

**MOL Manuscript # 98509**

## **PDZ Protein Regulation of GPCR Trafficking and Signaling Pathways**

Henry A. Dunn and Stephen S.G. Ferguson

J. Allyn Taylor Centre for Cell Biology, Robarts Research Institute, and the Department of  
Physiology and Pharmacology,

University of Western Ontario, London, Ontario N6A 5B7, Canada

## MOL Manuscript # 98509

Running Title: GPCR regulation by PDZ proteins

# *To whom correspondence should be addressed:*

Dr. Stephen S. G. Ferguson, Robarts Research Institute, University of Western Ontario, 100 Perth  
Dr., London, Ontario, Canada, N6A 5K8, Tel.: 519-931-5706; Fax: 519-931-5252;  
[ferguson@robarts.ca](mailto:ferguson@robarts.ca)

Body words: 6636

Figures: 2

Tables: 2

References: 228

Abstracts words: 187

Introduction words: 595

### Abbreviations:

GPCR, G protein-coupled receptor; PDZ, PSD-95, Disc large, Zona occludens-1; PSD, post-synaptic density; PSD-95/93, post-synaptic density protein of 95/93 kilodaltons; SAP97/102, synapse-associated protein of 97/102 kilodaltons; DLG5, discs, large homolog 5; CARD, caspase activation and recruitment domain; CARMA3, CARD and MAGUK domain-containing protein 3; MPP3, membrane protein, palmitoylated 3; CASK, calcium/calmodulin-dependent serine protein kinase; MAGI-1/2/3, membrane-associated guanylate kinase protein 1/2/3; NHERF1/2, Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor 1/2; PDZK1/2, PDZ domain-containing kidney protein 1/2; GIPC, GAIP interacting protein, C terminus; CAL, CFTR-associated ligand; PDZ-GEF1/2, PDZ domain-containing guanine nucleotide exchange factor; RGS3/12, regulator of g protein signaling; RH-RhoGEF, RGS-homology domain containing Rho guanine nucleotide exchange factor; LARG, leukemia-associated RhoGEF; PDZ-RhoGEF, PDZ domain-containing RhoGEF; SH3, SRC Homology 3 domain; Shank1/2/3, SH3 and multiple ankyrin repeat domains 1/2/3; Par3/6, Partitioning defective protein 3/6; MUPP1, multiple PDZ protein 1; nNOS, neuronal nitric oxide synthase; PICK1, protein interacting with PRKCA 1; SNX27, sorting nexin 27;  $\beta_{1/2}$ AR,  $\beta_{1/2}$  adrenergic receptors; 5-HT<sub>1-7</sub>R, serotonin 1-7 receptor; D<sub>1-3</sub>R, dopamine 1-3 receptor; CRFR1/2, corticotropin-releasing factor receptor 1/2; A<sub>1/2</sub>R, adenosine 1/2 receptor; VPAC1, vasoactive intestinal peptide receptors 1; mGluR, metabotropic glutamate receptor;  $\alpha_{1/2}$ AR,  $\alpha_{1/2}$  adrenergic receptor; TP, thromboxane A<sub>2</sub> receptor; h $\kappa$ -OR, human  $\kappa$  opioid receptor; SSTR, somatostatin receptor; PTH1R, parathyroid 1 receptor; CCR5, chemokine (C-C motif) receptor 5; PAFR, platelet-activating factor receptor; P2Y<sub>1/12</sub>R, purinergic P2Y receptors; hIPR, human prostacyclin receptor; CL1,  $\alpha$ -Latrotoxin receptor C1RL/latrophilin 1;  $\mu/\delta$ OR,  $\mu/\delta$  opioid receptor; GPR10, prolactin-releasing peptide receptor; GHRHR, growth hormone-releasing hormone receptor; LPA<sub>1/2</sub>R, lysophosphatidic acid 1/2 receptor; hLHR, human luteinizing hormone receptor; BAI1,

## MOL Manuscript # 98509

brain; MT<sub>1</sub>, melatonin 1 receptor; M<sub>1-4</sub>/mAChR1-4, muscarinic acetylcholine receptor 1-4 receptor; CB1, cannabinoid receptor 1; ET<sub>1</sub>R, endothelin <sub>1</sub> receptor; FSHR, follicle-stimulating hormone receptor; PAR1, protease-activated receptor 1; AT<sub>1</sub>R, angiotensin II receptor 1; OR2AG1, olfactory receptor 2AG1; GABA<sub>B</sub>, gamma-aminobutyric acid <sub>B</sub> receptor; B<sub>2</sub>R, bradykinin <sub>2</sub> receptor; GRPR, gastrin-releasing peptide receptor; GRHR, gonadotropin-releasing hormone receptor; C5aR, chemo-attractant C5a receptor; PAC1R, pituitary adenylate cyclase-activating polypeptide 1 receptor; S1PR2; sphingosine-1-phosphate receptor 2; GPR132, G2 accumulation protein/g protein-coupled receptor 132; H1R, histamine 1 receptor; MAS1, proto-oncogene mas; Mam2, pheromone p-factor receptor; ERK, extracellular signal-related kinase; IP<sub>3</sub>, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; Akt, protein kinase B; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; cfos; FAK, focal adhesion kinase; Fzd, frizzled; GPR37, G protein-coupled receptor 37 (endothelin receptor type B-like).

## MOL Manuscript # 98509

### Abstract

G protein-coupled receptors (GPCRs) contribute to the regulation of every aspect of human physiology and are the therapeutic targets for the treatment of numerous diseases. As a consequence, understanding the myriad of mechanisms controlling GPCR signaling and trafficking is essential for the development of new pharmacological strategies for the treatment of human pathologies. Of the many GPCR-interacting proteins (GIPs), PDZ domain-containing proteins appear most abundant and have similarly been implicated in disease mechanisms. PDZ proteins play an important role at regulating receptor and channel protein localization of synapses and tight junctions and function to scaffold intracellular signaling protein complexes. In the current study, we review the known functional interactions between PDZ domain-containing proteins and GPCRs, and provide insight into the potential mechanisms of action. These PDZ domain-containing proteins include the membrane-associated guanylate-like kinases (MAGUKs) (PSD-95, SAP97, PSD-93, SAP102, DLG5, CARMA3, MPP3, CASK, MAGI-1, MAGI-2, MAGI-3), NHERF proteins (NHERF1, NHERF2, PDZK1, PDZK2), Golgi-associated PDZ proteins (GIPC and CAL), PDZ-GEFs (PDZ-GEF1 and PDZ-GEF2), RGS-Homology-RhoGEFs (PDZ-RhoGEF and LARG), RGS3 and RGS12, spinophilin and neurabin-1, Shank proteins (Shank1, Shank2, Shank3), Par3 and Par6, MUPP1, Tamalin, nNOS, syntrophins, PICK1, syntenin-1 and SNX27.

## **MOL Manuscript # 98509**

### **Introduction**

In the central nervous system, G protein-coupled receptors (GPCRs) and ion channels are targeted at the membrane of dendritic post-synaptic terminals in and around a region termed the post-synaptic density (PSD) (Feng and Zhang, 2009; Neubig and Siderovski, 2002; Magalhaes et al., 2012). Each post-synaptic density is specifically organized such that dozens to hundreds of receptors are targeted to this specialized membrane domain via the interaction of scaffolding proteins with the receptors. These scaffold proteins containing multiple protein-protein interaction domains that allow them to interact with a multitude structural and signaling proteins holding them in close proximity with one another (Feng and Zhang, 2009). Of these scaffolding proteins, it is believed that PSD-95, Disc large, Zona occludens-1 (PDZ) domain-containing proteins are the most abundant, and often provide direct contact with both GPCRs and ion channels at the post-synaptic density (Cheng et al., 2006; Feng and Zhang, 2009). PDZ proteins are not only important for targeting GPCRs to synapses, but they an important role in regulating tight junctions and signaling protein complexes. In the current review, we will overview the growing understanding of the role PDZ domain-containing proteins in the regulation of GPCR subcellular localization, endocytosis, trafficking and signal transduction.

### **PDZ Domains**

PDZ domains are approximately 80-90 amino acid residues in size and represent the most common protein-protein interaction domain (Doyle et al., 1996; Feng and Zhang, 2009; Magalhaes et al., 2012). Although there are hundreds of unique PDZ domain sequences, they all contain a conserved glycine-leucine-glycine-phenylalanine (GLGF) sequence that provides the domain's folded, globular, cup-like structure that is capable of recognizing short, finger-like peptides (Harris and Lim, 2001). Because of this structure, PDZ domains appear best suited for

## MOL Manuscript # 98509

binding the distal regions of receptor carboxyl terminal tails, labelled the PDZ-binding motif (Kornau et al., 1995; Niethammer et al., 1996; Harris and Lim, 2001; Magalhaes et al., 2012). Interestingly, additional studies have identified internal PDZ ligands that, like a carboxyl terminal tail, project outwardly from the protein (Xu et al., 1998; Hillier et al., 1999; Christopherson et al., 1999; Fouassier et al., 2000; Harris and Lim, 2001; Paasche et al., 2005; Trejo, 2005). In this case, the internal PDZ-binding motif is manifest as a sharply folded, finger-like projection.

### PDZ-Binding Motifs

Although seemingly imperfect and likely biased against internal PDZ ligands (reviewed by Trejo, 2005), a simple classification system has evolved to identify potential PDZ-binding motifs and helps to predict potential PDZ domain-containing protein interactions (Songyang et al., 1997; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001; Vaccaro and Dente, 2002). Although there is some deliberation over how many classes of PDZ-binding motifs there are, it is most commonly limited to three classes (Sheng and Sala, 2001; Tonikian et al., 2008; Magalhaes et al., 2012). Class I PDZ-binding motifs are the most described class within the literature and are classified by their final 3 amino acid sequence of S/T-x- $\phi$ , where x indicates any amino acid and  $\phi$  indicates any hydrophobic amino acid (Songyang et al., 1997; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001; Vaccaro and Dente, 2002). However, valine, isoleucine, or leucine appear to be most common of the hydrophobic amino acids that contribute to the formation of a Class I PDZ binding motif (Songyang et al., 1997; Bezprozvanny and Maximov, 2001; Sheng and Sala, 2001; Vaccaro and Dente, 2002). Class II and III PDZ-binding motifs are not as well characterized and show slightly more ambiguous sequences: with class II having its final 3 amino acids as  $\phi$ -x- $\phi$ , and class III having  $\Psi$ -x- $\Psi$ , where  $\Psi$  represents any acidic amino acid residue (Sheng and Sala, 2001).

## MOL Manuscript # 98509

### GPCR-interacting PSD-95 Family PDZ Domain-Containing MAGUK Proteins

**PSD-95 (DLG4):** Post-Synaptic Density protein of 95 kDa (PSD-95) contains three PDZ domains, an SH3 domain, and a GK domain (Fig. 1) and is prototypically localized within the post-synaptic density (Sampedro et al., 1981; Cho et al., 1992). PSD-95 has been demonstrated to modulate both AMPA and NMDA receptor function, as well as a number of GPCRs. In regards to AMPA and NMDA receptors, it appears PSD-95 is important for enhancing and/or maintaining these receptors at the synaptic membrane, thereby potentiating receptor activation, channel opening, receptor-mediated currents and receptor trafficking (Elias et al., 2006; Elias and Nicoll, 2007). PSD-95 is able to indirectly bind and regulate AMPA receptors via a shared association with transmembrane AMPA receptor regulating proteins, such as stargazin (Chen et al., 2000). The  $\beta_1$ -adrenergic receptor ( $\beta_1$ AR) is the first GPCR to be reported as a PSD-95 interacting GPCR and PSD-95 is responsible for antagonizing  $\beta_1$ AR endocytosis in response to agonist activation, thereby stabilizing the receptor at the cell surface (Hu et al., 2000) (Table 1). Despite the potentiation of  $\beta_1$ AR membrane expression, this interaction appears to have no functional consequence on  $G\alpha_s$ -coupled signaling, as measured by cAMP accumulation (Hu et al., 2000). In contrast, PSD-95 interactions with the serotonin 2A receptor (5-HT<sub>2A</sub>R) facilitate  $G\alpha_q$ -coupled signaling by the receptor (Xia et al., 2003) (Table 2). PSD-95 has similarly been shown to antagonize the agonist-induced endocytosis of 5-HT<sub>2A</sub>R (Xia et al., 2003). G protein-coupled receptor kinase 5 phosphorylation also disrupts PSD-95 interactions with the  $\beta_1$ AR which is consistent with a PSD-95/ $\beta$ -arrestin competition model (Hu et al., 2002). Moreover, the recruitment of  $\beta$ -arrestin2 to the 5-HT<sub>2A</sub>R corresponds with the dissociation of PSD-95, suggesting competitive binding for 5-HT<sub>2A</sub>R with mechanistic implications for the regulation of endocytosis of PSD-95 associated GPCRs (Schmid and Bohn, 2010). Notably, PSD-95 is documented to have an opposing role in 5-HT<sub>2C</sub>R trafficking, where PSD-95 overexpression is suggested to suppress cell surface receptor expression and promote receptor endocytosis (Gavarini et al., 2006). This

## MOL Manuscript # 98509

decrease in receptor expression at the cell surface is correlated with enhanced desensitization of 5-HT<sub>2C</sub>R-mediated Ca<sup>2+</sup> accumulation (Gavarini et al., 2006). In PSD-95 null mice, 5-HT<sub>2C</sub>R-mediated cfos induction is impaired (Abbas et al., 2009). Despite significant sequence homology, PSD-95 appears to have opposing roles in regulating their trafficking and signaling pathways of the 5-HT<sub>2A</sub>R and 5-HT<sub>2C</sub>R (Xia et al., 2003; Gavarini et al., 2006). PSD-95 was recently suggested to form a complex with GPR30, AKAP5 and the PKA RII $\beta$  regulatory subunit thereby promoting GPR30 membrane localization and facilitating the constitutive inhibition of cAMP (Akama et al., 2013; Broselid et al., 2014). PSD-95 has also been reported to positively regulate dopamine 1 receptor (D<sub>1</sub>R) endocytosis and to inhibit D<sub>1</sub>R-mediated cAMP formation (Zhang et al., 2007). A more recent study suggests that PSD-95 contributes to D<sub>1</sub>R recycling and resensitization without influencing D<sub>1</sub>R-mediated G $\alpha_s$  activation (Sun et al., 2009). However, the methods and cellular contexts utilized to arrive at these conclusions in these various studies are not directly comparable. Nevertheless, this highlights the importance of considering the specific GPCR in question when determining the regulatory role of a PDZ domain-containing protein, as well as the endogenous trafficking and signaling machineries available within each specific cellular context.

**SAP97 (DLG1):** Although synapse-associated protein of 97 kDa (SAP97) shares ~60% sequence homology with PSD-95 (including three PDZ domains, an SH3 domain, a GK domain, and an additional L27 domain on the amino terminal), less is known about the role of SAP97 in regulating GPCR activity (Fig. 1). Nevertheless, SAP97 has been demonstrated to promote  $\beta_1$ AR phosphorylation via cyclic AMP-dependent protein kinase (PKA), despite having no effect on  $\beta_1$ AR-stimulated adenylyl cyclase activation and cAMP accumulation (Gardner et al., 2007). Additionally, SAP97 promotes recycling of the  $\beta_1$ AR by a mechanism that involves the formation of a complex between  $\beta_1$ AR, AKAP79 and PKA (Gardner et al., 2007; Nooh, et al., 2013; Nooh et al., 2014). In contrast, SAP97 promotes membrane stabilization of the corticotropin-releasing factor receptor 1 (CRFR1) by suppressing CRFR1 endocytosis (Dunn et al., 2013). Although



## MOL Manuscript # 98509

SAP97 does not contribute to the regulation of CRFR1-mediated cAMP accumulation via  $G\alpha_s$ , endogenous SAP97 is essential for CRF-mediated extracellular signal regulated kinase (ERK1/2) phosphorylation via the ERK1/2 signaling pathway (Dunn et al., 2013). In contrast, similar to what is observed for PSD-95-mediated enhancement of 5-HT<sub>2A</sub>R-stimulated inositol phosphate formation, the loss of endogenous SAP97 expression results in a reduction in 5-HT<sub>2A</sub>R-activated inositol accumulation via  $G\alpha_q$  (Xia et al., 2003, Dunn et al., 2014). However, SAP97 also suppresses 5-HT<sub>2A</sub>R endocytosis and facilitates 5-HT-mediated ERK1/2 phosphorylation. The role of endogenous SAP97 in facilitating CRFR1- and 5-HT<sub>2A</sub>R-stimulated ERK1/2 phosphorylation does not require interactions with the PDZ binding motifs of these receptors and knockdown of endogenous SAP97 also reduces CRFR2-mediated ERK1/2 phosphorylation (Dunn et al., 2013; Dunn et al., 2014). Since CRFR2 does not encode a PDZ binding motif, it is possible that SAP97 may play a global role in regulating GPCR-mediated ERK1/2 activity independent of receptor interactions.

**PSD-93 (DLG2) and SAP102 (DLG3):** Post-synaptic density protein of 93 kDa (PSD-93) contains three PDZ domains, an SH3 domain, and a GK domain (Fig. 1). Not a great deal is known about the role of PSD-93 in regulating GPCRs, but PSD-95 and PSD-93 have previously been demonstrated to compensate for one another (Sun and Turrigiano, 2011). Therefore, it is likely that both PSD-93 and PSD-95 may play similar roles with respect to GPCR regulation. PSD-95 and PSD-93 have been identified to interact with the somatostatin receptor 1 (SSTR1) and SSTR4 (Christenn et al., 2007) and have both been shown to inhibit NMDAR endocytosis (Lavezzari et al., 2003). Future studies are needed to examine the role of PSD-93 in the regulation of GPCR trafficking to determine whether its function overlaps with both PSD-95 and SAP97. Synapse-associated protein of 102 kDa (SAP102) contains three PDZ domains, an SH3 domain, and a GK domain (Fig. 1). SAP102 has been demonstrated to regulate adenosine A<sub>2A</sub> receptor (A<sub>2A</sub>R) mobility and promote A<sub>2A</sub>R-mediated ERK signaling (Thurner et al., 2014). SAP102 has

## MOL Manuscript # 98509

additionally been identified to regulate the trafficking of AMPA and NMDA receptors. Thus, it is of interest in the future to determine whether SAP102 plays a role similar to that of other MAGUK proteins in the regulation of GPCR activity.

