1 Title

- 2 The ELR-SOBIR1 complex functions as a two-component RLK to mount defense against
- 3 Phytophthora infestans
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19 Abstract

The ELICITIN RESPONSE (ELR) protein from Solanum microdontum can recognize INF1 20 elicitin of *Phytophthora infestans* and trigger defense responses. ELR is a receptor-like 21 protein (RLP) that lacks a cytoplasmic signaling domain and is anticipated to require 22 interaction with a signaling-competent receptor-like kinase (RLK). SUPPRESSOR OF BIR1-23 24 1 (SOBIR1) has been proposed as a general interactor for RLPs involved in immunity and as such, is a potential interactor for ELR. Here we investigate whether SOBIR1 is required for 25 response to INF1 and resistance to P. infestans and whether it associates with ELR. Our 26 results show that virus-induced gene silencing (VIGS) of SOBIR1 in Nicotiana benthamiana 27 28 leads to loss of INF1-triggered cell death and increased susceptibility to P. infestans. Using genetic complementation, we found that the kinase activity of SOBIR1 is required for INF1-29 triggered cell death. Co-immunoprecipitation experiments showed that ELR constitutively 30 associates with potato SOBIR1 in planta, forming a bi-partite receptor complex. Upon INF1 31 elicitation, this ELR-SOBIR1 complex recruits SOMATIC EMBRYOGENESIS RECEPTOR 32 KINASE 3 (SERK3) leading to downstream signaling activation. Overall, our study shows 33 that SOBIR1 is required for basal resistance to *P. infestans* and for INF1-triggered cell death, 34 35 and functions as an adaptor kinase for ELR.

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37 Keywords

BAK1/SERK3, cell death, ELICITIN RESPONSE (ELR) receptor-like protein (RLP), INF1
effector, late blight, pattern recognition receptor (PRR), *Phytophthora infestans*,
SUPPRESSOR OF BIR1-1 (SOBIR1).

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42 Introduction

Plants rely on cell surface, plasma membrane-spanning pattern-recognition receptors (PRRs) 43 as a first line of apoplastic defense against microbial pathogens. PRRs recognize microbial 44 molecules such as proteins that play a role in defense suppression (so-called effectors) or 45 structural components, referred to as microbe-associated molecular patterns (MAMPs) (Couto 46 and Zipfel, 2016). Two types of PRRs are recognized; those that have an intracellular kinase 47 domain, which are receptor-like kinases (RLKs), and those without such a domain, the 48 receptor-like proteins (RLPs). Leucine-rich repeat (LRR)-containing RLPs recognize MAMPs 49 or effectors. However, as they lack an obvious cytoplasmic signaling domain, they are 50 anticipated to require partner proteins for initiating defense signaling. The extent to which 51 RLP and RLKs associate to enable immune signaling remains poorly understood. 52

RLPs from diverse plant families (i.e. *Brassicaceae* and *Solanaceae*), involved in immunity 53 have been found to constitutively associate with the LRR-RLK SOBIR1 (SUPPRESSOR OF 54 BIR1-1), forming a heterodimeric complex (Gust and Felix, 2014; Liebrand et al., 2014; Bi et 55 al., 2016). Some examples are Ve1, Cf-2, Cf-4, Cf-9, RLP23, RLP30 and 56 57 RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1 (RBPG1, RLP42). 58 which are all RLPs involved in pathogen perception in tomato (Solanum lycopersicum, Sl) or Arabidopsis thaliana (At) (Liebrand et al., 2013; Zhang et al., 2013; Bi et al., 2014; Zhang et 59 al., 2014; Albert et al., 2015). SOBIR1 has been suggested to be a positive regulator of plant 60 defense, and overexpression of AtSOBIR1 in Arabidopsis resulted in a constitutive defense 61 phenotype (Gao et al., 2009). 62

Both in tomato and potato (*Solanum tuberosum*, *St*), two alleles of *SOBIR1* are present, named *SOBIR1* and *SOBIR1-like*, with putatively redundant functions (Liebrand et al., 2013; Liebrand et al., 2014). In several plant species, *SOBIR1* transcripts have been found to increase upon pathogen challenge or treatments with salicylic acid or pathogen elicitors (Liebrand et al., 2014; Peng et al., 2015). Moreover, *SI*SOBIR1 and *SI*SOBIR1-like, and *At*SOBIR1 were found to be required for resistance against several pathogens, including fungi and the oomycetes *Hyaloperonospora arabidopsidis* and *Phytophthora parasitica* (Liebrand et al., 2014; Zhang et al., 2014; Peng et al., 2015). It has been proposed that RLP-SOBIR1 complexes are functional equivalents of genuine RLKs in the sense that SOBIR1 provides the kinase domain that is lacking from the RLP (Gust and Felix, 2014; Liebrand et al., 2014; Bi et al., 2016).

Late blight, caused by the oomycete *Phytophthora infestans*, is the most threatening disease of 74 potato. So far, breeding for late blight resistance has been focused on the introduction of 75 cytoplasmic resistance (R) genes of the nucleotide-binding leucine-rich repeat (NLR) class. 76 However, the fast evolving RXLR effector repertoire of *P. infestans* has been shown to 77 promptly defeat any introduced R gene (Vleeshouwers et al., 2011), and resistance 78 mechanisms based on the recognition of more conserved effectors might provide a more 79 durable alternative (Du et al., 2015). Elicitins form a major class of conserved oomycete 80 effectors, having MAMP features, in *Phytophthora* and *Pythium* species (Derevnina et al., 81 2016). Recently, the RLP ELICITIN RESPONSE (ELR) was identified in the wild potato 82 83 species Solanum microdontum (Sm). ELR specifically recognizes elicitins of Phytophthora spp., and was shown to quantitatively enhance resistance against P. infestans in cultivated 84 potato (Du et al., 2015). ELR localizes at the plasma membrane, similar to other RLPs 85 involved in development and immunity (Du et al., 2015). Unlike other MAMP-triggered 86 responses, recognition of elicitins results in a swift cell death response in Solanum and 87 Nicotiana species (Chaparro-Garcia et al., 2011; Du et al., 2015). 88

Independent of whether the PRR is an LRR-RLP or LRR-RLK, it has been shown that upon elicitor perception there is recruitment of members of the SERK family of LRR-RLKs by the activated PRR (Chinchilla et al., 2007; Albert et al., 2015; Postma et al., 2016). The most well-studied member of this family is BAK1 (BRI1-ASSOCIATED KINASE 1), also known as SERK3 (SOMATIC EMPBRYOGENESIS RECEPTOR KINASE 3). Similar to SOBIR1, two SERK3 alleles are recognized in *Solanum*; SERK3a and SERK3b, with presumably
overlapping functions as well (Peng and Kaloshian, 2014). *Nicotiana benthamiana (Nb)*SERK3 was identified as a component required for INF1-induced cell death (Chaparro-Garcia
et al., 2011). In line with this, ELR was found to associate with SERK3/BAK1, and this
association was found to be stabilized upon INF1 elicitation (Du et al., 2015). However,
whether other co-regulatory RLKs mediate response to INF1 is unknown.

In this study, we employed VIGS and genetic complementation to test whether SOBIR1 is 100 required for resistance to P. infestans and INF1-induced cell death. We found that SOBIR1 is 101 required for resistance of N. benthamiana to various P. infestans isolates and is indispensable 102 for INF1-triggered cell death. By co-immunoprecipitation assays we found that SOBIR1 103 constitutively associates with ELR in planta, in contrast to the enhanced interaction with 104 SERK3. Furthermore, we demonstrate that the kinase domain of SOBIR1 is required for cell 105 death induction by INF1, while it is not required for interaction with ELR. Overall we provide 106 evidence that ELR functions in a tri-partite complex with SOBIR1 and SERK3 in order to 107 108 trigger downstream signaling upon elicitin perception. Additionally, we show that SOBIR1 supports ELR function, which, along with potential other RLPs also requiring SOBIR1 for 109 110 their function, mediates resistance to P. infestans.

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112 **Results**

113 SOBIR1 contributes to defense against *Phytophthora infestans*

Since SOBIR1 is required for the function of several RLPs and for basal resistance against pathogens, including *P. parasitica* (Peng et al., 2015), we hypothesized that SOBIR1 could be involved in plant defense against *P. infestans*. To test this, we employed a tobacco rattle virus (TRV)-mediated virus-induced gene silencing (VIGS) approach. *N. benthamiana* plants were agro-inoculated with *A. tumefaciens* carrying the TRV-*NbSOBIR1/-like* construct that has previously been shown to knock-down the expression of *NbSOBIR1* and *NbSOBIR1-like* (Liebrand et al., 2013). TRV-*GUS* (serving as a negative control) and TRV-*PDS* (targeting phytoene desaturase to monitor the onset of silencing), were included in the experiment (Kumagai et al., 1995; Liu et al., 2002).

