Review of Autophagy-related Approaches for Improving Nutrient Use Efficiency and Crop Yield Protection

Tamar Avin-Wittenberg[1], Frantisek Baluška[2], Peter V. Bozhkov[3], Pernilla H. Elander[3], Alisdair R. Fernie[4], Gad Galili[5], Ammar Hassan[2], Daniel Hofius[6], Erika Isono[7], Romain Le Bars[8], Céline Masclaux-Daubresse[9], Elena A. Minina[2], Hadas Peled-Zehavi[5], Núria Sánchez-Coll[10], Luisa M. Sandalio[11], Béatrice Satiat-Jeunemaitre[8], Agnieszka Sirko[12], Pilar S. Testillano[13], Henri Batoko[14¶]

[1]Department of Plant and Environmental Sciences, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Givat Ram, Jerusalem 9190401, Israel

[2]Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1 D-53115 Bonn, Germany

[3]Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, PO Box 7015, SE-75007 Uppsala, Sweden

[4]Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany

[5]Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot 76100 Israel

[6]Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center of Plant Biology, SE-75007 Uppsala, Sweden

[7]Department of Biology, University of Konstanz, Universitätsstrasse 10, 78464 Konstanz, Germany

[8]Cell Biology Pôle Imagerie-Gif, Institute of Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91 198, Gif-sur-Yvette, France.

[9]INRA-AgroParisTech, Institut Jean-Pierre Bourgin, UMR1318, ERL CNRS 3559, Saclay Plant Sciences, Versailles, France

[10]Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra-Cerdanyola del Valles 08193, Catalonia, Spain

[11]Departmento de Bioquímica, Biología Celular y Molecular de Plantas Experimental del Zaidín, CSIC, 18008 Granada, Spain
[12]Institute of Biochemitry and Biophysics Polish Academy of Sciences, ul. Pawinskiego 5A, 02-106 Warsaw, Poland
[13]Pollen Biotechnology of Crop Plants group, Centro de Investigaciones Biológicas, Biological Research Centre (CIB), C.S.I.C., Ramiro de Maeztu, 9. 28040 Madrid, Spain
[14]Université catholique de Louvain, Institute of Life Sciences, Croix du Sud 4, L7.07.14, 1348 Louvain-la-Neuve, Belgium
¶Corresponding author: henri.batoko@uclouvain.be

Abstract

Autophagy is a eukaryotic catabolic pathway essential for growth and development. In plants, it is activated in response to environmental cues or developmental stimuli. However, in contrast to other eukaryotic systems, we know comparatively little mechanistically, regarding the regulation of this important and complex pathway, or the full complement of the molecular players involved in it. In the framework of the COST (European Cooperation in Science and Technology) action TRANSAUTOPHAGY (2016-2020), we decided to review our current knowledge of autophagy responses in vascular plants, with emphasis on knowledge gaps. We also assess here the potential of translating the acquired knowledge to improve crop plant growth and development in a context of growing societal and environmental challenges for agriculture in the near future.

Introduction

During the life span of a eukaryotic cell, a catabolic pathway known as autophagy degrades dysfunctional or unnecessary cellular components as a way of recycling macromolecules' building blocks and ensuring cellular homeostasis (Klionsky *et al.*, 2016). In essence, autophagy consists in the translocation of cytoplasmic components (cargo) into the vacuole (yeast and plant) or the lysosome (animal) and their subsequent degradation (Li and Vierstra, 2012). In plants, autophagy is a central regulator of fitness, longevity and

fecundity, as well as a major housekeeping mechanism underpinning plant tolerance to various biotic and abiotic stresses (Minina *et al.*, 2018). Plant cells decrease their dependency on external sources of nutrients by recycling their contents *via* autophagy (Guiboileau *et al.*, 2013; Minina *et al.*, 2013b). Furthermore, autophagy increases viability of cells under stress conditions by a quick removal of damaged macromolecules and organelles (Bassham *et al.*, 2006; Li and Vierstra, 2012; Michaeli *et al.*, 2016), modulation of immune response and targeting of virulence factors or entire pathogens (Hafren *et al.*, 2017; Haxim *et al.*, 2017; Lenz *et al.*, 2011). Thus, autophagy defines important agricultural traits, i.e. tolerance to macro-nutrient depletion, drought, heat, oxidative and salt stress, as well as immune response to pathogen infection. Although most of the research so far has been performed in the model plant *Arabidopsis thaliana*, the involvement of autophagy in a variety of agricultural traits generates great interest in the development of tools for efficient modulation of autophagy in plants. In this manuscript, we will review the current knowledge regarding autophagy in plants, its functional mechanisms and physiological roles and highlight possible uses for autophagy manipulation as potential enhancers of plant yield and tolerance.

Types of autophagy in plants

Autophagy can be generically distinguished into microautophagy and macroautophagy (Galluzzi *et al.*, 2017). Other variants of autophagy such as chaperone-mediated autophagy (CMA) (Kaushik and Cuervo, 2012), secretory autophagy (Ponpuak *et al.*, 2015) in mammalian cells, and cytoplasm-to-vacuole transport (CVT) in yeast (Reggiori *et al.*, 2004) are cell type-specific, and have not been described so far in plant cells. Both microautophagy and macroautophagy can be selective or non-selective in plants. Microautophagy is characterized by a direct invagination of the tonoplast (vacuolar membrane) to take up the cellular components to be degraded. A well-described example in plants is the functional accumulation of anthocyanins through microautophagy-derived inclusion bodies in the plant vacuole (Chanoca *et al.*, 2015). Anthocyanins are a diverse family of flavonoid pigments synthesized in the cytoplasm, stored in the vacuole, acting as antioxidants and involved in plant tissues responses to environmental cues. These pigments

are stored in the vacuole as densely packed 3-10 μm vesicles generated through a microautophagy process (Chanoca *et al.*, 2015). The molecular mechanisms of membrane dynamics driving microautophagy are not well understood in plants, but seem not to require any of the gene products involved in macroautophagy. Macroautophagy (hereafter autophagy) is characterized by the *de novo* formation of a double membrane organelle, the autophagosome, wrapping defined cytoplasmic components for degradation. The initiation, elongation, maturation and fusion of the autophagosome with the vacuole is marshalled by a conserved set of proteins encoded by autophagy-related (*ATG*) genes (Tsukada and Ohsumi, 1993). Notably, plant-specific autophagic pathways as defined by the cargo type do exist. A well-described example is chlorophagy or the autophagic degradation of whole chloroplasts (up to 5-10 μm in size, mean volume of 20 μm^3) damaged by UV light (Izumi *et al.*, 2017). The molecular mechanism and the complement of ATG proteins involved in the formation of these uncommonly large autophagosomes may be specific to the plant kingdom.

Core autophagy complexes and their regulation in plants

Autophagy is a tightly regulated cellular response, which can be activated rapidly and transiently in eukaryotic cells. The formation of the autophagosome is a complex, dynamic, and stepwise process resulting in the engulfment of cytoplasmic material and its translocation to the vacuole. The molecular machinery that executes and regulates autophagy was first characterized in yeast (Tsukada and Ohsumi, 1993). About half of the more than 36 *ATG* genes characterized to date encode core autophagy proteins, and appear to be well conserved in most studied multicellular organisms, including plants (Galluzzi *et al.*, 2017; Klionsky *et al.*, 2016). Autophagy is a powerful catabolic process that needs to be quickly fine-tuned to fit the temporary requirements of cells under variable conditions. In animal cells, autophagy seems to oscillate with astonishingly high frequency (Nazio *et al.*, 2016). To prevent unwanted autophagic activity, most of the ATG proteins are synthesized in an inactive form and require activation by post-translational modification and recruitment into complexes (Ohsumi, 2014). Activation of autophagy is regulated by sensors of cellular nutrient state (Liu and Bassham, 2010) and stress (Minina *et al.*, 2013a; Wang *et al.*, 2015a; Yang *et al.*, 2016).The pre-autophagosomal membrane or phagophore

is initiated in response to a given internal or external cellular stimulus, then elongates and enwraps the cytoplasmic cargo. The closed phagophore matures into an autophagosome, and then fuses with the vacuole/lysosome. Each of the mentioned steps is under the control of specific autophagy complexes made of core autophagy proteins, whose assembly, subsequent subcellular localization and activity are directly or indirectly regulated by stress signaling pathways. Four main complexes are known to be required for autophagosome initiation and formation, namely the ATG1 complex, the VPS34 complex, the ATG9 complex, and the ATG8 conjugation systems (Figure 1).

The ATG1 complex is thought to be essential in transmitting stress signals to the site where the autophagosome will be formed, most likely at an organelle contact site involving the endoplasmic reticulum (ER) (Antonioli *et al.*, 2017; He and Klionsky, 2009; Nascimbeni *et al.*, 2017). In yeast and mammalian cells, the ATG1 complex is a trimeric heterocomplex made of a catalytic subunit (ATG1/ULK, a serine/threonine kinase), regulatory subunits (ATG13 and ATG101), and scaffold subunits (ATG11 or ATG17 in yeast, and FIP200/RB1CC1 RB1 inducible coiled-coil 1 in animals) (Galluzzi *et al.*, 2017). The structure, function and regulation of the ATG1 complex are not well understood in plants. The Arabidopsis genome, for example, encodes three full-length ATG1 proteins (ATG1a, locus AT3G61960; ATG1b, locus AT3G53960; ATG1c, locus AT2G37840), and a C-terminus truncated ATG1 variant called ATG1t (locus AT1G49180) (Suttangkakul *et al.*, 2011), whose function is not yet clear. The Arabidopsis genome also encodes two functional ATG13 homologues, ATG13a and ATG13b, and a single ATG101 (Suttangkakul *et al.*, 2011). Intriguingly, no functional or structural homologue of ATG17/FIP200 has been found yet in deciphered plant genomes. A potentially bifunctional protein containing structural domains related to yeast ATG11 and ATG17 is present in plants. In Arabidopsis, this protein was dubbed an ATG11 homologue since it is required for selective degradation of mitochondria *via* autophagy (Li *et al.*, 2014). However, whether the plant ATG11-related protein acts as a scaffold protein within a *bona fide* ATG1 complex remains to be clarified. If the plant ATG11-related protein functions only in selective autophagy, the plant scaffold protein required for non-selective autophagy is still to be identified.

The clustering and activation of the ATG1 complex at the phagophore initiates the recruitment of other autophagy complexes and in particular the class III VPS34 complex. The class III VPS34 complex involved in autophagy contains the catalytic subunit PI3kinase (PI3K), the regulatory subunits ATG6/Beclin-1 and ATG14, and the scaffold subunit VPS15. As compared to other multicellular organisms, plants have the peculiarity of expressing a single and essential PI3K of the class III type. The structure of the VPS34 complex involved in autophagy is not known in plants. Remarkably, ATG14 is absent in the plant lineage. Yeast ATG14 and its functional homologues in other eukaryotic systems only share resemblance at their N-terminal coil-coiled domain (~200 first amino acids), whereas the C-terminus of these proteins appearing highly divergent (Itakura *et al.*, 2008). ATG14 is known to determine the localization of the VPS34 complex, and to be required for both basal and induced autophagy in yeast and animals (Diao *et al.*, 2015; Fan *et al.*, 2011). Phosphorylation of ATG14 by the ATG1 kinase activates the catalytic activity of PI3K, which catalyzes the production of the membrane lipid PI3P (phosphatidylinositol-3-phosphate) essential for phagophore initiation and expansion (Baskaran *et al.*, 2014). Whether a functional counterpart of ATG14 exists in plants awaits experimental evidence.

The phagophore initiation and expansion requires input of specific lipids and proteins. The membrane source of these materials is still under debate, but they are more likely channeled to the site of autophagosome formation through ATG9-containing vesicles (Abada and Elazar, 2014; Karanasios *et al.*, 2016). ATG9 is the only transmembrane protein among all known ATG proteins (Reggiori *et al.*, 2004). The heterodimer complex ATG2-ATG18 regulates the polytopic ATG9 vesicle-mediated cycling and tethering to and from the growing phagophore. Although plants seem to encode single ATG9 and ATG2 homologues, a diversified multigenic family encodes the PI3P-binding ATG18-related proteins (up to 8 in Arabidopsis as compared to 1 in yeast and 4 in mammals). The resting cellular localization of ATG9 and the full complement of its interacting partners during autophagy-dependent membrane dynamics are not yet understood in plants.

The phagophore membrane expansion also requires the recruitment of lipidated ATG8-related protein. Soluble ubiquitin-like ATG8 becomes membrane-anchored through conjugation to the membrane lipid PE (phosphatidylethanolamine). This modification occurs through an ubiquitylation-like cascade regulated by the protease ATG4, the E1

activating enzyme ATG7, the E2 conjugating enzyme ATG3, and the E3 ligase complex comprising ATG5/ATG12/ATG16 (Pengo *et al.*, 2017; Sanchez-Wandelmer *et al.*, 2017). Apart from *bona fide* ATG16, whose plant orthologue has not yet been characterized, all the other components of this cascade are expressed and active in plants. ATG8-related members are relatively more diversified in plants, with some C-terminally truncated isoforms that are unique to plants (Bassham *et al.*, 2006; Li *et al.*, 2016a).

Autophagy is regulated at many steps through post-translational modification (PTM) such as ubiquitylation, phosphorylation, acetylation, glycosylation and lipidation of ATG proteins. Many ATG proteins in other organisms were shown to undergo complex and multi-layered regulation through PTM. ATG proteins can be differently modified at multiple sites whereby one type of PTM can depend on another modification. An example is the phosphorylation-dependent ubiquitylation that leads to degradation of target proteins(Lin *et al.*, 2002). By acting as a degradation signal, ubiquitylation not only regulate cargo of selective autophagy, but also the autophagy machinery itself. The abundance of mammalian ATG1/ULK1, ATG6/VPS30/beclin-1 (BECN1) and ATG12, for example, were reported to be regulated by ubiquitylation. ULK1 controls the autophagic flux together with ATG13 and is ubiquitylated by the E3 ligase NEDD4L (Nazio *et al.*, 2016) making NEDD4L a negative regulator of autophagy. BECN1, a positive regulator of autophagy induction, is a target of multiple E3 ligases (Shi and Kehrl, 2010; Xia *et al.*, 2013; Xu *et al.*, 2014). By removing the ubiquitin chains, deubiquitylating enzymes such as USP10, USP13, USP14 and USP19 can counteract the E3 ligase activity and rescue BECN1 from degradation, and thus act as positive regulators of autophagy (Jin *et al.*, 2016; Liu *et al.*, 2011; Xu *et al.*, 2016). These recent studies reveal molecular details of a tight regulation of autophagic activities through PTM. Since not all of these regulators are conserved in plants, whether and how PTM regulates plant ATG proteins awaits intensive future studies.

Maturation and fusion of the autophagosome with the lytic compartment involves vectorial movement of the matured autophagosome toward the vacuole in plants, and specific tethering of the autophagosome to the tonoplast. F-actin nucleating and branching ARP2/3 complex was shown in yeast to be associated to the autophagosome (Reggiori *et al.*, 2005). In mammalian cells, WASP homolog associated with actin, membranes and microtubules

(WHAMM), WAS protein family homolog (WASH) and junction-mediating and regulatory protein (JMY) were reported to regulate autophagy (Coutts and La Thangue, 2015; Kast *et al.*, 2015; King *et al.*, 2013; Xia *et al.*, 2013; Zavodszky *et al.*, 2014). In plants, only the WASP family verprolin (WAVE) homologous complex has been shown to be involved in autophagosome movement within the cytoplasm (Wang *et al.*, 2016a). One of the WAVE subunits, NAP1, changes its localization from the cytoplasm to ER membrane under mechanical stress (Wang *et al.*, 2016a). This localization change triggers the ARP2/3 dependent F-actin nucleation on the phagophore, which is important for its expansion and ultimately for the maturation of the autophagosome (Wang *et al.*, 2017a; Wang *et al.*, 2016a). Loss-of-function *nap1* mutant Arabidopsis seedlings (lacking a functional WAVE complex) form less autophagosomes and are more sensitive to salt and nitrogen-deficiency stresses (Wang *et al.*, 2017a; Wang *et al.*, 2016a) .

Selective autophagy in plants

Autophagy was initially considered a bulk, non-selective process. It later became evident that autophagy selectively degrades diverse cellular cargoes under various conditions (Anding and Baehrecke, 2017; Li and Vierstra, 2012; Michaeli *et al.*, 2016; Veljanovski and Batoko, 2014; Yang and Bassham, 2015). Selective autophagy typically utilizes cargo receptors that directly or indirectly bind specific cargo, and tether it to the forming autophagosome through interaction with core autophagy proteins (mainly ATG8) (Farre and Subramani, 2016; Kellner *et al.*, 2017; Zaffagnini and Martens, 2016). In mammals, multiple cargo receptors were identified, including p62/ SQSTM1 and NBR1 that were implicated in the selective autophagy of protein aggregates and organelles (Anding and Baehrecke, 2017; Zaffagnini and Martens, 2016). p62 and NBR1 bind both ubiquitin and the mammalian ATG8 homologue, LC3, thus docking ubiquitinated substrates to the autophagosome. Arabidopsis NBR1 and its tobacco homologue JOKA2 are functional hybrids of mammalian p62 and NBR1, capable of binding ATG8 and ubiquitin. Both were shown to play a role in nutrient deficiency and abiotic stress tolerance (Hafren *et al.*, 2017; Svenning *et al.*, 2011; Zhou *et al.*, 2013; Zhou *et al.*, 2014a; Zientara-Rytter *et al.*, 2011).

A fascinating cross talk between the major cellular degradation pathways, autophagy and

the proteasome, was uncovered with the discovery of both non-selective (starvation-induced) and selective autophagy mediated degradation of the 26S proteasome in Arabidopsis (Marshall *et al.*, 2015). While the proteasome subunit RPN10 was previously shown to facilitate the recognition of ubiquitinated targets, Marshall *et al.* found that it can also bind ATG8 (Marshall *et al.*, 2015). They further demonstrated that RPN10 is needed for inhibition-induced selective degradation of inactive 26S proteasome complexes (proteaphagy), suggesting a role for RPN10 as an autophagy cargo receptor. Intriguingly, in a recent study, mammalian p62 was shown to mediate selective starvation-induced autophagosomal uptake of proteasomes (Cohen-Kaplan *et al.*, 2016; Marshall *et al.*, 2016). Whether NBR1 or other plant cargo receptors have similar functions awaits further research.

Recent studies have demonstrated the selective degradation of peroxisomes by autophagy (Pexophagy). Peroxisomes are highly dynamic organelles, housing oxidative metabolic pathways, such as photorespiration and fatty acid β-oxidation, produce reactive oxygen species and contain important antioxidative components (Sandalio and Romero-Puertas, 2015). During seedling establishment, in a light-dependent manner, there is a functional transition from glyoxysomes, peroxisomes present in seeds and harboring the glyoxylate cycle and β-oxidation, to leaf type peroxisomes, containing photorespiration enzymes. Recent evidence shows that pexophagy takes place during this metabolic remodeling combined with peroxisomal protease LON2 activity (Young and Bartel, 2016). Pexophagy also mediates the turnover of peroxisomes damaged by H_2O_2 accumulation in old tissues, , under favorable and stress conditions, regulating the quality and number of peroxisomes (Shibata *et al.*, 2014; Shibata *et al.*, 2013). Pexophagy occurs at a higher rate in green tissues and appears to be more marked than other types of selective autophagy due to the highly oxidative peroxisomal metabolism (Yoshimoto *et al.*, 2014). Pexophagy is aided by autophagy receptors, although the plant autophagy receptor/adaptor protein linking ATG8 to damaged peroxisomes has not been identified. Some evidence, though controversial, suggests the involvement of NBR1 (Zhou *et al.*, 2013). Recently, 9 peroxines (PEXs, peroxisomal membrane proteins) have been identified as possible ATG8 binding proteins, two of which, AtPEX6 and AtPEX10, were shown to interact with ATG8 by bimolecular fluorescence complementation (BiFC) (Xie *et al.*, 2016). The signal involved in triggering

pexophagy has not yet been identified, although oxidized catalase has, controversially, been proposed as a possible candidate (Shibata *et al.*, 2013).

