

Figure S1: Characterization of *hy5* in response to higher ambient temperature

(A-C) Normalized root growth (27°C/21°C) in wild type and *hy5-221* under growth condition 2 (A) (see method section) or with roots grown in the dark (B) or with roots grown on medium not supplemented with sucrose (C). (D) Cumulative root meristem cell size in wild type, *hy5-221* and *phyAB*, 72 hours after temperature shift at 21°C or 27°C. (E) Normalized hypocotyl and root growth (27°/21°C) simultaneously measured on individual plants. (F) Normalized root growth (27°C/21°C) in wild type, *phyA*, *phyB* and *phyAB*. (G) Normalized root growth (27°C/21°C) in wild type, *pif7*, *pifQ* and *pif7 pifQ*. Statistics: Student's t-test (A-C,E), one-way ANOVA, Tukey HSD post-hoc test $P < 0.05$ (F,G). Red bar represents the mean (A-C,E-G)

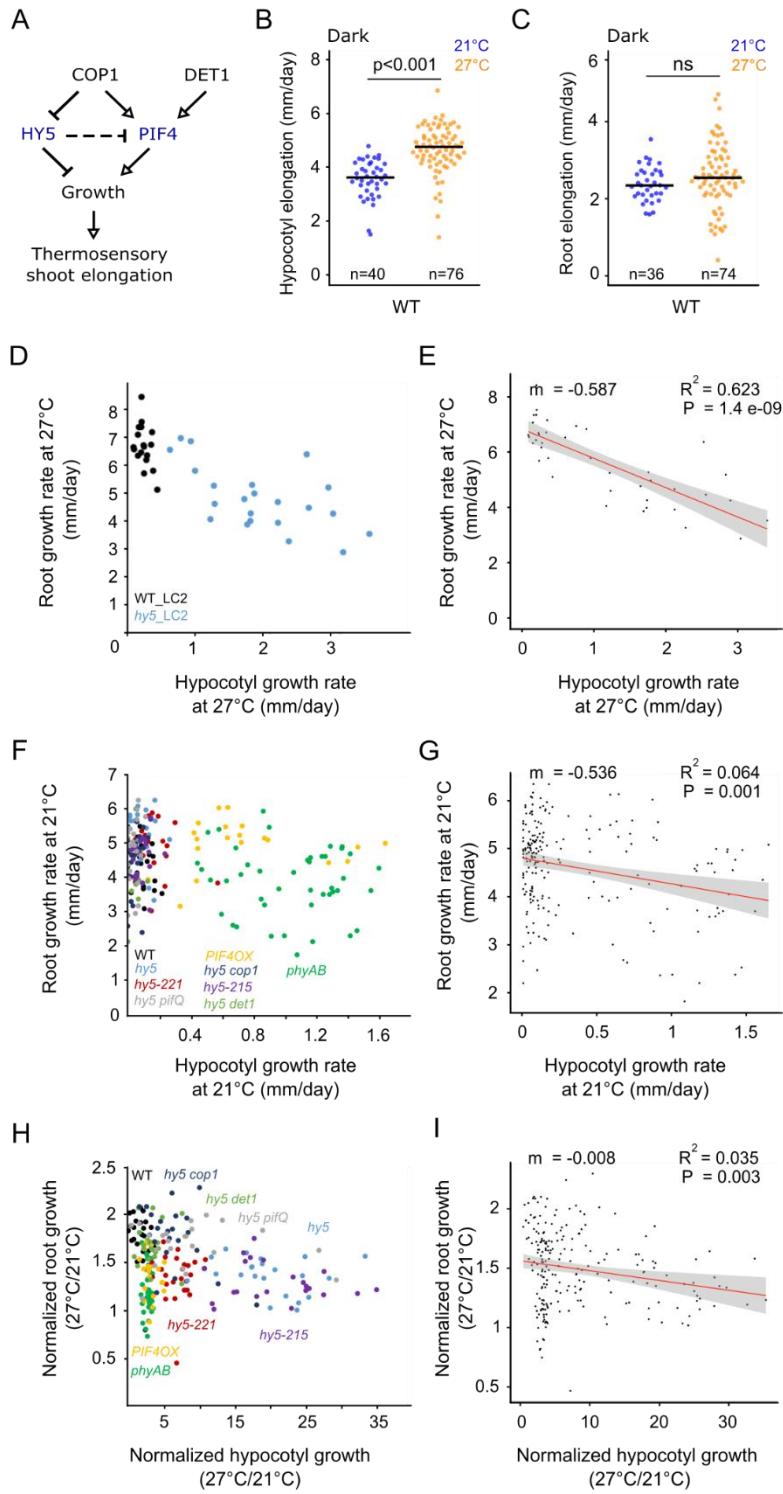


Figure S2: Characterization of the HY5-PIF module in response to higher ambient temperature

