



Supplemental Figure S1. Complementation of the *rha2a* mutant.

A, Quantification of cotyledon greening. Cotyledon greening percentage of the indicated genotypes on medium containing different concentrations of ABA was recorded at 5 d after the end of stratification. Data show the mean ± SD of three replicates. At least 100 seeds per genotype were measured in each replicate.

B, Root growth measurements. Seedling root length was measured at 5 d after the end of stratification. Relative root growth compared with that on ABA-free medium is indicated. Data show the mean \pm SD of three replicates. At least 30 seedlings per genotype were measured in each replicate.

Asterisks indicate the significance of the difference from the corresponding wild-type values determined by the Student's *t*-test (* $0.01 \le P < 0.05$, ** P < 0.01).

C, Photographs of seedlings at 5 d after the end of stratification. Twenty-four hours after stratification, germinated seeds were transferred from ABA-free medium to medium containing 0.5 µM ABA.





Seeds of different genotypes were pretreated with water (A) or 100 μ M fluridone (B) for 48 h at 4°C in the dark, washed, then sown on plates containing different concentrations of ABA and germination was scored after 3 days and expressed as a percent of total seed. Data show the mean ± SD of three replicates. At least 100 seedlings per genotype were measured in each replicate.



Supplemental Figure S3. Leaf water loss of the indicated genotypes.

Water loss during a 4-h period was measured using detached leaves from 4-week-old wild-type (Col-0), *rha2a* and 35S:*RHA2a* plants. Values are the means \pm SE of five individual plants per genotype. The experiment was replicated five times with similar results.

Bu et al., Supplemental Figure 4



Supplemental Figure S4. ABA response analysis of RHA2a overexpression in abi3-8.

A, Quantification of cotyledon greening. Cotyledon greening percentage of the indicated genotypes grown on medium containing different concentrations of ABA was recorded at 5 d after the end of stratification. Data show the mean \pm SD of three replicates. At least 100 seeds per genotype were measured in each replicate.

B, Root growth measurements. Seedling root length of the indicated genotypes grown on different concentrations of ABA was measured at 5 d after the end of stratification. Relative root growth compared with that on ABA-free medium is indicated. Data show the mean \pm SD of three replicates. At least 30 seedlings per genotype were measured in each replicate.

Asterisks in B indicate the significance of the difference from the corresponding *abi3-8* values determined by the Student's *t*-test (* $0.01 \le P < 0.05$, ** P < 0.01). Three independent experiments were conducted and similar results were obtained.

Bu et al., Supplemental Figure 5



Supplemental Figure S5. ABA response analysis of *RHA2a* overexpression in *abi4-1*.

A, Quantification of cotyledon greening. Cotyledon greening percentage of the indicated genotypes grown on medium containing different concentrations of ABA was recorded at 5 d after the end of stratification. Data show the mean \pm SD of three replicates. At least 100 seeds per genotype were measured in each replicate.

B, Root growth measurements. Seedling root length of the indicated genotypes grown on different concentrations of ABA was measured at 5 d after the end of stratification. Relative root growth compared with that on ABA-free medium is indicated. Data show the mean ± SD of three replicates. At least 30 seedlings per genotype were measured in each replicate.

Asterisks in B indicate the significance of the difference from the corresponding *abi4-1* values determined by the Student's *t*-test (* $0.01 \le P < 0.05$, ** P < 0.01). Three independent experiments were conducted and similar results were obtained.



Supplemental Figure S6. Transgenic plants overexpressing *RHA2aC89S* (*35S:RHA2aC89S*) do not show ABA-hypersensitive phenotype. A, RNA gel blot analysis showing the expression level of *RHA2a* in *35S:RHA2aC89S* 1# and *35S:RHA2aC89S* 2# is comparable to that in *35S:RHA2a* 7# plants. Two-week-old plants were collected for RNA extraction. Ten micrograms of RNA were loaded per lane. A duplicated gel stained with EtBr was used as loading control.

B, Quantification of cotyledon greening. Cotyledon greening percentage of the indicated genotypes on medium containing different concentrations of ABA was recorded at 5 d after the end of stratification. Data show the mean \pm SD of three replicates. At least 100 seeds per genotype were measured in each replicate.

C, Root growth measurements. Seedling root length was measured at 5 d after the end of stratification. Relative root growth compared with that on ABA-free medium is indicated. Data show the mean \pm SD of three replicates. At least 30 seedlings per genotype were measured in each replicate.

Asterisks indicate the significance of the difference from the corresponding wild-type values determined by the Student's *t*-test (* $0.01 \le P < 0.05$, ** P < 0.01).

D, Photographs of seedlings at 5 d after the end of stratification. Twenty-four hours after stratification, germinated seeds were transferred from ABA-free medium to medium containing $0.5 \,\mu M \,ABA$.