Quantitative RT-PCRs confirmed that NbSOBIR1 expression was significantly reduced in the 123 TRV-NbSOBIR1/-like-inoculated plants at three weeks post inoculation, while NbSOBIR1-like 124 was found, as expected, to be merely expressed (Fig. S1) (Liebrand et al., 2013). Leaves were 125 taken from the silenced plants and spot-inoculated with the fluorescent P. infestans isolate 126 127 88069td (expressing tdTomato red fluorescent protein). Lesion growth was visually examined five days after inoculation and we observed consistently larger lesions on the TRV-128 *NbSOBIR1/-like*-inoculated leaves as compared to plants inoculated with TRV-GUS (Figs. 1a, 129 S2). Molecular quantification of *P. infestans* biomass on inoculated *N. benthamiana* leaves by 130 measuring the abundance of *Pi* β -tubulin and *NbEF1a* as an internal standard, confirmed that a 131 much higher pathogen biomass was detected in TRV-*NbSOBIR1/-like*-inoculated leaves (Fig. 132 1b). Inoculation with two isolates currently causing late blight epidemics in the UK and the 133 Netherlands, UK3928A and Katshaar, respectively, resulted in a similar finding (Figs. 1b, 134 S2). This result indicates that the observed increase in plant susceptibility upon NbSOBIR1/-135 *like* silencing is not isolate-specific. Furthermore, these data show that SOBIR1 and SOBIR1-136 like clearly contribute to resistance against P. infestans. 137

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139 SOBIR1 is involved in INF1-induced cell death in *N. benthamiana*

INF1 and other elicitins are known to cause a *Nb*SERK3-dependent cell death in *N*. *benthamiana* (Kamoun et al., 1997; Chaparro-Garcia et al., 2011). To investigate whether
elicitin-triggered defense signaling in *N. benthamiana* also requires *NbSOBIR1*, we conducted

a VIGS experiment. For this, N. benthamiana plants were agro-inoculated with TRV-143 NbSOBIR1/-like. Agro-inoculation with TRV-NbSERK3a/b and TRV-GUS were included as 144 positive and negative controls, respectively, while TRV-PDS was included as a visual control 145 for the onset of silencing. At three weeks after the inoculation with TRV, plants were agro-146 infiltrated to express INF1 or empty vector (EV). After four days, the expected INF1-induced 147 148 cell-death was evident in the TRV-GUS-inoculated plants, but significantly decreased cell death was detected in leaves of TRV-NbSOBIR1/-like- and TRV-NbSERK3a/b-inoculated N. 149 benthamiana plants (Fig. 2a, b). Similar loss of INF1-triggered cell death was observed when 150 the same GUS- and NbSOBIR1/-like-silenced leaves were infiltrated with 1 µM of purified 151 152 INF1 protein (Fig. S3a, b). These data show that *NbSOBIR1* is required for the INF1-mediated cell death response in N. benthamiana. 153

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Synthetic SOBIR1 from S. microdontum can complement SOBIR1 from N. benthamiana 155 SOBIR1 is highly conserved in plants, but some degree of diversity occurs (Liebrand et al... 156 157 2014). We used PCR-based cloning to identify the homologs of SOBIR1 in S. microdontum, which is the source of ELR. We found that SmSOBIR1 and SmSOBIR1-like homologs share 158 97.9% and 99% amino acid similarity to StStSOBIR1 (Sotub06g029250.1.1) and StSOBIR1-159 like (Sotub03g023250.1.1), respectively (Fig. S4). To test whether SmSOBIR1 can 160 complement for INF1-induced cell death in NbSOBIR1-/like silenced N. benthamiana plants, 161 we generated a synthetic version that encodes the original wild-type (WT) SmSOBIR1 protein 162 sequence from S. microdontum (SmSOBIR1syn^{WT}), but which is not targeted by the TRV-163 NbSOBIR1/-like VIGS construct used in VIGS experiments with N. benthamiana (Liebrand et 164 al., 2013) (Figs S5a, S6). To verify that the synthetic gene drives expression of the expected 165 protein, we performed a western blot that confirmed the presence of Myc-tagged 166 SmSOBIR1syn^{WT} in plants silenced for NbSOBIR1/-like (Fig. S5b). Subsequently, we 167 performed a cell-death complementation experiment. For this, we silenced N. benthamiana 168

plants by agro-inoculation with TRV-NbSOBIR1/-like and took along TRV-GUS as a negative 169 and subsequently transiently co-expressed SmSOBIR1 (control) or the 170 control. SmSOBIR1syn^{WT} with INF1. We found that INF1-triggered cell death was not affected in the 171 TRV-GUS-inoculated plants, while in the TRV-NbSOBIR1/-like-inoculated plants INF1-172 triggered cell death was severely compromised. Interestingly, SmSOBIR1syn^{WT} co-expression 173 with INF1 restored the cell death response (Fig. 3). This observation indicates that 174 SmSOBIR1syn^{WT} complements for the loss of NbSOBIR1/-like in NbSOBIR1/-like-silenced 175 plants. 176

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178 SOBIR1 requires a functional kinase domain for mediating INF1-induced cell death

Next, we investigated whether kinase activity of SOBIR1 is required for cell death induction 179 by INF1. We cloned different versions of SmSOBIR1syn, in which the core catalytic aspartate 180 (D) of the conserved RD kinase motif is substituted by an asparagine (N) residue 181 (SmSOBIR1syn^{D473N}); a mutation that causes loss of kinase activity for all tested so-called 182 "RD" RLKs tested so far (Schwessinger et al., 2011; Liebrand et al., 2013). In addition, we 183 generated a SmSOBIR1syn mutant that completely lacks the kinase domain (with a deletion of 184 amino acids 333 to 625; SmSOBIR1syn^{Δ-kinase}) (Fig. S5a). Similar to SmSOBIR1syn^{WT}, by 185 using western blotting, we confirmed that the synthetic SmSOBIR1 mutant versions drive 186 production of the expected protein variants in plants silenced for NbSOBIR1/-like (Fig. S5b). 187 Next, we again proceeded to a cell-death complementation experiment by co-expressing the 188 kinase-inactive SmSOBIR1syn mutants with INF1 in NbSOBIR1/-like-silenced N. 189 benthamiana plants. We found that, unlike SmSOBIR1syn^{WT}, both SmSOBIR1syn^{D473N} and 190 SmSOBIR1syn^{Δ -kinase} failed to restore the INF1-triggered cell death response, indicating that 191 the kinase activity of SOBIR1 is required for INF1-triggered cell death (Fig. 3). 192

194 ELR associates with the co-regulatory RLK SOBIR1

In order to investigate whether ELR associates with SmSOBIR1/-like from S. microdontum, 195 we performed co-immunoprecipitation experiments. We generated constructs for transiently 196 expressing C-terminally Myc epitope-tagged SmSOBIR1 and SmSOBIR1-like and co-197 expressed them with ELR-eGFP in N. benthamiana. The RLP Cf-4 was included as a positive 198 199 control, and the RLK FLS2 and eGFP alone were used as negative controls (Liebrand et al., 2013). As shown in Fig. 4, immunopurification of ELR-eGFP, Cf-4-eGFP and FLS-2-GFP by 200 using GFP affinity beads and subsequent detection of co-purifying SmSOBIR1-myc and 201 202 SmSOBIR1-like-myc, indicates that, like Cf-4, ELR associates in planta with SmSOBIR1 and 203 SmSOBIR1-like, whereas no interaction of the two SOBIR1 homologs with FLS2 and eGFP was observed. 204

205

206 The SmSOBIR1 kinase domain is not required for interaction with ELR

To test whether the kinase domain of SOBIR1 is required for interaction with ELR, we coexpressed ELR with either an empty vector, SmSOBIR1syn^{WT} or the SmSOBIR1syn versions either lacking kinase activity or lacking the kinase domain (SmSOBIR1syn^{D473N} and SmSOBIR1syn^{Δ -kinase}, respectively), in *N. benthamiana*. Our results show that SOBIR1 kinase activity and the kinase domain itself are not required for its interaction with ELR, similar to what has been shown for the interaction between Cf-4 and SOBIR1, as in all cases the interaction with ELR remains intact (Fig. S7a) (Bi et al., 2016).

In addition to this, we noted an ELR-stabilizing effect when SOBIR1 is co-expressed with this RLP. To confirm this observation, we performed a similar experiment as above. For this, ELR and the different *Sm*SOBIR1 variants were transiently co-expressed in *N. benthamiana*, total protein was extracted at two days after agro-infiltration, and ELR accumulation was determined by western blotting and signal intensity quantification. In three independent experiments, we found that co-expression of the *Sm*SOBIR1 variants, all accumulating at

similar levels and interacting with ELR (Figs. S5b, S7a), led to an increase in the amounts of 220 ELR accumulating, as compared to co-expression with the empty vector (Fig. S8). 221 222 SmSOBIR1 co-expression with ELR was found to result in an up to 13-fold increase in ELR accumulation. Moreover, both the kinase-active and kinase-inactive versions of SOBIR1, in 223 addition to SmSOBIR1syn completely lacking the kinase domain, have a stabilizing effect on 224 225 ELR, though a bit lower as compared to SmSOBIR1syn. This indicates that the stabilization effect is likely not directly related to kinase activity of SOBIR1, but is rather based on ELR 226 interaction with SOBIR1. 227

Summarizing, these results show that the interaction between ELR and SOBIR1 is stabilizing the complex. Moreover, ELR association with SOBIR1 likely requires the extracellularjuxtamembrane, transmembrane and intracellular-juxtamembrane domains of ELR, as proposed for other RLPs (Fig. S8b) (Gust and Felix, 2014; Bi et al., 2016).

