Supplementary Information - Golonka et al.

Supplementary Figures

Supplementary Figure 1

PIF1	1	MHHFVPDFDTDDDYVNNHNSSLNHLPRKSITTMGEDDDLMELLWQNGQVVVQNQRLHTKKPSSSPPKLL	69
PIF2/PIL1	1	MEAKPLASSSSEPNMISPSSNIKPKLK-DEDYMELVCEN <mark>GQ</mark> ILAKIRRPKNNGSFQKQ-RRQ	61
PIF3	1	MPLFELFRLTKAKLESAQDRNPSPPVDEVVELVWENGQISTQSQSSRSRNIPPPQANSS	59
PIF4	1	MEHQGWSFEENYSLSTNRRSIRPQDELVELLWRDGQVVLQSQTHREQTQTQKQDHHE	57
PIF5	1	MEQVFADWNFEDNFHMSTNKRSIRPEDELVELLWRDGQVVLQSQARREPS-VQVQTHKQ	59
PIF6	1	MMFLPTDYCCRLS-DQEYMELVFENGQILAKGQRSNVSLHNQ-RTK	45
PIF7	1	MSNYGVKELTWENGQLTVHGLGDEVEPTTSNNPIWT	36
PIF8	1	MSQCVPNCHIDDTPAAATTTVRSTTAADIPILDYEVAELTWENGQLGLHGLGPPRVTASSTKYSTG	66
PIF1	70	PSMDPQQQPSSDQNLFIQEDEMTSWLHYPLR	100
PIF2/PIL1	62	SLLDLYETEYSEGFKKNIKILGDTQVVPVSQSKPQQDKET	100
PIF3	60	RAREIGNGSKTTMVDEIPMSVPSLMTGLSQDDDFVPWLNHH	100
PIF4	58	EALRSSTFLEDQETVSWIQYPPDEDPFEPDDFSSHFFSTMDPL	100
PIF5	60	ETLRKPNNIFLDNQETVQKPNYAALDDQETVSWIQYPPDDVI	100
PIF6	46	SIMDLYEAEYNEDFMKSIIHGGGGAITNLGDTOVVPQSHVAAAHETNMLESNKHVD	100
PIF7	37	QSLNGCETLESVVHQAALQQPSKFQLQSPNGPNHNYESKDGSCSRKRGYPQEMDRWFAVQEESH	100
PIF8	67	AGGTLESIVDQATRLPNPKPTDELVPWFHHRSSR	100

Sequence alignment of the N-terminal segments of the *A. thaliana* PIFs 1-8 according to Khanna *et al.*¹ Red color marks the N-terminal methionine; violet and gray color indicate strictly conserved and moderately conserved residues, respectively. Boxes highlight the conserved APB.A (red) and APB.B (blue) segments.



The initial rates of the recovery reaction of the *At*PhyB PCM following red-light exposure were determined in bacterial lysate in the presence of different *At*PIF variants and normalized to the reading obtained for the EYFP negative control. Data indicate mean \pm SEM of n = 3 independent biological replicates. See Fig. 2 for details.



Oligomeric state of the *At*PIF variants. (A) 10 μ M P6.100-EYFP were analyzed by size-exclusion chromatography, where the yellow lines represent the absorption at 513 nm. (B-U) As in (A), but for (B) P3; (C) P6; (D) P3.fus; (E); P6.fus; (F) P3.A; (G) P6.A; (H) P3.As; (I) P6.As; (J) P3.AA; (K) P6.AA; (L) P3.AAfus; (M) P6.AAfus; (N) P3.A19; (O) P6.A19; (P) P3.A14; (Q) P6.A14; (R) P3.A8; (S) P6.A8; (T) P3.B; (U) P6.B.



