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Supplementary Material

Phosphatidic acid binds to and regulates guanine nucleotide exchange factor 8 (GEF8) activity in *Arabidopsis*

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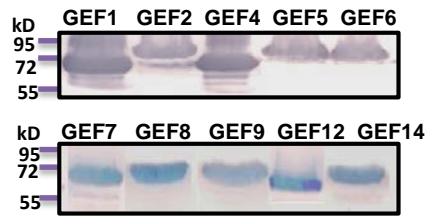


Fig. S1. Purification and immunoblotting of *Arabidopsis* recombinant GEF proteins.

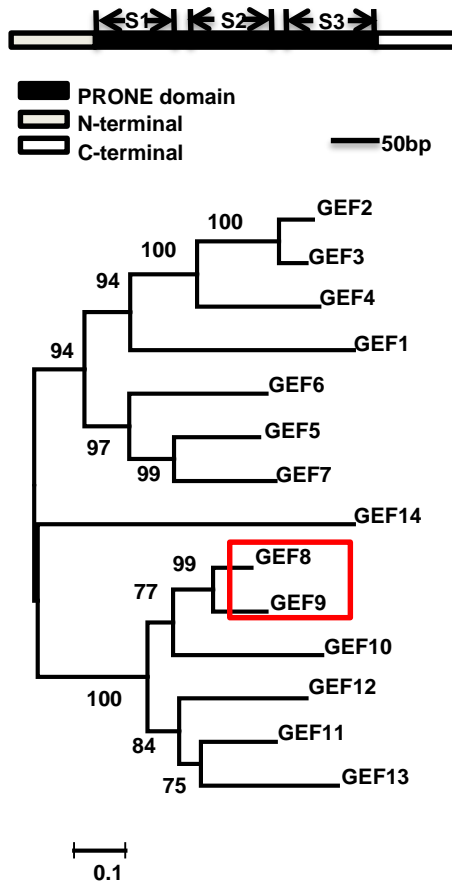


Fig. S2. Phylogenetic analysis of the GEF family in *Arabidopsis*. Top panel shows the schematic structures of GEFs, while the bottom panel shows the phylogenetic tree of GEF proteins using MEGA4.0 software.

