Supplementary Information for S. Chapman and A. Asthagiri

Supplementary Text

I. Dose-Response Properties as a Function of Ste5 Abundance

The dose-response data were fit to the Hill equation of the following form:

$$y - y_{\min} = y_{\max} \cdot \frac{x^{n_H}}{EC50_{\alpha}^{n_H} + x^{n_H}}$$

where y is the predicted pFUS1-GFP or phospho-MAP kinase response and x is the α-factor dose. The parameters determined by non-linear regression were $y_{\min}$ (the response corresponding to no pheromone), $y_{\max}$ (the response corresponding to a saturating pheromone dose), $EC50_{\alpha}$ (the dose that elicits half-maximal response), and n_H (the Hill coefficient). Data from each trial were individually fit to the Hill equation. The mean values and the standard error measurements for the fit parameter are listed in Table SIII-SV.

II. Signal Fidelity is Robust to Perturbation in Ste5 Expression

Signal crosstalk between the pheromone and high-osmolarity pathways is minimized in part through the use of two distinct scaffolds (Ste5 versus Pbs2, respectively). In addition, this scaffold-mediated fidelity is reinforced by mutual inhibition of pathway output (Figure S6A) (Bardwell, 2006; Bardwell et al., 2007; Hall et al., 1996; McClean et al., 2007; O'Rourke and Herskowitz, 1998). While mutual inhibition sharpens cell commitment to the proper response in the presence of a stimulus, our results raise the possibility that the baseline activation of Fus3/Kss1 in the absence of pheromone may inappropriately hamper the responsiveness of the high-osmolarity pathway.

To determine whether the basal activities of Fus3/Kss1 impede the high-osmolarity pathway, we measured sorbitol-mediated phosphorylation of Hog1, the high-osmolarity MAP kinase, in cells expressing different levels of Ste5. Our data show that the Hog1 signaling remains robust for all expression levels of Ste5 (Figure S6B, sorbitol). Thus, basal activation of Fus3/Kss1 does not inhibit the high-osmolarity pathway. Furthermore, this data shows that elevating Ste5 expression does not deplete the cellular pool of Ste11, allowing this upstream factor to remain available for the high-osmolarity response pathway. In fact, modulating the Ste5 expression level does not induce any inappropriate crosstalk between the pheromone and high osmolarity pathway: sorbitol treatment failed to activate pFUS1-GFP reporter above baseline levels (Figure S6C) and appropriately triggered Hog1 phosphorylation (Figure S6B). Meanwhile, pheromone stimulation did not activate Hog1, but did appropriately stimulate Fus3 and Kss1 activation (Figure S6B). Thus, across nearly 50-fold change in Ste5 expression level, signal fidelity is maintained.

Supplementary Tables

Table SI. Yeast strains

Strain	Description
CB011[1]	W303 *MAT**a**, ste5::KanR, bar1::NatR, far1Δ, mfa2::pFus1-GFP, his3, trp1, leu2, ura3*
EY1775[2]	W303 *MAT**a**, ste5::TRP1, bar1Δ, his3, trp1, leu2, ura3, ade2, can1*

[1] Strain kindly provided by Wendell Lim at UCSF (Bhattacharyya et al., 2006).
[2] Strain kindly provided by Elaine Elion at Harvard (Flotho et al., 2004).

Table SII. Plasmids

Name	Parent Vector	Promoter[1]	Description
pSC6-G	pRS416	GPD	Empty vector
pSC7-A	pRS416	ADH	STE5-13Myc[2]
pSC7-C	pRS416	CYC1	STE5-13Myc
pSC7-G	pRS416	GPD	STE5-13Myc
pSC7-T	pRS416	TEF	STE5-13Myc
pSC7-P	pRS416	STE5	STE5-13Myc
pSC10-G	pRS415	GPD	Empty vector
pSC11-A	pRS415	ADH	STE7-3HA[3]

[1] All promoters listed (except the native STE5 promoter) are from (Mumberg et al., 1995).
[2] The STE5 allele is from pSKM12 (Flotho et al., 2004). The 13Myc epitope tag is from pFA6a-13Myc-His3MX6 (Longtine et al., 1998).
[3] The STE7 allele is from pVS10 (van Drogen et al., 2001). The 3HA epitope tag from pFA6a-3HA-His3MX6 (Longtine et al., 1998).

