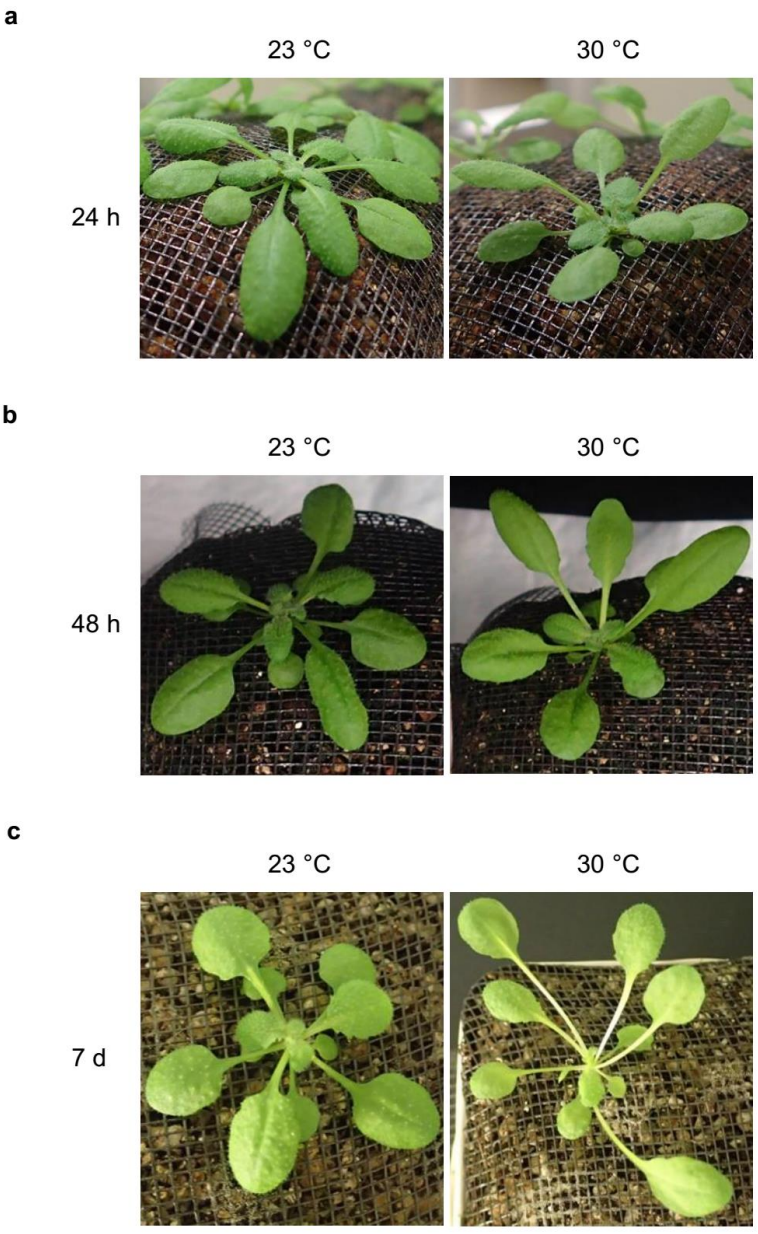
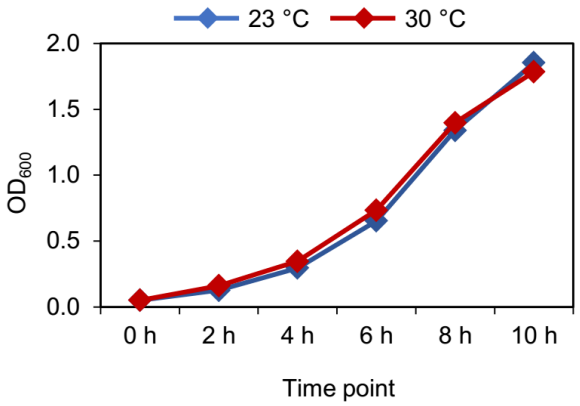
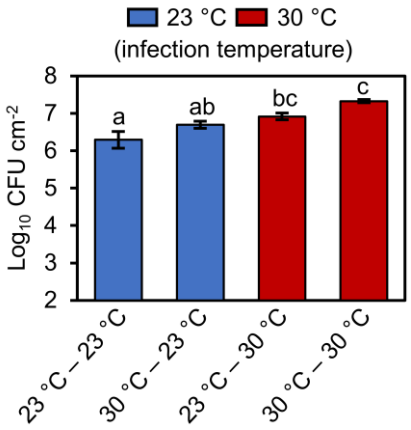
## Supplementary Figures



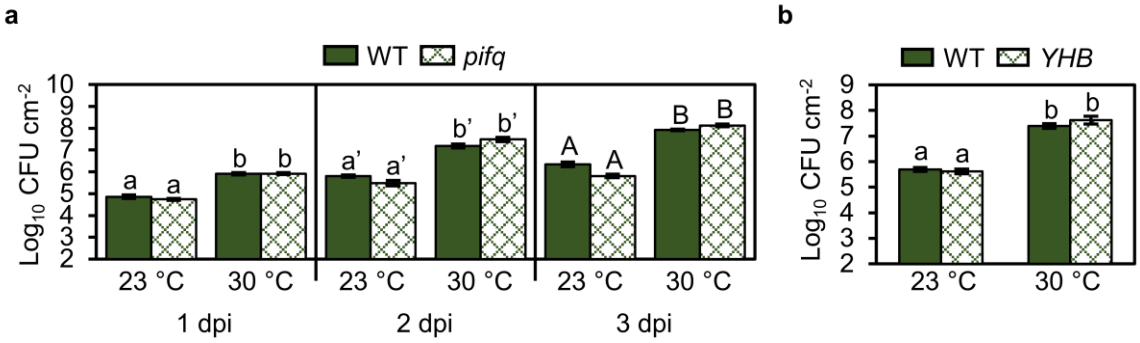

**Supplementary Figure 1. Morphological response of *Arabidopsis* plants to elevated temperature.** Four-week-old plants were shifted to test chambers at 23 °C or 30 °C. Pictures were taken (**a**) 24 h or (**b**) 48 h after temperature shift. (**c**) Two-week-old plants were shifted to test chambers at 23 °C or 30 °C. Pictures were taken 7 days after temperature shift.



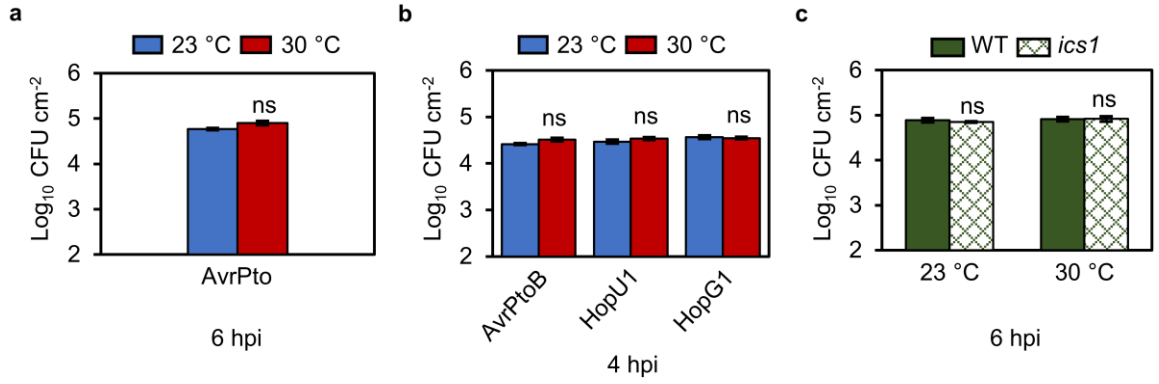

**Supplementary Figure 2. Effect of temperature on *Pst* DC3000 growth *in vitro*.** *Pst* DC3000 was grown in liquid culture at 23 °C or 30 °C. Diamonds indicate the mean reading of three biological replicates ± the standard error of the mean (s.e.m.). Data represent three independent experiments.



