THE PLANT CELL

The Plant Cell, 2025, **37**, koaf189

https://doi.org/10.1093/plcell/koaf189
Advance access publication 30 July 2025
Research Article

Evening complex proteins antagonize ABI3 and ABI5 to temporally regulate abscisic acid signaling and seed germination

Ruyu Tang,[1,2] Jiajia Yang,[1,2] Xiao Han,[1] Kunrong He,[3] Cuiping Zhang,[1] Milian Yang,[1] Juping Zhang,[1,2] Zhichong Huang,[1] Jingwen Ye,[1] Tingting Xu,[1,2] Chunlan Yu,[1] Jiancan Du,[1] Qiantang Fu,[1] Yanru Hu[1,4,5,*]

[1]CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
[2]College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
[3]State Key Laboratory of Tree Genetics and Breeding, Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China
[4]State Key Laboratory of Chemo and Biosensing, Hunan Key Laboratory of Plant Functional Genomics and Developmental Regulation, Hunan Research Center of the Basic Discipline for Cell Signaling, College of Biology, Hunan University, Changsha 410082, China
[5]Yuelushan Laboratory, Changsha 410128, China
*Author for correspondence: huyanru@hnu.edu.cn
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plcell/pages/General-Instructions) is Yanru Hu (huyanru@hnu.edu.cn).

Abstract

Seed germination and postgerminative growth are precisely regulated by multiple signals. In *Arabidopsis thaliana*, the phytohormone abscisic acid (ABA) suppresses these processes and several circadian clock-associated proteins mediate ABA responses. Nevertheless, whether seed germination is controlled by temporal signals under diurnal conditions remains obscure, as do the associated underlying molecular mechanisms. Here, we found that the germination of wild-type seeds varies with time of release from cold stratification (i.e. transferred to 22 °C) under diurnal conditions upon ABA, salinity, or osmotic stress exposure. Additionally, the evening complex (EC) components EARLY FLOWERING 3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) attenuate ABA signaling. Notably, time-dependent seed germination relies on these EC components and other core clock proteins. ELF3, ELF4, and LUX physically interact with and act genetically upstream of ABSCISIC ACID INSENSITIVE3 (ABI3) and ABI5, two crucial transcriptional activators of ABA signaling. ELF3, ELF4, and LUX repress the function and accumulation of ABI3 and ABI5. Consistent with these results, ABI3 and ABI5 are essential for the time-based modulation of seed germination. Our findings highlight the critical effects of temporal signals on seed germination and clarify the mechanism through which the EC components antagonize ABI3 and ABI5 to facilitate the crosstalk between the clock and ABA signaling pathways.

Introduction

The circadian clock is a conserved timekeeping mechanism that synchronizes cellular events with cyclical environmental signals to enhance the adaptive advantage of land plants (Dunlap 1999; Dodd et al. 2005; Pruneda-Paz and Kay 2010; Atkins and Dodd 2014; Grundy et al. 2015; Sanchez and Kay 2016; Fung-Uceda et al. 2018; Shalit-Kaneh et al. 2018; Simon et al. 2020; Xu et al. 2022). The core oscillator of the *Arabidopsis* (*Arabidopsis thaliana*) circadian clock consists of multiple transcription/translation-based negative feedback loops (Harmer 2009; Pokhilko et al. 2012; Carré and Veflingstad 2013; Hsu and Harmer 2014; Greenham and McClung 2015; Uehara et al. 2019; Webb et al. 2019; Nakamichi 2020; Simon et al. 2020). Two *MYB* gene family members, *CIRCADIAN CLOCK-ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), are expressed in the early morning (Wang and Tobin 1998; Harmer 2009). Both CCA1 and LHY repress the expression of *Pseudo Response Regulator* (*PRR*) genes (*PRR9*, *PRR7*, *PRR5*, and *PRR3*) and *TIMING OF CAB EXPRESSION1* (*TOC1*), which are genes that are sequentially expressed, reaching peak levels at specific times from dawn to dusk (Harmer et al. 2000; Matsushika et al. 2000; Strayer et al. 2000; Alabadi et al. 2001; Nakamichi et al. 2010; Farré and Liu 2013; Adams et al. 2015). The proteins encoded by these *PRR* genes bind to *CCA1* and *LHY* promoters and inhibit transcription (Alabadi et al. 2001; Perales and Más 2007; Nakamichi et al. 2010, 2012; Wang et al. 2010, 2013; Greenham and McClung 2015). Moreover, EARLY FLOWERING 3 (ELF3) physically interacts with ELF4 and LUX ARRHYTHMO (LUX; a single MYB domain-containing GARP transcription factor) to form the evening complex (EC), which is necessary for the modulation of circadian outputs, and suppress the expression of *PRR7* and *PRR9* genes, thereby indirectly relieving these PRR-mediated repression of *CCA1* and *LHY* expression (Helfer et al. 2011; Nusinow et al. 2011; Herrero et al. 2012; Mizuno et al. 2014).

The circadian clock regulates a wide range of developmental and physiological processes in *Arabidopsis*, such as hypocotyl growth, flowering time, anthocyanin biosynthesis, and stress tolerance-related responses (Dunlap 1999; Yamamoto et al. 2003; Dodd et al. 2005; Pruneda-Paz and Kay 2010; Atkins and Dodd 2014; Hsu and Harmer 2014; Grundy et al. 2015; Sanchez and Kay 2016; Webb et al. 2019; Simon et al. 2020;

Received April 02, 2025. Accepted June 13, 2025.

Wei et al. 2021; Yuan et al. 2021; He et al. 2022; Wang et al. 2023). Interestingly, there is accumulating evidence of the close interplay between the circadian clock and the phytohormone abscisic acid (ABA), which is a prominent signaling molecule inhibiting seed germination. For example, LHY produced at dawn modulates the transcription of several genes associated with ABA biosynthesis and downstream responses, thereby alleviating the inhibitory effect of ABA or salinity stress on seed germination (Adams et al. 2018; Liang et al. 2024). The central circadian clock proteins PRR5 and PRR7 physically interact with the transcription factor ABSCISIC ACID INSENSITIVE5 (ABI5) to stimulate ABA responses during seed germination (Yang et al. 2021). Consistently, the expression levels of several genes encoding ABA biosynthetic enzymes and signaling components exhibit circadian rhythmicity (Covington et al. 2008; Michael et al. 2008; Mizuno and Yamashino 2008; Fukushima et al. 2009; Penfield and Hall 2009; Seung et al. 2012; Liu et al. 2013a; Adams et al. 2018; Yang et al. 2021; Liang et al. 2024). Furthermore, several clock proteins, such as CCA1, LHY, LUX, and GIGANTEA (GI), integrate endogenous and/or environmental signals to mediate the release of dormancy. Impairment of these proteins leads to altered germination patterns under certain conditions, for instance alternating temperatures, low temperatures, and dry after-ripening (Penfield and Hall 2009; Footitt et al. 2011, 2017; Finch-Savage and Footitt 2017; Adams et al. 2018; Zha et al. 2020; Siemiatkowska et al. 2022). Despite the effects of clock components on the ABA pathway and seed dormancy/germination, whether seed germination is regulated by the temporal signal under diurnal conditions and the underlying molecular basis remain obscure.

Seed germination in response to specific modulatory cues is strictly controlled and requires the precise coordination of various positive and negative signaling regulators. Both ABI3 and ABI5 are crucial transcription factors that induce ABA-suppressed seed germination and postgerminative growth (Giraudat et al. 1992; Finkelstein 1994; Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000; Lopez-Molina et al. 2001, 2002; Nakamura et al. 2001; Brocard et al. 2002; Finkelstein et al. 2005; Carbonero et al. 2017; Li et al. 2024; Zhao et al. 2024). The expression of *ABI3* and *ABI5* mainly occurs in dry seeds and is highly induced by ABA (Giraudat et al. 1992; Finkelstein and Lynch 2000; Yu et al. 2015). The loss-of-function *abi3* and *abi5* mutants are considerably less sensitive to ABA than the wild-type (WT) control during seed germination (Koornneef et al. 1984; Giraudat et al. 1992; Finkelstein 1994; Finkelstein and Lynch 2000). Both ABI3 and ABI5 are precisely modified at the posttranslational level and stabilized in response to ABA (Kobayashi et al. 2005; Furihata et al. 2006; Fujii et al. 2007; Garcia et al. 2008; Fujii and Zhu 2009; Miura et al. 2009; Nakashima et al. 2009; Hu and Yu 2014; Albertos et al. 2015; Zhou et al. 2015a; Lynch et al. 2017; Ji et al. 2019; Nie et al. 2022; Varshney et al. 2023; Du et al. 2024). Notably, they are also critical for the convergence of ABA signaling and other signaling pathways (Lee et al. 2012; Guan et al. 2014; Lim et al. 2014; Kim et al. 2016; Yang et al. 2016; Hu et al. 2019; Guo et al. 2022; Li et al. 2022; Varshney et al. 2023; Huang et al. 2025). For example, CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) positively affects the ABA-induced accumulation of ABI5 in darkness, revealing a molecular link between the ABA and light signaling pathways (Peng et al. 2022). Other studies revealed ABI3 and ABI5 coordinate with JASMONATE ZIM-DOMAIN (JAZ) and AUXIN RESPONSE FACTOR16 (ARF16) proteins to integrate the ABA, jasmonic acid, and auxin signaling pathways (Liu et al. 2013b; Ju et al. 2019; Pan et al. 2020; He et al. 2023; Mei et al. 2023). Although there has recently been considerable progress in the characterization of ABI3- and ABI5-mediated ABA signaling, the exact mechanisms underlying how ABI3 and ABI5 transduce internal and external signals to modulate seed germination under certain physiological conditions remain to be elucidated.

In this study, we demonstrated a crucial regulatory effect of the temporal signal on ABA signaling and seed germination (as the output pathways) under diurnal conditions. Our findings also provided mechanistic insights into how the EC components ELF3, ELF4, and LUX directly interact with ABI3 and ABI5 to negatively modulate their abundance and transcriptional functions. The integration of the circadian clock and ABA signaling pathways may ensure that ABA signaling is maintained at an optimal level at the appropriate timing, thereby enhancing plant environmental adaptability.

Results

Arabidopsis seed germination in response to ABA varies with time of release from cold stratification contexts under diurnal conditions

Several circadian clock proteins influence ABA signaling and/or seed germination in *Arabidopsis* (Penfield and Hall 2009; Footitt et al. 2011, 2017; Finch-Savage and Footitt 2017; Adams et al. 2018; Zha et al. 2020; Yang et al. 2021), but there is a lack of experimental evidence of the temporal regulation of seed germination. An earlier study demonstrated that imbibition, but not release from cold stratification or its duration, provides a signal capable of resetting the circadian clock in *Arabidopsis* (Zhong et al. 1998). In the present study, to investigate the potential modulatory effects of temporal signals on seed germination under diurnal conditions, seeds were soaked in water for 30 min, surface-sterilized, sown on plates containing growth medium, and transferred to 4 °C at Zeitgeber time (ZT) 0 (dawn) for stratification in darkness for 72 h. Subsequently, seeds were transferred to 22 °C either at ZT0 or at ZT12 (12 h later than the ZT0 group), and their germination and cotyledon greening were compared. The WT seeds were assayed on half-strength Murashige and Skoog (MS) medium with or without 0.75 μM ABA under long-day (16-h light/8-h dark photoperiod) conditions. As expected, the presence of ABA decreased the germination and cotyledon greening percentages of the WT seeds, regardless of when they were released from cold stratification conditions (Fig. 1A and B). Interestingly, germination and seedling growth were faster for the ZT0-released seeds than for the ZT12-released seeds in the presence of ABA. The germination and cotyledon greening percentages were significantly higher for the ZT0-released seeds than for the ZT12-released seeds on medium containing ABA (Fig. 1A and B; Supplementary Fig. S1A).

To confirm these observations, we performed a reverse transcription quantitative real-time PCR (RT-qPCR) analysis to examine the transcript levels of several ABA-induced seed-expressed genes, including *LATE EMBRYOGENESIS ABUNDANT 1* (*EM1*), *EM6*, *RESPONSIVE TO ABA 18* (*RAB18*), and *RESPONSIVE TO DESICCATION 29B* (*RD29B*), in ABA-treated germinating WT seeds released from cold stratification conditions at ZT0 and ZT12. Notably, under long-day conditions, the transcript levels of *EM1*, *EM6*, *RAB18*, and *RD29B* in seeds released at ZT0 were significantly lower in the presence of ABA compared to those released at ZT12 (Fig. 1C). These differential transcript abundances likely reflect distinct germination states of the seeds, which may also arise from differential degradation of seed-expressed transcripts. To eliminate the effects of light on seed germination, we also

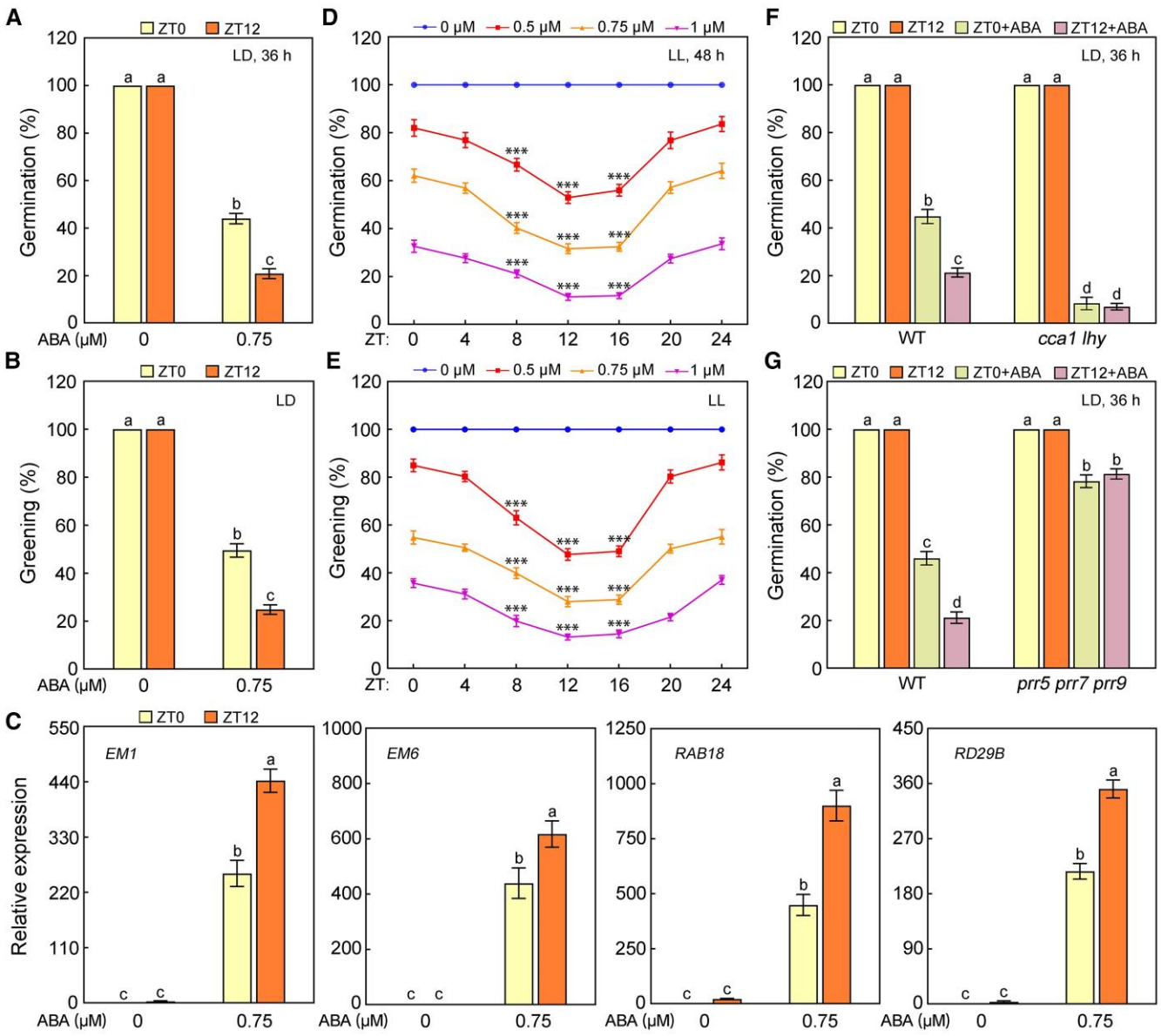

Figure 1. Seed germination upon ABA exposure varies with time of release from cold stratification contexts under diurnal conditions. **A)** Germination of the WT seeds on half-strength MS medium supplemented with or without 0.75 μM ABA under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT 0 or ZT12. Seed germination was recorded 36 h after stratification. In the medium without ABA (i.e. 0 μM), an equal volume of 10% (v/v) ethanol was added. Time is expressed was hours from dawn (ZT0). LD, long-day conditions. **B)** Cotyledon greening of the WT seeds on half-strength MS medium supplemented with or without 0.75 μM ABA under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12. Cotyledon greening was scored 5 d after stratification. **C)** RT-qPCR analysis of the ABA-induced expression of *EM1*, *EM6*, *RAB18*, and *RD29B* in WT seeds germinating on half-strength MS medium supplemented with or without 0.75 μM ABA. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds 36 h after stratification. The *PP2A* (AT1G13320) gene was used as a control. **D)** Germination of the WT seeds on half-strength MS medium supplemented with different concentrations of ABA under continuous light. The seeds were stratified at 4 °C and then transferred to 22 °C at different time points throughout the day (from ZT0 to ZT24), with each subsequent transfer (of the seeds) delayed by 4 h. Seed germination was recorded 48 h after stratification. LL, continuous light. **E)** Cotyledon greening of the WT seeds on half-strength MS medium supplemented with different concentrations of ABA under continuous light. The seeds were stratified at 4 °C and then transferred to 22 °C at different time points throughout the day. Cotyledon greening was scored 4 d after stratification. **F)** Germination of the WT and *cca1 lhy* seeds on half-strength MS medium supplemented with or without 0.75 μM ABA under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12. Seed germination was recorded 36 h after stratification. **G)** Germination of the WT and *prr5 prr7 prr9* seeds on half-strength MS medium supplemented with or without 0.75 μM ABA under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12. Seed germination was recorded 36 h after stratification. All experiments for germination and cotyledon greening analysis were performed 5 times by examining different batches of seeds. Each batch of seeds was pooled from more than 60 independent plants. For each biological replicate, more than 120 seeds were examined. Values presented in this figure are means ± standard deviation. Data shown in **(A–C)** and **(F–G)** were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). Data shown in **(D** and **E)** were analyzed by a one-way ANOVA using Tukey's HSD test. ***$P < 0.001$ (compared to ZT0). ABA, abscisic acid.

analyzed WT seed germination and cotyledon greening under continuous light. The WT seeds were transferred to 4 °C at ZT0 for stratification in darkness for 72 h and then relocated to a growth chamber set at 22 °C with continuous light at different time points throughout the day (from ZT0 to ZT24), with each subsequent transfer (of the seeds) delayed by 4 h. The germination

and cotyledon greening percentages of the ABA-treated WT seeds decreased following release from cold stratification conditions over the course of the day, with relatively lower levels at ZT12 and ZT16 (Fig. 1D and E, Supplementary Fig. S2A). Collectively, these results suggest that seed germination in response to ABA varies with time of release from cold stratification contexts under diurnal conditions.

Because CCA1/LHY and PRR proteins (i.e. PRR5, PRR7, and PRR9) mediate ABA signaling and seed germination (Adams et al. 2018; Yang et al. 2021), we speculated whether these core clock components contribute to the temporal modulation of seed germination. Hence, we examined the seed germination and cotyledon greening of the loss-of-function *cca1 lhy* double mutant and *prr5 prr7 prr9* triple mutant upon exposure to ABA. Compared with the WT seeds, the ZT0- and ZT12-released *cca1 lhy* seeds had significantly lower germination and cotyledon greening percentages on medium containing 0.75 μM ABA under long-day conditions (Fig. 1F, Supplementary Fig. S1B and C). Parallel experiments showed that germination and seedling growth were considerably faster for the *prr5 prr7 prr9* seeds released at ZT0 and ZT12 than for the corresponding WT seeds on medium supplemented with ABA (Fig. 1G, Supplementary Fig. S1D and E). More importantly, unlike the WT seeds, the ZT0- and ZT12-released *cca1 lhy* (or *prr5 prr7 prr9*) seeds had similar germination and cotyledon greening percentages in the presence of ABA (Fig. 1F and G; Supplementary Fig. S1B to E). Similar results were obtained when the *cca1 lhy* and *prr5 prr7 prr9* seeds were germinated and the resulting seedlings were grown under continuous light (Supplementary Fig. S1F to I). Hence, the time-dependent germination in response to ABA was abolished in *cca1 lhy* and *prr5 prr7 prr9* mutant seeds, reflecting the importance of CCA1/LHY and PRR proteins for the temporal control of seed germination. In alignment with this notion, the expression of *CCA1*, *LHY*, *PRR5*, *PRR7*, and *PRR9* exhibited rhythmic oscillation and ABA-responsive modulation during the initial phase of germination (Supplementary Fig. S2B to F; Penfield and Hall 2009; Yang et al. 2021).

Seed germination in salinity and osmotic stress situations is temporally modulated under diurnal conditions

To further clarify the effects of the temporal signal on seed germination, we investigated whether the temporal signal also mediates seed germination in particular natural contexts. Specifically, we first evaluated the performance of WT seeds released from cold stratification contexts at ZT0 and ZT12 under salinity and osmotic stress conditions. WT seeds were assayed on medium containing 150 mM NaCl or 300 mM mannitol under long-day conditions. The ZT0-released seeds had higher germination and cotyledon greening percentages than the ZT12-released seeds following the treatment with NaCl or mannitol (Fig. 2A to D, Supplementary Fig. S3A and B). Similarly, the germination and cotyledon greening of WT seeds under continuous light upon exposure to NaCl or mannitol were also examined. We initially stratified WT seeds at 4 °C for 72 h and then transferred them to 22 °C for germination at different time points throughout the day (from ZT0 to ZT24), with each subsequent transfer (of the seeds) delayed by 4 h. The percentages of seed germination and expanded green cotyledons decreased following release from cold stratification contexts over the course of the day, with lower levels at ZT12 and ZT16, under salinity and osmotic stress situations (Fig. 2E and F).

To further determine whether those core clock components (i.e. CCA1/LHY and PRR proteins) are involved in the temporal control of seed germination under salinity and osmotic stress conditions, we analyzed the seed germination and cotyledon greening of *cca1 lhy* and *prr5 prr7 prr9* mutants on medium containing NaCl or mannitol. Under long-day conditions, the germination and seedling growth were much lower for the *cca1 lhy* seeds released at ZT0 and ZT12 than for the corresponding WT controls on medium supplemented with 150 mM NaCl or 300 mM mannitol (Fig. 2G, Supplementary Fig. S3C to E). Compared with the WT seeds, the ZT0- and ZT12-released *prr5 prr7 prr9* seeds exhibited considerably higher germination and cotyledon greening percentages on medium containing NaCl or mannitol under long-day conditions (Fig. 2H, Supplementary Fig. S3F to H). Moreover, the timing of release from cold stratification contexts did not significantly affect the germination and cotyledon greening of *cca1 lhy* and *prr5 prr7 prr9* seeds in response to NaCl or mannitol (Fig. 2G and H, Supplementary Fig. S3C to H). In addition, parallel experiments showed that the *cca1 lhy* and *prr5 prr7 prr9* seeds behaved similarly under continuous light on medium supplemented with NaCl or mannitol (Supplementary Fig. S4A to H). Considered together, these results imply that *Arabidopsis* seed germination is temporally modulated in salinity and osmotic stress situations under diurnal conditions, and CCA1/LHY and PRR proteins are essential for this regulation.

