

1 **Sequence-unrelated long noncoding RNAs converged to modulate the activity of**
2 **conserved epigenetic machineries across kingdoms**

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20 **Running title: lncRNAs regulate Polycomb and DNA methylation**

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25

26 **SUMMARY**

27 RNA-DNA hybrid (R-loop)-associated long noncoding RNAs (lncRNAs), including the
28 Arabidopsis lncRNA *AUXIN-REGULATED PROMOTER LOOP (APOLO)*, are emerging as important
29 regulators of three-dimensional chromatin conformation and gene transcriptional activity. Here,
30 we showed that in addition to the PRC1-component LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1),
31 *APOLO* interacts with the methylcytosine-binding protein VARIANT IN METHYLATION 1 (VIM1), a
32 conserved homolog of the mammalian DNA methylation regulator UBIQUITIN-LIKE CONTAINING
33 PHD AND RING FINGER DOMAINS 1 (UHRF1). The *APOLO*-VIM1-LHP1 complex directly regulates
34 the transcription of the auxin biosynthesis gene *YUCCA2* by dynamically determining DNA
35 methylation and H3K27me3 deposition over its promoter during the plant thermomorphogenic
36 response. Strikingly, we demonstrated that the lncRNA *UHRF1 Protein Associated Transcript*
37 (*UPAT*), a direct interactor of UHRF1 in humans, can be recognized by VIM1 and LHP1 in plant
38 cells, despite the lack of sequence homology between *UPAT* and *APOLO*. In addition, we showed
39 that increased levels of *APOLO* or *UPAT* hamper VIM1 and LHP1 binding to *YUCCA2* promoter.
40 Collectively, our results uncover a new mechanism in which a plant lncRNA coordinates Polycomb
41 action and DNA methylation, and reveal that evolutionary unrelated lncRNAs may exert similar
42 functions across kingdoms.

43 INTRODUCTION

44 In eukaryotes, chromatin structure, composition and dynamics determine the three-
45 dimensional configuration of the genome and are critical for gene regulation, cell fate and function
46 (Yu and Ren, 2017; Doğan and Liu, 2018). Chromatin conformation and related transcriptional
47 states depend on coordinated shifts in DNA methylation, post-translational modifications of
48 histone tails and RNA interference (RNAi) pathways (Tamaru and Selker, 2001; Jaenisch and Bird,
49 2003; Matzke and Birchler, 2005). In mammalian genomes, DNA methylation is primarily observed
50 at CpG dinucleotides and is estimated to occur at ~70-80% of CpG sites throughout the genome
51 (Lister et al., 2009). A quarter of non-CG methylation is found in embryonic stem cells, while the
52 remaining unmethylated CpG sites are mostly found in dense clusters, near gene promoters,
53 referred to as CpG islands (Ramsahoye et al., 2000; Suzuki and Bird, 2008; Varley et al., 2013).
54 Unlike in mammals, DNA methylation in plants predominantly occurs on transposons and other
55 repetitive DNA elements, and exists in all possible sequence contexts: symmetric CpG and CpHpG
56 – where H is any base except G – or asymmetric CpHpH (Zhang et al., 2006; Johnson et al., 2007).
57 Non-CpG methylation is mainly distributed at heterochromatin regions, nevertheless, many
58 euchromatic genes exhibit cytosine methylation in their promoter, a feature likely correlated with
59 tissue specificity (Zhang et al., 2006).

60 In *Arabidopsis thaliana*, the establishment of *de novo* methylation in all sequence contexts
61 is catalyzed by DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), a plant homolog of
62 mammalian DNA (CYTOSINE-5)-METHYLTRANSFERASE 3 (DNMT3) a and b (Cao and Jacobsen,
63 2002; Law and Jacobsen, 2010). DRM2 is guided to chromatin by 24-nucleotide small interfering
64 RNAs (24nt siRNAs) as part of a pathway known as RNA-directed DNA methylation (RdDM; Cuerda-
65 Gil and Slotkin, 2016). RdDM involves two non-redundant plant specific RNA polymerases, Pol IV
66 and Pol V, in addition to the canonical RNA interference machinery, which requires the activity of
67 RNA-dependent RNA polymerase 2 (RDR2) and members of the Dicer and Argonaute families
68 (Wassenegger et al., 1994; Huettel et al., 2007; Pikaard et al., 2008; Law et al., 2010). Once
69 established, DNA methylation is maintained by three different pathways depending on the
70 sequence context. CpG methylation depends on DNA METHYLTRANSFERASE 1 (MET1), a homolog
71 of mammalian DNA METHYLTRANSFERASE 1 (DNMT1; Finnegan et al., 1996; Kankel et al., 2003).
72 CpHpG methylation relies on CHROMOMETHYLASE 3 (CMT3), a plant specific DNA
73 methyltransferase that associates with SU(VAR)3–9 HOMOLOG (SUVH) histone methyltransferases
74 and recognizes dimethylated histone 3 tails at lysine 9 (H3K9me2; Ebbs and Bender, 2006;

75 Henderson and Jacobsen, 2007; Johnson et al., 2007; Du et al., 2012; Stroud et al., 2014). Finally,
76 CpHpH methylation is maintained through persistent *de novo* methylation by DRM2 and by the
77 CMT3 homolog CHROMOMETHYLASE 2 (CMT2) that specifically reads the H3K9me2 mark (Stroud
78 et al., 2013, 2014; Zemach et al., 2013).

79 Beside the contribution of methyltransferases, the DNA methylation process involves
80 methylcytosine-binding proteins of the VARIANT IN METHYLATION (VIM/ORTH) family (Woo et al.,
81 2007, 2008). VIM/ORTH proteins are homologous to the mammalian UBIQUITIN-LIKE CONTAINING
82 PHD AND RING FINGER DOMAINS (UHRF) proteins known to regulate cytosine methylation
83 through the recruitment of DNMT1 to target loci (Bostick et al., 2007; Sharif et al., 2007; Kraft et
84 al., 2008; Bronner et al., 2019). In particular, UHRF1 functions as an epigenetic regulator
85 maintaining DNA methylation and histone modifications (Xue et al., 2019), and is stabilized by
86 direct interaction with the long noncoding RNA (lncRNA) *UPAT (UHRF1 Protein Associated*
87 *Transcript*; Taniue et al., 2016). VIM proteins are characterized by the presence of a PHD domain
88 recognizing trimethylated histone 3 tails at lysine 4 (H3K4me3; Li et al., 2006; Peña et al., 2006; Shi
89 et al., 2006; Wysocka et al., 2006), a SRA (SET [Su(var), Enhancer of Zeste, Trithorax] and RING
90 [Really Interesting New Gene] Associated) domain that can associate with methylated DNA (Unoki
91 et al., 2004; Johnson et al., 2007; Woo et al., 2007), and two RING domains conferring ubiquitin E3
92 ligase activity (Kraft et al., 2008). The Arabidopsis genome encodes five highly similar VIM proteins
93 – named VIM1 to 5 – and a related protein ORTH-LIKE1 (ORL1/VIM6; Kraft et al., 2008). VIM1,
94 VIM2 and VIM3 maintain MET1-mediated cytosine methylation at CpG dinucleotides throughout
95 the genome and have been reported to function entirely redundantly to mediate epigenetic
96 transcriptional silencing in collaboration with MET1 (Woo et al., 2008; Stroud et al., 2013; Shook
97 and Richards, 2014).

98 Interestingly, aberrant changes in active and repressive histone modifications have been
99 observed in *vim1/2/3* and *met1* mutants, supporting that VIM proteins coordinate DNA
100 methylation and histone modification (Soppe et al., 2002; Tariq et al., 2003; Deleris et al., 2012;
101 Kim et al., 2014). In Arabidopsis, the transcriptionally repressive mark histone H3 lysine 27
102 trimethylation (H3K27me3) is largely restricted to the transcribed regions of single genes,
103 exhibiting a global anti-correlated distribution with centromeric-enriched DNA methylation
104 (Mathieu et al., 2005; Johnson et al., 2007; Deleris et al., 2012). However, a loss of H3K27me3 was
105 also reported at gene bodies in *met1* mutants and at specific VIM1 targets located in euchromatic
106 regions in the *vim1/2/3* triple mutant (Deleris et al., 2012). Notably, the correlation between DNA

107 hypomethylation and H3K27me3 reduction in the *vim1/2/3* mutation was more prevalent in
108 promoter regions than in transcribed regions (Kim et al., 2014b). Collectively, these observations
109 hint at non-canonical mechanisms linking DNA methylation and repressive histone modifications
110 over specific transcriptionally active loci.

111 In *A. thaliana*, H3K27me3 is deposited by Polycomb group (PcG) proteins in euchromatic
112 regions containing protein-coding genes and is maintained by the plant Polycomb Repressive
113 Complex 1 (PRC1) component LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), a homolog of
114 mammalian HETEROCHROMATIN PROTEIN 1 (HP1; Gaudin et al., 2001; Hsieh et al., 2003; Turck et
115 al., 2007; Veluchamy et al., 2016). LHP1 capacity to localize and mediate epigenetic repression of
116 PcG target genes was shown to rely on its RNA-binding hinge region, suggesting that LHP1 activity
117 could be modulated by interacting RNAs (Berry et al., 2017). Consistently, it was shown that the
118 lncRNA *APOLO* (*AUXIN-REGULATED PROMOTER LOOP*) can regulate local chromatin conformation
119 by decoying LHP1 away from target loci. *APOLO* directly recognizes multiple distant and
120 independent auxin-related loci across the *A. thaliana* genome by short sequence complementarity
121 and the formation of DNA-RNA duplexes (R-loops; Ariel et al., 2014, 2020).

122 Here, we demonstrated that in addition to the PRC1 component LHP1, *APOLO* lncRNA
123 interacts *in vivo* with VIM1, the plant homolog of UHRF1, linking Polycomb and DNA methylation
124 machineries. RNA-sequencing analyses of *APOLO* over-expression and *vim1* mutant lines revealed
125 that *APOLO* and VIM1 control a large common set of genes related to the thermomorphogenic
126 response. In particular, the *APOLO*-VIM1-LHP1 complex directly targets the heat-responsive auxin-
127 biosynthetic gene *YUCCA2* and conjointly mediates cytosine methylation and H3K27me3
128 deposition at its promoter, representing a new epigenetic mechanism regulating the plant
129 response to warm temperatures. Strikingly, we also demonstrate that the lncRNA *UPAT*, known to
130 recognize UHRF1 in humans, can interact with VIM1 and LHP1 in plant cells despite the lack of
131 sequence homology between *UPAT* and *APOLO*. Furthermore, similarly to *APOLO* over-expression,
132 *UPAT* constitutive transcription in Arabidopsis plants precludes LHP1 and VIM1 binding to the
133 *YUCCA2* promoter region. Hence, sequence-unrelated lncRNAs may exert similar molecular
134 functions across kingdoms, integrating the epigenetic regulation of gene expression.

135

136 **RESULTS**

137 **Long noncoding RNA *APOLO* associates with the methylcytosine-binding protein VIM1 *in vivo***

138 In order to investigate the composition of the ribonucleoprotein complexes integrated by
139 the lncRNA *APOLO*, we performed an exploratory Chromatin isolation by RNA purification (ChIRP)
140 followed by protein precipitation and mass spectrometry. We used two independent sets of
141 biotinylated DNA probes against *APOLO* (ODD and EVEN sets) and LacZ-probes as a negative
142 control. Among the proteins identified by at least two positive hits of unique peptides in ODD and
143 EVEN, excluded from LacZ ChIRPs, we found the protein VARIANT IN METHYLATION 1 (VIM1,
144 AT1G57820; (Woo et al., 2007)). Transient expression of *GFP-VIM1* in *Nicotiana benthamiana* and
145 *Arabidopsis thaliana* leaves revealed that VIM1 is located in the cell nucleus, in agreement with
146 previous observations (Woo et al., 2007). Moreover, this localization was observed regardless of
147 the co-expression with nuclear-enriched *APOLO* transcripts (**Supplementary Figure 1A-B**). Anti-
148 GFP RNA immunoprecipitation (RIP) in *N. benthamiana* leaves transiently co-transformed with
149 *GFP-VIM1* and *APOLO*, or in stably transformed *Arabidopsis* seedlings over-expressing *GFP-VIM1*
150 (OE *VIM1-1*; **Figure 1A**; *VIM1* levels shown in **Supplementary Figure 2A**), revealed a high
151 enrichment of *APOLO* transcripts in VIM1 immunoprecipitated samples. These results indicate that
152 VIM1 is part of a novel ribonucleoprotein complex integrated by *APOLO* lncRNA.

