

1 **Polymorphic Inverted Repeats near coding genes**
2 **impact chromatin topology and phenotypic traits in**
3 ***Arabidopsis thaliana***

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26 **Abstract**

27 Transposons are mobile elements that are commonly silenced to protect
28 eukaryotic genome integrity. In plants, transposable elements (TEs) can be
29 activated during stress conditions and subsequently insert into gene-rich regions.
30 TE-derived inverted repeats (IRs) are commonly found near plant genes, where
31 they affect host gene expression with potentially positive effects on adaptation.
32 However, the molecular mechanisms by which these IRs control gene expression
33 is unclear in most cases. Here, we identify in the *Arabidopsis thaliana* genome
34 hundreds of IRs located near genes that are transcribed by RNA Polymerase II,
35 resulting in the production of 24-nt small RNAs that trigger methylation of the IRs.
36 The expression of these IRs is associated with drastic changes in the local 3D
37 chromatin organization, which alter the expression pattern of the hosting genes.
38 Notably, the presence and structure of many IRs differ between *A. thaliana*
39 accessions. Capture-C sequencing experiments revealed that such variation
40 changes short-range chromatin interactions, which translates into changes in
41 gene expression patterns. CRISPR/Cas9-mediated disruption of two of such IRs
42 leads to a switch in genome topology and gene expression, with phenotypic
43 consequences. Our data demonstrate that the insertion of an IR near a gene
44 provides an anchor point for chromatin interactions that can profoundly impact
45 the activity of neighboring loci. This turns IRs into powerful evolutionary agents
46 that can contribute to rapid adaptation.

47

48 **Introduction**

49 Transposable elements (TEs) are widely distributed among eukaryotic genomes.
50 In a process known as transposition, TEs move within the genome to different
51 locations, usually copying themselves as they 'jump' (Dubin et al., 2018). Plant
52 genomes are particularly rich in TEs and repetitive elements, which, for example,
53 account for 85% of the maize genome (Schnable et al., 2009). In plants, TEs are
54 commonly silenced through DNA methylation in a process known as RNA-
55 directed DNA methylation (RdDM), which maintains genome integrity (Matzke et
56 al., 2015). To trigger RdDM, short RNA Polymerase IV (RNAPIV)-dependent TE
57 transcripts are converted into double-stranded RNA (dsRNA) by the RNA-
58 dependent RNA polymerase 2 (RDR2) and then to 24 nt small interfering RNAs
59 (siRNAs) by DICER-Like 3 (DCL3) (Matzke et al., 2015; Zhou and Law, 2015).
60 ARGONAUTE 4 (AGO4)-loaded siRNAs then direct de novo methylation of the
61 TE loci by recognizing nascent RNAPV transcripts there. Such methylation
62 ultimately leads to nucleosome condensation and permanent silencing of the TE.
63 Still, massive bursts of TE amplification have occurred in plant genomes in
64 addition to the rarer, but continued movement of individual elements (Lu et al.,
65 2012; Maumus and Quesneville, 2014; SanMiguel et al., 1998). Stress can trigger
66 the activation of TEs and fuel transposition (Baduel et al., 2021; Tittel-Elmer et
67 al., 2010). TEs are thus significant contributors to genetic variation in plant
68 genomes (Baduel et al., 2021; Collier and Largaespada, 2007; Deragon et al.,

69 2008) and have been postulated as drivers of genome evolution and expansion,
70 as well as developmental plasticity and adaptation (Dubin *et al.*, 2018; Lisch,
71 2013).

72 There are two main classes of TEs: DNA transposons and retrotransposons. The
73 most abundant DNA transposons are miniature inverted-repeat TEs (MITEs),
74 while the most abundant retrotransposons are long terminal repeat
75 retrotransposons (LTRs) (Dubin *et al.*, 2018). MITEs exhibit characteristic
76 terminal inverted repeats (TIRs) and small direct repeats (target site duplications,
77 TSDs), but lack transposase sequences, making them non-autonomous
78 elements (Fattash *et al.*, 2013; Yang *et al.*, 2009). Many TE-derived inverted
79 repeated (IR) insertions may not be classified as MITE as they lack the above-
80 mentioned components of MITEs, either due to deletions after insertion, or
81 because they were generated through a different process. MITEs are commonly
82 situated near coding genes: for example, almost 60% of rice genes can be
83 associated with a MITE (Lu *et al.*, 2012), with the MITEs often changing the
84 expression of neighboring genes (Lu *et al.*, 2012; Underwood *et al.*, 2022; Wu *et*
85 *al.*, 2022; Xu *et al.*, 2020; Zhang *et al.*, 2016). Based on this, MITEs have been
86 proposed to play important roles in genome evolution and gene expression (Lu
87 *et al.*, 2012). One key feature of MITEs is that their transcripts can fold into
88 hairpin-shaped dsRNAs due to the extensive sequence complementarity
89 between IR arms. These dsRNA secondary structures are recognized and
90 processed by DCL3 to produce 24-nt siRNAs that trigger DNA methylation
91 without the need for RNAPIV/RDR2 activity (Ariel and Manavella, 2021;
92 Crescente *et al.*, 2022; Cuerda-Gil and Slotkin, 2016; Gagliardi *et al.*, 2019;
93 Sasaki *et al.*, 2014). Thus, transcripts of these MITEs can be initiated from
94 promoters of adjacent genes, triggering their RNA Polymerase II (RNAPII)-
95 dependent DNA methylation. At the sunflower *HaWRKY6* locus, the RNAPII-
96 mediated transcription of a MITE triggers the methylation of its coding region and
97 causes the formation of alternative regulatory short-range chromatin loops in the
98 locus that specifically change its expression (Gagliardi *et al.*, 2019).

99 Three-dimensional chromatin organization has recently emerged as a critical
100 feature determining genome functionality, fine-tuning gene expression and
101 developmental responses in plants (Domb *et al.*, 2022; Zhang and Wang, 2021).
102 Short-range chromatin loops reflect interaction between relatively close regions
103 of DNA, within a few kb, generally within a single locus or between adjacent loci
104 (Gagliardi and Manavella, 2020). Different from canonical regulation by mC
105 methylation in the linear DNA sequence, commonly associated with gene
106 repression, local three-dimensional chromatin organization can induce a plethora
107 of regulatory mechanisms, including transcriptional activation/repression,
108 transcription directionality, alternative splicing, the usage of cryptic termination
109 sites, impaired or enhanced RNAPII elongation, DNA replication and repair
110 (Gagliardi and Manavella, 2020; Grzechnik *et al.*, 2014; Sotelo-Silveira *et al.*,
111 2018).

112 In this study, we show that TE-derived IR elements located near genes in the
113 *Arabidopsis thaliana* genome can cause rearrangements of the chromatin
114 topology, promoting the formation of short-range chromatin loops. These
115 chromatin interactions, which depend on the production of IR-derived siRNAs and
116 *de novo* DNA methylation, often translate into changes in gene expression. The
117 presence of an IR and its associated chromatin loop near a gene does not cause
118 a uniform regulatory effect, and can either enhance or repress expression
119 depending on the locus and which regions within the locus are part of the loop.
120 Almost one third of the identified gene-associated IRs are not conserved among
121 a set of 216 *A. thaliana* natural accessions. Our data show that polymorphisms
122 in IRs near genes can be coupled with a change in the chromatin topology of the
123 region. These accession-related changes in chromatin landscapes controlled by
124 IRs can be linked to alteration in traits commonly associated with adaptation, such
125 as flowering. In proof-of-concept experiments, we used CRISPR/Cas9 genome
126 editing to mimic the situation in natural accessions lacking specific IRs, and
127 demonstrated that the IRs help to shape chromatin topology, which in turn can
128 control molecular and organismal phenotypes. We found that IRs downstream of
129 *PHYC* and *CRY1* cause the formation of repressive chromatin loops associated
130 with well-defined developmental phenotypes of some natural accessions.
131 Overall, our data demonstrate that TE-derived IRs can produce changes in
132 chromatin topology, gene expression, and ultimately phenotypic changes through
133 their capacity to trigger DNA methylation autonomously. Given the propensity of
134 TEs to activate during stress responses and the tendency of IRs to locate near
135 coding genes, our finding provides a scenario for these TEs to drive local
136 adaptation and domestication by supporting rapid and sometimes drastic
137 changes in 3D chromatin organization and gene activity after single-set
138 mutational events.

139

140 **Results**

141

142 **TE-derived IRs located near genes impact the local chromatin topology in** 143 ***Arabidopsis***

144 A TE-derived IR element located ~600 bp upstream of the *HaWRKY6* locus in
145 sunflower serves as an anchor point for the formation of two short-range
146 chromatin loops, and thereby promotes changes in local chromatin topology
147 (Gagliardi *et al.*, 2019). The ultimate outcome is the methylation of the locus, due
148 to 24-nt siRNAs produced after RNAPII-dependent transcription of these IRs. In
149 leaves, this leads to the formation of a repressive loop, while it promotes a
150 second, larger, loop that enhances transcription in cotyledons (Gagliardi *et al.*,
151 2019).

