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Apoptotic cell recognition receptors and scavenger receptors

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Summary

Phosphatidylserine recognition receptors are a highly diverse set of receptors grouped by their ability to recognize the 'eat-me' signal phosphatidylserine on apoptotic cells. Most of the phosphatidylserine recognition receptors dampen inflammation by inducing the production of anti-inflammatory mediators during phagocytosis of apoptotic corpses. However, many phosphatidylserine receptors are also capable of recognizing other ligands, with some receptors being categorized as scavenger receptors. It is now appreciated that these receptors can elicit different downstream events for particular ligands. Therefore, how phosphatidylserine recognition receptors mediate specific signals during recognition of apoptotic cells versus other ligands, and how this might help regulate the inflammatory state of a tissue is an important question that is not fully understood. Here, we revisit the work on signaling downstream of the phosphatidylserine recognition receptor BAI1, and evaluate how these and other signaling modules mediate signaling downstream from other receptors, including Stabilin-2, MerTK and αvβ5. We also propose the concept that phosphatidylserine recognition receptors could be viewed as a subset of scavenger receptors that are capable of eliciting anti-inflammatory responses to apoptotic cells.

Keywords

Phosphatidylserine; BAI1; scavenger receptors; apoptosis; signaling; apoptotic cells

Everyday, billions of cells in our body undergo the programmed cell death process known as apoptosis. The process of apoptotic cell death is crucial for normal tissue development as well as homeostasis within developed tissues. In fully developed tissues, aged and damaged members of the cellular community undergo apoptotic cell death(1). Subsequently, these dying cells are cleared by phagocytes, and the engulfing phagocytes then actively limit or dampen local tissue inflammation(2–5).

During apoptosis, dying cells expose 'eat-me' signals so that phagocytes can recognize and distinguish them from healthy counterparts. The most classic and well-studied 'eat-me' signal is phosphatidylserine (PtdSer). Normally, PtdSer is sequestered on the inner leaflet of the plasma membrane, and during apoptosis PtdSer is exposed on the outer leaflet of the plasma membrane, where it can interact with PtdSer receptors on phagocytes(6–8). Ligation

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of PtdSer receptors on phagocytes triggers phagocytic cup formation, which then facilitates the engulfment of the dying cell.

PtdSer recognition receptors and Scavenger receptors

PtdSer recognition receptors do not fall in to a single category or family of proteins as they do not share a common structure (Figure 1). Rather, PtdSer receptors come in a variety of different molecular formats, with the only commonality being their ability to recognize the ligand phosphatidylserine (either directly or indirectly). It should be noted that many, and potentially all PtdSer receptors, recognize multiple ligands and likely have roles other than apoptotic cell clearance(9–16). In fact, numerous PtdSer receptors have also been categorized as scavenger receptors (Table 1).

Scavenger receptors, like PtdSer receptors are a broad category of membrane proteins that are structurally distinct and evolutionarily ancient. The scavenger receptors were originally recognized and categorized on the basis of their ability to bind modified low-density lipoproteins (LDL)(3, 17). Currently, there are nine official classes of scavenger receptors. As new receptors are discovered and old receptors are re-categorized, additional classes are proposed. Scavenger receptor ligands are known to include altered self-ligands that are broadly termed damage associated molecular patterns (DAMPs). Many scavenger receptors are also capable of recognizing markers on pathogens that are referred to pathogen associated molecular patterns (PAMPs). The classification schema has been largely based on the general group of ligands recognized, which results in moderate structural variability within the same class. While many scavenger receptors have also been shown to recognize PtdSer, and receptors originally linked to PtdSer recognition now have other ligands, some PtdSer receptors, such as TIM-4 and BAI1 have remained excluded from scavenger receptor categorization. While BAI1, as a 7-transmembrane G-Protein coupled receptor, differs from most scavenger receptors in that it has more than one transmembrane domain, it does possess the ability to bind LPS as well as phosphatidylserine(9, 18). As the scavenger receptor category expands, this term of ‘scavenger’ may need to be revised given how many proteins possess this ‘scavenging’ function, yet it is a crucial characteristic and one that is relevant in the context of PtdSer receptors.

PtdSer receptors mediate what is often referred to as an “immunologically silent” apoptotic cell removal. However, the term ‘silence’ is a slight misnomer. While it is correct that apoptotic cell clearance does not elicit a pro-inflammatory response, apoptotic cell engulfment also elicits potent anti-inflammatory signaling in the phagocyte(4, 5, 19–21). This signaling ultimately leads to anti-inflammatory cytokine production and secretion (4, 5, 22, 23). Interestingly, while PtdSer receptors are typically viewed as anti-inflammatory, some also contribute to inflammatory responses in certain contexts (9, 24–26). It is unclear at present how these promiscuous receptors can mediate these diametrically opposite responses.

In part, the difficulty in recognizing both anti-inflammatory and pro-inflammatory responses downstream of phosphatidylserine receptors and scavenger receptors is due to issues of receptor nomenclature. For example, a review on scavenger receptors recently suggested

that the PtdSer receptor TIM-1 was a prototypical member of a novel class of scavenger receptor(27). However, the publications this suggestion was based on refer to TIM-1 as either KIM-1 (kidney injury molecule-1) or HAVCR1 (heptatis A virus cellular receptor 1) (14, 28). Additionally, the scavenger receptors FEEL 1 and 2 are also known as the PtdSer receptors Stablilin-1 and Stabilin-2 respectively. Additionally, Scavenger receptor type F family member 1 (SCARF1), which recognizes PtdSer via C1q, is also known as the scavenger receptor SREC-1(10, 29). A recent review proposed an overhaul to the nomenclature of scavenger receptors(30). While this may help clarify some of the issues, the greater hope is to re-conceptualize PtdSer receptors as a sub-class of scavenger receptors capable of eliciting anti-inflammatory responses to apoptotic cells. Many PtdSer receptors have already been classified or suggested as members of the scavenger receptor family (Table 1). However, other receptors, such as BAI1, meet the scavenger receptor criteria of being able to recognize DAMPs and PAMPs, but are yet to be recognized as a type of scavenger receptor(9).

A major question in the scavenger receptor field could just as easily be applied to the PtdSer receptor field: how do promiscuous receptors with a multitude of ligands elicit the appropriate response for a given ligand? The answer to this question is likely that “context matters” and context is almost certainly based on the receptor repertoire engaged during the recognition of a particular target or ligand. Additionally, multiple receptors might interact physically in a ‘signaling synapse’. Evidence for this hypothesis is increasing as more PtdSer receptors are found to act in concert e.g. Tim4 and β1 integrin, Stabilin-2 and αvβ5, MerTK and αvβ5(31–33). Alternatively, it is also possible that multiple receptors might be engaged individually and they intersect at the level of downstream cytoplasmic signaling pathways to elicit their effects.

Much of the early work on apoptotic cell clearance, prior to identification of specific PtdSer recognition receptors, was performed in model organisms. The mammalian homologs were subsequently identified, permitting signaling studies. The early genetic studies utilized *C. elegans* and *D. melanogaster* to identify specific genetic pathways that elicit apoptotic cell engulfment(34–38). In the years since their discovery, additional studies in the worm, fly, and mammal have elaborated these simple pathways into complex networks with multiple regulatory nodes and areas of signaling overlap. Here, we review the knowledge gained from signaling studies performed by many investigators (including our laboratory), identify new avenues of investigation toward understanding how specificity might be achieved in downstream signaling responses, and how PtdSer receptors might fit in the larger context of scavenger receptors.

Lessons from evolutionarily conserved signaling pathways

Initially, the molecular players involved in apoptotic cell recognition and clearance in mammals were a black box. However, cloning of the mammalian homologs of the genes linked to apoptotic cell clearance in a model organism, the nematode *C. elegans*, proved enlightening. Similarly, parallel studies in the fly *Drosophila melanogaster*, and complementary studies in other model organisms such as zebra fish, and mammalian systems have helped identify key players and pathways. Below, we detail some of the

studies that were initially performed in the nematode, enabled by the powerful genetic tools available for this organism, as well as the fact that cell death in the developing nematode is invariant (with specific cells undergoing stereotyped forms of developmental cell death) (39–41).

Genetic analyses and rescue studies of mutants have identified two partially redundant pathways involved in cell clearance in *C. elegans.* Most of these players have also been identified in mammals. The initial six genes discovered (*ced-1, ced-2, ced-5, ced-6, ced-7 and ced-10*) were grouped into two, partially redundant pathways, according to studies of double mutants. In these studies, single mutants in each pathway lead to an increase in the number of uncleared corpses, but mutation of multiple genes within the same pathway does not further enhance the phenotype. However, mutating genes in both pathways augments the number of uncleared corpses.

The first pathway involves the membrane protein CED-1 (whose homologs in mammals include MEGF-10, and also distantly LRP1), a second membrane protein CED-7 (ABCA1 and/or ABCA7 being the mammalian homologue(s)), and a cytoplasmic signaling protein CED-6 (mammalian homolog GULP) (34), (36), (42–45). The second pathway consisted of three cytoplasmic proteins CED-2, CED-5, CED-10, and CED-12, with the mammalian homologues being, respectively, CrkII, Dock180, and Rac (34) (43, 46–48). The identification of the members of the second pathway indicated a modality by which phagocytes may rearrange their cytoskeleton for corpse uptake. Dock180 (*ced*-5) was originally isolated as a Crk (*ced-2*) binding protein that caused cell membrane ruffling, which was later shown to be the result of downstream Rac1 (*ced-10*) activation. Their association with cell clearance, which requires membrane reorganization, indicated that the function of these proteins in regulating the cytoskeleton was likely conserved throughout evolution (49, 50). The initial genetic studies in *C. elegans* were extremely useful in identifying the key players involved in engulfment. The discovery of the mammalian homologues and the subsequent biochemical studies in mammalian systems have greatly helped our understanding of how these various molecular players work together during apoptotic cell engulfment.

Identification of ELMO1/Dock180 complex as a new type of Rac-GEF

Rac1 is a small GTPase that belongs to the Rho family of GTPases. The rate-limiting step in activation of Rho GTPases is the exchange of GDP for GTP, which requires their progression through the nucleotide-free transition state. This activation step is regulated by proteins that control the nucleotide binding state of the GTPases: Rho guanine nucleotide dissociation inhibitors (GDIs), Rho GTP hydrolysis-activating proteins (GAPs), and guanine nucleotide exchange factors (GEFs) (51). GEFs stabilize the Rho GTPases in the nucleotide free state and thus are essential for controlling the rate of GTPase activity(52). Despite the known involvement of Rac1 in apoptotic cell clearance, none of the other genes involved in apoptotic cell clearance were obvious candidates as a GEF for Rac. This was because all previously known GEFs for the Rho family GTPases possessed a DBL homology domain (DH) and a closely positioned pleckstrin-homology (PH) domain (often referred to as the DH-PH cassette) (52–55). Though Dock180 was found to associate with the nucleotide-free

form of Rac, Dock180 does not possess DH or PH domains(35, 50). In addition, Dock180 alone did not have RacGEF activity *in vitro*(56, 57). However, overexpression of Dock180 increased nucleotide exchange on Rac1 within cells, which suggested that there may be other missing players (50). The mechanism of Dock180 function in Rac nucleotide exchange was revealed when ELMO1, an additional member of this engulfment pathway, was identified.

The *C. elegans* gene, *ced-12*, and its mammalian homologue ELMO1 were found to function in the same engulfment pathway as CrkII, Dock180, and Rac(56). The worm CED-12 was also independently identified by two additional groups who showed that it was involved in apoptotic cell engulfment, cell migration, and gonadal morphogenesis(56, 58, 59).

Mammalian ELMO1 was found to bind to Dock180 and this interaction is evolutionarily conserved as CED-12 also binds CED-5 (the Dock180 homolog)(56, 58, 59). (Figure 2) (56, 58). In addition, Dock180 bound CrkII independent of its interaction with ELMO1; overexpression of all three proteins was found to drastically increase membrane ruffling in a Rac-dependent manner(56). These observations were critical as it placed the members of the second genetic pathway in the nematode, namely, CED-2, CED-5, and CED-12, in a physical complex that promotes CED-10/Rac activity.

Though the ELMO/Dock180/CrkII complex could promote Rac activity *in vivo*, neither ELMO, Dock180 or CrkII possessed the classic DH-PH domains found on other Rho GEFs. This finding prompted a series of deletion mutant studies of Dock180, which suggested that truncation at residue 1472 on Dock180 abolished the increase in Rac activation observed when Dock180 is overexpressed(57). Our laboratory then revealed that the deleted region was part of a larger domain, which was initially termed as ‘Docker’ (a Dock homology region involved in exchange on Rac) (57, 60). The identification of the Docker domain then helped identify a Dock180 superfamily of 11 proteins within the mammalian genome that contained the Dock-homology region (Dock1 through Dock11)(60). Of these, Dock1, 2, 3, 4, 6, 7 and 10 are capable of activating Rac1(61–66). In addition, there is direct evidence that Dock 1, 2, 3, and 4 interact with ELMO(56, 63, 67, 68).

However, the Docker domain alone was insufficient for Rac activation (56, 57). A series of studies that used a combination of biochemical and cellular approaches then revealed that ELMO1 and Dock180 form a bipartite GEF for Rac, as the expression of both proteins and their association was essential for *in vivo* GEF function(57, 69). ELMO promotes GEF activity at the Docker domain in at least three ways. First, ELMO1 helps recruit Dock180 to the plasma membrane to facilitate Rac1 activation (see below for the receptor upstream of this complex). Second, Dock180 normally exists in a ‘pretzel’ conformation where the SH3 domain of Dock180 sterically interferes with GEF function by directly binding to the Docker domain in *cis* (Figure 3A). ELMO binding to the SH3 domain of Dock180 alleviates steric auto-inhibition of Dock180 (Figure 3B) (70). Third, ELMO1 also contains a PH domain. The PH domain of ELMO binds to and stabilizes nucleotide-free Rac in *trans* with the docker domain of Dock180 (Figure 3B)(71). Thus, by ‘opening up’ Dock180 and by

stabilizing the Dock180/Rac1 interaction, ELMO1 acts as a key partner for the ELMO1/Dock180 complex to act as a bi-partite GEF for Rac.

