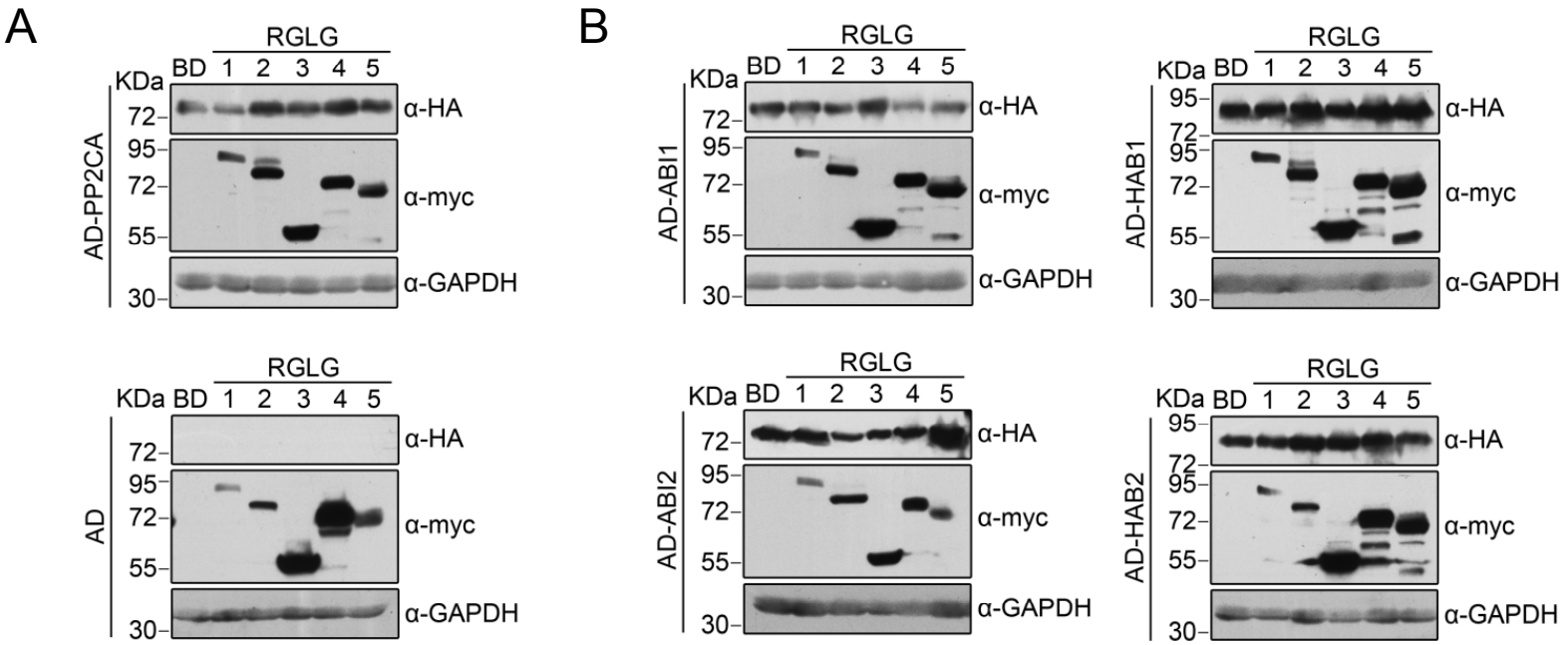
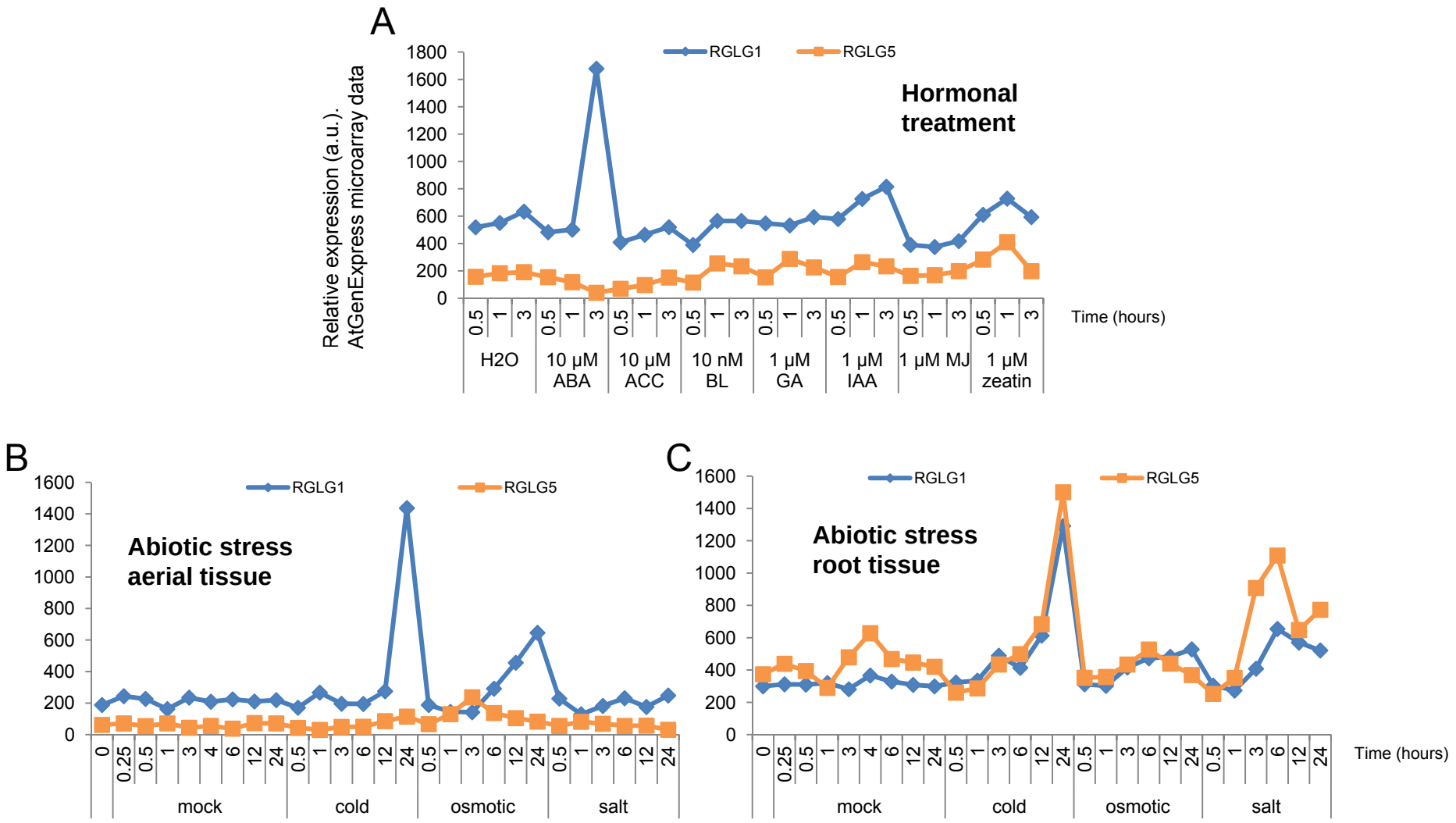