GEF8	MVAALERCLSAKSFNFRMFDSSSTRQCCSQTIIVVENGDSFIVESNTPESQNSDSFVSPVESLPMISFIRPGKR	78
GEF9	MVPSLERCISITSSFNLDIMFDSSPKKQCC.....QHELAEITTPESQTCDSLGGSPVETSRRPMTSRLIS..RR	67
Consensus	mv lerg s sfn rmfds k qq h e pesq ds spve s pm s l r	
GEF8	SERQCADLMMKDRFKLLLGEDMSGGKGVSSALALSNAITNLAASIFGEQTKLQPMPCDRCARWKKEIDWLLSVTD	156
GEF9	QDRQCSLMMKDRFKLLLGEDMSGGKGVSSALALSNAITNLAASIFGEQTKLQPMAPDRARWKKEIDWLLSVTD	145
Consensus	qq emkdrf klllgedm gggk gvssalalsnaitnlaasifgeqtklqpm dr arwkkeidwllsvtd	
GEF8	HIVEFVPSQCISKIGVCTEIMVTRQRGDLLMNIPALRKLDAMLIDTLDNFRGHNEFWYVSRDSEEGQCARNDRTDKW	234
GEF9	HIVEFVPSQCISKIGVCTEIMVTRQRGDLLMNIPALRKLDAMLIDTLDNFRGHNEFWYVSRDSEEGQCARNDRTDKW	223
Consensus	hiv efvpsqq sk gvcteimvtrqrgdllmnipalrkldamlidtdlnfrghnefwyvsrdseeg qarndrt dkw	
GEF8	WLFPVKVPPGGLSPPRRMLYFQKDSVQVQKAAMAINAQVLESEIPESYIISLPKNGRSLGDSYKSITEEWFDP	312
GEF9	WLFPVKVPPGGLSPPRRMLYFQKDSVQVQKAAMAINAQVLESEIPESYIISLPKNGRSLGDSYKSITEEWFDP	301
Consensus	wlppvkvpvp glse rrrml fqkdsv qvqkaamainaqvlsem ip syi slpkngr slgds yk siteewfdp	
GEF8	EQFLAMLDLSTEHKVLDKNRIEASVIWKRKLHDKDKSSWGSAVSLEKRELFEERAETILVLLKQKFFGLPQSSLD	390
GEF9	EQFLSTLLDSTEHKVLDKNRIEASVIWKRKLHDKDKSSWGSAVSLEKRELFEERAETILVLLKQKFFGLPQSSLD	379
Consensus	seqfl ld stehkvl dknrieas viwkrklh kd ksswgsavslekrelfeeraetilvllkqkffglpqqssld	
GEF8	ISKIKNNDVGCQAVLESYSRILESLAYIVMSRIEDVLYIDHLAKQITLLAEETSDDGGRTTETDSEASAGSSN SGEEA	466
GEF9	ISKIKNNDVGCQAVLESYSRILESLAYIVMSRIEDVLYIDHLAKQITLLAEETSDDGGRTTETDSEASAGSSN SGEEA	456
Consensus	iskiknkdvg qavlesysrilesl yt msri edvly d la kq eetsdgg tetds sagssn sgee	
GEF8	ERHDPHS...KTLLEFGWGDNSSKG.GDKETKSPNLTTPKKLSYLEKLENLNGFRSPKDR	522
GEF9	ERLESQNSSKTTLLEFGWGDNSSKGQSEKFKSPNLTTPKKLSYLEKLENLNGFRSPKDR	516
Consensus	ek h d p h s k t l l d f g w d n s s k g k p k s p n l t p k k l s y l e k l e n l n g f r s p k d r	

Fig. S3. Amino acid alignment of GEF8 and GEF9. Sequences that were identical between the two GEFs are highlighted in black. The conserved PRONE domain is indicated by black lines, and the main difference indicated by the red box.

