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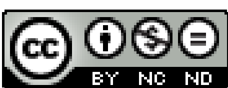

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Running title: Autophagy and Mammalian Viruses

Autophagy and Mammalian Viruses: Roles in Immune Response, Viral Replication and beyond

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Abstract

Autophagy is an important cellular catabolic process conserved from yeast to man. Double membrane vesicles deliver their cargo to the lysosome for degradation. Hence, autophagy is one of the key mechanisms mammalian cells deploy to rid themselves of intracellular pathogens including viruses. However, autophagy serves many more functions during viral infection. First, it regulates the immune response through selective degradation of immune components, thus preventing possibly harmful over-activation and inflammation. Additionally, it delivers virus-derived antigens to antigen loading compartments for presentation to T lymphocytes. Second, it might take an active part in the viral lifecycle by e.g. facilitating its release from cells. Lastly, in the constant arms race between host and virus, autophagy is often hijacked by viruses and manipulated to their own advantage. In this review, we will highlight key steps during viral infection in which autophagy plays a role. We have selected some exemplary viruses and will describe the molecular mechanisms behind their intricate relationship with the autophagic machinery, a result of host-pathogen co-evolution.

Keywords: Xenophagy, antiviral immunity, IL-1, antigen presentation, unconventional autophagy, viral evasion, herpes viruses, influenza virus, LC3 associated phagocytosis

1. The autophagic machinery in mammalian cells

1.1 General introduction to autophagy

Mammalian cells degrade biomolecules in a proteolytic vesicular compartment called the lysosome. Materials to be degraded include extracellular components taken up by endocytosis/phagocytosis as well as intracellular protein aggregates, damaged organelles or bulk cytoplasmic material. One of the mechanisms by which the latter constituents can reach the lysosome is called autophagy. Three types of autophagy can be distinguished. Microautophagy results from direct invaginations of the lysosome membrane which engulf cytoplasmic material (Kunz, Schwarz and Mayer, 2004). Secondly, during chaperone mediated autophagy, chaperone molecules act as molecular shuttles delivering specific cargo to the lysosome. Cargo is thought to translocate into the lysosome with the aid of a yet unknown transporter (reviewed in (Cuervo and Wong, 2014)). In this review, we will focus on the third type of autophagy termed macroautophagy. We will refer to it simply as autophagy from now on.

Characteristically, autophagic cargo becomes engulfed in a double membrane vesicle in the cytosol. The uptake of cargo into the autophagosome may either happen in bulk or in a very specific manner orchestrated by a variety of cargo receptors. The latter is referred to as selective autophagy. Further down the line the outer membrane of the autophagosome fuses with the lysosome and both inner autophagosomal membrane and cargo are degraded in the lysosome's proteolytic environment. After degradation of the cargo its molecular building blocks are shuttled out into the cytoplasm to serve biosynthesis. Providing building blocks for new biomolecules is also the reason why autophagy gets strongly upregulated during starvation. In general, autophagy regulation is highly responsive to environmental stress but also plays a protective role by disposing of protein aggregates, damaged organelles as well as intracellular pathogens. Hence, viruses are direct targets for degradation by autophagy. Should a viral infection manifest despite this early defense mechanism, autophagy again comes into play during antiviral immune responses. However, many viruses have evolved to evade these cellular defense mechanisms. More and more molecular details come to light with more than

200 articles published on 'autophagy and virus' in 2015 alone. Nevertheless, many lessons remain to be learned as the relationship between virus lifecycle and autophagy is a very complex one. Some viruses stimulate autophagy while others inhibit it. Additionally, viruses have learned to adapt to the degradative role of autophagy. Increasing knowledge on this intricate host/pathogen relationship might be exploited for novel therapy approaches in the future.

1.2 The autophagic machinery

The components of the autophagic machinery were first described in yeast (Thumm et al., 1994;Tsukada and Ohsumi, 1993), but many homologues exist in mammals. The core machinery that drives autophagy is comprised of more than 30 of the so called autophagy-related gene (ATG) products. One can distinguish the following key steps in the life cycle of an autophagosome: initiation, elongation, cargo uptake, closure/maturation and fusion with vesicles (including the lysosome) followed by destruction of the cargo (Figure 1). Distinct protein complexes coordinate these individual steps.

At the heart of autophagy initiation lays the ULK complex which sets off the formation of an autophagosomal membrane, called the phagophore or isolation membrane. The ULK complex is comprised of the Unc-51-like autophagy-activating kinases ULK1/2, FAK family kinase-interacting protein of 200 kDa (FIP200), ATG13 and ATG101 (Hara et al., 2008;Hosokawa et al., 2009a;Hosokawa et al., 2009b;Mercer, Kaliappan and Dennis, 2009). The phagophore may form at various sites in the cell often at or in vicinity of the ER (Carlsson and Simonsen, 2015). The ER-resident multi-membrane-spanning protein vacuole membrane protein (VMP) 1 was shown to be required and was suggested to mark the platform where phagophore formation initiates (Koyama-Honda et al., 2013). Autophagosome formation sites are characterized by enrichment in phosphatidylinositol phosphates, namely PI(3)P, which result from the action of another crucial protein complex, phosphoinositide-3-kinase (PI3K) complex III. This complex consists of the catalytic subunit of PI3K, vacuolar protein sorting (VPS) 34, VPS15, ATG14 and Beclin 1 (BECN1) (Itakura et al., 2008;Matsunaga et al., 2009;Sun

et al., 2008;Zhong et al., 2009). PI(3)P formation by the PI3K complex serves as a crucial binding platform for downstream effectors, which bind via their FYVE motifs. One such effector is double-FYVE-containing protein 1 (DFCP1), which can be used as a marker for autophagosome formation sites (Axe et al., 2008). Adjacent to DFCP1 the mammalian homologues of yeast ATG18 are recruited to PI(3)P, namely WD-repeat protein interacting with phosphoinositides (WIPI) 1-4 (Jeffries et al., 2004;Polson et al., 2010;Proikas-Cezanne et al., 2004). These will in turn recruit downstream members of the autophagic machinery (Dooley et al., 2014).

A growing phagophore requires membrane. Where this membrane comes from is still a matter of debate (Carlsson and Simonsen, 2015). A lot of evidence has been gathered showing that isolation membranes form in close proximity to the ER, which surrounds the phagophore in a structure called the omegasome (Axeet al., 2008;Hayashi-Nishino et al., 2009;Yla-Anttila et al., 2009). However, this structure might be the starting point of some special form of autophagy aimed at degrading parts of the ER itself (Khaminets et al., 2015;Mochida et al., 2015). Also mitochondria (Hailey et al., 2010;Hamasaki et al., 2013), the nuclear membrane (English et al., 2009) and recycling endosomes (Puri et al., 2013) among others have been proposed as possible sources of autophagosomal lipids/proteins/membrane. The only trans-membrane protein in the autophagic machinery is ATG9. In mammalian cells it is found at the omegasome, in the Golgi and on endosomes (Young et al., 2006). Upon autophagy induction ATG9 colocalises with ATG16L1 in recycling endosomes (Puriet al., 2013). Trafficking from there to the phagophore might provide membrane to the growing autophagosome (Lamb et al., 2016). Furthermore, regulated traffic of ATG9 vesicles from Golgi to the growing phagophore has been described (He et al., 2013). Retrieving ATG9 from the early autophagosome seems to be dependent on WIPI2 (Orsi et al., 2012), potentially through binding of WIPI2 to ATG2 as demonstrated in yeast (Reggiori et al., 2004).

In order for the phagophore to elongate, the lipidated form of the mammalian homologues of yeast ATG8 needs to be embedded in both inner and outer autophagosomal membrane (Xie, Nair and Klionsky, 2008). Mammalian homologues of ATG8 are divided into two clades: microtubule-

associated protein 1 light chain 3 (LC3) A/B/C and γ-aminobutyric acid receptor-associated protein (GABARAP), GABARAPL1/L2 (Shpilka et al., 2011), LC3B being the most extensively studied member. These ubiquitin-like proteins are conjugated to phosphatidylethanolamine (PE) in a fashion similar to the E1/E2/E3 ubiquitin ligase conjugation scheme. We will refer to all mammalian ATG8s as LC3 from now on unless specific roles are discussed.

LC3 is synthetized in pro-form and is freely available in the cytosol. Cleavage of the C-terminus of LC3 by ATG4B exposes a glycine residue needed for the conjugation reaction (Kirisako et al., 2000). ATG7 serves as the E1 enzyme activating both LC3 and ATG12. In the next step, LC3 and ATG12 are transferred to E2-like ATG3 and ATG10, respectively. Finally, ATG12 becomes isopeptide bonded to its substrate ATG5. This ATG12-ATG5 conjugate acts as an E3-like enzyme transferring LC3 to PE in the autophagosomal membrane (Ichimura et al., 2000;Mizushima et al., 1998). This last step is further promoted by ATG16L1 bound non-covalently to ATG5 in the ATG12-5 conjugate (Fujita et al., 2008). LC3-PE is important for the maturation of the autophagosome because it promotes tethering to other vesicles, hemifusion, trafficking and sealing of the autophagosome (Nakatogawa, Ichimura and Ohsumi, 2007;Pankiv et al., 2010;Weidberg et al., 2010). While LC3-PE on the inner membrane will be degraded together with the autophagic cargo in the lysosome, LC3-PE on the outer membrane can be recycled through the action of ATG4B (Kirisakoet al., 2000).

WIPIs seem to act as coordinators of these events. They are recruited early on to PI(3)P at the autophagosome formation site. WIPI1/2/4 are essential for autophagy (Lu et al., 2011;Polsonet al., 2010). All three are found at the autophagosome formation site, but only WIPI1 and 2 are incorporated into inner and outer autophagosomal membrane (Proikas-Cezanne and Robenek, 2011). WIPI2 binds ATG16L1 and hence recruits the ATG5/12/16L1 complex to the phagophore (Dooleyet al., 2014). In turn, LC3 bound to E2-like ATG3 is recruited to the E3-like ATG5/12/16L1 complex via interaction of ATG12 with ATG3 (Fujitaet al., 2008).

Recently, more and more findings suggest that the conventional route of autophagy initiation might differ from cell type to cell type and during development. These open questions are summarized in

(Lindqvist, Simon and Baehrecke, 2015), while some of the unconventional functions of ATG proteins are highlighted in point 5 of this review.

Movement of autophagosomes through the cell is mediated by FYVE and coiled-coil domain-containing FYCO1, which binds LC3 via a so-called LC3 interacting region (LIR) (Pankivet al., 2010). Furthermore, it can bind to PI(3)P and RAB7, a small GTPase enriched on late endosomes and autophagosomes. FYCO1 drives transport of vesicles along microtubules with the help of kinesin motors. Transport of autophagosomes in the opposite direction is most likely mediated by the adaptor protein RAB7 interacting lysosomal protein (RILP) and dynein motors (Kimura, Noda and Yoshimori, 2008;Liang et al., 2008;van der Kant et al., 2013).

The event of fusion between autophagosome and lysosome needs to be tightly regulated to avoid damage to the cell. The concerted action of various factors is required for tethering and fusion. Lipidated LC3 seems to be the key for these events to happen. Mature autophagosomes are decorated with RAB7 and a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) called Syntaxin 17 (STX17) (Itakura, Kishi-Itakura and Mizushima, 2012). An adaptor called Pleckstrin homology domain containing protein family member (PLEKHM) 1 is able to bind to LC3, RAB7 and STX17. PLEKHM1 recruits the tethering complex homotypic fusion and protein sorting (HOPS) complex which brings autophagosomes and lysosomes together (McEwan et al., 2015). STX17 interacts with an adaptor called synaptosomal-associated protein (SNAP) 29, which in turn links the entire machinery to the lysosomal SNARE vesicle-associated membrane protein (VAMP) 8 (Itakura, Kishi-Itakura and Mizushima, 2012;Jiang et al., 2014). Lately, it was shown that ATG14L alone is sufficient to bring STX17 and SNAP-29 in close proximity to mediate fusion (Diao et al., 2015).

Thus, autophagy provides an excellent example of *de novo* vesicle formation in the cell and its molecular machinery has developed to coordinate the required membrane trafficking events

1.3 Autophagic Cargo

Autophagy can engulf cargo in either an unspecific or a very selective manner. At basal level, bulk autophagy serves a kind of quality control function ensuring e.g. organelle homeostasis. In contrast, when a cell is deprived of nutrients, building blocks for biosynthesis need to be provided quickly and hence more phagophores are formed and autophagy flux is increased. Both bulk as well as selective degradation of cargo have been shown to link to nutrient deprivation. Alternatively, autophagy can degrade specific cargo in a highly selective manner in response to certain types of stress, e.g. damage to an organelle. Individual terms have been coined for this selective autophagy processes depending on the type of structure targeted: mitochondria (mitophagy), peroxisomes (pexophagy), pathogens (xenophagy), aggregated proteins (aggrephagy), to just name a few.

Selective autophagy is mediated by so called autophagy receptors (Table 1) which link cargo to LC3 on the phagophore. These receptors utilize LIR motifs to interact with LC3 while binding cargo through other moieties. LIR motifs are defined as Trp/Phe/Tyr-x-x-Leu/Ile/Val (reviewed in (Rogov et al., 2014)). The signal that is recognized on the cargo is ubiquitin in many cases. The best studied autophagy receptor that binds ubiquitin is p62, also called Sequestosome-1 (SQMST1) (Bjorkoy et al., 2005). However, recent reports have proposed other recognition signals such as lipids (Chu et al., 2013), lectins (Thurston et al., 2012) and methylated arginine (Li et al., 2013). Autophagic receptors tend to oligomerize which aids the clustering of cargo for uptake into the growing autophagosome.

Mitophagy is a well-studied example of selective autophagy in which different recognition motifs play a role. When mitochondria lose their functionality, their membrane potential drops. This depolarization causes an accumulation of PTEN-induced putative kinase (PINK) 1 at the outer mitochondrial membrane (OMM) (Narendra et al., 2008). PINK1 phosphorylates ubiquitin, which in turn activates the E3 ligase parkin (encoded by *PARK2* in humans) (Kane et al., 2014;Kazlauskaite et al., 2014;Koyano et al., 2014) to conjugate more ubiquitin to substrates in the OMM, which will be again phosphorylated by PINK1, amplifying the signal. Phosphorylated ubiquitin is recognized by the autophagy receptors optineurin (OPTN) and NDP52. These two receptors act redundantly and recruit members of the autophagy machinery, namely ULK1, DFCP1, WIPI1 and LC3 (Lazarou et al., 2015).

Most mitophagy related studies have triggered mitochondrial damage artificially. Whether the described mechanisms play a role in a physiological context remains to be proven. Not surprisingly, alternative mechanisms have been described involving the autophagy receptors NIX, BNIP3 and FUNDC1 (Liu et al., 2012;Novak et al., 2010;Quinsay et al., 2010). NIX and BNIP3 are related proteins that require serine phosphorylation adjacent to the LIR for their activity in promoting selective autophagy (Hamacher-Brady and Brady, 2016). In addition, mitochondria seem to be targets for autophagic degradation during nutrient deprivation (Kim and Lemasters, 2011), even so other organelles and cytoplasmic protein complexes might be degraded first (Dengjel et al., 2012). Failure of mitophagy can have a great impact on the cell, e.g. by altering immune regulation in the course of viral infections and compromising survival of terminally differentiated lymphocytes as discussed under point 2 and 3 of this review, respectively. The details of selective autophagy are beyond the scope of this review, but have recently been reviewed (Khaminets, Behl and Dikic, 2016;Sica et al., 2015).

The identification of more and more specific autophagy substrate recruitment mechanisms raises the question whether under any physiological circumstances at all, autophagy engulfs cytoplasmic material in a non-specific manner.

1.4 The regulation of autophagy

Autophagy has to be tightly regulated at many levels, foremost at the stage of phagophore formation. From a systemic point of view, autophagy is triggered when key metabolic molecules are low in abundance (reviewed by (Galluzzi et al., 2014). Various G protein-coupled receptors (GPCRs) at the plasma membrane are involved in sensing a drop in nutrient levels (e.g. amino acids or fatty acids) in the extracellular milieu (Wauson et al., 2014). Molecules in circulation can also affect autophagy. Insulin suppresses autophagy, while glucagon can trigger it (Kimball, Siegfried and Jefferson, 2004). Not surprisingly, starvation will cause a decrease in serum levels of insulin and

insulin-like growth factor 1, whereas levels of insulin-like growth factor binding protein 1 and glucagon increase (Cheng et al., 2014).

At the cellular level various cues can trigger autophagy, e.g. a drop in iron levels, depletion of amino acids, accumulation of NAD^+, and a decrease in overall energy balance leading to the accumulation of AMP. The latter is sensed by one of the key mediators of autophagy initiation, AMP-activated protein kinase (AMPK). AMPK activates the autophagy machinery by phosphorylating ULK1 (Egan et al., 2011) and BECN1 (Kim et al., 2013). In turn, ULK1 promotes autophagy initiation by phosphorylating BECN1 (Russell et al., 2013). Furthermore, AMPK plays a role in inactivating another central sensor of the energy state of the cell, namely mammalian target of rapamycin complex 1 (mTORC1) (Inoki et al., 2002). mTORC1 is composed of MTOR, RAPTOR, PRAS40, DEPTOR and mLST8. Under nutrient-rich conditions mTORC1 inactivates the ULK1 complex through direct interaction of RAPTOR and ULK1 leading to an inactivating phosphorylation of ULK1 and ATG13 (Ganley et al., 2009;Hosokawaet al., 2009a;Jung et al., 2009). At the same time mTORC1 stimulates cell growth through activation of transcription, protein and lipid synthesis.

