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Title Page

Gβγ pathways in cell polarity and migration linked to oncogenic GPCR signaling: potential relevance in tumor microenvironment

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Running Title Page

Running title: **Gβγ pathways in oncogenic GPCR signaling**

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Abstract

Cancer cells and stroma cells in tumors secrete chemotactic agonists that exacerbate invasive behavior, promote tumor-induced angiogenesis, and recruit protumoral bone marrow-derived cells. In response to shallow gradients of chemotactic stimuli recognized by G protein coupled receptors (GPCRs), Gβγ-dependent signaling cascades contribute to specify the spatiotemporal assembly of cytoskeletal structures that can dynamically alter cell morphology. This sophisticated process is intrinsically linked to the activation of Rho GTPases and their cytoskeletal-remodeling effectors. Thus, Rho guanine nucleotide exchange factors, the activators of these molecular switches, and their upstream signaling partners, are considered participants of tumor progression. Specifically, phosphoinositide-3 kinases (class-I PI3Ks, β and γ) and P-Rex1, a Rac-specific guanine nucleotide exchange factor, are fundamental Gβγ effectors in the pathways controlling directionally persistent motility. In addition, GPCR-dependent chemotactic responses often involve endosomal trafficking of signaling proteins, and coincidently, endosomes serve as signaling platforms for Gβγ. In preclinical murine models of cancer, inhibition of Gβγ attenuates tumor growth, whereas in cancer patients, aberrant overexpression of chemotactic Gβγ-effectors and recently-identified mutations in Gβ correlate with poor clinical outcome. Here we discuss emerging paradigms of Gβγ signaling in cancer, which are essential for chemotactic cell migration and represent novel opportunities to develop pathway-specific pharmacological treatments.

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Introduction

In tumor microenvironments, cells adjust their shape and move in response to multiple factors secreted by cancer cells and stromal cells (Friedl and Alexander, 2011; Roussos et al., 2011). Inherent intracellular forces are generated by actin polymerization and ATPase activity of myosin motors, and these mechanical responses are tightly controlled by receptor-dependent signaling mechanisms that define the precise spatiotemporal reorganization of the actin cytoskeleton (Alexander and Friedl, 2012; Devreotes and Horwitz, 2015). In particular, chemotactic G protein-coupled receptors (GPCRs) elicit G protein-dependent signaling pathways linked to metastatic dissemination of cancer cells, formation of new blood vessels, and recruitment of proangiogenic cells from the bone marrow, all of which are critical to maintain cancer progression (Figure 1) (Dorsam and Gutkind, 2007; Lappano and Maggiolini, 2011).

Current models of GPCR-dependent chemotactic cell migration have evolved from studies on fast-moving cells that migrate individually, such as *Dictyostelium* amoebae and neutrophils. These generic pathways mediating migration suggest the involvement of Gβγ-dependent effectors, particularly PI3Ks and phosphatidylinositol (3,4,5)-trisphosphate-dependent effectors, including guanine nucleotide exchange factors for Rho GTPases. Cytoskeletal reorganization is launched by actin nucleation sites generated by effectors of GTP-bound Rho GTPases; RhoA, Rac1 and Cdc42 are the best characterized members of this family of molecular switches (Figure 2). These Rho-GTPase-dependent downstream mechanisms will not be discussed here, but readers are encouraged to consult excellent reviews covering these topics (Burridge and Wennerberg, 2004; Charest and Firtel, 2007; Rickert et al., 2000; Ridley, 2015; Ridley et al., 2003; Van Haastert and Devreotes, 2004). General mechanisms of directional cell migration provide a basis to explore GPCR-dependent cell migration linked to cancer

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progression. However, in cancer settings, this process is further complicated by the influence of a plethora of stimuli in the microenvironment. Moreover, oncogenic pathways participating in chemotactic migration are affected by mutational changes, overexpression and differential repertoire of signaling systems which, among many other factors, eventually are integrated to facilitate individual and collective migration of cancer and protumoral cells (Figure 1).

We focus this review on the role of Gβγ signaling in cancer. Emphasis is given to upstream effectors leading to the activation of Rho GTPases in the pathways that control chemotactic cell migration. In this window of molecular interactions, the participation of class-I PI3Ks (β and γ) and P-Rex1, a Rac-specific guanine nucleotide exchange factor is considered critical. We highlight the properties of P-Rex1, known to be activated in concert by Gβγ and phosphatidylinositol (3,4,5)-trisphosphate (Welch, 2015; Welch et al., 2002), as a putative scaffold of chemotactic signaling. The proposed role of this multidomain effector is based on its reported interactions with sphingosine-1-phosphate receptors and the serine/threonine kinases mTOR and type I PKA (Chavez-Vargas et al., 2016; Hernandez-Negrete et al., 2007; Ledezma-Sanchez et al., 2010). Overall, the proven relevance of multiple chemokines in oncogenic settings has pointed to GPCRs and their signaling effectors as possible pharmacological targets in cancer research.

Protumoral chemotactic agonists

Multiple agonists play a driving role in cancer progression. Oncogenic chemokines attract and consequently diversify the repertoire of cells associated with proliferating tumoral cells. Once incorporated as cellular components of the tumor stroma, fibroblasts, endothelial and immune cells also contribute to increase the variety of secreted chemokines that not only

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stimulate the surrounding tissues, but also have a systemic effect: attracting cells from the bone marrow (Figure 1) (Kojima et al., 2010; Orimo et al., 2005; Scherz-Shouval et al., 2014; Wels et al., 2008). Tumor-associated bone marrow derived cells (BMDC) further support cancer progression via their ability to secrete chemokines, cytokines, growth factors, and proteins of the extracellular matrix (Gao et al., 2008; Nolan et al., 2007; Sekiguchi et al., 2011). Among BMDC's protumoral effects, preparing the ground where metastatic niches are eventually established is particularly significant (Gao et al., 2008; Kaplan et al., 2005; Lyden et al., 2001; Peinado et al., 2012; Psaila and Lyden, 2009; Qian et al., 2011).

Examples of protumoral GPCR agonists with chemotactic properties include CCL2/5/22/25, CXCL1/2/5/8(IL-8)/12(SDF-1), and CX3CL1 (Bronte and Bria, 2016; Roussos et al., 2011; Ugel et al., 2015). In addition, bioactive lipids such as sphingosine-1-phosphate (Liang et al., 2013) and LPA (Jonkers and Moolenaar, 2009; Liu et al., 2009) also stimulate pro-oncogenic migratory pathways. CXCL12 and CCL2 attract monocytes that promote tumor-induced angiogenesis and contribute to the maintenance of an immunosuppressive microenvironment, which allows transformed cells to evade anti-neoplasic immune responses (Kitamura et al., 2015; Qian and Pollard, 2010). Furthermore, CXCL12 recruits endothelial progenitors from the bone marrow (Orimo et al., 2005), promotes angiogenic responses mediated by Rho GTPases (Carretero-Ortega et al., 2010), and plays a positive role in tumor vascularization (Guleng et al., 2005). Multiple cancer cells overexpress CXCR4 receptors, putatively enabling them to metastasize in response to CXCL12. Examples include breast carcinoma (Muller et al., 2001; Yagi et al., 2011), gastric carcinoma (Yasumoto et al., 2006), pancreatic cancer (Marchesi et al., 2004; Xu et al., 2015), ovarian carcinoma (Barbolina et al., 2010) and oral cancers (Delilbasi et al., 2004; Uchida et al., 2007). Metastatic signaling

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mechanisms elicited by CXCR4 involve heterotrimeric Gi and G_{13} proteins. In these cases, chemotactic GPCRs activate Rho GTPases that control cell migration into the stroma of primary tumors and metastatic tissues. Therefore signaling pathways leading to the activation of Rho GTPases are considered central players in cancer settings (Dillenburg-Pilla et al., 2015; Tan et al., 2006; Yagi et al., 2011) (Figure 1). As an indicator of the emerging clinical importance of these pathways, a search for "chemokine and cancer" listed 108 studies in ClinicalTrials.gov.

The role of GPCRs, heterotrimeric G proteins, and their target RhoGEFs in tumor growth and metastasis. Cancer cells often hijack G protein signaling pathways, and this exacerbates the effect of oncogenes. Expressed in cancer and stroma cells in tumors, GPCRs act in concert with growth factor receptors, extracellular matrix proteins, and diverse agonists at various stages of oncogenic progression (Dorsam and Gutkind, 2007; Roussos et al., 2011) (Figure 1). Interestingly, these seven-transmembrane receptors further enhance the oncogenic potential of transformed cells by potentiating their paracrine effects. For instance, in highly metastatic breast cancer cells, Calcium Sensing Receptor (CaSR) promotes secretion of CCL22/MDC among other chemokines and growth factors that, *in vitro*, activate a chemotactic and angiogenic response. The mechanism involves transactivation of Epidermal Growth Factor Receptor and a positive loop based on the constitutive secretion of IL-6 (Hernandez-Bedolla et al., 2015). This GPCR also attenuates signaling effects of TGF-β, an important tumor suppressor. In particular, CaSR interferes with Smad2 phosphorylation and promotes its degradation. This inhibitory effect involves trafficking of CaSR in Rab11-positive endosomes (Organista-Juarez et al., 2013).

The oncogenic power of GTPase-deficient mutant Gα proteins, originally reported using transfected cellular models, has been confirmed in various types of human cancer. Examples include mutations in *GNAQ* (encoding Gαq), detected in almost 50% of ocular melanomas (Van

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Raamsdonk et al., 2009; Van Raamsdonk et al., 2010), and *GNAS* (encoding Gαs protein), initially characterized in pituitary tumors, and also identified in colon, hepatic, and pancreatic cancers (among others) using deep sequencing analysis, (Landis et al., 1989; O'Hayre et al., 2013).

To understand the mechanisms by which chemotactic GPCRs elicit local assembly of actin nucleation sites, it is critical to focus on Rho guanine nucleotide exchange factors (RhoGEFs) as effectors of heterotrimeric G proteins. Essentially, a polarized response marks the origin of cell protrusions to follow a chemotactic gradient. This is coordinated by Rho GTPases activated downstream of chemotactic GPCRs (Goicoechea et al., 2014; Kedziora et al., 2016; Vaque et al., 2013; Yagi et al., 2011). In this context, chemotactic stimuli eventually produce cytoskeletal structures as lamelipodia, filopodia and stress fibers, which are needed for cell protrusion and contraction. These examples of crosslinked fibers of polymerized actin and their interacting regulators are controlled by distinct Rho GTPases, such as Rac1, Cdc42 and RhoA (Figure 2) (Kozma et al., 1995; Nobes and Hall, 1995; Ridley and Hall, 1992; Ridley et al., 1992). Therefore, an instrumental role is anticipated for GPCR-regulated RhoGEFs, such as RH-RhoGEFs, P-REX1, and their upstream regulators as central controllers of the activation of Rho GTPases via the integration of second messengers, protein interactions and posttranslational modifications (Bracho-Valdes et al., 2011; Cook et al., 2014; Dillon et al., 2015; Hernandez-Negrete et al., 2007; Kim et al., 2011).

Receptors coupled to Gi, $G_{12/13}$ and $G_{q/11}$ activate Rho GTPases, either via Gβγ, in the case of Gi, or GTP-boud Gα subunits in the other cases (Figure 3). A group of RhoGEFs with a domain homologous to RGS, characteristic of proteins known as regulators of G protein signaling, are directly activated by $G\alpha_{12/13}$ (Aittaleb et al., 2010; Fukuhara et al., 2000; Fukuhara

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et al., 2001; Fukuhara et al., 1999; Hart et al., 1998; Kozasa et al., 2011; Vazquez-Prado and Gutkind, 2011), whereas p63RhoGEF and Trio are activated by $G\alpha_{q/11}$ (Lutz et al., 2005; Rojas et al., 2007). These G protein-regulated RhoGEFs are members of a superfamily of DH-RhoGEFs characterized by a catalytic domain with homology to *Dbl*, which is common among the 70 members of the biggest family of RhoGEFs (Cook et al., 2014; Rossman et al., 2005; Schmidt and Hall, 2002).

The group of RH-RhoGEFs (also been named RGS-RhoGEFs or RGL-RhoGEFs) includes p115RhoGEF/ARHGEF1 (Hart et al., 1998), PDZ-RhoGEF/ARHGEF11 (Fukuhara et al., 1999), and LARG/ARHGEF12 (Fukuhara et al., 2000) (Figure 3). The interaction between $G\alpha_{13}$ and the RGS-like domain, involving the switch-regions of $G\alpha_{13}$ and most of the GTPase domain (Vazquez-Prado et al., 2004), is reminiscent of the interaction between $G\alpha$ subunits and RGS proteins. Thus, no second messengers are required as intermediates in the activation of RhoA by these G proteins, but membrane localization of $G\alpha_{13}$ is essential (Vazquez-Prado et al., 2004). The RGS-like domains of PDZ-RhoGEF and p115RhoGEF interacting with $G\alpha_{13}$ or a chimeric $G\alpha_{13/i}$ GTPase, respectively, have been analyzed at the atomic level (Chen et al., 2008; Chen et al., 2005). These structures, together with multiple biochemical assays, support a model implying that $G\alpha_{13}$ putatively removes inhibitory intramolecular interactions and facilitates the establishment of stimulatory ones critical for exposing the catalytic DH domain. As a consequence, RH-RhoGEFs interact with RhoA, promoting exchange of GDP for GTP in this small GTPase (Aittaleb et al., 2010). In a murine model of breast cancer, an aberrant coupling of G_{13} to CXCR4 receptors has been linked to metastatic progression of tumors via RhoA-dependent cytoskeletal pathways regulating cell migration (Yagi et al., 2011).

