Supplementary Information

A conserved signaling axis integrates conflicting environmental drought and heat signals to control stomatal aperture in plants

This PDF file includes:

Extended Data Figures 1 to 26

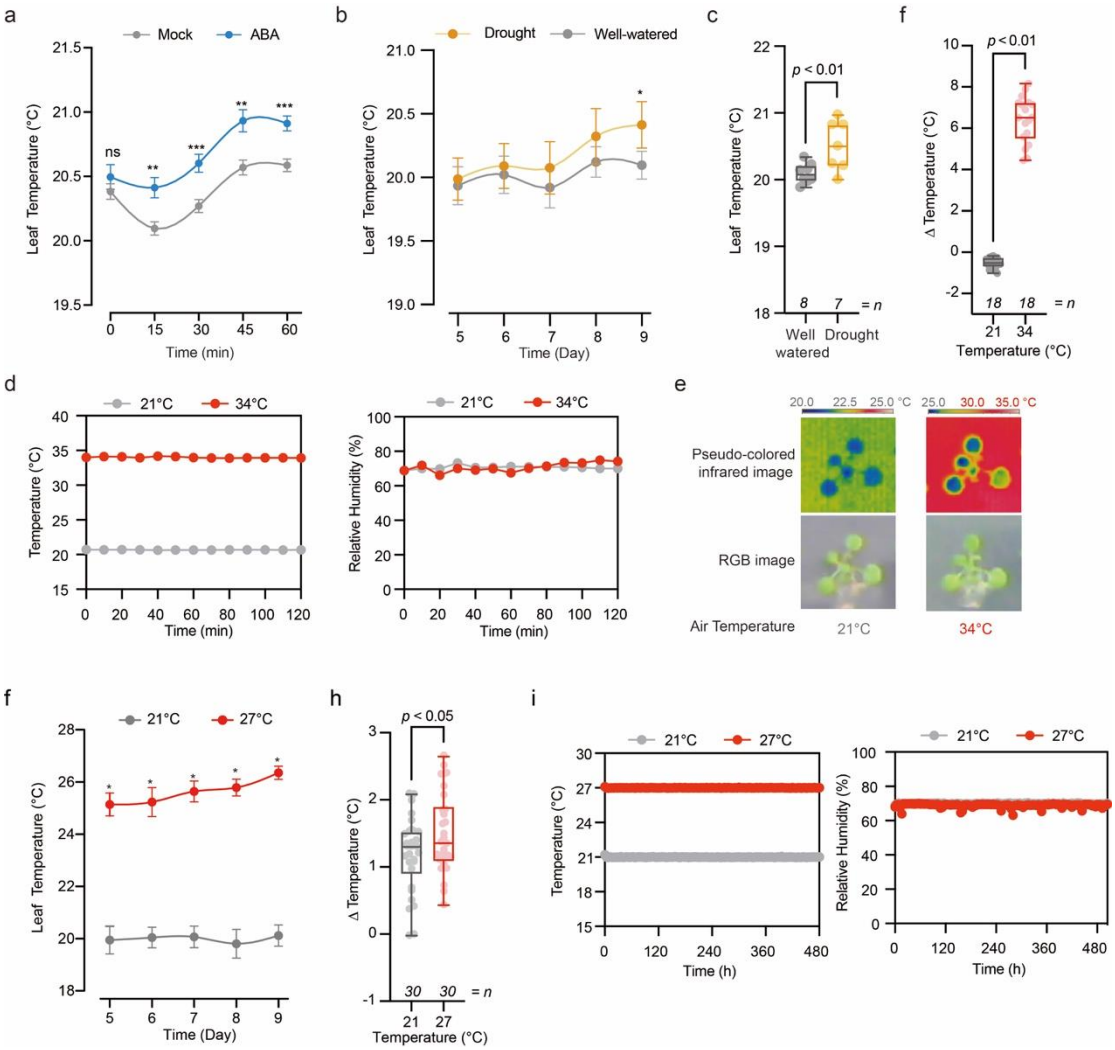
Extended Methods

References

Other Supplementary Materials for this manuscript include the following:

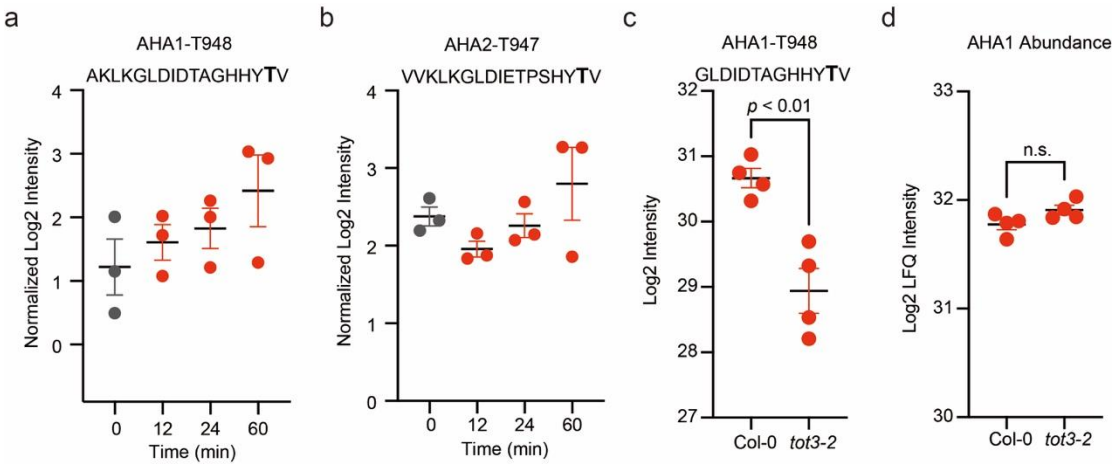
Extended Data Tables 1 to 6 (Excel format)

Extended Data Figures

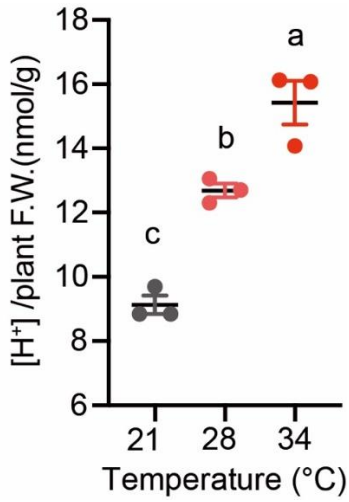


Extended Data Fig. 1. Leaf temperature of Arabidopsis Col-0 plants treated with ABA or exposed to high temperature. (a) Fit spline graph of average leaf temperature with the standard deviation of soil-grown Col-0 plants (10 leaf stage) at 21°C sprayed with 10 µM ABA in 0.025% (v/v) Silwet or Mock [0.025% (v/v) Silwet with EtOH]. For each treatment, the average temperature at each time point is calculated from eleven independent plants under each condition. The statistical difference is calculated with a Student's t-test pairwise at each time point between Mock and ABA. *, $p < 0.01$. **(b)** Fit spline graph of average leaf temperature with standard error of the mean of Col-0 under well-watered and progressive drought conditions. Col-0 (10 leaf stage) grown at 21 °C exposed to progressive drought or well-watered conditions. For each condition, the average temperature on each day was calculated from 8 independent plants. Statistical difference between well-watered and drought is calculated by Student's t-test within each day. *, $p < 0.01$. **(c)** Leaf temperature quantification of the Day 9 data shown in (b). The box plots display the average leaf temperature data recorded from 7-8 replicates (*n* below the plots) plants under each condition, with individual data points representing the average of all data points measured on Day 9 of each replicate plant, showing the

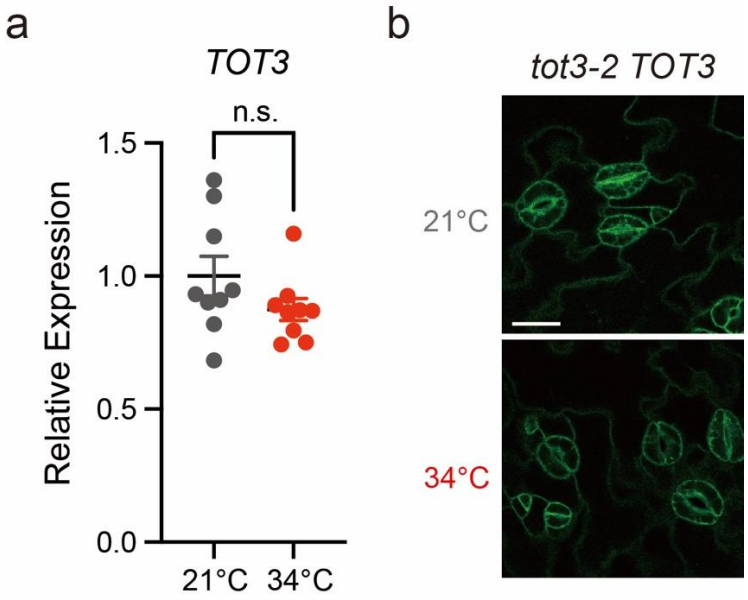
median with Tukey-based whiskers. Statistical difference between well-watered and drought is calculated by Student's t-test ($p < 0.01$). **(d)** Representative data logger values of experimental temperature treatment conditions (stable 21°C and 34 °C; with a largely stable relative humidity of experiment in the plates) associated with the experiment shown in Extended Data Fig. 1e-f, 3-8, 10-19, 23-25 and Fig. 1a-d, 2c-d, 2f-g, 3e-f. **(e-f)** Representative pseudo-colored infrared images and corresponding RGB images (e) of three-weeks-old Col-0 wild-type plants grown on half-strength MS agar plates under 21°C and exposed to 34°C for 2 hours. Red and blue color indicate higher and lower temperatures, respectively. Note that the thermal images have different color scales. (f) Quantification of the air-leaf temperature difference in (e). Box plot with individual data points shows the difference between air temperature and average leaf temperature (Δ temperature) of all true leaves per seedling. Value is derived from 18 independent seedlings from 6 replicated experiments with the standard error of the mean. Statistical differences between the two conditions were calculated by Student's t-test ($p < 0.01$). The number (n) of biological replicates at each time point is indicated below the plots. **(g-h)** Fit spline graph of average leaf temperature (g) with standard error of the mean of Col-0 wild-type at 27°C or 21°C. Col-0 (10-leaf stage) grown at 21°C in soil. Statistical difference between 21°C and 27°C is calculated by a Student's t-test within each day. *, $p < 0.01$. (h) Quantification of the leaf-air temperature difference in (g). Box plots with individual data points represent the distribution of the difference between air temperature and average temperature (Δ temperature) measured at all time points from day 5 to day 9, showing the median with Tukey-based whiskers. Statistical difference between 21°C and 27°C at each time point is calculated by a Student's t-test ($p < 0.05$). The number (n) of biological replicates at each timepoint is indicated below the plots. **(i)** Representative data logger values of temperature and relative humidity treatment conditions of plants in soil, for experiments shown in Extended Data Fig. 1a-c, 1g-h, 10c-d, 26 and Fig. 4 e-g.



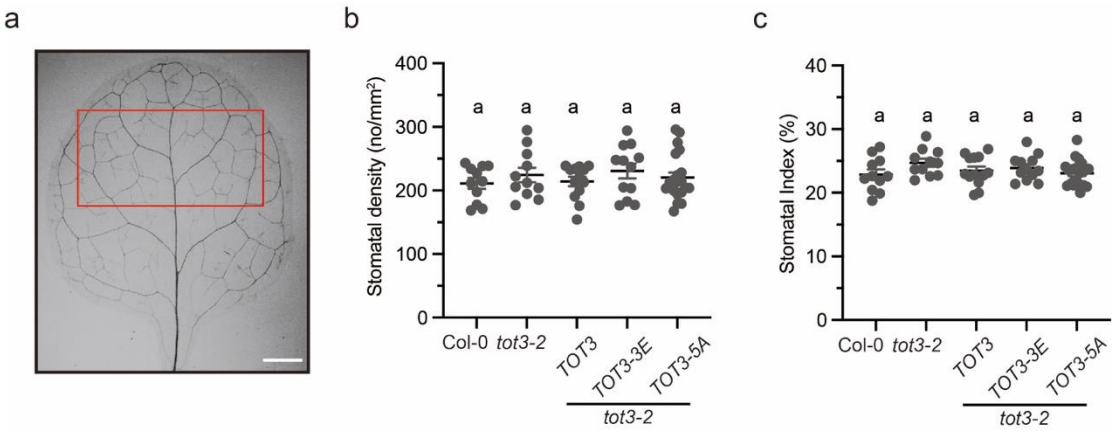
Extended Data Fig. 2. Phosphorylation of AHA1 and AHA2 at high temperature as detected by mass spectrometry. (a-b) Log2 phosphorylation intensity of indicated phosphopeptides from AHA1 and AHA2 under high temperature 28°C treatment in Col-0 wild-type. Phosphosites were selected by multi-sample test ($p < 0.01$) calculated in Perseus. **(c-d)** Log2 phosphorylation level (c) and Log2 protein label-free quantification (LFQ) intensity (d) of AHA1^{T948} in Col-0 wild-type and *tot3-2* mutant under 28°C. Scatter plots show the mean of individual values from biological replicates, with standard error of the mean. The *p*-value is calculated by Student's t-test. n.s. = not significant. Peptide sequences above the plots show the mass spectrometry-detected phosphopeptide, with identified phosphorylation residues shown in bold. Data in (a-c) taken from [1].



