## Phylogeny

• Member of the STE kinase group → germinal-centre kinase (GCK) family → GCK III sub-family together with MST4 and STK25 (rak2024developmentofselective pages 4-8).  
• Human MST3 catalytic domain shares ≈90 % sequence identity with MST4 and <20 % with Hippo paralogues MST1/2 (record2010structuralcomparisonof pages 1-3).  
• Canonical sequences display 93 % overall identity between human STK24, mouse Stk24 and rat Stk24 orthologs (qiu2023molecularmechanismsinvolved pages 1-3).  
• Distant orthologous relationship to Saccharomyces cerevisiae Sps1/Ste20 kinases, reflecting an ancestral Ste20 lineage (sugden2013sockmistsmask pages 1-2).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr ⇄ ADP + protein-L-Ser/Thr-phosphate (ko2010structuresofhuman pages 1-2).

## Cofactor Requirements

• Highest catalytic efficiency with Mn²⁺; Mg²⁺, Zn²⁺ or Co²⁺ can substitute with reduced activity (ko2010structuresofhuman pages 1-2, record2010structuralcomparisonof pages 1-3, sugden2013sockmistsmask pages 16-16).

## Substrate Specificity

• Phosphoproteomic profiling assigns a preference for hydrophobic-X-Thr-[Arg/Lys] motifs consistent with the broader STE20 consensus (rak2024developmentofselective pages 4-8).  
• Biochemical mapping defines DW(aHy)F X₀/₃ T\* (Hy)(Basic) or (aHy) X₀/₁ T\* (Hy)(Basic) where T\* is the phospho-acceptor threonine (sugden2013sockmistsmask pages 15-16).  
• Verified cellular substrates include NDR1/2 kinases (sugden2013sockmistsmask pages 15-16) and RhoA Ser26 controlling neuronal migration (qiu2023molecularmechanismsinvolved pages 9-10).

## Structure

• Two-domain organisation: N-terminal kinase domain aa 36–286 and C-terminal regulatory tail aa 287–443 containing a bipartite NLS (278–292) and NES (335–386) (qiu2023molecularmechanismsinvolved pages 1-3).  
• Crystal structures resolve active DFG-in conformations with adenine/ADP (PDB 3CKX) and Mn²⁺, revealing Lys53-Glu70 salt bridge, phosphorylated Thr178 in the activation loop, and Asp162 metal coordination (ko2010structuresofhuman pages 1-2).  
• Autophosphorylated structures with diverse inhibitors deposited under PDB 4QML–4QMZ (olesen2016discoveryofdiverse pages 6-8) and selective probes PDB 8BZJ, 8QLR–8QLT (rak2024developmentofselective pages 80-83).  
• Regulatory tail harbours cis-autophosphorylation site Thr328, caspase-3 cleavage motif AETD313, and an N-terminal myristoylation sequence influencing localisation (sugden2013sockmistsmask pages 15-16, qiu2023molecularmechanismsinvolved pages 3-7).

## Regulation

Post-translational modifications  
– Thr178 autophosphorylation required for catalytic activation (olesen2016discoveryofdiverse pages 3-4).  
– Thr328 autophosphorylation further enhances activity (sugden2013sockmistsmask pages 15-16).  
– Ser79 phosphorylation by CDK5 modulates neuronal migration signalling (qiu2023molecularmechanismsinvolved pages 3-7).  
– Thr18 phosphorylation on the brain-specific isoform MST3b by PKA augments axon growth (qiu2023molecularmechanismsinvolved pages 3-7).  
– PP2A within the STRIPAK complex dephosphorylates MST3 and restrains signalling (qiu2023molecularmechanismsinvolved pages 3-7, olesen2016discoveryofdiverse pages 3-4).  
– Caspase-3 cleavage at AETD313 yields a nuclear kinase fragment with >10-fold activity increase (wang2011stk24promotesmyogenic pages 28-32).  
– N-terminal myristoylation directs membrane versus nuclear partitioning of the cleaved fragment (qiu2023molecularmechanismsinvolved pages 3-7).

Allosteric / complex regulation  
• MO25 binding stimulates catalytic output (miller2019comprehensiveprofilingof pages 28-29).  
• STRIPAK assembly suppresses kinase activity via PP2A recruitment (olesen2016discoveryofdiverse pages 1-3).  
• Interaction with CCM3/PDCD10 targets MST3 to Golgi and centrosomes (sugden2013sockmistsmask pages 1-2).

## Function

Expression  
• Ubiquitous; highest levels in heart, skeletal muscle, pancreas and developing brain; neuron-restricted isoform MST3b (wang2011stk24promotesmyogenic pages 28-32, sugden2013sockmistsmask pages 2-3, qiu2023molecularmechanismsinvolved pages 1-3, qiu2023molecularmechanismsinvolved pages 9-10).

Biological roles and partners  
• Mediates oxidative-stress-induced apoptosis by activating JNK1/2 and p38 MAPKs (ko2010structuresofhuman pages 1-2).  
• Controls caspase-independent nuclear death pathway via AIFM1/ENDOG translocation (qiu2023molecularmechanismsinvolved pages 9-10).  
• Promotes radial neuronal migration and optic/radial nerve axon regeneration through RhoA Ser26 phosphorylation (qiu2023molecularmechanismsinvolved pages 9-10, getu2023themammaliansterile pages 3-4).  
• Regulates cell migration through STRIPAK-dependent modulation of paxillin/PTPN12 signalling (olesen2016discoveryofdiverse pages 1-3).  
• Phosphorylates NDR1/2 linking to Hippo-YAP pathway modulation (sugden2013sockmistsmask pages 15-16).  
• Influences epithelial sodium channel activity contributing to hypertension models (qiu2023molecularmechanismsinvolved pages 9-10).

Upstream modifiers  
CDK5, PKA, PP2A/STRIPAK (qiu2023molecularmechanismsinvolved pages 3-7).

Downstream substrates  
NDR1/2, RhoA, paxillin/PTPN12 (sugden2013sockmistsmask pages 15-16, qiu2023molecularmechanismsinvolved pages 9-10).

## Inhibitors

• Pyrido[2,3-d]pyrimidin-7(8H)-one derivatives MR24, MR26, MR30; cellular IC₅₀ 3–20 nM; co-crystal structures 8BZJ, 8QLR–8QLT; high kinome selectivity in NanoBRET assays (rak2024developmentofselective pages 80-83, rak2024developmentofselective pages 28-33).  
• Broad-spectrum panel of 14 type I inhibitors including staurosporine, bosutinib, danusertib; enzymatic IC₅₀ range 0.003–23 µM; structures 4QML–4QMZ (olesen2016discoveryofdiverse pages 3-4, olesen2016discoveryofdiverse pages 6-8).

## Other Comments

• Over-expression correlates with poor prognosis in gastric and breast cancers; knock-down reduces tumour proliferation (getu2023themammaliansterile pages 3-4).  
• Disruption of CCM3 interaction links MST3 to cerebral cavernous malformations (sugden2013sockmistsmask pages 1-2).  
• Functional involvement in ENaC-mediated hypertension provides a cardiovascular disease connection (qiu2023molecularmechanismsinvolved pages 9-10).

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