Title:

CREB-binding protein gene, *HAC701*, negatively regulates WRKY45-dependent immunity in rice

Running title:

HAC701-dependent immunity regulation

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SIGNIFICANCE

HAC701 is a member of CREB-binding protein (CBP) family that acts as transcriptional coactivator and acetyltransferase. However, little is known how it regulates innate immunity in plants. Herein we reported that rice *HAC701* suppresses WRKY45-dependent defense pathway. Our study showed that *HAC701* seemingly interacts genetically with *WRKY45* in rice to modulate immune responses against pathogens.

ABSTRACT

CREB-binding protein (CBP) is a known transcriptional coactivator and an acetyltransferase that functions in several cellular processes by regulating gene expression. However, how it functions in plant immunity remains unexplored. By characterizing *hac701*, we demonstrate that *HAC701* negatively regulates the immune responses in rice. *hac701* shows enhanced disease resistance against a bacterial pathogen, *Pseudomonas syringae* pv. *oryzae* (*Pso*), which causes bacterial halo blight of rice. Our transcriptomic analysis revealed that rice *WRKY45*, one of the main regulators of rice immunity, is upregulated in *hac701* and possibly conferring the resistance phenotype against *Pso*. The morphological phenotypes of *hac701* single mutants were highly similar to *WRKY45* overexpression transgenic lines reported in previous studies. In addition, we also compared the list of genes in these studies when *WRKY45* is overexpressed and chemically induced transiently with the differentially expressed genes (DEGs) in *hac701*, and found that they largely overlap. When we investigated for *cis*-elements found 1kb upstream of *WRKY45* gene and WRKY45-dependent DEGs, we found that *WRKY45* promoter contains the CRE motif, a possible target of HAC701-mediated regulation. Genome-wide H3K9 acetylation profiling showed depletion of acetylation at large set of genes in *hac701*. However, consistent with the upregulation of *WRKY45* gene expression, our ChIP-sequencing analysis demonstrated that regions of *WRKY45* promoter are enriched in H3K9 acetylation in *hac701* compared to the segregated wild type control in the mock condition. *WRKY45* promoter might be on the receiving end for possible genome-wide compensatory effects when a global regulator like *HAC701* is mutated. Finally, we show that *HAC701* may have roles in systemic immune signaling. We therefore propose that wild type *HAC701* negatively regulates *WRKY45* gene expression, thereby suppressing immune responses.

Keywords:

Oryza sativa, rice CREB-binding protein, *HAC701*, *WRKY45*, innate immunity, *Pseudomonas syringae* pv. *oryzae* (*Pso*), *Magnaporthe*

87 **INTRODUCTION**

88 Histone acetyltransferases (HATs) are a diverse group of histone- and non-histone-modifying
89 enzymes that contain multiple subunits to enable catalytic functions (1). These HATs primarily
90 function as scaffolding bridges of proteins to form the transcriptional regulatory complex
91 important for target recognition and subsequent substrate modification (1-3). The association
92 of HATs to its interacting proteins and the existing state of the epigenome dictates the dual-
93 switch functionality of either transcriptional activation or repression (1). They are considered
94 integrators or adaptors as they were shown to interact with DNA-binding activators and the
95 basal transcriptional machinery (4, 5).

96 In plant model Arabidopsis, HATs and histone deacetylases (HDACs) are grouped into
97 four (Figure 1A) and three families, respectively (6). HATs consists of CBP, TAF1/TAFII250,
98 GNAT, and MYST, while HDACs are RPD3/HDA1, HD2-like, and SIR2. A previous review
99 of HATs and HDACs in plants listed about 12 putative HAT and 18 HDAC proteins exhibiting
100 acetylation and deacetylation activities with differential site specificities (6). Work on rice
101 (*Oryza sativa*), however, has just begun with a preliminary investigation of eight histone
102 acetyltransferases (7, 8) and deacetylases (9).

103 Several studies have shown that histone acetylation and deacetylation control defense
104 signaling in plants in response to phytohormone or pathogen application (10-12). More
105 specifically, HATs and HDACs in rice model were shown to be responsive to abiotic stresses
106 and can be modulated by phytohormones, thus implicating a role in biotic stresses (7, 13).
107 Similar to Arabidopsis, HATs in rice are also classified into four families (Figure 1A) (7). In
108 contrary, rice HDACs are represented in only two families, RPD3/HDA1-like and SIR2-like
109 with no known rice member belonging to HD2 family (9).

110 *OsHAC701* was reported (14) earlier as a putative rice CBP-related acetyltransferase,
111 herein after referred to as *HAC701*, of the p300/CBP acetyltransferase (PCAT) family of
112 proteins. The Arabidopsis genome contains five CBP genes, while rice genome contains three
113 (Figure 1A), having broad acetyltransferase specificity on histones (7). Currently, there is no
114 clear consensus on the similarity of biological functions of Arabidopsis and rice homologs of
115 CBP family proteins. In animals, p300 and CBP are paralogs and originally described as
116 transcriptional coactivator that exhibit histone acetyltransferase activity on all four core
117 histones specifically at H4 N-terminal tail sites K5, K8, K12, and K16 (15, 16). Other sites
118 include H3 sites K14, 23 (17), K18, K27 (18, 19), and K56 (20); H2B sites are K12 and K15
119 (21). H3K9 is mainly acetylated by GCN5/PCAF (18), however acetylation by p300/CBP was
120 also reported (17, 22, 23). Its highly conserved function is mainly found in multicellular
121 organisms as it probably participates in complex physiological processes acting as a limiting
122 factor in various pathways due to its high cellular demand (14).

123 As HATs have been shown to be modulated by biotic stresses (24), we hypothesized
124 that it could be a potential system for studying rice immunity. Indeed, we show that *HAC701*
125 is involved in rice innate immunity. In this study, we have made CRISPR/Cas9 edited *hac701*
126 lines and showed that *HAC701* is apparently a negative regulator of immunity with the mutants
127 showing resistance against bacterial and fungal infection. Further transcriptomic investigation
128 showed that HAC701 possibly regulates *WRKY45* loci, one of the main regulators of rice
129 immunity pathways. In *hac701*, *WRKY45* and its direct and indirect gene targets were
130 upregulated upon pathogen inoculation. In addition, *hac701* phenocopies *WRKY45*-
131 overexpression (*WRKY45-ox*) lines, indicating a potential interaction between *HAC701* and
132 *WRKY45*-dependent immunity pathway in rice.

133

134 **RESULTS**

135 **Characterization of a rice acetyltransferase mutant, *HAC701*.**

136 We isolated null mutant lines by independently targeting two sites of *HAC701* gene (Fig. S1A-
137 C; Fig. S1D-I) using CRISPR/Cas9 editing technology. From the 11 positive *HAC701* lines
138 (Target 2), we have isolated four biallelic homozygous mutant lines (Fig. S1D), which we
139 utilized for experimentation including their segregating wild type siblings, 9-WT (Fig. 1B).
140 The phenotype in terms of effective grain number and tiller number of mostly all biallelic
141 homozygous mutants at T2 generation did not show impairment as compared to 9-WT (Fig.
142 S1F). In addition, the T3 lines did not show any visible growth or developmental defect in
143 height and photosynthesis (*e.g.* chlorosis phenotype) (Fig. S1G), respectively. However, the
144 weights of 1000-grain T3 and T4 generations of mutants were increased compared to 9-WT
145 (Fig. S1I). These results indicate that a loss of HAC701 does not cause severe developmental
146 defects in rice, and that HAC701 may have a positive role in seed production.

147 ***HAC701* mutant rice is resistant to pathogen infection.**

148 Our expression analysis of the rice HAT genes showed that *HAC701* is significantly up-
149 regulated by treatment of bacterial flg22 peptide, suggesting a potential role of HAC701 in
150 plant defense against pathogens (Fig. S2). To investigate the involvement of *HAC701* in basal
151 defense in rice, we inoculated 9-WT and *HAC701* mutant lines, *9-5*$^{-/-}$, *9-12a*$^{-/-}$, and *9-12b*$^{-/-}$ with
152 mock (10 mM $MgCl_2$) and *Pseudomonas syringae* pv. *oryzae* (*Pso*) (OD = 0.2; resuspended in
153 10 mM $MgCl_2$) for 72 h. *Pso* is a causative agent of halo blight in rice characterized by brown
154 lesions and yellow halo-like blotches on leaves (25). Infection of *Pso* showed that *hac701* was
155 resistant to *Pso*-treatment as compared to 9-WT (Fig. 1C; Fig. S3). These data suggest that
156 *HAC701* might be involved in the regulation of rice innate immunity acting in its capacity as a
157 transcriptional coactivator and/or as an acetyltransferase. We further tested whether *hac701*

158 also shows resistance phenotype when infected with a rice blast pathogen, *Magnaporthe*
159 *oryzae*. Although the data were not statistically significant, results showed a resistant
160 phenotype tendency in *hac701* (Fig. 1D). Overall, these indicate that *HAC701* plays a negative
161 role in rice innate immunity.

162 **Transcriptome profiling of the rice-*Pso* pathosystem.**

163 To profile the genome-wide effect of *HAC701* mutation in rice innate immunity, we performed
164 RNA-sequencing on mock- and *Pso*-treated 9-WT and *hac701* using local leaf tissues (Fig. S4;
165 Table S2). We first characterized the effect of *Pso* infection on 9-WT and analysis of the highly
166 variable genes in mock- and *Pso*-treated 9-WT plants showed gene clusters that were
167 dependent on *Pso* induction alone (Fig. S4; Fig. S5). The top 10 highly variable genes across
168 the 9-WT samples showed features involved in tolerance and/or resistance such as disease
169 resistance and stress tolerance mostly by catalysis of primary and secondary metabolism (Fig.
170 S5). This includes well-documented genes involved in plant resistance against pathogens, such
171 as tryptophan decarboxylase 1 (Os08g0140300) that catalyzes the production of serotonin via
172 conversion of tryptophan to tryptamine in rice (26), which has been implicated to confer
173 resistance in rice infected with *Bipolaris oryzae* (27). Another gene, naringenin 7-O-
174 methyltransferase (Os12g0240900), that catalyzes the production of rice phytoalexin
175 sakuranetin has been reported to participate in rice defenses via JA signaling (28, 29). Lignin
176 and phytoalexin encoding genes for instance laccases (*e.g.* Os12g0258700, Os11g0641500)
177 and cytochrome P450s (*e.g.* Os07g0218700, Os08g0508000) are metabolic products known to
178 be involved in plant defenses as well (30-32). Gene Ontology (GO) analysis of upregulated
179 genes in *Pso*-infected 9-WT plants revealed enrichment of genes involved in response to biotic
180 stimulus (GO terms: Response to wounding, Response to other organism, Cell wall
181 macromolecule catabolic process, *etc.*) (Fig. S6; Table S3). Both up and downregulated genes
182 showed enrichment of transcriptional machinery regulation, a possible consequence of

183 defense-related transcriptional reprogramming processes during bacterial invasion. GO
184 analysis also identified pathways associated with upregulated gene networks involved in plant
185 defense such as diterpene phytoalexin biosynthetic pathway and chorismate biosynthesis.
186 Thus, the rice-*Pso* pathosystem is a functional system to analyze defense responses of rice
187 against *Pso* infection.

188 **Potentiated *WRKY45* gene in *hac701* provides resistance against *Pseudomonas* and**
189 ***Magnaporthe* infections.**

190 To investigate the molecular basis of resistance phenotype observed in *Pso*-infected *hac701*,
191 we further analyzed our transcriptome data and found 141 upregulated genes in *hac701* (Fig.
192 S7A; Table S4). We refer to these genes as *HAC701*-repressed genes. GO analysis of *HAC701*-
193 repressed genes showed enrichment of processes involved in response to xenobiotic stimuli
194 and defense response (GO terms: Response to xenobiotic stimulus, Response to wounding,
195 Regulation of defense response, Jasmonic acid mediated signaling pathway, Defense response,
196 *etc.*). These genes were lowly expressed in the 9-WT background. On the other hand, 336
197 upregulated genes in the 9-WT background alone referred to as *HAC701*-independent genes
198 did not show a very distinct enrichment of genes in defense responses yet rather showed a more
199 general biological and physiological processes (GO terms: Mitochondrial respiratory chain
200 complex IV assembly, Alanine transport, Cellular response to heat, *etc.*) (Fig. S7A; Table S3).
201 We also looked into the features of 31 downregulated genes in the mutant background referred
202 to as *HAC701*-enhanced genes (Fig. S7B; Table S4). These genes were not normally
203 downregulated in the 9-WT background. *HAC701*-enhanced genes contained genes enriched
204 in photosynthetic and response to abiotic stress terms (GO terms: Glycerolipid biosynthetic
205 pathway, Response to cold, Photosynthesis, *etc.*). In addition, the remaining 46 downregulated
206 genes in the 9-WT background alone also showed a more general biological processes with no

207 hint of enrichment in defense response against pathogen infection (GO terms: Chloroplast
208 organization, Gluconeogenesis, Organelle fission, *etc.*) (Fig. S7B; Table S3).

209 We were intrigued what genes were affected in *HAC701* mutant background without
210 *Pso*-treatment. Thus, we analyzed the transcriptome of mock-treated *hac701*, and found a small
211 number of DEGs (98 genes; Table S5) mainly enriched in NADH dehydrogenase complex
212 assembly and photosynthesis. This is in contrast with the number of DEGs found in *Pso*-treated
213 *hac701* transcriptome, which yielded 660 genes (Table S6). These results simply indicate that
214 *Pso* induced the expression of substantial number of genes in *hac701*. To fully understand the
215 role of *HAC701* in *Pso*-treated conditions, we overlapped the list of genes derived from mock
216 and *Pso*-treated mutants, and found *WRKY45* (*OsWRKY45*) upregulated in the *hac701*
217 background in mock-treated *hac701* (Fig. 2A). *WRKY45* was also identified as one of the 141
218 upregulated genes in *Pso*-infected *HAC701* mutant (Fig. S7A). There was no candidate gene
219 found downregulated in *HAC701* mutant background alone (Fig. 2A). We then checked
220 whether there were differences in the upregulation strength of *WRKY45* in the *HAC701*
221 mutation alone, compared to *HAC701* mutation combined with *Pso*-treatment. Transcriptome
222 data revealed that *WRKY45* expression was increased by about 20% upon *Pso*-treatment (Fig.
223 2B). These results suggest that *WRKY45* expression is partially dependent on *HAC701*
224 mutation. In addition, *Pso*-treatment allowed the co-expression of genes downstream of
225 *WRKY45* pathway in a HAC701-dependent manner.

226 ***hac701* phenocopy *WRKY45* overexpression transgenic plants.**

227 To determine whether the upregulation of *WRKY45* in *hac701* is relevant to rice with
228 overexpressed *WRKY45*, we compared the DEGs in our *hac701* with *WRKY45* overexpression
229 (*WRKY45-ox*) lines previously reported (in these studies: 33, 34). Our results indicated a
230 striking similarity of targeted genes from rice samples despite the fact that they were inoculated

231 with two different types of pathogens, a bacterial (*Pso*; this project) and the fungal pathogen
232 *Magnaporthe* (33, 34) and minor differences in sampling time periods (Fig. 3). In addition, we
233 then analyzed whether benzothiadiazole-responsive genes (Fig. 3A; Table S6; Table S7)
234 would overlap with *HAC701* mutant-DEGs in *Pso*. It is known that benzothiadiazole (*i.e.*
235 BTH is a synthetic analog of salicylic acid, SA) robustly induces defense genes in rice (34, 35),
236 which usually contains high levels of SA. As a result, about 43.3% (286/660 genes; P<3.336e-
237 161) of *HAC701* mutant-DEGs in *Pso* overlapped with BTH-responsive genes, while about
238 12.4% (82/660 genes; P<2.368e-79) of the *HAC701* mutant-DEGs in *Pso* overlapped with
239 BTH- and WRKY45-regulated genes (Fig. 3B; Table S8). Furthermore, genes upregulated in
240 plants expressing DEX-induced myc-tagged WRKY45 protein in rice were mostly found in
241 *HAC701* mutant-DEGs in *Pso* (75% or 9/12 genes; Table S9). Our analysis also showed that
242 at most 9.1 % (60/660 genes; P<3.986e-15) of the *HAC701* mutant-DEGs in *Pso* were
243 regulated in BTH- and NPR1/NH1-dependent manner (Fig. 3B; Table S10). The rice
244 NPR1/NH1 is an independent immune signaling pathway that mediates gene responses through
245 BTH/SA-induction (Fig. S18). We then analyzed the 72 *HAC701* mutant-DEGs in *Pso* that
246 were exclusively regulated by BTH- and WRKY45 as well as the other 10 genes jointly
247 regulated by WRKY45 and NPR1/NH1 under BTH treatment (Fig. 3B-D). We found that most
248 of these *HAC701* mutant genes were genuine WRKY45-regulated genes (Fig. 3C-D) (33, 34).
249 Analysis of these WRKY45-dependent defense-related genes showed that in the presence of
250 *Pso*-infection, a number of glutathione-S-transferase (GST) gene, were more upregulated in 9-
251 WT than in *hac701* (Fig. 3D). These results were also consistent with the result that
252 *OsWRKY62*, a known negative regulator and direct target of *OsWRKY45* (34), had lower
253 transcription level in *HAC701* mutants (Fig. 3D). It is interesting that genes in JA biosynthesis,
254 signaling and perception tend to be more highly expressed in *HAC701* mutant than 9-WT (Fig.
255 3D). In addition, *HAC701* mutant genes that were found regulated in BTH and *WRKY45* indeed

256 contain W-box motifs (i.e. binding motifs for WRKY transcription factors, 36), raising more
257 the possibility that these genes are direct WRKY45 targets (72/82 or 87.8%; Table S11).
258 Meanwhile, all 10 genes that were commonly regulated in BTH, *WRKY45*, and *NPR1/NH1*
259 (Fig. 3) contain W-box motifs as well (10/10 or 100%; Table S12). Taken together, these results
260 indicate that upregulation of *WRKY45* expression as an outcome of *HAC701* mutation resulted
261 in expression profile of genes almost identical to overexpressing *WRKY45* transgenic lines as
262 well as to DEX-inducible *WRKY45* expression system. These also suggest that a significant
263 fraction of *HAC701* mutant DEGs are probably WRKY45-regulated, given the presence of W-
264 box motif.

265 Surprisingly, the upregulation of *WRKY45* expression in *hac701* did not result in any
266 visible morphological defects (Fig. S1G). This indicates that *WRKY45* does not pose growth
267 penalties, at least in this case where there is no pathogen applied.

268 ***WRKY45* promoter contains CRE motif.**

269 Rice HAC701 and two others, HAC703 and HAC704, belong to the CBP [Cyclic adenosine
270 monophosphate response element-binding protein (CREB) Binding Protein] family group of
271 proteins that is known to bind CREB transcription factor through its KIX domain (7, 37).
272 Therefore, we examined *in silico* the members of rice CBP family for the presence of KIX
273 domains using a KIX domain database (38). Our results showed that only HAC701 and
274 HAC703 proteins contain the highly conserved KIX domain (Fig. S8). Although we have not
275 identified CREB-like transcription factor candidates in rice so far, we further examined the
276 DNA motifs in the 1kb promoter regions of the 660 *HAC701* mutant-DEGs in *Pso* that might
277 potentially bind to KIX-CREB-like transcription factor. Our DNA motif analyses result
278 showed 49 significantly enriched motifs in promoters (Table S13; E-value <0.05). We then
279 used these motifs to compare against a database of known motifs and found a CRE motif when

280 the query sequence CGRCGRCG (E-value<1.5e-27) was used (Fig. S9A). The CRE motif is
281 a well-known sequence motif in animals usually bound by KIX-CREB transcription factor
282 complex (39). We then searched for genes that contain the CRE motif and found that only five
283 genes (0.76%) contain full CRE motif (Fig. S9B; Table S6). To our surprise, *WRKY45*
284 promoter contains the full CRE motif that could possibly be targeted via KIX domain and
285 CREB-like transcription factor-dependent regulation in rice system (Fig. S9C). These data
286 show that *WRKY45* promoter could possibly be *cis*-regulated by HAC701 through its KIX
287 domain, while the occurrence of CRE motif is not necessarily associated with other HAC701-
288 regulated genes.

