Virulence strategies of an insect herbivore and oomycete plant pathogen converge on host E3 SUMO ligase SIZ1 to suppress plant immunity

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Abstract

Plant parasites must colonise and reproduce on plants to survive. In most cases, active immune responses, triggered by (conserved) microbe-encoded molecules keep invaders at bay. Post-translational modifications (PTMs) of proteins are vital for contextual regulation and integration of plant immune responses. Pathogens and pests secrete proteins (effectors) to interfere with plant immunity through modification of host target functions and disruption of immune signalling networks. Importantly, molecular virulence strategies of distinct pathogens converge on a small set of regulators with central roles in plant immunity. The extent of convergence between pathogen and herbivorous insect virulence strategies is largely unexplored. Here we report that effectors from the oomycete pathogen, *Phytophthora capsici*, and the major aphid pest, *Myzus persicae* target the host immune regulator SIZ1, an E3 SUMO ligase. SIZ1-regulated immunity in Arabidopsis against bacterial pathogens is known to require the resistance protein SNC1, and signalling components PAD4 and EDS1. We show that SIZ1 functions as a negative regulator of plant immunity to aphids and an oomycete pathogen. However, this immune regulation is independent of SNC1, PAD4 and EDS1-signalling pointing to the presence of a novel SIZ1-mediated immune signalling route. Our results suggest convergence of distinct pathogen and pest virulence strategies on an E3 SUMO ligase that negatively regulates plant immunity.

Introduction

The plant immune system is complex, featuring different classes of receptors to detect pathogens and pests and initiate a multi-layered defence responses. Pattern recognition receptors (PRRs) recognize conserved pest and pathogen molecules, called pathogen associated molecular patterns (PAMPs), to activate immune responses and fight off the intruder (Jones and Dangl, 2006; Monaghan and Zipfel, 2012). Pathogens and pests deliver an arsenal of effector proteins inside their host to counter these and other plant defence pathways to promote effector-triggered susceptibility (ETS) through modulation of host protein activities. In addition, these effectors likely contribute to effective infection or infestation strategies by promoting the release of nutrients to support pathogen or pest growth. Another layer of plant immunity may be activated upon recognition of these effectors, or their activities, by nucleotide-binding leucine-rich repeat proteins (NLRs), which usually is associated with the activation of a Hypersensitive Response (HR). Given that plants carefully balance energy allocation between growth, development and reproduction, any effective immune responses need to be appropriate and controlled (Huot et al., 2014).

The identification of effector host targets and their effector-induced modification(s) can reveal the mechanistic basis of virulence and the biological processes that lead to susceptibility. Moreover, the identification of effector host targets for a range of pathogens pointed to convergence on key

host proteins. For example, Avr2 from the fungal pathogen *Cladosporium fulvum*, EPIC1 and EPIC2B from the oomycete *Phytophthora infestans*, and Gr-VAP1 from the plant-parasitic nematode *Globodera rostochiensis* target the same defense protease Rcr3^{pim} in tomato (Song et al., 2009; Lozano-Torres et al., 2012). In addition, the effector repertoires of distinct plant pathogens, such as the bacterium *Pseudomonas syringae*, oomycete *Hyaloperonospora arabidopsidis*, and the ascomycete *Golovinomyces orontii* disrupt key components of immune signalling networks (Mukhtar et al., 2011; Weßling et al., 2014). Specifically, transcription factor TCP14 is targeted by effectors from *P. syringae*, *H. arabidopsidis* and *Phytophthora capsici*, and contributes to plant immunity (Stam et al., 2013; Weßling et al., 2014). These findings suggest that molecular virulence strategies have evolved independently in distinct pathogens and converged on a small set of regulators with central roles in immunity.

While over the past decades our understanding of pathogen virulence strategies and susceptibility has increased dramatically, the extent with which host targets of plant-herbivorous insects overlap with other pathogens remains to be investigated. Effector biology has recently emerged as a new area in plant-herbivorous insect interactions research, leading to the identification of effector repertoires in several species (Carolan et al., 2009; Bos et al., 2010; Kaloshian and Walling, 2016; Thorpe et al., 2018; Rao et al., 2019), several host targets, and insights into their contribution to the infestation process (Rodriguez et al., 2017; Chaudhary et al., 2019; Wang et al., 2019; Xu et al., 2019). These studies support an extension of the effector paradigm in plant-microbe interactions to plant-herbivorous insect interactions. Whether plant pathogenic microbes and insects adopt similar strategies to attack and reprogram their host to redirect immune responses is yet to be determined.

Here, we show that *Myzus persicae* (aphid) effector, Mp64, and *P. capsici* (oomycete) effector CRN83_152 (also called PcCRN4) (Stam et al., 2013; Mafurah et al., 2015), associate with the immune regulator SIZ1 in the plant nucleus to promote susceptibility. SIZ1 is an E3 SUMO ligase involved in abiotic and biotic stress responses, including Salicylic Acid (SA)-mediated innate immunity and EDS1/PAD4-mediated Resistance gene signalling (Miura et al., 2005; Catala et al., 2007; Lee et al., 2007; Miura et al., 2007; Jin et al., 2008; Miura et al., 2009; Ishida et al., 2012; Lin et al., 2016). Additionally, SIZ1 regulates plant immunity partially through the immune receptor SNC1 (Gou et al., 2017) and controls the trade-off between SNC1-dependent immunity and growth in Arabidopsis at elevated temperature (Hammoudi et al., 2018). By using Arabidopsis knock-out lines and gene silencing in *N. benthamiana* we show that SIZ1 functions as a negative regulator of plant immunity to aphids and an oomycete pathogen. However, this immune regulation is independent of SNC1-signalling pointing to the presence of a novel SIZ1-mediated immune signalling route. Our results suggest that the effector target convergence principle can be extended to herbivorous insects and raise important questions about mechanisms of action.

75 **Results**

76

77 **Aphid effector Mp64 and oomycete effector CRN83_152 interact with SIZ1 in yeast and *in***
78 ***planta***

79 To gain novel insight into pathogen and pest effectors, we successfully applied yeast-two-hybrid
80 screens to identify candidate host targets (Rodriguez et al., 2017). We identified the E3 SUMO
81 ligase SIZ1 in screens against a *N. benthamiana* library (generated from aphid infested and *P.*
82 *capsici* infected leaves) with *M. persicae* (aphid) effector Mp64 and *P. capsici* (oomycete) effector
83 CRN83_152 as baits. Mp64 was screened against an estimated $5x10^6$ cDNAs and revealed 2
84 independent prey clones with an insert showing similarity to SIZ1, whilst the effector CRN83_152
85 screen of $4x10^6$ yeast transformants, identified 3 independent prey clones with an insert similar to
86 SIZ1. All putative interactors identified in the two effector screens are summarized in Table S1.
87 Since all NbSIZ1 (*N. benthamiana* SIZ1) prey clones from the Mp64 and CRN83_152 screens
88 were partial-length, we designed primers to amplify and clone the full-length NbSIZ1. Although we
89 were unable to amplify NbSIZ1 based on the two best BLAST hits against the *N. benthamiana*
90 genome database due to poor primer annealing at the 3' end, we successfully amplified NbSIZ1
91 sequences based on the 3' end of SIZ1 sequences from *N. attenuata* (XP_019237903) and *N.*
92 *tomentosiformis* (XP_018631066). The full-length NbSIZ1 sequence we cloned was identical to our
93 partial yeast prey clones and NbSIZ1 database sequences, except for a 27 amino acid insertion at
94 position 225-252. A direct comparison between NbSIZ1 and the well characterised AtSIZ1 showed
95 60% identity between proteins (Supplementary Fig.S1). Given that AtSIZ1 is well characterised
96 and helps regulate plant immunity (Miura et al., 2005; Catala et al., 2007; Lee et al., 2007;
97 Hammoudi et al., 2018), we included AtSIZ1 in our efforts to further validate effector-SIZ1
98 interactions and characterise the role of SIZ1 in immunity to aphids and oomycetes. It should be
99 noted that whilst Arabidopsis is a host for *M. persicae*, this plant species is not a natural host for *P.*
100 *capsici*. We first tested whether Mp64 and CRN83_152 interact with full-length NbSIZ1 and AtSIZ1
101 in yeast. Whilst yeast reporter assays showed interaction of Mp64 with both full-length SIZ1
102 versions (Supplementary Fig.S2), we were unable to obtain yeast co-transformants expressing
103 both CRN83_152 and full-length SIZ1 in repeated transformation experiments that included
104 transformation controls. We also included a mutant of CRN83_152, called CRN83_152_6D10
105 (Amaro et al., 2018), which does not trigger CRN-cell death activation but retains virulence activity,
106 in co-transformation experiments with similar results.

107 To test for *in planta* effector-SIZ1 interactions, we co-expresssed GFP-Mp64 and GFP-
108 CRN83_152_6D10 with either AtSIZ1-myc or NbSIZ1-myc in *N. benthamiana* (Fig. 1A and B).
109 Immunoprecipitation of both effectors resulted in the co-purification of NbSIZ1, suggestive of an
110 association *in planta* (Fig. 1B). Co-immunoprecipitation of the effectors with AtSIZ1 gave similar
111 results with the exception of CRN83_152, where no AtSIZ1 was detected (Fig. 1A). It should be

112 noted that CRN83_152 actives cell death in these assays, leading to protein degradation.
113 Altogether, our data confirm interaction of aphid effector Mp64 and *P. capsici* effector CRN83_152
114 with host E3 SUMO ligase SIZ1.