Chloroplasts represent an interesting case study for selective autophagy in plants, as they have unique turnover demands due to the photosynthetic electron chain and its oxidative byproducts. In addition, chloroplasts are the major nitrogen reservoir in mesophyll cells and thus important for nutrient recycling (Ishida *et al.*, 2014). Early studies suggested that the vacuole plays a role in chloroplast recycling (Minamikawa *et al.*, 2001; Wittenbach *et al.*, 1982) and autophagy-related and -unrelated pathways were later implicated in the degradation of chloroplast components (chlorophagy) (Ishida *et al.*, 2014; Izumi *et al.*, 2017; Martinez *et al.*, 2008; Michaeli *et al.*, 2014; Wang and Blumwald, 2014; Wang *et al.*, 2013; Xie *et al.*, 2016). Two types of vesicles, Rubisco containing bodies (RCBs) and ATG8-interacting protein1 (ATI1) bodies, were shown to participate in chlorophagy and are induced during senescence and abiotic stresses (Chiba *et al.*, 2003; Dong and Chen, 2013; Honig *et al.*, 2012; Ishida *et al.*, 2008; Michaeli *et al.*, 2014; Wada *et al.*, 2009; Yamane *et al.*, 2012). Interestingly, autophagy is also involved in the remobilization of transitory starch from chloroplasts to vacuoles via Small Starch Granules like structures (SSGLs) (Wang *et al.*, 2013). RCBs were characterized in Arabidopsis, tobacco, wheat and rice (Chiba *et al.*, 2003; Ishida *et al.*, 2008; Izumi *et al.*, 2015; Ono *et al.*, 2013; Prins *et al.*, 2008; Wada *et al.*, 2009). They were shown to deliver Rubisco and other stromal proteins to the vacuole, though their mode of cargo recognition is not known (Chiba *et al.*, 2003). ATI1 is a plant specific ATG8-binding protein localized in the ER and chloroplasts (Honig *et al.*, 2012; Michaeli *et al.*, 2014). In Arabidopsis, ATI1-labeled vesicles (ATI1-bodies) were shown to deliver plastid-targeted GFP to the vacuole. ATI1 can bind both stromal and membrane-bound chloroplast proteins, suggesting that the cargo of ATI1-bodies differ from that of RCBs (Michaeli *et al.*, 2014). Another difference is that RCBs are associated with chloroplast stromules, while ATI1 bodies initiate inside the chloroplast. In addition, the release of RCBs for the chloroplast is dependent on the ATG machinery , while ATI1 bodies bud from it in an ATG-independent manner, though their delivery to the vacuole requires active autophagy machinery (Ishida *et al.*, 2014; Michaeli *et al.*, 2014). Interestingly, two ESCRT-III subunit paralogs, were implicated in the delivery of RCBs to the vacuole, suggesting a cross talk between chlorophagy and endomembrane trafficking

events (Spitzer *et al.*, 2015)(Kalinowska and Isono, JXB review 2017, accepted for this issue). Another chlorophagy pathway involves the vacuolar delivery of entire shrunken chloroplasts (Wada *et al.*, 2009). This pathway is induced upon UV-B or high light treatments (Izumi *et al.*, 2017). Information regarding selective autophagy of other types of plastids is still limited, but there is evidence for RCB-like and entire plastid autophagy in roots of Arabidopsis and rice (Izumi *et al.*, 2015; Nakayama *et al.*, 2012).

Mitophagy, the selective degradation of mitochondria by autophagy, was only recently identified in plants with the characterization of an Arabidopsis ATG11-related protein. Similarly to yeast, the Arabidopsis ATG11-related protein participates in the selective clearance of mitochondria. Lack of ATG11-related protein in mutant Arabidopsis plant resulted in mitochondria accumulation (Li *et al.*, 2014). However, a plant homolog to the yeast ATG32, which recruits ATG11 to damaged mitochondria has not been identified (Anding and Baehrecke, 2017; Li *et al.*, 2014). Plants also lack homologues of animal mitophagy receptors such as the BCL2 interacting protein (BNIP) family members. ER-phagy (reticulophagy), the selective degradation of ER by autophagy, is induced by ER stress resulting from accumulation of unfolded or misfolded proteins in the ER, similarly to yeast and mammals (Anding and Baehrecke, 2017; Dikic, 2017; Liu *et al.*, 2012; Yang *et al.*, 2016). This process requires the ER stress sensor IRE1b, but the downstream factors remain unknown (Liu *et al.*, 2012). In Arabidopsis, as in other organisms, ribophagy, the autophagic degradation of rRNA, requires the nonspecific T2 endoribunuclease RNS2 (Bassham and MacIntosh, 2017; Floyd *et al.*, 2015; Floyd *et al.*, 2017; Hillwig *et al.*, 2011). A differential role was suggested for ATG5 and ATG9 in this process, but the exact mechanism of the selection of rRNA for degradation is still unknown (Floyd *et al.*, 2015)

Methods of monitoring and manipulating autophagy in plants

Monitoring autophagy in various systems has been previously described (Klionsky *et al.*, 2016). However, plant systems pose unique challenges requiring special modification. Here we summarize some of the methods commonly used to assess and modulate autophagy in plants, adding to some other excellent reviews on the topic (Bassham, 2015).

Monitoring autophagy in plants by biochemical analysis

Assessing the formation and degradation of autophagosomes can be performed using western blot analysis. Two main approaches exist for this analysis: (i) ATG8 lipidation assay and (ii) free GFP release assay from expressed GFP-ATG8 chimera.

ATG8 is incorporated to the growing phagophore membranes through a C-terminal post-translational modification (processing followed by lipidation). Assessing the rate of ATG8 lipidation can be used as a measure of autophagosome formation. The lipidated and non-lipidated forms of ATG8 can be separated by SDS-PAGE in presence of 6 M urea followed by western blotting (Chung *et al.*, 2009; Thompson *et al.*, 2005). Expression of GFP-ATG8 can be used to visualize autophagosomes using confocal microscopy, as discussed later in this section. In addition, it is also possible to monitor the release of free GFP after proteolysis of GFP-ATG8 in the vacuole. The level of free GFP released from ATG8 indicates the relative rate of autophagosome degradation and can be used as a measure of autophagic flux (Li *et al.*, 2015; Slavikova *et al.*, 2008). GFP-ATG8 degradation in the vacuole is drastically reduced by Concanamycin A (ConcA) treatment. ConcA increases the pH of the vacuolar lumen by inhibiting the activity of the vacuolar H^+-ATPase. Therefore, ConcA treatment can result in autophagosomal bodies accumulating in the vacuole, hence reducing the proteolysis of expressed GFP-ATG8.

Imaging approaches to study plant autophagy

Live imaging of autophagosomes in plants requires both specific reporters and an adequate light microscope (LM) configuration. Multiple organic dyes such as LysoTracker (Liu *et al.*, 2005) and Monodansylcadaverin (MDC) (Contento *et al.*, 2005), have been used to label autophagosomes, based on the presumed acidity of the autophagic interior. However, their selectivity for autophagic compartments is still questionable (Klionsky *et al.*, 2016; Mizushima, 2004). Fluorescently-tagged ATG proteins are more frequently used as autophagosome markers, allowing a specific identification of autophagosomes at different stages of their maturation (Le Bars *et al.*, 2014; Suttangkakul *et al.*, 2011).

Tracking autophagosome formation and dynamics within plant cells may be complicated because : i) the lifetime of the process is very short, ii) ATG proteins are only transiently associated with the autophagosomal membranes, iii) low expression levels of potential marker proteins and their high dynamics in certain cell types. To circumvent these

limitations, one possibility is to use a light microscope equipped with highly sensitive detectors and image acquisition at a high frame rate. These conditions are met using confocal laser scanning microscopes with a resonant scanner, or a spinning disk microscope whose high-speed acquisition rate can also contribute in lowering the phototoxic effect of the imaging process (Figure 2a).

It is worth mentioning that mechanical stress could arise from tissue preparation and mounting between the microscope slide and coverslip conducive of autophagy induction (Wang *et al.*, 2016a). Having a spacer between the coverslip and the slide and performing the microscopic observations immediately following mounting can alleviate these unwanted artefacts in the experimental design. Rootchip (Grossmann *et al.*, 2011) can be a good strategy to allow long-term observation of Arabidopsis roots without affecting autophagy.

Higher resolution autophagic structures can be visualized with transmission electron microscopy (TEM) (Figure 2b-c). Correlative light and electron microscopy (CLEM) protocols, allowing LM and TEM observations of the same sample can be used to combine the localization of ATG proteins with light microscopy and the identification of the labelled membranes with TEM (Marion *et al.*, 2017).

Indirect (using anti-GFP antibodies) (Figure 2b) or direct (using specific antibodies against plant ATG8) (Chung *et al.*, 2010) TEM immunogold labelling of ATG8 can provide ultrastructural details of ATG8 membrane-bound structures including autophagosomes. A convenient and feasible processing method for ATG8 immunogold labeling is freeze-substitution (FS) followed by cryoembedding in an acrylic resin (Figure 2c). Several protocols of FS have been developed, providing excellent ultrastructure and high sensitivity immunogold labelling of various antigens, including membrane-bound molecules (Andreu *et al.*, 2007; Bernal *et al.*, 2007; Derrien *et al.*, 2012; Segui-Simarro *et al.*, 2011; Segui-Simarro *et al.*, 2003). This strategy has revealed the localization of ATG8 in autophagosomes and autolysosomes in various plant cells and tissues such as maize aleurone (Reyes *et al.*, 2011), Arabidopsis root (Zhuang *et al.*, 2013), or *Brassica napus* tapetum (Figure 2c), a tissue with high autophagy activity during late pollen development (Hanamata *et al.*, 2014; Papini *et al.*, 2014). The development of antibodies against plant

ATG proteins, with high specificity and sensitivity, will help to identify the components and ultrastructural organization of autophagic structures in diverse plant cells and systems.

Approaches for manipulating plant autophagy

Since autophagy is a very dynamic process, it needs to be and is in fact tightly regulated at multiple levels: transcriptional, post-transcriptional, translational and post-translational (Feng *et al.*, 2015; Kraft *et al.*, 2008)

Targeting transcriptional regulation of plant *ATG* genes

Some ATG proteins are either actively incorporated into autophagosomes as their integral part or are engulfed together with the cargo destined for degradation (Nakatogawa, 2013; Nakatogawa *et al.*, 2012). Autophagy is constitutively active at the basal level in most types of plant cells, playing a housekeeping role. Hence, *ATG* genes are constitutively transcribed, albeit at lower levels (Pu *et al.*, 2017). Interestingly, expression of multiple plant *ATG* genes goes up under stress, e.g. under starvation conditions, coinciding with upregulation of autophagic activity (Chung *et al.*, 2010; Minina *et al.*, 2013b; Rose *et al.*, 2006). Thus, identification of master regulators influencing the expression of *ATG* genes is an important step towards the development of autophagy-modulating tools. Multiple transcription factors regulating *ATG* gene expression in animal cells have already been identified (Feng *et al.*, 2015). Although there is no doubt that such transcription factors also exist in plants, information about them is still very scarce. For example, it has been demonstrated that induced expression of Arabidopsis' *ATG* genes upon *Botrytis cinerea* infection is directly mediated by the transcriptional activator AtWRKY33 (Lai *et al.*, 2011). In addition, the tomato transcription factor HsFA1a binds the promoters of *ATG10* and *ATG18f* to activate their transcription upon drought stress (Wang *et al.*, 2015a).

To date, phenotypic studies of the role of autophagy in plants have been based on comparing the performance of either *ATG*-knockout or knockdown lines to wild–type plants under various unfavorable conditions (Kim *et al.*, 2012). All these studies collectively indicate a potential benefit of upregulated autophagy for stress tolerance and plant fitness. While ectopic overexpression of *ATG* genes in yeast did not seem to have an effect on autophagic activity (Ma *et al.*, 2007), a growing body of evidence indicates that

overexpression of *ATG* genes might be successfully used for upregulation of autophagy in other model organisms, including plants (Minina *et al.*, 2018; Pyo *et al.*, 2013; Scott *et al.*, 2007; Wang P *et al.*, 2016; Wang *et al.*, 2017b; Xia *et al.*, 2012). These results indicate that the level of the core ATG proteins are a limiting factor of autophagic activity in plant and animal cells, but not in yeast. A possible explanation of this phenomenon is the difference in the number of phagophore assembly sites (PAS), where the core ATG proteins are active. While yeast has a single PAS, animal and plant cells do not seem to have a limit in the number of PASes. Thus, availability of a higher amount of the core ATG proteins might stimulate the formation of a higher number of PASes, leading to increase formation of autophagosomes (Minina *et al.*, 2018). The predicted benefit of enhanced autophagy for plant fitness, fecundity, biomass and stress tolerance has been described in the recent studies (Minina *et al.*, 2018; Wang *et al.*, 2016b). Further development of this approach is required as there might be penalties for constitutive upregulation of plant autophagy in most of the tissues as well as benefits of tissue/organ-specific stimulation of autophagic activity.

Targeting post-transcriptional regulation of plant *ATG* genes

Although multiple examples of miRNA regulating autophagy are known for animal models (Feng *et al.*, 2015), almost nothing is known about post-transcriptional regulation of autophagy in plants. Indirect evidence of possible regulation of autophagy by miRNA *via* the stress sensor SnRK1 was demonstrated in the study by Confraria *et al* (Confraria *et al.*, 2013). So far, post-transcriptional silencing of plant *ATG* genes has only been implemented by using artificial *ATG*-specific RNAi constructs (Kim *et al.*, 2012).

Targeting translational regulation of plant ATG genes

Under stress conditions, autophagy degrades cytoplasmic content together with ribosomes, thus downregulating the translation of most mRNAs, including ATG mRNAs (Bassham and MacIntosh, 2017; Kraft *et al.*, 2008). Importantly, selective degradation of ribosomes, ribophagy, under normal conditions positively affects the efficacy of the translational machinery by controlling ribosome quality (Mathis *et al.*, 2017). Artificial modulation of autophagy at the translational level has not yet been attempted due to numerous challenges regarding the specificity of this approach.

453 Pharmacological modulation of plant autophagy

454 As compared to animals, pharmacological manipulation of autophagy in plant has not been
455 comprehensively tested in part due to poor cellular accessibility of many of the described
456 chemical modulators. Paradoxically, some of the natural chemicals tested for their
457 modulation of animal cell autophagy, are plant-derived and we know nothing about their
458 potential effect on plant autophagy (Fleming *et al.*, 2011; Vakifahmetoglu-Norberg *et al.*,
459 2015; Wang *et al.*, 2017c). There are several compounds that have been demonstrated to
460 either inhibit or stimulate plant autophagy ((Klionsky *et al.*, 2016), Table 1). Drug
461 treatment can be a quick and a relatively easy method to modulate autophagy activity in
462 plants. The important disadvantages of pharmacological modulation of plant autophagy are
463 potential off-target effects of the drugs currently available (Table 1), issues with drug
464 stability and tissue/cell-permeability. Nevertheless, this approach has a very important
465 practical benefit, as it may be applicable for agricultural purposes in the countries that do
466 not allow cultivation of genetically modified organisms.

467

468 **Autophagy responses to abiotic stress in plant**

469 Plant stress has been defined by Lichtenthaler (1996) as "any unfavorable condition or
470 substrate that affects or blocks a plant metabolism, growth or development". A common
471 feature of abiotic stresses such as high salinity, drought and osmotic stress is their ability
472 to induce, at the cellular level, a transient or permanent physiological water deficit,
473 conducive of energy limitation in plants. Low energy level in plant tissues is sensed by a
474 subfamily of serine/threonine kinases known as SnRK1 (SNF1-related kinase),
475 homologous to the yeast SNF1 (Sucrose Non-Fermenting-1) and the animal AMPK
476 (Adenosine MonoPhosphate-activated protein Kinase). Plant SnRK1 act as metabolite
477 sensors to constantly adapt metabolism to the supply of, and demand for, energy, and are
478 central integrators of a transcriptional network for stress and energy signaling (Bakshi *et*
479 *al.*, 2017; Emanuelle *et al.*, 2015; Jossier *et al.*, 2009; Nukarinen *et al.*, 2016). SnRK1-
480 dependent restoration of energy homeostasis and promotion of tolerance to adverse
481 conditions is partly achieved through an induction of catabolic processes and a general
482 repression of anabolism (Emanuelle *et al.*, 2015; Soto-Burgos and Bassham, 2017).

Multitudes of unrelated cellular pathways converge on the autophagy machinery to signal a diversity of stimuli. Indeed, activated SnRK1 induces the catabolic pathway autophagy by inhibiting its negative regulator TOR (Target Of Rapamycin) complex in plants (Chen *et al.*, 2017; Soto-Burgos and Bassham, 2017). A crucial feature of autophagy is that it is a highly regulated and dynamic process, able to sense intracellular stress within minutes and rapidly initiate an appropriate response to cope with the damage (Antonioli *et al.*, 2017). High salinity and osmotic stress induce autophagy in plant tissues within a couple of hours of incubation into stress-induction medium (Liu *et al.*, 2009; Vanhee *et al.*, 2011b). Accordingly, many core *ATG* genes are transcriptionally upregulated by various abiotic stresses (Bassham *et al.*, 2006; Wang *et al.*, 2015a; Zhou *et al.*, 2014a). Conversely, autophagy-deficient plants are more sensitive to abiotic stresses (Liu *et al.*, 2009). Recent evidence also suggest that ectopic overexpression of defined plant core *ATG* genes can confer tolerance to various types of stresses and improve growth performance under nutrient starvation conditions (Minina *et al.*, 2018; Wang *et al.*, 2017b)

The plant adaptation responses to abiotic stresses involve phytohormones-dependent signaling cascades, including that of the stress hormone abscisic acid (a growth negative regulator) and that of brassinosteroid (a growth promoting regulator) to reprogram its metabolism (Mair *et al.*, 2015). When subjected to an abiotic stress, plants have to balance between maintaining growth and competitiveness on the one hand, and ensuring survival on the other hand (Claeys and Inze, 2013). This delicate and vital process involves hormone-regulated master regulators, some of which have been characterized recently.

ABI1 (ABA insensitive 1) and PP2CA (protein phosphatase 2C-A) are negative regulators of ABA-dependent signaling, and the two phosphatases were shown to dephosphorylate and inactivate SnRK1. The repressive action of protein phosphatases, established negative regulators of the ABA signaling pathway, is blocked by their ABA-dependent interaction with ABA receptors (Emanuelle *et al.*, 2015). ABA-dependent signaling results in the expression of effector proteins regulating different aspects of plant physiology. The polytopic transmembrane protein TSPO is a multi-stress regulator, transiently induced by water-related stress and ABA treatment in plants (Guillaumot *et al.*, 2009). The induced Arabidopsis TSPO protein is also rapidly (within 48 hours) degraded, suggesting a time-limited role for it during stress. Plant TSPO may act as an autophagy cargo receptor for a

514 diverse set of cargo such as cytoplasmic free porphyrins and defined water channels
515 (Veljanovski and Batoko, 2014). AtTSPO interacts with a highly expressed plasma
516 membrane water channel, aquaporin PIP2;7, during osmotic stress. The aquaporin-TSPO
517 complex is targeted by autophagy for degradation in the vacuole, thus preventing PIP2;7
518 from reaching the plasma membrane and possibly protecting the cell from water loss
519 (Hachez *et al.*, 2014). However, constitutive expression of TSPO can be detrimental to
520 plant growth and development (Guillaumot *et al.*, 2009; Vanhee *et al.*, 2011a). This is
521 probably due to its intrinsic free heme binding capacity, and the consequence of this
522 cytoplasmic heme titration on ROS scavenger enzymes activity (Batoko *et al.*, 2015;
523 Vanhee *et al.*, 2011b). Enhanced ROS accumulation could generate ER-stress and chronic
524 UPR (Unfolded Protein Response) followed by cell death (Petrov *et al.*, 2015). The free
525 heme/porphyrin detoxification function of TSPO may be required only transiently, when
526 the plant cell needs to manage stress-induced ROS, and probably ROS-dependent signaling
527 events (Batoko *et al.*, 2015).

528 Plant TSPO is also upregulated by the growth-promoting hormone brassinosteroid (Nolan
529 *et al.*, 2017). Brassinosteroid (BR) plasma membrane receptor BRI1 (Brassinosteroid
530 Insensitive 1) and the downstream signaling components regulate the activity of the
531 transcription factor BES1 (BRI1-EMS Suppressor 1) (Li and Nam, 2002). BR inhibits the
532 activity of the kinase BIN2 that negatively regulates BES1 by phosphorylation. BES1
533 master transcriptional activity promotes plant growth, and its deregulation was shown
534 recently to enhance plant survival instead of growth during abiotic stress (Nolan *et al.*,
535 2017). During osmotic stress for example, BES1 is ubiquitylated and interacts with the
536 ubiquitin-binding receptor protein DSK2 (Dominant Suppressor of Kar2), a known
537 autophagy cargo receptor in higher eukaryotes (Lee *et al.*, 2013). BES1 is therefore
538 targeted for autophagy-mediated degradation as a response to abiotic stress. DSK2's
539 autophagy receptor activity is regulated by phosphorylation, the latter being catalyzed by
540 the BIN2 kinase. Loss-of-function *dsk2* mutant plants accumulate BES1 proteins, have
541 altered global gene expression profiles and compromised survival during abiotic stresses
542 (Nolan *et al.*, 2017). Consistently, constitutively active BR signaling mutant plants are
543 more sensitive to abiotic stress, suggesting that reducing growth during abiotic stress is a
544 vital mechanism for plant to survive during abiotic stresses. Although BES1 abundance

545 can be regulated by the ubiquitin proteasome system (Lin *et al.*, 2011), autophagy appears
546 to be a key pathway in achieving this tricky physiological and metabolic balance between
547 growth and survival.

