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Phytochrome-interacting transcription factors PIF4 and PIF5 induce leaf senescence in *Arabidopsis*

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Plants initiate senescence to shed photosynthetically inefficient leaves. Light deprivation induces leaf senescence, which involves massive transcriptional reprogramming to dismantle cellular components and remobilize nutrients. In darkness, intermittent pulses of red light can inhibit senescence, likely via phytochromes. However, the precise molecular mechanisms transducing the signals from light perception to the inhibition of senescence remain elusive. Here, we show that in *Arabidopsis*, dark-induced senescence requires phytochrome-interacting transcription factors PIF4 and PIF5 (PIF4/PIF5). ELF3 and phytochrome B inhibit senescence by repressing PIF4/PIF5 at the transcriptional and post-translational levels, respectively. PIF4/PIF5 act in the signalling pathways of two senescence-promoting hormones, ethylene and abscisic acid, by directly activating expression of *EIN3*, *ABI5* and *EEL*. In turn, PIF4, PIF5, EIN3, ABI5 and EEL directly activate the expression of the major senescence-promoting NAC transcription factor *ORESARA1*, thus forming multiple, coherent feed-forward loops. Our results reveal how classical light signalling connects to senescence in *Arabidopsis*.

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Leaf senescence, the final stage of leaf development, actively destabilizes intracellular organelles, including decomposition of macromolecules, to relocate nutrients into developing tissues or storage organs. Senescence occurs autonomously in an age-dependent manner, controlled by an innate genetic programme. However, unfavorable environmental stresses, such as darkness, drought, high temperature, high salinity and pathogen attacks, can also trigger senescence during leaf development[1]. For example, light deprivation induces senescence in lower leaves that are shaded by upper leaves[2].

Molecular genetic studies using *Arabidopsis thaliana* have identified many senescence-associated genes (SAGs), including genes involved in hormone signalling, transcriptional regulation and chlorophyll (Chl) catabolism[1,3]. The developmental and environmental signals of senescence commonly activate senescence-associated transcriptional factors (senTFs), which modulate the expression of SAGs[4]. To date, transcriptome analyses and molecular genetic studies, in *Arabidopsis* and other plants, have identified many senTFs[3,5]. In particular, the senTFs include a few plant-specific NAC genes, such as *ORESARA1* (*ORE1*, also known as *NAC092*)[5], indicating that the senescence-induced NAC transcription factors have important roles in modulating senescence. *ORE1* promotes senescence by regulating the expression of hundreds of SAGs that function in the breakdown of nucleic acids and proteins, and the transport of sugar[6,7]. Treatment with ethylene or abscisic acid (ABA) upregulates *ORE1* expression[8], suggesting a close relationship between hormone signalling and *ORE1*. Among the senescence-induced NACs, *NAC-LIKE, ACTIVATED BY AP3/PI* (*NAP/NAC029*) and *NAC016* also promote senescence, as demonstrated by the delayed senescence phenotype of *nap* and *nac016* mutants[9,10]. In contrast, *JUNGBRUNNEN 1* (*JUB1/NAC042*) and *VND-INTERACTING 2* (*VNI2/NAC083*) inhibit leaf senescence[11,12]. Thus, these observations show that the senescence-promoting and senescence-inhibiting NACs finely tune the expression of SAGs in response to developmental and environmental cues.

Plant hormones coordinate senescence in connection with senTFs, and ensure that senescence proceeds in an orderly fashion at the organismal level. Among plant hormones, ethylene promotes senescence, as demonstrated by accelerated senescence in ethylene-treated plants[13] and delayed senescence in mutants impaired in ethylene signalling, including mutants in the ethylene signalling component *ETHYLENE INSENSITIVE 2* (*EIN2*), the transcription factor *EIN3* and the ethylene receptor *ETHYLENE INSENSITIVE 1* (*ETR1*) (refs 14–16). ABA also induces senescence rapidly[17]. Mutants of *RECEPTOR-LIKE PROTEIN KINASE 1* (*RPK1*) and *SENESCENCE-ASSOCIATED GENE 113* (*SAG113*), which are insensitive to ABA, display delayed senescence phenotypes[18,19]. Senescence is rapidly induced by treatment with salicylic or jasmonic acids[20,21], but is extremely delayed by cytokinin treatment[22]. The exact molecular mechanisms of how these hormone signals coordinate the ageing or stress signals for leaf senescence are not fully understood.

Plant growth and development requires light[23], and plants require photoreceptors to adapt to ambient light conditions throughout development. Red light has long been considered to be a key negative signal for plant senescence, because pulses of red light substantially delay dark-induced senescence (DIS) in a few plant species, supposedly via phytochromes[24] and a low ratio of red/far-red light causes senescence in tobacco leaves[25]. Red light activates and far-red light inactivates phytochromes; darkness also causes slow inactivation of phytochromes[23]. Photoactivated phytochromes move from the cytosol to the nucleus, where they interact with and inactivate the negative regulators of light signalling, resulting in massive transcriptome changes[23]. In *Arabidopsis*, phyA and phyB regulate a wide range of light responses such as seed germination, seedling photomorphogenesis, hypocotyl gravitropism, shade avoidance and flowering time[23]. Phytochromes inactivate phytochrome-interacting bHLH transcription factors (PIFs), which inhibit light responses in the dark[26]. At the molecular level, active phytochromes directly interact with PIFs to induce their detachment from DNA[27], and promote their degradation by the 26S proteasome[28,29]. PIFs are also regulated at the transcriptional level; the EARLY FLOWERING 3 (ELF3)-ELF4-LUX ARRHYTHMO (LUX) complex directly associates with the promoters of *PIF4* and *PIF5* (*PIF4/PIF5*) and suppresses their expression in the regulation of circadian responses[30].

Although the regulatory function of the phytochrome-PIF signalling module has been intensively investigated in the light-responsive development of *Arabidopsis* seedlings, it is unknown if this signalling module has a role in plant senescence. Here, we show the molecular mechanism of how the phytochrome-PIF signalling module activates leaf senescence in *Arabidopsis*. We found that among the four PIFs, PIF4/PIF5 function as the essential, central transcriptional activators of DIS and that ELF3 and red light-activated phyB repress the senescence-promoting activity of PIF4/PIF5 at the transcriptional and post-translational levels, respectively. The two PIFs activate the expression of *ORE1* by forming multiple coherent feed-forward loops, together with two distinct classes of transcription factors, EIN3 in ethylene signalling and Group A bZIPs (ABA INSENSITIVE 5 (ABI5) and ENHANCED EM LEVEL (EEL)) in ABA signalling.

Results

The Pfr form of phyB inhibits leaf senescence. Phytochromes are red/far-red photoreceptors that can be interconverted to active Pfr and inactive Pr forms by red and far-red light irradiation, respectively[31]. To examine the effect of phytochrome-mediated light signalling on leaf senescence, 7-day-old *Arabidopsis* wild-type (WT) seedlings were incubated for 10-day in darkness (control), and in darkness with intermittent pulses of red light ($phyB_{on}$) or with pulses of red light followed by far-red light ($phyB_{off}$) (Fig. 1a, left panel). WT cotyledons turned yellow during dark incubation, a typical symptom of senescence in plants. When the light pulses were given, we found that the cotyledons stayed green in $phyB_{on}$ conditions but turned yellow in $phyB_{off}$ conditions (Fig. 1a, right panel), indicating that the Pfr form of phyB inhibits senescence. To investigate the role of phyB in senescence, we examined the phenotypes of 7-day-old seedlings of *phyB* mutants and *PHYB*-overexpressing (*PHYB*-OX) plants after 8 days of dark incubation (DDI). *phyB* mutants senesced faster and *PHYB*-OX plants senesced slower than WT (Fig. 1b,e,f). Similar to the seedling phenotypes, when attached or detached leaves of 3-week-old plants were incubated in darkness or individual leaves were covered with aluminium foil under long day conditions, *phyB* mutants senesced faster and *PHYB*-OX plants senesced slower than WT (Fig. 1c,d; Supplementary Fig. 1a and Supplementary Methods). Consistent with their visible phenotypes, *phyB* mutants had lower Chl levels, higher ion leakage rates, higher expression of two senescence marker genes (*SENESCENCE 4* (*SEN4*) and *SAG12*), and lower expression of a photosynthetic gene (*LIGHT HARVESTING CHLOROPHYLL A/B BINDING PROTEIN 1* (*Lhcb1*)) than WT seedlings or individually covered leaves of adult plants during dark incubation, while *PHYB*-OX had the opposite phenotypes, with the exception of ion leakage rate (Fig. 1e–g, Supplementary Fig. 1b–d). It appears that *PHYB* overexpression is not sufficient to retard the breakdown of membrane integrity of seedlings during DIS. In contrast, *phyA* mutants and *PHYA*-OX plants

