The phytochrome interacting proteins ERF55 and ERF58 repress light-induced seed germination in *Arabidopsis thaliana*

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Seed germination is a critical step in the life cycle of plants controlled by the phytohormones abscisic acid (ABA) and gibberellin (GA), and by phytochromes, an important class of photoreceptors in plants. Here we show that light-dependent germination is enhanced in mutants deficient in the AP2/ERF transcription factors ERF55 and ERF58. Light-activated phytochromes repress ERF55/ERF58 expression and directly bind ERF55/ERF58 to displace them from the promoter of *PIF1* and *SOM*, genes encoding transcriptional regulators that prevent the completion of germination. The same mechanism controls the expression of genes that encode ABA or GA metabolic enzymes to decrease levels of ABA and possibly increase levels of GA. Interestingly, ERF55 and ERF58 are themselves under transcriptional control of ABA and GA, suggesting that they are part of a self-reinforcing signalling loop which controls the completion of germination. Overall, we identified a role of ERF55/ERF58 in phytochrome-mediated regulation of germination completion.
light is one of the most important environmental cues for plants as it has a strong impact on growth and development. Plants have evolved different classes of photoreceptors to sense and respond to changes in the environment. Phytochromes are photoreceptors that perceive red (R) and far-red (FR) light. In the model plant Arabidopsis thaliana, phytochromes are represented by five members, of which two, phyA and phyB, have dominant functions. PhyA and phyB control the timing of developmental transitions such as seed germination, de-etiolation, and the induction of flowering, and play a key role in responses to canopy shade.

Seed germination is a critical step in the life cycle of plants. Seeds are relatively well protected against adverse environmental conditions, whereas seedlings emerging from germinated seeds are much more vulnerable. Proper timing of the completion of germination is therefore an important trait in crops and crucial for plant survival in natural environments. The phytohormones abscisic acid (ABA) and gibberellin (GA) control seed germination in opposite directions, with ABA inhibiting and GA promoting the completion of germination. ABA accumulates in seeds during seed maturation and is rapidly depleted during imbition. ABA INSENSITIVE 5 (ABI5) and DELLAs, including GA INSSENSITIVE (GAI), REPRESSOR OF GA1 (RGA), and RGA-LIKE 2 (RGL2), are important repressors of the completion of seed germination. ABI5 is a transcription factor that promotes ABA responses, whereas DELLAs inhibit GA signalling. High GA levels induce the degradation of DELLAs and thereby promote the completion of seed germination.

In Arabidopsis and many other species, light controls the completion of seed germination through phyA and phyB. The inactive state of phytochromes, Pr, has an absorption peak at 660 nm (R light), whereas the absorption peak of the physiologically active Pfr state is at 730 nm (FR light). Phytochromes reversibly convert between Pr and Pfr by absorption of light, with the relative proportion in the active state (Pfr/Ptot) being determined by the ratio of R and FR light in the environment. PhyB is active in light conditions with a high R:FR ratio, such as monochromatic R light or sunlight, and inactivated by monochromatic FR light or canopy shade, where the R:FR ratio is low. PhyA is primarily active in FR light and low-light conditions, where relative Pfr levels are low.

Previous studies showed that phyA and phyB mediate light-regulated seed germination partly by binding and thereby destabilizing the basic helix-loop-helix transcription factor PHYTOCHROME INTERACTING FACTOR 1 (PIF1). PIF1 directly associates with the promoter of GAI, RGA, and ABI5 to induce their expression. PIF1 also directly activates the transcription of SOMNUS (SOM), which enhances the expression of genes encoding GA catabolic or ABA anabolic enzymes; in contrast, the expression of genes encoding GA anabolic or ABA catabolic enzymes is repressed, thereby inhibiting the completion of germination. However, there is no evidence for a direct regulation of genes encoding ABA or GA metabolic enzymes by PIF1 or SOM. In addition to PIF1 and SOM, several other proteins play an important role in phyA and phyB regulated seed germination.

The AP2/ERF proteins represent a large transcription factor family in plants, with almost 150 members in Arabidopsis. This family includes members that orchestrate various biological functions, such as flower development and responses to hormones or abiotic stresses. Extensive redundancy probably exists within the AP2/ERF transcription factor family, and the genes whose expression AP2/ERFs control are often unknown.

ERF58 (ETHYLENE RESPONSE FACTOR 58) belongs to group A6 of the AP2/ERF transcription factor family, which consists of ERF53 to ERF62. ERF58 transcript levels are upregulated by ABA and heat stress; in vitro assays suggest ERF58 binds the dehydration responsive element (DRE; A/GCCGAC) and the coupling element CE1 (CCACG) within responsive promoters. However, growth and development of erf58 mutant plants does not differ from the wild-type and the function of endogenous ERF58 is still unknown. Overexpression of ERF58 leads to pleiotropic effects, including hypersensitivity to ABA during germination, reduced salt tolerance, and callus formation.

Here we show that ERF58 and its homologue ERF55 act redundantly during the phytochrome-controlled regulation of seed germination. Light-activated phyA and phyB repress the expression of ERF55 and ERF58, and interact with the DNA-binding domain of ERF55 and ERF58, preventing their association with the promoter of target genes. This results in altered expression of genes encoding regulators of seed germination and thereby promotes the completion of germination.

**Results**

**ERF58 interacts with phyA and phyB.** Evolution has shaped diverse seed germination strategies, with light serving as a germination trigger in positively photoblastic species. Light in the red and far-red spectral range is perceived by phytochromes. To identify phytochrome-dependent seed germination signalling pathways, we performed a yeast two-hybrid (Y2H) screen for proteins interacting with light-activated full-length phyA. We reported previously on SPA1, PCH1, COR27, and NOT9B as phyA-binding proteins identified in this screen. As ERF58, a member of group A6 of the AP2/ERF transcription factor family, was another protein identified in the screen for phyA-interacting proteins. In addition to phyA, phyB also interacts with ERF58 in Y2H assays, and we observed interaction of phyA and phyB also with most other AP2/ERFs of group A6 in yeast, including ERF55 (Fig. 1a, b; Supplementary Fig. 1a). As ERF55 and ERF58 are transcribed in mature seeds in contrast to other members of the A6 AP2/ERF subfamily, this report focuses on ERF55 and ERF58. When co-expressed in Nicotiana benthamiana leaves, HA-YFP-ERF58 and HA-YFP-ERF58 co-localised with phyA-NLS-CFP and phyB-CFP in photobodies, indicating they may interact with phyA and phyB in planta (Fig. 1c; Supplementary Fig. 1b).

Next, we performed co-immunoprecipitation (Co-IP) assays using a stable transgenic line expressing pERF58:HA-YFP-ERF58. Seedlings were grown for three days in constant darkness and irradiated with different light conditions as indicated in Fig. 1d. Endogenous phyA and phyB co-immunoprecipitated with HA-YFP-ERF58 when activated by light (Fig. 1d), confirming that ERF58 is in complex with phyA and phyB in planta. We were not able to obtain stable transgenic lines expressing tagged ERF55 and therefore experiments depending on such lines are currently not possible. Collectively, our results show that ERF58 interacts with phyA and phyB in plants, and we have evidence that other group A6 AP2/ERFs, including ERF55, bind phyA and phyB.