Identification of the receptor upstream of the ELMO1/Dock180/Rac1 module

Another key missing piece from the genetic studies in *C. elegans* was the lack of an obvious apoptotic cell recognition receptor upstream of the ELMO1/Dock180/Rac pathway. The mutational studies in mammalian cells and rescue studies in CED-12 deficient worms with the human orthologs revealed that N-terminus of ELMO is critical for membrane targeting and that likely was responsible for bringing the ELMO1/Dock180 complex to the membrane. This led to the search for binding proteins that may interact with the N-terminal region of ELMO1. Our laboratory used the N-terminus of ELMO in a yeast-two-hybrid screen and identified a membrane protein brain angiogenesis inhibitor 1 (BAI1) as a receptor upstream of ELMO(18).

BAI1 belongs to the type II adhesion GPCR family (recently classified as ADGRB1)(72). Human BAI1 is a 1584 amino acid protein with a large extracellular region, and a seven transmembrane region, followed by a long intracellular tail ((73)). The yeast 2-hybrid screen identified a small fragment of the cytoplasmic tail of BAI1 that interacted with ELMO. Following the identification of BAI1, our laboratory confirmed that it played a role in apoptotic cell clearance. When BAI1 was overexpressed, apoptotic cell engulfment was enhanced(18). However, if BAI1 was mutated such that it could not bind ELMO, it was unable to boost engulfment(18). Furthermore, co-transfection of dominant negative ELMO, Dock180 or Rac1 abolished the boost in apoptotic cell clearance conferred by BAI1 expression(18). These findings suggested that BAI1 functioned to promote apoptotic cell clearance upstream of the ELMO-Dock180-Rac1 module.

One of the key links between BAI1 and apoptotic cell recognition was that BAI1 could interact with phosphatidylserine (PtdSer) exposed on the surface of apoptotic cells(18). This interaction occurred via the type-1 thrombospondin repeats in the extracellular region of BAI1(18). Similarly, red blood cells exposing PtdSer can bind to thrombospondin in the endothelial matrix(74) (75). The observations that purified bacterial BAI1-TSR domains can bind purified PtdSer and that blocking PtdSer recognition abrogated the boost in clearance by BAI1 overexpression, suggested that BAI1 was a *bona fide* PtdSer receptor for apoptotic cell clearance (Figure 4) (18).

Additional branch points and regulators of this BAI1-ELMO1-Dock180-Rac1 pathway

A distinct role for Ced-2/CrkII during engulfment

Ced-2 and its mammalian ortholog CrkII were originally identified as promoters of engulfment as part of the pathway that includes Dock180, ELMO and Rac1(34, 76). CrkII is a member of the Crk adapter protein family, that associates with Dock180 (Figure 2)(77, 78). Overexpression of either Dock180 or CrkII boosts Rac activity, and co-expression of CrkII, Dock180 and ELMO further enhances Rac activation and increases the number of corpses that are engulfed(35, 43,46,49, 50, 56, 58). However, a direct physical interaction

between CrkII and Dock180 is not essential for engulfment of apoptotic cells either in the mammalian cells or in the nematode, yet MEF cells lacking CrkII alone are defective in engulfment (79). This suggests that CrkII and its signaling downstream represent a distinct 'submodule' within this pathway.

RhoG – a modulator of ELMO1/Dock180 mediated engulfment signals

The GTPase RhoG was initially reported to function upstream of other Rho family GTPases, Rac1 and Cdc42Hs(80–82). RhoG activity is regulated by the GEF, TRIO, and TRIO activation promotes RhoG-mediated Cdc42Hs and Rac1 activation (83). Importantly, RhoG also functions upstream of the ELMO-Dock180 complex in promoting Rac1 activation(82, 84, 85). RhoG interacts with the Armadillo (ARM) repeats on the N-terminal region of ELMO1 (Figure 2). Interaction between RhoG and ELMO promotes apoptotic cell engulfment(86). Importantly, the *C. elegans* orthologs of RhoG and TRIO (MIG-2 and UNC-73, respectively) also are part of the same pathway as CED-12, suggesting that this interaction/function is evolutionarily conserved(86). RhoG is important not only for apoptotic cell engulfment, but also for complement receptor and Fc receptor-mediated phagocytosis (Figure 5)(87). The ability of RhoG to interact with and stimulate multiple engulfment pathways suggests that it may function as a node to regulate upstream signals to facilitate activation of the appropriate pathways. Intriguingly, RhoG was found to play a crucial role in T cell endocytosis of T-cell receptor-MHC II complexes (a general process known as trogocytosis)(88). This finding further supports a broad role for RhoG in engulfment processes dependent on actin rearrangement.

RhoA – a negative regulator of apoptotic cell engulfment

The GTPase RhoA inhibits apoptotic cell engulfment via Rho kinase activation(Figure 5) (89). Consistent with its role as a brake on the engulfment machinery, the use of dominant negative RhoA or inhibition of Rho kinase (ROCK) promotes phagocytosis of apoptotic cells(89–92). However, RhoA is not a broad or general inhibitor of all phagocytic activity, as RhoA activation is critical for complement receptor mediated phagocytosis(93–96). Increased RhoA activity also promotes uptake of serum opsonized particles(97). However, RhoA was not determined to be a direct mediator of Fc-receptor mediated phagocytosis in an unbiased screen of GTPase function(87). This suggests that RhoA may indirectly regulate Fc receptor mediated phagocytosis while its role in complement receptor mediated phagocytosis is more direct.

The finding that RhoA activation promotes superoxide generation further supports a role for RhoA in the response to and clearance of pathogens (98–101). Intriguingly, the generation of superoxide may be sufficient to limit apoptotic cell clearance in professional phagocytes. A study on the effects of cigarette smoke found that smoking increased RhoA activity and superoxide formation in alveolar macrophages. These macrophages were impaired in their ability to phagocytose apoptotic cells and this was reversed upon treatment with a ROCK inhibitor or anti-oxidants, suggesting that oxidants (e.g. reactive oxygen species) may be sufficient to impair apoptotic cell engulfment(91). Of interest, our laboratory showed that phagocytes increase expression of the mitochondrial uncoupler protein 2 (UCP2) during apoptotic cell engulfment(102, 103). UCP2 overexpression enhances apoptotic cell

clearance, while UCP2 deficiency impairs apoptotic cell uptake(103). Although UCP2 has been shown to regulate mitochondrial production of ROS (104–106), modulating ROS did not directly influence apoptotic cell engulfment in non-professional phagocytes(103)

In vivo phenotypes of mice lacking specific engulfment genes

A multitude of knockout mouse lines have been generated to study the role of engulfment genes *in vivo*. Many groups have generated and studied knockouts of the engulfment genes BAI1, ELMO1, Dock180, CrkII, and Rac1. We summarize the phenotypes observed in these mouse strains below.

Deficiency of BAI1

Recently, our laboratory generated a global knockout of BAI1. We have seen distinct phenotypes from these mice so far. First, the BAI1 KO animals have smaller muscle fibers. *In vitro* studies suggest that this is the result of impaired myoblast fusion, where BAI1 mediated phosphatidylserine recognition plays a role in fusion between healthy myoblasts (107). Second, while peritoneal macrophages from BAI1 mice have impaired apoptotic cell clearance in vitro, mice lacking BAI1 do not display an increase in apoptotic cell corpses in the different tissues in steady state. However, when challenged with tissue injury, the BAI1-null mice have significantly more uncleared apoptotic cell corpses in thymus, testes, and in the colon (unpublished observations Lee CS et. al.) Third, when BAI1 KO mice were crossed to the low-density lipoprotein receptor KO mice to assess the role of BAI1 in the development of atherosclerosis, BAI1 KO mice on the LDLR KO background had increased numbers of apoptotic cells in atherosclerotic plaques. In addition these mice had an altered serum lipid profile that closely resembled the phenotype of mice deficient in macrophage expression of ABCA1(108, 109).

Loss of ELMO1

Our laboratory has also generated global, as well as conditional knockouts, of ELMO1. While the mice lacking ELMO1 globally were grossly normal, they displayed severe testicular pathology(110). Specifically, the architecture of the seminiferous tubules was disrupted, apoptotic germ cells accumulated, and the production of viable sperm was decreased(110). This was due to defective phagocytosis via the Sertoli cells that line the seminiferous epithelium(110). The Sertoli cells are critical for spermatogenesis as they serve both a nursing role for the developing spermatocytes and also phagocytose those germ cells that undergo defective meiosis or carry other developmental abnormalities(111, 112). The key role for ELMO1 in phagocytosis by Sertoli cells *in vivo* was confirmed by conditional Sertoli-specific deletion of ELMO1(110).

CrkII-null mice

The *crk* gene has two splice variants, CrkI and CrkII. Homozygous loss of Crk was found to be embryonic lethal due to defects in the vascular smooth muscle(113). However, when embryonic fibroblasts derived from the CrkII deficient animals are tested in phagocytosis, they were defective in engulfment of apoptotic cells (79). Testing the apoptotic cell

engulfment phenotype of mice carrying conditional tissue specific deletion of CrkII has not yet been reported.

Loss of Dock180

Genetic loss of Dock180 is embryonic lethal. While some mice do survive to birth, they rapidly develop cyanosis(114). This was due to underdevelopment of intercostal muscles and a thin diaphragm that was poorly attached(114). These defects in the musculature were due to a severe impairment of myoblast fusion in the developing embryo(114). In addition, these mice were found to have severe defects in cardiovascular development (115). We crossed mice with conditional deletion of Dock180 to the LysM cre to assess the phenotype of macrophage engulfment in the absence of Dock180. We observed that peritoneal macrophages from these mice did not have an engulfment defect (unpublished observations). However, Dock2, a homologue of Dock180 is expressed highly in hematopoietic cells (particularly in macrophages). Therefore, assessment of the role of Dock in professional phagocytes may be obscured by the presence of Dock2. The engulfment phenotype of mice lacking both Dock180 and Dock2 within phagocytes (such as macrophages) has not been determined.

Deficiency of Rac1

Given the central role played by Rac1 in cytoskeletal rearrangements in many contexts (including cell migration and phagocytosis), it was not surprising that the Rac1 null mice were lethal in the early stages of embryogenesis. This was due to the necessity of Rac1 in the formation of the three embryonic germ cell layers(116). Since then, the role of Rac1 *in vivo* has been studied through the generation of Rac1 conditional knockout mice. These mice have been used to assess the critical role of Rac1 in endothelial homeostasis, cardiovascular development, hematopoiesis, neutrophil migration and even bronchial epithelial cell homeostasis(117–121). In the context of phagocytosis, Rac1 is essential in macrophages such that loss of Rac1 specifically in peritoneal macrophages impairs their engulfment of apoptotic targets. Additionally, these mice exhibit a defect in ABCA1 upregulation downstream of apoptotic cell recognition (108). In the context of airways, mice specifically lacking Rac1 in the airway epithelial cells are defective in apoptotic cell clearance. These mice also have an exacerbated inflammatory response to airborne allergens such as house dust mite. This effect of Rac1 has been linked to decreased IL-10 in the bronchoalveolar lavage fluid, heightened levels of IL-33, and a subsequent increase in the activity of the type 2 innate lymphoid cells in the airways(121).

The phenotypes of the mice lacking specific engulfment genes detailed above support the notion that the signaling mediators downstream of phosphatidylserine receptors are involved in a multitude of processes. Global knockout of the receptor BAI1 and its immediate binding partner ELMO lead to relatively milder phenotypes compared to knockout of signaling molecules further downstream. This may be due to either redundancy of engulfment receptors and/or signaling through multiple receptors converging on the Crk/Dock180/Rac module to elicit various processes.

Crosstalk between originally defined engulfment pathways

CED-7, the *C. elegans* ortholog of ABCA1, was originally placed in an engulfment pathway downstream of CED-1 and CED-6(34, 48). Studies in mammalian cell lines and mice further confirmed that ABCA1, in addition to playing a role in cholesterol efflux, promoted phagocytosis of apoptotic cells(44, 122, 123). Indeed, one report found interaction between ABCA1 and MEGF10, a mammalian ortholog of CED-1, which further supported a role for ABCA1 in a single engulfment pathway (Figure 6)(44). However, ABCA1 was found to be part of a homeostatic response system to apoptotic cell engulfment in mammalian macrophages. Phosphatidylserine recognition by phagocytes leads to increases in ABCA1 expression, and this increase in ABCA1 subsequently leads to enhanced cholesterol efflux from the phagocyte(124). This is a mechanism by which phagocytes ingesting apoptotic cells could unburden themselves of the cholesterol ingested via the apoptotic meal. Additionally, studies of macrophages isolated from ABCA1$^{-/-}$ mice demonstrated that ABCA1 prevented oxidative injury and subsequent apoptotic cell death in phagocytes ingesting apoptotic cells(125). These findings supported a role for ABCA1 as a generalized component of the response to apoptotic cell engulfment rather than as a player in a single pathway.