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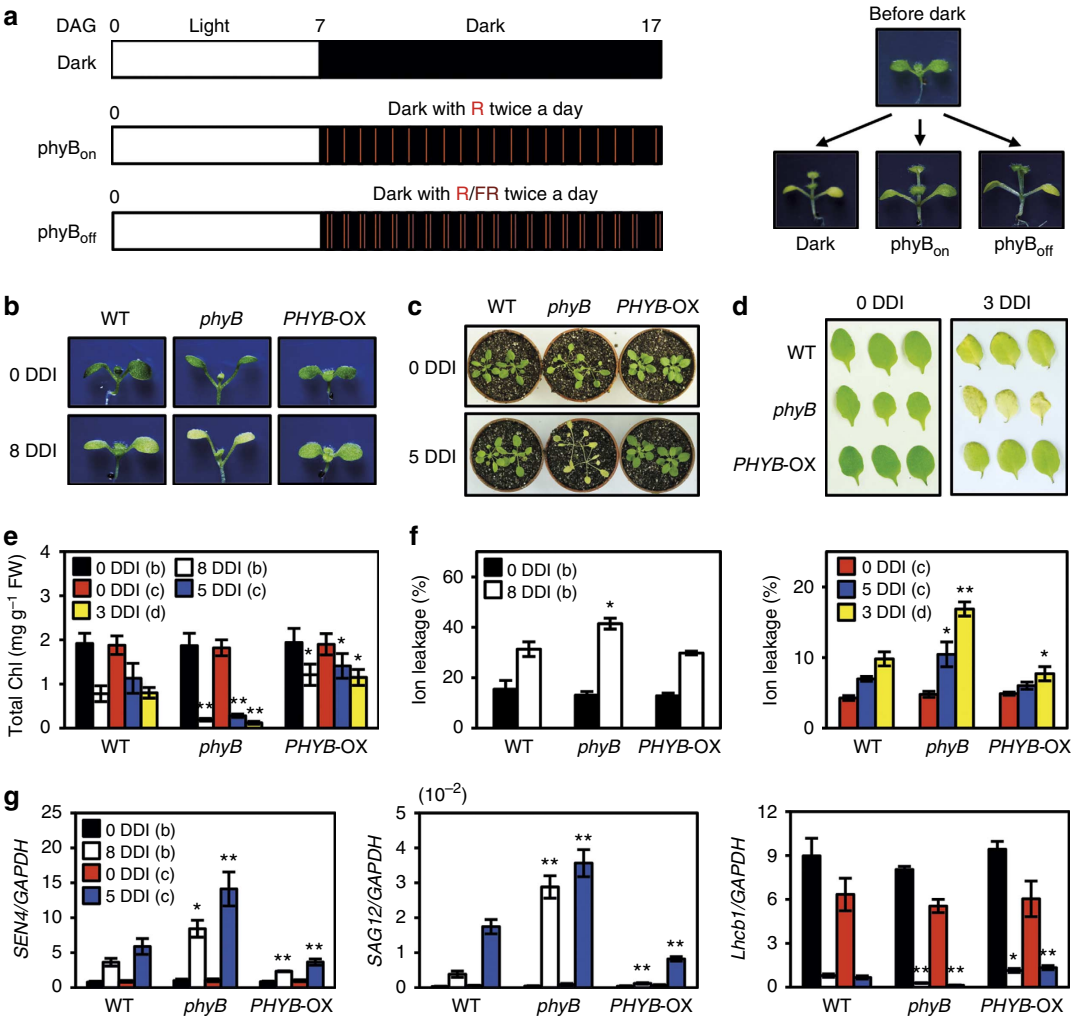

Figure 1 | PhyB suppresses senescence. (**a**) Suppression of senescence by treatment with intermittent red light pulses during dark incubation. Light-grown 7-day-old WT (Col-0 ecotype) seedlings were transferred to darkness for 10 days without any light (continuous darkness), with intermittent red light pulses ($phyB_{on}$) or with red light pulses immediately followed by far-red light pulses ($phyB_{off}$). R and FR indicate red light and far-red light pulses, respectively. (**b**–**f**) Suppression of senescence by phyB as judged by changes in leaf colour. Light-grown 7-day-old WT (Col-0), *phyB* (*phyB-9*) and *PHYB*-OX seedlings (**b**), and attached (**c**) or detached leaves (**d**) of 3-week-old WT, *phyB* and *PHYB*-OX plants were transferred to darkness for the indicated number of days. Total Chl levels (**e**) and ion leakage rates (**f**) in (**b**–**d**) were determined before and after dark treatment. (**g**) Expression levels of *SEN4*, *SAG12* and *Lhcb1* of WT, *phyB* and *PHYB*-OX in 7-day-old seedlings or 3-week-old plants during dark incubation. For quantitative real-time PCR, the relative expression levels were determined by normalizing to the transcript levels of *GAPDH*. *PHYB*-OX indicates the plants (Col) overexpressing *PHYB* under the control of 35S promoter. Mutant information is listed in Supplementary Table 3. Data are means ± s.d. of more than four independent biological replicates. $^{*}P<0.05$, $^{**}P<0.01$ (Student's *t*-test). DAG, day(s) after germination.

senesced at the same rate as WT (Supplementary Fig. 2). The results indicate that phyB is the major photoreceptor signalling to suppress senescence in *Arabidopsis*.

Mutations in *PIF4* and *PIF5* delay leaf senescence. We next investigated which phyB signalling components regulate DIS in *Arabidopsis* leaves. Previous work showed that *pif* quadruple (*pifQ*) mutants (*pif1 pif3 pif4 pif5*) show a constitutive photomorphogenic phenotype when grown in darkness, and phyB promotes light responses by repressing the activity of PIFs[32,33]. We found that *pifQ* mutants maintained green cotyledons at 10 DDI, while WT cotyledons turned completely yellow (Fig. 2a), indicating that PIFs promote senescence in light-deprived conditions. To determine which PIFs promote senescence, we examined the seedling phenotypes of four *pif* single mutants during dark incubation. The *pif1* and *pif3* mutants turned yellow at the same rate as WT, but *pif4* and *pif5* mutants, similar to *pifQ* mutants, senesced slower than WT (Fig. 2b). We also observed a similar delayed senescence pattern in the attached, detached or individually covered leaves of *pif4*, *pif5* and *pifQ* mutants (Fig. 2c,d; Supplementary Fig. 3a). Consistent with their visible phenotypes, the mutants had higher Chl levels, lower ion leakage rates, lower expression of *SEN4* and higher expression of *Lhcb1* than WT (Supplementary Figs 3b,c, 4 and 5). In addition, *pifQ* mutants maintained better-preserved chloroplast structure and *pif4* and *pif5* mutants retained higher levels of photosystem proteins (Supplementary Fig. 6 and Supplementary Methods). Moreover, *PIF4/PIF5* also promote age-dependent leaf senescence, as *pif4* and *pif5* mutant leaves senesced slower than WT leaves under long days (Supplementary Fig. 7), suggesting that *PIF4/PIF5* are the essential transcription factors that promote leaf senescence under not only dark-induced, but also under natural senescence conditions.

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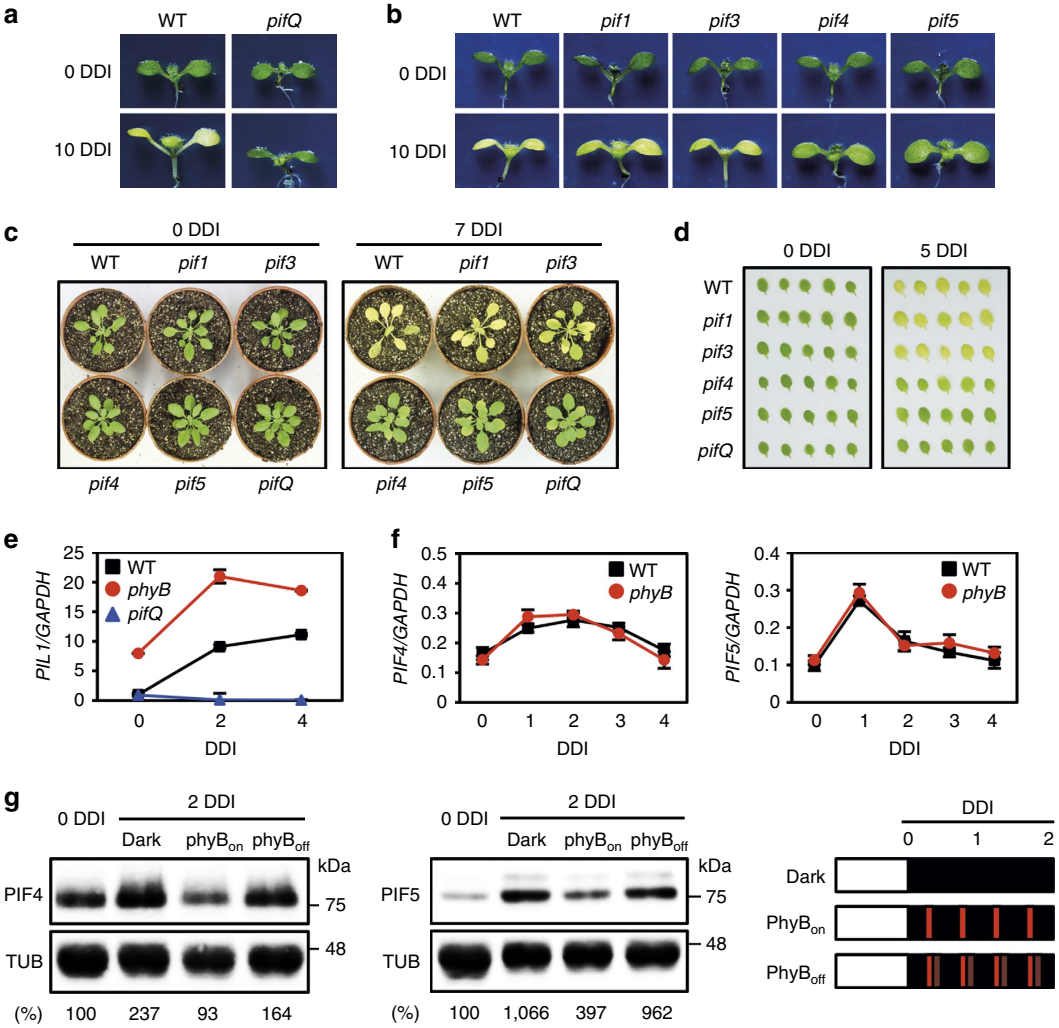

