Supplemental Data

The Role of Docking Interactions in Mediating Signaling Input, Output, and Discrimination in the Yeast MAPK Network

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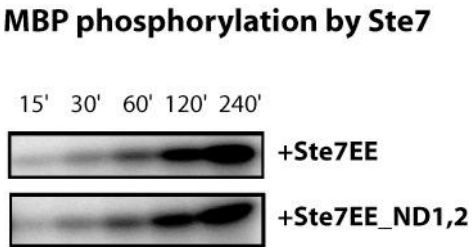

Figure S1. Ste7EE_ND1,2 (non-docking mutant) maintains full catalytic activity towards model substrate MBP (myelin basic protein).

Fus3 protein (2 μM) was incubated with 50 μM MBP in a kinase assay. Equal amounts of the reaction mixtures were taken at different time points after addition of radioactively labelled ATP and samples were run on SDS-PAGE. Gels were then dried and exposed to film.

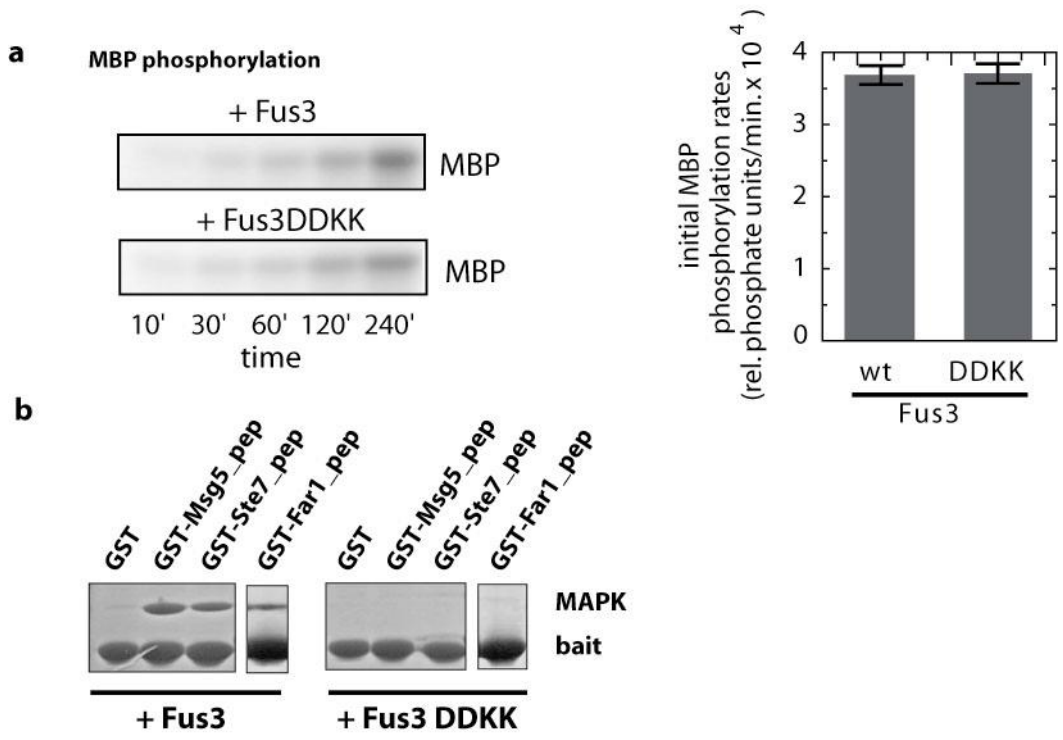

Figure S2. Mutation in the CD-site (Fus3DD-KK) can be used to probe docking interaction: mutation disrupts docking without altering core catalytic activity.

(a) Pre-phosphorylated Fus3 and Fus3DDKK phosphorylate MBP equally effectively. Autoradiogram and bar graph of initial phosphorylation rate are shown. DDKK mutations therefore only effect docking interactions, but not the intrinsic catalytic function. Error bars represent uncertainty in the fit to a first order polynomial equation for the linear part of the MBP phosphorylation curve.
(b) Mutations in the CD-site of Fus3 abolish interaction between the MAPK and its docking peptides. GST-pull down experiments with Fus3 and Fus3DDKK (D314K, D317K) using docking peptides from Msg5, Ste7 and Far1 as bait demonstrate that an intact CD-site is essential for interaction with all docking peptides from various MAPK partners.

Figure S3. Sequence alignment of Fus3 and Kss1 with mammalian MAP kinases.

Secondary structural elements from the Fus3 crystal structure are colored differently (α-helix: salmon; β-sheet: green; 3$_{10}$-helix: orange). Amino acid residues that play an important role in the catalytic reaction (Lys42, Glu59, Asp137, Asn142, and Asp 155) are colored in magenta. Residues that become phosphorylated upon MAPK activation are colored with red. Helix α1 corresponds to the catalytic "αC" helix (nomenclature based on the structure of PKA).

