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Supplemental information

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Figure S1.

Figure S1. Computational prediction of redox-sensitive sites in ERK1 and ERK2, related to Fig. 1. A. *Multiple sequence alignment of ERK2 and ERK1. Sequence alignments were done using Clustal Omega.* ***B-E****. Cy-Preds prediction of redox-sensitive Cys residues in unphosphorylated or doubly phosphorylated ERK1 and ERK2. The pdb ID used for the prediction is given in paratheses.*

Figure S2.

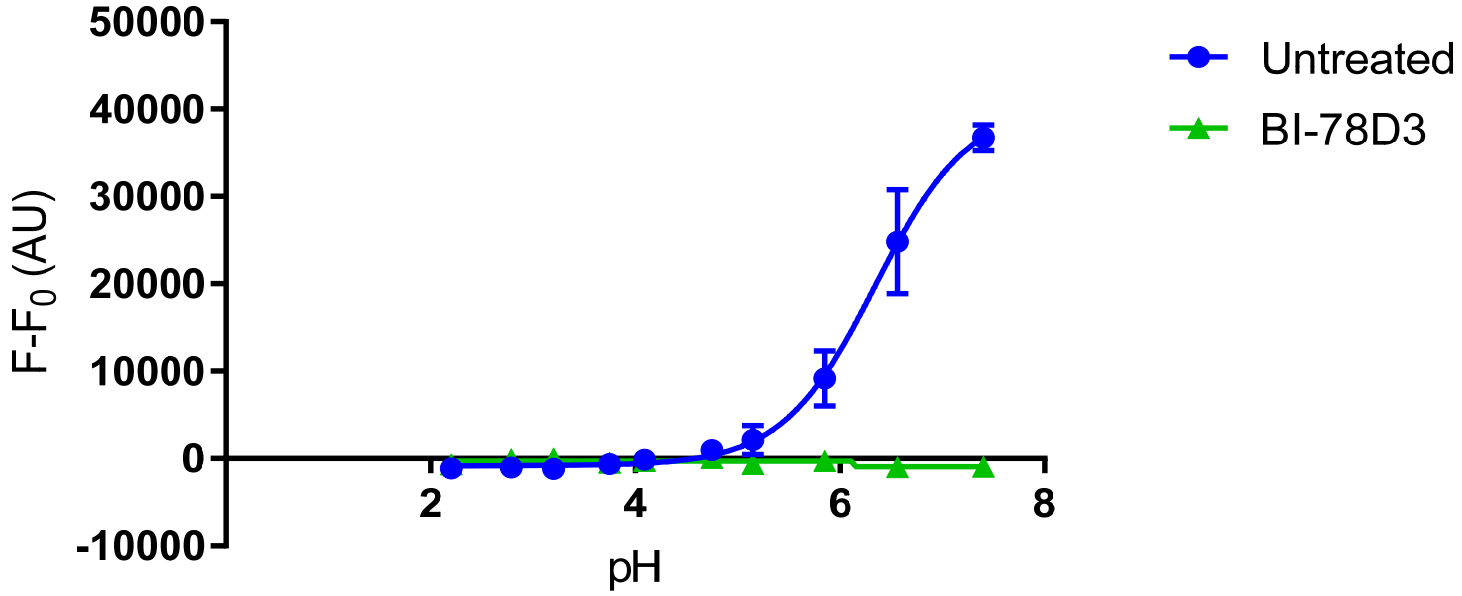


Figure S2. Bromobimane fluorescence labeling of recombinant ERK2, related to Fig. 1*. ERK2 was incubated with (green triangles) or without (blue circles) the C159-specific inhibitor, BI-78D3, for 60 minutes at room temperature. After incubation, the solution was incubated in the dark for an additional 60 minutes with monobromobimane in phosphate buffered saline at the indicated pH. Error bars represent the standard error about the mean of 3 independent experiments.*

Figure S3.

