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The LEAFY floral regulator displays pioneer transcription factor properties

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Short summary: LFY is a master floral regulator in Arabidopsis and has been suggested to act as a pioneer TF, a special class of TFs that are able to access closed chromatin regions and trigger gene expression. Here, we show that LFY fulfills several pioneer TF properties that may contribute to launch the floral gene expression program.

28 **Abstract**

29 Pioneer transcription factors (TFs) are a special category of TFs with the capacity to bind to
30 closed chromatin regions in which DNA is wrapped around histones and may be highly
31 methylated. Subsequently, pioneer TFs are able to modify the chromatin state to initiate gene
32 expression. In plants, LEAFY (LFY) is a master floral regulator and has been suggested to act
33 as a pioneer TF in Arabidopsis. Here, we demonstrate that LFY is able to bind both methylated
34 and non-methylated DNA using a combination of *in vitro* genome-wide binding experiments
35 and structural modeling. Comparisons between regions bound by LFY *in vivo* and chromatin
36 accessibility data suggest that a subset of LFY bound regions is occupied by nucleosomes. We
37 confirm that LFY is able to bind nucleosomal DNA *in vitro* using reconstituted nucleosomes.
38 Finally, we show that constitutive LFY expression in seedling tissues is sufficient to induce
39 chromatin accessibility in the LFY direct target genes, *APETALA1* and *AGAMOUS*. Taken
40 together, our study suggests that LFY possesses key pioneer TF features that contribute to
41 launch the floral gene expression program.

42 **Introduction**

43 Proper gene regulation is essential to all living organisms, controlling processes from basic
44 development to environmental response. Gene regulation requires the finely orchestrated
45 activity of transcription factors (TFs) that recognize specific DNA sequences in gene regulatory
46 regions and activate or repress transcription of their target genes. While the binding of most
47 TFs to DNA is restricted to accessible regions of the genome, a specific type of TF, called a
48 “pioneer”, is able to access its cognate binding site even in closed, nucleosome-rich chromatin
49 regions (Magnani et al., 2011; Iwafuchi-Doi and Zaret, 2014; Iwafuchi-Doi and Zaret, 2016;
50 Zaret, 2020). The ability to bind nucleosomal DNA *in vivo* and *in vitro* is a defining
51 characteristic of pioneer TFs and has been well-established for diverse mammalian pioneer TFs
52 (Fernandez Garcia et al., 2019). As DNA in closed chromatin regions is often highly
53 methylated, another emerging feature of pioneer TFs is their capability to bind DNA in a
54 methylation insensitive manner (Zhu et al., 2016; Mayran and Drouin, 2018). Some pioneer
55 TFs are even able to directly recruit DNA demethylases at methylated sites, thereby facilitating
56 the remodeling of closed regions (Iwafuchi-Doi, 2018).

57 Pioneer TFs are often master regulators controlling developmental transitions, with the
58 mammalian pluripotency factors Octamer binding TF (OCT4), SRY (sex determining region
59 Y)-box 2 (SOX2), and Kruppel-like factor 4 (KLF4) representing some of the most well-studied
60 (Soufi et al., 2015). These factors bind to closed chromatin regions and induce their opening or
61 remodeling, so that genes they contain can be activated by the pioneer TFs themselves or by
62 other TFs called settlers (Sherwood et al., 2014; Slattery et al., 2014). The modification of the
63 chromatin landscape by pioneer TF can be accomplished either directly by triggering DNA
64 detachment from nucleosomes (Dodonova et al., 2020; Michael et al., 2020), or indirectly by
65 the recruitment of ATP-dependent cellular machineries, such as chromatin remodelers that
66 remove or modify adjacent nucleosomes in order to prime downstream regulatory events (Hu
67 et al., 2011; King and Klose, 2017). Such capacity to modify DNA accessibility is another
68 defining feature of pioneer TFs (Iwafuchi-Doi and Zaret, 2014).

69 In plants, the only TF reported as pioneer TF so far is LEAFY COTYLEDON1 (LEC1), a seed
70 specific TF involved in embryonic epigenetic reprogramming (Tao et al., 2017). LEC1 was
71 shown to promote the initial establishment of an active chromatin state of its target gene in
72 silenced chromatin and activate its expression *de novo*. Pioneer TF activity was also suggested
73 for two types of factors controlling flower development, the MADS homeotic TFs (Pajoro et

74 al., 2014; Denay et al., 2017) and the master floral regulator, LEAFY (LFY) (Sayou et al.,
75 2016). The MADS TFs, including APETALA1 (AP1) and SEPALLATA3, were shown to be
76 able to access closed chromatin regions to specify floral organs, and were thus postulated to act
77 as pioneer TFs (Pajoro et al., 2014). However, mammalian MADS TFs do not seem to act as
78 pioneer factors and thus the identification of AP1 and SEP3 as potential pioneers remains
79 speculative (Sherwood et al., 2014). In contrast to the MADS TFs, one previous study suggest
80 that LFY may have pioneer activity (Sayou et al., 2016). LFY is a master regulator specifying
81 the floral identity of meristems. It directly induces the floral homeotic genes *API*, *APETALA3*
82 (*AP3*) and *AGAMOUS* (*AG*) (Parcy et al., 1998; Wagner et al., 1999; Lohmann et al., 2001;
83 Chae et al., 2008; Yamaguchi et al., 2013; Chahtane et al., 2013). *AG* and *AP3* are known to be
84 under the repression of Polycomb repressive complexes in seedlings (Goodrich et al., 1997;
85 Turck et al., 2007; Calonje et al., 2008). This suggests that their activation during flower
86 development requires modifications of their chromatin landscape and that the direct binding of
87 LFY to their regulatory regions might trigger. Consistent with this, LFY was suggested to be
88 able to access closed chromatin regions *in vivo* (Sayou et al., 2016). Moreover, LFY's role is
89 not confined to conferring a flower fate to meristems. It can also contribute to meristem
90 emergence (Moyroud et al., 2010; Chahtane et al., 2013; Yamaguchi et al., 2013), and together
91 with its co-regulators such as the homeodomain TF WUSCHEL or the F-Box protein
92 UNUSUAL FLORAL ORGANS, it can even induce floral meristem formation from root or
93 leaf tissue, respectively (Levin and Meyerowitz, 1995; Gallois et al., 2004; Risseeuw et al.,
94 2013). Taken together, these data indicate that LFY has the full capability of reprogramming
95 cell fate, a property often requiring pioneer activity. However, whether LFY is truly able to
96 directly bind closed chromatin regions and change their status has yet to be demonstrated.

