Supplementary Figure 1: 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 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, that is an insertion 497178 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,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 the same gene (*PRT6*). Germination potential of moist chilled seeds on 1/2 MS supplemented with ABA is indicated, following 7 days 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 the same gene (*PRT6*). Establishment of moist chilled seeds on water agarose supplemented with 1% (w/v) sucrose 7 days imbibition following 2 days 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.

Supplementary Figure 2: Physiological characteristics of *prt6-4* mutant seeds.
A. Endogenous ABA content of un-chilled L*er* and *prt6-4* seeds imbibed for 24h on water-agarose media at 24 ^{o}C. Seeds were stored for 1 month (AR1) or 24 months (AR24) prior to assay. Numbers above bars refer to percentage germination of seed lots. Seed material was harvested for ABA analysis as previously described (Mulholland, *et al.* (2003) Can ABA mediate responses of salinity stressed tomato. Environmental and Experimental Botany 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 % (w/v) 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 prior to assay for germination potential. Assayed on 1/2MS supplemented with ABA on 7 days imbibition, following 2 days 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.

Supplementary Figure 3. Mutants in the N-end rule pathway are not hypersensitive to ABA for root elongation.
Graphs A, B and C 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 six 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 (closed circles) 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.

Supplementary Figure 4: 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 which 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 signalling (*abi1-1*, *aba1-1*; L*er* background) and GA signalling (*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.

Supplementary Figure 5:
Bright field and confocal microscopy reveal oil body retention in hypocotyl epidermis and endosperm of N-end rule pathway mutants.

In each case both bright field and confocal micrographs of Nile Red stained material is shown.

Light-grown seedlings (5 days imbibed, grown on 1/2 MS) were treated with the lipophilic dye, Nile Red as previously described (Dietrich *et al*. (2008) Mol Biol Cell, 0.1091/mbc.E08-07-0745).

The presence of retained oil bodies is indicated by staining of lipid in localised areas within cells

by Nile Red.

White bars indicate 20 microns.

Supplementary Figure 6. 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 x MS medium containing 0.5 % (w/v) sucrose and 0.2 mg/ml 2,4-dichlorophenoxybutyric acid (2,4-DB) or
0.05 mg/ml 2,4-dichlorophenoxyacetic acid (2,4-D), as indicated. Root growth is inhibited
in all three genotypes in the presence of 2,4-D. *cts-1*, which is impaired in peroxisomal beta-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, prior to photographing.

Supplementary Figure 7:
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 0-24h imbibition.
Maximum values for each gene were:
PRT6:83 ATE1: 132 ATE2: 29

B. Data from A 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 in Penfield et al. 2006 Plant Cell
Maximum values for each gene were:
PRT6: 11.5 ATE1: 67 ATE2: 28

In all cases data were normalised using MAS5 with a scaling factor of 100. Intensity of colour
(yellow through red) indicates level of expression (low through high).