Supplementary Data

How activating mutations affect MEK1 regulation and function

Authors: Granton A. Jindal[1,2,3,†], Yogesh Goyal[1,2,3,†], John M. Humphreys[4], Eyan Yeung[2,3], Kaijia Tian[1,2], Victoria L. Patterson[2,3], Haixia He[4], Rebecca D. Burdine[3], Elizabeth J. Goldsmith[4,*], Stanislav Y. Shvartsman[1,2,3,*]

Affiliations:

[1]Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544.

[2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544.

[3]Department of Molecular Biology, Princeton University, Princeton, NJ 08544.

[4]Department of Biophysics, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, TX 75390-8816.

[†]These authors contributed equally to the work

*To whom correspondence should be addressed: elizabeth.goldsmith@utsouthwestern.edu, stas@princeton.edu.

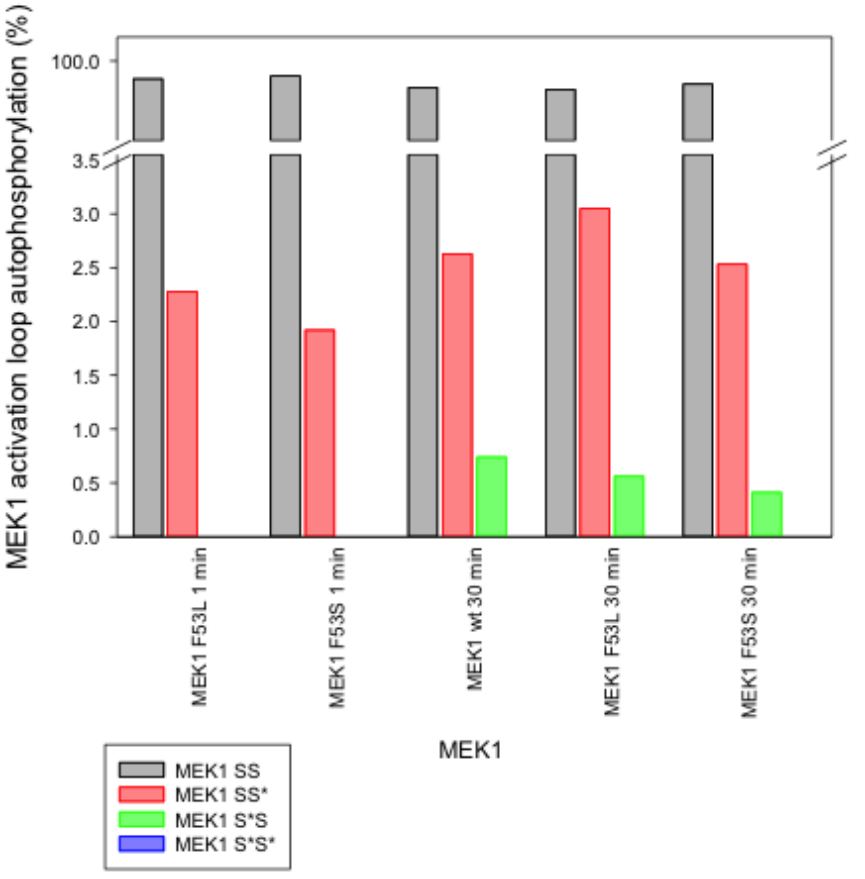

Figure S1. The activity of MEK1/F53L and MEK1/F53S does not involve autophosphorylation. Incubation of either WT-MEK1, MEK1/F53S, or MEK1/F53L with ERK2/K52M produces only small amounts of MEK1 SS*, as observed by mass spectrometry.

