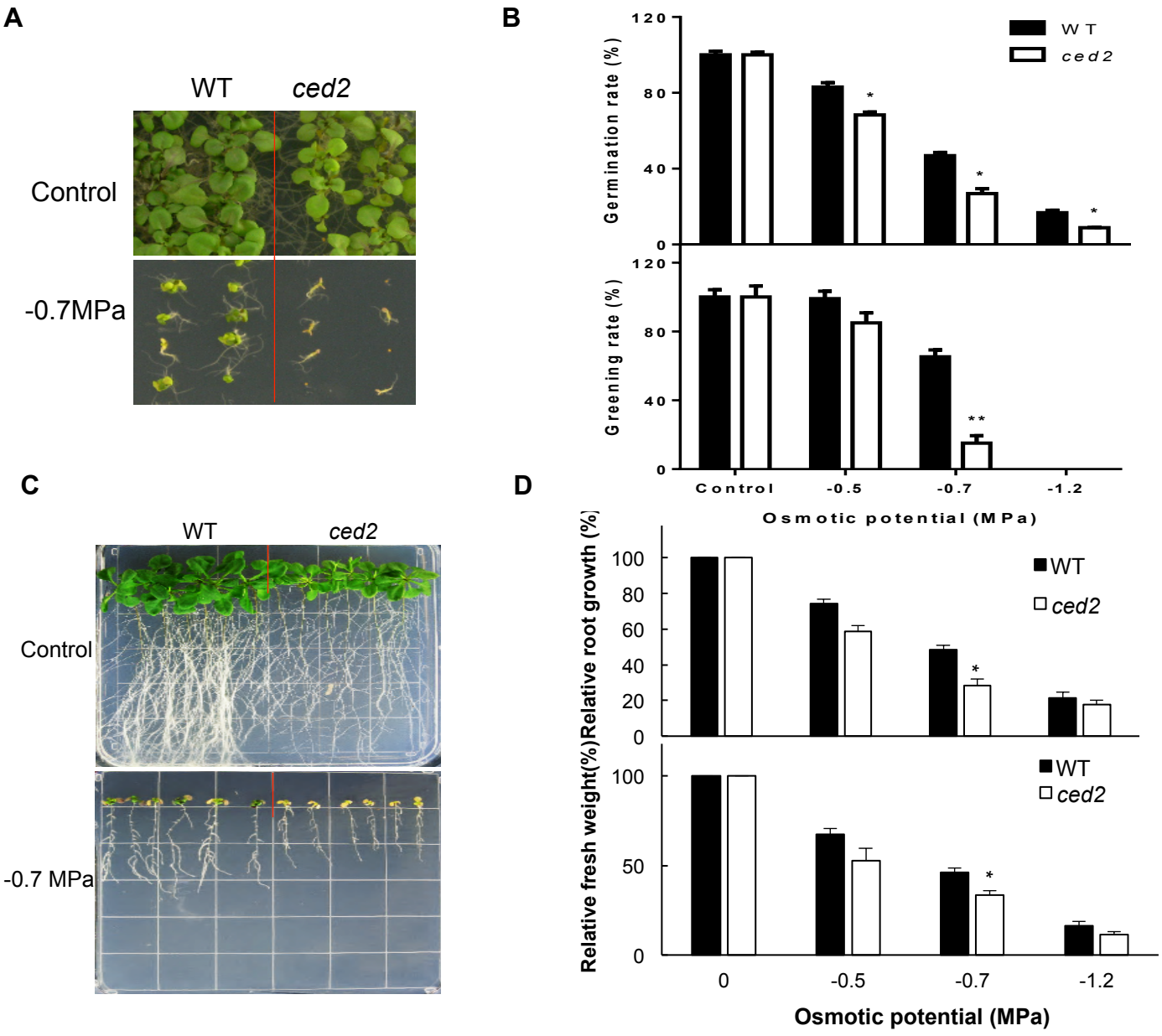
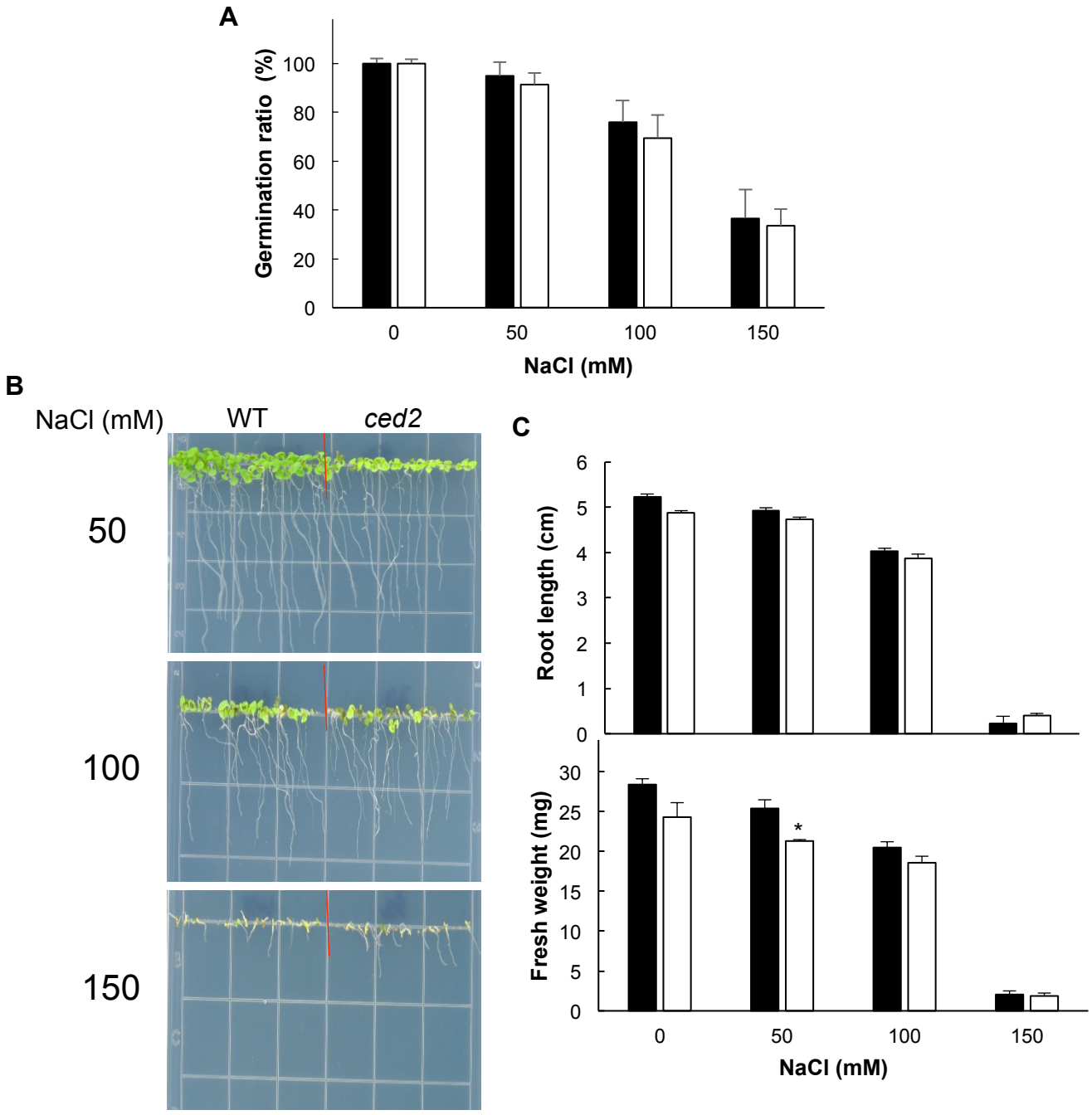
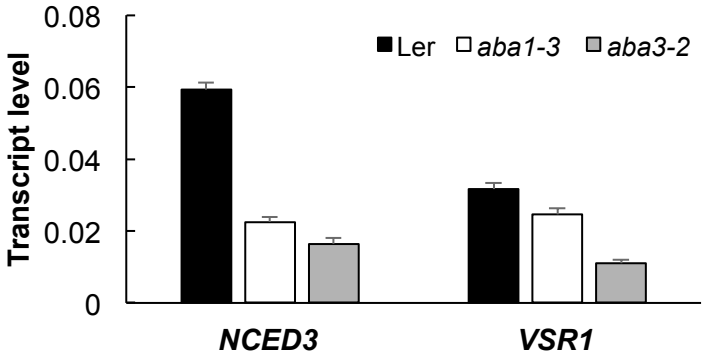


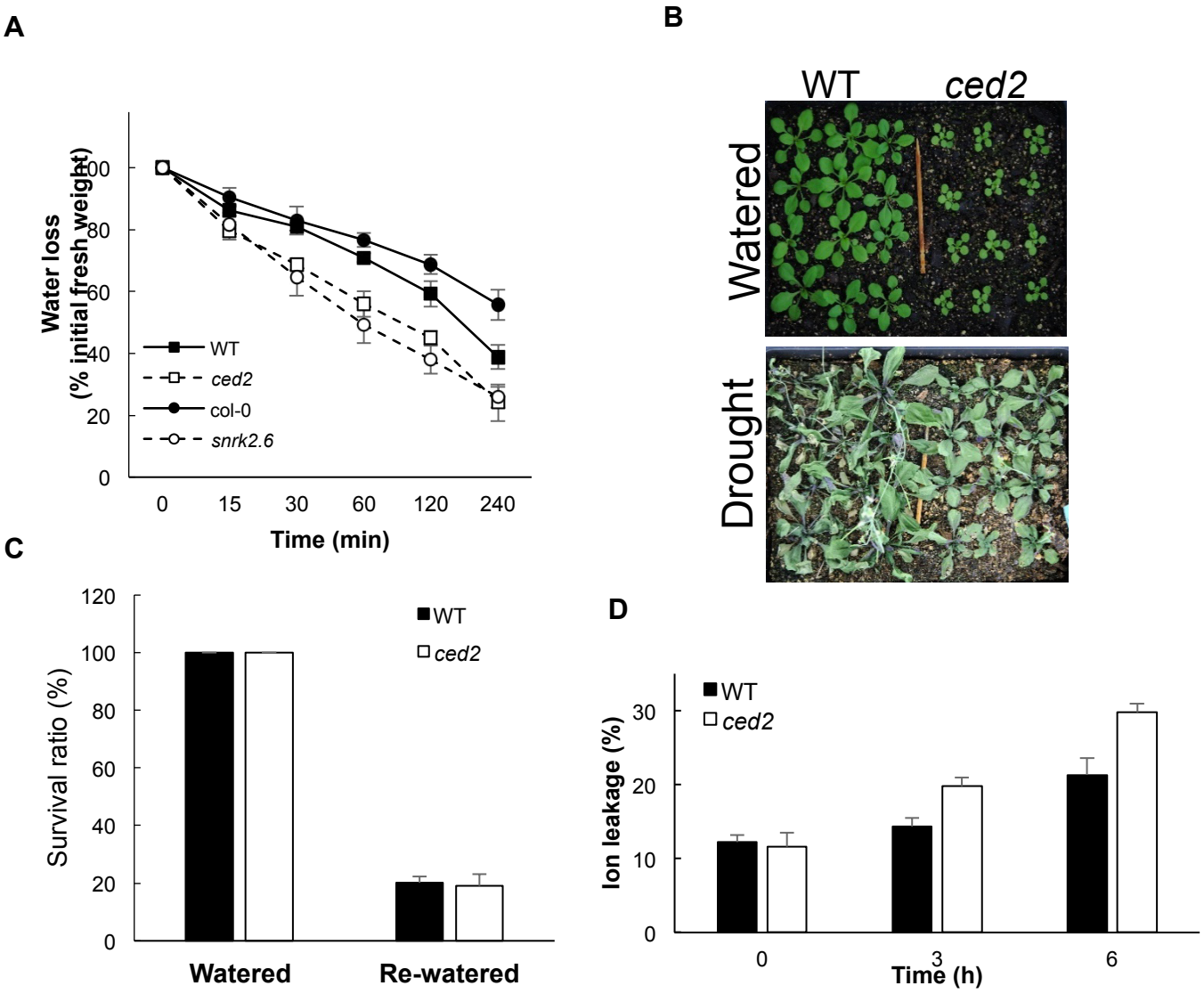
Supplemental Figure S1. Characterization of the *ced2* mutant. A, Seed germination and seedling greening of wild-type (WT) and *ced2* mutants on the control or PEG-infused agar plates. Four replicates were performed with similar results obtained. Representative