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Supplemental materials and methods

Screen and construction of yeast strains Strains from the library of S288c deletion strains (Winzeler et al, 1999; Research Genetics) were screened for FRE(Tec1)-lacZ activity in vegetatively growing cells that were grown in synthetic complete selective medium containing 2% dextrose. Mutants screened from the library included putative plasma membrane proteins, amino acid and sugar transporters, cell wall components, receptors, GTPases, guanine exchange factors, GTPase activating proteins, and numerous components of previously defined signal transduction pathways. Double mutant S288c strains constructed by crosses were made following standard procedures. Sterile parents harbored the wild type gene on a plasmid that was lost either before or after sporulation. Mating, **a** factor and α factor production and pheromone sensitivity tests were used to assess the phenotypes of meiotic progeny as previously described (Elion *et al*, 1990).

Construction of *ste5Δ241-336* The *ste5Δ241-336* mutation was made by two polymerase chain reactions (PCR) using primers 1 and 2, 3 and 4 respectively to generate two fragments using pYBS138 (*STE5 URA3 CEN*) as template. The two fragments were then annealed and a second PCR was done using primers 1 and 4 and the fragment was subcloned into the parent vector. Primer 1:
AAATGCATGCCTGCAGGTCGACTCTAGAGGATC,
Primer 2: TAAGGATAGAGTTGCTCCGGA AAT GCATTGAACGGCTTTG,
Primer 3: CAAAGCCGTTCAATGCATTTCCGGAGCAACTCT ATCCTTA,
Primer 4: AAGACTCGAGAATGAACTGCGTCTGGT.

Preparation of yeast extracts Yeast extracts were prepared as described (Elion *et al*, 1993) with the following modifications: cells were grown logarithmically to an OD_{600} of 0.8-1 and induced for 15 minutes with α factor where indicated. ~50 mls of cells were pelleted, washed once with ice water, then frozen in dry ice/ethanol. Pellets were thawed on ice, resuspended in 1.0 ml of buffer containing 10% glycerol, 25 mM Tris-Cl pH 7.4, 15 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1% Triton-X-100, 250 mM NaCl, 1mM NaN_3, 0.25 mM each meta- and ortho-vanadate, 0.1 μg/ml PMSF, 2mM benzamidine, 4mM 1,10-phenanthroline, 50mM NaF, 5 μg/ml each of pepstatin A, chymostatin, leupeptin, and peptin. Cells were broken with acid washed glass beads (Sigma or Biospec Products, Inc.). Cellular debris was removed by centrifugation at 3,500 RPM for 10 minutes at 4°C. The supernatant was slightly clarified by centrifugation at 14,000 RPM for 10 minutes at 4°C. Samples were immediately aliquoted and frozen. Protein concentrations were determined with the Bio-Rad protein assay.

Immunoblotting Samples were electrophoresed by SDS-PAGE on 10% polyacrylamide gels (acrylamide:bis-acrylamide at a ratio of 30:1) of 0.75-1.5 mm thickness using the Hoeffer vertical gel system. Immunoblots were blocked in 20 mM Tris-Cl pH 7.5, 150

mM NaCl, 0.1% Tween 20 (TBST) containing 5% nonfat milk (Carnation) for at least one hour at room temperature, then washed twice with TBST and incubated with primary antibody [anti-phospho p44/42 MAPK (Cell Signaling Techology, Inc., #9101), anti-Kss1 polyclonal anti-serum (Santa Cruz), anti-Fus3 polyclonal antiserum (Elion *et al*, 1993), anti-Tcm1 monoclonal antibody (gift of J. Warner, Albert Einstein College of Medicine)] in TBST-5% milk overnight at 4ºC or for 1 hour at room temperature, followed by three washings with TBST. Immunoblots were incubated with secondary antibody [rabbit anti-goat Ig-HRP, goat anti-mouse Ig-HRP and goat-anti-rabbit Ig-HRP] in TBST-5% milk for 1 hour at room temperature then washed three times with TBST and developed with the Amersham ECL kit. Immunoblots were stripped by incubating them in 50 mls of buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM TRIS pH 6.8) at 50°C with gentle shaking for 30 minutes and then washed twice with TBST.

Invasive growth assay Strains were grown on YPD agar plates at 30°C for three days and then washed gently with a fine stream of water as described (Roberts and Fink, 1994).

β-galactosidase assays Yeast cultures for extracts used in *lacZ*-assays were grown logarithmically to an OD_{600} of 0.5 without any dilution. Approximately 15 mls of cells were pelleted and used for preparation of yeast extracts, which was done with glass beads in 2ml tubes as described (Farley *et al*, 1999). β-galactosidase assays were performed at 30°C in triplicate (i.e. three different transformants) from which mean value and standard deviation were calculated.

Supplemental references

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Elion, E. A., Satterberg, B. and Kranz, J.E. (1993) FUS3 phosphorylates multiple components of the mating signal transduction cascade: evidence for STE12 and FAR1. *Mol. Biol. Cell* **4**: 495-510.

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Roberts, R. L. and Fink, G.R. (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes & Dev.* **8**: 2974-2985.

