1 **ER bodies are induced by *Pseudomonas syringae* and negatively regulate**
2 **immunity**

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4 **Running title:** Role of ER bodies in the defense response

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23 ABSTRACT

24 ER bodies are endoplasmic reticulum-derived organelles present in plants
 25 belonging to the *Brassicales* order. In *Arabidopsis thaliana*, ER bodies are
 26 ubiquitous in cotyledons and roots, and present only in certain cell types in
 27 rosette leaves. However, both wounding and jasmonic acid treatment induce
 28 the formation of ER bodies in leaves. Formation of this structure is dependent
 29 on the transcription factor *NA11*. The main components of the ER bodies are β -
 30 glucosidases (BGLUs), enzymes that hydrolyze specialized compounds. In
 31 *Arabidopsis*, PYK10 (BGLU23) and BGLU18 are the most abundant ER body
 32 proteins. In this work, we found that ER bodies are downregulated as a
 33 consequence of the immune responses induced by bacterial flagellin
 34 perception. *Arabidopsis* mutants defective in ER body formation show
 35 enhanced responses upon flagellin perception and enhanced resistance to
 36 bacterial infections. Furthermore, the bacterial toxin coronatine induces the
 37 formation of *de novo* ER bodies in leaves and its virulence function is partially
 38 dependent on this structure. Finally, we show that performance of the
 39 polyphagous beet armyworm herbivore, *Spodoptera exigua*, increases in plants
 40 lacking ER bodies. Altogether, we provide new evidence for the role of the ER
 41 bodies in plant immune responses.

42

43 **Keywords:** ER bodies, plant immunity, *Pseudomonas syringae*, *Spodoptera*
 44 *exigua*,

45 INTRODUCTION

46 ER bodies are rod-shaped endoplasmic reticulum (ER)-associated
 47 structures that are present in three families within the Brassicales order
 48 (Nakano et al., 2014a). ER bodies are constitutively present in roots and
 49 cotyledons of young plants (constitutive ER bodies), but their abundance
 50 decreases with senescence (Matsushima et al., 2002). In *Arabidopsis* rosette
 51 leaves, ER bodies are constitutively present in marginal cells, epidermal cells
 52 covering the midrib and extremely large pavement cells (Leaf ER bodies)
 53 (Nakazaki et al., 2019b). Furthermore, the formation of ER bodies in rosette
 54 leaves is induced by Methyl Jasmonate (MeJA) treatment or wounding
 55 (Inducible ER bodies) (Matsushima et al., 2002).

56 Formation of both constitutive and leaf ER bodies is dependent on the
 57 basic-helix-loop-helix transcription factor *NAI1* (Matsushima et al., 2004) and
 58 the essential protein *NAI2* (Yamada et al., 2008). Inducible ER bodies are
 59 formed in a *nai1* mutant, but differ in shape and composition with the ER bodies
 60 induced in wild-type Col-0 (Ogasawara et al., 2009). The main components of
 61 the constitutive ER bodies are the β -glucosidases (BGLU) PYK10 (BGLU23),
 62 BGLU21 and BGLU22 (Matsushima et al., 2003), while inducible-ER bodies
 63 also contain BGLU18 (Ogasawara et al., 2009). These myrosinases belong to a
 64 subfamily of 8 β -glucosidases (*i. e.* BGLU18 to BGLU25) (Xu et al., 2004) with
 65 ER-retention signals (Yamada et al., 2011). Leaf ER bodies mainly contain
 66 PYK10 and BGLU18 (Nakazaki et al., 2019b). PYK10 has been described as
 67 an atypical myrosinase that hydrolyzes Indole Glucosinolates (IG) (Nakano et
 68 al., 2017). Upon tissue damage, PYK10 associates in the cytosol with PYK10-
 69 binding protein 1 (PBP1), activating PYK10 function (Nagano et al., 2005). An

70 *Arabidopsis pyk10/blgu18* double mutant accumulates high amounts of the IG
 71 4-methoxyindol-3-ylmethylglucosinolate (4MI3G) after tissue damage (Nakazaki
 72 et al., 2019b). IGs and the products resulting from their breakdown have a role
 73 in the defense responses against microbial pathogens and herbivory (Bednarek
 74 et al., 2009; Clay et al., 2009; Millet et al., 2010; Müller et al., 2010; Johansson
 75 et al., 2014; Nakazaki et al., 2019b). IGs are also required for the establishment
 76 of beneficial interactions with the endophytic fungus *Piriformospora indica* by
 77 appropriately regulating the level of root colonization through *PYK10* (Sherameti
 78 et al., 2008; Lahrmann et al., 2015). Collectively, multiple independent studies
 79 have supported a role in defense responses for ER bodies (Yamada et al.,
 80 2011; Nakano et al., 2014b).