pictures are shown. (B) Quantitative evaluation of the germination rate (upper panel) and seedling greening rate (lower panel) of the wild-type and *ced2* seedlings on MS agar plates with different osmotic potentials at 4 days after sowing. Four replicates were performed each with about 100 seeds for each genotype. (C) Growth of seedlings on the control and PEG-infused agar plates. Seedlings were germinated and grown on MS agar plates for one week before being transferred to the indicated plates. Three replicates were performed with similar results obtained. Representative pictures are shown. (D) Quantitative evaluation of relative root growth (upper panel) and fresh weight (lower panel) on PEG-infused agar plates at two weeks after seed imbibition. Fresh weight or root length was measured and shown as a percentage relative to that on the normal MS medium. Three replicates were performed. Results in (B) and (D) are means ± SE.. *$p < 0.05$. **$p < 0.01$.



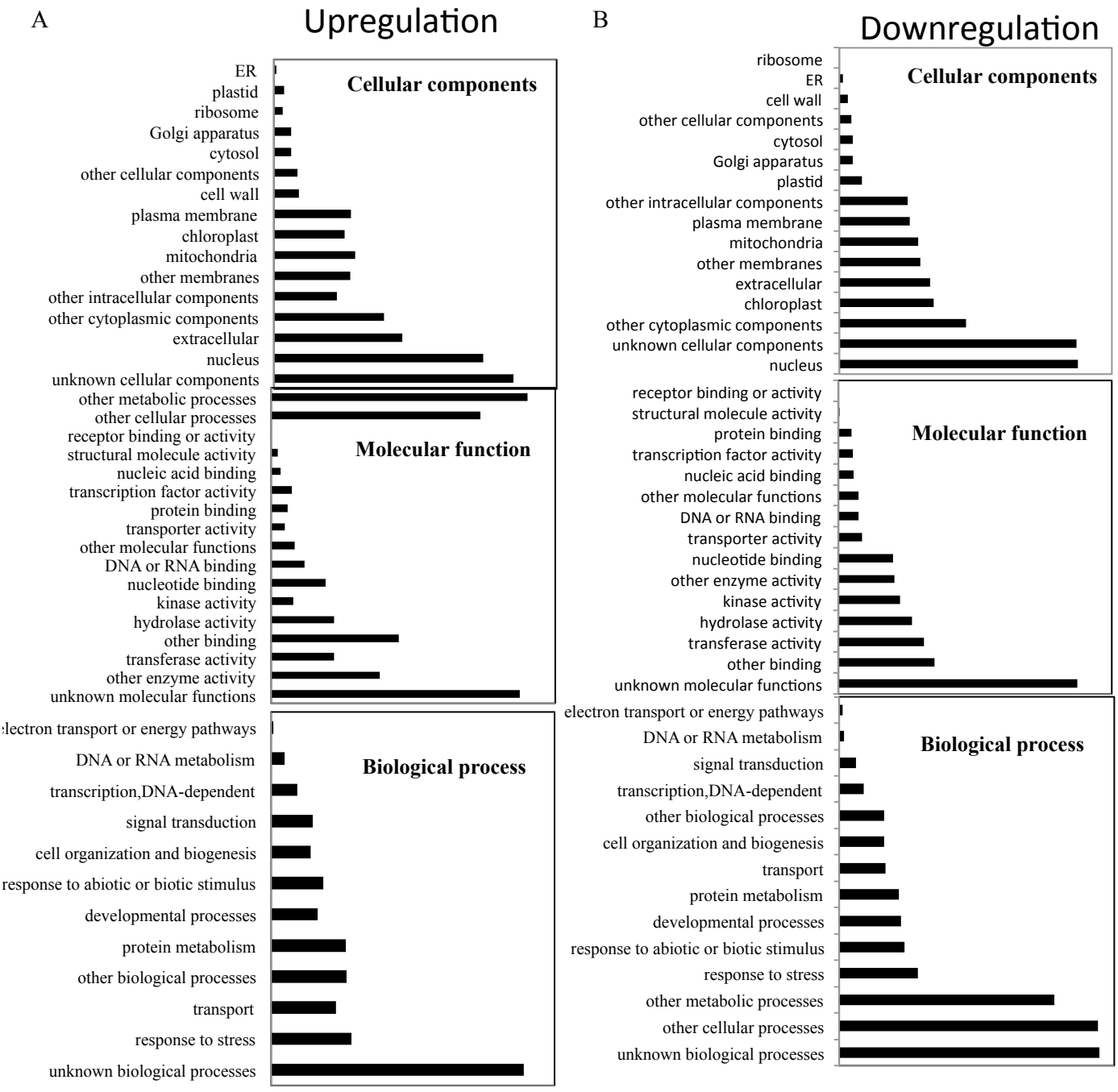
Supplemental Figure S2. Sensitivity of the *ced2* Mutant to salt stress. A, Germination rate of the wild-type and *ced2* seeds on MS agar plates supplemented with different concentrations of NaCl at four days after sowing. Four replicates were performed with about 100 seeds per