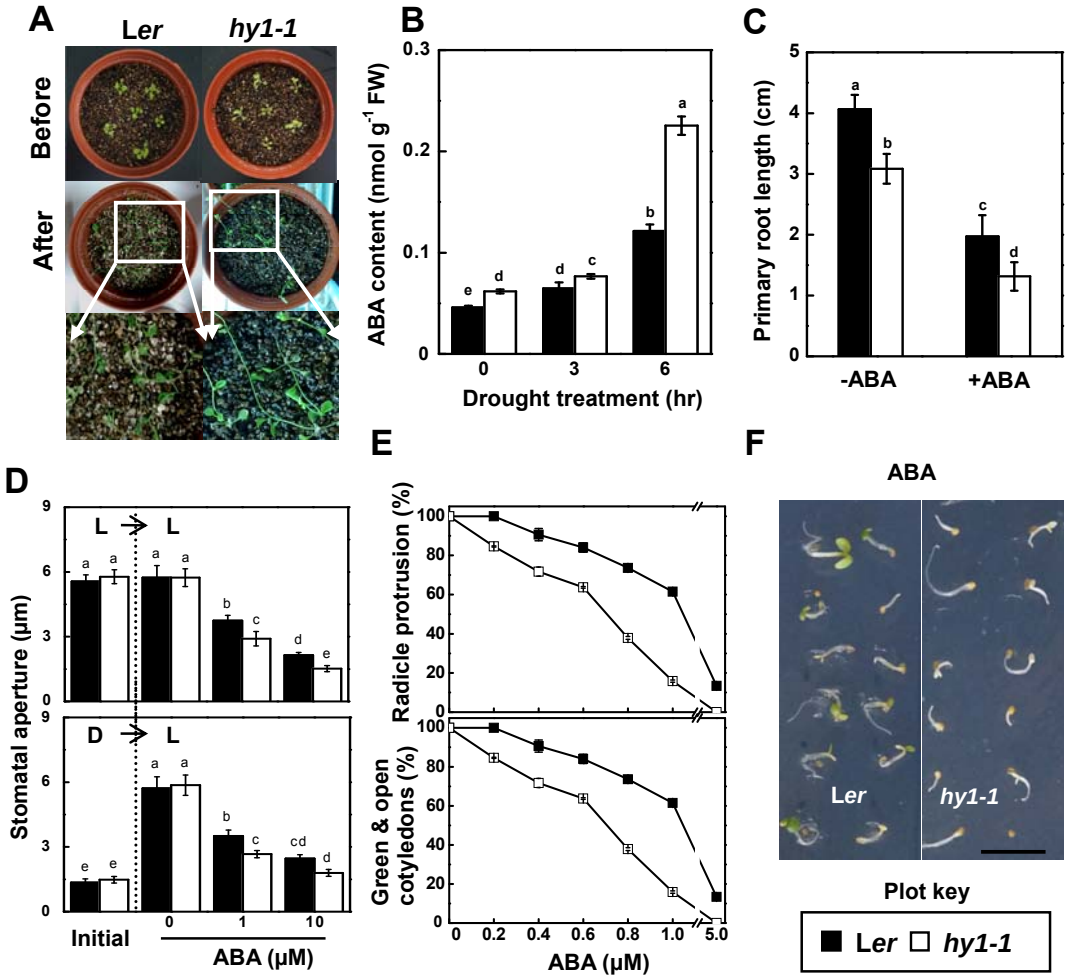
SUPPLEMENTAL DATA

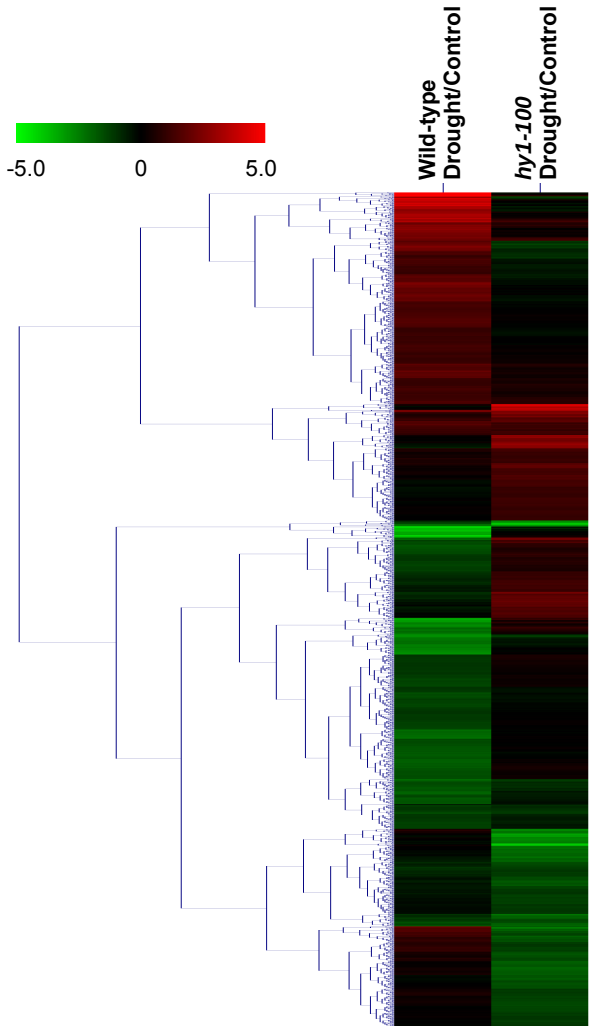
Supplemental Figure S1



Supplemental Figure S1. Phenotypes and ABA levels of *hy1-1* mutant in response to drought and ABA treatment. A, 20-day-old plants were cultured in pots before stopping irrigation. The pictures presented illustrate plants at day 0, and at day 15 after the application of drought stress. B, ABA contents in 5-day-old seedlings of L*er* or *hy1-1* mutant in response to drought stress for the indicated time. C, Primary root growth for each genotype 7 days after the transfer to the MS medium with or without

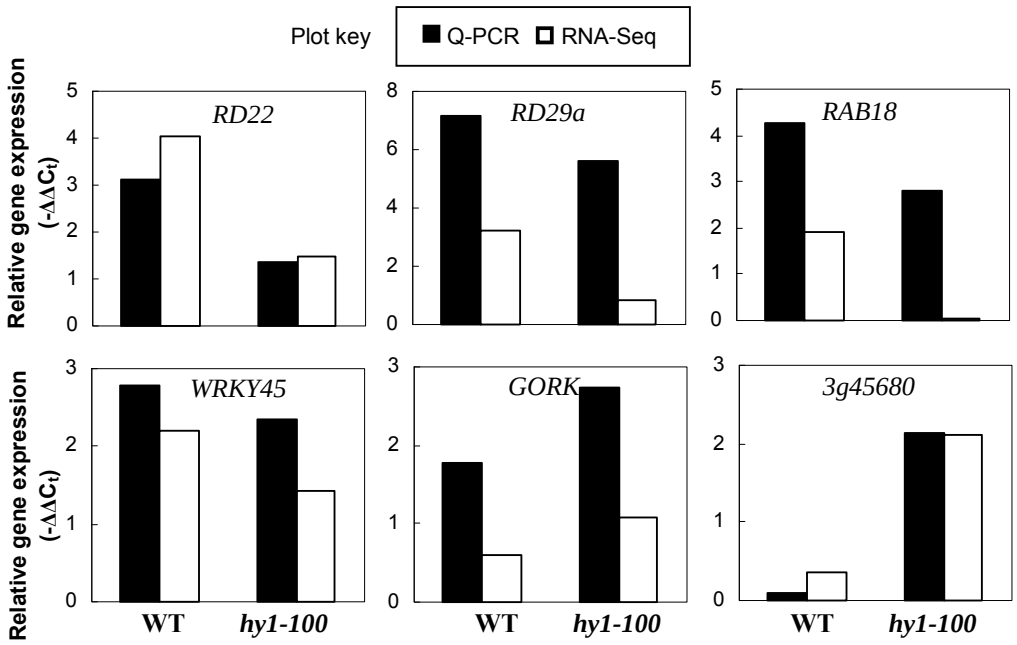
10 μM ABA (n = 15). D, ABA-induced stomatal closure (top panel) and inhibition of
11 stomatal opening (bottom panel), which was measured 2 hr after ABA treatment (n =
12 50). E, Radicle protrusion and green & open cotyledon rate (%) of each genotype
13 grown on MS medium containing the indicated ABA concentrations for 3 days or 5
14 days, respectively (n = 50). Pictures were also taken (F; 0.4 μM ABA for 5 days, bar =
15 1 cm). Plot key illustrated the genotypes for each bar shown in B-D. Data are mean ±
16 SE from at least three independent experiments. Differences among treatments were
17 analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to
18 Tukey's multiple range test.
19