115

116 **Nuclear aphid effector Mp64 enhances Arabidopsis susceptibility to *M. persicae***

117 While the nuclear PcCRN83_152 effector from *P. capsici* was previously shown to be essential for
118 pathogen virulence and promotes plant susceptibility (Stam et al., 2013; Mafurah et al., 2015), the
119 role of aphid effector Mp64, which was previously identified as a candidate effector in *A. pisum* and
120 *M. persicae* through bioinformatics pipelines (Carolan et al., 2011; Thorpe et al., 2016; Boulain et
121 al., 2018), in plant-aphid interactions is unknown. Mp64 is a protein of unknown function with a
122 predicted nuclear localisation based on ProteinPredict (Yachdav et al., 2014) and NLStradamus
123 (Nguyen Ba et al., 2009), and Mp64 homologs are present in other aphid species (Supplementary
124 Fig. S3). We investigated the subcellular localisation of Mp64 by confocal microscopy of *N.*
125 *benthamiana* leaves transiently expressing GFP-Mp64 (lacking the predicted signal peptide).
126 Imaging of epidermal cells expressing Mp64 revealed accumulation of GFP-Mp64 in the nucleus
127 and nucleolus, with no signal detectable in the cytoplasm (Fig. 2A). In addition, we observed dots
128 within the nucleoplasm corresponding to GFP-Mp64. Nuclear localisation of GFP-Mp64 was
129 confirmed upon co-localisation with the nuclear marker Histone 2B (H2B) (Fig. 2B).

130 To confirm whether Mp64 can affect host susceptibility to aphids, we generated Arabidopsis
131 transgenic lines expressing the mature Mp64 protein driven by the 35S promoter. Arabidopsis lines
132 expressing Mp64 (Supplementary Fig. S4A) showed no developmental or growth phenotypes
133 (Supplementary Fig. S4B) and were subjected to aphid fecundity assays. Two age-synchronized
134 *M. persicae* aphids were placed on transgenic Mp64 lines and Col-0 control plants, and progeny
135 was counted after 10 days. The average number of aphids on two independent transgenic Mp64
136 lines (25.6 and 29.5) was 31-34% higher than on the Col-0 control (Mann-Whitney U test, $p<0.05$;
137 Fig. 2C), indicating that Mp64 enhances Arabidopsis host susceptibility to *M. persicae*.

138 To test whether Mp64 also affects *P. capsici* infection, we transiently over-expressed Mp64 and a
139 vector control in *N. benthamiana* and challenged infiltration sites with a zoospore suspension
140 (Supplementary Fig. S5). In contrast to the *P. capsici* effector CRN83_152, aphid effector Mp64
141 did not enhance host susceptibility to *P. capsici*, suggesting that these effectors have distinct
142 virulence activities despite sharing SIZ1 as a virulence target.

143

144 **Effectors Mp64 and CRN83_152 co-localise with SIZ1 in the host nucleus**

145 Since both Mp64 and CRN83_152 are nuclear effectors, and their host interacting protein SIZ1 is
146 reported to localise and function in the plant nucleus (Miura et al., 2005), we determined whether

147 the effectors co-localise with SIZ1 in this subcellular compartment. We performed confocal imaging
148 of *N. benthamiana* leaves transiently co-expressing SIZ1-mRFP and GFP-effector fusions. In line
149 with previous reports, mRFP signal corresponding to both AtSIZ1 and NbSIZ1 was visible in the
150 plant nucleoplasm, along with distinct speckles in the nucleolus (Fig. 3; Supplementary Fig. S6). In
151 addition, GFP signal corresponding to GFP-Mp64 and CRN83_152 was detectable in the nucleus.
152 Mp64 or CRN83_152 did not affect the localisation of SIZ1 and similarly, SIZ1 did not affect the
153 localisation of the effectors (Fig. 3; Supplementary Fig. S6). Our data suggests that effectors Mp64
154 and CRN83_152 associate with SIZ1 in the host nucleus.

155

156 **Silencing of *NbSIZ1* reduces *N. benthamiana* host susceptibility to *P. capsici***

157 To assess the contribution of SIZ1 to immunity in a *P. capsici* host species, we made use of Virus
158 Induced Gene Silencing (VIGS) in *N. benthamiana* (Ratcliff et al., 2001; Lu et al., 2003). Our TRV-
159 *NbSIZ1* construct, designed to silence *NbSIZ1*, reduced transcripts levels by around 60%
160 compared with plants expressing the TRV-GFPfrag control (a fragment of GFP). Silenced plants
161 showed a slight reduction in growth compared with the TRV-GFPfrag control and cell death in
162 older leaves (Supplementary Fig. S7A and C). In our hands, VIGS assays based on TRV in *N.*
163 *benthamiana* are incompatible with aphid assays (TRV infection causes aphids to die), therefore,
164 we only performed infection assays with *P. capsici* on *NbSIZ1* silenced plants. Detached leaves
165 were used for *P. capsici* infection assays based on zoospore droplet inoculations, followed by
166 lesion size diameter measurements. *P. capsici* lesion size on *NbSIZ1* silenced leaves was
167 significantly reduced 2-4 days after inoculation when compared to control plants (Mann-Whitney U
168 test, $p<0.0001$; Fig. 4; Supplementary Fig. S6B). These results indicate that NbSIZ1 contributes to
169 host susceptibility to this oomycete plant pathogen, possibly by negatively regulating plant
170 immunity.

171

172 **Loss-of-function mutation *siz1-2* in Arabidopsis leads to enhanced resistance to *M.***
173 ***persicae* and *P. capsici***

174 Since AtSIZ1 negatively regulates plant innate immunity in Arabidopsis to the bacterial plant
175 pathogen *Pseudomonas syringae* pv. Tomato DC3000 (Pst) (Lee et al., 2007), we tested whether
176 this also applies to interactions with *M. persicae* and *P. capsici*. We performed aphid performance
177 assays, based on fecundity measurements, as well as *P. capsici* infection assays on the
178 Arabidopsis loss-of-function mutant *siz1-2*. Given that siz1-2 mutants have a dwarf phenotype,
179 associated with SA hyper-accumulation, we included Arabidopsis line *siz1-2*/NahG, in which this
180 phenotype is (partially) abolished (Lee et al., 2007). While Arabidopsis is a host for the aphid *M.*
181 *persicae,* only few *P. capsici* isolates infect Arabidopsis under controlled environmental conditions
182 and high levels of inoculum (Wang et al., 2013), suggesting that Arabidopsis is not a natural host.

183 Aphid performance assays showed a significant reduction in fecundity on the *siz1-2* and *siz1-*
184 *2*/*NahG* lines compared to the Col-0 (ANOVA, p<0.0001; Fig. 5A) and *NahG* controls (ANOVA,
185 p<0.01; Fig. 5A), respectively, with only few aphids surviving on the *siz1-2* line. The *siz1-2* reduced
186 susceptibility to aphids is largely maintained in the *NahG* background, implying that this phenotype
187 is largely independent of SA accumulation.

188 For *P. capsici* infection assays, plants were spray inoculated with a zoospore solution and the
189 percentage of symptomatic leaves was counted 10 days later. The percentage of symptomatic
190 *siz1-2* leaves was reduced by 83% compared with the Col-0 control (ANOVA, p<0.0001; Fig. 5B,
191 Supplementary Fig. S8). We did not observe a difference in *P. capsici* infection levels between the
192 *NahG* line and Col-0, but did note a slight reduction in infection on *siz1/NahG* compared to the
193 *NahG* background (ANOVA, p<0.01; Fig. 5B, Supplementary Fig. S8).

194

195 **Arabidopsis s*iz1-2* enhanced resistance to *M. persicae* and *P. capsici* is independent of**
196 **SNC1, EDS1 and PAD4**

197 EDS1, PAD4 and SNC1 are required for *siz1-2* enhanced resistance to *P. syringae* pv tomato
198 DC3000 (Lee et al., 2007; Gou et al., 2017; Hammoudi et al., 2018). To explore whether these
199 signalling components also contribute to *siz1-2* enhanced resistance to aphids and *P. capsici*, we
200 performed aphid infestation and infection assays on Arabidopsis *siz1-2/eds1-2*, *siz1-2/pad4-1* and
201 *siz1-2/snc1-11* double mutants.

202 Aphid infestation on the *siz1-2/eds1-2* mutant was reduced by 75% compared with the *eds1-2*
203 mutant (ANOVA, p<0.0001, Fig. 5C), and was comparable to *siz1-2* (Fig. 5C), suggesting that the
204 reduced susceptibility of *siz1-2* to aphids is independent of EDS1. In addition, aphid fecundity was
205 reduced on *siz1-2/pad4-1* by around 65% compared with the *pad4-1* mutant (ANOVA, p<0.0001,
206 Fig. 5C), and was comparable to *siz1-2* (Fig. 5C). These data suggest that *siz1-2* reduced
207 susceptibility to aphids is also independent of PAD4.