Figure 2 | PIF4 and PIF5 (PIF4/PIF5) promote senescence. (**a**–**d**) Delayed senescence of *pif4* and *pif5* mutants in darkness. WT and *pifQ* indicate wild-type (Col-0) and *pif1 pif3 pif4 pif5* quadruple mutants, respectively. For the assay, 7-day-old seedlings were grown in continuous light (**a**,**b**), and attached (**c**) or detached (4th or 5th) leaves (**d**) of 3-week-old plants grown under long days were incubated in darkness and photographed. Mutant information is listed in Supplementary Table 3. (**e**) *PIL1* expression in WT (black line), *phyB* mutants (*phyB-9*; red line), and *pifQ* mutants (blue line) during dark incubation. Means ± s.d. were calculated from at least four independent replicates. (**f**) *PIF4/PIF5* expression in WT (black lines) and *phyB* mutants (red lines) during dark incubation. (**e**,**f**) By reverse transcription and quantitative real-time PCR, relative transcript levels of *PIL1* (**e**) and *PIF4/PIF5* (**f**) were normalized to those of *GAPDH* (a control). (**g**) Stabilization of PIF4/PIF5 proteins in $phyB_{off}$ conditions. The 35S-driven PIF4/PIF5-Myc and tubulin (TUB; a control) were detected by anti-Myc and anti-tubulin antibodies, respectively. PIF4/PIF5 levels were normalized to TUB protein levels.

To gauge if PIF4/PIF5 transcription factors become active during dark incubation, we examined the expression of the PIF target gene *PHYTOCHROME-INTERACTING FACTOR 3-LIKE 1* (*PIL1*) during dark incubation, as PIFs directly regulate *PIL1* expression[34]. *PIL1* mRNA levels increased and plateaued at 2 DDI in WT (Fig. 2e). However, *PIL1* mRNA levels did not rise in *pifQ* mutants; in contrast, *phyB* mutants showed much higher *PIL1* mRNA levels than WT. The altered expression of *PIL1* in *pifQ* and *phyB* mutants provides indirect evidence that phyB negatively regulates the activity of PIF4/PIF5 in the light; the increased activity of PIF4/PIF5 in the prolonged darkness could be caused by the increase of *PIF4/PIF5* transcription, PIF4/PIF5 protein stability or both. To investigate this, we first examined the expression levels of *PIF4/PIF5* during dark incubation and found that their expression in *phyB* mutants was almost the same as in WT (Fig. 2f). This suggests that phyB does not regulate PIF4/PIF5 at the transcriptional level, but may possibly act at the post-translational level. Indeed, we found that in *PIF4*-OX and *PIF5*-OX plants, the levels of PIF4/PIF5 proteins increased in darkness (Fig. 2g). Intermittent pulses of red light ($PhyB_{on}$) inhibited the increase of PIF4/PIF5 proteins, but pulses of red light followed by far-red light ($PhyB_{off}$) did not, indicating that the PIF4/PIF5 proteins are stabilized when phyB converts to the inactive Pr form.

ELF3 inhibits leaf senescence by repressing *PIF4/PIF5*. A previous study showed that an ELF3–ELF4–LUX complex represses the expression of *PIF4/PIF5* in the evening phase[30]. Thus, we examined if ELF3 inhibits leaf senescence by repressing *PIF4/PIF5* transcription during dark incubation. We found that *elf3* mutants, similar to *PIF4*-OX and *PIF5*-OX plants, senesced much faster than WT in both detached and attached leaves of adult plants (Fig. 3a; Supplementary Fig. 8a), while *ELF3*-OX plants, similar to *pif4* and *pif5* mutants, exhibited delayed senescence (Fig. 3b), as shown by Chl levels (Fig. 3c), ion

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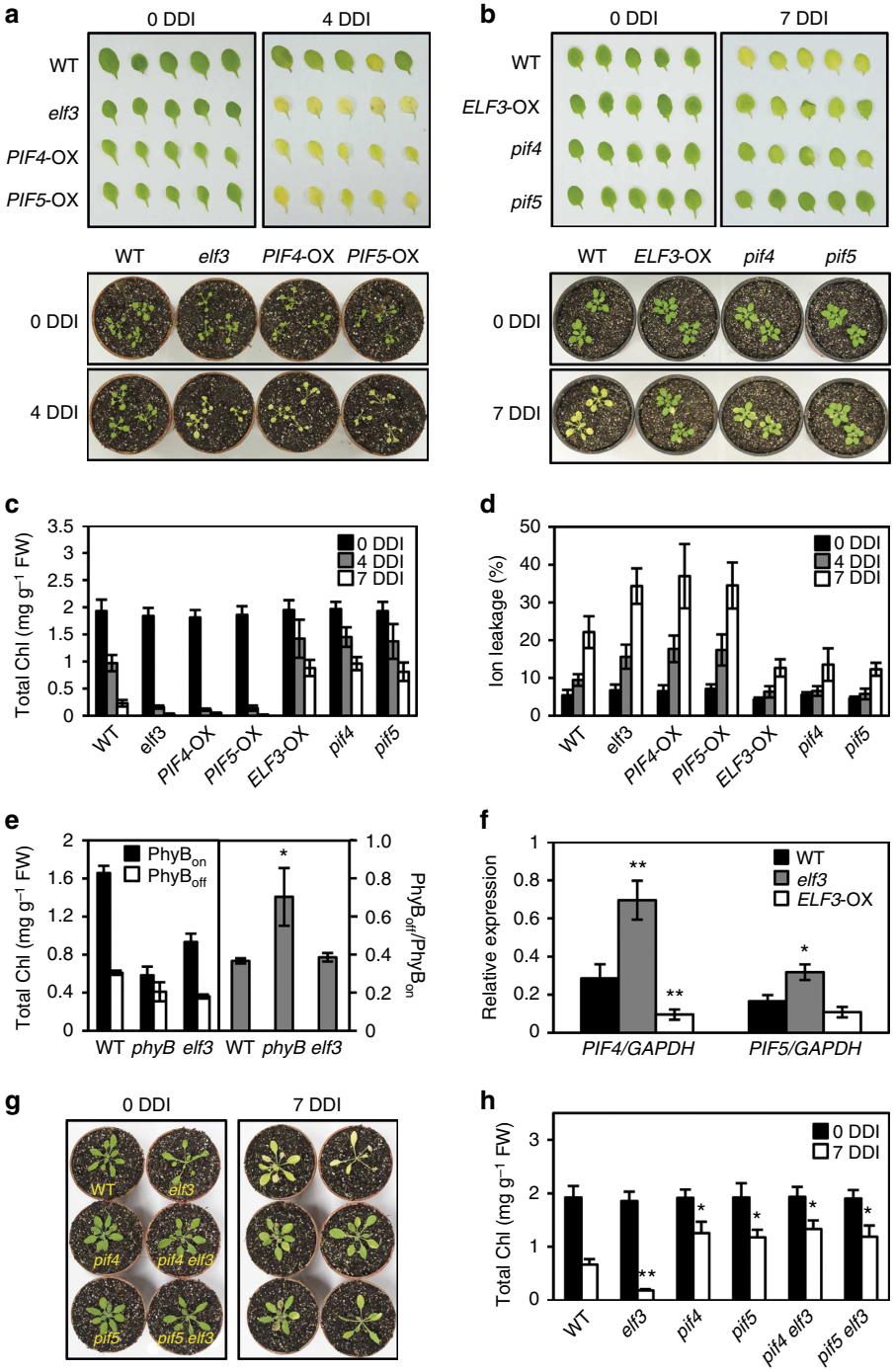