**DLG5:** DLG5 differs from the common topology of the PSD-95 subfamily of MAGUKs with the inclusion of an amino terminal caspase activation and recruitment domain (CARD), similar to CARMAs, and a fourth PDZ domain (de Mendoza et al., 2010) (Fig. 1). CARMA3 has been implicated in facilitating GPCR-induced activation of NF $\kappa$ B via lysophosphatidic acid, endothelin-1 and angiotensin II (Scudiero et al., 2014). Although there doesn't appear to be any examples of DLG5 in the direct regulation of GPCRs, DLG5 has been implicated in regulating synaptogenesis by enhancing the membrane localization of the transmembrane protein N-cadherin (Wang et al., 2014). DLG5 has also been demonstrated to scaffold atypical protein kinase C (PKC) isoforms and this provide a mechanism by which DLG5 contributes to the regulation of GPCR-mediated signaling (Nechiporuk et al., 2013).

### Other GPCR-interacting PDZ Domain-Containing MAGUK Proteins

**Membrane Palmitoylated Proteins and CASK (PALS3, LIN-2):** Membrane palmitoylated proteins (MPP1/p55, MPP2, MPP3, MPP4, MPP5/PALS1, MPP6/PALS2, and MPP7) are unified by the inclusion of a PDZ domain, SH3 domain, and GK domain (Fig. 1). Additionally, all but MPP1 have two amino terminal L27 domains, with MPP5 also including an amino terminal coiled-coil (CC) domain. MPP1-2 and MPP5-7 also include a HOOK domain between their SH3 and GK domains. Although MPP proteins are a relatively abundant group of MAGUK proteins, very little is known about their regulation of GPCR function. MPP3 has been demonstrated to promote the membrane stability of 5-HT<sub>2C</sub>R and prevent receptor desensitization (Gavarini et al., 2006). MPP1 has additionally been implicated in membrane organization, raft formation, and receptor tyrosine

## **MOL Manuscript # 98509**

kinase-mediated ERK signaling (Lach et al., 2012; Biernatowska et al., 2013). Thus, it is plausible that MPPs may generally promote the membrane organization of integral proteins including GPCRs.

Ca<sup>2+</sup>/Calmodulin-activated serine/threonine kinase (CASK) is very similar in topology to the MPPs with protein domains that include a catalytically active Ca<sup>2+</sup>/calmodulin-dependent kinase (CaMK) domain at the amino terminal followed by two L27 domains, a PDZ domain, a SH3 domain, and a GK domain (te Velthuis et al., 2007; Mukherjee et al., 2008) (Fig. 1). CASK forms a tripartite complex with PDZ domain-containing Mint1 and Veli proteins, but the role of Mint1 and Veli proteins in the regulation of GPCRs remains undetermined (Butz et al., 1998). Like MPP3, CASK has been shown to interact with 5-HT<sub>2C</sub>R (Gavarini et al., 2006; Bécamel et al., 2002; Bécamel et al., 2004). Although the functional consequence of this interaction on 5-HT<sub>2C</sub>R trafficking and signaling remains to be tested, CASK has been implicated in regulating the trafficking of the NMDAR and AMPAR, partly via its regulation of SAP97 conformation and receptor interactions (Jeyifous et al., 2009; Lin et al., 2013). Interestingly, CASK has been demonstrated to interact with PKA, PKC $\epsilon$  and regulator of G protein signaling 4 (RGS4), which may suggest a role for CASK in regulating GPCR-mediated signaling (Hong and Hsueh, 2006).

## **MAGI PDZ Protein Family**

Membrane-associated guanylate kinase with inverted orientation (MAGI) proteins include three proteins with an amino terminal PDZ domain followed by a GK domain, two tryptophan-tryptophan (WW) domains, and five more PDZ domains (Fig. 1). MAGI proteins differ from other MAGUK proteins in the exclusion of an SH3 domain (Dobrosotskaya et al., 1997). MAGI-1 colocalizes with brain angiogenesis inhibitor 1 receptor (BAI-1R) at the cell membrane via an interaction with the receptor carboxyl-terminal tail, and MAGI-3 interacts with BAI-1R to promote

## MOL Manuscript # 98509

ERK phosphorylation (Shiratsuchi et al., 1999; Stephenson et al., 2013). MAGI-3 promotes ERK and RhoA signaling mediated by the lysophosphatidic acid receptor 2 (LPA<sub>2</sub>R), but antagonizes ERK1/2 activation in response to the activation of either  $\beta_1$ AR or  $\beta_2$ AR (Zhang et al., 2007; He et al., 2006; Yang et al., 2010). MAGI-2 interacts with the  $\beta_1$ AR via its first PDZ domain and functions to promote  $\beta_1$ AR endocytosis without affecting  $\beta_1$ AR-mediated cAMP signaling (Xu et al., 2001). In contrast, MAGI-2 interactions with the vasoactive intestinal peptide receptor 1 (VPAC1) and functions to both inhibit VPAC1 endocytosis and suppress VPAC1-mediated cAMP signaling (Gee et al., 2009). MAGI-2 also promotes the cell surface expression of metabotropic glutamate receptor 1a (mGluR1a) via its association with the PDZ domain-containing protein tamalin (Sugi et al., 2007). Thus, similar to what has been reported for PSD-95 family PDZ proteins, the MAGI family of PDZ proteins contributes to the regulation of the endocytosis and cell signaling of a number of GPCRs, but the functional effects of these protein interactions has differential effects depending upon the GPCR studied.

### NHERF Family of PDZ Proteins

***NHERF1 (EBP50)***: Na<sup>+</sup>/H<sup>+</sup> Exchanger Regulatory Factor 1 (NHERF1), or ezrin/radixin/moesin (ERM)-Binding Protein 50 (EBP50), is a relatively small PDZ domain-containing protein characterized by two PDZ domains and a carboxyl terminal ezrin-binding domain (Fig. 2). NHERF1 represents one of the earliest PDZ proteins to be shown to interact with a GPCR (Hall et al., 1998). NHERF1 regulates Na<sup>+</sup>/H<sup>+</sup> exchange via its interaction with  $\beta_2$ AR without altering cAMP signaling, and has since been demonstrated to regulate a number of GPCRs. NHERF1 regulates the recycling of the  $\beta_2$ AR and its binding to the receptor is disrupted by G protein-coupled receptor kinase phosphorylation of the  $\beta_2$ AR at serine residue 411 (Cao et al., 1999). However, NHERF1 is reported to inhibit recycling of the parathyroid 1 receptor (PTH1R) (Wang

## MOL Manuscript # 98509

et al., 2007). NHERF1 also inhibits PTH1R desensitization and endocytosis, a function that appears to involve NHERF1-dependent inhibition of  $\beta$ -arrestin2 recruitment to the PTH1R (Wang et al., 2007; Wang et al., 2009). NHERF1 expression also enhances PTH1R-mediated cAMP signaling and couples PTHR1 to the activation of  $G\alpha_q$  (Wang et al., 2007; Wheeler et al., 2008; Wang et al., 2010). NHERF1 expression enhances cell surface expression of the  $\kappa$  opioid receptor inhibiting down-regulation and promoting receptor recycling (Li et al., 2002). In contrast, NHERF1 increases thromboxane receptor  $\beta$  (TP $\beta$ ) cell surface expression by blocking the internalization of the receptor (Rochdi and Parent, 2003). An additional mechanism by which NHERF1 may increase GPCR membrane targeting is via its competition with the cystic fibrosis transmembrane conductance regulator-associated ligand (CAL) to antagonize CAL-mediated retention of GPCRs in the Golgi (Bauch et al., 2014).

In contrast to the role of NHERF1 in antagonizing the endocytosis of the PTHR1 and TP $\beta$ , NHERF1 is reported to facilitate the endocytosis of a number of GPCRs. NHERF1 enhances CCR5 endocytosis and  $\beta$ -arrestin1 recruitment, thereby promoting the activation of ERK, Rho, and FAK signaling pathways, as well as potentially contribute to CCR5-mediated HIV-1 entry (Hammad et al., 2010; Kuang et al., 2012). NHERF1 overexpression also rescues the endocytosis of an internalization-defective platelet-activating factor receptor (PAFR) and antagonizes PAFR-mediated inositol phosphate formation (Dupré et al., 2012). Agonist activation of the P2Y<sub>12</sub> receptor results in the  $\beta$ -arrestin-dependent recruitment of NHERF1 to the receptor and promotes the formation of a P2Y<sub>12</sub> receptor/NHERF1 complex that does not require PDZ-binding motif interactions (Nisar et al., 2012). NHERF1 also regulated frizzled family receptor activity (Wheeler et al., 2011). Thus, NHERF1 appears to play an integral, but complex, role in regulating the endocytosis and recycling of a variety of different GPCRs.

## MOL Manuscript # 98509

**NHERF2:** The topology of NHERF2 is quite similar to NHERF1 as it shares 44% sequence homology with NHERF1 and contains two PDZ domains and a carboxyl terminal ezrin-binding domain (Ardura and Friedman, 2011) (Fig. 2). Similar to NHERF1, NHERF2 contributes to the regulation of the PTH1R (Mahon et al., 2002; Wang et al., 2010). NHERF2 functions to antagonize PTHR1 coupling to  $G\alpha_s$ -coupling, while concomitantly promoting the coupling of PTH1R to both the activation of  $G\alpha_q$  and  $G\alpha_i$  (Mahon et al., 2002; Wang et al., 2010). NHERF2 also interacts directly with PLC $\beta$  to enhance P2Y<sub>1</sub> receptor-mediated Ca<sup>2+</sup> signaling (Fam et al., 2005). Similarly, NHERF2 interacts with PLC $\beta$ 3 and the LPA<sub>2</sub>R allowing for the formation of a protein complex that directly links the receptor to PLC $\beta$ 3-mediated inositol phosphate signaling (Choi et al., 2010; Oh et al., 2004). NHERF2 and mGluR5 show overlapping expression in mouse brain at postsynaptic neuronal sites and astrocytic processes and NHERF2 prolongs the mGluR5-mediated Ca<sup>2+</sup> response (Paquet et al., 2006).

**PDZK1 (NHERF3) and PDZK2 (NHERF4):** PDZK1, formerly known as NHERF3, differs from NHERF1 and NHERF2 in structural topology by having four PDZ domains and no carboxyl terminal Ezrin-binding domain (Fig. 2). Nevertheless, PDZK1 has been implicated in regulating a subset of GPCRs. PDZK1 promotes the formation of a complex between SSTRs and PLC $\beta$ 3, similar to what is observed for the LPA<sub>2</sub>R (Choi et al., 2010; Oh et al., 2004), thereby facilitating somatostatin-stimulated PLC activation, Ca<sup>2+</sup> mobilization, and ERK1/2 phosphorylation (Kim et al., 2012). PDZK1 also functions to enhance human prostacyclin receptor (hIPR) cell surface localization and cAMP signaling and contributes to endothelial cell migration and angiogenesis (Turner et al., 2011). PDZK1 inhibits 5-HT<sub>2A</sub>R endocytosis and siRNA knockdown of PDZK1 results in reduced 5-HT<sub>2A</sub>R-mediated inositol phosphate accumulation, but is not involved in 5-HT<sub>2A</sub>R-stimulated ERK1/2 phosphorylation (Walther et al., 2015). However, PDZK1 interactions with 5-HT<sub>2A</sub>R do not appear to be required for its regulation of 5-HT<sub>2A</sub>R activity. In contrast,

## MOL Manuscript # 98509

although PDZK1 does not regulate CRFR1-mediated cAMP accumulation, unlike what is observed for the 5-HT<sub>2A</sub>R, PDZK1 facilitates CRFR1-mediated ERK1/2 phosphorylation. Similar to PDZK1, PDZK2 also has four PDZ domains and has been shown to regulate hIPR (Reid et al., 2012). Agonist activation of the hIPR increases PDZK2 association and results in PKA- and PKC-mediated phosphorylation of PDZK2 (Reid et al., 2012). Like PDZK1, PDZK2 also enhances hIPR cell surface expression and cAMP accumulation (Reid et al., 2012). Taken together, PDZK1 and PDZK2 appear to be important for regulating the trafficking of an increasing subset of GPCRs and may bias toward increased G<sub>αq</sub> signaling, similar to what is observed for both NHERF1 and NHERF2.

### PDZ Proteins that Regulate Golgi Trafficking

**GIPC (TIP-2, Synectin):** Regulator of G protein signaling G<sub>α</sub>-binding protein (RGS-GAIP)-interacting protein carboxyl terminus (GIPC) is a PDZ domain-containing protein with one PDZ domain that is implicated in the sorting of nascent proteins from the Golgi network (Liu et al., 2001) (Fig. 2). In regards to GPCRs, GIPC has been shown to target the D<sub>2</sub>R to endosomes and the Golgi apparatus (Jeanneteau et al., 2004). Furthermore, GIPC expression suppresses D<sub>3</sub>R G<sub>αi</sub>-coupling and prevents the D<sub>3</sub>R degradation (Jeanneteau et al., 2004). GIPC also plays a role in regulating both human luteinizing hormone receptor (hLHR) and LPA<sub>1</sub>R trafficking (Hirakawa et al., 2003; Varsano et al., 2012). The interaction of GIPC with the LPA<sub>1</sub>R is essential for LPA<sub>1</sub>R trafficking from APPL-positive signaling endosomes to EEA1-positive early endosomes (Varsano et al., 2012). Additionally, GIPC links the LPA<sub>1</sub>R to the Akt signaling pathway, cell proliferation, and cell motility (Varsano et al., 2012). GIPC also contributes to the suppression of β<sub>1</sub>AR-mediated ERK activation, but does affect β<sub>1</sub>AR-stimulated cAMP accumulation (Hu et al., 2003).

## MOL Manuscript # 98509

**CAL (GOPC, PIST):** CAL is also named Golgi-associated coiled-coil and PDZ domain-containing protein (GOPC), due to its common subcellular localization within the trans-Golgi network and structural topology consisting of two coiled-coil domains and one PDZ domain (Fig. 2). CAL is selectively localized to the trans-Golgi network in neurons, as well as other cell types, and interacts with Rab6a, a small GTPase implicated in Golgi-related trafficking pathways (Chen et al., 2012; Bergbrede et al., 2009; Valente et al., 2010). CAL reduces plasma membrane expression and recycling of the  $\beta_1$ AR, and interferes with both  $\beta_1$ AR-mediated ERK signaling and postendocytotic receptor degradation via the lysosome (He et al., 2004; Koliwer et al., 2015). CAL overexpression retains the SSTR5 in the Golgi apparatus, thereby reducing SSTR5 cell surface expression (Wente et al., 2005; Bauch et al., 2014). Additionally, CAL colocalizes with mGluR1a following agonist activation and its over-expression decreases mGluR1a-stimulated ERK signaling (Zhang et al., 2008). CAL is suggested to regulate mGluR5a function by increasing the expression of the receptor by a mechanism that involves the inhibition of mGluR5a ubiquitination (Cheng et al., 2010). Taken together, it appears CAL could have a regulatory role over the subcellular localization of a subset of GPCRs, perhaps by contributing to the post-translational modification of nascent and mature proteins that ultimately influence the sorting and trafficking fate.

### Additional GPCR-interacting PDZ Proteins

**Spinophilin (Neurabin-2) and Neurabin-1:** Both spinophilin/neurabin-2 and neurabin-1 contain an amino terminal actin-binding domain, a PP1 $\gamma$ -binding domain, a single PDZ domain, and a coiled-coil domain, with neurabin-1 also containing a carboxyl terminal SAM domain (Kelker et al., 2007) (Fig. 2). Spinophilin has been shown to interact with both the D2R and  $\alpha_2$ AR (Smith et al., 1999; Richman et al., 2001; Brady et al., 2003; Wang and Limbird, 2002; Wang et al., 2004).



## MOL Manuscript # 98509

However, these interactions appear to be mediated by the third intracellular loop domains of these GPCRs, as opposed to interactions with PDZ binding motifs. Spinophilin functions to promote the membrane localization and inhibit the endocytosis and desensitization of  $\alpha_2$ ARs by competing for  $\beta$ -arrestin2 binding (Wang et al., 2004). The interaction between spinophilin and  $\alpha_2$ AR is prevented by PKA-mediated phosphorylation of spinophilin that results in increased agonist-stimulated  $\alpha_{2A}$ AR endocytosis (Xu et al., 2008).  $\beta_2$ AR activation also stimulates PKA-mediated spinophilin phosphorylation to increase  $\alpha_{2A}$ AR-endocytosis (Cottingham et al., 2013). Conversely, spinophilin appears to promote RGS2-mediated inhibition of  $\alpha_2$ AR-evoked  $Ca^{2+}$  signaling and RGS2-mediated modulation of  $\alpha_1$ AR-NMDAR crosstalk (Wang et al., 2005; Liu et al., 2006). In spinophilin knockout mice,  $\alpha_{2A}$ AR exhibits increased G protein-coupling and sensitized responses to  $\alpha_{2A}$ AR agonists (Lu et al., 2010; Cottingham et al., 2012). Both spinophilin and neurabin-1 are implicated in the  $D_1$ R-dependent regulation of AMPAR, as well as long-term depression and potentiation, respectively (Allen et al., 2006). Spinophilin promotes prostacyclin receptor signaling via  $G_{\alpha_s}$  and influences both m1AChR and m3AChR activity by enhancing RGS8-mediated inhibition of the  $G_{\alpha_q}$ -coupled signaling (Ma et al., 2012; Fujii et al., 2008; Kurogi et al., 2009). Similarly, spinophilin recruits RGS4 to the m3AChR, and like RGS8, RGS4 antagonizes m3AChR inositol phosphate signaling (Ruiz de Azua et al., 2012). Spinophilin also promotes  $\mu$ -opioid receptor ( $\mu$ OR)-mediated signaling via  $G_{\alpha_i}$ , but inhibits  $\mu$ OR-mediated ERK activation, while facilitating  $\mu$ OR endocytosis (Charlton et al., 2008; Fourla et al., 2012).

