

1 **Title**

- 2 ● Full title (107/135 characters): Impairment of the cellulose degradation machinery  
3 enhances fungal virulence but limits reproductive fitness  
4 ● Short title (49/50 characters): Role of cellulose degradation in fungal infection

5

6 **Authors**

7 Francisco M. Gámez-Arjona<sup>1</sup>, Stefania Vitale<sup>2†</sup>, Aline Voxeur<sup>3</sup>, Susanne Dora<sup>1</sup>, Sascha  
8 Müller<sup>1</sup>, Gloria Sancho-Andrés<sup>1</sup>, Juan Carlos Montesinos<sup>1</sup>, Antonio Di Pietro<sup>2</sup>, Clara Sánchez-  
9 Rodríguez<sup>1\*</sup>

10

11 **Affiliations**

12 <sup>1</sup>Department of Biology, ETH Zurich, 8092 Zurich, Switzerland.

13 <sup>2</sup>Departamento de Genética, Campus de Excelencia Internacional Agroalimentario ceiA3,  
14 Universidad de Córdoba, 14014 Córdoba, Spain.

15 <sup>3</sup>Institut Jean-Pierre Bourgin, INRA, Centre National pour la Recherche Scientifique,  
16 AgroParisTech, Université Paris-Saclay, RD10, 78026, Versailles Cedex, France.

17

18 <sup>†</sup>Current address: IPSP-Instituto per la Protezione Sostenibile delle Piante, CNR, 80055  
19 Portici, Italy.

20

21 \* Corresponding author: [clara\\_sanchez@ethz.ch](mailto:clara_sanchez@ethz.ch)

22

23 **Abstract (144/150 words)**

24 Fungal endophytes grow in the apoplastic space, in constant contact with the plant cell wall  
25 (CW) that hinders microbe progression, while representing a source of nutrients. Although  
26 numerous fungal CW modifying proteins have been identified, their role during host  
27 colonization remains underexplored. Here we show that the root-infecting plant pathogen  
28 *Fusarium oxysporum* (Fo) does not require its complete arsenal of cellulases to infect the host  
29 plant. Quite the opposite, Fo mutants impaired in cellulose degradation become hypervirulent  
30 by enhancing the secretion of virulence factors. On the other hand, the reduction on cellulase  
31 activity had a severe negative effect on saprophytic growth and microconidia production during  
32 the final stages of the Fo infection cycle. These findings enhance our understanding on the  
33 function of plant CW degradation on the outcome of host-microbe interactions and reveal an  
34 unexpected role of cellulose degradation in a pathogen's evolutionary success.

35

36 **Teaser (123 /125 character):**

37 Unexpectedly, fungi compromised in their capacity to degrade plant cellulose are hypervirulent  
38 but impaired in sporulation.

## 39 MAIN TEXT (12,054/15,000 words)

### 40 Introduction

41 Most plant-microbe interactions are initially established at the apoplast. Here, the plant CW  
42 constitutes a source of nutrients and a physical barrier for the intruder, making its modification  
43 an important aspect of microbe colonization of the host. The main components of plant CWs  
44 are the para-crystalline fibres of cellulose embedded in a matrix of hemicellulose, pectin, lignin  
45 and suberin (1–3). Ions and wall remodeling proteins modify the interactions between CW  
46 polysaccharides *in muro* changing the physicochemical properties and, consequently, the  
47 structure and function of the CW (4, 5). The CW composition is not homogenous among  
48 different plant species, tissues or even within a single cell (6). This molecular complexity forces  
49 plant-colonizing microbes to metabolically regulate the secretion of a broad set of CW  
50 modifying proteins (CWMP) required to loosen and digest the plant CWs they encounter (7,  
51 8). The majority of these CWMPs are CW degrading enzymes (CWDEs), including glycosyl-  
52 hydrolases, lytic polysaccharide monoxygenases, pectate and pectin lyases, and esterases,  
53 which are further divided into different subgroups based on sequence similarity in the catalytic  
54 domain (9–13). Although all plant-colonizing microbes encode for CWMPs, it has been  
55 suggested that the role of these enzymes is more prominent in pathogens, which rely on their  
56 activity during host infection (14–18). However, so far the large functional redundancy of  
57 CWDEs has largely prevented a complete assessment of their role (19), since deletion of  
58 individual CWMEs genes do not generally affect the virulence of the pathogen (20). Fungi have  
59 evolved transcription factors that co-regulate the expression of CWMP groups based on the  
60 composition of their environment. Therefore, it has been suggested that targeting such master  
61 transcriptional regulators should allow the assessment of the role of a given group of CWMPs  
62 (21).

63 In their co-evolution journey, while microbes adapted to loosen and break the host CWs, plants  
64 evolved to perceive these degradation products as damage-associated molecular patterns  
65 (DAMPs). These DAMPs, together with microbe-associated molecular patterns (MAMPs) such  
66 as fungal chitin (22), activate the plant pattern-triggered immunity against the intruder (23, 24).  
67 The best characterized CW-derived DAMPs are pectin-derived oligogalacturonides (OGs),  
68 resulting from pectinase activity (25–28). Cellulose degradation products, i.e., like cellobiose,  
69 have also been shown to induce plant defense and are, therefore, considered potential  
70 DAMPs, although their release upon microbe colonization has not yet been proven (25–27).

71 The plant CW characteristics directly influence the communication between the two organisms.  
72 This is particularly relevant for plant response to microbes that mainly live in the apoplast, such  
73 as vascular fungi belonging to the *Fusarium oxysporum* (Fo) species complex, which are  
74 further classified into different formae speciales (ff.spp.) based on their host preference (29).  
75 Fo<sub>s</sub> are soil-borne microbes that colonize the roots of many plant species, being responsible  
76 for the devastation of many economically important crops throughout the world (29). Fo<sub>s</sub> are  
77 considered pathogenic when they cause plants to wilt and eventually die, which occurs  
78 because water flow and nutrient uptake are blocked by fungal proliferation inside the xylem.  
79 To reach the vascular system, Fo grows predominantly in the root apoplast (30, 31), making  
80 this fungus an ideal model to study CW-microbe interaction.

81 Cellulose is the most abundant and most recalcitrant component of the CW that provides  
82 mechanical strength to the plant cells thanks to its paracrystalline structure (7). Consequently,  
83 its synthesis is tightly regulated in response to intruders, including Fo (32–34) cellulose is one  
84 of the main targets of microbial CWMPs. Pathogens secrete a broad range of cellulolytic  
85 enzymes (glycosyl-hydrolases (GHs) and lytic polysaccharide monoxygenases (AAs)) (16),  
86 suggesting that this family of enzymes should be essential for infection. An optimal degradation  
87 of complex carbohydrates requires a hierarchical metabolic response. In the case of crystalline  
88 cellulose, AAs disrupt the outermost crystalline part of the cellulose fiber, thereby allowing the  
89 remaining cellulases to participate in the degradation process (9, 10, 35). The transcriptional

90 regulation of fungal cellulases has been reported to be dependent on a conserved zinc  
91 binuclear cluster transcription factor named CLR1. CLR1 regulates the expression of  
92 cellulolytic enzymes in *Neurospora crassa* and other ascomycetes and is, therefore, required  
93 for growth on cellulose as the sole carbon source (7, 36, 37).

94 Here we investigated the impact of cellulose degradation during microbe infection by  
95 identifying and targeting the orthologues of *N. crassa* CLR1 in *Fo* f.sp. *conglutinans* (Fo5176)  
96 and *lycopersici* (Fol4287), pathogens of *Arabidopsis thaliana* and *Solanum lycopersicum*,  
97 respectively (38, 39). Unexpectedly, our data show that crystalline cellulose degradation is not  
98 only dispensable, but disadvantageous, for *Fo* infection, as shown by the increased virulence  
99 displayed by *clr1* mutants. Using the model pathosystem *Arabidopsis thaliana*-Fo5176 ((33,  
100 34, 40, 41), we show that the *clr1* mutant compensates for cellulose degradation deficiency by  
101 increasing the secretion of various virulence factors to compromise plant immune responses.  
102 On the other hand, the reduction of cellulose degradation capacity severely compromised  
103 fungal metabolism during its saprophytic growth, with dramatic consequences for its  
104 microconidia production. Taken together, our findings expand the current understanding of  
105 plant-microbe interactions, showing that the cellulose is not the assumed central physical  
106 barrier against fungi, but a primary source of carbon to complete the pathogen's life cycle in  
107 the plant.

108  
109

## 110 Results

### 111 The transcriptional regulator CLR1 is required for efficient cellulose degradation by 112 Fo5176

113 To understand the role of *Fo* cellulases in root infection, we aimed to obtain a Fo5176 mutant  
114 impaired in cellulose degradation. Considering the large number of putative cellulases  
115 expressed by Fo5176 during root colonization (34) and their high functional redundancy, we  
116 chose to target the transcription factor CLR1, a master regulator of fungal cellulase expression  
117 previously identified in *N. crassa* (36). Using an *in silico* analysis, we found CLR1 orthologues  
118 in various *Fusarium* genomes, sharing a protein sequence identity above 70% and having the  
119 same DNA-binding domain sequence (Fig. 1A and 1B). Therefore, we generated a CLR1 null  
120 mutant in the previously described Fo5176 *pSIX1::GFP* background, which allows for  
121 monitoring Fo5176 colonization of the root vasculature (33, 41). We obtained deletion mutants  
122 lacking the entire CLR1 coding region (*clr1-1* and *clr1-2*) and a complemented strain (*clr1C*)  
123 generated by reintroducing the wild-type *pCLR1::CLR1* into the *clr1-1* mutant background (Fig.  
124 S1). We first determined whether *clr1* mutants were affected in stress response by growing  
125 them in control conditions (YPD), salt stress (NaCl), osmotic stress (Sorbitol), CW stress  
126 (Congo Red and Calcofluor) (42) as well as on minimal medium. There were no detectable  
127 differences in the colony phenotype of *clr1* mutants compared to the wild type (WT) in any of  
128 these conditions (Fig. S2). Next, we evaluated the role of CLR1 in cellulose degradation by *Fo*  
129 by testing the ability of *clr1-1*, *clr1C* and WT to utilize different carbon sources such as sucrose,  
130 cellobiose and cellulose. Fungal growth on sucrose was similar across all genotypes (Fig. 1C  
131 and S3). The *clr1-1* mutant exhibited a significant growth reduction on cellobiose and only  
132 showed residual growth on cellulose compared to WT or *clr1C* (Fig. 1C, 1D and S3). A similar  
133 phenotype was observed for *clr1-2* (Fig. S4A). These data are similar to those described in *N.*  
134 *crassa* (36), indicating that the essential function of CLR1 in cellulose catabolism is conserved  
135 across different ascomycetes.

### 136 Loss of CLR1 leads to faster colonization of the root cortex and xylem

137 To determine whether *Fo* requires cellulose degradation to infect its plant hosts, we performed  
138 plate infection assays as described before (41). We monitored root vascular colonization by  
139 following GFP signal presence in the root vasculature at different days post-transfer (dpt) to  
140 spore-containing plates. Unexpectedly, we counted a significantly higher number of vascular  
141 penetrations of *clr1-1* than WT at all time points, correlated with a higher expression of *FoSIX1*  
142 and an increased *in planta* fungal biomass at 7dpt measured by qRT-PCR, which was restored

143 to WT levels in the *clr1C* strain (Fig. 2A-C). A second isogenic mutant, *clr1-2*, also showed a  
144 higher capacity than WT to reach the host xylem (Fig. S4B). We therefore focused on the *clr1-1*  
145 mutant for further characterization. Using confocal microscopy, we observed that *clr1-1*  
146 advanced faster than WT through the epidermal apoplast as could be seen in the epidermis-  
147 cortex interface at 3dpt with a significant higher frequency than the WT fungus (Fig. 2D and  
148 2F). These results indicate that *clr1-1* is more efficient than WT in growing through the host  
149 CWs and reaching the xylem and might explain the higher levels of vascular invasion observed  
150 at later stages of the infection (Fig. 2A-C).

151 At 7dpt, when the fungus is colonizing the vasculature in all roots, we observed a reduced  
152 expression of genes required for cellulose degradation in *clr1-1* compared to WT including  
153 endoglucanase, glycosyl hydrolase family 5 member, two AA9 enzymes, and a periplasmic  $\alpha$ -  
154 glucanase, as well as for sugar metabolism such as a plasma membrane hexose transporter  
155 and an intracellular  $\alpha$ -glucanase (Fig 2G). These are orthologues of *N. crassa* genes  
156 previously reported to be regulated by CLR1 during growth on cellulose (43). Importantly, we  
157 detected a lower amount of residual cellulose in roots infected with WT compared to those  
158 infected with *clr1* (Fig. 2H), in line with the results of growth on cellulose (Fig. 1C). Taken  
159 together, our data reveal that CLR1 is required by Fo for efficient degradation of cellulose  
160 during root colonization but dispensable for reaching the plant vasculature.

### 161 **Arabidopsis defense response is delayed upon *clr1-1* infection compared to WT**

162 The reduction in cellulase activity observed in *clr1-1* might reduce the amount and/or nature  
163 of the CW-derived DAMPs generated by this mutant compared to the WTs. Therefore, the  
164 increased virulence of the cellulase-deficient *clr1* mutant might be a consequence of an  
165 impaired activation of plant immune responses. To address this hypothesis, we measured the  
166 expression of three genes previously reported to be activated in Arabidopsis roots infected by  
167 Fo5176; *At1g51890*, *WKRY45* and *WKRY53* (33, 44). At 2 and 3dpt, the expression of the  
168 three defense genes was lower in plants infected with *clr1* than WT (Fig. 3A). These  
169 differences progressively disappeared with the increased vascular colonization. At 7dpt, when  
170 the xylem was abundantly occupied by *clr1-1*, the plant defense gene expression was higher  
171 than in roots infected with WT (Fig. 3A). These findings suggested that the higher number of  
172 vascular penetrations observed upon *clr1-1* infection (Fig. 2A) might be a consequence of  
173 delayed activation of defense related genes during the early stages of the interaction.

174 We next tested whether external addition of CW-derived DAMPs could reduce the virulence  
175 of *clr1-1* to WT levels. We used cellobiose, a cellulose-degradation product confirmed to  
176 induce plant defense (27), to prime the root immune system. We pretreated plants for 25  
177 minutes with this molecule before transferring them to plates containing 2-day-old WT or *clr1-1*  
178 mycelium. We confirmed that cellobiose-induced root-defense priming returned the plant  
179 susceptibility of *clr1* to WT levels (Fig. 3B).

### 180 **Different amounts of CW degradation products accumulate upon *clr1-1* infection**

181 The reduced cellulose degradation and the concomitant absence or decrease of cellobiose in  
182 the apoplast during *clr1-1* infection might explain the delay in perception of this mutant by the  
183 root and its increased virulence. To test this idea, we measured the oligosaccharides derived  
184 from plant CW degradation by Fo5176 using HP-SEC-MS/MS (45). By employing different  
185 commercial standards to identify the different hexoses and saturated OGs (Fig. 4A), we  
186 identified cellobiose in Fo5176-infected but not mock-treated roots confirming its predicted role  
187 as DAMP. Our results showed a shift in the di-hexose profiles from sucrose- to cellobiose-  
188 enriched at 2dpt and 3dpt, respectively (Fig. 4B). From 3 dpt on the amount of di-hexoses,  
189 mainly cellobiose, was higher during infection with *clr1-1* compared with then WT (Fig. 4B and  
190 S5). The HP-SEC-MS results indicate that the impaired capacity of the root to detect *clr1-1*  
191 can not be explained by a reduced release of cellobiose by the mutant. Therefore, we studied  
192 the OGs produced by Fo5176 and identified acetylated OGs as a main product of plant CW  
193 degradation by both *clr1* and WT (Fig. 4B, 4D, 4E and S5). Moreover, less OGs were detected

194 during *clr1* infection compared to WT-infected roots, especially at 3 and 4dpt (Fig 4C-E and  
195 S5). This difference was statistically significant for Gal<sub>4</sub>Ac and Gal<sub>2</sub>ox (Fig. 4E and 4D). The  
196 decreased accumulation of OGs during the early stages of plant infection is in line with the  
197 compromised perception of *clr1-1* by the root. We detected significant differences in the  
198 amount of plant CW-degradation products in WT- and *clr1-1* infected roots, that might act as  
199 DAMPs. Therefore, we next tested whether WT-generated DAMPs can restore plant  
200 susceptibility to *clr1-1* by performing plate infection assays using the same number of  
201 microconidia concentration of the *clr1-1*, WT or both (50% WT+50% *clr1-1*). We expected to  
202 see a decrease in *clr1-1* virulence proportional to the concentration of WT spores in the mixed  
203 treatment if DAMP accumulation is the main reason for the differential plant susceptibility to  
204 *clr1-1* and WT6. By contrast, we found that the 50%-mixed infection displayed the same  
205 pathogenicity as *clr1-1* alone (Fig. S6A), observing a decrease in xylem penetration only when  
206 90% of the spores were WT (Fig. S6B). Our data indicate that although *clr1-1* infection  
207 generates different amounts of CW-degradation products than WT, they alone do not explain  
208 *clr1-1* higher pathogenicity.

### 209 ***clr1-1* secretes more virulence factors than WT during root colonization**

210 Pathogenicity relies on the ability of microorganisms to evade the MAMP- and DAMP-induced  
211 host defenses using secreted and cell-surface localised virulence factors (46). We performed  
212 proteomic analysis of the secretomes of WT and *clr1-1* during plant infection to determine  
213 whether differences in their profile of secreted virulence factors could explain the higher  
214 virulence of *clr1-1*. Seedlings growing in hydroponics were inoculated with either WT or *clr1-1*  
215 microconidia. 362 fungal proteins were identified, 75% with predicted secretory signal  
216 peptides. Of these, more than 50% belong to groups considered as virulence factors in other  
217 plant pathogens such as *F. graminearum* (46), including glycosyl hydrolases (26%),  
218 peptidases (13%), redox-related proteins (10%) and pectin and pectate lyases (4%) (Fig. 5A).

219 As expected, we found a significant reduction in the abundance of proteins required for  
220 cellulose degradation in the *clr1-1* secretome (Fig. 5B and Table 1), including GH5 (g17181),  
221 GH6 (g15944), and GH7 (g873) which are predicted to exhibit cellulolytic activity (47), as well  
222 as AA9s that make crystalline cellulose accessible to cellulases (10). These data confirm the  
223 downregulation of cellulase-related genes observed in *clr1-1* and explain its reduced capacity  
224 to degrade cellulose (Fig. 1C, 2D, 2F, 2G, and S3).

225 In contrast, we found an increased presence of other virulence factors in the secretome of *clr1-1*  
226 compared to that of WT (Fig. 5B and Table 1). Interestingly, *clr1-1* releases increased  
227 amounts of a protein (g2038) annotated as a cutinase, which was reported to be essential for  
228 virulence of necrotrophic fungal pathogens (48). Additionally, we found an increase in pectin  
229 and pectate lyases in the *clr1-1* secretome (49–51). Similarly, a set of 8 peptidases previously  
230 described as critical virulence factors (52), were more abundant in the *clr1-1* secretome. These  
231 included 4 subtilases and fungalysin, among others (Table 1). The subtilases are able to  
232 degrade plant proteins with antifungal activity, such as  $\beta$ 1-3 glucanases (53). The fungalysin  
233 proteins compromise host defense by cleaving class IV plant chitinases, preventing the release  
234 of the MAMP chitin and, as such, have been reported to be necessary for the virulence of  
235 several plant pathogenic filamentous ascomycetes, including *Ustilago maydis*, *F. oxysporum f*  
236 *sp. lycopersici* (Fol), *F. verticillioides* and *Colletotrichum graminicola* (54–57). Finally, we  
237 noticed an enrichment in a chitin deacetylase, whose orthologs impede chitin-triggered  
238 immunity in cotton by converting chitin oligomers into ligand-inactive molecules like chitosan  
239 (58). Our data indicate that *clr1-1* reduces the plant detection of its chitin, which could explain  
240 its increased virulence and the reduced host defense (Fig 5A). To test this possibility, we  
241 performed a chitin-dependent defense priming assay as previously done for cellobiose (Fig  
242 5b). Indeed, when roots were pre-treated with chitin, the plant susceptibility to *clr1* was  
243 indistinguishable from that observed against WT Fo5176 (Fig. 5C).

244 We next investigated the role of sugar availability on the up-regulation of the virulence factors  
245 enriched in the *clr1-1* secretome. We measured the expression of six of these genes (g16048,  
246 g2038,g7764,g1489,g15789 and g11385) in WT and *clr1* grown on sucrose media or on roots  
247 at 7dpt. We observed an increase in the expression in all the cases except with the subtilisin  
248 (g1489) in plants infected by *clr1-1* compared to WT, while no differences were observed  
249 between the strains grown on sucrose (Fig. 5D-E). These results suggest that *clr1-1* increases  
250 both the expression and secretion of various virulence factors to compensate for its decreased  
251 ability to utilize cellulose as a primary carbon source.

### 252 **CLR1 is essential to complete *F. oxysporum* life cycle**

253 Our data indicated that CLR1 is not required for Fo5176 to reach and enter the xylem and  
254 question the biological relevance of this transcription factor in Fo growth in planta. To shed  
255 light on this, we studied the temporal transcriptional regulation of *CLR1* in infected roots. *CLR1*  
256 expression increased significantly from 4dpt on, especially after 5dpt (Fig. 6A), growing  
257 exponentially until 7dpt, when Fo5176 had already proliferated in the vasculature. This  
258 suggests that cellulases might have a critical role in the final infection stages. We therefore  
259 explored CLR1 function during the final phase of the host-fungus interaction, saprophytic  
260 growth, which is an important part of the Fo life cycle. When Fo5176 was grown for 4 days on  
261 dead or living plants, we observed 3 times more expression of *CLR1* under saprophytic than  
262 under hemibiotrophic growth conditions (Fig. 6B). The last step of a fungal life cycle is the  
263 production of spores. Therefore, we tested the impact of CLR1 on microconidia production on  
264 dead seedlings. Our data showed that the WT and *clr1C* produced five times more  
265 microconidia than *clr1-1* (Fig. 6C). Taken together, these findings indicate that CLR1 is  
266 essential for saprophytic growth and spore production of Fo on dead plant tissue.

### 267 **The role of CLR1 in Fo infection is conserved among different pathosystems**

268 The *F. oxysporum* species complex contains different host specific plant pathogens, for some  
269 of which we also found CLR1 orthologues with a protein sequence similarity of 95.69% with  
270 the Fo5165 CLR1 and the same DNA-binding sequence (Fig. 1A and 1B). Thus, we asked  
271 whether the increased virulence displayed by *clr1-1* in the Arabidopsis-Fo5176 interaction is  
272 conserved among pathosystems. To answer this question, we generated a *CLR1* null mutant  
273 in the tomato-infecting Fo, Fo4287, and the corresponding *clr1C* complemented line (Fig. S7).  
274 Similar to Fo5176, tomato plants infected with Fo strains, a higher virulence of *clr1-1* mutant  
275 compared to the WT was observed, while *clr1C* was similar to WT (Fig. 7). These results  
276 suggest that CLR1 is not essential for *F. oxysporum* infection, both in Fo5176-Arabidopsis and  
277 Fo4782-tomato interactions.

278  
279

### 280 **Discussion**

281 Microbes need to loosen and digest the plant CWs in order to colonize their hosts. As the  
282 major and most recalcitrant CW component, cellulose is a key target for a plethora of  
283 transcriptionally coordinated microbial CWMPs (34, 36, 37, 59–63). In this work, we studied  
284 the function of cellulases in the life cycle of a plant fungal pathogen. The identification and  
285 characterization of CLR1 in Fo confirms the conservation of this transcription factor as a  
286 master regulator of cellulose degradation in ascomycetes (Fig. 1, fig. 2H and fig. S3) (8, 36,  
287 37, 43). Our work expands the current knowledge on the role of CLR1 during plant biomass  
288 degradation by demonstrating its function *in planta*. Interestingly, genes described in *N. crassa*  
289 as CLR1-dependent in cultures with cellulose as the only carbon source behaved similarly in  
290 Fo during root infection, suggesting that both metabolic situations require similar levels of  
291 CWMPs (Fig 2G; (37)). Unexpectedly, loss of CLR1 led to a significant increase in virulence  
292 both in the Arabidopsis and tomato pathogenic forms of Fo (Fig 2, fig. S4, and fig. 7). Although  
293 the *clr1* mutant is not completely impaired in cellulases secretion (Table 1), our data indicate  
294 that Fo not only does not need their CLR1-dependent cellulases, but benefit from their absence  
295 to infect their hosts. More precisely, we show here that *clr1* crosses the root epidermal layer

296 and reaches the vascular system of Arabidopsis roots faster, while degrading less crystalline  
297 cellulose, than WT (Fig. 2A-F, and H). This indicates that crystalline cellulose degradation is  
298 not a requirement for Fo to grow through the apoplast of the root layers and that the impairment  
299 of *clr1* in cellulose degradation is a consequence of the notable decrease in the expression  
300 and secretion of most, but not all cellulolytic enzymes (Fig. 2G, fig. 5B and 5E, and Table 1).