289 **Genome-wide investigation of histone modifications and enriched gene targets in *HAC701***
290 **mutant.**

291 The CREB-binding protein (CBP) is also known to regulate gene expression by acetylating
292 histone tails in both animals and plants (40). Thus, we performed chromatin
293 immunoprecipitation coupled to sequencing (ChIP-seq) analysis on histone markers associated
294 with active and repressive chromatin to examine the landscape of chromatin modifications in
295 *HAC701* mutant. We performed ChIP-seq analyses of H3K9 acetylation (H3K9ac), H3K27
296 acetylation (H3K27ac), H3K9 di-methylation (H3K9me2), and H3K9 tri-methylation
297 (H3K9me3) on untreated 9-WT and *9-12b*$^{-/-}$ mutant (Fig. S10; Table S14). Metaplot profiles of
298 these histone modifications were generated for genes, transposable elements (TEs), and simple
299 repeats (Fig. S11; Fig. S12). The enrichment profiles show that both H3K9ac and H3K27ac
300 accumulated in genes mainly along the transcriptional start site (TSS) and tapering through the
301 gene body regions. In contrast, we found that H3K9me2 and H3K9me3 were highly depleted
302 in the gene TSS region (Fig. S11A). As expected, H3K9 and H3K27 acetylation marks were
303 depleted in TEs, while H3K9 di- and tri-methylation marks showed enrichment (Fig. S11B).
304 In addition, enrichment analysis of simple repeats showed similar profiles with TEs in both

305 acetylation and methylation histone marks, although simple repeats tend to be highly enriched
306 in H3K9me2 at a narrow set of genomic loci (Fig. S12). These results showed that our ChIP-
307 seq profiles of histone modifications were largely consistent with the previous report on rice
308 ChIP-seq analysis (41).

309 The observed low enrichment of H3K9ac in *9-12b*$^{-/-}$ mutant is a probable indication of
310 mutation on *HAC701* gene (Fig. S11A). Therefore, we further analyzed the significantly
311 enriched or depleted regions in *9-12b*$^{-/-}$ by taking the enrichment ratio of 9-WT over *9-12b*$^{-/-}$
312 (wild type/*hac701*) in H3K9ac, H3K27ac, H3K9me2, and H3K9me3 modifications. These
313 analyses yielded 265 peaks in the H3K9ac fraction (Fig. 4A; Table S14). From the metaplot
314 analysis, it showed that *9-12b*$^{-/-}$ has depleted H3K9ac enrichment in the 265 regions identified
315 containing putatively 263 genes (Fig. 4A; Table S15), which include *HAC704* and *HAC701*
316 loci and a mediator gene, *MED11* (Fig. 4D; Fig. S13A). These 265 regions were not associated
317 with changes in other modifications in *9-12b*$^{-/-}$ mutant when compared to 9-WT (Fig. 4B).
318 These results suggest that HAC701 acetyltransferase may have a specificity to H3K9 site. It
319 also suggests that HAC701 probably autoacetylates its own promoter including another
320 member of CBP family, *HAC704*.

321 We then analyzed the GO enrichment features of the putative 263 genes found in these
322 265 regions with H3K9ac changes (Fig. 4C; Table S15). The results indicate that these genes
323 function in processes involved in the flow of genetic information, DNA methylation in the
324 CHH-context, circadian rhythm, organellar transport, cell cycle and reproduction,
325 ubiquitination, and in a number of biosynthetic processes. It is also perhaps not surprising that
326 histone acetylation was also enriched. In addition, enrichment of genes involving
327 environmental responses included heat stress and innate immunity were observed, in which
328 about 9% (23/263) were possibly involved in defense responses. A large fraction of these
329 defense genes were identified as candidate sensors or decoys (e.g. Cyclin A1, Thioredoxin,

330 Jacalin-like lectin, *etc.*) that may act as negative regulators of rice effector-triggered immunity
331 (ETI-immunity) (42) (Fig. 4E; Fig. S13B). The distribution of these 265 regions in H3K9ac
332 ChIP revealed that a large portion were found in genes (73.58%) followed by promoters
333 (18.11%) (Fig. S14). It is also interesting to note that H3K9ac in these identified regions was
334 found in transposable elements (7.55%). Overall, these results demonstrate that HAC701-
335 dependent acetylation of H3K9 histone site might modulate transcription of decoy genes that
336 are monitored by R proteins in rice immunity. Additionally, H3K9ac assessed in different
337 regions of the rice genome showed that it is found mostly in genes and promoters with high
338 similarity in the previous study (41).

339 We proceeded by analyzing the general distribution of H3K9ac, H3K27ac, H3K9me2,
340 and H3K9me3 peaks in intergenic and intragenic regions of rice genome in 9-WT and *9-12b*$^{-/-}$
341 mutant (Fig. 5A). A large proportion of H3K9ac and H3K27ac were concentrated on exonic
342 and intronic regions of the gene, while *HAC701* mutation resulted in the increase of enrichment
343 of H3K27ac genome-wide on all regions of the rice genome (Fig. 5A). It is also worth
344 mentioning that loss of *HAC701* increased the level of enrichment of these methylation
345 modifications in intergenic regions. These data suggest that the loss of *HAC701* leads to an
346 increase of a counter acetylation modification, H3K27ac, on a genome-wide scale.

347 To explain the disease resistance phenotype of *hac701* through WRK45-dependent
348 immune pathway, we investigated the genomic locus of rice *WRKY45* (Fig. 5B). The
349 upregulation of WRKY45 was accompanied by H3K9ac enrichment along the 1kb upstream
350 region of *WRKY45* gene in *9-12b*$^{-/-}$ (Fig. 5B, C). As the CRE motif was specifically enriched
351 in the promoter region of *WRKY45* (Fig. S9C), we compared the enrichment of H3K9ac and
352 H3K27ac in *9-12b*$^{-/-}$ *WRKY45* promoter. We found that H3K9ac, but not H3K27ac, was highly
353 enriched in this regulatory region (Fig. S15), while H3K27ac of *WRKY45* gene body was less
354 pronounced than H3K9ac. Overall, our ChIP-seq data are consistent with RNA-seq data in

355 showing that mutation in *HAC701* gene potentiated *WRKY45* locus by regulating the
356 enrichments of histone acetylation and methylation modifications on *WRKY45* promoter. We
357 also found that the CRE motif of *WRKY45* is modulated by H3K9ac and perhaps other
358 modifications, including those that are analyzed here.

359 **Systemic gene expression in rice-*Pso* pathosystem.** To explore the nature of systemic gene
360 expression in rice-*Pso* pathosystem, we performed RNA-sequencing on systemic or distal
361 tissues of 9-WT and *hac701*. The tissue samples were collected together with infected samples
362 (local samples) 72 hours after *Pso* infection without further bacterial treatment. Genome-wide
363 transcriptome analysis in systemic 9-WT samples showed that 24 genes were differentially
364 expressed, and among them were eight genes that were common to both local and systemic
365 tissues (Fig. S16A; Table S16). Comparative analysis also showed that although there were
366 substantial expression changes in systemic tissues, the number of DEGs was highly reduced in
367 systemic tissues than in local tissues. Among the eight DEGs, five of them showed altered
368 transcriptional expression from local to systemic tissues (Fig. S16B). These genes were
369 mannose-specific jacalin-related lectin/*OsJAC1* (Os12g0247700) (43), putative cytochrome
370 P450 (Os09g0275400) (44), ATPase (Os07g0187400), GDP-L-galactose phosphorylase
371 (Os12g0190000) (45), and terpene synthase (Os04g0344100), some with putative functions in
372 response to pathogen infection or stress. These results indicate that these genes were mostly
373 activated locally in the infection site and that any altered expression in distal tissues may
374 facilitate systemic form of resistance. To compare the effect of *HAC701* mutation on the
375 number of DEGs, we analyzed the MA-plots of systemic tissues of 9-WT and *hac701*. Our
376 results showed that the number of DEGs with significant expression (*i.e.* those that are in red)
377 was diminished in the mutant background implicating a possible role of *HAC701* in systemic
378 signaling in rice-*Pso* pathosystem (Fig. S17). Overall, these results indicate that systemic
379 tissues in *Pso*-challenged 9-WT plants have augmented transcriptional gene expression that

380 possibly aims to potentiate distal tissues on the onset of secondary pathogen attack. This also
381 indicates that *HAC701* might potentially regulate systemic defenses through an unknown
382 mechanism at distal non-infected site in preparation for future infection episodes.

383

384 **DISCUSSION**

385 Histone acetyltransferases or HATs and their complexes are involved in various biological
386 processes in the cell. Currently, studies have been focused on the roles of HATs specifically
387 members of the CREB-binding protein (CBP) family in regulating substrate specificity and
388 enzymatic activity such as acetylation (40). However, its involvement in molecular signaling
389 pathway leading to modulation of plant immunity remains to be explored. Therefore, it is
390 expected that the analysis of plant CBP will facilitate a broader understanding of regulation of
391 gene expression and protein function in plant immunity pathways.

392 In this study, we generated CRISPR/Cas9 mutants of the rice *HAC701* gene, a member
393 of the CBP family of HATs, since our results indicated its involvement in rice immunity. By
394 inoculating a rice compatible bacterial pathogen, *Pseudomonas syringae* pv. *oryzae* (*Pso*), we
395 showed that *hac701* are resistant to *Pso* proliferation and also showed resistance tendency
396 when infected with *Magnaporthe* (rice blast). These indicates that *HAC701* negatively
397 regulates rice immunity responses to *Pso* and *Magnaporthe* pathogens. When we analyzed our
398 transcriptome data, it showed that this negative regulation of immunity by *HAC701* is
399 attributable to suppression of a major immunity pathway in rice probably through WRKY45.
400 In *hac701*, *WRKY45* gene expression is upregulated and it seemed to be in a potentiated state
401 enabling a further increase of expression upon bacterial infection. In addition, both
402 morphological phenotypes and target genes in *hac701* mimic those in *WRKY45-ox* transgenic
403 lines possibly indicating a genetic interaction of *HAC701* and *WRKY45* genes. Lastly, we
404 showed in our ChIP-seq analysis that the promoter of *WRKY45* in *hac701* is enriched in

405 H3K9ac coinciding with a *cis* regulatory CRE motif that might be responsible for *WRKY45*
406 upregulation and control.

407 In Arabidopsis, the p300/CBP acetyltransferase gene, *HAC1*, has been shown to
408 positively regulate defense priming only in repetitively abiotic-stressed plants (46). *hac1-1* did
409 not show any immunity related responses prior to abiotic stress applications indicating that
410 Arabidopsis *HAC1* requires several abiotic stress stimulations to activate its immunity.
411 However, this is not the case in *HAC701* in rice. We showed that the rice *HAC701* did not need
412 abiotic stress stimulation to be transcribed and to effect pathogen and disease specific
413 phenotypes (Fig. 1; Fig. S2; Fig. S3). Rather, our results are consistent with the disease
414 phenotype exhibited by Arabidopsis mutant of GCN5, an acetyltransferase member of the
415 SAGA transcriptional coactivator complex catalyzing the H3K14 modification site and at the
416 same time influencing the acetylation of H3K9 (47). The *gcn5* showed upregulation of SA-
417 mediated immunity resulting in resistance against *Pseudomonas syringae* pv. *tomato* infection.
418 In our study, we found that WRKY45-dependent pathway confers resistance to *hac701*.
419 Consistent with the view that the rice hormone defense network does not support a
420 dichotomous role of salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) phytohormones in
421 regulating rice immunity (48), we found no evidence in this study that SA-mediated immunity
422 played a central role in *hac701* resistance phenotype. However, we found JA-related
423 differential gene expression as components of WRKY45-dependent defense-related genes
424 (Fig. 3D). Indeed, JA biosynthesis is known as a downstream target of rice WRKY45 (49). In
425 rice immunity model, both SA and JA/ET are considered effective in defense responses against
426 (hemi) biotrophic and necrotrophic pathogens (48, 50, 51). While SA-mediated *AtNPR1*
427 regulates majority, if not all, of Arabidopsis immune responses (52), *OsWRKY45* and
428 *OsNPR1/NH1* are mostly independent of each other (34; Fig. S18). Although, our results
429 suggest that *hac701* DEGs partially overlapped with NPR1/NH1-dependent pathways (Fig.

430 3B). Given that the rice system has a constitutive high levels of SA compared to most plants
431 including Arabidopsis and tobacco, and pathogen applications did not increase its basal SA
432 level (53, 54), our results are consistent with this report that SA in rice may not directly act on
433 pathogens, rather, it may function to potentiate endogenous defense pathways in which
434 *WRKY45* plays the central role. *OsWRKY45-ox* showed strong resistance against blast
435 pathogen, even though lacking the constitutive expression of defense genes prior to blast
436 infection (34). It could be that disruption of HAC701-dependent pathways through *HAC701*
437 mutation led to activation of WRKY45-dependent pathways in an already SA-potentiated rice
438 network, although this needs to be further examined in detail. Our results are also consistent
439 with the disease phenotype presented by non-sense mutation in *Med15* gene, a subunit of
440 Mediator complex (55). The *med15b.D* wheat mutants were resistant to stem rust, and the
441 authors attribute a part of this to segmental coregulation in which a certain portion of the
442 chromosome containing R genes (i.e. specifically NLR) was differentially expressed in wild
443 type compared to mutant. While we did not observe any form of segmental coregulation in our
444 analysis of *hac701*, we found that mutations in *HAC701* resulted in downregulation of ETI
445 components notably the decoys/sensors (Fig. 4E).

446 *OsHAC701*, *AtHAC1*, *AtGCN5*, and *TaMed15*, play a central role in regulating the
447 transcriptional machinery. They likely do this regulation by associating themselves and their
448 complexes to RNA polymerase II and transcription factors as shown by their homologous
449 counterparts in animals (4, 14, 56, 57). This is possible because they are multidomain genes
450 that have the capacity to form and maintain large complexes. Os*HAC701*, *AtHAC1*, and
451 *AtGCN5* are also involved in regulating abiotic stress responses suggesting that the complexity
452 and specificity of their activities are dependent on their interacting factors that recruit them to
453 the biological pathway itself (6, 7). Furthermore, majority of these genes, *OsHAC701*, *AtHAC1*,

454 and *TaMed15*, encode the KIX domain, a docking site for transcription factors reported to be
455 involved in plant immunity (58).

456 In line with the above results, we propose that HAC701 is a negative regulator of
457 WRKY45-dependent defense pathway in rice (Fig. S18). We suggest here two possible
458 mechanistic insights into HAC701 regulation of WRKY45 that need further experimental
459 evidence. First, similar to Med15, the HAC701-mediated immunity may require a loss or
460 truncation of HAC701 in regulating the transcription of *WRK45* locus. Given that the promoter
461 of *WRKY45* contains a rare CRE motif (Fig. S9), we assume that an unidentified CREB-like
462 transcription factor with regulatory potential is interacting with HAC701 through its KIX
463 domain (Fig. S8) and with the CRE motif of *WRKY45* promoter. This interaction may also
464 include other protein interactors specific to *WRKY45* locus regulation. From our results,
465 mutation of *HAC701* is enough to potentiate *WRKY45* and further addition of *Pso* enables
466 *hac701* to mount higher *WRKY45* expression compared to 9-WT. These suggest to us that
467 *WRKY45* locus is poised for activation as suggested by our ChIP-seq analysis of untreated
468 *hac701* (Fig. 5). However, the H3K9 acetylation we observed in *WRKY45* promoter might not
469 be catalyzed by HAC701 itself in *hac701*, but may come from other CBP family members (Fig.
470 1A; Fig. S1H) or other HATs. Although, we do not have ChIP-seq data for pathogen inoculated
471 *hac701*, our RNA-seq data suggest that the poised *WRKY45* through *HAC701* mutation is
472 enough to activate downstream defense likely through *WRK45*-dependent signaling. Second,
473 the decoys/sensors component that we found may act as negative regulators as predicted by the
474 Decoy model (Fig. 4E) (42, 59). It could be that HAC701 maintains the acetylation of these
475 decoys/sensors to equilibrium. Any disturbance in their expression could possibly lead to
476 activation of R genes monitoring these decoys/sensors, which could in effect activate
477 WRKY45-dependent immunity. Indeed, we found seven R genes upregulated in *HAC701*
478 mutation treated with *Pso* (Table S17). It is of course possible that these two mechanisms are

479 occurring simultaneously in conferring resistance, although finer details of these proposed
480 mechanisms are still unknown.

481 As HAC701 is a global transcriptional regulator, we found that it regulates a myriad
482 number of biological processes underscoring its importance in genome and epigenome control
483 (Fig. 4C). Among the enriched GOs, we found histone acetylation and innate immune
484 responses were affected in *hac701*. These suggest to us the intricate network in HAC701-
485 dependent regulation of immunity through its scaffolding and catalytic activity in the cell. It is
486 possible that any misregulation in *hac701* could lead to genome-wide functional compensation
487 events by activating other proteins acting on the same pathway as HAC701. This compensation
488 functionality has been shown in a PHD-finger protein, Enhanced Downy Mildew 2 (EDM2),
489 in NLR (i.e. a type of R protein) protein expression control as it affects plant fitness (60).
490 Therefore, it is also likely that the expression regulation of *WRKY45* locus as well as the
491 decoys/sensors may be subjected to compensatory regulation in the case of *HAC701* mutation.

492 The heterochromatic marker, H3K9me3, is common in animals, but rarely found in
493 Arabidopsis and in plants in general (61). Indeed, our analysis did not find the peaks in 9-WT
494 ChIP-seq, although we found 33 peaks in the *HAC701* mutant (Table S14). Most H3K9me3
495 enrichments were found in intergenic regions and in only two genes with intragenic
496 enrichments. Table S18 also shows genes nearest the H3K9me3 enrichment in the intergenic
497 regions. These results suggest that H3K9me3 is maybe a rare modification and is possibly
498 modulated by HAC701 presence and activity in certain genomic regions.

499 Trade-off between growth and defense in plants is a common occurrence and often
500 considered inevitable (62, 63). However, we were surprised to find that *hac701* in the T2 up
501 until T4 generations do not have readily visible penalties in height, tiller number, number of
502 effective grains, and grain weight (Fig. S1D-I). Previous studies showed that *WRKY45-ox* had

503 no obvious autoimmunity phenotype, although there was a slight decrease in height observed
504 as compared to wild type in response to growth conditions (33, 34). In this study, WRKY45-
505 dependent resistance against *Pso* was observed in *hac701* (Fig. 1C, Fig. S3). Furthermore, these
506 *HAC701* mutants also showed resistant tendencies against *Magnaporthe* infection (Fig. 1D).
507 *WRKY45-ox* treated with *Magnaporthe* showed resistance, while a BTH-induction in
508 knockdown *WRKY45* mutants did not rescue the *Magnaporthe* resistance and remained
509 susceptible (34). Together these suggest that morphological and disease phenotypes of *hac701*
510 resembled that of *WRKY45-ox* indicating that *HAC701* mutation indeed upregulates *WRKY45*
511 gene that prompts WRKY45-dependent defense pathway into action leading to resistance
512 against bacterial and fungal pathogens. It could be that the potentiated state of *WRKY45* in
513 *hac701* has modulated the expression of resistance from affecting these agronomic traits.
514 Nonetheless, the mechanistic basis for this trade-off, if it truly exists, is not well understood
515 and needs further investigation.

516 We would like to emphasize that at the moment we do not have strong evidence that
517 HAC701 specifically catalyzes H3K9 acetylation. Although, the H3K9ac antibody used in this
518 study detected H3K9ac decrease of numerous loci in *hac701* compared to 9-WT that are not
519 present in H3K27ac, histone target-specificity of HAC701 still needs to be verified. As
520 H3K9ac and H3K14ac were seemed co-regulated and were directly correlated to each other in
521 regulating their common target genes (47), we assume that the H3K14ac in our *hac701* would
522 show the same correlation as well.

523 HAC701 appears to be an ancient protein in the CBP histone acetyltransferase family
524 clade (Fig. 1A). The fact that *HAC701* mutation is not lethal, suggests that other CBP members
525 and arguably members of other histone acetyltransferase families might have redundant
526 functions at some regulatory level. However, our results showing that *hac701* has enhanced
527 disease resistance phenotype and reduction in acetylation demonstrate certain functional

528 specificities through HAC701-dependent pathways. Also, the fact that *hac701* showed
529 reduction in expressed genes at the systemic level (Fig. S17), indicates possible broader roles
530 of HAC701 other than localized modulation of rice innate immunity.

