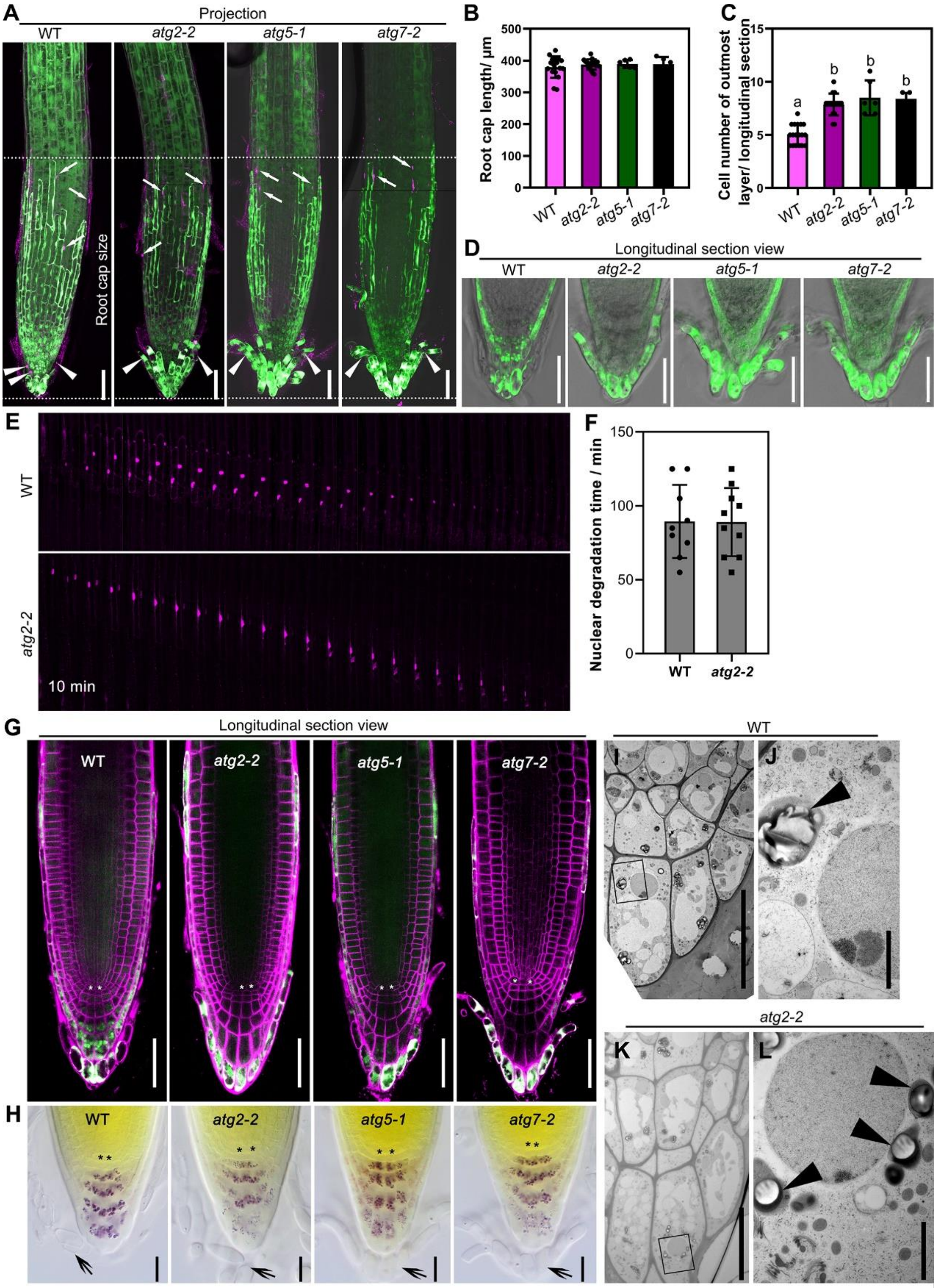

Figure S1 Distal LRC PCD and root cap development occur independent of autophagy. Related to Figure 2.

