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Supplementary Materials for

QUIRKY controls seed germination via precision degradation of ABI5

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The PDF file includes:

Figs. S1 to S12
Legends for tables S1 to S7

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S7

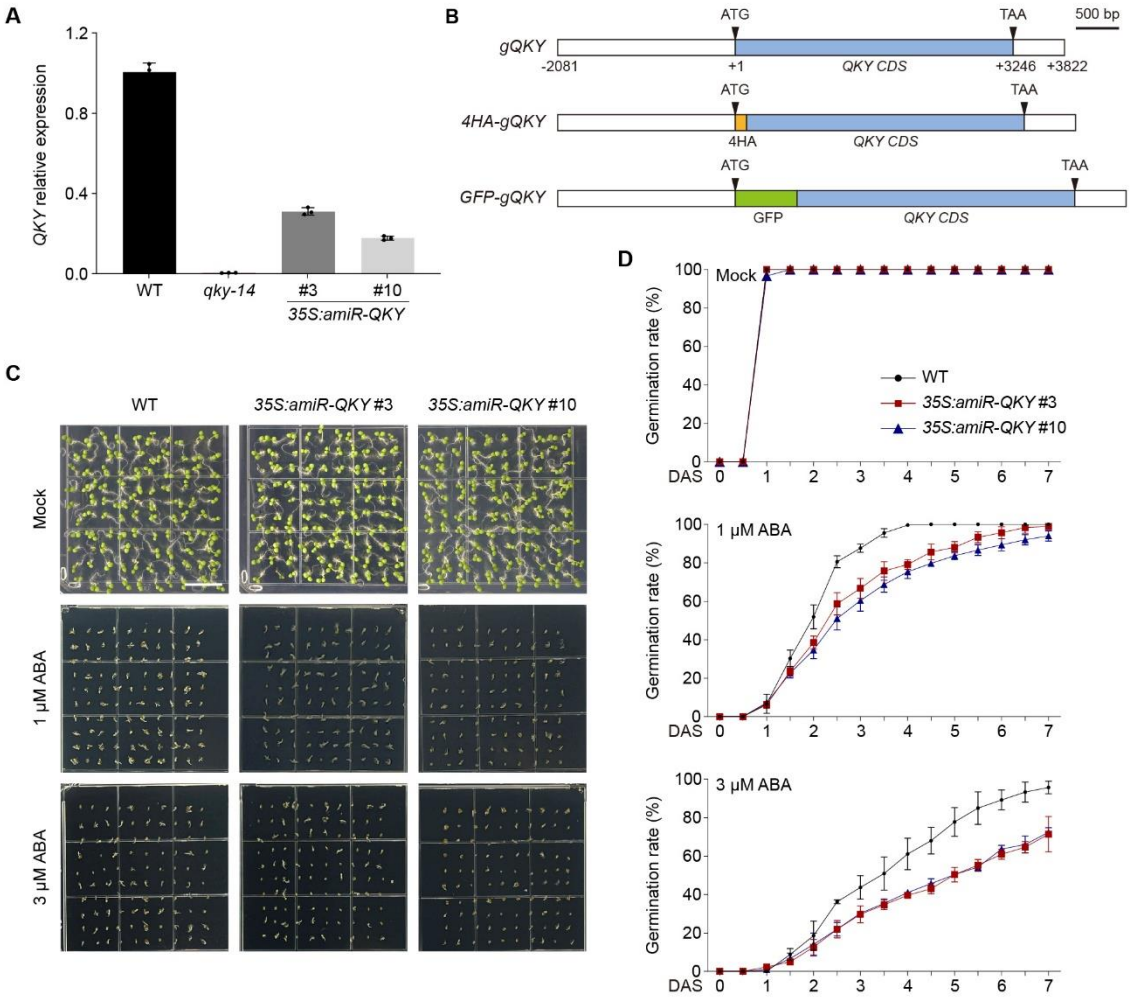

Fig. S1. *QKY* knockdown lines exhibit hypersensitivity to ABA during seed germination. (**A**) *QKY* expression was determined by quantitative real-time PCR in germinating seeds of wild-type (WT), *qky-14*, and *35S::amiR-QKY* lines 24 hours after stratification. Values are given as mean ± SD (n = 3). The expression level in WT was normalized to 1. (**B**) Schematic diagram of the *gQKY*, *4HA-gQKY*, and *GFP-gQKY* constructs. (**C**) A representative example of WT, *qky-14*, and *35S::amiR-QKY* lines 3 days after stratification on 1/2 MS plates containing 0, 1, 3 μM ABA. Scale bar, 1cm. (**D**) Germination rate of WT, *qky-14*, and *35S::amiR-QKY* lines treated with different concentrations of ABA (0, 1, 3 μM). Values are given as mean ± SD (n = 3), with approximately 100 seeds analyzed per replicate. DAS, days after stratification.