Supplemental Figure S2



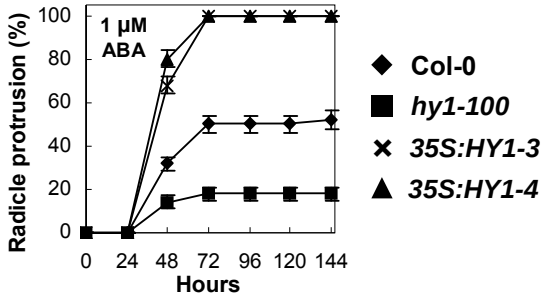
Supplemental Figure S2. Hierarchical cluster of all differentially expressed genes of RNA-Seq experiment. Distances were calculated using the Pearson similarity, and agglomeration was performed according to the Ward's minimum variance algorithm. The gradation from red to green represents strong up-regulation to strong down-regulation on a log scale.

Supplemental Figure S3



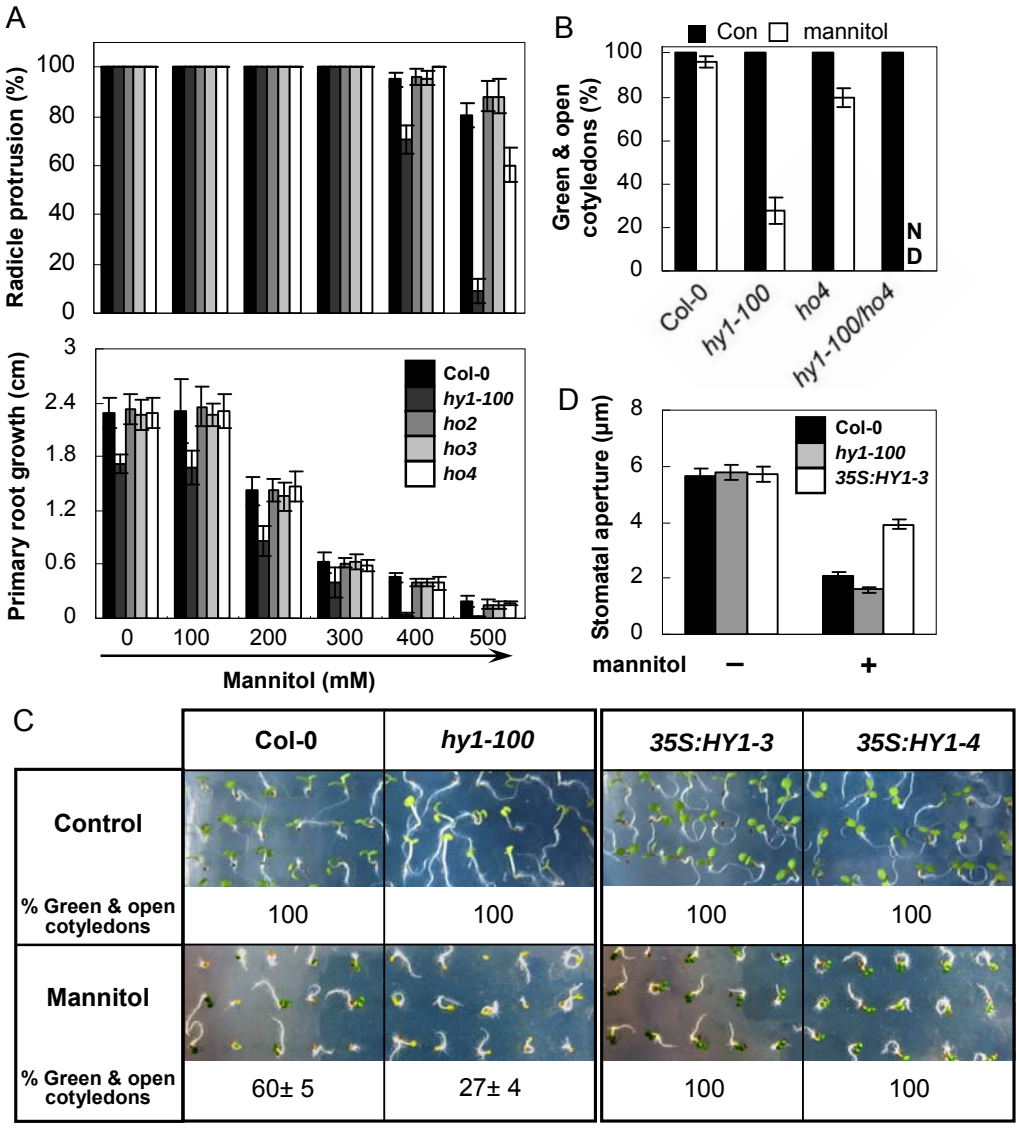
Supplemental Figure S3. Q-PCR validation for the fold-change of representative genes of wild-type and *HY1*-loss mutant detached leaves in response to desiccation for 3 hr. Expression of selected genes are presented relative to those of corresponding samples at 0 hr (100%). Values for Q-PCR are mean ± SE of at least three independent experiments.

