

Supplementary Material

Light-controlled affinity purification of protein complexes exemplified by the resting ZAP70 interactome

Maximilian Hörner, Julian Eble, O. Sascha Yousefi, Jennifer Schwarz, Bettina Warscheid, Wilfried Weber, Wolfgang W. A. Schamel



Supplementary Figure 1 | Characterization of PhyB* biotinylation and of the PhyB*-binding capacity of the NeutrAvidin agarose beads. Different amounts of PhyB* were incubated with 10 μ l of NeutrAvidin beads for 1 h. Afterwards, the beads were pelleted by centrifugation and the fluorescence of unbound PhyB* in the supernatant was measured. Comparing this value with the PhyB* fluorescence before the addition of the beads allowed to calculate the percentage of beads-bound PhyB*. All data are means \pm s.d. (n = 3).

Supplementary Material

Amino acid position in PIF6 (A. that	aliana)	1	15-17	18-33	34-36	
NP 001078329.1 PIF6 NP 182220.2 PIF3-1i] NP 179608.2 PIF1 [A AL187040.1 PIF1 [C. AAK09221.1 putative NP 001318965.1 PIF3 AL187041.1 PIF3 [C. AEX32796.1 PIF3 [M. EOY11780.1 PIF3 [M. EOY11780.1 PIF4 [A. AEE79871.1 PIF5 [A. AL187042.1 PIF5-1ikk AED97445.1 PIF7 [A.	<pre>(A. thaliana] ce 1 [A. thaliana] . thaliana] roseus] PIF [O. sativa] [A. thaliana] roseus] domestica] cative isoform 1 [T. cacao] thaliana] thaliana] e protein [C. roseus] thaliana]</pre>	MMFLPTDYCCR MISPSSNIKPK VVNNHNSSLNHLPRKS VSIPTTSSSLNRSI MAICST KLESAQDRNPS KLEASQCKTASPTI RVDSSQD KLDSSQDENPSCSTI YSLSTNRRSIR FHMSTNKRSIR FHMSTNKRSIR	LSDCYM LSDCYM ITTMGEDDIM KKTPMGEDIM DNLV KKTPMGEDIM PVDV VS DLSSVPNULV FENFL DLSFVEENFV PQDLV PENFV 	FENGOLLAKG VCENGOLLAKI WCDGOVVQN WENGOLSTOS WENGOLSTOS WENGOLMMQG WENGOVVQQ UENGOVVQQ LENGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ WRGOVVQQ	RSNV REHTKKPSSSPPK ORQASFKKSPVGGSEG ORQASFKSPVGGSEG OSSRSRNIP OSSRTRKSPSFNSILS OSSRTRKPSCNTLPSHO OSSRARTPACNSLPSHO OTHREQT	EIPTQQQSSAAGGTRDIR-
