



Deposited via The University of York.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/153864/>

Version: Accepted Version

---

**Article:**

Tong, Meixuezi, Lee, Kyounghee, Ezer, Daphne et al. (2020) The Evening Complex establishes repressive chromatin domains via H2A.Z deposition. *Plant Physiology*. pp. 612-625. ISSN: 0032-0889

<https://doi.org/10.1104/pp.19.00881>

---

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

1 **Short title:** EC-dependent H2A.Z deposition

2

3 **Corresponding authors:** Paloma Mas (paloma.mas@cragenomica.es), Philip A. Wigge  
4 (wigge@igzev.de), Pil Joon Seo (pjseo1@snu.ac.kr)

5

6 **The Evening Complex establishes repressive chromatin domains via H2A.Z**  
7 **deposition**

8

9 **Meixuezi Tong<sup>1,†</sup>, Kyounghee Lee<sup>2,†</sup>, Daphne Ezer<sup>1</sup>, Sandra Cortijo<sup>1</sup>, Jaehoon Jung<sup>1,2</sup>,**  
10 **Varodom Charoensawan<sup>1</sup>, Mathew S. Box<sup>1</sup>, Katja E. Jaeger<sup>1</sup>, Nozomu Takahashi<sup>3</sup>,**  
11 **Paloma Mas<sup>3,4,\*</sup>, Philip A. Wigge<sup>1,5,\*</sup>, Pil Joon Seo<sup>2,6,7,\*</sup>**

12

13 <sup>1</sup>Sainsbury Laboratory, University of Cambridge, 47 Bateman St., Cambridge CB2 1LR, UK

14 <sup>2</sup>Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Republic of  
15 Korea

16 <sup>3</sup>Center for Research in Agricultural Genomics (CRAG), Consortium CSIC-IRTA-UAB-UB,  
17 Parc de Recerca Universitat Autònoma de Barcelona (UAB), Bellaterra (Cerdanyola del  
18 Vallés), Barcelona, Spain

19 <sup>4</sup>Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain

20 <sup>5</sup>Leibniz-Institut für Gemüse- und Zierpflanzenbau (IGZ), Theodor-Echtermeyer-Weg 1,  
21 14979 Großbeeren, Germany

22 <sup>6</sup>Department of Chemistry, Seoul National University, Seoul 08826, Republic of Korea

23 <sup>7</sup>Plant Genomics and Breeding Institute, Seoul National University, Seoul 08826, Republic of  
24 Korea

25

26 †These authors contributed equally to this work.

27 \*Corresponding authors: P.M. (paloma.mas@cragenomica.es), P.W. (wigge@igzev.de), or  
28 P.J.S. (pjseo1@snu.ac.kr)

29

30 The author responsible for distribution of materials integral to the findings presented in this  
31 article in accordance with the policy described in the Instructions for Authors  
32 (www.plantphysiol.org) is: P.M. (paloma.mas@cragenomica.es), P.W. (wigge@igzev.de), or  
33 P.J.S. (pjseo1@snu.ac.kr).

34

35 **One sentence summary:** The Evening Complex interacts with the complex responsible for  
36 the deposition of the histone variant H2A.Z, creating repressive chromatin domains to repress  
37 a cohort of target genes in *Arabidopsis*.

38

39 **Author contributions:** PW, PM, and PJS participated in the design of the study and wrote the  
40 manuscript. MT, KL, and NT performed the molecular experiments. MT and DE performed  
41 the analysis of sequencing data. MT, SC, JJ, VC, and MSB performed large-scale time-course  
42 experiments. MT and KEJ performed ChIP-seq experiments. PW, PM, and PJS conceived the  
43 project. All authors read and approved the final manuscript.

44

45

46 **Abstract**

47 The Evening Complex (EC) is a core component of the *Arabidopsis* (*Arabidopsis thaliana*)  
48 circadian clock, which represses target gene expression at the end of the day and integrates  
49 temperature information to coordinate environmental and endogenous signals. Here we show  
50 that the EC induces repressive chromatin structure to regulate the evening transcriptome. The  
51 EC component ELF3 directly interacts with a protein from the SWI2/SNF2-RELATED  
52 (SWR1) complex to control deposition of H2A.Z-nucleosomes at the EC target genes. SWR1  
53 components display circadian oscillation in gene expression with a peak at dusk. In turn,  
54 SWR1 is required for the circadian clockwork, as defects in SWR1 activity alter morning-  
55 expressed genes. The EC-SWR1 complex binds to the loci of the core clock genes *PSEUDO-*  
56 *RESPONSE REGULATOR7 (PRR7)* and *PRR9* and catalyzes deposition of nucleosomes  
57 containing the histone variant H2A.Z coincident with the repression of these genes at dusk.  
58 This provides a mechanism by which the circadian clock temporally establishes repressive  
59 chromatin domains to shape oscillatory gene expression around dusk.

60

61 **Keywords** Chromatin remodeling, circadian clock, Evening Complex, H2A.Z, SWR1

62

## 63 **Introduction**

64 The circadian clock generates biological rhythms with a period of approximately 24 hours to  
65 coordinate plant growth and development with environmental cycles (Greenham and  
66 McClung, 2015). A large fraction of the *Arabidopsis* (*Arabidopsis thaliana*) transcriptome is  
67 circadian-regulated (Staiger and Green, 2011). Circadian transcription allows the molecular  
68 anticipation of the environmental cycles, which improves plant fitness and adaptation  
69 (Yerushalmi et al., 2011). Consistent with its adaptive function, the circadian clock is subject  
70 to multiple layers of regulation, which contribute towards accurate oscillations (Seo and Mas,  
71 2014).

72         Transcriptional regulation of circadian genes is a basic framework of clock  
73 architecture (Carre and Kim, 2002; Salome and McClung, 2004). The *Arabidopsis* central  
74 oscillator consists of a series of sequential regulatory loops composed of genes expressed at  
75 different times during the diurnal cycle. Morning-expressed genes such as *CIRCADIAN*  
76 *CLOCK-ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) repress the  
77 expression of evening-expressed genes like *TIMING OF CAB EXPRESSION 1/PSEUDO-*  
78 *RESPONSE REGULATOR 1* (*TOC1/PRR1*) (Alabadi et al., 2001), while in turn the TOC1  
79 protein represses *CCA1* and *LHY* during the night (Alabadi et al., 2001; Gendron et al., 2012;  
80 Huang et al., 2012; Pokhilko et al., 2013). Repression of *CCA1* and *LHY* throughout the day  
81 also occurs by the sequential action of additional members of the PRR family, including  
82 *PRR9*, *PRR7*, and *PRR5* (Nakamichi et al., 2010; Salome et al., 2010). Additional repressors  
83 of morning gene expression include the components of the Evening Complex (EC), *EARLY*  
84 *FLOWERING 3* (*ELF3*), *ELF4*, and *LUX ARRHYTHMO/PHYTOCLOCK 1* (*LUX/PCL1*)  
85 (Nusinow et al., 2011; Chow et al., 2012; Herrero et al., 2012).

86         The EC is a core component of the circadian clock, and *elf3* mutants are arrhythmic

87 under continuous light (Hicks et al., 1996; Lu et al., 2012). By binding to the promoters of  
88 hundreds of key regulators of circadian clock, photosynthesis, and phytohormone signaling,  
89 the EC is able to repress their expression (Ezer et al., 2017). Because the activity of the EC is  
90 reduced at warmer temperatures, and environmental sensing phytochromes co-bind target  
91 promoters, the EC is able to integrate environmental information into endogenous  
92 developmental programs (Ezer et al., 2017). However, the molecular mechanisms by which  
93 the EC represses gene expression are not known.

94 Histone variants influence chromatin structure and therefore transcription. H2A.Z is  
95 the most well-conserved histone variant, enriched near transcription start sites (TSSs)  
96 (Raisner et al., 2005; Raisner and Madhani, 2006) and influencing transcriptional activities of  
97 associated genes (Raisner et al., 2005). Effects of H2A.Z deposition are likely variable  
98 depending on chromatin context: H2A.Z deposition at promoters prevents the spread of  
99 heterochromatin in yeast and is associated with transcriptional inducibility (Guillemette et al.,  
100 2005), whereas in metazoans, H2A.Z has been shown to play a role in heterochromatin  
101 formation and maintenance (Rangasamy et al., 2003; Swaminathan et al., 2005). In plants,  
102 H2A.Z-nucleosomes confer transcriptional competence (Deal et al., 2007; To and Kim, 2014),  
103 and also appear to wrap DNA more tightly, facilitating inducible gene expression (Kumar and  
104 Wigge, 2010; Coleman-Derr and Zilberman, 2012).

105 The Arabidopsis genome encodes putative homologs of catalytic subunits of the  
106 Swi2/Snf2-Related (SWR1) / Swi2/Snf2-Related CBP Activator Protein (SRCAP) complex  
107 responsible for H2A.Z deposition, including PHOTOPERIOD-INDEPENDENT EARLY  
108 FLOWERING 1 (PIE1), ACTIN-RELATED PROTEIN 6 (ARP6), and SERRATED LEAVES  
109 AND EARLY FLOWERING (SEF) (March-Diaz and Reyes, 2009). The SWR1 complex  
110 associates extensively with chromatin and catalyzes H2A.Z exchange at genomic levels.

111 Consistently, SWR1-mediated chromatin remodeling is involved in diverse aspects of plant  
112 physiology and development, such as the floral transition, immune responses, and temperature  
113 sensing (Noh and Amasino, 2003; Deal et al., 2005; Kumar and Wigge, 2010). Here, we  
114 report that the EC associates with the SWR1 complex to repress the evening transcriptome.  
115 As a part of its biological impact, this complex shapes circadian oscillations by targeting  
116 clock genes such as *PRR7* and *PRR9* for H2A.Z deposition and gene repression. These results  
117 indicate that diurnal H2A.Z deposition provides a mechanism contributing to circadian gene  
118 expression in Arabidopsis.

119

## 120 **Results**

### 121 **ELF3 stabilizes nucleosome architecture at EC target genes**

122 To understand how the EC functions, we created a stringent list of direct EC targets  
123 (Supplemental Table S1), defined as genes whose promoters are bound by at least two EC  
124 proteins and which are mis-expressed at the end of the day in *elf3-1* (Ezer et al., 2017).  
125 Previously, we have seen that EC targets show the same pattern of mis-expression in both  
126 *elf3-1* and *lux-4*, when compared to wild type over a 24 h time course (Ezer et al., 2017). In  
127 both cases, there is minimal deviation from wild-type gene expression during the day, but  
128 maximal deviation during the evening and night-time, coinciding with the activity of the EC  
129 (Huang and Nusinow, 2016). Furthermore, the fold-increase in expression in *elf3-1* and *lux-4*  
130 is also conserved between target genes (Ezer et al., 2017). This indicates that the EC  
131 components control target gene expression possibly altogether. Thus, we next asked how the  
132 EC globally controls endogenous gene expression programs.

133         Since chromatin accessibility, which is related to gene responsiveness, is usually  
134 coordinated with transcriptional regulation, we investigated if EC targets have a distinctive

135 nucleosome structure, and if this is perturbed in *elf3-1*. Micrococcal nuclease (MNase)  
136 produces double-stranded cuts between nucleosomes, thus providing a simple method for  
137 obtaining information on the locations and arrangements of nucleosomes (Shu et al., 2013).  
138 We investigated the genome accessibility of the EC target loci at a range of time points using  
139 MNase digestion coupled with sequencing (MNase-seq). We observed high nucleosome  
140 occupancy for EC target genes (Fig. 1A-F), and this increased at ZT8 and ZT12 (Fig. 1B, C, E,  
141 and F), coinciding with maximal EC activity. Notably, a marked increase in chromatin  
142 accessibility was observed in the *elf3-1* mutant (Fig. 1B, C, E, and F), consistent with the EC  
143 repression of gene expression. The nucleosome occupancy in *elf3-1* was most similar to wild  
144 type at ZT0 (Fig. 1A and D), but decreased as the day progresses, showing the greatest loss at  
145 ZT12, by which time the gene body nucleosome occupancy was barely detectable (Fig. 1C  
146 and F). These results suggest that EC-dependent gene repression is induced in part by  
147 temporal stabilization of repressive chromatin structures.

148

#### 149 **Components of the SWR1 complex are necessary for EC function**

150 To identify genes mediating the connection between the EC and chromatin architecture, we  
151 surveyed the DIURNAL dataset (<http://diurnal.mocklerlab.org>) for transcripts having a  
152 similar expression pattern to the EC. Transcripts encoding histone variants as well as SWR1  
153 components required for H2A.Z deposition were included, and they were regulated in diurnal  
154 and circadian patterns (Supplemental Fig. S1A). To confirm these observations, we examined  
155 the circadian expression of genes associated with H2A.Z exchange by reverse transcription  
156 quantitative PCR (RT-qPCR) analysis. Genes encoding the SWR1 components, including  
157 *ARP6*, *PIE1*, and *SEF*, displayed circadian oscillation under free-running conditions with a  
158 peak around dusk (Fig. 2A-C), similar to *ELF3* (Fig. 2D). The transcript accumulation of

159 histone variant genes, *HTA8*, *HTA9*, and *HTA11*, however did not significantly oscillate during  
160 the circadian cycle in our conditions (Supplemental Fig. S1B).

161 To determine if H2A.Z-nucleosomes are important for EC function, we analyzed  
162 whether EC targets are mis-expressed in *arp6-1*, which is compromised in its ability to  
163 incorporate H2A.Z-nucleosomes (Kumar and Wigge, 2010). Expression of EC target genes  
164 over a diurnal cycle was analyzed using RNA-seq. These genes in this cluster showed a  
165 pattern of increased expression at the end of day and early night in *arp6-1* compared to wild  
166 type (Fig. 3A), a pattern consistent with reduced EC function in *arp6-1*.