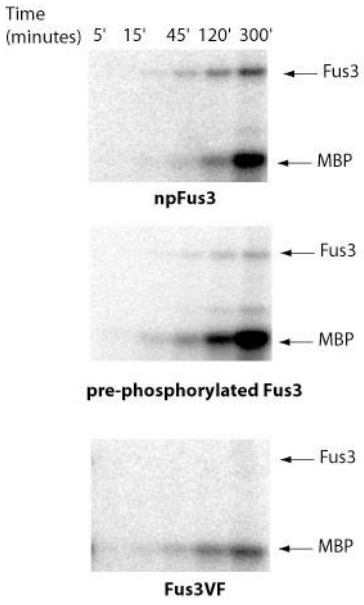

Figure S4. Comparison of catalytic activity of different Fus3 states and mutants: non-phosphorylated Fus3, pre-phosphorylated Fus3 and Fus3VF.

Fus3 protein (2 μM) was incubated with 50 μM MBP in a kinase assay. Equal amounts of the reaction mixtures were taken at different time points after addition of radioactively labelled ATP and samples were run on SDS-PAGE. Gels were then dried and exposed to film. The kinase activity of Fus3VF is reduced compared to non-phosphorylated Fus3 (npFus3). Kinase activity of npFus3 itself is reduced compared to pre-phosphorylated Fus3, as npFus3 first needs to be auto-activated in order to phosphorylate MBP (see radioactivity incorporation into Fus3 at top panel at later time points).

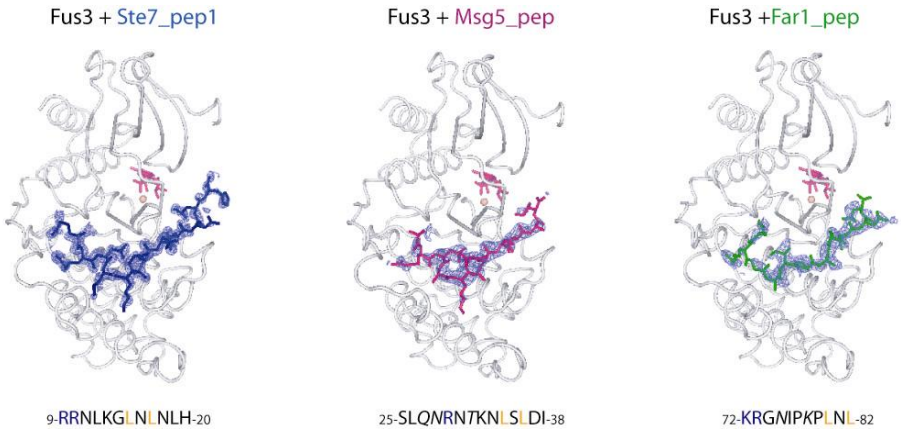

Figure S5. Crystal structures of Fus3/Ste7_pep1, Fus3/Msg5_pep and Fus3/Far1_pep complexes.

Peptides are shown with their σ_A-weighted simulated annealed omit maps calculated with the final models and contoured at 1σ. All docking peptides bind to the same region of Fus3. (Side chains of residues printed in italics are without electron density.)