301 Our previously reported transcriptomic data indicated that the days 2 and 3 of the root-Fo5176  
302 interaction are critical time points for disease development (34). The drastic decrease of  
303 sucrose levels detected in infected roots at these time points (Fig 4A-C) suggests that the  
304 initial steps of root colonization have an extraordinary impact on plant metabolism.  
305 Accordingly, we observed a significant impairment in plant response to *clr1* infection at these  
306 same time points (Fig 3A), indicating that the lack of a canonical set of CWDEs stunts the  
307 perception of the pathogen by the plant. However, although pre-treatment with cellobiose  
308 reduced the virulence of the *clr1* mutant (Fig3B), its detection in higher amounts in *clr1*-infected  
309 roots as compared to WT excludes this cellulose degradation product as the main reason for  
310 the delay in the perception of *clr1* by the plant. The high amounts of cellobiose detected in  
311 *clr1*-infected roots and the reduced capacity of *clr1* to grow on cellobiose as the only carbon  
312 source could be explained by the markedly reduced expression levels of the enzyme  $\beta$ -  
313 glucosidase, which is required for the final step of cellobiose hydrolysis (64). Importantly, the  
314 identification of cellobiose as a CW degradation product during microbial colonization confirms  
315 its predicted but never confirmed function as DAMP (27). Together with cellobiose, we  
316 identified various OGs in Fo-infected roots (Fig. 4B-D). Interestingly, most of the OGs identified  
317 in roots infected either with WT and *clr1* were acetylated, which differs from what was reported  
318 in leaves infected by the necrotrophic fungus *B. cinerea* (65). Aerial and soil-borne plant  
319 pathogens might thus use a different battery of CWDEs to adapt to the different CWs  
320 encountered in leaves and roots, with some functional similarities since certain OGs such as  
321 GalAox were detected in both infected organs. Acetylated OGs have been shown to impair the  
322 activity of some pectinases, eliciting the plant defense response (66, 67). Thus, the reduced  
323 amount of GalAc<sub>2</sub>ox and Gal<sub>4</sub>Ac detected in *clr1*-infected roots (Fig. 4D and E) might explain  
324 the delay in fungal perception by the plant .

325 Plant pathogens modulate their secretome to adapt to the host and the available nutrient  
326 sources (8, 68). Together with the decreased amount of cellulases in the *clr1* secretome, we  
327 found an enrichment of secreted virulence factors, which might partially explain the fact that  
328 co-infection with WT does not reduce the hypervirulence of the mutant (Fig. S6A and B).  
329 Intriguingly, the most enriched protein in the *clr1* secretome was a putative cutinase. Cutinases  
330 are essential for virulence of aerial plant pathogens (48) where the cuticle is a substantial  
331 barrier on the leaf and stem surfaces. In roots, only the root caps have been shown to have a  
332 cuticle (69). However, both pathogenic fungi and mutualistic mycorrhizae recruit the fatty acid  
333 biosynthesis program to facilitate host invasion. As reported for these microbes (34, 70), we  
334 observed an upregulation of the expression of these fatty acid biosynthesis genes in Fo during  
335 Arabidopsis root infection. In addition, cutinases from plant pathogens were shown to cleave  
336 suberin *in vitro* (71), a plant compound that prevents the spread of microbial pathogens (72).  
337 Because suberin blocks apoplastic transport both at the endodermis and at the root surface  
338 (73), the increased amounts of cutinase might explain the ability of *clr1* to cross the root  
339 epidermal layer and reach the xylem faster than WT (Fig 2).

340 We also found a set of peptidases enriched in the *clr1* secretome, some of which have been  
341 reported to affect host responses by degrading defense related proteins (52, 74). Among them,  
342 fungalysins have been reported to be involved in cleavage of plant chitinases and therefore be  
343 essential for the pathogenicity of different fungi, including Fol (56, 75). Interestingly, the *clr1*  
344 mutant also seems to undermine host defense responses by secreting more chitin  
345 deacetylases, which should decrease the amount of MAMPs available for plant perception  
346 (58). In fact, we found that pretreatment with chitin oligomers reduces *clr1* virulence to WT  
347 levels, implying that not only DAMP, but also MAMP perception is severely compromised

348 during *clr1* infection. The other group of virulence factors significantly enriched in the *clr1*  
349 secretome are the pectin and pectate lyases, which have a fundamental role in vascular wilt  
350 diseases (50). These enzymes generate unsaturated OGs that might be used efficiently as  
351 nutrients by Fo because most OGs detected in the secretomes were saturated. In line with  
352 this, the only unsaturated OG identified in the assay, GalA<sub>3</sub>Ac-H<sub>2</sub>Oox, was less abundant in  
353 the *clr1* secretome (Fig. S5). Interestingly, we did not find differences in the levels of  
354 polygalacturases (PGs) despite the higher levels of saturated OGs observed in infected roots.  
355 Most likely, the higher levels of pectin and pectate lyases detected might provide additional  
356 substrates for PGs, whose activity would not be limitant in this reaction. Indeed, the  
357 overaccumulation of subtilisin in the *clr1* secretome could create more cleavage sites for the  
358 PGs by activating type I pectin methyl esterases (76). The lower amount of saturated OGs  
359 observed in *clr1*-infected compared to WT-infected roots might thus be a consequence of (a)  
360 the higher cellulose degradation during WT infection that makes the cellulose-embedded  
361 pectin more accessible for deconstruction by pectinases or (b) the increased capacity of *clr1*  
362 to uptake, and use these OGs. We favour the second hypothesis considering that the majority  
363 of pectins is not bound to cellulose (77). Overall, the increase in the secretion of different  
364 virulence factors might explain the delay in the perception of *clr1* by the plant at the early  
365 stages of infection (Fig. 3Aa).

366 Sucrose was highly available for Fo at the beginning of its interaction with the root (2dpt), when  
367 Fo is entering the epidermal apoplast (34), while the CW-derived oligomers were not  
368 detectable (Fig. 4). At 3dpt when some hyphae have reached the cortical apoplast (Fig.2F  
369 (34)), sucrose levels had decreased dramatically and cellobiose and OGs were detectable,  
370 indicating that from 2 to 3 dpt the CW becomes the primary carbon source for the fungus. At  
371 this stage, energy acquisition might become limiting in *clr1* due to its reduced capacity to  
372 degrade cellulose. In this metabolic context, the enrichment in pectin and pectate lyases  
373 detected in the *clr1* secretome might represent a metabolic adaptation to exploit energy  
374 through the alternative carbon source pectin. Moreover, the higher levels of peptidases  
375 secreted by *clr1* could also provide additional carbon and nitrogen sources to the fungus (78).  
376 This hypothesis is supported by the fact that the increase in the expression of virulence factors  
377 in *clr1* compared to WT was only observed during growth *in planta* but not on carbon-rich  
378 axenic medium (Fig. 5D and E).

379 Finally, the composition of the medium strongly affected conidia production by Fo. A positive  
380 role of cellulose in conidia formation was reported previously (79), accordingly, *CLR1*  
381 expression was upregulated starting at 4dpt, when Fo has already entered the xylem (Fig. 6A  
382 and 2A), and remained significantly high when the fungus was growing on dead plants  
383 compared to alive ones (Fig. 6B). Thus, *CLR1* activation is likely a way to prepare the fungal  
384 metabolism for the next step in the Fo life cycle, i.e. formation of conidia on the decaying plant  
385 tissue which will spread the fungus to encounter new hosts. Indeed, we showed that *clr1* was  
386 severely compromised in asexual reproduction because microconidia formation on dead  
387 plants decreased drastically (Fig. 6B and 6C). Our data suggest that cellulose degradation is  
388 fundamental for the pathogen to fully exploit host resources for the production of dissemination  
389 structures.

390 In conclusion, we show for the first time that the impairment in cellulose degradation does not  
391 pose any obstacles for plant infection by the vascular pathogen Fo. We further demonstrate  
392 that this organism exhibits an extraordinarily high degree of metabolic plasticity that  
393 compensates the decrease in cellulase production during host colonization by triggering the  
394 expression of alternative CWDEs. However, the deficiency in obtaining energy from cellulose  
395 significantly decreases the capacity of the fungus to replicate, disseminate, and invade new  
396 hosts. Our findings imply that *CLR1* is highly conserved among ascomycetes because it is vital  
397 for saprophytic survival in natural environments. Evolutionary pressure thus makes the use of  
398 cellulases imperative for successful completion of the pathogen's life cycle.

399



400

## 401 **Material and Methods**

### 402 **Plant material and growth conditions.**

403 *Arabidopsis thaliana* ecotype Col-0 and tomato Monika (provided by Syngenta Seeds, Almeria,  
404 Spain) were used in the analysis of fungal pathogenicity. Growth conditions were 16-h light  
405 (24°)/8- h dark cycle at 21° C for all *Arabidopsis* experiments. In the case of plate experiments,  
406 seedlings were grown upright with ½ MS media (pH adjusted to 5.7 with KOH but without  
407 buffering). When the experiment was performed in a hydroponic system, the seeds were  
408 germinated on 2 mm foam plugs floating in 330 ml pots on ½ MS + 1% sucrose media at pH  
409 5.7 adjusted by KOH. The media was exchanged 6 days after germination to ½ MS and  
410 seedlings were further grown. Tomato cultivar susceptible cultivar Monika (provided by  
411 Syngenta Seeds, Almeria, Spain) planted in vermiculite, and maintained in a growth chamber  
412 (28 °C; photoperiod 14 h light/10 h dark)

### 413 **Fungal strains and culture conditions.**

414 *Fusarium oxysporum* strain 5176 (Fo5176) was originally isolated in Australia from infected  
415 *Arabidopsis* plants (38). The strains were routinely cultured in potato dextrose broth (PDB) at  
416 28°C with orbital shaking at 170 rpm. Where necessary the following antibiotics were added  
417 to the culture medium: hygromycin B (55 µg/ml), G418 (100 µg/ml) and phleomycin (5.5µg  
418 /ml). For microconidia collection, 3 to 5 day-old cultures were collected by filtration through a  
419 nylon filter (Monodur; mesh size 10 µm). Filtrates were centrifuged at 12000 g for 10 min, the  
420 pellet containing the microconidia was washed using deionized water, resuspended in water  
421 to reach the desired concentration.

422

### 423 **Identification of CLR1 in *Fusarium* species**

424 *Fusarium* genes encoding the predicted CLR1 proteins were identified by sequence similarity  
425 searches against the *Neurospora crassa* CLR1 (NCU07705) (36) using BLASTp  
426 (<http://blast.ncbi.nlm.nih.gov/>). The software CLC Genomic Workbench v.12 was used to  
427 perform the comparison analysis of CLR1 sequence in different ascomycetes.

### 428 **Fungal transformation.**

429 As described above, we identified the CLR1 proteins in Fo5176 (FOXB\_08021) and FoI4287  
430 (FOXG\_08626). Targeted gene deletion of the entire *CLR1* gene in the *Fo5176-pSIX1::GFP*  
431 background (33) and the *FoI4287-3XmClover3* genetic backgrounds was performed using the  
432 split-marker method (80) with the neomycin resistance cassettes following the protocol  
433 previously described (63). For the complementation of the *clr1* deletion mutants, a co-  
434 transformation with the native *CLR1* in the phleomycin resistance cassette was performed as  
435 reported (81). The oligonucleotides used to generate PCR fragments for gene replacement,  
436 complementation or identification of mutants are listed in Table 1. PCRs were routinely  
437 performed with the High-Fidelity Template PCR system (Roche Diagnostics, Barcelona,  
438 Spain) using an MJ Mini personal thermal cycler (Bio-Rad, Alcobendas, Spain). The amplified  
439 flanking sequences were PCR fused with partially overlapping truncated versions of the  
440 neomycin (Neo<sup>r</sup>). Transformants were purified by monoconidial isolation (82).

### 441 **Fungal Growth in different carbon sources.**

442 Fo5176 was grown on ½ MS media (Murashige and Skoog media, Difco). Carbon sources  
443 were added to 0.5% wt/vol. Conidia were inoculated into 3 ml liquid media at 10<sup>6</sup> conidia/mL  
444 and grown at 28° in dark and shaking (180 rpm), 3 days for sucrose and cellobiose (Fluka)  
445 and 7 days for cellulose (Sigmacell, cellulose type 50). After 1 day drying at 60°, the material  
446 was weighted or its nucleic acids were extracted. For the nucleic acid extraction, we followed  
447 a previous protocol (83). The nucleic acids were quantified by spectrophotometry using a  
448 NanoDrop.

### 449 **Fungal growth in *in vitro* stress conditions**

450 Drops containing serial dilution ( $1 \times 10^7$ ,  $1 \times 10^6$  and  $1 \times 10^5$ ) of freshly obtained microconidia were  
451 spotted on YPD agar plates supplemented with Congo red ( $5 \mu\text{g}/\text{mL}$ ) or  $5 \mu\text{g}/\text{mL}$  CalcoFluor  
452 White prepared in 0.5% KOH for testing cell wall stress as in (84). Minimal media was used  
453 for evaluating growth under nutrient deficiency, for (82)1M sorbitol was used to test  
454 hyperosmotic stress. Plates were incubated at  $28^\circ\text{C}$  for 2 days in general or 4 days for salt  
455 stress and Calcofluor white before imaging. .

#### 456 **Arabidopsis plant infection assays.**

457 Arabidopsis infection assays were performed as described previously (33, 41). In summary, 8  
458 days-old seedlings grown as described above were transplanted to plates with  $100 \mu\text{l}$  of  $10^7$   
459 microconidia/ml of Fo5176-pSIX1. The number of vascular penetrations per root was analyzed  
460 using the signal from the GFP under a stereomicroscope, when it showed a clear, linear and  
461 root central pattern. The experiment in which the plants were pretreated with cellobiose  $100$   
462  $\mu\text{M}$  or chitin  $100 \mu\text{g}/\text{ml}$  were based on (27). Roots of 10 days old seedlings were treated with  
463 cellobiose or chitin for 25 minutes, hereafter they were transferred on a  $\frac{1}{2}$  MS media plate with  
464 3 days-old *Fo* mycelia. The number of vascular penetrations were monitored as explained  
465 above.

#### 466 **Tomato plant infection assays.**

467 Tomato root infection with *FoI* was performed as previously described by (82). Briefly, 2 week-  
468 old seedlings were inoculated with *FoI* by immersing the roots into a fungal microconidia  
469 suspension ( $5 \times 10^5$  conidia/ml), planted in vermiculite, and maintained in a growth chamber  
470 ( $28^\circ\text{C}$ ; photoperiod 14 h light/10 h dark). Plant survival was recorded daily up to 29 days, as  
471 previously described (85), calculated by the Kaplan–Meier method, and compared among  
472 groups using the log-rank test. All infection assays included 10 plants per treatment.

#### 473 **Confocal microscopy**

474 Plates infection assays were performed as described above, at 3 dpt images were taken with  
475 a Zeiss LSM Axioobserver microscope, using the LD C-Apochromat 40x / 1.1 W Korr M27  
476 objective and Immersol W (Zeiss) between lens and coverslip. GFP (fungus) was excited at  
477 488 nm and emitted fluorescence was detected at 514 nm. The roots were stained with  
478 propidium iodide (PI;  $10 \mu\text{M}$ , Sigma-Aldrich) and imaged with 536 nm and 617 nm excitation  
479 and emission wavelength, respectively.

#### 480 **Cellulose, OGs and hexoses and quantification in infected roots.**

481 Plates infection assays were performed as indicated above. For cellulose quantification, roots  
482 were harvested at 7dpt and processed as described before to measure the crystalline cellulose  
483 (33, 86). To quantify OGs and hexoses, roots collected at different dpt were weighted, covered  
484 with 100% ethanol for several days and then dried using a freeze-dryer (Christ, Alpha 2-4).  
485 The roots were grinded in water (1ml), centrifuged (10 minutes at  $10,000g$ ) and the  
486 supernatant was collected to be analyzed via High-performance size-exclusion  
487 chromatography (HP-SEC-MS). The analysis was performed as indicated before (65). Briefly,  
488 samples were diluted at  $1 \text{ mg}/\text{ml}$  in ammonium formate  $50 \text{ mM}$ , formic acid  $0.1\%$ .  
489 Chromatographic separation was performed on an ACQUITY UPLC Protein BEH SEC Column  
490 ( $125\text{\AA}$ ,  $1.7 \mu\text{m}$ ,  $4.6 \text{ mm} \times 300 \text{ mm}$ , Waters Corporation, Milford, MA, USA). Elution was  
491 performed in  $50 \text{ mM}$  ammonium formate, formic acid  $0.1\%$  at a flow rate of  $400 \text{ l}/\text{min}$  and a  
492 column oven temperature of  $40^\circ\text{C}$ . The injection volume was set to  $10 \text{ l}$ . MS-detection was  
493 performed in negative mode with the end plate offset set voltage to  $500 \text{ V}$ , capillary voltage to  
494  $4000 \text{ V}$ , Nebulizer  $40 \text{ psi}$ , dry gas  $8 \text{ l}/\text{min}$  and dry temperature  $180^\circ\text{C}$ . Major peaks were  
495 annotated following accurate mass annotation, isotopic pattern and MS/MS analysis, as  
496 previously described (45).

#### 497 **Real-time quantitative PCR.**

498 LN2 frozen samples were grinded using a TissueLyser II (Quiagen) and glass beads.  $1 \mu\text{g}$  of  
499 total RNA/sample extracted by Isol-RNA lysis reagent (5 PRIME) was used to generate first-

500 strand cDNA using Thermo Scientific Maxima<sup>™</sup> H Minus cDNA Synthesis Master Mix with  
501 dsDNase (ThermoFisher), following manufacturer's instructions. Quantitative PCR reactions  
502 were performed in a LightCycler 480II apparatus (Roche) using Fast SYBR Green Master Mix  
503 (ThermoFisher) in a 10 µl reaction. Relative transcript levels were quantified with respect to a  
504 reference gene *GAPDH600B* for plants (87) (Czechowski et al. 2005) or *FoβTUB*  
505 (*FOXG\_06228*) for Fo5176 (33). The 2<sup>ΔCT</sup> method was used to quantify the relative expression  
506 of each gene. Primers are indicated in Table S1.

#### 507 **Protein identification in Fo5176 secretome .**

508 10-day old hydroponically-grown seedlings were treated with 20 µl of 10<sup>7</sup> microconidia/ml of  
509 either Fo5176 WT or *clr1*, as described before (34). At 3dpt., the roots were removed and the  
510 liquid media was collected. We next followed the protocol from (88) with some modifications:  
511 to remove big particles present in the media, we filtered it with a 45 microns sterile filter  
512 (Starlab) before concentration with . centricons (Amicon Ultra 3k, Merck Millipore) to 1.5 ml  
513 volume. 150 microliters of these 1.5 ml were sent to proteomic analysis.

514 For each sample, proteins were precipitated with trichloroacetic acid (TCA; Sigma-Aldrich) at  
515 a final concentration of 5% and washed twice with cold acetone. The dry pellets were dissolved  
516 in a 45 µl buffer (10 mM Tris + 2 mM CaCl<sub>2</sub>, pH 8.2). Reduction and alkylation of the proteins  
517 was performed by adding 2 mM of Tris(2-carboxyethyl) phosphin –hydrochlorid (TCEP) and  
518 15 mM of iodoacetamine (IAA). After 30 min at 60°C the samples were cooled to room  
519 temperature and 4 µg of Sequencing Grade Trypsin (Promega) for digestion were added. The  
520 digestion was carried out at 37°C for 4 hours. The samples were dried to completeness and  
521 re-solubilized in 20 µl of 3% acetonitrile, 0.1% formic acid for LC-MS/MS analysis. Before  
522 injection the samples were diluted 1:20 in the same solvent.

523 Mass spectrometry analysis was performed on an Orbitrap Fusion Lumos (Thermo Scientific)  
524 equipped with a Digital PicoView source (New Objective) and coupled to a M-Class UPLC  
525 (Waters). Solvent composition at the two channels was 0.1% formic acid for channel A and  
526 0.1% formic acid, 99.9% acetonitrile for channel B. For each sample 1 µL of diluted peptides  
527 were loaded on a commercial MZ Symmetry C18 Trap Column (100Å, 5 µm, 180 µm x 20 mm,  
528 Waters) followed by nanoEase MZ C18 HSS T3 Column (100Å, 1.8 µm, 75 µm x 250 mm,  
529 Waters). The peptides were eluted at a flow rate of 300 nL/min by a gradient from 5 to 22% B  
530 in 80 min, 32% B in 10 min and 95% B for 10 min. Samples were acquired in a randomized  
531 order. The mass spectrometer was operated in data-dependent mode (DDA) acquiring a full-  
532 scan MS spectra (300–1'500 m/z) at a resolution of 120'000 at 200 m/z after accumulation to  
533 a target value of 500'000. Data-dependent MS/MS were recorded in the linear ion trap using  
534 quadrupole isolation with a window of 0.8 Da and HCD fragmentation with 35% fragmentation  
535 energy. The ion trap was operated in rapid scan mode with a target value of 10'000 and a  
536 maximum injection time of 50 ms. Only precursors with intensity above 5'000 were selected  
537 for MS/MS and the maximum cycle time was set to 3 s. Charge state screening was enabled.  
538 Singly, unassigned, and charge states higher than seven were rejected. Precursor masses  
539 previously selected for MS/MS measurement were excluded from further selection for 20 s,  
540 and the exclusion window was set at 10 ppm. The samples were acquired using internal lock  
541 mass calibration on m/z 371.1012 and 445.1200. The mass spectrometry proteomics data  
542 were handled using the local laboratory information management system (LIMS) (89).

543 For protein identification and label free protein quantification, the acquired raw MS data were  
544 processed by MaxQuant (version 1.6.2.3), followed by protein identification using the  
545 integrated Andromeda search engine (90). Spectra were searched against a provided Fo5176  
546 database (40) concatenated to the Araport database  
547 ([https://www.arabidopsis.org/download/index-](https://www.arabidopsis.org/download/index-auto.jsp?dir=%2Fdownload_files%2FSequences%2FAraport11_blastsets)  
548 [auto.jsp?dir=%2Fdownload\\_files%2FSequences%2FAraport11\\_blastsets](https://www.arabidopsis.org/download_files%2FSequences%2FAraport11_blastsets), version 2020-06-  
549 18), concatenated to its reversed decoyed fasta database and common protein contaminants.  
550 Carbamidomethylation of cysteine was set as fixed modification, while methionine oxidation

551 and N-terminal protein acetylation were set as variables. Enzyme specificity was set to  
552 trypsin/P allowing a minimal peptide length of 7 amino acids and a maximum of two missed-  
553 cleavages. MaxQuant Orbitrap default search settings were used. The maximum false  
554 discovery rate (FDR) was set to 0.01 for peptides and 0.05 for proteins. Label free  
555 quantification was enabled and a 2 minutes window for match between runs was applied. In  
556 the MaxQuant experimental design template, each file is kept separate in the experimental  
557 design to obtain individual quantitative values. Protein fold changes were computed based on  
558 Intensity values reported in the proteinGroups.txt file. A set of functions implemented in the R  
559 package SRMServe (91) was used to filter for proteins with 2 or more peptides allowing for  
560 a maximum of 4 missing values, and to normalize the data with a modified robust z-score  
561 transformation and to compute p-values using the t-test with pooled variance. If all  
562 measurements of a protein are missing in one of the conditions, a pseudo fold change was  
563 computed replacing the missing group average by the mean of 10% smallest protein intensities  
564 in that condition.

### 565 **Fo5176 microconidia production in Arabidopsis.**

566 We performed plate infection assays as indicated above. 5 days after the plant was dead, we  
567 weighed the whole plant and we added 1.2 ml of H<sub>2</sub>O. The samples were shaken gently for 1  
568 minute and the plant material removed. The number of microconidia in solution counted with  
569 the Thoma cell counting chamber was shown per mg of dead material.

### 570 **Statistical analysis.**

571 Statistical analyses were performed and data plotted using GraphPad Prism 9.0.0 (GraphPad  
572 Software, Inc). Each figure legend indicates the statistical analysis which was performed and  
573 the level of significance.