Supplemental Figure S4



Supplemental Figure S4. Time course analysis of the radicle protrusion of wild-type, *HY1* loss- and gain-of-function mutants in response to 1 µM ABA. Data are mean ± SE of at least three independent experiments (n =50).

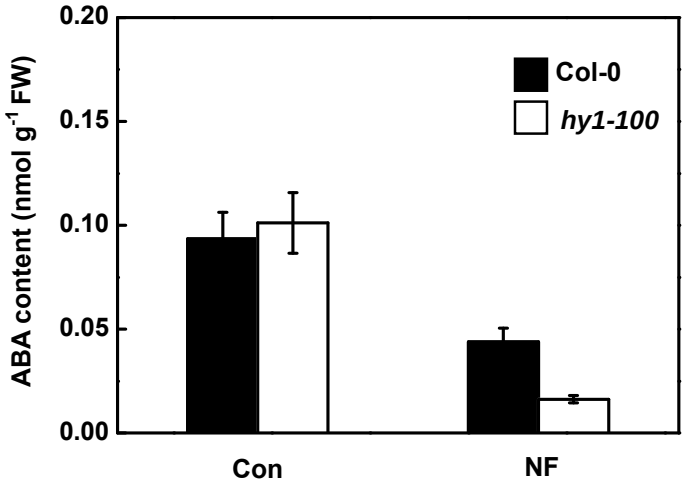
Supplemental Figure S5



Supplemental Figure S5. Osmotic phenotypic analyses of wild-type (Col-0), *hy1-100*, *ho2*, *ho3*, and *ho4* mutants, *hy1-100/ho4* and *HY1* overexpression lines *35S:HY1-3/4*. A, Dose-dependent germination rate and primary root growth inhibition in wild-type and each *ho* mutant induced by mannitol with increasing concentrations for 5 days (n = 50 or 15). B, Green & open cotyledon rate in wild-type (Col-0), *hy1-100*, *ho4*, and *hy1-100/ho4* seedlings with or without 400 mM mannitol for 8

