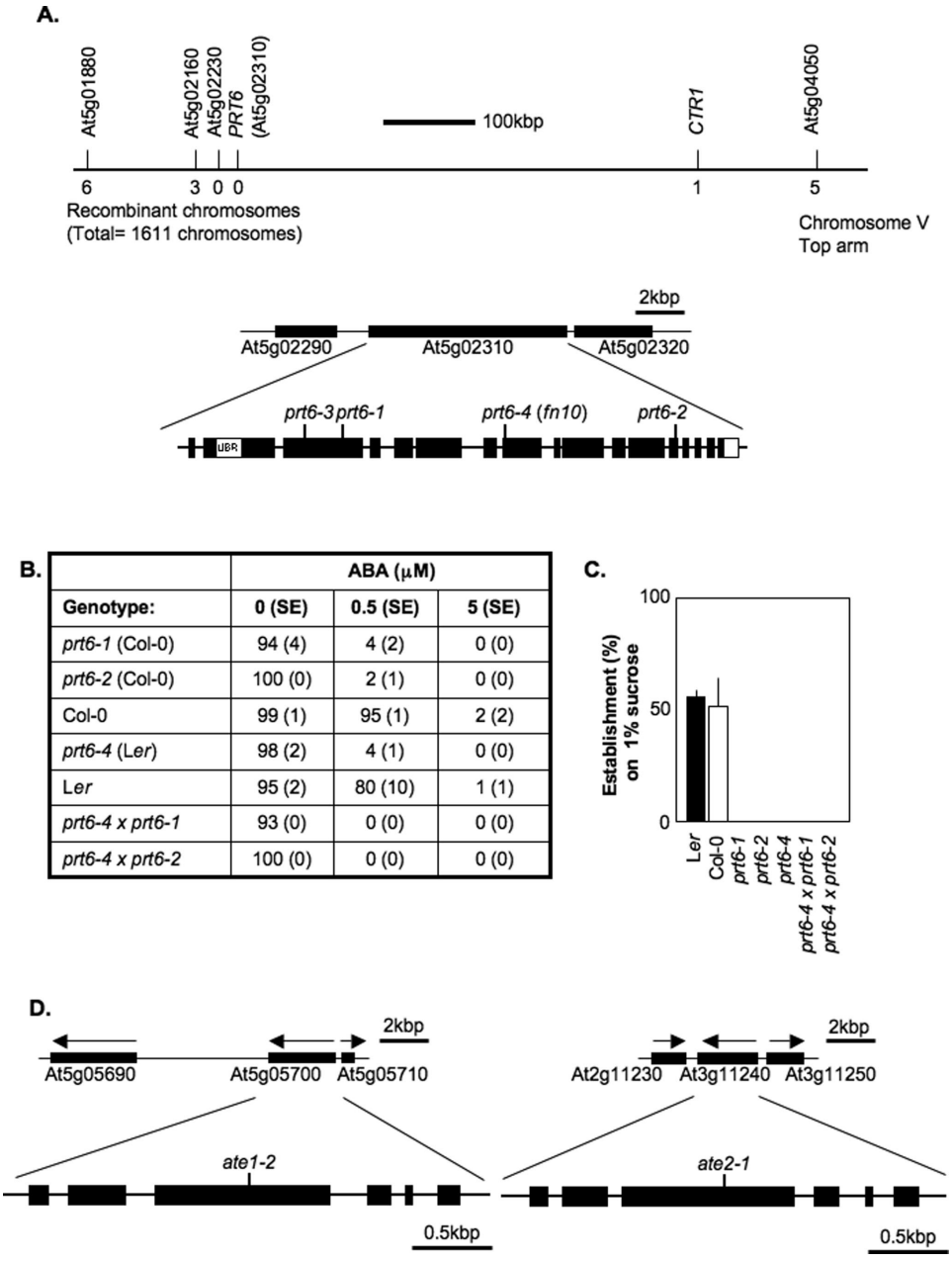
Supporting Information

Holman et al. 10.1073/pnas.0810280106



	ABA (μM)		
Genotype:	**0 (SE)**	**0.5 (SE)**	**5 (SE)**
prt6-1 (Col-0)	94 (4)	4 (2)	0 (0)
prt6-2 (Col-0)	100 (0)	2 (1)	0 (0)
Col-0	99 (1)	95 (1)	2 (2)
prt6-4 (L*er*)	98 (2)	4 (1)	0 (0)
L*er*	95 (2)	80 (10)	1 (1)
prt6-4 x prt6-1	93 (0)	0 (0)	0 (0)
prt6-4 x prt6-2	100 (0)	0 (0)	0 (0)