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ELR functions in a tri-partite complex with SOBIR1 and SERK3

It was previously shown that ELR associates with SERK3 and that this association is 234 235 specifically enhanced by INF1 elicitation. In order to investigate whether the observed 236 constitutive interaction of ELR with SOBIR1 is affected by INF1, we co-expressed ELR with 237 SOBIR1 and SERK3 in N. benthamiana. Two days after agroinfiltration, we infiltrated purified INF1 protein or the buffer in which INF1 was dissolved, and checked whether ELR 238 interacted with SOBIR1 and SERK3 through co-immunoprecipitation and western blotting 239 (Fig. 5). We found that, upon 15 min elicitation with 1 µM INF1 protein (see Fig. S3b), ELR 240 interaction with SERK3 is enhanced, as expected (Fig. 5) (Du et al., 2015). On the other hand, 241 ELR interaction with SOBIR1 was not affected by INF1 elicitation, indicating that the 242 interaction is constitutive and remains intact. These data show that ELR is present as a 243 constitutive two-component RLK with SOBIR1, and forms a tri-partite ELR-SOBIR1-SERK3 244 complex upon INF1 perception. 245

246 **Discussion**

In this study we show that basal resistance of *N. benthamiana* to *P. infestans* requires *Nb*SOBIR1. This is evident since *N. benthamiana* plants silenced for *NbSOBIR1* showed larger lesions and increased *P. infestans* biomass, for a range of different isolates. Our results are in line with the recent finding that resistance against *P. parasitica* of tomato requires SOBIR1 (Peng et al., 2015). Overall, our data support the importance of SOBIR1 in basal defense against pathogens, likely by supporting ELR function and potentially that of other LRR-RLPs as well (Liebrand et al., 2014).

ELR carries a short cytoplasmic tail without any obvious signaling domain and as such, it was 254 hypothesized that additional partner proteins are mediating signal transduction. Indeed, 255 SERK3/BAK1, a common interactor of LRR-containing PRRs, has been found to associate 256 with ELR (Du et al., 2015). Recently, the LRR-RLK SOBIR1 has been shown to 257 constitutively associate with a multitude of RLPs (Gust and Felix, 2014; Liebrand et al., 2014; 258 Zhang et al., 2014; Albert et al., 2015; Bi et al., 2016). In this study we provide evidence that 259 260 SOBIR1 is also required as a co-regulatory RLK for the response to the elicitin INF1. By gene 261 silencing we have demonstrated that the cell death response to INF1 in N. benthamiana 262 requires SOBIR1, similar to what has been described for ParA1 (Peng et al., 2015). In addition, by employing genetic complementation assays using synthetic SmSOBIR1, we found 263 that the INF1-triggered cell death response requires an active SmSOBIR1 kinase domain. This 264 indicates that the SOBIR1 kinase domain actively takes part in downstream defense signaling 265 initiated by INF1, which is in line with previous studies on ParA1, and Avr4 in Cf-4-266 expressing plants (Liebrand et al., 2013; Peng et al., 2015). However, the PRRs responsible 267 for elicitin-triggered responses in tomato cv. Summer Sweet and N. benthamiana have not 268 been cloned. Tomato is known to have a close ELR homolog, however, no obvious homologs 269 are found in N. benthamiana. Therefore, it remains unknown whether those responses are due 270 271 to the presence of a putative functional ELR orthologue (Peng et al., 2015).

Biochemical evidence from this work supports the observation that the INF1 receptor 272 physically associates with SOBIR1. Using co-immunoprecipitation, we found that ELR 273 associates in planta with both SmSOBIR1 and its close homolog SmSOBIR1-like and a 274 stabilized complex is formed. This association is constitutive, since it does not require INF1 275 276 and remains unaltered upon INF1 elicitation. ELR thus behaves similar to other RLPs 277 involved in immunity, such as Ve1, Cf-2, Cf-4, Cf-9, RLP23, RLP30 and RLP42 (Liebrand et al., 2013; Zhang et al., 2013; Bi et al., 2014; Zhang et al., 2014; Albert et al., 2015). 278 Moreover, we have consistently observed a stabilization effect of ELR when SOBIR1 was co-279 expressed. Although a different setup was used, this finding is complementary to the 280 281 observation of reduced Cf-4 and Ve1 protein levels when SOBIR1 was silenced (Liebrand et 282 al., 2013). For Cf-4 it was concluded that SOBIR1 potentially acts as a scaffold protein (Bi et al., 2016), in addition to being required for downstream signaling (Liebrand et al., 2013). For 283 both ELR and Cf-4, kinase activity of SOBIR1 is not required for stabilization, which is in 284 agreement with the hypothesis that the GxxxG dimerization motif present in the trans-285 membrane domains, in addition to the juxta membrane domains of ELR and SOBIR1, are 286 involved in their interaction as was proposed for many RLP-type LRR receptors (Gust and 287 Felix, 2014; Bi et al., 2016). 288

ELR has been shown to localize at the plasma membrane (Du et al., 2015). Therefore, it can 289 be assumed that interaction with SOBIR1/SERK3 is also occurring at this location, similar to 290 what was observed for Cf-4 (Postma et al., 2016). C. fulvum Avr4-induced endocytosis of 291 SOBIR1 in Cf-4-expressing plants has been recently shown in N. benthamiana (Postma et al., 292 2016). Therefore, it is likely that INF1 elicitation also causes endocytosis of the ELR-SOBIR1 293 294 complex. In agreement with this hypothesis, the elicitins cryptogein (from *P. cryptogea*) and ParA1 (from *P. parasitica*), have been shown to induce clathrin-mediated endocytosis of 295 SOBIR1 (Leborgne-Castel et al., 2008; Peng et al., 2015). Future studies should address 296 whether this endocytosis is SERK3-dependent as well (Postma et al., 2016). 297

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Studies on elicitin recognition in N. benthamiana have revealed the E3 ligase CYS, MET, 298 PRO, AND GLY PROTEIN 1 (CMPG1) to be an important component for the cell death 299 response triggered by INF1 (Bos et al., 2010). CMPG1 acts as a hub for signaling downstream 300 of both MAMP and effector perception, as it is required for the Cf-, Pto- and CELLULOSE-301 302 BINDING ELICITOR LECTIN (CBEL)-mediated cell death response as well (Gilroy et al., 303 2011). Despite the lack of molecular evidence showing interaction of CMPG1 with PRRs, we anticipate that the E3 ligase activity of CMPG1 modulates the first steps downstream of 304 effector perception, possibly through promoting ubiquitination (Gilroy et al., 2011). SOBIR1 305 306 seems to be heavily modified as it migrates as a smear on SDS gels as revealed by western blots (i.e. as shown in SOBIR1 bands in Figs. 4, 5), however the type of modifications and 307 their biological relevance have not been reported yet. P. infestans is known to secrete AVR3a, 308 an RXLR effector which suppresses INF1-triggered cell death. AVR3a is suppressing 309 MAMP-triggered responses in at least two ways; either by binding to CMPG1 or by blocking 310 receptor endocytosis via association with DYNAMIN-RELATED PROTEIN 2 (DRP2), a 311 protein involved in cellular trafficking (Bos et al., 2010; Chaparro-Garcia et al., 2015). With 312 the discovery that ELR functions in a complex with SERK3 and SOBIR1, it is now 313 appropriate to test whether CMPG1 is involved in trafficking of activated ELR-SOBIR1 and 314 whether AVR3a is interfering in this process. 315

Recently, the cloning of ELR gave insight into the molecular mechanisms involved in 316 apoplastic elicitin perception (Du et al., 2015; Derevnina et al., 2016). Understanding how 317 RLPs such as ELR function is a major step towards their deployment as resistance genes in 318 plant breeding. With our work we show that elicitin recognition and basal defense in 319 320 Solanaceae requires SOBIR1 which supports ELR function and immunity to P. infestans. ELR functions thus similar to Cf-4- and Cf-9- mediated recognition of Avr4 and Avr9. 321 respectively, and the downstream responses also appear to be similar (Liebrand et al., 2013; 322 323 Postma et al., 2016). We propose that, the ELR-SOBIR1 complex functions as a two component RLK that recruits SERK3 in an INF1-dependent manner. This tri-partite complex formation is reminiscent of the well-characterized RLKs FLS2 and EFR which, upon elicitation with their respective ligands flg22 and elf18, are associating with SERK3/BAK1 (Zipfel et al., 2006; Chinchilla et al., 2007; Boller and Felix, 2009). Since PRRs contribute to quantitative resistance and thus offer a lower selection pressure, stacking of ELR with other PRRs such as RLP23 could lead to a more durable resistance against the devastating pathogen *P. infestans* (Albert et al., 2015; Du et al., 2015).

331

332 Materials and methods

333 Plant material and growth conditions

Nicotiana benthamiana plants were grown from seeds and maintained in climate controlled greenhouse compartments at 22/18 °C and 16/8 h light day/night regime at 70% relative humidity. All protein expression and cell death assays were performed under these conditions.

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338 **Production and purification of INF1**

INF1 was produced from a stationary culture of *P. infestans* strain IPO-C, as previously 339 described (Chaparro-Garcia et al., 2011), with some minor modifications, Briefly, P. infestans 340 was grown for 4-5 weeks in liquid Plich medium. The mycelium was removed by passing 341 through filter paper and the culture medium was snap-frozen and freeze-dried. The resulting 342 powder was dissolved in 100 ml of 10 mM Tris-HCl, 10 mM NaCl (pH 7.4) buffer. The 343 solution was then dialyzed overnight (3.5 kDa cut off, Spectrum RC dialysis tubing), against 344 345 the same buffer, at 4°C. The resulting INF1-containing solution was loaded onto a 10 mM 346 Tris-HCl (pH 7.4)-equilibrated Q Sepharose Fast-Flow column (GE Healthcare). Column was 347 washed with three column volumes with the same equilibration buffer. Next, the column was eluted with a linear gradient of 0 - 500 mM NaCl in 10 mM Tris-HCl (pH 7.4) and fractions
of 10 ml were collected. The presence of INF1 in the different fractions was assayed by SDSPAGE, followed by CBB or silver staining. Protein concentration was estimated by BCA
assay (Thermo-Fisher Scientific). Purified INF1 was infiltrated in *N. benthamiana* leaves to
confirm cell-death inducing activity. Avr4 protein from *Cladosporium fulvum* has been
produced previously in *Pichia pastoris* (van den Burg et al., 2001).

