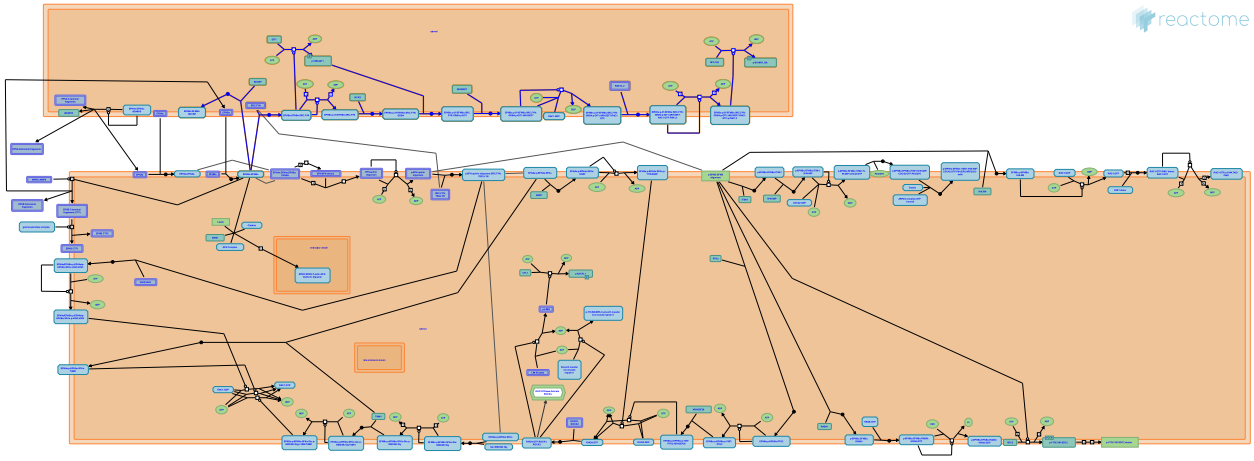
# Ephrin signaling



Garapati, P V., Ip, NY.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.



11/05/2024

# Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

## Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

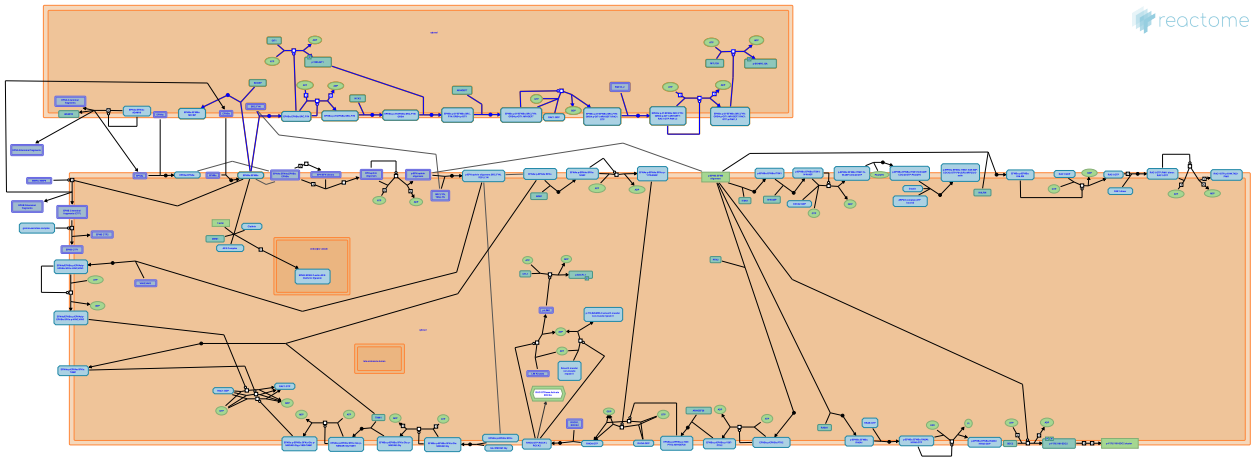
Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

## Ephrin signaling ↗

**Stable identifier:** R-HSA-3928664

**Compartments:** cytosol, plasma membrane



The interaction between ephrin (EFN) ligands and EPH receptors results not only in forward signaling through the EPH receptor, but also in 'reverse' signaling through the EFN ligand itself. Reverse signaling through EFNB is required for correct spine morphogenesis and proper path-finding of corpus callosum and dorsal retinal axons. The molecular mechanism by which EFNBs transduce a reverse signal involves phosphorylation of multiple, conserved tyrosines on the intracellular domain of B-type ephrins, facilitating binding of the SH2/SH3 domain adaptor protein GRB4 and subsequent cytoskeletal remodeling (Bruckner et al. 1997, Cowan & Henkemeyer 2001, Lu et al. 2001). The other mechanism of reverse signaling involves the C-terminus PSD-95/Dlg/ZO-1 (PDZ)-binding motif of EFNBs which recruits various PDZ domain containing proteins. Phosphorylation and PDZ-dependent reverse signaling by ephrin-B1 have each been proposed to play important roles in multiple contexts in development and disease (Bush & Soriano 2009).