One protein complex, on which autophagy regulation hinges, is the PI3K complex. As mentioned earlier, the core complex consists of the catalytic PI3K subunit VPS34, the regulatory PI3K subunit VPS15, ATG14 and BECN1 (Itakuraet al., 2008;Sunet al., 2008). This ATG14-containing complex acts early on in phagophore formation. BECN1 binding partners serve as regulators of this complex. Factors that positively regulate it are: e.g. VMP1 (the ER protein that marks the autophagosome formation site), or autophagy/beclin-1 regulator (AMBRA) 1 (Fimia et al., 2007). Binding partners that negatively regulate the ATG14 complex are: e.g. anti-apoptotic B cell CLL/lymphoma 2 (BCL2) and BCL-X_L (Pattingre et al., 2005), GAPR1 (Shoji-Kawata et al., 2013) or 14-3-3ε (Wang et al., 2012).

At later stages of autophagosome maturation, the PI3K complex comes into action again, but in a different conformation, replacing ATG14 with UV radiation resistance associated gene (UVRAG) (Itakuraet al., 2008). This UVRAG-containing complex is positively regulated by BECN1 binding to BIF-1 (Takahashi et al., 2007), while negative regulation involves BCL-2 and BCL-X_L (Pattingreet al., 2005).

The step of autophagosome maturation is inhibited by the third form of the PI3K complex which contains UVRAG and a negative regulator RUBICON at the same time (Matsunagaet al., 2009;Zhonget al., 2009). Given BECN1's central role in regulation of autophagy, it is not surprising that many viruses manipulate autophagy by attacking this protein in particular. More on this subject will be discussed under point 4 of this review. Recent work also suggests modes of autophagy regulation at the transcriptional level (Lee et al., 2014;Seok et al., 2014).

In this review, we will outline roles of autophagy during the immune response against viruses. Furthermore, we will highlight the many ways viruses manipulate the autophagic machinery during the constant arms race between pathogen and host and the role that autophagy plays during the release of viral particles from infected cells. Lastly, we will discuss some unconventional ways in which autophagic proteins are utilized in infected cells.

2. Autophagy in innate immune control of virus infections

Innate immune mechanisms serve as a first line of defense against invading viruses. On one hand, they consist of physical barriers that prevent the infection of host cells. On the other hand, germline encoded receptors recognize virus intrinsic features, so called pathogen-associated molecular patterns (PAMPs), and set off a cascade of transcriptional events that will lead to the production of anti-viral substances and immune modulating cytokines (Figure 2A). In turn, these cues will recruit immune cells, like natural killer (NK) cells, neutrophils and monocytes to the site of infection. These cells either directly kill infected cells or help to clear the site of dead cells. Ultimately, the triggering of an innate immune response will also aid the instruction of a more specialized adaptive immune response at later stages of infection (Figure 2B+C). The latter will be discussed under point 3 of this review.

With autophagy being one of the major degradative pathways in mammalian cells it is only natural to assume that it also plays an important role in disposing of intracellular pathogens. The selective removal of intruding pathogens is called xenophagy. Indeed, for many viral infections it was shown

that functional autophagy keeps viral titers at bay *in vivo* (Lee et al., 2010;Orvedahl et al., 2010;Shelly et al., 2009). Since viruses harbor PAMPs, a multitude of pattern recognition receptors (PRRs) is able to detect the commencing viral infection. Signaling through different classes of PRRs, namely toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and nucleotide oligomerization domain (NOD)-like receptors (NLRs), was shown to converge with the autophagy pathway. An autophagy stimulating role of PRRs was first described for TLR4 following infection with mycobacteria (Xu et al., 2007). To date many observations support the notion that PRRs trigger autophagy to initiate the degradation of viral particles. On the contrary, autophagy was also shown to play a role in keeping inflammation at bay via degradation of components of the innate immune systems or mitochondria, hence reducing the release of pro-inflammatory signals like mitochondrial DNA or reactive oxygen species (ROS) (Kimura et al., 2015;Tal et al., 2009). This concept is supported by the fact that a lack of autophagy in certain myeloid immune cells can lead to lung inflammation even in the absence of infection (Abdel Fattah et al., 2015;Kanayama, He and Shinohara, 2015;Lu et al., 2016). Examples displaying the dual role of autophagy in antiviral innate immune responses are given below and are summarized in Figure 2A.

2.1 Autophagy and pattern recognition receptors

Toll-like receptors are the best characterized group of PRRs. These membrane-bound receptors can be found on the plasma membrane (TLR1/2/4/5/6) or inside endosomal compartments (TLR3/7/8/9). The endosomal TLRs are mostly responsible for recognition of viral PAMPs, such as dsRNA (TLR3), ssRNA (TLR7/8) and DNA with unmethylated CpG (TLR9). They are mostly expressed by macrophages, conventional and plasmacytoid dendritic cells (pDC). TLRs act as homodimers. Signaling is mediated by adaptor molecules like myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF), which activate the transcription factors NF-κB, AP-1, and IRF3/7 leading to the expression of type I interferons (IFN) α and β and pro-inflammatory cytokines (reviewed in (Lee and Kim, 2007). Downstream so called IFN-regulated genes

(IRG) are transcriptionally activated or repressed leading to modulation of the immune response. Autophagy seems to play a role in TLR-mediated activation of type I IFN production. ATG5-deficient pDCs fail to upregulate type I IFNs following vesicular stomatitis virus (VSV) or Sendai virus infection (Lee et al., 2007). Autophagosomes seem to be required for delivery of cytosolic viral RNA to the lysosome where TLR7 triggering can take place. Similarily, autophagic proteins seem to facilitate the delivery of DNA to TLR9 containing compartments (Henault et al., 2012). However, this mechanism does not resemble conventional autophagy and will be further discussed under point 5 of this review.
The group of RLRs consists of three members, retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and LGP2. The latter is thought to play a rather regulatory role. RIG-I and MDA5 recognize different forms of viral RNA in the cytosol (Schlee, 2013). Both receptors interact with their common adaptor mitochondrial antiviral signaling (MAVS) via their caspase-recruiting domain (CARD) (Lee and Kim, 2007). MAVS is membrane bound and mostly found on mitochondria and peroxisomes. Downstream signaling leads to the activation of the transcription factors IRF3/7, NF-κB and ATF-2/c-Jun. In turn, these will switch on the transcription of type I IFNs and pro-inflammatory cytokines (Chiang, Davis and Gack, 2014). Two mechanisms have been proposed by which autophagy might influence RLR signaling. The ATG5/12/16L1 complex seems to interact with the CARDs of RIG-I and MAVS disrupting the signaling cascade and preventing the production of type I IFNs (Jounai et al., 2007). Alternatively, the role of basal autophagy in mitochondria homeostasis might prevent damaged mitochondria from accumulating and hence, keeps the level of MAVS signaling in check (Talet al., 2009). The latter finding is supported by the observation that measles virus hampers RLR signaling by actively triggering mitophagy which reduces the availability of MAVS (Xia et al., 2014).
A sensor of cytosolic dsDNA and cyclic nucleotides is stimulator of IFN genes (STING). STING activates the transcription factor IRF3 in endosomal compartments. IRF3 initiates the transcription of type I IFNs (Ishikawa and Barber, 2008). The activity of STING can be further enhanced when dsDNA is recognized by the sensor cGAMP synthase (cGAS). This enzyme synthetizes cyclic GMP-AMP (cGAMP)

upon detection of cytosolic DNA. The cyclic nucleotides further activate STING. The cytosolic DNA sensing pathway intersects with autophagy in different ways. STING has been implicated in upregulation of xenophagy in response to HSV-1 infection in bone marrow-derived dendritic cells (BM-DCs) (Rasmussen et al., 2011). In order to prevent excessive cGAS activity, a negative feedback loop exists. cGAS directly interacts with BECN1 leading to impairment of its nucleotidyl transferase activity. Less cGAMP available will dampen STING activity. Additionally, cGAS competes with RUBICON for BECN1 binding, thus releasing the PI3K complex from its inhibition and actively triggering autophagy. This is believed to aid in the degradation of cytosolic DNA and invading viruses (Liang et al., 2014). In a second negative feedback loop, cyclic nucleotides trigger the inactivation of AMPK, which therefore no longer represses ULK1. Active ULK1 phosphorylates STING leading to its degradation (Konno, Konno and Barber, 2013). Furthermore, STING was shown to rely on autophagic molecules like ATG9 for its trafficking (Saitoh et al., 2009). However, this process does not resemble conventional autophagy, but rather implies an unconventional usage of autophagy proteins (see point 5 of this review for more details).

Nod-like receptors comprise another class of cytosolic PRRs. This large group of proteins is characterized by their leucine-rich repeat (LRR) and NACHT domains. The NLRP subfamily contains a pyrin domain (PYD) in addition. The best studied member of this subfamily is NLRP3 which recognizes various ligands among which microbial PAMPs like viral RNA but also endogenous danger associated molecular patterns (DAMPs) like ROS. Similar to other NLRPs, NLRP3 can form inflammasomes, large protein complexes which contain an adaptor protein called ASC (apoptosis-associated speck-like protein containing a CARD). NLRP3 and ASC interact via their PYDs, while ASC interacts with pro-caspase 1 via their CARDs. The activation of the inflammasome requires two signals. Signal 1 is mediated by TLR, IL-1R or TNFR signaling and is thought to be provided constantly by commensal bacteria in the gut. Signal 1 triggers the expression of e.g. pro-IL1β and pro-IL18 as well as NLRP3 itself. Signal 2 is sensed by the NLRs and leads to autocatalysis of pro-caspase-1 cleavage into its active form followed by cleavage of pro-IL-1β and pro-IL-18 by active caspase-1. Mature IL-1β and IL-

18 are secreted and perform immune modulatory functions. Inflammasomes have been extensively reviewed by (Martinon, Mayor and Tschopp, 2009).

Autophagy regulates inflammasome activity to prevent possibly harmful overproduction of IL-1β or IL-18. A loss of autophagy leads to increased release of IL-1β in macrophages in response to the bacterial cell wall component LPS (Saitoh et al., 2008). ROS released from damaged mitochondria are another potent trigger of NLRP3 inflammasomes. Since autophagy plays an important role in mitochondria homeostasis, impairment of mitophagy was shown to be accompanied with increased IL-1β release (Nakahira et al., 2011;Zhou et al., 2011). Infection with influenza A virus (IAV) is a potent inducer of NLRP3 inflammasomes through sensing of viral ssRNA (Thomas et al., 2009), through proton channel function of matrix protein 2 (M2) in the *trans*-Golgi network (Ichinohe, Pang and Iwasaki, 2010) and through changes in mitochondrial membrane potential (Ichinohe et al., 2013). In addition, IAV is sensed by another member of the NLR family, NOD2. NOD2 activates receptor-interacting serine/threonine-protein kinase (RIPK) 2 which phosphorylates ULK1. Active ULK1 enhances the rate of mitophagy, thus preventing excessive inflammasome activation (Lupfer et al., 2013).

Another means by which autophagy regulates inflammasome activity is direct degradation. ASC becomes ubiquitinated after activation of NLRP3 and selective autophagy via p62 binding is initiated (Shi et al., 2012). Pro-IL-1β itself might also be subjected to autophagic degradation (Harris et al., 2011).

How IL-1β an IL-18 are secreted is still a matter of debate as they lack the leader peptide required for conventional secretion. Autophagy may play a role in their unconventional secretion (Dupont et al., 2011). This somehow contradicts the findings described above that propose a role of autophagy in restricting the amount of IL-1β released. One possible explanation is that under basal conditions autophagy limits IL-1β secretion, whereas induction of autophagy by e.g. starvation augments IL-1β secretion (Dupontet al., 2011). In a reconstituted system it was shown that IL-1β can be incorporated into the intermembrane space between inner and outer autophagosomal membrane (Zhang et al.,

2015). Hence, IL-1β might use the autophagosome as a shuttle for its secretion, the exact mechanism of which remains to be elucidated.

Taken together PRRs play a potent role in a first line of defense against viral infection by production of antiviral factors and immune modulating cytokines. Autophagy plays a strong regulatory role in these innate immune responses preventing excessive activation. Not surprisingly, some viruses have developed means to manipulate the autophagic machinery to prevent innate immune activation via PRR signaling and initiation of xenophagy. This aspect of host/virus crosstalk will be discussed under point 4 of this review.

2.2 Xenophagy – selective degradation of pathogens

Virus components can also be directly recognized and subjected to autophagic degradation (Table 1). One large family of genes with more than 100 members in humans is the tripartite motif (TRIM) protein family. One member, TRIM5α, acts as an anti-retroviral factor. It has been proposed that it restricts retroviral replication by recognizing capsids directly through its SPRY domain. Furthermore, TRIM5α binds BECN1 competing with negative regulators of BECN1 and hence, activates autophagy (Mandell et al., 2014). Recently, this view has been challenged, solely proposing a role for autophagy in turnover of basal TRIM5α levels, whereas autophagy deficiency did not impair retroviral restriction (Imam et al., 2016). The anti-retroviral activity of TRIM5α might also be proteasome mediated (Wu et al., 2006) or associated with its ability to increase innate immune signaling (Lascano et al., 2015). Recently, TRIM20 and 21 were shown to be involved in the autophagic degradation of inflammasome components and IRF3, respectively. This might represent yet another immune modulatory role for autophagy in response to viral and other infections (Kimuraet al., 2015).

Autophagy was also shown to limit the spread of Sindbis virus (Liang et al., 1998). A genome wide study has revealed a role for the E3 ligase SMURF1 in survival after Sindbis virus infection (Orvedahl et al., 2011). SMURF1 possibly ubiquitinates the capsid of Sindbis virus leading to recognition by p62,

as p62 was shown to recruit capsids to autophagosomes (Orvedahlet al., 2010). However, the exact mechanism remains unknown.

Lastly, in murine cells autophagic degradation of Chikungunya virus is mediated by p62 (Joubert et al., 2012), whereas autophagy seems to play a pro-viral role in human cells infected by Chikungunya possible due to some adaptation of the virus to its human host (Judith et al., 2013;Krejbich-Trotot et al., 2011). Xenophagy not only rids the cell of invading virus particles it may also deliver peptides for antigen presentation, which will boost the adaptive immune response, as discussed in the following paragraph.

In summary, autophagy plays a dual role in antiviral innate immune responses. Xenophagy triggered by various PRR signaling pathways keeps viral titers at bay, while selective autophagy degrades crucial components of the innate immunity signaling cascade to prevent excessive, possibly harmful immune activation.

3. Adaptive anti-viral immune responses mediated by autophagy

3.1. T cell monitoring of autophagic degradation products

In addition to autophagy's role in the early phase of viral infections, which are dominated by innate immune recognition as well as restriction of the infecting pathogen, autophagy also contributes to visualizing viruses to the adaptive immune system and sustaining adaptive lymphocyte differentiation and functions.

T cells detect peptides presented on major histocompatibility complex (MHC) molecules. Two main T cell lineages exist, namely cytotoxic $CD8^+$ T cells and helper $CD4^+$ T cells, which orchestrate adaptive immune responses (Kanno et al., 2012). $CD8^+$ T cells recognize octa- to nonameric peptides on MHC class I molecules, and $CD4^+$ T cells longer peptides with a nonameric core sequence that binds to MHC class II molecules. MHC class I molecules are primarily loaded with products of the cytosolic and nuclear multicatalytic protease complex, the proteasome, while MHC class II molecules primarily present peptides that are generated by lysosomal proteolysis (Trombetta and Mellman,

2005). Therefore, autophagy is expected to deliver antigens for MHC class II restricted antigen presentation, but it has become clear in recent years that it also affects MHC class I restricted antigen presentation (Figure 2B).

Consistent with a role of autophagy in targeting cytosolic proteins for MHC class II restricted antigen presentation, it was noted early on that peptides eluted from MHC class II molecules originate to 20-30% from nuclear and cytosolic source proteins (Chicz et al., 1993;Dengjel et al., 2005). This includes fragments of LC3, GABARAP and GABARAPL2 (Dengjelet al., 2005;Suri et al., 2008). Moreover, upon autophagy up-regulating starvation, MHC class II presentation of these cytosolic proteins increased by 50%, while membrane protein presentation remained unchanged (Dengjelet al., 2005). These findings strongly support a role of autophagy in delivering antigens to late endosomal compartments, namely MHC class II containing compartments (MIICs), in which lysosomal proteolysis generates ligands to be loaded onto MHC class II molecules. Indeed autophagosomes fuse frequently with MIICs in human B cells, dendritic cells and epithelial cell lines, as well as in mouse thymic epithelial cells (Kasai et al., 2009;Schmid, Pypaert and Münz, 2007). Furthermore, fusing proteins to the N-terminus of LC3B enhances MHC class II presentation of viral and tumor antigens up to 20-fold (Comber et al., 2011;Fonteneau et al., 2016;Jin et al., 2014;Schmid, Pypaert and Münz, 2007). Thus, MHC class II molecules present peptides of autophagic substrates, but does this also apply to viral antigens?

