The inhibitory effect of ABA on floral transition is mediated by ABI5 in *Arabidopsis*

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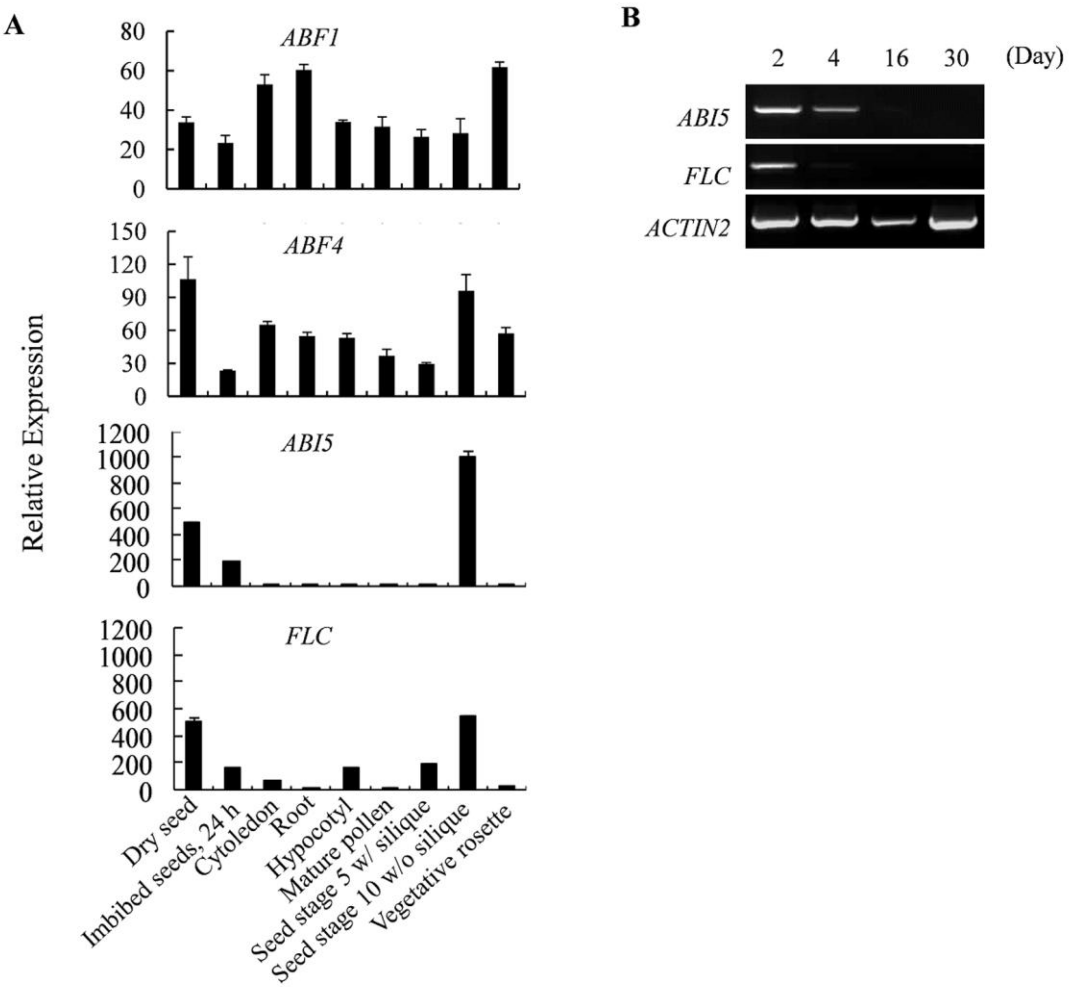
Supplementary material

