

Figure S1. Phylogenetic analysis of CTR1- and EDR- related proteins. The tree was constructed using a program Dendroblast (<http://www.dendroblast.com/>, Kelly and Maini, 2013) on broadly sampled amino acid sequences listed in Table S2.

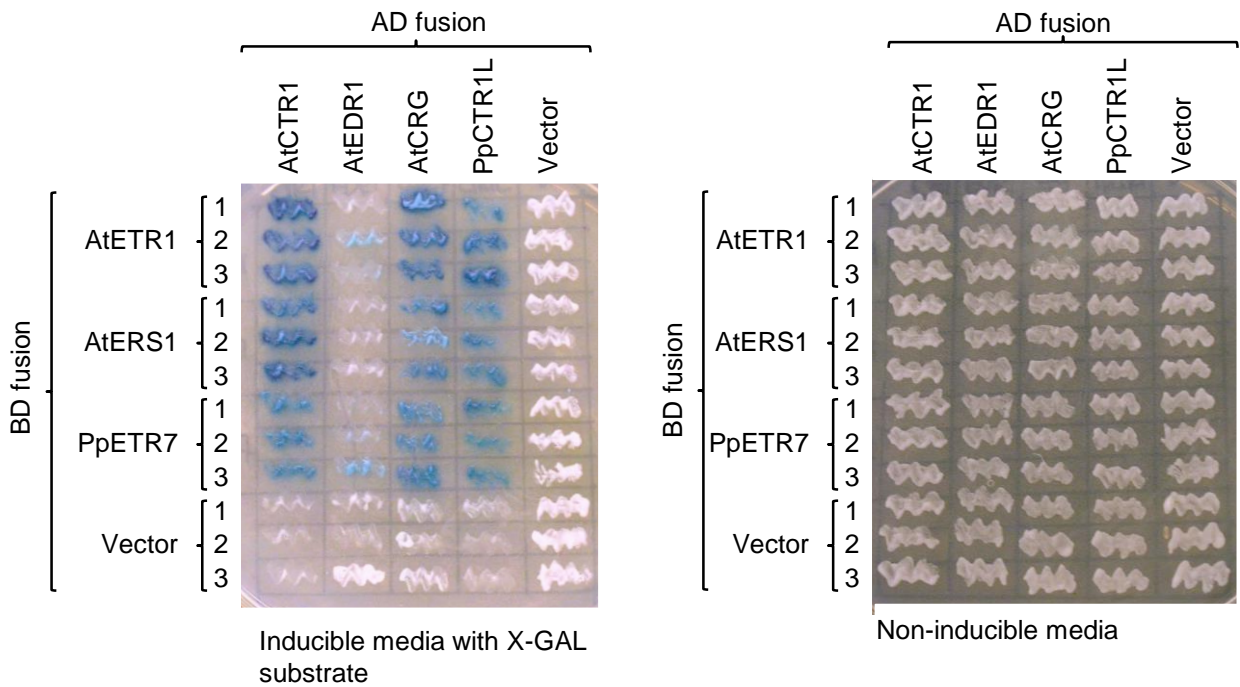
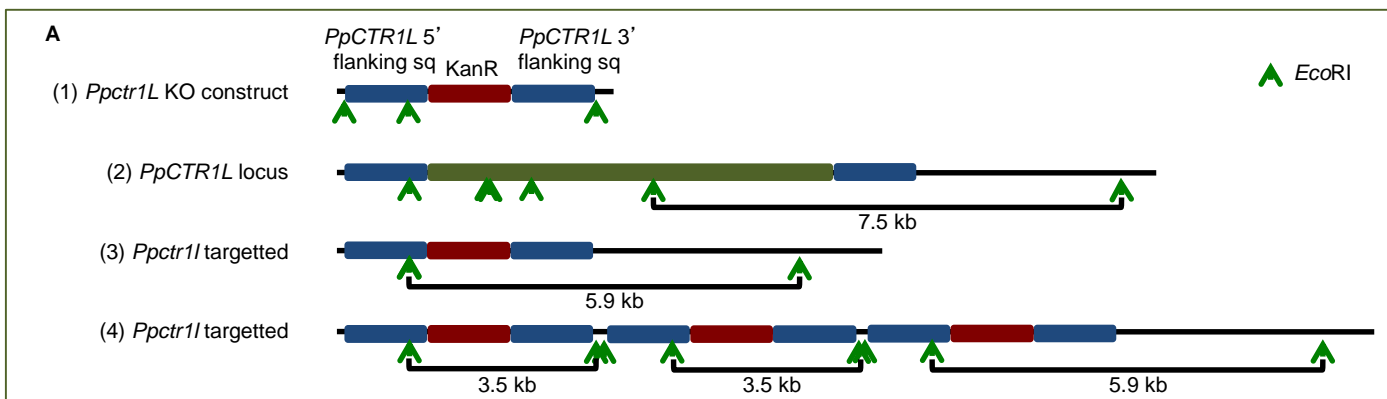


Figure S2. Yeast two hybrid plate assay.

