

## **New Phytologist Supporting Information**

Article title: **PHYTOCHROME INTERACTING FACTOR 7 is important for early responses to elevated temperature in Arabidopsis seedlings.**

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Article acceptance date: 5 November 2019

The following Supporting Information is available for this article:

**Fig. S1** Thermomorphogenic response requires both PIF4 and PIF7 for hypocotyl elongation in short-day (SD).

**Fig. S2** PIF4 and PIF7 regulate thermomorphogenic hypocotyl elongation downstream of phyB and cry1.

**Fig. S3** Relative expression of genes that were previously implicated in thermomorphogenesis.

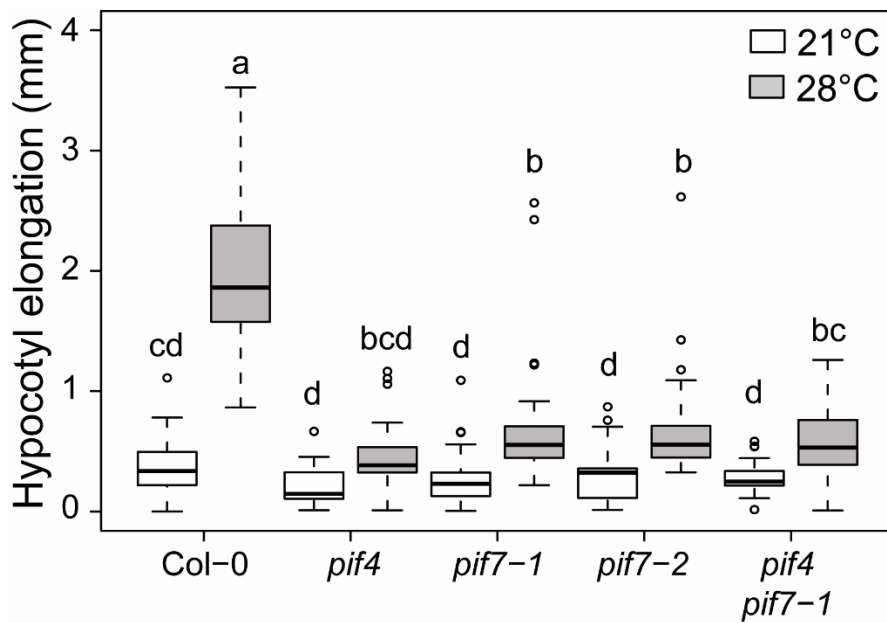
**Fig. S4** Relative expression of temperature-induced genes in Col-0 and *pif* mutants.

