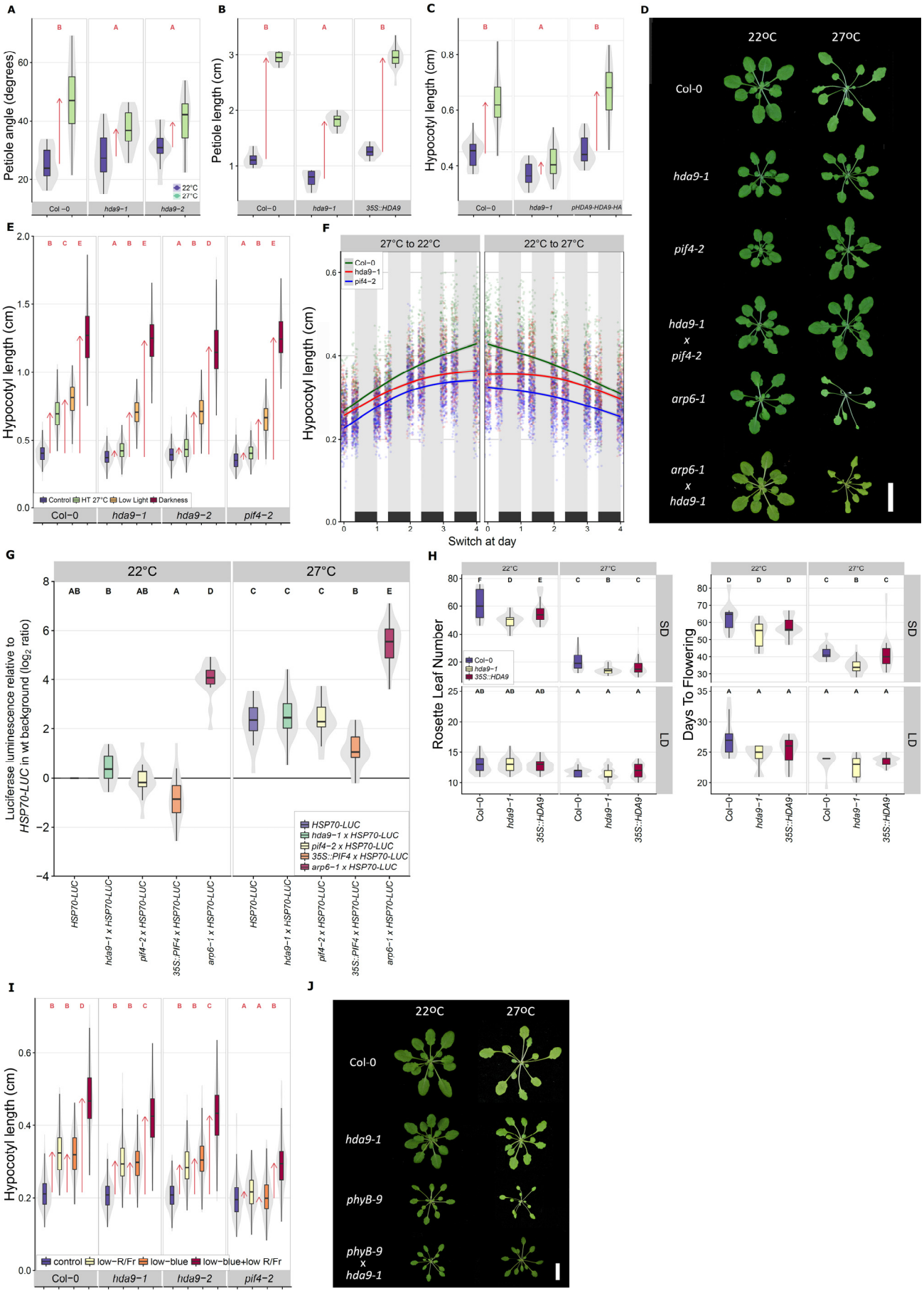
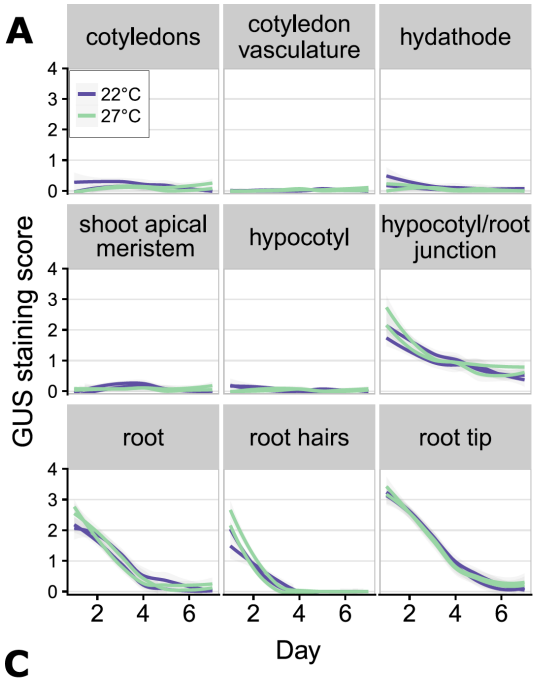
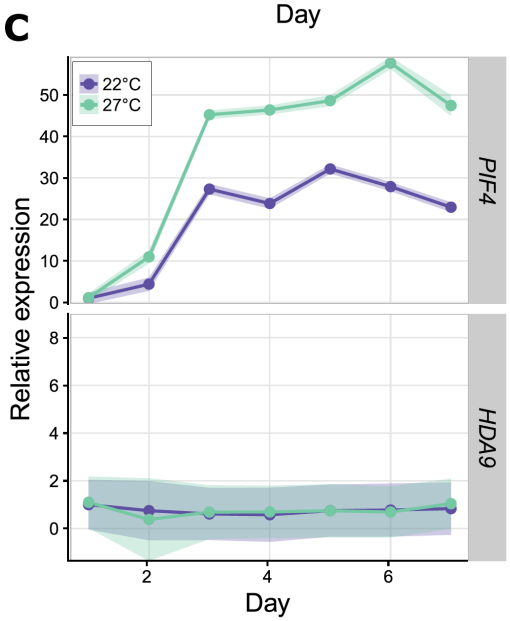
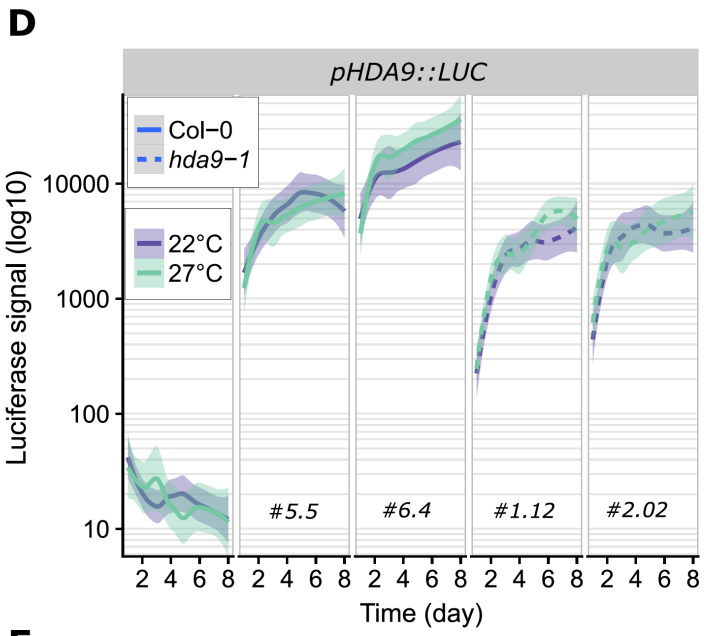

A
Petiole angle (degrees)
Col −0
hda9−1
hda9−2
22°C
27°C
B
Petiole length (cm)
Col−0
hda9−1
35S::HDA9
C
Hypocotyl length (cm)
Col−0
hda9−1
pHDA9-HDA9-HA
D
22ºC
27ºC
Col-0
hda9-1
pif4-2
hda9-1 x pif4-2
arp6-1
arp6-1 x hda9-1
E
Hypocotyl length (cm)
Control
HT 27°C
Low Light
Darkness
Col−0
hda9−1
hda9−2
pif4−2
F
27°C to 22°C
22°C to 27°C
Col−0
hda9−1
pif4−2
Hypocotyl length (cm)
Switch at day
G
22°C
27°C
Luciferase luminescence relative to HSP70-LUC in wt background (log2 ratio)
HSP70-LUC
hda9−1 x HSP70-LUC
pif4−2 x HSP70-LUC
35S::PIF4 x HSP70-LUC
arp6−1 x HSP70-LUC
H
Rosette Leaf Number
Days To Flowering
SD
LD
Col−0
hda9−1
35S::HDA9
I
Hypocotyl length (cm)
control
low−R/Fr
low−blue
low−blue+low R/Fr
Col−0
hda9−1
hda9−2
pif4−2
J
22ºC
27ºC
Col-0
hda9-1
phyB-9
phyB-9 x hda9-1

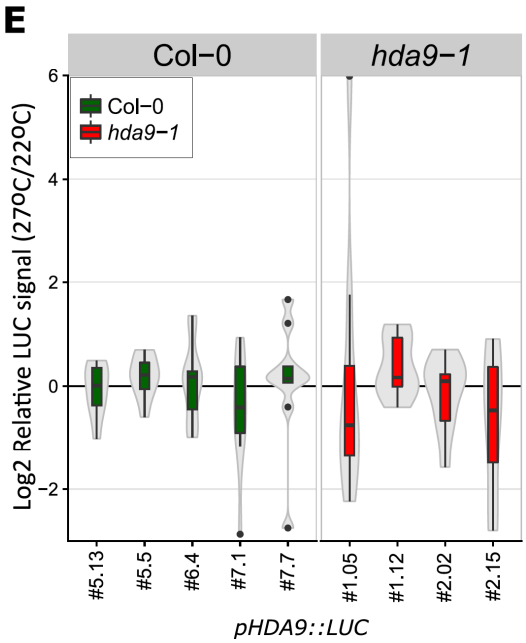
Figure S1. HDA9 defines a thermosignaling pathway. A,B, Quantification of **A,** petiole angle relative to the horizontal (hyponastic growth) and **B,** petiole length, of vegetative Col-0, *hda9-1* and *35S::HDA9* plants (~10 leaf-stage), at 22°C (purple) and 27°C (green). N= respectively **A**, 11-15, **B,** 6-16 plants per genotype and treatment. **C,** Hypocotyl lengths of 8 day-old Col-0 wild type, *hda9-1* and *pHDA9-HDA9-HA* seedlings at 22°C (purple) or 27°C (green). N=168-198 seedlings per genotype and treatment, divided over 5 replicates. **D,** Top photos of vegetative Col-0 wild type, *hda9-1, pif4-2* and *arp6-1* mutant plants and their derived double mutant combinations, grown at control (22°C; left column) or high temperature (27°C; right column). Note that the *arp6-1* and *arp6-1 hda9-1* mutants initiated bolting at 27°C. Scale bar = 2 cm. **E,** Hypocotyl lengths of 8 day-old Col-0 wild type, *hda9-1*, *hda9-2* and *pif4-2* mutants, grown at control white light conditions (~100 µMol m^{-2} s^{-1} PAR) at control temperature (22°C; purple), high temperature (27°C; green), or low light conditions (~10 µMol m^{-2} s^{-1} PAR; orange), all in short-day photoperiod conditions (8h light/16h dark) or in complete darkness (dark red boxes). N=146-262 seedlings per genotype and treatment, divided over 9 replicates. **F,** Best-fit curves (smooth curve over 3-point moving bins) through average hypocotyl lengths of Col-0 wild type (green line), *hda9-1* (red line) and *pif4-2* (blue line) seedlings, switched from 27°C to 22°C (left panel) or *vice versa* (right panel), at the indicated days, at dawn or dusk. Hypocotyl lengths were measured from all plants imaged at dusk of day 4. T=0h indicates the moment seeds were placed in the light to allow germination. Individual dots in the background represent individual seedlings. Black and gray-shaded vertical bars indicate the 16h-dark period and alternating white bars represent the 8h day time period. N=206-316 seedlings per genotype, treatment and timepoint, divided over 9 replicates. For statistical analyses (full anova) see *SI Appendix,* Supporting information data sets. **G,** Promoter activity of the *HSP70* temperature marker at 22°C (left panel) and 27°C (right panel), as quantified by luminescence detection of LUCIFERASE activity, corrected for total protein content, using *pHSP70::LUC* in the wild type (purple) and *hda9-1* (green), *pif4-2* (yellow), *35S::PIF4* (orange) and *arp6-1* (dark red) mutant genetic backgrounds. Data is normalized to *pHSP70::LUC* activity in the Col-0 wild type background at 22°C. N=16 replicates per genotype and treatment, pooled results of each ~25 seedlings per replicate. Note that *hda9-1* and *pif-4* mutations do not interfere with high temperature-induced *HSP70* induction, whereas *arp6-1* displays enhanced *HSP70* expression levels already in control temperature conditions*,* in line with earlier findings by Kumar and Wigge, 2010 (1). **H,** Flowering time expressed as left panels; rosette leaf number (N=21-45 plants per genotype and treatment, divided over 6 replicates) and right panels; days to flowering (N=15-26 plants per genotype and treatment, divided over 6 replicates), of Col-0 wild type (purple), *hda9-1* (yellow) and *35S::HDA9* overexpression line (dark red) in short day (SD; 8h photoperiod; upper panels) and long day (LD; 16h photoperiod; lower panels) conditions, at 22°C (left column per panel) and high temperatures (right column per panel). Different letters indicate significant different groups. **I,** Hypocotyl lengths of 8 day-old Col-0 wild type, *hda9-1*, *hda9-2* and *pif4-2* mutants, grown at control white light conditions (purple), low red-to-far red light (yellow), low blue light (orange) or a combination of low red-to-far red and low blue light (dark red), at 22°C in long-day photoperiod conditions (16h light/8h dark). N=201-253 seedlings per genotype and treatment, divided over 9 replicates. **J,** Top photos of vegetative Col-0 wild type, *hda9-1, phyB-9* and the derived *hda9-1 phyB-9* double mutant, grown at control (22°C; left) and high temperature (27°C). Scale bar = 2 cm. Boxes in panel **A-C,E,G-I** indicate the boundaries of the second and third quartile of the data distribution. Black bars within the boxes indicate the median and whiskers the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. Red arrows in panels **A-C,E,I** indicate the difference in mean hypocotyl length between treatment and control. Red letters indicate significant different groups between the hypocotyl responses (change) per panel ($P < 0.01$; two-sided t-test using the means and SD). **G,H,** Bold letters indicate significant different groups of **G,** absolute luciferase levels and **H,** leaf numbers respectively days ($P < 0.05$; TukeyHSD).


