

**a** Light spectra and fluence rate of high R/FR ratio and low R/FR ratio light conditions obtained with OceanOptics USB2000+ spectrometer in CLF plant growth incubator model AR-41L equipped with fluorescent light bulbs and supplemental far-red light-emitting diodes (LED). **b** Light intensities ( $\mu$ moles m<sup>-2</sup> s<sup>-1</sup>) of photosynthetically active radiation (PAR, between 400-700 nm), blue (400-500 nm), red (640-700 nm), far-red (700-760 nm) are listed on the table.



PIFs mediate flowering in low R/FR. Flowering time represented as number of days to flowering after sowing of plants growing under LD at 22°C in high and low R/FR **a** and the ratio of days to flowering in high *vs* low R/FR **b**. Plants were grown for 5 days high R/FR for complete de-etiolation and either kept in high R/FR or shifted to low R/FR on day 6 until the onset of flowering. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR<sup>-1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers. n represents the number of plants phenotyped.



Flowering time phenotype represented as total leaf number **a** and days to flowering **b** after bolting. Plants were grown under LD at 22°C in high R/FR for 5 days and either kept in the same condition or shifted to low R/FR on day 6 until the onset of flowering. **c** Ratio of total leaf number and days to flowering in high *vs* low R/FR represented in **a** and **b**. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR <sup>1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers.



**a** Days to flowering after sowing of Col-0, *phyB*, *phyB pif7*, *phyB pif4 pif5* and *phyB pif4 pif5* pif7 growing under SD at 22°C in high R/FR. Total leaf number **b** and days to flowering after sowing **c** of Col-0, *phyB*, *phyB pif4*, *phyB pif4 pif5* and *phyB pif4 pif5* pif7 mutants growing under LD at 22°C in high R/FR. **d** 25-day-old representative plants grown under LD. Scale bar correspond to 1 cm. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR <sup>1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers.



PIF4, PIF5 and PIF7 mediate flowering transition downstream of phyB in high and low R/FR. The flowering time phenotype is represented as total leaf number **a** and days to flowering **b** after bolting. Plants were grown under LD at 22°C in high R/FR for 5 days and either kept in the same condition or shifted to low R/FR until the onset of flowering. **c** Ratio of total leaf number and days to flowering in high *vs* low R/FR represented in **a** and **b**. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR<sup>1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers.



**a** Representative plants of *ft* single and *ft tsf* double mutant genotypes grown under LD at 22°C in high R/FR and low R/FR. Plants were grown under LD at 22°C in high R/FR for 5 days and either kept in the same condition or shifted to low R/FR until the onset of flowering. Flowering time phenotype was represented as total leaf number **b** and days to flowering after sowing **c**. **d** Ratio of total leaf number and days to flowering in high *vs* low R/FR represented in **b** and **c**. **e** Flowering time phenotype of Col-0, *phyB*, *tsf*, *phyB tsf*, *ft*, *phyB ft*, *ft tsf* and *phyB ft tsf* under LD at 22°C in high R/FR was represented as total leaf number after bolting. FR, supplemental far-red light. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR <sup>1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers. Scale bar correspond to 1 cm.



Short-term *FT* up-regulation in low R/FR. *FT* **a** and *TSF* **b** RNA-seq data of relative expression in hypocotyl and cotyledon samples before (0) and 15, 45, 90 and 180 minutes after shift from high to low R/FR. RNA-seq expression data was obtained from <sup>2</sup>. **c** *FT* transcript level using quantitative real-time PCR (RT-qPCR) of Col-0 and *pif4 pif5 pif7* plants growing under LD at 22°C. Plants were grown for 5 days in high R/FR and on day 6 either kept in standard high R/FR or shifted to low R/FR at ZT2. Samples were harvested 180 minutes after shift to low R/FR (ZT 5). FR, supplemental far-red light. Error bars represent the standard deviation of three biological and three technical replicates.



