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Negative regulation of ABA signaling by WRKY33 is critical for *Arabidopsis* immunity towards *Botrytis cinerea* 2100

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1 **Negative regulation of ABA signaling by WRKY33 is critical for**

2 ***Arabidopsis* immunity towards *B. cinerea* 2100**

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Abstract

The *Arabidopsis* mutant *wrky33* is highly susceptible to *Botrytis cinerea*. We identified >1680 *Botrytis*-induced WRKY33 binding sites associated with 1576 *Arabidopsis* genes. Transcriptional profiling defined 318 functional direct target genes at 14 h post inoculation. Comparative analyses revealed that WRKY33 possess dual functionality acting either as a repressor or as an activator in a promoter-context dependent manner. We confirmed known WRKY33 targets involved in hormone signaling and phytoalexin biosynthesis, but also uncovered a novel negative role of abscisic acid (ABA) in resistance towards *B. cinerea* 2100. The ABA biosynthesis genes *NCED3* and *NCED5* were identified as direct targets required for WRKY33-mediated resistance. Loss-of-WRKY33 function resulted in elevated ABA levels and genetic studies confirmed that WRKY33 acts upstream of *NCED3/NCED5* to negatively regulate ABA biosynthesis. This study provides the first detailed view of the genome-wide contribution of a specific plant transcription factor in modulating the transcriptional network associated with plant immunity.

Introduction

Necrotrophic fungi including *Botrytis cinerea, Fusarium oxysporum*, and *Alternaria brassicicola,* are the largest class of fungal phytopathogens causing serious crop losses worldwide (Łaźniewska *et al.*, 2010). These pathogens extract nutrients from dead host cells by producing a variety of phytotoxic compounds and cell wall degrading enzymes (Mengiste, 2012; Williamson *et al.*, 2007). *B. cinerea* has a broad host-range, causes pre- and postharvest disease, and is the second most agriculturally important fungal plant pathogen (Dean *et al.*, 2012).

Plant immunity towards *B. cinerea* appears to be under complex poorly understood genetic control (Rowe and Kliebenstein, 2008). Apart from the *Arabidopsis thaliana RESISTANCE TO LEPTOSPHAERIA MACULANS 3* (*RLM3*), no major *R-gene* has been associated with resistance to necrotrophs. However, over the past two decades numerous genes that influence the outcome of *B. cinerea* - host interactions have been identified (Mengiste, 2012). Among these are several transcription factors (TFs) consistent with the large transcriptional reprogramming observed in host cells upon *Botrytis* infection (Birkenbihl *et al.*, 2012; Birkenbihl and Somssich, 2011; Windram *et al.*, 2012). In *Arabidopsis* several MYB-type TFs regulate distinct host transcriptional responses towards *B. cinerea*. BOS1 (*BOTRYTIS SUSCEPTIBLE 1*)/MYB108 appears to restrict necrosis triggered by *B. cinerea*

61 and *A. brassicicola*, and loss-of-BOS1 function increased plant susceptibility

62 (Mengiste, 2012). In response to stress and *B. cinerea* infection, BOS1

63 physically interacts with and is ubiquitinated by BOI, a RING E3 ligase that

64 contributes to defense by restricting the extent of necrosis (Luo *et al.*, 2010).

65 MYB51 is involved in the transcriptional activation of indole glucosinolate

66 biosynthetic genes, which also contributes to resistance towards necrotrophs

67 (Kliebenstein *et al.*, 2005; Sánchez-Vallet *et al.*, 2010). In contrast, the

68 MYB-related genes *ASYMMETRIC LEAVES 1 (AS1)* and *MYB46* appear to

69 play a role in disease susceptibility as such mutants show increased disease

70 resistance towards *B. cinerea* (Nurmberg *et al.*, 2007; Ramírez *et al.*, 2011).

71

72 Ethylene and jasmonic acid (ET, JA) signaling are critical for host immunity to

73 necrotrophic pathogens, and several transcriptional activators and repressors

74 of the ET and JA pathways impact resistance to *B. cinerea* (Bari and Jones,

75 2009; Glazebrook, 2005). In particular the TFs ERF1, ORA59, ERF5, ERF6,

76 and RAP2.2, have regulatory functions in host susceptibility to this fungus.

77 (Berrocal-Lobo *et al.*, 2002; Moffat *et al.*, 2012; Pré *et al.*, 2008; Zhao *et al.*,

78 2012). Transgenic *Arabidopsis* lines over-expressing *ERF1* or *ORA59* confer

79 resistance to *B. cinerea* (Kazan and Manners, 2013) whereas *RNAi-ORA59*

80 silenced lines were more susceptible (Berrocal-Lobo *et al.*, 2002; Pré *et al.*,

81 2008). Both ERF1 and ORA59 appear to be key integrators of the ET- and

82 JA-signaling pathways (Pieterse *et al.*, 2009). In contrast, the bHLH

83 transcription factor MYC2/JIN1 is a master regulator of diverse JA-mediated

84 responses by antagonistically regulating two distinct branches of the JA

85 signaling pathway in response to necrotrophs (Kazan and Manners, 2013).

86

87 The WRKY family of TFs modulate numerous host immune responses

88 (Pandey and Somssich, 2009). In particular, WRKY33 is a key positive

89 regulator of host defense to both *A. brassicicola* and *B. cinerea* (Birkenbihl *et*

90 *al.*, 2012; Zheng *et al.*, 2006). WRKY33 was directly phosphorylated *in vivo* by

91 the MAP kinases MPK3 and MPK6 upon *B. cinerea* infection, and

92 subsequently activated *PAD3* expression by direct binding to its promoter (Mao

93 *et al.*, 2011). *PAD3* encodes a key biosynthetic enzyme required for the

94 production of the antimicrobial compound camalexin. Moreover, WRKY33

95 directly interacted with its own promoter, suggesting a positive feedback

96 regulatory loop on *WRKY33* expression. WRKY33 was also found to interact

97 with the VQ-motif containing protein MAP KINASE SUBSTRATE1

98 (MKS1/VQ21) and to form a ternary complex with the MAP kinases MPK4

99 within the nucleus of resting cells (Andreasson *et al.*, 2005; Qiu *et al.*, 2008).

100 Upon challenge with the hemibiotrophic pathogen *Pseudomonas syringae* or

101 upon elicitation by the Microbe-Associated Molecular Pattern (MAMP) flg22,

102 the active epitope of the bacterial flagella, activated MPK4 phosphorylates

103   MKS1 thereby releasing WRKY33 from the complex and leading to its

104   detection at the *PAD3* promoter.

105

106   We previously reported that activation of *Arabidopsis WRKY33* resulted in

107   rapid and massive host transcriptional reprogramming upon infection with *B.*

108   *cinerea* strain 2100 (Birkenbihl *et al.*, 2012). Compared to resistant wild-type

109   (WT) plants, susceptible *wrky33* mutants displayed early inappropriate

110   activation of salicylic acid (SA)-related host responses, elevated SA and JA

111   levels, and down-regulation of JA-associated responses at later infection

112   stages. Consistent with these results ChIP analysis demonstrated that

113   WRKY33 directly binds to the regulatory regions of *JAZ1* and *JAZ5*, two genes

114   encoding repressors of JA signaling, but also to the ERF class TF gene

115   *ORA59* involved in JA-ET crosstalk, and to two camalexin biosynthesis genes

116   *CYP71A13* and *PAD3* (Birkenbihl *et al.*, 2012). Although *pad3* plants are

117   susceptible to *B. cinerea* 2100, *wrky33* mutants are more highly susceptible.

118   Genetic studies revealed that altered SA responses at later infection stages

119   may contribute to the susceptibility of *wrky33* to *B. cinerea*, but were

120   insufficient for WRKY33-mediated resistance (Birkenbihl *et al.*, 2012). Thus,

121   WRKY33 apparently targets additional genes whose functions are critical for

122   establishing full WRKY33-dependent resistance towards this necrotroph.

123

124 In this paper we performed ChIP-seq and RNA-seq analyses to identify

125 WRKY33 regulated target genes in the *Arabidopsis thaliana* genome following

126 infection with *B. cinerea* 2100. The study uncovered numerous targets many of

127 which are associated with the regulation of hormonal signaling pathways.

128 Expression of the majority of WRKY33 direct targets is down-regulated upon

129 infection, but some, notably genes of camalexin biosynthesis, are strongly

130 up-regulated, indicating that WRKY33 is a dual-functional TF acting in a

131 promoter-context dependent manner. Subsequent genetic and hormonal

132 studies verified components of abscisic acid (ABA) biosynthesis as being

133 critical for WRKY33-dependent resistance towards this necrotrophic fungus.

134 This study provides the first genome-wide view of the gene regulatory network

135 underlying plant immunity governed by a host specific transcription factor.

136

137 **Results**

138 **Genome-wide detection of *Arabidopsis* WRKY33 binding sites in**

139 **response to *B. cinerea* 2100**

140 To gain a deeper insight into how WRKY33 regulates plant immunity towards

141 *B. cinerea* 2100, we performed ChIP-seq for genome-wide *in vivo* identification

142 of WRKY33 DNA-binding sites. For this, a transgenic *wrky33* null mutant

143 expressing an HA epitope-tagged *WRKY33* construct under the control of its

144 native promoter (P_{WRKY33}:*WRKY33-HA*) was used. This line complemented the

145   *B. cinerea* 2100 susceptibility phenotype of *wrky33* plants resulting in

146   resistance similar to WT Col-0 plants (Birkenbihl *et al.*, 2012). Rosette leaves

147   of 4-week old plants, mock treated or spray-inoculated with spores of *B.*

148   *cinerea* 2100, were collected at 14 hours post inoculation and used to perform

149   ChIP-seq. The 14 h timepoint was selected based on the induced

150   WRKY33-HA protein levels observed in western blots (*Figure 1A*). No

151   WRKY33-HA protein was detected in the absence of infection. Besides the

152   non-induced sample, we used identically treated WT plant tissue lacking

153   *WRKY33-HA* as an additional negative control. Two biological replicates each

154   were analyzed. The previously identified WRKY33 *in vivo* target genes,

155   *CYP71A13* and *PAD3*, were used to monitor by ChIP-qPCR specific

156   enrichment in samples used for library construction and sequencing

157   (Birkenbihl *et al*., 2012).

158   We identified 1684 high confidence WRKY33 binding sites common to both

159   replicates, which are associated with 1576 genes (*Figure 2C, Supplementary*

160   *file 1*). WRKY33 binding to all detected sites was dependent on prior infection

161   with *B. cinerea* 2100. Over 78 % of the identified peak regions were located in

162   promoter (1kb region upstream of the transcription start site) or 5' intergenic

163   regions (*Figure 1B*) and 15.4% were located near transcription termination

164   sites (TTS). Less than 1% and 5% of the peaks were located in exons and

165   intronic regions, respectively (*Figure 1B*). The genome-wide local distribution

166 of peak regions relative to genes showed clear accumulation of WRKY33

167 binding at about -300 bp from the transcription start sites (*Figure 1C*). The

168 fidelity of the ChIP-seq data was subsequently confirmed by ChIP-qPCR for a

169 number of genes (*Supplementary file 2*). Moreover, nearly all previously

170 reported WRKY33 *in vivo* targets including *PAD3, CYP71A13, ACS2, JAZ1,*

171 *ORA59, TRX-h5,* and *WRKY33* itself were successfully identified in our

172 ChIP-seq dataset (Birkenbihl *et al.*, 2012; Li *et al.*, 2012; Mao *et al.*, 2011).

173 Numerous studies have revealed that WRKY proteins specifically bind to a

174 DNA motif, TTGACT/C, termed the W-box (Rushton *et al.*, 2010), although

175 adjacent bases (W-box extended motifs) can also influence binding

176 (Ciolkowski *et al.,* 2008). Using the DREME/MEME software we determined

177 conserved consensus sequences within high confidence WRKY33 binding

178 sites across the genome. Of the 1684 identified WRKY33 binding regions, 80%

179 contained the well-established W-box motif (*Figure 1D*). We also found W-box

180 extended sequence motifs within the WRKY33 binding regions (*Figure 1 -*

181 *figure supplement 1A,B*). These W-box extended motifs also included the core

182 sequence GACTTTT (*Figure 1 - figure supplement 1C*), which was reported to

183 be bound by *Arabidopsis* WRKY70 and to be required for WRKY70-activated

184 gene expression (Machens *et al.*, 2014).

185 Apart from the W-box and W-box variants, we found one additional sequence

186 motif, T/GTTGAAT that occurs in 60% of the WRKY33 binding regions (*Figure*

187   *1D*). More than 48% (817 out of 1684) of WRKY33 binding peaks contained

188   both this new motif and the W-box (*Figure 1 - figure supplement 1D*). We

189   performed electrophoresis mobility shift assays (EMSA) using recombinant

190   WRKY33 protein to determine whether this newly identified DNA element is

191   bound by WRKY33. Two DNA oligonucleotide probes were synthesized whose

192   sequences were derived from two WRKY33 targets (*WAKL7, PROPEP3*)

193   containing either one or three copies of the motif, respectively. A previously

194   described DNA oligonucleotide containing three W-boxes (Mao *et al.*, 2011)

195   and a W-box mutated version hereof served as positive and negative controls.

196   A clear interaction (mobiltiy shift) was observed between WRKY33 and the

197   labeled W-box probe but not with W-boxmut and the two probes harboring the

198   T/GTTGAAT motifs (M-3 and M-7; *Figure 1 - figure supplement 2*). Specificity

199   of W-box binding was confirmed in competition experiments wherein only an

200   excess of the W-box probe was able to compete for binding of the protein.

201   Thus, T/GTTGAAT does not appear to be a WRKY33 direct binding site and its

202   functionality remains unclear.

203

204   **Differential expression of WRKY33 target genes upon *B. cinerea* 2100**

205   **infection**

206   ChIP-seq studies in different organisms have revealed that the majority of

207   binding sites bound by specific TFs *in vivo* do not result in altered expression

208   levels of associated genes (Chang *et al.*, 2013; Fan *et al.*, 2014; MacQuarrie *et*

209   *al.*, 2011). To investigate the impact of WRKY33 binding on target gene

210   expression, we performed RNA-seq and examined *WRKY33*-mediated gene

211   expression changes in mock and *B. cinerea* 2100 (14 hpi) treated 4-week old

212   *wrky33* and WT plants. Three independent biological replicates were

213   generated and analyzed allowing us to identify genes with consistently altered

214   expression after inoculation. In WT plants, the expression of 6101 genes was

215   altered 2-fold or more (P≤0.05) compared to non-infected plants, with 3048

216   genes being up-regulated and 3053 genes being down-regulated (*Figure 2A*).