97 Here, we address the pioneer activity of LFY *in vitro* and *in vivo*. Firstly, we determined
98 whether LFY binding was sensitive to DNA methylation. For this, we combined *in vitro* LFY
99 genome-wide binding data using methylated and unmethylated genomic DNA and structural
100 analysis. These experiments demonstrated that LFY binding is only mildly sensitive to DNA
101 methylation. In order to test whether LFY binding was compatible with the presence of
102 nucleosomes, we compared LFY binding data from chromatin immunoprecipitation sequencing
103 (ChIP-seq) and chromatin accessibility data. Based on these comparisons, we found that LFY
104 could access a number of closed chromatin regions and that LFY colocalizes with nucleosomes
105 in some regions *in vivo*. Using electrophoretic mobility shift assays (EMSA), we further showed
106 that LFY was able to directly bind nucleosomes *in vitro*. Finally, chromatin accessibility assays

107 demonstrated that LFY constitutive expression was sufficient to increase chromatin
108 accessibility in genomic regions including its known target genes *API* and *AG*. Taken together,
109 these data establish that LFY is able to act as a pioneer TF in the regulation of important target
110 genes critical for the establishment of floral fate.

111 **Results and discussion**

112 **LFY is weakly sensitive to DNA methylation**

113 Both the presence of nucleosomes and DNA methylation usually reduce TFs access and binding
114 to their target DNA (Yin et al., 2017; Klemm et al., 2019). DNA methylation in promoter
115 regions (including in euchromatin) is often associated with transcriptional silencing (Zhang et
116 al., 2006). This is also the case in the process of flowering and flower development (Yang et
117 al., 2015), suggesting a crosstalk between the DNA methylation landscape and TF action during
118 this process.

119 In order to assess the effect of DNA methylation on LFY binding, we applied DNA Affinity
120 Purification sequencing (DAP-seq) (O'Malley et al., 2016). Similar to ChIP-seq, this technique
121 allows the identification of the genomic regions bound by a TF but uses naked DNA and a
122 recombinant TF. We used Arabidopsis genomic DNA extracted from seedlings that was either
123 PCR amplified (ampDAP, DNA cleared of methylation) or not amplified (DAP, DNA retaining
124 methylation). Both experiments were performed in triplicates with high reproducibility
125 (Supplemental Figure 1; Supplemental Table 1). As controls, we used two TFs described as
126 methylation sensitive based on available ampDAP and DAP datasets (O'Malley et al., 2016)
127 (Supplemental Figure 2). For each genomic region bound by a given TF, we plotted the
128 DAP/ampDAP signal ratio as a function of i) the methylation density in the whole bound region
129 (based on Arabidopsis seedling methylation maps (Zhang et al., 2016) (Figure 1A-C).), and ii)
130 the number of methylated cytosines within the best TF binding site (TFBS), identified using
131 position weight matrices in each bound region (Figure 1D-F). Whereas an increased number of
132 methylated cytosines in the whole bound region or in the TFBS itself strongly decreases the
133 binding for the two methylation sensitive TFs in DAP relative to ampDAP, LFY binding was
134 only mildly affected (Figure 1A-F). Finally, we designed a specific procedure to compute the
135 effect of methylation on each individual cytosine possibly present in the best TFBS
136 (Supplemental Figure 3-5). In the case of LFY, we identified two positions where the binding
137 is increased by cytosine methylation (positions 4 on the forward DNA strand and 5 on the
138 reverse), and other positions (2,3,7,8 on the forward strand and 1,3,4,9 on the reverse) where

139 the binding is only mildly inhibited (Figure 1G). In contrast, methylation is inhibitory for the
140 two methylation sensitive TFs in most positions where a cytosine could possibly be present
141 (Figure 1H-I). We then examined this result in the context of the protein-DNA structural data.
142 It is known that DNA methylation inhibits the DNA binding of most TFs because the 5-methyl
143 group of methylcytosine often clashes with protein residues that are involved in specific base
144 readout (Yin et al., 2017). Some TFs, however, are not sensitive or even favor methylated DNA
145 because direct hydrophobic interactions form between the methyl group and the TF, as it is the
146 case for homeodomain TFs (Yin et al., 2017) or for some basic leucine zipper TFs (Weber et
147 al., 2019). In the case of LFY, the structural analysis of its DNA binding domain in complex
148 with DNA (Hamès et al., 2008) (PDB 2VY1 and 2VY2) provided a biochemical explanation
149 for the observed positive and negative effects (Supplemental Figure 6). In particular, the
150 hydrophobic contacts between LFY and DNA are likely to be enhanced by the presence of a
151 methyl group in positions 4 and 5 of the LFY binding site (LFYBS), consistent with the DAP
152 versus ampDAP analysis (Figure 1G). How much this weak sensitivity to DNA methylation
153 might help LFY to perform its master function during flowering remains to be determined.

