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PIF7-mediated epigenetic reprogramming promotes the transcriptional response to shade in *Arabidopsis*

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16 **Abstract**

17 For shade-intolerant plants, changes in light quality are taken as indicative of
18 competitions from neighbors and thus trigger shade avoidance syndrome
19 (SAS), with a series of morphological and physiological alterations that are
20 detrimental to plant health and consequently crop yield.

21 PHYTOCHROME-INTERACTING FACTOR 7 (PIF7) is a major transcriptional
22 regulator of SAS in *Arabidopsis*. Here, we show that PIF7 directly interacts
23 with the histone chaperone ANTI-SILENCING FACTOR 1 (ASF1). The
24 ASF1-deprived *asf1ab* mutant showed defective shade-induced hypocotyl
25 elongation. HISTONE REGULATOR HOMOLOG A (HIRA), which mediates
26 deposition of the H3.3 variant into chromatin, is also involved in SAS.

27 RNA/ChIP-sequencing analyses identified ASF1 directly regulating a subset of
28 target genes of PIF7. Furthermore, shade-elicited gene activation is
29 accompanied by H3.3 enrichment, which is mediated by the PIF7-ASF1-HIRA
30 regulatory module. Collectively, our data reveal that PIF7 recruits ASF1-HIRA
31 to increase H3.3 incorporation into chromatin to promote gene transcription,
32 thus enabling plants to effectively respond to environmental shade.

33 Key words: ASF1 / H3.3 incorporation / HIRA / PIF7 / shade avoidance

34 **Introduction**

35 Plants are sessile organisms that adapt to various strategies by altering their
36 growth and development in response to environmental changes. As
37 photosynthetic organisms, plants are particularly sensitive to local light
38 environments. Light influences every developmental transition, from seed
39 germination and seedling emergence to flowering, reproduction, and seed
40 formation. Shade-intolerant plants can detect the proximity and density of
41 neighboring vegetation through perception of a reduced ratio of red (660 nm)
42 to far red (730 nm) light, which triggers the shade avoidance syndrome (SAS)
43 that includes responses, such as an increase in hypocotyl and internode
44 elongation, extended petioles, and changes in leaf hyponasty (Casal, 2013;
45 Ruberti et al., 2012). Prolonged shade exposure can lead to early flowering,
46 reduced branching, and decreased seed yields (Fernandez-Milmanda and
47 Ballare, 2021; Galvao et al., 2019; Zhang et al., 2019). As an adaptive strategy,
48 SAS allows shaded plants to grow and compete with their neighbors at the
49 cost of grain yield or plant biomass.

50 In *Arabidopsis thaliana*, shade-light filtered through dense planting is
51 perceived by photoreceptors (mainly known as phytochromes). The
52 constitutive SAS phenotype of *phytochrome B* (*phyB*) mutant indicates that
53 phyB plays a dominant role in inhibiting SAS (Reed et al., 1993). Shade-light
54 drives the Pfr-to-Pr conversion of phyB, allowing dephosphorylation and
55 accumulation of PHYTOCHROME-INTERACTING FACTORS (PIFs), a family
56 of basic helix-loop-helix (bHLH) transcription factors (Leivar and Quail, 2011;
57 Paik et al., 2017). The activation of PIFs, especially PIF7, promotes
58 shade-induced gene expression and SAS (Galvao et al., 2019; Li et al., 2012;
59 Zhang et al., 2019). Numerous studies have been reported about the
60 transcriptional activity and function (Jiang et al., 2019; Li et al., 2012; Peng et
61 al., 2018; Willige et al., 2021; Yang et al., 2021), translation (Chung et al.,
62 2020), subcellular localization (Huang et al., 2018), and protein stability (Zhou

63 et al., 2021) of PIF7, which have helped on understanding the molecular
64 mechanisms of SAS.

65 Yeast two-hybrid (Y2H) assays, conducted in our previous study (Huang
66 et al., 2018), revealed that ANTI-SILENCING FUNCTION 1 (ASF1) is one of
67 the putative PIF7-interacting proteins. ASF1 is an evolutionarily conserved
68 histone chaperone of the H3/H4 family (English et al., 2006; Natsume et al.,
69 2007). The molecular functions and biochemical mechanisms of ASF1 have
70 been well studied in animals and yeast. At DNA replication forks, ASF1 plays
71 an important role in regulating the supply of H3.1 and H4 to the CHROMATIN
72 ASSEMBLY FACTOR 1 (CAF1) in chromatin assembly (Eitoku et al., 2008;
73 English et al., 2006; Otero et al., 2014). ASF1 also provides H3.3-H4 dimers to
74 HISTONE REGULATORY HOMOLOG A (HIRA) for DNA
75 replication-independent nucleosome assembly. While the canonical H3.1 is
76 incorporated during DNA replication, the histone variant H3.3 is mainly
77 incorporated upon gene activation and enriched in chromatin at actively
78 transcribed genes (Ahmad and Henikoff, 2002; McKittrick et al., 2004; Shu et
79 al., 2014; Stroud et al., 2012). Yeast cells lacking *ASF1* display increased
80 frequency of genome instability (Prado et al., 2004; Ramey et al., 2004).
81 Deficiency of *ASF1* in vertebrates and flies leads to embryonic lethality
82 (Moshkin et al., 2002; Schulz and Tyler, 2006).

83 Two ASF1-homologs (ASF1A and ASF1B) are found in *Arabidopsis*,
84 which play redundant roles and are shown to participate in S-phase DNA
85 replication-dependent chromatin assembly (Zhu et al., 2011). The
86 loss-of-function *asf1ab* double mutant exhibits defective thermo tolerance
87 (Weng et al., 2014) and early-flowering phenotypes (Zhao et al., 2021). Many
88 HIRA-regulated genes were found belonging to a broad class of responsive
89 genes to the environmental changes (Duc et al., 2017; Nie et al., 2014),
90 however, whether and how ASF1 and HIRA coordinately regulate the
91 transcription of specific genes in plants is still poorly understood.

92 In this study, we report that ASF1 and HIRA are required for
93 shade-induced hypocotyl elongation. Our results indicated that ASF1 is
94 recruited by PIF7 to bind to a cluster of shade-responsive genes. The
95 PIF7-ASF1-HIRA complex promotes transcription by depositing H3.3 into
96 chromatin at the target genes. Combined with previous reports (Peng et al.,
97 2018; Willige et al., 2021), our study brings out that PIF7 recruits different
98 chromatin modulators and conducts diverse types of histone variants (H3.3
99 and H2A.Z) and modifications to reprogram chromatin structure in response to
100 shade.

101

102 **Results**

103 **PIF7 interacts with ASF1A and ASF1B**

104 Before verifying the interaction between PIF7 and ASF1, we first determined
105 the effect of light on *ASF1* expression. In *Arabidopsis*, ASF1A and ASF1B
106 showed high sequence conservation (approximately 77.44% similarity)
107 (Appendix Fig S1A). Both *ASF1A* and *ASF1B* mRNA levels were found stable
108 in Col-0 and *pif7* under different light/shade/dark treatments (Appendix Fig
109 S1B), which is consistent with the published RNA sequencing (RNA-seq) data
110 (Appendix Fig S1C, GSE81202). To investigate the effects of light on ASF1A
111 and ASF1B protein abundance, 6-d-old white light-grown *35S::GFP-ASF1A*
112 and *35S::ASF1B-GFP* transgenic seedlings were transferred from white light
113 to shade for 2 h. The protein levels of ASF1A and ASF1B (Appendix Fig
114 S1D-E) were found barely changed after shade treatment. Together, these
115 observations indicate that light does not significantly affect the transcription
116 and protein levels of ASF1.

117 To test the interaction between PIF7 and ASF1, we used a luciferase
118 complementation imaging (LCI) assay. As shown in Fig 1A, cLUC-tagged PIF7
119 could interact with nLUC-tagged ASF1A or ASF1B when they were transiently
120 expressed in *Nicotiana benthamiana* leaf cells. In an *in vitro* pull-down assay,

121 SUMO-His-PIF7 was bound by GST-fused ASF1A or ASF1B (Fig 1B,
122 Appendix Fig S2A). In a semi-*in vivo* pull-down assay, GFP-ASF1A extracted
123 from *GFP-ASF1A* seedlings were found to be bound by TF-His-PIF7 proteins
124 purified from *E. coli* (Appendix Fig S2B). Interestingly, the dephosphorylated,
125 but not the phosphorylated, PIF7-Flash (9×Myc-6×His-3×Flag) extracted from
126 *35S:PIF7-Flash* (Li et al., 2012) seedlings, was found to be bound by the
127 GST-fused ASF1 (Fig 1C). Finally, to further examine the interaction between
128 ASF1 and PIF7 *in vivo*, we crossed the *GFP-ASF1A* line with *PIF7-Flash* line
129 and performed the co-immunoprecipitation (Co-IP) assays. As expected, we
130 found that GFP-ASF1A formed protein complexes with dephosphorylated
131 PIF7-Flash (Fig 1D, Appendix Fig S2C). Taken together, these data strongly
132 indicate that PIF7 physically interacts with ASF1, both *in vitro* and *in vivo*.

133 Because human ASF1 and HIRA form protein complexes (Tang et al.,
134 2006), we also tested the interactions of *Arabidopsis* HIRA with ASF1 and
135 PIF7. We first confirmed the interaction between *Arabidopsis* ASF1A and
136 HIRA by performing an *in vitro* pull-down assay using TF-His-HIRA and
137 GST-ASF1A (Fig 1E, Appendix Fig S2A). We further found that TF-His-HIRA
138 could also be pulled down by GFP-PIF7, albeit to a lesser extent (Appendix Fig
139 S2D). Lastly, GFP-ASF1A simultaneously precipitated both HIRA-HA and
140 PIF7-Flash when co-expressed in tobacco leaves (Fig 1F). Based on these
141 observations, we conclude that ASF1, HIRA and PIF7 together form a
142 multiple-protein complex.

143

144 **The histone chaperone ASF1 and HIRA positively regulate** 145 **shade-induced hypocotyl elongation**

146 To investigate the function of ASF1 in SAS, we examined the hypocotyl
147 phenotypes of the *asf1a-2*, *asf1b-1*, and *asf1a-2asf1b-1* (*asf1ab*) mutants (Zhu
148 et al., 2011). Although the single mutant *asf1a-2* and *asf1b-1* showed no
149 difference in shade-induced hypocotyl elongation compared to the wild-type

150 Col-0, the double mutant *asf1ab* displayed a shade-defective phenotype,
151 similar to that of *pif7-1* (Fig 2A). The *hira-1* was lesser shade-defective than
152 *asf1ab*. The *pif7-1*, *asf1ab*, and *hira-1* seedlings all showed reduced growth
153 and slower growth rates than Col-0 seedlings during the first 10 h after shade
154 treatment (Fig 2B-C), indicating that these components are involved in
155 promoting early shade response.

156 To determine the relationship of ASF1 and HIRA with phyB, we generated
157 *asf1ab * phyB-9* and *hira-1 * phyB-9* mutants by crossing. These combined
158 mutants showed shorter hypocotyls than *phyB* under white light conditions (Fig
159 2D), indicating that the function of the photoreceptor phyB partially relies on
160 ASF1 and HIRA.