81 Plants are constantly exposed to a variety of biotic and abiotic stresses.
 82 Surface localized plant pattern-recognition receptors (PRRs) act as one of the
 83 first lines of defense against pathogens and are able to recognize conserved
 84 pathogen features, such as flagellin, as non-self (Couto and Zipfel, 2016). One
 85 of the best-characterized PRRs is the flagellin receptor FLS2 (Flagellin Sensing
 86 2) which recognizes flg22, a 22 amino acid epitope of bacterial flagellin
 87 (Gómez-Gómez and Boller, 2000). Perception of flg22 by FLS2 induces a
 88 number of cellular events resulting in Pattern-Triggered Immunity (PTI), which
 89 restricts pathogen proliferation. Some of the cellular events that take place
 90 during PTI are the activation of MAP Kinases (MAPK) and calcium-dependent
 91 protein kinases, the production of reactive oxygen species (ROS), stomatal
 92 closure and the formation of callose (β -1,3-glucan) deposits in the cell wall
 93 (Henry et al., 2013). However, successful pathogens have evolved different

94 mechanisms to suppress defense responses and successfully colonize their
95 host.

96 *Pseudomonas syringae* is a Gram-negative bacterium that has been
97 extensively used to study plant immune signaling. *P. syringae* virulence
98 depends on the Type III Secretion System (T3SS), a complex nanomachine that
99 translocates proteins, called effectors, into the cytosol of the plant cell. Most of
100 the effectors suppress PTI acting on different cellular pathways (Macho, 2015).
101 Another virulence mechanisms used by bacterial pathogens to subvert plant
102 immunity is the production of phytotoxins. *P. syringae* pv. tomato DC3000 (*Pst*
103 DC3000) produces coronatine, a polyketide toxin that acts as a molecular mimic
104 of the plant hormone jasmonic acid (JA) (Weiler et al., 1994). Coronatine
105 induces stomata re-opening upon PRR perception to allow bacterial
106 colonization (Melotto et al., 2006; Melotto et al., 2008; Gimenez-Ibanez et al.,
107 2017), and also suppresses callose deposition and induces bacterial growth
108 within the apoplast (Geng et al., 2012).

109 In this work, we investigated the biological function of ER bodies during
110 plant immunity. We found that ER bodies are downregulated after flg22-
111 triggered immune activation but induced after *P. syringae* infection in a
112 coronatine-dependent manner. Furthermore, loci underlying ER body formation
113 act as negative regulators of immunity against bacterial pathogens but are
114 required for a complete response against the chewing insect *Spodoptera*
115 *exigua*. With these results, we report a new role of ER bodies in virulence of
116 phytopathogenic bacteria and their suppression by plant immune perception..

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119 RESULTS

120 ER bodies are rapidly inhibited upon flagellin perception

121 To investigate the role of ER body formation after plant pathogen perception,
 122 we used an *Arabidopsis* transgenic line expressing GFP with the ER-retention
 123 signal HDEL (GFP-HDEL), which allows the visualization of ER bodies
 124 (Mitsunashi et al., 2000). We vacuum-infiltrated 2 week-old *Arabidopsis* plants
 125 with either 10 μ M flg22 or water and visualized cotyledons three hours later by
 126 confocal microscopy. We were able to detect and quantify the ER bodies in
 127 cotyledons, where this structure is constitutively present, in mock-treated plants.
 128 However, after flg22 treatment, only a small number of ER bodies were
 129 detected (Fig. 1A). This decrease in ER body detection was statistically different
 130 compared to water-treated controls (Fig. 1A). To further investigate the
 131 disappearance of ER bodies within the same tissue, we monitored cotyledons
 132 for 120 minutes after either water (mock) or flg22 treatment. ER bodies lose
 133 their characteristic rod-shape within 60 minutes post-flg22 treatment, and the
 134 GFP signal is diffused throughout the cell at later time points (Fig. 1B).

135 To analyze the impact of the flg22 treatment in ER body formation at the
 136 transcriptional level, we analyzed the transcriptional expression of *NAI1* and
 137 *PYK10* under different biotic treatments using an available dataset
 138 (NASCArrays-120). We found that flg22 treatment reduces the expression level
 139 of *NAI1* and *PYK10* in an *FLS2*-dependent manner (Fig. 1C, Fig. S1), pointing
 140 to regulation of ER bodies at the transcriptional level as a consequence of
 141 activation of defense responses. However, inoculation with the virulent bacterial
 142 strain *Pst* DC3000 induces the expression of both *NAI1* and *PYK10* at 24h, and
 143 the same occurs with a strain expressing the avirulence effector AvrRpm1 (Fig.

144 1C). Interestingly, both the T3SS DC3000 mutant derivative $\Delta hrcC$ and the non-
 145 host strain 1448A reduced the accumulation of *NAI1* transcripts (Fig. 1C).
 146 Altogether, these results show a downregulation of ER bodies as a
 147 consequence of the activation of flg22-triggered immunity and suggest a role of
 148 this subcellular structure during the defense response against *P. syringae*.

149

150 ***Arabidopsis* mutants impacting ER body formation and contents exhibit** 151 **enhanced immune responses against bacterial pathogens**

152 After flg22 perception, many cellular events take place to restrict pathogen
 153 invasion, including activation of MAPKs and calcium dependent protein kinases
 154 minutes after perception, followed by ROS production, activation of defense
 155 gene expression and several hours later, callose deposition (Henry et al.,
 156 2013). To further characterize the role of the ER bodies in flg22-induced
 157 responses, we measured ROS production as an early event of PTI, in plants
 158 affected in the formation of ER bodies. We treated 3-week-old Col-0, *nai1-1*,
 159 *pyk10-1* and *pyk10-1/blgu21* plants, with 100 nM flg22 and measured the ROS
 160 burst. The total ROS produced in all the mutant backgrounds tested was higher
 161 than the ROS produced in Col-0 (Fig. 2A). This increase in ROS production in
 162 response to flg22 also occurs in both *bglu18-1* and *pbp1-1* mutants (Fig S2A).
 163 To analyze a late event of PTI, we treated four-week-old plants with flg22 and
 164 quantified callose deposition. Consistent with the ROS burst, the number of
 165 callose deposits was two times higher in ER bodies mutants compared to Col-0
 166 (Fig 2B). These data indicate that a loss of ER bodies or its main components
 167 induce an enhanced response to bacterial flg22, pointing to a negative role of
 168 ER bodies on PTI.