217   In *wrky33*, upon infection, the expression of 7441 genes was altered more than

218   2-fold, 3583 of them being up-regulated and 3858 down-regulated. A common

219   set of 4686 genes showed changes upon infection in both genotypes (*Figure*

220   *2A,B*). Comparative profiling of mock-treated plants identified 705 genes that

221   were differentially expressed between *wrky33* and WT in the absence of the

222   pathogen, 458 of them being up-regulated and 247 down-regulated (*Figure*

223   *2A*). Comparing the expression profiles of *B. cinerea*-infected *wrky33* and WT

224   plants (*wrky33 B.c* versus WT *B.c*), we identified 2765 differentially expressed

225   genes dependent on WRKY33, of which 1675 were up-regulated in the mutant

226   (termed WRKY33-repressed genes) and 1090 were down-regulated in the

227   mutant (termed WRKY33-induced genes; *Figure 2A,C*).

228   We then compared the WRKY33-dependent differentially expressed gene set

229 obtained by RNA-seq with the WRKY33 target gene set revealed by ChIP-seq.

230 This comparison identified 318 WRKY33-regulated target genes that were both

231 bound by WRKY33 and exhibited WRKY33-dependent altered gene

232 expression (*Figure 2C*). Of these, 240 (75%) were repressed upon infection

233 while 78 (25%) were induced (*Figure 2C,D*). We named those genes

234 WRKY33-repressed targets and WRKY33-induced targets, respectively.

235 Based on this analysis WRKY33 appears to have a prominent repressive role

236 on the transcription of many specific host genes indicating a negative

237 regulatory function of WRKY33 in mediating immunity to this pathogen. Genes

238 displaying altered expression in the *wrky33* mutant compared to WT but

239 showing no binding of WRKY33 at their respective gene loci were defined as

240 WRKY33-dependent non-targets (1435 WRKY33-repressed non-targets and

241 1012 WRKY33-induced non-targets; *Figure 2C*). The overlap between

242 observed WRKY33 binding and altered expression of the associated genes

243 upon fungal infection was around 20% (318 of 1576). This fraction is similar to

244 values reported for other plant transcription factors such as EIN3, HBI1 and

245 BES1 (Chang *et al.*, 2013; Fan *et al.*, 2014; Yu *et al.*, 2011).

246

247 **WRKY33 represses transcription of many plant immunity genes**

248 Compared to the entire genome the identified WRKY33-regulated targets were

249 significantly enriched in gene onthology (GO) categories involved in diverse

250   biological processes and molecular functions related to different forms of

251   stress, external and endogenous stimuli, signal transduction, transport,

252   metabolic processes and catalytic activity (P<0.05; *Figure 2 - figure*

253   *supplement 1*), and many of these genes are repressed upon *B. cinerea*

254   infection (*Figure 2E*). For example, genes related to "defense response" were

255   highly overrepresented among WRKY33-repressed targets (38%) and in the

256   WRKY33-repressed non-target sets (17%), suggesting that WRKY33 mainly

257   functions as a repressor of plant defense responses. However, it is important

258   to note that nearly 18% of the WRKY33-induced targets were associated to

259   defense responses compared to only 3% of the WRKY33-induced non-targets.

260   This indicates that WRKY33 can also acts as a direct activator of defense gene

261   expression, very likely in a promoter-context dependent manner. Particularly

262   prominent among the WRKY33-induced targets are genes associated with

263   responses to the phytohormone ethylene (ET; 21%).

264   Apart from hormonal pathways discussed below, genes associated with the

265   GO terms "cell death" or related to diverse "kinase activities" were markedly

266   enriched among WRKY33-repressed targets and non-targets (*Figure 2E*). 42

267   out of 318 WRKY33-regulated targets are involved in cell death, and 38 of

268   these appear to be repressed by WRKY33 (*Supplementary file 3*). This

269   WRKY33-mediated repression may be an important feature required to

270   reinforce resistance towards the necrotroph *B. cinerea* that depends on dead

271   host tissue to complete its life cycle. Furthermore, 41 of the

272   WRKY33-regulated target genes encode for various kinases, and again the

273   majority of these genes appear to be negatively regulated by WRKY33

274   (*Supplementary file 4*). For the WRKY33-regulated target *LecRK VI.2*, a critical

275   role in resistance against hemibiotrophic *P. syringae* pv. tomato DC3000 and

276   necrotrophic *Pectobacterium carotovorum* bacteria has been demonstrated

277   (Huang *et al.*, 2014; Singh *et al.*, 2012).

278   Several TF gene families involved in defense were targeted by WRKY33. In

279   total, WRKY33 binding was found at 133 TF gene loci. Predominant among

280   these are members of the AP2/ERFs, MYBs, WRKYs, and NACs families

281   (*Figure 3A*). However, expression of only 16% (21 of 133) of these genes was

282   directly modulated in a WRKY33-dependent manner after *B. cinerea* infection

283   (complete list see *Supplementary file 5*). WRKY factors are predicted to form a

284   highly interconnected regulatory sub-network (Llorca *et al.*, 2014). Indeed, 18

285   *WRKY* genes were identified as direct targets of WRKY33 (*Figure 3A*).

286   However, only 7 genes, *WRKY33, WRKY38, WRKY41, WRK48, WRKY50,*

287   *WRKY53* and *WRKY55,* showed altered expression upon WRKY33 binding at

288   14 hpi (*Figure 3B-G; Figure 3 - figure supplement 1, Supplementary file 5*).

289   Binding to the *WRKY33* promoter is consistent with reports suggesting a

290   positive autoregulatory feedback loop resulting in high-level accumulation of

291   WRKY33 in response to *B. cinerea* (Mao *et al.*, 2011). In addition,

292   WRKY33-dependent altered transcription of 18 other *WRKY* genes with no

293   detectable WRKY33 binding was observed following fungal infection,

294   indicating that these genes are indirectly regulated by WRKY33

295   (WRKY33-regulated non-targets; *Figure 3A*). WRKY33 function negatively

296   affected expression of most of these *WRKY* targets.

297

298   **WRKY33 modulates transcription of genes associated with hormonal**

299   **pathways**

300   Genes encoding components of pathways related to the key phytohormone

301   signaling molecules SA, JA, ET, and ABA were highly enriched in the

302   WRKY33-regulated gene set (*Figure 2E*). Genes involved in SA response were

303   overrepresented in WRKY33-repressed targets and non-targets (*Figure 2E*).

304   This is consistent with our previous transcriptomic profiling showing that

305   WRKY33 directly or indirectly repressed the expression of genes in SA

306   biosynthesis and SA-mediated signaling (Birkenbihl *et al.*, 2012). More than

307   80% (34 out of 42) of the SA-response targets are also associated with the GO

308   term "cell death" (*Figure 2 - figure supplement 2A*), suggesting that WRKY33

309   repression of the SA pathway is linked to modulation of host cell death

310   responses.

311

312   In contrast to SA signaling genes, genes responsive to ET were highly

313   enriched in the WRKY33-induced target dataset, among them are *ACS6*,

314   *ORA59* and *ERF5* (*Figure 2E*). ACS6 is involved in *Botrytis*-induced ethylene

315   production and plays an important role in plant immunity (Han *et al.*, 2010; Li *et*

316   *al.*, 2012). ORA59 and ERF5 belong to the AP2/ERF TF family with ORA59

317   acting as an integrator of JA and ET signaling and as a positive regulator of

318   resistance against *B. cinerea*, while ERF5 also regulates ET signaling and is a

319   key component of chitin-mediated immunity (Moffat *et al.*, 2012; Pré *et al.*,

320   2008). Genes responsive to JA and ABA were also overrepresented in our GO

321   term analysis, but in this case similar fractions of genes were identified among

322   WRKY33-induced   targets,   WRKY33-repressed   targets   and

323   WRKY33-repressed non-targets (*Figure 2E*). Some WRKY33-regulated

324   targets were enriched in more than one hormone response (*Figure 2 - figure*

325   *supplement 2B*), suggesting the involvement of WRKY33 in hormonal

326   co-regulation or crosstalk.

327   In conclusion, our global analysis revealed that WRKY33 influences various

328   hormonal responses upon infection with *B. cinerea* 2100, and that WRKY33

329   had both a positive and a negative functional relationship with a fraction of its

330   direct targets.

331

332   **WRKY33-dependent resistance to *B. cinerea* 2100 involves ABA**

333   Our previous genetic analyses excluded a major role of SA, JA and ET

334   signaling in WRY33-dependent resistance towards *B. cinerea* 2100 (Birkenbihl

335   *et al.*, 2012). Here, two additional genes, *GH3.2* and *GH3.3*, encoding

336   acyl-acid-amide synthetases capable of conjugating amino acids to JA and

337   auxin were identified as being WRKY33-repressed targets (*Figure 8 – figure*

338   *Supplement 3A*). GH3.3 controls JA homeostasis in seedlings, and *gh3.2*

339   mutants showed increased resistance to *B. cinerea* (González-Lamothe *et al.*,

340   2012; Gutierrez *et al.*, 2012). Thus, we generated *wrky33 gh3.2 gh3.3* triple

341   mutant plants but did not observe restoration of WT-like resistance towards *B.*

342   *cinerea* 2100, indicating that they are not critical for WRKY33-dependent

343   defense against this fungal strain (*Figure 8 – figure Supplement 3B*).

344   Interestingly, our global binding studies also revealed that WRKY33 binds to

345   the promoter region of *NCED3* and to the 3'UTR region of *NCED5* (*Figure*

346   *4A,D*), two major genes encoding 9-cis-epoxycarotenoid dioxygenase, a key

347   enzyme in the biosynthesis of ABA (Leng *et al.*, 2014). The precise role of ABA

348   in host defense remains enigmatic and ABA can positively or negatively impact

349   the outcome of plant-microbe interactions, depending on the pathogens'

350   lifestyle (Robert-Seilaniantz *et al.*, 2011). WRKY33 binding to both gene loci

351   was confirmed by ChIP-qPCR (*Figure 4C,F*). Transcript levels of *NCED3* and

352   *NCED5* both increased in the *wkry33* mutant upon *B. cinerea* infection

353   suggesting direct negative regulation by WRKY33 (*Figure 4B,E*). WRKY33

354   also bound to the *CYP707A3* promoter, a gene involved in ABA catabolism

355 (Leng *et al.*, 2014), but its expression decreased in the *wrky33* mutant and

356 increased in WT plants after infection indicative of positive regulation by

357 WRKY33 (*Figure 4 G-I*).

358 These results suggest that WRKY33 represses ABA levels during *B. cinerea*

359 2100 infection, and that this repressor function is an important component in

360 host resistance to this pathogen.

361

362 **Negative regulation of *NCED3* and *NCED5* by WRKY33 contributes to**

363 **resistance to *B. cinerea* 2100**

364 To clarify the involvement of ABA in WRKY33-mediated host defense to *B.*

365 *cinerea* 2100, we analyzed ABA mutants with respect to their phenotypes after

366 fungal infection. Previous reports have shown that *aba2-12* (Adie *et al.*, 2007),

367 *aba3-1* (Léon-Kloosterziel *et al.*, 1996)*,* and *nced3 nced5* (Frey *et al.*, 2012)

368 accumulated much less ABA than WT plants. Indeed, the *aba2-12, aba3-1,*

369 *nced3-2, nced5-2,* and *nced3 nced5* mutants were nearly as resistant as WT

370 plants *to B. cinerea* 2100 (*Figure 5B; Figure 5 - figure supplement 1*). To test

371 whether WT resistance towards this necrotroph is due to WRKY33-mediated

372 repression of *NCED3* and *NCED5* expression we generated *wrky33 nced3,*

373 *wrky33 nced5* double, and *wrky33 nced3 nced5* triple mutants, and tested their

374 infection phenotypes.

375 ABA deficiency severely affects plant growth leading to stunted phenotypes, as

376 observed in *nced3 nced5, aba2-12* and *aba3-1* mutants. A strong reduction of

377 rosette diameter was also observed in the *wrky33 nced3 nced5* mutant under

378 short day conditions (*Figure 5A*). However, unlike the *wrky33* mutant, the

379 *wrky33 nced3 nced5* triple mutant showed clear resistance to *B. cinerea* 2100

380 similar to WT plants (*Figure 5B*). In contrast, the *wrky33 nced3* and *wrky33*

381 *nced5* double mutants were as susceptible as *wrky33* (*Figure 5 - figure*

382 *supplement 2*). Consistent with the observed resistance phenotype, qPCR

383 analysis revealed strongly reduced fungal biomass in *wrky33 nced3 nced5*

384 compared to *wrky33* plants at 3 days post infection (*Figure 5C*). This clearly

385 indicates that increased expression levels of *NCED3* and *NCED5* in the

386 *wrky33* mutant contribute to susceptibility toward *B. cinerea* 2100, and that a

387 key function of WRKY33 in host immunity towards this pathogen is to repress

388 ABA biosynthesis.

389 Since *nced3 nced5* mutants have reduced ABA levels, we tested whether

390 exogenous application of ABA to the mutants could revert the resistant

391 phenotype. Indeed, application of ABA together with the fungal spore droplet to

392 leaves of the *wrky33 nced3 nced5* triple mutant partially rendered plants

393 susceptible to *B. cinerea* 2100 (*Figure 5D*). Similar tests on WT plants did not

394 alter host resistance (*Figure 5 - figure supplement 3*).

395 *CYP707A* mutants affecting ABA metabolism were reported to accumulate

396 more ABA than lines overexpressing ABA biosynthetic genes (Finkelstein,

397   2013). We therefore also tested the phenotypes of *cyp707a1, cyp707a2, and*

398   *cyp707a3* following infection. Interestingly, all of these mutants remained as

399   resistant as WT plants towards the fungus (*Figure 5 - figure supplement 4*).

400   Taken together, our genetic analysis combined with our ChIP-seq and

401   expression results strongly suggest that WRKY33-mediated control of *NCED3*

402   and *NCED5* expression plays a critical role in host resistance towards *B.*

403   *cinerea* 2100.

