Arabidopsis Transcription Factor TCP5 Controls Plant Thermomorphogenesis by Positively Regulating PIF4 Activity

HIGHLIGHTS
TCP5, TCP13, and TCP17 positively regulate plant thermomorphogenesis
TCP5 directly interacts with PIF4 transcription factor
TCPs and PIF4 share common downstream target genes
TCP5 promotes PIF4 activity at both transcriptional and protein levels

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Arabidopsis Transcription Factor TCP5 Controls Plant Thermomorphogenesis by Positively Regulating PIF4 Activity

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SUMMARY
Plants display thermomorphogenesis in response to high temperature (HT). PHYTOCHROME INTERACTING FACTOR 4 (PIF4) is a central integrator regulated by numerous negative regulators. However, the mechanisms underpinning PIF4 positive regulation are largely unknown. Here, we find that TEOSINTE BRANCHED 1/CYCLOIDEA/PCF 5 (TCP5), TCP13, and TCP17 transcription factors promote the activity of PIF4 at transcriptional and post-transcriptional levels. TCP5 is rapidly induced by HT treatment, and TCP5 protein stability increases under HT. The overexpression of TCP5 causes constitutive thermomorphogenic phenotypes, whereas the tcp5 tcp13 tcp17 triple mutant exhibits aberrant thermomorphogenesis. We demonstrate that TCP5 not only physically interacts with PIF4 to enhance its activity but also directly binds to the promoter of PIF4 to increase its transcript. TCP5 and PIF4 share common downstream targets. The tcp5 tcp13 tcp17 mutant partially restores the long hypocotyls caused by PIF4 overexpression. Our findings provide a layer of understanding about the fine-scale regulation of PIF4 and plant thermomorphogenesis.

INTRODUCTION
The strong 2018 summer heat waves across the Northern Hemisphere caused a high temperature (HT) of up to 30°C in some areas of the Arctic Circle and also severe losses in crop production. There is an urgent need to elucidate the molecular mechanisms by which plants adapt to HT. Plant thermomorphogenesis refers to changes in plant growth, development, and morphology under HT (Stavang et al., 2009). These changes, including hypocotyl elongation, petiole elongation, and hyponastic growth, are important for plant survival under HT (Quint et al., 2016; Wigge, 2013). The basic-helix-loop-helix (bHLH) transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4) integrates HT and other environmental cues with hormonal signals and acts as a central hub in the control of thermomorphogenesis (Franklin et al., 2011; Koini et al., 2009; Sun et al., 2012), and its activity is tightly regulated by numerous negative regulators (Bernardo-Garcia et al., 2014; Box et al., 2015; Delker et al., 2014; Foreman et al., 2011; Gangappa and Kumar, 2017; Jung et al., 2016; Legris et al., 2016; Ma et al., 2016; Nieto et al., 2015; Nusinow et al., 2011; Somers et al., 1991; Song et al., 2017; Tasset et al., 2018; Toledo-Ortiz et al., 2014; Zhang et al., 2017, 2018). At the transcriptional level, PIF4 transcripts are negatively regulated by the light-signaling component LONG HYPOCOTYL 5 (HYS) (Gangappa and Kumar, 2017; Toledo-Ortiz et al., 2014) and the evening complex component EARLY FLOWERING 3 (ELF3) (Nieto et al., 2015; Nusinow et al., 2011). At the protein level, the phosphorylation of PIF4, which is required for its degradation, is mediated by the BRASSINOSTEROID-INSENSITIVE 2 (BIN2) kinase in brassinosteroid (BR) signaling and by phytochrome B (phyB), which acts as both a photoreceptor and a thermosensor (Bernardo-Garcia et al., 2014; Jung et al., 2016; Legris et al., 2016; Song et al., 2017). Recently, BLADE-ON-PETIOLE (BOP) proteins, a component of CUL3BOP1/BOP2 (CULLIN3ABOP1/BOP2) E3 ubiquitin ligase complex, have been reported to mediate PIF4 degradation (Zhang et al., 2017). Several proteins, such as CRYPTOCHROME 1 (CRY1) (Ma et al., 2016), LONG HYPOCOTYL IN FAR-RED 1 (HFR1) (Foreman et al., 2011), and ELF3 (Box et al., 2015), repress PIF4 transcriptional activity by directly interacting with PIF4. However, to date, apart from BRASSINAZOLE RESISTANT 1 (BZR1) (Ibanez et al., 2018; Oh et al., 2012, 2014), very few PIF4 positive regulators have been found. Our previous work has found that Arabidopsis transcriptional repressor SPOROCYTELESS/NOZZLE (SPL/NZZ) inhibits the activity of CINCINNATA (CIN)-like TCP family and that the overexpression of TCP5 leads to aborted ovules (Wei et al., 2015). TCP proteins are a conserved, plant-specific class of transcription factors (Martin-Trillo and Cubas, 2010). They are further grouped into Class I and Class II based on their conserved TCP domains, which are responsible for DNA binding or protein-protein interactions (Martin-Trillo and Cubas, 2010). TCPs play essential roles in the control of plant development (Aggarwal et al.,...
Figure 1. TCP5 Regulates Plant Thermomorphogenesis

(A) Phenotypes of 10-day-old wild-type, 3Spro-TCP5-9, 3Spro-TCP13-6, and 3Spro-TCP17-12 seedlings grown continuously at 20°C for 7 days before being transferred to 28°C for 3 days.

(B) Statistical analysis of the hypocotyl lengths of the plants in (A).

(C) Phenotypes of 10-day-old wild-type, tcp5, tcp13, tcp17, and tcp5 tcp13 tcp17 triple mutant seedlings after HT treatment. (D) Statistical analysis of the hypocotyl lengths of the plants in (C).

(B and D) Data represent the mean ± SD of three biological replicates. Significant differences are indicated with *p < 0.05, n > 20, two-tailed Student’s t test; **p < 0.01; ***p < 0.001. The number above each column indicates fold changes of hypocotyl length relative to wild-type control under 20°C.

(E) Relative expression levels of TCP5 in wild-type after HT treatment for 1, 2, or 4 h. The expression levels of TCP5 were normalized to that of ACT8 and were relative to that in wild-type grown under normal temperatures. Data represent the mean ± SD of three biological replicates.

(F) Stability of TCP5 protein under HT treatment. 3Spro-TCP5-FLAG plants were treated with HT for 2 and 4 h.

(G and H) GUS staining of 10-day-old TCP5pro-GUS transgenic seedlings after treatment. Seven-day-old TCP5pro-GUS seedlings grown under continuous 20°C before being transferred to 20°C (G) or 28°C (H) for 3 additional days of growth.
RESULTS AND DISCUSSION
TCP5 Positively Regulates Plant Thermomorphogenesis

We previously found that the overexpression of TCP5 leads to aborted ovules (Wei et al., 2015). Surprisingly, we observed that most 35Spro-TCP5 overexpression lines (32/41) displayed constitutive thermomorphogenesis, including long hypocotyls, long petioles, and increased leaf hyponasty under normal temperatures (Figures 1A, 1B, and S1A–S1E), implying that TCP5 might play important roles in plant thermomorphogenesis. The protein alignments suggest that TCP5 is highly similar to TCP13 and TCP17, and that they form a small clade in the Class II TCP family (Figures S1F and S1G). To test whether TCP13 and TCP17 could be functionally redundant with TCP5, we overexpressed TCP13 or TCP17 using the Cauliflower Mosaic Virus (CaMV) 35S promoter and found that both 35Spro-TCP13 and 35Spro-TCP17 transgenic plants exhibited longer hypocotyls at 20°C and 28°C, resembling the constitutive thermomorphogenesis observed in 35Spro-TCP5 plants (Figures 1A and 1B). We then investigated the hypocotyl lengths of tcp5, tcp13, and tcp17 single mutants and of a tcp5tcp13tcp17 triple mutant under 28°C treatment for 3 days. The single mutants displayed no differences from the wild-type in hypocotyl length at 20°C or 28°C, whereas the triple mutant produced shorter hypocotyls than the wild-type under 28°C (Figures 1C and 1D). We further found that the petiole length of tcp5 tcp13 tcp17 was shorter than that of the wild-type under 28°C (Figures S1H and S1I), indicating that tcp5 tcp13 tcp17 mutants were insensitive to HT treatment. These results demonstrate that TCP5 has functional redundancy with TCP13 and TCP17 in the positive regulation of plant thermomorphogenesis.