208 In line with previous reports (Wang et al., 2013) the *eds1-2* and *pad4-1* mutants were less resistant
209 to *P. capsici* than Col-0 (ANOVA, p<0.0001, Fig. 5D, Supplementary Fig. S8), indicating EDS1 and
210 PAD4 contribute to Arabidopsis nonhost resistance to this pathogen. The percentage of
211 symptomatic *siz1-2/eds1-2* and *siz1-2/pad4-1* leaves was around 60% and 55% less compared to
212 the *eds1-2* (ANOVA, p<0.0001, Fig. 5D, Supplementary Fig. S8) and *pad4-1* (ANOVA, p<0.0001,
213 Fig. 5D, Supplementary Fig. S8) mutants, respectively. Similar to our aphid data, *siz1-2* enhanced
214 resistance to *P. capsici* is not fully dependent on EDS1 and PAD4, with *siz1-2* showing an
215 enhanced resistance phenotype in the *eds1-2* and *pad4-1* mutant background and when compared
216 to the appropriate controls (*eds1-2* and *pad4-1* respectively).

217 Aphid fecundity on *siz1-2/snc1-11* was approximately 85% reduced compared with the *snc1-11*
218 control (ANOVA, p<0.0001), and was comparable to *siz1-2* (Fig. 5E), suggesting that *siz1-2*
219 reduced susceptibility to aphids is independent of SNC1. The *siz1-2/snc1-11* double mutant also
220 showed enhanced resistance to *P. capsici*, with 55% less symptomatic leaves compared to the
221 *snc1-11* mutant (ANOVA, p<0.0001, Fig. 5B, Supplementary Fig. S8). The percentage of
222 symptomatic leaves on *siz1-2/snc1-11* was slightly higher compared with the *siz1-2* mutant
223 (ANOVA, p<0.05, Fig. 5B, Supplementary Fig. S7). With the *siz1-2* enhanced resistance to *P.*
224 *capsici* largely maintained in the *snc1-11* background, this phenotype is likely independent of the
225 immune receptor SNC1. Overall, *siz1-2* enhanced resistance to both *M. persicae* and *P. capsici* is
226 independent of signalling components previously implicated in SIZ1 immune functions.

227

228 **Arabidopsis *siz1-2* reduced susceptibility to aphids involves phloem resistance factors**

229 To shed light on the potential mechanism underlying reduced susceptibility to *M. persicae* in the
230 Arabidopsis *siz1-2* mutant, we monitored aphid probing and feeding behaviour using the Electrical
231 penetration graph (EPG) technique. Global analysis of all 80 recorded EPG parameters showed
232 that the feeding activities of *M. persicae* on *siz1-2* are not significantly different compared with
233 those on Col-0 control plants (MANOVA, p=0.054, Table 1.). However, feeding patterns within the
234 vascular tissue differed significantly as indicated by 17 relevant parameters (Table 1). The average
235 duration aphids spent on a single phloem salivation event (E1) on the *siz1-2* mutant was two times
236 longer, and the length of time aphids spent on phloem salivation and then phloem ingestion (E12)
237 was 3 times shorter than on the Col-0 control. Furthermore, aphids spent less time ingesting
238 phloem sap (E2) on the *siz1-2* mutant than on the Col-0 control, and the ratio of time aphids spent
239 ingesting phloem sap relative to the length of time probing plant tissue (E2/C) was significantly
240 lower (Mann-Whitney U test, p<0.05; Fig. 6). Additional aphid feeding parameters, which underline
241 vascular-related resistance against aphids are displayed in Table 1. Overall, aphids have more
242 difficulties feeding on phloem sap of the *siz1-2* mutant than that of Col-0 plants, suggesting that the
243 reduced susceptibility phenotype is at least partly associated with plant phloem factors.

244 **Discussion**

245 Pathogen infection strategies involve extensive modification of host cell biology which rely on the
246 modulation of hubs that control plant immunity. In this study, we show that effectors from an
247 herbivorous insect and oomycete plant pathogen target the host E3 SUMO ligase SIZ1. Our
248 findings suggest that the virulence strategies of two plant parasites with distinct evolutionary
249 histories and lifestyles, convergence on an important host immune component.

250 Our finding that SIZ1 is a key target of distinct plant parasites is in line with a recent study on the
251 cyst nematode *Globodera pallida,* which shows that effector GpRbp1 associates with potato SIZ1
252 *in planta* (Diaz-Granados et al., 2019). Importantly StSIZ1 emerged as a negative regulator of

253 immunity in plant-nematode interactions (Diaz-Granados et al., 2019), but the signalling
254 requirements for this immunity have not yet been reported. We propose that SIZ1 is a hub that
255 regulates immunity to a broad range of plant parasites, including herbivorous insects. Indeed,
256 Arabidopsis *siz1-2* plants show enhanced immunity not only upon pathogen infection as reported
257 here (Fig. 5) and previously (Lee et al., 2007) but also upon aphid infestation. In contrast to *siz1-2*
258 enhanced resistance to *P. syringae pv. tomato* DC3000, which is dependent on SA, EDS1, PAD4
259 and SNC1, we find that resistance to the aphid *M. persicae* and the oomycete *P. capsici* is largely
260 independent from these signalling components. These results point to an as yet unknown SIZ1-
261 dependent signalling pathway that regulates plant immunity. Although PAD4 has been reported to
262 play an important role in plant defence against *M. persicae* (Pegadaraju et al., 2007), in line with
263 Lei et al. (Lei et al., 2014), we did not observe an enhanced susceptibility phenotype of
264 Arabidopsis *pad4-1* in our aphid performance assays.

265 A reduction of SA levels in the *NahG* line did not enhance defence against the aphid *M. persicae*
266 (this study and previous reports (Pegadaraju et al., 2007; Lei et al., 2014)), nor did this reduce
267 nonhost resistance to the oomycete *P. capsici,* in contrast to an earlier report by Wang et al.
268 (Wang et al., 2013). However, we did observe a trend towards reduced resistance of the
269 transgenic NahG line to *P. capsici*, but this reduction was not statistically significant and may be
270 less pronounced due to differences in experimental set-up and infection conditions compared to
271 Wang et al (Wang et al., 2013). Arabidopsis defence to insect herbivores is mediated
272 predominantly through JA-signalling, whereas defence against (hemi-)biotrophic pathogens tend to
273 rely on SA-signalling (Howe and Jander, 2008; Pieterse et al., 2012). In the Arabidopsis-*M.*
274 *persicae* interaction, *siz1-2* reduced susceptibility is largely independent of SA accumulation, with
275 the *siz1-2/NahG* line being more resistant to aphids than the *NahG* control and Col-0 (Fig. 5).
276 Therefore, and in contrast to Lee et al.(Lee et al., 2007), SIZ1-regulated immunity to aphids is
277 independent of SA-signalling. Our EPG analyses of aphid probing and feeding behaviour on
278 Arabidopsis *siz1-2* suggest the presence of resistance factors that act against aphids in the
279 phloem. Phloem-mediated defences against aphids include the occlusion of sieve elements, which
280 prevents aphids from ingesting phloem sap, as well as deterrents in the phloem sap (Mayoral et
281 al., 1996; Will and van Bel, 2006; Medina-Ortega and Walker, 2015). Interestingly, the Arabidopsis
282 *siz1-2* mutant features changes in cell division, cell expansion and secondary cell wall formation,
283 including reduced secondary cell wall thickening (Miura et al., 2010; Liu et al., 2019). Aphid feeding
284 can trigger changes in cell wall composition that are associated with defences (Rasool et al.,
285 2017), and therefore changes in cell wall formation can be responsible for altered susceptibility.
286 However, reduced cell wall thickening most likely would lead to a reduction in defence against
287 aphids rather than an increase as observed in the *siz1-2* mutant, and we found no evidence of
288 altered stylet pathways or probing indicative of cell wall-associated defences.

289 With SIZ1 comprised of several conserved domain involved in different stress responses (Cheong
290 et al., 2009) it is possible that Mp64 and CRN83-152 target different protein regions and functions.
291 Arabidopsis and *N. benthamiana* SIZ1 domains include the SAP (scaffold attachment factor
292 A/B/acinus/PIAS) domain, PINIT (proline-isoleucine-asparagine-isoleucine-threonine) domain, an
293 SP-RING (SIZ/PIAS-RING) domain, SXS motif (serine-X-serine), and a PHD (plant
294 homeodomain). Functional analyses, using a set of (deletion) mutants revealed that these domains
295 contribute differently to the wide range of SIZ1 functions in both abiotic and biotic stress (Cheong
296 et al., 2009). The SP-RING domain of AtSIZ1 contributes to the nuclear localisation, SUMOylation
297 activity, as well as the regulation of SA levels and associated plant defence responses. This
298 domain is the suggested SIZ1 target site of the nematode effector GpRbp1 to interfere with SA-
299 mediated defences (Diaz-Granados et al., 2019). Our Arabidopsis-*M. persicae* interaction assays
300 though suggest that SIZ1 may also contribute to immunity in a SA-independent manner where
301 other domains may play an important role. Interestingly, SIZ1-mediated SUMOylation is involved in
302 regulating sugar signalling independent of SA (Castro et al., 2015; Castro et al., 2018), with the
303 *siz1-2* mutant showing reduced starch levels and increased expression of starch and sucrose
304 catabolic genes. Aphid infestation affects sugar metabolism as reflected for example by an
305 increase in sucrose and starch in infested Arabidopsis plants (Singh et al., 2011). With sugars in
306 phloem sap also being the main aphid food source, it will be interesting to further explore a
307 possible link between the role of SIZ1 in regulating sugar signaling and host susceptibility.

