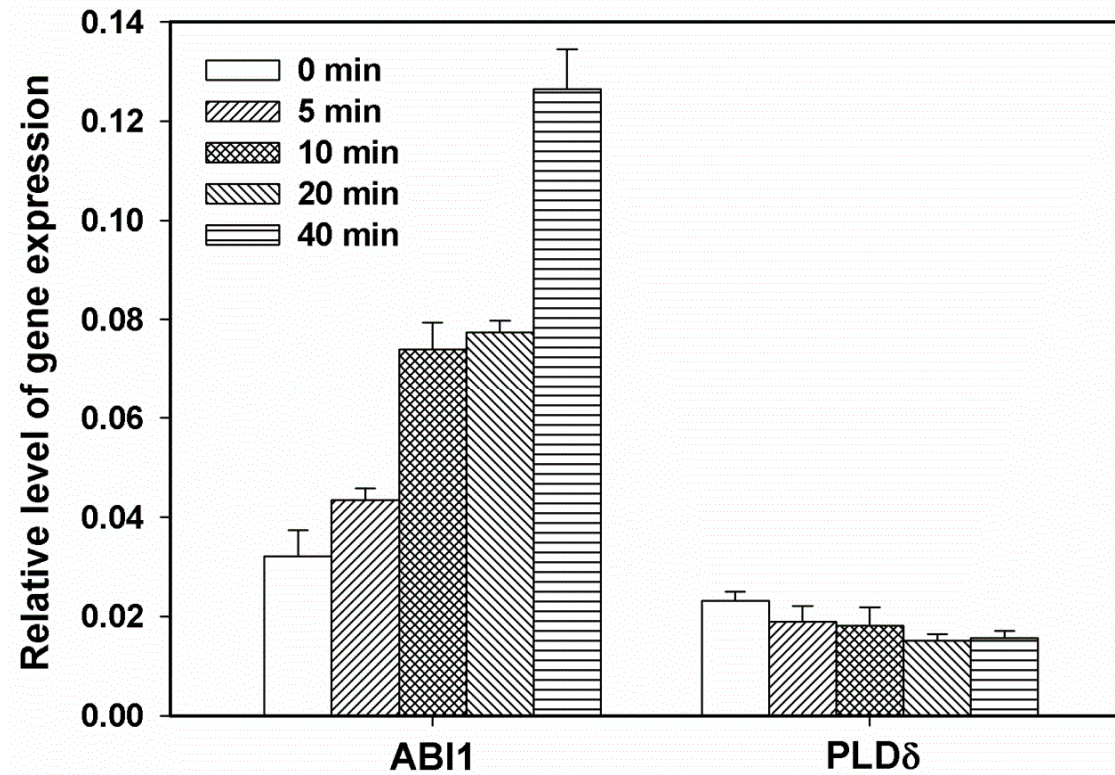
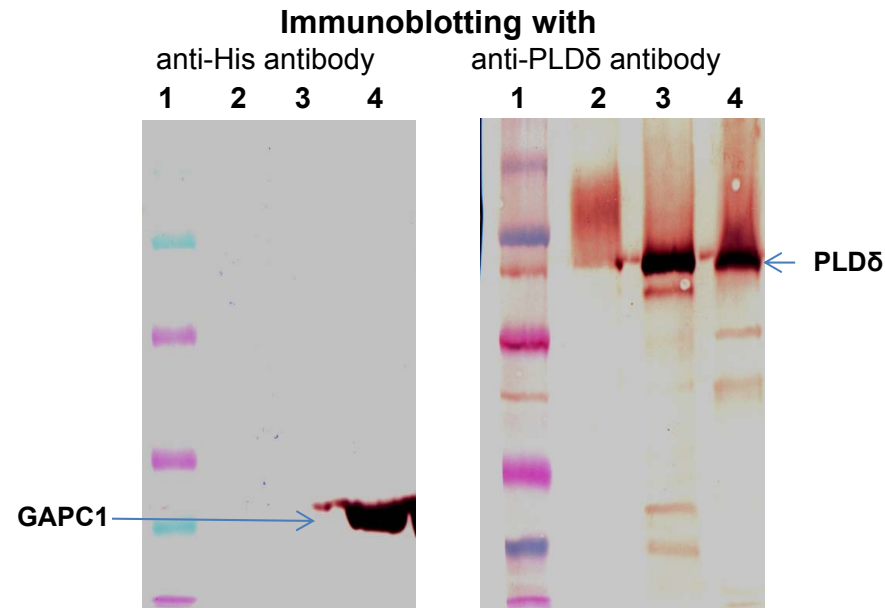


