## Phylogeny

• MAP4K5 (KHS1) is assigned to the STE group, germinal-center kinase subfamily 1 (GCK-I) within the human kinome (manning2002theproteinkinase pages 3-3).  
• Verified animal orthologs include Mus musculus Map4k5, Rattus norvegicus Map4k5, Drosophila melanogaster Sps1 and Caenorhabditis elegans mig-15, demonstrating conservation from invertebrates to mammals (manning2002theproteinkinase pages 7-8).  
• Plant homologs cluster in Clade III subclade IIIC, typified by Arabidopsis thaliana AtMAP4K5, indicating retention of MAP4K5-like kinases across the green lineage (pan2021acomprehensivephylogenetic pages 4-5).  
• Within the STE group, MAP4K5 is most closely related to MAP4K1-4 and MAP4K6-7, all sharing an N-terminal kinase domain followed by proline-rich/PEST and CNH regions characteristic of GCK-I enzymes (thiriet2013cytoplasmicproteinserinethreonine pages 4-7, marcotte2017germinal‐centerkinase‐likekinase pages 1-5).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr ⇌ ADP + protein-O-phospho-L-Ser/Thr (liu2022ste20phosphorylationof pages 5-6).

## Cofactor Requirements

Catalysis requires divalent metal ions; Mg²⁺ or Mn²⁺ are supplied in standard STE-kinase assay buffers (manning2002theproteinkinase pages 7-8, miller2019comprehensiveprofilingof pages 30-31).

## Substrate Specificity

• Direct phosphorylation of activation-loop threonines has been demonstrated for AMPKα1 T183, SIK1 T182, SIK2 T175 and SIK3 T221; substitution of these residues abolishes modification, indicating strict preference for a central Thr in an activation-loop context (liu2022ste20phosphorylationof pages 4-5, liu2022ste20phosphorylationof pages 8-9).  
• A global consensus peptide motif for MAP4K5 was not reported in the profiled STE20 kinase dataset (miller2019comprehensiveprofilingof pages 30-31).

## Structure

Domain organisation  
– N-terminal catalytic domain (~aa 1–300) executing phosphotransfer (marcotte2017germinal‐centerkinase‐likekinase pages 1-5).  
– Central proline-rich/PEST segment containing SH3-binding motifs and degrons (thiriet2013cytoplasmicproteinserinethreonine pages 4-7).  
– C-terminal citron-homology (CNH) domain and leucine-rich element mediating scaffold interactions (marcotte2017germinal‐centerkinase‐likekinase pages 1-5).

Three-dimensional organisation  
• The crystal structure of the isolated kinase domain reveals an activation-loop-swapped dimer; α-AL and α-EF helices from one protomer insert into the partner active site, stabilising an active configuration (marcotte2017germinal‐centerkinase‐likekinase pages 9-13).  
• Catalytic Lys45 forms the canonical salt bridge with Glu61 in the C-helix, and regulatory-spine residues Met64-His134-Ile137-Phe155 align in the active state (marcotte2017germinal‐centerkinase‐likekinase pages 9-13).  
• Activation-loop residue Ser170 contacts substrate; its phosphorylation enhances substrate binding and catalytic efficiency (marcotte2017germinal‐centerkinase‐likekinase pages 9-13).  
• A structured acidic C-terminal extension docks onto a basic groove of the neighbouring protomer, reminiscent of PIF-tide engagement in AGC kinases (marcotte2017germinal‐centerkinase‐likekinase pages 9-13).

## Regulation

Post-translational modifications  
• Ser170 phosphorylation is indispensable; dephosphorylated enzyme or S170A mutant retains ≤3 % activity (marcotte2017germinal‐centerkinase‐likekinase pages 25-30).  
• Ser174 is likewise critical; S174A abrogates phosphorylation of AMPKα1 and SIK3 (liu2022ste20phosphorylationof pages 5-6).  
• SARS-CoV-2 3CLpro cleaves MAP4K5, generating fragments with reduced kinase activity (pablos2021mechanisticinsightsinto pages 30-32).  
• Central PEST motifs are proposed degradation signals, although the cognate ubiquitin ligase is not identified (thiriet2013cytoplasmicproteinserinethreonine pages 4-7).

Conformational/allosteric control  
Activation-loop phosphorylation promotes and locks the swapped-dimer in an active conformation; loss of the phosphate destabilises dimer integrity and catalytic turnover (marcotte2017germinal‐centerkinase‐likekinase pages 9-13).

## Function

• Acts upstream of the stress-activated JNK pathway via TRAF2 engagement and selective binding to CRK/CRKL adaptors, integrating integrin and TNF-α signals (thiriet2013cytoplasmicproteinserinethreonine pages 4-7).  
• Functions as an alternative AMPK upstream kinase: over-expression in LKB1/CaMKK2-deficient HEK293T cells increases AMPKα T172 phosphorylation and downstream ACC1 activation (liu2022ste20phosphorylationof pages 5-6).  
• Directly activates SIK1/2/3 by phosphorylating their activation-loop threonines (liu2022ste20phosphorylationof pages 8-9).  
• Forms complexes with CRK, CRKL, NCK, GRB2, Abl kinases and SOS1/2, supporting assembly of multiprotein signalling platforms (thiriet2013cytoplasmicproteinserinethreonine pages 4-7).  
• Expression has been demonstrated in human embryonic kidney-derived HEK293T cells used for biochemical studies (liu2022ste20phosphorylationof pages 5-6).

## Inhibitors

• Compound 1: ATP-competitive inhibitor; IC₅₀ ≈ 110 nM against MAP4K5 kinase domain, forming hinge hydrogen bonds with Asp100 and occupying a pocket adjacent to catalytic Lys45 (marcotte2017germinal‐centerkinase‐likekinase pages 25-30).  
• Vemurafenib (PLX4032): binds MAP4K5 with 48 % residual activity at 100 nM in KINOMEscan profiling, indicating moderate affinity (klovekorn2021fromofftoontarget pages 1-8).

## Other Comments

• Proteolytic processing by SARS-CoV-2 3CLpro links MAP4K5 to host kinase remodeling during COVID-19 infection (pablos2021mechanisticinsightsinto pages 30-32).  
• Structural and functional similarities to other GCK-I kinases implicate MAP4K5 in inflammatory and oncogenic processes (marcotte2017germinal‐centerkinase‐likekinase pages 1-5).

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