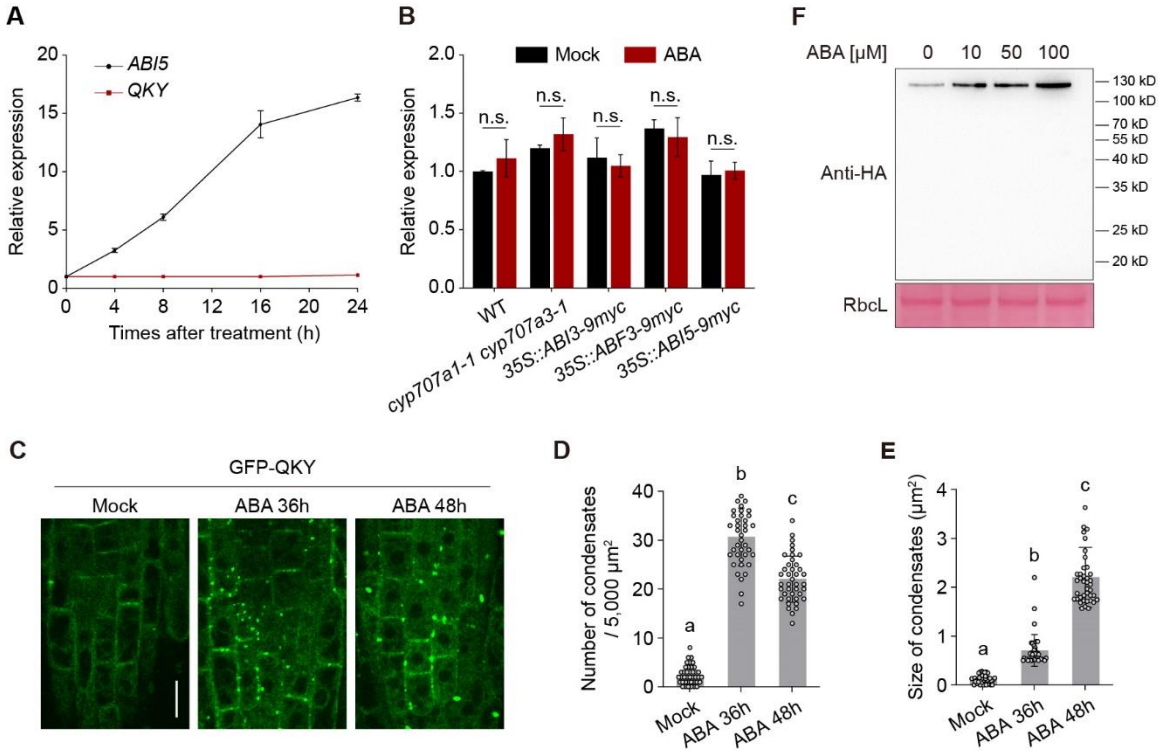

Fig. S2. The expression of *QKY* is not regulated by ABA. (**A**) Quantitative real-time PCR analysis of *ABI5* and *QKY* expressions in 2-day-old wild-type (WT) seedlings treated with 50 μM ABA for the indicated times. The expression levels at the starting point were normalized to 1. Error bars represent SD. (**B**) Quantitative real-time PCR analysis of *QKY* expression in germination seeds 24 hours after stratification of WT, *cyp707a1-1 cyp707a3-1*, *35S::ABI3-9myc*, *35S::ABF3-9myc*, and *35S::ABI5-9myc* lines with or without ABA treatment. The expression level in WT without ABA treatment was normalized to 1. Values are given as mean ± SD ($n = 3$). n.s. indicates no significant difference (two-tailed paired Student's *t*-test, n.s., $p > 0.05$). (**C**) Confocal images show GFP-QKY localization in root tip cells of *GFP-gQKY qky-14* transgenic lines two days poststratification with or without 3 μM ABA treatment for the indicated hours. GFP, green fluorescence protein. Scale bar, 10 μm. (**D** and **E**) Quantification of GFP-QKY condensate number (D) and size (E) shown in (C). For (D), each data point represents one image field (5000 μm²), with three or four fields analyzed per root from 10 independent plants. For (E), each data point signifies the condensate size calculated from at least 40 epidermal cells derived from 10 independent plants. Each data point represents one image field (5000 μm²) from the root tips of 10 independent plants, with four fields analyzed per root. Columns labeled with different letters represent statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's HSD test. Error bars represent SD. (**F**) Western blot analysis of 4HA-QKY protein abundance in *4HA-gQKY qky-14* seedlings two days poststratification treated with the indicated concentration of ABA for 4 hours (full gel photo of Figure 2F). Ponceau S staining (RbcL) was used as a loading control.