Indeed, it was found that the nuclear antigen 1 of Epstein Barr virus (EBNA1) is intracellularly processed by autophagy for MHC class II presentation (Münz et al., 2000;Paludan et al., 2005). This viral nuclear antigen limits its MHC class I presentation via its glycine-alanine (Gly-Ala) repeat, which inhibits its translation and blocks proteasomal degradation (Levitskaya et al., 1995;Yin, Manoury and Fahraeus, 2003). This inability to be degraded by proteasomes presumably makes EBNA1 a substrate of autophagy. Primarily cytosolic EBNA1 gets incorporated into autophagosomes, either directly after translation or during cell division when nuclear compartmentalization of EBNA1 is compromised (Leung et al., 2010). Mutation of the nuclear localization sequence of EBNA1 increases its

presentation on MHC class II molecules to specific CD4$^+$ T cell clones (Leunget al., 2010). Similarly, MHC class II presentation of ovalbumin after infection with ovalbumin transgenic herpes simplex virus (HSV) is sensitive to the virus' ability to inhibit autophagy via its BECN1 interacting ICP34.5 protein (Gobeil and Leib, 2012). Mutation of the Beclin-binding domain (BBD) of ICP34.5 increases MHC class II presentation of HSV encoded antigen. Along the same lines, autophagy (ATG5) deficiency in DCs, the initiators of most anti-viral adaptive immune responses, compromised CD4$^+$ T cell priming upon HSV infection in mice (Jiang et al., 2015;Leeet al., 2010). Accordingly, some of HSV-induced pathogenesis, primarily virus driven stromal keratitis, is attenuated in mice with autophagy deficient DCs (Jianget al., 2015). Thus, some viral proteins are presented on MHC class II molecules after autophagy.

However, in addition to MHC class II presentation, autophagy substrates can also contribute to MHC class I presentation to CD8$^+$ T cells, particularly under conditions of inhibition of the conventional MHC class I antigen processing pathway involving proteasomes and ER import via the transporter associated with antigen processing (TAP). It was noted that late during HSV infection *in vitro*, MHC class I antigen presentation of the viral glycoprotein B (gB) requires autophagy and lysosomal acidification (Englishet al., 2009). Interestingly, IFN-γ, but not IL-1β or heat shock exposure of the antigen presenting cells could overcome the requirement for autophagy in late HSV gB presentation on MHC class I molecules. Viral antigen processing for a second herpesvirus, human cytomegalovirus (HCMV), was also shown to require autophagy (Tey and Khanna, 2012). HCMV pUL138 presentation on MHC class I molecules by TAP deficient and HCMV infected cells required autophagy, and was compromised by pharmacological VPS34 inhibition or RNA silencing of *atg12* expression. Thus, intracellular viral antigen presentation on MHC class I molecules requires autophagy under conditions that viral immunoevasins block conventional MHC class I antigen processing via proteasomes and TAP.

A third pathway, by which autophagy supports antigen processing for MHC presentation is release of autophagic cargo for cross-presentation by neighbouring DCs on MHC class I molecules.

IAV infected mouse embryonic fibroblasts (MEFs) provided antigen more efficiently to human monocyte-derived DCs to stimulate IAV-specific $CD8^+$ T cells if apoptosis was compromised in the antigen donor cells (Uhl et al., 2009). Moreover, these IAV infected MEFs with deficient apoptosis also cross-primed IAV specific $CD8^+$ T cell responses more efficiently after injection into mice. Inhibition of autophagy by ATG5 down-regulation compromised this cross-presentation ability of apoptosis deficient MEFs. Indeed, vesicular release of ubiquitinated proteins, which serve as substrates for both proteasomes and autophagy, can be augmented by lysosomal and proteasomal inhibition (Li et al., 2011b). Thereby, proteasomal inhibition promotes the recruitment of ubiquitinated protein import into autophagosomes via p62 binding (Twitty et al., 2011). Autophagy competent cells indeed release vesicles that contain LC3 and ATG16L1 (Pallet et al., 2013), suggesting that under conditions inhibiting lysosomal degradation of autophagosomes, the inner autophagosomal membrane and its contents could be released into the supernatant. Vesicular fractions, whose secretion depended on macroautophagy, were found to be more efficient in delivering viral antigens, including EBV, HCMV and influenza proteins, to human monocytes and peripheral blood mononuclear cells for cross-presentation to specific $CD8^+$ T cells (Ye et al., 2014). Therefore, macroautophagy might package antigens for release and cross-presentation by neighbouring DCs.

Autophagy proteins seem to support antigen processing for MHC class II presentation and, in case of TAP inhibition, also MHC class I presentation. In addition they support cross-presentation on MHC class I molecules.

3.2. Lymphocyte differentiation and function requires autophagy

In addition to regulating antigen presentation, autophagy also influences the development of the adaptive immune system. Absence of ATG7 from the hematopoietic lineage abolishes development of T and B cells (Mortensen et al., 2011). Lymphocytes seem to be particularly sensitive to the accumulation of damaged mitochondria and the associated ROS production. Accordingly, the number

of thymic T cell precursors is severely compromised if they are deficient in ATG5 or BECN1 (Arsov et al., 2011;Pua et al., 2007). In the transition of T cells from the thymus to the periphery, T cells were found to significantly reduce their mitochondrial mass and could not do so in the absence of ATG7, hence accumulating ROS (Pua et al., 2009). For B cells a more subtle effect during development was noted. Particularly the more innate B1 cells were compromised in their development and maintenance by ATG5 deficiency in the B cell lineage (Miller et al., 2008). Thus, lymphocytes are sensitive to loss of mitophagy.

A second step during T cell differentiation, at which autophagy is required, is thymic selection. Thymic epithelial cells present self-proteins on MHC molecules in order to select for thymocytes that can interact with MHC molecules by low avidity interactions and to delete autoreactive thymocytes that too strongly interact with self (Kyewski and Klein, 2006). These have been found to be loaded in part via autophagy (Aichinger et al., 2013;Nedjic et al., 2008). Particularly, mitochondrial proteins and those that were targeted to autophagosomes by fusion to LC3B could delete T cell specificities from the thymocyte pool efficiently already at low transgene concentrations. Thus, autophagy supports thymocyte development by supporting MHC class II restricted self-protein presentation on thymic epithelial cells.

Finally, terminal differentiation of lymphocytes and the function of these mature lymphocyte populations also depend on autophagy. While the expansion of $CD8^+$ T cells in response to LCMV infection was not altered without ATG5 and ATG7, the primed memory T cells survived less well and thereby memory T cell responses were diminished (Xu et al., 2014). Similarly, after influenza A and murine cytomegalovirus (MCMV) infection memory $CD8^+$ T cells were less well maintained, if they lacked ATG7 (Puleston et al., 2014). Interestingly, the decreased levels of autophagy in aged $CD8^+$ T cells could be partially corrected by autophagy induction with spermidine, which improved memory responses by these T cells. ROS accumulation was again found in ATG5 deficient memory $CD8^+$ T cells after IAV infection and memory $CD8^+$ T cell responses could be partially rescued by ROS reduction *in vivo* (Schlie et al., 2015). Apart from T cells also B cells depend strongly on autophagy in their

terminal differentiation stages. Memory B cells that are established after IAV infection have been reported to depend on ATG7 in their maintenance (Chen et al., 2014). The second terminal differentiation stage of B cells for long-lived antibody secretion is plasma cells. Their numbers were found to be diminished after loss of ATG5 from the B cell lineage (Conway et al., 2013). In the absence of ATG5 in B cells their endoplasmic reticulum was expanded and an up-regulation of the unfolded protein response could be detected (Pengo et al., 2013). This is consistent with a role of autophagy in reducing expanded endoplasmic reticulum compartments (Bernales, McDonald and Walter, 2006;Khaminetset al., 2015). Thus, maintenance of lymphocyte memory, both at the level of memory lymphocyte populations and long-lived effector cells like plasma cells depends on autophagy. Particularly mitochondrial and endoplasmic reticulum turnover by autophagy seems to be important for these functions.

4. Autophagy manipulation by viruses

4.1. Inhibition of autophagosome formation by DNA viruses

Due to these important functions of autophagy in innate and adaptive immunity to virus infections, it is not surprising that viral pathogens manipulate this pathway for immune escape. In addition, they also often use the resulting membrane remodeling for their own benefit during replication (Figure 3). At two checkpoints of autophagy, viruses have been reported to interfere with this pathway. These are early during autophagosome formation and at the stage of autophagosome fusion with late endosomes or lysosomes (Münz, 2009).

Interestingly, especially herpesviruses are a rich source of autophagy inhibitors of the early checkpoint. The α-herpesvirus HSV encodes ICP34.5, which binds with its N-terminal domain to BECN1 to inhibit autophagosome formation (Orvedahl et al., 2007;Talloczy et al., 2002). In the absence of autophagy inhibition by ICP34.5, neurovirulence of HSV is attenuated in mice, while replication *in vitro* is not affected (Alexander et al., 2007;Orvedahlet al., 2007). ICP34.5 mediated inhibition of autophagy, however, promotes only HSV encephalitis in the adult, but not the newborn

brain (Wilcox et al., 2015). Interestingly, ICP34.5's binding to BECN1 might not exclusively affect autophagosome generation, but due to the additional involvement of BECN1 protein complexes in autophagosome maturation, might preferentially affect this later step of autophagy. In contrast to its inhibition of autophagosome formation in mouse neurons, ICP34.5 seems to arrest autophagosomes prior to lysosome fusion in DCs and neuroblastoma cells (Gobeil and Leib, 2012;Santana et al., 2012). Furthermore, the γ-herpesviruses Kaposi Sarcoma associated herpesvirus (KSHV) and murine γ-herpesvirus 68 (MHV-68) also encode viral Bcl-2 proteins acting as BECN1 interactors (Ku et al., 2008;Pattingreet al., 2005). This inhibition of autophagy supports chronic infection with MHV-68 (E et al., 2009). The β-herpesvirus HCMV also inhibits autophagy (Chaumorcel et al., 2012;Mouna et al., 2015). Its TRS1 and IRS1 proteins interact with BECN1 to inhibit autophagosome formation. HSV also encodes a second protein which inhibits autophagy, US11 (Lussignol et al., 2013). However, in contrast to ICP34.5, US11 does not interact with BECN1, but requires its dsRNA-dependent protein kinase PKR binding domain to block autophagy. Furthermore, KSHV also targets autophagy in a BECN1 independent manner. The K7 protein of the virus blocks autophagosome maturation and fusion with lysosomes (Liang et al., 2013). K7 achieves this block by interaction with RUBICON, the negative regulator of the UVRAG/BECN1 complex that modulates lysosomal fusion with autophagosomes. A third KHSV mechanism to modulate autophagy is targeting ATG3 and thereby inhibiting its function in LC3 conjugation to autophagosomal membranes (Lee et al., 2009). Viral FLICE-like inhibitor protein (vFLIP) binds to the LC3 conjugating enzyme ATG3 and blocks autophagosome formation. Also the vFLIP molecules of the γ-herpesvirus herpesvirus saimiri (HVS) and the poxvirus Molluscum contagiosum virus (MCV) were able to fulfill ATG3 mediated inhibition of autophagosome formation. Among the three KSHV encoded autophagy inhibitors (vBCL2, K7 and vFLIP), vBCL2 was required for lytic replication of KSHV, but this function did not require the ability of vBCL2 to inhibit autophagy (Gelgor et al., 2015;Liang et al., 2015). Nevertheless, these studies suggest that most herpesviruses, as a prominent group of DNA viruses, block autophagy either to

prevent their intracellular degradation during infection or to compromise autophagic antigen processing for MHC presentation. This at least applies to α-, β- and γ2-herpesviruses.

The notable exception among herpesviruses is the γ1-herpesvirus Epstein Barr virus (EBV), which seems to benefit from autophagy. The latent membrane protein 1 (LMP1) of EBV was shown to stimulate autophagic flux and thereby regulate its own degradation (Lee and Sugden, 2008). In the absence of autophagic degradation of LMP1, the overexpression of this oncogene compromises EBV transformed B cell growth. Also LMP2 was recently shown to induce autophagy, preventing cell death (Fotheringham and Raab-Traub, 2015). Autophagy up-regulation seems to ensure the survival of infected B cells during virus induced transformation to lymphoblastoid cell lines and counteract p53 induced apoptosis (McFadden et al., 2016;Pujals et al., 2015). In addition, EBV seems to also utilize autophagic membranes for its lytic replication (Granato et al., 2014;Nowag et al., 2014). This aspect of EBV's interaction with the autophagic machinery will be discussed in more detail under point 6. Thus, except for EBV, most herpesviruses compromise autophagy to escape innate and adaptive immune restriction.

4.2. Compromised autophagosome maturation by RNA viruses

In contrast to DNA viruses, which in their majority inhibit autophagic membrane formation, RNA viruses often induce the accumulation of autophagic membranes, irrespective of their replication in the cytosol or the nucleus. These membranes are often used as scaffolds for the viral replication complexes or support virus particle release. Indeed, the first virus, for which such an accumulation of double membrane vesicles was described is the picornavirus poliovirus (Dales et al., 1965). Poliovirus proteins 2BC and 3A the accumulation of double membrane vesicles and the virus is thought to replicate at these membranes (Jackson et al., 2005). These structures stain positive for LC3 and the lysosome associated membrane protein 1 (LAMP1). They are also partially acidified, because they can be visualized with monodansylcadaverine, which stains multilamellar acidified vesicles. RNA silencing of LC3 and ATG12 prevents the formation of these vesicles and decreases infectious Poliovirus

release. The LC3 positive double membrane vesicles in poliovirus infected cells do not seem to support viral genome replication, but support maturation of virus particles and release (Richards and Jackson, 2012). Indeed the non-lytic release of poliovirus was found to depend in part on the autophagic machinery with less viral spreading in cell cultures after LC3 silencing (Bird et al., 2014). *Vice versa* autophagy stimulation with the mTOR inhibitor rapamycin increased viral spreading. Similar findings seem to also apply to other picornaviruses. Infection with the rhinoviruses 2 and 14 leads to the accumulation of LC3 positive vesicles (Jacksonet al., 2005). Moreover, the proteins 2B, 2C and 3A of the food-and-mouth disease virus also associate with LC3 positive structures (O'Donnell et al., 2011). RNA silencing or pharmacological inhibition of the autophagic machinery decreased while mTOR inhibition increased infectious virus production. Finally, coxsackievirus B can be found in LC3 positive extracellular vesicles that often contain multiple virus particles surrounded by one LC3-PE containing membrane (Robinson et al., 2014). Thus, picornaviruses use mature autophagic vesicles to exit cells and spread in a non-lytic fashion to neighboring cells. Even so they are non-enveloped viruses, they use LC3-PE positive vesicles with often multiple virus particles inside for this spreading.

Another group of viruses that seem to be influenced by and regulate autophagy are alphaviruses. While Sindbis virus is mainly restricted in its infection by autophagy via p62 mediated import into autophagosomes, as mentioned above (Orvedahlet al., 2010;Orvedahlet al., 2011), Semliki Forest virus merely arrests autophagosome maturation (Eng et al., 2012). This arrest depends on the viral glycoprotein spike complex. However, inhibition or stimulation of autophagy does not change viral replication in culture. Finally, the alphavirus Chikungunya virus is at the same time restricted and uses part of the autophagic machinery (Judithet al., 2013). On one hand, p62 targets viral particles for autophagic degradation via binding to LC3B. On the other hand, human NDP52 binds to viral non-structural protein 2 (nsP2), on which the viral replication machinery assembles. Thereby, NDP52 enriches the viral replication complex at the *trans*-Golgi-network via its LC3C binding. In human cells, the net outcome of these interactions is that the core autophagic machinery

that allows LC3C lipidation supports Chikungunya virus replication. In mouse cells, however, autophagy mainly restricts Chikungunya virus replication. Thus different modules of the autophagic machinery play restrictive and supportive roles during alphavirus infections.

The pro-replicative role of the autophagic machinery prevails during flavivirus infections. Hepatitis C virus (HCV) requires ATGs 4, 5, 6 and 12 for efficient translation of the replication complex of the virus (Dreux et al., 2009). Moreover, viral replication seems to proceed on LC3 positive membranes (Sir et al., 2012). Inhibition of autophagosomal membrane formation inhibits HCV replication. The formation of these double membranes, on which HCV replicates, is also dependent on early autophagy proteins like Vps34 and DFCP1, which has led to the proposition that HCV actually replicates on omegasome structures (Mohl et al., 2016). Finally, even release of HCV was suggested to depend on autophagy (Shrivastava et al., 2015). The required inhibition of autophagic membrane degradation for HCV replication on these membranes and possibly for their support of exosomal viral release, seems to be mediated by RUBICON up-regulation (Wang, Tian and Ou, 2015). The non-structural protein NS4B seems to be sufficient to stimulate this RUBICON up-regulation. With Dengue virus another flavivirus also blocks autophagic membrane degradation and p62 overexpression leads to restriction of virus replication (Metz et al., 2015). Thus, autophagic membranes and their precursors, like the omegasomes, seem to primarily support flavivirus replication. The block that stabilizes these membranes might at the same time prevent virus degradation via autophagy.

Apart from the RNA virus families that replicate in the cytosol, regulation of autophagy has also been described for RNA viruses that replicate in the nucleus. One of the most prominent is the human immune deficiency virus (HIV). HIV-1's viral infectivity factor (Vif) binds directly to LC3 (Borel et al., 2015). This interaction inhibits autophagosome maturation. However, the virus encodes another inhibitor of autophagosome maturation. HIV's negative regulatory factor (Nef) also stabilizes autophagic membranes (Kyei et al., 2009). Preventing their degradation leads to efficient virus replication in macrophages. Nef seems to mediate this function through BECN1 binding (Kyeiet al., 2009). This interaction was proposed to activate mTOR, which phosphorylates transcription factor EB

(TFEB), retaining TFEB in the cytosol (Campbell et al., 2015). Nuclear TFEB augments lysosomal function via its transcription of lysosomal content and inhibition of this transcription attenuates autophagosome degradation. In addition to these effects on autophagy in the infected cell, HIV also induces autophagy *in trans* via binding of its envelope protein to CXCR4 (Espert et al., 2006). This autophagy induction in bystander $CD4^+$ T cells contributes to their cell death. Finally, as above, HIV seems to also benefit from inhibiting autophagosome degradation in order to counteract degradation of viral components that are essential for replication. Along these lines HIV's transactivator Tat is recruited via direct p62 binding to autophagosomes for degradation (Sagnier et al., 2015). Thus, HIV inhibits autophagosome maturation via Vif and Nef for more efficient replication in macrophages, but is restricted by autophagy in T cells.