Supplemental Figure 1. Immunoblot analysis verifies the expression of the AD-PP2C and BD-RGLG proteins in the Y2H assay.

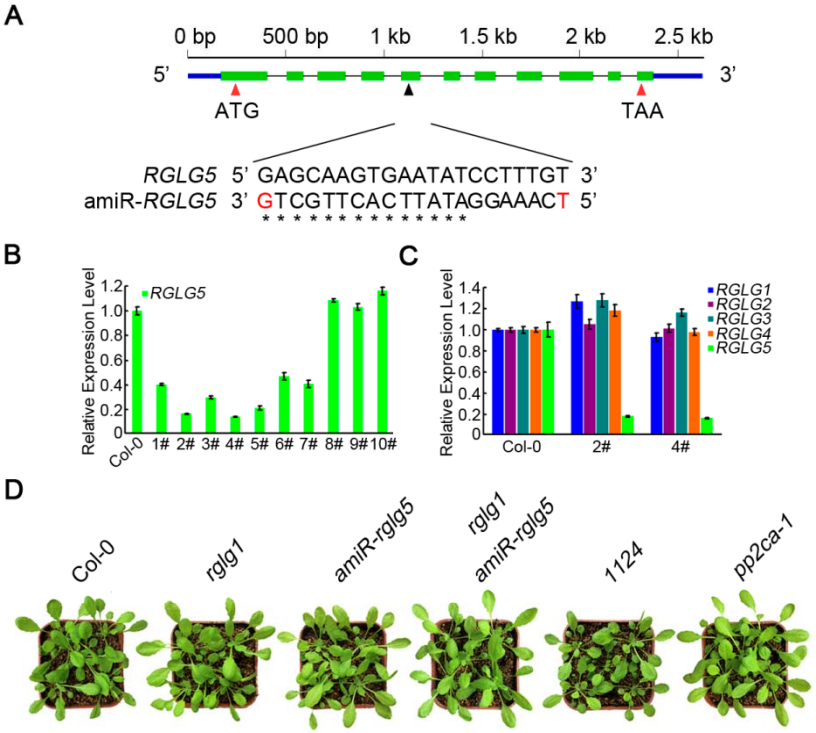
Total proteins were extracted from the yeast cells for immunoblot analysis. α-HA was used to detect AD-PP2Cs and α-myc was used to detect BD-RGLGs. GAPDH was used as a loading control. The RING domain of RGLG3 was deleted to avoid autoactivation and accordingly, its molecular mass is lower than the rest of RGLGs.



Supplemental Figure 2. Relative expression of *RGLG1* and *RGLG5* in seedlings.