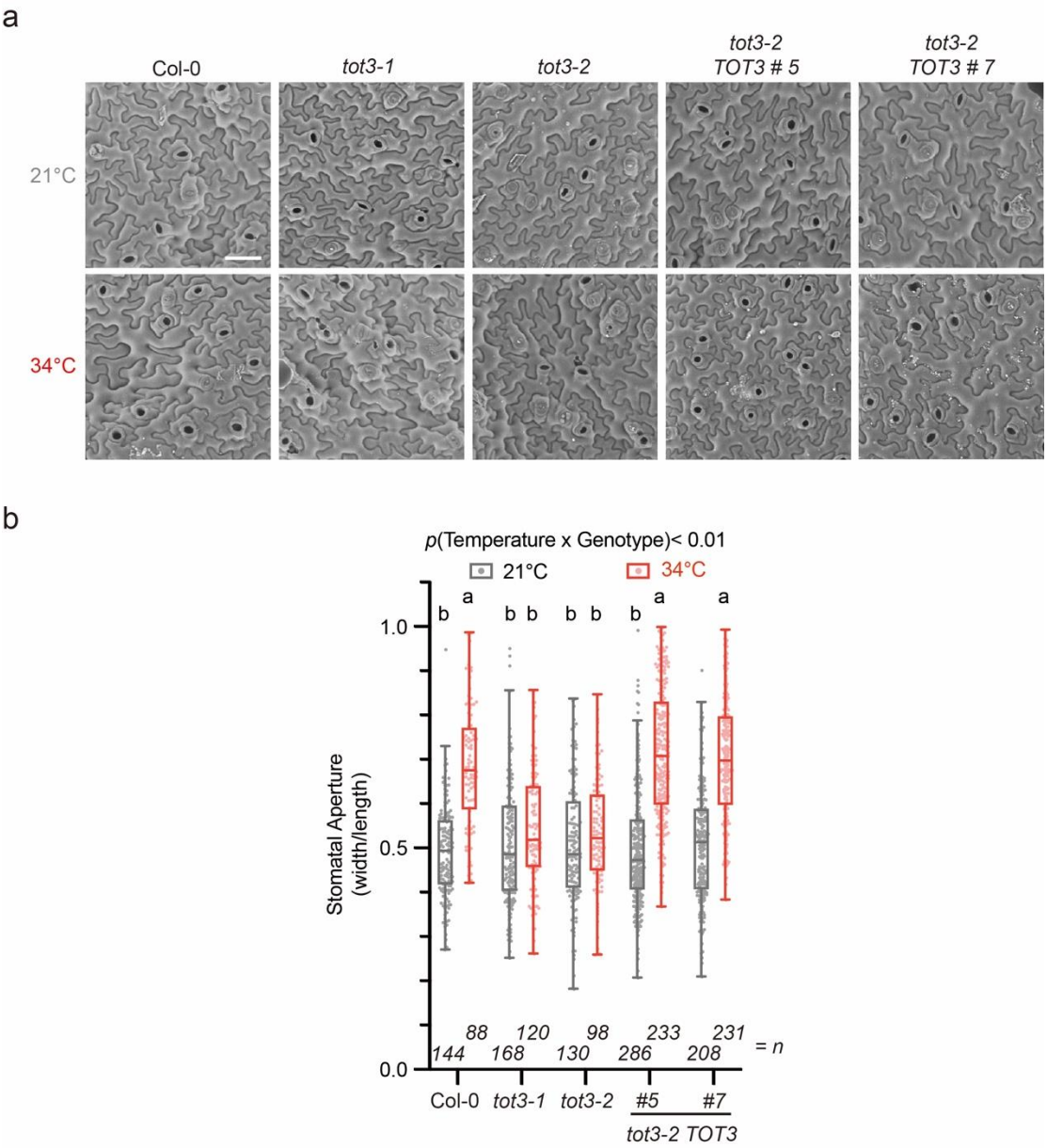
Extended Data Fig. 3. High temperature induces proton (H^+) release. Proton release in 3-week-old Col-0 wild-type plants, grown in liquid half-strength MS medium at 21°C that were moved to the indicated temperature for 8 h. The H^+ release was calculated as the difference between the initial and final pH and standardized to the total fresh weight of the plants (plant F.W.). The scatter plot shows the value of the mean from three biological replicates (using 5 seedlings for each replicate) under each temperature, with a standard error of the mean. Letters indicate significant differences based on one-way ANOVA ($p < 0.01$).



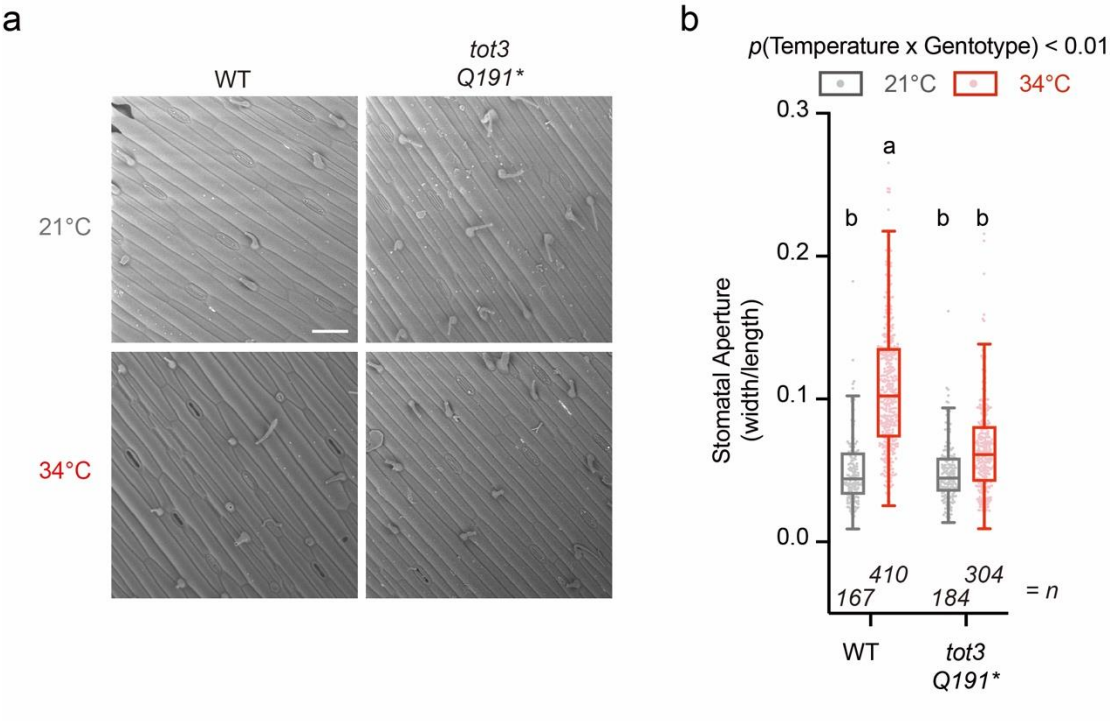
Extended Data Fig. 4. *TOT3* expression and TOT3 protein localization at high temperature. (a) Relative *TOT3* expression in 2-week-old Col-0 wild-type shoots exposed to 21°C or 34°C for 2 hours as measured by RT-qPCR. Scatter plots show the mean of individual values from nine biology replicates plants, with standard error of the mean. The *p*-value calculated by Student's t-test. n.s. = not significant. **(b)** Representative confocal microscopy image showing GFP-TOT3 localization in stomatal guard cells of a 7-day-old *tot3-2 pTOT3::GFP:TOT3 #5 (tot3-2 TOT3)* complementation line exposed to 21°C and 34°C for 2 hours. Scale bar = 25 µm.



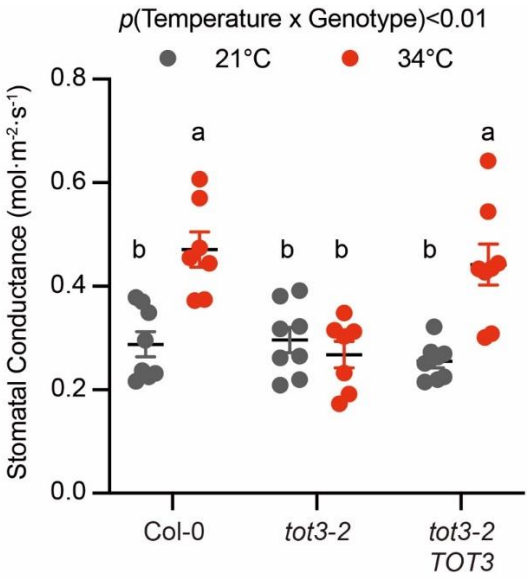
Extended Data Fig. 5. Stomatal density and index in *tot3-2*. (a) The leaf area used for quantification of stomata development and scanning electron microscope analyses in the 3rd true leaf of plants grown for 3 weeks at 21 °C is marked by a red rectangle. Scale bar = 1 mm. **(b-c)** Stomatal density (b) and stomatal index (c) of the third leaf from 3-week-old Col-0 wild-type, *tot3-2*, *tot3-2 pTOT3::GFP:TOT3 #5 (tot3-2 TOT3)*, *tot3-2 pTOT3::GFP:TOT3-3E #18 (tot3-2 TOT3-3E)* and *tot3-2 pTOT3::GFP:TOT3-5A # 1 (tot3-2 TOT3-5A)* seedlings grown at 21 °C. Scatter plots show the mean of individual values obtained from at least 10 independent plants from 3 replicated experiments, with standard error of the mean. Letters indicate no significant differences based on one-way ANOVA and Tukey's test ($p < 0.05$).



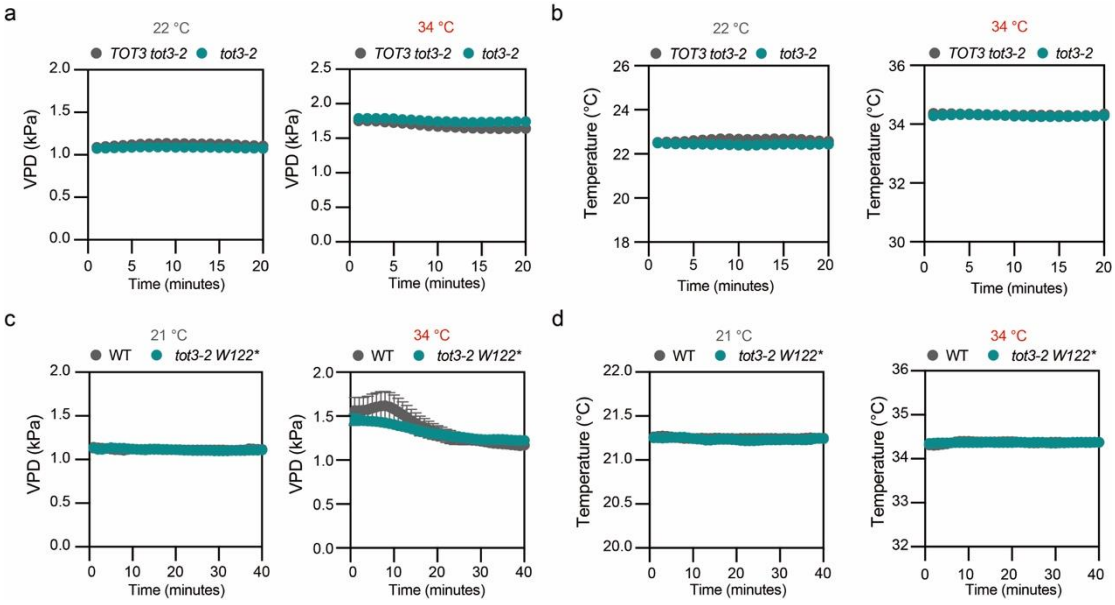
Extended Data Fig. 6. TOT3 regulates stomatal aperture under high temperature in Arabidopsis. (a-b) Representative scanning electron microscope (SEM) images of the Arabidopsis leaf epidermis, including stomata (a), and quantification of stomatal aperture (b) of the third leaf of three-week-old Arabidopsis Col-0 wild-type, *tot3-1* and *tot3-2* mutants, and *tot3-2 pTOT3::GFP:TOT3 #5* (*tot3-2 TOT3 #5)* and *#7* (*tot3-2 TOT3 #7*), exposed to 21°C or 34°C for 2 hours. Scale bar = 50 µm. Box plots with individual dots represent the distribution of all individual stomata measured, showing the median with Tukey-based whiskers. Letters indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$) between 21°C and 34°C. The *p*-value for the interaction (temperature x genotype) is shown at the top. The value under the boxes (*n*) indicates the number of stomata measured from 5-9 independent plants of each genotype.