Supplemental Figure 1. Confirmation of homozygous T-DNA insertion PLD mutants by PCR. PCR was conducted using genomic DNA extracted from plant leaves with a pair of gene specific primers (PLDα1RP+PLDα1LP for *PLDα1* and PLDδRP+PLDδLP for *PLDδ*) or a combination of a T-DNA left border primer (LBa1) and gene specific primers (PLDα1RP and PLDδRP). The presence of a T-DNA band and lack of a *PLDα1* or *PLDδ* band indicate that *pldδ* and *pldα1pldδ* are homozygous T-DNA mutants. The primers used for PCR are listed in Table S1.



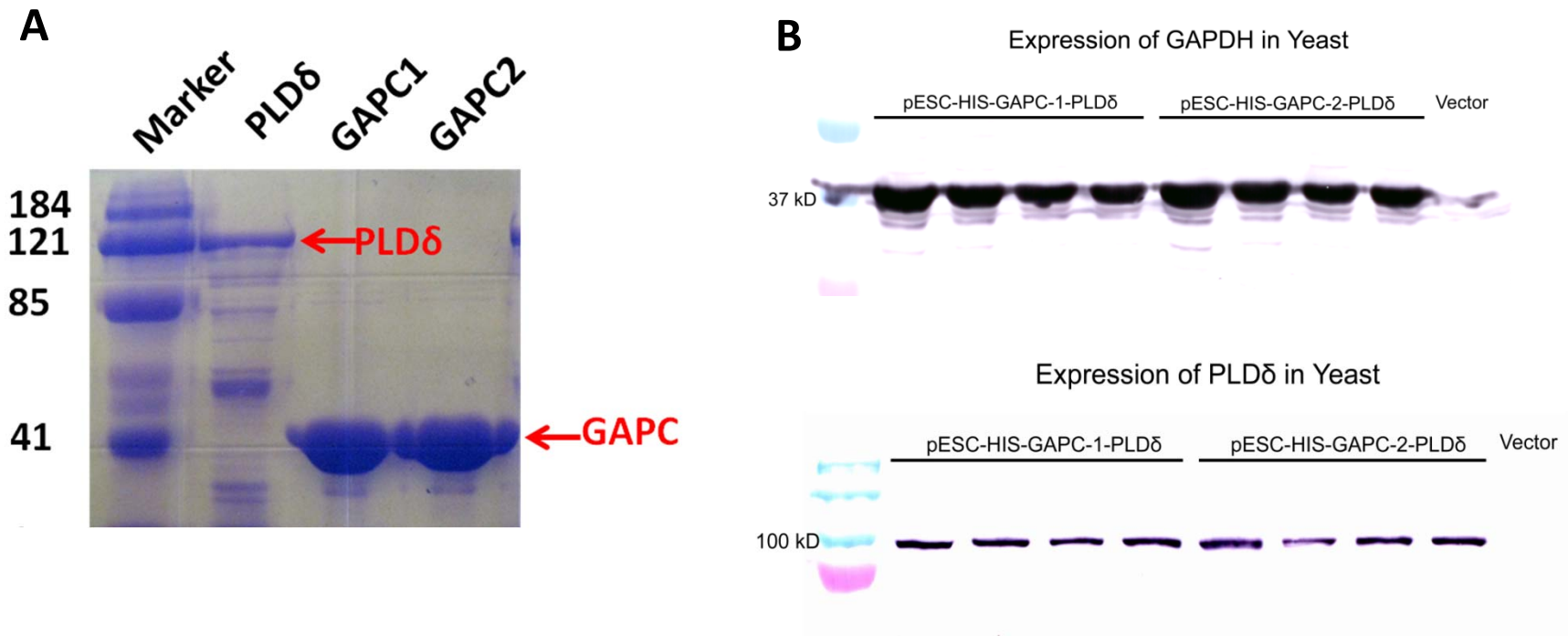
Supplemental Figure 2. Expression level of *PLDδ* in response to ABA.