154 Such computational analysis has the potential to be generalized to all TFs for which DAP and
155 ampDAP data are available. It represents a powerful complement to methylation-sensitive
156 SELEX (systematic evolution of ligands by exponential enrichment) analysis which was used
157 to detect the effect of methylation to TF-DNA binding using randomized DNA sequences (Yin
158 et al., 2017).

159 **A subset of LFY binding occurs at closed chromatin regions**

160 Next, we analyzed how *in vivo* factors (including the chromatin state) affect LFY DNA binding.
161 For this, we compared LFY binding *in vitro* and *in vivo* by plotting the coverage of LFY DAP-
162 seq peaks versus that of LFY ChIP-seq peaks. LFY ChIP-seq was obtained from *35S::LFY*
163 seedlings or floral meristems (Sayou et al., 2016; Goslin et al., 2017). This analysis identified
164 genome regions well bound in both experiments (Figure 2A; Supplemental Figure 7A; colored
165 in light purple to red). However, it also highlighted the existence of regions much better bound
166 *in vivo* (ChIP-specific regions colored in deep purple) or *in vitro* (DAP-specific regions colored
167 in orange). The existence of ChIP-specific regions indicated that LFY DNA binding might
168 increase due to interactions with *in vivo* factors. The presence of DAP-specific regions indicated
169 that the *in vivo* context inhibits LFY from binding to some genomic regions despite their high
170 affinity for LFY binding observed in DAP-seq.

171 To understand whether chromatin conformation could play a role in this inhibition, we analyzed
172 the chromatin state of each region using DNaseI-seq data obtained in two-week-old seedlings
173 (Zhang et al., 2012), a high DNaseI-seq signal being indicative of an open region (Figure 2B;
174 Supplemental Figure 7B). We found that many of the DAP-specific regions have a low DNaseI-
175 seq signal, typical of closed chromatin regions. This suggests that a closed chromatin state
176 inhibits LFY binding. However, as previously observed (Sayou et al., 2016), a number of
177 regions are bound in ChIP-seq despite low DNaseI-seq signal (right panels on Figure 2B and
178 Supplemental Figure 7B). Overall, this analysis suggests that while the closed chromatin
179 context is generally inhibitory for LFY binding, some closed chromatin regions can still be
180 bound. To analyze what type of closed regions are most likely to be bound, we analyzed the
181 upper and lower deciles of regions ranked based on their ChIP-seq signal, the upper decile
182 contains regions well bound in ChIP-seq whereas the lower has regions poorly bound in ChIP-
183 seq (but bound in DAP-seq). The distribution of nine chromatin states (as defined in the
184 literature (Sequeira-Mendes et al., 2014)) changes drastically between the two deciles (Figure
185 2C; Supplemental Figure 7C). Chromatin states 7, 8, and 9 (the most compacted states that
186 includes heterochromatin) are highly represented among regions better bound in DAP or in a
187 control set of unbound regions but they are less present in the ChIP-specific regions.
188 Conversely, states 1-5, which are closer to gene units or targets of Polycomb repression (state
189 5) are more frequently found in regions better bound in ChIP-seq than in DAP-seq. This
190 analysis underlines the fact that LFY can bind closed chromatin regions but not those with the
191 highest degree of compactness.

192 As closed chromatin regions are often occupied by nucleosomes, and since *in vivo* data suggests
193 that LFY might be able to bind some of these regions, we wondered whether LFY binding was
194 compatible with the presence of nucleosomes. To test this, we compared the position of LFY
195 ChIP-seq peaks with that of nucleosomes (based on MNase-seq data (Zhang et al., 2015)). We
196 found that nucleosomes were indeed enriched at the center of LFY ChIP-seq peaks in closed
197 regions (Figure 2D; Supplemental Figure 7D), but not in open ones (Figure 2E; Supplemental
198 Figure 7), suggesting that LFY might be able to directly bind nucleosomal DNA *in vivo*. We
199 mapped the LFYBS in nucleosome-occupied LFY ChIP-seq peaks that are also found in DAP-
200 seq experiments to ensure they contain a *bona fide* LFY dimer binding site. We found a slight
201 enrichment at the center of the nucleosome, around the dyad position which is a site commonly
202 bound by pioneer TFs (Figure 3A; Supplemental Figure 8) (Zaret, 2020). However, since these
203 genomic data are established on mixtures of tissues, they are not sufficient to firmly establish
204 that LFY is indeed able to bind nucleosomal DNA.