A
cotyledons
cotyledon vasculature
hydathode
shoot apical meristem
hypocotyl
hypocotyl/root junction
root
root hairs
root tip
22°C
27°C
GUS staining score
Day


B


C
22°C
27°C
PIF4
HDA9
Relative expression
Day


D
pHDA9::LUC
Col−0
hda9−1
22°C
27°C
#5.5
#6.4
#1.12
#2.02
Luciferase signal (log10)
Time (day)


E
Col−0
hda9−1
Log2 Relative LUC signal (27ºC/22ºC)
#5.13
#5.5
#6.4
#7.1
#7.7
#1.05
#1.12
#2.02
#2.15
pHDA9::LUC

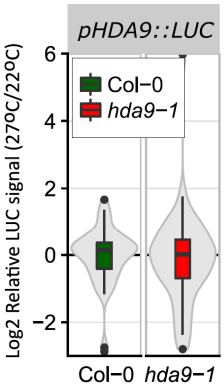

F
pHDA9::LUC
Col−0
hda9−1
Log2 Relative LUC signal (27ºC/22ºC)
Col−0
hda9−1

Figure S2. *HDA9* expression and promoter activity are not affected by high temperature. A, Spatial and temporal distribution of *HDA9* promoter activity in seedlings. Qualitative assessment scores of β-*glucuronidase* staining of *pHDA9::GUS* lines in various organs at 22°C (purple) and 27°C (green) are shown. Intensity and presence of blue staining was scored on a scale of 0 (absent) to 4 (saturated) from seed (day 1) to 7-day-old seedling. Two independent transformants were scored with similar results (note the presence of two lines per temperature treatment in each panel). N=56 per genotype, treatment and timepoint, divided over 4 replicates. For statistical analyses (full anova) see *SI Appendix,* Supporting information data sets. **B,** representative images of GUS-stained 2 day-old *pHDA9::GUS* plants. Scale bar = 200 µm. **C,** Relative expression of *PIF4* (upper panel) and *HDA9* (lower panel) at 22°C (purple) and 27°C (green) in Col-0 wild type at dawn, from seed (day 1) to seedling (day 7). N=3-4 replicates per genotype, per treatment per timepoint of each ~20 seedlings. **D-F,** *HDA9* promoter activity measured by luminescence detection of LUCIFERASE activity, corrected for total protein content, using independent *pHDA9-LUC* transformant lines in the Col-0 and *hda9-1* mutant background. **D,** LUC activity values, at 22°C (purple) and 27°C (green) at dawn, from seeds (t=0) to 7 day-old seedlings of transformants *#5.5* and *#6.4* in Col-0 (filled lines), and #*1.12* and #*2.02* in the *hda9-1* mutant (dashed lines) backgrounds. N=5-7 replicas, per genotype, per treatment per timepoint of ~20 seedlings each. Non-transgenic Col-0 wild type is included in panel **D** (most left panel) as negative control. **E,** Relative *pHDA9-LUC* induction (27°C/22°C) in 4 day-old seedlings of 5 independent transformants in the Col-0 (green) and 4 in *hda9-1* (red) mutant backgrounds, at dawn. N=8-10 replicas per genotype per treatment of ~20 seedlings each. **F,** Compiled average of relative induction of all transformants, as depicted in **E,** in the Col-0 (green) and *hda9-1* background (red). Shaded areas behind the lines indicate the SEM in panels**.** Boxes in panel **E** and **F**, indicate the boundaries of the second and third quartile of the data distribution. Black bars within the boxes indicate the median and whiskers the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. In panel **E** and **F** no significant differences in relative expression, nor between genotypes were detected. For statistical analyses (full anova) see *SI Appendix,* Supporting information data sets.

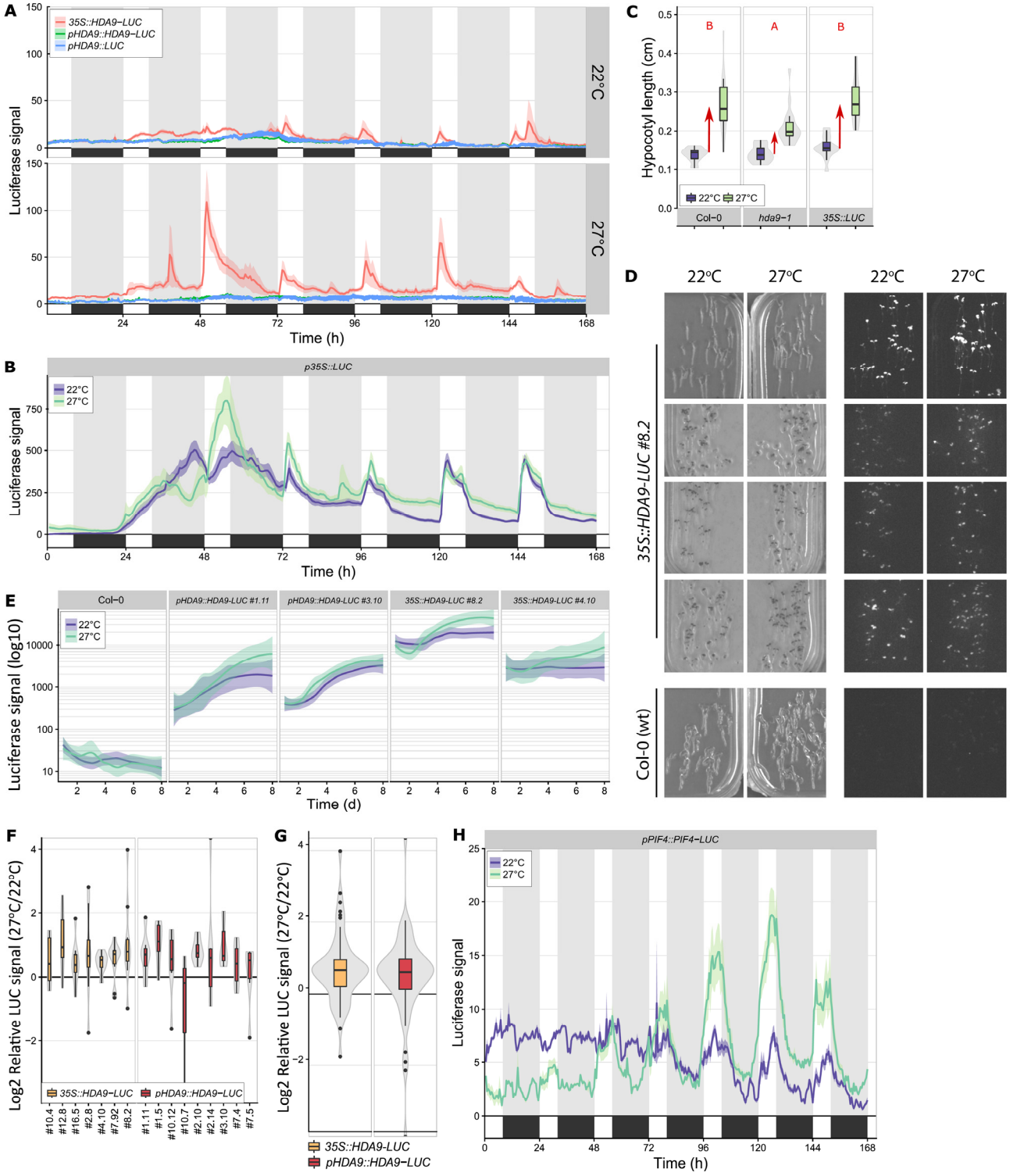

A
35S::HDA9−LUC
pHDA9::HDA9−LUC
pHDA9::LUC
Luciferase signal
22°C
27°C
Time (h)
B
p35S::LUC
22°C
27°C
Luciferase signal
Time (h)
C
Hypocotyl length (cm)
B
A
B
22°C 27°C
Col−0
hda9−1
35S::LUC
D
22°C
27°C
22°C
27°C
35S::HDA9-LUC #8.2
Col-0 (wt)
E
Col−0
pHDA9::HDA9-LUC #1.11
pHDA9::HDA9-LUC #3.10
35S::HDA9-LUC #8.2
35S::HDA9-LUC #4.10
22°C
27°C
Luciferase signal (log10)
Time (d)
F
Log2 Relative LUC signal (27°C/22°C)
35S::HDA9−LUC
pHDA9::HDA9−LUC
#10.4
#12.8
#16.5
#2.8
#4.10
#7.92
#8.2
#1.11
#1.5
#10.12
#10.7
#2.10
#2.14
#3.10
#7.4
#7.5
G
Log2 Relative LUC signal (27°C/22°C)
35S::HDA9-LUC
pHDA9::HDA9−LUC
H
pPIF4::PIF4−LUC
22°C
27°C
Luciferase signal
Time (h)

Figure S3. HDA9 protein levels are high under warm temperature conditions. A,B Circadian rhythmicity of **A,** HDA9 protein levels (*35S::HDA9-LUC* overexpression line *#4.10* (red) and *pHDA9::HDA9-LUC* line *#1.11* (green)) and *pHDA9* promoter activity (*pHDA9-LUC* line *#5.5* (blue)) and **B,** activity of the *35S CaMV* overexpression promoter (*p35S::LUC*), from seed (t=0h) to 7 day-old seedling (t=168h), at **A,** control 22^{o}C (upper panel) and high 27^{o}C temperature (lower panel) and **B,** 22^{o}C (purple line) and 27^{o}C (green line), as measured by continuous luminescence detection of LUCIFERASE activity. Shown are averages of N=6-19 individual seedings per genotype and treatment. Black/gray-shaded vertical boxes indicate the dark period (16h) and alternating white bands day time (8h). **C,** Hypocotyl lengths of 7 day-old Col-0 wild type, *hda9-1* and *35S::LUC* at 22^{o}C (purple) and 27^{o}C (green), measured as phenotypic trait control for the LUCIFERASE detection system (**A,B**). Red arrows indicate the difference in mean hypocotyl length between treatment and control. Red letters indicate significant different groups between the hypocotyl elongation responses (change) ($P < 0.05$; two-sided t-test using the means and SD). N=13-19 seedlings per genotype and treatment. **D,** Visualization of HDA9 protein stabilization by high temperature (27^{o}C) in the representative *35S::HDA9-LUC* transformant *#8.2* (Fig. 2A). LUCIFERASE signals were captured of 2 day-old seedlings at dawn, with a Hamamatsu ImaEM-X2 camera, with electron-multiplying (EM) gain. Left panels indicate bright field and right panels, LUCIFERASE luminescence detection at 22^{o}C (left) and 27^{o}C (right). Shown are four individual biological replicates and Col-0 wild type as negative control. **E,F,** HDA9 protein levels at 22^{o}C (purple) and 27^{o}C (green), corrected for total protein content, **E,** from seed (t=0h) to 7 day-old seedling in *pHDA9::HDA9-LUC* (transformants *#1.11* and *#3.10*) and *35S::HDA9-LUC* (transformants *#8.2* and *#4.10*) and **F,** relative induction (27^{o}C/22^{o}C) in 4 day-old seedlings of various independent *35S::HDA9-LUC* (orange) and *pHDA9::HDA9-LUC* (red) transformants, at dawn. N=5-7 replicas, per genotype, per treatment, per timepoint, of ~20 seedlings each. Non-transgenic Col-0 wild type (see also *SI Appendix,* Fig S2D) is included in panel **E** as negative control. **G,** Compiled average of relative induction of all transformants, as depicted in **F. H,** Close-up of PIF4 protein level rhythmicity, driven by the endogenous *PIF4* promoter at 22^{o}C (upper panel) and 27^{o}C (lower panel) as depicted in Fig. 2A. For details see legend panels **A** and **B**. Shaded areas behind the lines of panels **A,B,E,H** indicate SEM. Boxes in panels **C,F,G** indicate the boundaries of the second and third quartile of the data distribution. Black bars within the boxes indicate the median and whiskers the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. In panel **F** and **G** no significant differences between genotypes were detected. For statistical analyses (full anova) see *SI Appendix,* Supporting information data sets.