HT Regulates TCP5 at Transcriptional and Post-transcriptional Levels

To test whether the expression level of TCP5, TCP13, and TCP17 could be regulated by HT, we first treated wild-type Arabidopsis seedlings with HT in a time course (1, 2, or 4 h). Quantitative real-time PCR (qRT-PCR) assays showed that the transcripts of TCP5, TCP13, and TCP17 were all rapidly induced by HT (Figures 1E, S1J, and S1K). PIF4 is a central regulator in plant response to HT (Franklin et al., 2011; Koini et al., 2009; Sun et al., 2012). We then tested the expression levels of TCP5 in pif4 mutant with or without HT treatment. The results showed that the induction of TCP5 in HT was significantly reduced (Figure S1L). We further investigated TCP5 protein stability using 35Spro-TCP5-FLAG transgenic plants. The results indicated that TCP5 was more stable under HT than under normal conditions (Figure 1F). To investigate the expression pattern of TCP5, we generated a TCP5pro-GUS construct in which the GUS reporter gene was driven by the 2,400-bp TCP5 promoter. Fifteen TCP5pro-GUS transgenic lines showed similar GUS staining patterns, and strong GUS activity was observed in the cotyledons, hypocotyls, young leaf blades, and petioles (Figures 1G, 1I, 1K, and S1M–S1O). As the leaves grew, GUS staining gradually faded from the petioles and from the middle region of the blades to the leaf margins, which were mainly restricted to the leaf margins or serrations (Figures 1I, 1K, S1N, and S1O). GUS staining was also observed in the petals, anthers, pistils, and siliques (Figures S1P and S1Q). In addition, we also detected GUS signals in ovule and pollen grains in TCP5pro-GUS transgenic plants (Figures S1R and S1S). Furthermore, 28°C HT treatment for 3 days strengthened the GUS activity in the hypocotyl and cotyledons of TCP5pro-GUS-C14 plants (Figures 1G and 1H). Interestingly, HT treatment for 3 days led to strong GUS activity shifting from the leaf blades to the middle region of the blades to the leaf margins.
TCP5 Acts as a Transcriptional Activator

To determine the subcellular localization of TCP5, we generated TCP5pro-TCP5-GFP in which the TCP5 promoter was used to drive the TCP5-GFP fusion gene. We observed clear green fluorescence in the nuclei of hypocotyl cells of TCP5pro-TCP5-GFP transgenic plants, consistent with the function of TCP5 as a transcription factor (Figures S2A–S2D). We then used a GAL4-UAS-LUC reporter system (Guo et al., 2015; Tao et al., 2013) to test transactivation activity of TCPs. When the reporter was co-transformed with either GAL4DBD-PIF4 or GAL4DBD-TCP5, constructs in which the CaMV 35S promoter drives PIF4 or TCP5 fused to the sequence encoding the GAL4 DNA-binding domain (DBD), the LUC reporter gene was clearly activated, whereas in the negative controls, no fluorescence signal was observed (Figure S2E), indicating that TCP5 and PIF4 were transactivators. We further showed that the TCP domain was required for the transactivation activity of TCP5 because a truncated TCP5 with the TCP domain deleted could not activate the expression of reporter gene (Figure S2F). We also demonstrated that TCP13 and TCP17 were transactivators and that the TCP domain was required for their transactivation activity (Figures S2G and S2H).

TCP5 Interacts with PIF4

TCP5 and PIF4 Regulates Common HT-Induced Genes

To identify the downstream targets of TCP5 and provide more evidence supporting the association of TCP5 with PIF4, we conducted RNA sequencing (RNA-seq) transcriptome analysis using petioles or blades from wild-type grown at normal temperatures (20°C), and wild-type, tcp5 tcp13 tcp17 mutants, and pif4 mutants treated with HT (28°C) for 6 h. We identified 1,081 differentially expressed genes (DEGs) (false discovery rate < 0.01; fold change ≥ 2.0 or ≤ -2.0) (430 HT-induced genes and 651 HT-repressed genes) in petioles.
Figure 3. TCP5 and PIF4 Co-regulate Common Target Genes

(A) The Venn diagram indicates the numbers of differentially expressed genes (DEGs) coregulated by high temperature (HT), PIF4, and TCPs.
(B) Heatmap of 525 DEGs coregulated by HT and PIF4.
As the transcriptomic changes in petioles were more obvious than those in blades, we then focused on comparing the petiole transcriptomes of tcp5 tcp13 tcp17 or pif4 mutants with those of wild-type under HT (28°C). Among the 1,396 PIF4-regulated petiole genes (DEGs between pif4 mutants and wild-type under 28°C) (Table S2), more than 37% (525/1,396) of the genes were shared with HT-regulated genes (DEGs from wild-type with HT treatment) (Figure 3A), whereas almost 45% (224/491) of TCP-regulated petiole genes (DEGs between tcp5 tcp13 tcp17 mutant and wild-type under 28°C) (Table S2) were included in HT-regulated genes (Figure 3A), suggesting that both PIF4 and TCPs played important roles in thermomorphogenesis. Interestingly, approximately 72% (356/491) of TCP-regulated petiole genes were also regulated by PIF4 in petioles (Figure 3A), indicating that both PIF4 and TCPs played important roles in thermomorphogenesis.

Figure 3. Continued
(C) Heatmap of 356 DEGs coregulated by PIF4 and TCPs.
(D) Heatmap of 224 DEGs coregulated by PIF4 and TCPs.
(E) Trend lines of 208 DEGs upregulated by HT and PIF4 suggested that most of these genes were also regulated by TCPs. The gray lines represent the relative expression of each gene. The blue line represents the mean relative expression of all genes.
(F) qRT-PCR analysis of known PIF4 direct target genes in TCP5-inducible pER8-iTCP5 transgenic plants. The pER8-iTCP5 transgenic plants were treated with 50 μM estradiol or DMSO for 1, 2 or 4 h. The gene expression levels were normalized to that of ACT8 and are relative to that in pER8-iTCP5 treated with DMSO. The data represent the mean ± SD of three biological replicates.
(G) EMSA showed that TCP5 directly binds the promoter of PRE1.
(H) EMSA indicated that TCP5 directly binds the promoter of YUC8.
(I) Diagrams showing TCP5 binding motif in the promoter region of YUC8 and PRE1. Y1-F/Y1-R, P1-F/P1-R, and P2-F/P2-R indicate PCR primer pairs used in (J).
(J) ChiP-PCR assays showed that TCP5 bound to the promoter region of YUC8 and PRE1. 14-day-old wild-type (WT) and 35Spro-TCP5-FLAG plants grown under 20°C condition were collected for ChiP-PCR using the primer pairs as shown in (I). The data were first normalized to ACT8 and then were relative to that of input DNA samples. The data represent mean ± SD of three biological replicates.

See also Figure S3, Table S1, S2, and S3.

