Canonical and non-canonical autophagy pathways in microglia

Julia Jülg[a*], Laura Strohm[a*], Christian Behrends[a#]

[a]Munich Cluster for Systems Neurology, Medical Faculty, Ludwig–Maximilians–Universität München, Munich, Germany

*Julia Jülg and Laura Strohm contributed equally to this work.

#Address correspondence to

christian.behrends@mail03.med.uni-muenchen.de

Key Words:

canonical autophagy, non-canonical autophagy, LC3-associated endocytosis, LC3-associated phagocytosis, microglia, neurodegeneration

Abstract:

Besides the ubiquitin-proteasome-system, autophagy is a major degradation pathway within cells. It delivers invading pathogens, damaged organelles, aggregated proteins and other macromolecules from the cytosol to the lysosome for bulk degradation. This so-called canonical autophagy activity contributes to the maintenance of organelle, protein and metabolite homeostasis as well as innate immunity. Over the past years, numerous studies rapidly deepened our knowledge on the autophagy machinery and its regulation; driven by the fact that impairment of autophagy is associated with several human pathologies including cancer, immune diseases and neurodegenerative disorders. Unexpectedly, components of the autophagic machinery were also found to participate in various processes that did not involve lysosomal delivery of cytosolic constituents. These functions are hereafter defined as non-canonical autophagy. Regarding neurodegenerative diseases, most research was performed in neurons, while for a long-time microglia received considerably less attention. Concomitant with the notion that microglia greatly contribute to brain health, the understanding of the role of autophagy in microglia expanded. To facilitate an overview of the current knowledge, we present herein the fundamentals as well as the recent advances of canonical and non-canonical autophagy functions in microglia.

AUTOPHAGY PROCESSES

Maintaining cellular homeostasis involves controlled protein, organelle and metabolite degradation systems, which are conserved among all eukaryotic cells. Autophagy, Greek for

"self-eating", is an intracellular recycling process by which cytoplasmic material is targeted for lysosomal degradation (1). Depending on the type of cargo delivery to the lysosome three different autophagy processes can be classified: i) Microautophagy describes the internalization of smaller cytosolic portions by inward-budding vesicles from the lysosomal membrane (2), whereas in ii) chaperone-mediated autophagy, cytosolic proteins are specifically recognized by chaperones and taken up by the lysosome in a transporter dependent manner (2). Finally, iii) macroautophagy (hereafter abbreviated autophagy) is characterized by the engulfment of cytosolic constituents by double-membrane structures, termed autophagosomes, which fuse with lysosomes (Figure 1). Regardless of the delivery route, autophagic cargo is eventually digested by lysosomal enzymes and the degradation products are released to the cytosol as new building blocks (3).

During autophagy, autophagy-related (ATG) proteins regulate the formation of the autophagosome in a hierarchical manner through different phosphorylating and ubiquitin-like conjugating events (Figure 1). Initiation of autophagy requires assembly of the unc-51-like kinase 1 (ULK1) complex including its subunits ULK1, ATG13, ATG101 and RB1-inducible coiled-coil protein 1 (RB1CC1) with dephosphorylation of ATG13 being a main triggering factor (4). Following activation, ULK1 kinase phosphorylates and recruits the transmembrane protein ATG9 and class III phosphatidylinositol 3-kinase (PI3KC3) complex I, which in turn promote autophagosome biogenesis (5-7). Mammalian PI3KC3 complex I consists of a core composed of hVps34, hVps15, Beclin-1 (BECN1) and ATG14 as well as associated factors such as activating molecule in Beclin-1-regulated autophagy (AMBRA1) and nuclear receptor-binding factor 2 (NRBF2)(8, 9). PI3KC3 produces phosphatidylinositol 3-phosphate (PI3P) which then induces the formation of the omegasome, a membrane platform tightly associated with the endoplasmic reticulum (ER) that gives rise to a pre-autophagosomal structure termed phagophore or isolation membrane (10, 11). Enrichment of PI3P leads to the recruitment of WD repeat domain

phosphoinositide-interacting protein 2 (WIPI2) and FYVE domain-containing protein 1 (DFCP1) to the omegasome (11, 12). Subsequently, WIPI2 orchestrates the conjugation of ubiquitin-like human ATG8 (hATG8) proteins to phosphatidylethanolamine (PE) which anchors them to the nascent phagophore (12). The family of hATG8 proteins comprises seven members which are categorized in two protein subfamilies: i) the microtubule-associated proteins 1A/1B light chain 3 (LC3A, LC3B, LC3B2 and LC3C) and ii) the γ-aminobutyric acid receptor-associated proteins (GABARAP, GABARAPL1 and GABARAPL2) (13). This conjugation is exerted by a ubiquitin-like machinery where ATG7 is equivalent to an E1 activating-enzyme, ATG3 to an E2 conjugating-enzyme and the ATG12-ATG5-ATG16 complex to an E3 scaffolding-ligase (14). Upon lipidation to the concave phagophore membrane hATG8 proteins are retained in the autophagosome and degraded along with its engulfed cargo, whereas hATG8-PE conjugates on the outer membrane of autophagosomes are eventually removed by the family of ATG4 hydrolases (15). The dynamics of LC3 and GABARAP conjugation play a central role in autophagosome formation by controlling the tethering and hemifusion of membranes (16, 17). In addition, LC3 was found to participate in transporting autophagosomes to lysosomes by forming a complex with Rab7 and its effector protein FYCO1 which facilitates microtubule transport of vesicles (18).

Importantly, the hierarchical orchestration of ATG proteins is not absolutely mandatory for autophagic degradation of cytosolic content. BECN1-independent autophagy, for example, can be induced among other conditions by resveratrol and was shown to be insensitive to the knockdown of PI3KC3 complex components such as BECN1 or hVps34 (19). In the following years, other independent forms were discovered including the bypassing of ATG3, ATG5, ATG7 or ULK1 and ULK2 (20-22).

Most cell types exert basal levels of autophagy but can upregulate the pathway in response to a variety of stimuli such as nutrient deprivation. Thereby, autophagy provides the cell with metabolic building blocks for the synthesis of proteins, carbohydrates and lipids. Key energy

sensors are the mammalian target of rapamycin complex I (mTORC1) and the AMP-activated protein kinase (AMPK) which induce bulk autophagic degradation of cytosolic constituents according to the nutrient status of the cell (23). As a negative autophagy regulator, mTORC1 couples the sensing of amino acids, growth factors and metabolic stress to the phosphorylation-mediated repression of the ULK1 complex at high nutrient stages (24, 25). Decline of intracellular ATP levels triggers the activation of AMPK, which in turn positively controls ULK1 complex activation by direct phosphorylation of ULK1 and suppressing mTOR activity (26). The urge of the cell to overcome starvation and to promote cell survival requires autophagic activity to be elevated above basal levels and represents a rather non-selective pathway.

In contrast, selective autophagic degradation pathways exert cellular quality and quantity control through a number of soluble and membrane-bound receptors which mediate autophagic engulfment of specific substrates including protein aggregates, damaged organelles or pathogens (27-29). The ability of these autophagy receptors to recognize and tether their targets to the forming autophagosome is mediated by distinct functional domains. Here, polyubiquitination of cargo is a major signal for selective autophagy allowing the binding of cargo by receptors via their ubiquitin-binding domain (UBD) (30, 31). It remains unclear how different polyubiquitin chains confer specificity for different autophagy receptors. Several studies demonstrated lysine-63 ubiquitination to be predominantly associated with autophagic turnover. However, in autophagy-deficient mice several different types of polyubiquitin chains accumulate suggesting that substrate oligomerization rather than ubiquitin topology is the driving signal for receptor selectivity (32-34).

Apart from target recognition, autophagy receptors directly interact with members of the hATG8 family causing the autophagosome to zip around specific cargo (35). This interaction is mediated by the so-called LC3-interaction region (LIR), a characteristic linear motif among autophagy receptors and other hATG8-interacting proteins (36). Several autophagy receptors have been

identified to carry both, UBD and LIR, including sequestosome-1 (SQSTM1 also known as p62), next to BRCA1 gene 1 (NBR1), optineurin (OPTN), Calcium-binding and coiled-coil domain-containing protein 2 (CALCOCO2 alias NDP52), Tax1-binding protein 1 (TAX1BP1) and Toll-interacting protein (TOLLIP) (37). Genetic mutations within some of these autophagy receptor domains are linked to neurodegenerative diseases, thus assigning selective autophagy as a contributor to preserving neuronal homeostasis (38).

Throughout the brain and spinal cord, tissue maintenance and removal of cellular debris is accomplished by parenchymal macrophages, the microglia (39). Given the vital role of microglia in the central nervous system (CNS), this article discusses the latest findings regarding microglial autophagy in context of inflammatory response and different degradation pathways. Besides the degradation of cytosolic components by canonical autophagy, recently identified non-canonical pathways participate in the clearance of extracellular entities. Here, we focus on the molecular aspects of both autophagy pathway variants in microglia.

MICROGLIA

Microglia are the major resident immune cells of the central nervous system (CNS) that constitute up to 10 ~ 15 % of all CNS cells. They arise early in development and originate from a pool of primitive macrophages derived from the embryonic yolk sac. During development as well as during adulthood they contribute in multiple ways to overall brain function (40). Microglia are capable of orchestrating the tissue homeostasis by releasing inflammatory and neurotrophic factors as well as by phagocytosis (41, 42). Phagocytosis is the ingestion and digestion of extracellular particles, for example bacteria or dead cells. In an intact brain, microglial processes undergo rapid extensions and retractions thereby scanning their environment for tissue abnormalities (43). They can sense disturbances due to a variety of neurotransmitter and immune receptors as well as ion channels (44-50). As a response,

145 microglia can undergo multiple morphological and functional changes depending on micro-
146 environmental factors and migrate to the site of injury. These transformations include the
147 retraction of the processes accompanied by a more amoeboid appearance as well as changes in
148 signaling transduction, receptor expression and phagocytic capacity. When microglia are faced
149 with potentially harmful entities, they can recognize these through Toll-like receptors (TLRs),
150 triggering receptor expressed on myeloid cells 2 (TREM2) or other receptors like the mannose
151 receptor (51-53). The variety of activated receptors triggers different signaling pathways, which
152 initiate reorganization of the actin cytoskeleton and phagosome formation (54, 55). Firstly, the
153 target is engulfed by the plasma membrane leading to interiorization, also called ingestion (56).
154 Thereby, a membrane vesicle containing the particle, termed phagosome, buds inwards and
155 fuses with lysosomes (Figure 3A). This phagocytic mechanism serves for the clearance of all
156 kind of harmful targets. Furthermore, microglial activation can result in the release of a broad
157 range of pro- and anti-inflammatory factors as cytokines or chemokines (57, 58). Interestingly,
158 accumulating evidence indicates that autophagy plays a crucial role in the immune functions of
159 microglia.
160

161 **CANONICAL AUTOPHAGY IN MICROGLIA**

162 Canonical autophagy pathways follow a stepwise variable assembly of the autophagic
163 machinery. While in most cells there is no strict dependency on distinct components, autophagy
164 induction often includes the association of the ULK1 kinase complex, the recruitment of the
165 PI3KC3 complex, ATG9, PI3P effector proteins and the conjugation of hATG8s to PE on the
166 expanding phagophore. In this way, constituents from the cytosol are engulfed and delivered to
167 lysosomes. Whether these autophagy components are compulsory for microglial autophagy is
168 unknown. Within the CNS, autophagic activity has been mainly examined in neurons leaving

unanswered questions regarding microglia as the key players in neuronal immune response in the brain (59).