**a** Representative 50-day-old *ft*, *ft tsf* and *ft pif4 pif5 pif7* plants grown under LD at 22°C in low R/FR. Plants were grown under LD at 22°C in high R/FR for 5 days and either kept in the same condition or shifted to low R/FR on day 6 until the onset of flowering. Flowering time was represented as total leaf number **b** and days to flowering after sowing **c** in high and low R/FR. FR, supplemental far-red light. **d** Ratio of total leaf number and days to flowering after sowing in high *vs* low R/FR. **e** *TSF* mRNA levels in *ft* and *ft pif4 pif5 pif7* 19 days after sowing. Plants were grown under LD at 22°C in high R/FR for 5 days and either kept in the same condition or shifted to low R/FR on day 6. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR <sup>1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers. Scale bar correspond to 1 cm.



*FT* **a** and *TSF* **b** mRNA quantification using quantitative real-time PCR (RT-qPCR) of Col-0, *phyB*, *phyB pif7*, *phyB pif4 pif5* and *phyB pif4 pif5 pif7* plants growing under LD at 22°C. Plants were grown high R/FR at 22°C and samples were harvested 10 days after sowing at ZT 15-16. Error bars represent the standard deviation of three biological and three technical replicates.



*CO* mRNA level using quantitative real-time PCR (RT-qPCR) after shift from high to low R/FR in Col-0 and *pif4 pif5 pif7*. Plants were grown on soil for 5 days under LD at 22°C in high R/FR and samples were harvested at ZT 15-16 before (0) and 1, 2, 3, 5, and 7 days after shift to low R/FR. Error bars represent 2x SEM.



**a** Representative plants of *co* single and *co pif4 pif5 pif7* quadruple mutant were grown under LD at 22°C in high R/FR and low R/FR. Plants were grown under LD at 22°C in high R/FR for 5 days and either kept in the same condition or shifted to low R/FR on day 6 until the onset of flowering. Flowering time phenotype was represented as total leaf number **b** and days to flowering after bolting **c**. **d** Ratio of total leaf number and days to flowering of plants growing under high *vs* low R/FR represented in **b** and **c**. FR, supplemental far-red light. Letters represent the significance groups at p-value < 0.01 using ANOVA followed by Tukey honestly significant difference (HSD) test. n represent the number of plants phenotyped. Boxplots were created using the online BoxPlotR<sup>-1</sup>; center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range (IQR); dots, outliers. Scale bar correspond to 1 cm.



*pPIF7::PIF7-3HA-tPIF7* (*gPIF7*, labelled #27 on the figure) recues the *pif7-2* short hypocotyl phenotype **a** and the expression of marker genes *IAA29* and *YUC8* **b** in low R/FR. Transgene *gPIF7* expression was confirmed both using quantitative real time PCR (RT-qPCR) with both gene- and transgene-specific oligonucleotides **c** and western blot probed against  $\alpha$ -HA **d**. Error bars represent standard deviation of three biological replicates. FR, supplemental far-red light.



PIF4, PIF5 and PIF7 protein accumulation in high and low R/FR. Western-blot of *pif4-101/pPIF4::PIF4-3HA* **a**, *pif5-3/pPIF5::PIF5-3HA* **b** and *pif7-2/pPIF7::PIF7-3HA* **c**. Plants were grown on soil for 5 days in high R/FR and either kept in control high R/FR or shifted to low R/FR in day 6. Samples were harvested every 3 hours in high and low R/FR of 10-day-old plants for 24 hours. Western-blots were performed using anti-DET3 and anti-HA antibodies.



Short-term PIF7-HA protein accumulation by western-blot in 6-day-old *pif7-2/pPIF7::PIF7-3HA* plants in high and low R/FR **a**, and quantitative analysis over DET3 protein levels **b**. Plants were grown for 5 days in high R/FR and in day 6 either kept in control high R/FR or shifted to low R/FR at ZT2. Samples were harvested before (0) and 15, 45, 90 and 180 minutes after shift to low R/FR. White (high R/FR) and grey (low R/FR) bars correspond to the average protein levels of 3 biological replicates and at least 3 technical replicates relative to DET3. Red dashed line represent the PIF protein level ratio of low/high R/FR. Error bars represent standard deviation. FR, supplemental far-red light. Note that we observe 2 isoforms for PIF7 with more of the slower migrating one present after transfer to low R/FR.



WT ...(237) ttggtgCACGTGtacatacacctcttgggacttgatcattcgCATGTGacagta (340)..

