Selective autophagy: adding precision in plant immunity

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Plant immunity is antagonized by pathogenic effectors during interactions with bacteria, viruses or oomycetes. These effectors target core plant processes to promote infection. One such core plant process is autophagy, a conserved proteolytic pathway involved in ensuring cellular homeostasis. It involves the formation of autophagosomes around proteins destined for autophagic degradation. Many cellular components from organelles, aggregates, inactive or misfolded proteins have been found to be degraded via autophagy. Increasing evidence points to a high degree of specificity during the targeting of these components, strengthening the idea of selective autophagy. Selective autophagy receptors bridge the gap between target proteins and the forming autophagosome. To achieve this, the receptors are able to recognize specifically their target proteins in a ubiquitin-dependent or -independent manner, and to bind to ATG8 via canonical or non-canonical ATG8-interacting motifs. Some receptors have also been shown to require oligomerization to achieve their function in autophagic degradation. We summarize the recent advances in the role of selective autophagy in plant immunity and highlight NBR1 as a key player. However, not many selective autophagy receptors, especially those functioning in immunity, have been characterized in plants. We propose an in silico approach to identify novel receptors, by screening the Arabidopsis proteome for proteins containing features theoretically needed for a selective autophagy receptor. To corroborate these data, the transcript levels of these proteins during immune response are also investigated using public databases. We further highlight the novel perspectives and applications introduced by immunity-related selective autophagy studies, demonstrating its importance in research.

Introduction

Plant immunity centers around the plant-pathogen interface, where an intriguing arms race occurs at the molecular level. Plants defend themselves against pathogens using their innate immunity, where broad resistance to pathogens is conferred by pathogen-associated-molecular-pattern (PAMP)-triggered immunity (PTI), which involves recognition of conserved molecules shared by pathogens by surface-localized pattern-recognition receptors (PRRs) [1–3]. For a successful infection, plant pathogens such as bacteria, fungi or oomycetes colonize the plant extracellular space and secrete effector proteins into the host cell cytoplasm, which then target essential pathways in the plant [2,4,5]. Plant viruses are rather obligate intracellular parasites, but likewise utilize effectors for manipulating host processes from within the plant cell, and to facilitate cell-to-cell movement [6–8]. Effectors have been shown to target key plant processes such as PTI signaling, cytoskeleton localization and endocytic trafficking, in a process termed “effector-targeted pathways (ETP)” [4]. Effectors can in turn be recognized by nucleotide-binding leucine-rich repeat receptors (NLRs), which guard host immune components and induce effector-triggered immunity (ETI) [1–3].

As cellular processes are mediated to a great extent by proteins, plant proteostasis i.e. the homeostasis of the plant proteome is an important factor to consider in immunity. Proteostasis is the dynamic and
Selective autophagy receptors (SARs) bring their substrate to the nascent autophagosome ATG8 interaction. Substrate binding can be ubiquitin-dependent or -independent. The cargo for xenophagy and effectorphagy includes viral proteins and bacterial effectors. Protein aggregates are targeted in aggrephagy. Organelles such as endoplasmic reticulum (ER), proteasome, and mitochondria are the cargo for ER-phagy, proteaphagy and mitophagy respectively. Specific proteins such as FLS2 and BES1 have also been found to be targeted by SARs. Examples were highlighted based on data availability on ubiquitin-binding and AIM/LIR/UIM-containing. For a comprehensive review on receptors and their substrates see [19,20,25].

SARs can have ubiquitin-binding domains (UBD) that aids in substrate recognition. ATG8-, LC3- or ubiquitin- interacting motif (AIM/LIR/UIM) of the SAR contacts the LC3 docking site (LDS) on ATG8. Some SARs also contain domains that promote oligomerization.
constant interplay between protein synthesis and degradation, which can be influenced by factors such as protein translation, folding, localization, post-translational modifications, proteasome and autophagic activity [9]. A major hub where effectors converge for ETP are the main protein degradation pathways, autophagy and the ubiquitin–proteasome system (UPS) [5]. Both proteolytic pathways have been shown to be essential, where knockouts of some genes involved in these processes result in severe growth defects, especially under nutrient limiting conditions [10,11]. Autophagy also functions directly in immunity to limit infection [7,8,12]. It follows that targeting of autophagy and UPS pathways through ETP could be highly effective in perturbing host cell during infection, and indeed many examples of this has been described, as reviewed in Langin et al. [5].

**General autophagy**

Macroautophagy (henceforth autophagy) is essential for cellular homeostasis through intracellular constituent recycling and mediates degradation of large protein complexes, insoluble protein aggregates and dysfunctional organelles [11]. The mechanism behind autophagy is generally conserved in eukaryotes and involves the coordinated action of around 40 autophagy-related (ATG) genes to direct formation of the autophagosome, a double-membraned vesicle that envelops cytosolic material such as proteins and organelles destined for degradation [11,13,14]. In brief, during plant autophagy, ATG1 and ATG13 take in signals from Target of Rapamycin (TOR) kinase which is influenced by both developmental and nutritional signals, although it is to note that TOR-independence exists for oxidative or ER stress-related autophagy [15]. During TOR-dependent autophagy, TOR blocks autophagy by phosphorylating ATG13, which prevents its association with ATG1. Upon nutrient deficiency or other TOR-inactivating conditions, ATG13 is rapidly dephosphorylated which results in ATG1 binding. Additional hypo- and hyperphosphorylation events allow the assembly of the ATG1, ATG13 and the accessory subunits ATG11 and ATG101. The active ATG1 kinase complex then promotes the ATG9-mediated delivery of lipids and membrane sources derived from the ER to the developing phagophore. Nucleation and expansion of the phagophore also involves the VPS34 lipid kinase, which generates PI3P on the phagophore membrane. In parallel, a ubiquitin–like conjugation system (ATG5/ATG12/ATG16) drives the conjugation of ATG8 to PE, which decorates the autophagosome membrane. The autophagosome is further sealed by recruitment of SH3 domain-containing protein 2 (SH3P2) that stimulates phagophore curvature. Mature autophagosomes are delivered to the lytic vacuole for degradation by vacuolar hydrolases.

Many steps in this pathway have been shown to be modulated during plant–pathogen interactions, with excellent reviews summarizing the known examples [5,7,12]. Effectors from plant pathogens were also shown to target different components of the host autophagic machinery, strengthening the idea that autophagy is a key cellular process in immunity [16]. Autophagy has been shown play both pro- and anti-pathogen roles [7,8,12]. Thus, there must be a high degree of regulation behind plant-pathogen interactions. In this review, we focus on protein degradation via selective autophagy, and its role in adding specificity to plant immunity.