404

405   **WRKY33 controls hormone homeostasis in response to *B. cinerea***

406   Hormonal signaling appears to be affected in the susceptible *wrky33* mutant

407   compared with the resistant WT (*Figure 2E*), and *wrky33 nced3 nced5* triple

408   mutants restore WT-like resistance. Thus, we hypothesized that WRKY33

409   plays a critical regulatory role in hormone homeostasis. To test this we

410   measured hormonal levels in WT*, wrky33*, *nced3 nced5* and *wrky33 nced3*

411   *nced5* plants following infection with *B. cinerea* 2100 at various time points. It is

412   important to note that we have previously shown that up to 40 to 48 hpi no

413   differences in fungal biomass, hyphal expansion or other phenotypic criteria

414   were observed between resistant WT and susceptible *wrky33* plants

415   (Birkenbihl *et al.*, 2012).

416   As expected, ABA and SA levels increased strongly in susceptible *wrky33*

417   compared to resistant WT plants during fungal infection (*Figure 6A,B*).

418   However, JA and ACC (precursor of ET) levels also increased strongly in

419   *wrky33* compared to WT plants (*Figure 6 C,D*). Interestingly, the elevated SA

420   levels observed in *wkry33* appear to be a direct consequence of increased

421   ABA levels as SA levels were clearly reduced in the resistant ABA-deficient

422   *wrky33 nced3 nced5* compared to *wrky33* plants post infection. Moreover, the

423   levels of JA and ACC were also reduced in *wrky33 nced3 nced5* at later

424   infection stages. This suggests that ABA signaling exerts a positive role on the

425   biosynthesis of these other hormonal components.

426   Taken together, our data indicate that a key function of WRKY33 in *B. cinerea*

427   strain 2100 challenged WT plants is to limit ABA levels. Loss of *WRKY33*

428   function affects hormonal homeostasis in the plant during infection leading to

429   elevated ABA activity and subsequently resulting in altered hormone signaling.

430

431   **In *wrky33 nced3 nced5* plants, expression of many up-regulated genes in**

432   ***wrky33* is restored to WT-like levels**

433   Over 75% of WRKY33-regulated target genes showed elevated expression in

434   the susceptible *wrky33* mutant after *B. cinerea* 2100 infection (*Figure 2D*). To

435   test these genes for altered expression in resistant *wrky33 nced3 nced5* plants

436   we performed qRT-PCR analyses. The transcript levels of several highly

437   expressed SA-related genes observed in *wrky33* at 24 hpi decreased in the

438   *wrky33 nced3 nced5* plants, often returning to WT states. These included:

439   *ICS1, NPR1, NPR3, NPR4, TRX-h5* and *FMO1* (*Figure 7*). However, not all

440   SA-related genes were similarly affected as illustrated for *EDS1, PAD4,*

441   *NIMIN1, PR1* and *PR2*, whose expression levels remained significantly higher

442   than in WT (*Figure 7*). These results imply that simultaneous mutations of

443   *NCED3* and *NCED5* in the *wrky33* genotype partially impair SA biosynthesis

444   and signaling.

445   Reduced transcript levels were also observed for other genes in the *wrky33*

446   *nced3 nced5* mutant at 24 hpi such as the TF genes, *ERF1, NAC019,*

447   *NAC055, NAC061*, *NAC090, WRKY41, WRKY48, WRKY53* and *WRKY55*,

448   indicating a positive effect of ABA on their expression (*Figure 7*). In contrast,

449   expression of *WRKY38* and *WRKY50* increased in *wrky33 nced3 nced5* plants

450   to even higher levels than observed in *wrky33* (*Figure 7*) suggesting a negative

451   effect of ABA on these genes.

452   The *Botrytis*-induced expression of other ABA response genes including

453   *ACS2, BIR1, CDPK1, MPK11,* and *CRK36* was also restored to WT levels in

454   the *wrky33 nced3 nced5* mutant (*Figure 7*). Interestingly *CDPK1, MPK11,*

455   *CRK36* and *NPR3* are also responsive to SA and these genes are associated

456   with the GO term 'cell death', suggesting that ABA has a positive effect on cell

457   death responses (*Figure 2 – figure supplement 2A, Supplementary files 3+4*).

458   In summary, WRKY33 suppresses the expression of many of its target genes

459   by negatively regulating ABA responses. The subset of genes showing

460   restoration of WT-like expression levels in the resistant triple mutant constitute

461   prime candidates whose functions may be causal for WRKY33-mediated

462   resistance against this necrotrophic fungus.

463

464   **Discussion**

465   Signal transduction to the nucleus and a complex gene regulatory network

466   governing the massive transcriptional reprogramming in the host upon

467   pathogen perception, drives the plant immune response. TFs are key terminal

468   components of this signaling cascade and function by activating and

469   repressing the expression of numerous defense-associated genes. The TF

470   WRKY33 plays a major role in conferring resistance of *Arabidopsis* plants to

471   the fungal necrotroph *B. cinerea*. In the present work, combined genome-wide

472   binding studies and transcriptional analyses allowed us to identify WRKY33

473   binding sites within the *Arabidopsis* genome upon fungal infection, and to

474   correlate WRKY33 binding to altered transcriptional outputs. By including

475   appropriate mutants in this study we identified components of the ABA

476   hormonal pathway that act downstream of WRKY33 to mediate host

477   resistance.

478   Such a genome-wide analysis of *in vivo* target sites for a selected TF

479   expressed under its native promoter in intact pathogen-infected plant tissue

480   has not yet been reported. Thus, in a broader perspective, this study also sets

481   the framework for establishing a comprehensive gene regulatory network

482   model of plant immunity.

483

484   The number of high-affinity WRKY33 binding sites within the genome by far

485   exceeds the number of direct WRKY33 target genes affected in their

486   transcriptional response during *B. cinerea* infection. Overall, 80% of the

487   WRKY33 targets were not significantly differentially expressed in the *wrky33*

488   mutant upon infection compared to WT. This excess number of gene loci

489   bound by a given TF but unaffected in their expression is consistent with

490   previous ChIP-seq studies although the reasons for this discrepancy remain to

491   be elucidated (MacQuarrie *et al.*, 2011). One plausible explanation may be that

492   transcriptional activation/repression at specific promoter sites is context

493   dependent, and may require, apart from WRKY33 binding, additional diverse

494   signaling inputs. For example we detected strong enrichment of WRKY33

495   within the promoters of numerous genes coding for receptors of various

496   MAMPs and Damage-Associated Molecular Patterns (DAMPs) including

497   *FLS2*, *EFR*, *CERK1, PEPR1*, and *PEPR2*, but no altered expression of these

498   genes in the *wrky33* mutant upon *Botrytis* infection. *WRKY33* is also strongly

499   and rapidly induced during MAMP/DAMP-triggered immunity (Lippok *et al.*,

500   2007; Yamaguchi *et al.*, 2010), and thus a regulatory function of WRKY33 at

501   these promoters may require additional signals/co-factors only triggered during

502   MAMP/DAMP signaling. Additionally, spatial and temporal differences in the

503   activation of target genes need to be considered. The current paper provides a

504   snapshot of global WRKY33 function during *Botrytis* infection. Whether

505   WRKY33 binding results in temporally distinct transcription patterns, as was

506   observed for EIN3 in the ethylene response, remains to be investigated

507   (Chang *et al.*, 2013). However, such studies employing living pathogens on

508   intact host plants remain challenging due to the asynchrony of the infection

509   process at various cellular sites within the tissue.

510   Nevertheless, using conservative criteria for selecting differentially expressed

511   genes in *wrky33* mutants compared to WT plants after *B. cinerea* 2100

512   infection, about 2600 genes were identified that showed transcriptional up- or

513   down-regulation. The strikingly high number of modulated genes at early

514   infection stages (14hpi) highlights the importance of WRKY33 to initiate host

515   responses to this pathogen.

516

517   **WRKY33 regulation of ABA signaling is critical for host defense towards**

518   ***B. cinerea***

519   The role of ABA in biotic stress responses is complex and currently ill-defined.

520   The ability of *Arabidopsis* to restrict penetration by the non-host barley

521   pathogen *Blumeria graminis* was shown to be dependent on the NAC TF

522   ATAF1-mediated repression of ABA biosynthesis (Jensen *et al.*, 2008). In

523 contrast, overexpression of *ATAF1* resulted in enhanced susceptibility of

524 *Arabidopsis* plants to *B. cinerea* (Wang *et al.*, 2009). ATAF1 was shown to

525 directly bind to the *NCED3* promoter, which positively correlated with increased

526 *NCED3* expression and ABA levels (Jensen *et al.*, 2013). Moreover,

527 transcriptomic studies using 4-week old detached *Arabidopsis* leaves infected

528 with *B. cinerea* strain pepper revealed that genes involved in the suppression

529 of ABA accumulation and signaling were up-regulated at early infection stages

530 (Windram *et al.*, 2012). Our study clearly demonstrates that increased

531 expression of WRKY33 target genes associated with ABA biosynthesis

532 (*NCED3* and *NCED5*) are causal for the susceptibility of *wrky33* to *B. cinerea*

533 2100, and the ABA deficient *wrky33 nced3 nced5* mutant restored WT-like

534 resistance towards this necrotroph. Hence, our findings reveal a novel role of

535 WRKY33 in modulating host resistance to *B. cinerea* by suppressing ABA

536 accumulation/signaling (*Figure 8 – figure supplement 1*). Interestingly,

537 resistance to the necrotrophic fungus *Plectosphaerella cucumerina* is also

538 negatively impacted by ABA, and *wrky33-1* mutant plants exhibited an

539 enhanced susceptible phenotype towards this pathogen (Sánchez-Vallet *et al.*,

540 2012). Stimulating *NCED3* expression and ABA biosynthesis has also been

541 described as an important virulence strategy employed by the hemi-biotroph

542 *Pseudomonas syringae* DC3000 in *Arabidopsis* (de Torres‐Zabala *et al.*,

543 2007). Virulence to this pathogen is strongly reduced in ABA mutants. Whether

544   WRKY33 is involved in modulating ABA signaling during this host–bacterial

545   interaction, and whether *P. syringae* DC3000 suppresses *WRKY33* expression

546   is unknown.

547

548   In our experiments the expression of several NAC TF genes associated with

549   ABA regulation was affected upon *Botrytis* infection in a WRKY33-dependent

550   manner. In particular, increased expression of *NAC002* (*ATAF1*), *NAC019,*

551   *NAC055, NAC061, NAC068* (*NTM1*) and *NAC090* was observed in the *wrky33*

552   mutant. Like *ATAF1*, transgenic *Arabidopsis* lines overexpressing *NAC019* or

553   *NAC055* displayed enhanced susceptibility to *B. cinerea* (Bu *et al.*, 2008)*.* In

554   contrast, the *nac019 nac055* double mutant showed increased resistance to *B.*

555   *cinerea* compared with WT plants. ABA has been shown to induce *NAC019*

556   and *NAC055* expression (Jiang *et al.*, 2009; Zheng *et al.*, 2012). Whether any

557   of these NAC factors, apart from ATAF1, can also target the *NCED* genes and

558   thereby enhance ABA biosynthesis in the *wrky33* mutant remains to be tested.

559

560   ABA can repress SA-, ET-, and JA/ET-dependent signaling but also positively

561   affect some JA responses (Asselbergh *et al.*, 2008; Ton *et al.*, 2009). Our

562   genetic and phytohormone studies showed that elevated ABA levels in the

563   susceptible *wrky33* mutant resulted in concomitant increases in SA, JA, and

564   ACC levels upon *B. cinerea* 2100 infection, implying a positive effect of ABA on

565   these hormone signaling components. Increased SA levels per se in the

566   *wrky33* mutant however do not contribute to susceptibility as *wrky33 sid2*

567   double mutant plants are as susceptible to *B. cinerea* 2100 as the single

568   mutant (Birkenbihl *et al.*, 2012; *Figure 8 - figure supplement 1*). Interestingly

569   concurrent increases in ABA, JA/ET and SA have also been observed in the

570   interaction of *Arabidopsis* with *P. syringae* DC3000 and with the vascular

571   oomycete pathogen *Pythium irregular* (Adie *et al.*, 2007; de Torres‑Zabala *et*

572   *al.*, 2007). In the case of *P. irregular* however, host resistance correlated with

573   high ABA levels whereas ABA mutants were clearly more susceptible.

574   Our molecular analysis of *Botrytis*-challenged *wrky33* and *wrky33 nced3*

575   *nced5* plants confirmed that elevated ABA levels mainly activate

576   NPR1-dependent SA signaling while not affecting the upstream EDS1-PAD4

577   pathway (*Figure 7*). Increased ABA also activated *ACS2*, *ACS6*, *ERF1* and

578   *ORA59*, targets involved in ET/JA signaling. In the *wrky33 nced3 nced5* mutant

579   as in WT plants expression of *ACS6*, *ORA59,* but also *ERF5* and *PDF1.2* (data

580   not shown) was significantly reduced. However, as both genotypes are

581   resistant to *B. cinerea* these ET/JA components appear not to be essential in

582   maintaining plant resistance.

583

584   How elevated ABA levels trigger activation of these hormone signaling

585   cascades and specific TFs remains to be elucidated. The receptors for ABA

586   are known (Miyakawa *et al.*, 2013), but the molecular mechanisms linking

587   downstream ABA signaling to the other hormonal pathways requires further

588   investigation. It is conceivable that the increased ABA levels in *wrky33* during

589   *Botrytis* infection trigger the activation of currently unidentified downstream

590   ABA-response factors that bind to the ABA response elements (ABRE,

591   ACGTGG/T) or G-boxes (CACGTG) present in some gene promoters,

592   resulting in transcriptional activation. Indeed, several genes including *NAC019*,

593   *NAC061*, *FMO1*, *GH3.2* and *GH3.3* contain such conserved motifs, which

594   could respond to and be activated by ABA. In addition, the elevated levels of

595   SA in *wrky33* during *Botrytis* infection may in part be responsible for the strong

596   expression of the glutaredoxin gene *GRX480/ROXY19* observed in this mutant

597   (RNA-seq data in this study; Birkenbihl et al., 2012). GRX480 binds to class II

598   TGA factors, thereby activating TGA-regulated SA responses while preventing

599   their participation in JA mediated signaling (Zander *et al.*, 2014). Plants

600   ectopically overexpressing *GRX480* are susceptible to *B. cinerea* 2100

601   (Birkenbihl *et al*., 2012).