Fig. S1. Positional cloning of *prt6-4*. (*A*) Chromosomal positions of molecular markers used to map the position of the mutation represented by *prt6-4* (originally denoted in our genetic screen as fast neutron line 10; fn10). An F2 population was developed between L*er* and Col-0, and it was used to map the position of the mutation. Numbers of recombinant chromosomes in the mapping population are indicated. A diagrammatic representation of the *PRT6* gene (At5g02310) shows the position of the *prt6-4* (*fn10*) mutation, which is an insertion 497,178 kbp from the top of chromosome V into the *PRT6* gene. The positions of other previously identified *prt6* alleles are also shown (all T-DNA insertions). (*B* and *C*) Complementation analysis demonstrates that the *prt6-4* (*fn10*) mutation is in the same genetic complementation group as *prt6-1* and *prt6-2*. (*B*) Reduced ABA sensitivity cannot be restored in crosses between *prt6* alleles. Therefore, increased ABA sensitivity of all mutants is due to disruption of the same gene (*PRT6*). Germination potential of moist, chilled seeds on 1/2MS supplemented with ABA is indicated, following 7 days of imbibition. Data points represent mean values in percent ± standard error of the mean (SE). (*C*) Reduced sugar sensitivity cannot be restored in crosses between *prt6* alleles. Therefore, increased sugar sensitivity of all mutants is due to disruption of the same gene (*PRT6*). Establishment of moist chilled seeds on water-agarose supplemented with 1% (wt/vol) sucrose for 7 days of imbibition following 2 days of moist chilling. Data points represent mean values in percent ± standard error of the mean (SE). (*D*) Positions of TDNA insertions in genes *ATE1* and *ATE2*. *ate1-2* corresponds to SALK_023492, and *ate2-1* to SALK_040788.

