# Supporting Information

## Won et al. 10.1073/pnas.1016337108

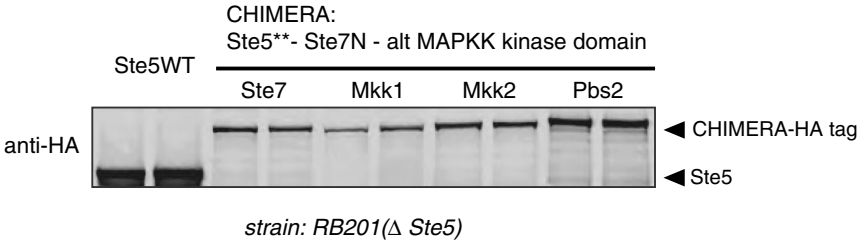


**Fig. S1.** Tethered alternative kinases are expressed in *Saccharomyces cerevisiae*. Expression level of alternative MAPKK chimeras detected by anti-HA-tag Western blot.

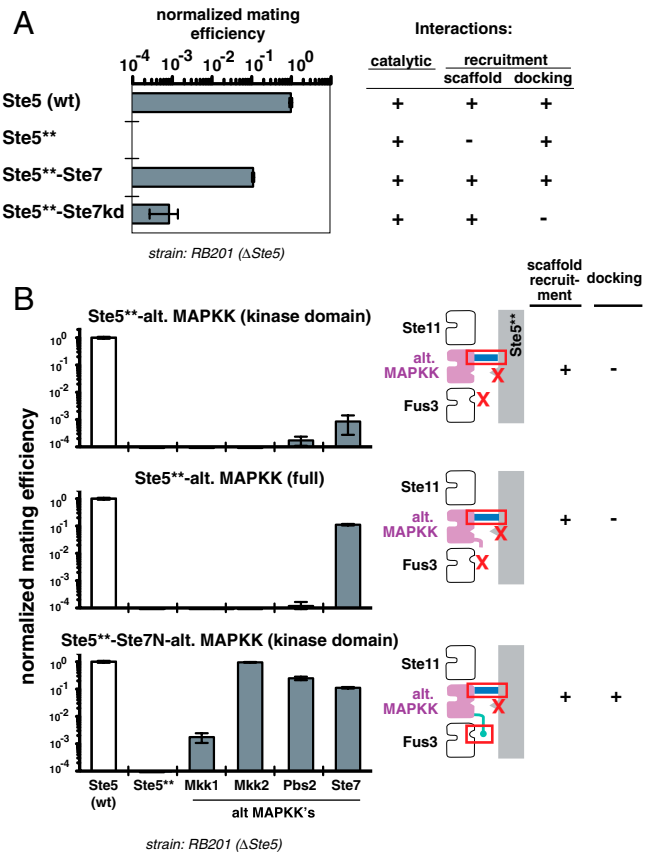


**Fig. S2.** Activity of chimeras at higher expression levels. Functional analysis of alternative MAPKK chimeras when expressed with stronger promoter (pADH1). Experiments were performed as described for Fig. 3.

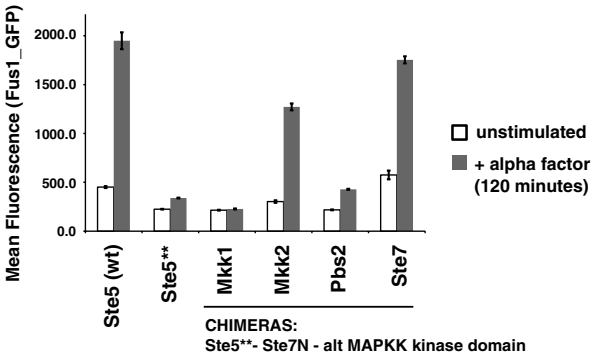


**Fig. S3.** Activation of mating transcriptional reporter (pFs1-GFP) by alternative MAPKK chimeras. Shown are the Fus1-GFP induction profiles of strains transformed with the tethered alternative kinases. Cultures were induced with 2 uM alpha factor for two hours. At each time point, cyclohexamine was added to each sample to inhibit protein synthesis. GFP levels analyzed using flow cytometry.

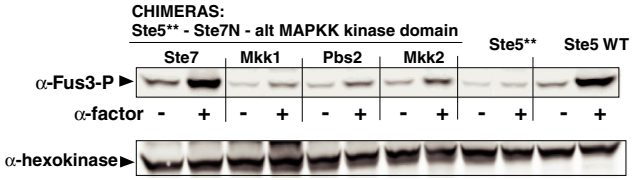


**Fig. S4.** Activation of mating MAPK Fus3 by alt MAPKK chimeras. Activation of mating MAPK Fus3 by alternative MAPK chimeras. Western blot analysis of Fus3 phosphorylation, as described in Fig. 4, but using MAPKK chimeric constructs that are expressed from the stronger Adh1 promoter (rather than native Ste5 promoter).