548

549 **The role of autophagy in plant-pathogen interactions**

550 Autophagy is a central regulator of plant innate immunity. It can either act as survival or
551 cell death pathway in response to invading microbes with different pathogenic (i.e.
552 biotrophic or necrotrophic) lifestyles. Because of the co-evolutionary battle with their
553 hosts, several pathogens have developed various countermeasures to suppress, evade or
554 subvert autophagy processes to the benefit of infection. In addition, some eukaryotic
555 microbes require their own autophagy machinery for successful pathogenesis (Hofius *et*
556 *al.*, 2017). Most studies demonstrating the role of autophagy in plant-microbe interactions
557 have considered autophagy as a largely unspecific ("bulk") process. However, recent
558 reports indicate that plants are able to explore selective autophagic mechanisms to
559 effectively fend off microbial intruders, whereas some pathogens overcome plant
560 immunity by hijacking autophagy pathways for selective removal of host components
561 (Clavel *et al.*, 2017; Dagdas *et al.*, 2016; Hafren *et al.*, 2017; Haxim *et al.*, 2017). In this
562 section, we will briefly discuss the role of autophagy in different immunity- and disease-
563 related contexts, including the hypersensitive response (HR) to avirulent pathogens as well
564 as infections with virulent fungal, viral, oomycete and bacterial species. More
565 comprehensive reviews on the topic are available from (Hofius *et al.*, 2017; Minina *et al.*,
566 2014; Zhou *et al.*, 2014b) and in this Special Issue on Plant Autophagy from Leary et al.
567 (Leary *et al.*, 2018).

568 Pathogen recognition by the plant immune system often results in HR, a localized form of
569 programmed cell death (PCD) activated by intracellular immune receptors [known as
570 resistance (*R*) genes] (Coll *et al.*, 2011). HR levels were reduced in autophagy-deficient
571 mutants infected with avirulent bacteria and oomycetes, or enhanced in autophagy-
572 stimulated transgenic plants upon virus challenge (Coll *et al.*, 2011; Hackenberg *et al.*,
573 2013; Han *et al.*, 2015; Munch *et al.*, 2014; Munch *et al.*, 2015). Hence, autophagy acts
574 locally as a positive regulator of HR. Autophagy mutants were also shown to display

575 unrestricted cell death upon HR induction (Liu *et al.*, 2005; Yoshimoto *et al.*, 2009)
576 suggesting that autophagy can contribute to the confinement of HR, thus minimizing
577 damage to healthy, non-infected tissue (Hofius *et al.*, 2011). This pro-survival effect of
578 autophagy might be linked to its homeostatic role in eliminating potentially noxious by-
579 products of systemic responses triggered during infection (Coll *et al.*, 2014; Hofius *et al.*,
580 2011; Munch *et al.*, 2014; Yoshimoto *et al.*, 2009).

581 An additional pro-survival role of autophagy in immunity has been revealed in the context
582 of plant defense against necrotrophs, which deliberately activate cell death to retrieve
583 nutrients from the host. Autophagy-deficient mutants displayed enhanced disease-
584 associated cell death and pathogen growth upon infection with different necrotrophic fungi
585 (Katsiarimpa *et al.*, 2013; Lai *et al.*, 2011; Lenz *et al.*, 2011; Li *et al.*, 2016b), whereas
586 plants with elevated level of autophagy showed increased resistance (Minina et al. 2018).
587 Besides restricting disease-associated necrotic cell death, autophagy may also contribute
588 to basal defense against necrotrophs by modulating hormone levels or eliminating toxic
589 cellular constituents induced as part of the disease response (Lai *et al.*, 2011). Some
590 necrotrophic fungi have therefore evolved mechanisms to overcome autophagy-mediated
591 defenses in plants. For example, secretion of the phytotoxin oxalic acid by *Sclerotinia*
592 *sclerotiorum* results in unrestricted host cell death via autophagy inhibition (Kabbage *et*
593 *al.*, 2013).

594 Intracellular pathogens in animals are often subject to direct targeting and elimination by
595 the autophagy machinery in a process referred to as xenophagy (Levine *et al.*, 2011;
596 Mostowy, 2013; Paul and Munz, 2016). In plants, viruses are the only pathogens with
597 intracellular replication, but the anti- and pro-viral functions of autophagy in host immunity
598 and viral pathogenesis have only recently begun to emerge (Clavel *et al.*, 2017). Most
599 strikingly, selective autophagy mechanisms were discovered as integral part of the innate
600 immune response against different DNA viruses. The cargo receptor NBR1 mediates
601 autophagic degradation of non-assembled capsid proteins and viral particles of *Cauliflower*
602 *mosaic virus* (CaMV), providing a first example of xenophagy in plants (Hafren *et al.*,
603 2017). Likewise, the virulence factor βC1 of *Cotton leaf curl Multan virus* (CLCuMuV) is
604 selectively targeted during infection (Haxim *et al.*, 2017). However, recruitment of this

viral suppressor of RNA silencing (VSR) to autophagosomes seems to involve direct interaction with ATG8 rather than distinct cargo receptors. The potyviral HCpro and cucumoviral 2b proteins, representing VSRs of RNA viruses, were also shown to undergo autophagic clearance but the link between their binding to the host protein rgsCaM and the autophagy machinery is unclear (Nakahara *et al.*, 2012). In contrast to the examples from DNA viruses, the biological relevance of the autophagic processes for antiviral immunity against RNA viruses remains to be shown.

As part of their counter defense, some viruses trigger the autophagic degradation of host antiviral RNA silencing pathway components (Cheng and Wang, 2017; Derrien *et al.*, 2012). In addition, virus-induced activation of bulk autophagy seems to benefit virus survival and particle production via suppression of disease-associated cell death and promotion of plant fitness (Hafren *et al.*, 2017). Hence, viral measures to interfere with xenophagic targeting may influence the pro-viral effects of bulk autophagy, implying a potential trade-off between suppression of antiviral autophagy and host survival.

The hemibiotrophic oomycete pathogen *Phytophthora infestans* was also shown to be targeted by NBR1-dependent autophagy processes as part of the host defense (Dagdas *et al.*, 2016). In turn, the *P. infestans* effector protein PexRD54 can outcompete the interaction of the NBR1 tobacco homolog Joka 2 with an ATG8 protein, which led to the speculation that *P. infestans* hijacks the autophagy pathway to selectively remove defense components or to recycle and deviate nutrients to the intracellular infection structures (Dagdas *et al.*, 2016). The role of autophagy during infection with the strictly biotrophic downy mildew oomycete and powdery mildew fungal species still remains unclear, probably because of the use of different autophagy-deficient mutant backgrounds and pathogen species or plant age-dependent alterations in cellular homeostasis and hormone signaling (Hofius *et al.*, 2009; Lenz *et al.*, 2011).

Similarly, the functions of autophagy during virulent bacterial infection are not well understood. There is the prevailing view that autophagy promotes plant susceptibility to infection with *Pseudomonas syringae* (Hofius *et al.*, 2017; Kwon *et al.*, 2013; Lenz *et al.*, 2011). The recent identification of the *Ralstonia solanacearum* AWR5 effector, which

inhibits the negative autophagy regulator TOR, further suggests that bacteria can exploit autophagy activation to enhance virulence (Popa *et al.*, 2016)

Autophagy as a facilitator of nutrient recycling and remobilization in plants

It is generally accepted that autophagy is involved in nutrient recycling and that it is induced under nutrient starvation. This role has been suspected since the early stages of autophagy research, when de Duve observed autophagosome structures in the livers of rats submitted to nutrient starvation (Deter *et al.*, 1967). Further, the possibility to induce autophagy for nutrient recycling in yeast using starvation was used by Oshumi and colleagues to set up a mutant screening strategy that permitted the discovery of the *ATG* genes (Takeshige *et al.*, 1992). In mice, the importance of autophagy in nutrient recycling was demonstrated by the strong impact of autophagic activity on newborn survival (Kuma *et al.*, 2004). In plants, hypersensitivity to carbon and nitrogen starvation has been established as a basic phenotype of *atg* mutants, characterized originally in Arabidopsis (Doelling *et al.*, 2002; Ishizaki *et al.*, 2005; Phillips *et al.*, 2008; Thompson *et al.*, 2005), but also shown in maize (Li *et al.*, 2015). However, our knowledge of the underlying molecular details of such interplay is limited.

Both carbon and nitrogen starvation are known to induce autophagy (Avila-Ospina *et al.*, 2016; Rose *et al.*, 2006). Expression of *ATG* genes was shown to increase upon carbon and nitrogen starvation in many plant species, including Arabidopsis, maize, tobacco, wheat and the model algae *Chlamydomonas reinhardtii*, as well as increased lipidation of ATG8 (Caldana *et al.*, 2011; Li *et al.*, 2015; Pei *et al.*, 2014; Perez-Perez *et al.*, 2010; Thompson *et al.*, 2005; Zientara-Rytter *et al.*, 2011). In addition, crossing *atg* mutants with starch deficient mutants was shown to exacerbate their starvation phenotype, demonstrating the tight link between autophagy and carbon supply under starvation (Izumi *et al.*, 2013). Links between autophagy and other nutrient deficiencies are less documented. Induction of some autophagy-related genes (*ATG8* and *Joka2*) in roots of tobacco plants grown in sulfur deficient conditions (Zientara-Rytter *et al.*, 2011) suggested that sulfur starvation induces autophagy activity. Indeed, it was recently shown in Arabidopsis that limited sulfur supply decreases soluble sugars, downregulates TOR activity, as demonstrated by downregulation

664 of its downstream target S6K, and increases level of the lipidated ATG8a (Dong *et al.*,
665 2017). Induction of autophagy under phosphorus starvation has also been suggested in the
666 model algae *Chlamydomonas reinhardtii* and marine algae *Emiliania huxleyi* (Couso *et al.*,
667 2017; Shemi *et al.*, 2016). In Arabidopsis, it has been proposed that in the absence of
668 phosphate, selective autophagy (with PUB9 as E3 ligase) is involved in degradation of
669 auxin accumulation repressor, leading to auxin accumulation and lateral roots growth (Deb
670 *et al.*, 2014).

671 We do not know what triggers the induction of autophagy-related genes during limitation
672 of certain nutrients, however the signal, at least for nitrogen, carbon and sulfur starvation,
673 is probably TOR-dependent (Dong *et al.*, 2017; Pu *et al.*, 2017; Rexin *et al.*, 2015).
674 Interestingly, the level of hydrogen sulfide, a recently identified negative regulator of
675 autophagy, drops during sulfur limitation, and, at least, in such conditions might be one of
676 the triggers (Gotor *et al.*, 2013; Laureano-Marin *et al.*, 2016).

677 It is considered that starvation induces non-specific autophagy (i.e. bulk degradation) of
678 cytoplasmic components for nutrient remobilization. In mammals, however, the selective
679 degradation of lipid bodies under starvation was demonstrated in hepathocytes (Singh *et*
680 *al.*, 2009). In addition, selective degradation of ald6p under nitrogen starvation has been
681 demonstrated in yeast (Onodera and Ohsumi, 2004). The diversity of the cytoplasmic
682 components dedicated for degradation by autophagy, including protein aggregates,
683 membranes, organelles, suggests that in addition to C and N molecules, many other mineral
684 nutrients could be released from the process. These compounds can then be used for the
685 cell's own metabolism, to sustain respiration for example (Barros *et al.*, 2017) or dedicated
686 for the whole organism after remobilization. Whether phosphate, iron, zinc, sulphur or
687 potassium can be recycled though autophagy is not documented. Also it is unknown
688 whether some selectivity exists in the cargos degraded by autophagy under starvation
689 conditions. It is likely, for example, that under dark conditions, chloroplasts could be
690 preferentially targeted and that autophagy could participate in starch degradation (Wang
691 and Liu, 2013; Wang *et al.*, 2015b). Under low nitrate availability, autophagy would
692 mainly participate in protein degradation but not starch degradation as proteins
693 accumulated in *atg* mutants while starch was depleted (Guiboileau *et al.*, 2013). Under

carbon starvation, the situation is singularly the opposite, with increased usage of free amino acids, presumably as an alternative carbon source for respiration (Avin-Wittenberg *et al.*, 2015).

At the whole plant level, autophagy is an essential process for nitrogen remobilization from leaf to seeds as shown by the ^{15}N pulse-chase experiments performed in Arabidopsis (Guiboileau *et al.*, 2012). Based on this Arabidopsis study, pulse-chase labelling strategy was used on maize *atg12* mutants that showed accordingly lower N mobilization to the seeds (Li *et al.*, 2015). Both studies thus confirmed that autophagy manages nutrient resources in source leaves and that its role for seed formation and seed filling is fundamental. In Arabidopsis, the composition of *atg* mutant seeds is strongly modified as their nitrogen content mainly relies on the post anthesis nitrate uptake rather than N remobilization from leaves (Guiboileau *et al.*, 2012). Because of their poor N remobilization capacity, *atg* mutant display lower yield and lower harvest index. Whether increasing autophagy activity in the source leaves during senescence could conversely increase plant performance in seed production and seed quality is then a major issue to be investigated.

While several studies have performed metabolic profiling of *atg* mutants (Avin-Wittenberg *et al.*, 2015; Barros *et al.*, 2017; Masclaux-Daubresse *et al.*, 2014), analysis of the changes in the metabolic fluxes are considerably less common. Metabolic flux analysis relies on determining the redistribution of label over time in order to estimate the atomic flux between pools of different metabolic species. Two approaches are commonly used: (i) radiolabeled isotopes, namely ^{14}C and ^{35}S and ^{3}H, and (ii) stable isotopes, such as ^{13}C and ^{15}N (Batista Silva *et al.*, 2016). Few works investigate primary metabolic fluxes in connection to autophagy and in plants. In the first study, etiolated wild type and *atg* mutant Arabidopsis seedlings were incubated in the presence of either uniformly labelled ^{14}C-glucose, positionally labelled ^{14}C glucoses or ^{13}C lysine in order to characterize the respiratory metabolism of these mutants (Avin-Wittenberg *et al.*, 2015). These revealed various effects, including lower protein synthesis and an accumulation of label in specific amino acids and TCA cycle intermediates. As mentioned above, the change in amino acid levels were different from that reported during nitrogen deficiency (Masclaux-Daubresse

et al., 2014). It would therefore be interesting to examine the impact of autophagy deficiency on metabolic fluxes in a range of conditions/tissues other than the etiolated seedling. These examples highlight the power of incorporating flux analyses into studies on plant autophagy, suggesting that their greater adoption will yield further insights into molecular and energetic mechanisms regulating and being modulated by autophagy.

Targeting autophagy in plant oil production

Plant oils play pivotal role in human nutrition and the potential for plant oils to replace fossil oil in chemical industry is likewise immense. To realize the full potential of using plant oils, it is crucial to optimize quantity and quality of the oil *in planta* using genetic and metabolic engineering. As in all eukaryotes, plants store their lipid reserves in specialized organelles, lipid droplets (LDs), which are especially abundant in seeds of oilseed crops. Recent research using animal and yeast systems has established that autophagy plays pivotal role in both breakdown and biogenesis of LDs (Singh *et al.*, 2009; Zhang *et al.*, 2009) and that LDs in return can regulate autophagy (Shpilka *et al.*, 2015). The process of autophagic degradation of LDs in the lysosome or lytic vacuole has been named "lipophagy" and shown to crosstalk in a number of ways with cytosolic lipolysis (Zechner *et al.*, 2017).

It has been shown that Arabidopsis mutants in beta-oxidation of fatty acids have greatly reduced seed oil content, demonstrating that turnover of lipids is an essential component for efficient seed oil accumulation (Lin *et al.*, 2004). Therefore, can manipulation of autophagy be used as a tool to improve oil crops? To-date, the evidence for the role of autophagy in biogenesis or degradation of LDs in plants is rather scarce and fragmented, encompassing only a few species. Thus, autophagy is required for the formation of LDs in tapetal cells and phospholipid editing in rice pollen (Kurusu *et al.*, 2014). Two cytological studies using electron microscopy have revealed micro- and macroautophagy-mediated engulfment of LDs in the algae *Auxenochlorella protothecoides* (Zhao *et al.*, 2014) and *Micrasterias denticulata* (Schwarz *et al.*, 2017) respectively. Finally, although autophagy does not seem to be critically required for Arabidopsis seed development, efficient mobilization of lipids upon seed germination under carbon-deprived conditions is at least partly dependent on autophagy (Avin-Wittenberg *et al.*, 2015). Clearly, more research is

754 needed to establish a solid platform for biotechnological application of autophagy in
755 regulating plant oil reserves (Elander *et al.*, 2018).

756

757 **Future perspectives**

758 The study of autophagy in plants has boomed in the last few years, and our understanding
759 of the function and regulation of this complex mechanism is steadily expanding. However,
760 much work is still needed in order to understand the many facets of autophagy and utilize
761 it for agricultural use. Primarily, it is very importance to continue deciphering the
762 mechanisms regulating autophagy in plants, as these are still only partially understood.
763 Better understanding of the regulation of autophagy will assist in the modulation of
764 autophagy on the field. In the field of selective autophagy, for example, information on the
765 role of selective autophagy in plant development is lagging behind. In addition, the cargo
766 receptors or other specificity factors involved in selective autophagy need to be further
767 identified and characterized and the role of ubiquitin-tagging in organelle degradation
768 elucidated. As a complementary approach, understanding the functional differences
769 between the different plant ATG8 isoforms would be very useful. Most of our
770 understanding of selective autophagy pathways is currently based on studies in model
771 plants, mainly Arabidopsis. More emphasis should be given to expanding the research to
772 crops and to possible specific differences in autophagy pathways and responses.

773 Translating the knowledge gained from model systems to crop plants is also a challenge
774 for understanding the interplay between autophagy and plant pathogens, which cause
775 devastating economical losses to farmers and threaten global food security. Future work
776 will help gaining additional insight into the molecular mechanisms that pathogens use to
777 exploit plant autophagy for their own benefit and deepen our understanding of the
778 autophagic components and pathways contributing to plant innate immune responses.

779 Development of artificial tools for modulating plant autophagy will allow us to control
780 crop fitness, stress-tolerance and productivity, eliminating the need in laborious and time-
781 consuming breeding process. Advances in CRISP/CAS9-based genetic editing tools and
782 high-throughput drug screens should facilitate manipulation of autophagy in crops. All this

may result in the production of crops with increased nutrient remobilization, able to cope better with nutrient starvation and increase efficacy of agriculture and its adjustability to the changing climate conditions, as well as stability under high pathogen pressure in the field.

Acknowledgements

The support of the COST action TRANSAUTOPHAGY (CA15138) is recognized. Research on autophagy in Bozhkov and Minina laboratory is supported by the Swedish Foundation for Strategic Research (SSF), Swedish Research Council (VR) and the Research Programme "Trees and Crops for the Future". Research in the Hofius laboratory is supported by the Swedish University of Agricultural Sciences (SLU), the Swedish Research Council (VR), and the Knut-and-Alice Wallenberg (KAW) foundation. Reaserach of the Avin-Wittenberg Group is supported by the Israeli Science Foundation, grant number 1899/16. The Testillano group is supported by project AGL2014-52028-R and AGL2017-82447-R funded by the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF/FEDER). Gad Galili and Hadas Zehavi are supported by The Israel Science Foundation (grant 612/16). Research in the Batoko laboratory was funded by the Wallonia-Brussels Federation Joint Research Action (ARC grant #11/16-036), the Belgian Funds for Scientific Research (FRS-FNRS) (CDR grant #19516174 and FRFC grant #6794930).

References

Abada A, Elazar Z. 2014. Getting ready for building: signaling and autophagosome biogenesis. *EMBO Rep* **15**, 839-852.

Anding AL, Baehrecke EH. 2017. Cleaning House: Selective Autophagy of Organelles. *Dev Cell* **41**, 10-22.

Andreu V, Collados R, Testillano PS, Risueno Mdel C, Picorel R, Alfonso M. 2007. In situ molecular identification of the plastid omega3 fatty acid desaturase FAD7 from soybean: evidence of thylakoid membrane localization. *Plant Physiol* **145**, 1336-1344.

Antonioli M, Di Rienzo M, Piacentini M, Fimia GM. 2017. Emerging Mechanisms in Initiating and Terminating Autophagy. *Trends Biochem Sci* **42**, 28-41.

Avila-Ospina L, Marmagne A, Soulay F, Masclaux-Daubresse C. 2016. Identification of Barley (Hordeum vulgare L.) Autophagy Genes and Their Expression Levels during Leaf Senescence, Chronic Nitrogen Limitation and in Response to Dark Exposure. *Agronomy-Basel* **6**.

Avin-Wittenberg T, Bajdzienko K, Wittenberg G, Alseekh S, Tohge T, Bock R, Giavalisco P, Fernie AR. 2015. Global analysis of the role of autophagy in cellular metabolism and energy homeostasis in Arabidopsis seedlings under carbon starvation. *Plant Cell* **27**, 306-322.

Bakshi A, Moin M, Kumar MU, Reddy AB, Ren M, Datla R, Siddiq EA, Kirti PB. 2017. Ectopic expression of Arabidopsis Target of Rapamycin (AtTOR) improves water-use efficiency and yield potential in rice. *Sci Rep* **7**, 42835.

Barros JA, Cavalcanti JHF, Medeiros DB, Nunes-Nesi A, Avin-Wittenberg T, Fernie AR, Araujo W. 2017. Autophagy deficiency compromises alternative pathways of respiration following energy deprivation. *Plant Physiol*.

Baskaran S, Carlson LA, Stjepanovic G, Young LN, Kim DJ, Grob P, Stanley RE, Nogales E, Hurley JH. 2014. Architecture and dynamics of the autophagic phosphatidylinositol 3-kinase complex. *Elife* **3**.

Bassham DC. 2015. Methods for analysis of autophagy in plants. *Methods* **75**, 181-188.

Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, Olsen LJ, Yoshimoto K. 2006. Autophagy in development and stress responses of plants. *Autophagy* **2**, 2-11.

Bassham DC, MacIntosh GC. 2017. Degradation of cytosolic ribosomes by autophagy-related pathways. *Plant Sci* **262**, 169-174.

