Review

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Putting the brakes on phagocytosis: "don't-eat-me" signaling in physiology and disease

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Abstract

Timely removal of dying or pathogenic cells by phagocytes is essential to maintaining host homeostasis. Phagocytes execute the clearance process with high fidelity while sparing healthy neighboring cells, and this process is at least partially regulated by the balance of "eat-me" and "don't-eat-me" signals expressed on the surface of host cells. Upon contact, eat-me signals activate "pro-phagocytic" receptors expressed on the phagocyte membrane and signal to promote phagocytosis. Conversely, don't-eat-me signals engage "anti-phagocytic" receptors to suppress phagocytosis. We review the current knowledge of don't-eat-me signaling in normal physiology and disease contexts where aberrant don't-eat-me signaling contributes to pathology.

Keywords 'don't-eat-me'; anti-phagocytic receptor; efferocytosis; ITIM; phagocytosis

Subject Categories Immunology; Membranes & Trafficking; Molecular Biology of Disease

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See the Glossary for abbreviations used in this article.

Introduction

Removal of unwanted or noxious cells is important for proper development, tissue integrity, and protection against pathogenic and immunogenic damage to the host (Arandjelovic & Ravichandran, 2015). Phagocytosis is a highly efficient process, and dying cells are rarely observed *in vivo* during homeostasis despite routine cellular turnover in several tissues (Elliott & Ravichandran, 2016). The "clearance crew" mediating phagocytosis can be divided into two broad categories: "professional phagocytes" and "non-professional phagocytes" (Arandjelovic & Ravichandran, 2015). Innate immune cells (e.g., immature dendritic cells, monocyte-derived macrophages, tissue-resident macrophages, and microglia) are considered professional phagocytes as they can sense and migrate toward target cells, as well as rapidly and successively internalize multiple targets (Elliott *et al,* 2009; Ariel & Ravichandran, 2016; Medina *et al,* 2020). Other tissue-resident cells (e.g., epithelial cells, fibroblasts, and endothelial cells) also play important roles in phagocytosis, albeit less efficiently, and are considered non-professional phagocytes (Wood *et al,* 2000; Monks *et al,* 2005; Juncadella *et al,* 2013; Arandjelovic & Ravichandran, 2015; Lee *et al,* 2016; Davies *et al,* 2018). The phagocytic response can be described in four phases: "smell", "taste", "ingestion", and "digestion/response"; and the molecular details promoting the clearance of dying cells have been excellently reviewed (Arandjelovic & Ravichandran, 2015; Morioka *et al,* 2019).

The phagocytic process must be tightly controlled, however, to prevent unwarranted removal of healthy cells. Regulation during the taste phase involves "integrated" recognition of eat-me signals and don't-eat-me signals expressed on the target cell. Eat-me signals include antibody and complement opsonins, exposed phosphatidylserine (PS), calreticulin, oxidized low-density lipoprotein, cell-bound thrombospondin (TSP), modified intracellular adhesion molecule ICAM-3, annexin I, and other modifications to surface proteins (Arandjelovic & Ravichandran, 2015). While living cells generally do not express eat-me signals, there are specific physiologic contexts when some living cells transiently express markers that partially mimic a dying cell such as during lymphocyte activation, skeletal muscle formation, and sperm–egg fusion (Elliott *et al,* 2005; Gardai *et al,* 2005; Hochreiter-Hufford *et al,* 2013; Hochreiter-Hufford *et al,* 2015; Rival *et al,* 2019). Thus, the expression of don't-eat-me signals provides an additional regulatory mechanism to prevent unwarranted clearance of "healthy" cells.

In this review, we discuss the current knowledge of don't-eat-me signaling in phagocytosis with an emphasis on anti-phagocytic receptors that mediate the recognition of don't-eat-me signals. Most known anti-phagocytic receptors are single-pass, type I transmembrane proteins belonging to the immunoglobulin (Ig) superfamily that contain one or more immunoreceptor tyrosine-based inhibitory motifs (ITIM) in their cytoplasmic tail (Daeron *et al,* 2008). The canonical ITIM consensus sequence is (I/V/L)x**Y**xx(L/V), where x can be any amino acid. Many anti-phagocytic receptors also have non-canonical "ITIM-like" motifs with a more divergent sequence (I/V/L/Sx**Y**xxL/V/I), as well as immunoreceptor tyrosine-based switch motifs (ITSM) and other protein-binding domains (Ravetch & Lanier, 2000; Daeron *et al,* 2008). In this review, we refer to all inhibitory motifs as ITIMs in an effort to reduce complexity. Anti-phagocytic

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Glossary

18F-FDG	fluorodeoxyglucose ^{18}F
Aβ	amyloid beta
AD	Alzheimer's disease
ADCP	antibody-dependent cellular phagocytosis
AIHA	autoimmune hemolytic anemia
B2M	beta-2 microglobulin
C3	complement component 3
cDC_2	classical dendritic cell 2
dLGN	dorsal lateral geniculate nucleus
F4/80	EGF-like module-containing mucin-like hormone receptor-like 1 (EMR1)
FAK	focal adhesion kinase
Fc	fragment crystallizable
Gas6	growth arrest-specific protein 6
Grb2	growth factor receptor-bound protein 2
ICAM-3	intracellular adhesion molecule-3
IFNγ	interferon gamma
Ig	immunoglobulin
IL-10	interleukin 10
IL-6	interleukin 6
ITIM	immunoreceptor tyrosine-based inhibitory motif
ITSM	immunoreceptor tyrosine-based switch motif
LILRB1	leukocyte immunoglobulin-like receptor B1
LRP1	low-density lipoprotein receptor-related protein 1
mAb	monoclonal antibody
MerTK	mer proto-oncogene tyrosine kinase
me^v	viable motheaten
MFG-E8	milk fat globule EGF factor VIII
MHC class I	major histocompatibility complex class I
MI	myocardial infarction
MIAT	myocardial infarction-associated transcript
MyD88	myeloid differentiation primary response 88
NF-κB	nuclear factor-kappa B
NK	natural Killer
NOD	non-obese diabetic
NSG	NOD scid gamma
PD-1	programmed cell death protein 1
PD-L1	programmed death ligand 1
PD-L2	programmed death ligand 2
PECAM-1	platelet endothelial cell adhesion molecule-1
PET/CT	positron emission tomography/computed tomography
PI3K	phosphoinositide 3-kinase
PLCγ	phospholipase C gamma
PS	phosphatidylserine
Pyk2	proline-rich tyrosine kinase 2
RGC	retinal ganglion cells
RhoA	Ras homolog family member A
RIG-I	retinoic acid-inducible gene I
SFK	Src family kinase
SH2	Src homology 2
SH3	Src homology 3
SHIP	Src homology 2 domain-containing inositol polyphosphate 5-phosphatase
SHP-1	Src homology region 2 domain-containing phosphatase-1
SHP-2	Src homology region 2 domain-containing phosphatase-2
Siglec	sialic acid-binding immunoglobulin-type lectin
SIRPα	signal regulatory protein α
SMCs	smooth muscle cells
SP-A	surfactant protein-A
SP-D	surfactant protein-D
Syk	spleen tyrosine kinase
TAM	tumor-associated macrophage
TGFβ	transforming growth factor β
TIDC	tumor-infiltrating dendritic cell
TLR	Toll-like receptor
TNFα	tumor necrosis factor α
TSP	thrombospondin

receptor activation induces tyrosine phosphorylation of the cytoplasmic ITIMs and binding of cytosolic SH2 domain-containing phosphatases, such as the SHP tyrosine phosphatases and the SHIP1/2 inositol phosphatases (Neel *et al,* 2003; Daeron *et al,* 2008). These phosphatases act as downstream effectors to mediate the inhibitory function of anti-phagocytic receptors, although their downstream substrates have been challenging to identify. In antibody-dependent cellular phagocytosis (ADCP) of opsonized targets, the FcγRIIb inhibitory receptor plays an important regulatory role via activation of SHIP1/2 (Aman *et al,* 2000; Getahun & Cambier, 2015). Most other anti-phagocytic receptors dependent on SHP-1 and/or SHP-2 tyrosine phosphatases for their inhibitory function, and we will focus our discussion on anti-phagocytic receptor signaling mediated by these tyrosine phosphatases (Getahun & Cambier, 2015). Structurally, SHP-1 and SHP-2 contain two N-terminal SH2 domains, a catalytic phosphatase domain and a C-terminal tail (Lorenz, 2009). Phosphorylation of two tandem ITIMs is thought to be required for SHP binding and activation downstream of anti-phagocytic receptors, but the exact molecular details are not entirely clear (Lorenz, 2009; Getahun & Cambier, 2015).

We will first discuss the CD47-SIRPα axis, as it represents one of the better characterized don't-eat-me checkpoints. Next, we will highlight what is known about other don't-eat-me checkpoints, and discuss several diseases with potential links to dysregulated phagocytosis as a result of aberrant anti-phagocytic signaling.

SIRPα as a prototypic anti-phagocytic receptor

Molecular characterization of SIRPα

SIRPα (also known as SIRPα1, PTPNS1, SHPS-1, BIT, p84, MFR, MyD-1, and CD172a) is a 115–120 kDa glycoprotein of the SIRP paired receptor family (Fujioka *et al,* 1996; Comu *et al,* 1997; Kharitonenkov *et al,* 1997; Yamao *et al,* 1997; Matozaki *et al,* 2009). It is expressed in most tissues and enriched on monocytes, macrophages, $CD8\alpha^-$ classical type II dendritic cells (cDC2), neutrophils, and osteoclasts, as well as microglia and neurons. It is also moderately expressed on fibroblasts, endothelial cells, and some epithelial cells (Adams *et al,* 1998; Veillette *et al,* 1998; Johansen & Brown, 2007). The extracellular region of SIRPα contains an N-terminal IgV domain followed by two IgC domains (Fig 1) (Fujioka *et al,* 1996; Kharitonenkov *et al,* 1997; Yamao *et al,* 1997). Species and tissue-specific differences in the molecular weight and ligand affinity of SIRPα are attributed to the highly polymorphic IgV domain and the varying number of N-linked glycosylation sites in the extracellular region (Yamao *et al,* 1997; Takenaka *et al,* 2007). Conversely, the cytoplasmic tail of SIRPα is highly conserved with four ITIMs and a proline-rich region predicted to bind SH3 domain-containing proteins (Fig 1) (Fujioka *et al,* 1996; Kharitonenkov *et al,* 1997; Yamao *et al,* 1997; Veillette *et al,* 1998). Although originally identified in 1990 as an adhesion protein on neurons, SIRPα was independently characterized as an ITIM-containing receptor and a substrate of activated

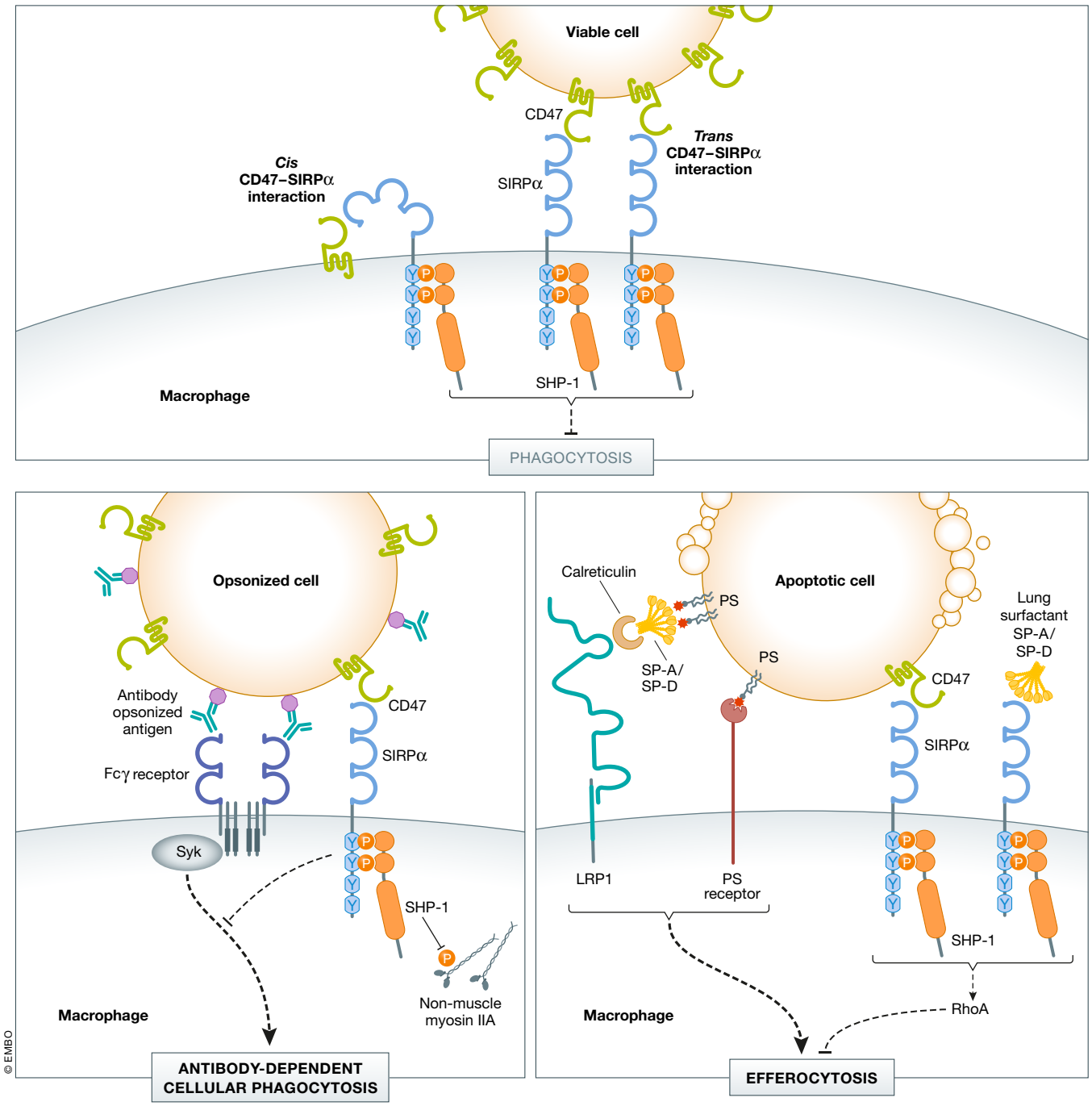

Figure 1. Inhibition of phagocytosis by SIRPα.