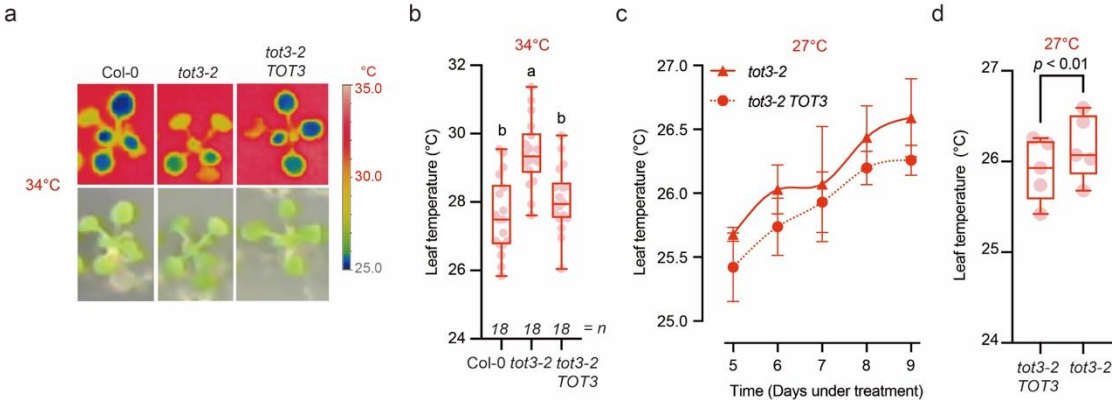
Extended Data Fig. 7. TOT3 regulates stomatal aperture under high temperature in wheat. (a) Representative SEM images of the leaf epidermis, including stomata, of the third leaf of indicated 3-week-old Cadenza wheat lines at 21°C transferred to 21°C or 34°C for 2 hours. F4 Wild-type (WT, selected from the backcrossing with the Cadenza TILLING line) and *tot3* Q191* mutant (Cadenza 0256 in the wheat TOT3 homeologue TraesCS7D01G232400) wheat lines were used. Scale bar = 100 µm. **(b)** Quantification of stomatal aperture of the 3rd leaf of 8-10 three-week-old wheat seedlings exposed to 21°C or 34°C for 2 hours. Box plots with individual dots represent the distribution of all individual stomata measured, showing the median with Tukey-based whiskers. Letters indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$) between 21°C and 34°C. The *p*-value for the interaction (temperature x genotype) is shown at the top. The value under the box plot indicates the number of stomata measured (*n*).



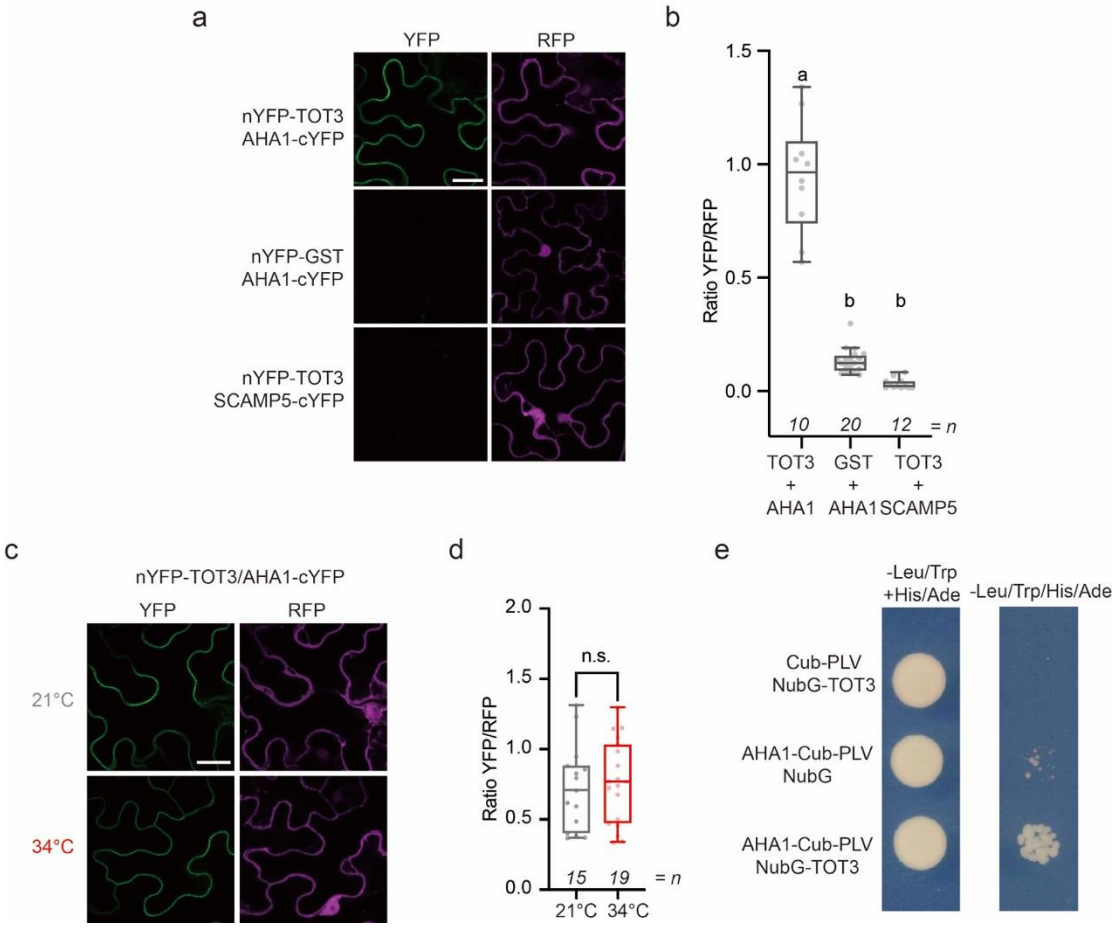
Extended Data Fig. 8. TOT3 regulates stomatal conductance in Arabidopsis under high temperature. Stomatal conductance of the 5th or 6th youngest leaf in 10 true leaf stage Col-0 wild-type, *tot3-2* and *tot3-2 pTOT3::GFP:TOT3 #5* (*tot3-2 TOT3*) plants, exposed to 21°C or 34°C for 2h under controlled relative humidity conditions measured with SC-1 Leaf Porometer. Scatter plots show the mean of individual values with the standard error of the mean. Letters above the boxplots indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$). The *p*-value for the interaction (temperature x genotype) is shown at the top.



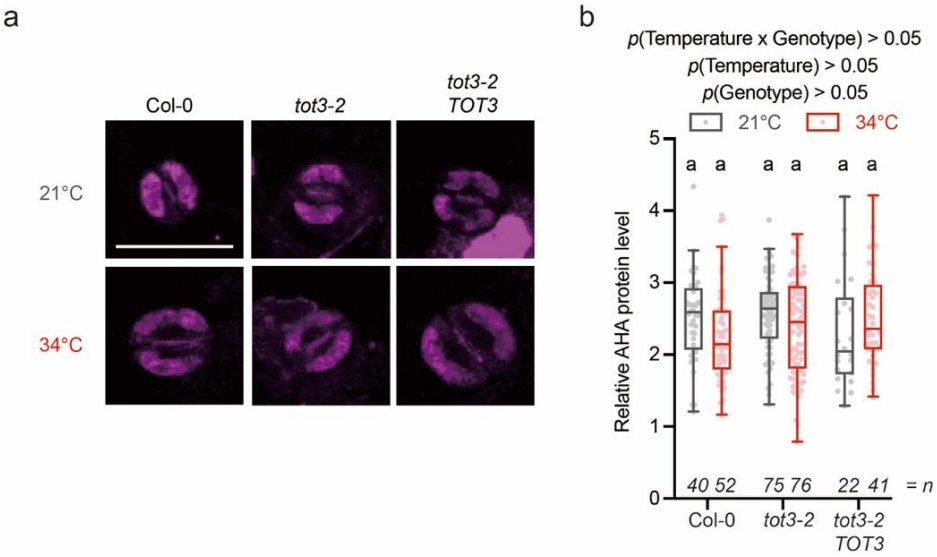
Extended Data Fig. 9. Data logger values of experimental conditions for stomatal conductance assays. (a-d) Stomatal conductance assays (see Fig. 1e-h) were performed at stable vapor pressure deficit (VPD) conditions at 21/22 °C and 34 °C for the Arabidopsis *tot3-2* mutant and the *tot3-2* complementation line *tot3-2 pTOT3::GFP:TOT3 #5* (*tot3-2 TOT3*) (a-b) and the wheat wild-type (WT, selected from the backcrossing with the Cadenza TILLING line) and *tot3 W122** mutant (Cadenza1716 in the wheat TOT3 homologue TraesCS7D01G232400) (c-d).



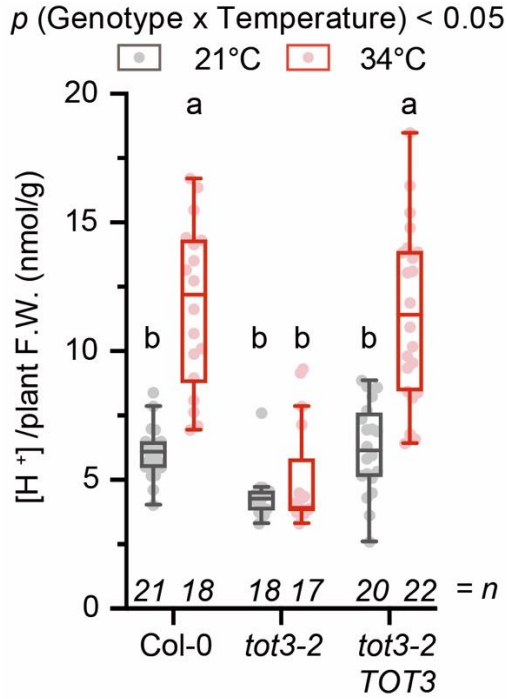
Extended Data Fig. 10. Leaf temperature of *tot3-2* mutant at high temperature. **(a-b)** Representative pseudo-colored infrared images (upper panels) and corresponding RGB images (lower panels) of plants (a) and leaf temperature quantification (b) of Col-0 wild-type, *tot3-2* and *tot3-2 pTOT3::GFP:TOT3* complementation line *#5 (tot3-2 TOT3)* at high temperature (34°C). For each replicate, all genotypes were grown on the same plate at 21°C for three weeks and exposed to 34°C for 2 hours. In total, 18 biological replicate plants (*n* under the box plot) from 6 different replicated experiments were analyzed. Red and blue pseudo colors indicate higher and lower temperature, respectively. Box plots show the average of 18 individual seedling temperature averages calculated from individual leaves showing the median with Tukey-based whiskers. Letters indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.01$). **(c-d)** Leaf temperature quantification of *tot3-2* mutant and *tot3-2 TOT3* that were continuously grown at 27°C. Data are shown as a time window (c) or an average over that time window (d). Fit spline graph (c) shows the average leaf temperature of 3-4 biological replicates of *tot3-2* and *tot3-2 TOT3* lines with standard error of the mean at the indicated day from day 5 to day 9. (d) The box plot shows the mean (line) of the leaf temperature for 5 consecutive days with Tukey-based whiskers. A single dot in the graph represents the average leaf temperature for a single day of all replicate plants. *p*-value indicates significant differences based on a paired Student's t-test.