TCP5 and PIF4 Shares Direct Target Genes including PRE1 and YUC8

To find the direct target genes of TCP5, we first analyzed whether the downstream genes of PIF4 and HT could also be regulated by TCPs. Among 525 genes coregulated by HT and PIF4, 208 genes were upregulated. We retrieved the expression data for these 208 genes in petioles from RNA-seq data (Table S3). H-cluster (hierarchical cluster) analysis showed that the expression levels of most genes in tcp5 tcp13 tcp17 mutants under HT were lower than those in wild-type under HT but higher than those in pif4 mutants under HT (Figure 3E), implying that TCPs could enhance PIF4 activity. These 208 genes include many PIF4 direct target genes, such as PRE1 (Bai et al., 2012; Oh et al., 2012), IAA19 (Sun et al., 2013), and YUC8 (Sun et al., 2012) (Figure S3G). We further used qRT-PCR to test the changes in the expression of known PIF4
Figure 4. TCP5 Regulates PIF4 Activity at Both the Transcriptional and Post-transcriptional Levels

(A and B) The overexpression of PIF4 rescued the aberrant hypocotyl elongation of the tcp5 tcp13 tcp17 triple mutant after HT treatment. (C) The hypocotyl length of wild-type, tcp5 tcp13 tcp17, 35Spro-PIF4-Myc, tcp5 tcp13 tcp17, and 35Spro-PIF4-Myc under 20°C and 28°C. Data represent the mean ± SD of three biological replicates, n = 20. Significant differences are indicated with ***p < 0.001. The number above each column indicates fold changes of hypocotyl relative to wild-type control under 20°C.

(D–F) qRT-PCR assays indicated the transcripts of (D) YUC8, (E) PRE1, and (F) IAA19 in plants from (A) and (B). Seven-day-old seedlings grown under 20°C and then transferred to 20°C or 28°C for 2 h before harvesting total RNAs. Data represent the mean ± SD of three biological replicates. Significant differences are indicated with ***p < 0.001.

(G) Transient dual-luciferase expression in tobacco leaves showed that TCP5, with PIF4, strengthened the activation of PRE1. (H) Transient dual-luciferase expression analysis in tobacco leaves indicated that TCP5, with PIF4, strengthened the activation of YUC8.

(I) The abundance of PIF4 protein in the tcp5 tcp13 tcp17, wild-type, or 35Spro-TCP5-FLAG backgrounds. Actin was used as an internal control. This experiment was repeated three times.

(J) EMSA showed that TCP5 directly bound the promoter of PIF4 gene.

(K) ChIP-PCR assays showed that TCP5 bound to the promoter region of PIF4. 14-day-old wild-type and 35Spro-TCP5-FLAG plants grown under 20°C were collected for ChIP-PCR using the primer pairs as presented in top panel. The data were first normalized to ACT78 and then relative to that in input DNA samples. The data represent the mean ± SD of three biological replicates.

(L) Transient expression assays demonstrated that TCP5 directly regulated the PIF4. The activity of REN was used as an internal control for normalization. The fluorescence ratio of LUC/REN represents the mean ± SD of five biological replicates. Significant differences are indicated with ***p < 0.001.

(M) A working model of TCPs in the regulation of thermomorphogenesis. HT induces TCP5 transcription and stabilizes TCP5. The TCP5 that accumulates under HT can directly promote PIF4 transcription and stabilize PIF4 protein. Moreover, TCP5 enhances PIF4 transactivation activity by interacting with PIF4. Many elongation- and auxin-related genes, such as YUC8 and PRE1, were activated to promote hypocotyl and petiole elongation during thermomorphogenesis.

Scale bars, 5 mm in (A and B). See also Figure S4.
and 4H), suggesting that TCP5 and PIF4 act together to strengthen the activation of their coregulated genes. We then investigated whether TCP5 could prevent the degradation of PIF4 protein. We crossed the 3Spro-PIF4-Myc plants with 3Spro-TCP5-FLAG plants and tcp5 tcp13 tcp17 mutant. We tested the abundance of PIF4-Myc protein and found that PIF4-Myc largely accumulated in 3Spro-TCP5-FLAG plant, but showed low abundance in the tcp5 tcp13 tcp17 mutants (Figure 4I), suggesting that TCPs may have protected the PIF4 protein from degradation. In addition to the promotion of PIF4 stability by TCP5, the expression level of PIF4 was strongly downregulated in tcp5 tcp13 tcp17 under HT in our RNA-seq data (Figure S4F). Using qRT-PCR, we confirmed that PIF4 was indeed highly repressed in tcp5 tcp13 tcp17 under HT (Figure S4G). To test whether TCP5 might directly regulate PIF4 gene, we used estradiol to treat pER8-iTCP5 transgenic plants and found that PIF4 transcripts rapidly increased (Figure S4H). We searched the promoter of PIF4 and found two possible TCP-binding sites. EMSA analysis and ChIP-qPCR indicated that the two binding sites were required for the direct binding of TCP5 to the promoter of PIF4, whereas mutations in the binding site disrupted TCP5 binding to the PIF4 promoter in EMSA (Figures 4J and 4K). We further used the PIF4 promoter or a PIF4-mutated promoter to drive the LUC reporter in PIF4pro-LUC or PIF4pro(mut)-LUC. Transient expression assays confirmed that TCP5 could directly bind to the promoter of PIF4 so that PIF4 expression could be activated (Figure 4L). These data suggest that TCP5 promotes the activity of PIF4 at both transcriptional and post-translation levels.

In this study, we demonstrate that TCP5 acts as an important component in the control of plant thermomorphogenesis by boosting PIF4 activity. We showed that TCP5 is rapidly induced by HT and that HT stabilizes TCP5 proteins. The overexpression of TCP5 causes constitutive thermomorphogenesis under normal growth temperature, whereas thermomorphogenesis is compromised in the tcp5 tcp13 tcp17 triple mutant. We propose a working model for the regulation of plant thermomorphogenesis by TCP5 (Figure 4M). HT promotes the transcription of TCP5 and the stability of TCP5 protein. On the one hand, TCP5 directly binds the PIF4 promoter and upregulates the transcriptional level of PIF4; on the other hand, TCP5 directly interacts with PIF4 to promote the stability and activity of PIF4 protein. TCP5 and PIF4 coactivate many HT-inducible genes to stimulate the growth of hypocotyls and petioles. Our data demonstrate that TCP5 acts as a positive regulator of plant response to HT. TCPs control the biosynthesis of multiple plant hormones (Barkoulas et al., 2007; Challa et al., 2016; Danisman, 2016; Gonzalez-Grandio et al., 2017; Guo et al., 2010; Lopez et al., 2015; Nicolas and Cubas, 2016; Schommer et al., 2008; Steiner et al., 2012; Wang et al., 2015), including auxin (Challa et al., 2016; Koyama et al., 2010), BRs (Guo et al., 2010), jasmonic acid (Schommer et al., 2008), salicylic acid (Wang et al., 2015), and abscisic acid (Gonzalez-Grandio et al., 2017). Our findings suggest that TCPs might function as important relays that convert environmental cues such as HT into endogenous signals during plant morphological response to ever-changing environments.

Limitations of the Study
In this study, we find that a clade of TCP transcription factors that include TCP5, TCP13, and TCP17 act as important positive regulators by boosting PIF4 activity at both transcriptional and protein levels during thermomorphogenesis. Although this study provides a mechanism for fine-tuning PIF4 activity during plant responses to HT, the relationships between TCPs and other PIF4 interactors including BZR1 and phyB are still unknown. The coordination of TCPs with other numerous regulators in the tight control of PIF4 activity and plant thermomorphogenesis needs to be subsequently studied in future.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.04.005.

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Author Contributions

X.H. and G.Q. designed all experiments, analyzed the data and wrote the manuscript. X.H. performed most of the experiments and collected the data. H.Y. generated the estradiol-inducible TCP5 construct and pER8-TC5 transgenic plants. H.Y., R.Y., Y.Y., and F.A. contributed to the generation of transgenic plants and the analyses of phenotypes and RNA-seq data.

Declaration of Interests

The authors declare no competing interests.

