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Supplemental Information

Direct Control of *SPEECHLESS* by PIF4 in the High-Temperature Response of Stomatal Development

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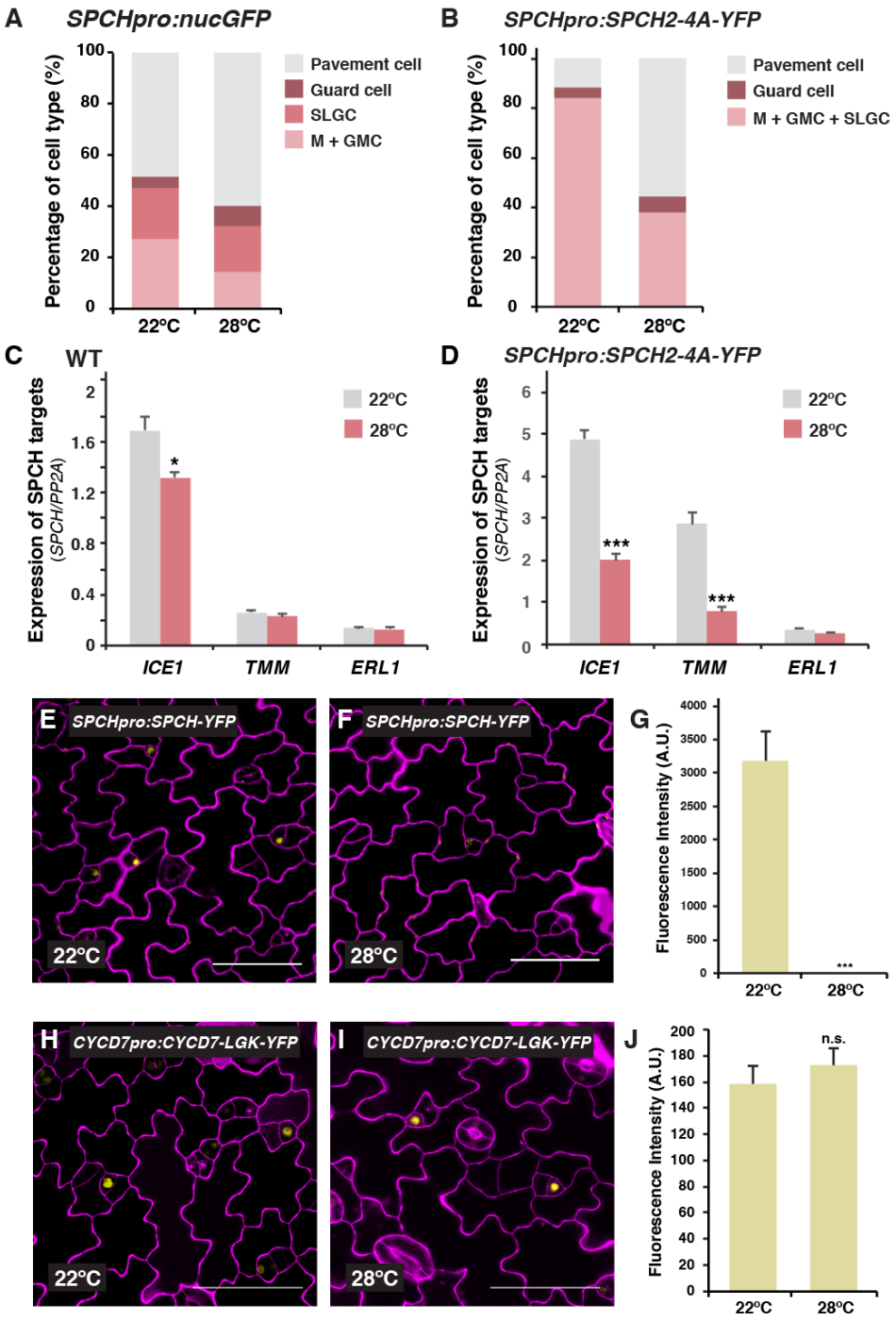