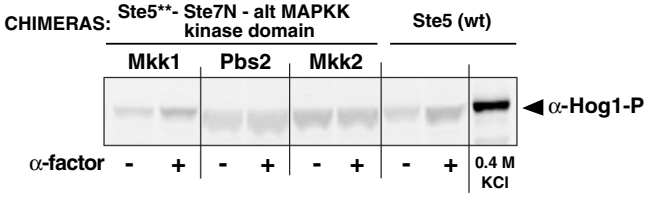


**Fig. S5.** Alternative MAPKK chimeras do not activate MAPK Hog1 upon alpha-factor stimulation. Osmolarity MAPK Hog1 is not activated by alternative MAPK chimeras upon alpha-factor stimulation. Western blot analysis of phospho-Hog1 as described in *Materials and Methods*. Positive control for Hog1 is shown (WT stimulated with 0.4 KCl).

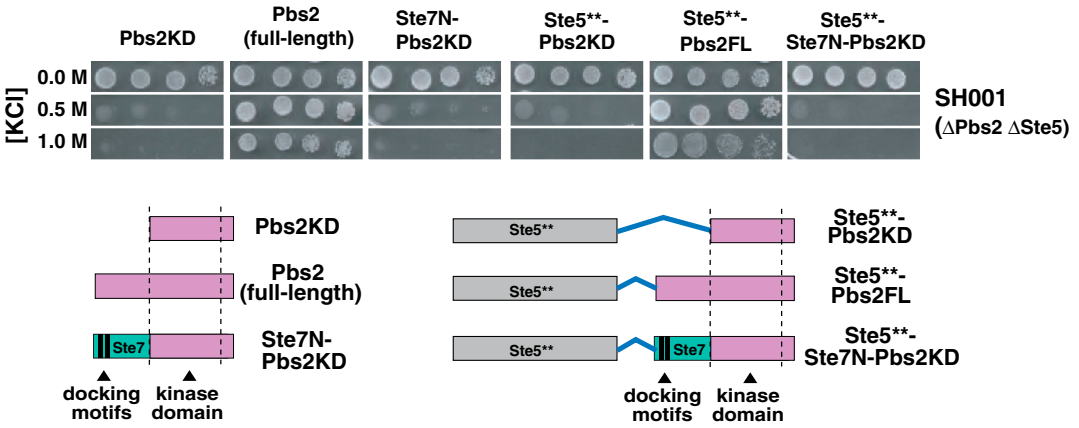


**Fig. S6.** Pbs2 chimera that is forced to adopt Ste7 identity no longer functions in the osmolarity response pathway. Pbs2 constructs were introduced into the osmosensitive strains AZ107 (Pbs2Δ) or SH001(pbs2Δ,ste5Δ), and their ability to complement osmosensitivity was determined. Cells were serially diluted and then spotted onto plates of selective medium with increasing concentrations of salt (0, 0.5, 1.0 M KCl).