161 To investigate the functional interactions between PIF7 and ASF1, we
162 crossed *asf1ab* and *hira-1* with *pif7-1*, respectively. As expected, the *asf1ab **
163 *pif7-1* and *hira-1 * pif7-1* mutants showed hypocotyl lengths that were similar
164 to that of *pif7-1* (Fig 2E), suggesting that the function of ASF1 and HIRA in
165 shade-induced hypocotyl growth depends on PIF7.

166 In addition to SAS, PIF7 is also important for early responses to elevated
167 temperature in *Arabidopsis* seedlings (Burko et al., 2022; Chung et al., 2020;
168 Fiorucci et al., 2020). We found defective hypocotyl elongation of *asf1ab* and
169 *hira-1* at 29°C (Appendix Fig S3), suggesting that the PIF7-ASF1-HIRA
170 complex may also play a role in thermomorphogenesis besides shade
171 response. Nonetheless, hereinafter, we focus on shade response to uncover
172 molecular mechanism underlying the PIF7-ASF1-HIRA regulation in hypocotyl
173 elongation.

174

175 **PIF7, ASF1, and HIRA are involved in shade-induced gene expression**

176 To investigate the roles of ASF1 and HIRA in shade-induced transcription, we
177 performed RNA-seq analysis in *asf1ab* and *hira-1* mutants, compared it with
178 *pif7-1* (Appendix Fig S4A-C). After 1 h shade stimuli, 642 shade-induced

179 genes were identified in Col-0 seedlings (fold change > 2 and *P*-value < 0.01,
180 Dataset EV1). The expression of these genes was compromised in *pif7-1*, as
181 well as in *asf1ab* and *hira-1* mutants to varying degrees (Fig 3A-B, Dataset
182 EV1). Specifically, there are 374, 222, and 258 genes showing reduced shade
183 induction in *pif7-1*, *asf1ab*, and *hira-1*, respectively (Fig 3C, Dataset EV2).
184 Hereinafter we called these genes as PIF7-regulated, ASF1-regulated and
185 HIRA-regulated shade-responsive genes, respectively. Furthermore, 132
186 common genes showed reduced shade induction in all three mutants (Fig 3C).
187 Gene ontology (GO) analysis revealed that these common genes were
188 enriched in functional categories, including auxin-activated signaling pathway,
189 response to auxin, and transcription DNA-templated (Fig 3D, Table EV1).

190 Remarkably, in response to shade stimuli, 77.2%, 66.7%, and 70.8% of
191 differentially expressed genes (DEGs) were down-regulated in *pif7-1*, *asf1ab*,
192 and *hira-1*, respectively, indicating a positive effect of PIF7, ASF1, and HIRA
193 on the transcriptional activation of these genes under shade (Appendix Fig
194 S4D, Dataset EV3). Among the identified DEGs, the expression patterns of
195 light related genes (*ATHB2*, and *NPY8*), hormone related genes (*PIN7*, *BIM1*,
196 and *ARL*), and developmental growth-related genes (*CSLC4*) were further
197 verified and confirmed using quantitative RT-PCR analysis (Appendix Fig S4E).
198 Based on the RNA-seq and qRT-PCR data, we conclude that PIF7, ASF1, and
199 HIRA act in transcriptional activation and regulate a substantial common
200 subset of shade-related genes.

201

202 **Shade increases ASF1-enrichment in the chromatin of target genes of** 203 **PIF7**

204 To investigate the chromatin-binding activity of ASF1 in response to shade
205 exposure, we performed chromatin immunoprecipitation sequencing
206 (ChIP-seq) analysis using anti-GFP antibodies on white light- and
207 shade-grown *GFP-ASF1A* transgenic *Arabidopsis* plants. Approximately 6,259

208 peaks (for 5,662 genes) under white light and 9,031 peaks (for 7,950 genes)
209 after 1 h of shade treatment were identified as high-confidence
210 GFP-ASF1A-enriched peaks (Fig 4A, fold change > 1.5, P -value < 0.01,
211 Dataset EV4). Examination of shade-induced GFP-ASF1A-enrichment
212 distributions revealed that 57.24% of the binding occurred at the coding exon,
213 and 29.91% of them were found at the promoter (Fig 4B, Dataset EV5).

214 The numbers of GFP-ASF1A-enriched peaks were slightly increased
215 under shade. Compared to that in white light conditions, the genome-wide
216 GFP-ASF1A-enrichment was increased in response to shade. Specifically, 2,701
217 peaks (for 2,566 genes) showed an increase (fold change > 1.5, P -value <
218 0.01; Dataset EV5), and these corresponding genes are called here after
219 referred to as shade-induced GFP-ASF1-bound genes. We found 161
220 shade-induced GFP-ASF1-bound genes (average density plot shown in
221 Appendix Fig S5A) that overlapped with DEGs in *asf1ab* (gene list in Dataset
222 EV3 and EV5), thus likely representing genes that are directly regulated by
223 ASF1 (GFP-ASF1A-targeted genes). GO analysis revealed that these genes
224 were over-represented by the “response to light stimulus,” and “response to
225 auxin,” were significantly enriched (Appendix Fig S5B, Table EV2).

226 Specifically, GFP-ASF1A enrichment was significantly increased under
227 shade in 642 shade-induced genes, but not at control genes (not regulated by
228 shade; Dataset EV2) (Fig 4C). Enhancement in enrichment was more
229 remarkable for 131 GFP-ASF1A bound genes (Appendix Fig S5C). These
230 results indicate that for a subset of shade-induced genes shade-mediated
231 gene activation is accompanied by ASF1 enrichment. The enrichment was
232 illustrated as integrative genomics viewer (IGV) screen shots for the selected
233 genes *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* (Fig 4D).

234 To examine the possible involvement of PIF7 in shade-induced
235 ASF1-bindings, we examined shade-induced GFP-ASF1A enrichment for the
236 182 PIF7-targeted (PIF7 bound and PIF7-regulated) shade-responsive genes

237 (Fig 4E, Dataset EV2). A shade-enhanced enrichment of GFP-ASF1A was
238 observed for 43 of these PIF7 targeted genes (Fig 4F, Dataset EV2), indicating
239 that their activation is associated with PIF7.

240 Then, we directly investigated the role of PIF7 in the binding of ASF1 to
241 target genes. For this, we used the anti-ASF1A antibody, which has been
242 proven to be effective in ChIP-PCR analyses (Weng et al., 2014).

243 Shade-induced enrichment of ASF1A at the selected genes *ATHB2*, *PIN7*,
244 *CSLC4*, *BIM1*, *ARL*, and *NPY8* was greatly reduced in *pif7-1* (Fig 4G) and
245 slightly reduced in *pifq* (Appendix Fig S5D), indicating that the recruitment of
246 ASF1A relies on PIFs, particularly PIF7.

247 Taken together, our results indicate that PIF7 recruits ASF1 for the
248 transcriptional activation of a subset of genes in response to shade.

249

250 **Shade induces H3.3 levels at a subset of shade-responsive genes**

251 Because ASF1 plays a role in the deposition of both H3.1 and H3.3 while HIRA
252 is considered as specific for H3.3 (Tagami et al., 2004), we speculated that
253 PIF7-ASF1-HIRA might regulate shade-induced gene expression by affecting
254 the balance of H3.1 and H3.3 assembly into chromatin. As a primary step, we
255 first performed ChIP-seq to examine the effect of shade exposure on the
256 genome-wide landscape distribution of H3.1 and H3.3. We selected HTR13 as
257 the representative for H3.1 and HTR5 for H3.3, both of which have been
258 described previously (Stroud et al., 2012). We generated *pHTR13::HTR13-HA*
259 and *pHTR5::HTR5-HA* transgenic lines and performed ChIP-seq analyses
260 using anti-HA antibodies, and obtained H3.1 and H3.3 density patterns (Fig 5,
261 Appendix Fig S6). Based on a cutoff fold change > 1.5 and *P*-value < 0.01, it
262 was found that HTR13 / HTR5 was enriched in up to 10,827 peaks (for 8,746
263 genes) / 14,623 peaks (for 12,125 genes) under white light, and 10,842 peaks
264 (for 8,527 genes) / 12,718 peaks (for 10,781 genes) after 1 h of shade
265 treatment (Dataset EV6 and Dataset EV7). The occupancy of HTR13 / HTR5

266 detected in this study showed good overlap with previously published datasets
267 obtained in Col-0 under white light (Wollmann et al., 2012) (Appendix Fig
268 S6A-B). Consistent with the previous studies (Stroud et al., 2012; Wollmann et
269 al., 2012), H3.1 was distributed across the gene body and associated with
270 transcriptionally silent regions in the genome (Appendix Fig S6C), whereas the
271 variant H3.3 was predominantly distributed towards the Transcriptional End
272 Site (TES) and positively correlated with the levels of gene expression
273 (Appendix Fig S6D).

274 Although the peak numbers of HTR13 and HTR5 were slightly reduced
275 under shade, it did not affect the global levels of histone H3.1 and H3.3 (Fig
276 5A-B, Appendix Fig S6E), which is consistent with the stable mRNA levels of
277 H3 genes (Appendix Fig S6F) and the protein levels of HTR13-HA and
278 HTR5-HA detected by western-blot analysis (Appendix Fig S6G). Interestingly,
279 for the 642 shade-induced genes, however, we found that shade increased
280 H3.3 level (Fig 5C) and no significantly changed the H3.1 level (Fig 5D),
281 indicating that shade-elicited gene activation is accompanied by H3.3
282 enrichment. As control, genes not regulated by shade (Dataset EV2) did not
283 show changes of H3.3 and H3.1 level under shade (Fig 5C-D). In parallel, we
284 also investigated whether shade-induced H3.3 enriched genes were
285 shade-responsive. Thus, the 1323 peaks (1246 protein-coding genes) that
286 displayed an increase in H3.3 level in response to shade (fold change > 1.5
287 and *P*-value < 0.01, Dataset EV8) were found to be transcriptionally slightly
288 induced under shade (Fig 5E, Dataset EV9). GO analysis revealed enrichment
289 of “response to auxin,” “transcription, DNA-templated,” and “brassinosteroid
290 mediated pathway,” related terms for the top 400 genes with gaining H3.3
291 incorporation and increasing expression upon shade in Col-0 (Fig 5F, Table
292 EV3), with the profiles of *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8*
293 showing as examples (Fig 5G).

294 To further examine the function of H3.3 in shade responses, we obtained
295 an H3.3 knockdown mutant (*h3.3kd*) (Wollmann et al., 2017). *h3.3kd* displayed
296 a defective phenotype for shade-induced hypocotyl elongation (Fig 5H), which
297 is largely similar to *asf1ab*. Moreover, the shade-induction of *ATHB2*, *PIN7*,
298 *CSLC4*, *BIM1*, *ARL*, and *NPY8* was impaired in *h3.3kd* (Fig 5I).

299 Taken together, our data indicate that activation of shade-induced genes
300 is associated with H3.3 enrichment and that H3.3 is required for proper a
301 subset of shade-induction gene transcription and hypocotyl elongation.