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355 Binary vectors for Agrobacterium tumefaciens-mediated transient transformation

Construction of pBin-KS-p35s::ELR-eGFP, pBin-KS-p35s::Cf-4-eGFP, pCAMBIA2300-356 pAtFLS2::SIFLS2-GFP and pTRV1 has been described (Liu et al., 2002; Liebrand et al., 357 2012; Liebrand et al., 2013; Du et al., 2015). For cloning of novel constructs, target gene 358 sequences were amplified from cDNA using Phusion proofreading polymerase (Thermo 359 Fisher Scientific), employing primers mentioned in Supplementary Table 1(Table S1). The 360 SmSOBIR1 and SmSOBIR1-like genes, as well as a PDS VIGS fragment (targeting phytoene 361 desaturase and used as a control for gene silencing) were amplified from S. microdontum 362 genotype 360-1 (source of ELR) cDNA. 363

A synthetic (syn) construct of *Sm*SOBIR1 carrying a D473N mutation (*Sm*SOBIR1syn^{D473N}) and synonymous nucleotide changes to enable expression in *TRV-NbSOBIR1/-like* VIGSed *N. benthamiana* was synthesized (Genscript) (Figs. S4a, S5). Using overlap extension PCR, the wild type version (*Sm*SOBIR1syn^{WT}) was reconstituted as described in Liebrand et al. (2013) (see Table S2, Fig. S4). A version of *Sm*SOBIR1syn (nucleotides 1 - 996) lacking the kinase domain (*Sm*SOBIR1syn^{Δ -kinase}) was amplified using *Sm*SOBIR1syn^{D473N} as a template.

A GUS VIGS fragment was amplified from the commercial vector pENTR-GUS (Invitrogen).
 SmSOBIR1, SmSOBIR1-like, SmSOBIR1syn as well as the VIGS fragments for GUS and
 SmPDS were cloned in pENTR/D-TOPO vector (Invitrogen). The inserts of all entry clones

were checked by sequencing and transferred to destination vectors by means of an LR 373 reaction using LR-Clonase II enzyme mix (Invitrogen), resulting in the following constructs: 374 pGWB20-p35s::SmSOBIR1-myc, pGWB20-p35s::SmSOBIR1-like-myc, 375 pGWB20p35s::SmSOBIR1svn^{WT}-mvc, pGWB20-p35s::SmSOBIR1svn^{D473N}-mvc, pGWB20-376 p35s::SmSOBIR1syn^{4-kinase}-myc (-myc refers to C-terminal 10x Myc), pTRV2-GUS and 377 pTRV2-PDS (Table S2). 378

- 379
- 380 Co-immunopurification and immunoblotting

The proteins under study were transiently expressed in young, fully-expanded leaves of 3 381 week-old N. benthamiana plants using Agrobacterium-mediated transient transformation 382 383 (agro-infiltration). At 2 d post agro-infiltration, leaves were collected, snap-frozen in liquid nitrogen and ground to a fine powder. Protein extraction was performed in modified RIPA 384 buffer, containing IGEPAL CA-630 as a sole detergent and supplemented with EDTA-free 385 protease inhibitor cocktail (Roche), as described previously (Liebrand et al., 2012; Liebrand et 386 al., 2013). Briefly, modified RIPA buffer was added at a ratio of 2 ml/g of frozen ground plant 387 material and mixed. The mixture was incubated at room temperature with occasional mixing. 388 until all frozen material was thawed. Then, 2 ml of protein extract was centrifuged for 30 min 389 390 at $13000 \times g$ to pellet plant debris. Clarified extracts, from which input samples were collected, were incubated with 15 µl RIPA-equilibrated GFP-Trap MA beads (50 % slurry, 391 Chromotek) for 1 h at 4°C. Beads were then washed 5 times with RIPA, after which they were 392 incubated at 95°C for 5 min in 100 µl 2x Laemli SDS buffer for 10 min, while 150 µl of input 393 394 sample was mixed with 50 µl 4x Laemli SDS buffer and also incubated at at 95°C for 5 min. In order to dissolve protein aggregates prior to SDS-PAGE, samples were mixed 1:1 with 8 M 395 urea and incubated at room temperature for 10-20 min. The samples were then again 396 397 incubated at 95°C for 5 min, centrifuged at 13000 \times g and proteins present in 35 μ l of the supernatant were separated by SDS-PAGE using TGX 4-20% gradient gels (BioRad). 398

Subsequently, proteins were transferred to a PVDF membrane using a MiniProtean wet 399 transfer system (BioRad), following standard procedures. Blots to be incubated with anti-GFP 400 antibodies were blocked in Tris-buffered saline with 0.05% Tween20 (TBS-T), containing 3% 401 bovine serum albumin (BSA), while blots to be incubated with α Myc and α HA were blocked 402 with TBS-T containing 5% skimmed milk. The antibodies were added in TBS-T solution at 403 404 the following dilutions: aGFP-HRP 1.5:5000 (Miltenyi Biotech), aHA-HRP 1.5:5000 (Miltenvi Biotech), αMyc-HRP 2.5:5000 (Santa Cruz-biotech). For chemo-luminescent signal 405 development, SuperSignal West-Femto substrate (ThermoFisher Scientific) was used. 406 Imaging of western blots was done using a G-BOX system (Syngene). 407

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409 VIGS assays

VIGS experiments were performed using wildtype *N. benthamiana* plants, as described
(Liebrand et al., 2013). Briefly, 2 week-old *N. benthamiana* seedlings were inoculated by
agro-infiltration (agro-inoculation) with 1:1 mixtures of pTRV1 (Liu et al., 2002) in
combination with pTRV2::*NbSOBIR1/-like* (Liebrand et al., 2013), pTRV2::*NbSERK3a/b*(Heese et al., 2007; Chaparro-Garcia et al., 2011), pTRV2::*GUS*, or pTRV2::*PDS*, at a final
OD₆₀₀ of 0.5 using a needleless syringe.

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417 Agrobacterium transient cell death assays

For INF1 cell death induction assays, young, fully expanded leaves of 4-5 week-old TRV-GUS- or TRV-*NbSOBIR1/-like*-inoculated *N. benthamiana* plants (i.e. 2-3 weeks after VIGS) were used. VIGSed leaves were agroinfiltrated with pCB302-3-*p35s-INF1* and pCB302-3p35s-EV (empty vector) (Du et al., 2015), at an OD₆₀₀ of 0.5. Leaves were visually examined for INF1-triggered cell-death at 4 d post-agroinfiltration. For genetic complementation assays using *Sm*SOBIR1syn variants, VIGSed plants were co-infiltrated with 1:1 mixtures of

Agrobacterium carrying pCB302-3-p35s-INF1, in combination with one of the following 424 pGWB20-p35s::SmSOBIR1svn^{WT}-mvc, pGWB20-p35s::SmSOBIR1-myc, 425 constructs: pGWB20-p35s::SmSOBIR1syn^{D473N}-myc or pGWB20-p35s::SmSOBIR1syn^{Δ -kinase}-myc, at a 426 final OD₆₀₀ of 0.5. Infiltrated leaves were observed for INF1-induced cell death at 5 d post-427 infiltration. In all cases, the percentage of cell death was quantified using scores of 0%, 25%, 428 429 50%, 75% and 100%, based on visual observation of the infiltrated area showing cell death when compared to the total area, as described previously (Du et al., 2014). 430

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432 Inoculations with *P. infestans*

Detached leaf assays with P. infestans on VIGSed N. benthamiana were performed as 433 described (Vleeshouwers et al., 1999). Briefly, leaves were placed with their petioles into tap 434 water-saturated floral foam (Oasis) with the abaxial side facing upwards. P. infestans 435 zoospore suspensions were prepared as described previously (Vleeshouwers et al., 1999) and 436 inoculations using these spores were performed twice per leaf, by pipetting 10 µl droplets of a 437 suspension of 1×10^5 zoospores/ml (in tap water) onto the abaxial side. At 5 dpi, pictures 438 were taken under normal or long wave UV-light. UV photos of the fluorescent isolate 88069td 439 (expressing tdTomato red fluorescent protein, a fluorescent protein consisting of a tandem 440 441 dimer of a dsRed monomeric mutant) (Whisson et al., 2007) were taken using the PathoScreen system (PhenoVation). 442

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444 RNA extraction and quantitative PCR analyses

RNA was isolated from TRV-inoculated *N. benthamiana* plants at 3 week post inoculation.
RNA extraction was performed using the RNAplant easy kit (Qiagen) and cDNA was
synthesized on 1 μg of total RNA using Superscript II (Invitrogen), according to
manufacturer's instructions. *NbSOBIR1* expression was quantified by RT-qPCR by using the

expression of *N. benthamiana ELONGATION FACTOR 1a* (*EF1a*) as a reference (Nicot et al., 2005). Quantitative RT-PCRs were performed on a CFX96 Real-Time System (BioRad). Gene expression data were normalized to the expression of *NbEF1a* and the $2^{-\Delta\Delta Ct}$ method was used for data analysis (Livak and Schmittgen, 2001). See Table S1 for the primers that were used.