**Selective autophagy**

Our early understanding of autophagy characterized it as a non-specific “bulk” process, where cell cytoplasmic contents are “randomly” engulfed and degraded. Although it was already suggested then that autophagy displays selectivity toward cargo [17], only with the characterization of selective autophagy receptors (SARs, abbreviation not to be confused with systemic acquired resistance) there is strong proof of substrate specificity in autophagy [18]. Receptors have been identified for the selective autophagy of many cellular components across a range of eukaryotic organisms, for example proteasomes (proteaphagy), endoplasmic reticulum (ER-phagy), intracellular pathogens ( xenophagy), protein aggregates (aggrephagy) and mitochondria (mitophagy) [19–22] (Figure 1). Particularly relevant to immunity, xenophagy of intracellular bacteria, viruses, protozoa and fungi have been well-described in mammalian systems, whereas for plants most research is directed at xenophagy of viruses [7,8,23,24]. Research on receptors and their identified substrates have been well-summarized for plants [20,25] and for the yeast model system [19]. Thus, in this review, we rather summarize common patterns behind domain organization of SARs and highlight recent advances in plant research regarding selective autophagy and immunity.

**The selective autophagy receptor**

One component underlying the mechanism of action behind selective autophagy is the receptor, and the study of these SARs has been a substantial driving force behind our understanding of selective autophagy. Despite the wide range of targets and functions, there are nonetheless common patterns that can be established in the mechanism of selective autophagy. In principle, SARs should be able to achieve two tasks. First—to recognize its substrate, either directly or via polyubiquitin chains on the substrate, and second—be able to bring the bound substrate to the
Figure 3. Differential gene expression of candidate SARs during immune responses represented in a heatmap

Expression values are represented as Log2FC, and the color key is indicated at the bottom. The dendrogram on the left clusters together genes with similar expression pattern. Columns correspond to the different dataset extracted from the public online database (http://ipf.sustech.edu.cn/pub/athlete/) as following: flg22 1h (PRJNA491484); flg22 2h (PRJNA429781); P. syringae pv. maculicola (Pma) (PRJNA390966); S. sclerotiorum (PRJNA418121) and B. cinerea (PRJNA276444).
Figure 4. Differential gene expression of ATG8 isoforms during immune responses represented in a heatmap

Expression values are represented as Log2FC, and the color key is indicated at the bottom. The dendrogram on the left clusters together isoforms with similar expression pattern. Columns correspond to the different dataset extracted from the public online database (http://ipf.sustech.edu.cn/pub/athma/) as following: flg22 1h (PRJNA491484); flg22 2h (PRJNA429781); P. syringae pv. maculicola (Pma) (PRJNA390966); S. sclerotiorum (PRJNA418121) and B. cinerea (PRJNA275444).
Both pathways depend on recognition of microbial effectors by receptors. SARs and NLRs are different by structure and domain organization. Both receptors inactivate the effector function either by inducing cell death (NMI) or by degrading the effector via autophagy (SMI) and leading to effector-triggered immunity.

autophagy machinery by binding ATG8, for example via ATG8-interacting motifs (AIMs), LC3-interacting motifs (LIR) or ubiquitin-interacting motifs (UIM) [19,20,25–27] (Figure 2).

**Ubiquitin-dependent substrate recognition**

Many SARs recognize polyubiquitinated substrates, and contain ubiquitin-binding domains for the recognition of their target substrate [26]. The post-translational modification ubiquitination, also known as ubiquitylation, is described as “eat me” signal due to its role in targeting proteins toward degradative processes [28]. Ubiquitin is conjugated on a lysine residue of a target protein based on the enzymatic cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligase (E3) enzymes [29]. The presence of internal lysine residues allows further conjugation of ubiquitin with itself to form polyubiquitinated chains which mark proteins for degradation [28], although targeting toward non-degradative processes such as the regulation of protein interactions and signal transduction also possible [29,30]. Domains known to bind ubiquitin include UBA (ubiquitin associated), UIM (ubiquitin-interacting motif), CUE (coupling of ubiquitin conjugation to endoplasmic reticulum degradation), TOM (target of Myb) and more as reviewed in [31].

One well-characterized plant SAR is NEIGHBOR OF BRCA1 gene 1 (NBR1), also known as Joka2 in solanaceous plants, which is a homolog of mammalian NBR1 and functionally similar to p62 [32,33]. NBR1 contains two UBA domains, but it was shown that only the C-terminal UBA domain of *A. thaliana* NBR1 could bind ubiquitin, and is thought to be involved in recognition of ubiquitinated targets for selective autophagy [32].

DOMINANT SUPPRESSOR OF KAR 2 (DSK2) is another SAR which acts as a ubiquitin receptor for BR1-EMS SUPPRESSOR 1 (BES1), the master regulator of the brassinosteroid (BR) pathway. Degradation of BES1 by DSK2 requires its UBA domain that can bind polyubiquitin chains, and AIM motif [34].
Similarly, proteasome subunit RPN10 which directs the 26S proteasome towards selective autophagy can bind both ubiquitin and ATG8 [35]. Additionally some proteasome subunits were found to be ubiquitinated thus strengthening RPN10 as a ubiquitin-dependent SAR [35,36].

Considering the role of ubiquitination as a degradation signal and the ubiquitin-binding ability of some SARs, many selective autophagy targets likely undergo ubiquitination. In fact, ubiquitinated proteins are observed to hyper-accumulate in nbr1 or atg5 mutants upon infection conditions shown to induce autophagy [37]. Thus, we hypothesize there are additional SARs with ubiquitin binding ability, and investigate this in more detail below.

**Ubiquitin-independent substrate recognition**

In plant-pathogen interactions, there is evolutionary pressure on the host side to recognize pathogenic components, and on the pathogen side to escape this recognition. Indirect substrate recognition via ubiquitination is intuitively an effective strategy in which xenophagy-related SARs can participate in the xenophagy of a wide range of molecules, from viral capsids to oomycete and bacterial effectors. However, there are exceptions where substrate recognition is still achieved independently of ubiquitin association.

The turnip mosaic virus (TuMV) subunit P4 is recognized by the *A. thaliana* NBR1, and this interaction is still observed with a truncated NBR1 variant missing its UBA domain, suggesting for ubiquitin-independence in the interaction [38].

A study from our lab showed that a bacterial effector XopL is recognized by NBR1 through both ubiquitin-dependent and independent mechanisms. We show through co-immunoprecipitation that XopL interacted with full-length NBR1 and UBA deletion mutants, but a higher molecular weight species hypothesized to be ubiquitinated XopL, only interacted with NBR1 when a functional UBA domain is present [39].

Both these examples of ubiquitin-independence during NBR1-mediated xenophagy are intriguing as NBR1 is the only xenophagy receptor identified so far in plants. Are there more similar examples to be discovered, and if so how would NBR1 recognize the diverse effectors and viral subunits that could be present within the host cell? Are there some conserved features such as structure, that can drive the recognition of these foreign molecules by xenophagy receptors? Indeed, this could be a possibility, given that plant ER-phagy receptors ATG8-interacting proteins 1 and 2 (ATI1/2) share structural similarity with other ER-phagy receptors, where their AIM is located at the end of long, intrinsically disordered cytosolic regions [40]. The structure to function relationship is proposed as the disordered domain could bridge the membrane of the rough ER to ATG8 on phagophores or endolysosomes [40].