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Supplemental Information

*Arabidopsis* Transcription Factor TCP5
Controls Plant Thermomorphogenesis
by Positively Regulating PIF4 Activity

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Figure S1. TCP5 and its close homologs participate in plant thermomorphogenesis, Related to Figure 1.
(A-C) 21-day-old wild-type (A), 35Spro-TCP5-Flag-9 (B) and 35S-TCP5- FLAG-16 (C) plants. The leaves grow upward in 35Spro-TCP5-Flag at 20°C. (D) Dissected leaves of 21-day-old wild-type and 35Spro-TCP5-Flag-9 plants at 20°C. (E) Quantitative analysis of length of the 4th and 5th petioles from wild type and 35Spro-TCP5-Flag-9. Significant differences are indicated with *** (P < 0.01, n>20, two-tailed Student’s t-test). The number above each column means fold changes of petiole length relative to wild type. (F) Protein alignment assays showing that TCP5, TCP13 and TCP17 share a conserved noncanonical bHLH domain. (G) Phylogenetic analysis of the full lengths of the class II TCP family in Arabidopsis. TCP5, TCP13 and TCP17 are grouped in a small subclade, suggesting that they have high similarity. The phylogenetic tree was constructed using MEGA 6.0 with default settings. (H) 26-day-old wild type, pif4 mutant and tcp5tcp13tcp17 mutant. 21-day-old plants grown in continuous 20°C before transferring to 20°C or 28°C for 5 additional days of growth. (I) Quantitative analysis of the length of the 5th petiole of wild type, pif4 mutant and tcp5tcp13tcp17 mutant. The data represent the mean ± SD of three biological replicates. Significant differences are indicated with * (P < 0.05, n>20) and *** (P < 0.001). The number above each column means fold changes of hypocotyl length relative to that of wild type under 20°C. (J) and (K) Relative expression levels of TCP13 (J) and TCP17 (K) in wild type after HT treatment for 1, 2 or 4 hours. The expression levels were normalized to that of ACT8 and were relative to that of wild-type control under 20°C. Data represent the mean ± SD of three biological replicates. (L) The expression changes of TCP5 in pif4 mutant under 20°C or 28°C. (M) GUS staining of 10-day-old TCP5pro-GUS transgenic seedlings. (N) GUS staining of 21-day-old TCP5pro-GUS transgenic plants. (O) GUS staining of 28-day-old TCP5pro-GUS transgenic plants. (P) GUS staining of inflorescence from 35-day-old TCP5pro-GUS transgenic plants. (Q) GUS staining of flowers from 35-day-old TCP5pro-GUS transgenic plants. (R) GUS staining of ovules from 35-day-old TCP5pro-GUS transgenic plants. (S) GUS staining of pollen grains from 35-day-old TCP5pro-GUS transgenic plants. Scale bars, 1 cm in (A-C), 1.5 mm in (D), 2 cm in (H), 1 mm in (M-Q), 50 µm in (R) and (S).
Figure S2. The subcellular localization of TCP5 and the transactivation activity of TCP5, TCP13 and TCP17, Related to Figure 2.

(A) Green fluorescence detected in the hypocotyls of TCP5pro-TCP5-GFP seedlings. (B) Nuclei stained with DAPI. (C) Bright field image of TCP5pro-TCP5-GFP hypocotyl. (D) Image merge of DAPI and GFP. (E) PIF4 and TCP5 act as transactivators. (F-H) TCP5, TCP13 and TCP17 work as transactivators, and their transactivation activity is dependent on the bHLH domains of TCP5 (F), TCP13 (G) and TCP17 (H). (I) Yeast two hybrid assays showed that TCP5 interacted with PIF1, PIF3, PIF5 and PIF7. Co-transformed yeast cells were grown on medium lacking Leu and Trp (SD-Leu-Trp) and selected on medium lacking Leu, Trp and His (SD-Leu-Trp-His) with or without 10 mM 3-AT at dilutions of 10 and 100 fold. The empty vector pDEST22 was used as a negative control. Scale bars, 10 µm in (A-D).
Figure S3. Transcriptomes regulated by HT in petioles and leaf blades, and the target genes regulated by TCPs, Related to Figure 3.
(A) Venn diagrams of genes upregulated by HT in petioles and leaf blades. (B) Venn diagrams of genes downregulated by HT in petioles and leaf blades. (C) Heat map of genes upregulated by HT in petioles and leaf blades. (D) Heat map of genes downregulated by HT in petioles and leaf blades. Scale bars indicate the fold changes of the DEGs. (E) GO analysis of genes up-regulated by both PIF4 and TCPs in petioles. (F) GO analysis of down-regulated by both PIF4 and TCPs in petioles. GO analysis were performed by DIVID 6.8 with default settings. (G) Diagrams of relative FPKM values of PIF4 direct target genes from RNA-seq data in WT at 20°C and 28°C, pif4 at 28°C and tcp5tcp13tcp17 at 28°C. (H) qRT-PCR analysis of the expression levels of TCPs- and PIF4-coregulated genes from hypocotyls. 7-day-old wild-type, pif4 mutant and tcp5tcp13tcp17 mutant seedlings grown under continuous 20°C before transferring to 28°C for additional 2 hours. ACT8 was used as an internal control. (I) qRT-PCR analysis of the expression levels of TCPs- and PIF4-coregulated genes from petioles. 21-day-old, pif4 and tcp5tcp13tcp17 mutant grown in continuous 20°C before transferring to 28°C for additional 2 hours. ACT8 was used as an internal control. (J) Estradiol- or mock-induced pER8-iTCP5-1 and pER8-iTCP5-4 transgenic plants. 10-day-old seedlings which were first grown for 3 days and then treated with 50 μM estradiol or DMSO for 7 days under 20°C. (K) Quantitative analysis of the hypocotyl lengths of pER8-iTCP5-1 and pER8-iTCP5-4 in (J). These data represent the mean ± SD of three biological replicates. Significant differences are indicated with *** (P < 0.001, n>20). The number above each column means fold changes of hypocotyl length relative to mock treatment. Scale bar=5 mm in (J).
Figure S4. Genetic and regulation analysis between TCP5 and PIF4. Related to Figure 4.

(A-E) Overexpression of TCP5 could not rescue the insensitivity of pif4 mutant in response to HT. The hypocotyl of wild type (A), pif4 (B), 35Spro-TCP5-Flag (C) and 35Spro-TCP5-Flag pif4-1 (D) and 35Spro-TCP5-Flag pif4-2 (E) under 20℃ and 28℃. (F) Diagrams of relative FPKM values of PIF4 gene from RNA-seq data in wild type at 20℃, wild type treated with 28℃, tcp5tcp13tcp17 treated with 28℃. (G) qRT-PCR analysis of the expression level of PIF4 gene from hypocotyls. 7-day-old wild-type and tcp5tcp13tcp17 seedlings grown under continuous 20℃ before transferring to 28℃ for 2 additional hours. ACT8 was used as an internal control. The data represent the mean ± SD of three biological replicates. (H) qRT-PCR analysis of PIF4 in TCP5-inducible pER8-iTCP5 transgenic plants. The pER8-iTCP5 transgenic plants were treated with 50 μM estradiol or DMSO for 2 hours. The gene expression levels were normalized to that of ACT8 and are relative to that in pER8-iTCP5 treated with DMSO. The data represent the mean ± SD of three biological replicates.
Table S4. The list of oligonucleotides used in this study. Related to Figure 1, 2, 3 and 4.