Batista Silva W, Daloso DM, Fernie AR, Nunes-Nesi A, Araujo WL. 2016. Can stable isotope mass spectrometry replace radiolabelled approaches in metabolic studies? *Plant Sci* **249**, 59-69.

Batoko H, Veljanovski V, Jurkiewicz P. 2015. Enigmatic Translocator protein (TSPO) and cellular stress regulation. *Trends Biochem Sci* **40**, 497-503.

Bernal M, Testillano PS, Alfonso M, del Carmen Risueno M, Picorel R, Yruela I. 2007. Identification and subcellular localization of the soybean copper P1B-ATPase GmHMA8 transporter. *J Struct Biol* **158**, 46-58.

Caldana C, Degenkolbe T, Cuadros-Inostroza A, Klie S, Sulpice R, Leisse A, Steinhauser D, Fernie AR, Willmitzer L, Hannah MA. 2011. High-density kinetic analysis of the metabolomic and transcriptomic response of Arabidopsis to eight environmental conditions. *Plant J* **67**, 869-884.

Chanoca A, Kovinich N, Burkel B, Stecha S, Bohorquez-Restrepo A, Ueda T, Eliceiri KW, Grotewold E, Otegui MS. 2015. Anthocyanin Vacuolar Inclusions Form by a Microautophagy Mechanism. *Plant Cell* **27**, 2545-2559.

Chen L, Su ZZ, Huang L, Xia FN, Qi H, Xie LJ, Xiao S, Chen QF. 2017. The AMP-Activated Protein Kinase KIN10 Is Involved in the Regulation of Autophagy in Arabidopsis. *Front Plant Sci* **8**, 1201.

Cheng X, Wang A. 2017. The Potyvirus Silencing Suppressor Protein VPg Mediates Degradation of SGS3 via Ubiquitination and Autophagy Pathways. *J Virol* **91**.

Chiba A, Ishida H, Nishizawa NK, Makino A, Mae T. 2003. Exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts by specific bodies in naturally senescing leaves of wheat. *Plant Cell Physiol* **44**, 914-921.

Chung T, Phillips AR, Vierstra RD. 2010. ATG8 lipidation and ATG8-mediated autophagy in Arabidopsis require ATG12 expressed from the differentially controlled ATG12A AND ATG12B loci. *Plant J* **62**, 483-493.
Chung T, Suttangkakul A, Vierstra RD. 2009. The ATG autophagic conjugation system in maize: ATG transcripts and abundance of the ATG8-lipid adduct are regulated by development and nutrient availability. *Plant Physiol* **149**, 220-234.
Claeys H, Inze D. 2013. The agony of choice: how plants balance growth and survival under water-limiting conditions. *Plant Physiol* **162**, 1768-1779.
Clavel M, Michaeli S, Genschik P. 2017. Autophagy: A Double-Edged Sword to Fight Plant Viruses. *Trends Plant Sci* **22**, 646-648.
Cohen-Kaplan V, Livneh I, Avni N, Fabre B, Ziv T, Kwon YT, Ciechanover A. 2016. p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome. *Proc Natl Acad Sci U S A* **113**, E7490-E7499.
Coll NS, Epple P, Dangl JL. 2011. Programmed cell death in the plant immune system. *Cell Death Differ* **18**, 1247-1256.
Coll NS, Smidler A, Puigvert M, Popa C, Valls M, Dangl JL. 2014. The plant metacaspase AtMC1 in pathogen-triggered programmed cell death and aging: functional linkage with autophagy. *Cell Death Differ* **21**, 1399-1408.
Confraria A, Martinho C, Elias A, Rubio-Somoza I, Baena-Gonzalez E. 2013. miRNAs mediate SnRK1-dependent energy signaling in Arabidopsis. *Front Plant Sci* **4**, 197.
Contento AL, Xiong Y, Bassham DC. 2005. Visualization of autophagy in Arabidopsis using the fluorescent dye monodansylcadaverine and a GFP-AtATG8e fusion protein. *Plant J* **42**, 598-608.
Couso I, Perez-Perez ME, Martinez-Force E, Kim HS, He Y, Umen JG, Crespo JL. 2017. Autophagic flux is required for the synthesis of triacylglycerols and ribosomal protein turnover in Chlamydomonas. *J Exp Bot*.
Coutts AS, La Thangue NB. 2015. Actin nucleation by WH2 domains at the autophagosome. *Nat Commun* **6**, 7888.
Crespo JL, Diaz-Troya S, Florencio FJ. 2005. Inhibition of target of rapamycin signaling by rapamycin in the unicellular green alga Chlamydomonas reinhardtii. *Plant Physiology* **139**, 1736-1749.
Dagdas YF, Belhaj K, Maqbool A, Chaparro-Garcia A, Pandey P, Petre B, Tabassum N, Cruz-Mireles N, Hughes RK, Sklenar J, Win J, Menke F, Findlay K, Banfield MJ, Kamoun S, Bozkurt TO. 2016. An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. *Elife* **5**.
Deb S, Sankaranarayanan S, Wewala G, Widdup E, Samuel MA. 2014. The S-Domain Receptor Kinase Arabidopsis Receptor Kinase2 and the U Box/Armadillo Repeat-Containing E3 Ubiquitin Ligase9 Module Mediates Lateral Root Development under Phosphate Starvation in Arabidopsis. *Plant Physiology* **165**, 1647-1656.
Deng KX, Dong P, Wang WJ, Feng L, Xiong FJ, Wang K, Zhang SM, Feng S, Wang BJ, Zhang JK, Ren MZ. 2017. The TOR Pathway Is Involved in Adventitious Root Formation in Arabidopsis and Potato. *Frontiers in Plant Science* **8**.
Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P. 2012. Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. *Proc Natl Acad Sci U S A* **109**, 15942-15946.

Deter RL, Baudhuin P, De Duve C. 1967. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J Cell Biol* **35**, C11-16.
Diao J, Liu R, Rong Y, Zhao M, Zhang J, Lai Y, Zhou Q, Wilz LM, Li J, Vivona S, Pfuetzner RA, Brunger AT, Zhong Q. 2015. ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. *Nature* **520**, 563-566.
Diaz-Troya S, Perez-Perez ME, Perez-Martin M, Moes S, Jeno P, Florencio FJ, Crespo JL. 2011. Inhibition of protein synthesis by TOR inactivation revealed a conserved regulatory mechanism of the BiP chaperone in Chlamydomonas. *Plant Physiol* **157**, 730-741.
Dikic I. 2017. Proteasomal and Autophagic Degradation Systems. *Annu Rev Biochem* **86**, 193-224.
Doelling JH, Walker JM, Friedman EM, Thompson AR, Vierstra RD. 2002. The APG8/12-activating enzyme APG7 is required for proper nutrient recycling and senescence in Arabidopsis thaliana. *J Biol Chem* **277**, 33105-33114.
Dong J, Chen W. 2013. The role of autophagy in chloroplast degradation and chlorophagy in immune defenses during Pst DC3000 (AvrRps4) infection. *PLoS One* **8**, e73091.
Dong P, Xiong FJ, Que YM, Wang K, Yu LH, Li ZG, Ren MZ. 2015. Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling pathways in Arabidopsis. *Frontiers in Plant Science* **6**.
Dong Y, Silbermann M, Speiser A, Forieri I, Linster E, Poschet G, Allboje Samami A, Wanatabe M, Sticht C, Teleman AA, Deragon JM, Saito K, Hell R, Wirtz M. 2017. Sulfur availability regulates plant growth via glucose-TOR signaling. *Nat Commun* **8**, 1174.
Elander PH, Minina EA, Bozhkov PV. 2018. Autophagy in turnover of lipid stores: trans-kingdom comparison. *J Exp Bot*.
Emanuelle S, Hossain MI, Moller IE, Pedersen HL, van de Meene AM, Doblin MS, Koay A, Oakhill JS, Scott JW, Willats WG, Kemp BE, Bacic A, Gooley PR, Stapleton DI. 2015. SnRK1 from Arabidopsis thaliana is an atypical AMPK. *Plant J* **82**, 183-192.
Fan W, Nassiri A, Zhong Q. 2011. Autophagosome targeting and membrane curvature sensing by Barkor/Atg14(L). *Proc Natl Acad Sci U S A* **108**, 7769-7774.
Farre JC, Subramani S. 2016. Mechanistic insights into selective autophagy pathways: lessons from yeast. *Nat Rev Mol Cell Biol* **17**, 537-552.
Feng Y, Yao Z, Klionsky DJ. 2015. How to control self-digestion: transcriptional, post-transcriptional, and post-translational regulation of autophagy. *Trends Cell Biol* **25**, 354-363.
Fleming A, Noda T, Yoshimori T, Rubinsztein DC. 2011. Chemical modulators of autophagy as biological probes and potential therapeutics. *Nat Chem Biol* **7**, 9-17.
Floyd BE, Morriss SC, MacIntosh GC, Bassham DC. 2015. Evidence for autophagy-dependent pathways of rRNA turnover in Arabidopsis. *Autophagy* **11**, 2199-2212.
Floyd BE, Mugume Y, Morriss SC, MacIntosh GC, Bassham DC. 2017. Localization of RNS2 ribonuclease to the vacuole is required for its role in cellular homeostasis. *Planta* **245**, 779-792.
Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, Choi AM, Chu CT, Codogno P, Colombo MI, Cuervo AM, Debnath J, Deretic V, Dikic I, Eskelinen EL, Fimia GM, Fulda S, Gewirtz DA, Green DR, Hansen M, Harper JW,

Jaattela M, Johansen T, Juhasz G, Kimmelman AC, Kraft C, Ktistakis NT, Kumar S, Levine B, Lopez-Otin C, Madeo F, Martens S, Martinez J, Melendez A, Mizushima N, Munz C, Murphy LO, Penninger JM, Piacentini M, Reggiori F, Rubinsztein DC, Ryan KM, Santambrogio L, Scorrano L, Simon AK, Simon HU, Simonsen A, Tavernarakis N, Tooze SA, Yoshimori T, Yuan J, Yue Z, Zhong Q, Kroemer G. 2017. Molecular definitions of autophagy and related processes. *EMBO J* **36**, 1811-1836.

Gotor C, Garcia I, Crespo JL, Romero LC. 2013. Sulfide as a signaling molecule in autophagy. *Autophagy* **9**, 609-611.

Grossmann G, Guo WJ, Ehrhardt DW, Frommer WB, Sit RV, Quake SR, Meier M. 2011. The RootChip: an integrated microfluidic chip for plant science. *Plant Cell* **23**, 4234-4240.

Guiboileau A, Avila-Ospina L, Yoshimoto K, Soulay F, Azzopardi M, Marmagne A, Lothier J, Masclaux-Daubresse C. 2013. Physiological and metabolic consequences of autophagy deficiency for the management of nitrogen and protein resources in Arabidopsis leaves depending on nitrate availability. *New Phytol* **199**, 683-694.

Guiboileau A, Yoshimoto K, Soulay F, Bataille MP, Avice JC, Masclaux-Daubresse C. 2012. Autophagy machinery controls nitrogen remobilization at the whole-plant level under both limiting and ample nitrate conditions in Arabidopsis. *New Phytol* **194**, 732-740.

Guillaumot D, Guillon S, Deplanque T, Vanhee C, Gumy C, Masquelier D, Morsomme P, Batoko H. 2009. The Arabidopsis TSPO-related protein is a stress and abscisic acid-regulated, endoplasmic reticulum-Golgi-localized membrane protein. *Plant J* **60**, 242-256.

Hachez C, Veljanovski V, Reinhardt H, Guillaumot D, Vanhee C, Chaumont F, Batoko H. 2014. The Arabidopsis abiotic stress-induced TSPO-related protein reduces cell-surface expression of the aquaporin PIP2;7 through protein-protein interactions and autophagic degradation. *Plant Cell* **26**, 4974-4990.

Hackenberg T, Juul T, Auzina A, Gwizdz S, Malolepszy A, Van Der Kelen K, Dam S, Bressendorff S, Lorentzen A, Roepstorff P, Lehmann Nielsen K, Jorgensen JE, Hofius D, Van Breusegem F, Petersen M, Andersen SU. 2013. Catalase and NO CATALASE ACTIVITY1 promote autophagy-dependent cell death in Arabidopsis. *Plant Cell* **25**, 4616-4626.

Hafren A, Macia JL, Love AJ, Milner JJ, Drucker M, Hofius D. 2017. Selective autophagy limits cauliflower mosaic virus infection by NBR1-mediated targeting of viral capsid protein and particles. *Proc Natl Acad Sci U S A* **114**, E2026-E2035.

Han S, Wang Y, Zheng X, Jia Q, Zhao J, Bai F, Hong Y, Liu Y. 2015. Cytoplastic Glyceraldehyde-3-Phosphate Dehydrogenases Interact with ATG3 to Negatively Regulate Autophagy and Immunity in Nicotiana benthamiana. *Plant Cell* **27**, 1316-1331.

Hanamata S, Kurusu T, Kuchitsu K. 2014. Roles of autophagy in male reproductive development in plants. *Front Plant Sci* **5**, 457.

Haxim Y, Ismayil A, Jia Q, Wang Y, Zheng X, Chen T, Qian L, Liu N, Wang Y, Han S, Cheng J, Qi Y, Hong Y, Liu Y. 2017. Autophagy functions as an antiviral mechanism against geminiviruses in plants. *Elife* **6**.

He C, Klionsky DJ. 2009. Regulation mechanisms and signaling pathways of autophagy. *Annu Rev Genet* **43**, 67-93.

Hillwig MS, Contento AL, Meyer A, Ebany D, Bassham DC, Macintosh GC. 2011. RNS2, a conserved member of the RNase T2 family, is necessary for ribosomal RNA decay in plants. *Proc Natl Acad Sci U S A* **108**, 1093-1098.
Hofius D, Li L, Hafren A, Coll NS. 2017. Autophagy as an emerging arena for plant-pathogen interactions. *Curr Opin Plant Biol* **38**, 117-123.
Hofius D, Munch D, Bressendorff S, Mundy J, Petersen M. 2011. Role of autophagy in disease resistance and hypersensitive response-associated cell death. *Cell Death Differ* **18**, 1257-1262.
Hofius D, Schultz-Larsen T, Joensen J, Tsitsigiannis DI, Petersen NH, Mattsson O, Jorgensen LB, Jones JD, Mundy J, Petersen M. 2009. Autophagic components contribute to hypersensitive cell death in Arabidopsis. *Cell* **137**, 773-783.
Honig A, Avin-Wittenberg T, Ufaz S, Galili G. 2012. A new type of compartment, defined by plant-specific Atg8-interacting proteins, is induced upon exposure of Arabidopsis plants to carbon starvation. *Plant Cell* **24**, 288-303.
Imamura S, Kawase Y, Kobayashi I, Shimojima M, Ohta H, Tanaka K. 2016. TOR (target of rapamycin) is a key regulator of triacylglycerol accumulation in microalgae. *Plant Signaling & Behavior* **11**.
Ishida H, Izumi M, Wada S, Makino A. 2014. Roles of autophagy in chloroplast recycling. *Biochim Biophys Acta* **1837**, 512-521.
Ishida H, Yoshimoto K, Izumi M, Reisen D, Yano Y, Makino A, Ohsumi Y, Hanson MR, Mae T. 2008. Mobilization of rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an ATG gene-dependent autophagic process. *Plant Physiol* **148**, 142-155.
Ishizaki K, Larson TR, Schauer N, Fernie AR, Graham IA, Leaver CJ. 2005. The critical role of Arabidopsis electron-transfer flavoprotein:ubiquinone oxidoreductase during dark-induced starvation. *Plant Cell* **17**, 2587-2600.
Itakura E, Kishi C, Inoue K, Mizushima N. 2008. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Molecular Biology of the Cell* **19**, 5360-5372.
Izumi M, Hidema J, Makino A, Ishida H. 2013. Autophagy contributes to nighttime energy availability for growth in Arabidopsis. *Plant Physiol* **161**, 1682-1693.
Izumi M, Hidema J, Wada S, Kondo E, Kurusu T, Kuchitsu K, Makino A, Ishida H. 2015. Establishment of monitoring methods for autophagy in rice reveals autophagic recycling of chloroplasts and root plastids during energy limitation. *Plant Physiol*.
Izumi M, Ishida H, Nakamura S, Hidema J. 2017. Entire Photodamaged Chloroplasts Are Transported to the Central Vacuole by Autophagy. *Plant Cell* **29**, 377-394.
Jiang Q, Zhao L, Dai J, Wu Q. 2012. Analysis of autophagy genes in microalgae: Chlorella as a potential model to study mechanism of autophagy. *Plos One* **7**, e41826.
Jin S, Tian S, Chen Y, Zhang C, Xie W, Xia X, Cui J, Wang RF. 2016. USP19 modulates autophagy and antiviral immune responses by deubiquitinating Beclin-1. *EMBO J* **35**, 866-880.
Jossier M, Bouly JP, Meimoun P, Arjmand A, Lessard P, Hawley S, Grahame Hardie D, Thomas M. 2009. SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. *Plant J* **59**, 316-328.

Kabbage M, Williams B, Dickman MB. 2013. Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of Sclerotinia sclerotiorum. *PLoS Pathog* **9**, e1003287.
Karanasios E, Walker SA, Okkenhaug H, Manifava M, Hummel E, Zimmermann H, Ahmed Q, Domart MC, Collinson L, Ktistakis NT. 2016. Autophagy initiation by ULK complex assembly on ER tubulovesicular regions marked by ATG9 vesicles. *Nat Commun* **7**, 12420.
Kast DJ, Zajac AL, Holzbaur EL, Ostap EM, Dominguez R. 2015. WHAMM Directs the Arp2/3 Complex to the ER for Autophagosome Biogenesis through an Actin Comet Tail Mechanism. *Curr Biol* **25**, 1791-1797.
Katsiarimpa A, Kalinowska K, Anzenberger F, Weis C, Ostertag M, Tsutsumi C, Schwechheimer C, Brunner F, Huckelhoven R, Isono E. 2013. The deubiquitinating enzyme AMSH1 and the ESCRT-III subunit VPS2.1 are required for autophagic degradation in Arabidopsis. *Plant Cell* **25**, 2236-2252.
Kaushik S, Cuervo AM. 2012. Chaperone-mediated autophagy: a unique way to enter the lysosome world. *Trends Cell Biol* **22**, 407-417.
Kellner R, De la Concepcion JC, Maqbool A, Kamoun S, Dagdas YF. 2017. ATG8 Expansion: A Driver of Selective Autophagy Diversification? *Trends Plant Sci* **22**, 204-214.
Kim SH, Kwon C, Lee JH, Chung T. 2012. Genes for plant autophagy: functions and interactions. *Mol Cells* **34**, 413-423.
King JS, Gueho A, Hagedorn M, Gopaldass N, Leuba F, Soldati T, Insall RH. 2013. WASH is required for lysosomal recycling and efficient autophagic and phagocytic digestion. *Molecular Biology of the Cell* **24**, 2714-2726.
Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, Adachi H, Adams CM, Adams PD, Adeli K, Adhihetty PJ, Adler SG, Agam G, Agarwal R, Aghi MK, Agnello M, Agostinis P, Aguilar PV, Aguirre-Ghiso J, Airoldi EM, Ait-Si-Ali S, Akematsu T, Akporiaye ET, Al-Rubeai M, Albaiceta GM, Albanese C, Albani D, Albert ML, Aldudo J, Algul H, Alirezaei M, Alloza I, Almasan A, Almonte-Beceril M, Alnemri ES, Alonso C, Altan-Bonnet N, Altieri DC, Alvarez S, Alvarez-Erviti L, Alves S, Amadoro G, Amano A, Amantini C, Ambrosio S, Amelio I, Amer AO, Amessou M, Amon A, An Z, Anania FA, Andersen SU, Andley UP, Andreadi CK, Andrieu-Abadie N, Anel A, Ann DK, Anoopkumar-Dukie S, Antonioli M, Aoki H, Apostolova N, Aquila S, Aquilano K, Araki K, Arama E, Aranda A, Araya J, Arcaro A, Arias E, Arimoto H, Ariosa AR, Armstrong JL, Arnould T, Arsov I, Asanuma K, Askanas V, Asselin E, Atarashi R, Atherton SS, Atkin JD, Attardi LD, Auberger P, Auburger G, Aurelian L, Autelli R, Avagliano L, Avantaggiati ML, Avrahami L, Awale S, Azad N, Bachetti T, Backer JM, Bae DH, Bae JS, Bae ON, Bae SH, Baehrecke EH, Baek SH, Baghdiguian S, Bagniewska-Zadworna A, Bai H, Bai J, Bai XY, Bailly Y, Balaji KN, Balduini W, Ballabio A, Balzan R, Banerjee R, Banhegyi G, Bao H, Barbeau B, Barrachina MD, Barreiro E, Bartel B, Bartolome A, Bassham DC, Bassi MT, Bast RC, Jr., Basu A, Batista MT, Batoko H, Battino M, Bauckman K, Baumgarner BL, Bayer KU, Beale R, Beaulieu JF, Beck GR, Jr., Becker C, Beckham JD, Bedard PA, Bednarski PJ, Begley TJ, Behl C, Behrends C, Behrens GM, Behrns KE, Bejarano E, Belaid A, Belleudi F, Benard G, Berchem G, Bergamaschi D, Bergami M, Berkhout B, Berliocchi L, Bernard A, Bernard M,