Microglial autophagy and intracellular aggregates

Accumulation of protein aggregates is a pathological hallmark in neurodegenerative diseases (60). Intracellular aggregates are predominantly found in neurons whereas they are remarkably less abundant in microglia. Interestingly, recent studies showed that extracellular aggregates, which reach the cytosol of microglia, can be cleared by autophagy.

Synucleinphagy, the degradation of neuron-released α-synuclein by selective autophagy in microglia, was discovered by Yue and colleagues studying *in vivo* and *in vitro* models (61). α-synuclein is thought to function in vesicular trafficking and is predominantly known due to its pathological contribution in Parkinson's disease (PD) where it aggregates in neuronal inclusions called Lewy bodies (62, 63). Following overexpression of human α-synuclein in transgenic mice, microglia are activated and engulf extracellular α-synuclein deposits (61). Binding of α-synuclein to TLR4 on the microglial cell surface is accompanied by a significant increase of p62 whereas levels of other autophagy receptors remain unchanged (61). This process is mediated by nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) which regulates the transcription of p62 (61). Upregulated p62 is thought to associate with internalized α-synuclein to promote its sequestration in autophagosomes (61). This selective binding is assumed to be mediated by p62's recognition of ubiquitinated α-synuclein (64). The mechanism of how α-synuclein is taken up by microglia and released into the cytosol remains unclear. Different from other TLR4 signaling, binding of α-synuclein does not stimulate TLR4 endocytosis, thus excluding phagocytosis or receptor-mediated endocytosis as internalization mechanism (61, 65). Alternatively, α-synuclein might permeate the microglial cell membrane in a lipid-raft dependent manner (66). When exposed to neuronal SH-SY5Y cells, biochemically generated α-synuclein

fibrils were shown to escape intracellular vesicles by rupturing their membrane after endocytosis (67). Consistent with this study, exogenous fibrillary α-synuclein is able to trigger lysosomal rupture following internalization by microglia-derived BV2 cells and primary microglia (68). In response to this damage, TANK-binding kinase 1 (TBK1), OPTN and LC3 are recruited to ubiquitin ear-marked rapture sites and drive the removal of irreparable lysosomes by autophagy (68).

Moreover, Yoon and colleagues described a function of autophagy in the clearance of extracellular β-amyloid (Aβ) fibrils using primary microglia and BV2 cells (69). Here, autophagy initiation is thought to be depending on AMPK and serine/threonine-protein kinase 11 (STK11) signaling (69, 70). OPTN and LC3B were found to coimmunoprecipitate with Aβ and deletion of OPTN resulted in higher intracellular Aβ concentrations (69). Accordingly, OPTN was suggested to be an autophagy receptor that recruits the autophagy machinery to cytosolic Aβ via binding to LC3B (69). How Aβ aggregates are selectively targeted by OPTN is not yet understood. However, OPTN was also found to associate with protein aggregates in a ubiquitin-independent manner and to mediate their autophagic clearance (71). While the uptake of extracellular Aβ can be accomplished by receptor-mediated phagocytosis (72, 73), Aβ might leak through the phagosomal membrane causing its release into the cytosol and thereby allowing the binding of OPTN for autophagic degradation (69).

Understanding the molecular dependencies between internalization of extracellular Aβ and microglial autophagy requires further investigations. For instance, TREM2 is capable of sensing Aβ through lipoproteins and mediates the phagocytic uptake of Aβ in microglia (74). Deletion of TREM2 in turn, has severe impact on mTOR signaling and causes increased activation of autophagy (75).

Microglial autophagy and pathogen defense

Autophagy is able to modulate the immune response as it functions as an intracellular defense mechanism by capturing cytosol invading pathogens and delivering them for lysosomal degradation (76), a selective autophagy pathway termed xenophagy. In macrophages, recognition of pathogen-associated molecular patterns and damage-associated molecular patterns stimulates phagocytosis and subsequent elimination of pathogens by autophagy (77). Comparable to selective degradation of other cytosolic cargo, xenophagy involves the core autophagic machinery, autophagosome formation and ubiquitination as "eat-me" signal. Several autophagy receptors (p62, NDP52 and OPTN) can target ubiquitinated pathogens or damaged pathogen-containing phagosomes to the autophagosome via binding to LC3 (29, 78-80). In addition, NDP52 interacts with galectin-8, a cytosolic lectin and danger receptor that recognizes cytosol accessible β-galactosides on damaged phagosomes (81, 82). This specific interaction represents an alternative way to selectively target invading bacteria for autophagic degradation.

As host cell invasion serves for microbe replication, several bacterial pathogens possess strategies to escape xenophagy. For instance, after infection of epithelial MDCK cells with the gram-negative bacterium *Shigella flexneri*, binding of the *Shigella* virulence factor VirG to ATG5 triggers autophagy induction (83). By secreting the bacterial effector IscB, *Shigella flexneri* is capable to conceal the presence of its virulence factor VirG (83). IscB binds VirG in a competitive manner, thereby ultimately preventing the autophagic capture of *Shigella flexneri* (83). Disrupting lysosomal degradation by inhibiting components of the xenophagy machinery is another strategy of pathogens to ensure bacterial multiplication and survival. During invasion of HEK 293T cells and primary macrophages, *Legionella pneumophila* utilizes the bacterial effector RavZ, a cysteine protease, to hydrolyze the linkage of LC3 to PE (84). This irreversible cleavage results in a conjugation-deficient LC3 protein and inhibits autophagy in the host cell (84, 85). Furthermore, the *Salmonella* Typhimurium virulence factors SseF and SseG inhibit autophagy

initiation by interfering with Rab1A signaling (86). Rab1A belongs to the family of small GTPases and is involved in translocation of the ULK1 kinase complex to the phagophore (87). In murine macrophage-like cells infected with SseF- or SseG-deficient *Salmonella* variants, bacterial replication was impaired whereas downregulation of Rab1A could restore this effect (86).

To which extent microglia deploy autophagy as pathogen defense mechanism and in what way bacterial effectors impact microglial autophagy remains largely elusive. Given that several bacterial pathogens are able to cross the blood-brain barrier and infect cells in the CNS, microglial xenophagy could serve as an important neuroprotective mechanism.

Microglial autophagy and proinflammatory response

Microglia-depending secretion of cytokines is a key event in regulating the stimulation of proinflammatory response after brain injury. Evidence arises that autophagy proteins act at the intersection of microglial inflammation by exhibiting either inductive or suppressive effects (69, 88, 89). In general, proinflammatory signaling is executed by the activation of the inflammasome, a cytoplasmic tripartite protein complex consisting of a sensory component for recognizing ligands, an adaptor component for the binding of caspases and caspases for proteolytic processing of cytokines (90).

Belonging to the inflammasome sensors, NACHT, LRR and PVD domains-containing protein 3 (NLRP3) can be activated in the presence of Aβ followed by oligomerization of the adaptor component apoptosis-associated speck-like protein containing a CARD (ASC) and the intrinsic cleavage of caspase-1 (CASP1) (91-93). These molecular events in turn, promote the secretion of the cytokine interleukine-1β (IL-β) and the proinflammatory response to Aβ plaques (Figure 2) (91). Studies on the Aβ-induced activation of the NLRP3 inflammasome indicate that dysfunctional autophagy enhances the inflammasomal activation state (69). For example, primary microglia treated with Aβ showed intensified cleavage of CASP1 and increased release

of IL-1β when the autophagy proteins LC3B and ATG7 were depleted (69). A constitutive and abnormal high inflammatory response can also lead to neuronal toxicity and cell damage. In this context, elevated autophagic activity points towards a reduction of Aβ-induced inflammation, thereby possibly promoting cell survival (69, 94). Which autophagy proteins directly participate in the regulation of microglial inflammation is poorly understood. Jendrach and colleagues describe a novel function of microglial autophagy in regulating NLRP3 inflammasome via BECN1 (88). In comparison to microglia from wild-type mice, the activation of BECN1 deficient microglia resulted in higher amounts of IL-1β secretion and NLRP3 protein levels (88). Furthermore, NLRP3 colocalizes with LC3 and NDP52 at autophagosomes (88). It is hypothesized that NDP52 recruits NLRP3 for autophagosomal turnover since downregulation of NDP52 leads to elevated levels of IL-1β (Figure 2) (88). In addition, Kehrl and colleagues showed a p62-dependent colocalization of ubiquitinated inflammasomes with autophagosomes in macrophages (95). Hence, microglia presumably modulate the activity of inflammasomes via autophagosomal degradation of NLRP3. Akira and colleagues demonstrate an ATG16L1-dependent regulation of endotoxin-induced inflammation in macrophages (89). ATG16L1 is essential for the formation and phagophore localization of the ATG12-ATG5-ATG16L1 E3 ligase scaffold and thus, for the lipidation of LC3 to the autophagosomal membrane (96). Upon endotoxic activation, ATG16L1-depleted macrophages elicit higher expression of IL-1β compared to wild-type macrophages (89). In what way this observation translates to microglia function needs to be further elucidated. However, clearly these findings suggest that autophagy has an important role in balancing microglial proinflammatory response.

Given the morphological changes accompanied by frequent encounter of invading pathogens and toxic aggregates, activation of microglia is in urgent need of high energy levels in order to maintain microglial function. Therefore, autophagy is not only critical for the regulation of proinflammatory response and elimination of extracellular aggregates but also needs to be

considered as catabolic process for permanently suppling microglia with essential nutrients and controlling intracellular protein and organelle quality.

NON-CANONICAL AUTOPHAGY IN MICROGLIA

In non-canonical autophagy processes, components of the autophagy machinery are deployed to fulfill functions, which do not involve lysosomal delivery of cytosolic entities. Initial indicators for these pathways in microglia were provided by Lucin and colleagues who were able to show that BECN1 is required for phagocytic uptake and subsequent degradation of Aβ. In *ex vivo* studies, deletion of BECN1 in microglia-derived BV2 cells exposed to APP transgenic mouse brain slices resulted in insufficient phagocytosis of Aβ (97). More importantly, isolated microglia from postmortem human AD brains exhibit reduced levels of BECN1 suggesting a link between autophagic regulation and phagocytic uptake of Aβ deposits (97). Recently, two pathways with key roles in myeloid cells deepened the understanding of non-canonical autophagy: LC3-associated phagocytosis (LAP) and LC3-associated endocytosis (LANDO) (98, 99). During LAP and LANDO autophagy machinery components are used to conjugate LC3 to the membrane of phagosomes and endosomes, respectively. Due to their important function in myeloid cells, these two pathways will be discussed below in more detail.