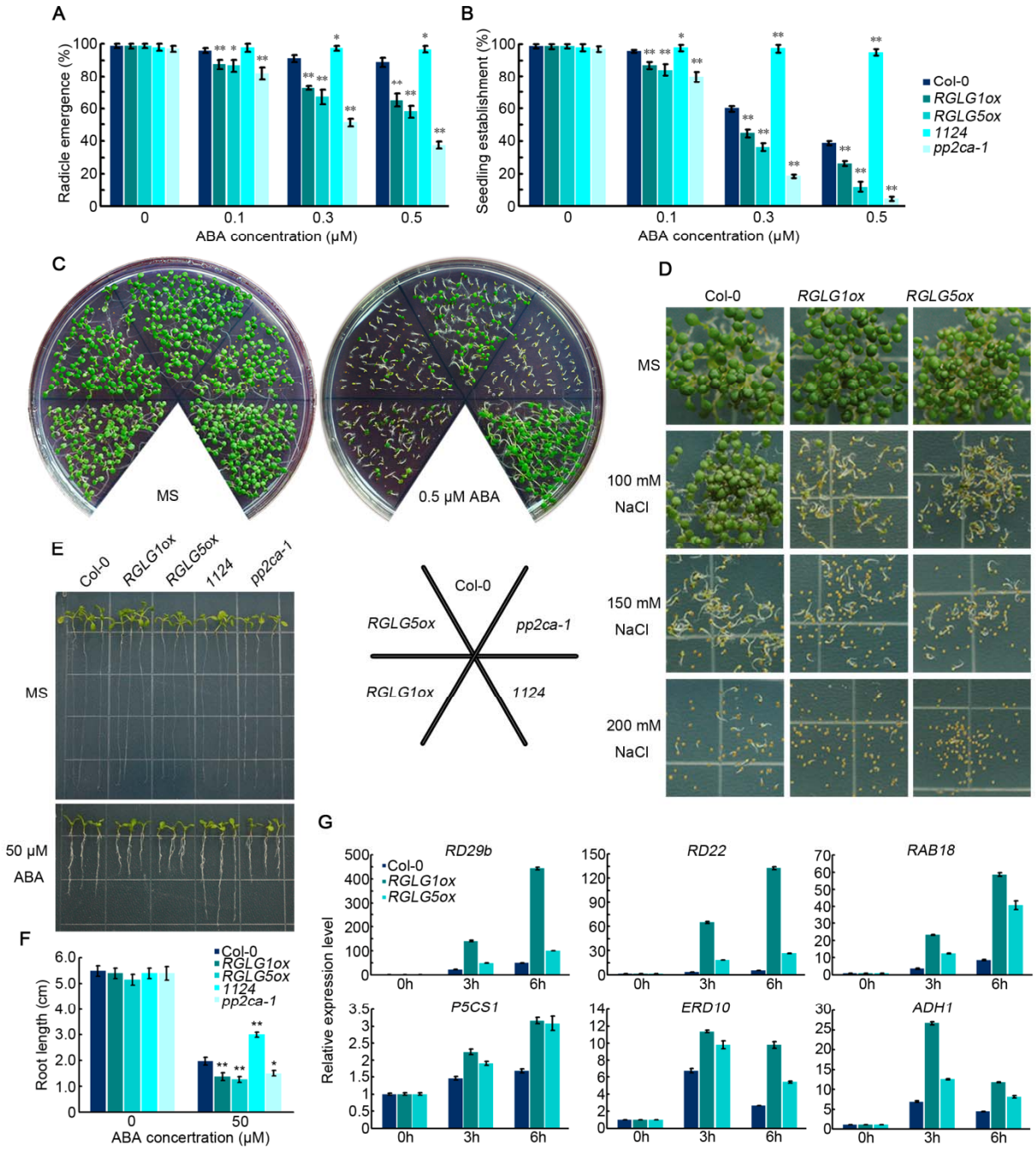
Seedlings were treated with different hormones **(A)** or submitted to cold (4ºC), osmotic (300 mM mannitol) or salt (150 mM NaCl) stress **(B, C)**. Expression data were obtained from Affymetrix microarrays for Arabidopsis deposited in AtGenExpress public database (Goda et al., 2008) and visualized using the AtGenExpress Visualization Tool located in http://weigelworld.org/resources.html.

Goda,H., et al., (2008). The AtGenExpress hormone and chemical treatment data set: experimental design, data evaluation, model data analysis and data access. *Plant J.* **55** , 526-542.