Recently, our laboratory reported that the increase in ABCA1 expression in engulfing phagocytes is rapidly upregulated via the BAI1-ELMO-Dock180-Rac module, independent of the LXR- pathway (Figure 6)(108). This finding was intriguing in that it clarified a phosphatidylserine dependent mechanism of ABCA1 upregulation and because it linked ABCA1 to the BAI1-ELMO-Dock180 pathway. The *C. elegans* engulfment pathways (CED-1, CED-6, CED-7 and CED-2, CED-5, CED-12, CED-10) were originally considered to be parallel, yet independent. However this notion was first challenged when it was found that both pathways intersected at CED10(126). The discovery that ABCA1 is transcriptionally regulated downstream of BAI1 signaling further supports the notion that these two pathways are partially redundant and mutually regulatory. Interestingly, the LDL receptor-related protein 1 (LRP1), another ortholog of CED-1, may upregulate ABCA1 expression and cholesterol efflux(127, 128), while LRP1 deficiency was shown to decrease ABCA1 expression.

Signaling pathways downstream of other phosphatidylserine receptors

The signaling pathways of several PtdSer receptors have recently become better understood. In this section, we will focus on three of these PtdSer recognition receptors and their signaling pathways, as we currently understand them: Stabilin-2, αvβ5 Integrin, and MerTK.

Stabilin-2

Stabilin-2, also referred to as the scavenger receptor FEEL-2, possesses multiple EGF-like repeat domains in its extracellular region that mediate direct interaction with PtdSer (Figure 1) (129). Upon binding to PtdSer on apoptotic cells, Stabilin-2 has been reported to promote corpse internalization via two independent but parallel pathways. In the first signaling pathway, stabilin-2 binds to the PTB domain of adapter protein GULP via an NPXY motif(130). Interaction of Stabilin-2 with GULP is then thought to elicit Rac1 activation as

the *C. elegans* ortholog of GULP, CED-6, activates the Rac1 ortholog CED-10(126). There is evidence in mammalian cells to suggest that GULP can activate Rac1, though this was shown to occur downstream of the scavenger receptor SR-B1, not stabilin-2(131). Alternatively, there is evidence that Stabilin-2 interacts with the integrin αvβ5 in the process of aged erythrocyte engulfment (33). As discussed below, αvβ5 integrin can signal via the ELMO-Dock180 module to activate Rac1, suggesting that Stabilin-2 might utilize this downstream signaling module via cooperation with αvβ5 integrin (132, 133).

Stabilin-2 is structurally similar to the *C. elegans* membrane receptor CED-1. Like CED-1, Stabilin-2 possesses multiple EGF repeats in its extracellular domain(134). CED-1 clusters around apoptotic cells on the phagocyte membrane and is crucial for clearance of apoptotic cells(34, 36, 135). CED-1 functions upstream of CED-6, the *C. elegans* ortholog of GULP(34, 128). While Stabilin-2 is not characterized as an ortholog of CED-1, CED-1 does have two mammalian orthologs: MEGF10 and LRP-1(128, 134). Interestingly, both MEGF10 and LRP-1 are capable of signaling via GULP, suggesting that this apoptotic cell recognition and signaling mechanism is evolutionarily conserved and utilized by a multitude of PtdSer receptors(128, 136).

αvβ5 Integrin

The integrin recognition of apoptotic cells is indirect and occurs through the soluble bridge, MFG-E8 or lactadherin, which links PtdSer on the apoptotic cell to integrins on the surface of phagocytes(137–140). Interestingly, αvβ5 integrin has been reported to use the ELMO-Dock180 signaling module to elicit Rac activation and apoptotic cell engulfment (Figure 6) (132, 133). When MFG-E8 bridges apoptotic cells to αvβ5 integrin, the cytoplasmic tail of β5 promotes signaling(132). FAK is phosphorylated and recruits $p130^{CAS}$ that binds to CrkII(132). CrkII then recruits Dock180 and triggers Rac1 activation(132, 133). Similarly, the *C. elegans* alpha integrin INA-1, also promotes apoptotic cell engulfment by signaling through CED-2, 5 and 10(141). Though signaling downstream of αvβ3 during apoptotic cell engulfment has not been characterized, αvβ3 signaling in a non-small cell lung carcinoma cell line has also been shown to upregulate Dock180 and Rac1 expression, suggesting that αvβ3 mediated engulfment might similarly depend on the ELMO-Dock180-Rac1 module (142).

MerTK

The receptor tyrosine kinase MerTK binds to phosphatidylserine via bridging molecules that include Gas6, ProteinS, Tubby and Tubby-like protein 1(32, 143–145). Bridging elicits dimerization of Mer and subsequent phosphorylation of the tyrosine kinase domain. While the signaling from this point is not completely solved, activation of MerTK drives a series of phosphorylation reactions. Bridging of MerTK to PtdSer stimulates phosphorylation of the tyrosine kinase domain of MerTK(146). This subsequently drives phosphorylation of phospholipase Cγ 2 (PLCγ2) (146, 147). PLCγ2 can then recruit $p130^{CAS}$ to the intracellular tail of β5 integrin and subsequently activate the CrkII-ELMO-Dock180 module(32). Interestingly, separate work has determined that all ELMO family members can be directly phosphorylated by the three TAM receptors (Tyro3, Axl and MerTK) and ELMO2 was found to be required for Axl-induced Rac activation(148). These data collectively suggest

that there are multiple engulfment receptors that utilize the ELMO-Dock180-Rac pathway to stimulate apoptotic cell engulfment. Furthermore, it seems that multiple pathways often interact and coalesce their downstream signaling around this pathway. Given these data, it would be interesting to determine whether BAI1 might also cooperate with integrins, perhaps via its extracellular RGD motif.

Signaling via scavenger receptors

Many scavenger receptors lack discernible signaling motifs in their intracellular domains. CD36, a class B scavenger receptor, is one of the best-characterized, yet its signaling modules are not completely understood. It is known however that CD36 is capable of eliciting a multitude of signaling responses that are ligand-dependent. For example, macrophages can recognize and bind both oxidized LDL (oxLDL) and β amyloid via CD36. However, oxLDL elicits downstream p38 MAPK activation while β amyloid stimulates p44 and p42 MAPK activation (149, 150). The ligand-specific CD36 responses are likely due to ligand-dependent activation of specific co-receptors and partners.

CD36 is known to interact with multiple cell surface receptors, including toll-like receptor (TLR) 2, TLR4, TLR6, β1 integrin, and β2 integrin(151–154). In addition, CD36 can promote αvβ5 activity, though it does not seem that direct interaction is required for this cooperation to occur(155). While CD36 is one of the best-characterized scavenger receptors, the use of co-receptors and the cooperation of multiple receptors in scavenger-mediated signaling is not atypical. The class-F scavenger receptor SREC-I modulates TLR-2 responses to *Klebsiella* and promotes TLR-4 signaling through NFκB and MAPK (156, 157). The class-A scavenger receptor MARCO is a component and modulator of TLR2 signaling pathways(158, 159). Another class-A scavenger receptor, SR-A1 interacts with the PtdSer receptor MerTK and is thought promote MerTK signaling during apoptotic cell uptake (160). The formation of multi-receptor ‘signalosomes’ is the most likely mechanism for generating such ligand-specific responses with a promiscuous receptor.

Challenges and opportunities

While our knowledge of the processes involved in apoptotic cell clearance has improved significantly since the first microscopic analysis of cell clearance in the nematode, there are still many interesting questions that need to be resolved. One of the most pressing questions is how individual receptors mediate diverse downstream responses. Answering this question requires a better understanding of the signaling intermediates downstream of the various receptors and the ‘nodes’ that modify the signals, either positively or negatively. The context in which a receptor engages a ligand likely influences how these ‘nodes’ affect signal cascades. An example reviewed above is RhoA, which negatively regulates apoptotic cell clearance mediated by phosphatidylserine receptors, but positively regulates complement receptor mediated phagocytosis. These clearance mechanisms are utilized under different physiological contexts, suggesting that RhoA is one such ‘context dependent’ node. We need to define other nodes that function downstream of specific PtdSer recognition receptor and scavenger receptors.

Another interesting aspect to this problem of multiple receptors with more than one ligand is that more than one receptor might be engaged at a given time. In a setting of inflammation, the simultaneous recognition of PAMPs and DAMPs likely influences the inflammatory response by the phagocyte. Under non-inflammatory settings, as in the case of apoptotic cell clearance, the absence of other inflammatory signals leads to an anti-inflammatory response by the phagocyte. This suggests that the production of anti-inflammatory mediators is the default response but an inflammatory milieu modifies the signal to elicit production of additional inflammatory mediators. A bias towards anti-inflammatory responses is logical as an unchecked or inappropriate inflammatory responses can drive auto-reactivity to self-antigens leading to autoimmune diseases. By gaining a better understanding of how signals are modified to generate inflammatory responses, we might be able to develop methods and pharmacological agents for dampening the inappropriate induction of inflammation.

Concurrent ligation and activation of multiple receptors could also be highly relevant for apoptotic cell clearance and subsequent responses. It is now being recognized that PtdSer receptors often act in concert to carry out apoptotic cell engulfment: TIM-4 uses integrin β1 as a co-receptor in the process of apoptotic cell clearance(31); MerTK recruits signaling complexes to the intracellular tail of integrin β5 during apoptotic cell recognition(32); Stabilin-2 and αvβ5 integrin cooperate in the process of aged-erythrocyte clearance (33); BAI1 and TIM-4 have been shown to function cooperatively in corpse removal in a zebrafish model, where BAI1 contributes to phagosome formation, while TIM-4 contributes to phagosome stabilization(161); CD36 promotes αvβ5 mediated uptake of photoreceptor outer segments(155). Further elucidation of how various PtdSer receptors interact to mediate apoptotic cell clearance is crucial to our understanding of how the phagocytic process is coordinated by different cell types and in different tissues. Fully appreciating how these various receptors communicate will help us understand the full orchestration of cell clearance in physiological states and how this process is modified in specific disease conditions.

A key next step for the scavenger receptor field (as well as for the many PtdSer recognition receptors) would be to define the specific downstream signaling pathways. In this context, multiple scavenger receptors have already been identified in *Drosophila*(162, 163). By using the powerful tools available in *Drosophila*, genetic screens could help identify the downstream signaling mediators and regulators of these scavenger receptors(164). These methods have already been employed to gain insight into the downstream signaling and regulation of apoptotic cell clearance by the *drosophila* receptors Croquemort and Draper(37, 38, 165, 166) suggesting their feasibility. Regulation and modification of promiscuous receptor activity is a crucial issue in biology. Often, the regulators and mediators that dampen pathway activity are far more complex than the activators. Better defining the context dependent activation and inactivation of scavenger receptors and phosphatidylserine receptors could help us gain greater insight in to their normal and altered functions, help classify them better, and most importantly manipulate them for therapeutic purposes in the future.

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References

1. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of Programmed Cell Death In Situ via Specific Labeling of Nuclear DNA Fragmentation. Journal of Cell Biology. 1992; 119:493–501. [PubMed: 1400587]
2. Duvall E, Wyllie AH, Morris RG. Macrophage recognition of cells undergoing programmed cell death (apoptosis). Immunology. 1985; 56:351–358. [PubMed: 3876985]
3. Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. PNAS. 1979; 76:333–337. [PubMed: 218198]
4. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-b, PGE2, and PAF. Journal of Clinical Investigation. 1998; 101:890–898. [PubMed: 9466984]
5. Voll R, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997; 390:350–351. [PubMed: 9389474]
6. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recogntion of removal by macrophages. The Journal of Immunology. 1992; 148:2207–2216. [PubMed: 1545126]
7. Fadok VA, et al. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. The Journal of Immunology. 1992; 149:4029–4035. [PubMed: 1281199]
8. Martin SJ, et al. Early Redistribution of Plasma Membrane Phosphatidylserine Is a General Feature of Apoptosis Regardless of the Initiating Stimulus: Inhibition by Overexpression of Bcl-2 and Abl. Journal of Experimental Medicine. 1995; 182:1545–1556. [PubMed: 7595224]
9. Das S, et al. Brain angiogenesis inhibitor 1 (BAI1) is a pattern recognition receptor that mediates macrophage binding and engulfment of Gram-negative bacteria. PNAS. 2011; 108:2136–2141. [PubMed: 21245295]
10. Tamura Y, et al. FEEL-1 and FEEL-2 Are Endocytic Receptors for Advanced Glycation End Products. Journal of Biological Chemistry. 2003; 278:12613–12617. [PubMed: 12473645]
11. Harris EN, Weigel JA, Weigel PH. The Human Hyaluronan Receptor for Endocytosis (HARE/Stabilin-2) Is a Systemic Clearance Receptor for Heparin. Journal of Biochemistry. 2008; 283:17341–17350.
12. Singh TD, et al. MEGF10 functions as a receptor for the uptake of amyloid-B. FEBS Letters. 2010; 584:3936–3942. [PubMed: 20828568]
13. Meyers JH, et al. TIM-4 is the ligand for TIM-1, and the TIM-1–TIM-4 interaction regulates T cell proliferation. Nature Immunology. 2005; 6:455–464. [PubMed: 15793576]
14. Ichimura T, Asseldonk EJPV, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV. Kidney injury molecule–1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. Journal of Clinical Investigation. 2008; 118:1657–1668. [PubMed: 18414680]
15. Gonias SL, Campana WM. LDL Receptor Related Protein-1A. The American Journal of Pathology. 2014; 184:18–27. [PubMed: 24128688]
16. Kim HY, et al. A polymorphism in TIM1 is associated with susceptibility to severe hepatitis A virus infection in humans. Journal of Clinical Investigation. 2011; 121:1111–1118. [PubMed: 21339644]