LC3-associated phagocytosis (LAP)

The LAP pathway makes use of components of the canonical autophagy machinery to conjugate LC3 to the phagosome (Figure 3B). Several membrane receptors can initiate this process, which is mainly studied in macrophages. Among others, these receptors include TLRs (1/2, 2/6 and 4) that are able to detect pathogen-associated patterns, immunoglobulin receptors that recognize antigens and pathogens opsonized with IgG, and T-cell immunoglobulin mucin receptor 4 that binds to phosphatidylserine on the surface of dead cells (100-103). Binding to the receptor

mediates the phagocytosis of the extracellular target. Upon complete engulfment of the cargo and the sealing of the phagosome, LAP effectors are recruited. However, it remains unclear how exactly this recruitment is triggered. The most upstream autophagy complex present in LAP is the PI3KC3 complex II, which shares the core subunits hVps34 and hVps15 with PI3KC3 complex I but instead of ATG14 it contains UV radiation resistance-associated gene protein (UVRAG) and Rubicon (RUBCN) (8, 9). While RUBCN is a known negative regulator of PI3KC3 during canonical autophagy (104), it is also compulsory for LAP (99). During LAP, RUBCN is on the one side essential for PI3KC3 mediated PI3P generation and on the other side, it recruits and stabilizes the NADPH oxidase-2 (NOX2) complex at the phagosome (99, 105). NOX2 activity itself as well as NOX induced reactive oxygen species (ROS) production are crucial for the following LC3 lipidation. However, the underlying mechanism remains unknown (99, 101, 106). PI3P generation and NOX2 association to the phagosome results in conjugation of LC3 to PE on the phagosomal membrane. Notably, lipidation of LC3 and GABARAP proteins during LAP differs in two important mechanistic aspects from its use in canonical autophagy: First, WIPI2 is not necessary but probably substituted by another, yet undiscovered effector protein (107). Second, the WD40 domain of ATG16L1 is indispensable (107, 108). One other main difference between canonical autophagy and LAP is the timing and the location of LC3 lipidation. In canonical autophagy, LC3 lipidation occurs during autophagosome formation on the inner and outer autophagosomal membrane (109). In contrast, LC3 conjugation during LAP occurs after formation of the single membrane phagosome, leading to LC3 lipidation on the outer leaflet only. The resulting structure is called LAPosome (103). Consequently, the function of conjugated LC3 is not entirely overlapping between canonical autophagy and LAP. However, a potential common role of LC3-PE in both pathways is to facilitate the fusion of the respective transient organelle with lysosomes, leading to the degradation of the engulfed material (99, 110, 111).

Due to the targeting of several pathogens, LAP is critical for fighting bacterial as well as fungal infection (106, 112-114). Martinez et al. highlighted an additional importance of LAP by showing ineffective efferocytosis, the phagocytosis of dead cells, accompanied by an increase in proinflammatory cytokines in LAP-deficient mice (115). Furthermore, LAP plays also a role in major histocompatibility complex (MHC) class II restricted antigen presentation. MHC class II proteins present antigens from extracellular proteins to $CD4^+$ T cells and are found on a number of professional antigen presenting cells including macrophages and microglia. Antigenic peptides derived from partially degraded exogenous particles taken up by phagocytosis or endocytosis are loaded on MHC class II molecules in late endosomes. Intriguingly, it was shown that recruitment of LC3 to the phagosome facilitates MHC class II presentation of fungal antigens in a murine macrophage cell line (116). This process is conserved in human macrophages in which a compromised fungal antigen presentation was reported when LAP was inhibited (117). Mechanistically, the coupling of LC3 to phagosomes is thought to result in a prolonged antigen presentation by MHC class II molecules in human macrophages.

So far, LAP has mainly been studied in macrophages. Although microglia are distinct from other macrophages, they share many features such as the capability to phagocyte and to release cytokines. Therefore, it was generally assumed that LAP occurs in a similar or even the same manner in microglia. This was recently confirmed by two independent studies, which observed a role of LAP in the uptake of zymosan in microglia (98, 118). However, the physiological targets of microglial LAP still remain unknown.

LC3-associated endocytosis (LANDO)

Remarkably, in microglia another uptake pathway was recently demonstrated to employ autophagy proteins and therefore - in analogy to LAP - termed LANDO (98). Endocytosis is a ubiquitous cellular process that is defined by the active uptake of extracellular materials as well

as components of the plasma membrane into the cytoplasm. It is important for many physiological processes including nutrient uptake and cell signaling. Generally, endocytic pathways are differentiated in clathrin-mediated and -independent whereat clathrin-independent endocytosis processes are classified as macropinocytosis and phagocytosis. In comparison to phagocytosis, clathrin-mediated endocytosis (CME) is common in all eukaryotic cells and describes the uptake of smaller cargo into clathrin-coated vesicles (Figure 3D) (119, 120). The early endosome matures to a late, multivesicular endosome which then finally fuses with lysosomes, resulting in the degradation of the cargo (121). This process is driven by a constant fusion and fission with other vesicles. Importantly, a considerable subset of the cargo escapes lysosomal degradation. For example, a large fraction of membrane components including receptors are often recycled back to the plasma membrane while other cargo is targeted to the trans-Golgi-network (122-124).

LANDO is characterized by LC3 conjugation to clathrin- and Rab5-positive endosomes (Figure 3C). As in LAP, the conjugation process is dependent on BECN1, VPS34, ATG5, ATG7 and RUBCN. In contrast to LAP, the absence of LC3 conjugation does not result in defects in cargo degradation but in reduced receptor recycling. Therefore, a secondary uptake of the cargo is diminished. So far, roles for LANDO have been observed for the recycling of the putative Aβ receptors TREM2, CD36 and TLR4 in microglia (98). It is conceivable that this non-canonical function of autophagic proteins plays not only a role for other receptors but also in other cell types. Notably, previous studies reporting a link between autophagy, retromer trafficking and receptor recycling are in line with the proposed role of LANDO. The retromer complex sorts endosomal cargo back to the plasma membrane, the trans-Golgi network or other compartments. Popovic et al. reported that induction of autophagy results in dissociation of the retromer-associated Rab GTPase-activating protein TBC1D5 from the retromer complex (125). This partition was observed to be due to an activation of Rab7a and resulted in enhanced

retromer receptor recycling (126, 127). Furthermore, Lucin et al. described a dependency of TREM2 as well as CD36 receptor recycling on BECN1 and VPS34 in microglia (97). Taken together, LANDO is a newly discovered non-canonical pathway in microglia involved in receptor recycling. Although, so far, evidence is limited to one report, several previous studies are in accordance with such a pathway. Clearly, there is a need for further investigation to dissect the mechanisms of LANDO, in microglia and other cell types, in detail.

RELEVANCE IN DISEASE

The human brain comprises only 2 % of total body weight, but consumes 20 % of total body oxygen (128). This highlights its relevance for the human body. Among others, it senses and coordinates information and controls body movements, personality as well as thoughts. Accordingly, disruption of brain functions has severe consequences as impaired mental abilities, memory damage, loss of muscle coordination and strength or vision and language impairment. Therefore, CNS repair and protection strategies are indispensable. The brain is not only protected by the skull and the blood-brain barrier, but a multitude of molecular mechanisms. Among others, these include enzymatic antioxidant systems, the ubiquitin-proteasome pathway, neuroinflammation and autophagy. Due to the role of autophagy in organelle and protein quality control, its impairment results in accumulation of aggregated proteins and damaged organelles, common hallmarks of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and PD. The essentiality of autophagic processes for the CNS is substantiated by the multiplicity of autophagic genes, which are known to be linked to neurological diseases. Ranging from autophagic receptors, regulators and adaptors (including OPTN, SQSTM1, TBK1, C9orf72, UBQLN2, HTT, PICALM) which are risk genes for neurodegenerative diseases to AMBRA1 that has been linked to autism and schizophrenia (129-139). Furthermore, mutations in the hATG8 binding protein Tectonin β-propeller containing

protein 2 (TECPR2) are known to result in hereditary spastic paraplegias while absence of the autophagic protein WD repeat domain phosphoinositide-interacting protein 4 (WIPI4) causes beta-propeller protein-associated neurodegeneration (140, 141). Although the value of autophagic processes for brain health is widely appreciated, they were mainly studied in neurons. However, the contribution of canonical as well as non-canonical autophagy functions in microglia increasingly receives more attention in several CNS diseases (Figure 4).

Relevance of microglial autophagy in diseases

As aging progresses, loss of protein homeostasis is a generic consequence (142). The tight regulation of inflammatory response and degradation of extracellular aggregates by microglial autophagy supports a link between disease development and impaired autophagic processes at different stages. During AD progression, alterations in AMPK signaling is a central issue (143, 144). As a homeostasis sensor, AMPK activates microglial autophagy in presence of Aβ plaques consequently leading to their lysosomal degradation (69). Inactivation of AMPK with compound C decreases Aβ clearance, thereby making AMPK a potential target for AD treatment. In PD, neuron-released α-synuclein and its accumulation in Lewy bodies results in degeneration of the dopaminergic system (145). Intriguingly, impaired microglial autophagy provokes a decrease of dopaminergic neurons when α-synuclein is expressed in mice (61). Hence, removal of extracellular α-synuclein by microglial autophagy is suggested to have a vital role in maintaining neuronal protection and function.

Modulation of proinflammatory response by autophagy was found to be crucial in Crohn's disease, where excessive production of cytokines results in chronic inflammations compromising the entire gastrointestinal tract (146, 147). Among patients suffering from Crohn's disease a genetic missense variant of ATG16L1 was identified (148). This disease-related polymorphism shows an elevated sensitivity to cleavage by caspase-3 which eventually leads to the

degradation of ATG16L1 (146, 149). Following ATG16L1 loss-of-function in macrophages, autophagic activity is reduced and as a consequence, levels of cytokines increase (146). Therefore, microglial autophagy could own a similar role in controlling proinflammatory response within the CNS.

Despite neurodegeneration and inflammatory diseases, microglia were shown to have a critical impact on neuropsychological behaviors (150). In autism spectrum disorders (ASD), impaired microglial autophagy pathways studied in mice led to defective synaptic pruning, which becomes visible by an abnormal high dendritic spine density (151). Synapsis formation and elimination is fundamental as it ensures proper brain development (152). Here, autophagy could have a modulatory role in neuronal connectivity by clearing excessive and dysfunctional synapses (151). Uncovering the molecular basis of autophagy pathways in microglia is an essential objective in order to provide a wide-ranging platform for neurotherapeutic approaches.