ELF3, *ELF4*, and *LUX* expression is repressed by ABA during seed germination

The EC proteins ELF3, ELF4, and LUX play crucial roles in the plant circadian clock; the disruption of any of these components leads to an arrhythmic circadian clock under constant light conditions (Helfer et al. 2011; Nusinow et al. 2011; Herrero et al. 2012; Mizuno et al. 2014; Sanchez and Kay 2016). Notably, ELF3 and LUX have been implicated as negative regulators of seed dormancy (Zha et al. 2020). The germination percentages of freshly harvested *elf3* and *lux* mutant seeds without cold stratification were lower than those of the WT, a finding that has also been confirmed under our experimental conditions (Zha et al. 2020; Supplementary Fig. S5). After ascertaining that seed germination is temporally modulated in response to ABA or under salinity and osmotic stress conditions, we further examined whether ELF3, ELF4, and LUX are involved in these processes. We first analyzed *ELF3*, *ELF4*, and *LUX* expression during seed germination. We cloned *ELF3*, *ELF4*, and *LUX* promoter fragments (*proELF3*, *proELF4*, and *proLUX*) upstream of the *β-glucuronidase* (*GUS*) reporter gene to generate reporter constructs (*ProELF3:GUS*, *ProELF4:GUS*, and *ProLUX:GUS*), which were inserted into WT plants. The GUS staining results revealed *ELF3*, *ELF4*, and *LUX* promoter activities in dry seeds and during seed germination (Fig. 3A). Additionally, GUS staining analysis showed *ELF3*, *ELF4*, and *LUX* expression in the endosperm and developing seeds (e.g. seeds at 5 d after pollination) (Supplementary Fig. S6). Together, these observations suggest that *ELF3*, *ELF4*, and *LUX* may be expressed continuously from seed development through germination. To assess the regulatory effect of ABA on *ELF3*, *ELF4*, and *LUX* transcription, we performed RT-qPCR analyses of *ELF3*, *ELF4*, and *LUX* transcription in germinating WT seeds with or without an ABA treatment. The expression of *ELF3*, *ELF4*, and *LUX* was rhythmic and downregulated by ABA during the early germination stage (Fig. 3B to D, Supplementary Fig. S2G to I). These results show *ELF3*, *ELF4*, and *LUX* are expressed in germinating seeds, and that their expression is suppressed by ABA.

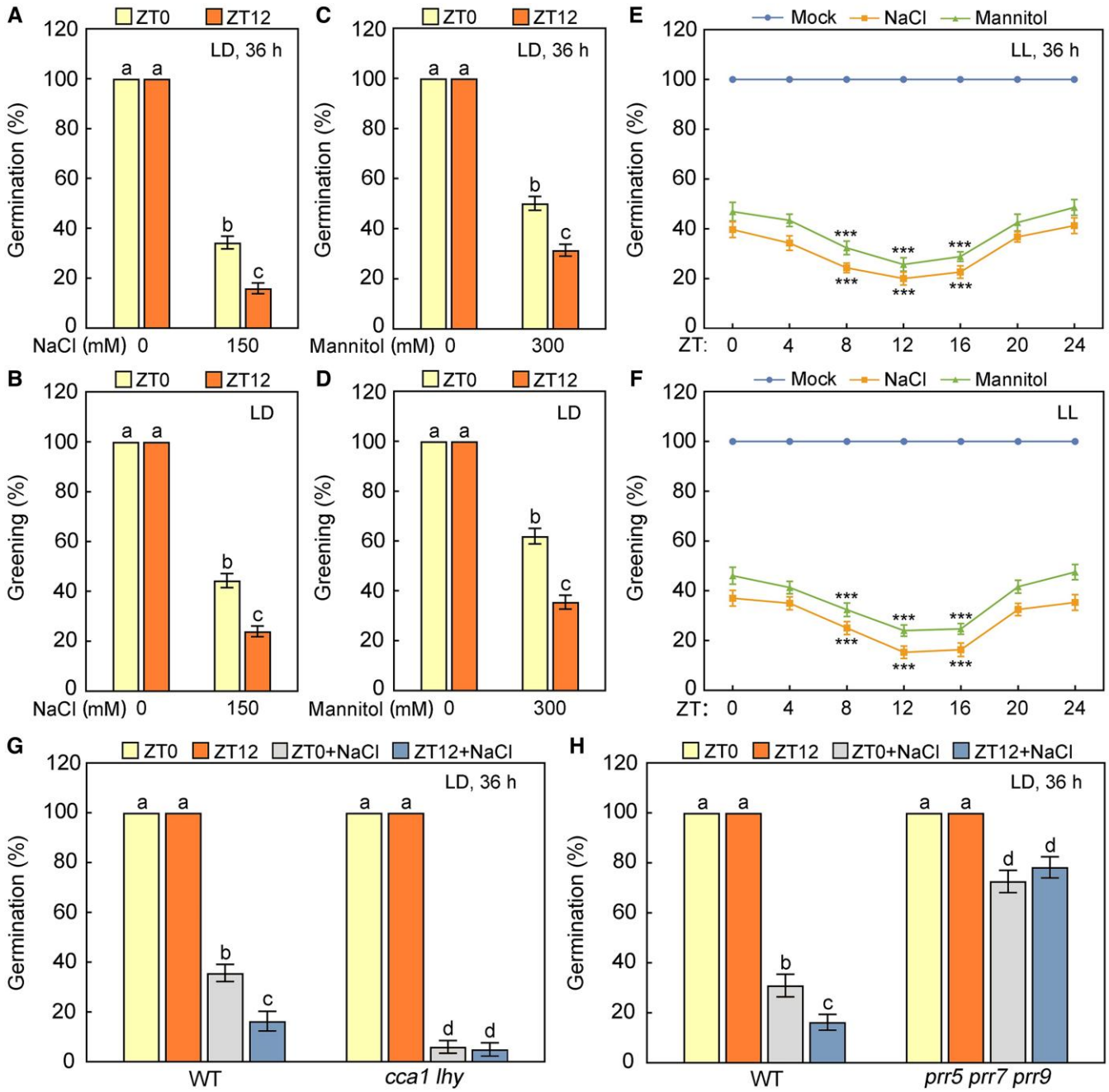

Figure 2. Seed germination in salinity and osmotic stress situations is temporally controlled under diurnal conditions. **A)** and **C)** Germination of the WT seeds on half-strength MS medium supplemented with or without 150 mM NaCl (A) or 300 mM mannitol **(C)** under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12. Seed germination was recorded 36 h after stratification. Time is expressed was hours from dawn (ZT0). LD, long-day conditions. **B)** and **D)** Cotyledon greening of the WT seeds on half-strength MS medium supplemented with or without 150 mM NaCl **(B)** or 300 mM mannitol **(D)** under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12. Cotyledon greening was scored 5 d after stratification. **E)** Germination of the WT seeds on half-strength MS medium supplemented with or without 150 mM NaCl or 300 mM mannitol under continuous light. The seeds were stratified at 4 °C and then transferred to 22 °C at different time points throughout the day (from ZT0 to ZT24), with each subsequent transfer (of the seeds) delayed by 4 h. Seed germination was recorded 36 h after stratification. LL, continuous light. **F)** Cotyledon greening of the WT seeds on half-strength MS medium supplemented with or without 150 mM NaCl or 300 mM mannitol under continuous light. The seeds were stratified at 4 °C and then transferred to 22 °C at different time points throughout the day. Cotyledon greening was scored 4 d after stratification. **G)** and **H)** Germination of the WT, *cca1 lhy* **(G)**, and *prr5 prr7 prr9* **(H)** seeds on half-strength MS medium supplemented with or without 150 mM NaCl or 300 mM mannitol under long-day conditions. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 or ZT12. Seed germination was recorded 36 h after stratification. All experiments for germination and cotyledon greening analysis were performed 5 times by examining different batches of seeds. Each batch of seeds was pooled from more than 60 independent plants. For each biological replicate, more than 120 seeds were examined. Values presented in this figure are means ± standard deviation. Data shown in **(A–D)** and **(G–H)** were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). Data shown in **(E** and **F)** were analyzed by a one-way ANOVA using Tukey's HSD test. ***, $P < 0.001$ (compared to ZT0).

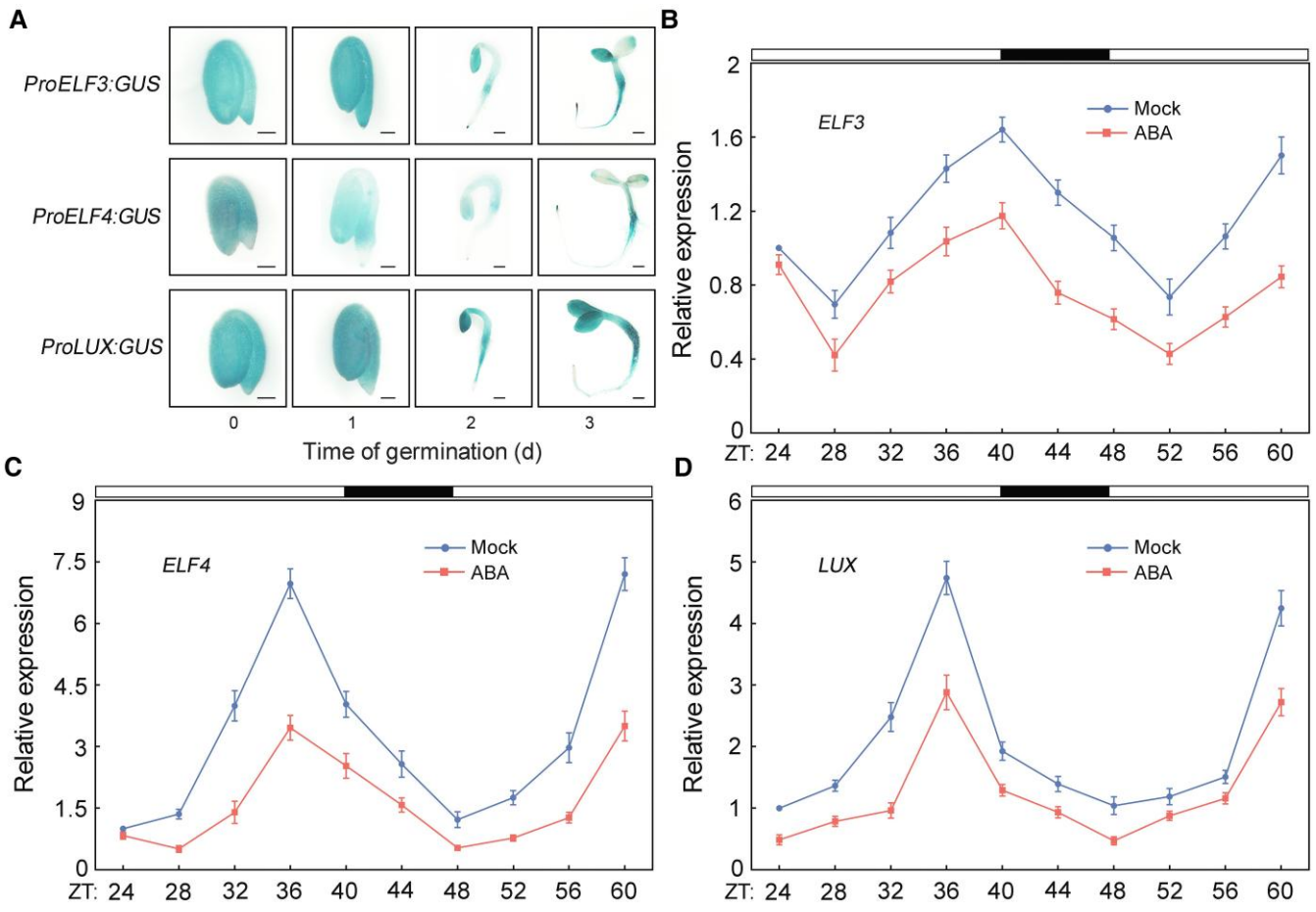

Figure 3. Expression patterns of *ELF3*, *ELF4*, and *LUX* in response to ABA during seed germination. **A)** GUS staining of *ProELF3:GUS*, *ProELF4:GUS*, and *ProLUX:GUS* transgenic seeds. Dry seeds (0 d) of the *proELF3:GUS*, *proELF4:GUS*, and *proLUX:GUS* transgenic lines were stripped of their seed coat prior to staining. The seeds were germinated on half-strength MS medium under long-day conditions for indicated times (0, 1, 2, or 3 d), and then the samples were harvested at ZT12 for staining. Experiments were performed 3 times with similar results. Bars = 100 μm for dry seeds (0 d) and 1d germinated seeds (1 d), 400 μm for 2d germinated seeds (2 d), and 500 μm for 3d germinated seeds (3 d). **B)** to **D)** RT-qPCR analysis of *ELF3* **(B)**, *ELF4* **(C)**, and *LUX* **(D)** expression in response to ABA. Total RNA was extracted from 5 different batches of germinating seeds (harvested from ZT24 to ZT60 after stratification) of WT with or without 0.75 μM ABA treatment grown under long-day conditions for indicated times. Time is expressed was hours from dawn (ZT0). In the mock treatment, an equal volume of 10% (v/v) ethanol was added. The *PP2A* (AT1G13320) gene was used as control. Values presented in this figure are means ± standard deviation. ABA, abscisic acid.

Disruption of ELF3, ELF4, or LUX results in germinating seed hypersensitivity to ABA and defective time-dependent germination

After demonstrating the inhibitory effect of ABA on *ELF3*, *ELF4*, and *LUX* expression during seed germination, we further investigated whether ELF3, ELF4, and LUX contribute to the regulation of ABA signaling and time-dependent seed germination. We first analyzed the seed germination of the loss-of-function *elf3-1*, *elf4-209*, and *lux-6* single mutants on half-strength MS medium supplemented with different ABA concentrations under long-day conditions. The *elf3-1*, *elf4-209*, and *lux-6* seeds released from cold stratification conditions at ZT12 (i.e. transferred to 22 °C) had substantially lower germination percentages than the WT seeds following treatments with different ABA concentrations (Fig. 4A). Moreover, in the presence of ABA, the cotyledon greening percentages were lower for the ZT12-released *elf3-1*, *elf4-209*, and *lux-6* seeds than for the WT seeds (Fig. 4B and C). To further elucidate how ELF3, ELF4, and LUX mediate ABA signaling and seed germination, we generated *elf3 lux* and *elf4 lux* double mutants and the *elf3 elf4 lux* triple mutant through genetic crosses. In response to ABA, the *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* double and triple mutant seeds released at ZT12 had lower germination and cotyledon greening percentages than the *elf3-1*, *elf4-209*, and *lux-6* single mutant seeds under long-day conditions (Fig. 4A to C). In accordance with these phenotypes, the transcript levels of the ABA-responsive genes *EM6* and *RAB18* were significantly higher in the germinating *elf3-1*, *elf4-209*, and *lux-6* seeds than in the germinating WT seeds after the ABA treatment, but they were even higher in the *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* seeds (Fig. 4D and E). Hence, ELF3, ELF4, and LUX may negatively modulate ABA signaling, thereby promoting seed germination.

To more precisely determine the ELF3, ELF4, and LUX functions affecting seed germination, we analyzed whether they also mediate seed germination under salinity and osmotic stress conditions. We examined the *elf3-1*, *elf4-209*, *lux-6*, and *elf3 elf4 lux* mutant seeds released at ZT12 on medium containing 150 mM NaCl or 300 mM mannitol. The *elf3-1*, *elf4-209*, *lux-6*, and *elf3 elf4 lux* mutant seeds on medium supplemented with NaCl or mannitol had much lower germination and cotyledon greening percentages than the WT controls under long-day conditions (Supplementary Fig. S7A to D). These observations imply that ELF3, ELF4, and LUX also stimulate seed germination under salinity and osmotic stress conditions.

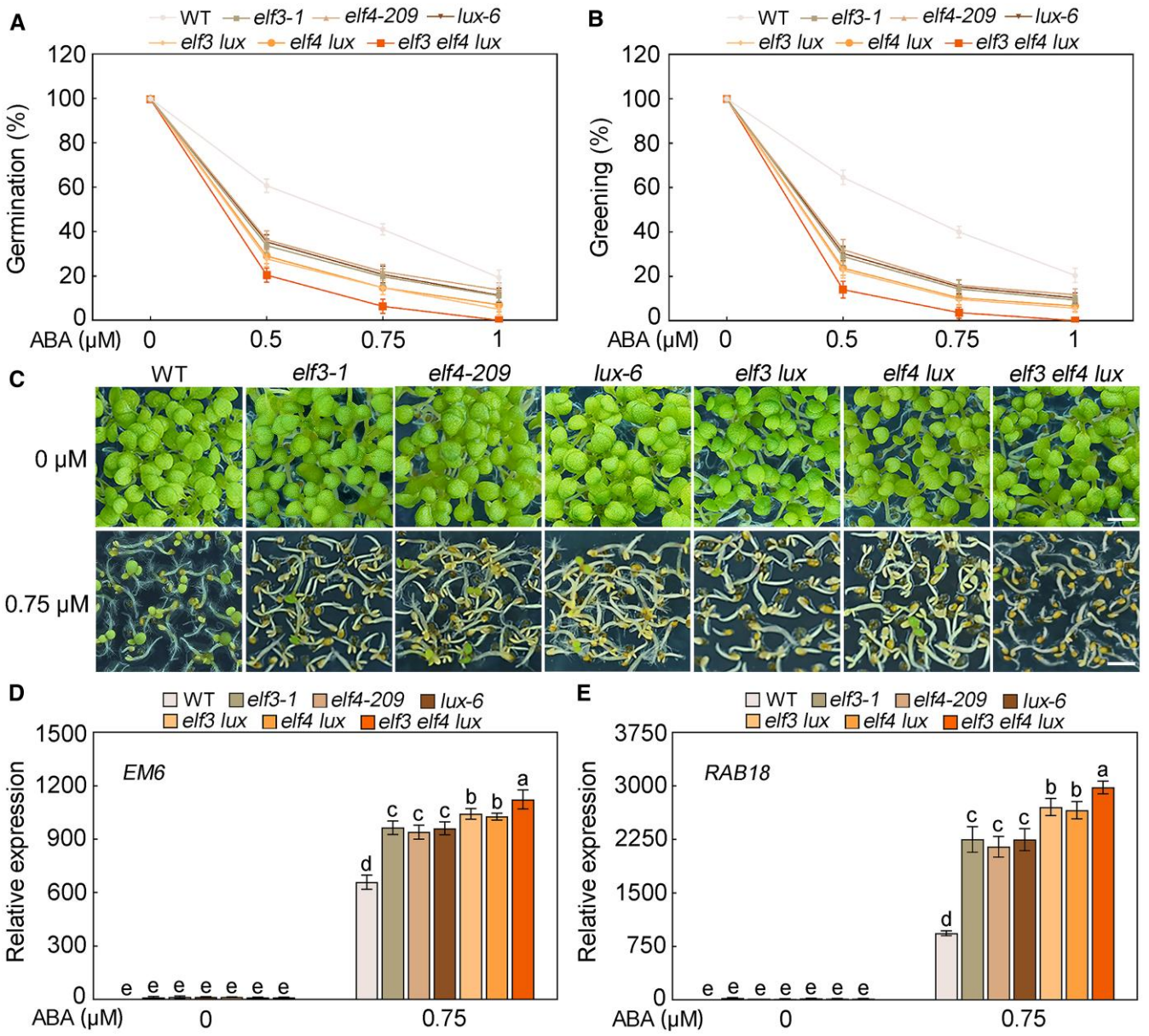

Figure 4. ABA responses of *elf3-1*, *elf4-209*, *lux-6*, *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* mutants during seed germination. **A)** Germination of the WT, *elf3-1*, *elf4-209*, *lux-6*, *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Seed germination was recorded 48 h after stratification on half-strength MS medium supplemented with different concentrations of ABA under long-day conditions. In the medium without ABA (i.e. 0 μM), an equal volume of 10% (v/v) ethanol was added. Time is expressed was hours from dawn (ZT0). **B)** Cotyledon greening of the WT, *elf3-1*, *elf4-209*, *lux-6*, *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Cotyledon greening was recorded 6 d after stratification on half-strength MS medium supplemented with different concentrations of ABA under long-day conditions. The experiments were performed 5 times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 60 individual plants. For each biological replicate, more than 120 seeds were examined. **C)** Seedlings of WT, *elf3-1*, *elf4-209*, *lux-6*, *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* mutants 6 d after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. Bar = 2.5 mm. **D)** and **E)** RT-qPCR analysis of the ABA-induced expression of *EM6* **(D)** and *RAB18* **(E)** in germinating WT, *elf3-1*, *elf4-209*, *lux-6*, *elf3 lux*, *elf4 lux*, and *elf3 elf4 lux* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.75 μM ABA on half-strength MS medium. The *PP2A* (AT1G13320) gene was used as a control. Values presented in this figure are means ± standard deviation. Data were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). ABA, abscisic acid.

Because mutations to ELF3, ELF4, and LUX resulted in defective germination in the presence of ABA or under salinity and osmotic stress situations, we analyzed the effects of ELF3, ELF4, and LUX on the temporal control of seed germination. We compared the germination and cotyledon greening of the *elf3-1*, *elf4-209*, *lux-6*, and *elf3 elf4 lux* seeds released at ZT0 with those of the seeds released at ZT12 on medium containing 0.75 μM ABA, 150 mM NaCl, or 300 mM mannitol. As anticipated, compared with the WT seeds, the *elf3-1*, *elf4-209*, *lux-6*, and *elf3 elf4 lux* seeds had considerably lower germination and cotyledon greening percentages on medium containing ABA, NaCl, or mannitol under long-day conditions, regardless of when the seeds were released from cold stratification conditions (Supplementary Fig. S7A to F). Interestingly, unlike the WT seeds, the ZT0- and ZT12-released

elf3-1, *elf4-209*, *lux-6*, or *elf3 elf4 lux* seeds had similar germination and cotyledon greening percentages after the treatment with ABA, NaCl, or mannitol (Supplementary Fig. S7A to F). Parallel experiments showed that the *elf3-1*, *elf4-209*, *lux-6*, and *elf3 elf4 lux* seeds behaved similarly under continuous light (Supplementary Fig. S7G to L). These results suggest that ELF3, ELF4, and LUX are essential for time-dependent seed germination in response to ABA or under salinity and osmotic stress conditions.

Overexpression of *ELF3*, *ELF4*, or *LUX* attenuates ABA signaling during seed germination

To further reveal the regulatory effects of ELF3, ELF4, and LUX on ABA signaling and seed germination, we generated transgenic plants overexpressing FLAG-tagged *ELF3* (*FLAG-ELF3-3*), *ELF4* (*FLAG-ELF4-2* and *FLAG-ELF4-7*), or *LUX* (*FLAG-LUX-6*) under the control of the cauliflower mosaic virus 35S promoter (*Pro35S*). Additionally, transgenic lines overexpressing *ELF3* (*ELF3-OE-1*; Ding et al. 2018) or *LUX* (*LUX-OE-58* from AraShare) were also analyzed. Similar to the results of previous studies (Doyle et al. 2002; McWatters et al. 2007; Yu et al. 2008; Yoshida et al. 2009; Hirohata et al. 2022), the *ELF3*-, *ELF4*-, or *LUX*-overexpressing plants flowered later than the WT controls. We subsequently analyzed these transgenic plants on medium containing various ABA concentrations. Germination and seedling growth were much faster for the *ELF3*-, *ELF4*-, or *LUX*-overexpressing seeds released at ZT12 than for the corresponding WT seeds in the presence of ABA (Fig. 5A to C). In accordance with these findings, the ABA-induced expression of *EM6* and *RAB18* was significantly lower in the germinating *ELF3*-, *ELF4*-, or *LUX*-overexpressing seeds than in the germinating WT seeds (Fig. 5D and E). Accordingly, the overexpression of *ELF3*, *ELF4*, or *LUX* apparently decreased the sensitivity of the germinating seeds to ABA. Considered together, these results further support the notion that the EC components ELF3, ELF4, and LUX inhibit ABA signaling and enhance seed germination in Arabidopsis.