## Literature references


Soriano, P., Bush, JO. (2009). Ephrin-B1 regulates axon guidance by reverse signaling through a PDZ-dependent mechanism. *Genes Dev., 23*, 1586-99. ↗


## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

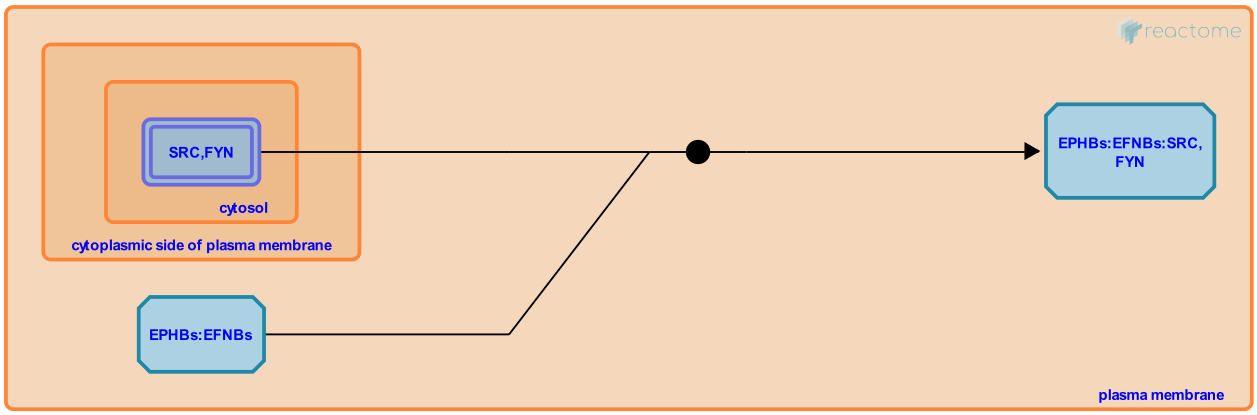
## EFNBs bind SFKs ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928615

**Type:** binding

**Compartments:** plasma membrane, cytosol




Src-family kinases (SFKs) are required for ephrinB (EFNB)-mediated axon guidance and angiogenic responses in endothelial cells. Following interaction with their cognate EPHB receptors and formation of circular tetramers and higher order clusters, EFNBs recruit SFKs and become tyrosine phosphorylated on the cytoplasmic tail. SFK recruitment and activation represents one of the first events in EFNB reverse signaling (Palmer et al. 2002).

**Followed by:** SFKs phosphorylate EFNBs

## Literature references

Klein, R., Zimmer, M., Deutsch, U., Eulenburg, V., Porthin, A., Palmer, A. et al. (2002). EphrinB phosphorylation and reverse signaling: regulation by Src kinases and PTP-BL phosphatase. *Mol. Cell, 9*, 725-37. ↗

## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

## SFKs phosphorylate EFNBs ↗

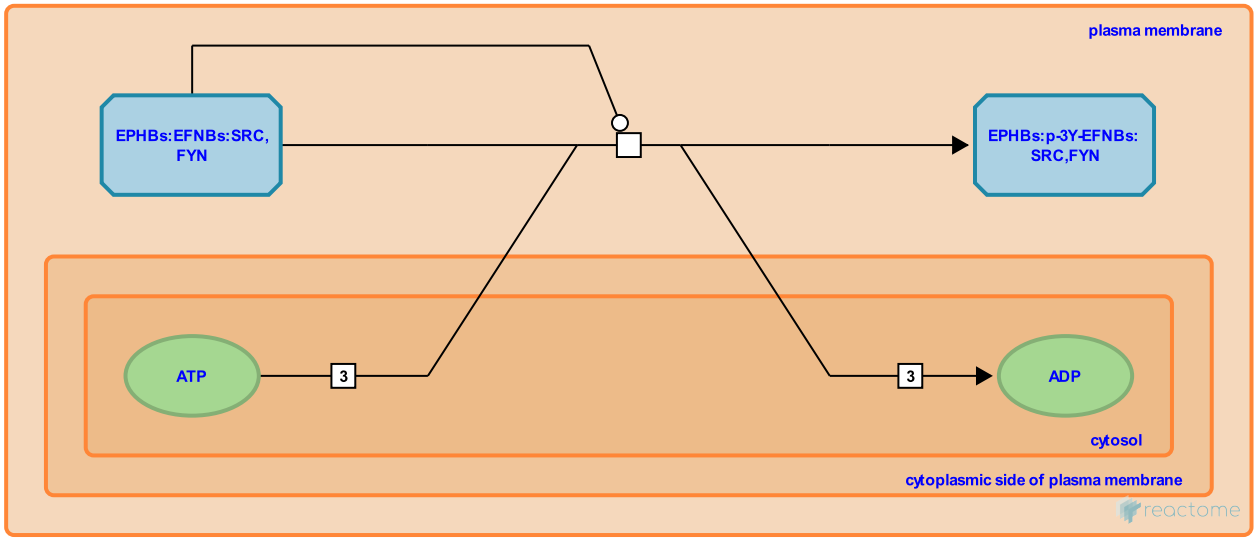
**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928580