**Fig. S5** PIF7 and PIF4 form homo- and hetero-dimers in yeast.

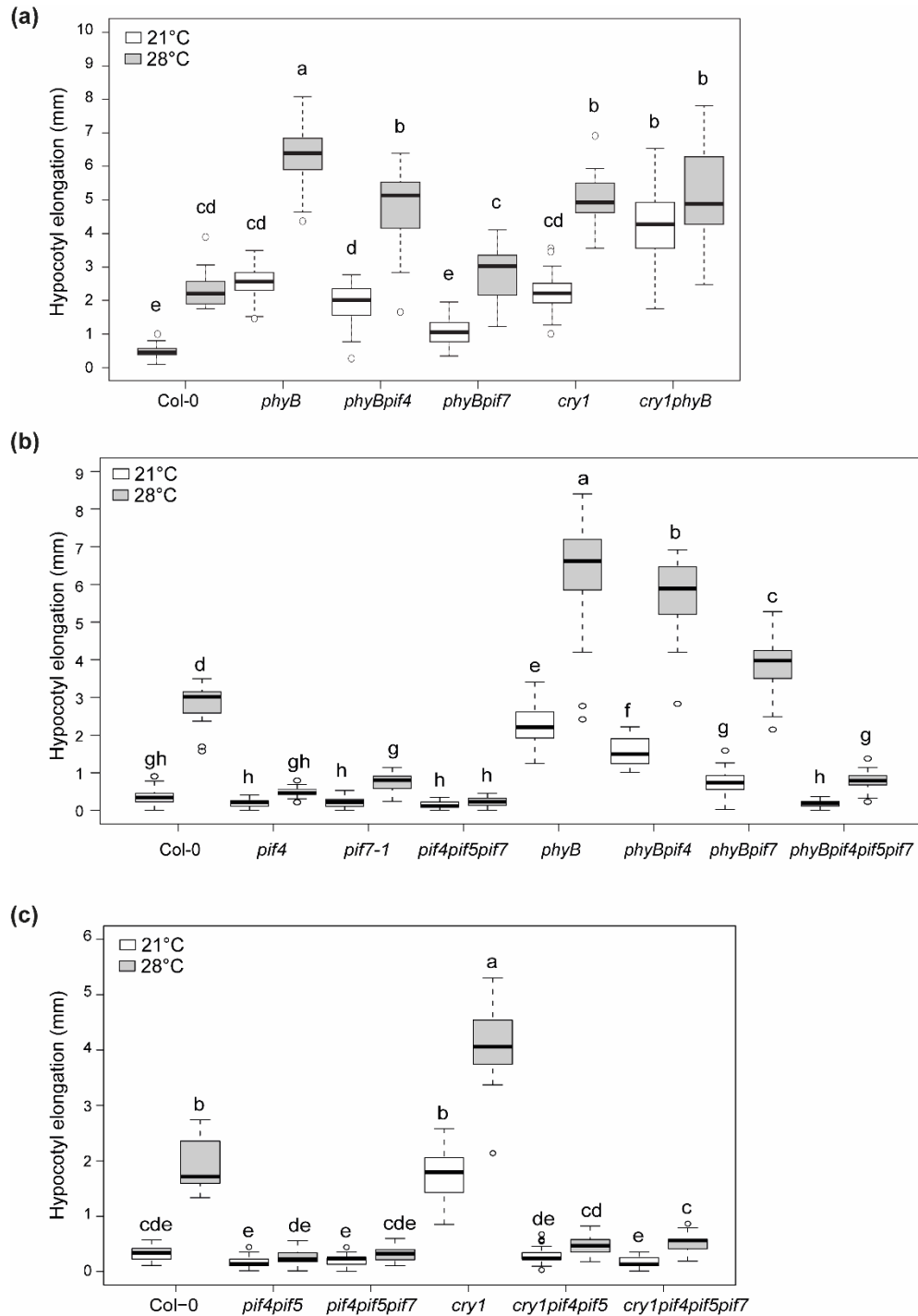
**Fig. S6** Regulation of the levels of both PIF7 isoforms in thermomorphogenesis.