37						80
SLHNORTKSI	MD-LYE-	AEYNEDFMKSIIHGGGG	AITN-LGDTO			VVP
SFQKQRRQSL	LD-LYE-	TEYSEGFKK	NIKI-LGDTQ			VVP
LLPSMDPQQQPSSI	DQNLFIQEDEMTSWLHYPLRD	DDFCSDLLF	SAAP-TATATAT	[VV]		SQV
SVETDAMAHQQQQQQ	-QQLFMHEDEMASWLHYPIDDSSFD-	RDIYSDLLYPPLST	SISS-TTTTTAT	I P	G	TNLPPREIRTTTTEIRP
SI	ASVLTGDDTETAAWFPDTLDD-ALE-	-KDLYTQLWR	SVTG-DAFPAAA	AA		AGP
TMVDE1PMSVPSI	MTGLSQDDDFVPWLNHH-PSL-	-DGYCSDFLR	DVSSPVTVNEQE	SDMAV		QRR
GREGEWUDGELDET DI GUDGO		UEVCUDEI P	EISG-VIGNEPS	CONTRACTOR	CCCCOVIDCNDNCAUEL	ACI OODNUCKUAI TCDADUSDD
GKEG-TIDSVISEIPMSVPSI	EMSLNODDEVUPWINYPVDO-SLO-	SEV-SDELP	FLSG-VAVNETS	THSNFASFDRR	SOSTRDSCTVSLNNG	AVEFOCNESKVETPADGE-APP
HHEEALRS-	STFLEDOETVSWIOYPPDED-PFEF	PDFSSHFFS	TMDP-LORPTSF	T		
PNNIFLDNOETVOK	PNYAALDDOETVSWIOYPPDDVID-PFE-	SEFSSHFFS	SIDH-LGGPE			KPRTIEETVKH
NSNNKQATTSCGNI	TATLIODNDTVSWIHCOVDD-PFD-	KEFSSNFLP	-VIP-LPTPOLE	D		PPHKOOIOHCRTHP
	WTQSLNGC <mark>-</mark> -TLE-		SVVHQAA	L		QQP
81						100
						100
QSHVAAAI						ETNMLESNKHVD
	20DKETN		EQP	INNNKKKLKS	SKIEF	CECCDLI CRANND
			V T(NEMNESRL	TOT	EDCDCCCCCVAV
SCHHADDDDLD_DDAADD			KI	INFRITE SKL-FK		EIGESSSKARK
KDGNESAP/	ASSSOYNGFOSHSLYGSDBAB	DLPSOOTNPDRFTOTOF	PLITSNKP-SLA	NESHELRPA	TFAK	TTNNNLH
RTGLLNPYLSOOGOTSVPS	SLGS-ISNAAMNSASHNOEISFGDSVC	GOLSAGGEVSMKAGKKN	EGSSNNGS-HLI	NFSHFSRPA	ALFRGNLOKTDGLTASCS	AGTEKMVKEEKVPATSSRIPM
RTGTSQLHSLSSQQSQASYPS	FRSRVSDTAGDNTSATHRDVGKNSTQ	I-SSAGGSPGMKILRPL	PVTPSNNNPSIV	NFSHFSRPA	ALVKANVQTNGVMAGSGS	SSMIRIANKDKFSAATSNNLP
RSGTSQLSTLPSQLCQTSSP	IRSRILENIGNSLGHTSTHHAIGGDSIG	VQASDGGLPGIKMQKQD	QVAPCNNT-VLM	INFSHFSRPA	ALVKASLQNISAIA	-SIERIGSKEKGSAASISDPA
KACPDPPPQVMPPP	KFRLT			-NSSSGIRE		
EAQAMAPPI	<pre></pre>					
STNFGPNPLPAPI	RFQS		DSN	INVRGGVSKCPN	VLLKA	DLGSSNSGPRPS
SKFQLQSP1	IGP			-NHNYESKD		

