Li et al., Supplemental Figure S1



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Mock treated

MG132 treated **Supplemental Figure S1.** Subcellular localization of the RHA2b and RHA2a.

A, ABA response of 35S:RHA2a-GFP and 35S:RHA2b-GFP transgenic Arabidopsis seedlings. Photographs of seedlings grew on medium containing 0 and 0.3 μ M ABA at 5 d after the end of stratification.

B, ABA response of 35S:RHA2a-GFP/rha2a and 35S:RHA2b-GFP/rha2b-1 seedlings. Photographs of seedlings grew on medium containing 0 and 0.5 µM ABA at 5 d after the end of stratification.

C, Photomicrographs of transgenic *Arabidopsis* roots harboring the 35S:RHA2b-GFP or 35S:RHA2a-GFP constructs. One-week-old plants treated (or mock treated) with 50 μ M MG132 for 6 h under dim light overnight were observed with fluorescence microscopy. Bars=50 μ m.



Supplemental Figure S2. Comparison of *RHA2b* and *RHA2a* expression.

A, ABA response of *pRHA2a:RHA2a-GUS/rha2a* and *pRHA2b:RHA2b-GUS/rha2b-1 Arabidopsis* seedlings. Photographs of seedlings grew on medium containing 0 and 0.5 μ M ABA at 5 d after the end of stratification.

B, Drought-induced *RHA2b* and *RHA2a* expression revealed by qRT-PCR. Two-week-old wild type seedlings were subjected to drought treatment for 0, 0.5, 1 and 3 h respectively. *ACTIN2* primers were used as an internal control. Data shown are mean \pm SD of three independent experiments.

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

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Supplemental Figure S3. Molecular analysis of *RHA2b/RHA2a* mutants.

A, Diagram of the *RHA2b* gene. The T-DNA inserted 168-bp upstream of the translation start site in Salk_014943 (*rha2b-1*).

B, Expression of *RHA2b* in *rha2b-1* revealed by qRT-PCR. *ACTIN2* primers were used as an internal control.

C, RT-PCR analysis showing reduced expression of *RHA2b* and no expression of *RHA2a* in the *rha2a rha2b-1* double mutant. *ACTIN2* primers were used as an internal control.

Asterisk in B indicate significance of the difference from the corresponding control values determined by Student's *t*-test (* $0.01 \leq P < 0.05$).

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Supplemental Figure S4. ABA responses of *RHA2b OE* 1# and *RHA2b OE* 9# plants in seed germination and postgerminative growth.

A, qRT-PCR analysis showing elevated expression of *RHA2b* in *RHA2b* overexpression transgenic lines. *ACTIN2* primers were used as an internal control.

B, qRT-PCR analysis showing elevated expression of *RHA2a* in *RHA2a* overexpression transgenic line 7. *ACTIN2* primers were used as an internal control.

C and D, Seed germination time course of *RHA2b OE* 1# and *RHA2b OE* 9# grown on medium without ABA (C) or containing 0.5 μ M ABA (D). Data shown are mean \pm SD of three replicates. At least 100 seeds per genotype were measured in each replicate.

E, Photographs of young seedlings at 5 d after the end of stratification. Seeds were germinated and allowed to grow on horizontal agar media containing 0 and 0.3 μ M ABA. The pictures were taken at 5 d after the end of stratification.

F, Cotyledon greening percentage of the seedlings described in (E). Values represent mean \pm SD of three replicates. At least 30 seedlings per genotype were measured in each replicate.

Asterisks in A, B and F indicate significance of the difference from the corresponding wild-type values determined by Student's *t*-test (** P < 0.01). At least three independent experiments were conducted and similar results were obtained.



Supplemental Figure S5. Expression of *ABI2* in the *abi2-2* (Salk_015166) mutant. RT-PCR analysis showing no expression of *ABI2* in *abi2-2* mutant. *ACTIN2* primers were used as an internal control.