169 To determine the role of the ER bodies in bacterial growth, we quantified *Pst*
 170 DC3000 titers in Col-0 and in the different mutant backgrounds (Fig. 2C). *Pst*
 171 DC3000 exhibited decreased growth (>1 log) in the *nai1-1* mutant compared to
 172 Col-0. Interestingly, both the *pyk10-1* and the *pyk10-1/bglu21* mutants exhibited
 173 a stronger decrease in bacterial growth, with 1.5 Log reduction compared to
 174 wild-type Col-0 (Fig 2C). No differences were detected between *pyk10* and
 175 *pyk10-1/bglu21*, indicating that *PYK10* is a critical gene involved in negative
 176 regulation of bacterial growth. Plants lacking *PBP1*, necessary for *PYK10*
 177 function, or *BGLU18*, the main component of inducible ER bodies, also showed
 178 enhanced resistance to bacterial infection (Fig, S2B).

179 A critical component controlling bacterial virulence is the ability to deliver
 180 effectors through the type III secretion system (Macho, 2015). In order to
 181 investigate the relationship between immune suppression and ER body
 182 formation, we used DC3000 $\Delta hrcC$, which is unable to translocate effectors to
 183 the plant cell, and thus, does not suppress PTI. We measured the growth of
 184 $\Delta hrcC$ in ER body mutants. In the *nai1-1* background, the growth of $\Delta hrcC$ was
 185 not significantly different from that in Col-0. However, we observed a slight but
 186 significant decrease in growth in the *pyk10-1/bglu21* background (Fig. 2D). The
 187 increased *P. syringae* growth restriction in *pyk10-1* and *pyk10-1/bglu21*
 188 backgrounds compared with *nai1* (Fig. 2C and 2D) could indicate a *NAI1*-
 189 independent regulation for *PYK10* and/or *BGLU21*. Altogether, these results
 190 show that the absence of ER bodies increases resistance against the bacterial
 191 pathogen *P. syringae*.

192

193 ***NAI1* contributes to the defense response against *S. exigua***

ER bodies have been proposed to participate in the defense response against herbivores (Yamada et al., 2011; Nakano et al., 2014b). A recent study demonstrated increased susceptibility of the double mutant *bglu18 pyk10* to the terrestrial isopod *Armadillidium vulgare* (Nakazaki et al., 2019b). The *pyk10/bglu21* mutant forms ER bodies, although they are larger and less numerous than those in wild-type plants (Nagano et al. 2009). We measured the performance of the generalist leaf-chewing herbivore *S. exigua* in different ER body related mutants. Performance assays have been widely used to study the defense mechanisms against herbivory (Cipollini et al., 2004; Chung et al., 2008; Van Oosten et al., 2008; Müller et al., 2010; Santamaría et al., 2017). We fed *S. exigua* larvae with Col-0 and the ER body mutants *nai1-1*, *pyk10-1* and *pyk10-1/bglu21* plants, and measured the weight of the larvae after 7 days. The fresh weight of *S. exigua* larvae was higher in *nai1-1* plants compared with the Col-0 control, confirming a role of ER bodies in the defense response against herbivore insects. Surprisingly, the performance of *S. exigua* in either *pyk10-1* or *pyk10-1/bglu21* plants was not different compared Col-0, suggesting a redundant role of *PYK10* and *BGLU21* with other myrosinases in this context. (Fig. 2E)

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213 **Coronatine induces the formation of ER bodies in rosette leaves**

Transcriptomic analyses indicate that inoculation with virulent *Pst* DC3000 induces the expression of *NAI1* and *PYK10* (Fig. 1C). To check if this increased expression results in *de novo* formation of ER bodies, we inoculated rosette leaves of *Arabidopsis* GFP-HDEL plants with a relatively high inoculum (5×10^7 CFU/mL) of *Pst* DC3000, the derivative $\Delta hrcC$ and a coronatine mutant (*cor*-,