(A) Regulatory model of thermosensory shoot elongation as proposed by (Delker et al., 2014). (B-C) Hypocotyl (B) or root (C) elongation of wild type plants grown in dark and shifted at 21°C or 27°C. (D-E) Relation between root and hypocotyl growth rate at 27°C under lower light intensity (growth condition 2: LC2) as shown with measurements on individual wild type (n=18), *hy5-221* (n=22), and after non-parametric regression analysis (E). (F-G) Relation between root and hypocotyl growth rate at 21°C as shown with measurements on individual wild type (n=20), *hy5-221* (n=19), *phyAB* (n=44), PIF4OX (n=20), *hy5* (n=22), *hy5 det1* (n=19), *hy5 cop1* (n=22), *hy5-215* (n=23), *hy5 pifQ* (n=20) plants (F) and after non-parametric regression analysis (G). (H-I) Relation between normalized root and hypocotyl growth (27°C/21°C) as shown with measurements on individual wild type (n=23), *hy5-221* (n=24), *phyAB* (n=43), PIF4OX (n=22), *hy5* (n=22), *hy5 det1* (n=20), *hy5 cop1* (n=22), *hy5-215* (n=23), *hy5 pifQ* (n=22) plants (H) and after non-parametric regression analysis (I). Shaded region in E, G, I indicates a point-wise 95% confidence interval on the fitted values (red line). Statistics: Student's t-test (B,C), linear regression method, Pearson correlation (E,G,H). Red bar represents the mean (B,C).

