## Phylogeny

CAMKV (Q8NCB2) is classified within the CAMK (Calcium/Calmodulin-dependent protein kinase) group of the human serine/threonine kinome (manning2002theproteinkinase pages 7-8, johnson2023anatlasof pages 4-4, simon2015molecularmechanismsof pages 8-10). The Manning et al. classification system, which organizes the human kinome into groups and families, identifies CAMK as one of the major kinase groups (manning2002theproteinkinase pages 1-2). Kinome-wide analysis based on phosphorylation-site motif selectivity further places CAMKV within the CAMK cluster (johnson2023anatlasof pages 4-5). Within this group, CAMKV is considered an atypical member, showing evolutionary divergence from canonical CAMK1 and CAMK2 family members (yu2024camkvkinasesignaling pages 38-40). Sequence alignment of the calmodulin binding domain (CBD) reveals conserved lysine and arginine residues among CAMK family members, indicating evolutionary conservation of this functional domain (yu2024camkvkinasesignaling pages 63-66). The phylogenetic classification scheme allows for the identification of orthologous groups across species including yeast, worm, fly, and human (manning2002theproteinkinase pages 1-2).

## Reaction Catalyzed

There are conflicting reports regarding the catalytic activity of CAMKV.

One body of research indicates that CAMKV is a catalytically active kinase. In this context, it has been demonstrated to catalyze the phosphorylation of specific protein substrates (yu2024camkvkinasesignaling pages 16-19, 25-27). The reaction is: ATP + [a protein] -> ADP + [a phosphoprotein] (yu2024camkvkinasesignaling pages 16-19).

Conversely, other studies report that CAMKV lacks detectable intrinsic kinase activity and functions as a pseudokinase or kinase-dead protein (rozen2024anoveldruggable pages 8-9, yu2024camkvkinasesignaling pages 82-85). This inactivity is attributed to mutations or deviations in critical catalytic residues and non-canonical conformations of structural elements required for catalysis (rozen2024anoveldruggable pages 8-9, yu2024camkvkinasesignaling pages 13-16). It was noted that recombinant CAMKV purified from bacteria lacks kinase activity, potentially due to the absence of post-translational modifications that are present in eukaryotic systems and essential for enzymatic function (yu2024camkvkinasesignaling pages 25-27).

## Cofactor Requirements

The initial activation of CAMKV kinase activity is dependent on its calmodulin-binding domain, but calmodulin is not required to maintain its kinase activity once initiated (yu2024camkvkinasesignaling pages 16-19, 25-27). Reports suggesting CAMKV is a pseudokinase note that its DFG motif, which is essential for coordinating Mg²⁺ ions in active kinases, is altered, impairing this function (yu2024camkvkinasesignaling pages 13-16, 82-85).

## Substrate Specificity

Hierarchical clustering based on amino acid motif selectivity places CAMKV within the CAMK kinome group, and kinases within the same group often share substrate motif preferences (johnson2023anatlasof pages 4-5). However, a specific consensus substrate motif for CAMKV is not defined in the provided literature.

Experimentally identified substrates and phosphorylation sites include: - The transcription factor CREB at Ser133 (yu2024camkvkinasesignaling pages 16-19, 25-27). - The transcription factor GATA2 at Ser182 and Ser192 (yu2024camkvkinasesignaling pages 19-22, 25-27).

Phosphoproteomic analyses also indicate CAMKV influences the phosphorylation of other kinases, including STK10 and RIOK1 (yu2024camkvkinasesignaling pages 25-27).

## Structure

CAMKV is composed of an N-terminal serine/threonine kinase-like domain and a C-terminal regulatory region (rozen2024anoveldruggable pages 8-9, yu2024camkvkinasesignaling pages 13-16). The C-terminal region is intrinsically disordered for approximately 200 amino acids and contains a calmodulin-binding domain (CBD) and a C-terminal repeat domain (CTD) (rozen2024anoveldruggable pages 8-9, yu2024camkvkinasesignaling pages 16-19). This CTD contains seven tandem octapeptide repeats with the motif D-X-X-X-T-P-A-T (rozen2024anoveldruggable pages 8-9). These repeats are critical for protein stability, as their deletion results in protein instability (rozen2023anoveldruggable pages 3-4, rozen2024anoveldruggable pages 9-11).

Structural predictions using AlphaFold 2 confirm a typical kinase fold for the N-terminal domain, despite conflicting reports on its catalytic competence (yu2024camkvkinasesignaling pages 82-85, rozen2024anoveldruggable pages 8-9).

Contradictory information exists regarding key catalytic features: - Reports describing CAMKV as inactive or a pseudokinase cite structural defects, including mutations in the DFG motif, an improperly positioned C-helix, and an activation loop locked in an inactive conformation (rozen2024anoveldruggable pages 8-9, yu2024camkvkinasesignaling pages 13-16, 82-85). - Reports describing CAMKV as an active kinase identify Lysine 53 (K53) as a residue essential for ATP binding and Threonine 183 (T183) as a critical phosphorylation site for kinase activation (yu2024camkvkinasesignaling pages 16-19). One study notes the catalytic lysine (Lys53) is present but the DFG motif is altered (yu2024camkvkinasesignaling pages 82-85).