Engagement of the CD47-SIRPα axis occurs in *cis* and in *trans*, which induces tyrosine phosphorylation of the ITIMs in the cytoplasmic tail of SIRPα. Phosphorylation at Y429 and Y453 of human SIRPα mediate binding of tyrosine phosphatase SHP-1 (Myers *et al*, 2020). Dephosphorylation of non-muscle myosin IIA is one proposed substrate of SHP-1, resulting in disassembly of the actomyosin cytoskeleton. (top) Viable cells express don't-eat-me signals such as CD47 and lack expression of eat-me signals, thus limiting phagocytic clearance. (bottom-left) Antibody-dependent cellular phagocytosis (ADCP) is also negatively regulated by the CD47-SIRPα axis (Zent & Elliott, 2017). (bottom-right) Efferocytosis by alveolar macrophages in the lung can be negatively regulated by SIRPα activation in response to binding CD47 and surfactant proteins, SP-A and SP-D. The mechanism of this suppression is thought to involve activation of the GTPase RhoA (Janssen *et al*, 2008).

receptor tyrosine kinases in response to various growth factors and mitogens (Chuang & Lagenaur, 1990; Fujioka *et al*, 1996; Noguchi *et al*, 1996; Comu *et al*, 1997; Kharitonenkov *et al*, 1997; Yamao *et al*, 1997; Veillette *et al*, 1998). Additionally, SIRPα is tyrosine phosphorylated in response to integrin-mediated adhesion to specific extracellular matrix components (Tsuda *et al*, 1998; Oh *et al*, 1999).

The tyrosine kinases responsible for direct phosphorylation of SIRPα have remained unclear and may be context-specific; however, Src family kinases (SFK), as well as focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2), have been implicated in SIRPα signaling (Takeda *et al,* 1998; Timms *et al,* 1999). Nonetheless, tyrosine phosphorylation of SIRPα at specific ITIMs permits the binding of SHP-1 or SHP-2 via their SH2 domains, which relieves repression on the catalytic phosphatase domain and allows for dephosphorylation of their respective substrates (Neel *et al,* 2003; Lorenz, 2009). It remains unclear whether these phosphatases compete for the same binding site(s), or bind distinct sites on SIRPα; however, current evidence supports the latter model (Takada *et al,* 1998; Myers *et al,* 2020). In addition to being a binding partner, SIRPα is also a substrate of these phosphatases (Timms *et al,* 1998). In professional phagocytes such as macrophages, SHP-1 predominantly mediates inhibitory signaling downstream of SIRPα, consistent with the abundant expression of SHP-1 in hematopoietic cells (Veillette *et al,* 1998; Lorenz, 2009; Abram & Lowell, 2017). Conversely, SHP-2 is ubiquitously expressed and predominately binds SIRPα in non-hematopoietic cells in response to stimulation with growth factors, mitogens, and cellular adhesion (Kharitonenkov *et al,* 1997; Tsuda *et al,* 1998; Barclay & Van den Berg, 2014). While SHP-2 can modulate cytokine receptor signaling and macrophage activation states, whether it has a direct role in regulating phagocytosis is unclear (Neel *et al,* 2003; Tao *et al,* 2014; Niogret *et al,* 2019).

The best-known ligand of SIRPα is CD47 (also known as integrin-associated protein, OV-3, and Rh-related protein), which is a conserved, ubiquitously expressed 45–55-kDa transmembrane glycoprotein belonging to the Ig superfamily (Brown *et al,* 1990; Lindberg *et al,* 1993; Lindberg *et al,* 1994; Reinhold *et al,* 1995; Jiang *et al,* 1999). CD47 has a single N-terminal extracellular IgV domain followed by five hydrophobic membrane-spanning segments and a short C-terminal cytoplasmic tail that is alternately spliced to form four isoforms (Fig 1) (Brown *et al,* 1990; Lindberg *et al,* 1993; Reinhold *et al,* 1995). CD47-SIRPα binding via their IgV domains regulates many cellular processes, including leukocyte transmigration, lymphocyte homeostasis, dendritic cell maturation and activation, and bone resorption (Latour *et al,* 2001; de Vries *et al,* 2002; Liu *et al,* 2002; Hagnerud *et al,* 2006; Van *et al,* 2006; Lundberg *et al,* 2007; Saito *et al,* 2010; Legrand *et al,* 2011; Maile *et al,* 2011; Sato-Hashimoto *et al,* 2011). As we will discuss in the following sections, it is also one of the most studied don't-eat-me checkpoints in suppressing phagocytosis.

CD47 is a marker of "self" for phagocytosis

Seminal work by Oldenborg *et al* (2000) established CD47 as a marker of "self" on erythrocytes to prevent their premature clearance from circulation. The average circulating lifespan of wild-type murine erythrocytes is between 40 and 60 days (Goodman & Smith, 1961; Ishikawa-Sekigami *et al,* 2006; Oldenborg, 2013). In stark contrast, CD47-negative erythrocytes were shown to be rapidly cleared from circulation within 24 h of transfusion into syngeneic wild-type recipient mice in a manner independent of complement and adaptive immunity. Rather, splenic red pulp macrophages were found to facilitate the clearance of CD47-negative erythrocytes, as splenectomy and depletion of macrophages using liposomal clodronate permitted their circulation in wild-type mice. Moreover, blockade of SIRPα on wild-type splenic macrophages enhanced the clearance of wild-type erythrocytes *ex vivo*. Since this work, several studies over the past two decades have shown that other cell types use CD47 to avoid phagocytosis, including platelets, lymphocytes, and hematopoietic stem cells (Blazar *et al,* 2001; Yamao *et al,* 2002; Olsson *et al,* 2005; Ahrens *et al,* 2006; Ishikawa-Sekigami *et al,* 2006; Jaiswal *et al,* 2009; Catani *et al,* 2011; Kuriyama *et al,* 2012). These data are consistent with the mild thrombocytopenia and lymphopenia observed in mice globally deficient in either CD47 or SIRPα expression (Lindberg *et al,* 1996; Yamao *et al,* 2002; Ishikawa-Sekigami *et al,* 2006; Li *et al,* 2012). Recent evidence suggests that the CD47-SIRPα axis also regulates neuronal pruning by microglia during postnatal development (Lehrman *et al,* 2018). Retinal ganglion cells (RGCs) extend axons in the dorsal lateral geniculate nucleus (dLGN) of the thalamus to synapse with relay neurons (Guido, 2008). RGC inputs need to be refined for proper development of eye-specific territories, and this process involves phagocytosis of RGC inputs by microglia in the dLGN (Guido, 2008; Schafer *et al,* 2012). CD47 was found enriched on active RGC inputs and its expression protected against microglial phagocytosis in a manner dependent on SIRPα (Lehrman *et al,* 2018). An observed reduction in CD47 on less active RGC inputs suggests that microglia use this marker, at least in part, to decide which inputs to remove (Lehrman *et al,* 2018). Together, these data support a role for the CD47-SIRPα axis as a brake on the phagocytosis of "self".

Given the importance of the CD47-SIRPα axis in protecting host cells from phagocytosis, it is confounding that mice deficient in either CD47 or SIRPα expression do not have severe developmental or homeostatic abnormalities (Lindberg *et al,* 1996; Li *et al,* 2012). Moreover, CD47-deficient mice are tolerant to transfused syngeneic CD47-negative bone marrow cells, suggesting a more complex role for this signaling axis than previously thought (Blazar *et al,* 2001). A phagocytic "licensing" role for CD47 has been hypothesized, analogous to licensing of natural killer (NK) cells for functional cytotoxicity. Inhibitory receptors expressed on the surface of NK cells must interact with MHC class I molecules on host cells in order for NK cells to acquire proper cytotoxic function (Jonsson & Yokoyama, 2009). Elegant bone marrow reconstitution studies suggest that CD47 expression on non-hematopoietic cells is important for regulating CD47-dependent phagocytosis (Wang *et al,* 2007). Endogenous and donor CD47-negative leukocytes were tolerated in CD47-deficient mice that were either partially or fully reconstituted with wild-type bone marrow (Wang *et al,* 2007). Conversely, wild-type recipient mice reconstituted with bone marrow lacking CD47 expression rapidly eliminated donor CD47-negative cells.

Senescent erythrocytes are known to be cleared by F4/80^{high} splenic red pulp macrophages of yolk-sac and fetal liver origin; however, bone marrow-derived monocytes can contribute to the F4/80^{high} splenic macrophage population to a certain degree (Schulz *et al,* 2012; Guilliams & van de Laar, 2015; Gonzalez & Castrillo, 2018). Interestingly, both wild-type mice and CD47-deficient mice reconstituted with wild-type bone marrow rapidly eliminated donor CD47-negative erythrocytes 24 weeks post-bone marrow transplantation (Wang *et al,* 2007). Perhaps, in this situation the donor monocyte-derived splenic macrophages contributed to the clearance of CD47-deficient erythrocytes. Alternatively, erythrocytes may lack expression of other don't-eat-me signals expressed on nucleated cells, thus making CD47-deficient erythrocytes more vulnerable to clearance. Mechanism notwithstanding, these findings suggest an

important role for CD47 in phagocyte development and/or effector function, with relevance for CD47-based immunotherapies currently attempted.

CD47-SIRPα axis in antibody-dependent cellular phagocytosis

After identifying CD47 as a marker of "self" on erythrocytes, Oldenborg *et al* (2001) showed that the CD47-SIRPα axis suppresses pro-phagocytic signaling downstream of activated Fcγ receptors and complement receptors. In this study, IgG-opsonized CD47-negative erythrocytes were completely absent from circulation within 8 h post-transfusion into wild-type syngeneic recipient mice, whereas unopsonized CD47-negative erythrocytes and IgG-opsonized wild-type erythrocytes remained for > 24 h. These data are consistent with the earlier onset and more severe autoimmune hemolytic anemia (AIHA) observed in CD47-deficient non-obese diabetic (NOD) mice, which are prone to autoimmune diseases (Oldenborg *et al,* 2002; Wong *et al,* 2014). Moreover, IgG-opsonized wild-type and CD47-negative erythrocytes were also eliminated from circulation within 8 h post-transfusion into viable motheaten (me^v/me^v) recipient mice, which have markedly reduced SHP-1 phosphatase activity (Shultz *et al,* 1984; Kozlowski *et al,* 1993; Shultz *et al,* 1993; Oldenborg *et al,* 2001). While target cell binding and Fcγ receptor activation appear mostly unaffected by CD47-induced inhibitory signaling, the mechanism of suppression at least partially involves regulation of cytoskeletal elements important for target cell internalization (Lowry *et al,* 1998; May & Machesky, 2001; Diakonova *et al,* 2002; Kant *et al,* 2002; Tsai & Discher, 2008; Gomez & Descoteaux, 2018). Global tyrosine phosphorylation is reduced in phagocytes following activation of CD47-SIRPα signaling, including reduced tyrosine phosphorylation of the non-muscle myosin IIA motor protein, which was previously shown to be a direct substrate of SHP-1 following B-cell activation (Fig 1) (Baba *et al,* 2003; Tsai & Discher, 2008). CD47-SIRPα-mediated inhibition of inside-out integrin activation may also reduce macrophage spreading around the bound target cell (Tsai & Discher, 2008; Morrissey *et al,* 2020). Phagocytic receptors are suggested to be restricted in lateral movement on the plasma membrane due to interactions with the underlying cytoskeleton (Freeman *et al,* 2018). SIRPα has been shown to form clusters near FcγRI receptors in resting macrophages, and engagement of the CD47-SIRPα axis promotes receptor clustering, whereas unligated SIRPα is suggested to be excluded from the phagocytic cup following FcγRI stimulation with IgG or disruption of filamentous actin (Lopes *et al,* 2017). Thus, co-ligation of pro-phagocytic receptors (e.g., IgG activation of Fcγ receptors) and anti-phagocytic receptors (e.g., CD47-SIRPα axis) may function to fine-tune phagocytosis in some contexts, perhaps to allow for proper antigen digestion and presentation. In the absence of anti-phagocytic receptor co-ligation, such as during the clearance of apoptotic cells as we will discuss in the next section, these phagocytic "brakes" are mostly excluded from the phagocytic synapse.

Studies investigating the role of the CD47-SIRPα axis in phagocytosis have largely focused on *trans* engagement of CD47 expressed on the target cell with SIRPα expressed on the phagocyte (Fig 1). It is important to note that many professional and non-professional phagocytes express both CD47 and SIRPα on their surface. Recent work investigating the potential for *cis* engagement of the CD47-SIRPα axis showed that loss of CD47 on macrophages also enhanced phagocytosis of unopsonized and IgG-opsonized erythrocytes *in vitro* (Hayes *et al,* 2020). Additionally, compared to wild-type macrophages, CD47-deficient macrophages bound more soluble CD47, presumably due to the lack of *cis* CD47-SIRPα interactions. Basal tyrosine phosphorylation of macrophage SIRPα was also reduced following deletion of macrophage CD47, and the loss of phosphorylation signal correlated with the loss of CD47 expression (Johansen & Brown, 2007; Hayes *et al,* 2020). Together, these data support a model whereby the SIRPα extracellular domains bend over to interact with the CD47 IgV domain in *cis* to influence the dynamics of cell clearance (Fig 1). Interestingly, other immune receptor-ligand pairs have been shown to engage in *cis*, suggesting that this may be a more general regulatory mechanism for immune cell function such as phagocytosis (Doucey *et al,* 2004). Whether the signaling strength downstream of SIRPα differs by binding CD47 *in cis* versus *in trans* remains unclear (Hayes *et al,* 2020). Localization and clustering of CD47-SIRPα signaling complexes near the phagocytic cup is clearly an important determining factor as to the potency of inhibitory signaling and the overall impact on phagocytosis. The conformational state of these molecules may also influence differences between *cis* and *trans* signaling downstream of SIRPα (Hayes *et al,* 2020).

Together, these data support an inhibitory role for the CD47-SIRPα axis in ADCP via activation of SHP-1 and subsequent inhibition of contractile forces necessary for internalization of the target cell.

CD47-SIRPα axis in apoptotic cell clearance

Over 200–300 billion cells die in the human body every day as a part of routine cellular turnover, and most of these cells are thought to die by apoptosis (Arandjelovic & Ravichandran, 2015). The clearance of apoptotic cells, also referred to as efferocytosis, is not only immunologically silent, but also actively immunosuppressive via the secretion of anti-inflammatory mediators such as lactate, IL-10, and TGFβ (Gardai *et al,* 2003; Morioka *et al,* 2018; Perry *et al,* 2019). An early event following induction of apoptosis is loss of phospholipid asymmetry and exposure of phosphatidylserine (PS), a potent eat-me signal for efferocytosis, as well as membrane blebbing (Fig 1) (Fadok *et al,* 2001). Given the importance of the CD47-SIRPα axis in suppressing other forms of phagocytosis, its potential role in regulating efferocytosis has been explored, albeit to a lesser extent. Reduced CD47 expression is observed on some cell types (e.g., neutrophils, fibroblasts) following induction of apoptosis *in vitro*, as well as on senescent erythrocytes (Gardai *et al,* 2005; Khandelwal *et al,* 2007; Lv *et al,* 2015). However, CD47 expression changes are not evident on other cell types such as Jurkat T cells and thymocytes during early stages of apoptotic cell death. Changes in the localization of CD47 on the plasma membrane of dying cells have also been observed such that fewer molecules of CD47 engage SIRPα within the phagocytic synapse, thus reducing the binding avidity and inhibitory signaling strength downstream of SIRPα (Gardai *et al,* 2005; Lv *et al,* 2015). Further, tyrosine phosphorylation of SIRPα has been shown to be reduced in macrophages co-cultured with apoptotic cells, suggesting less involvement of the CD47-SIRPα axis in regulating efferocytosis. Interestingly, the activation of SIRPα signaling by cross-linking or recombinant CD47 has been shown to reduce efferocytosis *in vitro* (Gardai *et al,* 2005; Janssen *et al,* 2008; Lv *et al,* 2015). Thus, the CD47-SIRPα axis is capable of inhibiting efferocytosis, at least in some conditions. "Brakes" for phagocytosis such as the CD47-SIRPα axis may largely be excluded from the phagocytic synapse during efferocytosis to prevent delayed

clearance of apoptotic cells, which can result in secondary necrosis and chronic inflammatory pathologies (Morioka *et al,* 2019).