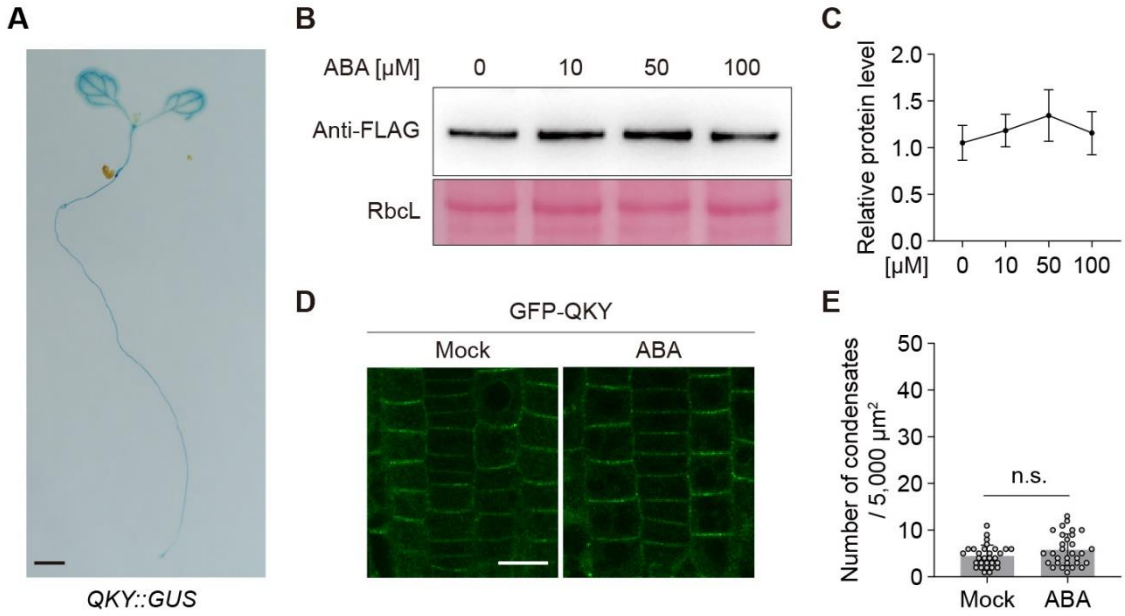

Fig. S3. QKY protein abundance is ABA-insensitive during later seedling development. (**A**) Representative histochemical GUS staining of *QKY::GUS* transgenic plants displays *QKY* expression in seedlings 7 days after germination. Scale bar, 1 mm. (**B**) Western blot analysis of 3FLAG-QKY abundance treated with the indicated concentration of ABA for 4 hours in 7-day-old *35S::3FLAG-QKY* seedlings. Ponceau S staining (RbcL) was used as a loading control. (**C**) Quantification of the relative 3FLAG-QKY protein abundance in (B). Values are given as mean ± SD (n = 3). The protein level of 3FLAG-QKY without ABA treatment is normalized to 1. (**D**) Confocal images show GFP-QKY localization in root tip cells of 7-day-old *GFP-gQKY qky-14* transgenic lines with or without 50 μM ABA treatment for 4 hours. GFP, green fluorescence protein. Scale bar, 5 μm. (**E**) Quantification of GFP-QKY condensate number shown in (D). Each data point represents one image field (5000 μm²) from the root tips of 10 independent plants, with three fields analyzed per root. Error bars represent SD. n.s. indicates no significant difference (two-tailed paired Student's t-test, n.s., $p > 0.05$).

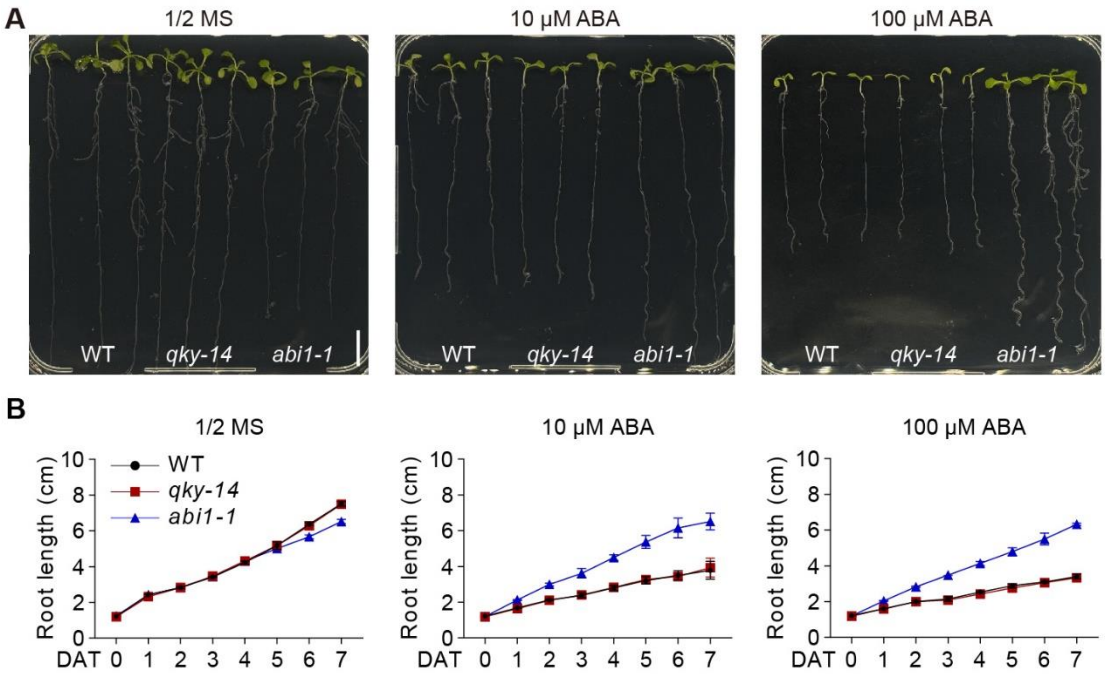

Fig. S4. The *qky-14* mutant does not show altered ABA sensitivity at the late seedling growth stage. (**A**) A representative example of WT, *qky-14*, and *abi1-1* seedlings treated with the indicated concentration of ABA (0, 10, 100 μM). 4-day-old seedlings grown in 1/2 MS plates were transferred to 1/2 MS plates containing different concentrations of ABA for 7 days. Scale bar, 1 cm. (**B**) Quantification of root length of WT, *qky-14*, and *abi1-1* seedlings after transferring to 1/2 MS plates containing different concentrations of ABA. DAT, days after transfer. At least 10 individual roots were analyzed per treatment. Values are given as mean ± SD.

