Phylogeny  
– Orthologs reported in Mus musculus (Tssk6), Bos taurus (Tssk6) and a single Drosophila melanogaster TSSK homolog whose deletion causes male sterility that can be partially rescued by human TSSKs (nayyab2025identificationoftssk1 pages 18-19).  
– Bayesian phylogenetic analyses cluster TSSK6 within the testis-specific serine/threonine kinase clade that branches from the AMPK-related subgroup of the CAMK group in the Manning kinome (salicioni2020testisspecificserinekinase pages 30-31, salicioni2020testisspecificserinekinase pages 31-32).

Reaction Catalyzed  
ATP + [protein] ⇄ ADP + [protein]-O-phospho-L-serine/threonine (salicioni2020testisspecificserinekinase pages 14-14).

Cofactor Requirements  
Catalytic activity is Mn²⁺-dependent; TSSK1/2 are Mg²⁺-specific, whereas the remaining family members, including TSSK6, require Mn²⁺ (salicioni2020testisspecificserinekinase pages 15-16).

Substrate Specificity  
– In vitro, TSSK6 phosphorylates the myelin basic protein–derived peptide GKGRGLSLARFAKK (salicioni2020testisspecificserinekinase pages 14-15).  
– Consensus motif resembles the basic residue–rich preference seen in AMPK-related kinases; a distinct Johnson 2023 consensus for TSSK6 has not yet been reported (salicioni2020testisspecificserinekinase pages 30-31).

Structure  
– Minimal two-segment architecture: N-terminal Ser/Thr kinase domain followed by a short C-terminal tail; overall protein length among the smallest in the human kinome (salicioni2020testisspecificserinekinase pages 16-17, salicioni2020testisspecificserinekinase pages 30-31).  
– Catalytic lysine in β3 strand (VAIK motif), HRD catalytic triad, and DFG magnesium-binding motif are conserved; the activation loop (T-loop) harbors the autophosphorylated threonine required for activity (salicioni2020testisspecificserinekinase pages 14-14).  
– AlphaFold model AF-Q9BXA6-F1 predicts a canonical bilobed kinase fold with an exposed cysteine adjacent to the ATP pocket that can be exploited for covalent inhibitor design (salicioni2020testisspecificserinekinase pages 20-21).  
– Surface patch engaging HSP90/HSP70 and the co-chaperone SIP is located on the C-terminal lobe, stabilizing the active conformation (salicioni2020testisspecificserinekinase pages 16-17).

Regulation  
Post-translational  
• Autophosphorylation on the T-loop threonine activates the kinase (salicioni2020testisspecificserinekinase pages 14-14).  
• HSP90 binding, facilitated by HSP70 and SIP, protects TSSK6 from ubiquitination and proteasomal degradation; pharmacological HSP90 inhibition abolishes catalytic activation (jha2013heatshockprotein pages 1-2, salicioni2020testisspecificserinekinase pages 16-17).

Allosteric/Conformational  
• Chaperone-mediated loading of the phosphorylated T-loop into the active site is mandatory; no upstream activating kinase is required (salicioni2020testisspecificserinekinase pages 14-14, salicioni2020testisspecificserinekinase pages 16-17).

Function  
Expression  
– Strictly testis-specific; highest levels in elongating and elongated spermatids, negligible in mature sperm (salicioni2020testisspecificserinekinase pages 16-17).

Biological roles  
– Component of sperm flagellar doublet microtubules, implicated in regulation of sperm motility (salicioni2020testisspecificserinekinase pages 30-31).  
– Governs DNA condensation during post-meiotic chromatin remodeling and the histone-to-protamine transition, partly via γH2AX formation (salicioni2020testisspecificserinekinase pages 31-32).  
– Essential for Izumo1 relocalization during the acrosome reaction; Tssk6-null sperm fail to fuse with oocytes despite normal ICSI-mediated activation (salicioni2020testisspecificserinekinase pages 19-20).  
– Tssk6-null male mice exhibit low sperm count, head and flagellar malformations, compromised motility and complete infertility (salicioni2020testisspecificserinekinase pages 19-20).

Interaction network  
– Directly associates with HSP90, HSP70 and SIP for maturation and stability (salicioni2020testisspecificserinekinase pages 16-17).  
– Functional interplay with Izumo1 during sperm–egg fusion is documented; additional microtubule protein substrates are inferred from flagellar localization (salicioni2020testisspecificserinekinase pages 30-31).

Inhibitors  
Family-oriented screening campaigns have yielded covalent and non-covalent scaffolds that exploit a conserved cysteine in the ATP pocket, but no TSSK6-selective chemical probe has been disclosed to date (salicioni2020testisspecificserinekinase pages 20-21).

Other Comments  
– TSSK6 deletion produces infertility in mice by haploinsufficiency, underscoring its critical role in spermatogenesis (salicioni2020testisspecificserinekinase pages 31-32).  
– No human infertility-linked point mutations in TSSK6 have been functionally characterized in the current literature set (salicioni2020testisspecificserinekinase pages 14-14).

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