531

532 **MATERIALS AND METHODS**

533 **Biological samples and plant growth conditions**. *Oryza sativa* ssp. *japonica* cv. Nipponbare
534 (wild type) plants and mutant lines were grown in commercial soil (Kumiai, JA Okinawa) at
535 30°C day/25°C night temperatures under a 12-h-light/12-h-dark photoperiod in an incubator
536 (BiOTRON, NK System). The lighting was supplied by white light at an intensity of 31,000
537 lx. Relative humidity was at 70%. For embryonic rescue, rice embryos were extracted from
538 wild-type immature seeds 10-14 days after flowering. Embryos were grown into plantlets in
539 Murashige and Skoog (MS) agar medium before being transferred to soil for further growth.

540 **Isolation and screening of *HAC701* mutant lines**. To generate *HAC701* CRISPR/Cas9
541 knockout mutant lines, two sgRNA *HAC701*-specific target sites were obtained from CRISPR-
542 P website and were used to synthesize primers for pRGEB31 (stable system) (64) (Table S1).
543 Briefly, the vectors were digested with BSA I, while primers were phosphorylated and
544 annealed to produce a DNA oligo duplex. The digested vectors were ligated to DNA oligo
545 duplex using T4 ligase. CRISPR/Cas9 vectors were introduced into *Agrobacterium* EHA105
546 and rice calli were transformed using the standard *Agrobacterium*-mediated transformation
547 procedure. For CRISPR/Cas9 T0 and T1 screening, leaf samples were collected and genomic
548 DNA was extracted using a standard CTAB protocol. Then, PCR-RFLP assay utilizing BseLI
549 restriction enzyme (Thermo Fisher Scientific) was used to detect the CRISPR/Cas9-engineered
550 mutations on *HAC701* targets (Table S1). Positive lines detected by PCR-RFLP assay were
551 further analyzed by sequencing for INDEL mutations using S2 and S5 forward primers (Table

552 S1). Four DNA amplicons per line were cloned and sequenced to determine the zygosity of the
553 lines. Biallelic mutations were found in a few T0 and T1 lines. First exon (Target 1) was
554 initially targeted in *HAC701* gene using a CRISPR/Cas9 vector construct containing a single-
555 guided RNA (S2) (Fig. S1A; Table S1). The isolation of the first generation (T0) *CRISPR*
556 *Cas9-HAC701-S2* lines yielded 90 positive independent lines, and among these, randomly
557 chosen representative lines were further genotyped to characterize the identified DNA
558 mutation. PCR and RFLP assays resulted in the isolation of monoallelic lines characterized
559 mostly by deletions and a few insertions (INDELS) on or surrounding the targeted site of
560 sgRNA (S2) (Fig. S1B-C; Table S1). The T1 *HAC701-S2* generation as observed from seeds
561 showed conservation of mutation directly from parental lines (Fig. S1B). Then, mutant lines
562 were isolated using the same technology targeting the fifth exon (Target 2) of the *HAC701*
563 gene using a construct that also contains a single guide RNA (S5) (Fig. S1D; Table S1). The
564 *CRISPR-Cas9-HAC701-S5* (T0) lines generated 11 positive independent lines of which three
565 were genotyped for verification of mutations (Fig. S1E). Second generation (T1) lines also
566 showed conservation of mutations (Fig. S1E). Similar to *HAC701-S2* lines, genotyping showed
567 INDELS in proximity to or on the target site.

568 **Phenotyping**. Positive lines containing biallelic homozygous mutations were phenotyped for
569 effective grain production by examining and counting the mature grains produced in each
570 panicle. Tiller number was counted one month after flowering. Chlorosis phenotype was used
571 as a proxy for any defect in photosynthesis and or apparatus. 1000-grain weight was measured
572 on seeds oven dried for 30-days at 37°C. Comparison of height and general morphological
573 structures were documented using photography.

574 **flg22 leaf disc assay**. flg22 peptide, a well-known inducer of plant innate immunity,
575 specifically of PTI was used to test which rice HATs respond to flg22 treatment. Leaf disc
576 assay (modified from 65, 66) was performed on fully expanded 30-d-old wild-type leaf samples

577 and treated with synthetic flg22 peptide (30-51 aa, Flic, *Pseudomonas aeruginosa*) (ADI, Inc.)
578 at different time periods and concentrations. Briefly, leaves were cut into about 5 mm sizes and
579 floated on the water for 24-h in growth chamber to remove the symptoms of wounding stress.
580 Leaves were then treated with PAMP solution in water at 15 ml falcon tubes with rotation
581 (Corning Science). After treatment, paper towel-dried leaves were frozen in liquid nitrogen.

582 **Pathogenesis assay**. Seeds were surface sterilized and imbibed in sterile water in the dark for
583 72 h before sowing on MS basal medium (Sigma Life Science). After 10 days, the plantlets
584 were transferred to soil and were grown for another 18 days until infection. *Pseudomonas*
585 *syringae* pv. *oryzae* (*Pso*) (MAFF No. 301530, NIAS Genebank) was grown in Luria-Bertani
586 (LB) broth (Sigma-Aldrich) at 28°C until OD = 0.2 and was resuspended in 10 mM $MgCl_2$.
587 The fourth leaf counting from the first true leaf was infected with *Pso* using needleless syringe
588 injected from the lower surface of the leaf. *Pso* leaf infiltration was performed 10 cm from the
589 tip of the leaf and was done 3x with approximately 1 cm space between the infiltration sites.
590 Infected plants were temporarily maintained outside the growth chamber for 2 h to allow drying
591 of the infected sites before returning to the chamber. Infected leaf samples were collected 3
592 days after *Pso* inoculation utilizing only the tissues comprising the spaces between the
593 infiltration sites. These tissues were grounded in sterile 10 mM $MgCl_2$ and the grounded tissue
594 suspensions were transferred to LB agar medium for incubation. The infected local tissues
595 were assayed with minor modifications as detailed in Liu, *et al.* (67). For log cfu per leaf disc
596 measurement, the grounded tissue suspensions were serially diluted six times to be able to
597 count with accuracy the *Pso* colonies on Luria-Bertani (LB) agar medium. *Pso* colonies were
598 counted from 4th until 6th serial dilution in several independent and genotypically dissimilar
599 *hac701* lines, *9-5*$^{-/}$, *9-12a*$^{-/-}$, and *9-12b*$^{-/-}$. The remaining samples of the fourth leaf were used
600 for RNA-sequencing analysis. For blast assay, the fifth leaf of 25-day-old plants were used for
601 infection of *Magnaporthe oryzae* (MAFF No. 101511, NIAS Genebank). Blast spores were

602 incubated 10 cm from the tip of the leaf (1st site) and at another site of the same leaf 10 cm
603 from the first site (2nd site). Infected plants were incubated for 10 days inside an incubator
604 (BiOTRON, NK System) and the length of blast infection was measured.

605 **Gene expression analysis**. Total RNA extraction was performed using RNeasy Plant Mini Kit
606 (Qiagen) or Maxwell® 16 LEV Plant RNA Kit (Promega). cDNA was synthesized using
607 Primescript II 1st Strand cDNA Synthesis Kit (Takara) according to manufacturer's
608 instructions. RT-qPCR assays were performed on three biologically independent samples or
609 as indicated. RT-qPCR was performed using SYBR Premix Ex Taq II (Tli RNaseH Plus)
610 (Takara) and was calculated following Pfaffl (68) by averaging the values relative to *ACT1*
611 control gene. Primer sequences are listed in Table S19.

612 **RNA-sequencing**. Total RNA was isolated from mock- and *Pseudomonas syringae* pv. *oryzae*
613 (*Pso*)-treated 9-WT wild type and *hac701* with Maxwell 16 LEV Plant RNA Kit (Promega)
614 run on the Maxwell 16 Instrument (Promega) and/or mirVana miRNA Isolation Kit (Invitrogen
615 by Thermo Fisher Scientific). The *hac701* lines, *9-5*$^{-/-}$ and *9-12b*$^{-/-}$, were lumped together as
616 two independent biological samples during analysis. To remove the contaminating genomic
617 DNA from RNA samples isolated using the mirVana miRNA Isolation Kit, RNA was treated
618 with DNase I (RNA free) (Nippon Gene) following the manufacturer's instructions. Samples
619 were submitted to OIST Sequencing Center for RNA quality checking, library preparation, and
620 paired-end mRNA-sequencing (PE mRNA-seq).

621 **Chromatin immunoprecipitation-sequencing**.

622 ChIP analysis of histone modifications in wild type and mutants were performed as follows:
623 One-month-old mature leaves of wild type (9-WT) and *hac701* (*9-12b*$^{-/-}$) were fixed in a
624 fixation buffer (10mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 M sucrose, 1 % formaldehyde)
625 for 10 min, followed by quenching with 125 mM Glycine for 5min. Nuclei isolation was

626 performed as previously described (69). Immuno-precipitation was performed for two
627 replicates for each genotype (about 1 g tissue/IP) by SimpleChIP Plus Kit (Cell Signaling
628 Technology) according to the manufacturer's instructions. Anti-Histone H3 antibody (Abcam
629 ab1791), Anti-acetyl Histone H3K9 antibody (Merck ABE18), Anti-Dimethyl Histone H3K9
630 antibody (Merck 05-1249), Anti-Trimethyl Histone H3K9 antibody (Merck 17-10242), Anti-
631 acetyl Histone H3K27 antibody (Abcam ab4729) were used for IPs. Dynabeads M-280 Sheep
632 Anti-Rabbit IgG (Invitrogen 11203D) or Anti-Mouse IgG (Invitrogen 11201D) was used for
633 purification of chromatin-antibody complex. Precipitated DNA samples were sequenced by
634 Hiseq 4000 in 150 bp paired-end mode in OIST Sequencing Center. For ChIP real-time PCR,
635 we used the "Percent Input Method" (Thermo Fisher Scientific) with 33% input value to
636 normalize the data for enrichment calculation. Three independent biological replicates were
637 performed for each sample.

638 **Data analysis**. For RNA-sequencing analysis, high quality reads were trimmed in order to
639 remove sequencing bias and adapter effects. Trimmed reads were then mapped to the *Oryza*
640 *sativa* spp. *japonica* genome (Os-Nipponbare-Reference-IRGSP-1.0) using Tophat (70, 71).
641 Custom R scripts were used to generate the RNA count table necessary to analyze the
642 differentially expressed genes (DEGs). Differential expression analysis was performed using
643 DESeq2 package (72). DEGs were selected at Benjamini-Hochberg adjusted p value of < 0.01.
644 To perform Gene Ontology (GO) analysis, gene lists were submitted to Enrichment Analysis
645 (73) web tool of the Gene Ontology Consortium (74-76). To create the Venn diagram, gene
646 sets were submitted to InteractiVenn web tool using *unions by list* to obtain overlapping
647 components of input datasets (77). For ChIP-sequencing analysis, raw reads were trimmed and
648 were mapped to *Oryza sativa* spp. *japonica* genome (Os-Nipponbare-Reference-IRGSP-1.0)
649 using Bowtie (78). Read alignments were visualized using Integrated Genome Browser (IGB)
650 (79). ChIP-enriched peaks were called by deriving a quantitative reproducibility score called

651 "irreproducibility discovery rate" (IDR) (73). Comparative analyses and quantitation of
652 enrichments between 9-WT and *hac701* (*9-12b*$^{-/-}$) were analyzed using a suite of tools and
653 utilities found in deepTools (80) and BEDTools (81). Peaks were positioned to genomic
654 locations (intergenic, genes, transposable elements, simple repeats) and within genes
655 (promoter, exon, intron, TSS, TTS). Table S14 showed the number of peaks identified for each
656 histone modifications in 9-WT and *hac701* (*9-12b*$^{-/-}$). Peak-to-gene assignment was performed
657 using Homer (v4.11) (82). To create the phylogenetic tree of Arabidopsis and rice histone
658 acetyltransferases, we used the UniProt amino acid sequences and reconstructed the
659 phylogenetic relationships using Phylogeny.fr (83, 84). For KIX domain analysis, we used
660 KIXBASE to perform multiple sequence alignment (38). To identify *cis*-elements on DNA
661 sequences, we used cister prediction software that utilizes hidden Markov model (85). For
662 motif analyses, we utilized the 1kb upstream promoter sequences of the sample genes. We used
663 Discriminative Regular Expression Motif Elicitation (DREME) (86) to discover the
664 significantly enriched motifs at a threshold of E-value<0.05 using shuffled input sequences as
665 control. These enriched motifs were used as query sequences in Tomtom (MEME suite 5.2.0)
666 to find similar motifs in published libraries. Libraries used were Eukaryotic DNA and
667 Vertebrates (*in vivo* and *in silico*).

668 **Data visualization**. Visualization of majority of the data was performed using Microsoft Excel
669 for Mac 2011 (version 14.7.1) and R packages: heatmaply, functions from *gplots*, and *ggplot2*.

670 **Data Repository**. For RNA-sequencing and ChIP-sequencing, raw data have been deposited
671 in the DDBJ Sequence Read Archive under accession ID (pending upon submission of data).
672 All other data can be found in the Supplemental Tables 1-19 in this manuscript.

673

674

675 **FIGURE LEGENDS**

676 **Figure 1** Disease phenotype of biallelic homozygous CRISPR/Cas9 lines targeting the fifth
677 exon of rice acetyltransferase gene, *HAC701*. (*A*) Phylogenetic relationships of members of
678 Arabidopsis and rice histone acetyltransferases. The numbers in red are branch support values.
679 All amino acid sequences are from UniProt database. (*B*) Upper panel: Alleles from four lines
680 of the second generation (T1) plants were identified by cloning and sequencing the PCR
681 products from *HAC701* target region using the primers found in Table S1. Lower panel: PCR
682 and RFLP assays of representative T1 generation lines. The mutant line *9-12a*$^{-/-}$ displaying a
683 wild type-like band was due to unaltered sequence order recognized by BseLI even after an
684 insertion event, however we confirmed by sequencing that an insertion mutation was present
685 in this line. (*C*) *Pseudomonas syringae* pv. *oryzae* (*Pso*) infection assay of *hac701*, *9-12b*$^{-/-}$ and
686 *9-5*$^{-/-}$ (T2), using *9-5*$^{+/+}$ segregated wild type line (9-WT) as control. Bacterial density
687 quantification uses values from 4th-6th serial dilutions. The mutant line *9-12b*$^{-/-}$ was grown from
688 the seeds of the third generation (T2), while *9-5*$^{-/-}$ line was embryonically rescued about 15
689 days post flowering from the second generation (T1) parental plants. All mock measurements
690 yielded zero bacterial growth for both wild type and mutant lines. Bars represent the 95%
691 confidence interval (CI) and compared to the wild type using two-tailed Student's *t*-test at
692 $P<0.05$ in *9-12b*$^{-/-}$; one-tailed Student's *t*-test at $P<0.05$ in *9-5*$^{-/-}$. (*D*) *Magnaporthe oryzae*
693 infection assay of *hac701*, *9-12b*$^{-/-}$ and *9-5*$^{-/-}$, using *9-5*$^{+/+}$ segregated wild type line (9-WT) as
694 control. Lesion length was measured on leaves after 10 dpi (days post infection). White length
695 indicator bar on the left panel is 10 mm. Bars on the right panel represent standard error (SE)
696 and compared to the wild type using *F*-test for variance and Student's *t*-test.

697

698 **Figure 2** The rice *WRK45* gene expression is induced in *hac701* lines. (*A*) Overlap analysis of
699 upregulated and downregulated genes in *hac701* background under mock and pathogen (*Pso*)-
700 treatment conditions (Table S5, S6). *OsWRKY45* is the only differentially expressed gene in
701 either the absence or presence of pathogen. Transcriptome data were normalized using mock
702 data sets for each genotype and condition. (*B*) *OsWRKY45* expression when *HAC701* is
703 mutated (+ sign). The addition of pathogen (+ sign) on *hac701* increased further the
704 *OsWRKY45* expression. Transcriptome data of *hac701*, *9-12b*$^{-/-}$ and *9-5*$^{-/-}$, under mock or *Pso*-
705 treated conditions were lumped as two independent biological samples for analysis (See Table
706 S2). hpi, hours after infection.

707

708 **Figure 3** *hac701* phenocopies *OsWRKY45* overexpression transgenic plants. (*A*) Overlap
709 analysis of differentially expressed genes (DEGs) in *hac701* background with benzothiadiazole
710 (BTH)-responsive genes (*i.e.* BTH is a salicylic acid analog) in wild type rice (Table S6, S7).
711 Significance value of the overlap data was tested using the hypergeometric distribution test.
712 (*B*) Overlap analysis of DEGs in *hac701* background with BTH-inducible and *OsWRKY45*-
713 dependent or *OsNPR1/NH1*-dependent genes (Table S8, S10). Significance values of the
714 overlap data were tested using the hypergeometric distribution test. (*C*) *OsWRKY45*-
715 dependent defense pathway components. (*D*) Gene expression of *OsWRKY45*-dependent
716 defense genes in 9-WT and *hac701*.

717

718 **Figure 4** Genome-wide H3K9 acetylation is depleted in *hac701*. (*A*) Heatmap showing the
719 accumulation of H3K9 acetylation on 265 peaks by taking the enrichment ratio of 9-WT and
720 *9-12b*$^{-/-}$ data (Wild type/mutant) (Table S15). (*B*) Heatmaps showing the accumulation of
721 H3K27 acetylation, H3K9 di-methylation, and H3K9 tri-methylation on 265 peaks by taking

722 the same enrichment ratio as (*A*). Heatmaps in (*A*) and (*B*) are representatives of two
723 biologically independent data showing similar enrichment results and number of peaks
724 identified. (*C*) GO enrichment analysis showing only the "Biological Process" of the 263 genes
725 found in the 265 peaks (Table S15). Numbers in enrichment indicate Fold Enrichment Value
726 using Fisher's Exact test with results for uncorrected $P<0.05$. (*D*) Enrichment scores of seven
727 representative genes showing depletion of H3K9 acetylation in *hac701*, *9-12b*$^{-/-}$. (*E*)
728 Enrichment scores of 10 representative putative decoy/sensor genes (42) showing depletion of
729 H3K9 acetylation in *hac701*, *9-12b*$^{-/-}$. Scores in (*D*) and (*E*) are averages of two biologically
730 independent ChIP-seq replicates with bars showing standard deviation (SD).

731

732 **Figure 5** *OsWRKY45* loci is enriched in H3K9 acetylation in *hac701*. (*A*) Enriched peaks on
733 histone modifications positioned to genomic locations (Intergenic, Promoter-TSS, Exon,
734 Intron, and TTS) in 9-WT and *hac701*, *9-12b*$^{-/-}$. All data were normalized to input. (*B*)
735 *OsWRKY45* depth graph of RNA-seq and H3K9 acetylation ChIP-seq showing the number of
736 reads in RPKM (Reads Per Kilobase of transcript, per Million mapped reads) in 9-WT and
737 *hac701*, *9-12b*$^{-/-}$. The red dotted box shows the 1kb upstream region of *OsWRKY45* with arrow
738 indicating the direction of transcription. (*C*) Upper panel: Gene map of *OsWRKY45* with arrow
739 showing the direction of transcription, gray bars are 5' and 3' untranslated regions (UTRs),
740 black bars are exons, and white bars are introns. Lower panel: 9-WT and *hac701* (*9-12b*$^{-/-}$)
741 mutant ChIP-qPCR showing enrichments of H3K9 acetylation and H3K9 di-methylation in
742 *OsWRKY45* locus. The significant difference in enrichment is computed using two-tailed
743 Student's *t*-test where asterisks: ***$P<0.01$, **$P<0.05$, and NS means Not Significant.