RNA was extracted from leaves sprayed with 100 μ M ABA with 0.01% Triton X-100. *PLDδ* transcript level was measured by real-time PCR normalized to *UBQ10*. The ABA response gene *ABI1* was used as a positive control. The experiment was repeated three times with similar results. Values are means \pm SE (n = 3) for one representative experiment. The primers for real-time PCR are listed in Table S1.



- Lane 1, Protein Kd standards
- Lane 2, Nickel-bead pulldown of *Arabidopsis* microsomal proteins without His-GAPC
- Lane 3, Total leaf microsomal extracts
- Lane 4, Nickel-bead-pulldown from microsomal proteins with added His-GAPC

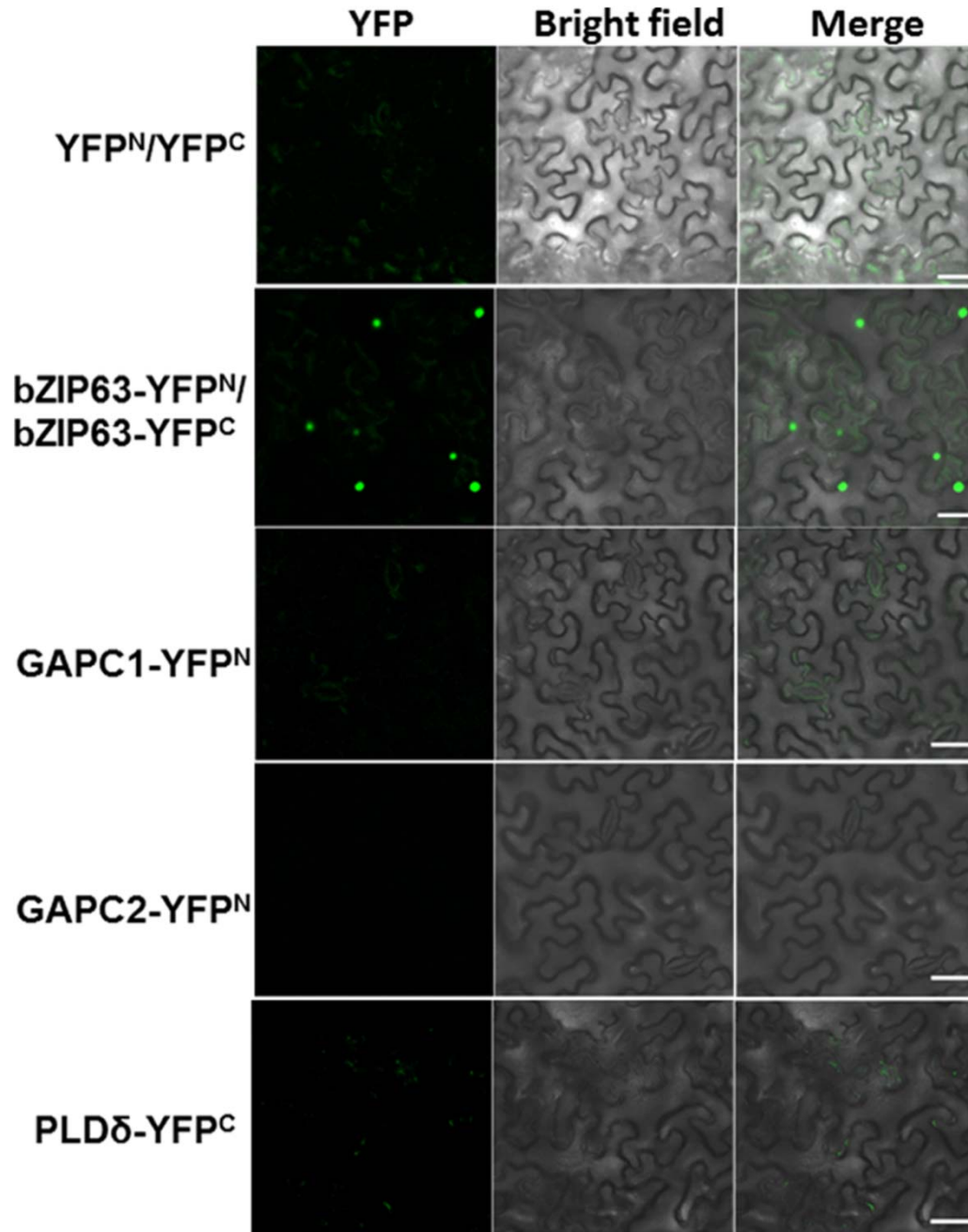
Supplemental Figure 3. PLD δ -GAPC association as identified by GAPC1 co-precipitation of PLD δ from microsomal proteins of *Arabidopsis* leaves. Immunoblotting using anti-PLD δ (*Right*) or anti-His-tag (*Left*) antibodies of GAPC1-copulldown from *Arabidopsis* leaf microsomal extracts. *Arabidopsis* GAPC1 was fused with a His-tag, purified from *E. coli*, and incubated with leaf microsomal proteins. Microsomal fraction was isolated and dissolved in buffer A containing 0.5% Triton X-100, following a procedure described previously (Wang and Wang, 2001). Precipitates with nickel-sepharose beads were washed five times in PBS containing 0.5% Triton X-100, subjected to 10% SDS-PAGE, immunoblotted with anti-His (*Left*) or anti-PLD (*Right*) antibodies, and visualized using alkaline phosphatase staining.