**Table S1** List of oligonucleotides used in this study.

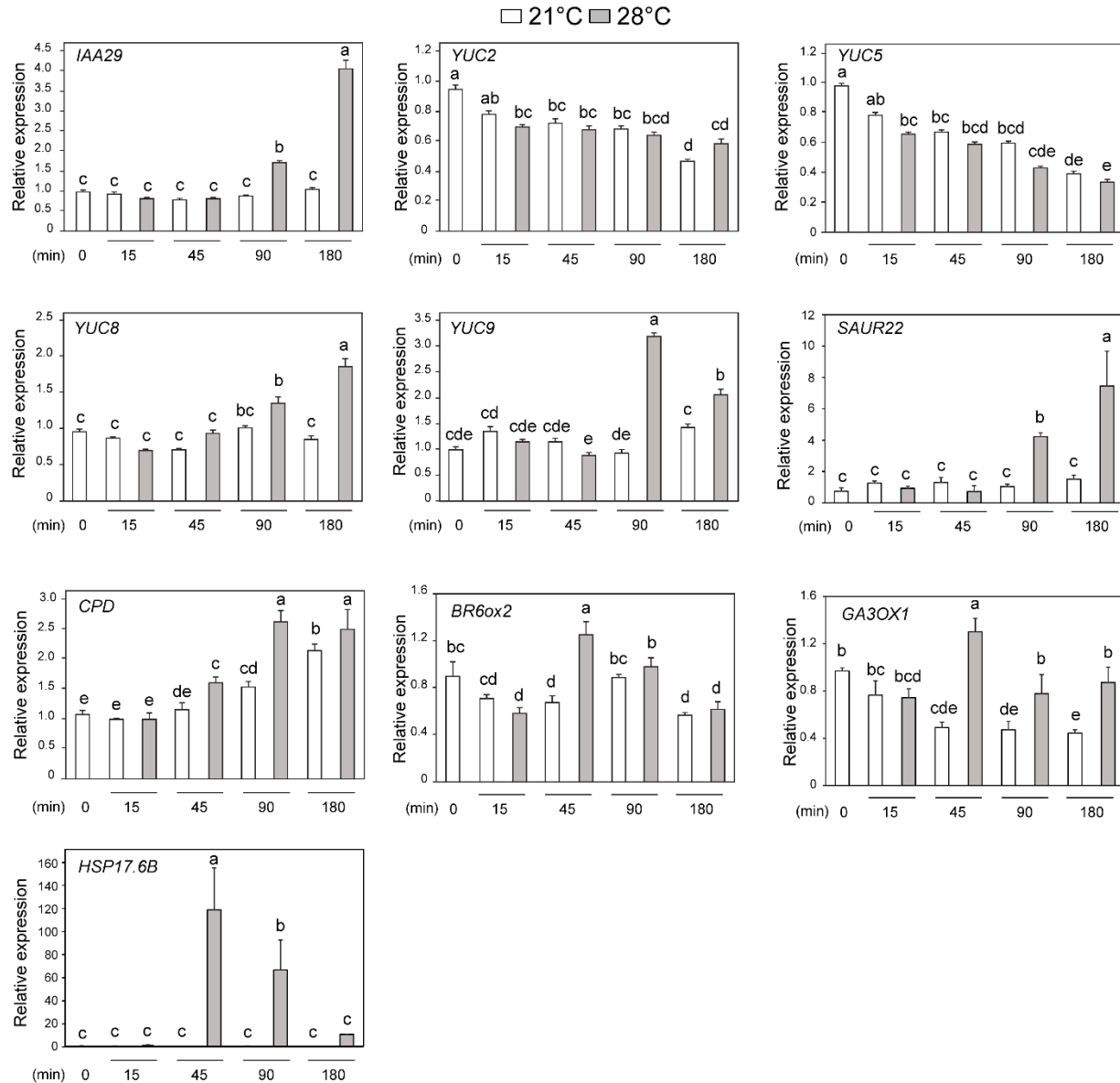
**Fig. S1.** Thermomorphogenic response requires both PIF4 and PIF7 for hypocotyl elongation in short-day (SD). Hypocotyl elongation of wild-type (Col-0) and *pif* mutants grown in SD (8h light, 16 hour dark) at 21°C for 4 days then either kept at 21°C or transferred to 28°C (at ZT2 on day 5) for five additional days. Elongation during the last 5 days is indicated. Different letters indicate significant difference (two-way ANOVA with Tukey's HSD test,  $P < 0.05$ ,  $n > 25$ ).



