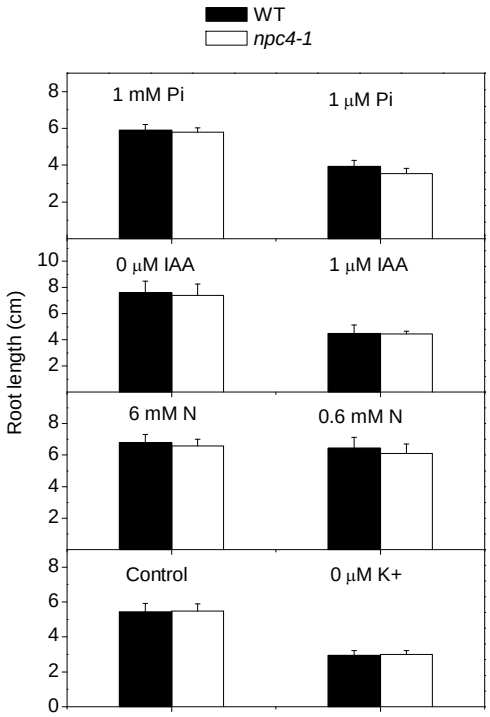
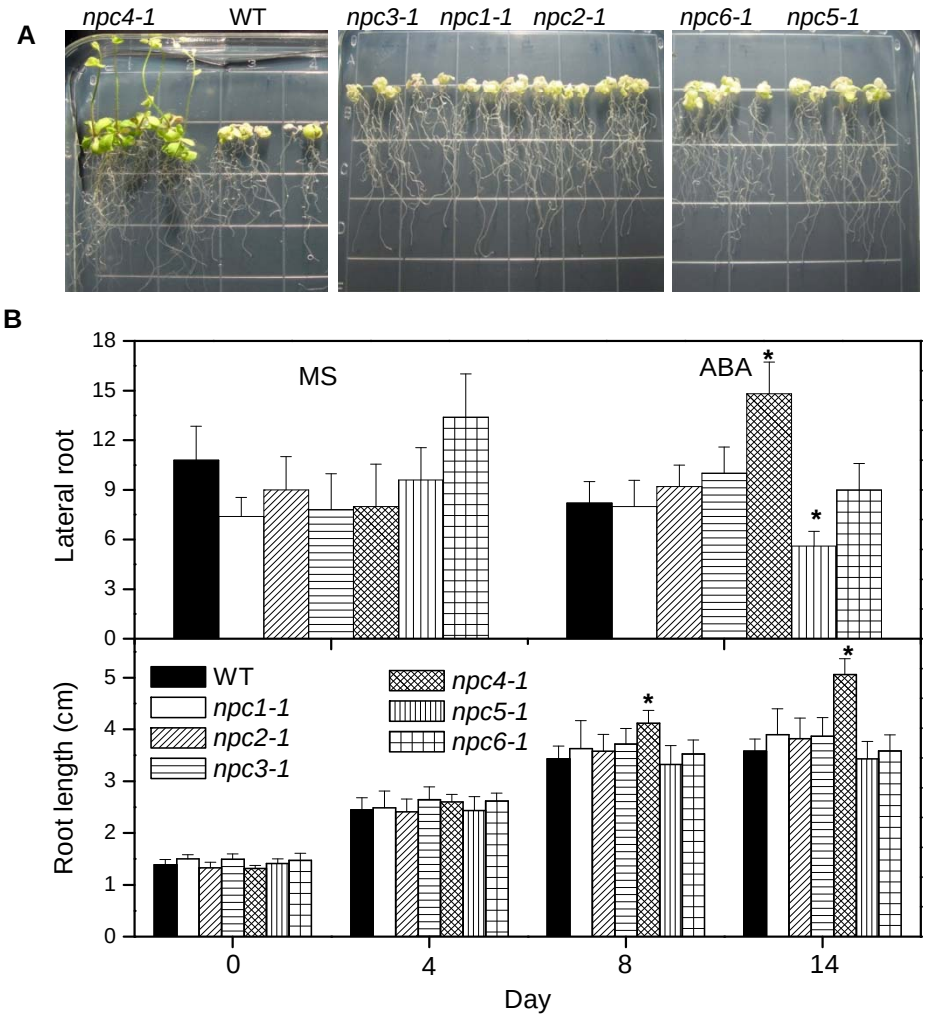


