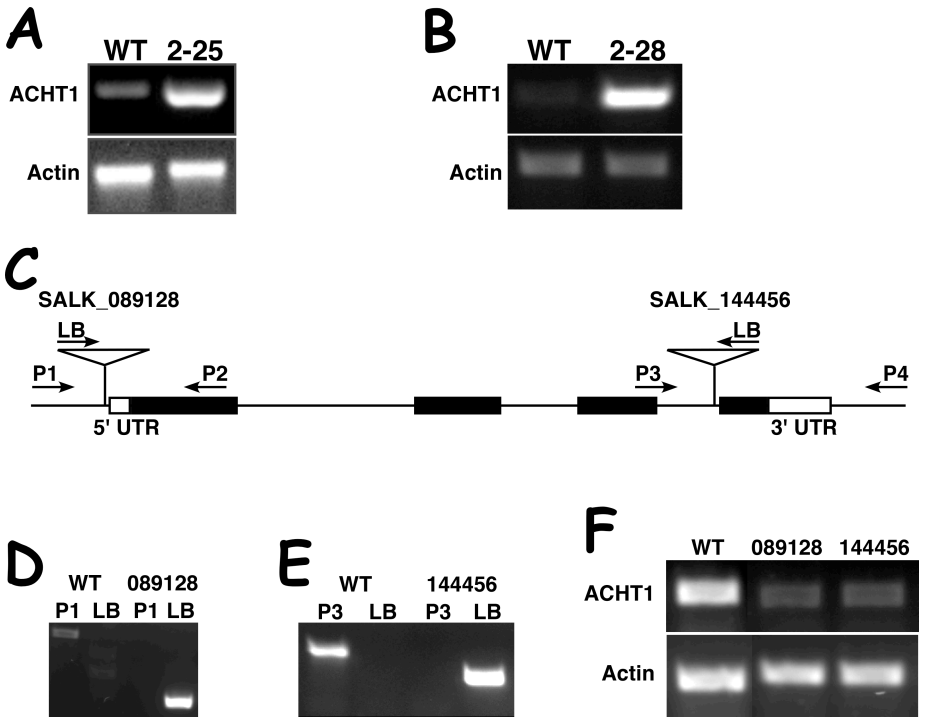
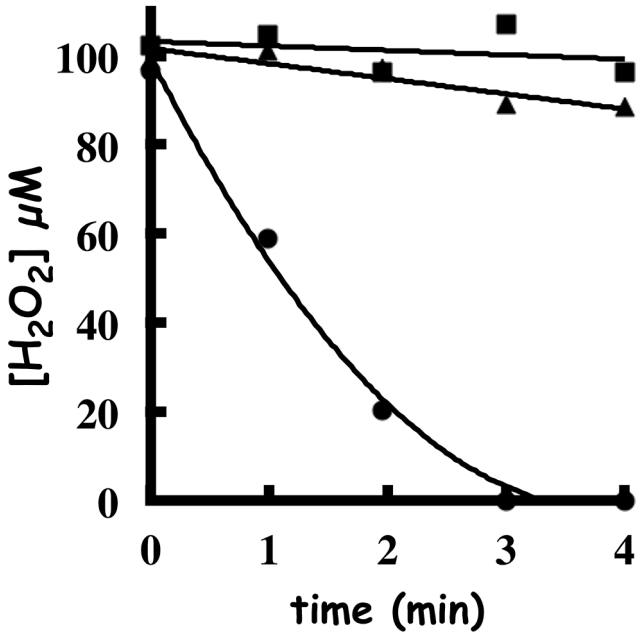
Supplemental Fig. 1



Characterization of plant lines with altered expression of ACHT1. A. ACHT1 expression is increased in transgenic plants expressing HA-tagged ACHT1 under the control of the 35S promotor. Total RNA was extracted from wild-type (WT) or transgenic (2-25) plants and was reverse transcribed into cDNA. PCR analysis of the cDNA using ACHT1-specific primers (forward primer, GCCAAACATGATTGACATTACCTCA; reverse primer, CTCAGTTTCTGGAACTTGGCAAGAGAGCAT) showed elevated amounts of ACHT1 transcript in the transgenic line. Actin was used as an internal control. Both ACHT1 and actin were amplified for 26 cycles. **B.** PCR analysis of a second independent transgenic line (2-28) showed similar results, demonstrating accumulation of elevated amounts of ACHT1 transcript relative to wild-type plants. **C.** The structure of *ACHT1*, showing the locations of the T-DNA insertions in the two lines used for phenotypic analysis, and the primers used for their PCR analysis. Black boxes, exons; white boxes, UTRs; lines, introns. Homozygous mutant plants were identified by genomic PCR using gene-specific primers P2 and either gene-specific primer P2 or the T-DNA left border primer, LBb1