308 Although our data support a key role for SIZ1 in plant immunity to *P. capsici* and *M. persicae* that is
309 targeted during infection and infestation, the mechanism by which Mp64 and CRN83-152 affect
310 SIZ1 is to be elucidated. The presence of host SIZ1 is required for infestation/infection as knock-
311 out of *AtSIZ1* and knock-down of *NbSIZ1* result in reduced host susceptibility phenotypes.
312 Therefore, we propose that Mp64 and CRN83-152 redirect SIZ1 function rather than inhibit it. In
313 line with this, our Arabidopsis transgenic lines expressing Mp64 do not show a reduced growth
314 phenotype similar to the *siz1-2* mutant (Supplementary Fig S4). Interestingly and in support of
315 these observations, Mp64 only enhances host susceptibility to aphids and not *P. capsici.* Given
316 that CRN83-152 does promote *P. capsici* virulence, we propose that while both virulence
317 strategies have converged onto SIZ1, their mechanisms of action are distinct. In this context, we
318 cannot rule out that additional candidate targets of Mp64 and CRN83-152 identified in our Y2H
319 screens (Supplementary Table S1) explain our observed differences in effector virulence activities.

320 As an E3 SUMO ligase, SIZ1 is required for SUMOylation of a range of substrates including
321 chromatin modifiers, coactivators, repressors, and transcription factors that are associated with
322 biotic and abiotic stress responses (Rytz et al., 2018). Similar to ubiquitination, SUMOylation
323 involves three key steps (Verma et al., 2018). First, the SUMO precursor is cleaved and the SUMO
324 moiety is linked to an SUMO-activating enzyme (E1). Activated SUMO is then transferred to the
325 SUMO-conjugating enzyme (E2), after which it is linked to target substrates with the help of

326 SUMO-ligases (E3). In the SUMO cycle, SUMO proteases are responsible for processing of the
327 SUMO precursor and release of SUMO from target substrates. SUMOylation of target proteins
328 plays an important role in plant immunity and is known to be targeted as part of bacterial plant
329 pathogen infection strategies (Verma et al., 2018)). For example, effector XopD from *Xanthomonas*
330 *campestris pv. vesicatoria* (*Xcv*) functions as a SUMO protease inside host cells to modulate host
331 defence signalling (Hotson et al., 2003; Kim et al., 2008). SUMOylation sites are predicted in Mp64
332 (Supplementary Fig. S3) and CRN83-152 (1 SUMO interaction motif: SVEKGANILSVEVPGCDVD;
333 2 SUMOylation sites: VKMLIEVKREVKSAS and WSHPQFEK*******) using prediction software
334 GPS-SUMO (Zhao et al., 2014). Possibly, these effectors are themselves SIZ1 substrates, and
335 their SUMOylation may be required for virulence activity. Our data suggest that modification of host
336 SUMOylation is a common strategy of plant parasites to enable host colonization, and that the
337 targeting strategies have evolved independently in distinct plant-feeding organisms including
338 herbivorous insects. In future work, a detailed analyse of changes in the SIZ1-dependent host
339 plant SUMOylome will be needed to understand how distinct plant parasites promote virulence
340 through SIZ1 targeting.
341

342 **Materials and Methods**

343 **Plants and growth conditions**

344 *Nicotiana benthamiana* plants were grown in a greenhouse with 16h of light and temperature of
345 25° during the daytime.

346 Transgenic Arabidopsis lines *siz1-2*, *eds1-2*, *pad4-1*, *NahG*, *siz1-2*/*NahG*, *siz1-2/eds1-2*, *siz1-*
347 *2/pad4-1* were kindly provided by Dr H.A. van den Burg, The University of Amsterdam. *Arabidopsis*
348 *thaliana* plants were grown in growth chambers with an 8h light/16h dark cycle at 22°/20°
349 (day/night), with a light intensity of 100-200 µmol/m^{-2} s^{-1} and relative humidity of 60%.

350

351 **Aphid rearing and *P. capsici* growth conditions**

352 *M. persicae* (JHI_genotype O (Thorpe et al., 2018)) was maintained on oil seed rape (*Brassica*
353 *napus*) plants in a Perspex growth chamber, with 12h light, and a temperature of 17°C and 50%
354 relative humidity.

355 *P. capsici* isolate LT1534 was maintained on V8 agar cubes at room temperature. For zoospore
356 collection, P. capsici LT1534 was grown on V8 agar plates at 25°.

357

358

359

360 **Plasmid construction**

361 The coding sequence of Mp64, lacking the region encoding the N-terminal signal peptide, was
362 amplified from *M. persicae* (JHI_genotype O) cDNA by PCR with gene-specific primers DONR-
363 Mp64_F and DONR-Mp64_Rev (Supplementary Table 2.) The amplicon was cloned into entry
364 vector pDONR207 (Invitrogen) using Gateway cloning technology. Cloning of the *Phythophthora*
365 *capsici* effector CRN83_152 and the CRN83_152_6D10 mutant was previously described (Stam et
366 al., 2013; Amaro et al., 2018). For *in planta* expression, both effectors were cloned into destination
367 vector pB7WGF2 (N-terminal GFP tag) (Karimi et al., 2002).

368 An entry clone carrying *AtSIZ1* was kindly provided by Dr H.A. van den Burg, The University of
369 Amsterdam. *NbSIZ1* was amplified from *N. benthamiana* cDNA with gene-specific primers NbSIZ1-
370 attB1 and NbSIZ1-attB2 or NbSIZ1-attB2-nostop (Supplementary Table 2). Amplicons were cloned
371 into entry vector pDONR207 (Invitrogen) using Gateway technology. For *in planta* expression,
372 *AtSIZ1* and *NbSIZ1* were cloned into destination vectors pB7FWG2 (C-terminal GFP tag)(Karimi et
373 al., 2002), pK7RWG2 (C-terminal mRFP tag, Karimi et al., 2005), and pGWB20 (C-terminal
374 10xMyc tag) (Nakagawa et al., 2007).

375

376 **Yeast-two-hybrid assays**

377 Yeast two hybrid screening of effectors against a *N. benthamiana* library was based on the
378 Dualsystems Y2H system (Dual Systems Biotech) and performed following the manufacturer's
379 instructions. Bait vectors (pLex-N) carrying effector sequences (lacking the signal peptide encoding
380 sequence) were transformed into yeast strain NMY51. Yeast cells expressing effectors were
381 transformed with a *N. benthamiana* prey library. This prey library was generated in pGAD-HA from
382 cDNA obtained from a combination of healthy leaves, leaves infected with *P. capsici*, and leaves
383 infested with aphids. Transformants were selected on synthetic dropout media plates lacking
384 leucine, histidine and tryptophan (-LTH) with addition of 2.5mM 3-amino-1,2,4-triazole (3-AT).
385 Yeast colonies selected on –LTH media were subjected to the β-galactosidase reporter assays
386 according to manufacturer's instructions (Dual Systems Biotech). The inserts of selected yeast
387 colonies were sequenced and analysed. The Mp64/CRN83_152-NbSIZ1 interaction was validated
388 in yeast by independent co-transformation experiments and reporter assays.

389

390 **Generation of Arabidopsis transgenic lines by floral dipping**

391 *Arabidopsis thaliana* wild type (Col-0) were grown in the greenhouse under long-day conditions
392 (16h of light) until flowering. The flowers of plant were dipped 3 times (one-week interval) in the
393 agrobacterium strain GV3101 suspension of OD_{600} =0.8-2 carrying binary plasmid pB7WG2-Mp64
394 or empty vector pB7WG2. T1 transformants were selected using 100µg/ml BASTA (Glufosinate-

395 ammonium) spray, and T2 seed were selected Murashige Skoog media containing 10µg/mL
396 BASTA. Homozygous T3 plants (predicted single insertion based on 3:1 segregation in T2) were
397 used for aphid performance experiments. Internal primers Mp64 (Mp64-int-F and Mp64-int-Rev)
398 were designed to confirm the presence of Mp64 in transgenic Arabidopsis by RT-PCR.

399

400 **Infection assays on *N. benthamiana* leaves transiently expressing effectors.**

401 *Phytophthora capsici* growth assays were performed on *N. benthamiana* leaves expressing
402 CRN83-152_6D10, Mp64 or the vector control upon agroinfiltration (OD600=0.3 each). Two days
403 after infiltration, leaves were drop inoculated with 5 *µ*L of zoospore solution (50,000 spores/mL)
404 from *P. capsici* strain LT1534. Lesion diameters were measured at 2 days post-inoculation.

