

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

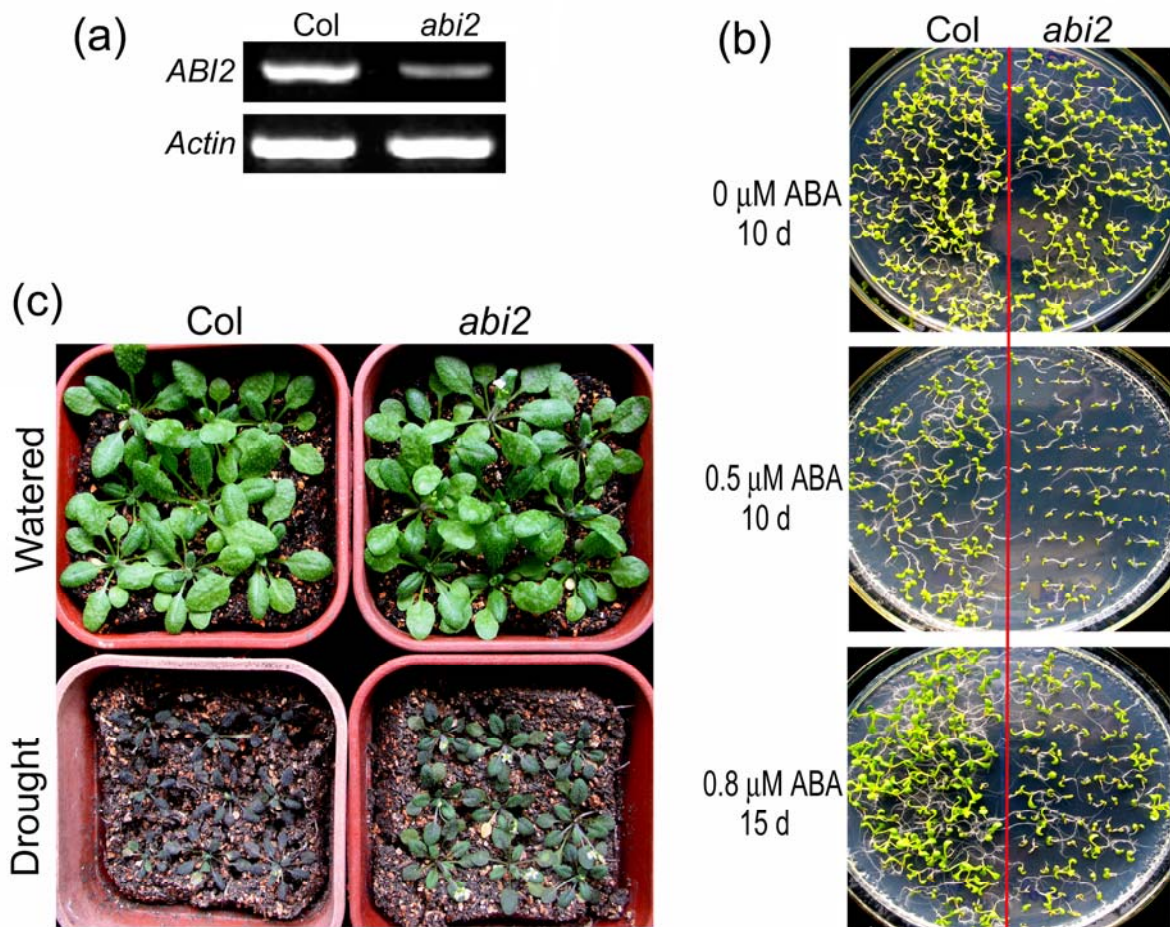
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**Supplementary Material**