ELF3, ELF4, and LUX physically interact with ABI3 and ABI5

Having demonstrated the regulatory effects of ELF3, ELF4, and LUX on ABA signaling and seed germination, we further investigated the underlying molecular mechanisms. Considering both ABI3 and ABI5 are transcription factors (essential regulators of ABA-repressed seed germination) that bind to several transcriptional modulators to integrate ABA signaling with other signaling pathways (Lim et al. 2014; Yu et al. 2015; Ju et al. 2019; Pan et al. 2020; Yang et al. 2021; Mei et al. 2023), we wondered whether they also directly associate with ELF3, ELF4, and/or LUX, thereby mediating the crosstalk between ABA and circadian clock signaling pathways. To test this possibility, we used a yeast two-hybrid system to examine the possible interactions of ELF3, ELF4, and LUX with ABI3 or ABI5. Specifically, sequences encoding the full-length ELF3, ELF4, and LUX proteins were ligated to the sequence encoding the Gal4 activation domain (AD) in the prey vector (AD-ELF3, AD-ELF4, and AD-LUX). The full-length *ABI3* and *ABI5* sequences were fused to the Gal4 DNA-binding domain (BD) of the bait vector to produce BD-ABI3 and BD-ABI5. In yeast (*Saccharomyces cerevisiae*) cells, ELF3, ELF4, and LUX strongly interacted with both ABI3 and ABI5 (Fig. 6A). Earlier research showed ABI4 is another transcription factor that functions as a critical stimulator of ABA signaling during seed germination (Finkelstein et al. 1998; Söderman et al. 2000; Chandrasekaran et al. 2020; Luo et al. 2024). Thus, the full-length *ABI4* sequence was also cloned and ligated to Gal4 BD of the bait vector to generate BD-ABI4. However, ABI4 did not interact with ELF3, ELF4, or LUX in yeast cells (Fig. 6A), indicative of the specificity of the physical associations between ELF3, ELF4, or LUX and ABI3/ABI5.

To identify the ABI3 and ABI5 regions critical for the interactions with ELF3, ELF4, and LUX, we performed directed yeast two-hybrid analyses. Sequences encoding two truncated ABI3 fragments and five truncated ABI5 fragments were produced and fused with Gal4 AD of the prey vector (Fig. 6B; Supplementary Fig. S8A). The yeast two-hybrid results indicated that the N-terminal domain of ABI3 (amino acids 1 to 416) mediates the ABI3–ELF3 interaction in yeast, whereas the full-length ABI3 is necessary for the interactions with ELF4 and LUX (Fig. 6B). Parallel analyses demonstrated that amino acids 350 to 442 (spanning the C-terminal bZIP domain) of ABI5 are sufficient for the interactions with ELF3, ELF4, and LUX. An ABI5 variant in which the N-terminal amino acids 1 to 349 were removed (AD-ABI5$^{350\text{-}442}$) was still able to interact with ELF3, ELF4, and LUX (Supplementary Fig. S8A). We conducted similar experiments to identify the ELF3 and LUX fragments crucial for the binding to ABI3 and ABI5. The results indicated that the middle region of ELF3 (amino acids 261 to 440) is responsible for the interactions with ABI3 and ABI5 in yeast (Supplementary Fig. S8B). More specifically, the amino acid residues ranging from 261 to 380 and those from 321 to 440 in the middle region of ELF3 are sufficient for interacting with ABI3 and ABI5, respectively (Supplementary Fig. S8C). For LUX, its middle region (amino acids 143 to 201) containing the GARP domain interacted with ABI3 in yeast, whereas the C-terminal fragment (amino acids 201 to 324) was required for the interaction with ABI5 (Supplementary Fig. S8D).

To confirm the interactions of ELF3, ELF4, and LUX with ABI3 and ABI5 in planta, we conducted bimolecular fluorescence complementation (BiFC) assays using *Nicotiana benthamiana* plants. The sequence encoding the N-terminal of the yellow fluorescent protein (nYFP) under the control of *Pro35S* was ligated to the full-length *ELF3*, *ELF4*, or *LUX* sequence to generate ELF3-nYFP, ELF4-nYFP, or LUX-nYFP. The sequence encoding the C-terminal YFP (cYFP) fragment under the control of *Pro35S* was fused with the full-length coding sequence of *ABI3* or *ABI5* to produce ABI3-cYFP or ABI5-cYFP. When ELF3-nYFP, ELF4-nYFP, or LUX-nYFP was transiently co-expressed with ABI3-cYFP or ABI5-cYFP in *N. benthamiana* leaves, strong YFP fluorescence was detected in the nucleus of transformed cells stained with 4′,6-diamidino-2-phenylindole (DAPI; Fig. 6C). No YFP signal was detected in the negative controls in which ELF3-nYFP, ELF4-nYFP, or LUX-nYFP was co-expressed with ABI3$^{417\text{-}720}$-cYFP (C-terminal amino acids 417 to 720 of ABI3 fused to cYFP) or ABI5$^{1\text{-}164}$-cYFP (N-terminal amino acids 1 to 164 of ABI5 fused to cYFP) (Fig. 6C; Supplementary Fig. S9A and B). Moreover, fluorescence was also undetectable when ELF3$^{1\text{-}261}$-nYFP (N-terminal amino acids 1 to 261 of ELF3 fused to nYFP) or LUX$^{1\text{-}143}$-nYFP (N-terminal amino acids 1 to 143 of LUX fused to nYFP) was co-expressed with ABI3-cYFP or ABI5-cYFP (Supplementary Fig. S9A and B).

Co-immunoprecipitation (CoIP) assay results provided evidence of the interaction between ELF3 and ABI3 or ABI5 in *Arabidopsis* simultaneously overexpressing *ELF3* and *ABI3* or *ABI5* (*FLAG-ELF3-3 MYC-ABI3* or *FLAG-ELF3-3 ABI5-MYC*; Fig. 6D). These plants were developed by introducing the *ELF3* overexpression construct into *ABI3*-overexpressing (*MYC-ABI3*; containing a functional 3MYC-ABI3 construct driven by *Pro35S*) and *ABI5*-overexpressing (*ABI5-MYC*; containing a functional ABI5-4MYC construct under

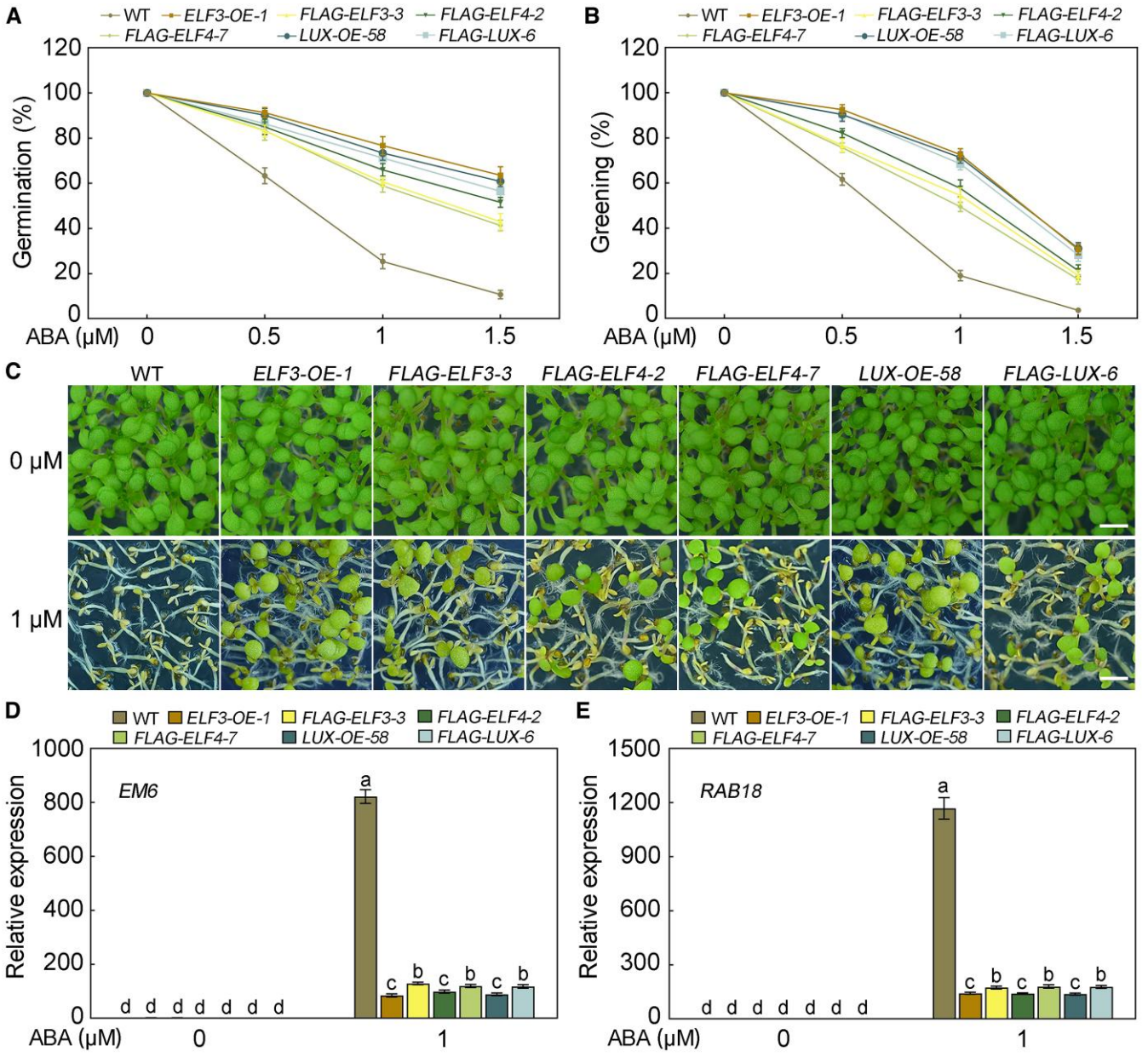

Figure 5. ABA responses of transgenic plants overexpressing *ELF3*, *ELF4*, or *LUX* during seed germination. **A)** Seed germination of the WT, *ELF3*-overexpressing plants (*ELF3-OE-1* and *FLAG-ELF3-3*), *ELF4*-overexpressing plants (*FLAG-ELF4-2* and *FLAG-ELF4-7*), and *LUX*-overexpressing plants (*LUX-OE-58* and *FLAG-LUX-6*). The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Seed germination was recorded 48 h after stratification on half-strength MS medium supplemented with different concentrations of ABA under long-day conditions. In the medium without ABA (i.e. 0 μM), an equal volume of 10% (v/v) ethanol was added. Time is expressed was hours from dawn (ZT0). **B)** Cotyledon greening of the WT, *ELF3-OE-1*, *FLAG-ELF3-3*, *FLAG-ELF4-2*, *FLAG-ELF4-7*, *LUX-OE-58*, and *FLAG-LUX-6* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Cotyledon greening was recorded 6 d after stratification on half-strength MS medium supplemented with different concentrations of ABA under long-day conditions. The experiments were performed 5 times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 60 individual plants. For each biological replicate, more than 120 seeds were examined. **C)** Seedlings of WT, *ELF3-OE-1*, *FLAG-ELF3-3*, *FLAG-ELF4-2*, *FLAG-ELF4-7*, *LUX-OE-58*, and *FLAG-LUX-6* 7 d after stratification on half-strength MS medium supplemented with 1 μM ABA under long-day conditions. Bar = 2.5 mm. **D)** and **E)** RT-qPCR analysis of the ABA-induced expression of *EM6* **(D)** and *RAB18* **(E)** in germinating WT, *ELF3-OE-1*, *FLAG-ELF3-3*, *FLAG-ELF4-2*, *FLAG-ELF4-7*, *LUX-OE-58*, and *FLAG-LUX-6* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 1 μM ABA on half-strength MS medium. The *PP2A* (AT1G13320) gene was used as a control. Values presented in this figure are means ± standard deviation. Data were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). ABA, abscisic acid.

the control of *Pro35S*) plants. These findings suggest that the EC components ELF3, ELF4, and LUX directly associate with the transcription factors ABI3 and ABI5 in the plant cell nucleus, implying that they may function together to mediate the convergence of clock and ABA signaling pathways during seed germination.

ELF3, ELF4, and LUX function upstream of ABI3 and ABI5 to modulate ABA signaling during seed germination

Both ABI3 and ABI5 are critical positive regulators of ABA signaling during seed germination. The loss-of-function *abi3* and

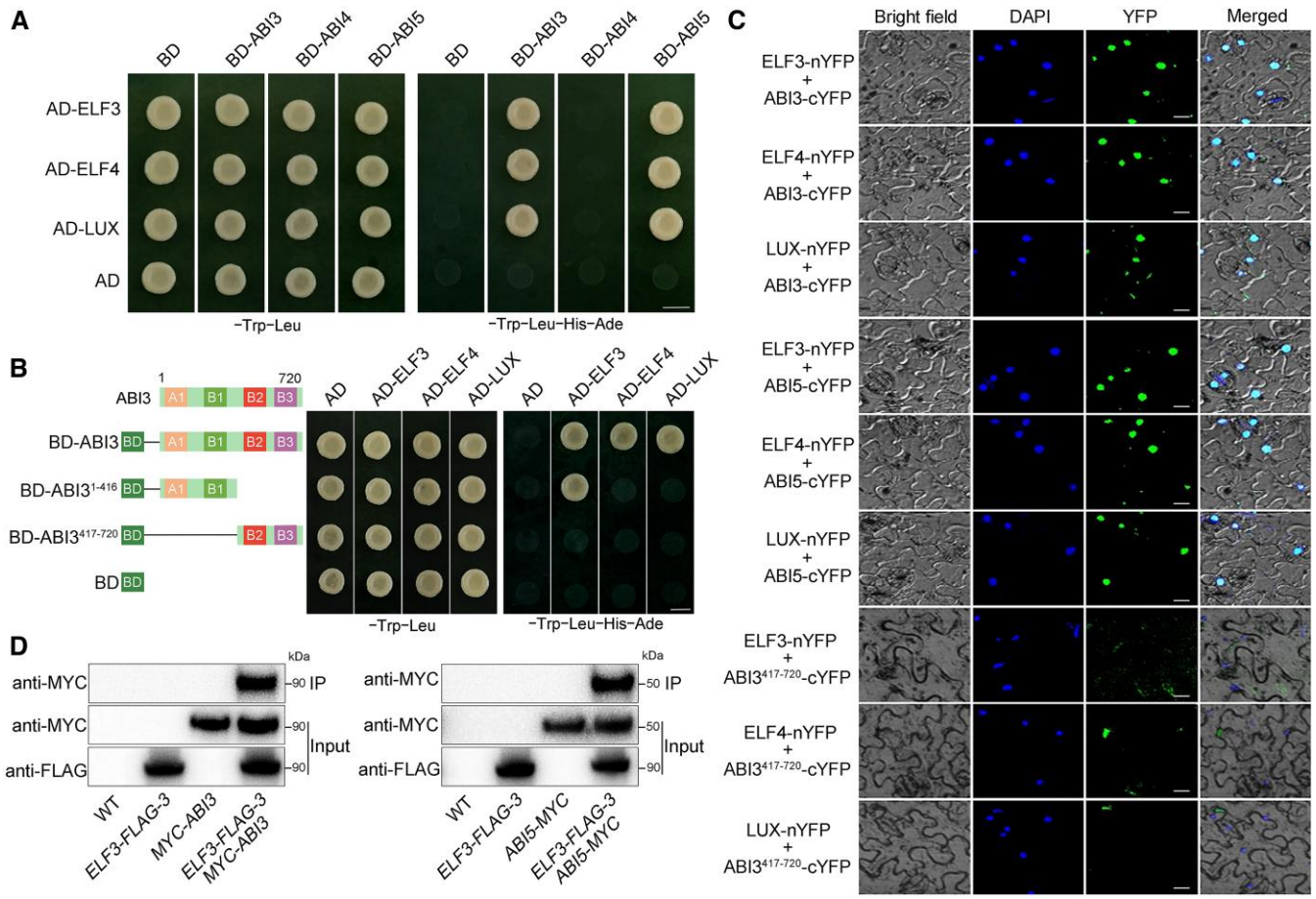

Figure 6. Physical interactions of ELF3, ELF4, or LUX with ABI3 and ABI5. **A)** Yeast two-hybrid assays. Interactions of ELF3, ELF4, or LUX with ABI3 and ABI5 were indicated by the ability of cells to grow on dropout medium lacking Leu, Trp, His, and Ade and containing 20 mM 3-aminotriazole after a 4-d incubation. No obvious interaction between ABI4 and ELF3, ELF4, or LUX was observed. pGBKT7 (BD) and pGADT7 (AD) were used as negative controls. Bar = 5 mm. **B)** Mapping the region(s) of ABI3 responsible for the interaction with ELF3, ELF4, or LUX. Left: Diagram of the full-length and truncated ABI3 constructs with specific deletions. Right: Interaction was indicated by the ability of cells to grow on dropout medium lacking Leu, Trp, His, and Ade and containing 15 mM 3-aminotriazole after a 4-d incubation. BD and AD were used as negative controls. Bar = 5 mm. **C)** BiFC assays. Fluorescence was detected in the nuclear compartment of transformed *N. benthamiana* cells, resulting from the complementation of ELF3-nYFP, ELF4-nYFP, or LUX-nYFP with ABI3-cYFP or ABI5-cYFP. No YFP signal was detected in the negative controls in which ELF3-nYFP, ELF4-nYFP, or LUX-nYFP was co-expressed with ABI3$^{417-720}$-cYFP (C-terminal amino acids 417 to 720 of ABI3 fused to cYFP). Nuclei are indicated by DAPI staining. Bars = 15 μm. **D)** CoIP assays. Total proteins were extracted from germinating seeds (3 d) of Arabidopsis simultaneously overexpressing *ELF3* and *ABI3* (*FLAG-ELF3-3 MYC-ABI3*) or *ELF3* and *ABI5* (*FLAG-ELF3-3 ABI5-MYC*) under the control of *Pro35S*. FLAG-fused ELF3 was immunoprecipitated using an anti-FLAG antibody (1:250) and the co-immunoprecipitated MYC-ABI3 (left) or ABI5-MYC (right) was detected using an anti-MYC antibody (1:10,000). The *FLAG-ELF3-3*, *MYC-ABI3*, and *ABI5-MYC* plants served as the controls. Protein input for FLAG-fused ELF3 in the immunoprecipitated complexes was also detected and is shown. Experiments were performed 3 times with similar results. DIC, differential interference contrast. DAPI, 4′,6-diamidino-2-phenylindole. YFP, yellow fluorescence protein. IP, immunoprecipitation.

abi5 mutant seeds are much less sensitive to ABA than WT seeds (Giraudat et al. 1992; Finkelstein 1994; Finkelstein and Lynch 2000; Nambara et al. 2002). Because of the confirmed physical interactions of ELF3, ELF4, or LUX with ABI3 and ABI5, all of which modulate ABA signaling-related delayed seed germination, we wondered whether the functions of ELF3, ELF4, and LUX influencing ABA signaling are associated with the functions of ABI3 and ABI5. For these analyses, we crossed *elf3-1*, *elf4-209*, and *lux-6* with *abi3-8* (*ABI3* loss-of-function mutant; Nambara et al. 2002) to generate the *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* double mutants. Similarly, *elf3-1*, *elf4-209*, and *lux-6* were crossed with *abi5-8* (*ABI5* loss-of-function mutant; Zhou et al. 2015a) to produce the *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* double mutants. The analysis of seed germination and/or cotyledon greening on medium containing either 0.75 μM or a higher concentration of 2 μM ABA revealed that compared with the WT seeds, the *elf3-1*, *elf4-209*, and *lux-6* seeds released from cold stratification conditions at ZT12 (i.e. transferred to 22 °C) were more sensitive to ABA, which was in contrast to the hyposensitivity of the *abi3-8* and *abi5-8* seeds to ABA (Figs. 7A to C and 8A to C, Supplementary Figs. S10A to C and S11A to C). Similar to the *abi3-8* seeds, the *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* double mutant seeds were also hyposensitive to 0.75 or 2 μM ABA, with much higher germination and cotyledon greening percentages than the WT, *elf3-1*, *elf4-209*, and *lux-6* seeds (Fig. 7A to C, Supplementary Fig. S10A to C). Parallel investigations showed that the germination and cotyledon greening percentages of the *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* double mutant seeds were similar to those of the *abi5-8* seeds in response to 0.75 or 2 μM ABA (Fig. 8A to C, Supplementary Fig. S11A to C). To verify the phenotypic observations, we analyzed the transcript levels of the ABA-responsive *EM6* and *RAB18* genes in ABA-treated germinating *elf3 abi3-8*, *elf4 abi3-8*, *lux abi3-8*, *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* seeds. The relative transcript levels of *EM6* and *RAB18* were substantially lower in the *elf3 abi3-8*, *elf4*

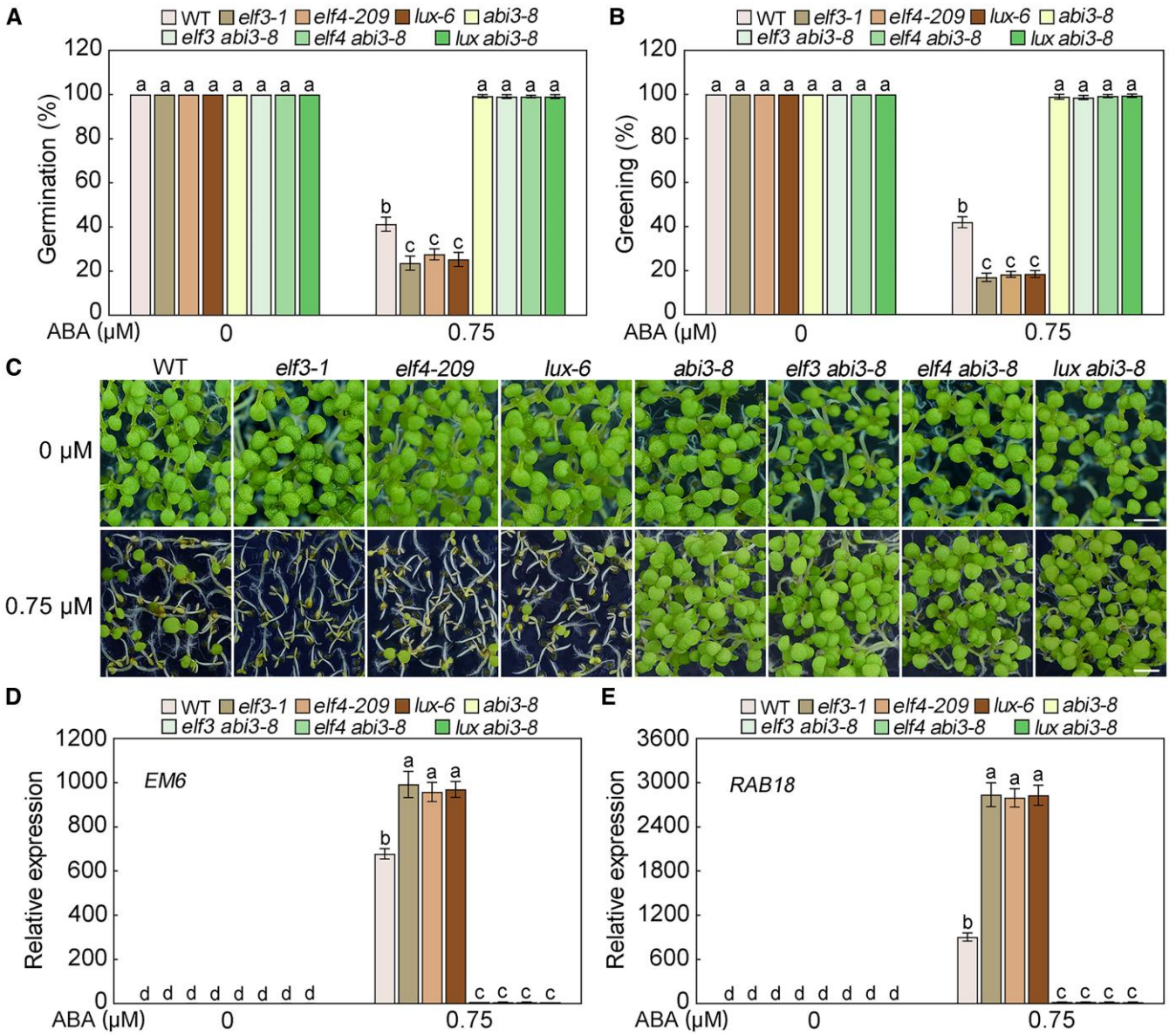

Figure 7. The ABA hypersensitivity of *elf3-1*, *elf4-209*, and *lux-6* mutants during seed germination is restored by the *ABI3* mutation. **A)** Germination of the WT, *elf3-1*, *elf4-209*, *lux-6*, *abi3-8*, *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Seed germination was recorded 48 h after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. In the medium without ABA (i.e. 0 μM), an equal volume of 10% (v/v) ethanol was added. Time is expressed was hours from dawn (ZT0). **B)** Cotyledon greening of the WT, *elf3-1*, *elf4-209*, *lux-6*, *abi3-8*, *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Cotyledon greening was recorded 6 d after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. The experiments were performed 5 times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 60 individual plants. For each biological replicate, more than 120 seeds were examined. **C)** Seedlings of WT, *elf3-1*, *elf4-209*, *lux-6*, *abi3-8*, *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* mutants 7 d after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. Bar = 2.5 mm. **D)** and **E)** RT-qPCR analysis of the ABA-induced expression of *EM6* **(D)** and *RAB18* **(E)** in germinating WT, *elf3-1*, *elf4-209*, *lux-6*, *abi3-8*, *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.75 μM ABA on half-strength MS medium. The *PP2A* (AT1G13320) gene was used as a control. Values presented in this figure are means ± standard deviation. Data were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). The statistical analyses described apply to all statistical analyses presented in this figure. ABA, abscisic acid.

abi3-8, *lux abi3-8*, *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* mutants than in the WT control and *elf3-1*, *elf4-209*, and *lux-6* mutants (Figs. 7D and E and 8D and E, Supplementary Figs. S10D and E and S11D and E). Accordingly, mutating *ABI3* or *ABI5* suppressed the ABA hypersensitivity of *elf3-1*, *elf4-209*, and *lux-6*, suggesting that *ABI3* and *ABI5* are epistatic to *ELF3*, *ELF4*, and *LUX* during the modulation of ABA-mediated seed germination inhibition.