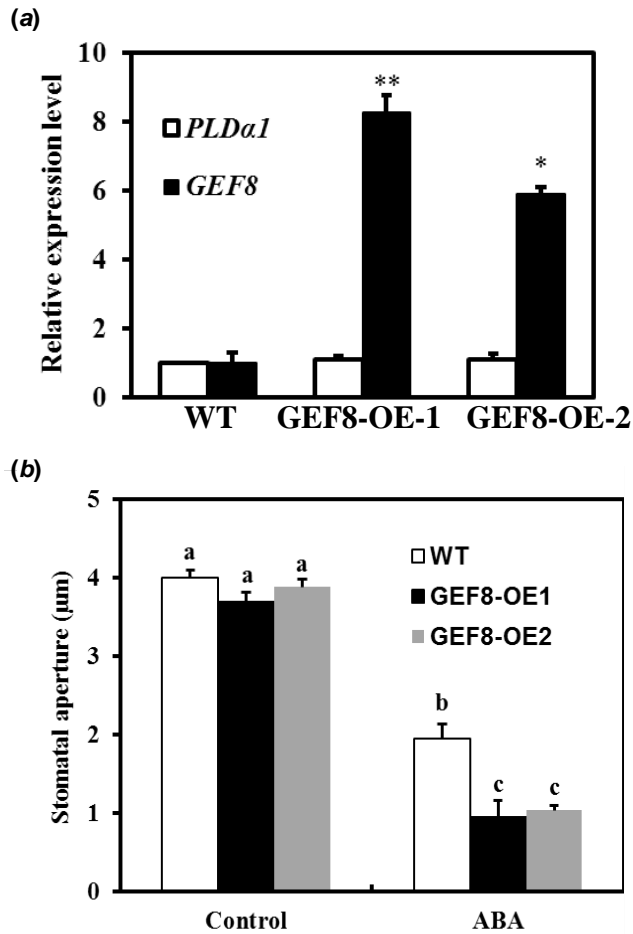


Fig. S4. Phenotype of GEF8-overexpressing lines after ABA treatment. (a) Detection of *PLDα1* and *GEF8* gene expression in GEF8-overexpressing transgenic plants (GEF8-OE) by qRT-PCR. The *Arabidopsis Actin 8* gene was used as an internal control. Asterisks mean that the average value is significantly different from that of the same gene in WT (* $P < 0.05$ and ** $P < 0.01$). (b) Applied ABA induced stomatal closure in WT and GEF8-overexpressing lines. Columns with different letters are significantly different from each other ($P < 0.05$).

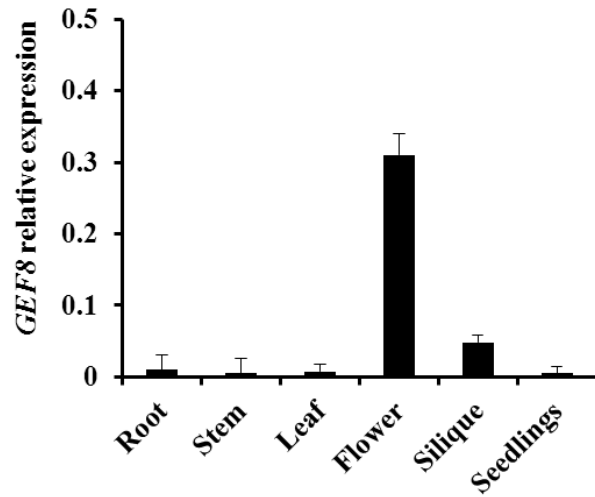


Fig. S5. qRT-PCR analysis of *Arabidopsis GEF8* expression in various tissues. The *Arabidopsis Actin 8* gene was used as an internal control to normalise the data.

