
Supplementary Information - Golonka *et al.*

Supplementary Figures

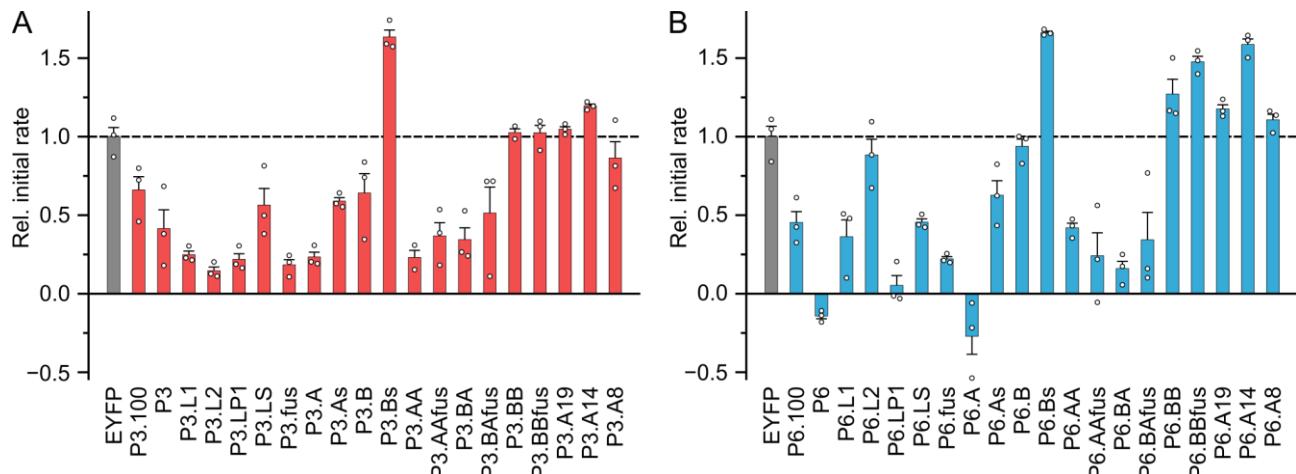
Supplementary Figure 1

PIF1	1	MHHFVPDFDTDDDVNNHNSSLNHLPRKSITTMGE	DDDLMEELLWQN	GQVVVQNQRLHTKKPSSSPKLL	69
PIF2/PIL1	1	-----MEAKPLASSSSEPNMISPSSNIKPPLK-	DEDYMELVCE	GQILAKIRRPKNNGSFQKQ-RRQ	61
PIF3	1	-----MPLFELFRLTAKLESAQDRNPSPPVDEVVELWEN	GQISTQSQSSRSRNIPPPQANSS	59	
PIF4	1	-----MEHQGWSFEENYSLSTNRRSIRPQDELVELLWRDGQVVVLQSQTHREQTQTKQDHHE	57		
PIF5	1	-----MEQVFADWNFEDNFHMSTNKRSIRPEDELVELLWRDGQVVVLQSQARREBS-VQVQTHKQ	59		
PIF6	1	-----MMFLPTDYCCRLS-DQEYMELVFENGQILAKGQR--SNVSLHNQ-RTK	45		
PIF7	1	-----MSNYGVKELTWENGQLTVHGLGDEVEPTTSNNPIWT	36		
PIF8	1	MSQCVPNCHIDDT PAAATTVRSTTAADIPIL--DYEVAELTWENGQLGLHGLGPPRVTASSTKYSTG		66	

PIF1	70	-----PSMDPQQQPSSD	ONLFIQEDEM	MTSWLHYPLR-----	100		
PIF2/PIL1	62	SLLDLYETEYSEGFKKNI-----	KILGDTQVV	PVSQSKPQODKET-----	100		
PIF3	60	RAREIGNGSKTTMVDEIPMSVPSLM	TG	LSQDDD	FVWLNH-----	100	
PIF4	58	EALR--SSTFLEDQETVSWI	QYPPDED	PFE	PDDFSSHFFSTM	DPL-----	100
PIF5	60	ETLRKPNNIFLDNQETVQKPNYAALDDQ--	ETVSWI	QYPPDDVI-----	100		
PIF6	46	SIMDLYEAEYNEDFMKSIIHGGGAITNL	GDTQVV	PQSHVAAA	HETTNMLESNKHVD-----	100	
PIF7	37	QSL---NGCETILESVVHQAA	LQOPSKFQLO	QSPNGPNHYESKDG	CSRKGYPQEMDRWF	AVQESH-----	100
PIF8	67	AGGTLESIVDQATRLPNPKPT-----	DELVPWF	HHRSSR-----		100	