**ERF55 and ERF58 repress the completion of seed germination.** To characterise the physiological function of ERF55 and ERF58, and compare it to the function of other group A6 AP2/ERFs, germination phenotypes of two independent ERF58 T-DNA insertion mutant alleles, erf58-1 and erf58-2 (Supplementary Fig. 2a, c), and one mutant allele for ERF55, erf55-1 (Supplementary Fig. 2b, d), ERF56, ERF57, ERF59, and ERF60 were examined. In addition, we examined germination also in ERF58ox lines, expressing HA-YFP-ERF58 under the strong, constitutive CaMV 3SS promoter (Supplementary Fig. 2e).
Under phyA-ON conditions, only phyA can induce the completion of germination of wild-type seeds, while under phyB-ON conditions, phyB is required for germination completion; under phyA- and phyB-OFF conditions, both phyA and phyB are inactive (Fig. 2a, b). Final cumulative germination percentages of erf55-1, erf58-1, and erf58-2 seeds were increased under phyB-ON conditions compared to the wild-type and other erf mutants (Fig. 2c, d; Supplementary Fig. 3a, b). Likewise, under phyA-ON conditions, erf55-1, erf58-1, and erf58-2 mutants had a higher percentage of germination completion than the wild-type (Fig. 2e, f; Supplementary Fig. 3c). In contrast, overexpression of ERF58 reduced the completion of germination compared to the wild-type under both phyA- and phyB-ON conditions (Fig. 2c–f; Supplementary Fig. 3b, c). Complementation of erf55-1 and erf58-2 by expression of pERF58:HA-YFP-ERF58 confirmed that defects in ERF55 and ERF58 are responsible for the increased final cumulative germination percentage of erf55-1 and erf58-2 (Supplementary Figs. 4 and 5). Unlike the pif1 mutant', erf55-1, erf58-1, and erf58-2 single mutants still required light for the completion of germination, but we observed that erf55-1 erf58-2 double mutant seeds completed germination in the dark (Fig. 2c–f). Taken together, we conclude

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**Fig. 1** ERF58 interacts with phyA and phyB. a Y2H protein-protein interaction assay. Full-length phyA or the N-terminal half of phyB (phyBN) fused to LexA and ERF58 fused to the GAL4 AD were expressed in yeast. Yeast cells were grown in chromophore (PCB)-supplemented medium, exposed to R or FR light for 5 min to convert phytochromes to Pfr or Pr, and incubated in the dark for another 4 h. β-Gal activity was then measured using an ONPG assay. Bars show mean β-Gal activity of three replicates ±SD. Different letters indicate significant differences as determined by two-way ANOVA followed by post-hoc Tukey’s HSD test; p < 0.05. b Y2H growth assay. phyA- or phyBN-LexA and AD-ERF58 were expressed in yeast. Yeast cells were grown on CSM -L-T plates or CSM -L-T-H plates supplemented with PCB. Plates were incubated in the dark (D) or R light (R). c Co-localisation of ERF58 with phyA and phyB in tobacco. p35S:HA-YFP-ERF58 and either p35S:PHYA-NLS-CFP or p35S:PHYB-CFP were transiently co-expressed in tobacco leaf epidermis cells by agroinfiltration; empty p35S:YFP and p35S:CFP vectors were used as control. YFP and CFP signals were detected by epifluorescence microscopy. Scale bar represents 5 μm. d Co-immunoprecipitation of phyA and phyB with ERF58. Stable transgenic Arabidopsis lines expressing pERF58:HA-YFP-ERF58 in Col-0 background were used for Co-IP; Col-0 was used as negative control. Three day old dark-grown seedlings were treated for 5 min with R light, for 2 h with FR light, for 2 h with FR light followed by 5 min R light, or kept in the dark and used for Co-IP. Eluate fractions were analysed by SDS-PAGE and immunoblotting with α-phyA, α-phyB, or α-HA antibodies. Experiments in b–d were repeated three times with similar results.
that ERF55 and ERF58 are negative regulators of the completion of seed germination required for suppression of germination completion in the dark.

**PhyA and phyB repress ERF55 and ERF58 expression.** Since ERF55 and ERF58 play a role in light-regulation of the completion of seed germination, we used RT-qPCR to test if ERF55 and ERF58 transcript levels in germinating seeds are altered in response to phyA- or phyB-specific light treatments (Supplementary Fig. 6a, b). Under both phyA-ON and phyB-ON conditions, ERF55 and ERF58 transcript abundance was reduced compared to phyA-OFF and phyB-OFF, depending on phyA and
Fig. 2 ERF55 and ERF58 inhibit the completion of seed germination. a, b Light treatments used to test for phyA- and phyB-dependent completion of seed germination. Seeds were imbibed for 1 h in the dark (D), treated for 5 min with FR light and then exposed to light conditions that induce the completion of seed germination either through phyA (phyA-ON: 2 days in D → 12 h in FR light → 5 days in D) or phyB (phyB-ON: 5 min in R light → 5 days in D). phyA and phyB are inactive under phyA-OFF and phyB-OFF conditions, respectively. Germination completion in white (W) light was used to confirm viability of seeds. c-f phyA- and phyB-dependent completion of seed germination. Germination completion of wild-type (Col-0), different mutants, and two independent ERF58ox lines (2IC and 2A) was tested under phyB-specific (c, d) or phyA-specific conditions (e, f). c, e Representative pictures of plates used for quantification of cumulative seed germination. d, f Bars show mean cumulative germination percentages of three replicates ±SD. Different letters indicate significant differences as determined by one-way ANOVA followed by post-hoc Tukey’s HSD test; p < 0.05.

ERF55 and ERF58 bind to the promoter of PIF1 and SOM. PIF1 and SOM negatively regulate the completion of seed germination downstream of phytochromes. We therefore quantified PIF1 and SOM expression in wild-type and erf55-1 erf58-2 seeds exposed to phyA- or phyB-specific light treatments. PIF1 and SOM transcript levels were reduced in erf55-1 erf58-2 compared to the wild-type, consistent with a role for ERF55 and ERF58 in suppressing the completion of germination (Fig. 3a, b).

To obtain further support for a function of ERF55 and ERF58 in promoting the expression of PIF1 and SOM, we used a transactivation assay in infiltrated tobacco leaves. HA-YFP-ERF55, HA-YFP-ERF58, or HA-YFP were co-expressed in tobacco leaves with PIF1 or SOM promoter:luciferase reporter constructs. All luminescence signals were normalised to p35S:GUS expression (Fig. 3c). The expression of ERF55 or ERF58 resulted in 2- to 4-fold increased activation of the promoter of PIF1 or SOM compared to the empty HA-YFP vector control (Fig. 3d, e). PIF1 and ABI5 were used as positive controls as they have previously been shown to promote PIF1 and SOM promoter activity.

Next, we used PIF1 and SOM promoter fragments to identify regions of these promoters that are sufficient to enhance the expression of the luciferase reporter when ERF55 or ERF58 are co-transformed. Different PIF1 and SOM promoter fragments resulted in activation of the luciferase reporter and therefore contain potential binding sites for ERF55 and ERF58 (Supplementary Figs. 7 and 8). We decided to use the −800…−1028 region of the PIF1 promoter and the −670…−840 region of the SOM promoter for further experiments.

To investigate if ERF55 and ERF58 directly associate with the promoter of PIF1 and SOM, we expressed them as MBP fusion proteins in E. coli, purified them, and used them for EMSAs (Electrophoretic mobility shift assays) with biotin-labelled PIF1 or SOM promoter fragments; an unlabelled fragment containing the DRE element to which ERF55 and ERF58 bind was used as competitor (Fig. 3f, g). Both ERF55 and ERF58 caused an upward shift of the PIF1 and SOM promoter fragments, suggesting ERF55 and ERF58 bind to them in vitro.

We then performed ChIP-qPCR using p35S:HA-YFP-ERF58 (ERF58ox) and p35S:YFP-HA transgenic lines. Chromatin was isolated from seeds germinating for 6 h and used for ChIP with α-GFP coupled magnetic beads followed by qPCR for quantification of PIF1 and SOM promoter fragments in the eluate fraction. PIF1 and SOM promoter fragments were strongly enriched in the ERF58ox sample compared to the YFP-HA negative control, suggesting that ERF58 associates with the PIF1 and SOM promoter in planta.