As another example of a RNA virus that replicates in the nucleus, the segmented RNA virus IAV also inhibits autophagosome maturation (Gannage et al., 2009). M2 of IAV blocks autophagosome degradation. This inhibition of autophagy leads to increased apoptotic cell death of infected cells. M2 also contains a LIR, which redirects LC3 to the cell membrane through which IAV buds (Beale et al., 2014). This M2 mediated redistribution of LC3 coupled membranes to the cell membrane allows filamentous budding of IAV, which seems to increase virus stability. M2 seems to require its proton channel activity to accumulate autophagic membranes in infected cells, which are then redistributed to the cell surface (Ren et al., 2015). Thus, IAV manipulates cell death of its host cells and redistributes LC3 bound membranes to its budding sites after inhibiting autophagosome maturation via M2. Finally, parainfluenza virus also blocks autophagosome degradation by preventing their fusion with lysosomes (Ding et al., 2014). The viral phosphoprotein binds to SNAP29 to block STX17 mediated autophagosome fusion with lysosomes. The resulting autophagosome accumulation support viral replication. Thus influenza and parainfluenza virus block autophagosome fusion with lysosomes, but how this in detail affects viral replication, still needs to be mechanistically better understood.

5. Unconventional use of autophagy proteins

Besides their role in orchestrating an important catabolic pathway, autophagic proteins may also take on alternative roles in cellular and anti-viral functions. As mentioned earlier, STING is an important sensor of cytosolic dsDNA of viral origin. While being an ER-resident protein under steady-state conditions, STING's activation coincides with its translocation to the Golgi and further localization at cytoplasmic punctuate structures where it interacts with TANK-binding kinase 1 (TBK1). These structures are also positive for the autophagic adaptor protein p62 and LC3, but they are not conventional autophagosomes as electron microscopy has revealed a single limiting membrane. Furthermore, these structures contain ATG9, the transmembrane autophagy protein required for membrane shuttling to the growing phagophore. In case of STING signaling, ATG9 seems to play an immune regulatory role, as its absence leads to enhanced translocation of STING from the Golgi to LC3 positive punctae, stronger assembly with TBK1 followed by phosphorylation of the transcription factor IRF3 and overproduction of type I IFNs (Figure 4A). This however, cannot be explained by aberrant conventional autophagy, since the loss of ATG7 and hence block of autophagosome formation does not alter STING signaling (Saitohet al., 2009).

Autophagic proteins seem to also aid another PRR, namely TLR9, by delivering DNA-based PAMPs to TLR9 containing compartments. This was shown for large DNA/auto-antibody immune complexes which bind Fc receptors and are taken up by pDCs via phagocytosis. Some proteins of the autophagic machinery are recruited to these phagosomes in a process that does not resemble conventional autophagy as the resulting structure is surrounded by a single membrane only (Henaultet al., 2012). This process has been described earlier and was termed LC3-associated phagocytosis (LAP) (Sanjuan et al., 2007) (Figure 4B). Recently, mechanistic details have been revealed highlighting which autophagic proteins are required for LAP (Martinez et al., 2015). Upon phagocytosis of pathogens or beads coupled with TLR2 ligands the PI3K complex containing RUBICON, but not ATG14 or AMBRA, is recruited to the phagosome in murine macrophages. The resulting PI(3)P production recruits the NADPH oxidase (NOX2) complex, which produces ROS. Together with PI(3)P, ROS are the signal for

recruitment of downstream autophagy effectors that facilitate the conjugation of LC3 to the phagosomal membrane. LAP proceeds independently of the pre-initiation ULK complex (Florey et al., 2011;Henaultet al., 2012;Martinez et al., 2011). LAP seems to enhance the rate of phagosome/lysosome fusion, thus ensuring efficient elimination of potential harmful pathogens (Martinezet al., 2011;Sanjuanet al., 2007). However, recent evidence in human macrophages and DC proposes a rather stabilizing role for LAP, enabling convergence with TLR containing endosomes (Henaultet al., 2012) or improved antigen delivery to MHC class II positive compartments for presentation to T cells (Romao et al., 2013). In addition to TLR2/4/6, Dectin-1 (Ma et al., 2012) and Fc receptors, also dying cells may trigger LAP via recognition of PE exposed at the cell surface by T cell immunoglobulin mucin protein 4 (TIM4) on the macrophage (Martinezet al., 2011). Influenza infected cells expose PE on their cell surface and can be taken up via phagocytosis (Shiratsuchi et al., 2000). A phagocytosis-like uptake mechanism has also been reported for HSV-1 (Clement et al., 2006). However, a direct role of LAP in virus elimination or in antiviral immune responses has not yet been demonstrated.

The complex formed by ATG5, ATG12 and ATG16L1, which acts as an E3-like enzyme in the conventional autophagy pathway (Figure 1), can also influence RLR-mediated signaling (Takeshita et al., 2008). ATG5/12/16L1 is able to interfere directly with CARD-mediated binding of RIG-I and the adaptor MAVS (Jounaiet al., 2007). The result of this interference is a reduction in type I IFN expression (Figure 2A). Furthermore, the ATG5/12/16L1 complex was shown to block the replication of norovirus. When type I IFN signaling is abrogated artificially or due to viral evasion strategies, IFNγ takes over and acts anti-virally preventing lethal infection. Loss of the ATG5/12/16L1 complex, but not other members of the conventional LC3 conjugation machinery, led to loss of the protective effect of IFNγ (Hwang et al., 2012).

A rather unusual role for autophagy is unconventional protein secretion as described for IL-1β under point 2 of this review (Figure 2A). Here, conventional autophagosomes seem to harbour leaderless proteins like IL-1β in the intermembrane space (Zhanget al., 2015). This mode of secretion seems to

also facilitate the release of endogenous DAMPs like high-motility group protein B1 (HMGB1), which might affect the immune response (Dupontet al., 2011), and acyl coenzyme A-binding protein (ACBP) in yeast (Duran et al., 2010;Manjithaya et al., 2010).

Lastly, autophagic proteins might also perform unconventional roles directly interfering with the viral lifecycle. UVRAG, a component of the PI3K complex, in which it binds BECN1, might alternatively bind to the C vacuolar protein sorting (C-VPS) tethering complex. In combination with C-VPS, UVRAG facilitates both autophagosome/lysosome and endosome/lysosome fusion (Lianget al., 2008). Recently, it was shown that this BECN1-independent function of UVRAG can also facilitate viral entry of VSV and IAV (Pirooz et al., 2014b). Upon infection, the pattern of UVRAG/C-VPS mediated SNARE pairing changes, favouring fusion of virus-containing endosomes with late endosomes rather than destructive lysosomes (Pirooz et al., 2014a). This poses a novel role for UVRAG in viral entry and evasion of lysosomal degradation irrespective of its role in conventional autophagy (Figure 4C).

6. Role of autophagy in virus particle release

A special case of unconventional use of the autophagy proteins is the export of viral particles from infected cells via autophagic membranes. This was first suspected for the picornavirus poliovirus and the term **A**utophagic exit **W**ith **O**ut **L**ysis (AWOL) was coined (Jacksonet al., 2005) (Figure 3). Apparently the release of poliovirus occurs from LC3 positive vesicular structures and the virus matures upon their acidification (Richards and Jackson, 2012). Indeed, release of secretory lysosomes by osteoclasts has previously been demonstrated to depend on the autophagic machinery (DeSelm et al., 2011). Since poliovirus release from autophagosomes or multivesicular amphisomes would result in viral particle release inside the inner autophagosomal membrane, this could protect the non-enveloped virus particles from the extracellular milieu and explain the more efficient spreading in cell culture from autophagy competent cells (Birdet al., 2014). However, such structures have so far only been observed for the closely related coxsackievirus B (Robinsonet al., 2014). Coxsackievirus particles have been found in extracellular vesicles, whose membrane is decorated with lipidated LC3.

Interestingly multiple viral particles were observed in individual vesicles by electron microscopy. These findings suggest that picornaviruses can be shed as packages in vesicles surrounded by the inner autophagosomal membrane. How these viruses get exposed to acidification within this inner autophagosomal membrane for their maturation, however, requires further investigation.

For enveloped viruses, especially those that acquire their membrane in the cytosol and not at the cell membrane, the autophagic machinery might provide the necessary means for envelope acquisition. Indeed, the γ-herpesvirus EBV stabilizes autophagic membranes upon reactivation from latency into lytic virus production (Granatoet al., 2014;Nowaget al., 2014). The production of these autophagic membranes is further improved by the up-regulation of LC3A, LC3B and ATG9B transcription by the immediate early lytic transcription factor Rta of EBV (Hung et al., 2014). Inhibition of autophagic membrane production decreases the production of infectious virus particles (Granatoet al., 2014;Nowaget al., 2014), but accumulated viral DNA and lytic EBV proteins in the cytosol (De Leo et al., 2015;Nowaget al., 2014). Moreover, viral DNA release was altered upon autophagy inhibition, elevated in some, but reduced in other studies (De Leoet al., 2015;Hunget al., 2014). The accumulation of constituents of EBV particles in the cytosol and increased DNA release, possibly after cell death due to autophagy inhibition, is consistent with a loss of efficient infectious virus packaging in the cytosol. In good agreement, lipidated LC3B was found in purified virus particles and LC3 could be visualized by immune electron microscopy on these virus particles (Nowaget al., 2014). Thus EBV might up-regulate autophagic membrane formation during lytic replication and block their degradation in order to use this membrane source during envelope acquisition in the cytosol. Its envelope seems to contain at least in part inner LC3 coupled autophagosomal membranes.

The two hepatitis viruses B and C also utilize autophagic membranes during viral particle release, even so they belong to completely different virus families, one being a DNA and the other a RNA virus. HCV uses the autophagic machinery to extensively remodel cytoplasmic membranes of its host cell and associates with the exosomal maker CD63 during virus release (Shrivastavaet al., 2015).

Inhibition of autophagy decreases total and exosome contained HCV RNA in the supernatant of replicating cells, and accumulates viral particles intracellularly in exosome like structures. Thus, HCV seems to be in part released in exosomes that originate from multivesicular bodies and require the autophagic machinery for their release. Indeed, ATG16L1 and LC3 have been found in vesicular fractions containing exosomes of autophagy competent cells (Palletet al., 2013). Similarly, hepatitis B virus (HBV) shedding into the supernatant of producer cells is diminished upon autophagy inhibition (Li et al., 2011a). As for HCV, HBV viral capsids get trapped within cells, suggesting a deficiency in cytosolic envelope acquisition in the absence of autophagy. However, the vesicular compartment by which HBV gets secreted is less well defined. However, multivesicular bodies (MVB) and exosomes might also play a role for HBV release.

In these MVBs the HIV is also thought to acquire some of its envelope in myeloid cells, while HIV buds through the cell membrane in T cells (Carter and Ehrlich, 2008;Jouvenet et al., 2006). Autophagy might participate in this viral budding into MVBs and exosome-like release of HIV. Indeed, it has been found that HIV blocks autophagosome maturation via its Nef protein (Kyeiet al., 2009). HIV's group-specific antigen (Gag) colocalizes with LC3 on these stabilized autophagic membranes. Pharmacological or siRNA mediated inhibition of their formation by RNA silencing of ATG6 or 7 decreases viral shedding into the supernatant of infected myeloid cells. These data suggest that autophagic membranes are required and interact with viral particles during release.

However, for HBV, HCV and HIV autophagic membranes in the viral envelope have not been described. Apart from EBV the only other virus for which an envelope of autophagic membranes has been found is the double stranded DNA coccolithovirus that infects the phytoplankton Emiliania huxleyi (Schatz et al., 2014). Autophagic membrane formation is up-regulated during viral replication and its inhibition blocks infectious virus shedding. Phytoplankton ATG8 was found in purified virus particles and ATG8 could be localized to the membrane of these large viruses. These findings suggest that coccolithoviruses might induce autophagic membrane formation for enveloping of these giant viruses that contain 400kb DNA genomes.

While for all the above discussed viruses autophagic membranes might directly contribute to either transport vesicles or the viral envelope, IAV seems to interfere with autophagosome degradation for a different purpose. IAV infection leads to the accumulation of autophagic LC3 positive membranes (Bealeet al., 2014;Gannageet al., 2009;Renet al., 2015). This membrane stock is then redirected to the plasma membrane by M2 and its direct binding to LC3 via its LIR motif (Bealeet al., 2014). Excess membrane that is delivered to the cell surface allows filamentous budding of IAV. Surprisingly, LC3 is not incorporated into the budding virus particles. However, in the absence of autophagic membrane formation and their LC3 mediated recruitment to the plasma membrane the produced virus particles are less robust and loose infectivity more rapidly than virus that was produced by autophagy competent cells. Thus, IAV uses the membrane remodeling functions of autophagy to provide more membrane to the cell surface, but how the lipids that are transported with LC3 get incorporated into viral particles, while LC3 is excluded, and how these lipids might render IAV more resilient to environmental stresses remains unclear.

7. Conclusions and future directions

Autophagy as one of the main catabolic pathways of eukaryotic cells interacts with virus infection and its immune control at multiple levels. It now has become apparent that innate as well as adaptive immune responses to virus infections are influenced by autophagy. Moreover, viruses manipulate autophagy for their immune escape, replication and release from infected cells, including recruitment of autophagic membranes to their envelopes. Recent years, however, have also provided evidence that not only canonical autophagy, but also other pathways that utilize just some modules of the molecular machinery of autophagy, influence virus infections and anti-viral immune responses. Therefore, the challenge for the future is to dissect, especially for the in vivo phenotypes of ATG deficiency, which of these are caused by canonical autophagy versus unconventional functions of ATGs. Only with a detailed understanding of this distinction, autophagy regulation can be explored for its anti-viral functions.

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References

Abdel Fattah, E., Bhattacharya, A., Herron, A., Safdar, Z., and Eissa, N. T. (2015). Critical role for IL-18 in spontaneous lung inflammation caused by autophagy deficiency. J Immunol 194, 5407.

Aichinger, M., Wu, C., Nedjic, J., and Klein, L. (2013). Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. J Exp Med 210, 287.

Alexander, D. E., Ward, S. L., Mizushima, N., Levine, B., and Leib, D. A. (2007). Analysis of the role of autophagy in replication of herpes simplex virus in cell culture. J Virol 81, 12128.

Arsov, I., Adebayo, A., Kucerova-Levisohn, M., Haye, J., MacNeil, M., Papavasiliou, F. N., Yue, Z., and Ortiz, B. D. (2011). A role for autophagic protein Beclin 1 early in lymphocyte development. J Immunol 186, 2201.

Axe, E. L., Walker, S. A., Manifava, M., Chandra, P., Roderick, H. L., Habermann, A., Griffiths, G., and Ktistakis, N. T. (2008). Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 182, 685.

Beale, R., Wise, H., Stuart, A., Ravenhill, B. J., Digard, P., and Randow, F. (2014). A LIR motif in influenza A virus M2 is required for virion stability. Cell Host & Microbe 15, 239-47.

Bernales, S., McDonald, K. L., and Walter, P. (2006). Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. PLoS Biol 4, e423.

Bird, S. W., Maynard, N. D., Covert, M. W., and Kirkegaard, K. (2014). Nonlytic viral spread enhanced by autophagy components. Proc Natl Acad Sci U S A 111, 13081.

Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H., and 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.

Borel, S., Robert-Hebmann, V., Alfaisal, J., Jain, A., Faure, M., Espert, L., Chaloin, L., Paillart, J. C., Johansen, T., and Biard-Piechaczyk, M. (2015). HIV-1 viral infectivity factor interacts with microtubule-associated protein light chain 3 and inhibits autophagy. Aids 29, 275.

Campbell, G. R., Rawat, P., Bruckman, R. S., and Spector, S. A. (2015). Human Immunodeficiency Virus Type 1 Nef Inhibits Autophagy through Transcription Factor EB Sequestration. PLoS Pathog 11, e1005018.

Carlsson, S. R., and Simonsen, A. (2015). Membrane dynamics in autophagosome biogenesis. J Cell Sci 128, 193.

Carter, C. A., and Ehrlich, L. S. (2008). Cell biology of HIV-1 infection of macrophages. Annu Rev Microbiol 62, 425.

Chaumorcel, M., Lussignol, M., Mouna, L., Cavignac, Y., Fahie, K., Cotte-Laffitte, J., Geballe, A., Brune, W., Beau, I., Codogno, P., and Esclatine, A. (2012). The human cytomegalovirus protein TRS1 inhibits autophagy via its interaction with Beclin 1. J Virol 86, 2571.

Chen, M., Hong, M. J., Sun, H., Wang, L., Shi, X., Gilbert, B. E., Corry, D. B., Kheradmand, F., and Wang, J. (2014). Essential role for autophagy in the maintenance of immunological memory against influenza infection. Nat Med 20, 503.

Cheng, C. W., Adams, G. B., Perin, L., Wei, M., Zhou, X., Lam, B. S., Da Sacco, S., Mirisola, M., Quinn, D. I., Dorff, T. B., Kopchick, J. J., and Longo, V. D. (2014). Prolonged fasting reduces IGF-1/PKA

to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell 14, 810.

Chiang, J. J., Davis, M. E., and Gack, M. U. (2014). Regulation of RIG-I-like receptor signaling by host and viral proteins. Cytokine Growth Factor Rev 25, 491.

Chicz, R. M., Urban, R. G., Gorga, J. C., Vignali, D. A., Lane, W. S., and Strominger, J. L. (1993). Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles. J Exp Med 178, 27.

