Identification of an important site for function of the type 2C protein phosphatase ABI2 in abscisic acid signalling in *Arabidopsis*

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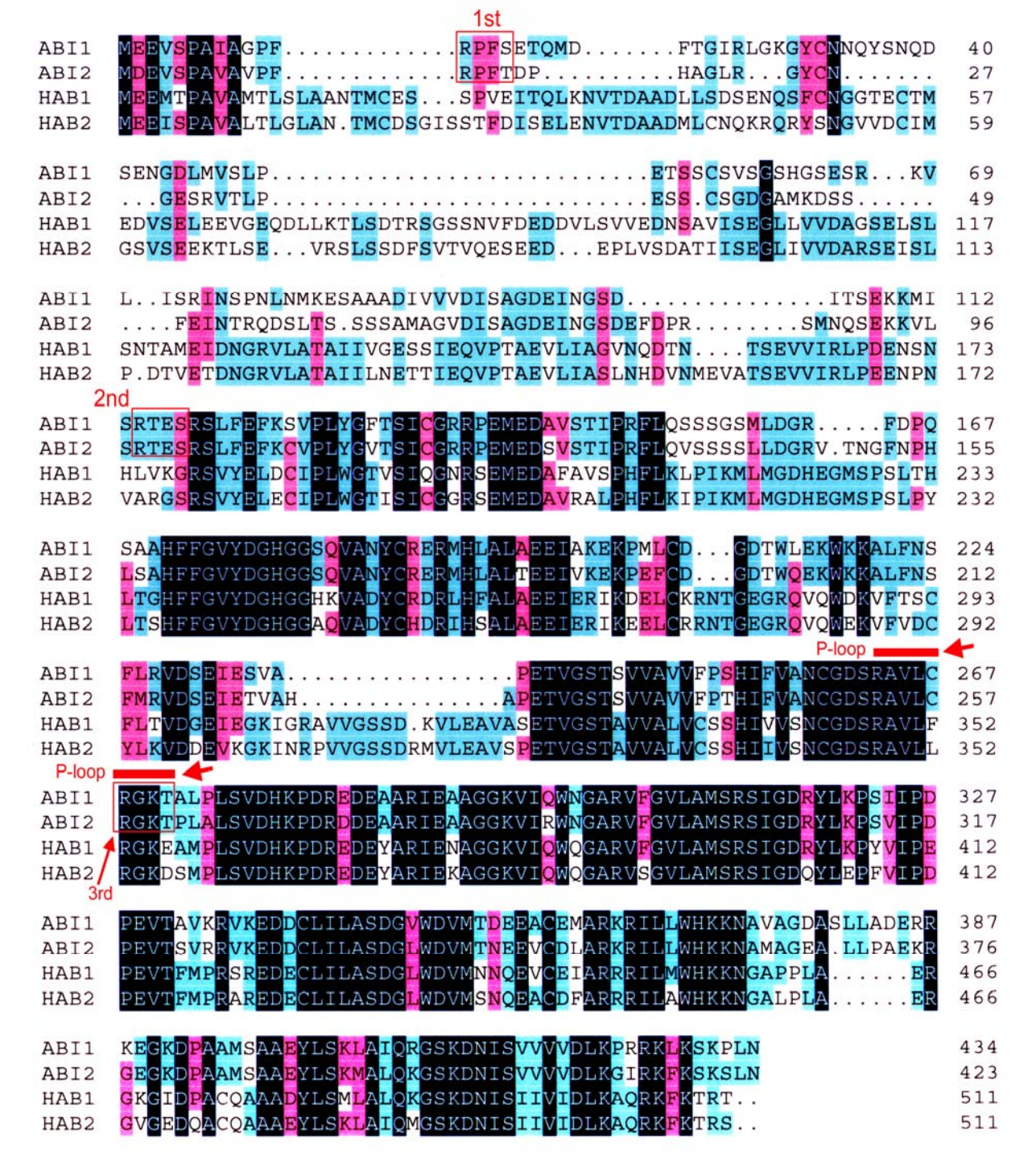
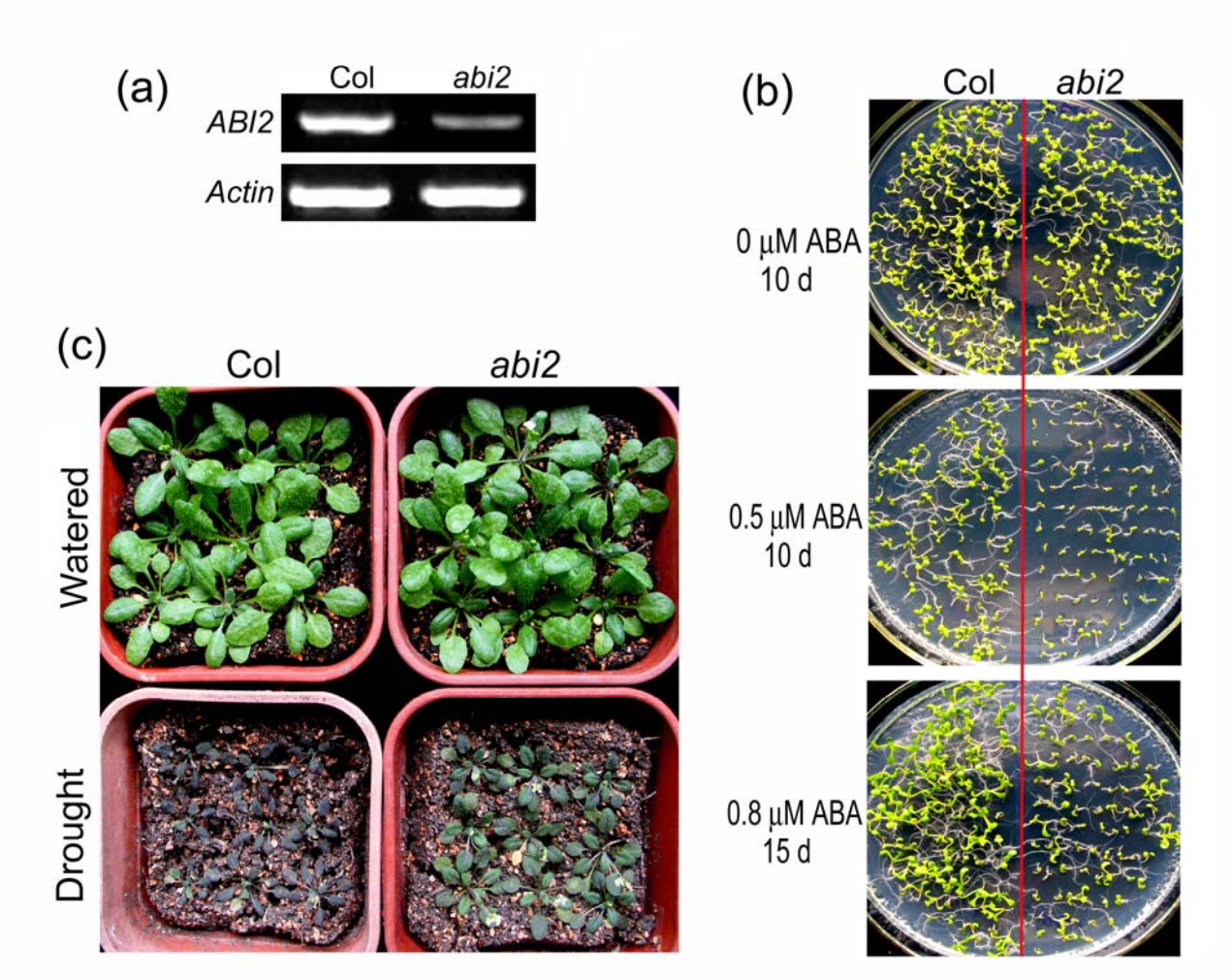
Supplementary Material