**Type:** transition

**Compartments:** plasma membrane, cytosol

**Inferred from:** Sfks phosphorylate EfnB1 (Gallus gallus)




The Src-family kinases (SFKs) SRC and FYN, colocalised with ephrinBs (EFNBs) at the membrane, are required for EFNB phosphorylation. The cytoplasmic domains of all three EFNBs consist of five conserved tyrosine residues of which three undergo phosphorylation. Electrospray tandem mass spectrometry and site-directed mutagenesis identified tyrosines 312, 317, and 331 (human 324, 329 and 343) of EFNB1 as phosphorylation sites (Kalo et al. 2001, Palmer et al. 2002).

**Preceded by:** EFNBs bind SFKs

**Followed by:** p-EFNB binds GRB4

## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

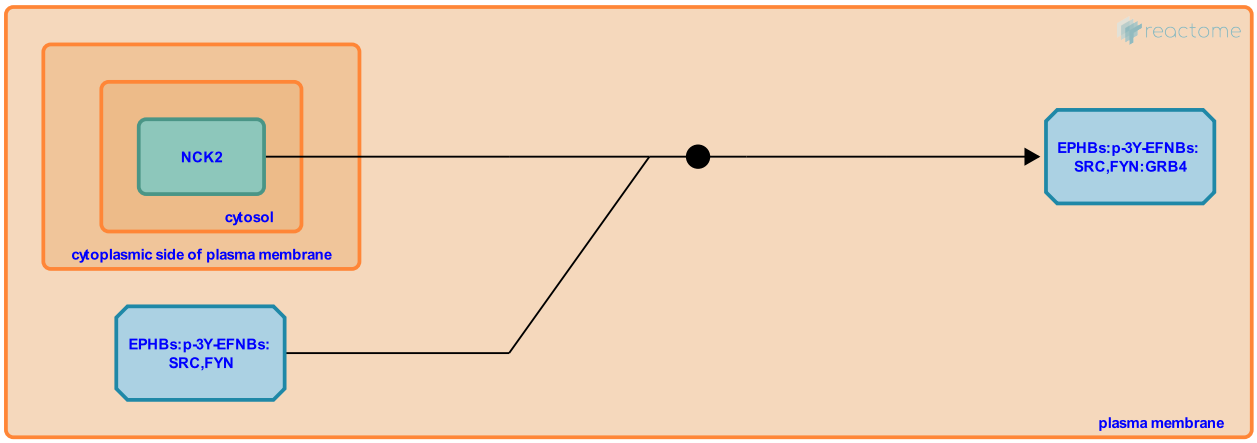
## p-EFNB binds GRB4 ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928639

**Type:** binding

**Compartments:** plasma membrane, cytosol




Unlike EPH receptors, ephrinBs (EFNBs) do not possess intrinsic catalytic activity and thus rely on the recruitment of signaling molecules to signal. Phosphorylated ephrinB (p-EFNB) provides docking site for the SH2/SH3 domain-containing adapter protein cytoplasmic protein NCK2 (NCK2 aka GRB4) (Cowan & Henkemeyer 2001). GRB4 is able to bind to phosphotyrosines in EFNBs through their SH2 domains and 'PxxP' motifs through their SH3 domains. It has been postulated that GRB4 acts as a bridge between EFNBs and G protein-coupled receptor kinase interacting protein (GIT) 1 and Rac at synapses (Segura et al. 2007).

**Preceded by:** SFKs phosphorylate EFNBs

**Followed by:** SFKs phosphorylate GIT1

## Literature references


Acker-Palmer, A., Essmann, CL., Weinges, S., Segura, I. (2007). Grb4 and GIT1 transduce ephrinB reverse signals modulating spine morphogenesis and synapse formation. *Nat. Neurosci., 10*, 301-10. ↗

Henkemeyer, M., Cowan, CA. (2001). The SH2/SH3 adaptor Grb4 transduces B-ephrin reverse signals. *Nature, 413*, 174-9. ↗


## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

## SFKs phosphorylate GIT1 ↗

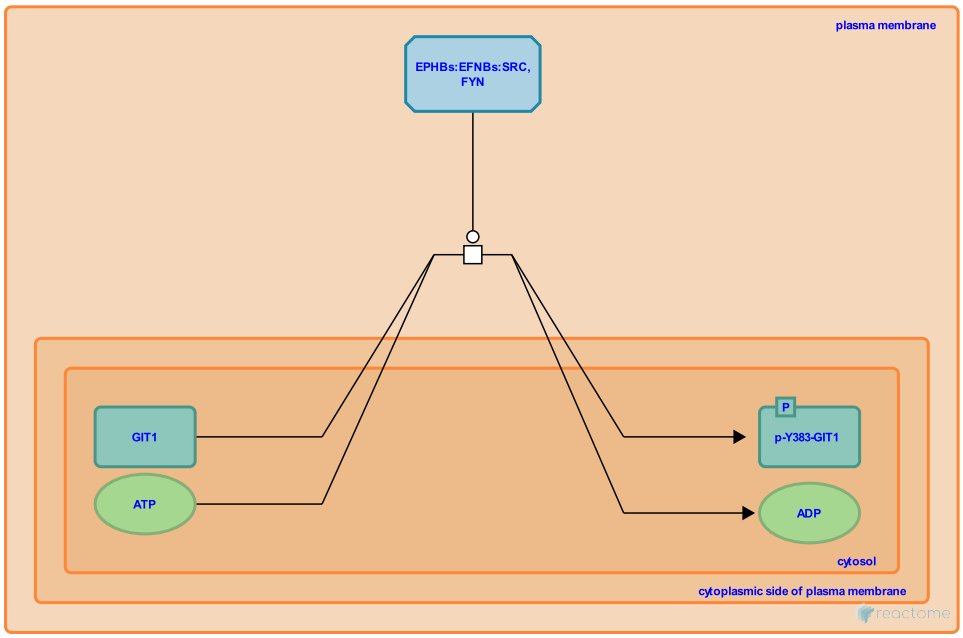
**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928594

**Type:** transition

**Compartments:** plasma membrane, cytosol

**Inferred from:** SFKs phosphorylate Git1 (Mus musculus)




G-protein-coupled receptor kinase-interacting protein 1 (GIT1) has been shown to be critical for spine morphogenesis and synapse formation through assembling and targeting multiprotein signaling complexes, which contain important actin regulators including PIX, Rac GEF, and PAK, between the sub-cellular compartments (Zhang et al. 2003). Activation of ephrinBs (EFNBs) by EPHB receptors may lead to the phosphorylation of GIT1 on Tyr392 (in humans Tyr385, based on sequence similarity). The Src-family kinases (SFKs) recruited to EFNB expression domains are involved in this phosphorylation. Tyr392 in the N-terminal of the SLD domain is required for GIT1s binding to the SH2 domain of GRB4 (Segura et al. 2007).

**Preceded by:** p-EFNB binds GRB4

**Followed by:** EFNB:GRB4 binds GIT1

## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

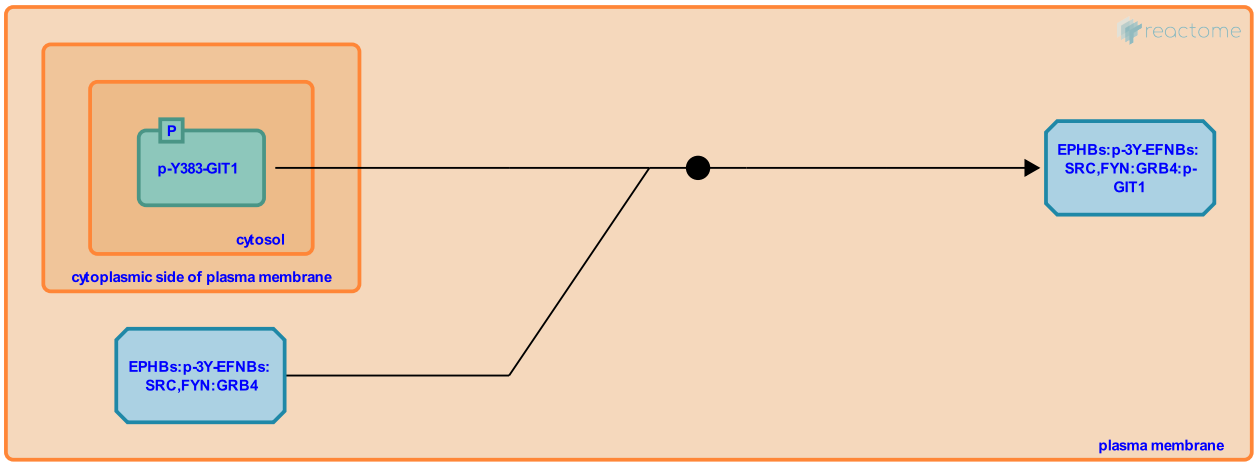
## EFNB:GRB4 binds GIT1 ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928614

**Type:** binding

**Compartments:** plasma membrane, cytosol




G-protein-coupled receptor kinase-interacting protein 1 (GIT1) is enriched in both presynaptic and postsynaptic terminals and after phosphorylation is targeted to the synapse by binding to cytoplasmic protein GRB4 (NCK2 aka GRB4). GRB4 binds by its SH2 domain to Tyr392 (Human Tyr383) in the synaptic localization domain (SLD) of GIT1. GIT1 acts as an adapter protein by providing docking site for beta-PIX (PAK interacting exchange factor) and serves to localize Rac activity (Segura et al. 2007, Zhang et al. 2003).