ELF3, ELF4, and LUX negatively modulate ABI3 and ABI5 functions

Because ELF3, ELF4, and LUX along with ABI3 and ABI5 mediate ABA responses during seed germination, we examined the molecular basis of their regulatory relationships. Previous studies indicated that several modulators affect ABI3 and/or ABI5 mainly by altering their functions via direct protein–protein interactions

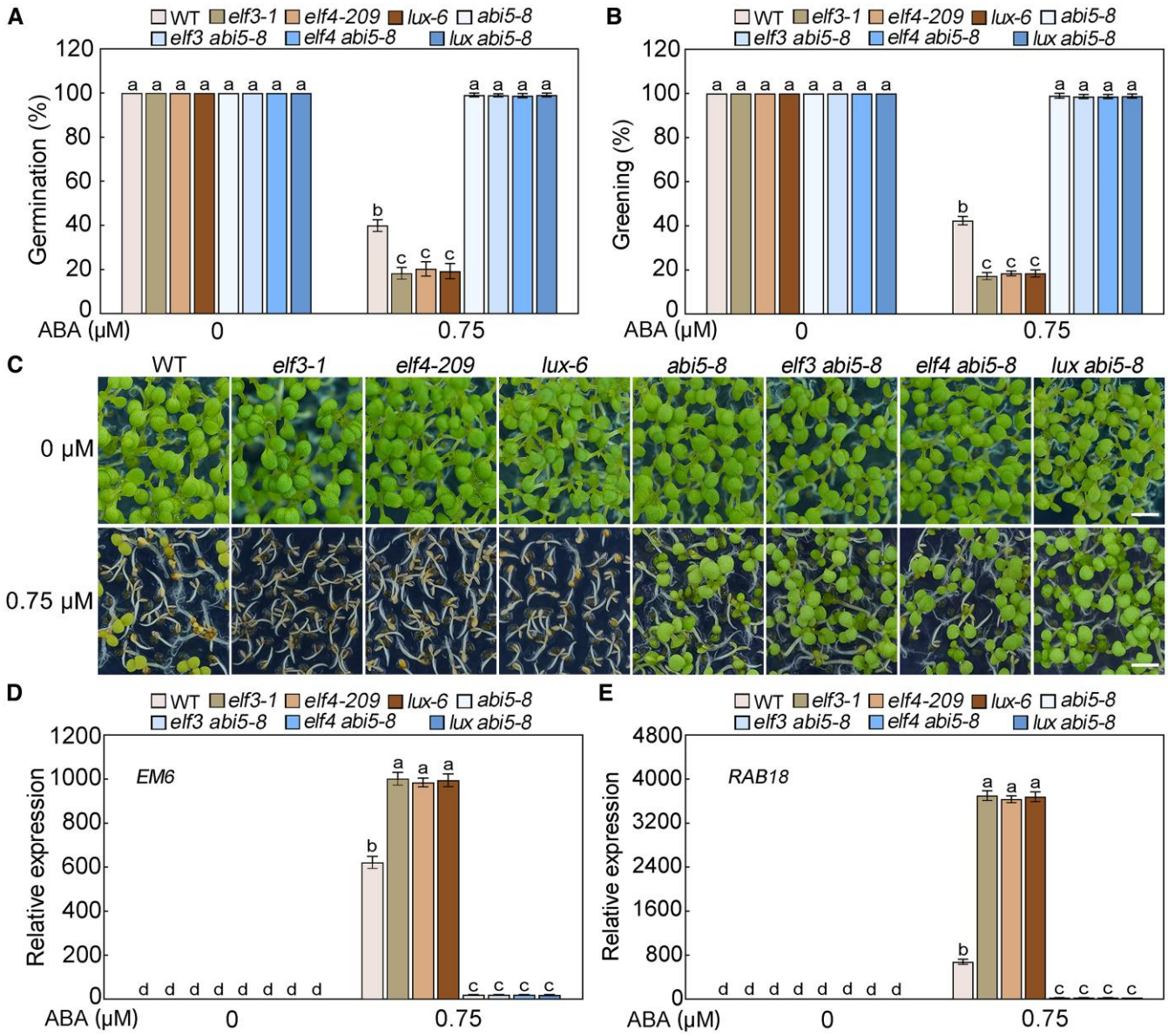

Figure 8. The ABA hypersensitivity of *elf3-1*, *elf4-209*, and *lux-6* mutants during seed germination is restored by the *ABI5* mutation. **A)** Germination of the WT, *elf3-1*, *elf4-209*, *lux-6*, *abi5-8*, *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Seed germination was recorded 48 h after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. In the medium without ABA (i.e. 0 μM), an equal volume of 10% (v/v) ethanol was added. Time is expressed was hours from dawn (ZT0). **B)** Cotyledon greening of the WT, *elf3-1*, *elf4-209*, *lux-6*, *abi5-8*, *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Cotyledon greening was recorded 6 d after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. The experiments were performed 5 times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 60 individual plants. For each biological replicate, more than 120 seeds were examined. **C)** Seedlings of WT, *elf3-1*, *elf4-209*, *lux-6*, *abi5-8*, *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* mutants 7 d after stratification on half-strength MS medium supplemented with 0.75 μM ABA under long-day conditions. Bar = 2.5 mm. **D)** and **E)** RT-qPCR analysis of the ABA-induced expression of *EM6* **(D)** and *RAB18* **(E)** in germinating WT, *elf3-1*, *elf4-209*, *lux-6*, *abi5-8*, *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.75 μM ABA on half-strength MS medium. The *PP2A* (AT1G13320) gene was used as a control. Values presented in this figure are means ± standard deviation. Data were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). The statistical analyses described apply to all statistical analyses presented in this figure. ABA, abscisic acid.

(Lim et al. 2014; Kim et al. 2016; Ju et al. 2019; Zhao et al. 2019; Pan et al. 2020; Yang et al. 2021, 2023; Guo et al. 2022; Mei et al. 2023). Hence, we investigated the possible regulatory effects of ELF3, ELF4, and LUX on ABI3 and ABI5 functions in *Arabidopsis* mesophyll protoplasts by performing dual-luciferase (LUC) reporter assays (Yoo et al. 2007). Because *EM6* is a downstream target of ABI3 and ABI5 associated with ABA signaling (Lopez-Molina and Chua 2000; Nakamura et al. 2001; Carles et al. 2002; Lopez-Molina et al. 2002), its promoter was cloned and ligated to the *LUC* gene to generate a reporter construct (Supplementary Fig. S12A). The effectors contained *ELF3*, *ELF4*, *LUX*, *ABI3*, *ABI5*, or *GFP* (encoding the green fluorescent protein) under the control of *Pro35S* (Supplementary Fig. S12A). We expressed ABI3 (or ABI5) with similar accumulation levels in the WT mesophyll protoplasts with or

without ELF3, ELF4, and/or LUX expression (Supplementary Fig. S12B and C). Compared with the effect of GFP alone, both ABI3 and ABI5 obviously increased the expression of *LUC* driven by the *EM6* promoter in WT mesophyll protoplasts treated with 5 μM ABA (Fig. 9A and B). However, in response to ABA, the *LUC* expression level was lower in the protoplasts co-expressing ELF3, ELF4, or LUX and ABI3 (or ABI5) than in the protoplasts co-expressing ABI3 (or ABI5) and GFP (Fig. 9A and B). Moreover, the simultaneous expression of ELF3, ELF4, and LUX with ABI3 (or ABI5) resulted in even lower *LUC* expression levels in the presence of ABA (Fig. 9A and B). These results suggest that ELF3, ELF4, and LUX repress ABI3 and ABI5 functions, thereby influencing the transcription of the downstream gene *EM6*.

To verify the regulatory effects of ELF3, ELF4, and LUX on ABI3 and ABI5, we examined whether loss-of-function mutations to ELF3, ELF4, and LUX alter ABI3 and ABI5 functions. We assessed the abilities of ABI3 and ABI5 to activate *EM6* transcription in *elf3-1*, *elf4-209*, and *lux-6* protoplasts. Following the ABA treatment, *LUC* expression under the control of the *EM6* promoter was much higher in *ABI3*- or *ABI5*-expressing *elf3-1*, *elf4-209*, and *lux-6* protoplasts than in the WT protoplasts (Fig. 9C and D, Supplementary Fig. S12D and E). These observations provide further evidence that the detrimental effects of ELF3, ELF4, and LUX on ABI3 and ABI5 functions modulate *EM6* expression in the presence of ABA. Additionally, our results demonstrated that the expression of *LUC*, driven by the *EM6* promoter, exhibited a moderate increase in *GFP*-expressing protoplasts of *elf3-1*, *elf4-209*, and *lux-6* mutants after ABA treatment, compared to WT protoplasts (Fig. 9C and D). This finding hints at the possibility that ELF3, ELF4, and LUX may associate with certain endogenous factors induced by ABA to regulate the expression levels of *EM6*.

Considering the EC components ELF3, ELF4, and LUX and ABI3/ABI5 have antagonistic effects on the ABA-induced expression of *EM6*, we analyzed whether EC directly mediates *EM6* transcription. Previous studies showed that in the ELF3-ELF4-LUX ternary transcriptional repression complex, LUX (i.e. transcription factor) is responsible for targeting the promoter regions through its specific DNA-BD (Helfer et al. 2011; Nusinow et al. 2011; Zhang et al. 2018). LUX directly associates with DNA via the corresponding LUX binding site (LBS; GATT/ACG) within its gene targets (Helfer et al. 2011; Zhang et al. 2019; Zha et al. 2020). Sequence analyses identified two putative LBS elements in the *EM6* promoter region (1,500 bp segment upstream of the translation start site), raising the possibility that LUX is capable of recognizing these motifs. However, our yeast one-hybrid assays revealed that LUX failed to interact with the *EM6* promoter region, despite positive control experiments showing that LUX could bind to the promoter region of *DELAY OF GERMINATION1* (*DOG1*) and ABI5 associated with the promoter region of *EM6* (Supplementary Fig. S13; Carles et al. 2002; Zha et al. 2020).

Although LUX does not bind to the *EM6* promoter, ELF3, ELF4, and LUX may affect the enrichment of ABI3 and/or ABI5 at the *EM6* promoter in response to ABA. To test this possibility, we performed chromatin immunoprecipitation (ChIP) assays involving *ABI3*- or *ABI5*-overexpressing *elf3-1*, *elf4-209*, and *lux-6* plants (i.e. *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC*). Both ABI3 and ABI5 were more enriched in the *EM6* promoter region (*pEM6-1* in Supplementary Table S1) in the ABA-treated germinating seeds when *ABI3* and *ABI5* were overexpressed in the *elf3-1*, *elf4-209*, and *lux-6* mutant backgrounds than when they were overexpressed in the WT background (Fig. 9E and F). These results imply that ELF3, ELF4, and LUX suppress the enrichment of ABI3 and ABI5 in the promoter region of downstream target genes (e.g. *EM6*).

ELF3, ELF4, and LUX destabilize ABI3 and ABI5

Previous studies showed that the accumulation of ABI3 and ABI5 is strictly controlled by multiple regulators and signaling pathways (Kobayashi et al. 2005; Zhang et al. 2005; Furihata et al. 2006; Fujii et al. 2007; Piskurewicz et al. 2008; Fujii and Zhu 2009; Nakashima et al. 2009; Lee et al. 2010; Guan et al. 2014; Hu and Yu 2014; Seo et al. 2014; Peng et al. 2022; Li et al. 2023). Thus, we investigated whether ELF3, ELF4, and LUX also modulate the abundance of ABI3 and ABI5 during seed germination. We analyzed the ABI3 and ABI5 protein levels in germinating *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds with or without an ABA treatment. As previously reported (Lopez-Molina et al. 2001; Piskurewicz et al. 2008; Chen et al. 2012; Li et al. 2023), the MYC-ABI3 and ABI5-MYC fusion proteins were stabilized by ABA in germinating *MYC-ABI3* and *ABI5-MYC* seeds, respectively (Fig. 9G and H). Interestingly, the MYC-ABI3 content was clearly higher in germinating *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, and *lux MYC-ABI3* seeds than in *MYC-ABI3* seeds with or without the ABA treatment (Fig. 9G). Similarly, ABI5-MYC was more abundant in the germinating *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds than in the germinating *ABI5-MYC* seeds in the absence or presence of ABA (Fig. 9H). Furthermore, when both ABA and the protein synthesis inhibitor cycloheximide (CHX) were applied simultaneously, the abundance of MYC-ABI3 and ABI5-MYC remained clearly higher in the *elf3-1*, *elf4-209*, and *lux-6* genetic backgrounds compared to the WT background (Fig. 9G and H). Notably, following the treatment with the 26S proteasome inhibitor MG132, both MYC-ABI3 and ABI5-MYC accumulated to similar levels in the WT, *elf3-1*, *elf4-209*, and *lux-6* genetic backgrounds (Fig. 9G and H). Collectively, these results suggest that ELF3, ELF4, and LUX negatively regulate the stability of ABI3 and ABI5.

To determine whether these observations were associated with altered *ABI3* or *ABI5* expression levels, we analyzed the transcript abundance of the exogenously introduced *3MYC-ABI3* and *ABI5-4MYC* transgenes in germinating seeds of their respective overexpression lines. Results revealed comparable *3MYC-ABI3* transcript levels in *ABI3*-overexpressing lines and *ABI5-4MYC* transcript levels in *ABI5*-overexpressing lines across WT, *elf3-1*, *elf4-209*, and *lux-6* seeds germinated on media with or without ABA (Supplementary Fig. S14A and B). Moreover, there were no significant changes in native *ABI3* or *ABI5* expression in the germinating *elf3-1*, *elf4-209*, and *lux-6* seeds (compared with the WT control) (Supplementary Fig. S14C and D), further supporting the few effects of ELF3, ELF4, and LUX on *ABI3* and *ABI5* expression. Accordingly, ELF3, ELF4, and LUX facilitate the degradation of ABI3 and ABI5 via the 26S proteasome pathway.

On the basis of the negative effects of ELF3, ELF4, and LUX on ABI3 and ABI5, we analyzed whether the ABA sensitivity of germinating *MYC-ABI3* and *ABI5-MYC* seeds was enhanced by mutations to *ELF3*, *ELF4*, or *LUX*. Compared with the *MYC-ABI3* seeds, the *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, and *lux MYC-ABI3* seeds released from cold stratification conditions at ZT12 had much lower germination and cotyledon greening percentages on medium containing 0.5 μM ABA (Fig. 10A to C). Likewise, seed germination and early seedling establishment were slower for *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* than for *ABI5-MYC* in response to ABA (Fig. 10A to C). Consistent with these findings,

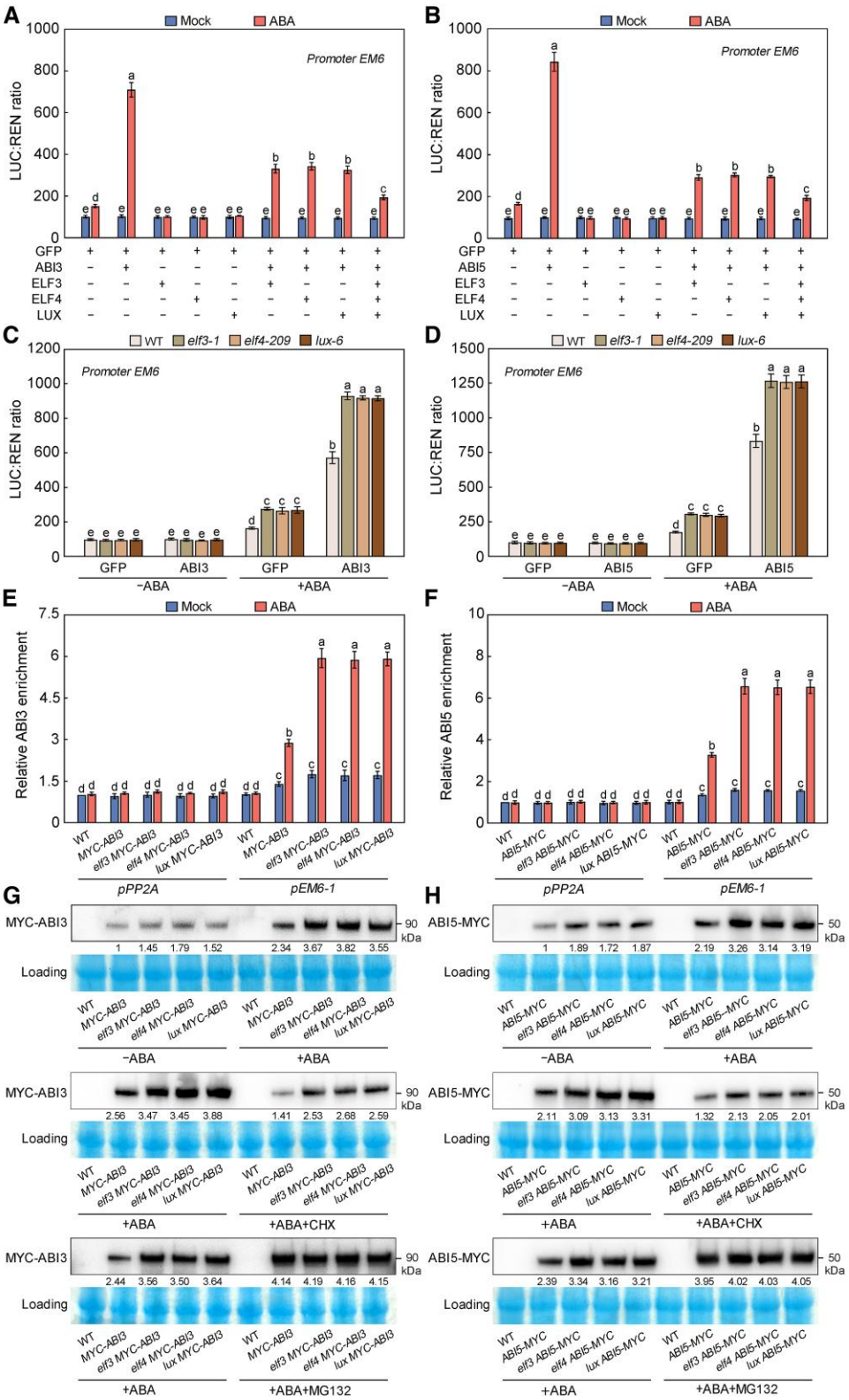

Figure 9. ELF3, ELF4, and LUX negatively influence the transcriptional functions and protein accumulation of ABI3 and ABI5. **A)** and **B)** Transient dual-luciferase reporter assays showing that ELF3, ELF4, and LUX antagonize ABI3 **(A)** and ABI5 **(B)** to modulate *EM6* expression upon 5 μM ABA treatment. Values are means ± standard deviation from 5 independent biological replicates using different batches of WT plants; each replicate was from different WT leaves of more than 50 plants. **C)** and **D)** Transient dual-luciferase reporter assays showing that activation of the *EM6* promoter by ABI3 **(C)** and ABI5 **(D)** was enhanced in the *elf3-1*, *elf4-209*, or *lux-6* mutant in response to 5 μM ABA. Values are means ± SD from 5 independent biological replicates using different batches of WT, *elf3-1*, *elf4-209*, or *lux-6* plants; each replicate was from different leaves of more than 50 plants. **E)** and **F)** ChIP-qPCR analysis of the enrichment of ABI3 **(E)** or ABI5 **(F)** in the promoter region of *EM6* (*pEM6-1*). For the analysis of ABI3 enrichment, the germinating WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, and *lux MYC-ABI3* seeds (3 d, harvested at ZT12) treated with 0.5 μM ABA on half-strength MS medium were used in ChIP assays. For the analysis of ABI5 enrichment, the germinating WT, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC*

(Continued)

the ABA-responsive *EM6* and *RAB18* genes were expressed at much higher levels in ABA-treated germinating *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds than in germinating *MYC-ABI3* and *ABI5-MYC* seeds (Fig. 10D and E). These results provide further genetic evidence that ELF3, ELF4, and LUX antagonize ABI3 and ABI5 to modulate ABA signaling-related delayed seed germination.

Considering the modulatory effects of ELF3, ELF4, and LUX (and other clock components) on ABI3 and ABI5, we speculated whether ABI3 and ABI5 are also involved in the temporal control of seed germination. Interestingly, unlike the WT seeds, the ZT0- and ZT12-released *abi3-8* or *abi5-8* seeds had similar germination and cotyledon greening percentages on medium containing 0.75 μM ABA under long-day conditions (Supplementary Fig. S15A to D). Hence, the time-dependent germination of ABA-treated *abi3-8* and *abi5-8* seeds was abolished, suggesting that ABI3 and ABI5 are crucial for the temporal control of seed germination in response to ABA.

The transcriptional regulatory relationships among the core components of the circadian clock persist during seed germination, irrespective of the presence or absence of ABA

According to earlier studies, the oscillatory mechanism of the circadian clock involves multiple transcriptional–translational negative feedback loops (Harmer 2009; Pokhilko et al. 2012; Carré and Veflingstad 2013; Hsu and Harmer 2014; Greenham and McClung 2015; Uehara et al. 2019; Nakamichi 2020; Simon et al. 2020). Because core clock proteins (e.g. LHY, PRR5, and ELF3) contribute to ABA signaling and seed germination (Adams et al. 2018; Zha et al. 2020; Yang et al. 2021; Figs. 4 and 5), we wondered whether these components sustain mutual regulation during seed germination in response to ABA. Thus, we first examined the *CCA1* and *LHY* transcript levels in germinating *prr5 prr7 prr9* and *elf3 elf4 lux* seeds. Compared with the corresponding expression levels in the WT controls, *CCA1* and *LHY* were expressed at higher levels in germinating *prr5 prr7 prr9* seeds, but at lower levels in germinating *elf3 elf4 lux* seeds, with or without the 0.75 μM ABA treatment (Fig. 11A and B, Supplementary Fig. S16A and B). These observations suggest that *CCA1* and *LHY* transcription is repressed by PRR5, PRR7, and PRR9, but stimulated by ELF3, ELF4, and LUX, during seed germination, regardless of the presence of ABA.

We also analyzed *PRR5* and *PRR7* expression in germinating *cca1 lhy* and *elf3 elf4 lux* mutant seeds treated with ABA. The *PRR5* and *PRR7* transcript levels were clearly higher in germinating *cca1 lhy* and *elf3 elf4 lux* seeds than in germinating WT seeds on medium with or without ABA (Fig. 11C and D, Supplementary Fig. S16C and D). Hence, *PRR5* and *PRR7* expression is downregulated by CCA1/LHY and EC components during seed germination. In addition, *ELF3* and *LUX* transcript levels were much higher in germinating *cca1 lhy* and *prr5 prr7 prr9* seeds than in the corresponding WT seeds in the absence (or presence) of ABA (Fig. 11E and F, Supplementary Fig. S16E and F). Considered together, these results suggest that transcriptional regulatory relationships among these core clock components persist in response to ABA during seed germination.