Relevance of microglial non-canonical autophagy in diseases

LANDO contributes to the clearance of Aβ plaques. Furthermore, an upregulation of proinflammatory cytokines was detected in LANDO-deficient cells, which resulted in microglia hyperactivation in mice containing several mutations associated with human familial AD. These mice also presented accelerated tau phosphorylation. In this model, the deficiency of microglial LANDO caused neuronal cell death accompanied by behavioral and memory impairment, emphasizing the role of microglia as well as LANDO in AD (98). Although the relevance of microglial LANDO has so far only been reported for AD, it is likely that this process targets other molecules or particles besides Aβ. Especially, since LANDO supports the recycling of the receptors CD36, TLR4 and TREM2, which can bind a broad range of ligands besides Aβ. Although LANDO is suggested to be a major player in Aβ clearance, it is not known to what extent this process is physiologically relevant. Still, it is tempting to speculate that stimulation of

LANDO could be a promising target for therapeutic interventions in neurodegenerative diseases especially in AD. However, it needs to be clarified to what extend increased LANDO is beneficial for microglia and at what point negative effects arise. In contrast to LANDO, to-date, LAP was not investigated in detail in microglia. Interestingly, LAP is firmly entangled with inflammation. Mice, which lack LAP-obligatory proteins release more proinflammatory cytokines and are defective in the clearance of apoptotic cells (115). Since microglia are responsible for the phagocytosis of apoptotic cells in the CNS and neuroinflammation is a common feature of neurodegenerative disorders, it is plausible that defective LAP could play an important role in the onset or progression of neurodegenerative diseases (153, 154). Another known function of LAP in macrophages is the elimination of pathogens. Several of these LAP-targeted pathogens, including *Streptococcus pneumoniae* and *Listeria monocytogenes*, can penetrate through the blood brain barrier into the CNS and are common causes for bacterial meningitis (155, 156). While it remains to be firmly established that microglial LAP is part of the defense against these pathogens in the CNS, increasing LAP activity in microglia could represent a potential target for anti-bacterial therapeutic approaches. However, due to the two-sided role of neuroinflammation in neurodegenerative diseases, both, a beneficial as well as a detrimental impact could result from an overactivation of LAP.

CONCLUSION

As resident immune cells of the brain and spinal cord, microglia accomplish vital functions including synaptic pruning, neurogenesis and immune response. Growing evidence indicates that microglia utilize autophagy to meet CNS homeostasis thereby strictly controlling proinflammatory response and removal of protein aggregates and damaged organelles from the cytosol. Compared to canonical autophagy, non-canonical pathways allow microglia to target also non-cytosolic entities for lysosomal degradation. LAP and LANDO are two non-canonical pathways,

which were shown to be present in microglia. While it was observed that microglial LANDO is crucial for the uptake of Aß, so far, the exact role of LAP in microglia remains unknown. Regarding the decline of protein function during aging and various neuronal diseases, these non-canonical features contribute to the maintenance of CNS homeostasis and their defect can have detrimental consequences. With regard to the energetic aspect, microglia may omit and exploit specific signaling pathways to activate autophagy, thereby recycling nutrients and sustaining energy. Taken together, canonical as well as non-canonical autophagy pathways play a key part in microglia functioning, most likely in complementary ways. Yet, several questions as the role of LAP in microglia and the detailed interplay between canonical and non-canonical autophagy remain unanswered and should be elucidated by further research.

CONTRIBUTION

J.J., L.S. and C.B. contributed to the drafting of the article. J.J. and L.S. wrote the manuscript and designed the figures. L.S. realized digital illustrations. All authors reviewed the final manuscript. Funding acquisition: C.B.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) within the frameworks of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198) and of the Collaborative Research Center 1177 (ID 259130777).

REFERENCES

1. Ohsumi Y. 1999. Molecular mechanism of autophagy in yeast, Saccharomyces cerevisiae. Philos Trans R Soc Lond B Biol Sci 354:1577-80; discussion 1580-1.

2. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. 2008. Autophagy fights disease through cellular self-digestion. Nature 451:1069-75.
3. Klionsky DJ, Ohsumi Y. 1999. Vacuolar import of proteins and organelles from the cytoplasm. Annu Rev Cell Dev Biol 15:1-32.
4. Shang L, Wang X. 2011. AMPK and mTOR coordinate the regulation of Ulk1 and mammalian autophagy initiation. Autophagy 7:924-6.
5. Russell RC, Tian Y, Yuan H, Park HW, Chang YY, Kim J, Kim H, Neufeld TP, Dillin A, Guan KL. 2013. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 15:741-50.
6. Zhou C, Ma K, Gao R, Mu C, Chen L, Liu Q, Luo Q, Feng D, Zhu Y, Chen Q. 2017. Regulation of mATG9 trafficking by Src- and ULK1-mediated phosphorylation in basal and starvation-induced autophagy. Cell Res 27:184-201.
7. Young AR, Chan EY, Hu XW, Kochl R, Crawshaw SG, High S, Hailey DW, Lippincott-Schwartz J, Tooze SA. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. J Cell Sci 119:3888-900.
8. Yu X, Long YC, Shen HM. 2015. Differential regulatory functions of three classes of phosphatidylinositol and phosphoinositide 3-kinases in autophagy. Autophagy 11:1711-28.
9. Young LN, Goerdeler F, Hurley JH. 2019. Structural pathway for allosteric activation of the autophagic PI 3-kinase complex I. Proc Natl Acad Sci U S A 116:21508-21513.
10. Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. 2009. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 5:1180-5.
11. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT. 2008. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 182:685-701.
12. Dooley HC, Razi M, Polson HE, Girardin SE, Wilson MI, Tooze SA. 2014. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. Mol Cell 55:238-52.
13. Slobodkin MR, Elazar Z. 2013. The Atg8 family: multifunctional ubiquitin-like key regulators of autophagy. Essays Biochem 55:51-64.
14. Sakoh-Nakatogawa M, Matoba K, Asai E, Kirisako H, Ishii J, Noda NN, Inagaki F, Nakatogawa H, Ohsumi Y. 2013. Atg12-Atg5 conjugate enhances E2 activity of Atg3 by rearranging its catalytic site. Nat Struct Mol Biol 20:433-9.
15. Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, Ohsumi M, Takao T, Noda T, Ohsumi Y. 2000. The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. J Cell Biol 151:263-76.
16. Nakatogawa H, Ichimura Y, Ohsumi Y. 2007. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell 130:165-78.
17. Yu ZQ, Ni T, Hong B, Wang HY, Jiang FJ, Zou S, Chen Y, Zheng XL, Klionsky DJ, Liang Y, Xie Z. 2012. Dual roles of Atg8-PE deconjugation by Atg4 in autophagy. Autophagy 8:883-92.
18. Pankiv S, Alemu EA, Brech A, Bruun JA, Lamark T, Overvatn A, Bjorkoy G, Johansen T. 2010. FYCO1 is a Rab7 effector that binds to LC3 and PI3P to mediate microtubule plus end-directed vesicle transport. J Cell Biol 188:253-69.
19. Scarlatti F, Maffei R, Beau I, Codogno P, Ghidoni R. 2008. Role of non-canonical Beclin 1-independent autophagy in cell death induced by resveratrol in human breast cancer cells. Cell Death Differ 15:1318-29.

20. Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, Komatsu M, Otsu K, Tsujimoto Y, Shimizu S. 2009. Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature 461:654-8.
21. Cheong H, Lindsten T, Wu J, Lu C, Thompson CB. 2011. Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. Proc Natl Acad Sci U S A 108:11121-6.
22. Chang TK, Shravage BV, Hayes SD, Powers CM, Simin RT, Wade Harper J, Baehrecke EH. 2013. Uba1 functions in Atg7- and Atg3-independent autophagy. Nat Cell Biol 15:1067-78.
23. Kim J, Kundu M, Viollet B, Guan KL. 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 13:132-41.
24. Ganley IG, Lam du H, Wang J, Ding X, Chen S, Jiang X. 2009. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 284:12297-305.
25. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. 2002. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell 110:163-75.
26. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R, Asara JM, Fitzpatrick J, Dillin A, Viollet B, Kundu M, Hansen M, Shaw RJ. 2011. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331:456-61.
27. Bjorkoy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T. 2005. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 171:603-14.
28. Kanki T, Klionsky DJ. 2008. Mitophagy in yeast occurs through a selective mechanism. J Biol Chem 283:32386-93.
29. Zheng YT, Shahnazari S, Brech A, Lamark T, Johansen T, Brumell JH. 2009. The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway. J Immunol 183:5909-16.
30. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. 2006. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441:880-4.
31. Matsumoto G, Wada K, Okuno M, Kurosawa M, Nukina N. 2011. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. Mol Cell 44:279-89.
32. Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR, Wooten MW. 2004. Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. Mol Cell Biol 24:8055-68.
33. Tan JM, Wong ES, Kirkpatrick DS, Pletnikova O, Ko HS, Tay SP, Ho MW, Troncoso J, Gygi SP, Lee MK, Dawson VL, Dawson TM, Lim KL. 2008. Lysine 63-linked ubiquitination promotes the formation and autophagic clearance of protein inclusions associated with neurodegenerative diseases. Hum Mol Genet 17:431-9.
34. Riley BE, Kaiser SE, Shaler TA, Ng AC, Hara T, Hipp MS, Lage K, Xavier RJ, Ryu KY, Taguchi K, Yamamoto M, Tanaka K, Mizushima N, Komatsu M, Kopito RR. 2010. Ubiquitin accumulation in autophagy-deficient mice is dependent on the Nrf2-mediated stress response pathway: a potential role for protein aggregation in autophagic substrate selection. J Cell Biol 191:537-52.
35. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Overvatn A, Bjorkoy G, Johansen T. 2007. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282:24131-45.
36. Noda NN, Kumeta H, Nakatogawa H, Satoo K, Adachi W, Ishii J, Fujioka Y, Ohsumi Y, Inagaki F. 2008. Structural basis of target recognition by Atg8/LC3 during selective autophagy. Genes Cells 13:1211-8.
37. Wild P, McEwan DG, Dikic I. 2014. The LC3 interactome at a glance. J Cell Sci 127:3-9.