Figure S1. Effect of high temperature on epidermal cell populations, the expression of SPCH targets and the expression of the translational reporter of *SPCH* and *CYCLIND7* (*CYCD7*), Related to Figure 1. (**A** and **B**) Quantification of epidermal cell types of 3-day-old abaxial cotyledons of *SPCHpro:nucGFP* (A) and *SPCHpro:SPCH2-4A-YFP* (B) grown at either 22°C or 28°C, using the same samples as in Figure 1C to J. For (B), due to the over-proliferation of the stomatal precursors in *SPCHpro:SPCH2-4A-YFP*, distinguishing meristemoids and SLGCs is challenging and thus they are grouped in one category. M: Meristemoid; GMC: Guard mother cell; SLGC: Stomatal-lineage ground cell. (**C** and **D**) Gene expression analysis of three gene targets of SPCH: *ICE1*, *TMM*, and ERL1 [S1] in WT (C) and *SPCHpro:SPCH2-4A-YFP* (D) by RT-qPCR. RNA was extracted from 3-day-old seedlings that were grown at 22°C (grey) or 28°C (red) before harvest. Values are mean +/- SEM, n = 3. Student's t-test (gene-specific comparison), ***, $p<0.001$; *, $p<0.05$. (**E** to **G**) Confocal images of 3-day-old abaxial cotyledons of *SPCHpro:SPCH-YFP* grown at either 22°C (E) or 28°C (F). Images were taken with the same acquisition setting, and at this setting, YFP signals from (E) were just below saturation while those from (F) were not detectable. Note that images were taken on a different microscope, which has a lower dynamic range, than those from Figure 1. Fluorescence intensity of the YFP-expressing cells are quantified (G) (**H** to **I**) Confocal images of 3-day-old abaxial cotyledons of *CYCD7pro:CYCD7-LGK-YFP* [S2,S3] grown at either 22°C (H) or 28°C (I). Fluorescence intensity of the YFP-expressing guard mother cells are quantified (J). The lower number of cells expressing this GMC marker is a secondary effect of fewer stomatal lineage cells being produced at 28°C compared to 22°C. Images were taken with the same laser and acquisition time settings. Cell outlines were visualized with propidium iodide (E, F, H and I; magenta). Scale bar, 50 μm. (G, J) Values are mean +/- SEM, n ≥ 7. Student's t-test, ***, $p<0.001$; n.s., not significant.