153

154 ***APOLO* and *VIM1* are important thermomorphogenesis regulators in *Arabidopsis thaliana***

155 Abnormal morphological phenotypes, including DNA methylation-dependent late
156 flowering, were previously reported for the *vim1/2/3* triple mutant in contrast to the *vim1* single
157 mutant exhibiting no evident phenotype (Woo et al., 2008). We thus wondered in what
158 developmental context the *APOLO*-VIM1 interaction may occur and exert a regulatory role over
159 target genes. By exploring the eFP *Arabidopsis* transcriptomic database (Winter et al., 2007), we
160 found that *VIM1*, *VIM2* and *VIM3* genes showed different transcriptional dynamics following heat
161 stress (38°C followed by recovery at 25°C; **Supplementary Figure 3A**, blue box). Therefore, we
162 evaluated the expression of these three genes and *APOLO* at 29°C, a warm temperature usually
163 faced by *Arabidopsis* in the wild (Parent and Tardieu, 2012). Remarkably, after 6h of exposure to
164 29°C, *APOLO* transcript levels decreased while *VIM1* transcript levels increased together with the
165 warmth-response markers *PIF4* and *YUC8* (**Figure 1B**; **Supplementary Figure 3B**). In contrast to
166 *VIM1*, the transcriptional accumulation of *VIM2* and *VIM3* was repressed or unaffected,
167 respectively (**Supplementary Figure 3C**).

168 To assess whether the ribonucleoprotein complex integrated by *APOLO* and *VIM1* is
169 involved in the response to warmth, we tested the effect of their deregulation on the

170 thermomorphogenesis response. Strikingly, two independent *APOLO* overexpression (OE) lines (OE
171 *APOLO*-1 and OE *APOLO*-2; (Ariel et al., 2020)), together with two independent *vim1* insertional
172 mutants resulting in a complete loss of function or knock-down of *VIM1* (*vim1*-2 and *vim1*-3,
173 respectively; **Supplementary Figure 2B-C**), exhibited an impaired hypocotyl elongation after four
174 days at 29°C (**Figure 1C-F**; **Supplementary Table 1**). Additionally, we observed a slight but
175 significant reduction of hypocotyl elongation at 29°C only in one out of two independent RNAi
176 *APOLO* lines (RNAi *APOLO*-1 and RNAi *APOLO*-2; Ariel et al., 2014; **Supplementary Figure 4A, C**;
177 **Supplementary Table 1**) and a minor but significant induction of hypocotyl elongation in OE *VIM1*
178 lines (OE *VIM1*-1 and OE *VIM1*-2; **Supplementary Figure 2A**; **Supplementary Figure 4B, D**;
179 **Supplementary Table 1**). Altogether, these results suggest that *APOLO* and *VIM1* regulate
180 thermomorphogenesis and incidentally indicate that *VIM1* is not redundant with *VIM2* or *VIM3* in
181 this developmental context.

182 To pinpoint molecular mechanisms that could explain impaired thermomorphogenesis
183 upon *APOLO* or *VIM1* deregulation, we profiled the transcriptomes of 4-day-old OE *APOLO*-1 and
184 *vim1*-3 seedlings grown at 23°C with RNA-Seq of 4-day-old wild-type (WT) seedlings grown at 23°C
185 and subjected or not to heat (29°C) for 6h. In WT seedlings, warmth caused the downregulation of
186 over 3,400 transcripts and the upregulation of approximately 2,650 transcripts (**Supplementary**
187 **Table 2**). Remarkably, 56% of the total set of up and downregulated transcripts at 29°C in WT were
188 already deregulated at 23°C in OE *APOLO*-1 and/or *vim1*-3 (3,403 genes over 6,073; **Figure 1G**),
189 whereas 52% of these transcripts (1,782 out of 3,403) were deregulated in both OE *APOLO*-1 and
190 *vim1*-3 at 23°C (**Figure 1G**, central intersection). Moreover, a heatmap analysis revealed that a
191 subset of up and downregulated genes in response to warmth in WT behave consistently in OE
192 *APOLO*-1 and *vim1*-3 at ambient temperature (**Figure 1H**). A GO analysis of upregulated transcripts
193 in WT in response to warmth revealed the enriched categories “Response to auxin”, “Cellular
194 response to auxin stimulus” and “Auxin-activated signaling pathway” (**Figure 1H**; **Supplementary**
195 **Figure 5**), in accordance with the role reported for auxin in thermomorphogenesis (Franklin et al.,
196 2011). Altogether, these transcriptomic analyses hint at a critical role for the *APOLO*-*VIM1*
197 complex in the transcriptional reprogramming of gene expression in response to warm
198 temperatures.

199 We then aimed at identifying the potential direct targets of the *APOLO*-*VIM1* complex
200 during the thermomorphogenic response. Given the well-known role of *VIM1* in DNA methylation,
201 we compared the list of hypo-methylated genes in *vim1* vs. WT identified by Bisulfite-Sequencing

202 analyses (BiS-Seq; Stroud et al., 2013), with potential *APOLO* direct targets identified by *APOLO*-
203 ChIRP-Seq (Ariel et al., 2020). Interestingly, among the candidate common targets we found
204 *YUCCA2* (*YUC2*, AT4G13260; **Supplementary Table 3**), a heat-responsive gene involved in auxin
205 biosynthesis (Mashiguchi et al., 2011; Sakata et al., 2010). By using a *proYUC2:GUS* transcriptional
206 fusion (Cheng et al., 2006), we observed that the *YUC2* promoter region is activated in the
207 hypocotyl of 4-day-old seedlings grown at 29°C (**Figure 2A**). In addition, *yuc2* loss-of-function
208 mutant seedlings (Cheng et al., 2006) exhibited a reduced hypocotyl elongation after 4 days at
209 29°C (**Figure 2B; Supplementary Table 1**), indicating that *YUC2* is required for a proper
210 thermomorphogenic response. Consistently, we observed that the transcriptional activation of
211 *YUC2* after 6h at 29°C was impaired in OE *APOLO-1* and *vim1-3* showing an altered hypocotyl
212 elongation (**Figure 2C**), further suggesting that the *APOLO-VIM1* complex directly regulates *YUC2*
213 to trigger the plant auxin-related thermomorphogenic response at warm temperatures.

214

215 ***APOLO* and *VIM1* directly mediate heat-dependent methylation at the *YUCCA2* promoter**

216 As *APOLO* recognizes its target loci by sequence complementarity and R-loop formation,
217 we first investigated the *APOLO* binding profile over the *YUC2* locus by ChIRP-Seq (Ariel et al.,
218 2020). Compared to the LacZ-probes used as a negative control, ODD and EVEN probes showed
219 specific binding of *APOLO* across the *YUC2* locus, notably to the exon 1, displaying six *APOLO*-
220 binding motifs “GAAGAA/TTCTTC” (**Figure 2D**, first three tracks in blue and light blue, binding
221 motifs indicated as red dots over the *YUC2* locus). *APOLO* ChIRP-qPCR over *YUC2* gene body in WT
222 and RNAi *APOLO-1* seedlings confirmed the specificity of the interaction (**Figure 2E**). Furthermore,
223 DNA-RNA immunoprecipitation followed by sequencing (DRIP-Seq; Xu et al., 2017) revealed that R-
224 loop formation occurs in the exon 1 of *YUC2*, in correlation with *APOLO* binding (**Figure 2D**, red
225 box), suggesting that *APOLO* directly recognizes *YUC2* through the formation of R-loops.
226 Consistently, DRIP-qPCR in WT and RNAi *APOLO-1* seedlings revealed that R-loop formation over
227 the *YUC2* locus depends on *APOLO* expression (**Figure 2F**).

228 Interestingly, a NRPE1 Pol V-subunit Chromatin immunoprecipitation (ChIP)-Seq (Johnson
229 et al., 2014) revealed that the *YUC2* promoter region, located 560 to 1010 bp upstream of *YUC2*
230 TSS, is directly regulated by Pol V in a MET1-dependent manner (**Figure 2D**, black box;
231 **Supplementary Figure 6A**). In addition, a small RNA-Seq profiling reported a temperature-
232 dependent accumulation of RdDM-related 24nt siRNAs over this locus (Gyula et al., 2018;
233 **Supplementary Figure 6B**), suggesting that DNA methylation in this regulatory region may

234 contribute to the temperature-induced transcriptional regulation of *YUC2*. We thus assessed VIM1
235 binding and DNA methylation at the *YUC2* promoter region in the same developmental stage as
236 our phenotypic characterization. GFP-VIM1 ChIP performed in 4-day-old seedlings grown at 23°C
237 and treated or not at 29°C for 6h, revealed that VIM1 binds to the region located 357 to 494 bp
238 upstream of *YUC2* TSS and that this binding is reduced at 29°C compared to 23°C (**Figure 2G**). In
239 addition, Bis-Seq analyses of WT seedlings grown at 23°C reported cytosine methylation in all
240 sequence contexts in the region located 360 to 610 bp upstream of *YUC2* TSS (**Figure 3A**;
241 **Supplementary Figure 7**). Remarkably, CpHpH methylation over this region is significantly reduced
242 in *vim1-3* (**Figure 3B**; **Supplementary Figure 7**), hinting at a critical role for VIM1 in the
243 maintenance of non-CpG methylation at the *YUC2* locus. Methylated DNA immunoprecipitation
244 (MeDIP) served to confirm these results and additionally revealed that DNA methylation is
245 reduced in this region in the WT after 6h at 29°C (**Figure 3C**). Furthermore, in OE *APOLO-1* and
246 *vim1-3*, DNA methylation levels resulted to be lower at 23°C and increased at 29°C, exhibiting the
247 opposite behavior to the WT (**Figure 3C**).

248 To further support the relevance of RdDM on *YUC2* regulation in the context of
249 thermomorphogenesis, we additionally characterized the physiological response to warm
250 temperatures of RdDM mutants *nrrpd2a* (a common subunit of Pol IV and Pol V), *rdr2-5*, *dcl3-1* and
251 *ago4-8*, as well as CpG methyltransferase mutant *met1-2* and non-CpG methyltransferase mutants
252 *cmt2-7* and *cmt3-11* (for a visual summary of their respective roles in RdDM, see **Supplementary**
253 **Figure 8A**). Hypocotyl elongation at 29°C was reduced in *nrrpd2a*, *met1-2*, *rdr2-5*, *dcl3-1* and *ago4-*
254 *8* backgrounds, whereas it was unaffected in *cmt3-11* and slightly enhanced in *cmt2-7*
255 backgrounds (**Figure 3D-F**; **Supplementary 8B-H**; **Supplementary Table 1**). In agreement, Bis-Seq
256 analyses (Stroud et al., 2013) revealed that RdDM and *met1-2* mutants showing impaired
257 thermomorphogenesis also displayed reduced CpHpH methylation levels in the *YUC2* regulatory
258 region (**Supplementary Figure 6C-D**, blue box), further supporting that epigenetic modifications
259 regulating DNA methylation at *YUC2* promoter are critical to modulate thermomorphogenesis.
260 Taken together, our results indicate that *APOLO* and VIM1 directly mediate DNA methylation at
261 *YUC2* promoter in response to warm temperatures, in a process likely controlled also by the RdDM
262 pathway.