38. Deng Z, Purtell K, Lachance V, Wold MS, Chen S, Yue Z. 2017. Autophagy Receptors and Neurodegenerative Diseases. Trends Cell Biol 27:491-504.
39. Li Q, Barres BA. 2018. Microglia and macrophages in brain homeostasis and disease. Nat Rev Immunol 18:225-242.
40. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M. 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841-5.
41. Miyamoto A, Wake H, Ishikawa AW, Eto K, Shibata K, Murakoshi H, Koizumi S, Moorhouse AJ, Yoshimura Y, Nabekura J. 2016. Microglia contact induces synapse formation in developing somatosensory cortex. Nat Commun 7:12540.
42. Hagemeyer N, Hanft KM, Akriditou MA, Unger N, Park ES, Stanley ER, Staszewski O, Dimou L, Prinz M. 2017. Microglia contribute to normal myelinogenesis and to oligodendrocyte progenitor maintenance during adulthood. Acta Neuropathol 134:441-458.
43. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8:752-8.
44. Noda M, Nakanishi H, Nabekura J, Akaike N. 2000. AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. J Neurosci 20:251-8.
45. Biber K, Laurie DJ, Berthele A, Sommer B, Tolle TR, Gebicke-Harter PJ, van Calker D, Boddeke HW. 1999. Expression and signaling of group I metabotropic glutamate receptors in astrocytes and microglia. J Neurochem 72:1671-80.
46. Kuhn SA, van Landeghem FK, Zacharias R, Farber K, Rappert A, Pavlovic S, Hoffmann A, Nolte C, Kettenmann H. 2004. Microglia express GABA(B) receptors to modulate interleukin release. Mol Cell Neurosci 25:312-22.
47. Town T, Jeng D, Alexopoulou L, Tan J, Flavell RA. 2006. Microglia recognize double-stranded RNA via TLR3. J Immunol 176:3804-12.
48. Bianco F, Fumagalli M, Pravettoni E, D'Ambrosi N, Volonte C, Matteoli M, Abbracchio MP, Verderio C. 2005. Pathophysiological roles of extracellular nucleotides in glial cells: differential expression of purinergic receptors in resting and activated microglia. Brain Res Brain Res Rev 48:144-56.
49. Lv M, Liu Y, Zhang J, Sun L, Liu Z, Zhang S, Wang B, Su D, Su Z. 2011. Roles of inflammation response in microglia cell through Toll-like receptors 2/interleukin-23/interleukin-17 pathway in cerebral ischemia/reperfusion injury. Neuroscience 176:162-72.
50. Kettenmann H, Hoppe D, Gottmann K, Banati R, Kreutzberg G. 1990. Cultured microglial cells have a distinct pattern of membrane channels different from peritoneal macrophages. J Neurosci Res 26:278-87.
51. Takahashi K, Rochford CD, Neumann H. 2005. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 201:647-57.
52. Blander JM, Medzhitov R. 2004. Regulation of phagosome maturation by signals from toll-like receptors. Science 304:1014-8.
53. Sung SS, Nelson RS, Silverstein SC. 1983. Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. J Cell Biol 96:160-6.
54. Yao H, Coppola K, Schweig JE, Crawford F, Mullan M, Paris D. 2019. Distinct Signaling Pathways Regulate TREM2 Phagocytic and NFkappaB Antagonistic Activities. Front Cell Neurosci 13:457.
55. Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K. 2007. UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446:1091-5.
56. Rabinovitch M. 1967. The dissociation of the attachment and ingestion phases of phagocytosis by macrophages. Exp Cell Res 46:19-28.

57. Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, Gulati G, Bennett ML, Sun LO, Clarke LE, Marschallinger J, Yu G, Quake SR, Wyss-Coray T, Barres BA. 2019. Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. Neuron 101:207-223 e10.
58. Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J, Marsh SE, Saunders A, Macosko E, Ginhoux F, Chen J, Franklin RJM, Piao X, McCarroll SA, Stevens B. 2019. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. Immunity 50:253-271 e6.
59. Hickey WF, Kimura H. 1988. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. Science 239:290-2.
60. Ross CA, Poirier MA. 2004. Protein aggregation and neurodegenerative disease. Nat Med 10 Suppl:S10-7.
61. Choi I, Zhang Y, Seegobin SP, Pruvost M, Wang Q, Purtell K, Zhang B, Yue Z. 2020. Microglia clear neuron-released alpha-synuclein via selective autophagy and prevent neurodegeneration. Nat Commun 11:1386.
62. Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, Caldwell KA, Caldwell GA, Rochet JC, McCaffery JM, Barlowe C, Lindquist S. 2008. The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. Proc Natl Acad Sci U S A 105:145-50.
63. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045-7.
64. Kuusisto E, Salminen A, Alafuzoff I. 2001. Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. Neuroreport 12:2085-90.
65. Liang Y, Chu H, Jiang Y, Yuan L. 2016. Morphine enhances IL-1beta release through toll-like receptor 4-mediated endocytic pathway in microglia. Purinergic Signal 12:637-645.
66. Park JY, Kim KS, Lee SB, Ryu JS, Chung KC, Choo YK, Jou I, Kim J, Park SM. 2009. On the mechanism of internalization of alpha-synuclein into microglia: roles of ganglioside GM1 and lipid raft. J Neurochem 110:400-11.
67. Flavin WP, Bousset L, Green ZC, Chu Y, Skarpathiotis S, Chaney MJ, Kordower JH, Melki R, Campbell EM. 2017. Endocytic vesicle rupture is a conserved mechanism of cellular invasion by amyloid proteins. Acta Neuropathol 134:629-653.
68. Bussi C, Peralta Ramos JM, Arroyo DS, Gallea JI, Ronchi P, Kolovou A, Wang JM, Florey O, Celej MS, Schwab Y, Ktistakis NT, Iribarren P. 2018. Alpha-synuclein fibrils recruit TBK1 and OPTN to lysosomal damage sites and induce autophagy in microglial cells. J Cell Sci 131.
69. Cho MH, Cho K, Kang HJ, Jeon EY, Kim HS, Kwon HJ, Kim HM, Kim DH, Yoon SY. 2014. Autophagy in microglia degrades extracellular beta-amyloid fibrils and regulates the NLRP3 inflammasome. Autophagy 10:1761-75.
70. Liang J, Shao SH, Xu ZX, Hennessy B, Ding Z, Larrea M, Kondo S, Dumont DJ, Gutterman JU, Walker CL, Slingerland JM, Mills GB. 2007. The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. Nat Cell Biol 9:218-24.
71. Korac J, Schaeffer V, Kovacevic I, Clement AM, Jungblut B, Behl C, Terzic J, Dikic I. 2013. Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates. J Cell Sci 126:580-92.
72. Bamberger ME, Harris ME, McDonald DR, Husemann J, Landreth GE. 2003. A cell surface receptor complex for fibrillar beta-amyloid mediates microglial activation. J Neurosci 23:2665-74.
73. Koenigsknecht J, Landreth G. 2004. Microglial phagocytosis of fibrillar beta-amyloid through a beta1 integrin-dependent mechanism. J Neurosci 24:9838-46.

74. Yeh FL, Wang Y, Tom I, Gonzalez LC, Sheng M. 2016. TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia. Neuron 91:328-40.
75. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, Loboda AA, Zhou Y, Cairns NJ, Kambal A, Loginicheva E, Gilfillan S, Cella M, Virgin HW, Unanue ER, Wang Y, Artyomov MN, Holtzman DM, Colonna M. 2017. TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. Cell 170:649-663 e13.
76. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. 2004. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 119:753-66.
77. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. 2012. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. Immunol Rev 249:158-75.
78. Thurston TL, Ryzhakov G, Bloor S, von Muhlinen N, Randow F. 2009. The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. Nat Immunol 10:1215-21.
79. Ravenhill BJ, Boyle KB, von Muhlinen N, Ellison CJ, Masson GR, Otten EG, Foeglein A, Williams R, Randow F. 2019. The Cargo Receptor NDP52 Initiates Selective Autophagy by Recruiting the ULK Complex to Cytosol-Invading Bacteria. Mol Cell 74:320-329 e6.
80. Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B, Korac J, Waidmann O, Choudhary C, Dotsch V, Bumann D, Dikic I. 2011. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. Science 333:228-33.
81. Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F. 2012. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482:414-8.
82. Kim BW, Hong SB, Kim JH, Kwon DH, Song HK. 2013. Structural basis for recognition of autophagic receptor NDP52 by the sugar receptor galectin-8. Nat Commun 4:1613.
83. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. 2005. Escape of intracellular Shigella from autophagy. Science 307:727-31.
84. Choy A, Dancourt J, Mugo B, O'Connor TJ, Isberg RR, Melia TJ, Roy CR. 2012. The Legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. Science 338:1072-6.
85. Park SW, Jeon P, Jun YW, Park JH, Lee SH, Lee S, Lee JA, Jang DJ. 2019. Monitoring LC3- or GABARAP-positive autophagic membranes using modified RavZ-based probes. Sci Rep 9:16593.
86. Feng ZZ, Jiang AJ, Mao AW, Feng Y, Wang W, Li J, Zhang X, Xing K, Peng X. 2018. The Salmonella effectors SseF and SseG inhibit Rab1A-mediated autophagy to facilitate intracellular bacterial survival and replication. J Biol Chem 293:9662-9673.
87. Webster CP, Smith EF, Bauer CS, Moller A, Hautbergue GM, Ferraiuolo L, Myszczynska MA, Higginbottom A, Walsh MJ, Whitworth AJ, Kaspar BK, Meyer K, Shaw PJ, Grierson AJ, De Vos KJ. 2016. The C9orf72 protein interacts with Rab1a and the ULK1 complex to regulate initiation of autophagy. EMBO J 35:1656-76.
88. Houtman J, Freitag K, Gimber N, Schmoranzer J, Heppner FL, Jendrach M. 2019. Beclin1-driven autophagy modulates the inflammatory response of microglia via NLRP3. EMBO J 38.
89. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, Tanaka K, Kawai T, Tsujimura T, Takeuchi O, Yoshimori T, Akira S. 2008. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature 456:264-8.
90. Sharma D, Kanneganti TD. 2016. The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. J Cell Biol 213:617-29.
91. Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT. 2008. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol 9:857-65.