Discussion

Successful seed germination under specific physiological conditions and the subsequent establishment of a young seedling require precisely coordinated regulatory responses to multiple endogenous and environmental cues. More specifically, ABA is an important phytohormone that suppresses seed germination and arrests seedling growth in *Arabidopsis* (Gubler et al. 2005; Finch-Savage and Leubner-Metzger 2006; Finkelstein et al. 2008; Cutler et al. 2010; Golldack et al. 2013; Nakashima and Yamaguchi-Shinozaki 2013; Sajeev et al. 2024). Recent research highlighted the close relationships between ABA and other regulatory signals influencing seed germination (Lim et al. 2014; Kim et al. 2016; Li et al. 2022). Intriguingly, there is accumulating evidence that the circadian clock helps control ABA biosynthesis and downstream responses (Nováková et al. 2005; Lee et al. 2006; Covington et al. 2008; Mizuno and Yamashino 2008; FukushiMa et al. 2009; Nakamichi et al. 2009; Penfield and Hall 2009; McAdam et al. 2011; Seung et al. 2012; Grundy et al. 2015; Adams et al. 2018; Yang et al. 2021; Liang et al. 2024). However, the temporal modulation of seed germination in response to ABA or under particular natural conditions has not been experimentally verified. In this study, we demonstrated that the temporal signal under diurnal conditions is crucial for modulating seed germination in response to ABA or under salinity and osmotic stress conditions (Figs. 1 and 2). Nevertheless, noncold-stratified WT seeds exhibited no variation in germination and cotyledon greening on half-strength MS medium in the absence of ABA, irrespective of the timing of germination initiation under diurnal conditions (Supplementary Fig. S17A and B). This observation implies that the baseline level of endogenous ABA has a minimal influence on the time-dependent germination under optimal conditions.

Multiple circadian clock proteins and the associated regulators affect time-controlled plant growth, development, and stress tolerance under various physiological and environmental conditions (Yamamoto et al. 2003; Bieniawska et al. 2008; Nakamichi et al.

Figure 9. (Continued)
seeds (3 d, harvested at ZT12) treated with 0.5 μM ABA on half-strength MS medium were used in ChIP assays. More than 500 germinating seeds for each sample were pooled for ChIP assays using an anti-MYC antibody. qPCR data from the ChIP assays with the *PP2A* (AT1G13320) promoter region sequence (*pPP2A*) were used as a negative control. Values are means ± SD from 5 independent biological replicates using different batches of seeds. Bars with different letters are significantly different from each other ($P < 0.05$). Data were analyzed by a two-way ANOVA using Tukey's HSD test. The statistical analyses described apply to all statistical analyses presented in this figure. **G)** and **H)** Immunoblot analyzing the accumulation of MYC-ABI3 **(G)** and ABI5-MYC **(H)** fusion proteins. The germinating WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, and *lux MYC-ABI3* seeds (4 d, harvested at ZT12) treated with 0.5 μM ABA on half-strength MS medium under long-day conditions were used for protein extraction. The germinating WT, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds (4 d, harvested at ZT12) treated with 0.5 μM ABA on half-strength MS medium under long-day conditions were used for protein extraction. In the medium without ABA (i.e. –ABA), an equal volume of 10% (v/v) ethanol was added. For treatments combining ABA with either the protein synthesis inhibitor CHX or the proteasome inhibitor MG132, seeds germinating on 0.5 μM ABA-containing medium were treated with 0.5 μM ABA plus 100 μM CHX or 50 μM MG132, respectively, for 6 h prior to protein extraction. The accumulation of MYC-ABI3 or ABI5-MYC fused protein was detected by immunoblotting with an anti-MYC antibody (1:10,000). Experiments were repeated 3 times with similar results. Relative protein levels of MYC-ABI3 or ABI5-MYC were quantified by ImageJ. ZT, Zeitgeber time. LUC, firefly luciferase. REN, *Renilla luciferase*. ABA, abscisic acid. MG132, carbobenzoxy-Leu-Leu-leucinal.

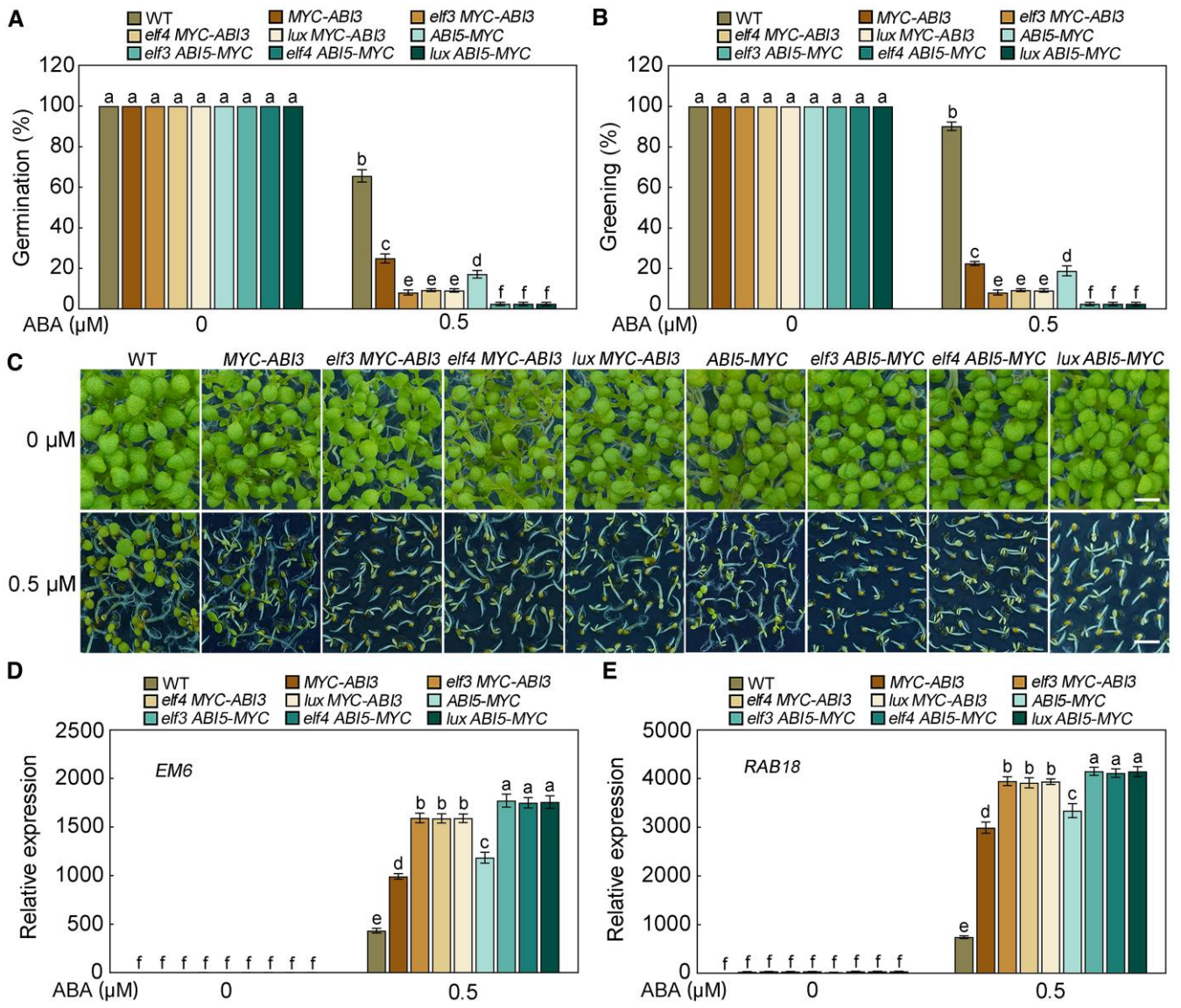

Figure 10. The ABA hypersensitivity of *ABI3*- or *ABI5*-overexpressing plants is enhanced by the *ELF3*, *ELF4*, or *LUX* mutation. **A)** Germination of the WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Seed germination was recorded 48 h after stratification on half-strength MS medium supplemented with 0.5 μM ABA under long-day conditions. In the medium without ABA (i.e. 0 μM), an equal volume of 10% (v/v) ethanol was added. Time is expressed was hours from dawn (ZT0). **B)** Cotyledon greening of the WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12. Cotyledon greening was recorded 7 d after stratification on half-strength MS medium supplemented with 0.5 μM ABA under long-day conditions. The experiments were performed 5 times by analyzing different batches of seeds. Each batch of seeds was pooled from more than 60 individual plants. For each biological replicate, more than 120 seeds were examined. **C)** Seedlings of WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* mutants 7 d after stratification on half-strength MS medium supplemented with 0.5 μM ABA under long-day conditions. Bar = 2.5 mm. **D)** and **E)** RT-qPCR analysis of the ABA-induced expression of *EM6* **(D)** and *RAB18* **(E)** in germinating WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* seeds. The seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.5 μM ABA on half-strength MS medium. The *PP2A* (AT1G13320) gene was used as a control. Values presented in this figure are means ± standard deviation. Data were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). The statistical analyses described apply to all statistical analyses presented in this figure. ABA, abscisic acid.

2009; Bhardwaj et al. 2011; Wang et al. 2011; Goodspeed et al. 2012; Shin et al. 2012; Korneli et al. 2014; Zhou et al. 2015b; Ezer et al. 2017; Lu et al. 2017; Joo et al. 2019; Zhang et al. 2019; Xu et al. 2022). For example, core clock components (e.g. CCA1/LHY and PRR proteins) along with light signaling modulators concurrently mediate the expression of *PHYTOCHROME INTERACTING FACTOR* (*PIF*) genes, especially *PIF4* and *PIF5*, to diurnally affect hypocotyl growth (Fujimori et al. 2004; Shen et al. 2007; Nusinow et al. 2011; Nakamichi et al. 2012; Liu et al. 2013a; Nieto et al. 2015; Soy et al. 2016; Zhu et al. 2016; Martín et al. 2018; Li et al. 2020). The PRR proteins redundantly and positively regulate the timing of floral initiation through the CONSTANS (CO)- and FLOWERING LOCUS T (FT)-dependent photoperiodic pathway (Nakamichi et al. 2005, 2007; Ito et al. 2008; Hayama et al. 2017). Notably, in terms of ABA responses, LHY directly represses the expression of *ABI5* as well as other ABA-responsive genes and

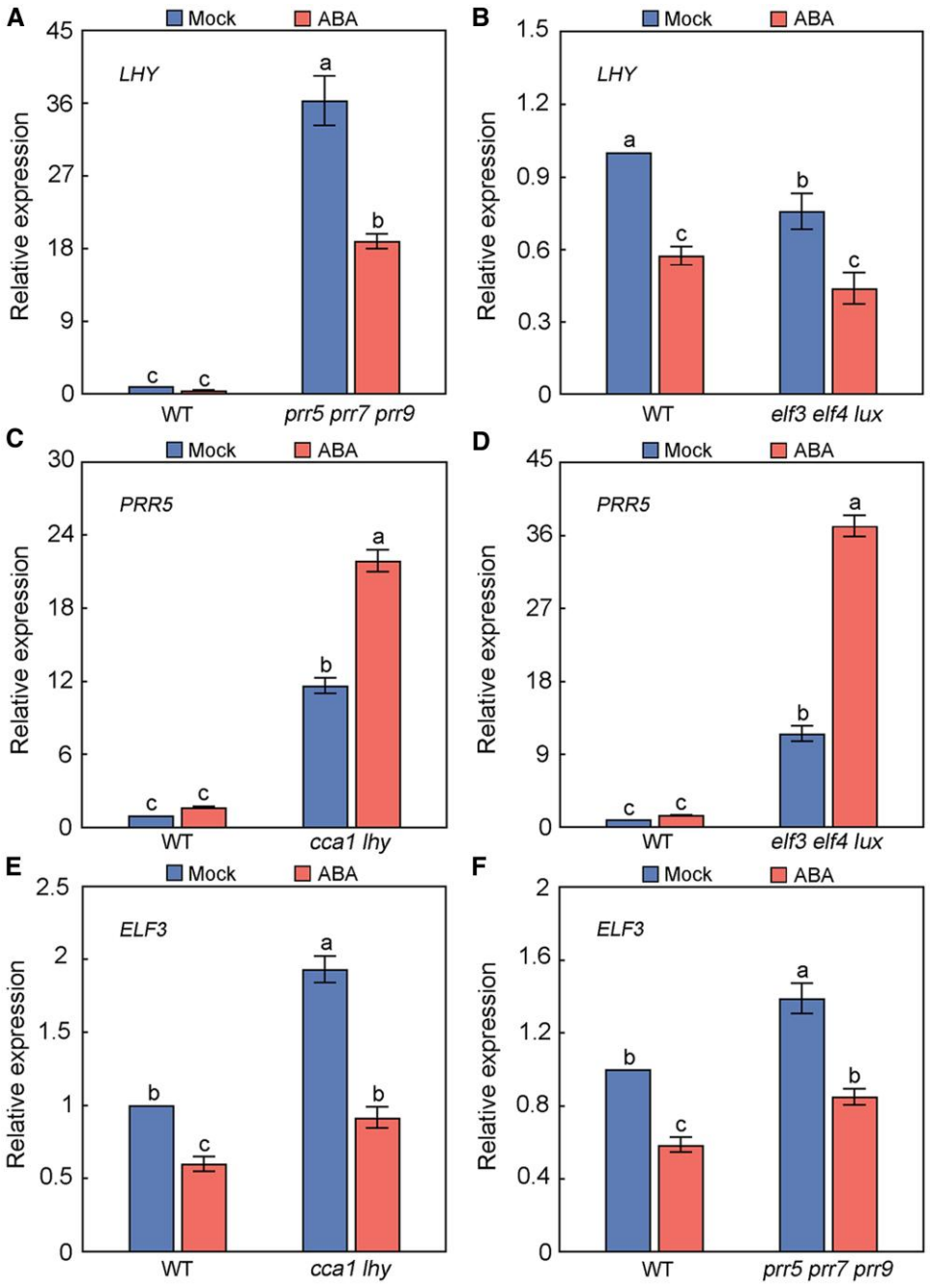

Figure 11. Expression of *LHY*, *PRR5*, and *ELF3* in response to ABA during seed germination. **A)** and **B)** RT-qPCR analysis of the expression of *LHY* in germinating WT, *prr5 prr7 prr9* **(A)**, and *elf3 elf4 lux* **(B)** seeds. The WT, *prr5 prr7 prr9*, and *elf3 elf4 lux* seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.75 μM ABA on half-strength MS medium. Time is expressed was hours from dawn (ZT0). **C)** and **D)** RT-qPCR analysis of the expression of *PRR5* in germinating WT, *cca1 lhy* **(C)**, and *elf3 elf4 lux* **(D)** seeds. The WT and *cca1 lhy* seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 under long-day conditions. For the analysis of *elf3 elf4 lux* mutant seeds, the WT and *elf3 elf4 lux* seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.75 μM ABA on half-strength MS medium. **E)** and **F)** RT-qPCR analysis of the expression of *ELF3* in germinating WT, *cca1 lhy* **(E)**, and *prr5 prr7 prr9* **(F)** seeds. The WT and *cca1 lhy* seeds were stratified at 4 °C and then transferred to 22 °C at ZT0 under long-day conditions. For the analysis of *prr5 prr7 prr9* mutant seeds, the WT and *prr5 prr7 prr9* seeds were stratified at 4 °C and then transferred to 22 °C at ZT12 under long-day conditions. Total RNA was extracted from 5 different batches of germinating seeds (48 h) treated with 0.75 μM ABA on half-strength MS medium. The *PP2A* (AT1G13320) gene was used as a control. Values presented in this figure are means ± standard deviation. Data were analyzed by a two-way ANOVA using Tukey's HSD test. Bars with different letters are significantly different from each other ($P < 0.05$). The statistical analyses described apply to all statistical analyses presented in this figure. ABA, abscisic acid.

attenuates the inhibitory effect of ABA on seed germination, whereas PRR5 and PRR7 enhance ABI5-mediated ABA signaling, thereby suppressing seed germination on ABA-containing medium (Adams et al. 2018; Yang et al. 2021). Despite the regulatory effects of LHY and PRR5/PRR7 on ABA responses, the possibility that the circadian clock and ABA coordinately control time-dependent seed germination remains to be explored in depth. In the current study, we observed that the temporal control of

germination was abolished in *cca1 lhy* and *prr5 prr7 prr9* mutant seeds in the presence of ABA, NaCl, or mannitol (Figs. 1F and G and 2G and H, Supplementary Figs. S1B to I, S3C to H, and S4A to H). Furthermore, the EC components ELF3, ELF4, and LUX negatively modulated the ABA-induced inhibition of seed germination; disrupting these proteins adversely affected time-dependent germination (Figs. 4 and 5, Supplementary Fig. S7). Hence, these core clock proteins are indispensable for the time-based regulation of seed germination after an exposure to ABA or under specific natural conditions.

The ABA signaling pathway is essential for seed germination inhibition, which involves profound changes in gene expression profiles associated with the interactions among multiple positive and negative modulators. After an increase in the ABA concentration is perceived by PYRABACTIN RESISTANCE (PYR)/PYR1-LIKE (PYL)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) proteins, the resulting disruption of type 2C PROTEIN PHOSPHATASE (PP2C) leads to the release of SUCROSE NONFERMENTING1-RELATED KINASE2 (SnRK2) from PP2C–SnRK2 complexes (Ma et al. 2009; Miyazono et al. 2009; Nishimura et al. 2009; Park et al. 2009; Santiago et al. 2009; Cutler et al. 2010). The released SnRK2 phosphorylate and stimulate downstream components (e.g. ABI5 and its homologs) to activate ABA-responsive genes (Kobayashi et al. 2005; Furihata et al. 2006; Fujii et al. 2007; Fujii and Zhu 2009; Nakashima et al. 2009; Cutler et al. 2010; Finkelstein 2013; Li et al. 2017; Chen et al. 2020). Among the regulators in the ABA pathway, ABI3 and ABI5 are two master transcriptional stimulators of ABA responses and function at key hubs that integrate ABA and other signals crucial for the precise modulation of seed germination (Finkelstein and Lynch 2000; Garcia et al. 2008; Miura et al. 2009; Lee et al. 2010; Zhou et al. 2015a; Kim et al. 2016; Varshney et al. 2023). In the present study, ELF3, ELF4, and LUX physically associated with ABI3 and ABI5 in yeast cells and in plants (Fig. 6A to D). Genetic analyses showed that ELF3, ELF4, and LUX function in an ABI3- and ABI5-dependent manner to disrupt ABA-induced delayed seed germination (Figs. 7 and 8). A mechanistic analysis indicated that ELF3, ELF4, and LUX negatively affect the stability of ABI3 and ABI5 as well as their functions (Fig. 9A to H). Interestingly, the regulation of ABI3/ABI5 by ELF3, ELF4, and LUX may also occur and be enhanced under specific natural conditions (e.g. under low ambient temperature conditions), as the enrichment of ABI3 and ABI5 was significantly greater in ABA-treated germinating seeds at 17 °C when ABI3 and ABI5 were overexpressed in the *elf3-1*, *elf4-209*, and *lux-6* mutant backgrounds compared to the WT background (Supplementary Fig. S18A and B). The importance of both ABI3 and ABI5 for the temporal control of seed germination in response to ABA was also revealed (Supplementary Fig. S15A to D). These results suggest that there is a previously unknown signaling module in which the clock-associated proteins ELF3, ELF4, and LUX directly repress the functions of the ABA-responsive transcription factors ABI3 and ABI5 through protein–protein interactions during the regulation of time-dependent seed germination.

Similar to ELF3, ELF4, and LUX, the core clock proteins CCA1 and LHY negatively modulate ABA responses, which leads to increased seed germination (Penfield and Hall 2009; Adams et al. 2018; Fig. 1F, Supplementary Fig. S1B and C). In accordance with the observed phenotypes, LHY binds directly to the promoter of multiple genes (e.g. *NCED3*, *ABI3*, *ABI5*, and *ABF3*), thereby regulating the transcription of these ABA biosynthesis/signaling-related genes and downstream ABA-responsive genes (Adams et al. 2018; Liang et al. 2024). However, unlike CCA1/LHY and the EC components, PRR proteins (i.e. PRR5, PRR7, and PRR9) potentiate ABA responses to inhibit seed germination (Yang et al. 2021). Both PRR5 and PRR7 enhance the ABI5 function via direct protein interactions to modulate target gene expression (Yang et al. 2021). Likewise, TOC1, which is also known as PRR1, reportedly associates with ABI3 in vivo and appears to positively mediate ABA signaling during seed germination (Kurup et al. 2000; Penfield and Hall 2009). In addition, LUX directly modulates the expression of *DOG1* and exerts a negative influence on seed dormancy (Zha et al. 2020). All these findings, together with the discoveries of the present study, point toward the interplay between circadian regulators and ABA-related pathways in controlling seed dormancy and germination. Furthermore, transcriptional modulatory relationships may persist among these core clock components in germinating seeds exposed to ABA (Fig. 11A to F, Supplementary Fig. S16A to F; Harmer 2009; Pokhilko et al. 2012; Hsu and Harmer 2014; Greenham and McClung 2015; Uehara et al. 2019; Nakamichi 2020; Simon et al. 2020). Consequently, we speculate that the effects of different core clock proteins on ABA-related pathways at multiple regulatory layers are carefully balanced to establish and maintain appropriate ABA signaling levels. This balance may be crucial for strictly controlling seed germination under particular natural conditions, especially during times of environmental stress. It is conceivable that this intricate regulation serves to enhance the plant's environmental adaptability, allowing it to fine-tune its germination timing in response to varying environmental cues. Interestingly, in addition to these core clock proteins, several critical regulators responsible for the circadian clock output, such as GI and CO, are also produced in seeds and/or mediate ABA responses (Putterill et al. 1995; Simon et al. 1996; Fowler et al. 1999; Samach et al. 2000; Kim et al. 2007; Sawa et al. 2007; Penfield and Hall 2009; Yu et al. 2023). Deciphering the potential relationships between other clock-associated components and key regulators of ABA signaling may clarify the circadian clock-mediated gating of ABA signaling networks during seed germination.