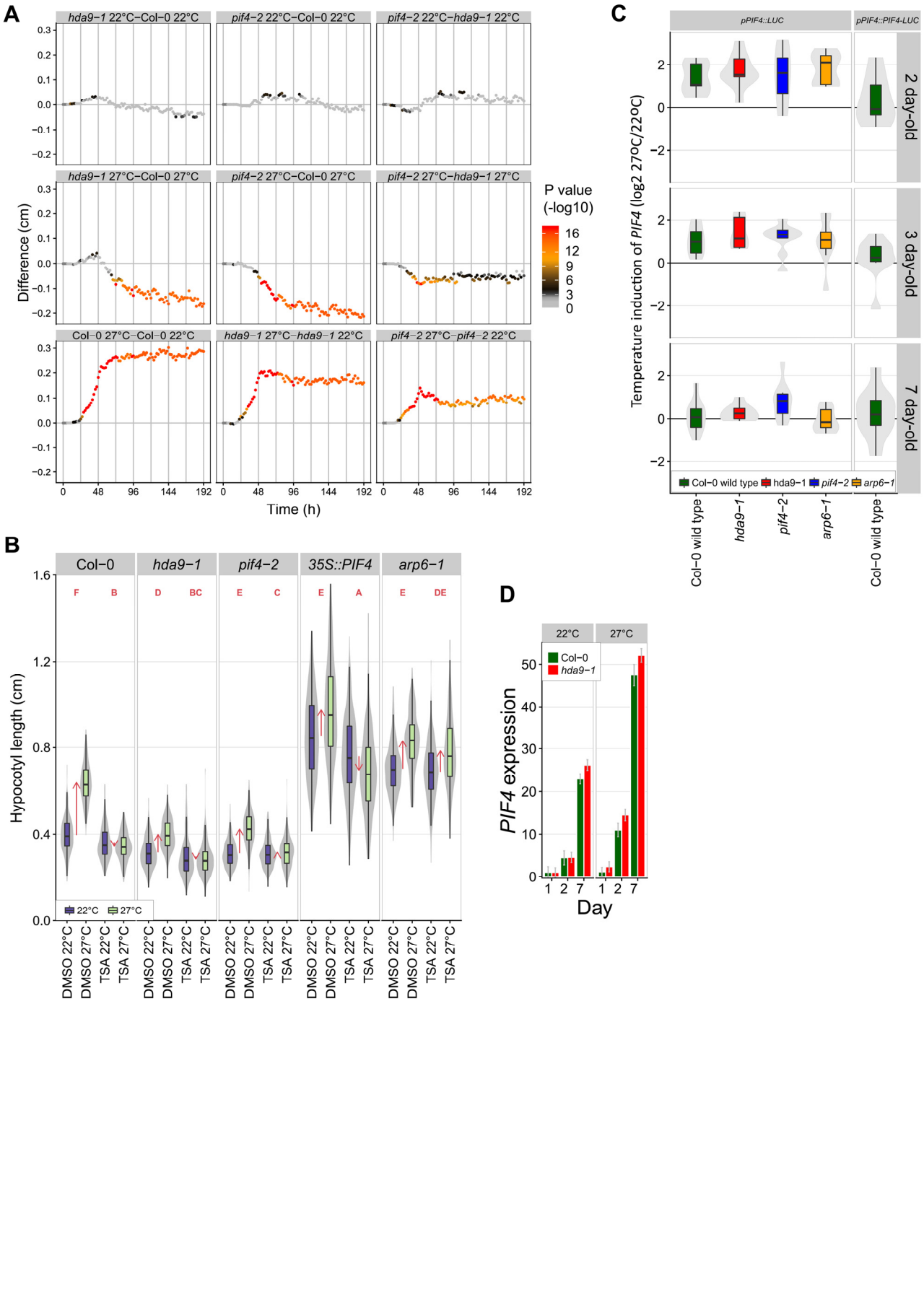

A
hda9−1 22°C−Col−0 22°C
pif4−2 22°C−Col−0 22°C
pif4−2 22°C−hda9−1 22°C
hda9−1 27°C−Col−0 27°C
pif4−2 27°C−Col−0 27°C
pif4−2 27°C−hda9−1 27°C
Col−0 27°C−Col−0 22°C
hda9−1 27°C−hda9−1 22°C
pif4−2 27°C−pif4−2 22°C
Difference (cm)
Time (h)
P value (-log10)
16
12
9
6
3
0
B
Col−0
hda9−1
pif4−2
35S::PIF4
arp6−1
F
B
D
BC
E
C
E
A
E
DE
Hypocotyl length (cm)
22°C
27°C
DMSO 22°C
DMSO 27°C
TSA 22°C
TSA 27°C
C
pPIF4::LUC
pPIF4::PIF4-LUC
2 day-old
3 day-old
7 day-old
Temperature induction of PIF4 (log2 27°C/22°C)
Col−0 wild type
hda9−1
pif4−2
arp6−1
D
22°C
27°C
Col−0
hda9−1
PIF4 expression
Day

Figure S4. The HDA9, PIF4, ARP6/H2A.Z thermosignaling module. A, Differences between hypocotyl growth profiles from seed (0h) to 8 day-old seedling (168h) of the indicated genotype and treatment comparisons presented in Fig. 2B. Statistical significance of the differences (Tukey HSD per time point) is indicated (-Log10 P-values) in color coding. Note that at control temperature conditions the tested genotypes do not significantly differ from each other (top row), that the *pif4-2* and *hda9-1* mutants behave similar and are less responsive than Col-0 wild type (middle row) and that responsiveness to high temperature is most pronounced in Col-0 wild type (lowest row). **B,** Hypocotyl lengths of 8 day-old Col-0 wild type, *hda9-1, pif4-2, 35S::PIF4* and *arp6-1* seedlings at 22^{o}C (purple) or 27^{o}C (green) in the absence (mock) and presence of the HDAC activity inhibitor Trichostatin-A (1 µM). Red letters indicate significant different groups between the hypocotyl responses (change) ($P<0.05$; two-sided t-test using the means and SD). N=164-254 per genotype and treatment, divided over 9 replicates. **C,** Relative induction of *PIF4* promoter activity and PIF4 protein levels by high temperature (27^{o}C/22^{o}C) in 2- (upper panel), 3- (middle panel) and 7 day-old seedlings (lower panel), as measured by luminescence detection of LUCIFERASE, using respectively; *pPIF4::LUC* (promoter activity) in the Col-0 wild type (green) and *hda9-1* (red), *pif4-2* (blue), *arp6-1* (orange) mutant genetic backgrounds and *pPIF4::PIF4-LUC* (protein) in the Col-0 wild type background (green). N=7-9 replicates per genotype and treatment, of each ~25 seedlings. **B,C,** boxes indicate the boundaries of the second and third quartile of the data distribution. Black bars within the boxes indicate the median and whiskers the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. **D,** Relative *PIF4* expression in 1-, 2- and 7 day-old Col-0 wild type (green) and *hda9-1* (red) seedlings at 22^{o}C (left panel) and 27^{o}C (right panel) as detected by quantitative RT-PCR. N=3-4 replicates per genotype and treatment, of each ~20 seedlings. Error bars indicate SEM. **A-C.** For statistical analyses (full anova and Tukey HSD) see *SI Appendix,* Supporting information data sets.

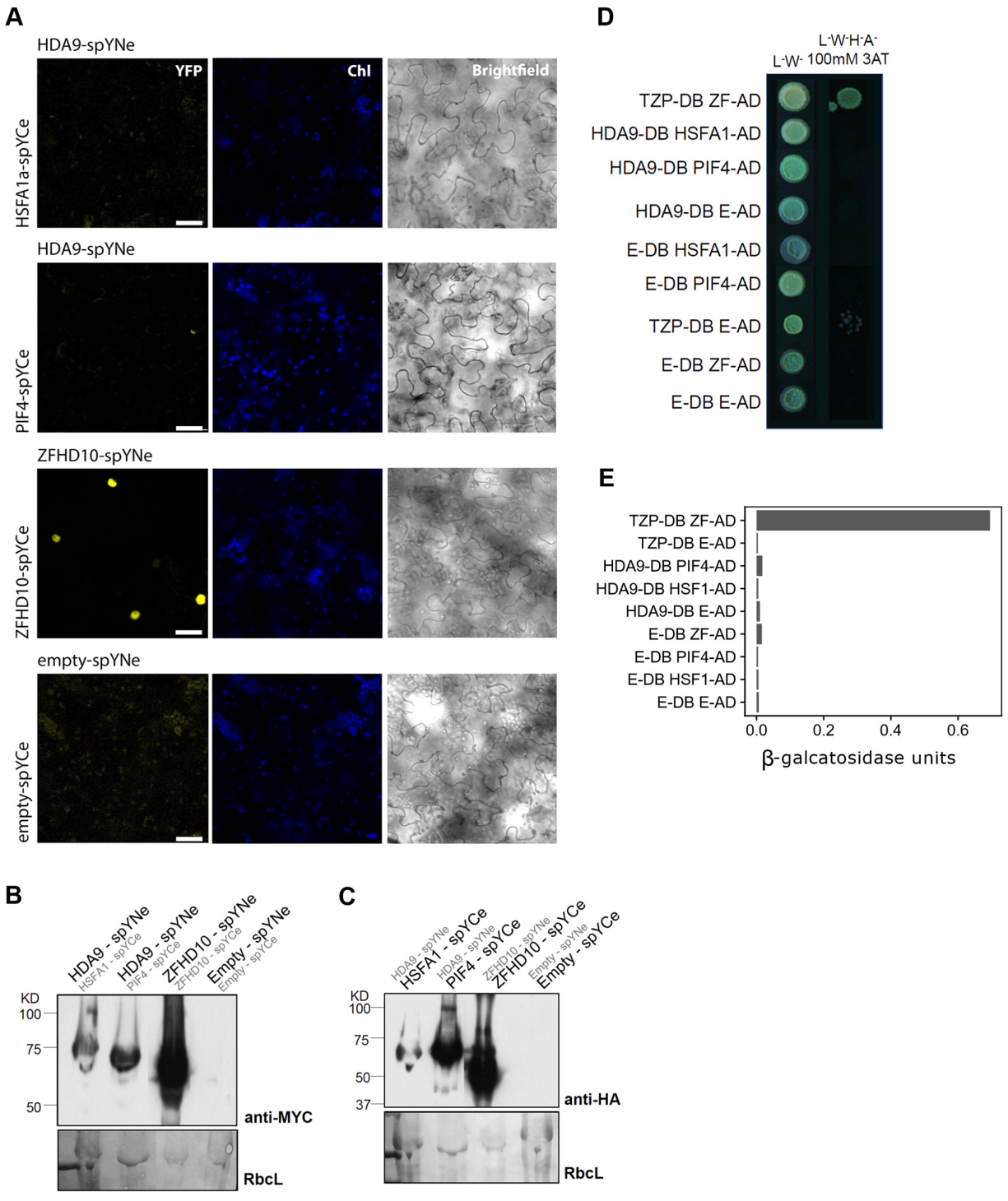

A
HDA9-spYNe
YFP
Chl
Brightfield
HSFA1a-spYCe
HDA9-spYNe
PIF4-spYCe
ZFHD10-spYNe
ZFHD10-spYCe
empty-spYNe
empty-spYCe
D
L-W-H-A-
L-W- 100mM 3AT
TZP-DB ZF-AD
HDA9-DB HSFA1-AD
HDA9-DB PIF4-AD
HDA9-DB E-AD
E-DB HSFA1-AD
E-DB PIF4-AD
TZP-DB E-AD
E-DB ZF-AD
E-DB E-AD
E
TZP-DB ZF-AD
TZP-DB E-AD
HDA9-DB PIF4-AD
HDA9-DB HSF1-AD
HDA9-DB E-AD
E-DB ZF-AD
E-DB PIF4-AD
E-DB HSF1-AD
E-DB E-AD
0.0
0.2
0.4
0.6
β-galcatosidase units
B
HDA9 - spYNe
HSFA1 - spYCe
HDA9 - spYNe
PIF4 - spYCe
ZFHD10 - spYNe
ZFHD10 - spYCe
Empty - spYNe
Empty - spYCe
KD
100
75
50
anti-MYC
RbcL
C
HDA9 - spYNe
HSFA1 - spYCe
HDA9 - spYNe
PIF4 - spYCe
ZFHD10 - spYNe
ZFHD10 - spYCe
Empty - spYNe
Empty - spYCe
KD
100
75
50
37
anti-HA
RbcL

Figure S5. HDA9 does not interact with HSFA1 nor with PIF4. A, Representative confocal images of Bimolecular Fluorescence Complementation (BiFC) analysis, assessing protein interactions between: HDA9 (HDA9-spYNe) and HSFA1 (HSFA1-spYCe; upper row) and PIF4 (PIF4-spYCe; second row), transiently co-expressed in *Nicotiana benthamiana* epidermal cells. ZINC-FINGER HOMEODOMAIN 10 (ZFHD10) homodimerization (ZFHD10-spYCe + ZFHD10-spyNe; third row) is included as a positive control, as published, see ref. (2) and empty vectors (lowest row) as negative control. Per interaction the YFP fluorescence complementation signal (left panel), chlorophyll autofluorescence signal (Chl; middle panel) and brightfield capture (right) are shown. Scale bars = 40 µm. **B,C,** Western blot analysis on total protein extracts from infiltrated *N. benthamiana* confirming the co-expression of all proteins tested in the BiFC experiment. **B,** An anti-HA antibody was used to detect spYCe fusions and **C,** an anti-cMYC for spYNe fusions. Ponceau staining of RbcL is shown as loading control. Note that the blots are compiled pictures, but per panel, all lanes are derived from the same gel and captured with identical intensity settings. **D,** Yeast-two-hybrid analysis of possible interactions between the indicated proteins fused to GAL4 DB or GAL4 AD. Yeast growth assays were performed on selective (L-W-H-A- 100mM 3AT) and non-selective (L-W-) media. Autoactivation of each protein was assessed by co-transformation with the respective empty GAL4 AD or DB empty plasmids (indicated with E). The known interaction between TANDEM ZINC-FINGER PLUS3 (TZP-DB) and ZFHD10 (ZF) is included as a positive control, as published, see ref. (2). **E,** Quantitative beta-galactosidase assay using Ortho-NitroPhenyl-β-Galactoside (ONPG), confirming the absence of interaction between HDA9 and HSFA1a and PIF4 with the approaches used and under the conditions tested**.**