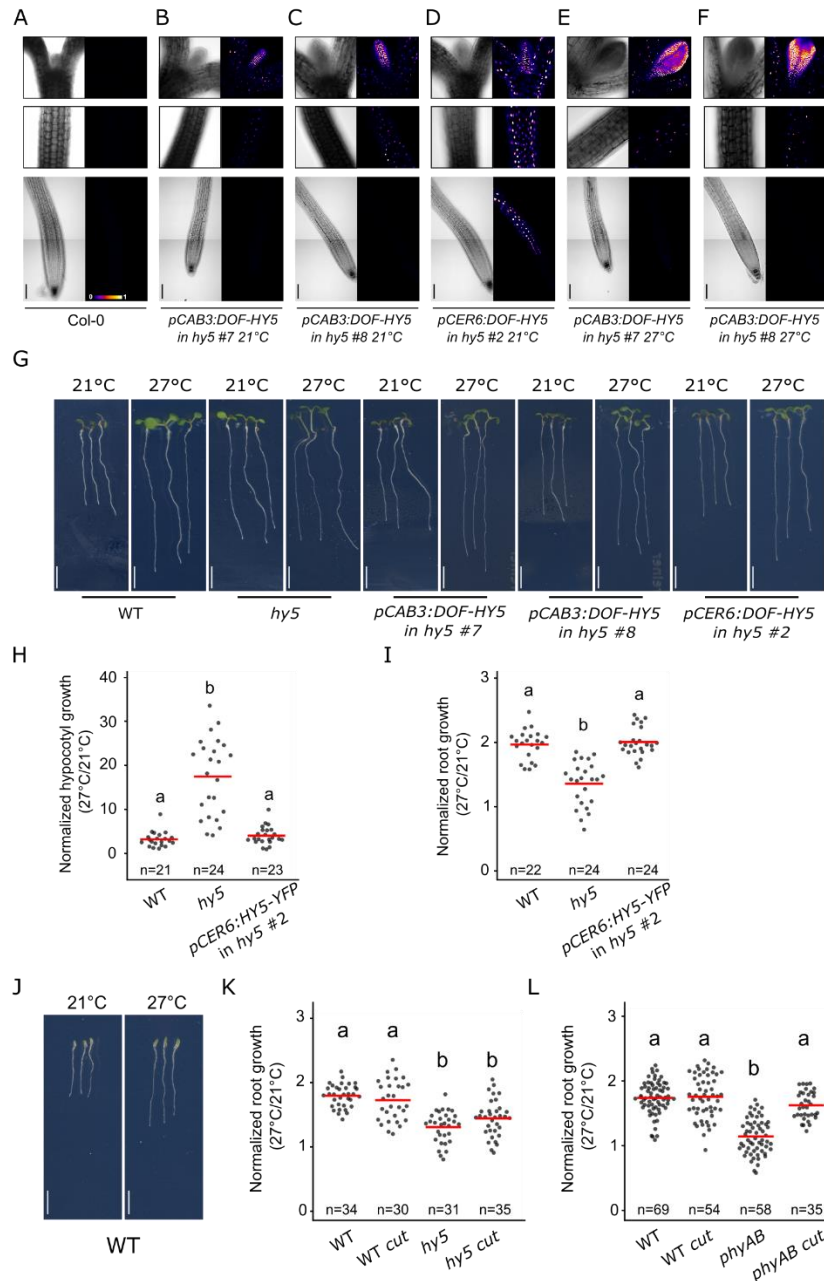


Figure S3: Characterization of HY5 chimera lines

(A-F) Brightfield or false color view of seedlings roots, hypocotyls and apical ends 6DAG in Col-0 (A), two independent lines of *hy5* carrying *pCAB3:DOF-HY5* (B,C), *hy5* carrying *pCER6:DOF-HY5* (D) and *pCAB3:DOF-HY5* lines at 27°C (E,F). (G) Wild type, *hy5*, two independent lines of *hy5* carrying *pCAB3:DOF-HY5*, *hy5* carrying *pCER6:DOF-HY5* seedlings 6DAG and 3 days after transfer at 21°C or 27°C. (H-I) Normalized hypocotyl (H) and root growth (I) (27°C/21°C) in wild type, *hy5* and *hy5* carrying *pCER6:DOF-HY5*. (J) Sectioned wild type seedlings 6DAG and 3 days after transfer at 21°C or 27°C. (K-L) Normalized root growth (27°C/21°C) of intact or sectioned wild type, *hy5* (K) and *phyAB* mutant seedlings (L). Statistics: One-way ANOVA, Tukey HSD post-hoc test $p < 0.05$ (H,I). Two-way ANOVA, Tukey HSD post-hoc test $p < 0.05$, (K,L). Red bar represents the mean (H,I,K,L). Scale bar: 5mm (G, K), 100µm (A-F).