302

303 **Shade-induced H3.3 enriched levels rely on PIF7-ASF1-HIRA regulatory** 304 **module**

305 Next, we examined the association of PIF7 and ASF1 functions with
306 shade-induced H3.3 incorporation. We checked the overlap between
307 shade-induced ASF1-bound genes, PIF7-bound genes (GSE156584) (Yang et
308 al., 2021) and H3.3 enriched genes (Appendix Fig S7A). Genome ontology
309 analysis of these genes showed that “regulation of transcription,
310 DNA-templated,” “regulation of cell size,” and “shade avoidance,” were
311 significantly enriched (Appendix Fig S7B, Table EV4). We also found that the
312 enrichments of H3.3 were amplified by shade at PIF7-regulated
313 shade-responsive genes, ASF1-regulated shade-responsive genes, and
314 HIRA-regulated shade-responsive genes (Appendix Fig S7C). Moreover,
315 shade effect on H3.3 enrichment was aggravated at PIF7-targeted
316 shade-responsive genes (Appendix Fig S7C). Consistently, the transcriptional
317 levels of top 400 genes with gaining H3.3 incorporation and increasing
318 expression upon shade in Col-0 were found compromised in *pif7-1*, *asf1ab*,
319 and *hira-1* (Appendix Fig S7D).

320 To further test the role of PIF7 in facilitating shade-induced H3.3
321 incorporation, we profiled H3.3 occupancy in the *pif7-1* mutant using
322 *pHTR5::HTR5-HA/pif7-1* transgenic lines under white light and shade

323 conditions (Fig 6A). HTR5 was enriched in up to 16,710 peaks (for 13,418
324 coding genes) under white light, and 16,363 peaks (for 13,158 coding genes)
325 after 1 h of shade treatment in *pif7-1* (with cutoff fold change > 1.5 and *P*-value
326 < 0.01, Dataset EV10). The peaks of H3.3 detected in *pif7-1* largely
327 overlapped with that in Col-0 (Appendix Fig S7E), suggesting that PIF7 does
328 not affect the distribution pattern of H3.3 in the genome.

329 The shade-induced enrichment of H3.3 in Col-0 was clearly faint in the
330 *pif7-1* mutant (Fig 6B). Then we calculated the shade-enhanced H3.3 levels
331 (enrichment levels of HTR5 under shade minus the enrichment levels of HTR5
332 under white light) in Col-0 and *pif7-1*. For 642 shade-induced genes,
333 shade-enhanced H3.3 enrichment was strongly compromised in the *pif7-1*
334 mutant (Fig 6C). Moreover, H3.3 enrichments at the PIF7-targeted
335 shade-responsive genes, at the ASF1-bound and PIF7 targeted
336 shade-responsive genes, as well as at the HIRA-regulated shade-responsive
337 genes were compromised in *pif7-1* compared to Col-0 (Fig 6D and Appendix
338 Fig S7F). These results suggest that shade-enhanced PIF7 activity is a
339 prerequisite for H3.3 deposition and subsequent gene activation.

340 To further verify whether shade-enhanced H3.3 levels are dependent on
341 the PIF7-ASF1-HIRA regulatory module, we assessed HTR5 occupancy at
342 multiple selected genes in Col-0, *pif7-1*, *asf1ab*, and *hira-1* by ChIP-PCR using
343 *pHTR5::HTR5-HA* transgenic lines. The levels of shade-induced HTR5
344 enrichment were reduced in these three mutants at the 3'-ends of *ATHB2*,
345 *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* (Fig 6E).

346 Taken together, these results corroborate the previously established
347 functions of ASF1-HIRA in H3.3 deposition, thus facilitating nucleosome
348 formation (Horard et al., 2018), and indicate that PIF7 recruits ASF1-HIRA to
349 increase H3.3 incorporation to activate transcription on a subset of
350 shade-induced genes.

351

352 Discussion

353 Plant growth and development are predominantly postembryonic and occur in
354 response to environmental cues. This developmental plasticity is thought to be
355 an adaptation to the sessile lifestyle of plants. SAS is an excellent example of
356 the phenotypic plasticity in the environment. Genetic and epigenetic factors
357 are intertwined in a complex regulatory network for environmental adaptation
358 (Xiao et al., 2017; Zhao et al., 2020). Transcription factors play an important
359 role in environmentally triggered transcriptional reprogramming events, in
360 addition to acting as transcriptional switches, they can also recruit chromatin
361 modulators to increase the precision of regulation (Zhao et al., 2020).

362 PIFs serve as regulatory hubs that integrate environmental cues into
363 transcriptional scenario. In this study, we found that PIF7 directly interacts with
364 ASF1 to promote the expression of shade-responsive genes by mediating
365 H3.3 enrichment at the target genes of PIF7. Under shade conditions, PIF7
366 can also recruit histone acetylases to promote H4K5ac in some shade-induced
367 genes, e.g., *YUC8*, *YUC9*, and *PAR1* (Peng et al., 2018). However, *YUC8*,
368 *YUC9*, and *PAR1* showed stable levels of H3.3 enrichment under shade
369 treatment (Appendix Fig S8A). PIF7 recruits histone acetylase and EIN6
370 ENHANCER (EEN) to regulate H3K9ac and H2A.Z profiles at PIF7 core genes,
371 e.g., *ATHB2* (Willige et al., 2021). The results of our study revealed that there
372 only a few genes showed more than one epigenetic change in response to
373 shade or low R:FR (Appendix Fig S8B), such as *ATHB2* showing enhanced
374 H3.3 and H3K9ac but reduced H2A.Z (Appendix Fig S8C). Therefore, PIF7
375 may recruit different chromatin modulators (e.g. ASF1, EEN, MRG1/2, and
376 histone acetylase) and conducts diverse types of histone variants (H3.3 and
377 H2A.Z) and modifications (H4K5ac and H3K9ac) in an interactive or
378 non-interactive manner, likely depending on the target gene context, to
379 reprogram chromatin structure under shade. These epigenetic changes might
380 occur at different speeds in response to shade, and/or in different genes

381 involved in different steps/pathways of regulation of cell proliferation/expansion.
382 The complexity of epigenetic regulatory mechanisms might be advantageous
383 for sedentary plants to precisely control gene transcription in response to
384 dynamic environmental changes.

385 Most binding peaks of PIF7 occur in the promoter region (Willige et al.,
386 2021; Yang et al., 2021), approximately 25.1% and 6.4% of PIF7 binding
387 events occur in exons and introns, respectively (Willige et al., 2021). Our
388 results indicated that ASF1 binding events were mainly enriched in gene
389 bodies (Fig 4B). Shade-induced binding events of ASF1 and H3.3 enrichment
390 mainly occur at the 3'-end of the gene bodies (Fig 4 and 5). So how does PIF7
391 regulate the distribution of H3.3 on the 3'-end of the gene bodies? One
392 hypothesis is that PIF7 bound in gene bodies can directly recruit the
393 ASF1-HIRA complex, thereby affecting the enrichment of H3.3 and regulating
394 gene expression. Another hypothesis is that genes can be configured as
395 looped structures (Kadauke and Blobel, 2009). Recent studies have described
396 that active phyB forms a repressive chromatin loop with
397 VIN3-LIKE1/VERNALIZATION 5 (VIL1/VRN5) in growth-promoting genes (Kim
398 et al., 2021). The enriched H3.3 at the 3'-end of *FLOWERING LOCUS C (FLC)*
399 can enhance active histone modifications (H3K4me3) at the 5'-end by gene
400 loop formation (Zhao et al., 2021). The three-dimensional genome chromatin
401 loop structure juxtapose regulatory elements to regulate transcription with high
402 efficiency (Kadauke and Blobel, 2009). However, whether PIF7-ASF1-HIRA
403 affects chromatin loops at shade-responsive genes remains to be investigated
404 further.

405 Our results suggest that ASF1 is involved in shade-induced hypocotyl
406 elongation mainly by activating gene expression, which is correlated with
407 transcriptional activation of PIF7 (Appendix Fig S4D). In this study, we found
408 that ASF1 activates shade-responsive gene expression mainly by promoting
409 the H3.3 enrichment. The ASF1-HIRA complex has been reported to promote

410 *FLC* gene expression by enhancing H3.3 levels during flowering (Zhao et al.,
411 2021). Incorporation of H3.3 into nucleosome likely modulates higher-order
412 chromatin folding, resulting in an open chromatin conformation (Chen et al.,
413 2013). Nucleosomes are unstable and prone to disassembly, and this
414 instability seems to facilitate the access of transcription factors or other
415 chromatin-associated factors to these regulatory sites (Grover et al., 2018; Jin
416 et al., 2009; Venkatesh and Workman, 2015). ASF1 can promote H3K56ac in
417 heat stress-related genes to induce gene expression under heat stress
418 conditions (Weng et al., 2014). H3.3 deposition has been reported to be
419 correlated with H3K4me3 and H3K36me3 levels, which are associated with
420 gene activation (Daury et al., 2006; Loyola et al., 2006; Zhao et al., 2021).
421 However, significant differences were not detected in either H3K4me3 or
422 H3K36me3 levels in *YUC8*, *PRE1*, and *IAA19* after 1 h of shade treatment
423 (Peng et al., 2018). It will be interesting to further explore the histone
424 modifications that accompany H3.3 under shade conditions.

425 In addition to the PIF7-ASF1-HIRA regulatory module, which plays pivotal
426 roles in SAS, PIF7, together with its homolog PIF4, are important for
427 thermomorphogenesis (Chung et al., 2020; Fiorucci et al., 2020). Shade
428 avoidance responses share phenotypic patterns similar to that of
429 thermomorphogenesis (Ballare and Pierik, 2017; Burko et al., 2022). Both
430 shade and elevated temperature can deactivate phyB, leading to increased
431 PIFs and elongation (Ballare and Pierik, 2017; Legris et al., 2016). Similar to
432 *pif4* or *pif7-1*, defective hypocotyl elongation was also found in *asf1ab* and
433 *hira-1* at 29 °C, suggesting a role of PIF7-ASF1-HIRA complex in
434 thermomorphogenesis (Appendix Fig S3). However, whether ASF1-HIRA
435 functions together with PIFs in thermomorphogenesis remains to be
436 investigated further.

437 Based on the results of our study, we propose a working model for how
438 ASF1 activates the transcription on a subset of PIF7 target genes (Fig 6F).

439 Shade induces the dephosphorylation of PIF7, and enhances PIF7
440 DNA-binding activity (Li et al., 2012; Willige et al., 2021; Yang et al., 2021).
441 Transcription factor PIF7 mainly binds to promoters and recruits other factors
442 to induce gene expression (Peng et al., 2018; Willige et al., 2021). One of
443 factors recruited by PIF7 is ASF1, which affect H3.3 enrichment with the help
444 of HIRA. Our results demonstrate that PIF7-ASF1-HIRA function on the
445 enrichment of H3.3 and promote gene expression, leading to morphological
446 changes in plants in response to shade.