Table SIII. Hill Equation Fit Parameters of pFUS1-GFP dose-response profiles

Promoter	y_{min} (a.u.)	y_{max} (a.u.)	$EC50_{\alpha}$ (nM)	n_H	Dynamic Range (S_{max}/S_{min})
STE5	0.03 ± 0.004	0.42 ± 0.01	9.0 ± 2.8	1.6 ± 0.1	20 ± 4.4
CYC	0.04 ± 0.01	0.60 ± 0.03	8.8 ± 2.3	1.8 ± 0.1	19 ± 4.6
ADH	0.08 ± 0.01	0.92 ± 0.03	8.7 ± 2.2	1.8 ± 0.2	13 ± 1.2
TEF	0.09 ± 0.01	0.90 ± 0.01	7.6 ± 2.2	1.7 ± 0.1	12 ± 0.9
GPD	0.08 ± 0.01	0.70 ± 0.04	9.4 ± 3.3	1.6 ± 0.1	9.1 ± 0.7

All mean values are indicated with ± S.E.M. (n=4).

Table SIV. Hill Equation Fit Parameters of phospho-Fus3 dose-response profiles

Promoter	y_{min} (a.u.)	y_{max} (a.u.)	$EC50_{\alpha}$ (nM)	n_H	Dynamic Range (S_{max}/S_{min})
STE5	0.05 ± 0.01	0.39 ± 0.02	2.9 ± 0.8	3.6 ± 0.6	8.9 ± 1.2
ADH	0.16 ± 0.03	0.99 ± 0.05	3.9 ± 0.5	3.6 ± 0.6	10 ± 3.3
GPD	0.15 ± 0.02	0.58 ± 0.03	4.4 ± 0.5	2.7 ± 0.5	5.5 ± 0.6

All mean values are indicated with ± S.E.M. (n=6).

Table SV. Hill Equation Fit Parameters of phospho-Kss1 dose-response profiles

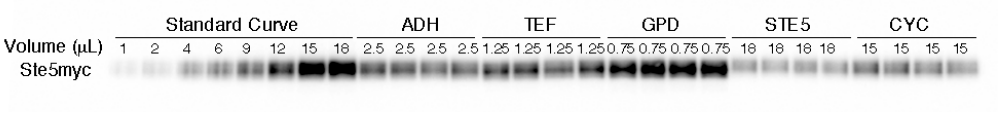
Promoter	y_{min} (a.u.)	y_{max} (a.u.)	$EC50_{\alpha}$ (nM)	n_H	Dynamic Range (S_{max}/S_{min})
STE5	0.12 ± 0.01	0.46 ± 0.02	2.0 ± 0.5	3.5 ± 0.5	5.3 ± 0.3
ADH	0.23 ± 0.03	0.89 ± 0.03	3.1 ± 0.4	4.3 ± 1.2	5.9 ± 1.4
GPD	0.18 ± 0.02	0.61 ± 0.02	3.5 ± 0.4	2.9 ± 0.5	5.1 ± 0.4

All mean values are indicated with ± S.E.M. (n=6).