574

575

### 576 **References**

- 577 1. P. Albersheim, A. G. Darvill, M. A. O'Neill, H. A. Schols, A. G. J. Voragen, in *Progress in*  
578 *Biotechnology*, J. Visser, A. G. J. Voragen, Eds. (Elsevier, 1996), vol. 14, pp. 47–55.
- 579 2. J. Graça, Suberin: the biopolyester at the frontier of plants. *Front Chem.* **3**, 62 (2015).
- 580 3. S. Naseer, Y. Lee, C. Lapierre, R. Franke, C. Nawrath, N. Geldner, Casparian strip  
581 diffusion barrier in Arabidopsis is made of a lignin polymer without suberin. *Proc. Natl.*  
582 *Acad. Sci. U. S. A.* **109**, 10101–10106 (2012).
- 583 4. C. T. Anderson, J. J. Kieber, Dynamic Construction, Perception, and Remodeling of Plant  
584 Cell Walls. *Annu. Rev. Plant Biol.* **71**, 39–69 (2020).
- 585 5. D. J. Cosgrove, Plant cell wall extensibility: connecting plant cell growth with cell wall  
586 structure, mechanics, and the action of wall-modifying enzymes. *J. Exp. Bot.* **67**, 463–476  
587 (2016).
- 588 6. H. Höfte, A. Voxeur, Plant cell walls. *Curr. Biol.* **27**, R865–R870 (2017).
- 589 7. N. L. Glass, M. Schmoll, J. H. D. Cate, S. Coradetti, Plant Cell Wall Deconstruction by  
590 Ascomycete Fungi. *Annu. Rev. Microbiol.* (2013), doi:10.1146/annurev-micro-092611-  
591 150044.
- 592 8. V. W. Wu, N. Thieme, L. B. Huberman, A. Dietschmann, D. J. Kowbel, J. Lee, S. Calhoun,  
593 V. R. Singan, A. Lipzen, Y. Xiong, R. Monti, M. J. Blow, R. C. O'Malley, I. V. Grigoriev, J.  
594 P. Benz, N. L. Glass, The regulatory and transcriptional landscape associated with carbon  
595 utilization in a filamentous fungus. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 6003–6013 (2020).
- 596 9. R. J. Quinlan, M. D. Sweeney, L. Lo Leggio, H. Otten, J.-C. N. Poulsen, K. S. Johansen,  
597 K. B. R. M. Krogh, C. I. Jørgensen, M. Tovborg, A. Anthonsen, T. Tryfona, C. P. Walter,  
598 P. Dupree, F. Xu, G. J. Davies, P. H. Walton, Insights into the oxidative degradation of  
599 cellulose by a copper metalloenzyme that exploits biomass components. *Proc. Natl. Acad.*

- 600 *Sci. U. S. A.* **108**, 15079–15084 (2011).
- 601 10. R. Zhang, Functional characterization of cellulose-degrading AA9 lytic polysaccharide  
602 monoxygenases and their potential exploitation. *Appl. Microbiol. Biotechnol.* **104**, 3229–  
603 3243 (2020).
- 604 11. A. B. Boraston, D. N. Bolam, H. J. Gilbert, G. J. Davies, Carbohydrate-binding modules:  
605 fine-tuning polysaccharide recognition. *Biochem. J.* **382**, 769–781 (2004).
- 606 12. B. Henrissat, A. Bairoch, New families in the classification of glycosyl hydrolases based  
607 on amino acid sequence similarities. *Biochem. J.* **293 ( Pt 3)**, 781–788 (1993).
- 608 13. P. Reignault, O. Valette-Collet, M. Boccara, The importance of fungal pectinolytic  
609 enzymes in plant invasion, host adaptability and symptom type. *Eur. J. Plant Pathol.* **120**,  
610 1–11 (2008).
- 611 14. K. Hématy, C. Cherk, S. Somerville, Host-pathogen warfare at the plant cell wall. *Curr.*  
612 *Opin. Plant Biol.* **12**, 406–413 (2009).
- 613 15. W. Underwood, The plant cell wall: a dynamic barrier against pathogen invasion. *Front.*  
614 *Plant Sci.* **3**, 85 (2012).
- 615 16. C. P. Kubicek, T. L. Starr, N. L. Glass, Plant cell wall-degrading enzymes and their  
616 secretion in plant-pathogenic fungi. *Annu. Rev. Phytopathol.* **52**, 427–451 (2014).
- 617 17. D. M. Gibson, B. C. King, M. L. Hayes, G. C. Bergstrom, Plant pathogens as a source of  
618 diverse enzymes for lignocellulose digestion. *Curr. Opin. Microbiol.* **14**, 264–270 (2011).
- 619 18. Z. Zhao, H. Liu, C. Wang, J.-R. Xu, Comparative analysis of fungal genomes reveals  
620 different plant cell wall degrading capacity in fungi. *BMC Genomics.* **14**, 274 (2013).
- 621 19. M. Kurašin, P. Våljamäe, Processivity of Cellobiohydrolases Is Limited by the Substrate.  
622 *J. Biol. Chem.* **286**, 169–177 (2011).
- 623 20. A. D. Pietro, M. P. Madrid, Z. Caracuel, J. Delgado-Jarana, M. I. G. Roncero, *Fusarium*  
624 *oxysporum*: exploring the molecular arsenal of a vascular wilt fungus. *Mol. Plant Pathol.*  
625 **4**, 315–325 (2003).
- 626 21. N. J. Tonukari, J. S. Scott-Craig, J. D. Walton, The *Cochliobolus carbonum* SNF1 gene is  
627 required for cell wall-degrading enzyme expression and virulence on maize. *Plant Cell.*  
628 **12**, 237–248 (2000).
- 629 22. T. Boller, Chemoperception of Microbial Signals in Plant Cells. *Annu. Rev. Plant Physiol.*  
630 *Plant Mol. Biol.* **46**, 189–214 (1995).
- 631 23. A. M. Husaini, A. Sakina, S. R. Cambay, Host-Pathogen Interaction in *Fusarium*  
632 *oxysporum* Infections: Where Do We Stand? *Mol. Plant. Microbe. Interact.* **31**, 889–898  
633 (2018).
- 634 24. S. Hou, Z. Liu, H. Shen, D. Wu, Damage-Associated Molecular Pattern-Triggered  
635 Immunity in Plants. *Front. Plant Sci.* **10**, 646 (2019).
- 636 25. S. Ferrari, D. V. Savatin, F. Sicilia, G. Gramegna, F. Cervone, G. D. Lorenzo,  
637 Oligogalacturonides: plant damage-associated molecular patterns and regulators of  
638 growth and development. *Front. Plant Sci.* **4**, 49 (2013).
- 639 26. A. Voxeur, H. Höfte, Pectin-derived immune elicitors in response to lignin modification in  
640 plants. *Proc. Natl. Acad. Sci. U. S. A.* **117** (2020), pp. 4442–4444.
- 641 27. C. de A. Souza, S. Li, A. Z. Lin, F. Boutrot, G. Grossmann, C. Zipfel, S. C. Somerville,  
642 Cellulose-Derived Oligomers Act as Damage-Associated Molecular Patterns and Trigger  
643 Defense-Like Responses. *Plant Physiol.* **173**, 2383–2398 (2017).
- 644 28. C. B. Michielse, M. Rep, Pathogen profile update: *Fusarium oxysporum*. *Mol. Plant Pathol.*  
645 **10**, 311–324 (2009).

- 646 29. T. R. Gordon, *Fusarium oxysporum* and the Fusarium Wilt Syndrome. *Annu. Rev.*  
647 *Phytopathol.* **55**, 23–39 (2017).
- 648 30. C. Hall, R. Heath, D. Guest, The infection process of *Fusarium oxysporum* f.sp.  
649 *vasinfectum* in Australian cotton. *Australas. Plant Pathol.* **42**, 1–8 (2013).
- 650 31. J. Nahalkova, J. Fatehi, C. Olivain, C. Alabouvette, Tomato root colonization by  
651 fluorescent-tagged pathogenic and protective strains of *Fusarium oxysporum* in  
652 hydroponic culture differs from root colonization in soil. *FEMS Microbiol. Lett.* **286**, 152–  
653 157 (2008).
- 654 32. C. Kesten, A. Menna, C. Sánchez-Rodríguez, Regulation of cellulose synthesis in  
655 response to stress. *Curr. Opin. Plant Biol.* **40**, 106–113 (2017).
- 656 33. C. Kesten, F. M. Gámez-Arjona, A. Menna, S. Scholl, S. Dora, A. I. Huerta, H.-Y. Huang,  
657 N. Tintor, T. Kinoshita, M. Rep, M. Krebs, K. Schumacher, C. Sánchez-Rodríguez,  
658 Pathogen-induced pH changes regulate the growth-defense balance in plants. *EMBO J.*  
659 **38**, e101822 (2019).
- 660 34. A. Menna, S. Dora, G. Sancho-Andrés, A. Kashyap, M. K. Meena, K. Sklodowski, D.  
661 Gasperini, N. S. Coll, C. Sánchez-Rodríguez, A primary cell wall cellulose-dependent  
662 defense mechanism against vascular pathogens revealed by time-resolved dual  
663 transcriptomics. *BMC Biol.* **19**, 1–20 (2021).
- 664 35. W. T. Beeson, C. M. Phillips, J. H. D. Cate, M. A. Marletta, Oxidative cleavage of cellulose  
665 by fungal copper-dependent polysaccharide monooxygenases. *J. Am. Chem. Soc.* **134**,  
666 890–892 (2012).
- 667 36. S. T. Coradetti, J. P. Craig, Y. Xiong, T. Shock, C. Tian, N. L. Glass, Conserved and  
668 essential transcription factors for cellulase gene expression in ascomycete fungi.  
669 *Proceedings of the National Academy of Sciences* (2012), doi:10.1073/pnas.1200785109.
- 670 37. J. P. Craig, S. T. Coradetti, T. L. Starr, N. Louise Glass, Direct target network of the  
671 *Neurospora crassa* plant cell wall deconstruction regulators *clr-1*, *clr-2*, and *xlr-1*. *MBio*  
672 (2015), doi:10.1128/mBio.01452-15.
- 673 38. L. F. Thatcher, D. M. Gardiner, K. Kazan, J. M. Manners, A highly conserved effector in  
674 *Fusarium oxysporum* is required for full virulence on *Arabidopsis*. *Mol. Plant. Microbe.*  
675 *Interact.* **25**, 180–190 (2012).
- 676 39. D. Nirmaladevi, M. Venkataramana, R. K. Srivastava, S. R. Uppalapati, V. K. Gupta, T.  
677 Yli-Mattila, K. M. Clement Tsui, C. Srinivas, S. R. Niranjana, N. S. Chandra, Molecular  
678 phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. *lycopersici*. *Sci.*  
679 *Rep.* **6**, 21367 (2016).
- 680 40. L. Fokkens, L. Guo, S. Dora, B. Wang, K. Ye, C. Sánchez-Rodríguez, D. Croll, A  
681 Chromosome-Scale Genome Assembly for the *Fusarium oxysporum* Strain Fo5176 To  
682 Establish a Model *Arabidopsis*-Fungal Pathosystem. *G3* . **10**, 3549–3555 (2020).
- 683 41. A. I. Huerta, C. Kesten, A. L. Menna, G. Sancho-Andrés, C. Sanchez-Rodriguez, In-Plate  
684 Quantitative Characterization of *Arabidopsis thaliana* Susceptibility to the Fungal Vascular  
685 Pathogen *Fusarium oxysporum*. *Curr Protoc Plant Biol.* **5**, e20113 (2020).
- 686 42. A. F. J. Ram, F. M. Klis, Identification of fungal cell wall mutants using susceptibility assays  
687 based on Calcofluor white and Congo red. *Nat. Protoc.* **1**, 2253–2256 (2006).
- 688 43. J. P. Craig, S. T. Coradetti, T. L. Starr, N. L. Glass, Direct Target Network of the  
689 *Neurospora crassa* Plant Cell Wall. *MBio.* **6**, 1–11 (2015).
- 690 44. S. Masachis, D. Segorbe, D. Turrà, M. Leon-Ruiz, U. Fürst, M. El Ghalid, G. Leonard, M.  
691 S. López-Berges, T. A. Richards, G. Felix, A. Di Pietro, A fungal pathogen secretes plant  
692 alkalizing peptides to increase infection. *Nature Microbiology* (2016),  
693 doi:10.1038/nmicrobiol.2016.43.

- 694 45. A. Voxeur, O. Habrylo, S. Guénin, F. Miart, M.-C. Soulié, C. Rihouey, C. Pau-Roblot, J.-  
695 M. Domon, L. Gutierrez, J. Pelloux, G. Mouille, M. Fagard, H. Höfte, S. Vernhettes,  
696 Oligogalacturonide production upon Arabidopsis thaliana-Botrytis cinerea interaction.  
697 *Proc. Natl. Acad. Sci. U. S. A.* **116**, 19743–19752 (2019).
- 698 46. M. E. Rauwane, U. V. Ogugua, C. M. Kalu, L. K. Ledwaba, A. A. Woldesemayat, K.  
699 Ntushelo, Pathogenicity and Virulence Factors of Fusarium graminearum Including  
700 Factors Discovered Using Next Generation Sequencing Technologies and Proteomics.  
701 *Microorganisms.* **8** (2020), doi:10.3390/microorganisms8020305.
- 702 47. M. Andlar, T. Rezić, N. Marđetko, D. Kracher, R. Ludwig, B. Šantek, Lignocellulose  
703 degradation: An overview of fungi and fungal enzymes involved in lignocellulose  
704 degradation. *Eng. Life Sci.* **18**, 768–778 (2018).
- 705 48. H. Ma, B. Zhang, Y. Gai, X. Sun, K.-R. Chung, H. Li, Cell-Wall-Degrading Enzymes  
706 Required for Virulence in the Host Selective Toxin-Producing Necrotroph Alternaria  
707 alternata of Citrus. *Front. Microbiol.* **10**, 2514 (2019).
- 708 49. C. A. Cuomo, U. Güldener, J.-R. Xu, F. Trail, B. G. Turgeon, A. Di Pietro, J. D. Walton,  
709 L.-J. Ma, S. E. Baker, M. Rep, G. Adam, J. Antoniw, T. Baldwin, S. Calvo, Y.-L. Chang,  
710 D. Decaprio, L. R. Gale, S. Gnerre, R. S. Goswami, K. Hammond-Kosack, L. J. Harris, K.  
711 Hilburn, J. C. Kennell, S. Kroken, J. K. Magnuson, G. Mannhaupt, E. Mauceli, H.-W.  
712 Mewes, R. Mitterbauer, G. Muehlbauer, M. Münsterkötter, D. Nelson, K. O'donnell, T.  
713 Ouellet, W. Qi, H. Quesneville, M. I. G. Roncero, K.-Y. Seong, I. V. Tetko, M. Urban, C.  
714 Waalwijk, T. J. Ward, J. Yao, B. W. Birren, H. C. Kistler, The Fusarium graminearum  
715 genome reveals a link between localized polymorphism and pathogen specialization.  
716 *Science.* **317**, 1400–1402 (2007).
- 717 50. Y. Yang, Y. Zhang, B. Li, X. Yang, Y. Dong, D. Qiu, A Verticillium dahliae Pectate Lyase  
718 Induces Plant Immune Responses and Contributes to Virulence. *Front. Plant Sci.* **9**, 1271  
719 (2018).
- 720 51. I. Kars, G. H. Krooshof, L. Wagemakers, R. Joosten, J. A. E. Benen, J. A. L. van Kan,  
721 Necrotizing activity of five Botrytis cinerea endopolygalacturonases produced in Pichia  
722 pastoris. *Plant J.* **43**, 213–225 (2005).
- 723 52. M. Monod, S. Capoccia, B. Léchenne, C. Zaugg, M. Holdom, O. Jousson, Secreted  
724 proteases from pathogenic fungi. *Int. J. Med. Microbiol.* **292**, 405–419 (2002).
- 725 53. I. E. Cota, R. Troncoso-Rojas, R. Sotelo-Mundo, A. Sánchez-Estrada, M. E. Tiznado-  
726 Hernández, Chitinase and  $\beta$ -1,3-glucanase enzymatic activities in response to infection  
727 by Alternaria alternata evaluated in two stages of development in different tomato fruit  
728 varieties. *Sci. Hortic.* **112**, 42–50 (2007).
- 729 54. J. M. Sanz-Martín, J. R. Pacheco-Arjona, V. Bello-Rico, W. A. Vargas, M. Monod, J. M.  
730 Díaz-Mínguez, M. R. Thon, S. A. Sukno, A highly conserved metalloprotease effector  
731 enhances virulence in the maize anthracnose fungus Colletotrichum graminicola. *Mol.*  
732 *Plant Pathol.* **17**, 1048–1062 (2016).
- 733 55. B. Ökmen, B. Kemmerich, D. Hilbig, R. Wemhöner, J. Aschenbroich, A. Perrar, P. F.  
734 Huesgen, K. Schipper, G. Doehlemann, Dual function of a secreted fungalysin  
735 metalloprotease in Ustilago maydis. *New Phytol.* **220**, 249–261 (2018).
- 736 56. M. K. Jashni, I. H. M. Dols, Y. Iida, S. Boeren, H. G. Beenen, R. Mehrabi, J. Collemare,  
737 P. J. G. M. de Wit, Synergistic Action of a Metalloprotease and a Serine Protease from  
738 Fusarium oxysporum f. sp. lycopersici Cleaves Chitin-Binding Tomato Chitinases,  
739 Reduces Their Antifungal Activity, and Enhances Fungal Virulence. *Mol. Plant. Microbe.*  
740 *Interact.* **28**, 996–1008 (2015).
- 741 57. T. A. Naumann, D. T. Wicklow, N. P. J. Price, Identification of a chitinase-modifying protein  
742 from Fusarium verticillioides: truncation of a host resistance protein by a fungalysin

- 743 metalloprotease. *J. Biol. Chem.* **286**, 35358–35366 (2011).
- 744 58. F. Gao, B. S. Zhang, J. H. Zhao, J. F. Huang, P. S. Jia, S. Wang, J. Zhang, J. M. Zhou,  
745 H. S. Guo, Deacetylation of chitin oligomers increases virulence in soil-borne fungal  
746 pathogens. *Nature Plants*. **5**, 1167–1176 (2019).
- 747 59. D. P. Roberts, T. P. Denny, M. A. Schell, Cloning of the *egl* gene of *Pseudomonas*  
748 *solanacearum* and analysis of its role in phytopathogenicity. *J. Bacteriol.* **170**, 1445–1451  
749 (1988).
- 750 60. H. Liu, S. Zhang, M. A. Schell, T. P. Denny, Pyramiding unmarked deletions in *Ralstonia*  
751 *solanacearum* shows that secreted proteins in addition to plant cell-wall-degrading  
752 enzymes contribute to virulence. *Mol. Plant. Microbe. Interact.* **18**, 1296–1305 (2005).
- 753 61. Q. Chen, S. Rehman, G. Smant, J. T. Jones, Functional analysis of pathogenicity proteins  
754 of the potato cyst nematode *Globodera rostochiensis* using RNAi. *Mol. Plant. Microbe.*  
755 *Interact.* **18**, 621–625 (2005).
- 756 62. B. Van Vu, K. Itoh, Q. B. Nguyen, Y. Tosa, H. Nakayashiki, Cellulases belonging to  
757 glycoside hydrolase families 6 and 7 contribute to the virulence of *Magnaporthe oryzae*.  
758 *Mol. Plant. Microbe. Interact.* **25**, 1135–1141 (2012).
- 759 63. N. Rispaill, A. Di Pietro, *Fusarium oxysporum* Ste12 controls invasive growth and virulence  
760 downstream of the Fmk1 MAPK cascade. *Mol. Plant. Microbe. Interact.* **22**, 830–839  
761 (2009).
- 762 64. X. Zang, M. Liu, Y. Fan, J. Xu, X. Xu, H. Li, The structural and functional contributions of  
763  $\beta$ -glucosidase-producing microbial communities to cellulose degradation in composting.  
764 *Biotechnol. Biofuels.* **11**, 51 (2018).
- 765 65. A. Voxeur, O. Habrylo, S. Guénin, Oligogalacturonide production upon *Arabidopsis*  
766 *thaliana*–*Botrytis cinerea* interaction. *Proceedings of the* (2019) (available at  
767 <https://www.pnas.org/content/116/39/19743.short>).
- 768 66. B. Randoux, D. Renard-Merlier, G. Mulard, S. Rossard, F. Duyme, J. Sanssené, J.  
769 Courtois, R. Durand, P. Reignault, Distinct defenses induced in wheat against powdery  
770 mildew by acetylated and nonacetylated oligogalacturonides. *Phytopathology.* **100**, 1352–  
771 1363 (2010).
- 772 67. G. Pogorelko, V. Lionetti, O. Fursova, R. M. Sundaram, M. Qi, S. A. Whitham, A. J.  
773 Bogdanove, D. Bellincampi, O. A. Zobotina, *Arabidopsis* and *Brachypodium distachyon*  
774 transgenic plants expressing *Aspergillus nidulans* acetyltransferases have decreased  
775 degree of polysaccharide acetylation and increased resistance to pathogens. *Plant*  
776 *Physiol.* **162**, 9–23 (2013).
- 777 68. S. W. McCotter, L. C. Horianopoulos, J. W. Kronstad, Regulation of the fungal secretome.  
778 *Curr. Genet.* **62**, 533–545 (2016).
- 779 69. A. Berhin, D. de Bellis, R. B. Franke, R. A. Buono, M. K. Nowack, C. Nawrath, The Root  
780 Cap Cuticle: A Cell Wall Structure for Seedling Establishment and Lateral Root Formation.  
781 *Cell.* **0** (2019), doi:10.1016/j.cell.2019.01.005.
- 782 70. Y. Jiang, W. Wang, Q. Xie, N. Liu, L. Liu, D. Wang, X. Zhang, C. Yang, X. Chen, D. Tang,  
783 E. Wang, Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and  
784 parasitic fungi. *Science.* **356**, 1172–1175 (2017).
- 785 71. R. Jablouné, M. Khalil, I. E. Ben Moussa, A.-M. Simao-Beauvoir, S. Lerat, R. Brzezinski,  
786 C. Beaulieu, Enzymatic Degradation of p-Nitrophenyl Esters, Polyethylene Terephthalate,  
787 Cutin, and Suberin by Sub1, a Suberinase Encoded by the Plant Pathogen *Streptomyces*  
788 *scabies*. *Microbes Environ.* **35** (2020), doi:10.1264/jsme2.ME19086.
- 789 72. A. Kashyap, M. Planas-Marquès, M. Capellades, M. Valls, N. S. Coll, Blocking intruders:  
790 inducible physico-chemical barriers against plant vascular wilt pathogens. *J. Exp. Bot.* **72**,



- 791 184–198 (2021).
- 792 73. I. Baxter, P. S. Hosmani, A. Rus, B. Lahner, J. O. Borevitz, B. Muthukumar, M. V.  
793 Mickelbart, L. Schreiber, R. B. Franke, D. E. Salt, Root suberin forms an extracellular  
794 barrier that affects water relations and mineral nutrition in Arabidopsis. *PLoS Genet.* **5**,  
795 e1000492 (2009).
- 796 74. S. Gottwald, B. Samans, S. Lück, W. Friedt, Jasmonate and ethylene dependent defence  
797 gene expression and suppression of fungal virulence factors: two essential mechanisms  
798 of Fusarium head blight resistance in wheat? *BMC Genomics.* **13**, 369 (2012).
- 799 75. T. A. Naumann, D. T. Wicklow, Chitinase modifying proteins from phylogenetically distinct  
800 lineages of Brassica pathogens. *Physiol. Mol. Plant Pathol.* **82**, 1–9 (2013).
- 801 76. F. Sénéchal, L. Graff, O. Surcouf, P. Marcelo, C. Rayon, S. Bouton, A. Mareck, G. Mouille,  
802 A. Stintzi, H. Höfte, P. Lerouge, A. Schaller, J. Pelloux, Arabidopsis PECTIN  
803 METHYLESTERASE17 is co-expressed with and processed by SBT3.5, a subtilisin-like  
804 serine protease. *Ann. Bot.* **114**, 1161–1175 (2014).
- 805 77. M. A. Atmodjo, Z. Hao, D. Mohnen, Evolving views of pectin biosynthesis. *Annu. Rev.*  
806 *Plant Biol.* **64**, 747–779 (2013).
- 807 78. R. G. T. Lowe, O. McCorkelle, M. Bleackley, C. Collins, P. Faou, S. Mathivanan, M.  
808 Anderson, Extracellular peptidases of the cereal pathogen Fusarium graminearum. *Front.*  
809 *Plant Sci.* **6**, 962 (2015).
- 810 79. R. D. Moura, L. A. M. de Castro, M. P. Culik, A. A. R. Fernandes, P. M. B. Fernandes, J.  
811 A. Ventura, Culture medium for improved production of conidia for identification and  
812 systematic studies of Fusarium pathogens. *J. Microbiol. Methods.* **173**, 105915 (2020).
- 813 80. N. L. Catlett, B.-N. Lee, O. C. Yoder, B. Gillian Turgeon, Split-Marker Recombination for  
814 Efficient Targeted Deletion of Fungal Genes. *Fungal Genet. Rep.* **50**, 9–11 (2003).
- 815 81. M. S. López-Berges, N. Rispaill, R. C. Prados-Rosales, A. Di Pietro, A nitrogen response  
816 pathway regulates virulence functions in Fusarium oxysporum via the protein kinase TOR  
817 and the bZIP protein MeaB. *Plant Cell.* **22**, 2459–2475 (2010).
- 818 82. A. Di Pietro, M. I. Roncero, Cloning, expression, and role in pathogenicity of pg1 encoding  
819 the major extracellular endopolygalacturonase of the vascular wilt pathogen Fusarium  
820 oxysporum. *Mol. Plant. Microbe. Interact.* **11**, 91–98 (1998).
- 821 83. U. Raeder, P. Broda, Rapid preparation of DNA from filamentous fungi. *Lett. Appl.*  
822 *Microbiol.* **1**, 17–20 (1985).
- 823 84. D. Segorbe, A. Di Pietro, E. Pérez-Nadales, D. Turrà, Three Fusarium oxysporum  
824 mitogen-activated protein kinases (MAPKs) have distinct and complementary roles in  
825 stress adaptation and cross-kingdom pathogenicity. *Mol. Plant Pathol.* **18**, 912–924  
826 (2017).
- 827 85. M. S. López-Berges, J. Capilla, D. Turrà, L. Schaffner, S. Matthijs, C. Jöchl, P. Cornelis,  
828 J. Guarro, H. Haas, A. Di Pietro, HapX-Mediated Iron Homeostasis Is Essential for  
829 Rhizosphere Competence and Virulence of the Soilborne Pathogen Fusarium oxysporum.  
830 *Plant Cell.* **24**, 3805–3822 (2012).
- 831 86. A. Menna, M. Fischer-Stettler, B. Pfister, G. S. Andrés, D. Holbrook-Smith, C. Sánchez-  
832 Rodríguez, Single-run HPLC Quantification of Plant Cell Wall Monosaccharides. *Bio*  
833 *Protoc.* **10**, e3546 (2020).
- 834 87. T. Czechowski, M. Stitt, T. Altmann, M. K. Udvardi, Genome-wide identification and testing  
835 of superior reference genes for transcript normalization in Arabidopsis. *Plant* (2005)  
836 (available at <https://academic.oup.com/plphys/article-abstract/139/1/5/6113402>).
- 837 88. T. Li, Y. Wu, Y. Wang, H. Gao, V. K. Gupta, X. Duan, H. Qu, Y. Jiang, Secretome Profiling

- 838 Reveals Virulence-Associated Proteins of *Fusarium proliferatum* during Interaction with  
839 Banana Fruit. *Biomolecules*. **9** (2019), doi:10.3390/biom9060246.
- 840 89. C. Türker, F. Akal, D. Joho, C. Panse, S. Barkow-Oesterreicher, H. Rehrauer, R.  
841 Schlapbach, in *Proceedings of the 13th International Conference on Extending Database*  
842 *Technology* (Association for Computing Machinery, New York, NY, USA, 2010), *EDBT*  
843 '10, pp. 717–720.
- 844 90. J. Cox, M. Mann, MaxQuant enables high peptide identification rates, individualized  
845 p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.*  
846 **26**, 1367–1372 (2008).
- 847 91. Wolski, Witold, Jonas Grossmann, and Christian Panse., SRMSservice - R-Package to  
848 Report Quantitative Mass Spectrometry Data (2018), (available at  
849 <http://github.com/protViz/SRMSservice>).