Figure 3 | ELF3 inhibits senescence. (**a**–**d**) Inhibition of senescence by ELF3 in darkness. Three-week-old detached leaves (upper) or whole plants (lower) grown in long days were transferred to complete darkness and photographed at 4 DDI (**a**) or at 7 DDI (**b**). Total Chl levels (**c**) and ion leakage rates (**d**) were measured at indicated DDIs. *elf3* is *elf3-8* (Supplementary Table 3). *PIF4*-OX, *PIF5*-OX and *ELF3*-OX indicate the plants overexpressing *PIF4*, *PIF5* and *ELF3* under the control of 35S promoter, respectively. (**e**) Independent regulation of senescence by ELF3 and phyB. The 7-day-old seedlings grown in continuous light conditions were transferred to darkness for 16 days with intermittent red ($PhyB_{on}$) or red/far-red light pulses ($PhyB_{off}$) as shown in Fig. 1a. $phyB_{off}/phyB_{on}$ indicates that total Chl levels of WT, *phyB* and *elf3* mutants in $PhyB_{off}$ conditions are normalized to those in $PhyB_{on}$ conditions. (**f**) Repression of *PIF4/PIF5* transcription by ELF3. The 7-day-old WT (black bars), *elf3* mutants (red bars) and *ELF3*-OX plants (blue bars) were transferred to darkness for 2 days. *PIF4/PIF5* expression was normalized to *GAPDH* expression. (**g**,**h**) *PIF4/PIF5* are epistatic to *ELF3*. Three-week-old plants grown in long days were transferred to darkness for 7 days (**g**) and total Chl levels were measured (**h**). DDI indicates day(s) of dark incubation. Data are means ± s.d. of at least four biological replicates. $^{*}P<0.05$, $^{**}P<0.01$ (Student's *t*-test).

leakage rates (Fig. 3d), the expression levels of *SEN4* and *Lhcb1* (Supplementary Fig. 5) and chloroplast structure and photosystem protein levels (Supplementary Fig. 6). ELF3 functions as one of the critical factors in light input into the circadian clock and in the regulation of flowering time[35,36]; therefore, we further examined whether senescence is also affected by mutations of other regulators of the circadian clock, flowering time and photomorphogenesis, such as *CIRCADIAN-CLOCK*

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ASSOCIATED 1 (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *ELF4*, *LUX*, *FLAVIN-BINDING, KELCH REPEAT, F-BOX 1* (*FKF1*), *CRYPTOCHROME 2* (*CRY2*), *GIGANTEA* (*GI*), *CONSTANS* (*CO*), *CONSTITUTIVE PHOTOMORPHOGENIC 1* (*COP1*) and *DE-ETIOLATED 1* (*DET1*) (refs 36,37). We found that none of the mutants for these regulators showed altered senescence phenotypes during dark incubation (Supplementary Fig. 8b). Although CRY1 was suggested to regulate PIF4/PIF5 for shade avoidance responses[38], we found that detached leaves of *cry1*, *cry2* and *cry1 cry2* double mutants did not show an early or delayed senescence phenotype during dark incubation (Supplementary Fig. 8c). These results indicate that among the components we tested, ELF3 functions as a unique negative regulator of senescence induction.

ELF3 acts as a phyB signalling component in seedling development[35]; we thus tested whether ELF3 inhibits senescence through red light-activated phyB signalling. Although both *phyB* and *elf3* mutants senesced faster than WT, *elf3* mutants still responded to red light pulses comparable to WT, whereas *phyB* mutants did not (Fig. 3e), indicating that ELF3 suppresses DIS independent of phyB. Since the expression of *PIF4/PIF5* increased during dark incubation independent of phyB activity (Fig. 2f), we measured the mRNA levels of *PIF4/PIF5* in *elf3* mutants and *ELF3*-OX plants at 2 DDI (Fig. 3f). We found that the *PIF4* expression was higher in *elf3* mutants than in WT, and lower in *ELF3*-OX plants. *PIF5* expression also increased more in *elf3* mutants. Furthermore, the *pif4 elf3* and *pif5 elf3* double mutants senesced much later than WT, but similar to *pif4* and *pif5* single mutants at 7 DDI (Fig. 3g,h), demonstrating that *pif4* and *pif5* are epistatic to *elf3*. Consistent with this, *ELF3*-OX *PIF4*-OX plants senesced faster than WT, but similar to *PIF4*-OX plants (Supplementary Fig. 9). These results indicate that ELF3 represses *PIF4/PIF5* transcription independent of phyB, while the Pfr form of phyB destabilizes PIF4/PIF5 proteins.

PIF4/PIF5 promote ethylene and ABA signalling. To investigate how PIF4/PIF5 promote DIS at the molecular level, we performed microarray analysis and identified 677 differentially expressed genes (DEGs) between WT and *pifQ* mutants at 2 DDI (WT/*pifQ*, twofold, false discovery rate (FDR) <0.05; Fig. 4a, left circle). We compared the DEGs of WT/*pifQ* with those of senescing WT at 2 DDI (DIS (2 days/0 days)) (Fig. 2a, right circle) from previously reported microarray data[39]. We found that the two sets share 132 DEGs ($P<10^{-72}$, hypergeometric test), showing a high correlation coefficient ($R=0.85$; Supplementary Fig. 10a). The common DEGs have enriched functional categories of ageing, leaf senescence, photosynthesis, Chl metabolism and light response (Supplementary Fig. 10b). Moreover, although the changes were less than twofold, Gene Set Enrichment Analysis (GSEA, see Methods) showed that the non-shared DEGs of senescing WT also significantly correlated with the changes in *pifQ* mutants (Supplementary Fig. 10c). The results indicate that PIF4/PIF5 function as the major regulators of SAG expression in senescing leaves during dark incubation.

To further explore how PIF4/PIF5 regulate the expression of SAGs, we tested whether the 132 shared DEGs include direct targets of PIF4/PIF5 transcription factors. Thirty two of the shared DEGs are PIF-direct targets ($P=0.0062$, hypergeometric test), according to previously reported PIF4/PIF5 chromatin immunoprecipitation (ChIP)-seq data[34,40]. Their promoters are enriched with the G-box element (CACGTG), a typical binding motif for PIF transcription factors[34,40–43] (Fig. 4b, upper panel). In contrast, the promoters of the indirectly regulated DEGs are enriched with ABRE-like elements (YACGT), the known binding sites of bZIP transcription factors, such as the positive ABA signalling component ABI5[44] (Fig. 4b, lower panel). These results suggest that PIF4/PIF5 directly activate the expression of many SAGs and also indirectly function through ABA signalling.

Ethylene signalling also promotes senescence[13–16]; we thus examined whether PIF4/PIF5 activate both ABA and ethylene signalling for inducing senescence. The GSEA indicated that both ABA- and ethylene-induced genes are downregulated in *pifQ* mutants at 2 DDI and are upregulated in senescing WT (Supplementary Table 1). The suppression of ABA and ethylene signalling in *pifQ* mutants might be partly due to the decreased expression of *EIN3* (a positive ethylene signalling component) and the Group A bZIP transcription factors *ABI5* and *EEL* (Fig. 4c,d,h–j), which are direct targets of PIF4/PIF5, as shown by ChIP assays (Fig. 4d). Consistent with the decreased expression of *ABI5*, *EEL* and *EIN3* in *pifQ* mutants, the bZIP- and EIN3-induced genes, which were identified from previously reported microarray data (see Methods), were significantly downregulated in *pifQ* mutants and upregulated in senescing WT (Supplementary Table 1). We found that *abi5, eel* and *ein3* mutations delayed senescence, and *abi5* and *eel* mutations showed additive effects (Fig. 4e–g), further supporting the hypothesis that decreased expression of *ABI5*, *EEL* and *EIN3* is closely associated with delayed senescence in *pifQ* mutants. Since ELF3 delays senescence by repressing the transcription of *PIF4/PIF5* (Fig. 3f), ELF3 should repress the expression of *ABI5*, *EEL* and *EIN3* during dark incubation. Indeed, their mRNA expression was severely downregulated in *ELF3*-OX plants and upregulated in *elf3* mutants (Fig. 4h–j). These results indicate that PIF4/PIF5 regulate senescence partly through the two bZIPs and EIN3.

PIF4/PIF5 activate *ORE1* through multiple feed-forward loops. We next investigated if PIF4, PIF5 and their direct target transcription factors (ABI5, EEL and EIN3) jointly affect the expression of known senescence-promoting regulators. To date, 3,744 senescence-associated loci in *Arabidopsis* have been curated[3], and 89 loci are known to promote senescence. Among the 89 senescence-promoting genes[3] (Supplementary Data 1), we found that *ORE1*, encoding a key senescence-promoting NAC transcription factor, was commonly induced by all three groups of transcription factors (Fig. 5a). *ORE1* expression was downregulated in *ELF3*-OX plants and *pifQ*, *abi5*, *eel* and *ein3* mutants, and upregulated in *elf3* mutants and *PIF4*-OX plants (Fig. 5b), indicating that *ORE1* is a target senTF acting downstream of *ELF3*, *PIF4, PIF5*, *ABI5*, *EEL* and *EIN3*. Consistent with this, *ore1* mutants, similar to *phyB* and *pif4* mutants, did not respond to red light pulse treatment during dark incubation (Fig. 5c,d). These results indicate that *ORE1* integrates phyB-mediated light signalling to promote senescence in light-deprived conditions.

