## Phylogeny

Experimentally validated orthologs exist in Homo sapiens, Mus musculus, Rattus norvegicus, Equus caballus, Gallus gallus and Danio rerio; all conserve the GFE motif that replaces the canonical DFG in active kinases (murphy2013thepseudokinasemlkl pages 2-3, goldberg2023emergingfunctionsof pages 4-6, unknownauthors2018smallmoleculeconjugation pages 13-18).  
Kinome-wide surveys anchored to the Manning 2002 framework classify MLKL in the pseudokinase subset of the “Other” group; sequence clustering places it closest to IRAK-like kinases yet clearly separate from the RIPK1–5 serine/threonine kinase family (byrne2017pseudokinasesupdateon pages 2-4, unknownauthors2022biochemicalandstructural pages 18-24).  
MLKL therefore represents a vertebrate-specific, catalytically inert branch of the eukaryotic kinome (goldberg2023emergingfunctionsof pages 11-12).

## Reaction Catalyzed

ATP + protein-Ser/Thr/Tyr → ADP + protein-Ser/Thr/Tyr-P (no phosphoryl-transfer activity detected; MLKL is a pseudokinase) (murphy2013thepseudokinasemlkl pages 1-2).

## Cofactor Requirements

Binds ATP in a cation-independent manner; no Mg²⁺ or Mn²⁺ requirement reported (byrne2017pseudokinasesupdateon pages 2-4).

## Substrate Specificity

Not applicable; no enzymatic substrates identified for this pseudokinase (murphy2013thepseudokinasemlkl pages 1-2).

## Structure

Domain organisation: N-terminal four-helix bundle (4HB, residues 1-~125) that executes membrane disruption; two brace helices (~126-180) transmit conformational signals; C-terminal pseudokinase domain (~181-464) functions as a regulatory switch (unknownauthors2018smallmoleculeconjugation pages 13-18, murphy2013thepseudokinasemlkl pages 9-9).  
3-D data: crystal structures are available for full-length mouse MLKL (PDB 4BTF) (murphy2013thepseudokinasemlkl pages 9-9), the human pseudokinase domain (PDB 4MWI) (lucet2017characterizationofligand pages 5-6) and a ligand-bound human construct (PDB 6O5Z) (pierotti2020potentinhibitionof pages 24-29).  
Catalytic/regulatory features: the VAIK lysine (K219) contacts Q343 on an activation-loop helix occupying the αC position; the HRD motif is absent and the DFG motif is replaced by GFE, abolishing Mg²⁺ coordination (murphy2013thepseudokinasemlkl pages 2-3).  
Unique elements: brace helices constrain the 4HB until phosphorylation-induced release; the activation-loop helix locks the pseudoactive site in an autoinhibited conformation (unknownauthors2018smallmoleculeconjugation pages 13-18, najafov2019tamkinasespromote pages 12-12).

## Regulation

Phosphorylation  
• Activating: RIPK3 phosphorylates Thr357 and Ser358 (human) or Ser345 (mouse), driving oligomerisation and membrane trafficking (unknownauthors2018smallmoleculeconjugation pages 13-18, najafov2019tamkinasespromote pages 12-12).  
• Inhibitory: Ser158 and Ser248 phosphorylation dampens necroptosis (unknownauthors2018smallmoleculeconjugation pages 13-18).  
Genetic activation  
• Pseudoactive-site mutations K219M, Q343A or phosphomimetic S345D trigger RIPK3-independent activity (murphy2013thepseudokinasemlkl pages 6-7).  
Protein cofactors  
• TAM kinases enhance MLKL oligomer formation (najafov2019tamkinasespromote pages 12-12).  
• HSP90–CDC37 chaperoning stabilises oligomers and supports membrane localisation (pierotti2020potentinhibitionof pages 29-32).

## Function

Expression: abundant in bone marrow, brain, heart, kidney, liver and lung (murphy2013thepseudokinasemlkl pages 4-5).  
Upstream signals: TNFR1 via RIPK1/RIPK3, TLR3/4-TRIF and ZBP1 pathways converge on RIPK3 to phosphorylate MLKL (murphy2013thepseudokinasemlkl pages 1-2, unknownauthors2018smallmoleculeconjugation pages 7-13).  
Mechanism: phosphorylation induces tetramerisation, translocation to the inner plasma-membrane leaflet, membrane disruption and Ca²⁺ influx; ESCRT-III components can repair MLKL-induced lesions (unknownauthors2018smallmoleculeconjugation pages 18-22, pierotti2020potentinhibitionof pages 29-32).  
Key interactors: RIPK1 (scaffold), RIPK3 (activating kinase), HSP90 (chaperone), ESCRT-III machinery (membrane repair) (pierotti2020potentinhibitionof pages 29-32).

## Inhibitors

Necrosulfonamide – covalently modifies Cys86 of human MLKL; blocks oligomerisation and membrane localisation (zhuang2020smallmoleculeinhibitorsof pages 27-29).  
GW806742X – non-covalent small molecule that disrupts MLKL function in human cells (pierotti2020potentinhibitionof pages 1-5).  
Compound 2 – nanomolar inhibitor binding MLKL, RIPK1 and RIPK3; protects mice in TNF-induced systemic inflammatory response syndrome (pierotti2020potentinhibitionof pages 24-29).  
Analogue 68 – high-potency covalent Cys86 binder; potent but cytotoxic (zhuang2020smallmoleculeinhibitorsof pages 27-29).

## Other Comments

Disease associations: necroptosis contributes to TNF-mediated SIRS (pierotti2020potentinhibitionof pages 1-5), ischemia-reperfusion injury and neurodegeneration (unknownauthors2018smallmoleculeconjugation pages 13-18) and has been implicated in ALS via RIPK-MLKL signalling (najafov2019tamkinasespromote pages 12-12).  
Pathogenic mutations: T357I and S358F alter the activation loop; F385I and L280P found in gastric cancer specimens; all enhance or dysregulate MLKL activation (murphy2013thepseudokinasemlkl pages 7-8, goldberg2023emergingfunctionsof pages 11-12).

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