The regulation of ABI3/ABI5-stimulated ABA signaling involves an intricate network of multiple transcriptional modulators that precisely control seed germination, such as GA-INSENSITIVE (GAI), BRI1-EMS-SUPPRESSOR1 (BES1), JAZ, and ARF10/ARF16 (Chen et al. 2012; Guan et al. 2014; Lim et al. 2014; Kim et al. 2016; Pan et al. 2018; Hu et al. 2019; Li et al. 2019; Zhao et al. 2019; Guo et al. 2022; Yang et al. 2023; Du et al. 2024). These findings along with the results of the current study suggest that the complex interplay among ABI3/ABI5 and other key regulators of diverse signaling pathways contributes to effective adaptive mechanisms in specific environmental contexts. In-depth analyses are required to reveal the specific mechanisms underlying the possible associations among ABI3/ABI5-binding modulators and to elucidate the biological significance of these relationships. Moreover, ABI3 and ABI5 are strictly regulated at the posttranslational level (Garcia et al. 2008; Miura et al. 2009; Dai et al. 2013; Albertos et al. 2015; Yu et al. 2015; Lynch et al. 2017). For example, SnRK2, BRASSINOSTEROID INSENSITIVE2 (BIN2), and PROTEIN KINASE SOS2-LIKE5 (PKS5) kinases phosphorylate and stabilize ABI5 in the presence of ABA, whereas several E3 ubiquitin ligases recruit ABI3 and/or ABI5 for degradation (Kobayashi et al. 2005; Zhang et al. 2005; Furihata et al. 2006; Fujii et al. 2007; Stone et al. 2007; Fujii and Zhu 2009; Miura et al. 2009; Nakashima et al. 2009; Lee et al. 2010; Liu and Stone 2010, 2014; Lyzenga et al. 2013; Hu and Yu 2014; Seo et al. 2014; Zhou et al. 2015a; Li et al. 2023). In this study, we determined that ELF3, ELF4, and LUX also facilitate the degradation of ABI3 and ABI5 through the proteasome pathway (Fig. 9G and H). However, the biochemical basis of the regulatory

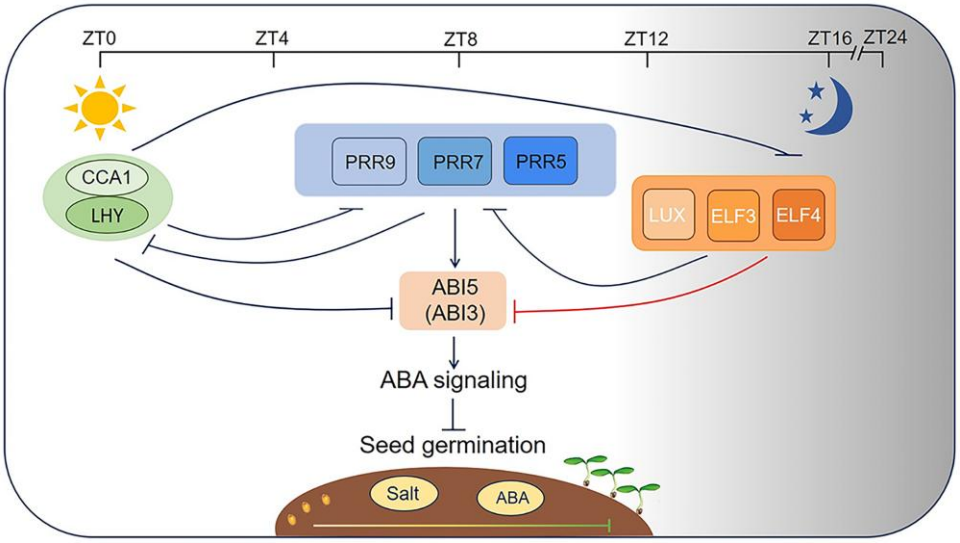

Figure 12. A simplified model involving core clock proteins and ABI3/ABI5 for the time-based regulation of ABA signaling-related delayed seed germination. When the ABA concentration increases, ABA induces the expression of *ABI3* and *ABI5* and stabilizes the encoding proteins to stimulate ABA signaling and suppress seed germination. LHY (and even CCA1) produced in the morning directly suppresses *ABI3* and *ABI5* transcription, which weakens ABA signaling. Both PRR5 and PRR7 physically associate with ABI5 and enhance its function, thereby stimulating ABA signaling. The EC components ELF3, ELF4, and LUX interact with and antagonize ABI3 and ABI5. The transcriptional regulatory relationships may persist among these core clock proteins in response to ABA during seed germination. The final outcome of these core clock proteins regulation is enhanced ABA signaling in the late afternoon (e.g. ZT12), therefore more effectively inhibiting seed germination. The red line indicates the main finding of this study. Pointed arrow indicates promotion or activation. Flat arrow indicates inhibition or repression. ABA, abscisic acid.

effects of ELF3, ELF4, and LUX on ABI3 and ABI5 accumulation will need to be more thoroughly characterized. Additionally, whether ELF3, ELF4, and LUX affect the interactions of ABI3 or ABI5 with kinases and/or E3 ubiquitin ligases will need to be determined. Moreover, investigating the structural basis of the physical associations among the EC components, ABI3/ABI5, kinases, and/or E3 ubiquitin ligases may further our understanding of the molecular mechanisms responsible for the effects of EC components on ABI3/ABI5 stability and functions.

The temporal signal and ABA are involved in modulating plant growth and stress responses via the synchronization of internal biological processes with external environmental changes. Considering the rhythmic regulation of ABA signaling, which results in greater inhibition of germination in the late afternoon for seeds undergoing stress (Figs. 1 and 2; Fukushima et al. 2009; Liu et al. 2013a; Adams et al. 2018; Yang et al. 2021), we speculate that the temporal control of ABA-mediated inhibition of seed germination may provide plants with an adaptive advantage, enabling them to coordinate and optimize their growth and responses to environmental conditions. For example, because ABA-enhanced stress tolerance involves the diversion of energy away from processes related to plant growth and development, a temporal gating mechanism underlying ABA signaling may enable plants to distribute energy appropriately during seed germination and seedling establishment. By temporally regulating ABA signaling and seed germination, plants may ensure radicle protrusion coincides with optimal environmental conditions of temperature, humidity, and light. This regulation may also allow sufficient time for the accumulation of stress-protective metabolites such as proline and anthocyanins prior to soil emergence, thereby enhancing their resilience against pathogens and other stressors in undisturbed ecosystems. Furthermore, appropriate germination during periods with suitable environmental conditions may augment photosynthesis and nutrient uptake, ultimately resulting in enhanced plant health and greater chances of survival.

To further elucidate the molecular mechanisms required for the temporal regulation of ABA signaling-related repressed seed germination, we propose a simplified model involving core clock proteins and ABI3/ABI5 (Fig. 12). When the ABA concentration increases, ABA induces the expression of *ABI3* and *ABI5* and stabilizes the encoding proteins to stimulate ABA signaling and suppress seed germination (Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000; Lopez-Molina et al. 2001, 2002). However, LHY (and even CCA1) produced in the morning directly suppresses *ABI3* and *ABI5* transcription, which weakens ABA signaling (Adams et al. 2018). Both PRR5 and PRR7 physically associate with ABI5 and enhance its function, thereby stimulating ABA signaling (Yang et al. 2021). Conversely, the EC components ELF3, ELF4, and LUX interact with and antagonize ABI3 and ABI5 (Figs. 6 and 9). The final outcome of these core clock proteins regulation is enhanced ABA signaling in the late afternoon (e.g. ZT12), therefore more effectively inhibiting seed germination (Fig. 1). Our results combined with the findings of previous studies provide evidence that the temporal signal and core clock proteins have critical regulatory effects on ABA signaling and seed germination (as the circadian output pathways), while also clarifying the molecular mechanism through which ELF3, ELF4, and LUX suppress ABI3 and ABI5 to facilitate the crosstalk between the clock and ABA signaling pathways.

Materials and methods

Materials and plant growth conditions

The phytohormone ABA was purchased from Sigma-Aldrich (catalog no. 862169-250MG). Common chemicals were purchased from Shanghai Sangon (Shanghai, China) and Taq DNA polymerase was from Takara Biotechnology (Dalian, China). WT and mutant *A. thaliana* plants used in this study had the Columbia (Col-0) genetic background. The *prr5 prr7 prr9*, *elf3-1*, *elf4-209*, and *lux-6* seeds were provided by Prof. Lei Wang (Institute of Botany, Chinese Academy of

Sciences). Seeds of *cca1 lhy* were provided by Prof. Xiaodong Xu (Henan University). The loss-of-function mutants *abi3-8* (Nambara et al. 2002) and *abi5-8* (Salk_013163C; Zhou et al. 2015a; Mei et al. 2023) were previously described, exhibiting dramatically reduced sensitivity to ABA compared to the WT during seed germination. The transgenic line *ABI5-MYC* (Chen et al. 2012) was provided by Prof. Chuanyou Li (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences). The *ELF3-OE-1* transgenic plants overexpressing *ELF3* were provided by Prof. Jianxiang Liu (Zhejiang University), whereas the *LUX-OE-58* transgenic plants overexpressing *LUX* (TPT_3.46640.1G) were purchased from AraShare (www.arashare.cn). To generate *FLAG-ELF3-3*, *FLAG-ELF4-5*, *FLAG-ELF4-7*, *FLAG-LUX-6*, and *MYC-ABI3* transgenic plants, the full-length cDNAs of *ELF3*, *ELF4*, and *LUX* fused to 2 FLAG tag sequences and the full-length cDNA of ABI3 fused to 3 MYC tag sequences were cloned into the binary vector pOCA30 in the sense orientation for the subsequent expression under the control of *Pro35S* (Hu et al. 2013). The *elf3 lux*, *elf4 lux*, *elf3 elf4 lux*, *elf3 abi3-8*, *elf4 abi3-8*, *lux abi3-8*, *elf3 abi5-8*, *elf4 abi5-8*, *lux abi5-8*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, *lux MYC-ABI3*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* plants were generated via genetic crosses. For long-day conditions, *Arabidopsis* plants were grown in a growth chamber at 22 °C with a 16-h light (100 μE m^{-2} s^{-1}; white fluorescent bulbs, full-spectrum light)/8-h dark photoperiod.

Determination of germination and greening

The dry seeds were soaked in water for 30 min, surface-sterilized, sown on plates containing growth medium, and transferred to 4 °C at ZT0 for stratification in darkness for 72 h. Subsequently, seeds were transferred to a growth chamber set at 22 °C with a 16-h light/8-h dark photoperiod either at ZT0 or at ZT12 (12 h later than the ZT0 group). To eliminate the effects of light on germination, the seeds after cold stratification in darkness for 72 h were relocated to a growth chamber set at 22 °C with continuous light at different time points throughout the day (from ZT0 to ZT24), with each subsequent transfer (of the seeds) delayed by 4 h. Seeds were considered to have germinated if the embryonic axis was detectable during a microscopic examination (i.e. protruding radicle). Seedling greening was assessed according to the appearance of green cotyledons. To examine the effects of ABA, NaCl, or mannitol on germination and greening, seeds were placed on half-strength MS medium containing ABA, NaCl, or mannitol in plates. For seed dormancy analysis, we meticulously selected plants with synchronously maturing early-stage siliques to ensure uniform developmental timing across all fresh seed samples. Seeds were directly sown without cold stratification on half-strength MS medium and then placed in the growth chamber set at 22 °C with a 16-h light/8-h dark photoperiod. All experiments were conducted more than 5 times using different batches of seeds as biological replicates. Each batch of seeds was pooled from more than 60 independent plants. For each biological replicate, more than 120 seeds were analyzed.

RNA extraction and RT-qPCR

The TRIzol reagent (Invitrogen) was used to extract total RNA from germinating seeds with or without an ABA treatment for a RT-qPCR analysis, which was performed as described previously (Han et al. 2020). Briefly, 1.0 μg DNase-treated RNA was reverse transcribed to cDNA in a 20 μL reaction volume containing an oligo-$(dT)_{18}$ primer and Moloney murine leukemia virus reverse transcriptase (Fermentas). Then, 1.0 μL cDNA was used for the RT-qPCR analysis, which was completed using a SYBR Premix Ex Taq kit (Takara) and a Roche LightCycler 480 real-time PCR system. At least 5 biological replicates per sample were prepared for the RT-qPCR analysis. The AT1G13320 gene, which encodes a subunit of Ser/Thr protein phosphatase 2A (PP2A) and is stably expressed in germinating seeds (Czechowski et al. 2005), was selected as the control. The RT-qPCR primers are listed in Supplementary Dataset 1.

GUS staining

Putative *ELF3*, *ELF4*, and *LUX* promoter fragments (*proELF3*, 2,485 bp; *proELF4*, 2,420 bp; *proLUX* 2,447 bp) were amplified from WT genomic DNA using gene-specific primers. The *proELF3:GUS*, *proELF4:GUS*, and *proLUX:GUS* constructs were cloned into the pOCA28 binary vector. The resulting recombinant vectors were inserted into WT plants. Dry seeds of the *proELF3:GUS*, *proELF4:GUS*, and *proLUX:GUS* transgenic lines were stripped of their seed coat prior to staining. The seeds were germinated (0, 1, 2, or 3 d) on half-strength MS medium, with samples collected and stained. The histochemical detection of GUS activity was performed as described previously (Chen et al. 2010). The primers used for cloning are listed in Supplementary Dataset 1.

Yeast two-hybrid assays

The full-length *ABI3*, *ABI4*, and *ABI5* coding sequences were cloned into pGBKT7 (Clontech) to generate bait vectors (BD-ABI3, BD-ABI4, and BD-ABI5) containing Gal4 BD. The full-length *ELF3*, *ELF4*, and *LUX* coding sequences were inserted into pGADT7 (Clontech) to produce prey vectors (AD-ELF3, AD-ELF4, and AD-LUX) with Gal4 AD. To identify specific regions critical for the interactions, multiple truncated *ABI3* and *ABI5* sequences were inserted into pGBKT7, whereas truncated *ELF3* and *LUX* sequences were cloned into pGADT7. Yeast two-hybrid assays were performed as described previously (Hu et al. 2019; Yang et al. 2021). Yeast strain AH109 cells were co-transformed with bait and prey vectors and then protein interactions were reflected by cell growth on dropout medium lacking Leu, Trp, His, and Ade at 3 d after plating. The primers used for cloning are listed in Supplementary Dataset 1.

BiFC assays

The cDNA sequences encoding the C-terminal 64-amino acid enhanced YFP (cYFP) and N-terminal 173-amino acid YFP (nYFP) fragments were amplified by PCR and inserted into separate pFGC5941 vectors to produce pFGC-cYFP and pFGC-nYFP constructs, respectively (Kim et al. 2008). The full-length cDNA sequence or a sequence encoding C-terminal amino acids 417 to 720 of ABI3 was cloned into pFGC-cYFP to produce a C-terminal in-frame fusion with cYFP (ABI3-cYFP or $ABI3^{417\text{-}720}$-cYFP). The full-length cDNA sequence or a sequence encoding N-terminal amino acids 1 to 164 of ABI5 was cloned into pFGC-cYFP to produce a C-terminal in-frame fusion with cYFP (ABI5-cYFP or $ABI5^{1\text{-}164}$-cYFP). The full-length *ELF3* sequence as well as the sequences encoding N-terminal amino acids 1 to 261 of ELF3, full-length *ELF4*, full-length *LUX*, and N-terminal amino acids 1 to 143 of LUX were inserted into pFGC-nYFP to generate an N-terminal in-frame fusion with nYFP (ELF3-nYFP, $ELF3^{1\text{-}261}$-nYFP, ELF4-nYFP, LUX-nYFP, and $LUX^{1\text{-}143}$-nYFP). The resulting recombinant plasmids were inserted into *Agrobacterium tumefaciens* strain GV3101 cells, which were used for the infiltration of WT *Nicotiana benthamiana* leaves as described previously (Hu et al. 2019). The leaves were analyzed 48 h postinfiltration, with YFP and DAPI fluorescence detected using the Fluoview FV1000 confocal laser scanning microscope (Olympus,

Tokyo, Japan). For DAPI staining, infected leaves were incubated in a 10 mM DAPI solution for 5 min prior to microscopic examination. YFP signals were detected using a 488 nm excitation wavelength (laser intensity set to 24%, gain value 1), with emission captured between 510 and 530 nm. DAPI signals were detected using a 405 nm excitation wavelength (laser intensity set to 15%, gain value 1), with emission captured between 420 and 440 nm. Experiments were performed at least 5 times using different batches of WT *N. benthamiana* plants; for each biological replicate, more than 12 *N. benthamiana* plants were infiltrated and more than 600 cells were analyzed. The primers used for cloning are listed in Supplementary Dataset 1.

CoIP assays

To verify the ELF3–ABI3 and ELF3–ABI5 interactions, total protein extracts were prepared from 0.75 μM ABA-treated germinating seeds (4 d) of transgenic *Arabidopsis* plants simultaneously overexpressing *ELF3* and *ABI3* (*FLAG-ELF3-3 MYC-ABI3*) or *ELF3* and *ABI5* (*FLAG-ELF3-3 ABI5-MYC*), which were generated by introducing the *ELF3* overexpression into *MYC-ABI3* or *ABI5-MYC* plants. The protein extraction was performed using extraction buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.1% (v/v) Triton X-100, 10% (v/v) glycerol, 1 mM PMSF, and 1× Roche Protease Inhibitor Cocktail. Immunoprecipitation experiments were conducted as previously described in Han et al. (2023) using Protein A/G Plus Agarose beads (Santa Cruz Biotechnology, catalog no. D1217) following the manufacturer's protocol. Briefly, cell lysates were precleared using the Protein A/G Plus Agarose beads, which were then incubated with an anti-FLAG antibody (catalog no. F7425, Sigma-Aldrich; 1:250) in the extraction buffer at 4 °C overnight. The beads were thoroughly washed twice with the extraction buffer and then the co-immunoprecipitated protein was detected by immunoblotting using an anti-MYC antibody (catalog no. A7470, Sigma-Aldrich; 1:10,000).

Transient transactivation assays

Full-length *ABI3*, *ABI5*, *ELF3*, *ELF4*, *LUX*, and *GFP* sequences were amplified by PCR and cloned into separate pGreenII 62-SK vectors to generate effectors (Hellens et al. 2005). The putative *EM6* promoter sequence (1,273 bp) was amplified by PCR and inserted into the pGreenII 0800-LUC vector to generate the reporter (Hellens et al. 2005). Different combinations of recombinant plasmids were used for the transformation of WT, *elf3-1*, *elf4-209*, or *lux-6 Arabidopsis* leaf mesophyll protoplasts as described previously (Sheen 2001). Transfected cells were cultured for 10 to 16 h with or without 5 μM ABA, after which the relative LUC activity was analyzed using a Dual-Luciferase Reporter Assay system (Promega, Madison, WI, USA), which measured the activities of firefly LUC and the internal control *Renilla reniformis* LUC (REN). The primers used for cloning are listed in Supplementary Dataset 1.

ChIP assays

ChIP assays were conducted essentially as described previously (Mukhopadhyay et al. 2008; Jiang et al. 2014). Briefly, WT, *MYC-ABI3*, *elf3 MYC-ABI3*, *elf4 MYC-ABI3*, and *lux MYC-ABI3* germinating seeds or WT, *ABI5-MYC*, *elf3 ABI5-MYC*, *elf4 ABI5-MYC*, and *lux ABI5-MYC* germinating seeds (4 d with or without a 0.75 μM ABA treatment) underwent a 1% formaldehyde cross-linking treatment prior to isolating their chromatin. Protein–DNA complexes were immunoprecipitated using an anti-MYC antibody, after which the precipitated DNA was purified using a PCR Purification kit (Qiagen) and subjected to a RT-qPCR analysis. To quantify the binding of ABI3 or ABI5 to the target promoter, a RT-qPCR analysis was performed according to a published procedure (Mukhopadhyay et al. 2008), using the *PP2A* (AT1G13320) promoter as an endogenous control. Relative quantitative values calculated using the $2^{(-\mathrm{DDCt})}$ method (Mukhopadhyay et al. 2008) represented the DNA-binding rate. Data were obtained from 5 biological replicates comprising different seed batches.

Yeast one-hybrid assays

Yeast one-hybrid assays were performed using the Matchmaker Yeast One-Hybrid System Kit (Clontech). Putative *EM6* and *DOG1* promoter fragments were cloned into the pAbAi vector to generate pAbAi-pEM6 constructs (pAbAi-pEM6.1 to pAbAi-pEM6.30, with pAbAi-EM6.24 and pAbAi-EM6.30 containing the identified LBS elements) and pAbAi-pDOG1.1. These constructs were linearized using *BstBI* and then inserted into Y1HGold yeast strain cells. The transformed cells were grown for 3 d on synthetic defined (SD)/−Ura medium in plates. Cells harboring pAbAi-pEM6 were transformed with the AD-LUX construct and then selected on SD/−Leu medium in plates. Co-transformed cells were cultured for 3 d on SD/−Leu medium supplemented with aureobasidin A (200 μg/L) in plates. Positive clones were spotted in several yeast concentrations from dilution of 10^0 ($OD_{600}=0.8$) to 10^{-3}. The primers used for cloning are listed in Supplementary Dataset 1.

Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) using Tukey's honest significant difference (HSD) as a post hoc test. Statistically significant differences were defined as those with $P<0.05$. Different lowercase letters above the columns in the figures indicate significant differences ($P<0.05$) among samples. The results of statistical analyses are shown in Supplementary Dataset 2.

Accession numbers

The genes discussed in this paper can be found in the *Arabidopsis* Genome Initiative database as follows: *ABI3*, AT3G24650; *ABI4*, AT2G40220; *ABI5*, AT2G36270; *ELF3*, AT2G25930; *ELF4*, AT2G40080; *LUX*, AT3G46640; *PRR5*, AT5G24470; *PRR7*, AT5G02810; *PRR9*, AT2G46790; *CCA1*, AT2G46830; *LHY*, AT1G01060; *EM1*, AT3G51810; *EM6*, AT2G40170; *RAB18*, AT1G43890; *RD29B*, AT5G52300; and *DOG1*, AT5G45830.

Acknowledgments

We thank Lei Wang (Institute of Botany, Chinese Academy of Sciences), Chuanyou Li (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences), Xiaodong Xu (Henan University), Jianxiang Liu (Zhejiang University), and Diqiu Yu (Yunnan University) for sharing research materials. We thank Arashare for the *LUX*-overexpressing seeds. We also thank the Central Laboratory of Xishuangbanna Tropical Botanical Garden and Kunming Institute of Botany, Chinese Academy of Sciences for technical supports. We thank Liwen Bianji (Edanz) (www.liwenbianji.cn) for editing the English text of a draft of this manuscript.

Author contributions

Y.H. and R.T. designed this study and wrote the article; R.T., J.Y., X.H., K.H., C.Z., M.Y., J.Z., Z.H., J.W.Y., T.X., C.Y., J.D., Q.F., and

Y.H. performed experiments and/or interpreted data. All authors approved the final article.

Supplementary data

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

Supplementary Figure S1. The time-dependent germination of *cca1 lhy* and *prr5 prr7 prr9* mutant seeds in response to ABA under long-day conditions or continuous light.

Supplementary Figure S2. The time-dependent germination of WT seeds in response to ABA under continuous light and the expression of several core clock genes during seed germination.

Supplementary Figure S3. The time-dependent germination of *cca1 lhy* and *prr5 prr7 prr9* mutant seeds exposed to salinity and osmotic stress under long-day conditions.

Supplementary Figure S4. The time-dependent germination of *cca1 lhy* and *prr5 prr7 prr9* mutant seeds exposed to salinity and osmotic stress under continuous light.

Supplementary Figure S5. The EC components inhibit seed dormancy.

Supplementary Figure S6. *ELF3*, *ELF4*, and *LUX* expression in the endosperm and developing seeds.

Supplementary Figure S7. The time-dependent germination of WT, *elf3-1*, *elf4-209*, *lux-6*, and *elf3 elf4 lux* seeds in response to ABA, salinity or osmotic stress under long-day or continuous light conditions.

Supplementary Figure S8. Yeast two-hybrid assays to analyze the interactions of ELF3, ELF4, and LUX with ABI3 and ABI5.

Supplementary Figure S9. BiFC assays to analyze the interactions of ELF3, ELF4, and LUX with ABI3 and ABI5.

Supplementary Figure S10. The responses of *elf3 abi3-8*, *elf4 abi3-8*, and *lux abi3-8* seeds to 2 μM ABA during germination.

Supplementary Figure S11. The responses of *elf3 abi5-8*, *elf4 abi5-8*, and *lux abi5-8* seeds to 2 μM ABA during germination.

Supplementary Figure S12. Accumulation of ABI3 and ABI5 in *Arabidopsis* leaf mesophyll protoplasts.

Supplementary Figure S13. Yeast one-hybrid assay testing LUX binding to the *EM6* promoter region.

Supplementary Figure S14. Effects of ELF3, ELF4, and LUX on *ABI3* and *ABI5* expression.

Supplementary Figure S15. The time-dependent germination of *abi3-8* and *abi5-8* mutant seeds was abolished upon ABA exposure under long-day conditions.

Supplementary Figure S16. Expression of *CCA1*, *PRR7*, and *LUX* in response to ABA during seed germination.

Supplementary Figure S17. Noncold-stratified WT seeds did not exhibit variation in germination and cotyledon greening on half-strength MS medium without ABA.

Supplementary Figure S18. The enrichment of ABI3 and ABI5 in the promoter region of *EM6* (*pEM6-1*) in ABA-treated germinating seeds at 17 °C.

Supplementary Table S1. The ABI3- and ABI5-enriched promoter sequence of *EM6* (*pEM6-1*).

Supplementary Dataset 1. Primers used for cloning and RT-qPCR.

Supplementary Dataset 2. ANOVA tables.

Funding

This work was supported by the Natural Science Foundation of Hunan Province (grant 2024JJ3007), the National Natural Science Foundation of China (grant 32270613 and 31922009), and the Yunnan Fundamental Research Projects (grant 202401AT070226, 202305AS350010, 202301AT070550, and 202201AS070051).

Conflict of interest statement. All authors state that they have no conflict of interest in relation on this research.

Data availability

All data supporting the findings of this study are available within the article and its supplementary materials.