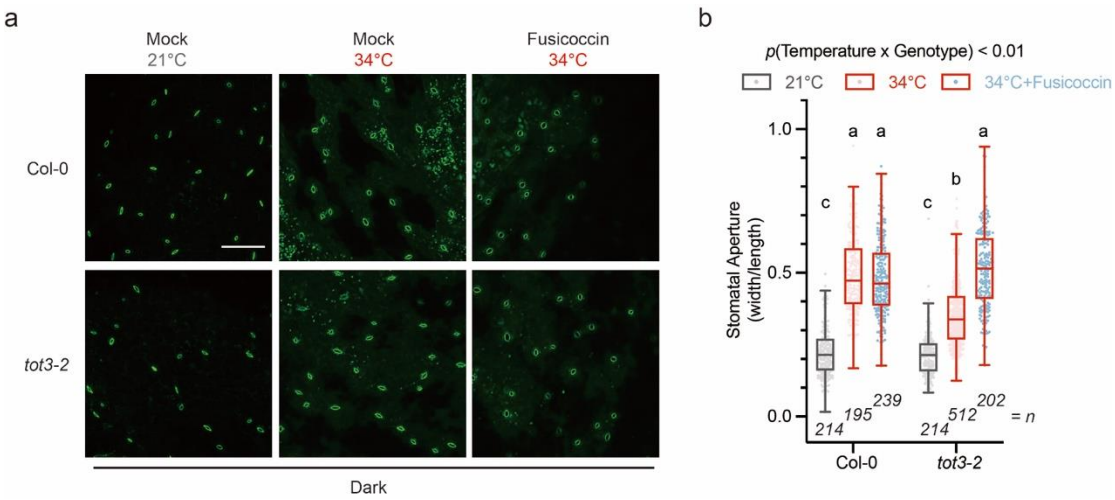
Extended Data Fig. 11. TOT3 interacts with AHA1. (a-b) Representative images (a) and intensity quantification (b) of bimolecular fluorescence complementation assay (BiFC) upon transient co-expression of *nYFP::TOT3* and *AHA1::cYFP* in *Nicotiana benthamiana* leaf epidermis cells. *nYFP::GST* with *AHA1::cYFP* and *nYFP::TOT3* with *SCAMP5::cYFP* pairs were used as negative controls. **(c-d)** Representative images (c) and intensity quantification (d) of BiFC transient co-expression of *nYFP::TOT3* and *AHA1::cYFP*, exposed to 21°C or 34°C for 2h. (a, c) RFP signals reflect the efficiency of transient expression. Scale bars = 25 µm. Quantification of the YFP/RFP fluorescence ratios (b) and (d) from the experiment in (a) and (c). Box plots of (b) and (d) with individual data points, from different infiltrated leaves, showing the median with Tukey-based whiskers. Letters in (b) indicate significant differences based on a one-way ANOVA. The *p*-value in (d) is calculated by Student's t-test. n.s. = not significant. The value below each boxplot (*n*) indicates the number of independent infiltrated leaves from 2 different replicated experiments. **(e)** Yeast-two-hybrid assays for TOT3 and AHA1. -Leu/Trp+His/Ade, control synthetic defined (SD) medium lacking Leu and Trp, but with His and Ade. -Leu/Trp/His/Ade, SD medium lacking Leu, Trp, His and Ade. The corresponding empty vectors (Empty-Cub-PLV and NubG-Empty) were used as negative controls.



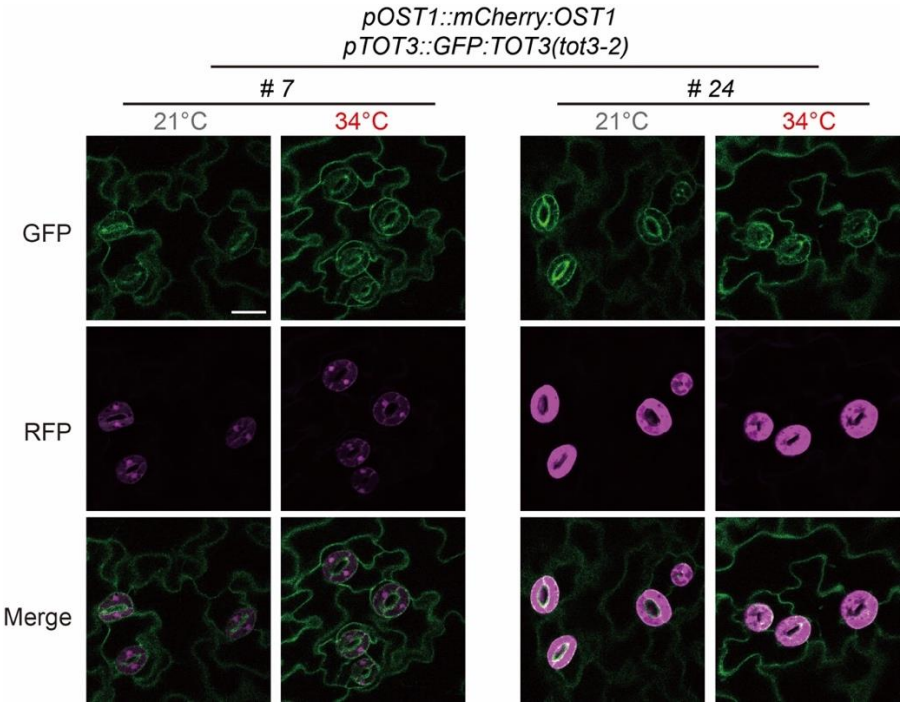
Extended Data Fig. 12. Immunohistochemical detection of the H$^+$-ATPase in the Arabidopsis epidermis under high temperature. **(a-b)** Representative fluorescence images (a) detected by an antibody against the H$^+$-ATPase catalytic domain [2] and intensity quantification (b) of immunohistochemical in the guard cells of Col-0 wild-type, *tot3-2*, and *tot3-2* complementation line *tot3-2 pTOT3::GFP:TOT3 #5* (*tot3-2 TOT3*), exposed to 21°C or 34°C for 15 minutes. Box plots with individual dots represent the distribution of all individual guard cells measured, showing the median with Tukey-based whiskers. Letters indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.05$) between 21°C and 34°C. The analysis *p*-value is shown at the top. The value below each box (*n*) indicates the number of measured stomata. Scale bar = 25 µm.



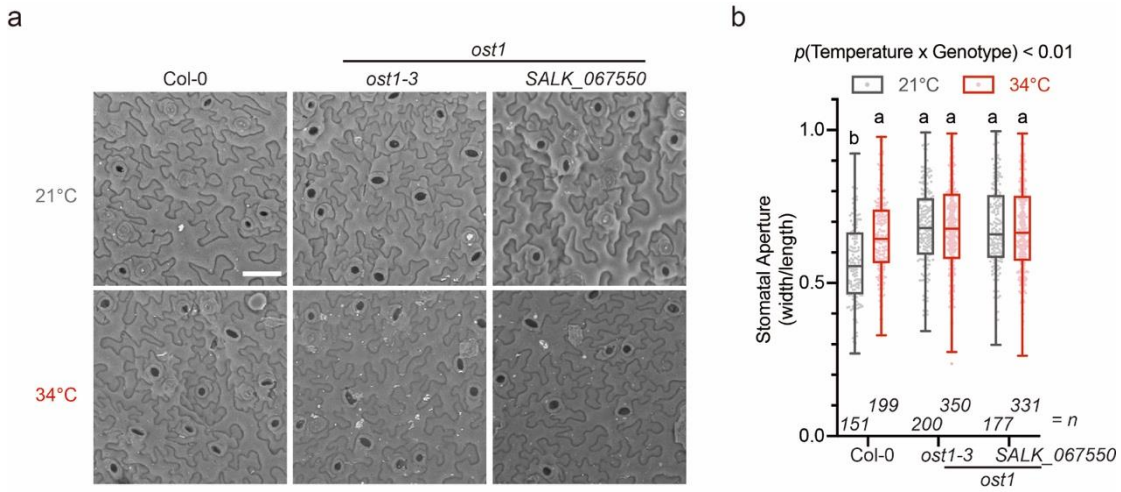
Extended Data Fig. 13. TOT3 regulates proton (H^+) release at high temperature. Three-week-old Col-0 wild-type, *tot3-2*, and *tot3-2* complementation line *tot3-2 pTOT3::GFP:TOT3 #5* (*tot3-2 TOT3*) grown in the liquid half-strength MS medium at 21°C, and then exposed to 21°C or 34°C for 8h. H^+ release was calculated by the difference between the initial and final pH and standardized to the total plants fresh weight of the plant (F.W.). Box plots show combined results from three experiments, resulting in individual values of 17-22 biological replicates (*n*) of each genotype, with standard error of the mean. Letters indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$) between 21°C and 34°C. The interaction (genotype × temperature) *p*-value is shown at the top.



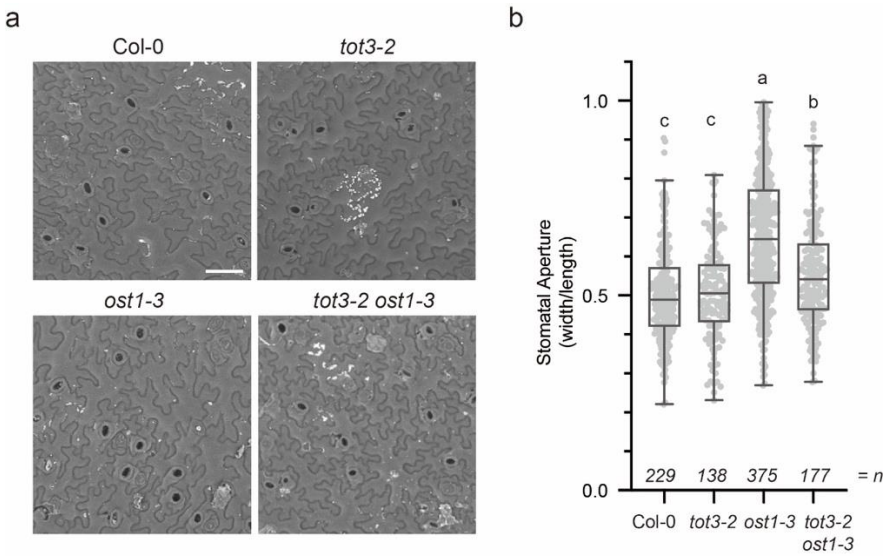
Extended Data Fig. 14. Fusicoccin treatment rescues the *tot3-2* stomatal phenotype at high temperature. (a) Representative autofluorescence confocal images of stomata from 3-week-old detached Col-0 wild-type and *tot3-2* leaves incubated in the stomatal buffer in darkness at 21°C or 34°C with DMSO (Mock) for 2 h, or 34°C with 10 µM fusicoccin for 2 h. Scale bar = 100 µm. **(b)** Quantification of stomatal aperture. Box plots with individual dots represent the distribution of all individual stomata measured, showing the median with Tukey-based whiskers. Letters above the boxplots indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$). The *p*-value for the interaction (temperature x genotype) is shown at the top. The value under the bars (*n*) indicates the number of stomata measured from at least thirteen independent leaves as biological replicates.



