# Regulatory Roles of Conserved Phosphorylation Sites in the Activation T-Loop of the MAP Kinase ERK1


Shenshen Lai[1] and Steven Pelech[1, 2]

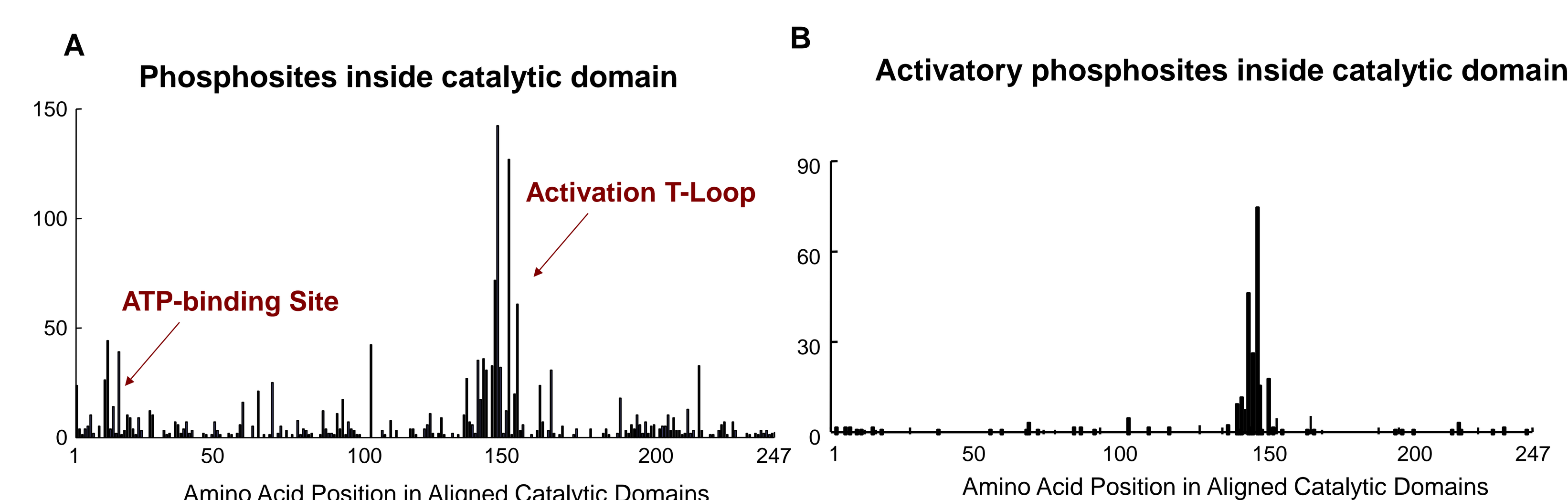
[1] Department of Medicine + Brain Research Centre, University of British Columbia, Vancouver, BC, Canada, [2] Kinexus Bioinformatics, Vancouver, BC, Canada


## Abstract


**The catalytic domains of most eukaryotic protein kinases are highly conserved. Phosphorylation of the activation T-loop, a variable region between kinase catalytic subdomains VII and VIII, is a common mechanism for stimulation of catalytic activity. The MAP kinase Extracellular signal-regulated kinase 1 (ERK1) serves as a paradigm for regulation of protein kinases in signaling modules. Our group investigated the possible roles of three conserved phosphosites in the activation loop of ERK1 flanking the well-documented pTEpY activating site. *In vitro* kinase assays with myelin basic protein (MBP) using the purified ERK1 phosphosite mutants supported the functional importance of T207 and Y210, but not T198 in regulating ERK1 catalytic activity. Single substitution of the T207 to glutamic acid abolished the activity of ERK1 without affecting the phosphorylation at TEY by MEK1. The Y210 site could be important for proper folding of ERK1 in this regulatory region, since the mutation of this residue caused decreases in protein solubility, and the Y210F mutant was not recognized by MEK1 for phosphorylation *in vitro*. Our data also indicated that ERK1 auto-phosphorylated T207, while the phosphorylation of Y210 was enhanced in presence of MEK1. We hypothesize that following the activation of ERK, subsequent slower phosphorylation of the flanking sites may result in autoinhibition of the kinase. Hyperphosphorylation within the kinase activation T-loop may serve as a general mechanism for protein kinase down-regulation after initial activation by their upstream kinases.**


## Background

**Figure 1. Distribution of phosphosites in human protein kinase catalytic domains**




Y axis - Number of confirmed phosphosites (A, total = 1942) and activation sites (B, total = 303) at each position in the alignment of 496 typical human protein kinase catalytic domains.