744

745

746 **TABLE LEGENDS**

747 None

748

749 **SUPPLEMENTARY FIGURE LEGENDS**

750 **Figure S1** Characterization of mutations generated by CRISPR/Cas9 editing on the first and
751 fifth exon of rice acetyltransferase gene, *HAC701*. (*A*) Schematic showing an sgRNA targeted
752 to the first exon of *HAC701* gene. (*B*) PCR and RFLP assays of representative T0 and T1
753 generation lines from leaf blade (Lb), panicle (Pa), and seed (S) DNA samples. +/+ and M
754 represent the zygosity of the line, where +/+ refers to wild type and M refers to monoallelic.
755 (*C*) Alleles from 12 T0 generation lines identified by cloning and sequencing the PCR products
756 from *HAC701* target regions using the primers found in Table S1. Similar line number indicates
757 that lines came from the same callus. For each line, four DNA amplicons were cloned and
758 sequenced and the fraction indicates the number of times the type of mutations were found in
759 each line. In case not indicated, it means wild type. The asterisk (*) indicates the most common
760 mutation found within and across different lines. (*D*) Upper panel: Schematic showing an
761 sgRNA targeted to the fifth exon of *HAC701*. Lower panel: Schematic showing the protein
762 domains of HAC701. (*E*) PCR and RFLP assays of representative T0 and T1 generation lines
763 from leaf blade DNA samples. M, B represent the zygosity of the line, where M refers to
764 monoallelic, and B refers to biallelic. T1 lines came from parental 2-3 line. (*F*) Phenotype of
765 T2 lines showing effective grains and tiller number. (*G*) Images of T3 *hac701* lines compared
766 to segregated wild type, 9-WT. (*H*) RT-qPCR levels of *HAC703* and *HAC704* genes in
767 *HAC701* mutation line background. Samples were treated hydroponically with HDAC
768 inhibitors (1μM Trichostatin A (TSA) and 100 μM Nicotinamide at final concentration) for
769 three days. Bars represent standard error (SE); n= 3. The significant difference in transcription
770 is computed using two-tailed Student's *t*-test where asterisks: *$P<0.01$. (*I*) Phenotype of T3
771 and T4 lines showing grain weight.

772 **Figure S2** flg22 induced the expression of rice pathogenesis-related and histone
773 acetyltransferase (HAT) gene, *HAC701*. Transcriptional levels of pathogenesis-related gene,
774 *PR10a*, and of eight HAT genes upon flg22 induction at concentrations in μM units. Data
775 shown are means ± SE; n= 3. The significant difference in transcription is computed using two-
776 tailed Student's *t*-test where asterisks: ***$P \leq 0.01$, **$P \leq 0.03$, * $P \leq 0.05$.

777

778 **Figure S3** *Pseudomonas syringae* pv. *oryzae* (*Pso*) infection assay in T2 *hac701* line, *9-12a*$^{-/-}$
779 . Values from 4th-6th serial dilutions were used for quantification. Segregated wild type line
780 was used as a control. Bars represent the 95% confidence interval (CI) and compared to wild
781 type using one-tailed Student's *t*-test at $P<0.05$.

782

783 **Figure S4** Sample distances of RNA-seq data after regularized-logarithm transformation
784 (rlog). Heatmap showing the Euclidean sample distance matrix of mock- and *Pseudomonas*
785 *syringae* pv. *oryzae* (*Pso*)-treated samples in two biologically independent replicates.

786

787 **Figure S5** Top 10 genes that are most highly variable in mock- and *Pso*-treated RNA-seq
788 samples. Gene descriptions were derived from Oryzabase: Integrated Rice Science Database
789 (NBRP) and Rice Genome Annotation Project (NSF). Two biologically independent RNA-seq
790 replicates are presented.

791

792 **Figure S6** Rice-*Pseudomonas syringae* pv. *oryzae* (*Pso*) pathosystem. (*A*) Enriched pathways
793 in the rice-*Pso* pathosystem. (*B*) Pie chart of the number of up- and down-regulated genes in
794 9-WT control 72h post *Pso* infection (Table S3). Two biologically independent RNA-seq data

795 in mock and *Pso* treatments are presented where differentially expressed genes (DEGs) were
796 selected at *P* adjusted value < 0.01.

797

798 **Figure S7** Features of differentially expressed genes (DEGs) in *Pseudomonas syringae* pv.
799 *oryzae* (*Pso*)-treated 9-WT and *hac701* transcriptomes. Overlap and GO-analysis of
800 upregulated (*A*) and downregulated (*B*) genes in *hac701* under *Pso* treatment (Table S3, S4).
801 Transcriptome data were normalized using mock data sets for each genotype. GO enrichment
802 analysis showing only the "Biological Process" of upregulated *HAC701*-repressed genes (141)
803 and HAC701-independent genes (336), downregulated *HAC701*-enhanced genes (31) and
804 HAC701-independent genes (46). Fold Enrichment Value uses Fisher's Exact test with results
805 for uncorrected *P*<0.05.

806

807 **Figure S8** Alignment of KIX domain found in different organisms. KIX domain in HAC701
808 (*A*) and HAC703 (*B*) proteins. Sc: *Saccharomyces cerevisiae*, Os: *Oryza sativa*, Hs: *Homo*
809 *sapiens*, Ce: *Caenorhabditis elegans*, Dm: *Drosophila melanogaster*, Mm: *Mus musculus*.

810

811 **Figure S9** *OsWRKY45* promoter contains a rare CRE motif. (*A*) A CRE motif (upper panel)
812 matches significantly to the query motif CGRCGRCG (lower panel). The query motif was
813 derived from discovered motifs (Table S13) in the 1 kb promoter regions of differentially
814 expressed genes (DEGs) in *hac701* treated with *Pso*. (*B*) 5 genes out of 660 differentially
815 expressed genes (DEGs) (0.76%) (Table S6) in the *hac701* contain the full CRE motif. Cister
816 parameter settings: default settings were used. *(C)* CRE motif cluster found in 1kb upstream
817 sequences of *OsWRKY45* start site. The red line indicates the probability of transcription factors
818 binding to these CRE motif sites. CRE motif is found most probably on the direct strand (+).

819 **Figure S10** Enrichment strength of ChIP signals in 9-WT input and histone modifications.

820

821 **Figure S11** Heatmaps of genome-wide histone modification enrichments in genes and
822 transposable elements (TEs) of rice 9-WT and *hac701* (*9-12b*$^{-/-}$) mutant. Enrichment of H3K9
823 acetylation, H3K9 di-methylation, H3K9 tri-methylation, H3K27 acetylation, and H3 in genes
824 (*A*) and TE (*B*) regions. Data represent one of the two biologically independent samples
825 showing similar enrichment results.

826 **Figure S12** Heatmaps of genome-wide histone modification enrichments in simple repeats of
827 rice 9-WT and *hac701* (*9-12b*$^{-/-}$) mutant. Enrichment of H3K9 acetylation, H3K9 di-
828 methylation, H3K9 tri-methylation, H3K27 acetylation, and H3 in simple repeat regions. Data
829 represent one of the two biologically independent samples showing similar enrichment results.

830

831 **Figure S13** Enrichment scores of additional representative genes showing differential
832 enrichments of H3K9 acetylation in *hac701* (*9-12b*$^{-/-}$) mutant. (*A*) Transcriptional coactivator
833 genes showing reduced or partially reduced H3K9 acetylation enrichments. (*B*) Putative
834 guardee genes showing differential enrichments of H3K9 acetylation. Scores are averages of
835 two biologically independent ChIP-seq replicates with bars showing standard deviation (SD).

836

837 **Figure S14** Distribution on genomic locations of 265 regions in H3K9 acetylation ChIP (wild
838 type/mutant).

839

840 **Figure S15** *OsWRKY45* depth graph of H3K9 and H3K27 acetylation ChIP-seq showing the
841 number of reads in RPKM (Reads Per Kilobase of transcript, per Million mapped reads) in 9-

842 WT and *hac701* (*9-12b*$^{-/-}$) mutant. The red dotted box shows the 1 kb upstream region of
843 *OsWRKY45* with arrow indicating the direction of transcription. Data show a representative
844 sample.

845

846 **Figure S16** Systemic gene expression analysis in rice-*Pseudomonas syringae* pv. *oryzae* (*Pso*)
847 pathosystem. (*A*) Differentially expressed genes (DEGs) in the local (870) and systemic (24)
848 tissues of plants infected with *Pso* (Table S16). Eight DEGs were found to be common in both
849 local and systemic tissues. (*B*) The expression of eight DEGs shown in log2 fold changes. For
850 local tissue analysis, two independent RNA-seq samples in both treatments (mock and *Pso*)
851 are presented. For systemic tissue analysis, two independent RNA-seq samples in mock and
852 three in *Pso* treatment are presented. The number of reads are in RPKM (Reads Per Kilobase
853 of transcript, per Million mapped reads) in 9-WT and *hac701*.

854

855 **Figure S17** MA-plot of differentially expressed genes (DEGs) in systemic tissues of 9-WT and
856 *hac701* infected with *Pseudomonas syringae* pv. *oryzae* (*Pso*). (*A*) and (*B*) plots showing
857 differences in measurements of differentially expressed genes (DEGs). *P* adjusted value is set
858 at <0.01. For 9-WT and *hac701* samples, two independent RNA-seq samples in mock treatment
859 and three in *Pso* treatment are presented.

860

861 **Figure S18** *OsWRKY45*-dependent defense pathway is suppressed by *HAC701*. Defense-
862 related genes regulated directly and indirectly by *OsWRKY45* are negatively regulated by
863 *HAC701* resulting in resistance phenotype of *hac701* through *OsWRKY45* upregulation.

864

865

866 **SUPPLEMENTARY TABLE LEGENDS**

867 **Table S1** sgRNA oligonucleotides used for CRISPR/Cas9 mutagenesis and primers for
868 genotyping. Bold sequences in the guide RNAs are the Protospacer Adjacent Motif (PAM).

869

870 **Table S2** Summary of total RNA-sequencing data in local tissues.

871

872 **Table S3** Wild type (9-WT) differentially expressed genes (DEGs) in *Pso*.

873

874 **Table S4** *HAC701* repressed and enhanced genes.

875

876 **Table S5** *HAC701* mutation differentially expressed genes (DEGs) in Mock (*HAC701mut*-
877 DEGs in Mock).

878

879 **Table S6** *HAC701* mutation differentially expressed genes (DEGs) in *Pso* (*HAC701mut*-DEGs
880 in *Pso*). The motifs were identified 1kb upstream sequence of the start codon.

881

882 **Table S7** BTH-responsive genes (24 hpi) in microarray experiments (34, 35) (Shimono et al.
883 2007, The Plant Cell & Sugano et al. 2010, Plant Molecular Biology). Overlap genes were
884 removed.

885

886 **Table S8** BTH-inducible & WRKY45-dependent genes (33) (Nakayama et al. 2013, BMC
887 Plant Biology).

888

889 **Table S9** Upregulated genes after DEX-induced *WRKY45* expression (33) (Nakayama et al.
890 2013, BMC Plant Biology).

891

892 **Table S10** BTH-inducible genes affected by rice *NPR1/NH1* knockdown (35) (Sugano et al.
893 2010, Plant Molecular Biology).

894

895 **Table S11** *HAC701* mutation differentially expressed genes (DEGs) found in BTH-WRKY45
896 regulated genes that contain W-box motifs. The motifs were identified 1 kb upstream sequence
897 of the start codon.

898 **Table S12** *HAC701* mutation differentially expressed genes (DEGs) found in BTH-WRKY45
899 and BTH-NPR1/NH1 regulated genes that contain W-box motifs. The motifs were identified
900 1 kb upstream sequence of the start codon.

901

902 **Table S13** Significantly enriched DNA motifs in HAC701 mutation differentially expressed
903 genes (DEGs) in *Pso* (HAC701mut-DEGs in *Pso*).

904

905 **Table S14** Rice ChIP-sequencing information of histone modifications.

906

907 **Table S15** Identified peaks in H3K9ac and their corresponding genes in 9-WT/*9-12b*$^{-/-}$ (wild
908 type/mutant). Colored boxes indicate the repeated genes.

909

910 **Table S16** Summary of total RNA-sequencing data in systemic tissues.

911

912 **Table S17** List of R genes upregulated in *hac701* in *Pso*.

913

914 **Table S18** Identified peaks in H3K9me3 and their corresponding genes in *9-12b*$^{-/-}$/Input
915 (mutant/Input). Colored boxes indicate repeated genes.

916

917 **Table S19** Oligonucleotides used for RT-qPCRs (1) and ChIP-qPCRs (2).

918

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931 **AUTHOR CONTRIBUTIONS**

932 NAE: Conceptualization, Data curation, Funding acquisition, Formal analysis, Investigation,
933 Visualization, Writing-original draft, Writing-review & editing

934 LNT: Data curation, Formal analysis, Writing-review & editing

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939 editing

940

941 **COMPETING INTERESTS**

942 The authors have declared that no competing interests exist.

943

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1126 efficient alignment of short DNA sequences to the human genome. *Genome biology*
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1129 analytics platform for genomics. *Bioinformatics* 32(14):2089-2095.

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1131 sequencing data analysis. *Nucleic Acids Res.* 44(W1):W160-165.

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1142 eukaryotic DNA. *Bioinformatics* 17:878-889.

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1145

1146

Figure 1

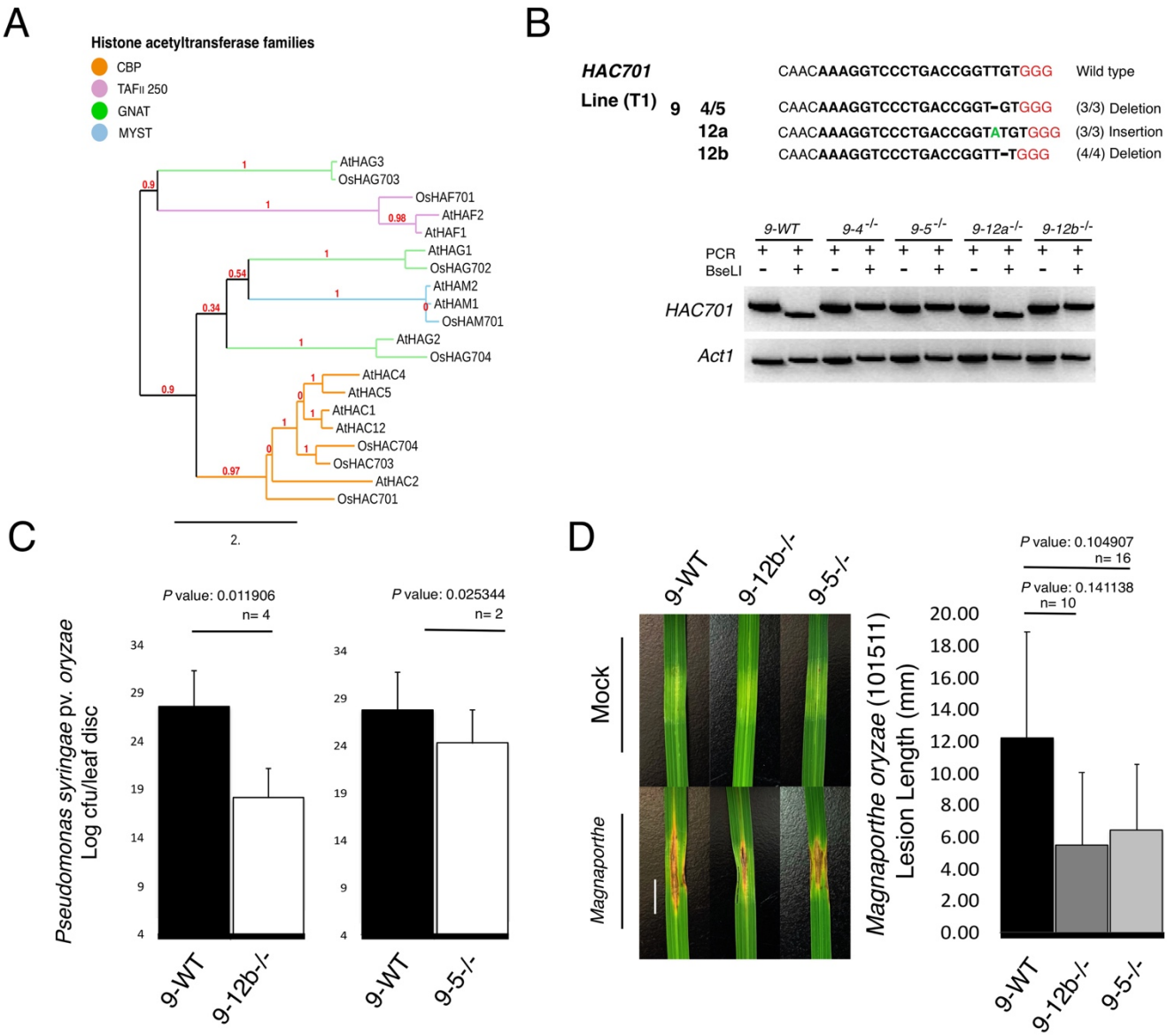

A
Histone acetyltransferase families
CBP
TAFII 250
GNAT
MYST
AtHAG3
OsHAG703
OsHAF701
AtHAF2
AtHAF1
AtHAG1
OsHAG702
AtHAM2
AtHAM1
OsHAM701
AtHAG2
OsHAG704
AtHAC4
AtHAC5
AtHAC1
AtHAC12
OsHAC704
OsHAC703
AtHAC2
OsHAC701
2.
B
HAC701
Line (T1)
9
4/5
12a
12b
CAACAAAGGTCCCTGACCGGTTGTGGG Wild type
CAACAAAGGTCCCTGACCGGT-GTGGG (3/3) Deletion
CAACAAAGGTCCCTGACCGGTATGTGGG (3/3) Insertion
CAACAAAGGTCCCTGACCGGTT-TGGG (4/4) Deletion
9-WT
9-4-/-
9-5-/-
9-12a-/-
9-12b-/-
PCR
BseLI
HAC701
Act1
C
Pseudomonas syringae pv. oryzae
Log cfu/leaf disc
P value: 0.011906
n= 4
P value: 0.025344
n= 2
9-WT
9-12b-/-
9-5-/-
D
9-WT
9-12b-/-
9-5-/-
Mock
Magnaporthe
Magnaporthe oryzae (101511)
Lesion Length (mm)
P value: 0.104907
n= 16
P value: 0.141138
n= 10

Figure 2

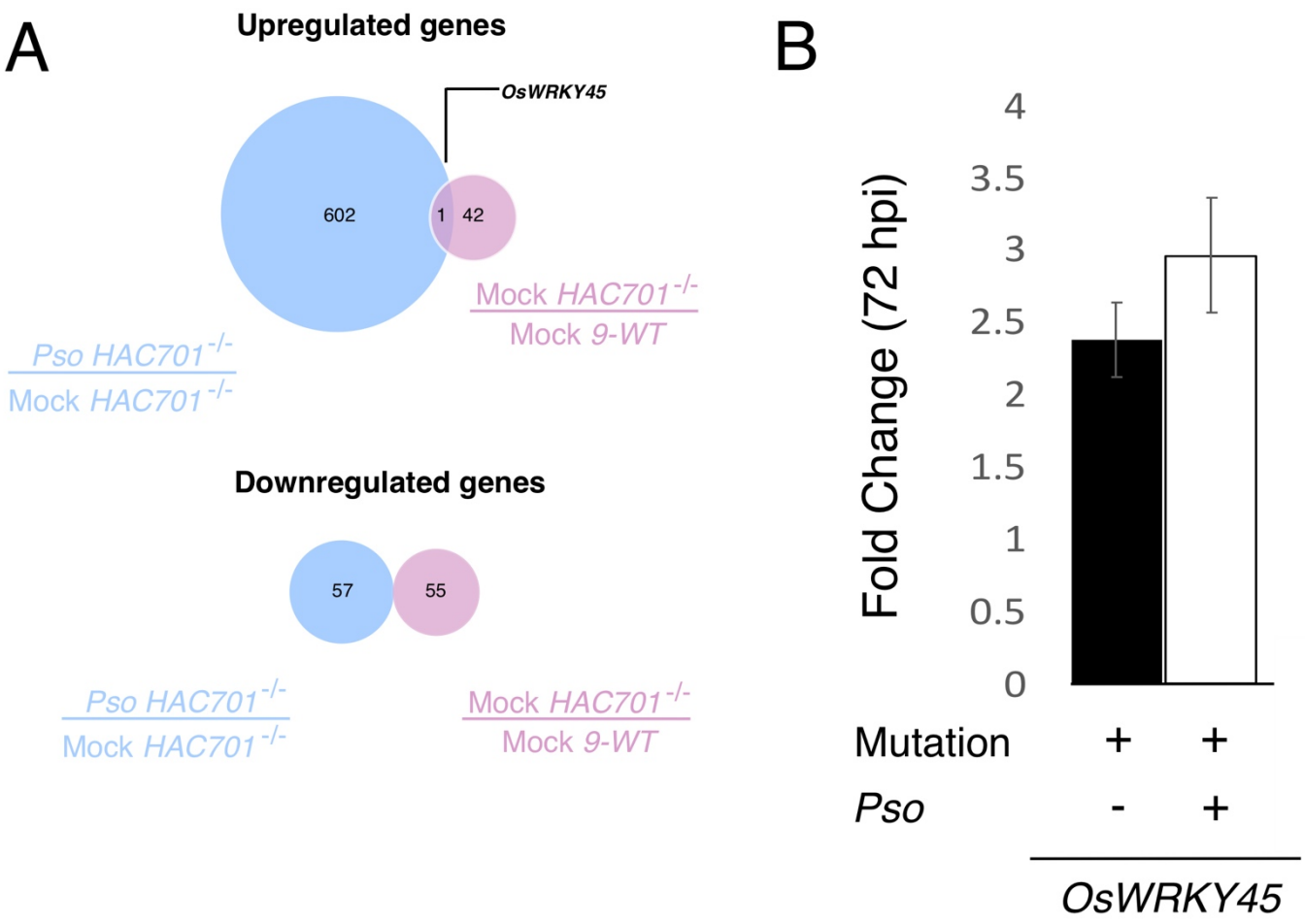

A
Upregulated genes
OsWRKY45
602
1
42
Pso HAC701-/-
Mock HAC701-/-
Mock HAC701-/-
Mock 9-WT
Downregulated genes
57
55
Pso HAC701-/-
Mock HAC701-/-
Mock HAC701-/-
Mock 9-WT
B
Fold Change (72 hpi)
4
3.5
3
2.5
2
1.5
1
0.5
0
Mutation + +
Pso - +
OsWRKY45