167 Since warm temperature reduces the ability of the EC to associate with target  
168 promoters (Box et al., 2015; Ezer et al., 2017), we investigated how these genes respond to  
169 27°C. As expected, EC targets were up-regulated in wild type at 27°C particularly around  
170 evening and night time (Fig. 3B), reflecting impaired EC function. This pattern was however  
171 strongly enhanced by the *arp6-1* mutation (Fig. 3B). These results indicate that the EC and  
172 H2A.Z-nucleosomes function to repress EC targets.

173

#### 174 **ELF3 physically interacts with SEF**

175 Having established a connection between the EC-dependent gene repression and H2A.Z-  
176 nucleosomes, we sought to investigate if there might be a direct physical interaction between  
177 the components involved in these processes. To test this possibility, we performed yeast-two-  
178 hybrid (Y2H) assays. Constructs of components of the SWR1 complex, ACTIN-RELATED  
179 PROTEIN 4 (ARP4), ARP6, SEF, SWR COMPLEX PROTEIN 2 (SWC2) and SWC5, fused  
180 with GAL4 DNA-binding domain (BD) and evening-expressed clock components fused with  
181 GAL4 activation domain (AD) were co-expressed in yeast cells. Cell growth on selective  
182 medium revealed that SEF interacts specifically with ELF3 (Fig. 4A and Supplemental Fig.

183 S2). Although ELF4 is also a member of the EC (Huang et al., 2016), we did not observe  
184 interactions of SEF with ELF4 in yeast cells (Fig. 4A). Since the PHOTOPERIOD-  
185 INDEPENDENT EARLY FLOWERING 1 (PIE1) protein is a catalytic core of SWR1  
186 complex, we also examined interactions between PIE1 and EC components. We had  
187 difficulties cloning the full-length PIE1 and instead assayed domains of PIE1. Our results  
188 showed that the SANT domain of PIE1 was able to interact with ELF3 (Supplemental Fig.  
189 S3). Together, the results indicate that PIE1, possibly along with SEF, participate in the  
190 physical interactions with ELF3.

191 To confirm the physical interaction *in vivo*, we carried out bimolecular fluorescence  
192 complementation (BiFC) assays using Arabidopsis protoplasts. The *SEF* cDNA sequence was  
193 fused in-frame to the 5'-end of a gene sequence encoding the N-terminal half of YFP, and the  
194 *ELF3* gene was fused in-frame to the 5'-end of a sequence encoding the C-terminal half of  
195 YFP. The fusion constructs were then transiently co-expressed in Arabidopsis protoplasts.  
196 Strong yellow fluorescence was detected in the nucleus of cells co-expressing the  
197 combination of SEF-ELF3 (Fig. 4B), while co-expression with empty vectors did not show  
198 visible fluorescence (Fig. 4B). To quantify the physical interaction, split Luciferase (Luc)  
199 assay was also employed. ELF3 was fused with the amino part of Luc (NLuc), and SEF was  
200 fused with carboxyl-part of Luc (CLuc) (Fig. 4C). Co-expression of ELF3-NLuc and SEF-  
201 CLuc in Arabidopsis protoplasts resulted in 2-fold increase of Luc activity, while co-  
202 expression of controls showed background-level Luc activities (Fig. 4C). The *in planta*  
203 interactions of ELF3 and SEF were confirmed by coimmunoprecipitation (Co-IP) assays  
204 using *Nicotiana benthamiana* cells transiently coexpressing 35S:*ELF3-GFP* and 35S:*SEF-*  
205 *MYC* fusion constructs. Because the full-length ELF3 protein is a large protein, it is difficult  
206 to express transiently in *N. benthamiana*. As an alternative, we designed fragments of ELF3

207 fused with GFP and used them to test physical interactions with SEF (Fig. 4D). Co-IP analysis  
208 revealed that the C-terminal region of ELF3 was responsible for interactions with SEF *in*  
209 *planta* (Fig. 4D). These results indicate that the EC directly interacts with the SWR1 complex,  
210 and this may facilitate the direct deposition of H2A.Z-nucleosomes at EC target genes.

211

### 212 **H2A.Z-nucleosomes are deposited at EC target loci**

213 Since EC target genes are mis-regulated in *arp6-1* and ELF3 and SEF interact directly, we  
214 investigated if H2A.Z-nucleosomes are enriched at EC target genes. HTA11 occupancy,  
215 assayed by ChIP-seq (Cortijo et al., 2017), showed a strong enrichment across the gene body  
216 of ELF3 targets (Fig. 5A). The occupancy was particularly high at the region surrounding the  
217 TSS at the presumptive +1 nucleosome, but was also markedly higher over the gene body of  
218 ELF3 targets (Fig. 5A). By comparison, randomly selected control genes showed H2A.Z  
219 enrichment around the TSS only (Fig. 5B and Supplemental Table S2).

220 We then compared the genome-wide association of ELF3 with H2A.Z. Consistent  
221 with the fact that the EC is recruited to target sites via LUX binding sites and G-box motifs  
222 (Ezer et al., 2017), we observed that these motifs were strongly enriched at ELF3-binding  
223 sites (Ezer et al., 2017). Notably, the footprint bound by ELF3 in the promoters of EC target  
224 genes, including *PRR7*, *PRR9* and *LUX*, was devoid of H2A.Z-nucleosome signal (Fig. 5C  
225 and Supplemental Fig. S4A-C), but the adjacent regions including the gene bodies were  
226 highly occupied with H2A.Z-nucleosomes (Fig. 5C and Supplemental Fig. S4A-C). This anti-  
227 phasing between H2A.Z occupancy and the EC was observed for all the EC targets (Fig. 5C  
228 and Supplemental Fig. S4A-C). H2A.Z-nucleosomes are refractory to transcription (Thakar et  
229 al., 2010), suggesting that the initial binding of the EC to the promoters of target genes may  
230 assist subsequent recruitment of H2A.Z-nucleosomes to repress EC target gene expression.

231 We thus further determined whether H2A.Z deposition is dependent on the EC. CHIP-  
232 seq assays showed that the H2A.Z occupancy was observed in EC-target genes particularly  
233 during night time (Fig. 5D and Supplemental Fig. S4D), whereas enrichment of H2A.Z-  
234 nucleosomes at those loci was compromised in the *elf3-1* mutant (Fig. 5E and Supplemental  
235 Fig. S4D). As a negative control, randomly selected control genes showed no temporal H2A.Z  
236 enrichment regardless of genetic background (Supplemental Fig. S4E). Further, we also  
237 obtained two additional lists of control genes: (1) genes bound by both ELF3 and LUX, but  
238 not up-regulated in *elf3-1* and *lux-4* mutants (Supplemental Table S3); (2) genes up-regulated  
239 in *elf3-1* and *lux-4*, but not bound by either ELF3 or LUX (Supplemental Table S4). Again,  
240 H2A.Z deposition was not diurnally regulated and influenced by ELF3 in both cases  
241 (Supplemental Figs. S5 and S6). These results indicate that the EC-SWR1 complex  
242 contributes to H2A.Z deposition at the EC target loci.

243

#### 244 **Temporal regulation of H2A.Z deposition at the *PRR7* and *PRR9* promoters underlies** 245 **proper circadian oscillation**

246 Given the essential role of the EC in the circadian clock and the direct physical and functional  
247 interaction between the EC and the SWR1 complex, SWR1 might be also important for  
248 proper circadian clock function. We used genetic mutants affected in the key SWR1 subunits,  
249 *arp6-1* and *sef-1*, and analyzed circadian function by monitoring the expression of a circadian  
250 output gene, *COLD*, *CIRCADIAN RHYTHM, AND RNA BINDING 2* (*CCR2*). RT-qPCR  
251 analysis revealed that the rhythmic amplitude of *CCR2* expression was significantly  
252 dampened in the two mutants (Supplemental Fig. S7). Similarly, the circadian expression of  
253 *CCR2* was also reduced in *hta9-1 hta11-2* double mutant (Supplemental Fig. S8), in a

254 comparable trend to that of SWR1 mutants. These results suggest that SWR1 activity and  
255 proper circadian exchange of H2A.Z-nucleosomes might be important for circadian function.

256 Next we aimed to decipher the clock factors that are direct targets of the SWR1  
257 complex. We thus employed *pHTA11:HTA11-GFP* transgenic plants and examined H2A.Z  
258 deposition at core clock gene promoters by ChIP assays with a GFP-specific antibody. ChIP-  
259 qPCR analysis showed that H2A.Z deposition specifically occurs at the gene bodies of the  
260 morning-expressed genes *PRR7* and *PRR9* (Fig. 6A), whereas other core clock genes did not  
261 show significant diurnal enrichment of the H2A.Z variant (Supplemental Fig. S9).  
262 Furthermore, H2A.Z accumulation was primarily observed around dusk at the *PRR7* and  
263 *PRR9* loci (Fig. 6A), which is consistent with the temporal expression patterns of the ELF3  
264 and SWR1 components (Fig. 2A-D).

265 We also generated transgenic plants over-expressing *SEF* (35S:*MYC-SEF*), in which  
266 the core component of the SWR1 complex, SEF, is fused in-frame to 6 copies of MYC-coding  
267 sequence. qPCR analysis following ChIP assays with an anti-MYC antibody showed that the  
268 SWR1 component binds to the *PRR7* and *PRR9* loci (Fig. 6B). These results support the  
269 specific association of SEF with the morning-expressed genes and agree with the pattern of  
270 H2A.Z deposition. H2A.Z-nucleosome deposition usually inhibits Pol II accessibility by  
271 stimulating closed chromatin formation (Kumar and Wigge, 2010). Consistent with this, Pol II  
272 recruitment was significantly increased at *PRRs* in the *sef-1* mutant (Fig. 6C), which has  
273 lower catalytic activity of H2A.Z deposition. These results indicate that H2A.Z deposition  
274 occurs around dusk at EC target loci such as *PRR7* and *PRR9* to repress their expression.

275

276 **ELF3 is necessary for H2A.Z deposition at *PRRs* in the control of circadian oscillation**

277 The SEF-ELF3 direct interaction suggests that there may be functional coordination in the  
278 temporal regulation of *PRR7* and *PRR9*. To investigate this, we employed *pELF3::ELF3-*  
279 *MYC/elf3-1* and *35S:MYC-ELF3* transgenic plants (Jang et al., 2015) and performed ChIP  
280 assays using an anti-MYC antibody. ChIP-qPCR analysis confirmed that ELF3 binds to the  
281 *PRR7* and *PRR9* loci (Fig. 7A), and this binding occurs preferentially at dusk (Supplemental  
282 Fig. S10). Consistent with the requirement of ELF4 and LUX in a functional EC, over-  
283 expression of *ELF3* still resulted in rhythmic binding.

284 Chromatin binding of ELF3 is causal for H2A.Z deposition and is independent of  
285 H2A.Z occupancy. We genetically crossed *pELF3::ELF3-MYC* transgenic plants with *arp6-1*,  
286 which lacks genome-wide H2A.Z deposition, and the *pELF3::ELF3-MYC x arp6-1* plants  
287 were used for ChIP assays using an anti-MYC antibody (Supplemental Fig. S11). As a result,  
288 ELF3 binding to *PRR* loci was comparable in both wild-type and *arp6-1* backgrounds  
289 (Supplemental Fig. S12), indicating that ELF3 binding is an active process to trigger H2A.Z  
290 deposition at cognate regions.

291 To determine the possible regulation of H2A.Z deposition by ELF3, we examined  
292 H2A.Z occupancy by ChIP-qPCR at *PRR7* and *PRR9* in *elf3-8*. As expected, H2A.Z  
293 deposition was reduced at these loci in *elf3-8*, particularly around dusk (Fig. 7B and  
294 Supplemental Fig. S13), indicating that ELF3 is both present on these promoters and  
295 facilitates the insertion or stability of repressive H2A.Z-nucleosomes (Fig. 7B). Consistently,  
296 binding of SEF was specifically observed at dusk, and its association to the *PRR7* and *PRR9*  
297 promoters occurred in an ELF3-dependent manner (Fig. 7C).

298 Consistent with the role of the EC and SWR1 complex in the repression of gene  
299 expression, the EC-SWR1 complex contributes to the declining phase of *PRR7* and *PRR9*  
300 expression during the evening and night period, when its expression is returning to basal

301 levels (Farre et al., 2005). As expected, Pol II accessibility of *PRR7* and *PRR9* was elevated at  
302 dusk in *elf3-8* (Fig. 7D). In further support of these results, expression of *PRR7* and *PRR9*  
303 was elevated during evening and night-time in *elf3-8* and H2A.Z-deficient *hta8 hta9 hta11*  
304 (*hta.z*) mutants (Fig. 7E). In addition, consistent with the repressing functions of *PRR7* and  
305 *PRR9* on *CCA1* and *LHY* (Nakamichi et al., 2010), expression of *CCA1* and *LHY* was  
306 significantly repressed during subjective morning in *elf3-8* and *hta.z* mutants (Supplemental  
307 Fig. S14). Clock-controlled *PHYTOCHROME-INTERACTING FACTOR 4* (*PIF4*) expression  
308 was also affected in *elf3-8* and *hta.z* mutants, and especially, its expression was derepressed  
309 during night period (Supplemental Fig. S14). To confirm the genetic interactions of EC and  
310 SWR1, we crossed *elf3-8* with 35S:*MYC-SEF* transgenic plants (Supplemental Fig. S15).  
311 35S:*MYC-SEF* transgenic plants displayed repressed rhythmic expression of *PRR7* and *PRR9*  
312 compared with wild-type plants, whereas arrhythmic expression was observed in *elf3-8* (Fig.  
313 7F). Notably, the 35S:*MYC-SEF/elf3-8* plants showed arrhythmicity similar to *elf3-8* (Fig. 7F),  
314 indicating that the SWR1 complex depends on the EC in the control of circadian oscillation.