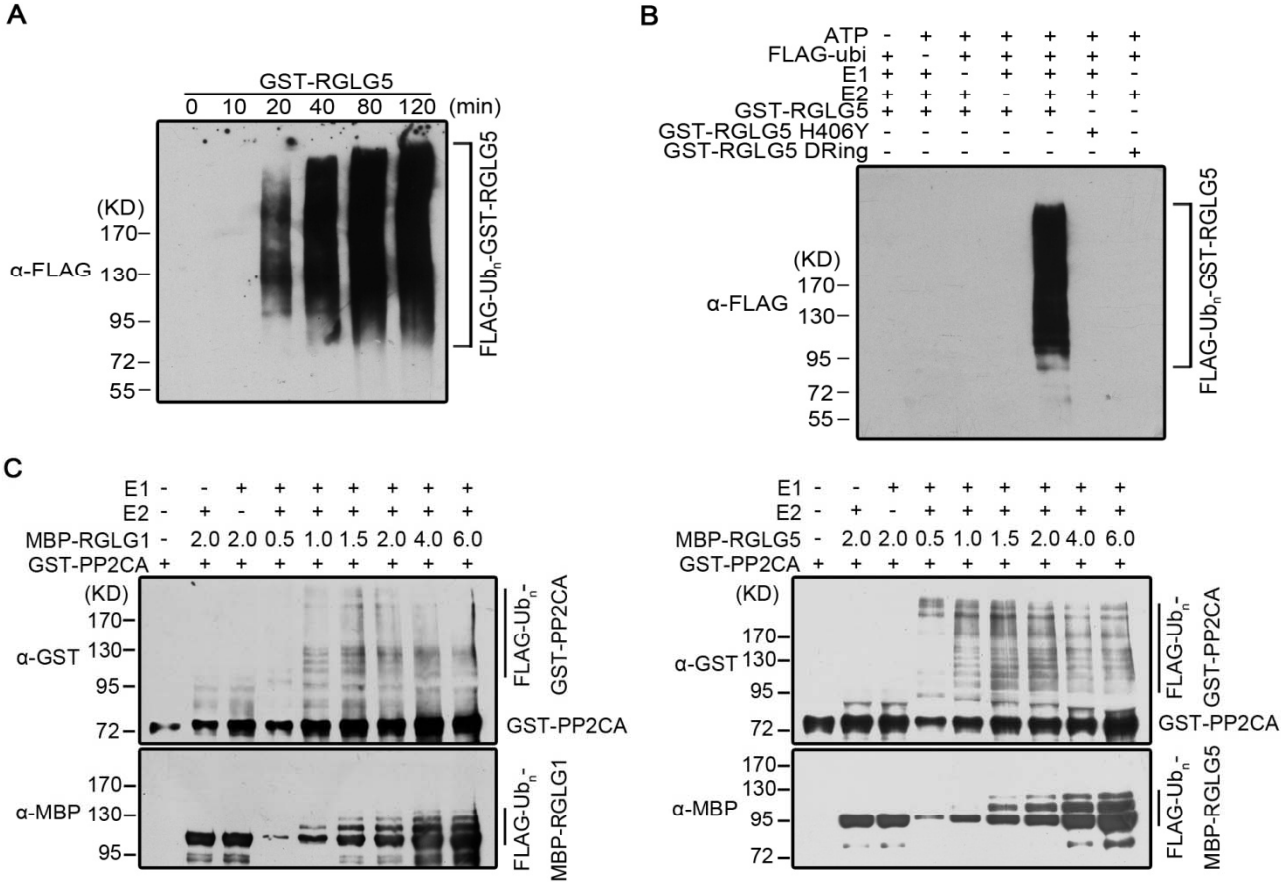
Supplemental Figure 3. *RGLG5* transcript levels were reduced in amiRNA transgenic plants compared to wild type.

(A) Diagram showing location and target sequence of the designed amiRNA in *RGLG5*.
(B) Decreased *RGLG5* transcript level in amiRNA transgenic lines compared to the wild type detected. Four-week-old *Arabidopsis* leaves were used for RNA extraction. Gene expression was examined using RT-qPCR. *UBQ10* was used as the internal control, and expression levels were normalized to that examined in Col-0. Data represent the mean ± SD of four technical replicas.
(C) Relative expression levels of five *RGLG* genes in selected *RGLG5* amiRNA transgenic lines. No significant effect on other *RGLGs* expression indicates specific downregulation of *RGLG5* in the checked amiRNA transgenic lines, and line 4 (4#) was chosen for use in this work. Four-week-old *Arabidopsis* leaves were used for RNA extraction. Gene expression was examined using RT-qPCR. *UBQ10* was used as the internal control, and expression levels were normalized to that examined in Col-0. Data represent the mean ± SD of four technical replicas.
(D) Photographs of 4-week-old plants under short-day conditions used in the phenotype analysis.


A
Radicle emergence (%)
ABA concentration (μM)
B
Seedling establishment (%)
ABA concentration (μM)
Col-0
RGLG1ox
RGLG5ox
1124
pp2ca-1
C
MS
0.5 μM ABA
D
Col-0
RGLG1ox
RGLG5ox
MS
100 mM NaCl
150 mM NaCl
200 mM NaCl
E
Col-0
RGLG1ox
RGLG5ox
1124
pp2ca-1
MS
50 μM ABA
F
Root length (cm)
ABA concertration (μM)
G
RD29b
RD22
RAB18
P5CS1
ERD10
ADH1
Relative expression level
0h
3h
6h