P. infestans biomass quantifications on infected leaves were performed using qPCR. Leaf discs (30 mm in diameter) were excised from the inoculation spots of each leaf at 5 dpi. Per treatment, leaf material was pooled, snap frozen and ground to a fine powder, independently for each biological repeat. Genomic DNA was extracted using the DNeasy Plant kit (Qiagen) and *P. infestans* biomass was quantified in a similar way as for gene expression, by comparing the relative abundance of *P. infestans* β -tubulin to that of *N. benthamiana EF1* α (see Table S1).

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470 Author contributions

E.D., M.H.A.J., S.K. and V.G.A.A.V. planned and designed the research. E.D. and D.W.,
performed experiments and analyzed the data. E.D., V.G.A.A.V., R.G.F.V. and M.H.A.J.,
wrote the manuscript.

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604 Figure legends

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Fig. 1. SOBIR1 is required for defense responses against *Phytophthora infestans*. Four 605 606 week-old Nicotiana benthamiana plants, inoculated with recombinant TRV carrying either an 607 NbSOBIR1/-like or GUS insert, were inoculated with zoospores obtained from the three P. infestans isolates Katshaar, UK3928A, or 88069td. (a) Fluorescent/visible overlay image of 608 mycelial growth of isolate 88069td expressing tdTomato red fluorescent protein on TRV-609 inoculated N. benthamiana at 5 dpi. (b) Relative biomass quantification of P. infestans 610 isolates on TRV-inoculated N. benthamiana leaves at 5 dpi. Quantification was performed by 611 RT-qPCR and by comparing the amplification of P. infestans β -tubulin to N. benthamiana 612 $EF1\alpha$ that was used as an internal standard. Error bars indicate the standard deviation of two 613 technical replicates of one representative experiment. The experiment was repeated three 614 times and gave similar results. 615

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Fig. 2. SOBIR1 is required for the cell death response triggered by INF1 elicitin in *Nicotiana benthamiana*. Four week-old *N. benthamiana* plants, inoculated with TRV-*NbSOBIR1/-like*, TRV-*GUS* (negative control) or TRV-*NbSERK3a/b* (positive control), were agro-infiltrated with INF1 or empty vector (EV) at the onset of *PDS* silencing (not shown). (a) Representative pictures of treated leaves at 4 dpi. (b) Cell death quantification of treated leaves at 4 dpi. Error bars indicate the standard error of three biological repeats (n = 64). Asterisks indicate significance at p<0.01 (Student's t-test).

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Fig. 3. Synthetic versions of *SmSOBIR1* complement the loss of INF1-triggered cell death in TRV-*NbSOBIR1/-like*-inoculated *Nicotiana benthamiana*. *Sm*SOBIR1 wild type (WT) (control), *Sm*SOBIR1syn^{WT}, *Sm*SOBIR1syn^{D473N} or *Sm*SOBIR1syn^{Δ -kinase}, were transiently co-expressed in TRV-*GUS* or TRV-*NbSOBIR1/-like*-inoculated plants, together with INF1. Cell death was scored at 5 dpi and representative pictures are shown. Results are an average of three independent biological repeats. The asterisk indicates significance at p<0.05 (One-way ANOVA, LSD test).

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Fig. 4. ELR forms a complex with SmSOBIR1 and SmSOBIR1-like. SmSOBIR1-myc and 633 SmSOBIR1-like-myc were transiently co-expressed in Nicotiana benthamiana leaves, 634 together with ELR-eGFP, Cf-4-eGFP (positive control), FLS2-GFP or eGFP (negative 635 controls), as indicated. Total protein was extracted and was subjected to immunoprecipitation 636 using GFP TrapMA beads to capture ELR, Cf-4 and FLS2. The immunopurified proteins 637 were detected with anti-GFP, while the interaction with SmSOBIR1-Myc or SmSOBIR1-like-638 639 Myc was assessed with anti-Myc. Ponceau S staining of RuBisCO indicates equal protein loading. This figure is representative for three biological repeats. 640

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642 Fig. 5. ELR forms a constitutive complex with SOBIR1, while it interacts with SERK3a

in an INF1-inducible manner. SmSOBIR1-myc and StSERK3a-HA were transiently co-643 expressed in Nicotiana benthamiana leaves, together with ELR-eGFP, Cf-4-eGFP or eGFP, 644 as indicated. Elicitation was performed at three days post infiltration with 1 µM purified INF1 645 protein (for ELR and eGFP) or 1 µM purified Avr4 (for Cf-4). Total protein was extracted and 646 was subjected to immunoprecipitation using GFP TrapMA beads to capture ELR, Cf-4 and 647 eGFP respectively. The immunopurified proteins were detected with anti-GFP, while the 648 interaction with SmSOBIR1-Myc or StSERK3a-HA, was assessed with anti-Myc or anti-HA, 649 respectively. Ponceau S staining of RuBisCO indicates equal protein loading. This figure is 650 651 representative for three biological repeats.

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653 Supplementary figure legends

Fig. S1. NbSOBIR1 is successfully silenced in TRV-NbSOBIR1/-like-inoculated Nicotiana 654 benthamiana. N. benthamiana plants were inoculated with TRV-GUS or TRV-NbSOBIR1/-655 *like* and the relative expression of *NbSOBIR1* was determined at three weeks post inoculation. 656 Quantitative RT-PCR data for *NbSOBIR1* were normalized to the expression of the *Nb*EF1 α 657 gene and a relative quantification was performed. Error bars indicate the standard deviation of 658 Molecular Plant-Microbe Interactions "First Look" paper • http://dx.doi.org/10.1094/MPMI-09-17-0217-R • posted 02/16/2018 This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ. three biological repeats. Asterisks indicate significance at p<0.01 (One-way ANOVA, LSD 659 test). 660 661 Fig. S2. SOBIR1 is required for resistance of Nicotiana benthamiana to Phytophthora 662 663 infestans. Four week-old Nicotiana benthamiana plants, inoculated with TRV-GUS or TRV-664 *NbSOBIR1/-like* (Liebrand et al., 2013), were inoculated with zoospores obtained from three different P. infestans isolates (Katshaar, UK3928A and 88069td). Images of representative 665 leaves were taken at 5 days post inoculation (dpi) under normal or UV light. 666 667 Fig. S3. SOBIR1 is required for the cell death response triggered by INF1 protein. Four 668 week-old Nicotiana benthamiana plants, inoculated with TRV-GUS or TRV-NbSOBIR1/-like 669

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(Liebrand et al., 2013), were infiltrated with 1 μ M INF1 protein. (a) Representative pictures from the INF1 protein infiltration taken at 4 days post infiltration. (b) SDS-PAGE and silver staining of the INF1 protein purified from Phytophthora infestans culture filtrate using anion exchange.

Fig. S4. Alignments of SmSOBIR1 and SmSOBIR1-like with StSOBIR1, StSOBIR1-like, 675 S/SOBIR1 and S/SOBIR1-like. Amino acid sequence alignment of SOBIR1 was created 676 using COBALT, and BOXSHADE was used to visualize conservation. Residues highlighted 677

in black are identical in >50% of the six sequences, while residues highlighted in grey are
residues with similar properties. The RD motif is highlighted by a red rectangle.

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Fig. S5. Designing of synthetic versions of SmSOBIR1 (SmSOBIR1syn) and in planta 681 protein detection. (a) Schematic overview of the synthetic constructs of SmSOBIR1 used in 682 this study. In red is the SmSOBIR1 sequence that is targeted by the TRV-NbSOBIR1/-like 683 construct. Protein (top) and gene models are scaled to represent the actual domain positions. 684 Alignments show the nucleotide differences between SmSOBIR1 and SmSOBIR1syn^{WT} 685 sequences, while the translated amino acid sequence remains identical. (b) Western blot 686 showing the detection of SmSOBIR1syn variants in TRV-GUS or TRV-NbSOBIR1/-like-687 688 inoculated plants. Wild type SmSOBIR1 (WT) and empty vector (EV) were used as controls.

689

Fig. S6. DNA alignment of *SmSOBIR1*, *SmSOBIR1syn^{WT}* and *SmSOBIR1-like* with the TRV-*NbSOBIR1/-like* VIGS fragments. DNA sequence alignment of SOBIR1 was created using MUSCLE, and BOXSHADE was used to visualize conservation. Nucleotides highlighted in black are identical in all sequences while residues highlighted in grey are residues identical in >50% of the sequences. The positions of *SmSOBIR1* that are targeted by the TRV-*NbSOBIR1/-like* construct are highlighted with a green or blue line, respectively, above the sequence.