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An alternative mechanism of activation of Rho GTPases, relevant to chemotactic and adhesion GPCRs signaling (Hamoud et al., 2014; Park et al., 2007; Stevenson et al., 2014; Watanabe et al., 2014), involves a second phylogenetically-conserved family of GEFs. This group of nonconventional GEFs is specific for Rac and Cdc42 GTPases. It is constituted by 11 DOCK proteins, characterized by their catalytic DHR2 domain, and 3 ELMO scaffolds, known as functional interactors of DOCK 1 to 5 proteins (Gadea and Blangy, 2014; Laurin and Cote, 2014). Diverse chemoattractants stimulate ELMO-2 translocation to the plasma membrane. This relocalization is mediated by Gβγ, which directly interacts with the amino-terminal region of ELMO (Wang et al., 2016). In vitro, the CXCL12-dependent chemotactic response of highly metastatic breast cancer cells requires the expression of ELMO-1/2 proteins, which seems to exert a redundant role. Interestingly, in invasive ductal breast carcinoma, ELMO-1 is overexpressed, whereas in a murine model of metastatic breast cancer, ELMO-1 knockdown prevented invasion to the lungs (Li et al., 2013). Moreover, in breast cancer cells, ELMO phosphorylation by Axl resulted in increased invasion (Abu-Thuraia et al., 2015). Consistent with the intervention of Axl in chemotactic GPCR signaling, in colon cancer cells, CXCL12 stimulated Axl expression. Furthermore, the effect of this chemokine on cell invasion is attenuated by Axl knockdown, indicating the existence of an aberrant positive loop that exacerbates the CXCR4-dependent invasive behavior of colon cancer cells (Heckmann et al., 2014).

The mechanisms by which Gβγ activates Rho GTPases appears to be diverse, and involve Gβγ-regulated RhoGEFs and other Gβγ effectors such as PI3Ks which, through phosphorylated lipid second messengers generated in the surroundings of Gβγ, activate PIP3-dependent RhoGEFs (Dbouk et al., 2012b; Donald et al., 2004; Dupre et al., 2009; Guzman-Hernandez et

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al., 2009; Rosenfeldt et al., 2004; Schwindinger and Robishaw, 2001; Vadas et al., 2013; Welch et al., 2002). As shown in Figure 3, different RhoGEFs interact with Gβγ and are likely activated by the versatile heterodimer, but follow-up investigations are necessary. Additional assays such as pulldowns of active-RhoGEFs using recombinant nucleotide-free mutant Rho GTPases (Garcia-Mata et al., 2006) might help to discern whether they are *sine qua non* Gβγ-effectors.

Intervention of various RhoGEFs in chemotactic signaling pathways is likely redundant. This is in part explained by their sensitivity to phosphoinositide-derived second messengers and, for certain groups, to the fact that those with equivalent conserved structures might be co-expressed. For instance, a recent systematic analysis assessing endothelial expression of 81 RhoGEFs (70 homologous to Dbl and 11 of the DOCK family) revealed that more than half, including RH-RhoGEFs and P-Rex1, are abundantly expressed in VEGF-stimulated cells as well as in tumor endothelial cells, supporting their potential participation in tumor angiogenesis (Hernandez-Garcia et al., 2015).

Availability of Gβγ for chemotactic signaling

A recent revolution in the field of GPCR structural analysis has consolidated a rather simplistic model to explain how multiple GPCRs transduce their signals through a small group of heterotrimeric G proteins (Tesmer, 2016); agonist-occupied seven transmembrane receptors are visualized as signaling funnels by which hundreds of ligands use common mechanisms of transduction. They expose an intracellular pocket that serves as a docking site occupied by the carboxyl-terminal tail of appropriate Gα subunits in complex with Gβγ. Following this interaction, Gα releases its bound GDP, incorporates GTP, and dissociates from Gβγ and the receptor, initiating signaling events at the plasma membrane (Tesmer, 2016). It is well accepted

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that this model explains multiple complex cellular processes. The emerging concept of biased agonism implicates a previously unnoticed sophisticated level of complexity. Current models of active-receptor conformation go beyond the originally postulated concept that functional receptors only existed as one of two possible conformations: active or inactive. Multiple lines of evidence suggest that various conformations of the receptors are able to promote heterotrimeric G protein-dependent and independent pathways (Liu et al., 2015b; Luttrell et al., 2015; Pupo et al., 2016).

In general terms, the canonical mechanism of heterotrimeric G protein signaling involves redistribution of GTP-bound Gα and Gβγ at the internal surface of the plasma membrane (Hepler, 2014; Lambert, 2008). There, they interact with their effectors, which are either integral plasma membrane proteins or cytosolic signaling partners recruited via direct interactions. This stage of the process is traditionally limited to the plasma membrane, where the cycle is reinitiated when the actions of both Gα and Gβγ are turned off.

The role of Gβγ in the general model of GPCR signal transduction has been established after some initial controversy. Thirty years of signaling history have taken Gβγ from an innocuous Gα-binding partner to a *bona fide* signal transducer that targets multiple effectors. The report by Eva Neer and David Clapham recognizing Gβγ as a powerful activator of ion channels (Logothetis et al., 1987) was initially received with some skepticism by the G-protein signaling community since the catalytic Gα subunits were in the spotlight of proposed transducing mechanisms. However, a key role for heterodimeric Gβγ is now well accepted (see below), in addition to the influence of heterodimeric Gβγ on Gα signaling apparent at various levels. Gβγ serves as a GDI (guanine-nucleotide dissociation inhibitor) that keeps the switch

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represented by Gα in the off-conformation. Gβγ is needed to reinitiate a cycle of heterotrimeric G proteins by reconstituting new heterotrimers once GTP has been hydrolyzed by Gα, and it is essential for heterotrimeric G protein coupling to agonist-occupied receptors (Tang et al., 2006).

As transducers, Gβγ heterodimers are among the most ubiquitous signaling proteins; five different Gβ and twelve Gγ are encoded in the human genome (Hurowitz et al., 2000). With the exception of Gβ5, which preferentially interacts with RGS 7 and 9 proteins, all Gβ subunits interact with different Gγ subunits, although association preferences do exist (Mervine et al., 2006). Thus, multiple possible combinations, mainly limited by their expression in different tissues, exist. Therefore, Gβγ heterodimers exhibit an enormous signaling repertoire, perhaps comparable with that of dynamically modulated signaling lipids such as PIP3, a regular coactivator of Gβγ effectors. The existence of many different Gβγ heterodimers and their redundant biochemical effects are evidence of their important role in eukaryotic evolution (Krishnan et al., 2015).

Although Gβγ signaling is expected to be activated downstream of every heterotrimeric G protein, research indicates that some heterotrimers are better providers of signaling-ready Gβγ heterodimers than others. It is well accepted that GPCR-dependent chemotactic events are mediated by Gβγ released from Gi, but not from Gs or Gq (Neptune et al., 1999). Such singularity in Gβγ signaling might be explained by heterogeneous abundance of different heterotrimers, unique kinetics of GTP hydrolysis by Gα subunits (influenced by different regulatory proteins) followed by recapture of free Gβγ, or even differential degrees of dissociation between GTP-bound Gα and Gβγ (Bondar and Lazar, 2014; Hepler, 2014).

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Spatiotemporal asymmetry defined by Gβγ availability sets the basis of chemotactic signaling (Jin et al., 2000). Recent optogenetic and chemical biology approaches confirmed that local availability of Gβγ is sufficient to control polarized cell migration (O'Neill and Gautam, 2014; Surve et al., 2014). Gβγ recruits cytosolic signaling proteins to the plasma membrane as a consequence of its dissociation from Gαi-GTP, which is elicited by chemotactic GPCRs. These include PI3Ks and RhoGEFs (Figures 2 and 3), the most relevant Gβγ effectors involved in cell migration. Initial translocation of signaling proteins to the plasma membrane is frequently correlated to their activation. However, it should not be assumed that every signaling protein recruited by Gβγ will be fully activated by this interaction. In some cases, Gβγ might only contribute to the translocation of signaling proteins or it might act as a coactivator in concert with lipid second messengers or additional signaling proteins. For instance, PI3Kβ requires simultaneous input by Gβγ and growth factor receptors (Houslay et al., 2016). This early array of signaling interactions is subjected to regulatory circuits that amplify the initial inputs. In particular, PI3Kβ is sensitive to GTP-bound Rac and Cdc42 GTPases. Thus, PIP3 stimulates Rho GEFs that activate Rac, Cdc42, and, consequently, their downstream effectors. Among them, PI3Kβ putatively closes a circuit that produces more PIP3 (Fritsch et al., 2013). Overall, integration of these signaling inputs is finally manifested by polarized cytoskeletal arrangements that promote cell propulsion, which sustains directional migration (Figure 2).

Structural analysis of heterodimeric Gβγ bound to different interactors assists in the understanding of how this non catalytic-signaling complex activates its effectors. For instance, in the case of GRK-2 (G protein-coupled receptor kinase-2), one of the few Gβγ effectors known at the structural level in complex with the heterodimer (Tesmer et al., 2005), GRK-2 is first

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recruited to the membrane by Gβγ. Then, the spatial orientation of GRK-2 is fine-tuned by lipid second messengers that fix the PH-domain in a position that orients the catalytic domain in the proper conformation for access to its substrates and regulators (Yang et al., 2016). This general mechanism may also be effective for other cytosolic Gβγ-effectors that integrate PIP3 and Gβγ signaling, such as P-Rex1. In contrast, the mechanism by which Gβγ activates effectors already positioned in the plasma membrane, such as the GIRK channels, serves as a model for understanding effector activation independent of the cytosol to membrane translocation step, which is common for most Gβγ-effectors. According to the structural analysis of tetrameric GIRK-2 channels, together with four Gβγ heterodimers, the orientation of Gβγ is similar to that observed in the ternary complex of agonist-occupied β2-adrenergic receptors coupled to nucleotide-free Gs (Rasmussen et al., 2011; Whorton and MacKinnon, 2013). In the channel, the intracellular domain of every one of its four subunits interacts with PIP2 and Gβγ, and this interaction fixes a pre-open channel conformation (Whorton and MacKinnon, 2013). It is interesting that the intracellular domains of the channel integrate phosphoinositides with Gβγ signaling. Thus, in cellular settings, a common theme in the regulation of Gβγ-effectors appears to be the concerted participation of lipid second messengers.

The number of Gβγ-regulated signaling molecules is still expanding and seems to be related to multiple possible protein-protein interacting motifs at the surface of Gβγ. A diversity of complementary experimental approaches has revealed this broad array of Gβγ-docking sites. Peptides, identified by phage-display as interactors of different regions of Gβγ, interfere with the activation of some Gβγ-effectors, but not others (Scott et al., 2001). Correspondingly, point-mutations at different surface residues of Gβγ affect a divergent pattern of Gβγ-regulated

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effectors (Ford et al., 1998). Comparison of different structures in which Gβγ is part of a complex with GIRK-2, GRK-2, $G\alpha_{i1}$, or Phosducin showed partial overlapping of the Gβγ-interacting surface, but specific differential areas of interaction were also recognized (Whorton and MacKinnon, 2013). Interestingly, a peptide corresponding to phosducin-like protein amino-terminal region competes with PI3Kγ for the interaction with Gβγ and attenuates the recruitment of this lipid kinase to the plasma membrane (Guzman-Hernandez et al., 2009). Moreover, this peptide inhibits AKT activation by sphingosine-1-phosphate in endothelial cells without affecting the simultaneous activation of ERK (Guzman-Hernandez et al., 2009). Furthermore, this peptide differentially inhibits chemotactic and angiogenic responses to sphingosine-1-phosphate without interfering with the effect of hepatocyte growth factor (Guzman-Hernandez et al., 2009). Altogether, these studies support the working hypothesis that differential inhibitors of Gβγ signaling, interfering with some effectors but not others, might reach clinical development with the final aim of interfering with aberrant Gβγ-dependent signaling pathways.

Gβγ-dependent activation of PI3K. The PI3K/AKT/mTOR signaling pathway plays a central role in cell migration, among other fundamental physiological processes (Bracho-Valdes et al., 2011). In order to participate in a chemotactic response, heterodimeric Gβγ exhibits its signaling interface, covered in the basal non-stimulated condition by Gα, to directly interact with the catalytic subunit of PI3Kβ (via a low affinity site, stabilized with membrane lipids) or with adaptors of PI3Kγ (Dbouk et al., 2012a; Thorpe et al., 2015; Vadas et al., 2013).

Although molecular structures of Gβγ/PI3Ks signaling complexes are not yet available, the interaction between Gβγ and PI3Kβ has been mapped to a non-conserved region, including 24 amino acids between the C2 and helical domains of p110β catalytic subunit (Dbouk et al.,

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2012a). Knock-in mice in which non-Gβγ-sensitive PI3Kβ was expressed revealed that this lipid kinase integrates signaling inputs by Gβγ and growth factor receptors (Houslay et al., 2016). The interaction of Gβγ with p110β is essential for Gi-dependent AKT activation, chemotaxis and *in vitro* transforming activity (Dbouk et al., 2012a). Interestingly, WDR26, an oncogenic scaffold overexpressed in breast cancer, facilitates PI3Kβ signaling by Gβγ (Ye et al., 2016).

The interaction between Gβγ and PI3Kγ is mediated by the regulatory p101 and p87/p84 subunits, and the interaction supports the association of the lipid kinase to the membrane where Gβγ then exerts a direct stimulatory effect on the catalytic subunit (Brazzatti et al., 2012; Shymanets et al., 2013). Gβγ stabilizes the conformation of the catalytic p110γ subunit, specifically the linker region between RBD and C2 exposed by interaction with lipids. The existence of these molecular contacts has been revealed by hydrogen–deuterium exchange mass spectrometry approaches (Vadas et al., 2013).

In Gβγ, the interface of interaction with PI3Ks (β and γ) seems to be partially different for each isoform (Dbouk et al., 2012a). PI3Kβ interacts with Gβγ in two regions. The first, located at the linker between the amino-terminal α-helix of Gβ and its first blade, appears to be exclusive for this lipid kinase. The second region corresponds to an already mapped hotspot in Gβ important for the interaction with various effectors, including PI3Kγ, at the second blade (Dbouk et al., 2012a; Shymanets et al., 2012). As formerly demonstrated, inhibition of Gβγ/PI3Kγ interactions with a construct corresponding to the amino-terminal region of phosducin-like protein prevents Gi-dependent chemotactic and angiogenic responses elicited by sphingosine-1-receptors (Guzman-Hernandez et al., 2009). These data raise the possibility that the interface between Gβγ and either PI3Kβ or PI3Kγ is an attractive target for therapeutics.