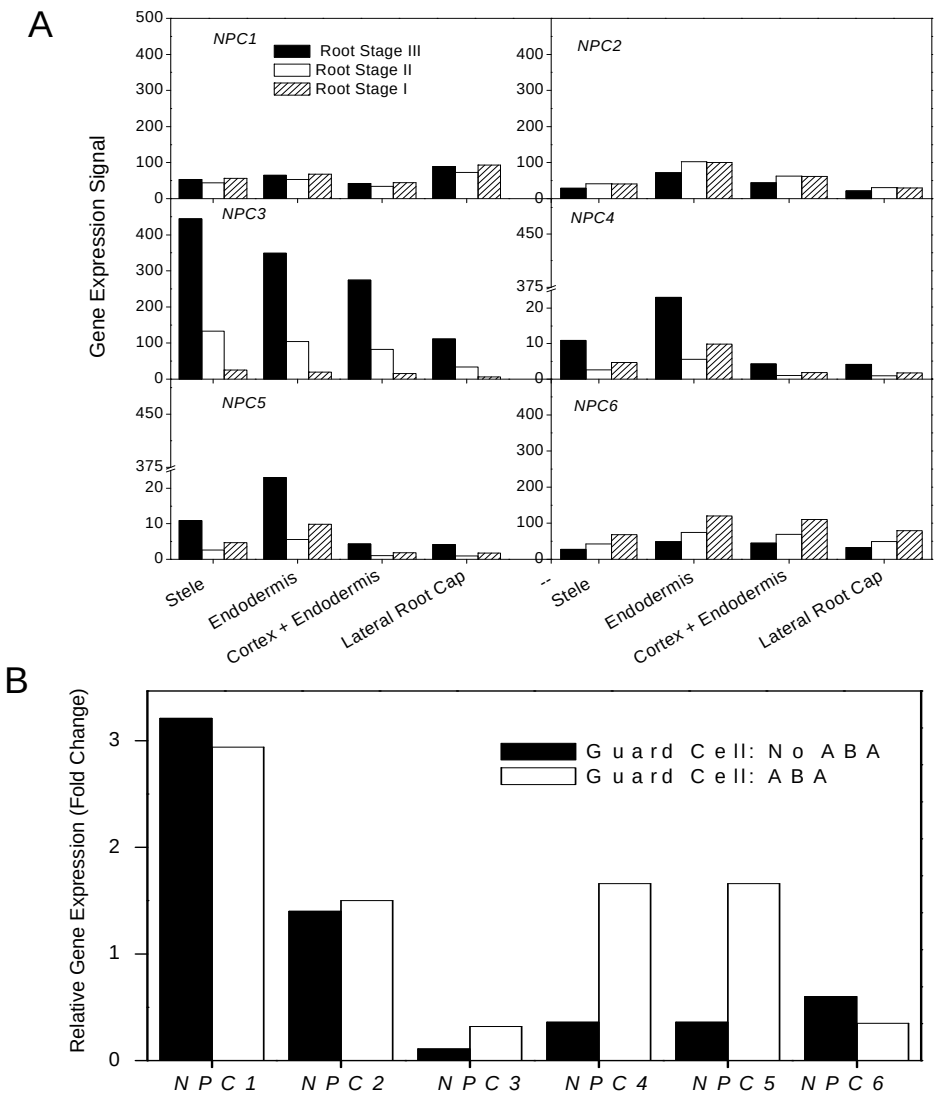
Supplemental Figure 1. Root length of *npc4-1* and WT seedlings grown in medium deficient in phosphate, potassium or nitrogen, or containing IAA.

For root growth assays, 5-day old seedlings were transferred to treatment media and grown under cool white fluorescent light of 100 ~mol $m^{-1}s^{-1}$ under 12-h light/ 12-h dark and 23°C/22°C cycles. Phosphate media were prepared as described by Lopez-Bucio et al. (2002). Measurements were taken after 10 days (*uppermost panel*). Values are means ± SD (n=15). For WT and *npc4-1* in response to IAA, five-day old seedlings were transferred to ½ MS media with or without IAA and the primary root growth was recorded after 7 days (*second panel*). Values are means ± SD (n=15). Nitrogen media were prepared as described by Hong et al. (2009). Measurements were taken after 8 days (*third panel*). Values are means ± SD (n=15). Potassium deficient media were prepared as described by Jung et al. (2009). Measurements were taken after 8 days (*lower panel*). Values are means ± SD (n=15).



