

Figure S1: Petiole length and leaf number of *spa* mutants.

Bar graphs show the petiole lengths (A) or leaf number for bolting (B) using *spa* mutants grown under 22 or 28°C. Leaf number for bolting was measured in LD (16L:8D) condition. SPA1-ox refers to *35S:TAP-SPA1*. Three *spaQ* background plants are indicated with - for *spaQ*, SPA1 for *35S:LUC-SPA1/spaQ*, and mSPA1 for *35S:LUC-mSPA1/spaQ*, respectively. The letters a-e indicate statistically significant differences between means of petiole lengths (A) or leaf number (B) ($P < 0.05$) based on one-way ANOVA analyses with Tukey's HSD test. Error bars indicate s.d. ($n=3$).

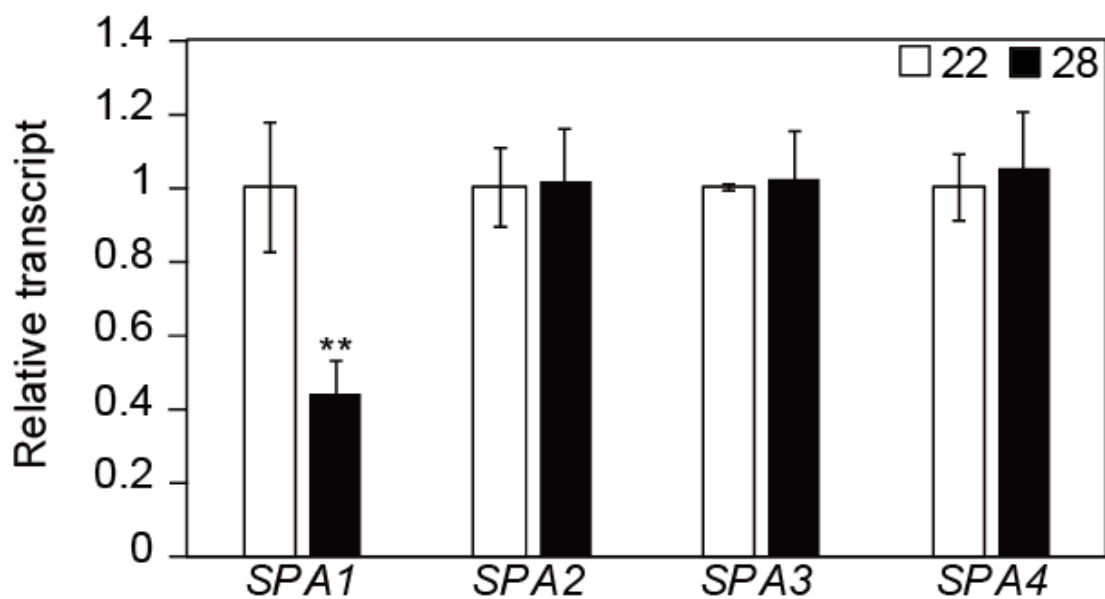


Figure S2: Relative transcript level of SPAs.

qPCR was performed to detect 4 SPAs transcriptional level. Samples were from Col-0 whole seedling grown for 5 days in 22°C and transferred to 22°C or 28°C for 4 hours. Three biological replicates were used in this study. Relative gene expression levels were normalized using expression levels of *ACT7*. Asterisks indicate statistical difference using Student's t-test; ** $p < 0.01$.