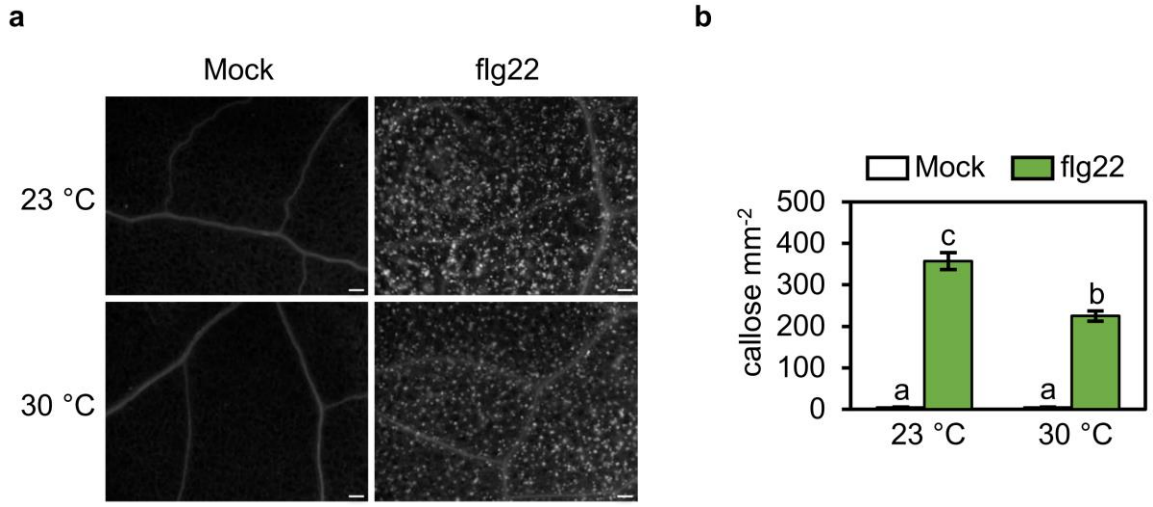

**Supplementary Figure 3. Disease outcomes of *Arabidopsis* plants with indicated temperature treatments before or after infection.** Bacterial growth in plants (n = 4) three days after syringe-infiltration with *Pst* DC3000. Plants were (i) acclimated and kept at 23 °C following infection (23 °C – 23 °C), (ii) acclimated at 30 °C and shifted to 23 °C (30 °C – 23 °C), (iii) acclimated at 23 °C and shifted to 30 °C following infection (23 °C – 30 °C), or (iv) acclimated and kept at 30 °C following infection (30 °C – 30 °C). Data represent three independent experiments, and are presented as the mean ± s.e.m., with n = biological replicates. Letters indicate statistical significance based on a two-factor ANOVA with Tukey's HSD post hoc analysis ($p$-value < 0.05); samples sharing letters are not significantly different.



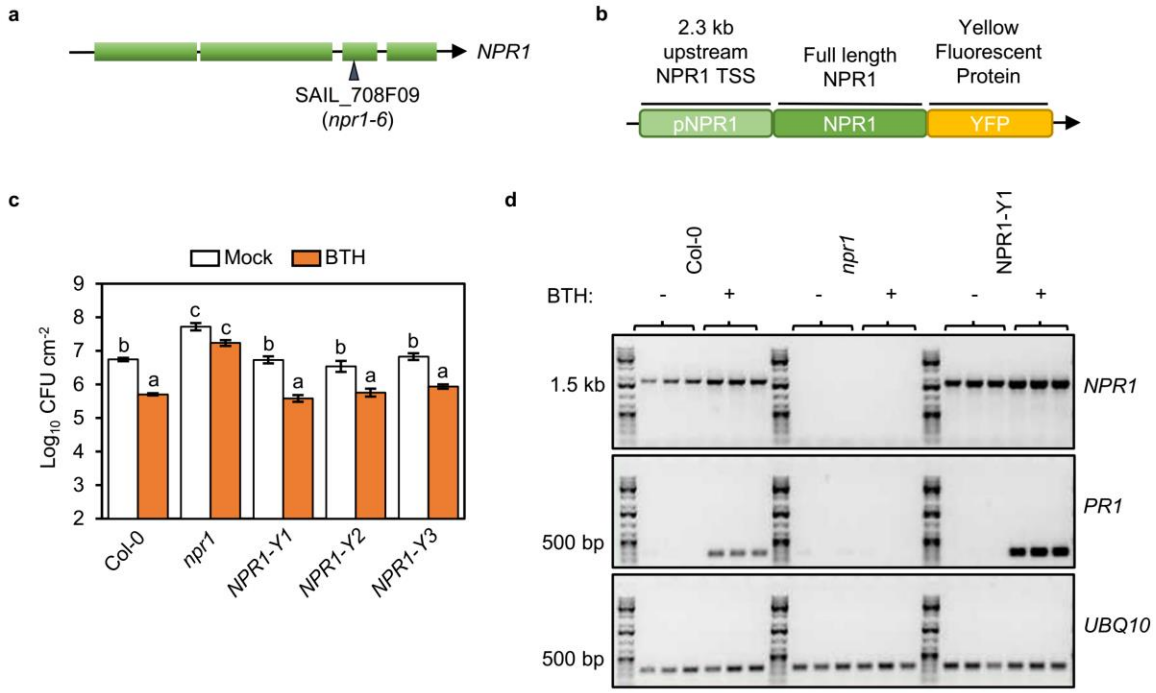

**Supplementary Figure 4. Enhanced growth of *Pst* DC3000 at 30 °C does not require PIFs or phyB.** (**a**) Bacterial growth in WT (Col-0) and *pifq* mutant plants (n = 4) one, two and three days after syringe-infiltration with *Pst* DC3000. (**b**) Bacterial growth in WT (L*er*) and *YHB* transgenic plants (n = 4) three days after syringe-infiltration with *Pst* DC3000. All data are representative of three independent experiments and are presented as the mean ± s.e.m., with n = biological replicates. Letters indicate statistical significance based on a two-factor ANOVA with Tukey's HSD post hoc analysis ($p$-value < 0.05); samples sharing letters are not significantly different. Data for each time point in (a) were analysed separately.



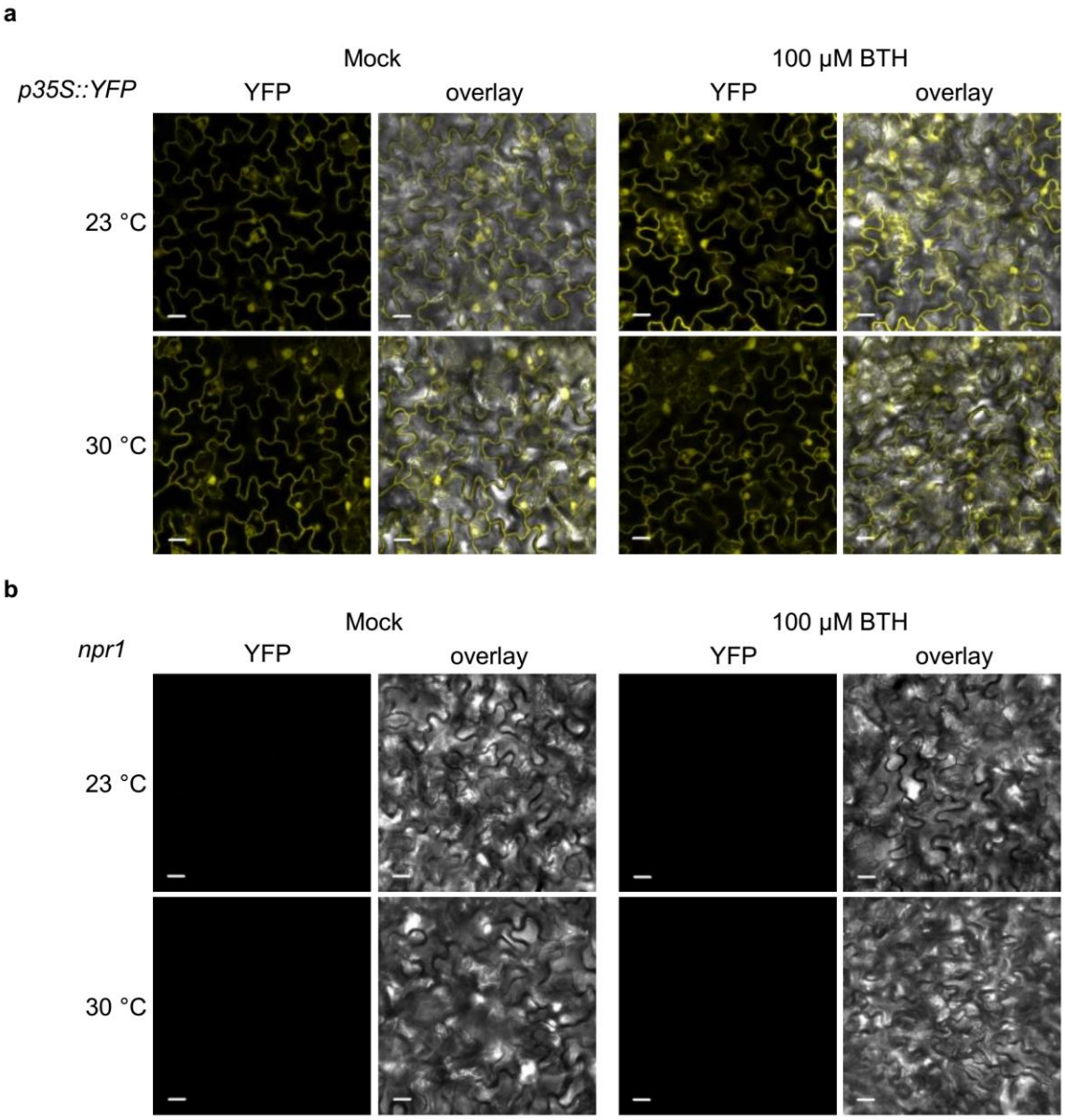

**Supplementary Figure 5. Elevated temperature does not affect bacterial growth *in planta* at 4 to 6 hpi.** Bacterial growth in WT plants (**a**) 6 h after syringe-infiltration with *Pst* DC3000 $P_{nptII}$*::avrPto-CyaA* (n = 4) (**b**) 4 h after syringe-infiltration with *Pst* DC3000($P_{tac}$*::avrPtoB-CyaA*) (n = 4), *Pst* DC3000($P_{tac}$*::HopU1-CyaA*) (n = 6),or *Pst* DC3000($P_{tac}$*::HopG1-CyaA*) (n = 6) strains. (**c**) Bacterial growth in WT and *ics1* mutant plants (n = 4) 6 h after syringe-infiltration with *Pst* DC3000($P_{nptII}$*::avrPto-CyaA*). Data are presented as the mean ± s.e.m. with n = biological replicates, and are representative of three independent experiments. "ns" indicates no statistical significance based on a Student's *t*-test (*p*-value > 0.05) of pairwise comparisons between samples at 23 °C vs. 30 °C (a and b) or between genotypes at each temperature (c).