602

603   **WRKY33 is a dual functional transcription factor**

604   In animals and humans, several TFs can act either as transcriptional activators

605   or repressors, depending on DNA binding sequences or interaction with

606   additional co-factors (Alexandre and Vincent, 2003; Berger and Dubreucq,

607 2012; Sakabe *et al.*, 2012; Zhu *et al.*, 2012). Moreover, many human TFs

608 function as repressors as often as they act as activators (Cusanovich *et al.*,

609 2014). In plants, few factors with dual functions have been unequivocally

610 characterized. In tomato, the transcriptional activator Pti4 can repress the

611 expression of *PR10-a* by forming a complex with the SEBP repressor

612 (Gonzalez-Lamothe *et al.*, 2008). In *Arabidopsis,* the TF WUSCHEL (WUS)

613 acts mainly as a repressor in stem cell regulation, but can function as an

614 activator of *AGAMOUS(AG)* during floral patterning (Ikeda *et al.*, 2009). Also

615 WRKY proteins can act as activators or repressors, and selected family

616 members in diverse plant species have been identified as key regulators in

617 diverse plant processes (Rushton *et al.*, 2010). WRKY53 can activate or

618 repress the expression of genes, depending on the nature of the target

619 promoter sequence (Miao *et al.*, 2004). *WRKY6* activates *PR1* expression

620 while suppressing the expression of its own gene, and that of its closely related

621 family member *WRKY42* (Robatzek and Somssich, 2002). Our data show that

622 also WRKY33 is a bi-functional TF that can act as an activator or as a

623 repressor in a promoter-context dependent manner (*Figure 8*). WRKY33

624 positively regulates genes involved in camalexin biosynthesis such as

625 *CYP71A13* and *PAD3* by directly binding to their promoter regions (Birkenbihl

626 *et al.*, 2012; Mao *et al.*, 2011). Our study confirmed these observations and

627 identified two additional camalexin biosynthetic genes, *AMT1* and *CYP71A12*

628   that are positively and directly regulated by WRKY33 (*Figure 8 – figure*

629   *supplement 2; Supplementary file 2*). Mutants of *CYP71A13* and *PAD3* are

630   susceptible to the necrotrophs *A. brassicicola* and *B. cinerea* (Nafisi *et al.*,

631   2007; Zhou *et al.*, 1999). Beyond this, several *Botrytis*-induced ET response

632   genes were targeted and positively regulated by WRKY33 (*Figure 2E*). Still,

633   WRKY33 had a negative regulatory relationship on the expression of >75% of

634   all targets implying that it mainly acts as a direct repressor of many defense

635   genes following pathogen challenge.

636   How WRKY33 exerts its dual regulatory functions mechanistically requires

637   further research. The simplest hypothesis would be that WRKY33 is recruited

638   to distinct repressor and activator complexes at defined promoter sites. For

639   instance, several WRKY33-interacting proteins containing a VQ motif have

640   been discovered that influence defense gene expression (Cheng *et al.*, 2012;

641   Lai *et al.*, 2011; Pecher *et al.*, 2014). VQ proteins appear to act as suppressors

642   of defense-related genes via their interaction with WRKY factors. Indeed, the

643   protein VQ4/MVQ1 appears to function as a negative regulator of WRKY-type

644   transcriptional activators including WRKY33. In a transient *Arabidopsis*

645   protoplast assay, stimulation by MAMPs resulted in degradation of VQ4/MVQ1

646   following MAPK-mediated phosphorylation enabling WRKY33 to activate

647   transcription of a defense-related reporter gene (Pecher *et al.*, 2014). Two

648   other proteins, SIB1 and SIB2, have been reported to interact with WRKY33

649 via their VQ motifs. This interaction was required to stimulate WRKY33 DNA

650 binding activity and very likely to positively regulate WRKY33-mediated

651 resistance to necrotrophic fungi (Lai *et al.*, 2011).

652 Interestingly, four VQ protein genes (*VQ8, VQ22/JAV1, VQ28, VQ33*) are also

653 direct targets for WRKY33 and show altered expression upon *Botrytis* infection

654 in *wrky33* compared to WT. VQ22/JAV1 functions as a negative regulator of

655 JA-mediated defenses, and transgenic VQ22/JAV1 RNAi lines showed

656 enhanced resistance to *B. cinerea* (Hu *et al.*, 2013). Thus, elevated levels of

657 these VQ proteins in *wrky33* may contribute to the suppression of JA signaling

658 and susceptibility to this fungus.

659 Finally, many members of the WRKY TF gene family, including *WRKY33* itself,

660 are also targets of WRKY33. The majority of *WRKY* genes are transcriptionally

661 activated during immune signaling (Pandey and Somssich, 2009).

662 Experimental and bioinformatics analyses have revealed that WRKY factors

663 form a complex and highly interconnected regulatory sub-network that is

664 positively and negatively affected by auto- and cross-regulation by various

665 WRKY factors. This WRKY web appears to be deeply interconnected to

666 various hormonal pathways at multiple levels, probably to ensure rapid and

667 efficient signal amplification while allowing for tighter control in limiting the

668 extent of the host immune response (Eulgem, 2006; Llorca *et al.*, 2014).

669

670   In summary, genome-wide binding analysis and transcriptional profiling have

671   identified potential targets of WRKY33, a key transcription factor involved in

672   mediating resistance towards the necrotroph *B. cinerea* 2100. This study

673   revealed that genes involved in ABA biosynthesis and directly regulated by

674   WRKY33 act at crucial nodes in this signaling cascade. Due to the complexity

675   of the highly interconnected hormonal signaling networks targeted by WRKY33

676   the precise molecular mechanisms underlying this resistance remain to be fully

677   elucidated. In this respect global transcriptional profiling of infected *wrky33*

678   *nced3 nced5* plants should prove extremely valuable to further narrow down

679   key genes and sub-signaling pathways required to re-establish WT-like levels

680   of resistance in this mutant.

681

682   **Materials and methods**

683   **Plant material and growth conditions**

684   For all experiments *Arabidopsis thaliana* ecotype Columbia (Col-0) was used.

685   Besides wild-type (WT), the following genotypes were employed: *wrky33*

686   (GABI_324B11)*, nced3-2, nced5-2, nced3 nced5, aba2-12, aba3-1,*

687   *cyp707a1-1, cyp707a2-1, cyp707a3-1, wrky33 sid2-1, wrky33 npr1-1, wrky33*

688   *wrky70, gh3.2, gh3.3-1.* The double or triple mutants; *wrky33 nced3, wrky33*

689   *nced5, wrky33 nced3 nced5, gh3.2 gh3.3-1* and *wrky33 gh3.2 gh3.3* were

690   generated by crossing single or double mutants followed by PCR-based

691 verification using appropriate primers (*Supplementary file 6*).

692 Plants were grown for 4 weeks under short-day conditions in closed cabinets

693 (Schneijder chambers: 16 h light/ 8 h dark cycle at 22-24°C, 60% relative

694 humidity) on 42mm Jiffy-7 pots (Jiffy) to prevent contaminations from garden

695 soil. Before sewing, the Jiffy pot peat pellets were re-hydrated in water

696 containing 0.1% liquid fertilizer Wuxal (Manna).

697

698 **Pathogen inoculation procedure**

699 *B. cinerea* strain 2100 was cultivated on potato dextrose plates at 22°C for 10

700 days. Spores were collected, washed, and frozen at -80°C in 0.8% NaCl at a

701 concentration of 10^7 spores mL^{-1}. For inoculation of *Arabidopsis* plants the

702 spores were diluted in Vogel buffer prepared as previously described

703 (Birkenbihl *et al.*, 2012). For droplet inoculations, 2 μl of 2.5×10^5 spores mL^{-1}

704 were applied to single leaves of 4-week old intact plants. Leaves were excised

705 from plants only for photographic documentation. The same spore

706 concentration was used for spray inoculations of 4-week old intact plants. For

707 mock treatment, Vogel buffer was used. Plants were kept prior to and during

708 infection under sealed hoods at high humidity.

709

710 **ChIP-seq assay**

711 4-week old WT plants or plants expressing *WRKY33-HA* from the native

712 *WRKY33* promoter (*P~WRKY33~:WRKY33-HA*) were spray inoculated or mock

713 treated for 14 h. ChIP assays were performed as previously described

714 (Birkenbihl *et al.*, 2012) following the modified protocol by Gendrel et al.

715 (Gendrel *et al.*, 2005), using rabbit polyclonal antibodies to HA (Sigma). ChIP

716 DNA was purified using a QIA quick PCR Purification kit (Qiagen) and

717 subjected to a linear DNA amplification (LinDA) protocol (Shankaranarayanan

718 *et al.*, 2011) which included two rounds of '*in vitro* transcription' by T7 RNA

719 polymerase. The resulting LinDA DNA was used to generate sequencing

720 libraries bearing barcodes using a NEBNext ChIP-Seq Library Pre Reagent

721 Set for Illumina kit (New England Biolabs). Sequencing was performed on

722 Illumina HiSeq2500 at the Max Planck Genome Centre Cologne and resulted

723 in about 10 million 100bp single-end reads per sample. ChIP-qPCR validation

724 of WRKY33 target genes was performed using gene specific primers

725 (*Supplementary file 7*).

726

727 **ChIP-seq data analysis**

728 Before mapping, remaining LinDA adapters and low quality sequences were

729 removed from the sequencing data using a two-step procedure. In this

730 procedure, first Bpm and t7-Bpm sites were trimmed from the 5' end using

731 cutadapt (version 1.2.1) (Martin, 2011) with options –e 0.2, -n 2 and –m 36

732 (otherwise default settings were used), and subsequently poly-A and poly-T

733 tails and low quality ends were trimmed and reads with overall low quality or

734 with less than 36 bases remaining after trimming were removed using

735 PRINSEQ lite (version 0.20.2) (Schmieder and Edwards, 2011) with options

736 –trim_qual_right/left 20, trim_tail_right/left 3 –min_len 36, -min_qual_mean 25.

737 After this pre-processing steps, the remaining high quality reads were mapped

738 to the *Arabidopsis thaliana* reference genome TAIR10

739 (*http://www.arabidopsis.org*) using Bowtie (version 0.12.7) (Langmead *et al.*,

740 2009) with options –best –m 1 to extract only uniquely mapped reads and

741 allowing two mismatches in the first mapping steps (default settings). The

742 ChIP-seq datasets used in this study have been deposited at the GEO

743 repository (GSE66289).

744 To identify genomic DNA regions enriched in sequencing reads in the ChIP

745 sample compared to input control as well as in inoculated compared to mock

746 treated samples ('peak regions'), the peak calling algorithm of the QuEST

747 program (version 2.4) (Valouev *et al.*, 2008) was applied using the transcription

748 factor mode (option "2"), with permissive parameter settings for the peak

749 calling (option "3"). Each of the two biological replicates was first analyzed

750 separately and additionally, to obtain more exact peak locations for the

751 consistent peaks, the mapped reads of the two replicates were pooled and

752 peaks were also called for the pooled samples. To annotate the peak location

753 with respect to annotated gene features in TAIR10 the annotatePeaks.pl

754 function from the Homer suite (Heinz *et al.*, 2010) was used with default

755 settings. To extract consistent peaks between the replicates, a custom R

756 (*http://www.r-project.org*) function (*Source code 1*) was used that identified

757 overlapping peak regions between the replicates. Two peak regions were

758 counted as overlapping, if they overlapped by at least 50% of the smaller

759 region and a peak region was counted as consistent, if it was found to be

760 overlapping between the two individual replicates as well as the pooled

761 sample.

762 To search for conserved binding motifs in the consistent WRKY33 binding

763 regions, for each consistent peak the 500 bp sequence surrounding the peak

764 maximum was extracted and submitted to the online version of MEME-ChIP

765 (Machanick and Bailey, 2011). MEME-Chip was run with default settings, but a

766 custom background model derived from the *Arabidopsis* genome was provided

767 and "Any number of repetitions" of a motif was allowed. For visualization,

768 prominent motifs identified within MEME-ChIP by either MEME or DREME

769 were chosen. To extract the number/percentage of peak regions that contain a

770 certain motif, the online version of FIMO (Grant *et al.*, 2011) was run with the

771 peak sequences and the motif of interest (MEME/DREME output) as input and

772 a p-value threshold of 0.001.

773

774 **RNA-seq assay**

775   Total RNA was extracted from mock treated (14 hpi) and *B. cinerea* infected

776   (14 hpi) 4-week old plants (WT and *wrky33*) with using the RNeasy Plant Mini

777   (Qiagen) according to the manufacturer's instructions, and mRNA sequencing

778   libraries were constructed with barcodes using the TrueSeq RNA Sample

779   Preparation Kit (Illumina). Three biological replicates were sequenced on

780   Illumina HiSeq2500 by the Max Planck Genome Centre Cologne, resulting in

781   25-45 million 100 bp single end reads per sample. Total reads were mapped to

782   the *Arabidopsis* genome (TAIR10) under consideration of exon-intron

783   structures using the splice-aware read aligner TopHat (version 2.0.10) (Kim *et*

784   *al.*, 2013) with settings –a 10 –g 10 and known splice sites provided based on

785   TAIR10 gene annotations. The RNA-seq datasets used in this study have been

786   deposited at the GEO repository (GSE66290).

787

788   **Statistical Analysis of RNA-seq**

789   The mapped RNA-seq reads were transformed into a count per gene using the

790   function coverageBed of the bedTools suite (Quinlan and Hall, 2010) with

791   option –split to consider exon-intron structures. Genes with less than 50 reads

792   in all samples together were discarded, and subsequently the count data of the

793   remaining genes was TMM-normalized and log2-transformed using functions

794   'calcNormFactors' (R package EdgeR) (Robinson *et al.*, 2010) and 'voom' (R

795   package limma) (Law *et al.*, 2014). To analyze differential gene expression

796 between genotypes (WT, *wrky33*) and treatments (mock treated, *B. cinerea*

797 infected), we fitted a linear model with the explanatory variable

798 "genotype_treatment" (i.e. including both genotype and treatment) using the

799 function lmFit (R package limma). Subsequently, we performed moderated

800 t-tests over the four contrasts of interest. Two contrasts compare *B. cinerea*

801 infected vs mock treated samples within each genotype and the other two

802 contrasts compare *wrky33* vs. WT Col-0 plants within each treatment. In all

803 cases, the resulting P values were adjusted for false discoveries due to

804 multiple hypothesis testing via the Benjamini–Hochberg procedure. For each

805 contrast, we extracted a set of significantly differentially expressed genes

806 between the tested conditions (adj. p value < 0.05, |log2FC≥1).