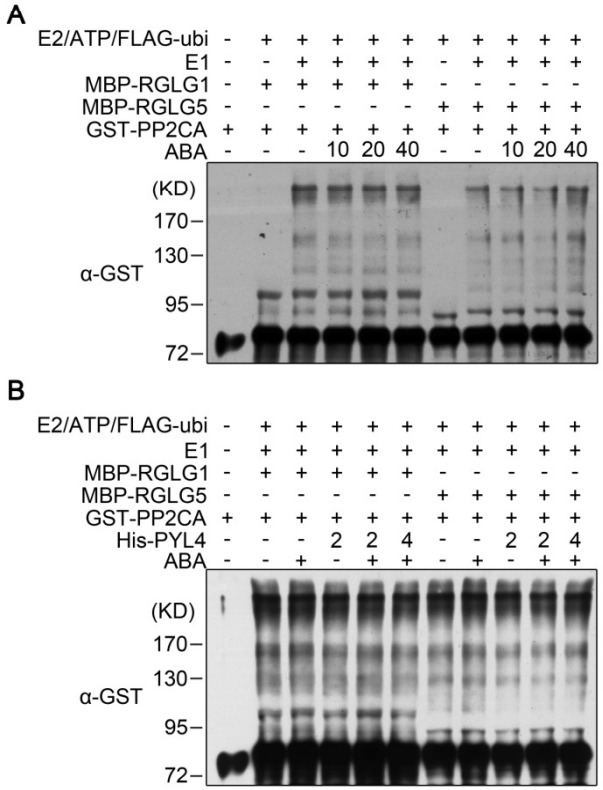
Supplemental Figure 4. ABA hypersensitivity in *Arabidopsis* transgenic lines overexpressing (OX) either *RGLG1* or *RGLG5*.

(A) Germination (radicle emergence) of wild type (Col-0), transgenic plants overexpressing *RGLG1* or *RGLG5*, *1124* and *pp2ca-1* mutants after 72-h growth on MS plates containing the indicated concentrations of ABA. Bars are SD of three replications. Asterisks indicate a significant difference from wild type (Student's t-test: *, $P < 0.05$; **, $P < 0.01$).
(B) Percentage of seeds that developed fully green expanded cotyledons (seedling establishment) after 7-day growth on MS plates containing the indicated concentrations of ABA. Bars are SD of three replications. Asterisks indicate a significant difference from wild type (Student's t-test: *, $P < 0.05$; **, $P < 0.01$).
(C) Photographs of representative seedlings taken 7 days after sowing on MS plates with or without 0.5 μM ABA.
(D) Enhanced sensitivity to salt-mediated inhibition of germination and seedling establishment. Germination and postgermination growth of *Arabidopsis* transgenic lines overexpressing *RGLG1* or *RGLG5* compared to Col-0 wt. Seeds were sown on MS plates containing the indicated concentrations of NaCl and photographs were taken after 7 days.
(E) Enhanced ABA-mediated inhibition of primary root growth in *RGLG1* or *RGLG5* OX seedlings compared to the wild type. Photographs were taken 10 days after transferring 5-day-old seedlings to MS plates supplemented without or with 50 μM ABA.
(F) Root length quantification of previous experiment. Bars indicate mean ± SD of thirty seedlings. Asterisks indicate a significant difference from wild type (Student's t-test: *, $P < 0.05$; **, $P < 0.01$).
(G) Enhanced induction of ABA-responsive genes is observed in *Arabidopsis* transgenic lines overexpressing either *RGLG1* or *RGLG5*. 7-day-old *Arabidopsis* seedlings were transferred to liquid MS medium containing 50 μM ABA and sampled at the indicated time points. Gene expression was examined using RT-qPCR. *UBQ10* was used as the internal control, and expression levels were normalized to that examined at time point 0 h. Data represent the mean ± SD of four technical replicates.

