Protein: Eukaryotic elongation factor-2 kinase  
Gene: EEF2K  
UniProt: O00418

## Phylogeny

• Atypical protein-kinase-like (PKL) supergroup member; classified within the α-kinase family on human kinome maps derived from Manning et al. 2002 (chitjian2018regulationofelongation pages 12-19)  
• Only Ca²⁺/calmodulin-dependent enzyme in the α-kinase clade (chitjian2018regulationofelongation pages 19-23)  
• Vertebrate orthologs: Homo sapiens, Mus musculus, Rattus norvegicus share 90-97 % sequence identity (ryazanov1997identificationofa pages 5-5)  
• Invertebrate ortholog: Caenorhabditis elegans retains ~40 % identity (ryazanov1997identificationofa pages 5-5)  
• Additional metazoan orthologs detected in Trichoplax adhaerens and diatom Thalassiosira pseudonana (middelbeek2010thealphakinasefamily pages 1-2)  
• Orthologs are absent in insects and fungi, indicating lineage-specific loss (piserchio2024revealingeef2kinase pages 14-17)  
• Clusters with Dictyostelium myosin heavy-chain kinases B/C within the α-kinase branch (middelbeek2010thealphakinasefamily pages 1-2)

## Reaction Catalyzed

ATP + [eEF2] Thr56 → ADP + [eEF2] Thr56-O-phosphate (karakas2020eukaryoticelongationfactor2 pages 1-2)

## Cofactor Requirements

• Activation requires Ca²⁺-bound calmodulin (chitjian2018regulationofelongation pages 19-23)  
• Catalysis depends on Mg²⁺ coordinated by Asp-284 in the DFG motif (chitjian2018regulationofelongation pages 19-23)  
• Acidic pH partially compensates for Ca²⁺ absence, sustaining activity (chitjian2018regulationofelongation pages 12-19)

## Substrate Specificity

• Canonical substrate: eEF2 Thr56 (karakas2020eukaryoticelongationfactor2 pages 1-2)  
• Consensus motif derived from chemical-genetic and peptide studies: acidic residue (E/D) at −2, Lys/Arg at +1 and +3 surrounding the phospho-threonine (lazarus2017discoveryofnew pages 6-7, crawley2008determinantsforsubstrate pages 1-2)  
• Strong threonine preference; targets residues embedded in α-helical segments (pavur2000mappingthefunctional pages 7-8)  
• Validated additional substrates: PP2A adaptor α4, NDRG1, and AMPKα Thr482 (lazarus2017discoveryofnew pages 4-6)

## Structure

Domain organization  
– 79-96: CaM-targeting helix with DXDXDG Ca²⁺-binding motif; Trp-85 anchors CaM (chitjian2018regulationofelongation pages 19-23)  
– 116-326: α-kinase catalytic domain containing GXGXXG glycine loop, catalytic Lys-170, and Asp-284 (chitjian2018regulationofelongation pages 19-23)  
– 327-489: Regulatory loop enriched in phosphorylation sites and intrinsically disordered (chitjian2018regulationofelongation pages 12-19)  
– 490-725: C-terminal SEL1-like helical repeats mediating substrate docking (pigott2012insightsintothe pages 1-2)

Three-dimensional information  
• CaM-bound active core (PDB 7SHQ) reveals dual-lobed α-kinase fold with autophospho-Thr348 occupying an allosteric phosphate pocket (klupt2023eef2kinhibitordesign pages 10-11)  
• ATP-competitive inhibitor A-484954 co-crystal structures (PDB 7S0U/7S0V) define the nucleotide pocket and drug-binding determinants (piserchio2023structureofthe pages 1-2)  
• Additional CaM complexes (PDB 8GM4/8GM5) capture conformational heterogeneity in the regulatory loop and C-helix (piserchio2023structureofthe pages 5-6)  
• AlphaFold2 full-length model corroborates domain arrangement and inter-domain contacts (klupt2023eef2kinhibitordesign pages 10-11)