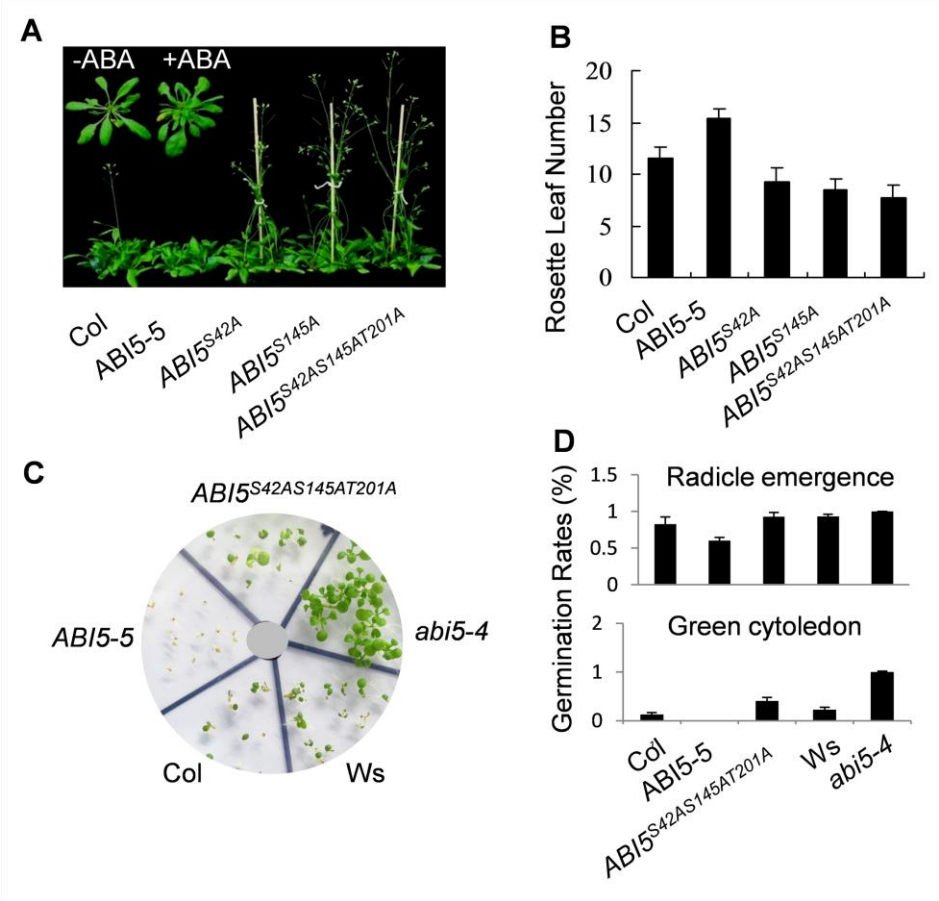
Supplementary Figures:

Fig. S1



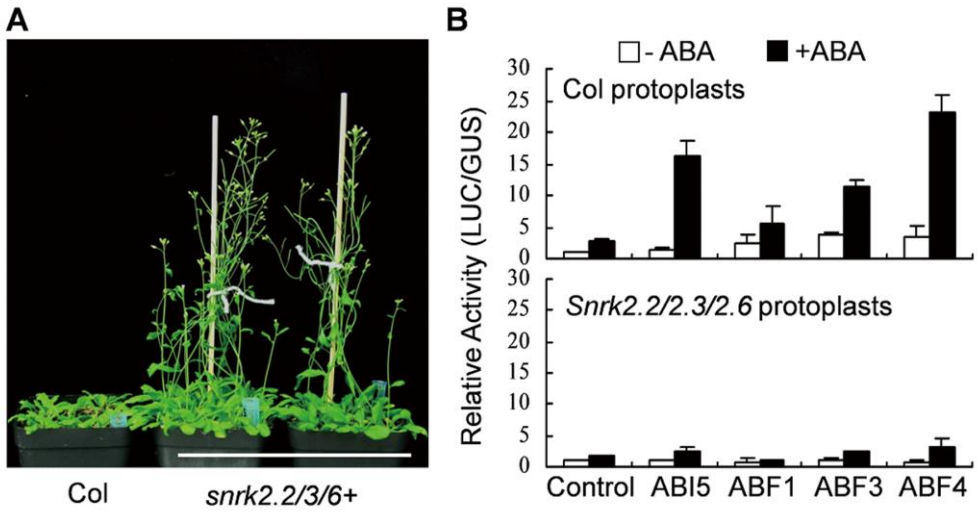
Supplementary Fig. S1. Comparisons of *ABFs*, *ABI5* and *FLC* expression patterns in *Arabidopsis*. (A) Public available microarray data illustrating similar expression patterns of *ABF1*, *ABF4*, *ABI5* and *FLC* in various tissues of *Arabidopsis* (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). (B) Comparative analyses of *ABI5* and *FLC* in Col seedlings (2, 4, 16, or 30 represents the day-of-germination). Expression level of *ACTIN2* represents the loading control.