850  
851

## 852 **Acknowledgements**

853 We thank all members of the Plant Cell Biology laboratory at ETHZ, especially Apolonio  
854 Huerta, Christopher Kesten, Oliver Terret, and Alexandra Menna, for technical support and  
855 fruitful scientific discussion. We are also very grateful to Niko Geldner (UNIL) for fruitful  
856 scientific discussions and to Paolo Nanni and Laura Kunz (Functional Genomics Center  
857 Zürich, FGCZ) for technical support during the generation and analysis of the proteomic data.  
858 We thank the ScopeM (ETH Zurich) for their service in the confocal microscopy.

859 **Funding:** The work described in this paper was supported by the Swiss National foundation  
860 to CSR (SNF 310030\_184769 to SD and GS), the Vontobel foundation to FMGA and CSR, and  
861 the Spanish Ministerio de Ciencia e Innovación (MICINN PID2019-108045RB-I00) to ADP.  
862 SV was supported by the Marie Curie ITN FUNGIBRAIN (FP7-PEOPLE-ITN-607963), and  
863 AV by a National Research Institute for Agriculture, Food and Environment tenure track grant.

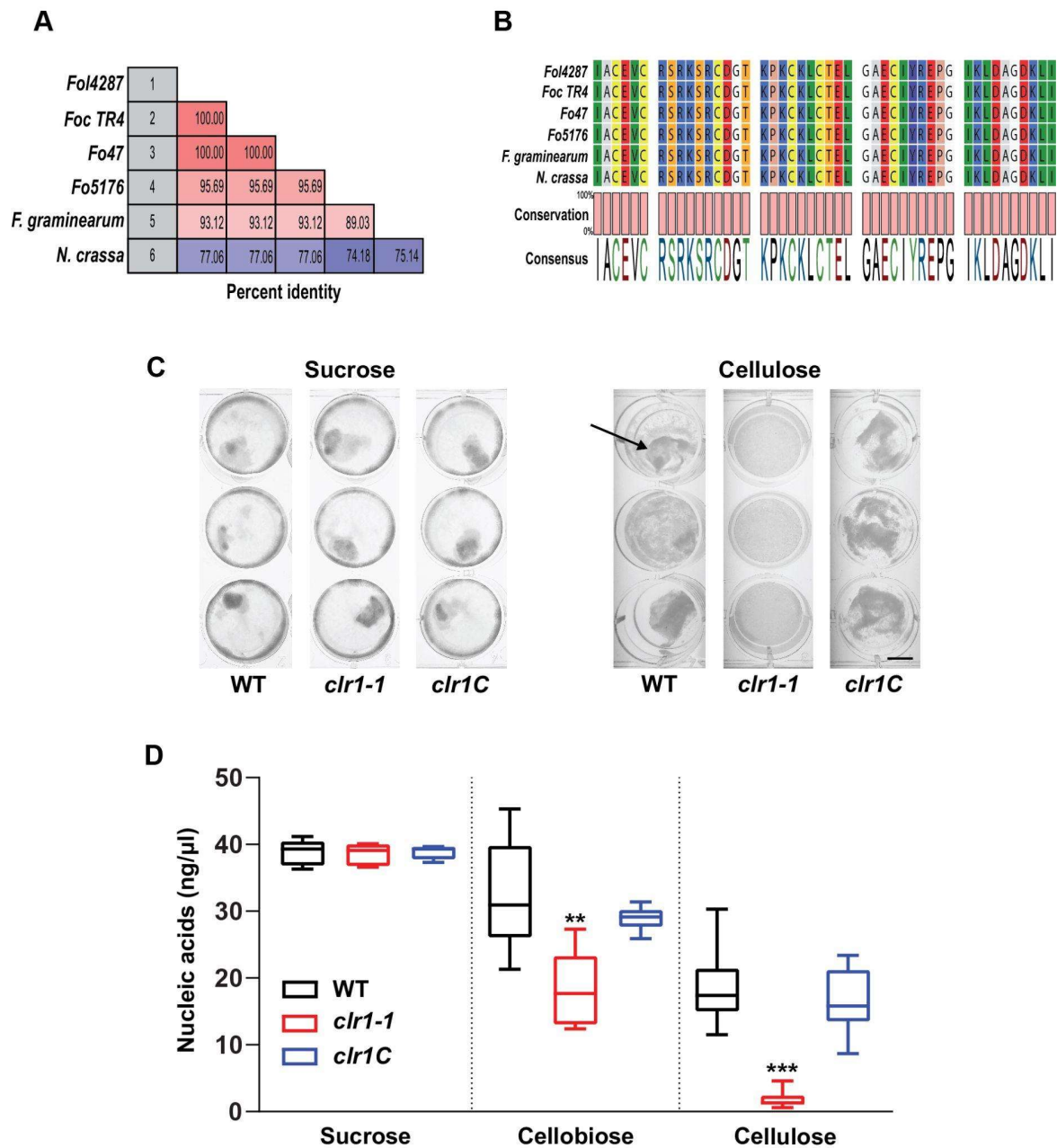
## 864 **Author contributions:**

865 Conceptualization: CSR  
866 Methodology: FMGA, SV, AV, SD, JCM  
867 Investigation: FMGA, SV, AV, SD, SM, GSA, JCM  
868 Visualization: FMGA, SV, AV, SD, GSA, JCM  
869 Supervision: CSR and ADP  
870 Writing—original draft: FMGA and CSR  
871 Writing—review & editing: FMGA, SV, AV, SD, JCM, ADP and CSR

872 **Competing interests:** All other authors declare they have no competing interests.

873 **Data and materials availability:** All data are available in the main text or the supplementary  
874 materials.

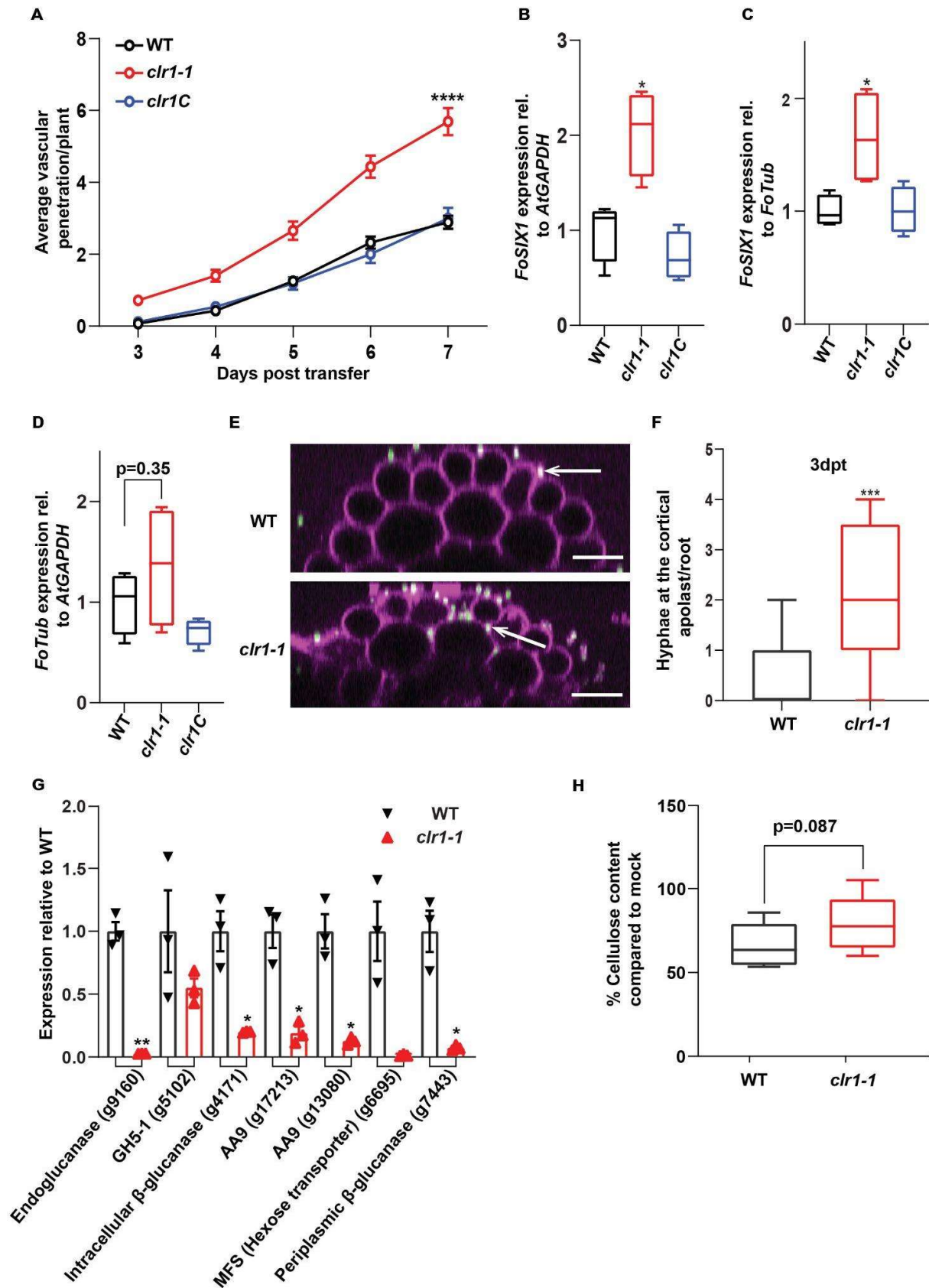
875 **Figures and Tables**



876

877 **Fig. 1. Cellulose degradation is regulated by CLR1 in *F. oxysporum* (Fo5176).**

878 **(A)** Pairwise comparison of 5 *Fusarium* species or *Fo* species complexes to identify potential  
 879 orthologous to the *Neurospora crassa* transcription factor. The percent of protein identity is  
 880 indicated. **(B)** Sequence alignment of the DNA-binding domains of the CLR1 proteins identified  
 881 in (A). The conservation grade is shown. Note the high conservation of the Zn(2)-C6 fungal-  
 882 type domain, ACEVCRSRKSRCDGTPKPKCKLCTELGAECIY, related to the DNA-binding  
 883 domain. **(C)** Representative picture of WT, *clr1-1* and *clr1C* growth on sucrose (left panel) or  
 884 cellulose (right panel). The arrow indicates the presence of mycelia. Scale bar, 1cm. **(D)**  
 885 Growth of WT, *clr1-1* and *clr1C* on different carbon sources measured as nucleic acid  
 886 concentration (ng/ $\mu$ l). The strains were growing on sucrose 0.5% or cellobiose 0.5% for 3 days,  
 887 or on cellulose 0.5% for 7 days. Shown are the box plots: centerlines show the medians; box  
 888 limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers extend to the minimum and maximum,  
 889  $n \geq 5$ . Asterisk indicated differences relative to WT. Welch's unpaired t-test; \*\*\*  $p$ -value  $< 0.001$ .  
 890 \*\*  $p$ -value  $\leq 0.01$ .

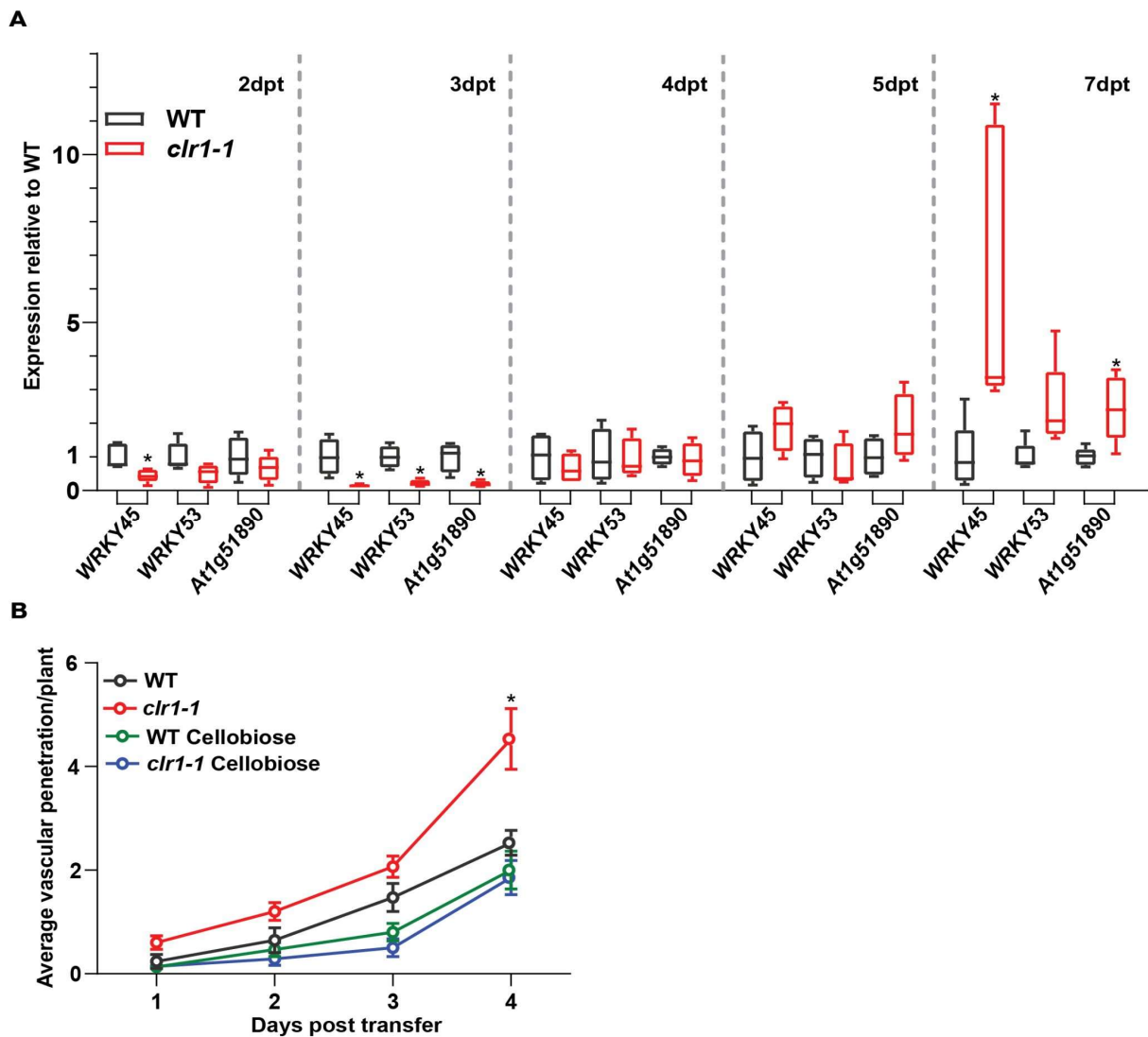


891

892 **Fig. 2. The lack of CLR1 increases Fo pathogenicity.**

893 (A) Cumulative Arabidopsis root vascular penetration by Fo at different days post-transfer (dpt)  
 894 to WT, *clr1-1* or *clr1C* microconidia-containing plates. Values are mean +/- SEM, N $\geq$  28 plants  
 895 from one representative experiment. The experiment was performed 3 times with similar

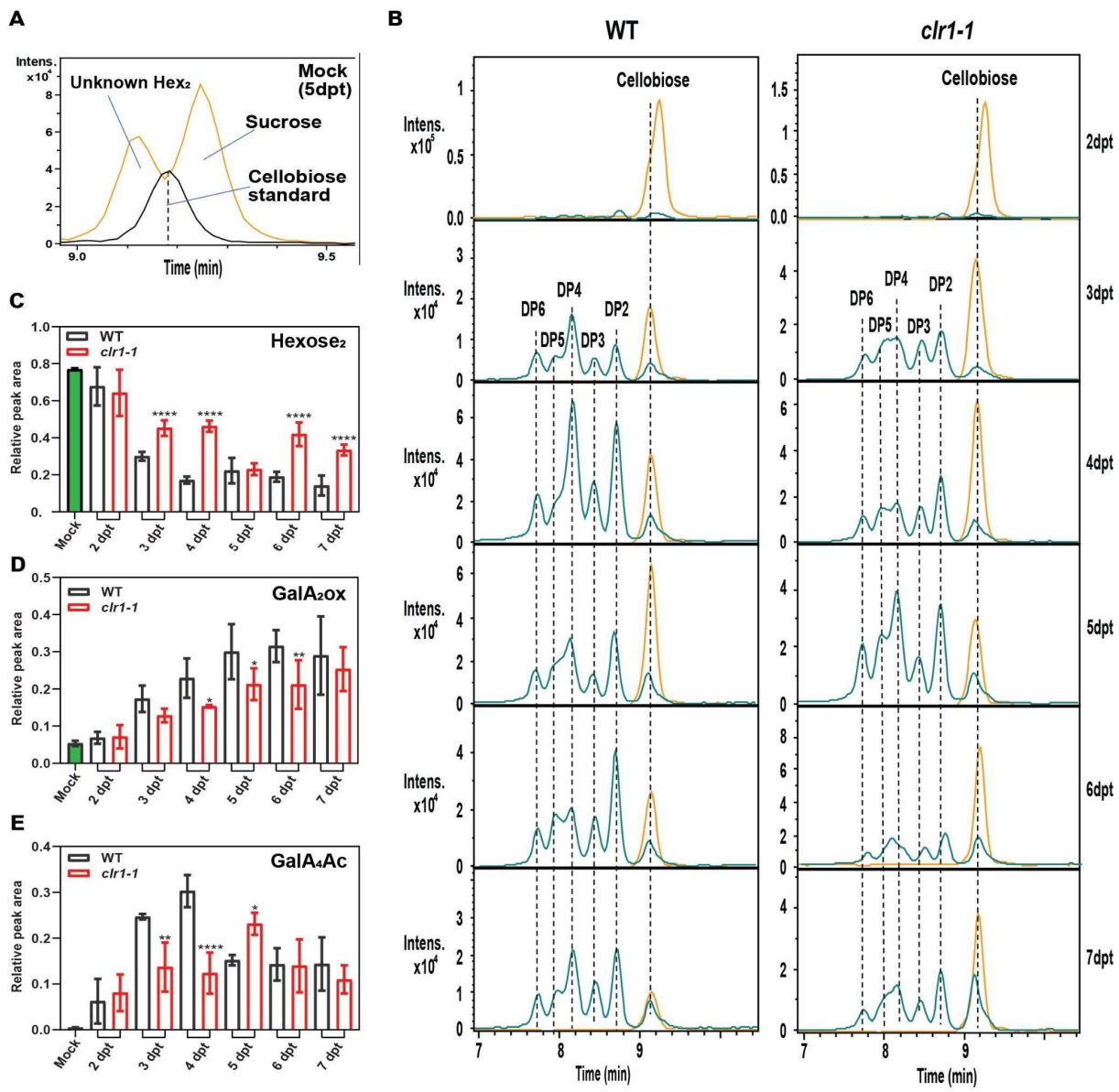
896 results. RM two-way ANOVA on vascular penetration rate:  $p < 0.0001$  (fungal genotype),  
897  $p < 0.0001$  (time),  $p \leq 0.0001$  (fungal genotype x time). Asterisk indicated a statistical difference  
898 with respect to WT at 7 dpt, Tukey's multiple comparison test, \*\*\*\*  $p < 0.0001$ . **(B)** and **(C)**  
899 *FoSIX1* expression relative to *AtGAPDH* or *FoTub*, respectively, in infected Arabidopsis roots  
900 at 7 dpt as in (A).. Shown are the box plots: centerlines show the medians; box limits indicate  
901 the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers extend to the minimum and maximum. N=4 biological  
902 replicates; Welch's unpaired t-test; \* p-value  $\leq 0.05$ . **(D)** Fungal biomass calculated as *FoTub*  
903 expression relative to *AtGAPDH*. Box plots shown as above. N=4 biological replicates. **(E)**  
904 Representative confocal image of WT or *clr1-1* hyphae (green) colonization of Arabidopsis  
905 roots stained with PI (magenta). Arrows point to hyphae. Scale bars: 20 $\mu$ m. **(F)** Number of WT  
906 or *clr1-1* hyphae able to reach the cortical apoplast as shown in (E). Data are represented on  
907 box plots as described above. N $\geq$ 12, Welch's unpaired t-test; \*\*\* p-value  $< 0.001$ . **(G)** WT and  
908 *clr1-1* gene expression in 7 dpt infected roots normalized to the fungal *FoTub* gene. Bars  
909 represent means  $\pm$  SEM, N=3 biological replicates (arrowheads). For each gene, the data  
910 were normalized to WT; Welch's unpaired t-test; \* p-value  $< 0.05$ . \*\* p-value  $< 0.01$ . **(H)** Cellulose  
911 content in WT and *clr1-1* infected roots at 7dpt represented as % of cellulose measured in 7  
912 dpt mock roots. Shown are the box plots as described above. N=8 biological replicates.  
913 Welch's unpaired t-test; p-value indicated.



914

915 **Fig. 3. The plant perceives later *clr1-1* than WT.**

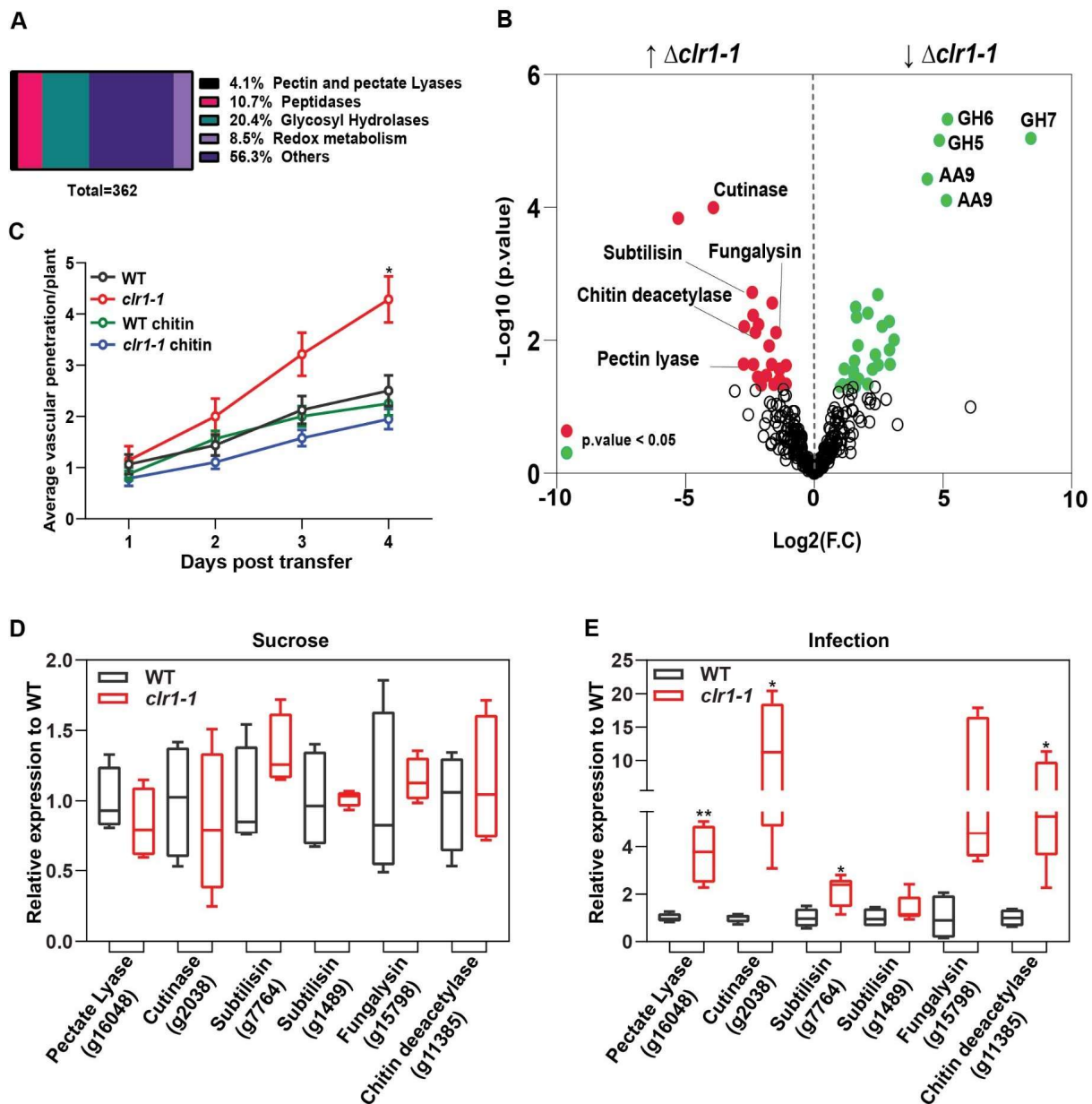
916 **(A)** Expression of *WRKY45*, *WRKY53*, and *At1g51890* relative to *AtGAPDH* in Arabidopsis  
 917 roots at 2, 3, 4, 5 or 7dpt to plates with WT or *clr1-1* microconidia. Shown are box plots:  
 918 centerlines show the medians; box limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers extend  
 919 to the minimum and maximum,  $n \geq 4$  biological replicates. For each gene, the data were  
 920 normalized to WT and the statistical differences are indicated by asterisks; Welch's unpaired  
 921 *t*-test; \*  $p$ -value  $< 0.05$ . **(B)** Cumulative Arabidopsis root vascular penetration by *Fo* at different  
 922 dpt to WT, *clr1-1* or *clr1C* hyphae-containing plates. Half of the plants were pretreated with  
 923 cellobiose for 25 minutes before being exposed to the fungus. Values are mean  $\pm$  SEM,  $N \geq 14$   
 924 from one representative experiment. The experiment was repeated three times with similar  
 925 results. RM two-way ANOVA on vascular penetration rate:  $p < 0.0001$  (fungal genotype and  
 926 treatment),  $p < 0.0001$  (time),  $p \leq 0.0001$  (fungal genotype x time). Asterisk indicates a statistical  
 927 difference with respect to WT at 7 dpt. Tukey's multiple comparisons test, \*  $p < 0.05$ .