Chu, C. T., Ji, J., Dagda, R. K., Jiang, J. F., Tyurina, Y. Y., Kapralov, A. A., Tyurin, V. A., Yanamala, N., Shrivastava, I. H., Mohammadyani, D., Qiang Wang, K. Z., Zhu, J., Klein-Seetharaman, J., Balasubramanian, K., Amoscato, A. A., Borisenko, G., Huang, Z., Gusdon, A. M., Cheikhi, A., Steer, E. K., Wang, R., Baty, C., Watkins, S., Bahar, I., Bayir, H., and Kagan, V. E. (2013). Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat Cell Biol 15, 1197.

Clement, C., Tiwari, V., Scanlan, P. M., Valyi-Nagy, T., Yue, B. Y., and Shukla, D. (2006). A novel role for phagocytosis-like uptake in herpes simplex virus entry. J Cell Biol 174, 1009.

Comber, J. D., Robinson, T. M., Siciliano, N. A., Snook, A. E., and Eisenlohr, L. C. (2011). Functional Macroautophagy Induction by Influenza A Virus Without a Contribution to MHC-Class II Restricted Presentation. J Virol 85, 6453.

Conway, K. L., Kuballa, P., Khor, B., Zhang, M., Shi, H. N., Virgin, H. W., and Xavier, R. J. (2013). ATG5 regulates plasma cell differentiation. Autophagy 9, 528.

Cuervo, A. M., and Wong, E. (2014). Chaperone-mediated autophagy: roles in disease and aging. Cell Res 24, 92.

Dales, S., Eggers, H. J., Tamm, I., and Palade, G. E. (1965). Electron Microscopic Study of the Formation of Poliovirus. Virology 26, 379.

De Leo, A., Colavita, F., Ciccosanti, F., Fimia, G. M., Lieberman, P. M., and Mattia, E. (2015). Inhibition of autophagy in EBV-positive Burkitt's lymphoma cells enhances EBV lytic genes expression and replication. Cell Death Dis 6, e1876.

Dengjel, J., Hoyer-Hansen, M., Nielsen, M. O., Eisenberg, T., Harder, L. M., Schandorff, S., Farkas, T., Kirkegaard, T., Becker, A. C., Schroeder, S., Vanselow, K., Lundberg, E., Nielsen, M. M., Kristensen, A. R., Akimov, V., Bunkenborg, J., Madeo, F., Jaattela, M., and Andersen, J. S. (2012). Identification of Autophagosome-associated Proteins and Regulators by Quantitative Proteomic Analysis and Genetic Screens. Mol Cell Proteomics 11, M111 014035.

Dengjel, J., Schoor, O., Fischer, R., Reich, M., Kraus, M., Muller, M., Kreymborg, K., Altenberend, F., Brandenburg, J., Kalbacher, H., Brock, R., Driessen, C., Rammensee, H. G., and Stevanovic, S. (2005). Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. Proc Natl Acad Sci U S A 102, 7922.

DeSelm, C. J., Miller, B. C., Zou, W., Beatty, W. L., van Meel, E., Takahata, Y., Klumperman, J., Tooze, S. A., Teitelbaum, S. L., and Virgin, H. W. (2011). Autophagy proteins regulate the secretory component of osteoclastic bone resorption. Dev Cell 21, 966.

Diao, J., Liu, R., Rong, Y., Zhao, M., Zhang, J., Lai, Y., Zhou, Q., Wilz, L. M., Li, J., Vivona, S., Pfuetzner, R. A., Brunger, A. T., and Zhong, Q. (2015). ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. Nature 520, 563.

Ding, B., Zhang, G., Yang, X., Zhang, S., Chen, L., Yan, Q., Xu, M., Banerjee, A. K., and Chen, M. (2014). Phosphoprotein of human parainfluenza virus type 3 blocks autophagosome-lysosome fusion to increase virus production. Cell Host Microbe 15, 564.

Dooley, H. C., Razi, M., Polson, H. E., Girardin, S. E., Wilson, M. I., and Tooze, S. A. (2014). WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12-5-16L1. Mol Cell 55, 238.

Dreux, M., Gastaminza, P., Wieland, S. F., and Chisari, F. V. (2009). The autophagy machinery is required to initiate hepatitis C virus replication. Proc Natl Acad Sci U S A 106, 14046.

Dupont, N., Jiang, S., Pilli, M., Ornatowski, W., Bhattacharya, D., and Deretic, V. (2011). Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta. Embo J 30, 4701.

Duran, J. M., Anjard, C., Stefan, C., Loomis, W. F., and Malhotra, V. (2010). Unconventional secretion of Acb1 is mediated by autophagosomes. J Cell Biol 188, 527.

E, X., Hwang, S., Oh, S., Lee, J. S., Jeong, J. H., Gwack, Y., Kowalik, T. F., Sun, R., Jung, J. U., and Liang, C. (2009). Viral Bcl-2-mediated evasion of autophagy aids chronic infection of gammaherpesvirus 68. PLoS Pathog 5, e1000609.

Egan, D. F., Shackelford, D. B., Mihaylova, M. M., Gelino, S., Kohnz, R. A., Mair, W., Vasquez, D. S., Joshi, A., Gwinn, D. M., Taylor, R., Asara, J. M., Fitzpatrick, J., Dillin, A., Viollet, B., Kundu, M., Hansen, M., and Shaw, R. J. (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331, 456.

Eng, K. E., Panas, M. D., Murphy, D., Karlsson Hedestam, G. B., and McInerney, G. M. (2012). Accumulation of autophagosomes in Semliki Forest virus-infected cells is dependent on expression of the viral glycoproteins. J Virol 86, 5674.

English, L., Chemali, M., Duron, J., Rondeau, C., Laplante, A., Gingras, D., Alexander, D., Leib, D., Norbury, C., Lippe, R., and Desjardins, M. (2009). Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. Nat Immunol 10, 480.

Espert, L., Denizot, M., Grimaldi, M., Robert-Hebmann, V., Gay, B., Varbanov, M., Codogno, P., and Biard-Piechaczyk, M. (2006). Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. J Clin Invest 116, 2161.

Fimia, G. M., 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., and Cecconi, F. (2007). Ambra1 regulates autophagy and development of the nervous system. Nature 447, 1121.

Florey, O., Kim, S. E., Sandoval, C. P., Haynes, C. M., and Overholtzer, M. (2011). Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. Nat Cell Biol 13, 1335.

Fonteneau, J. F., Brilot, F., Münz, C., and Gannage, M. (2016). The Tumor Antigen NY-ESO-1 Mediates Direct Recognition of Melanoma Cells by $CD4^+$ T Cells after Intercellular Antigen Transfer. J Immunol 196, 64.

Fotheringham, J. A., and Raab-Traub, N. (2015). Epstein-Barr virus latent membrane protein 2 induces autophagy to promote abnormal acinus formation. J Virol 89, 6940.

Fujita, N., Itoh, T., Omori, H., Fukuda, M., Noda, T., and Yoshimori, T. (2008). The Atg16L Complex Specifies the Site of LC3 Lipidation for Membrane Biogenesis in Autophagy. Mol Biol Cell 19, 2092.

Galluzzi, L., Pietrocola, F., Levine, B., and Kroemer, G. (2014). Metabolic control of autophagy. Cell 159, 1263.

Ganley, I. G., Lam du, H., Wang, J., Ding, X., Chen, S., and Jiang, X. (2009). ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 284, 12297.

Gannage, M., Dormann, D., Albrecht, R., Dengjel, J., Torossi, T., Ramer, P. C., Lee, M., Strowig, T., Arrey, F., Conenello, G., Pypaert, M., Andersen, J., Garcia-Sastre, A., and Münz, C. (2009). Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. Cell Host Microbe 6, 367.

Gelgor, A., Kalt, I., Bergson, S., Brulois, K. F., Jung, J. U., and Sarid, R. (2015). Viral Bcl-2 Encoded by the Kaposi's Sarcoma-Associated Herpesvirus Is Vital for Virus Reactivation. J Virol 89, 5298.

Gobeil, P. A., and Leib, D. A. (2012). Herpes simplex virus gamma34.5 interferes with autophagosome maturation and antigen presentation in dendritic cells. MBio 3, e00267.

Granato, M., Santarelli, R., Farina, A., Gonnella, R., Lotti, L. V., Faggioni, A., and Cirone, M. (2014). EBV blocks the autophagic flux and appropriates the autophagic machinery to enhance viral replication. J Virol 88, 12715.

Hailey, D. W., Rambold, A. S., Satpute-Krishnan, P., Mitra, K., Sougrat, R., Kim, P. K., and Lippincott-Schwartz, J. (2010). Mitochondria supply membranes for autophagosome biogenesis during starvation. Cell 141, 656.

Hamacher-Brady, A., and Brady, N. R. (2016). Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. Cell Mol Life Sci 73, 775.

Hamasaki, M., Furuta, N., Matsuda, A., Nezu, A., Yamamoto, A., Fujita, N., Oomori, H., Noda, T., Haraguchi, T., Hiraoka, Y., Amano, A., and Yoshimori, T. (2013). Autophagosomes form at ER-mitochondria contact sites. Nature 495, 389.

Hara, T., Takamura, A., Kishi, C., Iemura, S., Natsume, T., Guan, J. L., and Mizushima, N. (2008). FIP200, a ULK-interacting protein, is required for autophagosome formation in mammalian cells. J Cell Biol 181, 497.

Harris, J., Hartman, M., Roche, E., Zeng, S. G., O'Shea, A., Sharp, F. A., Lambe, E. M., Creagh, E. M., Golenbock, D. T., Tschopp, J., Kornfeld, H., Fitzgerald, K. A., and Lavellle, E. C. (2011). Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. J Biol Chem 286, 9587.

Hayashi-Nishino, M., Fujita, N., Noda, T., Yamaguchi, A., Yoshimori, T., and Yamamoto, A. (2009). A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat Cell Biol 11, 1433.

He, S., Ni, D., Ma, B., Lee, J. H., Zhang, T., Ghozalli, I., Pirooz, S. D., Zhao, Z., Bharatham, N., Li, B., Oh, S., Lee, W. H., Takahashi, Y., Wang, H. G., Minassian, A., Feng, P., Deretic, V., Pepperkok, R., Tagaya, M., Yoon, H. S., and Liang, C. (2013). PtdIns(3)P-bound UVRAG coordinates Golgi-ER retrograde and Atg9 transport by differential interactions with the ER tether and the beclin 1 complex. Nat Cell Biol 15, 1206.

Henault, J., Martinez, J., Riggs, J. M., Tian, J., Mehta, P., Clarke, L., Sasai, M., Latz, E., Brinkmann, M. M., Iwasaki, A., Coyle, A. J., Kolbeck, R., Green, D. R., and Sanjuan, M. A. (2012). Noncanonical

autophagy is required for type I interferon secretion in response to DNA-immune complexes. Immunity 37, 986.

Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., Guan, J. L., Oshiro, N., and Mizushima, N. (2009a). Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. Mol Biol Cell 20, 1981.

Hosokawa, N., Sasaki, T., Iemura, S., Natsume, T., Hara, T., and Mizushima, N. (2009b). Atg101, a novel mammalian autophagy protein interacting with Atg13. Autophagy 5, 973.

Hung, C. H., Chen, L. W., Wang, W. H., Chang, P. J., Chiu, Y. F., Hung, C. C., Lin, Y. J., Liou, J. Y., Tsai, W. J., Hung, C. L., and Liu, S. T. (2014). Regulation of autophagic activation by Rta of Epstein-Barr virus via the extracellular signal-regulated kinase pathway. J Virol 88, 12133.

Hwang, S., Maloney, N. S., Bruinsma, M. W., Goel, G., Duan, E., Zhang, L., Shrestha, B., Diamond, M. S., Dani, A., Sosnovtsev, S. V., Green, K. Y., Lopez-Otin, C., Xavier, R. J., Thackray, L. B., and Virgin, H. W. (2012). Nondegradative role of Atg5-Atg12/ Atg16L1 autophagy protein complex in antiviral activity of interferon gamma. Cell Host Microbe 11, 397.

Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Ohsumi, M., Noda, T., and Ohsumi, Y. (2000). A ubiquitin-like system mediates protein lipidation. Nature 408, 488.

Ichinohe, T., Pang, I. K., and Iwasaki, A. (2010). Influenza virus activates inflammasomes via its intracellular M2 ion channel. Nat Immunol 11, 404.

Ichinohe, T., Yamazaki, T., Koshiba, T., and Yanagi, Y. (2013). Mitochondrial protein mitofusin 2 is required for NLRP3 inflammasome activation after RNA virus infection. Proc Natl Acad Sci U S A 110, 17963.

Imam, S., Talley, S., Nelson, R. S., Dharan, A., O'Connor, C., Hope, T. J., and Campbell, E. M. (2016). TRIM5alpha degradation via autophagy is not required for retroviral restriction. J Virol in press.

Inoki, K., Li, Y., Zhu, T., Wu, J., and Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 4, 648.

Ishikawa, H., and Barber, G. N. (2008). STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455, 674.

Itakura, E., Kishi-Itakura, C., and Mizushima, N. (2012). The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell 151, 1256.

Itakura, E., Kishi, C., Inoue, K., and Mizushima, N. (2008). Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. Mol Biol Cell 19, 5360.

Jackson, W. T., Giddings, T. H., Jr., Taylor, M. P., Mulinyawe, S., Rabinovitch, M., Kopito, R. R., and Kirkegaard, K. (2005). Subversion of cellular autophagosomal machinery by RNA viruses. PLoS Biol 3, e156.

Jeffries, T. R., Dove, S. K., Michell, R. H., and Parker, P. J. (2004). PtdIns-specific MPR pathway association of a novel WD40 repeat protein, WIPI49. Mol Biol Cell 15, 2652.

Jiang, P., Nishimura, T., Sakamaki, Y., Itakura, E., Hatta, T., Natsume, T., and Mizushima, N. (2014). The HOPS complex mediates autophagosome-lysosome fusion through interaction with syntaxin 17. Mol Biol Cell 25, 1327.

Jiang, Y., Yin, X., Stuart, P. M., and Leib, D. A. (2015). Dendritic Cell Autophagy Contributes to Herpes Simplex Virus-Driven Stromal Keratitis and Immunopathology. MBio 6.

Jin, Y., Sun, C., Feng, L., Li, P., Xiao, L., Ren, Y., Wang, D., Li, C., and Chen, L. (2014). Regulation of SIV antigen-specific $CD4^+$ T cellular immunity via autophagosome-mediated MHC II molecule-targeting antigen presentation in mice. PLoS ONE 9, e93143.

Joubert, P. E., Werneke, S. W., de la Calle, C., Guivel-Benhassine, F., Giodini, A., Peduto, L., Levine, B., Schwartz, O., Lenschow, D. J., and Albert, M. L. (2012). Chikungunya virus-induced autophagy delays caspase-dependent cell death. J Exp Med 209, 1029.

Jounai, N., Takeshita, F., Kobiyama, K., Sawano, A., Miyawaki, A., Xin, K. Q., Ishii, K. J., Kawai, T., Akira, S., Suzuki, K., and Okuda, K. (2007). The Atg5 Atg12 conjugate associates with innate antiviral immune responses. Proc Natl Acad Sci U S A 104, 14050.

Jouvenet, N., Neil, S. J., Bess, C., Johnson, M. C., Virgen, C. A., Simon, S. M., and Bieniasz, P. D. (2006). Plasma membrane is the site of productive HIV-1 particle assembly. PLoS Biol 4, e435.

Judith, D., Mostowy, S., Bourai, M., Gangneux, N., Lelek, M., Lucas-Hourani, M., Cayet, N., Jacob, Y., Prevost, M. C., Pierre, P., Tangy, F., Zimmer, C., Vidalain, P. O., Couderc, T., and Lecuit, M. (2013). Species-specific impact of the autophagy machinery on Chikungunya virus infection. EMBO Rep 14, 534.

Jung, C. H., Jun, C. B., Ro, S. H., Kim, Y. M., Otto, N. M., Cao, J., Kundu, M., and Kim, D. H. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Mol Biol Cell 20, 1992.

Kanayama, M., He, Y. W., and Shinohara, M. L. (2015). The lung is protected from spontaneous inflammation by autophagy in myeloid cells. J Immunol 194, 5465.

Kane, L. A., Lazarou, M., Fogel, A. I., Li, Y., Yamano, K., Sarraf, S. A., Banerjee, S., and Youle, R. J. (2014). PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. J Cell Biol 205, 143.

Kanno, Y., Vahedi, G., Hirahara, K., Singleton, K., and O'Shea, J. J. (2012). Transcriptional and epigenetic control of T helper cell specification: molecular mechanisms underlying commitment and plasticity. Annu Rev Immunol 30, 707.

Kasai, M., Tanida, I., Ueno, T., Kominami, E., Seki, S., Ikeda, T., and Mizuochi, T. (2009). Autophagic compartments gain access to the MHC class II compartments in thymic epithelium. J Immunol 183, 7278.

Kazlauskaite, A., Kondapalli, C., Gourlay, R., Campbell, D. G., Ritorto, M. S., Hofmann, K., Alessi, D. R., Knebel, A., Trost, M., and Muqit, M. M. (2014). Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. Biochem J 460, 127.

Khaminets, A., Behl, C., and Dikic, I. (2016). Ubiquitin-Dependent And Independent Signals In Selective Autophagy. Trends Cell Biol 26, 6.

Khaminets, A., Heinrich, T., Mari, M., Grumati, P., Huebner, A. K., Akutsu, M., Liebmann, L., Stolz, A., Nietzsche, S., Koch, N., Mauthe, M., Katona, I., Qualmann, B., Weis, J., Reggiori, F., Kurth, I., Hubner, C. A., and Dikic, I. (2015). Regulation of endoplasmic reticulum turnover by selective autophagy. Nature 522, 354.