Fig. S2



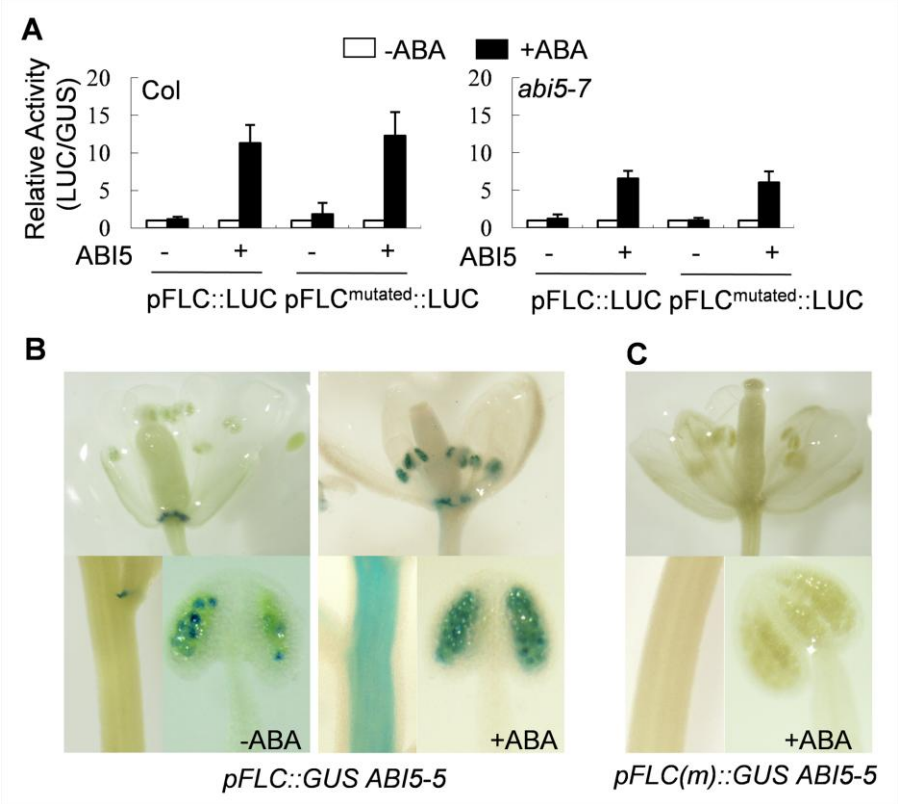
Supplementary Fig. S2. Comparisons of flowering phenotypes, total rosette leaf numbers, and seed germination phenotypes in wild-type and transgenic lines. (A) Comparisons of the flowering phenotype in 40-day-old seedlings (under long-day growth condition). Insert: ABA (100 μM) treated Col seedlings. (B) Comparisons of total rosette leaf numbers. Data represent the means ± SEs of three replicated experiments (n>20, each). (C) The seed germination phenotype was analyzed in indicated lines on ABA (1.0 μM) plate. Photo was taken at the 7th day after imbibitions. (D) Seed germination rates were quantified at the 7th day after imbibitions. Data represent the means ± SEs of three replicated experiments (n>30, each).

Fig. S3



Supplementary Fig. S3. SnRK2s are involved in regulations of *FLC* expression. (A) Early flowering phenotype of *snrk2.2/3/6+* triple mutant plants. Photo was taken after plants were growing for 35 days under the long day condition. (B) The transactivation of *FLC* by ABFs was dependent upon SnRK2s. Data represent means ± SDs of three replicated experiments. ABA (50 μM); Control (ethanol).