We also investigated the completion of seed germination of erf55-1 erf58-2 and SOM and PIF mutant or overexpression background (Supplementary Fig. 9). The set of seed batches used for the experiments in Supplementary Fig. 9 generally show a lower final cumulative germination percentage than seed batches used in other experiments due to different conditions during seed propagation. In phyA- and phyB-OFF conditions, we observed a low final cumulative germination percentage for the erf55-1 erf58-2 mutant and no seeds that completed germination for som-3; in contrast, final cumulative germination was almost 100% for the erf55-1 erf58-2 som-3 triple mutant (Supplementary Fig. 9a). Thus, ERF55/ERF58 possibly control the completion of seed germination also through a second SOM-independent pathway. In contrast to erf55-1 erf58-2, pif1-1 and erf55-1 erf58-2 pif1-1 mutants completed germination even under phyA- and phyB-OFF conditions (Supplementary Fig. 9b). Potential explanations for this observation are that PIF1 expression is only partially repressed in erf55-1 erf58-2 or that PIF1 could affect the completion of seed germination also independently of ERF55 and ERF58. Consistent with a model in which ERF55 and ERF58 are acting upstream of PIF1 and SOM to promote their transcription, we found that expression of PIF1 and SOM under the control of the constitutively active CaMV 35S promoter can partially complement the erf55-1 erf58-2 mutant (Supplementary Fig. 9a, b).

Overall, we conclude that PIF1 and SOM are direct targets of ERF58 and possibly ERF55 and that ERF55/ERF58 control the completion of seed germination through both PIF1/SOM-dependent and -independent mechanisms.

Phytochromes inhibit the DNA-binding activity of ERF55/ERF58. In seeds germinating under phyB-ON conditions, transcript levels of ERF55 and ERF58 are only reduced by about 50% when compared to seeds given a phyB-OFF treatment, and ERF58 protein stability is not or only weakly affected by light (Supplementary Fig. 6). Thus, we hypothesised that light might regulate ERF55/ERF58 also at other levels. Association with target promoters is another layer at which transcription factors can be controlled and therefore we used ChIP-qPCR to test if light has an effect on binding of ERF58 to the promoter of PIF1 or SOM. For this experiment, we slightly adapted the light treatment of seeds. Only 20 min before harvesting the samples for ChIP, a light treatment was given to activate phyB (phyB-ON-ON) in seeds previously exposed to phyB-OFF conditions or to inactivate phyB (phyB-OFF-ON) in seeds previously exposed to a phyB-ON-OFF treatment (Fig. 4a). The short time between the light treatment setting the final Pfr/Ptot level of phyB and harvesting the seeds for ChIP reduces indirect effects of light on the amount of ERF58 binding to target promoters and restricts the analysis to the influence of phyB because phyA is not yet present; immunoblot analysis furthermore confirmed that ERF58 protein levels in phyB-ON and phyB-OFF-ON as well as in phyB-ON and phyB-ON-Off conditions are very similar; in contrast, ERF58 protein levels are slightly higher in phyB-Off/phyB-Off-ON than phyB-ON/phyB-ON-Off (Fig. 4b).

ChIP-qPCR for seeds exposed to phyB-ON vs. phyB-ON-Off showed that association of ERF58 with the PIF1 and SOM promoter is strongly enhanced by the 10 min FR pulse in the phyB-ON-Off treatment, suggesting that inactivation of phyB
Fig. 3 ERF58 directly binds to the promoter of PIF1 and SOM to induce their expression. a, b RT-qPCR analysis of PIF1 (a) and SOM (b) expression in Col-0 and erf55-1 erf58-2 seeds germinating under phyA-ON/OFF or phyB-ON/OFF conditions. Light conditions are described in Supplementary Fig. 6a, b. ACT7 was used as an internal control. Values are means of three replicates ± SD. Different letters indicate significant differences as determined by two-way ANOVA followed by post-hoc Tukey’s HSD test; $p < 0.05$. c Constructs used for transactivation assays. d, e Transactivation assay in tobacco. Combinations of effector (p35S:HA-YFP-ERF58 or p35S:HA-YFP-ERF55; p35S:HA-YFP was used as negative control; p35S:HA-YFP-PIF1 and p35S:ABI5-YFP-HA were used as positive controls) and reporter constructs (pPIF1−1…−2000:LUC or pSOM−1…−2000:LUC) and p35S:GUS (used for normalisation) were transiently co-expressed in tobacco leaf epidermis cells by agro infiltration. LUC and GUS activity was measured in protein extracts from infiltrated leaves. Bars show mean relative LUC activity (LUC activity divided by GUS activity) of three replicates ± SD. Different letters indicate significant differences as determined by t-test and Holm–Bonferroni method; $p < 0.05$. f, g EMSAs. Biotin-labelled PIF1 and SOM promoter fragments (pPIF1−801…−1031 and pSOM−621…−840) were incubated with MBP-ERF58, MBP-ERF55, or MBP alone (negative control). Samples were analysed by native PAGE. Gels were blotted onto nylon membranes and signals were detected by streptavidin-coupled horseradish peroxidase and ECL. FP, free probe; Cold, excess of unlabelled DNA fragment containing the DRE motif to which ERF55/ERF58 bind. Experiments were repeated at least three times with similar results.
promotes binding of ERF58 to target promoters (Fig. 4c). In contrast, when comparing phyB-OFF vs. phyB-OFF-ON, the R pulse applied under phyB-OFF-ON conditions reduced binding of ERF58 to the promoter of *PIF1* and *SOM*, indicating that activation of phyB promotes dissociation of ERF58 from target promoters (Fig. 4d). Thus, association of ERF58 with promoters of target genes is regulated in a R/FR reversible manner, suggesting it is controlled by phytochromes. Since phyA is not present in seeds incubated on water for only few hours, light-regulation of ERF58 promoter association at this early time point is likely primarily conferred by phyB, while at later time points also phyA could play a role.

The rapid regulation of ERF58 promoter dissociation/association in response to R and FR pulses indicates this could be a direct effect of phytochromes binding to ERF58. We therefore used ERF58 truncations for Y2H assays to identify the domain of ERF58 interacting with phytochromes (Supplementary Fig. 10). We found that both the C-terminal domain and the AP2 domain
Fig. 4 Phytochromes inhibit the DNA-binding activity of ERF55 and ERF58. a Light conditions used in the ChIP experiment. Seeds were imbibed for 1 h in the dark (D) and treated for 5 min with FR light to inactivate phyB. Then, phyB was kept in the inactive state by incubation in the dark (phyB-OFF) or activated by 5 min R light before returning the seeds into darkness (phyB-ON). Twenty min before harvesting for ChIP, seeds in phyB-OFF conditions were exposed to R light for 10 min to activate phyB (phyB-OFF-ON), and seeds in phyB-ON conditions were treated with FR light for 10 min to inactivate phyB (phyB-ON-OFF). b HA-YFP-ERF58 protein levels in ERF58ox seeds. Col-0 p35S:HA-YFP-ERF58 (line 21C) seeds were treated as described in a. Total protein was then extracted and analysed by SDS-PAGE and immunoblotting with α-HA; α-ACT was used to detect ACTIN as loading control. Col-0 was included as a negative control. Signals detected by α-HA and α-ACT were quantified using ImageJ; numbers below the membrane show the HA/ACT ratio. c, d ChIP-qPCR. Seeds of Col-0 p35S:HA-YFP-ERF58 (line 21C) and Col-0 p35S:YFP-HA (negative control) were incubated and subject to light treatments as described in a. Chromatin was isolated and HA-YFP-ERF58 bound DNA fragments were purified using α-GFP coupled magnetic beads. PIF1 and SOM promoter fragments were incubated in the input and eluate fractions were detected using qPCR and specific primers. ACT7 was used for normalisation. Values show enrichment of PIF1 and SOM promoter fragments in the eluate fraction compared to the input fraction and are means of three replicates ±SD. e-h EMSAs. Biotin-labelled pPIF1 −801...−1031 (e, g) or pSOM −621...−480 (f, h) promoter fragments were incubated with MBP-ERF58 (e, f), MBP-ERF55 (g, h), or MBP alone (negative control), and light-activated phyA. Samples were analysed by native PAGE. Gels were blotted onto nylon membranes and signals were detected by streptavidin-coupled horseradish peroxidase and ECL. FP, free probe; Cold, excess of unlabelled DNA fragment containing the DRE motif to which ERF55/SOM bind. Zinc blot analysis of EMSA samples is shown in Supplementary Fig. 11b, c.

