Supporting Information

Nishiyama et al. 10.1073/pnas.1302265110

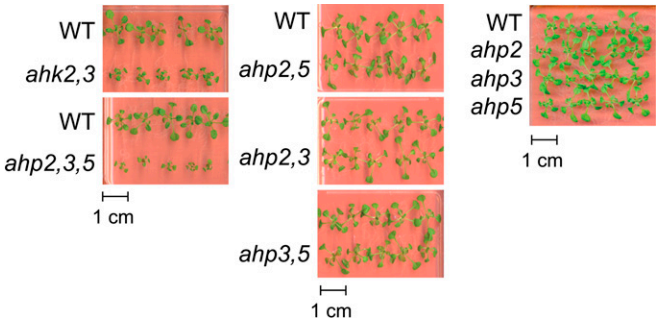

Fig. S1. Growth phenotype of the *Arabidopsis* histidine kinase *ahk2,3* mutant and all mutant combinations for *Arabidopsis* histidine phosphotransfer *AHP2*, *AHP3*, and *AHP5* genes. Plants were grown on germination medium plates for 10 d and were subsequently transferred to 0.5 × Murashige and Skoog (MS) for 5 d.

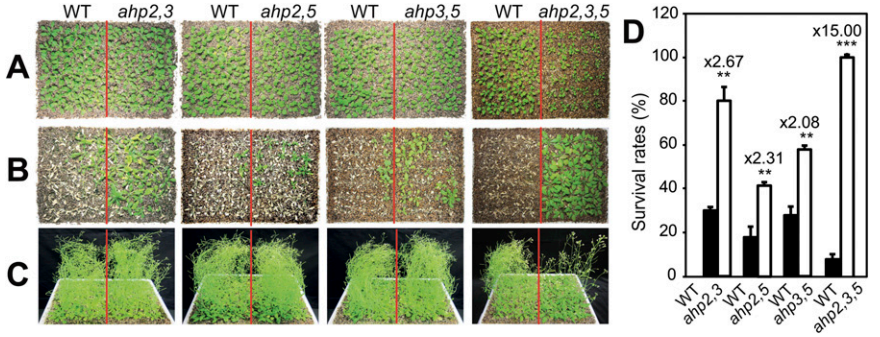

Fig. S2. Evaluation of the *Arabidopsis* histidine phosphotransfer *ahp2,3*, *ahp2,5*, and *ahp3,5* double mutant drought-tolerant phenotypes. (*A*) Two-week-old WT, *ahp2,3*, *ahp2,5*, and *ahp3,5* mutant plants were transferred from germination medium (GM) plates to soil and were grown for an additional week. (*B*) Three-week-old plants exposure to drought stress and rewatering was done when the best possible difference was observed between mutant and WT plants (between days 12 and 13). Plants were photographed 3 d subsequent to rewatering and after the removal of inflorescences. (*C*) For control purposes, 2-wk-old WT, *ahp2,3*, *ahp2,5*, and *ahp3,5* mutant plants were transferred from GM plates to trays and grown under well-watered conditions in parallel with the drought test as shown in Fig. 1*B*. (*D*) Survival rates and SEs (error bars) were calculated from the results of three independent experiments ($n = 30$ plants/genotype). For comparison purposes, the drought-tolerant phenotype of *ahp2,3,5* triple mutant (as shown in Fig. 1) is also displayed. Printed numbers represent the fold change over the WT. Asterisks indicate significantly higher survival rates than WT as determined by a Student's *t* test analysis (**$P < 0.01$; ***$P < 0.001$).