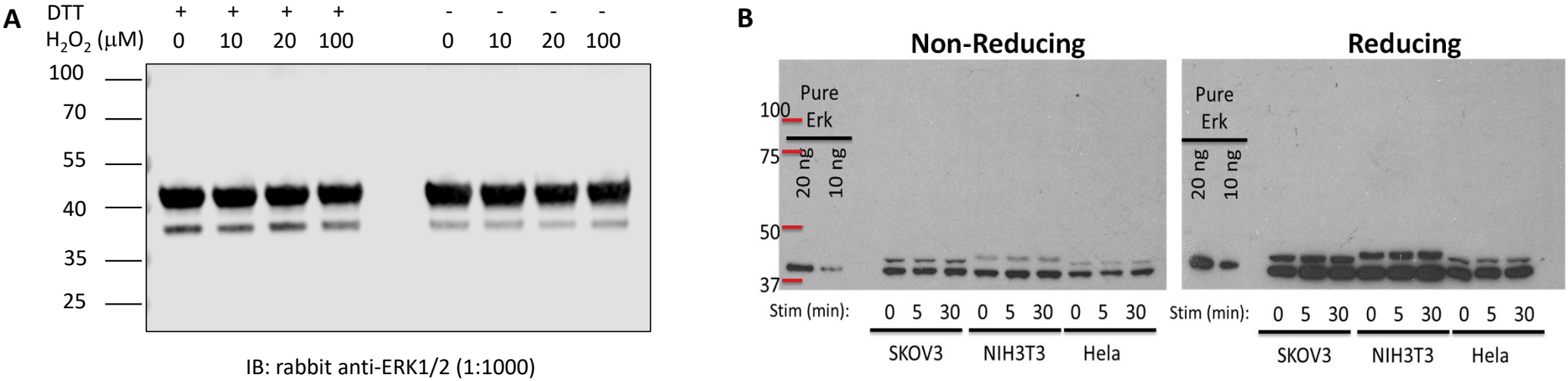


Fig. S3. Non-reducing PAGE, related to Fig. 1. A) *Purified ERK2 was treated with the indicated concentration of H^2O^2 for 10 minutes at room temperature. Excess H_2O_2 was then removed by catalase and the samples were resolved by SDS-PAGE under either reducing (left) or non-reducing (right) conditions. After electrophoresis, protein bands were visualized by western blotting using an anti-ERK1/2 antibody.* ***B)*** *Western blot analysis of endogenous ERK1/2 in cell lysates derived from three different cell lines following stimulation with lysophosphatidic acid (SK-OV3 cells), platelet-derived growth factor (NIH3T3 cells) or epidermal growth factor (HeLa cells) for the indicated time periods. Lysates were resolved under either reducing (left) or non-reducing (right) conditions and probed with an anti-ERK1/2 antibody.*

Figure S4.