ERF55 and ERF58 bind phyA and phyB. The AP2 domain is highly conserved in AP2/ERF transcription factors and required for the completion of seed germination2. We observed that the enzymes promote the completion of seed germination. In phyA-211 and phyB-9 triple mutants, we observed that ABA could be involved in ERF55/ERF58-mediated repression of the completion of seed germination. We therefore hypothesised that interaction of the inhibitory effect of ABA on germination completion is reduced in ERF55/ERF58 to target promoters and displacing promoter-binding to DNA. We therefore hypothesised that interaction of members of the AP2/ERF family (Fig. 5d, e). In EMSAs, ERF55 and ERF58 caused an upward shift of PIF1 and SOM promoter fragments, which is largely abolished when phyA is supplemented. Expression of phyB in E. coli was too low to obtain sufficient amounts for EMSAs. Overall, ChIP-qPCR and EMSA data are consistent with a mechanistic model in which light-activated phyA and phyB can bind ERF55/ERF58 to prevent their association with target promoters.

To genetically test if ERF55 and ERF58 act downstream of phyA and phyB, we generated erf55-1 erf58-2 phyA-211 and erf55-1 erf58-2 phyB-9 triple mutants. erf55-1 erf58-2 is almost fully epistatic over phyA-211 and phyB-9 and we observed a high final cumulative germination percentage for the two triple mutants in phyA- and phyB-OFF conditions (Figs. 2a, b and 4i, j). This finding is consistent with a model in which the repression of ERF55 and ERF58 activity by light-activated phytochromes promotes the completion of seed germination.

ERF55/ERF58 regulate genes encoding ABA metabolic enzymes. The phytohormone ABA is a key factor of ABA signal transduction30 and we found that ABI5 transcript levels in germinating seeds are lower in erf55-1 erf58-2 than the wild-type (Fig. 5a). Like the ABA2 promoter, the promoter of ABI5 also contains a DRE element (Fig. 6b, c). In EMSAs, both ERF55 and ERF58 bound a wild-type ABI5 promoter fragment containing the DRE motif to which ERF55/SOM bind. The completion of germination in ABI5−/− (line 21C) seeds was too low to obtain sufficient amounts for EMSAs. Therefore, we used the ChIP experiment with the promoter association (Fig. 5e, f; Supplementary Fig. 13a).

To confirm association of ERF55 with the ABA2 promoter in planta, we performed ChIP-qPCR. Similar to association with the PIF1 and SOM promoter, binding of ERF55 to the ABA2 promoter fragment containing the DRE element, while mutating the DRE element abolished promoter association (Fig. 6c, e; Supplementary Fig. 13a).

To confirm association of ERF55 with the ABA2 promoter in planta, we performed ChIP-qPCR. Similar to association with the PIF1 and SOM promoter, binding of ERF55 to the ABA2 promoter fragment containing the DRE element, while mutating the DRE element abolished promoter association (Fig. 6c, e; Supplementary Fig. 13a). Furthermore, using EMSA, we also observed association of ERF55 and ERF58 with NCED9 and AAO3 promoter fragments, which was strongly reduced when light-activated phyA was added to the assay (Supplementary Fig. 14).

ERF55 and ERF58 promote ABA downstream signalling. In addition to genes coding for ABA metabolic enzymes, genes that have a function in ABA downstream signalling are also targets potentially regulating the completion of seed germination3. ABI5 is a key factor of ABA signal transduction30 and we found that ABI5 transcript levels in germinating seeds are lower in erf55-1 erf58-2 than the wild-type (Fig. 6a). Like the ABA2 promoter, the promoter of ABI5 also contains a DRE element (Fig. 6b, c). In EMSAs, both ERF55 and ERF58 bound a wild-type ABI5 transcript levels of genes encoding ABA catabolic enzymes, and increasing ABA levels.

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promoter fragment, while inserting point mutations into the DRE element abolished binding (Fig. 6c, d; Supplementary Fig. 13b). Finally, we used ChIP-qPCR to confirm association of ERF58 with the ABI5 promoter in germinating seeds. We found that binding of ERF58 to the ABI5 promoter is reversibly regulated by R and FR light, with a R light pulse inhibiting binding and a FR light pulse promoting it (Fig. 6e). Collectively, these findings suggest that ERF55 and ERF58 enhance the expression of ABI5 and thereby promote ABA downstream signalling.

In agreement with a model in which ERF55 and ERF58 are acting upstream of ABI5 to promote its transcription, we found that expression of ABI5 under the control of the constitutively active CaMV 35S promoter partially complements the erf55-1 erf58-2 mutant phenotype (Supplementary Fig. 9c). In sets of seed batches with a low final cumulative germination percentage, few erf55-1 erf58-2 double mutant seeds completed germination in phyA- and phyB-OFF conditions, while abi5-8 did not complete germination at all under these conditions. In contrast, the final cumulative germination percentage for the erf55-1 erf58-2 abi5-8 triple mutant in phyA- or phyB-OFF conditions was much higher compared to the erf55-1 erf58-2 and abi5-8 parental lines, suggesting that ERF55 and ERF58 repress the completion of seed germination also partially independent of ABI5 (Supplementary Fig. 9c).
Fig. 5 ERF55 and ERF58 regulate the expression of genes encoding ABA metabolic enzymes and increase ABA levels. 

a. Completion of germination of wild-type (Col-0), erf55-1 erf58-2 double mutant, and ERF58ox (two independent lines) seeds on ddH2O and increasing concentrations of ABA. Seeds were exposed to W light for 6 h, and then incubated in the dark at 22 °C for 7 days before scoring for completion of germination. Data points show mean cumulative germination percentages of three replicates ±SD.

b. RT-qPCR analysis of ABA2 expression levels in Col-0 and erf55-1 erf58-2 seeds germinating under phyA-ON/OFF or phyB-ON/OFF conditions; data for other genes encoding ABA metabolic enzymes are shown in Supplementary Fig. 12. Light conditions are described in Supplementary Fig. 6a, b. ACT7 was used as an internal control. Values are means of three replicates ±SD.

c. Quantification of endogenous ABA levels in Col-0, erf55-1 erf58-2, and ERF58ox seeds after 24 h germination in the dark. Values are means of three replicates ±SD. Letters indicate levels of significance as determined by t-test and Holm-Bonferroni method; p < 0.05.

d, e. Schematic representation of the ABA2 promoter. The promoter fragment used for EMSA is shown in e. b EMSA. Biotin-labelled ABA2 −222...–52 promoter fragments containing a wild-type or mutated DRE motif were incubated with MBP-ERF58 or MBP alone (negative control). Samples were analysed by native PAGE. The gel was blotted onto a nylon membrane and signals were detected by streptavidin-coupled horseradish peroxidase and ECL. FP, free probe. The experiment was repeated six times with similar results. Data for ERF55 are shown in Supplementary Fig. 13a.

g. ChIP-qPCR. Seeds of Col-0 p35S:HA-YFP-ERF58 (line 21C) and Col-0 p35S:YFP-HA (negative control) were germinated and subject to light treatments as described in Fig. 4a. Chromatin was isolated and HA-YFP-ERF58 bound DNA fragments were purified using α-GFP coupled magnetic beads. qPCR and specific primers were used to detect the ABA2 −222...–52 promoter fragment in the eluate fraction. ACT7 was used for normalisation. Values show enrichment of the ABA2 promoter fragment in the eluate fraction compared to the input fraction and are means of three replicates ±SD. a, b, g Different letters indicate significant differences as determined by two-way ANOVA followed by post-hoc Tukey’s HSD test. a, b p < 0.05. g p < 0.01.