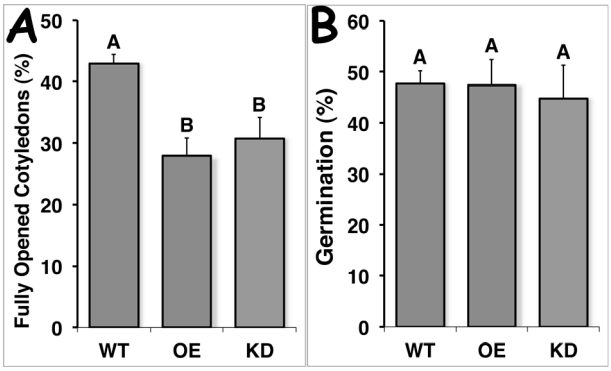
(LB), to analyze the T-DNA insertion in the SALK_089128 line **(D)** and gene-specific primers P4 and either gene-specific primer P3 or the T-DNA left border primer, LBb1 (LB), to analyze the T-DNA insertion in the SALK_144456 line **(E)**. The gene-specific primers amplified a product in the wild-type plants (WT), but the T-DNA insertion in homozygous mutant lines prevented PCR amplification with the gene-specific primers. PCR product was produced only when the T-DNA-specific primer was used. The position of the T-DNA insertion was determined by sequencing the PCR product obtained with either the P2 or P4 primers and the LBb1 primer and found to map at position 13255134 of chromosome 4 for the SALK_089128 insertion line, and position 13256518 of chromosome 4 for the SALK_144456 insertion line. **F.** ACHT1 expression is decreased in the T-DNA insertion mutant lines. Total RNA was extracted from WT plants or T-DNA insertion lines and was reverse transcribed into cDNA. PCR analysis of the cDNA using ACHT1 specific primers demonstrated a decrease in ACHT1 transcript in the T-DNA insertion lines. Actin was used as an internal control. Both ACHT1 and actin were amplified for 26 cycles. The results shown are representative of three independent experiments.

Supplementary Fig. 2



ACHT1 requires 2-cys Prx for peroxide reduction. The recombinant leaderless form of ACHT1 (2.5 μM) was incubated with H_2O_2 (100 μM) in the presence (circles) or absence (rectangles) of recombinant 2-Cys PrxA (5 μM). 2-Cys PrxA alone (triangles) was used as a control. The reaction was performed in the presence of 0.4 mM DTT, and the concentration of H_2O_2 was determined at different time points using the Peroxoquant reagent (Pierce).

Supplementary Fig. 3



Growth phenotype of plants with altered ACHT1 expression. A. Growth rate. ~ Fifty Seedlings of wild-type (WT), ACHT1 overexpressor (OE) and ACHT1 knock down (KD) plants were scored at 66 h post-stratification, and the percentage of plantlets with fully opened cotyledons was calculated. **B. Germination rate.** ~ Fifty seedlings of WT, OE and KD lines were scored at 24 h post-stratification, and the percentage of germination (determined by radicle emergence) was calculated.

Data represent mean and SE (n=12 for developmental scoring and n=8 for germination assays). Different letters on the bars represent means that are statistically different relative to WT plants based on the Tukey-Kramer HSD test using a *P*-value cutoff of 0.05.

Plants were grown at 20^0C/18^0C under 8/16 h light/dark cycle, using fluorescent white light at ~120 μE/m^2s. Results from two knock down lines (SALK_089128 and SALK_144456) were pooled together as well as the results from two over-expresser lines (2-25 and 2-28). The different lines are characterized in Supplemental Fig. 1 online.

Supplemental Table 1

Table 1: **2-Cys Prx peptides identified by MS/MS in immunopurified ACHT1 complexes**.

Peptide sequence	Mass	Charge
LSDYIGKK[1]	923.5198	2
SFGVLIHDQGIALR[1]	1525.849	3
LSKEYFSAI[1,2]	1057.557	3
APDFEAEAVFDQEFIK[1,2]	1855.875	2
AQADDLPLVGNK[1,2]	1240.653	2
KSGGLGDLNYPLISDVTK[1]	1877.002	2
GLFIIDKEGVIQHSTINNLGIGR[1,2]	2494.378	3
SGGLGDLNYPLISDVTK[1]	1748.907	2
APDFEAEAVFDQEFIK[1,2]	1855.875	2
TLQALQYIQENPDEVC(+57.02)PAGWKPGEK[1]	2871.397	3
AQADDLPLVGNKAPDFEAEAVFDQEFIK[1,2]	3077.51	3
SFGVLIPDQGIALR[2]	1485.843	2
TLQALQYVQENPDEVC(+57.02)PAGWKPGEK[2]	2857.381	3

[1]Peptide sequence found in 2-Cys PrxA
[2]Peptide sequence found in 2-Cys PrxB