17. Parthasarathy S, Printz DJ, Boyd D, Joy L, Steinberg D. Macrophage Oxidation of Low Density Lipoprotein Generates a Modified Form Recognized by the Scavenger Receptor. Arteriosclerosis, Thrombosis, and Vascular Biology. 1986; 6:505–510.
18. Park D, et al. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature. 2007; 450:430–434. [PubMed: 17960134]
19. Ipseiz N, et al. The Nuclear Receptor Nr4a1 Mediates Anti-Inflammatory Effects of Apoptotic Cells. The Journal of Immunology. 2014; 192:4852–4858. [PubMed: 24740500]
20. Reddy SM, et al. Phagocytosis of Apoptotic Cells by Macrophages Induces Novel Signaling Events Leading to Cytokine-Independent Survival and Inhibition of Proliferation: Activation of Akt and Inhibition of Extracellular Signal-Regulated Kinases 1 and 2. The Journal of Immunology. 2002; 169:702–713. [PubMed: 12097372]
21. Grau A, Tabib A, Grau I, Reiner I, Mevorach D. Apoptotic Cells Induce NF-κB and Inflammasome Negative Signaling. Plos ONE. 2015; 10:e0122440. [PubMed: 25822487]
22. Freire-de-Lima CG, Xiao Y-Q, Gardai SJ, Bratton DL, Schiemann WP, Henson PM. Apoptotic Cells, through Transforming Growth Factor-, Coordinately Induce Anti-inflammatory and Suppress Pro-inflammatory Eicosanoid and NO Synthesis in Murine Macrophages. Journal of Biological Chemistry. 2006; 281:38376–38384. [PubMed: 17056601]
23. McDonald PP, Fadok VA, Bratton DL, Henson PM. Transcriptional and Translational Regulation of Inflammatory Mediator Production by Endogenous TGF-b in Macrophages that Have Ingested Apoptotic Cells. The Journal of Immunology. 1999; 163:6164–6172. [PubMed: 10570307]
24. Murshid A, Borges TJ, Calderwood SK. Emerging roles for scavenger receptor SREC-I in immunity. Cytokine. 2015:1–5.
25. Uchida Y, et al. The emerging role of T cell immunoglobulin mucin-1 in the mechanism of liver ischemia and reperfusion injury in the mouse. Hepatology. 2009; 51:1363–1372. [PubMed: 20091883]
26. Gebhardt C, et al. RAGE signaling sustains inflammation and promotes tumor development. Journal of Experimental Medicine. 2008; 205:275–285. [PubMed: 18208974]
27. Canton J, Neculai D, Grinstein S. Scavenger receptors in homeostasis and immunity. Nature reviews Immunology. 2013; 13:621–634.
28. Feigelstock D, Thompson P, Mattoo P, Zhang Y, Kaplan GG. The Human Homolog of HAVcr-1 Codes for a Hepatitis A Virus Cellular Receptor. Journal of Virology. 1998; 72:6621–6628. [PubMed: 9658108]
29. He M, et al. Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells. EMBO Reports. 2011; 12:358–364. [PubMed: 21399623]
30. PrabhuDas M, et al. Standardizing Scavenger Receptor Nomenclature. The Journal of Immunology. 2014; 192:1997–2006. [PubMed: 24563502]
31. Flannagan RS, Canton J, Furuya W, Glogauer M, Grinstein S. The phosphatidylserine receptor TIM4 utilizes integrins as coreceptors to effect phagocytosis. Molecular Biology of the Cell. 2014; 25:1511–1522. [PubMed: 24623723]
32. Wu Y, Singh S, Georgescu M-M, Birge RB. A role for Mer tyrosine kinase in avb5 integrin-mediated phagocytosis of apoptotic cells. Journal of Cell Science. 2005; 118:539–553. [PubMed: 15673687]
33. Kim S, et al. Cross Talk between Engulfment Receptors Stabilin-2 and Integrin avb5 Orchestrates Engulfment of Phosphatidylserine-Exposed Erythrocytes. Molecular and Cellular Biology. 2012; 32:2698–2708. [PubMed: 22566688]
34. Ellis RE, Jacobson DM, Horvitz HR. Genes Required for the Engulfment of Cell Corpses During Programmed Cell Death in *Caenorhabditis elegans*. Genetics. 1991; 129:79–94. [PubMed: 1936965]
35. Nolan KM, Barrett K, Lu Y, Hu K-Q, Vincent S, Settleman J. Myoblast city, the Drosophila homolog of DOCK180/CED-5, is required in a Rac signaling pathway utilized for multiple developmental processes. Genes and Development. 1998; 12:3337–3342. [PubMed: 9808621]
36. Hedgecock EM, Sulston JE, Thomson JN. Mutations Affecting Programmed Cell Deaths in the Nematode Caenorhabditis elegans. Science. 1983; 220:1277–1279. [PubMed: 6857247]

37. Franc NC, Dimarcq JL, Lagueux M, Hoffmann J, Ezekowitz RA. Croquemort, a novel Drosophila hemocyte/macrophage receptor that recognizes apoptotic cells. Immunity. 1996; 4:431–443. [PubMed: 8630729]
38. Franc NC, Heitzler P, Ezekowitz RA, White K. Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. Science. 1999; 284:1991–1994. [PubMed: 10373118]
39. Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode Caenorhabditis elegans. Development. 1983; 100:64–119.
40. Sulston JE, Horvitz HR. Post-embryonic Cell Lineages of the Nematode *Caenorhabditis elegans*. Development. 1977; 56:110–156.
41. Sulston JE. Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. Philosophical transactions of the Royal Society of London. 1976; 275:287–297. [PubMed: 8804]
42. Callebaut I, Mignotte V, Souchet M, Mornon J-P. EMI domains are widespread and reveal the probable orthologs of the Caenorhabditis elegans CED-1 protein. Biochemical Journal. 2003; 300:619–623.
43. Reddien PW, Horvitz HR. CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in Caenorhabditis elegans. Nature Cell Biology. 2000; 2:131–136.
44. Hamon Y, et al. Cooperation between Engulfment Receptors: The Case of ABCA1 and MEGF10. Plos ONE. 2006; 1:e120. [PubMed: 17205124]
45. Wu H-H, et al. Glial precursors clear sensory neuron corpses during development via Jedi-1, an engulfment receptor. Nat Neurosci. 2009; 12:1534–1541. [PubMed: 19915564]
46. Wu Y-C, Horvitz HR. C. elegans phagocytosis and cell-migration protein CED-5 is similar to human DOCK180. Nature. 1998; 392:501–504. [PubMed: 9548255]
47. Liu QA, Hengartner MO. Human CED-6 encodes a functional homologue of the Caenorhabditis elegans engulfment protein CED-6. Current Biology. 1999; 9:1347–1350. [PubMed: 10574771]
48. Wu Y-C, Horvitz HR. The *C. elegans* Cell Corpse Engulfment Gene *ced-7* Encodes a Protein Similar to ABC Transporters. Cell. 1998; 93:951–960. [PubMed: 9635425]
49. Hasegawa H, et al. DOCK180, a Major CRK-Binding Protein, Alters Cell Morphology upon Translocation to the Cell Membrane. Molecular and Cellular Biology. 1996; 16:1–7. [PubMed: 8524284]
50. Kiyokawa E, Hashimoto Y, Kobayashi S, Sugimura H, Kurata T, Matsuda M. Activation of Rac1 by a Crk SH3-binding protein, DOCK180. Genes and Development. 1998; 12:3331–3336. [PubMed: 9808620]
51. Kaibuchi K, Kuroda S, Amano M. Regulation of the cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. Annual Reviews of Biochemistry. 1999; 68:459–486.
52. Erickson JW, Cerione RA. Structural Elements, Mechanism, and Evolutionary Convergence of Rho Protein–Guanine Nucleotide Exchange Factor Complexes. Biochemistry. 2004; 43:837–842. [PubMed: 14744125]
53. Hart MJ, Eva A, Evans T, Aaronson SA, Cerione RA. Catalysis of guanine nucleotide exchange on the CDC42Hs protein by the *dbl* oncogene product. Nature. 1991; 354:311–314. [PubMed: 1956381]
54. Ron D, et al. A region of proto-*dbl* essential for its transforming activity shows sequence similarity to a yeast cell cycle gene, *CDC24*, and the human breakpoint cluster gene, *bcr*. The New Biologist. 1991; 3:372–379. [PubMed: 2065022]
55. Hart MJ, et al. Cellular Transformation and Guanine Nucleotide Exchange Activity are Catalyzed by a Common Domain on the *dbl* Oncogene Product. Journal of Biological Chemistry. 1994; 269:62–65. [PubMed: 8276860]
56. Gumienny TL, et al. CED-12/ELMO, a Novel Member of the CrkII/Dock180/ Rac Pathway, Is Required for Phagocytosis and Cell Migration. Cell. 2001; 107:27–41. [PubMed: 11595183]
57. Brugnera E, et al. Unconventional Rac-GEF activity is mediated through the Dock180–ELMO complex. Nature Cell Biology. 2002; 4:574–582.
58. Wu Y-C, Tsai M-C, Cheng L-C, Chou C-J, Weng N-Y. CED-12 Acts in the Conserved CrkII/ DOCK180/Rac Pathway to Control Cell Migration and Cell Corpse Engulfment. Developmental Cell. 2001; 1:491–502. [PubMed: 11703940]

59. Zhou Z, Caron E, Hartwieg E, Hall A, Horvitz HR. The *C. elegans* PH Domain Protein CED-12 Regulates Cytoskeletal Reorganization via a Rho/Rac GTPase Signaling Pathway. Developmental Cell. 2001; 1:477–489. [PubMed: 11703939]
60. Côté J-F, Vuori K. Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. Journal of Cell Science. 2002; 115:4901–4913. [PubMed: 12432077]
61. Kunisaki Y, et al. DOCK2 is a Rac activator that regulates motility and polarity during neutrophil chemotaxis. The Journal of Cell Biology. 2006; 174:647–652. [PubMed: 16943182]
62. Sanz-Moreno V, et al. Rac activation and inactivation control plasticity of tumor cell movement. Cell. 2008; 135:510–523. [PubMed: 18984162]
63. Hiramoto K, Negishi M, Katoh H. Dock4 is regulated by RhoG and promotes Rac-dependent cell migration. Experimental Cell Research. 2006; 312:4205–4216. [PubMed: 17027967]
64. Miyamoto Y, Yamauchi J, Sanbe A, Tanoue A. Dock6, a Dock-C subfamily guanine nucleotide exchanger, has the dual specificity for Rac1 and Cdc42 and regulates neurite outgrowth. Experimental Cell Research. 2007; 313:791–804. [PubMed: 17196961]
65. Watabe-Uchida M, John KA, Janas JA, Newey SE, Van Aelst L. The Rac activator DOCK7 regulates neuronal polarity through local phosphorylation of stathmin/Op18. Neuron. 2006; 51:727–739. [PubMed: 16982419]
66. Ruiz-Lafuente N, et al. Dock10, a Cdc42 and Rac1 GEF, induces loss of elongation, filopodia, and ruffles in cervical cancer epithelial HeLa cells. Biol Open. 2015; 4:627–635. [PubMed: 25862245]
67. Stevenson C, et al. Essential Role of Elmo1 in Dock2-Dependent Lymphocyte Migration. The Journal of Immunology. 2014; 192:6062–6070. [PubMed: 24821968]
68. Namekata K, et al. Dock3 regulates BDNF-TrkB signaling for neurite outgrowth by forming a ternary complex with Elmo and RhoG. Genes Cells. 2012; 17:688–697. [PubMed: 22734669]
69. Grimsley CM, et al. Dock180 and ELMO1 Proteins Cooperate to Promote Evolutionarily Conserved Rac-dependent Cell Migration. Journal of Biological Chemistry. 2004; 279:6087–6097. [PubMed: 14638695]
70. Lu M, et al. A Steric-Inhibition Model for Regulation of Nucleotide Exchange via the Dock180 Family of GEFs. Current Biology. 2005; 15:371–377. [PubMed: 15723800]
71. Lu M, et al. PH domain of ELMO functions in trans to regulate Rac activation via Dock180. Nature Structural & Molecular Biology. 2004; 11:756–762.
72. Hamann J, et al. International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. Pharmacol. Rev. 2015; 67:338–367. [PubMed: 25713288]
73. Nishimori H, et al. A novel brain-specific p53-target gene, BAI1, containing thrombospondin type 1 repeats inhibits experimental angiogenesis. Oncogene. 1997; 15:2145–2150. [PubMed: 9393972]
74. Manodori AB, Barabino GA, Lubin BH, Kuypers FA. Adherence of phosphatidylserine-exposing erythrocytes matrix thrombospondin. Blood. 2000; 95:1293–1300. [PubMed: 10666202]
75. Gayen Betal S, Setty BNY. Phosphatidylserine-positive erythrocytes bind to immobilized and soluble thrombospondin-1 via its heparin-binding domain. Translational Research. 2008; 152:165–177. [PubMed: 18940719]
76. Tosello-Trampont A, Brugnera E, Ravichandran KS. Evidence for a conserved role for CrkII and Rac in engulfment of apoptotic cells. Journal of Biological Chemistry. 2001; 276:13797–13802. [PubMed: 11297528]
77. Akakura S, et al. C-terminal SH3 domain of CrkII regulates the assembly and function of the DOCK180/ELMO Rac-GEF. J. Cell. Physiol. 2005; 204:344–351. [PubMed: 15700267]
78. Feller SM. Crk family adaptors - signalling complex formation and biological roles. Oncogene. 2001; 20:6348–6371. [PubMed: 11607838]
79. Tosello-Trampont AC, Kinchen JM, Brugnera E, Haney LB, Hengartner MO, Ravichandran KS. Identification of two signaling submodules within the CrkII/ELMO/Dock180 pathway regulating engulfment of apoptotic cells. Cell Death and Differentiation. 2007
80. Gauthier-Rouvière C, Vignal E, Mériane M, Roux P, Montcourier P, Fort P. RhoG GTPase Controls a Pathway That Independently Activates Rac1 and Cdc42Hs. Molecular Biology of the Cell. 1998; 9:1379–1394. [PubMed: 9614181]