Fig. 6 ERF55 and ERF58 upregulate ABI5 expression. 

a. RT-qPCR analysis of ABI5 expression levels in Col-0 and erf55-1 erf58-2 seeds germinating under phyA-ON/OFF or phyB-ON/OFF conditions. Light conditions are described in Supplementary Fig. 6a, b. ACT7 was used as an internal control. Values show means of three replicates ±SD.

b. Schematic representation of the ABI5 promoter. The promoter fragment used for EMSA is shown in c.

c. DRE −234...–447 promoter fragments containing a wild-type or mutated DRE motif were incubated with MBP-ERF58 or MBP alone (negative control). Samples were analysed by native PAGE. The gel was blotted onto a nylon membrane and signals were detected by streptavidin-coupled horseradish peroxidase and ECL. FP, free probe. The experiment was repeated five times with similar results. Data for ERF55 are shown in Supplementary Fig. 13b.

d. ChIP-qPCR. Germinating seeds of Col-0 p35S:HA-YFP-ERF58 (line 21C) and Col-0 p35S:YFP-HA (negative control) were subject to light treatments as described in Fig. 4a. Chromatin was isolated and HA-YFP-ERF58 bound DNA fragments were purified using α-GFP coupled magnetic beads. qPCR and specific primers were used to detect the ABI5 −234...–447 promoter fragment in the eluate fraction. ACT7 was used for normalisation. Values show enrichment of the ABI5 promoter fragment in the eluate fraction compared to the input fraction and are means of three replicates ±SD. a, e Different letters indicate significant differences as determined by two-way ANOVA followed by post-hoc Tukey’s HSD test. a p < 0.05. e p < 0.01.
ERF55/ERF58 regulate genes encoding GA metabolic enzymes.

Light promotes the completion of germination in part by increasing the levels of bioactive gibberellins, which act antagonistically to ABA. Therefore, we tested completion of germination of wild-type, erf55-1 erf58-2, and ERF58ox seeds on medium containing different concentrations of the bioactive gibberellin GA3 or the gibberellin biosynthesis inhibitor paclobutrazol (PAC). We observed that the erf55-1 erf58-2 double mutant is hypersensitive to GA3 compared to the wild-type, while two independent ERF58ox lines are hyposensitive (Fig. 7a). In addition, wild-type and ERF58ox lines did not complete germination on medium containing PAC, whereas PAC inhibited the completion of germination of erf55-1 erf58-2 only at higher concentrations (Fig. 7b). Therefore, ERF55 and ERF58 could regulate the completion of seed germination partially through GA.

Using RT-qPCR, we then quantified transcript levels of genes encoding GA metabolic enzymes in germinating seeds. Expression of genes that encode GA anabolic enzymes (GA3ox1, GA3ox2) was upregulated in erf55-1 erf58-2 compared to the wild-type, while expression of genes encoding GA catabolic enzymes (GA2ox4, GA2ox2) was reduced (Fig. 7c; Supplementary Fig. 15). Similar to genes that encode GA metabolic enzymes, expression levels of most genes encoding GA metabolic enzymes that we tested differed more strongly between the wild-type and the erf55-1 erf58-2 double mutant under phyA/phyB-OFF conditions than under phyA/phyB-ON conditions. This supports a function of ERF55 and ERF58 in light-regulation of the expression of these genes.

Like ABA2, GA2ox4 is likely not a target of PIF1 and SOM but it is misregulated in erf55-1 erf58-2 and its promoter contains a DRE element (Fig. 7d, e)12. EMSAs with biotin-labelled wild-type and DRE mutant GA2ox4 promoter fragments showed that ERF55 and ERF58 associate with the GA2ox4 promoter depending on an intact DRE element (Fig. 7e, f; Supplementary Fig. 13c). Finally, we used ChIP-qPCR to show that ERF55 associates with the GA2ox4 promoter in germinating seeds. Similar to other promoters bound by ERF58, also binding to the GA2ox4 promoter was enhanced when phyB was in the inactive state, i.e. under phyB-OFF or phyB-ON-OFF conditions, while binding was lower under phyB-ON or phyB-ON-OFF conditions, where Ptr levels are high and phyB binds to ERF58 (Fig. 7g).

Overall we conclude that ERF55 and ERF58 affect the completion of seed germination also by regulation of genes encoding GA metabolic enzymes.

ABA and GA regulate the expression of ERF55 and ERF58.

Feedback regulation is not uncommon in signalling pathways. To test for potential feedback regulation of ERF55 and ERF58 by ABA or GA, we quantified expression of ERF55 and ERF58 in seeds germinating on medium containing ABA or GA3. Compared to mock treated seeds, ERF55 and ERF58 transcript levels were reduced in seeds germinating in the presence of GA3, while ABA enhanced the expression of ERF55 and ERF58 (Fig. 8a–d).

Interestingly, we found that ERF55 protein levels in germinating ERF58ox seeds increased in response to ABA. The ERF58ox line expresses HA-YFP-ERF58 under the control of the constitutively active CaMV 35S promoter, which is not regulated by ABA. Therefore, in addition to promoting ERF58 expression, ABA also increases ERF58 protein stability (Fig. 8e). In contrast, GA3 did not have an obvious effect on ERF58 protein stability (Fig. 8f).

Taken together, ABA upregulates ERF55 and ERF58 transcript levels and ERF58 protein stability, while GA downregulates ERF55 and ERF58 expression, suggesting ERF55 and ERF58 are under positive feedback regulation by ABA and GA.

Discussion

Seeds are generally much more tolerant to unfavourable environmental conditions than seedlings. Therefore, the completion of seed germination – a critical point of no return in the life cycle of plants – must be reliably controlled. Here, we have shown that the AP2/ERF transcription factors ERF55 and ERF58 control the completion of seed germination downstream of phytochromes at different levels. First, they regulate PIF1 and SOM, which are key transcriptional regulators of the completion of seed germination; second, they control the expression of genes encoding ABA or GA metabolic enzymes; and third, they promote ABA downstream signalling. Action of ERF55 and ERF58 at all three levels can contribute to inhibition of the completion of germination.

ERF58 and ERF55 belong to a group of AP2/ERFs not containing a repression domain and therefore possibly exclusively enhancing the expression of direct target genes16. In line with this, all genes that we identified as direct targets of ERF58 and likely ERF55 are downregulated in the erf55-1 erf58-2 mutant compared to the wild-type and all these genes repress the completion of germination. We also identified genes upregulated in erf55-1 erf58-2 that promote germination completion, but these genes might be indirectly regulated by ERF55 and ERF58 (Fig. 9a).

The completion of germination of erf55-1 erf58-2 seeds is not fully suppressed in the dark, i.e. under phyA- and phyB-OFF conditions. Therefore we propose a model in which light perceived by phyA and phyB downregulates ERF55 and ERF58 action. First, ERF55 and ERF58 transcript levels are reduced under phyA/phyB-ON conditions depending on phyA and phyB (Fig. 9a); in contrast, ERF58 protein stability appears not or only slightly affected by light. Second, using seeds germinating under phyB-ON conditions and exposed to a short FR light pulse, we have shown that converting phyB to the inactive Pr state leads to rapid association of ERF58 with target promoters. Conversely, a short R light pulse applied to seeds germinating under phyB-OFF conditions converts phyB to the active Pfr state and promotes the dissociation of ERF58 from target promoters. The R/FR reversible regulation of ERF58 target promoter association indicates that this effect depends on phyB. PhyA is not present in seeds imbied for only a few hours29, and it is likely that primarily phyB controls ERF58 target promoter association under the conditions used in the ChIP-qPCR experiments. However, EMSAs suggest that also phyA can regulate ERF58 and ERF55 target promoter binding, so phyA could use the same mechanism as phyB to control the association of ERF58 and possibly ERF55 with target promoters in planta. Phytochromes thus not only regulate the transcription of ERF55 and ERF58, but also control the binding of ERF55 and ERF58 to the promoter of target genes (Fig. 9a)29.

