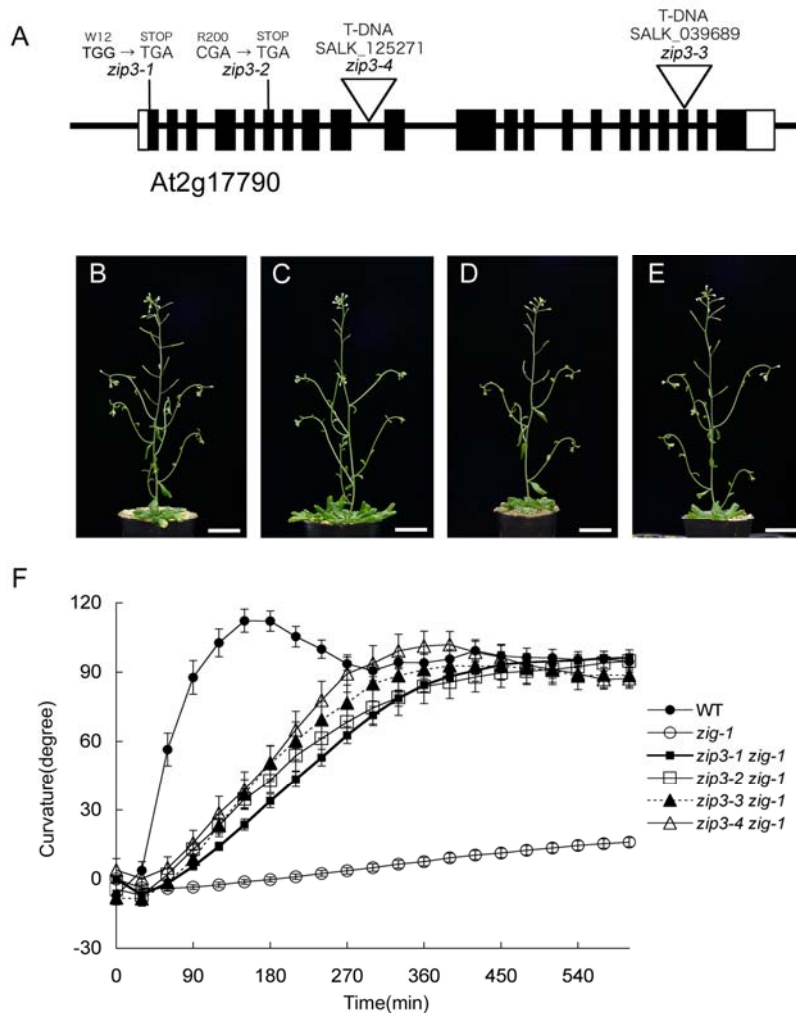


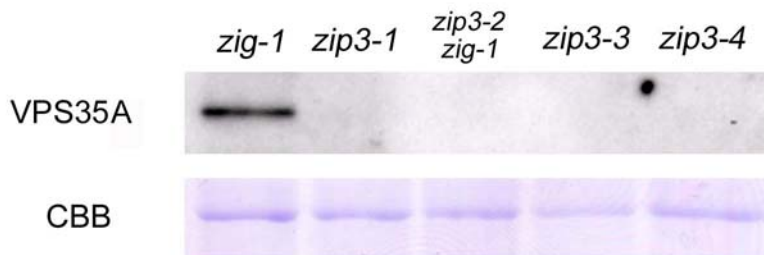
### Supplemental Figure 1. Morphological phenotype of *zip3-1 zig-1*.

We determined three morphological features to evaluate the suppression effect of *zip3-1* on the *zig-1* phenotype. **(A)** The angles between two adjacent internodes of the primary shoot in the *zig* mutant. N=12. **(B)** The internodal length in the second metamer. 7-8 week-old plants were used for measurement. N=12. **(C)** Leaf morphology. The fifth and sixth leaves from 4-week-old plants were used for the measurement. N=16. For all three features, the *zip3-1 zig-1* double mutant exhibited an intermediate phenotype between wild type and *zig-1*.