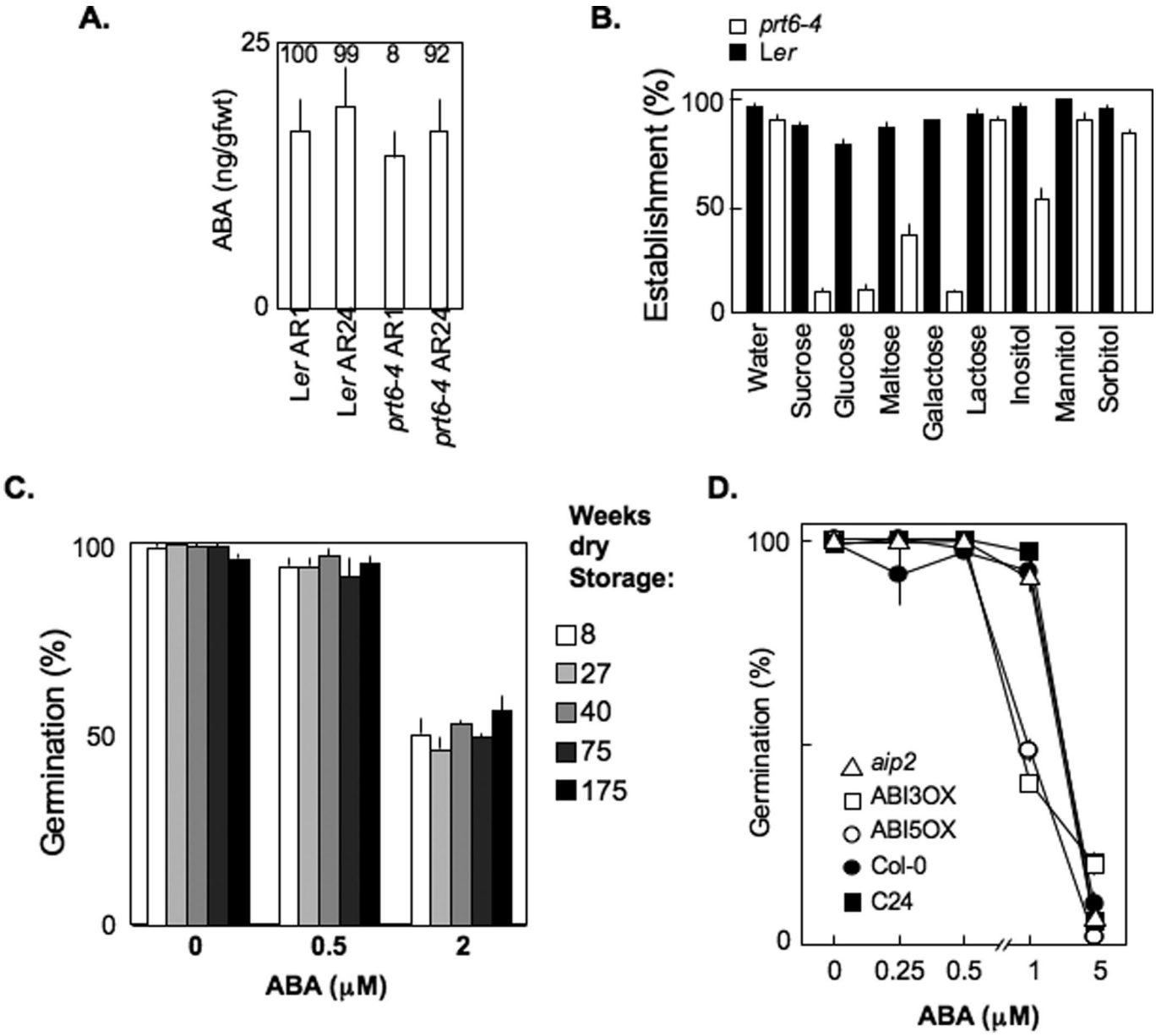


Fig. S2. Physiological characteristics of *prt6-4* mutant seeds. (*A*) Endogenous ABA content of unchilled L*er* and *prt6-4* seeds imbibed for 24 h on water-agarose media at 24 °C. Seeds were stored for 1 month (AR1) or 24 months (AR24) before assay. Numbers above bars refer to percentage germination of seed lots. Seed material was harvested for ABA analysis as previously described [Mulholland BJ, *et al.* (2003) *Environ Exp Bot* 50:17–28]. (*B*) Establishment of WT (black bars) and *prt6-4* (white bars) after imbibition for 7 days on water-agarose media supplemented with 1% (wt/vol) carbohydrate. (*C*) ABA sensitivity of endosperm rupture following after-ripening does not change in WT L*er* seeds. Seeds were stored for increasing periods of time before assay for germination potential. Assayed on 1/2MS supplemented with ABA on 7 days of imbibition, following 2 days of moist chilling. (*D*) ABA sensitivity of endosperm rupture of *aip2,* ABI3OX, and ABI5OX and WTs (respectively Col-0, C24, and Col-0). Seeds were assayed as for *C*. Data points represent mean values ± standard error.