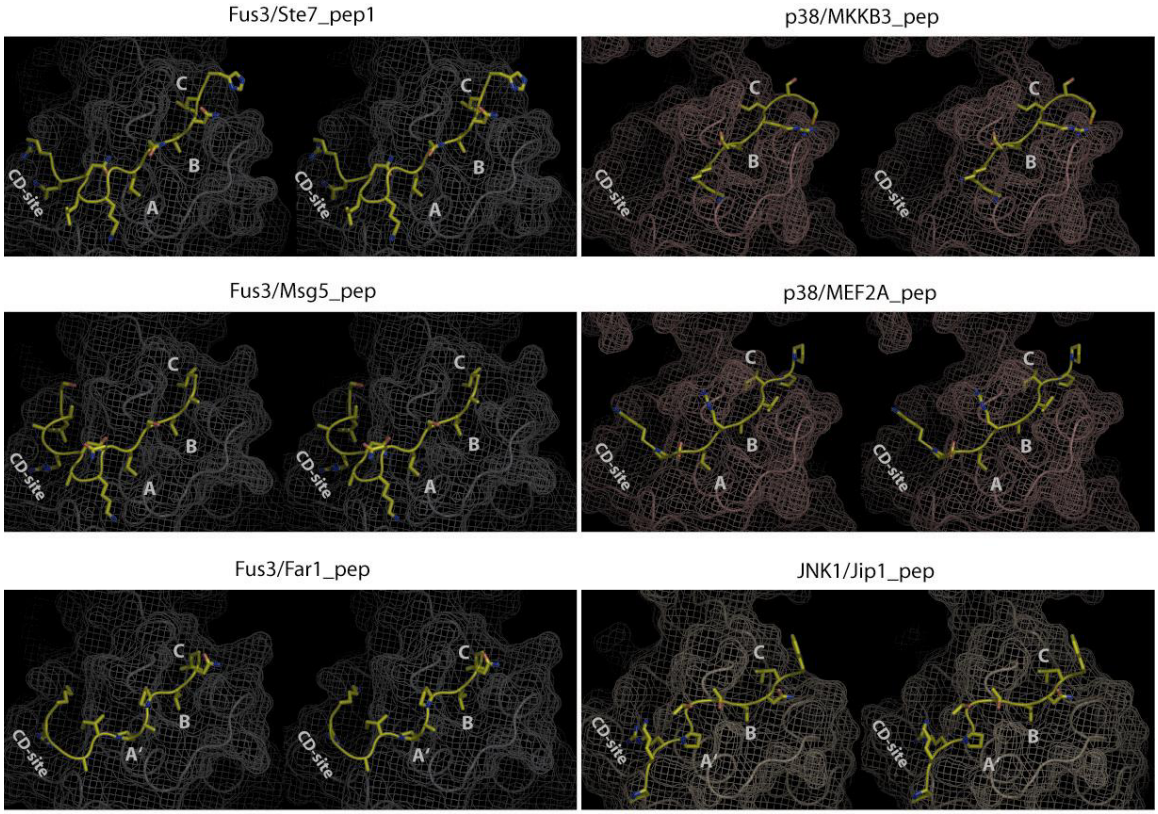

Figure S6. Structural comparison of Fus3 docking complexes with mammalian docking complexes.

Stereo-diagrams of all known MAPK docking complex structures. MAPK surfaces are depicted with mesh representation. Alignment of various docking motifs is given below. Note that some side chains on the docking peptides are not visible in the crystal structures. For example, the CD-site interactions in p38 are not visible. However, the basic motif at the N-terminus of the docking motifs is required for p38 binding, suggesting that these residues interact with the CD-site in a manner similar to that observed with the other MAPKs.

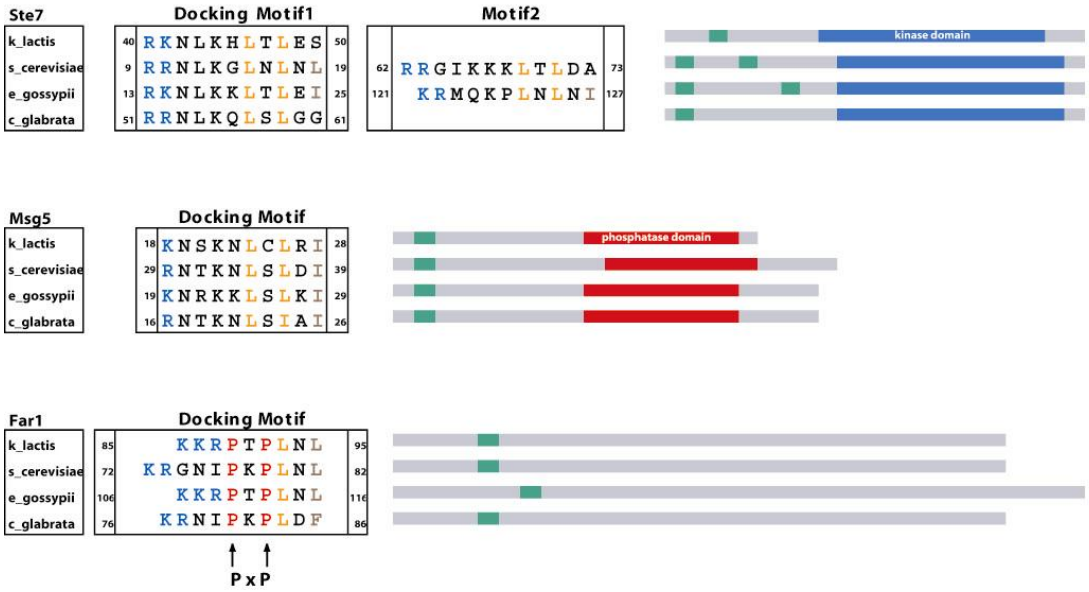

Figure S7. Conservation of docking motif sequences among several fungal species.

Comparison of fungal orthologs of Ste7, Msg5, and Far1 shows conservation of docking motifs, and unique conservation of the kinase selective PxP motif in Far1.

Strain	*Description*
MG40	W303 *MAT* ***a*** *, ste7::HIS3, bar1::NatR, far1* Δ*, mfa2::pFus1-GFP, his3, trp1, leu2, ura3*
MG20	W303 *MAT* ***a*** *, msg5::HIS3, kss1::KanR, bar1::NatR, mfa2::pFus1-LacZ, his3, trp1, leu2, ura3*
CB009	W303 *MAT* ***a*** *, bar1::NatR, far1* Δ*, mfa2::pFus1-GFP, trp1, leu2, ura3*

Table S1: Yeast strains used in this study.