Supplementary Figures

Figure S1

A



B

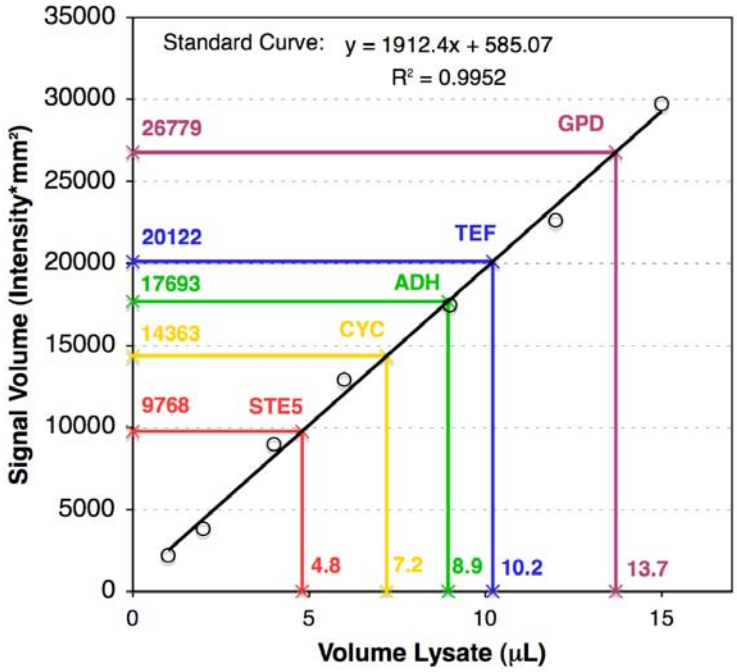


Figure S1. Quantitative Western blot of Ste5myc abundance. (A) Lysates of yeast expressing varying levels of Ste5myc were loaded in quadruplicate along with a standard curve in a single gel. Samples were differentially loaded by the volume indicated in order that all signals fall within the dynamic range of the standard curve. Whenever possible, lanes were loaded with a minimum of 15 µL total lysate using a filler lysate that lacked the antigenic protein of interest. Using Quantity1 software, boxes were drawn around the bands to obtain signal intensities (not shown). **(B)** Interpolation of quantitative Ste5 data from standard curve. The standard curve corresponding to the blot in Figure S1A was plotted and a linear fit was determined by regression. Mean signal intensities for the five yeast strains expressing varying amounts of Ste5 are displayed on the y-axis. The signal intensities were used to interpolate a corresponding volume of lysate from the standard curve. The interpolated values are indicated on the x-axis.