# Supplemental Data

		1st		
ABI1	MEEVSPAIAGPF.....RPFSETQMD.....FTGIRLGKGYC...NNQYSNQD		40	
ABI2	MDEVSPAVAVPF.....RPFETDP.....HAGLR...GYCN.....		27	
HAB1	MEEMTPAVAMTSLAANTMCES...SPVEITQLKNVTDAADLLSDSENQSF...CNGGTECTM		57	
HAB2	MEEISPAVALTLGLAN.TMCDSGISSTFDISELENVTDAADMLCNQKRQRY...SNGVVDCIM		59	
ABI1	SENGDLMVSLP.....ETSSCSVSGSHGSESR...KV		69	
ABI2	...GESRVTLP.....ESS.CSGDGAMKDSS.....		49	
HAB1	EDVSELEEVEGQDLLKTLSDTRSGSSNVFEDDVLVSVVEDNSAVISEGLLVVDAGSEL		117	
HAB2	GSVSEKTLSE...VRSLSSDFSVTVQSEED...EPLVSDATIISEGLIVVDARSEISL		113	
ABI1	L..ISRINSPNLNMKESAAADIVVVDISAGDEINGS...ITSEKKMI		112	
ABI2	...FEINTRQDSLIS.SSSAMAGVDISAGDEINGSDEFDPR...SMNQSEKKVL		96	
HAB1	SNTAMEIDNGRVLATAIIVGESSIEQVPTAEVLIAGVNQDTN...TSEVVIRLPDEN		173	
HAB2	P.DTVEITDNGRVLATAIILNETTIEQVPTAEVLIASLNHVDVNMEVATSEVVIRLPDEN		172	
		2nd		
ABI1	SRTESRSLFEFKSVPLYGFTSICGRRPEMEDAVSTIPRFLQSSSGSMLDGR....FDPQ		167	
ABI2	SRTESRSLFEFKCVPLYGVTISICGRRPEMEDSVSTIPRFLQVSSSLLDGRV.TNGFNPH		155	
HAB1	HLVKGRSVYELDCIPLWGTVSIQGNRSEMEDAFVSPHFLKLPKMLMGDHEGMSPSLTH		233	
HAB2	VARGSRSVYELDCIPLWGTISICGGRSEMEDAVRALPHFLKLPKMLMGDHEGMSPSLPY		232	
ABI1	SAAHFFGVYDGHGGSQVANYCRERMHLALAEIIEAKEKPMLCD...GDTWLEKWKALFNS		224	
ABI2	LSAHFFGVYDGHGGSQVANYCRERMHLALTEIIVKEKPEFCD...GDTWQEKWKALFNS		212	
HAB1	LTGHFFGVYDGHGKHVADYCRDRLHFALAEIIEIRIKDELCKRNTGEGRQVQWDFVFTSC		293	
HAB2	LTSHFFGVYDGHGGAQVADYCHDRIFHSALAEIIEIRIKDELCKRNTGEGRQVQWDFVDC		292	
				P-loop
ABI1	FLRVDSEIIESVA.....PETVVGSTSVVAVVFP...SHIFVANCGDSRAVLC		267	
ABI2	FMRVDSEIETVAH.....APETVVGSTSVVAVVFP...THIFVANCGDSRAVLC		257	
HAB1	FLTVDGEIEGKIGRAVVGSSD.KVLEAVAS...ETVVGSTAVVALVCS...SHIVVSNCGDSRAVLF		352	
HAB2	YLKVDDEVKGINRPVVGSSDRMVLEAVSPETVVGSTAVVALVCS...SHIIVSNCGDSRAVLL		352	
		P-loop		
ABI1	RGKTALPLSVDHKPDREDEAARIEAAGGKVIQWNGARVFGVLAMSRSIGDRYLKPSIIPD		327	
ABI2	RGKTPLALSVDHKPDRDDEAARIEAAGGKVIQWNGARVFGVLAMSRSIGDRYLKPSVIPP		317	
HAB1	RGKEAMPLSVDHKPDREDEYARIENAGGKVIQWQGARVFGVLAMSRSIGDRYLKPYVIPP		412	
HAB2	RGKDSMPLSVDHKPDREDEYARIEKAGGKVIQWQGARVSGVLAMSRSIGDQYLEPFVIPP		412	
		3rd		
ABI1	PEVTAVKRVKEDDCLILASDGLWVDMTDEEACEMARKRILLWHKKNVAGDASLLADERR		387	
ABI2	PEVTSVRRVKEDDCLILASDGLWVDMTNEFVCDLARKRILLWHKKNAMAGEA.LLPAEKR		376	
HAB1	PEVTFMPRSREDECLILASDGLWVDMNNEVCEIARRRILMWHKKNAGAPLA.....ER		466	
HAB2	PEVTFMPRAREDECLILASDGLWVDMNNEVCEIARRRILMWHKKNAGALPLA.....ER		466	
ABI1	KEGKDPAAMSAAEYLSKLAIQKRGSKDNISVVVVDLKP...RRK...KSKPLN		434	
ABI2	GEGKDPAAMSAAEYLSKMLAQKRGSKDNISVVVVDLKG...IRK...KSKSLN		423	
HAB1	GKGIDPACQAAADYLSMLALQKRGSKDNISIIVIDLKAQRKFKTRT..		511	
HAB2	GVGEDQACQAAAEYLSKLAIQKRGSKDNISIIVIDLKAQRKFKTRS..		511	