Key catalytic/regulatory elements  
• Allosteric phosphate pocket (Lys205-Arg252-Thr254) binds pThr348 to stabilize the active conformation (unknownauthors2018molecularsignalprocessing pages 12-16)  
• CaM binding re-orients the C-helix, forming the Lys170-Glu191 catalytic salt bridge (klupt2023eef2kinhibitordesign pages 10-11)  
• A ~150-residue flexible loop hampers crystallization and mediates multisite regulation (chitjian2018regulationofelongation pages 12-19)

## Regulation

Autophosphorylation  
– Thr348: obligatory for activity (klupt2023eef2kinhibitordesign pages 1-2)  
– Ser500: enhances Ca²⁺/CaM sensitivity (unknownauthors2018molecularsignalprocessing pages 21-25)

Activating upstream phosphorylation  
– Ser392, Ser398, Ser499 under stress signals elevate activity (karakas2020eukaryoticelongationfactor2 pages 1-2)

Inhibitory upstream phosphorylation  
– Ser78, Ser359, Ser366 phosphorylated by mTORC1-p70 S6K or ERK-p90 RSK diminish activity (wang2014eukaryoticelongationfactor pages 1-6)

Proteolytic control  
– Diphosphorylated Ser441/Ser445 creates a SCFβTrCP degron driving ubiquitination and rapid turnover (unknownauthors2018molecularsignalprocessing pages 29-33)  
– Hsp90 binding stabilizes the kinase, extending half-life (unknownauthors2018molecularsignalprocessing pages 29-33)

Other modifications  
– Pro98 hydroxylation disrupts CaM interaction, reducing activity (unknownauthors2018molecularsignalprocessing pages 29-33)

Environmental regulation  
– Acidic intracellular pH increases CaM affinity and can sustain activity without Ca²⁺ (chitjian2018regulationofelongation pages 12-19)

## Function

Expression  
• Broadly expressed; markedly elevated in breast, pancreatic, lung, esophageal and brain cancers where high levels correlate with poor prognosis (karakas2020eukaryoticelongationfactor2 pages 1-2)

Biological roles  
• Phosphorylation of eEF2 Thr56 slows ribosomal translocation, conserving ATP and amino acids during nutrient deprivation, hypoxia, DNA damage and ER stress (wang2017eukaryoticelongationfactor pages 1-3)  
• Promotes cell survival, proliferation, angiogenesis, migration and epithelial-mesenchymal transition in tumors (karakas2020eukaryoticelongationfactor2 pages 1-2)

Upstream regulators  
– AMPK activates EEF2K under energy stress (karakas2020eukaryoticelongationfactor2 pages 1-2)  
– mTORC1 and ERK–p90 RSK pathways provide inhibitory phosphorylation (wang2014eukaryoticelongationfactor pages 1-6)  
– CDK1 phosphorylates Ser359 during mitosis, raising Ca²⁺ requirement (unknownauthors2018molecularsignalprocessing pages 21-25)

Downstream substrates and partners  
– Primary: eEF2 Thr56 (karakas2020eukaryoticelongationfactor2 pages 1-2)  
– Additional: α4, NDRG1, AMPKα Thr482 (lazarus2017discoveryofnew pages 4-6)  
– Essential regulatory partner: calmodulin (chitjian2018regulationofelongation pages 19-23)

## Inhibitors

| Compound | Mechanism |
| --- | --- |
| NH125 | Active-site inhibitor |
| A-484954 | ATP-competitive; structural complex resolved |
| Rottlerin | Non-selective kinase inhibitor |
| TS-2 | 5,6-dihydro-4H-1,3-selenazine derivative |
| TS-4 | 5,6-dihydro-4H-1,3-selenazine derivative |
| CAM1 | De novo protein binder blocks CaM-binding helix |

## Other Comments

• Cancer-associated surface Arg→His/Cys missense mutations increase catalytic activity and eEF2 phosphorylation (unknownauthors2018molecularsignalprocessing pages 29-33)  
• Dysregulation implicated in Alzheimer’s disease and major depressive disorder (chitjian2018regulationofelongation pages 19-23)

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