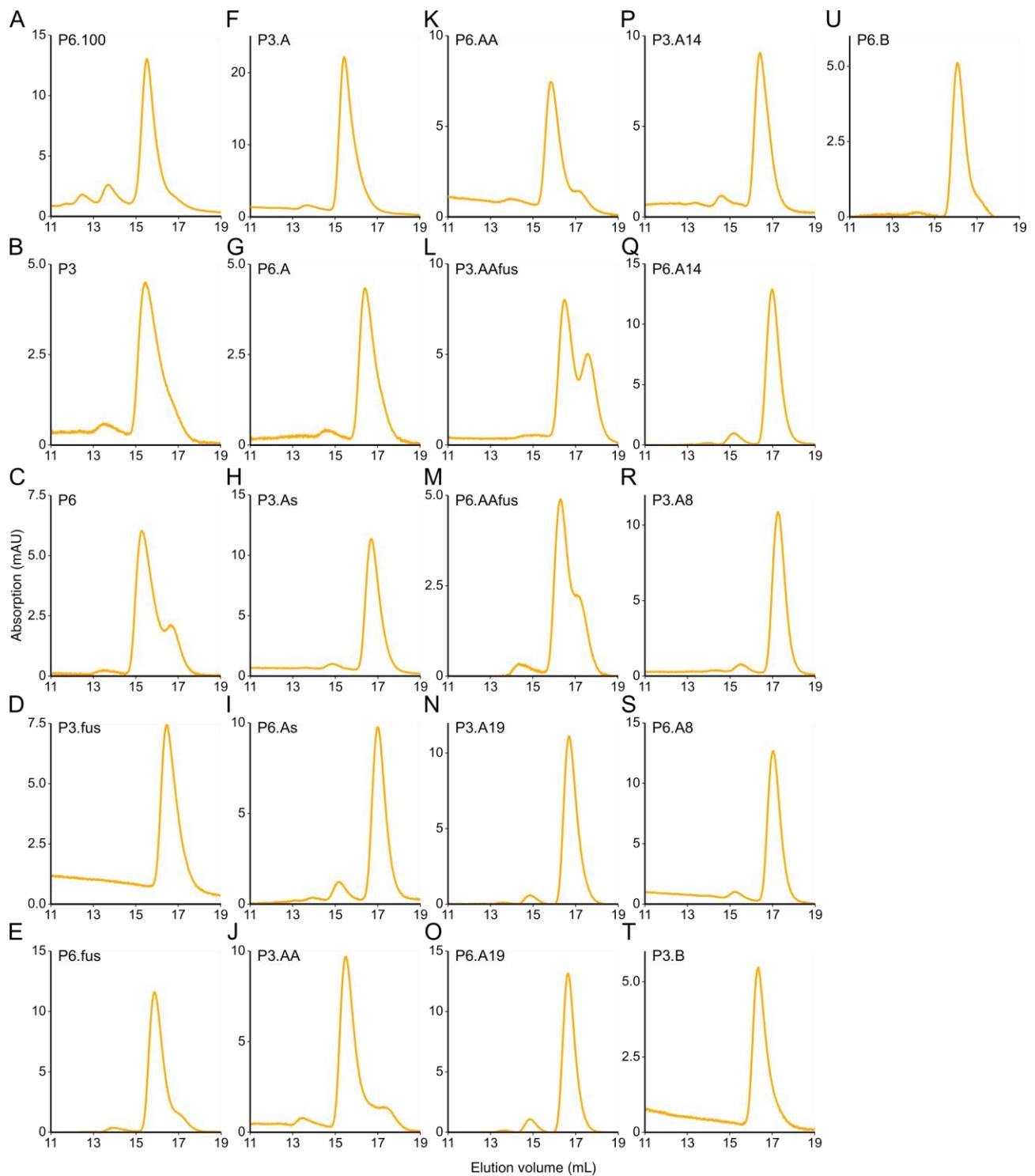
Sequence alignment of the N-terminal segments of the *A. thaliana* PIFs 1-8 according to Khanna *et al.*¹ Red color marks the N-terminal methionine; violet and gray color indicate strictly conserved and moderately conserved residues, respectively. Boxes highlight the conserved APB.A (red) and APB.B (blue)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