152 Because TE-derived IRs are frequently located near genes (Guo *et al.*, 2017), we
153 wondered whether the *HaWRKY6* case was just one example of a more general
154 phenomenon in plants. To evaluate if the insertion of an IR near a gene changes
155 local chromatin topology and gene expression, we first aimed to identify all IRs

156 neighboring protein-coding genes in the *A. thaliana* Col-0 reference genome.
157 Using *einverted* from the EMBOSS program suite (Rice et al., 2000), we found a
158 total of 885 IRs in the *A. thaliana* genome, 634 of them near annotated protein
159 coding genes (222 of which were located within 500 bp upstream or downstream
160 of a protein coding gene, a further 163 between 500 bp and 1,000 bp from a gene,
161 and 249 between 1,000 and 3,000 bp from a gene) (Fig. 1A). IRs were found
162 within 500 bp of 260 unique genes, within 500 to 1,000 bp of 215 unique genes,
163 and within 1,000 to 3,000 bp of 615 unique genes (Fig. 1A). These IRs have a
164 broad genome-wide distribution, with many located in the gene-rich chromosome
165 arms, and others in gene-poor/TE-rich pericentromeric regions (Fig. 1B).
166 Analyzing the overlap with annotated transposable elements (TEs) revealed that
167 68% of the IRs within 3,000 bp of a gene were clearly of TE origin, with most
168 (~44%) from the MuDR superfamily and 18% from the Helitron superfamily (Fig.
169 1C). These percentages remain invariable despite of the distance from the IR to
170 the hosting gene although a moderate enrichment is LTR/Gypsy TEs between
171 IRs not associated with genes (Fig. 1C). Using the plaNETseq dataset of RNAPII
172 associated nascent transcripts (Kindgren et al., 2020), we found that more than
173 half of the identified gene-associated IRs are transcribed by RNAPII (Fig. 1D).
174 Supporting the idea that these IRs are transcribed from promoters of nearby
175 genes, the fraction of IRs transcribed by RNAPII increases the closer the IRs are
176 to a gene (Fig. 1D). Conversely, IRs located far from annotated genes were less
177 likely to give rise to RNAPII transcripts. Instead, their transcription likely depends
178 on the canonical RNAPIV/RDR2 RdDM pathway (Fig. 1D).
179 With small RNA (sRNA)- and bisulfite (BS)-sequencing, and paralleling what we
180 observed in the case of the *HaWRKY6* adjacent IR, we found that most of the
181 identified IRs near genes produce RNAPII transcripts (Fig. 1D), giving rise to 24-
182 nt siRNAs (486/634 IRs 3000bp away from genes), and are associated with CHG
183 and CHH methylation (Fig. 1E). We often observed an additional peak of
184 methylation, which could represent a second anchor point for chromatin loop
185 formation, either on the opposite border or inside of many genes located near IRs
186 (Fig. S1), once more providing a scenario similar to that of the *HaWRKY6* locus
187 where two close-by methylated regions served as anchor points for regulatory
188 short-range chromatin loops.
189 To investigate whether the identified IRs, especially those producing siRNAs and
190 DNA methylation, impact local chromatin organization, we extracted RNA and
191 DNA from Col-0 wild-type plants, triple *dcl2*, *dcl3*, *dcl4* (*dcl234*) mutants (which
192 are impaired in 24-nt siRNA production) (Lu et al., 2006), and triple *drm1*, *drm2*,
193 *cmt3* (ddc) mutants (impaired in CHH methylation) (Kurihara et al., 2008), and
194 performed RNA-seq, sRNA-seq, BS-seq, and Capture-C. As the sequencing
195 depth required to detect short-range chromatin interaction through standard Hi-C
196 would be enormous, we selected 290 loci containing IRs and 40 control loci and
197 performed a Capture-C experiment to only focus on these regions and increase
198 the chances to detect local chromatin loops. A test mapping of Capture C reads
199 confirmed the enrichment on the captured regions compared to the input HiC

200 samples (Fig. S2A). Collectively, the designed probes cover ~1% of the genome.
201 After Capture-C we increased the ratio of reads mapping the targeted regions 40
202 times in average (Fig. S2B).
203 sRNA-seq revealed that 486 out of the 634 IRs 3000 bp from genes produce 24
204 nt siRNAs (Fig. 2A), with siRNA levels severely reduced in both *dcl234* and *ddc*
205 mutants, as expected for this class of small RNAs (Fig. 2A). Both CHG (~50%)
206 and CHH (~70%) methylated regions strongly overlapped with IRs within 500 bp
207 from genes (Fig. 1E), and methylation in both contexts is reduced in both in
208 *dcl234* and *ddc* for many IRs as expected by the reduction in siRNAs in these
209 genotypes (Fig. 2B). The proportion of IRs with reduced methylation in the
210 mutants is slightly higher for those within 500 bp of genes compared to the other
211 analyzed distance windows (Fig S2C). Changes in siRNAs and methylation were
212 correlated, consistent with a drop of siRNAs leading to reduced methylation in
213 RdDM impaired mutants (Fig. 2C). Altogether, these data suggest that a large
214 proportion of IRs located near genes and transcribed by RNAPII may be able to
215 trigger DNA methylation in *cis* through the non-canonical RdDM pathway.
216 As in the case of the *HaWRKY6* locus in sunflower, our data suggest that IRs
217 located near genes can act as regulatory elements changing the epigenetic
218 landscape of the region. In order to assess the impact of IR methylation on the
219 surrounding chromatin organization, we used the software CHESSE to compare
220 the structural similarity (SSIM) of the IR-host regions between wild type and
221 mutants (Galan et al., 2020). We also statistically determined the specific anchor
222 points of chromatin loops in each sample using capC-Map (Buckle et al., 2019)
223 in combination with peakC (Geeven et al., 2018). Analyzing individual loops, we
224 found clear alterations in the chromatin topology in several randomly picked loci
225 (Fig. 2D and S3). To compare the differential methylation-related changes in
226 loops formed near IRs, we calculated the SSIM for each captured region plus 10
227 kb on each end in wild type and siRNA- or methylation-deficient mutants. The
228 choice of a global similarity measure, the SSIM, to study short-range chromatin
229 changes, rather than comparing individual loops, aimed at increasing the power
230 to detect reliable differences, as random interactions increase with shorter
231 distances and increase the methodological background noise. Moreover, other
232 interactions, caused by dimerization of DNA-bound transcription factors or
233 nucleosome packing, can also impact such analyses. Chromatin organization of
234 the IR-containing loci, which should have SSIM values ~1 if similar between the
235 genotypes, were often changed in *ddc* and *dcl234* mutants (Fig. 2E), indicating
236 that (IR-triggered) methylation has a substantial effect on the local 3D topology.
237 Such differences are less pronounced when comparing *ddc* with *dcl234* as could
238 be expected from the methylation deficiency observed in both, therefore lacking
239 the anchor points for loop formation (Fig. 2E).
240 Changes in chromatin topology can affect gene expression (Domb *et al.*, 2022).
241 Given the alterations in chromatin topology that we found, we wondered whether
242 they impact gene expression. Formation of a chromatin loops may repress or

243 activate gene transcription, or even trigger production of alternative transcripts
244 (Gagliardi and Manavella, 2020).

245 We found 4,305 differentially expressed genes (DEGs) in the *ddc* mutant in com-
246 parison with Col-0, and 3,636 DEGs in *dcl234*, many of them overlapping be-
247 tween the two mutants (Fig. S4A). When we compared the SSIMs in regions with
248 DEGs with SSIMs in regions without DEGs we found topological differences
249 (lower SSIM) to be significantly increased in regions that included DEGs (Fig.
250 2F). We then split those differences into regions linked to differentially and not
251 differentially methylated IRs, which revealed greater topological differences in
252 *ddc* mutants for regions both including DEGs and differentially methylated IRs
253 (Fig. S4B). Methylation seemed to affect the SSIM correlation with DEGs less
254 clearly in *dcl234* (Fig. S4C). Loci with altered topology in *ddc* and *dcl234* included
255 both up- and downregulated genes (Fig. 2F). Thus, changes in chromatin topol-
256 ogy caused by an impaired RdDM machinery could be linked to opposite changes
257 in gene expression depending on the loci. This observation, also detected in rice
258 for genes adjacent to MITEs (Lu *et al.*, 2012), fits with short-range chromatin
259 loops affecting RNAPII activity in different ways, depending on which part of the
260 gene is included in the loop (Gagliardi and Manavella, 2020).

261 In summary, our data indicated that the insertion of an IR near a gene can trigger
262 changes in the local chromatin topology that ultimately affect gene expression in
263 a locus- and loop-dependent way.