References

Adams S, Grundy J, Veflingstad SR, Dyer NP, Hannah MA, Ott S, Carré IA. Circadian control of abscisic acid biosynthesis and signaling pathways revealed by genome-wide analysis of LHY binding targets. *New Phytol*. 2018:220(3):893–907. https://doi.org/10.1111/nph.15415

Adams S, Manfield I, Stockley P, Carré IA. Revised morning loops of the *Arabidopsis* circadian clock based on analyses of direct regulatory interactions. *PLoS One*. 2015:10(12):e0143943. https://doi.org/10.1371/journal.pone.0143943

Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA. Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. *Science*. 2001:293(5531):880–883. https://doi.org/10.1126/science.1061320

Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O. S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. *Nat Commun*. 2015:6(1):8669. https://doi.org/10.1038/ncomms9669

Atkins KA, Dodd AN. Circadian regulation of chloroplasts. *Curr Opin Plant Biol*. 2014:21:43–50. https://doi.org/10.1016/j.pbi.2014.06.008

Bhardwaj V, Meier S, Petersen LN, Ingle RA, Roden LC. Defence responses of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* are regulated by the circadian clock. *PLoS One*. 2011:6(10):e26968. https://doi.org/10.1371/journal.pone.0026968

Bieniawska Z, Espinoza C, Schlereth A, Sulpice R, Hincha DK, Hannah MA. Disruption of the *Arabidopsis* circadian clock is responsible for extensive variation in the cold-responsive transcriptome. *Plant Physiol*. 2008:147(1):263–279. https://doi.org/10.1104/pp.108.118059

Brocard IM, Lynch TJ, Finkelstein RR. Regulation and role of the *Arabidopsis ABSCISIC ACID-INSENSITIVE* 5 gene in abscisic acid, sugar, and stress response. *Plant Physiol*. 2002:129(4):1533–1543. https://doi.org/10.1104/pp.005793

Carbonero P, Iglesias-Fernández R, Vicente-Carbajosa J. The AFL subfamily of B3 transcription factors: evolution and function in angiosperm seeds. *J Exp Bot*. 2017:68(4):871–880. https://doi.org/10.1093/jxb/erw458

Carles C, Bies-Etheve N, Aspart L, Léon-Kloosterziel KM, Koornneef M, Echeverria M, Delseny M. Regulation of *Arabidopsis thaliana Em* genes: role of ABI5. *Plant J*. 2002:30(3):373–383. https://doi.org/10.1046/j.1365-313X.2002.01295.x

Carré I, Veflingstad SR. Emerging design principles in the *Arabidopsis* circadian clock. *Semin Cell Dev Biol*. 2013:24(5):393–398. https://doi.org/10.1016/j.semcdb.2013.03.011

Chandrasekaran U, Luo X, Zhou W, Shu K. Multifaceted signaling networks mediated by ABSCISIC ACID INSENSITIVE4. *Plant Commun*. 2020:1(3):100040. https://doi.org/10.1016/j.xplc.2020.100040

Chen K, Li GJ, Bressan RA, Song CP, Zhu J K, Zhao Y. Abscisic acid dynamics, signaling, and functions in plants. *J Integr Plant Biol*. 2020:62(1):25–54. https://doi.org/10.1111/jipb.12899

Chen L, Zhang L, Yu D. Wounding-induced WRKY8 is involved in basal defense in *Arabidopsis*. *Mol Plant Microbe Interact*. 2010:23(5):558–565. https://doi.org/10.1094/MPMI-23-5-0558

Chen R, Jiang H, Li L, Zhai Q, Qi L, Zhou W, Liu X, Li H, Zheng W, Sun J, et al. The *Arabidopsis* mediator subunit MED25 differentially regulates jasmonate and abscisic acid signaling through interacting with the MYC2 and ABI5 transcription factors. *Plant Cell*. 2012:24(7):2898–2916. https://doi.org/10.1105/tpc.112.098277

Covington MF, Maloof JN, Straume M, Kay SA, Harmer SL. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. *Genome Biol*. 2008:9(8):R130. https://doi.org/10.1186/gb-2008-9-8-r130

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. Abscisic acid: emergence of a core signaling network. *Annu Rev Plant Biol*. 2010:61(1):651–679. https://doi.org/10.1146/annurev-arplant-042809-112122

Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR. Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiol*. 2005:139(1):5–17. https://doi.org/10.1104/pp.105.063743

Dai M, Xue Q, Mccray T, Margavage K, Chen F, Lee J-H, Nezames CD, Guo L, Terzaghi W, Wan J, et al. The PP6 phosphatase regulates ABI5 phosphorylation and abscisic acid signaling in Arabidopsis. *Plant Cell*. 2013:25(2):517–534. https://doi.org/10.1105/tpc.112.105767

Ding L, Wang S, Song ZT, Jiang Y, Han JJ, Lu SJ, Li L, Liu JX. Two B-Box domain proteins, BBX18 and BBX23, interact with ELF3 and regulate thermomorphogenesis in Arabidopsis. *Cell Rep*. 2018:25(7):1718–1728. https://doi.org/10.1016/j.celrep.2018.10.060

Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ, Webb AA. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science*. 2005:309(5734):630–633. https://doi.org/10.1126/science.1115581

Doyle MR, Davis SJ, Bastow RM, McWatters HG, Kozma-Bognár L, Nagy F, Millar AJ, Amasino RM. The *ELF4* gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. *Nature*. 2002:419(6902):74–77. https://doi.org/10.1038/nature00954

Du C, Liu M, Yan Y, Guo X, Cao X, Jiao Y, Zheng J, Ma Y, Xie Y, Li H, et al. The U-box E3 ubiquitin ligase PUB35 negatively regulates ABA signaling through AFP1-mediated degradation of ABI5. *Plant Cell*. 2024:36(9):3277–3297. https://doi.org/10.1093/plcell/koae194

Dunlap JC. Molecular bases for circadian clocks. *Cell*. 1999:96(2):271–290. https://doi.org/10.1016/S0092-8674(00)80566-8

Ezer D, Jung J-H, Lan H, Biswas S, Gregoire L, Box MS, Charoensawan V, Cortijo S, Lai X, Stöckle D, et al. The evening complex coordinates environmental and endogenous signals in *Arabidopsis*. *Nat Plants*. 2017:3(7):17087. https://doi.org/10.1038/nplants.2017.87

Farré E, Liu T. The PRR family of transcriptional regulators reflects the complexity and evolution of plant circadian clocks. *Curr Opin Plant Biol*. 2013:16(5):621–629. https://doi.org/10.1016/j.pbi.2013.06.015

Finch-Savage WE, Footitt S. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *J Exp Bot*. 2017:68(4):843–856. https://doi.org/10.1093/jxb/erw477

Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. *New Phytol*. 2006:171(3):501–523. https://doi.org/10.1111/j.1469-8137.2006.01787.x

Finkelstein R. Abscisic acid synthesis and response. *Arabidopsis Book*. 2013:11:e0166. https://doi.org/10.1199/tab.0166

Finkelstein R, Gampala SS, Lynch TJ, Thomas TL, Rock CD. Redundant and distinct functions of the ABA response loci *ABA-INSENSITIVE(ABI)5* and *ABRE-BINDING FACTOR (ABF)3*. *Plant Mol Biol*. 2005:59(2):253–267. https://doi.org/10.1007/s11103-005-8767-2

Finkelstein R, Reeves W, Ariizumi T, Steber C. Molecular aspects of seed dormancy. *Annu Rev Plant Biol*. 2008:59(1):387–415. https://doi.org/10.1146/annurev.arplant.59.032607.092740

Finkelstein RR. Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. *Plant J*. 1994:5(6):765–771. https://doi.org/10.1046/j.1365-313X.1994.5060765.x

Finkelstein RR, Lynch TJ. The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell*. 2000:12(4):599–609. https://doi.org/10.1105/tpc.12.4.599

Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM. The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. *Plant Cell*. 1998:10(6):1043–1054. https://doi.org/10.1105/tpc.10.6.1043

Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE. Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. *Proc Natl Acad Sci U S A*.2011:108(50):20236–20241. https://doi.org/10.1073/pnas.1116325108

Footitt S, Ölçer-Footitt H, Hambidge AJ, Finch-Savage WE. A laboratory simulation of *Arabidopsis* seed dormancy cycling provides new insight into its regulation by clock genes and the dormancy-related genes *DOG1*, *MFT*, *CIPK23* and *PHYA*. *Plant Cell Environ*. 2017:40(8):1474–1486. https://doi.org/10.1111/pce.12940

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J. *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J*. 1999:18(17):4679–4688. https://doi.org/10.1093/emboj/18.17.4679

Fujii H, Verslues PE, Zhu JK. Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis*. *Plant Cell*. 2007:19(2):485–494. https://doi.org/10.1105/tpc.106.048538

Fujii H, Zhu JK. Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. *Proc Natl Acad Sci U S A*. 2009:106(20):8380–8385. https://doi.org/10.1073/pnas.0903144106

Fujimori T, Yamashino T, Kato T, Mizuno T. Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in *Arabidopsis thaliana*. *Plant Cell Physiol*. 2004:45(8):1078–1086. https://doi.org/10.1093/pcp/pch124

Fukushima A, Kusano M, Nakamichi N, Kobayashi M, Hayashi N, Sakakibara H, Mizuno T, Saito K. Impact of clock-associated *Arabidopsis* pseudo-response regulators in metabolic coordination. *Proc Natl Acad Sci U S A*. 2009:106(17):7251–7256. https://doi.org/10.1073/pnas.0900952106

Fung-Uceda J, Lee K, Seo PJ, Polyn S, De Veylder L, Mas P. The circadian clock sets the time of DNA replication licensing to regulate growth in *Arabidopsis*. *Dev Cell*. 2018:45(1):101–113.e4. https://doi.org/10.1016/j.devcel.2018.02.022

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozak K, Yamaguchi-Shinozaki K. Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proc Natl Acad Sci U S A*. 2006:103(6):1988–1993. https://doi.org/10.1073/pnas.0505667103

Garcia ME, Lynch T, Peeters J, Snowden C, Finkelstein R. A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. *Plant Mol Biol*. 2008:67(6):643–658. https://doi.org/10.1007/s11103-008-9344-2

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM. Isolation of the *Arabidopsis ABI3* gene by positional cloning.

Plant Cell. 1992:4(10):1251–1261. https://doi.org/10.1105/tpc.4.10.1251

Golldack D, Li C, Mohan H, Probst N. Gibberellins and abscisic acid signal crosstalk: living and developing under unfavorable conditions. *Plant Cell Rep*. 2013:32(7):1007–1016. https://doi.org/10.1007/s00299-013-1409-2

Goodspeed D, Chehab EW, Min-Venditti A, Braam J, Covington MF. Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. *Proc Natl Acad Sci U S A*. 2012:109(12):4674–4677. https://doi.org/10.1073/pnas.1116368109

Greenham K, McClung CR. Integrating circadian dynamics with physiological processes in plants. *Nat Rev Genet*. 2015:16(10):598–610. https://doi.org/10.1038/nrg3976

Grundy J, Stoker C, Carré IA. Circadian regulation of abiotic stress tolerance in plants. *Front Plant Sci*. 2015:6:648. https://doi.org/10.3389/fpls.2015.00648

Guan C, Wang X, Feng J, Hong S, Liang Y, Ren B, Zuo J. Cytokinin antagonizes abscisic acid-mediated inhibition of cotyledon greening by promoting the degradation of ABSCISIC ACID INSENSITIVE5 protein in Arabidopsis. *Plant Physiol*. 2014:164(3):1515–1526. https://doi.org/10.1104/pp.113.234740

Gubler F, Millar AA, Jacobsen JV. Dormancy release, ABA and pre-harvest sprouting. *Curr Opin Plant Biol*. 2005:8(2):183–187. https://doi.org/10.1016/j.pbi.2005.01.011

Guo J, Song R, Lu K, Zhang Y, Chen H, Zuo J, Li T, Li X, Liu W. Cycc1;1 negatively modulates ABA signaling by interacting with and inhibiting ABI5 during seed germination. *Plant Physiol*. 2022:190(4):2812–2827. https://doi.org/10.1093/plphys/kiac456

Han X, Kui M, Xu T, Ye J, Du J, Yang M, Jiang Y, Hu Y. CO interacts with JAZ repressors and bHLH subgroup IIId factors to negatively regulate jasmonate signaling in *Arabidopsis* seedlings. *Plant Cell*. 2023:35(2):852–873. https://doi.org/10.1093/plcell/koac331

Han X, Zhang M, Yang M, Hu Y. *Arabidopsis* JAZ proteins interact with and suppress RHD6 transcription factor to regulate jasmonate stimulated root hair development. *Plant Cell*. 2020:32(4):1049–1062. https://doi.org/10.1105/tpc.19.00617

Harmer SL. The circadian system in higher plants. *Annu Rev Plant Biol*. 2009:60(1):357–377. https://doi.org/10.1146/annurev.arplant.043008.092054

Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA. Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science*. 2000:290(5499):2110–2113. https://doi.org/10.1126/science.290.5499.2110

Hayama R, Sarid-Krebs L, Richter R, Fernández V, Jang S, Coupland G. PSEUDO RESPONSE REGULATORs stabilize CONSTANS protein to promote flowering in response to day length. *EMBO J*. 2017:36(7):904–918. https://doi.org/10.15252/embj.201693907

He Y, Sun S, Zhao J, Huang Z, Peng L, Huang C, Tang Z, Huang Q, Wang Z. UDP-glucosyltransferase OsUGT75A promotes submergence tolerance during rice seed germination. *Nat Commun*. 2023:14(1):2296. https://doi.org/10.1038/s41467-023-38085-5

He Y, Yu Y, Wang X, Qin Y, Su C, Wang L. Aschoff's rule on circadian rhythms orchestrated by blue light sensor CRY2 and clock component PRR9. *Nat Commun*. 2022:13(1):5869. https://doi.org/10.1038/s41467-022-33568-3

Helfer A, Nusinow DA, Chow BY, Gehrke AR, Bulyk ML, Kay SA. LUX ARRHYTHMO encodes a nighttime repressor of circadian gene expression in the *Arabidopsis* core clock. *Curr Biol*. 2011:21(2):126–133. https://doi.org/10.1016/j.cub.2010.12.021

Hellens RP, Allan AC, Friel EN, Bolitho K, Grafton K, Templeton MD, Karunairetnam S, Gleave AP, Laing WA. Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. *Plant Methods*. 2005:1(1):13. https://doi.org/10.1186/1746-4811-1-13

Herrero E, Kolmos E, Bujdoso N, Yuan Y, Wang M, Berns MC, Uhlworm H, Coupland G, Saini R, Jaskolski M, et al. EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the *Arabidopsis* circadian clock. *Plant Cell*. 2012:24(2):428–443. https://doi.org/10.1105/tpc.111.093807

Hirohata A, Yamatsuta Y, Ogawa K, Kubota A, Suzuki T, Shimizu H, Kanesaka Y, Takahashi N, Endo M. Sulfanilamide regulates flowering time through expression of the circadian clock gene LUX. *Plant Cell Physiol*. 2022:63(5):649–657. https://doi.org/10.1093/pcp/pcac027

Hsu PY, Harmer SL. Wheels within wheels: the plant circadian system. *Trends Plant Sci*. 2014:19(4):240–249. https://doi.org/10.1016/j.tplants.2013.11.007

Hu Y, Han X, Yang M, Zhang M, Pan J, Yu D. The transcription factor INDUCER OF CBF EXPRESSION1 interacts with ABSCISIC ACID INSENSITIVE5 and DELLA proteins to fine-tune abscisic acid signaling during seed germination in *Arabidopsis*. *Plant Cell*. 2019:31(7):1520–1538. https://doi.org/10.1105/tpc.18.00825

Hu Y, Jiang L, Wang F, Yu D. Jasmonate regulates the inducer of CBF expression-C-repeat binding factor/DRE binding factor1 cascade and freezing tolerance in *Arabidopsis*. *Plant Cell*. 2013:25(8):2907–2924. https://doi.org/10.1105/tpc.113.112631

Hu Y, Yu D. BRASSINOSTEROID INSENSITIVE2 interacts with ABSCISIC ACID INSENSITIVE5 to mediate the antagonism of brassinosteroids to abscisic acid during seed germination in *Arabidopsis*. *Plant Cell*. 2014:26(11):4394–4408. https://doi.org/10.1105/tpc.114.130849

Huang Z, Han X, He K, Ye J, Yu C, Xu T, Zhang J, Du J, Fu Q, Hu Y. Nitrate attenuates abscisic acid signaling via NIN-LIKE PROTEIN8 in *Arabidopsis* seed germination. *Plant Cell*. 2025:37(3):koaf046. https://doi.org/10.1093/plcell/koaf046

Ito S, Niwa Y, Nakamichi N, Kawamura H, Yamashino T, Mizuno T. Insight into missing genetic links between two evening-expressed pseudo-response regulator genes TOC1 and PRR5 in the circadian clock-controlled circuitry in *Arabidopsis thaliana*. *Plant Cell Physiol*. 2008:49(2):201–213. https://doi.org/10.1093/pcp/pcm178

Ji H, Wang S, Cheng C, Li R, Wang Z, Jenkins GI, Kong F, Li X. The RCC1 family protein SAB1 negatively regulates ABI5 through multidimensional mechanisms during postgermination in *Arabidopsis*. *New Phytol*. 2019:222(2):907–922. https://doi.org/10.1111/nph.15653

Jiang Y, Liang G, Yang S, Yu D. *Arabidopsis* WRKY57 functions as node of convergence for jasmonic acid- and auxin-mediated signaling in jasmonic acid-induced leaf senescence. *Plant Cell*. 2014:26(1):230–245. https://doi.org/10.1105/tpc.113.117838

Joo Y, Schuman MC, Goldberg JK, Wissgott A, Kim SG, Baldwin IT. Herbivory elicits changes in green leaf volatile production via jasmonate signaling and the circadian clock. *Plant Cell Environ*. 2019:42(3):972–982. https://doi.org/10.1111/pce.13474

Ju L, Jing Y, Shi P, Liu J, Chen J, Yan J, Chu J, Chen KM, Sun J. JAZ proteins modulate seed germination through interaction with ABI5 in bread wheat and *Arabidopsis*. *New Phytol*. 2019:223(1):246–260. https://doi.org/10.1111/nph.15757

Kim J, Kang H, Park J, Kim W, Yoo J, Lee N, Kim J, Yoon TY, Choi G. PIF1-interacting transcription factors and their binding sequence elements determine the *in vivo* targeting sites of PIF1. *Plant Cell*. 2016:28(6):1388–1405. https://doi.org/10.1105/tpc.16.00125

Kim KC, Lai Z, Fan B, Chen Z. *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell*. 2008:20(9):2357–2371. https://doi.org/10.1105/tpc.107.055566

Kim WY, Fujiwara S, Suh SS, Kim J, Kim Y, Han L, David K, Putterill J, Nam HG, Somers DE. ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. *Nature*. 2007:449(7160):356–360. https://doi.org/10.1038/nature06132

Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T. Abscisic acid-activated SNRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. *Plant J*. 2005:44(6):939–949. https://doi.org/10.1111/j.1365-313X.2005.02583.x

Koornneef M, Reuling G, Karssen CM. The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. *Physiol Plant*. 1984:61(3):377–383. https://doi.org/10.1111/j.1399-3054.1984.tb06343.x

Korneli C, Danisman S, Staiger D. Differential control of pre-invasive and post-invasive antibacterial defense by the *Arabidopsis* circadian clock. *Plant Cell Physiol*. 2014:55(9):1613–1622. https://doi.org/10.1093/pcp/pcu092

Kurup S, Jones HD, Holdsworth MJ. Interactions of the developmental regulator ABI3 with proteins identified from developing *Arabidopsis* seeds. *Plant J*. 2000:21(2):143–155. https://doi.org/10.1046/j.1365-313x.2000.00663.x

Lee JH, Yoon HJ, Terzaghi W, Martinez C, Dai M, Li J, Byun MO, Deng XW. DWA1 and DWA2, two *Arabidopsis* DWD protein components of CUL4-based E3 ligases, act together as negative regulators in ABA signal transduction. *Plant Cell*. 2010:22(6):1716–1732. https://doi.org/10.1105/tpc.109.073783

Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ, Hwang I. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell*. 2006:126(6):1109–1120. https://doi.org/10.1016/j.cell.2006.07.034

Lee KP, Piskurewicz U, Turečková V, Carat S, Chappuis R, Strnad M, Fankhauser C, Lopez-Molina L. Spatially and genetically distinct control of seed germination by phytochromes A and B. *Genes Dev*. 2012:26(17):1984–1996. https://doi.org/10.1101/gad.194266.112

Li B, Wang Y, Zhang Y, Tian W, Chong K, Jang J, Wang L. PRR5, 7 and 9 positively modulate TOR signaling-mediated root cell proliferation by repressing TANDEM ZINC FINGER1 in *Arabidopsis*. *Nucleic Acids Res*. 2019:47(10):5001–5015. https://doi.org/10.1093/nar/gkz191

Li K, Yang F, Miao Y, Song C. Abscisic acid signaling is involved in regulating the mitogen-activated protein kinase cascade module, AIK1-MKK5-MPK6. *Plant Signal Behav*. 2017:12(5):e1321188. https://doi.org/10.1080/15592324.2017.1321188

Li N, Zhang Y, He Y, Wang Y, Wang L. Pseudo response regulators regulate photoperiodic hypocotyl growth by repressing PIF4/5 transcription. *Plant Physiol*. 2020:183(2):686–699. https://doi.org/10.1104/pp.19.01599

Li R, Song Y, Wang X, Zheng C, Liu B, Zhang H, Ke J, Wu X, Wu L, Yang R, et al. OsNAC5 orchestrates OsABI5 to fine-tune cold tolerance in rice. *J Integr Plant Biol*. 2024:66(4):660–682. https://doi.org/10.1111/jipb.13585

Li Z, Li S, Jin D, Yang Y, Pu Z, Han X, Hu Y, Jiang Y. U-box E3 ubiquitin ligase PUB8 attenuates abscisic acid responses during early seedling growth. *Plant Physiol*. 2023:191(4):2519–2533. https://doi.org/10.1093/plphys/kiad044

Li Z, Luo X, Wang L, Shu K. ABSCISIC ACID INSENSITIVE 5 mediates light-ABA/gibberellin crosstalk networks during seed germination. *J Exp Bot*. 2022:73(14):4674–4682. https://doi.org/10.1093/jxb/erac200

Liang T, Yu S, Pan Y, Wang J, Kay SA. The interplay between the circadian clock and abiotic stress responses mediated by ABF3 and CCA1/LHY. *Proc Natl Acad Sci U S A*. 2024:121(7):e2316825121. https://doi.org/10.1073/pnas.2316825121

Lim S, Park J, Lee N, Jeong J, Toh S, Watanabe A, Kim J, Kang H, Kim DH, Kawakami N, et al. ABA-INSENSITIVE 3, ABA-INSENSITIVE 5, and DELLAs interact to activate the expression of SOMNUS and other high-temperature-inducible genes in imbibed seeds in *Arabidopsis*. *Plant Cell*. 2014:25(12):4863–4878. https://doi.org/10.1105/tpc.113.118604

Liu H, Stone SL. Abscisic acid increases *Arabidopsis* ABI5 transcription factor levels by promoting KEG E3 ligase self-ubiquitination and proteasomal degradation. *Plant Cell*. 2010:22(8):2630–2641. https://doi.org/10.1105/tpc.110.076075

Liu H, Stone SL. Regulation of ABI5 turnover by reversible post-translational modifications. *Plant Signal Behav*. 2014:9(1):e27577. https://doi.org/10.4161/psb.27577

Liu T, Carlsson J, Takeuchi T, Newton L, Farré EM. Direct regulation of abiotic responses by the *Arabidopsis* circadian clock component PRR7. *Plant J*. 2013a:76(1):101–114. https://doi.org/10.1111/tpj.12276

Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang H, Luan S, Li J, He Z. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated *ABI3* activation in *Arabidopsis*. *Proc Natl Acad Sci U S A*. 2013b:110(38):15485–11590. https://doi.org/10.1073/pnas.1304651110

Lopez-Molina L, Chua NH. A null mutation in a bZIP factor confers ABA-insensitivity in *Arabidopsis thaliana*. *Plant Cell Physiol*. 2000:41(5):541–547. https://doi.org/10.1093/pcp/41.5.541

Lopez-Molina L, Mongrand S, Chua NH. A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc Natl Acad Sci U S A*. 2001:98(8):4782–4787. https://doi.org/10.1073/pnas.081594298

Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH. ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J*. 2002:32(3):317–328. https://doi.org/10.1046/j.1365-313X.2002.01430.x

Lu H, McClung CR, Zhang C. Tick tock: circadian regulation of plant innate immunity. *Annu Rev Phytopathol*. 2017:55(1):287–311. https://doi.org/10.1146/annurev-phyto-080516-035451