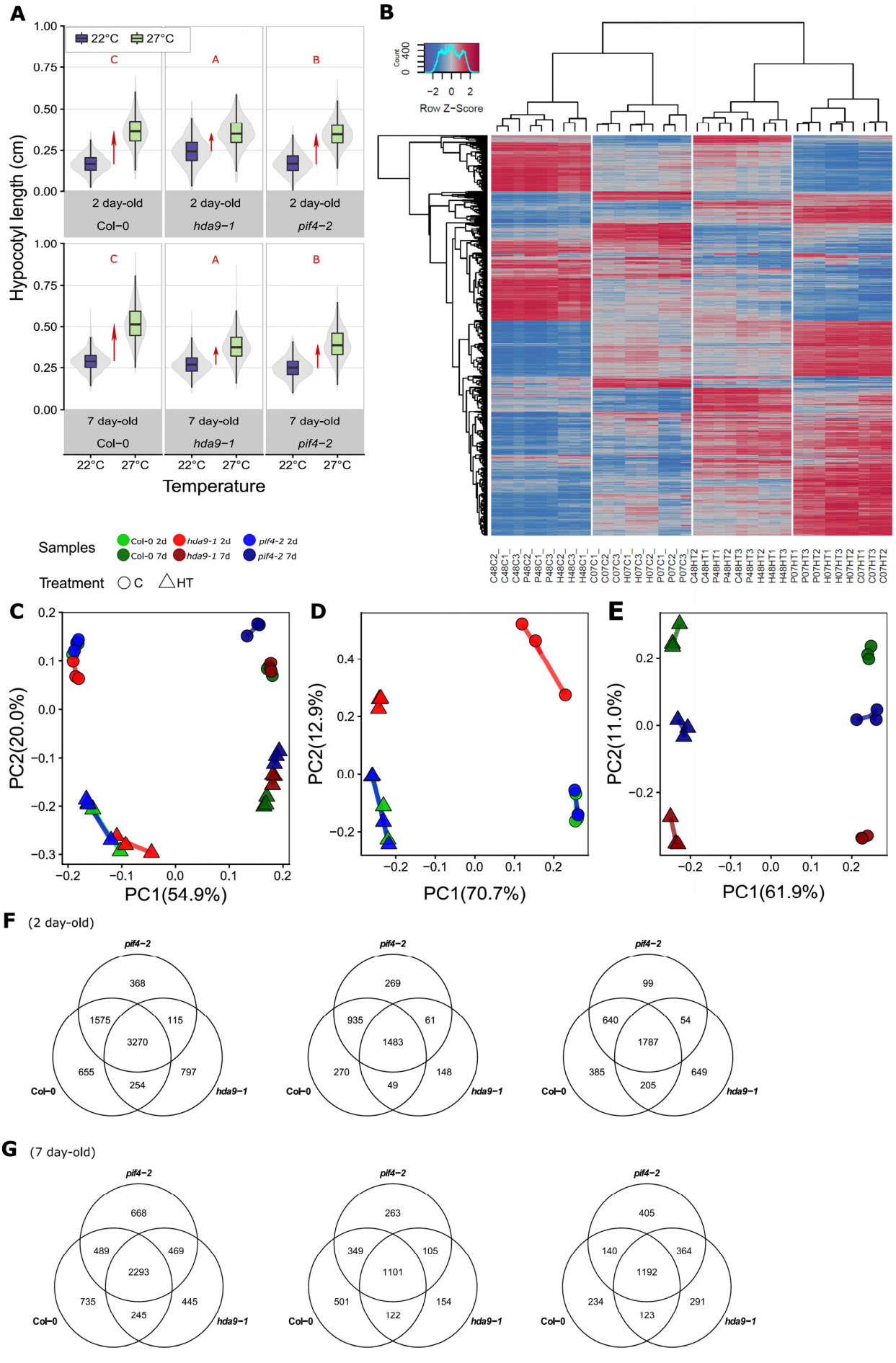

A
22°C
27°C
C
A
B
Hypocotyl length (cm)
1.00
0.75
0.50
0.25
0.00
2 day-old
Col−0
hda9−1
pif4−2
7 day-old
Col−0
hda9−1
pif4−2
22°C
27°C
Temperature
B
Count
0
400
−2
0
2
Row Z-Score
C48C2_
C48C1_
C48C3_
P48C2_
P48C1_
P48C3_
H48C2_
H48C3_
H48C1_
C07C1_
C07C2_
C07C3_
H07C1_
H07C3_
H07C2_
P07C1_
P07C2_
P07C3_
C48HT2
C48HT1
P48HT1
P48HT2
C48HT3
P48HT3
H48HT2
H48HT1
H48HT3
P07HT1
P07HT3
P07HT2
H07HT1
H07HT3
H07HT2
C07HT1
C07HT3
C07HT2
Samples
Col-0 2d
Col-0 7d
hda9-1 2d
hda9-1 7d
pif4-2 2d
pif4-2 7d
Treatment
C
HT
C
PC2(20.0%)
PC1(54.9%)
D
PC2(12.9%)
PC1(70.7%)
E
PC2(11.0%)
PC1(61.9%)
F
(2 day-old)
pif4−2
368
1575
115
3270
655
797
254
Col−0
hda9−1
269
935
61
1483
270
148
49
99
640
54
1787
385
649
205
G
(7 day-old)
668
489
469
2293
735
445
245
263
349
105
1101
501
154
122
405
140
364
1192
234
291
123

Figure S6. RNA-sequencing experiments. A, Hypocotyl lengths of 2 day-old (upper panel) and 7 day-old (lower panel) Col-0 wild type, *hda9-1* and *pif4-2* mutant seedlings, used for transcriptomics experiments, at 22^{o}C (purple) and 27^{o}C (green). Boxes indicate the boundaries of the second and third quartile of the data distribution. Black bars within the boxes indicate the median and whiskers the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. N=749-877 seedlings per genotype, treatment and time point, divided over 12 replicates. Red letters indicate significant different groups between the hypocotyl responses (change) (P<0.05; two-sided t-test). **B,** Euclidean distance hierarchically clustering of gene expression profiles. Columns represent biological samples (replicates) and rows indicate individual differentially expressed genes. Abbreviations: First letter indicates genotype (C = Col-0, P = *pif4-2,* H = *hda9-1*), 48 and 07 indicate respectively 2 day-old (48h) and 7 day-old seedlings. The following C or HT indicate control (22^{o}C) and high temperature (27^{o}C) respectively, followed by the sample number (1,2,3). Heat maps show all genes that are highly significantly affected by genotype and/or high temperature (1542; p < 1e-10; abs. log2 ratio > 0.5). The color range in the legend indicates Z-ranges from +3 (red, above average expression) to -3 (blue, below average expression). The dendrogram above the columns indicate relatedness among samples and the dendrogram linking the rows indicates similarity in expression patterns. **C,** First two axes of PCA on log2 ratio data of all detected transcripts. When all (both 2 and 7 day-old) samples are included; PC1 separates 2 day-old seedlings samples (light colored symbols) from 7 day-old seedlings (dark colored symbols) and PC2 separates 22^{o}C (circles) from 27^{o}C (triangles). **D,** when 2 day-old seedling samples are considered; PC1 separates 22^{o}C from 27^{o}C and PC2 separates *hda9-1* (red) from Col-0 (green) and *pif4-2* (blue). **E,** when 7 day-old seedling samples are considered; PC1 separates 22^{o}C from 27^{o}C and PC2 separates *hda9-1* from Col-0 and from *pif4-2*. Percentage of explained variance is indicated between brackets. **F,G,** VENN diagrams indicating overlap in genes affected by high temperature (comparison 22^{o}C vs 27^{o}C), between Col-0, *hda9-1* and *pif4-2* (Log2 differential expression cut-off at *p*=0.001 and absolute log2 expression ratio >0.5) in **F,** 2 day-old and **G,**7 day-old seedlings. Left diagrams; All differentially regulated genes, middle diagrams; upregulated by high temperature, right diagrams; downregulated by high temperature. Note that in 2 day-old seedlings, most genes specifically affected in *hda9-1* are down regulated opposed to the genes specifically affected in *pif4-2*, which are mostly upregulated. Auxin GO terms are enriched (Table 1) among the upregulated genes shared by *pif4-2* and Col-0 (**F,** middle panel; 935 genes), indicating that these genes do not respond in *hda9-1* upon high temperature treatment. In 7-day-old seedling (**G**), the overlap between Col-0 and *pif4-2* has gone down and *hda9-1* and *pif4-2* share more differentially-regulated genes compared to Col-0, that are mostly down regulated. For statistical analyses of transcriptomics data see Material and Methods section and *SI Appendix,* Supporting information data sets.

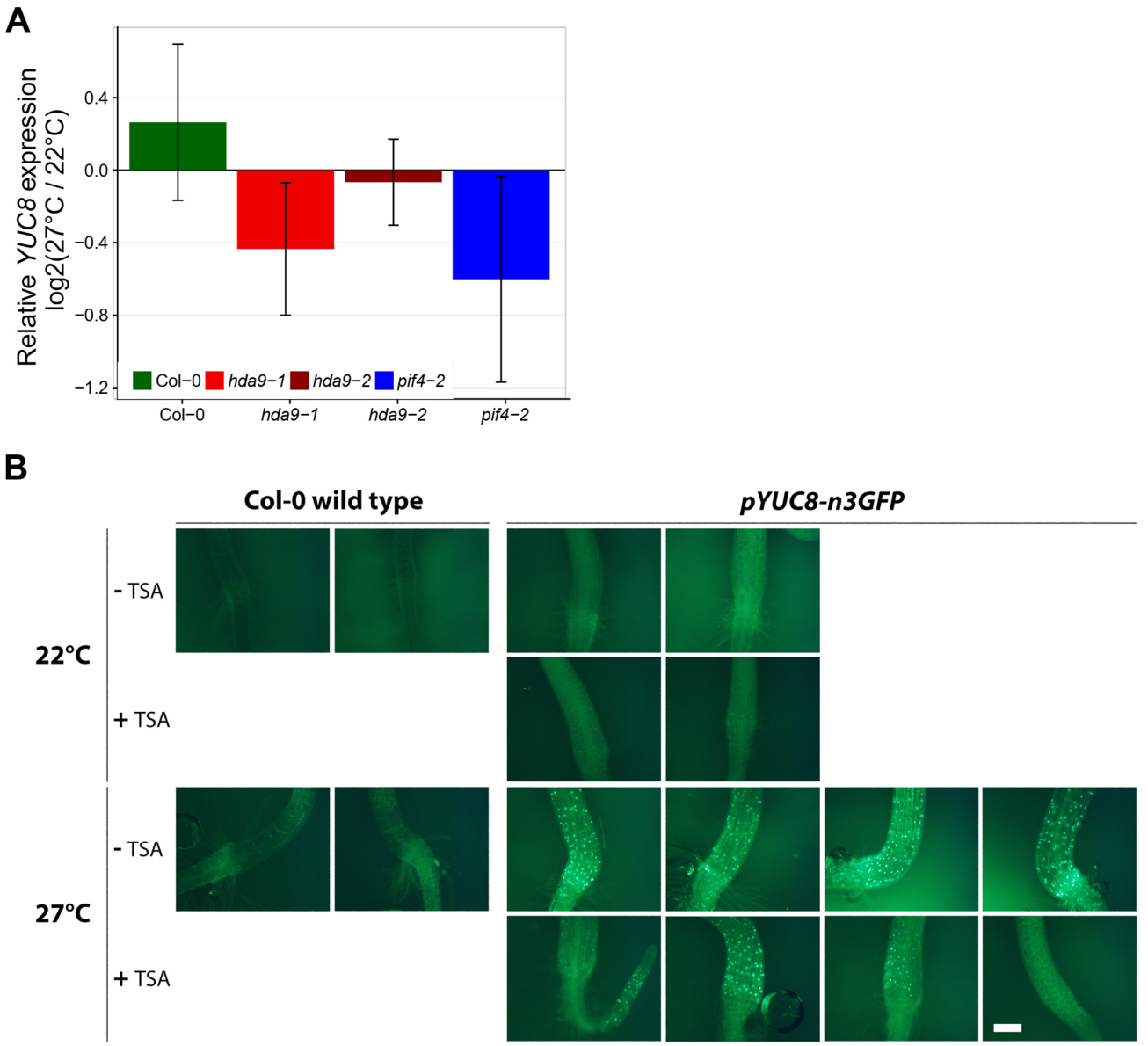


Figure S7. HDA9 modulates thermomorphogenesis in an auxin-dependent manner. A, Relative *YUC8* expression (27°C/22°C), 18-20h after transfer to high temperature (27°C) in 7 day-old Col-0 wild type (green), *hda9-1* (red), *hda9-2* (dark red) and *pif4-2* (blue) whole seedlings transiently transformed with *pYUC8:rLUC-35S:rLUC*. N=10, of each ~20 seedlings. **B,** Representative images of the root-shoot junction of 2 day-old Col-0 wild type and *pYUC8:n3GFP* at 22°C and 27°C in the presence and absence of Trichostatin-A (TSA; 1 µM). Scale bar = 200 µm.