**Table 1. Phosphosites from activation T-loops of protein-serine/threonine kinases**

| | 144 | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CDK1 | P | I | R | T | Y | T | H | E | V | V | T | L | W | Y | R | S | P | E |
| CDK2 | P | V | R | T | Y | T | H | E | V | V | T | L | W | Y | R | A | P | E |
| GSK3 | R | G | E | P | N | V | S | Y | I | C | S | R | Y | Y | R | A | P | E |
| ERK1 | H | T | G | F | L | T | E | Y | V | A | T | R | W | Y | R | A | P | E |
| p38 alpha | T | D | D | E | M | T | G | Y | V | A | T | R | W | Y | R | A | P | E |
| JNK1 | T | S | F | M | M | T | P | Y | V | V | T | R | Y | Y | R | A | P | E |
| PKC alpha | D | G | V | T | T | R | T | F | C | G | T | P | D | Y | I | A | P | E |
| PKC delta | G | E | S | R | A | S | T | F | C | G | T | P | D | Y | I | A | P | E |
| RSK1 | H | E | K | K | A | Y | S | F | C | G | T | I | E | Y | M | A | P | E |
| MAPKAPK2 | S | H | N | S | L | T | T | P | C | Y | T | P | Y | Y | V | A | P | E |
| CAMK1 | P | G | S | V | L | S | T | A | C | G | T | P | G | Y | V | A | P | E |
| PIM1 | K | D | T | V | Y | T | D | F | D | G | T | R | V | Y | S | P | P | E |
| AKT2 | D | G | A | T | M | K | T | F | C | G | T | P | E | Y | L | A | P | E |
| PLK1 | D | G | E | R | K | K | T | L | C | G | T | P | N | Y | I | A | P | E |

Green, confirmed activation sites;
Yellow, confirmed phosphosites with unknown function;
Grey, potential phosphosites based on similarities.