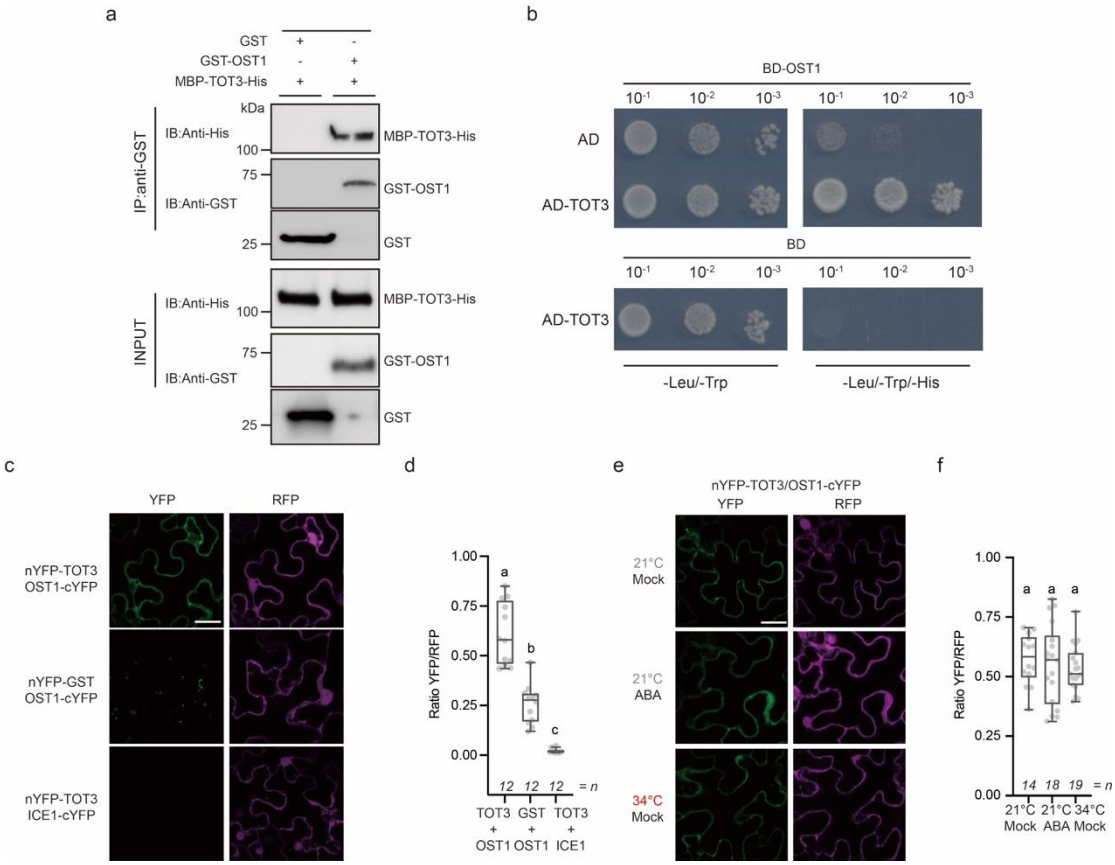
Extended Data Fig. 15. TOT3 and OST1 co-localize in stomatal guard cells in Arabidopsis. GFP and RFP signals (and their merge) in guard cells of two independent transgenic lines (*#7* and *#24*) carrying *pOST1::mCherry:OST1* and *pTOT3::GFP:TOT3* in the *tot3-2* mutant background. 7-day-old seedlings were used (that were grown at 21°C) that were exposed to 21°C or 34°C for 2 h. Leaves were immediately used to assess the signal by confocal microscopy. Scale bar = 50 µm.



Extended Data Fig. 16. OST1 regulates stomatal aperture in Arabidopsis. (a-b) Representative scanning electron microscope (SEM) images of the Arabidopsis leaf epidermis (a) and quantification of stomatal aperture (b) of the third leaf of 3-week-old Arabidopsis Col-0 wild-type, and knock-out mutants *ost1-3* and *ost1* mutant *SALK_067550*, grown at 21°C and exposed to 21°C or 34°C for 2 hours. Scale bar = 50 µm. Box plots with individual data points represent the distribution of all individual stomata measured (from the 3rd leaf of six to ten individual plants of each genotype), showing the median with Tukey-based whiskers. Letters indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$) between 21°C and 34°C. The *p*-value for the interaction (temperature x genotype) is shown at the top. The value under the bars (*n*) indicates the number of stomata measured.

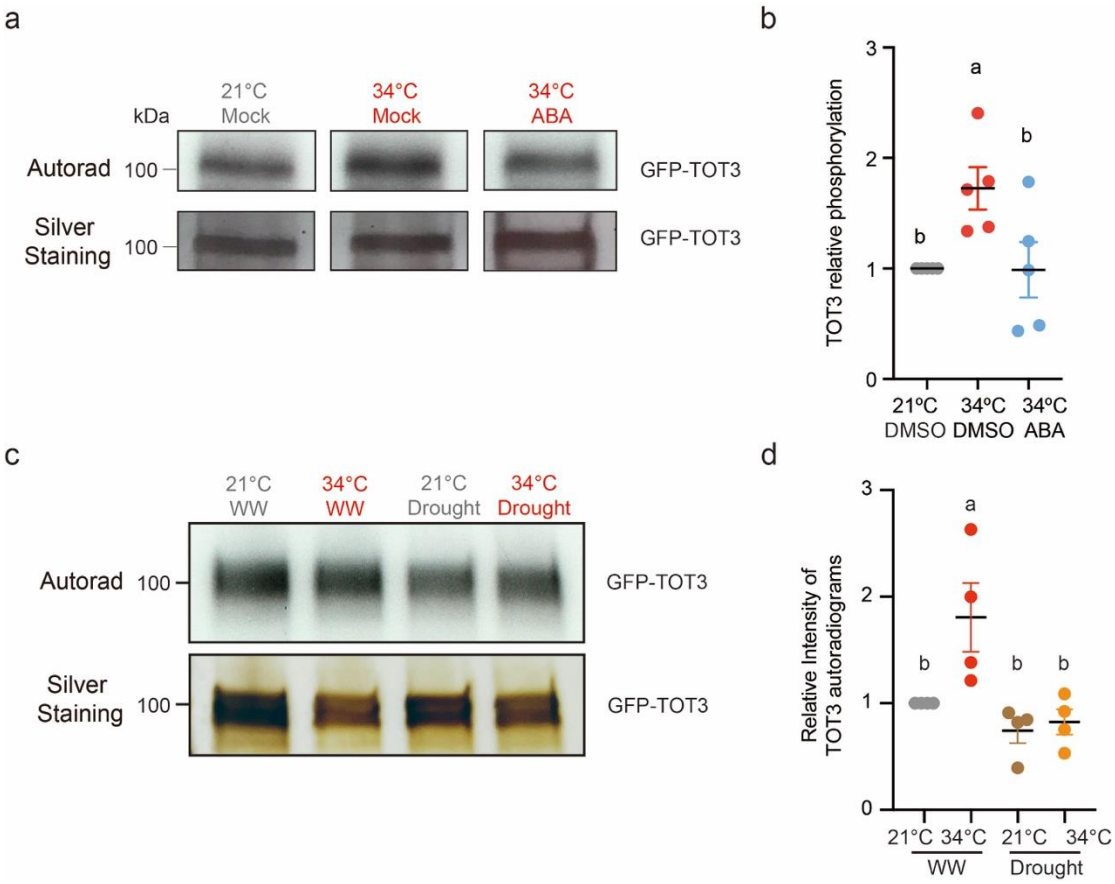


Extended Data Fig. 17. Partial reversion of the *ost1-3* constitutively open stomata phenotype in *tot3-2 ost1-3* double mutants. (a-b) Representative SEM images of the leaf epidermis (a) and quantification of stomatal aperture (b) in three-week-old indicated Arabidopsis Col-0 wild-type, *tot3-2* and *ost1-3* single mutants and *tot3-2 ost1-3* double mutant at 21°C. Box plots with individual data points represent the distribution of all individual stomata measured (from the 3rd leaf of 9-11 independent plants of each genotype), showing the median with Tukey-based whiskers. The letters above the boxplots indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.01$). The value under the bars (n) indicates the number of stomata measured. Scale bar = 50 μm.



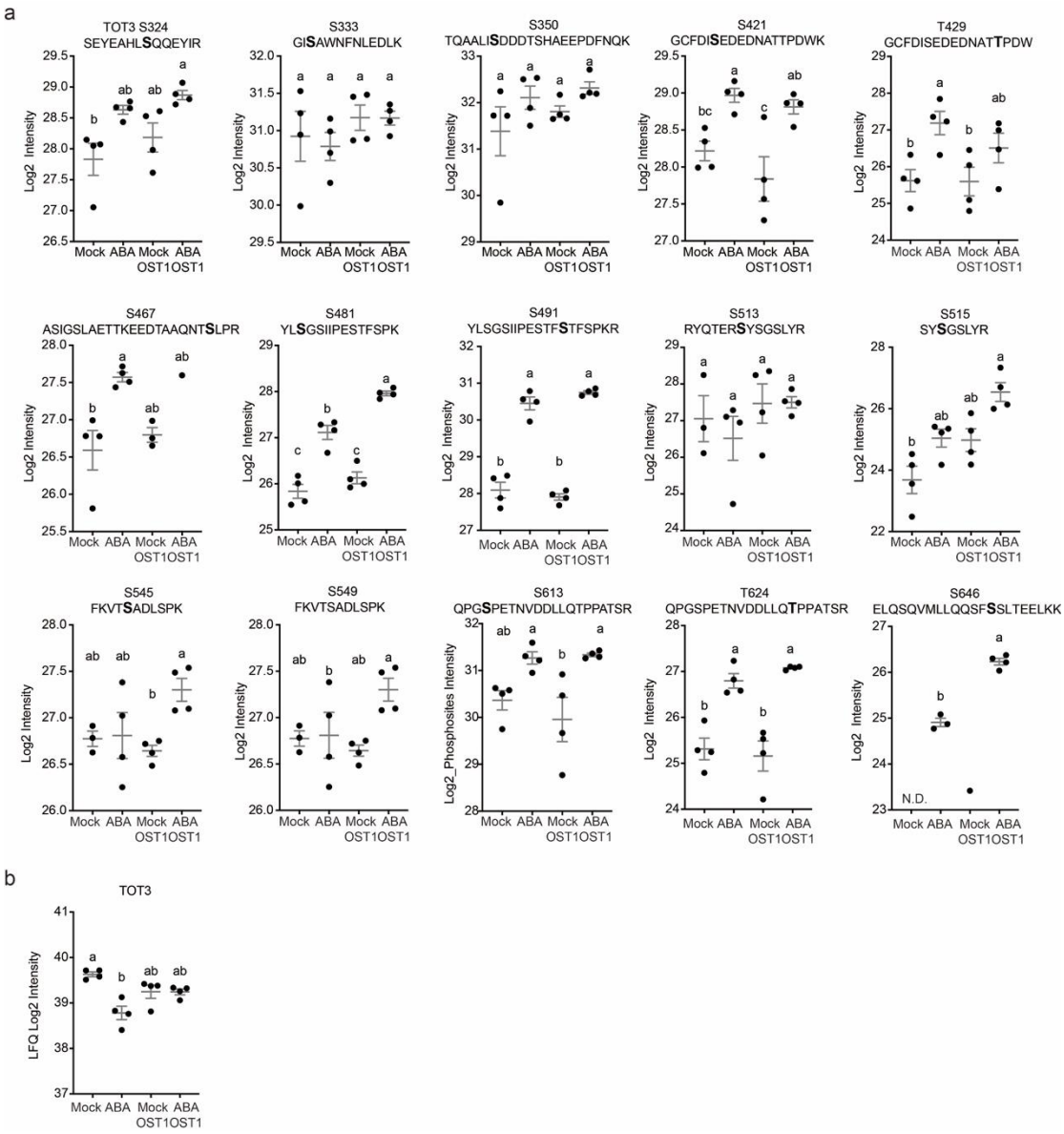
Extended Data Fig. 18. TOT3 interacts with OST1. (a) Representative results of α-GST co-immunoprecipitation of GST:OST1 and MBP:TOT3:His expressed in *E. coli*. The + indicates the tested combinations. Free GST protein was used as the negative control. The purified proteins in each combination were incubated together and enriched on GST beads. Anti-His was used for detection after GST-pulldown. The experiment was repeated three times with similar results. **(b)** Yeast-two-hybrid assays of OST1 and TOT3 interaction. Transformed yeast cultures were grown overnight in liquid synthetic defined (SD) medium lacking leucine (Leu) and tryptophan (Trp), and 10-fold serial dilutions of these cultures were dropped on either control medium lacking Leu and Trp, but with histidine (His) (SD: -Leu, -Trp, +His) or selective medium additionally lacking His (SD: -Leu, -Trp, -His). The corresponding empty vectors (BD: binding domain of Gal4; AD: activation domain of Gal4) were used as negative controls. Yeast growth was scored after 5 days at 30°C. **(c-f)** Representative images (c, e), and quantification (d, f) of the YFP/RFP fluorescence ratios of a bimolecular fluorescence complementation assay (BiFC). Representative images (c) and intensity quantification (d) of BiFC upon transient co-expression of *nYFP::TOT3* and *OST1::cYFP* in *Nicotiana benthamiana* leaf epidermis cells. The pairs of *nYFP::GST* with *OST1::cYFP* and *nYFP::TOT3* with *ICE1::cYFP*, were used as negative control. (e-f) Interaction between TOT3 and OST1 in response to ABA and heat treatment. Representative images (e) and intensity quantification (f) of BiFC transient co-expression of *nYFP::TOT3* and *OST1::cYFP,* exposed to 21°C or 34°C under mock or 50 µM ABA for 2h. Scale bar = 30 µm. The RFP signal reflects the efficiency of transient expression. Box plots with individual data points, from different infiltrated leaves, represent the

distribution of all individual replicates, showing the median with Tukey-based whiskers. Letters indicate significant differences based on one-way ANOVA. The value below each boxplot (*n*) indicates the number of independent leaves from two different experiments that were analyzed.