Light-activated phyA and phyB bind to the AP2-domain of ERF58 and thereby possibly directly block association of ERF58 with target promoters under phyA- and phyB-ON conditions (i.e. in light). Binding to phyA and phyB may be further stabilised by the interaction of phytochromes with the C-terminus of ERF58. Thus, in contrast to PIFs, which use dedicated motifs for binding phyA and phyB31, ERF58 can bind phyA and phyB through its DNA-binding domain. The AP2-domain is the hallmark of AP2/ERF transcription factors16 and binding to this domain could explain why most members of the A6 group of AP2/ERFs interact with phyA and phyB, and may also explain why CBF1, an AP2/ERF transcription factor from group A1, binds phyA and phyB32.

In addition, phytochromes might also bind members of other
Fig. 7 ERF55 and ERF58 regulate the expression of genes encoding GA metabolic enzymes. **a, b** Completion of germination of wild-type (Col-0), erf55-1 erf58-2 double mutant, and ERF58ox (two independent lines) seeds on ddH2O and increasing concentrations of GA3 (**a**) or the GA biosynthesis inhibitor PAC (**b**). Seeds were not exposed to light (**a**) or exposed to W light for 6 h (**b**); after 7 days incubation in the dark at 22 °C, seeds were scored for completion of germination. Data points show mean cumulative germination percentages of three replicates ±SD.

**c** RT-qPCR analysis of GA2ox4 expression levels in Col-0 and erf55-1 erf58-2 seeds incubated under phyA-ON/OFF or phyB-ON/OFF conditions; data for other genes encoding GA metabolic enzymes are shown in Supplementary Fig. 15. Light conditions are described in Supplementary Fig. 6a, b. ACT7 was used as an internal control. Values show means of three replicates ±SD.

**d, e** Schematic representation of the GA2ox4 promoter. The promoter fragment used for EMSA is shown in **e**. **f** EMSA. Biotin-labelled GA2ox4−1111…−1264 promoter fragments containing a wild-type or mutated DRE motif were incubated with MBP-ERF58 or MBP alone (negative control). Samples were analysed by native PAGE. The gel was blotted onto a nylon membrane and signals were detected by streptavidin-coupled horseradish peroxidase and ECL. FP, free probe. The experiment was repeated five times with similar results. Data for ERF55 are shown in Supplementary Fig. 13c.

**g** ChIP-qPCR. Germinating seeds of Col-0 p35S:HA-YFP-ERF58 (line 21C) and Col-0 p35S:YFP-HA (negative control) were subject to light treatments as described in Fig. 4a. Chromatin was isolated and HA-YFP-ERF58 bound DNA fragments were purified using α-GFP coupled magnetic beads. qPCR and specific primers were used to detect the GA2ox4−1111…−1264 promoter fragment in the eluate fraction. ACT7 was used for normalisation. Values show enrichment of the GA2ox4 promoter fragment in the eluate fraction compared to the input fraction and are means of three replicates ±SD. **a–c** p < 0.05. **g** p < 0.01.

**Different letters indicate significant differences as determined by two-way ANOVA followed by post-hoc Tukey’s HSD test.**
groups of the AP2/ERF family and put them under light control, offering huge potential for signal integration between light signalling and different signalling pathways controlled by AP2/ERFs.

Regulation of completion of seed germination is highly complex with different environmental cues that have to be integrated. Such multiple input/single output regulation is hardly possible with a simple linear pathway and requires complex regulatory networks. We identified ERF55 and ERF58 as additional nodes in the regulatory network controlling the completion of seed germination (Fig. 9b). The effect of removing a component from such a network is difficult to predict, since it is usually not known to what extent other parts of the network can compensate and to what extent the signal can be rerouted and take other paths through the network. Although we cannot yet fully answer these questions for the erf55 erf58 mutant, we have identified several genes encoding ABA anabolic or GA catabolic enzymes that are likely direct targets of ERF55 and ERF58, i.e. they are upregulated by ERF55 and ERF58, they contain a DRE element as potential ERF55/ERF58 binding site in their promoter, and both ERF55 and ERF58 associate with their promoter in ChIP-qPCR experiments and/or EMSA. In contrast, none of these genes has been identified as direct target of PIF1 or SOM; in a ChIP-chip approach, not a single gene encoding ABA anabolic or GA catabolic enzymes has been identified as direct target of PIF1, and the mechanism by which SOM regulates gene expression is still unclear. Here, however, we show that ERF55 and ERF58 have the potential to directly link light activation of phytochromes to regulation of a set of genes that encode ABA anabolic or GA catabolic enzymes.

Feedback regulation adds another layer of complexity to the regulatory network controlling the completion of seed germination. We found that ERF55 and ERF58 not only regulate genes encoding ABA or GA metabolic enzymes, but are themselves regulated by GA and in particular ABA, with GA reducing expression of ERF55 and ERF58, and ABA promoting it. There is evidence that PIF1 and SOM also are subject to positive feedback regulation by GA and ABA, which together with feedback regulation of ERF55 and ERF58 could insures rapid promotion of germination completion under favourable conditions and reliable inhibition of the completion of germination when conditions are unfavourable.

Taken together, we identified ERF55 and ERF58 as nodes in the regulatory network controlling the completion of seed germination. We propose a model in which light-activated phyA and phyB directly interact with ERF55 and ERF58 to prevent their association with the promoter of genes repressing germination completion. PhyA and phyB also reduce the expression of ERF55 and ERF58 in seeds exposed to light, thereby further promoting germination completion under phyA/phyB-ON conditions. The erf55-1 erf58-2 double mutant has a percentage of seeds capable of completing germination in the absence of light, highlighting the function of ERF55 and ERF58 in light regulation of the completion of seed germination and supporting the model proposed in Fig. 9.

Due to extensive redundancy, AP2/ERFs may not have been identified as light signalling components in classical loss-of-function screens. However, the fact that phyA and phyB bind to the AP2 domain of ERF58, which is highly conserved in all
AP2/ERF transcription factors, opens the possibility that responses mediated by a wide range of AP2/ERFs could be under light control by phytochromes.

**Methods**

**Vectors and cloning.** Details of plasmid constructs and primers used in this study can be found in Supplementary Tables 1, 2, and Supplementary Fig. 16.

**Plant material and growth conditions.** All Arabidopsis lines used in this study are in the Arabidopsis thaliana Columbia (Col-0; NASC N1092) background. The phyB-9 (NASC N6217)36; phyA-211 (NASC N6223)37; som-3 (SALK_008075; NASC N08075)38; and Col-0 p35S:SOM-FLAG39 have been described previously. erf55-1 (SALK_091212; NASC N68146); erf56 (SALK_139786; NASC N661174); erf57 (GK-098F06; NASC N409378); erf58-1 (SALK_139727; NASC N677156); erf58-2 (SAIL_1293_A03; NASC N848391); erf59 (SALK_020767; NASC N654090); erf60 (SALK_021999; NASC N656258); pif1-1 (SAIL_256_G07; NASC N66041); and abi5-8 (SALK_013163C; NASC N673861) were obtained from the NASC38–40. Col-0 pERF58:HA-YFP:ERF58, Col-0 p35S:HA-YFP:ERF58 (ERF58ox), Col-0 p35S:ABI5-YFP:HA, Col-0 p35S:YFP:ERF58; erf55-1 pERF58:ERF58, erf55-1 erf58-2 p35S:ABI5-YFP:HA, and erf55-1 erf58-2 p35S:HA-YFP-PIF1 were created by using the floral dip method41. The Col-0 p35S:YFP-HA line was described previously25. The erf55-1 erf58-2 phyA-211, erf55-1 erf58-2 phyB-9, erf55-1 erf58-2 pif1-1, erf55-1 erf58-2 abi5-8, erf55-1 erf58-2 p35S:SOM-FLAG, and erf58-2 pERF58:HA-YFP:ERF58 lines were generated by genetic crossing.

For propagation, Arabidopsis plants were grown in the greenhouse. Tobacco (Nicotiana benthamiana) plants were cultivated under 14 h day (26 °C)/10 h night (19 °C) cycles. Genotyping was performed as described in Supplementary Table 3 using primers listed in Supplementary Table 4.

