## Phylogeny

STK40 is phylogenetically classified as a pseudokinase within the human kinome, related to the Tribbles family (manning2002theproteinkinase pages 3-4, durzynska2017stk40isa pages 1-3). It is identified as a distant homolog of Tribbles-family pseudokinases, sharing approximately 19-21% sequence identity with Trib1, Trib2, and Trib3 (durzynska2017stk40isa pages 1-3). While one source classifies STK40 as an active kinase phylogenetically close to but distinct from the Tribbles-family (manning2002theproteinkinase pages 1-2), multiple other sources confirm its pseudokinase nature based on structural and biochemical evidence (durzynska2017stk40isa pages 1-3, tao2024pseudokinasestk40promotes pages 1-1). Within broader kinome classifications, STK40 is placed in the CAMK group and CAMK family (johnson2023anatlasof pages 4-4). The loss of catalytic function in STK40 is conserved across distant orthologs, including in frogs (durzynska2017stk40isa pages 6-8).

## Reaction Catalyzed

Serine/threonine kinases typically catalyze the transfer of the γ-phosphate from ATP to the hydroxyl group of serine or threonine residues on substrate proteins (durzynska2017stk40isa pages 15-19). ATP + a protein substrate → ADP + a phosphoprotein product However, STK40 is a pseudokinase that is enzymatically inactive (durzynska2017stk40isa pages 1-3). It lacks ATP-binding properties and therefore does not catalyze this phosphotransferase reaction (tao2024pseudokinasestk40promotes pages 1-1, durzynska2017stk40isa pages 1-3). This has been confirmed by ATP-binding assays and thermal shift assays, which demonstrate that STK40 does not bind ATP or its analogues (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 15-19).

## Cofactor Requirements

The catalytic activity of serine/threonine kinases typically requires a divalent metal ion cofactor, such as Mg²⁺, to coordinate ATP binding and facilitate catalysis (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 5-6). STK40 has no cofactor requirements because it is catalytically inactive (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 11-15). Its magnesium-binding DFG motif is replaced by an NFC sequence, which removes the critical aspartate residue needed for Mg²⁺ coordination (durzynska2017stk40isa pages 5-6).

## Substrate Specificity

There is no evidence for a defined substrate consensus motif for STK40 (johnson2023anatlasof pages 2-3, durzynska2017stk40isa pages 1-3). Its classification as a pseudokinase that lacks catalytic activity and the ability to bind ATP is consistent with the absence of defined substrate specificity (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 5-6, johnson2023anatlasof pages 1-2).

## Structure

The 3D structure of the STK40 kinase homology domain has been determined by X-ray crystallography to a resolution of 2.5 Å (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 3-5). The protein adopts a canonical kinase fold with N- and C-lobes (durzynska2017stk40isa pages 3-5). The KHD is followed by a C-terminal COP1-binding motif (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 11-15).

Despite being inactive, the N-lobe of STK40 resembles that of an active kinase, with the αC helix in an active-like conformation stabilized by a salt bridge (Lys66-Glu93) (durzynska2017stk40isa pages 3-5). However, several key features prevent catalytic activity. The ATP binding pocket is occluded by the substitution of an alanine with glutamine (Q64) in the β3 loop (durzynska2017stk40isa pages 5-6). The P-loop is divergent, and the DFG motif in the activation loop is replaced by an NFC sequence (durzynska2017stk40isa pages 5-6, durzynska2017stk40isa pages 11-15). The hydrophobic spine is disrupted, which results in STK40 adopting an inactive-like conformation (durzynska2017stk40isa pages 11-15, durzynska2017stk40isa pages 15-19). However, STK40 maintains hydrophobic spine packing that supports a stable, yet inactive, conformation (durzynska2017stk40isa pages 5-6).

