Supplemental information

ABI5–FLZ13 module transcriptionally represses growth-related genes to delay seed germination in response to ABA

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ABI5-FLZ13 Module Transcriptionally Represses Growth-related Genes to Delay Seed Germination in Response to ABA

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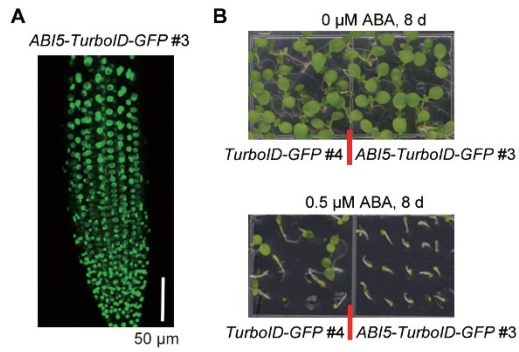
[2]Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University (SCNU), Guangzhou 510631, China

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A
ABI5-TurboID-GFP #3

50 µm

B

0 µM ABA, 8 d

TurboID-GFP #4 | *ABI5-TurboID-GFP* #3

0.5 µM ABA, 8 d

TurboID-GFP #4 | *ABI5-TurboID-GFP* #3

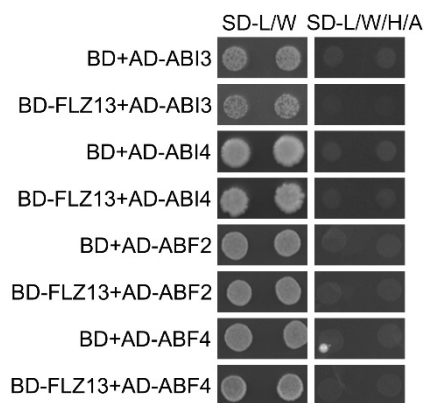**Supplemental Figure 1. Characterization of *ABI5-TurboID-GFP* transgenic plants.**

A, Confocal image showing the nuclear localization of ABI5-TurboID-GFP proteins in root cells of 5-day-old seedlings.

B, Photographs of seedlings of *TurboID-GFP* #4 and *ABI5-TurboID-GFP* #3 upon 0 or 0.5 µM ABA treatment for 8 days.

2
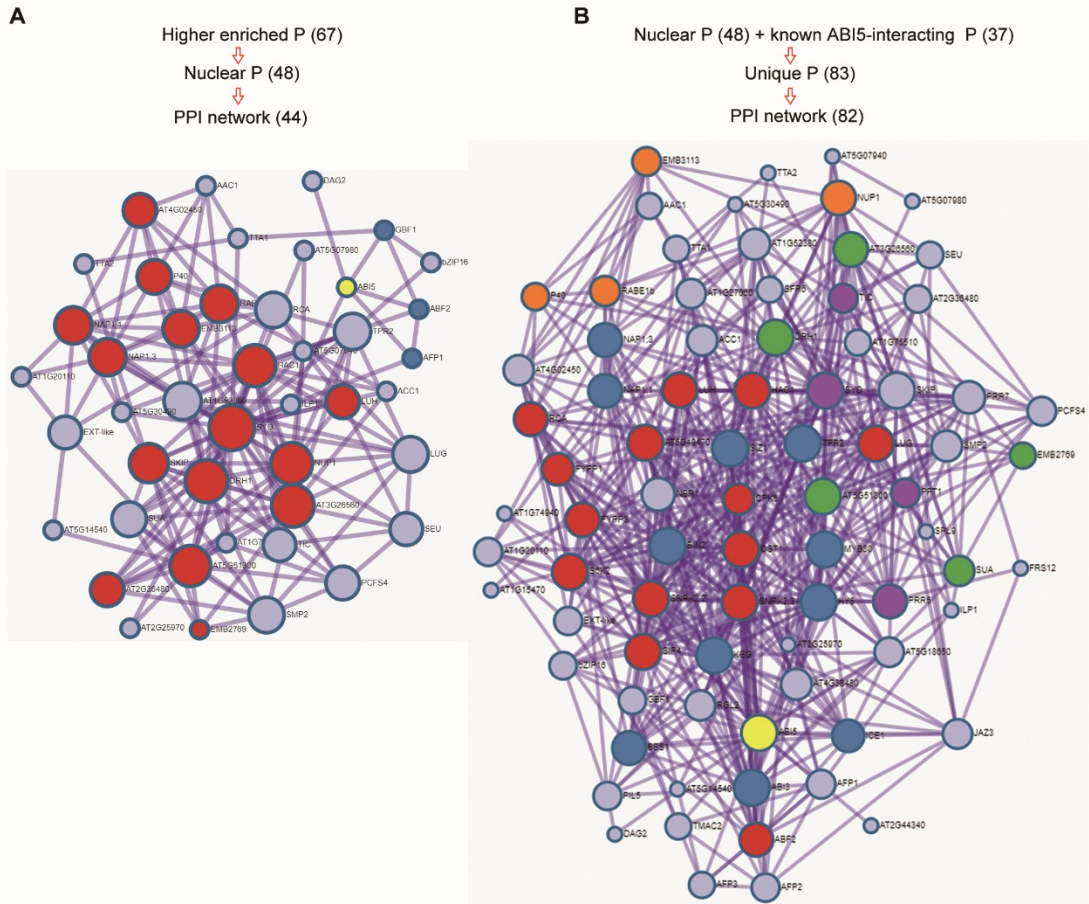
Supplemental Figure 2. Metascape visualization of ABI5 interactome networks. The numbers for the checked proteins are shown on the top. The protein-protein interaction (PPI) networks were generated on line (https://metascape.org/; Zhou et al., 2019). Each node represents a protein and each line represents the interlink between two proteins. ABI5 is highlighted in yellow. P, Protein.

