Supplementary Material for:

Molecular mechanism of ERK dephosphorylation by striatal-enriched protein tyrosine phosphatase (STEP)

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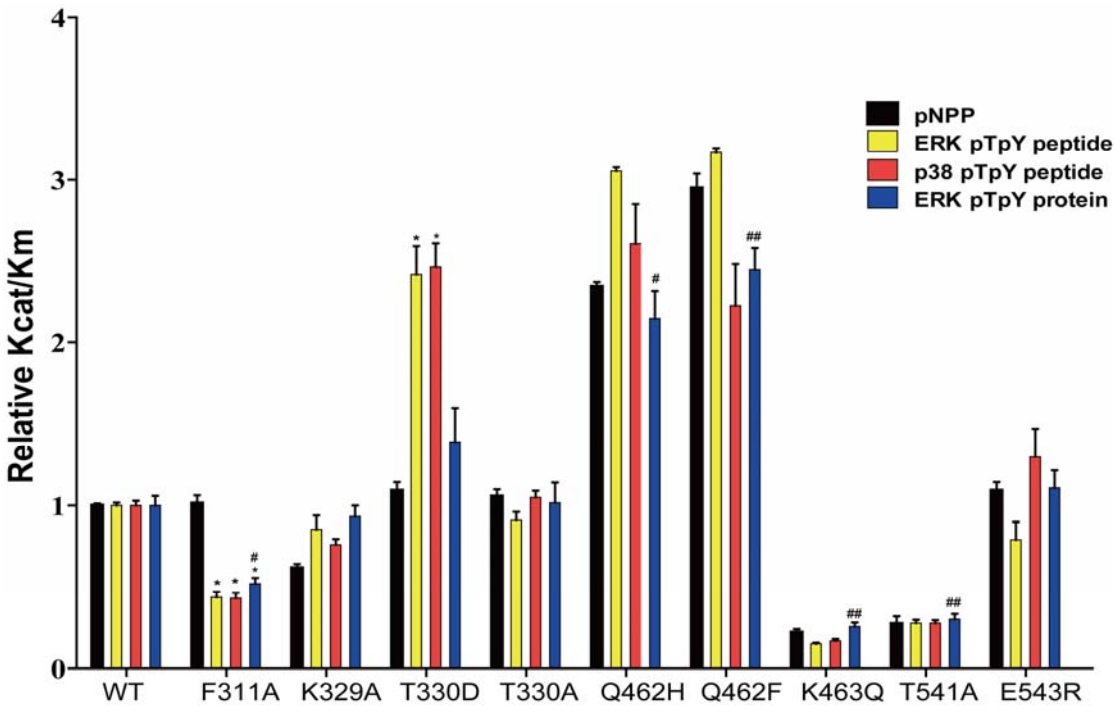
Supplemental-Figure S1: Bar graph and statistical analysis of the relative catalytic activity of STEP active-site mutants for four different substrates, pNPP, the phospho-peptides derived from phospho-ERK2-pT^{202}pY^{204} and pp-p38-pT^{180}pY^{182}, and full-length phospho-ERK protein, compared to wild-type STEP.



(*represents P<0.05 compared to each mutant relative catalytic activity for pNPP. # represents P<0.05, ##, P<0.01 compared to wild type catalytic activity for phospho-ERK protein. The data are the average of at least three independent measurements.)

Supplemental Materials and Methods

Molecular cloning and mutagenesis

The coding sequence of human STEP$_{46}$ (Swiss-Prot entry P54829) was

sub-cloned into the PET15b bacterial expression vector with an N-terminal hexa-histidine purification tag (MGSSHHHHHH), as described previously (Sun *et al.* 2007). The N-terminal deletions were generated by SLIC methods (Li & Elledge 2012). All point mutations of STEP were produced using the QuikChange Site-Directed Mutagenesis Kit (Stratagene,U.S.A). The PAGE-purified oligonucleotide primers used for cloning were obtained from Beijing Genomics Institute (China). All constructs were verified by DNA sequencing.

Protein expression and purification

The N-terminal His-tagged recombinant STEP proteins (wild-type and mutants) were expressed and purified as follows. BL21(DE3) cells were transformed with the expression plasmids and cultured in LB medium with vigorous shaking at 37°C. The culture temperature was adjusted to 18°C at OD_{600}=0.6, and expression was induced for 12 h with 0.4 mM IPTG at an OD_{600} of 0.8. The cells were harvested by centrifugation and re-suspended in lysis buffer (20 mM Tris [pH 8.0], 300 mM NaCl, 1 mM PMSF, and 5 mM imidazole). After centrifugation, the supernatant was incubated with Ni^{2+}-NTA resin with end-to-end mixing for 1 hour at 4°C. The beads were collected and washed with 20 ml wash buffer (20 mM Tris [pH 8.0], 300 mM NaCl, and 5 mM imidazole) and eluted with an imidazole gradient (20 mM Tris [pH 8.0], 300 mM NaCl, and 20-200 mM imidazole). The elute was diluted 10-fold in buffer A (20 mM Tris [pH 8.0], 50 mM NaCl, and 2 mM DTT) and loaded onto a 1-ml HiTrap Q FF column. The protein was eluted using a linear gradient from buffer A to 50% buffer B (20 mM Tris [pH 8.0], 1 M NaCl, and 2 mM DTT). The purified proteins were assessed by SDS-PAGE. The proteins were concentrated to 5–10 mg/ml using a 10-kDa cut-off concentrator (Millipore) and stored at -80°C after adjustment with 20% glycerol.

Preparation of phospho-ERK2 protein

Recombinant His-ERK2 and constitutively active MEK1(G7B) were expressed and purified using a procedure similar to that used for His-tagged STEP. Bisphosphorylated ERK2 was prepared and assessed as described previously (Zhao & Zhang 2001). ERK2 (purified, 1 mg/ml) was incubated with 0.1 mg/ml MEK1 in a reaction system of 20 mM HEPES (pH 7.4), 20 mM $Mg(OAc)_2$, 100 mM NaCl, 2 mM DTT, and 0.5 mM ATP. The reaction was incubated at 30°C for 90 min with occasional stirring. Phosphorylated ERK2 was loaded onto a Superdex-200 column (GE Healthcare) to remove the free ATP and then further purified using a Mono-Q column.

Western blotting

ERK2 (0.5 μM) was combined with 20 nM phosphatase in 50 mM 3-(N-morpholino)propanesulphonic acid (MOPS), 100 mM NaCl, 0.1 mM EDTA, and 1 mM DTT. The dephosphorylation of phospho-ERK2 by PPM1A was performed by adding 5 mM $MnCl_2$. Aliquots of the reaction were withdrawn at the indicated times and then added to tubes containing 2X sample loading buffer. The protein samples were then subjected to SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% BSA and incubated with the appropriate dilution of specific antibodies.

Generation of the docking model

The crystal structures of ERK (PDB ID: 2ERK) and STEP (PDB ID: 2CJZ) were combined together in the software programme HEX 6.3. The molecular minimisation was performed similar to the previous docking model of the PPM1A-phospho-ERK complex (Li *et al.* 2013), with 3000 iterations, in the software programme Desmond 3.1.

References

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