Supplemental Figure 4. Purification and immunoblotting of PLD δ and GAPCs produced in *E. coli* and yeast.

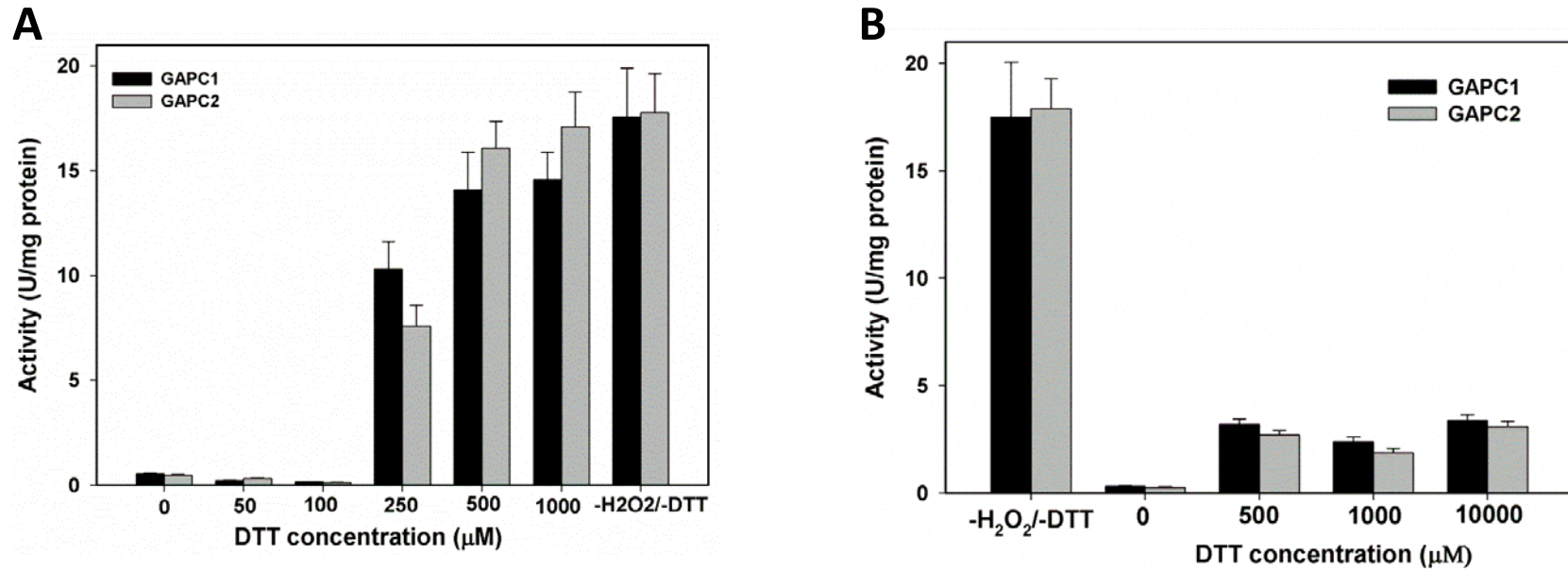
(A) Coomassie blue staining of purified PLD δ and GAPCs. PLD δ was expressed in *E. coli* as a GST fusion. GAPC1 and GAPC2 were fused with His tag and expressed in *E. coli*. PLD δ was purified using GST beads and GAPCs were purified using Ni-NTA agarose. Proteins were separated on a 10% SDS-PAGE gel followed by Coomassie blue staining.