Although not shown to be a SAR, exocyst subunit Exo70B2 was found to interact with ATG8 via two AIMS, and is subject to autophagic transport to the vacuole upon treatment with defense-related compounds salicylic acid (SA) and benzothiadiazole (BTH) or immunogenic peptide flg22 [41]. We speculate that Exo70B2 could function as a SAR, directing cargo toward autophagic degradation. Interestingly, Exo70B2 is phosphorylated by the immunity-related kinase MPK3 that enhances its interaction with ATG8 [41]. Thus, if Exo70B2 acts as a SAR, this phosphorylation could present a novel way in which SARs are regulated.

Although not related to immunity, plant mitophagy receptor, FRIENDLY (FMT) does not have UBA domain yet mediates the recycling of damaged mitochondria in Arabidopsis [42]. Furthermore, plant ER-phagy receptors ATI1/2, Sec62, C53, reticulon proteins 1 and 2 (Rtn1/2) and degrade specific proteins in the ER during proteotoxic stress [43–45] or dark-induced starvation [46]. These proteins do not have any predicted ubiquitin-binding functions, thus future work should confirm their ubiquitin-independence and characterize the mechanism.

For mammalian systems as well, under specific conditions receptors link their cargo to autophagosomes during mitophagy, pexophagy, and virophagy in a ubiquitin-independent manner [47]. Thus, is entirely possible that there are more examples of ubiquitin-independent selective autophagy involved in immunity.

**Motifs for ATG8 interaction**

Once a target substrate is bound, the subsequent role of a SAR is to bring its bound substrate to the autophagic machinery, usually the nascent autophagosome. To achieve this, canonical ATG8-interacting motif (AIM), otherwise known as LC3-interacting region (LIR), or the non-canonical ubiquitin-interacting motif (UIM) on a SAR is crucial, since the autophagosome is decorated with ATG8 proteins to facilitate this specific binding [48,49]. Mechanistically, this binding between the SAR and ATG8 occurs as the AIM/LIR contacts a hydrophobic patch on ATG8 known as the LDS docking site [50,51].

Plant NBR1 has a canonical AIM facilitating its interaction with ATG8 on autophagosomes [32,33]. Svenning et al. additionally showed that AtNBR1 during co-expression with AtATG8 or human GABARAPL2, required its AIM to be recognized as an autophagic substrate in HeLa cells [32].
Many other SARs have been found to contain AimS which are crucial for their function in selective autophagy, for example as mentioned above DSK2, RPN10 and potentially Exo70B2 [34,35,41].

RPN10 was additionally shown to have a UIM, which is a non-canonical motif for ATG8 interaction named for its similarity to existing UIM [35,52]. The authors further screened known ATG8-binding proteins for UIM, and identified three PUX proteins, thus raising the possibility that novel SARs with UIM are yet to be characterized [52].

The importance of the Aim in autophagy has inspired tools for Aim prediction to aid in discovery of novel autophagy targets [53,54]. This has potential in aiding in the identification of novel SARs, and will be discussed below.

Oligomerization

Oligomerization of SARs have been shown to be especially important during selective autophagy of aggregates [55,56]. This is because protein aggregates are present as biomolecular condensates, which are membraneless assemblies where certain cellular components such as biopolymers are concentrated [57]. Domains such as Phox and Bem1p (PB1) are present on some SARs which enable their oligomerization [58]. It is rather intuitive that SARs involved in aggrephagy would oligomerize, so that they more easily localize into condensates where their targets reside. Indeed, this idea holds true when we consider the receptors Dsk2 and Cue2, which play a role in both proteasome- and autophagy-dependent protein degradation [59]. Lu and colleagues designed artificial ubiquitin- and Atg8-binding receptors, with or without the ability to oligomerize, and found that only oligomeric receptors could function in aggrephagy. Although direct evidence was not shown, a previous study suggest that Joka2 aggregates contain its multimeric form, and that in the nucleus and cytoplasm it is in a monomeric form [60].

The ubiquitin-binding domains of SARs can also contribute to their oligomerization. For example, a PB1 domain is present on plant NBR1, which allows it to form oligomers with other molecules of itself, but this oligomerization is also partially dependent on its UBA domains [32,60]. In a similar way, human p62 is the main driver of ubiquitin condensate formation, but NBR1 also promotes the p62 condensation through its PB1 and UBA domains [61].

SAR oligomerization may have wider implications beyond aggrephagy, and may function generally in promoting the interaction with ATG8. It was shown that oligomerization of mammalian p62 has an important role in stabilizing the binding to LC3 on the autophagosome membrane [55,56]. Furthermore, the DIX domain of Dishevelled (Dvl) mediates its self-oligomerization, which is ultimately required for interaction with LC3 [62]. There is also increasing evidence that autophagosome formation during autophagy involves liquid-liquid phase separation, which can be promoted by the oligomerization of SARs [63].

Recent advances in selective autophagy in the context of plant immunity

Plant–viral interactions

The pro-plant role of xenophagy during plant–viral interactions is well-characterized. The SAR NBR1 functions in the targeted degradation of viral particles and proteins. For example, cauliflower mosaic virus (CaMV) capsid and P4 proteins, and Turnip mosaic virus (TuMV) HcPro have been shown to be removed by NBR1 [37,38]. In turn, TuMV evolved viral proteins VPg and 6K2 to counteract NBR1-driven host xenophagy by blocking the degradation of HcPro [64]. More recently, it has been reported that nuclear autophagy degrades geminivirus nuclear protein C1 to restrict viral infection in solanaceous plants [65]. In the present study, viral protein C1 harbors an AIM which facilitates its interaction with ATG8, leading to its degradation via autophagy in the cytoplasm. Considering that AIM-containing proteins can act as autophagy adaptors or receptors it is tempting to speculate whether C1, similar to Phytophtora infestans effector PexRD54 elaborated on below, functions to target nuclear proteins upon viral infection.

A recent study identified that autophagy core component ATG6/Beclin1 might act as a SAR, as it mediates the degradation of a TuMV RNA-dependent RNA polymerase N1b to restrict virus infection [66]. ATG6/Beclin1 was also shown to be involved in autophagy-like protein degradation of tobacco calmodulin-like protein rgs-CaM which bound to viral RNA silencing suppressor proteins, thus enhancing host antiviral RNAi [67]. Additionally, Jiang et al. identified a new cargo receptor NbP3IP with a previously unknown function, which specifically interacts with the P3 protein (VSR) of Rice stripe virus (RSV) and NbATG8f. These interactions mediate the selective degradation of the P3 protein and limit RSV infection [68].

Pro-viral effects of selective autophagy have also been described. P0 from Turnip yellows virus (TuYV) was found to manipulate selective autophagy for the degradation of ARGONAUTE1 (AGO1), exploiting AT11 and AT12 mediate the delivery of host AGO1 from the ER to the vacuole, ultimately down-regulating host antiviral RNA silencing [69].
Although the majority of the studies identified how SARs contribute to plant–virus interactions, further mechanistic details, such as oligomerization state of the SAR or whether substrate recognition is mediated by ubiquitin, remain still elusive.