Paradoxically, a pro-phagocytic role for CD47 has also been suggested in efferocytosis. In the presence of serum, CD47-deficient apoptotic cells were shown to be engulfed less efficiently than their wild-type counterparts *in vitro* (Tada *et al,* 2003; Nilsson & Oldenborg, 2009). CD47 is known to bind the C-terminal RFYVVM domain of thrombospondins (TSP), which are platelet-derived soluble coagulation factors, and TSP-1 promotes efferocytosis by acting as a molecular bridge between apoptotic cells and specific pro-phagocytic receptors (e.g., CD36 and αvβ3 integrin) (Savill *et al,* 1992; Arandjelovic & Ravichandran, 2015). Moreover, erythrocyte aging studies suggest that oxidative stress induces a conformational change in CD47 that permits TSP-1 binding via its C-terminal domain, suggesting that under specific conditions CD47 may be converted to an eat-me signal (Gao *et al,* 1996a; Gao *et al,* 1996b; Burger *et al,* 2012). In some contexts, ligation of CD47 has also been reported to induce a form of cell death that is phenotypically similar to apoptosis, including PS exposure, but often lacks nuclear changes and is caspase-independent (Oldenborg, 2013). It is not clear to what extent, if any, CD47-induced cell death contributes to cellular turnover or the clearance process.

Other don't-eat-me ligands for SIRPα: lung surfactant proteins

Surfactant proteins A and D (SP-A and SP-D, respectively) are oligomeric structures of the collectin family that are present in lung surfactant and participate in innate host defense (LeVine & Whitsett, 2001; Wright, 2005). In addition to modulating inflammation, SP-A and SP-D can facilitate phagocytosis of microorganisms and apoptotic cells by alveolar macrophages (Schagat *et al,* 2001; Vandivier *et al,* 2002; Reidy & Wright, 2003). The globular lectin domain of SP-A and SP-D binds the target cell, and the collagenous domain binds to pro-phagocytic calreticulin/low-density lipoprotein receptor 1 (LRP1) complexes on the phagocyte membrane. Paradoxically, the globular lectin domain of SP-A and SP-D has also been shown to interact with SIRPα on alveolar macrophages to suppress efferocytosis and pro-inflammatory cytokine production via tyrosine phosphorylation of SHP-1 and activation of the GTPase, RhoA (Fig 1) (Gardai *et al,* 2003; Tosello-Trampont *et al,* 2003; Janssen *et al,* 2008). Alveolar macrophages deficient in SHP-1 demonstrated enhanced efferocytosis *in vitro*, presumably due to loss of inhibitory signaling downstream of the SP-A/SP-D:SIRPα axis (Janssen *et al,* 2008). However, peritoneal macrophages lacking SHP-1 were not significantly affected in efferocytosis, suggesting tissue context differences (Janssen *et al,* 2008). In response to lung infection and trauma, SIRPα was recently shown to suppress alveolar macrophage phagocytosis and promote an immunosuppressive microenvironment conducive to secondary infection (Roquilly *et al,* 2020). Collectively, these observations support a role for SIRPα in suppressing alveolar macrophage function. The mechanisms regulating the dichotomous functions of SP-A and SP-D are not clear and may depend on other factors present in the lung environment.

Additional anti-phagocytic receptors

In addition to SIRPα, several other ITIM-containing receptors have also been suggested to inhibit phagocytosis via activation of SHP-1 and/or SHP-2 tyrosine phosphatases. Many of these receptors have been studied in the context of disease, and further studies are needed to assess the roles of these inhibitory receptors in modulating phagocytosis in normal physiology. Of note, CD31 (also known as PECAM-1) is an ITIM-containing receptor that has been shown to promote de-attachment of viable cells from phagocytes; however, its proposed mechanism differs from the anti-phagocytic receptors discussed in this review (Brown *et al,* 2002).

CD300a and CD300f

Inhibitory receptors of the CD300 family include CD300a and CD300f, and both contain a single extracellular IgV domain (Borrego, 2013). In humans and mice, these inhibitory receptors are primarily expressed on myeloid cells, and to varying levels on some lymphoid subsets (Borrego, 2013). Phosphatidylserine (PS) is thought to be a ligand for CD300a and CD300f as both receptors have been shown to bind apoptotic cells and PS-containing liposomes in a calcium-dependent manner (Choi *et al,* 2011; Nakahashi-Oda *et al,* 2012a; Nakahashi-Oda *et al,* 2012b; Simhadri *et al,* 2012). Additionally, the PS-binding proteins annexin V and milk-fat globule EGF factor VIII (MFG-E8) can block apoptotic cell binding to CD300a and CD300f. Other lipids have been suggested as ligands for these receptors, but further studies are warranted to clarify conflicting studies (Borrego, 2013).

CD300a

The cytoplasmic tail of human CD300a contains four ITIMs, whereas mouse CD300a contains two ITIMs and a third tyrosine associated with a tyrosine-based sorting motif (Fig 2) (Borrego, 2013). Activation of CD300a by PS induces SHP-1 binding and inhibits efferocytosis (Nakahashi-Oda *et al,* 2012a; Nakahashi-Oda *et al,* 2012b; Simhadri *et al,* 2012). The PS-dependent inhibitory function of this receptor is confounding since PS is a potent eat-me signal for efferocytosis. A potential explanation is that CD300a may be important to inhibit inflammatory responses to apoptotic cell uptake, albeit potentially at the expense of reduced efferocytosis (Simhadri *et al,* 2012). In support of this hypothesis, CD300a expression is induced on several types of immune cells in response to inflammatory stimuli and has been shown to inhibit MyD88 inflammatory cytokine production (Kim *et al,* 2012; Borrego, 2013). Additionally, activation of CD300a on human neutrophils was shown to suppress production of reactive oxygen species in response to FcγRIIa stimulation (Alvarez *et al,* 2008). Thus, CD300a expression is modulated on several immune cell types in response to infection and downregulates inflammatory responses to prevent excessive tissue damage. Whether CD300a plays a role in regulating efferocytosis in the absence of inflammatory stimuli such as during homeostatic cell clearance warrants further investigation.

CD300f

Both human and mouse CD300f have three cytoplasmic ITIMs, as well as binding motifs for PI3K and Grb2 signaling molecules (Fig 2) (Borrego, 2013). The role of CD300f in regulating phagocytosis is more complex. Studies suggest both anti-phagocytic and pro-phagocytic functions, depending on the cell type and activation context, perhaps due to its ability to bind SHP-1 and SHP-2 (Borrego, 2013). Activation of CD300f was shown to promote apoptotic

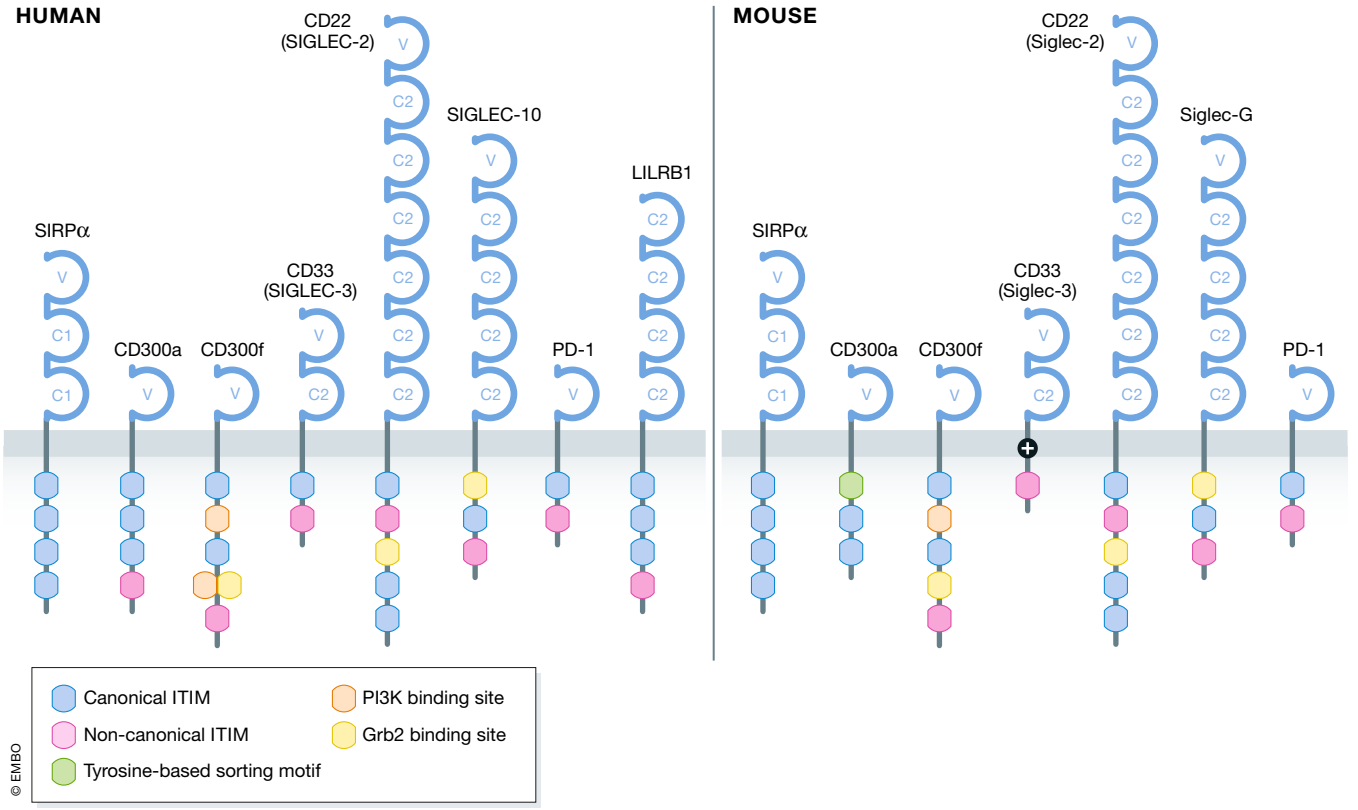

Figure 2. The structure of human and mouse anti-phagocytic receptors.

Several anti-phagocytic receptors have been identified in humans and mice. These are single-pass type I transmembrane receptors belonging to the immunoglobulin (Ig) superfamily and contain one or more immunoreceptor tyrosine-based inhibitory motifs (ITIM) in their cytoplasmic tails. Many of these receptors also have additional binding sites for signaling molecules that can endow these receptors with activation functions.

cell binding and internalization in a manner dependent on Y276 phosphorylation and p85α-PI3K signaling for phagocytic cup formation (Choi *et al,* 2011; Tian *et al,* 2014; Tian *et al,* 2016). Conversely, the other four tyrosines and expression of SHP-1 had an inhibitory effect on efferocytosis *in vitro*, and loss of CD300f enhanced efferocytosis and T-cell priming by dendritic cells, suggesting that it also functions as an anti-phagocytic receptor (Tian *et al,* 2014; Tian *et al,* 2016). Thus, the role of CD300f in modulating efferocytosis is complex and likely depends on interactions with other receptors, as well as available signaling molecules within different types of phagocytes.

Siglecs

Siglecs, or sialic acid-binding Ig-like lectins, are a large family of receptors primarily expressed on immune cells and have important roles in attenuating inflammatory responses to pathogen and damage-associated molecular patterns, mediating adhesion and phagocytosis, and regulating immune cell activation (Pillai *et al,* 2012). Many of these lectins bind specific sialic acid-containing glycoconjugates *in cis*, as well as *in trans*, to fine-tune responses following immune cell activation (Pillai *et al,* 2012). Below, we will focus our discussion on three Siglecs suggested to function as anti-phagocytic receptors.

CD33/Siglec-3

Human CD33 (also called Siglec-3) has an extracellular region consisting of one IgV domain and one IgC domain, and a cytoplasmic tail containing two ITIMs (Fig 2) (Ulyanova *et al,* 1999; Pillai *et al,* 2012). It is expressed on cells of the myeloid lineage and microglia (Pillai *et al,* 2012; Griciuc *et al,* 2013). Human CD33 binds α2,3- and α2,6-linked sialic acid-containing glycans (Brinkman-Vand der Linden *et al,* 2003). Activation of CD33 induces SHP-1 binding, and the membrane proximal ITIM is necessary and sufficient for SHP-1 activation (Ulyanova *et al,* 1999). Genome-wide association studies of genetic variants associated with Alzheimer's disease (AD) suggested a potential link to the CD33 loci (Hollingworth *et al,* 2011; Naj *et al,* 2011). Further analysis revealed a positive correlation in CD33 expression on microglia and the presence of insoluble Aβ42 levels and amyloid plaque burden in AD patients (Griciuc *et al,* 2013). Moreover, loss of CD33 in mouse models of AD was sufficient to reduce insoluble Aβ42 levels and amyloid plaque burden via enhanced uptake by microglia (Griciuc *et al,* 2013; Griciuc *et al,* 2019). These studies suggest CD33 as an anti-phagocytic receptor on microglia and its potential involvement in AD pathogenesis.

Unlike human CD33, the mouse ortholog has one ITIM and a positively charged lysine residue in the transmembrane region that

forms a putative binding site for signaling molecules associated with immune cell activation (Fig 2) (Brinkman-Vand der Linden *et al,* 2003; Pillai *et al,* 2012). Given these structural differences, divergent functions between human and mouse CD33 orthologs may exist and one study suggests that mouse CD33 may not regulate Aβ42 phagocytosis (Bhattacherjee *et al,* 2019). The reasons for these conflicting data are not clear.