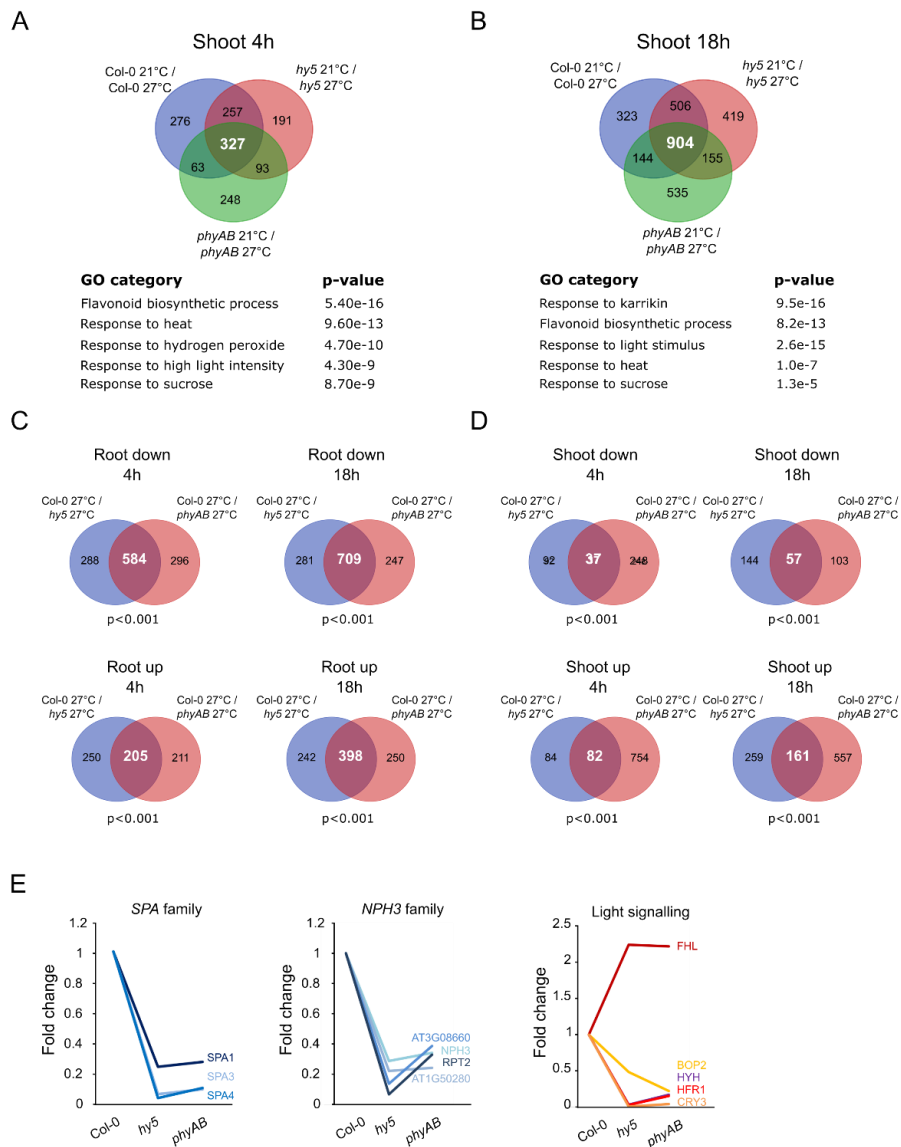


Figure S4: Transcriptional profiling of *hy5* and *phyAB* mutants

(A-B) Genes regulated 4 hours (A) or 18 hours (B) after temperature shift in wild type, *hy5*, *phyAB*, shoots. Gene ontologies (GO) characterize the biological processes enriched among the temperature-regulated genes that are shared between wild type, *hy5* and *phyAB*. (C-D) Commonly downregulated or upregulated genes in *hy5* and *phyAB* roots (C) or shoots (D) at 27°C, 4 or 18 hours after temperature shift. (E) Root expression change of genes co-regulated by HY5 and phytochromes. Statistics: p-value as calculated with AgrigoV2 (A,B), hypergeometric test (C,D).