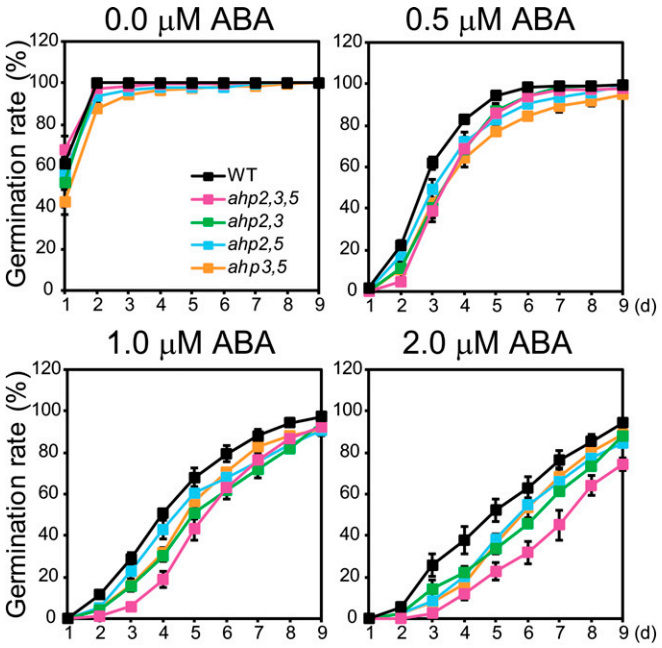

Fig. S3. Response of the *ahp* double and triple mutants to treatment with exogenous abscisic acid (ABA). Seeds were sown on germination medium-1% sucrose containing the indicated concentrations of ABA. Germination rates were quantified at the indicated time points by counting the number of open cotyledons. Error bards indicate the SEs that were calculated from the results of four independent experiments.

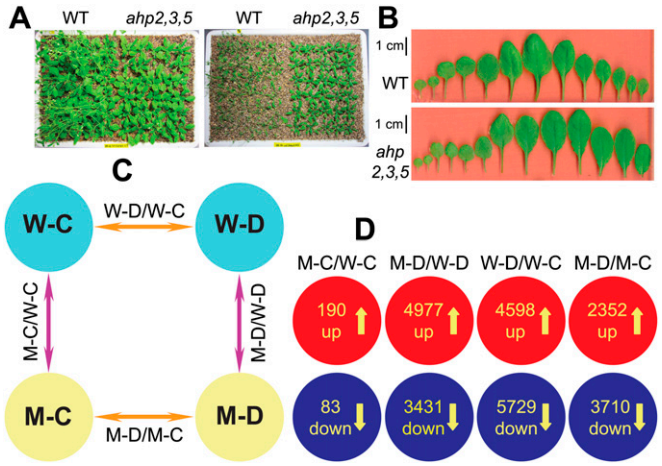

Fig. S4. Comparative transcriptome analysis of leaves of WT and *ahp2,3,5* plants under well-watered and drought stress conditions. (*A*) Three-week-old plants were continuously grown under well-watered conditions (control, *Left*) or exposed to drought stress (*Right*) for 10 d. Plants were photographed just before sample collections for microarray analysis. (*B*) Detached leaves from well-watered WT controls and *ahp2,3,5* triple mutants. (*C*) Diagrams showing experimental design and the comparisons. (*D*) Diagrams showing the compilation of genes with altered expression patterns in each comparison. Data were obtained from the results of three independent microarray experiments. W-D, WT-droughted; W-C, WT–well-watered control; M-D, *ahp2,3,5*–droughted; M-C, *ahp2,3,5*–well-watered control; W-D/W-C, WT-droughted versus WT–well-watered control; M-C/W-C, *ahp2,3,5*–well-watered control versus WT–well-watered control; M-D/M-C, *ahp2,3,5*-droughted versus *ahp2,3,5*–well-watered control; M-D/W-D, *ahp2,3,5*-droughted versus WT-droughted.