928

929 **Fig. 4. *clr1-1* infection generates different amounts of plant cell wall-derived molecules**  
 930 **than WT.**

931 **(A)** Extracted ion chromatograms for *m/z* 341 of mock root cell wall (yellow) water extract (5  
 932 dpt) and cellobiose commercial standard (black) analysed by HP-SEC-MS. Saccharose and  
 933 another di-hexose but no cellobiose are detected in cell wall water extract. **(B)** Representative  
 934 extracted ion chromatograms of oligogalacturonides (OGs; blue) and di-hexoses (yellow)  
 935 detected along 5 dpt to plates with WT or *clr1-1* microconidia. DP indicates the degree of  
 936 polymerization of OGs. The retention time of different standards of DP and cellobiose is  
 937 indicated with a vertical dotted line. **(C-E)** Kinetics of Hexose<sub>2</sub> (C), GalA<sub>2</sub>ox (D), and GalA<sub>4</sub>Ac  
 938 (E) produced in Arabidopsis roots by WT or *clr1-1* infection. Mock indicates the levels of  
 939 polysaccharides at 5 dpt. Bars are means  $\pm$  SEM, N=3 biological replicates as described in  
 940 (B) and 2 replicates for mock samples. Asterisk indicates statistical significance compared to  
 941 the WT on each dpt, two-way ANOVA with LSD Fisher test post hoc comparison; \*\*\*\*  
 942  $p < 0.0001$  \*\*  $p < 0.01$ , \*  $p < 0.05$ . OGs are named GalA<sub>x</sub>, GalA<sub>x</sub>Ac<sub>y</sub> or GalAox with the subscript  
 943 numbers indicating the degree of polymerization (x) and the number of acetylated groups (y);  
 944 "ox" indicate the presence of oxidized groups.



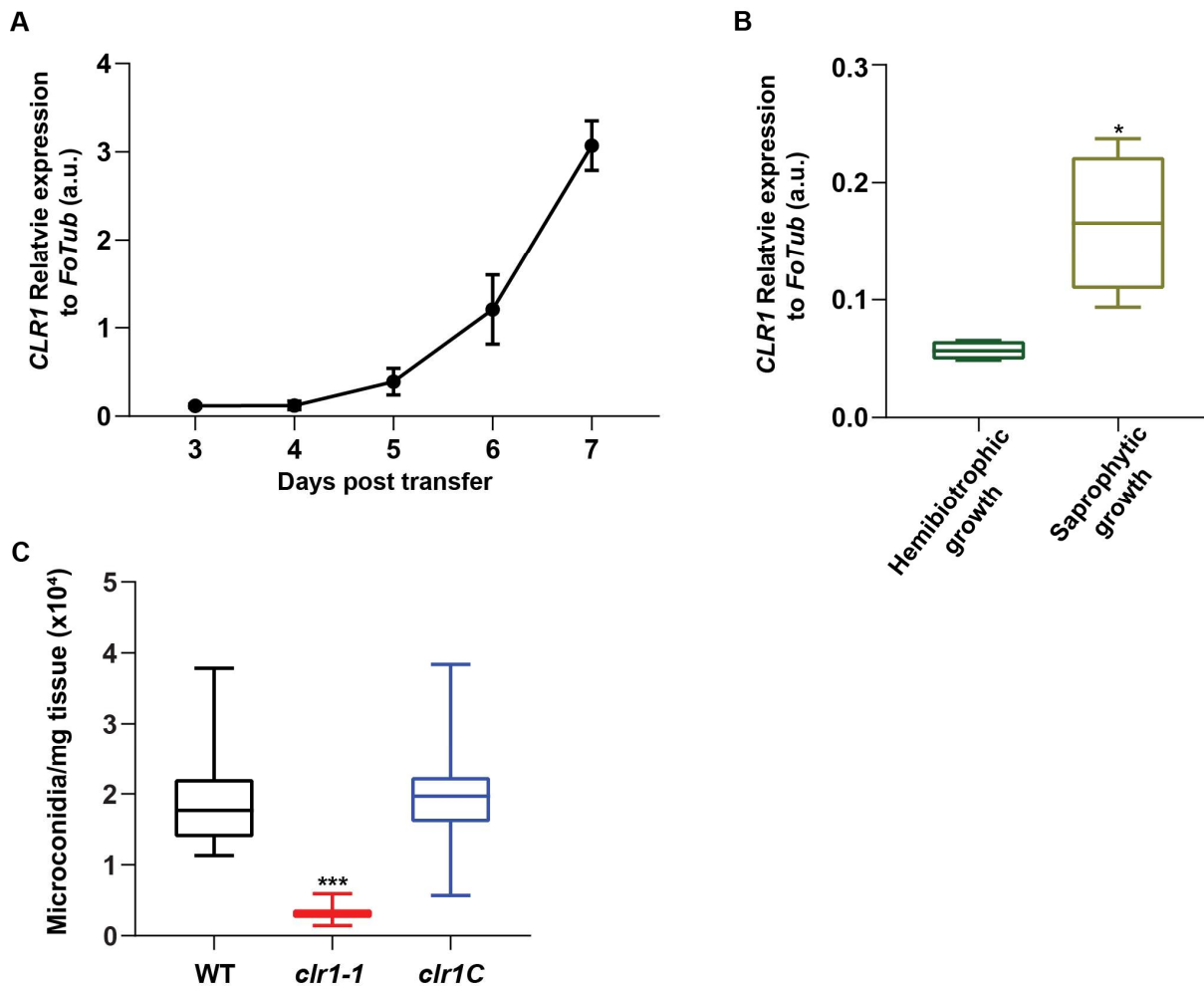
945

946 **Fig. 5. *clr1-1* secretes more virulence factors than WT during Arabidopsis root**  
 947 **infection.**

948 **(A)** Proteins identified in the secretome of WT Fo at 3 dpt of Arabidopsis seedlings in  
 949 hydroponic conditions. A total of 362 fungal proteins were identified in 4 independent replicates  
 950 and the percentages of the most representative family proteins found are presented. **(B)**  
 951 Volcano plot of differences in the abundance of proteins identified in the secretome of *clr1-1*  
 952 relative to the WT. Proteins significantly (*moderated T-test*, *p*-values < 0.05) less or more  
 953 present in the *clr1-1* secretome are shown in green or red, respectively. The name or family  
 954 of the most relevant proteins is indicated. **(C)** Cumulative Arabidopsis root vascular penetration  
 955 by Fo at different dpt to WT, *clr1-1* or *clr1C* hyphae-containing plates. Half of the plants were  
 956 pretreated with chitin for 25 minutes. Values are mean +/- SEM, N≥14 from one representative  
 957 experiment. The experiment was repeated three times with similar results. RM two-way  
 958 ANOVA on vascular penetration rate: *p*<0.001 (fungal genotype and treatment), *p*<0.0001  
 959 (time), *p* ≤ 0.0001 (fungal genotype x time). Asterisk indicates a statistical difference with  
 960 respect to WT at 7 dpt. Tukey's multiple comparisons test, \* *p*<0.05. **(D)** and **(E)** Gene  
 961 expression of 6 virulence factors relative to *FoTub* in WT or *clr1-1* grown 3 days in sucrose (D)  
 962 or at 3dpt in Arabidopsis roots (E). The genes were selected among the ones enriched in the

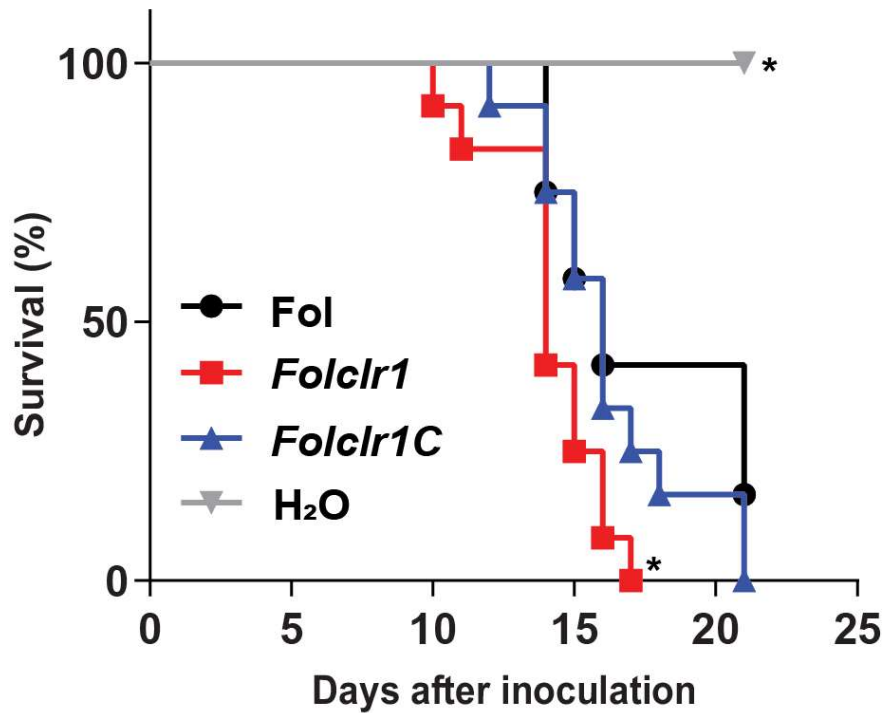


963 *clr1-1* secretome as shown in (B: : a pectate lyase (g16048), a cutinase (g2038), two subtilisins  
964 (g7764 and g1489), a fungalysin (g15798) and chitin deacetylase (g11385) Shown are box  
965 plots: center lines show the medians; box limits indicate the 25th and 75th percentiles;  
966 whiskers extend to the minimum and maximum,  $n \geq 4$ . For each gene, the data were  
967 normalized to WT and the statistical differences are indicated by asterisks. Welch's unpaired  
968 *t*-test; \* p-value<0.05. \*\* p-value<0.01.



969

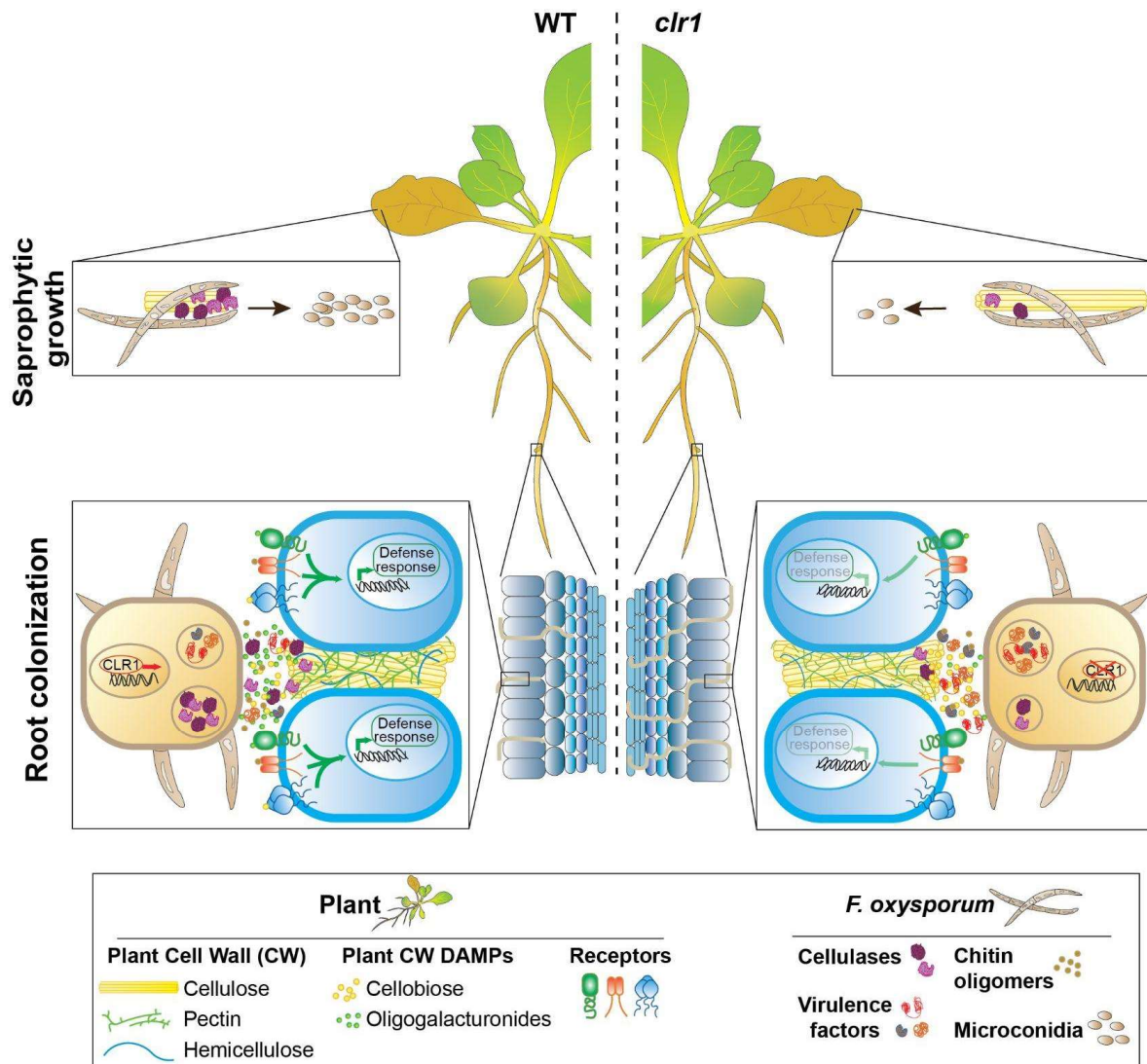
970 **Fig. 6. CLR1 is crucial for *Fo* saprophytic growth and spore production.**  
971 **(A)** *CLR1* expression analysis relative to *FoTub* in Arabidopsis infected roots at different dpt  
972 to WT microconidia containing plates. Values represent means +/- SEM from 3 biological  
973 replicates. **(B)** *CLR1* expression relative to *FoTub* in WT or *clr1-1* grown in alive or dead plants  
974 for 4 days. Shown are box plots: center lines show the medians; box limits indicate the 25th  
975 and 75th percentiles; whiskers extend to the minimum and maximum, N $\geq$ 4 biological  
976 replicates; Welch's unpaired *t*-test; \* p-value<0.05. **(C)** Microconidia production of WT, *clr1-1*  
977 and *clr1C* in dead aerial tissue of infected Arabidopsis plants. Box plots as described above.  
978 N $\geq$ 10 biological replicates. Asterisks indicate statistical difference relative to WT, Welch's  
979 unpaired *t*-test. \*\*\*p<0.001.



980

981 **Fig 7. The lack of CLR1 also increases *Fol4287* pathogenicity in tomato plants.**

982 Kaplan–Meier plot showing the survival of tomato plants grown in vermiculite and dip-  
983 inoculated or not (H<sub>2</sub>O) with *Fol*, *Folclr1* or *Folclr1C* microconidia. N=15 plants from one  
984 independent experiment. \**P* < 0.05 versus *Fol* alone according to log-rank test. The experiment  
985 was repeated 3 times with similar results.



986

987 **Fig. 8. Model of CLR1 role in the life cycle of *F. oxysporum* in planta.**

988 Bottom part: During root apoplast colonization, CLR1 is required to degrade and consume the  
 989 plant cellulose. In its absence, the *clr1* mutant secretes more virulence factors and produces  
 990 less oligogalacturonides, which are pectin-derived damage-associated molecular patterns  
 991 (DAMPs). The virulence factors secreted by *clr1* also reduce the production of chitin-  
 992 monomers perceived as microbe-associated molecular patterns by the plant. Consequently,  
 993 the host recognizes *clr1* less efficiently than WT and *clr1* can colonize faster the xylem. Green  
 994 arrows represent signalling cascade activation, their intensity reflects how much it is triggered.  
 995 Upper part: Once the plant is dead, Fo needs to grow saprophytically and needs CLR1 to use  
 996 cellulose as carbon source and produce microconidia.

997 **Table 1. Fo5176 secretome identified proteins**  
998 Proteins identified in *clr1-1* and WT Proteins identified in the secretome of WT and *clr1-1*  
999 infected roots at 3 dpt. Proteins differentially present in *clr1-1* secretome relative to WT with a  
1000 moderated T-test  $p < 0.05$  are highlighted in green and red for less or more abundant in the  
1001 mutant, respectively, as in Fig 5B. For the proteins without IPR description, no homologues  
1002 were found in the database to assign a putative activity.

Secretome Identified Proteins

gene_id	IPR_description	clr1-1	WT	log2FC	P.Value	Adjusted.P.Value
g15944	Glycoside hydrolase, family 6, conserved site, 1, 4-beta cellobiohydrolase, Cellulose-binding domain, funga	1.10	6.26	5.16	4.69E-06	0.001186
g873	Cellulose-binding domain, fungal, Glycoside hydrolase, family 7, Concanavalin A-like lectin/glucanase domi	-1.06	7.35	8.41	9.18E-06	0.001186
g17181	Glycoside hydrolase, family 5, Cellulose-binding domain, fungal, Cellulose-binding domain superfamily, Gly	0.44	5.28	4.84	9.83E-06	0.001186
g15991	Glycoside hydrolase, family 61	1.09	5.46	4.37	3.78E-05	0.003418
g13080	Glycoside hydrolase, family 61	-2.93	2.19	5.12	7.94E-05	0.005752
g2038	Alpha/Beta hydrolase fold, Cutinase, monofunctional, Cutinase/acetylxyylan esterase	4.10	0.18	-3.91	0.000101	0.006069
g5928	Copper amine oxidase, N2-terminal, Domain of unknown function DUF1965, Copper amine oxidase, Coppe	2.73	-2.56	-5.29	0.000145	0.007510
g7764	Peptidase S8/S53 domain superfamily, Peptidase S8, subtilisin-related, Peptidase S8, subtilisin, His-active s	0.34	-2.07	-2.41	0.001898	0.085018
g3515	Cyclophilin-type peptidyl-prolyl cis-trans isomerase domain, Cyclophilin-like domain superfamily, Cyclophil	-1.56	0.88	2.44	0.002114	0.085018
g4964	FAD/NAD(P)-binding domain superfamily, Glucose-methanol-choline oxidoreductase, N-terminal, Glucose-	4.68	3.05	-1.63	0.002753	0.096772
g4855	WD40/YVTN repeat-like-containing domain superfamily	4.60	6.17	1.57	0.003294	0.108405
g12830	GDSL lipase/esterase, SGNH hydrolase superfamily	-0.54	1.50	2.05	0.004027	0.116412
g12342	Glucose-methanol-choline oxidoreductase, C-terminal, Glucose-methanol-choline oxidoreductase, N-termi	1.37	-1.01	-2.37	0.004181	0.116412
g17742	Esterase, PHB depolymerase, Cellulose-binding domain, fungal, Alpha/Beta hydrolase fold	0.74	2.35	1.61	0.004642	0.120031
g3198	Glycoside hydrolase, family 29, Glycoside hydrolase superfamily, Alpha-L-fucosidase, metazoa-type	-2.16	0.73	2.89	0.005414	0.128718
g7360		0.02	-2.14	-2.16	0.005778	0.128718
g7411	Glucose-methanol-choline oxidoreductase, N-terminal, Glucose-methanol-choline oxidoreductase, C-termi	-1.99	-4.70	-2.71	0.006186	0.128718
g10128	FAD/NAD(P)-binding domain superfamily	-4.28	-1.68	2.61	0.0064	0.128718
g11385	Glycoside hydrolase/deacetylase, beta/alpha-barrel, NodB homology domain	1.70	-0.58	-2.28	0.00754	0.138344
g15798	Peptidase M36, fungalsin, FTP domain, Peptidase M4/M1, CTD superfamily	7.12	5.64	-1.48	0.007643	0.138344
g5082	Cellulose-binding domain superfamily, Cellulose-binding domain, fungal, Glycosyl hydrolases family 10, act	3.02	6.09	3.08	0.010274	0.177098
g16180	Pectin lyase fold/virulence factor, Pectinesterase, catalytic, Pectin lyase fold, Pectinesterase, Asp active siti	-1.93	-3.69	-1.76	0.012082	0.197791
g10757		-1.71	-0.05	1.66	0.012567	0.197791
g2525	LysM domain, LysM domain superfamily	-4.45	-1.55	2.90	0.014557	0.219565
g11976	Alpha/Beta hydrolase fold, Esterase, PHB depolymerase	1.72	4.06	2.34	0.017364	0.251429
g12120	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycoside hydrolase, family 43, Concar	1.43	2.96	1.53	0.021394	0.278408
g16048	Pectate lyase PlyH/PlyE-like, Pectin lyase fold, Pectin lyase fold/virulence factor	-2.25	-4.98	-2.73	0.022862	0.278408
g1489	Peptidase S8, subtilisin, Ser-active site, Peptidase S8, subtilisin, Asp-active site, Peptidase S8/S53 domain s	3.40	1.04	-2.36	0.022949	0.278408
g9438	FAD linked oxidase, N-terminal, FAD-binding domain, PCMH-type, FAD-binding, type PCMH-like superfamil	3.37	1.72	-1.65	0.023099	0.278408
g16997	Pectin lyase fold/virulence factor, Pectin lyase fold, PL-6 family, Right handed beta helix domain	1.07	-0.02	-1.09	0.023846	0.278408
g15934	GDSL lipase/esterase, SGNH hydrolase superfamily	-0.80	2.12	2.92	0.024253	0.278408
g15471	Glycoside hydrolase, family 32, Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycosi	-1.95	0.49	2.45	0.024611	0.278408
g15630	Proteinase K-like catalytic domain, Peptidase S8, subtilisin, Asp-active site, Peptidase S8/S53 domain supe	1.90	0.56	-1.34	0.026943	0.288834
g1832	Proteinase K-like catalytic domain, Peptidase S8, subtilisin, Asp-active site, Peptidase S8/S53 domain, Pept	3.79	2.44	-1.35	0.027505	0.288834
g9050		1.42	2.54	1.12	0.028448	0.288834
g6456	Heat shock protein 70 family, Heat shock protein 70kD, C-terminal domain superfamily, Heat shock protei	-4.46	-2.24	2.22	0.028724	0.288834
g6198	Glucanoyltransferase, Glycoside hydrolase superfamily	-1.44	0.04	1.49	0.03032	0.296647
g5964	Peptidase S10, serine carboxypeptidase, Alpha/Beta hydrolase fold, Serine carboxypeptidase, serine active	-0.11	-1.97	-1.86	0.033818	0.321392
g17041	Necrosis inducing protein	-1.80	-0.28	1.52	0.035176	0.321392
g9531	S1/P1 nuclease, Phospholipase C/P1 nuclease domain superfamily	-0.70	-2.89	-2.20	0.035513	0.321392
g12993	EMP46/EMP47, N-terminal lectin domain, Concanavalin A-like lectin/glucanase domain superfamily, Legun	-3.44	-1.71	1.72	0.038432	0.335491
g15873	Glycoside hydrolase, family 3, N-terminal domain superfamily, Glycoside hydrolase, family 3, N-terminal, F	2.15	0.80	-1.35	0.038924	0.335491
g15992	Tuberculosis necrotizing toxin	-2.78	-1.69	1.09	0.044578	0.338676
g8603	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycoside hydrolase, family 43	0.00	-1.35	-1.35	0.045103	0.338676
g6954	Rho protein GDP-dissociation inhibitor, Rho GDP-dissociation inhibitor domain superfamily, Immunoglobul	-4.17	-2.08	2.09	0.045306	0.338676
g4912	Serine proteases, trypsin family, histidine active site, Peptidase S1A, chymotrypsin family, Peptidase S1, PA	7.44	6.23	-1.21	0.045368	0.338676
g8786	GLEYA adhesin domain, PA14/GLEYA domain	1.44	0.34	-1.10	0.045649	0.338676
g17064	Endonuclease/exonuclease/phosphatase superfamily	1.41	-0.16	-1.56	0.045751	0.338676
g704	Ran GTPase, Small GTP-binding protein domain, P-loop containing nucleoside triphosphate hydrolase, Sma	-1.76	-0.41	1.35	0.046379	0.338676
g1768	Pepsin-like domain, Aspartic peptidase A1 family, Aspartic peptidase, active site, Aspartic peptidase domai	1.24	-0.82	-2.06	0.046778	0.338676
g7485		1.42	2.40	0.97	0.049228	0.341361
g10337		0.43	2.07	1.64	0.049459	0.341361
g551	Glycoside hydrolase superfamily	-2.99	-0.63	2.36	0.050274	0.341361
g837	YjgF/YER057c/UK114 family, RidA family, RidA, conserved site, RutC-like superfamily	-4.66	-3.15	1.51	0.050921	0.341361
g15627	Necrosis inducing protein	0.53	-0.66	-1.19	0.054819	0.359712
g17579	Isochorismatase-like superfamily, Isochorismatase-like	0.78	2.10	1.32	0.056291	0.359712
g7398	Short-chain dehydrogenase/reductase SDR, NAD(P)-binding domain superfamily	-1.14	-3.42	-2.28	0.05664	0.359712
g8556	Tyrosinase copper-binding domain, Uncharacterised domain, di-copper centre	-0.13	-3.21	-3.08	0.057801	0.360757
g6750	Glycoside hydrolase, family 61	1.57	2.52	0.95	0.060925	0.373812
g12184	Peroxiredoxin, C-terminal, Peroxiredoxin, AhpC-type, Thioredoxin-like superfamily, Thioredoxin domain, A	-2.68	-1.22	1.46	0.064091	0.386681
g14800	Glycoside hydrolase, family 61	0.15	-0.95	-1.10	0.067512	0.399896
g9034	Thioredoxin-like superfamily, Domain of unknown function DUF953, thioredoxin-like	-1.65	-2.82	-1.18	0.068491	0.399896
g1456	Cyclophilin-type peptidyl-prolyl cis-trans isomerase, conserved site, Cyclophilin-type peptidyl-prolyl cis-tra	-3.28	-1.20	2.07	0.075069	0.429309
g944	Inorganic pyrophosphatase superfamily, Inorganic pyrophosphatase	-4.66	-2.47	2.19	0.076033	0.429309
g6384	Glutathione reductase, eukaryote/bacterial, FAD/NAD(P)-binding domain superfamily, FAD/NAD-linked red	-3.20	-0.41	2.79	0.077086	0.429309
g8150	Alpha/Beta hydrolase fold, Alpha/beta hydrolase fold-1	-3.44	-2.09	1.35	0.079683	0.437049
g14988		-2.38	-4.11	-1.73	0.085775	0.463443
g14301	Inositol oxygenase	-3.04	-0.87	2.17	0.088056	0.468767
g8422	Heat shock protein 70kD, C-terminal domain superfamily, ATPase, nucleotide binding domain, Heat shock	-4.17	-1.82	2.36	0.089572	0.469929
g5368	Alpha/Beta hydrolase fold, Cutinase/acetylxyylan esterase, Cutinase, monofunctional	2.29	0.83	-1.46	0.091915	0.475333
g11902	RipA-like domain superfamily, Expansin, cellulose-binding-like domain superfamily, Expansin/pollen allergi	-0.02	-1.64	-1.63	0.093906	0.478790
g13461		7.39	6.06	-1.33	0.097666	0.491041
g2604		-5.15	0.92	6.08	0.100168	0.496722
g1758	Alpha carbonic anhydrase domain, Carbonic anhydrase, alpha-class, Alpha carbonic anhydrase domain sup	-0.32	0.47	0.79	0.104587	0.511626
g13593		2.00	3.47	1.47	0.111758	0.537084