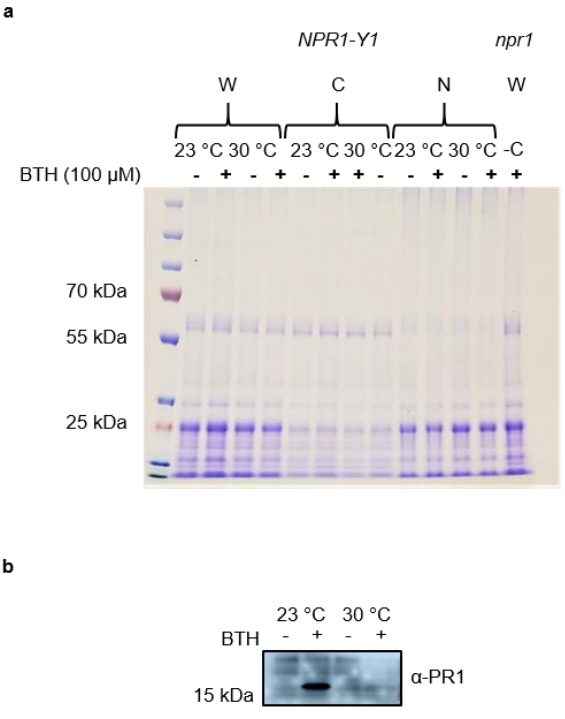

**Supplementary Figure 6. Flg22 treatment induces callose at elevated temperature.** (**a**) Representative images of callose accumulation 24 hpi with mock or flg22 of temperature acclimated plants (n = 6). Scale-bar length represents 100 µm. (**b**) Quantification of callose accumulation from plants treated as described in (a). Data are representative of three independent experiments. Graphical data are presented as the mean ± s.e.m., with n = biological replicates. Letters indicate statistical significance based on a two-factor ANOVA with Tukey's HSD post hoc analysis (*p*-value < 0.05); samples sharing letters are not significantly different.




**Supplementary Figure 7. Characterization of *Arabidopsis npr1-6* mutant and *pNPR1::NPR1-YFP* transgenic lines.** (**a**) Model of T-DNA insertion in *NPR1* for the SAIL_708F09 allele, named here *npr1-6*, and referred to as *npr1*. (**b**) Model of *pNPR1::NPR1-YFP* construct. The NPR1 promoter used was 2.3 kb upstream of the NPR1 transcriptional start site (TSS). (**c**) Bacterial growth in five-week-old mock- or BTH-pre-treated plants three days after syringe-infiltration with *Pst* DC3000. Data are presented as the mean (n = 3) ± s.e.m., and are representative of three independent experiments. Letters indicate statistical significance based on a two-factor ANOVA with Tukey's HSD post hoc analysis ($p$-value < 0.05); samples sharing letters are not significantly different. (**d**). Five-week-old plants (n = 3) were sprayed with mock or BTH 24 h prior to harvesting tissue for RNA extraction. Semi-quantitative gene expression analysis was used to determine the expression levels of *NPR1* (35 cycles) and *PR1* (25 cycles) with *UBQ10* (25 cycles) expression used as an internal control. Data are representative of two independent experiments. Primer sequences are provided in Supplementary Table 6.




**Supplementary Figure 8. Effect of elevated temperature and BTH on *p35S::YFP* and *npr1-6*.** Representative confocal microscopy images of (**a**) *p35S::YFP* and (**b**) *npr1* plants 24 h after spraying with mock or BTH. Images are of YFP (yellow) alone or YFP overlaid on Brightfield (grey-scale). Scale bar length represents 10 µm. Data are representative of three independent experiments with four biological replicates per experiment.

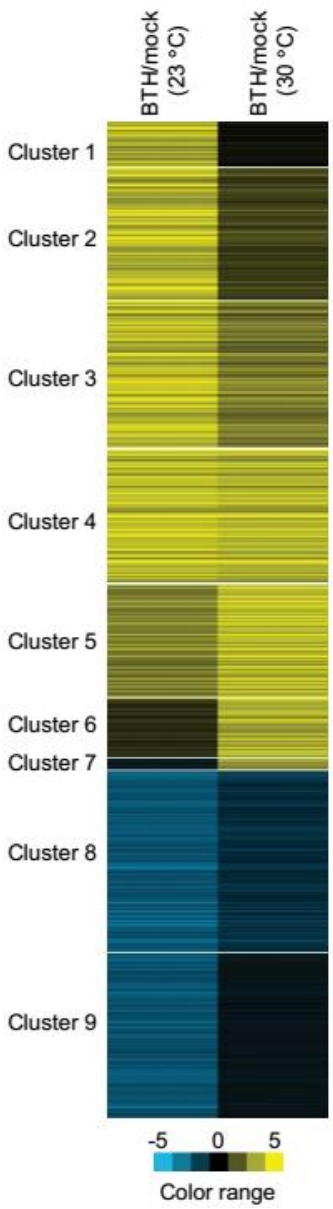



95

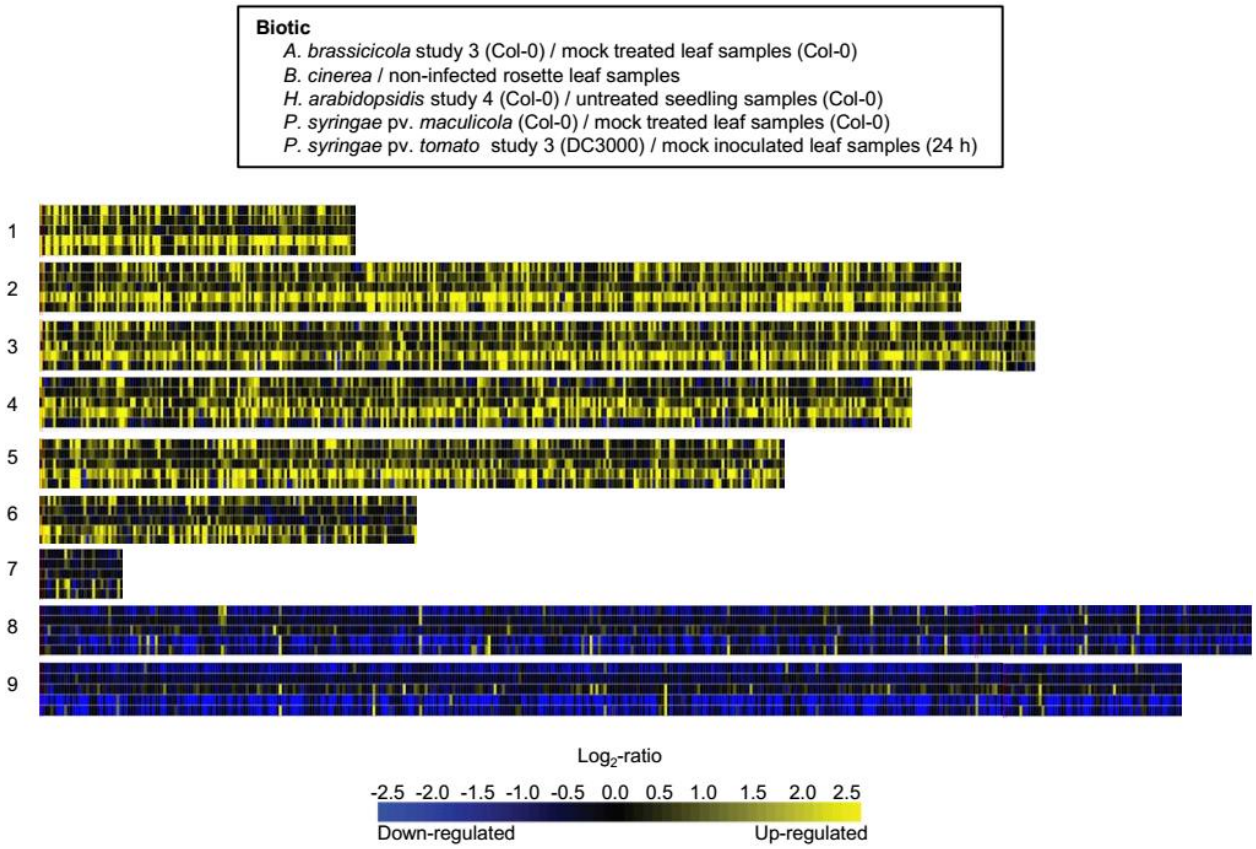
96 **Supplementary Figure 9. SDS polyacrylamide gel electrophoresis (PAGE) and western**
97 **blot of leaf proteins.** (**a**) Leaf protein samples of whole cell lysate (W), non-nuclear (C,
98 cytosolic) and nuclear (N) enriched fractions isolated from *NPR1-Y1* transgenic plants treated
99 with mock (-) or BTH (+) solutions at 23 °C or 30 °C were loaded in equal volumes (10 µl) and
100 run in a 4 – 12 % gradient SDS-PAGE gel. Whole cell lysate extracted from *npr1* plants treated
101 with BTH at 23 °C was used as the negative control for the NPR1-YFP band (for western
102 blotting shown in Fig. 4b). The gel was then stained with Coomassie to visualize protein bands
103 for assessment of equal loading. (**b**) Western blot of non-nuclear fraction isolated from leaves
104 pooled from four, temperature acclimated *NPR1-Y1* plants treated with mock (-) or BTH (+).
105 Equal volumes (10 µl) of each protein sample were loaded and run a 4 – 12 % gradient SDS-
106 PAGE gel. Following transfer, the PVDF membrane was probed using a α-PR1 primary antibody
107 (expected MW ~16 kD). Data are representative of three independent experiments.