Supplementary Figure 2



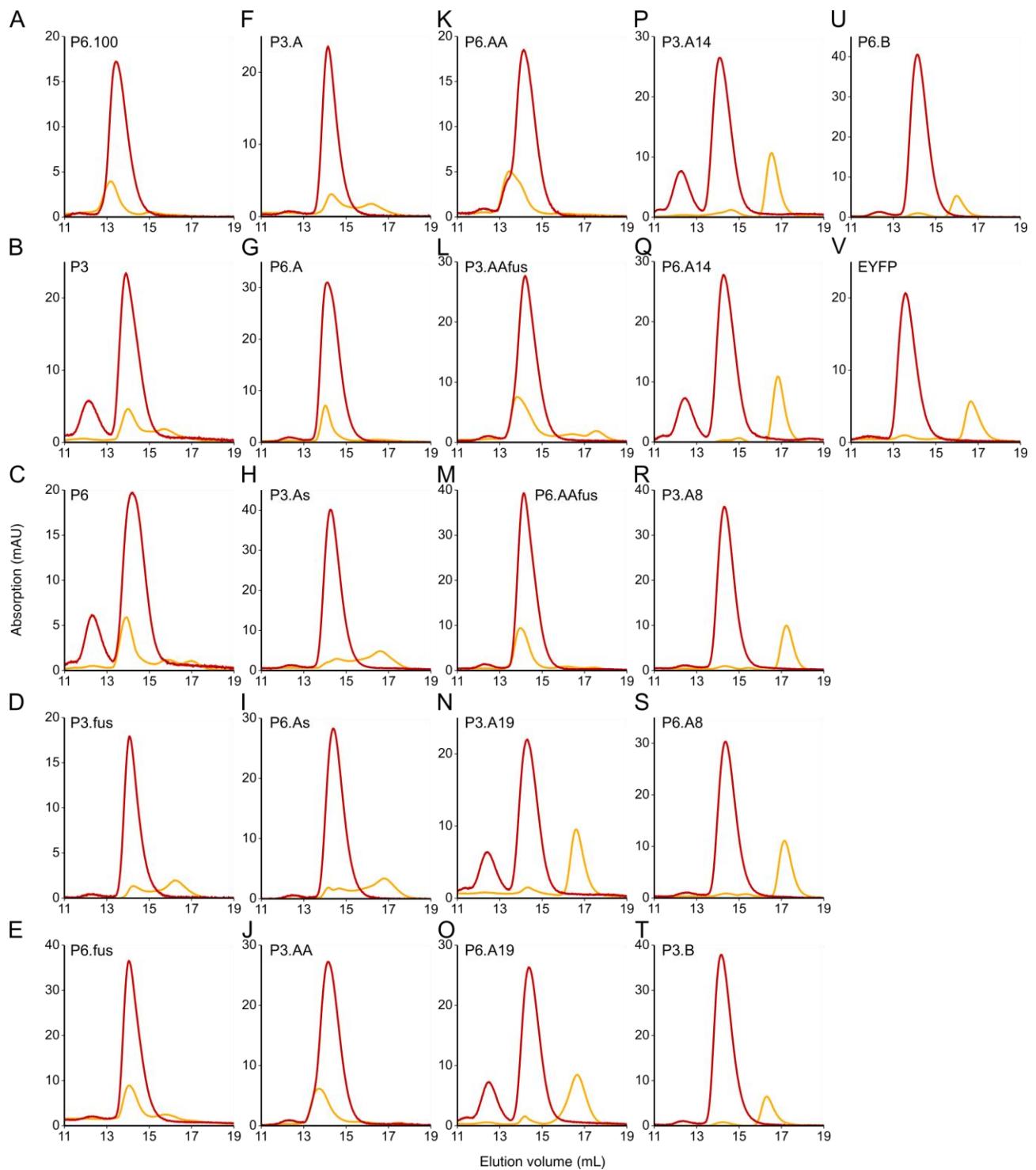
The initial rates of the recovery reaction of the AtPhyB PCM following red-light exposure were determined in bacterial lysate in the presence of different *AtPIF* variants and normalized to the reading obtained for the EYFP negative control. Data indicate mean \pm SEM of $n = 3$ independent biological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

