## Phylogeny

STK16 is conserved across eukaryotes and belongs to the Numb-associated kinase (NAK) family, which also includes AAK1, BIKE/BMP2K, and GAK (wang2019serinethreonineproteinkinase pages 1-3, wang2019tyr198isthe pages 7-9). According to the priority kinome classification by Manning et al., the NAK family is assigned to the ‘Other’ group of kinases, distinct from conventional kinase families (liu2017stk16regulatesactin pages 14-15, wang2019serinethreonineproteinkinase pages 1-3, tanaka2022degradationofstk16 pages 6-8, unknownauthors2016blockingmitoticexit pages 96-102, wang2019serinethreonineproteinkinase pages 9-11). Contradictory classifications place STK16 in the CAMK group (tanaka2022degradationofstk16 pages 6-8) or the AGC group (johnson2023anatlasof pages 3-4). STK16 shares approximately 25% sequence identity with Aurora kinase A (rangwala2022kinasesondouble pages 11-13).

## Reaction Catalyzed

The kinase catalyzes the ATP-dependent transfer of a phosphate group to serine or threonine residues on substrate proteins (alfahad2024virtualscreeningand pages 9-12, wang2019serinethreonineproteinkinase pages 5-7). It also undergoes autophosphorylation (liu2017stk16regulatesactin pages 14-15, wang2019serinethreonineproteinkinase pages 1-3). The enzyme acts as a dual-specificity kinase, able to phosphorylate serine/threonine residues and autophosphorylate on a tyrosine residue (rangwala2022kinasesondouble pages 11-13, wang2019tyr198isthe pages 7-9).

## Cofactor Requirements

Catalytic activity requires the presence of divalent metal ion cofactors, specifically Mg2+ or Mn2+ (alfahad2024virtualscreeningand pages 9-12, eswaran2008structureofthe pages 1-2, wang2019serinethreonineproteinkinase pages 1-3, tanaka2022degradationofstk16 pages 6-8).

## Substrate Specificity

Peptide library screens have identified a preferred consensus substrate motif as X-X-P/V/I-f-H/Y-T*-N/G-X-X-X, where ‘f’ or ‘Φ’ is an aliphatic residue and T* indicates the threonine phosphorylation site (eswaran2008structureofthe pages 1-2, wang2019serinethreonineproteinkinase pages 3-5). The kinase phosphorylates substrates primarily on threonine residues but also on serine (wang2019serinethreonineproteinkinase pages 3-5). According to the priority source, a substrate specificity profile for STK16 was derived from a comprehensive PSPA (positional scanning peptide array) analysis (johnson2023anatlasof pages 3-4).

## Structure

STK16 is a 305-amino acid protein composed of a kinase catalytic domain, a short N-terminal domain, and a C-terminus (wang2019serinethreonineproteinkinase pages 1-3). Its crystal structure (PDB: 2BUJ) reveals an atypical activation segment architecture characterized by a beta sheet and a large alpha-helical insertion (aEF), also known as the activation segment C-terminal helix (ASCH), a feature unique to the NAK family (eswaran2008structureofthe pages 1-2, eswaran2008structureofthe pages 10-10, wang2019serinethreonineproteinkinase pages 1-3). This architecture, stabilized by hydrophobic and polar interactions involving residues such as Arg147 in the HRD motif, maintains the kinase in a constitutively active conformation without requiring activation loop phosphorylation (wang2019serinethreonineproteinkinase pages 1-3, wang2019serinethreonineproteinkinase pages 3-5). In contrast, other reports describe the structure as a canonical kinase domain with a characteristic activation loop essential for regulation (alfahad2024virtualscreeningand pages 9-12, liu2017stk16regulatesactin pages 14-15). A flexible loop region comprising residues 98–106 is vital for ligand binding (alfahad2024virtualscreeningand pages 9-12).