(B) Immunoblotting of PLD δ and GAPCs expressed in yeast. PLD δ was co-expressed with GAPC1 or GAPC2 in yeast. Total protein (10 μ g) extracted from yeast was loaded on a 10% SDS-PAGE gel. PLD δ was immunoblotted with anti-FLAG antibody conjugated with alkaline phosphatase. GAPC1 and GAPC2 were immunoblotted with anti-cMyc antibody conjugated with alkaline phosphatase.



Supplemental Figure 5. Negative and positive control for BiFC.

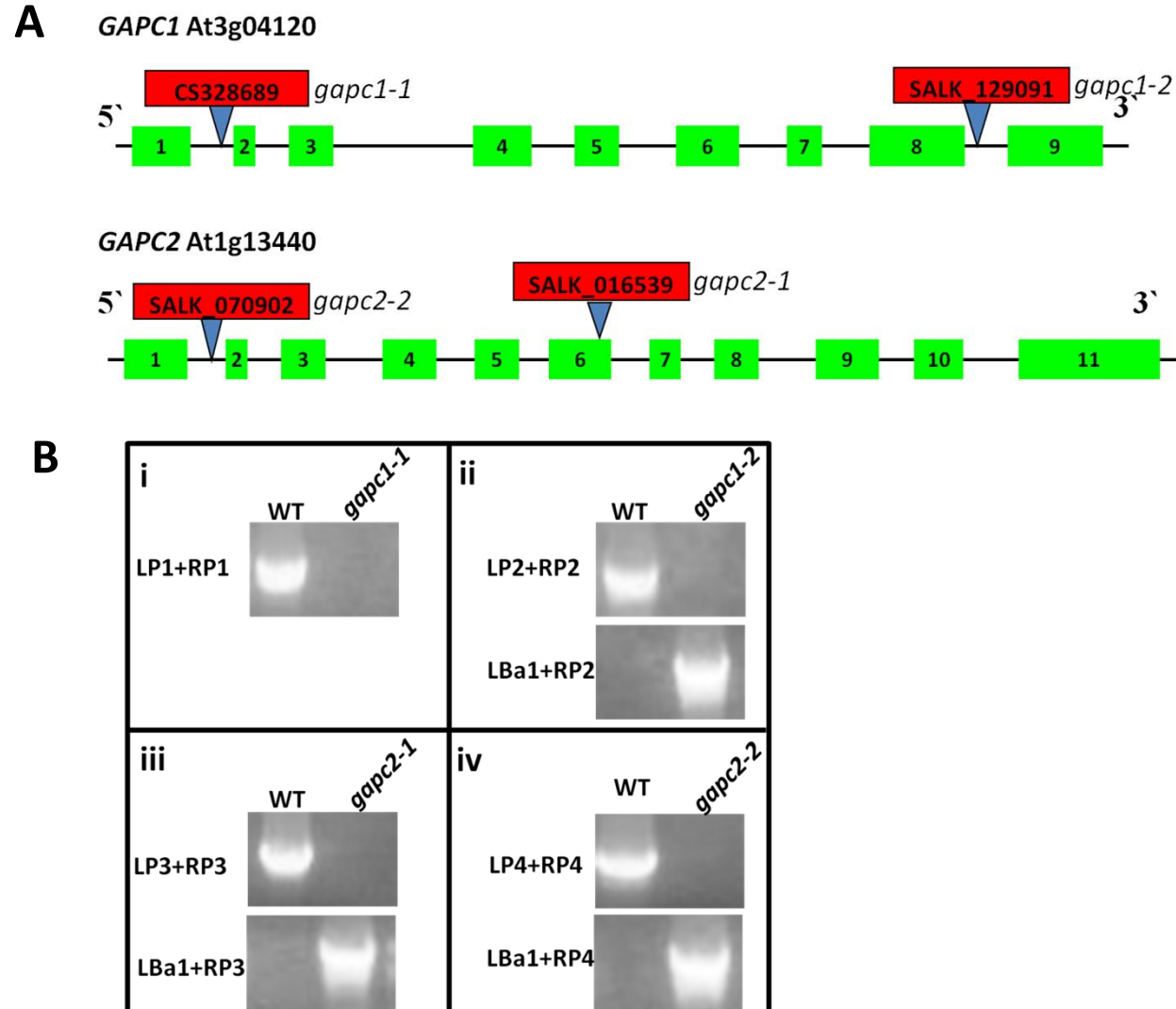
Empty vectors (YFP^N/YFP^C) were used as negative control and did not show YFP fluorescence. The positive control bZIP63-YFP^N was co-expressed with bZIP63-YFP^C. Green color (spot) in the lower panel represents YFP fluorescence, indicating formation of dimers of bZIP63 in the nucleus of plant cells. The constructs were co-transformed into tobacco leaves by infiltration. GAPC-YFP^N and PLDδ-YFP^C were transformed separately and did not generate YFP fluorescence on their own. Photographs were taken with a Zeiss LSM 510 confocal microscope. Scale bar = 50 μm.



Supplemental Figure 6. DTT protection of GAPC activity.

(A) GAPC activity in the presence of increasing concentrations of DTT followed by addition of 50 μM H₂O₂.