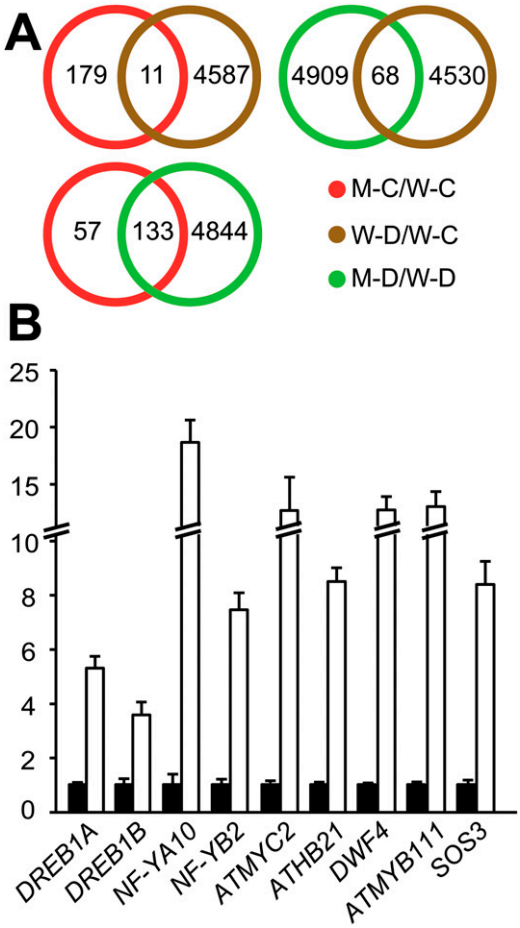

Fig. S5. (*A*) Venn diagram analysis showing the overlapping and nonoverlapping up-regulated gene sets between M-C/W-C and W-D/W-C, M-D/W-D and W-D/W-C, and M-C/W-C and M-D/W-D. W-D, WT-droughted; W-C, WT–well-watered control; M-D, *ahp2,3,5*–droughted; M-C, *ahp2,3,5*–well-watered control; W-D/W-C, WT-droughted versus WT–well-watered control; M-C/W-C, *ahp2,3,5*–well-watered control versus WT–well-watered control; M-D/W-D, *ahp2,3,5*–droughted versus WT-droughted. (*B*) Confirmation of microarray data by quantitative RT-PCR analysis. Nine genes were selected and their expression profiles were subsequently assessed by quantitative RT-PCR (qRT-PCR) in the leaf samples of droughted *ahp2,3,5* and droughted WT plants to verify the microarray data. Fold changes were calculated from the expression data obtained by qRT-PCR. cDNAs were obtained from three biological replicates.

