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Supplemental Information

DET1 and HY5 Control PIF4-Mediated Thermosensory Elongation Growth through Distinct Mechanisms

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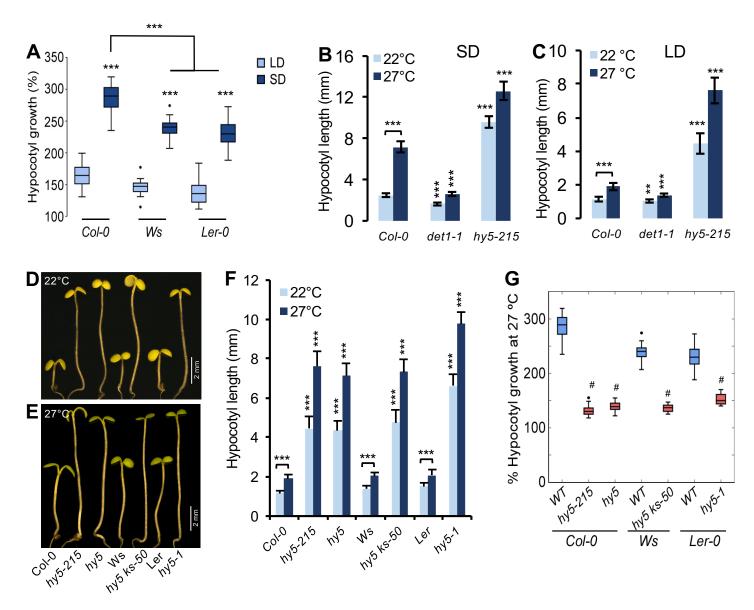


Figure S1. Thermosensory hypocotyl growth is photoperiod dependent. Related to Figure 1.

(A) Percent hypocotyl growth response (27 °C to 22 °C) of Col-0, Ws, and Ler-0 ecotypes grown under long-days (LD) and short-days (SD) for seven-days. Data shown is mean±SD (n≥20). ***P≤0.001 (Student's *t*-test) significantly different from LD in corresponding ecotypes or between indicated pairs as shown. (**B** and **C**) Hypocotyl length measurement data (mean±SD; n≥20) of Col-0, *det1-1*, and *hy5-215* mutants grown in 22 °C and 27 °C SD (B) and LD (C) for seven-days. (**D**-G) Representative seedling pictures of various *hy5* mutant alleles and their corresponding Wild-Types grown at constant 22 °C (D) and 27 °C (E) under short-days for seven-days. (**F** and **G**) Hypocotyl elongation measurement data (F) (mean±SD; n≥20) and % hypocotyl growth (27 °C to 22 °C) (G) of genotypes shown in (D) and (E). #P≤0.0001 (Student's *t*-test) significantly different from corresponding wild-type strains.

In Figure S1B, S1C, and S1F, ** $P \le 0.01$, *** $P \le 0.001$ (Student's *t*-test) significantly different from Col-0 in corresponding temperature conditions or between indicated pairs.

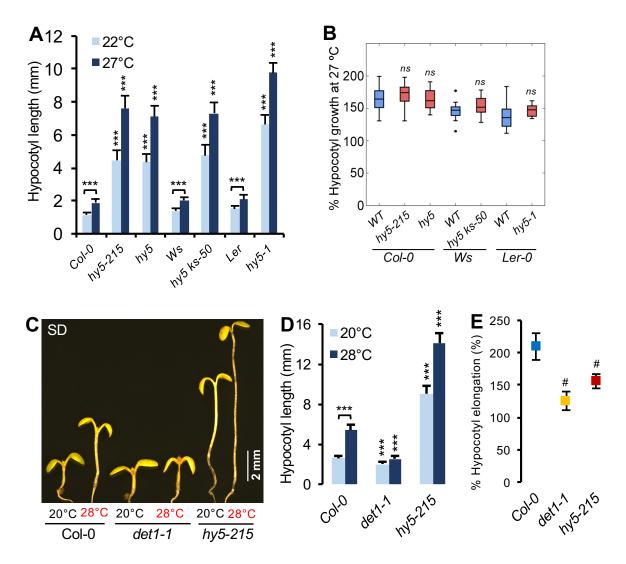


Figure S2. Control of thermosensory hypocotyl growth by DET1 and HY5. Related to Figure 1.

(**A** and **B**) Hypocotyl measurement data (A) (mean \pm SD; n \geq 20) and percent hypocotyl growth (B) of various *hy5* alleles grown at constant 22 and 27 °C under LD for seven-days. ***P \leq 0.001 (Student's *t*-test) significantly different from corresponding wild-types in corresponding temperature conditions or between indicated pairs. *ns*, not-significantly different from the respective wild-types. (**C-D**) Representative seedling hypocotyl picture (C), hypocotyl elongation measurement data (D) (mean \pm SD; n \geq 20). ***P \leq 0.001 (Student's *t*-test) significantly different from Col-0 in corresponding temperature conditions or between indicated pairs. (**E**) Relative hypocotyl response (%) of the genotypes shown in (C). *P \leq 0.001 (Student's *t*-test) significantly different from corresponding wild-type strains.