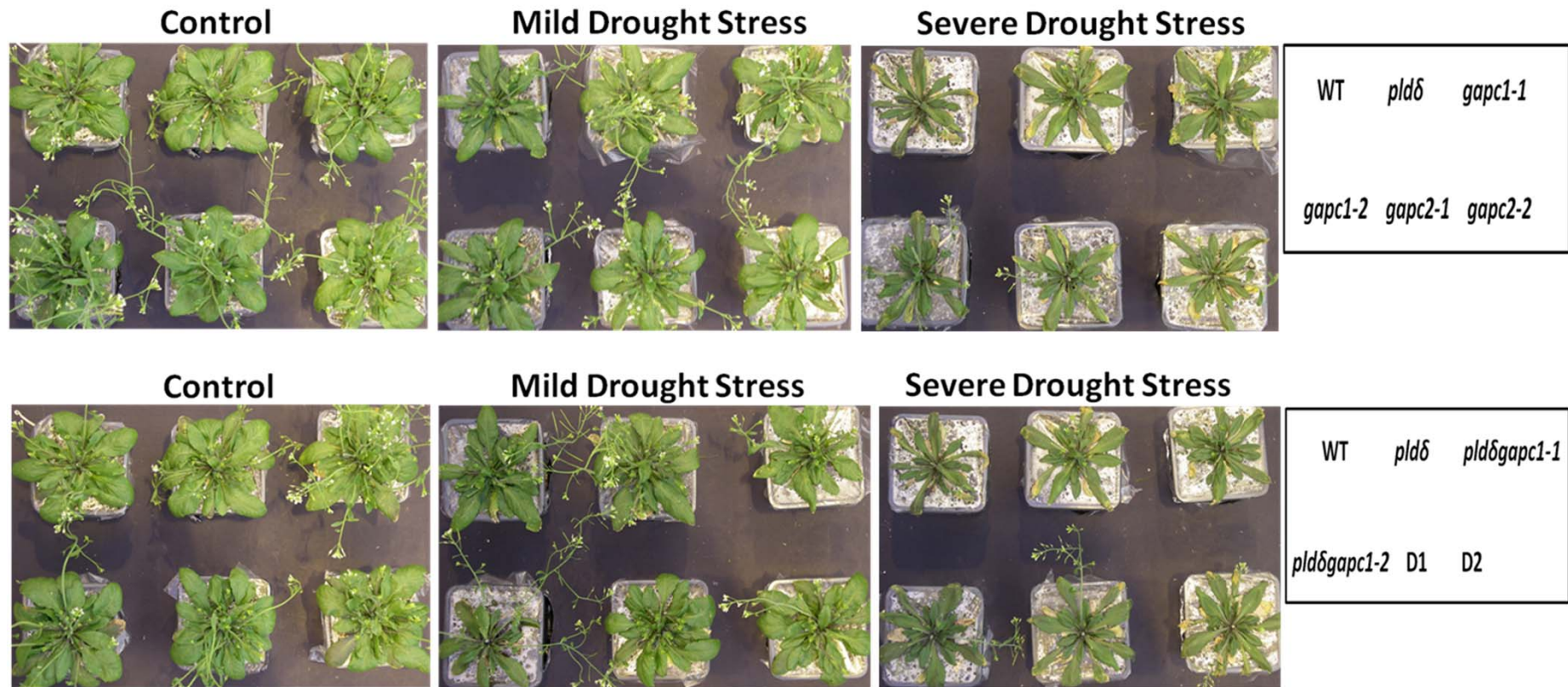
(B) DTT recovery of GAPC activity after H₂O₂ treatment for 10 min. Values are means \pm SE (n = 3).



Supplemental Figure 7. Isolation of *GAPC* T-DNA homozygous lines.

(A) Gene structure with the sites of T-DNA insertion. Two individual T-DNA insertion lines were isolated for *GAPC1* and *GAPC2*, respectively.

(B) PCR genotyping of mutants. All four lines were homozygous mutants as shown by the absence of gene-specific bands in mutants (LP+RP). PCR was done with genomic DNA and the primers are listed in Table S1. 7



Supplemental Figure 8. Growth phenotype of WT and GAPC and PLD δ mutants under control and drought conditions. Photos were taken at the end of experiment when plants were 6-week old. Three experiments were performed with similar results. D1 represents *gapc1-1gapc2-1* and D2 represents *gapc1-1gapc2-2*.