CD22/Siglec-2

CD22 (also called Siglec-2) has an extracellular region that contains an N-terminal IgV domain followed by six IgC domains, and the cytoplasmic tail contains four ITIMs and a Grb2 binding motif (Fig 2) (Pillai *et al,* 2012). It is highly expressed in B cells, as well as some myeloid-derived cells and microglia (Pillai *et al,* 2012). Mouse CD22 binds Neu5Gc glycans, and human CD22 binds both Neu5Ac and Neu5Gc glycans, although the specific glycoconjugates are not fully clear (Pillai *et al,* 2012). Activation of CD22 has been shown to bind and activate SHP-1, as well as a variety of other signaling molecules (Doody *et al,* 1995; Law *et al,* 1996; Blasioli *et al,* 1999; Poe *et al,* 2000; Otipoby *et al,* 2001). A recent study investigating age-related genes involved in microglial phagocytosis found CD22 enriched on microglia from aged mice and its expression negatively correlated with cognitive function (Pluvinage *et al,* 2019). CD22 was shown to inhibit microglial phagocytosis, presumably via activation of SHP-1, and loss of CD22 enhanced the clearance of several neurodegenerative factors, including myelin debris, Aβ oligomers, and α-synuclein fibrils *in vitro* and *in vivo* (Pluvinage *et al,* 2019). Thus, in addition to CD33, this study suggests that microglia also utilize CD22 as an anti-phagocytic receptor to restrict phagocytosis in the central nervous system, perhaps in early development as well as pathological contexts when there is an overabundance of cellular debris. Additional studies are needed to assess whether blockade of CD22 is of benefit in treating age-related cognitive decline and neurodegenerative diseases.

Siglec-10

Human Siglec-10, or mouse Siglec-G, has an extracellular region consisting of an N-terminal IgV domain followed by four IgC domains, and a cytoplasmic tail containing two ITIMs as well as a Grb2-binding motif (Fig 2) (Munday *et al,* 2001; Pillai *et al,* 2012). It is expressed on myeloid cells and some lymphocytes, and binds both SHP-1 and SHP-2 (Munday *et al,* 2001; Whitney *et al,* 2001; Pillai *et al,* 2012). CD24 is a sialylated glycosyl phosphoinositol-anchored protein and a cognate ligand of human Siglec-10, and mouse Siglec-G (Chen *et al,* 2009). Signaling through this axis suppresses NF-κB-mediated inflammation in response to damage-associated molecular patterns via SHP-1 activation (Chen *et al,* 2009; Chen *et al,* 2011). Additionally, NF-κB activation has been shown to increase Siglec-G expression in innate immune cells in response to viral infection, leading to retinoic acid-inducible gene I (RIG-I) degradation and attenuation of anti-viral responses in a manner involving SHP-2 (Chen *et al,* 2013). Mouse Siglec-G has also been shown to negatively regulate MHC class I-peptide cross-presentation by $CD8\alpha^{+}$ dendritic cells (Ding *et al,* 2016). Mechanistically, the activation of SHP-1 downstream of Siglec-G led to an increase in phagolysosomal pH such that MHC class I-peptide complex formation was impaired (Ding *et al,* 2016). Recently, CD24 was also identified as a don't-eat-me signal that is highly expressed on several cancers (Barkal *et al,* 2019). This study showed that tumor-associated macrophages (TAMs) from ovarian and breast tumors also expressed Siglec-10 (Barkal *et al,* 2019). Blockade or genetic depletion of either CD24 on tumor cells, or Siglec-10 on macrophages, enhanced tumor cell clearance *in vitro* and *in vivo,* presumably by disrupting *trans* engagement of this inhibitory axis (Barkal *et al,* 2019). Thus, in addition to dampening inflammatory responses, the CD24-Siglec-10 axis also regulates phagocytosis processes in professional phagocytes. Additional studies are needed to investigate the mechanism(s) driving increased expressed of CD24 on tumor cells, and Siglec-10 expression on TAMs, and if blockade of this signaling axis would provide enhanced anti-tumor immunity in cancers resistant to clinically available therapies. Future studies should also evaluate dysregulation of this don't-eat-me signaling axis in other contexts where impaired phagocytosis is evident such as in cardiovascular diseases.

PD-1

PD-1 (also known as programmed cell death protein 1 and CD279) is a well-known immune inhibitory receptor of the B7/CD28 family and has been extensively studied in suppressing T-cell activation, at least in part via SHP-2 (Okazaki *et al,* 2013; Rota *et al,* 2018; Marasco *et al,* 2020; Patsoukis *et al,* 2020). Structurally, it has one extracellular IgV domain and two cytoplasmic ITIMs (Fig 2) (Patsoukis *et al,* 2020). Its two cognate ligands are PD-L1 (also known as CD274 and B7-H1) expressed on non-lymphoid cells, and PD-L2 (also known as CD273 and B7-DC) expressed on antigen-presenting cells (Okazaki *et al,* 2013). In addition to being expressed on lymphocytes, PD-1 expression is induced in macrophages in response to infection, which is negatively associated with an inflammatory macrophage phenotype and pathogen clearance (Huang *et al,* 2009; Shen *et al,* 2016; Gordon *et al,* 2017). Upon microbial infection, Toll-like receptor/NF-κB activation induces PD-1 expression in macrophages, and PD-1 activation in turn suppresses NF-κB/p65-mediated inflammatory responses (Bally *et al,* 2015; Chen *et al,* 2016). In several cancers, a subset of TAMs and tumor-infiltrating dendritic cells (TIDCs) highly express PD-1, which negatively correlates with anti-tumor immune responses (Karyampudi *et al,* 2016; Gordon *et al,* 2017). Specifically, PD-1 expression on TAMs correlated with reduced phagocytosis of tumor cells, and inhibition of the PD-L1:PD-1 axis in lymphocyte-deficient tumor-bearing mice reduced tumor burden and extended survival, suggesting its potential role in don't-eat-me signaling (Gordon *et al,* 2017). Together, these studies establish a model whereby PD-1 is induced in innate immune cells to modulate their inflammatory state and act as a rheostat to prevent excessive inflammation and tissue damage, which can indirectly influence phagocytic activity to specific targets (Yurdagul *et al,* 2020). Additional studies are needed to assess whether PD-1 plays a more direct role in modulating phagocytic processes such as target cell internalization.

LILRB1

Human LILRB1 (leukocyte immunoglobulin-like receptor B1; also known as CD85J, ILT2, LIR-1) is an inhibitory receptor expressed on myeloid cells and subsets of lymphoid cells (Borges *et al,* 1997; Colonna *et al,* 1997; Fanger *et al,* 1998). Structurally, it has an extracellular region consisting of four IgC domains and a cytoplasmic tail containing four ITIMs (Fig 2) (Borges *et al,* 1997; Samaridis & Colonna, 1997). Tyrosine phosphorylation of the two membrane-distal

ITIMs permits SHP-1 binding and activation (Fanger *et al,* 1998; Bellon *et al,* 2002). LILRB1 binds MHC class I molecules expressed on all nucleated cells, and the invariant β_2-microglobulin subunit of MHC class I has been shown to be important for this interaction (Borges *et al,* 1997; Colonna *et al,* 1997; Fanger *et al,* 1998; Barkal *et al,* 2018). In a recent study, MHC class I expression was shown to protect some cancer cells from phagocytosis via engagement of LILRB1 (Barkal *et al,* 2018). Loss of either MHC class I or LILRB1 was shown to enhance tumor cell phagocytosis *in vitro* when combined with antibody opsonization to provide eat-me signaling through Fc receptor activation (Barkal *et al,* 2018). Additionally, loss of both MHC class I and CD47 don't-eat-me signals on tumor cells provided better anti-tumor immune responses in a manner dependent on macrophages, as well as contribution from adaptive immune cells *in vivo* (Barkal *et al,* 2018). This study highlights MHC class I as an additional don't-eat-me signal utilized by some cancers to avoid clearance. Activation of LILRB1-SHP1 inhibitory signaling has previously been shown to suppress early tyrosine phosphorylation events downstream of FcγRI signaling (Fanger *et al,* 1998). Thus, it is likely that engagement of the MHC class I-LILRB1 axis suppresses ADCP, and loss of this inhibitory axis allows for better uptake of opsonized cells.

Regulation gone awry: don't-eat-me signaling in disease

The expression of don't-eat-me signals by host cells is thought to prevent their unwarranted removal by neighboring phagocytes, and dysregulation of this innate immune checkpoint has pathological consequences. In addition to the studies discussed in the previous sections, below we highlight two disease categories where enhanced CD47 expression contributes to pathology.

Cancer

CD47 expression is upregulated in many cancers, including both hematologic and solid tumor malignancies, and its expression correlates with poor prognosis (Campbell *et al,* 1992; Jaiswal *et al,* 2009; Majeti *et al,* 2009; Willingham *et al,* 2012; Weiskopf *et al,* 2016; Michaels *et al,* 2018). The mechanisms contributing to enhanced CD47 expression differ among cancer subtypes, but aberrant transcriptional regulation is a commonality (Zhang *et al,* 2015; Case y *et al,* 2016; Betancur *et al,* 2017; Liu *et al,* 2017). Blocking the CD47-SIRPα axis was shown to promote phagocytosis of tumor cells *in vitro,* and tumor regression in animal models, when an eat-me signal was available to activate pro-phagocytic signaling (Fig 3). A first-in-human phase I trial evaluated the efficacy of a humanized anti-CD47 monoclonal antibody (called magrolimab or Hu5F9-G4) as monotherapy for advanced solid tumors and lymphomas, but limited anti-tumor responses were observed (Sikic *et al,* 2019; Veillette & Tang, 2019). Conversely, Advanti *et al* reported partial or complete responses in 50% of patients with relapsed/refractory non-Hodgkin's lymphoma enrolled in a small phase 1b trial where patients received co-administration of magrolimab and rituximab (an anti-CD20 monoclonal antibody). Additionally, 95% of responding patients were previously refractory to single agent rituximab treatment (Advani *et al,* 2018; Veillette & Tang, 2019). These studies provided the first clinical evidence that loss of CD47 don't-eat-me signaling promotes anti-tumor immunity at least partially via ADCP. Mild anemia and lymphopenia were the only low-grade adverse events observed with magrolimab treatment, suggesting promising tolerability to CD47 blockade, albeit larger trials with longer follow-ups are needed to fully assess treatment-related toxicities, including potential cognitive-related issues (Advani *et al,* 2018; Sikic *et al,* 2019; Veillette & Tang, 2019). Additional clinical trials investigating the efficacy and safety of immunotherapies targeting the CD47-SIRPα axis are currently underway for both hematologic and solid tumors, including their use as single agents and in combination with other checkpoint blockade therapies, as well as azacytidine—a hypomethylating agent (Feng *et al,* 2019). Blocking strategies include the use of several different anti-CD47 monoclonal antibodies, as well as a recombinant SIRPαFc fusion protein (TTI-621) consisting of the Fc region of human IgG1 and the extracellular IgV domain of human SIRPα (Feng *et al,* 2019). Altogether, these initial observations suggest that immunotherapies targeting the CD47-SIRPα axis to enhanced tumor cell ADCP are well-tolerated and demonstrate promising anti-tumor effects. Future studies investigating the efficacy of targeting other don't-eat-me signaling molecules are needed for cancers showing resistance to CD47 blockade (Barkal *et al,* 2018).

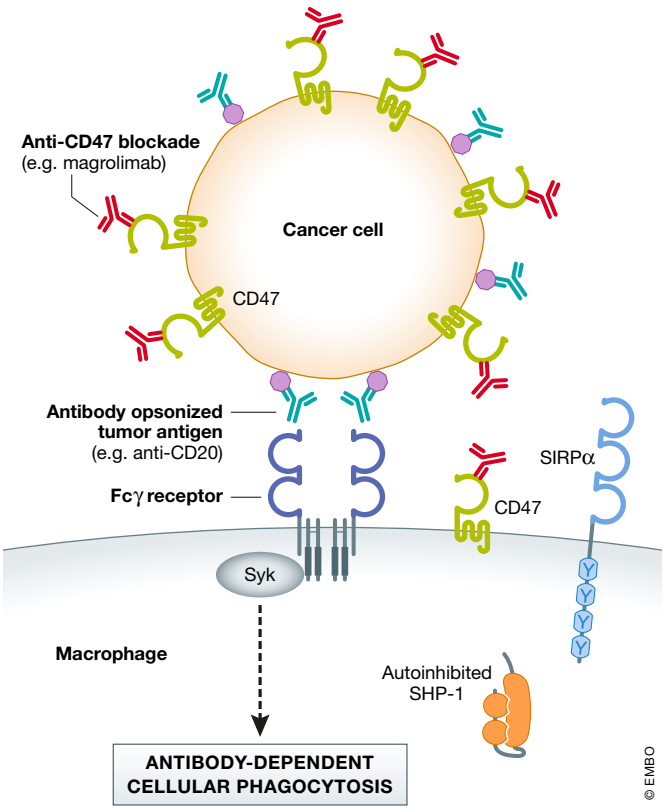

Figure 3. Therapeutically blocking the CD47-SIRPα axis to enhance cancer cell phagocytosis.

Many cancer cells have increased expression of CD47. Preclinical and clinical studies have shown promise in enhancing anti-tumor immunity when anti-CD47 blockade is used in combination with other immunotherapies such as the anti-CD20 monoclonal antibody Rituximab. This strategy provides an eat-me signal via activation of antibody-dependent cellular phagocytosis (ADCP) while concurrently blocking the CD47-SIRPα don't-eat-me signaling axis.

Cardiovascular disease

Atherosclerosis

Atherosclerosis is a major contributing factor to cardiovascular and cerebrovascular diseases, and mortality worldwide (Benjamin *et al,* 2019). In the early stages of atherosclerosis, modified circulating lipoproteins become trapped in the subendothelial space of arteries and stimulate inflammatory leukocyte recruitment into the vessel wall (Doran *et al,* 2019). A portion of macrophages that phagocytose entrapped lipoproteins become cholesterol-laden and undergo apoptosis (Yurdagul *et al,* 2018; Morioka *et al,* 2019). These dying cells are removed by other plaque-associated macrophages, but over time efferocytosis becomes impaired in diseased vessels and advanced plaques form. The accumulation of uncleared dying cells forms the "necrotic core" of advanced plaques, which are prone to rupture.