Fig. S4



Supplementary Fig. S4. ABRE-like and G-box motifs in *FLC* promoter are essential for *FLC* expression in responding to ABA. (A) ABI5 effect on *FLC* promoter activity was compared between the wild-type *FLC* promoter (pFLC::LUC) and the mutated *FLC* promoter (pFLCmutated::LUC). The mutation was created only at the ABRE-like element. Data represent means ± SEs of three replicated experiments. (B) & (C) ABA-induced GUS signal was analyzed in the plants of *pFLC::GUS ABI5-5* (B) and *pFLC(m)::GUS ABI5-5* (C) transgenic lines. The GUS signal was analyzed after treated with 100 μM ABA for 3 hours. Mutations created at ABRE-like element and G-box motifs (indicated as pFLC(m)::GUS) in *FLC* promoter showed no GUS signal.

Supplementary Tables:

Table S1

Table S1. Primers for identification of *snrk2.2/2.3/2.6* homozygous plants.

SnRK2.2	LP: 5’-CATGCCATGGAGATGGATCCGGCGACTAAT-3’ RP: 5’-GGAATTCTCAGAGAGCATAAACTATCTCTC-3’
SnRK2.3	LP: 5’-CATGCCATGGAGATGGATCGAGCTCCGGTG-3’ RP: 5’-GGAATTCTTAGAGAGCGTAAACTATCT-3’
SnRK2.6	LP: 5'-CATGCCATGGAGATGGATCGACCAGCAGTG-3' RP: 5'-GGAATTCTCACATTGCGTACACAATCT-3'

Table S2

Table S2. Primer sequences for plasmid construction.

ABF1-GFP	F: 5’-CCGCTCGAGATGGGTACTCACATTGATA-3’ R: 5’-GGGGTACCTCCCCTTCTTACCACGGACCG-3’
ABF3-GFP	F: 5’-CCGCTCGAGATGGGGTCTAGATTAAACT-3’ R: 5’-GGGGTACCTCCCTGGCGCAGAGGCTCCA-3’
ABF4-GFP	F: 5’-CCGCTCGAGATGGGAACTCACATCAAT-3’ R: 5’-GGGGTACCTCCCCATGGTCCGGTTAATG-3’
ABI5-HA	F: 5’-CCGCTCGAGTCCGGCGGCTTTTAAACTAT-3’ R: 5’-TCCCCGCGGTTAAGCGTAATCTGGAACATCGT-3’
ABI5-GFP	F: 5’-CCGCTCGAGTCCGGCGGCTTTTAAACTAT-3’ R: 5’-CCGCTCGAGGAGTGGACAACTCGGGTTCC-3’
ABI5^{S42A}-HA/GFP	F: 5’-AGACAATCCGCTATCTACTC-3’ R: 5’-GAGTAGATAGcGGATTGTCT-3’
ABI5^{S145A}-HA/GFP	F: 5’-CGACAAGGCGCTTTGACACT-3’ R: 5’-AGTGTCAAAGCGCCTTGTCG-3’
ABI5^{T201A}-HA/GFP	F: 5’-AGACAACCGGCTTTTGGAGA-3’ R: 5’-TCTCCAAAAGCCGGTTGTCT-3’
p35S::GFP-FLC	F: 5’-TCTTTAATTAAAGCCATGGGAAGAA-3’ R: 5’-TCAGAGCTCGATTTAAGGTGGCTAA-3’
pFLC::LUC	F: 5’-AACTGCAGTATTCGTGTTGCAAAATCGTAAA-3’ R: 5’-CATGCCATGGGGCTTCTCTCCGAGAGG-3’
pFLC(m)::LUC	F: 5’-AGACGCTCGTCGGGGGGTACAAAAGGCAATCTTGTCTT-3’ R: 5’-AACAGAAGTTTTGTGTGGGGGGAATAAGTGTAT-3’
p35S::SnRK2.6	F: 5’-CCCGGGATGGATCGACCAGCAGTGAGT-3’ R: 5’-ACTAGTCTCACATTGCGTACACAATC-3’

Table S3

Table S3. Plasmids used in this study.