263

264 **VIM1 and LHP1 cooperate to regulate temperature-dependent histone and DNA methylation at**
265 **the *YUCCA2* promoter**

266 Given that the plant PRC1 component LHP1 recognizes *APOLO in vivo* and co-regulates
267 common target loci across the Arabidopsis genome (Ariel et al., 2014, 2020), we explored whether
268 the *YUC2* locus was regulated by LHP1 and the related transcriptionally repressive mark
269 H3K27me3. ChIP-Seq analyses (Veluchamy et al., 2016) revealed that the *YUC2* locus is enriched in
270 LHP1 and H3K27me3 in standard growing conditions (**Figure 4A**), both drastically reduced at 29°C
271 over the promoter region (**Figure 4A**, black box; **Figure 4B-C**). Moreover, LHP1 binding to the *YUC2*
272 gene body was impaired by *APOLO* over-expression (**Figure 4A**, blue box; **Figure 4D**). Consistent
273 with an involvement of LHP1 in the thermomorphogenic response, 4-day-old *lhp1* loss-of-function
274 mutant seedlings (Veluchamy et al., 2016) exhibited a reduced hypocotyl elongation at 29°C and
275 no induction of *YUC2* after 6h at 29°C (**Figure 4E-F**; **Supplementary Table 1**). Taken together, these
276 results suggest that in addition to VIM1-dependent DNA methylation, the PcG-dependent histone
277 methylation machinery also directly regulates *YUC2* expression in response to warm temperatures.

278 Considering the similarities between VIM1 and LHP1 behaviors at the *YUC2* locus, we
279 wondered if VIM1-associated DNA methylation and LHP1-related H3K27me3 transcriptional
280 regulations were dependent on each other, and if the two proteins interacted *in vivo*. By
281 performing BiS-Seq of *lhp1* vs. WT seedlings at 23°C, we observed that the *YUC2* regulatory region
282 in the proximal promoter displayed lower levels of cytosine methylation in all sequence contexts
283 in the *lhp1* mutant (**Figure 5A-B**; **Supplementary Figure 7**, blue box), similarly to the pattern
284 observed in the *vim1-3* mutant (**Figure 3A-B**). Moreover, global DNA methylation resulted to be
285 restored at 29°C in *lhp1*, exhibiting opposite behavior to the WT (**Figure 5C**) and the same trend as
286 OE *APOLO-1* and *vim1-3* (**Figure 3C**). To further elucidate the link between LHP1 and VIM1, we
287 assessed the levels of H3K27me3 and LHP1 capacity to bind to the *YUC2* promoter region in 4-day-
288 old *vim1-3* seedlings. Notably, LHP1 binding and H3K27me3 levels were impaired in *vim1-3* (**Figure**
289 **5D-E**). In combination, these results indicate that while *lhp1* mutation affects DNA methylation of
290 a VIM1-target region, *vim1* mutation impairs LHP1 binding and H3K27me3 deposition on the same
291 locus, hinting at a cooperative interaction between these two epigenetic factors. Additionally, by
292 performing Bimolecular fluorescence complementation (BiFC) assays in *N. benthamiana* leaves
293 transiently transformed with *CYPF-VIM1* and *NYFP-LHP1* we observed an interaction between the
294 two proteins (**Figure 5F**; **Supplementary Figure 9**). Altogether, our results suggest that the lncRNA
295 *APOLO* associates with both a regulator of DNA methylation (VIM1) and a PcG component (LHP1),
296 which further cooperate to mediate DNA methylation and H3K27me3 deposition at a specific
297 *APOLO* target locus.

298

299 **Human lncRNA *UPAT* can exert similar regulatory functions as *APOLO* in *planta***

300 Arabidopsis VIM1 is a homolog of the mammalian methylcytosine-binding protein UHRF1
301 (Kraft et al., 2008; Feng et al., 2010). Interestingly, UHRF1 was shown to directly recognize *in vivo*
302 the human lncRNA *UPAT*, through its flexible spacer region positioned between the SRA and RING
303 domains (Fang et al., 2016; Taniue et al., 2016; Wang et al., 2018). This interaction stabilizes
304 UHRF1 by interfering with its ubiquitination and subsequent degradation (Taniue et al., 2016). In
305 order to determine if VIM1 and UHRF1 interaction with lncRNAs is conserved between plants and
306 animals, we delimited the minimal region of VIM1 that interacts with *APOLO*. We generated
307 independent GFP fusion constructs bearing different *VIM1* coding regions, according to Woo et al.,
308 2007 (**Figure 6A right panel**). All the construct-encoded proteins accumulated exclusively or
309 partially in the nucleus of *N. benthamiana* cells, with or without *APOLO* co-expression
310 (**Supplementary Figure 10A-B**). Similar to the reported interaction between UHRF1 and *UPAT*
311 (Taniue et al., 2016), an anti-GFP RIP performed in *N. benthamiana* leaves transiently co-
312 transformed with *APOLO* and GFP-VIM1 derivatives revealed that the two VIM1 portions
313 containing the full spacer region (GFP-SRA/RING2 and GFP-Spacer) bind to *APOLO in vivo*, although
314 with lower efficiency than the full-length VIM1 (**Figure 6A**). Thus, our results indicate that the
315 domain of VIM1 and UHRF1 involved in the interaction with lncRNAs is evolutionary conserved
316 between plants and animals.

317 Considering the common ability of the conserved VIM1 and UHRF1 proteins to interact
318 with lncRNAs, we wondered whether the lncRNA *UPAT*, exhibiting no apparent sequence
319 homology with *APOLO* (**Supplementary Figure 10C**), could interact with VIM1 and its partner LHP1
320 *in planta*. Strikingly, an anti-GFP RIP in *N. benthamiana* leaves transiently co-transformed with
321 *UPAT* and *GFP-UHRF1*, *GFP-VIM1* or *LHP1-GFP* demonstrated that *UPAT* is able to interact with
322 UHRF1, as well as with VIM1 and LHP1 in plant cells (**Figure 6B-C**).

323 We thus assessed if the constitutive expression of *UPAT* or *APOLO* could modulate VIM1
324 and LHP1 binding to *YUC2* promoter. CHIP-qPCR analyses performed in WT, *OE APOLO-1* and *OE*
325 *UPAT* Arabidopsis stable lines transiently transformed or not with *GFP-VIM1* (**Supplementary**
326 **Figure 1B**), revealed that both *APOLO* and *UPAT* over-expression precludes the interaction of VIM1
327 and LHP1 with *YUC2* promoter (**Figure 6D-E**). Furthermore, *UPAT* constitutive expression in stable
328 Arabidopsis seedlings resulted in decreased transcript levels of *YUC2* (**Supplementary Figure 11A-**
329 **B**). Altogether, our results revealed a functional interaction between sequence-unrelated long

330 noncoding transcripts and key epigenetic regulators, which is likely conserved across kingdoms
331 (Figure 6F for a model in plants).

332

333 **DISCUSSION**

334 **Epigenetic transcriptional reprogramming in the thermomorphogenic response**

335 When plants are exposed to warmer nonstressful average temperatures, some organs
336 grow and develop at a faster rate without affecting their final dimensions, whereas others suffer
337 morphological or developmental changes. The latest response is known as thermomorphogenesis
338 and includes petiole elongation, leaf hyponasty and auxin-dependent hypocotyl elongation (Gray
339 et al., 1998; Casal and Balasubramanian, 2019; Bellstaedt et al., 2019). These modifications of the
340 plant architecture rely on a deep transcriptional reprogramming determined by dynamic changes
341 of chromatin organization (Kumar and Wigge, 2010; Cortijo et al., 2017; Pajoro et al., 2017; Steffen
342 and Staiger, 2017; Susila et al., 2018; Tasset et al., 2018).

343 A strong correlation between temperature and DNA methylation was first established in
344 Swedish *A. thaliana* accessions, where higher levels of CpHpH methylation were observed in plants
345 grown at 16°C compared to 10°C (Dubin et al., 2015; Kawakatsu et al., 2016). Interestingly, CpHpH
346 methylation is conjointly regulated by CMT2 and the RdDM pathway (Stroud et al., 2013, 2014;
347 Zemach et al., 2013) and both have been involved in the heat response. It was shown that
348 *cmt2* mutants are more tolerant to heat-stress (Shen et al., 2014) and that the heat-stress
349 response relies on the integrity of the RdDM pathway (Popova et al., 2013; Naydenov et al., 2015).
350 In agreement, we observed that *cmt2* mutants displayed a slight but significantly increased
351 response to warm temperatures in contrast to RdDM mutants exhibiting hypocotyl elongation
352 defects. In addition, we observed that *met1* mutants exhibited the same developmental
353 phenotype as RdDM mutants. Accordingly, it was demonstrated that *met1* knockout causes a loss
354 of DNA methylation, a global loss of Pol V at its normal locations and a Pol V redistribution to sites
355 that become hypermethylated (Johnson et al., 2014). Furthermore, we observed a reduction in
356 CpHpH methylation at the *YUC2* promoter in the *vim1-3* mutant, linking the canonical CpG-related
357 machinery with the RdDM pathway.

358 Importantly, *met1* was also shown to suffer H3K9 hypermethylation at PcG target genes
359 and a redistribution of H3K27me3 to transposons (Deleris et al., 2012), hinting at a relation
360 between DNA methylation and PRC-dependent histone modifications. Consistently, we uncovered

361 here a new molecular link between the DNA methylation-associated protein VIM1 and the
362 H3K27me3-related protein LHP1, which can interact together and with the lncRNA *APOLO*. An
363 indirect relation between LHP1 and VIM1 had already been described through the histone
364 methyltransferase NtSET1, a common interactor. It was first shown in *Nicotiana tabacum* that
365 NtSET1 directly interacts with LHP1 (Yu et al., 2004) and it was then reported that NtSET1 can bind
366 to AtVIM1 *in planta* (Liu et al., 2007), although no link was established between LHP1 and DNA
367 methylation.

368 Although VIM1, VIM2 and VIM3 have been reported to function redundantly to mediate
369 epigenetic transcriptional silencing (Woo et al., 2008), we showed here that *vim1* single mutants
370 exhibit particular molecular and associated physiological phenotypes in response to warmth. We
371 related the hypocotyl elongation defect observed in *vim1* to the transcriptional activation of the
372 *YUC2* gene (Sakata et al., 2010; Mashiguchi et al., 2011). *YUC2* regulation in response to heat was
373 first observed in flowers, where heat shock (33°C) repressed its expression and caused plant male
374 sterility (Sakata et al., 2010). At milder temperatures (27°C), *YUC2* transcript levels were reported
375 to increase, in correlation with the demethylation of *YUC2* proximal promoter region targeted by
376 RdDM (Gyula et al., 2018), suggesting that cytosine methylation at the *YUC2* regulatory region is
377 critical for the control of its expression in response to heat. In addition to DNA methylation, a
378 correlation was established between the deposition of H3K27me3 and genes exhibiting either high
379 or low transcription rates under warm temperatures (Sidaway-Lee et al., 2014). Consistently, we
380 observed that the *lhp1* mutant displayed impaired hypocotyl elongation and showed no activation
381 of *YUC2* at 29°C. Interestingly, LHP1 was previously reported to exert a positive role in the
382 regulation of *YUCCA* genes under standard growth conditions. Indeed, *YUC5*, *YUC8* and *YUC9*
383 displayed an abnormal transcriptional activity in the *lhp1* mutant (Rizzardi et al., 2011), correlating
384 with a reduction of H3K27me3, as revealed by genome-wide approaches (Veluchamy et al.,
385 2016). In addition, LHP1 was found to localize to *YUC2*, whose expression was not affected by
386 the *lhp1* mutation at this developmental stage, in an auxin-dependent fashion (Rizzardi et al.,
387 2011).