We found that the *ORE1* promoter harbours three potential PIF binding sites (G-box motifs), several bZIP binding sites (ABRE motifs) and an EIN3 binding site (Fig. 5e), suggesting that *ORE1* is a direct target of not only PIF4/PIF5 but also of bZIPs and EIN3. To examine whether PIF4, PIF5, ABI5, EEL and EIN3 directly activate *ORE1* transcription, we next performed ChIP assays and found that all of the tested TFs bind to the *ORE1* promoter (Fig. 5f). Collectively, the direct activation of *ORE1* transcription by PIF4/PIF5 and by PIF4/PIF5-targeted transcription factors (ABI5, EEL and EIN3) indicates that these transcription factors establish multiple coherent feed-forward regulatory loops to induce leaf senescence; PIF4/PIF5 directly activate the expression of *ABI5, EEL* and *EIN3*, and in turn, PIF4, PIF5, ABI5, EEL and EIN3 directly activate *ORE1* transcription.

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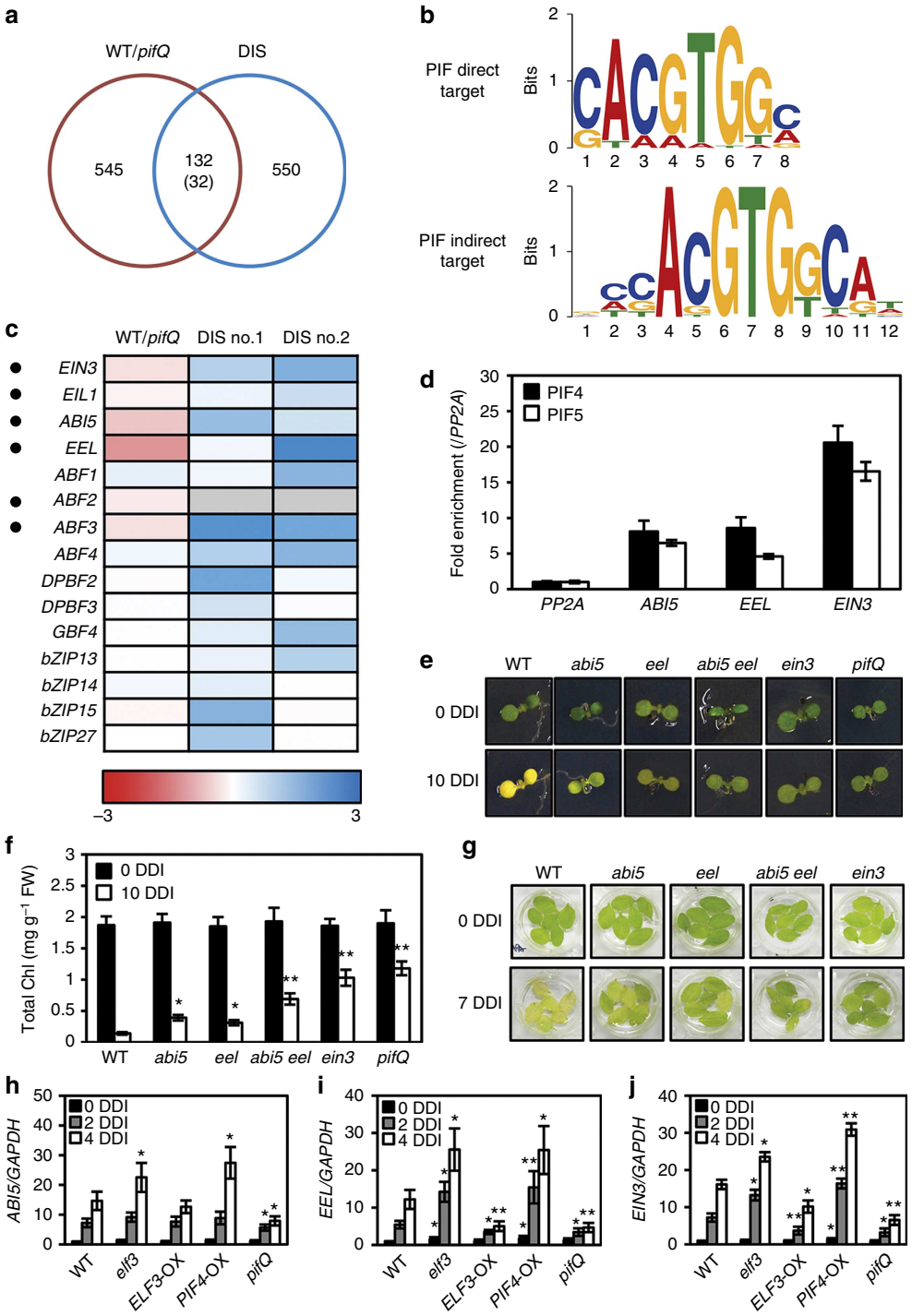

Figure 4 | PIF4/PIF5 directly induce bZIPs and *EIN3*. (**a**) Significant overlap between DEGs (twofold, FDR < 0.05) of PIF-induced genes (WT/*pifQ*, this study) and DIS genes (previous microarray data[39]). Light-grown 7-day-old WT and *pifQ* seedlings were transferred to darkness for 2 days (WT versus *pifQ*), and 4-week-old adult WT plants were transferred to darkness for 0 and 2 days (2 DDI versus 0 DDI). The number in parentheses indicates the PIF4/PIF5-direct target genes retrieved from the previous ChIP-seq database[34,40]. (**b**) Enrichment of the G-box motifs in the PIF-direct target promoters and the ABRE-like element in the PIF-indirect target promoters, identified by the MEME software using 500 bp promoter sequences. (**c**) Heat map showing the expression of *EIN3*, *EIL1* and group A bZIPs in the microarray analysis from this study (WT/*pifQ*) and two independent sets of DIS microarray data from previous studies[39,69]. Black circles indicate the PIF4/PIF5-direct target genes according to previous ChIP-seq analyses[34,40]. The scale bar (bottom) indicates fold changes as $\log_2$ values. The *ABF2* probe is absent in the DIS microarray (Affymetrix). (**d**) Direct binding of PIF4/PIF5 to the promoters of *ABI5*, *EEL* and *EIN3* by ChIP assays. Values were normalized to the values for *PP2A* as a non-binding control. Data are means ± s.d. of two biological replicates. (**e**–**g**) Delayed senescence of *abi5*, *eel*, *abi5 eel*, *ein3* and *pifQ* mutants. Light-grown 7-day-old seedlings were transferred to darkness for 10 days (**e**). Total Chl levels were measured at 10 DDI (**f**). Data are means ± s.d. of at least four biological replicates. Detached leaves of 3-week-old plants were transferred to darkness and photographed at 7 DDI (**g**). (**h**–**j**) Regulation of *ABI5*, *EEL* and *EIN3* expression by ELF3 and PIF4/5. The mRNA levels were determined by quantitative real-time PCR using light-grown 7-day-old seedlings transferred to darkness for 0, 2 and 4 DDI. *GAPDH* was used as a control. DDI indicates day(s) of dark incubation. Data are means ± s.d. of four biological replicates. *$P < 0.05$, **$P < 0.01$ (Student's *t*-test).

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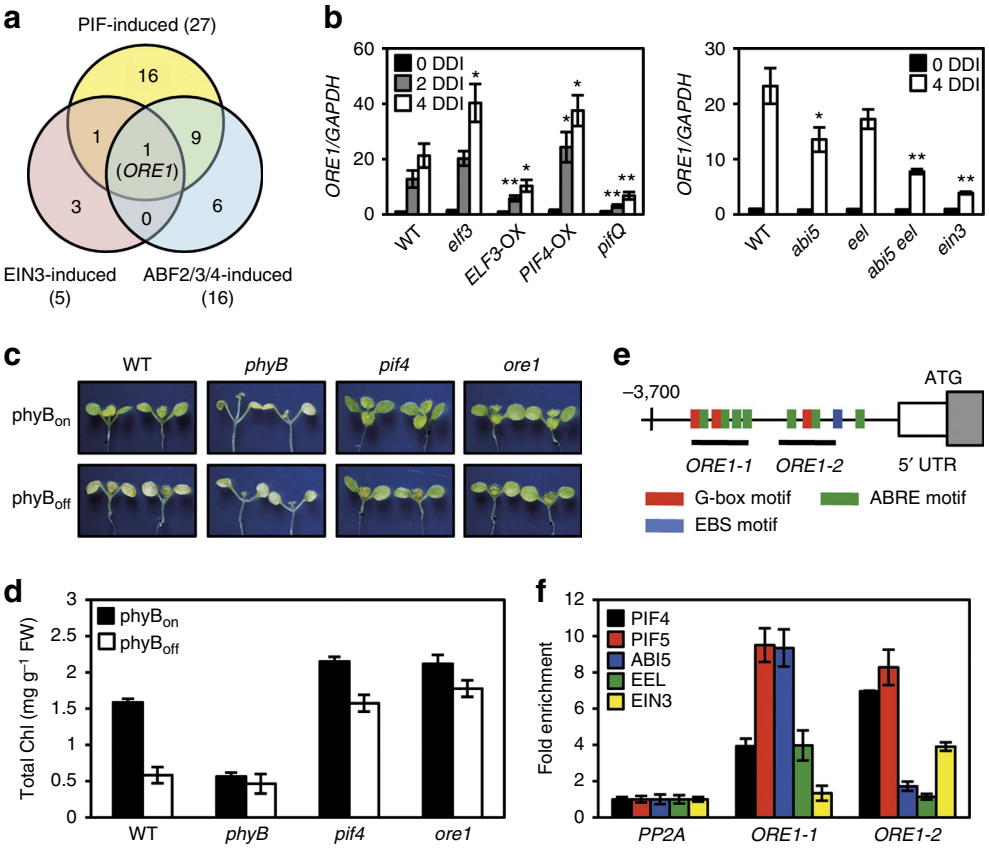