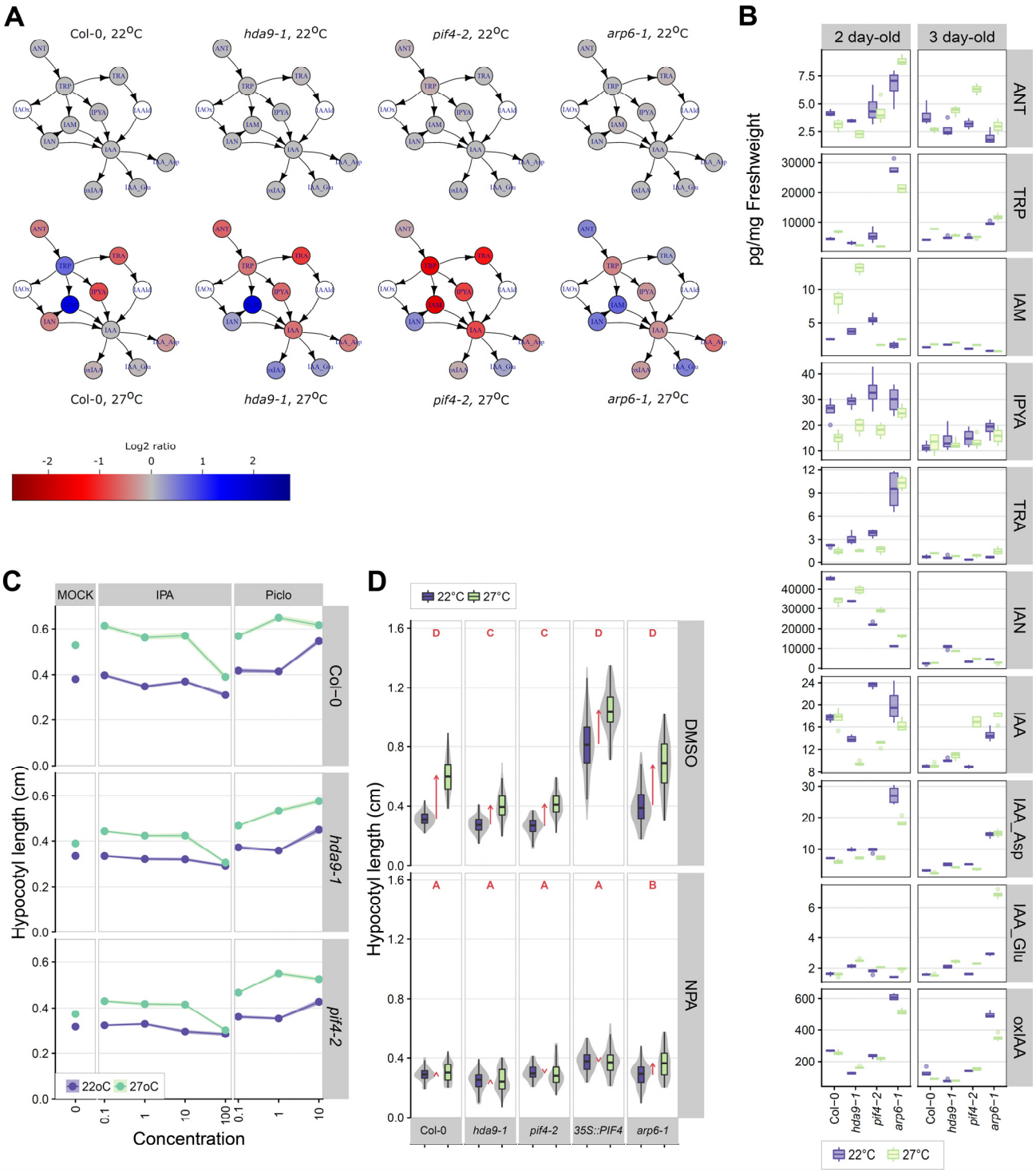

A
Col-0, 22°C
hda9-1, 22°C
pif4-2, 22°C
arp6-1, 22°C
Col-0, 27°C
hda9-1, 27°C
pif4-2, 27°C
arp6-1, 27°C
Log2 ratio
-2
-1
0
1
2
B
2 day-old
3 day-old
pg/mg Freshweight
ANT
TRP
IAM
IPYA
TRA
IAN
IAA
IAA_Asp
IAA_Glu
oxIAA
Col−0
hda9−1
pif4−2
arp6−1
22°C
27°C
C
MOCK
IPA
Piclo
Col−0
hda9-1
pif4-2
Hypocotyl length (cm)
22oC
27oC
Concentration
D
22°C
27°C
DMSO
NPA
Hypocotyl length (cm)
Col-0
hda9-1
pif4-2
35S::PIF4
arp6-1

Figure S8. HDA9 modulates thermomorphogenesis in an auxin-dependent manner. A, High temperature-induced changes in auxin metabolite levels in Col-0 wild type and *hda9-1, pif4-2* and *arp6-1* mutants, relative to control temperature (22°C). Blue and red indicate respectively higher and lower levels. Color intensities scale with log2 relative change, white symbols indicates not detectable. N=4 replicates per genotype and treatment, each of 10 mg (FW) 2 day-old seedlings. **B,** Absolute levels of auxin biosynthesis intermediates and catabolites, as indicated in panel **A** and Fig. 3C, at 22°C (purple) and 27°C (green) in 2 day-old (left column) and 3 day-old seedlings (right column). Abbreviations: ANT = Anthranilate, TRP = tryptophan, IAM = indole-3-acetamide, IPYA = indole-3-pyruvic acid, TRA = tryptamine, IAN = Indole-3 acetonitrile, IAA = indole-3-acetic acid, IAA_Asp = indole-3-acetyl aspartic acid conjugate, IAA_Glu = indole-3-acetyl glutamate acid conjugate, oxIAA = oxindole-3-acetic acid. **C,** Hypocotyl lengths of 8 day-old Col-0 wild type (upper row), *hda9-1* (middle row) and *pif4-2* (lower row) grown in the presence of different concentrations of IPyA, and Picloram at 22°C (purple) and 27°C (green). Mock treated plants are shown as reference in the most left column. Shaded areas behind the lines indicate SEM**.** N=80-200 seedlings per genotype, per treatment per concentration, divided over 9 replicates. For statistical analyses (full anova) see *SI Appendix,* Supporting information data sets. **D,** Hypocotyl lengths of 8 day-old Col-0 wild type, *hda9-1*, *pif4-2, 35S::PIF4,* and *arp6-1* in absence (mock; upper panel) and presence of 25 µM NPA at 22°C (purple) and 27°C (green). N=108-150 seedlings per genotype per treatment, divided over 3 replicates. Boxes in panels **B,D** indicate the boundaries of the second and third quartile of the data distribution. Black bars within the boxes indicate the median and whiskers the Q1 and Q4 values within 1.5 times the interquartile range. **D,** Violin plots behind the boxes designate phenotype distributions. Red arrows (**D**) indicate the difference in mean hypocotyl length between treatment and control. Red letters indicate significant different groups of hypocotyl responses (change) per panel ($P < 0.01$; two-sided t-test using the means and SD).

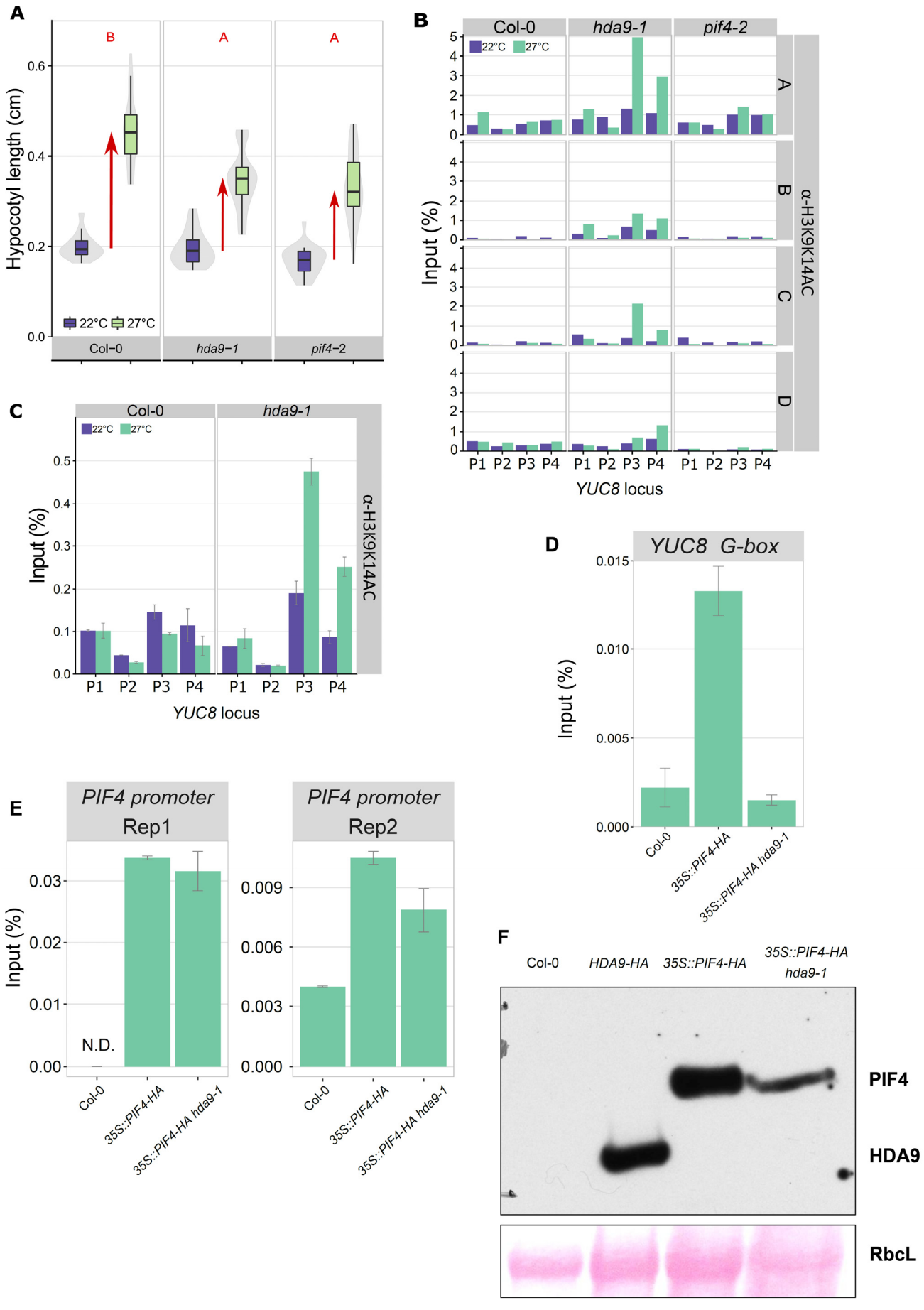

A
B
A
A
Hypocotyl length (cm)
0.6
0.4
0.2
0.0
22°C 27°C
Col−0
hda9−1
pif4−2
B
Col-0
hda9-1
pif4-2
22°C 27°C
Input (%)
A
B
C
D
α-H3K9K14AC
P1 P2 P3 P4 P1 P2 P3 P4 P1 P2 P3 P4
YUC8 locus
C
Col-0
hda9-1
22°C 27°C
Input (%)
α-H3K9K14AC
P1 P2 P3 P4 P1 P2 P3 P4
YUC8 locus
D
YUC8 G-box
Input (%)
0.015
0.010
0.005
0.000
Col-0
35S::PIF4-HA
35S::PIF4-HA hda9-1
E
PIF4 promoter
Rep1
PIF4 promoter
Rep2
Input (%)
0.03
0.02
0.01
0.00
N.D.
0.009
0.006
0.003
0.000
Col-0
35S::PIF4-HA
35S::PIF4-HA hda9-1
F
Col-0
HDA9-HA
35S::PIF4-HA
35S::PIF4-HA
hda9-1
PIF4
HDA9
RbcL

Figure S9. H3K9K14Ac levels and PIF4 binding at the *YUCCA8* locus. A, Hypocotyl lengths of 7 day-old Col-0 wild type, *hda9-1* and *pif4-2* mutants at 22°C (purple) or 27°C (green), to confirm effectiveness of high temperature treatment of seedlings used for ChIP experiments (in 2 day-old seedlings). N=16-27 seedlings per genotype per and treatment. Black bars within the boxes indicate the median and the whiskers indicate the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. Red arrows indicate the difference in mean hypocotyl length between treatment and control. Red letters indicate significant different groups of hypocotyl responses (change) per panel (P< 0.01; two-sided t-test using the means and SD). **B,** Four independent replicates of ChIP-qPCR analysis of H3K9K14Ac of the *YUC8* locus in the Col-0, *hda9-1* and *pif4-2* genetic backgrounds at 22°C (purple) or 27°C (green) in 2 day-old seedlings, depicted as fraction (%) of input. **C,** ChIP-qPCR analysis of H3K9K14Ac on the *YUC8* locus in 10 day-old Col-0 and *hda9-1* seedlings. **B,C,** P1 (-1374 bp), P2 (-657 bp), P3 (4 bp) and P4 (1813 bp) indicate tested positions relative to the transcriptional start site (0) and are derived from Lee et al. 2014 (3). **D,E,** ChIP-qPCR analysis of PIF4 binding to **D,** the G-box motif of the *YUC8* promoter **E,** the *PIF4* promoter (positive control; 2 replicates correspond to data presented in Fig. 4C (replicate 1) and Fig. S9D (replicate 2)), in Col-0 wild type (negative control), *35S::PIF4-HA* and *35S::PIF4-HA hda9-1,* 2 day-old seedlings at 27°C. N.D. indicates not detectable. **C-E,** Error bars represent SEM of 2 technical replicates. **F,** Western blot analysis of 2 day-old Col-0, *HDA9-HA* (positive control), *35S::PIF4-HA* and *35S::PIF4-HA hda9-1* seedlings, with antibodies raised against the HA epitope (50 mg pooled seedlings per genotype and treatment). Ponceau staining of RIBULOSE BIPHOSPHATE CARBOXYLASE LARGE CHAIN (RbcL) is shown as loading control.