Representation of PIF4 ChIP-seq reads mapped to the FT locus (At1g65480)<sup>3</sup>. Grey box represents high confidence PIF4 binding peak at the FT terminator and nucleotide sequence represent the WT DNA sequence. G-box (CACGTG) and PBE-box (CATGTG) are represented in uppercase.



Full western-blots shown in supplementary Figure 13a-c. **a.** PIF4-HA and DET3 loading control. **b.** PIF5-HA and DET3 loading control. **c.** PIF7-HA and DET3 loading control.



**Supplementary Figure 17** Full western-blot shown in supplementary Figure 14a.

Supplementary Table 1 – List of oligonucleotides used in this study.	
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Oligos used for cloning			
Construct	Backbone/Description	Oligonucleotide	Sequence (5'>3')
pASF-02	pBGW/pPIF7::PIF7-3HA:tPIF7		AGCATGCGACGTCGGGCCCTAATCAAAATTACTT
		oASF-25	ATATCAATGAAACTATATTTG
			CTGGAACGTCGTATGGGTAGTCATCTCTTTTCTC
		oVCG-165	ATGATTCGAAG
		NCC 1((	CTTCGAATCATGAGAAAAAGAGATGACTACCCAT
		0VCG-166	
		oVCG-167	
		oVCG-168	GTCCCGGACTATGCAGGATAGtctagcgacctagtattattg
			CGAGGGTACCCGGGGGATCCTGTGCATATGATCT
		oASF-26	ATTAACATTGAAG
pVG-24	pMAL-c2 TEV/MBP-PIF7_bHLH	oVCG-226 (SacI)	CGAGCTCGAATGGACGACGGGGACGAGCAG
		oVCG-192 (XbaI)	CCTCTAGACTTCATTGTTGGTGTTGGATGCTG
pVG-55	pGREENII-0800/pTSF::fLUC		CCCCCCTCGAGGTCGACGGGTTTTTTTCTCGGTC
		oVCG-440 (XhoI)	ATAAACG
			GGTGGCGGCCGCTCTAATTTATCTTGGATCTCAA
		oVCG-441 (Notl)	GTATCIC
pVG-84	pGREENII-0800/pTSF Ixmut::fLUC		
pVG-87	pGREENII-0800/pTSF 3xmut::fLUC	oVCG-374	CAATGCCCGGATTTATCTCTCTGT
		oVCG-559	GACCAGTTTCATTTGCTGACCAC
		oVCG-378	GTGGTCAGCAAATGAAACTG
		oASF-73	TTCTTATTCCAAAACCCACCAG
		oVCG-560	CTGGTGGGTTTTGGAATAAG
		oVCG-381	ATTTATCTTGGATCTCAAGTATCTC
			CCCCCCTCGAGGTCGACGGGTTTTTTTCTCGGTC
		oVCG-440 (XhoI)	ATAAACG
			GGTGGCGGCCGCTCTAATTTATCTTGGATCTCAA
		oVCG-441 (NotI)	GTATCTC
pAM-01	pCB308/pPIF4::GUS	SL-128 (BamHI)	ATCGGATCCGCCGCCAATCTGCCGACAAG
		SL-129 (BamHI)	ATCGGATCCGCTAGCGTCAGATCTCTGGAGACAT
pAM-03	pCB308/pPIF5::GUS	SL-132 (BamHI)	ATCGGATCCAAATACAACGACATCATTTG
			ATCGGATCCGCTAGCGTCAGATCTGTAAAGACA
		SL-133 (BamHI)	СТ
рМК-09	pCB308/pPIF7::GUS	MK-207 (XbaI)	CCGTCTAGAAAAGAGTCGAACAGGGAAAGTTC
		MK-208 (BamHI)	CCGGGATCCGTGTTACTTAGGCCGCACG

