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Rab GTPases in the Differential Processing of Phagocytosed Pathogens Versus Efferocytosed Apoptotic Cells

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1 # Rab GTPases in the Differential Processing of Phagocytosed Pathogens
2 # Versus Efferocytosed Apoptotic Cells
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15 # Abstract

16 Phagocytosis is an important feature of innate immunity in which invading microorganisms are engulfed,
17 killed and degraded – and in some immune cells, their antigens presented to adaptive immune system. A
18 closely related process, efferocytosis, removes apoptotic cells, and is essential for the maintenance of
19 homeostasis. Both phagocytosis and efferocytosis are tightly regulated processes that involve target
20 recognition and uptake through specific receptors, followed by endolysosomal trafficking and processing of
21 the internalized target. Central to the uptake and trafficking of these targets are the Rab family of small
22 GTPases, which coordinate the engulfment and trafficking of both phagocytosed and efferocytosed materials
23 through the endolysosomal system. Because of this regulatory function, Rab GTPases are often targeted by
24 pathogens to escape phagocytosis. In this review, we will discuss the shared and differential roles of Rab
25 GTPases in phagocytosis and efferocytosis.

26

27 **Keywords** Phagocytosis, Efferocytosis, Rab GTPase, Phagosome, Efferosome

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1. Overview of the Phagocytic and Efferocytic Process

Phagocytosis is an evolutionarily conserved mechanism which is thought to have arisen as a feeding mechanism for single-celled organisms such as amoeba (Yutin et al., 2009). In metazoans, phagocytosis plays a central role in both host defense and maintenance of tissue homeostasis. Phagocytosis is defined as the cellular uptake of microorganisms and other foreign particulates (>0.5 μm) into a plasma membrane-derived vacuole known as a phagosome (Esteban et al., 2015). In metazoans, phagocytosis not only targets foreign objects, but also functions in mediating the uptake and clearance of apoptotic cells via a separate but similar process known as efferocytosis (Gordon, 2016). Phagocytosis and efferocytosis consist of four main steps: recognition of the target particle, receptor signaling and internalization, phagosome formation, and target particle degradation through phagosome maturation (Nordenfelt and Tapper, 2011).

Phagocytosis and efferocytosis are performed predominantly by dedicated professional phagocytes such as monocytes, macrophages, neutrophils, dendritic cells, eosinophils and osteoclasts. However, in certain tissues and under specific circumstances, phagocytosis and efferocytosis can be conducted by non-professional phagocytes such as endothelial cells, epithelial cells and fibroblasts (Sarode et al., 2017). Generally, these non-professional phagocytes engage efferocytosis and not phagocytosis (Rosales and Uribe-Querol, 2017). Phagocytes recognize target particles through a variety of cell surface receptors, which upon binding to a cognate ligand initiate signaling cascades that result in engulfment of the target into an intracellular vacuole termed the phagosome or efferosome. These receptors can be divided into two general classes: opsonic and non-opsonic receptors. Opsonic receptors do not recognize their targets directly, instead relying on host-derived opsonins (e.g. antibodies, mannose-binding lectin, Gas6) that bind to the target (Kuhlman et al., 1989; Scott et al., 2001). Non-opsonic phagocytosis or efferocytosis occurs via direct ligation of a receptor on the phagocyte with a ligand on the target particle – e.g. binding of dectin-1 to β-glucans on fungal cell walls, or Tim-4 to phosphatidylserine (PtdSer) on apoptotic cells (Miyanishi et al., 2007; Taylor et al., 2007). These receptors often engage in cooperative signaling with non-phagocytic

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52 pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), to enhance target detection and
53 uptake (Yadav and Schorey, 2006; Shin et al., 2008).

54 Following ligation of the target particle, a signaling cascade is initiated by the phagocytic receptors,
55 resulting in an actin remodeling process that extends the phagocyte plasma membrane to engulf the target
56 particle (Kaplan, 1977; May et al., 2000). This engulfment process starts with the initial formation of a
57 phagocytic cup; an invagination of the plasma membrane beneath the target particle (Lee et al., 2007). The
58 distal margin of the cup is driven around the target by a combination of sequential engagement of
59 phagocytic receptors, organization of these receptors into a phagocytic synapse, and the coordinated
60 extension of the plasma membrane via actin reorganization (Freeman and Grinstein, 2014). For larger
61 particles, focal exocytosis delivers additional membrane to the phagocytic cup to ensure that sufficient
62 membrane is available for the complete envelopment of the target (Di et al., 2003). The resulting
63 phagosome/efferosome then proceeds through a tightly-regulated endolysosomal maturation pathway which
64 delivers the catabolic enzymes and other proteins that degrade the engulfed target. This degradative
65 pathway is mediated by the sequential fusion of the phagosome or efferosome with early endosomes, late
66 endosomes, and lastly lysosomes (Gordon et al., 1992; Berg et al., 1998).
67
68 Degradation of the engulfed target occurs predominantly during the phagolysosomal stage – i.e. after the
69 phagosome or efferosome has undergone fusion with lysosomes. Within the phagolysosome, a combination
70 of lysosomal hydrolases, acidification of the phagolysosomal compartment by vacuolar ATPases, and the
71 production of reactive oxygen species (ROS) via the NADPH oxidase complex, act together to degrade the
72 engulfed target (Jutras and Desjardins, 2005). In some cell types this is the final stage of phagocytosis, with
73 the resulting material thought to be either absorbed by the phagocyte, or expelled from the cell (Lancaster et
74 al., 2020). However, professional antigen presenting cells, notably dendritic cells and macrophages, add an
75 additional step to the phagocytic process – formation of the MHC II loading compartment (MIIC) (Uribe-
76 Querol and Rosales, 2017). The MIIC is derived from the phagolysosome, wherein short antigenic peptides

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77 derived from the phagocytosed target are loaded onto MHC II molecules. These are then exported to the
78 cell surface, where alongside costimulatory molecules and cytokines also produced by the professional
79 antigen presenting cell, they mediate activation and polarization of $CD4^+$ helper T cells, thereby initiating an
80 antigen-targeted adaptive immune response (Underhill and Goodridge, 2012). In contrast, phagolysosomes
81 containing innocuous materials such as degraded apoptotic cells, do not evolve into a MIIC. Rather, these
82 apoptotic cell contents are absorbed or otherwise disposed of by the phagocyte without initiation of MIIC
83 formation or the production of an adaptive immune response (Yin et al., 2016; Penteado et al., 2017; Yin et
84 al., 2017). Inappropriate processing of apoptotic cell-derived material has been associated with a range of
85 pathological processes including autoimmune diseases such as rheumatoid arthritis or systemic lupus
86 erythematosus, as well as chronic inflammatory disorders such as atherosclerosis (reviewed in Doran et al.,
87 2019).

88

89 All stages of phagocytosis and efferocytosis – from the engulfment of the target, through to the final
90 disposition of its remains – are tightly-regulated by members of the Rab (Ras related in brain) family of
91 small GTPases. Rab GTPases are the largest family of small GTPases, and serve as master regulators of
92 intracellular vesicle formation, trafficking and fusion (Stenmark, 2009). There are ~70 Rab subfamilies in
93 humans, many of which are associated with phagocytic and/or efferocytic processes (Murray et al., 2005;
94 Husebye et al., 2010; Flannagan et al., 2012). In this review, we discuss critical functions of Rab GTPases
95 in phagocytosis, efferocytosis, and in the differential processing of phagocytic versus efferocytic cargos.

96

97
2. Rab GTPases

98 Rab proteins are master regulators of vesicle formation, trafficking and fusion. To mediate these events,
99 Rabs interact with a broad range of effector proteins, with Rab activity tightly regulated through upstream
100 signaling (Mizuno-Yamasaki et al., 2012). Rab's function as nucleotide-dependent switches, possessing
101 both GDP-bound 'off' and GTP-bound 'on' forms, with conversion from the GTP-bound to GDP-bound

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state induced by the Rab's intrinsic GTPase activity (Toma-Fukai and Shimizu, 2019). Inactive GDP-bound Rabs are recognized by a Rab escort protein (REP), which serves both as a chaperone for the Rab protein, and recruits a geranylgeranyl transferase (GGT), which catalyzes the geranylgeranylation of the Rab protein (Alexandrov et al., 1994; Müller and Goody, 2018). This geranylgeranylated, GDP-bound Rab protein is then bound by Rab GDP dissociation inhibitor (GDI) and GDI displacement factor (GDF), which function together to target the Rab to the appropriate intracellular membrane. This targeting is generally via a guanine nucleotide-exchange factor (GEF) which is recruited to the target membrane, often via phosphoinositide-specific PH domains or other targeting motifs. Once recruited to the target membrane, the GEF catalyzes the displacement of bound GDP on the Rab for GTP, thereby activating the Rab (Oesterlin et al., 2012).

Upon Rab activation by its GEF, the Rab protein proceeds to recruit its effectors, with effector binding resulting in increased GEF activity. This Rab-GEF-effector positive feedback loop stabilizes the activated Rab protein on its target membrane, where the Rab can then mediate its activity via its effectors. There is a large plethora of Rab effectors, many of which are beyond the scope of this review. For a detailed review of Rabs and their effectors, see these excellent reviews (Hutagalung and Novick, 2011; Pylypenko et al., 2018). Depending on the Rab, the recruited effectors can mediate a number of cellular events, including the recruitment of dyneins and other proteins involved in the budding of vesicles off of target membranes, interactions with myosins and kinesins to mediate the intracellular movement of vesicles, and interactions with soluble NSF attachment protein receptors (SNAREs) to mediate vesicle-vesicle fusion (Söllner et al., 1993; Park and Loh, 2008). Rab proteins are inactivated by the activity of GTPase-activating proteins (GAP's), which stimulate the hydrolysis of GTP, converting the Rab protein back to its inactive GDP-bound state (Bollag and McCormick, 1991; al-Alawi et al., 1993; Li and Marlin, 2015).

