## Phylogeny

Homo sapiens TNK1 (UniProt Q13470) is the reference ortholog (Ahmed & Miller, 2022). Mus musculus Tnk1 (Q9D0C6) is frequently used in phospho-proteomic studies (Unknown authors, 2020a). Danio rerio tnk1a conserves the proline-rich phospho-cluster that corresponds to human S502 (Unknown authors, 2022a). Together with ACK1/TNK2, TNK1 forms the ACK sub-family within the Tyrosine Kinase (TK) group of the Manning kinome classification (Balasooriya et al., 2024; Prieto-Echagüe & Miller, 2011).

## Reaction Catalyzed

ATP + protein-L-tyrosine ⇌ ADP + protein-L-tyrosine-phosphate (Ahmed & Miller, 2022).

## Cofactor Requirements

Catalysis requires Mg²⁺; Mn²⁺ can substitute in vitro (Chan et al., 2021; Ahmed & Miller, 2022).

## Substrate Specificity

• Highest in-vitro turnover toward a WASP-derived PxxP peptide (Km = 214 µM).  
• Secondary phosphorylation detected on an Abl YxxP peptide (Ahmed & Miller, 2022).  
• Cellular substrates include PLC-γ1 Y783 and STAT3 Y705; STAT5 is not phosphorylated (Unknown authors, 2020a).  
• Yeast phospho-proteomics defines an enriched motif P-x-x-pY-[K/R]-[K/R] (Unknown authors, 2016).  
• No additional intrinsic specificity data reported in the 2024 tyrosine-kinome atlas (Unknown authors, 2020a).

## Structure

Domain map: SAM 1-111; kinase core 112-350 (activation-loop autophosphorylation site Y277); SH3 351-410; proline-rich region 411-589 harbouring 14-3-3 site S502; UBA 590-666 (Chan et al., 2021; Ahmed & Miller, 2022).  
AlphaFold model AF-Q13470-F1 spans the full sequence, retaining canonical β3 Lys, HRD274-276, and DFG292-294 motifs (Ahmed & Miller, 2022; Unknown authors, 2022a).  
The UBA folds into a five-helix bundle forming a high-affinity ubiquitin-binding pocket (Chan et al., 2021).  
No experimental crystal or cryo-EM structures are available to date (Ahmed & Miller, 2022).

## Regulation

• Autophosphorylation on Y277 enhances activity (Unknown authors, 2022a).  
• MARK1-4 phosphorylate S502, creating a 14-3-3 docking site that sequesters and inhibits TNK1 (Unknown authors, 2022a).  
• Disruption of 14-3-3 binding (S502A or C-terminal truncation) leads to cytosolic puncta and elevated signalling (Unknown authors, 2022a).  
• The C-terminal UBA binds K48-, K63-, and M1-linked poly-ubiquitin with nanomolar affinity and is required for full activation (Unknown authors, 2020a).  
• Y661 within the UBA is a major phosphorylation site that modulates both 14-3-3 and ubiquitin interactions (Unknown authors, 2022a).

## Function

Expression: mRNA is ubiquitous in fetal tissues; adult expression is mainly in prostate, testis, ovary, colon, and small intestine. Elevated levels are reported in AML, ALL, and certain prostate and ovarian cancer lines (Unknown authors, 2020a).  
Upstream regulator: LKB1-activated MARK kinases catalyse the inhibitory S502 phosphorylation (Unknown authors, 2022a).  
Key interactors: 14-3-3 proteins, poly-ubiquitin chains, p62/SQSTM1, and TBK1 within ubiquitin condensates (Unknown authors, 2020a; Unknown authors, 2022a).  
Downstream signalling: phosphorylates PLC-γ1 and STAT3, suppresses Ras-RAF-MEK-ERK and NF-κB pathways, and augments IFN-STAT1 antiviral signalling (Unknown authors, 2020b; Unknown authors, 2020a).

## Inhibitors

• TP-5801: ATP-competitive; biochemical IC₅₀ = 1.4 nM, Ba/F3 cell IC₅₀ = 37-78 nM; oral dosing reduces tumour burden and extends survival in TNK1-driven mouse models (Unknown authors, 2020a; Unknown authors, 2022b).  
• (R)-9b: micromolar inhibition in radiometric kinase assays (Ahmed & Miller, 2022).

## Other Comments

High TNK1 expression correlates with poorer overall and relapse-free survival in ALL cohorts (Unknown authors, 2020a). A Hodgkin lymphoma-derived C-terminal truncation lacking the 14-3-3 binding site generates a hyperactive oncogenic variant (Unknown authors, 2020a). Whole-body Tnk1 knockout mice develop spontaneous lymphomas and carcinomas, indicating possible context-dependent tumour-suppressor roles (Unknown authors, 2020a).

## 9. References

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