Yeast two-hybrid plate assay for interactions between AtCTR1, its related proteins and ethylene receptors from moss and Arabidopsis. C-terminal regions of the proteins were used in this assay to avoid transmembrane domains interfering with the assay. Three independent yeast lines transformed with the combination of AD- and BD-fusion constructs as indicated were streaked on the inducible media (left) and control media (right), both containing X-GAL.



B

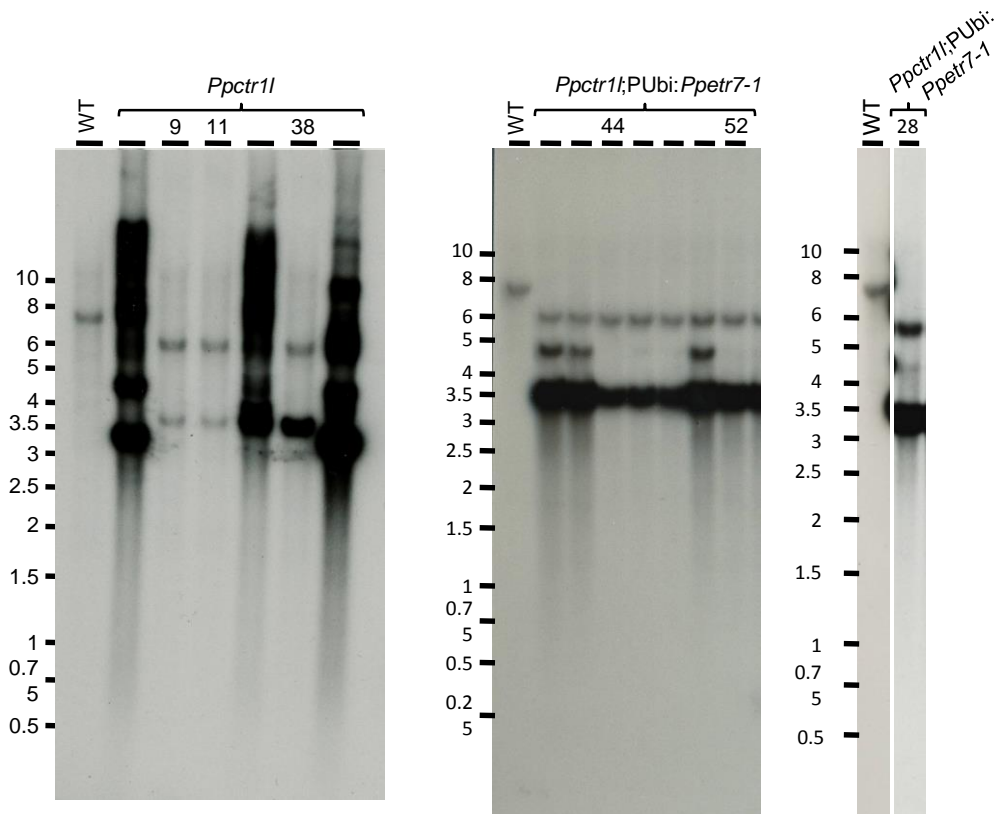


Figure S3. Characterization of *Ppctr1I* lines.

A, Schematic diagram of *Ppctr1I* knockout construct (1), the genomic *PpCTR1L* locus (2), the genomic *Ppctr1I* locus when the locus is replaced with the knockout construct via homologous recombination (3), and the genomic *Ppctr1I* locus when the locus is replaced with tandem repeats of the knockout constructs (4). Green arrows indicate EcoRI restriction sites with expected sizes of digests. B, DNA gel-blot analysis of genomic EcoRI digests of WT, *Ppctr1I* and *Ppctr1I;pUbi:Ppetr7-1* transgenic lines. The blot was hybridized with a radiolabelled fragment at the region 3' to the *PpCTR1L* 3' flanking sequence used in the knockout construct. Signals at 7.5 kb indicates WT *PpCTR1L* while those at 6.5 kb indicates that the locus was replaced with the knockout construct. Signals at 3.5 kb indicates the presence of tandem repeats of the construct in the transgenic line. Lines 9, 11 and 38 were selected for *Ppctr1I* transgenic lines in the further experiments, and lines 28, 44 and 52 were selected for *Ppctr1I;pUbi:Ppetr7-1* transgenic lines in the further experiments. Two photos on the right were taken from the gel blots which were run at the same time.