The interaction between spinophilin and opioid receptors appears to occur via the opioid receptor third intracellular loop and a conserved region of the carboxyl termini, proximal to the seventh transmembrane domain (Fourla et al., 2012). Interestingly, this region appears to correlate with a small helical region identified in many Class A Rhodopsin-like GPCRs as helix 8 (Huynh et al., 2009). This domain is suggested to run perpendicularly to the other 7 helical transmembrane domains and is initiated by an N-P-x-x-Y motif (Huynh et al., 2009). In examining

## MOL Manuscript # 98509

the amino acid sequences of Class A Rhodopsin-like GPCRs with this motif, a possible internal class I PDZ-binding motif, as characterized by a S/T-x- $\phi$  motif, may be present near this region (Trejo, 2005). Furthermore, homologous regions are found within  $\alpha_2$ ARs and D<sub>2</sub>R, which also interact with spinophilin via the third intracellular loop domain. Notably, a recent study has identified helix 8 of D<sub>2</sub>R to associate with the PDZ domain of GIPC (Sensoy and Weinstein, 2015). Future studies could look to investigate whether secondary interactions with spinophilin may occur within the  $\alpha_2$ ARs and D<sub>2</sub>R carboxyl terminal/helix 8, and whether these interactions require spinophilin's PDZ domain.

**Shank Proteins:** SH3 and multiple ankyrin repeat domains (Shank1-3) proteins are unified by the inclusion of multiple ankyrin repeat domains, a SH3 domain, a PDZ domain, and a sterile alpha motif (SAM) domain, however Shank2 lacks the ankyrin repeats (Fig. 2). Shank1B enhances mGluR1/5-mediated ERK1/2 and Ca<sup>2+</sup>-dependent signaling, and Shank3 is important for mGluR5-mediated ERK1/2 and CREB phosphorylation and subsequent mGluR5-mediated LTD (Sala et al., 2005; Verpelli et al., 2011). Furthermore, Shank3 prevents mGluR1-mediated inhibition of NMDAR via its association with Homer1A (Bertaso et al., 2010; Guo et al., 2004). Similarly, Shank1/3 modulates mAChR1- and D<sub>2</sub>R-mediated inhibition of L-type Ca<sup>2+</sup>-channels via Homer proteins (Olson et al., 2005). In regards to GPCR trafficking, Shank influences the clustering and subcellular localization of mGluR5 and calcium-independent alpha-latrotoxin/latrophilin 1 receptor (CL1) (Tu et al., 1999; Tobaben et al., 2000). Interestingly, a Shank/Homer1A complex can suppress NMDAR and AMPAR clustering and surface expression (Sala et al., 2003). Shank1 directly interacts with dynamin-2, which may provide insight into a mechanism of action in preventing GPCR-mediated crosstalk mechanisms and receptor surface expression (Okamoto et al., 2001). Future studies could look to investigate the role of Shank proteins in regulating GPCR trafficking and the crosstalk between GPCRs and ion channels.

## MOL Manuscript # 98509

**Par3 and Par6:** Partitioning defective (Par or PARD) proteins have been implicated in cellular polarization and Par3 and Par6 are PDZ domain-containing members of the Par family (Fig. 2) (Macara, 2004). Par3 is implicated as having a role in synaptogenesis as a consequence of its interaction with the BAI-1R (Duman et al., 2013). Additionally, Par3 has been shown to increase bradykinin receptor interactions with PLC $\beta$ 1 (Choi et al., 2010). Interestingly, both Par3 and Par6 interact and catalyze the activation of PLC $\beta$  downstream of heterotrimeric G proteins and form a complex with atypical PKCs (Cai et al., 2005; Joberty et al., 2000). Taken together, these observations suggest that Par3 and Par6 may contribute the regulation of GPCR-mediated G $\alpha_q$  signaling, as well as feedback receptor desensitization by atypical PKCs.

**MUPP1:** Multiple PDZ protein 1 (MUPP1) is one of the largest PDZ domain-containing proteins and is comprised of an amino terminal L27 domain followed by thirteen PDZ domains (Fig. 2). The interaction of MUPP1 with melatonin 1 receptor (MT $_1$ R) facilitates MT $_1$ R G $\alpha_i$ -coupling resulting in the inhibition of adenylyl cyclase activity (Guillaume et al., 2008). MUPP1 has also been shown to promote GABA $_B$  receptor-mediated Ca $^{2+}$  signaling, although MUPP1 knockdown prolongs the decay of the odorant receptor OR2AG1-mediated Ca $^{2+}$  response (Balasubramanian et al., 2007; Dooley et al., 2009). In regards to GPCR trafficking, MUPP1 increases the cell surface expression of the 5-HT $_{2A}$ R (Jones et al., 2009). Additionally, MUPP1 promotes the targeting of SSTR3 to tight junctions, thereby influencing transepithelial permeability (Liew et al., 2009; Vockel et al., 2010). Given that MUPP1 influences NMDA-dependent AMPA trafficking and clustering, it is likely that MUPP1 also regulates the trafficking of GPCRs that encode PDZ-binding motifs thereby contributing to GPCR-dependent regulation of synaptic activity (Krapivinsky et al., 2004).

## MOL Manuscript # 98509

**Tamalin (GRASP):** Tamalin, or general receptor for phosphoinositides (GRP1)-associated scaffold protein (GRASP), encodes a PDZ domain, a leucine zipper, and a class I PDZ-binding motif on the distal carboxyl terminal (Kitano et al., 2002; Kitano et al., 2003) (Fig. 2). Tamalin promotes the plasma membrane localization of mGluR1a, as well as the neuritic targeting of mGluR5 in hippocampal neurons (Kitano et al., 2002). Tamalin also interacts with mGluR2, mGluR3 and the GABA<sub>B2</sub>R, but the functional consequence of these interactions remain to be determined (Kitano et al., 2002). In the absence of mGluRs, or potentially other GPCR binding-partners, tamalin displays an auto-inhibitory confirmation caused by the interaction between the tamalin PDZ domain and tamalin PDZ-binding motif (Sugi et al., 2007). Upon mGluR1a binding to the tamalin PDZ domain, the tamalin PDZ-binding motif is free to associate with MAGI-2 to further enhance the membrane localization of mGluR1a (Sugi et al., 2007). PDZ-GEF1/2 also contain PDZ-binding motifs and future studies could look to determine whether they similarly exhibit auto-regulation (Ogawa et al., 2007; Kuiperij et al., 2003; Kuiperij et al., 2006).

**nNOS:** Neuronal nitric oxide synthase (nNOS) contains an amino terminal PDZ domain, a flavodoxin-like domain, and a flavin adenine dinucleotide (FAD)-binding domain (Fig. 2). nNOS, in conjunction with RGS17, has been demonstrated to complex with multiple GPCRs, including:  $\mu$ OR,  $\delta$ OR, 5-HT<sub>1A</sub>R, 5-HT<sub>2A</sub>R,  $\alpha_2$ AR, D<sub>1</sub>R, D<sub>2</sub>R, m2AChR, m4AChR, mGluR2, mGluR5, and cannabinoid receptor 1 (Sánchez-Blázquez et al., 2012). Activation of these receptors leads to the nNOS/NO-dependent recruitment of PKC $\gamma$  and Raf-1 to many of these GPCRs. nNOS also facilitates crosstalk between  $\mu$ OR and NMDAR (Rodríguez-Muñoz et al., 2008; Sánchez-Blázquez et al., 2010; Garzón et al., 2011). Interestingly, nNOS interacts with both PSD-95 and PSD-93, and is targeted to the neuromuscular junction via its interaction with PDZ protein  $\alpha$ -

## MOL Manuscript # 98509

syntrophin (Brenman et al., 1996; Adams et al., 2010). Although this nNOS interaction with PSD-95 is suggested to regulate NMDAR activity (Christopherson et al., 1999), it is yet to be determined whether these PDZ/PDZ protein interactions regulate GPCR function.

**Syntrophins:**  $\alpha$ -syntrophin,  $\beta_1$ -syntrophin and  $\beta_2$ -syntrophin all have an amino-terminal PH domain interrupted by a PDZ domain, followed by another PH domain and a syntrophin unique (SU) calmodulin-binding domain (Fig. 2) (Adams et al., 1995; Ahn et al., 1996; Chen et al., 2006). These syntrophins interact with  $\alpha_{1D}$ AR and collectively facilitate the functional expression of the receptor at the membrane, promoting  $\alpha_{1D}$ AR-mediated phosphatidylinositol hydrolysis, ERK1/2 phosphorylation and  $Ca^{2+}$  mobilization (Chen et al., 2006; Lyssand et al., 2008; Lyssand et al., 2010; Lyssand et al., 2011). Neither  $\gamma_1$ -syntrophin nor  $\gamma_2$ -syntrophin comparably bind  $\alpha_{1D}$ AR despite containing one PDZ domain and a PH domain, and their potential role in GPCR regulation remains uncertain (Chen et al., 2006).  $\alpha$ -syntrophin can additionally scaffold the PDZ protein nNOS and notably binds  $G\beta\gamma$  subunits via its PDZ domain (Brenman et al., 1996; Adams et al., 2010; Zhou et al., 2005).

**PICK1:** The protein interacting with C kinase 1 (PICK1) protein encodes one PDZ domain and an arfaptin homology domain/BAR (Bin/Amphiphysin/Rvs) domain involved in cell membrane interactions (Katsushima et al., 2013) (Fig. 2). PICK1 promotes the intracellular clustering of the prolactin-releasing peptide receptor, influences plasma membrane expression of the growth hormone-releasing hormone receptor (GHRHR) and antagonizes GHRHR-mediated cAMP signaling (Lin et al., 2001; Katsushima et al., 2013). PICK1 regulates PKC phosphorylation of mGluR7a, regulates the pre-synaptic clustering of mGluR7 and mediates stable mGluR7 cell surface expression (Dev et al., 2000; Boudin et al., 2000; Suh et al., 2008). mGluR7a knock-in

## MOL Manuscript # 98509

mice lacking a PDZ binding motif exhibit deficits in hippocampal-dependent spatial memory and are highly susceptible to the convulsant drugs, and the disruption of the mGluR7a-PICK1 complex induces epilepsy-like seizures (Zhang et al., 2008; Bertaso et al., 2008). Taken together, it appears PICK1 may be important for regulating the trafficking of a subset of GPCRs and may prove important in regulating GPCR-mediated signaling pathways. Notably, PICK1 can both homodimerize and heterodimerize with another PDZ domain-containing protein, syntenin-1 (Staudinger et al., 1997; Koroll et al., 2001).

**Syntenin-1:** Syntenin-1 contains two PDZ domains (Fig. 2) and has been found to self-associate, as well as heterodimerize with PICK1 and form a complex with mGluR7 (Koroll et al., 2001; Enz and Croci, 2003; Hirbec et al., 2002). Although PICK1 regulates mGluR7 phosphorylation, clustering, and membrane expression, it is not yet clear what role syntenin-1 may play in this regulation (Dev et al., 2000; Boudin et al., 2000; Suh et al., 2008). Nonetheless, syntenin-1 has been demonstrated to enhance the membrane expression of GPR37 (Dunham et al., 2009). In regards to signaling, syntenin-1 interacts with frizzled-7 (Fzd7) and promotes c-Jun phosphorylation, CDC42 activation, and PKC $\alpha$  recruitment to the membrane (Luyten et al., 2008). Syntenin-1 can also heterodimerize with syntenin-2, although little is known about the role of syntenin-2 in GPCR regulation (Koroll et al., 2001).

**SNX27:** Sorting nexin-27 (SNX27) differs from other sorting nexins through the inclusion of an amino terminal PDZ domain, followed by a Phox homology (PX) domain and a Ras-associating domain (Fig. 2). SNX27 interacts with both 5-HT<sub>4A</sub>R and  $\beta_2$ AR in early endosome antigen 1 (EEA1)-positive early endosomes (Joubert et al., 2004; Lauffer et al., 2010). Moreover, SNX27 is involved in regulating the recycling of the  $\beta_2$ AR,  $\beta_1$ AR, and SSTR5, thereby preventing receptor

## **MOL Manuscript # 98509**

degradation (Lauffer et al., 2010; Temkin et al., 2011; Nakagawa and Asahi, 2013; Bauch et al., 2014). The regulation of  $\beta_2$ AR recycling by SNX27 is dependent upon PX domain-mediated associations with the endosomal membrane (Lauffer et al., 2010). Furthermore, SNX27 interacts with the endosomal WASH complex to target the  $\beta_2$ AR to the retromer tubule for efficient recycling (Temkin et al., 2011). Taken together, it appears SNX27 is capable of promoting the endosomal sorting and recycling of a subset of GPCRs, a role that may be generalizable to several other PDZ motif-encoding GPCRs.

***PDZ-GEFs (RAPGEFs, CNrasGEF, RA-GEF):*** PDZ domain-containing guanine nucleotide exchange factors (PDZ-GEF1 and PDZ-GEF2) share approximately 56% sequence homology and include one or two cyclic nucleotide-binding domains, respectively, an N terminal Ras GEF domain, a PDZ domain, a Ras-associating domain, and Ras GEF catalytic domain within their molecular structure (Kuiperij et al., 2003; Kuiperij et al., 2006) (Fig. 2). Similar to the PDZ domain-containing protein tamalin, PDZ-GEF1/2 have also been reported to contain a class I PDZ-binding motif at their carboxyl termini, suggesting a capability for homo/hetero-oligomerization with PDZ domain-containing proteins, or perhaps even auto-regulatory capability via self-association (Ogawa et al., 2007; Kuiperij et al., 2003; Kuiperij et al., 2006). Our current understanding of PDZ-GEF2 regulation of GPCRs is poor, but PDZ-GEF1 couples the  $\beta_1$ AR to the activation of Ras (Pak et al., 2002). Furthermore, PDZ-GEF1 is essential for coupling the pituitary adenylate cyclase-activating polypeptide type I receptor to the ERK1/2 signaling pathway and the subsequent activation of neuritogenesis, with no effect on cAMP accumulation (Emery et al., 2013).

## MOL Manuscript # 98509

**RGS Proteins with PDZ domains (PDZ-RhoGEF, LARG, RGS3, and RGS12):** PDZ-RhoGEF and leukemia-associated RhoGEF (LARG) are members of the regulators of G protein signaling (RGS) homology domain-containing RhoGEF (RH-RhoGEF) subfamily and include an amino terminal PDZ domain, a RGS-homology domain, a RhoGEF domain, and a pleckstrin-homology (PH) domain (Fig. 2). LARG transduces  $G_{\alpha_{q/12/13}}$  activation into Rho activation via GPCRs such as the Mas receptor, G2 accumulation receptor, mACh1R, AT<sub>1</sub>R, sphingosine-1 phosphate receptor 2, histamine H1 receptor, thromboxane A2 receptor, and endothelin 1 receptor (Booden et al., 2002; Ying et al., 2006; Chiu et al., 2012; Del Galdo et al., 2013; Medlin et al., 2010; Pfreimer et al., 2012; Artamonov et al., 2013). Similarly, PDZ-RhoGEF is proposed contribute to gastrin-releasing peptide receptor-mediated activation of Rho/ROCK pathway via  $G_{\alpha_{13}}$  (Patel et al., 2014). Finally, both PDZ-RhoGEF and LARG have been implicated in sustaining Rho activation following thrombin and LPA receptor activation (Chikumi et al., 2002; Wang et al., 2004; Yamada et al., 2005). Interestingly, both proteins appear capable of homo- and hetero-dimerization (Chikumi et al., 2004).

RGS12 contains one PDZ domain, a phosphotyrosine-binding domain (PTB), a RGS domain, two Ras-binding domains, and a GoLoco motif (Fig. 2). The RGS12 PDZ domain binds to the interleukin-8 receptor B PDZ binding motif, but the functional consequence of this interaction is not well defined (Snow et al., 1998). Notably, RGS12 has been suggested to couple D<sub>2</sub>R to inward rectifier potassium channels Kir3.1/3.2 (Oxford and Webb, 2004). Regulator of G protein signaling 3 (RGS3) contains a membrane-targeting C2 domain, one PDZ domain, and an RGS domain (Fig. 2). RGS3 has been identified to inhibit  $G_{\alpha_q}$ - and  $G_{\alpha_i}$ -mediated signaling by acting as a GTPase-activating protein (Scheschonka et al., 2000). RGS3 antagonizes  $G_{\alpha_{q/11}}$  signaling via pheromone P factor receptor and mAChR3 activation and RGS3 promotes Ca<sup>2+</sup> oscillatory behaviour during submaximal mAChR3 activation (Ladds et al., 2007; Anger et al., 2004; Wang et al., 2002; Anger et al., 2007; Karakoula et al., 2008; Tovey and Willars, 2004).