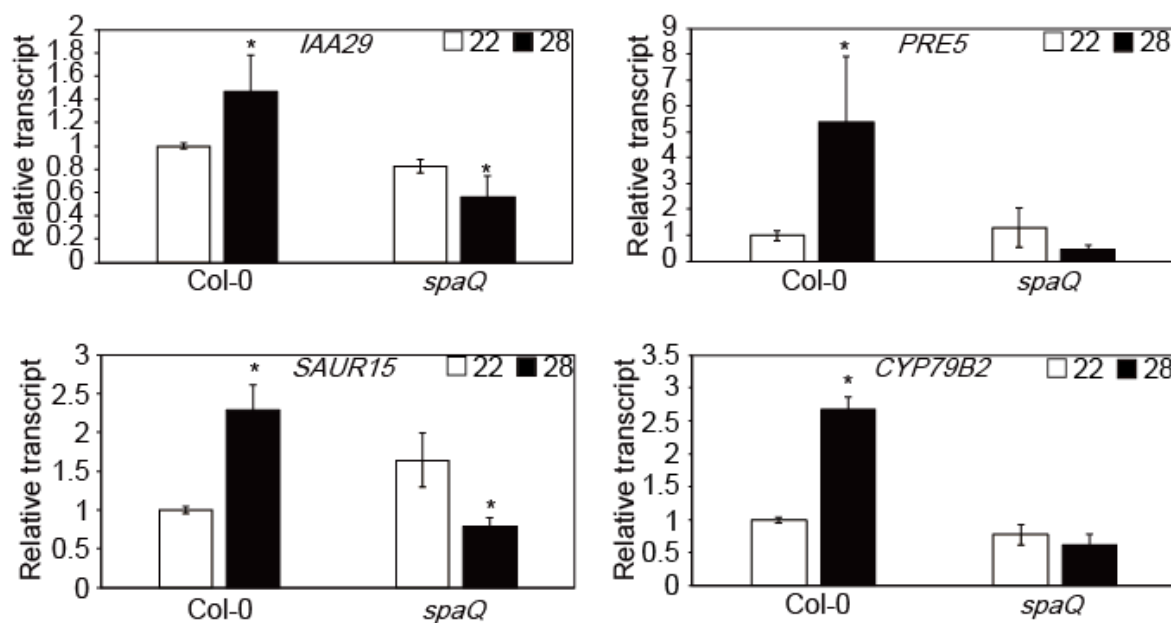
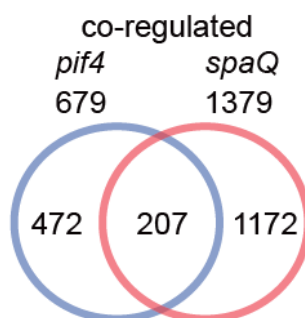


Figure S3: Relative transcript level of thermo-responsive genes.

qPCR was performed to detect transcriptional level of *IAA 29*, *PRE 5*, *SAUR 15*, and *CYP79B2*. Samples were from *Col-0* or *spaQ* mutant whole seedling grown for 5 days in 22°C and transferred to 22°C or 28°C for 4 hours. Three biological replicates were used in this study. Relative gene expression levels were normalized using expression levels of *ACT7*. Asterisks indicate statistical difference using Student's t-test; *p < 0.05.

A



B

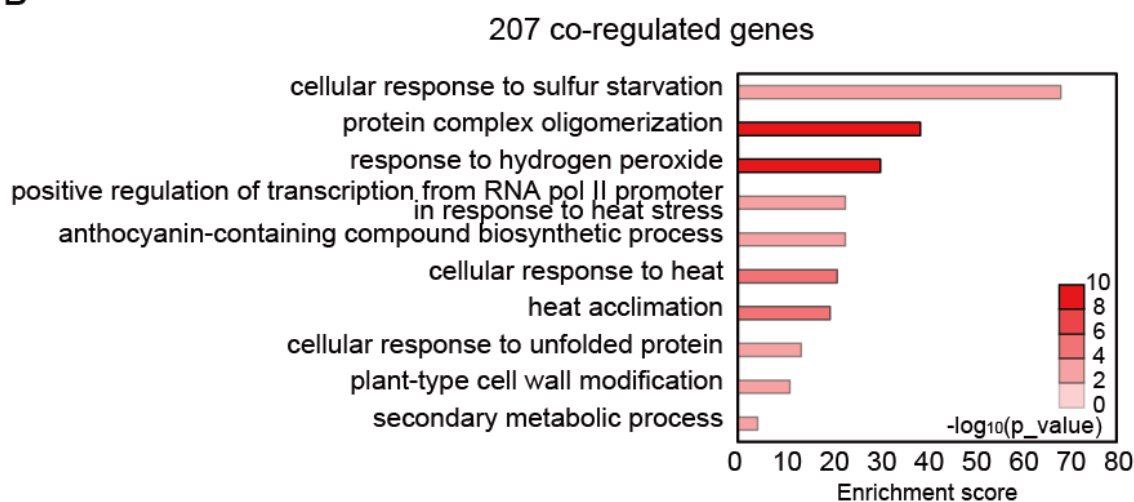


Figure S4: SPAs and PIF4 share co-regulated genes at high ambient temperature.