264

265 **The absence of IRs near genes between Arabidopsis accessions causes** 266 **natural variation in the local chromatin topology**

267 TEs are commonly silenced in plants as a means of protecting genome integrity.
268 However, under extreme stress conditions TE transcription can be reactivated,
269 giving TEs the potential to jump in the genome in a process that has been
270 proposed to help adaptation to new environments (Ito *et al.*, 2011; Tittel-Elmer *et*
271 *al.*, 2010). We wondered whether the IRs we were studying could have adaptive
272 potential to change the structure of the RNAs produced by a locus or their
273 expression, through changes in the 3D chromatin organization.

274 To test this hypothesis, we first used published TE polymorphism datasets (Stuart
275 *et al.*, 2016) to detect variations in the TE content of 216 *A. thaliana* genomes
276 (Schmitz *et al.*, 2013). Narrowing this down further to the 634 IRs located within
277 3,000 bp upstream or downstream of annotated genes in the Col-0 reference
278 genome, we found 193 of these 634 IRs to be variable between the 216
279 accessions studied (Fig. 3A). IRs without a clear TE origin are underrepresented
280 in the collection of polymorphic IRs (Fig. 3A). These 193 IRs are located within
281 3,000 bp of 368 annotated genes. This implies that each IR can influence two or
282 more adjacent genes in many cases, an observation that is not surprising given
283 the compact nature of *A. thaliana* genomes. To investigate the effect that the
284 variation in the IR content may have on local chromatin topology and gene
285 expression, we repeated the Capture-C, RNA-seq, BS-seq, and sRNA-seq
286 analyses in the Ba1 and Hod accessions. We selected these two accessions

287 based on the number of variable IRs present in each of them (46 and 31 IRs
288 present in Col-0 are missing in Hod and Ba-1, respectively, with 18 missing in
289 both accessions). With the Capture-C experiment, we captured 290 regions
290 comprising the genomic sequences around variable IRs which included adjacent
291 genes. The analysis of individual loci revealed that the presence of an IR at a
292 locus correlated with the accumulation of 24 nt siRNAs and with distinctive short-
293 range chromatin loops (Fig. 3B and S3).

294 We chose five potential candidate genes (*PHV*, *PHYC*, *P5CS1*, *PHR1*, *CRY1*) to
295 evaluate the effect of polymorphic IRs on loop formation and gene expression.
296 We corroborated by Sanger sequencing that each of the loci has IRs in Col-0, but
297 not in the indicated accessions (Fig. 4B, 4G, and S5). We then used Chromatin
298 Conformation Capture (3C) followed by qPCR to confirm and quantify the
299 formation of IR-dependent chromatin loops at these loci, and RT-qPCR to
300 measure correlation with gene expression in each locus (Fig. 3C, 4C, and 4I). We
301 detected alternative chromatin loops at *PHV*, *P5CS1*, and *PHR1* (Fig. 3C). In the
302 case of *PHV*, loop 1 appeared to form independently of the associated IR, but
303 loops 2 and 3 were missing in Ba-1, the accession without the IR, or in *dcl234*
304 mutants (Fig. 3C). For *P5CS1*, loop 2 appeared independently of the presence of
305 the IR while the formation of an intragenic loop 1 correlated with the presence of
306 the IR or a functional RdDM machinery (Fig. 3C). Both for *PHV* and *P5CS1* the
307 absence of a loop-triggering IR appeared to be associated with enhanced gene
308 expression (Fig. 3C). In the case of *PHR1*, we found that the formation of a loop
309 1 depends on the presence of the IR, which is missing in the Hod accessions, but
310 loop 2 was only formed when the IR is missing, probably reflecting a hierarchy of
311 chromatin interactions controlled by the IR (Fig. 3C). Contrary to the results with
312 *PHV* and *P5CS1*, the absence of the IR near *PHR1* caused repression of the
313 gene.

314 When we analyzed the genome-wide effects of insertional IR polymorphisms over
315 genome topology using CHESS, we also found a drastic change in chromatin
316 folding associated with the presence/absence of an IR (Fig. 3D). For this analysis
317 we calculated the difference between the SSIMs obtained from the comparison
318 of Ba-1 and Hod genome topology against Col-0, as recommended by the
319 authors of CHESS, to have a common reference to score differences between
320 these accessions (Galan *et al.*, 2020). As it can be observed in Figure 3D, the
321 difference in SSIMs are greater when Ba-1 shares the IR with Col-0, having a
322 higher SSIM, but it is absent in Hod resulting in a lower SSIM and an increased
323 positive difference.

324 These data suggest that the insertion of an IR near coding genes can have an
325 impact on local chromatin topology that affects gene expression. Aiming to
326 explore whether this could potentially have adaptive consequences, we asked
327 whether there is a correlation between the presence/absence of an IR near a
328 gene and well-defined phenotypic traits recorded in the Arapheno database
329 (Seren *et al.*, 2017). This analysis revealed an association between IR insertional
330 polymorphisms near some genes with a given phenotype related to flowering, a

331 typically adaptative trait (Fig. 3E). This observation suggests that insertion of an
332 IR near a gene can not only impact the local chromatin topology, but that it may
333 also have adaptive implications by changing the phenotypes controlled by the
334 adjacent gene.

335

336 **The insertion of an IR near genes causes strong phenotypic effects**

337 Our data suggest that the insertion of an IR near a gene can be a significant event
338 during adaptative evolution. However, this association may not be caused by the
339 IR itself, but by adjacent polymorphisms that may be in linkage disequilibrium with
340 the IR. In addition, even if the association is with the IR itself, it may not involve
341 changes in the genome topology. To study this possibility we selected two loci,
342 *PHYC* and *CRY1*, which display natural variation in the presence/absence of a
343 close-by IR that is associated with developmental phenotypes (Fig. 3E). Both
344 *PHYC* and *CRY1* associated IRs are located downstream of the transcription
345 termination site (TTS) (Fig. 4A and 4F), thus making it less likely that they act
346 directly on the promoter of the genes as general regulatory elements.

347 *PHYC* encodes a photoreceptor capable of sensing red light (R) and far-red light
348 (FR) and is implicated in several developmental transitions, such as flowering,
349 seed germination and hypocotyl elongation (Chen et al., 2014; Kippes et al.,
350 2020; Li et al., 2020; Nishida et al., 2013). In Col-0 an IR is located ~500 bp
351 downstream of the TTS of *PHYC*, while it is missing in several natural accessions,
352 including, for example, Ey15-2 (Fig. 4A and 4B). To test whether this IR impacts
353 *PHYC* expression and related phenotypes, we used a CRISPR/Cas9 strategy to
354 delete a fragment of the IR, with which we can disrupt dsRNA formation without
355 completely removing the IR sequence (Fig. 4A). Three homozygous lines were
356 obtained with a deletion of the IR fragment (Fig. 4B). Supporting a role for this IR
357 in modulating genome topology and gene expression, we detected a chromatin
358 loop encompassing the entire *PHYC* gene in Col-0 plants that was absent in the
359 CRISPR mutant lines and in the Ey15-2 accession, which lacks the IR (Fig. 4C).
360 The absence of the IR, both in Ey15-2 and in the CRISPR lines, correlated with
361 higher expression of *PHYC*, indicating that the IR promotes the formation of a
362 repressive loop in Col-0 (Fig. 4C). Both, the CRISPR lines and Ey15-2, had
363 altered developmental responses related to known *PHYC* functions, including
364 delayed flowering and shortened hypocotyls under a continuous red-light
365 treatment (Fig. 4D and 4E). Altogether, our data indicate that the presence of the
366 IR next to *PHYC* has a substantial impact on gene regulation, by enabling the
367 formation of a short-range chromatin loop that represses gene expression,
368 thereby contributing to the differential response of natural accessions to light
369 signals.

370 *CRY1* is a blue light receptor and participates predominately in the regulation of
371 blue-light inhibition of hypocotyl elongation and anthocyanin production (Ahmad
372 et al., 1998; Ahmad et al., 1995; Liu et al., 2022). Two IRs are located near the
373 gene, ~2,000 bp upstream of the transcription start site (TSS) and ~550 bp

374 downstream of the TTS (Fig. 4F). The second IR is variable among *A. thaliana*
375 accession, missing, for example, in Hod (Fig. 4G). Using CRISPR/Cas9
376 engineering, heterozygous lines could be obtained missing a fragment of the IR
377 located downstream of the TSS (with homozygous lines apparently not being
378 viable) (Fig. 4H). Similar to *PHYC*, we detected a chromatin loop that brings
379 together the borders of the *CRY1* gene only in those plants containing the IR and
380 that represses the expression of the gene (Fig. 4I). The absence of the IR, both
381 in the CRISPR lines or in the natural accession Hod, correlated with higher
382 expression levels of *CRY1* and shorter hypocotyls under blue light (Fig. 4I and
383 4J), a phenotype previously described in *CRY1* overexpressing lines (He et al.,
384 2019; Liu *et al.*, 2022).