219 *Pst* DC3118). We applied the bacterial suspension using a brush to avoid
 220 wounding tissue. Three days after inoculation, we observed the samples under
 221 the confocal microscope (Fig. 3A). No ER bodies were found in the mock-
 222 treated leaves, in agreement with the absence of ER bodies in certain rosette
 223 leaves (Matsushima et al., 2002; Nakazaki et al., 2019b). Interestingly, a large
 224 number of ER bodies was found in leaves inoculated with wild-type *Pst*
 225 DC3000. The distribution of the ER bodies was patchy, suggesting that only
 226 some cells were forming the structure. To investigate if the formation of ER
 227 bodies localized surrounding bacterial colonies, we generated a *Pst* DC3000
 228 derivative tagged with eYFP. Three days after inoculation, we observed a large
 229 number of ER bodies surrounding the bacterial microcolony in Col-0 (Fig. 3B,C)
 230 but only a few aberrant ER bodies in the *nai1-1* mutant (Fig. 3C), suggesting
 231 that ER bodies induced by *Pst* DC3000 are dependent on *NAI1*. To determine
 232 which bacterial factors are inducing the formation of ER bodies in rosette
 233 leaves, we inoculated Col-0 GFP-HDEL leaves with the $\Delta hrcC$ derivative and
 234 the *cor-* mutant, both tagged with eYFP. We were unable to detect $\Delta hrcC$ –
 235 eYFP microcolonies due to their extremely small size, consistent with their
 236 reduced growth *in planta*. Nevertheless, we did not detect ER bodies in leaves
 237 inoculated with the $\Delta hrcC$ mutant (Fig. 3A). Interestingly, the *cor-* eYFP mutant
 238 was unable to induce the formation of *de novo* ER bodies, consistent with the
 239 role of coronatine mimicking JA (Fig. 3). This result indicates that induction of
 240 ER bodies could be a virulence function of coronatine. Analyzing transcriptomic
 241 data of plants inoculated either with *Pst* DC3000 or the *cor-* mutant, we
 242 observed that many genes related to ER bodies, including *NAI1*, *PYK10* and
 243 *BGLU18*, were upregulated by *Pst* DC3000 but not after inoculation with *Pst*

244 DC3118 (*cor*-) (Fig. S3). To investigate the ability of coronatine to promote
 245 bacterial virulence in the absence of wild-type ER body formation, we measured
 246 the growth of *Pst* DC3000 and the *Pst cor*- mutant in Col-0, *nai1-1*, *pyk10-1* and
 247 *pyk10-1/bglu21*. In order to bypass the role of coronatine on re-opening stomata
 248 (Melotto et al., 2006; Melotto et al., 2008), we performed syringe inoculation in
 249 five-week old plant leaves. Four days after inoculation, a strong growth
 250 restriction was observed in the coronatine mutant compared with wild-type *Pst*
 251 DC3000 in Col-0 (Fig. 3D). In the ER body-defective backgrounds, a stronger
 252 growth restriction was observed for *Pst* DC3000 (Fig. 2C, Fig. 3D). However,
 253 the growth of the coronatine mutant was not significantly different between wild-
 254 type Col-0 and the ER body mutants.

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256

257 DISCUSSION

258 The main contents in ER bodies are β -glucosidases, enzymes which can
 259 produce toxic compounds to protect the plant from herbivore attack
 260 (Matsushima et al., 2003). Induction of ER bodies by wounding or by MeJA
 261 treatment suggests an association with plant defense (Nakano et al., 2014b).
 262 However, there are few experimental evidences of the association between ER
 263 bodies and plant immunity. Plants are infected by diverse pathogens that often
 264 utilize opposing virulence strategies and are differentially impacted by plant
 265 immune responses (e.g. chewing insects versus biotrophic/hemibiotrophic
 266 pathogens). In this study we demonstrate contrasting roles of ER bodies in the
 267 plant response to pathogenic bacteria and herbivores.

268 Previous studies have implicated ER bodies in plant protection against fungi
 269 and feeding insects. Sherameti et al. (2008) demonstrated that *PYK10* is
 270 required to protect plants against excessive root colonization by the endophytic
 271 fungus *Piriformospora indica*. Furthermore, Nakazaki et al. (2019b) showed that
 272 *bglu18 pyk10* double mutant plants are more susceptible than wild-type to the
 273 attack of the terrestrial isopod *Armadillidium vulgare* (pill-bug). In this work, we
 274 experimentally link the function of the ER bodies with the plant response
 275 against the bacterial pathogen *P. syringae* and the herbivore *S. exigua*. We
 276 found an increased weight acquisition of the generalist chewing herbivore *S.*
 277 *exigua* in *nai1-1* plants compared with Col-0, but not in *pyk10-1* and *pyk10-*
 278 *1/bglu21* mutants (Fig. 2E). *S. exigua* feeds on leaves, where constitutive ER
 279 bodies are absent (Matsushima et al., 2002). Constitutive leaf ER bodies, which
 280 are dependent on *NAI1*, localize in the edges of the leaves and mainly contain
 281 PYK10 and BGLU18 (Nakazaki et al., 2019b). The biogenesis and content of

282 leaf ER bodies is regulated by both Jasmonic Acid (JA) dependent and
 283 independent pathways (Nakazaki et al., 2019a). While *NAI1* expression, and
 284 therefore PYK10 accumulation is dependent on JA, BGLU18 accumulation in
 285 leaf ER bodies is independent on JA (Nakazaki et al., 2019a). Since the
 286 performance of *S. exigua* is not affected in *pyk10-1* mutant, the genetic analysis
 287 points to *BGLU18* as the responsible of the defense response against *S.*
 288 *exigua*. Furthermore, chewing induces JA accumulation (Rehrig et al., 2014)
 289 and this signal would potentially lead to the formation of the inducible ER
 290 bodies. The *nai1* mutant is compromised in formation of inducible local and
 291 systemic ER bodies. In the *nai1* mutant, inducible ER bodies are long and
 292 tubular and mainly observed in cells surrounding the wound site (Ogasawara et
 293 al., 2009). We also observed that *Pst* DC3000 was unable to induce ER body
 294 formation in the *nai1* mutant, indicating that the absence or altered
 295 composition/structure of ER bodies in *NAI1* suppresses growth of the
 296 hemibiotrophic pathogen *P. syringae*, while enhancing performance of a
 297 chewing herbivore.