205 **LFY binds nucleosomal DNA at specific sites *in vitro***

206 Next, we tested whether LFY has the capacity to bind nucleosomal DNA *in vitro*. We first
207 assembled nucleosomes using the Widom 601 strong nucleosome positioning sequence
208 (Lowary and Widom, 1998; McGinty and Tan, 2015), in which a LFYBS was inserted at
209 different positions (C1-C7 around the dyad and E1-E7 farther away) (Figure 3B; Supplemental
210 Table 2). With nucleosomes assembled with a LFYBS at position C2 and C7, we observed a
211 gel shift upon addition of LFY that is absent with LFYBS at positions C1, C3-C6, E1-E7 or
212 with no LFYBS, demonstrating that LFY binds nucleosomal DNA in a sequence specific
213 manner and only with a LFYBS present at specific positions (C2, located around the dyad, and
214 C7, located one helix turn apart from C2, with the LFYBS exposed to the outer nucleosome
215 surface (Figure 3B and C; Supplemental Figure 9)). This property is consistent with structural
216 data showing that LFY binds a single side of the DNA (Hamès et al., 2008) that needs to be
217 exposed to the outer surface (like C2 or C7) and not hidden by histones (C1, C3 to C6). It is
218 worth noting that LFY DNA binding presents structural similarities with that of the pioneer TF
219 FoxA in animals, whose helix-turn-helix DNA binding domain (DBD) mainly contacts the
220 major groove, and with a flexible loop contacting the minor groove, both of which are located
221 in the same side of the DNA (Zaret and Carroll, 2011; Fernandez Garcia et al., 2019). Using
222 the same methodology, as a negative control, we tested nucleosomal DNA binding of the TF
223 REGULATOR OF AXILLARY MERISTEMS 1 (RAX1), a direct downstream target of LFY
224 (Chahtane et al., 2013). We found that RAX1 cannot associate with nucleosomes even when its
225 binding site is exposed to the outer nucleosome surface and at the dyad (Supplemental Figure
226 10), suggesting that RAX1 is unlikely a pioneer TF. We also assembled nucleosomes with two
227 regions of the *API* gene, a known early activated LFY target (Parcy et al., 1998; Wagner et al.,
228 1999; Benlloch et al., 2011). These regions were taken from *API* first intron and *API* promoter
229 (annotated as *API* intron and *API* pro, respectively, in Figure 3D). They are both bound by
230 LFY *in vivo* (ChIP-seq (Moyroud et al., 2011; Winter et al., 2011; Sayou et al., 2016; Goslin et
231 al., 2017)) and *in vitro* (DAP-seq in Figure 3D), and with well-defined nucleosome signals from
232 MNase-seq in both seedlings and flower tissues (Zhang et al., 2015) (Figure 3D). We observed
233 that LFY was able to bind to these nucleosomes (Figure 3E and Supplemental Figure 11),
234 showing that LFY nucleosomal DNA binding also occurs within Arabidopsis genomic regions.

235 **LFY constitutive expression induce changes in chromatin accessibility and nucleosome**
236 **positioning**

237 One key characteristic feature of pioneer TFs is their ability to modify the status of closed
238 chromatin regions (Iwafuchi-Doi and Zaret, 2014). To test whether LFY is able to do so, we
239 examined whether it could alter chromatin accessibility when ectopically expressed in
240 seedlings. We selected regions bound by LFY in ChIP-seq (Figure 4A) (Sayou et al., 2016)
241 that are mapped with a nucleosome in wild-type seedlings but not in closed flower buds (Zhang
242 et al., 2015). These regions are adjacent to 496 genes including 54 that are upregulated by LFY
243 (induced by LFY overexpression or downregulated in *lfy* mutants (William et al., 2004; Schmid
244 et al., 2005; Winter et al., 2011)). Among these genes are present those encoding for early floral
245 regulators AP1, AG and ULTRAPETALA 1 (ULT1) (Moreau et al., 2016), as well as other
246 TFs and cell wall remodeling enzymes (Supplemental Table 4). We focused on these three well-
247 established floral regulators. Using Formaldehyde-Assisted Isolation of Regulatory Elements
248 (FAIRE)-qPCR that identifies accessible chromatin regions (including those depleted of
249 nucleosomes) and MNase-qPCR that locates the nucleosome (Figure 4 and Supplemental
250 Figure 12), we tested whether ectopic LFY expression (*35S::LFY*) could alter the local
251 chromatin as compared to two-week-old Col-0 seedlings where endogenous LFY is not yet
252 highly induced. Focusing on different types of regions (promoters or introns), we found that
253 LFY ectopic expression increased accessibility of these regions (Figure 4A) but not for three
254 control regions (*Actin2*, *AT2G38220* and *AT4G22285*) with poor accessibility in seedlings and
255 where LFY does neither bind *in vivo* nor *in vitro* (Figure 4B). The analysis of the *AP1* locus is
256 particularly interesting. This gene is a direct and early target of LFY and contains two LFY
257 binding peaks, one in its promoter (P2-P4 in *AP1*; Figure 4A) and one in its first intron (P1 in
258 *AP1*; Figure 4A). *AP1* promoter can be induced by LFY in seedling leaves, independently of
259 flower formation (Parcy et al., 1998). According to DNaseI-seq signal, *AP1* promoter is already
260 open in seedlings and with two nucleosomes detected by MNase-seq whereas the first intron is
261 closed (Figure 4A). We found that LFY expression triggers a strong accessibility increase in
262 the intron (3-fold, P1 in *AP1*, Figure 4A) and a more moderate increase in the promoter (P2 and
263 P3 in *AP1*; Figure 4A). We also found increased accessibility in *ULT1* promoter and *AG*
264 regulatory intron (Figure 4A). Consistent with FAIRE-qPCR, MNase-qPCR experiments on
265 the same regions gave comparable results, indicating that the increased accessibility is most
266 likely due to nucleosome depletion (Supplemental Figure 12). Our results are consistent with a
267 recent report using LFY induction in root explants, a system where *AP1* promoter appears in a
268 closed chromatin state and opens after LFY induction (Jin et al., 2020).

269 To conclude, we have obtained evidence that LFY shares some properties with pioneer TFs.
270 However, the pioneer function is likely a spectrum of activities: TFs that play central roles in
271 developmental transitions, such as LFY, are able to fulfill a pioneer role under certain chromatin
272 conditions or cellular contexts, for example in the presence of specific cofactors (Zaret, 2020)
273 and/or for a few distinct loci (Li et al., 2019). Taken together, our *in vitro* and *in vivo* results
274 demonstrate the essential properties of pioneer TFs- the competence to bind closed chromatin
275 and the ability to trigger subsequent opening of these closed regions- are properties of LFY in
276 the context of at least a few key floral regulatory targets.