Bernassola F, Bertolotti A, Bess AS, Besteiro S, Bettuzzi S, Bhalla S, Bhattacharyya S, Bhutia SK, Biagosch C, Bianchi MW, Biard-Piechaczyk M, Billes V, Bincoletto C, Bingol B, Bird SW, Bitoun M, Bjedov I, Blackstone C, Blanc L, Blanco GA, Blomhoff HK, Boada-Romero E, Bockler S, Boes M, Boesze-Battaglia K, Boise LH, Bolino A, Boman A, Bonaldo P, Bordi M, Bosch J, Botana LM, Botti J, Bou G, Bouche M, Bouchecareilh M, Boucher MJ, Boulton ME, Bouret SG, Boya P, Boyer-Guittaut M, Bozhkov PV, Brady N, Braga VM, Brancolini C, Braus GH, Bravo-San Pedro JM, Brennan LA, Bresnick EH, Brest P, Bridges D, Bringer MA, Brini M, Brito GC, Brodin B, Brookes PS, Brown EJ, Brown K, Broxmeyer HE, Bruhat A, Brum PC, Brumell JH, Brunetti-Pierri N, Bryson-Richardson RJ, Buch S, Buchan AM, Budak H, Bulavin DV, Bultman SJ, Bultynck G, Bumbasirevic V, Burelle Y, Burke RE, Burmeister M, Butikofer P, Caberlotto L, Cadwell K, Cahova M, Cai D, Cai J, Cai Q, Calatayud S, Camougrand N, Campanella M, Campbell GR, Campbell M, Campello S, Candau R, Caniggia I, Cantoni L, Cao L, Caplan AB, Caraglia M, Cardinali C, Cardoso SM, Carew JS, Carleton LA, Carlin CR, Carloni S, Carlsson SR, Carmona-Gutierrez D, Carneiro LA, Carnevali O, Carra S, Carrier A, Carroll B, Casas C, Casas J, Cassinelli G, Castets P, Castro-Obregon S, Cavallini G, Ceccherini I, Cecconi F, Cederbaum AI, Cena V, Cenci S, Cerella C, Cervia D, Cetrullo S, Chaachouay H, Chae HJ, Chagin AS, Chai CY, Chakrabarti G, Chamilos G, Chan EY, Chan MT, Chandra D, Chandra P, Chang CP, Chang RC, Chang TY, Chatham JC, Chatterjee S, Chauhan S, Che Y, Cheetham ME, Cheluvappa R, Chen CJ, Chen G, Chen GC, Chen G, Chen H, Chen JW, Chen JK, Chen M, Chen M, Chen P, Chen Q, Chen Q, Chen SD, Chen S, Chen SS, Chen W, Chen WJ, Chen WQ, Chen W, Chen X, Chen YH, Chen YG, Chen Y, Chen Y, Chen Y, Chen YJ, Chen YQ, Chen Y, Chen Z, Chen Z, Cheng A, Cheng CH, Cheng H, Cheong H, Cherry S, Chesney J, Cheung CH, Chevet E, Chi HC, Chi SG, Chiacchiera F, Chiang HL, Chiarelli R, Chiariello M, Chieppa M, Chin LS, Chiong M, Chiu GN, Cho DH, Cho SG, Cho WC, Cho YY, Cho YS, Choi AM, Choi EJ, Choi EK, Choi J, Choi ME, Choi SI, Chou TF, Chouaib S, Choubey D, Choubey V, Chow KC, Chowdhury K, Chu CT, Chuang TH, Chun T, Chung H, Chung T, Chung YL, Chwae YJ, Cianfanelli V, Ciarcia R, Ciechomska IA, Ciriolo MR, Cirone M, Claerhout S, Clague MJ, Claria J, Clarke PG, Clarke R, Clementi E, Cleyrat C, Cnop M, Coccia EM, Cocco T, Codogno P, Coers J, Cohen EE, Colecchia D, Coletto L, Coll NS, Colucci-Guyon E, Comincini S, Condello M, Cook KL, Coombs GH, Cooper CD, Cooper JM, Coppens I, Corasaniti MT, Corazzari M, Corbalan R, Corcelle-Termeau E, Cordero MD, Corral-Ramos C, Corti O, Cossarizza A, Costelli P, Costes S, Cotman SL, Coto-Montes A, Cottet S, Couve E, Covey LR, Cowart LA, Cox JS, Coxon FP, Coyne CB, Cragg MS, Craven RJ, Crepaldi T, Crespo JL, Criollo A, Crippa V, Cruz MT, Cuervo AM, Cuezva JM, Cui T, Cutillas PR, Czaja MJ, Czyzyk-Krzeska MF, Dagda RK, Dahmen U, Dai C, Dai W, Dai Y, Dalby KN, Dalla Valle L, Dalmasso G, D'Amelio M, Damme M, Darfeuille-Michaud A, Dargemont C, Darley-Usmar VM, Dasarathy S, Dasgupta B, Dash S, Dass CR, Davey HM, Davids LM, Davila D, Davis RJ, Dawson TM, Dawson VL, Daza P, de Belleroche J, de Figueiredo P, de Figueiredo RC, de la Fuente J, De Martino L, De Matteis A, De Meyer GR, De Milito A, De Santi M, de Souza W, De Tata V, De Zio D, Debnath J, Dechant R, Decuypere JP, Deegan S, Dehay B, Del Bello B, Del Re DP, Delage-Mourroux R, Delbridge LM, Deldicque L, Delorme-Axford E,

Deng Y, Dengjel J, Denizot M, Dent P, Der CJ, Deretic V, Derrien B, Deutsch E, Devarenne TP, Devenish RJ, Di Bartolomeo S, Di Daniele N, Di Domenico F, Di Nardo A, Di Paola S, Di Pietro A, Di Renzo L, DiAntonio A, Diaz-Araya G, Diaz-Laviada I, Diaz-Meco MT, Diaz-Nido J, Dickey CA, Dickson RC, Diederich M, Digard P, Dikic I, Dinesh-Kumar SP, Ding C, Ding WX, Ding Z, Dini L, Distler JH, Diwan A, Djavaheri-Mergny M, Dmytruk K, Dobson RC, Doetsch V, Dokladny K, Dokudovskaya S, Donadelli M, Dong XC, Dong X, Dong Z, Donohue TM, Jr., Doran KS, D'Orazi G, Dorn GW, 2nd, Dosenko V, Dridi S, Drucker L, Du J, Du LL, Du L, du Toit A, Dua P, Duan L, Duann P, Dubey VK, Duchen MR, Duchosal MA, Duez H, Dugail I, Dumit VI, Duncan MC, Dunlop EA, Dunn WA, Jr., Dupont N, Dupuis L, Duran RV, Durcan TM, Duvezin-Caubet S, Duvvuri U, Eapen V, Ebrahimi-Fakhari D, Echard A, Eckhart L, Edelstein CL, Edinger AL, Eichinger L, Eisenberg T, Eisenberg-Lerner A, Eissa NT, El-Deiry WS, El-Khoury V, Elazar Z, Eldar-Finkelman H, Elliott CJ, Emanuele E, Emmenegger U, Engedal N, Engelbrecht AM, Engelender S, Enserink JM, Erdmann R, Erenpreisa J, Eri R, Eriksen JL, Erman A, Escalante R, Eskelinen EL, Espert L, Esteban-Martinez L, Evans TJ, Fabri M, Fabrias G, Fabrizi C, Facchiano A, Faergeman NJ, Faggioni A, Fairlie WD, Fan C, Fan D, Fan J, Fang S, Fanto M, Fanzani A, Farkas T, Faure M, Favier FB, Fearnhead H, Federici M, Fei E, Felizardo TC, Feng H, Feng Y, Feng Y, Ferguson TA, Fernandez AF, Fernandez-Barrena MG, Fernandez-Checa JC, Fernandez-Lopez A, Fernandez-Zapico ME, Feron O, Ferraro E, Ferreira-Halder CV, Fesus L, Feuer R, Fiesel FC, Filippi-Chiela EC, Filomeni G, Fimia GM, Fingert JH, Finkbeiner S, Finkel T, Fiorito F, Fisher PB, Flajolet M, Flamigni F, Florey O, Florio S, Floto RA, Folini M, Follo C, Fon EA, Fornai F, Fortunato F, Fraldi A, Franco R, Francois A, Francois A, Frankel LB, Fraser ID, Frey N, Freyssenet DG, Frezza C, Friedman SL, Frigo DE, Fu D, Fuentes JM, Fueyo J, Fujitani Y, Fujiwara Y, Fujiya M, Fukuda M, Fulda S, Fusco C, Gabryel B, Gaestel M, Gailly P, Gajewska M, Galadari S, Galili G, Galindo I, Galindo MF, Galliciotti G, Galluzzi L, Galluzzi L, Galy V, Gammoh N, Gandy S, Ganesan AK, Ganesan S, Ganley IG, Gannage M, Gao FB, Gao F, Gao JX, Garcia Nannig L, Garcia Vescovi E, Garcia-Macia M, Garcia-Ruiz C, Garg AD, Garg PK, Gargini R, Gassen NC, Gatica D, Gatti E, Gavard J, Gavathiotis E, Ge L, Ge P, Ge S, Gean PW, Gelmetti V, Genazzani AA, Geng J, Genschik P, Gerner L, Gestwicki JE, Gewirtz DA, Ghavami S, Ghigo E, Ghosh D, Giammarioli AM, Giampieri F, Giampietri C, Giatromanolaki A, Gibbings DJ, Gibellini L, Gibson SB, Ginet V, Giordano A, Giorgini F, Giovannetti E, Girardin SE, Gispert S, Giuliano S, Gladson CL, Glavic A, Gleave M, Godefroy N, Gogal RM, Jr., Gokulan K, Goldman GH, Goletti D, Goligorsky MS, Gomes AV, Gomes LC, Gomez H, Gomez-Manzano C, Gomez-Sanchez R, Goncalves DA, Goncu E, Gong Q, Gongora C, Gonzalez CB, Gonzalez-Alegre P, Gonzalez-Cabo P, Gonzalez-Polo RA, Goping IS, Gorbea C, Gorbunov NV, Goring DR, Gorman AM, Gorski SM, Goruppi S, Goto-Yamada S, Gotor C, Gottlieb RA, Gozes I, Gozuacik D, Graba Y, Graef M, Granato GE, Grant GD, Grant S, Gravina GL, Green DR, Greenhough A, Greenwood MT, Grimaldi B, Gros F, Grose C, Groulx JF, Gruber F, Grumati P, Grune T, Guan JL, Guan KL, Guerra B, Guillen C, Gulshan K, Gunst J, Guo C, Guo L, Guo M, Guo W, Guo XG, Gust AA, Gustafsson AB, Gutierrez E, Gutierrez MG, Gwak HS, Haas A, Haber JE, Hadano S, Hagedorn M, Hahn DR, Halayko AJ, Hamacher-Brady A, Hamada K,

Hamai A, Hamann A, Hamasaki M, Hamer I, Hamid Q, Hammond EM, Han F, Han W, Handa JT, Hanover JA, Hansen M, Harada M, Harhaji-Trajkovic L, Harper JW, Harrath AH, Harris AL, Harris J, Hasler U, Hasselblatt P, Hasui K, Hawley RG, Hawley TS, He C, He CY, He F, He G, He RR, He XH, He YW, He YY, Heath JK, Hebert MJ, Heinzen RA, Helgason GV, Hensel M, Henske EP, Her C, Herman PK, Hernandez A, Hernandez C, Hernandez-Tiedra S, Hetz C, Hiesinger PR, Higaki K, Hilfiker S, Hill BG, Hill JA, Hill WD, Hino K, Hofius D, Hofman P, Hoglinger GU, Hohfeld J, Holz MK, Hong Y, Hood DA, Hoozemans JJ, Hoppe T, Hsu C, Hsu CY, Hsu LC, Hu D, Hu G, Hu HM, Hu H, Hu MC, Hu YC, Hu ZW, Hua F, Hua Y, Huang C, Huang HL, Huang KH, Huang KY, Huang S, Huang S, Huang WP, Huang YR, Huang Y, Huang Y, Huber TB, Huebbe P, Huh WK, Hulmi JJ, Hur GM, Hurley JH, Husak Z, Hussain SN, Hussain S, Hwang JJ, Hwang S, Hwang TI, Ichihara A, Imai Y, Imbriano C, Inomata M, Into T, Iovane V, Iovanna JL, Iozzo RV, Ip NY, Irazoqui JE, Iribarren P, Isaka Y, Isakovic AJ, Ischiropoulos H, Isenberg JS, Ishaq M, Ishida H, Ishii I, Ishmael JE, Isidoro C, Isobe K, Isono E, Issazadeh-Navikas S, Itahana K, Itakura E, Ivanov AI, Iyer AK, Izquierdo JM, Izumi Y, Izzo V, Jaattela M, Jaber N, Jackson DJ, Jackson WT, Jacob TG, Jacques TS, Jagannath C, Jain A, Jana NR, Jang BK, Jani A, Janji B, Jannig PR, Jansson PJ, Jean S, Jendrach M, Jeon JH, Jessen N, Jeung EB, Jia K, Jia L, Jiang H, Jiang H, Jiang L, Jiang T, Jiang X, Jiang X, Jiang X, Jiang Y, Jiang Y, Jimenez A, Jin C, Jin H, Jin L, Jin M, Jin S, Jinwal UK, Jo EK, Johansen T, Johnson DE, Johnson GV, Johnson JD, Jonasch E, Jones C, Joosten LA, Jordan J, Joseph AM, Joseph B, Joubert AM, Ju D, Ju J, Juan HF, Juenemann K, Juhasz G, Jung HS, Jung JU, Jung YK, Jungbluth H, Justice MJ, Jutten B, Kaakoush NO, Kaarniranta K, Kaasik A, Kabuta T, Kaeffer B, Kagedal K, Kahana A, Kajimura S, Kakhlon O, Kalia M, Kalvakolanu DV, Kamada Y, Kambas K, Kaminskyy VO, Kampinga HH, Kandouz M, Kang C, Kang R, Kang TC, Kanki T, Kanneganti TD, Kanno H, Kanthasamy AG, Kantorow M, Kaparakis-Liaskos M, Kapuy O, Karantza V, Karim MR, Karmakar P, Kaser A, Kaushik S, Kawula T, Kaynar AM, Ke PY, Ke ZJ, Kehrl JH, Keller KE, Kemper JK, Kenworthy AK, Kepp O, Kern A, Kesari S, Kessel D, Ketteler R, Kettelhut Ido C, Khambu B, Khan MM, Khandelwal VK, Khare S, Kiang JG, Kiger AA, Kihara A, Kim AL, Kim CH, Kim DR, Kim DH, Kim EK, Kim HY, Kim HR, Kim JS, Kim JH, Kim JC, Kim JH, Kim KW, Kim MD, Kim MM, Kim PK, Kim SW, Kim SY, Kim YS, Kim Y, Kimchi A, Kimmelman AC, Kimura T, King JS, Kirkegaard K, Kirkin V, Kirshenbaum LA, Kishi S, Kitajima Y, Kitamoto K, Kitaoka Y, Kitazato K, Kley RA, Klimecki WT, Klinkenberg M, Klucken J, Knaevelsrud H, Knecht E, Knuppertz L, Ko JL, Kobayashi S, Koch JC, Koechlin-Ramonatxo C, Koenig U, Koh YH, Kohler K, Kohlwein SD, Koike M, Komatsu M, Kominami E, Kong D, Kong HJ, Konstantakou EG, Kopp BT, Korcsmaros T, Korhonen L, Korolchuk VI, Koshkina NV, Kou Y, Koukourakis MI, Koumenis C, Kovacs AL, Kovacs T, Kovacs WJ, Koya D, Kraft C, Krainc D, Kramer H, Kravic-Stevovic T, Krek W, Kretz-Remy C, Krick R, Krishnamurthy M, Kriston-Vizi J, Kroemer G, Kruer MC, Kruger R, Ktistakis NT, Kuchitsu K, Kuhn C, Kumar AP, Kumar A, Kumar A, Kumar D, Kumar D, Kumar R, Kumar S, Kundu M, Kung HJ, Kuno A, Kuo SH, Kuret J, Kurz T, Kwok T, Kwon TK, Kwon YT, Kyrmizi I, La Spada AR, Lafont F, Lahm T, Lakkaraju A, Lam T, Lamark T, Lancel S, Landowski TH, Lane DJ, Lane JD, Lanzi C, Lapaquette P,

Lapierre LR, Laporte J, Laukkarinen J, Laurie GW, Lavandero S, Lavie L, LaVoie MJ, Law BY, Law HK, Law KB, Layfield R, Lazo PA, Le Cam L, Le Roch KG, Le Stunff H, Leardkamolkarn V, Lecuit M, Lee BH, Lee CH, Lee EF, Lee GM, Lee HJ, Lee H, Lee JK, Lee J, Lee JH, Lee JH, Lee M, Lee MS, Lee PJ, Lee SW, Lee SJ, Lee SJ, Lee SY, Lee SH, Lee SS, Lee SJ, Lee S, Lee YR, Lee YJ, Lee YH, Leeuwenburgh C, Lefort S, Legouis R, Lei J, Lei QY, Leib DA, Leibowitz G, Lekli I, Lemaire SD, Lemasters JJ, Lemberg MK, Lemoine A, Leng S, Lenz G, Lenzi P, Lerman LO, Lettieri Barbato D, Leu JI, Leung HY, Levine B, Lewis PA, Lezoualc'h F, Li C, Li F, Li FJ, Li J, Li K, Li L, Li M, Li M, Li Q, Li R, Li S, Li W, Li W, Li X, Li Y, Lian J, Liang C, Liang Q, Liao Y, Liberal J, Liberski PP, Lie P, Lieberman AP, Lim HJ, Lim KL, Lim K, Lima RT, Lin CS, Lin CF, Lin F, Lin F, Lin FC, Lin K, Lin KH, Lin PH, Lin T, Lin WW, Lin YS, Lin Y, Linden R, Lindholm D, Lindqvist LM, Lingor P, Linkermann A, Liotta LA, Lipinski MM, Lira VA, Lisanti MP, Liton PB, Liu B, Liu C, Liu CF, Liu F, Liu HJ, Liu J, Liu JJ, Liu JL, Liu K, Liu L, Liu L, Liu Q, Liu RY, Liu S, Liu S, Liu W, Liu XD, Liu X, Liu XH, Liu X, Liu X, Liu X, Liu Y, Liu Y, Liu Z, Liu Z, Liuzzi JP, Lizard G, Ljujic M, Lodhi IJ, Logue SE, Lokeshwar BL, Long YC, Lonial S, Loos B, Lopez-Otin C, Lopez-Vicario C, Lorente M, Lorenzi PL, Lorincz P, Los M, Lotze MT, Lovat PE, Lu B, Lu B, Lu J, Lu Q, Lu SM, Lu S, Lu Y, Luciano F, Luckhart S, Lucocq JM, Ludovico P, Lugea A, Lukacs NW, Lum JJ, Lund AH, Luo H, Luo J, Luo S, Luparello C, Lyons T, Ma J, Ma Y, Ma Y, Ma Z, Machado J, Machado-Santelli GM, Macian F, MacIntosh GC, MacKeigan JP, Macleod KF, MacMicking JD, MacMillan-Crow LA, Madeo F, Madesh M, Madrigal-Matute J, Maeda A, Maeda T, Maegawa G, Maellaro E, Maes H, Magarinos M, Maiese K, Maiti TK, Maiuri L, Maiuri MC, Maki CG, Malli R, Malorni W, Maloyan A, Mami-Chouaib F, Man N, Mancias JD, Mandelkow EM, Mandell MA, Manfredi AA, Manie SN, Manzoni C, Mao K, Mao Z, Mao ZW, Marambaud P, Marconi AM, Marelja Z, Marfe G, Margeta M, Margittai E, Mari M, Mariani FV, Marin C, Marinelli S, Marino G, Markovic I, Marquez R, Martelli AM, Martens S, Martin KR, Martin SJ, Martin S, Martin-Acebes MA, Martin-Sanz P, Martinand-Mari C, Martinet W, Martinez J, Martinez-Lopez N, Martinez-Outschoorn U, Martinez-Velazquez M, Martinez-Vicente M, Martins WK, Mashima H, Mastrianni JA, Matarese G, Matarrese P, Mateo R, Matoba S, Matsumoto N, Matsushita T, Matsuura A, Matsuzawa T, Mattson MP, Matus S, Maugeri N, Mauvezin C, Mayer A, Maysinger D, Mazzolini GD, McBrayer MK, McCall K, McCormick C, McInerney GM, McIver SC, McKenna S, McMahon JJ, McNeish IA, Mechta-Grigoriou F, Medema JP, Medina DL, Megyeri K, Mehrpour M, Mehta JL, Mei Y, Meier UC, Meijer AJ, Melendez A, Melino G, Melino S, de Melo EJ, Mena MA, Meneghini MD, Menendez JA, Menezes R, Meng L, Meng LH, Meng S, Menghini R, Menko AS, Menna-Barreto RF, Menon MB, Meraz-Rios MA, Merla G, Merlini L, Merlot AM, Meryk A, Meschini S, Meyer JN, Mi MT, Miao CY, Micale L, Michaeli S, Michiels C, Migliaccio AR, Mihailidou AS, Mijaljica D, Mikoshiba K, Milan E, Miller-Fleming L, Mills GB, Mills IG, Minakaki G, Minassian BA, Ming XF, Minibayeva F, Minina EA, Mintern JD, Minucci S, Miranda-Vizuete A, Mitchell CH, Miyamoto S, Miyazawa K, Mizushima N, Mnich K, Mograbi B, Mohseni S, Moita LF, Molinari M, Molinari M, Moller AB, Mollereau B, Mollinedo F, Mongillo M, Monick MM, Montagnaro S, Montell C, Moore DJ, Moore MN, Mora-Rodriguez R, Moreira PI, Morel E, Morelli