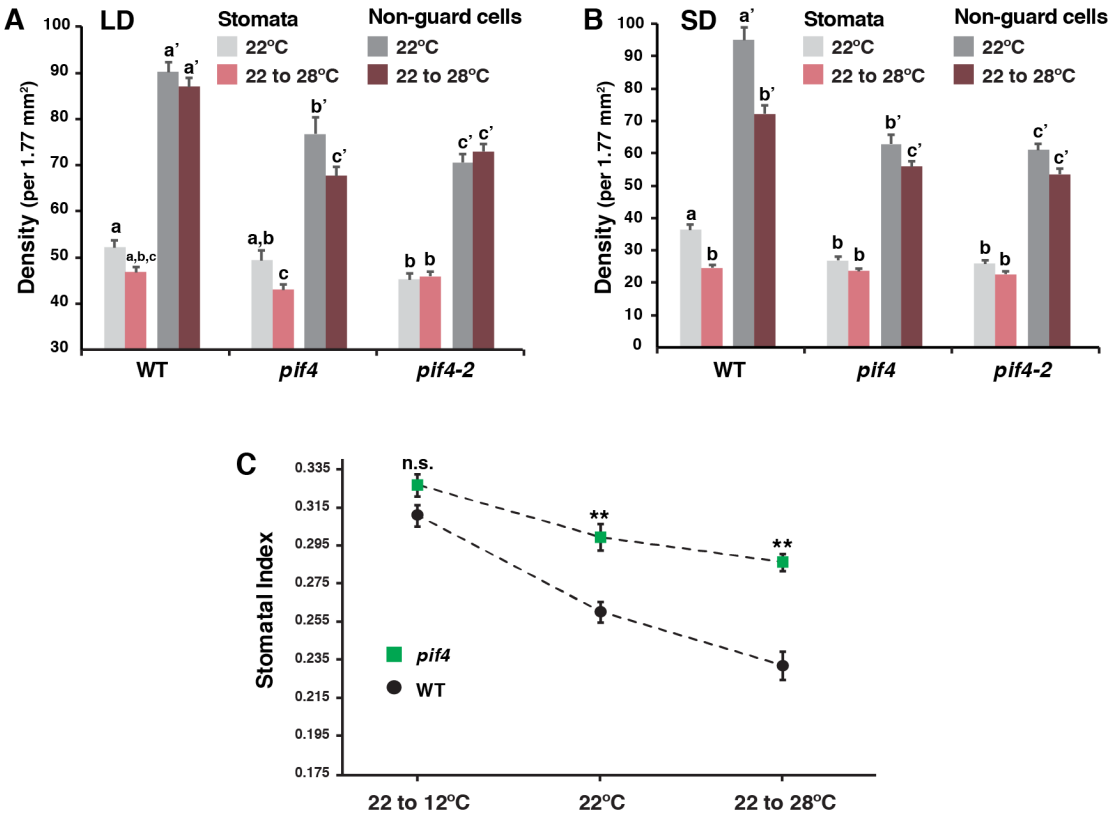

Figure S2. Number of stomatal and non-stomatal epidermal cells per area (density) of WT and *pif4* mutants grown at standard and high temperature, and the effect of temperature on stomatal development in WT and *pif4*, Related to Figure 2. (**A** and **B**) Quantification of stomata and non-stomatal epidermal cell densities in wild-type (WT), *pif4* and *pif4-2* abaxial cotyledons using the same plant samples as in Figure 2A and B (LD: long-day conditions; SD: short-day conditions). Values are mean +/- SEM, n ≥20. One-way ANOVA with post-hoc Tukey HSD (Data from "Stomata" and "Non-guard cells" were tested separately), $p<0.05$. (**C**) Quantification of stomatal indices of mature abaxial cotyledons of wild-type (WT; black) and *pif4* mutant (green) seedlings. Plants were grown at 22°C for 4 days before transfer to 12°C or 28°C or maintained at 22°C for 10 more days. Data for WT at 22°C and 22 to 28°C are repeated from Figure 1B for ease of comparison to *pif4* here. Values are mean +/- SEM, n ≥15. One-way ANOVA with post-hoc Tukey HSD (SIs of *pif4* were compared to the corresponding WT sample data under the same treatment), **, $p<0.01$; n.s., not significant.

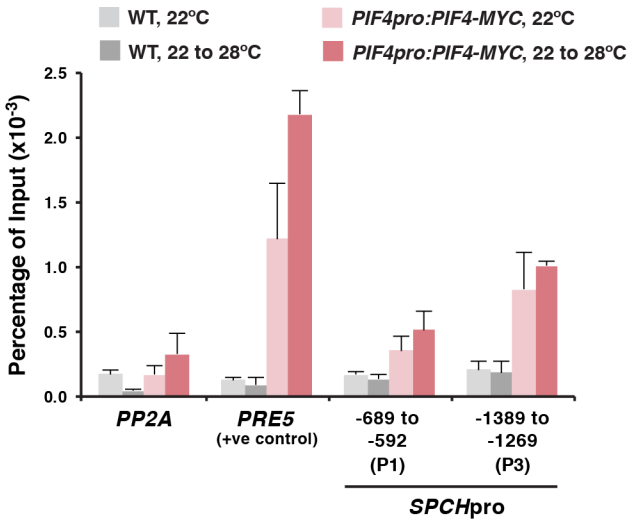

Figure S3. ChIP-qPCR assays performed with standard method showing enrichment of PIF4 at *SPCH* promoter, Related to Figure 3. ChIP-qPCR assays were performed on *PIF4pro:PIF4-Myc* and WT using an anti-Myc antibody. Plants were grown for 4 days at 22°C before transferred to 28°C for 4 h or kept at 22°C. Enrichment at the promoter of *SPCH*, notably around 1.3 kb upstream of the start codon, was observed in the PIF4 samples (rightmost). P1 and P3 denote the genomic location annotated in Figure 3D. *PRE5*, a known target of PIF4, was used as a positive control [S4]. *PP2A* represents a randomly selected genomic region as a specificity control. Values are mean +/- SEM (technical replicates), n = 3. Assay was repeated with similar results.