807

808 **Gene Ontology analysis**

809 GO term enrichment analysis on the gene sets of interest was performed using

810 the R package goseq (Young *et al.*, 2010) with custom GO term mappings

811 obtained from org.At.tairGO2ALLTAIRS within the R package (version 3.0.0)

812 org.At.tair.db: Genome wide annotation for Arabidopsis (Carlson, 2010). To

813 identify enriched GO terms, the Wallenius distribution was used to

814 approximate the null distribution and a probability weighting function was

815 applied to correct for potential count biases in the analyzed gene sets. The

816 resulting p values were adjusted for false discoveries due to multiple

817 hypothesis testing via the Benjamini-Hochberg procedure and for each subset

818 the significantly over-represented GO terms were extracted (adj. p value

819 <0.05). For the set of all WRKY33-regulated targets, the R package (version

820 2.18.0) topGO: Enrichment analysis for Gene Ontology (Alexa &

821 Rahnenführer, 2010) was used to visualize the GO sub-graphs induced by the

822 ten most significantly enriched GO terms in the category 'Biological Process'

823 and the five most significantly enriched GO terms in the category 'Molecular

824 Function', respectively.

825

826 **qRT-PCR**

827 Total RNA was isolated from leaves at 8, 14, 24, and 48 hpi with *B. cinerea*

828 spores as described above and reverse transcribed with oligo(dT) primer to

829 produce cDNA using the SuperScript First-Strand System for

830 Reverse-Transcription PCR following the manufacturer's protocol (Invitrogen).

831 cDNAs corresponding to 2.5 ng of total RNA was subjected to qPCR with

832 gene-specific primers (*Supplementary file 8*) using the SYBR Green reagent

833 (Bio-Rad). The qPCRs were performed on the iQ5 Multicolor Real-Time PCR

834 Detection System (Bio-Rad) with three biological replicates. The relative

835 expression was normalized to At4g26410 that was described as being highly

836 constant under varying stress conditions (Czechowski *et al.*, 2005). Data

837 shown are means ±SD from the three biological replicates.

838

Phytohormone measurements and quantification

Sample processing, data acquisition, instrumental setup, and calculations

were performed as described (Ziegler *et al.*, 2014). Instrument specific

parameters for the detection of SA are shown in the Table below.

$(3,4,5,6-D_4)$-SA was obtained from Campro Scientific (Veenendal, The

Netherlands) and used as internal standard for SA quantification (1.5 ng per

sample).

MS parameters for MRM-transition of salicylic acids (SA)

Hormone	MRM transitions	Declustering potential (DP), V	Entrance potential (EP), V	Cell entrance potential (CEP), V	Collision potential (CE), V	Cell exit potential (CEX), V
SA	**137→93**	-25	-5.5	-14	-22	0
	137→65	-25	-5.5	-14	-44	0
SA-D_4	**141→97**	-25	-5.5	-14	-22	0

Quantifier and qualifier transitions are indicated in bold and italics, respectively.

846

Quantification of fungal growth by qPCR

Quantification of fungal biomass relative to plant biomass by qPCR was

basically performed as previously described (Gachon and Saindrenan, 2004).

Leaves of the indicated *Arabidopsis* lines were inoculated with two 2 µl

droplets of *B. cinerea* spores and DNA extracted 3 days later from whole

leaves of similar fresh weight. The relative amounts of *B. cinerea* and

Arabidopsis DNA were determined by qPCR employing specific primers for

854   cutinase A and SKII, respectively.

855

856   **Expression of recombinant WRKY33 protein and EMSA**

857   Full-length WRKY33 protein fused with NusA and an 8xHis-tag was expressed

858   in the vector pMCSG48 in the *E. coli* strain BL21 (DE3) Magic (kindly provided

859   by Dr. Michal Sikorski and Marta Grzechowiak, Institute of Bioorganic

860   Chemistry, Poznan, Poland). Bacteria containing the *WRKY33* expression

861   construct or the empty vector were induced with 0,5 mM

862   isopropylthio-β-galactisude for 3 h at 18° C. The His-tagged protein was

863   purified using nickel affinity columns (QIAexpress Ni_NTA Fast Start, Qiagen,

864   Germany) following the instructions of the manufacturer, and subsequently

865   dialyzed against 20 mM Tris-HCl, pH 7,5 overnight at 4° C.

866   The following DNA oligonucleotides probes were synthesized and biotin

867   labeled by Sigma-Aldrich (Germany): W-box probe,

868   5'-CG<u>TTGACC</u>G<u>TTGACC</u>GAG<u>TTGACT</u>TTTTA-3'; W-boxmut,

869   5'-CGTTGA<u>A</u>CGTTGA<u>A</u>CGAGTTGA<u>A</u>TTTTTA-3'; M-3,

870   5'-AA<u>TTTGAAT</u>AATCAAAGATCTTCC<u>TTTGAAT</u>TACCT<u>ATTCAAC</u>AT-3'

871   (derived from the *PROPEP3* promoter sequence); and M-7,

872   5'-GTCCACGCTTG<u>TTTGAAT</u>TTTCAGCCTTTGCAGGCAAGGT-3' (derived

873   from the *WAKL7* promoter sequence). Two complementary strands of the

874   oligonucleotides were annealed by heating probes to 95°C for 3 min and then

875 allowing probes to cool to room temperature overnight. Freshly prepared

876 recombinant WRKY33 protein (1μg) was incubated with the DNA probe (20

877 fmol) for 20 min at room temperature using the LightShift Chemiluminescent

878 EMSA kit (Thermo Fisher) in the presence or absence of unlabeled competitor

879 DNA. The resulting protein-DNA complexes were electrophoresed on 5% (w/v)

880 polyacrylamide gels, and then transferred to N^+ nylon membranes in 0,5%

881 Trisborate/EDTA buffer at 380mA at 4° C for 60 min. Biotin labeled DNA

882 detection was done according to the instructions of the manufacturer (Thermo

883 Fisher). Bands were visualized using the BioRAD ChemiDoc[TM] MP Imaging

884 System.

885

900

901 **Competing interest**

902 The authors declare that no competing interests exist.

903

904 **Figure Legends**

905 **Figure 1.** Genome-wide identification of *Arabidopsis* WRKY33 binding sites.

906 (A) Western-blot analysis of WRKY33-HA protein levels after mock treatment

907 or spray-inoculation of P_{WRKY33}:*WRKY33-HA* transgenic plants with *B. cinerea*

908 2100 spores. Plant material selected for ChIP-seq is boxed. (B) Relative

909 binding-peak distribution across genomic regions. The 1 kb region upstream of

910 the transcription start site (TTS) is defined as promoter. The fraction of

911 nucleotides in the complete At genome associated with each annotation type is

912 included in the figure as background control (At genome). (C) Distribution of

913 identified WRKY33 binding sites relative to the TSS. (D) Conserved DNA

914 elements enriched within the 500 bp WRKY33 binding peak regions identified

915 by DREME motif search. The TTGACT/C motif represents the well-established

916 W box, whereas T/GTTGAAT is an identified new motif.

44

917 **Figure 2.** WRKY33 regulated direct target genes in response to *B. cinerea*

918 2100 infection. (A) Number of differentially expressed genes (≥2-fold; P≤0.05)

919 between WT and *wrky33* (*w33*) at 14 h after mock treatment (mo) or spray

920 inoculation with spores of *B. cinerea* 2100 (*B.c.*) identified by RNA-seq.

921 Indicated are total numbers (boxed) and numbers of up-regulated (   )

922 and down-regulated genes (   ) between treatments or genotypes. (B)

923 Venn diagram illustrating the total numbers and the number of common genes

924 affected in WT and *wrky33* 14 h post *B. cinerea* 2100 inoculation. (C) Venn

925 diagram showing the numbers of genes common to WRKY33-regulated genes

926 and WRKY33 target genes. (D) Percentage of WRKY33-repressed and

927 WRKY33-induced target genes (in total 318). (E) Enrichment of specific Gene

928 Ontology (GO) terms related to defense response, kinase activity, cell death,

929 and hormone responses among WRKY33-regulated target and non-target

930 genes (compared to the overall genome). The y-axis indicates the percentages

931 of genes associated to each GO category in each gene set. Asterisks indicate

932 significant enrichment (adj. p value <0.05) of genes associated to the

933 respective GO term within a gene set as determined by GO term enrichment

934 analysis with goseq (null distribution approximated as Wallenius distribution;

935 correction for potential count biases via probability weighting).

936

937 **Figure 3.** WRKY33 regulated transcription factor families commonly

938   associated with stress responses. (A) WRKY, MYB, NAC and AP2/ERF TF

939   family genes are dominant targets of WRKY33 after *B. cinerea* 2100 infection.

940   The total number of members for each TF family is given in parenthesis next to

941   name. The number of WRKY33 directly or indirectly regulated family members

942   are indicated. (B, C) Integrative Genomics Viewer (IGV) images of ChIP-seq

943   data revealing high infection-dependent WRKY33 binding at the promoters of

944   *Arabidopsis WRKY33* (B) and *WRKY41* (C). Images for mock and *B.c.*

945   treatment of both biological repetitions are shown (1 and 2). Structure of the

946   targeted genes are indicated below along with the position of all W box motifs

947   within the loci. Arrows indicate direction of transcription. (D, E) qRT-PCR

948   analysis of *B. cinerea* 2100-induced expression of *WRKY33* (D) and *WRKY41*

949   (E) in WT and *wrky33* mutant plants at indicated time points post fungal spore

950   application. All data were normalized to the expression of At4g26410 and fold

951   induction values of all genes were calculated relative to the expression level of

952   mock treated (mo) WT plants set to 1. Error bars represent SD of three

953   biological replicates. Asterisks indicate significant differences between WT and

954   *wrky33* (*, $P<0.05$; **, $P<0.001$; two-tailed *t*-test). (F, G) Validation of ChIP-seq

955   data by ChIP-qPCR showing WRKY33 binding to its own promoter region (F)

956   and to the *WRKY41* promoter (G). WRKY33-HA (33HA) plants were spray

957   inoculated with spores of *B. cinerea* 2100 (Bc) or mock treated (mo) for 14 h.

958   Input DNA before immune precipitation (IN) and immune-precipitated DNA

959   using an anti-HA antibody (IP) were analyzed by qPCR employing

960   gene-specific primer pairs (p) indicated in the IGV graph. Shown is the fold

961   enrichment of bound DNA relative to a non-bound DNA fragment from

962   At2g04450. As a control for primer efficiency purified genomic DNA was

963   included in the analysis. Each ChIP experiment was repeated at least twice

964   with similar results.

965

966   **Figure 4.** WRKY33 directly regulates target genes encoding ABA biosynthetic

967   (*NCED3*, *NCED5*) and metabolic (*CYP707A3*) enzymes by binding to their

968   promoters or 3'UTR after *B. cinerea* 2100 treatment. (A, D, G) IGV

969   visualization of ChIP-seq data revealing infection-dependent WRKY33

970   enrichment at the *Arabidopsis NCED3* (A), *NCED5* (D) and *CYP707A3* (G) loci

971   with features described in Figure 3. (B, E, H) qRT-PCR analysis of *B. cinerea*

972   2100-induced expression of *NCED3* (B) *NCED5* (E) and CYP707A3 (H) in WT

973   and *wrky33* as described in Figure 3. (C, F, I) Validation of the ChIP-seq data

974   for WRKY33 binding to the promoters of *NCED3* (C, with primer pairs p1 and

975   p2) and *CYP707A3* (I), and to the 3'UTR of *NCED5* (F) performed as

976   described the legend to Figure 3.

977

978   **Figure 5.** WRKY33 controls ABA-mediated plant susceptibility to *B. cinerea*

979   2100. (A) Growth phenotypes of WT, *wrky33*, *nced3 nced5*, and *wrky33 nced3*

980   *nced5 Arabidopsis* plants at 4 weeks under short day conditions. (B) *B. cinerea*

981   infection phenotypes 3 days post inoculation of WT, *wrky33*, *nced3*, *nced5*,

982   *nced3 nced5*, and *wrky33 nced3 nced5*. (C) *B. cinerea* biomass quantification

983   on indicated *Arabidopsis* genotypes. For fungal biomass determination the

984   relative abundance of *B. cinerea* and *Arabidopsis* DNA was determined by

985   qPCR employing specific primers for *Bc*Cutinase A and *At*SKII, respectively.

986   (D) Exogenous application of ABA (10µM) directly to infection droplets on

987   *wrky33 nced3 nced5* leaves partially renders plants susceptible to *B. cinerea*

988   2100. Upon completion of the infection experiments (3 dpi), leaves were

989   detached and photographed. For all infections, two 2 µl droplets containing

990   2.5×10^5 spores were applied to each leaf.

991

992   **Figure 6.** Hormone levels in different genotypes during *B. cinerea* 2100

993   infection. Concentrations of the hormones ABA (A), SA (B), ACC (C) and JA

994   (D) were measured at 8, 14, 24 and 40 hpi in leaves of indicated *Arabidopsis*

995   genotypes spray inoculated with spores of *B. cinerea* 2100. Mock treated

996   plants (mo, 14 h) served as a control. The data show the average values and

997   SDs of the combined data from three independent experiments with up to four

998   replicates each.

999

1000   **Figure 7.** Expression of numerous genes up-regulated in infected *wrky33*

48

1001 plants showing WT-like levels in the *wrky33 nced3 nced5* triple mutant.

1002 Heatmap showing expression levels of genes, differentially expressed in

1003 RNA-seq and analyzed by qRT-PCR in WT, *wrky33* (*w33*)*, nced3 nced5* (*n3n5*)

1004 and *wrky33 nced3 nced5* (*w33n3n5*) after mock treatment or 24 h post *B.*

1005 *cinerea* (*B.c.* 2100) infection. Genes showing high levels in *wrky33* but

1006 reduced levels in the triple mutant are boxed. All values were normalized to the

1007 expression of At4G26410.

1008

1009 **Figure 8.** Dual regulatory role of WRKY33 in modulating host defenses to *B.*

1010 *cinerea* 2100. WRKY33 positively regulates target genes involved in camalexin

1011 biosynthesis thereby contributing to host resistance towards *B. cinerea* 2100.

1012 Target genes involved in ET/JA biosynthesis and signaling can either be

1013 positively or negatively regulated by WRKY33. On the other hand, WRKY33

1014 negatively regulates ABA levels by i.e. directly targeting and repressing

1015 *NCED3* and *NCED5* expression*,* or inducing expression of *CYP707A3,* a gene

1016 involved in ABA metabolism. Thus, WRKY33 has both activator and repressor

1017 functions that may depend on promoter context. Red arrows indicate positive

1018 regulation whereas black bars indicate negative regulation. Curved red arrow

1019 highlights positive feedback regulation of WRKY33 on its own gene promoter.