## MOL Manuscript # 98509

RGS3 also antagonizes follicle-stimulating hormone receptor- and luteinizing hormone receptor-mediated inositol phosphate and cAMP accumulation (Castro-Fernandez et al., 2004). Furthermore, RGS3 has been demonstrated to suppress  $G\alpha_i$ -mediated signaling pathways via  $\mu$ OR, mAChR1, complement C5a receptor, and  $\beta_2$ AR, and even promote a  $G\alpha_s$  bias for  $\beta_2$ AR (Potenza et al., 1999; Anger et al., 2007; Nishiura et al., 2009; Chakir et al., 2011). In contrast, RGS3 was shown to inhibit gonadotropin-releasing hormone receptor-stimulated inositol phosphate signaling via  $G\alpha_q$ , but had no effect on cAMP signaling (Neill et al., 1997; Neill et al., 2001; Castro-Fernandez et al., 2002; Castro-Fernandez and Conn, 2002; Karakoula et al., 2008). Interestingly, RGS3 palmitoylation is increased following GRHR activation (Castro-Fernandez et al., 2002). Curiously, truncated RGS3 isoforms that have been reported to lack the amino terminal and PDZ domain have also demonstrated a role in influencing GPCR activity, including S1PR<sub>1-3</sub>, AT<sub>1</sub>R, ET<sub>1</sub>R, GRHR, 5-HT<sub>1A</sub>R, and mAChR2/3 (Druey et al., 1996; Cho et al., 2003; Castro-Fernandez et al., 2003; Jaén and Doupnik, 2005; Anger et al., 2004; Anger et al., 2007). Distinguishing the role of LARG, PDZ-RhoGEF, RGS3, and RGS12 PDZ domain interactions, as opposed to RGS domain interactions with heterotrimeric G proteins, in the regulation of GPCR signaling remains a challenge.

### Role PDZ Proteins in GPCR-regulated Physiology

**PSD-95 Family of MAGUK PDZ Proteins:** The PDZ domain-containing MAGUK proteins play an essential role in human neurophysiology and development. This is demonstrated in mouse knockout studies, where PSD-95 and PSD-93 double-knockout mice exhibit severe deficiencies in AMPA currents, and SAP97 knockout mice show neonatal lethality (Caruana and Bernstein, 2001; Howard et al., 2010). Of particular interest is the observation that PSD-95 is essential for hallucinogenic and atypical antipsychotic actions of 5-HT<sub>2A</sub>R and 5-HT<sub>2C</sub>R (Abbas et al., 2009). In addition to being involved atypical antipsychotic actions (Abbas et al., 2009), PDZ protein

## MOL Manuscript # 98509

interactions with GPCRs also appear important regulating stress and anxiety responses (Magalhaes et al., 2010). Pre-activation of the CRFR1 receptor sensitizes 5-HT<sub>2A</sub>R-stimulated IP formation dependent upon intact PDZ binding motifs in both receptors, receptor endocytosis and recycling (Magalhaes et al., 2010). Furthermore, the phenomenon can be blocked by a Tat-tagged fusion protein corresponding to the last 15 amino acids of the CRFR1 tail. In addition, pre-treatment of mice with sub-threshold doses of CRF into the prefrontal cortex sensitizes mouse anxiety responses to DOI treatment (Magalhaes et al., 2010). Thus, it is possible that PDZ protein interactions may serve as a good pharmaceutical target for the treatment of disease.

SAP102 is important during early synaptic development and SAP97 appears to be important in SSTR1-mediated growth cone dynamics, as evidenced by colocalization within the growth cone (Kim and Sheng, 2004; Elias et al., 2006; Cai et al., 2008). However, this role may not be limited to SAP97 and may include additional PDZ domain-containing proteins (Cai et al., 2008). PSD-95 plays a functional role in synaptic plasticity and contributes to GPCR-mediated regulation of both long-term potentiation, and long-term depression (Xu, 2011). Notably, SAP97 also modulates the ability to regulate AMPA and NMDA receptors by promoting synaptic trafficking of these receptors (Howard et al., 2010). Acute overexpression of SAP97 in hippocampal slice cultures restored synaptic transmission in PSD-95/PSD-93 double knockout mice, and long-term overexpression of SAP97 throughout development led to enhancements in synaptic transmission *in vivo* (Howard et al., 2010). This regulation of NMDAR- and AMPAR-mediated synaptic transmission is likely to also involve a role of GPCRs. PSD-95 is reported to have an important role in regulating the trafficking dynamics of D<sub>1</sub>R in striatal neurons, and this regulatory role may contribute to L-DOPA-induced dyskinesia (Porras et al., 2012). Thus, the role of PSD-95 in regulating D<sub>1</sub>R dynamics may be complicated by its ability to disrupt the formation of D<sub>1</sub>R/NMDAR complexes, a function which potentially may be directly associated with its role in the regulation of synaptic activity (Zhang et al., 2009). The association of PSD-95 with the

## MOL Manuscript # 98509

$\beta_1$ AR allows it to form a complex with the NMDAR and this may contribute to the regulation of synaptic activity by adrenergic ligands (Hu et al., 2000).

**Other PDZ Proteins:** There are a number other examples of PDZ proteins regulating GPCR-mediated regulation of physiological functions. In the immune system it has been found that the interaction of NHERF1 with complement component C3a receptor is required for C3a-mediated mast cell degranulation, NF $\kappa$ B-activation and chemokine production (Subramanian et al., 2012). CCR5 functions as a co-receptor for HIV-1 viral entry into mammalian cells by functioning as a cofactor for the entry of the virus (Henrich and Kuritzkes, 2013). NHERF1 interactions with CCR5 function to enhance actin filament rearrangement of host cells: a function that is essential to allow post-cell entry HIV-1 replication (Hammad et al., 2010; Kuang et al., 2012). PDZK1 interactions with hIPR selectively facilitate hIPR-dependent activation of endothelial migration and vascular angiogenesis *in vitro* (Turner et al., 2011). MUPP1, the largest of the PDZ domain-containing adaptor protein promotes the targeting of SSTR3 to tight junctions and consequently influences trans-epithelial permeability of skin cells (Liew et al., 2009; Vockel et al., 2010). Tamalin plays an important role in regulating mGluR signaling and tamalin knockout mice exhibit differences in their acute and adaptive responses to morphine administration. (Ogawa et al., 2007). Similarly, nNOS mediates a mechanism of crosstalk between  $\mu$ OR and NMDA receptors to regulate opioid tolerance and analgesia (Rodríguez-Muñoz et al., 2008; Sánchez-Blázquez et al., 2010; Garzón et al., 2011). PICK1 interactions with mGluR7a have been shown to be important for pre-synaptic mGluR7a clustering, and mGluR7a knock-in mice lacking a PDZ binding motif exhibit deficits in hippocampal-dependent spatial memory and the disruption of the mGluR7a-PICK1 complex induces epileptic-like seizures (Boudin et al., 2000; Zhang et al., 2008; Bertaso et al., 2008).  $\alpha$ -syntrophin and  $\beta_2$ -syntrophin knockout mice display normal systolic blood pressure and resting heart rate, however a double knockout prevents  $\alpha_{1D}$ AR-mediated blood pressure responses and

## **MOL Manuscript # 98509**

exhibits a distinct hypotonic phenotype at rest, thereby demonstrating the capability for PDZ protein compensation *in vivo* (Lyssand et al., 2008).

### **Concluding Remarks**

GPCRs are influential in the regulation of every aspect of human physiology. Therefore, any advancement in the understanding of how they can be regulated could contribute to the design and development of new pharmacological treatment and prevention strategies for a multitude of human diseases (Bockaert et al., 2010; Heng et al., 2013). Accordingly, it is becoming clear that PDZ proteins play an important role in the regulation of GPCR signaling and trafficking. Considering it is estimated that 20% of GPCRs have PDZ-binding motifs, and over 800 GPCRs have been identified in the human genome, it is safe to assume that this field is still in its infancy (Lee and Zheng, 2010; Fredriksson et al., 2003). Nevertheless, our growing understanding of the functional specificities and redundancies in PDZ regulation of GPCRs may lead to the development of new pharmacological compounds for precise modulation of GPCR activity. Such a strategy could be pertinent in the pharmacological treatment of a multitude of human pathologies including but not limited to mental illnesses, cystic fibrosis, and osteoporosis (Abbas et al., 2009; Magalhaes et al., 2010; Holcomb et al., 2014; Mahon, 2012).

## **MOL Manuscript # 98509**

### **Acknowledgements**

S. S. G. F. holds a Tier I Canada Research Chair in Molecular Neurobiology and is a Career Investigator of the Heart and Stroke Foundation of Ontario, Canada. H. A. D. is the recipient of a Jonathan and Joshua Memorial Scholarship in Mental Health Research.

**MOL Manuscript # 98509**

**Authorship Contribution:**

Henry A. Dunn and Stephen S. G. Ferguson wrote the manuscript.

## MOL Manuscript # 98509

### References

- Abbas AI, Yadav PN, Yao WD, Arbuckle MI, Grant SG, Caron MG and Roth BL (2009) PSD-95 is essential for hallucinogen and atypical antipsychotic drug actions at serotonin receptors. *J Neurosci* **29**:7124-7136.
- Adams ME, Anderson KN and Froehner SC (2010) The alpha-syntrophin PH and PDZ domains scaffold acetylcholine receptors, utrophin, and neuronal nitric oxide synthase at the neuromuscular junction. *J Neurosci* **30**:11004-11010.
- Adams ME, Dwyer TM, Dowler LL, White RA and Froehner SC (1995) Mouse alpha 1- and beta 2-syntrophin gene structure, chromosome localization, and homology with a discs large domain. *J Biol Chem* **270**:25859-25865.
- Ahn AH, Freener CA, Gussoni E, Yoshida M, Ozawa E and Kunkel LM (1996) The three human syntrophin genes are expressed in diverse tissues, have distinct chromosomal locations, and each bind to dystrophin and its relatives. *J Biol Chem* **271**:2724-2730.
- Akama KT, Thompson LI, Milner TA and McEwen BS (2013) Post-synaptic density-95 (PSD-95) binding capacity of G-protein-coupled receptor 30 (GPR30), an estrogen receptor that can be identified in hippocampal dendritic spines. *J Biol Chem* **288**:6438-6450.
- Allen PB, Zachariou V, Svenningsson P, Lepore AC, Centonze D, Costa C, Rossi S, Bender G, Chen G, Feng J, Snyder GL, Bernardi G, Nestler EJ, Yan Z, Calabresi P and Greengard P (2006) Distinct roles for spinophilin and neurabin in dopamine-mediated plasticity. *Neuroscience* **140**:897-911.
- Anger T, Klintworth N, Stumpf C, Daniel WG, Mende U and Garlich CD (2007) RGS protein specificity towards Gq- and Gi/o-mediated ERK 1/2 and Akt activation, in vitro. *J Biochem Mol Biol* **40**:899-910.
- Anger T, Zhang W and Mende U (2004) Differential contribution of GTPase activation and effector antagonism to the inhibitory effect of RGS proteins on Gq-mediated signaling in vivo. *J Biol Chem* **279**:3906-3915.
- Ardura JA and Friedman PA (2011) Regulation of G protein-coupled receptor function by Na<sup>+</sup>/H<sup>+</sup> exchange regulatory factors. *Pharmacol Rev* **63**:882-900.
- Artamonov MV, Momotani K, Stevenson A, Trentham DR, Derewenda U, Derewenda ZS, Read PW, Gutkind JS and Somlyo AV (2013) Agonist-induced Ca<sup>2+</sup> sensitization in smooth muscle: redundancy of Rho guanine nucleotide exchange factors (RhoGEFs) and response kinetics, a caged compound study. *J Biol Chem* **288**:34030-34040.
- Balasubramanian S, Fam SR and Hall RA (2007) GABAB receptor association with the PDZ scaffold Mupp1 alters receptor stability and function. *J Biol Chem* **282**:4162-4171.
- Bauch C, Koliwer J, Buck F, Honck HH and Kreienkamp HJ (2014) Subcellular sorting of the G-protein coupled mouse somatostatin receptor 5 by a network of PDZ-domain containing proteins. *PLoS One* **9**:e88529.
- Becamel C, Alonso G, Galeotti N, Demey E, Jouin P, Ullmer C, Dumuis A, Bockaert J and Marin P (2002) Synaptic multiprotein complexes associated with 5-HT(2C) receptors: a proteomic approach. *EMBO J* **21**:2332-2342.

## MOL Manuscript # 98509

Becamel C, Gavarini S, Chanrion B, Alonso G, Galeotti N, Dumuis A, Bockaert J and Marin P (2004) The serotonin 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors interact with specific sets of PDZ proteins. *J Biol Chem* **279**:20257-20266.

Bergbrede T, Chuky N, Schoebel S, Blankenfeldt W, Geyer M, Fuchs E, Goody RS, Barr F and Alexandrov K (2009) Biophysical analysis of the interaction of Rab6a GTPase with its effector domains. *J Biol Chem* **284**:2628-2635.

Bertaso F, Roussignol G, Worley P, Bockaert J, Fagni L and Ango F (2010) Homer1a-dependent crosstalk between NMDA and metabotropic glutamate receptors in mouse neurons. *PLoS One* **5**:e9755.

Bertaso F, Zhang C, Scheschonka A, de Bock F, Fontanaud P, Marin P, Huganir RL, Betz H, Bockaert J, Fagni L and Lerner-Natoli M (2008) PICK1 uncoupling from mGluR7a causes absence-like seizures. *Nat Neurosci* **11**:940-948.

Bezprozvanny I and Maximov A (2001) Classification of PDZ domains. *FEBS Lett* **509**:457-462.

Biernatowska A, Podkalicka J, Majkowski M, Hryniewicz-Jankowska A, Augoff K, Kozak K, Korzeniewski J and Sikorski AF (2013) The role of MPP1/p55 and its palmitoylation in resting state raft organization in HEL cells. *Biochim Biophys Acta* **1833**:1876-1884.

Bockaert J, Perroy J, Becamel C, Marin P and Fagni L (2010) GPCR interacting proteins (GIPs) in the nervous system: Roles in physiology and pathologies. *Annu Rev Pharmacol Toxicol* **50**:89-109.

Booden MA, Siderovski DP and Der CJ (2002) Leukemia-associated Rho guanine nucleotide exchange factor promotes G alpha q-coupled activation of RhoA. *Mol Cell Biol* **22**:4053-4061.

Boudin H, Doan A, Xia J, Shigemoto R, Huganir RL, Worley P and Craig AM (2000) Presynaptic clustering of mGluR7a requires the PICK1 PDZ domain binding site. *Neuron* **28**:485-497.

Brady AE, Wang Q, Colbran RJ, Allen PB, Greengard P and Limbird LE (2003) Spinophilin stabilizes cell surface expression of alpha 2B-adrenergic receptors. *J Biol Chem* **278**:32405-32412.

Brenman JE, Chao DS, Gee SH, McGee AW, Craven SE, Santillano DR, Wu Z, Huang F, Xia H, Peters MF, Froehner SC and Brecht DS (1996) Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. *Cell* **84**:757-767.

Broselid S, Berg KA, Chavera TA, Kahn R, Clarke WP, Olde B and Leeb-Lundberg LM (2014) G protein-coupled receptor 30 (GPR30) forms a plasma membrane complex with membrane-associated guanylate kinases (MAGUKs) and protein kinase A-anchoring protein 5 (AKAP5) that constitutively inhibits cAMP production. *J Biol Chem* **289**:22117-22127.

Butz S, Okamoto M and Sudhof TC (1998) A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. *Cell* **94**:773-782.

Cai C, Li H, Kangasniemi A, Pihlajamaa T, Von Ossowski L, Kerkela K, Schulz S, Rivera C and Keinänen K (2008) Somatostatin receptor subtype 1 is a PDZ ligand for synapse-associated protein 97 and a potential regulator of growth cone dynamics. *Neuroscience* **157**:833-843.

Cai Y, Stafford LJ, Bryan BA, Mitchell D and Liu M (2005) G-protein-activated phospholipase C-beta, new partners for cell polarity proteins Par3 and Par6. *Oncogene* **24**:4293-4300.



## MOL Manuscript # 98509

Cao TT, Deacon HW, Reczek D, Bretscher A and von Zastrow M (1999) A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. *Nature* **401**:286-290.

Caruana G and Bernstein A (2001) Craniofacial dysmorphogenesis including cleft palate in mice with an insertional mutation in the discs large gene. *Mol Cell Biol* **21**:1475-1483.

Castro-Fernandez C, Brothers SP and Michael Conn P (2003) A Galphas mutation (D229S) differentially effects gonadotropin-releasing hormone receptor regulation by RGS10, RGS3 and RGS3T. *Mol Cell Endocrinol* **200**:119-126.

Castro-Fernandez C and Conn PM (2002) Regulation of the gonadotropin-releasing hormone receptor (GnRHR) by RGS proteins: role of the GnRHR carboxyl-terminus. *Mol Cell Endocrinol* **191**:149-156.

Castro-Fernandez C, Janovick JA, Brothers SP, Fisher RA, Ji TH and Conn PM (2002) Regulation of RGS3 and RGS10 palmitoylation by GnRH. *Endocrinology* **143**:1310-1317.

Castro-Fernandez C, Maya-Nunez G and Mendez JP (2004) Regulation of follicle-stimulating and luteinizing hormone receptor signaling by. *Endocrine* **25**:49-54.

Chakir K, Depry C, Dimaano VL, Zhu WZ, Vanderheyden M, Bartunek J, Abraham TP, Tomaselli GF, Liu SB, Xiang YK, Zhang M, Takimoto E, Dulin N, Xiao RP, Zhang J and Kass DA (2011) Galphas-biased beta2-adrenergic receptor signaling from restoring synchronous contraction in the failing heart. *Sci Transl Med* **3**:100ra88.

Charlton JJ, Allen PB, Psifogeorgou K, Chakravarty S, Gomes I, Neve RL, Devi LA, Greengard P, Nestler EJ and Zachariou V (2008) Multiple actions of spinophilin regulate mu opioid receptor function. *Neuron* **58**:238-247.

Chen A, Gossling EK, Witkowski L, Bhindi A, Bauch C, Roussy G, Sarret P, Kreienkamp HJ and Stroh T (2012) Regional and subcellular distribution of the receptor-targeting protein PIST in the rat central nervous system. *J Comp Neurol* **520**:889-913.

Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Brecht DS and Nicoll RA (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**:936-943.

Chen Z, Hague C, Hall RA and Minneman KP (2006) Syntrophins regulate alpha1D-adrenergic receptors through a PDZ domain-mediated interaction. *J Biol Chem* **281**:12414-12420.

Cheng D, Hoogenraad CC, Rush J, Ramm E, Schlager MA, Duong DM, Xu P, Wijayawardana SR, Hanfelt J, Nakagawa T, Sheng M and Peng J (2006) Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. *Mol Cell Proteomics* **5**:1158-1170.

Cheng S, Zhang J, Zhu P, Ma Y, Xiong Y, Sun L, Xu J, Zhang H and He J (2010) The PDZ domain protein CAL interacts with mGluR5a and modulates receptor expression. *J Neurochem* **112**:588-598.

Chikumi H, Barac A, Behbahani B, Gao Y, Teramoto H, Zheng Y and Gutkind JS (2004) Homo- and hetero-oligomerization of PDZ-RhoGEF, LARG and p115RhoGEF by their C-terminal region regulates their in vivo Rho GEF activity and transforming potential. *Oncogene* **23**:233-240.

## MOL Manuscript # 98509

Chikumi H, Fukuhara S and Gutkind JS (2002) Regulation of G protein-linked guanine nucleotide exchange factors for Rho, PDZ-RhoGEF, and LARG by tyrosine phosphorylation: evidence of a role for focal adhesion kinase. *J Biol Chem* **277**:12463-12473.

Chiu WC, Juang JM, Chang SN, Wu CK, Tsai CT, Tseng YZ and Chiang FT (2012) Angiotensin II regulates the LARG/RhoA/MYPT1 axis in rat vascular smooth muscle in vitro. *Acta Pharmacol Sin* **33**:1502-1510.

Cho H, Harrison K, Schwartz O and Kehrl JH (2003) The aorta and heart differentially express RGS (regulators of G-protein signalling) proteins that selectively regulate sphingosine 1-phosphate, angiotensin II and endothelin-1 signalling. *Biochem J* **371**:973-980.

Cho KO, Hunt CA and Kennedy MB (1992) The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* **9**:929-942.

Choi JW, Lim S, Oh YS, Kim EK, Kim SH, Kim YH, Heo K, Kim J, Kim JK, Yang YR, Ryu SH and Suh PG (2010) Subtype-specific role of phospholipase C-beta in bradykinin and LPA signaling through differential binding of different PDZ scaffold proteins. *Cell Signal* **22**:1153-1161.

Christenn M, Kindler S, Schulz S, Buck F, Richter D and Kreienkamp HJ (2007) Interaction of brain somatostatin receptors with the PDZ domains of PSD-95. *FEBS Lett* **581**:5173-5177.

Christopherson KS, Hillier BJ, Lim WA and Brecht DS (1999) PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *J Biol Chem* **274**:27467-27473.

Cottingham C, Li X and Wang Q (2012) Noradrenergic antidepressant responses to desipramine in vivo are reciprocally regulated by arrestin3 and spinophilin. *Neuropharmacology* **62**:2354-2362.

Cottingham C, Lu R, Jiao K and Wang Q (2013) Cross-talk from beta-adrenergic receptors modulates alpha2A-adrenergic receptor endocytosis in sympathetic neurons via protein kinase A and spinophilin. *J Biol Chem* **288**:29193-29205.

de Mendoza A, Suga H and Ruiz-Trillo I (2010) Evolution of the MAGUK protein gene family in premetazoan lineages. *BMC Evol Biol* **10**:93-2148-10-93.

Del Galdo S, Vettel C, Heringdorf DM and Wieland T (2013) The activation of RhoC in vascular endothelial cells is required for the S1P receptor type 2-induced inhibition of angiogenesis. *Cell Signal* **25**:2478-2484.

Dev KK, Nakajima Y, Kitano J, Braithwaite SP, Henley JM and Nakanishi S (2000) PICK1 interacts with and regulates PKC phosphorylation of mGLUR7. *J Neurosci* **20**:7252-7257.

Dobrosotskaya I, Guy RK and James GL (1997) MAGI-1, a membrane-associated guanylate kinase with a unique arrangement of protein-protein interaction domains. *J Biol Chem* **272**:31589-31597.

Dooley R, Baumgart S, Rasche S, Hatt H and Neuhaus EM (2009) Olfactory receptor signaling is regulated by the post-synaptic density 95, *Drosophila* discs large, zona-occludens 1 (PDZ) scaffold multi-PDZ domain protein 1. *FEBS J* **276**:7279-7290.

Doyle DA, Lee A, Lewis J, Kim E, Sheng M and MacKinnon R (1996) Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. *Cell* **85**:1067-1076.

## MOL Manuscript # 98509

Druey KM, Blumer KJ, Kang VH and Kehrl JH (1996) Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. *Nature* **379**:742-746.

Dulin NO, Sorokin A, Reed E, Elliott S, Kehrl JH and Dunn MJ (1999) RGS3 inhibits G protein-mediated signaling via translocation to the membrane and binding to Galpha11. *Mol Cell Biol* **19**:714-723.

Duman JG, Tzeng CP, Tu YK, Munjal T, Schwechter B, Ho TS and Toliaf KF (2013) The adhesion-PCR BAI1 regulates synaptogenesis by controlling the recruitment of the Par3/Tiam1 polarity complex to synaptic sites. *J Neurosci* **33**:6964-6978.

Dunham JH, Meyer RC, Garcia EL and Hall RA (2009) GPR37 surface expression enhancement via N-terminal truncation or protein-protein interactions. *Biochemistry* **48**:10286-10297.

Dunn HA, Walther C, Godin CM, Hall RA and Ferguson SS (2013) Role of SAP97 protein in the regulation of corticotropin-releasing factor receptor 1 endocytosis and extracellular signal-regulated kinase 1/2 signaling. *J Biol Chem* **288**:15023-15034.

Dunn HA, Walther C, Yuan GY, Caetano FA, Godin CM and Ferguson SS (2014) Role of SAP97 in the Regulation of 5-HT2AR Endocytosis and Signaling. *Mol Pharmacol* **86**:275-283.

Dupre DJ, Rola-Pleszczynski M and Stankova J (2012) Rescue of internalization-defective platelet-activating factor receptor function by EBP50/NHERF1. *J Cell Commun Signal* **6**:205-216.

Elias GM, Funke L, Stein V, Grant SG, Bredt DS and Nicoll RA (2006) Synapse-specific and developmentally regulated targeting of AMPA receptors by a family of MAGUK scaffolding proteins. *Neuron* **52**:307-320.

Elias GM and Nicoll RA (2007) Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. *Trends Cell Biol* **17**:343-352.

Emery AC, Eiden MV, Mustafa T and Eiden LE (2013) Rapgef2 connects GPCR-mediated cAMP signals to ERK activation in neuronal and endocrine cells. *Sci Signal* **6**:ra51.

Enz R and Croci C (2003) Different binding motifs in metabotropic glutamate receptor type 7b for filamin A, protein phosphatase 1C, protein interacting with protein kinase C (PICK) 1 and syntenin allow the formation of multimeric protein complexes. *Biochem J* **372**:183-191.

Fam SR, Paquet M, Castleberry AM, Oller H, Lee CJ, Traynelis SF, Smith Y, Yun CC and Hall RA (2005) P2Y1 receptor signaling is controlled by interaction with the PDZ scaffold NHERF-2. *Proc Natl Acad Sci U S A* **102**:8042-8047.

Feng W and Zhang M (2009) Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. *Nat Rev Neurosci* **10**:87-99.

Fouassier L, Yun CC, Fitz JG and Doctor RB (2000) Evidence for ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50) self-association through PDZ-PDZ interactions. *J Biol Chem* **275**:25039-25045.

Fourla DD, Papakonstantinou MP, Vrana SM and Georgoussi Z (2012) Selective interactions of spinophilin with the C-terminal domains of the delta- and mu-opioid receptors and G proteins differentially modulate opioid receptor signaling. *Cell Signal* **24**:2315-2328.

Fredriksson R, Lagerstrom MC, Lundin LG and Schioth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* **63**:1256-1272.

## MOL Manuscript # 98509

Fujii S, Yamazoe G, Itoh M, Kubo Y and Saitoh O (2008) Spinophilin inhibits the binding of RGS8 to M1-mAChR but enhances the regulatory function of RGS8. *Biochem Biophys Res Commun* **377**:200-204.

Gardner LA, Naren AP and Bahouth SW (2007) Assembly of an SAP97-AKAP79-cAMP-dependent protein kinase scaffold at the type 1 PSD-95/DLG/ZO1 motif of the human beta(1)-adrenergic receptor generates a receptosome involved in receptor recycling and networking. *J Biol Chem* **282**:5085-5099.

Garzon J, Rodriguez-Munoz M, Vicente-Sanchez A, Bailon C, Martinez-Murillo R and Sanchez-Blazquez P (2011) RGSZ2 binds to the neural nitric oxide synthase PDZ domain to regulate mu-opioid receptor-mediated potentiation of the N-methyl-D-aspartate receptor-calmodulin-dependent protein kinase II pathway. *Antioxid Redox Signal* **15**:873-887.

Gavarini S, Becamel C, Altier C, Lory P, Poncet J, Wijnholds J, Bockaert J and Marin P (2006) Opposite effects of PSD-95 and MPP3 PDZ proteins on serotonin 5-hydroxytryptamine<sub>2C</sub> receptor desensitization and membrane stability. *Mol Biol Cell* **17**:4619-4631.

Gee HY, Kim YW, Jo MJ, Namkung W, Kim JY, Park HW, Kim KS, Kim H, Baba A, Yang J, Kim E, Kim KH and Lee MG (2009) Synaptic scaffolding molecule binds to and regulates vasoactive intestinal polypeptide type-1 receptor in epithelial cells. *Gastroenterology* **137**:607-17, 617.e1-4.

Guillaume JL, Daulat AM, Maurice P, Levoe A, Migaud M, Brydon L, Malpoux B, Borg-Capra C and Jockers R (2008) The PDZ protein mupp1 promotes Gi coupling and signaling of the Mt1 melatonin receptor. *J Biol Chem* **283**:16762-16771.

Guo W, Wei F, Zou S, Robbins MT, Sugiyo S, Ikeda T, Tu JC, Worley PF, Dubner R and Ren K (2004) Group I metabotropic glutamate receptor NMDA receptor coupling and signaling cascade mediate spinal dorsal horn NMDA receptor 2B tyrosine phosphorylation associated with inflammatory hyperalgesia. *J Neurosci* **24**:9161-9173.

Hall RA, Premont RT, Chow CW, Blitzer JT, Pitcher JA, Claing A, Stoffel RH, Barak LS, Shenolikar S, Weinman EJ, Grinstein S and Lefkowitz RJ (1998) The beta2-adrenergic receptor interacts with the Na<sup>+</sup>/H<sup>+</sup>-exchanger regulatory factor to control Na<sup>+</sup>/H<sup>+</sup> exchange. *Nature* **392**:626-630.

Hammad MM, Kuang YQ, Yan R, Allen H and Dupre DJ (2010) Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor-1 is involved in chemokine receptor homodimer CCR5 internalization and signal transduction but does not affect CXCR4 homodimer or CXCR4-CCR5 heterodimer. *J Biol Chem* **285**:34653-34664.

Harris BZ and Lim WA (2001) Mechanism and role of PDZ domains in signaling complex assembly. *J Cell Sci* **114**:3219-3231.

He J, Bellini M, Inuzuka H, Xu J, Xiong Y, Yang X, Castleberry AM and Hall RA (2006) Proteomic analysis of beta1-adrenergic receptor interactions with PDZ scaffold proteins. *J Biol Chem* **281**:2820-2827.

He J, Bellini M, Xu J, Castleberry AM and Hall RA (2004) Interaction with cystic fibrosis transmembrane conductance regulator-associated ligand (CAL) inhibits beta1-adrenergic receptor surface expression. *J Biol Chem* **279**:50190-50196.

Heng BC, Aubel D and Fussenegger M (2013) An overview of the diverse roles of G-protein coupled receptors (GPCRs) in the pathophysiology of various human diseases. *Biotechnol Adv* **31**:1676-1694.

## MOL Manuscript # 98509

Henrich TJ and Kuritzkes DR (2013) HIV-1 entry inhibitors: recent development and clinical use. *Curr Opin Virol* **3**:51-57.

Hillier BJ, Christopherson KS, Prehoda KE, Bredt DS and Lim WA (1999) Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. *Science* **284**:812-815.

Hirakawa T, Galet C, Kishi M and Ascoli M (2003) GIPC binds to the human lutropin receptor (hLHR) through an unusual PDZ domain binding motif, and it regulates the sorting of the internalized human choriogonadotropin and the density of cell surface hLHR. *J Biol Chem* **278**:49348-49357.

Hirbec H, Perestenko O, Nishimune A, Meyer G, Nakanishi S, Henley JM and Dev KK (2002) The PDZ proteins PICK1, GRIP, and syntenin bind multiple glutamate receptor subtypes. Analysis of PDZ binding motifs. *J Biol Chem* **277**:15221-15224.

Holcomb J, Spellmon N, Trescott L, Sun F, Li C and Yang Z (2014) PDZ Structure and Implication in Selective Drug Design against Cystic Fibrosis. *Curr Drug Targets*

Hong CJ and Hsueh YP (2006) CASK associates with glutamate receptor interacting protein and signaling molecules. *Biochem Biophys Res Commun* **351**:771-776.

Howard MA, Elias GM, Elias LA, Swat W and Nicoll RA (2010) The role of SAP97 in synaptic glutamate receptor dynamics. *Proc Natl Acad Sci U S A* **107**:3805-3810.

Hu LA, Chen W, Martin NP, Whalen EJ, Premont RT and Lefkowitz RJ (2003) GIPC interacts with the beta1-adrenergic receptor and regulates beta1-adrenergic receptor-mediated ERK activation. *J Biol Chem* **278**:26295-26301.

Hu LA, Chen W, Premont RT, Cong M and Lefkowitz RJ (2002) G protein-coupled receptor kinase 5 regulates beta 1-adrenergic receptor association with PSD-95. *J Biol Chem* **277**:1607-1613.

Hu LA, Tang Y, Miller WE, Cong M, Lau AG, Lefkowitz RJ and Hall RA (2000) beta 1-adrenergic receptor association with PSD-95. Inhibition of receptor internalization and facilitation of beta 1-adrenergic receptor interaction with N-methyl-D-aspartate receptors. *J Biol Chem* **275**:38659-38666.

Huynh J, Thomas WG, Aguilar MI and Pattenden LK (2009) Role of helix 8 in G protein-coupled receptors based on structure-function studies on the type 1 angiotensin receptor. *Mol Cell Endocrinol* **302**:118-127.

Jaen C and Doupnik CA (2005) Neuronal Kir3.1/Kir3.2a channels coupled to serotonin 1A and muscarinic m2 receptors are differentially modulated by the "short" RGS3 isoform. *Neuropharmacology* **49**:465-476.

Jeanneteau F, Diaz J, Sokoloff P and Griffon N (2004) Interactions of GIPC with dopamine D2, D3 but not D4 receptors define a novel mode of regulation of G protein-coupled receptors. *Mol Biol Cell* **15**:696-705.

Jeyifous O, Waites CL, Specht CG, Fujisawa S, Schubert M, Lin EI, Marshall J, Aoki C, de Silva T, Montgomery JM, Garner CC and Green WN (2009) SAP97 and CASK mediate sorting of NMDA receptors through a previously unknown secretory pathway. *Nat Neurosci* **12**:1011-1019.

Joberty G, Petersen C, Gao L and Macara IG (2000) The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat Cell Biol* **2**:531-539.

## MOL Manuscript # 98509

Jones KA, Srivastava DP, Allen JA, Strachan RT, Roth BL and Penzes P (2009) Rapid modulation of spine morphology by the 5-HT<sub>2A</sub> serotonin receptor through kalirin-7 signaling. *Proc Natl Acad Sci U S A* **106**:19575-19580.

Joubert L, Hanson B, Barthet G, Sebben M, Claeysen S, Hong W, Marin P, Dumuis A and Bockaert J (2004) New sorting nexin (SNX27) and NHERF specifically interact with the 5-HT<sub>4a</sub> receptor splice variant: roles in receptor targeting. *J Cell Sci* **117**:5367-5379.

Karakoula A, Tovey SC, Brighton PJ and Willars GB (2008) Lack of receptor-selective effects of either RGS2, RGS3 or RGS4 on muscarinic M<sub>3</sub>- and gonadotropin-releasing hormone receptor-mediated signalling through G $\alpha$  q/11. *Eur J Pharmacol* **587**:16-24.

Katsushima Y, Sato T, Yamada C, Ito M, Suzuki Y, Ogawa E, Sukegawa I, Sukegawa J, Fukunaga K and Yanagisawa T (2013) Interaction of PICK1 with C-terminus of growth hormone-releasing hormone receptor (GHRHR) modulates trafficking and signal transduction of human GHRHR. *J Pharmacol Sci* **122**:193-204.

Kelker MS, Dancheck B, Ju T, Kessler RP, Hudak J, Nairn AC and Peti W (2007) Structural basis for spinophilin-neurabin receptor interaction. *Biochemistry* **46**:2333-2344.

Kim E and Sheng M (2004) PDZ domain proteins of synapses. *Nat Rev Neurosci* **5**:771-781.

Kim JK, Kwon O, Kim J, Kim EK, Park HK, Lee JE, Kim KL, Choi JW, Lim S, Seok H, Lee-Kwon W, Choi JH, Kang BH, Kim S, Ryu SH and Suh PG (2012) PDZ domain-containing 1 (PDZK1) protein regulates phospholipase C-beta3 (PLC-beta3)-specific activation of somatostatin by forming a ternary complex with PLC-beta3 and somatostatin receptors. *J Biol Chem* **287**:21012-21024.