STK40 possesses unique structural features, including an extended loop insertion between β4 and β5 that contains a predicted nuclear localization signal (NLS), and a 10-amino acid insert in the hinge region that serves as a structural buttress (durzynska2017stk40isa pages 3-5, durzynska2017stk40isa pages 5-6).

## Regulation

STK40 function is primarily regulated by protein-protein interactions rather than by allosteric or post-translational modifications of its kinase domain (durzynska2017stk40isa pages 1-3). A unique insertion in the N-lobe between β4 and β5 contains a potential nuclear localization signal (NLS) that may be regulated by phosphorylation of nearby serine residues (durzynska2017stk40isa pages 6-8). The primary known regulatory mechanism is its function as an adaptor protein that binds the E3 ubiquitin ligase COP1 (durzynska2017stk40isa pages 1-3, tao2024pseudokinasestk40promotes pages 10-12).

## Function

STK40 expression levels increase during skeletal muscle differentiation in vitro in C2C12 myoblasts and in vivo in fetal mouse muscle (he2017serinethreoninekinase40 pages 1-2).

STK40 functions as a non-catalytic adaptor or scaffolding protein (durzynska2017stk40isa pages 1-3). Its primary interacting partner is the E3 ubiquitin ligase COP1 (RFWD2), which it binds via a C-terminal VPD/E motif (durzynska2017stk40isa pages 3-5, durzynska2017stk40isa pages 11-15). This interaction facilitates the K48-linked polyubiquitination and proteasomal degradation of specific substrates (tao2024pseudokinasestk40promotes pages 1-1). Substrates targeted for degradation by the STK40-COP1 complex include the transcription factors FOXO1 and FOXO4 (tao2024pseudokinasestk40promotes pages 1-1, tao2024pseudokinasestk40promotes pages 10-12). STK40 also regulates the protein level of histone deacetylase 5 (HDAC5), a corepressor of the myogenic transcription factor MEF2 (he2017serinethreoninekinase40 pages 1-2).

STK40 is involved in several signaling pathways and cellular processes: - **T-Cell Differentiation**: In CD4+ T cells, STK40 promotes the differentiation of TH1 and TH17 cells by mediating the degradation of FOXO1 and FOXO4, which in turn de-represses TH1/TH17 signature genes (tao2024pseudokinasestk40promotes pages 1-1, tao2024pseudokinasestk40promotes pages 10-12). - **Skeletal Muscle Differentiation**: It functions as a positive regulator of skeletal myogenesis by modulating the HDAC5-MEF2 transcriptional axis (he2017serinethreoninekinase40 pages 1-2). - **Stem Cell Differentiation**: Overexpression in embryonic stem cells induces differentiation through pathways that include Erk/MAPK (durzynska2017stk40isa pages 1-3). It also links the pluripotency factor Oct4 to the Erk/MAPK signaling pathway (durzynska2017stk40isa pages 10-11). - **Transcriptional Regulation**: STK40 acts as a negative regulator of NF-κB and p53-mediated gene transcription (tao2024pseudokinasestk40promotes pages 1-1, durzynska2017stk40isa pages 10-11). - **Adipogenesis**: Knockdown of STK40 enhances adipocyte differentiation through changes in C/EBPβ and C/EBPδ levels (durzynska2017stk40isa pages 1-3).

## Other Comments

STK40 is implicated in several diseases. Knockout in mice leads to perinatal lethality due to respiratory failure linked to defects in lung epithelial cell maturation (durzynska2017stk40isa pages 1-3, durzynska2017stk40isa pages 11-15). It is associated with psoriasis, where it is overexpressed, and esophageal squamous cell carcinoma via regulation by miR-31 (durzynska2017stk40isa pages 1-3). In the context of immunology, T cell-specific deletion of STK40 attenuates symptoms in mouse models of experimental autoimmune encephalomyelitis (EAE) and colitis, identifying it as a potential therapeutic target for autoimmune diseases (tao2024pseudokinasestk40promotes pages 1-1).

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