## Regulation

STK16 activity is regulated by post-translational modifications, including covalent lipid modifications and autophosphorylation (alfahad2024virtualscreeningand pages 9-12, wang2019serinethreonineproteinkinase pages 1-3). The kinase undergoes N-terminal myristoylation at Gly2, which is necessary for subsequent palmitoylation at cysteine residues that mediate Golgi membrane association (eswaran2008structureofthe pages 1-2, wang2019serinethreonineproteinkinase pages 3-5). The specific palmitoylation sites are reported differently as Cys6 and Cys8 (eswaran2008structureofthe pages 1-2, wang2019serinethreonineproteinkinase pages 3-5, rangwala2022kinasesondouble pages 11-13) or Cys13, Cys14, and Cys15 (wang2019serinethreonineproteinkinase pages 5-7).

Autophosphorylation is a key regulatory mechanism, and the kinase exhibits constitutive activity (wang2019serinethreonineproteinkinase pages 1-3, rangwala2022kinasesondouble pages 11-13). Mapped autophosphorylation sites on the activation segment include Thr185, Ser197, and Tyr198 (wang2019serinethreonineproteinkinase pages 5-7, wang2019tyr198isthe pages 1-3). Autophosphorylation at Tyr198 is essential for kinase activity, proper localization to the Golgi and cell membrane, and cell cycle progression (wang2019tyr198isthe pages 1-3, wang2019tyr198isthe pages 7-9). The dynamic phosphorylation state at Tyr198 regulates STK16’s subcellular localization (wang2019tyr198isthe pages 3-7). Degradation of STK16 occurs via the ubiquitin-proteasome system and is mediated by KCTD17 (tanaka2022degradationofstk16 pages 6-8).

## Function

STK16 is ubiquitously expressed, with high levels observed in the liver, kidney, testis, thymus, spleen, and heart muscle (eswaran2008structureofthe pages 1-2, wang2019serinethreonineproteinkinase pages 1-3, guinea2006nucleocytoplasmicshuttlingof pages 4-5). The kinase localizes primarily to the Golgi apparatus but shuttles to the nucleus upon Golgi disorganization (eswaran2008structureofthe pages 1-2, guinea2006nucleocytoplasmicshuttlingof pages 4-5). Known interacting partners include DRG1, ENO1, MAL2, actin, GlcNAcK, and WDR1 (alfahad2024virtualscreeningand pages 9-12, eswaran2008structureofthe pages 1-2, wang2019serinethreonineproteinkinase pages 1-3). It phosphorylates substrates such as DRG1 (at Thr100) and 4EBP1 (eswaran2008structureofthe pages 1-2, wang2019tyr198isthe pages 7-9).

STK16 participates in TGF-β signaling and is involved in regulating Golgi assembly, TGN protein secretion and sorting, actin dynamics, and cell cycle progression through G2/M, prometaphase, and cytokinesis (alfahad2024virtualscreeningand pages 9-12, liu2017stk16regulatesactin pages 12-14, wang2019serinethreonineproteinkinase pages 9-11). It directly binds actin and modulates its polymerization and depolymerization in a manner dependent on its concentration and kinase activity (liu2017stk16regulatesactin pages 12-14, liu2017stk16regulatesactin pages 12-14).

## Inhibitors

Experimentally investigated small molecule inhibitors include Neratinib, which exhibits a binding free energy (ΔG) of -36.61 kcal/mol (alfahad2024virtualscreeningand pages 9-12). Natural compounds NPC132329 and NPC160898 show inhibitory potential based on molecular dynamics simulations (alfahad2024virtualscreeningand pages 9-12). The selective experimental inhibitor STK16-IN-1 has been shown to reduce cancer cell proliferation and suppress kinase activity (liu2017stk16regulatesactin pages 2-4, wang2019serinethreonineproteinkinase pages 3-5, wang2019tyr198isthe pages 1-3).

## Other Comments

STK16 is implicated in cancer progression and is considered a therapeutic target (alfahad2024virtualscreeningand pages 9-12). It has been identified as a potential target to sensitize cancer cells to mitotic chemotherapies (unknownauthors2016blockingmitoticexit pages 96-102). Inhibition of STK16 is proposed as a therapeutic approach in oncology (wang2019serinethreonineproteinkinase pages 3-5).

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