line for each replicate. (B) Representative pictures of the wild-type (WT) and *ced2* seedlings grown under different concentrations of NaCl. (C) Quantitative evaluation of root growth and fresh weight of seedlings in (B) at two weeks after seed imbibition. Six plants were pooled and fresh weight or root length was measured. Three replicates were performed. In (A) and (C), Black bars, wild type; white bars, *ced2*. Results in (A) and (C) are means ± SE. *$p < 0.05$.



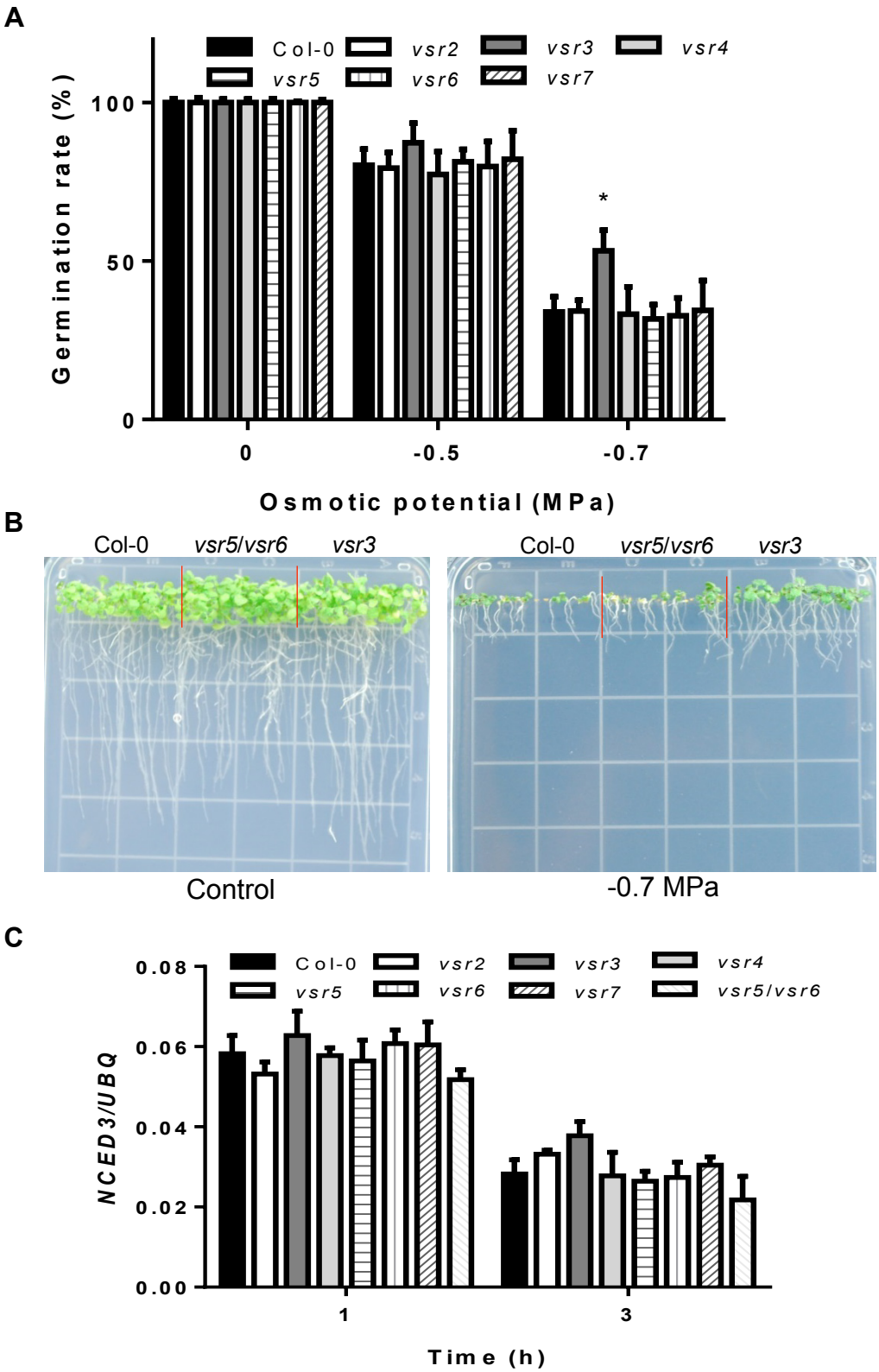
Supplemental Figure S3. *VSR1* expression is partly dependent on ABA.
Transcript level of *NCED3* and *VSR1* in Ler and two ABA-deficient mutants (*aba1-3* and *aba3-2*) under osmotic stress conditions (40% PEG, 6 h). Real-time RT-PCR quantifications were normalized to the expression of *UBQ3*. Error bars represent SE from three replicates.