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P-Rex1 as a potential chemotactic scaffold

Chemotactic GPCRs stimulate polarized actin polymerization, and effectors of Rho GTPases are essential activators of actin nucleation, implying that GPCRs must activate Rho GTPase regulators at a specific time and location to remodel the cytoskeleton (Hall, 2012). The ability of Gβγ to activate PI3Ks, which generate PIP3, together with the widely accepted concept that PH domains interact with this lipid second messenger (Moravcevic et al., 2012), set the basis for a general model of RhoGEF activation. Accordingly, polarized production of PIP3 potentially stimulates multiple RhoGEFs, most of which contain PH domains (Hernandez-Garcia et al., 2015; Rossman et al., 2005). However, other elements that impact the timing and magnitude of RhoGEF activation are likely integrated at the plasma membrane if a mechanism that is able to support persistent directional migration is involved. These include integral plasma membrane proteins, such as activated GPCRs (Ledezma-Sanchez et al., 2010) and free Gβγ that establish direct interactions with RhoGEFs and may have a role as docking sites and allosteric activators, likely maintaining RhoGEFs recruited at the plasma membrane. Thus, interacting molecules might control the temporal nature and levels of activity of different RhoGEFs.

During cell migration, chemotactic Gi-coupled receptors promote lamellipodial protrusions. These structures, dynamically formed at the leading edge of advancing polarized cells, are formed by effectors of Rac GTPases. Of particular interest, the P-Rex1 family of guanine nucleotide exchange factors, specific for Rac, is synergistically activated by Gβγ and PIP3 (Donald et al., 2004; Rosenfeldt et al., 2004; Welch, 2015; Welch et al., 2002). Structurally, this group is characterized by a multidomain architecture. This group of GEFs, including P-Rex1 and P-Rex2 (with two isoforms), represents the most common set of effectors by which Gβγ

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activates Rho GTPases, particularly Rac. However, other RhoGEFs have been reported as interactors and effectors of Gβγ (Figure 3).

The molecular characteristics expected for a chemotactic scaffold are present in the P-Rex family of GEFs. They contain six well defined domains, which in P-Rex1 are known to participate in different interactions with plasma membrane proteins, phosphoinositides, and serine/threonine kinases (Figure 4). In particular, P-Rex1 interacts with the carboxyl-terminal region of GPCRs such as S1P1 receptors. In endothelial cells expressing P-Rex1 PDZ domains, these receptors exhibit a sustained chemotactic response to sphingosine-1-phosphate. Due to their interaction with these domains, S1P1 receptors maintain their functionality at the plasma membrane, in contrast to those that are internalized in the absence of P-Rex1 PDZ domains (Ledezma-Sanchez et al., 2010). Furthermore, P-Rex1 is recognized and activated by mTORC2 (Hernandez-Negrete et al., 2007), a multimeric serine-threonine kinase that can directly be activated by PIP3 (Liu et al., 2015a). Moreover, mTORC2 is also recognized by Gβγ, which positively regulates its participation in GPCR-dependent activation of AKT (Robles-Molina et al., 2014). Although it is not yet known whether all of the P-Rex1 interactors mentioned above are simultaneous partners of this GEF, these proteins might be part of dynamic signaling complexes, where P-Rex1 is a central platform that putatively maintains directional cell migration.

P-Rex1 is inhibited by PKA (Chavez-Vargas et al., 2016; Mayeenuddin and Garrison, 2006; Urano et al., 2008), and accumulating evidence indicates that regulation of P-Rex1 by PKA occurs via a complex mechanism involving phosphorylation-dependent inhibitory interactions (Chavez-Vargas et al., 2016). Interestingly, the type I PKA regulatory subunit directly interacts with P-Rex1 PDZ domains. In addition, PKA catalytic subunit phosphorylates

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P-Rex1 at Ser-436 within the first DEP domain (Chavez-Vargas et al., 2016), whereas the carboxyl-terminal region of P-Rex1 is an indirect target of this kinase (Figure 4). As a consequence, P-Rex1 is inhibited by intramolecular interactions established between the phosphorylated domains and the catalytic DH-PH cassette (Chavez-Vargas et al., 2016).

In support of a spatiotemporal mechanism of reciprocal regulation between PKA and P-Rex1, experiments with fluorescently–tagged type I PKA regulatory subunit and P-Rex1 demonstrated that they translocate to the plasma membrane in response to CXCL12 (Chavez-Vargas et al., 2016). These results, together with the inhibitory effect of PKA-dependent P-Rex1 phosphorylation, indicate that type I PKA is a fine-tune control of P-Rex1 ready to modulate this GEF. In addition, the effect of cAMP on PKA dynamics suggests that the regulatory subunits of this kinase might also regulate P-Rex1 activity once the catalytic subunits are dissociated. Current efforts are addressing several intriguing hypothesis based on potential regulatory effects that go beyond P-Rex1 phosphorylation by PKA.

Gβγ/PI3K/mTORC2/P-Rex1 axis in oncogenesis

The fundamental role of Gβγ in GPCR-dependent chemotactic cell migration suggests that it plays a role in metastatic cancer; this is supported by preclinical evidence and cancer-related mutations in Gβ (Dbouk et al., 2012a; Tang et al., 2011; Yoda et al., 2015). The mechanism by which oncogenic activities of Gβγ are explained involves persistent activation of the PI3K/mTOR signaling pathway (Dbouk et al., 2012a; Khalil et al., 2016). Interestingly, Gβγ directly interacts with and activates PI3Ks (β and γ). Moreover, this signaling heterodimer also directly interacts with mTOR, facilitating GPCR signaling to AKT via mTORC2 (Robles-Molina et al., 2014), and it is well recognized that Gi-coupled GPCRs activate the PI3K/AKT/mTOR

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signaling pathways. Both type-I β and γ PI3Ks are activated by Gβγ, leading to mTORC1 activation downstream of AKT. Moreover, a prominent role for mTORC2 in GPCR-dependent chemotaxis (Liu et al., 2010) and angiogenesis has been suggested (Dada et al., 2008; Ziegler et al., 2016).

The participation of Gβγ in tumor growth was initially revealed through the use of a construct that encoded the carboxyl-terminal region of GRK2, a widely used inhibitor of Gβγ signaling. Expression of this Gβγ interactor prevented prostate tumor growth in a murine model (Bookout et al., 2003). Similarly, inhibiting Gβγ signaling in breast cancer cells prevented tumor cell migration and metastatic dissemination in nude mice (Tang et al., 2011). Very recently, oncogenic Gβγ mutants were identified in a patient with a subtype of acute leukemia infiltrated into the bone marrow (Yoda et al., 2015).

PI3Kγ plays a relevant role in cancer progression by controlling chemotactic responses of immune cells. In murine models, experimental inhibition of this lipid kinase prevents tumor growth and metastatic dissemination (Martin et al., 2011; Schmid et al., 2011). Mechanistically, its inhibition reprograms tumor-associated macrophages to activate cytotoxic T cells, which inhibit tumor progression in murine models of pancreatic ductal adenocarcinoma (Kaneda et al., 2016). Moreover, PI3Kγ plays a demonstrated role in Kaposi Sarcoma herpes virus vGPCR-dependent oncogenesis, in which mTOR plays a central role in the paracrine non-cell autonomous, oncogenic pathway (Martin et al., 2011). In preclinical models, it has been demonstrated that cell transformation is induced by over-expression of PI3Kγ, whereas its inhibition attenuates oncogenic processes (Brazzatti et al., 2012).

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GPCRs activate mTORC2; the macromolecular complex is an important upstream regulator of Rho GTPases and has been involved in GPCR-dependent chemotaxis (Dada et al., 2008; He et al., 2013; Hernandez-Negrete et al., 2007; Jacinto et al., 2004; Kuehn et al., 2011; Liu et al., 2010; Liu et al., 2014). Interestingly, mTORC2, the rapamycin-insensitive complex of mTOR, is known as the kinase that phosphorylates AKT at Ser-473, a critical residue whose phosphorylation is required to achieve the maximum activity of AKT (Sarbassov et al., 2005). Driving mutations and overexpression of type-I PI3Ks, as well as AKT, are frequently present in metastatic cancers with resistance to targeted therapies when the resistance occurs by a selection of clones in which mutation and aberrant expression of these proteins are linked to cancer dissemination (Juric et al., 2015).

Current findings implicate mTORC2 as a direct activator of Rac guanine nucleotide exchange factors, particularly P-Rex1, in response to GPCR activation acting through Gβγ. Gβγ interacts with mTORC2, putatively serving as a docking site to recruit it to the plasma membrane (Robles-Molina et al., 2014). As an interactor of mTOR, P-Rex1 was the first multi-domain protein with DEP domains known to be modulated by this multifunctional serine/threonine kinase (Hernandez-Negrete et al., 2007). Interestingly, an additional mTOR interactor containing DEP domains, named DEPTOR, behaves as an mTOR inhibitor whose expression is frequently reduced in multiple cancers (Peterson et al., 2009). Altogether, these findings suggest that P-Rex1 is a RacGEF that integrates PI3K and mTORC2 signaling outputs. Hypothetically, this might constitute a critical axis used by cells when recruited into the tumor stroma where they exert a paracrine effect stimulating tumor progression.

In this regard, P-Rex1 participates in the signaling cascade of CXCL12, which leads to Rac activation, cell migration, and an *in vitro* angiogenic effect (Carretero-Ortega et al., 2010).

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Moreover, the recently-demonstrated participation of mTORC2 in the angiogenic effect of CXCL12 (Ziegler et al., 2016), together with the known interaction between P-Rex1 and this multimeric serine/threonine kinase (Hernandez-Negrete et al., 2007), further support the relevance of the Gβγ/PI3K/mTORC2/P-Rex1 pathway in angiogenesis. Current efforts are oriented to directly assess the activation of these signaling pathways in BMDC responding to chemotactic agonists present in the tumor microenvironment and how these pathways contribute to the protumoral properties of BMDC.

In this context, it is interesting that the CXCL12/CXCR4 pathway activates PI3Kγ and promotes chemotaxis of cells from the bone marrow (Chavakis et al., 2008). P-Rex1 is activated by Gβγ and PIP3 downstream of G protein coupled receptors (Welch, 2015), in addition to activation by growth factor receptors (Montero et al., 2011; Sosa et al., 2010) and a direct interaction with mTORC2 (Hernandez-Negrete et al., 2007). These interactions hypothetically integrate a critical signaling pathway involved in cell migration to the recruitment of different cell populations to the tumor stroma.

The family of P-Rex1 and P-Rex2 RacGEFs has been implicated in cancer progression (Berger et al., 2012; Fine et al., 2009; Goel et al., 2016; Lindsay et al., 2011; Lissanu Deribe et al., 2016; Montero et al., 2011; Pandiella and Montero, 2013; Qin et al., 2009; Sosa et al., 2010; Waddell et al., 2015); P-Rex1 is overexpressed in several types of cancer and thus has been considered a prognostic marker of poor patient survival in human breast carcinoma (Sosa et al., 2010). In murine models, this RacGEF contributes to metastasis in prostate cancer (Qin et al., 2009) and melanoma (Lindsay et al., 2011), and has been linked to anti-angiogenic therapy resistance in prostate cancer (Goel et al., 2016).

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In the case of P-Rex2, this GEF mutation has been found in melanoma (Berger et al., 2012), and ectopic expression of the mutants accelerated oncogenesis of immortalized melanocytes in mice (Berger et al., 2012; Lissanu Deribe et al., 2016). An alternative mechanism by which this mutant RacGEF contributes to cancer is based on its resistance to regulation by PTEN, a tumor suppressor sensitive to inhibition by wild type P-Rex2 (Mense et al., 2015). An active area of research is how these observations can be translated into pharmacological strategies to inhibit Gβγ-dependent pathways in pathological settings such as cancer progression.

Endosomes as platforms of Gβγ signaling

Agonist-bound receptors activate GDP-bound heterotrimeric G proteins. As a consequence, signaling-ready Gβγ is released. The effects of time and space are now appreciated as critical factors defining new levels of complexity to GPCR signaling (Lohse and Hofmann, 2015). At the plasma membrane, different agonist-stabilized conformations can bias the activated pathways. Initial responses elicited at the plasma membrane are followed by a second wave of signaling by GPCR interactors such as arrestins. Independent trafficking routes for receptors and activated G proteins eventually lead to what has been considered a third wave of signaling at endosomal compartments, where novel effector systems may be locally activated (Murphy et al., 2009). The cell specific context of these waves of signaling elicits specific integrated actions. Various examples highlight the importance of endosomal trafficking, broadening possibilities of heterotrimeric G protein signaling in time and space. Gβγ, in particular, follows internalization routes that define the activation of signaling complexes at endosomal compartments (Garcia-Regalado et al., 2008), or alternatively, Gβγ can follow translocation routes independent of endosomal trafficking (Saini et al., 2007). LPA receptors promote Gβγ trafficking to endosomes

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where it assembles a PI3K/AKT signaling complex (Garcia-Regalado et al., 2008). This is a Gi-dependent process that involves Gβγ interaction with Rab11. Whether Gβγ signaling from endosomes contributes to chemotactic cell migration is currently unknown.

Gβγ activates ERK-signaling cascades

Multiple studies have revealed the diverse signaling pathways linking GPCR signaling to ERK activation. Rather unexpectedly, Gβγ activates ERK through Ras (Crespo et al., 1994), providing the first demonstration that GPCRs promote Gβγ-dependent proliferation involving protein interactions instead of diffusible second messengers.