315 In summary, the EC interaction with the SWR1 complex provides a mechanism to  
316 recruit transcriptionally repressive H2A.Z-nucleosomes at circadian regulated genes. In this  
317 way, targets may be repressed over the course of the night until the EC abundance declines  
318 and/or strong activation signals reactivate gene expression. In the case of circadian regulation,  
319 the *PRR7* and *PRR9* genes are under the temporal regulation of H2A.Z exchange. The EC-  
320 SWR1 complex is recruited to *PRR7* and *PRR9*, facilitating H2A.Z deposition to reduce gene  
321 expression during evening and night-time. This indicates a role for the EC in establishing  
322 circadian waves of gene expression.

323

## 324 Discussion

325 **EC-dependent coordination of chromatin structures**

326 The ELF3, ELF4, and LUX proteins form the tripartite EC complex and cooperatively  
327 regulate a variety of developmental processes. They are functionally intertwined in the gating  
328 of day-inducible genes (Hazen et al., 2005; Huang and Nusinow, 2016), and thus their genetic  
329 mutants share phenotypic alterations, such as elongated hypocotyls, early flowering, and  
330 altered circadian rhythms (Hicks et al., 2001; Hazen et al., 2005; Nusinow et al., 2011).  
331 Consistently, their binding sites largely overlap, and the EC controls target gene expression  
332 (Ezer et al., 2017). Here, we show that the EC recruits repressive chromatin domains to  
333 regulate the evening transcriptome. The EC interacts with the SWR1 complex and establishes  
334 transcriptionally-repressive H2A.Z-nucleosomes at its target genes. The high occupancy of  
335 H2A.Z-nucleosomes across the gene bodies of target genes provides an effective barrier to  
336 RNA Pol II, maintaining these genes in a repressed state.

337 Notably, mutants eliminating EC activity, like *elf3* and *lux*, exhibit an arrhythmic  
338 circadian clock. In contrast, SWR1 mutants are rhythmic but with reduced amplitude.  
339 Therefore, deposition of H2A.Z by the EC at core clock genes, such as *PRR7* and *PRR9*, is  
340 important for correct expression amplitude. However, circadian clock rhythmicity appears not  
341 to depend on this mechanism. Additionally, we cannot rule out the possibility that ELF3 may  
342 recruit additional chromatin modifiers and/or remodelers. The EC might be an important  
343 platform facilitating chromatin reconfiguration with multiple epigenetic modifications.

344 Temporal exchange of H2A.Z-nucleosomes provides a mechanism for diurnal gating  
345 of several physiological processes. For example, a majority of stress-responsive genes are  
346 gated primarily during day period (Seo and Mas, 2015), and consistently, a substantial number  
347 of stress-inducible genes that are responsive to drought, temperature, or pathogens are under  
348 the control of EC-dependent H2A.Z deposition in order to ensure their suppression during

349 night-time (Ezer et al., 2017). In addition, the circadian clock relies on a series of waves of  
350 transcriptional activation and repression. The recruitment of H2A.Z-nucleosomes provides a  
351 mechanism for the stable repression of target clock genes over the course of the night, which  
352 can be robustly activated the following day. Overall, the EC-SWR1 complex is a global  
353 transcriptional regulator that functions in the diurnal gating of many developmental and  
354 physiological processes and provides a more stable mechanism for maintaining repressive  
355 states during night-time.

356

### 357 **Chromatin-based regulation at the core of the circadian clock**

358 Time-of-day-dependent accumulation of chromatin marks such as H3ac and H3K4me3 occurs  
359 with the circadian transcript abundance of clock genes including *CCA1*, *LHY*, *TOC1*, *PRR7*,  
360 *PRR9*, and *LUX* (Hsu et al., 2013; Voss et al., 2015). Mechanistically, H3ac stimulates an  
361 open chromatin conformation (Song and Noh, 2012), whereas H3K4me3 inhibits the binding  
362 of clock repressor proteins to the core clock gene promoters, avoiding advanced circadian  
363 repressor binding (Malapeira et al., 2012). The SDG2/ATXR3 histone methyltransferase  
364 contributes to the H3K4me3 accumulation and thus controls the timing of clock gene  
365 expression, from activation to repression (Malapeira et al., 2012). Several additional  
366 chromatin modifiers, including HISTONE DEACETYLASE 6 (HDA6), HDA19, and  
367 JUMONJI C DOMAIN-CONTAINING PROTEIN 30/JUMONJI DOMAIN CONTAINING 5  
368 (JMJ30/JMJD5), are also connected with the circadian oscillation (Jones and Harmer, 2011;  
369 Lu et al., 2011; Wang et al., 2013), although the mechanisms behind circadian gene regulation  
370 remain to be determined.

371         The epigenetic regulation of core clock genes relies on a complex web of chromatin  
372 and clock components. For example, CCA1 facilitates repressive chromatin signatures to

373 regulate *TOC1* expression around dawn while histone deacetylases contribute to the declining  
374 phase of *TOC1* (Perales and Mas, 2007). Another MYB-like transcription factor known as  
375 REVEILLE 8/LHY-CCA1-LIKE 5 (RVE8/LCL5) favors H3 acetylation at the *TOC1*  
376 promoter, most likely by antagonizing CCA1 function throughout the day (Farinas and Mas,  
377 2011).

378 In this study, we identify a repressive chromatin state that shapes the rhythmic  
379 oscillations in gene expression. Circadian H2A.Z deposition underlies normal circadian  
380 oscillation, and *PRR7* and *PRR9* are primary targets of the SWR1 complex. H2A.Z-  
381 nucleosome deposition occurs around dusk, when the SWR1 components are highly  
382 expressed, to suppress gene expression. The interaction between the SWR1 complex and  
383 ELF3 provides a direct mechanism to facilitate H2A.Z exchange at cognate regions,  
384 contributing to precise oscillations in circadian gene expression. Since the *PRR7* and *PRR9*  
385 loci are also subjected to H3ac and H3K4me3 modifications (Malapeira et al., 2012), the  
386 higher-order combination of multiple chromatin modifications ultimately shapes the circadian  
387 waveforms of gene expression throughout the day-night cycle.

388 In mammals, rhythmical H2A.Z deposition at the promoters of CLOCK:BMAL1  
389 targets has been observed, although the underlying mechanism is not known (Menet et al.,  
390 2014). This suggests that H2A.Z-nucleosomes may have a conserved function in the  
391 eukaryotic circadian clock.

392

## 393 **Materials and Methods**

### 394 **Plant materials and growth conditions**

395 *Arabidopsis* (*Arabidopsis thaliana*) (Columbia-0 ecotype) was used for all experiments unless  
396 otherwise specified. *Arabidopsis* seeds were surface sterilized and sown on 0.7% (w/v) agar

397 plates containing half strengthened Murashige and Skoog media. After 3-day stratification at  
398 4°C, the seeds were grown in a Conviron reach-in chamber with  $170 \mu\text{mol m}^{-2} \text{s}^{-1}$  light  
399 intensity and 70% humidity under short day (8 h light/16 h dark), neutral day (12 h light/12 h  
400 dark), or long day (16 h light/8 h dark) conditions at 22°C or 27°C, as indicated in figure  
401 legends. The *arp6-1*, *arp6-3*, *elf3-1*, *elf3-8*, *hta9-1hta11-2*, *pHTA11:HTA11-FLAG*,  
402 *pHTA11:HTA11-GFP*, and *sef-1* plants were previously reported (March-Diaz et al., 2007;  
403 Kumar and Wigge, 2010; Coleman-Derr and Zilberman, 2012; Rosa et al., 2013; Nitschke et  
404 al., 2016). To produce transgenic plants overexpressing the *SEF* and *ELF3* genes, a full-length  
405 cDNA was subcloned into the binary pBA002 vector under the control of the CaMV 35S  
406 promoter. *Agrobacterium tumefaciens*-mediated *Arabidopsis* transformation was then  
407 performed.

408

#### 409 **MNase-seq and ChIP-seq experiments and analysis**

410 Approximately 1 g of 9-day-old *Arabidopsis* seedlings was harvested at the time points  
411 indicated in figure legends. The harvested seedlings were ground in liquid nitrogen to fine  
412 powder. The tissue powder was fixed for 10 min with 1% (v/v) formaldehyde (SIGMA,  
413 F8775) in Buffer 1 (0.4 M sucrose, 10 mM HEPES, 10 mM  $\text{MgCl}_2$ , 5 mM  $\beta$ -mercaptoethanol,  
414 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 X protease inhibitor (Roche,  
415 11836145001)). The reaction was quenched by adding glycine to a final concentration of 127  
416 mM. The homogenate was filtered through Mira-cloth twice and centrifuged to collect the  
417 pellet. The pellet was washed in Buffer 2 (0.24 M sucrose, 10 mM Tris-HCl (pH 8.0), 10 mM  
418  $\text{MgCl}_2$ , 5 mM  $\beta$ -mercaptoethanol, 0.1 mM PMSF and 1 X protease inhibitor, 1% v/v Triton)  
419 and then spun down in Buffer 3 (1.7 M sucrose, 10 mM Tris-HCl (pH 8.0), 10 mM  $\text{MgCl}_2$ , 5

420 mM  $\beta$ -mercaptoethanol, 0.1 mM PMSF and 1 X protease inhibitor, 0.15% v/v Triton). The  
421 nuclei pellet was then resuspended in Mnase Digestion Buffer (20 mM Tris-HCl (pH 8.0), 50  
422 mM NaCl, 1 mM DTT, 0.5% (v/v) NP-40, 1 mM CaCl<sub>2</sub>, 0.5 mM PMSF, 1 X protease  
423 inhibitor) and was flash frozen in liquid nitrogen twice to break the nuclear envelope.  
424 Chromatin was digested by adding Mnase (SIGMA, N3755) to a final concentration of 0.4  
425 U/ml for 12.5 min. The reaction was terminated by adding EDTA to a final concentration of 5  
426 mM. For ChIP-seq samples, H2A.Z in transgenic line *pHTA11:HTA11-FLAG* was  
427 immunoprecipitated by anti-FLAG M2 magnetic beads (SIGMA, M8823) and then eluted  
428 with 3XFLAG peptide (Bimake, B23112). After reverse crosslinking, the DNA was purified  
429 with SPRI beads. The libraries were constructed using TruSeq ChIP Sample Preparation Kit  
430 (Illumina, IP-202-1024) according to the manufacturer's instructions. The libraries were  
431 sequenced on an Illumina Nextseq 500 platform. The raw reads obtained from the sequencing  
432 facilities were analyzed using a combination of publicly available software and in-house  
433 scripts. We first assessed the quality of reads using  
434 FastQC:([www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)). Potential adaptor  
435 contamination and low quality trailing sequences were removed using Trimmomatic (Bolger  
436 et al., 2014). Then, the reads were mapped to the TAIR10 reference genome using Bowtie2  
437 (Langmead and Salzberg, 2012). Duplicates were removed with the Picard tools  
438 (<https://github.com/broadinstitute/picard>) and the read counts was normalised by the sample's  
439 genome-wide reads coverage. Nucleosome positioning and occupancy were determined using  
440 DANPOS (Chen et al., 2013). Nucleosome and H2A.Z average binding profiles and heatmaps  
441 were generated using deepTools (Ramirez et al., 2014).

442 The ‘randomly selected control genes’ were generated using ‘sample()’ function in R.  
443 The indices of the 52 genes were generated by the R code ‘index <- sample(33557, 52)’,  
444 where 33557 is the total number of Arabidopsis genes. The corresponding AGI gene names  
445 were generated by the R code ‘random52 <- all\_gene\_names[index,]’. In addition, we also  
446 obtained two additional lists of control genes with new filters: 1) genes bound by both ELF3  
447 and LUX, but not up-regulated in *elf3-1* and *lux-4* mutants (Supplemental Table S3), and 2)  
448 genes up-regulated in *elf3-1* and *lux-4*, but not bound by either ELF3 or LUX (Supplemental  
449 Table S4). The filter criteria for the former gene list are: A) having both ELF3 and LUX  
450 binding within 1000 bp upstream of the gene; and B) the log fold changes of TPM values  
451 compared with Col-0 at ZT16 are smaller than 0.5-fold in both *elf3-1* and *lux-4* mutants. The  
452 filter criteria for the latter gene list are : A) having no ELF3 or LUX binding within 1000 bp  
453 upstream of the gene; and B) the log fold changes of TPM values compared with Col-0 at  
454 ZT16 are larger than 1.5-fold in both *elf3-1* and *lux-4* mutants.

455

#### 456 **RNA-seq experiment and analysis**

457 Approximately 30 mg of 7-day-old Arabidopsis seedlings were harvested and their total RNA  
458 was extracted using the MagMAX-96 Total RNA Isolation kit (Ambion, AM1830) according  
459 to manufacturer’s instructions. Library preparation was performed using 1 µg of high integrity  
460 total RNA (RIN>8) using the TruSeq Stranded mRNA library preparation kit (Illumina, RS-  
461 122-2103) according to the manufacturer’s instructions. The libraries were sequenced on an  
462 Illumina Nextseq 500 platform.

463 For bioinformatics analysis, we first assessed the quality of reads using FastQC:  
464 ([www.bioinformatics.babraham.ac.uk/projects/fastqc/](http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)). Potential adaptor contamination and  
465 low quality trailing sequences were removed using Trimmomatic (Bolger et al., 2014), before

466 alignment to the TAIR10 transcriptome using Tophat (Trapnell et al., 2009). Potential optical  
467 duplicates resulting from library preparation were removed using the Picard tools  
468 (<https://github.com/broadinstitute/picard>), and the read counts were normalized by the  
469 sample's genome-wide reads coverage. Raw counts were determined by HTseq-count (Anders  
470 et al., 2015), and cufflinks was utilized to calculate Fragments Per Kilobase Million (FPKM),  
471 which was then converted into Transcripts Per Million (TPM).