## Results and Discussion

**Figure 2. Phosphorylation of GST-ERK1 by MEK1-ΔN3EE *in vitro***

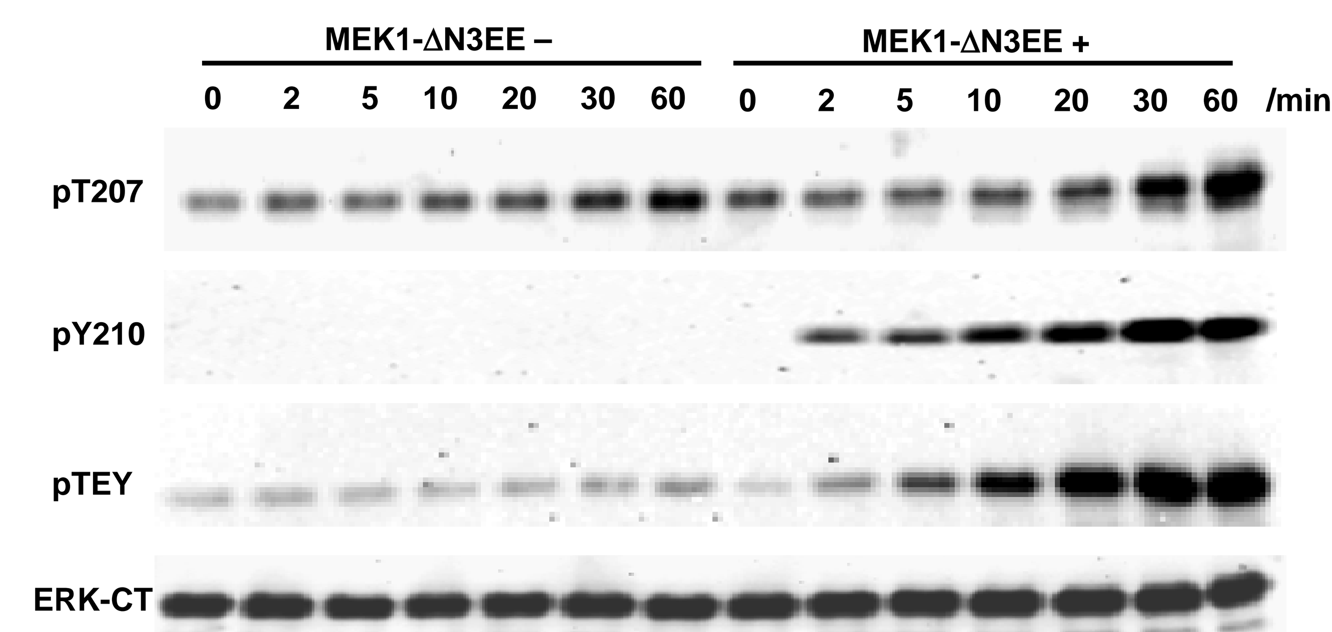



**Table 2. Mutants of ERK1 on flanking phosphosites T198, T207 and Y210**

| ERK1 | | 198 | | | | | | | | | 207 | | | 210 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WT | H | T | G | F | L | T | E | Y | V | A | T | R | W | Y | R | A | P | E |
| T198A | - | A | - | - | - | T | - | Y | - | - | T | - | - | Y | - | - | - | - |
| T207A | - | T | - | - | - | T | - | Y | - | - | A | - | - | Y | - | - | - | - |
| T207E | - | T | - | - | - | T | - | Y | - | - | E | - | - | Y | - | - | - | - |
| Y210F | - | T | - | - | - | T | - | Y | - | - | T | - | - | F | - | - | - | - |
| Y210E | - | T | - | - | - | T | - | Y | - | - | T | - | - | E | - | - | - | - |
| 2AF | - | A | - | - | - | T | - | Y | - | - | A | - | - | F | - | - | - | - |