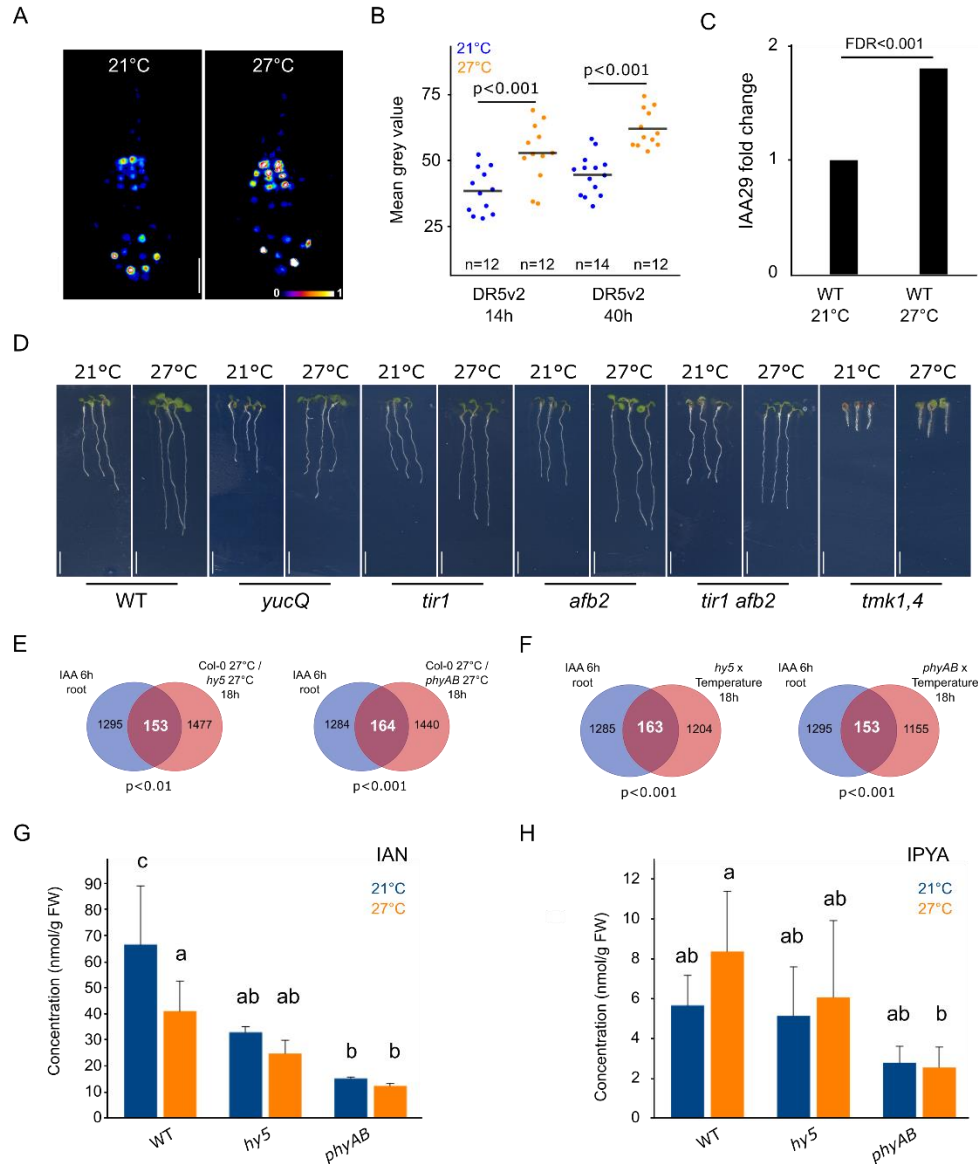
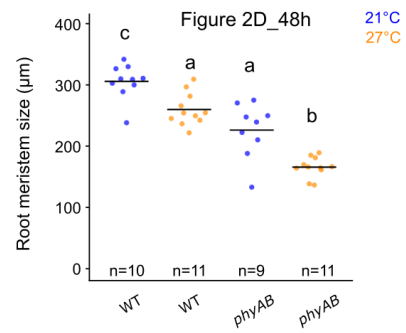
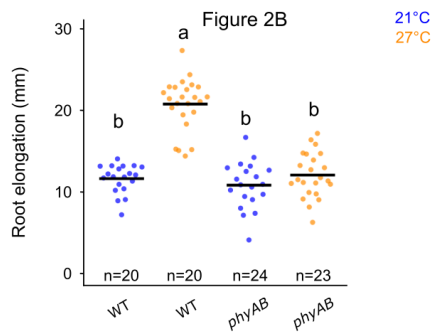
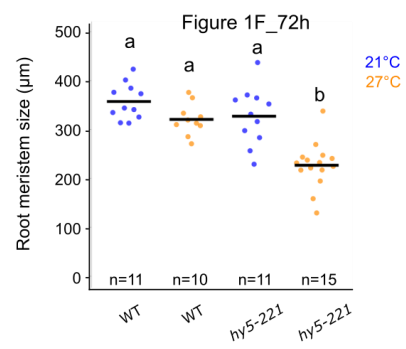
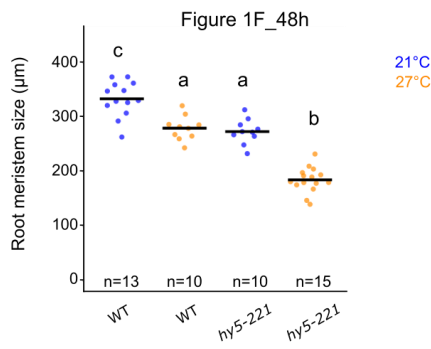
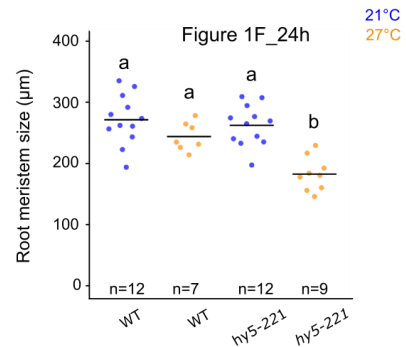
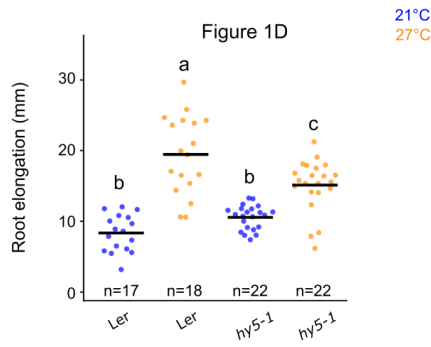
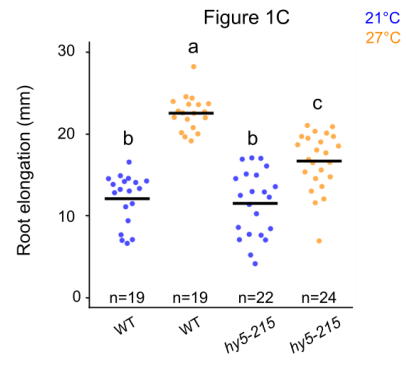
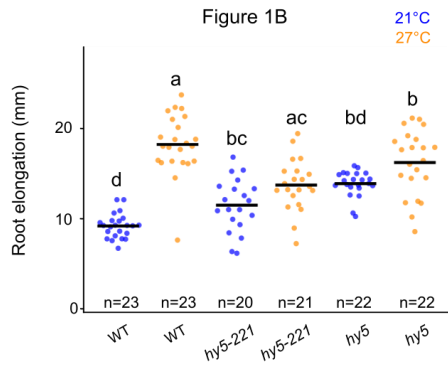