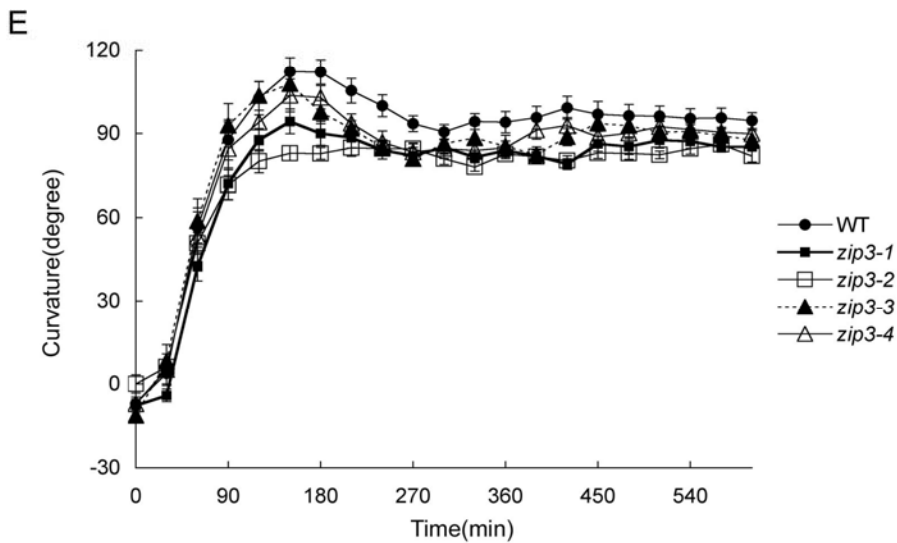
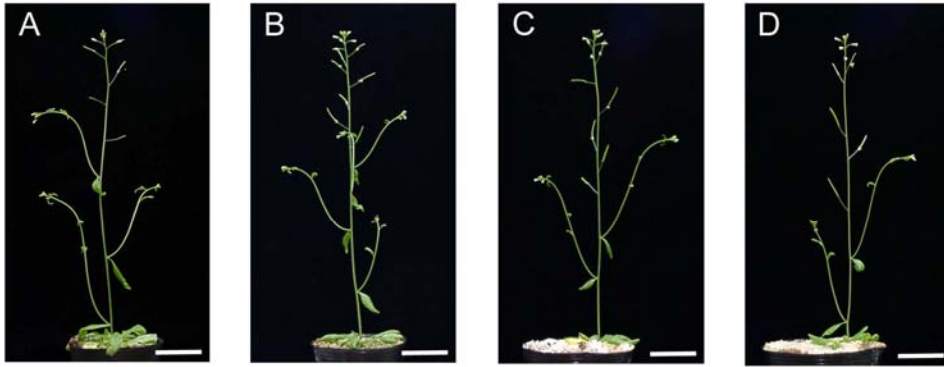
**Supplemental Figure 2. Phenotypes of alleles of *zip3 zig-1* double mutants.**

(A) Schematic structure of the *ZIP3* (*At2g17790*) gene and the position of mutations and T-DNA insertions. Boxes indicate exons and white boxes represent untranslated regions. Morphological phenotypes of 5-week-old plants of *zip3-1 zig-1* (B), *zip3-2 zig-1* (C), *zip3-3 zig-1* (D), and *zip3-4 zig-1* (E). Scale bars; 3 cm. (F) Gravitropic phenotypes. The gravitropic response of inflorescence stems of 5-week-old plants was gravi-stimulated by being placed horizontally at 23° C under dim non-directional light. 15 individuals of each genotype were examined. Bars represent SE.



**Supplemental Figure 3. Immunoblot analysis of ZIP3/VPS35A protein**

Proteins were extracted from 2-week-old seedlings from each genotype and subjected to SDS-PAGE, followed by immunoblot analysis using anti-VPS35A antibody. An approximately 90-kDa protein band was detected only in extracts prepared from the wild type plant. Lower panel shows CBB staining to indicate that a similar amount of protein was applied to each lane of the gel.