| Oligonucleotide name | sequence (5' - 3') |
|----------------------|--------------------|
| **Oligonucleotides for constructs** |
| PIF4-F | CACCATGGAACACCAAGGTGG |
| PIF4-R-NSC | TCCGTGTTCCAAAACGAGAACCGTC |
| PIF4-R-SC | CTATCCCTGGTGCTCAAAACGAGAACCGTC |
| TCP5-F | CACCATGAGATCAGGAGAATGTGATGAAGAG |
| TCP5-R-NSC | AGAATCTGATTCATTATCGC |
| TCP5-R-SC | TCAAGAATCTGATTACATTACGC |
| TCP5-R1 | GGACTATTTCAAGAATCTGATTCATTAT |
| TCP13-R-NSC | CATAATTGATCAGACATTTCCTCTCA |
| TCP13-R-SC | TCACATAGTGATCGACTTCCTCTCA |
| TCP17-F | CACCATGGAATAAAAAAGAAGATCA |
| TCP17-R-NSC | CTCGATATGCTGGTGGTGTGAG |
| TCP5pro-F1 | CACCTGCCAAATCCTGTCATACATTCCCAA |
| TCP5pro-R1 | CTCTTTAATTCACTCAACAGATCTTCA |
| TCP5pro-F2 | GGGGACAACTTTGTATAGAAAAAGGCTGCAATCCCTGTCATA |
| TCP5pro-R2 | GGACTGCTCTTTTGTACAAACTTGCACTTCCTCA |
| YUC8pro-F | GGGGACAACCTTTGTATAGAAAAAGGCTGCAATCCCTGTCATA |
| YUC8pro-R | GGGGACTGCTTTTTTGTACAAACTTGAAGTGTCTTTTAATAGT |
| PRE1pro-F | GGGGACAACCTTTGTATAGAAAAAGGCTGCAATCCCTGTCATA |
| PRE1pro-R | GGGGACAACCTTTGTATAGAAAAAGGCTGCAATCCCTGTCATA |
| PIF4pro(wt)-F | GGGGACAACCTTTGTATAGAAAAAGGCTGCAATCCCTGTCATA |
| PIF4pro(wt)-R | GGGGACAACCTTTGTATAGAAAAAGGCTGCAATCCCTGTCATA |
| PIF4pro(mut)-F | ATTAGTGGGACTGCTTTTGTACAAACTTGAAGTGTCTTTTAATAGT |
| Oligonucleotides for yeast two hybrid |
| PIF4ΔN53-F | CACCATGGAATCATGGAAGACACCGCTAAGAT |
| PIF4ΔC-R | TTAATGAAACCTTCAATGCTGATCCTCT |
| PIF4ΔN-F | CACCATGATCTGGAAGACACCGCTAAGAT |
| PIF4-bHLH-F | CACCATGAAATCTCCTCCGAAAGGAGAG |
| PIF4-bHLH-R | TTACACTTGGAAGCTTAAACTGGAAGT |
| TCP5ΔN1-F | CACCATGCGCTCTTCACTTCCACATGGGATTTAAC |
| TCP5ΔTCP-R | GAAATTCTGAGACCTCGAAGACATCCTGATTC |
| TCP5ΔTCP-F | GAATCCAAAGAATCGGCTGAGTCTCAGAAGACATTC |
| TCP5ΔN2-F | CACCATGGAATCAAGACACACACAGCAAGAAG |
| Oligonucleotides for genotyping |
| Oligonucleotides for qRT-PCR |
|-----------------------------|
| **ACTIN8-qRT-F** | TGTGCCAATCTACGAGGGTTT |
| **ACTIN8-qRT-R** | TTTCCCGCTCTGCTGTGT |
| **PIF4-qRT-F** | CCAGATCATCTCGGACCGGTTTG |
| **PIF4-qRT-R** | CTAGTGGTCAAACGAGAACCCT |
| **YUC6-qRT-F** | TGGCGACGCAAGTATGACCCTG |
| **YUC6-qRT-R** | TCCGGCTTTATGCAAACCTCCGA |
| **YUC8-qRT-F** | TGTATGCGGTTGAGTTACAGAG |
| **YUC8-qRT-R** | CCTTGAGCGTTTTGCGTCATTAGG |
| **PRE1-qRT-F** | GATCTGATAAGGCGATACGTCTC |
| **PRE1-qRT-R** | CATGAGTGGCTCTGATAAACGG |
| **PRE2-qRT-F** | CCGTCGTCCAAACACCGATCCA |
| **PRE2-qRT-R** | CTTTGAGCGTCTTGTGGAAGCTT |
| **IAA19-qRT-F** | GGTGACAATCTCGGAAATCAGTT |
| **IAA19-qRT-R** | CCCGGTAGCATTACGCCGATTTCA |
| **IAA29-qRT-F** | CAGGGCGATGAAACCAACATAT |
| **IAA29-qRT-R** | CTTCTGCAGCACTTCTCTATT |
| **ACS5-qRT-F** | GCAGATGTTATCTGCTTTT |
| **ACS5-qRT-R** | TTTTCGGCTTTGTTGTGTAAGCTT |
| **SAUR23-qRT-F** | ATTCAAACCTTCGACAAGAAGATGG |
| **SAUR23-qRT-R** | ACAAGGAAACAACCTCATACTC |
| **SAUR24-qRT-F** | GAGATATTGGTGCCCTGCTCATATT |
| **SAUR24-qRT-R** | CAAGAAGGAAGGAAAAAGGGCTC |
| **TAA1-qRT-F** | CACGGCTGTGCTGCTGTG |
| **TAA1-qRT-R** | AGACGTACGTTGCGAGTCTC |
| **TCP5-qRT-F** | GGGTTTAACACCAATCATCAACAA |
| **TCP5-qRT-R** | CGACAGTAACGTGTTACGAGATT |
| **TCP13-qRT-F** | CTGGTTACGGAACAGGATC |
| **TCP13-qRT-R** | AAATGTTTTGCGAAGACGAAGATGA |
| **TCP17-qRT-F** | GTTAAACGTACTGCTGCAATT |
| **TCP17-qRT-R** | GAACAGAAAGGTACCTTGGAG |
| **TCP5-SM-LP** | TGAATCTGTTTCTCCTCATCC |
| **TCP5-SM-RP** | CTCGAAAGCAGCAAAAGATGAC |
| **TCP13-SM-LP** | TTCTGTCTCAAGCTCAAGAC |
| **TCP13-SM-RP** | GACCGACGACATCCGATTAT |
| **TCP17-SALK-LP** | ATGGACCTTTGCTCGCATCAG |
| **TCP17-SALK-RP** | AAATCATCATCGGAGTTAGG |
| **PIF4-SALK-LP** | TCGTCGTATTTAATAAACCGCC |
| **PIF4-SALK-RP** | ATTTTGCCGAT TTTCGGAAC |
### Oligonucleotides for EMSA

| Oligonucleotide             | Sequence                                      |
|-----------------------------|-----------------------------------------------|
| TCP5-6*HIS-F                | CACCTCTAGAATGATATTTAGGTGACACTATAGAACAGACCACC |
| TCP5-6*HIS-R                | CTCGAGAAAAAAAAAAAAAAAAAAATTAGGATGATGATGATGA  |
| PIF4-EMSA-F(+3')            | ATGGGATATGGTCCATTACAAGTAGGCAACAATGGTCCACTAAT |
| PIF4-EMSA-R                 | ATAACAATTATTAGTGACCATTGGCCTATTGGTAAATGGACCA  |
| PIF4-EMSA-mut-F(+3')        | ATGGGATATGGTACCAAATGGAACATGGGATGAATTAATGGACCA |
| YUC8-EMSA-F(+3')            | TTCCTTCACGTGGCTTCCTCTCTGTTGGCCACAAGGAAT      |
| YUC8-EMSA-R                 | ATCTTTTGAGGACCAAGAGGAGAGCCAGTTGAGGAGAAGAAT   |
| YUC8-EMSA-mut-              | TCTTCCACGTGGCTTCCTCTCATTGGTCCACAAGGAAT       |
| PRE1-EMSA-F(+3')            | ATGTTGGGACATGGACGTAATGGGAGGGAAGTGGGAGAAGAAT |
| PRE1-EMSA-R                 | ATTGTTCGATTGGGCGGACAGAGGGAAGCCAGTGTTGAGAAGAAT |
| PRE1-EMSA-mut-              | ATGTTGGGACATGGGCAGGGAAGGGAAGCCAGTGTTGAGAAGAAT |

### Oligonucleotides for ChIP-PCR

| Oligonucleotide             | Sequence                                      |
|-----------------------------|-----------------------------------------------|
| YUC8-Y1-F                   | ATGTGGAGATGGCGGGACCAAGGAAA                   |
| YUC8-Y1-R                   | GGGTGATCTTTGAGGACAGAAGA                      |
| PRE1-P1-F                   | CAGCATGAACAGAGACATGGG                        |
| PRE1-P1-R                   | CGGCTTTAAAACATGTCGTGACAGA                    |
| PRE1-P2-F                   | ACACAATAACTAACCAGTATGGGAAACG                |
| PRE1-P2-R                   | AATAAACCTCGAATAGTGACG                       |
| PIF4-ChIP-F                 | TGATATACATTTACGAAAACCTTACG                  |
| PIF4-ChIP-R                 | GAAAAAGATAGTATGTTCTTC                    |
Transparent Methods.