472

### 473 **Reverse transcription quantitative PCR (RT-qPCR) analysis**

474 Total RNA was extracted using TRI reagent (TAKARA Bio, Singa, Japan) according to the  
475 manufacturer's recommendations. Reverse transcription (RT) was performed using Moloney  
476 Murine Leukemia Virus (M-MLV) reverse transcriptase (Dr. Protein, Seoul, South Korea)  
477 with oligo(dT18) to synthesize first-strand cDNA from 2 µg of total RNA. Total RNA samples  
478 were pretreated with an RNase-free DNase. cDNAs were diluted to 100 µL with TE buffer,  
479 and 1 µL of diluted cDNA was used for PCR amplification.

480 RT-qPCR reactions were performed in 96-well blocks using the Step-One Plus Real-  
481 Time PCR System (Applied Biosystems). The PCR primers used are listed in Supplemental  
482 Table S5. The values for each set of primers were normalized relative to the *EUKARYOTIC*  
483 *TRANSLATION INITIATION FACTOR 4A1* (*eIF4A*) gene (At3g13920). All RT-qPCR  
484 reactions were performed with biological triplicates using total RNA samples extracted from  
485 three independent replicate samples. The comparative  $\Delta\Delta\text{CT}$  method was employed to  
486 evaluate relative quantities of each amplified product in the samples. The threshold cycle  
487 (CT) was automatically determined for each reaction with the analysis software set using

488 default parameters. The specificity of the RT-qPCR reactions was determined by melting  
489 curve analysis of the amplified products using the standard method employed by the software.

490

#### 491 **Yeast two-hybrid assays**

492 Yeast two-hybrid (Y2H) assays were performed using the BD Matchmaker system (Clontech,  
493 Mountain View, CA, USA). The pGADT7 vector was used for the GAL4 AD fusion, and the  
494 pGBKT7 vector was used for GAL4 BD fusion. The yeast strain AH109 harboring the LacZ  
495 and His reporter genes was used. PCR products were subcloned into the pGBKT7 and  
496 pGADT7 vectors. The expression constructs were co-transformed into yeast AH109 cells and  
497 transformed cells were selected by growth on SD/-Leu/-Trp medium and SD/-Leu/-Trp/-His/-  
498 Ade. Interactions between proteins were analyzed by measuring  $\beta$ -galactosidase ( $\beta$ -Gal)  
499 activity using o-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) as a substrate.

500

#### 501 **Bimolecular fluorescence complementation (BiFC) assays**

502 The *ELF3* gene was fused in-frame to the 5' end of a gene sequence encoding the C-terminal  
503 half of EYFP in the pSATN-cEYFP-C1 vector (E3082). The *SEF* cDNA sequence was fused  
504 in-frame to the 5' end of a gene sequence encoding the N-terminal half of EYFP in the  
505 pSATN-nEYFP-C1 vector (E3081). Expression constructs were co-transformed into  
506 Arabidopsis protoplasts. Expression of the fusion constructs was monitored by fluorescence  
507 microscopy using a Zeiss LSM510 confocal microscope (Carl Zeiss, Jena, Germany).

508

#### 509 **Chromatin immunoprecipitation (ChIP) assays**

510 The epitope-tagged transgenic plant samples were cross-linked with 1% (v/v) formaldehyde,  
511 ground to powder in liquid nitrogen, and then sonicated. The sonicated chromatin complexes

512 were bound with corresponding antibodies. Anti-MYC (05-724, Millipore, Billerica, USA),  
513 anti-Pol II (sc-33754, Santa Cruz, Dallas, Texas, USA), anti-H2A.Z antibodies (ab4174,  
514 Abcam, Cambridge, UK), and salmon sperm DNA/protein A agarose beads (16-157,  
515 Millipore, Billerica, USA) were used for chromatin immunoprecipitation. DNA was purified  
516 using phenol/chloroform/isoamyl alcohol and sodium acetate (pH 5.2). The level of  
517 precipitated DNA fragments was quantified by quantitative PCR (qPCR) using specific  
518 primer sets (Supplemental Table S6). Values were normalized according to input DNA levels.  
519 Values for control plants were set to 1 after normalization against *eIF4a* for qPCR analysis.

520

### 521 **Split-luciferase assays**

522 The coding regions of *SEF* and *ELF3* were cloned into pcFLucC or pcFLucN vector. The  
523 recombinant constructs were cotransformed with into *Arabidopsis* protoplasts by polyethylene  
524 glycol-mediated transformation. The *pUBQ10::GUS* plasmid was also cotransformed as an  
525 internal control to normalize the LUC activity.

526

### 527 **Coimmunoprecipitation (Co-IP) assays**

528 *Agrobacterium tumefaciens* cells containing 35S:*SEF-MYC* and 35S:*ELF3-GFP* constructs  
529 were infiltrated to 3-week-old *Nicotiana benthamiana* leaves. *N. benthamiana* leaves were  
530 homogenized in protein extraction buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% v/v  
531 glycerol, 0.05% w/v Nonidet P-40, 2.5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and  
532 1 × complete cocktail of protease inhibitors). After protein extraction, anti-MYC antibodies  
533 (05-724, Millipore, Billerica, MA, USA) coupled to Protein-A sepharose beads (Sigma-  
534 Aldrich, St Louis, MO, USA) were mixed and incubated for 4 h at 4 °C. The precipitated  
535 samples were washed at least four times with the protein extraction buffer and then eluted by

536 1 × SDS–polyacrylamide gel electrophoresis (PAGE) loading buffer to perform SDS–PAGE  
537 with anti-MYC (1:2000 dilution; Millipore) or anti-GFP antibodies (1:1,000 dilution; sc-9996,  
538 Santa Cruz Biotech., Dallas, Texas, USA).

539

#### 540 **Statistical analysis**

541 Unless otherwise specified, quantitative data are presented as mean ± SD and significance  
542 was assessed by the two-tailed Student's *t* test.

543

#### 544 **Accession numbers**

545 The raw sequencing data reported in this work have been deposited in the NCBI Gene  
546 Expression Omnibus under accession number GSE109101.

547

#### 548 **Supplemental Data**

549 The following supplemental materials are available in the online version of this article.

550 **Supplemental Figure S1.** Diurnal expression of H2A.Z-related genes.

551 **Supplemental Figure S2.** Yeast-two-hybrid assays.

552 **Supplemental Figure S3.** Interaction of ELF3 with PIE1.

553 **Supplemental Figure S4.** H2A.Z occupancy on EC-target genes.

554 **Supplemental Figure S5.** H2A.Z enrichment at genes bound by both ELF3 and LUX, but not  
555 differentially expressed in *elf3-1* and *lux-4* mutants.

556 **Supplemental Figure S6.** H2A.Z enrichment at genes up-regulated in *elf3-1* and *lux-4*, but  
557 not bound by either ELF3 or LUX.

558 **Supplemental Figure S7.** Reduced rhythmic amplitude in genetic mutants of SWR1  
559 components.

560 **Supplemental Figure S8.** Influences on circadian clock in *hta9-1hta11-2*.  
561 **Supplemental Figure S9.** Deposition of H2A.Z at clock gene promoters.  
562 **Supplemental Figure S10.** Binding of ELF3 to the *PRR7* and *PRR9* promoters at ZT12.  
563 **Supplemental Figure S11.** Protein accumulation of ELF3 in *pELF3::ELF3-MYC* and  
564 *pELF3::ELF3-MYCxarp6-1*.  
565 **Supplemental Figure S12.** ELF3 binding to the *PRR* loci in *arp6-1* background.  
566 **Supplemental Figure S13.** H2A.Z deposition in *elf3-8* mutant throughout a day.  
567 **Supplemental Figure S14.** Expression of *CCA1*, *LHY*, and *PIF4* in *hta.z* and *elf3-8* mutants.  
568 **Supplemental Figure S15.** Protein accumulation of SEF in *35S:MYC-SEF* and *35S:MYC-*  
569 *SEFxelf3-8*.  
570 **Supplemental Table S1.** EC target gene list.  
571 **Supplemental Table S2.** 52 Randomly selected control genes.  
572 **Supplemental Table S3.** List of genes bound by both ELF3 and LUX, but not differentially  
573 expressed in *elf3-1* and *lux-4* mutants.  
574 **Supplemental Table S4.** List of genes up-regulated in *elf3-1* and *lux-4*, but not bound by  
575 either ELF3 or LUX.  
576 **Supplemental Table S5.** Primers used in RT-qPCR analysis.  
577 **Supplemental Table S6.** Primers used in ChIP assays.

578

579 **Acknowledgements:** We thank Dr. Hui Lan for bioinformatics data analysis. This work was  
580 supported by the Basic Science Research (NRF-2019R1A2C2006915) and Basic Research  
581 Laboratory (NRF-2017R1A4A1015620) programs provided by the National Research  
582 Foundation of Korea and by the Next-Generation BioGreen 21 Program (PJ01314501)  
583 provided by the Rural Development Administration to P.J.S. P.M. laboratory is funded by the

584 FEDER/Spanish Ministry of Economy and Competitiveness, by the Ramon Areces  
585 Foundation and by the Generalitat de Catalunya (AGAUR). P.M. laboratory also  
586 acknowledges financial support from the CERCA Program/Generalitat de Catalunya and by  
587 the Spanish Ministry of Economy and Competitiveness through the “Severo Ochoa Program  
588 for Centers of Excellence in R&D” 2016–2019 (SEV-2015-0533). Work in the lab of P.A.W.  
589 is supported by the Gatsby Foundation (GAT3273/GLB).

590

591

## 592 **Conflict of interest**

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

594

## 595 **Figure legends**

596 **Figure 1. Nucleosome occupancy in Evening Complex (EC) target genes in Col-0 and**  
597 ***elf3-1* at ZT0, 8, and 12.**

598 Average nucleosome signals (normalized Mnase-seq reads) on 52 EC target genes (see also  
599 Supplemental Table S1) at Zeitgeber Time 0 (ZT0), ZT8, and ZT12 (**A-C**) in Col-0 and *elf3-1*  
600 grown under long day conditions (LD). Heat map visualization of nucleosome signals over 52  
601 EC target genes on Col-0 and *elf3-1* at ZT0, ZT8, and ZT12 (**D-F**). Data are plotted from 1kb  
602 upstream of TSS to 1kb downstream of TES of EC target genes. Graphs show the results of  
603 two replicates. TSS: Transcription Start Site; TES: Transcription End Site.

604

605 **Figure 2. Coexpression of the SWR1 components with EC.**

606 **A-D** Circadian expression of SWR1 components and *ELF3*. Seedlings grown under neutral  
607 day conditions (ND, 12h light: 12h dark) for 2 weeks were transferred to continuous light

608 conditions (LL) at Zeitgeber Time 0 (ZT0). Whole seedlings were harvested from ZT24 to  
609 ZT68 to analyze transcript accumulation. Transcript levels of *ARP6* (A), *PIE1* (B), *SEF* (C),  
610 and *ELF3* (D) were determined by reverse transcription quantitative PCR (RT-qPCR). Gene  
611 expression values were normalized to the *EUKARYOTIC TRANSLATION INITIATION*  
612 *FACTOR 4A1* (*eIF4A*) expression. Biological triplicates were averaged. Bars represent the  
613 standard error of the mean. The white and grey boxes indicate the subjective day and night,  
614 respectively.

615

616 **Figure 3. Elevated expression of EC target genes in *arp6-1*.**

617 Heat map visualization of log fold changes of the expression levels of EC target genes in  
618 *arp6-1* at 22°C compared with Col-0 at 22°C grown under LDs (A) and in *arp6-1* at 27°C  
619 compared with Col-0 at 22°C grown under short days (SDs) (B). Values in (A) and (B)  
620 represent  $\log_2$  (TPM in *arp6-1* 22°C / TPM in Col-0 22°C) and  $\log_2$  (TPM in *arp6-1* 27°C /  
621 TPM in Col-0 22°C), respectively. The log fold change of the expression level of each gene  
622 was calculated by Z-score (mean = 0, standard deviation = 1). The heat map was generated  
623 using heatmap.2 function in R (version 3.2.5).

624

625 **Figure 4. Interactions of the SWR1 complex with EC.**

626 A yeast-two-hybrid (Y2H) assays. Y2H assays were performed with the SEF protein fused to  
627 the DNA-binding domain (BD) of GAL4 and evening-expressed clock components fused  
628 with the transcriptional activation domain (AD) of GAL4 for analysis of interactions.  
629 Interactions were examined by cell growth on selective media. -LWHA indicates Leu, Trp,  
630 His, and Ade drop-out plates. -LW indicates Leu and Trp drop-out plates. GAL4 was used as a  
631 positive control (P).

632 **B** BiFC assays. Partial fragments of YFP protein were fused with SEF and ELF3, and co-  
633 expressed in Arabidopsis protoplasts. Reconstituted fluorescence was examined by confocal  
634 microscopy. IDD14-RFP was used as a nucleus marker. Bars: 10  $\mu$ m.

635 **C** Interaction of ELF3-NLuc with SEF-CLuc. Partial fragments of Luciferase (NLuc and  
636 CLuc) were fused with ELF3 or SEF. The fusion constructs were coexpressed in Arabidopsis  
637 protoplasts and Luc activities were measured and normalized against total protein. Three  
638 independent biological replicates were averaged and statistically analyzed with Student's *t*-  
639 test ( $*P < 0.05$ ). Bars indicate the standard error of the mean.

640 **D** Co-IP assays. *Agrobacterium tumefaciens* cells containing 35S:*ELF3*<sup>N(1-345aa)</sup>-*GFP*,  
641 35S:*ELF3*<sup>C(346-695aa)</sup>-*GFP*, and 35S:*SEF-MYC* constructs were coinfiltrated to 3-week-old *N.*  
642 *benthamiana* leaves. Epitope-tagged proteins were detected immunologically using  
643 corresponding antibodies.

644

645 **Figure 5. ELF3 and H2A.Z occupancy of EC target genes.**

646 **A, B** H2A.Z enrichment (normalized HTA11-FLAG ChIP-seq reads) on 52 EC target genes  
647 (**A**) and control genes (**B**) was analyzed. For the class of 'control genes', a sample of 52 genes  
648 was randomly selected to compare their H2A.Z occupancy with EC target genes (see also  
649 Supplemental Table S2). (**A**) and (**B**) are plotted from 1kb upstream of the TSS to 1kb  
650 downstream of the TES of the corresponding genes.