92. Vajjhala PR, Mirams RE, Hill JM. 2012. Multiple binding sites on the pyrin domain of ASC protein allow self-association and interaction with NLRP3 protein. J Biol Chem 287:41732-43.
93. Martinon F, Burns K, Tschopp J. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 10:417-26.
94. Block ML, Zecca L, Hong JS. 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8:57-69.
95. Shi CS, Shenderov K, Huang NN, Kabat J, Abu-Asab M, Fitzgerald KA, Sher A, Kehrl JH. 2012. Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol 13:255-63.
96. Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, Yoshimori T. 2003. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. J Cell Sci 116:1679-88.
97. Lucin KM, O'Brien CE, Bieri G, Czirr E, Mosher KI, Abbey RJ, Mastroeni DF, Rogers J, Spencer B, Masliah E, Wyss-Coray T. 2013. Microglial beclin 1 regulates retromer trafficking and phagocytosis and is impaired in Alzheimer's disease. Neuron 79:873-86.
98. Heckmann BL, Teubner BJW, Tummers B, Boada-Romero E, Harris L, Yang M, Guy CS, Zakharenko SS, Green DR. 2019. LC3-Associated Endocytosis Facilitates beta-Amyloid Clearance and Mitigates Neurodegeneration in Murine Alzheimer's Disease. Cell 178:536-551 e14.
99. Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletier S, Gingras S, Orchard R, Guan JL, Tan H, Peng J, Kanneganti TD, Virgin HW, Green DR. 2015. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 17:893-906.
100. Henault J, Martinez J, Riggs JM, Tian J, Mehta P, Clarke L, Sasai M, Latz E, Brinkmann MM, Iwasaki A, Coyle AJ, Kolbeck R, Green DR, Sanjuan MA. 2012. Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. Immunity 37:986-997.
101. Huang J, Canadien V, Lam GY, Steinberg BE, Dinauer MC, Magalhaes MA, Glogauer M, Grinstein S, Brumell JH. 2009. Activation of antibacterial autophagy by NADPH oxidases. Proc Natl Acad Sci U S A 106:6226-31.
102. Martinez J, Almendinger J, Oberst A, Ness R, Dillon CP, Fitzgerald P, Hengartner MO, Green DR. 2011. Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells. Proc Natl Acad Sci U S A 108:17396-401.
103. Sanjuan MA, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, Komatsu M, Tanaka K, Cleveland JL, Withoff S, Green DR. 2007. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature 450:1253-7.
104. Kim YM, Jung CH, Seo M, Kim EK, Park JM, Bae SS, Kim DH. 2015. mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. Mol Cell 57:207-18.
105. Yang CS, Lee JS, Rodgers M, Min CK, Lee JY, Kim HJ, Lee KH, Kim CJ, Oh B, Zandi E, Yue Z, Kramnik I, Liang C, Jung JU. 2012. Autophagy protein Rubicon mediates phagocytic NADPH oxidase activation in response to microbial infection or TLR stimulation. Cell Host Microbe 11:264-76.
106. Lam GY, Cemma M, Muise AM, Higgins DE, Brumell JH. 2013. Host and bacterial factors that regulate LC3 recruitment to Listeria monocytogenes during the early stages of macrophage infection. Autophagy 9:985-95.
107. Fletcher K, Ulferts R, Jacquin E, Veith T, Gammoh N, Arasteh JM, Mayer U, Carding SR, Wileman T, Beale R, Florey O. 2018. The WD40 domain of ATG16L1 is required for its non-canonical role in lipidation of LC3 at single membranes. EMBO J 37.
108. Lystad AH, Carlsson SR, de la Ballina LR, Kauffman KJ, Nag S, Yoshimori T, Melia TJ, Simonsen A. 2019. Distinct functions of ATG16L1 isoforms in membrane binding and LC3B lipidation in autophagy-related processes. Nat Cell Biol 21:372-383.

109. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, Yoshimori T. 2000. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 19:5720-8.
110. Manil-Segalen M, Lefebvre C, Jenzer C, Trichet M, Boulogne C, Satiat-Jeunemaitre B, Legouis R. 2014. The C. elegans LC3 acts downstream of GABARAP to degrade autophagosomes by interacting with the HOPS subunit VPS39. Dev Cell 28:43-55.
111. Wang H, Sun HQ, Zhu X, Zhang L, Albanesi J, Levine B, Yin H. 2015. GABARAPs regulate PI4P-dependent autophagosome:lysosome fusion. Proc Natl Acad Sci U S A 112:7015-20.
112. Masud S, Prajsnar TK, Torraca V, Lamers GEM, Benning M, Van Der Vaart M, Meijer AH. 2019. Macrophages target Salmonella by Lc3-associated phagocytosis in a systemic infection model. Autophagy 15:796-812.
113. Oikonomou V, Moretti S, Renga G, Galosi C, Borghi M, Pariano M, Puccetti M, Palmerini CA, Amico L, Carotti A, Prezioso L, Spolzino A, Finocchi A, Rossi P, Velardi A, Aversa F, Napolioni V, Romani L. 2016. Noncanonical Fungal Autophagy Inhibits Inflammation in Response to IFN-gamma via DAPK1. Cell Host Microbe 20:744-757.
114. Herb M, Gluschko A, Schramm M. 2018. LC3-associated phagocytosis initiated by integrin ITGAM-ITGB2/Mac-1 enhances immunity to Listeria monocytogenes. Autophagy 14:1462-1464.
115. Martinez J, Cunha LD, Park S, Yang M, Lu Q, Orchard R, Li QZ, Yan M, Janke L, Guy C, Linkermann A, Virgin HW, Green DR. 2016. Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. Nature 533:115-9.
116. Ma J, Becker C, Lowell CA, Underhill DM. 2012. Dectin-1-triggered recruitment of light chain 3 protein to phagosomes facilitates major histocompatibility complex class II presentation of fungal-derived antigens. J Biol Chem 287:34149-56.
117. Romao S, Gasser N, Becker AC, Guhl B, Bajagic M, Vanoaica D, Ziegler U, Roesler J, Dengjel J, Reichenbach J, Munz C. 2013. Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. J Cell Biol 203:757-66.
118. Lee JW, Nam H, Kim LE, Jeon Y, Min H, Ha S, Lee Y, Kim SY, Lee SJ, Kim EK, Yu SW. 2019. TLR4 (toll-like receptor 4) activation suppresses autophagy through inhibition of FOXO3 and impairs phagocytic capacity of microglia. Autophagy 15:753-770.
119. Brodsky FM. 1988. Living with clathrin: its role in intracellular membrane traffic. Science 242:1396-402.
120. Pearse BM. 1976. Clathrin: a unique protein associated with intracellular transfer of membrane by coated vesicles. Proc Natl Acad Sci U S A 73:1255-9.
121. Mullock BM, Perez JH, Kuwana T, Gray SR, Luzio JP. 1994. Lysosomes can fuse with a late endosomal compartment in a cell-free system from rat liver. J Cell Biol 126:1173-82.
122. Goldstein JL, Brown MS, Anderson RG, Russell DW, Schneider WJ. 1985. Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. Annu Rev Cell Biol 1:1-39.
123. Lombardi D, Soldati T, Riederer MA, Goda Y, Zerial M, Pfeffer SR. 1993. Rab9 functions in transport between late endosomes and the trans Golgi network. EMBO J 12:677-82.
124. Mayor S, Presley JF, Maxfield FR. 1993. Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. J Cell Biol 121:1257-69.
125. Popovic D, Akutsu M, Novak I, Harper JW, Behrends C, Dikic I. 2012. Rab GTPase-activating proteins in autophagy: regulation of endocytic and autophagy pathways by direct binding to human ATG8 modifiers. Mol Cell Biol 32:1733-44.
126. Roy S, Leidal AM, Ye J, Ronen SM, Debnath J. 2017. Autophagy-Dependent Shuttling of TBC1D5 Controls Plasma Membrane Translocation of GLUT1 and Glucose Uptake. Mol Cell 67:84-95 e5.
127. Seaman MNJ, Mukadam AS, Breusegem SY. 2018. Inhibition of TBC1D5 activates Rab7a and can enhance the function of the retromer cargo-selective complex. J Cell Sci 131.

128. Mink JW, Blumenschine RJ, Adams DB. 1981. Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. Am J Physiol 241:R203-12.
129. Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, Ogier-Denis E. 2001. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem 276:35243-6.
130. Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, Miles JH, Wang CH, Stratton R, Pilarski R, Eng C. 2005. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. J Med Genet 42:318-21.
131. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, Ajroud K, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T. 2011. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477:211-5.
132. Fimia GM, Stoykova A, Romagnoli A, Giunta L, Di Bartolomeo S, Nardacci R, Corazzari M, Fuoco C, Ucar A, Schwartz P, Gruss P, Piacentini M, Chowdhury K, Cecconi F. 2007. Ambra1 regulates autophagy and development of the nervous system. Nature 447:1121-5.
133. Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Muller K, Marroquin N, Nordin F, Hubers A, Weydt P, Pinto S, Press R, Millecamps S, Molko N, Bernard E, Desnuelle C, Soriani MH, Dorst J, Graf E, Nordstrom U, Feiler MS, Putz S, Boeckers TM, Meyer T, Winkler AS, Winkelman J, de Carvalho M, Thal DR, Otto M, Brannstrom T, Volk AE, Kursula P, Danzer KM, Lichtner P, Dikic I, Meitinger T, Ludolph AC, Strom TM, Andersen PM, Weishaupt JH. 2015. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Nat Neurosci 18:631-6.
134. Kwok CT, Morris A, de Belleroche JS. 2014. Sequestosome-1 (SQSTM1) sequence variants in ALS cases in the UK: prevalence and coexistence of SQSTM1 mutations in ALS kindred with PDB. Eur J Hum Genet 22:492-6.
135. Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, de Vries R, Arias E, Harris S, Sulzer D, Cuervo AM. 2010. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 13:567-76.
136. Mitjans M, Begemann M, Ju A, Dere E, Wustefeld L, Hofer S, Hassouna I, Balkenhol J, Oliveira B, van der Auwera S, Tammer R, Hammerschmidt K, Volzke H, Homuth G, Cecconi F, Chowdhury K, Grabe H, Frahm J, Boretius S, Dandekar T, Ehrenreich H. 2017. Sexual dimorphism of AMBRA1-related autistic features in human and mouse. Transl Psychiatry 7:e1247.
137. Rietschel M, Mattheisen M, Degenhardt F, Genetic R, Outcome in P, Muhleisen TW, Kirsch P, Esslinger C, Herms S, Demontis D, Steffens M, Strohmaier J, Haenisch B, Breuer R, Czerski PM, Giegling I, Strengman E, Schmael C, Mors O, Mortensen PB, Hougaard DM, Orntoft T, Kapelski P, Priebe L, Basmanav FF, Forstner AJ, Hoffman P, Meier S, Nikitopoulos J, Moebus S, Alexander M, Mossner R, Wichmann H, Schreiber S, Rivandeneira F, Hofman A, Uitterlinden AG, Wienker TF, Schumacher J, Hauser J, Maier W, Cantor RM, Erk S, Schulze TG, Consortium SG-p, Craddock N, Owen MJ, O'Donovan MC, Borglum AD, Rujescu D, et al. 2012. Association between genetic variation in a region on chromosome 11 and schizophrenia in large samples from Europe. Mol Psychiatry 17:906-17.
138. Tian Y, Chang JC, Fan EY, Flajolet M, Greengard P. 2013. Adaptor complex AP2/PICALM, through interaction with LC3, targets Alzheimer's APP-CTF for terminal degradation via autophagy. Proc Natl Acad Sci U S A 110:17071-6.
139. Wong YC, Holzbaur EL. 2014. Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. Proc Natl Acad Sci U S A 111:E4439-48.