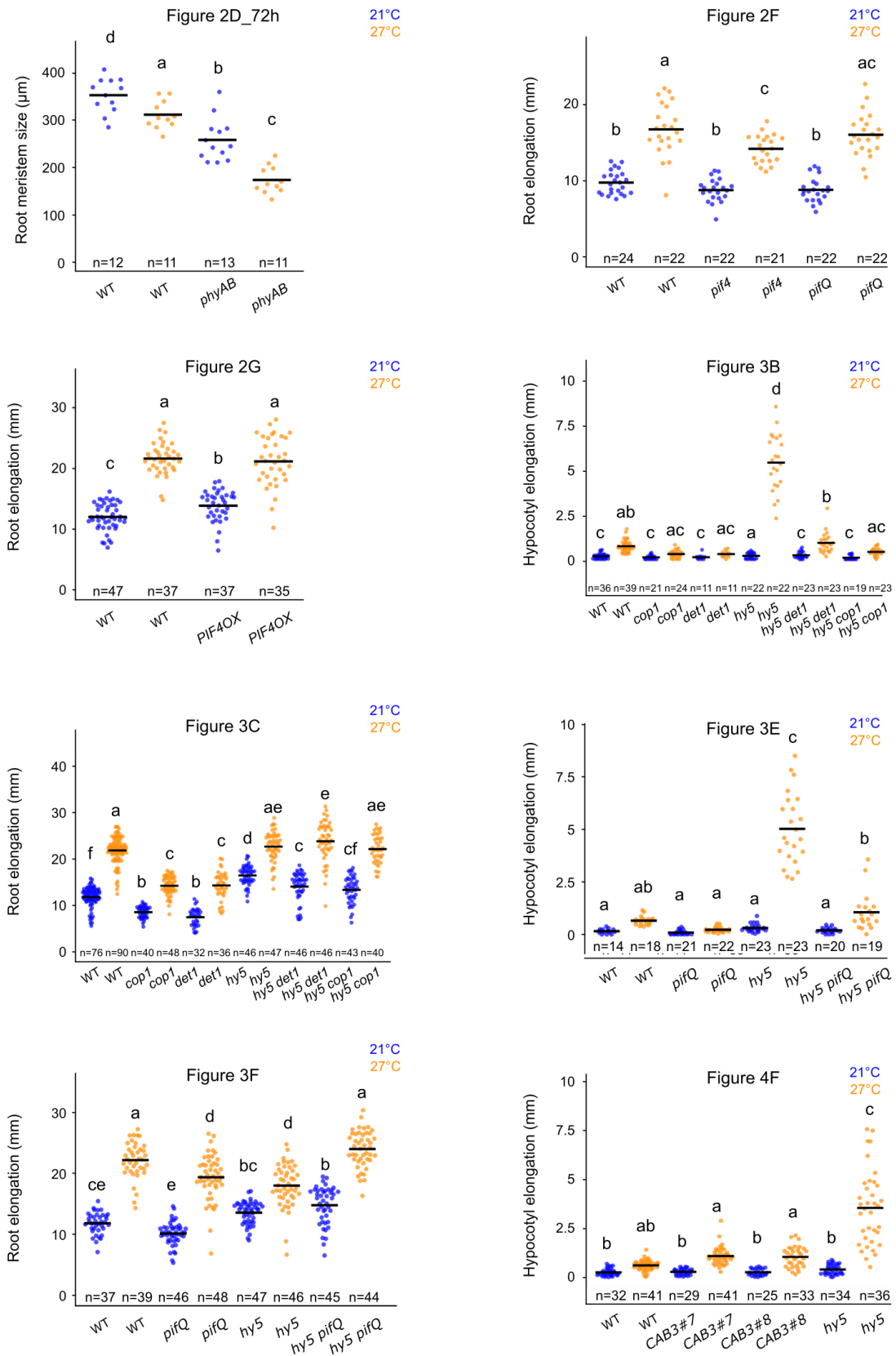
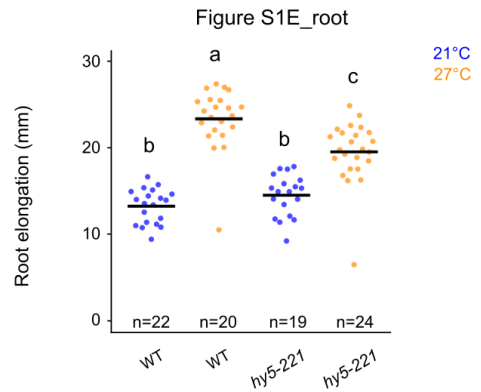
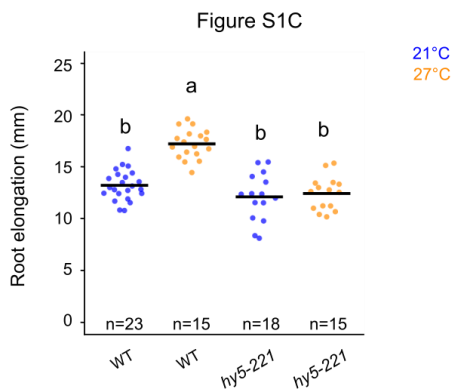
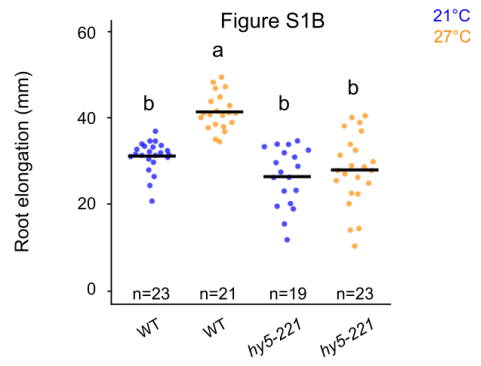
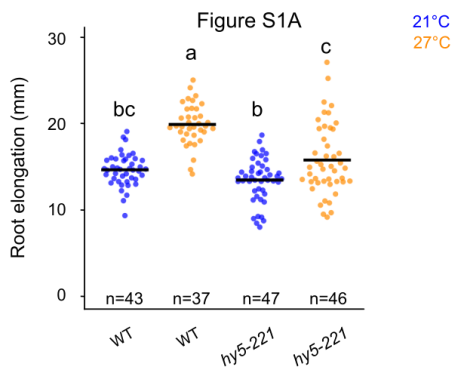
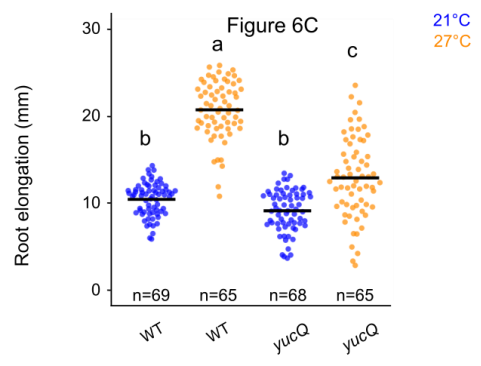
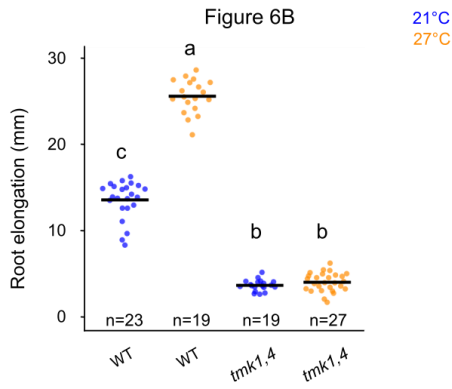
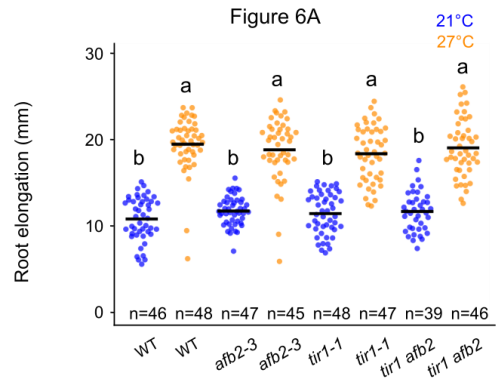
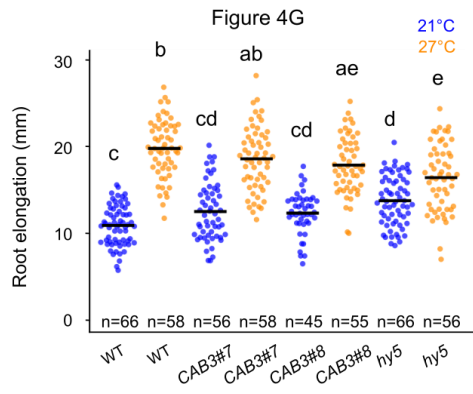