Supplementary Figure 3



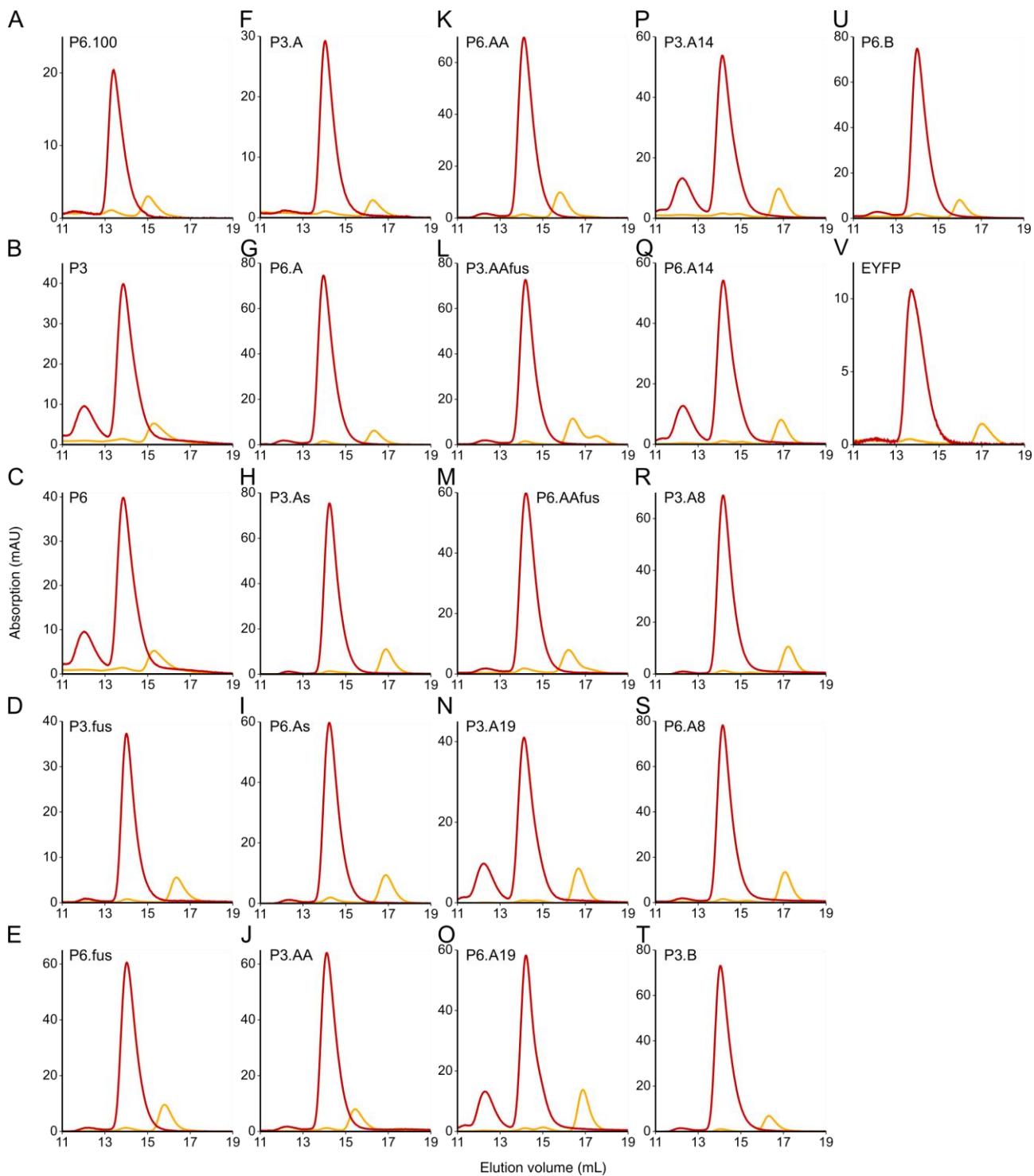
Oligomeric state of the AtPIF variants. (A) 10 μ M P6.100-EYFP were analyzed by size-exclusion chromatography, where the yellow lines represent the absorption at 513 nm. (B-U) As in (A), but for (B) P3; (C) P6; (D) P3.fus; (E) P6.fus; (F) P3.A; (G) P6.A; (H) P3.As; (I) P6.As; (J) P3-AA; (K) P6-AA; (L) 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.