697

Fig. S7. Kinase activity of SOBIR1 and its kinase domain are not required for its association with ELR. (a) ELR-eGFP was transiently co-expressed in *Nicotiana benthamiana* in combination with empty vector (EV) or *Sm*SOBIR1syn variants (*Sm*SOBIR1syn^{WT}, *Sm*SOBIR1syn^{D473N} or *Sm*SOBIR1syn^{Δ -kinase}). Total protein was extracted

and was subjected to immunoprecipitation using GFP TrapMA beads to capture ELR. The 702 immunopurified proteins were detected with anti-GFP, while the interaction with 703 SmSOBIR1syn variants was assessed with anti-Myc. Ponceau S staining of RuBisCO 704 indicates equal protein loading. This figure is representative for three biological repeats. (b) 705 Schematic representation of the putative interaction site between the extracellular-706 707 juxtamembrane (eJM), transmembrane (TM) and intracellular-juxtamembrane (iJM) domains of ELR and SmSOBIR1 based on Gust and Felix (2014) and Bi et al. (2016). Charged amino 708 acids are indicated by highlighting and a symbol according to charge (+ for positive or - for 709 negative charge). The GxxxG motif in the TM is also highlighted. 710

711

712 Fig. S8. SmSOBIR1 stabilizes ELR independently of its kinase activity. ELR-eGFP was transiently co-expressed in Nicotiana benthamiana with empty vector (EV), SmSOBIR1, 713 SmSOBIR1synWT, SmSOBIR1syn^{D473N} or SmSOBIR1syn^{Δ-kinase}. Total protein was extracted 714 and western blotting was performed with anti-GFP to visualize ELR accumulation. 715 Quantification of ELR band intensity was performed with imageJ. Relative band intensity 716 results were obtained by comparing the signal intensity of the SOBIR1 and SERK3a 717 agroinfiltrations to the EV agroinfiltration of each experiment. Ponceau S staining of 718 RuBisCO indicates equal protein loading. Data from three independent experiments are 719 720 shown.

721

722 Table S1. Constructs used in this study.

723

724 Table S2. Primers used in this study.



Fig. 1. SOBIR1 is required for defense responses against *Phytophthora infestans*. Four week-old *Nicotiana benthamiana* plants, inoculated with recombinant TRV carrying either an *NbSOBIR1/-like* or *GUS* insert, were inoculated with zoospores obtained from the three *P. infestans* isolates Katshaar, UK3928A, or 88069td. (a) Fluorescent/visible overlay image of mycelial growth of isolate 88069td expressing tdTomato red fluorescent protein on TRV-inoculated *N. benthamiana* at 5 dpi. (b) Relative biomass quantification of *P. infestans* isolates on TRV-inoculated *N. benthamiana* leaves at 5 dpi. Quantification was performed by RT-qPCR and by comparing the amplification of *P. infestans* β-tubulin to *N. benthamiana* EF1a that was used as an internal standard. Error bars indicate the standard deviation of two technical replicates of one representative experiment. The experiment was repeated three times and gave similar results.

83x40mm (300 x 300 DPI)



Fig. 2. SOBIR1 is required for the cell death response triggered by INF1 elicitin in *Nicotiana benthamiana*.
 Four week-old *N. benthamiana* plants, inoculated with TRV-*NbSOBIR1/-like*, TRV-*GUS* (negative control) or TRV-*NbSERK3a/b* (positive control), were agro-infiltrated with INF1 or empty vector (EV) at the onset of *PDS* silencing (not shown). (a) Representative pictures of treated leaves at 4 dpi. (b) Cell death quantification of treated leaves at 4 dpi. Error bars indicate the standard error of three biological repeats (n = 64). Asterisks indicate significance at p<0.01 (Student's t-test).

117x173mm (300 x 300 DPI)



Fig. 3. Synthetic versions of *Sm*SOBIR1 complement the loss of INF1-triggered cell death in TRV-*NbSOBIR1/-like*-inoculated *Nicotiana benthamiana*. *Sm*SOBIR1 wild type (WT) (control), *Sm*SOBIR1syn^{WT}, *Sm*SOBIR1syn^{D473N} or *Sm*SOBIR1syn^{Δ -kinase}, were transiently co-expressed in TRV-*GUS* or TRV-*NbSOBIR1/like*-inoculated plants, together with INF1. Cell death was scored at 5 dpi and representative pictures are shown. Results are an average of three independent biological repeats. The asterisk indicates significance at p<0.05 (One-way ANOVA, LSD test).

92x49mm (300 x 300 DPI)



Fig. 4. ELR forms a complex with *Sm*SOBIR1 and *Sm*SOBIR1-like. *Sm*SOBIR1-myc and *Sm*SOBIR1-like-myc were transiently co-expressed in *Nicotiana benthamiana* leaves, together with ELR-eGFP, Cf-4-eGFP (positive control), FLS2-GFP or eGFP (negative controls), as indicated. Total protein was extracted and was subjected to immunoprecipitation using GFP_TrapMA beads to capture ELR, Cf-4 and FLS2. The immunopurified proteins were detected with anti-GFP, while the interaction with *Sm*SOBIR1-like.Myc or *Sm*SOBIR1-like.Myc was assessed with anti-Myc. Ponceau S staining of RuBisCO indicates equal protein loading. This figure is representative for three biological repeats.

105x125mm (300 x 300 DPI)



Fig. 5. ELR forms a constitutive complex with SOBIR1, while it interacts with SERK3a in an INF1-inducible manner. SmSOBIR1-myc and StSERK3a-HA were transiently co-expressed in Nicotiana benthamiana leaves, together with ELR-eGFP, Cf-4-eGFP or eGFP, as indicated. Elicitation was performed at three days post infiltration with 1 μM purified INF1 protein (for ELR and eGFP) or 1 μM purified Avr4 (for Cf-4). Total protein was extracted and was subjected to immunoprecipitation using GFP_TrapMA beads to capture ELR, Cf-4 and eGFP respectively. The immunopurified proteins were detected with anti-GFP, while the interaction with SmSOBIR1-Myc or StSERK3a-HA, was assessed with anti-Myc or anti-HA, respectively. Ponceau S staining of RuBisCO indicates equal protein loading. This figure is representative for three biological repeats.

172x361mm (300 x 300 DPI)



Fig. S1. *NbSOBIR1* is successfully silenced in TRV-*NbSOBIR1/-like*-inoculated *Nicotiana benthamiana*. *N. benthamiana* plants were inoculated with TRV-*GUS* or TRV-*NbSOBIR1/-like* and the relative expression of *NbSOBIR1* was determined at three weeks post inoculation. Quantitative RT-PCR data for *NbSOBIR1* were normalized to the expression of the *NbEF1a* gene and a relative quantification was performed. Error bars indicate the standard deviation of three biological repeats. Asterisks indicate significance at p<0.01 (One-way ANOVA, LSD test).

108x69mm (300 x 300 DPI)



Fig. S2. SOBIR1 is required for resistance of *Nicotiana benthamiana* to *Phytophthora infestans*. Four weekold *Nicotiana benthamiana* plants, inoculated with TRV-*GUS* or TRV-*NbSOBIR1/-like* (Liebrand et al., 2013), were inoculated with zoospores obtained from three different *P. infestans* isolates (Katshaar, UK3928A and 88069td). Images of representative leaves were taken at 5 days post inoculation (dpi) under normal or UV light.

138x155mm (300 x 300 DPI)



Fig. S3. SOBIR1 is required for the cell death response triggered by INF1 protein. Four week-old *Nicotiana* benthamiana plants, inoculated with TRV-*GUS* or TRV-*NbSOBIR1/-like* (Liebrand et al., 2013), were infiltrated with 1 μM INF1 protein. (a) Representative pictures from the INF1 protein infiltration taken at 4 days post infiltration. (b) SDS-PAGE and silver staining of the INF1 protein purified from *Phytophthora* infestans culture filtrate using anion exchange.

62x36mm (300 x 300 DPI)

ffer.	SmSOBIR1_MCD360 StSOBIR1 SISOBIR1 SmSOBIR1-like_M StSOBIR1-like SISOBIR1-like	1 1 1 1 1	MASNFHF MASNFHF MTSNTHF MAFTASHIHL MAFTASHIHL MTFTASYIHL
rsion may di rsion may di	Sm SOBIR1_MCD360 SISOBIR1_ SISOBIR1 Sm SOBIR1-like_M SISOBIR1-like SISOBIR1-like	64 64 71 71 71	TQVLRVTRIV TQVLRVTRIV TQVVRVTRIV SYVLRVTRVI SYVLRVTRVV SNVLRVTRVV
I published vz. r	SmSOBIR1_MCD360	134	ELSSLTRLRL
	StSOBIR1	134	ELSSLNRLRV
	SISOBIR1	134	ELSALNRLRI
	SmSOBIR1-like_M	141	ELSSLVRLRT
	StSOBIR1-like	141	ELSSLVRLRT
	SISOBIR1-like	141	ELSSLVRLRI
ad. The fina	SmSOBIR1_MCD360	204	PVMSQIEHLS
	StSOBIR1	204	PVMSQIEHLS
	SISOBIR1	204	PVMSQIEHLS
	SmSOBIR1-like_M	211	PAVSQVEHLS
	StSOBIR1-like	211	PAVSQVEHLS
	SISOBIR1-like	211	PVVSQVEHLS
ted or prooffe	SmSOBIR1_MCD360	266	KLRSWFLGFL
	StSOBIR1	266	KLRSWFLGFL
	StSOBIR1	267	KLRSWFLGFL
	SmSOBIR1-like_M	280	KVGAWILGFF
	StSOBIR1-like	280	KVGAWILGFF
	StSOBIR1-like	280	KVGAWILGFF
vor.org.to.upe	SmSOBIR1_MCD360	336	MIGKGGCGEV
	StSOBIR1	336	MIGKGGCGEV
	SISOBIR1	349	MIGKGGCGEV
	SmSOBIR1-like_M	349	LIGQGGCGKV
	StSOBIR1-like	349	LIGQGGCGKV
	StSOBIR1-like	349	LIGQGGCGKV
t has not yet l	SmSOBIR1_MCD360 StSOBIR1 StSOBIR1 SmSOBIR1-like_M StSOBIR1-like StSOBIR1-like	406 406 406 419 393 393	LLAHMPRPDC LLAHMPRPDC LLAHMPRPDC LLAHMPRPDC LLAHMPRPDC
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	SISOBIR1	476	PANILLDDDM
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wed and a	SmSOBIR1_MCD360 StSOBIR1 SISOBIR1 SmSOBIR1-like_M StSOBIR1-like SISOBIR1-like	616 616 629 579 579	VRCMLTQIKH VRCMLTQIKH VRCMLTQIKH VRCMLMQIKH VRCMLMQIKH VRCMLMQIKH

Fig. S4. Alignments of SmSOBIR1 and SmSOBIR1-like with StSOBIR1, StSOBIR1-like, S/SOBIR1 and S/SOBIR1-like. Amino acid sequence alignment of SOBIR1 was created using COBALT, and BOXSHADE was used to visualize conservation. Residues which are identical in all sequences are highlighted in black, while those identical in >50% of the sequences are highlighted in grey.