**Supplementary Fig. S1.** Alignment of four members of the Arabidopsis PP2C family, ABI1, ABI2, HAB1 and 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 *abi2-1* T-DNA insertion mutant. (a) Semi-quantitative RT-PCR analysis indicates that *abi2-1* (indicated by *abi2*) is a knock-down mutant allele. The *abi2-1* 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'-GGGGTACCATTC AAGGATTTGCTCTTGAATTTCC-3'
I2A1F:	5'-CCATTCAGACCATTTCGCTGACCCTCACGCCGG-3'
I2A2F:	5'-CTTAGTAGAACAGAGGCTAGAAGTCTGTTTGAG-3'
I2A2R:	5'-CTCAAACAGACTTCTAGCCTCTGTTCTACTAAG-3'
I2A3F:	5'-GGTTTTGTGTTCGCGGCAAAGCGCCACTCGCGTTGTC-3'
I2A3R:	5'-GACAACGCGAGTGGCGCTTTGCCGCGACACAAAACC-3'
I2D1F:	5'-CCATTCAGACCATTTCGATGACCCTCACGCCGG-3'
I2D2F:	5'-CTTAGTAGAACAGAGGATAGAAGTCTGTTTGAG-3'
I2D2R:	5'-CTCAAACAGACTTCTATCCTCTGTTCTACTAAG-3'
I2D3F:	5'-GGTTTTGTGTTCGCGGCAAAGACCCACTCGCGTTGTC-3'
I2D3R:	5'-GACAACGCGAGTGGGTCTTTGCCGCGACACAAAACC-3'
I2F2:	5'-TGCAGTCGCTGTTCCATTCGACCCTCACGCCGGACTTAG-3'
I2F3:	5'-GAAGAAAGTACTTAGTAGAAGTCTGTTTGAG-3'
I2R3:	5'-CTCAAACAGACTTCTACTAAGTACTTTCTTC-3'
I2F4:	5'-GGGCGTTTTGTGTCCACTCGCGTTGTC-3'
I2R4:	5'-GACAACGCGAGTGGACACAAAACCGCCC-3'

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

Primer name	Sequence
<i>ABI2</i> forward:	5'-GGAATTCATGGACGAAGTTTCTCCTGC-3'
<i>ABI2</i> reverse:	5'-CCGCTCGAGTCAATTC AAGGATTTGCTC-3'
<i>PYL5</i> forward:	5'-GGAATTCATGAGGTCACCGGTGCAACT-3'
<i>PYL5</i> reverse:	5'-ACGCGTCGACTTATTGCCGGTTGGTACTTC-3'
<i>PYL9</i> forward:	5'-GGAATTCATGATGGACGGCGTTGAAG-3'
<i>PYL9</i> reverse:	5'-ACGCGTCGACTCACTGAGTAATGTCCTGAG-3'
<i>SnRK2.6</i> forward:	5'-CGGAATTCATGGATCGACCAGCAGTGAG-3'
<i>SnRK2.6</i> 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
<i>ABI2</i> forward:	5'-GGAATTCATGGACGAAGTTTCTCCTGC-3'
<i>ABI2</i> reverse:	5'-CCGCTCGAGTCAATTC AAGGATTTGCTC-3'
<i>PYL5</i> forward:	5'-GGAATTCATGAGGTCACCGGTGCAACT-3'
<i>PYL5</i> reverse:	5'-ACGCGTCGACTTATTGCCGGTTGGTACTTC-3'
<i>PYL9</i> forward:	5'-GGAATTCATGATGGACGGCGTTGAAG-3'
<i>PYL9</i> reverse:	5'-ACGCGTCGACTCACTGAGTAATGTCCTGAG-3'