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Mouse anti-MYC antibody | CMCTAG  | Cat. AT0023 |
| Mouse anti-FLAG antibody with HRP conjugating | Sigma-Aldrich | Cat. A8592 |
| Mouse anti-plant actin antibody | Sigma-Aldrich | Cat. A0480 |
| EZview™ red anti-c-Myc affinity gel | Sigma-Aldrich | Cat. E6654 |
| Anti-FLAG® M2 Magnetic Beads | Sigma-Aldrich | Cat. M8823 |
| Goat anti-mouse HRP-conjugated IgG | CWBIO | Cat. Cw0102 |
| **Bacterial and Virus Strains** |        |            |
| E.coli strain DH5α | N/A | N/A |
| A. tumefaciens GV3101 | N/A | N/A |
| Yeast strain AH109 | N/A | N/A |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| β-Estradiol | Sigma-Aldrich | Cat. E2758 |
| DAPI (4',6-diamidino-2-phenylindole dihydrochloride) | VECTOR | Cat. H-1200 |
| 3-AT (3-amino-1,2,4 triazole) | Sigma-Aldrich | Cat. A9126 |
| D-Luciferin | Sigma-Aldrich | Cat. L0594 |
| X-gluc (5-Bromo-4-chloro-3-indolyl β-D-glucuronide) | Sigma-Aldrich | Cat. B5285 |
| **Critical Commercial Assays** |        |            |
| First-strand cDNA reverse transcription kit | Promega | Cat. A3500 |
| Dual-luciferase reporter kit | Promega | Cat. E1910 |
| TNT SP6 high-yield wheat germ protein expression kit | Promega | Cat. L3260 |
| pENTR/D-TOPO Cloning Kit | Thermo Fisher | Cat. K240020 |
| Gateway LR Clonase II Enzyme mix | Thermo Fisher | Cat. 11791020 |
| LightShift™ Chemiluminescent EMSA kit | Thermo Fisher | Cat. 20148 |
| **Experimental Models: Organisms/Strains** |        |            |
| Arabidopsis: WT Col-0 | N/A | N/A |
| Arabidopsis: tcp5 | [29] | SM_3_29639 |
| Arabidopsis: tcp13 | [29] | SM_3_23151 |
| Arabidopsis: tcp17 | [29] | SALK_147288 |
| Arabidopsis: pif4 | [12] | SALK_140393C |
| 35Spro-PIF4-MYC/tcp5tcp13tcp17 | This study | N/A |
| 35Spro-TCP5-FLAG/pif4-1 | This study | N/A |
35Spro-TCP5-FLAG/pif4-2 This study N/A
35Spro-PIF4-MYC/35Spro-TCP5-FLAG This study N/A

Recombinant DNA

TCP5pro-GUS This study N/A
TCP5pro-TCP5-GFP This study N/A
35Spro-PIF4-Myc This study N/A
35Spro-TCP5-Flag This study N/A
pER8-iTCP5 This study N/A
35Spro-TCP13-Flag This study N/A
35Spro-TCP17-Flag This study N/A
PIF4pro-LUC This study N/A
PIF4mpro-LUC This study N/A
PRE1pro-LUC This study N/A
YUC8pro-LUC This study N/A

Software and Algorithms

ImageJ N/A https://imagej.net/
Excel (2016) Microsoft Office Excel N/A
Photoshop (cc2018) Adobe N/A
MEGA (6.0) N/A https://www.mega-software.net/
DIVID (6.8) N/A https://david.ncifcrf.gov/
BMK Cloud Biomarker Technology Co. https://www.biocloud.net/

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant materials and growth conditions

The Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used as the wild type in this study. T-DNA mutants and various transgenic plants were in the Col-0 background. The T-DNA insertion mutants were tcp5, tcp13, tcp17 (Efroni et al., 2008) and pif4 (Zhang et al., 2018). The transgenic plants are described below. Wild-type, mutant and transgenic seeds were first sterilized using 15% (v/v) sodium hypochlorite solution and then plated on half-strength Murashige and Skoog medium, which was supplemented with 20 µg/mL DL-phosphinothricin, 50 µg/mL kanamycin or 50 µg/mL hygromycin B if necessary. The seeds were then synchronized at 4°C for 3 days and then placed in a 20±1°C growth chamber with long-day (16-hour light and 8-hour dark) conditions for 7 days. Green seedlings were transferred to soil and put in a 20±1°C greenhouse or growth chamber under long-day conditions. Nicotiana benthamiana was grown in soil and put in the same greenhouse for transient expression assays. For high-temperature
treatment, 7-day-old seedlings or 21-day-old plants were transferred to a 28±1℃ growth chamber for 3 days or 5 days.

**Accession numbers**

Gene annotations and data in this article can be found in The Arabidopsis Information Resource (TAIR) database using the following accession numbers: TAIR: AT5G60970 (TCP5), TAIR: AT3G02150 (TCP13), TAIR: AT5G08070 (TCP17), TAIR: AT2G43010 (PIF4), TAIR: AT5G39860 (PRE1), and TAIR: AT4G28720 (YUCCA8).

**METHOD DETAILS**

**PCR analysis and gene expression assays**

All the primers used in this study are listed in Table S4.

The T-DNA insertion mutants were genotyped using the primers TCP5-SM-LP, TCP5-SM-RP and SM-P; TCP13-SM-LP, TCP13-SM-RP and SM-P; TCP17-SALK-LP, TCP17-SALK-RP and SALK-P; or PIF4-SALK-LP, PIF4-SALK-RP and SALK-P for tcp5, tcp13, tcp17 and pif4, respectively. PCR was performed for 30 cycles (94℃ for 20 s, 52-60℃ for 20 s, and 72℃ for 90 s).

To perform qRT-PCR, total RNA from seedlings, petioles or blades were extracted by TRlZol reagent (Invitrogen) and reverse-transcribed using reverse transcription kit (Promega) according to the manufacturer’s instructions. The products were used as the templates for qRT-PCR, which was conducted in an Applied Biosystems 7500 Fast Real-time PCR system with UltraSYBR Mixture (CWBIO, CW2601) as described in the manual. The PCR conditions were 95℃ for 10 min, then performed 40 cycles under conditions of 94℃ for 10 s, 58-60℃ for 15 s, and 72℃ for 25 s. Each experiment was repeated three times. ACT8 was used as an internal control. The relative expression levels were evaluated via the 2^(-ΔΔCT) (cycle threshold) method (Livak and Schmittgen, 2001).

**Generation of binary constructs and transgenic plants**

To examine the expression pattern of TCP5, the 2400-bp promoter upstream of the TCP5 start codon was amplified using primers TCP5pro-F1 and TCP5pro-R1 and then cloned into pENTR/D-TOPO (Invitrogen) to generate TCP5pro-TOPO. The TCP5pro-GUS vectors were generated via an LR reaction with TCP5pro-TOPO and pKGWFS7 (Ghent University).

To determine the subcellular localization of TCP5, the 2400-bp promoter upstream of the TCP5 start codon was amplified using primers TCP5pro-F2 and TCP5pro-R2 and then cloned into pDONR-P4P1R (Invitrogen) to generate TCP5pro-P4P1R. And the coding region of TCP5 without stop codon was amplified using primers TCP5-F and TCP5-R-NSC and then cloned into pENTR/D-TOPO (Invitrogen) to generate TCP5NSC-TOPO. The GREEN FLUORESCENT PROTEIN (GFP) gene was amplified.
from pK7WGF2 (Ghent University) using primers GFP-F and GFP-R and then cloned into pDONR-P2R-P3 (Invitrogen) to generate GFP-P2RP3. TCP5pro-TCP5-GFP was generated via an LR reaction with TCP5pro-P4P1R, TCP5NSC-TOPO, GFP-P2RP3 and pK7m34GW (Ghent University).