In addition, activation of PI3Kγ via Gβγ has been linked to the proliferative effect of GPCRs (Lopez-Ilasaca et al., 1997). In oncogenic settings, the GPCR signaling specificity of this lipid kinase has provided a rational basis for the treatment of Kaposi Sarcoma in preclinical models (Martin et al., 2011). In this case, a virally encoded GPCR that selectively activates PI3Kγ stimulates AKT and mTOR, generating a paracrine circuit directly linked to the carcinogenic effect. Intriguingly, in breast cancer cells P-Rex1 integrates PI3K signaling to ERK via a Rac/PAK/Raf/MEK pathway. This involves activating mutations of the catalytic subunit of PI3Kα and overexpression of HER2 growth factor receptors (Ebi et al., 2013).

Inhibition of Gβγ signaling offers pharmacological potential in cancer treatment

Aberrant chemotactic cell migration in cancer not only involves transformed cells but also multiple other types that constitute the stroma and those that enhance metastatic behavior. Since cancer cell dissemination is the most life threatening stage of cancer progression, pharmacological prevention of this aberrant process represents one of the most challenging yet

beneficial interventions to mechanistically fight cancer progression. GPCRs are essential in the normal and aberrant dissemination of multiple cells throughout the organism. Since Gβγ is a common actor of the chemotactic signaling pathways frequently altered in cancer, its ability to recognize multiple effectors via distinct interacting motifs may be pharmacologically targeted to prevent aberrant cell migration. Thus, characterization of chemotactic Gβγ-dependent signaling pathways offers unique opportunities to investigate promising pharmacological strategies with the ultimate goal to develop inhibitors that differentially block aberrant Gβγ-effectors. Keeping active those relevant to the physiological effects of this fundamental multitasking heterodimer, the aim is to inhibit those linked to cancer progression, related to inflammation, and involved in the recruitment of proangiogenic mediators that contribute to tumor angiogenesis and metastatic dissemination of tumor cells (Smrcka, 2013). Experimental evidence already shows that specific interactions between Gβγ and its effectors, along with the corresponding functional effects, can be blocked with small molecules selected by virtual docking (Bonacci et al., 2006). Moreover, inhibiting some of these specific interactions prevents invasive behavior of cancer cells (Tang et al., 2011). Therefore, further understanding of Gβγ-dependent signaling networks offers a growing potential for pharmacological intervention that aims to inhibit aberrant cell migration in cancer settings.

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Wrote or contributed to the writing of the manuscript: JVP, IBV, RDCV, and GRC.

References

Abu-Thuraia A, Gauthier R, Chidiac R, Fukui Y, Screaton RA, Gratton JP and Cote JF (2015) Axl phosphorylates Elmo scaffold proteins to promote Rac activation and cell invasion. *Mol Cell Biol* **35**(1): 76-87.

Aittaleb M, Boguth CA and Tesmer JJ (2010) Structure and function of heterotrimeric G protein-regulated Rho guanine nucleotide exchange factors. *Mol Pharmacol* **77**(2): 111-125.

Alexander S and Friedl P (2012) Cancer invasion and resistance: interconnected processes of disease progression and therapy failure. *Trends Mol Med* **18**(1): 13-26.

Barbolina MV, Kim M, Liu Y, Shepard J, Belmadani A, Miller RJ, Shea LD and Stack MS (2010) Microenvironmental regulation of chemokine (C-X-C-motif) receptor 4 in ovarian carcinoma. *Molecular cancer research : MCR* **8**(5): 653-664.

Berger MF, Hodis E, Heffernan TP, Deribe YL, Lawrence MS, Protopopov A, Ivanova E, Watson IR, Nickerson E, Ghosh P, Zhang H, Zeid R, Ren X, Cibulskis K, Sivachenko AY, Wagle N, Sucker A, Sougnez C, Onofrio R, Ambrogio L, Auclair D, Fennell T, Carter SL, Drier Y, Stojanov P, Singer MA, Voet D, Jing R, Saksena G, Barretina J, Ramos AH, Pugh TJ, Stransky N, Parkin M, Winckler W, Mahan S, Ardlie K, Baldwin J, Wargo J, Schadendorf D, Meyerson M, Gabriel SB, Golub TR, Wagner SN, Lander ES, Getz G, Chin L and Garraway LA (2012) Melanoma genome sequencing reveals frequent PREX2 mutations. *Nature* **485**(7399): 502-506.

Bonacci TM, Mathews JL, Yuan C, Lehmann DM, Malik S, Wu D, Font JL, Bidlack JM and Smrcka AV (2006) Differential targeting of Gbetagamma-subunit signaling with small molecules. *Science* **312**(5772): 443-446.

Bondar A and Lazar J (2014) Dissociated GalphaGTP and Gbetagamma protein subunits are the major activated form of heterotrimeric Gi/o proteins. *J Biol Chem* **289**(3): 1271-1281.

Bookout AL, Finney AE, Guo R, Peppel K, Koch WJ and Daaka Y (2003) Targeting Gbetagamma signaling to inhibit prostate tumor formation and growth. *J Biol Chem* **278**(39): 37569-37573.

Bracho-Valdes I, Moreno-Alvarez P, Valencia-Martinez I, Robles-Molina E, Chavez-Vargas L and Vazquez-Prado J (2011) mTORC1- and mTORC2-interacting proteins keep their multifunctional partners focused. *IUBMB Life* **63**(10): 896-914.

Brazzatti JA, Klingler-Hoffmann M, Haylock-Jacobs S, Harata-Lee Y, Niu M, Higgins MD, Kochetkova M, Hoffmann P and McColl SR (2012) Differential roles for the p101 and p84 regulatory subunits of PI3Kgamma in tumor growth and metastasis. *Oncogene* **31**(18): 2350-2361.

Bronte V and Bria E (2016) Interfering with CCL5/CCR5 at the Tumor-Stroma Interface. *Cancer Cell* **29**(4): 437-439.

Burridge K and Wennerberg K (2004) Rho and Rac take center stage. *Cell* **116**(2): 167-179.

Carretero-Ortega J, Walsh CT, Hernandez-Garcia R, Reyes-Cruz G, Brown JH and Vazquez-Prado J (2010) Phosphatidylinositol 3,4,5-triphosphate-dependent Rac exchanger 1 (P-Rex-1), a guanine nucleotide exchange factor for Rac, mediates angiogenic responses to stromal cell-derived factor-1/chemokine stromal cell derived factor-1 (SDF-1/CXCL-12) linked to Rac activation, endothelial cell migration, and in vitro angiogenesis. *Mol Pharmacol* **77**(3): 435-442.

Charest PG and Firtel RA (2007) Big roles for small GTPases in the control of directed cell movement. *Biochem J* **401**(2): 377-390.

Chavakis E, Carmona G, Urbich C, Gottig S, Henschler R, Penninger JM, Zeiher AM, Chavakis T and Dimmeler S (2008) Phosphatidylinositol-3-kinase-gamma is integral to homing functions of progenitor cells. *Circ Res* **102**(8): 942-949.

Chavez-Vargas L, Adame-Garcia SR, Cervantes-Villagrana RD, Castillo-Kauil A, Bruystens JG, Fukuhara S, Taylor SS, Mochizuki N, Reyes-Cruz G and Vazquez-Prado J (2016) Protein Kinase A (PKA) Type I

Interacts with P-Rex1, a Rac Guanine Nucleotide Exchange Factor: EFFECT ON PKA LOCALIZATION AND P-Rex1 SIGNALING. *J Biol Chem* **291**(12): 6182-6199.

Chen Z, Singer WD, Danesh SM, Sternweis PC and Sprang SR (2008) Recognition of the activated states of Galpha13 by the rgRGS domain of PDZRhoGEF. *Structure* **16**(10): 1532-1543.

Chen Z, Singer WD, Sternweis PC and Sprang SR (2005) Structure of the p115RhoGEF rgRGS domain-Galpha13/i1 chimera complex suggests convergent evolution of a GTPase activator. *Nat Struct Mol Biol* **12**(2): 191-197.

Cook DR, Rossman KL and Der CJ (2014) Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease. *Oncogene* **33**(31): 4021-4035.

Crespo P, Xu N, Simonds WF and Gutkind JS (1994) Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature* **369**(6479): 418-420.

Dada S, Demartines N and Dormond O (2008) mTORC2 regulates PGE2-mediated endothelial cell survival and migration. *Biochem Biophys Res Commun* **372**(4): 875-879.

Dbouk HA, Vadas O, Shymanets A, Burke JE, Salamon RS, Khalil BD, Barrett MO, Waldo GL, Surve C, Hsueh C, Perisic O, Harteneck C, Shepherd PR, Harden TK, Smrcka AV, Taussig R, Bresnick AR, Nurnberg B, Williams RL and Backer JM (2012a) G protein-coupled receptor-mediated activation of p110beta by Gbetagamma is required for cellular transformation and invasiveness. *Sci Signal* **5**(253): ra89.

Dbouk HA, Vadas O, Williams RL and Backer JM (2012b) PI3Kbeta downstream of GPCRs - crucial partners in oncogenesis. *Oncotarget* **3**(12): 1485-1486.

Delilbasi CB, Okura M, Iida S and Kogo M (2004) Investigation of CXCR4 in squamous cell carcinoma of the tongue. *Oral Oncol* **40**(2): 154-157.

Devreotes P and Horwitz AR (2015) Signaling networks that regulate cell migration. *Cold Spring Harb Perspect Biol* **7**(8): a005959.

Dillenburg-Pilla P, Patel V, Mikelis CM, Zarate-Blades CR, Doci CL, Amornphimoltham P, Wang Z, Martin D, Leelahavanichkul K, Dorsam RT, Masedunskas A, Weigert R, Molinolo AA and Gutkind JS (2015) SDF-1/CXCL12 induces directional cell migration and spontaneous metastasis via a CXCR4/Galphai/mTORC1 axis. *Faseb J* **29**(3): 1056-1068.

Dillon LM, Bean JR, Yang W, Shee K, Symonds LK, Balko JM, McDonald WH, Liu S, Gonzalez-Angulo AM, Mills GB, Arteaga CL and Miller TW (2015) P-REX1 creates a positive feedback loop to activate growth factor receptor, PI3K/AKT and MEK/ERK signaling in breast cancer. *Oncogene* **34**(30): 3968-3976.

Donald S, Hill K, Lecureuil C, Barnouin R, Krugmann S, John Coadwell W, Andrews SR, Walker SA, Hawkins PT, Stephens LR and Welch HC (2004) P-Rex2, a new guanine-nucleotide exchange factor for Rac. *FEBS Lett* **572**(1-3): 172-176.

Dorsam RT and Gutkind JS (2007) G-protein-coupled receptors and cancer. *Nat Rev Cancer* **7**(2): 79-94.

Dupre DJ, Robitaille M, Rebois RV and Hebert TE (2009) The role of Gbetagamma subunits in the organization, assembly, and function of GPCR signaling complexes. *Annu Rev Pharmacol Toxicol* **49**: 31-56.

Ebi H, Costa C, Faber AC, Nishtala M, Kotani H, Juric D, Della Pelle P, Song Y, Yano S, Mino-Kenudson M, Benes CH and Engelman JA (2013) PI3K regulates MEK/ERK signaling in breast cancer via the Rac-GEF, P-Rex1. *Proc Natl Acad Sci U S A* **110**(52): 21124-21129.

Fine B, Hodakoski C, Koujak S, Su T, Saal LH, Maurer M, Hopkins B, Keniry M, Sulis ML, Mense S, Hibshoosh H and Parsons R (2009) Activation of the PI3K pathway in cancer through inhibition of PTEN by exchange factor P-REX2a. *Science* **325**(5945): 1261-1265.

Ford CE, Skiba NP, Bae H, Daaka Y, Reuveny E, Shekter LR, Rosal R, Weng G, Yang CS, Iyengar R, Miller RJ, Jan LY, Lefkowitz RJ and Hamm HE (1998) Molecular basis for interactions of G protein betagamma subunits with effectors. *Science* **280**(5367): 1271-1274.

Friedl P and Alexander S (2011) Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell* **147**(5): 992-1009.

Fritsch R, de Krijger I, Fritsch K, George R, Reason B, Kumar MS, Diefenbacher M, Stamp G and Downward J (2013) RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. *Cell* **153**(5): 1050-1063.

Fukuhara S, Chikumi H and Gutkind JS (2000) Leukemia-associated Rho guanine nucleotide exchange factor (LARG) links heterotrimeric G proteins of the G(12) family to Rho. *FEBS Lett* **485**(2-3): 183-188.

Fukuhara S, Chikumi H and Gutkind JS (2001) RGS-containing RhoGEFs: the missing link between transforming G proteins and Rho? *Oncogene* **20**(13): 1661-1668.

Fukuhara S, Murga C, Zohar M, Igishi T and Gutkind JS (1999) A novel PDZ domain containing guanine nucleotide exchange factor links heterotrimeric G proteins to Rho. *J Biol Chem* **274**(9): 5868-5879.

Gadea G and Blangy A (2014) Dock-family exchange factors in cell migration and disease. *Eur J Cell Biol* **93**(10-12): 466-477.

Gao D, Nolan DJ, Mellick AS, Bambino K, McDonnell K and Mittal V (2008) Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science* **319**(5860): 195-198.

Garcia-Mata R, Wennerberg K, Arthur WT, Noren NK, Ellerbroek SM and Burridge K (2006) Analysis of activated GAPs and GEFs in cell lysates. *Methods Enzymol* **406**: 425-437.

Garcia-Regalado A, Guzman-Hernandez ML, Ramirez-Rangel I, Robles-Molina E, Balla T, Vazquez-Prado J and Reyes-Cruz G (2008) G protein-coupled receptor-promoted trafficking of Gbeta1gamma2 leads to AKT activation at endosomes via a mechanism mediated by Gbeta1gamma2-Rab11a interaction. *Mol Biol Cell* **19**(10): 4188-4200.

Goel HL, Pursell B, Shultz LD, Greiner DL, Brekken RA, Vander Kooi CW and Mercurio AM (2016) P-Rex1 Promotes Resistance to VEGF/VEGFR-Targeted Therapy in Prostate Cancer. *Cell Rep* **14**(9): 2193-2208.