447

448 **Materials and Methods**

449 **Genetic material and growth conditions.** All *Arabidopsis thaliana* plants
450 used in this study were of the Col-0 ecotype. The mutants used in this study
451 have been previously described: *pif7-1*, *phyB-9*, and *PIF7-Flash* (Li et al.,
452 2012); *pifq* (Yang et al., 2021); *asf1a*, *asf1b*, and *asf1ab* (Zhu et al., 2011);
453 *hira-1* (Nie et al., 2014). *HTR5-HA* transgenic plants were obtained by
454 transformation of Col-0, *pif7-1*, *asf1ab*, and *hira-1* with vectors containing the
455 *HTR5* genomic DNA. *HTR13-HA* transgenic plants were obtained by
456 transformation of Col-0 with vectors containing the *HTR13* genomic DNA.
457 Full-length *ASF1A* cDNA was amplified using PCR and ligated into
458 *pCambia1300* to generate *pCambia1300-GFP-ASF1A*. These constructs were
459 transferred into *Agrobacterium tumefaciens* strain *GV3101* (WEIDI, Shanghai,
460 China). Transgenic plants were screened on half-strength Murashige and
461 Skoog (1/2 MS) nutrient medium (Duchefa Biochemie, Haarlem, Netherlands)
462 containing hygromycin and confirmed using immunoblot analysis. We
463 generated *pif7-1 * asf1ab*, *pif7-1 * hira-1*, *phyB-9 * asf1ab*, *phyB-9 * hira-1*, and
464 *GFP-ASF1A * PIF7-Flash* plants via crossing. Double mutants were generated
465 by genetic crossing and were verified using phenotypic inspection, PCR
466 genotyping, and/or sequencing.

467 For phenotypic analysis, seeds were allowed to germinate on plates
468 containing 1/2 MS medium (Duchefa Biochemie, Haarlem, Netherlands) with 1%
469 agar (Sangon, Shanghai, China) and without sucrose. After stratification, the
470 plates were incubated in growth chambers under continuous white light (R,
471 $\sim 20 \mu\text{mol m}^{-2} \text{s}^{-1}$; B, $\sim 20 \mu\text{mol m}^{-2} \text{s}^{-1}$; FR, $\sim 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 4 d at 22 °C. The
472 plates were either left in white light or transferred to simulated shade (R, ~ 20
473 $\mu\text{mol m}^{-2} \text{s}^{-1}$; B, $\sim 20 \mu\text{mol m}^{-2} \text{s}^{-1}$; FR, $\sim 60 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 4 d before
474 measuring the hypocotyl length. To examine hypocotyl elongation at high
475 temperatures, plants were grown at 22 °C under white light (R, $\sim 20 \mu\text{mol m}^{-2}$
476 s^{-1} ; B, $\sim 20 \mu\text{mol m}^{-2} \text{s}^{-1}$; FR, $\sim 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 3-4 d, and were further shifted
477 to 29°C or maintained at 22°C for 4 d. *Nicotiana benthamiana* plants were
478 grown at 26 °C under long-day conditions with 16-h light.

479 **Hypocotyl measurement.** Quantitative measurements of hypocotyls were
480 performed on scanned images of seedlings using ImageJ software. At least 20
481 seedlings were used per treatment or genotype. Kinetics of hypocotyl growth
482 was measured by a commercial high-throughput imaging platform,
483 DynaPlant® (Microlens Technology, Beijing, <http://www.dynaplant.cn/en>).
484 Seedlings for kinetics measurement were sown on 1/2 MS medium containing
485 2% phytigel (Solarbio, P8170) and grown under continuous white light, and
486 then the plates were transferred to simulated shade. The images of hypocotyl
487 growth were captured by the DynaPlant® platform once every 15 min for each
488 seedling with the physical resolution of 1.2 μm per pixel. The lengths of new
489 hypocotyl growth in the time-series images were quantified by DynaPlant
490 Analysis software which was provided by the manufacturer. The values shown
491 indicate the means with SEMs.

492 **Firefly luciferase complementation imaging assays.** The fragments
493 encoding *PIF7* were amplified by PCR and ligated into a *pCAMBIA2300-nLUC*
494 vector to produce *nLUC-PIF7*. The coding regions of *ASF1A* or *ASF1B* were

495 amplified by PCR and ligated into *pCAMBIA2300-cLUC* to produce
496 *ASF1A-cLUC* or *ASF1B-cLUC*. The resulting constructs were transformed into
497 *Agrobacterium* strain *GV3101*. *Agrobacterium* cells harboring different
498 constructs were then infiltrated into *N. benthamiana* leaves. Three days after
499 infiltration, luciferin (Promega, USA) (2.5 mM, 0.1% Triton X-100) was spread
500 before LUC activity was monitored by a Tanon 5500 chemical luminescence
501 imaging system (Tanon, China).

502 **Protein pull-down assay.** For Figure 1B, in the GST pull-down assay, the
503 SUMO-His-PIF7 protein was incubated with pretreated GST-ASF1A or
504 GST-ASF1B beads for 2 h. GST was used as the negative control. The beads
505 were resuspended in SDS-PAGE loading buffer and analyzed by SDS-PAGE
506 and immunoblotting using the anti-His antibody (GNI4110-HS).

507 For Appendix Figure S2B, in a semi-*in vivo* pull-down assay, total protein
508 was extracted from *GFP-ASF1A* plants grown in duplicate under white light for
509 8 d using extraction buffer (100 mM Tris-HCl [pH 7.5], 300 mM NaCl, 2 mM
510 EDTA, 1% Triton X-100, 10% glycerol, and protease inhibitor cocktail). Protein
511 extracts were centrifuged at 20,000 × g for 10 min, and the resulting
512 supernatant was incubated with pretreated TF-His-PIF7 beads for 2 h. GFP
513 was used as the negative control. Beads were resuspended in SDS-PAGE
514 loading buffer, analyzed using SDS-PAGE, and followed by immunoblotting
515 using the anti-GFP antibody (GNI4110-GP). For Figure 1C, total protein was
516 extracted from *PIF7-Flash* plants grown in duplicate under white light for 8 d,
517 thereafter, one of the duplicates was treated with shade for 1 h, and the other
518 was maintained under white light for an additional 1 h. Protein extracts were
519 centrifuged at 20,000 × g for 10 min, and the resulting supernatant was
520 incubated with pretreated GST-ASF1A or GST-ASF1B beads for 2 h. GST was
521 used as the negative control. The beads were resuspended in SDS-PAGE
522 loading buffer and analyzed by SDS-PAGE and immunoblotting using the
523 anti-MYC antibody (GNI4110-MC).

524 **Co-IP assay.** Total protein extracts were prepared from *GFP-ASF1A* *
525 *PIF7-Flash* seedlings grown in duplicate under white light for 8 d; thereafter,
526 one of the duplicates was treated with shade for 1 h, and the other was
527 maintained under white light for an additional hour. A Co-IP assay was
528 performed as previously (Peng et al., 2018). IP was performed using anti-MYC
529 agarose beads. Input and IP-resulting fractions were analyzed by western
530 blotting using anti-MYC and anti-GFP antibodies.

531 **RNA-Seq Analysis.** RNA-seq was performed as previously described (Yang
532 et al., 2020). In brief, seedlings were grown under white light conditions for 6 d
533 and treated with 1 h shade. Three biological replicates were prepared for each
534 genotype of the plants grown under light and shade conditions. Total RNA was
535 extracted from snap-frozen tissues using TRIzol reagent according to the
536 manufacturer's instructions (Invitrogen). RNA libraries were constructed and
537 sequenced using Majorbio (<http://www.majorbio.com/>). Differential expression
538 analysis was performed using DESeq2 with $|\log_2(\text{foldchange})| > \log_2(2)$ and
539 $P\text{-value} < 0.01$.

540 **Quantitative RT-PCR analysis.** Approximately 100 mg of seedlings grown on
541 1/2 MS media supplemented with 1% agar under different light conditions were
542 collected in Eppendorf tubes, frozen in liquid nitrogen, and ground to a fine
543 powder. Three biological replicates were prepared for each genotype of the
544 plants grown under light and shade conditions. Total RNA was extracted using
545 a TRIzol kit (Promega, USA). Two micrograms of total RNA were reverse
546 transcribed using a First Strand cDNA Synthesis Kit (TIANGEN, China)
547 according to the manufacturer's instructions. The cDNAs were then subjected
548 to real-time qPCR using a CFX Connect Real-Time System (Bio-Rad, USA)
549 and SYBR Green qPCR Mix (Mei5 Biochem, China). Three biological
550 replicates per sample were used for the qRT-PCR analysis. The data are
551 presented as means with the SDs of three biological replicates normalized to
552 the expression of the reference gene *AT2G39960* (Li et al., 2012). The

553 comparative $\Delta\Delta C_t$ method was used to evaluate the relative quantities of each
554 amplified product in the samples. The specificity of the qRT-PCR reactions
555 was determined by melt curve analysis of the amplified products using the
556 standard method. Primers used are listed in Table EV5.

557 **ChIP-Seq Analysis.** ChIP-seq assays were performed as previously
558 described (Yang et al., 2020). For *GFP-ASF1A*, *HTR13-HA/Col-0*,
559 *HTR5-HA/Col-0*, and *HTR5-HA/pif7-1* ChIP-seq, two biological replicates were
560 prepared for each genotype of plants grown under light and shade conditions.
561 White light grown seedlings treated with 1 h shade was quickly plucked from
562 plates to perform ChIP assays. Approximately 4 g fresh seedlings were
563 collected and treated with Fix Buffer [0.4 M sucrose, 10 mM Tris-HCl (pH 8.0),
564 1 mM EDTA, 1 mM PMSF, 1% formaldehyde, 1 × protease inhibitor cocktail
565 tablet (Roche)] under vacuum for about 15 min. Fixation was quenched by
566 adding 0.125 M glycine and infiltrated for 7 min, after which the seedlings were
567 washed three times with cold water and rapidly frozen with liquid nitrogen. The
568 tissue was resuspended in extraction buffer 1 [0.4 M sucrose, 10 mM Tris-HCl
569 (pH 8.0), 10 mM MgCl₂, 5 mM β-ME, 0.1 mM PMSF, 1 × protease inhibitor
570 cocktail tablet] and mixed at 4°C for 20 min. The lysate was filtered through
571 Miracloth and centrifuged at 4,000 rpm for 20 min at 4°C. The precipitate was
572 resuspended in extraction buffer 2 [0.25 M Sucrose, 1% Triton X-100, 10 mM
573 Tris-HCl (pH 8.0), 10 mM MgCl₂, 5 mM β-ME, 0.1 mM PMSF, 1 × protease
574 inhibitor cocktail tablet] and centrifuged at 12,000 × g for 10 min at 4 °C. The
575 pellet was then resuspended in 300 μL extraction buffer 3 [1.7 M sucrose, 0.15%
576 Triton X-100, 10 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 5 mM β-ME, 0.1 mM
577 PMSF, 1 × protease inhibitor cocktail tablet]. Extraction buffer 3 (450 μL) was
578 layered and centrifuged at 16,000 × g for 1 h at 4 °C. Nuclei were sonicated in
579 300 μL Nuclei lysis buffer [1% SDS, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1
580 × protease inhibitor cocktail tablet], and DNA was sheared on a Bioruptor Next
581 Gen UCD-300 (Diagenode Co., Ltd., Toyama, Japan) for 15 cycles of 30/30 s