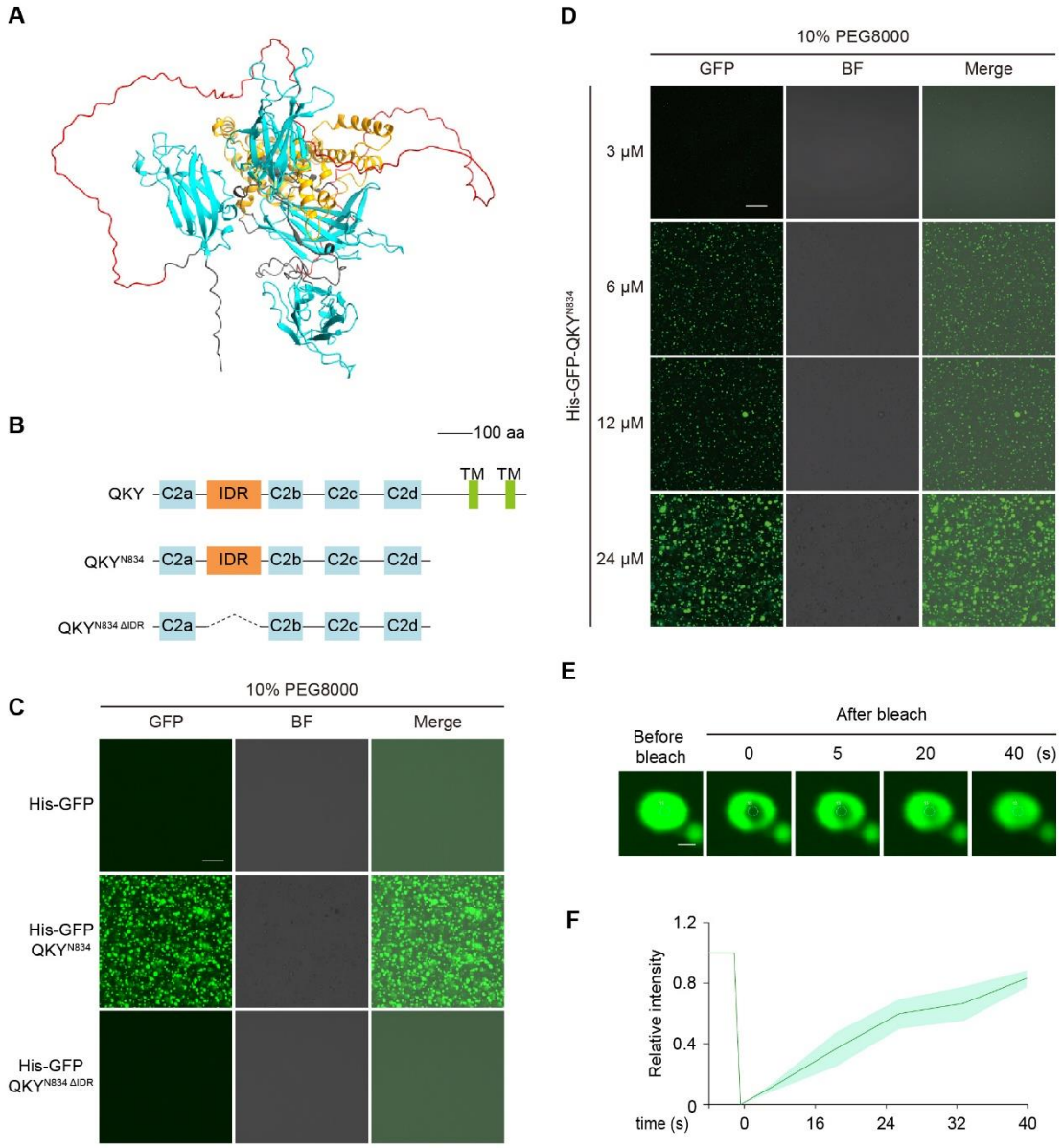

Fig. S5. QKY undergoes phase separation *in vitro*. (**A**) The predicted protein structure of QKY generated by AlphaFold3. The C2 domains are highlighted in blue, the intrinsically disordered regions (IDRs) are shown in red, and the transmembrane regions are indicated in yellow. (**B**) Schematic diagrams of QKY and its various derivatives. C2 domains, transmembrane region (TM), and IDRs are shown as blue, green, and orange boxes, respectively. (**C**) *In vitro* phase separation of His-GFP, His-GFP-QKY^{N834}, His-GFP-QKY$^{N834\ \Delta IDR}$ in the presence of PEG-8000. Scale bar, 20 μm. (**D**) *In vitro* phase separation of His-GFP-QKY^{N834} at the indicated concentrations in the presence of PEG-8000. Scale bar, 20 μm. (**E**) Fluorescence recovery after photobleaching (FRAP) of a GFP-QKY^{N834} droplet *in vitro*. The white circle indicates the bleached areas. Scale bar, 1.5 μm. (**F**) FRAP recovery plot of the subregion of the GFP-QKY^{N834} droplet shown in (E). Error bars represent SD.