Oligos used for genotyping			
Allele	Collection	Oligonucleotide	Sequence
phyB-9	Point mutation	PB9	GTGTCTGCGTTCTCAAAACG
		B9dCAPS	GTGGAAGAAGCTCGACCAGGCTTTG
ft-10	GK-290E08	oVCG-53	CCCATTTGGACGTGAATGTAGACAC
		oVCG-417	CGAGCAACATGTTTGAGCTAT
<i>co-101</i>	SAIL_24_H04	oVCG-61	TAGCATCTGAATTTCATAACCAATCTCGATACAC
		oVCG-143	AGCTCCCACACCATCAAACTTACTACATC
tsf-1	SALK_087522	oVCG-55	ACGTGGACTCTCGTAGCACAC
		oVCG-56	ATTTTGCCGATTTCGGAAC
pif3-1	SALK_030753	CF-230	AGGCATTCCCATACCCATTG
		oVCG-56	ATTTTGCCGATTTCGGAAC
pif4-101	SAIL_114_G06	SL-43	CAGACGGTTGATCATCTG
		oVCG-61	TAGCATCTGAATTTCATAACCAATCTCGATACAC
pif5-3 (pil6-1)	SALK_087012	SL-46	TCGCTCACTCGCTTACTTAC
		oVCG-56	ATTTTGCCGATTTCGGAAC

pif7-1	CS68809	SL-195	95 GTGGCAAGTTGGCTCTTAGG	
		SL-169	TGATAGTGACCTTAGGCGACTTTTGAACGC	
pif7-2	SAIL_622_G02	oASF-27	GGAGAGCCATAGAGTTGG	
		oVCG-61	TAGCATCTGAATTTCATAACCAATCTCGATACAC	

Oligos used for RT-qPCR				
Target	Primer Efficiency	Oligonucleotide	Sequence	
FT	2.00	oVCG-27	CCCTGCTACAACTGGAACAAC	
		oVCG-28	CACCCTGGTGCATACACTG	
TSF	2.00	MT-608	TGGAGATGTTCTTGATCCTTTC	
		MT-609	TAGATCCAAGCCATTAGTAACC	
CO	2.05	MT-596	AACGCCTGCACCGTGTATTG	
		MT-597	ACGCGATTGGCAGAGTGAAC	
PIF4	2.02	oVCG-246	TACCTCGATTTCCGGTTATGGATC	
		oVCG-247	GTTGTTGACTTTGCTGTCCCGC	
PIF5	2.01	oVCG-588	GAGCAGCTCGCTAGGTACATG	
		oVCG-589	GTTGTTGTTGCACGGTCTG	
PIF7	1.99	SL-194	AAAGGAGACGGCGTGATAGG	
		SL-195	GTGGCAAGTTGGCTCTTAGG	
PIL1	1.92	MT-125	TCAGACTCAGGCTACTTCTTTTACTCA	
		MT-126	TCCTCTATATTGCATTGCATCTTCTAA	
IAA29	1.94	MT-157	CTTCCAAGGGAAAGAGGGTGA	
		MT-158	TTCCGCAAAGATCTTCCATGTAAC	
YUC8	2.00	MT-271	GGCGGCTTGTCTCCATGAAC	
		MT-171	ACTGTTGACCTCGACGTTGTTG	
UBC	1.94	UBC-F	CAGTCTGTGTGTAGAGCTATCATAGCAT	
		UBC-R	AGAAGATTCCCTGAGTCGCAGTT	
YSL8	2.00	YSL8-F	TCATTCGTTTCGGCCATGA	
		YSL8-R	CTCAGCAACAGACGCAAGCA	
PIF7 - Pair 1	1.99	SL-194	AAAGGAGACGGCGTGATAGG	
		SL-195	GTGGCAAGTTGGCTCTTAGG	
PIF7 - Pair 2	1.97	oASF-52	GAACCACCCAAAGAAGCGTA	
		oASF-53	CTGATCCATACAGCGCCTTT	
PIF7 - Pair 3	1.94	oASF-63	TAGGTCGCTAGACTAATCTC	
		oASF-53	CTGATCCATACAGCGCCTTT	

## Supplementary Table 2 – DNA sequences.

## pTSF\_3xmut synthesis

## References

- Krzywinski M, Altman N. Visualizing samples with box plots. *Nat. Methods* 11, 119-120 (2014).
- 2. Kohnen MV, *et al.* Neighbor detection induces organ-specific transcriptomes, revealing patterns underlying hypocotyl-specific growth. *Plant Cell* **28**, 2889-2904 (2016).
- 3. Pedmale UV, *et al.* Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. *Cell* **164**, 233-245 (2016).