582 on/off. The mixture was then centrifuged at 13,200 rpm for 10 min at 4 °C. Four
583 times volume of ChIP Dilution Buffer [1.1% Triton X-100, 1.2 mM EDTA, 167
584 mM Tris-HCL (pH8.0), 167 mM NaCl, 1 x protease inhibitor cocktail tablet] was
585 added to the supernatant. The mixture was incubated overnight at 4 °C with
586 GFP antibody (ab290, Fig 4) and HA antibody (ab9110, Fig 5 and 6). Fifty
587 microliters of Protein A (Life Technologies) were washed with the ChIP dilution
588 buffer three times, and incubated for an additional 1 h. The mixture was then
589 centrifuged at 1,000 rpm for 2 min at 4°C. The beads were washed with 1 mL
590 of the following buffers for 5 min while rotating at 4 °C: 1 x low salt buffer [150
591 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8)],
592 1 x high-salt buffer [500 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA,
593 20 mM Tris-HCl (pH 8.0)], 1 x LiCl wash buffer [250 mM LiCl, 1% NP-40, 1%
594 sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl (pH 8.0)], and 2 x TE
595 buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. The immunocomplex was
596 eluted from the beads twice with elution buffer (1% SDS, 0.1 M NaHCO₃) and
597 incubated for 20 min with shaking at 65°C. A total of 20 µL of 5 M NaCl was
598 added, and crosslinking was reversed by incubation at 65°C overnight. Ten
599 microliters of 0.5 M EDTA, 20 µL 1 M Tris-HCl (pH 6.5–8.0), 2 µL 20 mg/mL
600 Proteinase K (Invitrogen), and 3 µL 10 mg/mL RNase A1 was added and the
601 mixture was incubated at 45°C for 1h. DNA was purified using the HiPure Gel
602 Pure DNA Mini Kit (Magen, D2111-02). Purified ChIP DNA was used to
603 prepare Illumina multiplexed sequencing libraries. Libraries were prepared
604 using the Illumina E7630S NEB/NEBNext® Library Quant Kit (Illumina).
605 Amplified libraries were size-selected using 2% gel to capture fragments
606 between 100 and 400 bp. The libraries were quantified using an Agilent 2100
607 Bioanalyzer (Agilent Technologies). Samples without antibodies were used as
608 input controls.

609 The sample library was sequenced on a NovaSeq6000 PE150 by
610 Shanghai Jiayin Biotechnology Ltd. (<http://www.rainbow-genome.com/>). A

611 quality distribution plot and base content distribution were generated using
612 FASTQC. Before read mapping, clean reads were obtained from the raw reads
613 by removing adaptor sequences. The clean reads were aligned to the
614 reference genome sequences using the BWA program. The bam file was
615 generated using the unique mapped reads as an input file and, using macs2
616 software for callpeak with cutoff fold change > 1.5 and *P*-value < 0.01. The
617 input file was the peak file and genome fasta file. The DNA sequence was
618 extracted according to the peak file, and the sequence was compared with the
619 motif database to obtain the motif. Read distributions (bigwig file of IP to
620 subtract input) across genes are presented as an average plot. For statistical
621 difference peak analysis, we merged the peak files of each sample using the
622 bedtools software. The counts of the reads over the bed were determined for
623 each sample using bedtools multicov. Differential accessible peak was
624 assessed using DESeq2. Regions were called differentially accessible with
625 cutoff fold change > 1.5 and *P*-value < 0.01. DAVID was used to identify gene
626 ontology enrichment in the ChIP-seq data.

627 **ChIP-PCR assay.** ChIP was performed as previously described (Yang et al.,
628 2020). For @ASF1 (Weng et al., 2014) ChIP, Col-0, *pif7-1*, and *pifq* seedlings
629 were grown under white light for 8 d and then treated with shade for 1 h. Two
630 biological replicates were prepared (Fig 4G and Appendix Fig S5D). For @HA
631 (ab91110) ChIP, *HTR5-HA/Col-0*, *HTR5-HA/pif7-1*, *HTR5-HA/asf1ab* and
632 *HTR5-HA/hira-1* seedlings were grown under white light for 8 d and then
633 treated with shade for 1 h. The seedlings were harvested and cross-linked for
634 15 min under vacuum in a cross-linking buffer (extraction buffer 1 with 1%
635 formaldehyde). Cross-linking was terminated with 125 mM glycine (pH 8.0)
636 under vacuum for 5 min, and the seedlings were washed three times in
637 double-distilled water and rapidly frozen. The bioruptor was used at high power
638 with 30 s on/30 s off cycles 15 times until the average chromatin size was
639 approximately 300 bp. An anti-HA affinity gel was used for IP. qRT-PCR was

640 performed using a kit from Takara to determine the enrichment of
641 immunoprecipitated DNA in the ChIP experiments.

642 **Data availability.** All mutants and transgenic lines can be requested from the
643 corresponding authors. RNA-seq and ChIP-seq data have been deposited in
644 the NCBI GEO database with accession number GSE189669
645 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE189669>) and
646 GSE193955
647 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193955>),
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649

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659

660 **Disclosure and competing interests statement**

661 The authors declare that they have no conflict of interest.

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796

797 **Figure legends**798 **Figure 1. The histone chaperones ASF1A and ASF1B interact with PIF7**

799 A. Interaction between PIF7 and ASF1A/ASF1B was detected using a
800 luciferase complementation imaging (LCI) assay. N-terminal and C-terminal
801 halves of LUC were fused to ASF1A/ASF1B and PIF7, respectively. Luciferin
802 was infiltrated before the LUC activity was monitored.

803 B. Interaction between PIF7 and ASF1A/ASF1B was detected using a
804 pull-down assay. SUMO-His-fused PIF7 and GST-ASF1A/ASF1B were
805 purified from *E. coli*.

806 C. Interaction between PIF7 and ASF1A/ASF1B was detected using a semi-*in*
807 *vivo* pull-down assay. GST-ASF1A/ASF1B were purified from *E. coli*.

808 PIF7-Flash protein from overexpressing *PIF7-Flash* seedlings grown under
809 white light or 1 h of shade treatment was detected using an anti-MYC antibody.

810 D. Interaction between PIF7 and ASF1A in *Arabidopsis* was detected using the
811 Co-IP assay. Anti-GFP Sepharose beads were used to precipitate
812 GFP-ASF1A from overexpressing *GFP-ASF1A * PIF7-Flash* seedlings grown
813 under white light or 1 h of shade treatment.

814 E. Interaction between HIRA and ASF1A was detected using a pull-down
815 assay. TF-His-HIRA and GST-ASF1A were purified from *E. coli*.

816 F. Interactions between ASF1A and PIF7/HIRA were detected in tobacco leaf
817 cells. The PIF7-Flash, HIRA-HA, and GFP-ASF1A (GFP as control) proteins
818 were co-expressed in tobacco leaf cells. Anti-GFP Sepharose beads were
819 used for Co-IP assay.

820 **Figure 2. The histone chaperones ASF1A and ASF1B positively regulate**
821 **shade-induced hypocotyl elongation**

822 A. Hypocotyl lengths of Col-0 (wild type), *pif7-1*, *asf1a*, *asf1b*, *asf1ab*, and
823 *hira-1* under white light and shade conditions. Seedlings were grown for 4 d

824 under continuous white light and either maintained under white light (WL, with
825 white box) or transferred to shade (SH, with grey box) for 4 d. The scale bar
826 represents 2 mm. Different letters indicate significant differences ($P < 0.01$)
827 calculated using one-way ANOVA with Tukey's honest significant difference
828 (HSD) test. At least 20 seedlings were used for each treatment or genotype.

829 B, C. Growth analysis of Col-0, *pif7-1*, *asf1ab*, and *hira-1* seedlings treated
830 with shade. New hypocotyl growth and real-time growth rates were recorded at
831 15-min intervals after shade treatment. I to IV representing four phases of
832 growth under shade.

833 D. Hypocotyl lengths of Col-0, *phyB-9*, *asf1ab*, *phyB-9 * asf1ab*, *hira-1*, and
834 *phyB-9 * hira-1* grown under white light. The scale bar represents 2 mm.
835 Different letters indicate significant differences ($P < 0.01$) calculated using
836 one-way ANOVA with Tukey's HSD test. At least 20 seedlings were used for
837 each treatment or genotype.

838 E. Hypocotyl lengths of Col-0, *pif7-1*, *asf1ab*, *pif7-1 * asf1ab*, *hira-1*, and *pif7-1*
839 ** hira-1* under white light and shade conditions. The scale bar represents 2 mm.
840 Different letters indicate significant differences ($P < 0.01$) calculated using
841 one-way ANOVA with Tukey's HSD test. At least 20 seedlings were used for
842 each treatment or genotype.

843 **Figure 3. The PIF7-ASF1-HIRA regulatory module activates the gene**
844 **expression under shade**

845 A. Heatmap representing the relative expression levels of shade-induced
846 genes in Col-0, *pif7-1*, *asf1ab*, and *hira-1*. The red and white rows indicate
847 RNA expression at high and low levels, respectively.

848 B. Boxplot displays the fold changes in shade-induced genes in Col-0, *pif7-1*,
849 *asf1ab*, and *hira-1* by comparing the transcript levels between white light and
850 shade conditions. Different letters indicate significant differences ($P < 0.05$) as
851 determined by one-way ANOVA.

852 C. Venn diagram shows the 132 PIF7-ASF1-HIRA co-regulated
853 shade-responsive genes.

854 D. Gene ontology (GO) analysis of 132 PIF7-ASF1-HIRA co-regulated
855 shade-responsive genes. For each point, the size is proportional to the number
856 of genes, and the colors represent the *P*-value.

857 **Figure 4. Shade increases ASF1-enrichment at chromatin of target genes**
858 **of PIF7**

859 A. Heatmap representing the enriched GFP-ASF1A binding peaks under white
860 light (WL) and shade (SH) conditions. Red and white rows indicate
861 GFP-ASF1A peaks at high and low levels, respectively.

862 B. Pie chart represents the genomic distribution of shade-induced GFP-ASF1A
863 binding events.

864 C. Average density plot represents the distribution profile of GFP-ASF1A for
865 shade-induced genes (left) and control genes (right). The *P*-value was
866 calculated in a window from 1kb upstream to TES by Welch's *t*-test.

867 D. Integrative genomics viewer (IGV) screenshots showing the distribution of
868 GFP-ASF1A enrichment at the *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8*
869 *loci*.

870 E. Average density plot representing the distribution profile of GFP-ASF1A in
871 PIF7-targeted shade-responsive genes. The *P*-value was calculated in a
872 window from 1kb upstream to TES by Welch's *t*-test.

873 F. Average density plot representing the distribution profile of GFP-ASF1A in
874 GFP-ASF1A bound and PIF7-targeted shade-responsive genes. The *P*-value
875 was calculated in a window from 1kb upstream to TES by Welch's *t*-test.

876 G. ChIP-PCR analysis of ASF1 enrichment using anti-ASF1A antibodies at the
877 *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8 loci*. Col-0 and *pif7-1* seedlings
878 were grown under continuous white light or transferred to the shade for 1 h.

879 Top panels show a schematic representation of the gene structures. The
880 bottom panels represent the effects of shade on ASF1A enrichment.
881 Shade-increased enrichment of ASF1A was calculated as input% SH minus
882 input% WL. The difference at the P2 locus in Col-0 was normalized to one for
883 each gene. Different letters indicate statistically significant differences ($P <$
884 0.05) by one-way ANOVA with Tukey's HSD test. The data shown are the
885 mean \pm SDs ($n = 3$, where n refers to technical replicates).