Luo X, Dai Y, Xian B, Xu J, Zhang R, Rehmani MS, Zheng C, Zhao X, Mao K, Ren X, et al. PIF4 interacts with ABI4 to serve as a transcriptional activator complex to promote seed dormancy by enhancing ABA biosynthesis and signaling. *J Integr Plant Biol*. 2024:66(5):909–927. https://doi.org/10.1111/jipb.13615

Lynch TJ, Erickson BJ, Miller DR, Finkelstein RR. ABI5-binding proteins (AFPs) alter transcription of ABA-induced genes via a variety of interactions with chromatin modifiers. *Plant Mol Biol*. 2017:93(4-5):403–418. https://doi.org/10.1007/s11103-016-0569-1

Lyzenga WJ, Liu H, Schofield A, Muise-Hennessey A, Stone SL. *Arabidopsis* CIPK26 interacts with KEG, components of the ABA signalling network and is degraded by the ubiquitin-proteasome system. *J Exp Bot*. 2013:64(10):2779–2791. https://doi.org/10.1093/jxb/ert123

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science*. 2009:324(5930):1064–1068. https://doi.org/10.1126/science.1172408

Martín G, Rovira A, Veciana N, Soy J, Toledo-Ortiz G, Gommers CMM, Boix M, Henriques R, Minguet EG, Alabadí D, et al. Circadian waves of transcriptional repression shape PIF-regulated photoperiod-responsive growth in *Arabidopsis*. *Curr Biol*. 2018:28(2):311–318.e5. https://doi.org/10.1016/j.cub.2017.12.021

Matsushika A, Makino S, Kojima M, Mizuno T. Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana:* insight into the plant circadian clock. *Plant Cell Physiol*. 2000:41(9):1002–1012. https://doi.org/10.1093/pcp/pcd043

McAdam SAM, Brodribb TJ, Ross JJ, Jordan GJ. Augmentation of abscisic acid (ABA) levels by drought does not induce short-term stomatal sensitivity to CO_2 in two divergent conifer species. *J Exp Bot*. 2011:62(1):195–203. https://doi.org/10.1093/jxb/erq260

McWatters HG, Kolmos E, Hall A, Doyle MR, Amasino RM, Gyula P, Nagy F, Millar AJ, Davis SJ. ELF4 is required for oscillatory properties of the circadian clock. *Plant Physiol*. 2007:144(1):391–401. https://doi.org/10.1104/pp.107.096206

Mei S, Zhang M, Ye J, Du J, Jiang Y, Hu Y. Auxin contributes to jasmonate-mediated regulation of abscisic acid signaling during seed germination in *Arabidopsis*. *Plant Cell*. 2023:35(3):1110–1133. https://doi.org/10.1093/plcell/koac362

Michael TP, Breton G, Hazen SP, Priest H, Mockler TC, Kay SA, Chory J. A morning-specific phytohormone gene expression program underlying rhythmic plant growth. *PLoS Biol*. 2008:6(9):e225. https://doi.org/10.1371/journal.pbio.0060225

Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM. Sumoylation of ABI5 by the *Arabidopsis* SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling. *Proc Natl Acad Sci U S A*. 2009:106(13):5418–5423. https://doi.org/10.1073/pnas.0811088106

Miyazono K, Miyakawa T, Sawano Y, Kubota K, Kang H-J, Asano A, Miyauchi Y, Takahashi M, Zhi Y, Fujita Y, et al. Structural basis of abscisic acid signalling. *Nature*. 2009:462(7273):609–614. https://doi.org/10.1038/nature08583

Mizuno T, Nomoto Y, Oka H, Kitayama M, Takeuchi A, Tsubouchi M, Yamashino T. Ambient temperature signal feeds into the circadian clock transcriptional circuitry through the EC night-time repressor in *Arabidopsis thaliana*. *Plant Cell Physiol*. 2014:55(5):958–976. https://doi.org/10.1093/pcp/pcu030

Mizuno T, Yamashino T. Comparative transcriptome of diurnally oscillating genes and hormone-responsive genes in *Arabidopsis thaliana:* insight into circadian clock-controlled daily responses to common ambient stresses in plants. *Plant Cell Physiol*. 2008:49(3):481–487. https://doi.org/10.1093/pcp/pcn008

Mukhopadhyay A, Deplancke B, Walhout AJM, Tissenbaum HA. Chromatin immunoprecipitation (ChIP) coupled to detection by quantitative real-time PCR to study transcription factor binding to DNA in *Caenorhabditis elegans*. *Nat Protoc*. 2008:3(4):698–709. https://doi.org/10.1038/nprot.2008.38

Nakamichi N. The transcriptional network in the *Arabidopsis* circadian clock system. *Genes (Basel)*. 2020:11(11):1284. https://doi.org/10.3390/genes11111284

Nakamichi N, Kiba T, Henriques R, Mizuno T, Chua NH, Sakakibara H. PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the *Arabidopsis* circadian clock. *Plant Cell*. 2010:22(3):594–605. https://doi.org/10.1105/tpc.109.072892

Nakamichi N, Kiba T, Kamioka M, Suzuki T, Yamashino T, Higashiyama T, Sakakibara H, Mizuno T. Transcriptional repressor PRR5 directly regulates clock-output pathways. *Proc Natl Acad Sci U S A*. 2012:109(42):17123–17128. https://doi.org/10.1073/pnas.1205156109

Nakamichi N, Kita M, Ito S, Yamashino T, Mizuno T. PSEUDO-RESPONSE REGULATORS, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of *Arabidopsis thaliana*. *Plant Cell Physiol*. 2005:46(5):686–698. https://doi.org/10.1093/pcp/pci086

Nakamichi N, Kita M, Niinum K, Ito S, Yamashin T, Mizoguchi T, Mizuno T. *Arabidopsis* clock-associated pseudo-response regulators PRR9, PRR7 and PRR5 coordinately and positively regulate flowering time through the canonical CONSTANS dependent photoperiodic pathway. *Plant Cell Physiol*. 2007:48(6):822–832. https://doi.org/10.1093/pcp/pcm056

Nakamichi N, Kusano M, Fukushima A, Kita M, Ito S, Yamashino T, Saito K, Sakakibara H, Mizuno T. Transcript profiling of an *Arabidopsis* PSEUDO RESPONSE REGULATOR arrhythmic triple mutant reveals a role for the circadian clock in cold stress response. *Plant Cell Physiol*. 2009:50(3):447–462. https://doi.org/10.1093/pcp/pcp004

Nakamura S, Lynch TJ, Finkelstein RR. Physical interactions between ABA response loci of *Arabidopsis*. *Plant J*. 2001:26(6):627–635. https://doi.org/10.1046/j.1365-313x.2001.01069.x

Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, Maruyama K, Yoshida T, Ishiyama K, Kobayashi M, et al. Three *Arabidopsis* SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. *Plant Cell Physiol*. 2009:50(7):1345–1363. https://doi.org/10.1093/pcp/pcp083

Nakashima K, Yamaguchi-Shinozaki K. ABA signaling in stress-response and seed development. *Plant Cell Rep*. 2013:32(7):959–970. https://doi.org/10.1007/s00299-013-1418-1

Nambara E, Suzuki M, Abrams S, McCarty DR, Kamiya Y, McCourt P. A screen for genes that function in abscisic acid signaling in *Arabidopsis thaliana*. *Genetics*. 2002:161(3):1247–1255. https://doi.org/10.1093/genetics/161.3.1247

Nie K, Zhao H, Wang X, Niu Y, Zhou H, Zheng Y. The MIEL1-ABI5/MYB30 regulatory module fine tunes abscisic acid signaling during seed germination. *J Integr Plant Biol*. 2022:64(4):930–941. https://doi.org/10.1111/jipb.13234

Nieto C, López-Salmerón V, Davière JM, Prat S. ELF3-PIF4 interaction regulates plant growth independently of the evening complex. *Curr Biol*. 2015:25(2):187–193. https://doi.org/10.1016/j.cub.2014.10.070

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED. Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. *Science*. 2009:326(5958):1373–1379. https://doi.org/10.1126/science.1181829

Nováková M, Motyka V, Dobrev PI, Malbeck J, Gaudinová A, Vanková R. Diurnal variation of cytokinin, auxin and abscisic acid levels in tobacco leaves. *J Exp Bot*. 2005:56(421):2877–2883. https://doi.org/10.1093/jxb/eri282

Nusinow DA, Helfer A, Hamilton EE, King JJ, Imaizumi T, Schultz TF, Farré EM, Kay SA. The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. *Nature*. 2011:475(7356):398–402. https://doi.org/10.1038/nature10182

Pan J, Hu Y, Wang H, Guo Q, Chen Y, Howe GA, Yu D. Molecular mechanism underlying the synergetic effect of jasmonate on abscisic acid signaling during seed germination in *Arabidopsis*. *Plant Cell*. 2020:32(12):3846–3865. https://doi.org/10.1105/tpc.19.00838

Pan J, Wang H, Hu Y, Yu D. *Arabidopsis* VQ18 and VQ26 proteins interact with ABI5 transcription factor to negatively modulate ABA response during seed germination. *Plant J*. 2018:95(3):529–544. https://doi.org/10.1111/tpj.13969

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, et al. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science*. 2009:324(5930):1068–1071. https://doi.org/10.1126/science.1173041

Penfield S, Hall A. A role for multiple circadian clock genes in the response to signals that break seed dormancy in

Arabidopsis. *Plant Cell*. 2009:21(6):1722–1732. https://doi.org/10.1105/tpc.108.064022

Peng J, Wang M, Wang X, Qi L, Guo C, Li H, Li C, Yan Y, Zhou Y, Terzaghi W, et al. COP1 positively regulates ABA signaling during *Arabidopsis* seedling growth in darkness by mediating ABA-induced ABI5 accumulation. *Plant Cell*. 2022:34(6):2286–2308. https://doi.org/10.1093/plcell/koac073

Perales M, Más P. A functional link between rhythmic changes in chromatin structure and the *Arabidopsis* biological clock. *Plant Cell*. 2007:19(7):2111–2123. https://doi.org/10.1105/tpc.107.050807

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. The gibberellic acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5 activity. *Plant Cell*. 2008:20(10):2729–2745. https://doi.org/10.1105/tpc.108.061515

Pokhilko A, Fernández AP, Edwards KD, Southern MM, Halliday KJ, Millar AJ. The clock gene circuit in *Arabidopsis* includes a repressilator with additional feedback loops. *Mol Syst Biol*. 2012:8(1):574. https://doi.org/10.1038/msb.2012.6

Pruneda-Paz JL, Kay SA. An expanding universe of circadian networks in higher plants. *Trends Plant Sci*. 2010:15(5):259–265. https://doi.org/10.1016/j.tplants.2010.03.003

Putterill J, Robson F, Lee K, Simon R, Coupland G. The CONSTANS gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell*. 1995:80(6):847–857. https://doi.org/10.1016/0092-8674(95)90288-0

Sajeev N, Koornneef M, Bentsink L. A commitment for life: decades of unraveling the molecular mechanisms behind seed dormancy and germination. *Plant Cell*. 2024:36(5):1358–1376. https://doi.org/10.1093/plcell/koad328

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G. Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. *Science*. 2000:288(5471):1613–1616. https://doi.org/10.1126/science.288.5471.1613

Sanchez SE, Kay SA. The plant circadian clock: from a simple timekeeper to a complex developmental manager. *Cold Spring Harb Perspect Biol*. 2016:8(12):a027748. https://doi.org/10.1101/cshperspect.a027748

Santiago J, Dupeux F, Round A, Antoni R, Par SY, Jamin M, Cutler SR, Rodriguez PL, Márquez JA. The abscisic acid receptor PYR1 in complex with abscisic acid. *Nature*. 2009:462(7273):665–668. https://doi.org/10.1038/nature08591

Sawa M, Nusinow DA, Kay SA, Imaizumi T. FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. *Science*. 2007:318(5848):261–265. https://doi.org/10.1126/science.1146994

Seo KI, Lee JH, Nezames CD, Zhong S, Song E, Byun MO, Deng XW. ABD1 is an *Arabidopsis* DCAF substrate receptor for CUL4-DDB1-based E3 ligases that acts as a negative regulator of abscisic acid 1651 signaling. *Plant Cell*. 2014:26(2):695–711. https://doi.org/10.1105/tpc.113.119974

Seung D, Risopatron JPM, Jones BJ, Marc J. Circadian clock-dependent gating in ABA signalling networks. *Protoplasma*. 2012:249(3):445–457. https://doi.org/10.1007/s00709-011-0304-3

Shalit-Kaneh A, Kumimoto RW, Filkov V, Harmer SL. Multiple feedback loops of the *Arabidopsis* circadian clock provide rhythmic robustness across environmental conditions. *Proc Natl Acad Sci U S A*. 2018:115(27):7147–7152. https://doi.org/10.1073/pnas.1805524115

Sheen J. Signal transduction in maize and *Arabidopsis* mesophyll protoplasts. *Plant Physiol*. 2001:127(4):1466–1475. https://doi.org/10.1104/pp.010820

Shen Y, Khanna R, Carle CM, Quail PH. Phytochrome induces rapid PIF5 phosphorylation and degradation in response to red-light activation. *Plant Physiol*. 2007:145(3):1043–1051. https://doi.org/10.1104/pp.107.105601

Shin J, Heidrich K, Sanchez-Villarreal A, Parker JE, Davis SJ. TIME FOR COFFEE represses accumulation of the MYC2 transcription factor to provide time-of-day regulation of jasmonate signaling in *Arabidopsis*. *Plant Cell*. 2012:24(6):2470–2482. https://doi.org/10.1105/tpc.111.095430

Siemiatkowska B, Chiara M, Badiger BG, Riboni M, D'Avila F, Braga D, Salem MAA, Martignago D, Colanero S, Galbiati M, et al. GIGANTEA is a negative regulator of abscisic acid transcriptional responses and sensitivity in *Arabidopsis*. *Plant Cell Physiol*. 2022:63(9):1285–1297. https://doi.org/10.1093/pcp/pcac102

Simon NML, Graham CA, Comben NE, Hetherington AM, Dodd AN. The circadian clock influences the long-term water use efficiency of *Arabidopsis*. *Plant Physiol*. 2020:183(1):317–330. https://doi.org/10.1104/pp.20.00030

Simon R, Igeño MI, Coupland G. Activation of floral meristem identity genes in *Arabidopsis*. *Nature*. 1996:384(6604):59–62. https://doi.org/10.1038/384059a0

Söderman EM, Brocard IM, Lynch TJ, Finkelstein RR. Regulation and function of the *Arabidopsis ABA-INSENSITIVE4* gene in seed and abscisic acid response signaling networks. *Plant Physiol*. 2000:124(4):1752–1765. https://doi.org/10.1104/pp.124.4.1752

Soy J, Leivar P, González-Schain N, Martín G, Diaz C, Sentandreu M, Al-Sady B, Quail PH, Monte E. Molecular convergence of clock and photosensory pathways through PIF3-TOC1 interaction and co-occupancy of target promoters. *Proc Natl Acad Sci U S A*. 2016:113(17):4870–4875. https://doi.org/10.1073/pnas.1603745113

Stone S, Williams LA, Farmer M, Vierstra RD, Callis J. KEEP ON GOING, a RING E3 ligase essential for *Arabidopsis* growth and development, is involved in abscisic acid signaling. *Plant Cell*. 2007:18(12):3415–3428. https://doi.org/10.1105/tpc.106.046532

Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, Más P, Panda S, Kreps JA, Kay SA. Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science*. 2000:289(5480):768–771. https://doi.org/10.1126/science.289.5480.768

Uehara TN, Mizutani Y, Kuwata K, Hirota T, Sato A, Mizoi J, Takao S, Matsuo H, Suzuki T, Ito S, et al. Casein kinase 1 family regulates PRR5 and TOC1 in the *Arabidopsis* circadian clock. *Proc Natl Acad Sci U S A*. 2019:116(23):11528–11536. https://doi.org/10.1073/pnas.1903357116

Varshney V, Hazra A, Rao V, Ghosh S, Kamble NU, Achary RK, Gautam S, Majee M. The *Arabidopsis* F-box protein SKP1-INTERACTING PARTNER 31 modulates seed maturation and seed vigor by targeting JASMONATE ZIM DOMAIN proteins independently of jasmonic acid-isoleucine. *Plant Cell*. 2023:35(10):3712–3738. https://doi.org/10.1093/plcell/koad199

Wang L, Fujiwara S, Somers DE. PRR5 regulates phosphorylation, nuclear import and subnuclear localization of TOC1 in the *Arabidopsis* circadian clock. *EMBO J*. 2010:29(11):1903–1915. https://doi.org/10.1038/emboj.2010.76

Wang L, Kim J, Somers DE. Transcriptional corepressor TOPLESS complexes with pseudoresponse regulator proteins and histone deacetylases to regulate circadian transcription. *Proc Natl Acad Sci U S A*. 2013:110(2):761–766. https://doi.org/10.1073/pnas.1215010110

Wang W, Barnaby JY, Tada Y, Li H, Tor M, Caldelari D, Lee DU, Fu XD, Dong X. Timing of plant immune responses by a central circadian regulator. *Nature*. 2011:470(7332):110–114. https://doi.org/10.1038/nature09766

Wang Y, Wu F, Lin Q, Sheng P, Wu Z, Jin X, Chen W, Li S, Luo S, Duan E, et al. A regulatory loop establishes the link between the circadian clock and abscisic acid signaling in rice. *Plant Physiol.* 2023:191(3):1857–1870. https://doi.org/10.1093/plphys/kiac548

Wang ZY, Tobin EM. Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) gene disrupts circadian rhythms and suppresses its own expression. *Cell.* 1998:93(7):1207–1217. https://doi.org/10.1016/S0092-8674(00)81464-6

Webb AA, Seki M, Satake A, Caldana C. Continuous dynamic adjustment of the plant circadian oscillator. *Nat Commun.* 2019:10(1):550. https://doi.org/10.1038/s41467-019-08398-5

Wei H, Wang X, He Y, Xu H, Wang L. Clock component OsPRR73 positively regulates rice salt tolerance by modulating OsHKT2;1-mediated sodium homeostasis. *EMBO J.* 2021:40(3):e105086. https://doi.org/10.15252/embj.2020105086

Xu X, Yuan L, Yang X, Zhang X, Wang L, Xie Q. Circadian clock in plants: linking timing to fitness. *J Integr Plant Biol.* 2022:64(4):792–811. https://doi.org/10.1111/jipb.13230

Yamamoto Y, Sato E, Shimizu T, Nakamich N, Sato S, Kato T, Tabata S, Nagatani A, Yamashino T, Mizuno T. Comparative genetic studies on the *APRR5* and *APRR7* genes belonging to the APRR1/TOC1 quintet implicated in circadian rhythm, control of flowering time, and early photomorphogenesis. *Plant Cell Physiol.* 2003:44(11):1119–1130. https://doi.org/10.1093/pcp/pcg148

Yang C, Li X, Chen S, Liu C, Yang L, Li K, Liao J, Zheng X, Li H, Li Y, et al. ABI5-FLZ13 module transcriptionally represses growth-related genes to delay seed germination in response to ABA. *Plant Commun.* 2023:4(6):100636. https://doi.org/10.1016/j.xplc.2023.100636

Yang M, Han X, Yang J, Jiang Y, Hu Y. The *Arabidopsis* circadian clock protein PRR5 interacts with and stimulates ABI5 to modulate abscisic acid signaling during seed germination. *Plant Cell.* 2021:33(9):3022–3041. https://doi.org/10.1093/plcell/koab168

Yang X, Bai Y, Shang J, Xin R, Tang W. The antagonistic regulation of abscisic acid-inhibited root growth by brassinosteroids is partially mediated via direct suppression of *ABSCISIC ACID INSENSITIVE 5* expression by BRASSINAZOLE RESISTANT 1. *Plant Cell Environ.* 2016:39(9):1994–2003. https://doi.org/10.1111/pce.12763

Yoo SD, Cho YH, Sheen J. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nat Protoc.* 2007:2(7):1565–1572. https://doi.org/10.1038/nprot.2007.199

Yoshida R, Fekih R, Fujiwara S, Oda A, Miyata K, Tomozoe Y, Nakagawa M, Niinuma K, Hayashi K, Ezura H, et al. Possible role of early flowering 3 (ELF3) in clock-dependent floral regulation by short vegetative phase (SVP) in *Arabidopsis thaliana*. *New Phytol.* 2009:182(4):838–850. https://doi.org/10.1111/j.1469-8137.2009.02809.x

Yu B, He X, Tang Y, Chen Z, Zhou L, Li X, Zhang C, Huang X, Yang Y, Zhang W, et al. Photoperiod controls plant seed size in a CONSTANS-dependent manner. *Nat Plants.* 2023:9(2):343–354. https://doi.org/10.1038/s41477-023-01350-y

Yu F, Wu Y, Xie Q. Precise protein post-translational modifications modulate ABI5 activity. *Trends Plant Sci.* 2015:20(9):569–575. https://doi.org/10.1016/j.tplants.2015.05.004

Yu JW, Rubio V, Lee N-Y, Bai S, Lee S-Y, Kim S-S, Liu L, Zhang Y, Irigoyen ML, Sullivan JA, et al. COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. *Mol Cell.* 2008:32(5):617–630. https://doi.org/10.1016/j.molcel.2008.09.026

Yuan M, Ngou BPM, Ding P, Xin XF. PTI-ETI crosstalk: an integrative view of plant immunity. *Curr Opin Plant Biol.* 2021:62:102030. https://doi.org/10.1016/j.pbi.2021.102030

Zha P, Liu S, Li Y, Ma T, Yang L, Jing Y, Lin R. The evening complex and the chromatin-remodeling factor PICKLE coordinately control seed dormancy by directly repressing *DOG1* in *Arabidopsis*. *Plant Commun.* 2020:1(2):100011. https://doi.org/10.1016/j.xplc.2019.100011

Zhang C, Gao M, Seitz NC, Angel W, Hallworth A, Wiratan L, Darwish O, Alkharouf N, Dawit T, Lin D, et al. LUX ARRHYTHMO mediates crosstalk between the circadian clock and defense in *Arabidopsis*. *Nat Commun.* 2019:10(1):2543. https://doi.org/10.1038/s41467-019-10485-6

Zhang X, Garreton V, Chua NH. The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation. *Genes Dev.* 2005:19(13):1532–1543. https://doi.org/10.1101/gad.1318705

Zhang Y, Wang Y, Wei H, Li N, Tian W, Chong K, Wang L. Circadian evening complex represses jasmonate-induced leaf senescence in *Arabidopsis*. *Mol Plant.* 2018:11(2):326–337. https://doi.org/10.1016/j.molp.2017.12.017

Zhao J, He Y, Zhang H, Wang Z. Advances in the molecular regulation of seed germination in plants. *Seed Biol.* 2024:3(1):e006. https://doi.org/10.48130/seedbio-0024-0005

Zhao X, Dou L, Gong Z, Wang X, Mao T. BES1 hinders ABSCISIC ACID INSENSITIVE5 and promotes seed germination in *Arabidopsis*. *New Phytol.* 2019:221(2):908–918. https://doi.org/10.1111/nph.15437

Zhong H, Painter JE, Salomé PA, Straume M, McClung CR. Imbibition, but not release from stratification, sets the circadian clock in *Arabidopsis* seedlings. *Plant Cell.* 1998:10(12):2005–2017. https://doi.org/10.1105/tpc.10.12.2005

Zhou M, Wang W, Karapetyan S, Mwimba M, Marqués J, Buchler NE, Dong X. Redox rhythm reinforces the circadian clock to gate immune response. *Nature.* 2015b:523(7561):472–476. https://doi.org/10.1038/nature14449

Zhou X, Hao H, Zhang Y, Bai Y, Zhu W, Qin Y, Yuan F, Zhao F, Wang M, Hu J, et al. SOS2-LIKE PROTEIN KINASE5, an SNF1-RELATED PROTEIN KINASE3-type protein kinase, is important for abscisic acid responses in *Arabidopsis* through phosphorylation of ABSCISIC ACID-INSENSITIVE5. *Plant Physiol.* 2015a:168(2):659–676. https://doi.org/10.1104/pp.114.255455

Zhu J, Oh E, Wang T, Wang Z. TOC1-PIF4 interaction mediates the circadian gating of thermoresponsive growth in *Arabidopsis*. *Nat Commun.* 2016:7(1):13692. https://doi.org/10.1038/ncomms13692