Supplementary Figure 2 | Multiple amino acid sequence alignment of phytochrome interaction factors (PIFs) from different plant species. The depicted PIF sequences (GenBank accession number are shown) were aligned by MAFFT (Katoh and Standley, 2013) (EMBL-EBI) with default parameter settings and displayed using BioEdit (Hall, 1999). Identities and similarities are highlighted as shaded amino acids (90% shade threshold, BLOSUM62 matrix). The amino acid positions are indicated with respect to *A. thaliana* PIF6.



Supplementary Figure 3 | Comparison of the light-controlled affinity purification with established purification methods. HEK-293T cells were transfected with plasmids (see Figure 2A) that encode GFP fused to the depicted different affinity tags and the untagged red fluorescent protein mCherry. During the purification process, fluorescence of GFP and of the background control mCherry was measured and is shown as percentage compared to the ones of the cell lysate. For each replicate, 4×10^6 cells were lysed with 500 µl of lysis buffer and proteins were purified with 50 µl of beads. FT, flow-through; W1-W4, wash 1-4. Data are means \pm s.d. (n = 3).

Supplementary Table 5 | Plasmids generated in this study. The following plasmids were used for the cloning: pcDNA3_m ζ -SBP (Molnar et al., 2012), pHB111 (Beyer et al., 2018), pLVX-IRES-ZsGreen1 (Clontech, Mountain View, CA), pMH212 (Hörner et al., 2014), pRS315 (Sikorski and Hieter, 1989). The sequences of the used oligonucleotides are depicted in Supplementary Table 6.

Plasmid	Description
рМН501	5'LTR-P _{CMV} - mCherry-IRES-mGFP_SBP-3'LTR mCherry was amplified from pMH212 using oligos oMH501 and oMH502; IRES was amplified from pLVX-IRES-ZsGreen1 using oligos oMH418 and oMH419; mGFP was amplified from pHB111 using oligos oMH505 and oMH507; SBP-tag was amplified from pcDNA3_mζ-SBP using oMH508 and oMH509; All four PCR products were Gibson-cloned into EcoRI/MluI digested pLVX- IRES-ZsGreen1.
рМН502	5'LTR-P _{CMV} - mCherry-IRES-mGFP_TEV _{CS} proteinA-3'LTR mCherry was amplified from pMH212 using oMH501 and oMH502; IRES was amplified from pLVX-IRES-ZsGreen1 using oMH418 and oMH419; mGFP was amplified from pHB111 using oligos oMH505 and oMH507; TEV _{CS} proteinA was amplified from pRS315 using oligos oMH511 and oMH512; All four PCR products were Gibson-cloned into EcoRI/MluI digested pLVX-IRES- ZsGreen1.
рМН503	5'LTR-P _{CMV} - mCherry-IRES-mGFP_PIF6(1-100)-3'LTR mCherry was amplified from pMH212 using oMH501 and oMH502; IRES was amplified from pLVX-IRES-ZsGreen1 using oMH418 and oMH419; mGFP_PIF6(1-100) was amplified from pHB111 using oligos oMH505 and oMH513; All three PCR products were Gibson-cloned into EcoRI/MluI digested pLVX-IRES-ZsGreen1.
рМН508	5'LTR-P _{CMV} - mCherry-IRES-mGFP_PIF6(18-36)-3'LTR mCherry was amplified from pMH212 using oMH501 and oMH502; IRES was amplified from pLVX-IRES-ZsGreen1 using oMH418 and oMH419; mGFP was amplified from pHB111 using oligos oMH505 and oMH507; PIF6(18-36) was amplified from pHB111 using oligos oMH536 and oMH537. All four PCR products were Gibson-cloned into EcoRI/MluI digested pLVX-IRES- ZsGreen1.
pMH511	5'LTR-P _{CMV} - ZAP70_PIF6(1-100)-IRES-ZsGreen1-3'LTR ZAP70 was amplified from pSV10.1 (Arthur Weiss, USA) using oligos oMH531 and oMH532; PIF6(1-100) was amplified from pHB111 using oligos oMH525 and oMH526; Both PCR products were Gibson-cloned into EcoRI/SpeI digested pLVX-IRES-ZsGreen1.
pMH512	5'LTR-P _{CMV} -mCherry-IRES-mGFP_PIF6(15-33)-3'LTR This plasmid was cloned as described for pMH508 except that oligos oMH541 and oMH542 were used for amplification of PIF6(15-33) from pHB111.
рМН513	5'LTR-P _{CMV} -mCherry-IRES-mGFP_PIF6(18-33)-3'LTR This plasmid was cloned as described for pMH508 except that oligos oMH536 and oMH542 were used for amplification of PIF6(18-33) from pHB111.
pMH516	5'LTR-P _{CMV} -mCherry-IRES-mGFP_PIF6(15-36)-3'LTR This plasmid was cloned as described for pMH508 except that oligos oMH541 and oMH537 were used for amplification of PIF6(15-36) from pHB111.
рМН521	5'LTR-P _{CMV} - ZAP70_PIF6(15-36)-IRES-ZsGreen1-3'LTR ZAP70 was amplified from pMH511 using oMH531 and oMH573; PIF6(15-36) was amplified from pMH516 using oMH563 and oMH572; Both PCR products were Gibson-cloned into EcoRI/SpeI digested pLVX-IRES-ZsGreen1.