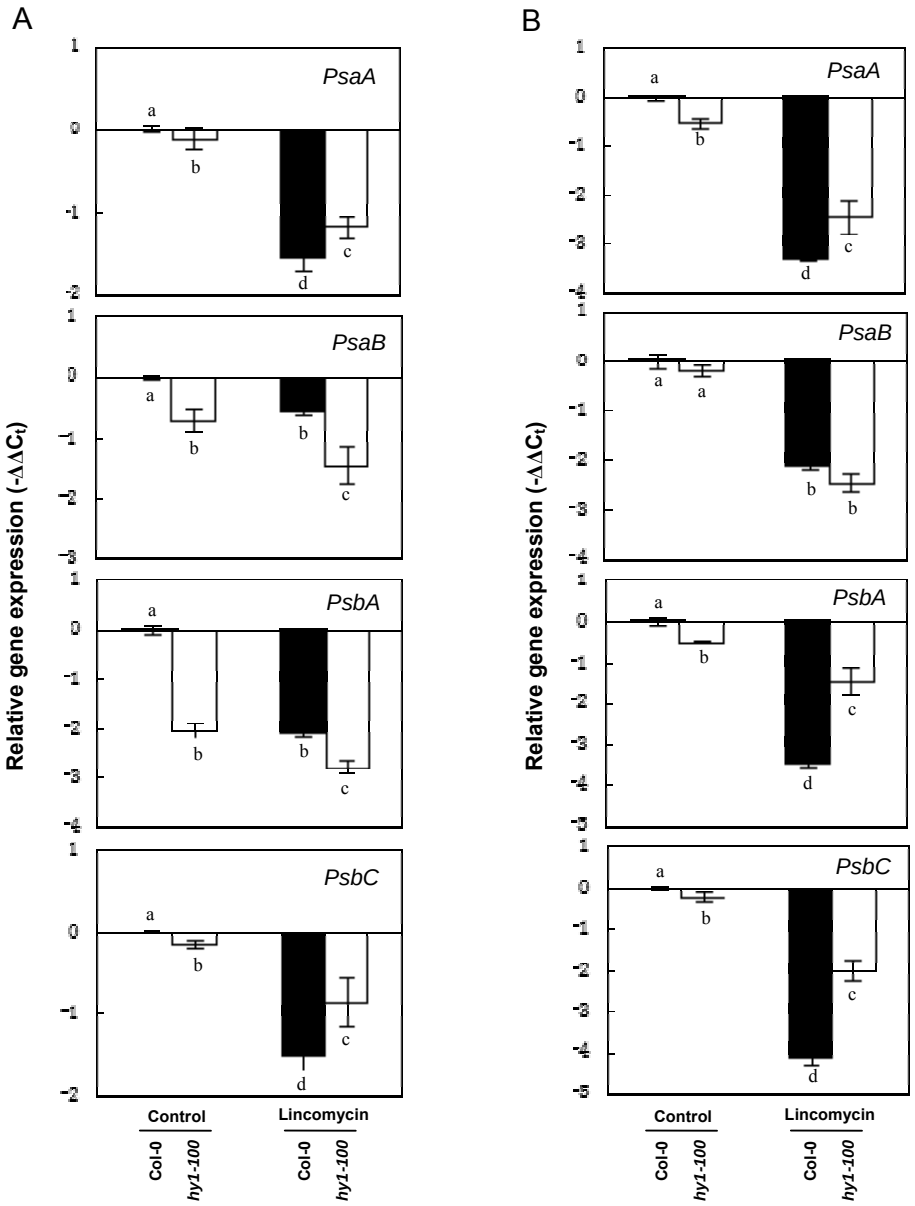
days (n = 50). C, Green & open cotyledon rate in wild-type (Col-0), *hy1-100* and *35S:HY1-3/1-4* seedlings with or without 400 mM mannitol for 5 days (n = 50). D, Mannitol-induced stomatal closure of the wild-type (Col-0), *hy1-100* and *35S:HY1-3* mutant plants (n = 50). ND, none detected. Data are means ± SE from at least three independent experiments.

Supplemental Figure S6



Supplemental Figure S6. ABA contents in wild-type and *hy1-100* mutant seedlings treated with or without norflurazon (NF) for 5 days. Seeds were sown on MS medium with or without norflurazon (5 μM), and collected 5 days later for ABA analysis. Seedlings without NF treatment were regarded as control (Con). Data are mean ± SE from at least three independent experiments.