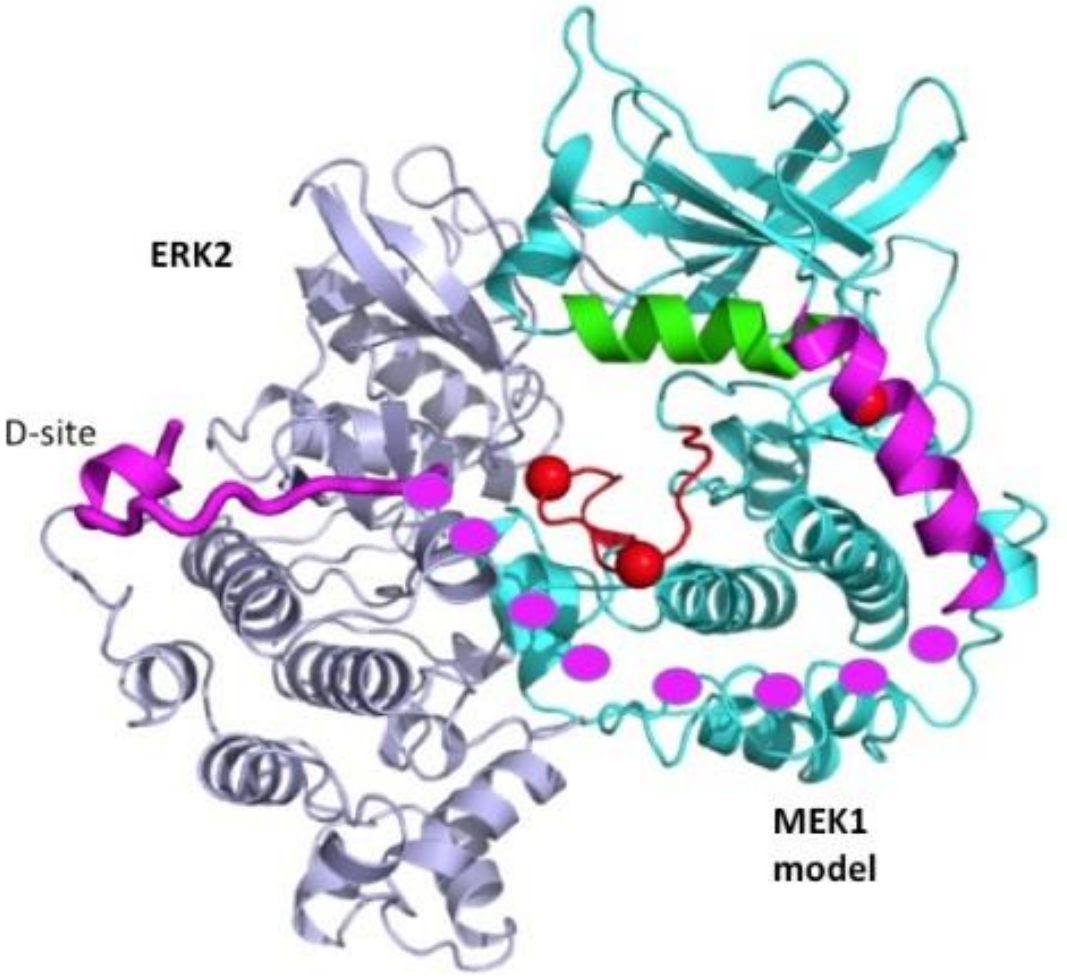

Figure S2. A model for the complex between MEK1 and ERK2. The model was constructed by using pdb file 2GPH (ERK2 with a docking peptide) (gray) and PKA (cyan) as a surrogate for active MEK1. These molecules were superimposed on a complex between p38 MAP kinase and MK2 (PDB file 4tyh). This is a good model to show how docking between the N-terminus of MEK1 and the ERK2 D-site might occur, but not a good model for active site interactions. Magenta dots show how the N-terminus of MEK1 (which is longer than PKA) might reach around to the docking site.