108

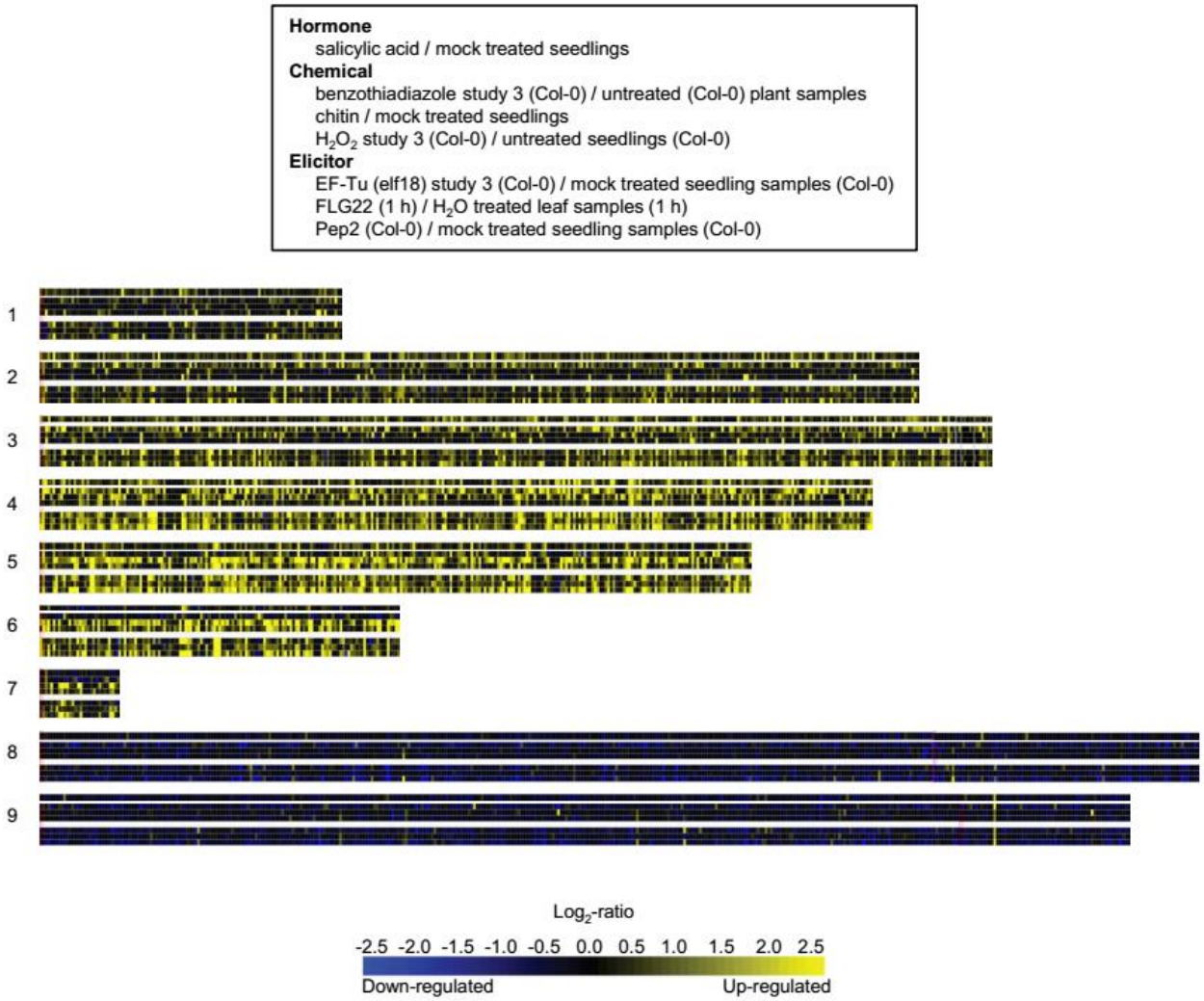
109



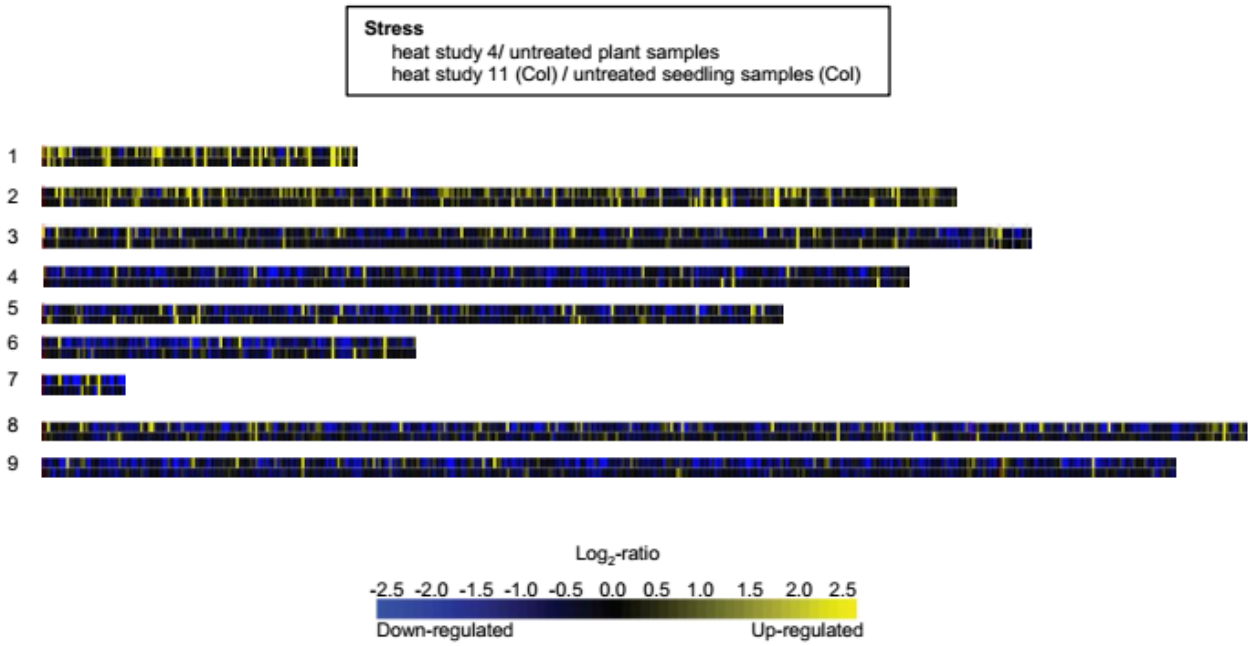

**Supplementary Figure 10. Effect of elevated temperature on the BTH regulated transcriptome.** Differentially expressed genes were identified from RNA-seq data and k-means clustering was conducted as described in Supplementary Note 1 and the main text. The heat map shows a visual representation of gene expression patterns based on fold change (BTH/mock) within each cluster. Down-regulation of expression between the mock- and BTH-treated plants at each temperature is denoted by the level of blue color, up-regulation by the level of yellow color and no change by black color as indicated by the color scale.