Supplementary Figure 4



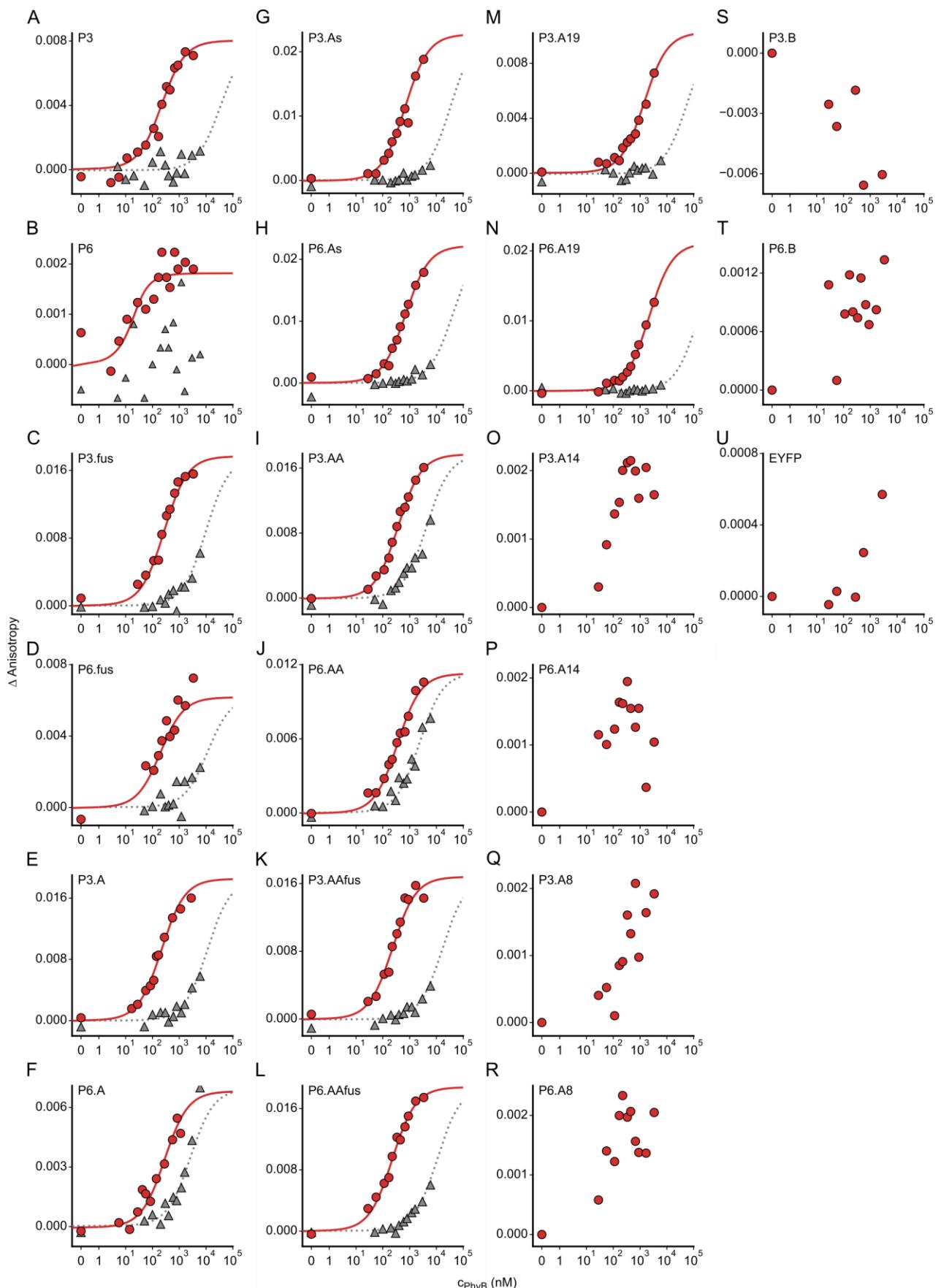
Light-dependent interactions of the AtPIF variants with the Pfr state of the AtPhyB PCM. (A) A mixture of 10 μ M P6.100-EYFP and 50 μ M AtPhyB PCM was exposed to red light and analyzed by size-exclusion chromatography, where the yellow and red lines represent the absorption at 513 and 650 nm, respectively. (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 AtPIF variants, with variants deriving from AtPIF3 and AtPIF6 shown in red and blue, respectively.

Supplementary Figure 5



Light-dependent interactions of the AtPIF variants with the Pr state of the AtPhyB PCM. (A) A mixture of 10 μ M P6.100-EYFP and 50 μ M AtPhyB 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 AtPIF variants, with variants deriving from AtPIF3 and AtPIF6 shown in red and blue, respectively.