(A) CLSM (z-stack) of root tips from 5 DAG seedlings of wild type, *atg2-2*, *atg5-1* and *atg7-2,* pulse labeled with FDA and PI. White arrowheads indicate PI-stained nuclei in distal LRC cells and white arrows indicate proximal LRC cells. Scale bars are 50 µm.

(B) Quantification of root cap size of wild type and *atg* mutants. Results are means ± SD. N = 17 for WT, 18 for *atg2-2*, 6 for *atg5-1*, 5 for *atg7-2*.

(C) Cell number quantification of the outmost root cap layer of wild type and *atg* mutants on longitudinal sections. Results are means ± SD. N = 18 for WT, 16 for *atg2-2*, 6 for *atg5-1* and 5 for *atg7-2*. Each mutant is significantly different from the wild type, as indicated by different letters (one-way ANOVA, Dunnett's multiple comparison test, $P < 0.05$).

(D) Longitudinal section of wild type and *atg* mutants, pulse labeled with FDA. Single optical section in (D) and maximal z section projection in (A) are generated from the same root. Scale bars are 50 µm.

(E) Kymograph showing nuclear degradation in *atg2-2* mutant compared with the wild type. Cells were imaged in 5 min intervals for 2 h after staining with PI (magenta).

(F) Quantification of the time of nuclear degradation shown in (A). Results are means ± SD. 10 cells from 4 roots counted for each genotype. There is not significant difference in wild type and *atg2-2* mutant (*t* test, $P > 0.05$).

(G) Longitudinal sections of Arabidopsis roots at 5 DAG pulse labeled with FDA (green) and FM4-64 (magenta). White asterisks indicate quiescent center cells (QC). Scale bars are 50 µm.

(H) Iodine-stained roots of the wild type and *atg* mutants at 5 DAG. Black asterisks indicate quiescent center cells (QC). Arrows point to the outermost root cap layer in which statoliths are degraded. Scale bars are 20 µm.

(I-F) TEM micrograph of columella cells in wild-type (I-J) and *atg2-2* mutant (K-L) at 5 DAG. Close-up area were shown for wild-type (J) and *atg2-2* (L).

Black arrowheads indicate statoliths containing bright starch granules. Scale bars are 20 µm (I and K), 2 µm (J and L).