SD-L/W   SD-L/W/H/A

BD+AD-ABI3

BD-FLZ13+AD-ABI3

BD+AD-ABI4

BD-FLZ13+AD-ABI4

BD+AD-ABF2

BD-FLZ13+AD-ABF2

BD+AD-ABF4

BD-FLZ13+AD-ABF4

35

36 **Supplemental Figure 3. Y2H analysis of the interaction between FLZ13 and other**

37 **key transcription factors involved in ABA signaling.**

38 Protein interaction were determined by growth of the yeast cells co-transformed with

39 various combinations of the plasmids on synthetic dropout medium lacking Leu and Trp

40 (SD-L/W) and lacking Leu, Trp, His, and adenine (SD-L/W/H/A) as indicated.
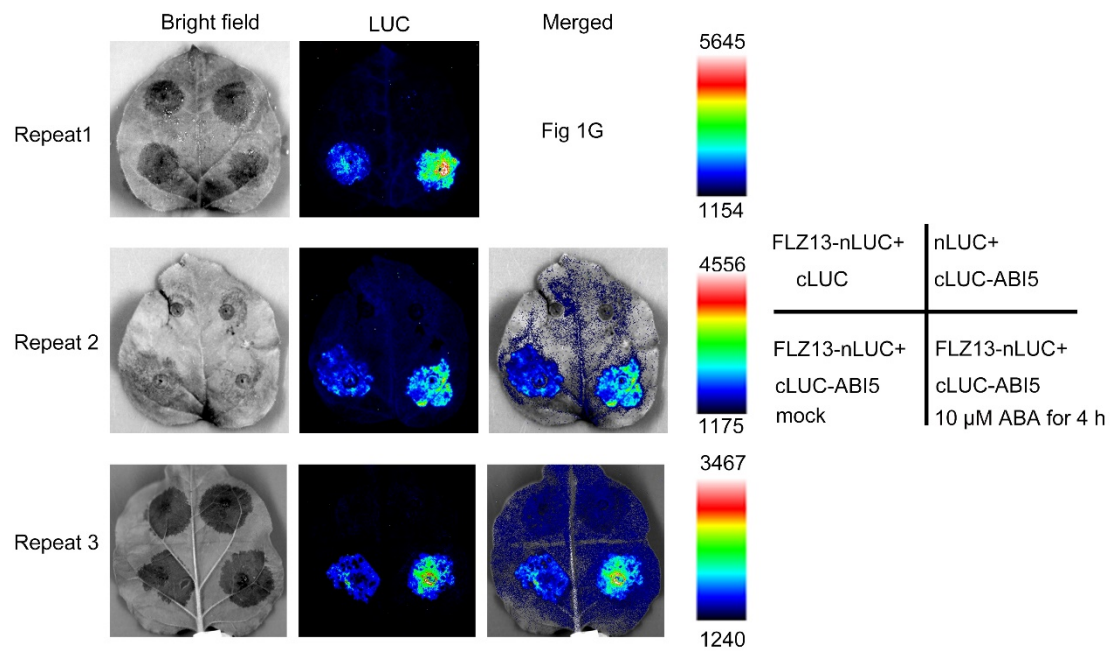
Supplemental Figure 4. Three biological replications of LCI experiments showing that ABA promotes FLZ13-ABI5 interaction.