**Plant–bacterial interactions**

During interactions with bacteria, plant autophagy can also function in a pro-plant or pro-bacterial manner. FLAGELLIN-SENSING 2 (FLS2), a receptor kinase which recognizes bacterial flagellin, was found to be degraded by selective autophagy [70]. Orosomucoid (ORM) proteins ORM1 and 2 act as SARs in the degradation of non-activated FLS2, which ensures sufficient amounts of activated FLS2 for signaling [70]. The pathway is hijacked for pro-pathogen effects, for example during *Pseudomonas syringae* infection, where it was found that a T3E HopM1 causes the targeted degradation of the host proteasome by inducing proteaphagy, which could involve the proteaphagy receptor RPN10 since *Pst* bacterial growth was elevated in the *rpn10* mutant [37].

As viruses are the only described intracellular pathogens in plants, we propose that xenophagy includes degradation of viruses, “virophagy” and of effectors, “effectorphagy”. During effectorphagy, bacterial effectors are recognized in the plant cell and degraded via the autophagy pathway, for example as in Leong et al. [39]. The study showed that NBR1/Joka2 interacts with and causes the autophagic degradation of a *Xanthomonas campestris pv. vesicatoria* (*Xcv*) effector XopL which are present in aggregates, providing a link between aggaphagy in immunity [39]. This is a novel example in plants of selective autophagy targeting bacterial effectors, and there is a possibility that NBR1 is involved in degradation of other bacterial effectors. Evidence for this include increased bacterial growth and disease progression of *Pseudomonas syringae* pathovar *tomato* DC3000 (*Pst*) in the *nbr1* mutant compared with WT, which indicates for a role of NBR1 in *Pst* infection [37]. The same study further shows that overexpression of NBR1 reduces water soaking caused by HopM1. Both of these results led the authors to speculate that NBR1 is involved in the degradation of other *Pst* effectors.

**Plant–oomycete interactions**

*Phytophtorainfestans*, the causal pathogen of potato late blight, was shown to secrete an effector PexRD54 which, via its AIM motif, associates with ATG8CL to antagonize Joka2, ultimately enhancing oomycete virulence [71]. Further studies showed that this interaction between oomycete effector and host SAR takes place at the extrahaustorial membrane (EHM), where defense-related Rab8a-vesicles labelled with Joka2 and ATG8CL accumulated [72,73]. Apart from providing more insight to the role of autophagy during plant–oomycete interactions, the authors additionally developed a tool for autophagy suppression. The AIM from PexRD54 was used to create an AIM peptide, which was shown to compete with ATG8-binding and thus blocks autophagy progression. The peptide has already been applied in competition assays to reinforce AIM-dependent ATG8 interactions identified in an immunoprecipitation coupled to mass spectrometry (IP-MS) screen [43]. It was also used to block autophagy by transient expression in *Nicotiana benthamiana*, as it is more specific to autophagy compared with other tools such as Concanamycin A which inhibits vacuolar degradation [39]. In summary, this highlights that studying the role of autophagy in the context of plant–microbe interaction may result in the discovery of novel tools to study the pathway in more detail.

**Novel perspectives on plant immunity introduced by selective autophagy research**

**Identification of new receptors**

We have established that SARs mediate substrate specificity during selective autophagy. Identifying and characterization of new SARs related to immunity can help advance our understanding of plant–pathogen interactions. We propose to identify new plant immunity-related SARs by mining the *Arabidopsis* database for proteins that bind to ubiquitin and/or ATG8, and further investigate these candidates by looking into their gene expression during infection using publicly available data.

Ubiquitin-binding proteins were identified based on the presence of characterized ubiquitin-binding domains (INTERPRO/PFAM/SIMART) or with the Gene Ontology annotation “ubiquitin-binding” (GO:0043130). From these 106 ubiquitin-binding proteins, we further cross-checked previously published screens for ATG8-interacting proteins [52,53]. We identified 25 ubiquitin-binding proteins that was also found in Marshall et al.’s UIM-interaction dataset [52], and 15 that were found in Jacomin et al.’s LIR prediction for *A. thaliana* proteome [53], with 5 proteins found in both datasets (Table 1). Among the identified receptors include the known SARs NBR1/Joka2, RPN10 and DSK2 with both ubiquitin-binding and ATG8-interacting abilities (Table 1).

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Table 1 Ubiquitin-binding proteins identified based on presence of ubiquitin-binding domains or Gene Ontology annotation

| Entry | Entry.name | Protein.names | Gene names | Length | Agi_Code | UBQ. binding prediction | ATGB_ Y2H | ILIR. prediction |
|-------|------------|---------------|------------|--------|----------|------------------------|------------|-----------------|
| Q9SIV2 | PSD2A_ARATH | 26S proteasome non-ATPase regulatory subunit 2 homolog A (26S proteasome regulatory subunit RPIN1a) (26S proteasome regulatory subunit S2 homolog A) | RPIN1A | 891 | AT2G20580 | GO |   |
| Q9ZT95 | R7SL1_ARATH | Ubiquitin domain-containing protein 7SL RNA1 (At7SL-1) | 7SL1 | 275 | AT4G02970 | GO |   |
| Q9ZQZ6 | R7SL2_ARATH | Ubiquitin domain-containing protein 7SL RNA2 (At7SL-2) (Protein ETERNALLY VEGETATIVE PHASE 1) | EVE1 | 263 | AT4G03350 | GO |   |
| Q9QZ4 | Q9QZ4_ARATH | Putative ubiquitin-like protein (Ubiquitin family protein) (Uncharacterized protein At4g03370) | At4g03370 | 295 | AT4G03370 | GO | + |
| Q9XIP2 | OTU6_ARATH | CIVARAN TUMOR DOMAIN-containing deubiquitinating enzyme 6 (OTU domain-containing protein 6) (EC 3.4.19.12) (Deubiquitinating enzyme OTU6) (Otubain-like deubiquitinate 1) | OTU6 | 505 | AT2G27350 | UBA |   |
| Q9SBD6 | PETS_ARATH | Polyprotein of EF-Ts, chloroplastic (150 kDa pro-protein) (Protein EMBRYO DEFECTIVE 2726) [Cleaved into: Plastid-specific ribosomal protein-7, chloroplastic; Elongation factor Ts, chloroplastic (EF-Ts)] | PETS | 953 | AT4G29060 | UBA-like |   |
| Q9SUG6 | PUX3_ARATH | Plant UBX domain-containing protein 3 (PUX3) (CDC48-interacting UBX-domain protein 3) | PUX3 | 302 | AT4G22150 | UBX | + |
| Q9SOD3 | Q9SOD3_ARATH | F1B13.13 protein (Ubiquitin system component C) (Uncharacterized protein At1g80040) | At1g80040 | 248 | AT1G80040 | CUE | + |
| Q9SLU5 | CKS2_ARATH | Cyclin-dependent kinases regulatory subunit 2 | CKS2 | 83 | AT2G27970 | GO |   |
| Q9SB64 | NBR1_ARATH | Protein NBR1 homolog (AtNBR1) (At4g24690) | NBR1 | 704 | AT4G24690 | UBA | + |
| Q9M9U7 | Q9M9U7_ARATH | A1g18760 (F6A14.13 protein) (Zinc finger, C3H4 type (RING finger) family protein) | A1g18760 | 224 | AT1G18760 | UIM |   |
| Q9M548 | DRM2_ARATH | DNA (cytosine-5)-methyltransferase DRM2 (EC 2.1.1.37) (Protein DOMAINS REARRANGED METHYLASE 2) | DRM2 | 626 | AT5G14620 | UBA |   |
| Q9M0X2 | Q9M0X2_ARATH | Ubiquitin-like superfamily protein | At4g05230 | 206 | AT4G05230 | GO |   |
| Q9M0X1 | Q9M0X1_ARATH | Ubiquitin-like superfamily protein | At4g05240 | 197 | AT4G05240 | GO |   |
| Q9M0X0 | Q9M0X0_ARATH | Ubiquitin-like superfamily protein | At4g05250 | 318 | AT4G05250 | GO |   |
| Q9M0W9 | Q9M0W9_ARATH | Ubiquitin-like superfamily protein | At4g05260 | 259 | AT4G05260 | GO |   |
| Q9M0W8 | Q9M0W8_ARATH | Ubiquitin-like superfamily protein | At4g05270 | 129 | AT4G05270 | GO |   |
| Q9M0W4 | Q9M0W4_ARATH | Ubiquitin-like superfamily protein | At4g05310 | 415 | AT4G05310 | GO |   |
| Q9M0N1 | PUX10_ARATH | Plant UBX domain-containing protein 10 (PUX10) | PUX10 | 480 | AT4G10790 | UBA/UBX | + |
| Q9L3Y5 | CID5_ARATH | Polyadenylate-binding protein-interacting protein 5 (Poly(A)-binding protein-interacting protein 5) (PAM2-containing protein CIDS) (Protein CTC-INTERACTING DOMAIN 5) (Protein INCREASED POLYPLOIDY LEVEL IN DARKNESS 1) | CID5 | 155 | AT5G11440 | CUE |   |
| Q9L3Y2 | NPL4_ARATH | DNA (cytosine-5)-methyltransferase NPL4-like protein 1 | Atg63000 | 413 | AT5G63000 | NPL4 |   |
| Q9L3E5 | DRM1_ARATH | DNA (cytosine-5)-methyltransferase DRM1 (EC 2.1.1.37) (Protein DOMAINS REARRANGED METHYLASE 2) | DRM1 | 624 | AT5G15380 | UBA |   |
| Q9L3R6 | DAR3_ARATH | Protein DAI-related 3 | DAR3 | 450 | AT5G86640 | DA1-like |   |
| Q9L3L6 | TOL3_ARATH | TOM-1-like protein 3 | TOL3 | 506 | AT1G21380 | VHS-GAT | + |
| Q9L3C6 | TOL2_ARATH | TOM-1-like protein 2 | TOL2 | 383 | AT1G06210 | VHS-GAT | + |
| Q9L3N8 | BUB31_ARATH | Mitotic checkpoint protein BUB3.1 (Protein BUB3.1) | BUB3.1 | 340 | AT3G19590 | WD40 |   |
| Q9L3S5 | Q9L3S5_ARATH | Cell division related protein-like (DnaJ and myb-like DNA-binding domain-containing protein) | At5g06110 | 663 | AT5G06110 | UBD |   |