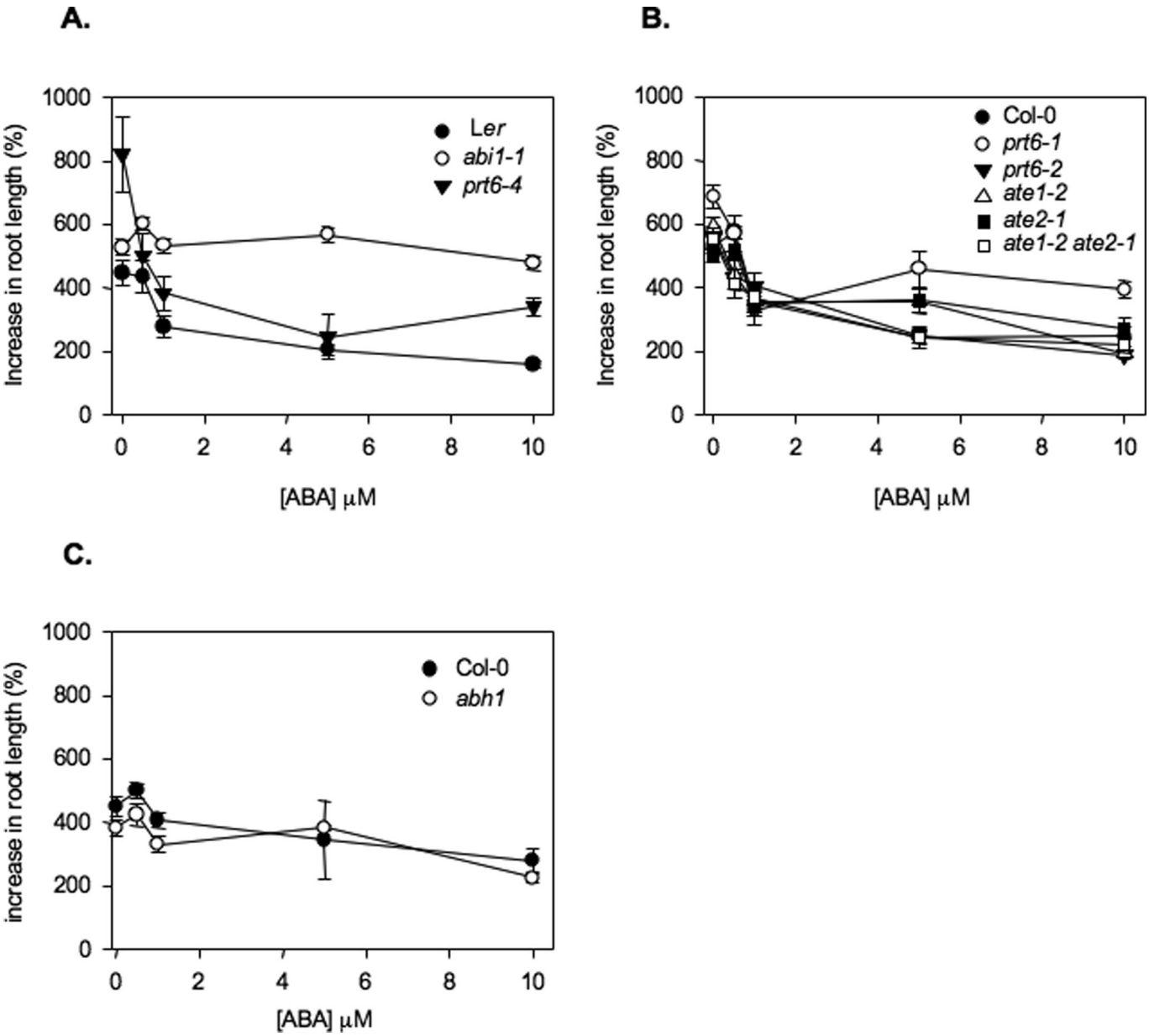


Fig. S3. Mutants in the N-end rule pathway are not hypersensitive to ABA for root elongation. Graphs show the percentage increase in root length of seedlings transferred to ABA following 5 days' imbibition on 1/2MS, assayed after incubation on ABA-supplemented media for 6 days. (*A*) The effect of exogenous ABA on the root elongation of *prt6-4* seedlings was similar to that of L*er*, with percentage increases in root length reducing with increasing concentrations of ABA. The *abi1-1* mutant seedlings (●) were insensitive to applied ABA. (*B*) The effect of exogenous ABA on the root elongation of *prt6-1,2, ate1-2, ate2-1,* and *ate1-2 ate2-1* double-mutant seedlings was similar to that of Col-0. (*C*) The *abh1* mutant and Col-0 seedlings had a similar level of sensitivity to applied ABA for root elongation. Data points represent mean values ± standard error.