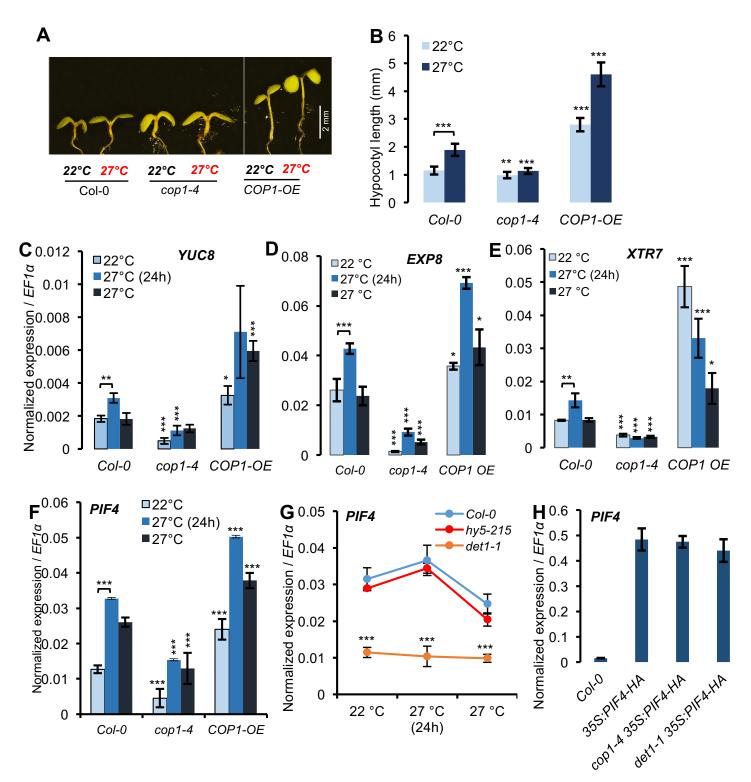


Figure S3. Thermosensory hypocotyl growth and the expression of elongation-related genes. Related to Figure 1 and Figure 3.

(A and B) Representative seedling hypocotyl picture (A) and measurement data (B) (mean \pm SD; n \geq 20) of Col-0, *cop1*-4, and *COP1-OE* lines in 22 °C and 27 °C for seven-days in LD. (C-F) Expression of growth responsive genes *YUC8* (C), *EXP8* (D), and *XTR7* (E); and *PIF4* (F) are downregulated in *cop1-4* mutant, but upregulated in *COP1-OE* compared to Col-0 as measured by qRT-PCR (mean \pm SD of three biological replicates) in seven-day-old seedlings grown constantly at 22 °C, after 24 h incubation at 27 °C, as well as continuous growth at 27 °C in short day conditions. (G) Expression of *PIF4* in Col-0, *hy5-215*, and *det1-1* as measured by qRT-PCR (mean \pm SD of three biological replicates) in seven-day-old seedlings grown at constant 22 °C and 27 °C, and 27 °C adapted seedlings for 24h in LD conditions. (H) Expression of *PIF4* in *35S:PIF4-HA*, *cop1-4 35S:PIF4-HA*, and *det1-1 35S:PIF4-HA* as measured by qRT-PCR (mean \pm SD of three biological replicates) in seven-day-old seedlings grown at constant 22 °C SD conditions. In Figure S3B-S3G, *P \leq 0.05, **P \leq 0.01, ***P \leq 0.001 (Student's *t*-test) significantly different from Col-0 in corresponding temperature conditions or between indicated pairs.

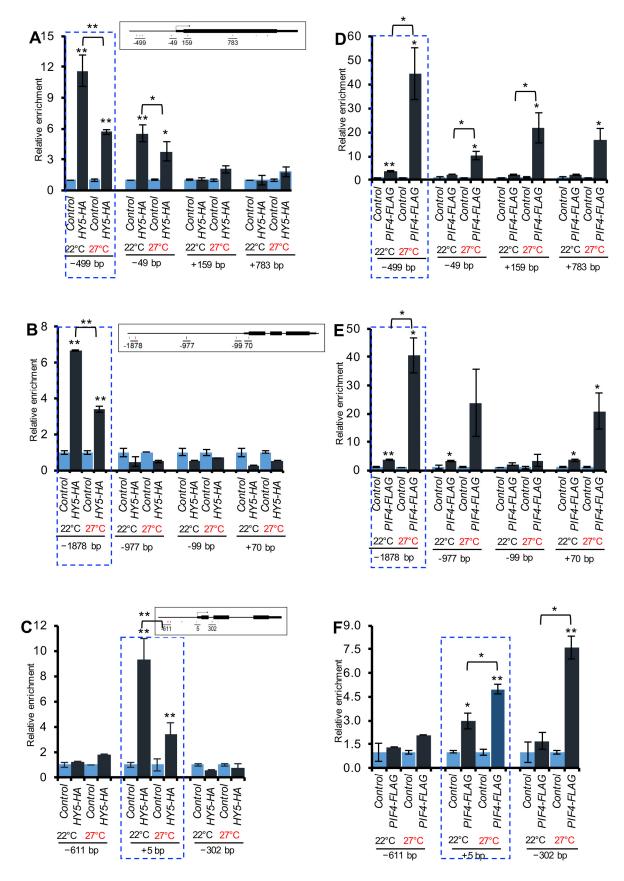


Figure S4. HY5 binds to the PIF4 targets. Related to Figure 4.