Figure 3

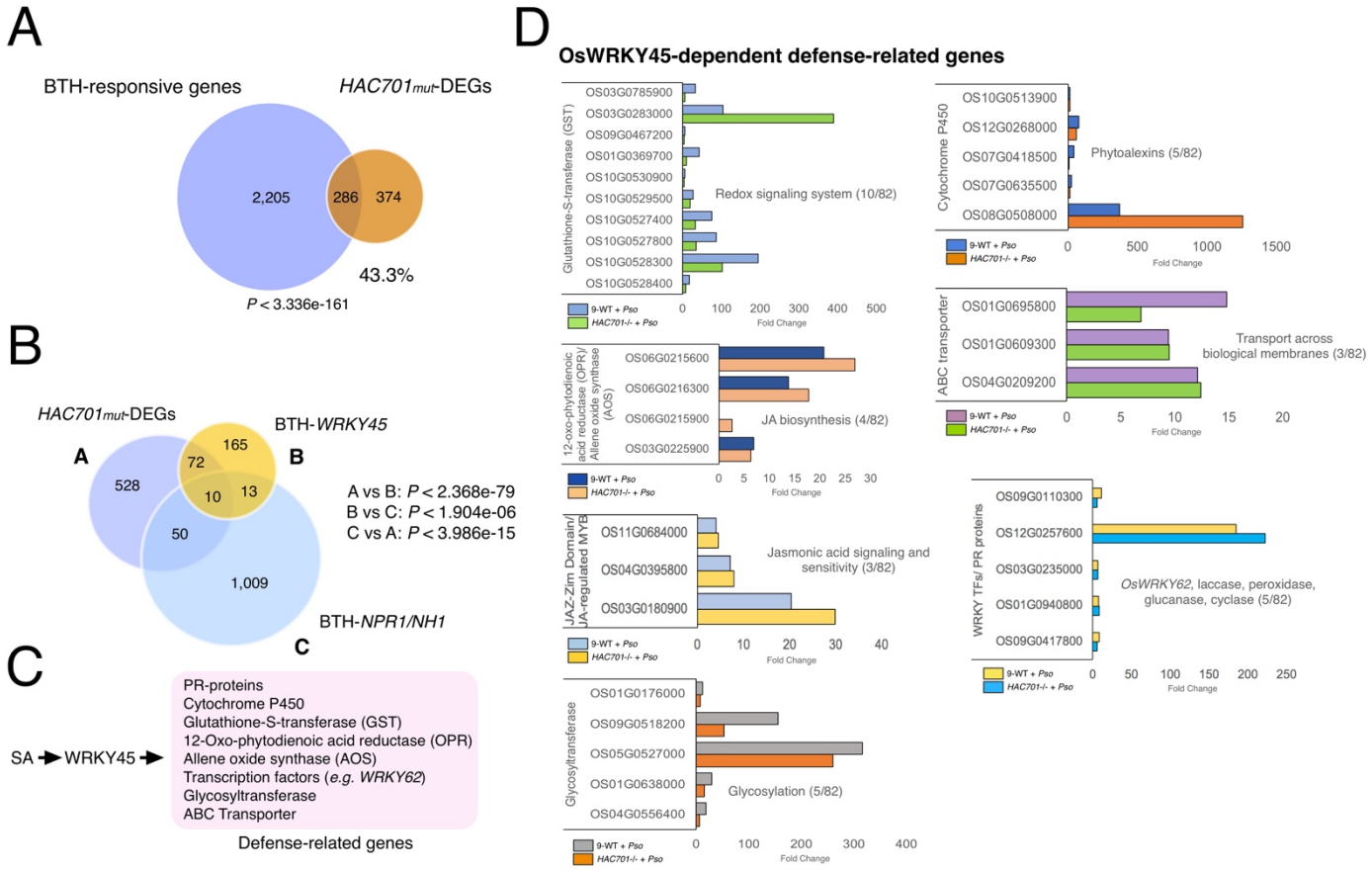

A
BTH-responsive genes
HAC701mut-DEGs
2,205
286
374
43.3%
P < 3.336e-161
B
HAC701mut-DEGs
BTH-WRKY45
A
B
C
528
72
165
10
13
50
1,009
A vs B: P < 2.368e-79
B vs C: P < 1.904e-06
C vs A: P < 3.986e-15
BTH-NPR1/NH1
C
SA → WRKY45 →
PR-proteins
Cytochrome P450
Glutathione-S-transferase (GST)
12-Oxo-phytodienoic acid reductase (OPR)
Allene oxide synthase (AOS)
Transcription factors (e.g. WRKY62)
Glycosyltransferase
ABC Transporter
Defense-related genes
D
OsWRKY45-dependent defense-related genes
Glutathione-S-transferase (GST)
OS03G0785900
OS03G0283000
OS09G0467200
OS01G0369700
OS10G0530900
OS10G0529500
OS10G0527400
OS10G0527800
OS10G0528300
OS10G0528400
Redox signaling system (10/82)
9-WT + Pso
HAC701-/- + Pso
0 100 200 300 400 500
Fold Change
Cytochrome P450
OS10G0513900
OS12G0268000
OS07G0418500
OS07G0635500
OS08G0508000
Phytoalexins (5/82)
0 500 1000 1500
12-oxo-phytodienoic acid reductase (OPR)/ Allene oxide synthase (AOS)
OS06G0215600
OS06G0216300
OS06G0215900
OS03G0225900
JA biosynthesis (4/82)
0 5 10 15 20 25 30
ABC transporter
OS01G0695800
OS01G0609300
OS04G0209200
Transport across biological membranes (3/82)
0 5 10 15 20
JAZ-Zim Domain/ JA-regulated MYB
OS11G0684000
OS04G0395800
OS03G0180900
Jasmonic acid signaling and sensitivity (3/82)
0 10 20 30 40
WRKY TFs/ PR proteins
OS09G0110300
OS12G0257600
OS03G0235000
OS01G0940800
OS09G0417800
OsWRKY62, laccase, peroxidase, glucanase, cyclase (5/82)
0 50 100 150 200 250
Glycosyltransferase
OS01G0176000
OS09G0518200
OS05G0527000
OS01G0638000
OS04G0556400
Glycosylation (5/82)
0 100 200 300 400

Figure 4

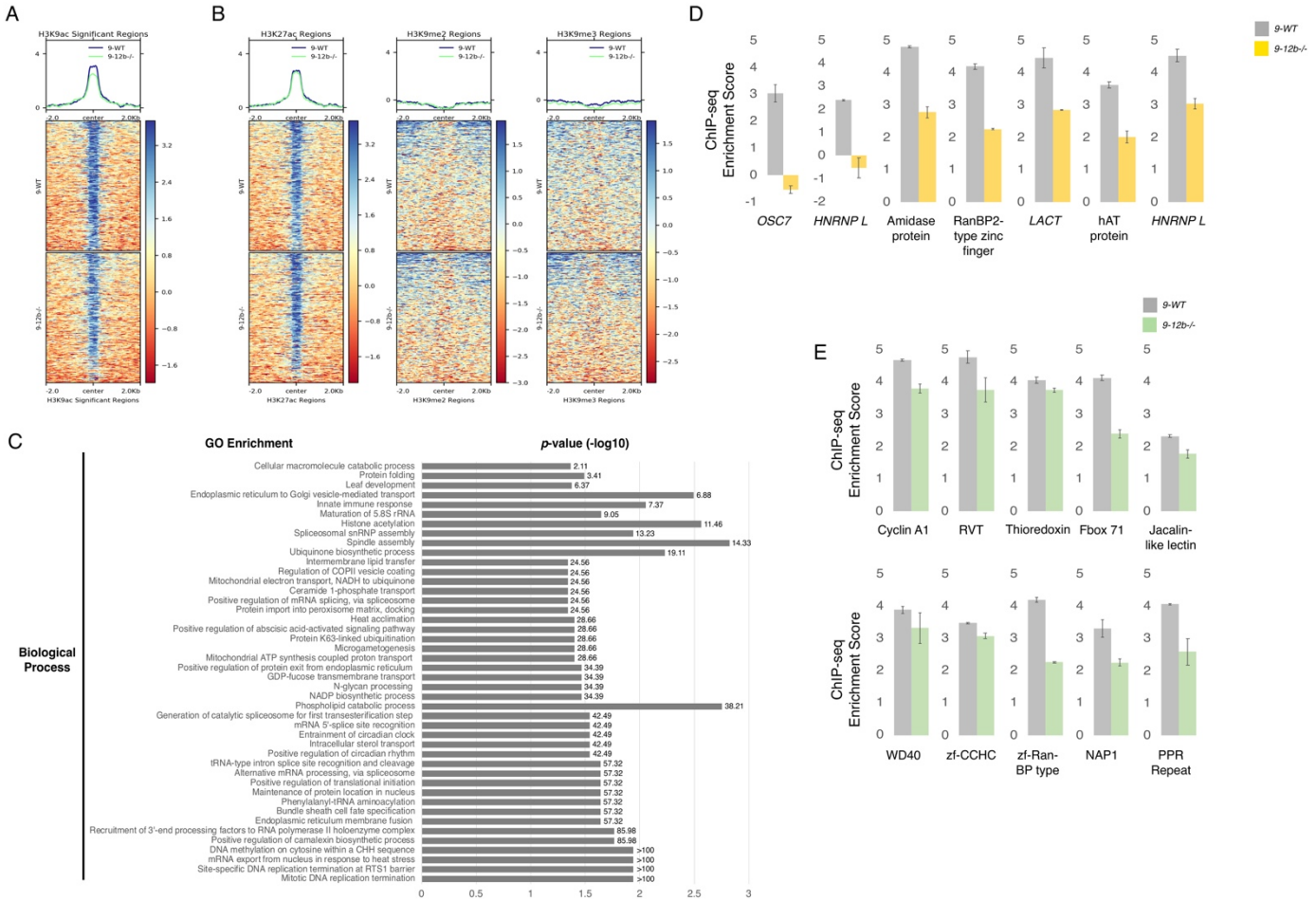
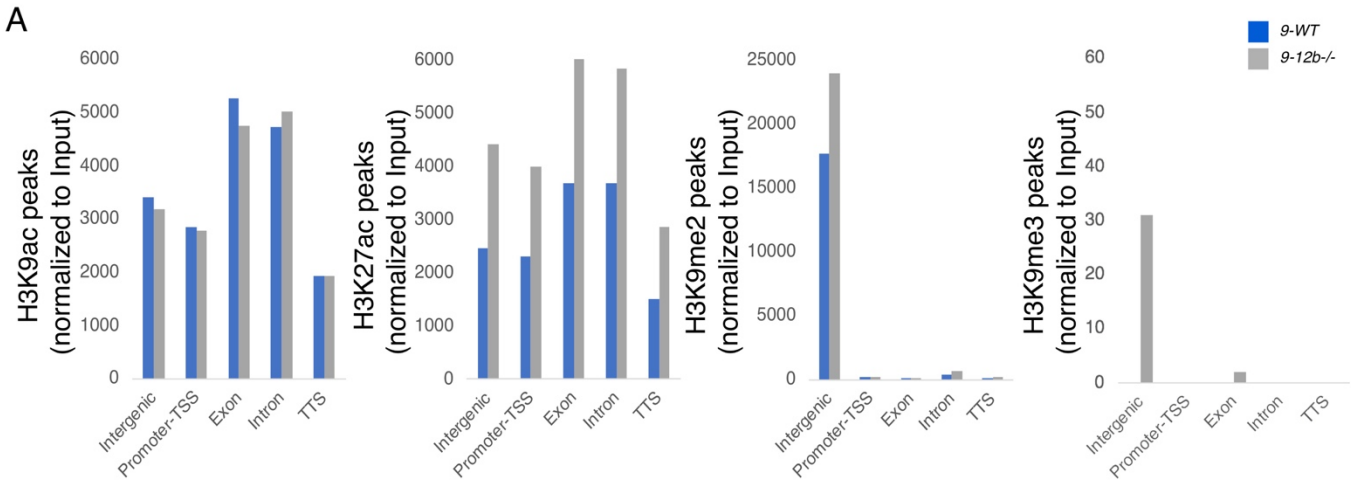
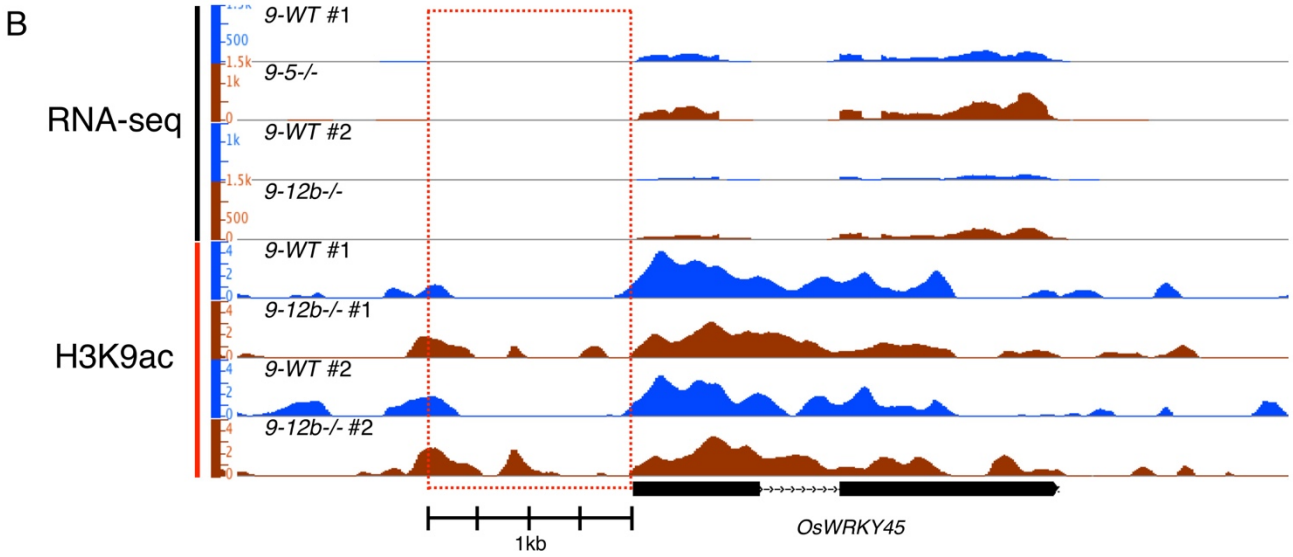
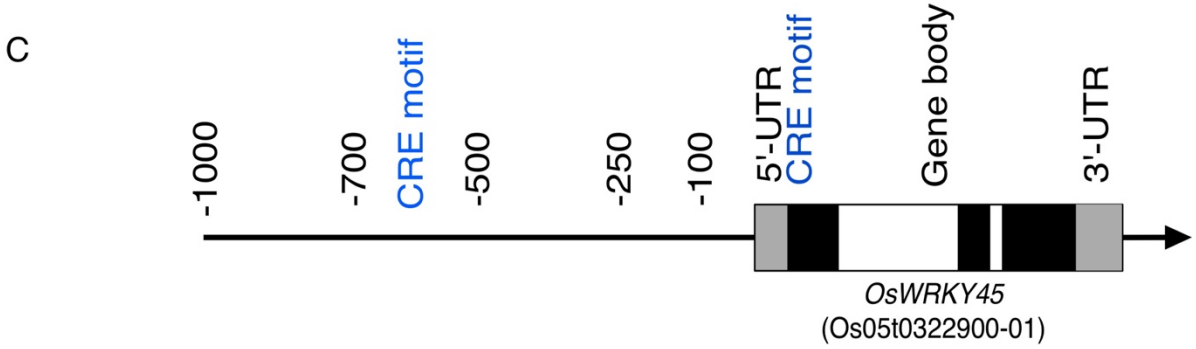

A
H3K9ac Significant Regions
9-WT
9-12b-/-
B
H3K27ac Regions
H3K9me2 Regions
H3K9me3 Regions
C
GO Enrichment
p-value (-log10)
Biological Process
Cellular macromolecule catabolic process 2.11
Protein folding 3.41
Leaf development 6.37
Endoplasmic reticulum to Golgi vesicle-mediated transport 6.88
Innate immune response 7.37
Maturation of 5.8S rRNA 9.05
Histone acetylation 11.46
Spliceosomal snRNP assembly 13.23
Spindle assembly 14.33
Ubiquinone biosynthetic process 19.11
Intermembrane lipid transfer 24.56
Regulation of COPII vesicle coating 24.56
Mitochondrial electron transport, NADH to ubiquinone 24.56
Ceramide 1-phosphate transport 24.56
Positive regulation of mRNA splicing, via spliceosome 24.56
Protein import into peroxisome matrix, docking 24.56
Heat acclimation 28.66
Positive regulation of abscisic acid-activated signaling pathway 28.66
Protein K63-linked ubiquitination 28.66
Microgametogenesis 28.66
Mitochondrial ATP synthesis coupled proton transport 28.66
Positive regulation of protein exit from endoplasmic reticulum 34.39
GDP-fucose transmembrane transport 34.39
N-glycan processing 34.39
NADP biosynthetic process 34.39
Phospholipid catabolic process 38.21
Generation of catalytic spliceosome for first transesterification step 42.49
mRNA 5'-splice site recognition 42.49
Entrainment of circadian clock 42.49
Intracellular sterol transport 42.49
Positive regulation of circadian rhythm 42.49
tRNA-type intron splice site recognition and cleavage 57.32
Alternative mRNA processing, via spliceosome 57.32
Positive regulation of translational initiation 57.32
Maintenance of protein location in nucleus 57.32
Phenylalanyl-tRNA aminoacylation 57.32
Bundle sheath cell fate specification 57.32
Endoplasmic reticulum membrane fusion 57.32
Recruitment of 3'-end processing factors to RNA polymerase II holoenzyme complex 85.98
Positive regulation of camalexin biosynthetic process 85.98
DNA methylation on cytosine within a CHH sequence >100
mRNA export from nucleus in response to heat stress >100
Site-specific DNA replication termination at RTS1 barrier >100
Mitotic DNA replication termination >100
D
ChIP-seq Enrichment Score
9-WT
9-12b-/-
OSC7
HNRNP L
Amidase protein
RanBP2-type zinc finger
LACT
hAT protein
HNRNP L
E
ChIP-seq Enrichment Score
Cyclin A1
RVT
Thioredoxin
Fbox 71
Jacalin-like lectin
WD40
zf-CCHC
zf-Ran-BP type
NAP1
PPR Repeat

Figure 5


A
9-WT
9-12b-/-
H3K9ac peaks (normalized to Input)
H3K27ac peaks (normalized to Input)
H3K9me2 peaks (normalized to Input)
H3K9me3 peaks (normalized to Input)
Intergenic
Promoter-TSS
Exon
Intron
TTS


B
RNA-seq
H3K9ac
9-WT #1
9-5-/-
9-WT #2
9-12b-/-
9-WT #1
9-12b-/- #1
9-WT #2
9-12b-/- #2
1kb
OsWRKY45


C
-1000
-700
CRE motif
-500
-250
-100
5'-UTR
CRE motif
Gene body
3'-UTR
OsWRKY45
(Os05t0322900-01)