Figure S2

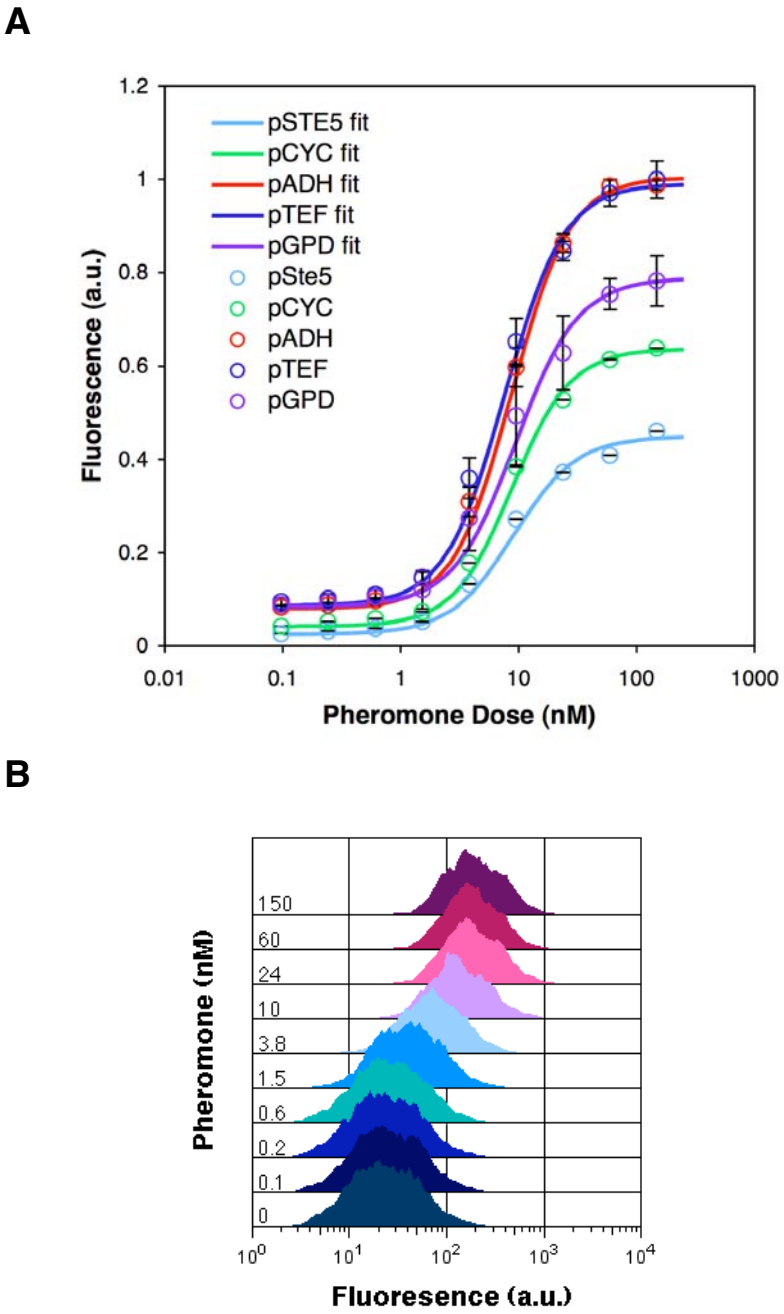


Figure S2. Dose-response curves of pFUS1-GFP as a function of Ste5 abundance. (A) Dose-response curves of pFUS1-GFP reporter data fit to the Hill equation. Open circles are pFUS1-GFP data points and solid lines represent the fit to the Hill equation. Error bars on the data points denote standard error (n=4). See Supplementary text for more details. **(B)** Yeast cells expressing Ste5 from an *ADH* promoter were induced with α-factor for 2.5 h. The pFUS1-GFP reporter response was measured by flow cytometry. Histograms of GFP fluorescence are shown for various α-factor doses. The unimodal distribution reveals a graded response to pheromone.