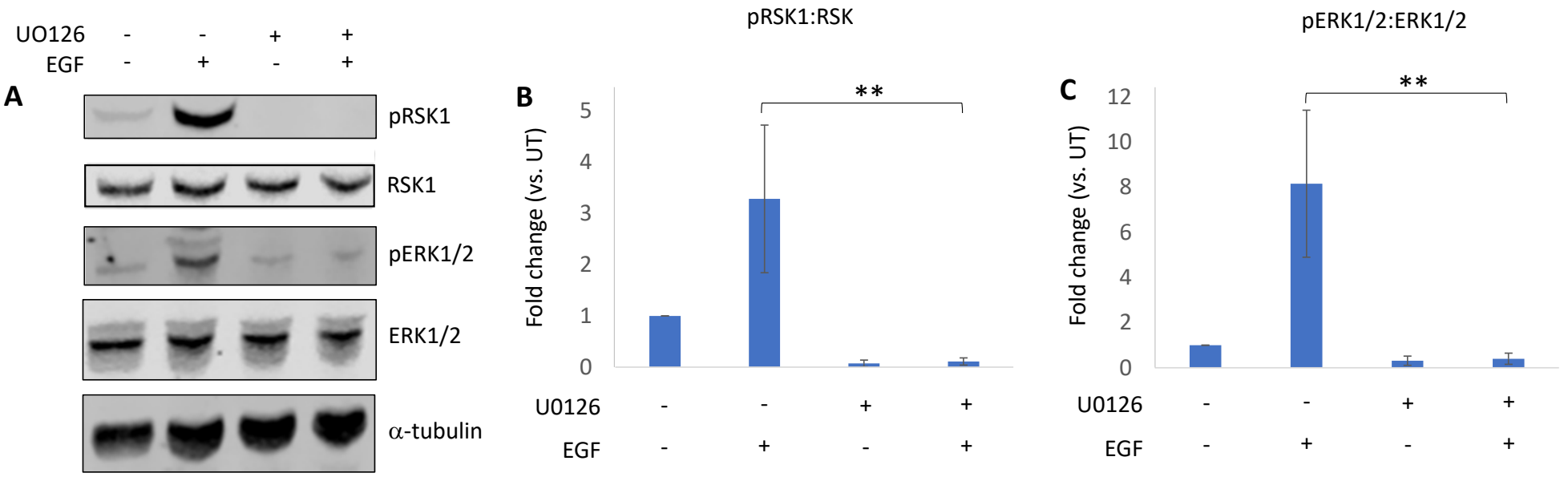


Fig. S4. Dependence of RSK1 phosphorylation on MAPK signaling in HeLa cells, related to Fig. 6. A. *Representative western blot of HeLa cell lysates from cells pre-treated with the MEK1 inhibitor, UO126, or vehicle alone followed by incubation with or without EGF for 10 minutes before lysis.* ***B.*** *Average normalized response of western blots. Error bars represent standard error about the mean of three independent experiments (n = 3). **: $p < 0.01$.*

Figure S5.

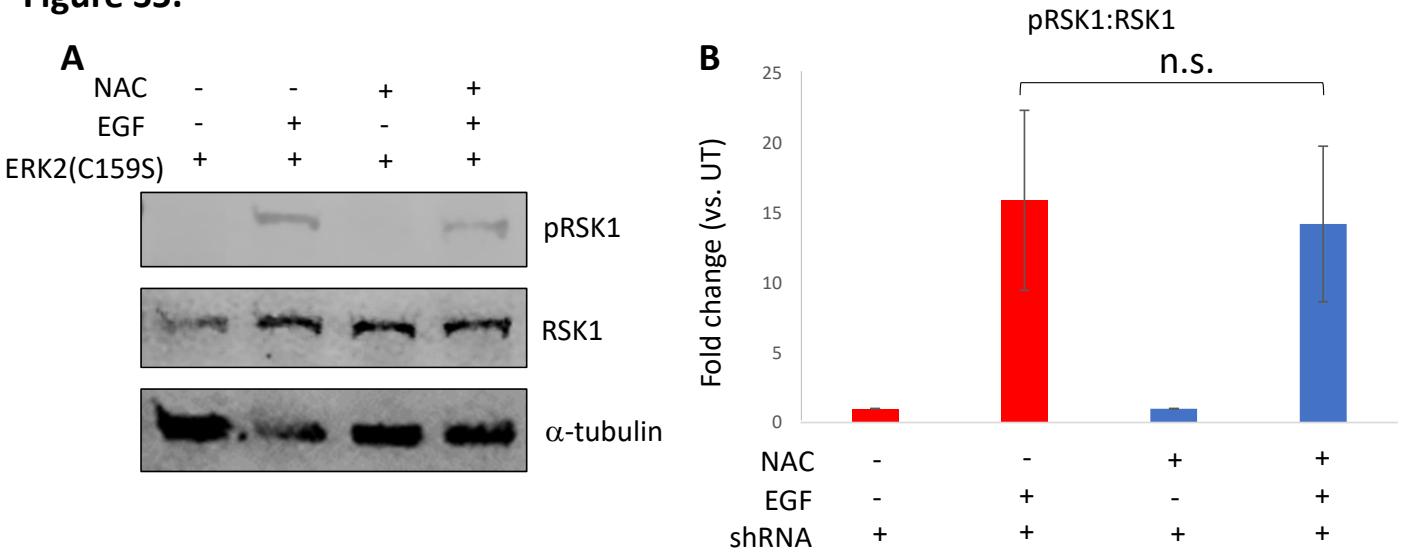


Fig. S5. Overexpression of ERK2(C159S) abolishes NAC-dependent changes in ERK2-mediated phosphorylation of RSK1, related to Fig. 6. A. *Representative western blot of HeLa cell lysates from cells that were transfected with the mammalian expression vector, pcDNA3.1, encoding ERK2(C159S) under the control of the strong CMV promoter. Transfected cells were serum-starved before being treated with N-acetyl-cysteine (NAC) for 45 minutes followed by stimulation with epidermal growth factor (EGF) or vehicle alone for 10 minutes. Lysates were probed with the indicated antibodies.* ***B.*** *Average normalized response of western blots. Error bars represent standard error about the mean of three independent experiments (n = 3). n.s.: not significant.*

Figure S6.