**Preceded by:** SFKs phosphorylate GIT1

**Followed by:** GIT1 binds bPIX

## Literature references


Acker-Palmer, A., Essmann, CL., Weinges, S., Segura, I. (2007). Grb4 and GIT1 transduce ephrinB reverse signals modulating spine morphogenesis and synapse formation. *Nat. Neurosci., 10*, 301-10. ↗

Webb, DJ., Zhang, H., Horwitz, AF., Asmussen, H. (2003). Synapse formation is regulated by the signaling adaptor GIT1. *J. Cell Biol., 161*, 131-42. ↗


## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

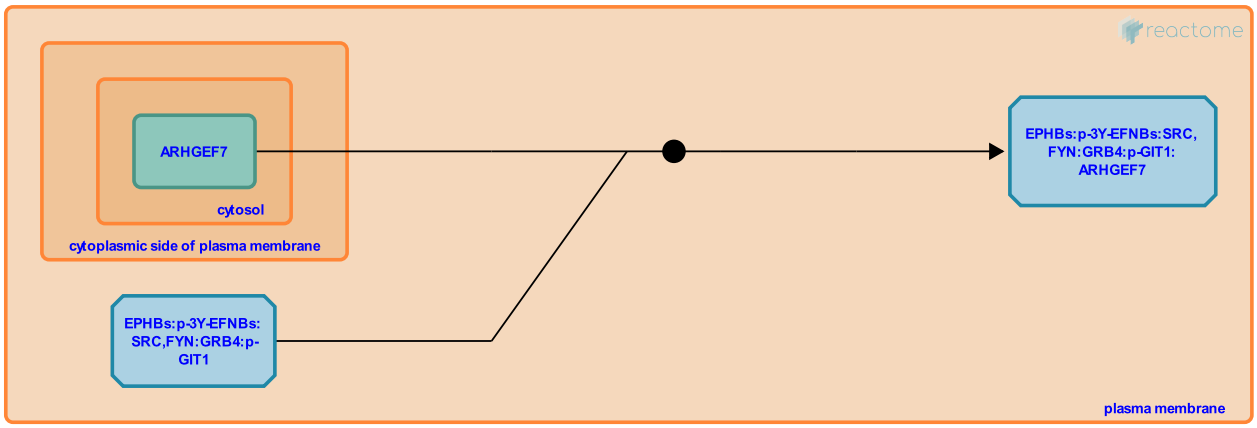
## GIT1 binds bPIX ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928631

**Type:** binding

**Compartments:** plasma membrane, cytosol




p-21-activated kinase (PAK) interacting exchange factor (beta-PIX, bPIX aka ARHGEF7) is a Rac guanine nucleotide exchange factor that acts downstream of GIT1 to affect spine morphology and synapse formation. Endogenous bPIX is present in hippocampal neurons and is targeted to the synapses by binding to GIT1. bPIX serves as an exchange factor for Rac and a binding protein for a Rac effector, PAK (Zhang et al. 2003).

**Preceded by:** EFNB:GRB4 binds GIT1

**Followed by:** bPIX exchanges GTP for GDP on RAC, activating it

## Literature references

Webb, DJ., Zhang, H., Horwitz, AF., Asmussen, H. (2003). Synapse formation is regulated by the signaling adaptor GIT1. *J. Cell Biol., 161*, 131-42. ↗

## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

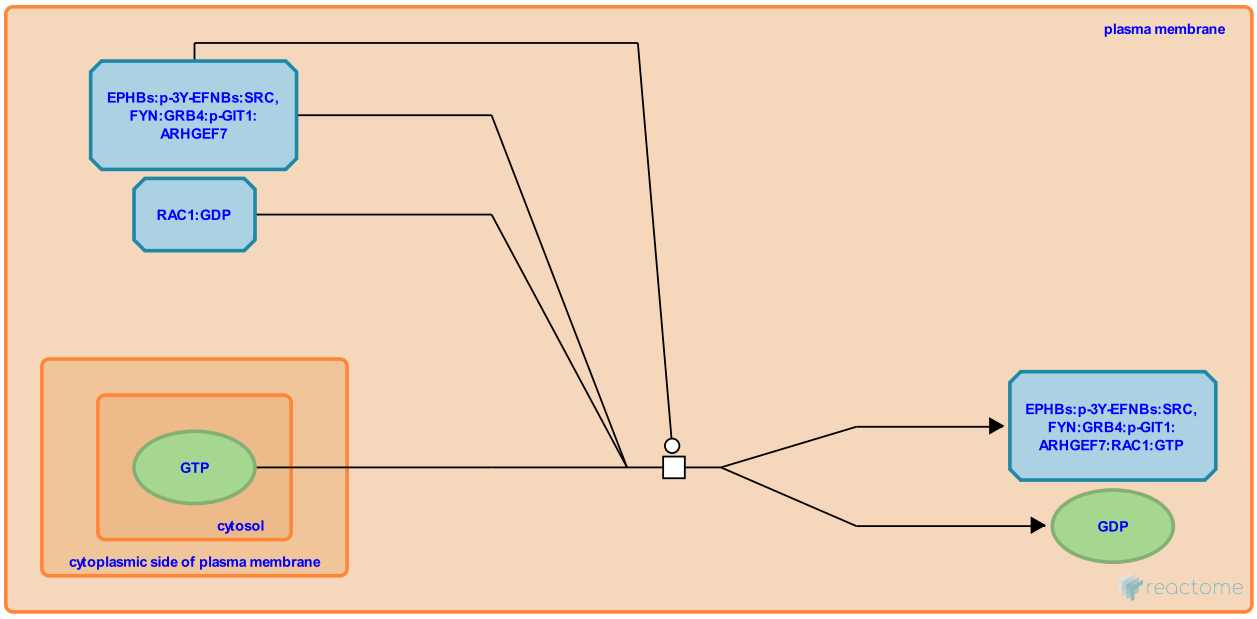
## bPIX exchanges GTP for GDP on RAC, activating it ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928633

**Type:** transition

**Compartments:** plasma membrane




p-21-activated kinase (PAK) interacting exchange factor (beta-PIX, bPIX aka ARHGEF7) a Rac GEF localised at the dendritic spines, activates Rac by exchanging GDP for GTP.

**Preceded by:** GIT1 binds bPIX

**Followed by:** PAK binds RAC and bPIX

## Literature references


Webb, DJ., Zhang, H., Horwitz, AF., Asmussen, H. (2003). Synapse formation is regulated by the signaling adaptor GIT1. *J. Cell Biol., 161*, 131-42. ↗


## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

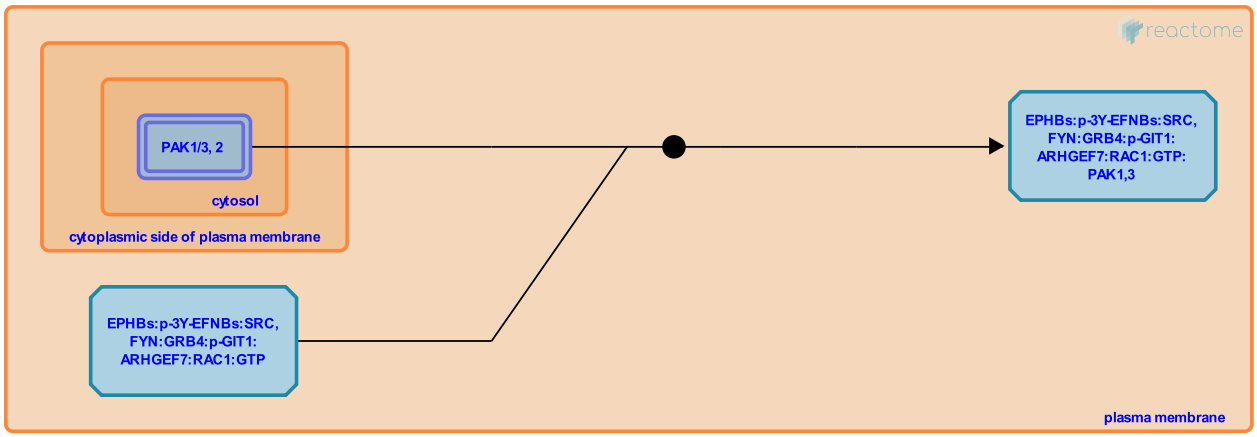
## PAK binds RAC and bPIX ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928641

**Type:** binding

**Compartments:** plasma membrane, cytosol




Serine/threonine-protein kinases PAK1, 2 and 3 (PAK1,2,3) serve as downstream effectors of RACs in regulating spine and synapse formation. Once activated, Ras-related C3 botulinum toxin substrate 1 (RAC1) binds to a variety of downstream effector proteins. Among the most well characterized effectors are PAKs. In addition to binding active RAC, PAKs also directly bind p-21-activated kinase (PAK) interacting exchange factor (beta-PIX, bPIX aka ARHGEF7) (Zhang et al. 2005).