277 **Methods**

278 Detailed methods are available in Supplemental Information (SI).

279 **DAP-seq and AmpDAP-seq**

280 The input library of ampDAP-seq was PCR amplified from Col-0 genomic DNA constructed
281 according to published protocol (O'Malley et al., 2016; Bartlett et al., 2017; Lai et al., 2020).
282 For the DAP-seq input library, genomic DNA was extracted from two-weeks-old seedlings of
283 a *35S::LFY* line (pCA26 #15) (Sayou et al., 2016) grown on 0.5 x Murashige and Skoog
284 medium in long-day conditions. The LFY protein was produced using an *in vitro*
285 transcription/translation system (Promega L3260). DAP-seq was carried out according to
286 published protocol with minor modifications (O'Malley et al., 2016; Bartlett et al., 2017). The
287 immunoprecipitated DNA fragments were PCR amplified for 20 cycles, and purified using
288 AMPure XP magnetic beads (Beckman). Individual libraries were pooled with equal molarity,
289 and sequenced on Illumina HiSeq (Genewiz). Both DAP-seq and ampDAP-seq were performed
290 in triplicates.

291 **Bioinformatic analyses**

292 Regions bound by LFY *in vitro*, to methylated and non-methylated genomic DNA, were
293 detected from DAP-seq data generated in this study as described previously (Lai et al., 2020).
294 *In vivo* LFY bound regions were obtained from ChIP-seq data from two weeks-old seedling
295 *35S::LFY* tissue (Sayou et al., 2016) and inflorescence tissue of *35S::LFY-GR ap1 cal* (Goslin
296 et al., 2017). DNA accessibility, nucleosomes position and probability of methylcytosines were
297 obtained from processed DNaseI-seq, MNase-seq and bisulfite sequencing data (Zhang et al.,
298 2012, Zhang et al., 2015, Zhang et al., 2016), respectively. Microarray analyses were performed

299 on published data of LFY overexpression lines (GEO: GSE911) (William et al., 2004), and
300 (GEO: GSE28062) (Winter et al., 2011) and data from *lfy* lines (GEO: SE576) (Schmid et al.,
301 2003) and available on AtGenExpress (Schmid et al., 2005). *Ad hoc* Python, R and Shell scripts
302 developed in our laboratory are available at [https://github.com/Bioinfo-LPCV-](https://github.com/Bioinfo-LPCV-RDF/TF_genomic_analysis)
303 RDF/TF_genomic_analysis.

304 **Nucleosome reconstruction and Electrophoretic mobility shift assay (EMSA)**

305 The recombinant AtLFY Δ 40 was produced in *E. coli* Rosetta2 (DE3) strain (Novagen) and
306 purified by nickel affinity purification (GE Healthcare). The production and purification of
307 histones was carried out according to published protocols (Shim et al., 2012). The nucleosome
308 assembly was performed by salt dilution method (Okuwaki et al., 2005) using the corresponding
309 DNA probes (Supplemental Tables 2 and 3). Nucleosomes of interest were incubated with 500
310 μ M AtLFY Δ 40 for 1 hour at room temperature. The EMSAs were run on 5 % non-denaturing
311 polyacrylamide gels for one hour at 4 °C at 120 V, and visualized by Cy5 signal on ChemiDoc
312 MP Imager (BIO-RAD).

313 **FAIRE-qPCR**

314 FAIRE-qPCR was performed on two-week-old seedlings of Col-0 and *35S::LFY* (Sayou et al.,
315 2016). 1 g of plant material was crosslinked by formaldehyde for 15 min using vacuum
316 infiltration. Crosslinking was quenched by adding glycine solution to 0.125 M. Nuclei were
317 isolated using NI buffer (see SI) and then resuspended in 1 mL of FAIRE Lysis Buffer (see SI).
318 The crosslinked DNA was sheared to an average size of 200 - 300 bp using Covaris S220. An
319 aliquot was used as control DNA and directly treated with RNase A+T1 cocktail enzyme mix
320 (Thermo Fisher Scientific) followed by proteinase K treatment and reverse crosslinked. The
321 non-de-crosslinked samples were treated as for control DNA and subject to a
322 phenol/chloroform extraction. DNA was then quantified using Qubit dsDNA HS kit (Thermo
323 Fisher Scientific) and the ratio between nucleosome-free DNA versus total DNA was
324 determined by qPCR analysis using 20 ng of template DNA for each reaction.

325 **Accession Numbers**

326 LFY DAP-seq sequencing data from this article can be found in the NCBI GEO data libraries
327 under accession numbers GSE160013.

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333

334 **Authors contributions**

335 F.P., C.Z., and R.D. designed and supervised the project, R.B.M., J.L., A.S., and L.T. performed
336 bioinformatics analyses, L.G., G.V., J.L.M., H.D., E.T. and E.B.H. performed biochemical
337 analyses, X.L. performed DAP-seq, Y.H. performed FAIRE-qPCR and MNase-qPCR
338 supervised by M.B. and D.L., F.P., C.Z., and X.L. wrote the paper with the help of all authors.