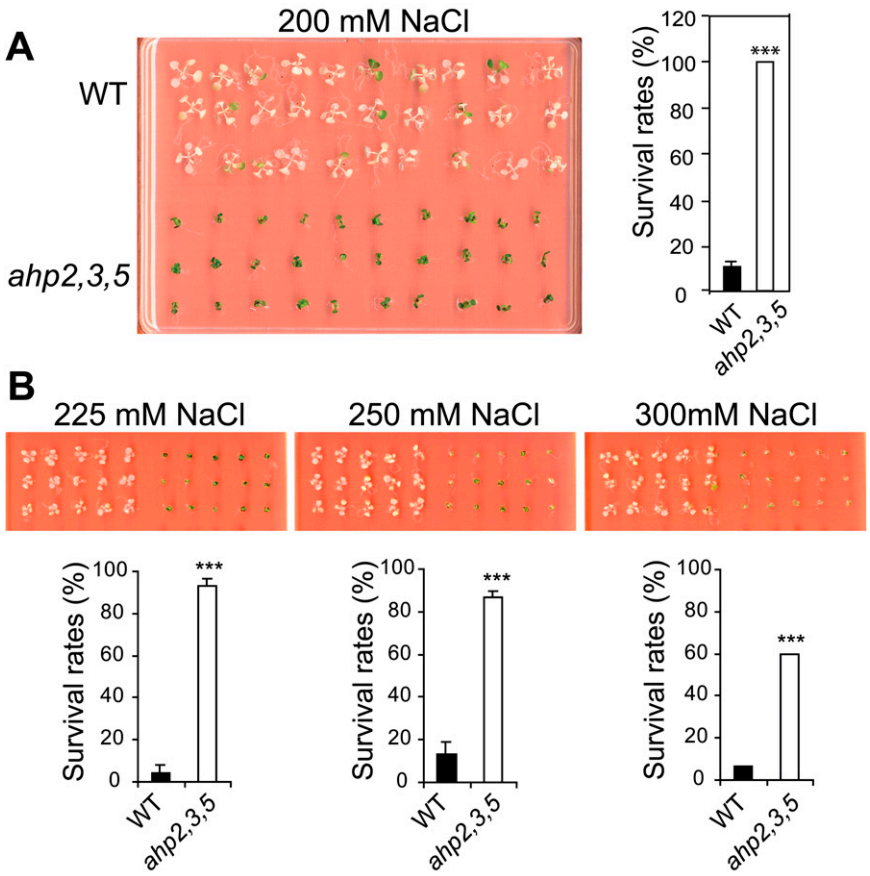

Fig. S6. Salt-tolerant phenotype of the *ahp2,3,5* mutant plants. (*A*) Plants were grown on germination medium (GM) plates for 10 d and were then transferred to 0.5 × Murashige and Skoog (MS) plates containing 200 mM NaCl for 6 d. Survival rates and SEs (error bars) were calculated from the results of three independent experiments (n = 30 plants/genotype). (*B*) Plants were grown on GM plates for 10 d and were subsequently transferred to 0.5 × MS plates containing the indicated concentrations of NaCl for 4 d (225 mM) or 3 d (250 and 300 mM). Survival rates and SEs (error bars) were calculated from the results of three independent experiments (n = 15 plants/genotype). Asterisks indicate significantly higher survival rates than WT as determined by a Student's t test analysis (***$P < 0.001$).

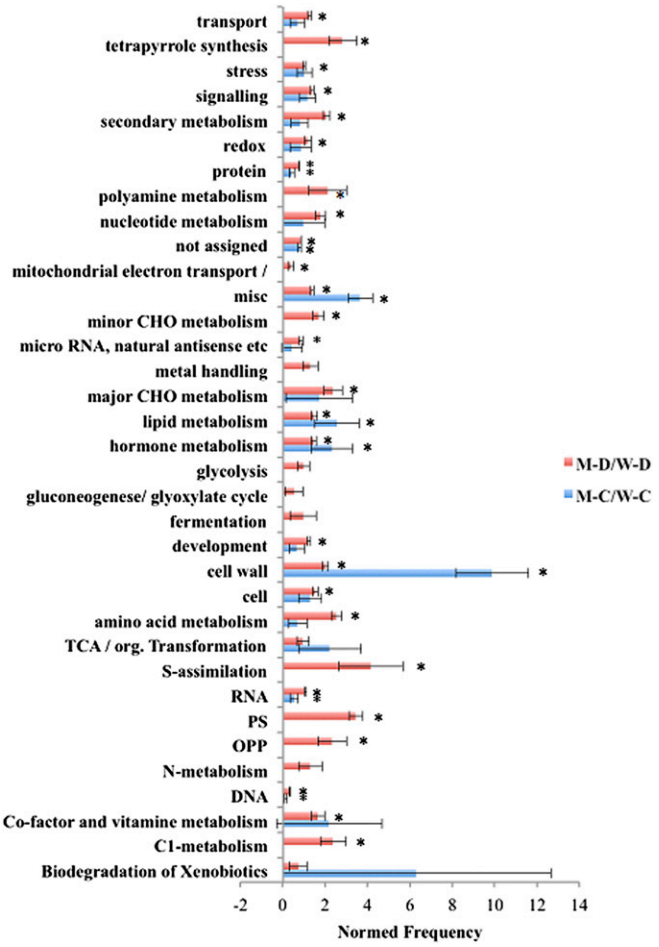

Fig. S7. MAPMAN-based functional classification of differentially expressed transcripts in *ahp2,3,5* plants under well-watered and drought stress conditions. MAPMAN classification of the transcripts using the web-tool Classification Superviewer (http://bar.utoronto.ca) with normalized class score option. The asterisk indicates a *P* value < 0.05. CHO, carbohydrate; OPP, oxidative pentose phosphate; PS, photosynthesis; TCA, tricarboxylic acid; M-C/W-C, *ahp2,3,5*–well-watered control versus WT–well-watered control; M-D/W-D, *ahp2,3,5*–droughted versus WT-droughted.