886 **Figure 5. Shade-enriched H3.3 levels at its target genes**

887 A, B. Heatmaps representing genome-wide H3.1 and H3.3 enrichment levels
888 from 2 kb upstream of the TSS to 2 kb downstream of the TES under white
889 light (WL) and shade (SH) conditions. Gradient colors represent enrichment
890 levels. The red and white rows indicate H3.1 / H3.3 enrichment at high and low
891 levels, respectively. TSS: transcription start site; TES: transcription end site.

892 C. Average density plots representing the distribution profiles of H3.3 for
893 shade-induced genes and control genes. The P -value was calculated in a
894 window from 1kb upstream to TES by Welch's t -test.

895 D. Average density plots representing the distribution profiles of H3.1 for
896 shade-induced genes and control genes. The P -value was calculated in a
897 window from TSS to TES by Welch's t -test.

898 E. The box-plot represents the expression levels of 1246 protein-coding genes
899 that displayed increased H3.3 levels in response to shade. The paired t -test
900 was used for statistical analysis.

901 F. GO analysis of the top 400 genes related to H3.3 enrichment and
902 shade-induced transcription. For each point, the size is proportional to the
903 number of genes, and the colors represent the P -value.

904 G. IGV screenshots show the distribution of H3.3 and H3.1 enrichment levels
905 at the *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* loci.

906 H. Hypocotyl lengths of Col-0, *pif7-1*, and *h3.3kd* under white light and shade
907 conditions. The scale bar represents 2 mm. Different letters indicate significant
908 differences ($P < 0.01$) calculated using one-way ANOVA with Tukey's HSD test.
909 At least 20 seedlings were used for each treatment or genotype.

910 I. Relative expression levels of *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8*
911 in Col-0, *pif7-1*, and *h3.3kd* seedlings grown under white light or shade
912 conditions. The seedlings were grown under white light for 6 d, and then
913 maintained under white light, or transferred to shade for 1 h. Different letters
914 indicate statistically significant differences ($P < 0.05$) by one-way ANOVA with
915 Tukey's HSD test. The data shown are the mean \pm SDs ($n = 3$, n refers to
916 biological replicates).

917 **Figure 6. Shade-enriched H3.3 levels at its target genes probably depend**
918 **on PIF7-ASF1**

919 A. Heatmap representing genome-wide H3.3 enrichment levels from 2 kb
920 upstream of the TSS to 2 kb downstream of the TES in *pif7-1* under white light
921 (WL) and shade (SH) conditions. Gradient colors represent enrichment levels.
922 The red and white rows indicate H3.3 enrichment at high and low levels,
923 respectively.

924 B. Heatmap representing shade-increased enrichment of H3.3 at 1246
925 shade-induced H3.3 genes under Col-0 and *pif7-1* background.
926 Shade-increased enrichment of H3.3 was calculated by enrichment levels of
927 H3.3 under shade minus enrichment levels of H3.3 under white light.

928 C. Average density plots representing the distribution profile of H3.3 at
929 shade-induced genes (left) and control genes (right) in Col-0 and *pif7-1*
930 background. Enhanced H3.3 levels (enrichment levels of H3.3 under shade
931 minus enrichment levels of H3.3 under white light) are visualized around the
932 TES after shade exposure. The P -value was calculated in a window from 1kb
933 upstream to TES by Welch's t -test.

934 D. Average density plots representing the distribution profile of H3.3 at
935 PIF7-targeted shade-responsive genes (left), and GFP-ASF1A bound and
936 PIF7-targeted shade-responsive genes (right) in Col-0 and *pif7-1* background.
937 Enhanced H3.3 levels (enrichment levels of H3.3 under shade minus
938 enrichment levels of H3.3 under white light) are visualized around the TES
939 after shade exposure. The *P*-value was calculated in a window from 1kb
940 upstream to TES by Welch's *t*-test.

941 E. ChIP-PCR analysis of HTR5 (H3.3) levels at the *ATHB2*, *PIN7*, *CSLC4*,
942 *BIM1*, *ARL*, and *NPY8* loci. Top panels show a schematic representation of the
943 gene structures. The bottom panels represent the effects of shade on H3.3
944 enrichment. Shade-increased enrichment of H3.3 was calculated as the input%
945 SH minus input% WL. The difference at the P2 locus in Col-0 was normalized
946 to one for each gene. Different letters indicate statistically significant
947 differences ($P < 0.05$) by one-way ANOVA with Tukey's HSD test. The data
948 shown are the means \pm SDs ($n = 3$, where n refers to biological replicates).

949 F. Proposed model for shade-induced H3.3 enrichment and active
950 transcription at the targets of PIF7. Under shade conditions, dephosphorylated
951 PIF7 binds to its targets and recruits the ASF1-HIRA complex. Consequently,
952 gene-body-localized H3.3 is enhanced and target genes of PIF7 are activated,
953 leading to hypocotyl elongation.

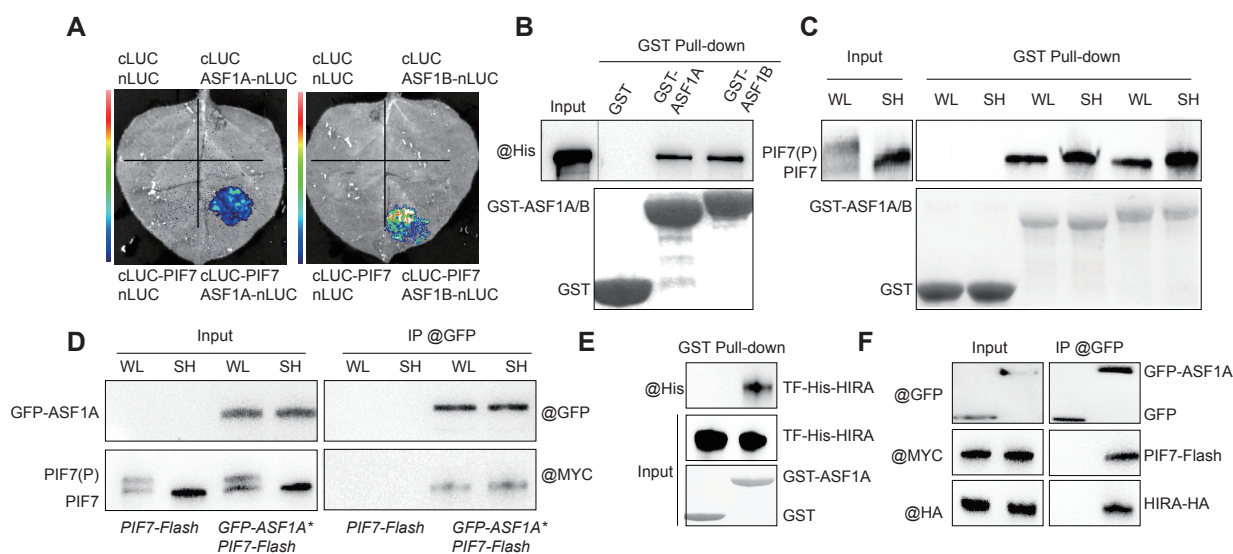


Figure 1. The histone chaperones ASF1A and ASF1B interact with PIF7

A. Interaction between PIF7 and ASF1A/ASF1B was detected using a luciferase complementation imaging (LCI) assay. N-terminal and C-terminal halves of LUC were fused to ASF1A/ASF1B and PIF7, respectively. Luciferin was infiltrated before the LUC activity was monitored.

B. Interaction between PIF7 and ASF1A/ASF1B was detected using a pull-down assay. SUMO-His-fused PIF7 and GST-ASF1A/ASF1B were purified from *E. coli*.

C. Interaction between PIF7 and ASF1A/ASF1B was detected using a semi-*in vivo* pull-down assay. GST-ASF1A/ASF1B were purified from *E. coli*. PIF7-Flash protein from overexpressing *PIF7-Flash* seedlings grown under white light or 1 h of shade treatment was detected using an anti-MYC antibody.

D. Interaction between PIF7 and ASF1A in *Arabidopsis* was detected using the Co-IP assay. Anti-GFP Sepharose beads were used to precipitate GFP-ASF1A from overexpressing *GFP-ASF1A * PIF7-Flash* seedlings grown under white light or 1 h of shade treatment.

E. Interaction between HIRA and ASF1A was detected using a pull-down assay. TF-His-HIRA and GST-ASF1A were purified from *E. coli*.

F. Interactions between ASF1A and PIF7/HIRA were detected in tobacco leaf cells. The PIF7-Flash, HIRA-HA, and GFP-ASF1A (GFP as control) proteins were co-expressed in tobacco leaf cells. Anti-GFP Sepharose beads were used for Co-IP assay.

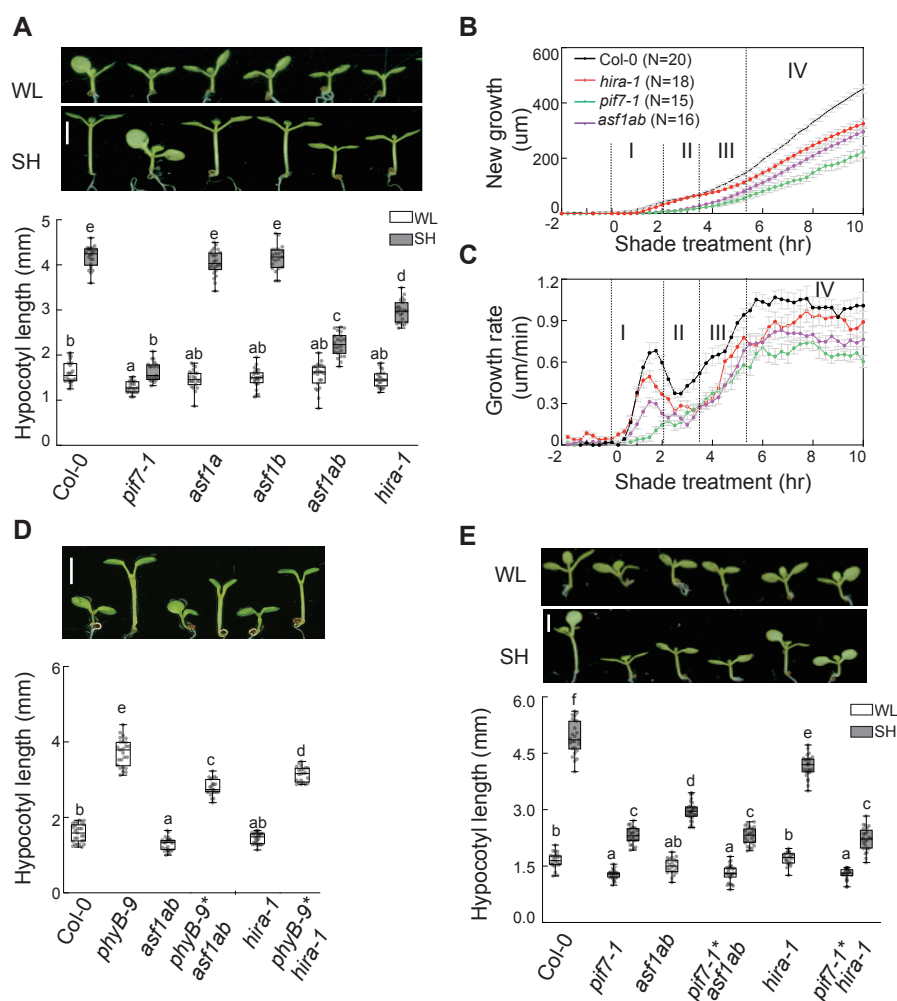


Figure 2. The histone chaperones ASF1A and ASF1B positively regulate shade-induced hypocotyl elongation

A. Hypocotyl lengths of Col-0 (wild type), *pif7-1*, *asf1a*, *asf1b*, *asf1ab*, and *hira-1* under white light and shade conditions. Seedlings were grown for 4 d under continuous white light and either maintained under white light (WL, with white box) or transferred to shade (SH, with grey box) for 4 d. The scale bar represents 2 mm. Different letters indicate significant differences ($P < 0.01$) calculated using one-way ANOVA with Tukey's honest significant difference (HSD) test. At least 20 seedlings were used for each treatment or genotype.