140. Stadel D, Millarte V, Tillmann KD, Huber J, Tamin-Yecheskel BC, Akutsu M, Demishtein A, Ben-Zeev B, Anikster Y, Perez F, Dotsch V, Elazar Z, Rogov V, Farhan H, Behrends C. 2015. TECPR2 Cooperates with LC3C to Regulate COPII-Dependent ER Export. Mol Cell 60:89-104.
141. Saitsu H, Nishimura T, Muramatsu K, Kodera H, Kumada S, Sugai K, Kasai-Yoshida E, Sawaura N, Nishida H, Hoshino A, Ryujin F, Yoshioka S, Nishiyama K, Kondo Y, Tsurusaki Y, Nakashima M, Miyake N, Arakawa H, Kato M, Mizushima N, Matsumoto N. 2013. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat Genet 45:445-9, 449e1.
142. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. 2009. Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78:959-91.
143. Vingtdeux V, Giliberto L, Zhao H, Chandakkar P, Wu Q, Simon JE, Janle EM, Lobo J, Ferruzzi MG, Davies P, Marambaud P. 2010. AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism. J Biol Chem 285:9100-13.
144. Zimmermann HR, Yang W, Kasica NP, Zhou X, Wang X, Beckelman BC, Lee J, Furdui CM, Keene CD, Ma T. 2020. Brain-specific repression of AMPKalpha1 alleviates pathophysiology in Alzheimer's model mice. J Clin Invest doi:10.1172/JCI133982.
145. Xu J, Kao SY, Lee FJ, Song W, Jin LW, Yankner BA. 2002. Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease. Nat Med 8:600-6.
146. Murthy A, Li Y, Peng I, Reichelt M, Katakam AK, Noubade R, Roose-Girma M, DeVoss J, Diehl L, Graham RR, van Lookeren Campagne M. 2014. A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. Nature 506:456-62.
147. Xavier RJ, Podolsky DK. 2007. Unravelling the pathogenesis of inflammatory bowel disease. Nature 448:427-34.
148. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J, Gunther S, Prescott NJ, Onnie CM, Hasler R, Sipos B, Folsch UR, Lengauer T, Platzer M, Mathew CG, Krawczak M, Schreiber S. 2007. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 39:207-11.
149. Lassen KG, Kuballa P, Conway KL, Patel KK, Becker CE, Peloquin JM, Villablanca EJ, Norman JM, Liu TC, Heath RJ, Becker ML, Fagbami L, Horn H, Mercer J, Yilmaz OH, Jaffe JD, Shamji AF, Bhan AK, Carr SA, Daly MJ, Virgin HW, Schreiber SL, Stappenbeck TS, Xavier RJ. 2014. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. Proc Natl Acad Sci U S A 111:7741-6.
150. Koyama R, Ikegaya Y. 2015. Microglia in the pathogenesis of autism spectrum disorders. Neurosci Res 100:1-5.
151. Kim HJ, Cho MH, Shim WH, Kim JK, Jeon EY, Kim DH, Yoon SY. 2017. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. Mol Psychiatry 22:1576-1584.
152. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT. 2011. Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456-8.
153. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. 2019. Neuroinflammation as a Common Feature of Neurodegenerative Disorders. Front Pharmacol 10:1008.
154. Witting A, Muller P, Herrmann A, Kettenmann H, Nolte C. 2000. Phagocytic clearance of apoptotic neurons by Microglia/Brain macrophages in vitro: involvement of lectin-, integrin-, and phosphatidylserine-mediated recognition. J Neurochem 75:1060-70.

155. Gluschko A, Herb M, Wiegmann K, Krut O, Neiss WF, Utermohlen O, Kronke M, Schramm M. 2018. The beta2 Integrin Mac-1 Induces Protective LC3-Associated Phagocytosis of Listeria monocytogenes. Cell Host Microbe 23:324-337 e5.
156. Ogawa M, Takada N, Shizukuishi S, Tomokiyo M, Chang B, Yoshida M, Kakuta S, Tanida I, Ryo A, Guan JL, Takeyama H, Ohnishi M. 2020. Streptococcus pneumoniae triggers hierarchical autophagy through reprogramming of LAPosome-like vesicles via NDP52-delocalization. Commun Biol 3:25.

Figure Legends:

Figure 1: The autophagy pathway.

The energy sensors mTORC1 and AMPK control autophagy activation via the ULK1 complex. Following activation, the ULK1 and PI3KC3 complex regulate the formation of the omegasome and cargo is then recruited by autophagic receptors to the phagophore. Finally, the maturated autophagosome fuses with lysosomes to autolysosomes and the cargo is degraded.

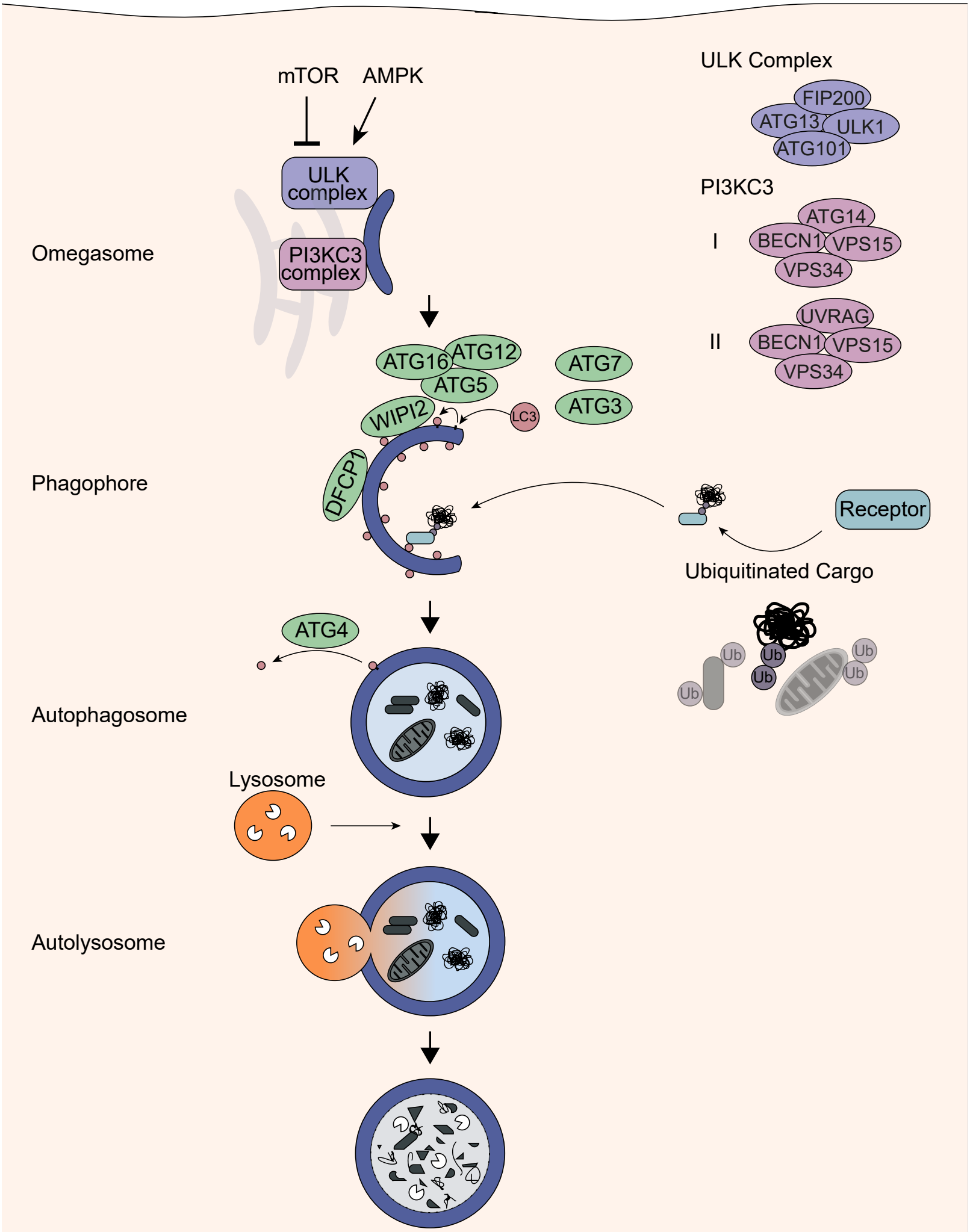
Figure 2: Selective autophagy as possible regulator of inflammasome activity in microglia.

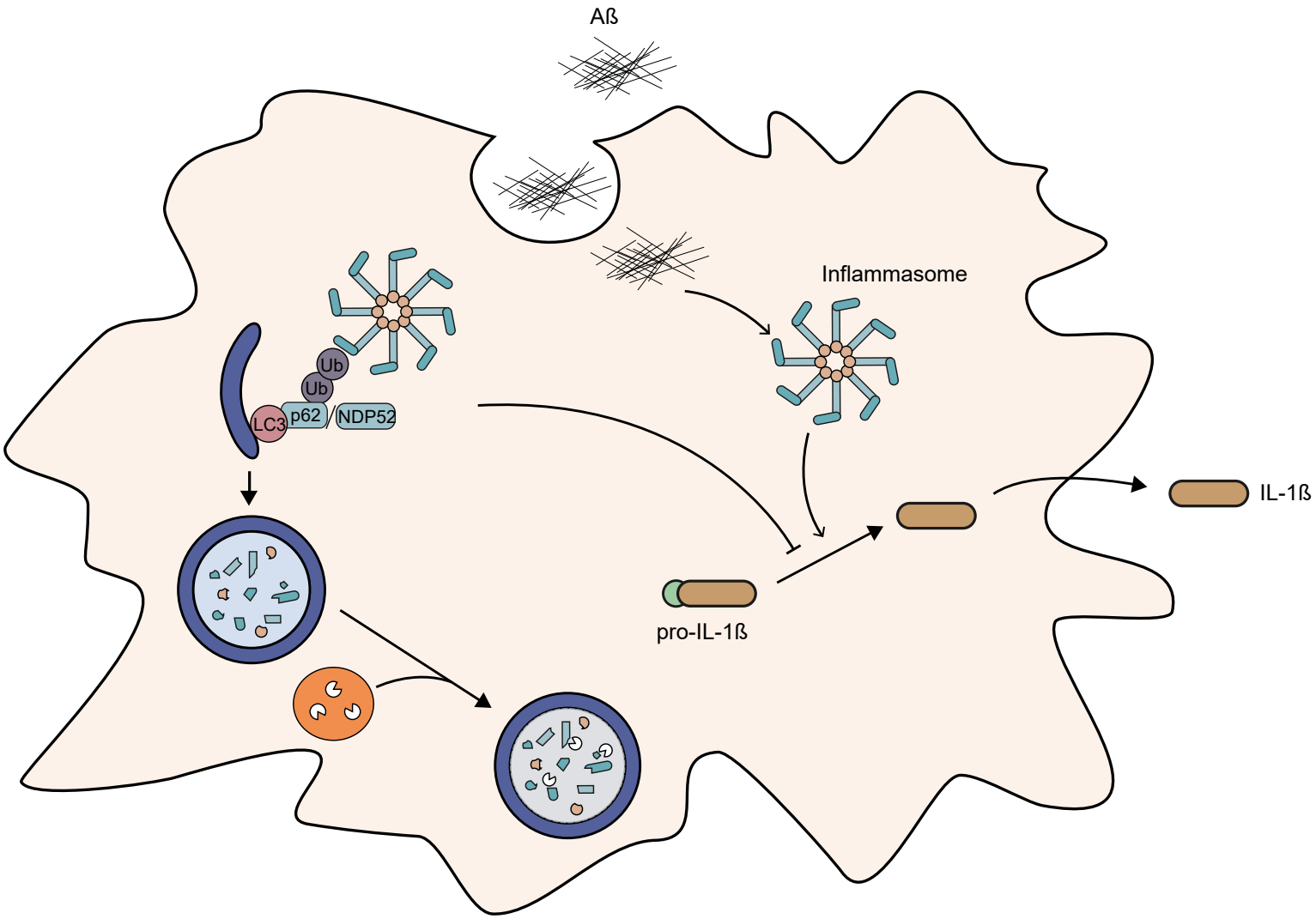
Presence of Aβ causes activation of proinflammatory response and release of the cytokine IL-1β. Autophagy receptors p62 and NDP52 might recognize and target ubiquitinated inflammasomes for lysosomal degradation thereby controlling Aβ-induced inflammation and survival of the cell.