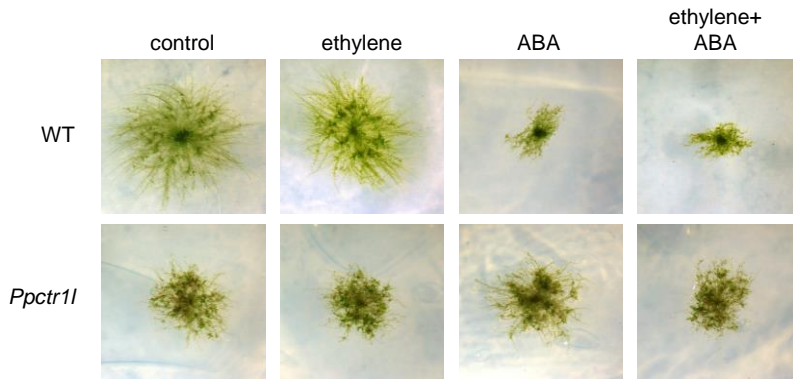


Figure S4. Ethylene does not alter ABA response in *P. patens*. 14 days-old WT and *Ppctr1l* moss grown on the media containing 0 or 10 μM ABA were transferred on the 5th day following inoculation to an airflow chamber filled with air (control, ABA) or with air containing 3 $\mu\text{l}\cdot\text{L}^{-1}$ ethylene (ethylene, ethylene+ABA).

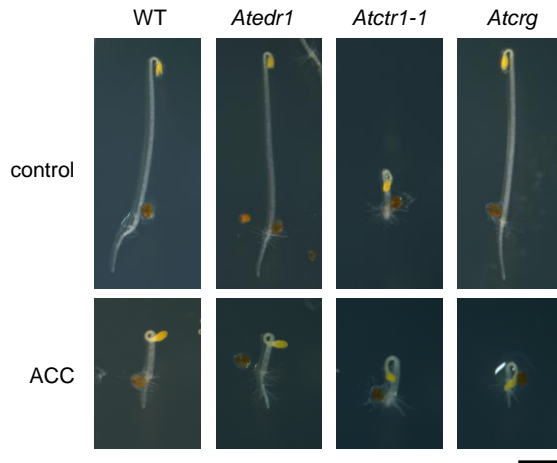


Figure S5. Triple response assay. Seedlings of WT, *Atedr1*, *Atctr1-1* and *Atcrg* were grown on the media containing 0 or 10 μM ACC in dark for three days. Typical triple response was observed for all the seedling in the presence of ACC but not in the absence of ACC, except for *Atctr1-1*.

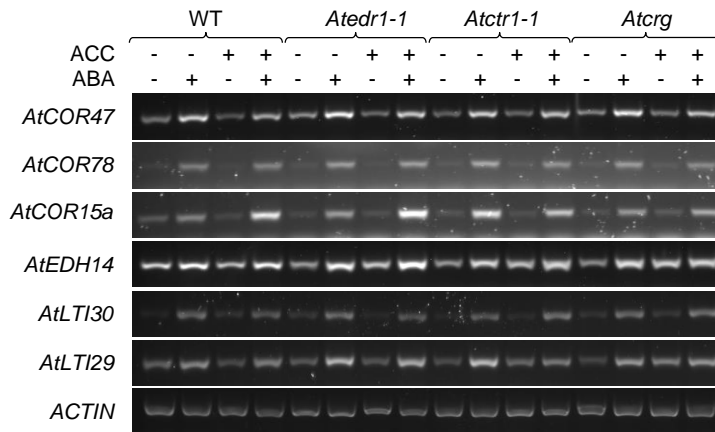


Figure S6. ABA-inducible gene expression.

Semi-quantitative RT-PCR showing levels of ABA-regulated transcripts in WT, *Atctr1-1*, *Atedr1-1* and *Atcrg* mutant. RNA was harvested from 10 day-old Arabidopsis seedlings grown on the media containing 0 or 20 μ M ACC and/or 0 or 10 μ M ABA.