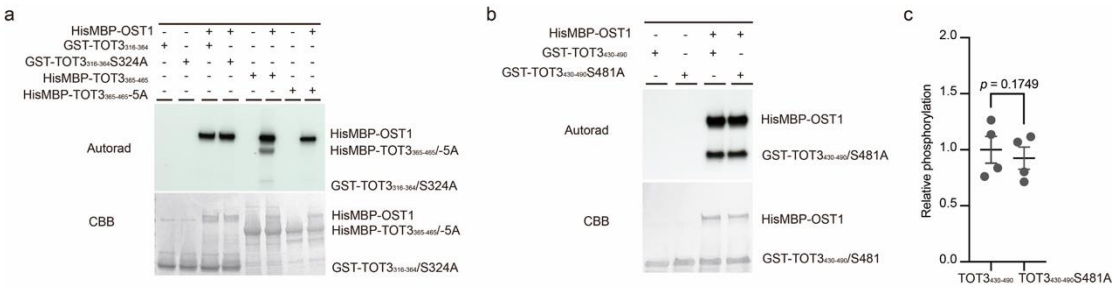


Extended Data Fig. 19. High temperature increases TOT3 kinase activity, and ABA/Drought represses TOT3 kinase activity. (a-b) Representative *in vitro* phosphorylation assay autoradiograms (with [γ-32P]ATP) of recombinant TOT3 (a) under high temperature and ABA treatment, using *tot3-2 pTOT3::GFP:TOT3 #5* plants with corresponding quantification (b). Two-week-old plants were transferred to half-strength MS medium plates with 50 μM ABA or DMSO under 21°C or 34°C for one hour. The lower gels show silver staining as the loading control. (b) Scatter plots show the mean of the individual value of five biological replicates (colored dots). Relative phosphorylation of target protein is calculated by the ratio between the intensity of autoradiograms and protein amount under each condition. The ratio of 21°C DMSO was set to one to calculate the ratio of other treatments in each replicate. Letters indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.05$) between each indicated condition. **(c-d)** Representative *in vitro* phosphorylation assay autoradiograms (with [γ-32P]ATP) of recombinant TOT3 under high temperature, drought treatment and combined heat and drought condition, using 10-leaf stage *tot3-2 pTOT3::GFP:TOT3 #5* plants exposed to drought or well-watered (WW) conditions at 21°C (c). For drought, the shoot sample was collected when the pot weight was dried down to 30% of the initial weight. Plants under WW or drought conditions were separately exposed to 21°C (21°C WW and 21°C Drought) or 34°C (34°C WW and 34°C Drought) for 2 h and the leaves were collected for protein extraction and kinase assays. The lower gel shows silver staining of protein level as the loading control. (d) Relative phosphorylation of TOT3 is calculated by the ratio between the intensity of

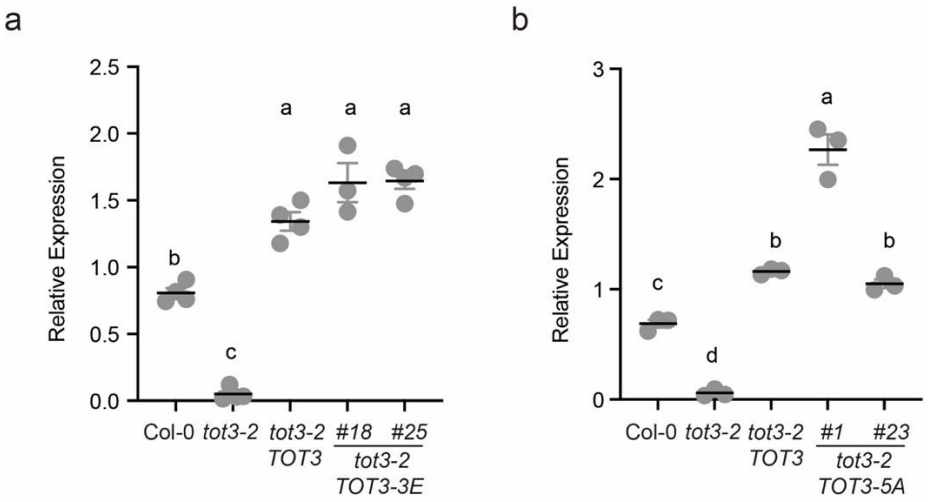
GFP-TOT3 autoradiograms and protein amount under each condition. The ratio of 21°C WW was set to one to calculate the ratio of other groups treatment in each replicate. The experiment was repeated four times with similar results. Scatter plots show the mean of the individual value of four biological replicates (colored dots). Letters indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.05$) between each indicated condition.



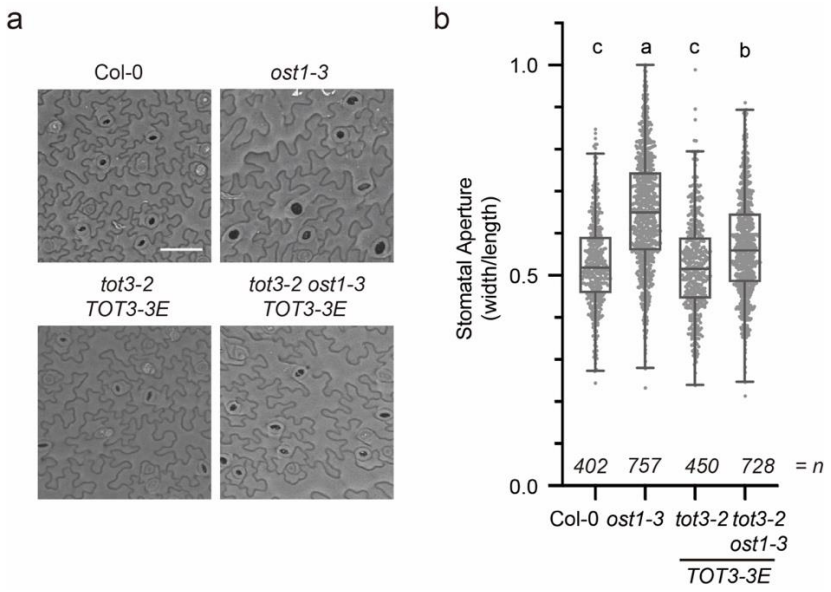
Extended Data Fig. 20. **Phosphosites detected from transiently expressed TOT3 in *Nicotiana benthamiana* tobacco leaves at indicated conditions, after pull-down and mass spectrometry. (a-b)** Log2 phosphorylation intensity of TOT3 phosphopeptides in response to ABA treatment or OST1 co-expression (a) and TOT3 protein level under these conditions (b). *Nicotiana benthamiana* leaves transiently expressing *GFP:TOT3* with *RFP:OST1* or *RFP* (control) were treated with 50 µM ABA or DMSO (Mock) for 2 hours. Scatter plots show the average of four biological replicates (black dots), with a standard error of the mean. N.D. = not detected. Letters indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.05$) between each indicated condition. Peptide sequences above the bar charts show the mass spectrometry-detected phosphopeptides with relevant phosphorylated residues shown in bold.



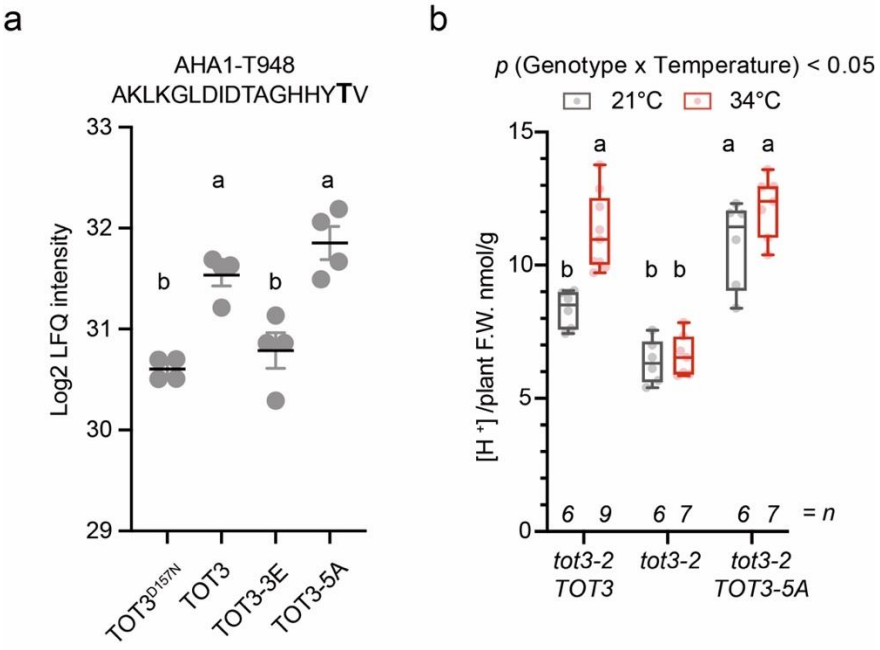
Extended Data Fig. 21. Analysis of TOT3-S324 and TOT3-S481 as OST1 target phosphosites and role of TOT3-S481 in controlling stomatal aperture under high temperature. (a-b) *In vitro* phosphorylation assay autoradiograms (with [γ-32P]ATP) of recombinant TOT3 fragments 316-364)(a) and 430-490 (b) comprising S324 and S481 phosphosites incubated with recombinant OST1. The lower gel shows coomassie brilliant blue (CBB) staining as loading control. **(c)** Quantification of relative phosphorylation in (b). Relative phosphorylation of target protein is calculated by the ratio between the intensity of autoradiograms and protein amount under each condition. The ratio of the average of $TOT3_{430\text{-}490}$ was set to one to calculate the ratio of each replicate value. Scatter plots show the mean of individual values from 4 independent replicates, with standard error of the mean. The *p*-value is calculated by Student's t-test.



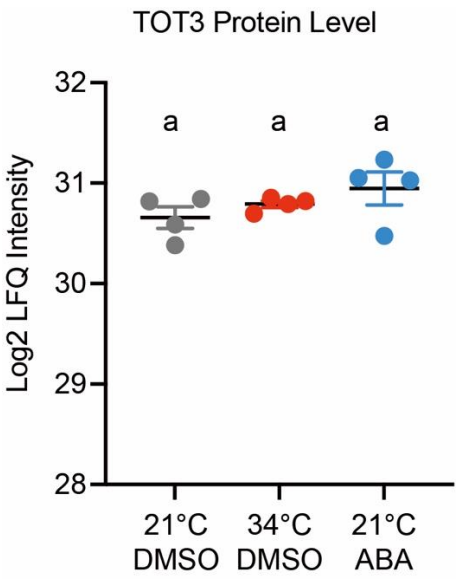
Extended Data Fig. 22. *TOT3* expression in TOT3 phosphomimetic and phosphomutant transgenic lines. (a-b) Relative *TOT3* expression as measured by RT-qPCR in 2-week-old *tot3-2* plants expressing a phosphomimetic TOT3 protein variant [*tot3-2 pTOT3::GFP:TOT3-3E (tot3-2 TOT3-3E #18* and *tot3-2 TOT3-3E #25)*] (a) and phosphomutant TOT3 protein variant [*tot3-2 pTOT3::GFP:TOT3-5A (tot3-2 TOT3-5A #1* and *tot3-2 TOT3-5A #23)*] (b), compared with Col-0, *tot3-2*, and a *tot3-2* complementation line expressing *pTOT3::GFP:TOT3 (tot3-2 TOT3)*. Scatter plots show individual values from at least three biological replicates, with a standard error of the mean. Letters indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.01$) between genotypes.