Light-dependent interactions of the *At*PIF variants with the Pfr state of the *At*PhyB PCM. (A) A mixture of 10 μ M P6.100-EYFP and 50 μ M *At*PhyB PCM was exposed to red light and analyzed by size-exclusion chromatography, where the yellow and red lines represent the absorption at 513 and 650 nm, respectively. (B-V) As in (A), but instead of P6.100-EYFP for (B) P3; (C) P6; (D) P3.fus; (E); P6.fus; (F) P3.A; (G) P6.A; (H) P3.As; (I) P6.As; (J) P3.AA; (K) P6.AA; (L) P3.AAfus; (M) P6.AAfus; (N) P3.A19; (O) P6.A19; (P) P3.A14; (Q) P6.A14; (R) P3.A8; (S) P6.A8; (T) P3.B; (U) P6.B; (V) EYFP. The schematics in the graphs indicate the composition of the *At*PIF variants, with variants deriving from *At*PIF3 and *At*PIF6 shown in red and blue, respectively.



Light-dependent interactions of the *At*PIF variants with the Pr state of the *At*PhyB PCM. (A) A mixture of 10 μ M P6.100-EYFP and 50 μ M *At*PhyB PCM was exposed to far-red light and analyzed by size-exclusion chromatography, where the yellow and red lines represent the absorption at 513 and 650 nm, respectively. (B-V) As in (A), but instead of P6.100-EYFP for (B) P3; (C) P6; (D) P3.fus; (E); P6.fus; (F) P3.A; (G) P6.A; (H) P3.As; (I) P6.As; (J) P3.AA; (K) P6.AA; (L) P3.AAfus; (M) P6.AAfus; (N) P3.A19; (O) P6.A19; (P) P3.A14; (Q) P6.A14; (R) P3.A8; (S) P6.A8; (T) P3.B; (U) P6.B; (V) EYFP. The schematics in the graphs indicate the composition of the *At*PIF variants, with variants deriving from *At*PIF3 and *At*PIF6 shown in red and blue, respectively.



Quantitative analyses of the light-dependent protein:protein interaction between *At*PIF variants and the *At*PhyB PCM. (A) Titration of 20 nM P3-EYFP with increasing concentrations of dark-adapted

(gray) or red-light-exposed *At*PhyB PCM (red), as monitored by anisotropy of the EYFP fluorescence. Data points show averages of three biological replicates. The lines denote fits to single-site binding isotherms. (B-U) As in (A), but instead of P3-EYFP for (B) P6; (C) P3.fus; (D) P6.fus; (E) P3.A; (F) P6.A; (G) P3.As; (H) P6.As; (I) P3.AA; (J) P6.AA; (K) P3.AAfus; (L) P6.AAfus; (M) P3.A19; (N) P6.A19; (O) P3.14; (P) P6.14; (Q) P3.8; (R) P6.8; (S) P3.B; (T) P6.B; (U) EYFP.



Harnessing the *At*PIF variants for the light-dependent regulation of gene expression in mammalian cells. (A) SEAP expression was determined for the diverse *At*PIF6 variants and normalized to the constitutive expression of *Gaussia* luciferase. Black and red bars denote mean normalized SEAP expression \pm SEM for *n* = 4 independent biological replicates under dark conditions or red light, respectively. The numbers above the bars indicate the factor difference between dark and red-light conditions for a given *At*PIF6 variant. (B) As panel (A) but for the *At*PIF3 variants. (C) As panel (A) but for the *At*PIF1 variants.



Light-dependent regulation of gene expression in mammalian cells. The experiment was conducted as described in Fig. 5 but the cells were incubated in darkness for 48 h (black bars) or for 24 h under 20 μ mol m⁻² s⁻¹ 660-nm light, followed by 20 μ mol m⁻² s⁻¹ 740-nm light for another 24 h (brown). (A) SEAP expression was determined for the diverse *At*PIF6 variants and normalized to the constitutive expression of *Gaussia* luciferase. Bars denote mean normalized SEAP expression ± SEM for *n* = 4 independent biological replicates. (B) As panel (A) but for the *At*PIF3 variants. (C) As panel (A) but for the *At*PIF1 variants.