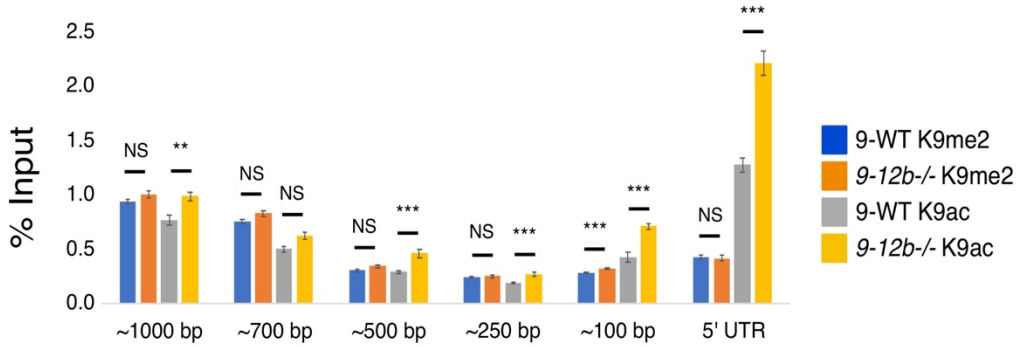

% Input
9-WT K9me2
9-12b-/- K9me2
9-WT K9ac
9-12b-/- K9ac
~1000 bp
~700 bp
~500 bp
~250 bp
~100 bp
5' UTR
NS
**
***

Figure S1

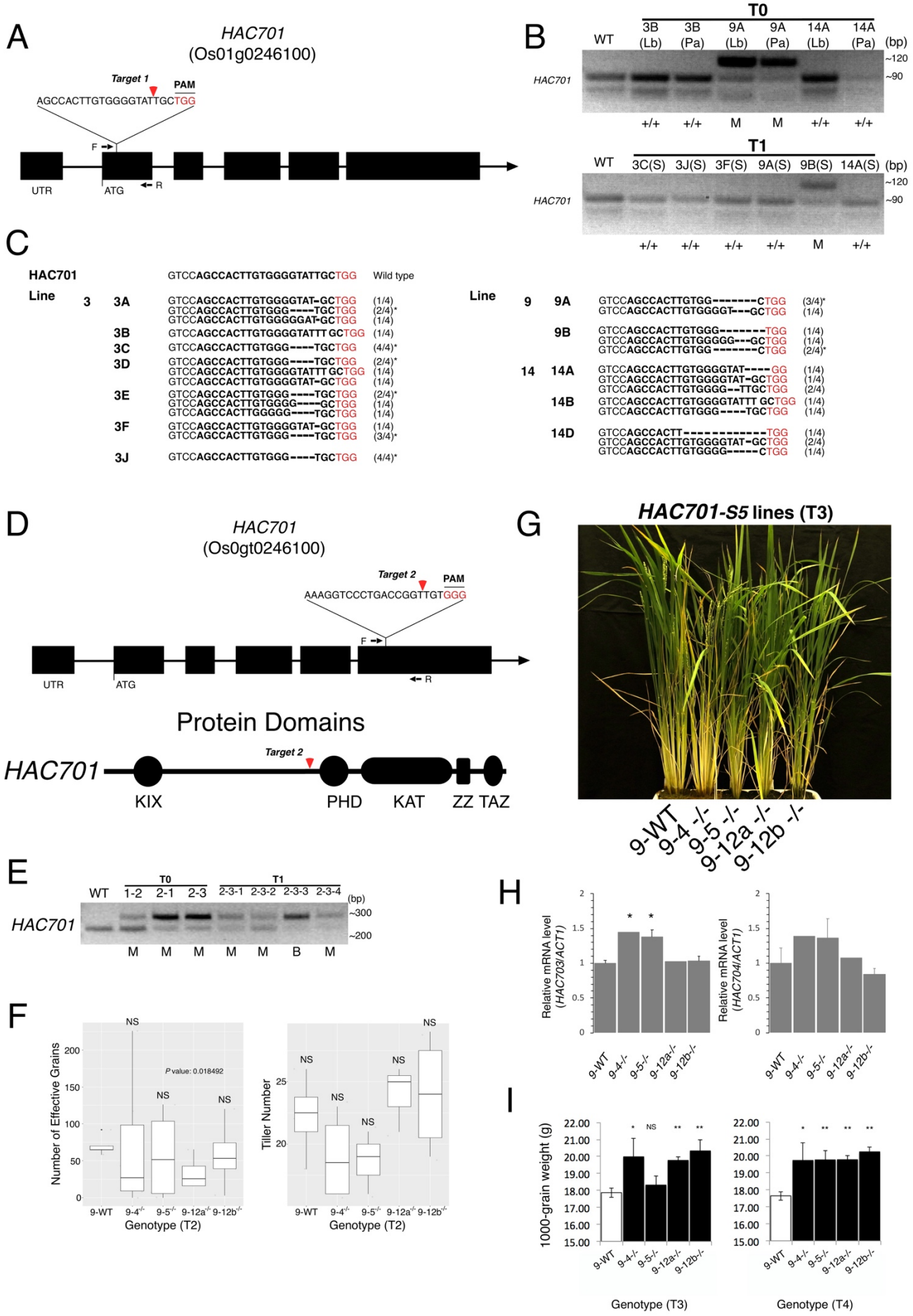

A
HAC701
(Os01g0246100)
Target 1
PAM
AGCCACTTGTGGGGTATTGCTGG
F
R
UTR
ATG
B
T0
WT
3B (Lb)
3B (Pa)
9A (Lb)
9A (Pa)
14A (Lb)
14A (Pa)
(bp)
~120
~90
HAC701
+/+ +/+ M M +/+ +/+
T1
WT
3C(S) 3J(S) 3F(S) 9A(S) 9B(S) 14A(S)
(bp)
~120
~90
HAC701
+/+ +/+ +/+ +/+ M +/+
C
HAC701 GTCCAGCCACTTGTGGGGTATTGCTGG Wild type
Line
3 3A GTCCAGCCACTTGTGGGGTAT-GCTGG (1/4)
GTCCAGCCACTTGTGGG----TGCTGG (2/4)*
GTCCAGCCACTTGTGGGGGAT-GCTGG (1/4)
3B GTCCAGCCACTTGTGGGGTATTT GCTGG (1/4)
3C GTCCAGCCACTTGTGGG----TGCTGG (4/4)*
3D GTCCAGCCACTTGTGGG----TGCTGG (2/4)*
GTCCAGCCACTTGTGGGGTATTT GCTGG (1/4)
GTCCAGCCACTTGTGGGGTAT-GCTGG (1/4)
3E GTCCAGCCACTTGTGGG----TGCTGG (2/4)*
GTCCAGCCACTTGTGGGG----GCTGG (1/4)
GTCCAGCCACTTGGGGG----TGCTGG (1/4)
3F GTCCAGCCACTTGTGGGGTAT-GCTGG (1/4)
GTCCAGCCACTTGTGGG----TGCTGG (3/4)*
3J GTCCAGCCACTTGTGGG----TGCTGG (4/4)*
Line
9 9A GTCCAGCCACTTGTGG-------CTGG (3/4)*
GTCCAGCCACTTGTGGGGT---GCTGG (1/4)
9B GTCCAGCCACTTGTGGG-------TGG (1/4)
GTCCAGCCACTTGTGGGGG---GCTGG (1/4)
GTCCAGCCACTTGTGG -------CTGG (2/4)*
14 14A GTCCAGCCACTTGTGGGGTAT----GG (1/4)
GTCCAGCCACTTGTGGGGTAT-GCTGG (1/4)
GTCCAGCCACTTGTGGGG--TTGCTGG (2/4)
14B GTCCAGCCACTTGTGGGGTATTT GCTGG (1/4)
GTCCAGCCACTTGTGGG----TGCTGG (1/4)
14D GTCCAGCCACTT----------TGG (1/4)
GTCCAGCCACTTGTGGGGTAT-GCTGG (2/4)
GTCCAGCCACTTGTGGGG-----CTGG (1/4)
D
HAC701
(Os0gt0246100)
Target 2
PAM
AAAGGTCCCTGACCGGTTGTGGG
F
R
UTR
ATG
Protein Domains
HAC701
Target 2
KIX
PHD
KAT
ZZ TAZ
G
HAC701-S5 lines (T3)
9-WT
9-4 -/-
9-5 -/-
9-12a -/-
9-12b -/-
E
T0
T1
WT
1-2 2-1 2-3
2-3-1 2-3-2 2-3-3 2-3-4
(bp)
~300
~200
HAC701
M M M M M B M
F
Number of Effective Grains
P value: 0.018492
NS
Genotype (T2)
Tiller Number
Genotype (T2)
9-WT
9-4-/-
9-5-/-
9-12a-/-
9-12b-/-
H
Relative mRNA level (HAC703/ACT1)
Relative mRNA level (HAC704/ACT1)
I
1000-grain weight (g)
Genotype (T3)
Genotype (T4)

Figure S2

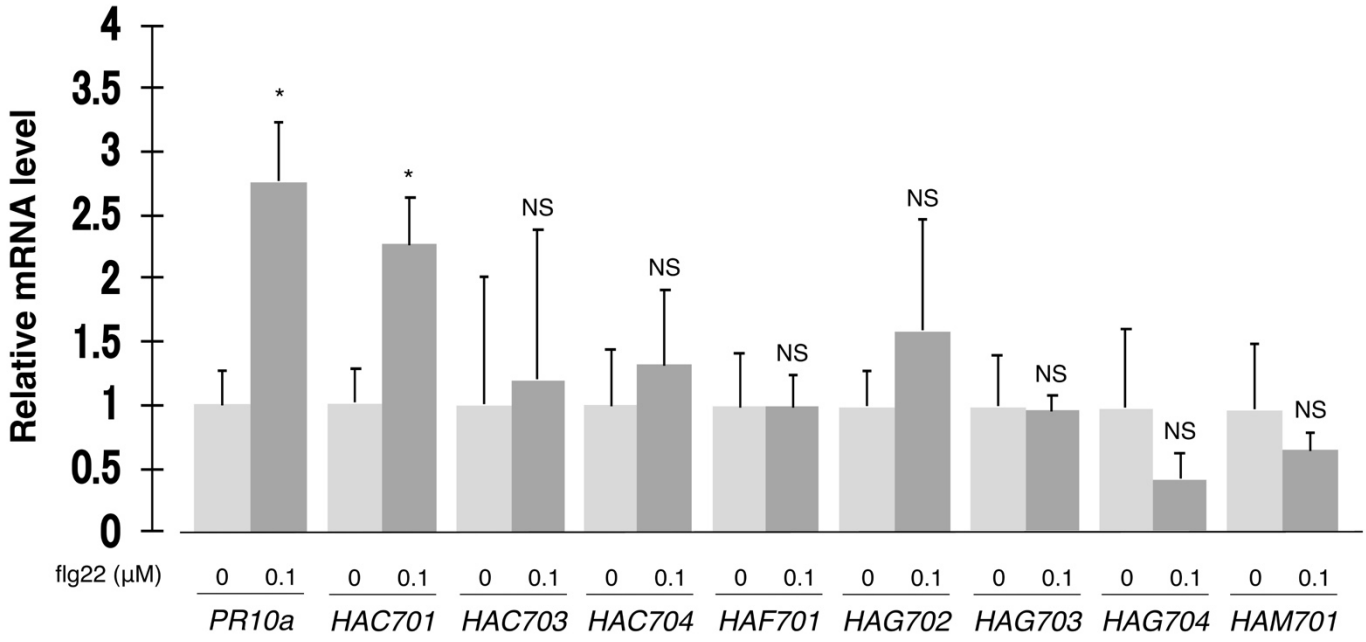

Relative mRNA level
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
NS
NS
NS
NS
NS
NS
NS
flg22 (μM)
0 0.1
PR10a
HAC701
HAC703
HAC704
HAF701
HAG702
HAG703
HAG704
HAM701

Figure S3

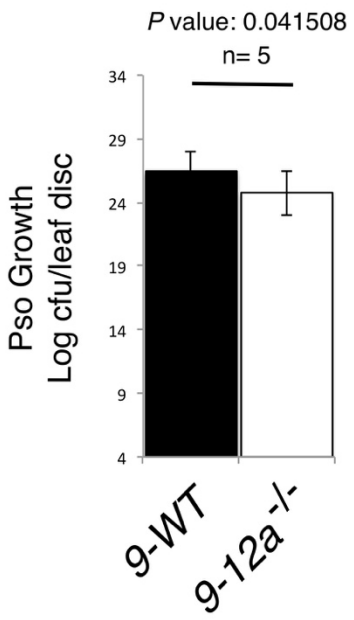

P value: 0.041508
n= 5
Pso Growth
Log cfu/leaf disc
34
29
24
19
14
9
4
9-WT
9-12a -/-

Figure S4

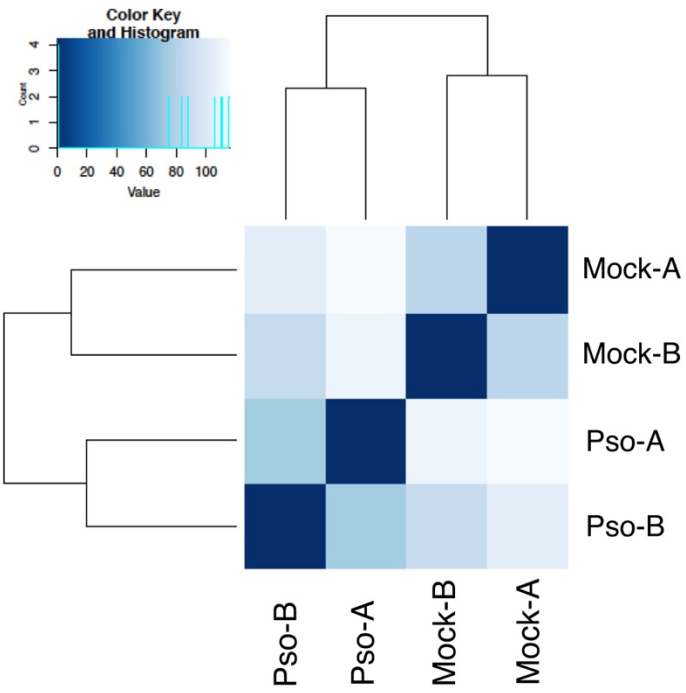
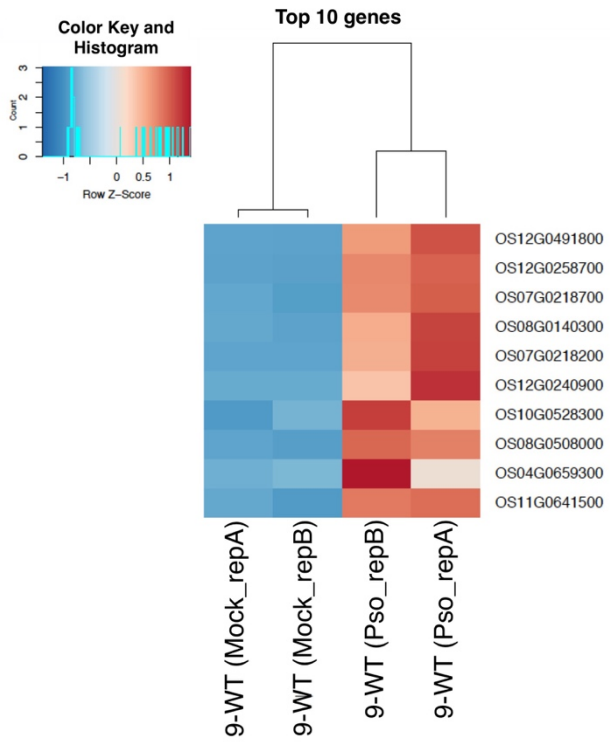

Color Key
and Histogram
Count
0
1
2
3
4
0
20
40
60
80
100
Value
Mock-A
Mock-B
Pso-A
Pso-B
Pso-B
Pso-A
Mock-B
Mock-A

Figure S5



Gene ID	Description
OS12G0491800	Kaurene synthase-like 10
OS12G0258700	Laccase 29
OS07G0218700	Cytochrome P450 family protein
OS08G0140300	Tryptophan decarboxylase 1
OS07G0218200	Terpene synthase 3
OS12G0240900	Naringenin 7-O-methyltransferase
OS10G0528300	Tau glutathione S-transferase 4
OS08G0508000	Cytochrome P450 76M2
OS04G0659300	Root meander curling
OS11G0641500	Laccase 23

Figure S6

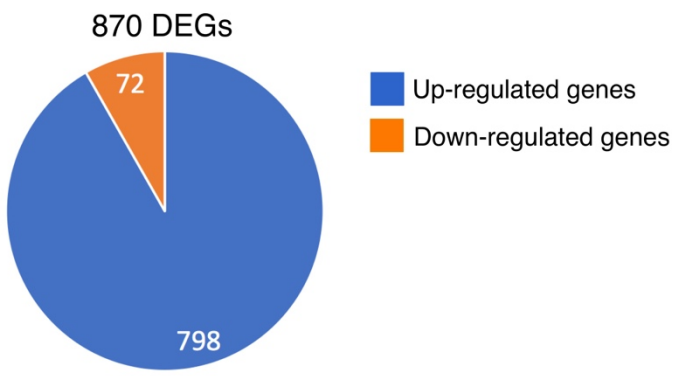
A

Highly enriched terms upon *Pseudomonas syringiae pv. oryzae* infection in 9-WT rice

Up-regulated terms	Fold Enrichment	raw P-value
Cell wall macromolecule catabolic process	54.94	4.22E-07
Adventitious root development	54.94	3.54E-02
Lignin biosynthetic process	54.94	3.54E-02
Sphingolipid biosynthetic process	54.94	3.54E-02
Gibberellin biosynthetic process	54.94	3.54E-02
Chitin catabolic process	54.94	4.22E-07
Response to wounding	54.94	3.54E-02
Response to other organism	54.94	2.70E-08
ent-kaurene oxidation to kaurenoic acid	54.94	3.54E-02
Diterpene phytoalexin precursor biosynthetic process pathway	41.21	1.89E-04
Shikimate biosynthetic process	36.63	3.08E-03
Chorismate biosynthetic process	36.63	3.08E-03
Carbohydrate transport	18.31	8.32E-03
Carbohydrate metabolic process	5.78	6.62E-03
Electron transport chain	5.49	1.17E-03
Regulation of transcription, DNA templated	2.79	2.43E-02

Down-regulated terms	Fold Enrichment	raw P-value
Regulation of transcription, DNA templated	10.26	1.69E-02