Three independent experiments of LCI showing the interaction between FLZ13 and ABI5, and the promoting effects of ABA on this interaction. *Agrobacteria* harboring FLZ13-nLUC was co-infiltrated with *Agrobacteria* harboring cLUC-ABI5 into *N. benthamiana* leaves. The FLZ13-nLUC/cLUC and cLUC-ABI5/nLUC pairs were used as negative controls. After 36 h co-infiltration, leaves were treated with mock and 10 µM ABA for 4 h followed by luminescence imaging.

50
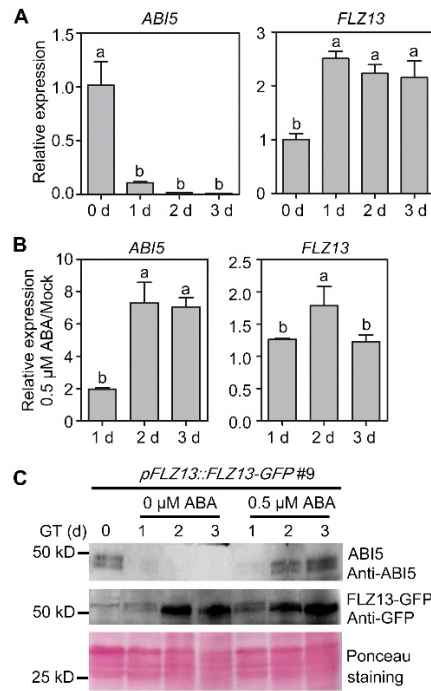
Supplemental Figure 5. Expression pattern of *FLZ13* and *ABI5* during seed germination with or without ABA treatment.

A, Quantitative RT-PCR assay showing the expression of *FLZ13* and *ABI5* in dry and germinating seeds. *Actin2* was used as the internal control. Data are presented as mean ± SD (n = 3 technical replicates). The different letters above each bar indicate statistically significant differences determined by one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.05$). This experiment was repeated twice and similar results were obtained.

B, Quantitative RT-PCR assay showing the expression of *FLZ13* and *ABI5* in germinating seeds in response to ABA treatment. The data are presented as the ratio of ABA to Mock treatment. *Actin2* was used as the internal control. Data are presented as mean ± SD (n = 3 technical replicates). The different letters above each bar indicate statistically significant differences determined by one-way ANOVA followed by Tukey's multiple comparison test ($P < 0.05$). This experiment was repeated twice and similar results were obtained.