Figure S5: Auxin homeostasis during root thermomorphogenesis

(A) False color view of root tips expressing *pDR5v2:3xYFP-NLS* grown at 21°C or 27°C. (B) Quantification of DR5v2 signal in seedlings 14 hours or 40 hours after transfer at 21°C or 27°C. (C) Relative IAA29 expression detected in wild type roots 4 hours after transfer at 21°C or 27°C. (D) Wild type, *yucQ*, *tir1*, *afb2*, *tir1 afb2* and *tmk1,4* mutant seedlings 6DAG and 3 days after transfer at 21°C or 27°C. (E) Differentially regulated genes in *hy5* and *phyAB* roots at 27°C, 18 hours after temperature shift that are auxin responsive (Omelyanchuk et al., 2017). (F) Overlap between genes whose transcriptional response changes in *hy5* or *phyAB* and that are auxin responsive. (G-H) Indole-3-acetonitrile (G) and indole-3-pyruvic acid (H) concentration in 6DAG wild type, *hy5-221* and *phyAB* roots 12 hours after transfer at 27°C. Statistics: Student t-test (B), false discovery rate (FDR) as calculated by EdgeR (C), hypergeometric test (E-F), Two-way ANOVA, Tukey HSD post-hoc test $p < 0.05$ (G-H). Scale bar: 5mm (D). Black bar represents the mean (B).