**Supplemental Figure 4. Phenotypes of alleles of *zip3* single mutants.**

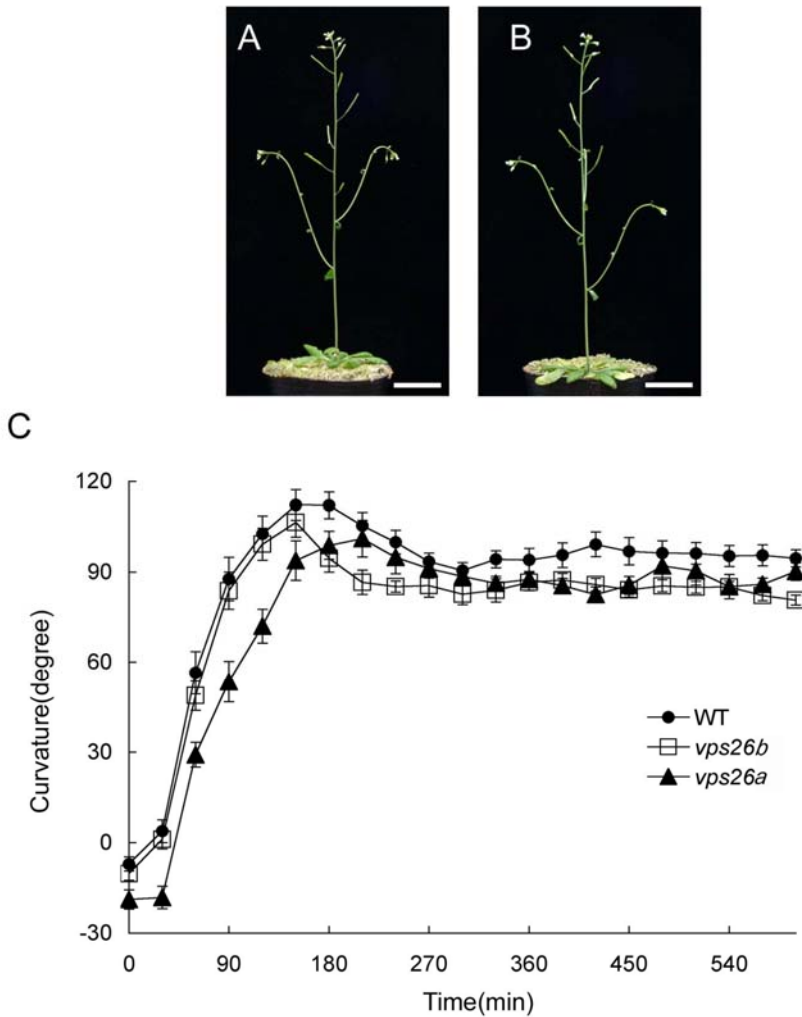
5-week-old plants of *zip3-1* (A), *zip3-2* (B), *zip3-3* (C), and *zip3-4* (D). Scale bars; 3 cm.

(E) Gravitropic phenotypes. Gravitropic response of inflorescence stems of 5-week-old plants was gravi-stimulated by being placed horizontally at 23° C under dim non-directional light. 15 individuals of each genotype were examined. Bars represent SE.



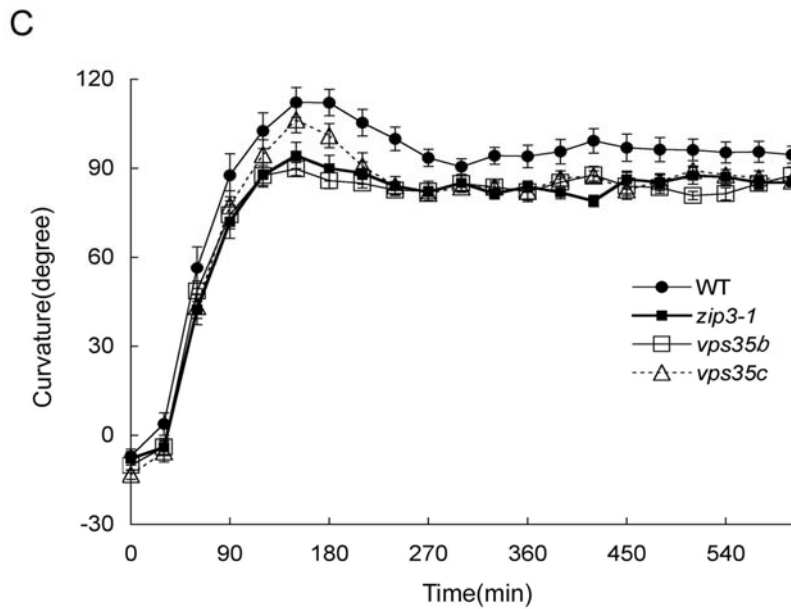
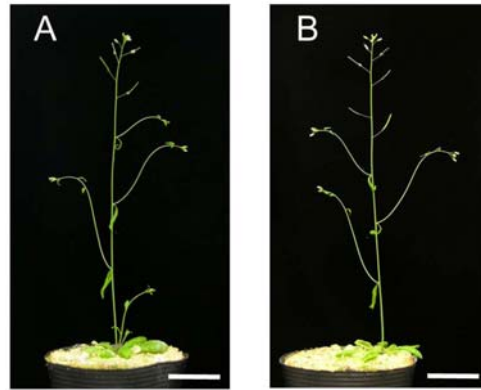
**Supplemental Figure 5. Morphological phenotypes of *mag1-1* and *mag1-1 zig-1* mutants.**

5-week-old plants of *mag1-1* (A), *mag1-1 zig-1* (B). Scale bars; 3 cm.