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3. Particle Uptake

Phagocytic uptake is a receptor-mediated and actin-driven process, requiring significant reorganization of the actin cytoskeleton beneath the forming phagocytic cup (Lee et al., 2007). The importance of membrane remodeling during phagocytosis in terms of both lipid composition changes and delivery of new membrane to the site of particle binding is well characterized (reviewed in Rougerie et al., 2013). The extension of the plasma membrane around the target particle requires that new membrane be added to the growing phagocytic cup. This addition of membrane occurs through the focal exocytosis of intracellular vesicles derived from the Golgi and endocytic system (Vashi et al., 2017). This exocytic process is regulated by Arf6, a GTPase which regulates trafficking to and from recycling endosome compartments (Aikawa and Martin, 2003; Vashi et al., 2017). While Arf6 is not itself a Rab GTPase, it is a member of the larger small GTPase family, and functions in a similar manner to Rab GTPases (Chardin et al., 1996; Donaldson and Jackson, 2011). While Arf6 is the primary mediator of focal exocytosis at the phagocytic cup, its activity is regulated by Rab GTPases that also localize to the phagocytic cup. These Rabs are recruited by the presence of specific phosphatidylinositol species generated by phagocytic signaling (reviewed in Gutierrez, 2013). Specifically, Rab8a, 8b 10, 13, 27a and 35 are recruited by phosphatidylinositol-3,4,5-*tris*phosphate (PIP_3), which is generated in the phagocytic cup from plasma membrane phosphatidylinositol-4,5-*bis*phosphate ($PI(4,5)P_2$) (Tamura et al., 2009). This lipid phosphorylation is performed by receptor-activated type I phosphatidylinositol-3-kinases (PI3Ks) whose activity is stimulated by their binding to phosphorylated (activated) phagocytic receptors (Byekova et al., 2010; Yeo et al., 2016; Demirdjian et al., 2018). While multiple Rabs are recruited to the forming cup, knockdown of only Rab13 or Rab35 inhibits phagocytosis, suggesting that the other Rabs recruited to the phagocytic cup are either functionally redundant, or that their recruitment is dispensable for phagocytosis (Yeo et al., 2016). Rab35 directly controls focal exocytosis at the phagocytic cup through mediating recruitment of the Arf6 GAP ACAP2, thus controlling the activity of Arf6 (Egami et al., 2011). The role of Rab13 is less well defined, but studies have suggested that Rab13 is recruited to the phagocytic cup by the Rab35 effector MICAL-L1, and that Rab13 may be required for the

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151 focal exocytosis of phagocytic receptor-bearing vesicles during phagocytic cup formation (Kobayashi et al., 152 2014). In addition, Rab13, via its effector MICAL-L2, recruits the actin-regulatory GTPase RhoA, and 153 therefore may also contributes to RhoA-mediated actin reorganization at the phagocytic cup (Ioannou and 154 McPherson, 2016). While the roles of these Rab GTPases at the forming phagocytic cup remain only 155 partially elucidated, it is clear that these proteins play a critical regulatory role necessary for the extension 156 of the plasma membrane around the phagocytic target.

157

158 4. Phagosome Maturation

159 Once phagocytic targets are internalized, they are confined within a plasma membrane-derived vesicle 160 known as a phagosome which undergoes maturation through fusion with an increasingly acidic series of 161 endolysosomal vesicles, resulting in the degradation of the phagocytosed cargo (Lukacs et al., 1990; Canton 162 et al., 2014). Some pathogens modulate Rab activity to promote their intracellular growth, typically by 163 blocking or altering the phagosome maturation pathway. Bacteria including *Mycobacterium tuberculosis,* 164 *Helicobacter pylori, Salmonella,* and *Chlamydia* all disrupt phagosome maturation through the selective 165 retention or exclusion of Rabs on vacuolar membranes (Brumell and Scidmore, 2007), highlighting the 166 importance of Rab-mediated regulation of phagosome maturation in pathogen killing. Phagosome 167 maturation is a step-wise process, in which the phagosome progresses through early, late and 168 phagolysosomal maturation stages (**Figure 1**), with each stage and the transition between stages regulated 169 by Rab GTPases. More than 20 Rab proteins have been detected on phagosomes (**Table 1**), with the known 170 roles of these GTPases reviewed below.

171

172 *4.1 Early Phagosome*

173 Early phagosomes are characterized by the presence of Rab5 on the phagosomal membrane. Initially, the 174 GEF Rabex-5 is recruited to early phagosomes by Rab22; an early endosome Rab GTPase which is targeted 175 to the phagosome via a currently unknown mechanism (Roberts et al., 2006). Active Rab5 recruits

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176 additional Rabex-5 as well as another Rab5 effector, Rabaptin-5, which enhances the activity of Rabex-5,
177 generating a positive feedback loop for Rab5 activation (Zhu et al., 2007). During phagosome closure,
178 $PI(4,5)P_2$ and PIP_3 are dephosphorylated by lipid phosphatases such as PTEN and INPP5, producing an
179 early phagosome bearing phosphatidylinositol as its predominant inositide species (Kamen et al., 2007;
180 Serezani et al., 2012; T. Segawa et al., 2014). One of the first activities conducted by Rab5 on the early
181 phagosome is activation of the type III PI3K Vps34 that generates phosphatidylinositol 3-phosphate
182 (PI(3)P) on the cytosolic leaflet of early phagosomes (Kinchen et al., 2008). PI(3)P serves to dock and
183 activate Rab5 effectors including endosomal early antigen 1 (EEA1) and rabenosyn-5 (Simonsen et al.,
184 1998; Nielsen et al., 2000). Rab5 and its effectors then mediate the formation of the large class C core
185 vacuole/endosomes tethering (CORVET) complex, comprised of a core of Vps11, 16, 18, 33 plus the
186 CORVET-specific Vps3 and 8 subunits (Balderhaar and Ungermann, 2013). This complex mediates the
187 tethering of early endosomes to the maturing phagosome, through bridging active Rab5 on the phagosome
188 to active Rab5 on the early endosome. This bridging of an early endosome to the phagosome sets the stage
189 for fusion, which is initiated by the SNARE syntaxin 13, with fusion requiring SNARE activation by Rab5
190 and NSF (Collins et al., 2002; Becken et al., 2010). Fusion with early endosomes begins the delivery of
191 vacuolar ATPases to the phagosome, which reduces the pH of the phagosome from that of the extracellular
192 milieu (pH 7.4) to a pH of 6.5 (Davies et al., 1994; Flannagan et al., 2009).
193
194 Other Rab proteins that are involved in the regulation of early phagosome maturation include Rab22a and
195 Rab14. As discussed above, Rab22a, which is highly homologous to Rab5, induces recruitment of Rabex-5
196 and Rab5 activation, promoting fusion of early endosomes with the nascent phagosome (Zhu et al., 2009).
197 Rab14 also has been shown to play a role in the regulation of fusion between the phagosome and early
198 endosomes (Gutierrez, 2013). While the role of these Rab GTPases are not as defined as the roles of Rab5,
199 their function is critical for phagosome maturation, and indeed, mycobacterial pathogens manipulate Rab14

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200 and Rab22 to block phagosome maturation at the early (e.g. minimally microbicidal) phagosome stage
201 (Kyei et al., 2006; Roberts et al., 2006).
202

203 *4.2 Late Phagosome*

204 Following fusion with early endosomes, Rab5 triggers the next stage of phagosome maturation by
205 facilitating its exchange with Rab7 (Vieira et al., 2003). The transition of Rab5 to Rab7 is driven by Mon1
206 and Ccz1, which together serve as the Rab7 GEF (Nordmann et al., 2010). Recruitment of the Mon1/Ccz1
207 complex terminates Rab5 activation by displacing Rabex-5 from the early phagosome, while also recruiting
208 and activating Rab7 (Kinchen and Ravichandran, 2010; Poteryaev et al., 2010). Rab7, through its effectors
209 Rab-Interacting Lysosomal Protein (RILP) and oxysterol-binding protein related-protein 1 (ORP1L),
210 recruits dynein/dynactin to the phagosome, causing the phagosome to migrate along microtubules to the
211 perinuclear region (Toyohara and Inaba, 1989; Blocker et al., 1998; Johnson et al., 2016). Here, Rab7 seeds
212 the formation of the HOPS complex which shares the same core as the CORVET complex (Vps11, 16, 18
213 and 33), plus bears the HOPS-specific effectors Vps39 and 41 (Harrison et al., 2003; Johansson et al.,
214 2007). Like the CORVET complex, the HOPS complex serves as a bridge between the phagosome and
215 intracellular vesicles, but differs in that the HOPS complex tethers vesicles bearing Rab7, allowing the
216 phagosome to fuse with late endosomes and lysosomes (Balderhaar and Ungermann, 2013). These fusion
217 events deliver the hydrolytic enzymes required for killing and degradation of the phagocytosed pathogen, as
218 well as additional vacuolar ATPases, reducing the luminal pH to 5.0 or lower, thereby activating these
219 hydrolases (Johnson et al., 2016). Following fusion with late endosomes and lysosomes the phagosome
220 matures into a fully microbicidal phagolysosome (Pauwels et al., 2017).
221

222 *4.3 Phagolysosome*

223 In the last phagosome maturation stage, late phagosomes fuse with lysosomes to become phagolysosomes,
224 which are regarded as the ultimate microbicidal organelle (Harrison et al., 2003). Phagolysosomes, through

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a number of mechanisms, drive the killing and degradation of phagocytosed microorganisms. Defects in the process of phagolysosome formation and maturation cause a number of negative consequences. For instance, mutations in lysosome-associated membrane protein-2 (LAMP2) recruitment to the late phagosome is associated with impaired fusion of phagosomes with lysosomes, and contributes to the development of severe periodontitis early in life due to reduced bacterial killing by phagocytes such as neutrophils (Beertsen et al., 2008).