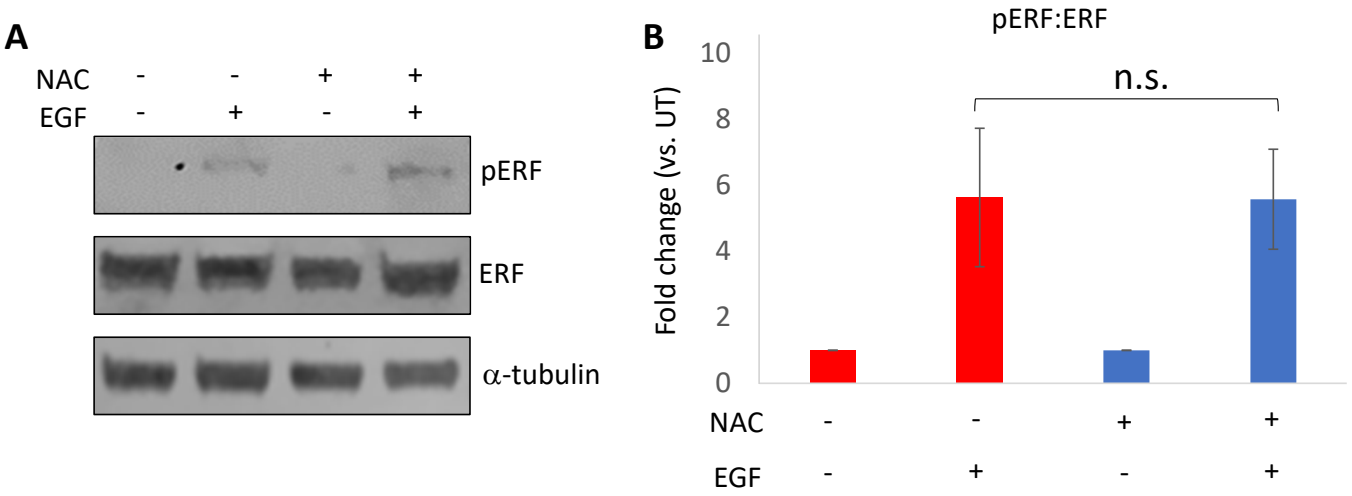
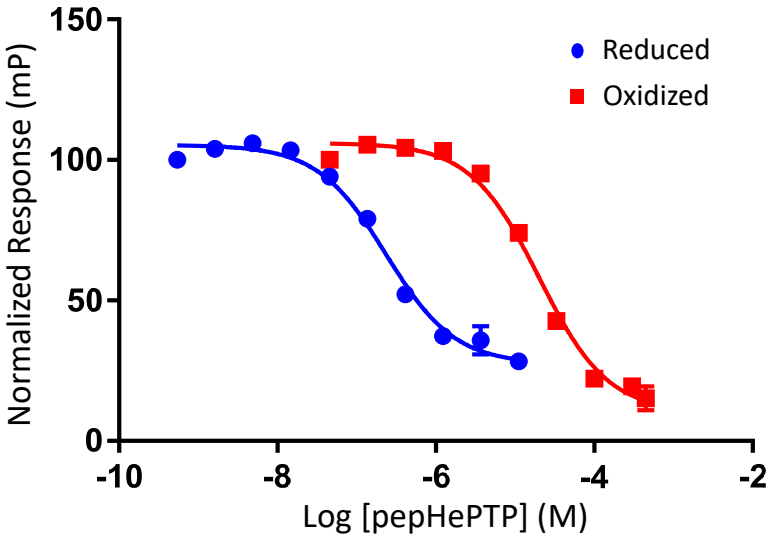


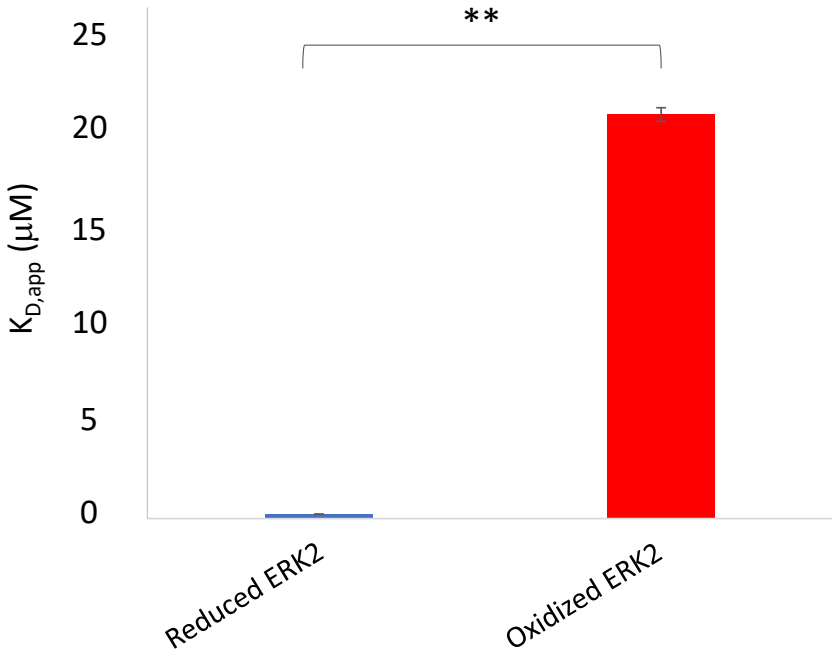
Fig. S6. NAC treatment does not affect ERK1/2-mediated phosphorylation of the F-site substrate, ERF, related to Fig. 6. A. *Representative western blot of HeLa cell lysates treated with N-acetyl-cysteine (NAC) for 45 minutes followed by stimulation with epidermal growth factor (EGF) or vehicle alone for 10 minutes. Lysates were probed with the indicated antibodies.* ***B.*** *Average normalized response of western blots. Error bars represent standard error about the mean of three independent experiments (n = 3). n.s.: not significant.*