388 It was recently shown in *Arabidopsis* that the 5' untranslated region of several mRNAs may
389 adopt alternative hairpin conformations under warm cycling daytime temperatures, modulating
390 translation efficiency. It was proposed that mRNA thermoswitches enhancing protein synthesis
391 may constitute a conserved mechanism enabling plants to respond rapidly to high temperatures
392 (Chung et al., 2020). In contrast, the potential role of lncRNAs as thermosensors in higher

393 organisms remains unexplored. In this work, we showed that *APOLO* participates in the
394 thermomorphogenic response in Arabidopsis. Interestingly, *APOLO* transcriptional accumulation
395 increases in response to cold (Moison et al., 2021), whereas we showed here that it decreases
396 under warm temperatures. Further research will be needed to uncover the effect of temperature
397 on the structure of *APOLO* and other lncRNAs that determines their molecular role and
398 contributes to the plant adaptation to environmental constraints.

399

400 **R-loop-associated long noncoding RNAs modulate DNA methylation and histone modifications**

401 RNA-DNA hybrids (R-loops) have been identified as important regulators of chromatin
402 conformation and gene transcriptional regulation (Fazzio, 2016; Crossley et al., 2019). *APOLO* is
403 known to recognize its multiple *trans* targets by sequence complementarity and R-loop formation.
404 Upon interaction, it decoys LHP1, shaping local chromatin 3D conformation and subsequently
405 modulating gene transcriptional activity (Ariel et al., 2020). Consistently, we showed here that
406 *APOLO* recognizes *YUC2* gene body through the formation of R-loops.

407 Interestingly, *APOLO* additionally interacts with VIM1 to co-regulate DNA methylation at
408 the *YUC2* promoter, mediating *YUC2* transcriptional response to warm temperatures. Although the
409 involvement of RNA Pol II-lncRNAs in the regulation of DNA methylation in plants remains largely
410 unknown, previous reports have linked lncRNA activity to promoter methylation in mammals,
411 through the interaction with protein partners (Chalei et al., 2014) or the formation of R-loops
412 (Arab et al., 2014, 2019). Notably, it was reported that DNMT1-interacting lncRNAs can promote
413 or block local DNA methylation (Mohammad et al., 2010; Di Ruscio et al., 2013; Chalei et al., 2014),
414 whereas it was proposed that R-loops formed by nascent transcripts can preclude DNA
415 methylation from promoter regions by repelling DNA methyltransferases (Ginno et al., 2012;
416 Grunseich et al., 2018).

417 In addition to DNA methylation, a link between R-loops and the activity of PRC complexes
418 has also been proposed. In mammals, it was shown that R-loops can form at a subset of PcG target
419 genes, resulting in alternative regulatory outputs depending on the interplay between the DNA-
420 RNA duplex and PRC components (Skourti-Stathaki et al., 2019). In this work, we showed that the
421 over-accumulation of *APOLO* can titer VIM1 and LHP1 away from the *YUC2* promoter region. It is
422 thus tempting to hypothesize that R-loops formed by *APOLO* at the *YUC2* locus stoichiometrically
423 modulate VIM1 and LHP1 recruitment to DNA in response to changes in ambient temperature. At
424 23°C, a precise amount of *APOLO* over the *YUC2* locus may guide the LHP1-VIM1 complex to the

425 target region. In contrast, at 29°C *APOLO* transcript levels decrease, hindering VIM1 and LHP1
426 efficient recognition of *YUC2*. On the other hand, over-accumulation of *APOLO* can decoy LHP1
427 and VIM1 away from the *YUC2* locus, explaining the similar hypocotyl elongation phenotypes of
428 OE *APOLO*, *vim1* and *lhp1*, as well as the similar DNA methylation levels observed at the *YUC2*
429 promoter in these three backgrounds. The re-methylation of the *YUC2* promoter in OE *APOLO*,
430 *vim1* and *lhp1* at warm temperatures, in contrast to WT, might be due to altered RdDM activity
431 when any component of the regulatory complex is affected. Thereby, we propose that *APOLO*
432 functions as a key mediator of the PRC and DNA methylation machineries, hinting at a
433 stoichiometric factor fine-tuning the activity of R-loops-related lncRNAs.

434

435 **Long noncoding RNAs as key mediators of the Polycomb and DNA methylation machineries**

436 Connections between Polycomb and DNA methylation machineries have been reported in
437 few studies in mammals. DNMT1 was shown to directly interact with HP1 to mediate silencing of
438 euchromatic genes (Smallwood et al., 2007) and its enzymatic activity is also stimulated by direct
439 interaction with UHRF1 (Nady et al., 2011; Rothbart et al., 2012; Nishiyama et al., 2013;
440 Bashtrykov et al., 2014; Zhao et al., 2016). Interestingly, HP1 and UHRF1 have been shown to co-
441 exist on chromatin (Rose and Klose, 2014), although their direct interaction has not been proven.
442 Here, we showed that LHP1 and VIM1 interact *in planta*, suggesting that a direct interaction
443 between their homologs HP1 and UHRF1 may occur in mammals. As in mammals, it was shown in
444 Arabidopsis that VIM1 recruits MET1 to hemi-methylated DNA (Feng et al., 2010; Yao et al., 2012;
445 Kim et al., 2014; Shook and Richards, 2014), hinting at the existence of analogous complexes
446 formed by MET1/DNMT1, VIM1/UHRF1 and LHP1/HP1 in plants and animals.

447 Remarkably, DNMT1, UHRF1 and HP1 were all shown to interact with coding or long
448 noncoding RNAs (Piacentini et al., 2009; Di Ruscio et al., 2013; Taniue et al., 2016), whereas in
449 plants only LHP1 was shown to bind to RNAs *in vitro* (Berry et al., 2017) and *in vivo* (Ariel et al.,
450 2014, 2020). Notably, UHRF1 can interact with the lncRNA *UPAT* whose scaffold function prevents
451 UHRF1 from ubiquitination and proteasomal degradation (Taniue et al., 2016). Knockdown of
452 *UPAT* results in a drastic decrease in the levels of DNMT1 protein and a reduction of
453 hemimethylation at its target genes (Taniue et al., 2016). Here, we showed that *UPAT* can interact
454 with VIM1 and LHP1 in plant cells, and high amounts of *UPAT* can decoy VIM1 and LHP1 from
455 *YUC2* promoter, as the sequence-unrelated transcript *APOLO*.

456 Although a global anti-correlation is observed in the Arabidopsis genome between DNA
457 methylation and H3K27me3 deposition, characterizing heterochromatic and euchromatic regions
458 respectively (Johnson et al., 2007; Mathieu et al., 2005; Deleris et al., 2012; Antunez-Sanchez et
459 al., 2020), DNA hypomethylation was associated to H3K27me3 reduction at specific genes in *met1*
460 and *vim1/2/3* mutants (Deleris et al., 2012; Kim et al., 2014b). Consistently, our results indicate
461 that *APOLO* lncRNA interacts with LHP1 and VIM1 to regulate *YUC2*, constituting a lncRNA-
462 mediated non-canonical cooperative interaction between Polycomb and DNA methylation
463 machineries. Although other lncRNAs have been linked to thermomorphogenesis or light
464 dependent hypocotyl elongation, their mechanisms of action or target recognition remain
465 unknown (Wang et al., 2014; Severing et al., 2018). Considering that the sequence-unrelated
466 lncRNA *UPAT* mimicked the action of *APOLO* over the chromatin-binding capacity of the VIM1-
467 LHP1 complex, it is likely that additional yet-uncovered lncRNAs exert a similar role as *APOLO* in
468 Arabidopsis.

469 Altogether, our results hint at a mechanism possibly conserved across kingdoms, which
470 may rely on the structure rather than the sequence of long noncoding transcripts. A deeper
471 knowledge about the molecular basis behind lncRNA-related regulatory mechanisms will likely
472 push back the frontiers of human therapeutics and will allow the design of innovative strategies
473 for sustainable agriculture in a climate change context.

474

475 **METHODS**

476

477 **Data availability**

478 RNA-sequencing data have been deposited in GEO with the accession code XXX. Bisulfite-
479 sequencing data have been deposited in the European Nucleotide Archive (ENA) under project
480 XXX.

481

482 **Plant material and growth conditions**

483 All the *Arabidopsis thaliana* lines used were in the Columbia-0 (Col-0) background. *vim1-2*
484 (SALK_050903) and *vim1-3* (SALK_149277c) seeds were obtained from the Arabidopsis Biological
485 Resource Center (<http://www.arabidopsis.org/>). Mutant plants were genotyped by PCR using
486 specific primers to amplify the endogenous locus and T-DNA borders (primers used are listed in
487 Supplementary Table 4). *VIM1* and *UPAT* over-expression (OE) transgenic plants were generated

488 through *Agrobacterium tumefaciens* (strain EHA105)-mediated transformation (Clough and Bent,
489 2008). Two independent lines of transformants harboring *GFP-VIM1* or *UPAT* were selected on
490 MS/2 medium supplemented with kanamycin (40µg/mL). *VIM1* expression levels were measured
491 by RT-qPCR (primers used are listed in Supplementary Table 4).

492 Seeds were surface-sterilized by treatments with 70% EtOH and 5% hypochlorite-1% SDS,
493 washed and stratified at 4°C for 2 days to obtain homogeneous germination. Seedlings were
494 grown at 23°C or 29°C on solid half-strength Murashige and Skoog (MS/2) medium (Duchefa),
495 under long day conditions (16h light, 95µE.m⁻².sec⁻¹/8h dark). For phenotypic characterization of
496 hypocotyl elongation, seedlings were grown for 3h at 23°C and transferred to 29°C or maintained
497 at 23°C for 4 days. Hypocotyl lengths were measured using the ImageJ software. For RNA-
498 sequencing, chromatin immunoprecipitation and methylated DNA immunoprecipitation assays
499 performed in Arabidopsis stable lines, seedlings were grown for 4 days at 23°C and transferred to
500 29°C or maintained at 23°C for 6h. For chromatin isolation by RNA purification and DNA-RNA
501 Immunoprecipitation, seedlings were grown for 11 days at 23°C.

502

503 Cloning procedures

504 The entire coding region of *VIM1* (AT1G57820), *VIM1* derivatives (PHD/RING1, SRA/RING2,
505 SRA, RING2; Woo et al., 2007), *LHP1* (AT5G17690) and the lncRNA *UPAT* (Taniue et al., 2016) were
506 amplified by PCR on genomic DNA or pBluescript:UPAT vector (Taniue et al., 2016) respectively,
507 and cloned into the Gateway entry vector pENTR/D-TOPO (Invitrogen). Entry clones were
508 recombined by Gateway technology (LR reaction) into the pK7WGF2, pK7FWG2 or pK7WG2
509 vectors containing respectively a p35S-GFP-GW, a p35S-GW-GFP or a p35S-GW cassette
510 (<http://www.psb.ugent.be/gateway/index.php>). pENTR/VIM1, pENTR/SRA/RING2 and
511 pENTR223/LHP1 were recombined by LR reaction into the pGPTVII.Bar-C/NYFP vectors containing
512 respectively a p35S-CYFP or a p35S-NYFP cassette (<http://www.psb.ugent.be/gateway/index.php>).
513 The spacer of *VIM1* and the entire coding region of *UHRF1* were amplified by PCR on cDNA and
514 pGE-HIS-MBP *UHRF1* plasmid respectively, and cloned into the GreenGate entry vector pGGC000.
515 Destination vectors were constructed associating modules: 35S promoter, GFP, Ubiquitin 10
516 terminator and pNOS:BastaR,tNOS, according to Lampropoulos et al. (2013). All Gateway
517 constructs were subsequently transformed into the *A. tumefaciens* strain EHA105 and GreenGate
518 constructs into the *A. tumefaciens* strain ASE containing a pSOUP helper plasmid.