B



9-WT in *Pso* treatment

Figure S7

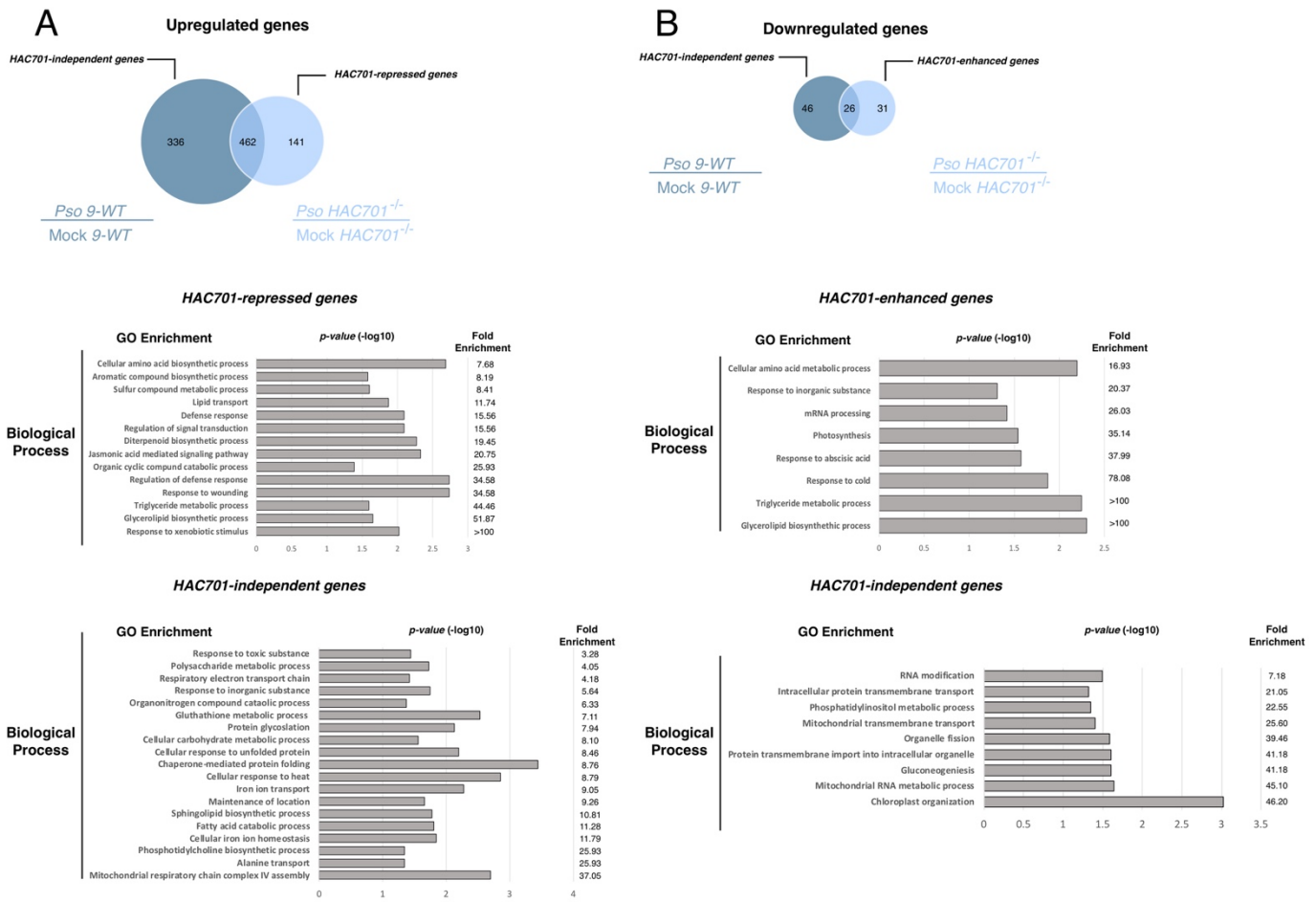
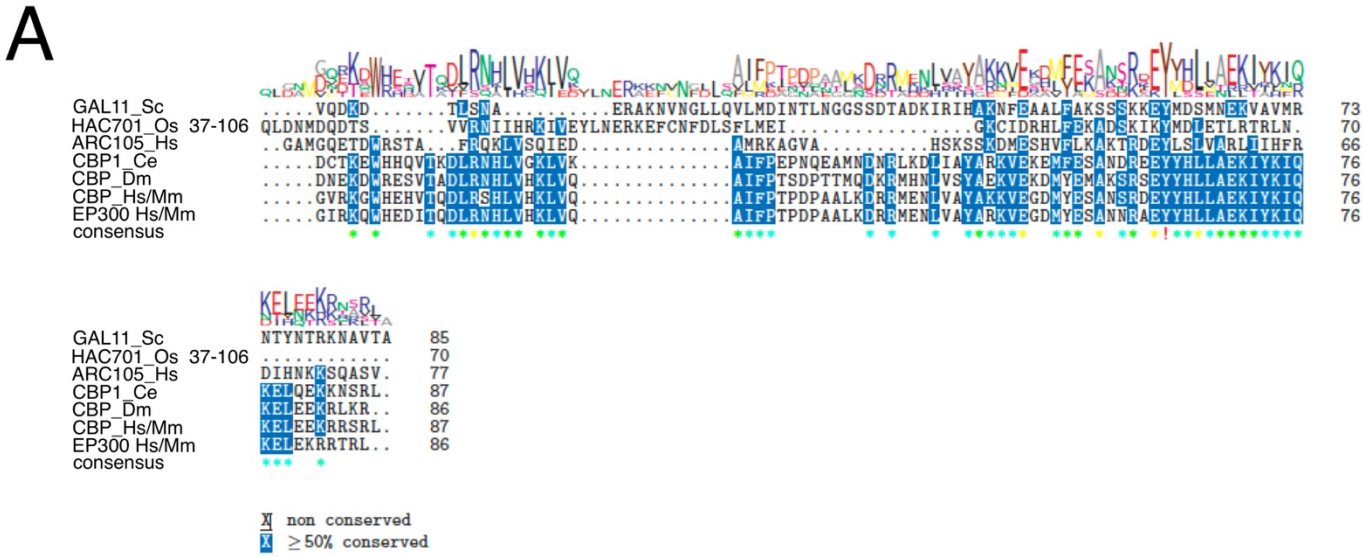

A
Upregulated genes
HAC701-independent genes
HAC701-repressed genes
336
462
141
Pso 9-WT
Mock 9-WT
Pso HAC701-/-
Mock HAC701-/-
B
Downregulated genes
HAC701-independent genes
HAC701-enhanced genes
46
26
31
Pso 9-WT
Mock 9-WT
Pso HAC701-/-
Mock HAC701-/-
HAC701-repressed genes
GO Enrichment
p-value (-log10)
Fold Enrichment
Biological Process
Cellular amino acid biosynthetic process 7.68
Aromatic compound biosynthetic process 8.19
Sulfur compound metabolic process 8.41
Lipid transport 11.74
Defense response 15.56
Regulation of signal transduction 15.56
Diterpenoid biosynthetic process 19.45
Jasmonic acid mediated signaling pathway 20.75
Organic cyclic compund catabolic process 25.93
Regulation of defense response 34.58
Response to wounding 34.58
Triglyceride metabolic process 44.46
Glycerolipid biosynthetic process 51.87
Response to xenobiotic stimulus >100
0 0.5 1 1.5 2 2.5 3
HAC701-enhanced genes
GO Enrichment
p-value (-log10)
Fold Enrichment
Biological Process
Cellular amino acid metabolic process 16.93
Response to inorganic substance 20.37
mRNA processing 26.03
Photosynthesis 35.14
Response to abscisic acid 37.99
Response to cold 78.08
Triglyceride metabolic process >100
Glycerolipid biosynthethic process >100
0 0.5 1 1.5 2 2.5
HAC701-independent genes
GO Enrichment
p-value (-log10)
Fold Enrichment
Biological Process
Response to toxic substance 3.28
Polysaccharide metabolic process 4.05
Respiratory electron transport chain 4.18
Response to inorganic substance 5.64
Organonitrogen compound cataolic process 6.33
Gluthathione metabolic process 7.11
Protein glycoslation 7.94
Cellular carbohydrate metabolic process 8.10
Cellular response to unfolded protein 8.46
Chaperone-mediated protein folding 8.76
Cellular response to heat 8.79
Iron ion transport 9.05
Maintenance of location 9.26
Sphingolipid biosynthetic process 10.81
Fatty acid catabolic process 11.28
Cellular iron ion homeostasis 11.79
Phosphotidylcholine biosynthetic process 25.93
Alanine transport 25.93
Mitochondrial respiratory chain complex IV assembly 37.05
0 1 2 3 4
HAC701-independent genes
GO Enrichment
p-value (-log10)
Fold Enrichment
Biological Process
RNA modification 7.18
Intracellular protein transmembrane transport 21.05
Phosphatidylinositol metabolic process 22.55
Mitochondrial transmembrane transport 25.60
Organelle fission 39.46
Protein transmembrane import into intracellular organelle 41.18
Gluconeogeniesis 41.18
Mitochondrial RNA metabolic process 45.10
Chloroplast organization 46.20
0 0.5 1 1.5 2 2.5 3 3.5

Figure S8


A
GAL11_Sc
HAC701_Os 37-106
ARC105_Hs
CBP1_Ce
CBP_Dm
CBP_Hs/Mm
EP300 Hs/Mm
consensus
X non conserved
X ≥50% conserved

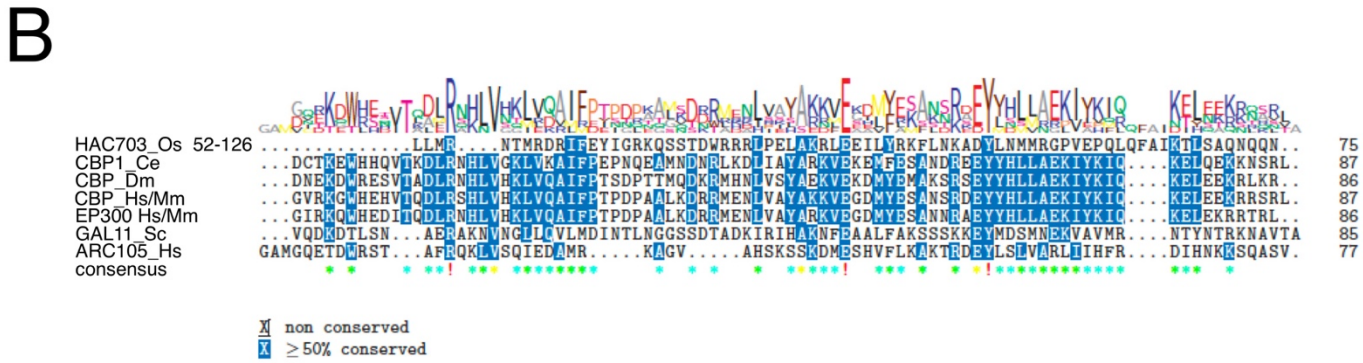
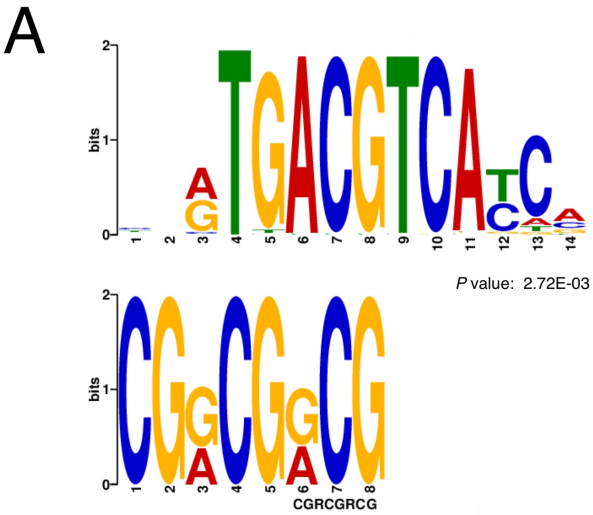
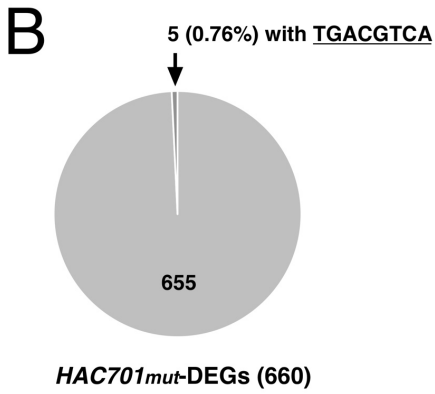
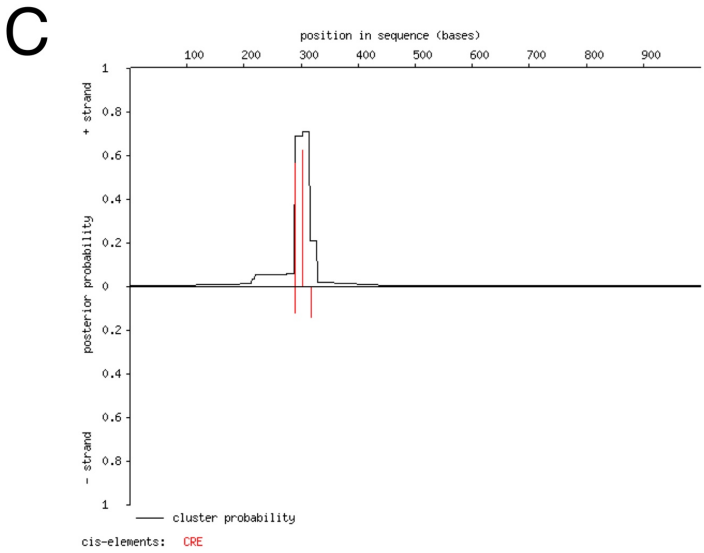

B
HAC703_Os 52-126
CBP1_Ce
CBP_Dm
CBP_Hs/Mm
EP300 Hs/Mm
GAL11_Sc
ARC105_Hs
consensus
X non conserved
X ≥50% conserved

Figure S9







Possible functional cis-elements

Type	Position	Strand	Sequence	Probability
CRE	303-314	+	cgtgacgtcatc	0.62
CRE	289-300	+	gctgacgtcgcc	0.56
CRE	317-328	-	gttgtcgtcccc	0.14
CRE	289-300	-	gctgacgtcgcc	0.12

Figure S10

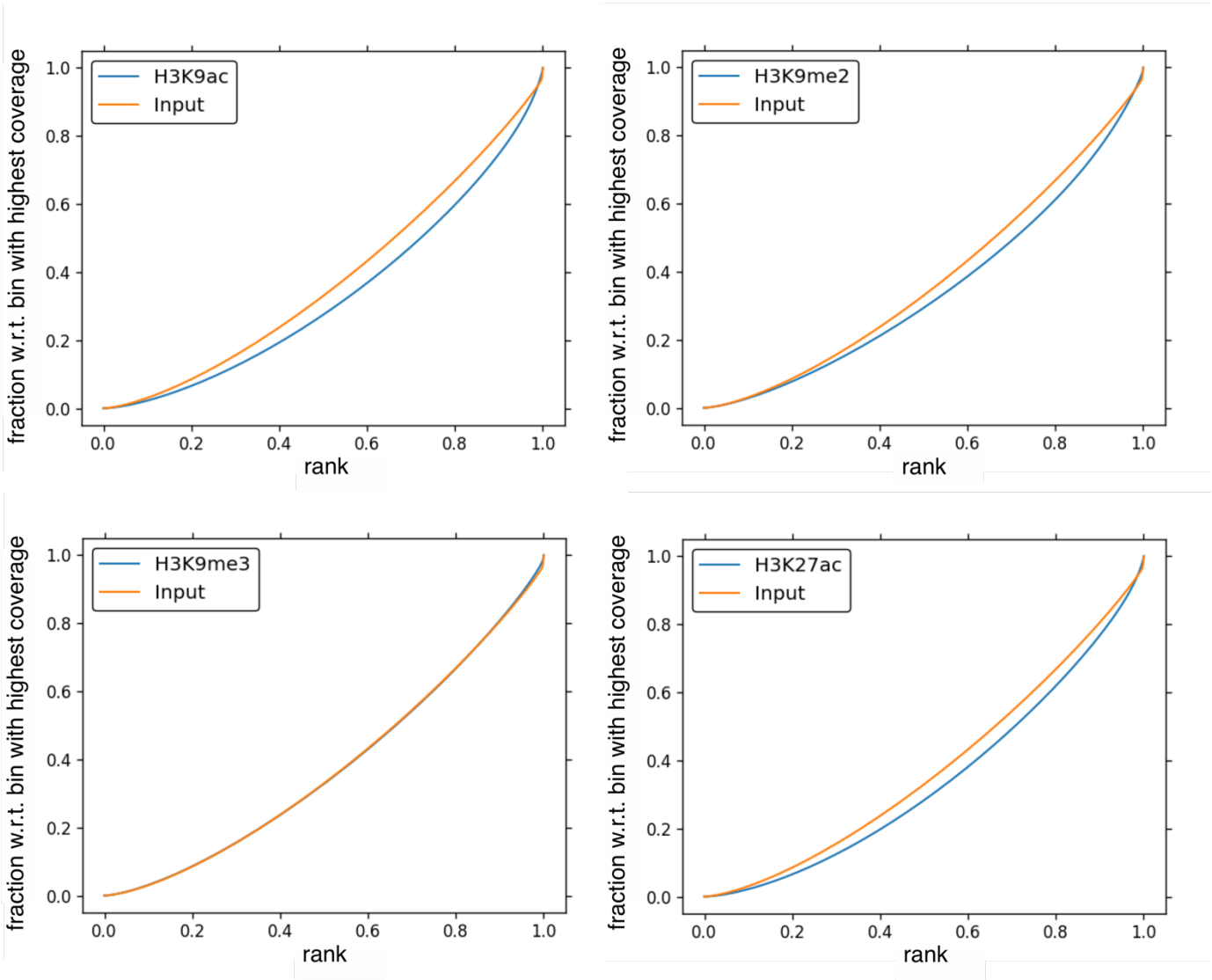

H3K9ac
Input
H3K9me2
Input
H3K9me3
Input
H3K27ac
Input
fraction w.r.t. bin with highest coverage
rank
0.0
0.2
0.4
0.6
0.8
1.0

Figure S11

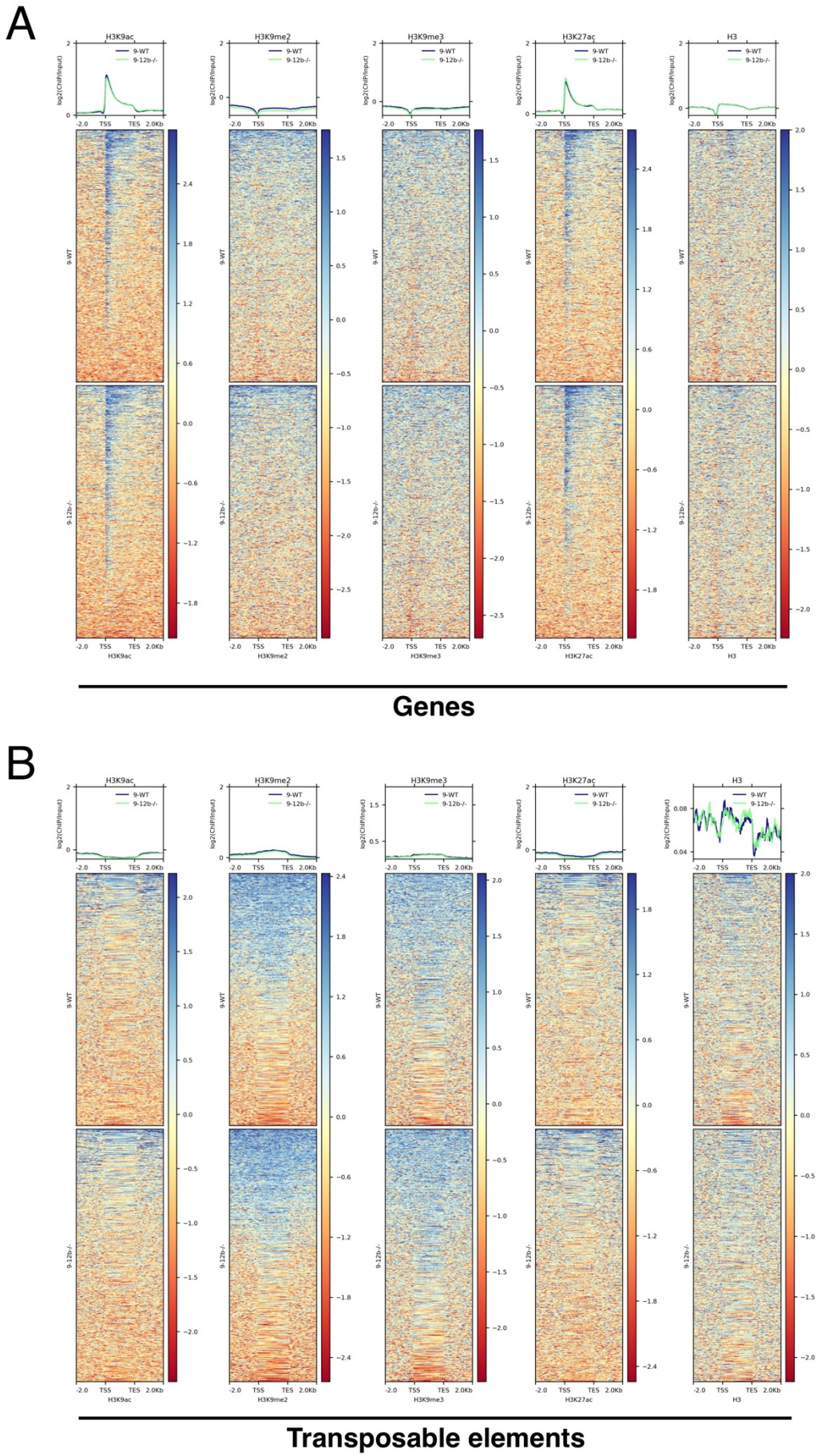
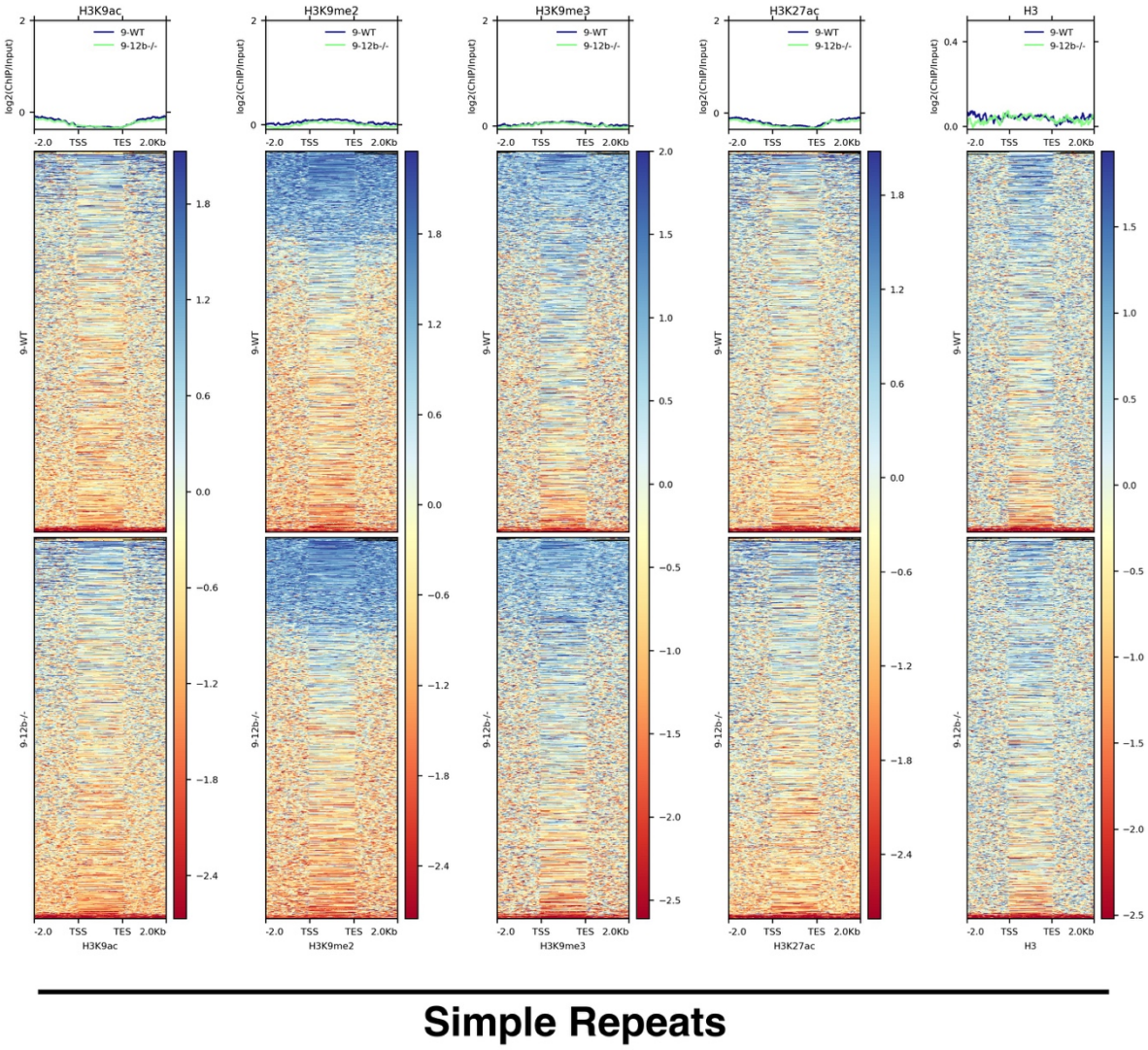