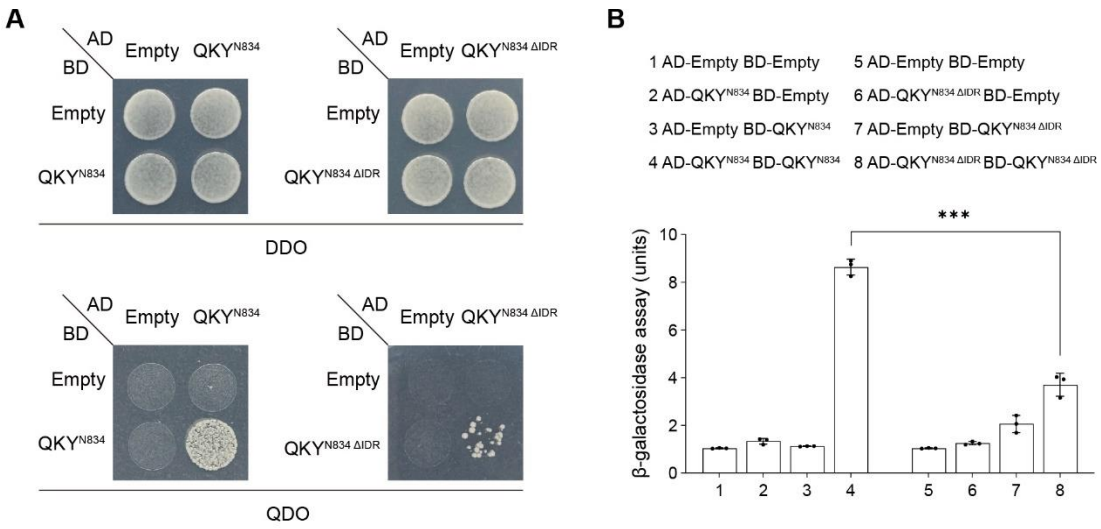

Fig. S6. The IDR contributes to the self-interaction of QKY. (**A**) Yeast two-hybrid assays testing the self-interaction of QKY^{N834} and QKY$^{N834\ \Delta IDR}$. (**B**) Quantification of protein interactions shown in (A) by β-galactosidase activity assays. Values are given as mean ± SD (n = 3). The asterisks indicate statistically significant differences (two-tailed paired Student's t-test, *** $p < 0.001$).

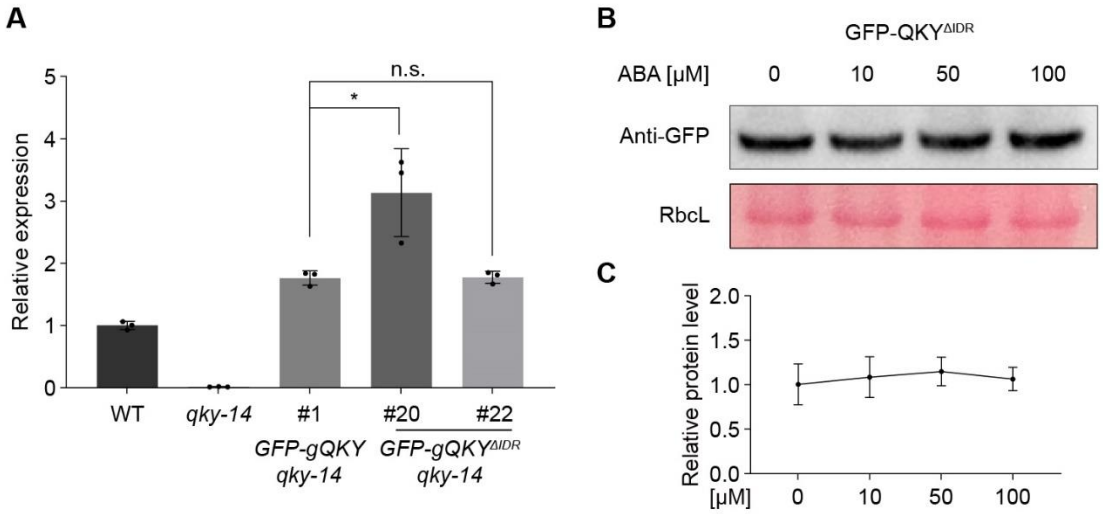

Fig. S7. The IDR of QKY is required for ABA-induced protein accumulation. (**A**) *QKY* expression was determined by quantitative real-time PCR in germinating seeds of wild-type (WT), *qky-14*, *GFP-gQKY qky-14*, and *GFP-gQKY*$^{\Delta IDR}$ *qky-14* lines 24 hours after stratification. Values are given as mean ± SD (n = 3). The expression level in WT was normalized to 1 (two-tailed paired Student's t-test, n.s., $p > 0.05$; * $p < 0.05$). (**B**) Western blot analysis of GFP-QKY$^{\Delta IDR}$ protein abundance treated with ABA at indicated concentrations (0, 10, 50, 100 μM) for 4 hours in *GFP-gQKY*$^{\Delta IDR}$ *qky-14* seedling two days poststratification. Ponceau S staining (RbcL) was used as a loading control. (**C**) Quantification of the relative GFP-QKY$^{\Delta IDR}$ protein abundance in (B). Values are given as mean ± SD (n = 3). The protein level without ABA treatment was normalized to 1. Error bars represent SD.