**Germination assay.** Seeds used in the same experiment were obtained from plants grown at the same time in the same conditions and harvested at the same time. Differences during plant cultivation and seed set resulted in seed batches with

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**Fig. 9 Light regulation of the completion of seed germination by ERF55 and ERF58.**

**a** Model for regulation of the completion of seed germination by ERF55/ERF58 and phytochromes. In phyA/B-OFF conditions (e.g. in the dark), ERF55 and ERF58 bind to the promoter of genes that repress the completion of seed germination and enhance their expression (direct targets; shown in orange). Genes that promote the completion of seed germination can be repressed by ERF55/ERF58 through upregulation of intermediate factors (shown in grey) but they are possibly not direct targets of ERF55/ERF58. Indirect targets of ERF55/ERF58 are shown in green and may either promote (light green) or repress (dark green) completion of germination. Light-activated phytochromes repress the expression of ERF55 and ERF58 in phyA/B-ON conditions and also bind ERF55 and ERF58 to prevent their association with the promoter of various target genes that repress the completion of germination. Solid lines indicate direct regulation; dashed lines indicate indirect regulation; →, positive regulation; ⊣, negative regulation; thick arrows, strong regulation; thin arrows, weak regulation; other pathways → completion of germination.

**b** Simplified regulatory network for light regulation of the completion of seed germination. Regulatory interactions of ERF55/ERF58 are shown in red; other regulatory interactions are shown in black. Solid lines indicate direct regulation; dashed lines indicate indirect regulation or regulatory steps for which it is not known whether they are direct or indirect; →, positive regulation; ⊣, negative regulation.

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**ARTICLE**

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Quantification of transcript levels by RT-qPCR. Total RNA was extracted using a modified version of the method described by Malnoy et al.\(^\text{11}\). One hundred mg seed samples were ground to fine powder using a mortar and pestle for at least one month at room temperature before using them for qPCR. Final cumulative germination percentage was calculated as follows:\(^\text{11,46}\). Sixty seeds were surface sterilised and sown on filter paper (Macherey-Nagel; Cat. no. MN 615) soaked with 4.5 ml ddH\(_2\)O or on ½× MS plates/1.5% agar and exposed to W light for 5 or 16 h and then transferred to the dark at 22 °C for another 5 days (phyA-ON). The relative luciferase activity was calculated as ratio of luciferase luminescence/GUS transcription kit (Thermo Fisher Scientific, Waltham, MA, USA; Cat. no. 4368814), and qPCR was carried out using the SensiFAST\textsuperscript{TM} SYBR Hi-ROX kit (Bioline Meridian Bioscience, London, UK; Cat. no. BIO-52077). Reverse transcription into cDNA was performed using the High Capacity Reverse Transcription kit (Bioline Meridian Bioscience, London, UK; Cat. no. BIO-92005). Each experiment was performed in triplicate with three biological replicates. Gene expression levels were normalised to ACT7 (A1tg32200 or ACT7 (A15g099810). The primers used for RT-qPCR can be found in Supplementary Table 5.

**Protein expression and purification**

For expression of MBP, MBP-ERF58, MBP-ERF55, and MBP-FHY1 (163–202), the plasmids pMAL, pMALARF, pMALERF55, or pMAL-FHY1 (163–202) were transformed into the E. coli strain BL21(DE3)-RIL. For expression of MBP-ERF55, MBP-ERF58, and MBP-FHY1, transformed cells were inoculated into 10 ml LB medium supplemented with 100 μg/ml Ampicillin (Duchefa; Cat. no. A0104), grown overnight at 37 °C, and diluted according to the manufacturer’s instructions (NEB, Ipswich, MA, USA; Cat. no. E8021L). Proteins were eluted with elution buffer (20 mM Tris-HCl pH 7.4, 0.2 M NaCl, 1 mM EDTA, 10 mM maltose).

Expression of MBP-FHY1 (163–202) for subsequent affinity purification of phyA was done as described above with the following modifications. IPTG was added to 2 mM final concentration, induction was performed at 37 °C for 2 h, and pelleted bacteria were resuspended in 45 ml 50 mM KPO\(_4\) pH 7.8, 20 mM NaCl, 1 mM EDTA, 2 mM DTT, protease inhibitors (Sigma-Aldrich; Cat. no. I3911; 1 vial per litre), and subsequently divided into 5 x 10 ml and frozen at −20 °C until the day of phyA purification.

Expression of photoactive phyA-FLAG in E. coli.pDS5A-PHYA-FLAG-pPB MKII was transformed into the E. coli strain BL21(DE3)-RIL. Transformed cells were inoculated into 10 ml LB medium supplemented with 30 μg/ml Kanamycin (Duchefa; Cat. no. K0126), grown at 28 °C for 2 h, and diluted into 11 Terrific Broth (25 g l\(^{-1}\) yeast extract, 10 g l\(^{-1}\) tryptone, 10 g l\(^{-1}\) peptone, 1.2% [v/v] glycerol, 1.2% [v/v] 100 mM KPO\(_4\), pH 7.4) at 1:100. The cell suspension was grown at 37 °C for 5 h until OD\(_{600}\) = 1. The temperature was then decreased to 16 °C and 50 ml 100 mM Malate/100 mM Glutamate solution was added to the cell culture. After 1 h, the culture was induced with 2 mM IPTG, covered with black cloth and incubated overnight at 16 °C in the dark. The cell culture was centrifuged at 3800 x g for 30 min at 4 °C. The cell pellet was resuspended in 0.5 M EDTA (pH 8.0) and 0.05 M PMSF, and Lysozyme (Carl-Roth, Karlsruhe, Germany; Cat. no. 5295.1) was added to a final concentration of 1 mg ml\(^{-1}\). After stirring for 30 min on ice, the cells were lysed by ultra-sonication on ice (three times for 2 min).

The cell lysate was centrifuged at 30,000 x g for 45 min at 4 °C. The supernatant was then applied to the amyllose resin and the resin was centrifuged according to the manufacturer’s instructions (NEB, Ipswich, MA, USA; Cat. no. E8021L). Proteins were eluted with elution buffer (20 mM Tris-HCl pH 7.4, 0.2 M NaCl, 1 mM EDTA, 10 mM maltose).

**Yeast interaction assay**

To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible. To evaluate completion of seed germination in presence of GA, 60 surface-sterilised seeds were sown on ½× MS plates/1.5% agar supplemented with 20 μM 3-(3-Chlorosulfonyl)phenylboronic acid (CSM-L-T-H), and grown at 28 °C until colonies were visible.
eluted at 1 ml min \(^{-1}\). Fractions containing protein as determined by nanodrop (Thermo Fischer Scientific) were pooled, DTT added to 2 mM and Protease inhibitors (Sigma-Aldrich; Cat. no. 11991) added, followed by concentration using a vivaspin 6 ml centrifugal concentrator (100 kDa cut-off PES, prewashed with ddH2O and column wash buffer; Sartorius; Cat. no. VS0642) at 4000 x g, 4 °C for 10 min.

Electrophoretic mobility shift assay
Electrophoretic mobility shift assays (EMSAs) were done according to the LightShift Chemiluminescence EMSA kit manual (Thermo Fischer Scientific, Waltham, MA, USA; Cat. no. 201144) with modifications. Biotinylated probes were used in this study. Biotin-labelled wild-type probes were obtained by PCR or by annealing complementary oligonucleotides (Supplementary Table 6) using primers listed in Supplementary Table 7. Gels were exposed to 20 μl × binding buffer (10 mM Tris-HCl pH 8.0, 150 mM KCl, 5 mM EDTA pH 8.0, 0.1% Triton-X 100, 12.5% glycerol, 0.2 mM DTT [freshly added before use]) supplemented with 0.25 mg ml\(^{-1}\) BSA (NEB, Cat. no. B9001S) at 24 °C for 20 min. Then biotin-labelled probes (50 nM final concentration) were added to the respective binding reactions. Incubation at 24 °C for 10 min, followed by 60% non-denaturing polyacrylamide gels, which were run in 0.5× Tris-Borate-EDTA buffer at 100 V. Gels were then blotted onto a nylon membrane at 380 mA (~100 V) for 30 min and DNA was immediately crosslinked to the membrane at 120 mJ cm\(^{-2}\) using a UV crosslinker. The membrane was then blocked overnight at 4 °C in 30 ml 0.5% BSA buffer and incubated under gentle shaking for 15 min at room temperature in 0.5% BSA buffer containing 1:300,000 diluted Stabilised Streptavidin-Horseradish Peroxidase Conjugate (Thermo Fischer Scientific, Waltham, MA, USA; Cat. no. 21124). After washing the membrane twice or three times with PBS and once with PBS, Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare, Pittsburgh, IL, USA; cat. no. RPN2223) was applied onto the membrane and luminescence signals were detected using a CCD camera.

