Phylogeny  
TNNI3K orthologs have been identified in Homo sapiens, Mus musculus and Xenopus laevis, and sequence conservation extends across vertebrate lineages and even to several invertebrate species possessing cardiac tissue, indicating an ancient origin (zhao2003cloningandcharacterization pages 4-7, gan2020theprevalenti686t pages 1-2).  
Within the human kinome the enzyme belongs to the MAP kinase kinase kinase (MAPKKK) group, mixed-lineage kinase subfamily, and shares highest sequence similarity with integrin-linked kinase (ILK) (tang2013overexpressionoftnni3k pages 1-2, zhao2003cloningandcharacterization pages 2-3, milano2015tnni3kincardiovascular pages 2-3).

Reaction Catalyzed  
ATP + protein ⇌ ADP + protein-O-phosphate; the enzyme transfers the γ-phosphate to serine, threonine or tyrosine residues on target proteins (milano2015tnni3kincardiovascular pages 1-2, tang2013overexpressionoftnni3k pages 1-2).

Cofactor Requirements  
Catalytic activity requires a divalent metal ion cofactor, typically Mg²⁺ or Mn²⁺ (gan2020theprevalenti686t pages 1-2).

Substrate Specificity  
A consensus phosphorylation motif has not been defined; the kinase has not yet been profiled in large-scale motif screens (gan2020theprevalenti686t pages 1-2).  
Cardiac troponin I is a proposed interacting substrate, although in-cell phosphorylation has not been confirmed (zhao2003cloningandcharacterization pages 1-2, gan2020theprevalenti686t pages 1-2).  
Autophosphorylation occurs on multiple residues, including Y24, T399, Y416, Y425, T622 (activation loop), S737, S739, S741, Y771, Y804, T805 and Y812 (tang2013overexpressionoftnni3k pages 22-24).

Structure  
Domain organisation: N-terminal ankyrin-repeat region (7–10 repeats) mediating protein interactions; central bilobed kinase domain; C-terminal serine-rich domain functioning as a negative regulatory segment (tang2013overexpressionoftnni3k pages 1-2).  
Key catalytic features within the kinase domain include the invariant Lys490 required for ATP binding—substitution K490R abolishes activity—and the conserved HRD and DFG motifs typical of protein kinases (tang2013overexpressionoftnni3k pages 5-7, tang2013overexpressionoftnni3k pages 9-10).  
The activation loop contains autophosphorylation site Thr622 (tang2013overexpressionoftnni3k pages 9-10).  
An X-ray crystal structure of the isolated kinase domain is available (PDB 4YFI) and served as the template for mutation modelling studies (ramzan2021anovelmissense pages 4-5).

Regulation  
Autophosphorylation on Tyr, Ser and Thr residues is essential for catalytic competence; mutation of Lys490 eliminates both autophosphorylation and enzymatic activity (tang2013overexpressionoftnni3k pages 5-7).  
The C-terminal serine-rich domain exerts autoinhibitory control by restraining further phosphorylation (tang2013overexpressionoftnni3k pages 1-2).  
Peroxiredoxin-3 binds the ankyrin and kinase domains and suppresses kinase activity (lal2014troponiniinteractingprotein pages 2-3).

Function  
Expression is highly restricted to cardiomyocytes, with strongest transcript and protein levels in the interventricular septum and apex; negligible expression is detected in non-cardiac tissues (zhao2003cloningandcharacterization pages 1-2, lal2014troponiniinteractingprotein pages 1-2).  
Subcellular localisation encompasses the sarcomeric Z-disc, perinuclear space and nucleus (tang2013overexpressionoftnni3k pages 1-2, zhao2003cloningandcharacterization pages 1-2).  
Documented interactors include cardiac troponin I, α-actin, myosin-binding protein C and peroxiredoxin-3 (zhao2003cloningandcharacterization pages 4-7, lal2014troponiniinteractingprotein pages 2-3).  
The kinase modulates protein kinase A signalling and β-adrenergic contractile reserve in cardiomyocytes (gan2020theprevalenti686t pages 1-2).  
Catalytically active overexpression accelerates pressure-overload cardiomyopathy, whereas kinase-dead variants do not, demonstrating that enzymatic activity drives pathological remodelling (tang2013overexpressionoftnni3k pages 1-2).  
Loss-of-function alleles impair calcium handling and contractility and lead to concentric ventricular remodelling (gan2020theprevalenti686t pages 1-2).  
The kinase contributes to ischemia-reperfusion injury via p38 MAPK-dependent mitochondrial dysfunction (lal2014troponiniinteractingprotein pages 1-2).  
Natural variation in Tnni3k expression influences cardiac conduction intervals and cardiomyocyte ploidy in mice (pham2021thediverseroles pages 6-8).

Inhibitors  
Potent small-molecule inhibitors have been reported: GSK854 (IC₅₀ < 10 nM, highly selective), GSK329 (IC₅₀ < 10 nM), GSK114 (IC₅₀ ≈ 25 nM, ~40-fold selectivity over B-Raf) and compound 6O (IC₅₀ ≈ 410 nM). These inhibitors reduce infarct size, fibrosis and adverse cardiac remodelling in mouse ischemia-reperfusion models (pham2021thediverseroles pages 9-11).

Other Comments  
Pathogenic variants include p.Gly526Asp and p.Thr539Ala in the kinase domain, both reducing autophosphorylation; p.Glu768Lys in the serine-rich domain, which enhances autophosphorylation; the common hypomorphic p.Ile686Thr allele (~38 % of wild-type activity); and p.Ser511Pro, which causes recessive cardiac conduction disease (pham2021thediverseroles pages 8-9, gan2020theprevalenti686t pages 1-2, ramzan2021anovelmissense pages 4-5, theis2014tnni3kmutationin pages 6-8).

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