81. Katoh H, et al. Small GTPase RhoG Is a Key Regulator for Neurite Outgrowth in PC12 Cells. Molecular and Cellular Biology. 2000; 20:7378–7387. [PubMed: 10982854]
82. Katoh H, Hiramoto K, Negishi M. Activation of Rac1 by RhoG regulates cell migration. Journal of Cell Science. 2006; 119:56–65. [PubMed: 16339170]
83. Blangy A, Vignal E, Schmidt SAD, Gauthier-Rouvière C, Fort P. TrioGEF1 controls Rac- and Cdc42-dependent cell structures through the direct activation of RhoG. Journal of Cell Science. 2000; 113:729–739. [PubMed: 10652265]
84. Katoh H, Negishi M. RhoG activates Rac1 by direct interaction with the Dock180-binding protein Elmo. Nature. 2003; 424:461–464. [PubMed: 12879077]
85. Kim J-Y, Oh MH, Bernard LP, Macara IG, Zhang H. The RhoG/ELMO1/Dock180 Signaling Module Is Required for Spine Morphogenesis in Hippocampal Neurons. Journal of Biological Chemistry. 2011; 286:37615–37624. [PubMed: 21900250]
86. deBakker CD, et al. Phagocytosis of Apoptotic Cells Is Regulated by a UNC-73/TRIO-MIG-2/RhoG Signaling Module and Armadillo Repeats of CED-12/ELMO. Current Biology. 2004; 14:2208–2216. [PubMed: 15620647]
87. Tzircotis G, Braga VMM, Caron E. RhoG is required for both Fc R- and CR3-mediated phagocytosis. Journal of Cell Science. 2011; 124:2897–2902. [PubMed: 21878497]
88. Martínez-Martín N, et al. T Cell Receptor Internalization from the Immunological Synapse Is Mediated by TC21 and RhoG GTPase-Dependent Phagocytosis. Immunity. 2011; 35:208–222. [PubMed: 21820331]
89. Tosello-Trampont AC, Nakada-Tsukui K, Ravichandran KS. Engulfment of Apoptotic Cells Is Negatively Regulated by Rho-mediated Signaling. Journal of Biological Chemistry. 2003; 278:49911–49919. [PubMed: 14514696]
90. Vandivier RW, et al. Dysfunctional cystic fibrosis transmembrane conductance regulator inhibits phagocytosis of apoptotic cells with proinflammatory consequences. AJP: Lung Cellular and Molecular Physiology. 2009; 297:L677–L686. [PubMed: 19633071]
91. Richens TR, et al. Cigarette Smoke Impairs Clearance of Apoptotic Cells through Oxidant-dependent Activation of RhoA. Am J Respir Crit Care Med. 2009; 179:1011–1021. [PubMed: 19264974]
92. Nakaya M, Tanaka M, Okabe Y, Hanayama R, Nagata S. Opposite Effects of Rho Family GTPases on Engulfment of Apoptotic Cells by Macrophages. Journal of Biological Chemistry. 2006; 281:8836–8842. [PubMed: 16439364]
93. Caron E, Hall A. Identification of Two Distinct Mechanisms of Phagocytosis Controlled by Different Rho GTPases. Science. 1998; 282:1717–1721. [PubMed: 9831565]
94. Olazabal IM, Caron E, May RC, Schilling K, Knecht DA, Machesky LM. Rho-Kinase and Myosin-II Control Phagocytic Cup Formation during CR, but Not FcyR, Phagocytosis. Current Biology. 2002; 12:1413–1418. [PubMed: 12194823]
95. Wiedemann A, Patel JC, Lim J, Tsun A, van Kooyk Y, Caron E. Two distinct cytoplasmic regions of the 2 integrin chain regulate RhoA function during phagocytosis. The Journal of Cell Biology. 2006; 172:1069–1079. [PubMed: 16567504]
96. Shi Y, et al. Protein-tyrosine kinase Syk is required for pathogen engulfment in complement-mediated phagocytosis. Blood. 2006; 107:1–10.
97. Kim JG, et al. Ras-related GTPases Rap1 and RhoA Collectively Induce the Phagocytosis of Serum-opsonized Zymosan Particles in Macrophages. Journal of Biological Chemistry. 2012; 287:5145–5155. [PubMed: 22194606]
98. Kim J-S, et al. Downstream components of RhoA required for signal pathway of superoxide formation during phagocytosis of serum opsonized zymosans in macrophages. Experimental and molecular medicine. 2005; 37:575–587. [PubMed: 16391519]
99. Moon M-Y, et al. Involvement of small GTPase RhoA in the regulation of superoxide production in BV2 cells in response to fibrillar Aβ peptides. Cellular Signalling. 2013; 25:1861–1869. [PubMed: 23707391]
100. Kim JS, Diebold BA, Kim JI, Kim J, Lee JY, Park JB. Rho Is Involved in Superoxide Formation during Phagocytosis of Opsonized Zymosans. Journal of Biological Chemistry. 2004; 279:21589–21597. [PubMed: 14970220]

101. Li Y, et al. Small GTPases Rap1 and RhoA regulate superoxide formation by Rac1 GTPases activation during the phagocytosis of IgG-opsonized zymosans in macrophages. Free Radical Biology and Medicine. 2012; 52:1796–1805. [PubMed: 22330068]
102. Garabuczi É, Sarang Z, Szondy Z. Glucocorticoids enhance prolonged clearance of apoptotic cells by upregulating liver X receptor, peroxisome proliferator-activated receptor-δ and UCP2. BBA - Molecular Cell Research. 2014; 1853:573–582. [PubMed: 25523142]
103. Park D, et al. Continued clearance of apoptotic cells critically depends on the phagocyte Ucp2 protein. Nature. 2011; 477:220–224. [PubMed: 21857682]
104. Arsenijevic D, et al. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nature Genetics. 2000; 26:435–439. [PubMed: 11101840]
105. de Bilbao F, et al. Resistance to cerebral ischemic injury in UCP2 knockout mice: evidence for a role of UCP2 as a regulator of mitochondrial glutathione levels. J Neurochem. 2004; 89:1283–1292. [PubMed: 15147521]
106. Růžička M, et al. Recruitment of mitochondrial uncoupling protein UCP2 after lipopolysaccharide induction. The International Journal of Biochemistry & Cell Biology. 2005; 37:809–821. [PubMed: 15694840]
107. Hochreiter-Hufford AE, et al. Phosphatidylserine receptor BAI1 and apoptotic cells as new promoters of myoblast fusion. Nature. 2013; 497:263–267. [PubMed: 23615608]
108. Fond AM, Lee CS, Schulman IG, Kiss RS, Ravichandran KS. Apoptotic cells trigger a membrane-initiated pathway to increase ABCA1. Journal of Clinical Investigation. 2015
109. Bi X, et al. Myeloid cell-specific ATP-binding cassette transporter A1 deletion has minimal impact on atherogenesis in atherogenic diet-fed low-density lipoprotein receptor knockout mice. Arteriosclerosis, Thrombosis, and Vascular Biology. 2014; 34:1888–1899.
110. Elliott MR, et al. Unexpected requirement for ELMO1 in clearance of apoptotic germ cells in vivo. Nature. 2011; 467:333–337. [PubMed: 20844538]
111. Griswold MD. The central role of Sertoli cells in spermatogenesis. Semin. Cell Dev. Biol. 1998; 9:411–416. [PubMed: 9813187]
112. Nakagawa A, Shiratsuchi A, Tsuda K, Nakanishi Y. In vivo analysis of phagocytosis of apoptotic cells by testicular Sertoli cells. Mol. Reprod. Dev. 2005; 71:166–177. [PubMed: 15791597]
113. Park T-J, Boyd K, Curran T. Cardiovascular and craniofacial defects in Crk-null mice. Molecular and Cellular Biology. 2006; 26:6272–6282. [PubMed: 16880535]
114. Laurin M, Fradet N, Blangy A, Hall A, Vuori K, Côté J-F. The atypical Rac activator Dock180 (Dock1) regulates myoblast fusion in vivo. PNAS. 2008; 105:15446–15451. [PubMed: 18820033]
115. Sanematsu F, et al. DOCK180 Is a Rac Activator That Regulates Cardiovascular Development by Acting Downstream of CXCR4. Circulation Research. 2010; 107:1102–1105. [PubMed: 20829512]
116. Sugihara K, et al. Rac1 is required for the formation of three germ layers during gastrulation. Oncogene. 1998; 17:3427–3433. [PubMed: 10030666]
117. Fiedler LR. Rac1 regulates cardiovascular development and postnatal function of endothelium. Cell Adh Migr. 2009; 3:143–145. [PubMed: 19287203]
118. Tan W, Palmby TR, Gavard J, Amornphimoltham P, Zheng Y, Gutkind JS. An essential role for Rac1 in endothelial cell function and vascular development. The FASEB Journal. 2008; 22:1829–1838.
119. Glogauer M, et al. Rac1 deletion in mouse neutrophils has selective effects on neutrophil functions. J. Immunol. 2003; 170:5652–5657. [PubMed: 12759446]
120. Gu Y, et al. Hematopoietic cell regulation by Rac1 and Rac2 guanosine triphosphatases. Science. 2003; 302:445–449. [PubMed: 14564009]
121. Juncadella IJ, et al. Apoptotic cell clearance by bronchial epithelial cells critically influences airway inflammation. Nature. 2012; 493:547–551. [PubMed: 23235830]
122. Hamon Y, et al. ABC1 promotes engulfment ofapoptotic cells and transbilayer redistribution of phosphatidylserine. Nature Cell Biology. 2000; 2:399–406.

123. Luciani M-F, Chimini G. The ATP binding cassette transporter ABC1, is required for the engulfment of corpses generated by apoptotic cell death. EMBO J. 1996; 15:226–235. [PubMed: 8617198]

124. Kiss RS, Elliott MR, Ma Z, Marcel YL, Ravichandran KS. Apoptotic Cells Induce a Phosphatidylserine-Dependent Homeostatic Response from Phagocytes. Current Biology. 2006; 16:2252–2258. [PubMed: 17113390]

125. Yvan-Charvet L, et al. ABCA1 and ABCG1 Protect Against Oxidative Stress-Induced Macrophage Apoptosis During Efferocytosis. Circulation Research. 2010; 106:1861–1869. [PubMed: 20431058]

126. Kinchen JM, et al. Two pathways converge at CED-10 to mediate actin rearrangement and corpse removal in C. elegans. Nature. 2005; 434:93–99. [PubMed: 15744306]

127. Zhou L, Choi HY, Li W-P, Xu F, Herz J. LRP1 Controls cPLA2 Phosphorylation, ABCA1 Expression and Cellular Cholesterol Export. Plos ONE. 2009; 4:e6853. [PubMed: 19718435]

128. Su HP, et al. Interaction of CED-6/GULP, an Adapter Protein Involved in Engulfment of Apoptotic Cells with CED-1 and CD91/Low Density Lipoprotein Receptor-related Protein (LRP). Journal of Biological Chemistry. 2002; 277:11772–11779. [PubMed: 11729193]

129. Park SY, Kim SY, Jung MY, Bae DJ, Kim IS. Epidermal Growth Factor-Like Domain Repeat of Stabilin-2 Recognizes Phosphatidylserine during Cell Corpse Clearance. Molecular and Cellular Biology. 2008; 28:5288–5298. [PubMed: 18573870]

130. Park S-Y, Kang K-B, Thapa N, Kim S-Y, Lee S-J, Kim I-S. Requirement of Adaptor Protein GULP during Stabilin-2-mediated Cell Corpse Engulfment. Journal of Biological Chemistry. 2008; 283:10593–10600. [PubMed: 18230608]

131. Osada Y, Sunatani T, Kim IS, Nakanishi Y, Shiratsuchi A. Signalling Pathway Involving GULP, MAPK and Rac1 for SR-BI-Induced Phagocytosis of Apoptotic Cells. Journal of Biochemistry. 2009; 145:387–394. [PubMed: 19122200]

132. Albert ML, Kim J-I, Birge RB. avB5 integrin recruits the CrkII–Dock180–Rac1 complex for phagocytosis of apoptotic cells. Nature Cell Biology. 2000; 2:899–905.

133. Akakura S, et al. The opsonin MFG-E8 is a ligand for the αvβ5 integrin and triggers DOCK180-dependent Rac1 activation for the phagocytosis of apoptotic cells. Experimental Cell Research. 2004; 292:403–416. [PubMed: 14697347]

134. Suzuki E, Nakayama M. The mammalian Ced-1 ortholog MEGF10/KIAA1780 displays a novel adhesion pattern. Experimental Cell Research. 2007; 313:2451–2464. [PubMed: 17498693]

135. Zhou Z, Hartwieg E, Horvitz HR. CED-1 Is a Transmembrane Receptor that Mediates Cell Corpse Engulfment in. Cell. 2001; 104:43–56. [PubMed: 11163239]

136. Scheib JL, Sullivan CS, Carter BD. Jedi-1 and MEGF10 Signal Engulfment of Apoptotic Neurons through the Tyrosine Kinase Syk. Journal of Neuroscience. 2012; 32:13022–13031. [PubMed: 22993420]

137. Shi J, Heegaard CW, Rasmussen JT, Gilbert GE. Lactadherin binds selectively to membranes containing phosphatidyl-l-serine and increased curvature. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2004; 1667:82–90.

138. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. Nature. 2002; 417:182–187. [PubMed: 12000961]

139. Andersen MH, Berglund L, Rasmussen JT, Petersen TE. Bovine PAS-6/7 Binds avb5 Integrin and Anionic Phospholipids through Two Domains. Biochemistry. 1997; 36:5441–5446. [PubMed: 9154926]

140. Andersen MH, Graversen H, Fedosov SN, Petersen TE, Rasmussen JT. Functional Analyses of Two Cellular Binding Domains of Bovine Lactadherin. Biochemistry. 2000; 39:6200–6206. [PubMed: 10821695]

141. Hsu T-Y, Wu Y-C. Engulfment of Apoptotic Cells in *C. elegans* Is Mediated by Integrin a/SRC Signaling. Current Biology. 2010; 20:477–486. [PubMed: 20226672]

142. Shah PP, Fong MY, Kakar SS. PTTG induces EMT through integrin αVβ3-focal adhesion kinase signaling in lung cancer cells. Oncogene. 2011; 31:3124–3135. [PubMed: 22081074]

143. Caberoy NB, Zhou Y, Li W. Tubby and tubby-like protein 1 are new MerTK ligands for phagocytosis. EMBO J. 2010; 29:3898–3910. [PubMed: 20978472]

144. Burstyn-Cohen T, Lew ED, Través PG, Burrola PG, Hash JC, Lemke G. Genetic Dissection of TAM Receptor-Ligand Interaction in Retinal Pigment Epithelial Cell Phagocytosis. Neuron. 2012; 76:1123–1132. [PubMed: 23259948]

145. Lew ED, et al. Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities. eLife. 2014; 3

146. Tibrewal N, et al. Autophosphorylation Docking Site Tyr-867 in Mer Receptor Tyrosine Kinase Allows for Dissociation of Multiple Signaling Pathways for Phagocytosis of Apoptotic Cells and Down-modulation of Lipopolysaccharide-inducible NF-kB Transcriptional Activation. Journal of Biological Chemistry. 2008; 283:3618–3627. [PubMed: 18039660]

147. Todt JC, Hu B, Curtis JL. The receptor tyrosine kinase MerTK activates phospholipase C gamma2 during recognition of apoptotic thymocytes by murine macrophages. Journal of Leukocyte Biology. 2004; 75:705–713. [PubMed: 14704368]

148. Abu-Thuraia A, et al. Axl Phosphorylates Elmo Scaffold Proteins To Promote Rac Activation and Cell Invasion. Molecular and Cellular Biology. 2014; 35:76–87. [PubMed: 25332238]

149. Rahaman SO, Lennon DJ, Febbraio M, Podrez EA, Hazen SL, Silverstein RL. A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. Cell Metabolism. 2006; 4:211–221. [PubMed: 16950138]

150. Moore KJ, et al. A CD36-initiated signaling cascade mediates inflammatory effects of beta-amyloid. J. Biol. Chem. 2002; 277:47373–47379. [PubMed: 12239221]

151. Stewart CR, et al. CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. Nature Immunology. 2010; 11:155–161. [PubMed: 20037584]

152. Davis SP, Lee K, Gillrie MR, Roa L, Amrein M, Ho M. CD36 recruits $\alpha_5\,\beta_1$ integrin to promote cytoadherence of P. falciparum-infected erythrocytes. PLoS Pathog. 2013; 9:e1003590. [PubMed: 24009511]

153. Stuart LM, et al. Response to Staphylococcus aureus requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. The Journal of Cell Biology. 2005; 170:477–485. [PubMed: 16061696]

154. Heit B, et al. Multimolecular signaling complexes enable Syk-mediated signaling of CD36 internalization. Developmental Cell. 2013; 24:372–383. [PubMed: 23395392]

155. Finnemann SC, Silverstein RL. Differential Roles of CD36 and avb5 Integrin in Photoreceptor Phagocytosis by the Retinal Pigment Epithelium. Journal of Experimental Medicine. 2001; 194:1289–1298. [PubMed: 11696594]

156. Jeannin P, et al. Complexity and complementarity of outer membrane protein A recognition by cellular and humoral innate immunity receptors. Immunity. 2005; 22:551–560. [PubMed: 15894273]

157. Murshid A, Gong J, Prince T, Borges TJ, Calderwood SK. Scavenger Receptor SREC-I Mediated Entry of TLR4 into Lipid Microdomains and Triggered Inflammatory Cytokine Release in RAW 264.7 Cells upon LPS Activation. Plos ONE. 2015; 10:e0122529. [PubMed: 25836976]

158. Bowdish DME, et al. MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and Mycobacterium tuberculosis. PLoS Pathog. 2009; 5:e1000474. [PubMed: 19521507]

159. Kissick HT, Dunn LK, Ghosh S, Nechama M, Kobzik L, Arredouani MS. The scavenger receptor MARCO modulates TLR-induced responses in dendritic cells. Plos ONE. 2014; 9:e104148. [PubMed: 25089703]

160. Todt JC, Hu B, Curtis JL. The scavenger receptor SR-A I/II (CD204) signals via the receptor tyrosine kinase Mertk during apoptotic cell uptake by murine macrophages. Journal of Leukocyte Biology. 2008; 84:510–518. [PubMed: 18511575]

161. Mazaheri F, et al. Distinct roles for BAI1 and TIM-4 in the engulfment of dying neurons by microglia. Nature Communications. 2014; 5:1–11.

162. Kiefer C, Sumser E, Wernet MF, Lintig, Von J. A class B scavenger receptor mediates the cellular uptake of carotenoids in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 2002; 99:10581–10586. [PubMed: 12136129]

163. Pearson A, Lux A, Krieger M. Expression cloning of dSR-CI, a class C macrophage-specific scavenger receptor from Drosophila melanogaster. Proc. Natl. Acad. Sci. U.S.A. 1995; 92:4056–4060. [PubMed: 7732030]
164. Silva EA, Burden J, Franc NC. In vivo and in vitro methods for studying apoptotic cell engulfment in Drosophila. Meth. Enzymol. 2008; 446:39–59. [PubMed: 18603115]
165. Cuttell L, et al. Undertaker, a Drosophila Junctophilin, links Draper-mediated phagocytosis and calcium homeostasis. Cell. 2008; 135:524–534. [PubMed: 18984163]
166. Silva E, Au-Yeung HW, Van Goethem E, Burden J, Franc NC. Requirement for a Drosophila E3-ubiquitin ligase in phagocytosis of apoptotic cells. Immunity. 2007; 27:585–596. [PubMed: 17936033]
167. Lanoue V, et al. The adhesion-GPCR BAI3, a gene linked to psychiatric disorders, regulates dendrite morphogenesis in neurons. Molecular Psychiatry. 2013; 18:943–950. [PubMed: 23628982]
168. Hamoud N, Tran V, Croteau LP, Kania A, Cote JF. G-protein coupled receptor BAI3 promotes myoblast fusion in vertebrates. PNAS. 2014; 111:3745–3750. [PubMed: 24567399]
169. Curtiss ML, et al. Fyn binds to and phosphorylates T cell immunoglobulin and mucin domain-1 (Tim-1). Molecular Immunology. 2011; 48:1424–1431. [PubMed: 21513984]
170. Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. Nature. 2007; 450:435–439. [PubMed: 17960135]
171. Lu Q, et al. Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. Nature. 1999; 398:723–728. [PubMed: 10227296]
172. Seitz HM, Camenisch TD, Lemke G, Earp HS, Matsushima GK. Macrophages and Dendritic Cells Use Different Axl/Mertk/Tyro3 Receptors in Clearance of Apoptotic Cells. The Journal of Immunology. 2007; 178:5635–5642. [PubMed: 17442946]
173. Zagórska A, Través PG, Lew ED, Dransfield I, Lemke G. Diversification of TAM receptor tyrosine kinase function. Nature Immunology. 2014; 15:920–928. [PubMed: 25194421]
174. Adachi H, Tsujimoto M. FEEL-1, a Novel Scavenger Receptor with in Vitro Bacteria-binding and Angiogenesis-modulating Activities. Journal of Biological Chemistry. 2002; 277:34264–34270. [PubMed: 12077138]
175. Kzhyshkowska J, Gratchev A, Goerdt S. Stabilin-1, a homeostatic scavenger receptor with multiple functions. Journal of Cellular and Molecular Medicine. 2006; 10
176. Politz O, et al. Stabilin-1 and-2 constitute a novel family of fasciclin-like hyaluronan receptor homologues. Journal of Biochemical Society. 2002; 362:155–164.
177. Park SY, et al. Stabilin-1 mediates phosphatidylserine-dependent clearance of cell corpses in alternatively activated macrophages. Journal of Cell Science. 2009; 122:3365–3373. [PubMed: 19726632]
178. Park S-Y, Kim S-Y, Kang K-B, Kim I-S. Adaptor protein GULP is involved in stabilin-1-mediated phagocytosis. Biochemical Journal. 2010; 398:467–472.
179. Hirose Y, et al. Inhibition of Stabilin-2 elevates circulating hyaluronic acid levels and prevents tumor metastasis. PNAS. 2012; 109:4263–4268. [PubMed: 22371575]
180. Park SY, et al. Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. Cell Death and Differentiation. 2007; 15:192–201. [PubMed: 17962816]
181. Zhou B, Weigel JA, Fauss L, Weigel PH. Identification of the Hyaluronan Receptor for Endocytosis (HARE). Journal of Biological Chemistry. 2000; 275:37733–37741. [PubMed: 10952975]
182. Bu H-F, et al. Milk fat globule–EGF factor 8/lactadherin plays a crucial role in maintenance and repair of murine intestinal epithelium. Journal of Clinical Investigation. 2007
183. Lauber K, et al. Milk fat globule-EGF factor 8 mediates the enhancement of apoptotic cell clearance by glucocorticoids. Cell Death and Differentiation. 2013; 20:1230–1240. [PubMed: 23832117]
184. Savill J, Dransfield I, Hogg N, Haslett C. Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. Nature. 1990; 343:170–173. [PubMed: 1688647]

185. Berwin B, Delneste Y, Lovingood RV, Post SR, Pizzo SV. SREC-I, a Type F Scavenger Receptor, Is an Endocytic Receptor for Calreticulin. Journal of Biological Chemistry. 2004; 279:51250–51257. [PubMed: 15371419]

186. Tamura Y, et al. Scavenger Receptor Expressed by Endothelial Cells I (SREC-I) Mediates the Uptake of Acetylated Low Density Lipoproteins by Macrophages Stimulated with Lipopolysaccharide. Journal of Biological Chemistry. 2004; 279:30938–30944. [PubMed: 15145948]

187. Ramirez-Ortiz ZG, et al. The scavenger receptor SCARF1 mediates the clearance of apoptotic cells and prevents autoimmunity. Nature Immunology. 2013; 14:917–926. [PubMed: 23892722]

188. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. Journal of Clinical Investigation. 2001; 108:779–784. [PubMed: 11560943]

189. Wang X, et al. Caenorhabditis elegans transthyretin-like protein TTR-52 mediates recognition of apoptotic cells by the CED-1 phagocyte receptor. Nature Cell Biology. 2010; 12:655–664.

190. Chung W-S, et al. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature. 2013; 504:394–400. [PubMed: 24270812]

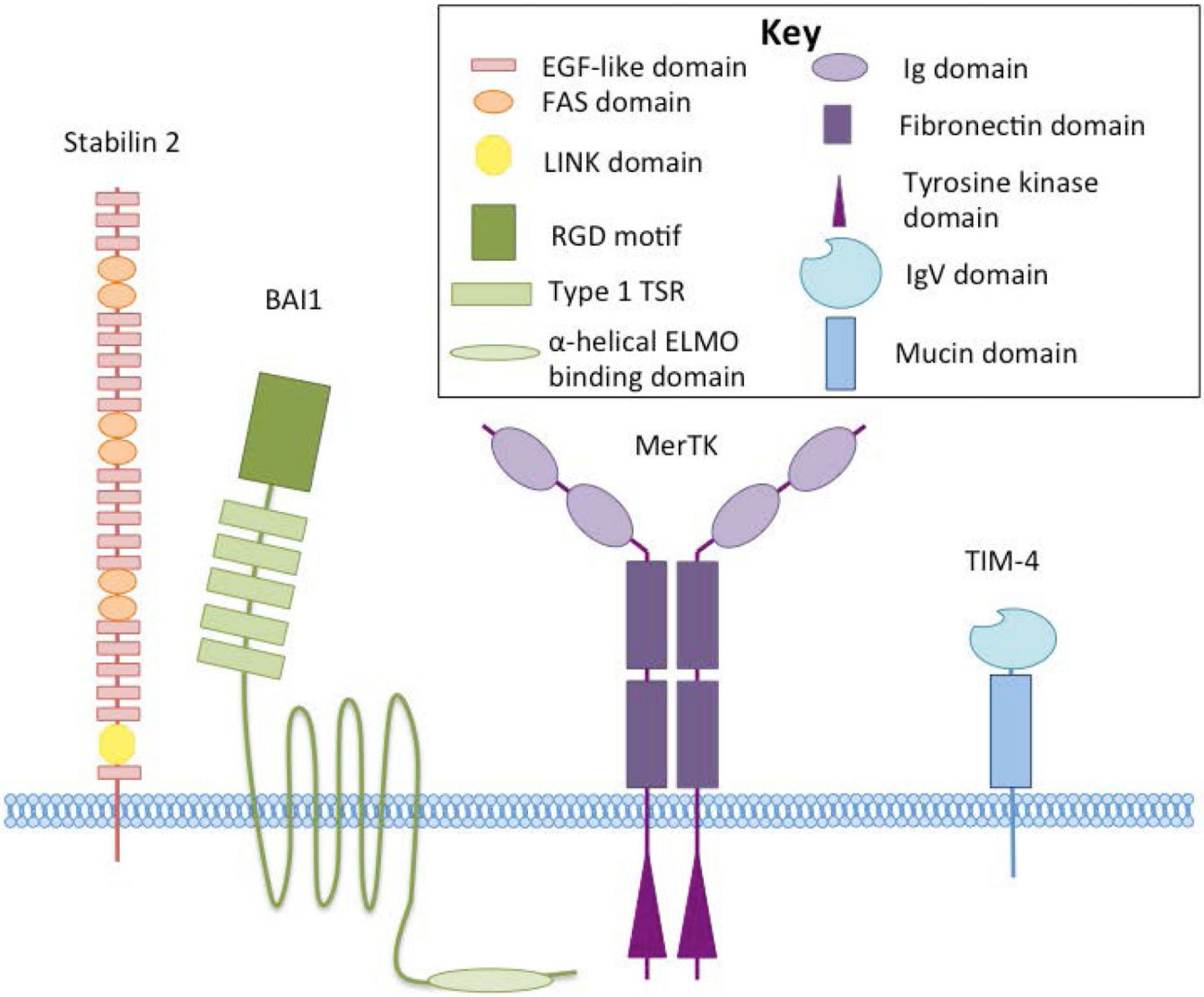

Figure 1. Distinct structural features of phosphatidylserine recognition receptors

Stabilin-2, TIM-4, BAI1 bind PtdSer directly while MerTK recognizes phosphatidylserine indirectly. Their general structural domains and motifs are indicated. Stabilin-2 belongs to the scavenger receptor FEEL family, which also includes Stabilin-1 (FEEI1) that can also bind phosphatidylserine. BAI1 represents the BAI family of 7-transmembrane phosphatidylserine receptors that belong to the adhesion family of G-protein coupled receptors. MerTK is representative of the TAM family of phosphatidylserine receptors, which utilize bridging molecules (Gas6 or protein S) to bind phosphatidylserine. TIM-4 is representative of the TIM family of proteins. TIM-1 and TIM-3 are also capable of binding phosphatidylserine.