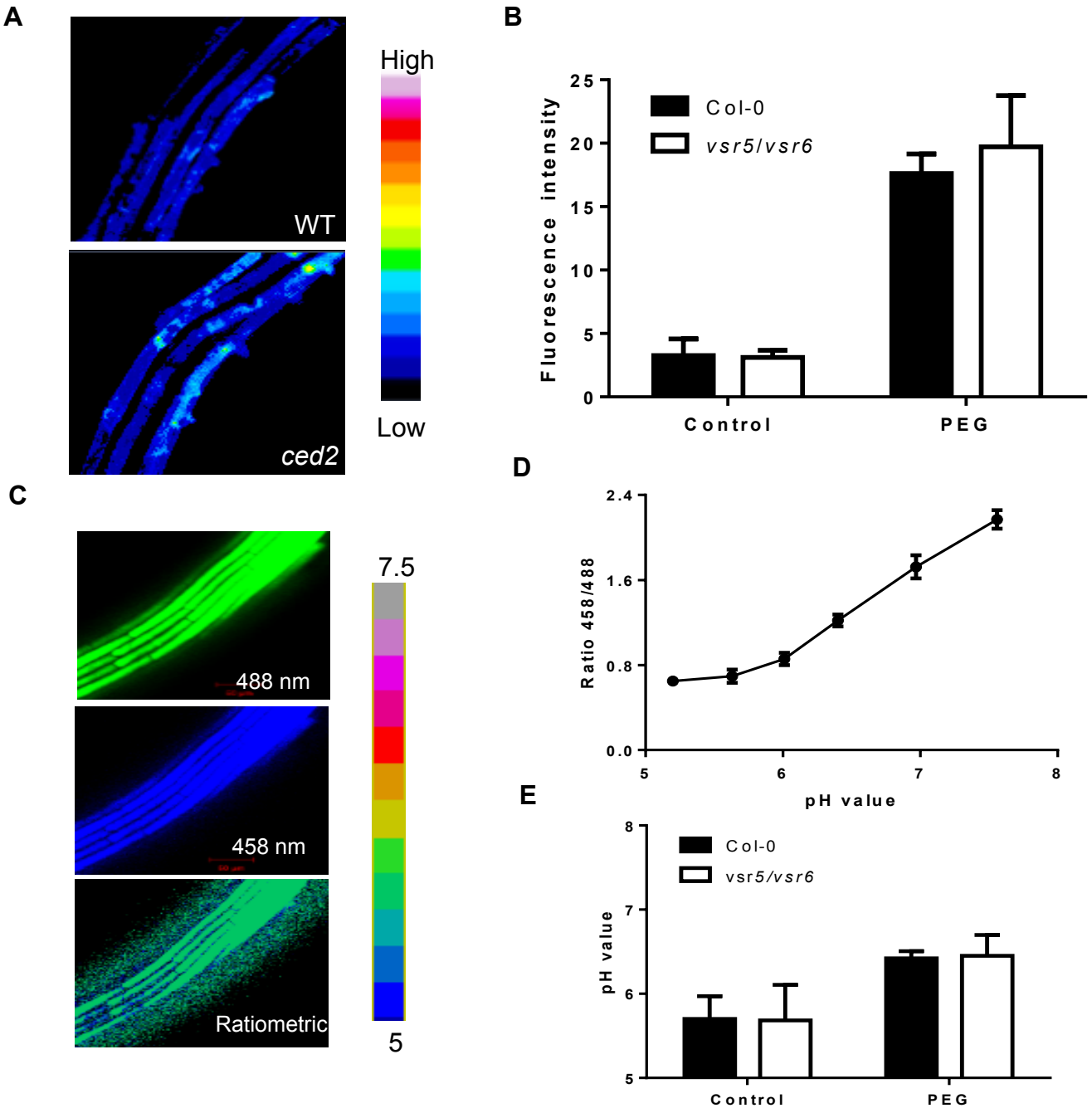
Supplemental Figure S4. Water loss rate and drought tolerance of the *ced2* mutant. A, Water loss rates of detached *ced2* and wild-type (WT) leaves. The *snrk2.6* mutant and its wild-type Columbia (Col-0) are shown as controls. Data are means ± SE (*n* =4). B, Comparison of drought tolerance between wild-type and *ced2* plants. Soil-grown plants were supplied with sufficient water for 2 weeks (Watered), and water was then withheld for 14 d (Drought). Representative picture was shown. C, Drought survival rates of the wild-type and *ced2* seedlings shown in (E). Four replicates were performed. Results are means ± SE. D, Electrolyte leakage of the wild-type (WT) and *ced2* mutant plants under the control or 40% PEG treatments. Two-week-old plants were transferred to plates containing 40% PEG solution and incubated for the indicated time. Data are means and SE from four replicates.



