## Phylogeny

Orthologs: Mus musculus Map3k8, Rattus norvegicus Tpl2, Danio rerio map3k8 paralogs, and the Drosophila melanogaster MAP3K “slpr” (slipper) (manning2002theproteinkinase pages 2-3, manning2002theproteinkinase pages 3-3, guan2023functionsofmap3ks pages 13-14).  
Kinome position: STE group → MAP3K family → MAP3K8/Tpl2 subfamily (manning2002theproteinkinase pages 2-3).  
Distinctive evolutionary feature: the Gly-rich loop contains Pro145 instead of the canonical glycine, a substitution unique among human kinases (gantke2011regulationandfunction pages 10-12).

## Reaction Catalyzed

ATP + [MAP2K]-Ser/Thr-OH ⇌ ADP + [MAP2K]-Ser/Thr-O-PO₃²⁻ (gantke2011regulationandfunction pages 2-4).

## Cofactor Requirements

Catalysis requires divalent metal ions; in vitro activity is supported by 5 mM Mg²⁺ + 2 mM Mn²⁺ (chan2005trafdependentassociationof pages 1-2). Independent peptide-array profiling employed 10 mM MgCl₂ (parikh2009comparisonofpeptide pages 2-3).

## Substrate Specificity

Verified protein substrates: MEK1/2, MKK4, MEK5, MKK6; MKK7 is not phosphorylated (gantke2011regulationandfunction pages 2-4, chiu2024map3k8isa pages 7-8).  
Peptide-array mapping reveals a stringent preference for phenylalanine at −3/−2/−1 and +1 and for lysine at basic positions surrounding the phosphorylated Ser/Thr; most peptides remain unmodified, indicating narrow specificity (parikh2009comparisonofpeptide pages 6-7, parikh2009comparisonofpeptide pages 7-9).  
No consensus motif for MAP3K8 was reported in the kinome-wide atlas by Johnson et al.; therefore a definitive linear motif is presently undefined (parikh2009comparisonofpeptide pages 3-5).

## Structure

Domain architecture: N-terminal regulatory segment harboring a nuclear-export sequence; central bilobed kinase domain; C-terminal inhibitory degron (residues 435-457) (collins2018theiκbproteinbcl3 pages 6-9, gantke2011regulationandfunction pages 2-4).  
Crystallography: a 2.6 Å structure of the catalytic core (COT/Tpl2) displays an atypical fold with a flexible P-loop insert (gutmann2015thecrystalstructure pages 1-1).  
Catalytic motifs: Lys-Glu salt bridge, HRD catalytic triad, DFG Mg²⁺-binding motif, and the unique Pro145 in the Gly-rich loop (bayliss2015theysand pages 20-25, gantke2011regulationandfunction pages 10-12).  
Activation loop: Thr290 autophosphorylation site; Ser400 phosphorylation creates a 14-3-3 docking site (gantke2011regulationandfunction pages 7-9, gutmann2015thecrystalstructure pages 1-1).  
Hydrophobic R- and C-spines align in the active state; the C-terminal tail folds back to suppress activity until proteolysis removes the degron (bayliss2015theysand pages 1-4, gantke2011regulationandfunction pages 2-4).  
Quaternary assembly: physiological folding and stability require a stoichiometric complex with NF-κB1 p105 and ABIN-2 (gantke2011regulationandfunction pages 1-2, webb2019abin2ofthe pages 2-3).

## Regulation

Sequestration: In resting cells, MAP3K8 is inhibited within the p105–ABIN-2 ternary complex (gantke2011regulationandfunction pages 1-2).  
Activation: IKKβ phosphorylates p105, causing K48-linked ubiquitination and proteasomal degradation that liberates MAP3K8 (collins2018theiκbproteinbcl3 pages 1-6).  
Autophosphorylation/Trans-phosphorylation: Thr290 (autocatalytic) and Ser400 (auto- or trans-phosphorylation) are required for full activity and 14-3-3 binding (gantke2011regulationandfunction pages 7-9).  
Nuclear turnover: After stimulus-induced nuclear translocation, MAP3K8 is polyubiquitinated and degraded; BCL-3 accelerates this process, lowering the MAPK activation threshold (collins2018theiκbproteinbcl3 pages 13-17).  
Metabolic modulation: Extracellular L-arginine enhances Thr290/Ser400 phosphorylation and boosts signalling amplitude (gantke2011regulationandfunction pages 7-9).  
C-terminal degron: residues 435-457 target the kinase for proteasomal destruction; truncations stabilize the protein and confer constitutive activity (gantke2011regulationandfunction pages 2-4).

## Function

Expression: Abundant in macrophages and other myeloid cells; inducible in airway epithelium and adipocytes (chiu2024map3k8isa pages 7-8, gantke2011regulationandfunction pages 1-2).  
Upstream stimuli: TLR2/4/9 ligands, IL-1β, TNF, CD40, TRAF6; activation routes require the MyD88–IKK axis (gantke2011regulationandfunction pages 1-2, chiu2024map3k8isa pages 7-8).  
Downstream signalling: Dominant MEK1/2→ERK1/2 cascade; context-dependent activation of MKK4→JNK and MKK3/6→p38 (chiu2024map3k8isa pages 7-8, gantke2011regulationandfunction pages 2-4).  
Cytokine and chemokine control: indispensable for LPS-induced TNF; modulates IL-1β, IL-6, IL-8, IL-10, IL-12, CCL2, CXCL8, and related chemokines (gantke2011regulationandfunction pages 7-9, chiu2024map3k8isa pages 7-8).  
Metabolism: couples IL-1β/TNF signalling to ERK-dependent lipolysis in adipocytes (gantke2011regulationandfunction pages 1-2).  
Immune polarization: required for macrophage M2 lipid-metabolic programming and limits Schistosoma-induced Th2 fibrosis (kannan2016tpl2regulatesmacrophage pages 20-22).  
Antiviral interface: Foot-and-mouth disease virus VP1 blocks Thr290 phosphorylation, dampening IRF3-mediated IFN-β induction (guan2023functionsofmap3ks pages 5-7).

## Inhibitors

• C34: ATP-competitive probe that suppresses MAP3K8-dependent cytokine production in macrophages (kannan2016tpl2regulatesmacrophage pages 20-22).  
• Quinoline-3-carbonitrile series; analogue IIIa is the most potent inhibitor in biochemical assays targeting Tpl2 (hu2007inhibitionoftpl2 pages 1-1).  
• 8-Substituted-4-anilino-6-aminoquinoline-3-carbonitriles demonstrate selective Tpl2 inhibition with in-vivo anti-inflammatory efficacy (unknownauthors2023combinationtherapiestargeting pages 199-201).  
Quantitative IC₅₀ values were not provided in the cited excerpts.

## Other Comments

Oncogenic activation: C-terminal truncations that delete the degron enhance stability and transforming potential in lymphoid models (gantke2011regulationandfunction pages 10-12).  
Disease linkage: Elevated MAP3K8 activity is implicated in rheumatoid arthritis, Crohn’s disease, colitis-associated cancer, melanoma, breast carcinoma and inflammatory bowel disease (gantke2011regulationandfunction pages 10-12, webb2019abin2ofthe pages 10-10).

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