Plasmids Names	Cloning Sites	Vectors	References
ProUBQ10-GUS	HindIII/BamHI	Pro35S-GUS	Wu *et al*., 1997
pBA-ABI5-HA	XhoI/SacI	pBA002	Kost *et al*., 1998
pBA-ABI5^{S42A}-HA	XhoI/SacI	pBA002	Kost *et al*., 1998
pBA-ABI5^{S145A}-HA	XhoI/SacI	pBA002	Kost *et al*., 1998
pBA-ABI5^{S42AS145AT201A}-HA	XhoI/SacI	pBA002	Kost *et al*., 1998
p35S::ABI5-GFP	XhoI/KpnI	pGFP2	Kost *et al*., 1998
p35S::ABI5^{S42A}-GFP	XhoI/KpnI	pGFP2	Kost *et al*., 1998
p35S::ABI5^{S145A}-GFP	XhoI/KpnI	pGFP2	Kost *et al*., 1998
p35S::ABI5^{S42AS145A}-GFP	XhoI/KpnI	pGFP2	Kost *et al*., 1998
p35S::ABI5^{S42AS145AT201A}-GFP	XhoI/KpnI	pGFP2	Kost *et al*., 1998
p35S::FLC-GFP	XhoI/KpnI	pGFP2	Kost *et al*., 1998
pFLC::LUC	NcoI/PstI	pARR6::LUC	Hwang and Sheen 2001
pFLC(m)::LUC	NcoI/PstI	pARR6::LUC	Hwang and Sheen 2001
p35S::ABF1-GFP	XhoI/KpnI	pGFP2	Kost *et al.*, 1998
p35S::ABF3-GFP	XhoI/KpnI	pGFP2	Kost *et al.*, 1998
p35S::ABF4-GFP	XhoI/KpnI	pGFP2	Kost *et al.*, 1998
p35S::SnRK2.6	XmaI/SpeI	p35S	Kost *et al.*, 1998

Table S4

Table S4. Primer sequences for analysis of enrichment of DNA fragments in chromatin immunoprecipitation assay.

DNA1	F: 5’-AATATCTGGCCCGACGAA-3’ R: 5’-ATTTGATCCTCAGGTTTGGG-3’
DNA2	F: 5’-TTGAAAGTCTTTGTAGGTTTGGTT-3’ R: 5’-CCCAAATCTTTGGCTACCAT-3’
DNA3	F: 5’-AAATGTTTGTGTGGCTCCAA-3’ R: 5’-CGATATTGGTGATTGGTATTAACTTT-3’
DNA4	F: 5’-TTTGTGTTAATCTCCCGAACA-3’ R: 5’-GTGTTACCAAAGTCGTGCCTAC-3’
DNA5	F: 5’- AATATCTGGCCCGACGAA-3’ R: 5’- ATTTGATCCTCAGGTTTGGG-3’
DNA6	F: 5’-GCAATAGTTCAATCCGTATCGTA-3’ R: 5’-CCGGAGATACTAAGCGTT-3’

Table S5

Table S5. Primer sequences for qRT-PCR experiments.

ABI5	F: 5’-GAAGAGAATGCGCAGCTAAA-3’ R: 5’-TTGTGCCCTTGACTTCAAAC-3’
FCA	F: 5’-GTTCGAACGAGAGCAACAGA-3’ R: 5’-GCTGCTGAACTTGTTGTGGT-3’
FT	F: 5’-ATGGTGGATCCAGATGTTCC-3’ R: 5’-AGTGCACAAACCAATGGAGA-3’
FLC	F: 5’-GGCGATAACCTGGTCAAGAT-3’ R: 5’-TTTGACTGATGATCCAAGGC-3’
SOC1	F: 5’-CTCTTGGGAGAAGGCATAGG-3’ R: 5’-CACATACCTTTCTTGCTCGAAT-3’
β-ACTIN8	F: 5’- AGTGGTCGTACAACCGGTATTGT-3’ R: 5’- GAGGATAGCATGTGGAAGTGAGAA-3’