Figure S7.

A



B



C

Interaction pair	$K_{D,app}$ (M) ± SE
Reduced ERK2-pepHePTP	$2.27 \times 10^{-7} \pm 3.08 \times 10^{-9}$
Oxidized ERK2-pepHePTP	$2.08 \times 10^{-5} \pm 3.15 \times 10^{-7}$

Fig. S7. Impact of oxidation of ERK2 on its interaction with pepHePTP, related to Figure 6. A. *Fluorescence polarization competition assays using oxidized (red) and reduced (blue) ERK2.* ***B.*** *Average $K_{D,app}$ of oxidized (red) and reduced (blue) ERK2 for unlabeled pepHePTP. Error bars represent the standard error about the mean of three independent experiments done in duplicate. **p < 0.01.*

Figure S8.

A

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ERK1     NDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLIN-TTCDLKICDFGLARIA-DPE-HD 197
ERK2     NDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLN-TTCDLKICDFGLARVA-DPD-HD 177
ERK5     LEHVRYFLYQLLRGLKYMHSAQVIHRDLKPSNLLVN-ENCELKIGDFGMARGLCTSP-AE 213
ERK7     DIHKRCIFYQLLRATKFIHSGRVIHRDQKPANVLLD-AACRVKLCDFGLARSLSDFPEGP 170
JNK1     HERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVK-SDCTLKILDFGLARTAGT----- 178
JNK2     HERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVK-SDCTLKILDFGLARTACT----- 178
JNK3     HERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVK-SDCTLKILDFGLARTAGT----- 216
p38α     DDHVQFLIYQILRGLKYIHSADIIHRDLKPSNLAVN-EDCELKILDFGLARHTDD----- 177
p38β     DEHVQFLVYQLLRGLKYIHSAGIIHRDLKPSNVAVN-EDCELRILDFGLARQADE----- 184
p38γ     EDRIQFLVYQMLKGLKYIHAAGVIHRDLKPGNLAVN-EDCELKILDFGLARQADS----- 180
p38δ     EEKVQYLVYQMLKGLKYIHSAGIVHRDLKPGNLAVN-EDCELKILDFGLARHTDA----- 177
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B

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Human        LMETDLYKLLK-TQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKI 165
Chimpanzee   LMETDLYKLLK-TQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKI 165
Rat          LMETDLYKLLK-TQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKI 163
Mouse        LMETDLYKLLK-TQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKI 163
Zebrafish    LMETDLYKLLK-TQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKI 174
Drosophila   LMETDLYKLLK-TQRLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNKTCDLKI 178
Yeast        LMQTDLHRVIS-TQMLSDDHIQYFIYQTLRAVKVLHGSNVIHRDLKPSNLLINSNCDLKV 153
Arabidopsis  LMDTDLHQIIKSSQVLSNDHCQYFLFQLLRGLKYIHSANILHRDLKPGNLLVNANCDLKI 174
```

Figure S8. Conservation of ERK2^{C159} Among Mitogen-Activated Protein Kinase Family Members, related to Discussion. A) *Multiple sequence alignment of* Rattus norvegicus *MAPK family members. Identical residues are highlighted in green while ERK2^{C159} is boxed in red.* ***B)*** *Multiple sequence alignment of ERK2 orthologs from human to Arabidopsis. Highlighting is as in A. All alignments were done using Clustal-Omega (EMBL-EBI).*