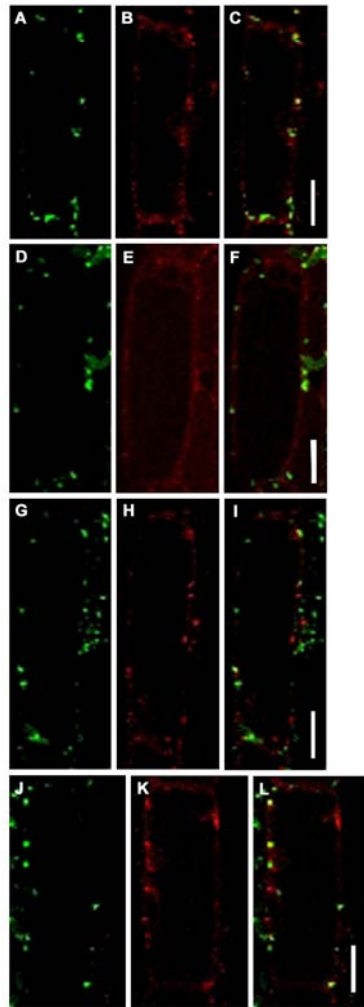
**Supplemental Figure 6. Phenotypes of *vps26* single mutants.**

The 5-week-old plants of *vps26a* (A) and *vps26b* (B). Scale bars; 3 cm. (C) Gravitropic phenotypes. Gravitropic response of inflorescence stems of 5-week-old plants were gravi-stimulated by being placed horizontally at 23° C under dim non-directional light. 15 individuals of each genotype were examined. Bars represent SE.



**Supplemental Figure 7. Phenotypes of *vps35b* and *vps35c* single mutants.**

5-week-old plants of *vps35b* (A) and *vps35c* (B). Scale bars; 3 cm. (C) Gravitropic phenotypes. Gravitropic response of inflorescence stems of 5-week-old plants was gravitropically stimulated by being placed horizontally at 23° C under dim non-directional light. 15 individuals of each genotype were examined. Bars represent SE.



**M**

	WT	<i>zig-1</i>	<i>zip3-1 zig-1</i>	<i>zip3-1</i>
GFP (VTI12)	10.52±0.66	12.76±0.84	11.98±0.64	11.76±0.60
RFP (ARA6)	12.98±0.80	12.86±0.82	10.02±0.60	12.44±0.64
merge	3.64±0.34	2.72±0.29	2.28±0.21	2.42±0.23

(n = 50)

### Supplemental Figure 8. Localization of GFP-VTI12 and ARA6-mRFP in endodermal cells

Confocal images of GFP-VTI12 and ARA6-mRFP in endodermal cells. (A-C) wild type, (D-F) *zig-1*, (G-I) *zip3-1 zig-1*, (J-L) *zip3-1*. VTI12 and Ara6 were observed with GFP (A, D, G, H) and RFP fluorescence (B, E, H, K), respectively. *zip3-1 zig-1/VTI12pro:GFP-VTI12* and *WT/SCRpro:ARA6-mRFP* lines were crossed, and four genotypes of wild type, *zig-1*, *zip3-1 zig-1* and *zip3-1* bearing both constructs homozygously were isolated. (M) Quantitative analysis of GFP-, RFP- and both signal- positive dots. The number of dots in images of endodermal cell as shown in A to L was counted. Values represent means  $\pm$  SE. Fifty cells of each genotype were examined.