519

520 **Transient expression and confocal microscopy**

521 Leaves of *Nicotiana benthamiana* were transiently transformed as described in Waadt and
522 Kudla (2008). For RNA immunoprecipitation, *GFP-VIM1* or derivatives (*GFP-PHD/RING1*, *GFP-*
523 *SRA/RING2*, *GFP-SRA*, *GFP-RING2* and *GFP-Spacer*) were co-transformed with p35S-*APOLO* (Ariel et
524 al., 2020), or *GFP-UHRF1*, *GFP-VIM1* or *LHP1-GFP* were co-transformed with p35S-*UPAT*. For sub-
525 cellular localization, *VIM1* protein or derivatives were co-transformed or not with p35S-*APOLO*.
526 For bimolecular fluorescence complementation, *YFPC-VIM1* or *YFPC-SRA/RING2* were co-
527 transformed with *YFPN-LHP1*. Leaves were harvested or cells were analyzed 2 days after
528 infiltration using a Zeiss confocal microscope equipped with Plan-Apochromat 10x/NA 0,45/M27
529 or Plan-Apochromat 20x/NA 0,8/r M27 US-VIS-IR dry lens. eGFP and eYFP fluorescence was
530 excited with 488 nm and 514 nm argon laser lines and emission recorded between 490nm and
531 580nm and 520nm and 600nm respectively. Image acquisitions and analyses were performed on
532 the IPS2 Imaging Facility.

533 Leaves of 3-week-old *Arabidopsis thaliana* were transiently transformed as described in
534 Zhang et al., 2020. Transformation with *GFP-VIM1* was carried out in leaves > 0.5 cm in length, in
535 10 to 15 plants per genotype (WT, OE *APOLO-1* and OE *UPAT*). Leaves were crosslinked and
536 harvested (for ChIP) or cells were analyzed 3 days after infiltration, using a Leica TCS SP8 confocal
537 laser scanning microscope. eGFP was excited at 488 nm (intensity=8%) and emission recorded
538 between 495nm and 530nm for GFP and 610nm and 670nm (gain 650) for chlorophyll
539 fluorescence. Images were processed using the Fiji software (Schindelin et al., 2012).

540

541 **Chromatin isolation by RNA purification and Mass spectrometry**

542 ChIRP-qPCR was performed as previously described (Ariel et al., 2014, 2020).
543 Formaldehyde-crosslinked seedlings were ground and nuclei were isolated and sonicated using a
544 water bath Bioruptor Pico (Diagenode; 30s on/ 30s off pulses, at high intensity for 10 cycles).
545 *APOLO*-associated chromatin was purified using 100pmol of two independent sets of primers
546 (ODD and EVEN) and a negative control (primers matching the LacZ RNA), respectively (Ariel et al.,
547 2014, 2020). Primers used are listed in Supplementary Table 4.

548 A method adapted from the ChIRP protocol (Chu et al., 2012) was developed to allow
549 identification of plant nuclear proteins bound to specific lncRNAs, as described in Rigo et al., 2020.
550 Briefly, plants were *in vivo* crosslinked and cell nuclei were purified and extracted through
551 sonication. The resulting supernatant was hybridized against biotinylated complementary

552 oligonucleotides that tile the lncRNA of interest and putative lncRNA-containing protein
553 complexes were isolated using magnetic streptavidin beads. Co-purified ribonucleoprotein
554 complexes were eluted and used to purify RNA or proteins, which were later subject to
555 downstream assays for identification and quantification.

556 *Crosslinking and ribonucleoprotein complexes purification*

557 For protein extraction, approximately 250 g of 7-day-old Col-0 plants grown on solid half-
558 strength MS medium was irradiated three times with UV using a CROSSLINKERCL-508 (Uvitec) at
559 0.400 J/ cm². For RNA extraction, 10g of 7-day-old Col-0 plants grown on solid MS/2 medium was
560 crosslinked under vacuum for 15min with 37ml of 1% (v/v) formaldehyde. The reaction was
561 stopped by adding 2.5ml of 2M glycine and seedlings were rinsed with Milli-Q purified water. For
562 both crosslinking methods, 6g of the fixed material was ground in liquid nitrogen (representing 15
563 ml of plant material ground to fine dust) and added to 50ml tubes with 25 ml of extraction buffer
564 1– (the nuclei were prepared starting with 30 tubes; buffer 1: 10mM Tris–HCl pH8, 0.4M sucrose,
565 10mM MgCl₂, 5mM β-mercaptoethanol, 1ml/30g of sample powder Plant Protease Inhibitor Sigma
566 P9599). The solution was then filtered through Miracloth membrane (Sefar) into a new tube and
567 5ml of extraction buffer 2 (10mM Tris–HCl pH8, 0.25M sucrose, 10mM MgCl₂, 5mM β-
568 mercaptoethanol, 1%Triton X-100, 10 μl 100 μl PMSF) was added. The solution was then
569 centrifuged, the supernatant discarded and the pellet was resuspended in 500 μl of extraction
570 buffer 3 (10mM Tris–HCl pH8, 1.7M sucrose, 2mM MgCl₂, 5mM β-mercaptoethanol, 0.15% Triton
571 X-100, 50μl protease inhibitor) and layered on top of 600 μl of fresh extraction buffer 3 in a new
572 tube. After centrifugation at 13000rpm for 2min at 4°C to pellet the nuclei, the supernatant was
573 discarded and the pellet resuspended in 300μl of nuclei lysis buffer (50mM Tris–HCl pH7, 1% SDS,
574 10mM EDTA, 1mM DTT, 50μl protease inhibitor, 10μl RNase inhibitor per tube) to degrade nuclear
575 membranes. Samples were sonicated three times in refrigerated Bioruptor Plus (Diagenode), 30
576 cycles 30sec ON/30sec OFF in a Diagenode TPX microtube M-50001. After centrifugation, the
577 supernatant was transferred to a new tube and diluted two times volume in hybridization buffer
578 (50mM Tris–HCl pH7, 750 mM NaCl, 1% SDS, 15% formamide, 1mM DTT, 50μl protease inhibitor,
579 10μl RNase inhibitor). One hundred pmol of probes against *APOLO* (ODD and EVEN set of probes,
580 Ariel et al., 2014, 2020) and the corresponding negative set against LacZ were added to samples
581 and incubated 4h at 50°C in a thermocycler. Samples were transferred to tubes containing
582 Dynabeads- Streptavidin C1 (Thermo Fisher Scientific) and incubated 1h at 50°C. Then, samples
583 were placed on a magnetic field and washed three times with 1ml of wash buffer (2× SSC, 0.5%

584 SDS, 1 mM DTT, 100µl protease inhibitor). Protein purification samples for protein extraction were
585 DNase-treated according to the manufacturer (Thermo Scientific). After addition of 1.8ml of TCA-
586 acetone (5ml 6.1N TCA + 45ml acetone + 35µl β-mercaptoethanol), samples were incubated
587 overnight at -80°C. After centrifugation at 20000rpm for 20min at 4°C, the supernatant was
588 discarded and 1.8ml of acetone wash buffer (120ml acetone, 84µl β-mercaptoethanol) was added
589 to the samples. Then, samples were incubated 1h at -20°C and centrifuged again at 20000 rpm for
590 20min at 4°C. The supernatant was discarded, and the dry pellet was used for mass spectrometry
591 analyses.

592

593 **DNA-RNA Immunoprecipitation**

594 DNA-RNA Immunoprecipitation (DRIP) was performed as described in Ariel et al., 2020.
595 Non-crosslinked seedlings were used for nuclei purification and samples were sonicated using a
596 water bath Bioruptor Pico (Diagenode; 30s on/ 30s off pulses, at high intensity for 4 cycles).
597 Chromatin samples were incubated with 50 ml of washed Protein G Dynabeads pre-coated with
598 1mg of S9.6 antibody (Millipore MABE1095) for 16h at 4°C. Samples treated with RNaseH
599 (Invitrogen) for 2h at 37°C were used for DRIP in parallel as a negative control. After washing, DNA
600 was recovered for qPCR over R-loop related loci (primers used are listed in Supplementary Table
601 4).

602

603 **RNA-sequencing analysis**

604 Total RNA was prepared from 4-day-old *A. thaliana* wild-type (WT), OE *APOLLO-1* (Ariel et
605 al., 2014) and *vim1-3* seedlings using the QIAGEN RNeasy plant mini kit and treated with DNase
606 (Fermentas). Three independent biological replicates were produced per genotype. After RNA
607 extraction, libraries were constructed using the Tru-Seq Stranded mRNA Sample Prep kit
608 (Illumina®). Sequencing was carried out at the IPS2. The Illumina NextSeq500 technology was used
609 to perform 75-bp sequencing. A minimum of 15 million of single end reads by sample was
610 generated. RNA-seq preprocessing included trimming library adapters and quality controls were
611 performed with Trimmomatic (Bolger et al., 2014). Paired end reads with Phred Quality Score
612 Qscore > 20 and read length > 30 bases were kept, and ribosomal RNA sequences were removed
613 with SortMeRNA (Kopylova et al., 2012). Processed reads were aligned using STAR with the
614 following arguments: `--alignIntronMin 20 --alignIntronMax 3000 --outSAMtype BAM`
615 `SortedByCoordinate --alignIntronMax 3000 --outSAMtype BAM SortedByCoordinate --`

616 alignIntronMax 3000 --outSAMtype BAM SortedByCoordinate. Read overlapping exons per genes
617 were counted using the featureCounts of the subreads package using the GTF annotation files
618 from the Araport11 project (Cheng et al., 2017). Significance of differential gene expression was
619 estimated using DEseq2 (Love et al., 2014) and the FDR correction of the p-value was used during
620 pairwise comparison between genotypes. A gene was declared differentially expressed if its
621 adjusted p-value (FDR) was < 0.01. Heatmap was generated using log2 transformed fold change
622 values compared WT 23°C and computed with the pheatmap R (Kolde, 2019).

623

624 **Bisulfite-sequencing analysis**

625 Genomic DNA (1µg) was prepared from 4-day-old WT and *vim1-3*, or 14-day-old WT and
626 *lhp1* seedlings, respectively. For WT vs. *vim1-3*, bisulfite conversion, BS-seq libraries and
627 sequencing (paired-end 100 nt reads) were performed by BGI Tech Solutions (Hong Kong). For WT
628 vs. *lhp1*, bisulfite conversion was performed with the Premium Whole Genome Bisulfite
629 Sequencing kit (Diagenode) and libraries (paired-end 100 nt reads) were sequenced at the IPS2
630 platform. Adapter and low-quality sequences were trimmed using Trimming Galore v0.3.3.
631 Mapping was performed on TAIR10 genome annotation using Bismark v0.14.2 (Krueger and
632 Andrews, 2011) and the parameters: --bowtie2, -N 1, -p 3 (alignment); --ignore 5 --ignore_r2 5 --
633 ignore_3prime_r2 1 (methylation extractor). Only uniquely mapping reads were retained. The
634 methylKit package v0.9.4 (Akalin et al., 2012) was used to calculate differential methylation in 100
635 bp bp non-overlapping windows (DMRs). Significance of calculated differences was determined
636 using Fisher's exact test and Benjamin-Hochberg (BH) adjustment of p-values (FDR<0.05) and
637 methylation difference cutoffs of 40% for CG, 20% for CHG and 20% for CHH. Differentially
638 methylated windows within 100bp or 20bp of each other were merged to form larger DMRs. Only
639 100bp windows with at least six cytosines covered by a minimum of 6 (CG and CHG) and 10 (CHH)
640 reads in all libraries were considered.