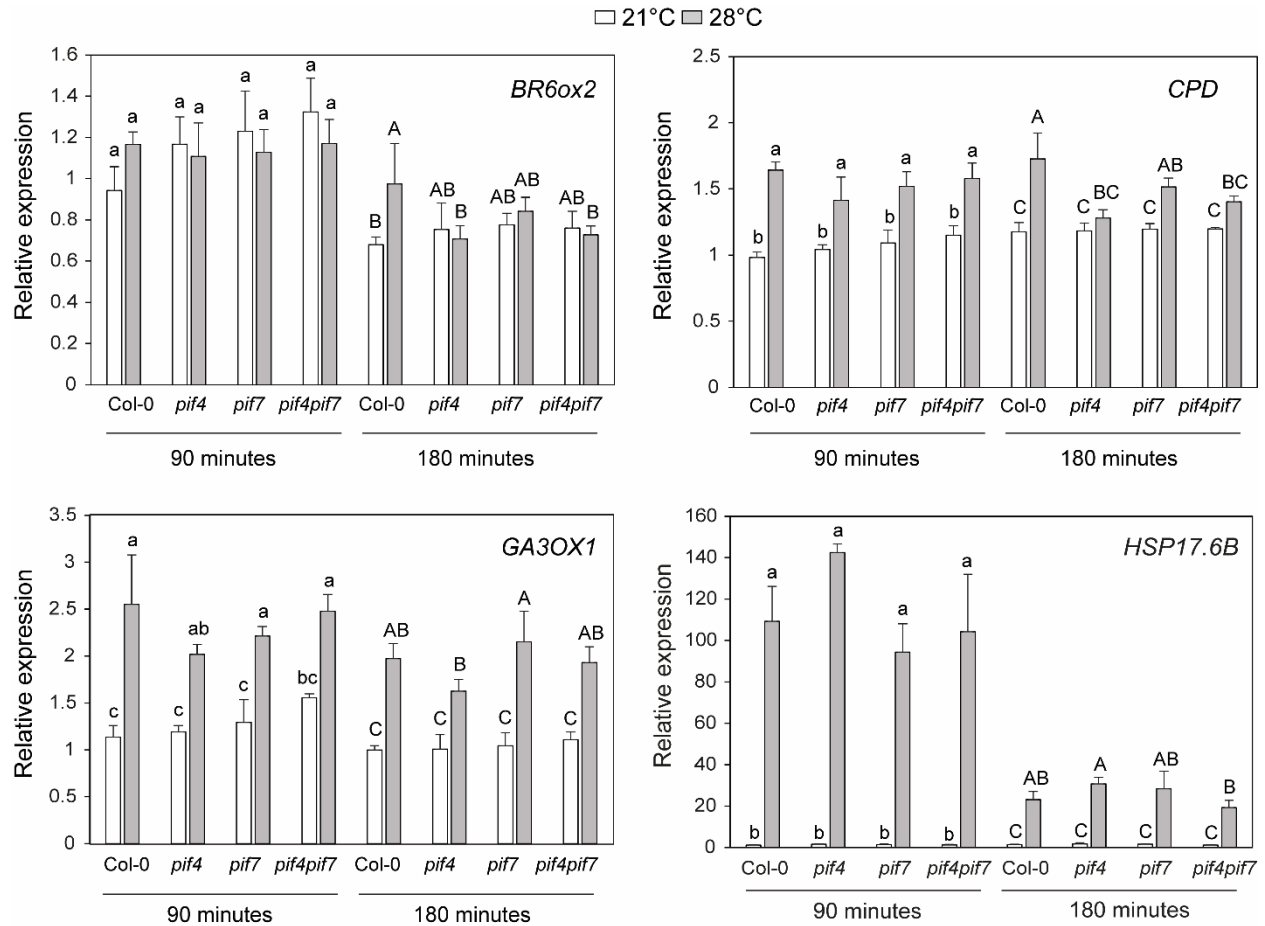
**Fig. S2** PIF4 and PIF7 regulate thermomorphogenic hypocotyl elongation downstream of phyB and cry1. (a-c) Hypocotyl elongation of indicated genotypes grown in LD at 21°C for 4 days then either kept at 21°C or transferred to 28°C for three additional days. Elongation during the last 3 days is indicated. Different letters indicate significant difference (two-way ANOVA with Tukey's HSD test,  $P < 0.05$ ,  $n > 25$ ).



