## Phylogeny

• Kinome hierarchy: CMGC group → DYRK-related clade → HIPK sub-branch (agnew2019thecrystalstructure pages 1-2).  
• Vertebrate paralogues: HIPK1, HIPK3, HIPK4; kinase-domain identity HIPK1 vs HIPK2 ≈93 % (kaltheuner2021abemaciclibisa pages 1-2).  
• Representative orthologs: Mus musculus Hipk2, Danio rerio hipk2, Xenopus laevis hipk2, Drosophila melanogaster minibrain (Mnb) and Saccharomyces cerevisiae Yak1 (hofmann2000humanhomeodomaininteractingprotein pages 2-5).  
• Bayesian comparison of 1 498 HIPK sequences against 14 296 CMGC kinases highlights HIPK-specific residues in the αC-β4 loop and CMGC-insert that phylogenetically separate HIPKs from DYRKs and CLKs (agnew2019thecrystalstructure pages 25-26).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr ⇌ ADP + protein-L-Ser/Thr-phosphate (agnew2019thecrystalstructure pages 18-20).

## Cofactor Requirements

Catalysis is Mg²⁺-dependent, consistent with CMGC kinase chemistry (kaltheuner2021abemaciclibisa pages 1-2).

## Substrate Specificity

• Consensus motifs determined biochemically: S/T-P and S/T-P-X-P, indicating strong proline preference at +1 and optional +3 positions (saul2013hipk2kinaseactivity pages 1-2).  
• Autophosphorylation specificity: Tyr354 (human Tyr361 in crystal structure) within an S-x-Y motif characteristic of DYRK/HIPK kinases (agnew2019thecrystalstructure pages 2-3).

## Structure

Domain organisation  
1 – ≈330: N-terminal kinase domain; 331 – ≈480: homeoprotein-interacting domain (HID); ≈481 – ≈740: PEST/speckle-retention sequence (SRS) with SUMO-binding motif; ≈741 – ≈915: autoinhibitory domain (AID); ≈916 - end: SQA-rich tail (kaltheuner2021abemaciclibisa pages 1-2, agnew2019thecrystalstructure pages 2-3).

3-D architecture  
• Crystal structure at 2.2 Å (kinase domain + CX-4945, PDB reported in primary study) adopts an active helix-C-in/DFG-in conformation with a complete regulatory spine (agnew2019thecrystalstructure pages 3-4).  
• Activation loop: pTyr361 hydrogen-bonds to Arg368 (CMGC arginine) and Gln363, locking the active state (agnew2019thecrystalstructure pages 22-25).  
• Unique CMGC-insert: extended helix H, short helix M and β-hairpin bearing auto-pSer441; pSer441 interacts with Arg437 to stabilise the insert (agnew2019thecrystalstructure pages 7-8).  
• HIPK-specific αC-β4 loop expands the N-lobe surface and contacts the CMGC-insert, a signature feature absent from DYRK1A/2 (agnew2019thecrystalstructure pages 8-9).  
• Catalytic residues: Lys228 (β3), Glu243 (helix C), Asp346-Phe347-Gly348 (DFG), His365-Arg368 (HRD-equivalent) (agnew2019thecrystalstructure pages 3-4).

## Regulation

Post-translational modifications  
• Cis-autophosphorylation: Tyr354/Tyr361 and Ser357 within the activation loop are indispensable for catalytic activity and substrate affinity (saul2013hipk2kinaseactivity pages 1-2).  
• Additional autophosphorylation: Ser441 in the CMGC-insert modulates monomer–dimer equilibrium (agnew2019thecrystalstructure pages 8-9).  
• SUMOylation: Lys25 by PIAS1 supports speckle retention and transcriptional repression (agnew2019thecrystalstructure pages 3-4).  
• Acetylation: Lys10 and multiple kinase-domain lysines by CBP/p300 enhance oxidative-stress signalling (agnew2019thecrystalstructure pages 3-4).  
• Ubiquitination: Siah-1 and WSB-1 target HIPK2 for proteasomal degradation under basal conditions (agnew2019thecrystalstructure pages 1-2, kuwano2016homeodomaininteractingproteinkinase2 pages 3-5).  
• Caspase-6 cleavage at Asp916 & Asp977 removes the AID, increasing kinase activity during genotoxic stress (kuwano2016homeodomaininteractingproteinkinase2 pages 3-5).  
Upstream regulation  
• ATM and ATR phosphorylate and stabilise HIPK2 in the DNA-damage response (kuwano2016homeodomaininteractingproteinkinase2 pages 1-3).

## Function

Expression and localisation  
• Predominantly nuclear; concentrates in subnuclear speckles via the SRS (kuwano2016homeodomaininteractingproteinkinase2 pages 1-3).  
• Broad tissue distribution; notable expression in cardiomyocytes where it supports basal ERK signalling (guo2019cardiomyocytehipk2maintains pages 21-23).

Signalling roles  
• DNA damage/apoptosis: phosphorylates p53 Ser46 to drive pro-apoptotic transcription (agnew2019thecrystalstructure pages 1-2).  
• Wnt/β-catenin: phosphorylates β-catenin leading to β-TrCP-dependent degradation (agnew2019thecrystalstructure pages 2-3).  
• TGF-β/JNK: cooperates with DAXX to activate JNK after TGF-β stimulation (agnew2019thecrystalstructure pages 1-2).  
• Hypoxia: acts as transcriptional co-suppressor of HIF-1α (agnew2019thecrystalstructure pages 1-2).  
Principal substrates/interactors: PML, EP300, CTBP1, SMAD1, POU4F1/Brn3a, CREB1, CBX4, RUNX1, HMGA1, ZBTB4, DAZAP2, Pin1 (agnew2019thecrystalstructure pages 13-15, saul2013hipk2kinaseactivity pages 7-7).

## Inhibitors

• CX-4945: ATP-competitive; co-crystallised with the kinase domain (agnew2019thecrystalstructure pages 22-25).  
• Abemaciclib: potent nanomolar inhibitor of HIPK2, HIPK3 and DYRK1A (kaltheuner2021abemaciclibisa pages 1-2).  
• TBID: selective inhibitor; IC₅₀ = 0.33 µM (cozza2014synthesisandproperties pages 1-2).  
• D-115893: cellular inhibitor identified in activity assays (saul2013hipk2kinaseactivity pages 8-9).

## Other Comments

• Cancer: HIPK2 is amplified or overexpressed in cervical cancer, tonsillar squamous cell carcinoma and pilocytic astrocytoma; loss enhances tumorigenesis (agnew2019thecrystalstructure pages 13-15).  
• Fibrosis: drives TGF-β/Smad3-dependent renal and pulmonary fibrosis and keloid formation (agnew2019thecrystalstructure pages 13-15).  
• Neurodegeneration: promotes ER-stress-mediated neuronal death in Alzheimer’s disease and ALS models (agnew2019thecrystalstructure pages 12-13).  
• Hematological disease: pathogenic missense variant R868W and del(7q) events in myelodysplastic syndrome reduce HIPK2 dosage (agnew2019thecrystalstructure pages 13-15, hofmann2000humanhomeodomaininteractingprotein pages 2-5).

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