641

642 **RNA immunoprecipitation assay**

643 RNA immunoprecipitation (RIP) assays were performed on transiently transformed *N.*
644 *benthamiana* leaves as described in Sorenson and Bailey-Serres (2015), or in 2-week-old *A.*
645 *thaliana* OE *VIM1.1* seedlings as described in Bardou et al. (2014), using anti GFP (Abcam ab290)
646 and anti-IgG (Abcam ab6702). RIP was performed using Invitrogen Protein A Dynabeads.
647 Precipitated RNAs were prepared using TRI Reagent (Sigma-Aldrich), treated with DNase

648 (Fermentas) and subjected to RT-qPCR (High Capacity cDNA Reverse Transcription Kit (Thermo);
649 primers used are listed in Supplementary Table 4). Total RNAs were processed in parallel and
650 considered as the input sample.

651

652 **Chromatin immunoprecipitation assay**

653 Chromatin immunoprecipitation (ChIP) assays were performed using anti GFP (Abcam
654 ab290), anti LHP1 (Covalab pab0923-P), anti H3K27me3 (Diagenode pab-195-050) and anti-IgG
655 (Abcam ab6702), as described in Ariel et al., 2020. Crosslinked chromatin was sonicated using a
656 water bath Bioruptor Pico (Diagenode; 30sec ON/30sec OFF pulses; 10 cycles; high intensity). ChIP
657 was performed using Invitrogen Protein A Dynabeads. Precipitated DNA was recovered using
658 Phenol:Chloroform:Isoamlic Acid (25:24:1; Sigma) and subjected to RT-qPCR (primers used are
659 listed in Supplementary Table 4). Untreated sonicated chromatin was processed in parallel and
660 considered as the input sample.

661

662 **Methylated DNA immunoprecipitation**

663 Methylated DNA immunoprecipitation (MedIP) assays were performed using anti 5-mC
664 (Diagenode Mab-081-100) and anti-IgG (Abcam ab6702), as described in Nagymihály et al. (2017).
665 Genomic DNA (1µg) was sonicated using a water bath Bioruptor Pico (Diagenode; 30s on/30s off
666 pulses; 4 cycles; high intensity). MedIP was performed using Invitrogen Protein G Dynabeads.
667 Precipitated DNA was recovered using Phenol:Chloroform:Isoamlic Acid (25:24:1; Sigma) and
668 subjected to RT-qPCR (primers used are listed in Supplementary Table 4). Untreated sonicated
669 genomic DNA was processed in parallel and considered as the input sample.

670

671 **RT-qPCR**

672 RT-qPCR were performed using the LightCycler 480 SYBR Green I Master Kit on a StepOne
673 Plus apparatus (Applied Biosystems) using standard protocols (40 to 45 cycles, 60°C annealing).
674 *PP2A* (AT1G13320) was used for normalization (primers used are listed in Supplementary Table 4).

675

676 **Histochemical staining**

677 Four-day-old *pYUCCA2:GUS* transgenic seedlings (Cheng et al., 2006) grown at 23°C or 29°C
678 were infiltrated with GUS staining solution (0.1M Phosphate buffer pH 7.5; 100mM K₃Fe(CN)₆;
679 100mM K₄Fe(CN)₆; 20% Triton X-100; 50mM X-Gluc) under vacuum and subsequently incubated at

680 37°C for 12h. Stained tissues were cleared in 70% EtOH for 24h at 37°C and observed under a light
681 microscope.

682

683 **Statistical analyses**

684 Statistical analyses were performed with non-parametric tests, Mann–Whitney when n=2
685 independent samples, and Kruskal- Wallis test when n>2 independent samples.

686

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696

697 **Author contributions**

698 FA and MC conceived the project; FA, CFF, MC, LQ and MB designed the experiments; CFF, AC,
699 JSRP, DL and FA performed the experiments; LQ, TB and LL analyzed the data; MFL, MM, LF, DG
700 and MB contributed materials and methods; CFF and FA wrote the paper.

701

702 **Declaration of interests**

703 The authors declare no competing interests.

704

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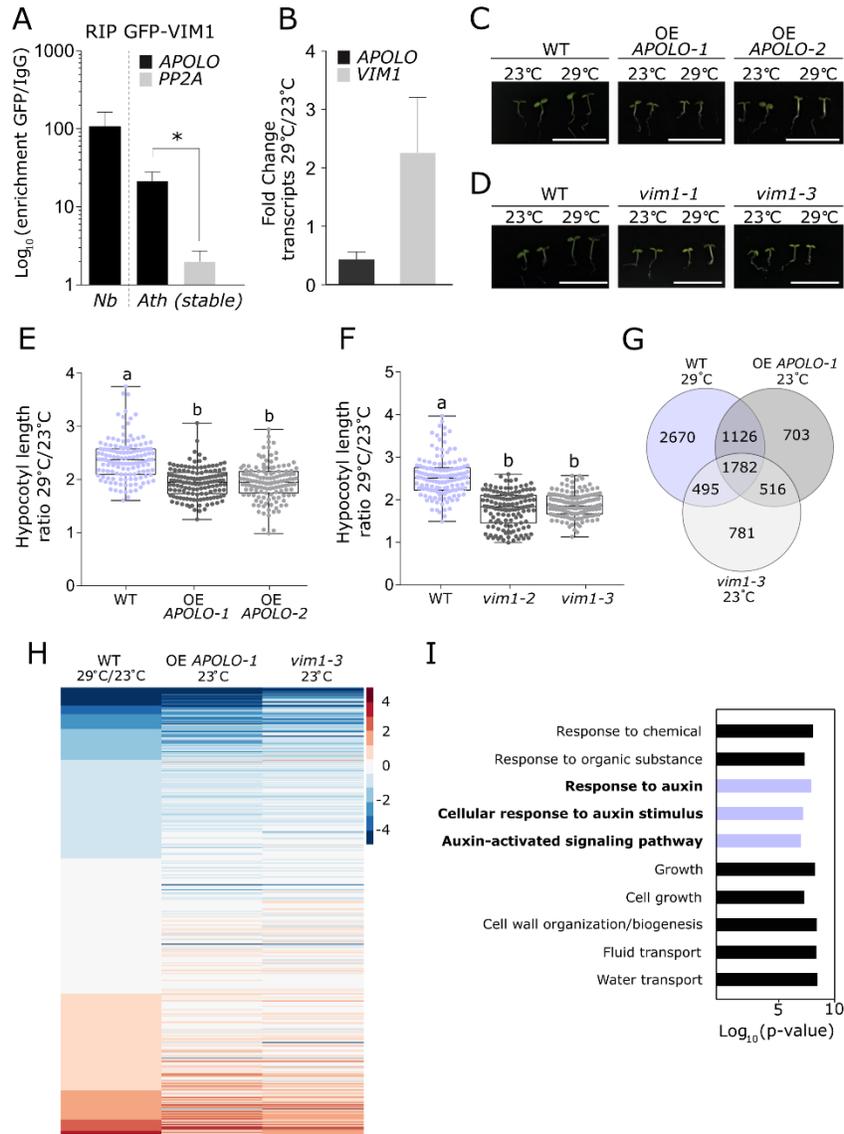
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1055 **Figures and Legends**

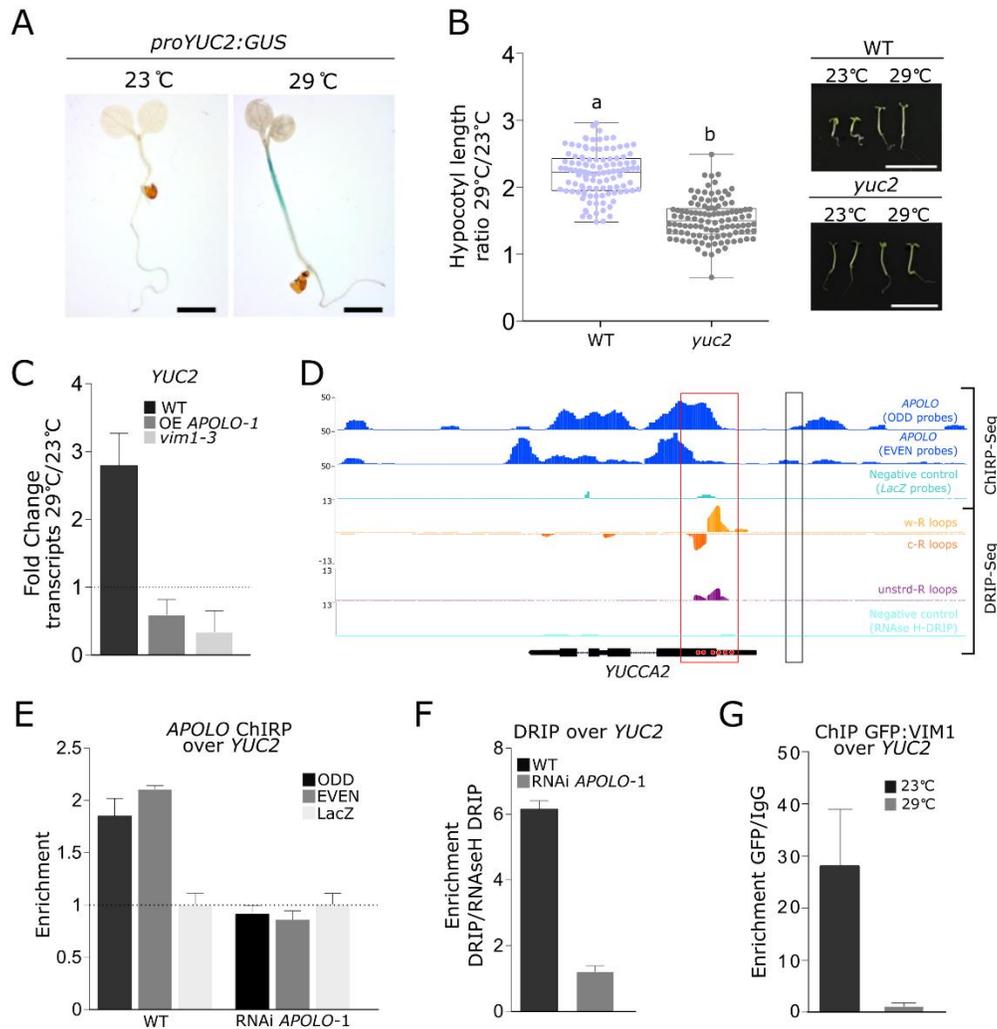


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1057 **Figure 1: The lncRNA *APOLO* and the methylcytosine-binding protein *VIM1* are**
 1058 **thermomorphogenesis regulators**

1059 (A) RNA immunoprecipitation (RIP) assay in *Nicotiana benthamiana* leaves transiently co-
 1060 transformed with *APOLO* and *GFP-VIM1* translational fusion expressed under the control of the
 1061 35S-CaMV promoter, or in *Arabidopsis thaliana* 2-week-old *VIM1* over-expression (OE *VIM1-1*)
 1062 seedlings. Results are expressed as the log₁₀ of the enrichment ratio of the GFP IP over the IgG IP.
 1063 The non-specific background level of RNA precipitation (*PP2A*) is also shown in Arabidopsis. (B)
 1064 Fold change of *APOLO* and *VIM1* expression levels in relation to 23 °C control conditions in 4-day-
 1065 old wild-type (WT) seedlings treated with heat (29°C) for 6h. (C-D) Representative morphological
 1066 phenotypes of 4-day-old *APOLO* over-expression (OE *APOLO-1*, OE *APOLO-2*) (in C) or *vim1* mutant
 1067 (*vim1-2*, *vim1-3*) (in D) seedlings and their associated WT grown at 23°C or 29°C. Bars, 1cm. (E-F)

