SUPPLEMENTARY INFORMATION

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

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SUPPLEMENTARY FIGURES

Supplementary Fig. 1 Group A6 ERFs interact with phyA and phyB. **a** Y2H growth assay. phyA- or the N-terminal half of phyB (phyBN) fused to LexA and ERFs from group A6 of the AP2/ERF transcription factor family fused to the GAL4 AD 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). **b** Co-localisation of ERF55 with phyA and phyB in tobacco. *p35S:HA-YFP-ERF55* and either *p35S:PHYA-NLS-CFP* or *p35S:PHYB-CFP* were transiently co-expressed in tobacco leaf epidermis cells by agro infiltration; empty *p35S:YFP* and *p35S:CFP* vectors were used as control. YFP and CFP signals were detected by epifluorescence microscopy. The YFP/CFP control is also shown in **Fig. 1c**. Scale bar represents 5 μm. Experiments were repeated three times with similar results.

 \overline{a}

Supplementary Fig. 2 *ERF58* and *ERF55* T-DNA insertion and ERF58 overexpression lines. **a, b** Diagrams showing the position of the T-DNA insertions in the *ERF58* locus in *erf58-1* and *erf58-2* **(a)**, and in the *ERF55* locus in *erf55-1* **(b)**. Black box, exon; white boxes, UTRs; arrow above boxes, transcription start site; arrows below boxes, primers used for RT-qPCR in **c, d**. **c, d** RT-qPCR to detect *ERF58* **(c)** and *ERF55* **(d)** transcript levels in 4 day old darkgrown wild-type and *erf58-1*, *erf58-2* **(c)** or *erf55-1* **(d)** mutant seedlings. *ACT7* was used as an internal control. Values are means 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. **e** HA-YFP-ERF58 protein levels in two independent ERF58ox lines (2A and 21C). Seeds were germinated for 12 hours in the dark. Total protein was 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 negative control. Signals detected by α-HA and α-ACT were quantified using ImageJ; numbers below the membrane show the HA/ACT ratio. The experiment was repeated thee times with similar results.

Supplementary Fig. 3 ERF55 and ERF58 are negative regulators of the completion of seed germination. **a-c** Final cumulative seed germination of different genotypes was quantified under phyA- **(c)** or phyB-specific **(a, b)** conditions. Light treatments (phyA-ON/OFF, phyB-ON/OFF) are described in **Fig. 2a, b**. Bars represent 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.

Supplementary Fig. 4 Complementation of the *erf58-2* mutant by expression of *pERF58:HA-YFP-ERF58*. **a-d** Test of completion of germination for wild-type (Col-0), *erf58-2*, and two independent *erf58-2 pERF58:HA-YFP-ERF58* lines (A, B) under phyA-ON/OFF **(a, b)** or phyB-ON/OFF **(c, d)** conditions. Light treatments (phyA-ON/OFF, phyB-ON/OFF) are described in **Fig. 2a, b**. **b, d** Bars show mean cumulative germination percentages of three replicates ±SD. **e** Schematic representation of the *pERF58:HA-YFP-ERF58* transgene. Arrows indicate primers used for RT-qPCR in **f**. **f** RT-qPCR analysis of transgene expression

in complementing lines. Total RNA was extracted from seeds germinating for 12 hours in the dark and used for RT-qPCR with transgene-specific primers. *ACT7* was used as an internal control. Values are means of three replicates ±SD. **b, d, f** Different letters indicate significant differences as determined by one-way ANOVA followed by post-hoc Tukey's HSD test; p<0.05.

Supplementary Fig. 5 Complementation of the *erf55-1* mutant by expression of *pERF55:ERF55*. **a-b** Test of completion of germination for wild-type (Col-0), *erf55-1*, and two independent *erf55-1 pERF55:ERF55* lines (A, B) under phyA-ON/OFF **(a)** or phyB-ON/OFF **(b)** conditions. Light treatments (phyA-ON/OFF, phyB-ON/OFF) are described in **Fig. 2a, b**. Bars show mean cumulative germination percentages of three replicates ±SD. **c** Schematic representation of the *pERF55:ERF55* transgene. Arrows indicate primers used for RT-qPCR in **d**. **d** RT-qPCR analysis of transgene expression in complementing lines. Total RNA was extracted from seeds germinating for 12 hours in the dark and used for RT-qPCR with transgene-specific primers. *ACT7* was used as an internal control. Values are means of three replicates ±SD. **a, b, d** Different letters indicate significant differences as determined by oneway ANOVA followed by post-hoc Tukey's HSD test; p<0.05.