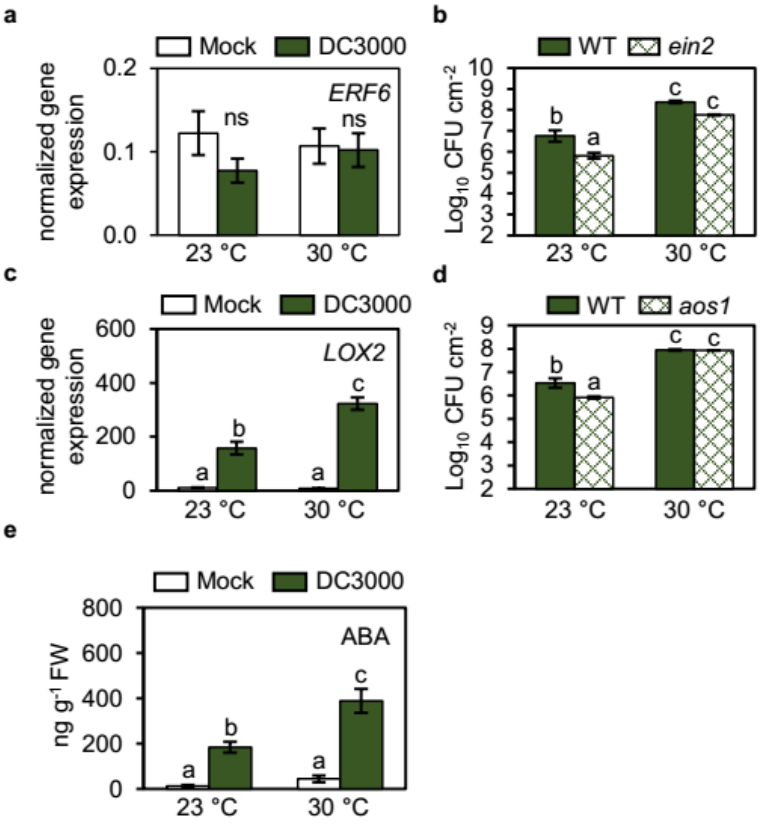

**Supplementary Figure 11. Genevestigator analysis using publicly available microarray expression data of genes in response to biotic stress.** Of the pathogens listed under biotic stress, *Alternaria brassicicola* and *Botrytis cinerea* are necrotrophic pathogens, *Hyaloperonospora arabidopsidis* is an obligate biotrophic pathogen and both strains of *Pseudomonas syringae* are hemi-biotrophic pathogens. Numbers to the left of each panel indicate the cluster to which that set of genes is assigned. Expression values are $\log_2$ ratios of treated vs. untreated or mock controls, with down-regulation of expression denoted by the level of blue color, up-regulated denoted by the level of yellow color and no change in expression denoted by black color as indicated by the color scale.



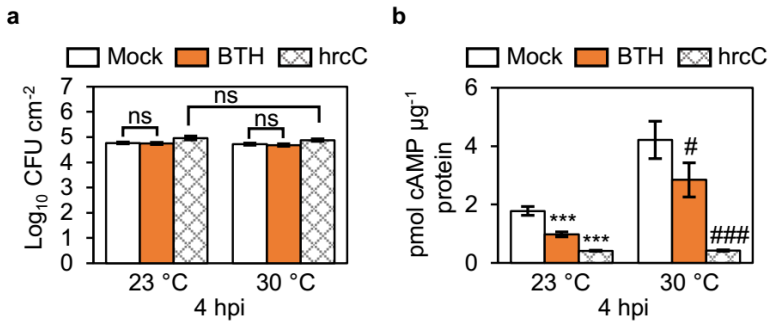

**Supplementary Figure 12. Genevestigator analysis using publicly available microarray expression data genes in response to SA or PAMP elicitors.** Numbers to the left of each panel indicate the cluster to which that set of genes is assigned. Expression values are $\log_2$ ratios of treated vs untreated or mock controls, with down-regulation of expression denoted by the level of blue color, up-regulated denoted by the level of yellow color and no change in expression denoted by black color as indicated by the color scale.



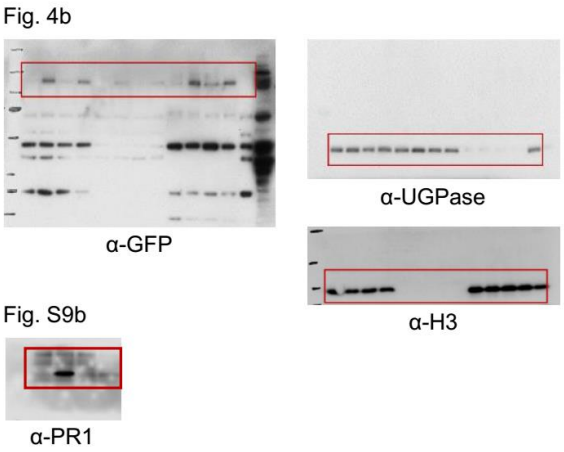

**Supplementary Figure 13. Genevestigator analysis using publicly available microarray expression data of genes in response to heat stress.** Numbers to the left of each panel indicate the cluster to which that set of genes is assigned. Expression values are $\log_2$ ratios of treated vs untreated or mock controls, with down-regulation of expression denoted by the level of blue color, up-regulated denoted by the level of yellow color and no change in expression denoted by black color as indicated by the color scale.




**Supplementary Figure 14. Hormone pathways known to antagonize SA are not involved in elevated temperature-associated enhanced susceptibility.** (**a**) ET and (**c**) JA marker gene expression (n = 3) in plants 24 h after vacuum-infiltration with mock or *Pst* DC3000. qPCR was used for gene expression analysis, with expression of *ERF6* and *LOX2* normalized to the expression of *PP2AA3*. Bacterial growth in WT, (**b**) *ein2* and (**d**) *aos1* mutant plants (n = 4) three days after vacuum-infiltration with *Pst* DC3000. (**e**) ABA metabolite levels in plants (n = 4) 24 h after vacuum-infiltration with mock or *Pst* DC3000. All data are representative of three independent experiments and are presented as the mean ± s.e.m., with n = biological replicates. Letters indicate statistical significance based on a two-factor ANOVA with Tukey's HSD post hoc analysis ($p$-value < 0.05); samples sharing letters are not significantly different. "ns" indicates no statistical significance.




**Supplementary Figure 15. Bacterial populations and effector translocation in plants treated with mock or BTH.** (**a**) Bacterial growth in plants (n = 4) 4 hpi; tissue was collected from the same plants as were used to measure cAMP data presented in Fig. 7g. Plants were pre-treated with mock or BTH 24 h before syringe-infiltration with *Pst* DC3000($P_{nptII}$::*avrPto-CyaA*). Additional mock-treated plants (n = 4 for each temperature) were infiltrated with *hrcC*$^-$ ($P_{nptII}$::*avrPto-CyaA*) as a negative control. (**b**) Translocation of bacterial effector proteins in plants (n = 4) pre-treated with mock or BTH 24 h before syringe-infiltration with *Pst* DC3000($P_{nptII}$::*avrPto-CyaA*) or *hrcC*$^-$($P_{nptII}$::*avrPto-CyaA* strains). Tissue was collected at 4 hpi for quantification of cAMP, which was normalized by total protein. Data are presented as the mean ± s.e.m. with n = biological replicates. Data in (b) and Fig. 7g are two of three independent experiments. In (a), "ns" indicates no statistical significance based on a Student's *t*-test (*p*-value > 0.05). Symbols in (b) denote statistical significance based on a one-factor ANOVA with Dunnett's post hoc analysis (***, ### *p*-value < 0.001, # *p*-value < 0.05) using the mock-treated sample at each temperature as the means for comparison.




**Supplementary Figure 16. Uncropped western blot images.** Uncropped scans of western blots shown in Fig. 4b and Supplementary Fig. 9b. Red boxes indicate cropped sections used in figures. Images were edited to remove saturation to show in grayscale. Marker lines were drawn on some X-ray scans to show bands in PAGE ruler to determine band size. Last lane in first blot (α-GFP) has the Precision Plus Protein™ WesternC™ Standard (Bio Rad).

## Supplementary Tables

**Supplementary Table 1. Functional annotations for differentially expressed genes based on 23 °C Mock vs. 30 °C Mock analysis.**

| Category | GO term | Up at 30 °C | Down at 30 °C |
|---|---|---|---|
| **Biotic Stress** | Defence | | 30 |
| | Chitin | | 16 |
| | Fungus | | 13 |
| | Bacterium | | 10 |
| | SA | | 6 |
| | ET | | 12 |
| | JA | | 11 |
| | Cell wall | | 10 |
| **Abiotic stress** | Abiotic stress | 17 | 22 |
| | Response to oxygen | 10 | 38 |
| | Temperature/heat | 14 | |

The Gene ontology (GO) term is provided in the second column. Subsequent columns provide the number of genes for the corresponding GO term that are either up- or down-regulated at 30 °C relative to expression levels observed at 23 °C. There is some redundancy of genes across categories (e.g. all temperature genes are also annotated as involved in abiotic stress). GO analysis was conducted using DAVID (https://david.ncifcrf.gov/). For data used to compile this table, see Supplementary Data 2.