405

406 **Pathogen and pest infection/infestation assays on Arabidopsis**

407 Two 2-day old *M. persicae* nymphs (age-synchronized) were placed on 4-6-week old Arabidopsis
408 plants. The plants were placed in a large plastic tube sealed with a mesh lid and placed in a growth
409 cabinet (8h of light, 22/20° for day/night, 60% humidity). The numbers of aphids per plant were
410 counted 10 days post infestation.

411 *P. capsici* isolate LT1534 was grown in V8 agar plate for 3 days in the dark at 25° and exposed to
412 continuous light for 2 days to stimulate sporulation. Sporangia was dislodged and collected with
413 ice-cold water. The sporangia suspension was incubated under light for 30-45min to promote
414 zoospore release. For Arabidopsis infection, 4-6 weeks old plants were spray-inoculated with a
415 spore suspension of 100,000 spores/ml. The percentage of infected leaves was scored 8 days
416 after spray inoculation.

417 Statistical analyses were carried out by using R studio Version 1.2.5001 running R-3.6.1. A linear
418 mixed effects model, with experimental block and biological replicate incorporated as random
419 factors, was used for aphid fecundity assays. A linear mixed effects model, with biological
420 replicates as a random factor, was used for *P. capsici* infection assays. ANOVA was used to
421 analyse the final models, by using emmeans package calculating the Least Squares Means as a
422 post hoc test.

423

424 **Virus-induced gene silencing assays**

425 Tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) was used to silence *NbSIZ1*
426 in *N. benthamiana*. The VIGS construct was generated by cloning a 249-bp fragment of *NbSIZ1*,
427 amplified with primers Sumo_Vigs_Phusion_Frag3_F and Sumo_Vigs_Phusion_Frag3_R
428 (Supplementary Table S2). To generate a TRV-only control, a GFP fragment was amplified using
429 the primers eGFP_Fw and eGFP_Rv. Amplified fragments were cloned into the Tobacco Rattle

430 Virus vector (pTRV2) (Lu et al., 2003) using the In-Fusion HD cloning kit (Clontech).
431 Agrobacterium strains containing desired pTRV2 constructs were co-infiltrated with strains carrying
432 pTRV1 at OD_{600}=0.5 into 4-leaf-stage *N. benthamiana* plants. Three weeks post infiltration, leaves
433 at the same position of different plants were detached for quantification of *NbSIZ1* transcripts by
434 qRT-PCR and *P.capsici* infection assays. Six independent plants were used for each VIGS
435 construct in each replicated experiment, with a total of three replicated experiments. For infection
436 assays on silenced plants, leaves were drop-inoculated with 5μL of zoospore suspension (50,000
437 spores per mL) of *P. capsici* strain LT1534, or for data corresponding to Supplementary Fig S7
438 with 10ul of zoospore suspension (100,000). Lesion diameter was recorded 2-3 days post
439 inoculation. Data analyses was carried out by using R studio Version 1.2.5001 running R-3.6.1.
440 Group comparison was conducted by Mann-Whitney U test for non-normally distributed data.
441

442 **Confocal microscopy**

443 Agrobacterium strain carrying desired constructs were infiltrated individually or in combination in *N.*
444 *benthamiana* plants with an OD_{600} of 0.1. *N. benthamiana* cells were imaged at 1-2 days post
445 infiltration using a Leica TCS SP2 AOBS, Zeiss 710 confocal microscopes with HC PL FLUOTAR
446 63X0.9 and HCX APO L U-V 40X0.8 water-dipping lenses. GFP was excited with 488 nm from an
447 argon laser, and emissions were detected between 500 and 530 nm. The excitation wavelength for
448 mRFP was 561 nm and emissions were collected from 600 to 630 nm. *N. benthamiana* Histone
449 (H2B) fused to mRFP was used as a nuclear marker (Goodin et al., 2007). Single optical section
450 images or z-stacks images were collected from leaf cells those have relatively low expression level
451 to minimize the potential artefacts. Images were projected and processed using the Image J 1.52p-
452 Fiji (Wayne Rasband, National Institute of Health, USA). Further figure generation was processed
453 with Adobe Photoshop CS5.1.

454

455 **Protein Extractions and Co-Immunoprecipitation Assays**

456 Agrobacterium strain GV3101 expressing N-terminal GFP-tagged
457 Mp64/CRN83_152/CRN83_152_6D10 and C-terminal 10xMyc-tagged AtSIZ1/NbSIZ1 were co-
458 infiltrated in *N. benthamiana* leaves with an OD_{600} of 0.3 with addition of p19 (OD_{600}=0.1). Leaf
459 samples were harvested 48 hours post infiltration. For detection of protein expression in total
460 extracts, Laemmli loading buffer (addition of 10mM DTT) was directly added ground leaf samples
461 followed by SDS-PAGE and western blotting. For CO-IP, Equal amounts of plant material was
462 extracted in GTEN buffer (10% glycerol, 25 mM Tris-HCl PH=7.5, 150 mM NaCl, 1mM EDTA)
463 supplemented with protease inhibitor (S8820, Sigma-Aldrich), 2% PVPP, 0.1% NP-40 detergent
464 and fresh 10mM DTT. Samples were incubated for 10 min on ice. The lysate was centrifuged at
465 14460g for 3 times, 4min/each time and then the supernatant was subjected to CO-IP with GFP-

466 Trap®-M magnetic beads (Chromotek) for affinity binding of GFP-fused proteins. Western blotting
467 was performed with the monoclonal GFP antibody raised in mouse (Sigma-Aldrich, cat. no. G1546)
468 and a monoclonal anti cMyc antibody raised in mouse (Santa Cruz, cat. no. SC-40) at 1:3000
469 dilution, followed by anti-mouse Ig-HRP antibody (Sigma-Aldrich, cat. no. A9044) at 1:5000 dilution
470 to detect corresponding epitopes.

471

472 **RNA extraction and Quantitative RT-PCR**

473 Total RNA was extracted by using RNeasy Mini Kit (Qiagen) with a following DNase I treatment
474 (Invitrogen™) from VIGS *N. benthamiana*. 1 µg RNA was reverse-transcribed using SuperScript III
475 reverse transcriptase (Sigma-Aldrich, UK) following the manufacturer's protocol. RT-qPCR
476 experiment was designed following the MIQE guidelines (Bustin et al., 2009) with gene specific
477 primers (Supplementary Table S2). Herein EF1α (accession number: TC19582 (At5g60390)) and
478 PP2A (accession number: TC21939 (At1g13320)) (Liu et al., 2012) were used as reference genes.
479 For PCR amplification, GoTaq® RT-qPCR system (Promega, UK) was used on a StepOne™ Real-
480 Time PCR Machine (Applied Biosystems, UK). In each reaction, 12.5 µl reactions with a final
481 concentration of 1x GoTaq® qPCR Master Mix, 1µM of each primer, 1.4 mM MgCl2, 2.4 µM CXR
482 reference dye and a cDNA quantity of approx. 25 ng. The PCR program was set as follows: 95° for
483 15 mins followed by 40 cycles of 15s at 95°, 30s at 60°, and 30s at 72°. A melting curve was
484 generated at the end of the PCR program and $2^{-\Delta\Delta Ct}$ value (Livak and Schmittgen, 2001) was
485 calculated to determine the relative expression of *NbSIZ1*. Three technical replicates were
486 performed in each run and three biological replicates were carried out throughout the experiment.

487

488 **Electrical penetration graph (EPG) monitoring of aphid feeding**

489 We used the EPG technique (Tjallingii, 1978) (monitor the probing and feeding behaviour of *M.*
490 *persicae* on Arabidopsis Col-0 and the *siz1-2* mutant using a Giga-4 DC-EPG device with 1 Giga Ω
491 resistance (EPG systems, The Netherlands). Experimental set up was described by Leybourne et
492 al. (2019)(Leybourne et al., 2019). Software Stylet +D was used for data collection (EPG systems,
493 The Netherlands). A total of 12 and 15 successful recordings were obtained from aphids feeding
494 on Col-0 and *siz1-2* Arabidopsis plants, respectively. Different waveform patterns were observed,
495 such as np (non-probing), C (stylet penetration/pathway), pd (intercellular punctures), E1 (saliva
496 secretion into phloem), E2 (saliva secretion and passive phloem ingestion), F (mechanical
497 penetration difficulty) or G (xylem ingestion) phases (Tjallingii, 1988; Alvarez et al., 2006). R studio
498 Version 1.2.5001 running R-3.6.1 was used for statistical analyses. A permutated MANOVA was
499 fitted to the dataset for global assessing of aphid feeding behavior, and Mann-Whitney U test was
500 used to carry out pairwise comparisons.

501

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510 Institute under license PH\6\2015. The authors declare no conflict of interest.