Supplementary Fig. 6 PhyA and phyB repress *ERF55* and *ERF58* expression. **a, b** Light treatments used to test for phyA- and phyB-dependent regulation of *ERF55* and *ERF58* expression in germinating seeds. Seeds were imbibed for 1 hour in the dark (D), treated for 5 min with FR light and then exposed to light conditions that specifically activate phyA (phyA-ON: 2 days in D \rightarrow 12 hours in FR light \rightarrow 12 hours in D) or phyB (phyB-ON: 5 min in R light

 \rightarrow 12 hours in D). phyA and phyB are inactive under phyA-OFF and phyB-OFF conditions. Time points for harvesting samples for testing HA-YFP-ERF58 protein stability **(e, f)** are indicated in grey. **c, d** RT-qPCR analysis of *ERF58* **(c)** and *ERF55* expression **(d)** in seeds germinating under phyA-ON/OFF or phyB-ON/OFF conditions. *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. **e, f** HA-YFP-ERF58 protein stability. Col-0 *p35S:HA-YFP-ERF58* seeds (line 21C) were germinated under phyA-ON/OFF **(e)** or phyB-ON/OFF **(f)** conditions followed by 0, 1, 2, 3, 6, or 12 hours incubation in the dark as indicated in panel **a**. Col-0 was used as negative control. Total protein was extracted and analysed by SDS-PAGE and immunoblotting with α-HA; α-ACT was used to detect ACTIN as loading control. Signals detected by α-HA and α-ACT were quantified using ImageJ; numbers below the membrane show the HA/ACT ratio. Experiments were repeated three times with similar results.

a

Effectors

 $\mathbf b$

Supplementary Fig. 7 ERF55 and ERF58 activate the *PIF1* promoter. **a** Schematic representation of effector, reporter, and control constructs used for transient transactivation assays in tobacco. Effector and control constructs were driven by the *35S* promoter; *PIF1* promoter fragments were used to drive expression of firefly luciferase. *GUS* driven by the *35S* promoter was used as an internal control. **b** Diagram depicting the *PIF1* promoter and promoter fragments (*pPIF1-A*, *pPIF1-B*, *pPIF1-C*, *pPIF1-D*, *pPIF1-E*, *pPIF1-F*) used in the transactivation assay. Arrows indicate primers used for ChIP-qPCR and for PCR amplification of probes for EMSAs **(Fig. 3f, g; Fig. 4c, d, e, g)**. **c, d** Transactivation assay in tobacco. The effector constructs *p35S:HA-YFP-ERF58* **(c)** or *p35S:HA-YFP-ERF55* **(d)**, reporter constructs (*pPIF1* promoter fragments driving expression of *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 two-way ANOVA followed by post-hoc Tukey's HSD test; p<0.05.

a **Effectors** $p35S$ HA-YFP-ERF58 $p35S$ HA-YFP-ERF55 $p35S$ HA-YFP **Internal control** p35S \overline{GUS} Reporter **Firefly Luciferase** 0SOM $\mathbf b$ Promoter of SOM -670 -840 -510 -330 ATG... pSOM-D pSOM-C pSOM-B pSOM-A -680 -1000 -500 -830 $\mathbf d$ $\mathbf c$ pSOM:LUC pSOM:LUC 5 **YFP** \overline{b} **YFP** Relative luciferase activity Relative luciferase activity **ERF55 ERF58** 4 3 3 $\overline{2}$ яŀ $\overline{2}$ p 1 PSOMILA ... 1000) OM-A SOM-B RIVE DAVS psom(1.1.1 ps) Ω Pool Print **Property** Psomic
Angiomic PSOM/D PSOM/D

Supplementary Fig. 8 ERF55 and ERF58 activate the *SOM* promoter. **a** Schematic representation of effector, reporter, and control constructs used for transient transactivation assays in tobacco. Effector and control constructs were driven by the *35S* promoter; *SOM* promoter fragments were used to drive expression of firefly luciferase. *GUS* driven by the *35S* promoter was used as an internal control. **b** Diagram depicting the *SOM* promoter and promoter fragments (*pSOM-A*, *pSOM-B*, *pSOM-C*, *pSOM-D*) used in the transactivation

assay. Arrows indicate primers used for ChIP-qPCR and for PCR amplification of probes for EMSAs **(Fig. 3f, g; Fig. 4c, d, f, h)**. **c, d** Transactivation assay in tobacco. The effector constructs *p35S:HA-YFP-ERF58* **(c)** or *p35S:HA-YFP-ERF55* **(d)**, reporter constructs (*pSOM* promoter fragments driving expression of *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 two-way ANOVA followed by post-hoc Tukey's HSD test; p<0.05.

Supplementary Fig. 9 Completion of seed germination of *erf55-1 erf58-2* in *SOM*, *PIF1*, or *ABI5* mutant or overexpression background. **a-c** Completion of seed germination of indicated genotypes was tested under phyA- and phyB-specific conditions. Light treatments (phyA-ON/OFF, phyB-ON/OFF) are described in **Fig. 2a, b**. The same data set is shown for Col-0 and *erf55-1 erf58-2* in **a-c**. Bars represent mean cumulative germination percentages of three replicates ±SD. Different letters indicate significant differences as determined by twoway ANOVA followed by post-hoc Tukey's HSD test; p<0.05.