Kim, I., and Lemasters, J. J. (2011). Mitochondrial degradation by autophagy (mitophagy) in GFP-LC3 transgenic hepatocytes during nutrient deprivation. Am J Physiol Cell Physiol 300, C308.

Kim, J. Y., Zhao, H., Martinez, J., Doggett, T. A., Kolesnikov, A. V., Tang, P. H., Ablonczy, Z., Chan, C. C., Zhou, Z., Green, D. R., and Ferguson, T. A. (2013). Noncanonical autophagy promotes the visual cycle. Cell 154, 365.

Kimball, S. R., Siegfried, B. A., and Jefferson, L. S. (2004). Glucagon represses signaling through the mammalian target of rapamycin in rat liver by activating AMP-activated protein kinase. J Biol Chem 279, 54103.

Kimura, S., Noda, T., and Yoshimori, T. (2008). Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. Cell Struct Funct 33, 109.

Kimura, T., Jain, A., Choi, S. W., Mandell, M. A., Schroder, K., Johansen, T., and Deretic, V. (2015). TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. J Cell Biol 210, 973.

Kirisako, T., Ichimura, Y., Okada, H., Kabeya, Y., Mizushima, N., Yoshimori, T., Ohsumi, M., Takao, T., Noda, T., and 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.

Konno, H., Konno, K., and Barber, G. N. (2013). Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. Cell 155, 688.

Koyama-Honda, I., Itakura, E., Fujiwara, T. K., and Mizushima, N. (2013). Temporal analysis of recruitment of mammalian ATG proteins to the autophagosome formation site. Autophagy 9, 1491.

Koyano, F., Okatsu, K., Kosako, H., Tamura, Y., Go, E., Kimura, M., Kimura, Y., Tsuchiya, H., Yoshihara, H., Hirokawa, T., Endo, T., Fon, E. A., Trempe, J. F., Saeki, Y., Tanaka, K., and Matsuda, N. (2014). Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature 510, 162.

Krejbich-Trotot, P., Gay, B., Li-Pat-Yuen, G., Hoarau, J. J., Jaffar-Bandjee, M. C., Briant, L., Gasque, P., and Denizot, M. (2011). Chikungunya triggers an autophagic process which promotes viral replication. Virol J 8, 432.

Ku, B., Woo, J. S., Liang, C., Lee, K. H., Hong, H. S., E, X., Kim, K. S., Jung, J. U., and Oh, B. H. (2008). Structural and biochemical bases for the inhibition of autophagy and apoptosis by viral BCL-2 of murine gamma-herpesvirus 68. PLoS Pathog 4, e25.

Kunz, J. B., Schwarz, H., and Mayer, A. (2004). Determination of four sequential stages during microautophagy in vitro. J Biol Chem 279, 9987.

Kyei, G. B., Dinkins, C., Davis, A. S., Roberts, E., Singh, S. B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., Federico, M., Panganiban, A., Vergne, I., and Deretic, V. (2009). Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. J Cell Biol 186, 255.

Kyewski, B., and Klein, L. (2006). A central role for central tolerance. Annu Rev Immunol 24, 571.

Lamb, C. A., Nuhlen, S., Judith, D., Frith, D., Snijders, A. P., Behrends, C., and Tooze, S. A. (2016). TBC1D14 regulates autophagy via the TRAPP complex and ATG9 traffic. Embo J 35, 281.

Lascano, J., Uchil, P. D., Mothes, W., and Luban, J. (2015). TRIM5 Retroviral Restriction Activity Correlates with the Ability To Induce Innate Immune Signaling. J Virol 90, 308.

Lazarou, M., Sliter, D. A., Kane, L. A., Sarraf, S. A., Wang, C., Burman, J. L., Sideris, D. P., Fogel, A. I., and Youle, R. J. (2015). The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature 524, 309.

Lee, D. Y., and Sugden, B. (2008). The latent membrane protein 1 oncogene modifies B-cell physiology by regulating autophagy. Oncogene 27, 2833.

Lee, H. K., Lund, J. M., Ramanathan, B., Mizushima, N., and Iwasaki, A. (2007). Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 315, 1398.

Lee, H. K., Mattei, L. M., Steinberg, B. E., Alberts, P., Lee, Y. H., Chervonsky, A., Mizushima, N., Grinstein, S., and Iwasaki, A. (2010). In vivo requirement for Atg5 in antigen presentation by dendritic cells. Immunity 32, 227.

Lee, J. M., Wagner, M., Xiao, R., Kim, K. H., Feng, D., Lazar, M. A., and Moore, D. D. (2014). Nutrient-sensing nuclear receptors coordinate autophagy. Nature 516, 112.

Lee, J. S., Li, Q., Lee, J. Y., Lee, S. H., Jeong, J. H., Lee, H. R., Chang, H., Zhou, F. C., Gao, S. J., Liang, C., and Jung, J. U. (2009). FLIP-mediated autophagy regulation in cell death control. Nat Cell Biol 11, 1355.

Lee, M. S., and Kim, Y. J. (2007). Signaling pathways downstream of pattern-recognition receptors and their cross talk. Annu Rev Biochem 76, 447.

Leung, C. S., Haigh, T. A., Mackay, L. K., Rickinson, A. B., and Taylor, G. S. (2010). Nuclear location of an endogenously expressed antigen, EBNA1, restricts access to macroautophagy and the range of CD4 epitope display. Proc Natl Acad Sci U S A 107, 2165.

Levitskaya, J., Coram, M., Levitsky, V., Imreh, S., Steigerwald-Mullen, P. M., Klein, G., Kurilla, M. G., and Masucci, M. G. (1995). Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. Nature 375, 685.

Li, J., Liu, Y., Wang, Z., Liu, K., Wang, Y., Liu, J., Ding, H., and Yuan, Z. (2011a). Subversion of cellular autophagy machinery by hepatitis B virus for viral envelopment. J Virol 85, 6319.

Li, S., Yang, P., Tian, E., and Zhang, H. (2013). Arginine methylation modulates autophagic degradation of PGL granules in C. elegans. Mol Cell 52, 421.

Li, Y., Wang, L. X., Pang, P., Cui, Z., Aung, S., Haley, D., Fox, B. A., Urba, W. J., and Hu, H. M. (2011b). Tumor-derived autophagosome vaccine: mechanism of cross-presentation and therapeutic efficacy. Clin Cancer Res 17, 7047.

Liang, C., Lee, J. S., Inn, K. S., Gack, M. U., Li, Q., Roberts, E. A., Vergne, I., Deretic, V., Feng, P., Akazawa, C., and Jung, J. U. (2008). Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. Nat Cell Biol 10, 776.

Liang, Q., Chang, B., Brulois, K. F., Castro, K., Min, C. K., Rodgers, M. A., Shi, M., Ge, J., Feng, P., Oh, B. H., and Jung, J. U. (2013). Kaposi's sarcoma-associated herpesvirus K7 modulates Rubicon-mediated inhibition of autophagosome maturation. J Virol 87, 12499.

Liang, Q., Chang, B., Lee, P., Brulois, K. F., Ge, J., Shi, M., Rodgers, M. A., Feng, P., Oh, B. H., Liang, C., and Jung, J. U. (2015). Identification of the Essential Role of Viral Bcl-2 for Kaposi's Sarcoma-Associated Herpesvirus Lytic Replication. J Virol 89, 5308.

Liang, Q., Seo, G. J., Choi, Y. J., Kwak, M. J., Ge, J., Rodgers, M. A., Shi, M., Leslie, B. J., Hopfner, K. P., Ha, T., Oh, B. H., and Jung, J. U. (2014). Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. Cell Host Microbe 15, 228.

Liang, X. H., Kleeman, L. K., Jiang, H. H., Gordon, G., Goldman, J. E., Berry, G., Herman, B., and Levine, B. (1998). Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol 72, 8586.

Lindqvist, L. M., Simon, A. K., and Baehrecke, E. H. (2015). Current questions and possible controversies in autophagy. Cell Death Discov 1, pii.

Liu, L., Feng, D., Chen, G., Chen, M., Zheng, Q., Song, P., Ma, Q., Zhu, C., Wang, R., Qi, W., Huang, L., Xue, P., Li, B., Wang, X., Jin, H., Wang, J., Yang, F., Liu, P., Zhu, Y., Sui, S., and Chen, Q. (2012). Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. Nat Cell Biol 14, 177.

Lu, Q., Yang, P., Huang, X., Hu, W., Guo, B., Wu, F., Lin, L., Kovacs, A. L., Yu, L., and Zhang, H. (2011). The WD40 repeat PtdIns(3)P-binding protein EPG-6 regulates progression of omegasomes to autophagosomes. Dev Cell 21, 343.

Lu, Q., Yokoyama, C. C., Williams, J. W., Baldridge, M. T., Jin, X., DesRochers, B., Bricker, T., Wilen, C. B., Bagaitkar, J., Loginicheva, E., Sergushichev, A., Kreamalmeyer, D., Keller, B. C., Zhao, Y., Kambal, A., Green, D. R., Martinez, J., Dinauer, M. C., Holtzman, M. J., Crouch, E. C., Beatty, W., Boon, A. C., Zhang, H., Randolph, G. J., Artyomov, M. N., and Virgin, H. W. (2016). Homeostatic Control of Innate Lung Inflammation by Vici Syndrome Gene Epg5 and Additional Autophagy Genes Promotes Influenza Pathogenesis. Cell Host Microbe 19, 102.

Lupfer, C., Thomas, P. G., Anand, P. K., Vogel, P., Milasta, S., Martinez, J., Huang, G., Green, M., Kundu, M., Chi, H., Xavier, R. J., Green, D. R., Lamkanfi, M., Dinarello, C. A., Doherty, P. C., and Kanneganti, T. D. (2013). Receptor interacting protein kinase 2-mediated mitophagy regulates inflammasome activation during virus infection. Nat Immunol 14, 480.

Lussignol, M., Queval, C., Bernet-Camard, M. F., Cotte-Laffitte, J., Beau, I., Codogno, P., and Esclatine, A. (2013). The herpes simplex virus 1 Us11 protein inhibits autophagy through its interaction with the protein kinase PKR. J Virol 87, 859.

Ma, J., Becker, C., Lowell, C. A., and Underhill, D. M. (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.

Mandell, M. A., Jain, A., Arko-Mensah, J., Chauhan, S., Kimura, T., Dinkins, C., Silvestri, G., Munch, J., Kirchhoff, F., Simonsen, A., Wei, Y., Levine, B., Johansen, T., and Deretic, V. (2014). TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. Dev Cell 30, 394.

Manjithaya, R., Anjard, C., Loomis, W. F., and Subramani, S. (2010). Unconventional secretion of Pichia pastoris Acb1 is dependent on GRASP protein, peroxisomal functions, and autophagosome formation. J Cell Biol 188, 537.

Martinez, J., Almendinger, J., Oberst, A., Ness, R., Dillon, C. P., Fitzgerald, P., Hengartner, M. O., and Green, D. R. (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.

Martinez, J., Malireddi, R. K., Lu, Q., Cunha, L. D., Pelletier, S., Gingras, S., Orchard, R., Guan, J. L., Tan, H., Peng, J., Kanneganti, T. D., Virgin, H. W., and Green, D. R. (2015). Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 17, 893.

Martinon, F., Mayor, A., and Tschopp, J. (2009). The inflammasomes: guardians of the body. Annu Rev Immunol 27, 229.

Matsunaga, K., Saitoh, T., Tabata, K., Omori, H., Satoh, T., Kurotori, N., Maejima, I., Shirahama-Noda, K., Ichimura, T., Isobe, T., Akira, S., Noda, T., and Yoshimori, T. (2009). Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol 11, 385.

McEwan, D. G., Popovic, D., Gubas, A., Terawaki, S., Suzuki, H., Stadel, D., Coxon, F. P., Miranda de Stegmann, D., Bhogaraju, S., Maddi, K., Kirchof, A., Gatti, E., Helfrich, M. H., Wakatsuki, S., Behrends, C., Pierre, P., and Dikic, I. (2015). PLEKHM1 Regulates Autophagosome-Lysosome Fusion through HOPS Complex and LC3/GABARAP Proteins. Mol Cell 57, 39.

McFadden, K., Hafez, A. Y., Kishton, R., Messinger, J. E., Nikitin, P. A., Rathmell, J. C., and Luftig, M. A. (2016). Metabolic stress is a barrier to Epstein-Barr virus-mediated B-cell immortalization. Proc Natl Acad Sci U S A.

Mercer, C. A., Kaliappan, A., and Dennis, P. B. (2009). A novel, human Atg13 binding protein, Atg101, interacts with ULK1 and is essential for macroautophagy. Autophagy 5, 649.

Metz, P., Chiramel, A., Chatel-Chaix, L., Alvisi, G., Bankhead, P., Mora-Rodriguez, R., Long, G., Hamacher-Brady, A., Brady, N. R., and Bartenschlager, R. (2015). Dengue Virus Inhibition of

Autophagic Flux and Dependency of Viral Replication on Proteasomal Degradation of the Autophagy Receptor p62. J Virol 89, 8026.

Miller, B. C., Zhao, Z., Stephenson, L. M., Cadwell, K., Pua, H. H., Lee, H. K., Mizushima, N. N., Iwasaki, A., He, Y. W., Swat, W., and Virgin, H. W. t. (2008). The autophagy gene ATG5 plays an essential role in B lymphocyte development. Autophagy 4, 309.

Mizushima, N., Noda, T., Yoshimori, T., Tanaka, Y., Ishii, T., George, M. D., Klionsky, D. J., Ohsumi, M., and Ohsumi, Y. (1998). A protein conjugation system essential for autophagy. Nature 395, 395.

Mochida, K., Oikawa, Y., Kimura, Y., Kirisako, H., Hirano, H., Ohsumi, Y., and Nakatogawa, H. (2015). Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. Nature 522, 359.

Mohl, B. P., Bartlett, C., Mankouri, J., and Harris, M. (2016). Early events in the generation of autophagosomes are required for the formation of membrane structures involved in hepatitis C virus genome replication. J Gen Virol.

Mortensen, M., Soilleux, E. J., Djordjevic, G., Tripp, R., Lutteropp, M., Sadighi-Akha, E., Stranks, A. J., Glanville, J., Knight, S., SE, W. J., Kranc, K. R., and Simon, A. K. (2011). The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. J Exp Med 208, 455.

Mouna, L., Hernandez, E., Bonte, D., Brost, R., Amazit, L., Delgui, L. R., Brune, W., Geballe, A. P., Beau, I., and Esclatine, A. (2015). Analysis of the role of autophagy inhibition by two complementary human cytomegalovirus BECN1/Beclin 1-binding proteins. Autophagy, 0.

Münz, C. (2009). Enhancing immunity through autophagy. Annu Rev Immunol 27, 423.

Münz, C., Bickham, K. L., Subklewe, M., Tsang, M. L., Chahroudi, A., Kurilla, M. G., Zhang, D., O'Donnell, M., and Steinman, R. M. (2000). Human $CD4^+$ T lymphocytes consistently respond to the latent Epstein-Barr virus nuclear antigen EBNA1. J Exp Med 191, 1649.

Nakahira, K., Haspel, J. A., Rathinam, V. A., Lee, S. J., Dolinay, T., Lam, H. C., Englert, J. A., Rabinovitch, M., Cernadas, M., Kim, H. P., Fitzgerald, K. A., Ryter, S. W., and Choi, A. M. (2011). Autophagy

proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12, 222.

Nakatogawa, H., Ichimura, Y., and Ohsumi, Y. (2007). Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell 130, 165.

Narendra, D., Tanaka, A., Suen, D. F., and Youle, R. J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183, 795.

Nedjic, J., Aichinger, M., Emmerich, J., Mizushima, N., and Klein, L. (2008). Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. Nature 455, 396.

Novak, I., Kirkin, V., McEwan, D. G., Zhang, J., Wild, P., Rozenknop, A., Rogov, V., Lohr, F., Popovic, D., Occhipinti, A., Reichert, A. S., Terzic, J., Dotsch, V., Ney, P. A., and Dikic, I. (2010). Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep 11, 45.

Nowag, H., Guhl, B., Thriene, K., Romao, S., Ziegler, U., Dengjel, J., and Münz, C. (2014). Macroautopphagy proteins assist Epstein Barr virus production and get incorporated into the virus particles. EBioMedicine 1, 116.

O'Donnell, V., Pacheco, J. M., LaRocco, M., Burrage, T., Jackson, W., Rodriguez, L. L., Borca, M. V., and Baxt, B. (2011). Foot-and-mouth disease virus utilizes an autophagic pathway during viral replication. Virology 410, 142.

Orsi, A., Razi, M., Dooley, H. C., Robinson, D., Weston, A. E., Collinson, L. M., and Tooze, S. A. (2012). Dynamic and transient interactions of Atg9 with autophagosomes, but not membrane integration, are required for autophagy. Mol Biol Cell 23, 1860.

Orvedahl, A., Alexander, D., Talloczy, Z., Sun, Q., Wei, Y., Zhang, W., Burns, D., Leib, D., and Levine, B. (2007). HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. Cell Host & Microbe 1, 23.

Orvedahl, A., MacPherson, S., Sumpter, R., Jr., Talloczy, Z., Zou, Z., and Levine, B. (2010). Autophagy protects against Sindbis virus infection of the central nervous system. Cell Host Microbe 7, 115.

Orvedahl, A., Sumpter, R., Jr., Xiao, G., Ng, A., Zou, Z., Tang, Y., Narimatsu, M., Gilpin, C., Sun, Q., Roth, M., Forst, C. V., Wrana, J. L., Zhang, Y. E., Luby-Phelps, K., Xavier, R. J., Xie, Y., and Levine, B. (2011). Image-based genome-wide siRNA screen identifies selective autophagy factors. Nature 480, 113.