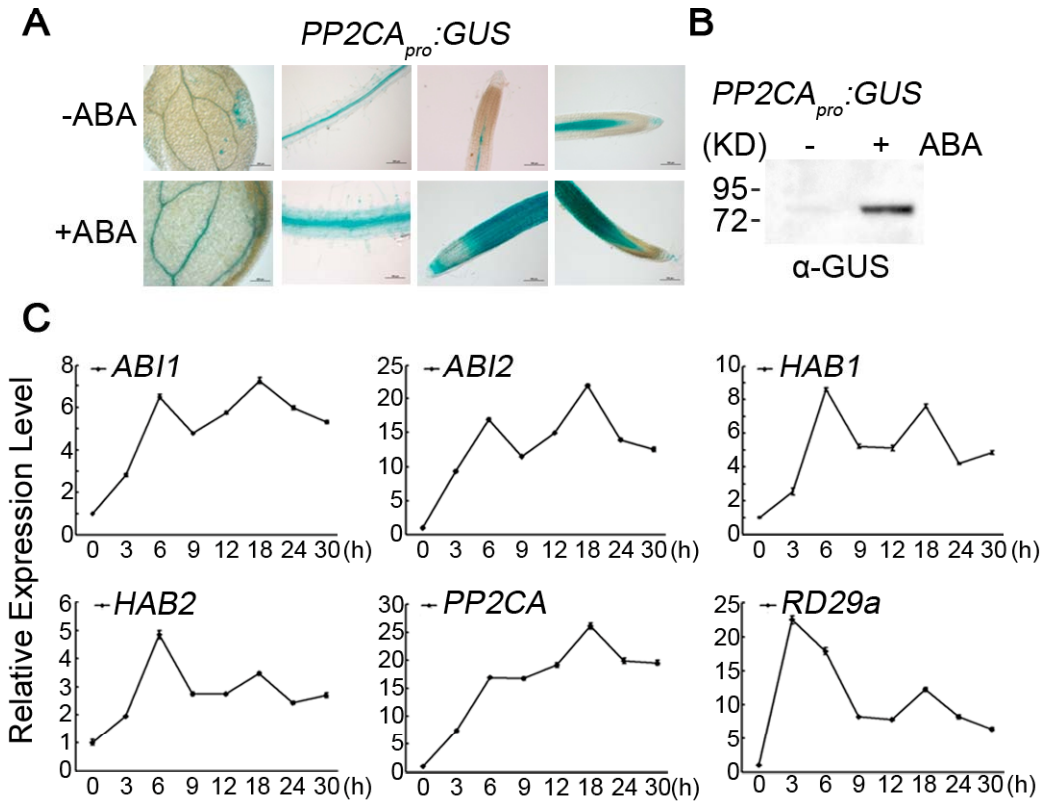


Supplemental Figure 5. E3 ligase activity of RGLG5 and *in vitro* ubiquitination of PP2CA by RGLG1 and RGLG5.

(A) Self-ubiquitination of GST-RGLG5 indicating ubiquitin ligase activity of RGLG5. The ubiquitination assay was performed in the presence of E1, E2**,** ATP and FLAG-ubiquitin, and sampled at the indicated time points. Ubiquitinated bands were detected using FLAG antibody.
(B) Loss of ubiquitination activities of GST-RGLG5 by deleting or mutating its RING-domain. Ubiquitination assay was carried out as in **(A)**. FLAG antibody was used to detect the ubiquitinated bands.
(C) *In vitro* ubiquitination of PP2CA by RGLG1 (left) and RGLG5 (right). Purified GST-PP2CA was incubated with ATP, FLAG-Ubiquitin, E1, E2 and serial concentrations of MBP-RGLG1 or RGLG5. The ubiquitinated products were examined by anti-GST or anti-MBP antibodies. IB using anti-MBP detects self-ubiquitination of MBP-RGLG1 and MBP-RGLG5.

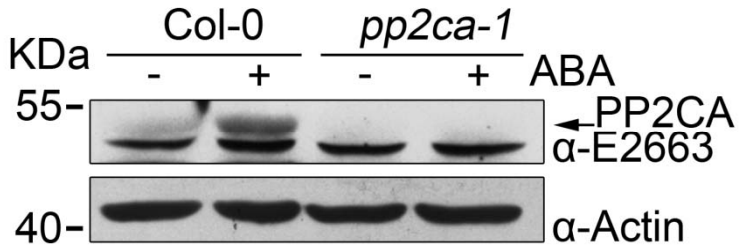


Supplemental Figure 6. *In vitro* ubiquitination of PP2CA by RGLG1 and RGLG5 is not affected by ABA and PYL4.
(A) *In vitro* ubiquitination of PP2CA in the presence of serial concentrations of ABA. Ubiquitination assay was performed as described in **Figure 5A**. 10, 20 and 40 μM ABA was added together with other reaction components, respectively. The ubiquitination of PP2CA was analyzed by western blot using anti-GST antibody.
(B) *In vitro* ubiquitination of PP2CA in the presence of ABA, PYL4 or both. The ubiquitination level of PP2CA was not influenced by addition of 10 μM ABA, PYL4 or ABA plus serial concentrations of PYL4, respectively.



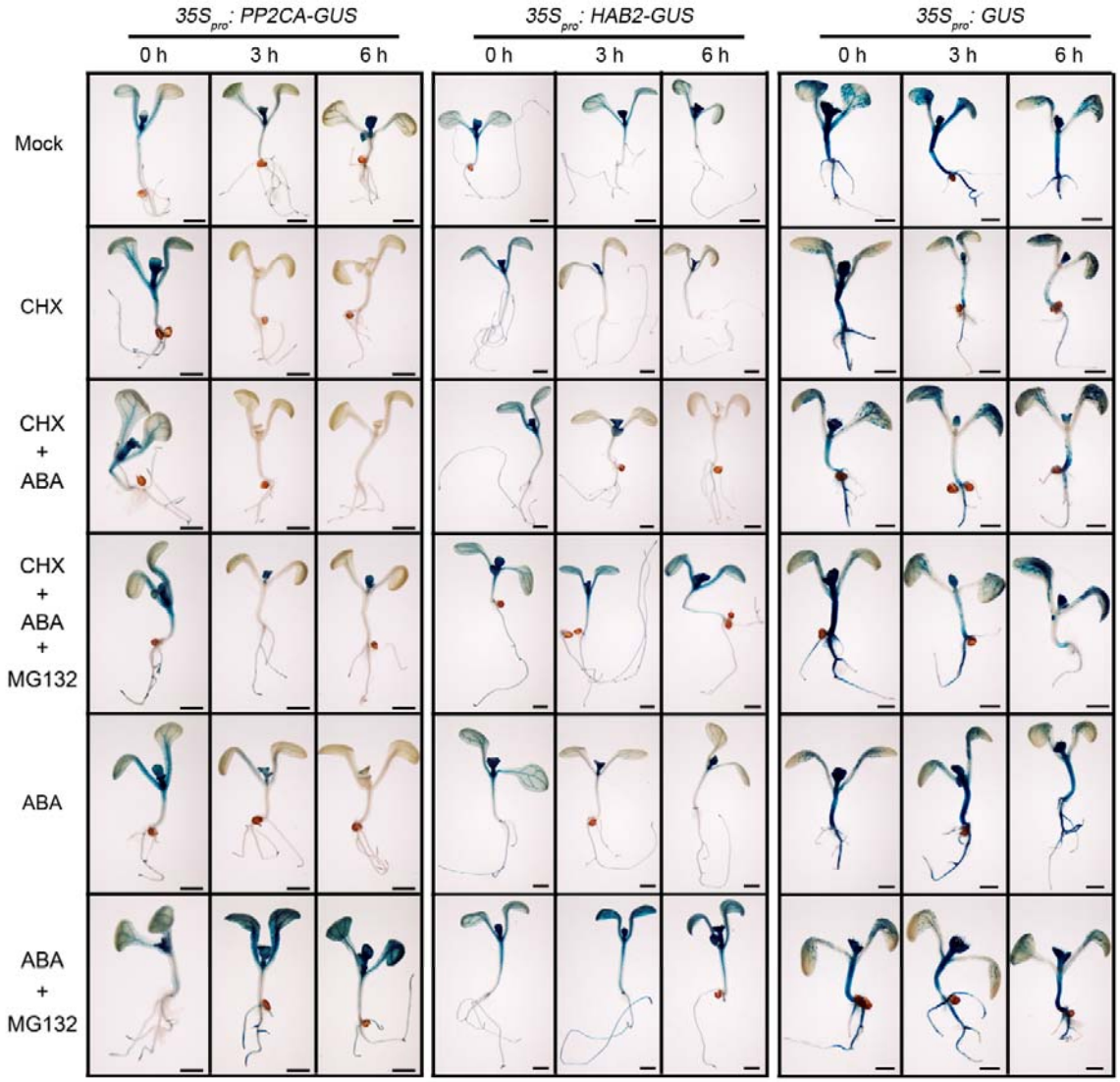
Supplemental Figure 7. Expression of the *PP2CA* promoter is strongly induced by ABA.