The recruitment of large numbers of V-ATPase molecules on the phagolysosomal membrane is responsible for establishing the highly acidic environment of the phagolysosomal lumen that drives pathogen killing and degradation (Marshansky and Futai, 2008). Fusion of lysosomes results in the delivery of hydrolytic enzymes including glycosidases, lipases, DNases and proteases such as cathepsins to the phagolysosomes. Cathepsins and other lysosomal proteases require low pH for optimal activity, and therefore work synergistically with vATPase-mediated acidification of the phagolysosome (Strasser et al., 1999; Pauwels et al., 2017) . Phagolysosomes also contribute to microorganism destruction through concentrating ROS and reactive nitrogen species (RNS) which have microbiocidal properties (Castrillo et al., 2001; Winterbourn et al., 2006). Finally, previous studies have shown that antimicrobial peptides including defensins and cathelicidins are delivered to the phagolysosomes to disrupt the integrity of phagocytized pathogens (Selsted et al., 1993; Larrick et al., 1994; Bensch et al., 1995).

Rab7, 20, 22b, 32, 34, 38 and 43 are all known to be involved in the delivery of hydrolases such as cathepsin D to the phagosome (Seto et al., 2011). Rab27 has been reported to function on later phagolysosomes by regulation of phagosomal pH and NADPH production, thus promoting antigen cross-presentation (Jancic et al., 2007). Additionally, Rab34 promotes phagolysosome biogenesis through interaction with RILP (Wang and Hong, 2002).

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4.4 MHC II Loading Compartment Formation

Professional antigen presenting cells add an additional step to phagosome maturation – conversion of the phagolysosome into an MIIC. The trafficking events driving this process are not well understood, in part because the pathway allowing for newly synthesized MHC II to be delivered to the phagolysosome remains unclear (Saric et al., 2015). MIIC formation begins with MHC II transcription and assembly of MHC II along with the invariant chain in the endoplasmic reticulum. The invariant chain binds to the antigen binding grove of the MHC II heterodimer, preventing the inadvertent loading of peptide prior to the complex reaching the phagolysosome (ten Broeke et al., 2013). Several studies have suggested that this complex is exported to the cell surface, and is then endocytosed and delivered to the MIIC via the canonical endolysosomal trafficking route (Schmid et al., 2007; Blander, 2018). This model is predicated on the observation that MHC II and invariant chain can be found on the cell surface; however, this complex is not observed colocalizing with endosomal markers such as Rab5, leading to the alternative hypothesis that the MHC II/invariant chain complex is instead delivered via direct Golgi-to-phagolysosome trafficking (Dörfel et al., 2005; Schmid et al., 2007). It is unclear at this time as to the extent these two trafficking routes contribute to the transport of MHC II to the phagolysosome, and consequentially, the Rab GTPases regulating this process remain to be described (Yin et al., 2016; Penteado et al., 2017). Regardless, once delivered to the phagolysosome, the invariant chain is cleaved by lysosomal proteases, leaving a small fragment in the MHC II antigen binding grove. Chaperones then mediate the exchange of this fragment for a phagolysosome-derived peptide, thereby loading the MHC II molecule (Stumptner and Benaroch, 1997; Landsverk et al., 2009). The antigen/MHC II complex is then exported to the cell surface, allowing for antigen presentation to CD4$^+$ T helper cells (Rock et al., 2016).

4.5 Recycling Endosome

While not directly involved in engulfment and killing of bacterial pathogens, the recycling endosome plays a critical role in phagocytosis. The recycling endosome is a highly dynamic system of endosomal structures

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275 and associated regulatory molecules that is responsible for recycling of internalized phagocytic receptors
276 back to the cell surface to permit rapid, sequential clearance of multiple phagocytic targets (reviewed in
277 Goldenring, 2015). Unsurprisingly, Rab GTPases are involved in multiple components of the recycling
278 endosome system. Rab11 and Rab14 are known to regulate membrane receptor transport from the recycling
279 endosome to the cell surface in phagocytic cells (Agola et al., 2011). Of these, Rab11 is the best studied and
280 mediates the transport of vesicles from the trans-Golgi network to the recycling endosome, and from there
281 to the cell surface (Horgan and McCaffrey, 2012). In particular, Rab11 activity is required for FcR-
282 mediated phagocytosis, as macrophages expressing an inactive form of Rab11 have impaired FcR-mediated
283 phagocytosis and reduced cell surface FcR expression (Cox et al., 2000). Rab10 is another critical regulator
284 of phagosome recycling, and is recruited to the phagosomal membrane prior to the acquisition of Rab5.
285 Here, Rab10 plays an indispensable role in facilitating the recycling of phagosomal GPI-linked proteins
286 back to the plasma membrane (Cardoso et al., 2010). Rab10 overexpression partially rescues phagosomal
287 maturation arrest induced by *Mycobacteria*, suggesting Rab10 is a potential target that can restore normal
288 phagosome maturation during infection (Cardoso et al., 2010).
289

290 ## 5. Efferosome Maturation

291 The recognition, uptake and degradation of apoptotic cells through efferocytosis is a crucial mechanism in
292 the maintenance of tissue homeostasis (Henson, 2017). In most cases, efferocytosis is performed by
293 professional phagocytic cells—macrophages in particular are responsible for apoptotic cell clearance in
294 many tissues of the body (Gordon and Plüddemann, 2018). Efferocytosis removes apoptotic cells from the
295 body, thus preventing apoptotic cells from undergoing secondary necrosis – a pro-inflammatory process due
296 to the release of proinflammatory cytosolic contents (Wickman et al., 2013; Henson, 2017; Morioka et al.,
297 2019) . Efferocytosis shares many parallels with phagocytosis, and utilizes many of the same Rab GTPases
298 and vesicular trafficking events to engulf and degrade apoptotic cells (**Figure 1**). However, even in
299 professional antigen presenting cells, efferosomes do not mature into a MIIC, thus limiting the presentation

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300 of apoptotic cell derived antigens (e.g. self-antigens) to the adaptive immune system (Yin et al., 2016,
301 2017).
302
303 Although the process of apoptotic cell recognition, binding of the phagocyte to the apoptotic cell and
304 subsequent apoptotic cell internalization and degradation is similar to the processes utilized during the
305 phagocytosis of pathogens, efferocytosis utilizes a distinct set of receptors that recognize and bind to unique
306 chemical motifs present on the surface of apoptotic cells. Although we provide a brief overview of
307 apoptotic cell recognition and internalization below, these topics have recently been reviewed in detail by
308 Flannagan *et al.* and Penberthy *et al.* (Flannagan et al., 2012; Penberthy and Ravichandran, 2016).
309
310 *5.1 Recognition and uptake of efferocytic targets*
311 Efferocytosis is initiated upon recognition of apoptotic cells by phagocytes. Efferocytic cells are capable of
312 recognizing specific ligands on the surface of the apoptotic cells, called "eat-me signals" (Elliott and
313 Ravichandran, 2016). A number of eat-me signals have now been identified, including exposure of PtdSer
314 on the outer leaflet of the plasma membrane of apoptotic cells, alterations in the glycosylation patterns on
315 the apoptotic cell surface, changes in ICAM-1 epitopes on the cell surface, and exposure of the endoplasmic
316 protein calreticulin on the cell surface (reviewed in Green et al., 2016; Nagata, 2018). Of these eat-me
317 signals, PtdSer exposure is the best understood. In healthy cells, PtdSer is sequestered on the cytoplasmic
318 face of the plasma membrane through the activity of flippase enzymes (Kimani et al., 2014). During
319 apoptosis, the amount of PtdSer exposed on the cell surface increased by more than 280-fold, comprising up
320 to 10% of the exofacial lipid content (Borisenko et al., 2003). This large change in PtdSer distribution is
321 driven by two caspase-mediated processes: the cleavage-induced inactivation of the flippases ATP11A and
322 ATP11C, and the parallel cleavage-induced activation of the scramblase XRK8 (Suzuki et al., 2013; K.
323 Segawa et al., 2014; Suzuki et al., 2016).

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324 Exposed eat-me signals on the surface of apoptotic cells are recognized by specialized efferocytic receptors
325 (Freeman and Grinstein, 2014; Gordon and Plüddemann, 2018). Most known efferocytic receptors function
326 by binding to PtdSer, either directly or through one or more opsonins. Among the efferocytic receptors that
327 bind directly to PtdSer are members of the TIM family of receptors (including TIM-1, TIM-3 and TIM-4),
328 brain angiogenesis inhibitor 1 (BAI1), Stabilin-2, members of the CD300 family of receptors, and the
329 receptor for advanced glycation end products (RAGE) (Park et al., 2007; Miyanishi et al., 2007; Park et al.,
330 2008; He et al., 2011; Nakahashi-Oda et al., 2012). Opsonin-dependent efferocytosis receptors include
331 multiple integrins - recognizing apoptotic cells via MFG-E8 and sCD93, and the TAM family of receptors -
332 recognizing apoptotic cells via the opsonins Gas6 and Protein S (Savill et al., 1990; Meer et al., 2014;
333 Blackburn et al., 2019).
334
335 Following recognition and binding, the phagocyte engulfs the apoptotic cell. The exact nature of this uptake
336 mechanism is unclear, with *in vitro* experiments often observing the uptake of intact cells (Yin et al., 2016,
337 2017), while *in vivo* studies often observe a piecemeal disassembly of the apoptotic cell – often by multiple
338 phagocytes (Wang et al., 2017). Regardless, the signaling and engulfment mechanisms appear to closely
339 parallel pathogen phagocytosis: initial binding and receptor activation leads to the protrusions of the
340 phagocyte's plasma membrane around the target cell, culminating in engulfment of the apoptotic cell (or
341 cell fragment) into an intracellular efferosome (Karaji and Sattentau, 2017).
342
343
344 *5.2 Early Efferosome*
345 Following closure of the phagocytic cup, the apoptotic cell becomes fully internalized within the
346 efferosome, a plasma membrane-derived vacuole analogous to the phagosome (Flannagan et al., 2012). The
347 efferosome undergoes a series of highly-regulated biochemical modifications to efficiently degrade the
348 internalized apoptotic cell (Kinchen and Ravichandran, 2008). Similar to phagosome maturation,
349 efferosome maturation is characterized by both sequential fusion with early and then late endosomes, along

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350 with progressive acidification of the efferosome lumen (Kinchen and Ravichandran, 2008). The regulation
351 of the early stages of efferosome maturation is similar to that involved in early phagosome maturation and
352 is characterized by the recruitment of the small GTPase Rab5 to the efferosome surface (Kinchen and
353 Ravichandran, 2008). To date no studies have investigated the role of focal exocytosis by Rab13 and Rab35
354 during apoptotic cell engulfment, meaning that the earliest known efferosome maturation event following
355 apoptotic cell internalization is the recruitment of Rab5 to the nascent efferosome (Kitano et al., 2008).
356 However, in *C. elegans* Rab35 mutants do not effectively clear apoptotic cells and have delayed Rab5
357 accumulation on the efferosome surface, suggesting a similar role for Rab35 in efferocytosis and
358 phagocytosis (Haley et al., 2018).