**Table S1. Plasmids used in this study**

| | Name | Description | Promoter | Ste5**Fusion Junction | Parent Plasmid |
|---|---|---|---|---|---|
| Ste5**-kinase domain fusions | | | | | |
| | pAW301 | Ste5**_Ste7KD | AdhI | .....HMDYI **GS** LVQLG ..... | pRS316 |
| | pAW302 | Ste5**_Mkk1KD | AdhI | .....HMDYI **GS** IETLG ..... | pRS316 |
| | pAW303 | Ste5**_Mkk2KD | AdhI | .....HMDYI **GS** ITTLG ..... | pRS316 |
| | pAW304 | Ste5**_Pbs2KD | AdhI | .....HMDYI **GS** LEFLD ..... | pRS316 |
| | pAW327 | Ste5**_Ste7KD | Ste5 | .....HMDYI **GS** LVQLG ..... | pRS316 |
| | pAW328 | Ste5**_Mkk1KD | Ste5 | .....HMDYI **GS** IETLG ..... | pRS316 |
| | pAW329 | Ste5**_Mkk2KD | Ste5 | .....HMDYI **GS** ITTLG ..... | pRS316 |
| | pAW330 | Ste5**_Pbs2KD | Ste5 | .....HMDYI **GS** LEFLD ..... | pRS316 |
| Ste5**-full length kinase fusions | | | | | |
| | pAW550/305 | Ste5**_Ste7FL | Adh1 | .....HMDYI GS MFQRR ... | pRS316 |
| | pAW306 | Ste5**_Mkk1FL | AdhI | .....HMDYI **GS** MASLF ..... | pRS316 |
| | pAW307 | Ste5**_Mkk2FL | AdhI | .....HMDYI **GS** MASMF ..... | pRS316 |
| | pAW308 | Ste5**_Pbs2FL | AdhI | .....HMDYI **GS** MEDKF ..... | pRS316 |
| | pAW331 | Ste5**_Ste7FL | Ste5 | .....HMDYI GS MFQRR ... | pRS316 |
| | pAW332 | Ste5**_Mkk1FL | Ste5 | .....HMDYI **GS** MASLF ..... | pRS316 |
| | pAW333 | Ste5**_Mkk2FL | Ste5 | .....HMDYI **GS** MASMF ..... | pRS316 |
| | pAW334 | Ste5**_Pbs2FL | Ste5 | .....HMDYI **GS** MEDKF ..... | pRS316 |
| Ste5 or Ste5**, no fusion | | | | | |
| | pAW312 | Ste5** | AdhI | none | pRS316 |
| | pAW553/313 | Ste5WT | Adh1 | none | pRS316 |
| | pAW554/339 | Ste5WT | Ste5 | none | pRS316 |
| | pAW338 | Ste5** | Ste5 | none | pRS316 |
| Ste5**-Ste7N-alternative kinase domain fusions | | | | | |
| | pAW309 | Ste5**_Ste7NMkk1KD | AdhI | .....HMDYI **GS** MFQRR ..... | pRS316 |
| | pAW551/310 | Ste5**Ste7NMkk2KD | Adh1 | .....HMDYI **GS** MFQRR ..... | pRS316 |
| | pAW311 | Ste5**_Ste7NPbs2KD | AdhI | .....HMDYI **GS** MFQRR ..... | pRS316 |
| | pAW335 | Ste5**_Ste7NMkk1KD | Ste5 | .....HMDYI **GS** MFQRR ..... | pRS316 |
| | pAW552/336 | Ste5**Ste7NMkk2KD | Ste5 | .....HMDYI **GS** MFQRR ..... | pRS316 |
| | pAW337 | Ste5**_Ste7NPbs2KD | Ste5 | .....HMDYI **GS** MFQRR ..... | pRS316 |
| | Name | Description | Promoter | Ste5**Fusion Junction | Parent Plasmid |
| unfused full length kinase | | | | | |
| | pAW350 | Ste7 | Adh1 | none | pRS314 |
| Ste7N_kinase domain | | | | | |
| | pAW382 | Ste7NMkk1KD | AdhI | none | pRS314 |
| | pAW385 | Ste7NMkk2KD | AdhI | none | pRS314 |
| | pAW388 | Ste7NPbs2KD | AdhI | none | pRS314 |
| Integrating plasmids | | | | | |
| | pAW501 | Ste7 | AdhI | .....HMDYI **GS** MFQRR ..... | pNH605 |
| | pAW502 | Ste5**_Ste7NMkk1KD | AdhI | .....HMDYI **GS** MFQRR ..... | pNH605 |
| | pAW503 | Ste5**_Ste7NMkk2KD | AdhI | .....HMDYI **GS** MFQRR ..... | pNH605 |
| | pAW504 | Ste5**_Ste7NPbs2KD | AdhI | .....HMDYI **GS** MFQRR ..... | pNH605 |
| | pAW505 | Ste5** | AdhI | none | pNH605 |
| | pAW506 | Ste5WT | AdhI | none | pNH605 |
| | | KD = KinaseDomain; FL = Fulllength | | | |

**Table S2. Strains used in this study**

| | |
|---|---|
| RB201 | *W303 MATa, trp1, leu2, ura3, his3, can1R, ADE+* |
| | *mfa2::FUS1-LacZ, ste5::KanR* |
| CB011 | *W303 MATa, ste5::KanR, bar1::NatR, far1D, mfa2::pFUS1-GFP, his3, trp1, leu2, ura3* |
| Maya12 | *alpha mating typelys1-* |
| RB203 | *W303 MATa, ste5::KanR, ste11::TRP1, mfa2::FUS1-LacZ, leu2, ura3, his3, can1R, ADE+* |
| RB211 | *W303 MATa, ste5::LEU2, fus3::KanR, kss1::NatR* |
| | *trp1, ura3, his3, can1R, ADE+* |
| | *mfa2::FUS1-LacZ* |
| AZ107 | *pbs2::KAN, W303(his3, trp1, ura3, leu2)* |
| SH001 | *pbs2::KanR, ste5::LEU2, Fus1::lacZ(leu2D)* |