Goicoechea SM, Awadia S and Garcia-Mata R (2014) I'm coming to GEF you: Regulation of RhoGEFs during cell migration. *Cell Adh Migr* **8**(6): 535-549.

Guleng B, Tateishi K, Ohta M, Kanai F, Jazag A, Ijichi H, Tanaka Y, Washida M, Morikane K, Fukushima Y, Yamori T, Tsuruo T, Kawabe T, Miyagishi M, Taira K, Sata M and Omata M (2005) Blockade of the stromal cell-derived factor-1/CXCR4 axis attenuates in vivo tumor growth by inhibiting angiogenesis in a vascular endothelial growth factor-independent manner. *Cancer Res* **65**(13): 5864-5871.

Guzman-Hernandez ML, Vazquez-Macias A, Carretero-Ortega J, Hernandez-Garcia R, Garcia-Regalado A, Hernandez-Negrete I, Reyes-Cruz G, Gutkind JS and Vazquez-Prado J (2009) Differential inhibitor of Gbetagamma signaling to AKT and ERK derived from phosducin-like protein: effect on sphingosine 1-phosphate-induced endothelial cell migration and in vitro angiogenesis. *J Biol Chem* **284**(27): 18334-18346.

Hall A (2012) Rho family GTPases. *Biochem Soc Trans* **40**(6): 1378-1382.

Hamoud N, Tran V, Croteau LP, Kania A and Cote JF (2014) G-protein coupled receptor BAI3 promotes myoblast fusion in vertebrates. *Proc Natl Acad Sci U S A* **111**(10): 3745-3750.

Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG, Sternweis PC and Bollag G (1998) Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. *Science* **280**(5372): 2112-2114.

He Y, Li D, Cook SL, Yoon MS, Kapoor A, Rao CV, Kenis PJ, Chen J and Wang F (2013) Mammalian target of rapamycin and Rictor control neutrophil chemotaxis by regulating Rac/Cdc42 activity and the actin cytoskeleton. *Mol Biol Cell* **24**(21): 3369-3380.

Heckmann D, Maier P, Laufs S, Li L, Sleeman JP, Trunk MJ, Leupold JH, Wenz F, Zeller WJ, Fruehauf S and Allgayer H (2014) The disparate twins: a comparative study of CXCR4 and CXCR7 in SDF-1alpha-induced gene expression, invasion and chemosensitivity of colon cancer. *Clin Cancer Res* **20**(3): 604-616.

Hepler JR (2014) G protein coupled receptor signaling complexes in live cells. *Cell Logist* **4**: e29392.

Hernandez-Bedolla MA, Carretero-Ortega J, Valadez-Sanchez M, Vazquez-Prado J and Reyes-Cruz G (2015) Chemotactic and proangiogenic role of calcium sensing receptor is linked to secretion of multiple cytokines and growth factors in breast cancer MDA-MB-231 cells. *Biochim Biophys Acta* **1853**(1): 166-182.

Hernandez-Garcia R, Iruela-Arispe ML, Reyes-Cruz G and Vazquez-Prado J (2015) Endothelial RhoGEFs: A systematic analysis of their expression profiles in VEGF-stimulated and tumor endothelial cells. *Vascul Pharmacol* **74**: 60-72.

Hernandez-Negrete I, Carretero-Ortega J, Rosenfeldt H, Hernandez-Garcia R, Calderon-Salinas JV, Reyes-Cruz G, Gutkind JS and Vazquez-Prado J (2007) P-Rex1 links mammalian target of rapamycin signaling to Rac activation and cell migration. *J Biol Chem* **282**(32): 23708-23715.

Houslay DM, Anderson KE, Chessa T, Kulkarni S, Fritsch R, Downward J, Backer JM, Stephens LR and Hawkins PT (2016) Coincident signals from GPCRs and receptor tyrosine kinases are uniquely transduced by PI3Kbeta in myeloid cells. *Sci Signal* **9**(441): ra82.

Hurowitz EH, Melnyk JM, Chen YJ, Kouros-Mehr H, Simon MI and Shizuya H (2000) Genomic characterization of the human heterotrimeric G protein alpha, beta, and gamma subunit genes. *DNA Res* **7**(2): 111-120.

Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A and Hall MN (2004) Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol* **6**(11): 1122-1128.

Jin S and Exton JH (2000) Activation of RhoA by association of Galpha(13) with Dbl. *Biochem Biophys Res Commun* **277**(3): 718-721.

Jin T, Zhang N, Long Y, Parent CA and Devreotes PN (2000) Localization of the G protein betagamma complex in living cells during chemotaxis. *Science* **287**(5455): 1034-1036.

Jonkers J and Moolenaar WH (2009) Mammary tumorigenesis through LPA receptor signaling. *Cancer Cell* **15**(6): 457-459.

Juric D, Castel P, Griffith M, Griffith OL, Won HH, Ellis H, Ebbesen SH, Ainscough BJ, Ramu A, Iyer G, Shah RH, Huynh T, Mino-Kenudson M, Sgroi D, Isakoff S, Thabet A, Elamine L, Solit DB, Lowe SW, Quadt C, Peters M, Derti A, Schegel R, Huang A, Mardis ER, Berger MF, Baselga J and Scaltriti M (2015) Convergent loss of PTEN leads to clinical resistance to a PI(3)Kalpha inhibitor. *Nature* **518**(7538): 240-244.

Kaneda MM, Cappello P, Nguyen AV, Ralainirina N, Hardamon CR, Foubert P, Schmid MC, Sun P, Mose E, Bouvet M, Lowy AM, Valasek MA, Sasik R, Novelli F, Hirsch E and Varner JA (2016) Macrophage PI3Kgamma drives pancreatic ductal adenocarcinoma progression. *Cancer discovery* **6**(8): 870-885.

Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S and Lyden D (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* **438**(7069): 820-827.

Kedziora KM, Leyton-Puig D, Argenzio E, Boumeester AJ, van Butselaar B, Yin T, Wu YI, van Leeuwen FN, Innocenti M, Jalink K and Moolenaar WH (2016) Rapid Remodeling of Invadosomes by Gi-coupled Receptors: DISSECTING THE ROLE OF Rho GTPases. *J Biol Chem* **291**(9): 4323-4333.

Khalil BD, Hsueh C, Cao Y, Abi Saab WF, Wang Y, Condeelis JS, Bresnick AR and Backer JM (2016) GPCR Signaling Mediates Tumor Metastasis via PI3Kbeta. *Cancer research* **76**(10): 2944-2953.

Kim EK, Yun SJ, Ha JM, Kim YW, Jin IH, Yun J, Shin HK, Song SH, Kim JH, Lee JS, Kim CD and Bae SS (2011) Selective activation of Akt1 by mammalian target of rapamycin complex 2 regulates cancer cell migration, invasion, and metastasis. *Oncogene* **30**(26): 2954-2963.

Kitamura T, Qian BZ and Pollard JW (2015) Immune cell promotion of metastasis. *Nat Rev Immunol* **15**(2): 73-86.

Kojima Y, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-Porath I, Onder TT, Wang ZC, Richardson AL, Weinberg RA and Orimo A (2010) Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proc Natl Acad Sci U S A* **107**(46): 20009-20014.

Kozasa T, Hajicek N, Chow CR and Suzuki N (2011) Signalling mechanisms of RhoGTPase regulation by the heterotrimeric G proteins G12 and G13. *J Biochem* **150**(4): 357-369.

Kozma R, Ahmed S, Best A and Lim L (1995) The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol Cell Biol* **15**(4): 1942-1952.

Krishnan A, Mustafa A, Almen MS, Fredriksson R, Williams MJ and Schioth HB (2015) Evolutionary hierarchy of vertebrate-like heterotrimeric G protein families. *Mol Phylogenet Evol* **91**: 27-40.

Kuehn HS, Jung MY, Beaven MA, Metcalfe DD and Gilfillan AM (2011) Prostaglandin E2 activates and utilizes mTORC2 as a central signaling locus for the regulation of mast cell chemotaxis and mediator release. *J Biol Chem* **286**(1): 391-402.

Lambert NA (2008) Dissociation of heterotrimeric g proteins in cells. *Sci Signal* **1**(25): re5.

Landis CA, Masters SB, Spada A, Pace AM, Bourne HR and Vallar L (1989) GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature* **340**(6236): 692-696.

Lappano R and Maggiolini M (2011) G protein-coupled receptors: novel targets for drug discovery in cancer. *Nat Rev Drug Discov* **10**(1): 47-60.

Laurin M and Cote JF (2014) Insights into the biological functions of Dock family guanine nucleotide exchange factors. *Genes Dev* **28**(6): 533-547.

Ledezma-Sanchez BA, Garcia-Regalado A, Guzman-Hernandez ML and Vazquez-Prado J (2010) Sphingosine-1-phosphate receptor S1P1 is regulated by direct interactions with P-Rex1, a Rac guanine nucleotide exchange factor. *Biochem Biophys Res Commun* **391**(4): 1647-1652.

Li H, Yang L, Fu H, Yan J, Wang Y, Guo H, Hao X, Xu X, Jin T and Zhang N (2013) Association between Galphai2 and ELMO1/Dock180 connects chemokine signalling with Rac activation and metastasis. *Nat Commun* **4**: 1706.

Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang WC, Hait NC, Allegood JC, Price MM, Avni D, Takabe K, Kordula T, Milstien S and Spiegel S (2013) Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. *Cancer Cell* **23**(1): 107-120.

Lindsay CR, Lawn S, Campbell AD, Faller WJ, Rambow F, Mort RL, Timpson P, Li A, Cammareri P, Ridgway RA, Morton JP, Doyle B, Hegarty S, Rafferty M, Murphy IG, McDermott EW, Sheahan K, Pedone K, Finn AJ, Groben PA, Thomas NE, Hao H, Carson C, Norman JC, Machesky LM, Gallagher WM, Jackson IJ, Van Kempen L, Beermann F, Der C, Larue L, Welch HC, Ozanne BW and Sansom OJ (2011) P-Rex1 is required for efficient melanoblast migration and melanoma metastasis. *Nat Commun* **2**: 555.

Lissanu Deribe Y, Shi Y, Rai K, Nezi L, Amin SB, Wu CC, Akdemir KC, Mahdavi M, Peng Q, Chang QE, Hornigold K, Arold ST, Welch HC, Garraway LA and Chin L (2016) Truncating PREX2 mutations activate its GEF activity and alter gene expression regulation in NRAS-mutant melanoma. *Proc Natl Acad Sci U S A* **113**(9): E1296-1305.

Liu L, Das S, Losert W and Parent CA (2010) mTORC2 regulates neutrophil chemotaxis in a cAMP- and RhoA-dependent fashion. *Dev Cell* **19**(6): 845-857.

Liu L, Gritz D and Parent CA (2014) PKCbetaII acts downstream of chemoattractant receptors and mTORC2 to regulate cAMP production and myosin II activity in neutrophils. *Mol Biol Cell* **25**(9): 1446-1457.

Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, Wang B, Blenis J, Cantley LC, Toker A, Su B and Wei W (2015a) PtdIns(3,4,5)P3-Dependent Activation of the mTORC2 Kinase Complex. *Cancer discovery* **5**(11): 1194-1209.

Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, Zhang F, Yu S, Stephens LC, Cui X, Murrow G, Coombes K, Muller W, Hung MC, Perou CM, Lee AV, Fang X and Mills GB (2009) Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. *Cancer Cell* **15**(6): 539-550.

Liu Y, Yang Y, Ward R, An S, Guo XX, Li W and Xu TR (2015b) Biased signalling: the instinctive skill of the cell in the selection of appropriate signalling pathways. *Biochem J* **470**(2): 155-167.

Logothetis DE, Kurachi Y, Galper J, Neer EJ and Clapham DE (1987) The beta gamma subunits of GTP-binding proteins activate the muscarinic K+ channel in heart. *Nature* **325**(6102): 321-326.

Lohse MJ and Hofmann KP (2015) Spatial and Temporal Aspects of Signaling by G-Protein-Coupled Receptors. *Mol Pharmacol* **88**(3): 572-578.

Lopez-Ilasaca M, Crespo P, Pellici PG, Gutkind JS and Wetzker R (1997) Linkage of G protein-coupled receptors to the MAPK signaling pathway through PI 3-kinase gamma. *Science* **275**(5298): 394-397.

Luttrell LM, Maudsley S and Bohn LM (2015) Fulfilling the Promise of "Biased" G Protein-Coupled Receptor Agonism. *Mol Pharmacol* **88**(3): 579-588.

Lutz S, Freichel-Blomquist A, Yang Y, Rumenapp U, Jakobs KH, Schmidt M and Wieland T (2005) The guanine nucleotide exchange factor p63RhoGEF, a specific link between Gq/11-coupled receptor signaling and RhoA. *J Biol Chem* **280**(12): 11134-11139.

Lutz S, Shankaranarayanan A, Coco C, Ridilla M, Nance MR, Vettel C, Baltus D, Evelyn CR, Neubig RR, Wieland T and Tesmer JJ (2007) Structure of Galphaq-p63RhoGEF-RhoA complex reveals a pathway for the activation of RhoA by GPCRs. *Science* **318**(5858): 1923-1927.

Lyden D, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R and Rafii S (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* **7**(11): 1194-1201.

Marchesi F, Monti P, Leone BE, Zerbi A, Vecchi A, Piemonti L, Mantovani A and Allavena P (2004) Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. *Cancer research* **64**(22): 8420-8427.

Martin D, Galisteo R, Molinolo AA, Wetzker R, Hirsch E and Gutkind JS (2011) PI3Kgamma mediates kaposi's sarcoma-associated herpesvirus vGPCR-induced sarcomagenesis. *Cancer Cell* **19**(6): 805-813.

Masia-Balague M, Izquierdo I, Garrido G, Cordomi A, Perez-Benito L, Miller NL, Schlaepfer DD, Gigoux V and Aragay AM (2015) Gastrin-stimulated Galpha13 Activation of Rgnef Protein (ArhGEF28) in DLD-1 Colon Carcinoma Cells. *J Biol Chem* **290**(24): 15197-15209.