298 *Pst* DC3000 induces the formation of ER bodies in leaves. Since the flagellin
 299 peptide flg22 downregulates the formation of constitutive ER bodies and we
 300 have shown that ER bodies negatively regulate PTI, we hypothesize that *P.*
 301 *syringae*-induction of ER bodies is a virulence strategy to suppress host
 302 immune responses. The two main virulence factors of *P. syringae* pv. tomato
 303 are the phytotoxin coronatine and the T3SS effectors. The bacterial phytotoxin
 304 coronatine, which mimics the function of JA in the plant (Bender et al., 1999),
 305 induces the formation of ER bodies in leaves (Fig 3A,B). JA is perceived in the
 306 plant through degradation of JASMONATE ZIM-DOMAIN (JAZ) transcriptional

307 repressors and JAZ proteins suppress the expression of ER body components
 308 as well as indole glucosinolates (Guo et al., 2018; Ghorbel et al., 2021). Thus,
 309 JA or pathogen JA-mimics can regulate the abundance of this specialized
 310 organelle to induce or suppress defense depending on pathogen type. The
 311 most studied coronatine virulence function is to re-open stomata upon PAMP-
 312 induced closure (Melotto et al., 2006; Melotto et al., 2008). However, coronatine
 313 also has been shown to suppress immunity once the bacteria colonize the
 314 apoplast (Fig. 3C; (Geng et al., 2012)). The T3SS defective mutant $\Delta hrcC$ is not
 315 able to induce the formation of ER bodies, which would indicate an effect of
 316 T3Es on the induction of ER bodies. However, we cannot rule out that the
 317 absence of ER bodies is due to an insufficient amount of coronatine produced
 318 by the $\Delta hrcC$ microcolonies.

319 Bacterial flg22 perception leads to a down regulation of the ER bodies at both
 320 transcriptional and post-transcriptional levels (Fig. 1 and S1). We found that
 321 *Arabidopsis* mutants defective in either ER body biogenesis or composition are
 322 more resistant to pathogenic bacteria (Fig. 2 and S2). The main components of
 323 ER bodies are myrosinases, enzymes that modify glucosinolates to initiate the
 324 glucosinolate breakdown, which will result in different products depending on
 325 the biochemical properties of the modifier protein and the chemical nature of the
 326 glucosinolate side chain (Wittstock and Burow, 2010; Sugiyama and Hirai,
 327 2019). Those products can function as defense compounds or as signaling
 328 molecules.

329 PYK10/BGLU23 is an atypical myrosinase similar to PENETRATION 2 (PEN2,
 330 BGLU26) that hydrolyzes indole glucosinolates (IGs) (Nakano et al., 2017;
 331 Nakazaki et al., 2019b). PEN2-mediated conversion of 4-methoxyindol-3-

ylmethylglucosinolate (4MI3G) into glucosinolate-derived products plays an important role in the defense response against fungi (Bednarek et al., 2009). Interestingly, *PEN2* is required for callose deposition upon flg22 perception and for resistance against *Pst* DC3000 infection in seedlings grown in liquid media (Clay et al., 2009; Johansson et al., 2014). In contrast, in adult plants grown in soil, callose deposition and *Pst* DC3000 growth is not affected in the *pen2-1* mutant (Geng et al., 2012). The *pen2-1* mutant grown in soil exhibits enhanced resistance to *P. syringae* pv. *maculicola* (Stahl et al., 2016). These apparently contradictory results indicate that PEN2 activity is dependent on age and/or the metabolic state of the plant. 4MI3G overaccumulates in homogenized tissue of *bglu18/pyk10* mutants indicating that one or both of these β -glucosidases hydrolyze 4MI3G (Nakazaki et al., 2019). Our results indicate that the single *pyk10-1* mutant and the double *pyk10-1/bglu21* mutant accumulate an increased number of callose deposits in response to flg22 treatment and are more resistant to *Pst* DC3000. Although both PEN2 and PYK10 seem to have the same substrate (4MI3G), their respective mutants differ on phenotypes such as flg22-induced callose deposition and resistance to *P. syringae* *Pst* DC3000 (Clay et al., 2009; Johansson et al., 2014). Apart from the above-mentioned effect related to growth conditions, one of the reasons explaining these differences could be the different compartmentalization of both PEN2 and PYK10. While PEN2 is localized in peroxisome and mitochondria (Lipka et al., 2005; Fuchs et al., 2016), PYK10 localizes in ER bodies (Matsushima et al., 2003). Furthermore, myrosinase activity is regulated by different Myrosinase-Binding Proteins and Myrosinase-Associated Proteins, as well as different specifier proteins, which lead to the formation of different breakdown products

357 (Wittstock et al., 2016). This variety of compounds can have a direct or indirect
 358 effect on the plant defense response (Wittstock et al., 2016). It is possible that
 359 mutants lacking ER bodies or core components of ER bodies also exhibit
 360 heightened basal defense, resulting in enhanced ROS and callose production.