(A-F) ChIP analysis showing binding of HY5 (A-C) and PIF4 (D-F) to the PIF4 targets such as YUC8 (A and D), XTR7 (B and E), EXP8 (C and F) as measured by by qRT-PCR (mean±SD of two biological replicates) in seven-day-old seedlings grown at constant 22 °C, and 27 °C adapted seedlings for 24 h in short day conditions. Insets in A, B and C show gene diagram along with position of G-box (blue line) and/or E-box (red line) elements present in the promoters, and position of primer pairs used for Q-PCR. *P \leq 0.05, **P \leq 0.01, ***P \leq 0.001 (Student's t-test) significantly different from wild-type (Control) in corresponding temperature conditions or between indicated pairs. Marked area with the blue dotted line indicate the regions that are shown in the Figure 4A-4C and 4E-4G, respectively for HY5-HA and PIF4-FLAG.

Table S1. Oligo nucleotide sequences used in the study. Related to Figure 1, Figure 3 and Figure 4

Name	Oligo No.	Oligo Sequence (5'3')	Purpose
Primers used for gene expression analysis			
EXP8-F	633	CTCTTTCCGAAGAGTACCATGT	Q-PCR
EXP8-R	634	GTGTACGTCTCCTGCTCCTC	Q-PCR
YUC8-F	615	CGATGAGACCAGTGGCTTGT	Q-PCR
YUC8-R	616	TTTTCTCCCGTAGCCACCAC	Q-PCR
PIF4-F	766	ACCTCAGAGACGGTTAAGCC	Q-PCR
PIF4-R	767	TGGAGGAGGCATGACTTGAG	Q-PCR
ATHB2-F	667	CCGTCGGCTACAAAAAGAAG	Q-PCR
ATHB2-R	668	GAAGGCACATGGTCAAAGT	Q-PCR
XTR7-F	1127	CGGCTTGCACAGCCTCTT	Q-PCR
XTR7-R	1128	TCGGTTGCCACTTGCAATT	Q-PCR
Primers used for Cl YUC8-F (-499 bp)	1294	GGGAATGGGTTTGATGTGGA	Q-PCR
YUC8-R (-499 bp)	1295	GGGTGATTCTTTGTGGGACC	Q-PCR
<i>YUC8-F</i> (-49 bp)	1293	TCGTCGAGTGGAAAAATATTCA	Q-PCR
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YUC8-R (-49 bp)	1297	TGGAAATGGTTTTGAATTTGG	Q-PCR
<i>YUC8-F</i> (+159 bp)	1298	GGAGAATATGTTTCGTTTGATGG	Q-PCR
YUC8-R (+159 bp)	1299	CTAACCCGACGGTCCAG	Q-PCR
<i>YUC8-F</i> (+783 bp)	1300	AGTTTCTCTTGACCTAGCAAACC	Q-PCR
YUC8-R (+783 bp)	1301	AACATCTTCATTGCAAGCTCAA	Q-PCR
<i>EXP8-F</i> (-611 bp)	1286	AAACCTTCAACAAAAATGTGAGG	Q-PCR
EXP8-R (-611 bp)	1287	ACATGCAAATGTTGCGGTTA	Q-PCR
<i>EXP8-F</i> (-611 bp)	1288	GGCACAATTATTCACTCGAGTAC	Q-PCR
<i>EXP8-R</i> (-611 bp)	1289	AGGAGGGTTAGTTATTTCGGT	Q-PCR
<i>EXP8-F</i> (-611 bp)	1290	GGGTACGTATGACCAATCCCA	Q-PCR
<i>EXP8-R</i> (-611 bp)	1291	GTGTTCGTCCCGTAACCTTG	Q-PCR
<i>XTR7-R</i> (-1878 bp)	1336	GGATTTCATTGTATTTGCATGCC	Q-PCR
<i>XTR7-R</i> (-1878 bp)	1337	TAGTGCTACATCCGACGTGT	Q-PCR
<i>XTR7-R</i> (-977 bp)	1338	TGGTTTCAATAGACGATCCTTGT	Q-PCR
<i>XTR7-R</i> (-977 bp)	1339	CGTCGACCACACCATTCTCT	Q-PCR
<i>XTR7-R</i> (-99 bp)	1340	GGAATTTTAGAGATTTTCTCTCATCTG	Q-PCR
<i>XTR7-R</i> (-99 bp)	1341	GCATTGGGAAGCTAAGACCA	Q-PCR
<i>XTR7-R</i> (+70 bp)	1342	ACACCAAAACACAAAGCTCTCA	Q-PCR
<i>XTR7-R</i> (+70bp)	1343	CAAGAAGAACAGTCGCCACG	Q-PCR