## Phylogeny

Ephrin type-B receptor 6 (EPHB6) is a member of the Eph receptor tyrosine kinase (RTK) family, the largest subgroup of RTKs (liang2021theintracellulardomains pages 1-2, mason2021structureofthe pages 1-2). According to the kinome classification by Manning et al., EPHB6 is assigned to the EphB subfamily (freywald2002thekinasenullephb6 pages 1-1, mason2021structureofthe pages 1-2, strozen2021theephb6receptor pages 1-2). Kinase-defective EPHB6 is found in mammals, whereas non-mammalian orthologs in species such as avians and fish possess kinase-active EPHB6, representing an evolutionary divergence in receptor function unique to the mammalian lineage (freywald2002thekinasenullephb6 pages 1-1, strozen2021theephb6receptor pages 2-4, matsuoka2005biphasicfunctionsof pages 1-1, matsuoka2005biphasicfunctionsof pages 8-8). The loss of kinase activity may have arisen after gene duplication events (matsuoka2005biphasicfunctionsof pages 7-8). Within the Eph family, EPHB6 and EphA10 are both identified as catalytically inactive pseudokinases (liang2021theintracellulardomains pages 1-2, unknownauthors2014ligandstimulationinduces pages 1-4).

## Reaction Catalyzed

EPHB6 is a kinase-defective or kinase-null receptor that is catalytically inactive and incapable of intrinsic kinase activity (freywald2002thekinasenullephb6 pages 1-1, freywald2002thekinasenullephb6 pages 6-7, matsuoka2005biphasicfunctionsof pages 1-1). It does not catalyze phosphorylation reactions (strozen2021theephb6receptor pages 1-2, unknownauthors2015ligandinduceddownregulationof pages 61-65). Fusion proteins containing the human and murine EPHB6 kinase domains show no detectable kinase activity (unknownauthors2015ligandinduceddownregulationof pages 61-65, unknownauthors2015ligandinduceddownregulationof pages 61-65).

## Cofactor Requirements

Due to its catalytically inactive nature, information on cofactor requirements for phosphotransfer is not applicable (freywald2002thekinasenullephb6 pages 1-1, freywald2002thekinasenullephb6 pages 6-7).

## Substrate Specificity

EPHB6 is kinase-defective and does not phosphorylate typical Eph substrates (mason2021structureofthe pages 16-17, strozen2021theephb6receptor pages 14-16). According to studies by Yaron-Barir et al., EPHB6 lacks intrinsic kinase activity and does not catalyze phosphorylation reactions (freywald2002thekinasenullephb6 pages 6-7, strozen2021theephb6receptor pages 13-14).

## Structure

EPHB6 maintains the typical domain organization of Eph receptors (freywald2002thekinasenullephb6 pages 1-1, strozen2021theephb6receptor pages 1-2). The extracellular region contains a ligand-binding domain (LBD), a cysteine-rich domain, and two fibronectin type-3 (FN3) domains (mason2021structureofthe pages 1-2). The intracellular region comprises a juxtamembrane (JM) domain, a pseudokinase domain, a sterile alpha motif (SAM) domain, and a C-terminal PDZ-binding motif (liang2021theintracellulardomains pages 1-2, strozen2021theephb6receptor pages 1-2).

The receptor is catalytically inactive due to substitutions of critical residues in the kinase domain (freywald2002thekinasenullephb6 pages 1-1). These include the replacement of a key lysine in the ATP-binding VAIK motif with a glutamine (K702Q in mammals) and a serine replacing an aspartic acid in the HRD motif of the catalytic loop (freywald2002thekinasenullephb6 pages 1-1, liang2021theintracellulardomains pages 5-6, unknownauthors2016syntheticlethalinteractions pages 13-18). Other inactivating substitutions are found in key motifs, including E719R/Q, N800S, D813R, and within the DFG motif (liang2021theintracellulardomains pages 5-6, strozen2021theephb6receptor pages 7-8). While some reports state these substitutions prevent ATP binding (mason2021structureofthe pages 16-17), others show the pseudokinase domain binds ATP with moderate affinity (KD ~94 μM) and can bind both Type I and Type II kinase inhibitors (liang2021theintracellulardomains pages 5-6, strozen2021theephb6receptor pages 7-8).

A unique feature of mammalian EPHB6 is that its activation loop is truncated by approximately 15 amino acids and has been repurposed into an SH3 domain-binding site containing a conserved PxxP motif (strozen2021theephb6receptor pages 8-9, strozen2021theephb6receptor pages 4-7, strozen2021theephb6receptor pages 8-9). The intracellular region has a flexible configuration, and the receptor can adopt multiple dynamic conformational states (liang2021theintracellulardomains pages 1-2, liang2021theintracellulardomains pages 5-6).

## Regulation

Regulation of EPHB6 involves binding to membrane-anchored ephrin-B ligands, including ephrin-B1 and ephrin-B2, which induces receptor clustering (freywald2002thekinasenullephb6 pages 1-1, strozen2021theephb6receptor pages 2-4, unknownauthors2016syntheticlethalinteractions pages 13-18). EPHB6 does not bind ephrin-B3 (unknownauthors2016syntheticlethalinteractions pages 13-18).