(A) Photographs showing GUS expression driven by $PP2CA_{pro}$ in roots and cotyledons of 5-d-old seedlings. ABA treatment (10 μM for 3 h) strongly induces expression of GUS.
(B) Immunoblot analysis using anti-GUS antibody was performed to quantify GUS protein levels.
(C) Transcriptional profiles of *PP2C* genes in response to ABA. Seven-day-old *Arabidopsis* seedlings were transferred in liquid MS medium containing 50 μM ABA and sampled at the indicated time points. Gene expression was examined using RT-qPCR. ABA-responsive *RD29a* expression indicated the effectiveness of ABA treatment. *UBQ10* was used as the internal control, and expression levels of the indicated transcripts were normalized to those measured at time point 0. Data represent the mean ± SD of four technical replicates.



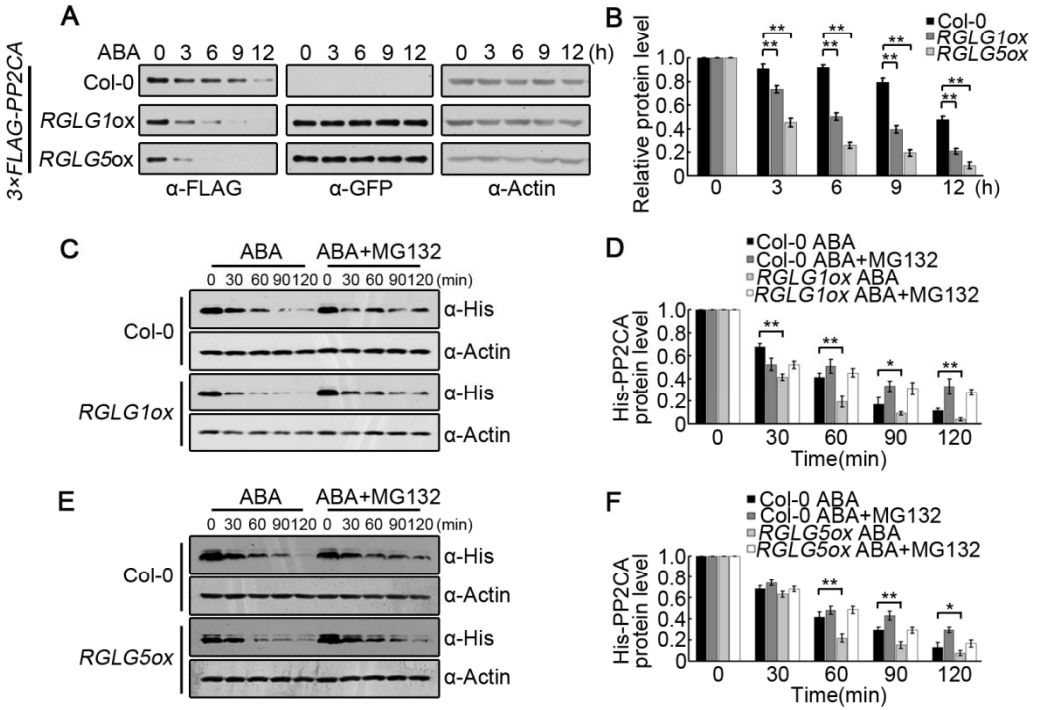
Supplemental Figure 8. α-E2663 is a specific antibody for PP2CA.