Supplementary Fig. 10 The AP2 domain of ERF58 interacts with phyA and phyB. **a** Diagram depicting the domain structure of ERF58; numbers indicate amino acid positions. **b** Y2H growth assay. Full-length phyA or the N-terminal half of phyB (phyBN) fused to LexA and different ERF58 fragments (numbers indicate amino acid positions) fused to the GAL4 AD 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 2 μ mol m⁻² s⁻¹ R light. The experiment was repeated three times with similar results.

 \mathbf{a}

Supplementary Fig. 11 Pr/Pfr absorption spectra and zinc blot analysis for recombinant purified phyA. **a** Pr and Pfr absorption spectra of recombinant phyA. Absorption spectra of purified recombinant phyA-FLAG after saturating irradiation with far-red light (Pr) or red light (Pfr) were measured. Assuming a Pfr/Prot ratio of 0.87 after saturating irradiation with red light, the spectrum for Pfr was calculated. **b, c** Zinc blot for EMSA samples shown in **Fig. 4e, f**. Bilin-linked polypeptides in the presence of Zinc-acetate form a complex that results in orange fluorescence when viewed under UV light¹. Here, we used this method to confirm that chromophore-bound phyA is present in the respective EMSA samples. EMSA samples were analysed on PAGE gels supplemented with Zinc-acetate and fluorescence signals were detected by exposing the PAGE gels to UV light. Cold, excess of unlabelled DNA fragment containing the DRE motif to which ERF55/ERF58 bind. The experiment was repeated 3 times with similar results.

Supplementary Fig. 12 ERF55 and ERF58 regulate the expression of genes encoding ABA metabolic enzymes. **a-j** RT-qPCR was used to quantify expression of genes encoding ABA anabolic **(a-d, f-i)** or catabolic enzymes **(e, j)** in wild-type (Col-0) and *erf55-1 erf58-2* seeds germinating under phyA-ON/OFF **(a-e)** or phyB-ON/OFF conditions **(f-j)**. Light conditions are described in **Supplementary Fig. 6a, b**. *ACT7* was used as an internal control. Values show 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.

Supplementary Fig. 13 ERF55 binds to the promoter of *ABA2*, *ABI5*, and *GA2ox4*. **a-c** EMSAs. Biotin-labelled *ABA2 -52…-222* **(a)**, *ABI5 -234…-447* **(b)**, or *GA2ox4 -1111…-1264* **(c)** promoter fragments containing a wild-type or mutated DRE motif were incubated with 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. Experiments were repeated six **(a)**, four **(b)**, or three times **(c)** with similar results.

Supplementary Fig. 14 PhyA prevents the association of ERF55 and ERF58 with *NCED9* and *AAO3* promoter fragments. **a, b** EMSAs. Biotin-labelled *NCED9 -611...-633* **(a)** or *AAO3 -421...-453* **(b)** promoter fragments containing a DRE motif were incubated with MBP-ERF55, MBP-ERF58, or MBP alone (negative control). Light-activated photoactive phyA was added where indicated. Samples were analysed by native PAGE. Gels were blotted onto nylon membranes and signals were detected by streptavidin-coupled horseradish peroxidase and ECL. Cold, excess of unlabelled DNA fragment containing the DRE motif to which ERF55/ERF58 bind; FP, free probe. Experiments were repeated three times with similar results.

Supplementary Fig. 15 ERF55 and ERF58 regulate the expression of genes encoding GA metabolic enzymes. **a-f** RT-qPCR was used to quantify expression of genes encoding GA catabolic **(a, d)** or anabolic enzymes **(b, c, e, f)** in wild-type (Col-0) and *erf55-1 erf58-2* seeds germinating under phyA-ON/OFF **(a-c)** or phyB-ON/OFF conditions **(d-f)**. Light conditions are described in **Supplementary Fig. 6a, b**. *ACT7* was used as an internal control. Values show 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.

Supplementary Fig. 16 Plasmid maps for pDSLEXAv3 (a) and pDS15A-PHYA-FLAG-PφB **(b)**; see **Supplementary Data 1** and **2** for nucleotide sequence.

SUPPLEMENTARY TABLES

Supplementary Table 1 Cloning of plasmid constructs.

Supplementary Table 2 Primers used for cloning of plasmid constructs.

Supplementary Table 3 Genotyping of mutants used in this study.

Supplementary Table 5 Primers used for qPCR.

Supplementary Table 6 Probes for EMSA experiments.

Supplementary Table 7 Primers used for generation of probes for EMSA experiments.

Supplementary Table 8 Primers used for ChIP-qPCR.

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