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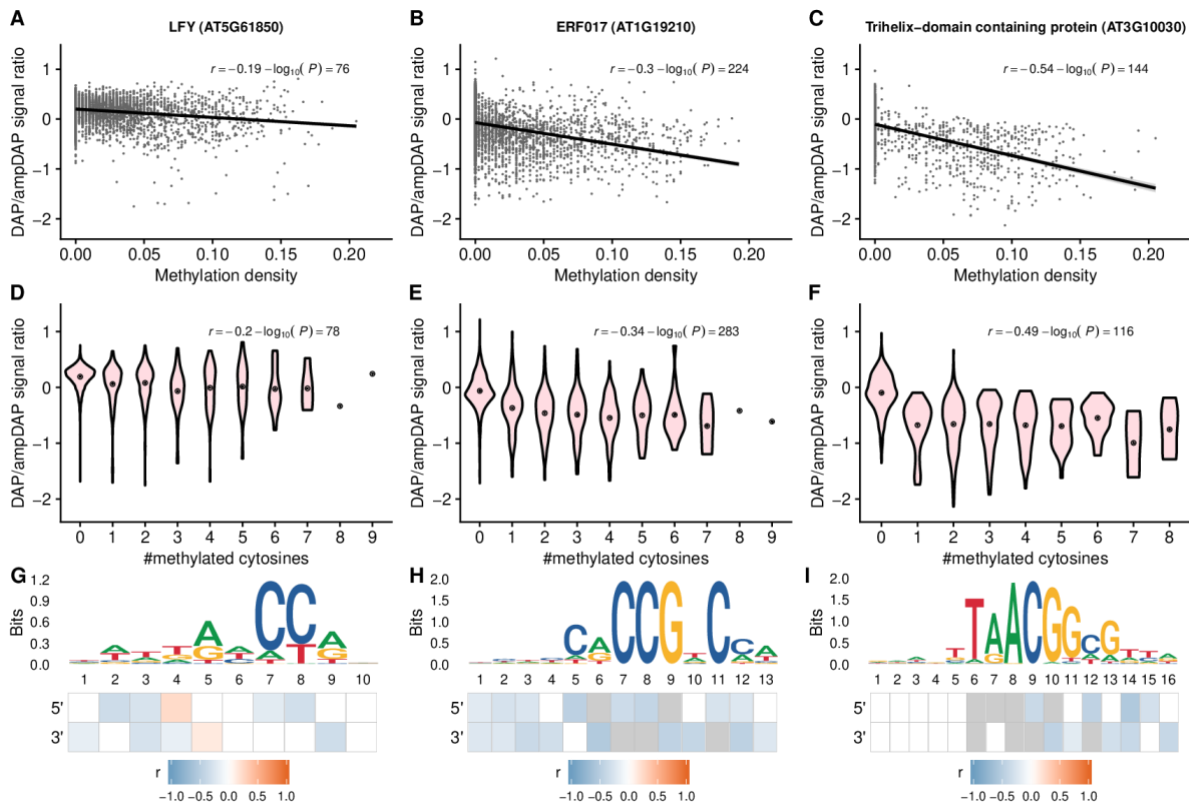
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524

525 Figure Legends



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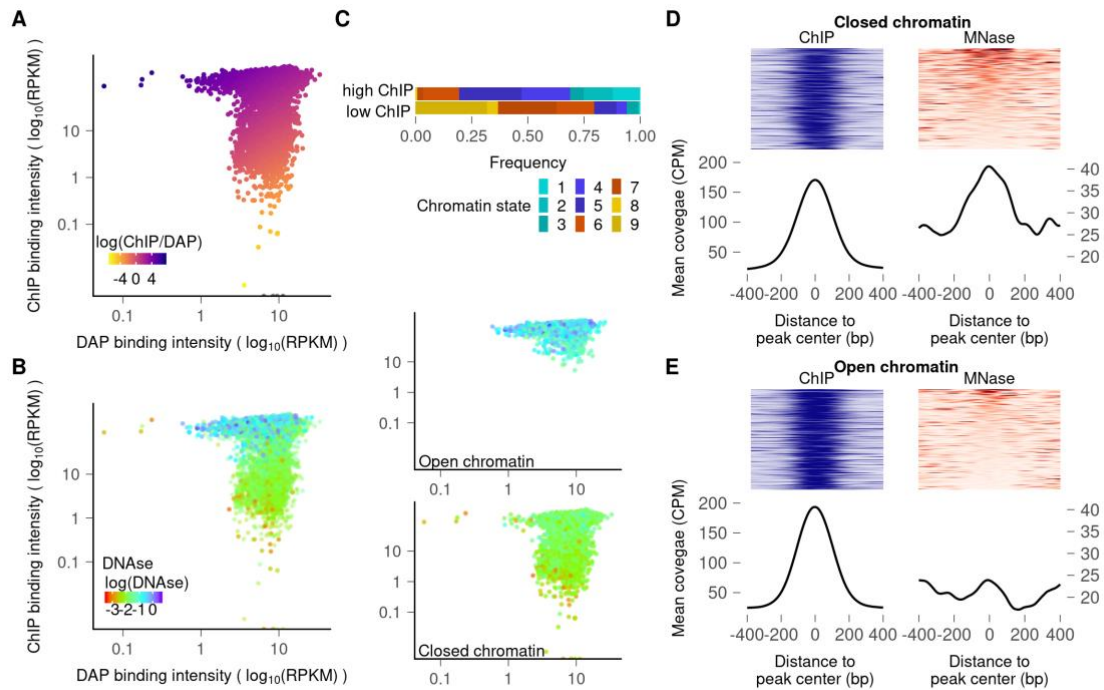
527 **Figure 1: Cytosine methylation has a mild effect on LFY DNA-binding intensity.**

528 Effect of cytosine methylation on DNA binding for three transcription factors: LFY (left),
529 ERF017 (middle) and a trihelix-domain containing protein (right). (A-C) Biplots between the
530 DAP/ampDAP signal ratio (peak normalized read coverage in the DAP experiment divided to
531 that in the ampDAP experiment) in a log₁₀ scale and methylation density (proportion of
532 cytosines with a probability of methylation greater than 0.5) within transcription factor bound
533 regions. The increasing methylation density has weaker effect on LFY than on the two other
534 TFs. (D-F) Violin plots of DAP/ampDAP signal ratio in a log₁₀ scale as a function of the number
535 of methylated cytosines in the best TF binding site (TFBS) of each bound region. LFY binding
536 is barely affected by the increased number of methylated cytosines. (G-I) Binding site sequence