Figure S3

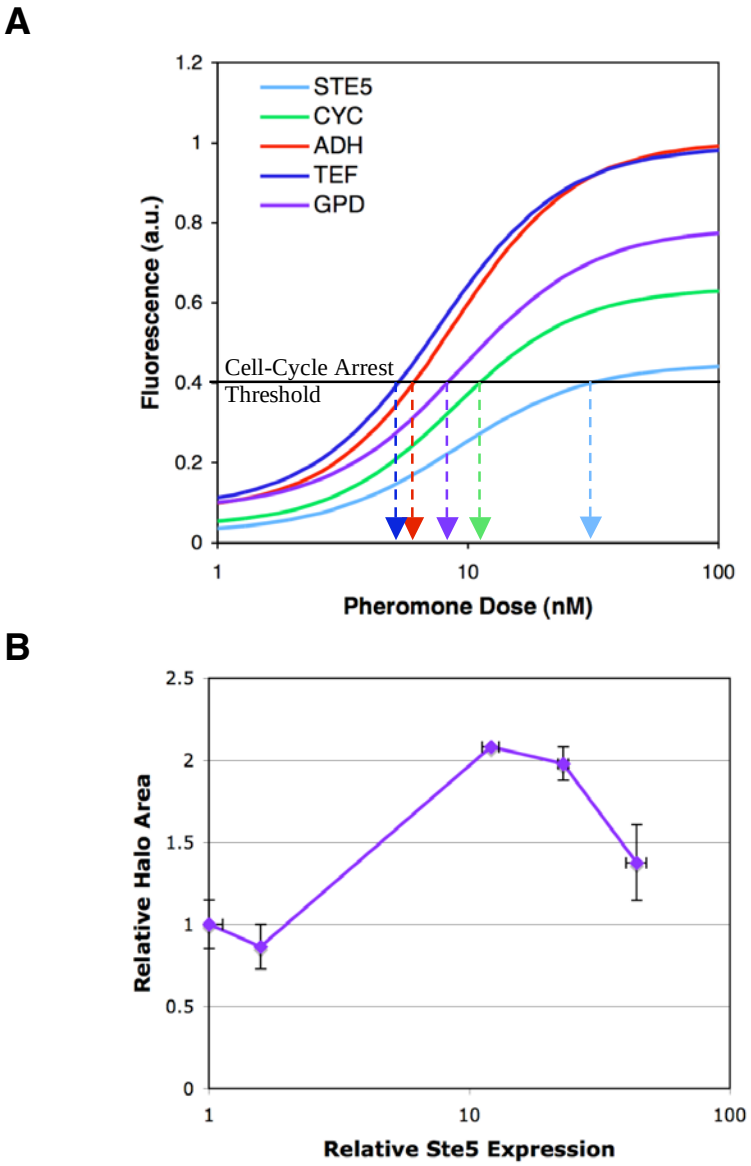


Figure S3. Optimal Ste5 expression maximizes phenotypic response in the halo assay. (A) Dose response curves of the FUS1 reporter is shown for the yeast strains expressing different levels of Ste5. The curves are based on the fit to the Hill equation described in Supplemental Text I and Table SIII. The solid horizontal line depicts our hypothesis that below a threshold level of signaling, the mating pathway no longer induces cell cycle arrest. The threshold is arbitrarily depicted here as 0.4 a.u. for illustratitive purposes. Since Ste5 expression alters the dose response, the amount of pheromone at which the system dips below the threshold is predicted to be a function of Ste5 expression level. Thus, according to our hypothesis, yeast cells expressing the optimum level of Ste5 (pADH) will undergo cell cycle arrest even at low doses of pheromone, and the area of the halo will be largest for this optimized strain. **(B)** Quantitation of the halo assay response displayed in Figure 2B. Halo area varies biphasically with respect to Ste5 expression level. Error bars denote S.E.M. (n=3).

Figure S4

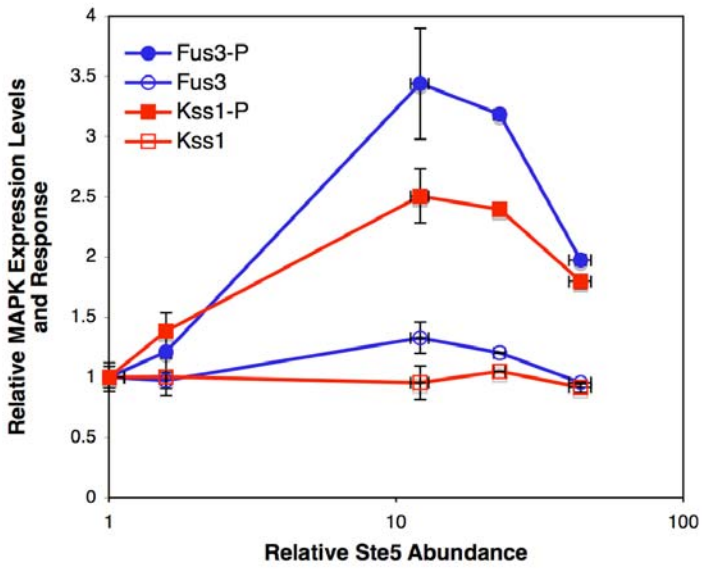


Figure S4. Fus3 and Kss1 expression vary minimally with Ste5 abundance. Fus3 and Kss1 expression levels were determined by quantitative immunoblot. Phospho-Fus3 (Fus3-P) and phospho-Kss1 (Kss1-P) responses to stimulation with 1.2 μM pheromone are shown for comparison. Phospho-Fus3 and Fus3 levels were measured from identical lysates that were stimulated with pheromone. Because the phospho-Kss1 isoform of the Kss1 doublet partially merges with a heavy background band, Kss1 expression was quantified using lysates with no pheromone stimulation. Expression levels were normalized by an equal loading control. Error bars denote S.E.M. (n=3).