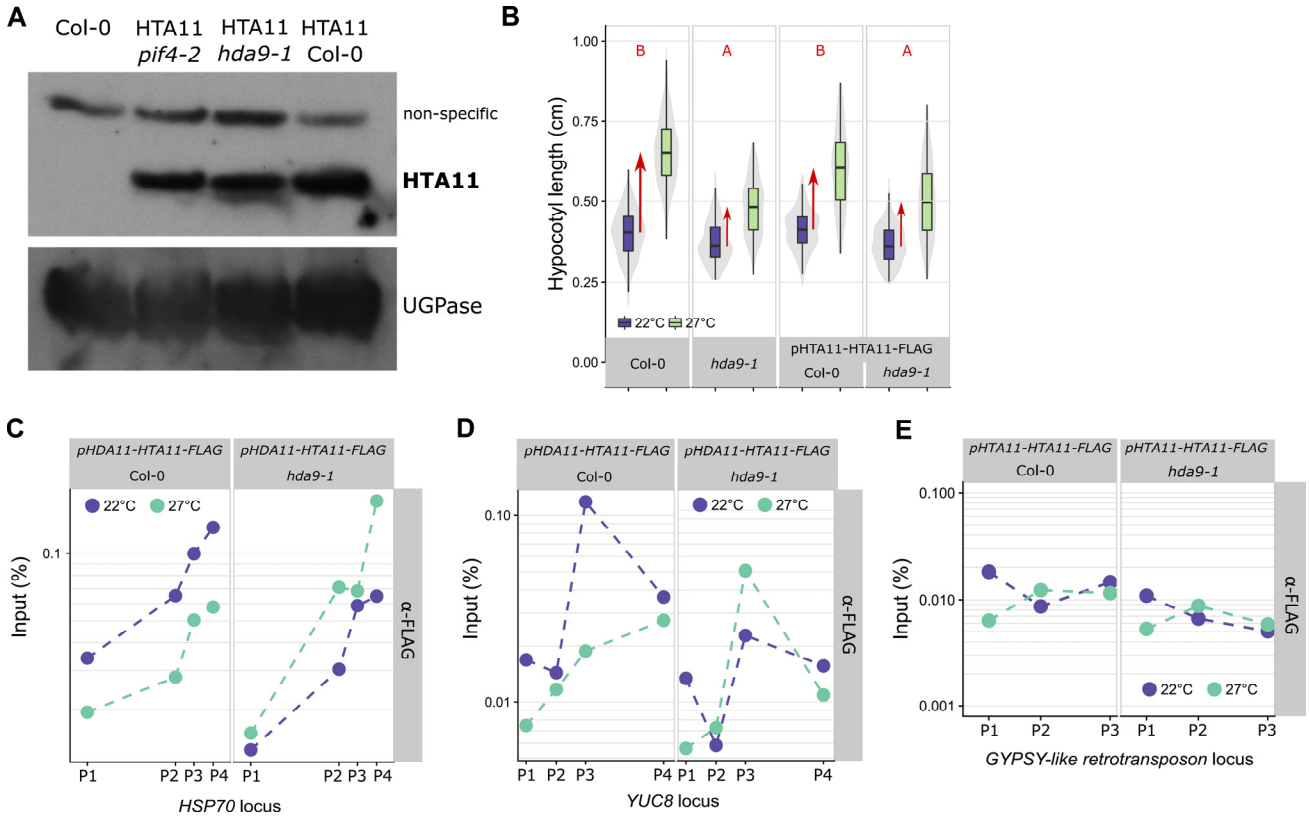
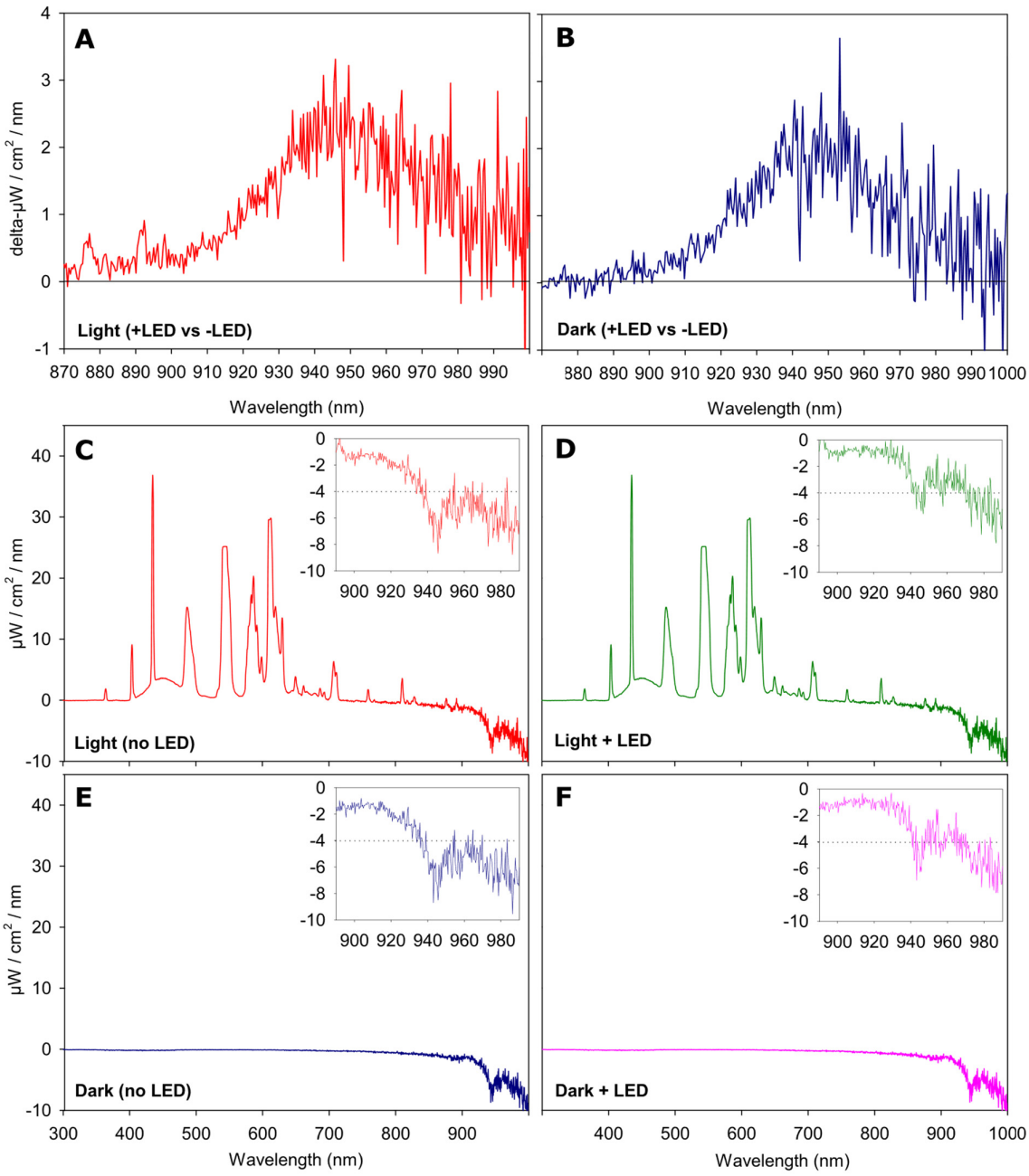


Figure S10. H2A.Z occupancy and H3K9K14Ac levels at the *YUCCA8* locus. A, Western blot analysis of 2 day-old Col-0, *hda9-1* and *pif4-2* seedlings harboring the *pHTA11-HTA11-FLAG* construct, grown at 22°C or 27°C, with antibodies raised against the FLAG epitope (50 mg pooled seedlings per genotype and treatment), indicating that the *hda9-1* nor *pif4-2* mutation affect H2A.Z (HTA11) levels. As loading control anti-UGPase was used. **B,** Hypocotyl lengths of 7 day-old Col-0 wild type, *hda9-1* and *pHTA11-HTA11-FLAG* (H2A.Z) immune-tagged lines in the wild type and *hda9-1* mutant background at 22°C (purple) or 27°C (green). N=172-347 seedlings per genotype per treatment, divided over 7 replicates. Black bars within the boxes indicate the median and the whiskers indicate the Q1 and Q4 values within 1.5 times the interquartile range. Violin plots behind the boxes designate phenotype distributions. Red arrows indicate the difference in mean hypocotyl length between treatment and control. Red letters indicate significant different groups of hypocotyl responses (change) per panel (P< 0.01; two-sided t-test using the means and SD). **C-E,** independent replicates of H2A.Z (HTA11) enrichment at different chromatin regions of **C,** *HSP70* and **D,** *YUCCA8* and **H,** the *GYPSY*-like transposon (*At4g07700*) locus (negative control), all in 2 day-old *pHTA11-HTA11-FLAG* lines in the Col-0 wild type and *hda9-1* mutant genetic backgrounds, depicted as fraction (%) of input. Tested chromatin regions are **C,** P1 (-1374 bp), P2 (-657 bp), P3 (4 bp) and P4 (1813 bp), **D,** P1 (-359 bp), P2 (4 bp), P3 (80 bp) and P4 (159 bp) and **E,** P1 (-73 bp), P2 (1 bp), P3 (100 bp) relative to the transcriptional start site (0) and are derived from Lee et al. 2014 (3) (*YUC*8) and Kumar & Wigge, 2010 (1) (*HSP70, GYSPSY*).


A
B
C
D
E
F
delta-µW / cm² / nm
µW / cm² / nm
Light (+LED vs -LED)
Dark (+LED vs -LED)
Light (no LED)
Light + LED
Dark (no LED)
Dark + LED
Wavelength (nm)

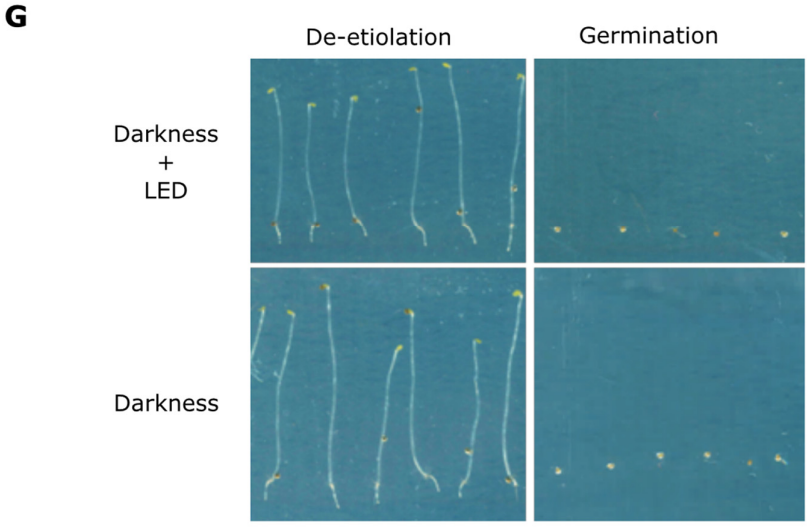

G
De-etiolation
Germination
Darkness + LED
Darkness

Figure S11. Validation of continuous hypocotyl elongation measurement experimental setup. A,B, Differential of the light spectra in the 870-1000 nm wavelength band (with-without supplemented LED light; 940 ± 10 nm) during **a,** day time and **B,** in darkness. **C-F,** show the full spectra (200-1000 nm) during **C,D,** day time **C,** without and **D,** with supplemental LED lighting and **E,F,** in the night period **E,** without and **F,** with supplemented LED light. Insets in panel **C-F** show a close-up of the wavelength band between 890 en 990 nm. **G,** Validation of the effect of supplemented LED lighting (940 ± 10 nm) on seed germination and seedling de-etiolation. Shown are scans of seeds and de-etiolated seedlings, exposed for 7 days with (upper panels) and without (control; lower panels) supplemented LED lighting in otherwise dark conditions. To induce germination, seedlings (left panels, de-etiolated) were exposed to white light for 6 hours prior to LED exposure, while non-germinated seeds (right panels) were transferred directly to darkness.