**Supplementary Table 2. Distribution of differentially expressed genes in response to BTH by group and cluster.**

**a**

| | Cluster → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | A | 121 | 344 | 318 | 160 | 12 | 1 | 0 | | | 956 |
| | B | 12 | 36 | 97 | 221 | 266 | 87 | 6 | | | 725 |
| | C | 0 | 0 | 1 | 1 | 40 | 80 | 30 | | | 152 |
| | D | | | | | | | | 365 | 445 | 810 |
| | E | | | | | | | | 149 | 28 | 177 |
| | Total | 133 | 380 | 416 | 382 | 318 | 168 | 36 | 514 | 473 | |

**b**

| | Cluster → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | A | 91% | 91% | 76% | 42% | 4% | 1% | | | | 52% |
| | B | 9% | 9% | 23% | 58% | 84% | 52% | 17% | | | 40% |
| | C | | | | | 13% | 48% | 83% | | | 8% |
| | D | | | | | | | | 71% | 94% | 82% |
| | E | | | | | | | | 29% | 6% | 18% |
| | Total | 7% | 21% | 23% | 21% | 17% | 9% | 2% | 52% | 48% | |

Number (**a**) or percentage (**b**) of DEGs in each group and cluster. Groups A through C are defined as genes induced by BTH at 23 °C (genes in clusters 1 – 7) that show lower expression at 30 °C (Group A), similar expression levels at 30 °C (Group B) or higher expression at 30 °C (Group C). Groups D and E are defined as genes suppressed by BTH at 23 °C (genes in clusters 8 and 9) that show higher expression levels at 30 °C. Percentage totals are calculated based on the total number of genes within the cluster or group divided by the total number of induced (1,833) or suppressed (987) genes. For data used to compile this table, see Supplementary Data 1.

**Supplementary Table 3. Functional annotations for differentially expressed genes based on BTH vs. Mock analysis.**

**a**

| Category | GO term | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| Biotic Stress | Defence | | | 40 | 53 | 54 | 21 | | | |
| | Cell death | | | 20 | 20 | 21 | | | | |
| | Bacterium | 7 | 13 | 15 | 17 | 14 | | | | |
| | Chitin | | | | 16 | 32 | 20 | 3 | | |
| | Callose | | | | | | | | | |
| | SA/SAR | | | 10 | 19 | | | | | |
| | ET | | | | | 9 | 17 | | | |
| | JA | | | | | 8 | | | | |
| Abiotic stress | Abiotic stress | | | | | | 17 | | | |
| | ABA | | | | 13 | | 7 | | | |
| | heat | 7 | 8 | | | | | | | |
| Growth | Chloroplast part | | | | | | | | 34 | 45 |
| | photosynthesis | | | | | | | | | 26 |
| | auxin | | | | | | | | 10 | 12 |

**b**

| Category | GO term | A | B | C | D |
|---|---|---|---|---|---|
| Biotic Stress | Defence | 70 | 53 | 77 | |
| | Cell death | 29 | 20 | 24 | |
| | Bacterium | 35 | 17 | 17 | |
| | Chitin | | 16 | 55 | |
| | Callose | 4 | | 5 | |
| | SA/SAR | 17 | 16 | | |
| | ET | | | 28 | |
| | JA | | | 13 | |
| Abiotic stress | Abiotic stress | | | 41 | 65 |
| | ABA | | 13 | | |
| | heat | 17 | | | |
| Growth | Chloroplast part | | | | 79 |
| | photosynthesis | | | | 30 |
| | auxin | 5 | | | 52 |

The GO term is provided in the second column. Subsequent columns provide the number of genes within each cluster (**a**) or group (**b**) containing the corresponding GO term. GO analysis was conducted using DAVID (https://david.ncifcrf.gov/). For data used to compile this table, see Supplementary Data 3 and 4.

**Supplementary Table 4. Differentially expressed genes involved in SA biosynthesis and signalling.**

| Process | AGI Number | Gene Name | Cluster | Group | References |
|---|---|---|---|---|---|
| Positive regulation of SA biosynthesis | AT3G52430 | PAD4 | 3 | A | 1-3 |
| | AT3G48090 | EDS1 | 4 | A | 4, 5 |
| | AT3G20600 | NDR1 | 5 | B | 6 |
| | AT5G13320 | PBS3 | 2 | A | 7, 8 |
| | AT5G26920 | CBP60G | 4 | A | 9 |
| | AT1G73805 | SARD1 | 4 | A | 9 |
| | AT4G18170 | WRKY28 | 5 | B | 10 |
| | AT2G46400 | WRKY46 | 4 | A | 10 |
| | AT1G74710 | ICS1, SID2 | 1 | A | 11, 12 |
| | AT4G39030 | EDS5, SID1 | 1 | A | 11 |
| | AT4G14400 | ACD6 | 4 | B | 13 |
| | AT2G13810 | ALD1 | 2 | A | 14, 15 |
| | AT1G64280 | NPR1, SAI1, NIM1 | 4 | B | 2 |
| Negative regulation of SA biosynthesis | AT2G40750 | WRKY54 | 4 | B | 16 |
| | AT3G56400 | WRKY70 | 4 | A | 16 |
| | AT1G29690 | CAD1 | 5 | B | 17 |
| | AT2G39660 | BIK1 | 4 | B | 18 |
| | AT1G28380 | NSL1 | 5 | B | 19 |
| | AT1G64280 | NPR1, SAI1, NIM1 | 4 | B | 2, 20 |
| | AT1G52890 | ANAC019 | 5 | B | 21 |
| | AT3G15500 | ANAC055 | 5 | B | 21 |
| | AT4G27410 | ANAC072 | 5 | B | 21 |
| | AT1G32640 | MYC2 | 7 | C | 21 |
| Positive role in SA signalling/SAR | AT1G64280 | NPR1, SAI1, NIM1 | 4 | B | 20, 22, 23 |
| | AT5G55170 | SUMO3 | 4 | B | 24 |
| | AT1G22070 | TGA3 | 3 | A | 25, 26 |
| | AT5G06960 | TGA5, OBF5 | 2 | A | 27 |
| | AT4G31800 | WRKY18 | 4 | A | 16 |
| | AT4G23810 | WRKY53 | 5 | C | 16 |
| | AT2G40750 | WRKY54 | 4 | B | 16 |
| | AT3G56400 | WRKY70 | 4 | A | 16 |
| | AT2G13810 | ALD1 | 2 | A | 14, 28 |
| | AT1G19250 | FMO1 | 3 | A | 29 |
| | AT2G14610 | PR1 | 2 | A | 30 |
| | AT3G57260 | PR2, BGL2 | 4 | A | 30 |
| | AT1G75040 | PR5 | 4 | A | 30 |
| Negative role in SA signalling/SAR | AT5G45110 | NPR3 | 4 | B | 31, 32 |
| | AT1G02450 | NIMIN1 | 4 | A | 33, 34 |
| | AT3G25882 | NIMIN-2 | 4 | A | 33, 34 |

| | | | | | |
|---|---|---|---|---|---|
| | AT4G31800 | WRKY18 | 4 | A | 35 |
| | AT5G22570 | WRKY38 | 4 | B | 16, 36 |
| | AT3G01080 | WRKY58 | 4 | A | 16 |
| | AT2G25000 | WRKY60 | 3 | A | 35 |
| | AT5G01900 | WRKY62 | 3 | A | 36, 37 |
| | AT5G04340 | ZAT6 | 6 | B | 38 |

Genes are identified based on their Arabidopsis Genome Initiative (AGI) number and commonly used gene name. Cluster numbers are based on k-means clustering. Group classifications are based on expression level differences (2-fold cut-off) between the BTH-treated samples at 23 °C and 30 °C. See Supplementary Data 1 for spreadsheet containing full list of DEGs.