385

386 Discussion

387 How organisms can adapt to a rapidly changing environment is one of the most
388 interesting questions in evolutionary biology (Barrett and Schluter, 2008;
389 Hermisson and Pennings, 2005). SNPs have been the main focus of many
390 genomic studies aiming to assess the evolutionary potential of mutations, but TEs
391 can be particularly powerful actors in rapid adaptation, as single transposition
392 events can have potentially wide-ranging consequences on gene expression and
393 derived phenotypes (Baduel *et al.*, 2021). On one side, the broad distribution of
394 TEs across the genome facilitates the generation of chromosomal
395 rearrangements through ectopic recombination. Even more significantly, the
396 mobilization of TEs can disrupt, modify or even change the expression of genes
397 in various ways that could generate a favorable adaptative trait (Dubin *et al.*,
398 2018; Friedli and Trono, 2015). New alleles caused by TE insertions have been
399 proposed to guarantee a consistent supply of potentially adaptable variants in
400 response to the environment (Baduel *et al.*, 2021). Still, many TEs inserted within
401 gene-rich areas are quickly purged, according to population genomic surveys of
402 TE polymorphisms (Quadrona *et al.*, 2016). This is in agreement with
403 transposition tending to produce alleles with negative effects.

404 Contrary to autonomous TEs, MITEs were found to be distributed on
405 chromosome arms in plants, highly associated with genes, and frequently
406 transcribed with adjacent genes (Kuang *et al.*, 2009; Lu *et al.*, 2012; Oki *et al.*,
407 2008). In agreement with this, our study identified numerous TE-derived IRs near
408 coding genes in *A. thaliana*. Different from rice, where more than half of the genes
409 are associated with MITEs (Lu *et al.*, 2012), fewer genes are associated with IRs
410 in *A. thaliana*. This observation is not surprising as Arabidopsis is an outlier
411 regarding TE content among plants, with only 15% of its genome represented by
412 TEs. In comparison, they account for 85% of maize and up to ~40% of rice
413 genomes (Arabidopsis Genome, 2000; Li *et al.*, 2017; Schnable *et al.*, 2009).

414 The association between MITEs and protein coding genes suggested that these
415 TEs may play essential roles in genome evolution. Current evidence suggests
416 that siRNA-triggered TE methylation tends to cause the repression of neighboring
417 genes (Hollister and Gaut, 2009; Hollister *et al.*, 2011). However, in the case of

418 MITEs, both positive and negative effects on the expression of host genes have
419 been reported (Gagliardi *et al.*, 2019; Underwood *et al.*, 2022; Wu *et al.*, 2022;
420 Xu *et al.*, 2020; Zhang *et al.*, 2016). The weak correlation between methylation of
421 MITEs and the expression of adjacent genes can now be better explained in the
422 light of our findings showing that IRs located near genes affect the local chromatin
423 organization. While methylation of DNA on its own is expected to have primarily
424 repressive effects, short-range chromatin loops could produce many different
425 outcomes, including transcriptional repression/activation and production of
426 alternative mRNAs (Gagliardi and Manavella, 2020).

427 MITEs have the potential to transpose into various locations in the genome
428 resulting in the presence/absence (insertional) polymorphisms between
429 genotypes (Lu *et al.*, 2012; Lyons *et al.*, 2008). Such polymorphism can be
430 caused by the insertion or excision of a MITE from a locus. However, it is
431 unknown which of these scenarios contributes more to the genetic variation within
432 a species. Here, we show that ~30% of the IRs located within 3,000bp of protein
433 coding genes present insertional polymorphism between 216 *A. thaliana* natural
434 accessions. Our results also indicate that such natural variation of the gene-
435 associated IR content can cause changes in chromatin organization that could
436 be considered "3D polymorphisms". Our experiments using not only association
437 between IR polymorphisms and gene expression changes, but also
438 CRISPR/Cas9-editing to demonstrate a causal relationship in several cases,
439 show that the insertion of an IR near a gene can have a profound impact on
440 chromatin organization, gene expression, and associated phenotypes. This
441 phenomenon can potentially boost a plant's capacity to adapt to a rapidly
442 changing environment. Because IRs can coopt the promoters of adjacent genes
443 to produce RNAPII-derived siRNAs through a stem-loop dsRNA intermediate,
444 they can act as autonomous regulatory elements, drastically changing the
445 chromatin landscape of a locus upon insertion. These characteristics turn IRs into
446 powerful elements during adaptative evolution.

447 Modelling has suggested faster generation of large-effect alleles due to
448 larger transposition rates in specific populations in response to global warming
449 (Baduel *et al.*, 2021). Consistent with this scenario, and in a world of a rapidly
450 changing climate, the discovery of IRs as elements shaping the 3D chromatin
451 organization and driving genome adaptation is of great interest. The manipulation
452 of IRs, and in consequence, genome topology, can potentially become a powerful
453 biotechnological tool to improve crop adaptation without the need to incorporate
454 exogenous DNA or alter coding sequences in plants.

455

456 **Material and methods**

457 **Plant material and growth conditions**

458 *Arabidopsis thaliana* accessions Col 0, Hod, Ba1, EY15-2, *dcl234* (Lu *et al.*
459 *et al.*, 2006), and *ddc* (Kurihara *et al.*, 2008) mutants and CRISPR mutant lines
460 were grown at 23°C in long day (16/8 hours light/dark). Blue and red-light
461 experiments were carried out as follows: seeds were sowed on petri dishes with

462 humidified filter paper and stratified in the dark for 5 days, and then transferred
463 to white light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$) at 23°C for 3 hours and subsequently transferred
464 to red ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$) or blue light ($5 \mu\text{mol m}^{-2} \text{s}^{-1}$) in LED chambers. Hypocotyl
465 measurements were taken at four days using ImageJ (<http://rsb.info.nih.gov/ij>).

466 To obtain plants with genomic fragments deletions a CRISPR/Cas9 vector
467 toolbox (Wu et al., 2018) was used. Specific sgRNAs, described in Supplemental
468 Table S1, were designed to obtain the IRs deletions. Col-0 plants were
469 transformed using the floral dip method, T1 plants were selected based on the
470 presence of red fluorescence in seeds under a fluorescent dissecting microscope
471 (Leica, Solms, Germany), non-fluorescent T2 seeds missing the transgenes were
472 grown and genotyped by PCR to identify effective deletions.

473 For genomic DNA extraction 100 mg of fresh plant material was ground in
474 700 μl of extraction buffer (200 mM Tris-HCl pH8, 25 mM EDTA, 0.5% SDS)
475 and precipitated with isopropanol. PCR was performed using primers detailed in
476 Table S1. RNA was extracted using TRIzolTM Reagent (Invitrogen) following
477 manufacturer's recommendations.

478 The presence of the IR in different *A. thaliana* accessions was determine
479 by amplification of the IR region with flanking primers with Q5 polymerase,
480 followed by Sanger sequencing. For the deletion of the IRs neighboring *PHYC*
481 and *CRY1*, pairs of sgRNAs targeting each locus were cloned in a CRISPR/Cas9
482 super module (SM) vector as described (Wu et al., 2018). Briefly, sgRNAs
483 targeting flanking regions of the IR were designed using the CRISPR-P Web Tool
484 (Lei et al., 2014). Each sgRNA was introduced into the shuffle vectors by overlap
485 PCR with Q5 Hi-Fidelity polymerase followed by digestion of the original vector
486 with *DpnI* (Thermo Scientific). A destination vector harboring *UBQ10* promoter,
487 pcoCas9, proLacZ:LacZ between both sgRNAs targeting each IR, and
488 At2S3:mCherry for fluorescence selection in seeds were generated with the
489 Green Gate assembly system. Destination vectors were transformed into Col-0
490 plants, and red fluorescence-positive seeds were isolated as hemizygous seeds.
491 Transgene-free T2 offspring without seed fluorescence were chosen, and plants
492 were tested by PCR and Sanger sequencing to identify IR deletion lines. All
493 primers used are listed in Table S1

494

495 **IR detection**

496 Inverted repeats were identified in the Col-0 *Arabidopsis thaliana* genome
497 using einverted, from the EMBOSS program suite (Rice et al., 2000). The
498 parameters used were: maximum repeat of 1000, a gap penalty of 8, a minimum
499 score threshold of 150, a match score of 3, and a mismatch score of -4.

500

501 **RNA-seq**

502 RNA-seq library preparation was performed as described (Cambiagno et
503 al., 2021). An in-house scaled-down version of Illumina's TruSeq reaction was
504 used. mRNA was purified with NEBNext Poly(A) Magnetic Isolation Module (New
505 England Biolabs, Ipswich, MA) and heat fragmented with Elute-Prime-Fragment

506 buffer (5x first-strand buffer, 50 ng/ml random primers). For first- and second-
507 strand synthesis SuperScript II Reverse Transcriptase (Thermo Fisher) and DNA
508 polymerase I (NEB) were used, respectively. T4 DNA polymerase, Klenow DNA
509 polymerase, T4 polynucleotide kinase (NEB), and Klenow Fragment (30/50 exo-)
510 (NEB) were used for end repair and A-tailing. Ligation of universal adapters
511 compatible with Nextera barcodes i7 and i5 was performed with T4 DNA Ligase
512 (NEB), and Q5 Polymerase (NEB) was used for PCR enrichment using Nextera
513 i7 and i5 barcodes. SPRI beads were used for DNA purification in each step and
514 size selection of the library preps. 2 x 150 bp paired-end reads were generation
515 on the Illumina HiSeq 3000 platform.