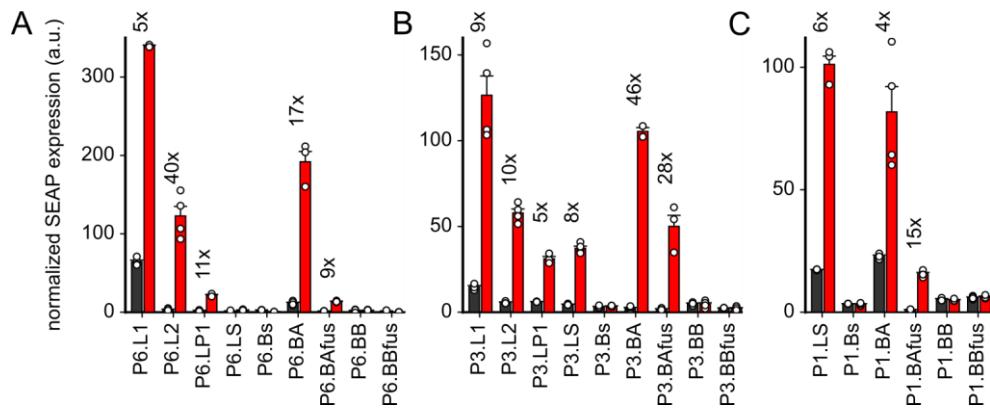
Supplementary Figure 6



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

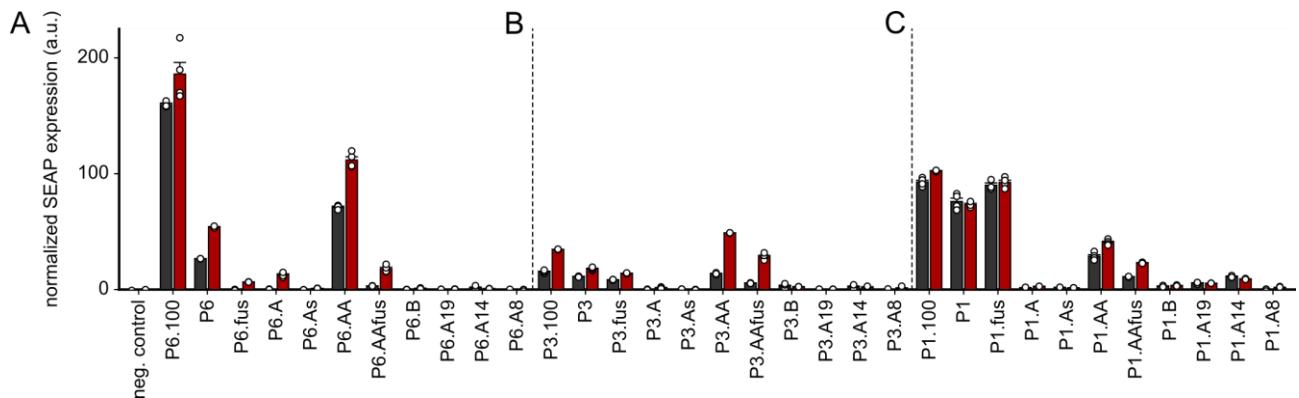
(gray) or red-light-exposed *AtPhyB* PCM (red), as monitored by anisotropy of the EYFP fluorescence. Data points show averages of three biological replicates. The lines denote fits to single-site binding isotherms. (B-U) As in (A), but instead of P3-EYFP for (B) P6; (C) P3.fus; (D) P6.fus; (E) P3.A; (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.

Supplementary Figure 7



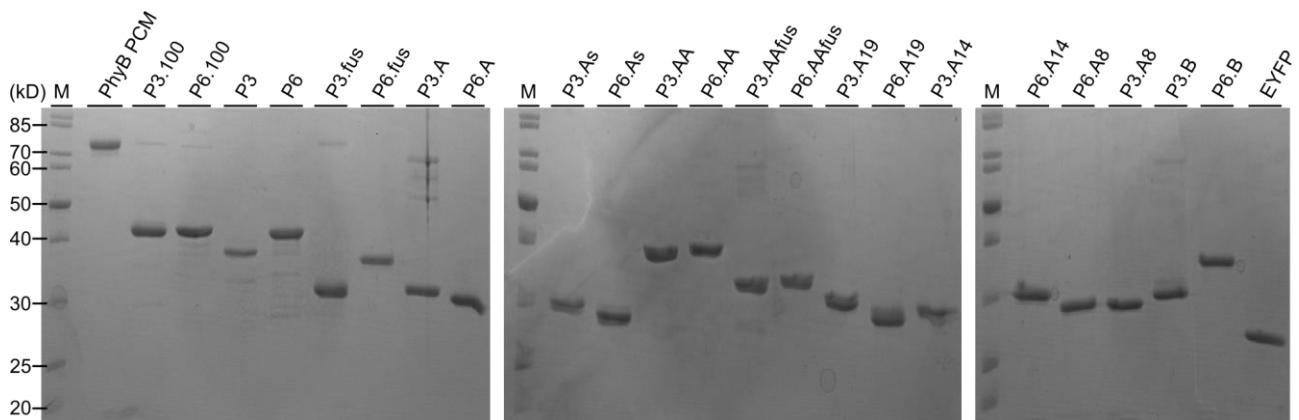
Harnessing the *AtPIF* variants for the light-dependent regulation of gene expression in mammalian cells. (A) SEAP expression was determined for the diverse *AtPIF6* variants and normalized to the constitutive expression of *Gaussia* luciferase. Black and red bars denote mean normalized SEAP expression ± 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 *AtPIF6* variant. (B) As panel (A) but for the *AtPIF3* variants. (C) As panel (A) but for the *AtPIF1* variants.