C, Immunoblot analysis of FLZ13-GFP and ABI5 proteins in response to ABA treatment during seed germination. GT: germination time.
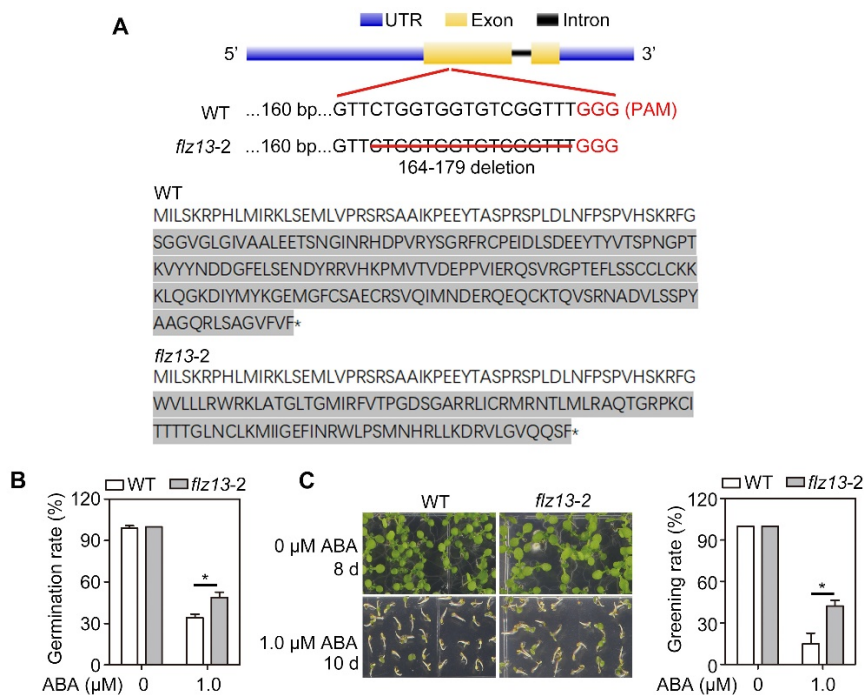
67
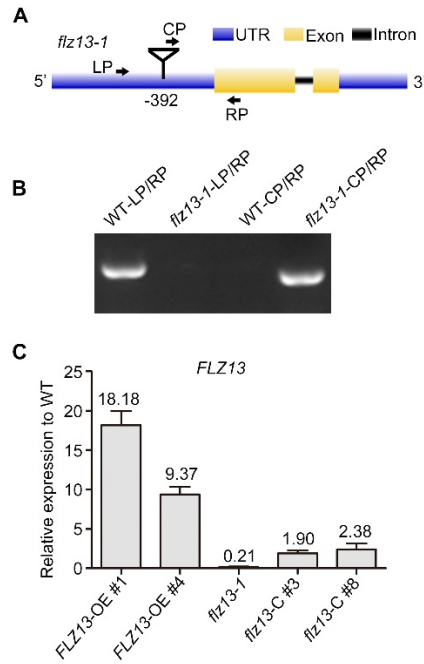
Supplemental Figure 6. Characterization of *FLZ13*-related plant materials.

A, Schematic gene structure of *FLZ13*. Triangle indicates the T-DNA insertion site in *flz13-1* mutant. Arrows indicate primers used for genotyping of *flz13-1* mutant. LP, left primer; CP, common primer; RP, right primer; UTR, untranslated regions.

B, Gel electrophoresis of genotyping PCR with WT and *flz13-1* DNA using primers indicated in panel A.

C, Quantitative RT-PCR showing the gene expression of *FLZ13* in the indicated genetic background. Data are presented as mean ± SD (*n* = 3 technical replicates). *Actin2* was used as the internal control. The expression level in WT was set as 1.0. The numbers on the bars indicate the fold change.

A

WT ...160 bp...GTTCTGGTGGTGTCGGTTTGGG (PAM)

flz13-2 ...160 bp...GTTCTGGTGGTGTCGGTTTGGG
164-179 deletion

WT
MILSKRPHLMIRKLSEMLVPRSRSAAIKPEEYTASPRSPLDLNFPSPVHSKRFG
SGGVGLGIVAALEETSNGINRHDPVRYSGRFRCPEIDLSDEEYTYVTSPNGPT
KVYYNDDGFELSENDYRRVHKPMVTVDEPPVIERQSVRGPTEFLSSCCLCKK
KLQGKDIYMYKGEMGFCSAECRSVQIMNDERQEQCKTQVSRNADVLSSPY
AAGQRLSAGVFVF*

flz13-2
MILSKRPHLMIRKLSEMLVPRSRSAAIKPEEYTASPRSPLDLNFPSPVHSKRFG
WVLLLRWRKLATGLTGMIRFVTPGDSGARRLICRMRNTLMLRAQTGRPKCI
TTTTGLNCLKMIIGEFINRWLPSMNHRLLKDRVLGVQQSF*

B

C

0 μM ABA
8 d

1.0 μM ABA
10 d

Supplemental Figure 7. Characterization of the *flz13*-2 mutant.