516 The analysis started by quality trimming and filtering the raw reads with
517 Trimmomatic version 0.36; (Bolger et al., 2014). They were then aligned to the
518 Arabidopsis thaliana genome (TAIR10) using STAR version 2.5.2b, (Dobin, 2013
519 #73), which was guided by the gene and exon annotation from Araport V11
520 201606, (Pasha et al., 2020). Samtools version 1.; (Li et al., 2009) was then used
521 to keep only primary alignments with a minimum MAPQ of 3. Read quality before
522 and after trimming was analyzed with FastQC (version 0.11.5;
523 <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and, together with
524 mapping efficiency, they were summarized with MultiQC version 1.7 (Ewels et
525 al., 2016). Read counts on each gene were then calculated with featureCounts
526 version 1.6.2 (Liao et al., 2014). This pipeline was run with the aid of the
527 Snakemake workflow engine (Koster and Rahmann, 2012). Gene counts were
528 used to identify differentially expressed genes with DESeq2 (Love et al., 2014);
529 R Core Team 2022) filtering out genes with counts below 10 in all samples.

530

531 **sRNA-seq**

532 For sRNA-seq library preparation, 1 µg of total RNA was used as input for
533 the TruSeq sRNA Library Preparation kit (Illumina) as described in the TruSeq
534 RNA Sample Preparation v2 Guide (Illumina). BluePippin System (Sage Science)
535 was used for sRNA library size selections. Sequencing was performed on the
536 Illumina HiSeq 3000 platform.

537 The small RNA reads generated were first cut to remove 3' adapters using
538 cutadapt (version 1.9.1) and their quality checked using FastQC (version 0.11.4,
539 <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and MultiQC
540 (Ewels et al., 2016). They were then mapped with bowtie (version 1.1.2;
541 (Langmead et al., 2009)) to *A. thaliana* rRNA, tRNA, snoRNA and snRNA from
542 RFAM (version 14.1, (Kalvari et al., 2018)). Unmapped reads were then mapped,
543 also with bowtie, to the *A. thaliana* genome. Statistical analyses were performed
544 in the R statistical programming environment (R Core Team, 2022) and graphics
545 were produced with the ggplot package.

546 IRs which had 10 or more 24 nucleotide reads in its entire region were
547 considered to have potential sRNA production. Changes in sRNA levels were
548 calculated first calculating reads per million (RPM) mapping reads to the genome

549 in each library, then averaging this value for all replicates in each IR, and then
550 calculating the log₂ Fold Change between the RPM in the mutant versus Col-0.

551

552 **Bisulfite treatment of DNA and library preparation**

553 For BS-seq, DNA extraction was performed with DNeasy plant Mini Kit
554 (QUIAGEN). DNA was sheared to 350 bp by g by Covaris ultrasonication.
555 Libraries were generated with Illumina TrueSeq DNA Nano Kit. After adaptor
556 ligation, libraries were bisulfite converted with the EpiTec Plus DNA Bisulfite
557 Conversion Kit (QUIAGEN). Library enrichment was done using Kapa Hifi Uracil+
558 DNA polymerase (Kapa Biosystems, USA). Paired-end reads (2 x 150 bp) were
559 generation on the HiSeq 3000 platform (Illumina).

560 The analysis of these reads started by quality trimming and filtering them
561 with Trimmomatic (version 0.36; (Bolger *et al.*, 2014)). Then we used the Bismark
562 program (Krueger and Andrews, 2011) to perform the mapping of the reads to
563 the *A. thaliana* Col-0 genome, internally done with Bowtie2 (Langmead and
564 Salzberg, 2012), the deduplication of the alignments and the extraction of the
565 methylation results in the three contexts: CG, CHG and CHH. This output was
566 then analyzed in R (R core team, 2022) with the methylKit package (Akalin *et al.*,
567 2012). Only Cytosines with at least 4 reads were considered, and each sample
568 was segmented with methSeg and methylation levels were calculated for those
569 including at least 4 Cs. For Col-0, segments were collapsed for replicates using
570 the mergeGRangesData function from the BRGenomics package
571 (<https://rdr.io/bioc/BRGenomics/>) and IRs with repeats overlapping segments
572 with more than 10 or more percent of CHG or CHH methylation were considered
573 methylated.

574 Differential methylation in the *ddc* and *dcl234* mutants was also calculated
575 with the methylKit package. First replicates were combined with the unite function
576 and then differential methylation calculated with the calculateDiffMeth function,
577 correcting for overdispersion with the MN method, using a q-value threshold of
578 0.1 and a differential threshold of 15 %. Then IRs with repeats overlapping any
579 of these differential segments was considered differentially methylated.

580

581 **Capture-C assay**

582 For Capture-C, Hi-C was performed as described (Liu, 2017). Briefly, we
583 collected 1.5 g of plant tissue, and fixed them with 1% formaldehyde. Nuclei were
584 isolated and finally washed with NEB buffer #3. Nuclei penetration was done by
585 resuspending the pellet in 150 µl 0.5% SDS and incubating them at 62 °C for 5
586 min. After that, 435 ul of water and 75 ul of 10% Triton X-100 were added and
587 incubated 37 °C for 15 min. NEB buffer #3 was added to 1X, and 50 U of DpnII
588 to digest the chromatin over night at 37°C. Incubating the digested chromatin with
589 10 U Klenow, dTTP, dATP, dGTP, and biotin-14-dCTP at 37°C for 2 h, cohesive
590 ends were filled. Blunt-end ligation of chromatin was performed by adding blunt-
591 end ligation buffer to 1X and 20 U of T4 DNA ligase at room temperature for 4 h.
592 Nuclei were lysed with SDS buffer (50 mM Tris-HCl, 1% SDS, 10 mM EDTA,

593 pH 8.0) and incubated with 10 µg proteinase K at 55°C for 30 min. To reverse the
594 crosslinking, NaCl was added to reach 0.2 M and the samples were incubated at
595 65 °C overnight. Hi-C DNA was purified by Phenol-Chloroform-IAA method and
596 RNase A treated. Hi-C DNA was sheared to 500 bp with a Covaris E220
597 sonicator. DNA was purified and size selected (longer than 300 bp) using Ampure
598 beads. Unligated biotin was removed in a reaction with 0.1 mM dTTP, 0.1 mM
599 dATP and 5 U T4 DNA polymerase incubated at 20°C for 30 min. DNA was
600 purified with Ampure beads and end-repair and adaptor ligation were performed
601 with the NEBNext® Ultra™ II DNA Library Prep Kit by following the
602 manufacturer's instructions. Biotin affinity purification was then performed by
603 using Dynabeads MyOne Streptavidin C1 beads (Invitrogen). Library
604 amplification was done with Ultra II Q5 Master Mix with universal and selected
605 index primers.

606 For the Capture step, hybridization capture was performed with the
607 MyBaits system (Arbor Biosciences) following the manufacturer's instructions.
608 Baits of 80 nucleotides were designed on each end of the digestion fragments
609 corresponding to the captured regions. These regions included all genes within
610 3000 bp of the IR and the spacer region up to the IR, excluding it. When a region
611 was surrounded by 2 IRs, it was considered a single captured region.

612 Finally, Capture-C DNA was paired-end sequenced (2 × 150 bp reads) on
613 an Illumina HiSeq 3000. The resulting reads were processed with capC-MAP
614 (Buckle *et al.*, 2019), which performs the *in silico* genome digestion, read
615 alignment, the pile-up of interactions, and can generate normalized, binned and
616 smoothed profiles of interaction for each target. For this the Col-0 genome was
617 used, an exclusion zone of 500 bp, a bin size of 500 bp and a step of 250 bp. The
618 results were then processed with the R package peakC (Geeven *et al.*, 2018) to
619 determine statistically significant interactions. These loops were visualized with
620 the aid of the R package and Gviz (Hahne and Ivanek, 2016).

621 For the SSIM calculation, raw pileups were first normalized with FAN-C
622 (Kruse *et al.*, 2020), using the VC-SQRT method on 1000 bp bins. The SSIM
623 value was obtained on a region comprising the captured region extended by 10
624 kb on both extremes, and using a relative window size of 0.1.

625

626 **Data processing, plotting and statistical analysis**

627 Data obtained in the different analysis of the sequencing experiments was
628 further processed and statistically analyzed in R (R Core Team, 2022) using a
629 diversity of packages. Genomic information was handled using GenomicRanges
630 (Lawrence *et al.*, 2013), Biostrings
631 (<https://bioconductor.org/packages/Biostrings>) GenomicInteractions (Harmston
632 *et al.*, 2015) and rtracklayer (Lawrence *et al.*, 2009) packages.