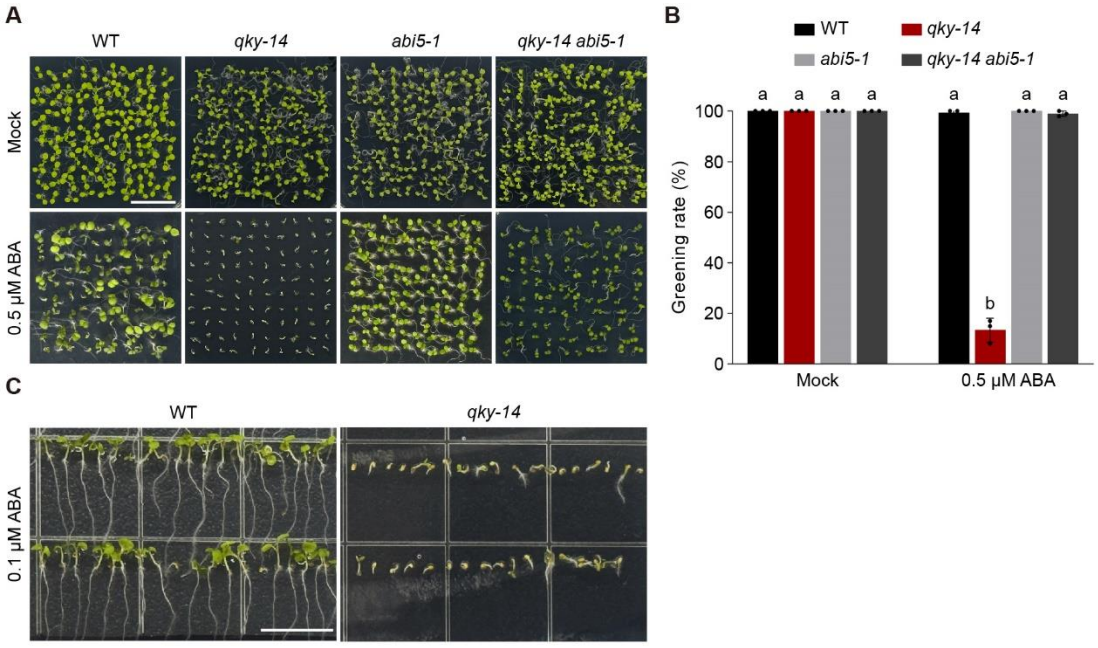

Fig. S8. QKY genetically interacts with ABI5. (**A**) WT, *qky-14*, *abi5-1*, and *qky-14 abi5-1* seeds were germinated on 1/2 MS plates with or without 0.5 μM ABA, and images were taken 7 days (Mock) or 12 days (ABA treatment) after stratification. Scale bar, 1 cm. (**B**) Cotyledon greening rate of WT, *qky-14*, *abi5-1*, and *qky-14 abi5-1* plants 12 days after stratification germinated on 1/2 MS plates containing 0.5 μM ABA. $n = 3$ biological replicates, with approximately 100 seeds analyzed per replicate. Columns labeled with different letters represent statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's HSD test. Error bars represent SD. (**C**) Representative image of WT and *qky-14* seedlings germinated on 1/2 MS plates containing 0.1 μM ABA for 7 days. Scale bar, 1 cm.

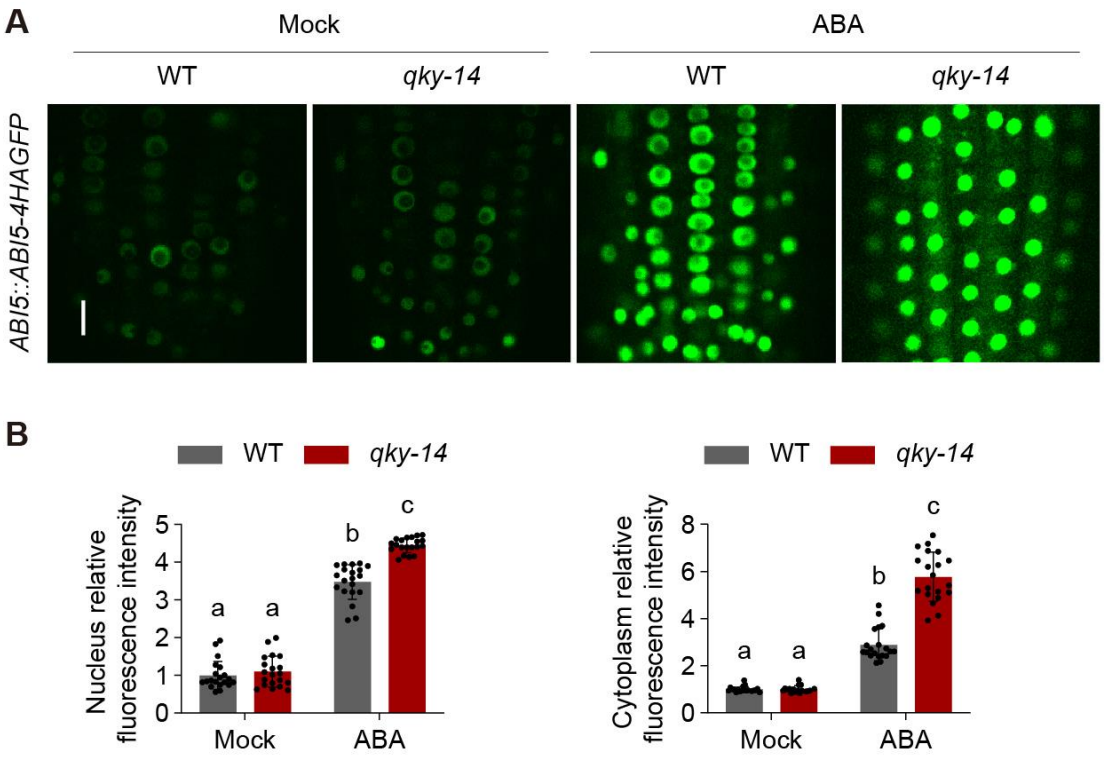

Fig. S9. QKY regulates ABI5 protein degradation. (**A**) Confocal images of ABI5-GFP in root tip cells of *ABI5::ABI5-4HAGFP* seedlings in WT and *qky-14* mutant two days poststratification, and then treated with 3 μM ABA for 2 days. The experiments were conducted three times, and representative images are shown. HA, hemagglutinin tag; GFP, green fluorescent protein. Scale bar, 10 μm. (**B**) Quantification of nucleus and cytoplasm fluorescence intensity of ABI5-GFP signals in the radicle tip cells for *ABI5::ABI5-4HAGFP* or *ABI5::ABI5-4HAGFP qky-14* as shown in (A). Fluorescence intensity is indicated as the mean gray value, representing the average signal intensity in the nucleus and cytoplasm. Data represent fluorescence measurements from at least 20 epidermal cells derived from 10 independent seedlings per genotype. The fluorescence intensity of ABI5-GFP in *ABI5::ABI5-4HAGFP* seedlings under mock treatment was normalized to 1. Values are given as mean ± SD. Columns labeled with different letters represent statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's HSD test.