359

360 The stages and timing of the early efferosome maturation are very similar to phagocytosis. Indeed, our
361 group has directly compared these processes, and aside from a slight delay in the Rab5-Rab7 transition on
362 efferosomes, the early maturation steps appear identical (Yin et al., 2016). Many of the other events in
363 phagosome maturation have been observed on efferosomes, including the Mon1/Ccz1 mediated exchange
364 of Rab5 for Rab7, lysosome fusion, and efferosome acidification (Kinchen and Ravichandran, 2008).
365 However, while phagocytosis results in the induction of an inflammatory immune response and presentation
366 of antigens from the internalized pathogen, efferocytosis is immunologically silent and does not result in
367 antigen presentation (McColl et al., 2009; Birge et al., 2016). Therefore, key differences must exist in the
368 regulation of the late maturation of efferosomes compared to phagosomes to achieve these disparate
369 outcomes.

370

371 *5.3 Late Efferosome*

372 As in phagocytosis, replacement of Rab5 by Rab7 marks the transition from the early efferosome to the late
373 efferosome (Kinchen and Ravichandran, 2008). Rab7 then recruits effectors that have crucial roles in
374 driving apoptotic cell degradation. These mediators include RILP and ORP1L, which as in the late

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375 phagosome, interact with the dynein/dynactin motor complex to traffic late efferosomes to the perinuclear
376 region (Harrison et al., 2003; Yin et al., 2017). Perinuclear localization is necessary for efficient fusion with
377 lysosomes and the delivery of lysosomal hydrolases to the efferosome (Cantalupo et al., 2001; Johnson et
378 al., 2016). RILP further mediates v-ATPase assembly on the efferosome surface and drives efferosome
379 acidification and activation of lysosomal hydrolases to complete degradation of the internalized apoptotic
380 cell (De Luca et al., 2015). Thus far, late efferosome maturation closely resembles phagosome maturation.
381 However, it is at this point where the two processes diverge (**Figure 1**).
382
383 While pathogen-bearing phagolysosomes mature into a MIIC, efferosomes do not, and instead late
384 efferosomes interact with the recycling endosome system (Yin et al., 2017). The small GTPase Rab17 is an
385 essential regulator of this process (Yin et al., 2016, 2017; Ono et al., 2020). Rab17 has previously been
386 characterized in the literature as a regulator of apical-to-basolateral transport during transcytosis in hepatic
387 cells, and in the exocytosis melanosomes from melanocytes (Hunziker and Peters, 1998; Zacchi et al.,
388 1998; Beaumont et al., 2011). Following apoptotic cell internalization, Rab17 is recruited to the maturing
389 efferosome in parallel with Rab5, but unlike Rab5, Rab17 persists on the efferosome throughout the entire
390 efferosome maturation process (Yin et al., 2016). Following lysosome fusion in the perinuclear region,
391 Rab17 mediates the outwards migration of the efferosome towards the cell periphery. Here, the efferosome
392 intermixes with recycling endosomes, presumably to complete the absorption of the apoptotic cell or to
393 exocytose the degraded apoptotic cell debris (Yin et al., 2017). Recent work by Ono and colleagues has
394 presented evidence that Rabex-5 and another Rab17-interacting protein, ALS, recruit Rab17 to the
395 efferosome surface, with Rabex-5 acting as a Rab17 GEF (Mori et al., 2012; Ono et al., 2020). When a
396 dominant negative Rab17 mutant is expressed, trafficking of degraded apoptotic cell contents to the
397 recycling endosome does not occur, and instead, the apoptotic cell containing phagolysosome remains in
398 the perinuclear area where it accumulates markers of an MIIC (Yin et al., 2016, 2017; Ono et al., 2020).
399 Clearly, late efferosome maturation diverges from late phagosome maturation, with the selective retention

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400 of Rab17 to efferosomes playing a central role in the trafficking of efferocytosed apoptotic cell contents
401 away from the MIIC and into the recycling endosome system, and in doing so, limits the loading of self-
402 antigens onto MHC II and their subsequent presentation to CD4^{+} T cells.
403
404 *5.4 Recycling endosome*
405 In addition to aiding in the final dispossession of apoptotic cells, the recycling endosome system plays other
406 important roles during efferocytosis. For example, Chen *et al.* demonstrated that in *C. elegans* mutants
407 lacking sorting nexins involved in vesicle trafficking within the recycling endosome system had impaired
408 recycling of the efferocytic opsonin MFG-E8, resulting in lower levels of extracellular MFG-E8, poorer
409 apoptotic cell opsonization, and a resulting increase in the numbers of uncleared apoptotic cells during
410 germline development (Chen et al., 2010). Recruitment of Rab17 to the recycling endosome occurs in a
411 Rab11-dependent manner, suggesting that Rab11 activity may be critical to transport of degraded apoptotic
412 cell contents from the late efferosome to the recycling endosome compartment (Ono et al., 2020). However,
413 Rab11 activity may oppose efferocytosis under certain circumstances. Jiang *et al.* showed that reduction of
414 Rab11 expression enhances the clearance of apoptotic neutrophils by macrophages in a murine model of
415 airway inflammation (Jiang et al., 2017). They suggest that this was due to Rab11-mediate recycling of the
416 metalloproteinase ADAM17, which when localized to the cell surface cleaveed the efferocytic receptor
417 CD36 (Jiang et al., 2017). Another Rab protein involved in the regulation of apoptotic cell clearance and
418 endocytic recycling is Rab2 (Mangahas et al., 2008). Rab2 was initially identified through a loss-of-
419 function screen as playing a role in apoptotic cell clearance in *C. elegans,* and was proposed to be a direct
420 regulator of efferosome maturation. However, subsequent evidence has suggested that Rab2 is instead
421 involved in targeting proteins to the recycling endosome system. In phagocytic cells, defective Rab2
422 activity results in the aberrant trafficking of endosome-associated proteins such as RME-1, resulting in their
423 mis-localization to the cell surface (Lu et al., 2008; Mangahas et al., 2008).
424

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425 # 6. Conclusion

426 Phagocytosis and efferocytosis are highly conserved processes that involve the binding of a target particle,
427 internalization of the target into a phagosome or efferosome, and the maturation of the
428 phagosome/efferosome through sequential fusion with endosomes and lysosomes. However, while
429 phagocytosis is involved in pathogen elimination and results in inflammation and antigen presentation,
430 efferocytosis removes apoptotic cells and is immunologically silent. As central regulators of membrane and
431 vesicular trafficking, the Rab family of small GTPases play crucial roles in the regulation of the maturation
432 processes of phagosomes and efferosomes, and help determine the fate of phagocytic and efferocytic cargo
433 **(Figure 1).** Unsurprisingly, pathogens often target Rab GTPases and their effectors to evade phagocytic
434 killing, and Rab GTPases are putative targets for treating autoimmunity through manipulating the
435 presentation of self-antigens (Lam et al., 2016). Future research is crucial for improving our understanding
436 of how pathogens evade phagocytic killing, and how the aberrant presentation of apoptotic cell-derived
437 antigens contribute to autoimmune disease.

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438 # Tables

439 **Table 1.** Rab GTPases involved in the regulation of phagosome and efferosome maturation.
440

Rab GTPases	Function	Pathway	Reference
Rab1	ER to Golgi transport	Phagocytosis	(Saraste, 2016)
Rab2	ER to Golgi transport	Phagocytosis/ Efferocytosis	(Saraste, 2016)
Rab5	Required from nascent phagosome to Rab7(+) stage	Phagocytosis/ Efferocytosis	(Mottola, 2014)
Rab7	Required from Rab5(+) to LAMP	Phagocytosis/ Efferocytosis	(Bröcker et al., 2012)
Rab11	Transport from Golgi to polarized membrane, v-ATPase transport	Phagocytosis	(Jing and Prekeris, 2009)
Rab14	Phagosome and early endosome fusion, trafficking between early endosomes and Golgi	Phagocytosis/ Efferocytosis	(Yeo et al., 2016)
Rab17	Efferosome to recycling endosome trafficking	Efferocytosis	(Yin et al., 2016)
Rab20	Required for late phagosome	Phagocytosis	(Egami and Araki, 2012)
Rab22	Trans-Golgi-endosome transport	Phagocytosis	(Weber and Faris, 2018)
Rab35	Required for early phagosome	Phagocytosis/ Efferocytosis	(Yeo et al., 2016)
Rab39	Required for late phagosome	Phagocytosis	(Yeo et al., 2016)

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Figure Caption

Figure 1. Role of Rab GTPases during phagosome and efferosome maturation. Model illustrating the role of Rab GTPases in phagocytosis and efferocytosis. Both processes involve maturation of the phagosome or efferosome through sequential fusion with early endosomes (EE), late endosomes (LE) and lysosomes (LY). In phagocytosis, following lysosomal fusion, the phagolysosome matures into a MHC II loading compartment through delivery of newly synthsized MHC II to the phagolysosome. In contrast, in efferocytosis, the late efferosome breaks into smaller vesicles which traffick to the cell periphery where they undergo fusion with the recycling endosome system (RE).