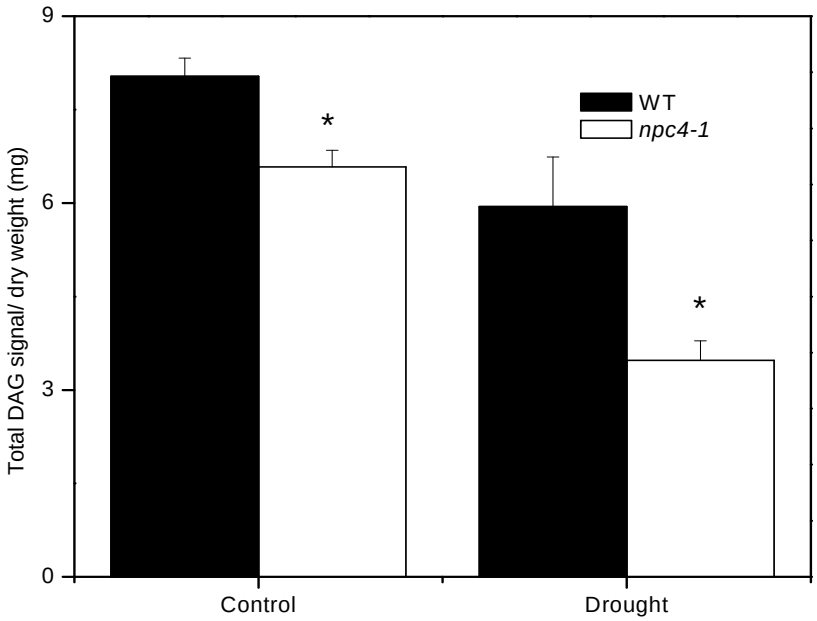
Supplemental Figure 2. Phenotype of six NPC knockout mutants and WT seedlings in response to ABA.

(A) Seedlings on ½ MS media containing ABA. Picture was taken two weeks after five-day old seedlings were transferred to medium containing 25 µM ABA. (**B**) Lateral root number and root length on ½ MS media or media supplemented with 25 µM ABA. *Upper Panel*, Lateral roots were scored after two weeks on media with or without ABA. Values are means +/- SD (n = 5). *Lower Panel*, primary root length was measured at indicated days after transfer. Day 0 refers to the day of transfer and before exposure to ABA. Values are means +/- SD (n = 15). Plants were grown under cool white fluorescent light of 100 ~mol $m^{-1}s^{-1}$ under 16-h light/ 8-h dark and 23°C/22°C cycles for 2 weeks. * Significant at $P < 0.05$ compared with the WT based on Student's *t* test.



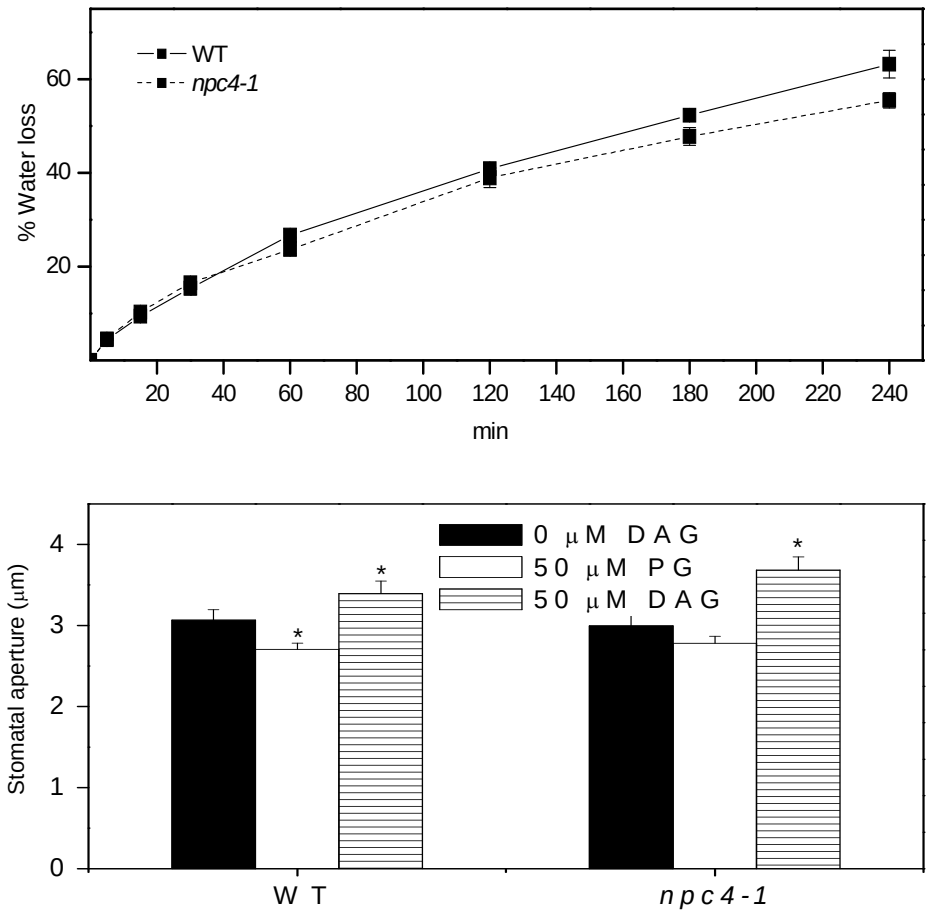
Supplemental Figure 3. Expression of *NPC*s in root cells and guard cells in response to ABA.