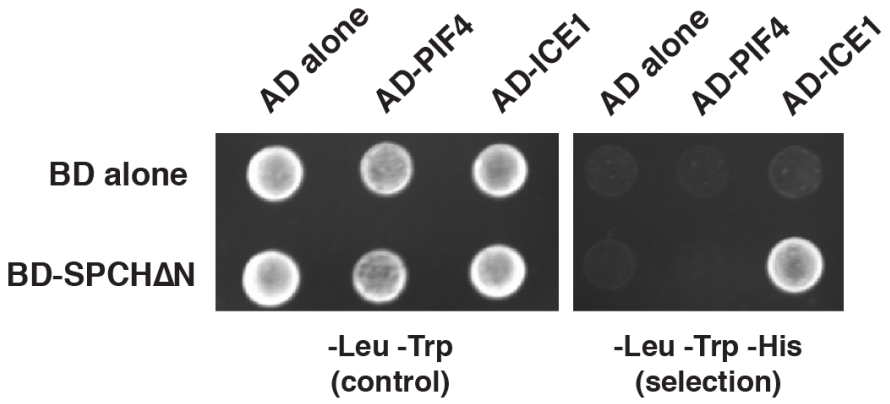

Figure S4. Yeast two-hybrid assay between SPCH and PIF4, Related to Figure 4. No protein interaction between SPCH and PIF4 was detected in our yeast two-hybrid assay (no growth on selection medium). The ICE1-SPCHΔN (91 a.a. to 294 a.a.) pair was used as a positive control [S5]. Assay plates were supplemented with 5 mM 3-AT.

Table S2. List of primers for RT-qPCR and ChIP-qPCR, Related to STAR methods

Primers for RT-qPCR		(all written from 5' to 3')		
Gene name	**AGI code**	**Forward primer**	**Reverse primer**	**Amplicon size (bp)**
ACTIN2	AT3G18780	TCTTCCGCTCTTTCTTTCCAAGC	ACCATTGTCACACACGATTGGTTG	77
SPCH	AT5G53210	TCCTTCACCGCCTGTTCTAAGC	TGAATCTGGTGGTGGTTGATGCG	69
PP2A	AT1G13320	CAAGTGAACCAGGTTATTGGGA	ATAGCCAGACGTACTCTCCAG	101
ICE1	AT3G26744	GGGTTTGCCTTGGATGTTTT	ATCATACCAGCATACCCTGC	110
TMM	AT1G80080	AGCTGAGGCTCAACGATAACA	CCTCAGCTTTCTCCTCATCCT	83
ERL1	AT5G62230	CGCATAACTTGCGGGAATTTG	AGTCCTTGTGCAGCTCCAACC	226

Primers for ChIP-qPCR		(all written from 5' to 3')		
Name in this study	**Distance from ATG of *SPCH* (bp)**	**Forward primer**	**Reverse primer**	**Amplicon size (bp)**
P1	-592	CACGATTGAGGCGCTAAAA	CAATCCCGGTTTCCAAGTAT	98
P2	-953	TGCCATCTCATCAGGTGTTT	CTTCTTTCCCGAGCCATACA	105
P3	-1269	TTTTGAAAGGGAAAGTTCAAATG	GATGTCATACTAGACGTGCCTCA	90
5'	-2667	TCATCTGTCAAAAGCATGTCGT	GTCACGGTGGTGAAGTTATGAA	89
5"	-3166	ACTGCGACCACTTATTGGGTTT	TGGAATAATTTAAGCTCTCTTTCTC TC	85
3'	+3000	ACATCATGTTTGGGATGTGAGA	TTGTGGTTTAAGTTGCATATTCC	102

Supplemental References

[S1] Lau OS, Davies KA, Chang J, Adrian J, Rowe MH, Ballenger CE, et al. Direct roles of SPEECHLESS in the specification of stomatal self-renewing cells. Science 2014;345:1605–9. doi:10.1126/science.1256888.

[S2] Adrian J, Chang J, Ballenger CE, Bargmann BOR, Alassimone J, Davies KA, et al. Transcriptome dynamics of the stomatal lineage: birth, amplification, and termination of a self-renewing population. Dev Cell 2015;33:107–18. doi:10.1016/j.devcel.2015.01.025.

[S3] Matos JL, Lau OS, Hachez C, Cruz-Ramírez A, Scheres B, Bergmann DC. Irreversible fate commitment in the Arabidopsis stomatal lineage requires a FAMA and RETINOBLASTOMA-RELATED module. Elife 2014;3::e03271. doi:10.7554/eLife.03271.

[S4] Oh E, Zhu J-Y, Wang Z-Y. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol 2012;14:802–9. doi:10.1038/ncb2545.

[S5] Kanaoka MM, Pillitteri LJ, Fujii H, Yoshida Y, Bogenschutz NL, Takabayashi J, et al. SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to arabidopsis stomatal differentiation. Plant Cell 2008;20:1775–85. doi:10.1105/tpc.108.060848.