## Proposed EC/sub-subclass

Not applicable – EphB6 is a catalytically inactive receptor tyrosine pseudokinase (Freywald et al., 2002; Liang et al., 2021).

## Accepted name

Ephrin type-B receptor 6

## Synonyms

EphB6; EphB6 receptor; kinase-defective EphB6

## Phylogeny

EphB6 belongs to the EphB subfamily of Eph receptor tyrosine kinases, the largest RTK sub-group (Freywald et al., 2002; Mason et al., 2021; Strozen et al., 2021). Mammalian EphB6 orthologues have lost catalytic activity, whereas avian and fish orthologues remain kinase-active, indicating a lineage-specific inactivation that likely followed gene-duplication events (Matsuoka et al., 2005; Strozen et al., 2021). EphB6 and EphA10 constitute the two catalytically inactive members within the Eph family (Liang et al., 2021).

## Reaction catalyzed

None. Mammalian EphB6 lacks intrinsic kinase activity and does not catalyze ATP-dependent phosphorylation reactions (Freywald et al., 2002; Strozen et al., 2021).

## Cofactor requirements

Not applicable – no phosphotransfer activity has been detected (Freywald et al., 2002).

## Substrate specificity

Because the receptor is kinase-dead, no Eph-typical substrates are phosphorylated by EphB6 (Mason et al., 2021; Strozen et al., 2021; Freywald et al., 2002).

## Structure

EphB6 displays the canonical Eph architecture: an extracellular ligand-binding domain, cysteine-rich region and two FN3 repeats, followed by a single-pass transmembrane helix and an intracellular region comprising a juxtamembrane segment, pseudokinase domain, SAM domain and C-terminal PDZ-binding motif (Mason et al., 2021; Liang et al., 2021; Strozen et al., 2021).  
Catalytic motifs are disrupted (e.g., K702Q in the VAIK motif, Ser in place of the HRD Asp), and additional substitutions occur in VAIK, HRD and DFG motif-adjacent residues (Liang et al., 2021; Strozen et al., 2021). Despite inactivation, the pseudokinase binds ATP with moderate affinity (K\_D ≈ 94 µM) and accommodates type I/II kinase inhibitors (Liang et al., 2021).  
A mammalian-specific activation loop truncation (~15 aa) contains a conserved PxxP SH3-binding motif (Strozen et al., 2021). The intracellular domains adopt multiple dynamic conformations (Liang et al., 2021).

## Regulation

• Ligand binding: Membrane-bound ephrin-B1 or ephrin-B2 triggers EphB6 clustering (Freywald et al., 2002; Strozen et al., 2021). Ephrin-B3 is not bound (Unknown Authors, 2016).  
• Trans-phosphorylation: Upon hetero-oligomerization with catalytically active Eph receptors (EphB1, EphB4, etc.) or Src-family kinases (e.g., Fyn), conserved juxtamembrane tyrosines Y645 (JX1) and Y651 (JX2) are phosphorylated (Freywald et al., 2002; Liang et al., 2021).  
• Post-translational modifications: inducible tyrosine phosphorylation; potential acetylation (Liang et al., 2021).  
• Protein interactions: Hsp90 binds the receptor basally; ligand stimulation breaks this interaction, promoting clathrin/Rab5-dependent internalization and lysosomal degradation (Unknown Authors, 2014; Mason et al., 2021). SAM-domain-mediated oligomerization can relieve autoinhibition (Strozen et al., 2021).

## Function

Expression: detected in brain, thymus, pancreas, kidney, vascular smooth muscle, endothelial and neuronal cells, T-lymphocytes and monocytes (Freywald et al., 2002; Strozen et al., 2021).  
Roles: acts as a signaling scaffold regulating cell adhesion, migration and cytoskeletal dynamics; exhibits ligand-concentration-dependent biphasic effects on adhesion/migration (Matsuoka et al., 2005).  
Signaling partnerships: forms complexes with EphB1/2/4, EphA2, Src, Abl, Vav3, c-Cbl, AF6 and ErbB RTKs (Freywald et al., 2002; Liang et al., 2021; Unknown Authors, 2021). Downstream pathways influenced include JNK, MAPK, Rho GTPases, JAK/STAT3 and PI3K/Akt (Strozen et al., 2021). Additional roles are reported in T-cell receptor modulation, vascular contractility, pain transmission and lysosomal function (Strozen et al., 2021; Unknown Authors, 2015).

## Inhibitors

The EphB6 pseudokinase domain can bind both type I and type II ATP-competitive kinase inhibitors (Liang et al., 2021).

## Other comments

EphB6 dysregulation is linked to cancer. Often acting as a metastasis suppressor, its expression is frequently lost via promoter hypermethylation in aggressive breast, lung, colorectal, prostate, melanoma and neuroblastoma tumors; loss correlates with poor prognosis in colorectal cancer (Strozen et al., 2021; Unknown Authors, 2021). Conversely, elevated EphB6 expression is associated with poor outcomes in tongue squamous cell carcinoma and selected leukemia and colorectal contexts (Unknown Authors, 2015; 2021). In triple-negative breast cancer, EphB6 suppresses invasion yet promotes proliferation of tumor-initiating cells (Liang et al., 2021). Mutations destabilizing the receptor or altering the SAM linker can enhance metastasis, migration and chemoresistance (Strozen et al., 2021).

## References

Freywald, A., Sharfe, N., & Roifman, C. (2002). The kinase-null EphB6 receptor undergoes transphosphorylation in a complex with EphB1. Journal of Biological Chemistry, 277, 3823–3828. https://doi.org/10.1074/jbc.M108011200

Liang, L.-Y., Roy, M., Horne, C. R., Sandow, J., Surudoi, M. G., Dagley, L. F., Young, S. N., Dite, T. A., Babon, J., Janes, P. W., Patel, O., Murphy, J. M., & Lucet, I. (2021). The intracellular domains of the EphB6 and EphA10 receptor tyrosine pseudokinases function as dynamic signalling hubs. Biochemical Journal, 478, 3351–3371. https://doi.org/10.1042/BCJ20210572

Mason, E. O., Goldgur, Y., Robev, D., Freywald, A., Nikolov, D., & Himanen, J. (2021). Structure of the EphB6 receptor ectodomain. PLOS ONE, 16, e0247335. https://doi.org/10.1371/journal.pone.0247335

Matsuoka, H., Obama, H., Kelly, M. L., Matsui, T., & Nakamoto, M. (2005). Biphasic functions of the kinase-defective EphB6 receptor in cell adhesion and migration. Journal of Biological Chemistry, 280, 29355–29363. https://doi.org/10.1074/jbc.M500010200

Strozen, T. G., Sharpe, J. C., Harris, E. D., Uppalapati, M., & Toosi, B. M. (2021). The EphB6 receptor: Kinase-dead but very much alive. International Journal of Molecular Sciences, 22, 8211. https://doi.org/10.3390/ijms22158211

Unknown Authors. (2014). Ligand stimulation induces clathrin- and Rab5-dependent downregulation of the kinase-dead EphB6 receptor preceded by the disruption of EphB6-Hsp90 interaction.

Unknown Authors. (2015). Ligand-induced downregulation of the kinase-dead EphB6 receptor.

Unknown Authors. (2015). High expression of EphB6 protein in tongue squamous cell carcinoma is associated with a poor outcome.

Unknown Authors. (2016). Synthetic lethal interactions of EPHB6 in breast cancer cells.

Unknown Authors. (2021). Analysis of the crosstalk between the EphB6 and ErbB2 receptors in breast cancer cells.

Unknown Authors. (2021). Functional crosstalk between EphB6 and EGFR.