To obtain the PIF4 and TCP5 overexpression lines, PIF4 without stop codon was amplified with PIF4-F and PIF4-R-NSC and cloned into pENTR/D-TOPO to generate PIF4NSC-TOPO. The 35Spro-PIF4-Myc or 35Spro-TCP5-Flag constructs were generated via an LR reaction with PIF4NSC-TOPO and pK7MYCGW2 (Li et al., 2016) or with TCP5NSC-TOPO and pK7FLAGGW2 (Li et al., 2016).

To obtain estrogen-inducible TCP5 transgenic plants, TCP5 was amplified using primer TCP5-F1, which had an Xho I site, and primer TCP5-R1, carrying a Spe I site. The PCR products and pER8 vectors were digested by Xho I and Spe I. The pER8-iTCP5 vectors were generated by ligating Xho I-Spe I-digested PCR products with the Xho I-Spe I-digested pER8 vector.

To generate 35Spro-TCP13-Flag and 35Spro-TCP17-Flag, TCP13 and TCP17 were amplified using primers TCP13-F and TCP13-R-NSC and primers TCP17-F and TCP17-R-NSC, respectively. The two genes were cloned into pENTR/D-TOPO to generate TCP13NSC-TOPO and TCP17NSC-TOPO. The 35Spro-TCP13-Flag and 35Spro-TCP17-Flag constructs were generated via an LR reaction between TCP13NSC-TOPO or TCP17NSC-TOPO and pK7FLAGGW2.

To test TCP5 binding to the PIF4 promoter, the PIF4 promoter was amplified using primers PIF4pro(wt)-F and PIF4pro(wt)-R and then cloned into pDONR-P4P1R to generate PIF4pro-P4P1R. pENTR/D-FLUC was generated as described previously (Guo et al., 2015). The PIF4pro-LUC reporter was generated via LR reactions with PIF4pro-P4P1R, pENTR/D-FLUC and pH7m24GW (Ghent University). To generate the PIF4 mutated promoter, mutations in the cis-element (5′-TGGTCC-3′ mutated to 5′-TGGGTG-3′) were included in primers PIF4pro(mut)-R and PIF4pro(mut)-F. PIF4pro(wt)-F and PIF4pro(mut)-R or PIF4pro(mut)-F and PIF4pro(wt)-R were used to amplify the two halves of the PIF4 mutated promoter. The two halves were then mixed together and used as PCR templates with primers PIF4pro(wt)-F and PIF4pro(wt)-R to obtain the PIF4 mutated promoter. The PIF4 mutated promoter was cloned into pDONR-P4P1R to generate PIF4mpro-P4P1R. The PIF4mpro-LUC reporter was generated via an LR reaction with PIF4mpro-P4P1R, pENTR/D-FLUC and pH7m24GW.

To generate PRE1pro-LUC and YUC8pro-LUC, the PRE1 and YUC8 promoters were amplified using PRE1pro-F and PRE1pro-R or YUC8pro-F and YUC8pro-R. The fragments were cloned into pDONR-P4P1R to generate PRE1pro-P4P1R or YUC8pro-P4P1R. The PRE1pro-LUC and YUC8pro-LUC reporter vectors were generated via an LR reaction with PRE1pro-P4P1R or YUC8pro-P4P1R, pENTR/D-FLUC and pH7m24GW.

The binary constructs were transformed into Agrobacterium strain GV3101 by electroporation. Transgenic Arabidopsis were generated using the floral dip method.
For genetic analysis, 35Spro-PIF4-Myc tcp5tcp13tcp17 was obtained by crossing 35Spro-PIF4-Myc with tcp5tcp13tcp17. The 35Spro-PIF4-Myc 35Spro-TCP5-Flag line was obtained by crossing 35Spro-PIF4-Myc with 35Spro-TCP5-Flag.

**Staining and microscopy**

Tissues from TCP5pro-GUS lines were fixed in 90% acetone overnight at 4°C and then washed three times using 1× phosphate buffer (pH 6.0). The samples were then immersed into GUS staining buffer solution (1 mg/mL X-gluc in phosphate buffer) and placed under vacuum for 2 hours. The samples were kept in a 37°C incubator for 12~24 hours and then transferred into 75% ethanol to stop staining before observation.

To observe subcellular localization, 7-day-old TCP5pro-TCP5-GFP seedlings were treated with DAPI staining solution for 5-10 min and observed using a Leica TCS SPE confocal microscope. The GFP signal was observed at 488 nm, and the DAPI signal was observed at 340 nm.

**The measurement of hypocotyl and petiole lengths**

Pictures of hypocotyls or petioles were first taken using a Canon digital camera, and their lengths were then measured using ImageJ software. To analyze hypocotyl elongation under HT, 7-day-old seedlings growing at 20°C were transferred to 28°C for further growing 3 days before being photographed. To analyze petiole elongation under HT, 21-day-old plants growing under 20°C were transferred to 28°C for further growing 5 days before being photographed. The experiments were repeated three times. The data were then analyzed using Microsoft Excel, and statistical significance was determined using Student’s t test (n > 20).

**Yeast two-hybrid assays**

To test the PIF4 and TCPs interaction, prey constructs PIF4-AD or TCPs-AD were generated via LR reactions between PIF4-TOPO or TCPs-TOPO and pDEST22 (Invitrogen). To generate the bait constructs, a truncated PIF4Δ53 with the first 53 amino acids of the N-terminus deleted was used because full-length PIF4 can activate the reporter in yeasts. PIF4Δ53 was amplified from PIF4-TOPO plasmids using primers PIF4Δ53-F and PIF4-R-SC and cloned into pENTR/D-TOPO to generate PIF4Δ53-TOPO. The bait constructs DBD-PIF4Δ53 and DBD-TCPs were generated via an LR reaction between PIF4Δ53-TOPO or TCP-TOPO and pDEST32 (Invitrogen). Each bait construct was cotransformed with each prey construct or empty pDEST22 into yeast strain AH109 (Clontech).

To further determine the PIF4 region responsible for the interaction with TCP5, truncated *PIF4* fragments were amplified using primers PIF4-F and PIF4ΔC-R, PIF4ΔN-F and PIF4-R-SC or PIF4-bHLH-F and PIF4-bHLH-R and were then cloned
into pENTR/D-TOPO to generate PIF4ΔC-TOPO, PIF4ΔN-TOPO and PIF4-bHLH-TOPO. The prey constructs PIF4ΔC-AD, PIF4ΔN-AD and PIF4-bHLH-AD were generated via an LR reaction between PIF4ΔC-TOPO, PIF4ΔN-TOPO or PIF4-bHLH and pDEST22. The DBD-TCP5 vector was cotransformed with each prey construct or empty pDEST22 into yeast strain AH109. The bait DBD-TCP5 was cotransformed with each prey construct or empty pDEST22 into yeast strain AH109.

To determine the region in TCP5 responsible for the interaction with PIF4, truncated TCP5 fragments were amplified using primers TCP5ΔN1-F and TCP5-R, TCP5-F and TCP5ΔTCP-R, TCP5ΔTCP-F and TCP5-R, and TCP5ΔN2-F and TCP5-R and were then cloned into pENTR/D-TOPO to generate TCP5ΔN1-TOPO, TCP5ΔTCP-TOPO and TCP5ΔN2-TOPO. The bait constructs were generated via an LR reaction between pDEST32 and TCP5ΔN1-TOPO, TCP5ΔTCP-TOPO or TCP5ΔN2-TOPO. Each bait construct was cotransformed with PIF4-AD or empty pDEST22 into yeast strain AH109.

The yeast was grown in a 30°C incubator for 3 days. Selection was conducted using SD-Leu-Trp-His (GeneStar) medium with or without 10 mM 3-AT.