Supplemental Data



Supplementary Fig. S1. Alignment of four members of the Arabidopsis PP2C family, ABI1, ABI2, HAB1and HAB2, which are involved in ABA signaling. Alignment was performed with DNAMAN software. Numbers on the right column indicate numbers of amino acid residues in the predicted sequences. Gaps, indicated by dots (….), were introduced to maximize alignment. Three putative phosphorylation sites of ABI1 and ABI2 by CDPK are indicated with red letters 1st , 2nd, and 3rd. The potential ATP/GTP-binding site motif or phosphate-binding loop (P-loop: AVLCRGKT), conserved in ABI1 and ABI2, is also indicated.



Supplementary Fig. S2. Characterization of the *abi2-t1* T-DNA insertion mutant. (a) Semi-quantitative RT-PCR analysis indicates that *abi2-t1* (indicated by *abi2*) is a knock-down mutant allele. The *abi2-t1* mutation results in ABA hypersensitivity in ABA-induced inhibition of seed germination and postgermination growth arrest (b), and enhances drought tolerance (c). Drought treatment: Plants were grown on soil until they were 3-week old, and then drought was imposed by withdrawing irrigation for one-half of the plants until the lethal effects were observed on most of these plants, whereas the other half were grown under a standard irrigation regime as a control.

Supplementary Table S1. Primers used to create ABI2 mutations/deletions.

Primer name	Sequence
I2F1:	5’-GCTCTAGAATGGACGAAGTTTCTCCTGCAGTCGCTG-3’
I2R1:	5’-GGGGTACCATTCAAGGATTTGCTCTTGAATTTCC-3’
I2A1F：	5’-CCATTCAGACCATTCGCTGACCCTCACGCCGG-3’
I2A2F：	5’-CTTAGTAGAACAGAGGCTAGAAGTCTGTTTGAG-3’
I2A2R：	5’-CTCAAACAGACTTCTAGCCTCTGTTCTACTAAG-3’
I2A3F：	5’-GGTTTTGTGTCGCGGCAAAGCGCCACTCGCGTTGTC-3’
I2A3R：	5’-GACAACGCGAGTGGCGCTTTGCCGCGACACAAAACC-3’
I2D1F：	5’-CCATTCAGACCATTCGATGACCCTCACGCCGG-3’
I2D2F：	5’-CTTAGTAGAACAGAGGATAGAAGTCTGTTTGAG-3’
I2D2R：	5’-CTCAAACAGACTTCTATCCTCTGTTCTACTAAG-3’
I2D3F：	5’-GGTTTTGTGTCGCGGCAAAGACCCACTCGCGTTGTC-3’
I2D3R：	5’-GACAACGCGAGTGGGTCTTTGCCGCGACACAAAACC-3’
I2F2:	5’-TGCAGTCGCTGTTCCATTCGACCCTCACGCCGGACTTAG-3’
I2F3:	5’-GAAGAAAGTACTTAGTAGAAGTCTGTTTGAG-3’
I2R3:	5’-CTCAAACAGACTTCTACTAAGTACTTTCTTC-3’
I2F4:	5’-GGGCGGTTTTGTGTCCACTCGCGTTGTC-3’
I2R4:	5’-GACAACGCGAGTGGACACAAAACCGCCC-3’

Supplementary Table S2. Primers used for yeast two-hybrid constructs.

Primer name	Sequence
ABI2 forward:	5’-GGAATTCATGGACGAAGTTTCTCCTGC-3’
ABI2 reverse:	5’-CCGCTCGAGTCAATTCAAGGATTTGCTC-3’
PYL5 forward:	5’-GGAATTCATGAGGTCACCGGTGCAACT-3’
PYL5 reverse:	5’-ACGCGTCGACTTATTGCCGGTTGGTACTTC-3’
PYL9 forward:	5’-GGAATTCATGATGGACGGCGTTGAAG-3’
PYL9 reverse:	5’-ACGCGTCGACTCACTGAGTAATGTCCTGAG-3’
SnRK2.6 forward:	5’-CGGAATTCATGGATCGACCAGCAGTGAG-3’
SnRK2.6 reverse:	5’-CGGGATCCTCACATTGCGTACACAATC-3’

Supplementary Table S3. Primers used for production of PYL5, PYL 9, ABI2 and mutated ABI2 proteins in *E. coli*.

Primer name	Sequence
ABI2 forward:	5’-GGAATTCATGGACGAAGTTTCTCCTGC-3’
ABI2 reverse:	5’-CCGCTCGAGTCAATTCAAGGATTTGCTC-3’
PYL5 forward:	5’-GGAATTCATGAGGTCACCGGTGCAACT-3’
PYL5 reverse:	5’-ACGCGTCGACTTATTGCCGGTTGGTACTTC-3’
PYL9 forward:	5’-GGAATTCATGATGGACGGCGTTGAAG-3’
PYL9 reverse:	5’-ACGCGTCGACTCACTGAGTAATGTCCTGAG-3’