1068 Boxplots showing hypocotyl length quantification ratio at 29°C over 23°C of 4-day-old OE *APOLO-1*,
1069 OE *APOLO-2* (**E**) or *vim1-2*, *vim1-3* (**F**) seedlings and their associated WT. Values are represented by
1070 colored points. (**G**) Venn diagram of differentially expressed transcripts in WT treated with heat
1071 (29°C) for 6h, and in untreated OE *APOLO-1* and *vim1-3* seedlings. (**H**) Heatmap showing \log_2 (Fold
1072 Change) compared to WT 23°C. A correlation of up- and down-regulated genes in WT in response
1073 to 29°C is observed for OE *APOLO-1* and *vim1-3* at 23°C. (**I**) Gene Ontology (GO) enrichment
1074 analysis of upregulated transcripts in WT treated with heat. Top ten GO categories with more
1075 significant p-values are shown. Violet bars: auxin-related pathways. The ShinyGO Browser is
1076 located at bioinformatics.sdstate.edu and published in Ge and Jung (2018).
1077 In (**A**), bars represent average \pm SD (n = 3 biological replicates). The asterisk indicates significant
1078 difference based on a t test ($\alpha < 0.05$). In (**B**), transcript levels are normalized relatively to the
1079 untreated control to show fold changes. Bars represent average \pm SD (n = 3 biological replicates).
1080 In (**E-F**), results are the mean of three biological replicates and letters indicate significant
1081 differences compared to WT, based on a Kruskal-Wallis test ($\alpha = 0.05$; n \geq 134).
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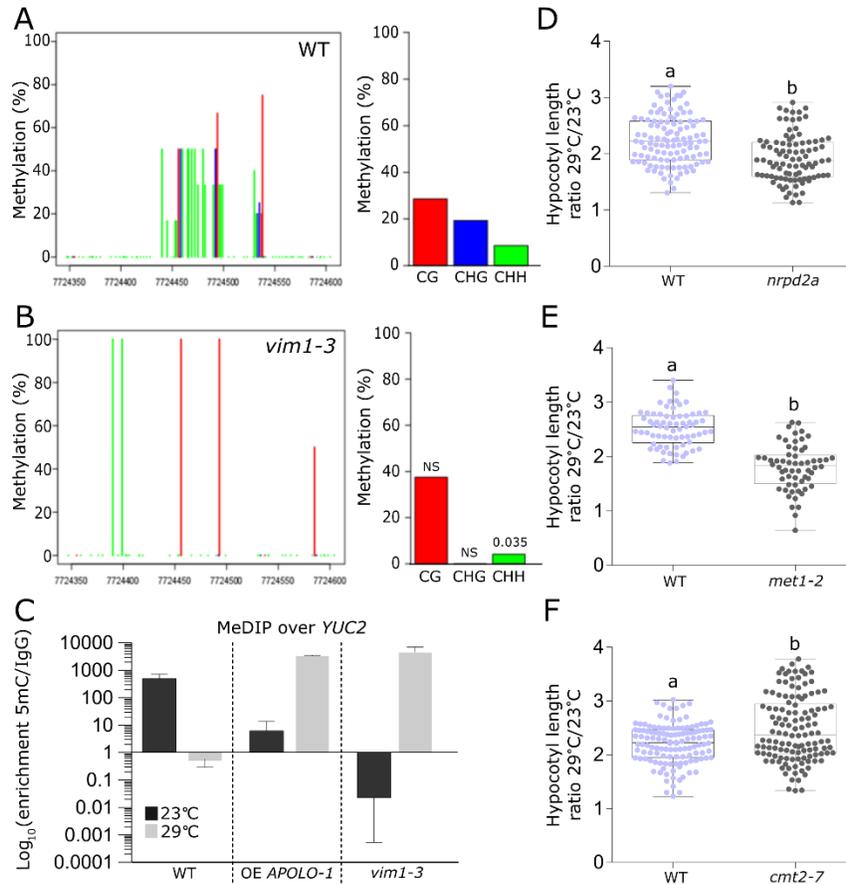


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1084 **Figure 2: The thermomorphogenesis-related gene *YUCCA2* is directly co-regulated by *APOLO* and**
1085 ***VIM1***

1086 (A) Histochemical localization of GUS activity in 4-day-old seedlings containing the *pYUCCA2:GUS*
1087 construct, grown at 23°C or 29°C. Scale bars, 0.1cm. (B) Boxplots showing hypocotyl length
1088 quantification ratio at 29°C over 23°C of 4-day-old *yuc2* seedlings and their associated wild-type
1089 (WT). Values are represented by colored points. Representative morphological phenotypes are
1090 shown on the right. Scale bars, 1 cm. (C) *YUCCA2* (*YUC2*) transcript levels in 4-days-old WT, OE
1091 *APOLO-1* and *vim1-3* seedlings treated or not with heat (29°C) for 6h. (D) Epigenetic profile at the
1092 *YUC2* locus. Tracks 1 to 3 (Ariel et al. 2020): *APOLO* recognition by chromatin isolation by RNA
1093 purification (ChIRP)-Sequencing, using ODD (Track 1) and EVEN (Track 2) sets of probes against
1094 *APOLO*. ChIRP negative control using LacZ probes is shown in Track 3. Tracks 4 to 7 (Xu et al. 2017):
1095 R-loop formation by DNA:RNA immunoprecipitation (DRIP)-Sequencing, on Watson (Track 4), Crick
1096 strand (Track 5) or unstranded sequencing (Track 6). DRIP negative control after RNaseH
1097 treatment is shown in Track 7. Gene annotation is shown at the bottom. On the *YUCCA2* schematic
1098 representation in the bottom, red dots indicate the presence of six GAAGAA/TTCTTC boxes which
1099 may mediate *APOLO* recognition according to Ariel et al. 2020. (E) *APOLO* association to DNA of
1100 the *YUC2* locus by ChIRP-qPCR in WT and RNAi *APOLO-1* plants. The background level was

1101 determined using a set of probes against LacZ RNA. **(F)** RNA-DNA hybrid (R-loop) formation at the
1102 *YUC2* locus by DRIP-qPCR in WT and RNAi *APOLO-1* plants. **(G)** Chromatin immunoprecipitation
1103 (ChIP)-qPCR analysis of VIM1 binding at the *YUC2* promoter in 4-day-old *VIM1* over-expression (OE
1104 *VIM1-1*) seedlings treated or not with heat (29°C) for 6h.
1105 In **(A)**, one representative picture out of ten stained seedlings is shown. In **(B)**, results are the
1106 mean of three biological replicates and letters indicate significant differences compared to WT,
1107 based on a Mann-Whitney test ($\alpha = 0.05$; $n \geq 110$). In **(C)**, transcript levels are normalized relatively
1108 to the untreated control to show fold changes. Bars represent average \pm SD ($n = 3$ biological
1109 replicates). In **(E-F)**, bars represent average \pm SD ($n = 3$ biological replicates). In **(G)**, results are
1110 expressed as the enrichment ratio of the GFP IP over the IgG IP. Bars represent SD ($n = 2$ technical
1111 replicates). One representative experiment out of two biological replicates is shown.
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Figure 3: *APOLLO* and *VIM1* mediate heat-dependent methylation at the *YUCCA2* promoter

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(A-B) Quantification of DNA methylation at the *YUCCA2* (*YUC2*) promoter (Chr4:7,724,346-

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7,724,604) by bisulfite (BiS)-Sequencing, in the three sequence contexts CG (red bars), CHG (blue

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bars) and CHH (green bars), in 4 day-old wild-type (WT) (in A) or *vim1-3* (in B) mutant seedlings

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grown at 23°C. (C) Methylated DNA immunoprecipitation (MeDIP)-qPCR analysis at the *YUC2*

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promoter in 4-day-old *APOLLO* over-expression (OE *APOLLO-1*) or *vim1-3* mutant seedlings treated

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or not with heat (29°C) for 6h. (D-F) Boxplots showing hypocotyl length quantification at 29°C over

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23°C of 4-day-old *nrpd2a* (in D), *met1-2* (in E) or *cmt2-7* (in F) mutant seedlings and their

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associated WT. Values are represented by colored points.

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In (A-B), statistically significant differences between WT and *vim1-3* mutant were calculated using

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the Fisher's exact test. NS stands for not significant. In (C), results are expressed as the Log_{10} of the

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enrichment ratio of the 5mC IP over the IgG IP. Bars represent standard deviation (n = 2 biological

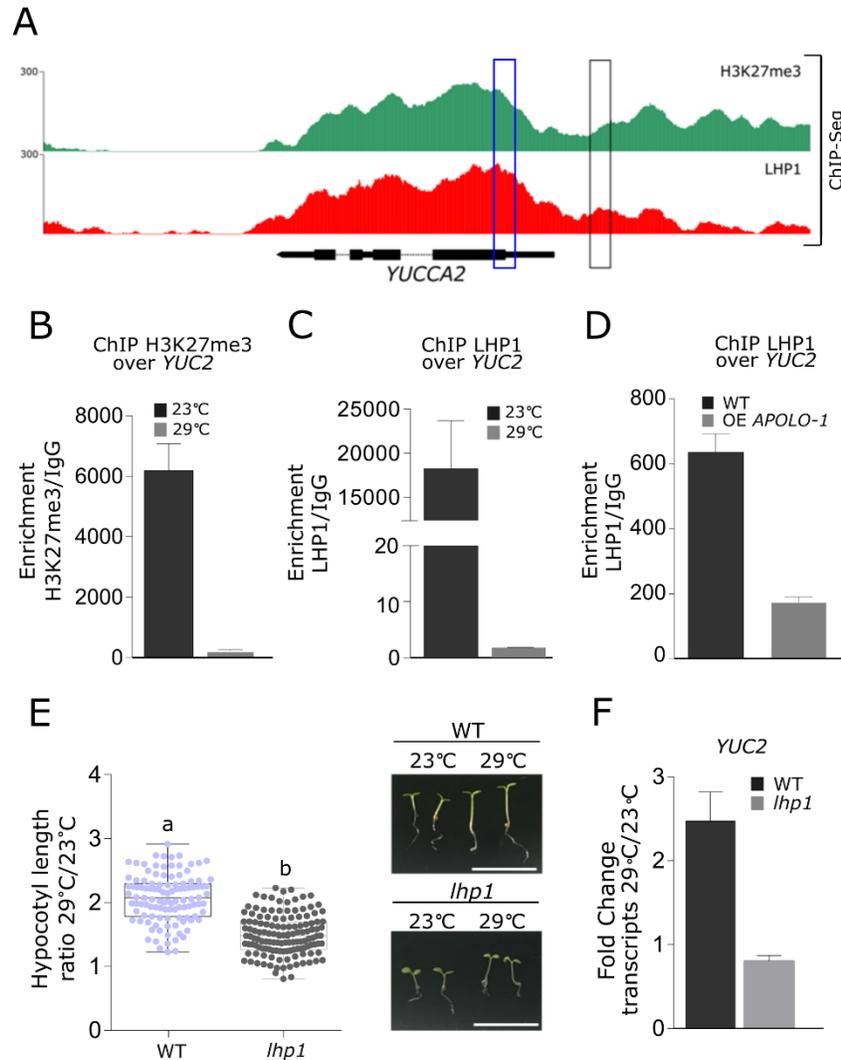
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replicates). In (D-F), results are the mean of three biological replicates and letters indicate