Figure 5 | PIF4/PIF5 induce *ORE1* through multiple feed-forward loops. (**a**) Identification of *ORE1* as a common target of PIFs, bZIPs and EIN3. Among the literature-curated senescence-promoting genes[3], DEGs (FDR < 0.05) were identified from the microarray data for PIF-induced genes (this study), ABF2/3/4-induced genes[67] and EIN3-induced genes (GSE21762). (**b**) Regulation of *ORE1* expression by ELF3, PIF4/PIF5, ABI5, EEL and EIN3. *ORE1* mRNA levels were determined by quantitative real-time PCR at 0 (black bars), 2 (grey bars) and 4 (white bars) DDIs. *GAPDH* was used as a control. Data are means ± s.d. of at least four independent biological replicates. (**c**,**d**) Senescence induction in $phyB_{off}$ conditions requires *ORE1*. Light-grown 7-day-old seedlings were transferred to darkness for 16 days with intermittent red light pulses ($phyB_{on}$) or red/far-red light pulses ($phyB_{off}$) as shown in Fig. 1a and total Chl levels (**d**) were measured to determine the degree of senescence. Data are means ± s.d. of four biological replicates. (**e**) A diagram of the *ORE1* promoter showing the positions of G-box elements (red boxes), ABRE-like elements (green boxes), EIN3 binding sites (EBS; blue box) and two ChIP amplicons (*ORE1-1* and *ORE1-2*; black horizontal lines). (**f**) Direct binding of PIF4, PIF5, ABI5, EEL and EIN3 to the *ORE1* promoter *in vivo*. Enrichment of *ORE1* promoter by PIF4 (black), PIF5 (red), ABI5 (blue), EEL (green) and EIN3 (yellow) were examined by ChIP-qPCR. Values were normalized to the values for *PP2A*, a non-binding control. Data are means ± s.d. of two biological replicates. $^{*}P < 0.05$, $^{**}P < 0.01$ (Student's *t*-test).

ABI5 and EEL directly promote Chl degradation. PIF4, PIF5 and their direct target transcription factors (EIN3 and bZIPs) may have other senescence-related targets in addition to *ORE1*. Microarray data analysis indicated that among the SAGs, PIF4, PIF5 and bZIPs induce four Chl catabolism-associated genes, including *STAYGREEN 1* (*SGR1*, also known as *NYE1*) and *NON-YELLOW COLORING 1* (*NYC1*) (refs 45,46) (Supplementary Table 2). *SGR1* and *NYC1* expression was downregulated in *ELF3*-OX plants and *pifQ* mutants, and upregulated in *elf3* mutants and *PIF4*-OX plants (Fig. 6a). We also found the bZIP binding motif (ABRE motif) in the promoters of *SGR1* and *NYC1* (Fig. 6b). Consistent with the presence of ABREs, ChIP assays revealed that ABI5 and EEL directly bind to the promoters of *SGR1* and *NYC1* (Fig. 6c) and the expression levels of *SGR1* and *NYC1* were significantly downregulated in *abi5 eel* double mutants during DIS (Fig. 6d). Although the promoters of *SGR1* and *NYC1* also contain a putative PIF binding motif (G-box motif; Supplementary Fig. 11a), PIF4/PIF5 did not bind to the G-box motifs of *SGR1* and *NYC1* (Supplementary Fig. 11b). The results indicate that PIF4/PIF5 indirectly promote Chl degradation through their target bZIPs during leaf senescence.

Discussion

Light deprivation initiates leaf senescence in plants. In this study, we reveal the molecular genetic pathway by which light deprivation accelerates senescence, via increased levels of *PIF4/PIF5* mRNAs and PIF4/PIF5 proteins, which are negatively regulated by ELF3 and phyB, respectively (Fig. 7). The increased activity of PIF4/PIF5 promotes the expression of *ORE1* not only by directly binding to its promoter but also indirectly through EIN3 and bZIPs (ABI5 and EEL). These TFs, in turn, activate the expression of hundreds of SAGs that mediate the highly ordered decomposition of cellular components, such as Chls and photosynthetic proteins in chloroplasts, finally leading to leaf senescence[1,3,4].

We used plate-grown young seedlings rather than adult plants for the majority of the senescence assays, because it is easier to synchronize the growth of seedlings and to reduce unknown variation caused by environmental fluctuations. However, as we have shown, *phyB* and *elf3* mutants senesce early, while *pif4*, *pif5* and *pifQ* mutants senesce later, not only in seedlings but also in detached, attached or individually covered leaves of adult plants, suggesting that the senescence pathways identified in seedlings are likely important for senescence at other stages of

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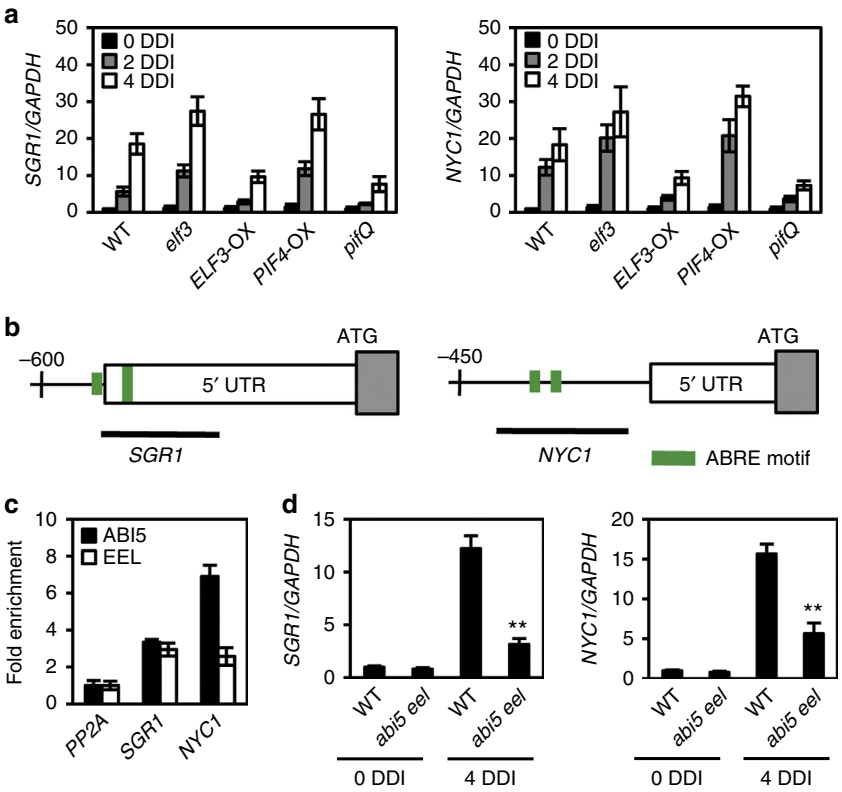

Figure 6 | bZIPs directly induce *SGR1* and *NYC1* for Chl breakdown. (**a**) Gene expression analysis of *SGR1* and *NYC1* in light-grown 7-day-old WT (Col), *elf3* (*elf3-8*), *ELF3*-OX, *pifQ* and *PIF4*-OX plants at 0, 2 and 4 DDI. (**b**) The promoter regions of *SGR1* and *NYC1* showing the position of ABRE motifs (green boxes) and ChIP amplicons (black thick underlines). (**c**) ChIP assays showing direct binding of ABI5 and EEL to the promoters of *SGR1* and *NYC1*. The *PP2A* locus was used as a non-binding control. Data are means ± s.d. of two independent biological replicates. (**d**) Decreased expression of *SGR1* and *NYC1* in *abi5 eel* double mutants. Gene expression was measured by quantitative real-time PCR using light-grown 7-day-old seedlings transferred to darkness for 0 and 4 days. The relative mRNA levels of *SGR1* and *NYC1* were normalized to *GAPDH* expression as a control. Data are means ± s.d. of four independent biological replicates. $^{**}P<0.01$ (Student's *t*-test).

development. Overall, our data show that PIF4/PIF5 promote senescence throughout development, while phyB and ELF3 repress PIF4/PIF5 to prevent precocious senescence in the presence of light. Our study revealed the role of PIF4/PIF5 in senescence in relation to light signalling, and other works showed that PIF4 also mediates high temperature signalling to promote hypocotyl growth[47]. Thus, additional work is required to investigate whether other signals from abiotic stresses, such as high temperature, also regulate senescence through PIF4/PIF5.