Figure S5

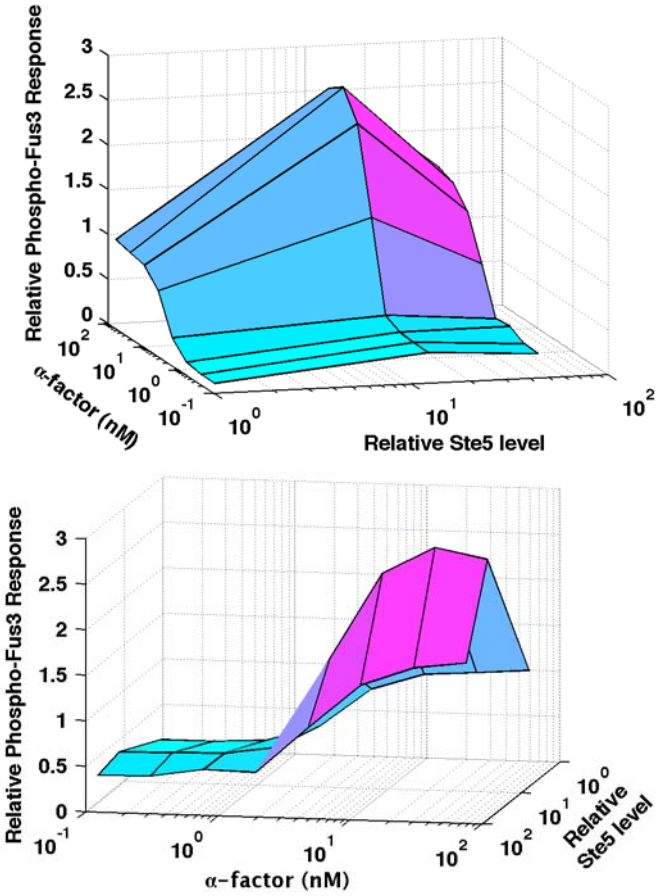


Figure S5. Sensitivity analysis of phospho-Fus3 response to perturbation in scaffold abundance. Cells were induced with α-factor for 5 min. and phoshpo-Fus3 was measured by quantitative immunoblot. The relative phospho-Fus3 response, normalized by total Fus3 expression, is shown for the various Ste5 abundance levels and α-factor doses. Two different views of the surface plot are shown.