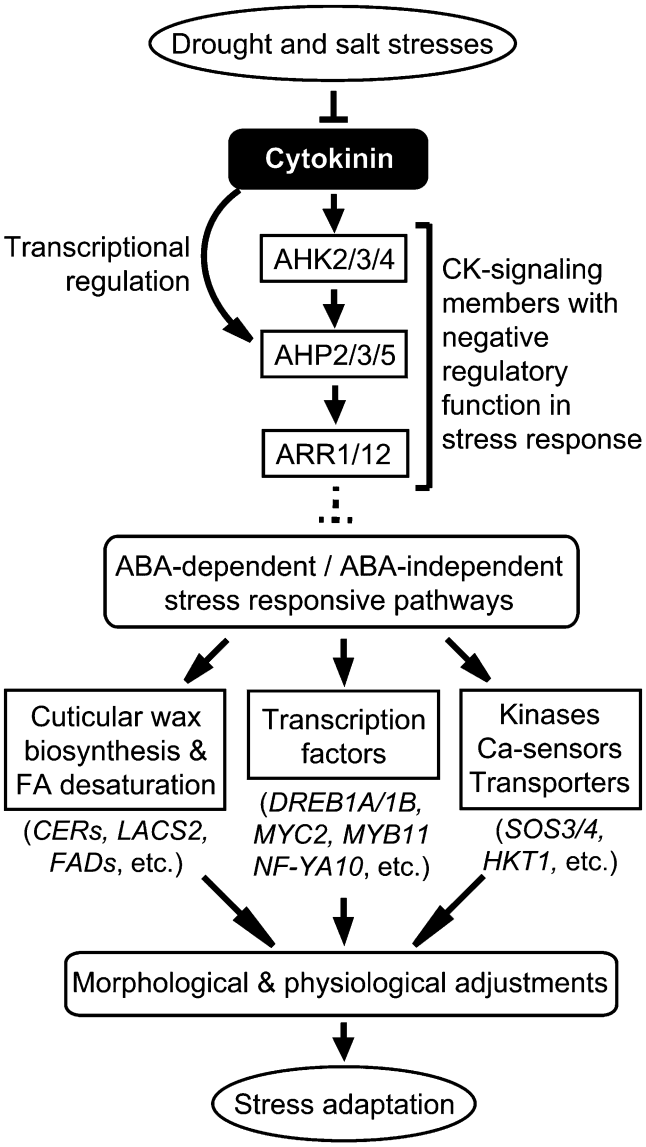

Fig. S8. During stress, a yet-unknown mechanism is activated to reduce endogenous cytokinin (CK) levels. This in turn leads to the repression of CK-related signaling mediated by at least *Arabidopsis* histidine kinase AHK2, AHK3, and AHK4 negative regulators (1); *Arabidopsis* histidine phosphotransfer AHP2, AHP3, and AHP5 negative regulators (this work); and type-B *Arabidopsis* response regulator ARR1 and ARR12 negative regulators (2). As a result, the inhibitory effect of this signaling on expression of stress-responsive and/or exogenous abscisic acid (ABA)-responsive downstream genes is attenuated (dotted bar), leading to appropriate morphological and physiological adjustments to enhance plant adaptation to stressful conditions.

1. Tran LS, et al. (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in Arabidopsis. *Proc Natl Acad Sci USA* 104(51):20623–20628.
2. Mason MG, et al. (2010) Type-B response regulators ARR1 and ARR12 regulate expression of AtHKT1;1 and accumulation of sodium in Arabidopsis shoots. *Plant J* 64(5):753–763.

Dataset S1. Microarray analysis of differential expression profiles of WT and *ahp2,3,5* plants under well-watered and drought stress conditions

Dataset S1 (XLSX)

Dataset S2. Lists of up-regulated genes from three different comparisons

Dataset S2 (XLSX)

(*A*) List of up-regulated genes (fold change ≥ 2) in *ahp2,3,5*–well-watered versus WT–well-watered; (*B*) in *ahp2,3,5*-droughted versus WT-droughted; (*C*) and in WT-droughted versus WT–well-watered treatments.

Dataset S3. Lists of down-regulated genes from two different comparisons

Dataset S3 (XLSX)

(*A*) List of down-regulated genes (fold change ≤ −2) in *ahp2,3,5*–well-watered versus WT–well-watered; (*B*) and in *ahp2,3,5*–droughted versus WT-droughted.

Dataset S4. Lists of the overlapping up-regulated genes between each pair of comparisons

Dataset S4 (XLSX)

(*A*) List of overlapping up-regulated genes (fold change ≥ 2) between *ahp2,3,5*-C/WT-C and WT-D/WT-C; (*B*) between *ahp2,3,5*-D/WT-D and WT-D/WT-C; and (*C*) between *ahp2,3,5*-C/WT-C and *ahp2,3,5*-D/WT-D.

Dataset S5. Lipid metabolism-related genes with altered transcript level in *ahp2,3,5* plants relative to WT under well-watered and drought stress conditions

Dataset S5 (XLSX)

Dataset S6. Primers used in this study

Dataset S6 (XLSX)