Tabel S1. Change in expression (Log2) of selected auxin biosynthesis, perception and signaling genes

	Gene ID	Gene name	AGI	Differential expression (log2) at high temperature						*p* values					
				Col-0 2d	*hda9-1* 2d	pif4 2d	Col-0 7d	hda9-1 7d	pif4 7d	Col-0 2d	hda9-1 2d	*pif4-2* 2d	Col-0 7d	*hda9-1* 7d	pif4-2 7d
Biosynthesis	***YUC1***	*YUCCA 1*	AT4G32540	0,02	0,00	0,04	-0,23	-0,23	0,01	0,998190673	0,99999986	0,960539042	0,34067949	0,326945013	0,999997527
	YUC2	*YUCCA2*	AT4G13260	0,17	0,10	0,02	-0,47	-0,68	-0,29	0,64487078	0,947754859	0,999917325	0,000639173	1,48769E-05	0,02592619
	YUC3	*YUCCA 3*	AT1G04610	-0,55	-0,21	-0,41	-0,13	-0,28	-0,39	0,003929979	0,453401478	0,031002277	0,479906994	0,016261655	0,001509193
	YUC4	*YUCCA4*	AT5G11320	-0,22	-0,08	-0,17	-0,22	-0,10	-0,34	0,204287142	0,948396786	0,421491036	0,5352816	0,961701654	0,150460331
	YUC5	*YUCCA5*	AT5G43890	-0,04	-0,22	-0,05	-0,39	0,12	0,04	0,993085283	0,071640335	0,970641054	0,024716764	0,850765953	0,999048117
	YUC6	*YUCCA6*	AT5G25620	0,45	0,22	0,39	0,05	-0,11	-0,39	5,82398E-05	0,020105247	0,000202448	0,998848113	0,976537	0,165703727
	YUC7	*YUCCA7*	AT2G33230	-0,86	-1,07	-0,75	0,00	-0,10	-0,03	4,00791E-05	4,03294E-06	0,000149805	1	0,412947149	0,984207795
	YUC8	*YUCCA8*	AT4G28720	1,10	0,54	0,94	0,72	0,26	1,04	2,33434E-05	0,012302895	0,000112454	0,00048614	0,289430953	1,40669E-05
	YUC9	*YUCCA9*	AT1G04180	-0,10	0,04	-0,04	-0,39	0,02	0,61	0,990991545	0,999928694	0,99984795	0,042100246	0,999973119	0,001713324
	YUC10	*YUCCA10*	AT1G48910												
	AMI1	*AMIDASE-LIKE PROTEIN 1*	AT1G08980	1,47	1,32	1,54	0,95	0,89	1,30	8,93358E-08	3,09197E-07	5,16911E-08	1,7384E-08	3,8444E-08	4,88216E-10
	TAA1	*TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*	AT1G70560	-0,19	-0,26	-0,03	-0,78	-0,86	-0,59	0,746397948	0,435745511	0,999882595	0,008733045	0,004412822	0,052813261
	TAR2	*TRYPTOPHAN AMINOTRANSFERASE RELATED 2*	AT4G24670	-0,94	-0,84	-0,81	-0,46	-0,18	-0,57	0,000334485	0,000953796	0,001329928	0,017461434	0,650651157	0,0039612
	TAR1	*TRYPTOPHAN AMINOTRANSFERASE RELATED 1*	AT1G23320												
	CYP79B2	*CYTOCHROME P450, FAMILY 79, SUBFAMILY B POLYPEPTIDE 2*	AT4G39950	-0,74	-0,99	-0,66	-1,25	-0,82	-1,36	0,007317158	0,000663341	0,016914019	3,93058E-05	0,001951639	1,60896E-05
	CYP79B3	*CYTOCHROME P450, FAMILY 79, SUBFAMILY B POLYPEPTIDE 3*	AT2G22330	0,12	0,02	0,21	-0,89	-0,66	-1,01	0,742669014	0,999858669	0,223S3107	2,30927E-06	5,58307E-05	5,77769E-07
	NIT1	*NITRILASE 1*	AT3G44310	-0,32	-0,30	-0,31	0,37	0,52	0,34	0,000830771	0,001374637	0,001144692	0,009250535	0,000530616	0,014542923
	NIT2	*NITRILASE 2*	AT3G44300	1,14	0,44	0,60	0,05	0,05	0,02	0,097585245	0,858575406	0,634329759	0,995374477	0,997484011	0,999976369
	NIT3	*NITRILASE 3*	AT3G44320	-0,82	-0,98	-0,83	-0,13	-0,22	-0,27	0,000110051	1,76888-05	9,56357E-05	0,802786293	0,347059798	0,185152062
	CYP71A13	*CYTOCHROME P450, FAMILY 71, SUBFAMILY A, POLYPEPTIDE 13*	AT2G30770	-0,05	0,33	-0,09	-0,67	-0,43	-0,41	0,998410552	0,193235582	0,981759265	0,000587742	0,021425404	0,026311652
	CYP71B15	*CYTOCHROME P450, FAMILY 71, SUBFAMILY B, POLYPEPTIDE 15*	AT3G26830	0,24	-0,13	0,04	-0,48	-0,46	-0,53	0,506051458	0,92543458	0,999711457	0,155255698	0,18247003	0,106564158
	CYP83B1	*CYTOCHROME P450, FAMILY 83, SUBFAMILY B, POLYPEPTIDE 1*	AT4G31500	-0,42	-0,53	-0,33	-0,31	-0,04	-0,60	0,073655704	0,019991149	0,216268131	0,246361954	0,999489184	0,005941817
perception	***TIR1***	*TRANSPORT INHIBITOR RESPONSE 1*	AT3G62980	0,67	0,58	0,68	0,34	0,40	0,48	1,03455E-05	5,10152E-05	8,64979E-06	0,04141265	0,017081376	0,004133009
	AFB4	*AUXIN SIGNALING F-BOX 4*	AT4G24390	0,22	0,17	0,18	0,02	-0,07	0,03	0,032978983	0,109049784	0,082414793	0,999480873	0,891321474	0,998076779
	AFB5	*AUXIN F-BOX PROTEIN 5*	AT5G49980	0,12	0,09	0,18	0,15	0,05	0,17	0,104844057	0,310041974	0,009639128	0,008935263	0,767830455	0,003378184
	AFB3	*AUXIN SIGNALING F-BOX 3*	AT1G12820	0,53	0,51	0,56	0,35	0,27	0,28	1,2477E-06	1,64305E-06	7,07785E-07	1,11094E-06	1,6213E-05	1,45514E-05
	AFB2	*AUXIN SIGNALING F-BOX 2*	AT3G26810	0,50	0,38	0,46	0,22	0,10	0,30	2,19577E-07	3,90442E-06	4,65713E-07	0,007694799	0,398579874	0,000798779
	AFB1	*AUXIN SIGNALING F BOX PROTEIN 1*	AT4G03190	0,00	-0,09	-0,04	0,14	-0,03	0,17	1	0,355373118	0,926133748	0,411591019	0,998302377	0,240012314
AUX/IAA	***IAA1/AXR5***	*INDOLE-3-ACETIC ACID INDUCIBLE / AUXIN RESISTANT 5*	AT4G14560	0,32	0,36	0,37	0,71	0,46	0,83	0,047395657	0,021554929	0,018408276	0,000874208	0,024842607	0,000203842
	IAA2	*INDOLE-3-ACETIC ACID INDUCIBLE 2*	AT3G23030	1,00	0,66	0,94	1,03	0,90	0,99	0,000380532	0,012074666	0,000686184	6,44512E-06	2,56271E-05	1,02636E-05
	IAA3/SHY2	*INDOLE-3-ACETIC ACID INDUCIBLE 3 / SHORT HYPOCOTYL*	AT1G04240	1,00	0,29	0,96	0,14	-0,07	0,02	0,000257593	0,439868867	0,000386559	0,853775731	0,987178953	0,99998401
	IAA4	*INDOLE-3-ACETIC ACID INDUCIBLE 4*	AT5G43700	0,59	0,47	0,55	0,46	0,10	0,17	3,74711E-06	3,93698E-05	7,98604E-06	0,043069659	0,976694139	0,780905824
	IAA5	*INDOLE-3-ACETIC ACID INDUCIBLE 5*	AT1G15580	-0,16	-0,17	0,16	-0,04	-0,07	-0,06	0,376259927	0,328149604	0,405481358	0,953532258	0,684720929	0,825543317
	IAA6/SHY1	*INDOLE-3-ACETIC ACID 6/SHORT HYPOCOTYL 1*	AT1G52830	2,21	0,63	2,24	0,13	-0,05	0,30	2,10698E-06	0,085854073	1,80686E-06	0,891403591	0,998429331	0,228504864
	IAA7/AXR2	*INDOLE-3-ACETIC ACID 7 / AUXIN RESISTANT 2*	AT3G23050	1,67	0,66	1,61	0,86	0,61	0,63	1,45321E-05	0,037443593	2,13356E-05	2,55384E-06	9,53034E-05	6,8977E-05
	IAA9	*INDOLE-3-ACETIC ACID INDUCIBLE 9*	AT5G65670	0,12	0,03	0,08	0,43	0,15	0,09	0,070033384	0,967977832	0,28562344	0,017030456	0,737201805	0,956856394
	IAA10	*INDOLEACETIC ACID-INDUCED PROTEIN 10*	AT1G04100	0,04	-0,12	-0,06	0,10	-0,17	-0,14	0,992635333	0,62724323	0,953490855	0,798465368	0,283030072	0,464276878
	IAA11	*INDOLE-3-ACETIC ACID INDUCIBLE 11*	AT4G28640	0,23	0,14	0,33	0,12	-0,17	-0,25	0,008532611	0,159501062	0,000496329	0,701625138	0,371199789	0,095162632
	IAA12/BDL	*INDOLE-3-ACETIC ACID INDUCIBLE 12 / BODENLOS*	AT1G04550	-0,39	-0,34	-0,33	-0,34	-0,67	-0,30	5,96774E-06	2,15994E-05	3,26102E-05	0,005147949	9,87543E-06	0,013615107
	IAA13	*AUXIN-INDUCED PROTEIN 13*	AT2G33310	0,31	0,33	0,26	0,60	0,31	0,39	0,005116858	0,0029338	0,019839045	7,46511E-07	0,00061114	7,99731E-05
	IAA14/SLR	*INDOLE-3-ACETIC ACID INDUCIBLE 14 / SOLITARY ROOT*	AT4G14550	1,22	0,89	1,15	0,00	-0,17	0,03	6,10827E-06	0,000153829	1,1254E-05	0,99999997	0,186621382	0,994532826
	IAA15	*INDOLE-3-ACETIC ACID INDUCIBLE 15*	AT1G80390												
	IAA16	*INDOLEACETIC ACID-INDUCED PROTEIN 16*	AT3G04730	0,73	0,64	0,63	0,43	0,34	0,22	5,12139E-05	0,000188355	0,000212716	3,62789E-05	0,000361523	0,016134072
	IAA17/AXR3	*INDOLE-3-ACETIC ACID INDUCIBLE 17/AUXIN RESISTANT 3*	AT1G04250	0,92	0,31	0,78	-0,03	-0,47	-0,49	7,70479E-06	0,070561339	3,96234E-05	0,997842555	0,000247519	0,000156818
	IAA18	*INDOLE-3-ACETIC ACID INDUCIBLE 18*	AT1G51950	0,40	0,36	0,38	0,34	0,41	0,50	0,002469122	0,005789391	0,003791875	0,000453591	6,25365E-05	8,93295E-06
	IAA19/MSG2	*INDOLE-3-ACETIC ACID INDUCIBLE 19 / MASSUGU 2*	AT3G15540	1,35	0,20	1,41	0,41	0,00	0,25	0,000245576	0,905609897	0,000166449	0,132926824	1	0,579428947
	IAA20	*INDOLE-3-ACETIC ACID INDUCIBLE 20*	AT2G46990	1,03	0,72	1,00	1,13	0,20	0,22	8,49141E-09	4,8137E-07	1,08363E-08	0,000205436	0,814792547	0,772565962
	IAA21/NPH4	*NDOLE-3-ACETIC ACID INDUCIBLE 21 / NON-PHOTOTROPHIC HYPOCOTYL / AUXIN RESPONSE FACTOR 7*	AT5G20730	0,53	0,15	0,65	0,11	0,16	0,11	0,000106462	0,348627616	1,33998E-05	0,927921822	0,776974118	0,939455642
	IAA22/ARF19/ARF11	*INDOLE-3-ACETIC ACID INDUCIBLE 22/AUXIN RESPONSE FACTOR 19 -11*	AT1G19220	-0,07	-0,05	-0,01	-0,13	-0,29	-0,25	0,940039012	0,973720318	0,999963208	0,969211647	0,541539147	0,669789655
	IAA24ARF5/MP	*;INDOLE-3-ACETIC ACID INDUCIBLE 24 / AUXIN RESPONSE FACTOR 5 / MONOPTEROS*	AT1G19850	0,04	0,01	0,14	-0,41	-0,61	-0,49	0,97982729	0,999998759	0,234582571	0,033895689	0,001995148	0,011526778
	IAA27	*INDOLE-3-ACETIC ACID INDUCIBLE 27*	AT4G29080	0,39	0,25	0,22	0,14	-0,38	-0,06	0,021490133	0,229432367	0,310466487	0,868031987	0,077107092	0,996822077
	IAA28	*;INDOLE-3-ACETIC ACID INDUCIBLE 28*	AT5G25890	-0,15	-0,29	-0,28	-0,43	-0,57	-0,73	0,81218923	0,219834743	0,247964635	0,010463581	0,001052462	0,000121811
	IAA29	*INDOLE-3-ACETIC ACID INDUCIBLE 29*	AT4G32280	1,23	0,95	0,99	0,09	0,24	0,86	3,90284E-05	0,000464763	0,000305206	0,924990838	0,166800166	8,82103E-06
	IAA30	*INDOLE-3-ACETIC ACID INDUCIBLE 30*	AT3G62100	-0,01	0,17	0,32	0,14	-0,09	-0,04	0,999999698	0,613074042	0,086045515	0,750219054	0,959376033	0,999282429
	IAA31	*INDOLE-3-ACETIC ACID INDUCIBLE 31*	AT3G17600	-1,03	-0,32	-0,88	0,01	-0,02	-0,14	0,000146799	0,301754127	0,000683829	0,999988033	0,999791444	0,665757375
	IAA32	*INDOLE-3-ACETIC ACID INDUCIBLE 32*	AT2G01200	-0,04	0,11	0,16	0,45	0,08	0,11	0,999960798	0,992832059	0,953882903	3,50324E-05	0,745453922	0,387473242
	IAA33	*INDOLE-3-ACETIC ACID INDUCIBLE 33*	AT5G57420	-0,20	-0,10	-0,13	0,01	-0,01	0,01	0,21648932	0,828082125	0,610058468	0,999997811	0,999997147	0,999975434
	IAA34	*INDOLE-3-ACETIC ACID INDUCIBLE 34*	AT1G15050	-0,44	0,25	-0,46	0,86	-0,02	0,58	0,048975759	0,42523599	0,039714808	1,19208E-05	0,999836806	0,000604137
Auxin Response Factors	***ARF1***	*AUXIN RESPONSE FACTOR 1*	AT1G59750	0,24	0,17	0,27	0,21	0,11	0,11	0,001215085	0,020007418	0,000349845	0,503031251	0,932516605	0,934199382
	ARF10	*AUXIN RESPONSE FACTOR 10*	AT2G28350	0,10	-0,08	0,21	-0,20	-0,31	-0,33	0,365057544	0,64522073	0,010154812	0,633884902	0,219477968	0,179944703
	ARF11	*AUXIN RESPONSE FACTOR 11*	AT2G46530	0,30	0,13	0,44	-0,22	-0,40	-0,29	0,054058733	0,702644522	0,003762109	0,51984224	0,073386761	0,282956721
	ARF12	*AUXIN RESPONSE FACTOR 12*	AT1G34310												
	ARF13	*AUXIN RESPONSE FACTOR 13*	AT1G34170												
	ARF14	*AUXIN RESPONSE FACTOR 14*	AT1G35540												
	ARF16	*AUXIN RESPONSE FACTOR 16*	AT4G30080	0,03	-0,26	0,15	-0,31	-0,43	-0,47	0,994318787	0,002955847	0,105825067	0,005168467	0,000269305	0,000132941
	ARF17	*AUXIN RESPONSE FACTOR 17*	AT1G77850	-0,28	-0,46	-0,27	-0,04	-0,13	0,00	0,027626729	0,00063191	0,033862074	0,994825732	0,491212878	0,999999759
	ARF18	*AUXIN RESPONSE FACTOR 18*	AT3G61830	0,18	0,31	0,26	-0,14	-0,19	-0,16	0,802724062	0,330146846	0,512616927	0,856573758	0,63345138	0,778433583
	ARF2	*AUXIN RESPONSE FACTOR 2*	AT5G62000	0,50	0,51	0,54	0,05	0,06	0,19	7,34956E-08	5,93276E-08	3,16195E-08	0,976362724	0,910183369	0,097561865
	ARF20	*AUXIN RESPONSE FACTOR 20*	AT1G35240	0,00	-0,04	-0,02	-0,01	-0,16	-0,20	0,999999997	0,993856741	0,999550524	0,999875185	0,1308389	0,043512293
	ARF21	*AUXIN RESPONSE FACTOR 21*	AT1G34410												
	ARF22	*AUXIN RESPONSE FACTOR 22*	AT1G34390												
	ARF23	*AUXIN RESPONSE FACTOR 23*	AT1G43950												
	ARF3	*AUXIN RESPONSE TRANSCRIPTION FACTOR 3*	AT2G33860	-0,33	-0,36	-0,23	0,39	0,36	0,35	0,011446139	0,005123716	0,092635212	0,001483072	0,002638363	0,003559547
	ARF4	*AUXIN RESPONSE FACTOR 4*	AT5G60450	-0,59	-0,67	-0,50	-0,28	-0,35	-0,16	0,000141028	3,76189E-05	0,000604619	0,021517733	0,004241961	0,307844296
	ARF6	*AUXIN RESPONSE FACTOR 6*	AT1G30330	0,14	0,08	0,31	0,62	0,58	0,50	0,62762907	0,939788458	0,041079579	0,003630603	0,006104189	0,016700386
	ARF8	*AUXIN RESPONSE FACTOR 8*	AT5G37020	-0,36	-0,65	-0,15	-0,41	-0,51	-0,55	0,013928627	7,9059E-05	0,553263796	0,002048008	0,000275503	0,000129021
	ARF9	*AUXIN RESPONSE FACTOR 9*	AT4G23980	0,49	0,31	0,59	0,03	-0,13	-0,03	5,79511E-06	0,000495233	6,77469E-07	0,983014619	0,159240337	0,993606722
SMALL AUXIN UP RNA	***SAUR15***	*SMALL AUXIN UPREGULATEDRNA 15*	AT4G38850	0,60	0,58	0,57	-0,15	-0,38	-0,57	0,001077463	0,001445419	0,001790054	0,708439188	0,030810849	0,00153352
	SAUR19/24	*SMALL AUXIN UP RNA 19/24*	AT5G18010	1,36	0,81	1,47	0,40	0,21	0,39	3,48118E-05	0,003845352	1,6061E-05	0,349331914	0,88521037	0,387014813
	SAUR20	*SMALL AUXIN UP RNA 20*	AT5G18020	1,19	0,57	1,24	-0,02	-0,26	-0,34	0,000262289	0,066148467	0,000175479	0,999997336	0,820496409	0,618458662
	SAUR21	*SMALL AUXIN UP RNA 21*	AT5G18030	1,10	0,60	1,25	0,11	-0,05	0,20	3,91142E-05	0,008206164	1,0611E-05	0,970305883	0,998977164	0,719384654
	SAUR22	*SMALL AUXIN UP RNA 22*	AT5G18040	0,57	0,57	0,54	1,22	1,57	1,40	0,000318959	0,00030099	0,000522908	3,83033E-07	2,16115E-08	8,48074E-08
	SAUR23	*SMALL AUXIN UP RNA 23*	AT5G18050	1,51	0,79	1,49	0,59	0,44	0,43	3,69523E-06	0,001993234	4,24299E-06	0,054385716	0,209475349	0,223752617
	SAUR24	*SMALL AUXIN UP RNA 24*	AT5G18060	1,23	0,69	1,21	0,19	0,09	0,09	1,66697E-05	0,003609114	1,99724E-05	0,84318709	0,991692599	0,992796621
	SAUR26	*SMALL AUXIN UP RNA 26*	AT5G18080	0,99	0,40	0,87	0,15	-0,05	0,03	0,000899406	0,258662309	0,002780248	0,945524678	0,999646838	0,999967794
	SAUR27	*SMALL AUXIN UP RNA 27*	AT3G03850	0,95	0,38	1,04	0,13	-0,11	-0,19	0,000204478	0,130185583	7,92368E-05	0,902676181	0,956296955	0,679264341
	SAUR28	*SMALL AUXIN UP RNA 28*	AT3G03840	0,84	0,27	1,02	0,19	-0,20	-0,20	0,000891914	0,455746138	0,000149342	0,944923916	0,940944868	0,936304929
	SAUR29	*SMALL AUXIN UP RNA 29*	AT3G03830	0,99	0,57	1,15	0,28	-0,14	0,29	0,001162788	0,061563729	0,000294122	0,418812151	0,917573717	0,385184799
	SAUR29	*SMALL AUXIN UP RNA 29*	AT3G03820	0,92	0,22	0,81	0,00	-0,23	-0,14	3,64114E-05	0,407425406	0,000130534	0,999999996	0,565748181	0,909183781
	SAUR44	*SMALL AUXIN UPREGULATED RNA 44*	AT5G03310	0,91	1,12	1,02	1,53	1,33	1,59	1,94696E-06	1,8603E-07	5,48362E-07	0,000265114	0,000988335	0,000185899
	SAUR63	*SMALL AUXIN UP RNA 6*	AT1G29440	1,22	0,58	1,19	-0,14	-0,22	-0,21	1,79891E-06	0,00278145	2,43959E-06	0,7875033	0,401236545	0,468490046
	SAUR68	*SMALL AUXIN UPREGULATED 68*	AT1G29510	1,08	0,83	1,21	0,15	-0,14	0,24	6,8908E-06	9,71307E-05	2,08949E-06	0,906770852	0,917817602	0,585997276
	SAUR75	*SMALL AUXIN UP RNA 75*	AT5G27780												