Figure S6

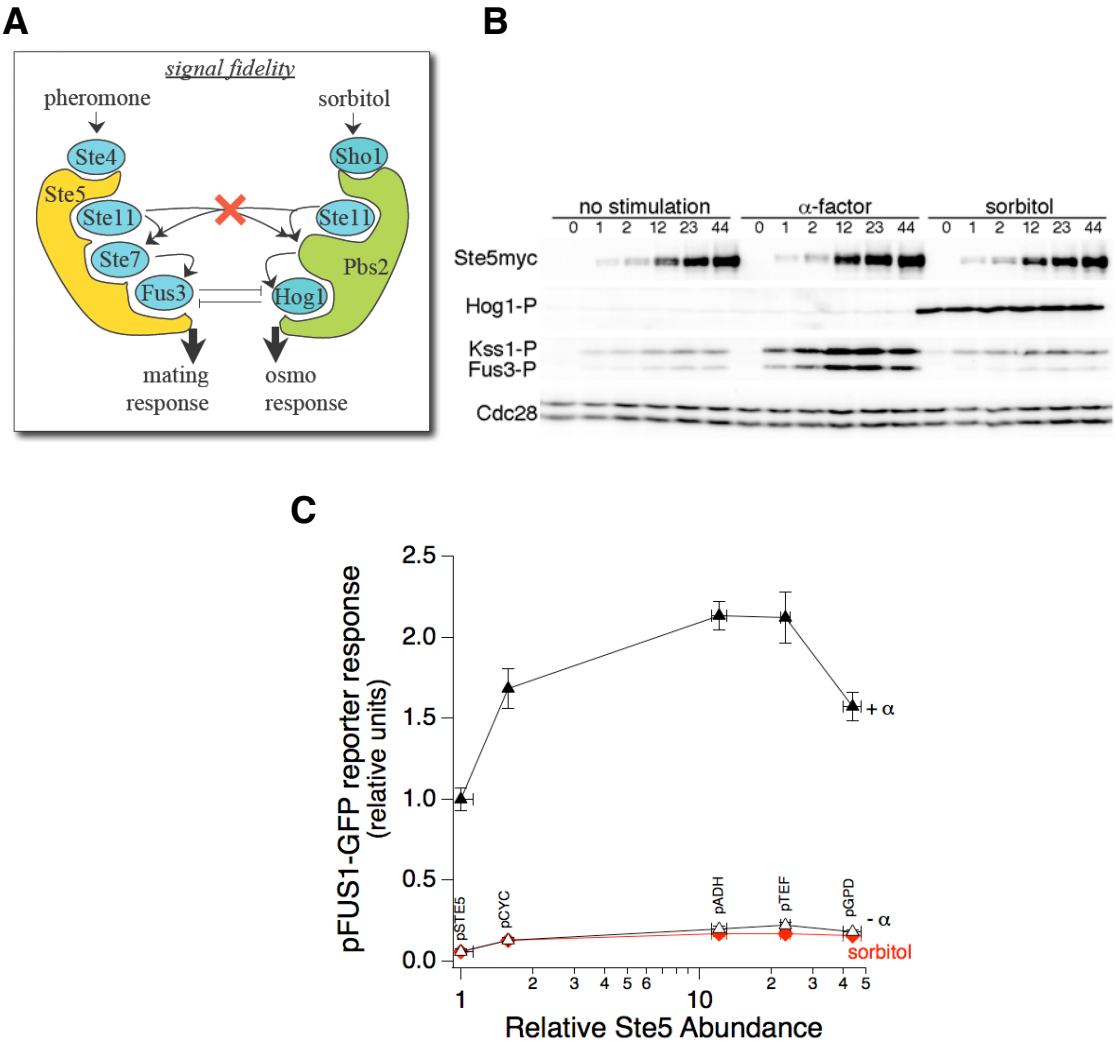


Figure S6. Signal fidelity is robust to perturbations in Ste5 abundance. (A) The fidelity of input-output response may be compromised by the presence of excess Ste5. Ste11 is a common component of the high-osmolarity pathway (right) and the mating pathway (left). Inappropriate exchange of Ste11 may cause high-osmolarity to trigger mating signals, or vice versa. Mutual inhibitory mechanisms between the two pathways prevent co-activation due to upstream leakiness. **(B)** Baseline and induction of MAP kinase signaling. Yeast expressing varying levels of Ste5 were left unstimulated or stimulated with α-factor or sorbitol for 15 minutes. The phosphorylation of Hog1, Kss1, and Fus3 were monitored by Western blot. Relative Ste5 expression is indicated above the gel lanes. Blots are indicative of 2 independent trials. **(C)** Baseline and induction of the mating transcriptional reporter. Yeast expressing varying levels of Ste5 were left unstimulated (black, open triangles) or stimulated with α-factor (black, solid triangles) or sorbitol (red diamonds) for 2.5 hours. The pFUS1-GFP reporter response was measured by flow cytometry. Error bars denote S.E.M. (n=3).

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