361 In conclusion, we have demonstrated the involvement of ER body-dependent
 362 regulation of immunity to bacteria and induction of ER bodies as a pathogen
 363 virulence strategy. Further work will shed light on how this novel function of ER
 364 bodies influences bacterial-plant interactions and pave the way for the
 365 discovery of the role of indole-glucosinolates breakdown on the response
 366 against bacterial invasion in plants.

367 **Experimental procedures**

368 **Plant material and growth conditions**

369 *A. thaliana* Col-0, the transgenic line expressing GFP-HDEL (Hayashi et al.,
370 2001) and the mutants *nai1-1* (Matsushima et al., 2003), *pyk10-1* (Nagano et
371 al., 2008), *pyk10-1/bglu21* (Nagano et al., 2009), *bglu18-1* (Ogasawara et al.,
372 2009) and *pbp1-1* (Nagano et al., 2005) were grown in a controlled environment
373 chamber at 23°C, 70% relative humidity, and a 10h/14h light/dark photoperiod
374 with light intensity of 100µE.m⁻².S⁻¹. All seeds were stratified for 3-4 days at 4°C
375 before sowing into soil.

376

377 **Bacterial strains, growth conditions and pathogen assays**

378 *Pseudomonas syringae* pv. *tomato* DC3000 (Cuppels, 1986) and the derivative
379 strains $\Delta hrcC$ and *cor*⁻ (*cma*- *cfa*-; (Brooks et al., 2004)) were grown at 28°C in
380 Lysogeny Broth (LB) medium (Bertani, 1951). Antibiotics were used when
381 appropriate, at the following concentrations: Rifampicin (100 µg/ml), kanamycin
382 (25 µg/ml) and gentamycin (10 µg/ml).

383 The *Pst* DC3000, $\Delta hrcC$ and *cor*⁻ eYFP (enhanced yellow fluorescent protein)
384 derivatives were generated using a Tn7 delivery system as previously described
385 (Rufian et al., 2018)

386 For pathogen assays, five-week-old *Arabidopsis* plants were inoculated with a
387 *P. syringae* suspension of 5x10⁴ CFU/mL prepared in 10 mM MgCl₂. Three
388 leaves per plant were syringe infiltrated and five plants were used for each
389 experiment. Four days after inoculation, three leaf discs of 1 cm of diameter
390 (one per leaf were ground in 1 mL 10 mM MgCl₂, and serial dilutions were
391 plated.

392

393 **Transcriptomic analyses**

394 For the analysis of NAI1 and PYK10 expression under different biotic stresses,
395 we used transcriptomic data available in eFP Browser. Specifically, we used the
396 dataset AtGenExpress: Response to virulent, avirulent, type III secretion
397 system deficient and nonhost bacteria (NASCArrays-120). 5-week-old Col-0
398 plants were infiltrated with the corresponding *P. syringae* strain. Samples were
399 taken 24h after treatment and RNA was isolated and hybridized to the ATH1
400 GeneChip. The data were normalized by GCOS normalization, TGT 100.

401

402 **flg22-related assays**

403 For ROS burst assays, leaf discs were collected using a cork borer of 5mm
404 diameter from three-week-old *Arabidopsis* plants and floated overnight in
405 deionized water. The next day, the water was replaced with an assay solution
406 containing 17 mg/ml luminol (Sigma), 10 mg/ml horse radish peroxidase
407 (Sigma) and 100 nM flg22 (Genscript). Luminescence was measured using
408 Tristar multimode reader (Berthold technology).

409 Callose deposits were quantified from four-week-old *Arabidopsis* leaves
410 infiltrated with 10 μ M flg22 solution. Eighteen hours after treatment, leaves were
411 detached and incubated 15 minutes at 65°C in 5 ml of alcoholic lactophenol (1
412 volume of phenol: glycerol: lactic acid: water (1:1:1:1) and 2 volumes of
413 ethanol). Leaves were transferred to fresh alcoholic lactophenol and incubated
414 overnight at room temperature. After two washes with 50% ethanol, leaves were
415 stained with aniline blue and callose deposits were visualized under a

416 fluorescence microscope. Quantification of callose deposits was performed
417 using the Fiji distribution of ImageJ.

418

419 **Confocal microscopy**

420 Two-week old *Arabidopsis* GFP-HDEL plants were vacuum infiltrated with either
421 water or 100 nM flg22. Three hours after the treatment, cotyledons were
422 detached and observed under a Zeiss LSM710 confocal microscope equipped
423 with a LDC-apochromat 40x/1.1W Korr M27 water-immersion objective (NA
424 1.1). GFP was excited at 488 nm and emission collected at 500-550 nm. For
425 monitoring of the dynamics of the ER bodies, cotyledons were treated as above
426 and visualized using a Leica SP8 confocal microscope (Leica Microsystems
427 GmbH, Wetzlar, Germany). GFP was excited at 488 nm and emission collected
428 at 500-550 nm.

429 For visualization of bacterial-induced ER bodies, five-week old *Arabidopsis*
430 GFP-HDEL plants were brush-inoculated with a 5×10^7 CFU/mL suspension of
431 the indicated strain (Fig. 3A). Three days after inoculation, leaves were
432 observed as described above.