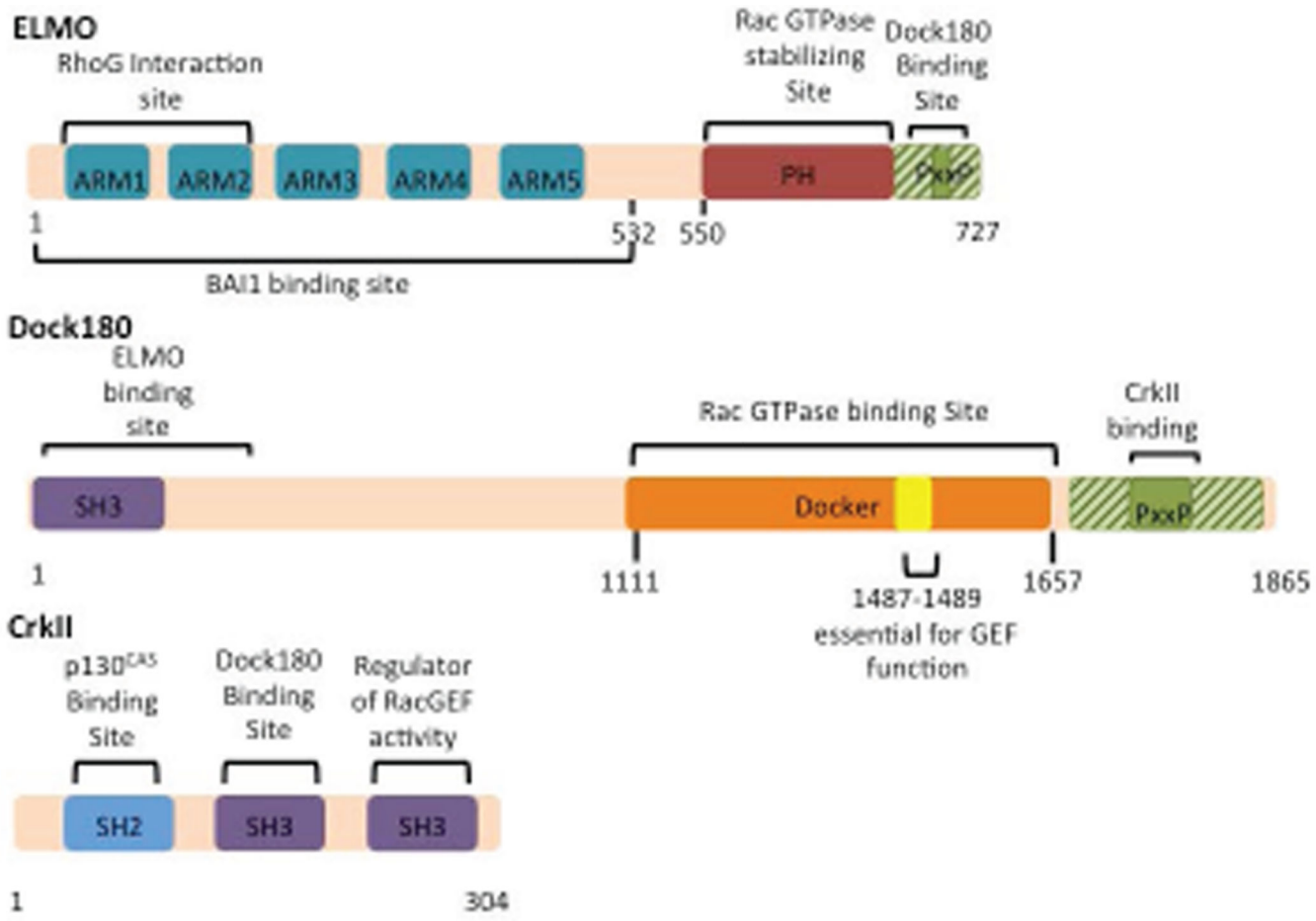

Figure 2. Domains and binding sites within ELMO, Dock180, and CrkII

ELMO, via its PxxP motif and a region within its PH domain, interacts with the N-terminal SH3 and adjoining regions of Dock180. Similarly a PxxP motif on Dock180 binds to an SH3 domain on CrkII. Dock180 possesses that a Docker domain that is important for binding to nucleotide free Rac and this binding between Dock180 and Rac is stabilized by the domain of ELMO.

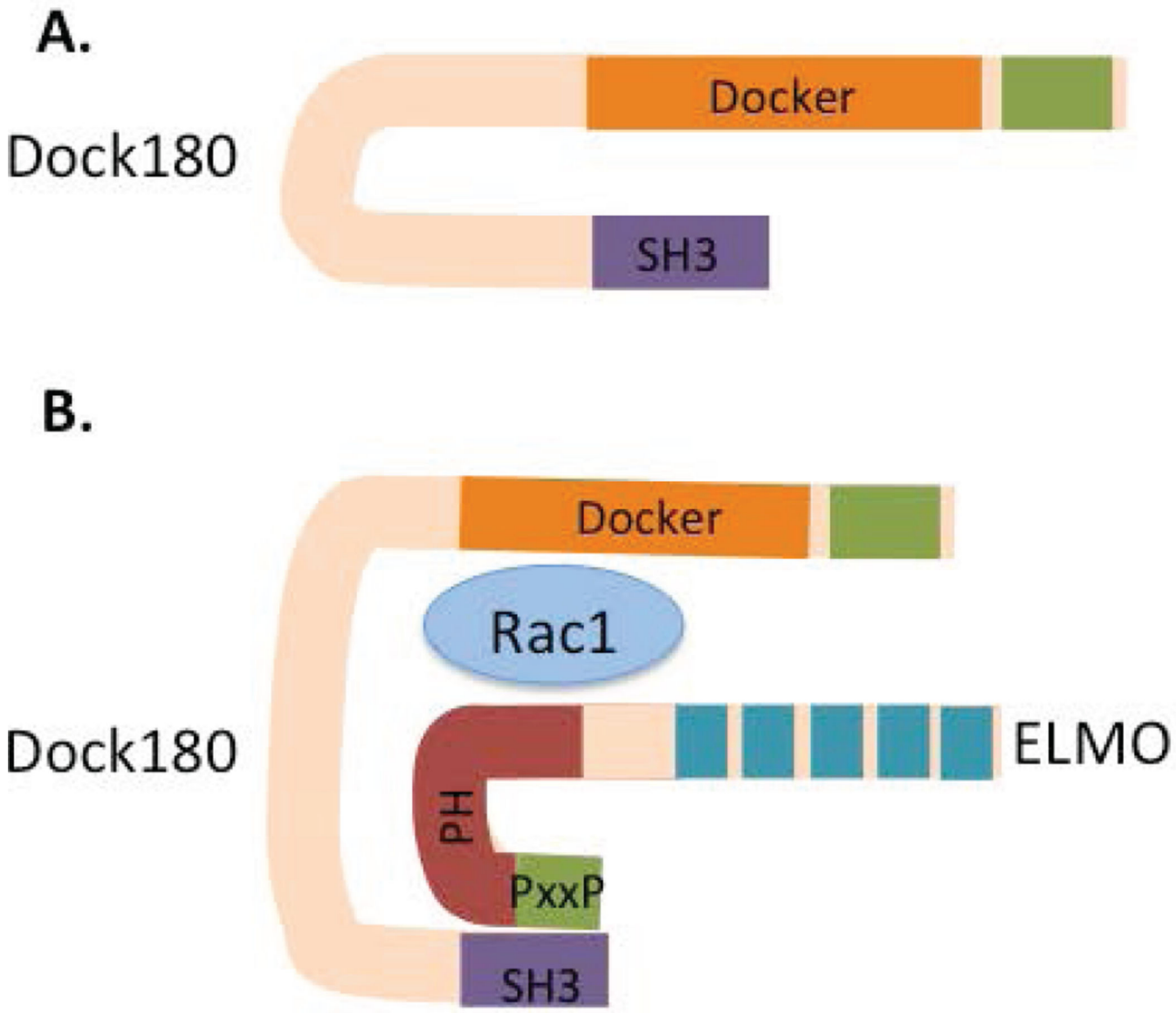

Figure 3. Schematic of ELMO influence on Dock180 and Rac

A. The SH3 domain (purple) on the N-terminal domain of Dock180 can bind to and inhibit the Docker domain (orange) activity in cis. B. The PxxP motif in the proline rich domain of ELMO binds to the SH3 domain of Dock180 to alleviate the steric autoinhibition. The docker domain (orange) is then 'opened' to interact with nucleotide-free Rac1. ELMO further stabilizes this Dock180 association with nucleotide free Rac in trans via its PH domain (red). The ARMADILLO repeats on ELMO in blue interact with RhoG and participate in binding to BAI1.

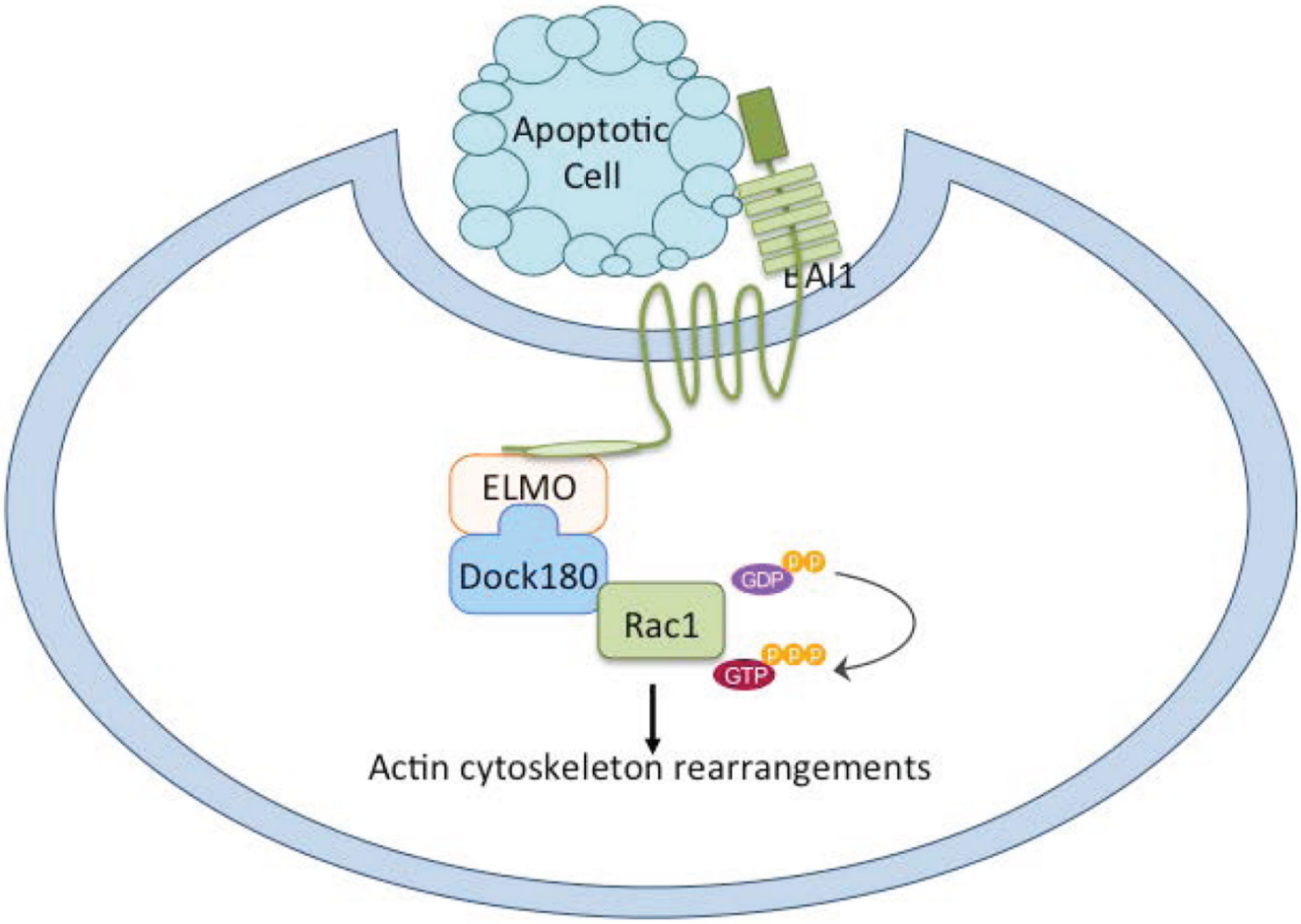

Figure 4. Schematic of BAI1 signaling

BAI1 binds PtdSer on an apoptotic cell via the 5 type1 thromobospondin repeats in its extracellular domain. BAI1 interacts with the N-terminal domain of ELMO via an alpha-helical domain in its intracellular tail. ELMO recruits Dock180 and promotes GEF activity. The GEF activity of the ELMO-Dock180 complex promotes Rac1 association with GTP and thereby mediates actin cytoskeletal reorganization during engulfment.