651 **C** ELF3 and H2A.Z average binding plot on 52 EC target genes.

652 **D, E** H2A.Z enrichment in wild type (**D**) and *elf3-1* mutant (**E**) at various time points under  
653 LD conditions.

654

655 **Figure 6. H2A.Z deposition at *PRR7* and *PRR9* loci by the SWR1 complex.**

656 In (A) to (C), fragmented DNA was eluted from the protein-DNA complexes and used for  
657 qPCR analysis. Enrichment was normalized relative to *eIF4A*. Three independent biological  
658 replicates were averaged, and the statistical significance of the measurements was determined.  
659 Bars indicate the standard error of the mean.

660 **A** Accumulation of H2A.Z at clock gene loci. Two-week-old plants grown under ND were  
661 used for ChIP analysis with anti-GFP antibody. Gene structures are presented (upper panel).  
662 Underbars represent the amplified genomic regions. Statistically significant differences  
663 between ZT0 and ZT12 samples are indicated by asterisks (Student's *t*-test, \**P* < 0.05, \*\**P* <  
664 0.01).

665 **B** Binding of SEF to clock gene promoters. Two-week-old 35S:*MYC-SEF* transgenic plants  
666 grown under ND were harvested at ZT12. Statistically significant differences between wild-  
667 type and 35S:*MYC-SEF* plants are indicated by asterisks (Student's *t*-test, \**P* < 0.05).

668 **C** Recruitment of Pol II at *PRRs* in *sef-1*. Two-week-old plants grown under ND were  
669 harvested at ZT12 and used for ChIP analysis with an anti-N-terminus of Arabidopsis Pol II  
670 antibody. qPCR was performed with a primer pair amplifying B region of each gene promoter  
671 (see also **Fig. 6A**). Statistically significant differences between WT and *sef-1* plants are  
672 indicated by asterisks (Student's *t*-test, \**P* < 0.05).

673

674 **Figure 7. H2A.Z exchange at *PRR7* and *PRR9* loci by ELF3.**

675 **A** Binding of ELF3 to *PRR* promoters. Two-week-old *pELF3::ELF3-MYC/elf3-1* seedlings  
676 grown under ND were harvested at ZT12 and used to conduct ChIP assays. Statistically  
677 significant differences between Col-0 and *pELF3::ELF3-MYC/elf3-1* plants are indicated by  
678 asterisks (Student's *t*-test, \**P* < 0.05, \*\*\**P* < 0.001).

679 **B** H2A.Z deposition at clock gene promoters in *elf3-8*. Two-week-old plants grown under ND  
680 were used for ChIP analysis with anti-H2A.Z antibody. Statistically significant differences  
681 between Col-0 and *elf3-8* plants are indicated by asterisks (Student's *t*-test, \**P* < 0.05, \*\*\**P* <  
682 0.001).

683 **C** SEF binding to the *PRR* loci in *elf3-8* background. Two-week-old plants grown under ND  
684 were used for ChIP analysis with anti-MYC antibody. Statistically significant differences  
685 between ZT0 and ZT12 samples are indicated by asterisks (Student's *t*-test, \**P* < 0.05).

686 **D** Recruitment of Pol II at clock gene promoters in *elf3-8*. Two-week-old plants grown under  
687 ND were harvested at ZT12 and used for ChIP analysis with an anti-N-terminus of  
688 Arabidopsis Pol II antibody. In (A) to (D), fragmented DNA was eluted from the protein-DNA  
689 complexes and used for qPCR analysis. Enrichment was normalized relative to *eIF4A*. Three  
690 independent biological replicates were averaged, and the statistical significance of the  
691 measurements was determined by Student's *t*-test (\**P* < 0.05). Bars indicate the standard error  
692 of the mean.

693 **E** Circadian expression of *PRR7* and *PRR9* in *elf3-8* and *h2a.z*.

694 **F** *PRR* expression in 35S:MYC-SEF/*elf3-8*. In (E) and (F), seedlings grown under ND  
695 conditions for 2 weeks were transferred to LL at ZT0.

696

#### 697 LITERATURE CITED

698 **Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA** (2001) Reciprocal regulation between  
699 TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science* **293**: 880-883

700 **Anders S, Pyl PT, Huber W** (2015) HTSeq--a Python framework to work with high-throughput sequencing  
701 data. *Bioinformatics* **31**: 166-169

702 **Bolger AM, Lohse M, Usadel B** (2014) Trimmomatic: a flexible trimmer for Illumina sequence data.  
703 *Bioinformatics* **30**: 2114-2120

704 **Box MS, Huang BE, Domijan M, Jaeger KE, Khattak AK, Yoo SJ, Sedivy EL, Jones DM, Hearn TJ, Webb**  
705 **AA, Grant A, Locke JC, Wigge PA** (2015) ELF3 controls thermoresponsive growth in Arabidopsis.  
706 *Curr Biol* **25**: 194-199

707 **Carre IA, Kim JY** (2002) MYB transcription factors in the Arabidopsis circadian clock. *J Exp Bot* **53**: 1551-  
708 1557

709 **Chen K, Xi Y, Pan X, Li Z, Kaestner K, Tyler J, Dent S, He X, Li W** (2013) DANPOS: dynamic analysis of  
710 nucleosome position and occupancy by sequencing. *Genome Res* **23**: 341-351

711 **Chow BY, Helfer A, Nusinow DA, Kay SA** (2012) ELF3 recruitment to the PRR9 promoter requires other  
712 Evening Complex members in the Arabidopsis circadian clock. *Plant Signal Behav* **7**: 170-173

713 **Coleman-Derr D, Zilberman D** (2012) Deposition of histone variant H2A.Z within gene bodies regulates  
714 responsive genes. *PLoS Genet* **8**: e1002988

715 **Cortijo S, Charoensawan V, Brestovitsky A, Buning R, Ravarani C, Rhodes D, van Noort J, Jaeger KE,**  
716 **Wigge PA** (2017) Transcriptional Regulation of the Ambient Temperature Response by H2A.Z  
717 Nucleosomes and HSF1 Transcription Factors in Arabidopsis. *Mol Plant* **10**: 1258-1273

718 **Deal RB, Kandasamy MK, McKinney EC, Meagher RB** (2005) The nuclear actin-related protein ARP6 is a  
719 pleiotropic developmental regulator required for the maintenance of FLOWERING LOCUS C  
720 expression and repression of flowering in Arabidopsis. *Plant Cell* **17**: 2633-2646

721 **Deal RB, Topp CN, McKinney EC, Meagher RB** (2007) Repression of flowering in Arabidopsis requires  
722 activation of FLOWERING LOCUS C expression by the histone variant H2A.Z. *Plant Cell* **19**: 74-83

723 **Ezer D, Jung JH, Lan H, Biswas S, Gregoire L, Box MS, Charoensawan V, Cortijo S, Lai X, Stockle D,**  
724 **Zubieta C, Jaeger KE, Wigge PA** (2017) The evening complex coordinates environmental and  
725 endogenous signals in Arabidopsis. *Nat. Plants* **3**: 17087

726 **Farinas B, Mas P** (2011) Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian  
727 control of histone acetylation. *Plant J* **66**: 318-329

728 **Farre EM, Harmer SL, Harmon FG, Yanovsky MJ, Kay SA** (2005) Overlapping and distinct roles of PRR7  
729 and PRR9 in the Arabidopsis circadian clock. *Curr Biol* **15**: 47-54

730 **Gendron JM, Pruneda-Paz JL, Doherty CJ, Gross AM, Kang SE, Kay SA** (2012) Arabidopsis circadian  
731 clock protein, TOC1, is a DNA-binding transcription factor. *Proc Natl Acad Sci U S A* **109**: 3167-3172

732 **Greenham K, McClung CR** (2015) Integrating circadian dynamics with physiological processes in plants. *Nat*

733 Rev Genet **16**: 598-610

734 **Guillemette B, Bataille AR, Gevry N, Adam M, Blanchette M, Robert F, Gaudreau L** (2005) Variant histone  
735 H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome  
736 positioning. PLoS Biol **3**: e384

737 **Hazen SP, Schultz TF, Pruneda-Paz JL, Borevitz JO, Ecker JR, Kay SA** (2005) LUX ARRHYTHMO  
738 encodes a Myb domain protein essential for circadian rhythms. Proc Natl Acad Sci U S A **102**: 10387-  
739 10392

740 **Herrero E, Kolmos E, Bujdoso N, Yuan Y, Wang M, Berns MC, Uhlworm H, Coupland G, Saini R,**  
741 **Jaskolski M, Webb A, Goncalves J, Davis SJ** (2012) EARLY FLOWERING4 recruitment of EARLY  
742 FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock. Plant Cell **24**: 428-443

743 **Hicks KA, Albertson TM, Wagner DR** (2001) EARLY FLOWERING3 encodes a novel protein that regulates  
744 circadian clock function and flowering in Arabidopsis. Plant Cell **13**: 1281-1292

745 **Hicks KA, Millar AJ, Carre IA, Somers DE, Straume M, Meeks-Wagner DR, Kay SA** (1996) Conditional  
746 circadian dysfunction of the Arabidopsis early-flowering 3 mutant. Science **274**: 790-792

747 **Hsu PY, Devisetty UK, Harmer SL** (2013) Accurate timekeeping is controlled by a cycling activator in  
748 Arabidopsis. Elife **2**: e00473

749 **Huang H, Alvarez S, Bindbeutel R, Shen Z, Naldrett MJ, Evans BS, Briggs SP, Hicks LM, Kay SA,**  
750 **Nusinow DA** (2016) Identification of Evening Complex Associated Proteins in Arabidopsis by Affinity  
751 Purification and Mass Spectrometry. Mol Cell Proteomics **15**: 201-217

752 **Huang H, Nusinow DA** (2016) Into the Evening: Complex Interactions in the Arabidopsis Circadian Clock.  
753 Trends Genet **32**: 674-686

754 **Huang W, Perez-Garcia P, Pokhilko A, Millar AJ, Antoshechkin I, Riechmann JL, Mas P** (2012) Mapping  
755 the core of the Arabidopsis circadian clock defines the network structure of the oscillator. Science **336**:  
756 75-79

757 **Jang K, Lee HG, Jung SJ, Paek NC, Seo PJ** (2015) The E3 Ubiquitin Ligase COP1 Regulates Thermosensory  
758 Flowering by Triggering GI Degradation in Arabidopsis. Sci Rep **5**: 12071

759 **Jones MA, Harmer S** (2011) JMJD5 Functions in concert with TOC1 in the arabidopsis circadian system. Plant  
760 Signal Behav **6**: 445-448

761 **Kumar SV, Wigge PA** (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in

762 Arabidopsis. Cell **140**: 136-147

763 **Langmead B, Salzberg SL** (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods **9**: 357-359

764 **Lu SX, Knowles SM, Webb CJ, Celaya RB, Cha C, Siu JP, Tobin EM** (2011) The Jumonji C domain-  
765 containing protein JMJ30 regulates period length in the Arabidopsis circadian clock. Plant Physiol **155**:  
766 906-915

767 **Lu SX, Webb CJ, Knowles SM, Kim SH, Wang Z, Tobin EM** (2012) CCA1 and ELF3 Interact in the control  
768 of hypocotyl length and flowering time in Arabidopsis. Plant Physiol **158**: 1079-1088

769 **Malapeira J, Khaitova LC, Mas P** (2012) Ordered changes in histone modifications at the core of the  
770 Arabidopsis circadian clock. Proc Natl Acad Sci U S A **109**: 21540-21545

771 **March-Diaz R, Garcia-Dominguez M, Florencio FJ, Reyes JC** (2007) SEF, a new protein required for  
772 flowering repression in Arabidopsis, interacts with PIE1 and ARP6. Plant Physiol **143**: 893-901

773 **March-Diaz R, Reyes JC** (2009) The beauty of being a variant: H2A.Z and the SWR1 complex in plants. Mol  
774 Plant **2**: 565-577

775 **Menet JS, Pescatore S, Rosbash M** (2014) CLOCK:BMAL1 is a pioneer-like transcription factor. Genes Dev  
776 **28**: 8-13

777 **Nakamichi N, Kiba T, Henriques R, Mizuno T, Chua NH, Sakakibara H** (2010) PSEUDO-RESPONSE  
778 REGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. Plant Cell  
779 **22**: 594-605

780 **Nitschke S, Cortleven A, Iven T, Feussner I, Havaux M, Riefler M, Schumling T** (2016) Circadian Stress  
781 Regimes Affect the Circadian Clock and Cause Jasmonic Acid-Dependent Cell Death in Cytokinin-  
782 Deficient Arabidopsis Plants. Plant Cell **28**: 1616-1639

783 **Noh YS, Amasino RM** (2003) PIE1, an ISWI family gene, is required for FLC activation and floral repression  
784 in Arabidopsis. Plant Cell **15**: 1671-1682

785 **Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farre EM, Kay SA** (2011) The  
786 ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature **475**:  
787 398-402

788 **Perales M, Mas P** (2007) A functional link between rhythmic changes in chromatin structure and the  
789 Arabidopsis biological clock. Plant Cell **19**: 2111-2123

790 **Pokhilko A, Mas P, Millar AJ** (2013) Modelling the widespread effects of TOC1 signalling on the plant

791 circadian clock and its outputs. *BMC Syst Biol* **7**: 23

792 **Raisner RM, Hartley PD, Meneghini MD, Bao MZ, Liu CL, Schreiber SL, Rando OJ, Madhani HD** (2005)

793 Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell* **123**:

794 233-248

795 **Raisner RM, Madhani HD** (2006) Patterning chromatin: form and function for H2A.Z variant nucleosomes.