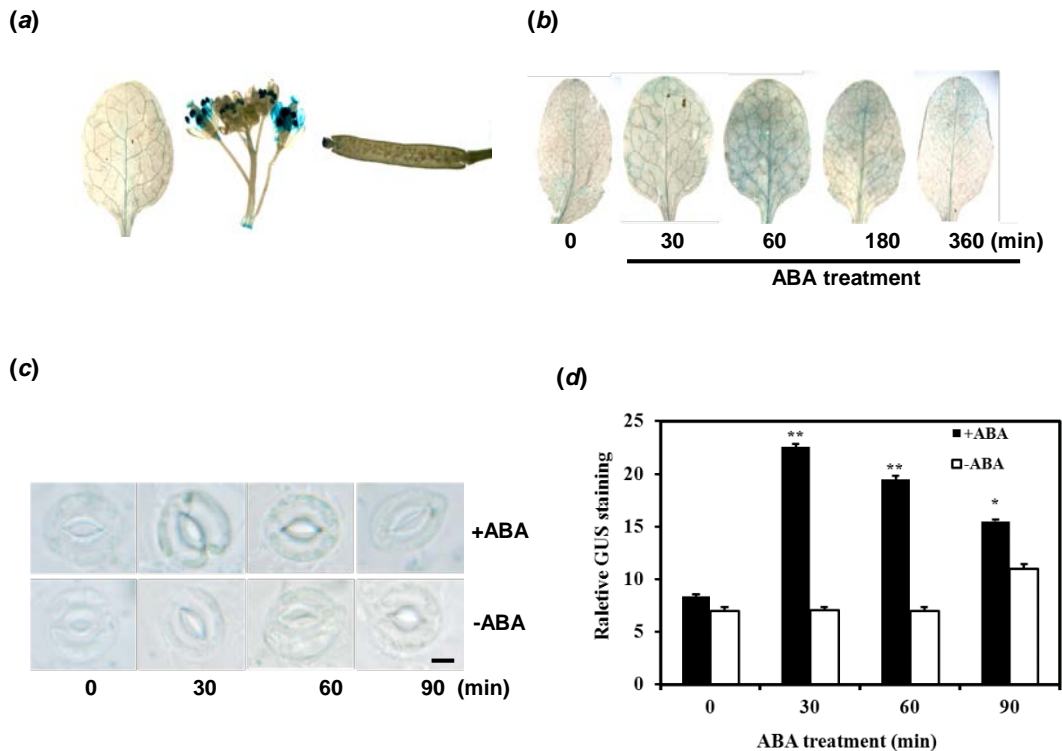


Fig. S6. Expression pattern of *GEF8* in various tissues. (a) Expression patterns of *GEF8* revealed by histochemical analysis of the *GEF8* promoter ($P_{GEF8} : GUS$). (b) ABA enhanced *GEF8* expression in $P_{GEF8} : GUS$ transgenic leaves. (c) ABA enhanced *GEF8* expression in $P_{GEF8} : GUS$ transgenic guard cells. Bar = 15 μm . (d) Quantification of GUS-staining intensity in guard cells of $P_{GEF8} : GUS$ transgenic plants. Data shown in (c) were quantified using Image J software ($n > 40$ cells from three samples). Asterisks in (d) mean that the average value is significantly different from that of the control at the same conditions (* $P < 0.05$ and ** $P < 0.01$).

Table S1. Primers used for PCR, site-directed mutagenesis, pull-down, BIFC and transgenic plant construction