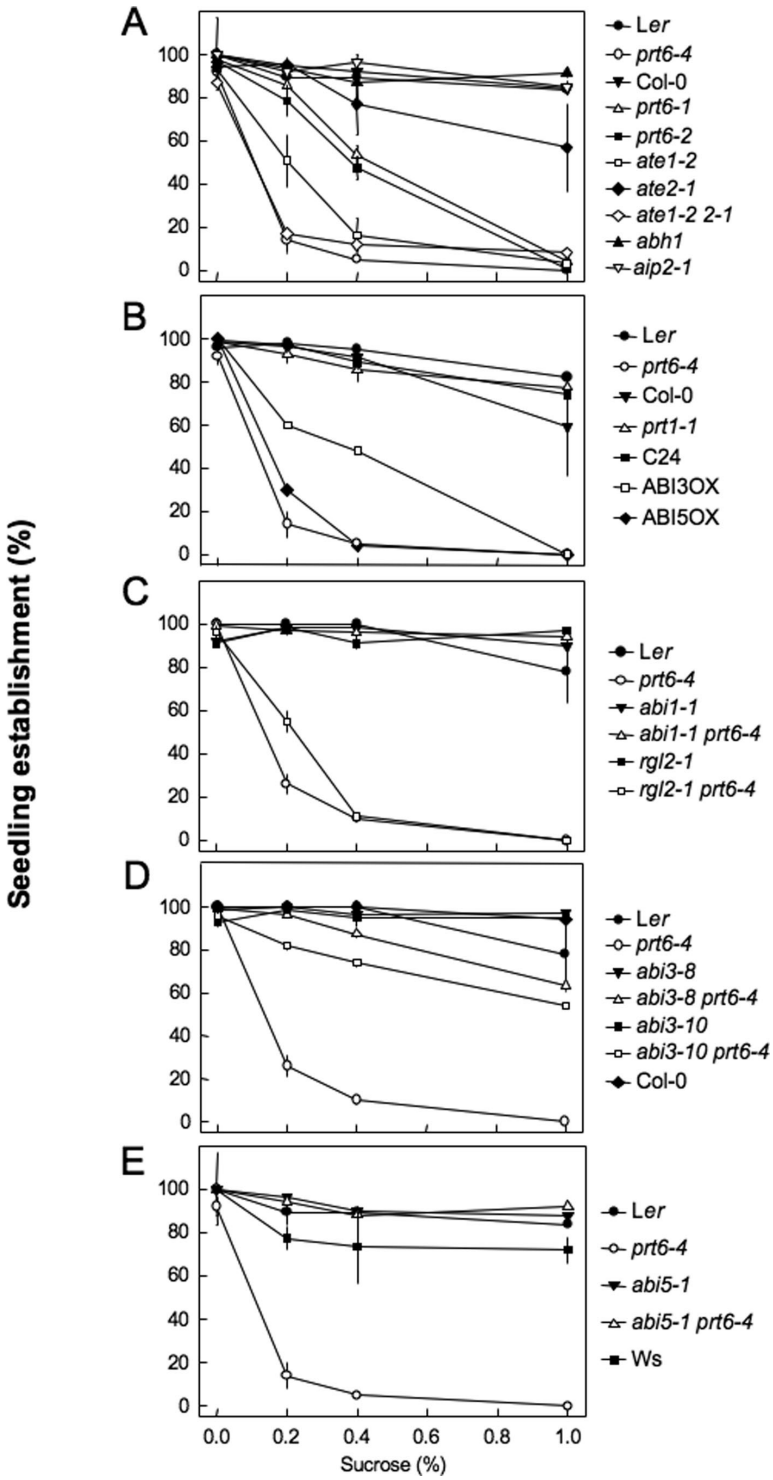


Fig. S4. Effect of exogenous sucrose on seedling establishment of single and double mutants. Seeds were plated on water-agarose media containing sucrose, as indicated. Establishment was scored as the presence/absence of green cotyledons after 7 days. Data represent means ± SE of the mean. (*A*) N-end rule pathway mutants (*prt6-4*, L*er* background; *prt6-1*, *prt6-2*, *ate1-2*, *ate2-1*; Col-0 background) and the ABA-hypersensitive mutants, *abh1* and *aip2-1* (Col-0). *abh1* and *aip2-1* are insensitive to exogenous sucrose, *ate2-1* shows modest sensitivity, whereas establishment of *prt6-1*, *prt6-2*, *prt6-4*, *ate1-2*, and the *ate1-2 ate2-1* double mutant is sensitive to applied sugar. (*B*) Seedling establishment of the N-end rule pathway mutant *prt1-1* is insensitive to exogenous sucrose, as are transgenics that express ABI3 and ABI5 under the control of the *35SCaMV* promoter (*ABI3OX,* Col-0; *ABI5OX*, C24 background, respectively). L*er* and *prt6-4* are shown for comparison. (*C*) Genetic interactions of *prt6-4* with components of ABA synthesis and signaling (*abi1-1*, *aba1-1*; L*er* background) and GA signaling (*rgl2*; L*er* background). (*D*) Genetic interactions of *prt6-4* with *ABI3*. *abi3-8* and *abi3-10* alleles are in the Col-0 background. (*E*) Genetic interactions of *prt6-4* with *ABI5*. *abi5-1* is in the Ws background. *ABI5* is epistatic to *PRT6* in this assay.