796 *Curr Opin Genet Dev* **16**: 119-124

797 **Ramirez F, Dundar F, Diehl S, Gruning BA, Manke T** (2014) deepTools: a flexible platform for exploring

798 deep-sequencing data. *Nucleic Acids Res* **42**: W187-191

799 **Rangasamy D, Berven L, Ridgway P, Tremethick DJ** (2003) Pericentric heterochromatin becomes enriched

800 with H2A.Z during early mammalian development. *EMBO J* **22**: 1599-1607

801 **Rosa M, Von Harder M, Cigliano RA, Schlogelhofer P, Mittelsten Scheid O** (2013) The Arabidopsis SWR1

802 chromatin-remodeling complex is important for DNA repair, somatic recombination, and meiosis. *Plant*

803 *Cell* **25**: 1990-2001

804 **Salome PA, McClung CR** (2004) The Arabidopsis thaliana clock. *J Biol Rhythms* **19**: 425-435

805 **Salome PA, Weigel D, McClung CR** (2010) The role of the Arabidopsis morning loop components CCA1, LHY,

806 PRR7, and PRR9 in temperature compensation. *Plant Cell* **22**: 3650-3661

807 **Seo PJ, Mas P** (2014) Multiple layers of posttranslational regulation refine circadian clock activity in

808 Arabidopsis. *Plant Cell* **26**: 79-87

809 **Seo PJ, Mas P** (2015) STRESSing the role of the plant circadian clock. *Trends Plant Sci* **20**: 230-237

810 **Shu H, Gruissem W, Hennig L** (2013) Measuring Arabidopsis chromatin accessibility using DNase I-

811 polymerase chain reaction and DNase I-chip assays. *Plant Physiol* **162**: 1794-1801

812 **Song HR, Noh YS** (2012) Rhythmic oscillation of histone acetylation and methylation at the Arabidopsis central

813 clock loci. *Mol Cells* **34**: 279-287

814 **Staiger D, Green R** (2011) RNA-based regulation in the plant circadian clock. *Trends Plant Sci* **16**: 517-523

815 **Swaminathan J, Baxter EM, Corces VG** (2005) The role of histone H2Av variant replacement and histone H4

816 acetylation in the establishment of Drosophila heterochromatin. *Genes Dev* **19**: 65-76

817 **Thakar A, Gupta P, McAllister WT, Zlatanova J** (2010) Histone variant H2A.Z inhibits transcription in

818 reconstituted nucleosomes. *Biochemistry* **49**: 4018-4026

819 **To TK, Kim JM** (2014) Epigenetic regulation of gene responsiveness in Arabidopsis. *Front Plant Sci* **4**: 548

820 **Trapnell C, Pachter L, Salzberg SL** (2009) TopHat: discovering splice junctions with RNA-Seq.  
821 *Bioinformatics* **25**: 1105-1111

822 **Voss U, Wilson MH, Kenobi K, Gould PD, Robertson FC, Peer WA, Lucas M, Swarup K, Casimiro I,**  
823 **Holman TJ, Wells DM, Peret B, Goh T, Fukaki H, Hodgman TC, Laplaze L, Halliday KJ, Ljung**  
824 **K, Murphy AS, Hall AJ, Webb AA, Bennett MJ** (2015) The circadian clock rephases during lateral  
825 root organ initiation in *Arabidopsis thaliana*. *Nat Commun* **6**: 7641

826 **Wang L, Kim J, Somers DE** (2013) Transcriptional corepressor TOPLESS complexes with pseudoresponse  
827 regulator proteins and histone deacetylases to regulate circadian transcription. *Proc Natl Acad Sci U S A*  
828 **110**: 761-766

829 **Yerushalmi S, Yakir E, Green RM** (2011) Circadian clocks and adaptation in *Arabidopsis*. *Mol Ecol* **20**: 1155-  
830 1165

831

Figure 1

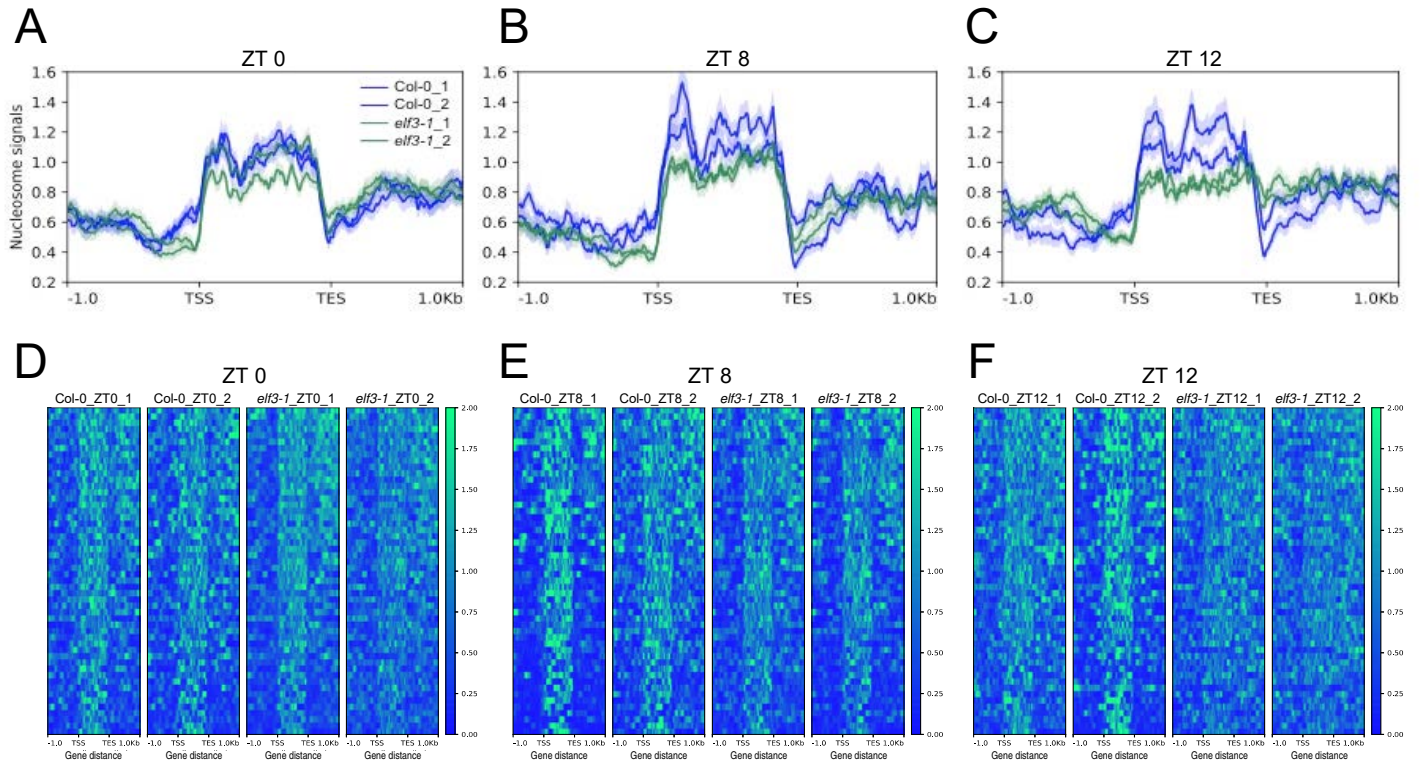


Figure 2

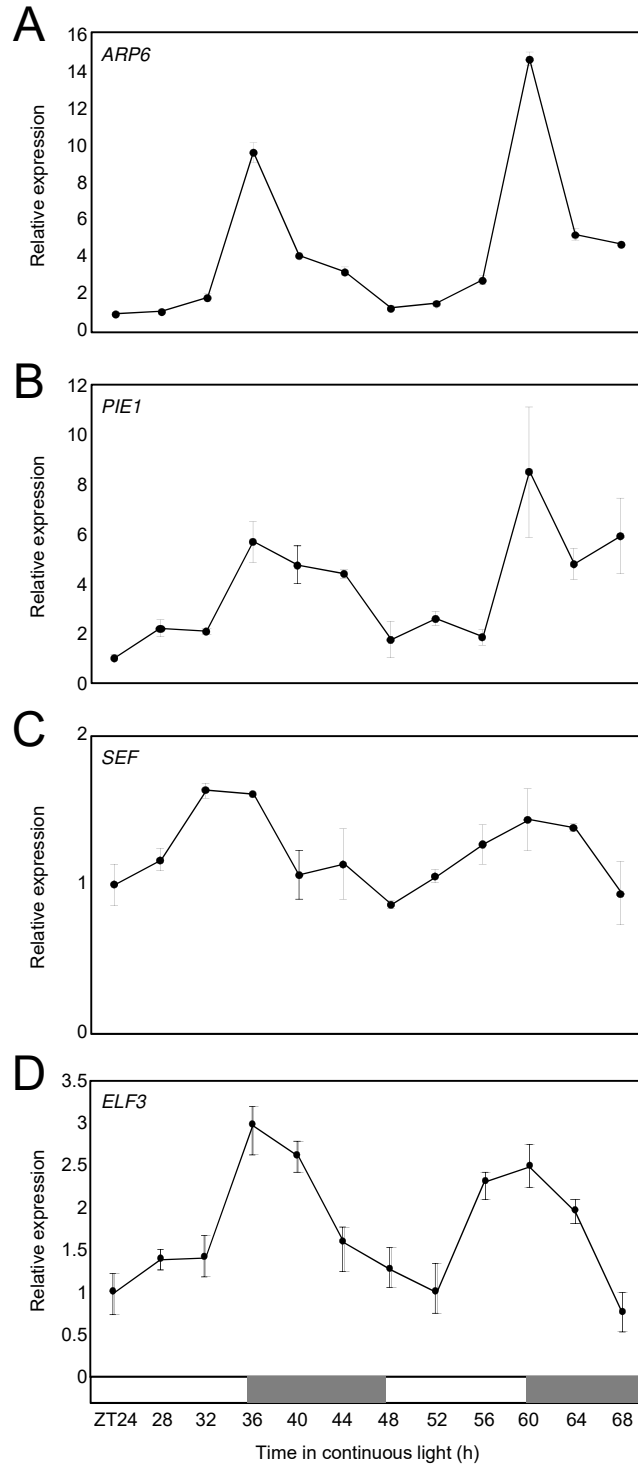
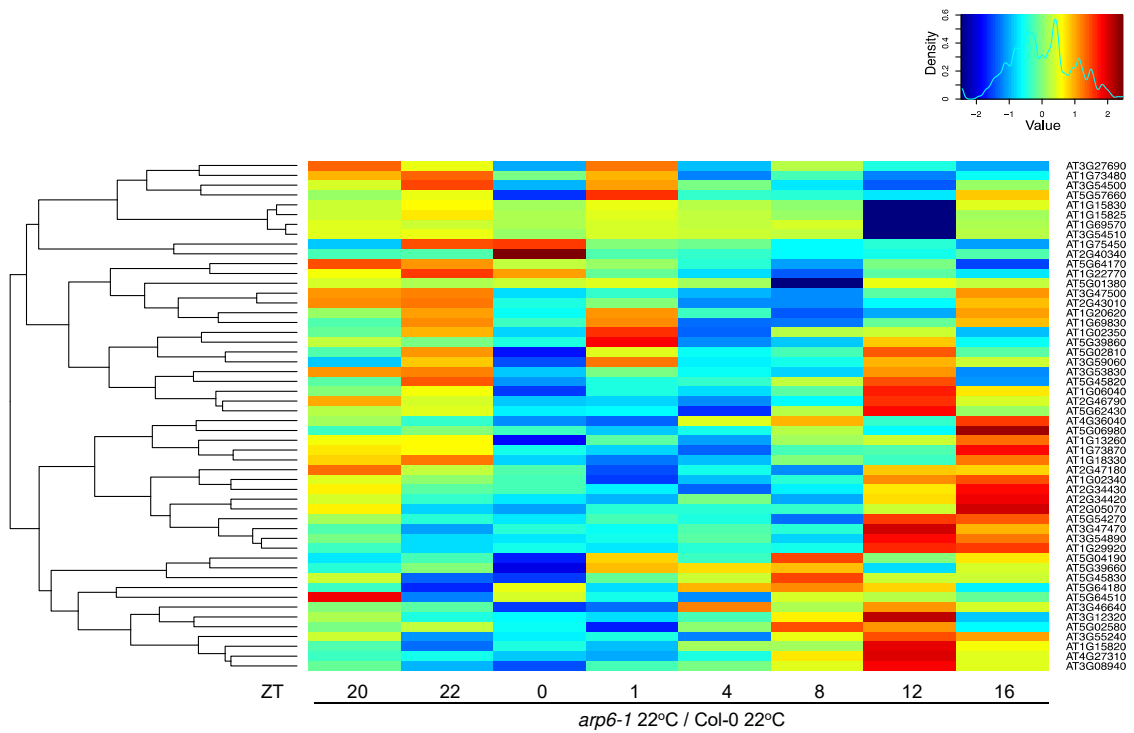
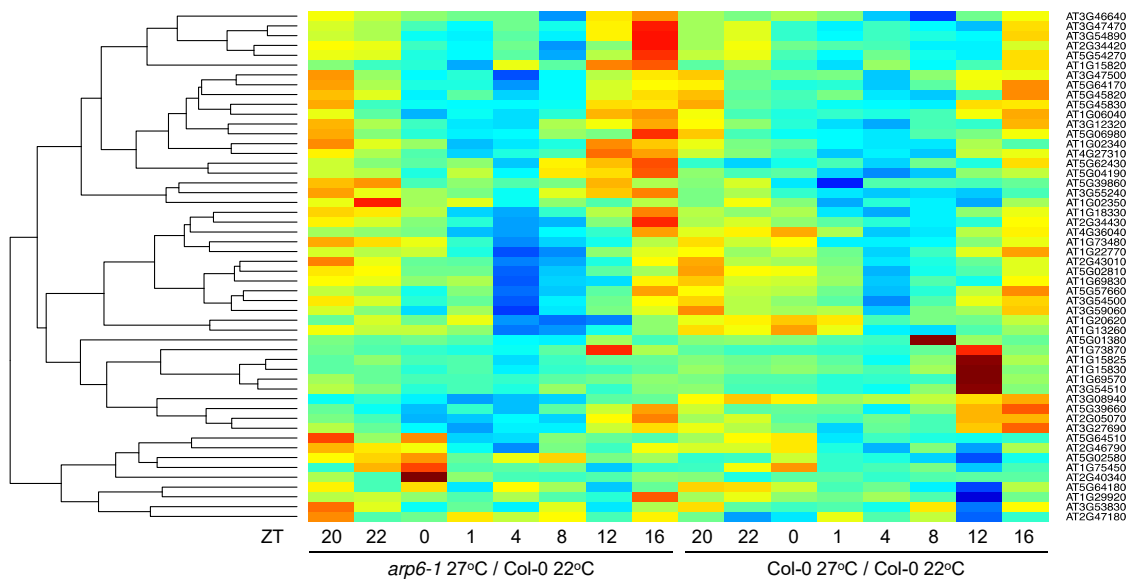