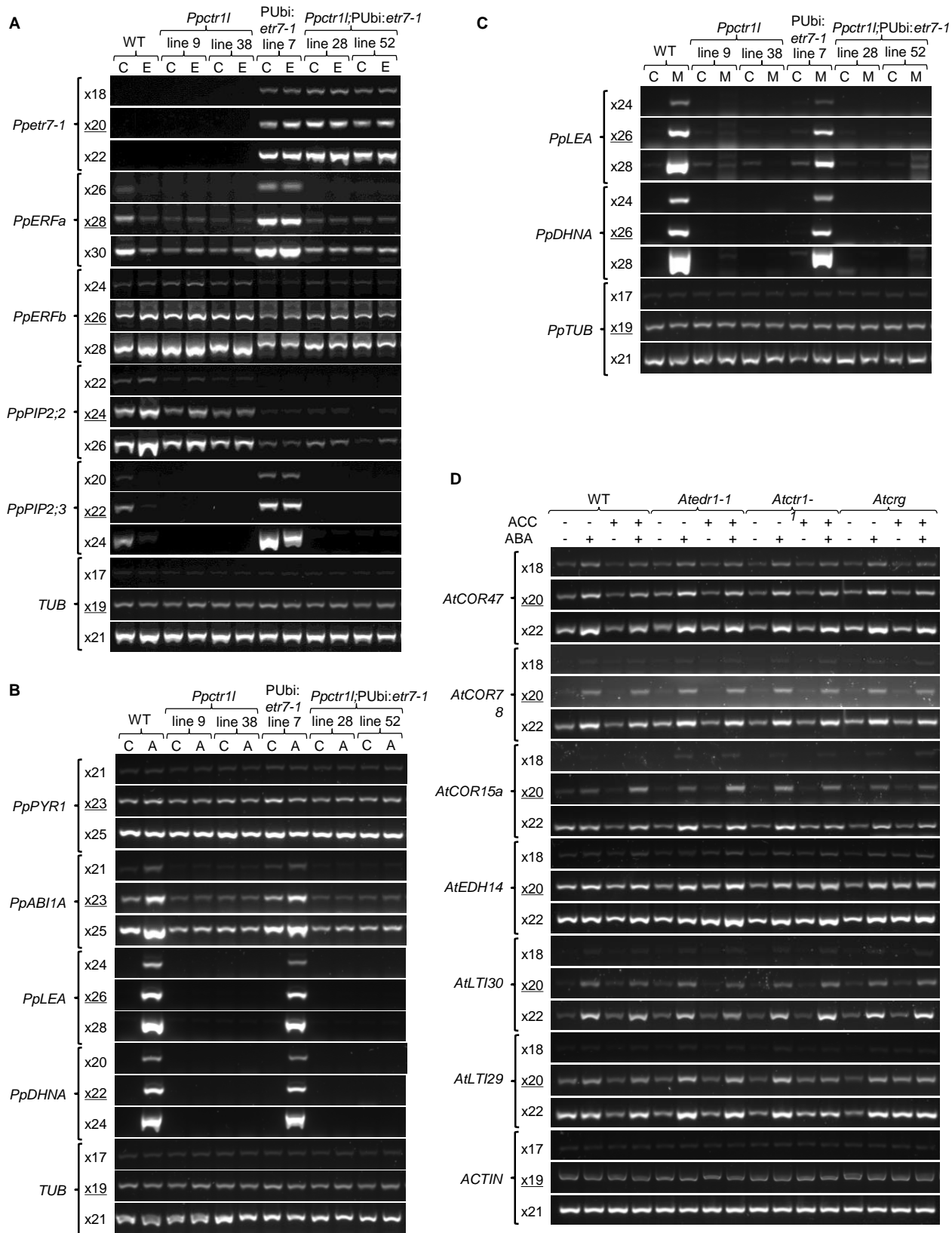


Figure S7. Full result of semi-quantitative RT-PCR analysis with amplification status at +/- 2 cycles. For each transcript accumulation levels studied in the Figures 3C, 5C, 5D and S6, amplification status at three different stages of PCR at the intervals of 2 cycles is shown in A, B, C and D respectively. Gel photos with underlined cycle numbers were included in the figures mentioned above, and the increasing levels of amplification from -2 cycle stage to +2 cycle stage shows that the data were taken from the log phase of PCR amplification.