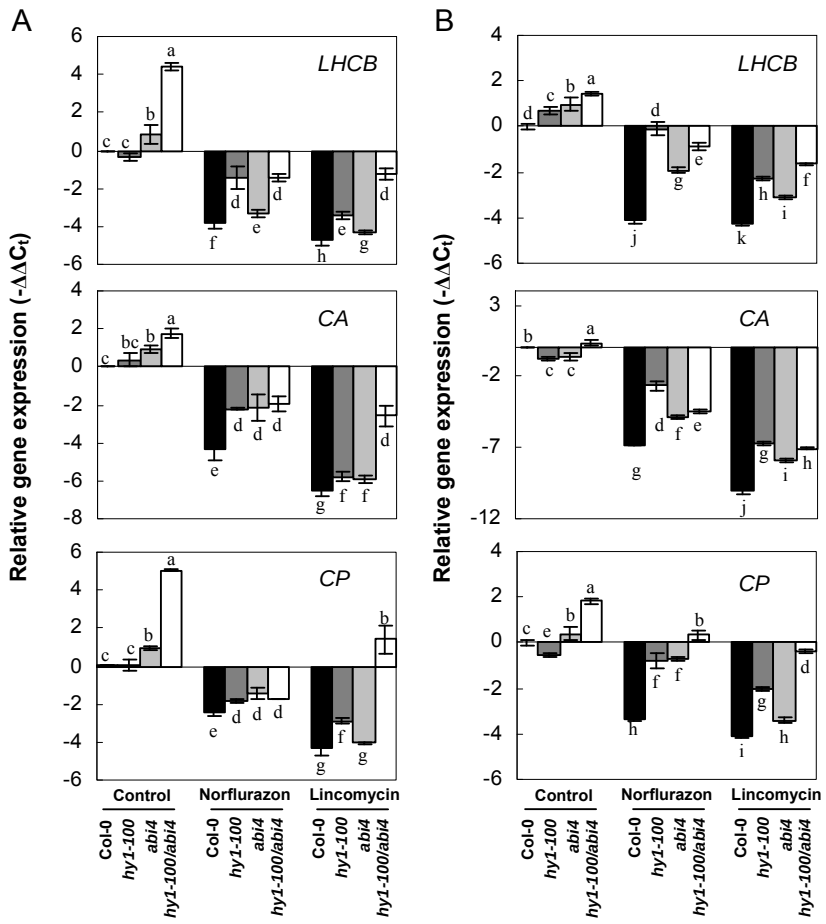
Supplemental Figure S7



Supplemental Figure S7. Impact of lincomycin treatments on gene expression in seedlings of wild-type (black bar) and *hy1-100* (white bar). Related gene included: *PSI P700 apoprotein A1* (*PsaA*, Atcg00350), *PSI P700 apoprotein A2* (*PsaB*, Atcg00340), *Photosystem II protein D1* (*PsbA*, Atcg00020), and *PSII 43 kDa protein* (*PsbC*, Atcg00280). A, Seeds were sown on MS medium with or without 500 μM lincomycin, and collected 5 d later for Q-PCR analysis. B, 5-day-old seedlings were

transferred to MS medium with or without 500 μM lincomycin, and collected 5 days later for Q-PCR analysis. Seeds or seedlings without chemical treatments were regarded as control. Expressions of selected genes are presented relative to corresponding wild-type control samples. Values are mean ± SE of at least three independent experiments. Differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test.