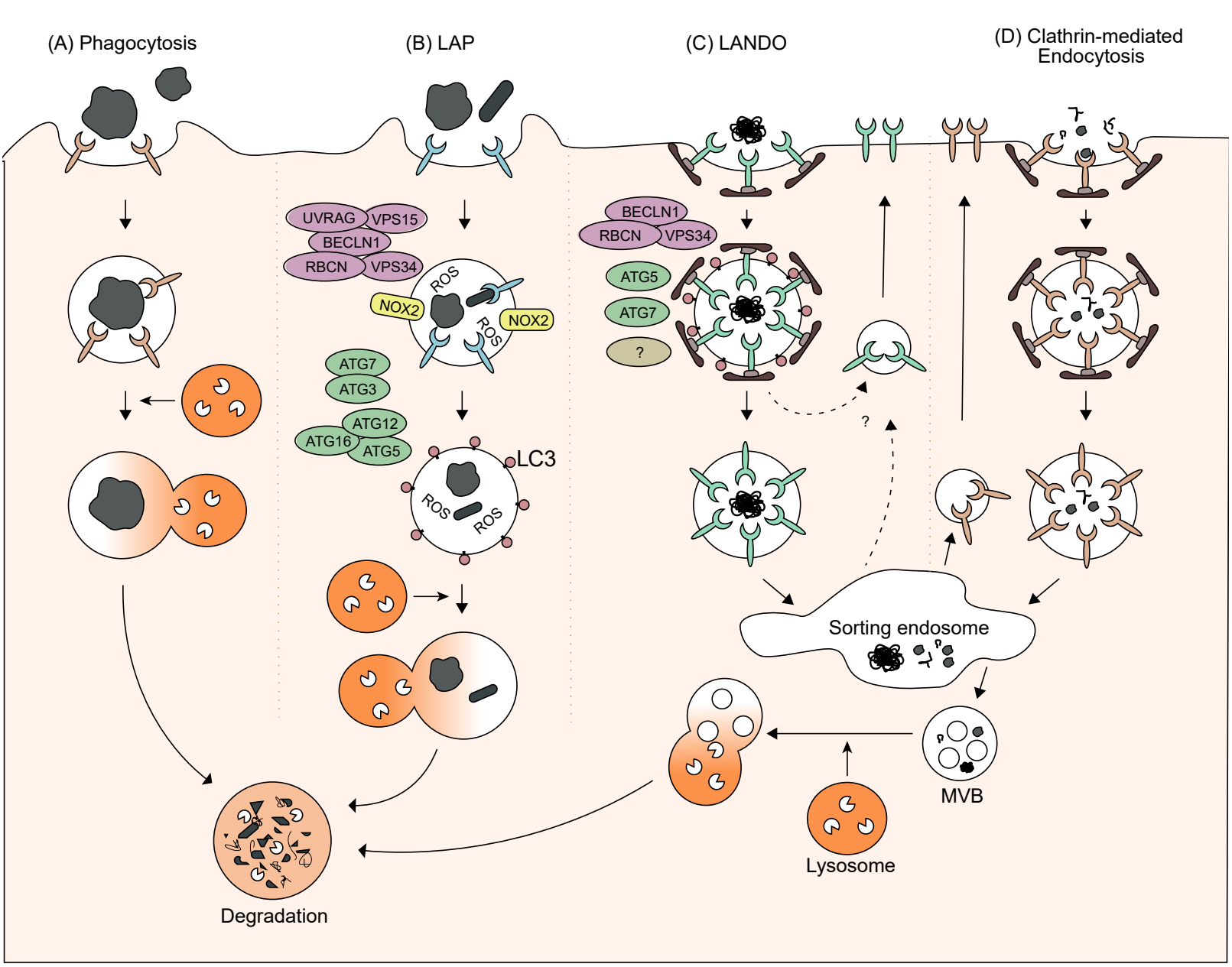
Figure 3: An overview of phagocytosis, LAP, LANDO and clathrin-mediated endocytosis.

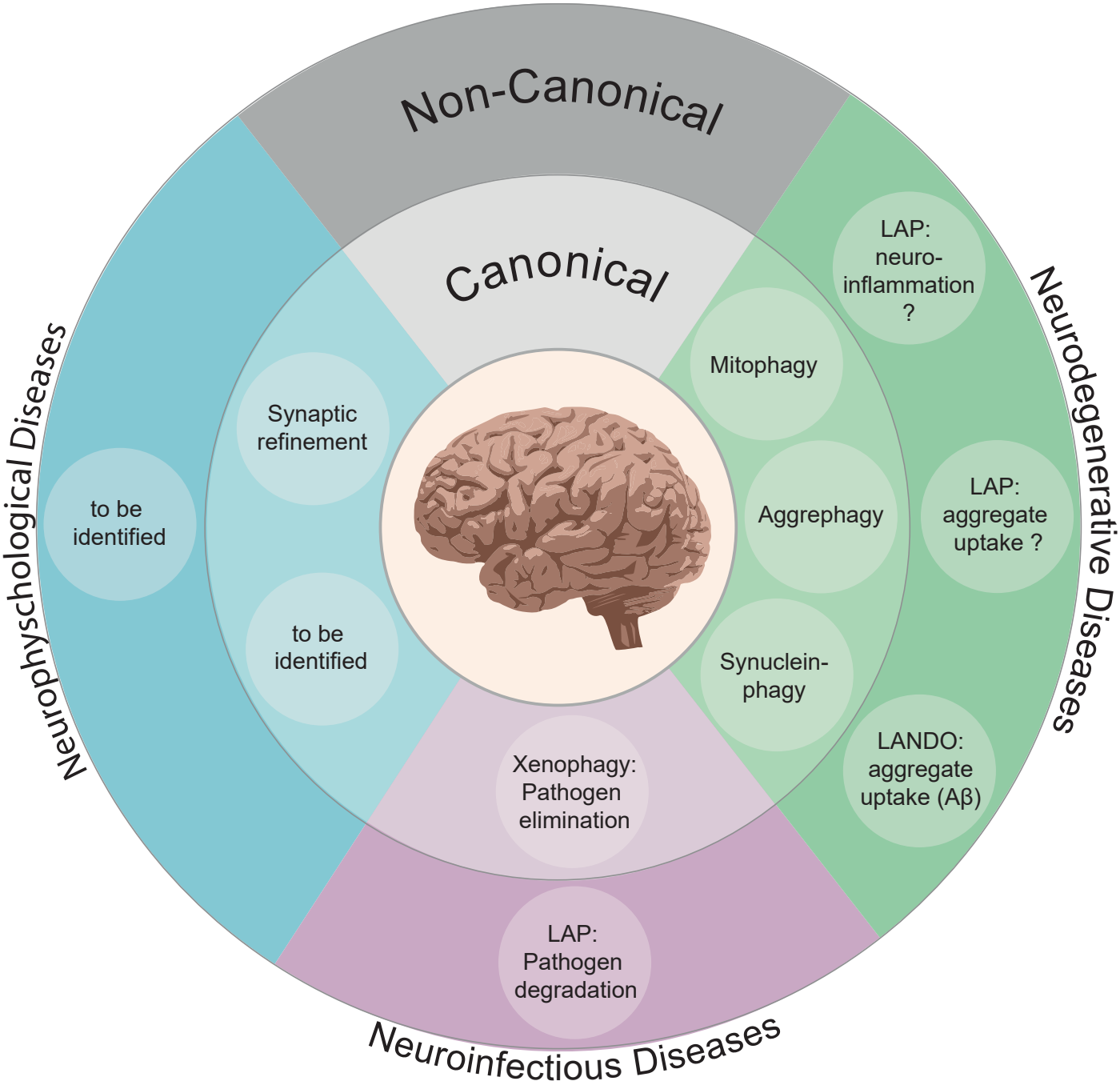
(A) During phagocytosis, large extracellular particles are recognized by specific receptors, engulfed by the plasma membrane and finally internalized by phagosomes. (B) When LC3 is recruited via the PI3KC3 II complex and other autophagy proteins to the phagosome before its fusion with the lysosome, the pathway is referred to as LAP. (C) When conjugation of LC3 by

977 autophagy proteins to Rab5- and clathrin-positive endosomes is necessary for receptor
978 recycling, the pathway is called LANDO. (D) Clathrin-mediated endocytosis describes the uptake
979 of smaller extracellular cargo into clathrin-coated vesicles. After uncoating, the nascent vesicle is
980 trafficked further within the cell for example to the sorting endosome.
981
982 **Figure 4: Canonical and non-canonical autophagy functions associated to**
983 **neurological diseases.**
984 The inner circle presents canonical and the outer circle non-canonical autophagy processes. So
985 far, these pathways were most extensively studied in neurodegenerative diseases. However,
986 only little is known about their functions in neuropsychological diseases. Further aspects still
987 need to be identified. Concerning neuroinfectious diseases, either canonical xenophagy or LAP
988 can eliminate the invading particle, depending on its pathogenic characteristics.
989


mTOR
AMPK
ULK complex
PI3KC3 complex
Omegasome
ATG16
ATG12
ATG5
ATG7
ATG3
WIPI2
LC3
DFCP1
Phagophore
Receptor
Ubiquitinated Cargo
Ub
ATG4
Autophagosome
Lysosome
Autolysosome
ULK Complex
FIP200
ATG13
ULK1
ATG101
PI3KC3
I
ATG14
BECN1
VPS15
VPS34
II
UVRAG
BECN1
VPS15
VPS34


Aß
Inflammasome
Ub
Ub
LC3
p62
NDP52
pro-IL-1ß
IL-1ß


(A) Phagocytosis
(B) LAP
(C) LANDO
(D) Clathrin-mediated Endocytosis
UVRAG
VPS15
BECLN1
RBCN
VPS34
ROS
NOX2
ATG7
ATG3
ATG12
ATG16
ATG5
LC3
BECLN1
RBCN
VPS34
ATG5
ATG7
?
Sorting endosome
MVB
Lysosome
Degradation


Non-Canonical
Canonical
LAP: neuro-inflammation ?
Mitophagy
Synaptic refinement
Aggrephagy
LAP: aggregate uptake ?
to be identified
to be identified
Synuclein-phagy
LANDO: aggregate uptake (Aβ)
Xenophagy: Pathogen elimination
LAP: Pathogen degradation
Neurophyschological Diseases
Neurodegenerative Diseases
Neuroinfectious Diseases