Kitano J, Kimura K, Yamazaki Y, Soda T, Shigemoto R, Nakajima Y and Nakanishi S (2002) Tamalin, a PDZ domain-containing protein, links a protein complex formation of group 1 metabotropic glutamate receptors and the guanine nucleotide exchange factor cytohesins. *J Neurosci* **22**:1280-1289.

Kitano J, Yamazaki Y, Kimura K, Masukado T, Nakajima Y and Nakanishi S (2003) Tamalin is a scaffold protein that interacts with multiple neuronal proteins in distinct modes of protein-protein association. *J Biol Chem* **278**:14762-14768.

Koliwer J, Park M, Bauch C, von Zastrow M and Kreienkamp HJ (2015) The Golgi-associated PDZ Domain Protein PIST/GOPC Stabilizes the beta1-Adrenergic Receptor in Intracellular Compartments after Internalization. *J Biol Chem* **290**:6120-6129.

Kornau HC, Schenker LT, Kennedy MB and Seeburg PH (1995) Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* **269**:1737-1740.

Koroll M, Rathjen FG and Volkmer H (2001) The neural cell recognition molecule neurofascin interacts with syntenin-1 but not with syntenin-2, both of which reveal self-associating activity. *J Biol Chem* **276**:10646-10654.

Krapivinsky G, Medina I, Krapivinsky L, Gapon S and Clapham DE (2004) SynGAP-MUPP1-CaMKII synaptic complexes regulate p38 MAP kinase activity and NMDA receptor-dependent synaptic AMPA receptor potentiation. *Neuron* **43**:563-574.

Kuang YQ, Pang W, Zheng YT and Dupre DJ (2012) NHERF1 regulates gp120-induced internalization and signaling by CCR5, and HIV-1 production. *Eur J Immunol* **42**:299-310.

## MOL Manuscript # 98509

Kuiperij HB, de Rooij J, Rehmann H, van Triest M, Wittinghofer A, Bos JL and Zwartkuis FJ (2003) Characterisation of PDZ-GEFs, a family of guanine nucleotide exchange factors specific for Rap1 and Rap2. *Biochim Biophys Acta* **1593**:141-149.

Kuiperij HB, Rehmann H and Zwartkuis FJ (2006) Biochemistry of the Rap-specific guanine nucleotide exchange factors PDZ-GEF1 and -2. *Methods Enzymol* **407**:174-186.

Kurogi M, Nagatomo K, Kubo Y and Saitoh O (2009) Effects of spinophilin on the function of RGS8 regulating signals from M2 and M3-mAChRs. *Neuroreport* **20**:1134-1139.

Lach A, Grzybek M, Heger E, Korycka J, Wolny M, Kubiak J, Kolondra A, Boguslawska DM, Augoff K, Majkowski M, Podkalicka J, Kaczor J, Stefanko A, Kuliczowski K and Sikorski AF (2012) Palmitoylation of MPP1 (membrane-palmitoylated protein 1)/p55 is crucial for lateral membrane organization in erythroid cells. *J Biol Chem* **287**:18974-18984.

Ladds G, Goddard A, Hill C, Thornton S and Davey J (2007) Differential effects of RGS proteins on G alpha(q) and G alpha(11) activity. *Cell Signal* **19**:103-113.

Lauffer BE, Melero C, Temkin P, Lei C, Hong W, Kortemme T and von Zastrow M (2010) SNX27 mediates PDZ-directed sorting from endosomes to the plasma membrane. *J Cell Biol* **190**:565-574.

Lee HJ and Zheng JJ (2010) PDZ domains and their binding partners: structure, specificity, and modification. *Cell Commun Signal* **8**:8-811X-8-8.

Li JG, Chen C and Liu-Chen LY (2002) Ezrin-radixin-moesin-binding phosphoprotein-50/Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor (EBP50/NHERF) blocks U50,488H-induced down-regulation of the human kappa opioid receptor by enhancing its recycling rate. *J Biol Chem* **277**:27545-27552.

Liew CW, Vockel M, Glassmeier G, Brandner JM, Fernandez-Ballester GJ, Schwarz JR, Schulz S, Buck F, Serrano L, Richter D and Kreienkamp HJ (2009) Interaction of the human somatostatin receptor 3 with the multiple PDZ domain protein MUPP1 enables somatostatin to control permeability of epithelial tight junctions. *FEBS Lett* **583**:49-54.

Lin EI, Jeyifous O and Green WN (2013) CASK regulates SAP97 conformation and its interactions with AMPA and NMDA receptors. *J Neurosci* **33**:12067-12076.

Lin SH, Arai AC, Wang Z, Nothacker HP and Civelli O (2001) The carboxyl terminus of the prolactin-releasing peptide receptor interacts with PDZ domain proteins involved in alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor clustering. *Mol Pharmacol* **60**:916-923.

Liu TF, Kandala G and Setaluri V (2001) PDZ domain protein GIPC interacts with the cytoplasmic tail of melanosomal membrane protein gp75 (tyrosinase-related protein-1). *J Biol Chem* **276**:35768-35777.

Liu W, Yuen EY, Allen PB, Feng J, Greengard P and Yan Z (2006) Adrenergic modulation of NMDA receptors in prefrontal cortex is differentially regulated by RGS proteins and spinophilin. *Proc Natl Acad Sci U S A* **103**:18338-18343.

Lu R, Chen Y, Cottingham C, Peng N, Jiao K, Limbird LE, Wyss JM and Wang Q (2010) Enhanced hypotensive, bradycardic, and hypnotic responses to alpha2-adrenergic agonists in spinophilin-null mice are accompanied by increased G protein coupling to the alpha2A-adrenergic receptor. *Mol Pharmacol* **78**:279-286.

## MOL Manuscript # 98509

Luyten A, Mortier E, Van Campenhout C, Taelman V, Degeest G, Wuytens G, Lambaerts K, David G, Bellefroid EJ and Zimmermann P (2008) The postsynaptic density 95/disc-large/zona occludens protein syntenin directly interacts with frizzled 7 and supports noncanonical Wnt signaling. *Mol Biol Cell* **19**:1594-1604.

Lyssand JS, DeFino MC, Tang XB, Hertz AL, Feller DB, Wacker JL, Adams ME and Hague C (2008) Blood pressure is regulated by an alpha1D-adrenergic receptor/dystrophin signalosome. *J Biol Chem* **283**:18792-18800.

Lyssand JS, Lee KS, DeFino M, Adams ME and Hague C (2011) Syntrophin isoforms play specific functional roles in the alpha1D-adrenergic receptor/DAPC signalosome. *Biochem Biophys Res Commun* **412**:596-601.

Lyssand JS, Whiting JL, Lee KS, Kastl R, Wacker JL, Bruchas MR, Miyatake M, Langeberg LK, Chavkin C, Scott JD, Gardner RG, Adams ME and Hague C (2010) Alpha-dystrobrevin-1 recruits alpha-catulin to the alpha1D-adrenergic receptor/dystrophin-associated protein complex signalosome. *Proc Natl Acad Sci U S A* **107**:21854-21859.

Ma P, Cierniewska A, Signarvic R, Cieslak M, Kong H, Sinnamon AJ, Neubig RR, Newman DK, Stalker TJ and Brass LF (2012) A newly identified complex of spinophilin and the tyrosine phosphatase, SHP-1, modulates platelet activation by regulating G protein-dependent signaling. *Blood* **119**:1935-1945.

Macara IG (2004) Par proteins: partners in polarization. *Curr Biol* **14**:R160-2.

Madsen KL, Thorsen TS, Rahbek-Clemmensen T, Eriksen J and Gether U (2012) Protein interacting with C kinase 1 (PICK1) reduces reinsertion rates of interaction partners sorted to Rab11-dependent slow recycling pathway. *J Biol Chem* **287**:12293-12308.

Magalhaes AC, Dunn H and Ferguson SS (2012) Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. *Br J Pharmacol* **165**:1717-1736.

Magalhaes AC, Holmes KD, Dale LB, Comps-Agrar L, Lee D, Yadav PN, Drysdale L, Poulter MO, Roth BL, Pin JP, Anisman H and Ferguson SS (2010) CRF receptor 1 regulates anxiety behavior via sensitization of 5-HT2 receptor signaling. *Nat Neurosci* **13**:622-629.

Mahon MJ (2012) The parathyroid hormone receptorsome and the potential for therapeutic intervention. *Curr Drug Targets* **13**:116-128.

Mahon MJ, Donowitz M, Yun CC and Segre GV (2002) Na(+)/H(+) exchanger regulatory factor 2 directs parathyroid hormone 1 receptor signalling. *Nature* **417**:858-861.

Medlin MD, Staus DP, Dubash AD, Taylor JM and Mack CP (2010) Sphingosine 1-phosphate receptor 2 signals through leukemia-associated RhoGEF (LARG), to promote smooth muscle cell differentiation. *Arterioscler Thromb Vasc Biol* **30**:1779-1786.

Mukherjee K, Sharma M, Urlaub H, Bourenkov GP, Jahn R, Sudhof TC and Wahl MC (2008) CASK Functions as a Mg<sup>2+</sup>-independent neurexin kinase. *Cell* **133**:328-339.

Nakagawa T and Asahi M (2013) Beta1-Adrenergic Receptor Recycles Via a Membranous Organelle, Recycling Endosome, by Binding with Sorting Nexin27. *J Membr Biol* **246**:571-579.

Nechiporuk T, Klezovitch O, Nguyen L and Vasioukhin V (2013) Dlg5 maintains apical aPKC and regulates progenitor differentiation during lung morphogenesis. *Dev Biol* **377**:375-384.



## MOL Manuscript # 98509

Neill JD, Duck LW, Sellers JC, Musgrove LC and Kehrl JH (2001) A regulator of G protein signaling, RGS3, inhibits gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) secretion. *BMC Cell Biol* **2**:21.

Neill JD, Duck LW, Sellers JC, Musgrove LC, Scheschonka A, Druey KM and Kehrl JH (1997) Potential role for a regulator of G protein signaling (RGS3) in gonadotropin-releasing hormone (GnRH) stimulated desensitization. *Endocrinology* **138**:843-846.

Neubig RR and Siderovski DP (2002) Regulators of G-protein signalling as new central nervous system drug targets. *Nat Rev Drug Discov* **1**:187-197.

Niethammer M, Kim E and Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *J Neurosci* **16**:2157-2163.

Nisar SP, Cunningham M, Saxena K, Pope RJ, Kelly E and Mundell SJ (2012) Arrestin scaffolds NHERF1 to the P2Y12 receptor to regulate receptor internalization. *J Biol Chem* **287**:24505-24515.

Nishiura H, Nonaka H, Revollo IS, Semba U, Li Y, Ota Y, Irie A, Harada K, Kehrl JH and Yamamoto T (2009) Pro- and anti-apoptotic dual functions of the C5a receptor: involvement of regulator of G protein signaling 3 and extracellular signal-regulated kinase. *Lab Invest* **89**:676-694.

Nooh MM, Chumpia MM, Hamilton TB and Bahouth SW (2014) Sorting of beta1-adrenergic receptors is mediated by pathways that are either dependent on or independent of type I PDZ, protein kinase A (PKA), and SAP97. *J Biol Chem* **289**:2277-2294.

Nooh MM, Naren AP, Kim SJ, Xiang YK and Bahouth SW (2013) SAP97 controls the trafficking and resensitization of the beta-1-adrenergic receptor through its PDZ2 and I3 domains. *PLoS One* **8**:e63379.

Ogawa M, Miyakawa T, Nakamura K, Kitano J, Furushima K, Kiyonari H, Nakayama R, Nakao K, Moriyoshi K and Nakanishi S (2007) Altered sensitivities to morphine and cocaine in scaffold protein tamalin knockout mice. *Proc Natl Acad Sci U S A* **104**:14789-14794.

Oh YS, Jo NW, Choi JW, Kim HS, Seo SW, Kang KO, Hwang JI, Heo K, Kim SH, Kim YH, Kim IH, Kim JH, Banno Y, Ryu SH and Suh PG (2004) NHERF2 specifically interacts with LPA2 receptor and defines the specificity and efficiency of receptor-mediated phospholipase C-beta3 activation. *Mol Cell Biol* **24**:5069-5079.

Okamoto PM, Gamby C, Wells D, Fallon J and Vallee RB (2001) Dynamin isoform-specific interaction with the shank/ProSAP scaffolding proteins of the postsynaptic density and actin cytoskeleton. *J Biol Chem* **276**:48458-48465.

Olson PA, Tkatch T, Hernandez-Lopez S, Ulrich S, Ilijic E, Mugnaini E, Zhang H, Bezprozvanny I and Surmeier DJ (2005) G-protein-coupled receptor modulation of striatal CaV1.3 L-type Ca<sup>2+</sup> channels is dependent on a Shank-binding domain. *J Neurosci* **25**:1050-1062.

Oxford GS and Webb CK (2004) GoLoco motif peptides as probes of Galpha subunit specificity in coupling of G-protein-coupled receptors to ion channels. *Methods Enzymol* **390**:437-450.

Paasche JD, Attramadal T, Kristiansen K, Oksvold MP, Johansen HK, Huitfeldt HS, Dahl SG and Attramadal H (2005) Subtype-specific sorting of the ETA endothelin receptor by a novel endocytic recycling signal for G protein-coupled receptors. *Mol Pharmacol* **67**:1581-1590.

## MOL Manuscript # 98509

Pak Y, Pham N and Rotin D (2002) Direct binding of the beta1 adrenergic receptor to the cyclic AMP-dependent guanine nucleotide exchange factor CNrasGEF leads to Ras activation. *Mol Cell Biol* **22**:7942-7952.

Patel M, Kawano T, Suzuki N, Hamakubo T, Karginov AV and Kozasa T (2014) Galpha13/PDZ-RhoGEF/RhoA signaling is essential for gastrin-releasing peptide receptor-mediated colon cancer cell migration. *Mol Pharmacol* **86**:252-262.

Paquet M, Asay MJ, Fam SR, Inuzuka H, Castleberry AM, Oller H, Smith Y, Yun CC, Traynelis SF and Hall RA (2006) The PDZ scaffold NHERF-2 interacts with mGluR5 and regulates receptor activity. *J Biol Chem* **281**:29949-29961.

Pfreimer M, Vatter P, Langer T, Wieland T, Gierschik P and Moepps B (2012) LARG links histamine-H1-receptor-activated Gq to Rho-GTPase-dependent signaling pathways. *Cell Signal* **24**:652-663.

Porras G, Berthet A, Dehay B, Li Q, Ladepeche L, Normand E, Dovero S, Martinez A, Doudnikoff E, Martin-Negrier ML, Chuan Q, Bloch B, Choquet D, Boue-Grabot E, Groc L and Bezard E (2012) PSD-95 expression controls L-DOPA dyskinesia through dopamine D1 receptor trafficking. *J Clin Invest* **122**:3977-3989.

Potenza MN, Gold SJ, Roby-Shemkowitz A, Lerner MR and Nestler EJ (1999) Effects of regulators of G protein-signaling proteins on the functional response of the mu-opioid receptor in a melanophore-based assay. *J Pharmacol Exp Ther* **291**:482-491.

Reid HM, Turner EC, Mulvaney EP, Hyland PB, McLean C and Kinsella BT (2012) Interaction of the human prostacyclin receptor and the NHERF4 family member intestinal and kidney enriched PDZ protein (IKEPP). *Biochim Biophys Acta* **1823**:1998-2012.

Richman JG, Brady AE, Wang Q, Hensel JL, Colbran RJ and Limbird LE (2001) Agonist-regulated Interaction between alpha2-adrenergic receptors and spinophilin. *J Biol Chem* **276**:15003-15008.

Rochdi MD and Parent JL (2003) Galphaq-coupled receptor internalization specifically induced by Galphaq signaling. Regulation by EBP50. *J Biol Chem* **278**:17827-17837.

Rodriguez-Munoz M, de la Torre-Madrid E, Sanchez-Blazquez P, Wang JB and Garzon J (2008) NMDAR-nNOS generated zinc recruits PKCgamma to the HINT1-RGS17 complex bound to the C terminus of Mu-opioid receptors. *Cell Signal* **20**:1855-1864.

Ruiz de Azua I, Nakajima K, Rossi M, Cui Y, Jou W, Gavrilova O and Wess J (2012) Spinophilin as a novel regulator of M3 muscarinic receptor-mediated insulin release in vitro and in vivo. *FASEB J* **26**:4275-4286.

Sala C, Futai K, Yamamoto K, Worley PF, Hayashi Y and Sheng M (2003) Inhibition of dendritic spine morphogenesis and synaptic transmission by activity-inducible protein Homer1a. *J Neurosci* **23**:6327-6337.

Sala C, Roussignol G, Meldolesi J and Fagni L (2005) Key role of the postsynaptic density scaffold proteins Shank and Homer in the functional architecture of Ca<sup>2+</sup> homeostasis at dendritic spines in hippocampal neurons. *J Neurosci* **25**:4587-4592.

Sampedro MN, Bussineau CM and Cotman CW (1981) Postsynaptic density antigens: preparation and characterization of an antiserum against postsynaptic densities. *J Cell Biol* **90**:675-686.

## MOL Manuscript # 98509

Sanchez-Blazquez P, Rodriguez-Munoz M, Bailon C and Garzon J (2012) GPCRs promote the release of zinc ions mediated by nNOS/NO and the redox transducer RGS22 protein. *Antioxid Redox Signal* **17**:1163-1177.

Sanchez-Blazquez P, Rodriguez-Munoz M and Garzon J (2010) Mu-opioid receptors transiently activate the Akt-nNOS pathway to produce sustained potentiation of PKC-mediated NMDAR-CaMKII signaling. *PLoS One* **5**:e11278.