433 For visualization of bacterial microcolonies, five-week old *Arabidopsis* GFP-
434 HDEL plants were syringe infiltrated with a 5×10^4 CFU/mL suspension and
435 leaves were visualized using the Leica SP5 II confocal microscope (Leica
436 Microsystems GmbH, Wetzlar, Germany). Images of eYFP and GFP were
437 sequentially obtained using the following conditions (excitation/ emission):
438 eYFP (514 nm/ 525 to 600 nm), GFP (488/ 500 to 525 nm). Z series imaging
439 was taken at 1 μ m interval. Image processing was performed using Leica LAS

440 AF (Leica Microsystems). ER bodies were quantified using Fiji distribution of
441 ImageJ software.

442

443 ***S. exigua* performance**

444 *S. exigua* eggs were kindly provided by Dr. Salvador Herrero (U. Valencia).

445 After eclosion, *S. exigua* larvae were fed with an artificial diet (Elvira et al.,

446 2010). Three days after eclosion, five larvae of approximately same size were

447 transferred to a four-to-five week old *Arabidopsis* plant grown in Jiffy-7 pods.

448 Larvae were transfer to new plants every 24 hours to avoid food privation.

449 Seven days after plant feeding, fresh weight of the larvae was measured. For

450 each experiment, five replicates were performed (25 larvae per genotype).

451

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461

462 **Figure legends**

463 **Figure 1. ER bodies are downregulated after activation of PTI.**

464 **(A)** Constitutive ER bodies disappear after flg22 treatment. Two-week-old
 465 *Arabidopsis* GFP-HDEL plants were vacuum infiltrated with water or 100 nM
 466 flg22. Cotyledons were observed in a Zeiss LSM710 confocal microscope three
 467 hours after treatment. Red autofluorescence correspond to chloroplasts. For ER
 468 body quantification, three fields from three independent cotyledons were used.
 469 ER bodies were quantified using Fiji. The experiment was repeated four times
 470 with similar results and a mean value $\pm$ SE of all quantifications is shown.
 471 Statistical differences were determined with a two-tailed t-test comparing water
 472 treatment with flg22. Three asterisks indicate $p < 0.001$. Scale bar: 20 μ m. **(B)**
 473 Two-week-old *Arabidopsis* GFP-HDEL plants were vacuum infiltrated with water
 474 or 100 nM flg22. The infiltrated cotyledons were monitored for 120 minutes
 475 taken photos every 5 minutes with a Leica SP8 confocal microscope. **(C)**
 476 Expression patterns of *nai1* and *pyk10* genes under different biotic stress. Data
 477 was obtained from available microarray in eFP Browser. Five week-old Col-0
 478 plant leaves were syringe-infiltrated with either a 1 μ M flg22 solution (using
 479 water as mock treatment) or the indicated bacterial strain at 10^8 cfu/ml (using 10
 480 mM $MgCl_2$ as mock treatment). Samples were taken 24 hours after treatment.
 481 Fold change relative to mock treatment is shown.

482

483 **Figure 2. ER body mutants exhibit enhanced resistance against *P.*** 484 ***syringae*.**

485 **(A)** flg22-induced ROS burst in Col-0, *nai1-1*, *pyk10-1* and *pyk10-1/bglu21*.
 486 Three-week-old *Arabidopsis* leaf discs were treated with 100 nM flg22 and ROS

487 was quantified using a luminescence based assay. The left graph represent the
488 dynamics of the ROS produced in the different genotypes and the right graph
489 represent the total relative lights units (RLU) detected over a 30 minutes period.
490 Error bars indicate SD, $n = 16$. Statistically differences were determined with
491 one-way ANOVA ($\alpha = 0.05$) with Tukey's multiple comparisons test and different
492 letters indicate statistical significance. The experiment was repeated four times
493 with similar results and a representative experiment is shown. **(B)** Quantification
494 of flg22-induced callose deposition in Col-0, *nai1-1*, *pyk10-1* and *pyk10-*
495 *1/bglu21*. Four-week-old plants were infiltrated with 10 μ M flg22 and callose
496 deposits were visualized under an epifluorescence microscope eighteen hours
497 after treatment. Four images were taken per leaf and nine leaves were used per
498 experiment. The mean number of deposits was quantified and is included $\pm$ SE
499 in the bottom-right corner of each representative image. The experiment was
500 repeated three times with similar results, and a representative experiment is
501 shown. White bar: 100 μ m. **(C)** Growth of *P. syringae* pv. *tomato* DC3000 in
502 Col-0, *nai1-1*, *pyk10-1* and *pyk10-1/bglu21*. Five-week-old plant leaves were
503 syringe inoculated with a suspension of 5×10^4 CFU/mL. Four days after
504 inoculation, bacteria were recovered and quantified. Values from three
505 independent replicates are shown ($n=18$). Different symbols represent individual
506 values from different replicates. Statistically differences were determined with
507 by ANOVA ($\alpha = 0.05$) with Tukey's multiple comparisons test and different
508 letters indicate statistical significance. **(D)** Growth of the *P. syringae* pv. *tomato*
509 Δ *hrcC* derivative in Col-0, *nai1-1*, and *pyk10-1/bglu21*. Five-week-old plant
510 leaves were syringe inoculated with a suspension of 5×10^4 CFU/mL. Four days
511 after inoculation, bacteria were recovered and quantified. Values from three