Primer	Sequence
GEF1-S	5'-GTCGACTTAATCTCTTTCCGGCGTC-3'
GEF1-A	5'-TCCGAATTCATGGGGAGCTTATCTTCTGAG-3'
GEF2-S	5'-TGGGGATCCATGGAGAATTTGCCAAATCAC-3'
GEF2-A	5'-CGCCTCGAGTCATTCTTCTCCTCTCATTGT-3'
GEF4-S	5'-TTGGGATCCATGGAGAGTTCTTCCAATTCC-3'
GEF4-A	5'-TTCCCGGGCTAATCATCTCTGTTTCTCA-3'
GEF5-S	5'-CGCGGATCCATGGAGAATTTAGTGAAGAGC-3'
GEF5-A	5'-TCGCCCGGGTTAAGAGACAGTGTACTTTGG-3'
GEF6-S	5'-CGCGGATCCATGGAGGATAATAGCTGTATC-3'
GEF6-A	5'-TCCGTCGACTCAACCCCGGAGATAATTG-3'
GEF7-S	5'-TCCGGATCCATGGATGGTTCGTCGGAAAA-3'
GEF7-A	5'-GCCCTCGAGTCAAATCCCAGGATCAAGGT-3'
GEF8-S	5'-TTGGAATTCATGGTTGCAGCGTTGGAACGA-3'
GEF8-A	5'-GGGCTCGAGTTAATGCCTATCTTTGGGACTTC-3'
GEF9-S	5'-TCGGGATCCATGGTTCATCGTTGGAACG-3'
GEF9-A	5'-TCGGTCGACTCAATGCCTATCTTTAGGGCTC-3'
GEF12-S	5'-GGCGAATTCATGGTTCGTGCTTCCGGAAC-3'
GEF12-A	5'-GTCGACTATCAATGCCGTGCCGTTGGAC-3'
GEF14-S	5'-GTCGACTCAAGGAGAAGTATCAGAAG-3'
GEF14-A	5'-CGGGATTCAAGGAGAAGTATCAGAAGGC-3'
ROP1-S	5'-GGAATTCATGAGCGCTTCGAGGTTTCGT-3'
ROP1-A	5'-CCGCTCGAGTCATAGAATGGAGCATGCCT-3'
ROP4-S	5'-GGAATTCATGAGCGCTTCGAGGTTTCAT-3'
ROP4-A	5'-CCGCTCGAGTTACAAAATGGAGCAGGCT-3'
ROP6-S	5'-CGGGATCCATGAGTGCTTCGAGGTTTATAAAG-3'
ROP6-A	5'-CGGGATCCATGAGTGCTTCGAGGTTTATAAAG-3'
ROP7-S	5'-GGAATTCATGAGCACAGCAAGATTCAT-3'
ROP7-A	5'-CCGCTCGAGTTATAGGAAAAAGCATATTC-3'
ROP10-S	5'-GGAATTCATGGCTTCGAGTGCTTCAA-3'
ROP10-A	5'-CCGCTCGAGTCAATTCTTCCCACACAG-3'
GEF8-1-S	5'-CGGGATCCATGGTTGCAGCGTTGGAAC-3'
GEF8-84-A	5'-GGAATTCGCTTGTGTCTCTCAGATCTTTTT-3'
GEF8-85-S	5'-CGGGATCCGATATGGAGATGATGAAAGACAGGT-3'
GEF8-218-A	5'-GGAATTCATCTCTTGAGACATACAAAACCTCG-3'
GEF8-219-S	5'-CGGGATCCCTCAGAAGAAGGGCAGCAAGCAAG-3'
GEF8-348-A	5'-GGAATTCCTTTGTGTGAAGCTTCCCTCTTCC-3'
GEF8-349-S	5'-CGGGATCCGGACACCAAATCTTCGTGGGGC-3'
GEF8-434-A	5'-GGAATTCATAGTCCAATGTATCTGTGTAAAGA-3'
GEF8-435-S	5'-CGGGATCCAAGCAAACCTTTATTGGCTGAAGAAA-3'
GEF8-523-A	5'-GGAATTCCTTAATGCCTATCTTTGGGACTTCTA-3'
GEF8-K13A-S	5'-GAGGACTAAGCGCTTCCGCGTCATTCAACTTTAAAG-3'
GEF8-K13A-A	5'-CTTTTAAAGTTGAATGACGCGGAAGCGCTTAGTCCCTC-3'
GEF8-K18A-S	5'-CTTCAAATCATTCAACTTTGCGAGAATGTTTGATTTCATCTTC-3'
GEF8-K18A-A	5'-GAAGATGAATCAAACATTCTCGCAAAGTTGAATGATTGGAAG-3'
GEF8-K13/18A-S	5'-CGCGTCATTCAACTTTGCGAGAATGTTTGATTTCATC-3'
GEF8-K13/18A-A	5'-GATGAATCAAACATTCTCGCAAAGTTGAATGACGCG-3'
GEF8OX-S	5'-CCGCTCGAGATTCTTCCCACACAGAATGTT-3'
GEF8OX-A	5'-GGAATTCATGGTTGCAGCGTTGGAACG-3'
GEF8-SPYCE-S	5'-CGGGATCCATGGTTGCAGCGTTGGAACGA-3'
GEF8-SPYCE-A	5'-GAAGGC CATGCCTATCTTTGGGACTTC-3'
ROP7-SPYNE-S	5'-CGGGATCCATGAGCACAGCAAGATTCAT-3'
ROP7-SPYNE-A	5'-CCGCTCGAGTAGGAAAAAGCATATTCTTG-3'
ROP10-SPYNE-S	5'-ACGCGTCGACATGGCTTCGAGTGCTTCAA-3'
ROP10-SPYNE-A	5'-CCGCTCGAGATTCTTCCCACACAGAATGTT-3'
GEF8-RtPCR-S	5'-GCAGATATGGAGATGATGAAAGACA-3'
GEF8-RtPCR-A	5'-GGAAAGAGCCAGAGCAGAAGATAC-3'
actin8-S	5'-TGTTGGACTCTGGTGATGGTGTGT-3'
actin8-A	5'-ATTTCCCGTTCTGCTGTTGTGGTG-3'
GEF8-PRO-S	5'-CGGGATCCGGTACGACAACGTTTCGCTTGTT-3'
GEF8-PRO-A	5'-ACGCGTCGACGGAACCGAGAAAACAACCTGATT-3'
PLDα1-RtPCR-S	5'-CATTGTATTCTGTGCCCTCGTAACCC-3'
PLDα1-RtPCR-S	5'-GAACAAGGAGTGGAAACGGAGTGTCG-3'