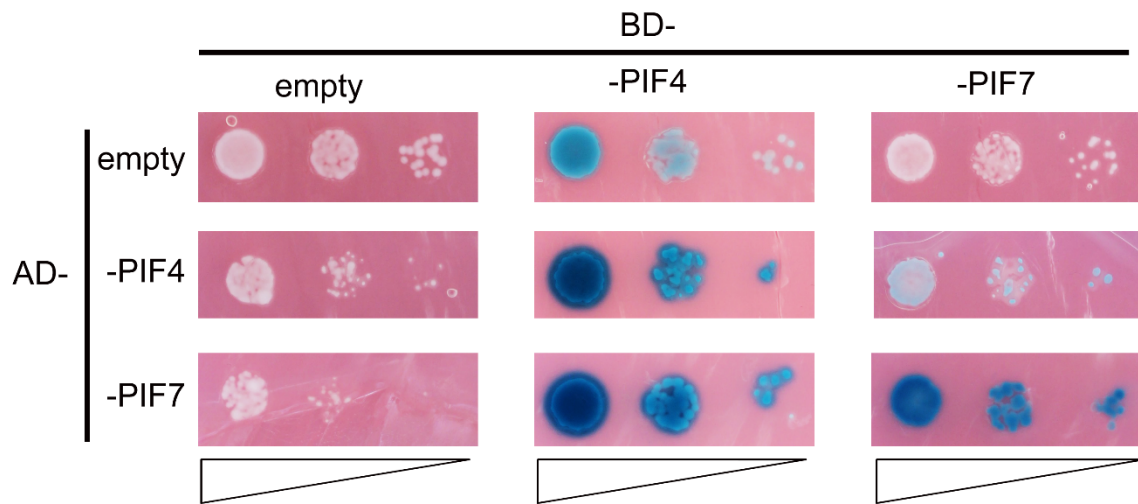
**Fig. S3** Relative expression of genes that were previously implicated in thermomorphogenesis. Seedlings were grown as indicated in Fig. 2a. Gene expression values were calculated as fold induction relative to a Col-0 sample at 21°C, t = 0. n = 3 (biological) with 3 technical replicas for each RNA sample. Data are mean, error bar indicates 2XSE. Different letters indicate significant difference (two-way ANOVA with Tukey's HSD test,  $P < 0.05$ ).



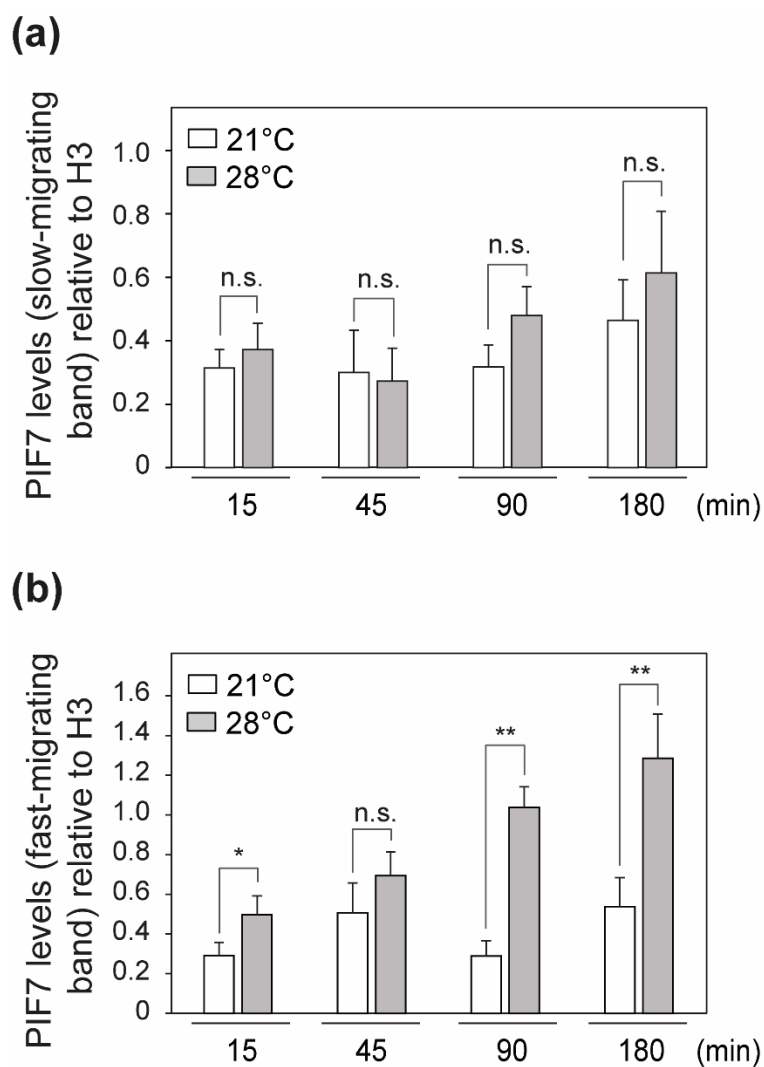
**Fig. S4** Relative expression of temperature-induced genes in Col-0 and *pif* mutants. Seedlings were grown as indicated in Fig. 2a. Gene expression values were calculated as fold induction relative to a Col-0 sample at 21°C, t = 90 min. n = 3 (biological) with 3 technical replicas for each RNA sample. Data are mean, error bar indicates 2XSE. Different letters indicate significant differences within timepoints (p<0.05).