1020

1021 **Figure 1 - figure supplement 1**. Conserved DNA elements within the 500 bp

1022 WRKY33 binding peak summit regions identified by MEME. (A) W-box with 5'

1023 extended motifs. (B) W-box with 3' extended motifs. (C) Additional conserved

1024 sequence GACTT/ATTC element. (D) Venn diagram illustrating the number of

1025 overlapping peaks containing both the W-box and the newly identified motif

1026 T/GTTGAAT.

1027

1028 **Figure 1 – figure supplement 2**

1029 WRKY33 does not bind to the G/TTTGAAT motif. EMSA was performed using

1030 recombinant WRKY33 and biotin labeled DNA probes. A DNA oligonucleotide

1031 containing 3 W-box elements (Mao *et al.*, 2011) or a W-box mutated version

1032 (W-boxmut) hereof served as a positive and as a negative control, respectively

1033 (A). The 45 bp M-3 was derived from the *PROPEP3* (At5g64905) gene

1034 promoter and contained three copies of the G/TTTGAAT motif, whereas the 40

1035 bp M-7 was derived from the *WAKL7* (At1g16090) gene promoter and

1036 contained one G/TTTGAAT motif. No binding of WRKY33 was observed to the

1037 W-boxmut probe and to both the M-3 and M-7 probes (B). Specificity of W-box

1038 binding was shown by competition assays using 250-fold (W-box) and 500-fold

1039 (W-box; M-3, M-7) excess of unlabeled probes. Protein lysates derived from

1040 IPTG-induced bacteria harboring the empty expression vector pMCSG48

1041 served as an additional control.

1042

1043 **Figure 2 - figure supplement 1**. GO graph visualization of top GO terms

1044 enriched among WRKY33-regulated WRKY33 target genes. (A) GO sub-graph

1045 induced by the top 10 GO terms in the category 'Biological Process'. (B) GO

1046 sub-graph induced by the top 5 GO terms in the category 'Molecular Function'.

1047 Boxes indicate the 10 (A) and 5 (B) most significant terms, respectively. Box

1048 color represents the relative significance, ranging from dark red (most

1049 significant) to light yellow (least significant). GO term enrichment was

1050 determined using goseq, with the null distribution approximated as Wallenius

1051 distribution and correcting for potential count biases via probability weighting.

1052

1053 **Figure 2 – figure supplement 2.** Analysis of WRKY33-regulated target genes

1054 from GO-terms hormone responses and cell death. (A) Venn diagram showing

1055 the overlap between the WRKY33-regulated target genes responsive to SA

1056 and associated with cell death. (B) The overlap of WRKY33-regulated target

1057 genes associated with the hormone pathways SA, ET, ABA and JA.

1058

1059 **Figure 3 - figure supplement 1**. Validation of WRKY33 directly regulated

1060 *WRKY* genes. Shown for each of the *WRKY* genes *38* (A)*, 48* (B)*, 50* (C)*,

1061 *53*(D)*,* and *55* (E), are; i) the ChIP-seq data, visualized by the IGV browser,

1062 revealing strong infection-dependent WRKY33 enrichment at the

1063 corresponding promoter, ii) qRT-PCR analysis of *B. cinerea* 2100-induced

1064 expression of the respective *WRKY* gene in WT and *wrky33* at indicated time

1065 points post spore inoculation, and iii) ChIP-qPCR confirmation of WRKY33

1066 binding to the respective *WRKY* promoter. Amplicons used are indicated in the

1067 IGV images (p, p1, p2, p3). For detailed descriptions on how qRT-PCR and

1068 ChIP-qPCR were performed see legend to Figure 3.

1069

1070 **Figure 5 - figure supplement 1.** *B. cinerea* 2100 infection phenotypes of

1071 mature 4-week old leaves derived from *aba2-12 and aba3-1* mutant plants.

1072 Pictures were taken 3 days post inoculation of two 2 μl droplets containing

1073 $2.5x10^5$ spores to each leaf (leaves were detached after completion of the

1074 experiment for photographic purposes only).

1075

1076 **Figure 5 - figure supplement 2.** *B. cinerea* 2100 infection phenotypes of

1077 mature 4-week old leaves derived from *wrky33 nced3* and *wrky33 nced5*

1078 mutant plants. Pictures were taken 3 days post inoculation of two 2 μl droplets

1079 containing $2.5x10^5$ spores to each leaf (leaves were detached after completion

1080 of the experiment for photographic purposes only).

1081

1082 **Figure 5 – figure supplement 3.** Phenotype of WT Col-0 plants treated with

1083 ABA. Infection droplets with ABA (10μM; red arrows) or without ABA (black

1084 arrows) were applied to 4-week old leaves. No alteration of the WT resistant

1085   phenotype was observed. Picture was taken 3 days post inoculation of two 2 μl

1086   droplets containing 2.5×10^5 spores to each leaf.

1087

1088   **Figure 5 - figure supplement 4.** *B. cinerea* 2100 infection phenotypes of

1089   mature 4-week old leaves derived from *cyp707a1, cyp707a2* and *cyp707a3*

1090   mutant plants. Pictures were taken 3 days post inoculation of two 2 μl droplets

1091   containing 2.5×10^5 spores to each leaf (leaves were detached after completion

1092   of the experiment for photographic purposes only).

1093

1094   **Figure 8 - figure supplement 1.** Schematic representation showing of

1095   WRKY33-dependent host immunity towards the necrotroph *B. cinerea* 2100

1096   through repressing the ABA network. In WT *Arabidopsis* Col-0 plants (upper

1097   panel), *B. cinerea* inoculation induces *WRKY33* expression and protein

1098   accumulation that controls ABA levels by repressing the ABA biosynthetic

1099   target genes *NCED3* and *NCED5*, and by inducing the ABA metabolic gene

1100   *CYP707A3*. WRKY33 also directly or indirectly represses expression of other

1101   genes in different hormone signaling pathways as well as genes encoding

1102   WRKY, and NAC TFs. WRKY33 also positively affects resistance towards *B.*

1103   *cinerea* 2100 by directly targeting and inducing the expression of several genes

1104   involved in camalexin biosynthesis.

1105   In the *wrky33* mutant (lower panel) *B. cinerea* infection fails to activate host

1106   camalexin biosynthesis genes and increases ABA levels by up-regulation of

1107   *NCED3* and *NCED5*, which activates downstream ABA-dependent genes and

1108   ABA signaling. Among the ABA-dependent genes are genes involved in SA

1109   signaling, WRKY TFs, NAC TFs, and genes associated with auxin or JA

1110   conjugation indicating that ABA may acts as a key sub-node in

1111   WRKY33-dependent host immunity to *B. cinerea*.

1112   Whereas WT-like resistance is restored in the *wrky33 nced3 nced5* triple

1113   mutant, all currently tested mutants of WRKY33 regulated genes acting

1114   downstream of ABA failed to restore resistance in the *wrky33* mutant

1115   background (marked by an asterisk). Thus, additional crucial genes causal for

1116   WRKY33-dependent resistance to *B. cinerea* 2100 remain to be discovered, as

1117   do ABA signaling components affecting *WRKY33* expression (indicated by the

1118   question marks). The solid lines with arrows indicate induction or positive

1119   modulation, the bar heads indicate suppression. Direct WRKY33 targets are

1120   indicated by the green color.

1121

1122   **Figure 8 – figure supplement 2.** *AMT1* and *CYP71A12* expression is

1123   positively regulated by WRKY33. (A-B) Integrative Genomics Viewer (IGV)

1124   images of ChIP-seq data revealing high infection-dependent WRKY33 binding

1125   at the promoters of *AMT1* (A) and *CYP71A12* (B). (C-D) qRT-PCR analyses of

1126   *AMT1* (C) and *CYP71A12* (D) in WT and *wrky33* at the indicated time points

1127 after *B. cinerea* 2100 spore inoculation. qRT-PCR data were normalized to the

1128 expression of At4g26410. Error bars represent SD of three biological replicates

1129 (n=3). Asterisks indicate significant differences between WT and *wrky33* (*,

1130 *P*<0.05; **, *P*<0.001; two-tailed *t*-test).

1131

1132 **Figure 8 – figure supplement 3**. The role of *GH3* genes in

1133 WRKY33-mediated host resistance to *B. cinerea* 2100. (A) Expression levels

1134 of *GH3.2* and *GH3.3* in WT and *wrky33* plants determined by qRT-PCR at the

1135 indicated time points after *B. cinerea* 2100 spore inoculation. Data were

1136 normalized to the expression of At4g26410. Error bars represent SD of three

1137 biological replicates (n=3). Asterisks indicate significant differences between

1138 WT and *wrky33* (*, *P*<0.05; **, *P*<0.001; two-tailed *t*-test). (B) *B. cinerea* 2100

1139 infection phenotypes of WT, *wrky33, gh3.2, gh3.3, gh3.2 gh3.3* and *wrky33*

1140 *gh3.2 gh3.3* plants are shown at 3 days post infection. Two 2 μl droplets

1141 containing 2.5×10^5 spores were applied to each leaf.

1142

1143 **Supplementary file 1.** Summary of identified WRKY33 binding sites 14 h post

1144 *B. cinerea* 2100 inoculation.

1145 **Supplementary file 2.** List of confirmed WRKY33 target genes.

1146 **Supplementary file 3.** List of WRKY33 regulated target genes involved in cell

1147 death.

1148   **Supplementary file 4.** List of WRKY33 regulated target genes associated in

1149   the GO category "kinase activity".

1150   **Supplementary file 5.** List of WRKY33 target genes encoding transcription

1151   factors.

1152   **Supplementary file 6.** List of primers used for genotyping.

1153   **Supplementary file 7.** List of primers used for ChIP-qPCR.

1154   **Supplementary file 8.** List of primers used for qRT-PCR.

1155

1156   **Source code 1.** R function to identify overlapping peak regions.

1157

1158   **References**

1159   Adie BAT, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano J-J,

1160   Schmelz EA , Solano R. 2007. ABA is an essential signal for plant

1161   resistance to pathogens affecting JA biosynthesis and the activation of

1162   defenses in Arabidopsis. *The Plant Cell* **19**:1665-1681.

1163   Alexandre C , Vincent J-P. 2003. Requirements for transcriptional repression

1164   and activation by Engrailed in *Drosophila* embryos. *Development*

1165   **130**:729-739. 10.1242/dev.00286

1166   Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NHT, Zhu S,

1167   Qiu, J.-L., Micheelsen P, Rocher A, Petersen M, Newman M-A, Nielsen

1168   HB, Hirt H, Somssich I, Mattsson O , Mundy J. 2005. The MAP kinase

substrate MKS1 is a regulator of plant defense responses. *The EMBO Journal* **24**:2579-2589.

Asselbergh B, De Vleesschauwer D , Höfte M. 2008. Global switches and fine-tuning - ABA modulates plant pathogen defense. *Molecular Plant-Microbe Interactions* **21**:709-719.

Bari R , Jones J. 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology* **69**:473-488.

Berger N , Dubreucq B. 2012. Evolution goes GAGA: GAGA binding proteins across kingdoms. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression* **1819**:863-868. http://dx.doi.org/10.1016/j.bbagrm.2012.02.022

Berrocal-Lobo M, Molina A , Solano R. 2002. Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi. *The Plant Journal* **29**:23-32.

Birkenbihl RP, Diezel C , Somssich IE. 2012. Arabidopsis WRKY33 is a key transcriptional regulator of hormone and metabolic responses towards *Botrytis cinerea* infection. . *Plant Physiology* **159**:266-285.

Birkenbihl RP , Somssich IE. 2011. Transcriptional plant responses critical for resistance towards necrotrophic pathogens. *Frontiers in Plant Science* **2**:76.

Bu Q, Jiang H, Li C-B, Zhai Q, Zhang J, Wu X, Sun J, Xie Q , Li C. 2008. Role

1190   of the *Arabidopsis thaliana* NAC transcription factors ANAC019 and

1191   ANAC055 in regulating jasmonic acid-signaled defense responses. *Cell*

1192   *Research* **18**:756-767.

1193   Chang K, Zhong S, Weirauch M, Hon G, Pelizzola M, Li H, Huang S-s, Schmitz

1194   R, Urich MA, Kuo D, Nery J, Qiao H, Yang A, Jamali A, Chen H, Ideker

1195   T, Ren B, Bar-Joseph Z, Hughes T , Ecker J. 2013. Temporal

1196   transcriptional response to ethylene gas drives growth hormone

1197   cross-regulation in Arabidopsis. *eLife* **2** 00675. DOI:

1198   10.7554/eLife.00675

1199   Cheng Y, Zhou Y, Yang Y, Chi Y-J, Zhou J, Chen J-Y, Wang F, Fan B, Shi K,

1200   Zhou Y-H, Yu J-Q , Chen Z. 2012. Structural and functional analysis of

1201   VQ motif-containing proteins in *Arabidopsis* as interacting proteins of

1202   WRKY transcription factors. *Plant Physiology* **159**:810-825.

1203   Ciolkowski I, Wanke D, Birkenbihl R , Somssich IE. 2008. Studies on

1204   DNA-binding selectivity of WRKY transcription factors lend structural

1205   clues into WRKY-domain function. *Plant Molecular Biology* **68**:81-92.

1206   Cusanovich DA, Pavlovic B, Pritchard JK , Gilad Y. 2014. The functional

1207   consequences of variation in transcription factor binding. *PLoS*

1208   *Genetics* **10**:e1004226. 10.1371/journal.pgen.1004226

1209   Czechowski T, Stitt M, Altmann T, Udvardi MK , Scheible W-R. 2005.

1210   Genome-wide identification and testing of superior reference genes for

1211   transcript normalization in *Arabidopsis*. *Plant Physiology* **139**:5-17.

1212   de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW,

1213   Rodriguez Egea P, Bögre L , Grant M. 2007. *Pseudomonas syringae* pv.

1214   *tomato* hijacks the *Arabidopsis* abscisic acid signalling pathway to

1215   cause disease. *The EMBO Journal* **26**:1434-1443.

1216   Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu

1217   PD, Rudd JJ, Dickman M, Kahmann R, Ellis J , Foster GD. 2012. The

1218   Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant*

1219   *Pathology* **13**:414-430. 10.1111/j.1364-3703.2011.00783.x

1220   Eulgem T. 2006. Dissecting the WRKY web of plant defense regulators. *PLoS*

1221   *Pathogens* **2**:e126.