IA IA IS PAHRNA PAHRNA

PIQ PVQ PTE PTE

IPY IPS VPP VPP

RNK RKN HNK

L N L N L N L N L N

PAPAPV PAPAPV QAPGPS QAPGPS

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TEEDTKALNKKMRQVKSEIQIVGQI TEEDTKALNKKMRQVKSEIQIVGQI LTEEDSKAMNKKMRQVKSEIQIVGQI LTEEDSKAMNKKMRQVKSEIKIVGQI LTEEDSK

AQARLNLYPUDHAALLLVQKDLGI VQARLNLYPODHAALLLVQKDLGI VQARLNLYPDHAALLLVQKDLGI VQAKLNLYSPDHSALLLVQKGLGI VQAKLNLYSPDHSALLLVQKGLGI

L KEL SL S S S

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LAENSTRSNQIS-LAENSTRSNQIS-LAENSTRISAMGF LAENERRPRHTP-LAENERRPRHTP-LAENATRPRHTP-

LLFKLVMFFVRR LLFKMVMFFVRR VLFKVLMFLIRG VLFKVLMFLIRG VLFKVLMFLIRG

HI

SPMDAAE SPMDAAE PRDAAE PRDAAE

SEMSLVKWEINVMISDDPKIAIDPKLIGNGVEECMLLVLKIACFCTL SEMSLVKWENVMISDDPKIAIDPKLIGNGVEECMLLVLKIACFCTL SEMSLVKWENVMISDDPKIAIDPKLIGNGVDECMLLVLKIACFCTL SEMSLVKWENVMISDPNRAIDPKLIMSNGMEDCMLLVLKIACFCTL SEMSLVKWENVMISDPNRAIDPKLIMSNGMEDMLLVLKIACFCTL SEMSLVKWENVMISEDPNRAIDPKLMSNGMEDCMLLVLKIACFCTL

KII KMI

HYLVYEYMKNGS HYLVYEYMKNGS HYLVYEYMKNGS

ARMA

MF

VP SVP APPER

GKND GNND RND RND SG

231x299mm (300 x 300 DPI)



Fig. S5. Designing of synthetic versions of *Sm*SOBIR1 (*Sm*SOBIR1syn) and *in planta* protein detection. (a) Schematic overview of the synthetic constructs of *Sm*SOBIR1 used in this study. In red is the *Sm*SOBIR1 sequence that is targeted by the TRV-*NbSOBIR1/-like* construct. Protein (top) and gene models are scaled to represent the actual domain positions. Alignments show the nucleotide differences between *Sm*SOBIR1 and *Sm*SOBIR1syn^{WT} sequences, while the translated amino acid sequence remains identical. (b) Western blot showing the detection of *Sm*SOBIR1syn variants in TRV-*GUS* or TRV-*NbSOBIR1/-like*-inoculated plants. Wild type *Sm*SOBIR1 (WT) and empty vector (EV) were used as controls.

178x135mm (300 x 300 DPI)

SRage 110f47360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	1 1 1 1	ATCGCTTCAAATTTCCACTTTTTTCTCTCCTATACCTTGTGACCCTTTTCCTTTTTGCTCAAG
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	62 1 62 71 1	CAAGACTGAATCTTTATCCACAAGATCATGCTGCACTTTTGCTTGTTCAAAAAGACTTGGGCATCATTGC AATCTTTATCCACCAGATCATGCTGCACTTTTGCTTGTCCAAAAGACTTGGGCATCCAAGG CAAGACTGAACCTGTACCCACAAGACCACGCAGCTCTGTTGCTTGTACAGAAGGACTTGGGCATCATTGC CAAAACTCAACCTTTACTCACCTGATCACAGTGCTCTTTTGCTTGTCCAAAAAGCCTTAGGCATCCCIGC
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	132 63 132 141 1	TCTTAACAACCCCTGTACGCTCGCAGGAATATCCTGCGACCGTAGACCGGGTAACAGA TCAACGCATTGCACTTTGCAACTCTGCAACGAATATCCTGTGAAAGGCGAAAGGCAAACAGA TCTTAACAACCCCTGTACGCTCGCAGGAATATCCTGCGACCGTAGACGGGTAACAGA TCACCGCAATGCTCTTGAGAACCCATGCAACTCTGTTGGAATAACATGAAAAACGACTCACAAACAA
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	190 124 190 211 1	ACACAAGTTCTGAGAGTTACCCGTATTGTATTCAGATCCAATGGATTGAAGGGAACTTTGTCTTCTGCTA ACACAATTGTTGAGAGTCACCCGTATTGACTTCAGATCCACTGGATTGACTGGAACTTTATCTCCTGCCA ACACAAGTTCTGAGAGTTACCCGTATTGTATT
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	260 194 260 281 1	TTGGCAAACTCTCTGAGCTCAAAGAGCTTTCTTCTTTCCGACAATCAACTATCTGAACAAATCCCAGTTCA TTGGCAAAACTTTCTGGGATCCT TTGGCAAACTCTCTGAGCTCAAAGAGCTTTCTCTTTCCGACAATCAACTATCTGAACAAATCCCAGTTCA TTGGGAGGCTTTCTGAGCTCAAAGAACTGTCCCTCCAAAACAACAACAACTCTTTGACAGAATACCAACTGA
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	330 330 351 1	GATTCTTGATTGTCGGAAATTGGAGATTCTTGAAGAACCAATTTTCTGGGAAGATTCCGTAT GATTCTTGATTGTCGGAAATTGGAGATTCTTGAACTTCAAACAAA
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	400 400 421 53	GAATTGTCATCTTTAACCCGTTTTAAGCCTAGTTGACTTTTCATCGAATGAGTTTTCTGGGAATCTTCATT GAATTGTCATCTTTAACCCGTTTAAGCCTAGTTGACTTTTCATCGAATGAGTTTTCTGGGAATCTTCATT GAATTATCATCTCTAGTCCGCCTTCGTACCTTGACCTTGGCTCTAATGAGTTATCTGGGAACCTGAACT GAATTATCATCTCTTGTCCGCCTTCGAATCCTTGACCTCTCTAATGAATTATCAGGGAACCTCAATT
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	470 470 491 123	TCTTGAAGTACTTTCCTAATTTGGAAAAAACTGTCTCTGGCTGACAATATGTTCACTGGAAAAATACCCTT TCTTGAAGTACTTTCCTAATTTGGAAAAAACTGTCTCTGGCTGACAATATGTTCACTGGAAAAAATACCCTT TCTTGAAATACTTTCCTAACCTTGAAAAACTGTCCCTTGCTGATAACATGTTTACTGGCAGAATACCTCA TCTTGAAATACTTTCCCAACCTTGAAAAATTTGTCCCTTGCTGATAACATGTTCATTGACAAAATACCTCA
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COBIR1_MCD360 COBIR1_VIGS COBIR1Syn COBIR1Syn COBIR1-like_M COBIR1-like_V COBIR1-like_V COBIR1-like_V	869 869 911	TG <mark>CTATTCAAG</mark> CTCGTA <mark>ATGTT</mark> CTTCCTAAGA <mark>AGGGGAA</mark> AGAACCATAATTCAAGTTTAACGATATTTAG TGCTATTCAAGCTCGTAATGTTCTTCCTAAGAAGGGGAAAGAACCATAATTCAAGTTTAACGATATTTAG TCCTCTTCAAGGTGCTTATGTTTTTAATCAGAGGGATCACAAATCATTCAGGCTTAACAATTTTCAG
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SmSOBIRI_MCD360 NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	1149 1149 1188	GAAAATGCGTCAAGTTAAATCAGAAATTCAAAATTGTAGGTCAAATCAGACACCGGAATCTGCTTCCATTA GAAAATGCGTCAAGTTAAATCAGAAATTCAAAATTGTAGGTCAAATCAGACACCGGAATCTGCTTCCATTA GAAAATGCGCCAGATTAAATCAGAAATCAAAATTGTAGGTCAAATCAGACACCGGAATTTGCTTCCCCCTA
SmSOBIRI_MCD360 NbSOBIRI_VIGS SmSOBIRIsyn SmSOBIRI-like_M NbSOBIRI-like_V	1219 1219 1258	CTGGCCCATATGCCAAGGCCAGACTGTCATTACTTGGTGTATGAATATATGAAAAATGGGAGTTTACAGG CTGGCCCATATGCCAAGGCCAGACTGTCATTACTTGGTG CTGGCACATATGCCAAGACCAGACTGCCATTACTTGGTC TATGAGTACATGAAAAATGGGAGCTTACAGG ATATCCTCCACCAAGACCAGACC
SmSOBIR1_MCD360 NbSOBIR1_VIGS SmSOBIR1-like_M NbSOBIR1-like_V SmSOBIR1_MCD360	1289 1289 1328	ATATCCTGCAGCAAGTCACAGAAGGCACAAGAGAATTAGATTGGTTGG
NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V SmSOBIR1_MCD360	1359 1398 1429	GATAGCTGCTGGTCTTGGAGTATCTCCATATAAACCATACTCAACGCATAATTCACAGAGATCTAAAGCCA AATAGCTGCTGGACTCGAGTATCTCCCATATAAATCATACTCACCGGTATAATTCACAGAGATCTAAAGCCA GCAAATATCCTACTTGATGATGACATGGAAGCTCGAATAGCTGATTTTGGGCTTGCAAAGGCAGTTCCAG
NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V SmSOBIR1_MCD360	1429 1468 1499	GCAAATATCCTACTTGATGATGACATGGAAGCTCGAATAGCTGATTTTGGCCTTGCAAAGGCAGTTCCAG GGCAATGTCCTCCTTGATGATGACATGGAAGCTCGAATTGCAGATTTTGGCCTTGCAAAGGCTGTCCCAG ATGCTCATACACATATTACGACTTCAAATGTTGCAGGAACTCTCGGATATATTGCACCAGAATATCATCA
NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V SmSOBIR1_MCD360	1499 1538 1569	ATGCTCATACACATATTACGACTTCAAATGTTGCAGGAACTGTGGGATATATTGCACCAGAATATCATCA ATGCTCATACACATATTACAACTTCAAATGTGGCAGGAACTATAGGATACATCGCTCCAGAATATCATCA GACACTGAAGTTTACAGCAGGTGTGATATATACAGCTTCGGTGGTGCTGCTAGCTGTGTTGGTTATCGGA
NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V SmSOBIR1 MCD360	1569 1608 1639	GACACTCAAGTTTACAGACAAGTGTGATATATACAGCTTCGGTGTGCTGCTGGTGTGTGT
NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V SmSOBIR1_MCD360	1639 1678 1709	AAGGCTCCATCGGATGAATTTTTTCCAACATACTTCTGAGATGAGTTTAGTTAACTGGCTGAGAAATGTAA AAGCTTCCATCTGATGAGTTCTTCCACAACATTCTGAGATGAGTTTAGTGAAATGGATGAGAAATGTCA TGACTTCTGATGATCCTAAAAATAGCAATTGATCCTAAGCTGATAGCAAATGGATATGACCACAAATGCT
NbSOBIR1_VIGS SmSOBIR1syn SmSOBIR1-like_M NbSOBIR1-like_V	1709 1748	TGACTTCTGATGATCCTAAAATAGCAATTGATCCTAAGCTGATAGGAAATGGATATGAGGAGCAAATGCT TGACTTCTGATGATCCAAACAGAGCAATTGATCCAAAGCTGATCGGTAATGGAAATGAGGACCAAATGCT