537 motif for each TF and the methylation effect on each individual position. For LFY, a single half
538 of the symmetric motif is shown. Heatmaps show the Pearson's correlation coefficient (r)
539 between the DAP/ampDAP signal ratio in a \log_{10} scale and the probability of methylation at
540 each position of the best TFBSs. Blank positions have a high false discovery rate ($> 5\%$) and
541 grey indicates positions with less than ten cytosines in the dataset. Correlation are tested on
542 both sides of a symmetric motif (G) or on both strands for non-symmetric motifs (H-I).
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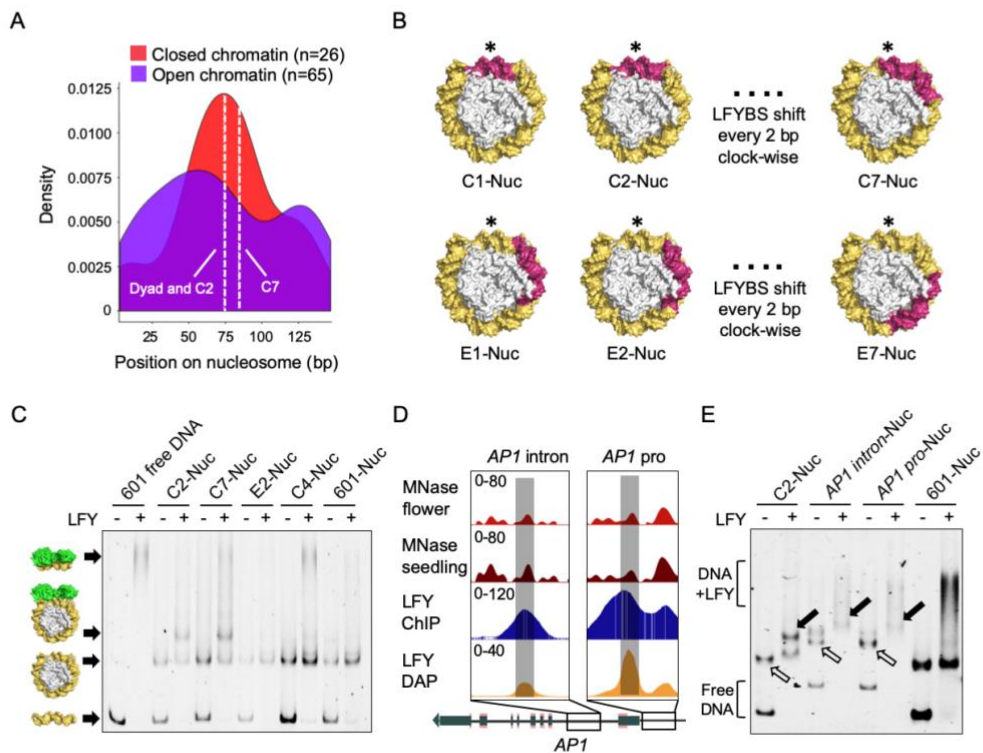
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546

547 **Figure 2: LFY is able to bind nucleosomes in closed chromatin regions.**

548 (A) Plots comparing the LFY binding intensities (peak coverages) in ChIP-seq (Y-axis) vs
549 DAP-seq (X-axis) experiments. Heat map is based on the ChIP-seq/DAP-seq intensity ratio.
550 (B) Overlay of DNaseI signal (heat map) on LFY bound regions. The two panels on the right
551 show the same regions split into open (upper panel) and closed (lower panel) chromatin states.
552 (C) Distribution of chromatin states 1 to 9 according to (Sequeira-Mendes et al., 2014) for the
553 first and last decile of LFY bound regions based on ChIP-seq signal and for a comparable set
554 of regions not bound by LFY. (D-E) MNase signal around ChIP-seq peak centers in closed (D)
555 or open (E) chromatin regions. Upper panels show ChIP-seq and MNase-seq coverage for each
556 peak ordered based on MNase-seq signal. Lower panels represent the mean coverage. Regions
557 above the dotted lane show a MNase signal in their center indicative of the presence of a
558 nucleosome (see methods).