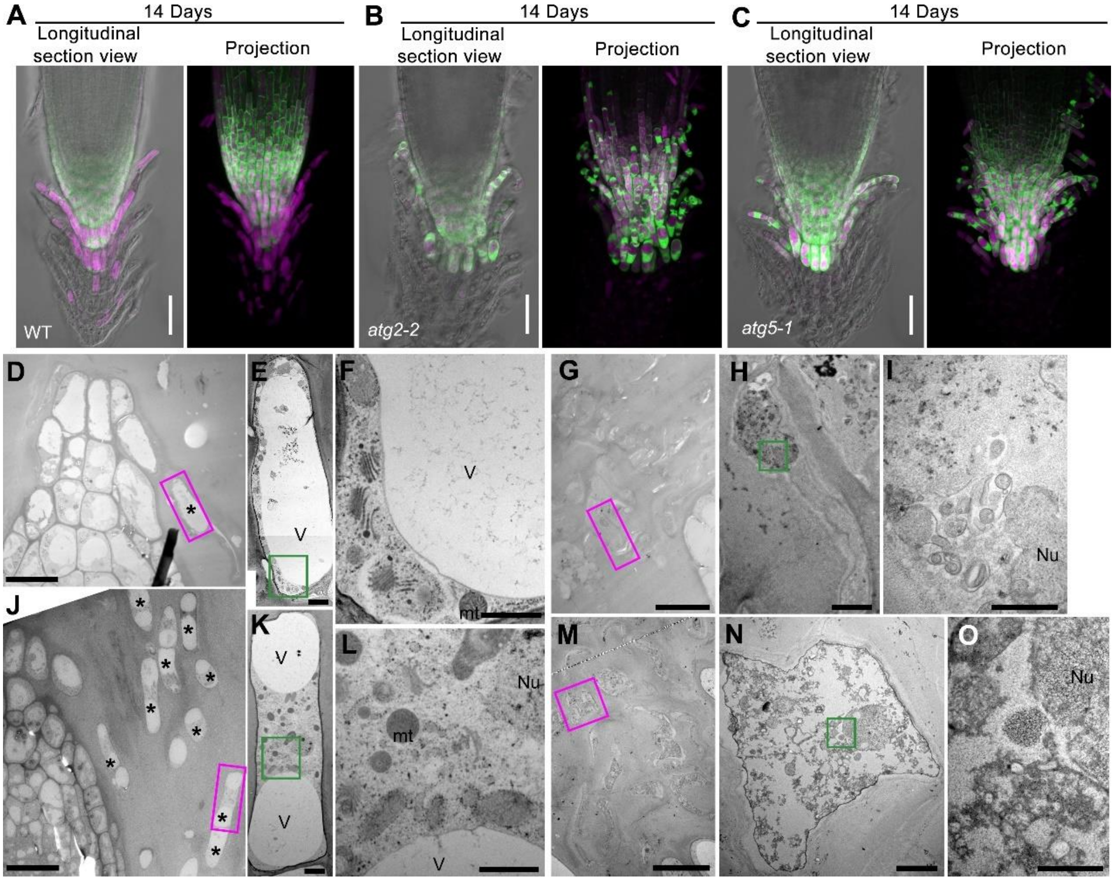

Figure S2 Autophagy regulates PCD onset in proximal LRC cells and corpse clearance in columella cells. Related to Figure 2.

(A-C) pUBQ10::ToIM expression in the wild type (A), *atg2-2* (B), and *atg5-1* (C) was analyzed at 14 DAG. Cytoplasmic EGFP signal is shown in green, and vacuolar mRFP signal is shown in magenta. Scale bars are 50 μm.

(D-O) TEM of wild type (D-I) and *atg2-2* (J-O) seedlings at 14 DAG. Closeups of the wild type (E) and *atg2-2* (K) show viable proximal LRC cells that are indicated by magenta frames in (D) and (J), respectively; closeups of the wild type (F) and *atg2-2* (L) indicated by the green frames in (E) and (K), respectively. Closeups of the wild type (H) and *atg2-2* (N) showing dead columella cells indicated by magenta frames in (G) and (M), respectively; closeups of the wild type (I) and *atg2-2* (O) indicated by green frames in (H) and (N), respectively. The asterisks indicate alive proximal LRC cells. V: vacuole, mt: mitochondria, Nu: nucleus or nuclear remnant. Scale bars are 20 μm for D, G, J and M, 2 μm for E, H, K, and N, 1 μm for F and L, and 500 nm for I and O.