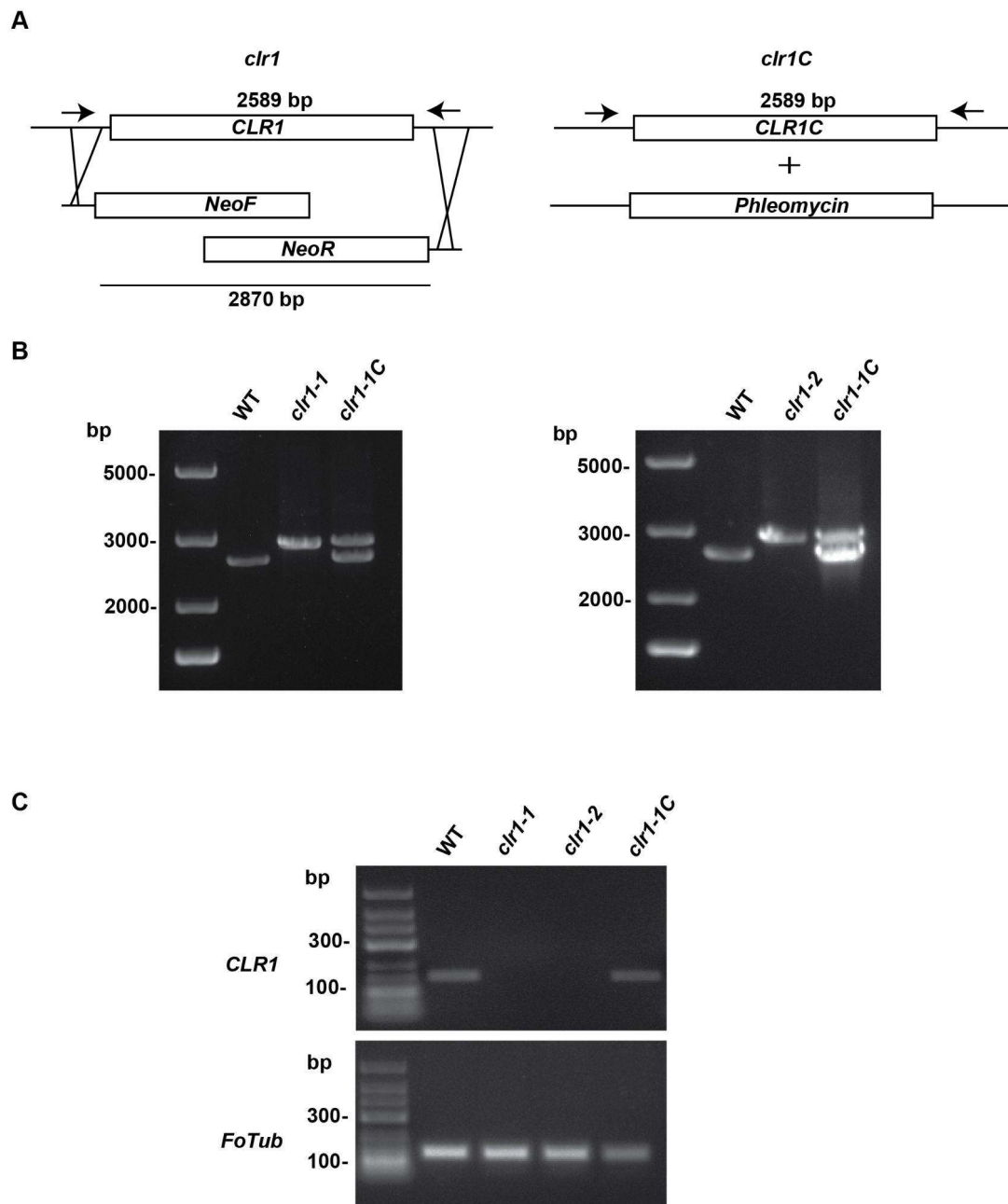
g3337	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycoside hydrolase, family 62, arabin	3.82	5.32	1.50	0.115926	0.537084
g4548	ACP-like superfamily, Phosphopantetheine binding ACP domain, Trimeric LpxA-like superfamily, AMP-depe	-1.20	-2.32	-1.12	0.117796	0.537084
g11299	Immunoglobulin-like fold, Glycoside hydrolase family 3 C-terminal domain superfamily, Glycoside hydrolas	2.91	2.08	-0.83	0.118335	0.537084
g15930	Beta-xylosidase, C-terminal Concanavalin A-like domain, Glycoside hydrolase, family 43, Concanavalin A-lik	3.63	4.36	0.72	0.118371	0.537084
g10243		0.38	-0.37	-0.76	0.118882	0.537084
g4447	Peptidase M28, PA domain, Peptidase M28, SGAP-like	-0.31	0.93	1.24	0.121824	0.537084
g17329	Major facilitator superfamily, Alpha/Beta hydrolase fold, Peptidase S9, prolyl oligopeptidase, catalytic dom	-0.91	-0.03	0.88	0.12213	0.537084
g9446	Oxidoreductase, C-terminal, Oxidoreductase, N-terminal, NAD(P)-binding domain superfamily	-2.45	-1.51	0.94	0.123896	0.537084
g15661	Uncharacterised domain, di-copper centre, Tyrosinase copper-binding domain	-1.33	-2.36	-1.03	0.124627	0.537084
g8037	Glycoside hydrolase superfamily, Glycoside hydrolase family 17	2.58	0.02	-2.57	0.131398	0.555750
g5019	Alpha-L-fucosidase, metazoa-type, Glycoside hydrolase superfamily, Glycosyl hydrolase, all-beta, Alpha-L-f	2.00	0.76	-1.24	0.136941	0.555750
g1132	Uncharacterised protein family, glycosyl hydrolase catalytic domain, Glycoside hydrolase superfamily	-0.01	1.11	1.12	0.137409	0.555750
g15834	Chorismate mutase II, prokaryotic-type, Chorismate mutase domain superfamily, Chorismate mutase type	-3.81	-5.14	-1.33	0.138957	0.555750
g13117	Peptidase S53, activation domain, Peptidase S8/S53 domain superfamily, Sedolisin domain, Peptidase S8/S	0.58	-0.91	-1.50	0.139461	0.555750
g16179	Pectin lyase fold, Glycoside hydrolase, family 28, Pectin lyase fold/virulence factor	-0.99	-2.33	-1.34	0.140138	0.555750
g15705	Pectin lyase fold/virulence factor, Pectin lyase fold	3.91	4.82	0.91	0.141106	0.555750
g16885	Six-hairpin glycosidase superfamily, Beta-L-arabinofuranosidase, GH127	-1.78	-3.59	-1.82	0.14124	0.555750
g7119	SUN family	-4.54	-2.81	1.73	0.143716	0.559411
g14313	Transcription factor, GTP-binding domain, Translation elongation factor EFTu-like, domain 2, P-loop contai	-0.55	-1.47	-0.92	0.146658	0.564788
g10908	Glycosyl hydrolase family 32, N-terminal, Glycosyl hydrolase, five-bladed beta-propellor domain superfami	4.65	5.29	0.65	0.151147	0.575951
g16076	Glucose-methanol-choline oxidoreductase, N-terminal, Glucose-methanol-choline oxidoreductase, C-termi	3.83	3.07	-0.76	0.155742	0.582728
g160	Leucine-rich repeat, Leucine-rich repeat domain superfamily, Leucine-rich repeat, typical subtype	-0.59	0.23	0.82	0.157808	0.588934
g10104	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Concanavalin A-like lectin/glucanase d	2.19	4.02	1.83	0.165521	0.602413
g1461	Cutinase/acetylxylan esterase, Alpha/Beta hydrolase fold	2.97	3.80	0.83	0.167525	0.602413
g11425	Six-hairpin glycosidase superfamily, Glycoside hydrolase, family 37, Six-hairpin glycosidase-like superfamili	-1.98	-3.47	-1.49	0.168331	0.602413
g11287		1.42	0.45	-0.97	0.169097	0.602413
g4077	Farnesyl pyrophosphate synthase-like, Polyprenyl synthetase, conserved site, Isoprenoid synthase domain	-5.35	-3.19	2.15	0.169741	0.602413
g1642	Concanavalin A-like lectin/glucanase domain superfamily, Glycoside hydrolase family 16	1.84	2.83	0.98	0.172731	0.604016
g8629	Glycoside hydrolase, family 27, Aldolase-type TIM barrel, Alpha galactosidase, C-terminal beta sandwich do	1.88	2.81	0.93	0.17353	0.604016
g15670	Chitin-binding, type 1, conserved site, Chitin-binding, type 1, Glycosyl hydrolases family 18 (GH18) active s	-2.83	-4.74	-1.91	0.181262	0.624922
g1682	SGNH hydrolase superfamily, Rhamnogalacturonan acetyltransferase RhgT-like, SGNH hydrolase-type esteras	1.58	0.80	-0.78	0.186037	0.629416
g16522	DNA/RNA polymerase superfamily, Reverse transcriptase domain, Ribonuclease H superfamily, Endonuclei	-5.77	-2.54	3.24	0.186043	0.629416
g2432		0.56	-0.22	-0.78	0.193975	0.644992
g4902	Glycoside hydrolase, family 27, Galactose-binding-like domain superfamily, Glycosyl hydrolase, all-beta, Al	1.43	0.04	-1.39	0.19421	0.644992
g10652	Allergen Asp f 4	3.68	5.04	1.36	0.201376	0.662710
g14783	Glycoside hydrolase, family 43, Galactose-binding-like domain superfamily, Glycosyl hydrolase, five-bladed	3.55	2.64	-0.91	0.205739	0.667896
g10105	Concanavalin A-like lectin/glucanase domain superfamily, Glycoside hydrolase, family 32, Glycosyl hydrola	3.52	4.58	1.06	0.207311	0.667896
g8299	Galactose-binding-like domain superfamily, SGNH hydrolase superfamily, CtCE2-like domain, SGNH hydrol	4.35	4.93	0.58	0.208487	0.667896
g7394	Glycoside hydrolase, family 61	0.32	-0.49	-0.80	0.214457	0.670561
g8868		-2.28	-3.36	-1.07	0.218331	0.670561
g352	Peptidase S28, Alpha/Beta hydrolase fold	3.04	2.44	-0.60	0.21845	0.670561
g10771	Expansin, cellulose-binding-like domain superfamily, RipA-like domain superfamily	4.22	3.44	-0.78	0.219194	0.670561
g7502	Glycoside hydrolase superfamily, Glycoside hydrolase, family 3, N-terminal, Glycoside hydrolase family 3 C	3.75	3.07	-0.68	0.220085	0.670561
g2577	Peptidase, metallopeptidase, Peptidase M12A, Metallopeptidase, catalytic domain superfamily	0.49	1.12	0.63	0.221246	0.670561
g16090	Berberine/berberine-like, FAD-binding domain, PCMH-type, FAD-binding, type PCMH-like superfamily, FAD	-1.37	-2.26	-0.89	0.222285	0.670561
g7661	Glycoside hydrolase family 16, Concanavalin A-like lectin/glucanase domain superfamily, Glycoside hydrol	1.24	2.02	0.78	0.222609	0.674745
g17217	Glycosyl hydrolase, family 13, catalytic domain, Alpha-amylase-like, Glycoside hydrolase superfamily	7.28	6.51	-0.78	0.2274	0.674745
g6054	SurE-like phosphatase/nucleotidase superfamily, Survival protein SurE-like phosphatase/nucleotidase	3.14	2.39	-0.76	0.231102	0.680154
g8752	Galactose mutarotase-like domain superfamily, Glycoside hydrolase-type carbohydrate-binding, Aldose 1-	-0.33	-1.24	-0.91	0.233396	0.681365
g10423	Aspartic peptidase domain superfamily, Peptidase family A1 domain, Aspartic peptidase A1 family, Pepsin-	-2.77	-1.70	1.08	0.236887	0.686025
g4469	Cellulose/chitin-binding protein, N-terminal	1.54	0.88	-0.66	0.23936	0.687685
g15672	LysM domain, LysM domain superfamily	-2.54	-1.37	1.17	0.242751	0.691936
g6006	Glycoside hydrolase family 12, Concanavalin A-like lectin/glucanase domain superfamily, Glycoside hydrol	1.47	0.75	-0.73	0.249115	0.701318
g5159	Lactonase, 7-bladed beta propeller, WD40/YVTN repeat-like-containing domain superfamily, Cytochrome c	1.31	2.09	0.77	0.251158	0.701318
g9752	FAD-binding domain, PCMH-type, FAD linked oxidase, N-terminal, FAD-binding, type PCMH-like superfamil	3.61	3.05	-0.56	0.251854	0.701318
g8983		-3.35	-1.84	1.51	0.255368	0.705674
g867	Superoxide dismutase, copper/zinc binding domain, Superoxide dismutase (Cu/Zn) / superoxide dismutase	-0.65	-1.32	-0.67	0.267753	0.709457
g15445	FAD-binding, type PCMH-like superfamily, FAD linked oxidase, N-terminal, FAD-binding domain, PCMH-ty	-0.79	-1.46	-0.67	0.272844	0.709457
g637	Lumazine/riboflavin synthase superfamily, Lumazine synthase, Lumazine/riboflavin synthase	-2.70	-1.93	0.77	0.273407	0.709457
g7298	Glycoside hydrolase family 16, Concanavalin A-like lectin/glucanase domain superfamily	6.79	6.26	-0.53	0.273536	0.709457
g7659	Pectate lyase, Pectin lyase fold, Pectin lyase fold/virulence factor	3.84	3.35	-0.49	0.273641	0.709457
g457	Enolase, Enolase, conserved site, Enolase, C-terminal TIM barrel domain, Enolase-like, N-terminal, Enolase	-4.20	-2.82	1.38	0.273857	0.709457
g16950	Pectin lyase fold/virulence factor, Pectate lyase PlyH/PlyE-like, Pectin lyase fold	0.47	-0.71	-1.17	0.275663	0.709457
g8754	Cellulose/chitin-binding protein, N-terminal	2.74	0.76	-1.97	0.277382	0.709457
g16623		6.27	5.00	-1.27	0.278097	0.709457
g9543	EGF-like, conserved site, EGF-like domain	-3.54	-4.74	-1.20	0.278935	0.709457
g17325	Acyl transferase/acyl hydrolase/lysophospholipase, Lysophospholipase, catalytic domain	-3.60	-2.83	0.78	0.28036	0.709457
g235	Cutinase/acetylxylan esterase, Cutinase, monofunctional, Alpha/Beta hydrolase fold	0.37	1.02	0.65	0.282088	0.709457
g4767	Galactose oxidase-like, Early set domain, Galactose oxidase, central domain superfamily, Carbohydrate-bin	-2.04	-1.13	0.92	0.282215	0.709457
g11199	Class I glutamine amidotransferase-like, DJ-1/Pfpl	-1.50	-2.65	-1.16	0.287281	0.717212
g16962	Cell wall mannoprotein 1	2.73	2.25	-0.48	0.29632	0.724674
g3965	Peptidase S10, serine carboxypeptidase, Alpha/Beta hydrolase fold, Serine carboxypeptidase, serine active	-3.04	-4.06	-1.02	0.296857	0.724674
g2649	CAP domain, Golgi-associated plant pathogenesis-related protein 1, SCP domain, Cysteine-rich secretory p	0.56	-0.05	-0.61	0.296925	0.724674
g15939	Glycoside hydrolase family 11/12, Glycoside hydrolase family 11, active site 1, Glycosyl hydrolases family 1	5.91	6.59	0.69	0.298277	0.724674
g9845	Glycoside hydrolase 131, catalytic N-terminal	3.11	2.60	-0.51	0.306576	0.726461
g10911	Pectin lyase fold/virulence factor, Pectate lyase, Pectin lyase fold	1.50	0.60	-0.90	0.30668	0.726461
g7852	Class I glutamine amidotransferase-like, DJ-1/Pfpl	2.19	0.69	-1.50	0.307031	0.726461
g83	Domain of unknown function DUF1996	-3.82	-2.75	1.07	0.307601	0.726461
g8701	Peptidase M14, carboxypeptidase A	0.22	-0.69	-0.92	0.311883	0.726461

g9317	Transaldolase/Fructose-6-phosphate aldolase, Transaldolase type 1, Transaldolase, active site, Aldolase-ty	0.89	1.97	1.07	0.311893	0.726461
g183	Glycoside hydrolase superfamily, Glycoside hydrolase, family 5	1.01	0.45	-0.57	0.313061	0.726461
g12871	Thioredoxin domain, Thioredoxin-like superfamily	-1.89	-2.41	-0.52	0.324239	0.738856
g6139	Nucleoside diphosphate kinase, Nucleoside diphosphate kinase-like domain superfamily, Nucleoside diph	-0.82	-0.18	0.64	0.326933	0.738856
g3321	Glycoside hydrolase superfamily, Chitinase II, Chitinase insertion domain superfamily, Endochitinase-like s	0.09	-0.96	-1.05	0.329671	0.738856
g4355	Pectin lyase fold/virulence factor, Pectin lyase fold, Pectate lyase superfamily protein	6.02	5.47	-0.55	0.330138	0.738856
g5029	Rhamnogalacturonan acetyltransferase RhgT-like, SGNH hydrolase-type esterase domain, SGNH hydrolase su	0.80	1.67	0.88	0.330284	0.738856
g9884	NADP-dependent oxidoreductase domain, NADP-dependent oxidoreductase domain superfamily, Aldo/ket	1.83	2.33	0.50	0.330648	0.738856
g10729	Peptidase S10, serine carboxypeptidase, Alpha/Beta hydrolase fold	-1.27	-1.97	-0.70	0.339023	0.748118
g6878	FERM/acyl-CoA-binding protein superfamily, Acyl-CoA-binding protein, ACBP, Acyl-CoA binding protein su	0.25	-0.71	-0.96	0.342724	0.748118
g4892	Glycoside hydrolase superfamily, Glycoside hydrolase, family 5	6.09	5.50	-0.59	0.345224	0.748118
g12384	DinB/YfiT-like putative metalloenzymes, Protein of unknown function DUF1993	-2.98	-2.08	0.89	0.346441	0.748118
g766	Cytochrome b5-like heme/steroid binding domain superfamily, FMN-dependent dehydrogenase, FMN hyd	-3.11	-2.26	0.85	0.346861	0.748118
g6772	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily	-3.05	-4.71	-1.66	0.350004	0.748118
g923	Kre9/Knh1 family	1.15	1.71	0.56	0.353625	0.748118
g8590	X8 domain, Glycoside hydrolase superfamily, Glucanosyltransferase	4.08	4.80	0.72	0.354715	0.748118
g8248	Aspartic peptidase A1 family, Aspartic peptidase domain superfamily, Peptidase family A1 domain, Asparti	-0.39	-1.17	-0.78	0.356781	0.748118
g7590	Peptidase G1, Peptidase G1 superfamily, Concanavalin A-like lectin/glucanase domain superfamily	-0.30	1.09	1.40	0.357129	0.748118
g12235	Glycoside hydrolase superfamily, Glycoside hydrolase, family 5	-0.83	-1.50	-0.67	0.357526	0.748118
g1171	Heat shock protein 70kD, C-terminal domain superfamily, Heat shock protein 70 family, Heat shock protei	0.65	1.70	1.05	0.36672	0.755502
g9227	Carbohydrate-binding WSC	0.99	0.44	-0.55	0.367511	0.755502
g7081	Chaperone DnaK, Heat shock protein 70kD, peptide-binding domain superfamily, Heat shock protein 70 fa	-4.01	-3.36	0.65	0.368259	0.755502
g3363	Chitinase insertion domain superfamily, Endochitinase-like superfamily, Chitinase II, LysM domain, Glycosi	-0.99	-2.66	-1.67	0.369403	0.755502
g6504	Tyrosinase copper-binding domain, Uncharacterised domain, di-copper centre	-0.53	0.05	0.58	0.372599	0.757757
g15857	Metallopeptidase, catalytic domain superfamily	0.62	-0.01	-0.63	0.375991	0.760385
g16113	Beta-Galactosidase/glucuronidase domain superfamily, Glycosyl hydrolases family 2, sugar binding domain	1.08	0.59	-0.49	0.378247	0.760696
g8231	Glycosyl hydrolase family 32, C-terminal, Glycoside hydrolase, family 32, Glycosyl hydrolase, five-bladed b	2.93	3.65	0.72	0.404074	0.792555
g11972	Glycosyl hydrolase family 53, Glycoside hydrolase superfamily	1.36	2.06	0.70	0.411034	0.792555
g16729	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycoside hydrolase, family 43	4.31	3.84	-0.47	0.411188	0.792555
g13716	Glycoside hydrolase family 10 domain, Cellulose-binding domain, fungal, Cellulose-binding domain superfa	-2.35	-1.94	0.41	0.411872	0.792555
g3016	Peptidase, metallopeptidase, Peptidase M12A, Metallopeptidase, catalytic domain superfamily	6.20	6.67	0.47	0.412039	0.792555
g4763	Glycosyl hydrolase family 32, C-terminal, Glycosyl hydrolase family 32, N-terminal, Glycosyl hydrolase, five	1.35	1.74	0.38	0.413228	0.792555
g13297		-2.12	-2.90	-0.78	0.413507	0.792555
g14816	Metallo-beta-lactamase, Ribonuclease Z/Hydroxyacylglutathione hydrolase-like	-1.49	-0.95	0.54	0.413768	0.792555
g15867	Alpha/beta hydrolase fold-1, Alpha/Beta hydrolase fold, Peptidase S33 tripeptidyl aminopeptidase-like, C-t	-1.85	-1.24	0.60	0.413921	0.792555
g555	Thioredoxin-like superfamily, Protein disulphide isomerase, Disulphide isomerase, Thioredoxin domain, Th	0.91	1.37	0.45	0.415982	0.792555
g6631	Glycoside hydrolase family 17, Glycoside hydrolase superfamily	-2.30	-2.96	-0.66	0.422837	0.798634
g4476	Peptidase family A1 domain, Aspartic peptidase domain superfamily, Aspartic peptidase A1 family, Aspergi	-4.05	-3.27	0.78	0.423585	0.798634
g3073	Pectin lyase fold/virulence factor, Pectin lyase fold, Glycoside hydrolase, family 28, Parallel beta-helix repe	2.74	1.36	-1.39	0.429932	0.800784
g1020	S-adenosylmethionine synthetase superfamily, S-adenosylmethionine synthetase, central domain, S-aden	-2.98	-2.01	0.97	0.430242	0.800784
g3990	Concanavalin A-like lectin/glucanase domain superfamily, Glycoside hydrolase family 16	0.52	0.97	0.45	0.431791	0.800784
g13513		-3.99	-3.39	0.61	0.434033	0.800784
g13614	Cellulose-binding domain, fungal, Cellulose-binding domain superfamily	-1.63	-2.30	-0.66	0.435786	0.800784
g16649		-1.17	-2.37	-1.19	0.441534	0.803507
g8761	SGNH hydrolase superfamily, GDSL lipase/esterase	0.99	0.49	-0.50	0.441707	0.803507
g14481	Glycoside hydrolase, family 28, Pectin lyase fold, Pectin lyase fold/virulence factor	-2.39	-1.77	0.62	0.450854	0.814020
g11320	Cyclin-like, Palmitoyl protein thioesterase, Cyclin-like superfamily, Cyclin/Cyclin-like subunit Ssn8, Alpha/B	-2.68	-1.98	0.70	0.451984	0.814020
g16195	Tetrahelic helical domain superfamily, CHAT domain	-1.61	-2.28	-0.67	0.460042	0.822299
g6459	Endonuclease/exonuclease/phosphatase superfamily	-3.99	-3.13	0.86	0.463385	0.822299
g14329	WD40/YVTN repeat-like-containing domain superfamily, Cytochrome cd1-nitrite reductase-like, haem d1 c	2.76	3.17	0.41	0.464622	0.822299
g6295		1.66	0.91	-0.75	0.466982	0.822299
g15989	Carboxylesterase type B, conserved site, Alpha/Beta hydrolase fold, Carboxylesterase type B, active site, C	4.47	4.87	0.40	0.471606	0.822299
g16857		-3.17	-2.54	0.62	0.473054	0.822299
g12195	Peptidase family A1 domain, Aspartic peptidase domain superfamily, Aspartic peptidase A1 family	-3.71	-2.61	1.10	0.473113	0.822299
g3972	Glycoside hydrolase family 16, Concanavalin A-like lectin/glucanase domain superfamily	0.88	1.34	0.46	0.474917	0.822299
g12093	Glycoside hydrolase family 16, Concanavalin A-like lectin/glucanase domain superfamily	-1.14	-0.50	0.63	0.477025	0.822299
g14024	Leucine-rich repeat domain superfamily	-1.03	-0.52	0.51	0.480106	0.823667
g8504	Protein of unknown function DUF3455	-0.57	-1.28	-0.71	0.483609	0.823667
g14243	Armado-like helical, Ribosomal protein L19/L19e, Gcn1, N-terminal, Ribosomal protein L19/L19e superfa	-0.50	0.91	1.42	0.484644	0.823667
g12277	Alpha/Beta hydrolase fold, Carboxylesterase type B, active site, Carboxylesterase, type B	-0.17	-0.72	-0.55	0.486969	0.823752
g15710		-1.16	-0.75	0.42	0.489851	0.824772
g16104	Peptidase S8/S53 domain, Peptidase S8, subtilisin, His-active site, Peptidase S8/S53 domain superfamily, P	6.16	5.70	-0.46	0.493861	0.825399
g8486	EF-hand domain pair, EF-hand domain, EF-Hand 1, calcium-binding site, Calmodulin	-2.44	-1.75	0.69	0.495765	0.825399
g519	Beta-hexosaminidase, eukaryotic type, N-terminal, Glycoside hydrolase family 20, catalytic domain, Beta-h	-0.47	-1.43	-0.96	0.497064	0.825399
g4593	NodB homology domain, Glycoside hydrolase/deacetylase, beta/alpha-barrel	5.35	4.25	-1.10	0.500773	0.827761
g6835	Pex, N-terminal, Glycoside hydrolase family 16, Carbohydrate-binding WSC, Zinc finger, RING/FYVE/PHD-ty	0.00	0.38	0.38	0.511013	0.840848
g15003	Alpha/Beta hydrolase fold, Esterase, PHB depolymerase	1.29	1.68	0.39	0.514859	0.843343
g16906	Alpha/Beta hydrolase fold, Tannase/feruloyl esterase	-1.78	-2.57	-0.79	0.52321	0.849087
g5013	Peptidase M14, carboxypeptidase A	5.19	4.54	-0.65	0.523617	0.849087
g11706	Peptidase S8 propeptide/proteinase inhibitor I9 superfamily	2.54	2.12	-0.42	0.52708	0.849087
g4777		3.74	3.02	-0.72	0.52854	0.849087
g7610	Dimeric alpha-beta barrel, Antibiotic biosynthesis monooxygenase domain	-3.73	-3.14	0.59	0.530093	0.849087
g6361	Cupredoxin, Multicopper oxidase, type 3, Multicopper oxidases, conserved site, Multicopper oxidase, type	-0.94	-0.59	0.36	0.533042	0.850049
g900	Galactose oxidase/Kelch, beta-propeller, Kelch-type beta propeller	-3.48	-2.94	0.54	0.539878	0.851774
g12817		-4.83	-4.40	0.43	0.546377	0.863704
g10240	Alpha carbonic anhydrase domain, Alpha carbonic anhydrase domain superfamily, Carbonic anhydrase, pr	1.70	1.33	-0.37	0.555704	0.874630
g9271	Ubiquitin-like domain superfamily, Ubiquitin conserved site, Ubiquitin-like domain, Ubiquitin domain	-1.21	-0.91	0.29	0.566007	0.886989
g10632	Phytanoyl-CoA dioxygenase	-2.99	-2.47	0.52	0.571211	0.891287
g7682		-2.26	-1.88	0.39	0.577051	0.894970



