## Phylogeny

TSSK1B (UniProt Q9BXA7; also termed STK22A) is a member of the Ca²⁺/calmodulin-regulated protein kinase (CAMK) group within the testis-specific serine/threonine kinase (TSSK) family and has also been placed in the broader AMPK superfamily (Manning et al., 2002; Salicioni et al., 2020). The TSSK family is evolutionarily conserved and required for male reproduction (Nayyab et al., 2025). TSSK1B is a primate-specific paralogue that likely arose by retrotransposition and resides on human chromosome 5, whereas human TSSK2 maps to 22q11 (Nayyab et al., 2025; Salicioni et al., 2020). In mice, the orthologous Tssk1 and Tssk2 genes are adjacent on chromosome 16 (Xu et al., 2008).

## Reaction Catalyzed

ATP + [target protein] ⇌ ADP + phospho-[protein] (Salicioni et al., 2020).

## Cofactor Requirements

Catalytic activity requires a divalent cation. Mg²⁺ is preferred, whereas Mn²⁺ supports little or no activity (Salicioni et al., 2020; UnknownAuthors, 2011).

## Substrate Specificity

Kinase-substrate profiling indicates a preference for basic residues at the −3 and −2 positions relative to the Ser/Thr phosphorylation site (Johnson et al., 2023; Salicioni et al., 2020). TSSK1B phosphorylates the AMARA peptide (AMARAASAAALARRR) and the testis-specific protein TSKS; candidate phospho-sites on TSKS include Ser-281, Ser-285 (in vivo target of TSSK2), and Ser-288 (Basu, 2009; UnknownAuthors, 2014; Xu et al., 2008).

## Structure

The protein contains a canonical bilobal kinase fold with an N-terminal lobe bearing the αC-helix and a C-terminal lobe that houses the activation segment; AlphaFold modelling supports a flexible activation loop (Salicioni et al., 2020; UnknownAuthors, 2011).

## Regulation

Activity is modulated by post-translational phosphorylation. Autophosphorylation of Thr172 within the activation (T)-loop is essential for full activity (Basu, 2009; Salicioni et al., 2020). Interaction with PPP1CC2 (via the scaffold TSKS) and with HSP70/HSP90 cochaperones further influences stability and activity (Salicioni et al., 2020; UnknownAuthors, 2014).

## Function

Expression is restricted to testis, with localisation in post-meiotic spermatids and mature sperm (Salicioni et al., 2020; Nayyab et al., 2025). TSSK1B supports spermatogenesis, influencing sperm morphology, motility, flagellogenesis, mitochondrial sheath formation, and chromatoid-body remodelling (Salicioni et al., 2020). It phosphorylates TSKS and indirectly associates with PPP1CC2 (Salicioni et al., 2020). Although overlapping with TSSK2, the two kinases are not fully redundant; human TSSK1B partially rescues germ-cell defects in a Drosophila model where TSSK2 does not (Nayyab et al., 2025).

## Inhibitors

Low-nanomolar ATP-competitive inhibitors with pyrrolopyrimidine or pyrimidine scaffolds have been reported, including the pyrimidine compound TAE684 and related analogues (Salicioni et al., 2020; Nayyab et al., 2025). Current compounds lack adequate kinase selectivity.

## Other Comments

Deletion of the 5q22.2–q23.1 region containing TSSK1B is linked to asthenoteratozoospermia, and missense variants occur in ~10 % of screened infertile men (Nayyab et al., 2025). Combined deletion of Tssk1 and Tssk2 in mice causes male infertility (Xu et al., 2008). Because of its testis-restricted expression and essential role in fertility, TSSK1B is under investigation as a non-hormonal male contraceptive target (Salicioni et al., 2020).

## 9. References

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