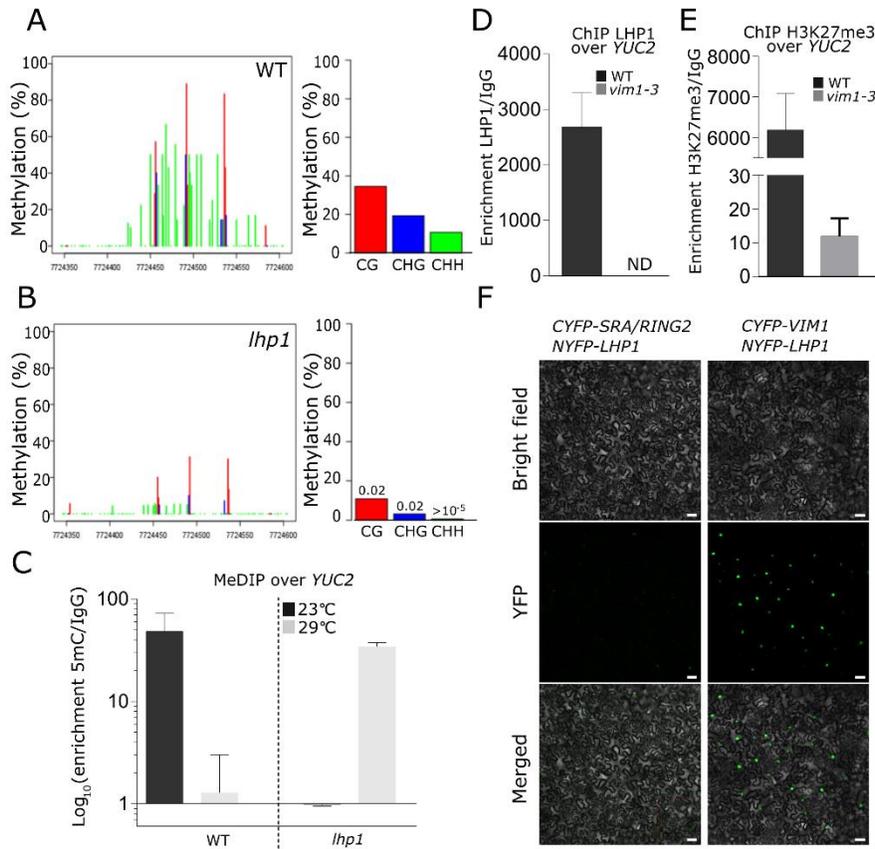
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significant differences compared to WT, based on a Mann-Whitney test ($\alpha = 0.05$; n \geq 60).

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 1130 **(A)** Epigenetic landscape at the *YUCA2* (*YUC2*) locus. Track 1: H3K27me3 deposition by ChIP-
 1131 sequencing. Track 2: LHP1 binding by ChIP-sequencing (Veluchamy et al. 2016). Gene annotation is
 1132 shown in the bottom. **(B)** ChIP-qPCR analysis of H3K27me3 deposition at the *YUC2* promoter in
 1133 4-day-old wild-type (WT) seedlings treated or not with heat (29°C) for 6h. **(C)** ChIP-qPCR analysis of
 1134 LHP1 binding at the *YUC2* promoter in 4-day-old WT seedlings treated or not with heat (29°C) for
 1135 6h. **(D)** ChIP-qPCR analysis of LHP1 binding at the *YUC2* gene body in WT and *APOLO-1* over-
 1136 expression (OE *APOLO-1*) plants. **(E)** Boxplots showing hypocotyl length quantification ratio at 29°C
 1137 over 23°C of 4-day-old *lhp1* seedlings and their associated WT. Values are represented by colored
 1138 points. Representative morphological phenotypes are shown on the right. Scale bars, 1 cm. **(F)**
 1139 *YUC2* transcript levels in 4-days-old WT and *lhp1* seedlings treated or not with heat (29°C) for 6h.
 1140 In **(B-C)**, results are expressed as the enrichment ratio of the H3K27me3 IP **(B)** or LHP1 IP **(C)** over
 1141 the IgG IP. Bars represent standard deviation (n = 2 technical replicates). One representative
 1142 experiment out of three biological replicates is shown. In **(D)**, bars represent average ± SD (n = 3
 1143 biological replicates). In **(E)**, results are the mean of three biological replicates and letters indicate
 1144 significant differences compared to WT, based on a Mann-Whitney test ($\alpha = 0.05$; n ≥ 111). In **(F)**,
 1145 transcript levels are normalized relatively to the untreated control to show fold changes. Bars
 1146 represent average ± SD (n = 3 biological replicates).



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Figure 5: VIM1 and LHP1 co-regulate histone and DNA methylation at the *YUCA2* promoter

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(A-B) Quantification of DNA methylation at the *YUCA2* (*YUC2*) promoter (Chr4:7,724,346-

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7,724,604) by bisulfite (BiS)-sequencing, in the three sequence contexts CG (red bars), CHG (blue

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bars) and CHH (green bars), in wild-type (WT) (A) or *lhp1* (B) mutant seedlings. (C) Methylated

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DNA immunoprecipitation (MeDIP)-qPCR analysis at the *YUC2* promoter in 4-day-old *lhp1* mutant

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seedlings treated or not with heat (29°C) for 6h. (D-E) Chromatin immunoprecipitation (ChIP)-qPCR

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analyses of LHP1 binding (in D) and H3K27me3 deposition (in E) at the *YUC2* promoter in 4-day-old

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WT and *vim1-3* mutant seedlings. ND stands for not detected. (F) Bimolecular Fluorescence

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Complementation (BiFC) assay in transiently transformed *Nicotiana benthamiana* leaves. CYFP was

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fused to VIM1 or SRA/RING2 and NYFP was fused to LHP1. In both panels, bright-field images

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(top), YFP fluorescence (middle) and merged images (bottom) are shown. Scale bars, 50µm.

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In (A-B), statistically significant differences between WT and *lhp1* mutant were calculated using

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the Fisher's exact test. In (C), results are expressed as the enrichment ratio of the 5mC IP over the

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IgG IP. Bars represent standard deviation (n = 2 biological replicates). In (D-E), results are

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expressed as the enrichment ratio of the LHP1 (in D) or H3K27me3 IP (in E) over the IgG IP. Bars

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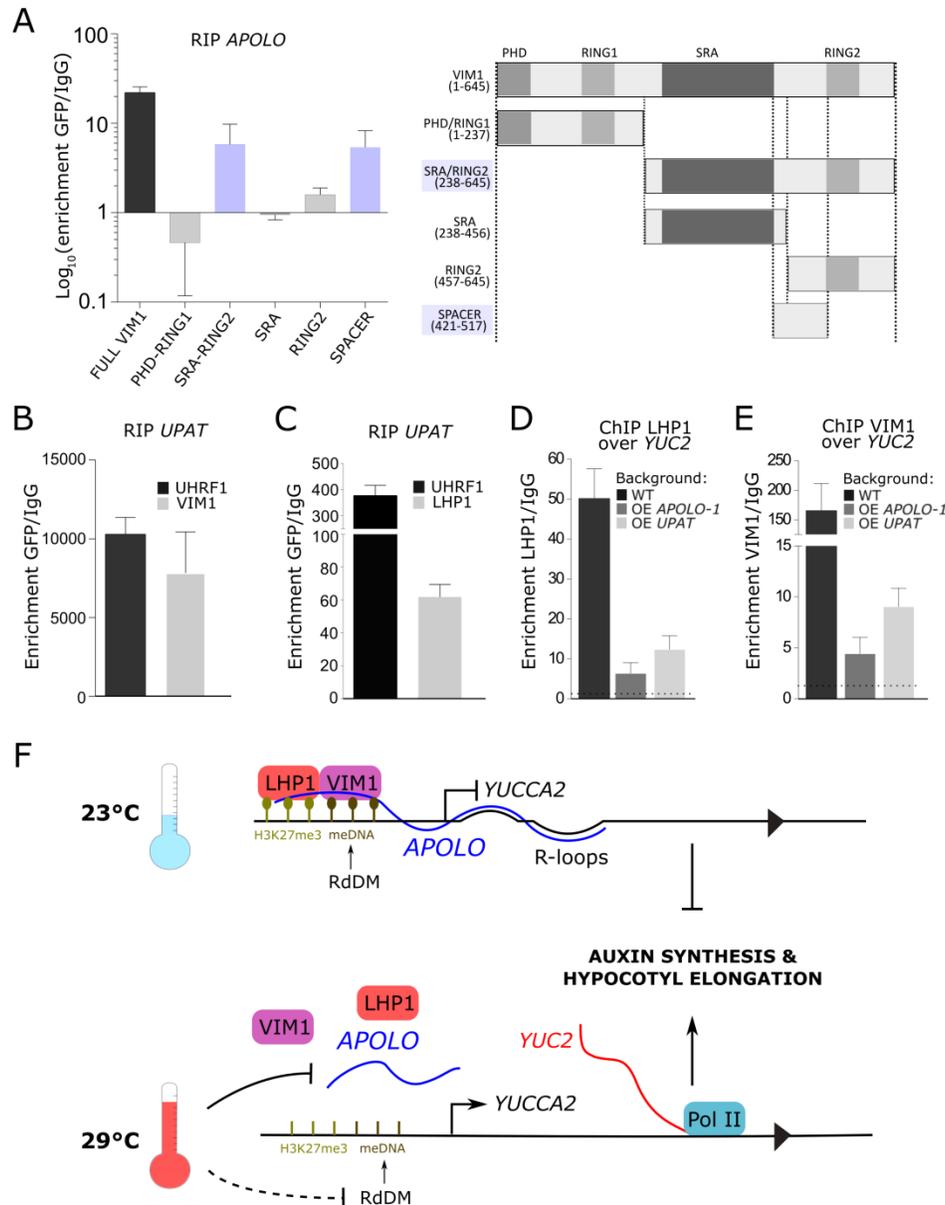
represent standard deviation (n = 2 technical replicates). One representative experiment out of

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three biological replicates is shown. In (F), one representative picture out of six biological

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replicates is shown.



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Figure 6: The UHRHF1-interacting lncRNA *UPAT* binds to VIM1 and LHP1 in plant cells and decoys the complex away from chromatin

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(A) RNA immunoprecipitation (RIP) assay in *Nicotiana benthamiana* leaves transiently co-transformed with *APOL* and translational fusions expressing GFP-VIM1 or derivatives (GFP-PHD/RING1, GFP-SRA/RING2, GFP-SRA, GFP-RING2, GFP-SPACER) under the control of the 35S-CaMV promoter. Results are expressed as the Log₁₀ of the enrichment ratio of the GFP IP over the IgG IP. Schematic representation of VIM1 protein and derivatives tested for RIP is shown on the right. Amino acid coordinates are indicated between brackets. (B-C) RIP assay in *N. benthamiana* leaves transiently co-transformed with *UPAT* and GFP-UHRF1, GFP-VIM1 (B) or LHP1-GFP (C) translational fusions expressed under the control of the 35S-CaMV promoter. Results are expressed as the enrichment ratio of the GFP IP over the IgG IP. (D-E) Chromatin immunoprecipitation (ChIP)-qPCR analysis of LHP1 (in D) and VIM1 (in E) binding at the *YUC2* promoter in wild-type (WT), *APOL* over-expression (OE *APOL-1*) and *UPAT* over-expression (OE

1180 *UPAT*) plants transiently transformed or not with *GFP-VIM1*. **(F)** Model for the regulation of *YUC2*
1181 expression in response to heat by the *APOLO*-*VIM1*-*LHP1* complex: at 23°C, *APOLO* lncRNA
1182 recognizes the *YUCCA2* (*YUC2*) locus by sequence complementarity and R-loop formation. *APOLO*
1183 interacts with *VIM1* and *LHP1* over the *YUC2* promoter region, which exhibits RdDM and
1184 H3K27me3 deposition, blocking *YUC2* transcription. meDNA (light brown balls) and H3K27me3
1185 (dark brown balls) are cooperatively maintained by the *VIM1*-*LHP1* complex. At 29°C, *APOLO*
1186 transcript levels decrease and *VIM1*-*LHP1* binding to the *YUC2* promoter region is reduced.
1187 Conjointly, 24nt siRNAs accumulation is decreased, impairing RdDM over the *YUC2* promoter. As a
1188 result, *YUC2* transcriptional activity increases. *YUC2* participates in auxin synthesis, promoting
1189 hypocotyl elongation in response to heat.
1190 In **(A-F)**, bars represent average \pm SD (n = 3 biological replicates).

1191