MB, Moreno S, Morgan MJ, Moris A, Moriyasu Y, Morrison JL, Morrison LA, Morselli E, Moscat J, Moseley PL, Mostowy S, Motori E, Mottet D, Mottram JC, Moussa CE, Mpakou VE, Mukhtar H, Mulcahy Levy JM, Muller S, Munoz-Moreno R, Munoz-Pinedo C, Munz C, Murphy ME, Murray JT, Murthy A, Mysorekar IU, Nabi IR, Nabissi M, Nader GA, Nagahara Y, Nagai Y, Nagata K, Nagelkerke A, Nagy P, Naidu SR, Nair S, Nakano H, Nakatogawa H, Nanjundan M, Napolitano G, Naqvi NI, Nardacci R, Narendra DP, Narita M, Nascimbeni AC, Natarajan R, Navegantes LC, Nawrocki ST, Nazarko TY, Nazarko VY, Neill T, Neri LM, Netea MG, Netea-Maier RT, Neves BM, Ney PA, Nezis IP, Nguyen HT, Nguyen HP, Nicot AS, Nilsen H, Nilsson P, Nishimura M, Nishino I, Niso-Santano M, Niu H, Nixon RA, Njar VC, Noda T, Noegel AA, Nolte EM, Norberg E, Norga KK, Noureini SK, Notomi S, Notterpek L, Nowikovsky K, Nukina N, Nurnberger T, O'Donnell VB, O'Donovan T, O'Dwyer PJ, Oehme I, Oeste CL, Ogawa M, Ogretmen B, Ogura Y, Oh YJ, Ohmuraya M, Ohshima T, Ojha R, Okamoto K, Okazaki T, Oliver FJ, Ollinger K, Olsson S, Orban DP, Ordonez P, Orhon I, Orosz L, O'Rourke EJ, Orozco H, Ortega AL, Ortona E, Osellame LD, Oshima J, Oshima S, Osiewacz HD, Otomo T, Otsu K, Ou JH, Outeiro TF, Ouyang DY, Ouyang H, Overholtzer M, Ozbun MA, Ozdinler PH, Ozpolat B, Pacelli C, Paganetti P, Page G, Pages G, Pagnini U, Pajak B, Pak SC, Pakos-Zebrucka K, Pakpour N, Palkova Z, Palladino F, Pallauf K, Pallet N, Palmieri M, Paludan SR, Palumbo C, Palumbo S, Pampliega O, Pan H, Pan W, Panaretakis T, Pandey A, Pantazopoulou A, Papackova Z, Papademetrio DL, Papassideri I, Papini A, Parajuli N, Pardo J, Parekh VV, Parenti G, Park JI, Park J, Park OK, Parker R, Parlato R, Parys JB, Parzych KR, Pasquet JM, Pasquier B, Pasumarthi KB, Patschan D, Patterson C, Pattingre S, Pattison S, Pause A, Pavenstadt H, Pavone F, Pedrozo Z, Pena FJ, Penalva MA, Pende M, Peng J, Penna F, Penninger JM, Pensalfini A, Pepe S, Pereira GJ, Pereira PC, Perez-de la Cruz V, Perez-Perez ME, Perez-Rodriguez D, Perez-Sala D, Perier C, Perl A, Perlmutter DH, Perrotta I, Pervaiz S, Pesonen M, Pessin JE, Peters GJ, Petersen M, Petrache I, Petrof BJ, Petrovski G, Phang JM, Piacentini M, Pierdominici M, Pierre P, Pierrefite-Carle V, Pietrocola F, Pimentel-Muinos FX, Pinar M, Pineda B, Pinkas-Kramarski R, Pinti M, Pinton P, Piperdi B, Piret JM, Platanias LC, Platta HW, Plowey ED, Poggeler S, Poirot M, Polcic P, Poletti A, Poon AH, Popelka H, Popova B, Poprawa I, Poulose SM, Poulton J, Powers SK, Powers T, Pozuelo-Rubio M, Prak K, Prange R, Prescott M, Priault M, Prince S, Proia RL, Proikas-Cezanne T, Prokisch H, Promponas VJ, Przyklenk K, Puertollano R, Pugazhenthi S, Puglielli L, Pujol A, Puyal J, Pyeon D, Qi X, Qian WB, Qin ZH, Qiu Y, Qu Z, Quadrilatero J, Quinn F, Raben N, Rabinowich H, Radogna F, Ragusa MJ, Rahmani M, Raina K, Ramanadham S, Ramesh R, Rami A, Randall-Demllo S, Randow F, Rao H, Rao VA, Rasmussen BB, Rasse TM, Ratovitski EA, Rautou PE, Ray SK, Razani B, Reed BH, Reggiori F, Rehm M, Reichert AS, Rein T, Reiner DJ, Reits E, Ren J, Ren X, Renna M, Reusch JE, Revuelta JL, Reyes L, Rezaie AR, Richards RI, Richardson DR, Richetta C, Riehle MA, Rihn BH, Rikihisa Y, Riley BE, Rimbach G, Rippo MR, Ritis K, Rizzi F, Rizzo E, Roach PJ, Robbins J, Roberge M, Roca G, Roccheri MC, Rocha S, Rodrigues CM, Rodriguez CI, de Cordoba SR, Rodriguez-Muela N, Roelofs J, Rogov VV, Rohn TT, Rohrer B, Romanelli D, Romani L, Romano PS, Roncero MI, Rosa JL, Rosello A, Rosen KV, Rosenstiel P, Rost-Roszkowska M, Roth KA, Roue G, Rouis M, Rouschop KM, Ruan DT, Ruano D,

Rubinsztein DC, Rucker EB, 3rd, Rudich A, Rudolf E, Rudolf R, Ruegg MA, Ruiz-Roldan C, Ruparelia AA, Rusmini P, Russ DW, Russo GL, Russo G, Russo R, Rusten TE, Ryabovol V, Ryan KM, Ryter SW, Sabatini DM, Sacher M, Sachse C, Sack MN, Sadoshima J, Saftig P, Sagi-Eisenberg R, Sahni S, Saikumar P, Saito T, Saitoh T, Sakakura K, Sakoh-Nakatogawa M, Sakuraba Y, Salazar-Roa M, Salomoni P, Saluja AK, Salvaterra PM, Salvioli R, Samali A, Sanchez AM, Sanchez-Alcazar JA, Sanchez-Prieto R, Sandri M, Sanjuan MA, Santaguida S, Santambrogio L, Santoni G, Dos Santos CN, Saran S, Sardiello M, Sargent G, Sarkar P, Sarkar S, Sarrias MR, Sarwal MM, Sasakawa C, Sasaki M, Sass M, Sato K, Sato M, Satriano J, Savaraj N, Saveljeva S, Schaefer L, Schaible UE, Scharl M, Schatzl HM, Schekman R, Scheper W, Schiavi A, Schipper HM, Schmeisser H, Schmidt J, Schmitz I, Schneider BE, Schneider EM, Schneider JL, Schon EA, Schonenberger MJ, Schonthal AH, Schorderet DF, Schroder B, Schuck S, Schulze RJ, Schwarten M, Schwarz TL, Sciarretta S, Scotto K, Scovassi AI, Screaton RA, Screen M, Seca H, Sedej S, Segatori L, Segev N, Seglen PO, Segui-Simarro JM, Segura-Aguilar J, Seki E, Sell C, Seiliez I, Semenkovich CF, Semenza GL, Sen U, Serra AL, Serrano-Puebla A, Sesaki H, Setoguchi T, Settembre C, Shacka JJ, Shajahan-Haq AN, Shapiro IM, Sharma S, She H, Shen CK, Shen CC, Shen HM, Shen S, Shen W, Sheng R, Sheng X, Sheng ZH, Shepherd TG, Shi J, Shi Q, Shi Q, Shi Y, Shibutani S, Shibuya K, Shidoji Y, Shieh JJ, Shih CM, Shimada Y, Shimizu S, Shin DW, Shinohara ML, Shintani M, Shintani T, Shioi T, Shirabe K, Shiri-Sverdlov R, Shirihai O, Shore GC, Shu CW, Shukla D, Sibirny AA, Sica V, Sigurdson CJ, Sigurdsson EM, Sijwali PS, Sikorska B, Silveira WA, Silvente-Poirot S, Silverman GA, Simak J, Simmet T, Simon AK, Simon HU, Simone C, Simons M, Simonsen A, Singh R, Singh SV, Singh SK, Sinha D, Sinha S, Sinicrope FA, Sirko A, Sirohi K, Sishi BJ, Sittler A, Siu PM, Sivridis E, Skwarska A, Slack R, Slaninova I, Slavov N, Smaili SS, Smalley KS, Smith DR, Soenen SJ, Soleimanpour SA, Solhaug A, Somasundaram K, Son JH, Sonawane A, Song C, Song F, Song HK, Song JX, Song W, Soo KY, Sood AK, Soong TW, Soontornniyomkij V, Sorice M, Sotgia F, Soto-Pantoja DR, Sotthibundhu A, Sousa MJ, Spaink HP, Span PN, Spang A, Sparks JD, Speck PG, Spector SA, Spies CD, Springer W, Clair DS, Stacchiotti A, Staels B, Stang MT, Starczynowski DT, Starokadomskyy P, Steegborn C, Steele JW, Stefanis L, Steffan J, Stellrecht CM, Stenmark H, Stepkowski TM, Stern ST, Stevens C, Stockwell BR, Stoka V, Storchova Z, Stork B, Stratoulias V, Stravopodis DJ, Strnad P, Strohecker AM, Strom AL, Stromhaug P, Stulik J, Su YX, Su Z, Subauste CS, Subramaniam S, Sue CM, Suh SW, Sui X, Sukseree S, Sulzer D, Sun FL, Sun J, Sun J, Sun SY, Sun Y, Sun Y, Sun Y, Sundaramoorthy V, Sung J, Suzuki H, Suzuki K, Suzuki N, Suzuki T, Suzuki YJ, Swanson MS, Swanton C, Sward K, Swarup G, Sweeney ST, Sylvester PW, Szatmari Z, Szegezdi E, Szlosarek PW, Taegtmeyer H, Tafani M, Taillebourg E, Tait SW, Takacs-Vellai K, Takahashi Y, Takats S, Takemura G, Takigawa N, Talbot NJ, Tamagno E, Tamburini J, Tan CP, Tan L, Tan ML, Tan M, Tan YJ, Tanaka K, Tanaka M, Tang D, Tang D, Tang G, Tanida I, Tanji K, Tannous BA, Tapia JA, Tasset-Cuevas I, Tatar M, Tavassoly I, Tavernarakis N, Taylor A, Taylor GS, Taylor GA, Taylor JP, Taylor MJ, Tchetina EV, Tee AR, Teixeira-Clerc F, Telang S, Tencomnao T, Teng BB, Teng RJ, Terro F, Tettamanti G, Theiss AL, Theron AE, Thomas KJ, Thome MP, Thomes PG, Thorburn A, Thorner J, Thum T, Thumm M, Thurston TL, Tian L, Till A, Ting JP,

Titorenko VI, Toker L, Toldo S, Tooze SA, Topisirovic I, Torgersen ML, Torosantucci L, Torriglia A, Torrisi MR, Tournier C, Towns R, Trajkovic V, Travassos LH, Triola G, Tripathi DN, Trisciuoglio D, Troncoso R, Trougakos IP, Truttmann AC, Tsai KJ, Tschan MP, Tseng YH, Tsukuba T, Tsung A, Tsvetkov AS, Tu S, Tuan HY, Tucci M, Tumbarello DA, Turk B, Turk V, Turner RF, Tveita AA, Tyagi SC, Ubukata M, Uchiyama Y, Udelnow A, Ueno T, Umekawa M, Umemiya-Shirafuji R, Underwood BR, Ungermann C, Ureshino RP, Ushioda R, Uversky VN, Uzcategui NL, Vaccari T, Vaccaro MI, Vachova L, Vakifahmetoglu-Norberg H, Valdor R, Valente EM, Vallette F, Valverde AM, Van den Berghe G, Van Den Bosch L, van den Brink GR, van der Goot FG, van der Klei IJ, van der Laan LJ, van Doorn WG, van Egmond M, van Golen KL, Van Kaer L, van Lookeren Campagne M, Vandenabeele P, Vandenberghe W, Vanhorebeek I, Varela-Nieto I, Vasconcelos MH, Vasko R, Vavvas DG, Vega-Naredo I, Velasco G, Velentzas AD, Velentzas PD, Vellai T, Vellenga E, Vendelbo MH, Venkatachalam K, Ventura N, Ventura S, Veras PS, Verdier M, Vertessy BG, Viale A, Vidal M, Vieira HL, Vierstra RD, Vigneswaran N, Vij N, Vila M, Villar M, Villar VH, Villarroya J, Vindis C, Viola G, Viscomi MT, Vitale G, Vogl DT, Voitsekhovskaja OV, von Haefen C, von Schwarzenberg K, Voth DE, Vouret-Craviari V, Vuori K, Vyas JM, Waeber C, Walker CL, Walker MJ, Walter J, Wan L, Wan X, Wang B, Wang C, Wang CY, Wang C, Wang C, Wang C, Wang D, Wang F, Wang F, Wang G, Wang HJ, Wang H, Wang HG, Wang H, Wang HD, Wang J, Wang J, Wang M, Wang MQ, Wang PY, Wang P, Wang RC, Wang S, Wang TF, Wang X, Wang XJ, Wang XW, Wang X, Wang X, Wang Y, Wang Y, Wang Y, Wang YJ, Wang Y, Wang Y, Wang YT, Wang Y, Wang ZN, Wappner P, Ward C, Ward DM, Warnes G, Watada H, Watanabe Y, Watase K, Weaver TE, Weekes CD, Wei J, Weide T, Weihl CC, Weindl G, Weis SN, Wen L, Wen X, Wen Y, Westermann B, Weyand CM, White AR, White E, Whitton JL, Whitworth AJ, Wiels J, Wild F, Wildenberg ME, Wileman T, Wilkinson DS, Wilkinson S, Willbold D, Williams C, Williams K, Williamson PR, Winklhofer KF, Witkin SS, Wohlgemuth SE, Wollert T, Wolvetang EJ, Wong E, Wong GW, Wong RW, Wong VK, Woodcock EA, Wright KL, Wu C, Wu D, Wu GS, Wu J, Wu J, Wu M, Wu M, Wu S, Wu WK, Wu Y, Wu Z, Xavier CP, Xavier RJ, Xia GX, Xia T, Xia W, Xia Y, Xiao H, Xiao J, Xiao S, Xiao W, Xie CM, Xie Z, Xie Z, Xilouri M, Xiong Y, Xu C, Xu C, Xu F, Xu H, Xu H, Xu J, Xu J, Xu J, Xu L, Xu X, Xu Y, Xu Y, Xu ZX, Xu Z, Xue Y, Yamada T, Yamamoto A, Yamanaka K, Yamashina S, Yamashiro S, Yan B, Yan B, Yan X, Yan Z, Yanagi Y, Yang DS, Yang JM, Yang L, Yang M, Yang PM, Yang P, Yang Q, Yang W, Yang WY, Yang X, Yang Y, Yang Y, Yang Z, Yang Z, Yao MC, Yao PJ, Yao X, Yao Z, Yao Z, Yasui LS, Ye M, Yedvobnick B, Yeganeh B, Yeh ES, Yeyati PL, Yi F, Yi L, Yin XM, Yip CK, Yoo YM, Yoo YH, Yoon SY, Yoshida K, Yoshimori T, Young KH, Yu H, Yu JJ, Yu JT, Yu J, Yu L, Yu WH, Yu XF, Yu Z, Yuan J, Yuan ZM, Yue BY, Yue J, Yue Z, Zacks DN, Zacksenhaus E, Zaffaroni N, Zaglia T, Zakeri Z, Zecchini V, Zeng J, Zeng M, Zeng Q, Zervos AS, Zhang DD, Zhang F, Zhang G, Zhang GC, Zhang H, Zhang H, Zhang H, Zhang H, Zhang J, Zhang J, Zhang J, Zhang J, Zhang JP, Zhang L, Zhang L, Zhang L, Zhang L, Zhang MY, Zhang X, Zhang XD, Zhang Y, Zhang Y, Zhang Y, Zhang Y, Zhang Y, Zhao M, Zhao WL, Zhao X, Zhao YG, Zhao Y, Zhao Y, Zhao YX, Zhao Z, Zhao ZJ, Zheng D, Zheng XL, Zheng X, Zhivotovsky B, Zhong Q, Zhou GZ, Zhou G, Zhou H, Zhou SF, Zhou XJ, Zhu H, Zhu

H, Zhu WG, Zhu W, Zhu XF, Zhu Y, Zhuang SM, Zhuang X, Ziparo E, Zois CE, Zoladek T, Zong WX, Zorzano A, Zughaier SM. 2016. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* **12**, 1-222.
Kraft C, Deplazes A, Sohrmann M, Peter M. 2008. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat Cell Biol* **10**, 602-610.
Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N. 2004. Analysis of autophagy induced in neonates using autophagy-deficient mice. *Molecular Biology of the Cell* **15**, 211a-211a.
Kurusu T, Koyano T, Hanamata S, Kubo T, Noguchi Y, Yagi C, Nagata N, Yamamoto T, Ohnishi T, Okazaki Y, Kitahata N, Ando D, Ishikawa M, Wada S, Miyao A, Hirochika H, Shimada H, Makino A, Saito K, Ishida H, Kinoshita T, Kurata N, Kuchitsu K. 2014. OsATG7 is required for autophagy-dependent lipid metabolism in rice postmeiotic anther development. *Autophagy* **10**, 878-888.
Kwon SI, Cho HJ, Kim SR, Park OK. 2013. The Rab GTPase RabG3b positively regulates autophagy and immunity-associated hypersensitive cell death in Arabidopsis. *Plant Physiol* **161**, 1722-1736.
Lai Z, Wang F, Zheng Z, Fan B, Chen Z. 2011. A critical role of autophagy in plant resistance to necrotrophic fungal pathogens. *Plant J* **66**, 953-968.
Laureano-Marin AM, Moreno I, Romero LC, Gotor C. 2016. Negative Regulation of Autophagy by Sulfide Is Independent of Reactive Oxygen Species. *Plant Physiology* **171**, 1378-1391.
Le Bars R, Marion J, Le Borgne R, Satiat-Jeunemaitre B, Bianchi MW. 2014. ATG5 defines a phagophore domain connected to the endoplasmic reticulum during autophagosome formation in plants. *Nat Commun* **5**, 4121.
Leary AY, Sanguankiattichai N, Duggan C, Tumtas Y, Pandey P, Segretin ME, Linares JS, Savage ZD, Yow RJ, T.O. B. 2018. Modulation of plant autophagy during pathogen attack. *J Exp Bot*.
Lee DY, Arnott D, Brown EJ. 2013. Ubiquilin4 is an adaptor protein that recruits Ubiquilin1 to the autophagy machinery. *EMBO Rep* **14**, 373-381.
Lenz HD, Haller E, Melzer E, Kober K, Wurster K, Stahl M, Bassham DC, Vierstra RD, Parker JE, Bautor J, Molina A, Escudero V, Shindo T, van der Hoorn RA, Gust AA, Nurnberger T. 2011. Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. *Plant J* **66**, 818-830.
Levine B, Mizushima N, Virgin HW. 2011. Autophagy in immunity and inflammation. *Nature* **469**, 323-335.
Li F, Chung T, Pennington JG, Federico ML, Kaeppler HF, Kaeppler SM, Otegui MS, Vierstra RD. 2015. Autophagic recycling plays a central role in maize nitrogen remobilization. *Plant Cell* **27**, 1389-1408.
Li F, Chung T, Vierstra RD. 2014. AUTOPHAGY-RELATED11 plays a critical role in general autophagy- and senescence-induced mitophagy in Arabidopsis. *Plant Cell* **26**, 788-807.
Li F, Vierstra RD. 2012. Autophagy: a multifaceted intracellular system for bulk and selective recycling. *Trends Plant Sci* **17**, 526-537.
Li J, Nam KH. 2002. Regulation of brassinosteroid signaling by a GSK3/SHAGGY-like kinase. *Science* **295**, 1299-1301.