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Conflict of Interest

The authors declare no commercial or financial conflict of interest.

HISTOLOGY AND HISTOPATHOLOGY (non-edited manuscript)

456 # References

457 Agola J.O., Jim P.A., Ward H.H., Basuray S and Wandinger-Ness A. (2011). Rab GTPases as
458 regulators of endocytosis, targets of disease and therapeutic opportunities. Clin. Genet. 80, 305–
459 318.

460 Aikawa Y and Martin T.F.J. (2003). ARF6 regulates a plasma membrane pool of
461 phosphatidylinositol(4,5)bisphosphate required for regulated exocytosis. J. Cell Biol. 162, 647-
462 659

463 al-Alawi N., Xu G., White R., Clark R., McCormick F and Feramisco J.R. (1993). Differential
464 regulation of cellular activities by GTPase-activating protein and NF1. Mol. Cell. Biol. 13,
465 2497-2503

466 Alexandrov K., Horiuchi H., Steele-Mortimer O., Seabra M.C and Zerial M. (1994). Rab escort
467 protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their
468 target membranes. EMBO J. 13, 5262–5273.

469 Balderhaar H.J and kleine Ungermann C. (2013). CORVET and HOPS tethering complexes –
470 coordinators of endosome and lysosome fusion. J. Cell Sci. 126, 1307-1316

471 Beaumont K.A., Hamilton N.A., Moores M.T., Brown D.L., Ohbayashi N., Cairncross O., Cook A.L.,
472 Smith A.G., Misaki R., Fukuda M., Taguchi T., Sturm R.A and Stow J.L. (2011). The
473 Recycling Endosome Protein Rab17 Regulates Melanocytic Filopodia Formation and
474 Melanosome Trafficking. Traffic 12, 627–643.

475 Becken U., Jeschke A., Veltman K and Haas A. (2010). Cell-free fusion of bacteria-containing
476 phagosomes with endocytic compartments. Proc. Natl. Acad. Sci. 107, 20726.

477 Beertsen W., Willenborg M., Everts V., Zirogianni A., Podschun R., Schröder B., Eskelinen E.-L. and
478 Saftig P. (2008). Impaired Phagosomal Maturation in Neutrophils Leads to Periodontitis in
479 Lysosomal-Associated Membrane Protein-2 Knockout Mice. J. Immunol. 180, 475.

HISTOLOGY AND HISTOPATHOLOGY (non-edited manuscript)

480 Bensch K.W., Raida M., Mägert H.-J., Schulz-Knappe P and Forssmann W.-G. (1995). hBD-1: a novel
481 β-defensin from human plasma. FEBS Lett. 368, 331–335.
482 Berg T.O., Fengsrud M., Strømhaug P.E., Berg T and Seglen P.O. (1998). Isolation and
483 Characterization of Rat Liver Amphisomes: EVIDENCE FOR FUSION OF
484 AUTOPHAGOSOMES WITH BOTH EARLY AND LATE ENDOSOMES. J. Biol. Chem.
485 273, 21883–21892.
486 Birge R.B., Boeltz S., Kumar S., Carlson J., Wanderley J., Calianese D., Barcinski M., Brekken R.A.,
487 Huang X., Hutchins J.T., Freimark B., Empig C., Mercer J., Schroit A.J., Schett G and
488 Herrmann M. (2016). Phosphatidylserine is a global immunosuppressive signal in efferocytosis,
489 infectious disease, and cancer. Cell Death Differ. 23, 962–978.
490 Blackburn J.W.D., Lau D.H.C., Liu E.Y., Ellins J., Vrieze A.M., Pawlak E.N., Dikeakos J.D and Heit
491 B. (2019). Soluble CD93 is an apoptotic cell opsonin recognized by $\alpha_x \beta_2$. Eur. J. Immunol. 1–
492 23.
493 Blander J.M. (2018). Regulation of the Cell Biology of Antigen Cross-Presentation. Annu. Rev.
494 Immunol. 36, 717–753.
495 Blocker A., Griffiths G., Olivo J.C., Hyman A.A and Severin F.F. (1998). A role for microtubule
496 dynamics in phagosome movement. J. Cell Sci. 111, 303-312.
497 Bollag G and McCormick F. (1991). Differential regulation of rasGAP and neurofibromatosis gene
498 product activities. Nature 351, 576–579.
499 Borisenko G.G., Matsura T., Liu S.X., Tyurin V.A., Jianfei J., Serinkan F.B and Kagan V.E. (2003).
500 Macrophage recognition of externalized phosphatidylserine and phagocytosis of apoptotic
501 Jurkat cells - Existence of a threshold. Arch. Biochem. Biophys. 413, 41–52.
502 Bröcker C., Kuhlee A., Gatsogiannis C., kleine Balderhaar H.J., Hönscher C., Engelbrecht-Vandré S.,
503 Ungermann C and Raunser S. (2012). Molecular architecture of the multisubunit homotypic

HISTOLOGY AND HISTOPATHOLOGY (non-edited manuscript)

504 fusion and vacuole protein sorting (HOPS) tethering complex. Proc. Natl. Acad. Sci. 109, 1991-
505 1996.
506 Brumell J.H and Scidmore M.A. (2007). Manipulation of rab GTPase function by intracellular bacterial
507 pathogens. Microbiol. Mol. Biol. Rev. MMBR 71, 636–652.
508 Byekova Y.A., Powell R.R., Welter B.H and Temesvari L.A. (2010). Localization of
509 phosphatidylinositol (3,4,5)-trisphosphate to phagosomes in entamoeba histolytica achieved
510 using glutathione S-transferase- and green fluorescent protein-tagged lipid biosensors. Infect.
511 Immun. 78, 125–137.
512 Cantalupo G., Alifano P., Roberti V., Bruni C.B and Bucci C. (2001). Rab-interacting lysosomal
513 protein (RILP): The Rab7 effector required for transport to lysosomes. EMBO J. 20, 683–693.
514 Canton J., Khezri R., Glogauer M and Grinstein S. (2014). Contrasting phagosome pH regulation and
515 maturation in human M1 and M2 macrophages. Mol. Biol. Cell 25, 3330–3341.
516 Cardoso C.M.P., Jordao L and Vieira O.V. (2010). Rab10 Regulates Phagosome Maturation and Its
517 Overexpression Rescues Mycobacterium-Containing Phagosomes Maturation. Traffic 11, 221–
518 235.
519 Castrillo A., Pennington D.J., Otto F., Parker P.J., Owen M.J and Boscá L. (2001). Protein Kinase Cϵ Is
520 Required for Macrophage Activation and Defense Against Bacterial Infection. J. Exp. Med.
521 194, 1231–1242.
522 Chardin P., Paris S., Antonny B., Robineau S., Béraud-Dufour S., Jackson C.L and Chabre M. (1996).
523 A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. Nature
524 384, 481–484.
525 Chen D., Xiao H., Zhang K., Wang B., Gao L., Jian Y., Qi X., Sun J., Miao L and Yang C. (2010).
526 Retromer is required for apoptotic cell clearance by phagocytic receptor recycling. Science 327,
527 1261–1264.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

528 Collins R.F., Schreiber A.D., Grinstein S and Trimble W.S. (2002). Syntaxins 13 and 7 Function at
529 Distinct Steps During Phagocytosis. J. Immunol. 169, 3250-3256.
530 Cox D., Lee D.J., Dale B.M., Calafat J and Greenberg S. (2000). A Rab11-containing rapidly recycling
531 compartment in macrophages that promotes phagocytosis. Proc. Natl. Acad. Sci. 97, 680-685.
532 Davies J.M., Hunt I and Sanders D. (1994). Vacuolar H(+)-pumping ATPase variable transport
533 coupling ratio controlled by pH. Proc. Natl. Acad. Sci. U. S. A. 91, 8547–8551.
534 De Luca M., Cogli L., Progida C., Nisi V., Pascolutti R., Sigismund S., Di Fiore P.P and Bucci C.
535 (2015). RILP regulates vacuolar ATPase through interaction with the V1G1 subunit. J. Cell Sci.
536 128, 2565–2565.
537 Demirdjian S., Hopkins D., Sanchez H., Libre M., Gerber S.A and Berwin B. (2018).
538 Phosphatidylinositol-(3,4,5)-Trisphosphate Induces Phagocytosis of Nonmotile Pseudomonas
539 aeruginosa. Infect. Immun. 86, e00215-00218.
540 Di A., Nelson D.J., Bindokas V., Brown M.E., Libunao F and Palfrey H.C. (2003). Dynamin regulates
541 focal exocytosis in phagocytosing macrophages. Mol. Biol. Cell 14, 2016–2028.
542 Donaldson J.G and Jackson C.L. (2011). ARF family G proteins and their regulators: roles in
543 membrane transport, development and disease. Nat. Rev. Mol. Cell Biol. 12, 362–375.
544 Doran A.C., Yurdagul A and Tabas I. (2019). Efferocytosis in health and disease. Nat. Rev. Immunol.
545 254-267.
546 Dörfel D., Appel S., Grünebach F., Weck M.M., Müller M.R., Heine A and Brossart P. (2005).
547 Processing and presentation of HLA class I and II epitopes by dendritic cells after transfection
548 with in vitro–transcribed MUC1 RNA. Blood 105, 3199–3205.
549 Egami Y and Araki N. (2012). Rab20 Regulates Phagosome Maturation in RAW264 Macrophages
550 during Fc Gamma Receptor-Mediated Phagocytosis. PLOS ONE 7, e35663.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