**Figure 3. Phosphorylation of ERK1 mutants and their kinase activity towards MBP**

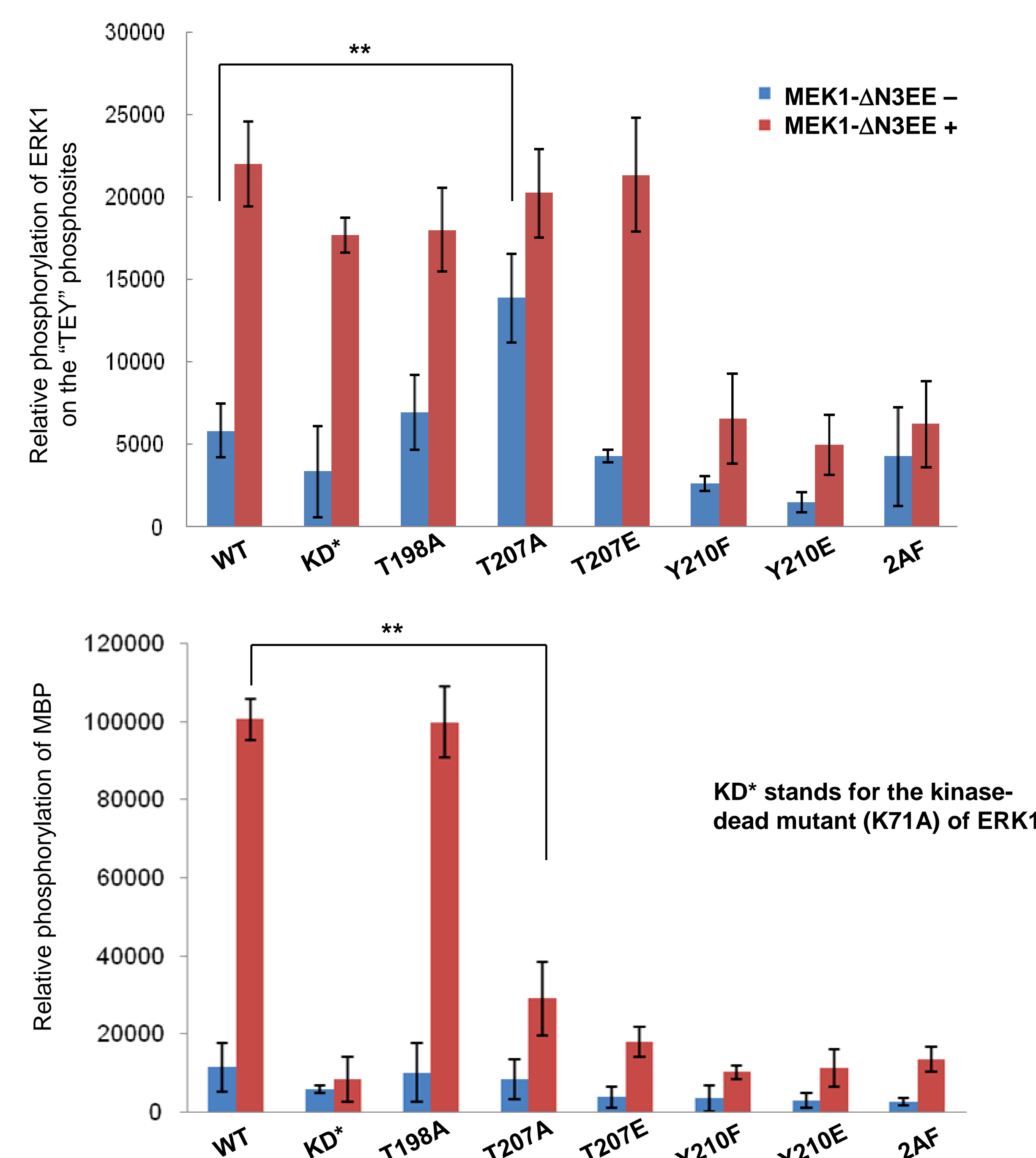
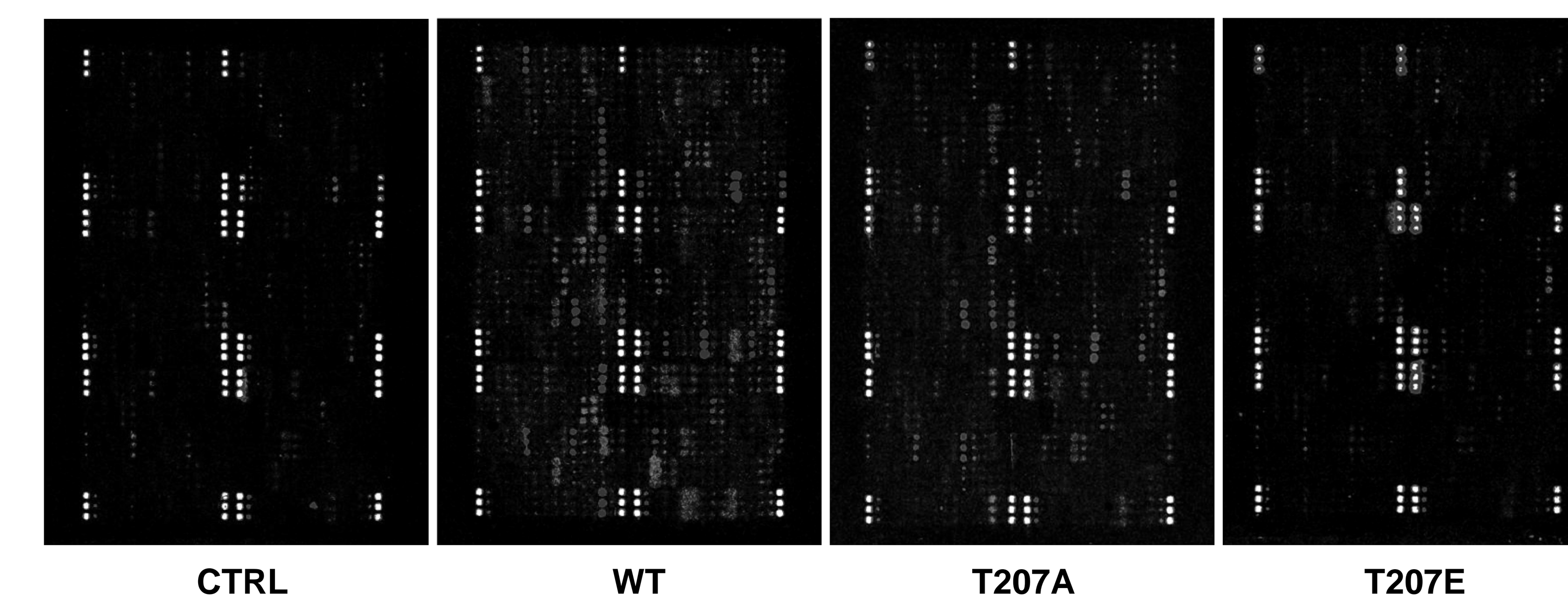
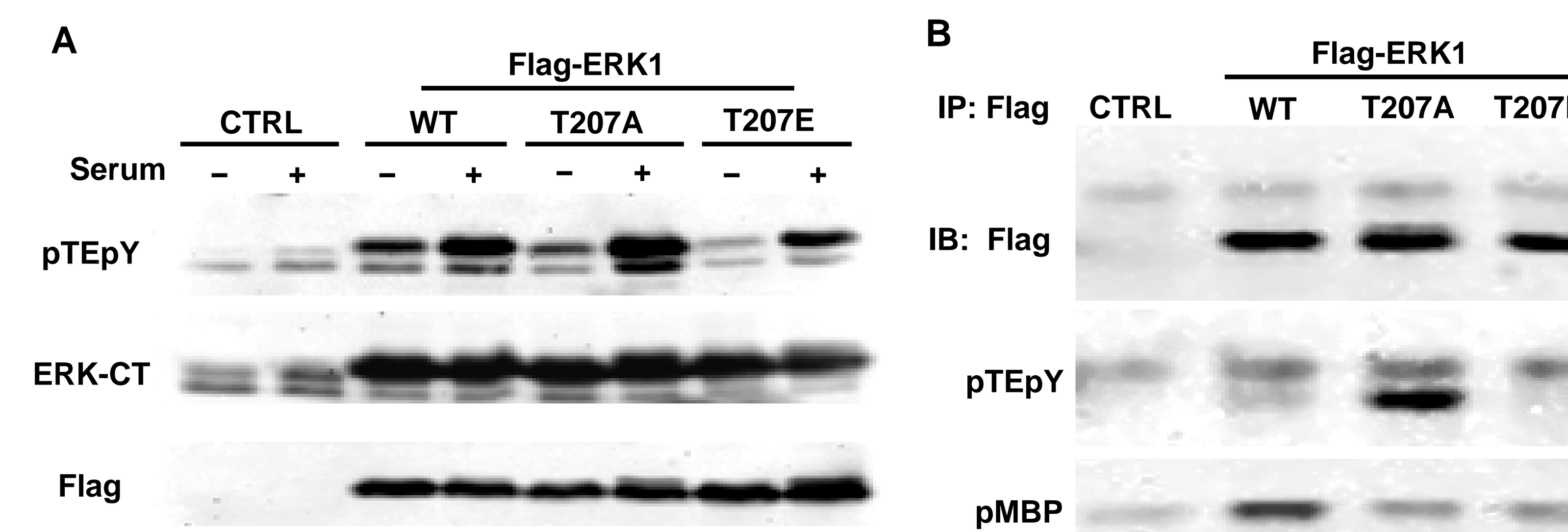