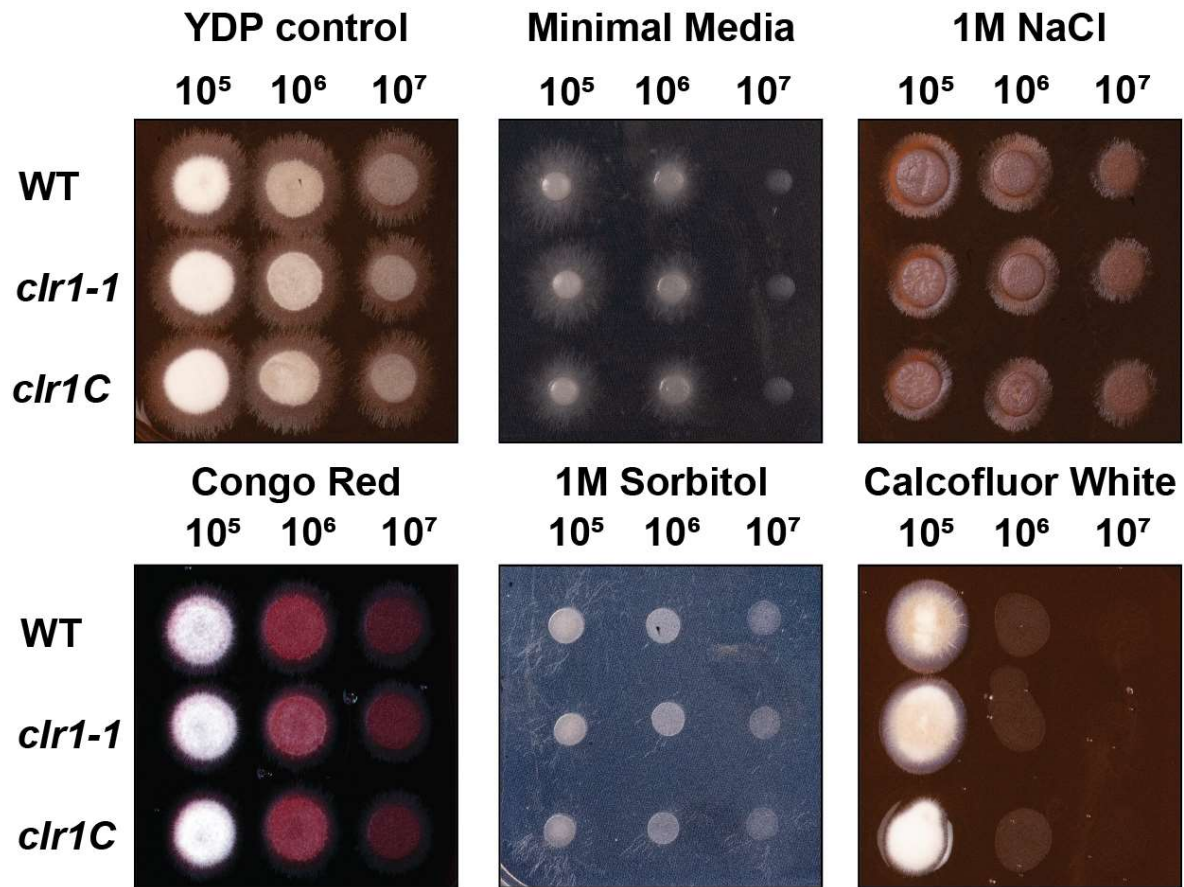
g2502		3.41	3.17	-0.24	0.578517	0.894970
g13805	Protein of unknown function DUF3455	2.66	1.98	-0.68	0.581939	0.896433
g1220		-2.91	-2.32	0.59	0.586365	0.897274
g11415	Signaling mucin MSB2	1.59	1.93	0.34	0.589665	0.897274
g9319	Superoxide dismutase, copper/zinc, binding site, Superoxide dismutase (Cu/Zn) / superoxide dismutase co	2.09	1.78	-0.31	0.591307	0.897274
g10601	Aldo/keto reductase, Aldo/keto reductase, conserved site, NADP-dependent oxidoreductase domain, NAD	-0.95	-1.25	-0.31	0.595198	0.897274
g8790	Peptidase M28	4.52	4.11	-0.41	0.595602	0.897274
g17816	Glycoside hydrolase, family 27, Galactose-binding-like domain superfamily, Alpha galactosidase, C-termina	2.22	2.75	0.54	0.597356	0.897274
g7410	SGNH hydrolase-type esterase domain, SGNH hydrolase superfamily	7.64	7.86	0.22	0.600215	0.897842
g11761	Aspartic peptidase domain superfamily, Aspartic peptidase A1 family, Aspartic peptidase, active site, Pepti	-1.31	-0.99	0.31	0.612028	0.911256
g14799	Glycoside hydrolase family 10 domain, Glycoside hydrolase superfamily	1.46	1.06	-0.40	0.620129	0.911256
g11136	FAD linked oxidase, N-terminal, FAD-binding, type PCMH-like superfamily, FAD-binding domain, PCMH-typ	7.03	6.77	-0.26	0.621141	0.911256
g10913	Isochorismatase-like, Isochorismatase-like superfamily	-1.19	-1.52	-0.32	0.622534	0.911256
g13290		-4.03	-3.56	0.48	0.625204	0.911256
g10055	Sialidase superfamily	6.56	6.28	-0.28	0.627663	0.911256
g1204	FAD/NAD(P)-binding domain superfamily, Thioredoxin reductase, Pyridine nucleotide-disulphide oxidorede	-0.20	-1.31	-1.10	0.629267	0.911256
g11945		2.13	1.65	-0.48	0.629356	0.911256
g12780	WD40/YVTN repeat-like-containing domain superfamily	1.98	1.68	-0.30	0.635177	0.911256
g7792	Peptidase M28, M28 Zn-Peptidase Glutaminy Cyclase	-1.52	-1.17	0.36	0.642805	0.911256
g10659	Sec7 domain, Armadillo-type fold, Sec7, C-terminal domain superfamily, Mon2, dimerisation and cyclophil	-2.68	-3.09	-0.41	0.642815	0.911256
g9512	Nuclear transport factor 2, eukaryote, Nuclear transport factor 2, NTF2-like domain superfamily	-3.00	-2.60	0.40	0.643777	0.911256
g5053	Alpha/Beta hydrolase fold, Peptidase S9, prolyl oligopeptidase, catalytic domain	0.41	-0.02	-0.43	0.647311	0.911256
g1631	Concanavalin A-like lectin/glucanase domain superfamily, Glycoside hydrolase family 16	1.35	1.03	-0.31	0.648049	0.911256
g6181	Necrosis inducing protein	4.17	3.97	-0.20	0.648989	0.911256
g12201	Lysophospholipase, catalytic domain, Glycoside hydrolase superfamily, Acyl transferase/acyl hydrolase/lys	0.29	-0.18	-0.46	0.649459	0.911256
g4080		2.86	3.16	0.29	0.653682	0.913641
g1291	Aldehyde dehydrogenase domain, Aldehyde dehydrogenase, C-terminal, Aldehyde dehydrogenase, N-terr	0.13	-0.11	-0.24	0.656215	0.913653
g12967	Alpha/beta hydrolase fold-1, Alpha/Beta hydrolase fold	4.68	4.93	0.25	0.661163	0.915406
g15019	Zinc finger, MYND-type, Alpha/Beta hydrolase fold, Cutinase/acetyl xylan esterase	5.29	5.60	0.31	0.662531	0.915406
g15861		0.85	0.56	-0.29	0.665749	0.916354
g390	FKBP-type peptidyl-prolyl cis-trans isomerase domain	-2.12	-2.55	-0.43	0.677692	0.925858
g1027	Kre9/Knh1 family	3.61	4.17	0.56	0.680597	0.925858
g11276	PA domain, Peptidase M28, Peptidase M28, SGAP-like	2.11	1.76	-0.35	0.687312	0.925858
g7350	Pectin lyase fold, Parallel beta-helix repeat, Glycoside hydrolase, family 28, Pectin lyase fold/virulence fact	1.95	2.19	0.24	0.687768	0.925858
g13053	Haem peroxidase, Fungal ligninase, Peroxidase, active site, Haem peroxidase superfamily	-2.73	-3.04	-0.31	0.689261	0.925858
g12382	S-adenosyl-L-methionine-dependent methyltransferase, Methyltransferase domain 25	-3.16	-3.53	-0.37	0.691619	0.925858
g536	Ribosome maturation protein SBDS, N-terminal domain superfamily, Ribosome maturation protein SBDS, t	-2.87	-2.53	0.34	0.691829	0.925858
g2478		3.64	3.44	-0.19	0.695967	0.925858
g9067	Serine/threonine-protein kinase TOR, FATC domain, PIK-related kinase, FAT, Armadillo-type fold, FKBP12-r	-1.19	-1.47	-0.28	0.69863	0.925858
g4585	Amidase, Amidase signature domain, Amidase signature (AS) superfamily	3.52	3.28	-0.24	0.702047	0.925858
g7515	Kelch-type beta propeller, Kelch repeat type 1	-1.44	-1.67	-0.23	0.704676	0.925858
g15722	Pectate lyase, Pectin lyase fold/virulence factor, Pectin lyase fold	-0.85	-1.05	-0.20	0.7063	0.925858
g14893	Peptidase M43, pregnancy-associated plasma-A, Metallopeptidase, catalytic domain superfamily	0.33	0.58	0.25	0.707202	0.925858
g6817		1.57	1.30	-0.28	0.71365	0.925858
g1621	Peptidase S8 propeptide/proteinase inhibitor I9, Peptidase S8, subtilisin-related, Peptidase S8 propeptide/	-2.26	-2.50	-0.24	0.714829	0.925858
g6251	Glycoside hydrolase, family 61	1.53	0.87	-0.66	0.714922	0.925858
g13864	RipA-like domain superfamily	1.71	2.02	0.31	0.716133	0.925858
g6145	Domain of unknown function DUF1996	2.44	2.16	-0.28	0.720075	0.926109
g16949	Peptidase S8/S53 domain superfamily, Peptidase S8, subtilisin-related, Peptidase S8/S53 domain, Peptidas	0.29	0.55	0.25	0.721444	0.926109
g9676	RNA recognition motif domain, RNA-binding domain superfamily, Nucleotide-binding alpha-beta plait dom	0.19	0.40	0.20	0.728791	0.929303
g1961	Alpha/Beta hydrolase fold, Carboxylesterase type B, active site, Carboxylesterase, type B	5.05	4.86	-0.19	0.7303	0.929303
g15912		4.40	4.66	0.26	0.732603	0.929303
g786	Translation elongation factor EF1B, beta/delta subunit, guanine nucleotide exchange domain, Translation i	-1.68	-1.38	0.30	0.734201	0.929303
g3852	P-loop containing nucleoside triphosphate hydrolase, ATPase, F1/V1/A1 complex, alpha/beta subunit, nucl	-3.23	-2.97	0.26	0.740402	0.933887
g5020	Endonuclease/exonuclease/phosphatase superfamily, Endonuclease/exonuclease/phosphatase	0.69	0.91	0.23	0.759093	0.948523
g5140	Alpha/Beta hydrolase fold, Carboxylesterase, type B	5.05	5.19	0.13	0.761934	0.948523
g4369	Glycoside hydrolase, family 3, N-terminal, Glycoside hydrolase family 3 C-terminal domain superfamily, Im	3.59	3.45	-0.14	0.764366	0.948523
g7743	Glycoside hydrolase family 17, Glycoside hydrolase superfamily	1.73	1.50	-0.23	0.764457	0.948523
g2494		3.80	3.96	0.16	0.767287	0.948523
g15891	Alpha/Beta hydrolase fold, Carboxylesterase type B, active site, Carboxylesterase, type B, Carboxylesterase	-0.18	0.07	0.25	0.769484	0.948523
g8260		-1.64	-2.08	-0.44	0.773514	0.948523
g563		-2.16	-2.35	-0.18	0.774865	0.948523
g12121	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Carbohydrate binding module family 6	-0.17	0.19	0.36	0.777946	0.948523
g4480	Alpha/Beta hydrolase fold, Alpha/beta hydrolase fold-1	3.69	3.96	0.27	0.779519	0.948523
g8214	Cupredoxin, Multicopper oxidase, copper-binding site, Fungal multicopper oxidase, cupredoxin domain 3,	-0.72	-1.08	-0.36	0.780829	0.948523
g8296	Berberine/berberine-like, FAD linked oxidase, N-terminal, FAD-binding domain, PCMH-type, FAD-binding, t	7.77	7.62	-0.16	0.784747	0.948893
g10261	Histidine phosphatase superfamily, Histidine phosphatase superfamily, clade-1	-3.98	-3.78	0.20	0.786375	0.948893
g8677	Protein of unknown function DUF1348, NTF2-like domain superfamily	-2.29	-2.47	-0.18	0.791069	0.949646
g13282		-3.74	-3.40	0.35	0.792462	0.949646
g9786	MmgE/PrpD, MmgE/PrpD superfamily, 2-methylcitrate dehydratase PrpD, MmgE/PrpD superfamily, domai	1.69	1.49	-0.20	0.794869	0.949646
g17881	Glycoside hydrolase family 16, Glycoside hydrolase, family 16, CRH1, predicted, Concanavalin A-like lectin/	1.15	1.35	0.20	0.799972	0.952598
g11805	UROD/MetE-like superfamily, Cobalamin-independent methionine synthase, Cobalamin-independent met	-2.40	-2.73	-0.33	0.812579	0.961358
g4570		-1.78	-1.63	0.15	0.812639	0.961358
g16861	Glycoside hydrolase, family 43, Alpha-L-arabinofuranosidase, Glycosyl hydrolase, five-bladed beta-propell	2.30	2.58	0.28	0.816069	0.962271
g14975	Pectin lyase fold, Glycoside hydrolase, family 28, Pectin lyase fold/virulence factor, Parallel beta-helix repe	1.01	1.33	0.32	0.820206	0.963335
g13481		1.12	1.37	0.25	0.822295	0.963335
g17120		0.91	0.76	-0.15	0.82521	0.963377
g8638	5'-Nucleotidase, C-terminal domain superfamily, 5'-Nucleotidase, C-terminal, Metallo-dependent phosph	-3.83	-4.03	-0.20	0.833997	0.963377
g452	SUN family	3.45	3.29	-0.16	0.8347	0.963377

g7601	Cytochrome b5-like heme/steroid binding domain, Succinate dehydrogenase/fumarate reductase flavopro	-2.05	-1.86	0.19	0.835162	0.963377
g7052	Peptidase S16, active site, PUA-like superfamily, Peptidase S16, Lon proteolytic domain, Lon, substrate-bin	-1.64	-1.43	0.21	0.838278	0.963377
g17239	Catalase-peroxidase haem, Haem peroxidase, Haem peroxidase superfamily, Peroxidase, active site, Perox	4.15	4.27	0.12	0.838298	0.963377
g16905	Uncharacterised protein family UPF0311	3.22	3.07	-0.14	0.843093	0.964348
g223	Autophagy-related protein 27	-3.70	-3.90	-0.19	0.84528	0.964348
g12827	Endochitinase-like superfamily, Chitinase II, Glycoside hydrolase family 18, catalytic domain, Chitin-binding	7.85	8.00	0.15	0.853637	0.964348
g17201	Alpha-L-arabinofuranosidase B, arabinose-binding domain, Alpha-L-arabinofuranosidase B, catalytic, Alpha	5.20	5.10	-0.10	0.854237	0.964348
g14035	Glucanosyltransferase, Glycoside hydrolase superfamily	0.44	0.54	0.10	0.85507	0.964348
g12925	Cellulose-binding domain, fungal, Glycoside hydrolase, family 45, Cellulose-binding domain superfamily, RI	2.67	2.54	-0.13	0.855126	0.964348
g4431	Peptidase M28, SGAP-like, Peptidase M28, PA domain	-2.50	-2.39	0.12	0.857985	0.964567
g10876	Glycoside hydrolase, family 61	0.51	0.38	-0.13	0.866524	0.967102
g10917	Phospholipase A2 domain superfamily, Phospholipase A2, prokaryotic/fungal	3.86	3.78	-0.08	0.867204	0.967102
g17675		-2.76	-2.62	0.14	0.868255	0.967102
g9447	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycosyl hydrolase family 32, C-termin	-1.26	-1.36	-0.10	0.876335	0.969683
g4900	FAD linked oxidase, N-terminal, FAD-binding, type PCMH-like superfamily, FAD-binding domain, PCMH-typ	-1.71	-1.60	0.11	0.876633	0.969683
g16730	Glycoside hydrolase, family 43, Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycosi	1.84	1.92	0.07	0.878608	0.969683
g3863		-0.84	-0.92	-0.08	0.881762	0.970207
g1468		-0.53	-0.67	-0.14	0.888325	0.974465
g6907	FKBP-type peptidyl-prolyl cis-trans isomerase domain	1.22	1.15	-0.07	0.892856	0.976477
g4291		-0.03	0.15	0.18	0.899257	0.980516
g8359	Glycoside hydrolase, family 61	0.85	0.95	0.10	0.902145	0.980710
g14769	Six-hairpin glycosidase-like superfamily, Carbohydrate binding module family 20, Glucoamylase, Carbohyd	0.22	-0.02	-0.24	0.908532	0.983856
g450	Aldolase-type TIM barrel, Glycoside hydrolase superfamily, Glycoside-hydrolase family GH114, TIM-barrel	-1.23	-1.14	0.09	0.913761	0.983856
g16746	Galactose-binding-like domain superfamily, Beta-glucuronidase, C-terminal, Six-hairpin glycosidase-like sup	4.16	4.05	-0.11	0.914325	0.983856
g8785	Tyrosinase copper-binding domain, Uncharacterised domain, di-copper centre	0.88	0.75	-0.13	0.91591	0.983856
g16097	CAP superfamily, CAP domain, Cysteine-rich secretory protein-related, Golgi-associated plant pathogenesi	3.68	3.63	-0.05	0.921828	0.987284
g15470	Glycosyl hydrolase, five-bladed beta-propellor domain superfamily, Glycosyl hydrolase family 32, N-termin	-3.09	-2.92	0.17	0.926366	0.989217
g12126	Glycosyl hydrolase, family 88, Six-hairpin glycosidase superfamily, Six-hairpin glycosidase-like superfamily	-0.61	-0.56	0.05	0.929867	0.990034
g4263	Triosephosphate isomerase, Triosephosphate isomerase, active site, Aldolase-type TIM barrel, Triosephos	-1.12	-1.05	0.07	0.933194	0.990496
g7674	Complex I intermediate-associated protein 30, mitochondrial, NADH:ubiquinone oxidoreductase intermed	2.79	2.75	-0.04	0.935773	0.990496
g12240	Chitinase insertion domain superfamily, Chitinase II, Glycoside hydrolase family 18, catalytic domain, Glycc	4.45	4.50	0.05	0.945301	0.996426
g10854	FAD-binding domain, PCMH-type, FAD linked oxidase, N-terminal, FAD-binding, type PCMH-like superfamil	-3.82	-3.75	0.07	0.947367	0.996426
g17018		-2.17	-2.13	0.04	0.954959	0.996426
g8818	Peptidase S10, serine carboxypeptidase, Serine carboxypeptidases, histidine active site, Alpha/Beta hydrol	3.87	3.92	0.05	0.955215	0.996426
g17204	SMP-30/Gluconolactonase/LRE-like region, Six-bladed beta-propeller, TolB-like	-1.64	-1.60	0.04	0.960286	0.996426
g9171	Glycoside hydrolase, family 5, Glycoside hydrolase superfamily	3.19	3.23	0.05	0.963219	0.996426
g10884	Peptidase family A1 domain, Aspartic peptidase domain superfamily, Aspartic peptidase A1 family	1.47	1.50	0.03	0.968041	0.996426
g15794	Cutinase/acetylxylan esterase, Alpha/Beta hydrolase fold, Cutinase, monofunctional	1.88	1.87	-0.02	0.973331	0.996426
g6847	Phycocyanin domain, Cupredoxin	-2.27	-2.29	-0.02	0.974563	0.996426
g16281		-1.32	-1.28	0.04	0.97489	0.996426
g15816	Chloroperoxidase-like superfamily, Chloroperoxidase	4.63	4.66	0.03	0.979486	0.996426
g4544	PA14/GLEYA domain, GLEYA adhesin domain	-0.57	-0.54	0.03	0.979565	0.996426
g7572	SGNH hydrolase-type esterase domain, SGNH hydrolase superfamily	-0.32	-0.33	-0.02	0.980325	0.996426
g4411		-1.46	-1.48	-0.02	0.980455	0.996426
g2339	Haem peroxidase, Catalase-peroxidase haem, Haem peroxidase superfamily, Peroxidase, active site, Perox	1.15	1.17	0.02	0.991257	0.996426
g7868	Glucosyltransferase, Glucoamylase, CBM20 domain, Carbohydrate binding module family 20, Six-hairpin glycosi	6.71	6.72	0.01	0.991651	0.996426
g17213	Cellulose-binding domain, fungal, Cellulose-binding domain superfamily, Glycoside hydrolase, family 61	1.27	1.25	-0.01	0.991814	0.996426
g805	Rhamnogalacturonan lyase, domain III, Rhamnogalacturonase B, Rhamnogalacturonase B, N-terminal, Gal	0.92	0.91	-0.01	0.992343	0.996426
g6411	Formin, GTPase-binding domain, Formin, FH2 domain superfamily, Armadillo-like helical, Formin, FH2 dom	0.80	0.79	-0.01	0.993674	0.996426
g14330	Glycoside hydrolase, family 16, CRH1, predicted, Glycoside hydrolase family 16, Concanavalin A-like lectin/	1.18	1.18	0.00	0.997772	0.997772