551 Egami Y., Fukuda M and Araki N. (2011). Rab35 regulates phagosome formation through recruitment
552 of ACAP2 in macrophages during FcγR-mediated phagocytosis. J. Cell Sci. 124, 3557-3567.
553 Elliott M.R and Ravichandran K.S. (2016). The dynamics of apoptotic cell clearance. Dev. Cell 38,
554 147–160.
555 Esteban M.Á., Cuesta A., Chaves-Pozo E and Meseguer J. (2015). Phagocytosis in Teleosts.
556 Implications of the New Cells Involved. Biology 4, 907–922.
557 Flannagan R.S., Cosío G and Grinstein S. (2009). Antimicrobial mechanisms of phagocytes and
558 bacterial evasion strategies. Nat. Rev. Microbiol. 7, 355–366.
559 Flannagan R.S., Jaumouillé V and Grinstein S. (2012). The Cell Biology of Phagocytosis. Annu. Rev.
560 Pathol. Mech. Dis. 7, 61–98.
561 Freeman S.A and Grinstein S. (2014). Phagocytosis: receptors, signal integration, and the cytoskeleton.
562 Immunol. Rev. 262, 193–215.
563 Goldenring J.R. (2015). Recycling endosomes. Curr. Opin. Cell Biol. 35, 117–122.
564 Gordon P.B., Høyvik H and Seglen P.O. (1992). Prelysosomal and lysosomal connections between
565 autophagy and endocytosis. Biochem. J. 283, 361–369.
566 Gordon S. (2016). Phagocytosis: An Immunobiologic Process. Immunity 44, 463–475.
567 Gordon S and Plüddemann A. (2018). Macrophage Clearance of Apoptotic Cells: A Critical
568 Assessment. Front. Immunol. 9, 127. 1-9.
569 Green D.R., Oguin T.H and Martinez J. (2016). The clearance of dying cells: table for two. Cell Death
570 Differ. 23, 915–926.
571 Gutierrez M.G. (2013). Functional role(s) of phagosomal Rab GTPases. Small GTPases 4, 148–158.
572 Haley R., Wang Y and Zhou Z. (2018). The small GTPase RAB-35 defines a third pathway that is
573 required for the recognition and degradation of apoptotic cells, PLoS Genetics. 1-34

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

574 Harrison R.E., Bucci C., Vieira O.V., Schroer T.A and Grinstein S. (2003). Phagosomes Fuse with Late
575 Endosomes and/or Lysosomes by Extension of Membrane Protrusions along Microtubules:
576 Role of Rab7 and RILP. Mol. Cell. Biol. 23, 6494-6506.

577 He M., Kubo H., Morimoto K., Fujino N., Suzuki T., Takahasi T., Yamada M., Yamaya M., Maekawa
578 T., Yamamoto Y and Yamamoto H. (2011). Receptor for advanced glycation end products
579 binds to phosphatidylserine and assists in the clearance of apoptotic cells. EMBO Rep. 12, 358–
580 364.

581 Henson P.M. (2017). Cell Removal : Efferocytosis. Annu. Rev. Cell Dev. Biol. 33, 1–18.

582 Horgan C.P and McCaffrey M.W. (2012). Rab GTPases and microtubule motors. Biochem. Soc. Trans.
583 39, 1202–1206.

584 Hunziker W., Peters P.J. (1998). Rab17 localizes to recycling endosomes and regulates receptor-
585 mediated transcytosis in epithelial cells. J. Biol. Chem. 273, 15734–15741.

586 Husebye H., Aune M.H., Stenvik J., Samstad E., Skjeldal F., Halaas Ø., Nilsen N.J., Stenmark H., Latz
587 E., Lien E., Mollnes T.E., Bakke O and Espevik T. (2010). The Rab11a GTPase Controls Toll-
588 like Receptor 4-Induced Activation of Interferon Regulatory Factor-3 on Phagosomes.
589 Immunity 33, 583–596.

590 Hutagalung A.H and Novick P.J. (2011). Role of Rab GTPases in membrane traffic and cell
591 physiology. Physiol. Rev. 91, 119–149.

592 Ioannou M.S and McPherson P.S. (2016). Regulation of Cancer Cell Behavior by the Small GTPase
593 Rab13. J. Biol. Chem. 291, 9929–9937.

594 Jancic C., Savina A., Wasmeier C., Tolmachova T., El-Benna J., Dang P.M.-C., Pascolo S., Gougerot-
595 Pocidalo M.-A., Raposo G., Seabra M.C and Amigorena S. (2007). Rab27a regulates
596 phagosomal pH and NADPH oxidase recruitment to dendritic cell phagosomes. Nat. Cell Biol.
597 9, 367–378.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

598 Jiang C., Liu Z., Hu R., Bo L., Minshall R.D., Malik A.B and Hu G. (2017). Inactivation of Rab11a
599 GTPase in Macrophages Facilitates Phagocytosis of Apoptotic Neutrophils. J. Immunol.
600 1601495. 1660-1672

601 Jing J and Prekeris R. (2009). Polarized endocytic transport: the roles of Rab11 and Rab11-FIPs in
602 regulating cell polarity. Histol. Histopathol. 24, 1171–1180.

603 Johansson M., Rocha N., Zwart W., Jordens I., Janssen L., Kuijl C., Olkkonen V.M and Neefjes J.
604 (2007). Activation of endosomal dynein motors by stepwise assembly of Rab7–RILP–
605 p150Glued, ORP1L, and the receptor βlll spectrin. J. Cell Biol. 176, 459–471.

606 Johnson D.E., Ostrowski P., Jaumouillé V and Grinstein S. (2016). The position of lysosomes within
607 the cell determines their luminal pH. J. Cell Biol. 212, 677–692.

608 Jutras I and Desjardins,M. (2005). PHAGOCYTOSIS: At the Crossroads of Innate and Adaptive
609 Immunity. Annu. Rev. Cell Dev. Biol. 21, 511–527.

610 Kamen L.A., Levinsohn J and Swanson J.A. (2007). Differential association of phosphatidylinositol 3-
611 kinase, SHIP-1, and PTEN with forming phagosomes. Mol. Biol. Cell 18, 2463–2472.

612 Kaplan G. (1977). Differences in the Mode of Phagocytosis with Fc and C3 Receptors in Macrophages.
613 Scand. J. Immunol. 6, 797–807.

614 Karaji N and Sattentau Q.J. (2017). Efferocytosis of Pathogen-Infected Cells. Front. Immunol. 8, 1863–
615 1863.

616 Kimani S.G., Geng K., Kasikara C., Kumar S., Sriram G., Wu Y and Birge R.B. (2014). Contribution
617 of defective PS recognition and efferocytosis to chronic inflammation and autoimmunity. Front.
618 Immunol. 5, 1–9.

619 Kinchen J.M., Doukoumetzidis K., Almendinger J., Stergiou L., Tosello-Trampont A., Sifri C.D.,
620 Hengartner M.O and Ravichandran K.S. (2008). A pathway for phagosome maturation during
621 engulfment of apoptotic cells. Nat. Cell Biol. 10, 556–566.

HISTOLOGY AND HISTOPATHOLOGY (non-edited manuscript)

622 Kinchen J.M and Ravichandran K.S. (2010). Identification of two evolutionarily conserved genes
623 regulating processing of engulfed apoptotic cells. Nature 464, 778–782.
624 Kinchen J.M and Ravichandran, K.S. (2008). Phagosome maturation: going through the acid test. Nat.
625 Rev. Mol. Cell Biol. 9, 781–795.
626 Kitano M., Nakaya M., Nakamura T., Nagata S and Matsuda M. (2008). Imaging of Rab5 activity
627 identifies essential regulators for phagosome maturation. Nature 453, 241–245.
628 Kobayashi H., Etoh K., Ohbayashi N and Fukuda M. (2014). Rab35 promotes the recruitment of Rab8,
629 Rab13 and Rab36 to recycling endosomes through MICAL-L1 during neurite outgrowth. Biol.
630 Open 3, 803–814.
631 Kuhlman M., Joiner K and Ezekowitz R.A. (1989). The human mannose-binding protein functions as
632 an opsonin. J. Exp. Med. 169, 1733–1745.
633 Kyei G.B., Vergne I., Chua J., Roberts E., Harris J., Junutula J.R and Deretic V. (2006). Rab14 is
634 critical for maintenance of Mycobacterium tuberculosis phagosome maturation arrest. EMBO J.
635 25, 5250–5259.
636 Lam T., Kulp D.V., Wang R., Lou Z., Taylor J., Rivera C.E., Yan H., Zhang Q., Wang Z., Zan H.,
637 Ivanov D.N., Zhong G., Casali P and Xu Z. (2016). Small Molecule Inhibition of Rab7 Impairs
638 B Cell Class Switching and Plasma Cell Survival To Dampen the Autoantibody Response in
639 Murine Lupus. J. Immunol. Baltim. Md 1950 197, 3792–3805.
640 Lancaster C., Fountain A., Somerville E., Sheth J., Dayam R.M., Jacobelli V., Somerville A.,
641 Terebiznik M and Botelho R.J. (2020). Phagosome resolution regenerates lysosomes and
642 maintains the degradative capacity in phagocytes. bioRxiv 2020.05.14.094722.
643 Landsverk O.J.B., Bakke O and Gregers T.F. (2009). MHC II and the Endocytic Pathway: Regulation
644 by Invariant Chain. Scand. J. Immunol. 70, 184–193.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