A, The CRISPR/Cas9 gene editing mutant of *FLZ13*. *flz13*-2 harbors a 16-nucleotides deletion which is predicted to encode a truncated protein. UTR, untranslated regions; PAM, protospacer adjacent motif.

B, Germination rates of WT and *flz13-2* treated with the indicated concentrations of ABA. The germination rates were recorded 3 d after plating on 1/2 MS medium. Data are presented as mean ± SD (n = 3 biological replicates). The asterisk indicates statistically significant differences (*P < 0.05) determined by Student's t-test.

C, Photographs of seedlings (left) and greening rates (right) of WT and *flz13-2* upon 0 or 1.0 μM ABA treatment as recorded at the indicated time points. Data is mean ± SD (n = 3 biological replicates). The asterisk indicates statistically significant differences (*P < 0.05) determined by Student's t-test.
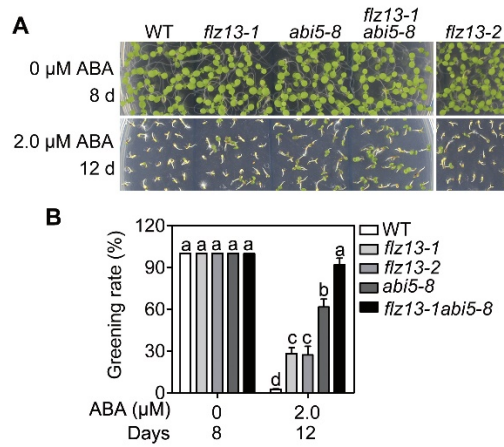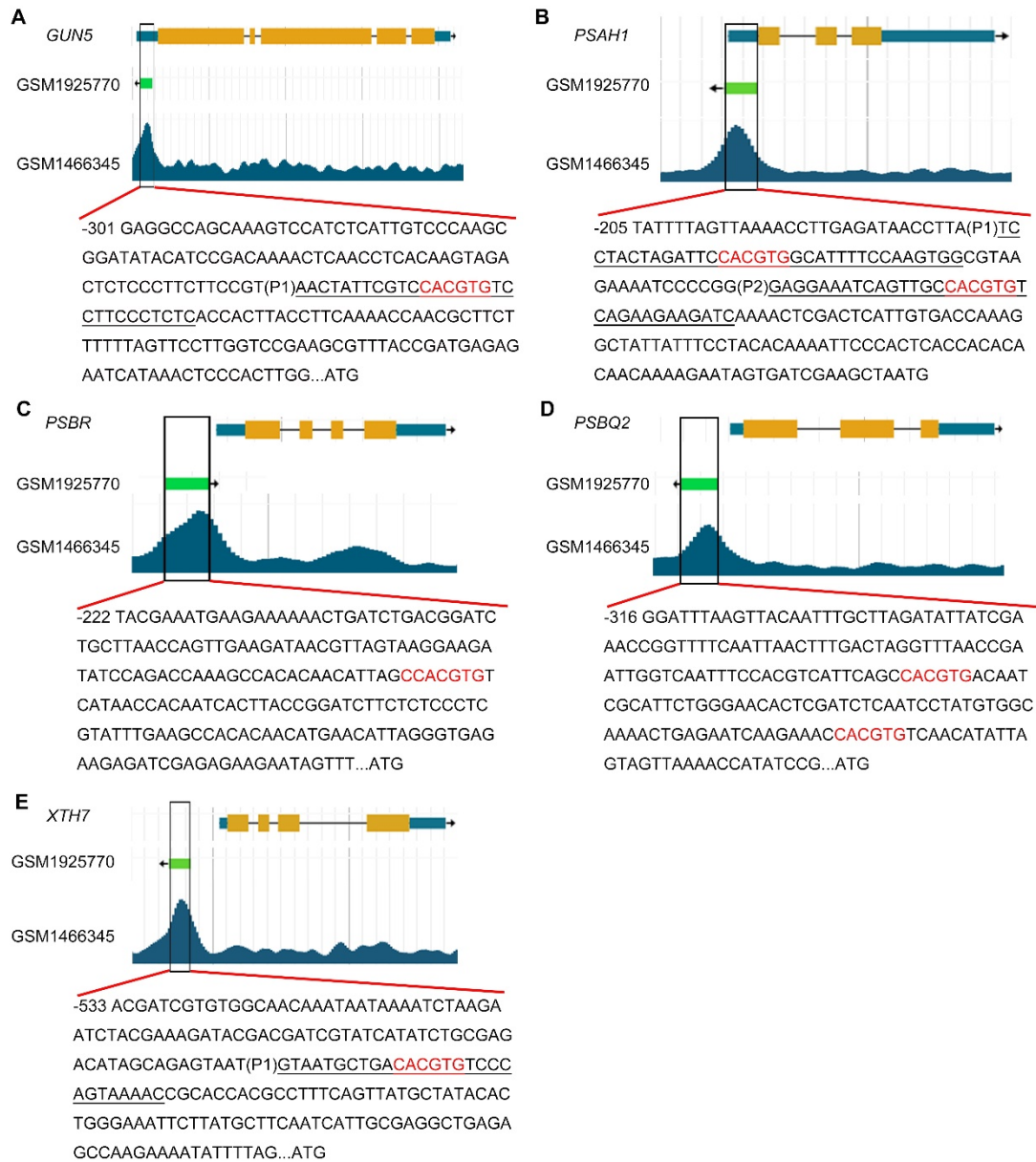
Supplemental Figure 8. ABA response of *flz13* and *abi5* mutants.