**Figure 4. Substrate specificity of ERK1 T207 mutants on peptide microarrays**




GST-ERK1 and its mutants were activated by MEK1-ΔN3EE before incubated with kinase substrate peptide microarray at 30°C for 2 hours. MEK1 activity was suppressed by UO126. One field (CTRL) was incubated with MEK1-ΔN3EE/UO126 only.

**Figure 5. Phosphorylation and kinase activity of ERK1 T207 mutants in HEK293 cells**




Flag-tagged wild-type ERK1 and T207 mutants were overexpressed in HEK293 cells. Stimulation with 10% fetal bovine serum for 10 minutes led to activation of the MAPK cascade and phosphorylation of ERK1/2 at the TEY site (A). Flag-ERK1 was immunoprecipitated with Flag tag antibody and assayed for MBP phosphorylation *in vitro* (B).

## Conclusion

**Based on our results, we propose the Y210 phosphosite of ERK1 becomes hyperphosphorylated after the phosphorylation of the "TEY" site by MEK1, which inhibits its catalytic activity. The T207 site also appears to be inhibitory, and arises from autophosphorylation. Our findings contribute to an improved understanding of the activation of MAPKs and protein kinases in general. Hyper-phosphorylation within the kinase activation T-loop following the initial activation by upstream kinases may serve as a general mechanism for protein kinase inhibition to prevent prolonged signaling.**

## Acknowledgement


This study is supported by Kinexus Bioinformatics Corporation. We thank Drs. Hong Zhang, Jane Shi, Gary Yalloway for helpful advice, Dr. Dirk Winkler for synthesizing all the peptides for antibody production and validation, and James Hopkins for assistance in the lab.