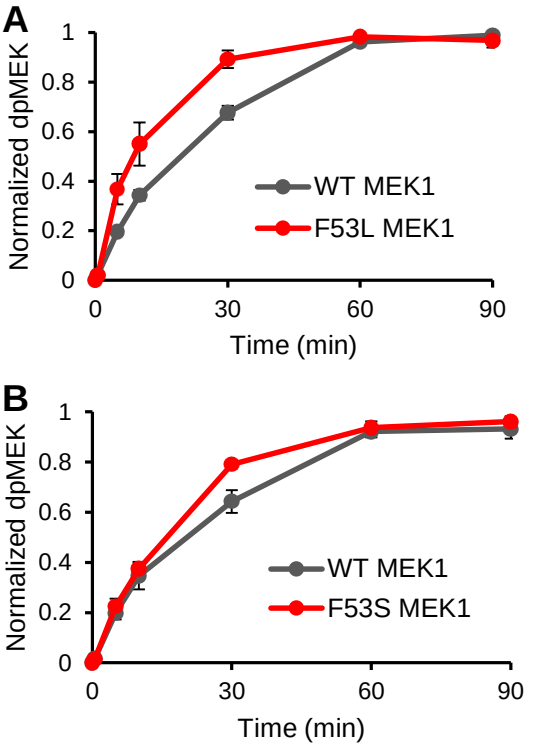

Figure S3. Activating MEK mutations enhance the rate of MEK1 phosphorylation by Raf. (A, B) Phosphorylation of MEK1/F53L and MEK1/F53S occurs faster than phosphorylation of wild-type MEK1. The reaction was run at a Raf:MEK ratio of 1:50. The normalized dpMEK is an average of 3 experimental replicates. Error bars, s.e.m.

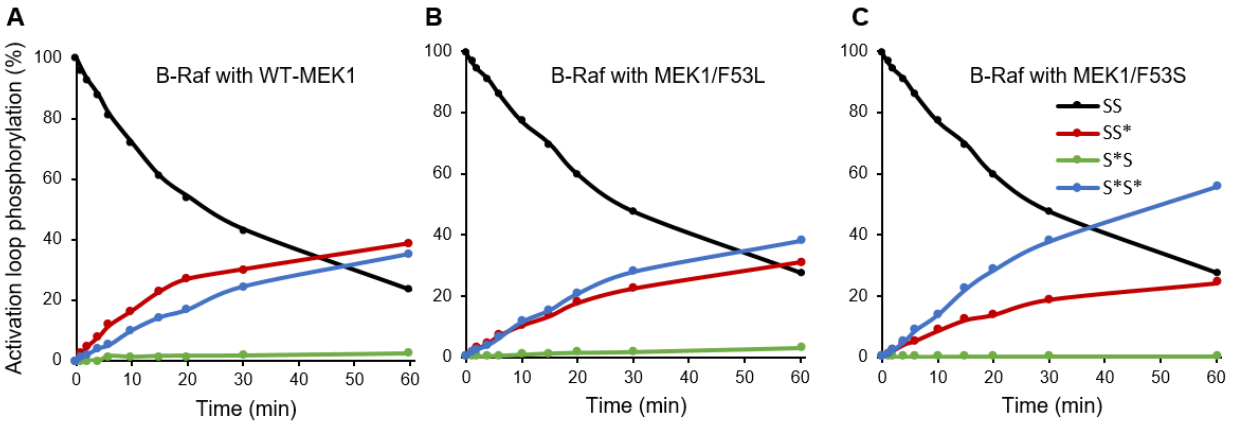

Figure S4. Order of phosphorylation of MEK1/F53S and MEK1/F53L by B-Raf. Activation loop phosphorylation by B-Raf of the MEK1 species indicated was followed over time using LC-MS. **(A)** B-Raf phosphorylates MEK1 through the intermediate MEK1/SS*. **(B, C)** B-Raf phosphorylated the MEK1/F53 mutants with an order and mechanism like wild-type.

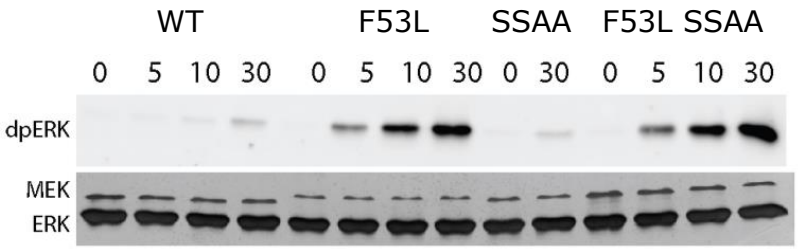

Figure S5. Alanine substitutions at S218 and S222 do not affect MEK1/F53L activity. Alanine substitutions at S218 and S222 do not affect MEK1/F53L intrinsic activity, which is much higher than wild-type MEK1. A western blot for comparing ERK T*Y* formed by incubating wild-type ERK2 with unphosphorylated wild-type MEK1, wild-type MEK1 SSAA, MEK1/F53L, and MEK1/F53L SSAA. MEK1 (2 μM) and ERK2 (10 μM) at a ratio of 1:5.