(A) Venn diagrams show co-regulated genes in *pif4* vs *spaQ* mutant at high ambient temperature response. (B) Gene Ontology (GO) analysis of 207 co-regulated genes. DEGs from *pif4* were derived from Ding et al., 2018. *spaQ* were grown under constant light condition (22°C and 28°C for 24 hr) whereas *pif4* were grown under long day condition (22°C and 29°C for 24 hr).

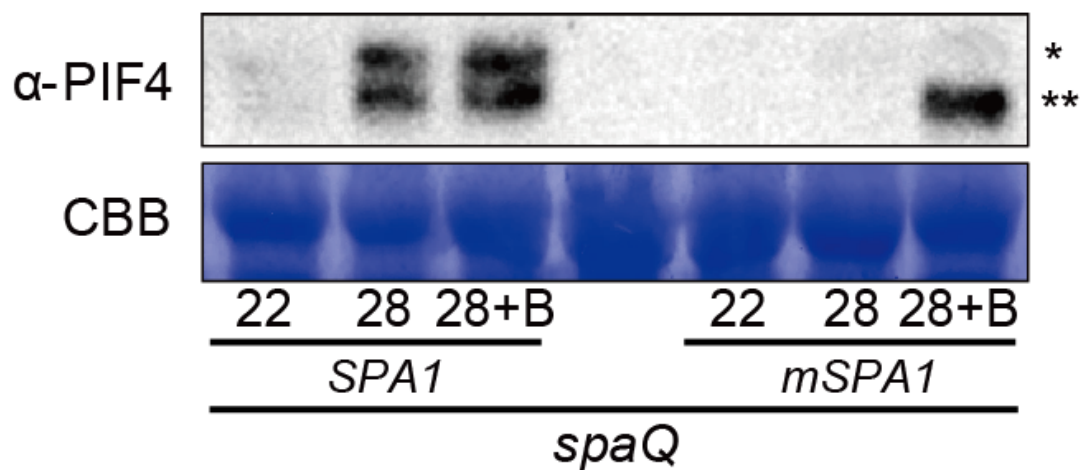


Figure S5: Relative expression level of *PIF4*

Proteasome inhibitor treatment experiment using *spaQ* transgenic lines. Three *spaQ* background plants are indicated with blank for *spaQ*, *SPA1* for *35S:LUC-SPA1/spaQ*, and *mSPA1* for *35S:LUC-mSPA1/spaQ*, respectively. B stands for bortezomib. Single asterisk (*) shows phosphorylated form and double asterisks (**) show unphosphorylated form.