The identified pathway links light signalling to senescence and consists of multiple coherent feed-forward loops, which likely function to make the pathway robust. In conditions with sufficient light, ELF3 and phyB convey light information to PIF4/PIF5, resulting in the reduction of PIF4/PIF5 mRNA and protein levels (Figs 2g and 3f). Under prolonged darkness, however, the process reverses and PIF4/PIF5 mRNA and protein levels increase. The increased PIF4/PIF5 activate the expression of *EIN3* and two bZIPs (*ABI5* and *EEL*) by directly associating with their promoters (Fig. 4c,d,h–j), thus affecting regulators of ethylene and ABA signalling, respectively. In turn, the increased EIN3 and bZIPs directly activate the expression of *ORE1* (Fig. 5f), a major senescence-promoting NAC transcription factor that regulates hundreds of SAGs. Notably, not only EIN3 and bZIPs but also PIF4/PIF5 directly bind to the *ORE1* promoter to activate its expression (Fig. 5f), indicating that PIF4/PIF5 form coherent feed-forward loops to activate the expression of *ORE1* (Fig. 7). Such coherent feed-forward loops make a pathway less prone to being disturbed by ephemeral environmental fluctuations[48]. Similar coherent feed-forward loops between EIN2 and *ORE1* were previously proposed[5]; EIN2 activates the expression of *ORE1* both by repressing the expression of *miR164*, which cleaves the *ORE1* mRNA, and by a *miR164*-independent pathway. EIN2 also inhibits the expression of *miR164* through EIN3, which directly binds to the *miR164* promoter[17]. Since we showed that EIN3 directly binds to the *ORE1* promoter to activate its expression (Fig. 5b,f), these results suggest that a previously proposed *miR164*-independent pathway likely functions through *EIN3* and thus *EIN3*, *miR164*, and *ORE1* form a coherent feed-forward loop. Such a loop implies that the cascade of coherent feed-forward loops regulates the expression of *ORE1* during senescence. We note that EIN3 also directly binds to the promoter of *NAP*, a major senescence-promoting NAC transcription factor and one of the 89 senescence-promoting genes[3] (Supplementary Data 1), and induces *NAP* transcription[49], indicating that EIN3 activates the expression of both *ORE1* and *NAP*.

Our study showed that ELF3 represses senescence independently of its previously reported roles in light signalling. A previous study showed that ELF3 interacts with phyB to regulate the phyB signal transduction pathway[35]. Although ELF3 represses senescence as phyB does, the *elf3* mutants still respond to red light pulse treatment similar to WT, indicating that ELF3 regulates senescence independent of phyB activity[35,50]. The fact that ELF3 represses the expression of *PIF4/PIF5* mRNA and phyB promotes the degradation of PIF4/PIF5 proteins further supports the independent roles of ELF3 and phyB in senescence (Figs 2g and 3f). Our study also suggests that ELF3 represses

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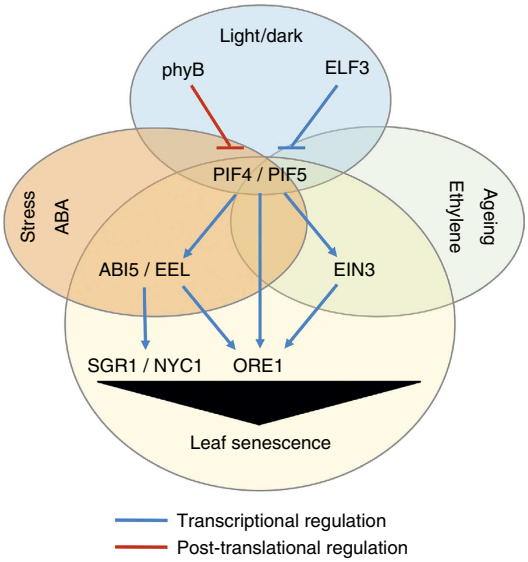

Figure 7 | A proposed model for PIF4/PIF5-induced leaf senescence. PIF4/PIF5 promote DIS by forming multiple coherent feed-forward loops with bZIPs (*ABI5* and *EEL*), *EIN3* and *ORE1*, all of which are inhibited by phyB and ELF3.

senescence independently of its roles in circadian clock, flowering time and diurnal control of hypocotyl elongation. First, ELF3 is required to maintain the circadian clock under continuous light[51] and its mRNA expression is directly repressed by CCA1 for the regulation of the diurnal control of hypocotyl length and flowering time[52]. Besides *elf3* mutants, however, the circadian-clock mutants including *cca1*, *lhy1* and *det1* do not exhibit an altered senescence phenotype in darkness (Supplementary Fig. 8b), indicating that the dysfunction of the circadian clock *per se* does not cause the early senescence symptoms of *elf3* mutants. Second, ELF3 interacts with COP1, which allows COP1 to destabilize GI downstream of CRY2 to control flowering time[36]. However, *cop1*, *gi*, *fkf1*, *co* and *cry2* mutants do not show any senescence-associated phenotype in darkness, suggesting that the flowering-time defect in *elf3* mutants is irrelevant to the early senescence in darkness. Third, ELF3 forms an evening complex with ELF4 and LUX and represses the expression of *PIF4/PIF5* mRNAs for diurnal control of hypocotyl elongation[30]. Unlike the *elf3* mutants, *elf4* and *lux* mutants senesce at the same rate as WT in darkness, indicating that ELF3 acts as the transcription repressor of *PIF4/PIF5* in the absence of ELF4 or LUX. Taken together, our data suggest that the inhibition of senescence represents a novel function of ELF3.

The increase of *PIF4/PIF5* expression independent of phyB (Fig. 2f) raises the possibility that other light signalling components also suppress senescence by repressing the expression of *PIF4/PIF5* in connection with ELF3. A previous study showed that ELF3 is destabilized when light-grown seedlings were transferred to darkness[35]. It is not known, however, which light signalling components regulate ELF3 stability. A few possibilities exist. First, the inactivation of other phytochromes might be responsible for the destabilization of ELF3, which leads to the upregulation of *PIF4/PIF5* expression in the dark, but the role of phyA in senescence remains inconclusive. We showed that the *Arabidopsis phyA* mutants do not show any senescence phenotype in our conditions (Supplementary Fig. 2). Unlike our analysis, however, other studies have reported two contradictory roles of phyA in senescence: the overexpression of oat phyA delayed senescence in tobacco[53], whereas the pea *phyA* mutants exhibited delayed senescence[54]. These contrasting results could be due to the different senescence conditions (dark-induced versus natural senescence) or different plant species (*Arabidopsis* versus tobacco versus pea). Alternatively, phyA proteins from different plant species might have different functions. For example, *Arabidopsis* phyA does not mediate the red light-low fluence response (LFR)[55], whereas monocot phyA mediates the red light-LFR even when it is expressed in *Arabidopsis*[56]. Thus, the suppression of senescence by oat phyA could be due to its unique ability to mediate the LFR, a function usually reserved for phyB[57]. The delayed, rather than early, senescence of pea *phyA* mutants also excludes pea phyA as the repressor of *PIF4/PIF5* mRNA expression in the dark. Currently, the role of three minor phytochromes (phyC, phyD and phyE) in senescence remains to be examined. Second, the activation of the expression of *PIF4/PIF5* during senescence might require the absence of blue light, rather than presence of red light. Previous studies showed that blue light pulses inhibit DIS in papaya[58]. In soybean, CRY2a interacts with and inhibits a CRY-interacting CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 1 (CIB1) transcription factor that promotes leaf senescence by directly upregulating the expression of *WRKY53* (ref. 59). Since low red to far-red ratio also promotes leaf senescence in soybean[60], both phytochrome and cryptochrome signals can delay leaf senescence in soybean. However, we found that cryptochrome signalling does not regulate senescence in *Arabidopsis* (Supplementary Fig. 8c). Since blue light is perceived not only by CRY but also by phototropins and Light–Oxygen–Voltage-sensing-domain containing F-box proteins[23], our data do not exclude the possibility that other blue light receptors repress senescence through ELF3. Further investigation is needed to determine which light signals regulate the expression of *PIF4/PIF5* mRNA through ELF3 during senescence.

The inclusion of ABI5 and EIN3 in the PIF4/PIF5-initiated feed-forward loops suggests that the identified regulatory loops integrate signals from light, abiotic stress and ageing, to regulate senescence (Fig. 7). Previous studies showed that ABA signalling increases the activity of ABI5 and its homologues, Group A bZIPs, in response to abiotic stresses, both at the transcriptional and post-transcriptional levels[44,61] (AtGenExpress; abiotic stress). Activated ABI5 and its homologues trigger a subset of ABA responses, including senescence (Fig. 4e–g), seedling growth arrest and abiotic stress tolerance[61,62]. Our data indicate that PIF4/PIF5 also activate the expression of *ABI5* and its homologues at the transcriptional level, by directly binding to their promoters (Fig. 4c,d,h–j). Light decreases the activity of PIF4/PIF5; this finding indicates that light and abiotic stresses antagonistically regulate the expression of *ABI5* and its homologues. This finding also indicates that the integration of light and abiotic stress signalling determines the output of the PIF4/PIF5-initiated coherent feed-forward loops. Similarly, the PIF4/PIF5-initiated coherent feed-forward loops likely integrate developmental stage signals through EIN3, as shown by a gradual increase of *EIN3* expression during ageing, and the consequent promotion of age-dependent senescence[16]. In conclusion, identification of EIN3 and ABI5 as constituents of the loops in our model suggests that light signalling (represented by PIF4/PIF5) interacts with abiotic stress signalling (ABI5 and EEL)[62] and developmental age signalling (EIN3)[16] to regulate senescence (Fig. 7). The interplay among these signals provides the mechanism by which plants decide when to senesce in response to ageing and dynamic environmental conditions.