Figures and Tables

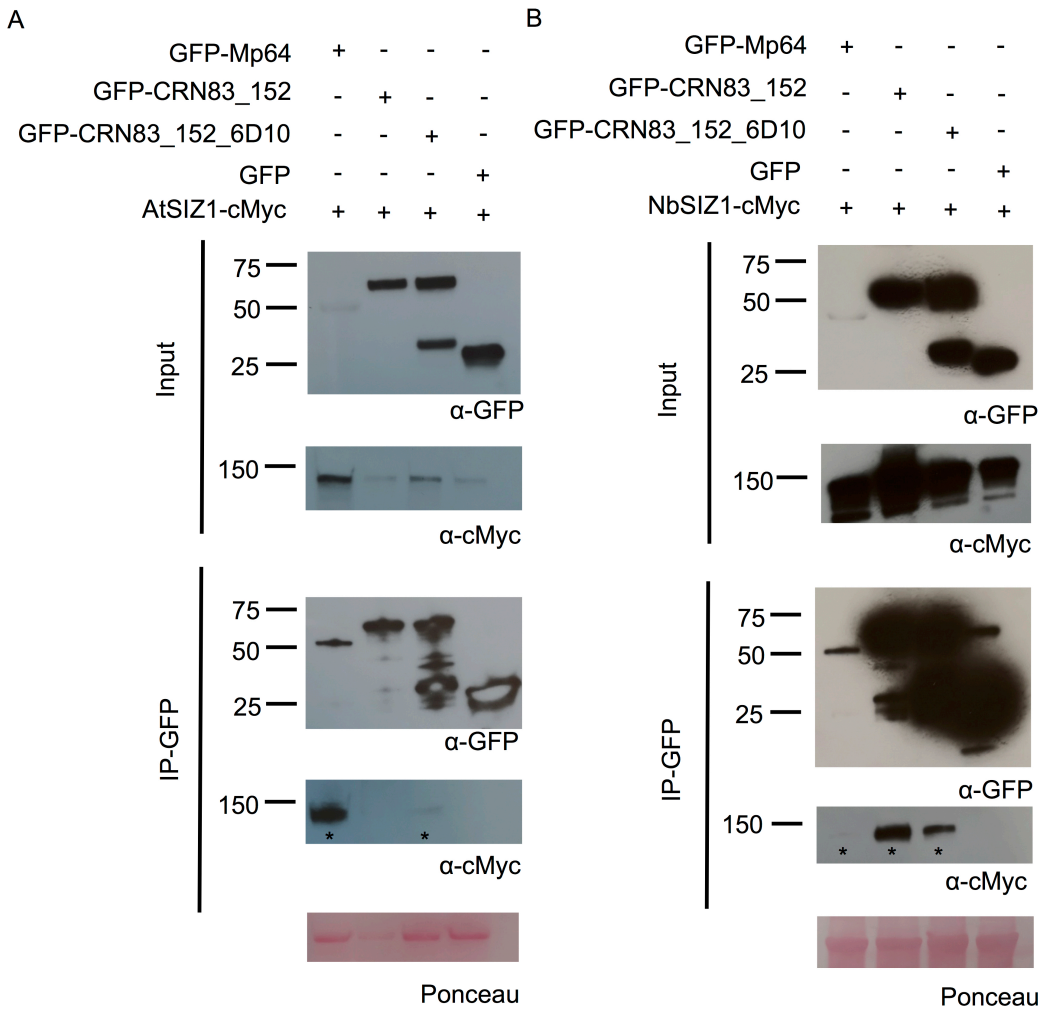

Fig. 1. *Myzus persicae* effector Mp64 and *Phytophthora capsici* effector CRN83_152 associate with SIZ1 from Arabidopsis and *Nicotiana benthamiana*.

(A) Immunoprecipitation (IP) of protein extracts from agroinfiltrated leaves using GFP-Trap confirmed that AtSIZ1-cMyc(10x) associates with GFP-Mp64 and GFP-CRN83_152_6D10, but not with the GFP control.

(B) Immunoprecipitation (IP) of protein extracts from agroinfiltrated leaves using GFP-Trap confirmed that NbSIZ1-cMyc(10x) associates with GFP-Mp64, GFP-CRN83_152 and GFP-CRN83_152_6D10, but not with the GFP control. Protein size markers are indicated in kD, and protein loading is shown upon ponceau staining of membranes. Experiments were repeated at least three times with similar results.

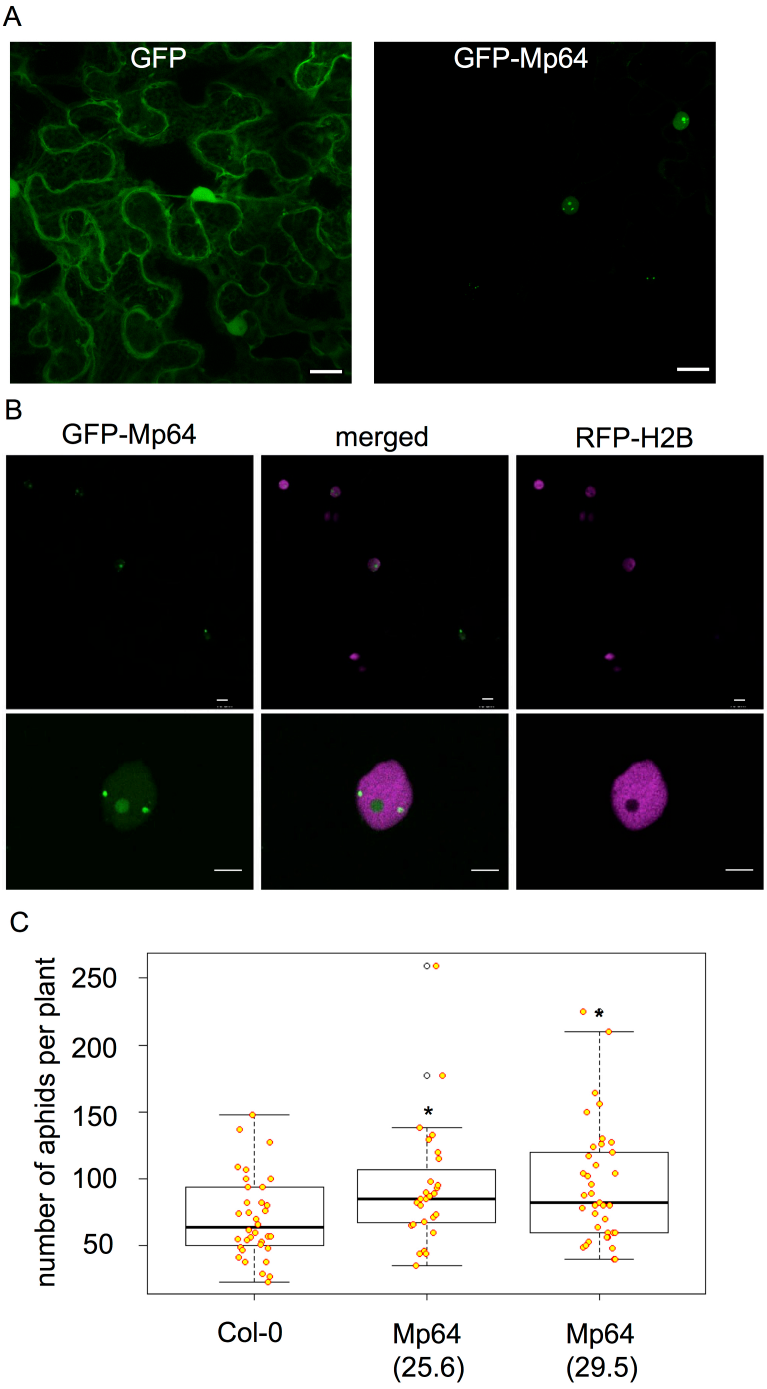

Fig. 2. Constitutive ectopic expression of nuclear aphid effector Mp64 in Arabidopsis enhances susceptibility to *Myzus persicae*.
(A) Confocal images showing localisation of aphid effector Mp64 in host *Nicotiana benthamiana*. Leaves transiently expressing GFP-Mp64 or GFP alone were used for confocal imaging 2 days after agroinfiltration. Scale bar represents 20µm.
(B) Confocal images showing nuclear localisation of aphid effector Mp64 in host *Nicotiana benthamiana*. Leaves transiently expressing GFP-Mp64 and histone marker RFP-Histone 2B (H2B), a nuclear marker, were used for confocal imaging 2 days after agroinfiltration. Images in the upper panel were collected as z-stack projection and those in the lower panel were collected as single optical sections through nuclei of cells ectopically overexpressing Mp64. Scale bar represents 10µm.
(C) Two Arabidopsis transgenic lines, 25.6 and 29.5, were challenged with two apterous adult aphids. Total numbers of aphids per plant were counted 10 days post infestation. The boxplot displays the distribution of datapoints from three independent biological replicates (n=10 per

replicate). The black line within the box represents the median. The top and bottom edges of the box indicate upper quantile and lower quantile. The datapoints out of the upper and lower extreme of the whisker are outliers. Asterisks denote significant difference between treatments and control (Mann-Whitney U test, $p<0.05$).