Mayeenuddin LH and Garrison JC (2006) Phosphorylation of P-Rex1 by the cyclic AMP-dependent protein kinase inhibits the phosphatidylinositiol (3,4,5)-trisphosphate and Gbetagamma-mediated regulation of its activity. *J Biol Chem* **281**(4): 1921-1928.

Meiri D, Marshall CB, Mokady D, LaRose J, Mullin M, Gingras AC, Ikura M and Rottapel R (2014) Mechanistic insight into GPCR-mediated activation of the microtubule-associated RhoA exchange factor GEF-H1. *Nat Commun* **5**: 4857.

Mense SM, Barrows D, Hodakoski C, Steinbach N, Schoenfeld D, Su W, Hopkins BD, Su T, Fine B, Hibshoosh H and Parsons R (2015) PTEN inhibits PREX2-catalyzed activation of RAC1 to restrain tumor cell invasion. *Sci Signal* **8**(370): ra32.

Mervine SM, Yost EA, Sabo JL, Hynes TR and Berlot CH (2006) Analysis of G protein betagamma dimer formation in live cells using multicolor bimolecular fluorescence complementation demonstrates preferences of beta1 for particular gamma subunits. *Mol Pharmacol* **70**(1): 194-205.

Montero JC, Seoane S, Ocana A and Pandiella A (2011) P-Rex1 participates in Neuregulin-ErbB signal transduction and its expression correlates with patient outcome in breast cancer. *Oncogene* **30**(9): 1059-1071.

Moravcevic K, Oxley CL and Lemmon MA (2012) Conditional peripheral membrane proteins: facing up to limited specificity. *Structure* **20**(1): 15-27.

Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E and Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**(6824): 50-56.

Murphy JE, Padilla BE, Hasdemir B, Cottrell GS and Bunnett NW (2009) Endosomes: a legitimate platform for the signaling train. *Proc Natl Acad Sci U S A* **106**(42): 17615-17622.

Neptune ER, Iiri T and Bourne HR (1999) Galphai is not required for chemotaxis mediated by Gi-coupled receptors. *J Biol Chem* **274**(5): 2824-2828.

Nishida K, Kaziro Y and Satoh T (1999) Association of the proto-oncogene product dbl with G protein betagamma subunits. *FEBS Lett* **459**(2): 186-190.

Niu J, Profirovic J, Pan H, Vaiskunaite R and Voyno-Yasenetskaya T (2003) G Protein betagamma subunits stimulate p114RhoGEF, a guanine nucleotide exchange factor for RhoA and Rac1: regulation of cell shape and reactive oxygen species production. *Circ Res* **93**(9): 848-856.

Nobes CD and Hall A (1995) Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* **81**(1): 53-62.

Nolan DJ, Ciarrocchi A, Mellick AS, Jaggi JS, Bambino K, Gupta S, Heikamp E, McDevitt MR, Scheinberg DA, Benezra R and Mittal V (2007) Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. *Genes Dev* **21**(12): 1546-1558.

O'Hayre M, Vazquez-Prado J, Kufareva I, Stawiski EW, Handel TM, Seshagiri S and Gutkind JS (2013) The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. *Nat Rev Cancer* **13**(6): 412-424.

O'Neill PR and Gautam N (2014) Subcellular optogenetic inhibition of G proteins generates signaling gradients and cell migration. *Mol Biol Cell* **25**(15): 2305-2314.

Organista-Juarez D, Carretero-Ortega J, Vicente-Fermin O, Vazquez-Victorio G, Sosa-Garrocho M, Vazquez-Prado J, Macias-Silva M and Reyes-Cruz G (2013) Calcium-sensing receptor inhibits TGF-beta-signaling by decreasing Smad2 phosphorylation. *IUBMB Life* **65**(12): 1035-1042.

Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL and Weinberg RA (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**(3): 335-348.

Pandiella A and Montero JC (2013) Molecular pathways: P-Rex in cancer. *Clin Cancer Res* **19**(17): 4564-4569.

Park D, Tosello-Trampont AC, Elliott MR, Lu M, Haney LB, Ma Z, Klibanov AL, Mandell JW and Ravichandran KS (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* **450**(7168): 430-434.

Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, Garcia-Santos G, Ghajar C, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan J, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J and Lyden D (2012) Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med* **18**(6): 883-891.

Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, Gray NS and Sabatini DM (2009) DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* **137**(5): 873-886.

Psaila B and Lyden D (2009) The metastatic niche: adapting the foreign soil. *Nat Rev Cancer* **9**(4): 285-293.

Pupo AS, Duarte DA, Lima V, Teixeira LB, Parreiras ESLT and Costa-Neto CM (2016) Recent updates on GPCR biased agonism. *Pharmacol Res*. doi: 10.1016/j.phrs.2016.01.031

Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA and Pollard JW (2011) CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* **475**(7355): 222-225.

Qian BZ and Pollard JW (2010) Macrophage diversity enhances tumor progression and metastasis. *Cell* **141**(1): 39-51.

Qin J, Xie Y, Wang B, Hoshino M, Wolff DW, Zhao J, Scofield MA, Dowd FJ, Lin MF and Tu Y (2009) Upregulation of PIP3-dependent Rac exchanger 1 (P-Rex1) promotes prostate cancer metastasis. *Oncogene* **28**(16): 1853-1863.

Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK and Kobilka BK (2011) Crystal structure of the beta2 adrenergic receptor-Gs protein complex. *Nature* **477**(7366): 549-555.

Rickert P, Weiner OD, Wang F, Bourne HR and Servant G (2000) Leukocytes navigate by compass: roles of PI3Kgamma and its lipid products. *Trends Cell Biol* **10**(11): 466-473.

Ridley AJ (2015) Rho GTPase signalling in cell migration. *Curr Opin Cell Biol* **36**: 103-112.

Ridley AJ and Hall A (1992) The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* **70**(3): 389-399.

Ridley AJ, Paterson HF, Johnston CL, Diekmann D and Hall A (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**(3): 401-410.

Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT and Horwitz AR (2003) Cell migration: integrating signals from front to back. *Science* **302**(5651): 1704-1709.

Robles-Molina E, Dionisio-Vicuna M, Guzman-Hernandez ML, Reyes-Cruz G and Vazquez-Prado J (2014) Gbetagamma interacts with mTOR and promotes its activation. *Biochem Biophys Res Commun* **444**(2): 218-223.

Rojas RJ, Yohe ME, Gershburg S, Kawano T, Kozasa T and Sondek J (2007) Galphaq directly activates p63RhoGEF and Trio via a conserved extension of the Dbl homology-associated pleckstrin homology domain. *J Biol Chem* **282**(40): 29201-29210.

Rosenfeldt H, Vazquez-Prado J and Gutkind JS (2004) P-REX2, a novel PI-3-kinase sensitive Rac exchange factor. *FEBS Lett* **572**(1-3): 167-171.

Rossman KL, Der CJ and Sondek J (2005) GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nat Rev Mol Cell Biol* **6**(2): 167-180.

Roussos ET, Condeelis JS and Patsialou A (2011) Chemotaxis in cancer. *Nat Rev Cancer* **11**(8): 573-587.

Saini DK, Kalyanaraman V, Chisari M and Gautam N (2007) A family of G protein betagamma subunits translocate reversibly from the plasma membrane to endomembranes on receptor activation. *J Biol Chem* **282**(33): 24099-24108.

Sarbassov DD, Guertin DA, Ali SM and Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**(5712): 1098-1101.

Scherz-Shouval R, Santagata S, Mendillo ML, Sholl LM, Ben-Aharon I, Beck AH, Dias-Santagata D, Koeva M, Stemmer SM, Whitesell L and Lindquist S (2014) The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. *Cell* **158**(3): 564-578.

Schmid MC, Avraamides CJ, Dippold HC, Franco I, Foubert P, Ellies LG, Acevedo LM, Manglicmot JR, Song X, Wrasidlo W, Blair SL, Ginsberg MH, Cheresh DA, Hirsch E, Field SJ and Varner JA (2011) Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3kgamma, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* **19**(6): 715-727.

Schmidt A and Hall A (2002) Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev* **16**(13): 1587-1609.

Schwindinger WF and Robishaw JD (2001) Heterotrimeric G-protein betagamma-dimers in growth and differentiation. *Oncogene* **20**(13): 1653-1660.

Scott JK, Huang SF, Gangadhar BP, Samoriski GM, Clapp P, Gross RA, Taussig R and Smrcka AV (2001) Evidence that a protein-protein interaction 'hot spot' on heterotrimeric G protein betagamma subunits is used for recognition of a subclass of effectors. *Embo J* **20**(4): 767-776.

Sekiguchi H, Ii M, Jujo K, Yokoyama A, Hagiwara N and Asahara T (2011) Improved culture-based isolation of differentiating endothelial progenitor cells from mouse bone marrow mononuclear cells. *PLoS One* **6**(12): e28639.

Shymanets A, Ahmadian MR, Kossmeier KT, Wetzker R, Harteneck C and Nurnberg B (2012) The p101 subunit of PI3Kgamma restores activation by Gbeta mutants deficient in stimulating p110gamma. *Biochem J* **441**(3): 851-858.

Shymanets A, Prajwal, Bucher K, Beer-Hammer S, Harteneck C and Nurnberg B (2013) p87 and p101 subunits are distinct regulators determining class IB phosphoinositide 3-kinase (PI3K) specificity. *J Biol Chem* **288**(43): 31059-31068.

Smrcka AV (2013) Molecular targeting of Galpha and Gbetagamma subunits: a potential approach for cancer therapeutics. *Trends Pharmacol Sci* **34**(5): 290-298.

Sosa MS, Lopez-Haber C, Yang C, Wang H, Lemmon MA, Busillo JM, Luo J, Benovic JL, Klein-Szanto A, Yagi H, Gutkind JS, Parsons RE and Kazanietz MG (2010) Identification of the Rac-GEF P-Rex1 as an essential mediator of ErbB signaling in breast cancer. *Mol Cell* **40**(6): 877-892.

Stevenson C, de la Rosa G, Anderson CS, Murphy PS, Capece T, Kim M and Elliott MR (2014) Essential role of Elmo1 in Dock2-dependent lymphocyte migration. *J Immunol* **192**(12): 6062-6070.

Surve CR, Lehmann D and Smrcka AV (2014) A chemical biology approach demonstrates G protein betagamma subunits are sufficient to mediate directional neutrophil chemotaxis. *J Biol Chem* **289**(25): 17791-17801.

Tan W, Martin D and Gutkind JS (2006) The Galpha13-Rho signaling axis is required for SDF-1-induced migration through CXCR4. *J Biol Chem* **281**(51): 39542-39549.

Tang W, Tu Y, Nayak SK, Woodson J, Jehl M and Ross EM (2006) Gbetagamma inhibits Galpha GTPase-activating proteins by inhibition of Galpha-GTP binding during stimulation by receptor. *J Biol Chem* **281**(8): 4746-4753.

Tang X, Sun Z, Runne C, Madsen J, Domann F, Henry M, Lin F and Chen S (2011) A critical role of Gbetagamma in tumorigenesis and metastasis of breast cancer. *J Biol Chem* **286**(15): 13244-13254.

Tesmer JJ (2016) Hitchhiking on the heptahelical highway: structure and function of 7TM receptor complexes. *Nat Rev Mol Cell Biol* **17**(7): 439-450.

Tesmer VM, Kawano T, Shankaranarayanan A, Kozasa T and Tesmer JJ (2005) Snapshot of activated G proteins at the membrane: the Galphaq-GRK2-Gbetagamma complex. *Science* **310**(5754): 1686-1690.

Thorpe LM, Yuzugullu H and Zhao JJ (2015) PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer* **15**(1): 7-24.

Uchida D, Onoue T, Tomizuka Y, Begum NM, Miwa Y, Yoshida H and Sato M (2007) Involvement of an autocrine stromal cell derived factor-1/CXCR4 system on the distant metastasis of human oral squamous cell carcinoma. *Molecular cancer research : MCR* **5**(7): 685-694.

Ueda H, Nagae R, Kozawa M, Morishita R, Kimura S, Nagase T, Ohara O, Yoshida S and Asano T (2008) Heterotrimeric G protein betagamma subunits stimulate FLJ00018, a guanine nucleotide exchange factor for Rac1 and Cdc42. *J Biol Chem* **283**(4): 1946-1953.

Ugel S, De Sanctis F, Mandruzzato S and Bronte V (2015) Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages. *J Clin Invest* **125**(9): 3365-3376.

Urano D, Nakata A, Mizuno N, Tago K and Itoh H (2008) Domain-domain interaction of P-Rex1 is essential for the activation and inhibition by G protein betagamma subunits and PKA. *Cell Signal* **20**(8): 1545-1554.

Vadas O, Dbouk HA, Shymanets A, Perisic O, Burke JE, Abi Saab WF, Khalil BD, Harteneck C, Bresnick AR, Nurnberg B, Backer JM and Williams RL (2013) Molecular determinants of PI3Kgamma-mediated activation downstream of G-protein-coupled receptors (GPCRs). *Proc Natl Acad Sci U S A* **110**(47): 18862-18867.

Van Haastert PJ and Devreotes PN (2004) Chemotaxis: signalling the way forward. *Nat Rev Mol Cell Biol* **5**(8): 626-634.

Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS and Bastian BC (2009) Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature* **457**(7229): 599-602.

Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, Obenauf AC, Wackernagel W, Green G, Bouvier N, Sozen MM, Baimukanova G, Roy R, Heguy A, Dolgalev I, Khanin R, Busam K, Speicher MR, O'Brien J and Bastian BC (2010) Mutations in GNA11 in uveal melanoma. *N Engl J Med* **363**(23): 2191-2199.

Vaque JP, Dorsam RT, Feng X, Iglesias-Bartolome R, Forsthoefel DJ, Chen Q, Debant A, Seeger MA, Ksander BR, Teramoto H and Gutkind JS (2013) A genome-wide RNAi screen reveals a Trio-regulated Rho GTPase circuitry transducing mitogenic signals initiated by G protein-coupled receptors. *Mol Cell* **49**(1): 94-108.

