDI for Gpa2p
HDA2	YDR295C	++	-	-	Subunit of a trichostatin A-sensitive class II histone deacetylase complex
APM3	YBR288C	+	-	-	Mu3-like subunit of the clathrin associated protein complex (AP-3)

Each strain was screened in duplicate; The phenotypes are categorized as follows: wild type-like, -; weak, +; medium, ++; strong, +++
*Only these single deletion mutants were validated by reconstructing the strain.

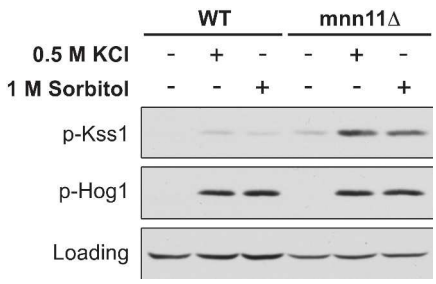

Figure S1. Deletion of *MNN11* results in hyperactivation of Kss1 in response to osmotic stress. Activation of Kss1 and Hog1; Wild-type and *mnn11*Δ cells were stimulated with either 0.5 M KCl or 1 M sorbitol for 5 min. Cell lysates were resolved by 10% SDS-PAGE. Phospho-Kss1 (p-Kss1) and phospho-Hog1 (p-Hog1) were detected by immunoblotting with phospho-p44/42 and phospho-p38 antibodies, which recognize the dually phosphorylated and activated forms of Kss1 and Hog1. G6PDH served as a loading control.

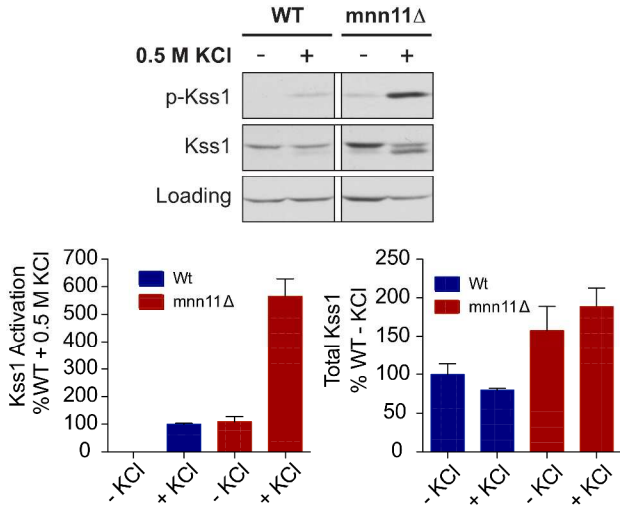

Figure S2. Deletion of *MNN11* results in 2-fold overexpression of Kss1. The 2-fold higher expression of Kss1 and phospho-Kss1 coincide wih the higher basal transcription activity observed in the deletion strain (Figure 2C). WT and *mnn11*Δ cells were stimulated with 0.5 M KCl for 5 min. Cell lysates were resolved by 10% SDS-PAGE. Specific antibodies were used to detect the dually phosphorylated, fully activated forms of Kss1 (p-Kss1). Total Kss1 abundance was determined with Kss1 specific antibodies. Note that total Kss1 is seen as a doublet, in which the bottom band is the activated form as previously published (*1*). G6PDH served as a loading control. The bottom panels show quantitation of band intensity. Error bars represent ± SEM.

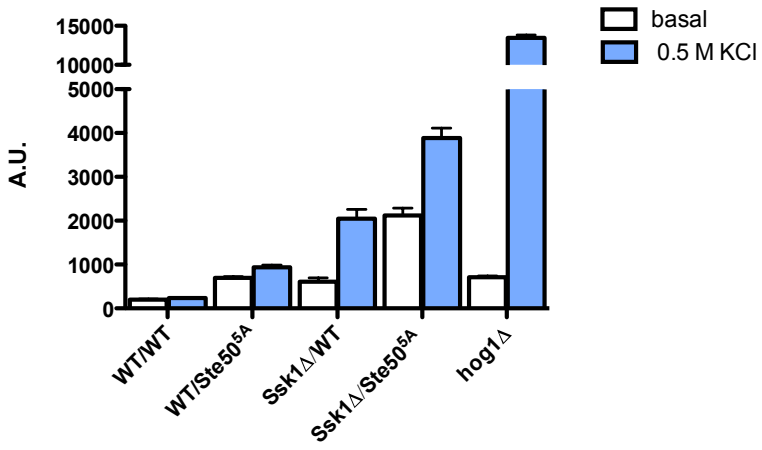

Figure S3. Ste50 phosphorylation limits Kss1 activation by the Sho1 branch of the HOG pathway. Transcriptional activation (β-galactosidase activity) was measured spectrofluorometrically in *ste50*Δ (WT) and *ste50*Δ*ssk1*Δ mutant cells, transformed with either pRS316-STE50 or pRS316-ste50^{5A} and a pheromone-inducible reporter (pRS423-FUS1-lacZ). Transcription was induced by the addition of 0.5 M KCl. Data are the mean ± SE of 4 individual colonies measured in triplicate. A.U., arbitrary units.

Reference

1. Yu, R. C., Pesce, C. G., Colman-Lerner, A., Lok, L., Pincus, D., Serra, E., Holl, M., Benjamin, K., Gordon, A., and Brent, R. (2008) Negative feedback that improves information transmission in yeast signalling, *Nature 456*, 755-761.