Figure 3

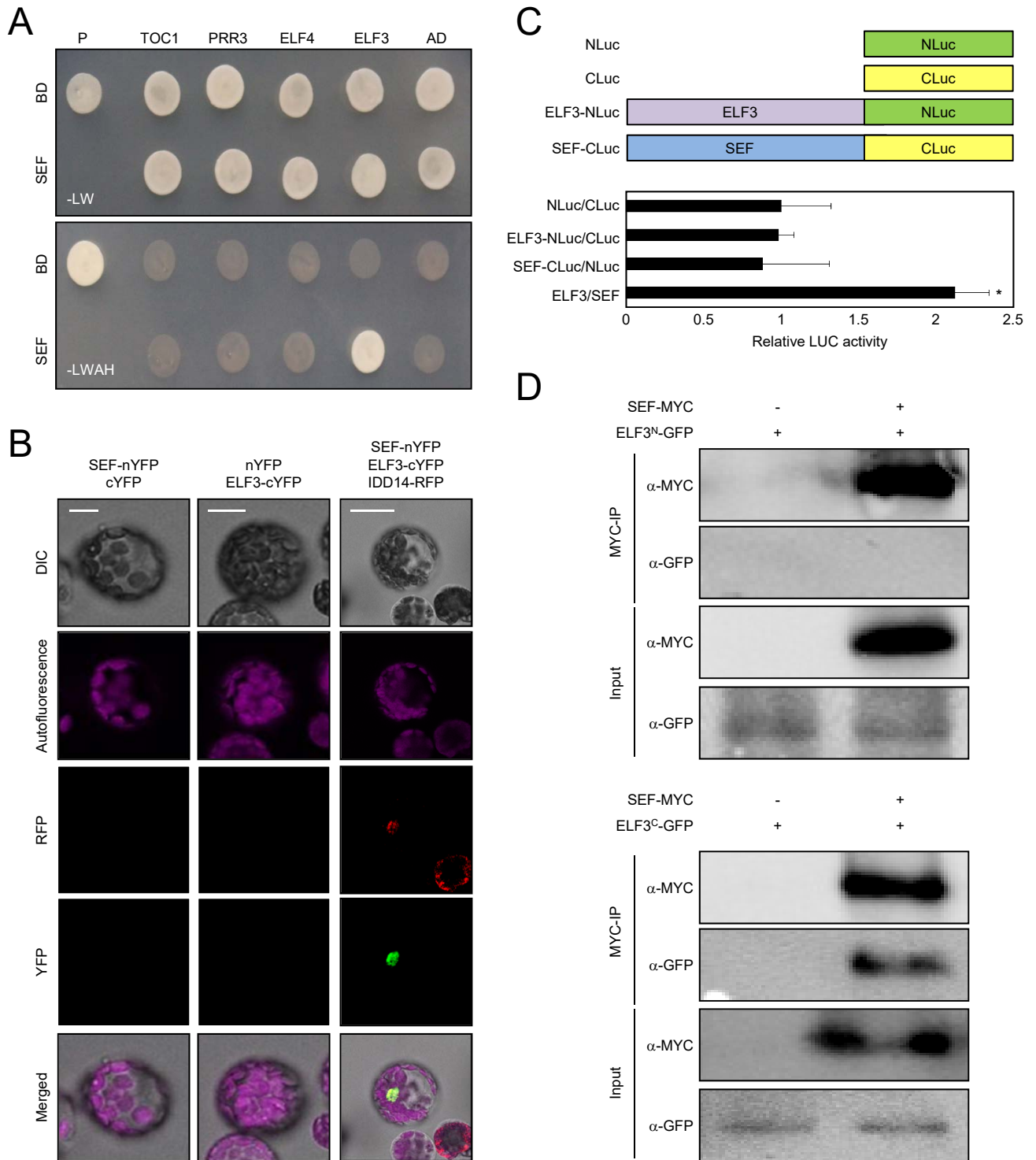
A



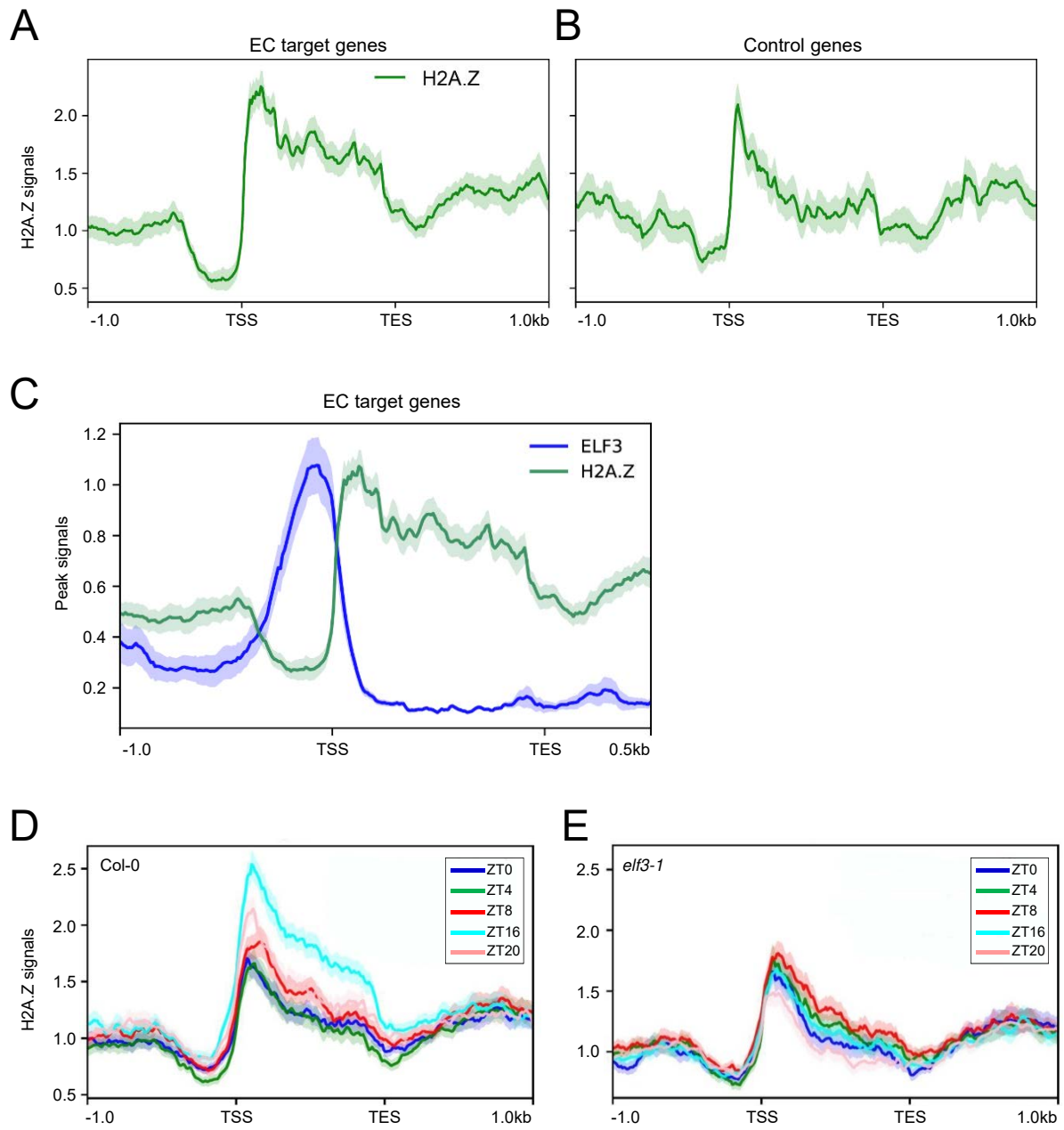
B



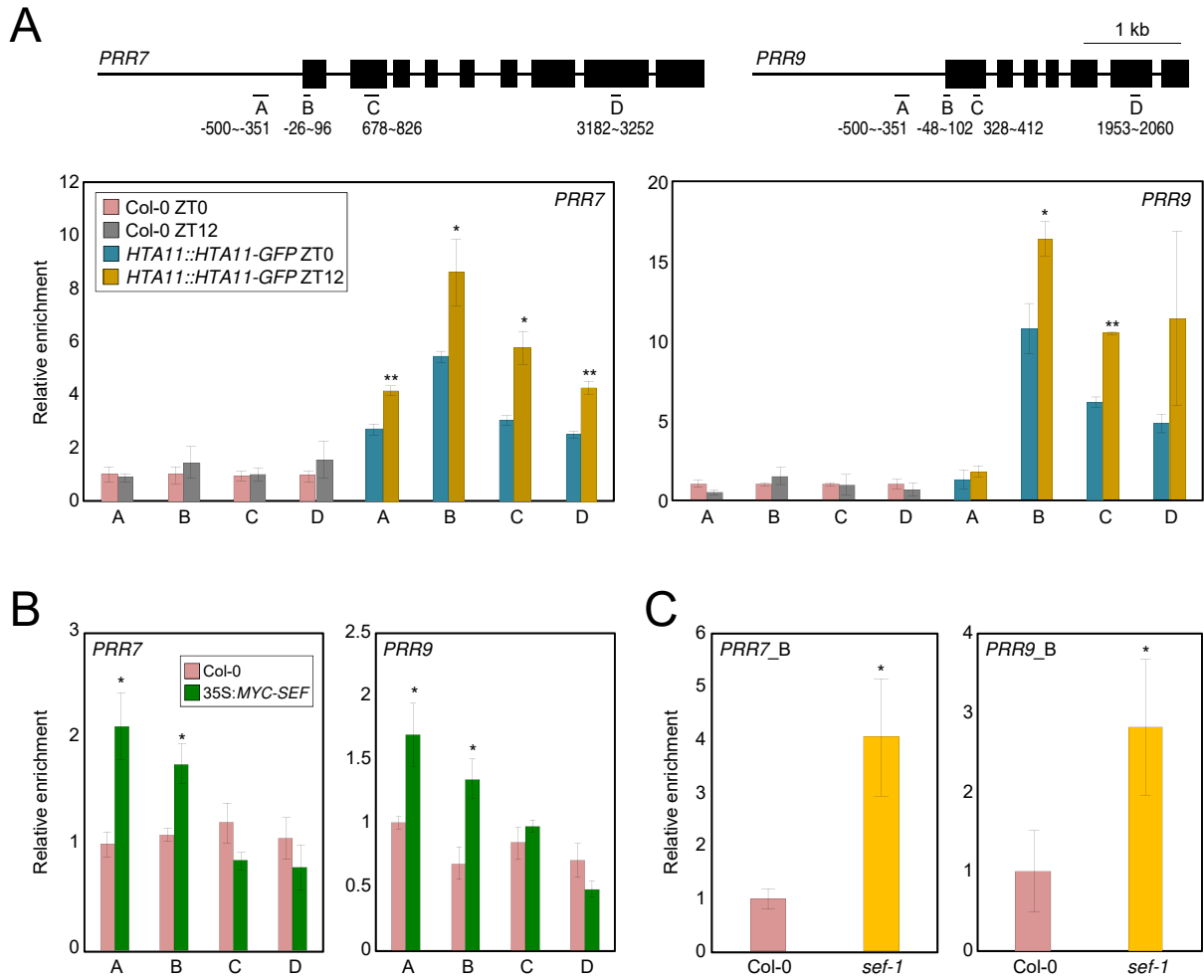
**Figure 4**



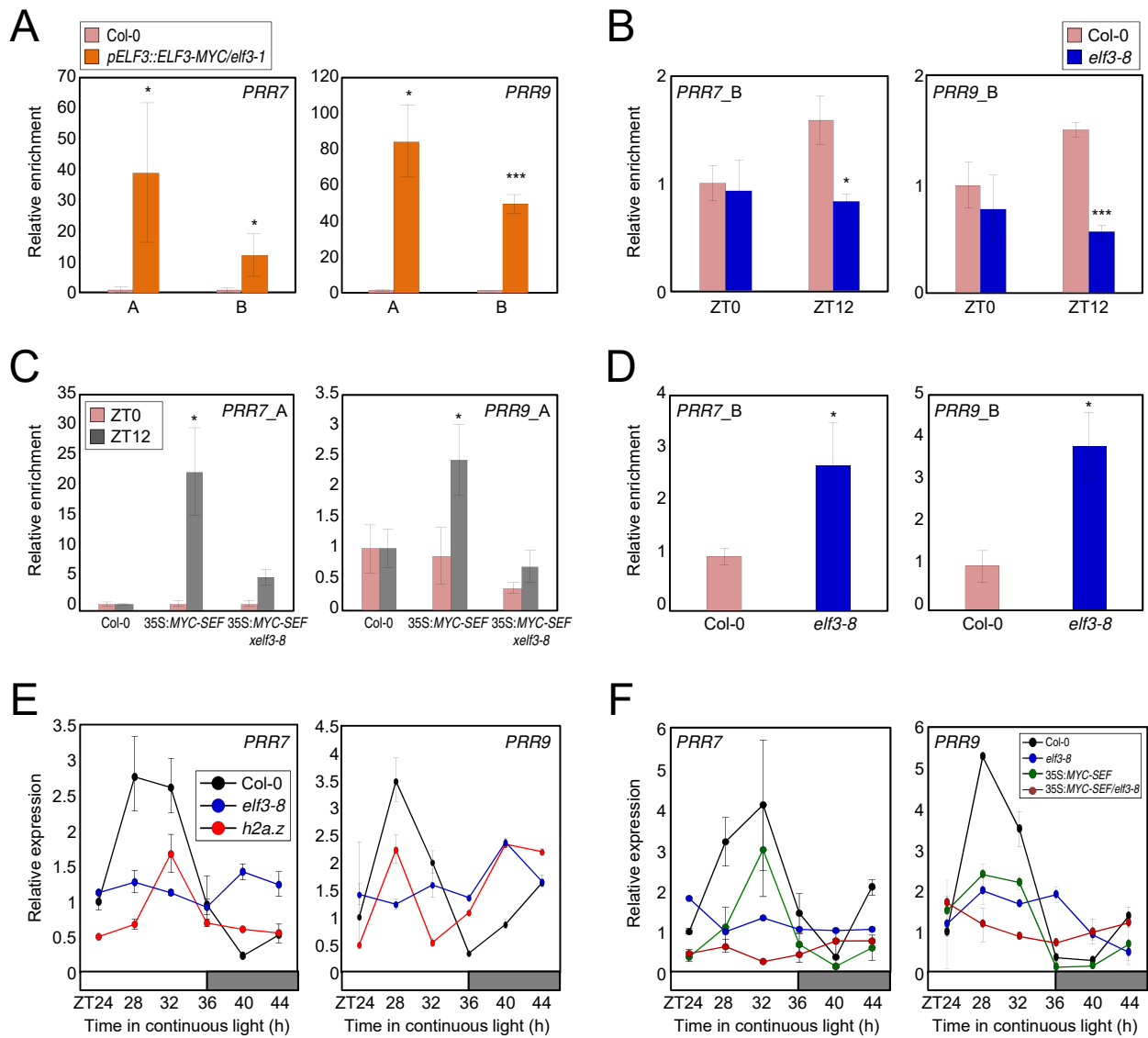
**Figure 5**



# Figure 6



# Figure 7



## Parsed Citations

**Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. Science 293: 880-883**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Anders S, Pyl PT, Huber W (2015) HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics 31: 166-169**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114-2120**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Box MS, Huang BE, Domijan M, Jaeger KE, Khattak AK, Yoo SJ, Sedivy EL, Jones DM, Hearn TJ, Webb AA, Grant A, Locke JC, Wigge PA (2015) ELF3 controls thermoresponsive growth in Arabidopsis. Curr Biol 25: 194-199**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Carre IA, Kim JY (2002) MYB transcription factors in the Arabidopsis circadian clock. J Exp Bot 53: 1551-1557**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Chen K, Xi Y, Pan X, Li Z, Kaestner K, Tyler J, Dent S, He X, Li W (2013) DANPOS: dynamic analysis of nucleosome position and occupancy by sequencing. Genome Res 23: 341-351**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Chow BY, Helfer A, Nusinow DA, Kay SA (2012) ELF3 recruitment to the PRR9 promoter requires other Evening Complex members in the Arabidopsis circadian clock. Plant Signal Behav 7: 170-173**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Coleman-Derr D, Zilberman D (2012) Deposition of histone variant H2AZ within gene bodies regulates responsive genes. PLoS Genet 8: e1002988**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Cortijo S, Charoensawan V, Brestovitsky A, Buning R, Ravarani C, Rhodes D, van Noort J, Jaeger KE, Wigge PA (2017) Transcriptional Regulation of the Ambient Temperature Response by H2AZ Nucleosomes and HSF1 Transcription Factors in Arabidopsis. Mol Plant 10: 1258-1273**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Deal RB, Kandasamy MK, McKinney EC, Meagher RB (2005) The nuclear actin-related protein ARP6 is a pleiotropic developmental regulator required for the maintenance of FLOWERING LOCUS C expression and repression of flowering in Arabidopsis. Plant Cell 17: 2633-2646**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Deal RB, Topp CN, McKinney EC, Meagher RB (2007) Repression of flowering in Arabidopsis requires activation of FLOWERING LOCUS C expression by the histone variant H2AZ. Plant Cell 19: 74-83**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Ezer D, Jung JH, Lan H, Biswas S, Gregoire L, Box MS, Charoensawan V, Cortijo S, Lai X, Stockle D, Zubieta C, Jaeger KE, Wigge PA (2017) The evening complex coordinates environmental and endogenous signals in Arabidopsis. Nat. Plants 3: 17087**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Farinas B, Mas P (2011) Functional implication of the MYB transcription factor RVE8/LCL5 in the circadian control of histone acetylation. Plant J 66: 318-329**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Farre EM, Harmer SL, Harmon FG, Yanovsky MJ, Kay SA (2005) Overlapping and distinct roles of PRR7 and PRR9 in the Arabidopsis circadian clock. Curr Biol 15: 47-54**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Gendron JM, Pruneda-Paz JL, Doherty CJ, Gross AM, Kang SE, Kay SA (2012) Arabidopsis circadian clock protein, TOC1, is a DNA-binding transcription factor. Proc Natl Acad Sci U S A 109: 2167-2172**

- Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Greenham K, McClung CR (2015) Integrating circadian dynamics with physiological processes in plants. Nat Rev Genet 16: 598-610**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Guillemette B, Bataille AR, Gevry N, Adam M, Blanchette M, Robert F, Gaudreau L (2005) Variant histone H2AZ is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol 3: e384**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hazen SP, Schultz TF, Pruneda-Paz JL, Borevitz JO, Ecker JR, Kay SA (2005) LUX ARRHYTHMO encodes a Myb domain protein essential for circadian rhythms. Proc Natl Acad Sci U S A 102: 10387-10392**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Herrero E, Kolmos E, Bujdoso N, Yuan Y, Wang M, Berns MC, Uhlworm H, Coupland G, Saini R, Jaskolski M, Webb A, Goncalves J, Davis SJ (2012) EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock. Plant Cell 24: 428-443**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hicks KA, Albertson TM, Wagner DR (2001) EARLY FLOWERING3 encodes a novel protein that regulates circadian clock function and flowering in Arabidopsis. Plant Cell 13: 1281-1292**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hicks KA, Millar AJ, Carre IA, Somers DE, Straume M, Meeks-Wagner DR, Kay SA (1996) Conditional circadian dysfunction of the Arabidopsis early-flowering 3 mutant. Science 274: 790-792**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hsu PY, Devisetty UK, Harmer SL (2013) Accurate timekeeping is controlled by a cycling activator in Arabidopsis. Elife 2: e00473**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Huang H, Alvarez S, Bindbeutel R, Shen Z, Naldrett MJ, Evans BS, Briggs SP, Hicks LM, Kay SA, Nusinow DA (2016) Identification of Evening Complex Associated Proteins in Arabidopsis by Affinity Purification and Mass Spectrometry. Mol Cell Proteomics 15: 201-217**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Huang H, Nusinow DA (2016) Into the Evening: Complex Interactions in the Arabidopsis Circadian Clock. Trends Genet 32: 674-686**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Huang W, Perez-Garcia P, Pokhilko A, Millar AJ, Antoshechkin I, Riechmann JL, Mas P (2012) Mapping the core of the Arabidopsis circadian clock defines the network structure of the oscillator. Science 336: 75-79**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Jang K, Lee HG, Jung SJ, Paek NC, Seo PJ (2015) The E3 Ubiquitin Ligase COP1 Regulates Thermosensory Flowering by Triggering GI Degradation in Arabidopsis. Sci Rep 5: 12071**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Jones MA, Harmer S (2011) JMJD5 Functions in concert with TOC1 in the arabidopsis circadian system. Plant Signal Behav 6: 445-448**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Kumar SV, Wigge PA (2010) H2AZ-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell 140: 136-147**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357-359**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Lu