(A) Expression of the NPCs in root cells as reported by http://bar.utoronto.ca. Col-0 Arabidopsis plants were grown under 16-h light/ 8-h dark conditions on MS with 4.5% sucrose. Roots cells were isolated by protoplasting and fluorescence-activated cell sorting from 6-day old plants. RNA was hybridized to ATH1 GeneChips normalized by GCOS, (n=3). Results from Bimbaum et al. (2003) Science 302:1956; Nawy et al. (2005) Plant Cell 17: 1908. (**B**) RNA was hybridized to ATH1 GeneChip from guard cell protoplasts of 5-week old Arabidopsis Col-0 grown under 16-h light/8-h dark. The data were normalized by GCOS and measurements were taken in duplicates. Results from Yang et al. (2008) Plant Methods 4:6 as reported by http://bar.utoronto.ca. Note that the database data failed to distinguish *NPC4* and *NPC5* due to high sequence similarity. However, our data using gene specific primers showed that *NPC5* was mostly expressed in inflorescences, and its level of expression in leaves and roots was much lower than that of *NPC4* (refer to Figure 3). Thus, the combined NPC4/5 transcript level in leaves and roots may result mostly from the *NPC4* expression.



Supplemental Figure 4. Drought effect on DAG content in WT and *npc4-1*.

Water was withheld from four-week old soil grown Arabidopsis plants for 10 days; leaves were excised and total lipids were extracted as described by Welti et al. (2002). Briefly, excised tissue was immersed immediately in 3 ml hot isopropanol containing 0.01% butylated hydroxytoluene at 75°C. Samples were kept at 75°C for 15 min and 1.5 ml of chloroform and 0.6 ml of water were added. Samples were placed on a shaker for 1 h, and the solvent was transferred to a new tube. The samples were re-extracted using chloroform:methanol (2:1) at least 4 times for 30 min each time on a shaker. The lipid extracts were combined and washed with 1 M KCl followed by washing with 1 ml water. The solvent was evaporated under a stream of nitrogen gas and the remaining tissue was oven-dried at 100°C and weighed. DAG content was analyzed using ESI-MS/MS. * Denotes that the difference between WT and mutant is significant with $P < 0.05$ as determined by Student's *t* test. Values are means +/- SE (n = 5).



Supplemental Figure 5. Water loss from detached leaves and DAG effect on stomatal opening
(A) Fresh weight changes from detached leaves. Detached leaves of six-week-old plants were exposed to cool white light (125 mol $m^{-1}s^{-1}$) at 23°C. Leaves were weighed at various time intervals, and loss of fresh weight (%) was used to indicate water loss. Values are means +/- SD (n = 5). Experiment was performed at least three times with similar results. **(B)** The effect of DAG nad PG on stomatal opening in WT and *npc4-1***.** Experiment was performed as described by Lee and Assmann (1991). Briefly, epidermal peels of fully expanded leaves from 4-week old Arabidopsis plants were kept in darkness for 3 h to promote closure. Peels were then placed under cool white light (150 μmol $m^{-1}s^{-1}$) for 2 h and 8:0/8:0 DAG or 8:0/8:0 PG was added every 30 min. Stomatal aperture was visualized under a light microscope and measured using Image Pro software. * Denotes that the difference between control and treatment is significant with $P < 0.05$ as determined by Student's *t* test. Values are means +/- SE (n = 25-40).