Methods

Plant materials and growth conditions. *Arabidopsis thaliana* plants were grown at 22 to 24 °C under long days (16-h light/8-h dark) in a growth room equipped

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with cool-white fluorescent light (90 to 100 μmol m^{-2} s^{-1}) for general growth and seed harvest. For phenotypic analysis, surface-sterilized seeds were plated on Murashige and Skoog (MS) agar plates (half-strength MS, 0.8% phytoagar and 0.05% MES, pH 5.7) and stratified for 3 days at 4 °C in darkness before being transferred to the growth room. For DIS, 7-day-old seedlings, detached (4th or 5th) rosette leaves of 3-week-old plants with 3 mM MES buffer (pH 5.8) or whole plants were incubated in complete darkness for the indicated periods. For quantitative reverse transcriptase PCR and dark treatment with or without light pulse irradiance, 7-day-old seedlings grown on MS agar plates grown under continuous light (100 μmol m^{-2} s^{-1}) were incubated in darkness with or without 5 min of red light (13 μmol m^{-2} s^{-1}) followed by darkness or 5 min of far-red light (3.2 μmol m^{-2} s^{-1}) for the indicated periods.

Plasmid construction and plant transformation. For *Arabidopsis* transgenic plants expressing genes under the control of the 35S promoter, the full-length *PHYB*, *PIF4*, *PIF5*, *ABI5*, *EEL* and *EIN3* cDNAs were amplified with specific primers (Supplementary Table 4), cloned into the binary vectors, pBI121 (*PIF4* and *PIF5*) and pCAMBIA1300, bearing either GFP (*PHYB*), Myc (*PIF4* and *PIF5*) or FLAG (*ABI5*, *EEL* and *EIN3*) tag, and transformed into WT (Col-0) by *Agrobacterium*-mediated transformation. *35S:FLAG-ABI5* and *35S:FLAG-EEL* are hypersensitive to ABA and *35S:EIN3-FLAG* is hypersensitive to ethylene (Supplementary Fig. 12).

Measurement of Chl pigments. For measurement of total Chl concentration, Chl pigments were extracted with 80% ice-cold acetone from leaf tissues of plants grown under long days. The Chl concentration was determined using an ultraviolet/visible spectrophotometer according to the previous method[63].

Reverse transcription and quantitative real-time PCR. Total RNA from plant tissues was isolated using the Total RNA Extraction Kit (iNtRON Biotechnology, Korea). For RT, first-strand cDNAs were prepared with 5 μg of total RNA using M-MLV reverse transcriptase and oligo$(dT)_{15}$ primer (Promega) and diluted to 100 μl with water. Gene expression levels were determined by qPCR analysis. Twenty microlitres of qPCR mixture contained 2 μl of first-strand cDNAs, 10 μl of 2X QuantiTect SYBR Green I Master (Roche) and 0.25 μM of the forward and reverse primers for each gene. The qPCR analysis was performed using the Light Cycler 2.0 (Roche Diagnostics). Relative expression levels of each gene were normalized to mRNA levels of glyceraldehyde phosphate dehydrogenase (*GADPH*) as a loading control. The gene-specific primers for qPCR are listed in Supplementary Table 4.

Measurement of ion leakage rate. To measure ion leakage rate caused by DIS, approximately 10 whole seedlings or 10 rosette leaves in each condition were placed in a tube with 6 ml of 0.4 M mannitol solution. The tubes were placed at room temperature for 3 h with gentle shaking. Conductivity of incubated solution was measured before and after boiling for 10 min, using an electroconductivity metre (CON6 METRE, LaMOTTE Co., USA). The ion leakage rate was calculated by the ratio of initial to total conductivity.

SDS-PAGE and immunoblot analysis. PIF4-Myc and PIF5-Myc seedlings were collected and immediately frozen in liquid nitrogen under a dim green light. The seedlings were then ground in liquid nitrogen and homogenized in denaturing buffer (100 mM NaH_2PO_4, 10 mM Tris-HCl, 8 M urea, pH 8.0) by vigorous vortexing. The debris was removed by centrifugation at 20,000 *g* for 10 min at 4 °C. For immunoblot analysis, the supernatants were separated on an 8% SDS-polyacrylamide gel, and the proteins were transferred to a nitrocellulose membrane (Hybond ECL, Amersham) using transfer buffer (5.8 g l^{-1} Tris base, 29 g l^{-1} glycine, 20% methanol and 0.01% SDS). For protein detection, rabbit polyclonal anti-Myc antibody (Santa Cruz, USA) with 1/1,000 dilution for PIF4-Myc and PIF5-Myc and mouse monoclonal anti-tubulin antibody (Sigma, USA) with 1/10,000 dilution for a loading control in PBS buffer containing 0.05% Tween 20 were used. Blots were washed three times with the same buffer and then incubated with appropriate secondary antibodies. After washing three times, the horseradish peroxidase activity of secondary antibodies was detected using an ECL detection kit (AbFRONTIER, Korea). Uncropped blot images of Fig. 2g are shown in Supplementary Fig. 13.

Microarray analysis. For microarray analysis, 7-day-old light-grown seedlings of WT and *pifQ* mutants were incubated in the dark for 2 days before RNA extraction. Total RNA was extracted using the Spectrum Plant Total RNA kit (Sigma-Aldrich, USA). Three biological replicates of WT and *pifQ* mutants were grown and sampled for RNA purification. Two μg of total RNA was used for probe synthesis. The Agilent Arabidopsis Genome 44 K chip (version 4) was used. The analysis of microarray data was done in R version 2.15.0. The limma package was used for background correction and for normalization within arrays and between arrays[64]. Then, a linear model was fitted using *lmFit* followed by statistical calculation using *ebayes*. FDR less than 0.05 was applied to identify DEGs. Microarray data were deposited in the NCBI Gene Expression Omnibus (GEO) with accession number GSE52646. For the identification of DEGs of WT DIS and hormonal transcription factor-activated genes, raw data deposited in NCBI GEO and EMBL-EBI were obtained and processed as described above.

ChIP assay. The plants overexpressing *PIF4-Myc*, *PIF5-Myc*, *ABI5-FLAG*, *EEL-FLAG* and *EIN3-FLAG* under the cauliflower mosaic virus *35S* promoter (see Plasmid construction and plant transformation) were grown in continuous white light for 7 days and then transferred to darkness for 3 days before cross-linking for 20 min with 1% formaldehyde under vacuum. Chromatin complexes were isolated and sonicated with slight modification[43]. Anti-Myc monoclonal antibody (mouse, Cell Signaling) or anti-FLAG polyclonal antibody (rabbit, Sigma), and Protein A agarose/salmon sperm DNA (Millipore) were used for immunoprecipitation. After reverse cross-linking and protein digestion, DNA was purified by QIAquick PCR Purification Kit (Qiagen).

GSEA. GSEA[65] was conducted with Software GSEA v2.08 (Broad Institute, MIT) according to the online user guide (http://www.broadinstitute.org/gsea). ABA and ACC-responsive gene sets were generated based on previously processed microarray data[66]. Sets of genes activated by ABI5, ABF2/3/4 and EIN3 were identified from public microarray data from previous studies[67,68] and from NCBI GEO deposition (GSE21762) with twofold criteria (FDR < 0.05) as described in Microarray Analysis. The set of genes activated by ABI5 was further selected for genes whose expression is induced by ABA treatment (AtGenExpress). Parameters were set as gene_set for permutation type, 1,500 for maximal size of gene set and default for other parameters.

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Author contributions

Y.S., J.J., M.-Y.K. and J.K. performed the experiments. N-C.P. and G.C. designed the study and supervised the work. All authors discussed the results and made substantial contributions to the manuscript.

Additional information

Accession codes: Gene information from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases as the following accession numbers: *ABI5*, At1g16540; *CO*, At5g15840; *CRY1*, At4g08920; *CRY2*, At1g04400; *DET1*; At4g10180; *EEL*, At1g30230; *EIN3*, At3g20770; *ELF3*, At2g25930; *ELF4*, At2g40080; *GAPDH*, At3g26650; *Lhcb1*, At1g29910; *LUX*, At3g46640; *NYC1*, At4g13250; *ORE1*,

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At5g39610; *PHYA*, At1g09570; *PHYB*, At2g18790; *PIF1*, At2g20180; *PIF3*, At1g09530; *PIF4*, At2g43010; *PIF5*, At3g59060; *PIL1*, At2g46970; *PP2A*, At1g13320; *RGA*, At2g01570; *SAG12*, At5g45890; *SEN4*, At4g30270; *SGR1*, At4g22920. Microarray data were deposited in the NCBI Gene Expression Omnibus (GEO) with accession number GSE52646.

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