1222   Fan M, Bai M-Y, Kim J-G, Wang T, Oh E, Chen L, Park CH, Son S-H, Kim S-K,

1223   Mudgett MB , Wang Z-Y. 2014. The bHLH transcription factor HBI1

1224   mediates the trade-off between growth and pathogen-associated

1225   molecular pattern–triggered immunity in *Arabidopsis*. *The Plant Cell*

1226   **26**:828-841. 10.1105/tpc.113.121111

1227   Finkelstein R. 2013. Abscisic acid synthesis and response. *The Arabidopsis*

1228   *Book* **11**:e0166. 10.1199/tab.0166

1229   Frey A, Effroy D, Lefebvre V, Seo M, Perreau F, Berger A, Sechet J, To A,

1230   North HM , Marion-Poll A. 2012. Epoxycarotenoid cleavage by NCED5

1231   fine-tunes ABA accumulation and affects seed dormancy and drought

1232   tolerance with other NCED family members. *The Plant Journal*

1233   **70**:501-512. 10.1111/j.1365-313X.2011.04887.x

1234 Gachon C , Saindrenan P. 2004. Real-time PCR monitoring of fungal

1235   development in Arabidopsis thaliana infected by Alternaria brassicicola

1236   and Botrytis cinerea. *Plant Physiology and Biochemistry* **42**:367-371.

1237 Gendrel A-V, Lippman Z, Martienssen R , Colot V. 2005. Profiling histone

1238   modification patterns in plants using genomic tiling microarrays. *Nature*

1239   *Methods* **2**:213-218.

1240 Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic

1241   and necrotrophic pathogens. *Annual Reviews of Phytopathology*

1242   **43**:205-227.

1243 Gonzalez-Lamothe R, Boyle P, Dulude A, Roy V, Lezin-Doumbou C, Kaur GS,

1244   Bouarab K, Despres C , Brisson N. 2008. The transcriptional activator

1245   Pti4 is required for the recruitment of a repressosome nucleated by

1246   repressor SEBF at the potato *PR-10a* gene. *The Plant Cell*

1247   **20**:3136-3147.

1248 González-Lamothe R, El Oirdi M, Brisson N , Bouarab K. 2012. The

1249   conjugated auxin indole-3-acetic acid - aspartic acid promotes plant

1250   disease development. *The Plant Cell* **24**:762-777.

1251 Grant C, Bailey T , Noble W. 2011. FIMO: scanning for occurrences of a given

1252   motif. *Bioinformatics* **27**:1017 - 1018.

1253   Gutierrez L, Mongelard G, Floková K, Păcurar DI, Novák O, Staswick P,

1254   Kowalczyk M, Păcurar M, Demailly H, Geiss G , Bellini C. 2012. Auxin

1255   controls *Arabidopsis* adventitious root initiation by regulating jasmonic

1256   acid homeostasis. *The Plant Cell Online* **24**:2515-2527.

1257   10.1105/tpc.112.099119

1258   Han L, Li GJ, Yang KY, Mao G, Wang R, Liu Y , Zhang S. 2010.

1259   Mitogen-activated protein kinase 3 and 6 regulate *Botrytis*

1260   *cinerea*-induced ethylene production in *Arabidopsis*. *The Plant Journal*

1261   **64**:114-127.

1262   Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C,

1263   Singh H , Glass CK. 2010. Simple combinations of lineage-determining

1264   transcription factors prime *cis*-regulatory elements required for

1265   macrophage and B cell identities. *Molecular Cell* **38**:576-589.

1266   http://dx.doi.org/10.1016/j.molcel.2010.05.004

1267   Hu P, Zhou W, Cheng Z, Fan M, Wang L , Xie D. 2013. JAV1 controls

1268   jasmonate-regulated plant defense. *Molecular Cell* **50**:504-515.

1269   Huang P-Y, Yeh Y-H, Liu A-C, Cheng C-P , Zimmerli L. 2014. The *Arabidopsis*

1270   LecRK-VI.2 associates with the pattern-recognition receptor FLS2 and

1271   primes *Nicotiana benthamiana* pattern-triggered immunity. *The Plant*

1272   *Journal* **79**:243-255. 10.1111/tpj.12557

1273   Ikeda M, Mitsuda N , Ohme-Takagi M. 2009. Arabidopsis WUSCHEL is a

1274   bifunctional transcription factor that acts as a repressor in stem cell

1275   regulation and as an activator in floral patterning. *The Plant Cell*

1276   **21**:3493-3505. 10.1105/tpc.109.069997

1277   Jensen MK, Hagedorn PH, De Torres-Zabala M, Grant MR, Rung JH, Collinge

1278   DB , Lyngkjaer MF. 2008. Transcriptional regulation by an NAC

1279   (NAM–ATAF1,2–CUC2) transcription factor attenuates ABA signalling

1280   for efficient basal defence towards *Blumeria graminis* f. sp. *hordei* in

1281   Arabidopsis. *The Plant Journal* **56**:867-880.

1282   10.1111/j.1365-313X.2008.03646.x

1283   Jensen MK, Lindemose S, Masi Fd, Reimer JJ, Nielsen M, Perera V, Workman

1284   CT, Turck F, Grant MR, Mundy J, Petersen M , Skriver K. 2013. ATAF1

1285   transcription factor directly regulates abscisic acid biosynthetic gene

1286   *NCED3* in *Arabidopsis thaliana*. *FEBS Open Bio* **3**:321-327.

1287   http://dx.doi.org/10.1016/j.fob.2013.07.006

1288   Jiang H, Li H, Bu Q , Li C. 2009. The RHA2a-interacting proteins ANAC019

1289   and ANAC055 may play a dual role in regulating ABA response and

1290   jasmonate response. *Plant Signaling & Behavior* **4**:464-466.

1291   Kazan K , Manners JM. 2013. MYC2: The master in action. *Molecular Plant*

1292   **6**:686-703

1293   Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R , Salzberg S. 2013.

1294   TopHat2: accurate alignment of transcriptomes in the presence of

1295   insertions, deletions and gene fusions. *Genome Biology* **14**:R36.

1296 Kliebenstein DJ, Rowe HC , Denby KJ. 2005. Secondary metabolites influence

1297   Arabidopsis/Botrytis interactions: variation in host production and

1298   pathogen sensitivity. *The Plant Journal* **44**:25-36.

1299 Lai Z, Li Y, Wang F, Cheng Y, Fan B, Yu J-Q , Chen Z. 2011. Arabidopsis sigma

1300   factor binding proteins are activators of the WRKY33 transcription factor

1301   in plant defense. *The Plant Cell* **23**:3824-3841.

1302 Langmead B, Trapnell C, Pop M , Salzberg S. 2009. Ultrafast and

1303   memory-efficient alignment of short DNA sequences to the human

1304   genome. *Genome Biology* **10**:R25.

1305 Law C, Chen Y, Shi W , Smyth G. 2014. voom: precision weights unlock linear

1306   model analysis tools for RNA-seq read counts. *Genome Biology*

1307   **15**:R29.

1308 Łaźniewska J, Macioszek V, Lawrence C , Kononowicz A. 2010. Fight to the

1309   death: *Arabidopsis thaliana* defense response to fungal necrotrophic

1310   pathogens. *Acta Physiologiae Plantarum* **32**:1-10.

1311 Leng P, Yuan B, Guo Y , Chen P. 2014. The role of abscisic acid in fruit

1312   ripening and responses to abiotic stress. *Journal of Experimental*

1313   *Botany*:in press. 10.1093/jxb/eru204

1314 Léon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE,

1315   Schwartz SH, Zeevaart JAD , Koornneef M. 1996. Isolation and

characterization of abscisic acid-deficient Arabidopsis mutants at two
new loci. *The Plant Journal* **10**:655-661.
10.1046/j.1365-313X.1996.10040655.x

Li G, Meng X, Wang R, Mao G, Han L, Liu Y , Zhang S. 2012. Dual-level
regulation of ACC synthase activity by MPK3/MPK6 cascade and its
downstream WRKY transcription factor during ethylene induction in
Arabidopsis. *PLoS Genetics* **8**:e1002767.

Lippok B, Birkenbihl RP, Rivory G, Brümmer J, Schmelzer E, Logemann E ,
Somssich IE. 2007. Expression of *AtWRKY33* encoding a
pathogen-/PAMP-responsive WRKY transcription factor is regulated by
a composite DNA motif containing W box elements. . *Molecular
Plant-Microbe Interactions* **20**:420-429.

Llorca CM, Potschin M , Zentgraf U. 2014. bZIPS and WRKYs: two large
transcription factor families executing two different functional strategies.
Frontiers in Plant Science **5**:169. 10.3389/fpls.2014.00169

Luo H, Laluk K, Lai Z, Veronese P, Song F , Mengiste T. 2010. The Arabidopsis
Botrytis Susceptible1 Interactor defines a subclass of RING E3 ligases
that regulate pathogen and stress responses. *Plant Physiology*
154:1766-1782.

Machanick P , Bailey T. 2011. MEME-ChIP: motif analysis of large DNA
datasets. *Bioinformatics* **27**:1696 - 1697.

1337   Machens F, Becker M, Umrath F , Hehl R. 2014. Identification of a novel type

1338   of WRKY transcription factor binding site in elicitor-responsive

1339   *cis*-sequences from *Arabidopsis thaliana*. *Plant Molecular Biology*

1340   **84**:371-385. 10.1007/s11103-013-0136-y

1341   MacQuarrie KL, Fong AP, Morse RH , Tapscott SJ. 2011. Genome-wide

1342   transcription factor binding: beyond direct target regulation. *Trends in*

1343   *Genetics* **27**:141-148. http://dx.doi.org/10.1016/j.tig.2011.01.001

1344   Mao G, Meng X, Liu Y, Zheng Z, Chen Z , Zhang S. 2011. Phosphorylation of a

1345   WRKY Transcription Factor by Two Pathogen-Responsive MAPKs

1346   Drives Phytoalexin Biosynthesis in Arabidopsis. *The Plant Cell*

1347   **23**:1639-1653.

1348   Martin M. 2011. Cutadapt removes adapter sequences from high-throughput

1349   sequencing reads. *EMBnet.journal* **17**:10-12. 10.14806/ej.17.1.200

1350   pp. 10-12

1351   Mengiste T. 2012. Plant immunity to necrotrophs. *Annual Review of*

1352   *Phytopathology*   **50**:267-294.

1353   doi:10.1146/annurev-phyto-081211-172955

1354   Miao Y, Laun T, Zimmermann P , Zentgraf U. 2004. Targets of the WRKY53

1355   transcription factor and its role during leaf senescence in *Arabidopsis*.

1356   *Plant Molecular Biology* **55**:853-867.

1357   Miyakawa T, Fujita Y, Yamaguchi-Shinozaki K , Tanokura M. 2013. Structure

1358   and function of abscisic acid receptors. *Trends in Plant Science*

1359   **18**:259-266.

1360   Moffat CS, Ingle RA, Wathugala DL, Saunders NJ, Knight H , Knight MR. 2012.

1361   ERF5 and ERF6 play redundant roles as positive regulators of

1362   JA/Et-mediated defense against *Botrytis cinerea* in Arabidopsis. *PLoS*

1363   *ONE* **7**:e35995.

1364   Nafisi M, Goregaoker S, Botanga CJ, Glawischnig E, Olsen CE, Halkier BA ,

1365   Glazebrook J. 2007. Arabidopsis cytochrome P450 monooxygenase

1366   71A13 catalyzes the conversion of indole-3-acetaldoxime in camalexin

1367   synthesis. *The Plant Cell* **19**:2039-2052.

1368   Nurmberg PL, Knox KA, Yun B-W, Morris PC, Shafiei R, Hudson A , Loake GJ.

1369   2007. The developmental selector *AS1* is an evolutionarily conserved

1370   regulator of the plant immune response. *Proceedings of the National*

1371   *Academy of Sciences of the United States of America*

1372   **104**:18795-18800.

1373   Pandey SP , Somssich IE. 2009. The role of WRKY transcription factors in

1374   plant immunity. *Plant Physiology* **150**:1648-1655.

1375   Pecher P, Eschen-Lippold L, Herklotz S, Kuhle K, Naumann K, Bethke G, Uhrig

1376   J, Weyhe M, Scheel D , Lee J. 2014. The *Arabidopsis thaliana*

1377   mitogen-activated protein kinases MPK3 and MPK6 target a subclass of

1378   'VQ-motif'-containing proteins to regulate immune responses. *New*

1379   *Phytologist* **203**:592-606. 10.1111/nph.12817

1380   Pieterse CMJ, Leon-Reyes A, Van der Ent S , Van Wees SCM. 2009.

1381   Networking by small-molecule hormones in plant immunity. *Nature*

1382   *Chemical Biology* **5**:308-316.

1383   Pré M, Atallah M, Champion A, De Vos M, Pieterse CMJ , Memelink J. 2008.

1384   The AP2/ERF domain transcription factor ORA59 integrates jasmonic

1385   acid and ethylene signals in plant defense. *Plant Physiology*

1386   **147**:1347-1357.

1387   Qiu J-L, Fiil B-K, Petersen K, Nielsen HB, Botanga CJ, Thorgrimsen S, Palma

1388   K, Suarez-Rodriguez MC, Sandbech-Clausen S, Lichota J, Brodersen

1389   P, Grasser KD, Mattsson O, Glazebrook J, Mundy J , Petersen M. 2008.

1390   *Arabidopsis MAP kinase 4 regulates gene expression through*

1391   *transcription factor release in the nucleus*. *The EMBO Journal*

1392   **27**:2214-2221.

1393   Quinlan A , Hall I. 2010. BEDTools: a flexible suite of utilities for comparing

1394   genomic features. *Bioinformatics* **26**:841 - 842.

1395   Ramírez V, Agorio A, Coego A, García-Andrade J, Hernández MJ, Balaguer B,

1396   Ouwerkerk PBF, Zarra I , Vera P. 2011. MYB46 modulates disease

1397   susceptibility to *Botrytis cinerea* in *Arabidopsis*. *Plant Physiology*

1398   **155**:1920-1935.

1399   Robatzek S , Somssich IE. 2002. Targets of AtWRKY6 regulation during plant

1400   senescence and pathogen defense. *Genes & Development*

1401   **16**:1139-1149.

