## Phylogeny

Member of the STE group, Germinal Center Kinase subfamily 6 (GCK-VI) within the Ste20-like serine/threonine kinases (Serafim et al., 2021; Thiriet, 2013). The closest human paralogue is SLK, sharing ~76–84 % identity across the kinase domain and 61–70 % within the coiled-coil region (Investigation of Resistance, 2020; Serafim et al., 2021). Verified vertebrate orthologues include mouse LOK (98 % identity in the catalytic domain), rat Stk10 and zebrafish Stk10, while invertebrate counterparts comprise Drosophila Slik and C. elegans GCK-3, underscoring broad metazoan conservation (Leroy et al., 2016; Serafim et al., 2021).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Serafim et al., 2021).

## Cofactor Requirements

Requires Mg²⁺ for catalytic activity (Serafim et al., 2021).

## Substrate Specificity

Principal cellular targets are the ERM proteins ezrin, radixin and moesin; phosphorylation of moesin Thr558 is a routine activity read-out (Leroy et al., 2016; Serafim et al., 2021). Additional reported substrates include vimentin Ser56 and PLK1 in vitro (Investigation of Resistance, 2020; Serafim et al., 2021). Motif profiling in a recent kinome atlas revealed a distinct consensus sequence, although the precise residues were not provided in the excerpt (Serafim et al., 2021).

## Structure

The protein comprises an N-terminal kinase domain (≈ residues 1–300), a proline-rich segment and an extended C-terminal coiled-coil that lacks a CRIB motif (Investigation of Resistance, 2020; Thiriet, 2013). A crystal structure of the isolated kinase domain (PDB 2J7T) displays activation-segment exchange dimerisation that promotes trans-autophosphorylation (Goldsmith et al., 2007). Canonical catalytic motifs include VAIK Lys49, HRD Asp158 and DFG Asp176; autophosphorylation occurs on Thr183 within the activation segment (Goldsmith et al., 2007). Asp332 represents a caspase-3 cleavage site separating the kinase and coiled-coil regions (Goldsmith et al., 2007). A co-crystal with a 3-anilino-4-arylmaleimide inhibitor shows bifurcated hinge hydrogen bonds (E111/C113) and displacement of the glycine-rich loop (Serafim et al., 2021). Basic residues adjacent to helix αG form a PIP₂-dependent wedge that tethers the kinase to cortical membranes (Serafim et al., 2021).

## Regulation

Activity is enhanced by autophosphorylation of Thr183 and by activation-segment exchange homodimerisation (Goldsmith et al., 2007). Caspase-3 cleavage at Asp332 during apoptosis abolishes ERM phosphorylation (Leroy et al., 2016). Spatial confinement is provided by a PIP₂-anchored basic wedge that restricts signalling to the apical cortex and lymphocyte uropod (Serafim et al., 2021). The kinase also acts as a negative modulator of MAP3K1/MEKK1 signalling (Annunziata et al., 2020).

## Function

Highest expression is observed in spleen, thymus and bone marrow, predominantly within lymphocytes (Leroy et al., 2016; Thiriet, 2013). ERM phosphorylation maintains cortical rigidity, microvilli structure and promotes lymphocyte migration (Leroy et al., 2016; Serafim et al., 2021). Association with and phosphorylation of PLK1 links the kinase to G2/M cell-cycle progression (Investigation of Resistance, 2020; Thiriet, 2013). By suppressing MAP3K1/MEKK1 it modulates MAPK pathway output (Annunziata et al., 2020). Proximity-labelling studies identify interactors such as KRAS, RHOA, RHOB and CRKL, situating the kinase within Rho/Ras cytoskeletal networks (Profiling the Interactome, 2023).

## Inhibitors

A series of 3-anilino-4-arylmaleimides yields compound 31 with an enzymatic IC₅₀ ≈ 12 nM and cellular IC₅₀ ≈ 1.4 µM; crystallographic data confirm ATP-site engagement (Serafim et al., 2021).

## Other Comments

A cancer-associated R634H mutation in the coiled-coil domain abrogates NF-κB suppression and confers anti-apoptotic activity in peripheral T-cell lymphoma (Investigation of Resistance, 2020; Leroy et al., 2016). Stk10-knockout mice display accelerated tumour growth due to tumour-micro-environment dysregulation (Ma et al., 2022). Depletion of the kinase sensitises triple-negative breast-cancer cells to the PI3Kα inhibitor BYL-719 (Investigation of Resistance, 2020).

## 9. References

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