Li W, Chen M, Wang E, Hu L, Hawkesford MJ, Zhong L, Chen Z, Xu Z, Li L, Zhou Y, Guo C, Ma Y. 2016a. Genome-wide analysis of autophagy-associated genes in foxtail millet (Setaria italica L.) and characterization of the function of SiATG8a in conferring tolerance to nitrogen starvation in rice. *BMC Genomics* **17**, 797.
Li Y, Kabbage M, Liu W, Dickman MB. 2016b. Aspartyl Protease-Mediated Cleavage of BAG6 Is Necessary for Autophagy and Fungal Resistance in Plants. *Plant Cell* **28**, 233-247.
Lin HK, Wang L, Hu YC, Altuwaijri S, Chang C. 2002. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. *EMBO J* **21**, 4037-4048.
Lin Y, Cluette-Brown JE, Goodman HM. 2004. The peroxisome deficient Arabidopsis mutant sse1 exhibits impaired fatty acid synthesis. *Plant Physiol* **135**, 814-827.
Lin YL, Sung SC, Tsai HL, Yu TT, Radjacommare R, Usharani R, Fatimababy AS, Lin HY, Wang YY, Fu H. 2011. The defective proteasome but not substrate recognition function is responsible for the null phenotypes of the Arabidopsis proteasome subunit RPN10. *Plant Cell* **23**, 2754-2773.
Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, Cai Y, Norberg HV, Zhang T, Furuya T, Jin M, Zhu Z, Wang H, Yu J, Li Y, Hao Y, Choi A, Ke H, Ma D, Yuan J. 2011. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell* **147**, 223-234.
Liu Y, Bassham DC. 2010. TOR is a negative regulator of autophagy in Arabidopsis thaliana. *PLoS One* **5**, e11883.
Liu Y, Burgos JS, Deng Y, Srivastava R, Howell SH, Bassham DC. 2012. Degradation of the endoplasmic reticulum by autophagy during endoplasmic reticulum stress in Arabidopsis. *Plant Cell* **24**, 4635-4651.
Liu Y, Schiff M, Czymmek K, Talloczy Z, Levine B, Dinesh-Kumar SP. 2005. Autophagy regulates programmed cell death during the plant innate immune response. *Cell* **121**, 567-577.
Liu Y, Xiong Y, Bassham DC. 2009. Autophagy is required for tolerance of drought and salt stress in plants. *Autophagy* **5**, 954-963.
Ma J, Jin R, Dobry CJ, Lawson SK, Kumar A. 2007. Overexpression of autophagy-related genes inhibits yeast filamentous growth. *Autophagy* **3**, 604-609.
Mair A, Pedrotti L, Wurzinger B, Anrather D, Simeunovic A, Weiste C, Valerio C, Dietrich K, Kirchler T, Nagele T, Vicente Carbajosa J, Hanson J, Baena-Gonzalez E, Chaban C, Weckwerth W, Droge-Laser W, Teige M. 2015. SnRK1-triggered switch of bZIP63 dimerization mediates the low-energy response in plants. *Elife* **4**.
Marion J, Le Bars R, Satiat-Jeunemaitre B, Boulogne C. 2017. Optimizing CLEM protocols for plants cells: GMA embedding and cryosections as alternatives for preservation of GFP fluorescence in Arabidopsis roots. *J Struct Biol* **198**, 196-202.
Marshall RS, Li F, Gemperline DC, Book AJ, Vierstra RD. 2015. Autophagic Degradation of the 26S Proteasome Is Mediated by the Dual ATG8/Ubiquitin Receptor RPN10 in Arabidopsis. *Mol Cell* **58**, 1053-1066.
Marshall RS, McLoughlin F, Vierstra RD. 2016. Autophagic Turnover of Inactive 26S Proteasomes in Yeast Is Directed by the Ubiquitin Receptor Cue5 and the Hsp42 Chaperone. *Cell Rep* **16**, 1717-1732.

Martinez DE, Costa ML, Gomez FM, Otegui MS, Guiamet JJ. 2008. 'Senescence-associated vacuoles' are involved in the degradation of chloroplast proteins in tobacco leaves. *Plant J* **56**, 196-206.
Masclaux-Daubresse C, Clement G, Anne P, Routaboul JM, Guiboileau A, Soulay F, Shirasu K, Yoshimoto K. 2014. Stitching together the Multiple Dimensions of Autophagy Using Metabolomics and Transcriptomics Reveals Impacts on Metabolism, Development, and Plant Responses to the Environment in Arabidopsis. *Plant Cell* **26**, 1857-1877.
Mathis AD, Naylor BC, Carson RH, Evans E, Harwell J, Knecht J, Hexem E, Peelor FF, 3rd, Miller BF, Hamilton KL, Transtrum MK, Bikman BT, Price JC. 2017. Mechanisms of In Vivo Ribosome Maintenance Change in Response to Nutrient Signals. *Mol Cell Proteomics* **16**, 243-254.
Matsuoka K, Higuchi T, Maeshima M, Nakamura K. 1997. A vacuolar-type H+-ATPase in a nonvacuolar organelle is required for the sorting of soluble vacuolar protein precursors in tobacco cells. *Plant Cell* **9**, 533-546.
Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C. 2002. Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 6422-6427.
Michaeli S, Galili G, Genschik P, Fernie AR, Avin-Wittenberg T. 2016. Autophagy in Plants--What's New on the Menu? *Trends Plant Sci* **21**, 134-144.
Michaeli S, Honig A, Levanony H, Peled-Zehavi H, Galili G. 2014. Arabidopsis ATG8-INTERACTING PROTEIN1 is involved in autophagy-dependent vesicular trafficking of plastid proteins to the vacuole. *Plant Cell* **26**, 4084-4101.
Minamikawa T, Toyooka K, Okamoto T, Hara-Nishimura I, Nishimura M. 2001. Degradation of ribulose-bisphosphate carboxylase by vacuolar enzymes of senescing French bean leaves: immunocytochemical and ultrastructural observations. *Protoplasma* **218**, 144-153.
Minina EA, Bozhkov PV, Hofius D. 2014. Autophagy as initiator or executioner of cell death. *Trends Plant Sci* **19**, 692-697.
Minina EA, Filonova LH, Fukada K, Savenkov EI, Gogvadze V, Clapham D, Sanchez-Vera V, Suarez MF, Zhivotovsky B, Daniel G, Smertenko A, Bozhkov PV. 2013a. Autophagy and metacaspase determine the mode of cell death in plants. *J Cell Biol* **203**, 917-927.
Minina EA, Moschou PN, Vetukuri RR, Sanchez-Vera V, Cardoso C, Liu Q, Elander PH, Dalman K, Beganovic M, Yilmaz JL, Marmon S, Shabala L, Suarez MF, Ljung K, Novák O, Shabala S, Stymne S, Hofius D, Bozhkov PV. 2018. Transcriptional stimulation of rate-limiting components of the autophagic pathway improves plant fitness *J Exp Bot*.
Minina EA, Sanchez-Vera V, Moschou PN, Suarez MF, Sundberg E, Weih M, Bozhkov PV. 2013b. Autophagy mediates caloric restriction-induced lifespan extension in Arabidopsis. *Aging Cell* **12**, 327-329.
Mizushima N. 2004. Methods for monitoring autophagy. *Int J Biochem Cell Biol* **36**, 2491-2502.
Montane MH, Menand B. 2013. ATP-competitive mTOR kinase inhibitors delay plant growth by triggering early differentiation of meristematic cells but no developmental patterning change. *Journal of Experimental Botany* **64**, 4361-4374.

Moriyasu Y. 1995. Examination of the contribution of vacuolar proteases to intracellular protein degradation in Chara corallina. *Plant Physiol* **109**, 1309-1315.
Mostowy S. 2013. Autophagy and bacterial clearance: a not so clear picture. *Cell Microbiol* **15**, 395-402.
Mukae K, Inoue Y, Moriyasu Y. 2015. ATG5-knockout mutants of Physcomitrella provide a platform for analyzing the involvement of autophagy in senescence processes in plant cells. *Plant Signaling & Behavior* **10**, e1086859.
Munch D, Rodriguez E, Bressendorff S, Park OK, Hofius D, Petersen M. 2014. Autophagy deficiency leads to accumulation of ubiquitinated proteins, ER stress, and cell death in Arabidopsis. *Autophagy* **10**, 1579-1587.
Munch D, Teh OK, Malinovsky FG, Liu Q, Vetukuri RR, El Kasmi F, Brodersen P, Hara-Nishimura I, Dangl JL, Petersen M, Mundy J, Hofius D. 2015. Retromer contributes to immunity-associated cell death in Arabidopsis. *Plant Cell* **27**, 463-479.
Nakahara KS, Masuta C, Yamada S, Shimura H, Kashihara Y, Wada TS, Meguro A, Goto K, Tadamura K, Sueda K, Sekiguchi T, Shao J, Itchoda N, Matsumura T, Igarashi M, Ito K, Carthew RW, Uyeda I. 2012. Tobacco calmodulin-like protein provides secondary defense by binding to and directing degradation of virus RNA silencing suppressors. *Proc Natl Acad Sci U S A* **109**, 10113-10118.
Nakatogawa H. 2013. Two ubiquitin-like conjugation systems that mediate membrane formation during autophagy. *Essays Biochem* **55**, 39-50.
Nakatogawa H, Ohbayashi S, Sakoh-Nakatogawa M, Kakuta S, Suzuki SW, Kirisako H, Kondo-Kakuta C, Noda NN, Yamamoto H, Ohsumi Y. 2012. The autophagy-related protein kinase Atg1 interacts with the ubiquitin-like protein Atg8 via the Atg8 family interacting motif to facilitate autophagosome formation. *J Biol Chem* **287**, 28503-28507.
Nakayama M, Kaneko Y, Miyazawa Y, Fujii N, Higashitani N, Wada S, Ishida H, Yoshimoto K, Shirasu K, Yamada K, Nishimura M, Takahashi H. 2012. A possible involvement of autophagy in amyloplast degradation in columella cells during hydrotropic response of Arabidopsis roots. *Planta* **236**, 999-1012.
Nascimbeni AC, Giordano F, Dupont N, Grasso D, Vaccaro MI, Codogno P, Morel E. 2017. ER-plasma membrane contact sites contribute to autophagosome biogenesis by regulation of local PI3P synthesis. *EMBO J* **36**, 2018-2033.
Nazio F, Carinci M, Valacca C, Bielli P, Strappazzon F, Antonioli M, Ciccosanti F, Rodolfo C, Campello S, Fimia GM, Sette C, Bonaldo P, Cecconi F. 2016. Fine-tuning of ULK1 mRNA and protein levels is required for autophagy oscillation. *J Cell Biol* **215**, 841-856.
Nolan TM, Brennan B, Yang M, Chen J, Zhang M, Li Z, Wang X, Bassham DC, Walley J, Yin Y. 2017. Selective Autophagy of BES1 Mediated by DSK2 Balances Plant Growth and Survival. *Dev Cell* **41**, 33-46 e37.
Nukarinen E, Nagele T, Pedrotti L, Wurzinger B, Mair A, Landgraf R, Bornke F, Hanson J, Teige M, Baena-Gonzalez E, Droge-Laser W, Weckwerth W. 2016. Quantitative phosphoproteomics reveals the role of the AMPK plant ortholog SnRK1 as a metabolic master regulator under energy deprivation. *Sci Rep* **6**, 31697.
Oh-ye Y, Inoue Y, Moriyasu Y. 2011. Detecting autophagy in Arabidopsis roots by membrane-permeable cysteine protease inhibitor E-64d and endocytosis tracer FM4-64. *Plant Signaling & Behavior* **6**, 1946-1949.
Ohsumi Y. 2014. Historical landmarks of autophagy research. *Cell Res* **24**, 9-23.

Ono Y, Wada S, Izumi M, Makino A, Ishida H. 2013. Evidence for contribution of autophagy to rubisco degradation during leaf senescence in Arabidopsis thaliana. *Plant Cell Environ* **36**, 1147-1159.
Onodera J, Ohsumi Y. 2004. Ald6p is a preferred target for autophagy in yeast, Saccharomyces cerevisiae. *J Biol Chem* **279**, 16071-16076.
Papini A, Mosti S, van Doorn WG. 2014. Classical macroautophagy in Lobivia rauschii (Cactaceae) and possible plastidial autophagy in Tillandsia albida (Bromeliaceae) tapetum cells. *Protoplasma* **251**, 719-725.
Paul P, Munz C. 2016. Autophagy and Mammalian Viruses: Roles in Immune Response, Viral Replication, and Beyond. *Adv Virus Res* **95**, 149-195.
Pei D, Zhang W, Sun H, Wei X, Yue J, Wang H. 2014. Identification of autophagy-related genes ATG4 and ATG8 from wheat (Triticum aestivum L.) and profiling of their expression patterns responding to biotic and abiotic stresses. *Plant Cell Rep*.
Pengo N, Agrotis A, Prak K, Jones J, Ketteler R. 2017. A reversible phospho-switch mediated by ULK1 regulates the activity of autophagy protease ATG4B. *Nat Commun* **8**, 294.
Perez-Perez ME, Florencio FJ, Crespo JL. 2010. Inhibition of target of rapamycin signaling and stress activate autophagy in Chlamydomonas reinhardtii. *Plant Physiol* **152**, 1874-1888.
Petrov V, Hille J, Mueller-Roeber B, Gechev TS. 2015. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front Plant Sci* **6**, 69.
Phillips AR, Suttangkakul A, Vierstra RD. 2008. The ATG12-conjugating enzyme ATG10 Is essential for autophagic vesicle formation in Arabidopsis thaliana. *Genetics* **178**, 1339-1353.
Ponpuak M, Mandell MA, Kimura T, Chauhan S, Cleyrat C, Deretic V. 2015. Secretory autophagy. *Curr Opin Cell Biol* **35**, 106-116.
Popa C, Li L, Gil S, Tatjer L, Hashii K, Tabuchi M, Coll NS, Arino J, Valls M. 2016. The effector AWR5 from the plant pathogen Ralstonia solanacearum is an inhibitor of the TOR signalling pathway. *Sci Rep* **6**, 27058.
Prins A, van Heerden PD, Olmos E, Kunert KJ, Foyer CH. 2008. Cysteine proteinases regulate chloroplast protein content and composition in tobacco leaves: a model for dynamic interactions with ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) vesicular bodies. *J Exp Bot* **59**, 1935-1950.
Pu YT, Luo XJ, Bassham DC. 2017. TOR-Dependent and -Independent Pathways Regulate Autophagy in Arabidopsis thaliana. *Frontiers in Plant Science* **8**.
Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, Jung S, Jung YK. 2013. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat Commun* **4**, 2300.
Qin X, Zhang RX, Ge S, Zhou T, Liang YK. 2017. Sphingosine kinase AtSPHK1 functions in fumonisin B1-triggered cell death in Arabidopsis. *Plant Physiol Biochem* **119**, 70-80.
Reggiori F, Monastyrska I, Shintani T, Klionsky DJ. 2005. The actin cytoskeleton is required for selective types of autophagy, but not nonspecific autophagy, in the yeast Saccharomyces cerevisiae. *Molecular Biology of the Cell* **16**, 5843-5856.
Reggiori F, Wang CW, Nair U, Shintani T, Abeliovich H, Klionsky DJ. 2004. Early stages of the secretory pathway, but not endosomes, are required for Cvt vesicle and

autophagosome assembly in Saccharomyces cerevisiae. *Molecular Biology of the Cell* **15**, 2189-2204.
Ren MZ, Venglat P, Qiu SQ, Feng L, Cao YG, Wang E, Xiang DQ, Wang JH, Alexander D, Chalivendra S, Logan D, Mattoo A, Selvaraj G, Datla R. 2012. Target of Rapamycin Signaling Regulates Metabolism, Growth, and Life Span in Arabidopsis. *Plant Cell* **24**, 4850-4874.
Rexin D, Meyer C, Robaglia C, Veit B. 2015. TOR signalling in plants. *Biochem J* **470**, 1-14.
Reyes FC, Chung T, Holding D, Jung R, Vierstra R, Otegui MS. 2011. Delivery of prolamins to the protein storage vacuole in maize aleurone cells. *Plant Cell* **23**, 769-784.
Rose TL, Bonneau L, Der C, Marty-Mazars D, Marty F. 2006. Starvation-induced expression of autophagy-related genes in Arabidopsis. *Biol Cell* **98**, 53-67.
Sanchez-Wandelmer J, Kriegenburg F, Rohringer S, Schuschnig M, Gomez-Sanchez R, Zens B, Abreu S, Hardenberg R, Hollenstein D, Gao J, Ungermann C, Martens S, Kraft C, Reggiori F. 2017. Atg4 proteolytic activity can be inhibited by Atg1 phosphorylation. *Nat Commun* **8**, 295.
Sandalio LM, Romero-Puertas MC. 2015. Peroxisomes sense and respond to environmental cues by regulating ROS and RNS signalling networks. *Ann Bot* **116**, 475-485.
Schwarz V, Andosch A, Geretschlager A, Affenzeller M, Lutz-Meindl U. 2017. Carbon starvation induces lipid degradation via autophagy in the model alga Micrasterias. *J Plant Physiol* **208**, 115-127.
Scott RC, Juhasz G, Neufeld TP. 2007. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. *Curr Biol* **17**, 1-11.
Segui-Simarro JM, Corral-Martinez P, Corredor E, Raska I, Testillano PS, Risueno MC. 2011. A change of developmental program induces the remodeling of the interchromatin domain during microspore embryogenesis in Brassica napus L. *J Plant Physiol* **168**, 746-757.
Segui-Simarro JM, Testillano PS, Risueno MC. 2003. Hsp70 and Hsp90 change their expression and subcellular localization after microspore embryogenesis induction in Brassica napus L. *J Struct Biol* **142**, 379-391.
Sequera-Mutiozabal MI, Erban A, Kopka J, Atanasov KE, Bastida J, Fotopoulos V, Alcazar R, Tiburcio AF. 2016. Global Metabolic Profiling of Arabidopsis Polyamine Oxidase 4 (AtPAO4) Loss-of-Function Mutants Exhibiting Delayed Dark-Induced Senescence. *Frontiers in Plant Science* **7**, 173.
Shemi A, Schatz D, Fredricks HF, Van Mooy BA, Porat Z, Vardi A. 2016. Phosphorus starvation induces membrane remodeling and recycling in Emiliania huxleyi. *New Phytol* **211**, 886-898.
Shi CS, Kehrl JH. 2010. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal* **3**, ra42.
Shibata M, Oikawa K, Yoshimoto K, Goto-Yamada S, Mano S, Yamada K, Kondo M, Hayashi M, Sakamoto W, Ohsumi Y, Nishimura M. 2014. Plant autophagy is responsible for peroxisomal transition and plays an important role in the maintenance of peroxisomal quality. *Autophagy* **10**, 936-937.

Shibata M, Oikawa K, Yoshimoto K, Kondo M, Mano S, Yamada K, Hayashi M, Sakamoto W, Ohsumi Y, Nishimura M. 2013. Highly oxidized peroxisomes are selectively degraded via autophagy in Arabidopsis. *Plant Cell* **25**, 4967-4983.
Shpilka T, Welter E, Borovsky N, Amar N, Mari M, Reggiori F, Elazar Z. 2015. Lipid droplets and their component triglycerides and steryl esters regulate autophagosome biogenesis. *EMBO J* **34**, 2117-2131.
Singh R, Kaushik S, Wang YJ, Xiang YQ, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. 2009. Autophagy regulates lipid metabolism. *Nature* **458**, 1131-U1164.
Slavikova S, Ufaz S, Avin-Wittenberg T, Levanony H, Galili G. 2008. An autophagy-associated Atg8 protein is involved in the responses of Arabidopsis seedlings to hormonal controls and abiotic stresses. *J Exp Bot* **59**, 4029-4043.
Soto-Burgos J, Bassham DC. 2017. SnRK1 activates autophagy via the TOR signaling pathway in Arabidopsis thaliana. *PLoS One* **12**, e0182591.
Spitzer C, Li F, Buono R, Roschzttardtz H, Chung T, Zhang M, Osteryoung KW, Vierstra RD, Otegui MS. 2015. The Endosomal Protein CHARGED MULTIVESICULAR BODY PROTEIN1 Regulates the Autophagic Turnover of Plastids in Arabidopsis. *Plant Cell* **27**, 391-402.
Suttangkakul A, Li F, Chung T, Vierstra RD. 2011. The ATG1/ATG13 protein kinase complex is both a regulator and a target of autophagic recycling in Arabidopsis. *Plant Cell* **23**, 3761-3779.
Svenning S, Lamark T, Krause K, Johansen T. 2011. Plant NBR1 is a selective autophagy substrate and a functional hybrid of the mammalian autophagic adapters NBR1 and p62/SQSTM1. *Autophagy* **7**, 993-1010.
Takatsuka C, Inoue Y, Matsuoka K, Moriyasu Y. 2004. 3-methyladenine inhibits autophagy in tobacco culture cells under sucrose starvation conditions. *Plant and Cell Physiology* **45**, 265-274.
Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. 1992. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* **119**, 301-311.
Thompson AR, Doelling JH, Suttangkakul A, Vierstra RD. 2005. Autophagic nutrient recycling in Arabidopsis directed by the ATG8 and ATG12 conjugation pathways. *Plant Physiol* **138**, 2097-2110.
Tsukada M, Ohsumi Y. 1993. Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. *FEBS Lett* **333**, 169-174.
Vakifahmetoglu-Norberg H, Xia HG, Yuan J. 2015. Pharmacologic agents targeting autophagy. *J Clin Invest* **125**, 5-13.
Vanhee C, Guillon S, Masquelier D, Degand H, Deleu M, Morsomme P, Batoko H. 2011a. A TSPO-related protein localizes to the early secretory pathway in Arabidopsis, but is targeted to mitochondria when expressed in yeast. *J Exp Bot* **62**, 497-508.
Vanhee C, Zapotoczny G, Masquelier D, Ghislain M, Batoko H. 2011b. The Arabidopsis multistress regulator TSPO is a heme binding membrane protein and a potential scavenger of porphyrins via an autophagy-dependent degradation mechanism. *Plant Cell* **23**, 785-805.
Veljanovski V, Batoko H. 2014. Selective autophagy of non-ubiquitylated targets in plants: looking for cognate receptor/adaptor proteins. *Front Plant Sci* **5**, 308.