Supplementary Figure 9



Analysis of the purified *At*PIF3/6-EYFP proteins and the *At*PhyB PCM by denaturing polyacrylamide gel electrophoresis.

Supplementary Tables

Supplementary Table 1. Amino-acid sequences of the *At*PIF variants used in this study.

Name	Sequence ^a				
	MHHFVPDFDT	DDDYVNNHNS	SLNHLPRKSI	TTMGEDDDLM	EL <mark>LWQN</mark> GQVV
P1.100	VQNQRLHTKK	PSSSPPKLLP	SMDPQQQPSS	DQNLFIQEDE	MTSWLHYPLR
P3.100	MPLFELFRLT	KAKLESAQDR	NPSPPVDEVV	EL <mark>VWEN</mark> GQIS	TQSQSSRSRN
	IPPPQANSSR	AREIGNGSKT	TMVDEIPMSV	PSLMTGLSQD	DDFVPWLNHH
P6.100	MMFLPTDYCC	RLSDQEYM <mark>EL</mark>	VFENGQILAK	<mark>GQ</mark> RSNVSLHN	QRTKSIMDLY
	EAEYNEDFMK	SIIHGGGGAI	TNLGDTQVVP	QSHVAAAHET	NMLESNKHVD
P1	MDDDLM <mark>EL</mark> LW	QN <mark>GQ</mark> VVVQNQ	RLHTKKPSSS	PPKLLPCMDP	QQQPSSDQNL
	FIQEDEMTSW	LHYPLR			
P3	MVDEVV <mark>EL</mark> VW	EN <mark>GQ</mark> ISTQSQ	SSRSRNIPPP	QANSSRAREI	GNGSKTTMVD
	EIPMSVPSLM	TGLSQDDDFV	PWLNHH		
P6	MDQEYM <mark>EL</mark> VF	EN <mark>GQ</mark> ILAKGQ	RSNVSLHNQR	TKSIMDLYEA	EYNEDFMKSI
	IHGGGGAITN	LGDTQVVPQS	HVAAAHETNM	LESNKHVD	
P3.L1	MVDEVV <mark>EL</mark> VW	EN <mark>GQ</mark> ISTQSQ	SSRSRRAREI	GNGSKTTMVD	EIPMSVPSLM
	TGLSQDDDFV	PWLNHH			
P6.L1	MDQEYM <mark>el</mark> vf	EN <mark>GQ</mark> ILAKGQ	RSNMDLYEAE	YNEDFMKSII	HGGGGAITNL
	GDTQVVPQSH	VAAAHETNML	ESNKHVD		
P3.L2	MVDEVV <mark>el</mark> vw	EN <mark>GQ</mark> ISTQSQ	SSRSRNIPPP	QANSSRAREI	GNGSKTTMTG
	LSQDDDFVPW		D GNU / GT UNIOD		
P6.L2	MDQEYMELVF	EN <mark>GQ</mark> ILAKGQ	RSNVSLHNQR	TKSIMDLYEA	EINEDALINL
	GDTQVVPQSH	VAAAHETNML	ESNKHVD		
P3.LP1		EN <mark>GQ</mark> ISTQSQ	SSRSRKPSSS	PPKLLPCMDP	QQQPSSDMTG
			DONKDOCODD		
P6.LP1		EN <mark>GÖ</mark> T PAVGŐ	RSNRPSSSPP	KTTLCWDLŐŐ	QP55DATINL
	MUDEVUELVW	FNCOISTOSO	SSRSPDSACS	ACSACMTCIS	
P3.LS		TH <mark>QÖ</mark> TDTÖDÖ	55K5KD5AG5	AGSAGIIIGIIS	QUUDE VI MIII
	MDOEYMELVE	EN <mark>GO</mark> TLAKGO	RSNDSAGSAG	SAGATTNIGD	ΤΟΛΛΡΟSHVA
P6.LS	AAHETNMLES	NKHAD TW <mark>GÕ</mark> TTWGÕ	Rondonoono	SHOTITINED	10001001011
D 4 6					
P1.fus	MDDDLM <mark>EL</mark> LW	QN <mark>GQ</mark> VVVQNQ	RLHTKQNLFI	QEDEMTSWLH	YPLR
P3.fus	MVDEVV <mark>el</mark> vw	EN <mark>GQ</mark> ISTQSQ	SSRSRMTGLS	QDDDFVPWLN	НН
	MDOEYM <mark>EL</mark> VF	EN <mark>GO</mark> TLAKGO	RSNATTNIGD	TOVVPOSHVA	AAHETNMLES
P6.fus	NKHVD			-2	
D1 4					
PI.A	MDDDTWETTM	ŨИ <mark>ĊŎ</mark> ĸĸĸŎИŎ	RLHTKKPSSS	PPKLLP	
P3.A	MVDEVV <mark>el</mark> vw	EN <mark>GQ</mark> ISTQSQ	SSRSRNIPPP	QANSSRAREI	GN
P6 A	MDOEYM <mark>EL</mark> VF	EN <mark>GO</mark> TLAKGO	RSNVSLHNOR	TKSIMDLYEA	
P1.B	MSMDPQQQPS	SDQNLFIQED	EMTSWLHYPL	R	
P3.B	MGSKTTMVDE	IPMSVPSLMT	GLSQDDDFVP	WLNHH	
P6.B	MEYNEDFMKS	IIHGGGGAIT	NLGDTQVVPQ	SHVAAAHETN	MLESNKHVD
P1.As	MDDDLM <mark>EL</mark> LW	QN <mark>GQ</mark> VVVQNQ	RLHTK		