**Fig. S5** PIF7 and PIF4 form homo- and hetero-dimers in yeast. Yeast two-hybrid  $\beta$ -galactosidase assay testing the interactions between full-length PIF7 and PIF4 fused to either GAL4 binding domain (BD-) or GAL4 activation domain (AD-). Yeast co-transformed with the indicated vectors were spotted on SD-LW medium (10x serial dilutions from OD0.1 to OD0.001) and grown for two days at 30°C before an X-gal-containing agarose overlay. Plates were kept at 37°C in darkness and pictures were taken after 5h (all combinations with BD-PIF4) and 22h (all the others). Empty pGBKT7 and pGADT7 vectors are used as negative controls.



**Fig. S6** Regulation of the levels of both PIF7 isoforms in thermomorphogenesis. (a) Slow-migrating and (b) fast-migrating isoforms of PIF7-HA protein detected with anti-HA antibody from total protein extracts after the indicated time points at 21°C and 28°C in 5-days-old LD-grown PIF7-HA seedlings treated at ZT2. The HA signal was quantified and normalized to H3 signal (n = 6). Data are mean, error bar indicates SE. Asterisks indicate significant difference ( $p$  values) between 28°C and 21°C samples at a given timepoint (Student's t-test, \* < 0.05, \*\* < 0.01), n.s. non significant.



**Table S1** List of oligonucleotides used in this study.

Oligos used for genotyping			
Allele	Collection	Oligonucleotide	Sequence
<i>phyB-9</i>	Point mutation	PB9	GTGTCTGCGTTCTCAAACG
		B9dCAPS	GTGGAAGAAGCTCGACCAGGCTTTG
<i>cry1-304</i>	Deletion	CF586	GGTAGGGTTTCTAGGTGGTGGCTC
		CF587	GGTGAAGAAGAGGAGACTCAGGG
<i>yuc2-1</i>	SALK_030199	oJM1845	TTCTTGCAATTTCTCGCTCTACG
		MT440	AACCCGTGGCGAGTATAATG
<i>yuc5-3</i>	GT6160	oJM1203	CGGACTCTAATCAAAGTCCC
		oJM1204	GGAGATTTCAAACACTAGATTTG
<i>yuc8-1</i>	CS110939	oJM1206	CATCCTCTCCACGTGGCTTCC
		oJM1207	GAAGTACGCTTCGTCGGGTAC
<i>yuc9-1</i>	SAIL_871G01	oJM1199	GCTCGTAAGCAAACAAAACACTG
		oJM1200	GAAGGAAATGCCCAATGAGAC
<i>pif4-101</i>	SAIL_114_G06	SL-43	CAGACGGTTGATCATCTG
		oVCG-61	TAGCATCTGAATTTATAACCAATCTCGATACAC
<i>pif5-3 (pil6-1)</i>	SALK_087012	SL-46	TCGCTCACTCGCTTACTTAC
		oVCG-56	ATTTTGCCGATTCGGAAC
<i>pif7-1</i>	CS68809	SL-195	GTGGCAAGTTGGCTCTTAGG
		SL-169	TGATAGTGACCTTAGCGACTTTTGAACGC
<i>pif7-2</i>	SAIL_622_G02	oASF-27	GGAGAGCCATAGAGTTGG
		oVCG-61	TAGCATCTGAATTTATAACCAATCTCGATACAC