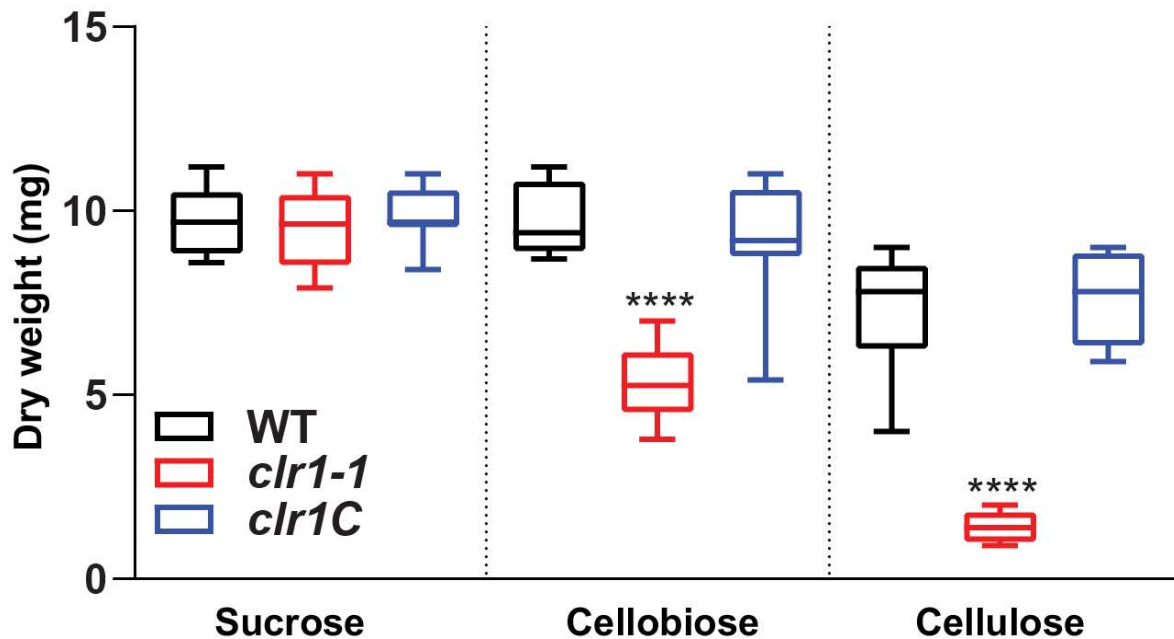
**Fig. S1. Identification of Fo5176 *clr1* mutants and *clr1C* complemented.**

(A) Left panel, schematic representation of targeted *CLR1* gene replacement with the neomycin cassette (*Neo<sup>r</sup>*) using the split-marker method. Right panel, complementation of the *clr1* mutants by co-transformation with the phleomycin resistance cassette (*Phleo<sup>r</sup>*) and the WT *CLR1* gene. (B) PCR screening to identify *clr1* and *clr1C* mutant strains. Genomic DNA of the indicated fungal strains was amplified by using the primer pair ForCLR1genotyping and RevCLR1genotyping (indicated with arrows in panel A). The expected PCR fragments for *clr1* or *clr1C* mutants are 2589 or 2870 bp, respectively. (C) *CLR1* expression measured by Rt-PCR in the indicated strains using the primers pair CLR1rtpcrfor and CLR1rtpcrrev. *FoTub* expression was used as reference.



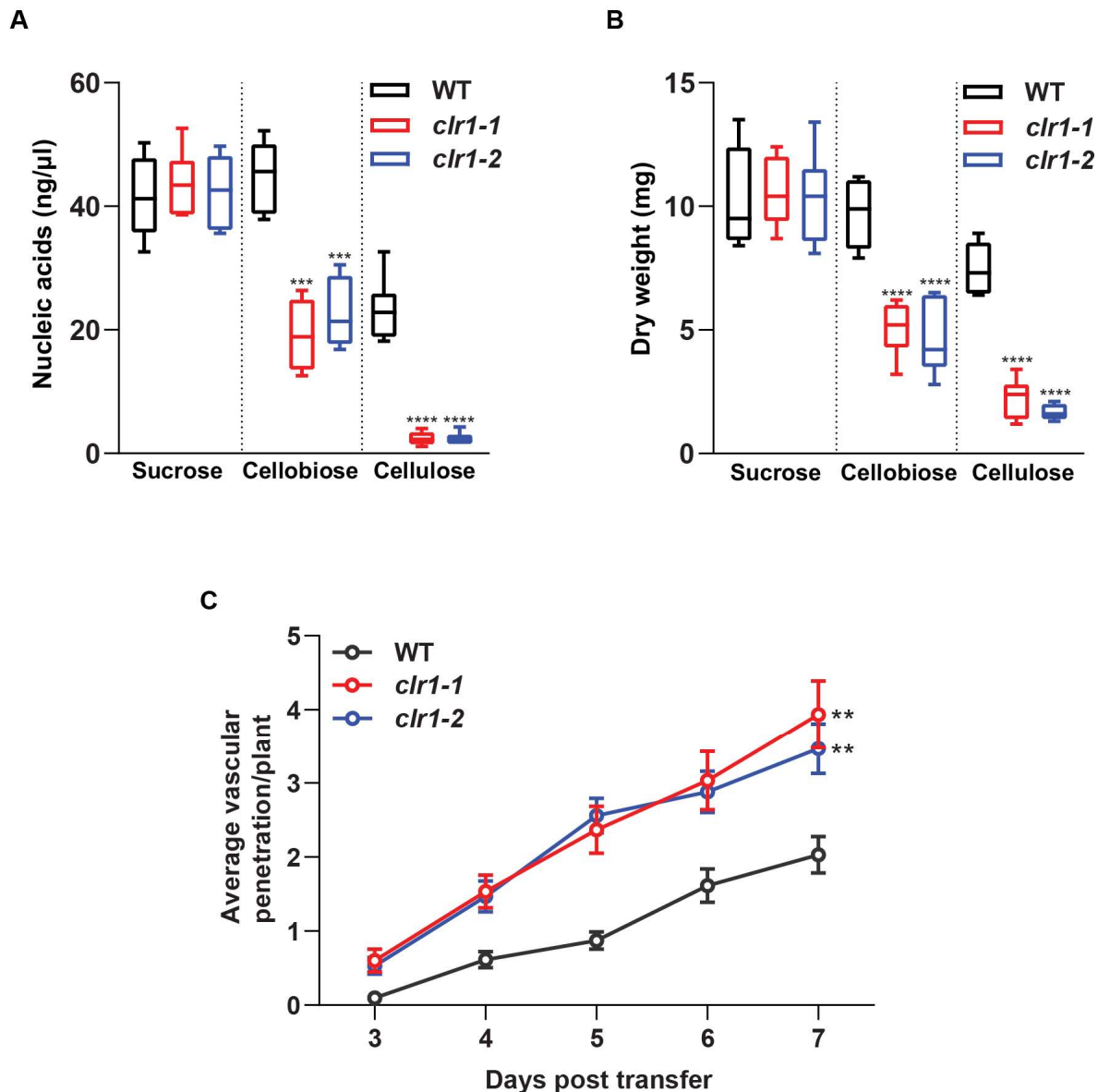
**Fig. S2. *CLR1* deletion does not alter *Fo* growth on different stress media.**

Representative images of WT, *clr1-1*, and *clr1C* growth in YFP control media and in various stress media: Minimal Media (nutrient limitation), YDP with 1 M NaCl (salt stress), YDP with 50  $\mu$ g/mL Congo Red or YDP with 40  $\mu$ g/mL Calcofluor White (cell wall perturbations) or 1 M of Sorbitol (osmotic stress). The experiment was repeated 2 times with similar results. Plates were spot-inoculated with 20  $\mu$ l of decimated dilution from  $10^7$  microconidia/ml.



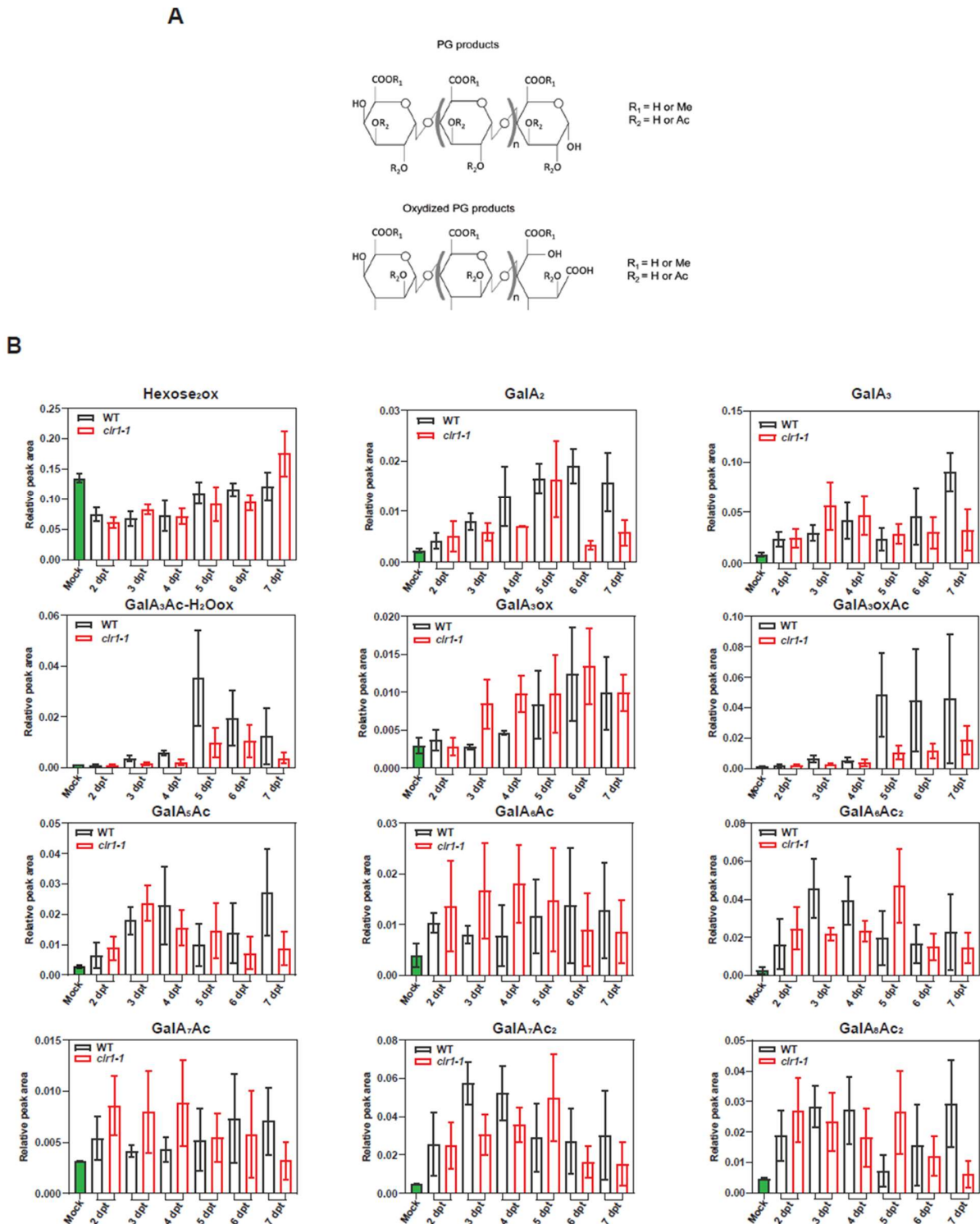
**Fig. S3. The lack of CLR1 impairs *Fo* growth in different carbon sources.**

Growth WT, *clr1-1* and *clr1C* on sucrose 0.5% or cellobiose 0.5% for 3three days, or on cellulose 0.5% for 7 days measured as dry weight (mg). Shown are the box plots: centerlines show the medians; box limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers extend to the minimum and maximum, N=9. Asterisk indicated differences relative to WT. Welch's unpaired t-test; \*\*\*\* p-value  $\leq 0.0001$ .



**Fig S4. A second independent CLR1 deletion mutant, *clr1-2*, displayed the same phenotype than *clr1-1*.**

(A) and (B) Growth of WT, *clr1-1* and *clr1-2* on sucrose 0.5% or cellobiose 0.5% for 3 days, or on cellulose 0.5% for 7 days measured as dry weight (mg) (A) and nucleic acid concentration (ng/ml) (B). Shown are the box plots: centerlines show the medians; box limits indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles; whiskers extend to the minimum and maximum, N≥5. Welch's unpaired t-test; asterisks show differences with respect to WT.\*\*\*p-value≤0.001, \*\*\*\*p-value ≤0.0001. (C) Cumulative Arabidopsis root vascular penetration by Fo at different days post-transfer (dpt) to WT, *clr1-1* or *clr1-2* microconidia plates. Values are mean +/- SEM, N≥30 plants from one representative experiment. The experiment was performed 3 times with similar results. RM two-way ANOVA on vascular penetration rate: p<0.0001 (fungal genotype), p<0.0001 (time), p ≤ 0.01 (fungal genotype x time). Asterisk indicated a statistical difference with respect to WT at 7 dpt, Tukey's multiple comparison test, \*\* p<0.01.

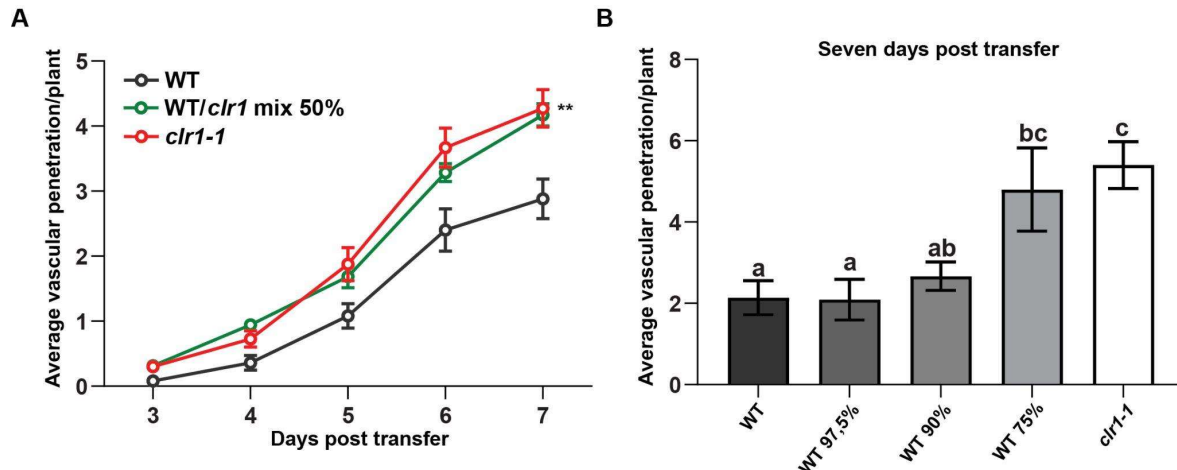


**Fig. S5. Kinetics of Hexoses and OGs identified in Arabidopsis roots infected with WT or *clr1-1*.**

(A) Examples of the modifications observed in the detected OGs: acetylated or methylated residues and oxidized PG products. (B) Kinetics of Hexoses<sub>2</sub> and OGs identified by HP-SEC-MS

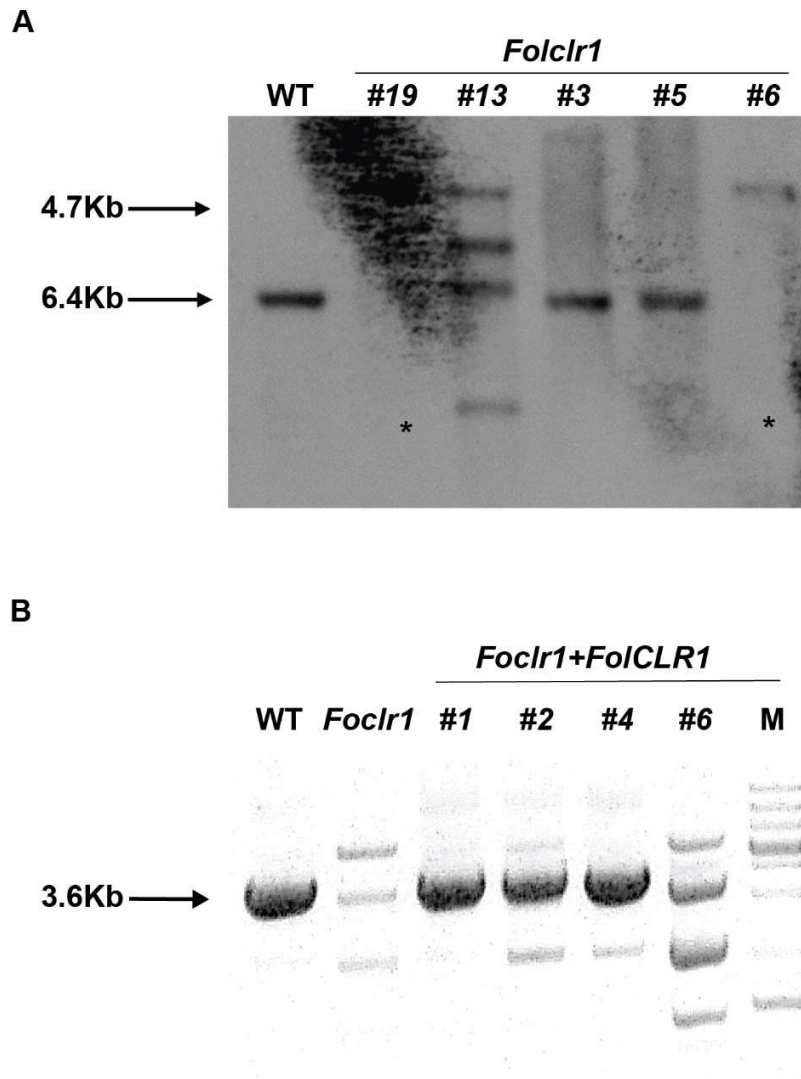
in infected roots at different days post transfer (dpt ) to WT or *clr1-1* microconidia. Bars represent means  $\pm$  SEM from 3 biological replicates (2 for mock samples). No significant differences were observed in the *clr1-1* samples compared to WT ones using two-way ANOVA with LSD Fisher test post hoc comparison at any dpt. OGs are named GalA<sub>x</sub>Ac<sub>y</sub>, indicating the subscript numbers indicate the degree of polymerization (x) and the number of acetylated groups (y). “ox” indicate the presence of oxidized groups, respectively.





**Fig. S6. *clr1-1* virulence is not compensated by WT coinfection**

**(A)** Cumulative *Arabidopsis* root vascular penetration by *Fo* at different days post-transfer (dpt) to plates with WT, *clr1-1* or a 50/50 WT/*clr1-1* microconidia mix. Values are mean  $\pm$  SEM,  $N \geq 25$  plants from one representative experiment. The experiment was performed 3 times with similar results. RM two-way ANOVA on vascular penetration rate:  $p < 0.001$  (fungal genotype),  $p < 0.0001$  (time),  $p \leq 0.01$  (fungal genotype x time). Asterisk indicated a statistical difference with respect to WT at 7 dpt. Tukey's multiple comparisons test, \*\*  $p < 0.01$ . **(B)** Root vascular penetration at 7 dpt to plates with WT, *clr1-1* or a mix with 97,5%, 90%, or 75% WT microconidia complemented to 100% with *clr1-1* microconidia. Bars represent means  $\pm$  SEM,  $N \geq 22$ , from one independent experiment. The experiment was performed 2 times with similar results. One-way Anova was performed and Tukey's multiple comparisons test; letters indicate statistical difference between samples;  $p < 0.05$ .



**Fig. S7. Identification of *Fol4287 clr1* and *clr1C* mutants.**

**(A)** Southern blot hybridization analysis of the WT and of five indicated *clr1* transformants. Genomic DNA was digested with the *HindIII* restriction enzyme and hybridized with a probe obtained by using the primer pair CLR1TERFOR and CLR1TERREV. 6.4 Kb indicates WT fragment whereas 4.7 the cassette insertion. Transformants marked with an asterisk indicate strains where targeted replacement of the indicated gene by homologous integration of a single copy of the *Neo<sup>r</sup>* resistance cassette occurred. **(B)** PCR screening to identify *clr1C* mutant strains. Genomic DNA of the indicated fungal strains was amplified by using the primer pair CLR1PROMFORNEST and CLR1revgene. The expected PCR fragment for *clr1C* mutants is of 3600 bp.

**Table S1. Primers used in this study.**

Primer	Sequence 5' → 3'	USE
gpdA15B	GGATCCCGAGACCTAATACAGCCCCT	HygBr cassette/Phleor
trppter8B	GGATCCAAACAAGTGTACCTGTGCATTC	
Neo G	TGCCCTGAATGAACTGCAAGA	cassette/Neor cassette
Neo Y	CCAAGTTCTTCAGCAATATCAC	
CLR1PromFor	TCCAAGTAAGCATCAAGTCCG	<i>CLR1</i> Knockout/ Complementation
CLR1PromForNest	GGTCTTTCTCCTCTCGTGGC	
CLR1PromRev	TTTACCCAGAATGCACAGGTACACTTGTTT AGAAACGGTATCGAGGAACGG	
CLR1TerFor	TGGTCGTTGTAGGGGCTGTATTAGGTCTCT TCTGGGGTGATATCCTGGG	
CLR1TerRevNest	TTTTGAAATGGCCAGGGGGGT	
CLR1TerRev	AGAGTAGAGAAGATGAACGGAA	
CLR1 rev gene	ATGATGAGCAGCGTTTGGGAG	
CLR1 rev nest 2	TTCATGTGGGTCATCGAACTC	
For CLR1 genotyping	GCCGCTACACCAACTCAACT	
Rev CLR1 genotyping	TGCTGGGCTCAACATCCAACCAA	
CLR1_qPCR-Fwd	GCTTGCCCTGTGGATTTGAA	
CLR1_qPCR-REV	TCCAGGCTTGTTGTTGATGC	
At1g51890_qPCR-Fwd	CTAGCCGACTTTGGGCTATC	
At1g51890_qPCR-Rev	CCAGTTTGTCTGTAATACTCAGG	
WRKY45_qPCR-Fwd	GAACAATCCATTCCCAGGAG	
WRKY45_qPCR-Rev	GGAGGGAAGATGTGCATTTGTG	
WRKY53_qPCR-Fwd	GCGACAAGACACCAGAGTCA	
WRKY53_qPCR-Rev	ACCGTTGGATTGAACCAGTC	
FoSIX1_qPCR-Fwd	TCAAGAGGCTGCGGTTGG	
FoSIX1_qPCR-Rev	GACGCTCAGGGCGACATA	
FoβTUB_qPCR-Fwd	AACTCCGATGAGACCTTCTG	
FoβTUB_qPCR-Rev	GACATGACAGCAGAAACGAG	
GAPDH_qPCR-Fwd	AGGTGGAAGAGCTGCTTCCTTC	

GAPDH_qPCR-Rev	GCAACACTTTCCCAACAGCCT	
g16048_qPCR-Fwd	GCAGTACCGTGTGATCATGG	
g16048_qPCR-Rev	CATCGAGAACACTGCTTCCA	
g2038_qPCR-Fwd	TGGCAATAAGATTGCTGCTG	
g2038_qPCR-Rev	GGTTTATGCCGTTTCCTTGAA	
g7764_qPCR-Fwd	TATCGAGGCCGATACCAAAG	
g7764_qPCR-Rev	AGGTGTCCTGGTTGACCTTG	
g1489_qPCR-Fwd	GCACCGTCCTTGACATCTTT	
g1489_qPCR-Rev	ACCAGCAATGTGGGGAGTAG	
G9160_qPCR-Fwd	TTGCGCCAAGAAGTGTATCC	
G9160_qPCR-Rev	TGAAGACGAAGCTTGTTGCC	
G4171_qPCR-Fwd	TGACCGCAAGAAATCCGATG	
G4171_qPCR-Rev	TTGGGCTTGAATTCCTCACG	
G5102_qPCR-Fwd	TCGCCAACGATGTCAACATC	
G5102_qPCR-Rev	AGCCTTTATCGTGCCATGAC	
g17213_qPCR-Fwd	GCTGGTTCAAGATCAAGGACTG	qPCR
g17213_qPCR-Rev	ATATGCGCCGTTGGTAATGC	
G13080_qPCR-Fwd	ACGGACCTGTTGAGGATGTC	
G13080_qPCR-Rev	GACGGGTCCAACATGAGAGT	
G6695_qPCR-Fwd	CTCTGCCAAAGTTCCTCCAG	
G6695_qPCR-Rev	CTGCTGAGAGGTGTTGACCA	
G7443_qPCR-Fwd	TGATGCTGAGTGGGAGTCTG	
G7443_qPCR-Rev	TGAAGAATCACAGGCACTCG	
g11385_qPCR-Fwd	GGCTGAGTTCCTCAAGATCG	
q11385_qPCR-Rev	TGATGACGTGGTATCCGAGA	
g15798_qPCR-Fwd	CGTCCTGATAGTCCTTCGCT	
G15798_qPCR-Rev	CCTACACCACCCTGACCATT	