Oligo	Sequence $(5' \rightarrow 3')$
oMH418	ACTAGTTCTAGAGCGGCCGC
oMH419	CATATTATCATCGTGTTTTTCAAAGGAAAACC
oMH501	CACCGACTCTACTAGAGGATCTATTTCCGGTGAATTCGCCACCATGGTGAGCAAGGGCGA
	GGAGGATAAC
oMH502	AGGGAGAGGGGGGGGGGGGCGGGCCGCCCCCCAGAACTAGTTTACTTGTACAGCTCGTCCAT
	GCCGC
oMH505	GGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGTGAGCAAGGGCGAGGAGCTG
oMH507	ACCAGCAGAACCTGCGGAGCC
oMH508	CTGTACAAGGGCTCCGCAGGTTCTGCTGGTATGGACGAGAAGACCACCGGCTGGAG
oMH509	CACAAATTTTGTAATCCAGAGGTTGATTGTTCCAGACGCGTTCAGGGCTCCCTCTGGCCC
	TGGG
oMH511	CTGTACAAGGGCTCCGCAGGTTCTGCTGGTGTCGACGGATCCGAGAATCTTTATTTTCAG
oMH512	CACAAATTTTGTAATCCAGAGGTTGATTGTTCCAGACGCGTCTAAAGAGCCGCGGAATTC
	GCG
oMH513	CACAAATTTTGTAATCCAGAGGTTGATTGTTCCAGACGCGTTCAGTCAACATGTTTATTGC
	TTTCCAACATGTTTGTTTC
oMH525	GGCTCCGCAGGTTCTGCTGGT
oMH526	GAGAGGGGGGGGGGATCCGCGGCCGCTCTAGAACTAGTCAGTC
	AACATGTTTGTTTC
oMH531	GACACCGACTCTACTAGAGGATCTATTTCCGGTGAATTCGCCACCATGCCAGACCCCGCG
	GCGCAC
oMH532	GAACATCATACCAGCAGAACCTGCGGAGCCGGCACAGGCAGCCTCAGCC
oMH536	CTGTACAAGGGCTCCGCAGGTTCTGCTGGTATGGAGCTTGTGTTTGAGAATGGCC
oMH536	CTGTACAAGGGCTCCGCAGGTTCTGCTGGTATGGAGCTTGTGTTTGAGAATGGCC
oMH537	CACAAATTTTGTAATCCAGAGGTTGATTGTTCCAGACGCGTTCAAACGTTGGATCTTTGG
	CCCTTTGC
oMH537	CACAAATTTTGTAATCCAGAGGTTGATTGTTCCAGACGCGTTCAAACGTTGGATCTTTGG
	CCCTTTGC
oMH541	CTGTACAAGGGCTCCGCAGGTTCTGCTGGTCAAGAGTATATGGAGCTTGTGTTTGAGAAT
	G
oMH542	CACAAATTTTGTAATCCAGAGGTTGATTGTTCCAGACGCGTTCATCTTTGGCCCTTTGCAA
	GAATCTGG
oMH563	CAAGAGTATATGGAGCTTGTGTTTGAGAATG
oMH572	GAGAGGGGGGGGGGATCCGCGGCCGCTCTAGAACTAGTCAAACGTTGGATCTTTGGCCCTTT
	GC
oMH573	CATTCTCAAACACAAGCTCCATATACTCTTGACCAGCAGAACCTGCGGAGCC

Supplementary Table 6 | Sequences of the oligonucleotides used in this study.

Supplementary Tables 1-4 are available as separate files.

References

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