1402 Robert-Seilaniantz A, Grant M , Jones JDG. 2011. Hormone crosstalk in plant

1403   disease and defense: More than just JASMONATE-SALICYLATE

1404   antagonism. *Annual Review of Phytopathology* **49**:317-343.

1405 Robinson MD, McCarthy DJ , Smyth GK. 2010. edgeR: a Bioconductor

1406   package for differential expression analysis of digital gene expression

1407   data. *Bioinformatics* **26**:139-140. 10.1093/bioinformatics/btp616

1408 Rowe HC , Kliebenstein DJ. 2008. Complex genetics control natural variation

1409   in *Arabidopsis thaliana* resistance to *Botrytis cinerea*. *Genetics*

1410   **180**:2237-2250.

1411 Rushton PJ, Somssich IE, Ringler P , Shen QJ. 2010. WRKY transcription

1412   factors. *Trends in Plant Science* **15**:247-258.

1413 Sakabe NJ, Aneas I, Shen T, Shokri L, Park S-Y, Bulyk ML, Evans SM ,

1414   Nobrega MA. 2012. Dual transcriptional activator and repressor roles of

1415   TBX20 regulate adult cardiac structure and function. *Human Molecular*

1416   *Genetics* **21**:2194-2204. 10.1093/hmg/dds034

1417 Sánchez-Vallet A, López G, Ramos B, Delgado-Cerezo M, Riviere M-P,

1418   Llorente F, Fernández PV, Miedes E, Estevez JM, Grant M , Molina A.

1419   2012. Disruption of abscisic acid signaling constitutively activates

1420   *Arabidopsis* resistance to the necrotrophic fungus *Plectosphaerella*

1421   *cucumerina. Plant Physiology* **160**:2109-2124. 10.1104/pp.112.200154

1422   Sánchez-Vallet A, Ramos B, Bednarek P, López G, Piślewska-Bednarek M,

1423   Schulze-Lefert P , Molina A. 2010. Tryptophan-derived secondary

1424   metabolites in *Arabidopsis thaliana* confer non-host resistance to

1425   necrotrophic *Plectosphaerella cucumerina* fungi. *The Plant Journal*

1426   **63**:115-127. 10.1111/j.1365-313X.2010.04224.x

1427   Schmieder R , Edwards R. 2011. Quality control and preprocessing of

1428   metagenomic   datasets.   *Bioinformatics*   **27**:863-864.

1429   10.1093/bioinformatics/btr026

1430   Shankaranarayanan P, Mendoza-Parra M-A, Walia M, Wang L, Li N, Trindade

1431   LM , Gronemeyer H. 2011. Single-tube linear DNA amplification (LinDA)

1432   for robust ChIP-seq. *Nature Methods* **8**:565-567.

1433   Singh P, Kuo Y-C, Mishra S, Tsai C-H, Chien C-C, Chen C-W,

1434   Desclos-Theveniau M, Chu P-W, Schulze B, Chinchilla D, Boller T ,

1435   Zimmerli L. 2012. The lectin receptor kinase-VI.2 is required for priming

1436   and positively regulates *Arabidopsis* pattern-triggered immunity. *The*

1437   *Plant Cell* **24**:1256-1270.

1438   Ton J, Flors V , Mauch-Mani B. 2009. The multifaceted role of ABA in disease

1439   resistance.   *Trends   in   Plant   Science*   **14**:310-317.

1440   http://dx.doi.org/10.1016/j.tplants.2009.03.006

1441   Valouev A, Johnson DS, Sundquist A, Medina C, Anton E, Batzoglou S, Myers

1442   RM , Sidow A. 2008. Genome-wide analysis of transcription factor

1443   binding sites based on ChIP-Seq data. *Nature Methods* **5**:829-834.

1444 Wang Xe, Basnayake BMVS, Zhang H, Li G, Li W, Virk N, Mengiste T , Song F.

1445   2009. The Arabidopsis ATAF1, a NAC transcription factor, is a negative

1446   regulator of defense responses against necrotrophic fungal and

1447   bacterial pathogens. *Molecular Plant-Microbe Interactions*

1448   **22**:1227-1238.

1449 Williamson B, Tudzynski B, Tudzynski P , Van Kan JAL. 2007. *Botrytis cinerea*:

1450   the cause of grey mould disease. *Molecular Plant Pathology* **8**:561-580.

1451 Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ,

1452   Penfold CA, Baxter L, Breeze E, Kiddle SJ, Rhodes J, Atwell S,

1453   Kliebenstein DJ, Kim Y-s, Stegle O, Borgwardt K, Zhang C, Tabrett A,

1454   Legaie R, Moore J, Finkenstadt B, Wild DL, Mead A, Rand D, Beynon J,

1455   Ott S, Buchanan-Wollaston V , Denby KJ. 2012. Arabidopsis defense

1456   against *Botrytis cinerea*: chronology and regulation deciphered by

1457   high-resolution temporal transcriptomic analysis. *The Plant Cell*

1458   **24**:3530-3557.

1459 Yamaguchi Y, Huffaker A, Bryan AC, Tax FE , Ryan CA. 2010. PEPR2 is a

1460   second receptor for the Pep1 and Pep2 peptides and contributes to

1461   defense responses in *Arabidopsis*. *The Plant Cell* **22**:508-522.

1462 Young M, Wakefield M, Smyth G , Oshlack A. 2010. Gene ontology analysis for

1463   RNA-seq: accounting for selection bias. *Genome Biology* **11**:R14.

1464 Yu X, Li L, Zola J, Aluru M, Ye H, Foudree A, Guo H, Anderson S, Aluru S, Liu

1465   P, Rodermel S , Yin Y. 2011. A brassinosteroid transcriptional network

1466   revealed by genome-wide identification of BESI target genes in

1467   *Arabidopsis thaliana*. *The Plant Journal* **65**:634-646.

1468 Zander M, Thurow C , Gatz C. 2014. TGA transcription factors activate the

1469   salicylic acid-suppressible branch of the ethylene-induced defense

1470   program by regulating *ORA59* expression. *Plant Physiology*

1471   **165**:1671-1683. 10.1104/pp.114.243360

1472 Zhao Y, Wei T, Yin K-Q, Chen Z, Gu H, Qu L-J , Qin G. 2012. Arabidopsis

1473   RAP2.2 plays an important role in plant resistance to *Botrytis cinerea*

1474   and ethylene responses. *New Phytologist* **195**:450-460.

1475   10.1111/j.1469-8137.2012.04160.x

1476 Zheng X-y, Spivey Natalie W, Zeng W, Liu P-P, Fu Zheng Q, Klessig Daniel F,

1477   He Sheng Y , Dong X. 2012. Coronatine promotes *Pseudomonas*

1478   *syringae* virulence in plants by activating a signaling cascade that

1479   inhibits salicylic acid accumulation. *Cell Host & Microbe* **11**:587-596.

1480   10.1016/j.chom.2012.04.014

1481 Zheng Z, Qamar SA, Chen Z , Mengiste T. 2006. Arabidopsis WRKY33

1482   transcription factor is required for resistance to necrotrophic fungal

1483   pathogens. *The Plant Journal* **48**:592-605.

1484   Zhou N, Tootle TL , Glazebrook J. 1999. Arabidopsis *PAD3*, a gene required for

1485   camalexin biosynthesis, encodes a putative cytochrome P450

1486   monooxygenase. *The Plant Cell* **11**:2419-2428.

1487   Zhu X, Wang Y, Pi W, Liu H, Wickrema A , Tuan D. 2012. NF-Y recruits both

1488   transcription activator and repressor to modulate tissue- and

1489   developmental stage-specific expression of human γ-globin gene. *PLoS*

1490   *ONE* **7**:e47175. 10.1371/journal.pone.0047175

1491   Ziegler J, Qwegwer J, Schubert M, Erickson JL, Schattat M, Bürstenbinder K,

1492   Grubb CD , Abel S. 2014. Simultaneous analysis of apolar

1493   phytohormones and 1-aminocyclopropan-1-carboxylic acid by high

1494   performance liquid chromatography/electrospray negative ion tandem

1495   mass spectrometry via 9-fluorenylmethoxycarbonyl chloride

1496   derivatization. *Journal of Chromatography A* **1362**:102-109.

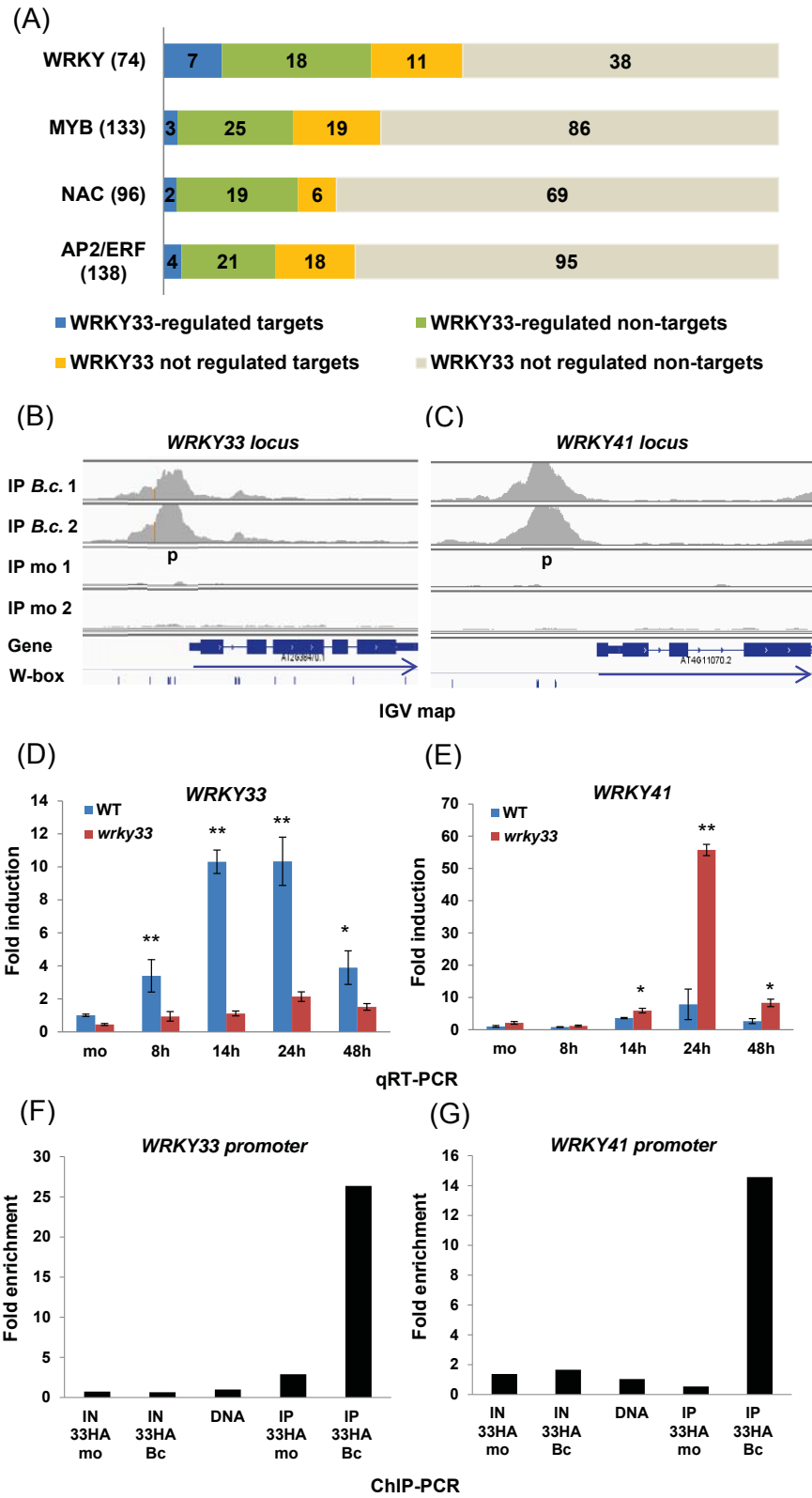1497   http://dx.doi.org/10.1016/j.chroma.2014.08.029

1498

1499

(A)

mock | *B. cinerea* 2100
14h | 14 h | 24h | 48h

α-HA

Ponceau S

(C)

Frequency

Distance to TSS

(B)

Fraction per Annotation type(%)

■ ChIP peaks
□ At genome

3UTR TTS Exon Intron Interg Promo 5UTR

(D)

bits

TTGAC T/C

P=5.5e-76

bits

T/G TTGAAT

P=4.9e-7

Figure 1

Figure 2

Figure 3

(A)

NCED3 locus

IP *B.c.* 1
IP *B.c.* 2
IP mo 1
IP mo 2
Gene
W-box

p1   p2

IGV map

(B)

NCED3

WT
wrky33

Fold induction

mo   8h   14h   24h   48h

qRT-PCR

(C)

NCED3 promoter

NCED3 ChIP p1
NCED3 ChIP p2

Fold enrichment

IN 33HA mo   IN 33HA Bc   DNA   IP 33HA mo   IP 33HA Bc

ChIP-qPCR

(D)

NCED5 locus

IP *B.c.* 1
IP *B.c.* 2
IP mo 1
IP mo 2
Gene
W-box

p1

IGV map

(E)

NCED5

WT
wrky33

Fold induction

mo   8h   14h   24h   48h

qRT-PCR

(F)

NCED5 3'UTR region

Fold enrichment

IN 33HA mo   IN 33HA Bc   DNA   IP 33HA mo   IP 33HA Bc

ChIP-qPCR

(G)

CYP707A3 locus

IP *B.c.* 1
IP *B.c.* 2
IP mo 1
IP mo 2
Gene
W-box

p2   p1

IGV map

(H)

CYP707A3

WT
wrky33

Fold induction

mo   8h   14h   24h   48h

qRT-PCR

(I)

CYP707A3 promoter

CYP707A3 ChIP p1
CYP707A3 ChIP p2

Fold enrichment

IN 33HA mo   IN 33HA Bc   DNA   IP 33HA mo   IP 33HA Bc

ChIP-qPCR

1

Figure 4

(A)

(B)

WT   wrky33   nced3

nced5   nced3 nced5   wrky33 nced3 nced5

3 dpi

(C)

fungal biomass 3dpi

$BcCutA/AtSKII$

WT   wrky33   nced3   nced5   n3n5   w33n3n5

(D)

3 dpi

wrky33 nced3 nced5
B.c. 2100 with ABA (10µM)

Figure 5

Figure 6

Figure 7

Figure 8