SX, Knowles SM, Webb CJ, Celaya RB, Cha C, Siu JP, Tobin EM (2011) The Jumonji C domain-containing protein JM30 regulates period length in the Arabidopsis circadian clock. Plant Physiol 155: 906-915**  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Lu SX, Webb CJ, Knowles SM, Kim SH, Wang Z, Tobin EM (2012) CCA1 and ELF3 Interact in the control of hypocotyl length and flowering time in Arabidopsis. Plant Physiol 158: 1079-1088**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Malapeira J, Khaitova LC, Mas P (2012) Ordered changes in histone modifications at the core of the Arabidopsis circadian clock. Proc Natl Acad Sci U S A 109: 21540-21545**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**March-Diaz R, Garcia-Dominguez M, Florencio FJ, Reyes JC (2007) SEF, a new protein required for flowering repression in Arabidopsis, interacts with PIE1 and ARP6. Plant Physiol 143: 893-901**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**March-Diaz R, Reyes JC (2009) The beauty of being a variant: H2AZ and the SWR1 complex in plants. Mol Plant 2: 565-577**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Menet JS, Pescatore S, Rosbash M (2014) CLOCK:BMAL1 is a pioneer-like transcription factor. Genes Dev 28: 8-13**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Nakamichi N, Kiba T, Henriques R, Mizuno T, Chua NH, Sakakibara H (2010) PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. Plant Cell 22: 594-605**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Nitschke S, Cortleven A, Iven T, Feussner I, Havaux M, Riefler M, Schumling T (2016) Circadian Stress Regimes Affect the Circadian Clock and Cause Jasmonic Acid-Dependent Cell Death in Cytokinin-Deficient Arabidopsis Plants. Plant Cell 28: 1616-1639**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Noh YS, Amasino RM (2003) PIE1, an ISM family gene, is required for FLC activation and floral repression in Arabidopsis. Plant Cell 15: 1671-1682**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farre EM, Kay SA (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature 475: 398-402**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Perales M, Mas P (2007) A functional link between rhythmic changes in chromatin structure and the Arabidopsis biological clock. Plant Cell 19: 2111-2123**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Pokhilko A, Mas P, Millar AJ (2013) Modelling the widespread effects of TOC1 signalling on the plant circadian clock and its outputs. BMC Syst Biol 7: 23**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Raisner RM, Hartley PD, Meneghini MD, Bao MZ, Liu CL, Schreiber SL, Rando OJ, Madhani HD (2005) Histone variant H2AZ marks the 5' ends of both active and inactive genes in euchromatin. Cell 123: 233-248**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Raisner RM, Madhani HD (2006) Patterning chromatin: form and function for H2AZ variant nucleosomes. Curr Opin Genet Dev 16: 119-124**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Ramirez F, Dundar F, Diehl S, Gruning BA, Manke T (2014) deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Res 42: W187-191**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Rangasamy D, Berven L, Ridgway P, Tremethick DJ (2003) Pericentric heterochromatin becomes enriched with H2AZ during early mammalian development. EMBO J 22: 1599-1607**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Rosa M, Von Harder M, Cigliano RA, Schlogelhofer P, Mittelsten Scheid O (2013) The Arabidopsis SWR1 chromatin-remodeling complex is important for DNA repair, somatic recombination, and meiosis. Plant Cell 25: 1990-2001**

Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

**Salome PA, McClung CR (2004) The Arabidopsis thaliana clock. J Biol Rhythms 19: 425-435**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Salome PA, Weigel D, McClung CR (2010) The role of the Arabidopsis morning loop components CCA1, LHY, PRR7, and PRR9 in temperature compensation. Plant Cell 22: 3650-3661**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Seo PJ, Mas P (2014) Multiple layers of posttranslational regulation refine circadian clock activity in Arabidopsis. Plant Cell 26: 79-87**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Seo PJ, Mas P (2015) STRESSing the role of the plant circadian clock. Trends Plant Sci 20: 230-237**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Shu H, Grissem W, Hennig L (2013) Measuring Arabidopsis chromatin accessibility using DNase I-polymerase chain reaction and DNase I-chip assays. Plant Physiol 162: 1794-1801**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Song HR, Noh YS (2012) Rhythmic oscillation of histone acetylation and methylation at the Arabidopsis central clock loci. Mol Cells 34: 279-287**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Staiger D, Green R (2011) RNA-based regulation in the plant circadian clock. Trends Plant Sci 16: 517-523**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Swaminathan J, Baxter EM, Corces VG (2005) The role of histone H2Av variant replacement and histone H4 acetylation in the establishment of Drosophila heterochromatin. Genes Dev 19: 65-76**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Thakar A, Gupta P, McAllister WT, Zlatanova J (2010) Histone variant H2AZ inhibits transcription in reconstituted nucleosomes. Biochemistry 49: 4018-4026**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**To TK, Kim JM (2014) Epigenetic regulation of gene responsiveness in Arabidopsis. Front Plant Sci 4: 548**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25: 1105-1111**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Voss U, Wilson MH, Kenobi K, Gould PD, Robertson FC, Peer WA, Lucas M, Swarup K, Casimiro I, Holman TJ, Wells DM, Peret B, Goh T, Fukaki H, Hodgman TC, Laplaze L, Halliday KJ, Ljung K, Murphy AS, Hall AJ, Webb AA, Bennett MJ (2015) The circadian clock rephases during lateral root organ initiation in Arabidopsis thaliana. Nat Commun 6: 7641**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Wang L, Kim J, Somers DE (2013) Transcriptional corepressor TOPLESS complexes with pseudoresponse regulator proteins and histone deacetylases to regulate circadian transcription. Proc Natl Acad Sci U S A 110: 761-766**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)

**Yerushalmi S, Yakir E, Green RM (2011) Circadian clocks and adaptation in Arabidopsis. Mol Ecol 20: 1155-1165**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only Title Only Author and Title](#)