A combination of aberrations contributes to impaired efferocytosis in atherosclerotic lesions. These include reduced surface expression of pro-phagocytic receptors such as LRP1 and the Mer proto-oncogene tyrosine kinase (MerTK) via proteolytic cleavage and receptor degradation (Fig 4) (Doran *et al,* 2019). Additionally, enhanced CD47 expression was observed in human lesions, and blockade of CD47 in murine atherosclerotic models showed fewer plaque-associated dead cells and smaller necrotic cores (Kojima *et al,* 2016; Gerlach *et al,* 2020). One mechanism contributing to enhanced CD47 expression on plaque cells involved NF-κB

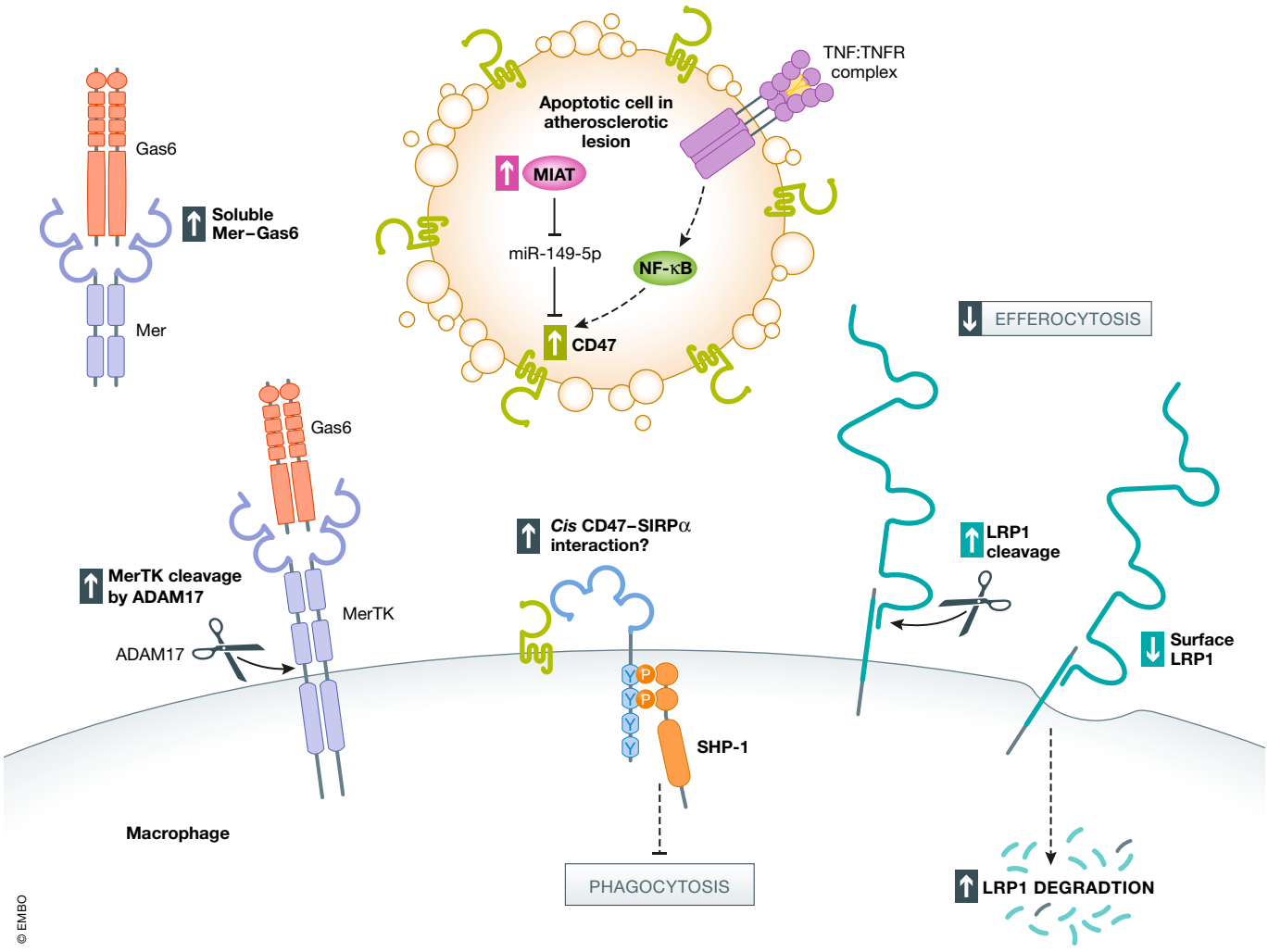

Figure 4. Impaired efferocytosis in cardiovascular disease.

Several mechanisms contribute to impaired efferocytosis in cardiovascular diseases, such as atherosclerosis (Doran *et al,* 2019). Tumor necrosis factor (TNF) signaling through the TNF receptor activates the nuclear factor-κB pathway leading to enhanced CD47 expression on apoptotic cells in atherosclerotic lesions. Enhanced expression of the "myocardial infarct-associated transcript" (MIAT) long non-coding RNA inhibits processing of the microRNA, miR-149-5p, and in turn, results in increased CD47 expression. CD47 can bind SIRPα *in cis* on the phagocyte surface to inhibit phagocytosis. Loss of pro-phagocytic receptors on the phagocyte surface including LRP1 and MerTK contributes to impaired efferocytosis. Metalloproteinase ADAM17 cleaves MerTK, resulting in soluble Mer that may act to sequester growth arrest-specific protein 6 (Gas6) needed for recognition of phosphatidylserine (PS) on the surface of apoptotic cells. Parts of this figure were adapted from Doran *et al* (2019).

activation downstream of tumor necrosis factor (TNF) signaling (Kojima *et al,* 2016). In agreement with this finding, other reports in a variety of disease contexts have also linked pro-inflammatory signaling to increased CD47 expression, suggesting that CD47 is induced in response to some inflammatory conditions to fine-tune inflammatory responses (Betancur *et al,* 2017; Cui *et al,* 2020; Tal *et al,* 2020; Wang *et al,* 2020). It is possible that increased CD47 expression on plaque-associated phagocytes may also contribute to impaired efferocytosis via *cis* engagement of the CD47-SIRPα axis (Fig 4) (Hayes *et al,* 2020; Wang *et al,* 2020). Increased expression of the long non-coding RNA called "myocardial infarction-associated transcript" (MIAT) was also observed in advanced lesions (Ye *et al,* 2019). MIAT was shown to negatively regulate the microRNA miR-149-5p, thereby relieving repression of CD47 expression (Fig 4) (Ye *et al,* 2019). Knockdown of MIAT *in vitro* and in murine atherosclerosis promoted efferocytosis, reduced plaque necrosis, and improved plaque stability, presumably via reduced CD47 expression (Ye *et al,* 2019).

Potential therapeutic benefit in blocking CD47 for atherosclerosis was suggested based on a retrospective analysis of 9 patients enrolled on the Advanti trial (Jarr *et al,* 2021). Reduced inflammation in the carotid arteries was observed by 18F-FDG PET/CT scans following combination treatment with magrolimab and rituximab. Future trials directly evaluating the efficacy of blocking CD47 for treating atherosclerosis are needed. It should also be noted that one study using CD47-deficient mice with hypercholesterolemia showed larger atherosclerotic plaques compared with wild-type mice, and this phenotype was associated with enhanced lymphocyte activation, particularly INFγ-producing $CD90^{+}$ NK cells (Engelbertsen *et al,* 2019). Thus, CD47 appears to play a more general role in controlling both innate and adaptive immune responses, and detailed studies are needed to evaluate both on-target and off-target effects of CD47 blockade.

Myocardial infarction

Heart failure after myocardial infarction (MI) is another major source of cardiac-related mortality (Frangogiannis, 2012). Efficient clearance of dying cardiomyocytes and timely resolution of acute inflammation following myocardial infarction is critical to cardiac repair (Frangogiannis, 2012; Doran *et al,* 2019). Cleavage of the MerTK receptor is one mechanism of impaired cardiomyocyte efferocytosis (Wan *et al,* 2013; DeBerge *et al,* 2017). Cardiomyocytes associated with infarcted tissue also express more CD47, and acute blockade of CD47 or SIRPα during reperfusion injury could enhance cardiomyocyte clearance and reduce infarct size and collagen content (Zhang *et al,* 2017). The mechanisms underlying enhanced CD47 expression on cardiomyocytes in infarcted tissue remain unclear, and additional studies are needed to assess the benefit of blocking the CD47-SIRPα axis following ischemic injury.

While professional phagocytes are important for reparative responses post-myocardial infarct, non-professional phagocytes such as cardiac myofibroblasts can assist in the clearance process (Nakaya *et al,* 2017). The physiological response to myofibroblast efferocytosis in the damaged myocardium remains unclear. It is also not known if efferocytosis by non-professional phagocytes such as cardiac myofibroblasts is regulated by don't-eat-me signaling, and future studies are needed to explore this area of phagocytosis research in more depth.

Conclusions

Efficient clearance of dying and pathogenic cells is critical to preventing unnecessary inflammation and maintaining immune quiescence. However, this process must be tightly controlled to avoid aberrant removal of healthy cells. Regulation during the taste phase of phagocytosis involves phagocyte recognition of eat-me and don't-eat-me signals expressed on host cells. In general, it is thought that don't-eat-me signaling raises the activation threshold needed for phagocytosis to occur.

In ADCP, evidence supports a role for the CD47-SIRPα axis, and other don't-eat-me checkpoints, in fine-tuning the clearance response. Many anti-phagocytic receptors play important roles in controlling inflammatory responses by suppressing cytokine and TLR signaling, and in some cases, antigen presentation for the activation of adaptive immune responses. Additional studies are needed to decipher how don't-eat-me signaling directly regulates phagocytosis, and this includes identifying the cellular substrates of SHP-1 and SHP-2 following anti-phagocytic receptor activation. Specifically, whether regulation beyond suppression of integrin activation and the actomyosin cytoskeleton occurs needs to be defined (see also Box 1).

Box 1: In need of answers

- What is (are) the mechanism(s) by which anti-phagocytic receptors regulate phagocytosis? Specifically, what are the substrates of SHP-1 and SHP-2 regulating phagocytosis?
- Is there specificity in which phagocytes are regulated by particular anti-phagocytic receptors?
- Do anti-phagocytic receptors cooperate or compensate, and in which tissue contexts?
- Which types of anti-phagocytic receptors are important for regulating ADCP versus efferocytosis?
- Does dysregulation of phagocytosis via aberrant don't-eat-me signaling contribute to fibrosis?
- What are the long-term consequences of therapeutically blocking anti-phagocytic receptors?

During efferocytosis, exclusion of some anti-phagocytic receptors from the phagocytic synapse may reduce the activation threshold to allow for efficient removal of dying cells. Additionally, efferocytosis is immunologically silent and can actively promote anti-inflammatory responses. Engagement of anti-phagocytic receptors may contribute to suppressing inflammation in response to the uptake of apoptotic cells. The degree of regulation on efferocytosis by anti-phagocytic receptors is less understood and requires further investigation.

Several new don't-eat-me checkpoints have been identified in recent years, specifically in cancers. Similarly, several pro-phagocytic receptors have been identified for efferocytosis, and while initially thought to be a matter of redundancy, evidence suggests that pro-phagocytic receptor expression is likely tissue and phagocyte-specific (Penberthy *et al,* 2017). Is there similar specificity for anti-phagocytic receptors? Do anti-phagocytic receptors regulate phagocytosis by non-professional phagocytes? Studies aimed at genetically deleting individual anti-phagocytic receptors in specific phagocyte populations will be useful to address these questions (see also Box 1).

Lastly, we and others have reviewed several examples of how impaired phagocytosis contributes to pathology (Doran *et al,* 2019; Feng *et al,* 2019; Morioka *et al,* 2019). Currently available studies evaluating the efficacy and safety of blocking the CD47-SIRPα axis have shown promising results. Additional studies are needed to evaluate the long-term safety and efficacy of these therapies, as well as therapeutically targeting other don't-eat-me checkpoints (see also Box 1). Finally, are there other diseases where aberrant don't-eat-me signaling contributes to pathology? Recent studies suggest that CD47 and PD-L1 are upregulated in pulmonary fibrosis, but it is unclear from these studies if dysregulated phagocytosis contributes to fibrotic pathologies (Wernig *et al,* 2017; Cui *et al,* 2020).

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Abram CL, Lowell CA (2017) Shp1 function in myeloid cells. *J Leukoc Biol* 102: 657 – 675

Adams S, van der Laan LJW, Vernon-Wilson E, de Lavalette CR, Dopp EA, Dijkstra CD, Simmons DL, van den Berg TK (1998) Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. *J Immunol* 161: 1853 – 1859

Advani R, Flinn I, Popplewell L, Forero A, Bartlett NL, Ghosh N, Kline J, Roschewski M, LaCasce A, Collins GP *et al* (2018) CD47 blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma. *N Engl J Med* 379: 1711 – 1721

Ahrens N, Pagenkopf C, Kiesewetter H, Salama A (2006) CD47 is expressed at normal levels in patients with autoimmune haemolytic anaemia and/or immune thrombocytopenia. *Transfus Med* 16: 397 – 402

Alvarez Y, Tang X, Coligan JE, Borrego F (2008) The CD300a (IRp60) inhibitory receptor is rapidly up-regulated on human neutrophils in response to inflammatory stimuli and modulates CD32a (Fc gamma IIa) mediated signaling. *Mol Immunol* 45: 253 – 258

Aman MJ, Walk SF, March ME, Su HP, Carver DJ, Ravichandran KS (2000) Essential role for the C-terminal noncatalytic region of SHIP in Fc gamma RIIB1-mediated inhibitory signaling. *Mol Cell Biol* 20: 3576 – 3589

Arandjelovic S, Ravichandran KS (2015) Phagocytosis of apoptotic cells in homeostasis. *Nat Immunol* 16: 907 – 917

Ariel A, Ravichandran KS (2016) 'This way please': apoptotic cells regulate phagocyte migration before and after engulfment. *Eur J Immunol* 46: 1583 – 1586

Baba T, Fusaki N, Shinya N, Iwamatsu A, Hozumi N (2003) Myosin is an *in vivo* substrate of the protein tyrosine phosphatase (SHP-1) after mIgM cross-linking. *Biochem Biophs Res Commun* 304: 67 – 72

Bally APR, Lu PY, Tang Y, Austin JW, Scharer CD, Ahmed R, Boss JM (2015) NF-kappa B regulates PD-1 expression in macrophages. *J Immunol* 194: 4545 – 4554

Barclay AN, Van den Berg TK (2014) The interaction between signal regulatory protein alpha (SIRPalpha) and CD47: structure, function, and therapeutic target. *Annu Rev Immunol* 32: 25 – 50

Barkal AA, Weiskopf K, Kao KS, Gordon SR, Rosental B, Yiu YY, George BM, Markovic M, Ring NG, Tsai JM *et al* (2018) Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. *Nat Immunol* 19: 76 – 84

Barkal AA, Brewer RE, Markovic M, Kowarsky M, Barkal SA, Zaro BW, Krishnan V, Hatakeyama J, Dorigo O, Barkal LJ *et al* (2019) CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* 572: 392 – 396

Bellon T, Kitzig F, Sayos J, Lopez-Botet M (2002) Mutational analysis of immunoreceptor tyrosine-based inhibition motifs of the Ig-like transcript 2 (CD85j) leukocyte receptor. *J Immunol* 168: 3351 – 3359

Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR *et al* (2019) Heart disease and stroke statistics-2019 update: a report from the American Heart Association. *Circulation* 139: e56 – e528