NbSOBIR1-like_V





Fig. S7. Kinase activity of SOBIR1 and its kinase domain are not required for its association with ELR. (a) ELR-eGFP was transiently co-expressed in *Nicotiana benthamiana* in combination with empty vector (EV) or *Sm*SOBIR1syn variants (*Sm*SOBIR1syn^{WT}, *Sm*SOBIR1syn^{D473N} or *Sm*SOBIR1syn^{Δ-kinase}). Total protein was extracted and was subjected to immunoprecipitation using GFP_TrapMA beads to capture ELR. The immunopurified proteins were detected with anti-GFP, while the interaction with SmSOBIR1syn variants was assessed with anti-Myc. Ponceau S staining of RuBisCO indicates equal protein loading. This figure is representative for three biological repeats. (b) Schematic representation of the putative interaction site between the extracellular-juxtamembrane (eJM), transmembrane (TM) and intracellular-juxtamembrane (iJM) domains of ELR and SmSOBIR1 based on Gust and Felix (2014) and Bi et al. (2016). Charged amino acids are indicated by highlighting and a symbol according to charge (+ for positive or - for negative charge). The GxxxG motif in the TM is also highlighted.

179x156mm (300 x 300 DPI)

(a)



	ELR-eGFP +					
Relative band	EV	SmSOBIR1	SmSOBIR1syn	SmSOBIR1syn	SmSOBIR1syn	StSERK3a
intensity	EV	-Myc	-Myc	D473N-Myc	^{∆-kinase} -Myc	-HA
Experiment 1	1.0×	4.6×	13.0×	6.4×	5.7×	1.9×
Experiment 2	1.0×	-	12.2×	-	-	-
Experiment 3	1.0×	-	-	6.2×	-	-

Fig. S8. SmSOBIR1 stabilizes ELR independently of its kinase activity. ELR-eGFP was transiently co-expressed in Nicotiana benthamiana with empty vector (EV), SmSOBIR1, SmSOBIR1syn^{WT},
 SmSOBIR1syn^{D473N} or SmSOBIR1syn^{Δ-kinase}. Total protein was extracted and western blotting was performed with anti-GFP to visualize ELR accumulation. Quantification of ELR band intensity was performed with imageJ. Relative band intensity results were obtained by comparing the signal intensity of the SOBIR1 and SERK3a agroinfiltrations to the EV agroinfiltration of each experiment. Ponceau S staining of RuBisCO indicates equal protein loading. Data from three independent experiments are shown.

98x57mm (300 x 300 DPI)

Purpose	Construct	Binary vector	Notes	A. tumefaciens strain	References
death assays	INF1	pCB302-3		AGL1	(Du <i>et al</i> ., 2015)
	Empty vector	pCB302-3		AGL1	(Du <i>et al</i> ., 2015)
Ce	Empty-Myc	pGWB20	Empty vector C' 10× Myc	AGL1	This study
Protein fusions	ELR-eGFP	pBin-KS- GWY-35s- eGFP	C' eGFP	C58C1	(Du <i>et al.</i> , 2015)
	Cf-4-eGFP	pBin-KS- GWY-35s- eGFP	C' eGFP	C58C1	(Liebrand <i>et al.</i> , 2012)
	<i>St</i> SERK3a-HA	pGWB14	С' 3× НА	GV3101	(Du <i>et al</i> ., 2015)
	Empty-eGFP	pBin-KS- GWY-35s- eGFP	Empty vector, C' eGFP	AGL1	This study
	SmSOBIR1- Myc	pGWB20	C' 10× Myc	AGL1	This study
	SmSOBIR1- like-Myc	pGWB20	C' 10× Myc	AGL1	This study
	SmSOBIR1syn -Myc	pGWB20	C' 10× Myc	AGL1	This study
	SmSOBIR1syn	pGWB20	C' 10× Myc	AGL1	This study

Table S1. Constructs used in this study.

	D473N-Myc				
	<i>Sm</i> SOBIR1syn △—kinase-Myc	pGWB20	C' 10× Myc	AGL1	This study
Gene silencing (VIGS)	pTRV1		TRV-VIGS	GV3101	(Liu <i>et al</i> ., 2002)
	pTRV2-GUS		TRV-VIGS	AGL1	This study
	pTRV2-StPDS		TRV-VIGS	AGL1	This study
	pTRV2- NbSERK3a/3b		TRV-VIGS	C58C1	(Heese <i>et</i> <i>al.</i> , 2007)
	pTRV2- NbSOBIR1/- like		TRV-VIGS	C58C1	(Liebrand <i>et al.</i> , 2013)

Primer name	Purpose	Sequence 5'-3'	References
StSOBIR Fwd	Cloning	CACCATGGCTTCAAATTTCCACTTTTTTCT	This study
StSOBIR Rev	Cloning	ATGCTTGATCTGAGTTAACATGCA	This study
StSOBIR-like Fwd	Cloning	CACCATGGCCTTCACAGCCTCACA	This study
StSOBIR-like Rev	Cloning	ATGCTTGATCTGCATCAACATGC	This study
GUS 1-240 VIGS Fwd	Cloning	CACCCATGGTCCGTCCTGTAGAAA	This study
GUS 1-240 VIGS Rev	Cloning	GCCCAACCTTTCGGTATAAA	This study
<i>SmSOBIR1^{∆-kinase}</i> Rev	Cloning	TGCTACTCCATCTTCCATCTCT	This study
<i>Sm/NbPDS</i> VIGS Fwd	Cloning	CACCAACTAAACCATTGGAGATTGTTATTG	This study
<i>Sm/NbPDS</i> VIGS Rev	Cloning	TTAATCCCTAATTCTCCAAACAGG	This study
<i>NbSOBIR1</i> qRT- PCR Fwd	qPCR	CTTAGAAAAACTCTCTTTAGC	(Liebrand <i>et al.</i> , 2013)
<i>NbSOBIR1</i> qRT- PCR Rev	qPCR	TATGGATTGGAGTGACATTATG	(Liebrand <i>et al.</i> , 2013)
NbSOBIR1-like	qPCR	GCAATTGTAGTACCAGTACAC	(Liebrand

qRT-PCR Fwd			et al.,
			2013)
<i>NbSOBIR1-like</i> qRT-PCR Rev	qPCR	AATCAATGGACTGAAAAC	(Liebrand <i>et al.</i> , 2013)
<i>Piβ-tubulin</i> qPCR Fwd	qPCR	GGTCGTGGAGCCCTATAACG	This study
<i>Piβ-tubulin</i> qPCR Rev	qPCR	GTCACCATAAGTGGGGGGGGG	This study
<i>St/NbEF1α</i> qPCR Fwd	qPCR	TGACCAAGATTGACAGGCGT	This study
<i>St/NbEF1α</i> qPCR Rev	qPCR	GCAAAACGACCCAATGGTGG	This study