A, Photographs of seedlings of WT, *flz13-1*, *flz13*-2, *abi5-8*, and *flz13-1 abi5-8* upon 0 or 2.0 µM ABA treatment at the indicated time points.

B, Cotyledon greening rates of WT, *flz13-1*, *flz13*-2, *abi5-8*, and *flz13-1 abi5-8* upon various concentration ABA treatment at the indicated time points. Data are presented as mean ± SD (n = 3 biological replicates for 0 µM ABA treatment and n = 4 biological replicates for 2.0 µM ABA treatment). The different letters above each bar indicate statistically significant differences determined by one-way ANOVA followed by Tukey's multiple comparison test (P < 0.05).

A GUN5

GSM1925770

GSM1466345

-301 GAGGCCAGCAAAGTCCATCTCATTGTCCCAAGC
GGATATACATCCGACAAAACTCAACCTCACAAGTAGA
CTCTCCCTTCTTCCGT(P1)<u>AACTATTCGTC</u>CACGTGTC
<u>CTTCCCTCTC</u>ACCACTTACCTTCAAAACCAACGCTTCT
TTTTTAGTTCCTTGGTCCGAAGCGTTTACCGATGAGAG
AATCATAAACTCCCACTTGG...ATG

B PSAH1

GSM1925770

GSM1466345

-205 TATTTTAGTTAAAACCTTGAGATAACCTTA(P1)<u>TC</u>
<u>CTACTAGATTC</u>CACGTGGCATTTTCCAAGTGGCGTAA
GAAAATCCCCGG(P2)<u>GAGGAAATCAGTTGC</u>CACGTGT
<u>CAGAAGAAGATC</u>AAAACTCGACTCATTGTGACCAAAG
GCTATTATTTCCTACACAAAATTCCCACTCACCACACA
CAACAAAAGAATAGTGATCGAAGCTAATG

C PSBR

GSM1925770

GSM1466345

-222 TACGAAATGAAGAAAAAACTGATCTGACGGATC
TGCTTAACCAGTTGAAGATAACGTTAGTAAGGAAGA
TATCCAGACCAAAGCCACACAACATTAGCCACGTGT
CATAACCACAATCACTTACCGGATCTTCTCTCCCTC
GTATTTGAAGCCACACAACATGAACATTAGGGTGAG
AAGAGATCGAGAGAAGAATAGTTT...ATG