**Preceded by:** bPIX exchanges GTP for GDP on RAC, activating it

**Followed by:** PAKs autophosphorylate

## Literature references

Webb, DJ., Zhang, H., Horwitz, AF., Asmussen, H., Niu, S. (2005). A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. *J. Neurosci., 25*, 3379-88. ↗

## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

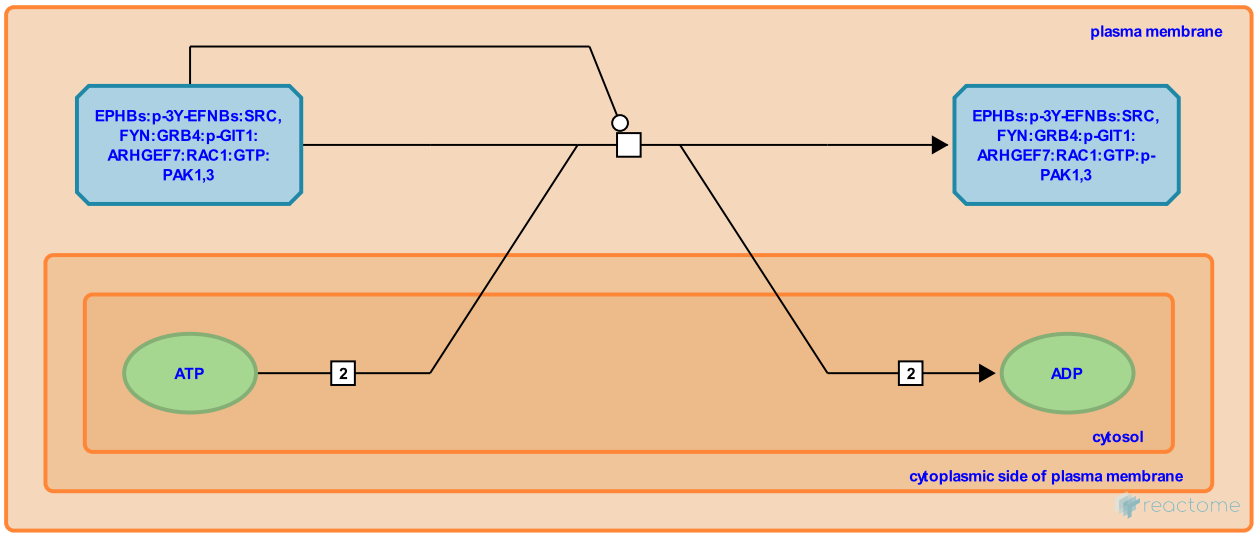
## PAKs autophosphorylate ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928625

**Type:** transition

**Compartments:** plasma membrane, cytosol




PAK is also locally activated in the synapse. PAK1 needs autophosphorylation for complete activation. PAK1 is autophosphorylated at several sites, but serine 144 (S144) in the GTPase-binding domain and threonine 423 (T423) in the activation loop are the two conserved sites that regulate the catalytic activity (Bokoch 2003).

**Preceded by:** PAK binds RAC and bPIX

**Followed by:** PAKs phosphorylate MLC

## Literature references


Bokoch, GM. (2003). Biology of the p21-activated kinases. *Annu. Rev. Biochem., 72*, 743-81. ↗

Webb, DJ., Zhang, H., Horwitz, AF., Asmussen, H., Niu, S. (2005). A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. *J. Neurosci., 25*, 3379-88. ↗


## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

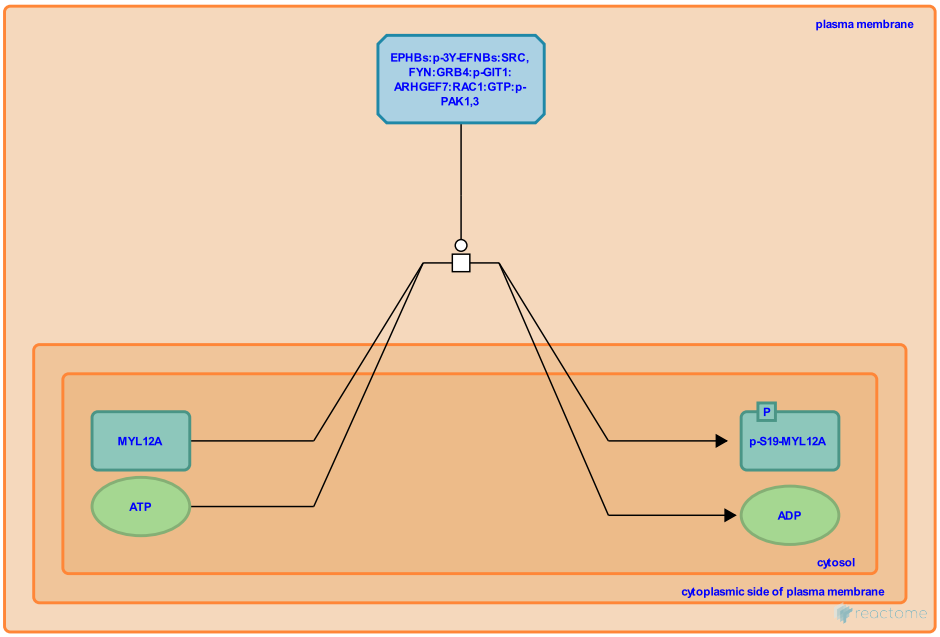
## PAKs phosphorylate MLC ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-3928640

**Type:** transition

**Compartments:** plasma membrane, cytosol




Myosin II regulatory light chain (MLC) acts downstream of serine/threonine-protein kinase PAK (PAK) to mediate its effect on spine morphogenesis and excitatory synapse formation. PAK directly phosphorylates MLC on serine 19. MLC phosphorylation could promote dendritic spine morphogenesis by stabilizing the actin network at this site (Zhang et al. 2005).