633 Plots summarizing information were mostly performed with the ggplot and
634 ggpubr packages. Plots of genomic regions were produced with the Gviz (Hahne
635 and Ivanek, 2016) package. Circular plots were generated with ciclize (Gu *et al.*,
636 2014).

637

638 **Data availability**

639 All sequencing data were deposited at the European Nucleotide Archive
640 (<https://www.ebi.ac.uk/ena/>) public repository with accession PRJEB53956.

641

642 **3C assay and RT-PCR**

643 3C assay was performed as described (Gagliardi *et al.*, 2019). For
644 detection of loops at *PHV* and *PHYC*, EcoRI (NEB) overnight digestion was
645 performed; for *P5CS1*, *CRY1*, and *PHR1*, XbaI (NEB) was used. For DNA
646 ligation, 100 U of highly concentrated T4 DNA ligase (Thermo) were used at 22°C
647 for 5 h in a 4 mL volume. Reverse crosslinking and proteinase K treatment
648 (Invitrogen) were performed, and phenol/chloroform method was used for DNA
649 purification. For interaction frequency measurement, qPCR was performed using
650 *ACTIN2* as housekeeping gene. All primers used are listed in Table S1.

651 For quantitative RT-PCR, 1 µg of total RNA was used for reverse
652 transcription reactions using RevertAid RT Reverse Transcription Kit (Thermo
653 Fisher Scientific). qPCR was performed using SYBR green (Thermo Scientific
654 Maxima SYBR Green qPCR Master Mix (2x)). Three biological replicates were
655 used to calculate the standard error of the mean. Standard error of the mean
656 (SEM) was calculated using propagation of error of the $2^{-\Delta\Delta C_t}$ values and
657 expressed in figures as two times the SEM. Statistical significance was tested
658 using a two-tailed, unpaired Student's t-test. All primers used are listed in Table
659 S1.

660

661

662 **Author contribution**

663 A.L.A. performed the majority of the analyses. D.A.C. prepared the
664 libraries for sequencing and Capture-C experiments. P.L.L. and H.A.B. helped
665 with the design of the Capture-C probes. P.L.L. performed the Capture
666 experiment. R.M. and D.A.C. validated the chromatin loops formations and
667 created the CRISPR/CAS9 mutant lines. R.M. characterized the CRISPR/Cas9
668 mutant lines and performed validation experiments. A.L.A., D.A.C., P.A.M., and
669 D.W. conceived this study; P.A.M. and D.W. supervised the work and secured
670 project funding; A.L.A., R.M., D.W., and P.A.M. wrote the manuscript.

671

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679

680 **Footnotes**

681 The authors declare no competing interest.

682

683 **References**

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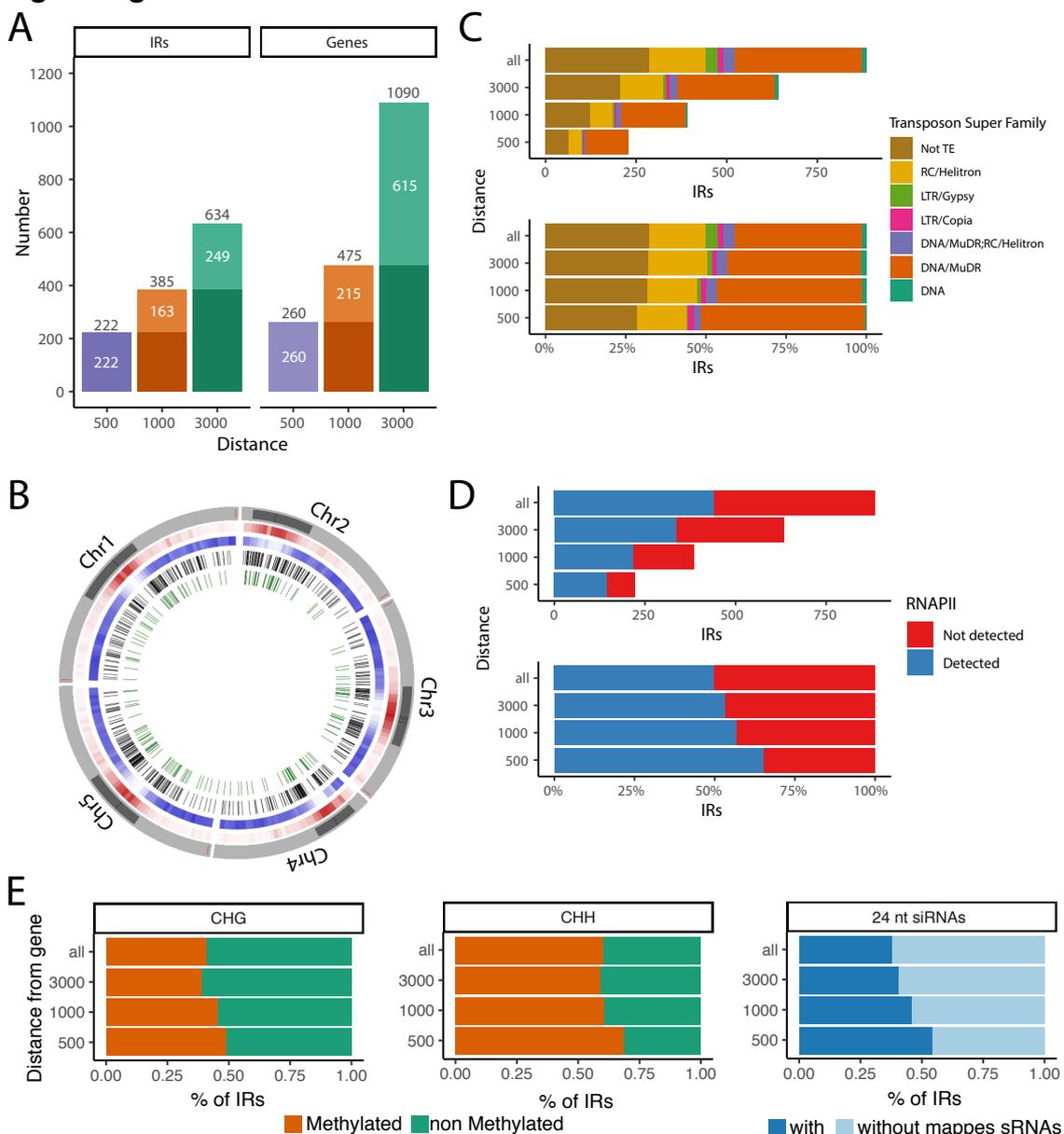
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968 **Figure legends**



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970 **Figure 1. IRs distribution near annotated genes in the *A. thaliana* Col-0**
 971 **reference genome.**

972 **A.** Number of IRs detected in the Col-0 *genome* within 500, 1,000 and 3,000 bp
 973 from annotated genes (left), and the number of associated genes within the same
 974 size window from the identified IRs (right). White numbers indicate the number of
 975 IR and genes exclusive of the 0-500, 501-1000, and 1001-3000 bp windows while
 976 black numbers show the cumulative count.

977 **B.** Distribution of IRs. Outermost track, the five Arabidopsis chromosomes with
 978 the pericentromeric regions highlighted in darker gray. Then, TE density in 500
 979 kb windows shown in red, and gene density in the same bins, in blue. In both red
 980 and blue tracks, darker color indicates higher density. The inner two tracks show

981 the distribution of all IRs (black bars) or only IRs within 3,000 bp upstream or
982 downstream of annotated protein coding genes (green bars).