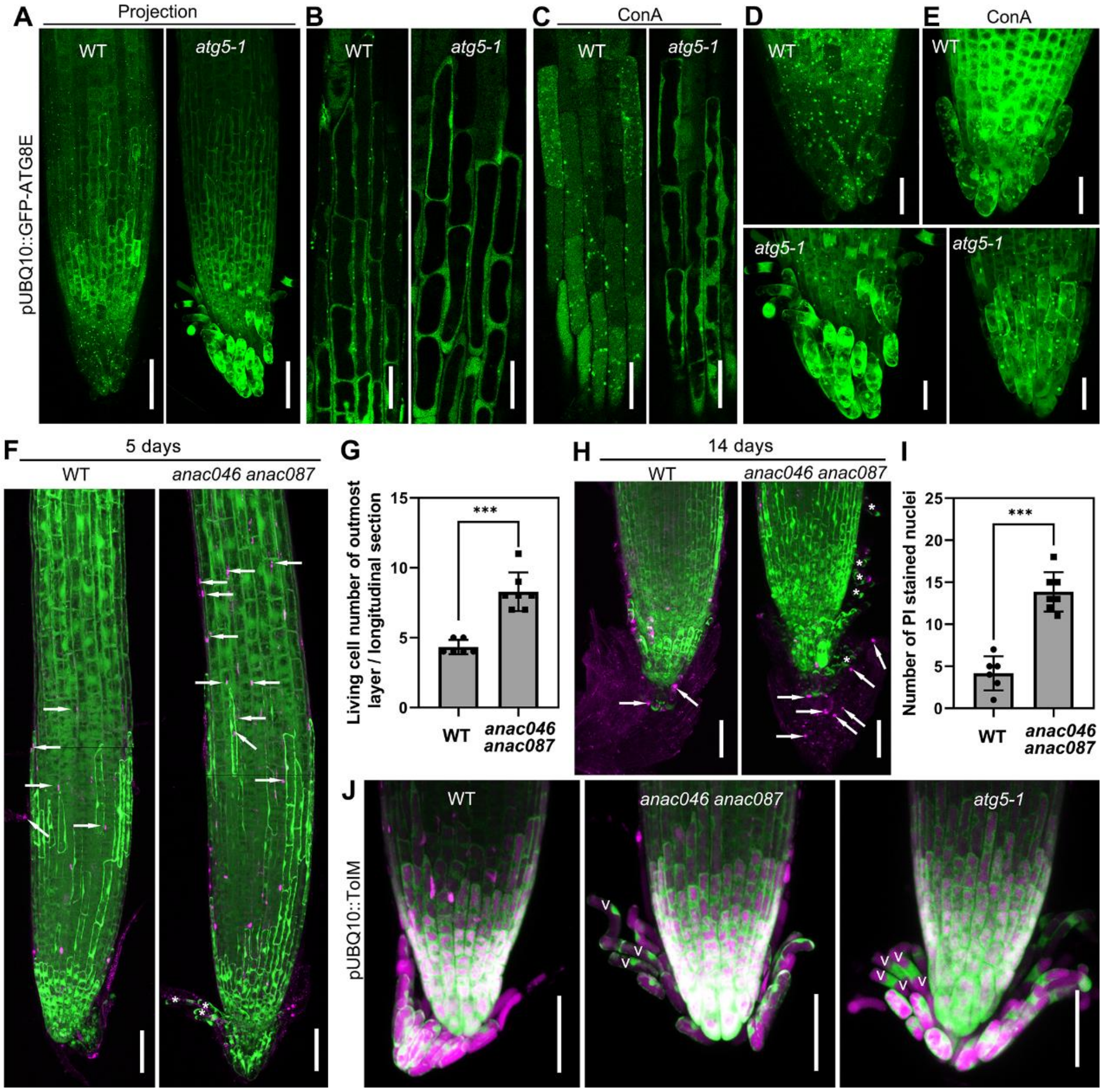

Figure S3 Expression of pUBQ10::GFP-ATG8E in the wild type and *atg5-1*, and phenotypic analyses of the *anac046 anac087* mutant. Related to Figure 3.

(A) CLSM (z-stack projection) of root tips of wild type and *atg5-1* seedlings at 5 DAG expressing GFP-ATG8E. Scale bars are 50 μm.

(B-C) Distal LRC cells of wild type and *atg5-1* seedlings at 5 DAG expressing GFP-ATG8E (B), treated with 1 μM ConA for 8 h (C). Scale bars are 20 μm.

(D-E) Proximal LRC and columella cells of wild type (top row) and *atg5-1* (bottom row) seedlings at 5 DAG expressing GFP-ATG8E (D), treated with 1 μM ConA for 8 h (E). z-stack projection. Scale bars are 50 μm.

(F) CLSM (z-stack projection) of root tips of wild type and *anac046 anac087* mutant seedlings at 5 DAG, stained with FDA and PI. White arrows point to cell corpses in distal LRC cells. Asterisks indicate viable proximal LRC cells. Scale bars are 50 μm.

(G) Quantification of viable cells in the outermost root cap cell layer of wild type and *anac046 anac087* mutant seedlings at 5 DAG counted on longitudinal root sections. Results are means ± SD. N = 6 roots for WT, 7 roots for *anac046 anac087*. *** indicates a significant difference (*t* test, $P < 0.001$).

(H) CLSM (z-stack projection) of root tips from 14 DAG seedlings of wild type and *anac046 anac087* mutant, stained with FDA and PI. White arrows point cell corpse in columella cells. Asterisks point alive proximal LRC cells. Scale bars are 50 μm.