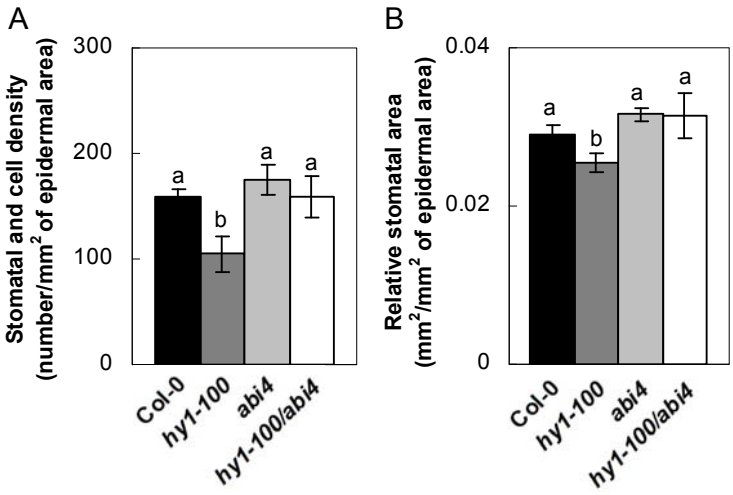
Supplemental Figure S8



Supplemental Figure S8. Impact of norflurazon and lincomycin treatments on *LHCB*, *CA*, and *CP* transcript levels in seedlings of wild-type, *hy1-100*, *abi4*, and *hy1-100/abi4*. A, Seeds were sown on MS medium with or without 5 μM norflurazon or 500 μM lincomycin, and collected 5 days later for Q-PCR analysis. B, 5-day-old seedlings were transferred to MS medium with or without 5 μM norflurazon or 500 μM lincomycin, and collected 5 days later for Q-PCR analysis. Seeds or seedlings without chemical treatments were regarded as control. Expressions of selected genes are presented relative to corresponding wild-type control samples. Values are mean ±

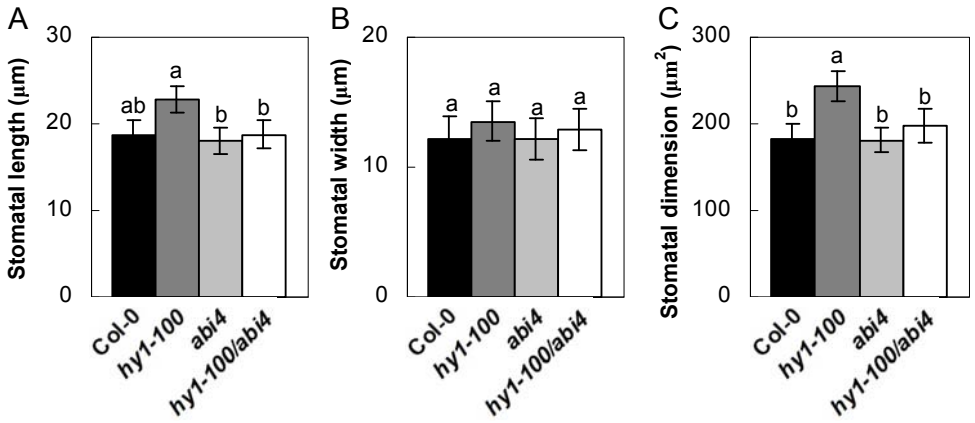
SE of at least three independent experiments. Differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test.

Supplemental Figure S9



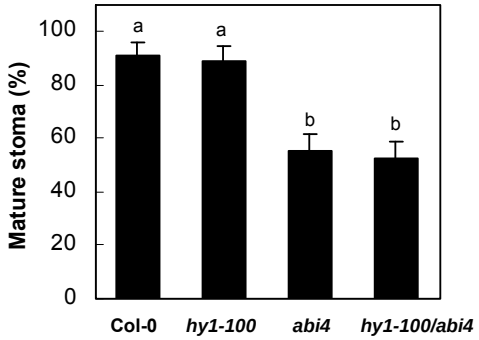
Supplemental Figure S9. Comparisons of stomatal and cell density (A) and relative stomatal area (B) in adaxial epiderm of the wild-type, *hy1-100*, *abi4*, and *hy1-100/abi4* mutants. 4-week-old plants were used (n = 50). Data are means ± SE from at least three independent experiments. Differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test (n =50).

Supplemental Figure S10



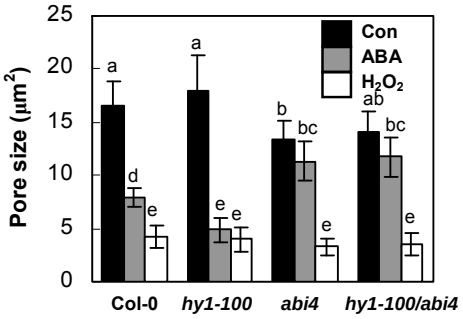
Supplemental Figure S10. Comparisons of stomatal length (A), stomatal width (B), and stomatal dimension (C) in adaxial epiderm of the wild-type, *hy1-100*, *abi4*, and *hy1-100/abi4* mutants. 4-week-old plants were used (n = 50). Data are means ± SE of 100 stoma from 10 plants. Differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test.