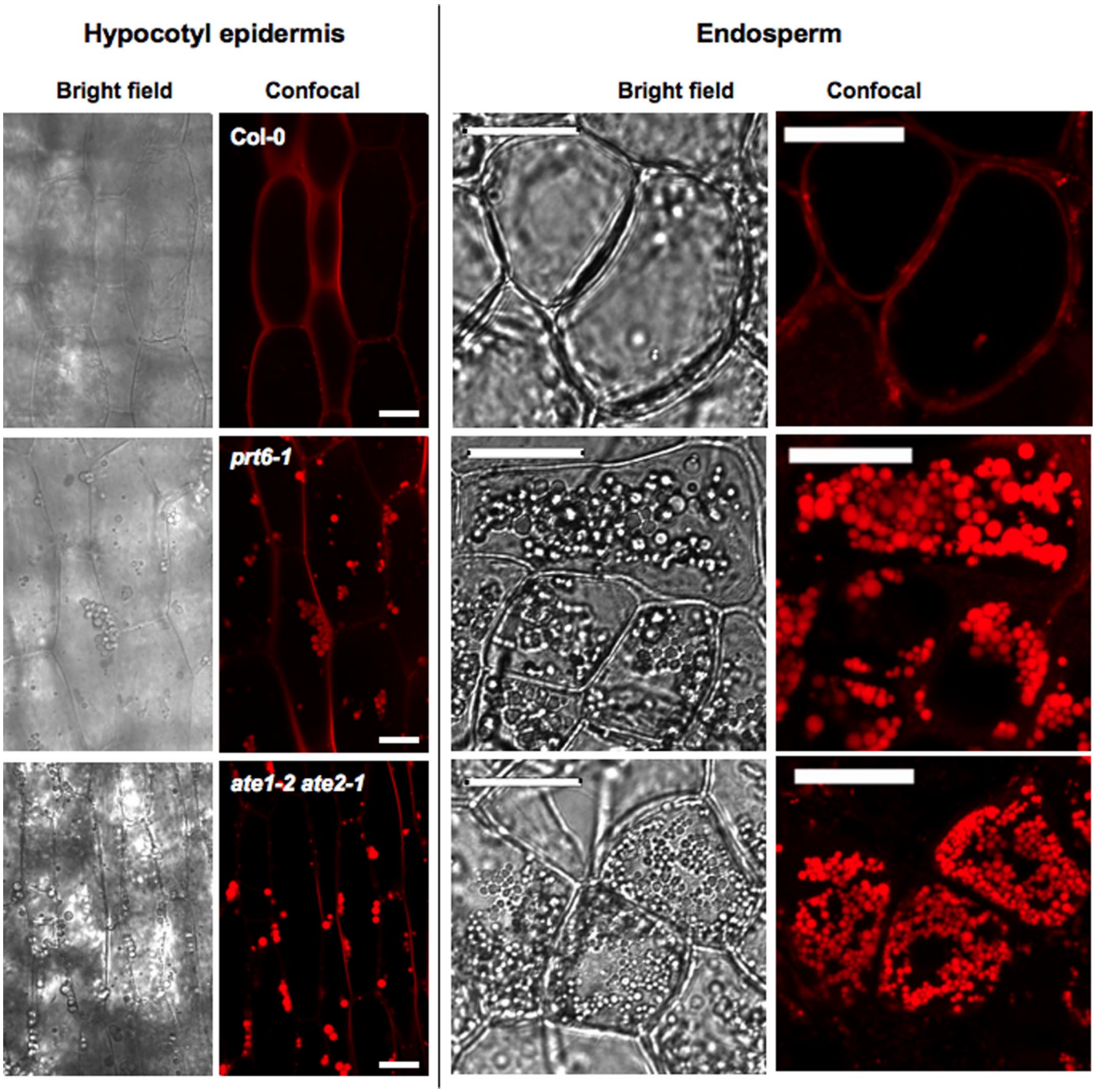


Fig. S5. Brightfield and confocal microscopy reveal oil body retention in hypocotyl epidermis and endosperm of N-end rule pathway mutants. In each case, both brightfield and confocal micrographs of Nile Red-stained material is shown. Light-grown seedlings (5 days imbibed, grown on 1/2MS) were treated with the lipophilic dye, Nile Red as previously described [Dietrich D, et al. (2009) *Mol Biol Cell* 20:530–543]. The presence of retained oil bodies is indicated by staining of lipid in localized areas within cells by Nile Red. (Scale bars: 20 μm.)

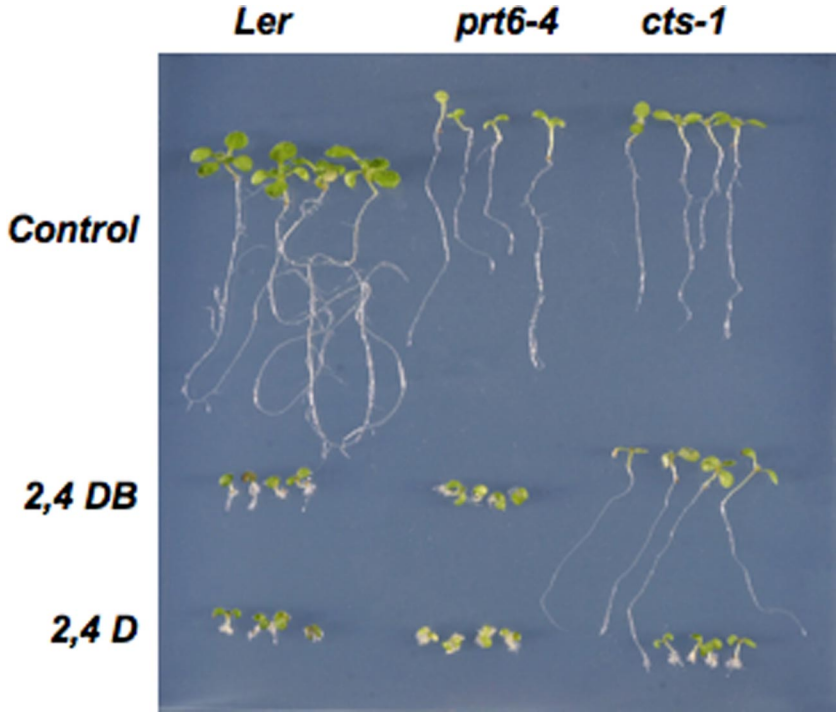


Fig. S6. The *prt6-4* mutant is sensitive to 2,4-DB. Seeds of *prt6-4*, *cts-1*, and the respective wild type, L*er*, were plated on 0.5× MS medium containing 0.5% (wt/vol) sucrose and 0.2 mg/mL 2,4-DB or 0.05 mg/mL 2,4-dichlorophenoxyacetic acid (2,4-D), as indicated. Root growth is inhibited in all 3 genotypes in the presence of 2,4-D. *cts-1*, which is impaired in peroxisomal β-oxidation and unable to convert 2,4-DB to 2,4-D, is able to develop roots and shoots on media supplemented with 2,4-DB. In contrast, the *prt6-4* mutant shows a similar stunted phenotype to the wild type. Seedlings were rearranged on a fresh plate before photographing.