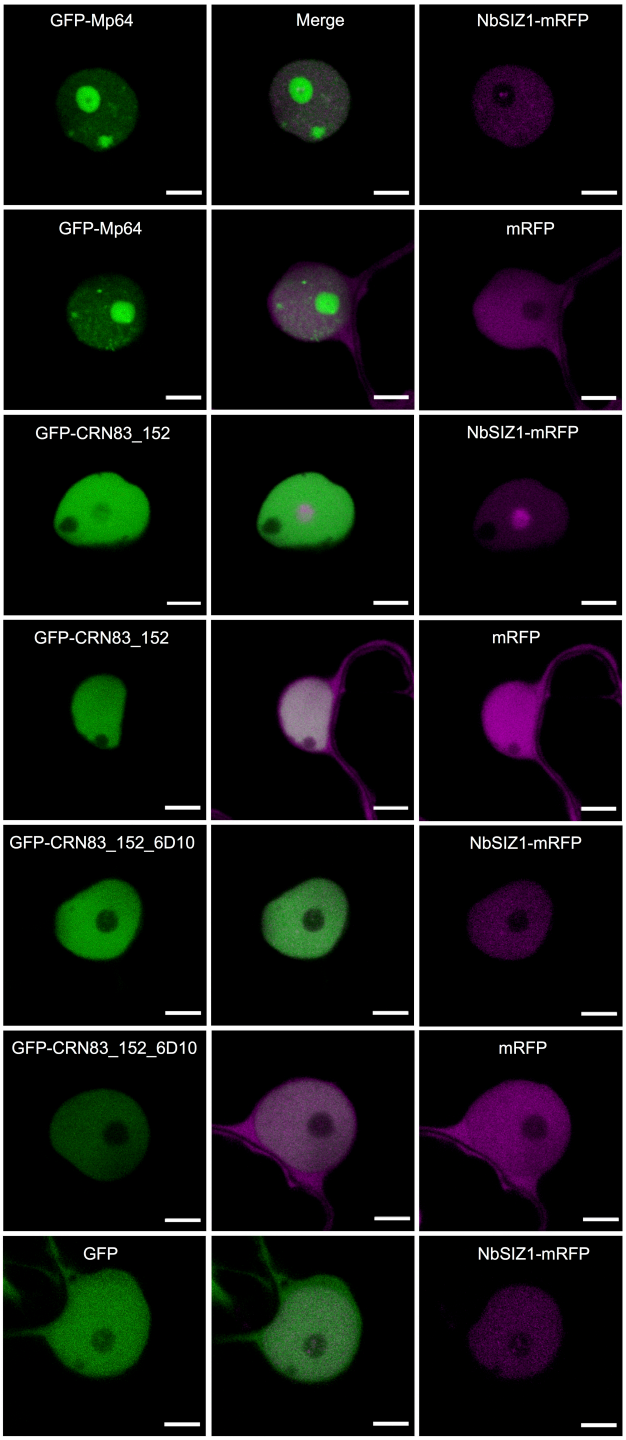

Fig. 3. Effectors Mp64 and CRN83_152 co-localize with NbSIZ1 in the host nucleus.
Leaves transiently expressing GFP-Mp64, GFP-CRN83_152 or GFP-CRN83_152_6D10 in combination with RFP or NbSIZ1-RFP were used for confocal imaging 2 days after agroinfiltration. Images show single optical sections through nuclei co-expressing the GFP-effector with NbSIZ1-RFP or RFP control. Scale bars represent 5 µm.

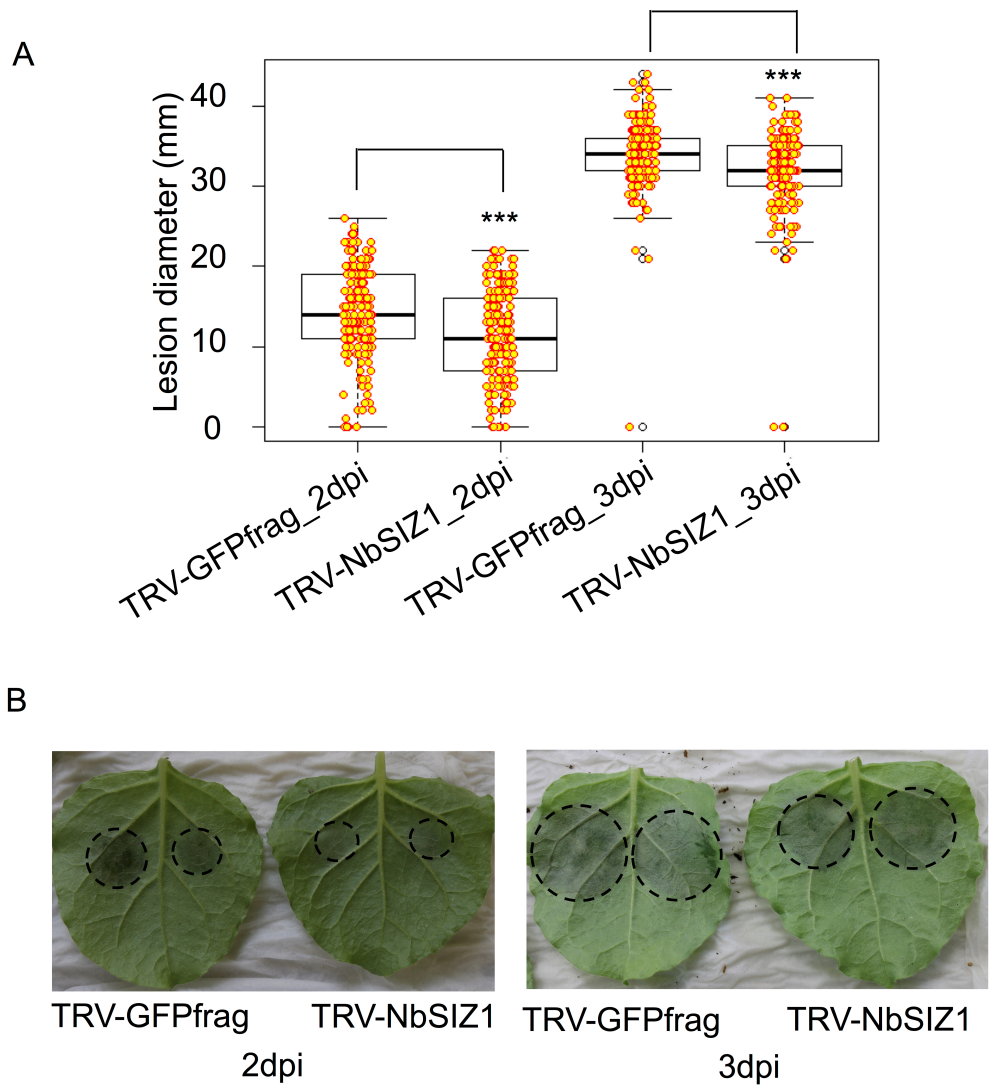

Fig. 4. Virus-induced gene silencing of *NbSIZ1* reduces host susceptibility to *Phytophthora capsici*.

(A) Boxplot showing the lesion diameter of *P. capsici* infection sites on *Nicotiana benthamiana* control (TRV-GFPfrag) or *NbSIZ1*-silenced plants (TRV-NbSIZ1). 5ul of zoospore suspension (50,000 spores/ml) was drop-inoculated on *N. benthamiana* leaves. Data was collected 2 and 3 days post inoculation (dpi) from three biological replicates (n=48 per biological replicate). Asterisks denote significant difference between the GFPfrag control and *NbSIZ1*-silenced plants (Mann-Whitney U test, $p<0.0001$).

(B) Representative images of *NbSIZ1*-silenced and GFPfrag control leaves 2 days and 3 days post inoculation (dpi) with *P. capsici* zoospores.