Supplemental Figure S11



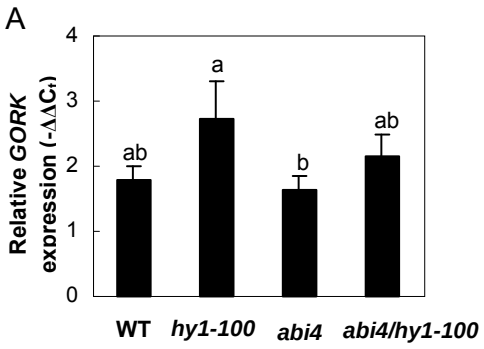
Supplemental Figure S11. Comparisons of percentage of mature stoma in leaves of the wild-type, *hy1-100*, *abi4*, and *hy1-100/abi4* mutants. 4-week-old plants were used. The ratio of ostiole length/stoma length higher than 1/3 was regarded as mature stoma. Data are means ± SE of 100 stoma from 10 plants. Differences were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test.

Supplemental Figure S12



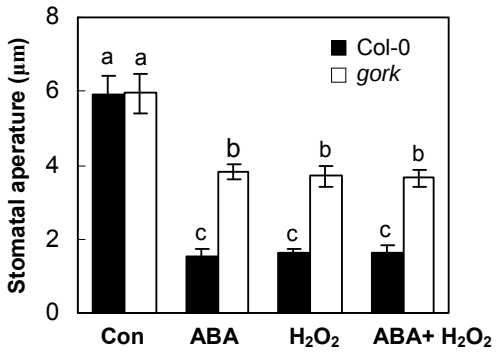
Supplemental Figure S12. Comparisons of stomal pore size in leaves of the wild-type, *hy1-100*, *abi4*, and *hy1-100/abi4* mutants. 4-week-old plants were used. Arabidopsis leaves of each ecotype were treated with ABA (10 μM) or H_2O_2 (100 μM) in MES-KCl buffer for 2 hr. Data are means ± SE of 100 stoma from 10 plants. Differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test.

Supplemental Figure S13.



Supplemental Figure S13. ABA-induced *GORK* gene expression in 4-week-old wild-type, *hy1-100*, *abi4* and *abi4/hy1-100* mutant leaves. Relative *GORK* gene expression were measured 1 hr after ABA treatment (100 μM), taking the expression level of each ecotype of the ABA-free control sample as 100%. Data are mean ± SE from at least three independent experiments. Differences among treatments were analyzed by one-way ANOVA, taking $P < 0.05$ level as significant according to Tukey's multiple range test.

Supplemental Figure S14



Supplemental Figure S14. Relative stomatal aperture of *gork* mutant plants in repsonses to ABA or H_2O_2. Epidermal fragments of wild-type (Col-0) or *gork* mutant plants were incubated in MES buffer in the presence of ABA (10 μM) or H_2O_2 (50 μM) alone or their combinations for 2 hr. The control (Con) means a treatment with MES-KCl buffer only. Data are presented as means ± SE of 30 guard cells. Bars with different letters are significantly different at $P < 0.05$ level according to Tukey's multiple comparison.

MATERIALS AND METHODS

Analysis of noncovalently bound heme content

Noncovalently bound heme was extracted as described (Weller et al., 1996). The heme concentration was measured by the heme ELISA kit according to the manufacturer's instructions (DongSongBo Industry Biotechnology Co., Ltd, Beijing, China).

LITERATURE CITED

Weller JL, Terry MJ, Rameau C, Reid JB, Kendrick RE (1996) The phytochrome-deficient *pcd1* mutant of pea is unable to convert heme to biliverdin IXα. Plant Cell 8: 55–67