B, C. Growth analysis of Col-0, *pif7-1*, *asf1ab*, and *hira-1* seedlings treated with shade. New hypocotyl growth and real-time growth rates were recorded at 15-min intervals after shade treatment. I to IV representing four phases of growth under shade.

D. Hypocotyl lengths of Col-0, *phyB-9*, *asf1ab*, *phyB-9* asf1ab*, *hira-1*, and *phyB-9* hira-1* grown under white light. The scale bar represents 2 mm. Different letters indicate significant differences ($P < 0.01$) calculated using one-way ANOVA with Tukey's HSD test. At least 20 seedlings were used for each treatment or genotype.

E. Hypocotyl lengths of Col-0, *pif7-1*, *asf1ab*, *pif7-1* asf1ab*, *hira-1*, and *pif7-1* hira-1* under white light and shade conditions. The scale bar represents 2 mm. Different letters indicate significant differences ($P < 0.01$) calculated using one-way ANOVA with Tukey's HSD test. At least 20 seedlings were used for each treatment or genotype.

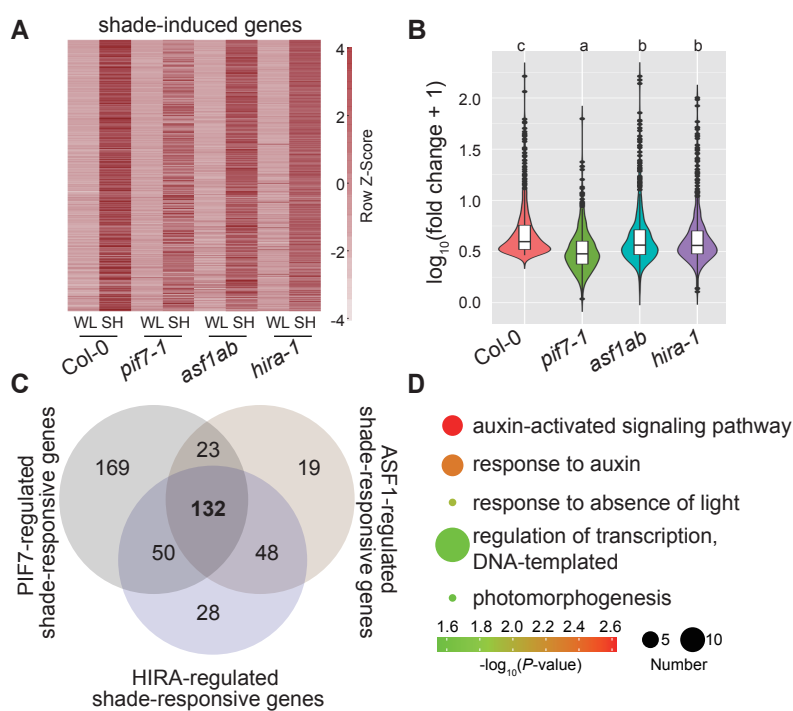


Figure 3. The PIF7-ASF1-HIRA regulatory module activates the gene expression under shade

A. Heatmap representing the relative expression levels of shade-induced genes in *Col-0*, *pif7-1*, *asf1ab*, and *hira-1*. The red and white rows indicate RNA expression at high and low levels, respectively.

B. Boxplot displays the fold changes in shade-induced genes in *Col-0*, *pif7-1*, *asf1ab*, and *hira-1* by comparing the transcript levels between white light and shade conditions. Different letters indicate significant differences ($P < 0.05$) as determined by one-way ANOVA.

C. Venn diagram shows the 132 PIF7-ASF1-HIRA co-regulated shade-responsive genes.

D. Gene ontology (GO) analysis of 132 PIF7-ASF1-HIRA co-regulated shade-responsive genes. For each point, the size is proportional to the number of genes, and the colors represent the P -value.

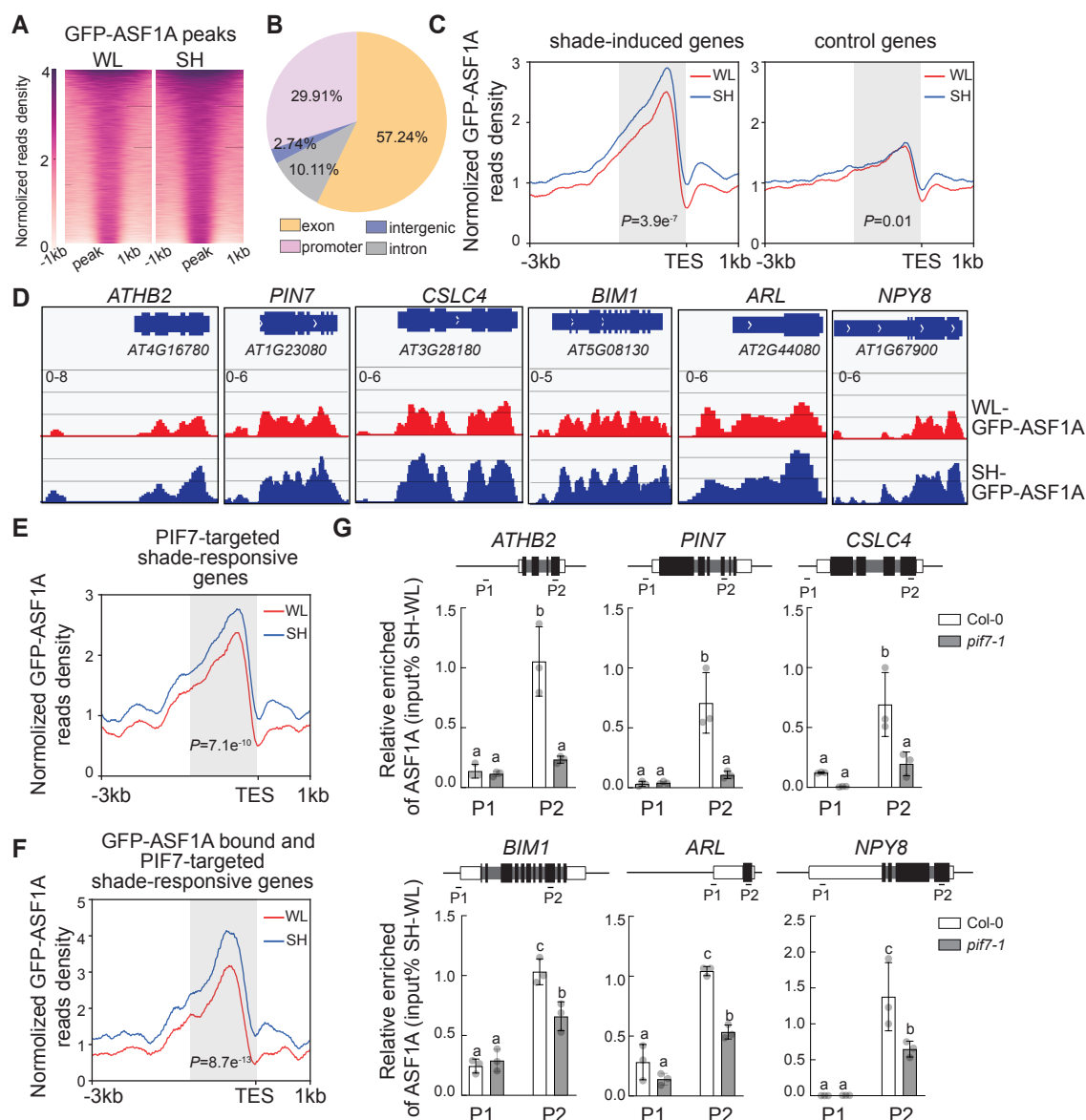


Figure 4. Shade increases ASF1-enrichment at chromatin of target genes of PIF7

A. Heatmap representing the enriched GFP-ASF1A binding peaks under white light (WL) and shade (SH) conditions. Red and white rows indicate GFP-ASF1A peaks at high and low levels, respectively.

B. Pie chart represents the genomic distribution of shade-induced GFP-ASF1A binding events.

C. Average density plot represents the distribution profile of GFP-ASF1A for shade-induced genes (left) and control genes (right). The P -value was calculated in a window from 1kb upstream to TES by Welch's t -test.

D. Integrative genomics viewer (IGV) screenshots showing the distribution of GFP-ASF1A enrichment at the *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* loci.

E. Average density plot representing the distribution profile of GFP-ASF1A in PIF7-targeted shade-responsive genes. The P -value was calculated in a window from 1kb upstream to TES by Welch's t -test.

F. Average density plot representing the distribution profile of GFP-ASF1A in GFP-ASF1A bound and PIF7-targeted shade-responsive genes. The P -value was calculated in a window from 1kb upstream to TES by Welch's t -test.

G. ChIP-PCR analysis of ASF1 enrichment using anti-ASF1A antibodies at the *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* loci. Col-0 and *pif7-1* seedlings were grown under continuous white light or transferred to the shade for 1 h. Top panels show a schematic representation of the gene structures. The bottom panels represent the effects of shade on ASF1A enrichment. Shade-increased enrichment of ASF1A was calculated as input% SH minus input% WL. The difference at the P2 locus in Col-0 was normalized to one for each gene. Different letters indicate statistically significant differences ($P < 0.05$) by one-way ANOVA with Tukey's HSD test. The data shown are the mean \pm SDs ($n = 3$, where n refers to technical replicates).