A
H3K9ac
H3K9me2
H3K9me3
H3K27ac
H3
9-WT
9-12b-/-
log2(ChIP/Input)
-2.0 TSS TES 2.0Kb
Genes
B
Transposable elements

Figure S12



Simple Repeats

Figure S13

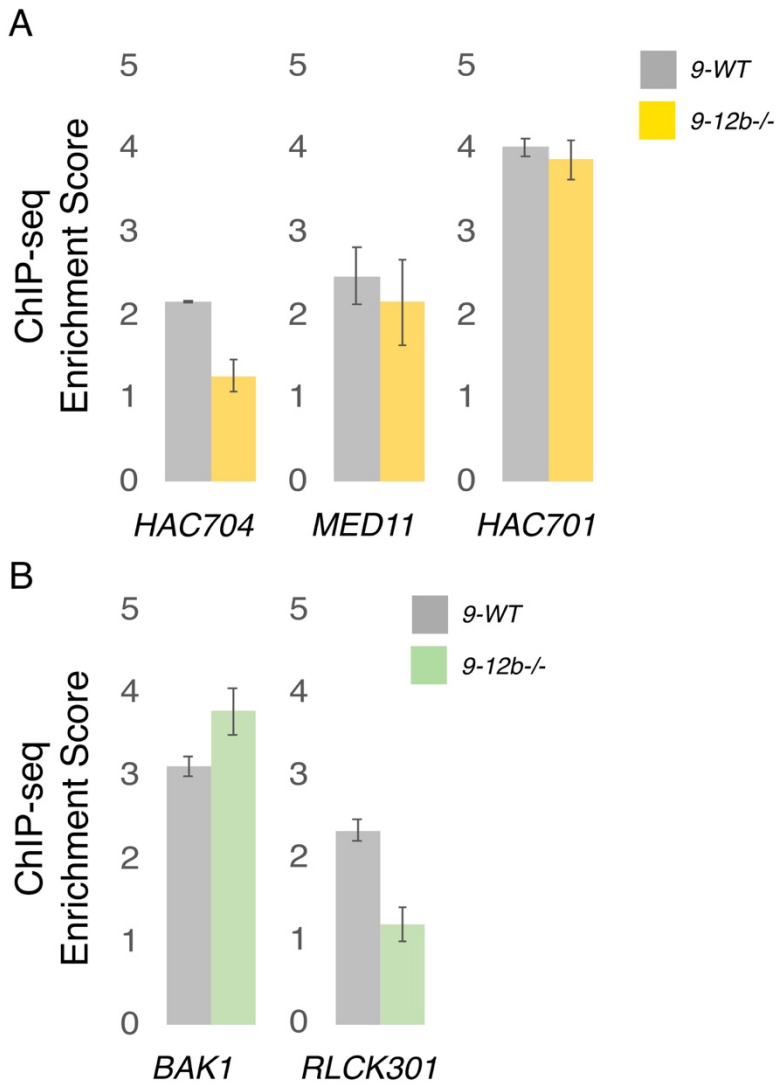

A
ChIP-seq
Enrichment Score
0
1
2
3
4
5
HAC704
MED11
HAC701
9-WT
9-12b-/-
B
ChIP-seq
Enrichment Score
BAK1
RLCK301
9-WT
9-12b-/-

Figure S14

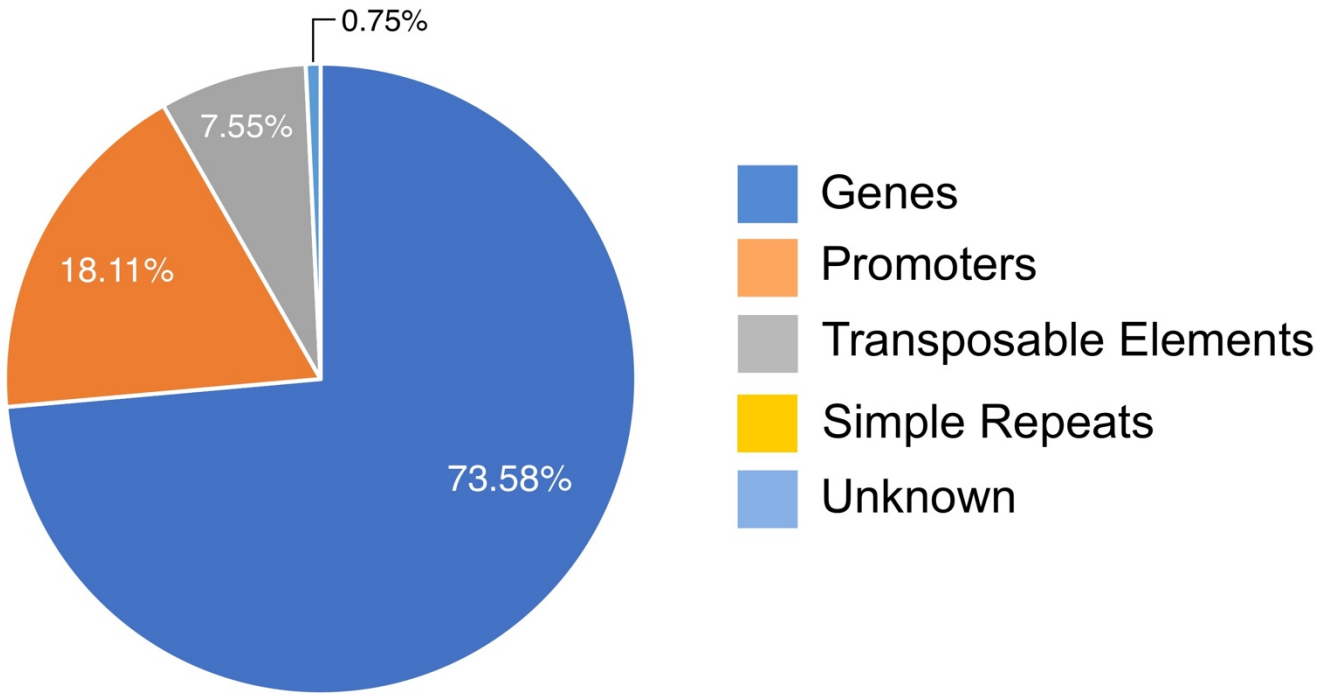

0.75%
7.55%
18.11%
73.58%
Genes
Promoters
Transposable Elements
Simple Repeats
Unknown

Figure S15

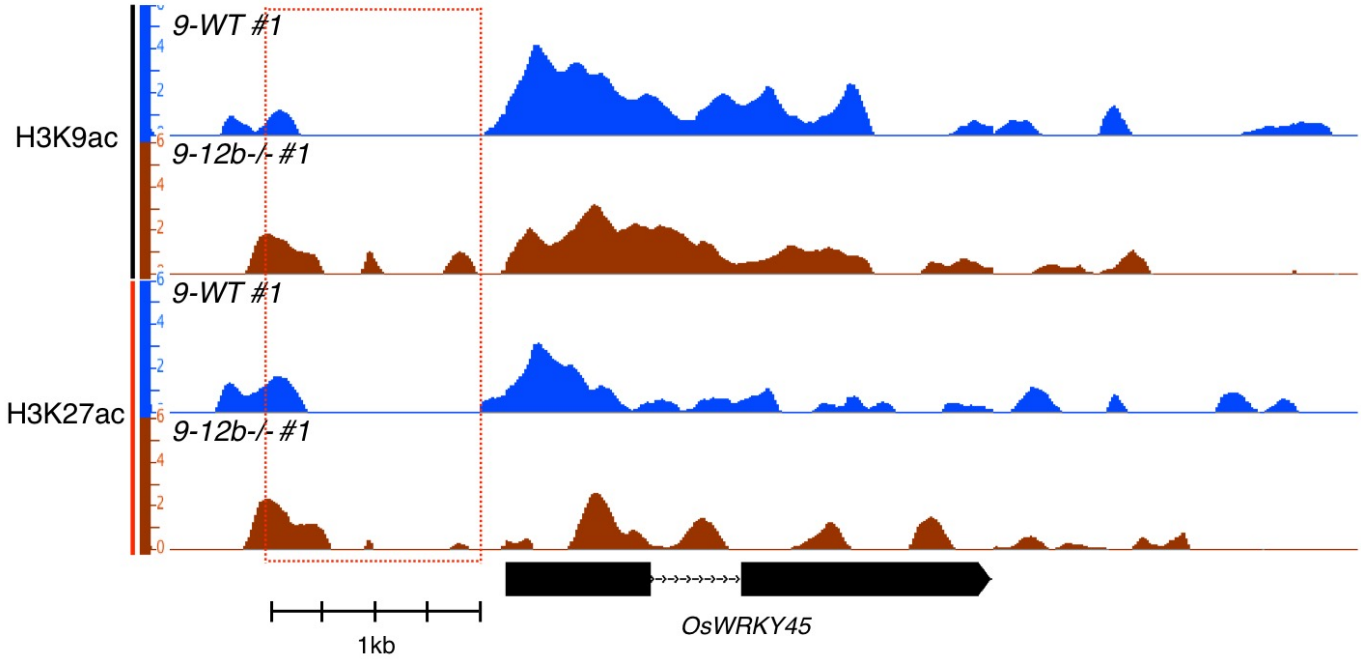


Figure S16

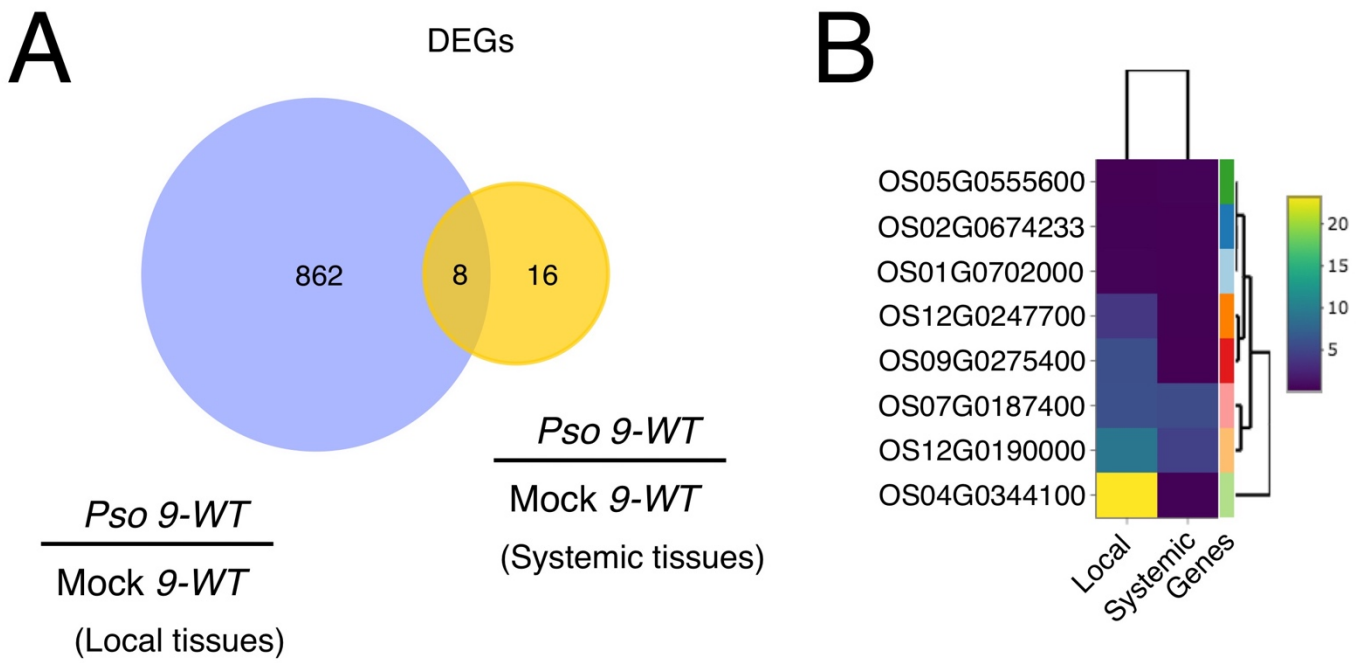
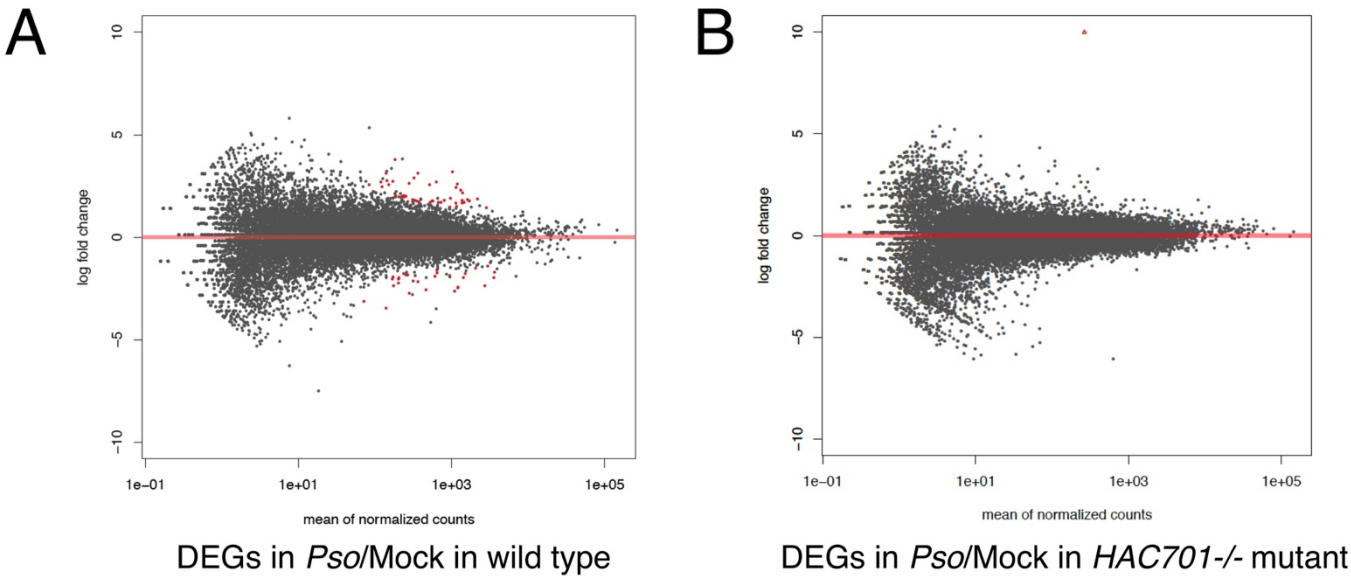
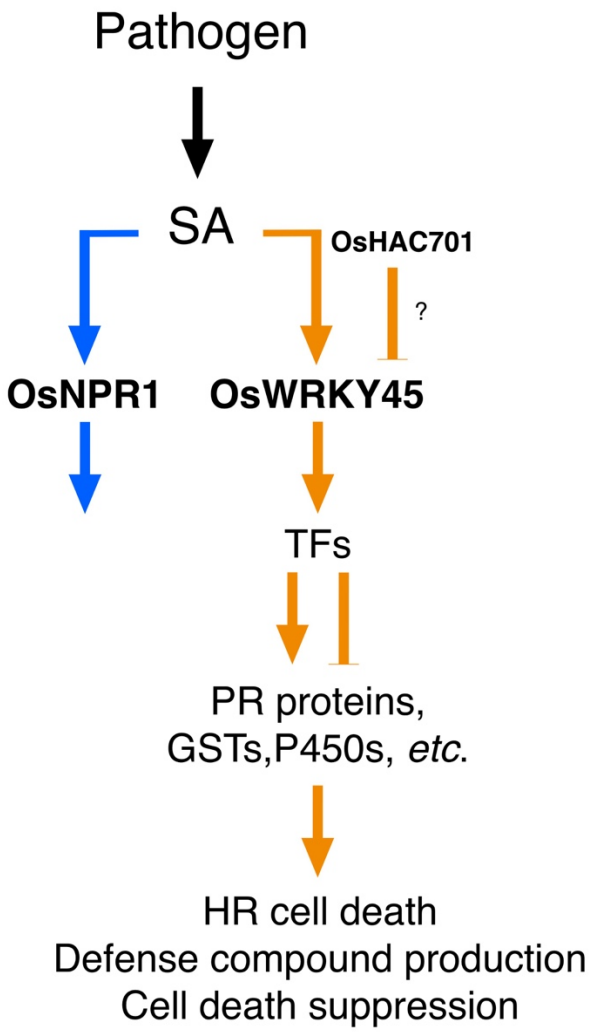

A
DEGs
862
8
16
Pso 9-WT
Mock 9-WT
(Local tissues)
Pso 9-WT
Mock 9-WT
(Systemic tissues)
B
OS05G0555600
OS02G0674233
OS01G0702000
OS12G0247700
OS09G0275400
OS07G0187400
OS12G0190000
OS04G0344100
Local
Systemic
Genes
20
15
10
5

Figure S17



Systemic tissues

Figure S18


Pathogen
SA
OsHAC701
?
OsNPR1
OsWRKY45
TFs
PR proteins,
GSTs,P450s, *etc.*
HR cell death
Defense compound production
Cell death suppression