Scheschonka A, Dessauer CW, Sinnarajah S, Chidiac P, Shi CS and Kehrl JH (2000) RGS3 is a GTPase-activating protein for g(ialpha) and g(qalpha) and a potent inhibitor of signaling by GTPase-deficient forms of g(qalpha) and g(11alpha). *Mol Pharmacol* **58**:719-728.

Schmid CL and Bohn LM (2010) Serotonin, but not N-methyltryptamines, activates the serotonin 2A receptor via a ss-arrestin2/Src/Akt signaling complex in vivo. *J Neurosci* **30**:13513-13524.

Scudiero I, Vito P and Stilo R (2014) The three CARMA sisters: so different, so similar: a portrait of the three CARMA proteins and their involvement in human disorders. *J Cell Physiol* **229**:990-997.

Sensoy O and Weinstein H (2015) A mechanistic role of Helix 8 in GPCRs: Computational modeling of the dopamine D2 receptor interaction with the GIPC1-PDZ-domain. *Biochim Biophys Acta* **1848**:976-983.

Sheng M and Sala C (2001) PDZ domains and the organization of supramolecular complexes. *Annu Rev Neurosci* **24**:1-29.

Shiratsuchi T, Futamura M, Oda K, Nishimori H, Nakamura Y and Tokino T (1998) Cloning and characterization of BAI-associated protein 1: a PDZ domain-containing protein that interacts with BAI1. *Biochem Biophys Res Commun* **247**:597-604.

Smith FD, Oxford GS and Milgram SL (1999) Association of the D2 dopamine receptor third cytoplasmic loop with spinophilin, a protein phosphatase-1-interacting protein. *J Biol Chem* **274**:19894-19900.

Snow BE, Hall RA, Krumins AM, Brothers GM, Bouchard D, Brothers CA, Chung S, Mangion J, Gilman AG, Lefkowitz RJ and Siderovski DP (1998) GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. *J Biol Chem* **273**:17749-17755.

Songyang Z, Fanning AS, Fu C, Xu J, Marfatia SM, Chishti AH, Crompton A, Chan AC, Anderson JM and Cantley LC (1997) Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* **275**:73-77.

Stephenson JR, Paavola KJ, Schaefer SA, Kaur B, Van Meir EG and Hall RA (2013) Brain-specific angiogenesis inhibitor-1 signaling, regulation, and enrichment in the postsynaptic density. *J Biol Chem* **288**:22248-22256.

Subramanian H, Gupta K and Ali H (2012) Roles for NHERF1 and NHERF2 on the regulation of C3a receptor signaling in human mast cells. *PLoS One* **7**:e51355.

Sugi T, Oyama T, Muto T, Nakanishi S, Morikawa K and Jingami H (2007) Crystal structures of autoinhibitory PDZ domain of Tamalin: implications for metabotropic glutamate receptor trafficking regulation. *EMBO J* **26**:2192-2205.

## MOL Manuscript # 98509

Suh YH, Pelkey KA, Lavezzari G, Roche PA, Huganir RL, McBain CJ and Roche KW (2008) Corequirement of PICK1 binding and PKC phosphorylation for stable surface expression of the metabotropic glutamate receptor mGluR7. *Neuron* **58**:736-748.

Sun P, Wang J, Gu W, Cheng W, Jin GZ, Friedman E, Zheng J and Zhen X (2009) PSD-95 regulates D1 dopamine receptor resensitization, but not receptor-mediated Gs-protein activation. *Cell Res* **19**:612-624.

Sun Q and Turrigiano GG (2011) PSD-95 and PSD-93 play critical but distinct roles in synaptic scaling up and down. *J Neurosci* **31**:6800-6808.

te Velthuis AJ, Admiraal JF and Bagowski CP (2007) Molecular evolution of the MAGUK family in metazoan genomes. *BMC Evol Biol* **7**:129.

Temkin P, Lauffer B, Jager S, Cimermancic P, Krogan NJ and von Zastrow M (2011) SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. *Nat Cell Biol* **13**:715-721.

Thurner P, Gsandtner I, Kudlacek O, Choquet D, Nanoff C, Freissmuth M and Zezula J (2014) A two-state model for the diffusion of the A2A adenosine receptor in hippocampal neurons: agonist-induced switch to slow mobility is modified by synapse-associated protein 102 (SAP102). *J Biol Chem* **289**:9263-9274.

Tobaben S, Sudhof TC and Stahl B (2000) The G protein-coupled receptor CL1 interacts directly with proteins of the Shank family. *J Biol Chem* **275**:36204-36210.

Tonikian R, Zhang Y, Sazinsky SL, Currell B, Yeh JH, Reva B, Held HA, Appleton BA, Evangelista M, Wu Y, Xin X, Chan AC, Seshagiri S, Lasky LA, Sander C, Boone C, Bader GD and Sidhu SS (2008) A specificity map for the PDZ domain family. *PLoS Biol* **6**:e239.

Tovey SC and Willars GB (2004) Single-cell imaging of intracellular Ca<sup>2+</sup> and phospholipase C activity reveals that RGS 2, 3, and 4 differentially regulate signaling via the Galphaq/11-linked muscarinic M3 receptor. *Mol Pharmacol* **66**:1453-1464.

Trejo J (2005) Internal PDZ ligands: novel endocytic recycling motifs for G protein-coupled receptors. *Mol Pharmacol* **67**:1388-1390.

Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P, Doan A, Aakalu VK, Lanahan AA, Sheng M and Worley PF (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**:583-592.

Turner EC, Mulvaney EP, Reid HM and Kinsella BT (2011) Interaction of the human prostacyclin receptor with the PDZ adapter protein PDZK1: role in endothelial cell migration and angiogenesis. *Mol Biol Cell* **22**:2664-2679.

Vaccaro P and Dente L (2002) PDZ domains: troubles in classification. *FEBS Lett* **512**:345-349.

Valente C, Polishchuk R and De Matteis MA (2010) Rab6 and myosin II at the cutting edge of membrane fission. *Nat Cell Biol* **12**:635-638.

Varsano T, Taupin V, Guo L, Bateria OY, Jr and Farquhar MG (2012) The PDZ protein GIPC regulates trafficking of the LPA1 receptor from APPL signaling endosomes and attenuates the cell's response to LPA. *PLoS One* **7**:e49227.

Verpelli C, Dvoretzkova E, Vicidomini C, Rossi F, Chiappalone M, Schoen M, Di Stefano B, Mantegazza R, Broccoli V, Bockers TM, Dityatev A and Sala C (2011) Importance of Shank3

## MOL Manuscript # 98509

protein in regulating metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses. *J Biol Chem* **286**:34839-34850.

Vockel M, Breitenbach U, Kreienkamp HJ and Brandner JM (2010) Somatostatin regulates tight junction function and composition in human keratinocytes. *Exp Dermatol* **19**:888-894.

Walther C, Caetano FA, Dunn HA and Ferguson SS (2015) PDZK1/NHERF3 Differentially Regulates Corticotropin-releasing Factor Receptor 1 and Serotonin 2A Receptor Signaling and Endocytosis. *Cell Signal*

Wang B, Ardura JA, Romero G, Yang Y, Hall RA and Friedman PA (2010) Na/H exchanger regulatory factors control parathyroid hormone receptor signaling by facilitating differential activation of G(alpha) protein subunits. *J Biol Chem* **285**:26976-26986.

Wang B, Bisello A, Yang Y, Romero GG and Friedman PA (2007) NHERF1 regulates parathyroid hormone receptor membrane retention without affecting recycling. *J Biol Chem* **282**:36214-36222.

Wang B, Yang Y, Abou-Samra AB and Friedman PA (2009) NHERF1 regulates parathyroid hormone receptor desensitization: interference with beta-arrestin binding. *Mol Pharmacol* **75**:1189-1197.

Wang Q and Limbird LE (2002) Regulated interactions of the alpha 2A adrenergic receptor with spinophilin, 14-3-3zeta, and arrestin 3. *J Biol Chem* **277**:50589-50596.

Wang Q, Liu M, Kozasa T, Rothstein JD, Sternweis PC and Neubig RR (2004) Thrombin and lysophosphatidic acid receptors utilize distinct rhoGEFs in prostate cancer cells. *J Biol Chem* **279**:28831-28834.

Wang Q, Liu M, Mullah B, Siderovski DP and Neubig RR (2002) Receptor-selective effects of endogenous RGS3 and RGS5 to regulate mitogen-activated protein kinase activation in rat vascular smooth muscle cells. *J Biol Chem* **277**:24949-24958.

Wang Q, Zhao J, Brady AE, Feng J, Allen PB, Lefkowitz RJ, Greengard P and Limbird LE (2004) Spinophilin blocks arrestin actions in vitro and in vivo at G protein-coupled receptors. *Science* **304**:1940-1944.

Wang SH, Celic I, Choi SY, Riccomagno M, Wang Q, Sun LO, Mitchell SP, Vasioukhin V, Huganir RL and Kolodkin AL (2014) Dlg5 regulates dendritic spine formation and synaptogenesis by controlling subcellular N-cadherin localization. *J Neurosci* **34**:12745-12761.

Wang X, Zeng W, Soyombo AA, Tang W, Ross EM, Barnes AP, Milgram SL, Penninger JM, Allen PB, Greengard P and Muallem S (2005) Spinophilin regulates Ca<sup>2+</sup> signalling by binding the N-terminal domain of RGS2 and the third intracellular loop of G-protein-coupled receptors. *Nat Cell Biol* **7**:405-411.

Wente W, Stroh T, Beaudet A, Richter D and Kreienkamp HJ (2005) Interactions with PDZ domain proteins PIST/GOPC and PDZK1 regulate intracellular sorting of the somatostatin receptor subtype 5. *J Biol Chem* **280**:32419-32425.

Wheeler DS, Barrick SR, Grubisha MJ, Brufsky AM, Friedman PA and Romero G (2011) Direct interaction between NHERF1 and Frizzled regulates beta-catenin signaling. *Oncogene* **30**:32-42.

Wheeler D, Garrido JL, Bisello A, Kim YK, Friedman PA and Romero G (2008) Regulation of parathyroid hormone type 1 receptor dynamics, traffic, and signaling by the Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor-1 in rat osteosarcoma ROS 17/2.8 cells. *Mol Endocrinol* **22**:1163-1170.

## MOL Manuscript # 98509

Xia Z, Gray JA, Compton-Toth BA and Roth BL (2003) A direct interaction of PSD-95 with 5-HT<sub>2A</sub> serotonin receptors regulates receptor trafficking and signal transduction. *J Biol Chem* **278**:21901-21908.

Xu J, Chen Y, Lu R, Cottingham C, Jiao K and Wang Q (2008) Protein kinase A phosphorylation of spinophilin modulates its interaction with the alpha 2A-adrenergic receptor (AR) and alters temporal properties of alpha 2AAR internalization. *J Biol Chem* **283**:14516-14523.

Xu J, Paquet M, Lau AG, Wood JD, Ross CA and Hall RA (2001) beta 1-adrenergic receptor association with the synaptic scaffolding protein membrane-associated guanylate kinase inverted-2 (MAGI-2). Differential regulation of receptor internalization by MAGI-2 and PSD-95. *J Biol Chem* **276**:41310-41317.

Xu W (2011) PSD-95-like membrane associated guanylate kinases (PSD-MAGUKs) and synaptic plasticity. *Curr Opin Neurobiol* **21**:306-312.

Xu XZ, Choudhury A, Li X and Montell C (1998) Coordination of an array of signaling proteins through homo- and heteromeric interactions between PDZ domains and target proteins. *J Cell Biol* **142**:545-555.

Yamada T, Ohoka Y, Kogo M and Inagaki S (2005) Physical and functional interactions of the lysophosphatidic acid receptors with PDZ domain-containing Rho guanine nucleotide exchange factors (RhoGEFs). *J Biol Chem* **280**:19358-19363.

Yang X, Zheng J, Xiong Y, Shen H, Sun L, Huang Y, Sun C, Li Y and He J (2010) Beta-2 adrenergic receptor mediated ERK activation is regulated by interaction with MAGI-3. *FEBS Lett* **584**:2207-2212.

Ying Z, Jin L, Palmer T and Webb RC (2006) Angiotensin II up-regulates the leukemia-associated Rho guanine nucleotide exchange factor (RhoGEF), a regulator of G protein signaling domain-containing RhoGEF, in vascular smooth muscle cells. *Mol Pharmacol* **69**:932-940.

Zhang CS, Bertaso F, Eulenburg V, Lerner-Natoli M, Herin GA, Bauer L, Bockaert J, Fagni L, Betz H and Scheschonka A (2008) Knock-in mice lacking the PDZ-ligand motif of mGluR7a show impaired PKC-dependent autoinhibition of glutamate release, spatial working memory deficits, and increased susceptibility to pentylentetrazol. *J Neurosci* **28**:8604-8614.

Zhang H, Wang D, Sun H, Hall RA and Yun CC (2007) MAGI-3 regulates LPA-induced activation of Erk and RhoA. *Cell Signal* **19**:261-268.

Zhang J, Cheng S, Xiong Y, Ma Y, Luo D, Jeromin A, Zhang H and He J (2008) A novel association of mGluR1a with the PDZ scaffold protein CAL modulates receptor activity. *FEBS Lett* **582**:4117-4124.

Zhang J, Vinuela A, Neely MH, Hallett PJ, Grant SG, Miller GM, Isacson O, Caron MG and Yao WD (2007) Inhibition of the dopamine D1 receptor signaling by PSD-95. *J Biol Chem* **282**:15778-15789.

Zhang J, Xu TX, Hallett PJ, Watanabe M, Grant SG, Isacson O and Yao WD (2009) PSD-95 uncouples dopamine-glutamate interaction in the D1/PSD-95/NMDA receptor complex. *J Neurosci* **29**:2948-2960.

Zhou YW, Oak SA, Senogles SE and Jarrett HW (2005) Laminin-alpha1 globular domains 3 and 4 induce heterotrimeric G protein binding to alpha-syntrophin's PDZ domain and alter intracellular Ca<sup>2+</sup> in muscle. *Am J Physiol Cell Physiol* **288**:C377-88.

**MOL Manuscript # 98509**

**Footnotes:**

This work is supported by a Canadian Institutes of Health Research grant [MOP-62738] to S. S. G. F.

## MOL Manuscript # 98509

**Figure 1: Molecular topology of protein-protein interaction domains found in MAGUK family PDZ proteins.** CaMKII, Ca<sup>2+</sup>/Calmodulin-dependent kinase domain; CARD, Caspase activation and recruitment domain; CC, coiled-coiled domain; GK, Guanylate kinase-like domain; L27, L27 domain; PDZ, PDZ domain; SH3, Src homology 3 domain WW, tryptophan-tryptophan domain.

**Figure 2: Molecular topology of other PDZ domain-containing proteins that interact with GPCRs.** ABD, actin binding domain; ANK, ankyrin repeat domain; AH, Arfaptin homology domain; C2, C2 domain; CC, coiled-coiled domain; cNBD, Cyclic nucleotide binding domain; EBD, Ezin-binding domain; FAD, FAD-binding domain; FDX, flavodoxin-like domain; G, Golocco motif; GEF-CD, Ras GEF catalytic domain; GEF-N, Ras-like GEF, N-terminal domain; L27, L27 domain; PDZ, PDZ domain; PB1, Phox/Bem1 domain; PH, pleckstrin homology domain; PH<sub>A/B</sub>, interrupted pleckstrin homology domain; PP1, PP1-binding domain; PTB, phosphotyrosine-binding domain; PX, Phox-homolgy domain; RA, Ras association domain; RB, Ras-binding domain; RGS, RGS domain; RGSL, RGS-like domain; RhoGEF, RhoGEF domain; SAM, Sterile alpha motif; SU, syntrophin unique domain; SH3, Src homology 3 domain.