Supplementary Figure 8



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 $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ 660-nm light, followed by $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ 740-nm light for another 24 h (brown). (A) SEAP expression was determined for the diverse AtPIF6 variants and normalized to the constitutive expression of *Gaussia* luciferase. Bars denote mean normalized SEAP expression \pm SEM for $n = 4$ independent biological replicates. (B) As panel (A) but for the AtPIF3 variants. (C) As panel (A) but for the AtPIF1 variants.

Supplementary Figure 9



Analysis of the purified *AtPIF3/6*-EYFP proteins and the *AtPhyB* PCM by denaturing polyacrylamide gel electrophoresis.

Supplementary Tables

Supplementary Table 1. Amino-acid sequences of the AtPIF variants used in this study.

Name	Sequence ^a
P1.100	MHHFVPDFDT DDDYVNNHNS SLNHLPRKSI TTMGEDDDLM ELLWQNGQVV VQNQLRLHTKK PSSSPPKLLP SMDPQQQPS DQNLFIQED MTSQLHYPLR
P3.100	MPLFELFRLT KAKLESAQDR NPSPPVDEVV ELVWENGQIS TQSQSSRSRN IPPPQANSSR AREIGNGSKT TMVDEIPMSV PSLMTGLSQD DDFVPWLNNH
P6.100	MMFLPTDYCC RLSDQEYMEEL VFENGQILAK GQRSNVSLHN QRTKSIMDLY EAEYNEDFMK SIIHGGGGAI TNLGDQVVP QSHVAAAHEH NMLESNKHVD
P1	MDDDLMELLW QNGQVVVQNO RLHTKKPSSS PPKLLPCMDP QQQPSSDQNL FIQEDEMITSW LHYPLR
P3	MVDEVVELVW ENGQIYSTQSQ SSRSRNIPPP QANSSRAREI GNGSKTTMVD EIPMSVPSLM TGLSQDDDFV PWLNHH
P6	MDQEYMEELVF ENGQIILAKGQ RSNVSLHNQR TKSIMDLYEA EYNEDFMKSI IHGGGGAITN LGDTQVVPQSH HVAAAHETNM LESNKHVD
P3.L1	MVDEVVELVW ENGQIYSTQSQ SSRSRRAREI GNGSKTTMVD EIPMSVPSLM TGLSQDDDFV PWLNHH
P6.L1	MDQEYMEELVF ENGQIILAKGQ RSNMDLYEAE YNEDFMKSI HGGGGAITNL GDTQVVPQSH VAAAHETNML ESNKHVD
P3.L2	MVDEVVELVW ENGQIYSTQSQ SSRSRNIPPP QANSSRAREI GNGSKTTMTG LSQDDDFV PWLNHH
P6.L2	MDQEYMEELVF ENGQIILAKGQ RSNVSLHNQR TKSIMDLYEA EYNEDAITNL GDTQVVPQSH VAAAHETNML ESNKHVD
P3.LP1	MVDEVVELVW ENGQIYSTQSQ SSRSRKPSSS PPKLLPCMDP QQQPSSDMTG LSQDDDFV PWLNHH
P6.LP1	MDQEYMEELVF ENGQIILAKGQ RSNKPSSSSP KLLPCMDPQQ QPSSDAITNL GDTQVVPQSH VAAAHETNML ESNKHVD
P3.LS	MVDEVVELVW ENGQIYSTQSQ SSRSRDSAGS AGSAGMTGLS QDDDFVPWLNNH
P6.LS	MDQEYMEELVF ENGQIILAKGQ RSNDSDAGSAG SAGAITNLGD TQVVPQSHVA AAHETNMLES NKHVD
P1.fus	MDDDLMELLW QNGQVVVQNO RLHTKQNLFI QEDEMITSWLH YPLR
P3.fus	MVDEVVELVW ENGQIYSTQSQ SSRSRMTGLS QDDDFVPWLNNH
P6.fus	MDQEYMEELVF ENGQIILAKGQ RSNAITNLGD TQVVPQSHVA AAHETNMLES NKHVD
P1.A	MDDDLMELLW QNGQVVVQNO RLHTKKPSSS PPKLLP
P3.A	MVDEVVELVW ENGQIYSTQSQ SSRSRNIPPP QANSSRAREI GN
P6.A	MDQEYMEELVF ENGQIILAKGQ RSNVSLHNQR TKSIMDLYEA
P1.B	MSMDPQQQPS SDQNLFIQED EMTSQLHYPL R
P3.B	MGSKTTMVD EIPMSVPSLMT GLSQDDDFV PWLNHH
P6.B	MEYNEDFMKS IIHGGGGAIT NLGDTQVVPQ SHVAAAHETN MLESNKHVD
P1.As	MDDDLMELLW QNGQVVVQNO RLHTK