Vera VS, Kenchappa CS, Landberg K, Bressendorff S, Schwarzbach S, Martin T, Mundy J, Petersen M, Thelander M, Sundberg E. 2017. Autophagy is required for gamete differentiation in the moss Physcomitrella patens. *Autophagy*. In press.
Wada S, Ishida H, Izumi M, Yoshimoto K, Ohsumi Y, Mae T, Makino A. 2009. Autophagy plays a role in chloroplast degradation during senescence in individually darkened leaves. *Plant Physiol* **149**, 885-893.
Wang P, Sun X, Wang N, Jia X, F. M. 2016. Ectopic expression of an autophagy-associated MdATG7b gene from apple alters growth and tolerance to nutrient stress in Arabidopsis thaliana. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 1-15.
Wang P, Hawkins TJ, Hussey PJ. 2017a. Connecting membranes to the actin cytoskeleton. *Curr Opin Plant Biol* **40**, 71-76.
Wang P, Richardson C, Hawes C, Hussey PJ. 2016a. Arabidopsis NAP1 Regulates the Formation of Autophagosomes. *Curr Biol* **26**, 2060-2069.
Wang P, Sun X, Jia X, Ma F. 2017b. Apple autophagy-related protein MdATG3s afford tolerance to multiple abiotic stresses. *Plant Sci* **256**, 53-64.
Wang P, Sun X, Jia X, Wang N, Gong X, Ma F. 2016b. Characterization of an Autophagy-Related Gene MdATG8i from Apple. *Front Plant Sci* **7**, 720.
Wang P, Zhu L, Sun D, Gan F, Gao S, Yin Y, Chen L. 2017c. Natural products as modulator of autophagy with potential clinical prospects. *Apoptosis* **22**, 325-356.
Wang S, Blumwald E. 2014. Stress-induced chloroplast degradation in Arabidopsis is regulated via a process independent of autophagy and senescence-associated vacuoles. *Plant Cell* **26**, 4875-4888.
Wang Y, Cai S, Yin L, Shi K, Xia X, Zhou Y, Yu J, Zhou J. 2015a. Tomato HsfA1a plays a critical role in plant drought tolerance by activating ATG genes and inducing autophagy. *Autophagy* **11**, 2033-2047.
Wang Y, Liu YL. 2013. Autophagic degradation of leaf starch in plants. *Autophagy* **9**, 1247-1248.
Wang Y, Yu B, Zhao J, Guo J, Li Y, Han S, Huang L, Du Y, Hong Y, Tang D, Liu Y. 2013. Autophagy contributes to leaf starch degradation. *Plant Cell* **25**, 1383-1399.
Wang Y, Zheng XY, Yu BJ, Han SJ, Guo JB, Tang HP, Yu AYL, Deng HT, Hong YG, Liu YL. 2015b. Disruption of microtubules in plants suppresses macroautophagy and triggers starch excess-associated chloroplast autophagy. *Autophagy* **11**, 2259-2274.
Wittenbach VA, Lin W, Hebert RR. 1982. Vacuolar localization of proteases and degradation of chloroplasts in mesophyll protoplasts from senescing primary wheat leaves. *Plant Physiol* **69**, 98-102.
Xia P, Wang S, Du Y, Zhao Z, Shi L, Sun L, Huang G, Ye B, Li C, Dai Z, Hou N, Cheng X, Sun Q, Li L, Yang X, Fan Z. 2013. WASH inhibits autophagy through suppression of Beclin 1 ubiquitination. *EMBO J* **32**, 2685-2696.
Xia T, Xiao D, Liu D, Chai W, Gong Q, Wang NN. 2012. Heterologous expression of ATG8c from soybean confers tolerance to nitrogen deficiency and increases yield in Arabidopsis. *PLoS One* **7**, e37217.
Xie Q, Tzfadia O, Levy M, Weithorn E, Peled-Zehavi H, Van Parys T, Van de Peer Y, Galili G. 2016. hfAIM: A reliable bioinformatics approach for in silico genome-wide identification of autophagy-associated Atg8-interacting motifs in various organisms. *Autophagy* **12**, 876-887.

Xu C, Feng K, Zhao X, Huang S, Cheng Y, Qian L, Wang Y, Sun H, Jin M, Chuang TH, Zhang Y. 2014. Regulation of autophagy by E3 ubiquitin ligase RNF216 through BECN1 ubiquitination. *Autophagy* **10**, 2239-2250.
Xu D, Shan B, Sun H, Xiao J, Zhu K, Xie X, Li X, Liang W, Lu X, Qian L, Yuan J. 2016. USP14 regulates autophagy by suppressing K63 ubiquitination of Beclin 1. *Genes Dev* **30**, 1718-1730.
Yamane K, Mitsuya S, Taniguchi M, Miyake H. 2012. Salt-induced chloroplast protrusion is the process of exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts into cytoplasm in leaves of rice. *Plant Cell Environ* **35**, 1663-1671.
Yang X, Bassham DC. 2015. New Insight into the Mechanism and Function of Autophagy in Plant Cells. *Int Rev Cell Mol Biol* **320**, 1-40.
Yang X, Srivastava R, Howell SH, Bassham DC. 2016. Activation of autophagy by unfolded proteins during endoplasmic reticulum stress. *Plant J* **85**, 83-95.
Yano K, Yanagisawa T, Mukae K, Niwa Y, Inoue Y, Moriyasu Y. 2015. Dissection of autophagy in tobacco BY-2 cells under sucrose starvation conditions using the vacuolar H(+)-ATPase inhibitor concanamycin A and the autophagy-related protein Atg8. *Plant Signaling & Behavior* **10**, e1082699.
Yoshimoto K, Jikumaru Y, Kamiya Y, Kusano M, Consonni C, Panstruga R, Ohsumi Y, Shirasu K. 2009. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in Arabidopsis. *Plant Cell* **21**, 2914-2927.
Yoshimoto K, Shibata M, Kondo M, Oikawa K, Sato M, Toyooka K, Shirasu K, Nishimura M, Ohsumi Y. 2014. Organ-specific quality control of plant peroxisomes is mediated by autophagy. *J Cell Sci* **127**, 1161-1168.
Young PG, Bartel B. 2016. Pexophagy and peroxisomal protein turnover in plants. *Biochim Biophys Acta* **1863**, 999-1005.
Zaffagnini G, Martens S. 2016. Mechanisms of Selective Autophagy. *J Mol Biol* **428**, 1714-1724.
Zavodszky E, Seaman MN, Moreau K, Jimenez-Sanchez M, Breusegem SY, Harbour ME, Rubinsztein DC. 2014. Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. *Nat Commun* **5**, 3828.
Zechner R, Madeo F, Kratky D. 2017. Cytosolic lipolysis and lipophagy: two sides of the same coin. *Nat Rev Mol Cell Biol* **18**, 671-684.
Zhang R, Meng Z, Zhou T, Deng Y, Feng L, Wang Y, Sun G, Guo S, Ren M. 2013. ScFKBP12 bridges rapamycin and AtTOR in Arabidopsis. *Plant Signaling & Behavior* **8**, e26115.
Zhang R, Patena W, Armbruster U, Gang SS, Blum SR, Jonikas MC. 2014. High-Throughput Genotyping of Green Algal Mutants Reveals Random Distribution of Mutagenic Insertion Sites and Endonucleolytic Cleavage of Transforming DNA. *Plant Cell* **26**, 1398-1409.
Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, Jin S. 2009. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. *Proc Natl Acad Sci U S A* **106**, 19860-19865.
Zhao L, Dai J, Wu Q. 2014. Autophagy-like processes are involved in lipid droplet degradation in Auxenochlorella protothecoides during the heterotrophy-autotrophy transition. *Front Plant Sci* **5**, 400.

Zhou J, Wang J, Cheng Y, Chi YJ, Fan B, Yu JQ, Chen Z. 2013. NBR1-mediated selective autophagy targets insoluble ubiquitinated protein aggregates in plant stress responses. *PLoS Genet* **9**, e1003196.
Zhou J, Wang J, Yu JQ, Chen Z. 2014a. Role and regulation of autophagy in heat stress responses of tomato plants. *Front Plant Sci* **5**, 174.
Zhou J, Yu JQ, Chen Z. 2014b. The perplexing role of autophagy in plant innate immune responses. *Mol Plant Pathol* **15**, 637-645.
Zhuang X, Wang H, Lam SK, Gao C, Wang X, Cai Y, Jiang L. 2013. A BAR-domain protein SH3P2, which binds to phosphatidylinositol 3-phosphate and ATG8, regulates autophagosome formation in Arabidopsis. *Plant Cell* **25**, 4596-4615.
Zientara-Rytter K, Lukomska J, Moniuszko G, Gwozdecki R, Surowiecki P, Lewandowska M, Liszewska F, Wawrzynska A, Sirko A. 2011. Identification and functional analysis of Joka2, a tobacco member of the family of selective autophagy cargo receptors. *Autophagy* **7**, 1145-1158.

Table 1. Tools for plant autophagy modulation

			Confirmation of the expected effect on autophagy		
Type of regulation	**Effect on autophagy**	**Suggest mechanism of action/target**	**Algae**	**Mosses**	**Seed plants**
Genetic regulation					
Knockout of *ATG* gene(s)	Inhibition		Possibly (Zhang *et al.*, 2014)	Yes (Mukae *et al.*, 2015), (Vera *et al.*, 2017)	Yes (Kim *et al.*, 2012)
Knockdown of *ATG* gene(s)	Inhibition		-	-	Yes (Kim *et al.*, 2012)
Overexpression of *ATG* gene(s)	Stimulation		-	-	Possibly (Xia *et al.*, 2012), (Wang *et al.*, 2017; Wang *et al.*, 2016)
Pharmacological regulation					
Rapamycin	Stimulation	An inhibitor of TOR kinase	Yes (Crespo *et al.*, 2005)	Reference to personal communication in (Menand *et al.*, 2002)	In Arabidopsis it requires the presence of FKBP12 (Ren *et al.*, 2012), (Zhang *et al.*, 2013)
AZD8055	Stimulation	TOR kinase active site inhibitor	Yes (Imamura *et al.*, 2016)	-	Yes (Dong *et al.*, 2015)
Torin1	Stimulation	TOR active site inhibitor	Yes (Imamura *et al.*, 2016)	-	Yes (Montane and Menand, 2013)
KU63794	Stimulation	TOR active site inhibitor	-	-	Yes, especially in combination with rapamycin (Deng *et al.*, 2017)

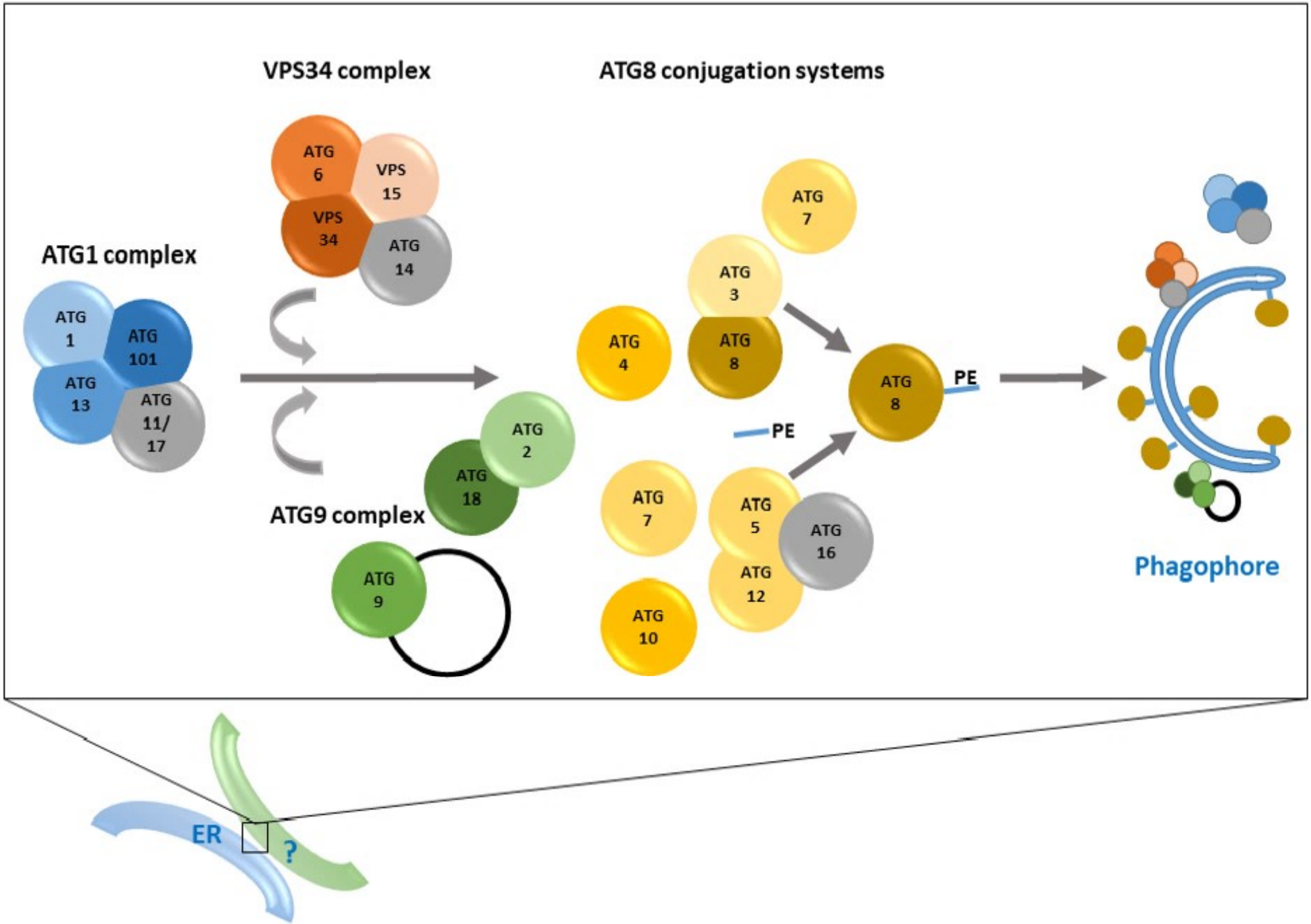
3-MA (3-methyladenine)	Inhibition, but also might lead to enhancement	A pan Phosphatidylinosytol-3 kinase (PI3K) inhibitor. Can persistently inhibit class III PI3K and transiently inhibit class I PI3K.	Yes (Jiang *et al.*, 2012)	-	Yes (Takatsuka *et al.*, 2004), (Wang *et al.*, 2013)
Wortmannin	Inhibition	A pan Phosphatidylinosytol-3 kinase (PI3K) inhibitor. Inhibit class I and III PI3K with the same efficacy.	-	-	Yes (Takatsuka *et al.*, 2004)
LY294002	Inhibition, but also might lead to enhancement	A pan Phosphatidylinosytol-3 kinase (PI3K) inhibitor. Inhibits activity of class I and class III PI3Ks and additionally influences Ca^{2+} homeostasis.	-	-	Yes (Takatsuka *et al.*, 2004)
Bafilomycin A1	Inhibition	A specific inhibitor of vacuolar H^+-ATPase	-	-	Yes. In BY-2 it gives a weaker effect than Concanamycin A (Matsuoka *et al.*, 1997)
Concanamycin A	Inhibition	A specific inhibitor of vacuolar H^+-ATPase	-	Yes (Mukae *et al.*, 2015)	Yes (Matsuoka *et al.*, 1997; Yano *et al.*, 2015)
E-64c/d	Inhibition	A cysteine-protease inhibitor, blocks degradation of autophagic cargo and ATG4 activity	Possibly (Moriyasu, 1995)	Yes (Mukae *et al.*, 2015)	Yes (Oh-ye *et al.*, 2011; Yano *et al.*, 2015)
BTH (benzothiadiazole)	Stimulation	Acts as analog of salicylic acid	-	-	Yes (Yoshimoto *et al.*, 2009)
Fumonisin B1	Stimulation	An inhibitor of sphingosine N-acyltransferase	-	-	Possibly (Qin *et al.*, 2017)

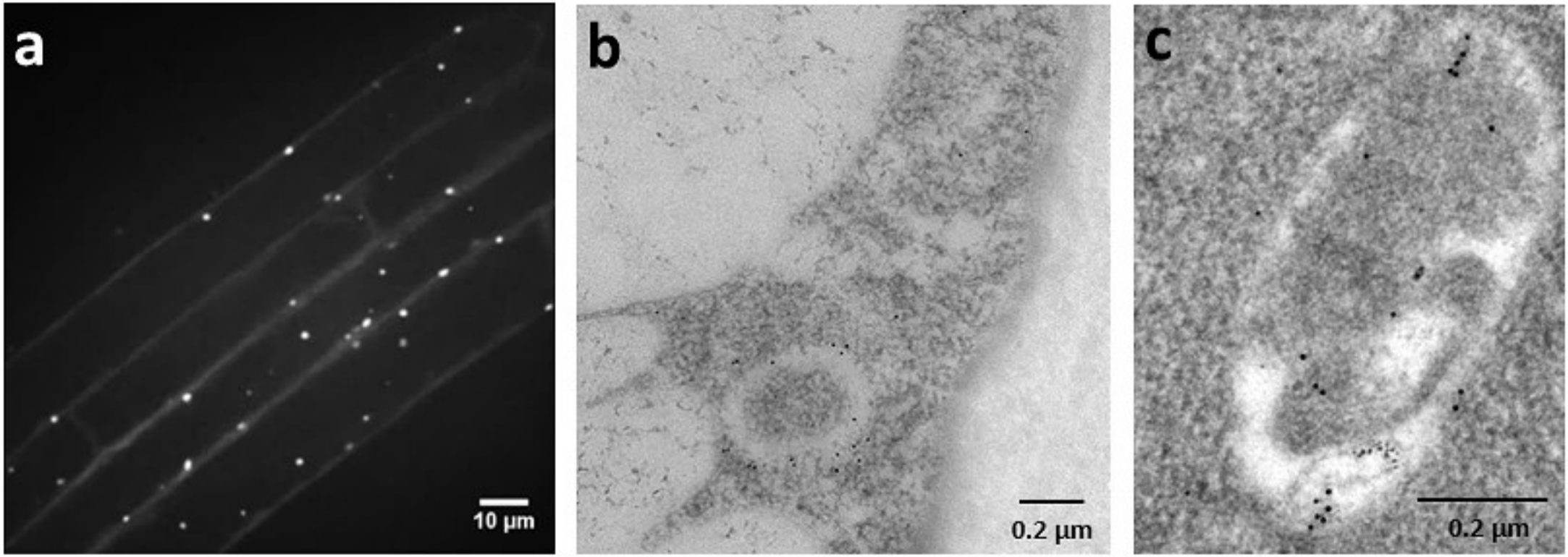
Tunicamycin	Stimulation	Induces ER stress	Possibly (Diaz-Troya *et al.*, 2011)	-	Yes (Yang *et al.*, 2016)
Polyamines	Stimulation	Spermidine was suggested to influence expression of *ATG* genes by changing chromatin structure	-	-	Possibly (Sequera-Mutiozabal *et al.*, 2016)

"-", no published data is yet available

Figure 1: Autophagic response initiation in plants: complexes in complexity. In response to a stimulus, the ATG1 complex is formed and targeted to an organelle contact site involving the endoplasmic reticulum (ER) and an unknown organelle (?). The ATG1 complex activates and recruits the VPS34 complex resulting in local PI3P (phosphatidylinositol-3-Phosphate) synthesis and enrichment within the organelles contact site. ATG9-containing vesicles (black circle) are docked to the contact site by ATG9 interaction with ATG2-ATG18 dimers, site-localized through ATG18 binding to PI3P, the input of membrane lipids and defined proteins contributing in the formation of the phagophore. The phagophore membranes are decorated with enzymatically processed and lipidated (conjugation to PE, phosphatidylethanolamine) ATG8. This process is facilitated by components of the ATG8 conjugation systems. Putative subunits of the various complexes not yet characterized in plant are illustrated in grey.

Figure 2. Imaging of plant autophagic structures and subcellular localization of ATG8 by different microscopy approaches. (a) Live imaging of ATG8-GFP reporter proteins in Arabidopsis roots observed by spinning disk confocal microscope. (b) TEM micrograph of the same plant tissues immunolabelled for ATG8 -GFP detection using anti-GFP antibodies. Note the gold particles on the autophagosome membrane. (c) TEM immunogold labelling with anti-ATG8 antibodies of a tapetal cell of *Brassica napus*.


VPS34 complex
ATG8 conjugation systems
ATG1 complex
ATG 6
VPS 15
VPS 34
ATG 14
ATG 1
ATG 101
ATG 13
ATG 11/ 17
ATG 2
ATG 18
ATG9 complex
ATG 9
ATG 7
ATG 3
ATG 4
ATG 8
PE
ATG 8
PE
ATG 7
ATG 5
ATG 16
ATG 12
ATG 10
Phagophore
ER
?


a
10 µm
b
0.2 µm
c
0.2 µm