Supplemental Table: Primer list

Purpose	Gene	Primer Sequence
T-DNA confirmation	T-DNA	Lba1: 5'-TGGTTCACGTAGTGGGCCATCG-3'
<i>plda1</i> screening	<i>PLDα1</i>	PLD α 1RP: 5'-CAAGGCTGCAAAGTTTCTCTG-3' PLD α 1LP: 5'-ATTAAGTGCAGGGCATTGATG-3'
<i>pldδ</i> screening	<i>PLDδ</i>	PLD δ RP: 5'-TCCGTTTGACCAGATCCATAG-3' PLD δ LP: 5'-TTGCGATTATTACCAACAGCC-3'
<i>gapc1-1</i> screening	<i>GAPC1</i>	RP1: 5'-CTGATGCCGAAAACAGTGACT-3' LP1: 5'-CTAGATCATTCCGACCCCTC-3'
<i>gapc1-2</i> screening	<i>GAPC1</i>	RP2: 5'-CGAAAACGACAAATTCAGACC-3' LP2: 5'-GAAGGTTGTTATCTCTGCCCC-3'
<i>gapc2-1</i> screening	<i>GAPC2</i>	RP3: 5'-AGTGTTACGGTCAGTGGAAG-3' LP3: 5'-GGTTAGGACTGAGGGTCCTTG-3'
<i>gapc2-2</i> screening	<i>GAPC2</i>	RP4: 5'-TGTCTGCAACAAATCGATACC-3' LP4: 5'-AATGGTTGGAGTAATGTTGCT G-3'
<i>GAPC1</i> cloning/ expression in <i>E.coli</i>	<i>GAPC1</i>	Forward: 5'-GCGGGATCCATGGCTGACAAGAAGA-3' Reverse: 5'-GCGAAGCTTTTAGGCCTTTGACATGT-3'
<i>GAPC2</i> cloning/ expression in <i>E.coli</i>	<i>GAPC2</i>	Forward: 5'-GCGGGATCCATGGCTGACAAGAAGATCAGA-3' Reverse: 5'-GCGAAGCTTTTAGGCCTTTGACATGTGAA-3'
<i>GAPC1</i> cloning/ expression in yeast	<i>GAPC1</i>	Forward: 5'-GCGGATCCGATGGCTGACAAGAAGATTAGG-3' Reverse: 5'-GCGGGCCCTTGGCCTTTGACATGTGGACGAT-3'
<i>GAPC2</i> cloning/ expression in yeast	<i>GAPC2</i>	Forward: 5'-GCGGATCCGATGGCTGACAAGAAGATCAGA-3' Reverse: 5'-GCGGGCCCTTGGCCTTTGACATGTGAACG-3'
<i>PLDδ</i> cloning/ expression in yeast	<i>PLDδ</i>	Forward: 5'-GCCCGGGTATGGCGGAGAAAGTATCGGA-3' Reverse: 5'-GCGTCGACTTACGTGGTTAAAGTGTGCAAG-3'
<i>GAPC1</i> BiFC	<i>GAPC1</i>	Forward: 5'-GCG TCTAGAATGGCTGACAAGAAG AT-3' Reverse: 5'-CGC GGTACCGGCCTTTGACATGTG GA-3'
<i>GAPC2</i> BiFC	<i>GAPC2</i>	Forward: 5'-GCGTCTAGAATGGCTGACAAGAAGATCAGA-3' Reverse: 5'-CGCGGTACCGGCCTTTGACATGTGAACG-3'
<i>PLDδ</i> BiFC	<i>PLDδ</i>	Forward: 5'-GCGGGCGCGCCATGGCGGAGAAAGT-3' Reverse: 5'-CGCCCCGGGCGTGGTTAAAGTGTCA-3'
<i>pldδ</i> Complementation	<i>PLDδ</i>	Forward: 5'-TGGATGGATTTATGGATCAGT-3' Reverse: 5'-GGGTGCAAAATGTAGAGATCG-3'
RT-PCR	<i>GAPC1</i>	GAPC1-3'UTR: 5'-GAPCCCTATCATTCGAGATCTGCTTC-3'
RT-PCR	<i>GAPC2</i>	GAPC2-3'UTR: 5'-TCAACCACACACAACTCTCG-3'
RT-PCR	<i>18S</i> <i>rRNA</i>	Forward: 5'-TGGTCTTAATTGGCCGGGTC-3' Reverse: 5'-CTAAGAACGGCCATGCACCAC-3'
Realtime PCR	<i>PLDδ</i>	Forward: 5'-TGGGCGCATACCAACCTAATCA-3' Reverse: 5'-TGGCTCCACAACTCATCTCCA-3'
Realtime PCR	<i>ABI1</i>	Forward: 5'-TGTGGTGGTGGTTGATTTGAAGCC-3' Reverse: 5'-GCCTCAGTTCAAGGGTTTGCTCTT-3'
Realtime PCR	<i>UBQ10</i>	Forward: 5'-CACACTCCAATTGGTCTTGCGT-3' Reverse: 5'-TGGTCTTCCGGTGAGACTCTTCA-3'