Oligos used for RT-qPCR			
Target	Primer Efficiency	Oligonucleotide	Sequence
<i>UBC</i>	1.94	UBC-F	CAGTCTGTGTGATAGCTATCATAGCAT
		UBC-R	AGAAGATTCCTGAGTCGCAGTT
<i>YSL8</i>	2.00	YSL8-F	TCATTGTTTTCGCCATGA
		YSL8-R	CTCAGCAACAGACGCAAGCA
<i>PIF4</i>	2.02	oVCG-246	TACCTCGATTTCCGGTTATGGATC
		oVCG-247	GTTGTTGACTTTGCTGTCCCGC
	1.78	SL63	TTCTCCTCCCCTTCTTCTC
		SL64	AGGTTCAGGACTGGACTTAG
<i>PIF7</i>	2.01	oVCG-588	GAGCAGCTCGCTAGGTACATG
		oVCG-589	GTTGTTGTTGCACGGTCTG
<i>YUC2</i>	1.94	MT-437	AACTCCGGATGGAAGTTTG
		MT-438	CCCGAAAGTCGATATACCTAGC
<i>YUC5</i>	1.9	MT-459	TGGAGCTAGTAGACGGTCTAG
		MT-460	GAAACGGCGATTTCCGGAAC
<i>YUC8</i>	2.0	MT-271	GGCGGCTTGTCTCCATGAAC



		PH-171	GATGAACTGACGCTTCGTCG
YUC9	2.0	MT-297	GCTAACCACAATGCAATTAC
		MT-298	CATCACTGAGATTCCAAATG
IAA29	1.94	MT-157	CTTCCAAGGGAAAGAGGGTGA
		MT-158	TTCCGCAAAGATCTTCCATGTAAC
BR6ox2	2.05	oVCG-740	GTGAGCGGTTTCGTCAGTTC
		oVCG-741	GGTAACGATCTTGTATTCCGG
CPD	2.06	oVCG-726	GCACTTCAACCTTGGAGA
		oVCG-727	CAGAGAGTGCAACCTAGCC
HSP17.6B	2.21	YI578	CAGGTTAAGGCTGCGATGGA
		YI579	AGCCTTAGGCACCGTAACAG
GA3OX1	1.88	YI622	TACCGACTCCACCTCCTAA
		YI623	GACCCAACCAAGATCATCGC
SAUR22	1.99	MT515	GTATGAGAGTGGCACTAAG
		MT516	GCTCTGGTGAGAAGTCTAC

#### Oligos used for ChIP-qPCR

Target	Primer Efficiency	Oligonucleotide	Sequence
IAA29 peak (G-box)	1.86	MK54	ACATTACGCCACGAGTAG
		MK55	GATCAACCAAGCAGAAGAG
IAA29 control	1.92	MK60	GGGATGTTACATGGAAGTAAG
		MK61	ATGAACAGATTCCGCAAAG
YUC8 peak (G-box)	1.97	oASF213	GGAATGGGTTTGTATGTGGAA
		oASF214	GATTCTTGTGGGACCAACG
YUC8 control	2.03	MK34	AGCTGGCCTATGAAATAAC
		MK35	AGTGGACGATCAATTCTC

#### Oligos used for cloning (Two-hybrid vectors)

Plasmid	Strategy	Oligonucleotide	Sequence
pAD-PIF7 (pVG20)	Digestion of amplified fragment and pGADT7 with EcoRI and BamHI followed by T4 ligation.	oVCG-193 (EcoRI)	TGAATTCCAaTCGAATTATGGAGTTAAAGAG
		oVCG-194 (BamHI)	CGATGGATCCCCTAATCTCTTTTCTCATGA
pAD-PIF4 (pVG22)	Blunt T4 ligation of amplified fragment into SmaI digested pGADT7	33265	GAACACCAAGGTTGGAGT
		33183	CTAGTGGTCCAAACGAGAAC
pBD-PIF7 (pASF13)	InFusion cloning between NcoI-linearized	oASF205	AGGACCTGCATATGGCCATGTGCAATTATGGAGTTAAAGAGCTCACA
		oASF206	CCGGGAATTCGGCCTCCATGCTAATCTCTTTTCTCATGATTCCAAGAAGCTTGAAG
pBD-PIF4 (pASF14)	pGBKT7 and PCR amplified PIF7/PIF4.	oASF207	AGGACCTGCATATGGCCATGGAACACCAAGGTTGGAGTTTTG
		oASF208	CCGGGAATTCGGCCTCCATGCTAGTGGTCCAAACGAGAACCG