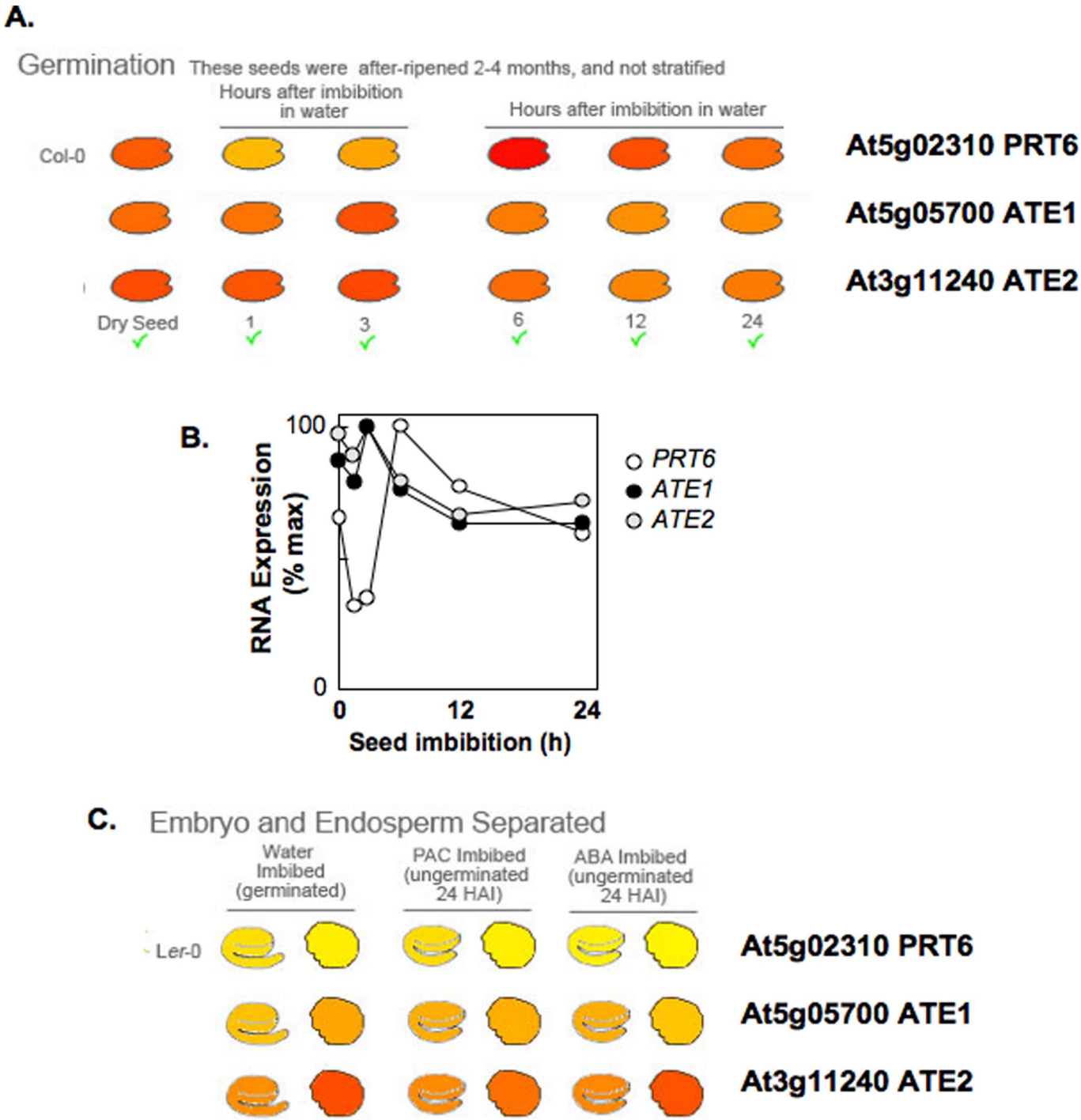


Fig. S7. Relative expression of RNA for *PRT6*, *ATE1*, and *ATE2* during seed germination. Values of RNA expression and images of seeds were taken from the eFP browser (http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi). (*A*) Expression during germination at 0–24 h of imbibition. Maximum values for each gene were PRT6: 83, ATE1: 132, and ATE2: 29. (*B*) Data from *A* are expressed as relative to maximum expression of each gene. (*C*) Expression data for isolated embryo (first image in each case) or endosperm, for untreated seeds or seed treated with PAC or ABA, as described [Penfield S, et al. (2006) *Plant Cell* 18:1887–1899]. Maximum values for each gene were PRT6: 11.5, ATE1: 67, and ATE2: 28. In all cases, data were normalized by using MAS5 with a scaling factor of 100. Intensity of color (yellow through red) indicates level of expression (low through high).