Footnote: Change in expression (Log2) of selected auxin biosynthesis, perception and signaling genes (AUX/IAA, Auxin response factors (ARFs) and Small Auxin UP RNA (SAURs), in response to high temperature treatment (27°C) in 2 day-old (2d) and 7 day-old (7d) Col-0 wild type, *hda9-1* and *pif4-2* mutant seedlings. Indicated are: Gene ID, Gene name, Arabidopsis Genome Initiative (AGI) identifier (www.arabidopsis.org). Positive values with green shading, and negative values with red shading under the heading: '*Differential expression (log2) at high temperature'*, indicate up and downregulation respectively, by high temperature treatment (27°C vs. 22°C), within the genotype. Significance of the transcriptional regulation is indicated under the heading '*p values'*, where significant values (P< 0.05) are indicated in red letters. Missing values indicates that no reads corresponding to the gene were detected. *YUCCA8* is highlighted by thick borders and orange shading.

Table S2. Oligonucleotides used in this study

Gene		**Fw primer**	**Rev primer**	**Selection marker**	**Ref.**
ARP6	*WT allele*	GTTCTTCCTGATGGTGTTACACATA	GGCATGAGTTTATAGCTCGGACAAT		(4)
arp6-1	*T-DNA insert*	TAGCATCTGAATTTCATAACCAATCTCGA (SAIL LB3)	GGCATGAGTTTATAGCTCGGACAAT	*BASTA*	(4)
HDA9	*WT allele*	TTCTGAATATTACCTTAACATCGCC	CGGCGGAACCATAGGTAAAT		
hda9-1	*T-DNA insert*	TTCTGAATATTACCTTAACATCGCC	ATTTTGCCGATTTCGGAAC (SALK LBb1.3)		
pHTA11-HTA11-FLAG		CAGCCGTCTACACAGCTTCA	CTTATCGTCGTCATCCTTGTAATC	*KANA*	
HSP70:LUC	LUCIFERASE	TTGGGCGCGTTATTTATCGG	TAGGATCTCTGGCATGCGAG	*BASTA*	
PIF4	*WT allele*	AATACATTTTGCAGGCAATCG	CGTAATGAAGTTGCACGTTTACTC		
pif4-2	*T-DNA insert*	TAGCATCTGAATTTCATAACCAATCTCGA (SAIL LB3)	CGTAATGAAGTTGCACGTTTACTC	*BASTA*	
phyB-9	CAPS marker *	AGCTAGTGGAAGAAGCTCGATGAGGCCTTG	ACCGTCACATTTCACTAAGTCCATGGTACT		(5)
35S:PIF4-HA		AGAAATGGCTAGTGGAAGATG	GGCTTCGTCTAAAATCGAAG		(6)

Footnote: Primers used for genotyping. * *Note that phyB-9 is a point mutation that can be detected by PCR followed by Eco130I/StyI digestion and restriction fragment length analysis*

HDA9-PROM-Fw	Box 1	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTGGGCGTGTTGAAGAAGAT
HDA9-PROM-Rev	Box 1	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGTCTTAAAGAAGAAAAGACACGAAGA
HDA9-PROM-Fw	Box 2	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGTGGGCGTGTTGAAGAAGAT
HDA9-PROM-Rev	Box 2	GGGGACCACTTTGTACAAGAAAGCTGGGTGGTCTTAAAGAAGAAAAGACACGAAGA
HDA9 CDS-stop-Fw	Box 2	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGTTGATTTCGTACGATCTTCGT
HDA9 CDS-stop-Rev	Box 2	GGGGACCACTTTGTACAAGAAAGCTGGGTGCGATAACGATGCGTCAGACC

Footnote: *GATEWAY* cloning primers used for generation of plants transformation vectors. HDA9 gene or promoter-specific sequences are underlined.

HDA9-attB1- Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCATGCGTTCCAAGGACAAAATC
HDA9-attB2-Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCTGACGCATCGTTATCGTTG
HSFA1-attB1-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTTTGTAAATTTCAAATACTTCTCTTTC
HSFA1-attB2-Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCTAGTGTTCTGTTTCTGATGTGAG
PIF4-AttB1-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGAACACCAAGGTTGGA
PIF4-AttB2-Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTGGTCCAAACGAGAACC

Footnote: *GATEWAY* cloning primers used for generation of BiFC and Y2H vectors. Gene-specific sequences are underlined.

HDA9 Fw:	*At3g44680*	TTAAAACACCATCCCCGTGT
HDA9 Rev	*At3g44680*	TTCCTTAACATCGCCTGTCC
YUC8 Fw:	*At4g28720*	AAACGCTCAAGGGGTTCTCTTCG
YUC8 Rev:	*At4g28720*	CACGCACAACACCCTTTGATTCG
PIF4-Fw:	*At2g43010*	TCAGATGCAGCCGATGGAGATG
PIF4-Rev:	*At2g43010*	CGACGGTTGTTGACTTTGCTGTC
YLS8 Fw:	*At5g08290*	TTACTGTTTCGGTTGTTCTCCATTT
YLS8 Rev:	*At5g08290*	CACTGAATCATGTTCGAAGCAAGT
SK42 Fw:	*At1G57870*	ACAGAGGCTGATGAGGAGAGAGAC
SK42 Rev:	*At1G57870*	ATCACATGGCCAGGTTCTGCAC

Footnote: Primers used for qRT-PCR, with AGI codes

YUC8 G-box Fw:	TCCTCATCCTCTCCACGTGG	
YUC8 G-box Rev:	GTGGGACCAACGAGAGGAAG	
PIF4 promoter Fw:	CACTGATTCCAACACAATGTCC	Ref. (7)
PIF4 promoter Rev	GGTACAGACAGAAAGTGACAGGAG	Ref. (7)

Footnote: Primers used for PIF4 G-Box and *PIF4* promoter binding ChIP-PCR

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