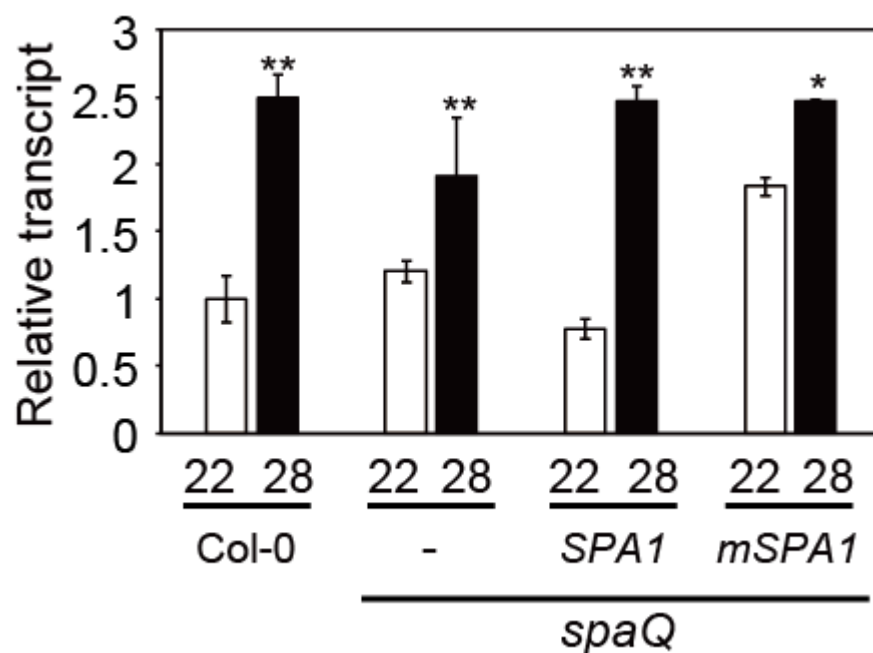


Figure S6: Relative expression level of *PIF4*

Transcription level of *PIF4* using qPCR analysis. qPCR samples were from Col-0, *spaQ*, *35S:SPA1-LUC/spaQ*, and *35S:mSPA1-LUC/spaQ* whole seedling grown for 5 days in 22°C and transferred to 22°C or 28°C for additional 24 hours. Three biological repeats were performed. Relative gene expression levels were normalized using expression levels of *ACT7*. Asterisks indicate statistical difference using Student's t-test; * $p < 0.05$, ** $p < 0.01$.

Table S1: primers used in this study.

Oligonucleotides	
<i>SPA1-F</i> AGGGCTGGCAAGTTTGAGCAT	{Gangappa, 2017 #677}
<i>SPA1-R</i> ACAAATTCAGGAGGAGTTTCC	{Gangappa, 2017 #677}
<i>SPA2-F</i> ACAAATGAGGTTTATGCCTATCACC	This study
<i>SPA2-R</i> CATATTCGATCTTTTTCTCCAGCAA	This study
<i>SPA3-F</i> GAGACTAACGAGGTTTTTCGTGTACC	This study
<i>SPA3-R</i> CGTAGACGACTGTCCTCGCCAGCAG	This study
<i>SPA4-F</i> ACTATAGACCCTGTGTCGGAACCTCG	This study
<i>SPA4-R</i> AACTAAGGTCGACGACTGTCCCCGC	This study
<i>SAUR20-F</i> AACTTGAATCTTTTCATACATCTTCAGAAGA	{Franklin, 2011 #410}
<i>SAUR20-R</i> TAACTAGGAAGAAAAATGTTGGCTCATC	{Franklin, 2011 #410}
<i>At4g33720-F</i> GACGAGCAATTTGACTACGATTATG	This study
<i>At4g33720-R</i> GTTGCAAGTGATAAAGGTTTGACCA	This study
<i>At1g09380-F</i> GAAGAGAACTCTACACCGGCACGT	This study
<i>At1g09380-R</i> TTCACTCTTGACTTTATGGTTTTGT	This study
<i>IAA29-F</i> ATAGCAAGAAAAGTGGATATCAAGC	This study
<i>IAA29-R</i> AAGTAGCCAGTCACCCTCTTCCCT	This study
<i>PRE5-F</i> AACGGCGTCGTTCTGATAAG	{Oh, 2012 #421}
<i>PRE5-R</i> CATGAGTAAGCTTCTAATCACGG	{Oh, 2012 #421}
<i>SAUR15-F</i> AAGAGGATTCATGGCGGTCTATG	{Oh, 2012 #421}
<i>SAUR15-R</i> GTATTGTTAAGCCGCCATTGG	{Oh, 2012 #421}
<i>CYP79B2-F</i> AGTACCGGGAAAAGAGGTTGTGCGG	This study
<i>CYP79B2-R</i> CAGAAACATATCGTGACTAGACTCC	This study
<i>ACT7-F</i> TCCATGAAACAACCTTACAACCTCCATCA	{Sun, 2013 #739}
<i>ACT7-R</i> CATCGTACTCACTCTTTGAAATCCACA	{Sun, 2013 #739}
<i>LexA-PIF4-F</i> AAAAGAATTCAAATGGAACACCAAGGTTGGAGTTT	This study
<i>LexA-PIF4-R</i> AAAACCTCGAGCTAGTGGTCCAAACGAGAACCGT	This study

Table S2. Antibodies used in this study.

Antibody	source	Catalog number
Anti-Myc	Cell signaling	#2276S
Anti-phyB	Dr. Peter Quail	N/A
Anti-MBP	New England Biolabs	E8032S
Anti-GST-HRP conjugate	GE Healthcare Bio-Sciences	RPN1236
ChIP grade Anti-Myc	Abcam	Ab32
Anti-GFP	Abcam	ab6556
Anti-GFP	Thermo Fisher Scientific	A11120
Anti-PIF4	Agrisera	AS12 1860
Anti-LUC	Thermofisher	A11120