Continued over
Table 1 Ubiquitin-binding proteins identified based on presence of ubiquitin-binding domains or Gene Ontology annotation (Continued)

| Entry     | Entry.name | Protein.names | Gene. names | Length | Agi. Code | UBQ. binding. Prediction | ATG8. Y2H | iLIR. prediction |
|-----------|------------|---------------|-------------|--------|-----------|-------------------------|-----------|------------------|
| Q8LH3     | Q8LH3ARATH | AT3g18860/MCB22.3 (Transducin family protein / WD-40 repeat family protein) | ELC | 398 | AT3G12400 | UEV | + | + |
| Q8LH3     | ELC_ARATH  | Protein ELC (AtELC) (ESCRT-I complex subunit VPS23 homolog 1) (Protein VACUOLAR PROTEIN SORTING 23A) (Vacular protein-sorting-associated protein 23 homolog 1) | ELC | 398 | AT3G12400 | UEV | + | + |
| Q8LFL3    | TOL1_ARATH | TOM1-like protein 1 | TOL1 | 407 | AT5G16880 | VHS-GAT | | |
| Q8LTE3    | RBL20_ARATH | Rhomboid-like protein 20 (AtRBL20) | RBL20 | 293 | AT3G56740 | UBA | | |
| Q9FMQ0    | Q9FMQ0ARATH | AT5g12120/MCX9.8 (At5g12120) (Ubiquitin-associated/translation elongation factor EF1B protein) (Uncharacterized protein At5g12120) | MCM9.8 | 619 | AT5G12120 | UBA | + | |
| Q9FLZ3    | KIN12_ARATH | SNF1-related protein kinase catalytic subunit alpha KIN12 (AKIN12) (EC 2.7.11.1) (AKIN alpha-3) (AKINalpha3) (SNF1-related kinase 1.3) (SnRK1.3) | KIN12 | 494 | AT5G39440 | UBA | | |
| Q9FKZ1    | DRL42_ARATH | Probable disease resistance protein At5g66900 | At5g66900 | 809 | AT5G06690 | GO | | |
| Q9FKZ1    | DRL43_ARATH | Probable disease resistance protein At5g66910 | At5g66910 | 815 | AT5G06691 | GO | | |
| Q9FKN7    | DAR4_ARATH | Protein DA1-related 4 (Protein CHILLING SENSITIVE 3) | DAR4 | 1613 | AT3G17890 | DA1-like | | |
| Q9FJX9    | DAR7_ARATH | Protein DA1-related 7 | DAR7 | 560 | AT5G06610 | UIM | + | |
| Q9FJX9    | DAR6_ARATH | Protein DA1-related 6 | DAR6 | 644 | AT5G06620 | UIM | + | |
| Q9FFQ0    | TOL5_ARATH | TOM1-like protein 5 | TOL5 | 447 | AT5G06360 | VHS-GAT | | |
| Q9FF81    | VPS36_ARATH | Vacuolar protein sorting-associated protein 36 (AtVPS36) (ESCRT-II complex subunit VPS36) | VPS36 | 440 | AT5G04920 | VPS36 | | |
| Q9FDZ8    | Q9FDZ8ARATH | At1g73440 (Calmodulin-like protein) (Uncharacterized protein T9L24.51) | T9L24.51 | 254 | AT1G73440 | UIM | + | |
| Q9C9Y1    | TOL8_ARATH | TOM1-like protein 8 | TOL8 | 607 | AT3G08790 | VHS | | |
| Q9C9U5    | SIS8_ARATH | Probable serine/threonine-protein kinase SIS8 (EC 2.7.11.1) (MAPKK kinase SIS8) (Protein SUGAR INSENSITIVE 8) | SIS8 | 1030 | AT1G73660 | UIM | + | |
| Q9C701    | BUB32_ARATH | Mitotic checkpoint protein BUB3.2 (Protein BUDDING UNINHIBITED BY BENZYMIDAZOL 3,2) | BUB3.2 | 339 | AT1G49910 | WD40 | | |
| Q9C5H4    | MTV1_ARATH | Protein MODIFIED TRANSPORT TO THE VACUOLE 1 | MTV1 | 690 | AT3G16270 | VHS-GAT | | |
| Q9C5G7    | PUX13_ARATH | Plant UBX domain-containing protein 13 (PUX13) | PUX13 | 525 | AT4G23040 | UBA/UBX | + | |
| Q9ASS2    | FREE1_ARATH | Protein FREE1 (FYVE domain protein required for endosomal sorting 1) (FYVE domain-containing protein 1) | FREE1 | 601 | AT1G20110 | Experimentally proven | + | |
| Q94JZ8    | PUX7_ARATH | Plant UBX domain-containing protein 7 (PUX7) | PUX7 | 468 | AT1G14570 | UMA/UBA/UBX | + | |
| Q93ZS8    | Q93ZS6_ARATH | At3g05090/T12H1.5 (Transducin/WD40 repeat-like superfamily protein) (Uncharacterized protein At3g05090) | LRS1 | 753 | AT3G05090 | WD40 | | |
| Q8W4Q5    | RIN3_ARATH | E3 ubiquitin protein ligase RIN3 (EC 2.3.2.27) (RING3-type E3 ubiquitin transferase RIN3) (RPM1-interacting protein 3) | RIN3 | 577 | AT5G51450 | CUE | | |
| Q8W4F0    | DAR1_ARATH | Protein DA1-related 1 | DAR1 | 553 | AT4G36860 | UIM/DA1 | + | |
| Q8VZS6    | GIP1_ARATH | GIP1-interacting protein 1 | GIP1 | 567 | AT3G13222 | UBA-like | | |
| Q8VY8     | RIN2_ARATH | E3 ubiquitin protein ligase RIN2 (EC 2.3.2.27) (ARM receptor-like protein 1A (RING-type E3 ubiquitin transferase RIN2) (RPM1-interacting protein 2) | RIN2 | 578 | AT4G25230 | CUE | | |
| Q8WWF1    | SH3P2_ARATH | SH3 domain-containing protein 2 (AtSH3P2) | SH3P2 | 368 | AT4G34660 | SH3 | + | |
| Q8RWY8    | Q8RWY8_ARATH | Nucleic acid binding protein (Uncharacterized protein At1g72750) | At1g72750 | 1075 | AT1G72750 | GO | + | |
| Q8RWU7    | PUX4_ARATH | Plant UBX domain-containing protein 4 (PUX4) (CDC48-interacting UBX-domain protein 4) | PUX4 | 303 | AT4G04210 | UBX | + | |