P3.As	MVDEVV ELVW EN GQI STQS Q SSRSR
P6.As	M DQEY MELVF EN GQI LAKG Q RSN
P1.Bs	M QNLFIQED EMTSWLHYPL R
P3.Bs	M MT GLSQDDDFVP WLNH
P6.Bs	M AIT NLGDTQVV PQ SHVAAAHETN MLESNKHVD
P1.AA	M DDD LMELLW QN GQVVVQ RLHTKKPSS PPKLLPCMDP QQQPSSDDDD LME LLW QNGQ VVVQNQLRLHT KMTSWLHYPL R
P3.AA	M VDEVV ELVW EN GQI STQS Q SSRSRNIPPP QANSSRAREI GNGSKTTMVD EIPMSVPSLV DEVV EI WEN GQI STQS QSS RS R FVPWLNH H
P6.AA	M DQEY MELVF EN GQI LAKG Q RSNVSLHNQR TKSIMDLYEA EYNEDFMKSI IHGGGGDQEY MELVFENGQI LAKGQRSNTN MLESNKHVD
P1.AAfus	M DDD LMELLW QN GQVVVQ RLHTKDDDL M ELLW QNGQVV VQNQRLH TKM TSWLHYPLR
P3.AAfus	M VDEVV ELVW EN GQI STQS Q SSRSRVDEVV E LVWEN GQIS TQSQSSRSRF VPWLNH
P6.AAfus	M DQEY MELVF EN GQI LAKG Q RSND QEYME VF E NG QI LAK G Q RSNTNM LE SNKHVD
P1.BB	M QNLFIQED E KPSSSPPKLL PCMDPQQQPS SD QNLFIQED EMTSWLHYPL R
P3.BB	M MTGLSQDDD NIPPPQANS S RAREIGNGSK TTMVDEIPMS VPSLMTGLSQ DDDFVPWLNH H
P6.BB	M AITNLGDT Q VSLHNQRTKS IMDLYAEAYN EDFMKSIIHG GGGAITNLGD TQVVPQSHVA AAHETNM LE S NKHVD
P1.BBfus	M QNLFIQED E QNLFIQED EM TSWLHYPLR
P3.BBfus	M MTGLSQDDD MTGLSQDDDF VPWLNH
P6.BBfus	M AITNLGDT Q AITNLGDT QV VPQSHVAAA ETNM LESNKH VD
P1.BA	M QNLFIQED E KPSSSPPKLL PCMDPQQQPS SDDDD LMELL W QNGQVVVQ QRLHTKMTSW LH YPL R
P3.BA	M MTGLSQDDD NIPPPQANS S RAREIGNGSK TTMVDEIPMS VPSLVDEVV E I LVWEN GQIST QSQSSRSRFV PWLNH
P6.BA	M AITNLGDT Q VVPQSHVAAA HEVSLHNQRT KSIMDLYEAE YNEDFMKSI HGGGGDQEY M ELVFENG QIL AKGQRSNTNM LESNKHVD
P1.BAfus	M QNLFIQED E DDD LMELLW Q NGQVVVQ NQR LHTKMTSWLH YPLR
P3.BAfus	M MTGLSQDDD VDEVV ELVWE N NGQI STQS Q S SRSRFVPWLHNHH
P6.BAfus	M AITNLGDT Q VVPQSHVAAA HED QEYME I LV F E NG QI LAKG Q RSNTNM LE NKHVD
P1.19	M DDD LMELLW QN GQVVVQ Q
P3.19	M VDEVV ELVW EN GQI STQS Q
P6.19	M DQEY MELVF EN GQI LAKG Q
P1.14	M DDD LMELLW QN GQV
P3.14	M VDEVV ELVW EN GQI

P6.14	M DQEY M ELVF EN GQI
P1.8	M EIL LWQN GQ
P3.8	M ELV WEN GQ
P6.8	M EIL VFEN GQ

^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).