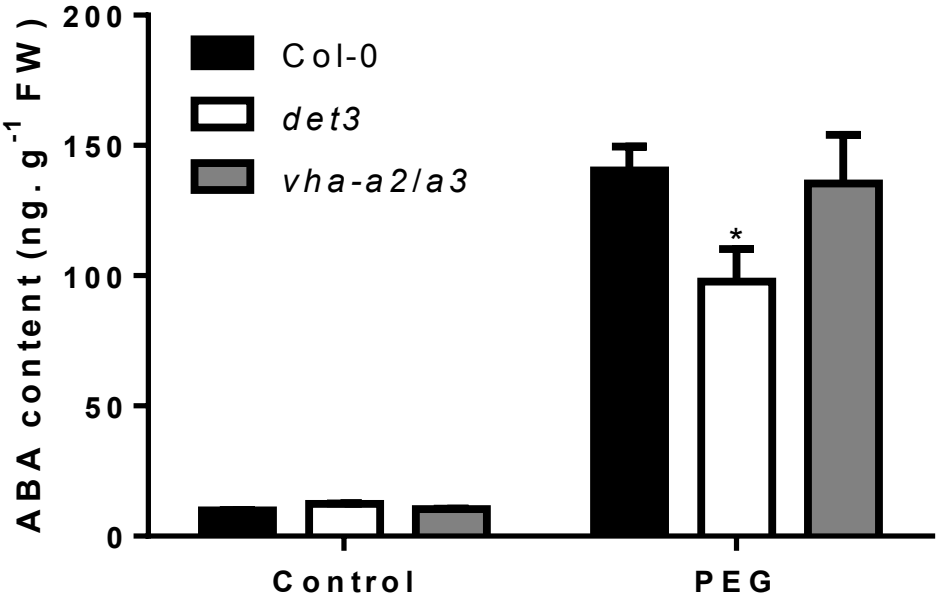
Supplemental Figure S5. Gene Ontology of genes with altered expression in *ced2* under osmotic stress conditions. A, Upregulated and B, downregulated genes from the *ced2* microarray data were analyzed with Gene Ontology Annotations database (Berardini et al. 2004; http://Arabidopsis.org/tools/bulk/go/).



Supplemental Figure S6. Characterization of other *vsr* mutants. A, Germination rate of Col-0 and other *vsr* mutants. Seeds were directly sown on MS agar plates with various osmotic potentials and germination (radicle emergence) was scored 3 day later. B, Morphology of 10-day-old Col-0, *vsr3* and *vs5/vsr6* seedlings on the control or -0.7 MPa PEG-infused MS agar plates. C, Transcript level of *NCED3* in Col-0 and other *vsr* mutants *(vsr2, vsr3, vsr4, vsr5, vsr6* and *vsr7* and *vsr5*/*vsr6)* under 40% PEG treatment for the indicated time. Real-time RT-PCR quantifications were normalized to the expression of *UBQ3*. Error bars represent SE from three replicates.



Supplemental Figure S7. Membrane potential and vacuolar pH. A, Representative images of the membrane potential in mature roots of the wild type and *ced2*. B, Calcium flux in *vsr5*/*vsr6* in response to 40% PEG treatment. C, Representative images of vacuolar pH in mature roots of the wild-type plants. D, In situ calibration curve. Four-day-old seedlings were incubated in the 10 μM BCECF-AM in darkness for 1 h, and then immersed in each pH equilibration buffers for 15 min. The fluorescence was obtained with dye loaded roots excited at 458 and 488 nm. Twenty images were taken from 10 different seedlings. E. Vacuolar pH in *vsr5*/*vsr6* in response to 40% PEG treatment.



Supplemental Figure S8. ABA accumulation in *det3* and *vha-a2*/*a3* mutants. Two-week-old seedlings were transferred to plates containing 40% PEG solution and incubated for 3 h before ABA measurement. Data are means and SE from 4 replicates.