2-week-old Col-0 and *pp2ca-1* etiolated seedlings were treated without (-) or with (+) 50 μM ABA for 6 h in darkness. Then total proteins were extracted and equal amounts of each treatment were subjected to western blot analysis. α-E2663 was used to detect endogenous PP2CA with a dilution of 1:3500. α-Actin was analyzed as a loading control. Endogenous PP2CA (arrow) was detected in Col-0 and it was markedly induced by ABA treatment, whereas it was absent in *pp2ca-1* mutant (-/+ABA).



Supplemental Figure 9. GUS staining of seedlings expressing PP2CA-GUS or HAB2-GUS reveals ABA-induced degradation of the phosphatases via the 26S proteasome pathway.

Representative photographs of transgenic *Arabidopsis* seedlings constitutively expressing PP2CA-GUS, HAB2-GUS or GUS (negative control). For drug treatment, 7-day-old seedlings were transferred to liquid MS medium supplemented without (Mock) or with 100 μM CHX, 50 μM ABA, or the indicated combinations lacking or containing 50 μM MG132. Samples were taken at the indicated time points after treatment and subjected to GUS staining. Bars = 1 mm.



Supplemental Figure 10. Overexpression of *RGLG1* or *RGLG5* enhances ABA-promoted degradation of FLAG-PP2CA. ABA-promoted degradation of His-PP2CA in cell-free assays is enhanced in protein extracts prepared from RGLG1 or RGLG5 overexpressing plants.

(A) FLAG-PP2CA protein dynamics in Col-0 versus *RGLG1* or *RGLG5* overexpressing (ox) backgrounds in response to ABA. Assays were performed as described in **Figure 7C**.
(B) The signal intensity in **(A)** was measured by Image J software. The relative protein levels of FLAG-PP2CA in different genetic backgrounds at time point 0 h were set as "1". Bars indicate the mean ± SD from three replications. Student's t tests were used to determine significant levels of the indicated comparisons. **, $P < 0.01$; *, $P < 0.05$.
(C) *In vitro* cell-free degradation of PP2CA in Col-0 versus *RGLG1ox* extracts. Purified His-PP2CA was incubated for the indicated time period in the presence of ABA or ABA+MG132 with protein extracts prepared from 7-day-old wild type (Col-0) or *RGLG1ox* seedlings. Actin was analyzed as a loading control.
(D) Three-independent experiments as in **(C)** were performed and the signal intensity was determined by Image J software. The protein levels of His-PP2CA at 0 min were defined as 1. Bars indicate SD calculated from three replicas.
(E) *In vitro* cell-free degradation of PP2CA in Col-0 versus *RGLG5ox* extracts. Assays were performed as in **(C)**.
(F) Three-independent experiments as in **(E)** were performed and the signal intensity was determined by Image J software. Bars indicate SD calculated from three replicas.

Treatment	Protein name	Peptides identified by MS	Peptide score/ p
Mock	PP2CA	VLGVLAMSR	45.56/0.00017
		VLGVLAMSR	44.05/0.00024
		VLGVLAMSR	40.57/0.00053
		VIYWDGAR	34.86/0.0027
		RLDLLPSIK	27.61/0.0049
		QSSDNVSVVVVDLR	29.04/0.012
		QSSDNVSVVVVDLR	26.91/0.02
		QSSDNVSVVVVDLR	52.89/5.80E-05
ABA + MG132	PP2CA	VLGVLAMSR	53.72/2.60E-05
		VLGVLAMSR	52.81/3.20E-05
		VLGVLAMSR	40.73/0.00081
		VIYWDGAR	31.8/0.0055
		RLDLLPSIK	30.99/0.0022
		RLDLLPSIK	33.59/0.0012
		ECNLVVNGATR	48.42/8.80E-05
		IVADSAVAPPLENCR	56.49/2.30E-05
		QSSDNVSVVVVDLRK	23.53/0.035
		DMEDAVSIHPSFLQR	44.75/0.00015
		DMEDAVSIHPSFLQR	27.49/0.0089
		MDKEVSQRECNLVVNGATR	26.01/0.017
	RGLG1	GAGAGDDSDAAHNACSDAALLLTK	37.14/0.00079
		GAGAGDDSDAAHNACSDAALLLTK	69.76/4.90E-07
		IPNFEPSVPPYPFESK	24.87/0.026
		IPNFEPSVPPYPFESK	37.65/0.0014
		IPNFEPSVPPYPFESK	22.6/0.042
		IPNFEPSVPPYPFESK	36.16/0.0019
		ISDNYSSLLQVSEALGR	57.76/1.40E-05

Supplemental Table 1. List of peptides identified for PP2CA and RGLG1 in anti-FLAG immunoprecipitates obtained from *$35S_{pro}$:3×FLAG-PP2CA Arabidopsis* seedlings mock-treated or treated with ABA and MG132. For mock treatment, two-week-old seedlings were transferred to liquid MS medium for 30 h. For MG and ABA treatment, 50 μM MG132 was added for 24 h and then 50 μM ABA was added for additional 6 h.