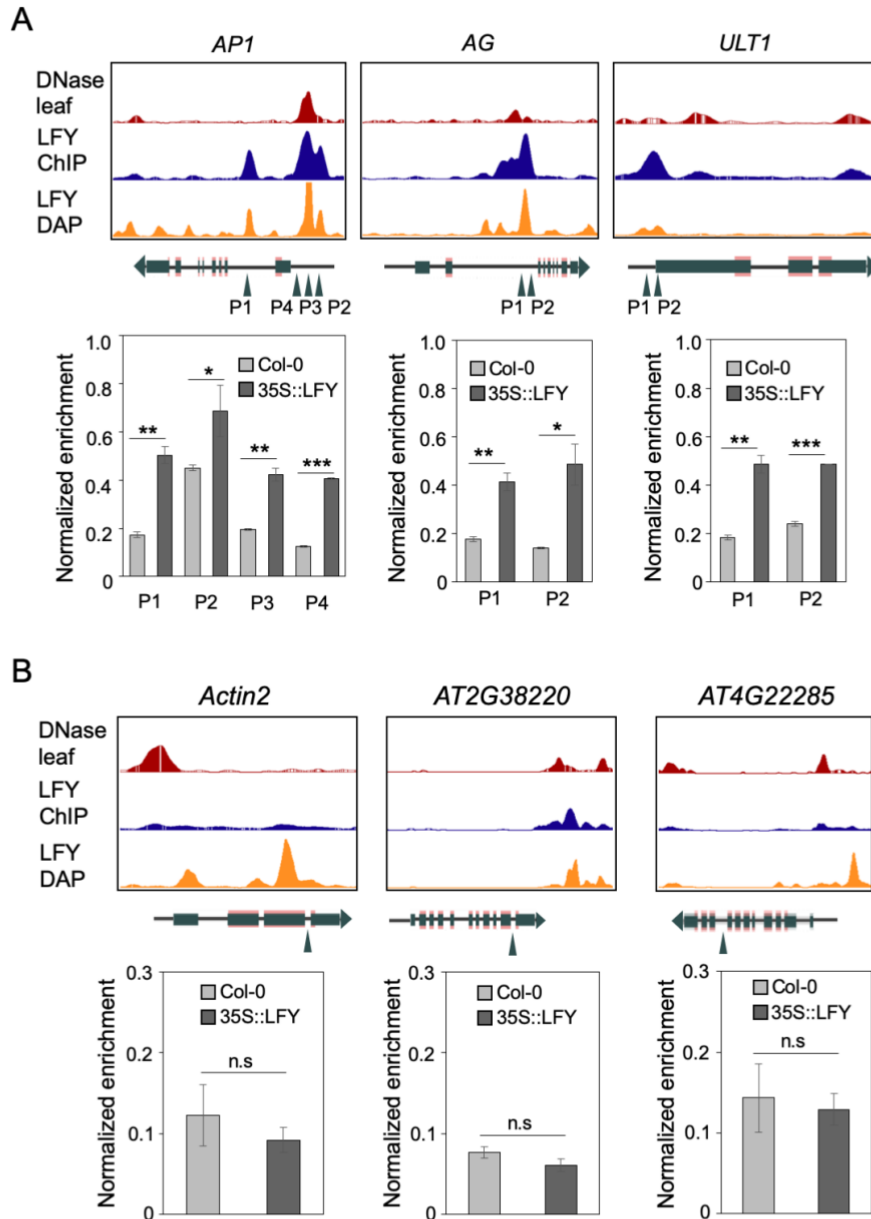


559

560 **Figure 3: LFY binds nucleosomes *in vitro*.**

561 (A) Density plot of the LFY best binding site present in ChIP-seq peaks along a canonical 147-
 562 bp nucleosomal sequence in open and closed chromatin contexts for flower tissues. An
 563 enrichment for LFY binding sites (LFYBS) around the dyad position (the center of the

564 nucleosomal DNA) is observed in closed chromatin regions. C2 (at dyad) and C7 positions are
565 indicated. Alternative plots for different datasets and thresholds for binding sites selection are
566 reported in Supplemental Figure 8. (B) Design of Widom 601 sequences (yellow orange) with
567 a LFYBS (warm pink) inserted at different positions (central C1-C7 (top) and external E1-E7
568 (bottom)) on nucleosome (PDB: 3UT9 (Chua et al., 2012). * indicates the dyad.
569 (C) Representative EMSA showing LFY binding to 601 nucleosomes with a LFYBS at
570 positions C2 (labelled C2-Nuc) and C7, but not at E2, C4 or 601 nucleosome without a LFYBS
571 (refer to Supplemental Figure 9 for the screening of LFY nucleosomal DNA binding at all other
572 positions). Free DNA (C2 in the first 2 lanes, or present in the nucleosomal preparations) is
573 shifted at the very top of the gels. 601-Nuc is made with wild-type 601 sequence (without a
574 LFYBS): only free DNA is shifted due to non-sequence specific interactions with LFY. Cartoon
575 on the side from bottom to top are free DNA, nucleosome alone, LFY-nucleosome complex
576 and free DNA-LFY complex. (D) Genomic snapshots of LFY DAP-seq, ChIP-seq (seedlings
577 tissue), and MNase-seq (seedlings and closed flower buds) at the *API* loci. *API* intron and *API*
578 pro sequences used to assemble nucleosomes in (E) are highlighted in grey. Both regions are
579 bound in DAP and ChIP, and with well-defined nucleosome signals, lower in floral tissue than
580 in seedlings. (E) EMSA showing LFY binding to nucleosomes assembled with *API* intron and
581 *API* pro sequences. AP1-intron-Nuc and AP1-pro-Nuc are longer than 601 due to the presence
582 of amplification primers. Note some free 601 DNA is shifted despite the absence of LFYBS in
583 the last lane. The hollow and solid arrows indicate the position of reconstituted nucleosomes
584 and the shifted nucleosomes signals, respectively.



585

586 **Figure 4: LFY constitutive expression increases chromatin accessibility.**

587 (A) (Top) Genomic snapshots of chromatin accessibility (DNaseI-seq from 2-week-old Col-0

588 seedlings (Zhang et al., 2012)), LFY binding *in vitro* (DAP-seq using genomic DNA from 2-

589 week-old *35::LFY* seedlings), *in vivo* (ChIP-seq of 2-week-old *35::LFY* seedlings (Sayou et al.,

590 2016)) at *API*, *AG* and *ULT1* loci. The regions tested in FAIRE-qPCR are indicated by triangle
591 arrows (P1-4 labels). (Bottom) FAIRE-qPCR of the indicated regions are performed in 2-week-
592 old seedlings of Col-0 (pale gray) and *35S::LFY* (dark gray), respectively. Error bars represent
593 means \pm standard deviation. Significance test is performed by one-tailed students' t-test,
594 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s, not significant. (B) (Top) genome browser snapshots of
595 three genomic regions devoid of LFY binding and poorly accessible in 2-week-old seedlings.
596 (Bottom) FAIRE-qPCR on the indicated regions. Significance test is performed as per (A). The
597 FAIRE-qPCR is performed by two biological replicates, with three technical replicates for each.
598 The enrichment is normalized by input DNA in each experiment.
599