D PSBQ2

GSM1925770

GSM1466345

-316 GGATTTAAGTTACAATTTGCTTAGATATTATCGA
AACCGGTTTTCAATTAACTTTGACTAGGTTTAACCGA
ATTGGTCAATTTCCACGTCATTCAGCCACGTGACAAT
CGCATTCTGGGAACACTCGATCTCAATCCTATGTGGC
AAAACTGAGAATCAAGAAACCACGTGTCAACATATTA
GTAGTTAAAACCATATCCG...ATG

E XTH7

GSM1925770

GSM1466345

-533 ACGATCGTGTGGCAACAAATAATAAAATCTAAGA
ATCTACGAAAGATACGACGATCGTATCATATCTGCGAG
ACATAGCAGAGTAAT(P1)<u>GTAATGCTGA</u>CACGTGTCCC
<u>AGTAAAAC</u>CGCACCACGCCTTTCAGTTATGCTATACAC
TGGGAAATTCTTATGCTTCAATCATTGCGAGGCTGAGA
GCCAAGAAAATATTTTAG...ATG

102

103 **Supplemental Figure 9. Five selected target genes of ABI5.**

104 The raw data were downloaded from http://bioinfo.sibs.ac.cn/plant-regulomics/ (Ran et al.,

105 2020) and edited in photoshop. The primers used for EMSA analysis are underlined. The
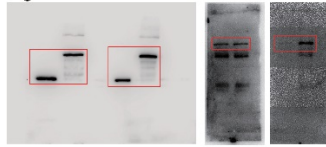
106 sequences of G-box are highlighted in red.
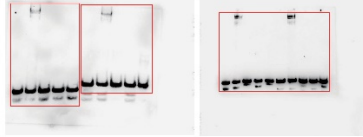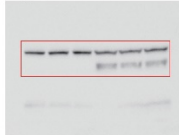
Figure 1C
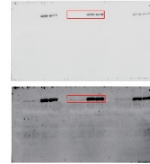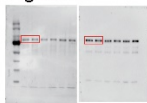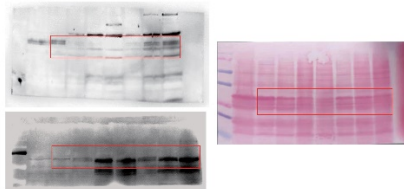
Figure 5F

Figure 6C　　　　　Figure 6D

Figure 6E

Supplemental Figure 5C

107

108　**Supplemental Figure 10. Original images for Western blots.** The red boxes mark the

109　sheared regions presented in the corresponding figures.

11

Supplemental Tables

The following materials are available in the uploaded Excel file.

Supplemental Table 1. List of the higher enriched proteins in ABI5-TurboID-GFP vs. TurboID-GFP comparison.

Supplemental Table 2. List of the 48 nuclear-localized proteins of 67 identified higher enriched ones in Supplemental Table 1.

Supplemental Table 3. List of the 37 known ABI5-interacting proteins.

Supplemental Table 4. DEGs in response to ABA treatment in WT germinating seeds.

Supplemental Table 5. Expression of known ABA-induced marker genes in the RNA-seq data.

Supplemental Table 6. DEGs in ABI5-OE seeds compared to WT upon ABA treatment.

Supplemental Table 7. DEGs in FLZ13-OE seeds compared to WT upon ABA treatment.

Supplemental Table 8. Expression of 567 ABA, FLZ13 and ABI5 co-regulated genes.

Supplemental Table 9. GO enrichment of 496 ABA/ABI5/FLZ13 co-repressed genes.

Supplemental Table 10. Target genes of ABI5.

Supplemental Table 11. List of the 35 ABI5 target genes co-regulated by ABA/ABI5/FLZ13.

Supplemental Table 12. Expression of the 500 ABA/ABI5/FLZ13 synchronously co-regulated genes in WT, *FLZ13*-OE, and *FLZ13*-OE/*abi5-8* plants upon ABA treatment.

Supplemental Table 13. Expression of the 500 synchronously ABA/ABI5/FLZ13 co-regulated genes in WT, *ABI5*-OE, and *ABI5*-OE/*flz13-1* plants upon ABA treatment.

Supplemental Table 14. List of primers used in this study.

Supplemental Table 15. Original data for bar charts production and statistical analysis.