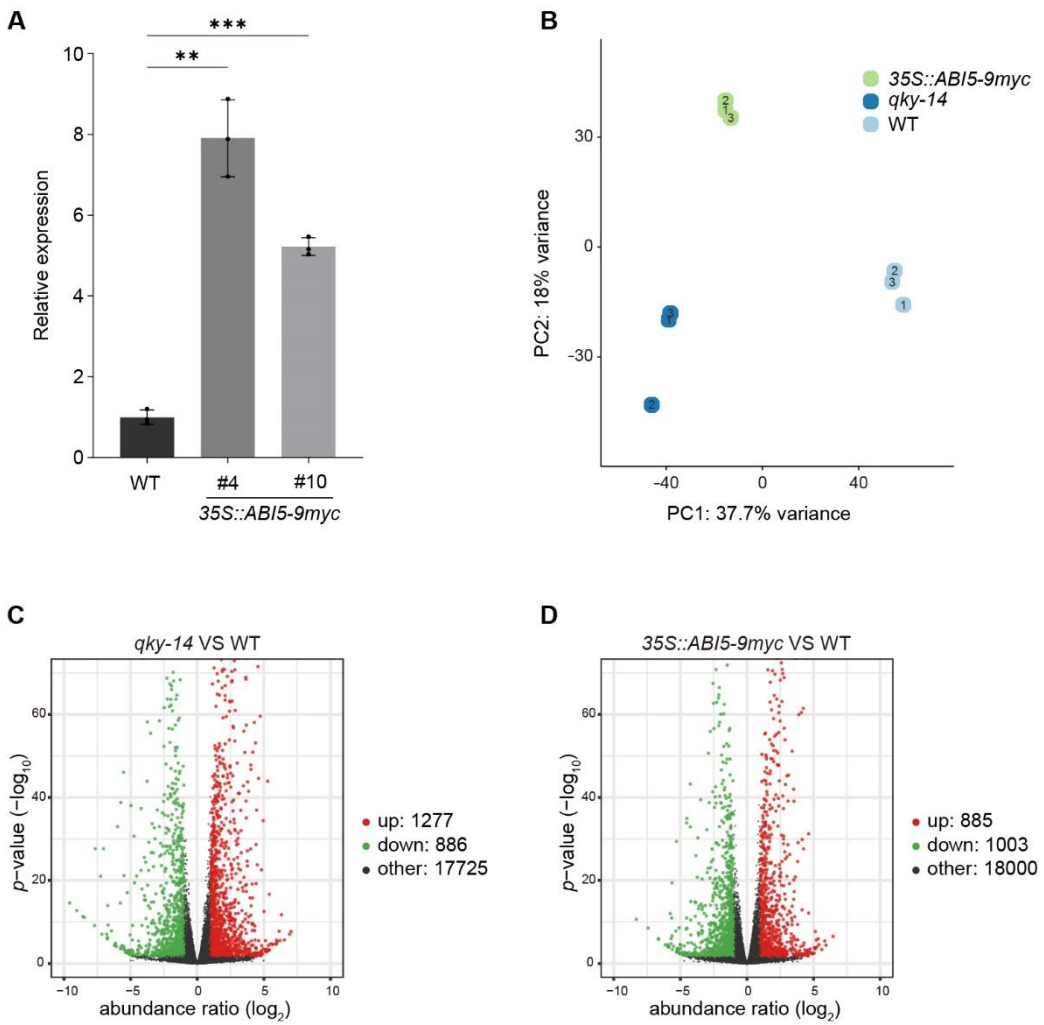

Fig. S10. Genome-wide analysis of gene expression changes in *qky-14* and *35S::ABI5-9myc* with ABA treatment. (**A**) Quantitative real-time PCR analysis of *ABI5* expression in germinating seeds of WT and *35S::ABI5-9myc* lines 24 hours after stratification. Values are given as mean ± SD (n = 3). The expression level in WT was normalized to 1. The asterisks indicate statistically significant differences (two-tailed paired Student's t-test, ** $p < 0.01$; *** $p < 0.001$). (**B**) Principal component analysis (PCA) plot of gene expression data obtained from RNA-seq for three biological replicates of WT, *qky-14*, and *35S::ABI5-9myc* samples treated with 100 μM ABA for 4 hours. (**C**) Volcano plot displaying differentially expressed genes between WT and *qky-14*, highlighting significantly upregulated (red) and downregulated (green) transcripts. (**D**) Volcano plot displaying differentially expressed genes between WT and *35S::ABI5-9myc*, highlighting significantly upregulated (red) and downregulated (green) transcripts.