**Preceded by:** PAKs autophosphorylate

## Literature references


Goeckeler, ZM., Masaracchia, RA., Wysolmerski, RB., Chew, TL. (1998). Phosphorylation of non-muscle myosin II regulatory light chain by p21-activated kinase (gamma-PAK). *J. Muscle Res. Cell. Motil., 19*, 839-54. ↗

Webb, DJ., Zhang, H., Horwitz, AF., Asmussen, H., Niu, S. (2005). A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. *J. Neurosci., 25*, 3379-88. ↗


## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

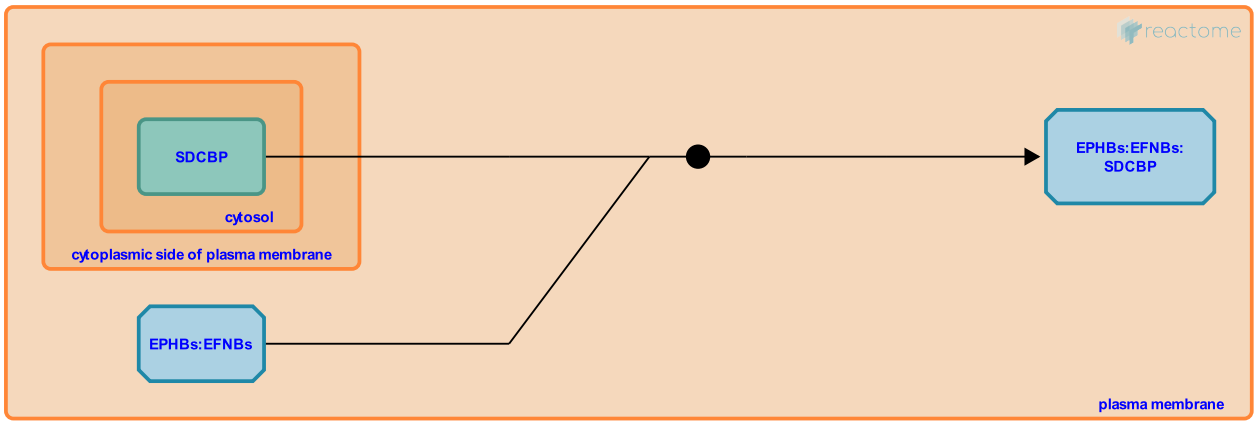
## EFNBs bind SDCBP ↗

**Location:** Ephrin signaling

**Stable identifier:** R-HSA-4093329

**Type:** binding

**Compartments:** plasma membrane, cytosol




Activation of ephrin-B1 (EFNB1) and ephrin-B2 (EFNB2) by postsynaptic EPHB receptors initiates presynaptic differentiation and causes increases in the density of presynaptic release sites. The EPHB-EFNB interaction recruits the adaptor protein syntenin-1 (SDCBP) through the PDZ-binding domain of EFNBs. SDCBP colocalizes with EFNB1 and EFNB2 at synaptic contacts and knockdown of EFNBs leads to a reduction in the number of synaptic contacts. SDCBP provides a direct link by which EFNB can associate with a protein complex involved in the recruitment and regulation of presynaptic vesicles. McClelland et al. suggest a model whereby SDCBP recruits multidomain scaffolding molecules that enables the clustering of synaptic vesicles including ERC2/CAST1, RIM1 and Rab synaptic vesicle proteins (McClelland et al. 2009).

## Literature references

McClelland, AC., Dalva, MB., Kayser, MS., Sheffler-Collins, SI. (2009). Ephrin-B1 and ephrin-B2 mediate EphB-dependent presynaptic development via syntenin-1. *Proc. Natl. Acad. Sci. U.S.A., 106*, 20487-92. ↗

## Editions

| | | |
|---|---|---|
| 2013-07-23 | Authored, Edited | Garapati, P V. |
| 2014-05-19 | Reviewed | Ip, NY. |

# Table of Contents


Introduction 1

Ephrin signaling 2

- EFNBs bind SFKs 3
- SFKs phosphorylate EFNBs 4
- p-EFNB binds GRB4 5
- SFKs phosphorylate GIT1 6
- EFNB:GRB4 binds GIT1 7
- GIT1 binds bPIX 8
- bPIX exchanges GTP for GDP on RAC, activating it 9
- PAK binds RAC and bPIX 10
- PAKs autophosphorylate 11
- PAKs phosphorylate MLC 12
- EFNBs bind SDCBP 13

Table of Contents 14