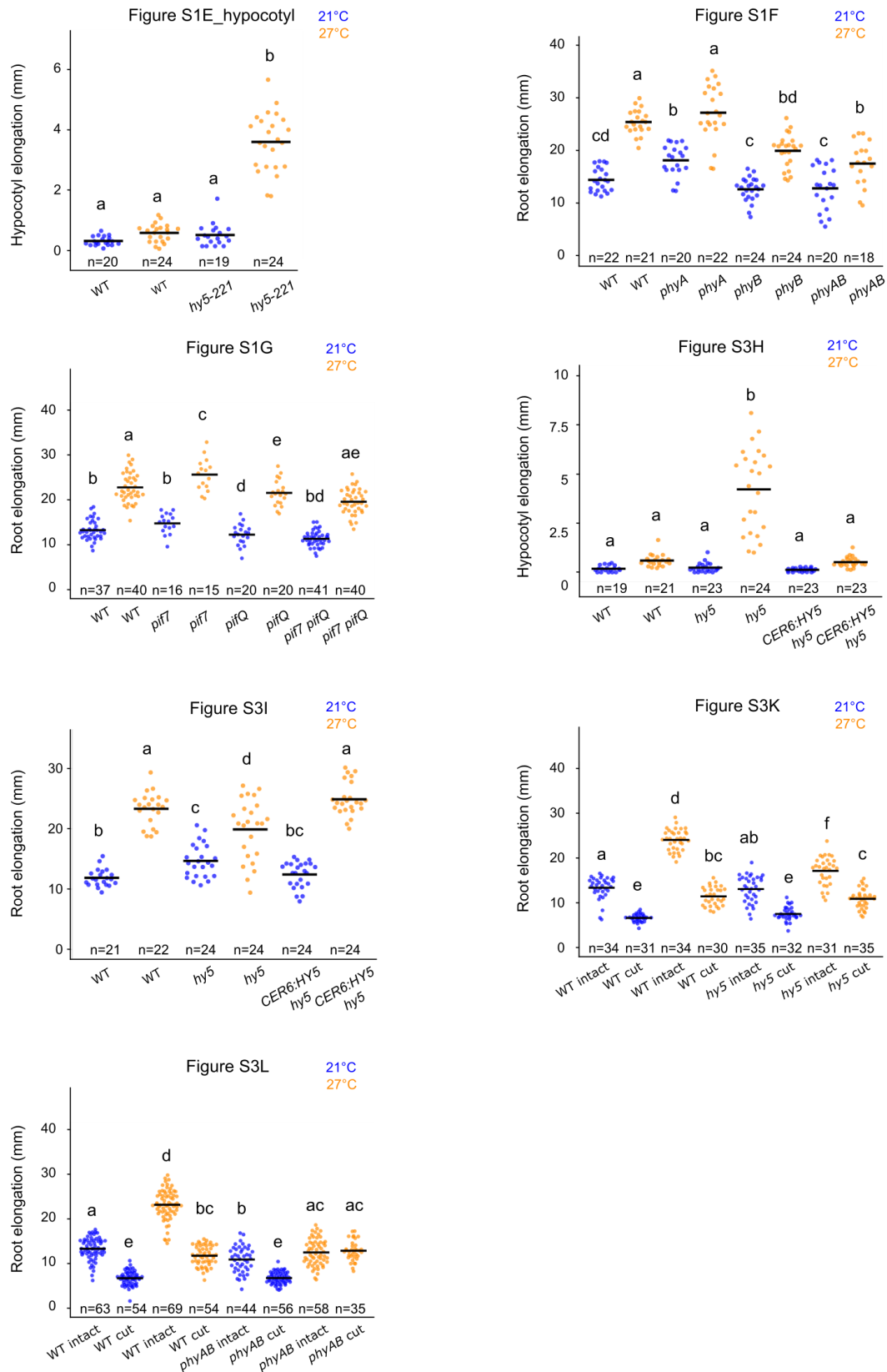


Figure S6: Root measurements at individual temperatures

Statistics: Two-way ANOVA, Tukey HSD post-hoc test $p < 0.05$. Three-way ANOVA, Tukey HSD post-hoc test $p < 0.05$ (FigS3K, FigS3L)

Table S1: Source data for figures.

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