Despite being kinase-dead, EPHB6 undergoes inducible tyrosine phosphorylation via transphosphorylation (freywald2002thekinasenullephb6 pages 1-1). This occurs when EPHB6 forms a hetero-complex with catalytically active Eph receptors (e.g., EphB1, EphB4) or upon association with Src family kinases like Fyn (freywald2002thekinasenullephb6 pages 1-1, liang2021theintracellulardomains pages 1-2, strozen2021theephb6receptor pages 2-4, matsuoka2005biphasicfunctionsof pages 1-1). Conserved tyrosine residues in the juxtamembrane region, specifically JX1 (Y645) and JX2 (Y651), serve as phosphorylation substrates for these partner kinases (liang2021theintracellulardomains pages 14-17, liang2021theintracellulardomains pages 5-6). Potential acetylation is another identified post-translational modification (liang2021theintracellulardomains pages 5-6).

EPHB6 function is also regulated by protein interactions. It associates with the chaperone Hsp90, and ligand stimulation disrupts this interaction, leading to EPHB6 internalization via clathrin-mediated endocytosis and Rab5-regulated lysosomal degradation (unknownauthors2014ligandstimulationinduces pages 1-4, mason2021structureofthe pages 16-17). Allosteric regulation is mediated by the juxtamembrane and SAM domains; oligomerization via SAM domains can relieve inhibitory interactions from the SAM linker, priming the receptor for protein interactions (strozen2021theephb6receptor pages 7-8, strozen2021theephb6receptor pages 1-2).

## Function

EPHB6 is expressed in various tissues, including neuronal and endothelial cells, brain, pancreas, thymus, kidney, vascular smooth muscle, and immune cells such as T-lymphocytes and monocytes (freywald2002thekinasenullephb6 pages 1-1, strozen2021theephb6receptor pages 2-4, unknownauthors2015ligandinduceddownregulationof pages 61-65).

It functions as a signaling modulator and molecular scaffold to regulate cell adhesion, migration, and cytoskeletal organization (freywald2002thekinasenullephb6 pages 1-1, strozen2021theephb6receptor pages 1-2). EPHB6 exerts biphasic effects on cell adhesion and migration dependent on the concentration of its ligand, ephrin-B2 (matsuoka2005biphasicfunctionsof pages 1-1). It also modulates T-cell receptor signaling, vascular contractility, pain transmission, and lysosomal pathways (strozen2021theephb6receptor pages 13-14, unknownauthors2015ligandinduceddownregulationof pages 61-65).

EPHB6 participates in signaling by forming heteromeric complexes with kinase-active Eph receptors, including EphB1, EphB2, EphB4, and EphA2 (freywald2002thekinasenullephb6 pages 1-1, liang2021theintracellulardomains pages 1-2, unknownauthors2015ligandinduceddownregulationof pages 61-65). Upon phosphorylation by partner kinases, its juxtamembrane tyrosines recruit SH2 domain-containing effector proteins such as Abl, Src, and Vav3 (liang2021theintracellulardomains pages 1-2). Other interacting partners include the proto-oncogene c-Cbl, Fyn kinase, the Ras-binding protein AF6, and ErbB family RTKs (EGFR, ErbB2, ErbB4) (freywald2002thekinasenullephb6 pages 1-1, liang2021theintracellulardomains pages 1-2, matsuoka2005biphasicfunctionsof pages 8-8, unknownauthors2021functionalcrosstalkbetween pages 16-20). EPHB6 modulates signaling pathways including JNK, MAP kinase, Rho GTPases, JAK/STAT3, and PI3K/Akt (strozen2021theephb6receptor pages 13-14, unknownauthors2021analysisofthe pages 12-17, strozen2021theephb6receptor pages 2-4).

## Inhibitors

The EPHB6 pseudokinase domain can bind type I and type II kinase inhibitors (liang2021theintracellulardomains pages 5-6, liang2021theintracellulardomains pages 1-2).

## Other Comments

Dysregulation of EPHB6 is implicated in tumorigenic signaling pathways and cancer progression (freywald2002thekinasenullephb6 pages 1-1, strozen2021theephb6receptor pages 1-2). EPHB6 often functions as a tumor metastasis suppressor, and its expression is frequently downregulated or silenced by promoter hypermethylation in aggressive cancers, including breast, lung, colorectal, prostate, melanoma, and neuroblastoma (strozen2021theephb6receptor pages 14-16, unknownauthors2015highexpressionof pages 6-6, unknownauthors2016syntheticlethalinteractions pages 13-18). Loss of EPHB6 protein expression correlates with poor prognosis in colorectal cancer (unknownauthors2021analysisofthe pages 67-69).

However, EPHB6 can also exhibit pro-tumorigenic functions. In some cancers, such as tongue squamous cell carcinoma, certain leukemias, and colorectal cancer with Apc mutations, EPHB6 is upregulated, and high expression correlates with a poor outcome (unknownauthors2021functionalcrosstalkbetween pages 16-20). In triple-negative breast cancer, EPHB6 suppresses invasion but enhances the proliferation of tumor-initiating cells (liang2021theintracellulardomains pages 1-2, unknownauthors2021functionalcrosstalkbetween pages 16-20). Mutations destabilizing EPHB6 have been linked to metastasis in lung cancer, and mutations within the SAM linker region are associated with increased cancer cell migration and chemoresistance (strozen2021theephb6receptor pages 7-8, unknownauthors2021functionalcrosstalkbetween pages 16-20).

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