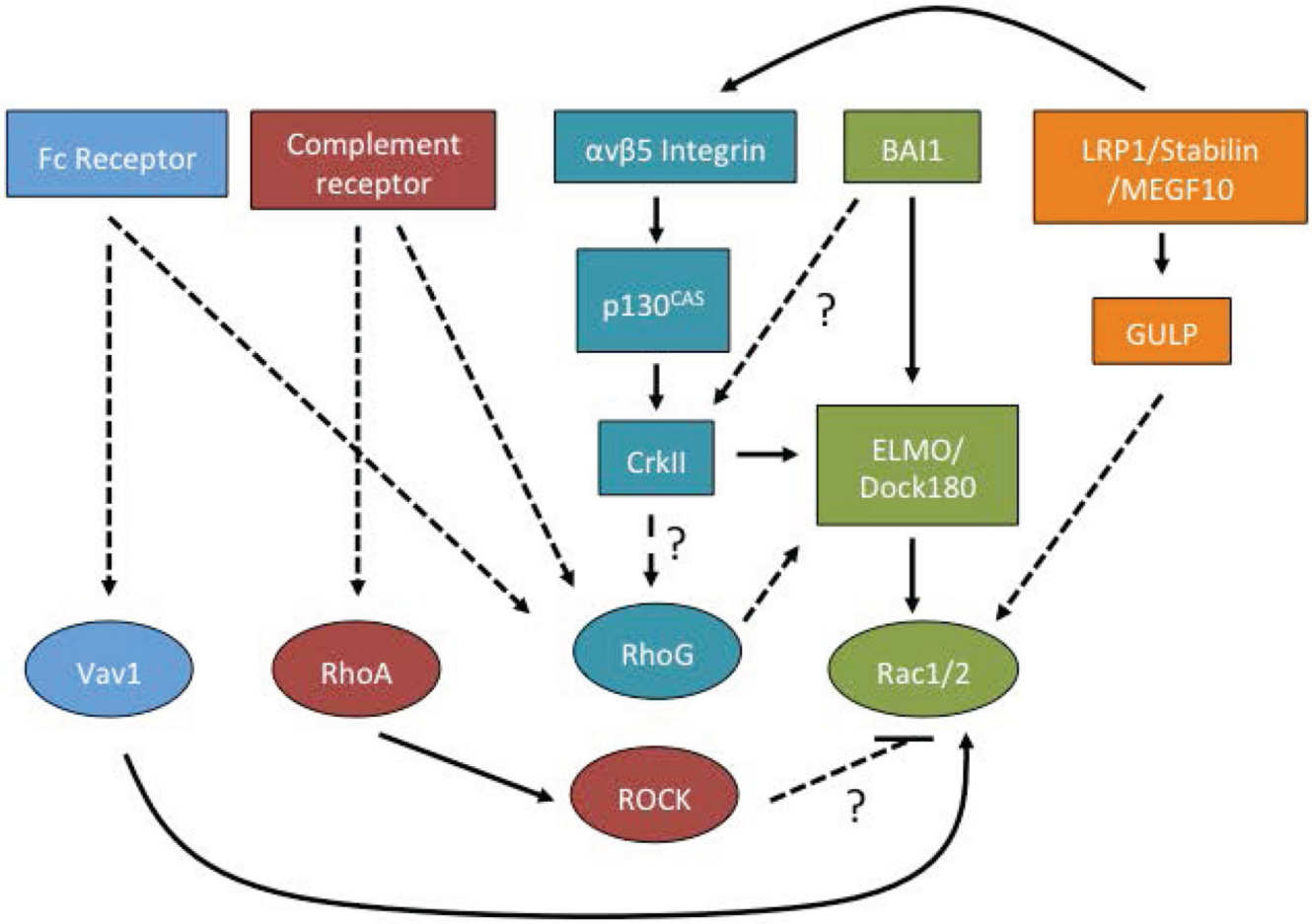

Figure 5. A network of signaling shows how multiple engulfment pathways intersect and influence each other

Apoptotic cell engulfment via integrins, BAI1 and CED-1 homologs utilize Rac1 to facilitate actin re-arrangement. The integrin pathway and CED-1 pathway can elicit ELMO/Dock180 activation via cooperation and via CrkII. Complement receptor mediated phagocytosis relies on RhoA activation. This pathway inhibits other engulfment pathways by activating Rho kinase (ROCK). RhoG promotes activity of multiple engulfment pathways. *Dashed arrows indicate indirect or less clearly defined interaction (s)*.

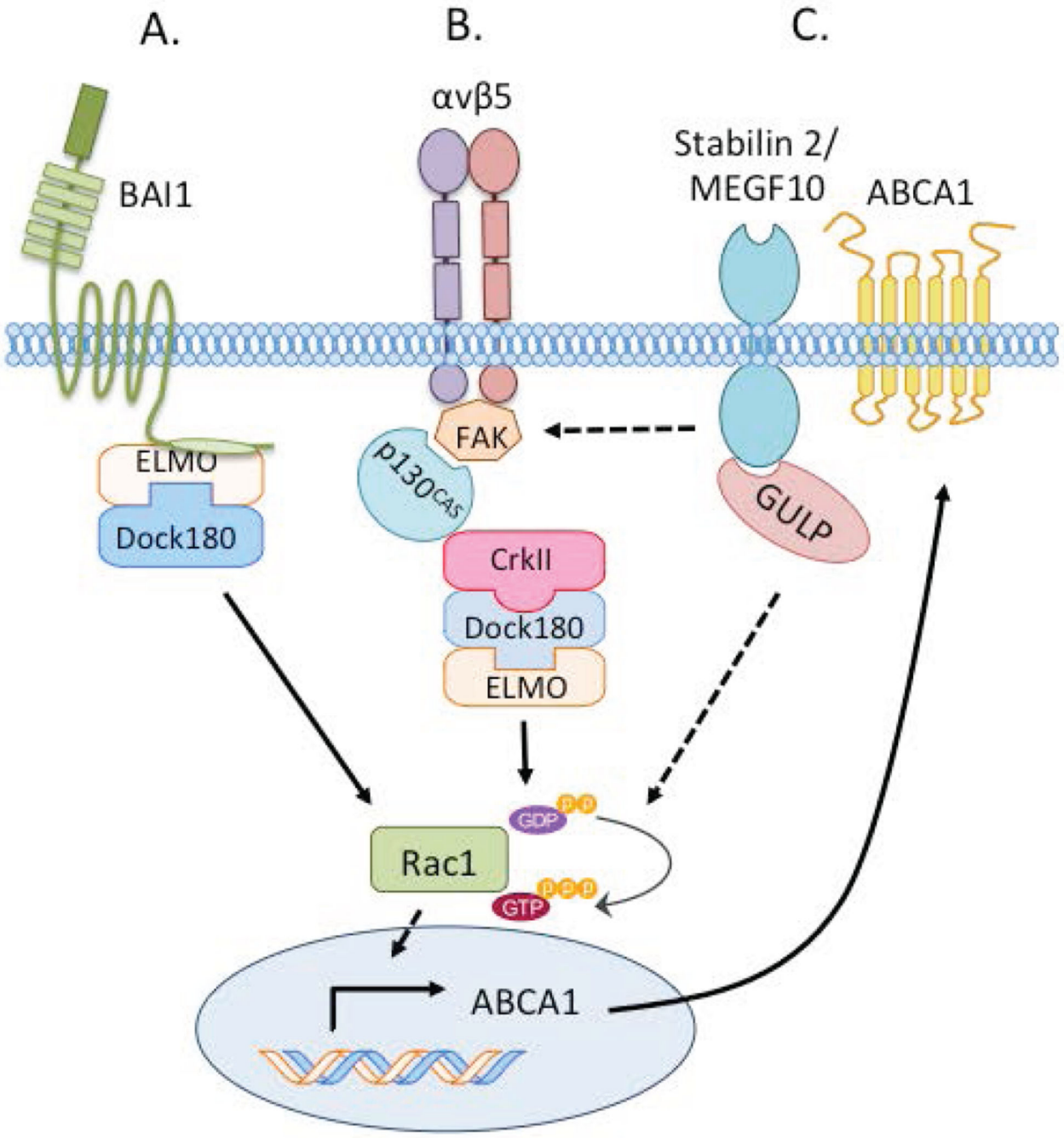

Figure 6. Multiple engulfment pathways utilize similar signaling components and influence each other

A. BAI1 engulfment pathway promotes ABCA1 transcription for cholesterol efflux at the cell membrane. B. Integrin mediated engulfment similarly relies on Dock180 and ELMO to elicit Rac1 activation. It is unclear whether this pathway also elicits ABCA1 transcription. C. Stabilin-2 can cooperate with integrins to promote RBC engulfment. Alternatively, stabilin-2 and its homolog MEGF10 can utilize the adapter GULP to elicit Rac1 activation.

ABCA1 is important for MEGF10 mediated engulfment but it is unknown how ABCA1 promotes GULP activity and binding to MEGF10.

Phosphatidylserine receptors: signaling and scavenging functions

Name(s)	PtdSer Binding mechanism	Signaling	Scavenging activity?	Current scavenger designation	Ref
Brain angiogenesis inhibitor 1 (BAI1)[#], adhesion G-protein coupled receptor 1	Direct binding via type 1 thrombospondin repeats	ELMO-Dock180-Rac1 module	Recognizes LPS on gram negative bacteria via thrombospondin repeats	None	(9, 18)
Brain angiogenesis inhibitor 3 (BAI3)[#], adhesion G-protein coupled receptor 3	Not characterized	ELMO-Dock180-Rac1 module	None characterized	None	(167, 168)
T-cell immunoglobulin and mucin receptor 1[#] **(TIM-1)**, Hepatitis C-virus receptor (HAVCR1), kidney injury molecule 1 (KIM1)	Direct binding via binding pocket in immunoglobulin domain	Possess tyrosine phosphorylation sites in intracellular domain – may signal via Fyn kinase	Binds to native and oxidized LDL, binds to hepatitis A virus	Proposed as a new class, J – characterized by immunoglobulin and mucin domains	(14, 16, 27, 28, 169)
T-cell immunoglobulin and mucin receptor 4[#] **(TIM-4)**, SMUCKLER	Direct binding via binding pocket within immunoglobulin domain	No signaling domain – reported to utilize integrins as co-receptors for signaling	None characterized	None	(31, 170)
Tyro3[#]	Indirect binding via Gas6, Protein S	Possess a tyrosine kinase domain, but precise substrates not yet defined	None characterized	None	(145, 171)
Axl[#]	Indirect binding via Gas6	Possess a tyrosine kinase domain, but precise substrates not yet defined; Can phosphorylate ELMO to elicit Rac1 activation	None characterized	None	(148, 172, 173)
MerTK[#]	Indirect binding via Gas6, Protein S, Tubby and Tubby-like protein	May associate with integrins for signaling – links to FAK phosphorylation and to ELMO-Dock180-Rac1 module	None characterized	None	(32, 143)
Stabilin 1[#] (FEEL-1)	Binds directly via EGF-like domain.	Signals via GULP to elicit Rac1 activation	Receptor for hyaluronic acid, oxLDL, AGE, gram negative bacteria, gram positive bacteria	Class H	(10, 174–178)
Stabilin 2[#], FEEL-2, HARE	Binds directly via EGF-like domain	Signals via GULP to elicit Rac1 activation	Receptor for hyaluronic acid, heparin, AGE, oxLDL, gram negative bacteria, gram positive bacteria	Class H	(10, 11, 129, 130, 179–181)
αvβ3[*†#]	Indirectly binds via MFGE8 – also called lactadherin	Known to link to CrkII, Dock180, and Rac in other systems	None characterized	None	(138, 182–184)
αvβ5[*†#]	Indirectly binds via MFGE8 also called lactadherin	Signals via FAK to ELMO-Dock180-Rac module	None characterized	None	(132, 138, 182–184)
Scavenger receptor type F family member 1 (SCARF1), SRECI	Reported to engage PtdSer via C1q.	Not defined for engulfment	Binds acetylated LDL and calreticulin	Class F	(185–187)

Name(s)	PtdSer Binding mechanism	Signaling	Scavenging activity?	Current scavenger designation	Ref
Low-density lipoprotein receptor-related protein 1 (LRP1)[*][∓][#], CD91, α macroglobulin receptor 2	Not defined, however the ortholog CED-1 binds via bridging protein TTR-52	Signals via GULP to elicit Rac1 activation	Over 40 ligands including lipoprotein and heat shock proteins	Although referred to in the literature as a 'scavenger' no official classification	(128, 188, 189)
MEGF10[*][∓][#]	Not defined, however the ortholog CED-1 binds via bridging protein TTR-52	Signals via GULP and Syk to elicit Rac1 activation	Binds β amyloid	Class F	(12, 134, 136, 189, 190)

[#]Receptors marked with this symbol possess a homolog in zebrafish.

[*]Receptors marked with this symbol possess a *C. elegans ortholog.*

[∓]Receptors marked with this symbol possess a *D. melanogaster* ortholog.