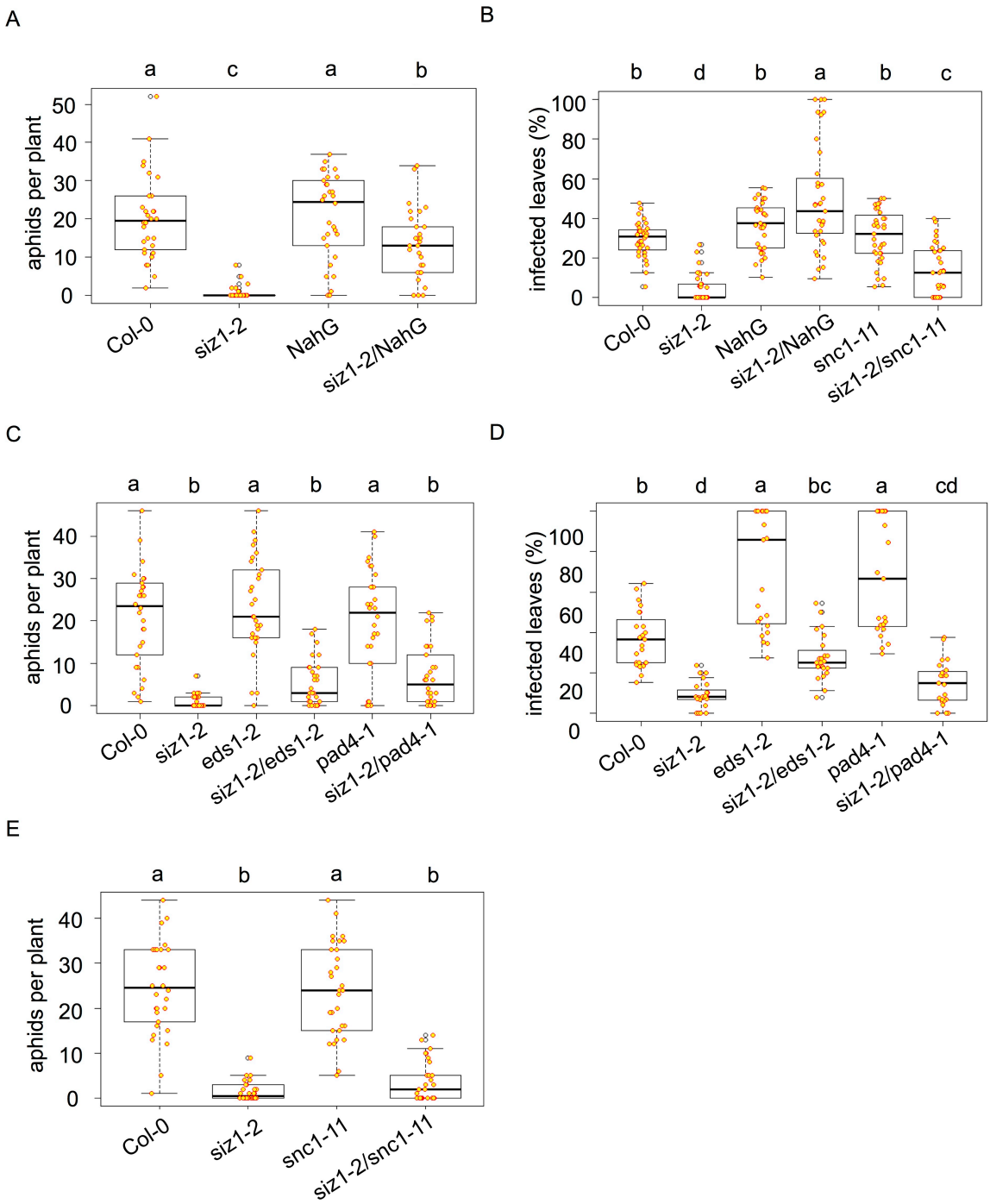

Fig. 5. The Arabidopsis *siz1-2* mutant shows reduced susceptibility to *Myzus persicae* and enhanced resistance to *Phytophthora capsici*.

For aphid infestation assays (A, C, E) plants were infested with two 2-day old nymphs and the aphid populations were counted 10 days later. For *P. capsici* infection assays (B, D) plants were spray-inoculated with a zoospore suspension of 100,000 spores/ml and the percentage of symptomatic leaves was recorded 8 days later. A linear mixed effects model with experimental block and biological replicate as random factors was fitted in dataset from aphid fecundity assays. A linear mixed model with biological replicate as random effect was used in dataset of *P. capsici* infection assays. ANOVA was used to analyse the final models and a post-hoc test was performed

by calculating the Least Squares Means by using an emmeans package in R. Different letters denote significant differences within a set of different plant genotypes.

(A) Arabidopsis mutant *siz1-2* is less susceptible to *M. persicae* (aphids) than the Col-0 control with significantly less aphids recorded on the mutant versus control plants ($p<0.0001$), including in the *NahG* background ($p<0.01$).

(B) Arabidopsis mutant *siz1-2* shows enhanced resistance to *P. capsici* compared with the Col-0 control, with significantly less infected leaves on the mutant versus control plants ($p<0.0001$). In the *NahG* background, *siz1-2* is associated with increased infection compared to the *NahG* control ($p<0.01$). The *siz1-2/snc1-11* mutant shows enhanced resistance to *P. capsici* compared with the *snc1-11* mutant, with significantly less leaves infected on the double mutant ($p<0.0001$).

(C) Arabidopsis mutants *siz1-2/esd1-2* and *siz1-2/pad4-1* are less susceptible to *M. persicae* (aphids) than the *eds1-2* and *pad4-1* mutants, respectively, with significantly less aphids recorded on the double compared with the single knock-out mutants ($p<0.0001$).

(D) Arabidopsis mutants *siz1-2/eds1-2* and *siz1-2/pad4-1* are more resistant to *P. capsici* than the *eds1*-2 and *pad4-1* mutants, respectively, with significantly less leaves infected on the double compared with the single knock-out mutants ($p<0.0001$).

(E) Arabidopsis mutant *siz1-2/snc1-11* is less susceptible to *M. persicae* (aphids) than the compared *snc1-11* mutant, with significantly less aphids recorded on the double mutant ($p<0.0001$).

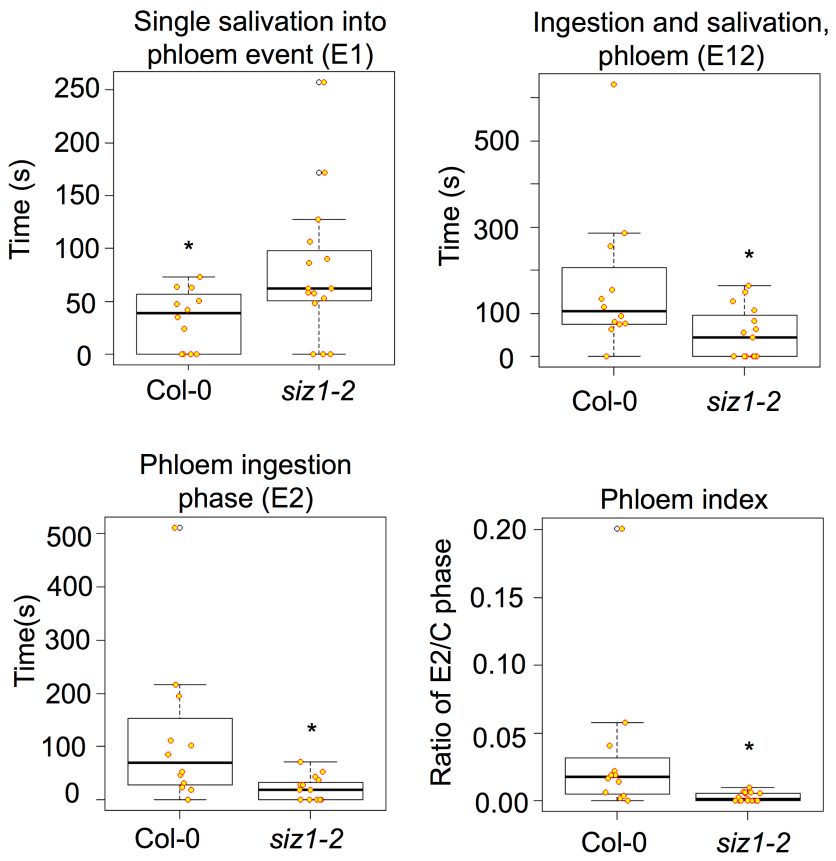

Fig. 6. Aphids show reduced phloem ingestion on the Arabidopsis *siz1-2* mutant. Boxplots show significant EPG parameters indicative of reduced phloem interaction on Arabidopsis *siz1-2* compared with Col-0 plants. E1 phase indicates the phase where aphids secrete saliva into the phloem, E2 phase indicates the phase where aphids ingest phloem sap, E12 phase indicates the phase where aphids either secrete into/ingest phloem, and E2/C ratio indicates the ratio of the time aphids spend on ingesting phloem sap relative to time spend on stylet pathway activities (intercellular). Data was analysed in R studio Version 1.2.5001 running R-3.6.1. Asterisks denote significant difference between the *siz1-2* mutant and Col-0 control (Mann-Whitney U test, $p<0.05$)

Table 1. Overview of significant EPG parameters based on Mann-Whitney U test ($p<0.05$). E1, salivation into phloem; E2, phloem ingestion; E12, fractions of E1 within periods of E1 and E2 waveforms; C, stylet pathway phase, mainly intercellular.

EPG parameter	Hypothesised location of resistance factor	Number of individuals which produced waveform		P value (Mann–Whitney U test)
		Col-0	siz1-2	
All parameters (Global analysis)	All tissue	-	-	0.054
Average duration single E1 period (s)	Phloem	8/12	12/15	0.046
Maximum duration of single E1 period (s)	Phloem	8/12	12/15	0.036
Number of E1 fraction periods	Phloem	11/12	8/15	0.016
Number of E12 periods	Phloem	11/12	8/15	0.017
Average duration of E12 (s)	Phloem	11/12	8/15	0.018
Median duration of E12 (s)	Phloem	11/12	8/15	0.034
Total duration of E12 (s)	Phloem	11/12	8/15	0.006
Maximum duration of E12 (s)	Phloem	11/12	8/15	0.012
Number of E2 periods	Phloem	11/12	8/15	0.017
Average duration of E2 phase (s)	Phloem	11/12	8/15	0.005
Median duration of E2 phase (s)	Phloem	11/12	8/15	0.008
Total duration of E2 phase (s)	Phloem	11/12	8/15	0.003
Maximum duration of E2 phase (s)	Phloem	11/12	8/15	0.005
Time from first E1 phase until first E2 phase (s)	Phloem	12/12	15/15	0.037
Ratio of E2 phase to C phase (%)	Phloem	11/12	8/15	0.002
Ratio of number of E1 fractions to number of E12 (%)	Phloem	11/12	8/15	0.016
Ratio of E2 to total duration after 1st E2 start (%)	Phloem	11/12	8/15	0.006

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