Supplemental Table 1. Primers for identifying NPC-KO mutants and real-time PCR for genes in ABA response.

	Gene	Primer Sequence
Primers for identifying NPC- KO mutants	*NPC1*	Forward 5'-GTCTGCTCCTGCTTCCAGTGC-3' Reverse 5'-TCTGGTTTTGAATCCCCCTTAGC-3'
	NPC2	Forward 5'-GCTTGCCTTGCTTTGCATGAT-3' Reverse 5'- TCTCCGAGTTCGCTGTTTTCG-3'
	NPC3	Forward 5'-CGTGGACAGTTGGACACATGC-3' Reverse 5'- TTCGGGATCTAAACCGGGAAA-3'
	NPC4	Forward 5'-AGCTACCCAACTACGTCGTGGTCG-3' Reverse 5'- GGTTGAGCAGCATTTGATGCTTC-3'
	NPC5	Forward 5'- TCACCTCCCCTTTCTCTTGCC-3' Reverse 5'- TTGTGGTTGAACAACGTTGGTATG-3'
	NPC6	Forward 5'- GAATCCAAGGCGAAACCATAATGGT-3' Reverse 5'- CAGTAAACCCGACAATAAACGGT-3'
Real time PCR primers for ABA responsive genes	*NPC1*	Forward 5'-ATATGTGCCCTCTGCATGTACCCA-3' Reverse 5'-ATGGAGGCCATACCCGACAATCAT-3
	NPC2	Forward 5'-AGAGCTTGTACAACTAGCTGCGGT-3' Reverse 5'-AGCCATACGACCAGCCTCTAAGAA-3'
	NPC3	Forward 5'-CTGATTCAAGCAGCGGCTGTACTA-3' Reverse 5'-AGCCTTCTTGGACTCACCATGGAA-3'
	NPC4	Forward 5'-AGCATCAAATGCTGCTGCTCAACC-3' Reverse 5'-TCCACCCACACACAAGAGAAGTGA-3'

	NPC5	Forward 5'-GGAGACTACAAAAACGAAGAATTGCTAT-3' Reverse 5'-GTGATGGTGGTGGTTTCACAACATTAT-3'
	NPC6	Forward 5'-GCGGTTCTCAATGGTGATCACTTCCT-3' Reverse 5'-GGCACCAAGCTTCATTGCCTCTTTAC-3'
	ABI1	Forward 5'-TGTGGTGGTGGTTGATTTGAAGCC-3' Reverse 5'-GCCTCAGTTCAAGGGTTTGCTCTT-3'
	ABI2	Forward 5'-AAGTGTGCGATTTGGCTCGGAAAC-3' Reverse 5'-TCCGGCCATCGCGTTCTTCTTAT-3'
	RAB18	Forward 5'-GCAGTCGCATTCGGTCGTTGTATT-3' Reverse 5'-ACAACACACATCGCAGGACGTACA-3'
	RD29B	Forward 5'-ACAATCACTTGGCACCACCGTT-3' Reverse 5'-AACTCACTTCCACCGGAATCCGAA-3'
	RCN1	Forward 5'-GCGAAGCTGCAGCAAACAACCTAA-3' Reverse 5'-CTGCATTGCCCATTCAGGACCAAA-3'
	OST1	Forward 5'-TGGAGGAAGACTTAGAGAGCGACCTT-3' Reverse 5'-TGCGTACACAATCTCTCCGCTACT-3'
	ERA1	Forward 5'-TGTGGCTCAGCACGCTTGGTTAAA-3' Reverse 5'-ACCCATAATGTCGCGAGTCAAAGG-3'
	UBQ10	Forward 5'-CACACTCCACTTGGTCTTGCGT-3' Reverse 5'-TGGTCTTTCCGGTGAGACTCTTCA-3'

Supplemental References

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