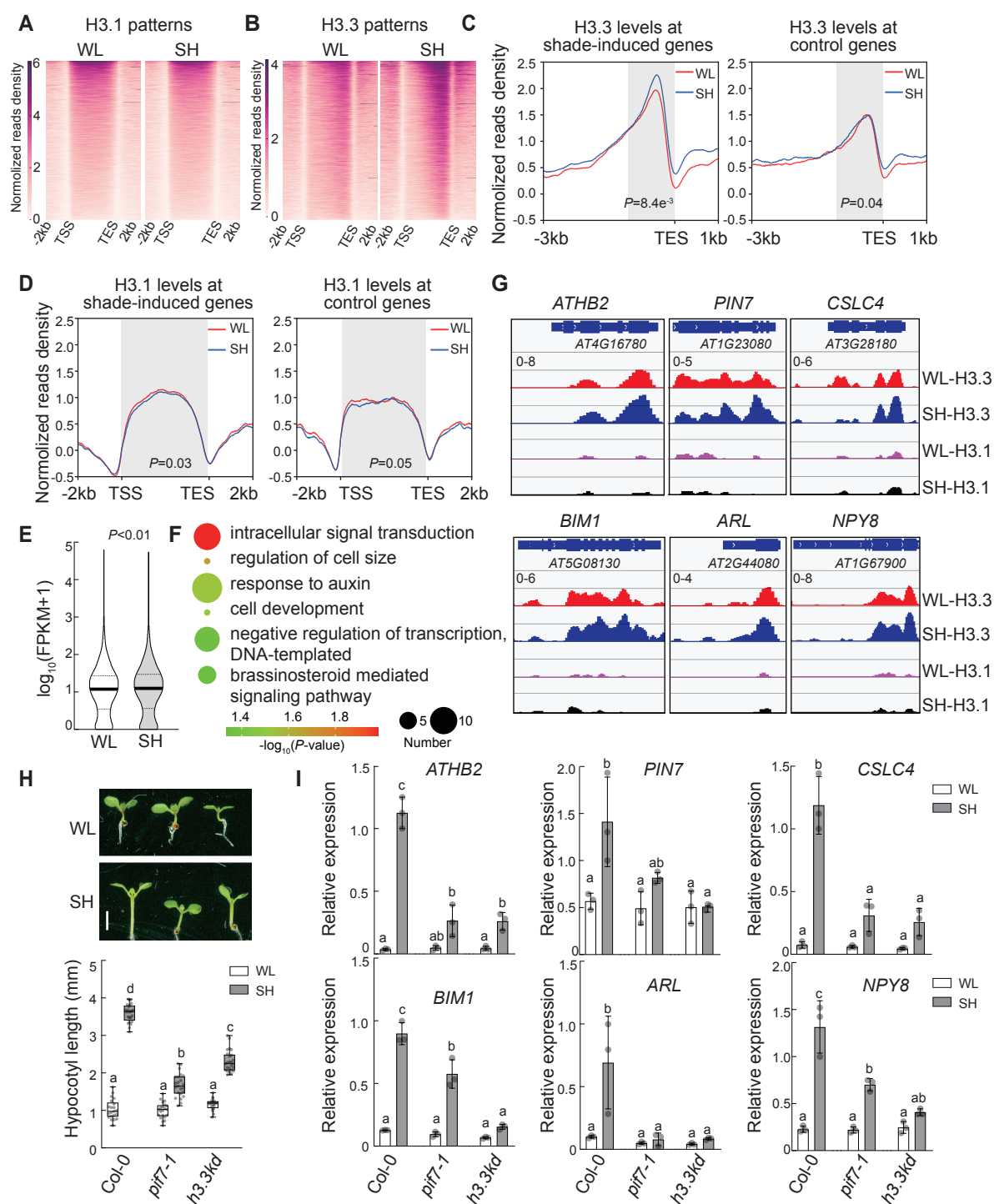


Figure 5. Shade-enriched H3.3 levels at its target genes

A, B. Heatmaps representing genome-wide H3.1 and H3.3 enrichment levels from 2 kb upstream of the TSS to 2 kb downstream of the TES under white light (WL) and shade (SH) conditions. Gradient colors represent enrichment levels. The red and white rows indicate H3.1 / H3.3 enrichment at high and low levels, respectively. TSS: transcription start site; TES: transcription end site.

C. Average density plots representing the distribution profiles of H3.3 for shade-induced genes and control genes. The P -value was calculated in a window from 1kb upstream to TES by Welch's t -test.

D. Average density plots representing the distribution profiles of H3.1 for shade-induced genes and control genes. The P -value was calculated in a window from TSS to TES by Welch's t -test.

E. The box-plot represents the expression levels of 1246 protein-coding genes that displayed increased H3.3 levels in response to shade. The paired t -test was used for statistical analysis.

F. GO analysis of the top 400 genes related to H3.3 enrichment and shade-induced transcription. For each point, the size is proportional to the number of genes, and the colors represent the P -value.

G. IGV screenshots show the distribution of H3.3 and H3.1 enrichment levels at the *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* loci.

H. Hypocotyl lengths of Col-0, *pif7-1*, and *h3.3kd* under white light and shade conditions. The scale bar represents 2 mm. Different letters indicate significant differences ($P < 0.01$) calculated using one-way ANOVA with Tukey's HSD test. At least 20 seedlings were used for each treatment or genotype.

I. Relative expression levels of *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* in Col-0, *pif7-1*, and *h3.3kd* seedlings grown under white light or shade conditions. The seedlings were grown under white light for 6 d, and then maintained under white light, or transferred to shade for 1 h. Different letters indicate statistically significant differences ($P < 0.05$) by one-way ANOVA with Tukey's HSD test. The data shown are the mean \pm SDs ($n = 3$, n refers to biological replicates).

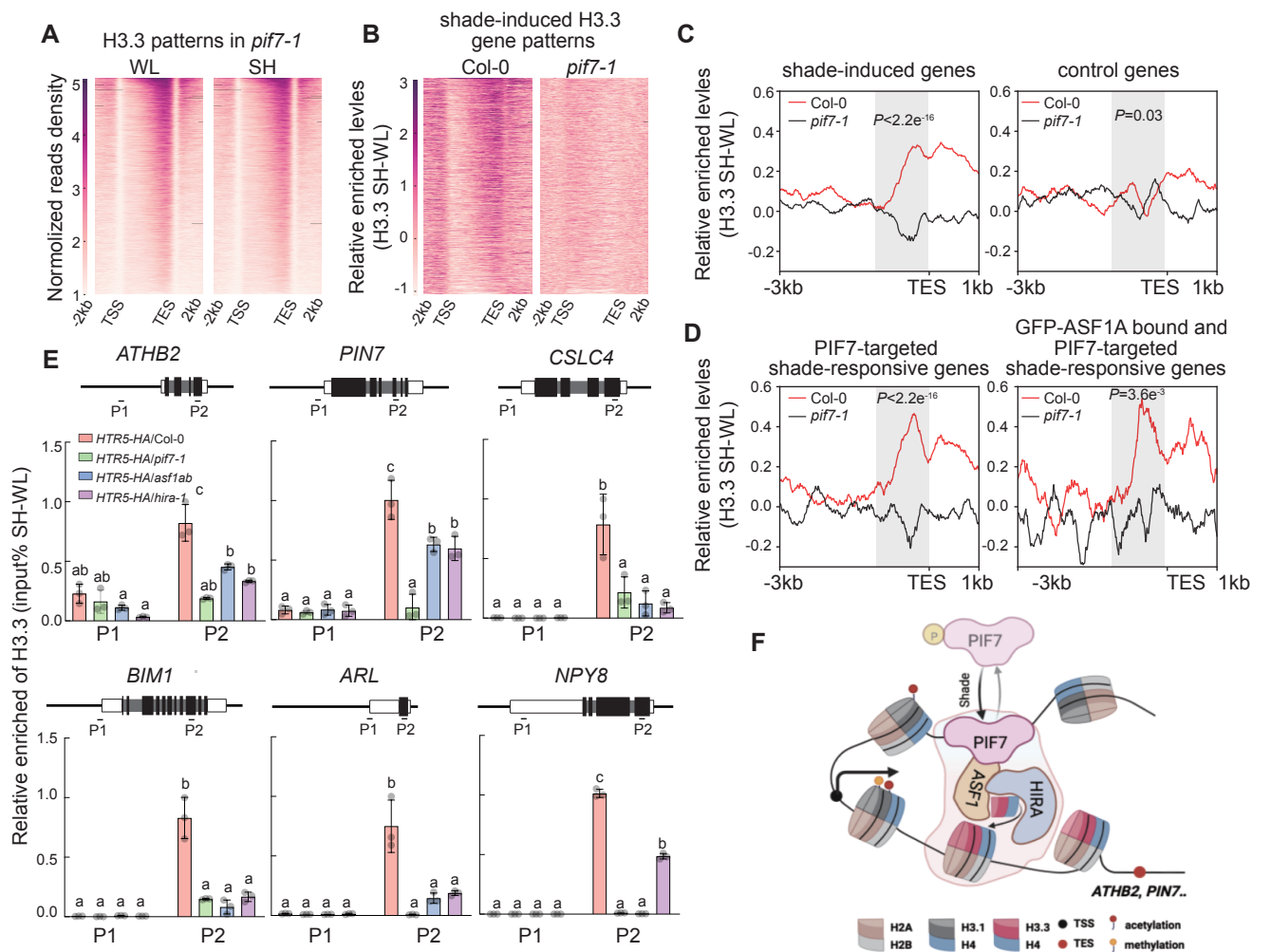


Figure 6. Shade-enriched H3.3 levels at its target genes probably depend on PIF7-ASF1

A. Heatmap representing genome-wide H3.3 enrichment levels from 2 kb upstream of the TSS to 2 kb downstream of the TES in *pif7-1* under white light (WL) and shade (SH) conditions. Gradient colors represent enrichment levels. The red and white rows indicate H3.3 enrichment at high and low levels, respectively.

B. Heatmap representing shade-increased enrichment of H3.3 at 1246 shade-induced H3.3 genes under Col-0 and *pif7-1* background. Shade-increased enrichment of H3.3 was calculated by enrichment levels of H3.3 under shade minus enrichment levels of H3.3 under white light.

C. Average density plots representing the distribution profile of H3.3 at shade-induced genes (left) and control genes (right) in Col-0 and *pif7-1* background. Enhanced H3.3 levels (enrichment levels of H3.3 under shade minus enrichment levels of H3.3 under white light) are visualized around the TES after shade exposure. The P -value was calculated in a window from 1kb upstream to TES by Welch's t -test.

D. Average density plots representing the distribution profile of H3.3 at PIF7-targeted shade-responsive genes (left), and GFP-ASF1A bound and PIF7-targeted shade-responsive genes (right) in Col-0 and *pif7-1* background. Enhanced H3.3 levels (enrichment levels of H3.3 under shade minus enrichment levels of H3.3 under white light) are visualized around the TES after shade exposure. The P -value was calculated in a window from 1kb upstream to TES by Welch's t -test.

E. ChIP-PCR analysis of HTR5 (H3.3) levels at the *ATHB2*, *PIN7*, *CSLC4*, *BIM1*, *ARL*, and *NPY8* loci. Top panels show a schematic representation of the gene structures. The bottom panels represent the effects of shade on H3.3 enrichment. Shade-increased enrichment of H3.3 was calculated as the input% SH minus input% WL. The difference at the P2 locus in Col-0 was normalized to one for each gene. Different letters indicate statistically significant differences ($P < 0.05$) by one-way ANOVA with Tukey's HSD test. The data shown are the means \pm SDs ($n = 3$, where n refers to biological replicates).

F. Proposed model for shade-induced H3.3 enrichment and active transcription at the targets of PIF7. Under shade conditions, dephosphorylated PIF7 binds to its targets and recruits the ASF1-HIRA complex. Consequently, gene-body-localized H3.3 is enhanced and target genes of PIF7 are activated, leading to hypocotyl elongation.