645 Larrick J.W., Hirata M., Zheng H., Zhong J., Bolin D., Cavaillon J.M., Warren H.S and Wright S.C.
646 (1994). A novel granulocyte-derived peptide with lipopolysaccharide-neutralizing activity. J.
647 Immunol. 152, 231.
648 Lee W.L., Mason D., Schreiber A.D and Grinstein S. (2007). Quantitative analysis of membrane
649 remodeling at the phagocytic cup. Mol. Biol. Cell 18, 2883–2892.
650 Li, G., Marlin, M.C., 2015. Rab family of GTPases. Methods Mol. Biol. Clifton NJ 1298, 1–15.
651 Lu Q., Zhang Y., Hu T., Guo P., Li W and Wang X. (2008). C. elegans Rab GTPase 2 is required for
652 the degradation of apoptotic cells. Development 135, 1069–1080.
653 Lukacs G.L., Rotstein O.D and Grinstein S. (1990). Phagosomal acidification is mediated by a
654 vacuolar-type H(+)-ATPase in murine macrophages. J. Biol. Chem. 265, 21099–21107.
655 Mangahas P.M., Yu X., Miller K.G and Zhou Z. (2008). The small GTPase Rab2 functions in the
656 removal of apoptotic cells in Caenorhabditis elegans. J. Cell Biol. 180, 357–373.
657 Marshansky V and Futai M. (2008). The V-type H+-ATPase in vesicular trafficking: targeting,
658 regulation and function. Curr. Opin. Cell Biol. 20, 415–426.
659 May R.C., Caron E., Hall A and Machesky L.M. (2000). Involvement of the Arp2/3 complex in
660 phagocytosis mediated by FcγR or CR3. Nat. Cell Biol. 2, 246–248.
661 McColl A., Bournazos S., Franz S., Perretti M., Morgan B.P., Haslett C and Dransfield I. (2009).
662 Glucocorticoids Induce Protein S-Dependent Phagocytosis of Apoptotic Neutrophils by Human
663 Macrophages. J. Immunol. 183, 2167-2175.
664 Meer J.H.M. Van Der Poll T. Van Der Veer and C. Van (2014). TAM receptors, Gas6, and protein S:
665 roles in inflammation and hemostasis. Blood 123, 2460–2470.
666 Miyanishi M., Tada K., Koike M., Uchiyama Y., Kitamura T and Nagata S. (2007). Identification of
667 Tim4 as a phosphatidylserine receptor. Nature 450, 435–439.

HISTOLOGY AND HISTOPATHOLOGY (non-edited manuscript)

668 Mizuno-Yamasaki E., Rivera-Molina F., Novick P. (2012). GTPase Networks in Membrane Traffic.
669 Annu. Rev. Biochem. 81, 637–659.
670 Mori Y., Matsui T., Furutani Y., Yoshihara Y and Fukuda M. (2012). Small GTPase Rab17 regulates
671 dendritic morphogenesis and postsynaptic development of hippocampal neurons. J. Biol. Chem.
672 287, 8963–8973.
673 Morioka S., Maueröder C and Ravichandran K.S. (2019). Living on the Edge: Efferocytosis at the
674 Interface of Homeostasis and Pathology. Immunity 1149–1162.
675 Mottola G. (2014). The complexity of Rab5 to Rab7 transition guarantees specificity of pathogen
676 subversion mechanisms. Front. Cell. Infect. Microbiol. 4, 180. 1-4
677 Müller M.P and Goody R.S. (2018). Molecular control of Rab activity by GEFs, GAPs and GDI. Small
678 GTPases 9, 5–21.
679 Murray R.Z., Kay J.G., Sangermani D.G and Stow J.L. (2005). A Role for the Phagosome in Cytokine
680 Secretion. Science 310, 1492-1495.
681 Nagata S. (2018). Apoptosis and Clearance of Apoptotic Cells. Annu Rev Immunol 361829, 1–18.
682 Nakahashi-Oda C., Tahara-Hanaoka S., Honda S. ichiro Shibuya K and Shibuya A. (2012).
683 Identification of phosphatidylserine as a ligand for the CD300a immunoreceptor. Biochem.
684 Biophys. Res. Commun. 417, 646–650.
685 Nielsen E., Christoforidis S., Uttenweiler-Joseph S., Miaczynska M., Dewitte F., Wilm M., Hoflack B
686 and Zerial M. (2000). Rabenosyn-5, a novel Rab5 effector, is complexed with hVPS45 and
687 recruited to endosomes through a FYVE finger domain. J. Cell Biol. 151, 601–612.
688 Nordenfelt P and Tapper H. (2011). Phagosome dynamics during phagocytosis by neutrophils. J.
689 Leukoc. Biol. 90, 271–284.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

690 Nordmann M., Cabrera M., Perz A., Bröcker C., Ostrowicz C., Engelbrecht-Vandré S and Ungermann
691 C. (2010). The Mon1-Ccz1 Complex Is the GEF of the Late Endosomal Rab7 Homolog Ypt7.
692 Curr. Biol. 20, 1654–1659.
693 Oesterlin L.K., Goody R.S and Itzen A. (2012). Posttranslational modifications of Rab proteins cause
694 effective displacement of GDP dissociation inhibitor. Proc. Natl. Acad. Sci. 109, 5621.
695 Ono S., Otomo A., Murakoshi S., Mitsui S., Sato K., Fukuda M and Hadano S. (2020). ALS2, the small
696 GTPase Rab17-interacting protein, regulates maturation and sorting of Rab17-associated
697 endosomes. Biochem. Biophys. Res. Commun. 523, 908–915.
698 Park D., Tosello-Trampont A.C., Elliott M.R., Lu M., Haney L.B., Ma Z., Klibanov A.L., Mandell J.W
699 and Ravichandran K.S. (2007). BAI1 is an engulfment receptor for apoptotic cells upstream of
700 the ELMO/Dock180/Rac module. Nature 450, 430–434.
701 Park J.J and Loh Y.P. (2008). Minireview: How Peptide Hormone Vesicles Are Transported to the
702 Secretion Site for Exocytosis. Mol. Endocrinol. 22, 2583–2595.
703 Park S.Y., Jung M.Y., Kim H.J., Lee S.J., Kim S.Y., Lee B.H., Kwon T.H., Park R.W., Kim I.S.
704 (2008). Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. Cell
705 Death Differ. 15, 192–201.
706 Pauwels A.-M., Trost M., Beyaert R and Hoffmann E. (2017). Patterns, Receptors, and Signals:
707 Regulation of Phagosome Maturation. Trends Immunol. 38, 407–422.
708 Penberthy K.K and Ravichandran K.S. (2016). Apoptotic cell recognition receptors and scavenger
709 receptors. Immunol. Rev. 269, 44–59.
710 Penteado L. de A., Dejani N.N., Verdan F.F., Orlando A.B., Niño V.E., Dias F.D.N., Salina A.C.G and
711 Medeiros A.I. (2017). Distinctive role of efferocytosis in dendritic cell maturation and
712 migration in sterile or infectious conditions. Immunology 151, 304–313.

HISTOLOGY AND HISTOPATHOLOGY (non-edited manuscript)

713 Poteryaev D., Datta S., Ackema K., Zerial M and Spang A. (2010). Identification of the Switch in
714 Early-to-Late Endosome Transition. Cell 141, 497–508.
715 Pylypenko O., Hammich H., Yu I.-M and Houdusse A. (2018). Rab GTPases and their interacting
716 protein partners: Structural insights into Rab functional diversity. Small GTPases 9, 22–48.
717 Roberts E.A., Chua J., Kyei G.B and Deretic V. (2006). Higher order Rab programming in
718 phagolysosome biogenesis. J. Cell Biol. 174, 923–929.
719 Rock K.L., Reits E and Neefjes J. (2016). Present Yourself! By MHC Class I and MHC Class II
720 Molecules. Trends Immunol. 37, 724–737.
721 Rosales C., and Uribe-Querol E. (2017). Phagocytosis: A Fundamental Process in Immunity. BioMed
722 Res. Int. 2017, 9042851–9042851.
723 Rougerie P., Miskolci V and Cox D. (2013). Generation of membrane structures during phagocytosis
724 and chemotaxis of macrophages: role and regulation of the actin cytoskeleton. Immunol. Rev.
725 256, 222–239.
726 Saraste J. (2016). Spatial and Functional Aspects of ER-Golgi Rabs and Tethers. Front. Cell Dev. Biol.
727 4, 28–28.
728 Saric A., Hipolito V.E.B., Kay J.G., Canton J., Antonescu C.N and Botelho R.J. (2015). mTOR
729 controls lysosome tubulation and antigen presentation in macrophages and dendritic cells. Mol.
730 Biol. Cell 27, 321–333.
731 Sarode G.S., Sarode S.C., Maniyar N., Sharma N.K and Patil S. (2017). Carcinogenesis-relevant
732 biological events in the pathophysiology of the efferocytosis phenomenon. Oncol. Rev. 11,
733 343–343.
734 Savill J., Dransfieldt I., Hoggt N and Haslett C. (1990). Vitronectin receptor-mediated phagocytosis of
735 cells undergoing apoptosis. Nature 343, 170–173.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