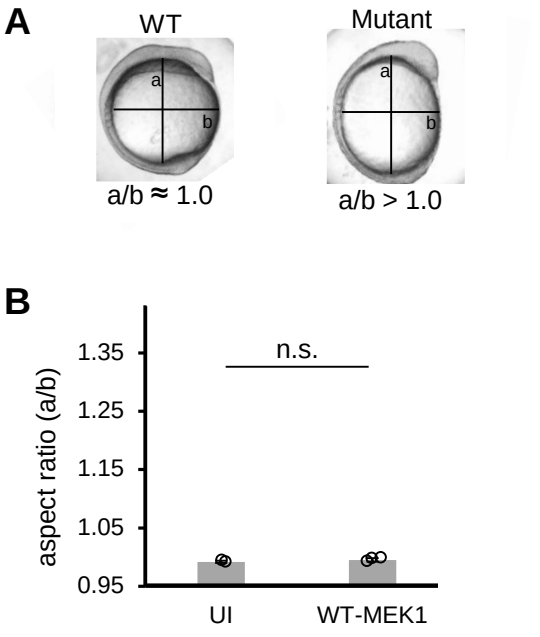

Figure S6. Overexpression of tagged MEK1 does not affect oval shape of the zebrafish embryo. (A) Images of a wild-type embryo and an embryo injected with a MEK mutant at 11 hours post fertilization with major (a) and minor (b) axes marked. The degree of oval shape of the embryo is measured as a/b. **(B)** The tagged WT-MEK1 mRNA upon injection does not lead to an oval embryo phenotype. N_{UI} = 57 (2 biological replicates), $N_{WT\text{-}MEK1}$ = 94 (3 biological replicates). Pairwise Student's t-test (two-sided, homoscedastic) was performed to compare between groups: n.s. not significant. Error bars, s.d.

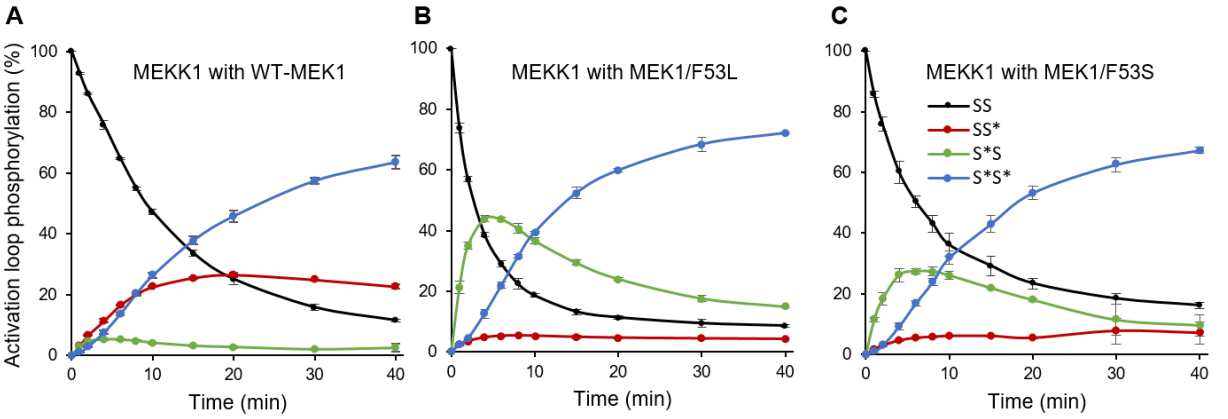

Figure S7. Order of phosphorylation of MEK1/F53S and MEK1/F53L by MEKK1. Activation loop phosphorylation by MEKK1 of the MEK1 species indicated was followed over time using LC-MS. To generate progress curves on similar scales as to Figure S4, 60-fold more MEKK1 than B-Raf was used with equal concentrations of MEK1. **(A)** MEKK1 phosphorylates MEK1 through the intermediate MEK1/SS*. **(B, C)** MEKK1 phosphorylation of the MEK1/F53 mutants showed a reversal of the phosphointermediate (MEK1/S*S (green) instead of MEK1/SS* (red)) in comparison to B-Raf.