**Supplementary Table 5. Transcription factors implicated in regulation of genes in BTH vs. Mock analyses.**

**a**

| cluster 2 | cluster 3 | cluster 4 | cluster 5 | cluster 6 | cluster 8 | cluster 9 |
|---|---|---|---|---|---|---|
| TGA5 | | TGA5 | TGA5 | CAMTA2 | PIF1 | PIF1 |
| TGA6 | | TGA6 | TGA6 | CAMTA3 | PIF3 | PIF3 |
| TGA2 | | | | BZR1 | PIF4 | PIF4 |
| ANAC55 | ANAC55 | ANAC55 | ANAC55 | ABF1 | PIF5 | PIF5 |
| | | WRKY48 | | ABF2 | EDT1 | EDT1 |
| | | WRKY33 | WRKY33 | ABF3 | MYC2 | MYC2 |
| | WRKY51 | WRKY51 | WRKY51 | ABI5 | MYC3 | MYC3 |
| | WRKY60 | WRKY60 | WRKY60 | | MYC4 | ATHB6 |
| | WRKY40 | WRKY40 | WRKY40 | | JAM2 | |
| WRKY47 | WRKY47 | WRKY47 | WRKY47 | | BIM2 | |
| WRKY8 | WRKY8 | WRKY8 | WRKY8 | WRKY8 | BIM3 | |
| WRKY12 | WRKY12 | WRKY12 | WRKY12 | WRKY12 | BEE2 | |
| WRKY14 | WRKY14 | WRKY14 | WRKY14 | WRKY14 | | |
| WRKY17 | WRKY17 | WRKY17 | WRKY17 | WRKY17 | | |
| WRKY21 | WRKY21 | WRKY21 | WRKY21 | WRKY21 | | |
| WRKY23 | WRKY23 | WRKY23 | WRKY23 | WRKY23 | | |
| WRKY30 | WRKY30 | WRKY30 | WRKY30 | WRKY30 | | |
| WRKY38 | WRKY38 | WRKY38 | WRKY38 | WRKY38 | | |
| WRKY45 | WRKY45 | WRKY45 | WRKY45 | WRKY45 | | |
| WRKY57 | WRKY57 | WRKY57 | WRKY57 | WRKY57 | | |
| WRKY62 | WRKY62 | WRKY62 | WRKY62 | WRKY62 | | |
| WRKY75 | WRKY75 | WRKY75 | WRKY75 | WRKY75 | | |
| | WRKY2 | WRKY2 | WRKY2 | WRKY2 | | |
| | WRKY11 | WRKY11 | WRKY11 | WRKY11 | | |
| | WRKY15 | WRKY15 | WRKY15 | WRKY15 | | |
| | WRKY18 | WRKY18 | WRKY18 | WRKY18 | | |
| | WRKY25 | WRKY25 | WRKY25 | WRKY25 | | |
| | WRKY43 | WRKY43 | WRKY43 | WRKY43 | | |
| | WRKY63 | WRKY63 | WRKY63 | WRKY63 | | |

**b**

| Group A | Group B | Group C | Group D |
|---|---|---|---|
| TGA5 | CAMTA2 | CAMTA2 | TCP4 |
| TGA6 | CAMTA3 | CAMTA3 | TCP15 |
| NTL9 | NTL9 | GBF1 | TCP16 |
| | CCA1 | GBF3 | TCP23 |
| | RVE1 | GBF4 | GBF4 |
| | RVE6 | ABI5 | ABI5 |

| | | | |
|---|---|---|---|
| | ABF3 | ABF3 | |
| WRKY2 | WRKY2 | ABF1 | ABF1 |
| WRKY8 | WRKY8 | AREB1 | AREB1 |
| WRKY11 | WRKY11 | | MYC3 |
| WRKY12 | WRKY12 | MYC2 | MYC2 |
| WRKY14 | WRKY14 | MYC4 | MYC4 |
| WRKY15 | WRKY15 | JAM1 | JAM1 |
| WRKY17 | WRKY17 | JAM2 | JAM2 |
| WRKY18 | WRKY18 | | PIF1 |
| WRKY21 | WRKY21 | PIF3 | PIF3 |
| WRKY23 | WRKY23 | PIF4 | PIF4 |
| WRKY25 | WRKY25 | | PIF5 |
| WRKY30 | WRKY30 | BZR1 | BZR1 |
| WRKY38 | WRKY38 | HY5 | BIM2 |
| WRKY40 | WRKY40 | EDT1 | BIM3 |
| WRKY43 | WRKY43 | BEE2 | CIB5 |
| WRKY45 | WRKY45 | BES1 | HBI1 |
| WRKY47 | WRKY47 | SPT | SPT |
| WRKY51 | WRKY51 | | UNE10 |
| WRKY60 | WRKY60 | | SPL8 |
| WRKY57 | WRKY57 | WRKY57 | AGL8 |
| WRKY62 | WRKY62 | WRKY62 | SEP4 |
| WRKY75 | WRKY75 | WRKY75 | SEP3 |
| WRKY63 | WRKY63 | | BPE |
| | WRKY1 | | |
| | WRKY27 | | |
| | WRKY29 | | |
| | WRKY33 | | |
| | WRKY48 | | |

Analysis of Motif Enrichment (AME; http://meme-suite.org/tools/ame) was conducted by cluster (**a**) and by group (**b**) as described in Supplementary Note 1. For each analysis, a subset of TFs, as implicated by enrichment of the motif to which they bind, was compiled based on known relevance to SA, ABA, JA, and growth-related processes. No enriched motifs were identified for clusters 1 and 7. See Supplementary Data 5 for html files with actual data output for each analysis.

**Supplementary Table 6. Primer sequences.**

| AGI Number (Gene name) | Primer name | Primer sequence (5'-3') | Purpose |
|---|---|---|---|
| NA | SAIL_LB3 | TAGCATCTGAATTTCATAACCAATCTCGATACAC | Genotyping |
| AT1G64280 (*NPR1*) | SAIL708F09_LP | ATTTGTTTGAAGCACACCTGC | Genotyping |
| | SAIL708F09_RP | CTCTCAAAGGCCGACTATGTG | Genotyping |
| NA | SALK_LBb1.3 | ATTTTGCCGATTTCGGAAC | Genotyping |
| AT1G32640 (*MYC2*) | MYC2_GT_LP | GCTACAACCAACGATGAATC | Genotyping |
| | MYC2_GT_RP | TCATCAACAGCGTCATCCGA | Genotyping |
| NA | GABI Kat_LB1 | ATAACGCTGCGGACATCTACATT | Genotyping |
| AT5G46760 (*MYC3*) | MYC3_GT_LP | GTTAGATCAGCTGCGAATGATTCGG | Genotyping |
| | MYC3_GT_RP | CTCCGACTTTCGTCATCAAAGCAAC | Genotyping |
| AT4G17880 (*MYC4*) | MYC4_GT_LP | GGATCCATGTCTCCGACGAATGTTCAAGTA | Genotyping |
| | MYC4_GT_RP | TCTCTCACAACTTGATCCAGCTAA | Genotyping |
| AT1G64280 (*NPR1*) | NPR1_F | agaattcATGGACACCACCATTGATGGA | Cloning |
| | NPR1_R | agtcgacCCGACGACGATGAGAGARTTTAC | Cloning |
| *NPR1* promoter | pNPR1_F | cgcggccgcTCGTTTGTTTTCCGTTTTGTTCTGA | Cloning |
| | pNPR1_R | agaattcCAACAGGTTCCGATGAATTGAAAT | Cloning |
| AT1G64280 (*NPR1*) | NPR1-NT F | AGGATCCATGGACACCACCATTGATGG | RT-PCR |
| | NPR1-CT R | AGCGGCCGCTCACCGACGACGATGAGAGA | RT-PCR |
| At2g14610 (*PR1*) | PR1-RT-F | GTTCACAACCAGGCACGA | RT-PCR |
| | PR1-RT-R | CACCTCACTTTGGCACATCC | RT-PCR |
| AT4G05320 (*UBQ10*) | UBQ10-RT-F | ACCCTCCACTTGGTCCTCA | RT-PCR |
| | UBQ10-RT-R | AGTTTTCCCAGTCAACGTCTT | RT-PCR |
| At1G13320 (*PP2AA3*) | PP2AA3_qRT_F1 | GGTTACAAGACAAGGTTCACTC | qPCR |
| | PP2AA3_qRT_R1 | CATTCAGGACCAAACTCTTCAG | qPCR |
| At2g14610 (*PR1*) | PR1_qRT_F1 | GGCTAACTACAACTACGCTG | qPCR |
| | PR1_qRT_R1 | TCTCGTTCACATAATTCCCAC | qPCR |
| At1g74710 (*ICS1*) | ICS1_qRT_F2 | ACTTACTAACCAGTCCGAAAGACGA | qPCR |
| | ICS1_qRT_R2 | ACAACAACTCTGTCACATATACCGT | qPCR |
| AT5G03280 (*EIN2*) | EIN2_qRT_F1 | TGGGGAAAGTACACTTACGTTCTCAA | qPCR |
| | EIN2_qRT_R1 | ATTCCGTTAGCTGAAGTCGGACT | qPCR |
| AT4G17490 (*ERF6*) | ERF6_qRT_F1 | AGAGGAGAAGAGGCATTACAGAG | qPCR |
| | ERF6_qRT_R1 | GAGCCAAACACGAGTTCCAC | qPCR |
| AT4G17880 (*MYC2*) | MYC2_qRT_F1 | GAAACTCCAAATCAAGAACCAG | qPCR |
| | MYC2_qRT_R1 | ATCTTCACTTCAATCTCCATCC | qPCR |
| At3g45140 (*LOX2*) | LOX2_qRT_F | GCCGTTAATAAGCAAACTAGAC | qPCR |
| | LOX2_qRT_R | TTCTTTAGAGCCTCATCAACTG | qPCR |
| AT3G14440 (*NCED3*) | NCED3_qRT_F1 | AAAGCCATCGGTGAGCTTCA | qPCR |
| | NCED3_qRT_R1 | GCAGCTCTGGCGTAGAATAGC | qPCR |