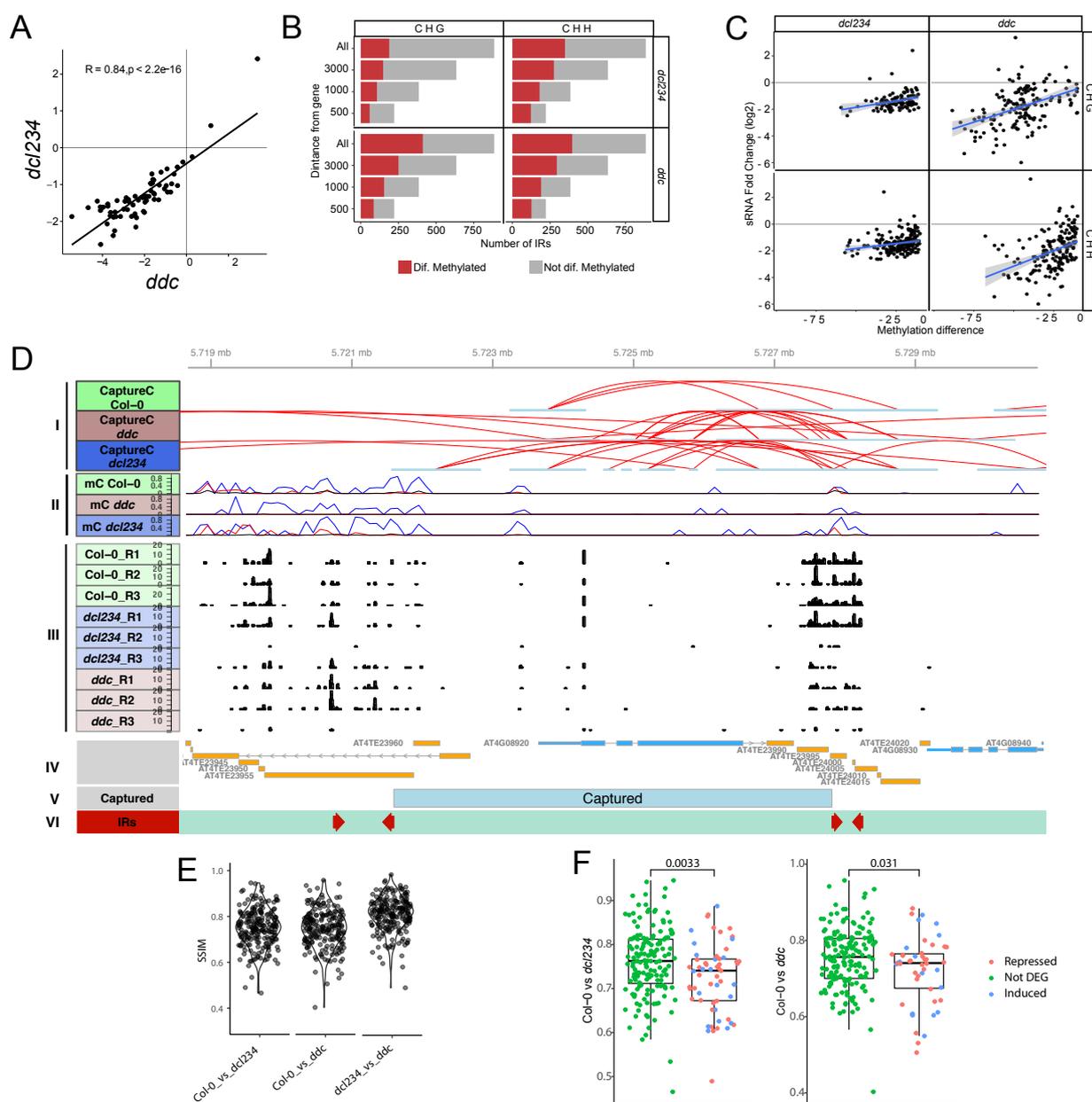
983 **C.** Classification of the identified IRs between annotated TE super families, only
984 showing super families with at least five overlapping IRs. The
985 “DNA/MuDR;RC/Helitron” category indicates IRs overlapping with TEs from both
986 superfamilies. The upper panel shows the total number of IR in each superfamily
987 while the bottom panel displays the percentage of each superfamily identified in
988 each distance window from annotated genes.

989 **D.** Total number (up) or percentage (down) of IR nascent transcripts associated
990 with RNAPII as detected by plaNet-Seq (Kindgren *et al.*, 2020). IRs producing
991 transcripts associated with RNAPII are marked in blue and IRs without RNAPII
992 transcripts in red.

993 **E.** Percentage of gene-adjacent IRs with cytosine methylation in the CHG (left)
994 and CHH (center) contexts detected in at last 10% of the mapping reads and with
995 24-nt siRNAs mapping to the IR sequence (at least 10 reads in at least one
996 replicate).

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Figure 2. IR near genes produce siRNAs, trigger DNA methylation and alter the local chromatin 3D organization.

A. Changes in siRNA levels mapping to IRs in the *ddc* and *dcl234* mutants with respect to Col-0. The axes represent log₂ Fold Change of siRNA reads per million (RPM) over each IR. The correlation (R) is shown on the upper left corner.

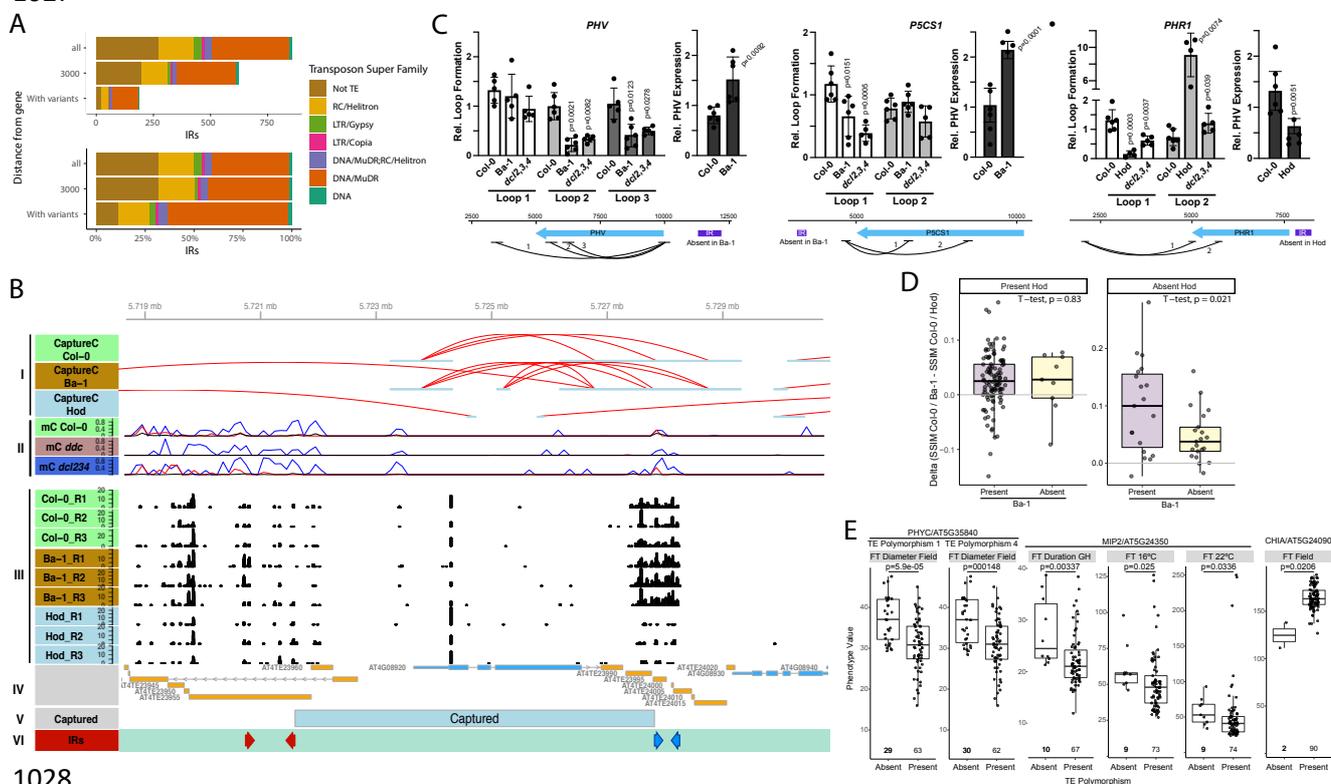
B. Number of gene-associated IRs showing differential DNA methylation in the CHG and CHH contexts in the *ddc* and *dcl234* mutants with respect to Col-0.

C. Scatter plots showing the relation between changes in siRNA levels and DNA methylation over each gene-associated IRs in both mutants, *ddc* and *dcl234*. The blue lines show the linear regression while the gray shades show the confidence interval.

D. Region of the Col-0 genome containing the *CRY1* locus displaying the epigenetic and topological profile. (I) Chromatin interactions as detected by Capture-C experiments and shown by red lines indicating interacting fragments.

1014 (II) Cytosine DNA methylation in CG (blue), CHG (red) and CHH (black) contexts.
 1015 (III) 24 nt siRNAs mapping to the genomic regions as determined by sRNA
 1016 sequencing in biological triplicates of WT plants and *dcl234* and *ddc* mutants. (IV)
 1017 Annotated genes (cyan) and transposons (yellow) in the region. (V) Region
 1018 captured by the probes designed for the CaptureC experiment. (IV) IRs identified
 1019 in this region of the genome.
 1020 E. Structural similarity (SSIM) of captured regions in *dcl234* and *ddc* mutants with
 1021 respect to Col-0 or to each other.
 1022 F. SSIM of captured regions in *dcl234* and *ddc* mutants with respect to Col-0
 1023 grouped by regions including differentially regulated genes (DEGs) or regions
 1024 without DEGs (NotDEGs). The colors of the dots represent whether they are not
 1025 differentially expressed, upregulated, or downregulated.