**Protein extraction and immunoblotting**

Plant tissues were ground in liquid nitrogen and then suspended in native buffer (50 mM Tris-MES, pH 8.0, 0.5 M sucrose, 1 mM MgCl2, 10 mM EDTA, 1 mM PMSF) with 5 mM DL-dithiothreitol (DTT) and 1×complete protease inhibitor. The mixtures were kept on ice for 30 min and then centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant was combined with 5×loading buffer (60 mM Tris-HCl, 20% glycerol, 8% SDS, 0.5% Coomassie Brilliant Blue, 2% mercaptoethanol and 10 mM DTT) and then loaded onto 12% SDS/PAGE gels for electrophoresis.

To confirm the interaction between PIF4 and TCPs via co-immunoprecipitation assays, 35Spro-PIF4-Myc and 35Spro-TCPs-Flag were coinfiltrated into tobacco leaves for transient expression as described previously. Total protein from tobacco leaves or transgenic Arabidopsis transformed with both 35S-PIF4-Myc and 35S-TCP5-Flag was extracted and incubated with anti-Flag beads overnight. Anti-Myc or anti-Flag antibodies were used to detect TCP5-Flag or PIF4-Myc. The membranes were incubated in anti-Myc or anti-Actin antibodies diluted 5,000-fold in 3% milk. Anti-Flag antibody conjugated with HRP was diluted 10,000-fold and incubated with the membrane in 3% milk. The secondary goat anti-mouse HRP-conjugated IgG was diluted 5,000-fold. Stable peroxide solution and luminol/enhancer solution (Thermo Scientific, SJ257615) were used for HRP-based detection.

**Firefly luciferase complementation imaging**

To test the PIF4 interaction with TCPs *in vivo*, PIF4-nLUC was generated via an LR reaction between PIF4-TOPO and pCB1300-nLUC-GW (Zhang et al., 2017). cLUC-
TCPs constructs were generated via an LR reaction between TCPs-TOPO and pCB1300-cLUC-GW (Zhang et al., 2017). PIF4-nLUC and cLUC-TCPs were transformed into Agrobacterium strain GV3101. Different combinations of plasmids were coinfiltrated into tobacco leaves. The plants were grown for 72 hours under long-day conditions. The tobacco leaves were sprayed with 100 mM D-luciferin and kept in the dark for 15 min. The fluorescence was detected using a low-light cooled charge-coupled device (CCD) imaging apparatus (NightOWL II LB983) with indiGO software.

**Transient expression analysis in tobacco leaves**

The reporter constructs including PRE1pro-LUC, YUC8pro-LUC, PIF4pro-LUC, and PIF4mpro-LUC and the constructs 35Spro-TCP5-Flag and 35Spro-PIF4-Myc were as described above. The control construct, 35Spro-Flag, was generated via an LR reaction between 3Flag-TOPO and pB7FLAGGW2. The constructs were transformed into Agrobacterium strain GV3101. Each reporter was cotransformed into tobacco leaves with pCambia-1300-P19, 35Spro-REN (Guo et al., 2015; Zhang et al., 2017), 35Spro-TCP5-Flag and/or 35Spro-PIF4-Myc, or the control constructs. After incubation for 3 days, the tobacco leaves were treated with luciferin and observed using a CCD imaging apparatus. The firefly (*Photinus pyralis*) and Renilla (*Renilla reniformis*) luciferase activities were measured using dual-luciferase reporter kits with GloMax® 20/20 luminometer (Promega). The fluorescence intensity of REN was used as an internal control. At least three biological repeats were performed.

For dual-luciferase reporter assays of PIF4pro-LUC or PIF4mpro-LUC and TCP5, experimental groups combined with PIF4pro-LUC or PIF4mpro-LUC and 35Spro-3×Flag or PIF4pro-LUC and 35Spro-TCP5-Flag or PIF4mpro-LUC and 35Spro-TCP5-Flag were cotransformed with pCambia-1300-P19 and 35S-REN into tobacco leaves. After 3 days of incubation, the tobacco leaves were treated with luciferin and observed using a CCD imaging apparatus.

**Electrophoretic mobility shift assay (EMSA)**

An 84-bp probe containing possible TCP binding motifs (5′-GGACCA-3′) in the *PRE1* promoter and its corresponding control probe, in which only the motif (5′-GGACCA-3′) was mutated to 5′-CACCCA-3′, were synthesized and labeled with biotin at the 3′-end by a company (Invitrogen). A 41-bp probe containing possible TCP binding motifs (3′-GGACCA-5′) in the *YUC8* promoter and its corresponding control probe, in which the motif was mutated to 3′-CAGCCA-5′, were synthesized and labeled with biotin at the 3′-end. A 52-bp probe containing possible TCP binding motifs (5′-GGACCA-3′) in the *PIF4* promoter and its corresponding control probe, in which the motif was mutated to 5′-CACCCA-3′, were synthesized and labeled with biotin at the 3′-end. The TCP5-His protein was expressed using a TNT SP6 high-yield wheat germ protein expression system in vitro according to the manufacturer’s instructions. The double-stranded
probes were acquired by annealing equimolar concentrations of both complementary oligoes in annealing buffer (10 mM Tris, pH 7.5, 1 mM EDTA, and 50 mM NaCl). EMSAs were performed using a LightShift™ Chemiluminescent EMSA kit according to the manual. Reaction mixtures containing TCP5 protein and probes were incubated at room temperature for 30 minutes, and the products were loaded onto 12% nondenaturing polyacrylamide gels.

RNA-seq analysis
Wild type, pif4 and tcp5tcp13tcp17 plants were grown at 20°C for 3 weeks and then transferred to a 28°C growth chamber for 3 days. Total RNA was extracted from the petioles of wild type, pif4 or tcp5tcp13tcp17 plants treated with HT for 3 days and from the petioles of the control, which was the wild type continually grown under 20°C. RNA-seq was performed on a HiSeq Illumina Hisequation 2000 platform in the Biomedical Pioneering Innovation Center (BIOPIC) of Peking University. The clean reads were separated from random adapters and low-quality reads. The RNA-seq data were analyzed using a platform from Biomarker Technology Co. (Beijing, China, https://www.biocloud.net/). To find differentially expressed genes (DEGs), HISAT2 (http://ccb.jhu.edu/software/htsat2/index.shtml) was used to align the reads to the TAIR10 and StringTie (https://ccb.jhu.edu/software/stringtie/index.shtml) was used to normalize and calculate the FPKM (fragments per kilobase of exon per million fragments mapped) values as the expression value of each gene. To find the DEGs, RNA-seq data from two samples were analyzed using EBseq (R package version 1.20.0). The false discovery rate (FDR) < 0.01 and fold change ≥2 or ≤ -2 were set as the threshold for significantly differential expression. Venn diagrams were made using VENNY (http://bioinfogp.cnb.csic.es/tools/venny/) with default settings. Heat maps were made using a platform from Biomarker Technology Co. GO analysis were made using DIVID 6.8 (Database for Annotation, Visualization and Integrated Discovery, https://david.ncifcrf.gov/) with default settings.

Chromatin immunoprecipitation PCR (ChIP-PCR)
For ChIP-PCR assays, wild-type control and 35Spro-TCP5-Flag transgenic plants were grown under 20°C for 14 days after germination. Chromatins were sonicated to chip DNA into 200 bp to 500 bp fragments, and then were enriched by Anti-FLAG® M2 Magnetic Beads (Sigma). The DNA fragments was used as templates for qRT-PCR analysis. Relative levels of IP/Input was first normalized by ACTIN8 (ACT8), and then was compared with input DNA samples.

QUANTIFICATION AND STATISTICAL ANALYSIS
Statistical analyses were performed using Microsoft Office Excel 2016. Details of the statistical tests applied, including the statistical methods, number of replicates, mean and error bar details and significances, are indicated in the relevant figure legends. All replicates are biological, unless otherwise noted in the figure legend.