independent replicates are shown ($n=18$). Different symbols represent individual values from different replicates. Statistically differences were determined by ANOVA ($\alpha= 0.05$) with Tukey's multiple comparisons test and different letters indicate statistical significance. (E) Three-week-old *Arabidopsis* Col-0, *nai1-1*, *pyk10-1* and *pyk10-1/bglu21* plants were challenged with three-days-old *S. exigua* larvae. Seven days after feeding, fresh weight of each larva was measured. Bars represent the mean weight $\pm$ SE ($n=20$). Statistically differences were determined with one-way ANOVA ($\alpha= 0.05$) with Tukey's multiple comparisons test and different letters indicate statistical significance. The experiment was repeated three times with similar results, and a representative experiment is shown.

523

Figure 3. Coronatine induces formation of ER bodies in leaves and its virulence function is compromised in mutants impaired in ER body formation.

(A-C) Confocal microscope images of bacterial micro-colonies (yellow) and ER bodies (green). Five-week-old *Arabidopsis* GFP-HDEL plants were brush-inoculated with a suspension containing 5×10^7 CFU/mL (A) or syringe inoculated with a bacterial suspension containing 5×10^4 CFU/mL (B, C). Three days after inoculation, leaf sections were visualized under the confocal microscope. A representative z-stack projection image is shown. ER bodies were quantified from the green channel using Fiji. Bars represent the mean number of ER bodies in (A) or the number of ER bodies surrounding a single bacterial micro colony in (B, C) $\pm$ SE. Red autofluorescence correspond to chloroplasts. The experiments were repeated three times with similar results,

537 and the mean of all experiments is shown. ER bodies were quantified using Fiji
538 and statistically differences were determined with one-way ANOVA ($\alpha = 0.05$)
539 with Tukey's multiple comparison test in (A) and different letters indicate
540 statistical significance. In (B, C) data were analyzed by a t-test ($\alpha = 0.001$).
541 White bar: 20 μm (A), 100 μm (B), 50 μm (C). (D) Growth of *P. syringae* pv.
542 *tomato* DC3000 and its derivative coronatine mutant (*cor* -, DC3118) in Col-0,
543 *nai1-1*, *pyk10-1* and *pyk10-1/bglu21*. Five-week-old plant leaves were syringe
544 inoculated with a bacterial suspension of 5×10^4 CFU/mL. Four days after
545 inoculation, bacteria were recovered and quantified. Values from three
546 independent replicates are shown ($n=12$). Different symbols represent individual
547 values from different replicates. Statistically differences were determined by
548 ANOVA ($\alpha = 0.05$) with Tukey's multiple comparisons test and different letters
549 indicate statistical significance.

550

551 **Supplemental Figure Legends**

552 **Figure S1. Downregulation of *NAI1* and *PYK10* after flg22 treatment.**

553 Expression pattern of *NAI1* and *PYK10* after flg22 treatment (5, 10, 30, 90 and
554 180 minutes) in Col-0 and *fls2* plants. Data were obtained from Bjornson et al.
555 (2021). Fourteen-day old Arabidopsis Col-0 or *fls2* seedlings were immersed in
556 1 μM flg22 and samples were taken at the indicated time points.

557

558 **Figure S2. *bglu18-1* and *pbp1-1* show enhanced resistance to *P. syringae***

559 (A) Flg22-induced ROS burst in Col-0, *bglu18-1*, and *pbp1-1*. Three-week-old
560 *Arabidopsis* leaf discs were treated with 100 nM flg22 and ROS was quantified

561 using a luminescence based assay. Error bars indicate $\pm$ SE, $n = 16$. The
 562 experiment was repeated twice with similar results and a representative
 563 experiment is shown. **(B)** Growth of *P. syringae* pv. *tomato* DC3000 in Col-0,
 564 *bglu18-1*, and *pbp1-1*. Five-week-old plant leaves were syringe inoculated with
 565 a suspension of 5×10^4 CFU/mL. Four days after inoculation, bacteria were
 566 recovered and quantified. Values for each individual plant are shown ($n=6$).
 567 Bars represent the mean value $\pm$ SE. Statistically differences were determined
 568 with one-way ANOVA ($\alpha= 0.05$) with Tukey's multiple comparisons test and
 569 different letters indicate statistical significance. The experiment was repeated
 570 twice with similar results and a representative experiment is shown.

571

572 **Figure S3. Coronatine induce the expression of ER body and indole**
 573 **glucosinolate related genes.**

574 (A) Transcriptomic analysis of several genes related with ER bodies formation
 575 or indole-glucosinolate metabolism. Bars show Log_2 signal values from
 576 microarrays datasets. Data was obtained from the study by Thilmony et al.
 577 (2006), available in Genevestigator. *Arabidopsis* Col-5 leaves were inoculated
 578 with water (Mock), or a 10^6 cfu/ml suspension of *Pst* DC3000 or *Pst* DC3118
 579 (cor). Samples were taken 24 hours after inoculation. (B) Coexpression
 580 analyses of PYK10 (At3G09260). Analyses were performed using ATTED-II
 581 software.

582

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