Betancur PA, Abraham BJ, Yiu YY, Willingham SB, Khameneh F, Zarnegar M, Kuo AH, McKenna K, Kojima Y, Leeper NJ *et al* (2017) A CD47-associated super-enhancer links pro-inflammatory signalling to CD47 upregulation in breast cancer. *Nat Commun* 8: 14802

Bhattacherjee A, Rodrigues E, Jung J, Luzentales-Simpson M, Enterina JR, Galleguillos D, St. Laurent CD, Nakhaei-Nejad M, Fuchsberger FF, Streith L *et al* (2019) Repression of phagocytosis by human CD33 is not conserved with mouse CD33. *Commun Biol* 2: 450

Blasioli J, Paust S, Thomas ML (1999) Definition of the sites of interaction between the protein tyrosine phosphatase SHP-1 and CD22. *J Biol Chem* 274: 2303 – 2307

Blazar BR, Lindberg FP, Ingulli E, Panoskaltsis-Mortari A, Oldenborg PA, Iizuka K, Yokoyama WM, Taylor PA (2001) CD47 (integrin-associated protein) engagement of dendritic cell and macrophage counterreceptors is required to prevent the clearance of donor lymphohematopoietic cells. *J Exp Med* 194: 541 – 549

Borges L, Hsu ML, Fanger N, Kubin M, Cosman D (1997) A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. *J Immunol* 159: 5192 – 5196

Borrego F (2013) The CD300 molecules: an emerging family of regulators of the immune system. *Blood* 121: 1951 – 1960

Brinkman-Vand der Linden ECM, Angata T, Reynolds SA, Powell LD, Hedrick SM, Varki A (2003) CD33/Siglec-3 binding specificity, expression pattern, and consequences of gene deletion in mice. *Mol Cell Biol* 23: 4199 – 4206

Brown E, Hooper L, Ho T, Gresham H (1990) Integrin-associated protein: a 50-kD plasma membrane antigen physically and functionally associated with integrins. *J Cell Biol* 111: 2785 – 2794

Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J (2002) Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. *Nature* 418: 200 – 203

Burger P, Hilarius-Stokman P, de Korte D, van den Berg TK, van Bruggen R (2012) CD47 functions as a molecular switch for erythrocyte phagocytosis. *Blood* 119: 5512 – 5521

Campbell IG, Freemont PS, Foulkes W, Trowsdale J (1992) An ovarian tumor-marker with homology to vaccinia virus contains an Igv-like region and multiple transmembrane domains. *Cancer Res* 52: 5416 – 5420

Casey Sc, Tong L, Li Y, Do R, Walz S, Fitzgerald Kn, Gouw Am, Baylot V, Gutgemann I, Eilers M *et al* (2016) MYC regulates the antitumor immune response through CD47 and PD-L1. *Science* 352: 227 – 231

Catani L, Sollazzo D, Ricci F, Polverelli N, Palandri F, Baccarani M, Vianelli N, Lemoli RM (2011) The CD47 pathway is deregulated in human immune thrombocytopenia. *Exp Hematol* 39: 486 – 494

Chen GY, Tang J, Zheng P, Liu Y (2009) CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. *Science* 323: 1722 – 1725

Chen G-Y, Chen Xi, King S, Cavassani KA, Cheng J, Zheng X, Cao H, Yu H, Qu J, Fang D *et al* (2011) Amelioration of sepsis by inhibiting sialidase-mediated disruption of the CD24-SiglecG interaction. *Nat Biotechnol* 29: 428 – 435

Chen W, Han C, Xie B, Hu X, Yu Q, Shi L, Wang Q, Li D, Wang J, Zheng P *et al* (2013) Induction of Siglec-G by RNA viruses inhibits the innate immune response by promoting RIG-I degradation. *Cell* 152: 467 – 478

Chen W, Wang J, Jia L, Liu J, Tian Y (2016) Attenuation of the programmed cell death-1 pathway increases the M1 polarization of macrophages induced by zymosan. *Cell Death Dis* 7: e2115

Choi SC, Simhadri VR, Tian LJ, Gil-Krzewska A, Krzewski K, Borrego F, Coligan JE (2011) Cutting edge: mouse CD300f (CMRF-35-Like Molecule-1) recognizes outer membrane-exposed phosphatidylserine and can promote phagocytosis. *J Immunol* 187: 3483 – 3487

Chuang W, Lagenaur CF (1990) Central-nervous-system antigen-P84 can serve as a substrate for neurite outgrowth. *Dev Biol* 137: 219 – 232

Colonna M, Navarro F, Bellon T, Llano M, Garcia P, Samaridis J, Angman L, Cella M, LopezBotet M (1997) A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med* 186: 1809 – 1818

Comu S, Weng W, Olinsky S, Ishwad P, Mi Z, Hempel J, Watkins S, Lagenaur CF, Narayanan V (1997) The murine P84 neural adhesion molecule is SHPS-1, a member of the phosphatase-binding protein family. *J Neurosci* 17: 8702 – 8710

Cui L, Chen SY, Lerbs T, Lee JW, Domizi P, Gordon S, Kim YH, Nolan G, Betancur P, Wernig G (2020) Activation of JUN in fibroblasts promotes pro-fibrotic programme and modulates protective immunity. *Nat Commun* 11: 2795

Daeron M, Jaeger S, Du Pasquier L, Vivier E (2008) Immunoreceptor tyrosine-based inhibition motifs: a quest in the past and future. *Immunol Rev* 224: 11 – 43

Davies SP, Reynolds GM, Stamataki Z (2018) Clearance of apoptotic cells by tissue epithelia: a putative role for hepatocytes in liver efferocytosis. *Front Immunol* 9: 44

DeBerge M, Yeap XY, Dehn S, Zhang S, Grigoryeva L, Misener S, Procissi D, Zhou X, Lee DC, Muller WA *et al* (2017) MerTK cleavage on resident cardiac macrophages compromises repair after myocardial ischemia reperfusion injury. *Circ Res* 121: 930 – 940

Diakonova M, Bokoch G, Swanson JA (2002) Dynamics of cytoskeletal proteins during Fcgamma receptor-mediated phagocytosis in macrophages. *Mol Biol Cell* 13: 402 – 411

Ding Y, Guo Z, Liu Y, Li X, Zhang Q, Xu X, Gu Y, Zhang Y, Zhao D, Cao X (2016) The lectin Siglec-G inhibits dendritic cell cross-presentation by impairing MHC class I-peptide complex formation. *Nat Immunol* 17: 1167 – 1175

Doody GM, Justement LB, Delibrias CC, Matthews RJ, Lin JJ, Thomas ML, Fearon DT (1995) A role in B-cell activation for Cd22 and the protein-tyrosine-phosphatase Shp. *Science* 269: 242 – 244

Doran AC, Yurdagul Jr A, Tabas I (2019) Efferocytosis in health and disease. *Nat Rev Immunol* 20: 254 – 267

Doucey MA, Scarpellino L, Zimmer J, Guillaume P, Luescher IF, Bron C, Held W (2004) Cis association of Ly49A with MHC class I restricts natural killer cell inhibition. *Nat Immunol* 5: 328 – 336

Elliott JI, Surprenant A, Marelli-Berg FM, Cooper JC, Cassady-Cain RL, Wooding C, Linton K, Alexander DR, Higgins CF (2005) Membrane phosphatidylserine distribution as a non-apoptotic signalling mechanism in lymphocytes. *Nat Cell Biol* 7: 808 – 816

Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P *et al* (2009) Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature* 461: 282 – 286

Elliott MR, Ravichandran KS (2016) The dynamics of apoptotic cell clearance. *Dev Cell* 38: 147 – 160

Engelbertsen D, Autio A, Verwilligen RAF, Depuydt MAC, Newton G, Rattik S, Levinsohn E, Saggu G, Jarolim P, Wang H *et al* (2019) Increased lymphocyte activation and atherosclerosis in CD47-deficient mice. *Sci Rep* 9: 10608

Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL (2001) Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. *J Biol Chem* 276: 1071 – 1077

Fanger NA, Cosman D, Peterson L, Braddy SC, Maliszewski CR, Borges L (1998) The MHC class I binding proteins LIR-1 and LIR-2 inhibit Fc receptor-mediated signaling in monocytes. *Eur J Immunol* 28: 3423 – 3434

Feng M, Jiang W, Kim BYS, Zhang CC, Fu YX, Weissman IL (2019) Phagocytosis checkpoints as new targets for cancer immunotherapy. *Nat Rev Cancer* 19: 568 – 586

Frangogiannis NG (2012) Regulation of the inflammatory response in cardiac repair. *Circ Res* 110: 159 – 173

Freeman SA, Vega A, Riedl M, Collins RF, Ostrowski PP, Woods EC, Bertozzi CR, Tammi MI, Lidke DS, Johnson P *et al* (2018) Transmembrane pickets connect Cyto- and pericellular skeletons forming barriers to receptor engagement. *Cell* 172: 305 – 317.e310

Fujioka Y, Matozaki T, Noguchi T, Iwamatsu A, Yamao T, Takahashi N, Tsuda M, Takada T, Kasuga M (1996) A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing protein tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. *Mol Cell Biol* 16: 6887 – 6899

Gao AG, Lindberg FP, Dimitry JM, Brown EJ, Frazier WA (1996a) Thrombospondin modulates alpha(v)beta(3) function through integrin-associated protein. *J Cell Biol* 135: 533 – 544

Gao AG, Lindberg FP, Finn MB, Blystone SD, Brown EJ, Frazier WA (1996b) Integrin-associated protein is a receptor for the C-terminal domain of thrombospondin. *J Biol Chem* 271: 21 – 24

Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, Henson PM (2003) By binding SIRP alpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 115: 13 – 23

Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, Bratton DL, Oldenborg PA, Michalak M, Henson PM (2005) Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell* 123: 321 – 334

Gerlach BD, Marinello M, Heinz J, Rymut N, Sansbury BE, Riley CO, Sadhu S, Hosseini Z, Kojima Y, Tang DD *et al* (2020) Resolvin D1 promotes the targeting and clearance of necroptotic cells. *Cell Death Differ* 27: 525 – 539

Getahun A, Cambier JC (2015) Of ITIMs, ITAMs, and ITAMis: revisiting immunoglobulin Fc receptor signaling. *Immunol Rev* 268: 66 – 73

Gomez CP, Descoteaux A (2018) Moesin and myosin IIA modulate phagolysosomal biogenesis in macrophages. *Biochem Biophys Res Commun* 495: 1964 – 1971

Gonzalez A, Castrillo A (2018) Origin and specialization of splenic macrophages. *Cell Immunol* 330: 151 – 158

Goodman JW, Smith LH (1961) Erythrocyte life span in normal mice and in radiation bone marrow chimeras. *Am J Physiol* 200: 764 – 770

Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, Gupta R, Tsai JM, Sinha R, Corey D *et al* (2017) PD-1 expression by

tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 545: 495 – 499

Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, Hooli B, Choi SH, Hyman BT, Tanzi RE (2013) Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron* 78: 631 – 643

Griciuc A, Patel S, Federico AN, Choi SH, Innes BJ, Oram MK, Cereghetti G, McGinty D, Anselmo A, Sadreyev RI *et al* (2019) TREM2 acts downstream of CD33 in modulating microglial pathology in Alzheimer's disease. *Neuron* 103: 820 – 835.e827

Guido W (2008) Refinement of the retinogeniculate pathway. *J Physiol* 586: 4357 – 4362

Guilliams M, van de Laar L (2015) A Hitchhiker's guide to myeloid cell subsets: practical implementation of a novel mononuclear phagocyte classification system. *Front Immunol* 6: 406

Hagnerud S, Manna PP, Cella M, Stenberg A, Frazier WA, Colonna M, Oldenborg PA (2006) Deficit of CD47 results in a defect of marginal zone dendritic cells, blunted immune response to particulate antigen and impairment of skin dendritic cell migration. *J Immunol* 176: 5772 – 5778

Hayes BH, Tsai RK, Dooling LJ, Kadu S, Lee JY, Pantano D, Rodriguez PL, Subramanian S, Shin JW, Discher DE (2020) Macrophages show higher levels of engulfment after disruption of *cis* interactions between CD47 and the checkpoint receptor SIRPα. *J Cell Sci* 133: jcs237800

Hochreiter-Hufford AE, Lee CS, Kinchen JM, Sokolowski JD, Arandjelovic S, Call JA, Klibanov AL, Yan Z, Mandell JW, Ravichandran KS (2013) Phosphatidylserine receptor BAI1 and apoptotic cells as new promoters of myoblast fusion. *Nature* 497: 263 – 267

Hochreiter-Hufford AE, Arandjelovic S, Ravichandran KS (2015) Using phosphatidylserine exposure on apoptotic cells to stimulate myoblast fusion. *Methods Mol Biol* 1313: 141 – 148

Hollingworth P, Harold D, Sims R, Gerrish A, Lambert J-C, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V *et al* (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* 43: 429 – 435

Huang X, Venet F, Wang Yl, Lepape A, Yuan Z, Chen Y, Swan R, Kherouf H, Monneret G, Chung C-s *et al* (2009) PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc Natl Acad Sci USA* 106: 6303 – 6308

Ishikawa-Sekigami T, Kaneko Y, Okazawa H, Tomizawa T, Okajo J, Saito Y, Okuzawa C, Sugawara-Yokoo M, Nishiyama U, Ohnishi H *et al* (2006) SHPS-1 promotes the survival of circulating erythrocytes through inhibition of phagocytosis by splenic macrophages. *Blood* 107: 341 – 348

Jaiswal S, Jamieson CH, Pang WW, Park CY, Chao MP, Majeti R, Traver D, van Rooijen N, Weissman IL (2009) CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* 138: 271 – 285

Janssen WJ, McPhillips KA, Dickinson MG, Linderman DJ, Morimoto K, Xiao YQ, Oldham KM, Vandivier RW, Henson PM, Gardai SJ (2008) Surfactant proteins A and D suppress alveolar macrophage phagocytosis via interaction with SIRP alpha. *Am J Respir Crit Care Med* 178: 158 – 167

Jarr KU, Nakamoto R, Doan BH, Kojima Y, Weissman IL, Advani RH, Iagaru A, Leeper NJ (2021) Effect of CD47 blockade on vascular inflammation. *N Engl J Med* 384: 382 – 383

Jiang P, Lagenaur CF, Narayanan V (1999) Integrin-associated protein is a ligand for the P84 neural adhesion molecule. *J Biol Chem* 274: 559 – 562

Johansen ML, Brown EJ (2007) Dual regulation of SIRP alpha phosphorylation by integrins and CD47. *J Biol Chem* 282: 24219 – 24230

Jonsson AH, Yokoyama WM (2009) Natural killer cell tolerance: licensing and other mechanisms. *Adv Immunol* 101: 27 – 79