**Supplemental Table 1. Localization of amyloplasts in the endodermal cell.**

<b>Position</b>	<b>WT</b>	<b><i>zig-1</i></b>	<b><i>zip3-1 zig-1</i></b>	<b><i>zip3-1</i></b>
<b>Top</b>	<b>0.06±0.03</b>	<b>3.2±0.35</b>	<b>0.22±0.07</b>	<b>0.04±0.03</b>
<b>Middle</b>	<b>0.18±0.08</b>	<b>2.0±0.51</b>	<b>0.36±0.12</b>	<b>0.24±0.09</b>
<b>Bottom</b>	<b>5.16±0.24</b>	<b>2.94±0.28</b>	<b>3.78±0.29</b>	<b>4.8±0.33</b>

Localization of amyloplasts in the endodermal cell. An endodermal cell is divided into three areas (top, middle, bottom), and then the number of amyloplasts (mean  $\bar{X}$  } SD) located at each area was counted, based on the image of longitudinal sections shown in Figure 3 A -D. Fifty cells of each genotype were examined.

**Supplemental Table 2. Quantitative analysis of the cytological phenotype of living endodermal cells based on the observation as shown in Figure 3 H-K.**

	WT	<i>zig-1</i>	<i>zip3-1 zig-1</i>	<i>zip3-1</i>
Öü	<b>6.42±0.46</b>	<b>4.12±0.39</b>	<b>5.66±0.48</b>	<b>6.6±0.63</b>
Ö†	<b>3.04±0.34</b>	<b>0.16±0.07</b>	<b>1.78±0.23</b>	<b>4.44±0.49</b>
Ö°	<b>0.46±0.21</b>	<b>4.14±0.39</b>	<b>2.1±0.30</b>	<b>0.38±0.14</b>
Ö€	<b>35</b>	<b>5</b>	<b>29</b>	<b>33</b>
Ö£	<b>0</b>	<b>13</b>	<b>6</b>	<b>0</b>

Quantitative analysis of the cytological phenotype of living endodermal cells based on the observation as shown in Figure 3 H-K. Time-lapse imaging for 5 min with 15 seconds-intervals were performed. Five characteristics of amyloplasts and vacuolar membrane were quantitatively observed in living endodermal cells (I to V). I; Average number of amyloplasts in an endodermal cell in a focal plane at a timepoint (time 0). II; Average number of amyloplasts that appeared to be enclosed by the vacuolar membrane within I. III; Average number of amyloplasts that hardly move during a 5 min-observation within I. IV; Number of cells in which transvacuolar strands arose during 5 min-observation, indicating vacuolar membrane dynamics. V; Number of cells in which abnormal clusters of vesicles were found during 5 min-observation. Fifty cells were observed for each genotype and then analyzed.

**Supplemental Table 3.****Primer sets for cloning and RT-PCR**

Primer name	Sequence
gZIP3-F	5'CCCGGGAAATGTTAGATGCTTC-3'
gZIP3-R	5'CCCGGGTTTACATTTTCGAGCAGC-3'
cZIP3-F	5'GTTCCCGGGATGATCGCAGACGGATCAGA-3'
cZIP3-R	5'GTTCCCGGGTACTTTGATCGCCTGGTATC-3'
cVPS35B-F	5'CTTGGATCCATGAGAACGCTCGCCGGAGTAG-3'
cVPS35B-R	5'CTTGGATCCTCACAGCTTGATAGGGTCATA-3'
cVPS35C-F	5'CTTGGATCCATGATCGCCGACGACGATGAG-3'
cVPS35C-R	5'CTTGGATCCTCATTCAAACCATTCATTTTG-3'
cVPS35B-F2	5'AAGAGTTCTTCTCTGTTAAGACG-3'
cVPS35B-R2	5'TGTGTCTCGGATTTAAGTCACAGC-3'
cVPS35C-F2	5'ATTCTGCAAGACTCCTAAGGAAGC-3'
cVPS35C-R2	5'TCTTCTTGCTGTCTCTGAAACTCG-3'
cVPS26A-F1	5'GTCTCTCTCCGATTGAAATAAACC-3'
cVPS26A-R2	5'TGCATACAGAAAGTCTTGAAAAGG-3'
cVPS26B-F1	5'ATTACGCTCTTAACATCAGCTTGG-3'
cVPS26B-R2	5'ATCTGAGTACACAAAGTCTAAAGC-3'
ACT8 Df	5'GAGAGATTCAGGTGCCAG -3'
ACT8 Dr	5'AGAGCGAGAGCGGGTTTTCA -3'
pSCR(-30)D	5'TCTTACCTTATTTATAACCTAGGC-3'
cVPS35B-R1	5'GATACATTCTGGGCAATATGTTGC-3'
cZIP3-R2	5'ATACAGTCTATTAGGTAATATTGG-3'
cVPS35C-R1	5'ATTCATGTGTGCATCACCATCTCC-3'