## Supplementary Notes

### Supplementary Note 1

**RNA sequencing and data analysis.** Extracted RNA was checked for purity using a BioAnalyzer Agilent 2100 and three biological replicates of each treatment type were selected based on having a RNA Integrity Number (RIN) score around 7. The final twelve samples were submitted to the Research Technology Support Facility at Michigan State University for preparation of next-generation sequencing libraries. Pooled samples were loaded on two lanes of an Illumina HiSeq 2500 Rapid Run flow cell (v1) and sequenced in a 1 x 50 bp single end format using Rapid SBS reagents. Base calling was done by Illumina Real Time Analysis (RTA) v1.18.61 and output of RTA was de-multiplexed and converted to FastQ format using Illumina Bcl2fastq v1.8.4. Due to inadvertent exclusion of sample 6, a second pool was generated and run on one lane of a Rapid Run flow cell, and sample 6 was also run individually on a MiSeq flow cell.

RNA-seq reads were cleaned and trimmed using Trimmomatic[39] and were aligned to the *Arabidopsis thaliana* genome assembly (TAIR10) using the STAR alignment program, allowing only unique alignments[40]. Read counts were obtained for each gene using the featureCounts function from the Rsubread package in R[41], and subsequent count data were normalized using TMM using the limma package in R[42]. Genes with average counts less than 10 across all samples were discarded. The count data were normalized with normalization factors calculated by the function calcNormFactors in the package edgeR in R, and log-transformed by the function voom in the package limma in R to yield $\log_2$ counts per million[42]. The expression data were fit to a linear model (treatment:temp + replicate) by using the function lmFit in the limma package in R. The eBayes function in the limma package in R was used for variance shrinkage in calculation of the *p*-values, which were then used to calculate the Storey's *q*-values using the qvalue function in the qvalue package in R. Differentially expressed genes were identified with a

275 *q*-value < 0.01. Results were then filtered for those genes that exhibited a $\log_2$-fold change
276 greater than 2. Gene ontology analysis was done for genes in each cluster using The Database
277 for Annotation, Visualization and Integrated Discovery (DAVID)[43]. For motif enrichment analysis,
278 the 1,000 bp upstream of the transcription start sites of the selected genes were tested for
279 enrichment of the known *cis* elements using AME[44]. The heatmap was generated with
280 CLUSTER using k-Means (k=9), and visualized by TREEVIEW[45].
281

**Supplementary Note 2**

**Confirmation of a T-DNA knock-out allele for NPR1.** We identified a *npr1* mutant allele, SAIL_708F09, containing a T-DNA insertion in the third exon of the *NPR1* gene (Supplementary Fig. 7a). RT-PCR was used to confirm this allele has a complete loss of *NPR1* transcript and loss of *PR1* gene induction by BTH (Supplementary Fig. 7d). Bacterial growth in mock- and BTH-treated plants was assessed to confirm enhanced susceptibility relative to WT and loss of BTH-mediated protection (Supplementary Fig. 7c). We named this allele *npr1-6*.

**Preparation, selection and complementation analysis of transgenic lines.** The full length coding sequence of *NPR1* without the stop codon was PCR-amplified using NPR1_F and NPR1_R primers (Supplementary Table 6) and cloned into *EcoR*I/*Xho*I sites of pENJAZ9C[46] to create pEN*NPR1C*, a Gateway compatible entry vector. Next, a 2.3 kb DNA fragment containing the *NPR1* promoter was PCR-amplified using pNPR1_F and pNPR1_R primers (Supplementary Table 6) and cloned into *Not*I/*EcoR*I sites of pEN*NPR1C* to create pEN*pNPR1C::NPR1*. Then, the *pNPR1::NPR1* construct was transferred by LR recombination into the binary expression vector pGWB540 to create the *pNPR1::NPR1-YFP* construct (Supplementary Fig. 7b). The correct construct was confirmed by sequencing and introduced into *Agrobacterium tumefaciens* (GV3101) by electroporation. GV3101 clones containing the gene fusion construct were selected on LB medium containing rifampicin (Rif, 100 mg $L^{-1}$), spectinomycin (50 mg $L^{-1}$), and gentamycin (25 mg $L^{-1}$) antibiotics and used to transform *npr1-6* by floral dipping[47]. T1 seeds were plated on ½X Murashige and Skoog, 5 mM MES, 0.7 % Bacto agar plates (1/2 MMS) containing hygromycin and resistant seedlings were transplanted to soil. Ten T1 plants were selected for protein extraction, and transgene expression was assessed using Western blot analysis using an α-GFP primary antibody (1:5,000, Abcam, data not shown). T2 seeds were collected from the ten T1 lines and ~100 seeds each were sown on ½ MMS plus 1 % sucrose (1/2 MMSS) plates containing hygromycin (25 mg $L^{-1}$) to ascertain segregation ratios. Resistant T2 seedlings from lines exhibiting a 3:1 segregation ratio were then screened for induction by

BTH using confocal microscopy (data not shown). Lines showing strong induction were transplanted to soil. Homozygous T3 lines were selected by screening for 100 % resistance to hygromycin. BTH protection assays were conducted in three independent lines to confirm complementation of the *npr1-6* knockout mutation (Supplementary Fig. 7c). RT-PCR was also used to confirm recovery of *NPR1* expression and BTH-induction of *PR1* in the NPR1-Y1 line used for experiments (Supplementary Fig. 7d).

For generation of *p35S::YFP* transgenic plants, the GATEWAY cassette in pEARLEYGATE104[48] was removed by XmaI digestion, and the resulting linearized vector was re-ligated to create *pJYP35S:YFP*. This construct was used for Agrobacterium-mediated transformation of Arabidopsis Col-0 wild-type plants by floral dipping[47]. A homozygous T3 line with the single T-DNA insertion was selected for the further experiments.

**Supplementary Note 3**

***Pst* DC3000 Inoculum Preparation.** *Pst* DC3000 was streaked from a frozen glycerol stock onto a LM (10.0 g Bacto Tryptone, 6.0 g Bacto yeast extract, 1.5 g $K_2HPO_4$, 0.6 g NaCl, 0.4 g $MgSO_4$ * 7 $H_2O$ $L^{-1}$) + Rif (100 mg $L^{-1}$) plate and grown in the dark for two days at room temperature until single colonies were formed. Colonies from this plate were streaked onto a fresh LM+Rif plate and grown in the dark for one day at room temperature, after which 100 µl sterile LM media was added to the plate and the cells were spread evenly and kept in the dark at room temperature overnight to form a lawn. Cells were scraped from this lawn plate and re-suspended in 0.25 mM $MgCl_2$ by incubating at room temperature for 5 min and then vortexing vigorously. A DU800 Spectrophotometer (Beckman Coulter, Inc, Fullerton, California) was used to measure the optical density (OD) of the culture at an absorbance wavelength of 600 nm ($OD_{600}$), and an inoculation culture was prepared by first adjusting the starting culture to 1 x $10^8$ CFU $ml^{-1}$ ($OD_{600}$ of approximately 0.1) and then preparing 1:10 dilutions to reach the desired inoculum concentration of ~1 – 3 x $10^6$ CFU $ml^{-1}$ ($OD_{600}$ of approximately 0.001). For vacuum-infiltration, Silwet (0.005%) was added to the culture to enhance wetting of the leaves. Serial dilutions of inoculum were plated to determine the actual CFU $ml^{-1}$ of culture used in each experiment.

**Disease and BTH protection assays.** Following temperature acclimation and chemical treatments, syringe- or vacuum-infiltration was used to inoculate plants as previously described[49]. Following infiltration, plants were immediately returned to the test chambers where the leaves were allowed to dry until leaves returned to the pre-infiltration appearance before covering with transparent domes to maintain high humidity. Bacterial quantification was done by harvesting and grinding leaf discs in 0.25 mM $MgCl_2$ and preparing serial dilutions, which were then plated on modified Luria-Bertani liquid medium (LM, 10.0 g Bacto tryptone, 6.0 g Bacto yeast extract, 1.5 g $K_2HPO_4$, 0.6 g NaCl, 0.4 g $MgSO_4 \cdot 7$ $H_2O$ $L^{-1}$ $H_2O$) + Rifampicin (Rif, 100

345 mg $ml^{-1}$) plates and kept for 24 h at 30 °C. CFUs were counted and the CFU $cm^{-2}$ calculated as

346 (CFUs * total dilution)/(vol plated)/leaf area harvested.

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