P3.As	MVDEVV <mark>EL</mark> VW	EN <mark>GQ</mark> ISTQSQ	SSRSR		
P6.As	MDQEYM <mark>EL</mark> VF	EN <mark>GQ</mark> ILAKGQ	RSN		
P1.Bs	MQNLFIQED E	EMTSWLHYPL H	२		
P3.Bs	MMT GLSQDDI	DFVP WLNHH			
P6.Bs	MAIT NLGDTÇ	QVVPQ SHVAA	AHETN MLESNE	KHVD	
P1.AA	MDDDLM <mark>EL</mark> LW LM <mark>EL</mark> LWQN <mark>GQ</mark>	QN <mark>GQ</mark> VVVQNQ VVVQNQRLHT	RLHTKKPSSS KMTSWLHYPL	PPKLLPCMDP R	QQQPSSDDDD
P3.AA	MVDEVV <mark>EL</mark> VW EIPMSVPSLV	EN <mark>GQ</mark> ISTQSQ DEVV <mark>EL</mark> VWEN	SSRSRNIPPP GQISTQSQSS	QANSSRAREI RSRFVPWLNH	GNGSKTTMVD H
P6.AA	MDQEYM <mark>EL</mark> VF IHGGGGDQEY	EN <mark>GQ</mark> ILAKGQ M <mark>EL</mark> VFEN <mark>GQ</mark> I	RSNVSLHNQR LAKGQRSNTN	TKSIMDLYEA MLESNKHVD	EYNEDFMKSI
P1.AAfus	MDDDLM <mark>EL</mark> LW TSWLHYPLR	QN <mark>GQ</mark> VVVQNQ	RLHTKDDDLM	EL <mark>LWQN</mark> GQVV	VQNQRLHTKM
P3.AAfus	<mark>M</mark> VDEVV <mark>EL</mark> VW VPWLNHH	EN <mark>GQ</mark> ISTQSQ	SSRSRVDEVV	EL <mark>VWEN<mark>GQ</mark>IS</mark>	TQSQSSRSRF
P6.AAfus	MDQEYM <mark>EL</mark> VF SNKHVD	EN <mark>GQ</mark> ILAKGQ	RSNDQEYM <mark>EL</mark>	VFEN <mark>GQ</mark> ILAK	GQRSNTNMLE
P1.BB	<mark>M</mark> QNLFIQEDE R	KPSSSPPKLL	PCMDPQQQPS	SDQNLFIQED	EMTSWLHYPL
P3.BB	<mark>M</mark> MTGLSQDDD DDDFVPWLNH	NIPPPQANSS H	RAREIGNGSK	TTMVDEIPMS	VPSLMTGLSQ
P6.BB	<mark>M</mark> AITNLGDTQ TQVVPQSHVA	VSLHNQRTKS AAHETNMLES	IMDLYEAEYN NKHV <mark>D</mark>	EDFMKSIIHG	GGGAITNLGD
P1.BBfus	MQNLFIQEDE	QNLFIQEDEM	TSWLHYPLR		
P3.BBfus	MMTGLSQDDD	MTGLSQDDDF	VPWLNHH		
P6.BBfus	MAITNLGDTQ	AITNLGDTQV	VPQSHVAAAH	ETNMLESNKH	VD