Zinc blot assay. Zinc blot assays were performed as described in Kami et al.\(^{49}\) with slight modifications. Samples prepared for the EMSA experiment were run on native gels containing 1 mM Znacetate in running buffer supplemented with 1 mM Znacetate. Fluorescence signals (caused by complex formation of zinc ions and the chromophore of phytomochromes) were detected on a UV-illuminator using a camera equipped with a red light filter.

In vivo co-immunoprecipitation assay. Six hundred μl Col-0 pERF58::YFP-ERF58 seeds were sown on different 1/6 MS plates/15% agar. The plates were incubated for 2 days at 4 °C in the dark, exposed for 8 h to 10 μmol m\(^{-2}\) s\(^{-1}\) R light (600 nm) at 22 °C to induce germination, returned to the dark, and incubated for another 64 h at 22 °C. Then, seedlings on different plates were exposed to different light intensities (at 22 °C). D. plates were kept in the dark; S. plates were exposed to 20 μmol m\(^{-2}\) s\(^{-1}\) R light for 3 min; 2. plates were exposed to 10 μmol m\(^{-2}\) s\(^{-1}\) R light for 2 h; 3. plates were exposed to 10 μmol m\(^{-2}\) s\(^{-1}\) R light for 10 min. Total protein was then extracted and used for co-immunoprecipitation. Native co-immunoprecipitation buffer contained 100 mM phosphate buffer pH 7.8, 150 mM NaCl, 1 mM KCl, 1 mM EDTA pH 8.0, 1% PEG 4000, 0.5% Triton X-100, 50 mM MgCl\(_2\) (Sigma-Aldrich; Cat. no. 474790), 1× Protease inhibitor Cocktail (Sigma-Aldrich; Cat. no. 11991), and 1× Complete Protease Inhibitor Cocktail (Sigma-Aldrich; Cat. no. 04693159001). For each sample, 50 μl of α-GFP MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany; Cat. no. 130-091-125) were added to the extracts, followed by 2 h of incubation in darkness with gently shaking. Immunoprecipitated complexes were washed twice with native extraction buffer and three times with native extraction buffer without KCl, and eluted in 2× SDS loading buffer (100 mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 0.05% bromophenol blue). Samples were then analysed by SDS-PAGE and immunoblotting. α-HA and α-actin (Sigma-Aldrich, St. Louis, MO, USA; Cat. no. A0480, monoclonal, mouse; 1:3,000 dilution) antibodies were used to detect proteins. Blots were quantified according to Enderle et al.\(^{56}\) using ImageJ.

ChIP assay. ChIP assays were performed based on the protocol by Yamaguchi et al.\(^{32}\) with some modifications. Col-0 pSS53:HAYPF-ERF58 and Col-0 pSS53:HAYPF- STABLE transgenic lines were used for ChIP. One gram (corresponding to ~1.5 ml of seeds germinating under conditions described in the respective figures and treated with 3% H2O2) was washed at 15,000 × g for 10 min at room temperature. The supernatant was collected and transferred to a clean 2 ml microcentrifuge tube containing four volumes of acetone. After careful mixing, proteins were precipitated for 20 min at −20 °C. Samples were then centrifuged at 15,100 × g for 10 min at 4 °C. The supernatant was discarded and the complexes were resuspended in 150 μl of acetone (100 mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 0.05% bromophenol blue). Samples were then analysed by SDS-PAGE and immunoblotting. α-HA and α-actin (Sigma-Aldrich, St. Louis, MO, USA; Cat. no. A0480, monoclonal, mouse; 1:3,000 dilution) antibodies were used to detect proteins. Blots were quantified according to Enderle et al.\(^{56}\) using ImageJ.

Measurement of ABA content in germinating seeds. Dry seeds (Col-0, cfr5-1 cfr5-2, ERF58ox 21C; 3 replicates per genotype; 1.5 g dry seeds per replicate) were incubated on ddH2O for 24 h. Excess water was drained off, seeds were frozen in liquid nitrogen, and stored at −80 °C until shipment to collaborators performing the ABA measurement. The ABA content in the seeds was measured as follows. The tubes containing the frozen germinating seeds were defrosted at 10 °C in an ultrasonic bath for 2× (2× 10 min). For each replicate, 2× 100 μl of 1× ABA extraction buffer were weighed and transferred into separate 2 ml safelock tubes containing a 5 mm steel ball. The seeds were frozen in liquid nitrogen and ground to powder using a Retch mill (4× 15 s; 25 Hz) with intermittent cooling in liquid nitrogen. All solvents used in the following steps were preheated at 8 °C. Three hundred sixty μl ABA extraction buffer containing 100 mM DTT and ABA (150 μl) were added to each sample. The samples were then mixed and incubated for 1 min in an ultrasonic bath at 10 °C and then for 10 min at 10 °C in a thermoshaker at 950 rpm. After adding 200 μl CHCl\(_3\), the samples were incubated again for 1 min in an ultrasonic bath at 10 °C followed by 10 min at 10 °C in a thermoshaker at 950 rpm. Then, 400 μl water were added to each sample, then 1 min before incubating them again in an ultrasonic bath and thermomixer under the above-described conditions. After 10 min centrifugation at 10 °C, 18,600 x g, 20 μl supernatant were diluted 1/5 with water and measured using targeted LCMS.

The LCMS profiling analysis was performed using a Micro-LC MS (Trap and Elute) and a QTRAP6500 + (Sciex) operated in MRM mode (MRMs ABA (1) quantifier ion (m/z) Q1/Q3 263/153, declustering potential DP −40 V, collision energy CE −15V; ABA (2) (m/z) Q1/Q3 263/219, DP −40 V, CE −20V; D6-ABA (1) quantifier ion (m/z) Q1/Q3 269/159; D6-ABA (2) (m/z) Q1/Q3 269/225). Chromatographic separation was achieved on a Luna Omega Polar C18 column (0.3 × 50 mm, Phenomenex) and a Luna C18 column (0.3 × 100 mm, Phenomenex) with a column temperature of 35 °C. The following binary gradient was applied for the main column at a flow rate of 10 μl min\(^{-1}\): 0–0.2 min, isotropic 90% A; 0.2–2 min, linear from 90% A to 30% A: 2–4.5 min, linear from 30% A to 10% A; 4.5–5 min, linear from 10% A to 5% A; 5–5.5 min, isotropic 5% A; 5.5–5.5 min, linear from 5% A to 90% A; 5.5–6 min, isotropic 90% A (A: water, 0.1% q. formic acid, B: acetonitrile, 0.1% q. formic acid). The samples were then concentrated on the trap column using the following conditions: flow rate 50 μl min\(^{-1}\); 0–1.5 min isotropic 95% A; 1.5 min start gradient; 1.5–1.7 min isotropic 95% A. The injection volume was 50 μl. Analytes were ionised using an Optiflow Turbo V spray source (AB SCIEX) and a QTRAP 6500+ with a Triple Quad detector (AB SCIEX) with a column temperature of 25 °C; scan time 3 ms. The ABA content in a replicate was calculated as the mean of the two samples taken from the respective replicate. Three biological replicates were measured.
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