Vazquez-Prado J and Gutkind JS (2011) RGS-RhoGEFs and other RGS multidomain proteins as effector molecules in GPCR-dependent and GPCR-independent cell signaling, in *G Protein-Coupled Receptors Structure, Signaling and Physiology* (Siehler S and Milligan G eds) pp 159-188, Cambridge University Press, New York.

Vazquez-Prado J, Miyazaki H, Castellone MD, Teramoto H and Gutkind JS (2004) Chimeric G alpha i2/G alpha 13 proteins reveal the structural requirements for the binding and activation of the RGS-like (RGL)-containing Rho guanine nucleotide exchange factors (GEFs) by G alpha 13. *J Biol Chem* **279**(52): 54283-54290.

Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MC, Robertson AJ, Fadlullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Wilson PJ, Markham E, Cloonan N, Anderson MJ, Fink JL, Holmes O, Kazakoff SH, Leonard C, Newell F, Poudel B, Song S, Taylor D, Wood S, Xu Q, Wu J, Pinese M, Cowley MJ, Lee HC, Jones MD, Nagrial AM, Humphris J, Chantrill LA, Chin V,

Steinmann AM, Mawson A, Humphrey ES, Colvin EK, Chou A, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Pettitt JA, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Jamieson NB, Graham JS, Niclou SP, Bjerkvig R, Grutzmann R, Aust D, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Falconi M, Zamboni G, Tortora G, Tempero MA, Gill AJ, Eshleman JR, Pilarsky C, Scarpa A, Musgrove EA, Pearson JV, Biankin AV and Grimmond SM (2015) Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* **518**(7540): 495-501.

Wang Y, Xu X, Pan M and Jin T (2016) ELMO1 Directly Interacts with Gbetagamma Subunit to Transduce GPCR Signaling to Rac1 Activation in Chemotaxis. *J Cancer* **7**(8): 973-983.

Wang Z, Kumamoto Y, Wang P, Gan X, Lehmann D, Smrcka AV, Cohn L, Iwasaki A, Li L and Wu D (2009) Regulation of immature dendritic cell migration by RhoA guanine nucleotide exchange factor Arhgef5. *J Biol Chem* **284**(42): 28599-28606.

Watanabe M, Terasawa M, Miyano K, Yanagihara T, Uruno T, Sanematsu F, Nishikimi A, Cote JF, Sumimoto H and Fukui Y (2014) DOCK2 and DOCK5 act additively in neutrophils to regulate chemotaxis, superoxide production, and extracellular trap formation. *J Immunol* **193**(11): 5660-5667.

Welch HC (2015) Regulation and function of P-Rex family Rac-GEFs. *Small GTPases* **6**(2): 49-70.

Welch HC, Coadwell WJ, Ellson CD, Ferguson GJ, Andrews SR, Erdjument-Bromage H, Tempst P, Hawkins PT and Stephens LR (2002) P-Rex1, a PtdIns(3,4,5)P3- and Gbetagamma-regulated guanine-nucleotide exchange factor for Rac. *Cell* **108**(6): 809-821.

Wels J, Kaplan RN, Rafii S and Lyden D (2008) Migratory neighbors and distant invaders: tumor-associated niche cells. *Genes Dev* **22**(5): 559-574.

Whorton MR and MacKinnon R (2013) X-ray structure of the mammalian GIRK2-betagamma G-protein complex. *Nature* **498**(7453): 190-197.

Xu Q, Wang Z, Chen X, Duan W, Lei J, Zong L, Li X, Sheng L, Ma J, Han L, Li W, Zhang L, Guo K, Ma Z, Wu Z, Wu E and Ma Q (2015) Stromal-derived factor-1alpha/CXCL12-CXCR4 chemotactic pathway promotes perineural invasion in pancreatic cancer. *Oncotarget* **6**(7): 4717-4732.

Yagi H, Tan W, Dillenburg-Pilla P, Armando S, Amornphimoltham P, Simaan M, Weigert R, Molinolo AA, Bouvier M and Gutkind JS (2011) A synthetic biology approach reveals a CXCR4-G13-Rho signaling axis driving transendothelial migration of metastatic breast cancer cells. *Sci Signal* **4**(191): ra60.

Yang P, Homan KT, Li Y, Cruz-Rodriguez O, Tesmer JJ and Chen Z (2016) Effect of Lipid Composition on Membrane Orientation of the G protein-coupled Receptor Kinase 2-Gbetagamma Complex. *Biochemistry* **55**(20): 2841-2848.

Yasumoto K, Koizumi K, Kawashima A, Saitoh Y, Arita Y, Shinohara K, Minami T, Nakayama T, Sakurai H, Takahashi Y, Yoshie O and Saiki I (2006) Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. *Cancer research* **66**(4): 2181-2187.

Ye Y, Tang X, Sun Z and Chen S (2016) Upregulated WDR26 serves as a scaffold to coordinate PI3K/ AKT pathway-driven breast cancer cell growth, migration, and invasion. *Oncotarget* **7**(14): 17854-17869.

Yoda A, Adelmant G, Tamburini J, Chapuy B, Shindoh N, Yoda Y, Weigert O, Kopp N, Wu SC, Kim SS, Liu H, Tivey T, Christie AL, Elpek KG, Card J, Gritsman K, Gotlib J, Deininger MW, Makishima H, Turley SJ, Javidi-Sharifi N, Maciejewski JP, Jaiswal S, Ebert BL, Rodig SJ, Tyner JW, Marto JA, Weinstock DM and Lane AA (2015) Mutations in G protein beta subunits promote transformation and kinase inhibitor resistance. *Nat Med* **21**(1): 71-75.

Ziegler ME, Hatch MM, Wu N, Muawad SA and Hughes CC (2016) mTORC2 mediates CXCL12-induced angiogenesis. *Angiogenesis*.

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Footnotes

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Legends for Figures

Figure 1. GPCR signaling to Rho GTPases plays a critical role in tumor microenvironment. Cancer progression relies on the communication among different cell populations recruited into the tumor stroma. These include fibroblasts and endothelial cells from surrounding tissues and bone marrow derived cells (BMDC), such as macrophages, leukocytes, monocytes, endothelial progenitor cells, and other myeloid precursors. Chemokines secreted by stromal and cancer cells are key mediators of inflammation, angiogenesis, and immunosuppression, contributing to tumor growth, invasion and metastasis. Among them, several GPCR agonists in the tumor microenvironment activate signaling pathways to Rho GTPases via Gα and Gβγ subunits and their RhoGEF effectors. The leading edge of migrating cells contributing to tumor progression is amplified in the inset to highlight the role of Gβγ as an activator of RhoGEFs that regulate actin cytoskeleton dynamics leading chemotactic cell migration.

Figure 2. GPCR-dependent chemotaxis in individual migrating cells. A general model of chemotactic cell migration is based on studies of fast-moving individual cells, such as neutrophils and *Dictyostelium discoideum* amoebas (Devreotes and Horwitz, 2015). Accordingly, a chemotactic gradient is sensed by GPCRs located at the proximity of incoming stimuli (A). Then, Gβγ is locally released, recruiting PI3K and RhoGEFs (B). These effectors of Gβγ promote a spatiotemporal restricted activation of Rho GTPases resulting in polarized morphological changes based on the dynamics of the actin cytoskeleton (C). In this context, cell migration occurs as an integrated effect of protrusions, adhesion and contraction at the advancing front, followed by rear edge retraction. Maintained cell migration involves translocation of signaling proteins (D). The process is positively influenced by vesicle trafficking (Murphy et al.,

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2009). In this regard, Gβγ trafficking, mediated by its interaction with Rab11, activates a PI3K-AKT signaling axis at early endosomes (EE) (Garcia-Regalado et al., 2008). Whether Gβγ-trafficking leads to endosomal activation of RhoGEFs is currently being investigated.

Figure 3. RhoGEFs identified as Gα or Gβγ interactors. GPCRs activate Rho GTPases via direct interactions between Gβγ, GTP-bound $G\alpha_{12/13}$ or $G\alpha_q$ with the indicated RhoGEFs. These RhoGEF have a modular organization characterized by the presence of a catalytic DH (*Dbl*-homology) domain followed by a PH (Pleckstrin-homology) domain predicted with the SMART analysis tool (http://smart.embl-heidelberg.de/). The list of GPCR-regulated RhoGEFs is based on studies showing, at least by coimunoprecipitation or other equivalent assays, interaction between the indicated G proteins and RhoGEFs. $G\alpha_{12/13}$-regulated RhoGEFs: p115RhoGEF/ARHGEF1 (Hart et al., 1998), LARG/ARHGEF12 (Fukuhara et al., 2000), PDZ-RhoGEF/ARHGEF11 (Fukuhara et al., 1999), MCF2/Dbl/ARHGEF21 (Jin and Exton, 2000), ARHGEF2/GEF-H1 (Meiri et al., 2014) and ARHGEF28/RGNEF (Masia-Balague et al., 2015). $G\alpha_q$-regulated RhoGEFs: p63RhoGEF/ARHGEF25 (Lutz et al., 2005; Lutz et al., 2007), TRIO/ARHGEF23 (Rojas et al., 2007; Vaque et al., 2013), and ARHGEF28/RGNEF (Masia-Balague et al., 2015). Gβγ-regulated RhoGEFs: P-Rex1 (Welch et al., 2002) and P-Rex2 (Donald et al., 2004), PLEKHG2 (Ueda et al., 2008), p114RhoGEF/ARHGEF18 (Niu et al., 2003), ARHGEF5/TIM (Wang et al., 2009) and MCF2/Dbl/ARHGEF21(Nishida et al., 1999).

As shown at the bottom, the cycle of Rho GTPases is regulated by GEFs, GAPs and GDIs. G protein regulated RhoGEFs activate Rho GTPases by promoting exchange of GDP for GTP. GTPase-Activating Proteins (GAPs) are negative regulators that help the GTPase to hydrolyze GTP to GDP. Guanine Nucleotide Dissociation Inhibitors (GDIs) inhibit GDP

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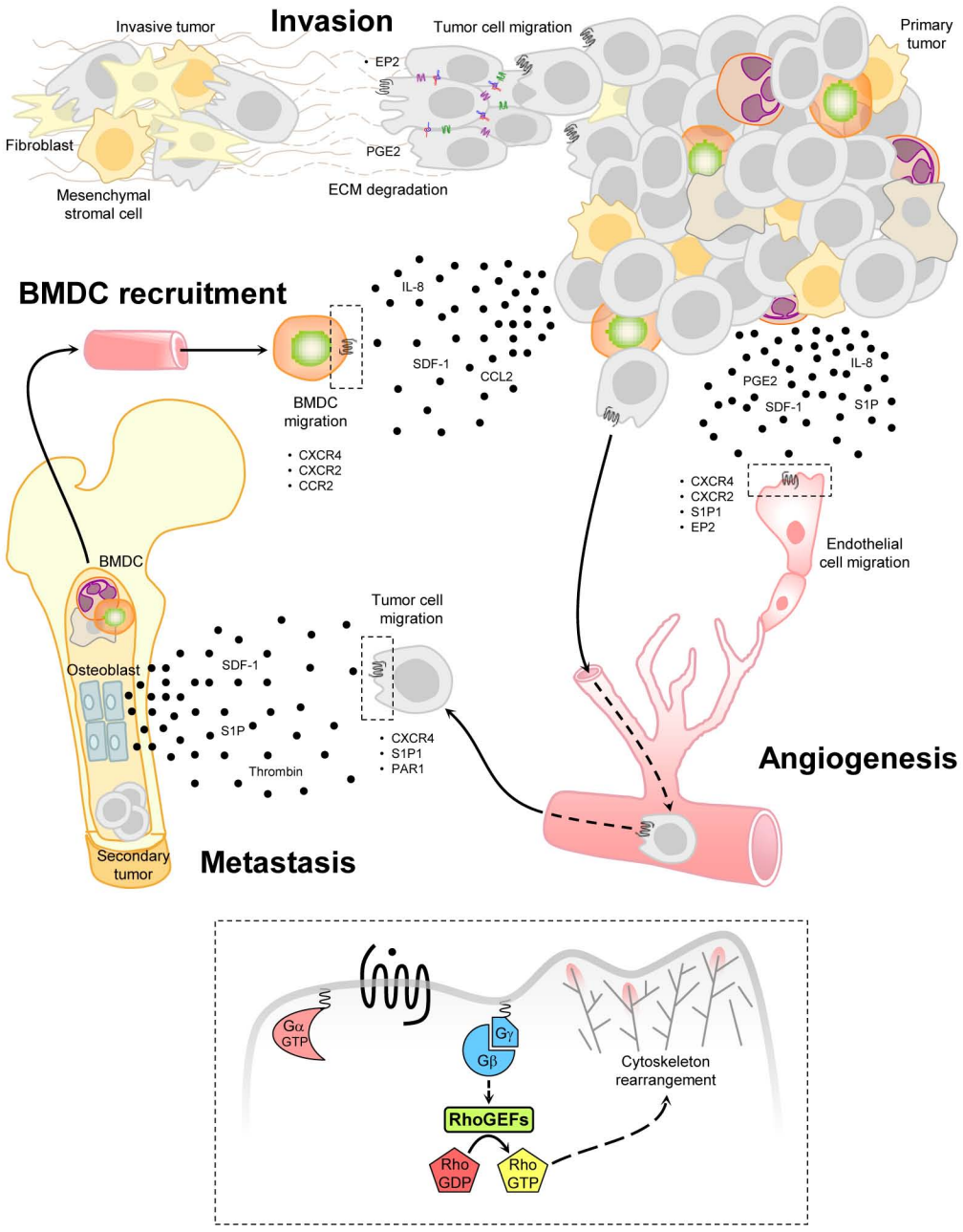
dissociation, keeping the GTPase in an inactive state.