**MOL Manuscript # 98509**

**Table 1:** Effect of PDZ proteins on GPCR trafficking

PDZ Protein	Trafficking Function	GPCR	Reference
<b>PSD-95</b>	↓ endocytosis ↑ recycling ↑ membrane localization ↑ endocytosis	β <sub>1</sub> AR, 5-HT <sub>2A</sub> AR D <sub>1</sub> R GPR30 5-HT <sub>2C</sub> R, D <sub>1</sub> R	Hu et al., 2000; Xia et al 2003 Sun et al., 2009 Akama et al., 2013; Broselid et al., 2014 Gavarini et al., 2006; Zhang et al., 2007
<b>SAP97</b>	↓ endocytosis ↑ recycling	CRFR1, 5-HT <sub>2A</sub> AR β <sub>1</sub> AR	Dunn et al., 2013; Dunn et al., 2014 Gardner et al., 2007
<b>SAP102</b>	↓ mobility	A <sub>2A</sub> AR	Thurner et al., 2014
<b>MPP3</b>	↑ membrane localization	5-HT <sub>2C</sub> R	Gavarini et al., 2006
<b>MAGI-2</b>	↓ endocytosis ↑ endocytosis ↑ membrane localization	VPAC1 β <sub>1</sub> AR mGluR1a	Gee et al., 2009 Xu et al., 2001 Sugi et al., 2007
<b>NHERF1</b>	↓ endocytosis ↑ recycling ↑ membrane localization ↑ microvilli localization ↑ cytoskeletal localization ↑ endocytosis	β <sub>2</sub> AR, TPβ β <sub>2</sub> AR , hκ-OR SSTR5, PTH1R 5-HT <sub>4A</sub> AR Fzd4 CCR5, PAFR, P2Y <sub>12</sub> R	Wang et al., 2007; Rochdi and Parent, 2003 Cao et al., 1999; Li et al., 2002 Bauch et al., 2014; Wheeler et al., 2008 Joubert et al., 2004 Wheeler et al., 2011 Hammad et al., 2010; Dupré et al., 2012; Nisar et al., 2012
<b>PDZK1</b>	↓ endocytosis ↑ membrane localization	5-HT <sub>2A</sub> AR hIPR	Walther et al., 2015 Turner et al., 2011
<b>PDZK2</b>	↑ membrane localization	hIPR	Reid et al., 2012
<b>Shank1</b>	↑ clustering	mGluR5, CL1	Tu et al., 1999; Tobaben et al., 2000
<b>Spinophilin</b>	↓ endocytosis ↑ endocytosis	α <sub>2</sub> AR μOR	Brady et al., 2003 Charlton et al., 2008
<b>MUPP1</b>	↑ membrane localization ↑ tight junction localization	5-HT <sub>2A</sub> AR SSTR3	Jones et al., 2009 Liew et al., 2009
<b>Tamalin</b>	↑ membrane localization ↑ neurite localization	mGluR1 mGluR5	Kitano et al., 2002; Sugi et al., 2007 Kitano et al., 2002
<b>Syntrophins</b>	↑ membrane localization	α <sub>1D</sub> AR	Chen et al., 2006; Lyssand et al., 2008; Lyssand et al., 2011
<b>PICK1</b>	↑ intracellular clustering ↓ recycling	GPR10 GHRHR	Lin et al., 2001; Madsen et al., 2012 Katsushima et al., 2013
<b>Syntenin-1</b>	↑ membrane localization	GPR37	Dunham et al., 2009
<b>SNX27</b>	↑ recycling	β <sub>2</sub> AR, β <sub>1</sub> AR, SSTR5	Lauffer et al., 2010; Temkin et al., 2011; Nakagawa and Asahi, 2013; Bauch et al., 2014
<b>GIPC</b>	↑ endosome/golgi localization ↑ trafficking to early endosome ↑ membrane stability	D <sub>2</sub> R, D <sub>3</sub> R LPA <sub>1</sub> R hLHR	Jeanneteau et al., 2004 Varsano et al., 2012 Hirakawa et al., 2003
<b>CAL</b>	↓ membrane localization ↓ recycling ↑ golgi localization	β <sub>1</sub> AR, SSTR5 β <sub>1</sub> AR SSTR5	He et al., 2004; Koliwer et al., 2015; Bauch et al., 2014 Koliwer et al., 2015 Wente et al., 2005; Bauch et al., 2014

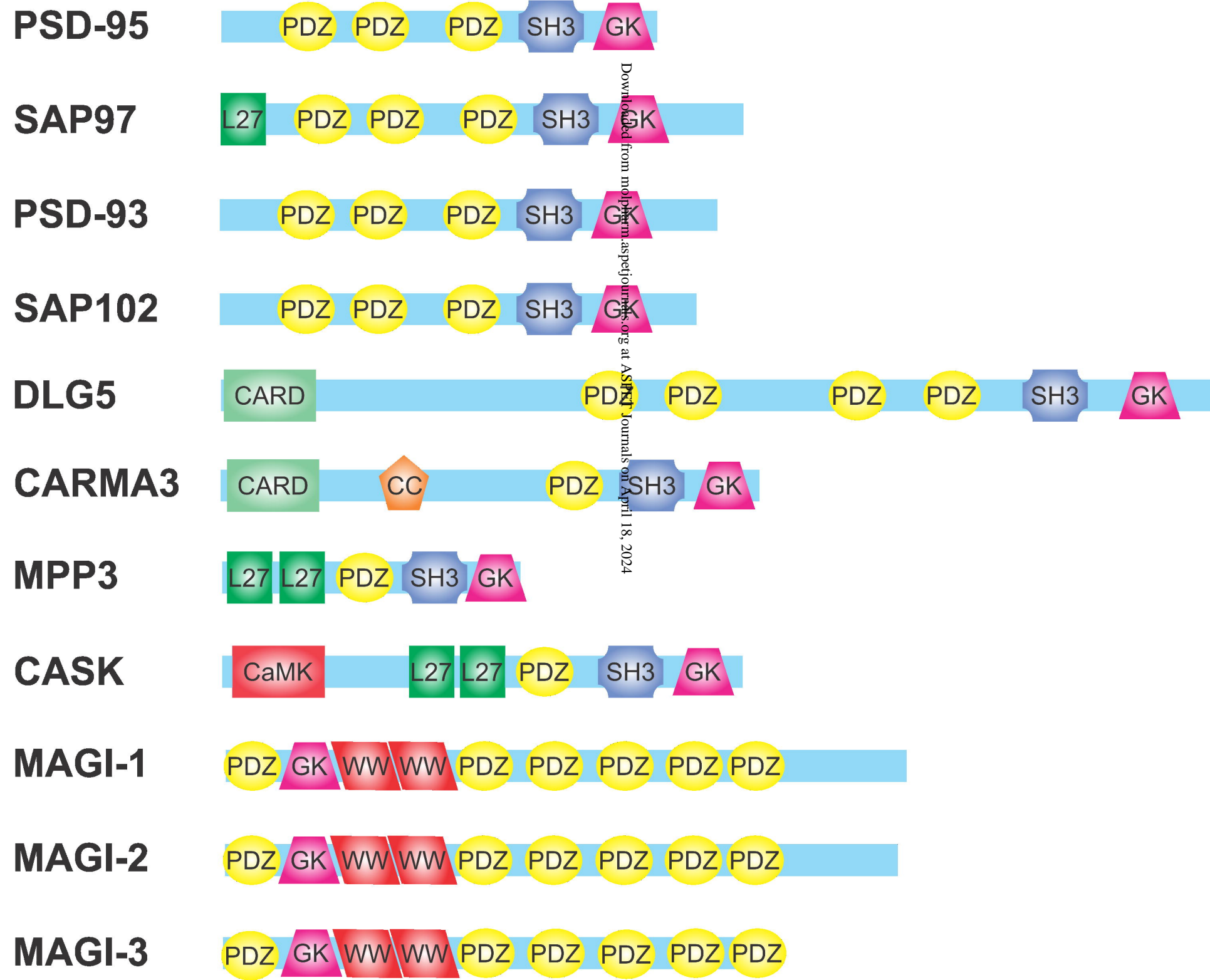
**MOL Manuscript # 98509**

**Table 2: Effect of PDZ proteins on GPCR Signaling**

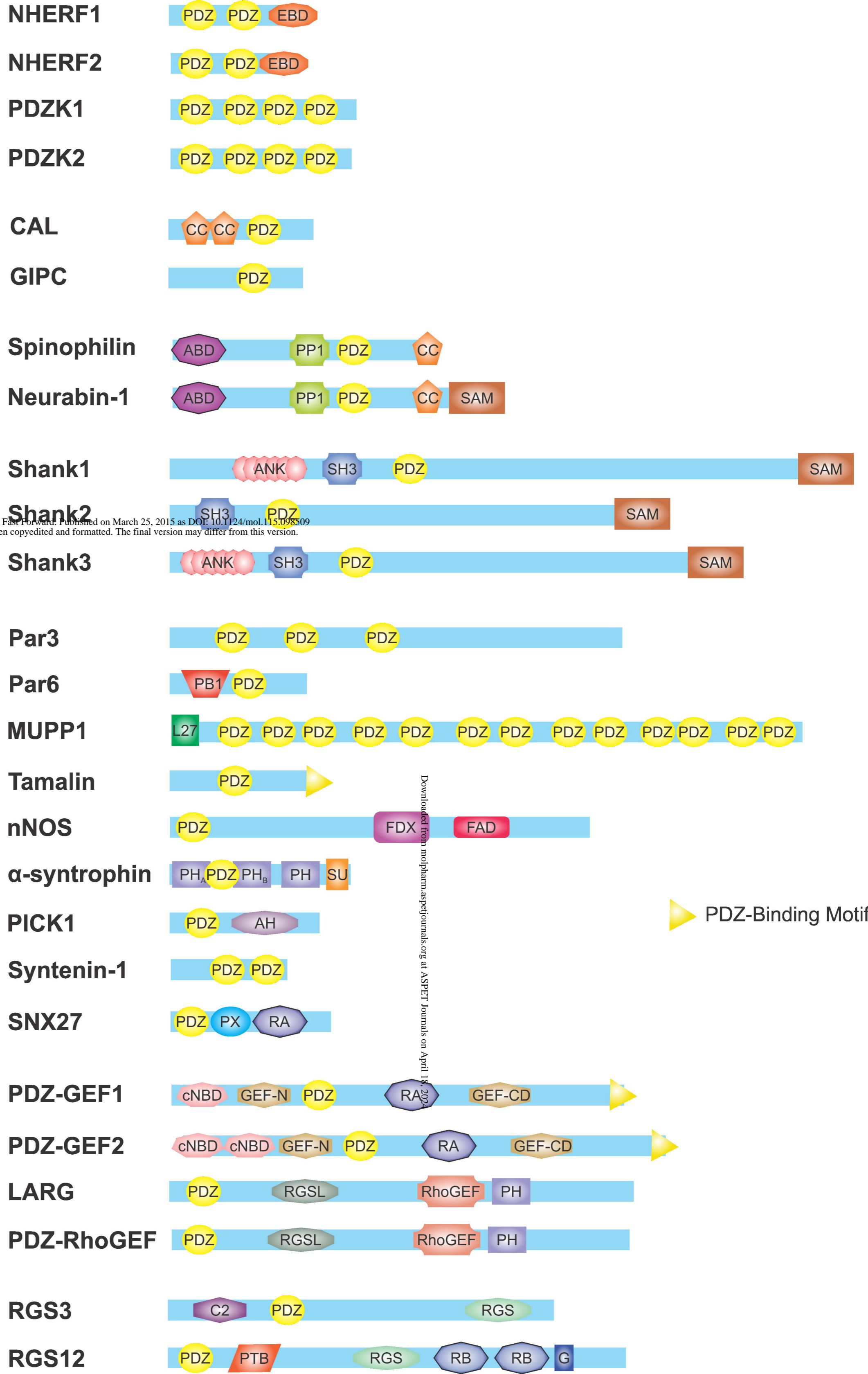
PDZ Protein	Signaling Function	GPCR	Reference
<b>PSD-95</b>	↑ IP <sub>3</sub> ↑ c-fos ↑ desensitization of Ca <sup>2+</sup> ↓ or = cAMP	5-HT <sub>2A</sub> R 5-HT <sub>2C</sub> R 5-HT <sub>2C</sub> R D1R	Xia et al., 2003 Abbas et al., 2009 Gavarini et al., 2006 Zhang et al., 2007; Sun et al., 2009
<b>SAP97</b>	↑ IP <sub>3</sub> ↑ ERK	5-HT <sub>2A</sub> R CRFR1, CRFR2, 5-HT <sub>2A</sub> R	Dunn et al., 2014 Dunn et al., 2013; Dunn et al., 2014
<b>SAP102</b>	↑ ERK	A <sub>2</sub> R	Thurner et al., 2014
<b>MPP3</b>	↓ desensitization of Ca <sup>2+</sup>	5-HT <sub>2C</sub> R	Gavarini et al., 2006
<b>MAGI-2</b>	↓ cAMP	VPAC1	Gee et al., 2009
<b>MAGI-3</b>	↑ ERK ↑ Rho ↓ ERK	BAI1, LPA <sub>2</sub> R LPA <sub>2</sub> R β <sub>1</sub> AR, β <sub>2</sub> AR	Stephenson et al., 2013; Zhang et al., 2007 Zhang et al., 2007 He et al., 2006; Yang et al., 2010
<b>NHERF1</b>	↓ desensitization of cAMP ↓ cAMP ↑ Gα <sub>q</sub> coupling and activation ↑ ERK ↑ FAK, ↑ Rho ↓ β-catenin	PTH1R PTH1R PTH1R CCR5 CCR5 Fzd2/4	Wang et al., 2007 Wheeler et al., 2008; Wang et al., 2007 Wang et al., 2010 Hammad et al., 2010; Kuang et al., 2012 Kuang et al., 2012 Wheeler et al., 2011
<b>NHERF2</b>	↑ Gα <sub>q</sub> coupling and PLC activation ↑ Gα <sub>i</sub> coupling and ↓ AC activation ↑ PLC interaction ↑ IP <sub>3</sub> and ERK ↑ Ca <sup>2+</sup>	PTH1R PTH1R P2Y <sub>1</sub> , LPA <sub>2</sub> R LPA <sub>2</sub> R P2Y <sub>1</sub> , mGluR5	Wang et al., 2010; Mahon et al., 2002 Wang et al., 2010; Mahon et al., 2002 Fam et al., 2005; Choi et al., 2010 Oh et al., 2004 Fam et al., 2005; Paquet et al., 2006
<b>PDZK1</b>	↑ PLC interaction and activation ↑ IP <sub>3</sub> ↑ Ca <sup>2+</sup> ↑ ERK ↑ cAMP	SSTR5 SSTR5, 5-HT <sub>2A</sub> R SSTR5 SSTR5, CRFR1 hIPR	Kim et al., 2012 Kim et al., 2012; Walther et al., 2015 Kim et al., 2012 Turner et al., 2011; Walther et al., 2015 Turner et al., 2011
<b>PDZK2</b>	↑ cAMP	hIPR	Reid et al., 2012
<b>Shank1</b>	↑ Ca <sup>2+</sup> and ERK	mGluR1/5	Sala et al., 2005
<b>Shank3</b>	↑ ERK and CREB phosphorylation	mGluR5	Verpelli et al., 2011
<b>Par3</b>	↑ PLC interaction	B <sub>2</sub> R	Choi et al., 2010
<b>Spinophilin</b>	↓ Ca <sup>2+</sup> ↓ Gα <sub>i</sub> coupling ↑ cAMP ↑ Gα <sub>i</sub> coupling ↓ or ↑ ERK ↑ Gα <sub>i</sub> coupling ↓ ERK	α <sub>2</sub> AR, M <sub>1</sub> R, M <sub>3</sub> R α <sub>2</sub> AR, A <sub>1</sub> R IPR μOR μOR δOR δOR	Wang et al., 2005; Fujii et al., 2008; Kurogi et al., 2009; Ruiz de Azua et al., 2012 Lu et al., 2010; Chen et al., 2012 Ma et al., 2012 Charlton et al., 2008; Fouria et al., 2012 Charlton et al., 2008; Fouria et al., 2012 Fouria et al., 2012 Fouria et al., 2012
<b>MUPP1</b>	↓ Gα <sub>i</sub> coupling ↑ Ca <sup>2+</sup> ↑ Ca <sup>2+</sup> decay	MT <sub>1</sub> R GABA <sub>B</sub> OR2AG1	Guillaume et al., 2008 Balasubramanian et al., 2007 Dooley et al., 2009
<b>nNOS</b>	↑ PKC interaction	μOR, 5-HT <sub>1A</sub> R, 5-HT <sub>2A</sub> R, D <sub>2</sub> R, M <sub>4</sub> R, CB1R	Sanchez-Blazquez et al., 2012
<b>Syntrophins</b>	↑ IP <sub>3</sub> , Ca <sup>2+</sup> and ERK	α <sub>1D</sub> AR	Lyssand et al., 2008; Lyssand et al., 2011
<b>PICK1</b>	↓ cAMP	GHRHR	Katsushimi et al., 2013
<b>Syntenin-1</b>	↑ c-Jun, CDC42, and PKCα	Fzd7	Luyten et al., 2008
<b>GIPC</b>	↓ Gα <sub>i</sub> coupling ↑ Akt ↓ ERK	D <sub>3</sub> R LPA <sub>1</sub> R β <sub>1</sub> AR	Jeannotte et al., 2004 Varsano et al., 2012 Hu et al., 2003
<b>CAL</b>	↓ ERK	mGluR1, β <sub>1</sub> AR	Zhang et al., 2008; Koliwer et al., 2015
<b>PDZ-GEF1</b>	↑ Ras ↑ ERK	β <sub>1</sub> AR PAC1R	Pak et al., 2002 Emery et al., 2013
<b>LARG</b>	↑ Rho	AT <sub>1</sub> R, S1PR2, ET <sub>1</sub> R, M <sub>1</sub> R, GPR132, H1R, TP, MAS1	Booden et al., 2002; Ying et al., 2006; Chiu et al., 2010; Del Galdo et al., 2013; Medlin et al., 2010; Pireimer et al., 2012; Artamonov et al., 2013
<b>PDZ-RhoGEF</b>	↑ Rho	GRPR	Patel et al., 2014
<b>RGS3</b>	↓ Gα <sub>q</sub> and Gα <sub>11</sub> activation ↓ IP <sub>3</sub>  ↓ DAG ↓ Ca <sup>2+</sup> ↓ ERK ↓ Akt ↓ cAMP ↓ Gα <sub>i</sub> -mediated signaling ↑ Gα <sub>s</sub> -mediated signaling	Mam2 M <sub>3</sub> R, GRHR, LHR, FSHR, PAR1  M <sub>3</sub> R, GRHR M <sub>3</sub> R, ET <sub>1</sub> R M <sub>2</sub> R, M <sub>3</sub> R, C5aR, ET <sub>1</sub> R M <sub>2</sub> R, M <sub>3</sub> R LHR, FSHR μOR, D <sub>2</sub> R, β <sub>2</sub> AR β <sub>2</sub> AR	Ladds et al., 2007 Anger et al., 2004; Tovey and Willars, 2004; Karakoula et al., 2008; Castro-Fernandez et al., 2004; Neill et al., 1997; Neill et al., 2001; Castro-Fernandez and Conn, 2002; Castro-Fernandez et al., 2002; Chen et al., 2014; Karakoula et al., 2008 Tovey and Willars, 2004; Dulin et al., 1999 Anger et al., 2007; Wang et al., 2002; Nishura et al., 2009; Dulin et al., 1999 Anger et al., 2007 Castro-Fernandez et al., 2004 Potenza et al., 1999; Chakir et al., 2011 Chakir et al., 2011

**MOL Manuscript # 98509**

# Figure 1



# Figure 2



Molecular Pharmacology Fast Forward. Published on March 25, 2015 as DOI: 10.1124/mol.115.098509  
 This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from molpharm.aspetjournals.org at ASPET Journals on April 18, 2024

▶ PDZ-Binding Motif