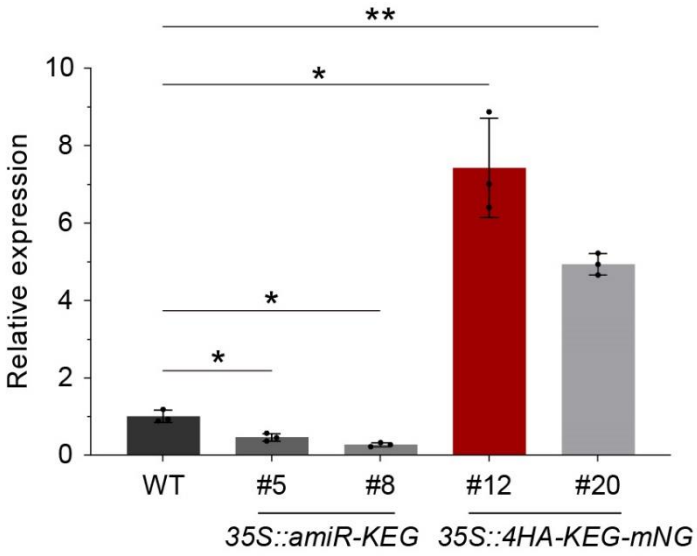

Fig. S11. Expression analysis of *KEG* in various plants. Quantitative real-time PCR analysis of *KEG* expression in germinating seeds of WT, *35S::amiR-KEG*, and *35S::4HA-KEG-mNG* lines 24 hours after stratification. Values are given as mean ± SD ($n = 3$). The mRNA expression level in WT was normalized to 1. The asterisks indicate statistically significant differences (two-tailed paired Student's *t*-test, * $p < 0.05$, ** $p < 0.01$).

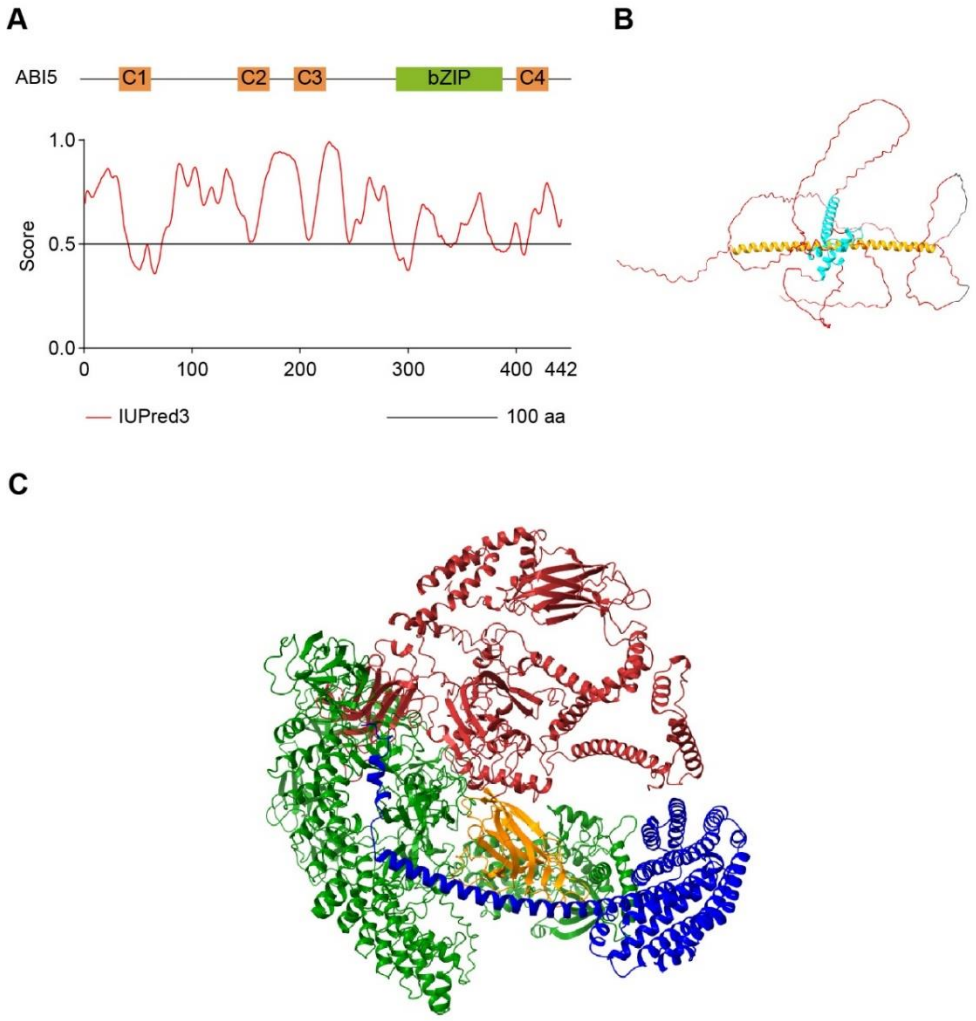

Fig. S12. Structural analysis of ABI5 and QKY-ABI5-KEG complex. (**A**) Schematic diagrams of ABI5 structure. aa, amino acid. Intrinsically disordered regions (IDRs), predicted by IUPred3, are displayed below. (**B**) The predicted structure of ABI5 generated by AlphaFold3, highlighting the C1, C2, and C3 domains in blue, the IDRs in red, and the bZIP domain in yellow. (**C**) The predicted structural model of QKY-KEG-ABI5 complex generated by AlphaFold3. QKY is shown in brown (with the C2c domain highlighted in dark orange), ABI5 in blue, and KEG in green.

Captions for Supplemental Tables

Table S1. Differentially expressed genes identified in *qky-14* compared with WT under ABA treatment.
Table S2. Differentially expressed genes identified in *35S::ABI5-9myc* compared with WT under ABA treatment.
Table S3. Co-upregulated genes identified in both *qky-14* and *35S::ABI5-9myc* compared with WT under ABA treatment.
Table S4. Co-downregulated genes identified in both *qky-14* and *35S::ABI5-9myc* compared with WT under ABA treatment.
Table S5. KEGG enrichment analysis of co-upregulated genes in both *qky-14* and *35S::ABI5-9myc* compared with WT under ABA treatment.
Table S6. KEGG enrichment analysis of co-downregulated genes in both *qky-14* and *35S::ABI5-9myc* compared with WT under ABA treatment.
Table S7. List of primers used in this study.