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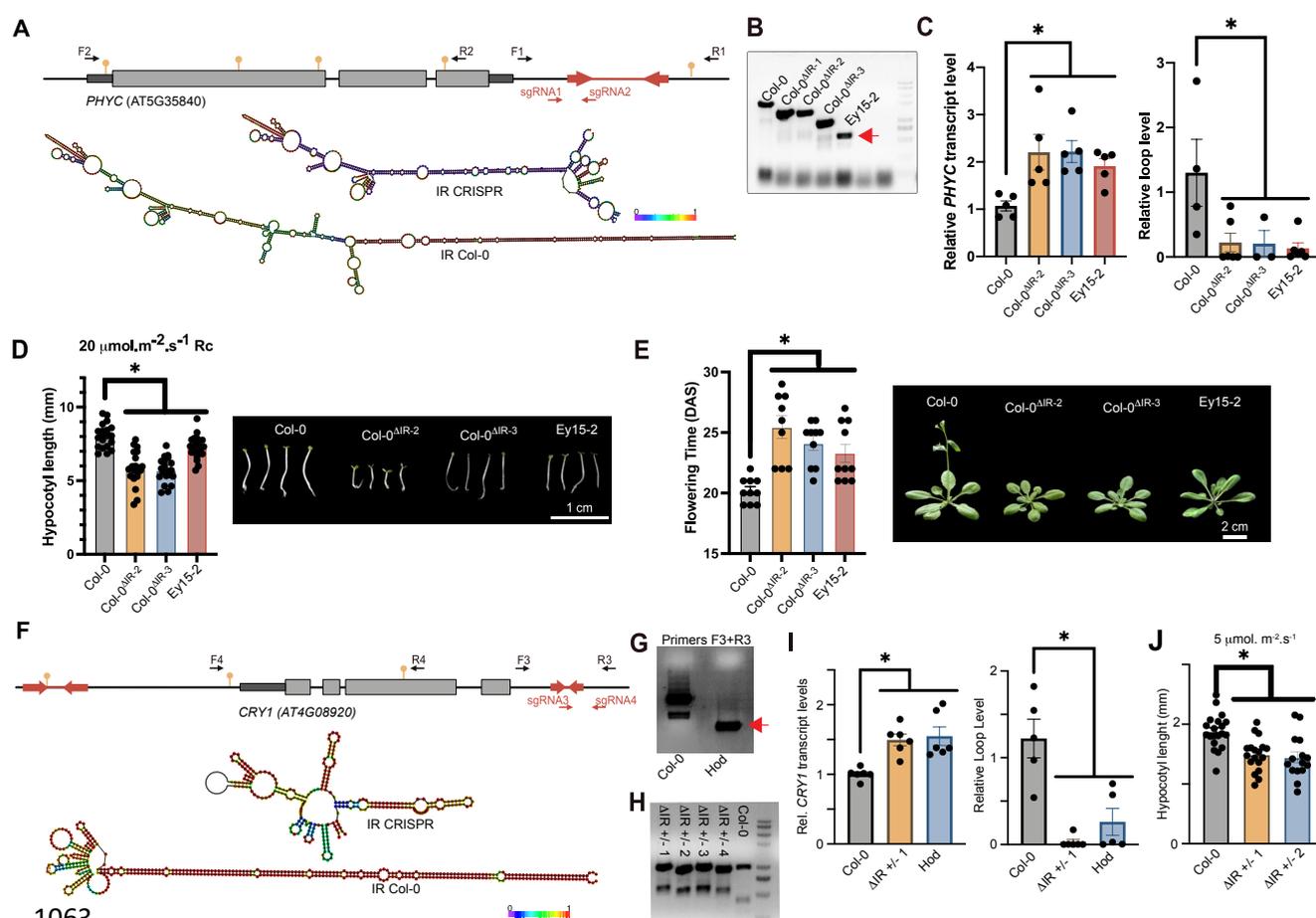


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 1029 **Figure 3. Insertional polymorphisms of IRs near genes cause natural**
 1030 **variation in short-range chromatin topology.**

1031 **A.** Number of IRs and polymorphic IRs associated with genes (upper panel).
 1032 Percentage of IRs and polymorphic IRs by annotated TE superfamilies (lower
 1033 panel). Only superfamilies with at least five overlapping IRs are displayed. The
 1034 “DNA/MuDR;RC/Helitron” category indicates IRs overlapping with TEs from both
 1035 superfamilies.

1036 **B.** Region of the Col-0 genome containing the *CRY1* locus with its epigenetic and
 1037 topological profile. (I) Chromatin interactions as detected by Capture-C
 1038 experiments from Col-0 and Ba-1 plants (both contain the IR) and Hod plants
 1039 (which has a deletion polymorphism in the same IR). Red lines indicate

1040 interacting fragments. (II) Cytosine DNA methylation in CG (blue), CHG (red) and
1041 CHH (black) contexts. (III) 24 nt siRNAs mapping to the genomic regions as
1042 determined by sRNA sequencing in biological triplicates of wild-type Col-0, Ba-1
1043 and Hod plants. (IV) Annotated genes (cyan) and transposons (yellow) in the
1044 region. (V) Region captured by the probes designed for the Capture-C
1045 experiment. (IV) IRs identified in this region of the genome. The IR is red is
1046 conserved between the three genotypes while the IR in blue is missing in Hod.
1047 **C.** RT-qPCR with total RNA, and Chromatin Conformation Capture (3C) qPCT
1048 experiments to quantify the formation of specific chromatin loops as well as the
1049 expression of the affected loci in WT, Ba-1, Hod plants and *dcl234* and *ddc*
1050 mutants. Data are presented as mean values +/- SD. p-values were calculated
1051 with two-tailed unpaired Student's t-Test with Welch's correction. n>4 biologically
1052 independent samples. A diagram on the bottom of each gene shows the
1053 chromatin loops amplified and confirmed by Sanger sequencing for each locus.
1054 **D.** Delta of the structural similarity (SSIM) calculated with CHESS for the
1055 contrasts Col-0 vs Ba-1 and Col-0 vs Hod for each captured region. Each
1056 captured region is classified depending on whether the associated IR is present
1057 or absent in the three analyzed accessions.
1058 **E.** Association of flowering phenotypes (FT), including rosette diameter upon
1059 flowering, flowering duration, and flowering time at different temperatures either
1060 in plants grown in the field or green house (GH), with the presence or absence of
1061 an IR near selected genes.
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Figure 4. Insertional polymorphisms of IRs near *PHYC* and *CRY1* cause changes in the loci topology, expression and associated phenotypes.

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A. *PHYC* locus diagram showing the position of the IR, restriction sites for EcoRI used for 3C experiments (yellow pins), the sgRNAs used for CRISPR/Cas9 deletion (sgRNA1 and sgRNA2), and primer positions. Predicted secondary RNA structures for the IR transcript in Col-0 plants or in the CRISPR-edited plants are shown using a color scale corresponding to base pairing probability 0 (purple) to 1 (red).

B. PCR using F1-R1 primers on genomic DNA, confirming partial deletion of the IR in the CRISPR lines and complete absence of the IR in Ey15-2 accession. Red arrowhead indicates the band sequenced to confirm the absence of the polymorphic IR in the Ey15-2 accession.

C. *PHYC* relative expression as measured by RT-qPCR and normalized against *ACT2* (left panel). Quantification of the formation of a short-range chromatin loop by 3C-qPCR analysis using EcoRI and F2-R2 primers (right panel). Data represents individual values + SEM of five independent biological replicates.

D. Hypocotyl length under Continuous Red light (Rc) regimen. Seedlings were grown under Rc light $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ for four days before measurement.

E. Flowering time for the different genotypes expressed as days after sowing (DAS). Data represents individual values \pm SD, significant differences are indicated as *, for $P < 0.05$ in a one-way ANOVA.

1085 **F.** *CRY1* locus diagram showing the position of the IRs, restriction sites for XbaI
1086 (yellow pins), the sgRNAs used for CRISPR/Cas9 deletion (sgRNA3 and
1087 sgRNA4), and primer positions. On the bottom are the secondary RNA structures
1088 as predicted by RNAfold for the Col-0 wildtype IR transcript and the CRISPR-
1089 edited outcome of the same IR. Color scale shows base pair probability 0 (purple)
1090 to 1 (red).

1091 **G and H.** PCR using F3-R3 primers upon genomic DNA showing successful
1092 partial deletion of the IR in CRISPR heterozygous lines and complete absence in
1093 Hod plants. Red arrowhead indicates the band sequenced to confirm the absence
1094 of the polymorphic IR in the Hod accession.

1095 **I.** *CRY1* expression analysis measured by RT-qPCR normalized against *ACT2*
1096 on the left. Quantification of a chromatin loop by 3C-qPCR using XbaI and F4-R4
1097 primers (right panel). Data represents average values of three independent
1098 biological replicates with SEM.

1099 **J.** Hypocotyl length in continuous blue light. Seedlings were grown under blue
1100 light $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ for four days before measured.

1101 In all cases data represent individual values \pm SD, significant differences are
1102 indicated as *, for $P < 0.05$ in a one-way ANOVA.

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