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Table 1 Ubiquitin-binding proteins identified based on presence of ubiquitin-binding domains or Gene Ontology annotation (Continued)

| Entry | Entry.name | Protein.names | Gene. names | UBQ. binding. Prediction | ATG8_Y2H | ILIR. prediction |
|-------|------------|---------------|-------------|-------------------------|----------|-----------------|
| Q8LG98 | OTU1_ARATH | CHRAN TUMOR DOMAIN-containing deubiquitinating enzyme 1 (OTU domain-containing protein 1) (EC 3.4.19.12) (Deubiquitinating enzyme OTU1) | OTU1 | 306 | AT1G28120 | GO |
| Q8LG11 | Q8LG11_ARATH | Prolin-rich cell wall protein-like (Ubiquitin-associated/translation elongation factor EF1B protein) | At5g53330 | 221 | AT5G53330 | UBA |
| Q8L6E0 | TOL9_ARATH | TOM1-like protein 9 | TOL9 | 675 | AT4G32780 | VHS-QAT |
| Q8L6F1 | UBP14_ARATH | Ubiquitin carboxyl-terminal hydrolase 14 (EC 3.4.19.12) (Deubiquitinating enzyme 14) (ATUBP14) (TITAN-6 protein) (Ubiquitin thioesterase 14) (Ubiquitin-specific-processing protease 14) | UBP14 | 797 | AT3G20630 | UBA |
| Q8HG0T4 | UPL2_ARATH | E3 ubiquitin-protein ligase UPL2 (Ubiquitin-protein ligase 2) (EC 2.3.2.26) (HECT-type E3 ubiquitin transferase UPL2) | UPL2 | 3658 | AT1G70320 | UIM/UBA |
| Q8GY23 | UPL1_ARATH | E3 ubiquitin-protein ligase UPL1 (Ubiquitin-protein ligase 1) (EC 2.3.2.26) (HECT-type E3 ubiquitin transferase UPL1) | UPL1 | 3681 | AT1G55860 | UIM/UBA |
| Q84WJ0 | DARS_ARATH | Protein D1-related 5 | DARS | 702 | AT5G66630 | DA1-like |
| Q84L33 | RD23B_ARATH | Ubiquitin receptor RAD23b (ARRAD23b) (Putative DNA repair protein RAD23-1) (RAD23-like protein 1) (ARRAD23-1) | RAD23B | 371 | AT1G79650 | UBA |
| Q84L3E | RD23A_ARATH | Probable ubiquitin receptor RAD23a (ARRAD23a) (Putative DNA repair protein RAD23-2) (RAD23-like protein 2) (ARRAD23-2) | RAD23A | 368 | AT1G16190 | UBA |
| Q84L3I | RD23C_ARATH | Ubiquitin receptor RAD23c (ARRAD23c) (Putative DNA repair protein RAD23-3) (RAD23-like protein 3) (ARRAD23-3) | RAD23C | 419 | AT3G02540 | UBA |
| Q84L3O | RD23D_ARATH | Ubiquitin receptor RAD23d (ARRAD23d) (Putative DNA repair protein RAD23-4) (RAD23-like protein 4) (ARRAD23-4) | RAD23D | 378 | AT5G38470 | UBA |
| Q7Y17S | PU5X_ARATH | Plant UBX domain-containing protein 5 (PU5X) | PU5X | 421 | AT4G15410 | UBA/UBX |
| Q6NQK0 | TOL4_ARATH | TOM1-like protein 4 | TOL4 | 446 | AT1G76970 | VHS-QAT |
| Q6NQH9 | CID6_ARATH | Polyadenylate-binding protein-interacting protein 6 (PABP-interacting protein 6) (Poly(A)-binding protein-interacting protein 6) (PAM2-containing protein CID6) (Protein CTC-INTERACTING DOMAIN 6) | CID6 | 175 | AT5G25540 | CUE |
| Q3YF75 | EFTS_ARATH | Elongation factor Ts, mitochondrial (EF-Ts) (EF-TMII) | EFTS | 395 | AT4G11120 | UBA-ike |
| Q4V3D3 | PU9X_ARATH | Plant UBX domain-containing protein 9 (PU9X) | PU9X | 469 | AT4G00752 | UIM/UBA-ike/UBX |
| Q38997 | KIN10_ARATH | SNF1-related protein kinase catalytic subunit alpha KIN10 (AKIN10) (EC 2.7.11.1) (AKIN alpha-2) (AKINalpha2) (SNF1-related kinase 1.1) (SnRK1.1) | KIN10 | 512 | AT3G01090 | UBA |
| Q38942 | RAE1_ARATH | Protein RAE1 (RNA export factor 1) | RAE1 | 349 | AT1G80670 | WD40 |
| Q0WSN2 | DAR2_ARATH | Protein D1A-related 2 (Protein LATERAL ROOT DEVELOPMENT 3) | DAR2 | 528 | AT2G39830 | UBA |
| Q0WL28 | Q0WL28_ARATH | Ubiquitin system component Cue protein (Uncharacterized protein At1g27750) | At1g27750 | 656 | AT1G27752 | CUE |
| P92958 | KIN11_ARATH | SNF1-related protein kinase catalytic subunit alpha KIN11 (AKIN11) (EC 2.7.11.1) (AKIN alpha-1) (AKINalpha1) (SNF1-related kinase 1.2) (SnRK1.2) | KIN11 | 512 | AT3G29160 | UBA |
| P95034 | PSMD4_ARATH | 26S proteasome non-ATPase regulatory subunit 4 homolog (26S proteasome regulatory subunit RP4N10) (ARRPN10) (26S proteasome regulatory subunit SSA homolog) (Multiubiquitin chain-binding protein 1) (AMCB1) | RP4N10 | 386 | AT4G38630 | UBA |