Juncadella IJ, Kadl A, Sharma AK, Shim YM, Hochreiter-Hufford A, Borish L, Ravichandran KS (2013) Apoptotic cell clearance by bronchial epithelial cells critically influences airway inflammation. *Nature* 493: 547 – 551

Kant AM, De P, Peng XD, Yi TL, Rawlings DJ, Kim JS, Durden DL (2002) SHP-1 regulates Fc gamma receptor-mediated phagocytosis and the activation of RAC. *Blood* 100: 1852 – 1859

Karyampudi L, Lamichhane P, Krempski J, Kalli KR, Behrens MD, Vargas DM, Hartmann LC, Janco JM, Dong H, Hedin KE *et al* (2016) PD-1 blunts the function of ovarian tumor-infiltrating dendritic cells by inactivating NF-kappaB. *Cancer Res* 76: 239 – 250

Khandelwal S, van Rooijen N, Saxena RK (2007) Reduced expression of CD47 during murine red blood cell (RBC) senescence and its role in RBC clearance from the circulation. *Transfusion* 47: 1725 – 1732

Kharitonenkov A, Chen ZJ, Sures I, Wang HY, Schilling J, Ullrich A (1997) A family of proteins that inhibit signalling through tyrosine kinase receptors. *Nature* 386: 181 – 186

Kim EJ, Lee SM, Suk K, Lee WH (2012) CD300a and CD300f differentially regulate the MyD88 and TRIF-mediated TLR signalling pathways through activation of SHP-1 and/or SHP-2 in human monocytic cell lines. *Immunology* 135: 226 – 235

Kojima Y, Volkmer JP, McKenna K, Civelek M, Lusis AJ, Miller CL, Direnzo D, Nanda V, Ye JQ, Connolly AJ *et al* (2016) CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis. *Nature* 536: 86 – 90

Kozlowski M, Mlinaric-Rascan I, Feng GS, Shen R, Pawson T, Siminovitch KA (1993) Expression and catalytic activity of the tyrosine phosphatase PTP1C is severely impaired in motheaten and viable motheaten mice. *J Exp Med* 178: 2157 – 2163

Kuriyama T, Takenaka K, Kohno K, Yamauchi T, Daitoku S, Yoshimoto G, Kikushige Y, Kishimoto J, Abe Y, Harada N *et al* (2012) Engulfment of hematopoietic stem cells caused by down-regulation of CD47 is critical in the pathogenesis of hemophagocytic lymphohistiocytosis. *Blood* 120: 4058 – 4067

Latour S, Tanaka H, Demeure C, Mateo V, Rubio M, Brown EJ, Maliszewski C, Lindberg FP, Oldenborg A, Ullrich A *et al* (2001) Bidirectional negative regulation of human T and dendritic cells by CD47 and its cognate receptor signal-regulator protein-alpha: down-regulation of IL-12 responsiveness and inhibition of dendritic cell activation. *J Immunol* 167: 2547 – 2554

Law CL, Sidorenko SP, Chandran KA, Zhao ZH, Shen SH, Fischer EH, Clark EA (1996) CD22 associates with protein tyrosine phosphatase 1C, Syk, and phospholipase C-gamma 1 upon B cell activation. *J Exp Med* 183: 547 – 560

Lee CS, Penberthy KK, Wheeler KM, Juncadella IJ, Vandenabeele P, Lysiak JJ, Ravichandran KS (2016) Boosting apoptotic cell clearance by colonic epithelial cells attenuates inflammation *in vivo*. *Immunity* 44: 807 – 820

Legrand N, Huntington ND, Nagasawa M, Bakker AQ, Schotte R, Strick-Marchand H, de Geus SJ, Pouw SM, Bohne M, Voordouw A *et al* (2011) Functional CD47/signal regulatory protein alpha (SIRP(alpha)) interaction is required for optimal human T- and natural killer- (NK) cell homeostasis *in vivo*. *Proc Natl Acad Sci USA* 108: 13224 – 13229

Lehrman EK, Wilton DK, Litvina EY, Welsh CA, Chang ST, Frouin A, Walker AJ, Heller MD, Umemori H, Chen CF *et al* (2018) CD47 protects synapses from excess microglia-mediated pruning during development. *Neuron* 100: 120 – 134

LeVine AM, Whitsett JA (2001) Pulmonary collectins and innate host defense of the lung. *Microbes Infect* 3: 161 – 166

Li LX, Atif SM, Schmiel SE, Lee SJ, McSorley SJ (2012) Increased susceptibility to *Salmonella* infection in signal regulatory protein alpha-deficient mice. *J Immunol* 189: 2537 – 2544

Lindberg FP, Gresham HD, Schwarz E, Brown EJ (1993) Molecular cloning of integrin-associated protein: an immunoglobulin family member with multiple membrane-spanning domains implicated in alpha v beta 3-dependent ligand binding. *J Cell Biol* 123: 485 – 496

Lindberg FP, Lublin DM, Telen MJ, Veile RA, Miller YE, Doniskeller H, Brown EJ (1994) Rh-related antigen Cd47 is the signal-transducer integrin-associated protein. *J Biol Chem* 269: 1567 – 1570

Lindberg FP, Bullard DC, Caver TE, Gresham HD, Beaudet AL, Brown EJ (1996) Decreased resistance to bacterial infection and granulocyte defects in IAP-deficient mice. *Science* 274: 795 – 798

Liu Y, Buhring HJ, Zen K, Burst SL, Schnell FJ, Williams IR, Parkos CA (2002) Signal regulatory protein (SIRP alpha), a cellular ligand for CD47, regulates neutrophil transmigration. *J Biol Chem* 277: 10028 – 10036

Liu F, Jiang CC, Yan XG, Tseng H-Y, Wang CY, Zhang YY, Yari H, La T, Farrelly M, Guo ST *et al* (2017) BRAF/MEK inhibitors promote CD47 expression that is reversible by ERK inhibition in melanoma. *Oncotarget* 8: 69477 – 69492

Lopes FB, Balint S, Valvo S, Felce JH, Hessel EM, Dustin ML, Davis DM (2017) Membrane nanoclusters of FcgammaRI segregate from inhibitory SIRPalpha upon activation of human macrophages. *J Cell Biol* 216: 1123 – 1141

Lorenz U (2009) SHP-1 and SHP-2 in T cells: two phosphatases functioning at many levels. *Immunol Rev* 228: 342 – 359

Lowry MB, Duchemin AM, Robinson JM, Anderson CL (1998) Functional separation of pseudopod extension and particle internalization during Fc gamma receptor-mediated phagocytosis. *J Exp Med* 187: 161 – 176

Lundberg P, Koskinen C, Baldock PA, Lothgren H, Stenberg A, Lerner UH, Oldenborg PA (2007) Osteoclast formation is strongly reduced both *in vivo* and *in vitro* in the absence of CD47/SIRPalpha-interaction. *Biochem Biophys Res Commun* 352: 444 – 448

Lv Z, Bian Z, Shi L, Niu S, Ha B, Tremblay A, Li L, Zhang X, Paluszynski J, Liu M *et al* (2015) Loss of cell surface CD47 clustering formation and binding avidity to SIRPalpha facilitate apoptotic cell clearance by macrophages. *J Immunol* 195: 661 – 671

Maile LA, DeMambro VE, Wai C, Lotinun S, Aday AW, Capps BE, Beamer WG, Rosen CJ, Clemmons DR (2011) An essential role for the association of CD47 to SHPS-1 in skeletal remodeling. *J Bone Miner Res* 26: 2068 – 2081

Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs Jr KD, van Rooijen N, Weissman IL (2009) CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* 138: 286 – 299

Marasco M, Berteotti A, Weyershaeuser J, Thorausch N, Sikorska J, Krausze J, Brandt Hj, Kirkpatrick J, Rios P, Schamel Ww *et al* (2020) Molecular mechanism of SHP2 activation by PD-1 stimulation. *Sci Adv* 6: eaay4458

Matozaki T, Murata Y, Okazawa H, Ohnish H (2009) Functions and molecular mechanisms of the CD47-SIRP alpha signalling pathway. *Trends Cell Biol* 19: 72 – 80

May RC, Machesky LM (2001) Phagocytosis and the actin cytoskeleton. *J Cell Sci* 114: 1061 – 1077

Medina CB, Mehrotra P, Arandjelovic S, Perry JSA, Guo Y, Morioka S, Barron B, Walk SF, Ghesquière B, Krupnick AS *et al* (2020) Metabolites released from apoptotic cells act as tissue messengers. *Nature* 580: 130 – 135

Michaels AD, Newhook TE, Adair SJ, Morioka S, Goudreau BJ, Nagdas S, Mullen MG, Persily JB, Bullock TNJ, Slingluff Jr CL *et al* (2018) CD47 blockade as an adjuvant immunotherapy for resectable pancreatic cancer. *Clin Cancer Res* 24: 1415 – 1425

Monks J, Rosner D, Geske FJ, Lehman L, Hanson L, Neville MC, Fadok VA (2005) Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. *Cell Death Differ* 12: 107 – 114

Morioka S, Perry JSA, Raymond MH, Medina CB, Zhu Y, Zhao L, Serbulea V, Onengut-Gumuscu S, Leitinger N, Kucenas S *et al* (2018) Efferocytosis induces a novel SLC program to promote glucose uptake and lactate release. *Nature* 563: 714 – 718

Morioka S, Maueroder C, Ravichandran KS (2019) Living on the edge: efferocytosis at the interface of homeostasis and pathology. *Immunity* 50: 1149 – 1162

Morrissey MA, Kern N, Vale RD (2020) CD47 Ligation repositions the inhibitory receptor SIRPA to suppress integrin activation and phagocytosis. *Immunity* 53: 1 – 13

Munday J, Kerr S, Ni J, Cornish AL, Zhang JQ, Nicoll G, Floyd H, Mattei MG, Moore P, Liu D *et al* (2001) Identification, characterization and leucocyte expression of Siglec-10, a novel human sialic acid-binding receptor. *Biochem J* 355: 489 – 497

Myers DR, Abram CL, Wildes D, Belwafa A, Welsh AMN, Schulze CJ, Choy TJ, Nguyen T, Omaque N, Hu Y *et al* (2020) Shp1 loss enhances macrophage effector function and promotes anti-tumor immunity. *Front Immunol* 11: 576310

Naj AC, Jun G, Beecham GW, Wang L-S, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK *et al* (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* 43: 436 – 441

Nakahashi-Oda C, Tahara-Hanaoka S, Honda S, Shibuya K, Shibuya A (2012a) Identification of phosphatidylserine as a ligand for the CD300a immunoreceptor. *Biochem Biophs Res Commun* 417: 646 – 650

Nakahashi-Oda C, Tahara-Hanaoka S, Shoji M, Okoshi Y, Nakano-Yokomizo T, Ohkohchi N, Yasui T, Kikutani H, Honda S-I, Shibuya K *et al* (2012b) Apoptotic cells suppress mast cell inflammatory responses via the CD300a immunoreceptor. *J Exp Med* 209: 1493 – 1503

Nakaya M, Watari K, Tajima M, Nakaya T, Matsuda S, Ohara H, Nishihara H, Yamaguchi H, Hashimoto A, Nishida M *et al* (2017) Cardiac myofibroblast engulfment of dead cells facilitates recovery after myocardial infarction. *J Clin Invest* 127: 383 – 401

Neel BG, Gu HH, Pao L (2003) The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. *Trends Biochem Sci* 28: 284 – 293

Nilsson A, Oldenborg PA (2009) CD47 promotes both phosphatidylserine-independent and phosphatidylserine-dependent phagocytosis of apoptotic murine thymocytes by non-activated macrophages. *Biochem Biophs Res Commun* 387: 58 – 63

Niogret C, Birchmeier W, Guarda G (2019) SHP-2 in lymphocytes' cytokine and inhibitory receptor signaling. *Front Immunol* 10: 2468

Noguchi T, Matozaki T, Fujioka Y, Yamao T, Tsuda M, Takada T, Kasuga M (1996) Characterization of a 115-kDa protein that binds to SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in Chinese hamster ovary cells. *J Biol Chem* 271: 27652 – 27658

Oh ES, Gu HH, Saxton TM, Timms JF, Hausdorff S, Frevert EU, Kahn BB, Pawson T, Neel BG, Thomas SM (1999) Regulation of early events in integrin signaling by protein tyrosine phosphatase SHP-2. *Mol Cell Biol* 19: 3205 – 3215

Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T (2013) A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. *Nat Immunol* 14: 1212 – 1218

Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP (2000) Role of CD47 as a marker of self on red blood cells. *Science* 288: 2051 – 2054

Oldenborg PA, Gresham HD, Lindberg FP (2001) CD47-signal regulatory protein alpha (SIRP alpha) regulates Fc gamma and complement receptor-mediated phagocytosis. *J Exp Med* 193: 855 – 861

Oldenborg PA, Gresham HD, Chen Y, Izui S, Lindberg FP (2002) Lethal autoimmune hemolytic anemia in CD47-deficient nonobese diabetic (NOD) mice. *Blood* 99: 3500 – 3504

Oldenborg PA (2013) CD47: a cell surface glycoprotein which regulates multiple functions of hematopoietic cells in health and disease. *ISRN Hematol* 2013: 614619

Olsson M, Bruhns P, Frazier WA, Ravetch JV, Oldenborg PA (2005) Platelet homeostasis is regulated by platelet expression of CD47 under normal conditions and in passive immune thrombocytopenia. *Blood* 105: 3577 – 3582

Otipoby KL, Draves KE, Clark EA (2001) CD22 regulates B cell receptor-mediated signals via two domains that independently recruit Grb2 and SHP-1. *J Biol Chem* 276: 44315 – 44322

Patsoukis N, Wang Q, Strauss L, Boussiotis VA (2020) Revisiting the PD-1 pathway. *Sci Adv* 6: eabd2712

Penberthy KK, Rival C, Shankman LS, Raymond MH, Zhang J, Perry JSA, Lee CS, Han CZ, Onengut-Gumuscu S, Palczewski K *et al* (2017) Context-dependent compensation among phosphatidylserine-recognition receptors. *Sci Rep* 7: 14623

Perry JSA, Morioka S, Medina CB, Iker Etchegaray J, Barron B, Raymond MH, Lucas CD, Onengut-Gumuscu S, Delpire E, Ravichandran KS (2019) Interpreting an apoptotic corpse as anti-inflammatory involves a chloride sensing pathway. *Nat Cell Biol* 21: 1532 – 1543

Pillai S, Netravali IA, Cariappa A, Mattoo H (2012) Siglecs and immune regulation. *Annu Rev Immunol* 30: 357 – 392

Pluvinage JV, Haney MS, Smith BAH, Sun J, Iram T, Bonanno L, Li L, Lee DP, Morgens DW, Yang AC *et al* (2019) CD22 blockade restores homeostatic microglial phagocytosis in ageing brains. *Nature* 568: 187 – 192