## Regulation

CAMKV is regulated by post-translational modifications, allosteric interactions, and protein stability elements.

**Phosphorylation:** - CAMKV is a direct substrate of DYRK3, which phosphorylates it within its C-terminal disordered region (rozen2023anoveldruggable pages 3-4, rozen2024anoveldruggable pages 8-9). Threonine residues T387 and T427, located within the tandem octapeptide repeats, are canonical DYRK phosphorylation sites (rozen2024anoveldruggable pages 9-11). DYRK3-mediated phosphorylation regulates CAMKV’s ability to undergo liquid-liquid phase separation (rozen2023anoveldruggable pages 3-4). - Phosphorylation at Threonine 183 (T183) is critical for CAMKV’s own kinase activation (yu2024camkvkinasesignaling pages 16-19, 25-27). The T183E phosphomimetic mutant is constitutively active (yu2024camkvkinasesignaling pages 16-19).

**Allosteric and Conformational Regulation:** - The calmodulin-binding domain (CBD) is required for the initial activation of CAMKV kinase activity (yu2024camkvkinasesignaling pages 16-19, 25-27). - The C-terminal domain appears to have an inhibitory role, as a truncated isoform (CAMKV-S) lacking 31 C-terminal amino acids is more potent than the full-length protein (CAMKV-FL) (yu2024camkvkinasesignaling pages 16-19).

**Protein Stability:** - The seven tandem octapeptide repeats in the C-terminal domain are essential for CAMKV protein stability and expression (rozen2023anoveldruggable pages 3-4, rozen2024anoveldruggable pages 9-11).

## Function

**Expression and Localization:** - CAMKV expression is highly enriched in neuroblastoma (NB) cells and brain tissues (rozen2024anoveldruggable pages 8-9, rozen2023anoveldruggable pages 1-3). It is also highly expressed in neuroblasts from embryos and fetal adrenal glands (yu2024camkvkinasesignaling pages 19-22). - In NB cells, CAMKV shows a predominantly homogeneous cytosolic distribution (rozen2023anoveldruggable pages 3-4). During cell division, endogenous CAMKV localizes to the mitotic spindle (rozen2023anoveldruggable pages 3-4). Overexpressed CAMKV can form dynamic aggregates upon inhibition of DYRK3 (rozen2023anoveldruggable pages 3-4).

**Signaling and Biological Roles:** - CAMKV is a direct transcriptional target of the MYCN and MYC oncogenes, which bind to its promoter and drive expression (yu2024camkvkinasesignaling pages 19-22, 44-46). - The DYRK3/CAMKV signaling module is implicated in the regulation of the mitotic spindle, which is essential for NB cell proliferation and tumor growth (rozen2023anoveldruggable pages 1-3). - As a kinase, CAMKV directly phosphorylates and activates the transcription factor CREB at Ser133, promoting NB cell proliferation (yu2024camkvkinasesignaling pages 1-5, 16-19). It also phosphorylates GATA2 to promote proliferation (yu2024camkvkinasesignaling pages 19-22). - CAMKV knockdown impairs NB cell proliferation and alters the expression of genes involved in neuronal function, axon guidance, protein translation, and apoptosis (rozen2023anoveldruggable pages 3-4, yu2024camkvkinasesignaling pages 19-22). - Beyond cancer, CAMKV has been implicated in neuronal functions such as dendritic spine maintenance and activity-dependent bulk endocytosis (yu2024camkvkinasesignaling pages 1-5).

**Interacting Partners:** - CAMKV interacts directly with the upstream kinase DYRK3 (rozen2023anoveldruggable pages 3-4, rozen2024anoveldruggable pages 8-9). - CAMKV interacts directly with its substrate CREB in NB cells (yu2024camkvkinasesignaling pages 16-19).

## Inhibitors

Small molecule inhibitors targeting CAMKV have been shown to suppress tumor growth and improve survival in neuroblastoma xenograft models (yu2024camkvkinasesignaling pages 1-5, 25-27). Molecular docking studies have been performed with compounds such as K-252a and OTSSP167 in the ATP-binding pocket of CAMKV (yu2024camkvkinasesignaling pages 82-85).

## Other Comments

**Disease Association:** - High CAMKV expression correlates with worse patient survival, high-risk disease, and advanced tumor stages in neuroblastoma (NB) (rozen2023anoveldruggable pages 3-4, yu2024camkvkinasesignaling pages 19-22). Its expression is positively correlated with MYCN/MYC expression in NB tumors (rozen2023anoveldruggable pages 3-4). - CAMKV has also been reported as a prognostic biomarker in human endometrial carcinoma (yu2024camkvkinasesignaling pages 1-5).

**Isoforms:** - At least two isoforms exist in NB cells due to alternative splicing: a full-length form (CAMKV-FL, 501 amino acids) and a shorter, truncated form (CAMKV-S) that lacks 31 amino acids at the C-terminus (yu2024camkvkinasesignaling pages 13-16, 16-19). CAMKV-S exhibits more potent kinase activity toward CREB and a greater ability to enhance cell proliferation compared to CAMKV-FL (yu2024camkvkinasesignaling pages 16-19).

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