Continued over
Table 1 Ubiquitin-binding proteins identified based on presence of ubiquitin-binding domains or Gene Ontology annotation (Continued)

| Entry      | Entry.name | Protein.names                                                                 | Gene. names | Length | Agi. Code | UBQ. binding Prediction | ATG8. Y2H | iLIR. prediction |
|------------|------------|------------------------------------------------------------------------------|-------------|--------|-----------|------------------------|-----------|------------------|
| P0DK4      | PUX14_ARATH| Putative plant UBX domain-containing protein 14 (PUX14)                      | PUX14       | 417    | AT4G14250 | UBX                    | +         |                  |
| P0C7Q8     | DA1_ARATH  | Protein DA1 (Protein SUPPRESSOR OF LARGE SEED AND ORGAN PHENOTYPES OF DA1-1) | DA1         | 532    | AT1G19270 | UIM                    | +         |                  |
| O82264     | NPL42_ARATH| NPL4-like protein 2                                                           | At2g47970   | 413    | AT2G47970 | UBQ-like/MPN           | +         |                  |
| O81015     | O81015_ARATH| At2g26920 (Ubiquitin-associated/translation elongation factor EF1B protein) (Uncharacterized protein At2g26920) | At2g26920     | 646    | AT2G26920 | UBA                    | +         |                  |
| O80996     | BRIZ2_ARATH| BRAP2 RING ZnF UBP domain-containing protein 2 (EC 2.3.2.27)                 | BRIZ2       | 479    | AT2G26000 | ZnFUBP-like            |          |                  |
| O80910     | TOL6_ARATH | TOM1-like protein 6                                                           | TOL6        | 671    | AT2G38410 | VHS-GAT                |          |                  |
| O65506     | O65506_ARATH| Disease resistance protein (TIR-NBS-LRR class) (Putative disease resistance protein) | At4g08140     | 1607   | AT4G36140 | GO                     |          |                  |
| O48726     | RPN13_ARATH| 26S proteasome regulatory subunit RPN13 (AIP113) (26S proteasome non-ATPase regulatory subunit 13) | RPN13       | 300    | AT2G26590 | PH                     |          |                  |
| O48696     | O48696_ARATH| AAA-type ATPase family protein (F36.23 protein)                              | At1g24920   | 525    | AT1G24920 | UBA                    |          |                  |
| O23249     | CKS1_ARATH | Cyclin-dependent kinases regulatory subunit 1 (CKS1-A1)                      | CKS1        | 87     | AT2G27960 | GO                     |          |                  |
| F4KE5S     | F4KE5S_ARATH| Ubiquitin system component Cue protein                                        | At5g32440   | 265    | AT5G32440 | CUE                    |          |                  |
| F4KC6N     | F4KC6N_ARATH| Ubiquitin receptor RAD23 (DNA repair protein RAD23)                          | At5g16090   | 171    | AT5G16090 | UBA                    |          |                  |
| F4KAU9     | TOL7_ARATH | TOM1-like protein 7                                                           | TOL7        | 542    | AT5G01760 | VHS-GAT                |          |                  |
| F4JPR7     | PLX8_ARATH | Plant UBX domain-containing protein 8 (PLX8) (Ara4-interacting protein) (Suppressor of Arabidopsis-induced defect of ypt1) (SAY1) | PLX8        | 564    | AT4G11740 | UIM/UBA-like/UBX       | + +       |                  |
| F4J85      | F4J85_ARATH| Ubiquitin family protein                                                      | At4g03860   | 322    | AT4G03860 | GO                     |          |                  |
| F4XN6      | PLX6_ARATH | Plant UBX domain-containing protein 6 (PLX6)                                | PLX6        | 435    | AT3G21660 | UBX                    | + +       |                  |
| F4TTP6     | F4TTP6_ARATH| Ubiquitin-like superfamily protein                                           | At2g32350   | 242    | AT2G32350 | GO                     |          |                  |
| F41241     | BUB33_ARATH| Mitotic checkpoint protein BUB3.3 (Protein BUDDING UNINHIBITED BY BENZYMIDAZOL 3.3) | BUB3.3       | 314    | AT1G69400 | WD40                   |          |                  |
| F4KXZ1     | BRO1_ARATH | Vacuolar-sorting protein BRO1 (BRO domain-containing protein 1) (AtBRO1)      | BRO1        | 846    | AT1G15130 | BRO1                   |          |                  |
| A4FVR1     | GIP1L_ARATH| GBF-interacting protein 1-like (Protein GIP1L-ike)                          | GIP1L       | 575    | AT1G58820 | UBA-like               |          |                  |
| ADA1P8AW60 | ADA1P8AW60| Ubiquitin-associated (UBA)/TS-N                                             | At1g04850   | 324    | AT1G04850 | UBA                    |          |                  |

The list of proteins were cross-checked with previously published screens for ATG8-interacting proteins conducted by Marshall et al. [52] and Jacomin et al. [53] and indicated if they were found in these screens.

Expression level of candidate receptors were then analyzed using data available from public Arabidopsis RNAseq libraries [74]. In brief, five representative datasets were used for the analysis based on following criteria: (1) Dataset contains only one treatment. (2) There are at least three biological replicates in control and treated conditions. (3) Datasets are non-redundant. FPKM values for each gene and replicate were extracted from the five datasets, and subsequent Log2 Fold-change (Log2FC) and false discovery rate (FDR) for each gene comparing treated samples...
to control samples were assessed using Rstudio software (https://www.rstudio.com/). Only, genes with FDR < 0.05 were considered statistically significant. Log2FC of statistically significant genes are displayed as a heatmap with a dendrogram representing genes sorting based on expression pattern similarity from the different dataset (Figure 3).

As NBR1 is well-characterized, we first investigated the data on NBR1 presented by the heatmap. Notably, expression of NBR1 displayed a high positive log fold change during infection with P. syringae pv. maculicola (Pma) but showed no significant expression differences during flg22 treatment or Sclerotinia sclerotiorum and Botrytis cinerea infection (Figure 3).