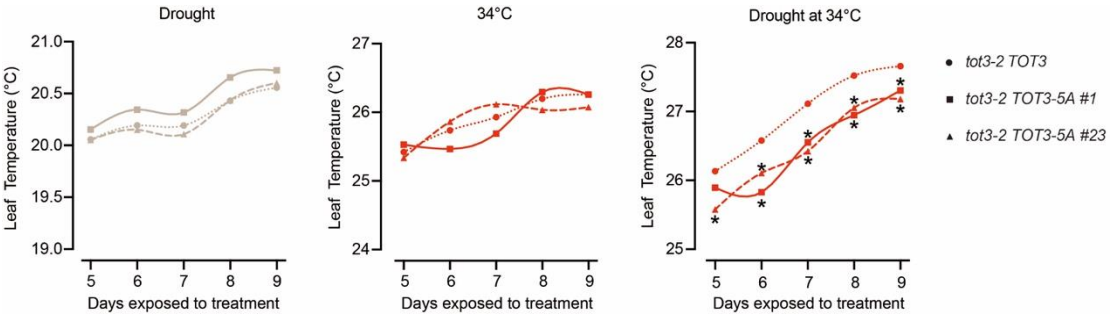
Extended Data Fig. 23. The TOT3 phosphomimetic protein variant (GFP:TOT-3E) partially restores the *ost1-3* open stomata phenotype. (a-b) Representative SEM images of the leaf epidermis (a) and quantification of stomatal aperture (b) in three-week-old Arabidopsis Col-0 wild-type, *tot3-2 pTOT3::GFP:TOT3-3E #18* (*tot3-2 TOT3-3E*), *ost1-3* and *ost1-3 tot3-2 pTOT3::GFP:TOT3-3E #18* (*ost1-3 tot3-2 TOT3-3E*) at 21°C. Box plots with individual dots represent the distribution of all individual stomata measured (from 3rd leaf of 11-14 independent plants of each genotype), showing the median with Tukey-based whiskers. Letters above the boxplots indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.01$). The value under the bars (*n*) indicates the number of stomata measured. Scale bar = 50 µm.



Extended Data Fig. 24. The TOT3-5A protein variant results in higher AHA phosphorylation and activity levels. (a) Log2 phosphorylation intensity of an AHA1 phosphopeptide that contains T948, as detected through mass spectrometry on AHA1 co-expressed with TOT3 or indicated mutant TOT3 variants in *Nicotiana benthamiana* leaves. Bar diagrams show the average of four biological replicates, with standard error of the mean. Letters indicate significant differences based on one-way ANOVA and Tukey's test ($p < 0.01$). The protein sequence above the bar charts shows the mass spectrometry-detected phosphopeptide. The relevant phosphorylated residue is indicated in bold. **(b)** Three-week-old *tot3-2, tot3-2 pTOT3::GFP:TOT3 #5 (tot3-2 TOT3)* and *tot3-2 pTOT3::GFP:TOT3-5A #1* (*tot3-2* TOT3-*5A*) grown in liquid half-strength MS media at 21°C, and then exposed to 21°C or 34°C for 8h. H^+ release was calculated by the difference between the initial and final pH and standardized to the total plants fresh weight. (F.W.). Box plots with the individual show the median with Tukey-based whiskers. Letters indicate significant differences based on two-way ANOVA and Tukey's test ($p < 0.01$) between 21°C and 34°C. The interaction (genotype × temperature) *p*-value is shown at the top. The value under the bars (*n*) indicates the number of independent plants measured.



Extended Data Fig. 25. **TOT3 protein level under high temperature and ABA treatment in Arabidopsis.** Two-week-old *tot3-2 pTOT3::GFP:TOT3 #5* plants treated with DMSO or 50 μM ABA at 21°C or 34°C in liquid half-strength MS for one hour. The shoot was collected for GFP-TOT3 IP-Mass Spectrometry. Log2 LFQ intensity of enriched TOT3 under each condition is shown as scatter plots showing the mean of individual log2 values from 4 independent plants, with standard error of the mean. Letters indicate significant differences based on Student's t-test ($p < 0.05$).



Extended Data Fig. 26. Leaf temperature of Arabidopsis *TOT3 tot3-2* and *TOT3-5A tot3-2* lines exposed to high temperature and/or drought. Average leaf temperature with fit spline under progressive drought, heat (34°C), or combined drought with heat (34°C) at the indicated day of *tot3-2* expressing *pTOT3::GFP:TOT3 (tot3-2 TOT3), pTOT3::GFP:TOT3-5A (tot3-2 TOT3-5A #1* and *tot3-2 TOT3-5A #23).* The day average leaf temperature for each mutant was calculated by taking the average temperature of all replicate plants across all time points measured on each day. For heat, drought and combined heat and drought treatment, 5, 3, 3 *TOT3 tot3-2* plants, 8, 4, 4 *TOT3-5A tot3-2 #1* plants, and 6, 3, 3 TOT3-*5A tot3-2 #23* plants were analyzed, respectively. The graph was plotted using all the data of all biological replicates. Statistical difference is calculated between *TOT3 tot3-2* and *TOT3-5A tot3-2 #1* or *TOT3-5A tot3-2 #23* by Student's t-test pairwise at each time point. *: $p < 0.01$.

Extended Methods

High temperature experiments

For the *in vitro* Arabidopsis stomatal aperture imprints and thermal imaging of Arabidopsis seedlings, intact plants were grown on square petri plates with half-strength MS. To avoid a position effect, for each replicate petri plate, the different genotypes were sown in a different region of the petri plate. The plate was sealed with micropore tape (Micropore Surgical Tape, 3M, USA). After 2 days of stratification at 4°C, the plates were moved to a growth cabinet (TC 445S, Tintometer GmbH) under long-day conditions (16 h light/8 h dark, 90 µmol m^{-2} s^{-1} photosynthetically active radiation and 70% relative humidity (RH)) and grown for 3 weeks at 21 °C. After 3 weeks, the plates were immediately moved into a growth cabinet (TC 445S, Tintometer GmbH) with identical environmental conditions (70% RH and 90 µmol m^{-2} s^{-1} photosynthetically active radiation), at either 21 °C or 34 °C for 2 hours (Extended Data Fig. 1d). The 3rd true leaf was used for taking leaf imprints and for thermal imaging (PI 200 thermal camera, Germany). For wheat stomatal aperture imprints, uniformly germinated seeds were selected and grown in plastic pots with Jiffy 7c pellets (Jiffy Products International AS, Norway) in a growth cabinet (TC 445S, Tintometer GmbH) with 70% RH, 16 h light/8 h dark and 90 µmol m^{-2} s^{-1} light. After 3 weeks, the plants were immediately moved into a growth cabinet (TC 445S, Tintometer GmbH) with identical environmental conditions of 70% RH and 90 µmol m^{-2} s^{-1} photosynthetically active radiation, at either 21 °C or 34 °C for 2 h. The middle area of 3rd leaf was used for imprints.

For high temperature treatment and thermal imaging of Arabidopsis plants on soil, seeds were sown in standard 9x9 cm pots containing a 1:2 mix of potting soil:perlite (Primasta BV, Asten, The Netherlands) and stratified at 4 °C in darkness for four days. The pots were subsequently placed in a climate-controlled growth room set at 21 °C, 70% RH and 120 µmol m^{-2} s^{-1} light intensity (PAR) at plant height provided by LED lights (Valoya BX120c4, NS1), under 8 h photoperiod/16 h darkness (Extended Data Fig. 1i). Seedlings at the two-true-leaf stage (ca. two weeks after sowing) were transferred to Jiffy 7c coco pellets (Jiffy Products International AS, Norway) that were pre-soaked in water after 50 mL of nutrient solution [3] was added. Thereafter, the nutrient solution was applied two, six and eight days after transplanting (10 mL, 20 mL and 10 mL, respectively) and pellets were watered every two days until the plants reached the developmental stage of 10 true leaves. When at the 10-leaf stage, the plant-containing pellets were fitted in a plastic cup and randomly assigned per genotype to either the 21°C treatment group or the 27 °C group, and subsequently placed in a randomized block design (matrix) of 7 x 10 plants. A FLIR A655sc High Resolution LWIR thermal imaging (IR) camera was vertically mounted above the plants, equipped with a 13.1 mm FoV 45°x 33.7° hawkeye IR lens and connected to a laptop. Thermal images were taken every 15 min during the photoperiod and darkness period for 3 weeks

continuously using FLIR ResearchIR Max software (version 4, FLIR Inc., USA).

For wheat stomatal aperture imprints, uniformly germinated seeds were selected and grown in plastic pots with Jiffy 7c pellets (Jiffy Products International AS, Norway) in a growth cabinet (TC 445S, Tintometer GmbH) with 70% RH (Extended Data Fig. 1d), 16 h light/8 h dark and 90 µmol m^{-2} s^{-1} light. After 3 weeks, the plants were immediately moved into a growth cabinet (TC 445S, Tintometer GmbH) with identical environmental conditions of 70% RH and 90 µmol m^{-2} s^{-1} photosynthetically active radiation, at either 21 °C or 34 °C for 2 h. The middle area of the 3rd leaf was used for imprints.

Drought experiments

Drought experiments were performed as previously described [4]. Seeds were sown on standard 9 x 9 cm pots containing a 1:2 mix of potting soil:perlite (Jiffy Products International BV, Zwijndrecht, The Netherlands) and stratified at 4°C in darkness for four days. The pots were subsequently placed in a climate-controlled growth room set at 21°C (day and night), 70% RH, 120 µmol m^{-2} s^{-1} light intensity (PAR) at plant height provided by LED lights (Valoya BX120c4, NS1), under 8 h photoperiod/16 h darkness (Extended Data Fig. 1i). The seedlings were randomized in the matrix of plants without having the key to genotypes versus plant number along. Seedlings at the two-leaf stage (ca. two weeks after sowing) were transferred to Jiffy 7c coco pellets (Jiffy Products International BV, Zwijndrecht, The Netherlands) that were pre-soaked in water after 50 mL of the nutrient solution was added. Thereafter, the nutrient solution was applied two, six and eight days after transplanting (10 mL, 20 mL and 10 mL, respectively) and pellets were watered every two days until the plants reached the developmental stage of 10 true leaves. When at the 10-leaf stage, the plant-containing pellets were fitted in a plastic cup and randomly assigned per genotype to either the well-watered (WW) treatment group or the drought-subjected (Drought) group, and subsequently placed in a randomized block design (matrix) of 7 x 10 plants. A FLIR A655sc High Resolution LWIR thermal imaging (IR) camera was vertically mounted above the plants, equipped with a 13.1 mm FoV 45° x 33.7° hawkeye IR lens and connected to a laptop. Thermal images were taken every 15 minutes during the photoperiod and darkness period for 3 weeks continuously using FLIR ResearchIR Max software (version 4, USA). Plants subjected to the well-watered group received water every two days by applying it to the cup fitting the pellets. The plants subjected to the drought group were subjected to progressive soil drying by withholding watering. The plants were scored every day (including the weekends) shortly after the start of the photoperiod as follows: turgid plants versus dying/shriveled plants (= wilted) to record the days until wilting occurred [4]. While recording the pot weight during the dry-down, after three weeks [4], the experiment was stopped, and plants were cut at the rosette base and photographed from the top with a standard RGB photo camera.

Stomatal conductance analyses

For Fig. 1e-f and Extended Data Fig. 9, ten-leaf stage Arabidopsis plants, grown on Jiffy coco pellets as indicated above for the drought experiments (see *Drought experiments*), were randomly divided into two groups and placed in a growth chamber at 21°C and 27°C. For the measurements the cuvette temperature was at 22°C and 34°C. Measurements of CO_2 and H_2O gas exchange of Arabidopsis leaves was made on up to 4-5 different plants per line, divided between two measurement temperatures (22 and 34 °C). Leaf stomatal conductance (g_s) and other gas exchange parameters were measured using an open gas exchange system with a 2 cm^2 leaf cuvette with an integrated red–blue LED light source (LI6800, LI-COR, Lincoln, NE, USA). Each leaf was clamped in the cuvette and exposed to an irradiance of 110 µmol photons m^{-2} s^{-1} (80-20% red-blue), an ambient [CO_2] of 400 µmol mol^{-1} and an airflow of 150 µmol sec^{-1}. Leaf temperature was maintained at either 22 or 34 °C and a leaf-to-air vapor pressure deficit (VPDL) of 1.1 and 1.8 kPa was realized, respectively. Conditions were chosen to ensure high accuracy (large delta H_2O and CO_2) and environmental stability (leaf temperature and humidity) during measurement. Recordings were taken every 60 seconds for a maximum period of 30 minutes, monitoring the acclimation of g_s to the given conditions. Subsequently, the maximum g_s during this period was determined from the 15-20 min when environmental condition stability was reached.

For Extended Data Fig. 8, ten-leaf stage Arabidopsis plants, grown on Jiffy Coco pellet at 21°C as indicated above for drought experiments (see *Drought experiments*), were transferred 2 h after dawn (ZT=2) to an identical cabinet set at either 21 °C or 34 °C with 70% RH and 120 µmol m^{-2} s^{-1} light intensity for 2 h. Stomatal conductance was measured using a SC-1 Leaf Porometer (Decagon Devices, Inc., Pullman, USA) on the 5^{th} or 6^{th} youngest leaves. The measurements were conducted on the abaxial side of the leaf and the porometer was equilibrated to ambient temperature and humidity between readings. Seven to eight independent plants were measured for each genotype.