(I) Quantification of PI-positive (dead but non-degraded) cells in columella cells of wild type and *anac046 anac087* mutant seedlings, as shown in (H). Results are means ± SD. N = 6 roots for WT, 7 roots for *anac046 anac087*. *** indicates a significant difference (*t* test, $P < 0.001$).

(J) Tonoplast integrity marker in wild type, *anac046 anac087* mutant and *atg5-1* mutant seedlings at 5 DAG. V: vacuole. Scale bars are 50 μm.

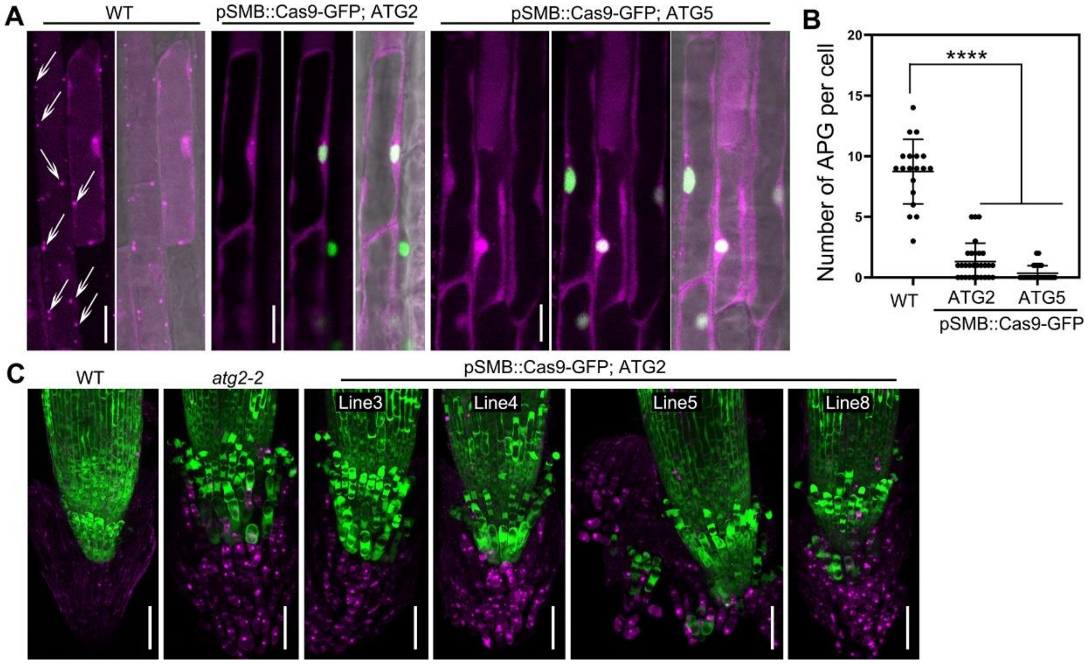

Figure S4 Root cap specific CRISPR-TSKO of ATG mimics the *atg* phenotype. Related to Figure 4.

(A) CLSM of LRC cells from 4 DAG seedlings expressing p35S::mCherry-ATG8E in wild type, and in the CRISPR-TSKO lines pSMB::Cas9-GFP;ATG2 and pSMB::Cas9-GFP;ATG5. mCherry signal is shown in magenta, GFP signal is shown in green. White arrows indicate autophagosomes. Scale bars are 20 μm.

(B) Quantification of autophagosome (APG) in LRC cells. Results are means ± SD. N = 19 cells from 6 roots for WT, 29 cells from 9 roots for pSMB::Cas9-GFP;ATG2, and 26 cells from 9 roots for pSMB::Cas9-GFP;ATG5. **** indicates a significant difference (*t* test, $P < 0.0001$).

(C) CLSM (z-stack projection) of root tips of seedlings carrying a pSMB::NLS-GFP construct at 14 DAG. From left to right: wild type (WT), *atg2-2*, and four independent lines carrying a root cap-specific CRISPR-TSKO construct targeting ATG2 (pSMB::Cas9-GFP;ATG2), pulse labeled with FDA (green) and PI (magenta). Scale bars are 50 μm.