736 Schmid D., Pypaert M and Münz C. (2007). Antigen-Loading Compartments for Major
737 Histocompatibility Complex Class II Molecules Continuously Receive Input from
738 Autophagosomes. Immunity 26, 79–92.
739 Scott R.S., McMahon E.J., Pop S.M., Reap E.A., Caricchio R., Cohen P.L., Earp H.S and Matsushima
740 G.K. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature 411,
741 207–211.
742 Segawa K., Kurata S., Yanagihashi Y., Brummelkamp T.R., Matsuda F and Nagata S. (2014). Caspase-
743 mediated cleavage of phospholipid flippase for apoptotic phosphatidylserine exposure. Science
744 344, 1164–1168.
745 Segawa T., Hazeki K., Nigorikawa K., Morioka S., Guo Y., Takasuga S., Asanuma K and Hazeki O.
746 (2014). Inpp5e increases the Rab5 association and phosphatidylinositol 3-phosphate
747 accumulation at the phagosome through an interaction with Rab20. Biochem. J. 464, 365–375.
748 Selsted M.E., Tang Y.Q., Morris W.L., McGuire P.A., Novotny M.J., Smith W., Henschen A.H and
749 Cullor J.S. (1993). Purification, primary structures, and antibacterial activities of beta-defensins,
750 a new family of antimicrobial peptides from bovine neutrophils. J. Biol. Chem. 268, 6641–
751 6648.
752 Serezani C.H., Kane S., Medeiros A.I., Cornett A.M., Kim S.-H., Marques M.M., Lee S.-P., Lewis C.,
753 Bourdonnay E., Ballinger M.N., White E.S and Peters-Golden M. (2012). PTEN Directly
754 Activates the Actin Depolymerization Factor Cofilin-1 During PGE2-Mediated Inhibition of
755 Phagocytosis of Fungi. Sci. Signal. 5, ra12. 1-21.
756 Seto S., Tsujimura K and Koide Y. (2011). Rab GTPases Regulating Phagosome Maturation Are
757 Differentially Recruited to Mycobacterial Phagosomes. Traffic 12, 407–420.
758 Shin D.-M., Yang C.-S., Yuk J.-M., Lee J.-Y., Kim K.H., Shin S.J., Takahara K., Lee S.J and Jo E.-K.
759 (2008). Mycobacterium abscessus activates the macrophage innate immune response via a

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

760 physical and functional interaction between TLR2 and dectin-1. Cell. Microbiol. 10, 1608–
761 1621.
762 Simonsen A., Lippe R., Christoforidis S., Gaullier J.-M., Brech A., Callaghan J., Toh B.-H., Murphy
763 C., Zerial M and Stenmark H. (1998). EEA1 links PI(3)K function to Rab5 regulation of
764 endosome fusion. Nature 394, 494–498.
765 Söllner T., Bennett M.K., Whiteheart S.W., Scheller R.H and Rothman J.E. (1993). A protein
766 assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic
767 vesicle docking, activation, and fusion. Cell 75, 409–418.
768 Stenmark H. (2009). Rab GTPases as coordinators of vesicle traffic. Nat. Rev. Mol. Cell Biol. 10, 513–
769 525.
770 Strasser J.E., Newman S.L., Ciraolo G.M., Morris R.E., Howell M.L and Dean G.E. (1999). Regulation
771 of the Macrophage Vacuolar ATPase and Phagosome-Lysosome Fusion by Histoplasma
772 capsulatum. J. Immunol. 162, 6148-6154.
773 Stumptner P and Benaroch P. (1997). Interaction of MHC class II molecules with the invariant chain:
774 role of the invariant chain (81–90) region. EMBO J. 16, 5807–5818.
775 Suzuki J., Denning D.P., Imanishi E., Horvitz H.R and Nagata S. (2013). Xk-Related Protein 8 and
776 CED-8 Promote Phosphatidylserine Exposure in Apoptotic Cells. Science 341, 403-406.
777 Suzuki J., Imanishi E and Nagata S. (2016). Xkr8 phospholipid scrambling complex in apoptotic
778 phosphatidylserine exposure. Proc. Natl. Acad. Sci. 113, 9509-9514.
779 Tamura N., Hazeki K., Okazaki N., Kametani Y., Murakami H., Takaba Y., Ishikawa Y., Nigorikawa
780 K and Hazeki O. (2009). Specific role of phosphoinositide 3-kinase p110α in the regulation of
781 phagocytosis and pinocytosis in macrophages. Biochem. J. 423, 99–108.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

782 Taylor P.R., Tsoni S.V., Willment J.A., Dennehy K.M., Rosas M., Findon H., Haynes K., Steele C.,
783 Botto M., Gordon S and Brown G.D. (2007). Dectin-1 is required for beta-glucan recognition
784 and control of fungal infection. Nat. Immunol. 8, 31–38.
785 ten Broeke T., Wubbolts R and Stoorvogel W. (2013). MHC class II antigen presentation by dendritic
786 cells regulated through endosomal sorting. Cold Spring Harb. Perspect. Biol. 5, a016873. 1-21.
787 Toma-Fukai S and Shimizu T. (2019). Structural Insights into the Regulation Mechanism of Small
788 GTPases by GEFs. Mol. Basel Switz. 24, 3308. 1-30.
789 Toyohara A and Inaba, K. (1989). Transport of phagosomes in mouse peritoneal macrophages. J. Cell
790 Sci. 94, 143-153.
791 Underhill D.M and Goodridge H.S. (2012). Information processing during phagocytosis. Nat. Rev.
792 Immunol. 12, 492–502.
793 Uribe-Querol E and Rosales, C. (2017). Control of Phagocytosis by Microbial Pathogens. Front.
794 Immunol. 8, 1368–1368.
795 Vashi N., Andrabi S.B.A., Ghanwat S., Suar M and Kumar D. (2017). Ca(2+)-dependent Focal
796 Exocytosis of Golgi-derived Vesicles Helps Phagocytic Uptake in Macrophages. J. Biol. Chem.
797 292, 5144–5165.
798 Vieira O.V., Bucci C., Harrison R.E., Trimble W.S., Lanzetti L., Gruenberg J., Schreiber A.D., Stahl
799 P.D and Grinstein S. (2003). Modulation of Rab5 and Rab7 Recruitment to Phagosomes by
800 Phosphatidylinositol 3-Kinase. Mol. Cell. Biol. 23, 2501-2514.
801 Wang J., Hossain M., Thanabalasuriar A., Gunzer M., Meininger C and Kubes P. (2017). Visualizing
802 the function and fate of neutrophils in sterile injury and repair. Science 358, 111-116.
803 Wang T and Hong W. (2002). Interorganellar regulation of lysosome positioning by the Golgi
804 apparatus through Rab34 interaction with Rab-interacting lysosomal protein. Mol. Biol. Cell 13,
805 4317–4332.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

806 Weber M.M and Faris R. (2018). Subversion of the Endocytic and Secretory Pathways by Bacterial
807 Effector Proteins. Front. Cell Dev. Biol. 6, 1-17.
808 Wickman G.R., Julian L., Mardilovich K., Schumacher S., Munro J., Rath N., Zander S.A., Mleczak
809 A., Sumpton D., Morrice N., Bienvenut W.V and Olson M.F. (2013). Blebs produced by actin–
810 myosin contraction during apoptosis release damage-associated molecular pattern proteins
811 before secondary necrosis occurs. Cell Death Differ. 20, 1293–1305.
812 Winterbourn C.C., Hampton M.B., Livesey J.H and Kettle A.J. (2006). Modeling the Reactions of
813 Superoxide and Myeloperoxidase in the Neutrophil Phagosome: IMPLICATIONS FOR
814 MICROBIAL KILLING. J. Biol. Chem. 281, 39860–39869.
815 Yadav M and Schorey J.S. (2006). The beta-glucan receptor dectin-1 functions together with TLR2 to
816 mediate macrophage activation by mycobacteria. Blood 108, 3168–3175.
817 Yeo J.C., Wall A.A., Luo L and Stow J.L. (2016). Sequential recruitment of Rab GTPases during early
818 stages of phagocytosis. Cell. Logist. 6, e1140615. 1-2.
819 Yin C., Argintaru D and Heit B. (2017). Rab17 mediates intermixing of phagocytosed apoptotic cells
820 with recycling endosomes. Small GTPases 0, 1–9.
821 Yin C., Kim Y., Argintaru D and Heit, B. (2016). Rab17 mediates differential antigen sorting following
822 efferocytosis and phagocytosis. Cell Death Dis. 7, e2529-12.
823 Yutin N., Wolf M.Y., Wolf Y.I and Koonin E.V. (2009). The origins of phagocytosis and
824 eukaryogenesis. Biol. Direct 4, 9–9.
825 Zacchi P., Stenmark H., Parton R.G., Orioli D., Lim F., Giner A., Mellman I., Zerial M and Murphy C.
826 (1998). Rab17 regulates membrane trafficking through apical recycling endosomes in polarized
827 epithelial cells. J. Cell Biol. 140, 1039–1053.
828 Zhu H., Liang Z and Li G. (2009). Rabex-5 Is a Rab22 Effector and Mediates a Rab22-Rab5 Signaling
829 Cascade in Endocytosis. Mol. Biol. Cell 20, 4720–4729.

HISTOLOGY AND HISTOPATHOLOGY
(non-edited manuscript)

830 Zhu H., Zhu G., Liu J., Liang Z., Zhang X.C and Li G. (2007). Rabaptin-5-independent Membrane
831 Targeting and Rab5 Activation by Rabex-5 in the Cell. Mol. Biol. Cell 18, 4119–4128.

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833 Fig 1. Role of Rab GTPases during phagosome and efferosome maturation. Model illustrating
834 the role of Rab GTPases in phagocytosis and efferocytosis. Both processes involve maturation of the
835 phagosome or efferosome through sequential fusion with early endosomes (EE), late endosomes (LE)
836 and lysosomes (LY). In phagocytosis, following lysosomal fusion, the phagolysosome matures into a
837 MHC II loading compartment through delivery of newly synthsized MHC II to the phagolysosome. In
838 contrast, in efferocytosis, the late efferosome breaks into smaller vesicles which traffick to the cell
839 periphery where they undergo fusion with the recycling endosome system (RE).

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Phagocytosis
Efferocytosis
Exocyotsis?
Nutrient Recovery?
Rab5
Rab35
EE
EE
Rab7
Rab5
Rab7
Rab17
Rab5
Rab7
Rab35
Rab11
EE
Rab5
EE
Rab17
Rab7
Rab17
RE
Rab17
Rab7
LE
LE
Rab17
Rab14
Rab7
LE
LE
Rab14
Rab39
Rab20
Golgi
Rab22
MHC II
Rab2
Rab1
Rab17
Rab17
Rab7
Rab17
Rab17
Rab7
ER
LY
LY
LY
LY
LY
Rab39
LY
LY
Nucleus
Inflammation &
Antigen Presentation
Anti-inflammatory &
Immunologically Silent