For Fig. 1g-h and Extended Data Fig. 9, three-week-old wheat plants, grown in soil under the above-mentioned wheat growth conditions (see *Plant materials and growth conditions*), were transferred to a growth cabinet (TC 445S, Tintometer GmbH) with identical environmental conditions of 70% ± 10% RH and 90 µmol m^{-2} s^{-1} photosynthetically active radiation, at either 21 °C or 34 °C for 2h (Extended Data Fig. 1d). Leaf stomatal conductance and other parameters were measured by a LI-6400XT (LI-COR, Lincoln, NE, USA) under the following conditions: 110 µmol photons m^{-2} s^{-1} (90-10% red-blue), ambient CO_2 of 400 µmol mol^{-1} and vapor pressure deficit (VPD) of 1.15 kPa with a flow rate range from 30 - 250 µmol s^{-1} (variable as our LI-6400XT does not allow to fix this additional parameter in combination with the other fixed parameters). Recordings were taken every 60 s to monitor the acclimation of gas exchange to the given conditions. Subsequently, the maximum gas exchange was determined from the last 20-40 min when environmental condition stability was reached. Six to eight independent plants were measured for each genotype.

Immunohistochemical detection of AHA and the phosphorylation of the AHA penultimate residue in guard cells

Fully expanded rosette leaves (from 6-week-old plants) were harvested and cut into small pieces with a blender described previously[5]. The mixture was filtered with 200 μm nylon mesh (PROSEP, Belgium) to enrich for guard cell. The collected guard cells were kept in a buffer (containing 10 mM MES-KOH and 50 mM KCl, pH 6.15) and exposed to 21°C or 34°C for 15 mins in a growth cabinet (TC 445S, Tintometer GmbH) under otherwise identical environmental conditions (70% RH and 90 μmol m^{-2} s^{-1} photosynthetically active radiation). The samples were collected and fixed in 4% (w/v) freshly prepared paraformaldehyde in fixation buffer [50 mM PIPES-NaOH (pH 7.0), 5 mM $MgSO_4$, 5 mM EGTA] for 1 hour at room temperature in vacuum. After fixation, the samples were transferred to the 30-well rack of the robot and subjected to 6 wash steps (3 times in PBS pH 7.4+ 0.1% Triton X-100 (PBS-T); 3 times in distilled water + 0.1% Triton X-100; 5 min for each wash), cell wall digestion (1.5% Driselase in PBS, 30 min at 37˚C), 3 times washes (PBS-T, 5min), permeabilization (3% NP-40, 10% DMSO in PBS; 30 min room temperature), 3 times washes (PBS-T, 5 min), blocking solution (3% BSA in PBS, 1h 37˚C), primary antibody solution (primary antibodies anti-$pThr^{947}$ or anti-H^+-ATPase diluted in blocking solution at 37˚C for 4h), 5 times washes (PBS-T, 5 min), secondary antibody solution (secondary antibodies diluted in blocking solution, 3h, 37˚C), and 5 times washes (PBS-T, 5 min). The dilutions of the primary antibodies were rabbit anti-H^+-ATPase (1:1000) and rabbit anti-$pThr^{947}$ (1:1000). The dilutions of the secondary antibodies used was AlexaFluor555 donkey anti-rabbit (1:600) (A-31572, ThermoFisher).

Quantitative analysis of stomatal density and index

The third leaf of 3-week-old Arabidopsis plants was cleared in cold 90% (v/v) acetone and mounted in 80% (v/v) lactic acid on a microscope slide. To avoid major veins, abaxial epidermal cells at the center of the leaf blade (Extended Data Fig. 5a) were drawn with a microscope equipped with differential interference contrast optics (DM LB with 403 and 633 objectives: Leica). Photographs of scanned cell drawings were used to measure epidermal cell area and numbers with a script as described [6]. Stomatal density (number of small cells/mm^2) and stomatal index (number of stomata/total number of epidermal cells) were calculated.

Bimolecular fluorescence complementation and quantification.

Tobacco (*N. benthamiana*) leaves were infiltrated with *Agrobacterium tumefaciens* C58 cells containing a pBiFCt-2in1-NC vector expressing indicated combinations of genes (Extended Data Table 6). The vector also contains an internal RFP marker for expression control. After 3 days, leaf disks were collected for confocal imaging using a Leica SP8 confocal microscope. Images were captured with a hybrid detector (HyD) at 488 nm laser excitation and 500 nm-540 nm long-pass emission for YFP and 561 nm

laser excitation and 590–640 nm long-pass emission for RFP. Gating technology was applied for autofluorescence removal.

Protein extraction, co-immunoprecipitation, and Western blot

In brief, the plant material was suspended with cold extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40 (v/v), 10% glycerol (v/v), 10 mM EDTA, 1 mM DTT, 1 mM sodium molybdate, 1 mM NaF and, 1 mini tablet of Roche Complete protease inhibitor per 10 mL buffer). The samples were centrifuged twice for 20 min at 20,817 g at 4°C to remove debris. Depending on the experiment, each sample was mixed with 50 µL of either magnetic GFP-Trap beads (Chromotek, USA) or RFP-Trap beads (Chromotek, USA) or Anti-HA magnetic beads (Thermo Scientific, USA) and incubated at 4°C for 2 h with rotation. The supernatant was removed from the beads using a magnetic rack. Beads were subsequently washed three times, each with 1 mL wash buffer (20 mM Tris-HCl pH 7.5; 150 mM NaCl). Samples were mixed with Laemmli sample buffer (Bio-Rad, USA) and NuPAGE™ Sample Reducing Agent (Thermo Scientific, USA) and incubated at 70°C for 10 min. Proteins were separated on 4–20% SDS-PAGE stain-free protein gel (Bio-Rad Laboratories, Inc., USA), then transferred onto a Trans-Blot® Turbo™ Mini PVDF packs (Bio-Rad Laboratories, Inc., USA). Depending on the experiment, the membrane containing the proteins was incubated with anti-GFP-HRP (Chromotek, USA) (1:5000), anti-RFP (Chromotek, USA) (1:2000), anti-His at 1:2000 dilution (Qiagen, Germany) and anti-HA (Chromotek, USA) (1:2000) and mouse anti-mouse antibody at 1:10000 dilution (Cytiva, USA). The proteins were detected by using a ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, Inc., USA). Ponceau (Sigma-Aldrich, USA) or Coomassie stain (Abcam, UK) staining were used as the sample loading control.

Recombinant protein purification

One liter of BL21 (DE3) cell culture was grown at 37°C until an OD_{600} of 0.4-0.6 was reached. Protein expression was induced by adding isopropyl ß-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM. The culture was grown further overnight at 18°C. The bacteria pellet was collected by centrifugation and lysed in 20 mL extraction buffer (50 mM Tris-HCl pH 8, 150 mL NaCl, 5 mM EDTA, 1 mM DTT, 10% (v/v) glycerol, 0.5% (v/v) NP-40 and 1 mini tablet of Roche Complete protease inhibitor per 10 mL buffer) and sonicated on ice. The lysate was cleared by centrifuging at 10,000 g for 30 min at 4°C. The supernatant was incubated with His-Tag Purification Resin (Cube Biotech GmbH, Germany) or glutathione-Sepharose beads (Sigma-Aldrich, USA) at 4°C for 2 h with rotation. The supernatant containing unbound proteins was separated and removed from the beads by centrifugation at 500 g. The pelleted beads were washed three times with 5 mL wash buffer each. The wash buffer contained 50 mM Tris-HCl pH 8, and 150 mM NaCl. For His-fusion proteins, the wash buffer also contained 10 mM imidazole to remove unspecifically bound proteins. The proteins were eluted from the beads with elution

buffer containing 50 mM Tris-HCl pH 8, 150 mM NaCl and 10 mM reduced glutathione or 200 mM imidazole for GST-fusion proteins or His-fusion proteins, respectively. The eluted proteins were concentrated using an ultrafiltration unit (Sartorius, Germany).

Radioactive kinase assay of TOT3 under combined heat and drought treatment

Briefly, plants were grown in a climate-controlled growth chamber set at 21°C, 70% relative humidity, 120 µmol m^{-2} s^{-1} light intensity under 8 h photoperiod/16 h darkness until the 10-leaf stage was reached. The plants were then divided randomly into two groups with an equal amount of plants of each genotype per group. One group received watering throughout the experiment (well-watered, WW) and the other group was withheld from water (drought). Pot weight was recorded daily until the weight reached 30% of the initial weight (well-watered). The plants were further divided into two groups (21°C-well-watered and 34°C-well watered) and (21°C-drought and 34°C-drought) and were moved into a growth chamber with identical environmental conditions (70% RH and 90 µmol m^{-2} s^{-1} photosynthetically active radiation), at 21°C or 34°C for 2 hours. All leaves were collected for protein extraction and kinase assays. Enriched proteins were incubated in kinase reaction buffer (10 mM Tris-HCl, pH 7.5, 20mM MnCl2, 100 mM MgCl2, and 5mM DTT) in the presence of 5 µCi [γ32P]-ATP (NEG502A001MC; Perkin-Elmer) at 30°C for 60 min. The reactions were terminated by adding NuPAGE LDS sample buffer (Invitrogen) and NuPAGE sample reducing agent (Invitrogen), separated by 4-20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained. Gels were dried and radioactivity was detected by autoradiography on a photographic film with a FLA 5100 phosphor imager (Fujifilm).

Phosphopeptide enrichment and LC-MS/MS analysis

In brief, after drought treatment, samples were collected and homogenized in 5 ml protein extraction buffer (50 mM Tris-HCl pH 8, 0.1 M KCl, 5 mM EDTA, 500 mM DTT, 30% sucrose and 1 Complete Ultra EDTA-free Protease Inhibitor Cocktail Tablet (Roche) and 1 Phosphatase Inhibitor Cocktail Tablet PhosSTOP (Roche) per 50 mL buffer). The samples were sonicated on ice and centrifuged at 4 °C for 15 min at 2500g to remove debris. Methanol/chloroform precipitation of proteins was performed on the supernatant. The protein pellets were resuspended in 8 M urea in 50 mM triethylammonium bicarbonate (TEAB) buffer (pH 8). Alkylation of cysteine residues was performed in the dark by adding tris(carboxyethyl)phosphine (TCEP, Pierce) and 30 mM iodoacetamide (Sigma-Aldrich) to a final concentration of 15 mM. The sample buffer was exchanged on Illustra NAP columns (GE Healthcare Life Sciences) to 50 mM TEAB buffer (pH 8). Three milligrams of the proteins were pre-digested with EndoLysC (Wako Chemicals) at an enzyme-to-substrate ratio of 1:500 (w:w) for 4 h, followed by digestion with trypsin overnight at an enzyme-to-substrate ratio of 1:100 (w:w). The digest was acidified to pH 3 with trifluoroacetic acid (TFA) and desalted using SampliQ C18 SPE cartridges (Agilent) according to the manufacturer's

guidelines, vacuum dried and resuspended in 80% (v/v) acetonitrile, 5% (v/v) TFA. The re-suspended peptides were incubated with 1 mg MagReSyn® Ti-IMAC microspheres (ReSyn Biosciences) for 20 min at room temperature with continuous mixing. The microspheres were washed once with 60% acetonitrile, 1% TFA, 200 mM NaCl and twice with wash 60% acetonitrile, 1% TFA. The bound phosphopeptides were eluted with three volumes (80 μL) of elution buffer (40% acetonitrile, 5% NH4OH), immediately followed by acidification to pH 3 using 100% formic acid. The peptides were vacuum dried and stored at −20 °C until LC-MS/MS analysis [7].

Extended Data Tables

Extended Data Table 1. List of identified phosphosites and protein of AHA1 from tobacco infiltration IP-Mass spectrometry.

Extended Data Table 2. List of identified phosphosites and protein of TOT3 from tobacco infiltration IP-Mass spectrometry.

Extended Data Table 3. List of identified phosphosites and proteins from GFP-TOT3 IP- Mass spectrometry data in Arabidopsis exposed to high temperature or ABA.

Extended Data Table 4. List of identified phosphosites of TOT3 from phosphoprofiling of Arabidopsis seedlings treated with progressing drought stress (our data) or mannitol [8].

Extended Data Table 5. Primers used in this study.

Extended Data Table 6. Plasmids generated and used in this study.

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