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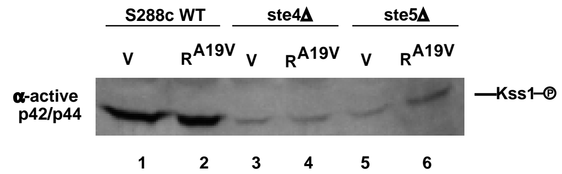
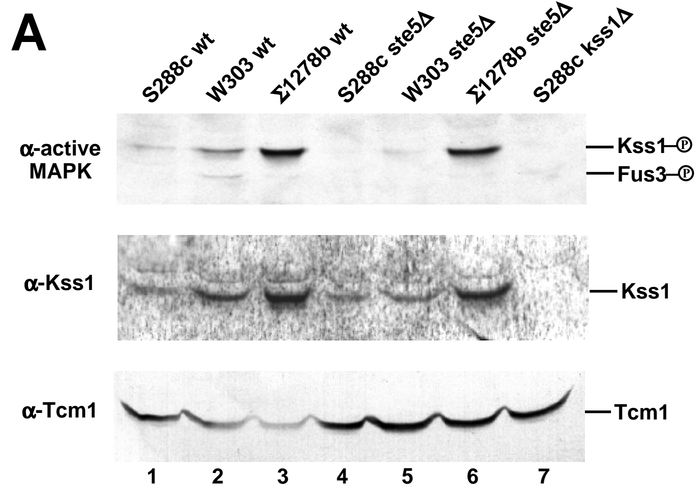
Legends to the supplemental data

Supplemental Figure 1. Effect of Ras2^{A19V} on Kss1 activity. *RAS2^{A19V}* was constitutively expressed from its own promoter in liquid selective medium containing 2% glucose. A slight increase in Kss1 activation was detected in the presence of Ras2^{A19V}. Iodine staining confirmed lower glycogen levels in strains that express Ras2^{A19V}.

Supplemental Figure 2. Dependence on Ste5 correlates with low levels of basal signaling. A. Higher level of Kss1 activity and Kss1 protein in Σ1278b compared to S288c and W303a. B. *FRE(Tec1)-lacZ* expression in Σ1278b and W303a strains. C. Relative level of Kss1 activity and Kss1 protein in progeny from a cross between Σ1278b *ste5Δ* and S288c *ste5Δ* parents. Bypass of the *ste5Δ* mutation correlates with high basal activation of Kss1 and high basal levels of Kss1 protein. Two tetrads are shown. The parents were first transformed with a *STE5 URA3* plasmid and then mated. Diploids were passaged over 5-fluoro-orotic acid to select for cells that had lost the plasmid prior to sporulation. Cells were grown in liquid medium containing 2% dextrose.



Supplemental Figure 1


A
S288c wt
W303 wt
Σ1278b wt
S288c ste5Δ
W303 ste5Δ
Σ1278b ste5Δ
S288c kss1Δ
α-active MAPK
Kss1–Ⓟ
Fus3–Ⓟ
α-Kss1
Kss1
α-Tcm1
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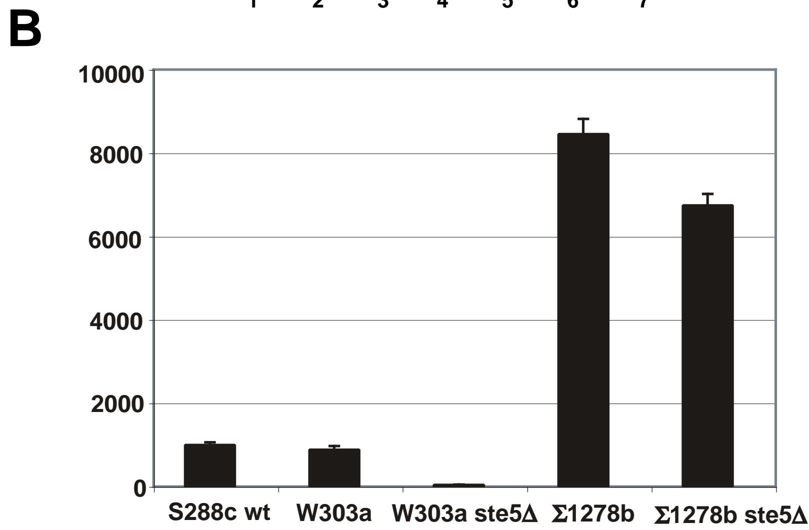
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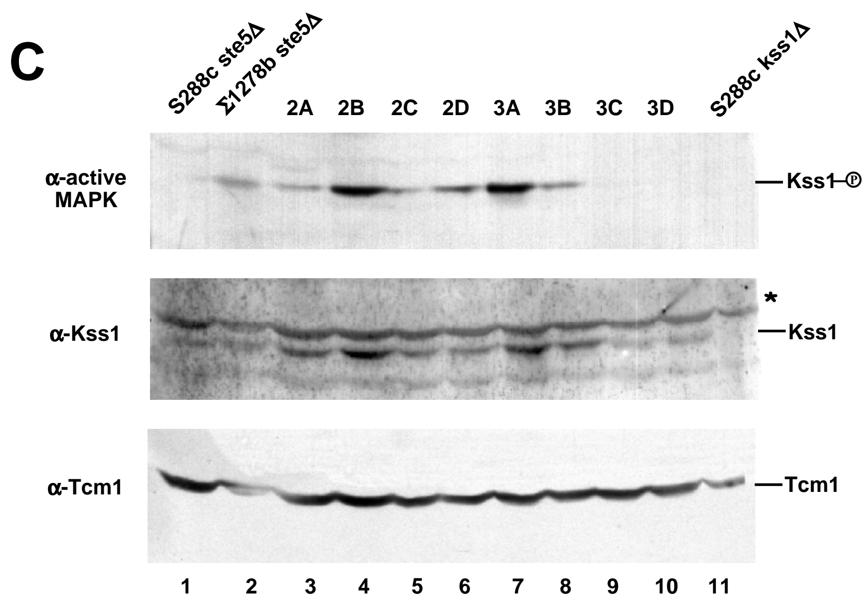
C
S288c ste5Δ
Σ1278b ste5Δ
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S288c kss1Δ
α-active MAPK
Kss1–Ⓟ
α-Kss1
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Kss1
α-Tcm1
Tcm1
1 2 3 4 5 6 7 8 9 10 11