Pallet, N., Sirois, I., Bell, C., Hanafi, L. A., Hamelin, K., Dieude, M., Rondeau, C., Thibault, P., Desjardins, M., and Hebert, M. J. (2013). A comprehensive characterization of membrane vesicles released by autophagic human endothelial cells. Proteomics 13, 1108.

Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T., and Münz, C. (2005). Endogenous MHC class II processing of a viral nuclear antigen after autophagy. Science 307, 593.

Pankiv, S., Alemu, E. A., Brech, A., Bruun, J. A., Lamark, T., Overvatn, A., Bjorkoy, G., and 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.

Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X. H., Mizushima, N., Packer, M., Schneider, M. D., and Levine, B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 122, 927.

Pengo, N., Scolari, M., Oliva, L., Milan, E., Mainoldi, F., Raimondi, A., Fagioli, C., Merlini, A., Mariani, E., Pasqualetto, E., Orfanelli, U., Ponzoni, M., Sitia, R., Casola, S., and Cenci, S. (2013). Plasma cells require autophagy for sustainable immunoglobulin production. Nat Immunol 14, 298.

Pirooz, S., He, S., O'Connell, D., Khalilzadeh, P., Yang, Y., and Liang, C. (2014a). Viruses customize autophagy protein for efficient viral entry. Autophagy 10, 1355.

Pirooz, S. D., He, S., Zhang, T., Zhang, X., Zhao, Z., Oh, S., O'Connell, D., Khalilzadeh, P., Amini-Bavil-Olyaee, S., Farzan, M., and Liang, C. (2014b). UVRAG is required for virus entry through combinatorial interaction with the class C-Vps complex and SNAREs. Proc Natl Acad Sci U S A 111, 2716.

Polson, H. E., de Lartigue, J., Rigden, D. J., Reedijk, M., Urbe, S., Clague, M. J., and Tooze, S. A. (2010). Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy 6, 506.

Proikas-Cezanne, T., and Robenek, H. (2011). Freeze-fracture replica immunolabelling reveals human WIPI-1 and WIPI-2 as membrane proteins of autophagosomes. J Cell Mol Med 15, 2007.

Proikas-Cezanne, T., Waddell, S., Gaugel, A., Frickey, T., Lupas, A., and Nordheim, A. (2004). WIPI-1alpha (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy. Oncogene 23, 9314.

Pua, H. H., Dzhagalov, I., Chuck, M., Mizushima, N., and He, Y. W. (2007). A critical role for the autophagy gene Atg5 in T cell survival and proliferation. J Exp Med 204, 25.

Pua, H. H., Guo, J., Komatsu, M., and He, Y. W. (2009). Autophagy is essential for mitochondrial clearance in mature T lymphocytes. J Immunol 182, 4046.

Pujals, A., Favre, L., Pioche-Durieu, C., Robert, A., Meurice, G., Le Gentil, M., Chelouah, S., Martin-Garcia, N., Le Cam, E., Guettier, C., Raphael, M., Vassilev, L. T., Gaulard, P., Codogno, P., Lipinski, M., and Wiels, J. (2015). Constitutive autophagy contributes to resistance to TP53-mediated apoptosis in Epstein-Barr virus-positive latency III B-cell lymphoproliferations. Autophagy 11, 2275.

Puleston, D. J., Zhang, H., Powell, T. J., Lipina, E., Sims, S., Panse, I., Watson, A. S., Cerundolo, V., Townsend, A. R., Klenerman, P., and Simon, A. K. (2014). Autophagy is a critical regulator of memory CD8(+) T cell formation. Elife 3.

Puri, C., Renna, M., Bento, C. F., Moreau, K., and Rubinsztein, D. C. (2013). Diverse autophagosome membrane sources coalesce in recycling endosomes. Cell 154, 1285.

Quinsay, M. N., Thomas, R. L., Lee, Y., and Gustafsson, A. B. (2010). Bnip3-mediated mitochondrial autophagy is independent of the mitochondrial permeability transition pore. Autophagy 6, 855.

Rasmussen, S. B., Horan, K. A., Holm, C. K., Stranks, A. J., Mettenleiter, T. C., Simon, A. K., Jensen, S. B., Rixon, F. J., He, B., and Paludan, S. R. (2011). Activation of autophagy by alpha-herpesviruses in myeloid cells is mediated by cytoplasmic viral DNA through a mechanism dependent on stimulator of IFN genes. J Immunol 187, 5268.

Reggiori, F., Tucker, K. A., Stromhaug, P. E., and Klionsky, D. J. (2004). The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. Dev Cell 6, 79.

Ren, Y., Li, C., Feng, L., Pan, W., Li, L., Wang, Q., Li, J., Li, N., Han, L., Zheng, X., Niu, X., Sun, C., and Chen, L. (2015). Proton Channel Activity of Influenza A Virus Matrix Protein 2 Contributes to Autophagy Arrest. J Virol 90, 591.

Richards, A. L., and Jackson, W. T. (2012). Intracellular vesicle acidification promotes maturation of infectious poliovirus particles. PLoS Pathog 8, e1003046.

Robinson, S. M., Tsueng, G., Sin, J., Mangale, V., Rahawi, S., McIntyre, L. L., Williams, W., Kha, N., Cruz, C., Hancock, B. M., Nguyen, D. P., Sayen, M. R., Hilton, B. J., Doran, K. S., Segall, A. M., Wolkowicz, R., Cornell, C. T., Whitton, J. L., Gottlieb, R. A., and Feuer, R. (2014). Coxsackievirus B exits the host cell in shed microvesicles displaying autophagosomal markers. PLoS Pathog 10, e1004045.

Rogov, V., Dotsch, V., Johansen, T., and Kirkin, V. (2014). Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. Mol Cell 53, 167.

Romao, S., Gasser, N., Becker, A. C., Guhl, B., Bagajic, M., Vanoaica, L. D., Ziegler, U., Roesler, J., Dengjel, J., Reichenbach, J., and Münz, C. (2013). Essential autophagy proteins stabilize pathogen containing phagosomes for prolonged MHC class II antigen processing. J Cell Biol 203, 757.

Russell, R. C., Tian, Y., Yuan, H., Park, H. W., Chang, Y. Y., Kim, J., Kim, H., Neufeld, T. P., Dillin, A., and Guan, K. L. (2013). ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. Nat Cell Biol 15, 741.

Sagnier, S., Daussy, C. F., Borel, S., Robert-Hebmann, V., Faure, M., Blanchet, F. P., Beaumelle, B., Biard-Piechaczyk, M., and Espert, L. (2015). Autophagy restricts HIV-1 infection by selectively degrading Tat in CD4+ T lymphocytes. J Virol 89, 615.

Saitoh, T., Fujita, N., Hayashi, T., Takahara, K., Satoh, T., Lee, H., Matsunaga, K., Kageyama, S., Omori, H., Noda, T., Yamamoto, N., Kawai, T., Ishii, K., Takeuchi, O., Yoshimori, T., and Akira, S. (2009). Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. Proc Natl Acad Sci U S A 106, 20842.

Saitoh, T., Fujita, N., Jang, M. H., Uematsu, S., Yang, B. G., Satoh, T., Omori, H., Noda, T., Yamamoto, N., Komatsu, M., Tanaka, K., Kawai, T., Tsujimura, T., Takeuchi, O., Yoshimori, T., and Akira, S. (2008). Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature 456, 264.

Sanjuan, M. A., Dillon, C. P., Tait, S. W., Moshiach, S., Dorsey, F., Connell, S., Komatsu, M., Tanaka, K., Cleveland, J. L., Withoff, S., and Green, D. R. (2007). Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature 450, 1253.

Santana, S., Bullido, M. J., Recuero, M., Valdivieso, F., and Aldudo, J. (2012). Herpes simplex virus type I induces an incomplete autophagic response in human neuroblastoma cells. J Alzheimers Dis 30, 815.

Schatz, D., Shemi, A., Rosenwasser, S., Sabanay, H., Wolf, S. G., Ben-Dor, S., and Vardi, A. (2014). Hijacking of an autophagy-like process is critical for the life cycle of a DNA virus infecting oceanic algal blooms. New Phytol 204, 854.

Schlee, M. (2013). Master sensors of pathogenic RNA - RIG-I like receptors. Immunobiology 218, 1322.

Schlie, K., Westerback, A., DeVorkin, L., Hughson, L. R., Brandon, J. M., MacPherson, S., Gadawski, I., Townsend, K. N., Poon, V. I., Elrick, M. A., Cote, H. C., Abraham, N., Wherry, E. J., Mizushima, N., and Lum, J. J. (2015). Survival of effector CD8^{+} T cells during influenza infection is dependent on autophagy. J Immunol 194, 4277.

Schmid, D., Pypaert, M., and Münz, C. (2007). MHC class II antigen loading compartments continuously receive input from autophagosomes. Immunity 26, 79.

Seok, S., Fu, T., Choi, S. E., Li, Y., Zhu, R., Kumar, S., Sun, X., Yoon, G., Kang, Y., Zhong, W., Ma, J., Kemper, B., and Kemper, J. K. (2014). Transcriptional regulation of autophagy by an FXR-CREB axis. Nature 516, 108.

Shelly, S., Lukinova, N., Bambina, S., Berman, A., and Cherry, S. (2009). Autophagy is an essential component of Drosophila immunity against vesicular stomatitis virus. Immunity 30, 588.

Shi, C. S., Shenderov, K., Huang, N. N., Kabat, J., Abu-Asab, M., Fitzgerald, K. A., Sher, A., and Kehrl, J. H. (2012). Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol 13, 255.

Shiratsuchi, A., Kaido, M., Takizawa, T., and Nakanishi, Y. (2000). Phosphatidylserine-mediated phagocytosis of influenza A virus-infected cells by mouse peritoneal macrophages. J Virol 74, 9240.

Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G. R., Zou, Z., Kinch, L., Wilkins, A. D., Sun, Q., Pallauf, K., MacDuff, D., Huerta, C., Virgin, H. W., Helms, J. B., Eerland, R., Tooze, S. A., Xavier, R., Lenschow, D. J., Yamamoto, A., King, D., Lichtarge, O., Grishin, N. V., Spector, S. A., Kaloyanova, D. V., and Levine, B. (2013). Identification of a candidate therapeutic autophagy-inducing peptide. Nature 494, 201.

Shpilka, T., Weidberg, H., Pietrokovski, S., and Elazar, Z. (2011). Atg8: an autophagy-related ubiquitin-like protein family. Genome Biol 12, 226.

Shrivastava, S., Devhare, P., Sujijantarat, N., Steele, R., Kwon, Y. C., Ray, R., and Ray, R. B. (2015). Knockdown of autophagy inhibits infectious hepatitis C virus release by exosomal pathway. J Virol.

Sica, V., Galluzzi, L., Bravo-San Pedro, J. M., Izzo, V., Maiuri, M. C., and Kroemer, G. (2015). Organelle-Specific Initiation of Autophagy. Mol Cell 59, 522.

Sir, D., Kuo, C. F., Tian, Y., Liu, H. M., Huang, E. J., Jung, J. U., Machida, K., and Ou, J. H. (2012). Replication of hepatitis C virus RNA on autophagosomal membranes. J Biol Chem 287, 18036.

Sun, Q., Fan, W., Chen, K., Ding, X., Chen, S., and Zhong, Q. (2008). Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 105, 19211.

Suri, A., Walters, J. J., Rohrs, H. W., Gross, M. L., and Unanue, E. R. (2008). First Signature of Islet {beta}-Cell-Derived Naturally Processed Peptides Selected by Diabetogenic Class II MHC Molecules. J Immunol 180, 3849.

Takahashi, Y., Coppola, D., Matsushita, N., Cualing, H. D., Sun, M., Sato, Y., Liang, C., Jung, J. U., Cheng, J. Q., Mule, J. J., Pledger, W. J., and Wang, H. G. (2007). Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. Nat Cell Biol 9, 1142.

Takeshita, F., Kobiyama, K., Miyawaki, A., Jounai, N., and Okuda, K. (2008). The non-canonical role of Atg family members as suppressors of innate antiviral immune signaling. Autophagy 4, 67.

Tal, M. C., Sasai, M., Lee, H. K., Yordy, B., Shadel, G. S., and Iwasaki, A. (2009). Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. Proc Natl Acad Sci U S A 106, 2770.

Talloczy, Z., Jiang, W., Virgin, H. W. t., Leib, D. A., Scheuner, D., Kaufman, R. J., Eskelinen, E. L., and Levine, B. (2002). Regulation of starvation- and virus-induced autophagy by the eIF2alpha kinase signaling pathway. Proc Natl Acad Sci U S A 99, 190.

Tey, S. K., and Khanna, R. (2012). Autophagy mediates transporter associated with antigen processing-independent presentation of viral epitopes through MHC class I pathway. Blood 120, 994.

Thomas, P. G., Dash, P., Aldridge, J. R., Jr., Ellebedy, A. H., Reynolds, C., Funk, A. J., Martin, W. J., Lamkanfi, M., Webby, R. J., Boyd, K. L., Doherty, P. C., and Kanneganti, T. D. (2009). The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity 30, 566.

Thumm, M., Egner, R., Koch, B., Schlumpberger, M., Straub, M., Veenhuis, M., and Wolf, D. H. (1994). Isolation of autophagocytosis mutants of Saccharomyces cerevisiae. FEBS Lett 349, 275.

Thurston, T. L., Wandel, M. P., von Muhlinen, N., Foeglein, A., and Randow, F. (2012). Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482, 414.

Trombetta, E. S., and Mellman, I. (2005). Cell biology of antigen processing in vitro and in vivo. Annu Rev Immunol 23, 975.

Tsukada, M., and Ohsumi, Y. (1993). Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett 333, 169.

Twitty, C. G., Jensen, S. M., Hu, H. M., and Fox, B. A. (2011). Tumor-derived autophagosome vaccine: induction of cross-protective immune responses against short-lived proteins through a p62-dependent mechanism. Clin Cancer Res 17, 6467.

Uhl, M., Kepp, O., Jusforgues-Saklani, H., Vicencio, J. M., Kroemer, G., and Albert, M. L. (2009). Autophagy within the antigen donor cell facilitates efficient antigen cross-priming of virus-specific $CD8^+$ T cells. Cell Death Differ 16, 991.

van der Kant, R., Fish, A., Janssen, L., Janssen, H., Krom, S., Ho, N., Brummelkamp, T., Carette, J., Rocha, N., and Neefjes, J. (2013). Late endosomal transport and tethering are coupled processes controlled by RILP and the cholesterol sensor ORP1L. J Cell Sci 126, 3462.

Wang, L., Tian, Y., and Ou, J. H. (2015). HCV induces the expression of Rubicon and UVRAG to temporally regulate the maturation of autophagosomes and viral replication. PLoS Pathog 11, e1004764.

Wang, R. C., Wei, Y., An, Z., Zou, Z., Xiao, G., Bhagat, G., White, M., Reichelt, J., and Levine, B. (2012). Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 338, 956.

Wauson, E. M., Dbouk, H. A., Ghosh, A. B., and Cobb, M. H. (2014). G protein-coupled receptors and the regulation of autophagy. Trends Endocrinol Metab 25, 274.

Weidberg, H., Shvets, E., Shpilka, T., Shimron, F., Shinder, V., and Elazar, Z. (2010). LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis. Embo J 29, 1792.

Wilcox, D. R., Wadhwani, N. R., Longnecker, R., and Muller, W. J. (2015). Differential reliance on autophagy for protection from HSV encephalitis between newborns and adults. PLoS Pathog 11, e1004580.

Wu, X., Anderson, J. L., Campbell, E. M., Joseph, A. M., and Hope, T. J. (2006). Proteasome inhibitors uncouple rhesus TRIM5alpha restriction of HIV-1 reverse transcription and infection. Proc Natl Acad Sci U S A 103, 7465.

Xia, M., Gonzalez, P., Li, C., Meng, G., Jiang, A., Wang, H., Gao, Q., Debatin, K. M., Beltinger, C., and Wei, J. (2014). Mitophagy enhances oncolytic measles virus replication by mitigating DDX58/RIG-I-like receptor signaling. J Virol 88, 5152.

Xie, Z., Nair, U., and Klionsky, D. J. (2008). Atg8 Controls Phagophore Expansion during Autophagosome Formation. Mol Biol Cell 19, 3290.

Xu, X., Araki, K., Li, S., Han, J. H., Ye, L., Tan, W. G., Konieczny, B. T., Bruinsma, M. W., Martinez, J., Pearce, E. L., Green, D. R., Jones, D. P., Virgin, H. W., and Ahmed, R. (2014). Autophagy is essential for effector CD8(+) T cell survival and memory formation. Nat Immunol 15, 1152.

Xu, Y., Jagannath, C., Liu, X. D., Sharafkhaneh, A., Kolodziejska, K. E., and Eissa, N. T. (2007). Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity 27, 135.

Ye, W., Xing, Y., Paustian, C., van de Ven, R., Moudgil, T., Hilton, T. L., Fox, B. A., Urba, W. J., Zhao, W., and Hu, H. M. (2014). Cross-presentation of viral antigens in dribbles leads to efficient activation of virus-specific human memory T cells. J Transl Med 12, 100.

Yin, Y., Manoury, B., and Fahraeus, R. (2003). Self-inhibition of synthesis and antigen presentation by Epstein-Barr virus-encoded EBNA1. Science 301, 1371.

Yla-Anttila, P., Vihinen, H., Jokitalo, E., and Eskelinen, E. L. (2009). 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 5, 1180.

Young, A. R., Chan, E. Y., Hu, X. W., Kochl, R., Crawshaw, S. G., High, S., Hailey, D. W., Lippincott-Schwartz, J., and Tooze, S. A. (2006). Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. J Cell Sci 119, 3888.