Figure 4. P-Rex1 as a putative chemotactic scaffold. Directional cell migration is the most conspicuous consequence of a precise choreography of Rho GTPases activated at the right time and place by chemotactic GPCR-regulated RhoGEFs. This sophisticated process is putatively more efficient with the intervention of multidomain proteins acting as chemotactic scaffolds. Accumulating evidences suggest that P-Rex1, a Rac1-specific GEF, which has been associated with metastatic processes, has the inherent attributes to perform this role. Besides its reported interactions with phosphoinositides and Gβγ (Welch, 2015), it also interacts with additional signaling proteins having a relevant role in migratory responses. S1P1 receptor carboxyl-terminal tail interacts with P-Rex1-PDZ domains, maintaining a longer chemotactic effect due to an internalization delay (Ledezma-Sanchez et al., 2010). Gβγ and P-Rex1 interact with mTORC2, a multimeric serine/threonine kinase involved in AKT activation and cell migration (Hernandez-Negrete et al., 2007; Robles-Molina et al., 2014). Finally, type I PKA interacts with P-Rex1-PDZ domains, phosphorylates the GEF at its first DEP domain and exerts an indirect effect on the carboxyl-terminal region (via a putative PKA-regulated kinase), resulting in inhibitory intramolecular interactions (Chavez-Vargas et al., 2016). In conclusion, P-Rex1 is a putative chemotactic scaffold that integrates the actions of diverse signaling proteins including receptors, G protein subunits, lipid second messengers and important serine-threonine kinases such as mTOR (mechanistic target of rapamycin) and PKA (cyclic AMP-dependent protein kinase).

Figure 1


Invasion
Invasive tumor
Tumor cell migration
Primary tumor
• EP2
Fibroblast
PGE2
Mesenchymal stromal cell
ECM degradation
BMDC recruitment
IL-8
SDF-1
CCL2
BMDC migration
• CXCR4
• CXCR2
• CCR2
PGE2
IL-8
SDF-1
S1P
• CXCR4
• CXCR2
• S1P1
• EP2
Endothelial cell migration
BMDC
Tumor cell migration
Osteoblast
SDF-1
S1P
Thrombin
• CXCR4
• S1P1
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Angiogenesis
Secondary tumor
Metastasis
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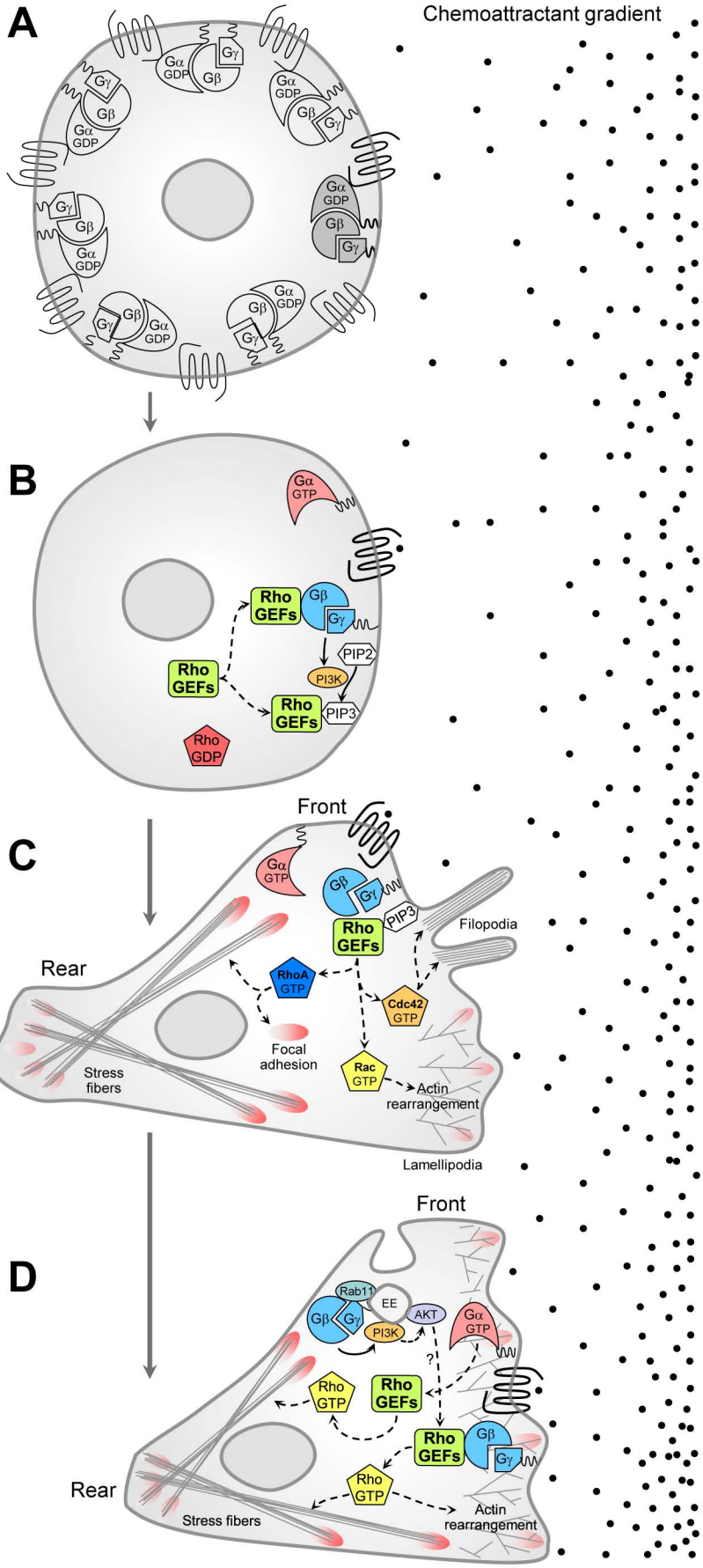
Gγ
Gβ
Cytoskeleton rearrangement
RhoGEFs
Rho GDP
Rho GTP


A
Chemoattractant gradient
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Gβ
Gγ
B
Gα GTP
Rho GEFs
PIP2
PI3K
PIP3
Rho GDP
C
Front
Filopodia
Rear
RhoA GTP
Cdc42 GTP
Rac GTP
Focal adhesion
Stress fibers
Actin rearrangement
Lamellipodia
D
Front
Rab11
EE
AKT
PI3K
Rho GTP
Rear
Stress fibers
Actin rearrangement

Figure 3

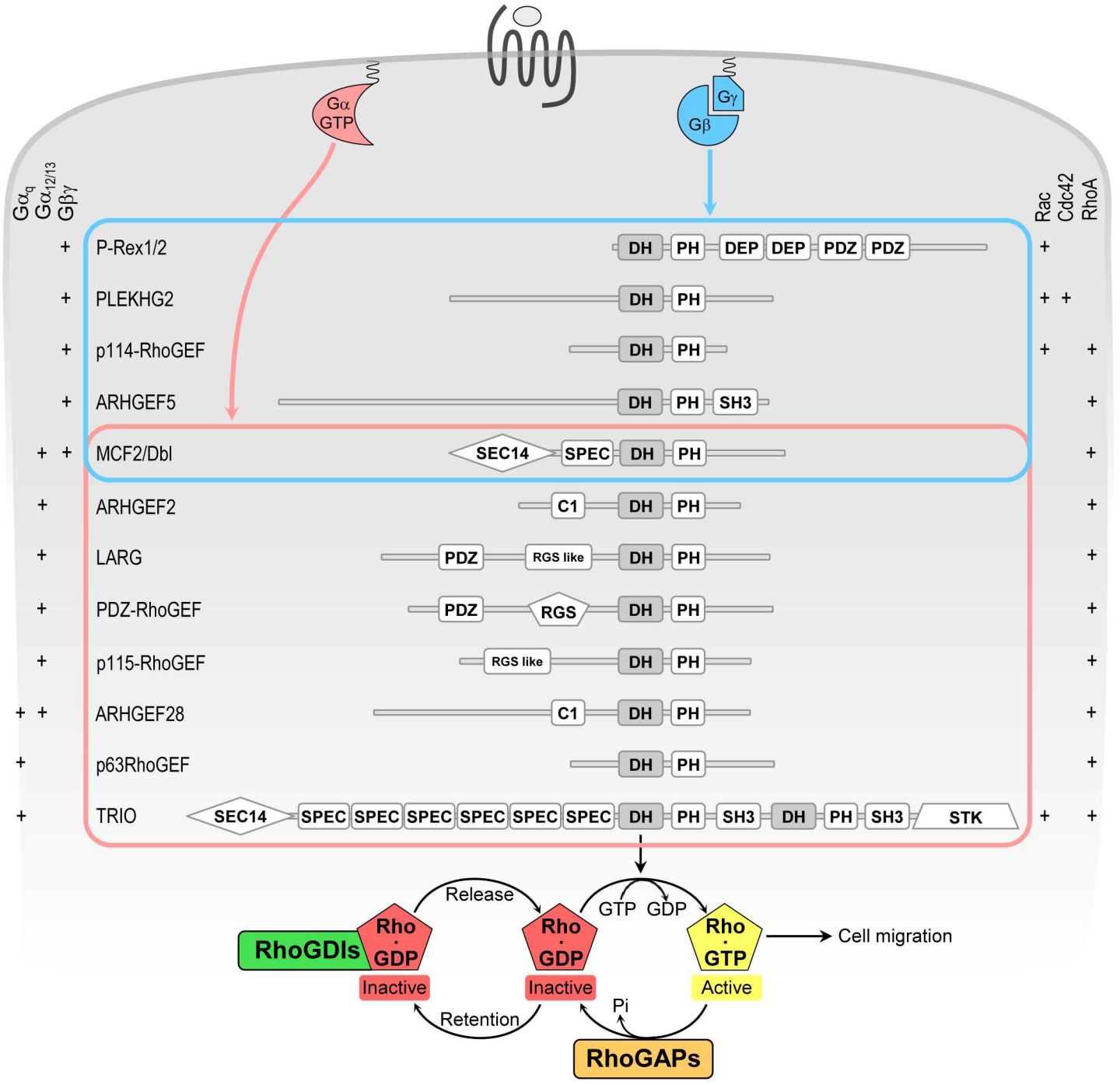
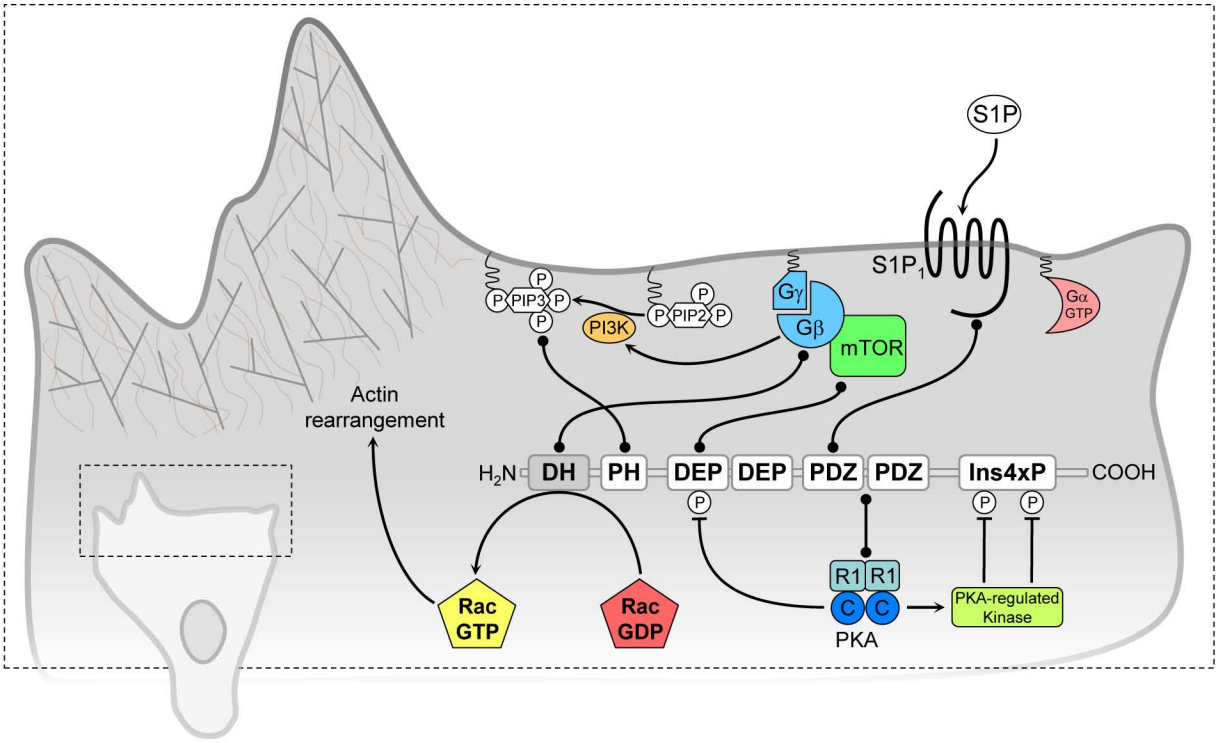

Gα GTP
Gγ
Gβ
Gαq
Gα12/13
Gβγ
Rac
Cdc42
RhoA
P-Rex1/2
DH PH DEP DEP PDZ PDZ
PLEKHG2
DH PH
p114-RhoGEF
DH PH
ARHGEF5
DH PH SH3
MCF2/Dbl
SEC14 SPEC DH PH
ARHGEF2
C1 DH PH
LARG
PDZ RGS like DH PH
PDZ-RhoGEF
PDZ RGS DH PH
p115-RhoGEF
RGS like DH PH
ARHGEF28
C1 DH PH
p63RhoGEF
DH PH
TRIO
SEC14 SPEC SPEC SPEC SPEC SPEC SPEC DH PH SH3 DH PH SH3 STK
Release
GTP GDP
RhoGDIs
Rho · GDP
Inactive
Rho · GDP
Inactive
Rho · GTP
Active
Cell migration
Retention
Pi
RhoGAPs

Figure 4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mTOR
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P
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DH
PH
DEP
DEP
PDZ
PDZ
Ins4xP
COOH
R1
R1
C
C
PKA
PKA-regulated
Kinase
Rac
GTP
Rac
GDP