We draw focus to the proteins which display expression patterns evidently different from NBR1, as they could be novel immune-related SARs with specialized functions different from the well-characterized NBR1. For example, the subset of proteins ATMCB1, VPS36, LRS1, SH3P2 and RIN2 were up-regulated during infection with S. sclerotiorum and B. cinerea, and could be novel ubiquitin- and/or AIM-binding receptors with functions in immunity. In contrast, there is a high negative log fold change in the expression of proteins AT1G24290, DM17, ATG4G00752, and AT1G73660 during S. sclerotiorum and B. cinerea infection (Figure 3). This indicates for a possibility that these proteins could be targeted during infection, perhaps because they as well play a role in immunity.

S. sclerotiorum and B. cinerea are necrotrophic in contrast with the hemibiotrophic P. syringae. Past studies have shown autophagy plays different roles during immunity against biotrophic and necrotrophic pathogens, due in part to its cross-talk with salicylic-acid signalling which mediates resistance against biotrophic pathogens [75]. It is therefore plausible that these putative SARs which respond specifically to necrotrophic pathogens could mediate resistance in a novel manner.

We have identified here many ubiquitin- and/or ATG8-binding receptors with significant expression differences upon flg22 treatment or pathogen challenge. Further investigation should be directed at validating the role of these proteins as SARs, and characterizing how they mediate pathogen interactions and immune responses. The molecular mechanisms that drive their substrate recognition and interaction with autophagic machinery should also be studied so we gain more insight into these interactions.

**ATG8 isoforms**

We have discussed in depth how SARs can promote the specificity of substrate degradation via autophagy. On the other hand, recent research has shown that different ATG8 isoforms can also add specificity to autophagy. This was already hinted at in earlier publications where Arabidopsis NBR1 showed different levels of interaction with different members from the ATG8 family of proteins [32]. A study into potato ATG8 isoforms showed that the isoforms display differential gene expression under a range of conditions, and interact with distinct sets of proteins with varying degrees of overlap, which suggests some degree of functional specialization of the isoforms [76]. In addition, a yeast-two-hybrid screen revealed differential preference of A. thaliana ATG8 isoforms to associate with the UIM [52]. In an immunity context, Leong et al. found that in N. benthamiana transcript levels of NbATG8-2 increased earlier than that of NbATG8-1 during Xcv infection [39]. P. infestans effector PexRD54 also showed a preference for ATG8CL over ATG8IL [72].

To strengthen the idea that ATG8 isoforms add specificity during autophagic responses in immunity, we analyzed publicly available data on the gene expression of A. thaliana ATG8 isoforms during responses to immunogenic peptide flg22 and pathogens Pma, B. cinerea, and S. sclerotiorum (Figure 4). The analysis method and datasets used are the same as above for identification of putative novel SARs. We found differential gene expression patterns of the ATG8 isoforms during these conditions. Notably, the ATG8 isoforms show different expression patterns compared to each other and under different pathogenic contexts. As previously mentioned, potato ATG8 isoforms show differential gene expression and interact with distinct subsets of proteins, which suggests functionalization [76]. We build upon this hypothesis and apply it to our analysis of A. thaliana ATG8 isoforms, and suggest that the differential expression patterns in ATG8 isoforms strengthen the idea that the isoforms have specific functions. Some ATG8 isoforms could be grouped according to the similarity in which they behave. For example, ATG8B, ATG8E, and ATG8I were up-regulated during Pma infection and but did not show significant log fold change during S. sclerotiorum or B. cinerea infection. ATG8A and ATG8H responded to both hemibiotrophic Pma and necrotrophic S. sclerotiorum. In contrast, ATG8C and ATG8G displayed negative log fold change during infection with S. sclerotiorum. These patterns, where a subset of isoforms could specifically respond to the different pathogenic contexts, are similar to the patterns presented by putative ubiquitin- and/or ATG8-binding proteins analyzed above. This strongly supports a scenario where receptor and ATG8 works together to add specificity during immunity.
Spatiotemporal control
Studies in selective autophagy and immunity have also revealed the importance of spatiotemporal regulation in cellular processes. Infection and autophagy are dynamic processes involving many players which constantly display changes in expression, localization, and stability. Dagdas et al. showed that during P. infestans infection, the effector PexRD54 caused a diversion of Joka2-ATG8CL defense-related vesicles to haustoria [72]. Leong et al. further showed that the protein accumulation of NBR1 occurs earlier than its increase in transcription [39]. In the same study, it was also shown that knockdown of NBR1 expression via virus-induced gene silencing (VIGS) reduced Xcv growth at 3 days-post-inoculation (dpi) compared with WT but not at 6dpi, thus indicating for a role in selective autophagy during the earlier steps of infection [39].

Conclusions
Selective autophagy research has contributed much to our understanding of plant–pathogen interactions. During infection, autophagy is targeted by pathogens due to its central role in plant homeostasis but can also function in clearing pathogenic components and reducing infection. The SARs mediate selective autophagy, and studying these receptors gives us more mechanistic insight into how selective autophagy interacts with immunity. We have highlighted many excellent studies which have shed more light on the role of selective autophagy during immunity. There are nevertheless still many questions to answer, giving great potential for further investigation.

In a classical immunity concept of the “zig-zag” model, gene-for-gene resistance is mediated by cell surface PRRs and cytoplasmic NLRs, which recognize pathogenic components in an extracellular and intracellular manner respectively [1,3]. In this review, we have highlighted the role of SARs in recognizing pathogenic components and their downstream effects within the cell. We draw parallels between NLRs-mediated immunity (NMI) and the role of immune-related SARs reviewed here, and propose that SARs can confer immunity to pathogens through SAR-mediated immunity (SMI) which results in effectorphagy (Figure 5). In both scenarios, effectors and/or their downstream effects are recognized by intracellular proteins with various features leading to effector-triggered immunity (ETI). In fact, Deretic et al. proposed, in the context of mammalian SARs, that they could represent a new paradigm in innate immunity as a new class of PRRs [77]. We support this view, and further emphasize the mechanistic details behind SAR function—how substrate recognition can be both ubiquitin-dependent and -independent, and how SARs directly bridge their substrate to the autophagosome thus immediately targeting them for degradation. This allows SARs to recognize a wide range of pathogenic effectors that are more directly involved in modulation of cellular processes.

Summary
1. Due to its central role in ensuring plant cellular homeostasis, autophagy is targeted by plant pathogens during infection
2. Selective autophagy has been found to play pro-plant or pro-pathogen roles during immunity
3. Similarities can be found across selective autophagy receptor domain organization and function
4. Selective autophagy receptors are emerging as key players in plant interaction with bacteria, viruses, and oomycetes.
5. The interplay between selective autophagy and pathogenic effectors can present new perspectives on the molecular mechanisms behind plant–pathogen interactions

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution
J.X.L. and S.Ü. wrote the manuscript. J.X.L. S.Ü. and G.L designed figures. G.L. performed in silico analysis to identify novel SARs.

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Abbreviations
CaMV, cauliflower mosaic virus; ETI, effector-triggered immunity; NMI, NLR-mediated immunity; PAMP, pathogen-associated-molecular-pattern; PRR, pattern-recognition receptor; PTI, PAMP-triggered immunity; SAR, selective autophagy receptor; SMI, SAR-mediated immunity; TuMV, Turnip mosaic virus; TuYV, Turnip yellows virus; VIGS, virus-induced gene silencing.

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