P1.BA	MQNLFIQEDE QRLHTKMTSW	KPSSSPPKLL LHYPLR	PCMDPQQQPS	SDDDDLM <mark>EL</mark> L	WQN <mark>GQ</mark> VVVQN
P3.BA	MMTGLSQDDD <mark>L</mark> VWEN <mark>GQ</mark> IST	NIPPPQANSS QSQSSRSRFV	RAREIGNGSK PWLNHH	TTMVDEIPMS	VPSLVDEVV <mark>E</mark>
P6.BA	MAITNLGDTQ HGGGG <mark>DQEYM</mark>	VVPQSHVAAA <mark>EL</mark> VFEN <mark>GQ</mark> IL	HEVSLHNQRT AKGQRSNTNM	KSIMDLYEAE LESNKHVD	YNEDFMKSII
P1.BAfus	MQNLFIQEDE	DDDLM <mark>EL</mark> LWQ	N <mark>GQ</mark> VVVQNQR	LHTKMTSWLH	YPLR
P3.BAfus	MMTGLSQDDD	VDEVV <mark>EL</mark> VWE	N <mark>GQ</mark> ISTQSQS	SRSRFVPWLN	НН
P6.BAfus	<mark>M</mark> AITNLGDTQ NKHVD	VVPQSHVAAA	HEDQEYM <mark>EL</mark> V	FEN <mark>GQ</mark> ILAKG	QRSNTNMLES
P1.19	MDDDLM <mark>EL</mark> LW	QN <mark>GQ</mark> VVVQNQ			
P3.19	MVDEVV <mark>EL</mark> VW	EN <mark>GQ</mark> ISTQSQ			
P6.19	MDQEYM <mark>EL</mark> VF	EN <mark>GQ</mark> ILAKGQ			
P1.14	MDDDLM <mark>EL</mark> LW	QN <mark>GQ</mark> V			
P3.14	MVDEVV <mark>EL</mark> VW	EN <mark>GQ</mark> I			

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P6.14	MDQEYM <mark>el</mark> vf en <mark>gq</mark> i
P1.8	M <mark>el</mark> lwQN <mark>GQ</mark>
P3.8	M <mark>el</mark> vwen <mark>gq</mark>
P6.8	M <mark>el</mark> vfen <mark>gq</mark>

^a Red color marks the N-terminal methionine; violet and gray color indicates strictly conserved and moderately conserved residues, respectively.

Supplementary Reference

1. Khanna, R. et al. A Novel Molecular Recognition Motif Necessary for Targeting Photoactivated Phytochrome Signaling to Specific Basic Helix-Loop-Helix Transcription Factors. *Plant Cell* **16**, 3033–3044 (2004).