Poe JC, Fujimoto M, Jansen PJ, Miller AS, Tedder TF (2000) CD22 forms a quaternary complex with SHIP, Grb2, and Shc - A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux. *J Biol Chem* 275: 17420 – 17427

Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. *Science* 290: 84 – 89

Reidy MF, Wright JR (2003) Surfactant protein A enhances apoptotic cell uptake and TGF-beta 1 release by inflammatory alveolar macrophages. *Am J Physiol-Lung C* 285: L854 – L861

Reinhold MI, Lindberg FP, Plas D, Reynolds S, Peters MG, Brown EJ (1995) *In vivo* expression of alternatively spliced forms of integrin-associated protein (CD47). *J Cell Sci* 108(Pt 11): 3419 – 3425

Rival CM, Xu W, Shankman LS, Morioka S, Arandjelovic S, Lee CS, Wheeler KM, Smith RP, Haney LB, Isakson BE *et al* (2019) Phosphatidylserine on viable sperm and phagocytic machinery in oocytes regulate mammalian fertilization. *Nat Commun* 10: 4456

Roquilly A, Jacqueline C, Davieau M, Molle A, Sadek A, Fourgeux C, Rooze P, Broquet A, Misme-Aucouturier B, Chaumette T *et al* (2020) Alveolar macrophages are epigenetically altered after inflammation, leading to long-term lung immunoparalysis. *Nat Immunol* 21: 636 – 648

Rota G, Niogret C, Dang AT, Barros CR, Fonta NP, Alfei F, Morgado L, Zehn D, Birchmeier W, Vivier E *et al* (2018) Shp-2 Is dispensable for establishing T cell exhaustion and for PD-1 signaling *in vivo*. *Cell Rep* 23: 39 – 49

Saito Y, Iwamura H, Kaneko T, Ohnishi H, Murata Y, Okazawa H, Kanazawa Y, Sato-Hashimoto M, Kobayashi H, Oldenborg PA *et al* (2010) Regulation by SIRPalpha of dendritic cell homeostasis in lymphoid tissues. *Blood* 116: 3517 – 3525

Samaridis J, Colonna M (1997) Cloning of novel immunoglobulin superfamily receptors expressed on human myeloid and lymphoid cells: structural evidence for new stimulatory and inhibitory pathways. *Eur J Immunol* 27: 660 – 665

Sato-Hashimoto M, Saito Y, Ohnishi H, Iwamura H, Kanazawa Y, Kaneko T, Kusakari S, Kotani T, Mori M, Murata Y *et al* (2011) Signal regulatory protein alpha regulates the homeostasis of T lymphocytes in the spleen. *J Immunol* 187: 291 – 297

Savill J, Hogg N, Ren Y, Haslett C (1992) Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest* 90: 1513 – 1522

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74: 691 – 705

Schagat TL, Wofford JA, Wright JR (2001) Surfactant protein A enhances alveolar macrophage phagocytosis of apoptotic neutrophils. *J Immunol* 166: 2727 – 2733

Schulz C, Perdiguero EG, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SEW, Pollard JW *et al* (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 336: 86 – 90

Shen L, Gao Y, Liu YY, Zhang BY, Liu QQ, Wu J, Fan L, Ou QF, Zhang WH, Shao LY (2016) PD-1/PD-L pathway inhibits M.tb-specific CD4(+) T-cell functions and phagocytosis of macrophages in active tuberculosis. *Sci Rep* 6: 38362

Shultz LD, Coman DR, Bailey CL, Beamer WG, Sidman CL (1984) Viable Motheaten, a new allele at the Motheaten locus. 1. PATHOLOGY. *Am J Pathol* 116: 179 – 192

Shultz LD, Schweitzer PA, Rajan TV, Yi TL, Ihle JN, Matthews RJ, Thomas ML, Beier DR (1993) Mutations at the Murine Moth-Eaten Locus are within the hematopoietic-cell protein-tyrosine-phosphatase (Hcph) gene. *Cell* 73: 1445 – 1454

Sikic BI, Lakhani N, Patnaik A, Shah SA, Chandana SR, Rasco D, Colevas AD, O'Rourke T, Narayanan S, Papadopoulos K *et al* (2019) First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. *J Clin Oncol* 37: 946 – 953

Simhadri VR, Andersen JF, Calvo E, Choi SC, Coligan JE, Borrego F (2012) Human CD300a binds to phosphatidylethanolamine and phosphatidylserine, and modulates the phagocytosis of dead cells. *Blood* 119: 2799 – 2809

Tada K, Tanaka M, Hanayama R, Miwa K, Shinohara A, Iwamatsu A, Nagata S (2003) Tethering of apoptotic cells to phagocytes through binding of CD47 to Src homology 2 domain-bearing protein tyrosine phosphatase substrate-1. *J Immunol* 171: 5718 – 5726

Takada T, Matozaki T, Takeda H, Fukunaga K, Noguchi T, Fujioka Y, Okazaki I, Tsuda M, Yamao T, Ochi F *et al* (1998) Roles of the complex formation of SHPS-1 with SHP-2 in insulin-stimulated mitogen-activated protein kinase activation. *J Biol Chem* 273: 9234 – 9242

Takeda H, Matozaki T, Fujioka Y, Takada T, Noguchi T, Yamao T, Tsuda M, Ochi F, Fukunaga K, Narumiya S *et al* (1998) Lysophosphatidic acid-induced association of SHP-2 with SHPS-1: roles of RHO, FAK, and a SRC family kinase. *Oncogene* 16: 3019 – 3027

Takenaka K, Prasolava TK, Wang JCY, Mortin-Toth SM, Khalouei S, Gan OI, Dick JE, Danska JS (2007) Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. *Nat Immunol* 8: 1313 – 1323

Tal MC, Torrez Dulgeroff LB, Myers L, Cham LB, Mayer-Barber KD, Bohrer AC, Castro E, Yiu YY, Lopez Angel C, Pham E *et al* (2020) Upregulation of CD47 is a host checkpoint response to pathogen recognition. *MBio* 11: e01293–20

Tao B, Jin W, Xu J, Liang Z, Yao J, Zhang Y, Wang K, Cheng H, Zhang X, Ke Y (2014) Myeloid-specific disruption of tyrosine phosphatase Shp2 promotes

alternative activation of macrophages and predisposes mice to pulmonary fibrosis. *J Immunol* 193: 2801 – 2811

Tian LJ, Choi SC, Murakami Y, Allen J, Morse HC, Qi CF, Krzewski K, Coligan JE (2014) p85 alpha recruitment by the CD300f phosphatidylserine receptor mediates apoptotic cell clearance required for autoimmunity suppression. *Nat Commun* 5: 3146

Tian L, Choi SC, Lee HN, Murakami Y, Qi CF, Sengottuvelu M, Voss O, Krzewski K, Coligan JE (2016) Enhanced efferocytosis by dendritic cells underlies memory T-cell expansion and susceptibility to autoimmune disease in CD300f-deficient mice. *Cell Death Differ* 23: 1086 – 1096

Timms JF, Carlberg K, Gu HH, Chen HY, Kamatkar S, Nadler MJS, Rohrschneider LR, Neel BG (1998) Identification of major binding proteins and substrates for the SH2-containing protein tyrosine phosphatase SHP-1 in macrophages. *Mol Cell Biol* 18: 3838 – 3850

Timms JF, Swanson KD, Marie-Cardine A, Raab M, Rudd CE, Schraven B, Neel BG (1999) SHPS-1 is a scaffold for assembling distinct adhesion-regulated multi-protein complexes in macrophages. *Curr Biol* 9: 927 – 930

Tosello-Trampont A-C, Nakada-Tsukui K, Ravichandran KS (2003) Engulfment of apoptotic cells is negatively regulated by Rho-mediated signaling. *J Biol Chem* 278: 49911 – 49919

Tsai RK, Discher DE (2008) Inhibition of "self" engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. *J Cell Biol* 180: 989 – 1003

Tsuda M, Matozaki T, Fukunaga K, Fujioka Y, Imamoto A, Noguchi T, Takada T, Yamao T, Takeda H, Ochi F *et al* (1998) Integrin-mediated tyrosine phosphorylation of SHPS-1 and its association with SHP-2. Roles of Fak and Src family kinases. *J Biol Chem* 273: 13223 – 13229

Ulyanova T, Blasioli J, Woodford-Thomas TA, Thomas ML (1999) The sialoadhesin CD33 is a myeloid-specific inhibitory receptor. *Eur J Immunol* 29: 3440 – 3449

Van VQ, Lesage S, Bouguermouh S, Gautier P, Rubio M, Levesque M, Nguyen S, Galibert L, Sarfati M (2006) Expression of the self-marker CD47 on dendritic cells governs their trafficking to secondary lymphoid organs. *EMBO J* 25: 5560 – 5568

Vandivier RW, Ogden CA, Fadok VA, Hoffmann PR, Brown KK, Botto M, Walport MJ, Fisher JH, Henson PM, Greene KE (2002) Role of surfactant proteins D, D, and C1q in the clearance of apoptotic cells *in vivo* and *in vitro*: calreticulin and CD91 as a common collectin receptor complex. *J Immunol* 169: 3978 – 3986

Veillette A, Thibaudeau E, Latour S (1998) High expression of inhibitory receptor SHPS-1 and its association with protein-tyrosine phosphatase SHP-1 in macrophages. *J Biol Chem* 273: 22719 – 22728

Veillette A, Tang Z (2019) Signaling regulatory protein (SIRP)α-CD47 blockade joins the ranks of immune checkpoint inhibition. *J Clin Oncol* 37: 1012 – 1014

de Vries HE, Hendriks JJA, Honing H, de Lavalette CR, van der Pol SMA, Hooijberg E, Dijkstra CD, van den Berg TK (2002) Signal-regulatory protein alpha-CD47 interactions are required for the transmigration of monocytes across cerebral endothelium. *J Immunol* 168: 5832 – 5839

Wan E, Yeap XY, Dehn S, Terry R, Novak M, Zhang S, Iwata S, Han X, Homma S, Drosatos K *et al* (2013) Enhanced efferocytosis of apoptotic cardiomyocytes through myeloid-epithelial-reproductive tyrosine kinase links acute inflammation resolution to cardiac repair after infarction. *Circ Res* 113: 1004 – 1012

Wang H, Madariaga ML, Wang S, Van Rooijen N, Oldenborg PA, Yang YG (2007) Lack of CD47 on nonhematopoietic cells induces split macrophage tolerance to CD47null cells. *Proc Natl Acad Sci USA* 104: 13744 – 13749

Wang Y, Nanda V, Direnzo D, Ye J, Xiao S, Kojima Y, Howe KL, Jarr KU, Flores AM, Tsantilas P *et al* (2020) Clonally expanding smooth muscle cells promote atherosclerosis by escaping efferocytosis and activating the complement cascade. *Proc Natl Acad Sci USA* 117: 15818 – 15826

Weiskopf K, Jahchan NS, Schnorr PJ, Cristea S, Ring AM, Maute RL, Volkmer AK, Volkmer JP, Liu J, Lim JS *et al* (2016) CD47-blocking immunotherapies stimulate macrophage-mediated destruction of small-cell lung cancer. *J Clin Invest* 126: 2610 – 2620

Wernig G, Chen SY, Cui L, Van Neste C, Tsai JM, Kambham N, Vogel H, Natkunam Y, Gilliland DG, Nolan G *et al* (2017) Unifying mechanism for different fibrotic diseases. *Proc Natl Acad Sci USA* 114: 4757 – 4762

Whitney G, Wang SL, Chang H, Cheng KY, Lu P, Zhou XD, Yang WP, McKinnon M, Longphre M (2001) A new siglec family member, siglec-10, is expressed in cells of the immune system and has signaling properties similar to CD33. *Eur J Biochem* 268: 6083 – 6096

Willingham SB, Volkmer JP, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, Wang J, Contreras-Trujillo H, Martin R, Cohen JD *et al* (2012) The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. *Proc Natl Acad Sci USA* 109: 6662 – 6667

Wong AS, Mortin-Toth S, Sung M, Canty AJ, Gulban O, Greaves DR, Danska JS (2014) Polymorphism in the innate immune receptor SIRPalpha controls CD47 binding and autoimmunity in the nonobese diabetic mouse. *J Immunol* 193: 4833 – 4844

Wood W, Turmaine M, Weber R, Camp V, Maki RA, McKercher SR, Martin P (2000) Mesenchymal cells engulf and clear apoptotic footplate cells in macrophageless PU.1 null mouse embryos. *Development* 127: 5245 – 5252

Wright JR (2005) Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 5: 58 – 68

Yamao T, Matozaki T, Amano K, Matsuda Y, Takahashi N, Ochi F, Fujioka Y, Kasuga M (1997) Mouse and human SHPS-1: molecular cloning of cDNAs and chromosomal localization of genes. *Biochem Biophys Res Commun* 231: 61 – 67

Yamao T, Noguchi T, Takeuchi O, Nishiyama U, Morita H, Hagiwara T, Akahori H, Kato T, Inagaki K, Okazawa H *et al* (2002) Negative regulation of platelet clearance and of the macrophage phagocytic response by the transmembrane glycoprotein SHPS-1. *J Biol Chem* 277: 39833 – 39839

Ye ZM, Yang S, Xia YP, Hu RT, Chen S, Li BW, Chen SL, Luo XY, Mao L, Li Y *et al* (2019) LncRNA MIAT sponges miR-149-5p to inhibit efferocytosis in advanced atherosclerosis through CD47 upregulation. *Cell Death Dis* 10: 138

Yurdagul A, Doran AC, Cai BS, Fredman G, Tabas IA (2018) Mechanisms and consequences of defective efferocytosis in atherosclerosis. *Front Cardiovasc Med* 4: 86

Yurdagul Jr A, Subramanian M, Wang X, Crown SB, Ilkayeva OR, Darville L, Kolluru GK, Rymond CC, Gerlach BD, Zheng Z *et al* (2020) Macrophage metabolism of apoptotic cell-derived arginine promotes continual efferocytosis and resolution of injury. *Cell Metab* 31: 518 – 533.e510

Zent CS, Elliott MR (2017) Maxed out macs: physiologic cell clearance as a function of macrophage phagocytic capacity. *FEBS J* 284: 1021 – 1039

Zhang H, Lu H, Xiang L, Bullen JW, Zhang C, Samanta D, Gilkes DM, He J, Semenza GL (2015) HIF-1 regulates CD47 expression in breast cancer cells to promote evasion of phagocytosis and maintenance of cancer stem cells. *Proc Natl Acad Sci USA* 112: E6215 – E6223

Zhang S, Yeap XY, DeBerge M, Naresh NK, Wang K, Jiang Z, Wilcox JE, White SM, Morrow JP, Burridge PW *et al* (2017) Acute CD47 blockade during ischemic myocardial reperfusion enhances phagocytosis-associated cardiac repair. *JACC Basic Transl Sci* 2: 386 – 397