Zhang, M., Kenny, S., Ge, L., Xu, K., and Schekman, R. (2015). Translocation of interleukin-1beta into a vesicle intermediate in autophagy-mediated secretion. Elife 4, e11205.

Zhong, Y., Wang, Q. J., Li, X., Yan, Y., Backer, J. M., Chait, B. T., Heintz, N., and Yue, Z. (2009). Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. Nat Cell Biol 11, 468.

Zhou, R., Yazdi, A. S., Menu, P., and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. Nature 469, 221.

Tables

Table 1 Receptors for selective autophagy (table is not exhaustive)

Receptor	Cargo	Recognition Signal	Reference
Optineurin	Mitochondria	phospho-ubiquitin	Lazarou Nature 2015
	Bacteria	Ubiquitin	Wild Science 2011
NDP52	Mitochondria	phospho-ubiquitin	Lazarou Nature 2015
	Bacteria	Ubiquitin	Thurston Nat Immun 2009
	damaged endosomes	Galectin-8	Thurston Nat 2012
p62	Bacteria	Ubiquitin	Zheng JI 2009

NIX/BNIP3	Mitochondria	Ser phosphorylation adjacent to LIR	Hamacher-Brady CellMol LifeSci 2015
NBR1	Peroxisomes	Ubiquitin	Deosaran J Cell Sci 2013
FAM134B	ER	direct	Khaminets Nature 2015
SMURF1	Sindbis virus and HSV	unknown	Orvedahl Nature 2011
TRIM5α	HIV	capsid protein p24	Mandell Dev Cell 2014

Figure Legends

Figure 1 Overview of the autophagic machinery. Autophagosome formation initiates at a structure called omegasome at the ER based on nutrient availability. Two protein complexes drive phagophore formation, namely ULK and PI3K complex. The membrane source for elongation of the phagophore is unknown. LC3 becomes conjugated to lipids in the autophagosomal membrane in an ubiquitin-like conjugation cascade. Autophagosomes can take up cytoplasmic material in bulk or in a very selective manner mediated by autophagic receptors like p62, which binds to ubiquitinated cargo. Autophagosomes move bidirectionally along microtubules owing to the action of different RAB7-binding adaptor molecules. After closure of the autophagosome, LC3 coupled to the outer membrane can get recycled through cleavage by ATG4B. The entire process of autophagosome maturation and fusion is controlled by two variants of the PI3K complex. Fusion to the lysosome is mediated by an adaptor molecule, a tethering complex and SNARE proteins. Degraded cargo is shuttled out into the cytoplasm to serve biosynthesis. ER: endoplasmatic reticulum, PE: phosphatidylethanolamine.

Figure 2 Role of Autophagy during anti-viral immune responses. (A) Viral PAMPs are recognized by various PRRs in the host cell leading to the activation of various transcription factors. PRR signaling often leads to the induction of selective autophagy of the virus (xenophagy). Autophagy also plays a regulatory role preventing an excessive activation of the innate immune response. Lastly, autophagy can aid in the unconventional secretion of IL-1β and IL-18. (B) Autophagy, LC3 associated phagocytosis (LAP) and autophagy assisted exocytosis contribute to antigen processing for MHC

presentation. Late endosomal MHC loading compartments receive input from autophagosomes. This leads to MHC class II presentation of autophagic cargo, and even to MHC class I presentation, if classical MHC class I loading in the ER is compromised. LAP phagosomes and secreted vesicles that are dependent on autophagy for their exocytosis also gain access to these MHC loading compartments. (C) Different immune system components act during different phases of viral infection. The early innate immune response is characterized by the production of type I interferons and pro-inflammatory cytokines and by mobilization of e.g. natural killer cells. The adaptive immune response that follows is dominated by $CD8^+$ cytotoxic T cell activity and circulating virus-specific antibodies.

Figure 3 Autophagy manipulation by viruses. Viruses interfere either with autophagosome generation or degradation. While herpesviruses escape their degradation by inhibiting autophagosome formation, many RNA viruses stabilize autophagic membranes for their replication and to facilitate their release from infected cells.

Figure 4 Unconventional use of autophagy proteins. (A) ATG9 plays an unconventional role in inhibiting STING's translocation from the Golgi to cytoplasmic, LC3-positive vesicles. This immune regulatory function prevents the over-activation of TBK1 and IRF3. (B) LC3-associated phagocytosis is triggered by engagement of various surface receptors with their respective ligand. Downstream these single-membrane LC3-positive vesicles may recruit TLR9 containing vesicles or may fuse with antigen loading compartments or lysosomes. (C) UVRAG is a member of the PI3K complex that facilitates autophagosome maturation. In context of the C-VPS complex it mediates early to late endosome transition and various fusion events downstream. Viral evasion strategies lead to increased fusion with late endosome preventing viral degradation in the lysosome. DNA-IC: DNA immune complex, MIIC: MHC class II containing compartment, EE: early endosome, LE: late endosome

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NBR1	Peroxisomes	Ubiquitin	Deosaran J Cell Sci 2013
FAM134B	ER	direct	Khaminets Nature 2015
SMURF1	Sindbis virus and HSV	unknown	Orvedahl Nature 2011
TRIM5α	HIV	capsid protein p24	Mandell Dev Cell 2014

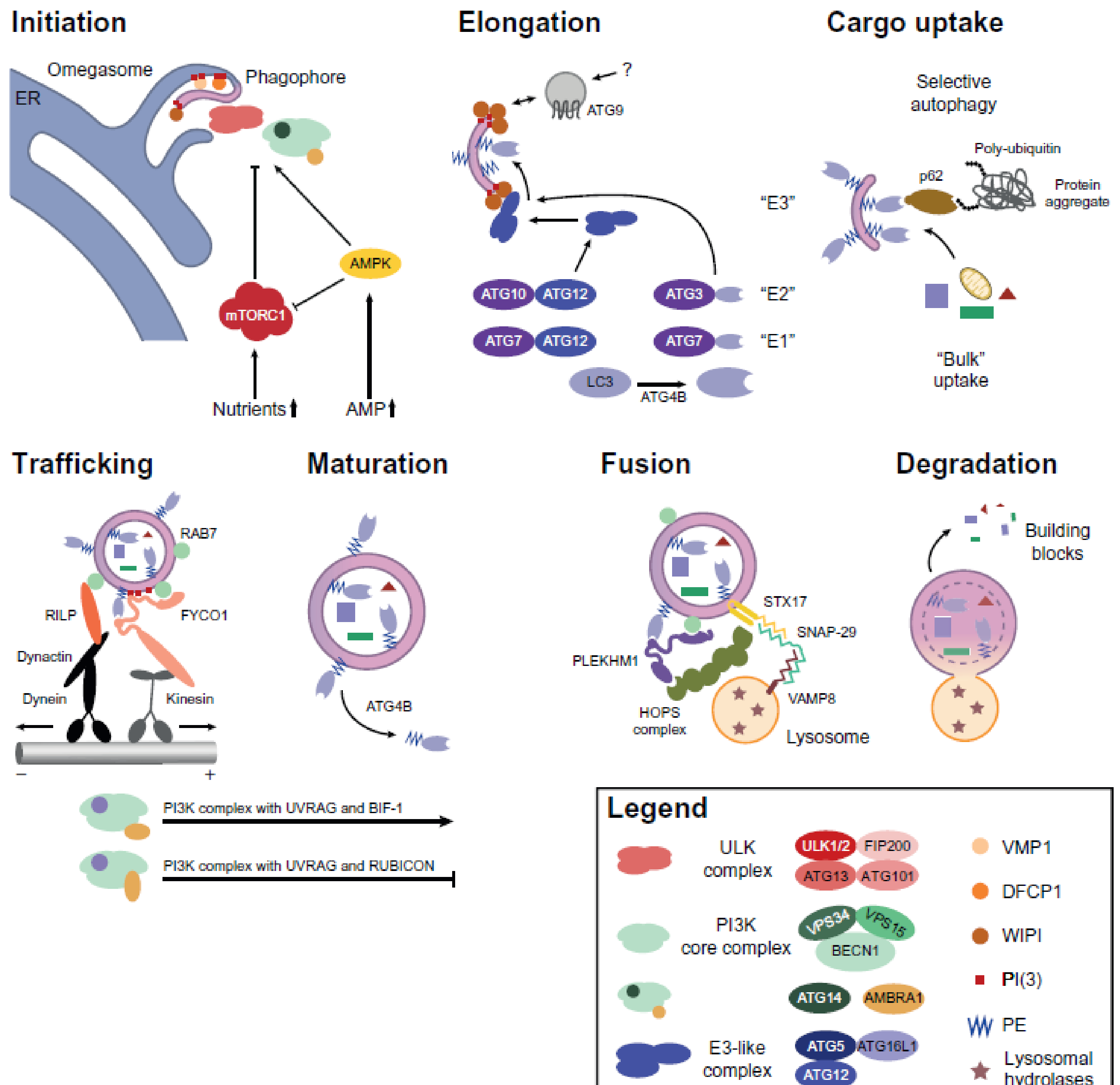

Figure 1

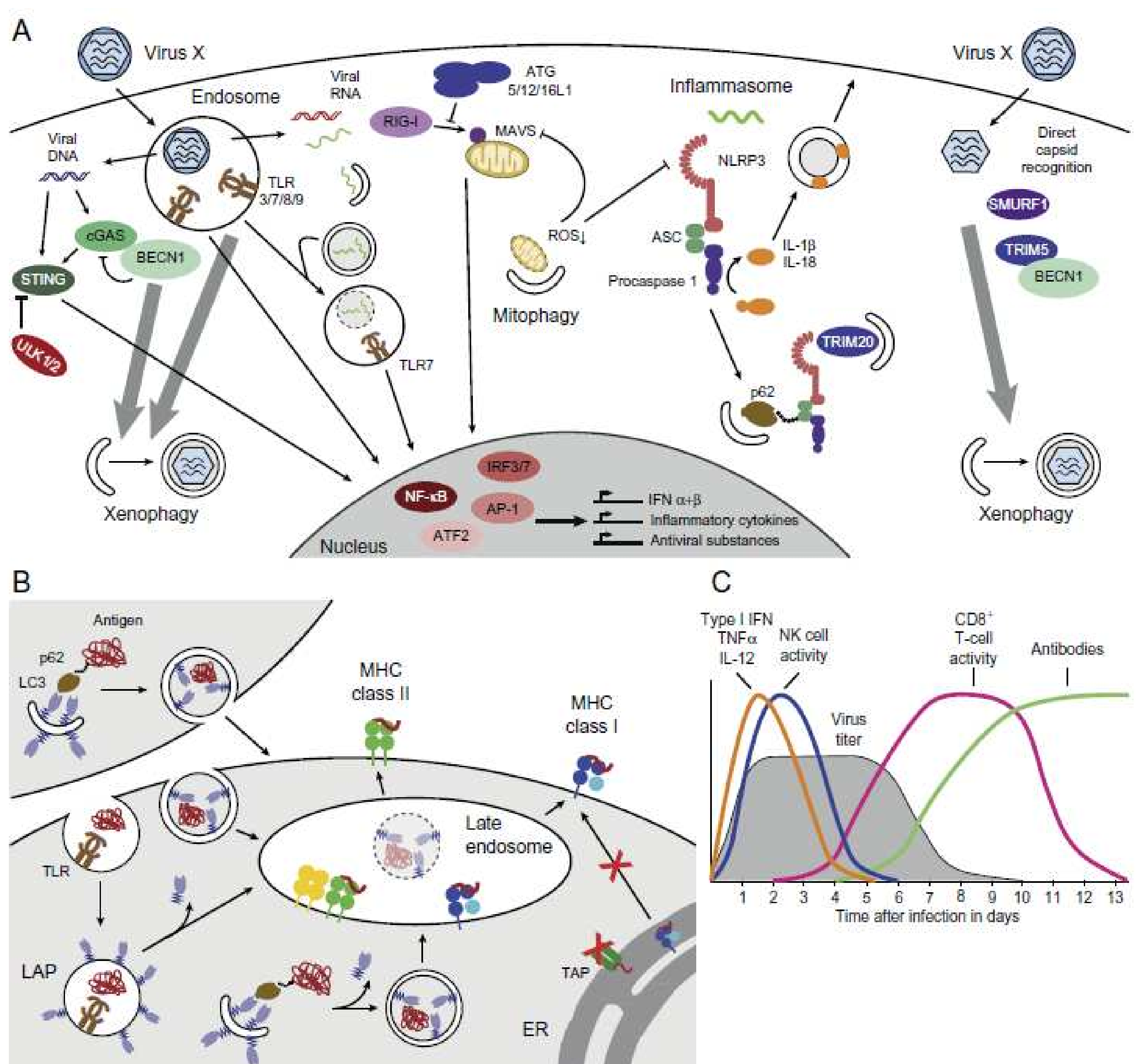

Figure 2

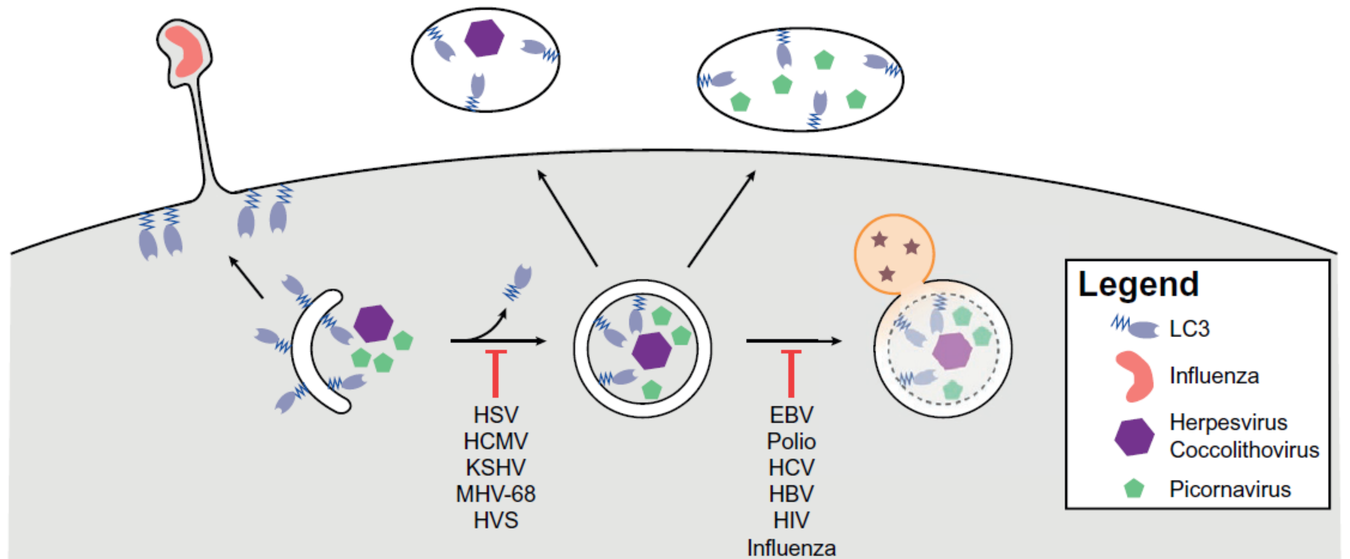

Figure 3

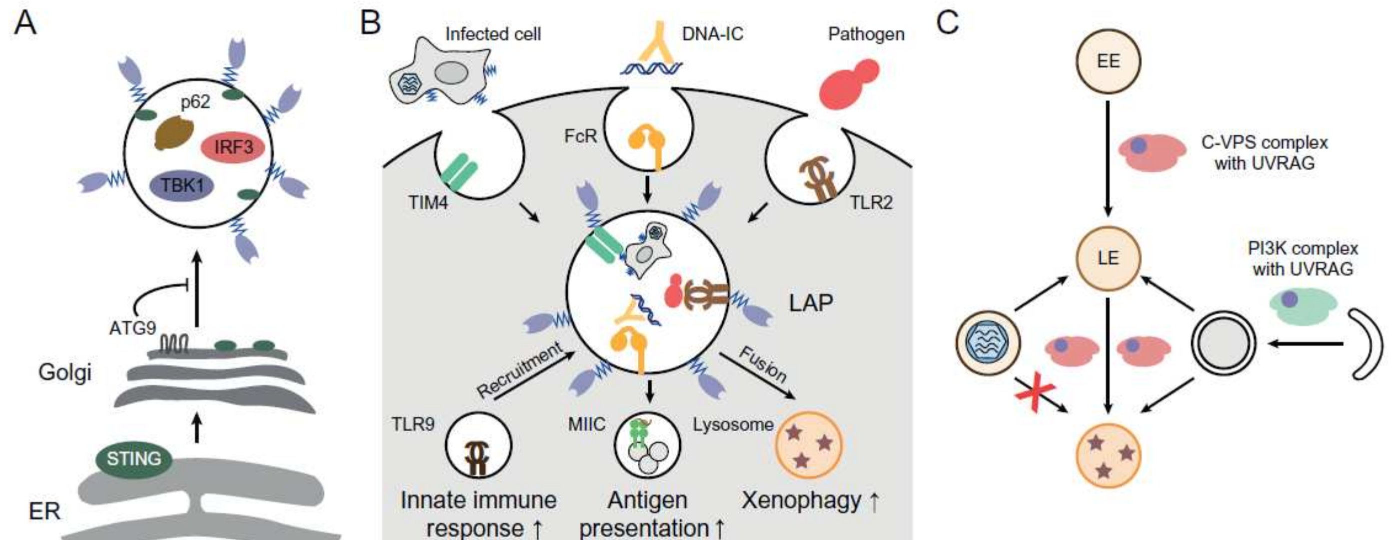

Figure 4