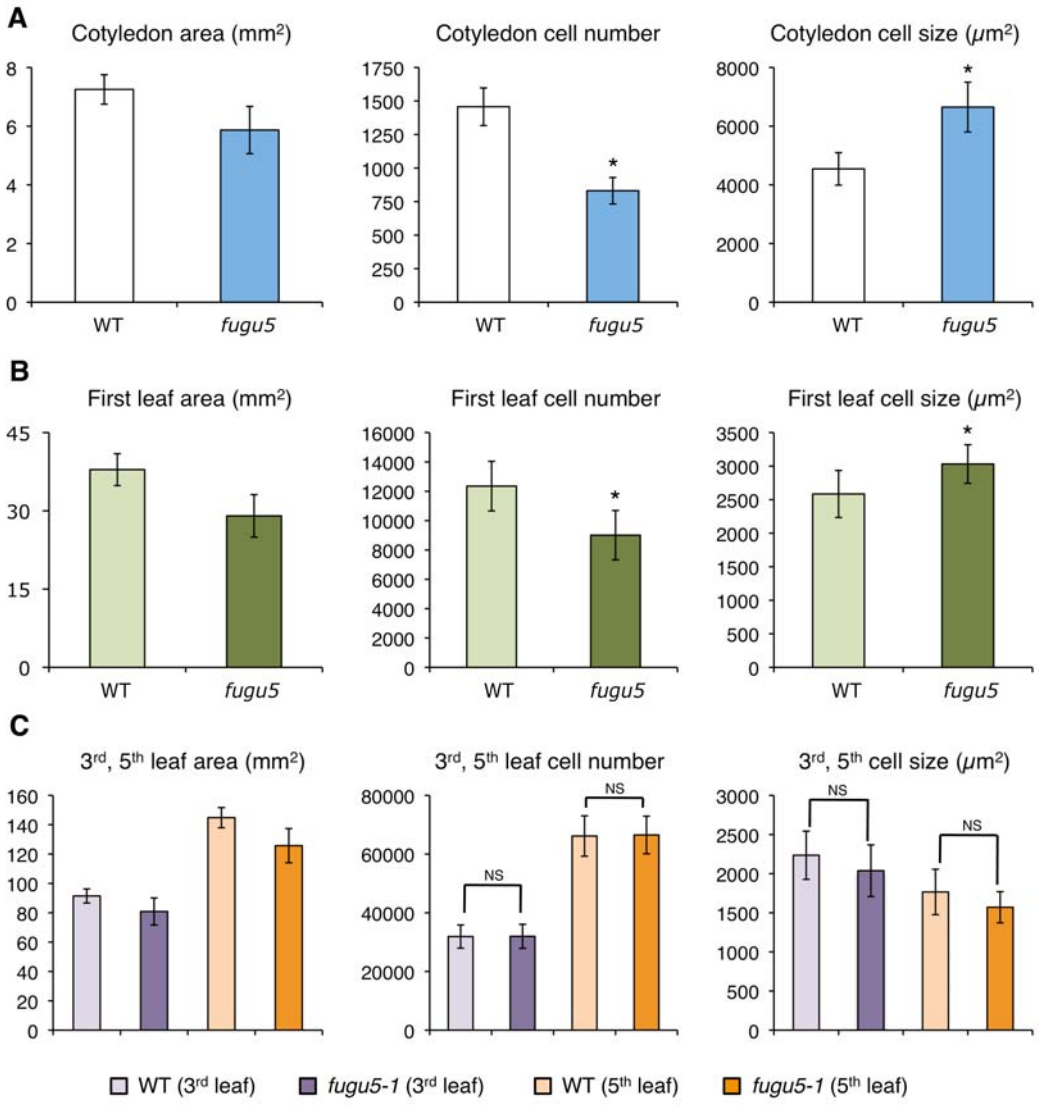
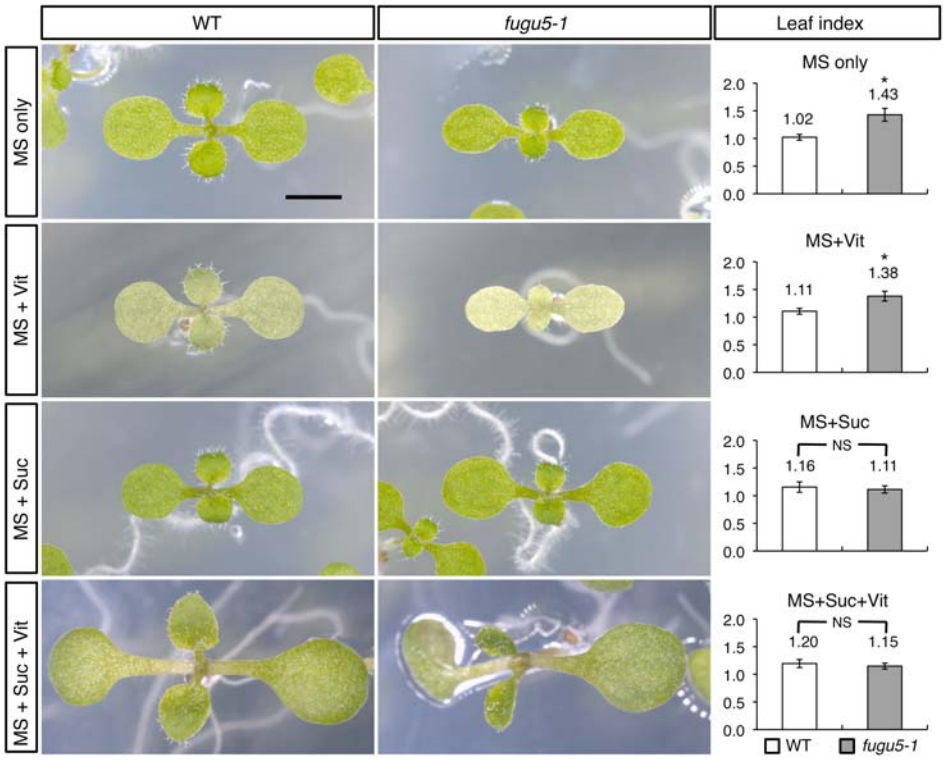


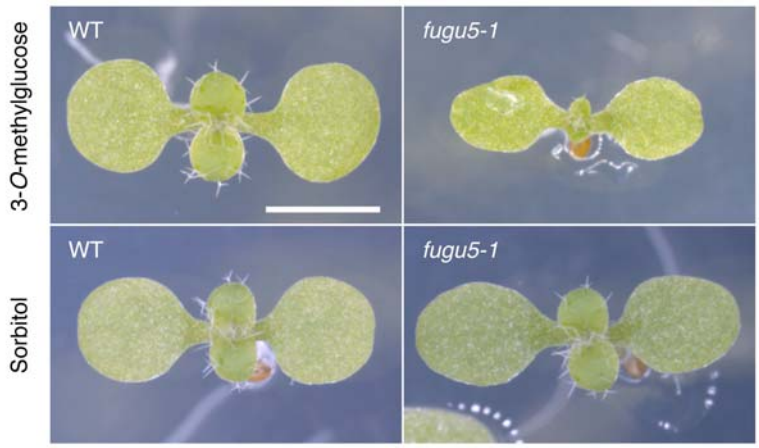
Supplemental Figure 1. Compensation in *fugu5* is restricted to cotyledons and first leaves.

(A-C) Cellular phenotypes in cotyledons and rosette leaves at different positions are affected differently. (A) Cotyledons, (B) First leaves, and (C) Third and fifth leaves from WT and *fugu5-1* mutants plants grown on rockwool for 30 DAS were collected and their average areas, cell numbers and cell sizes were determined. (A-C) Data are means and standard deviation ($n = 8$). NS, no significant difference among the two genotypes (WT and *fugu5-1*). Asterisk, $P < 0.01$.



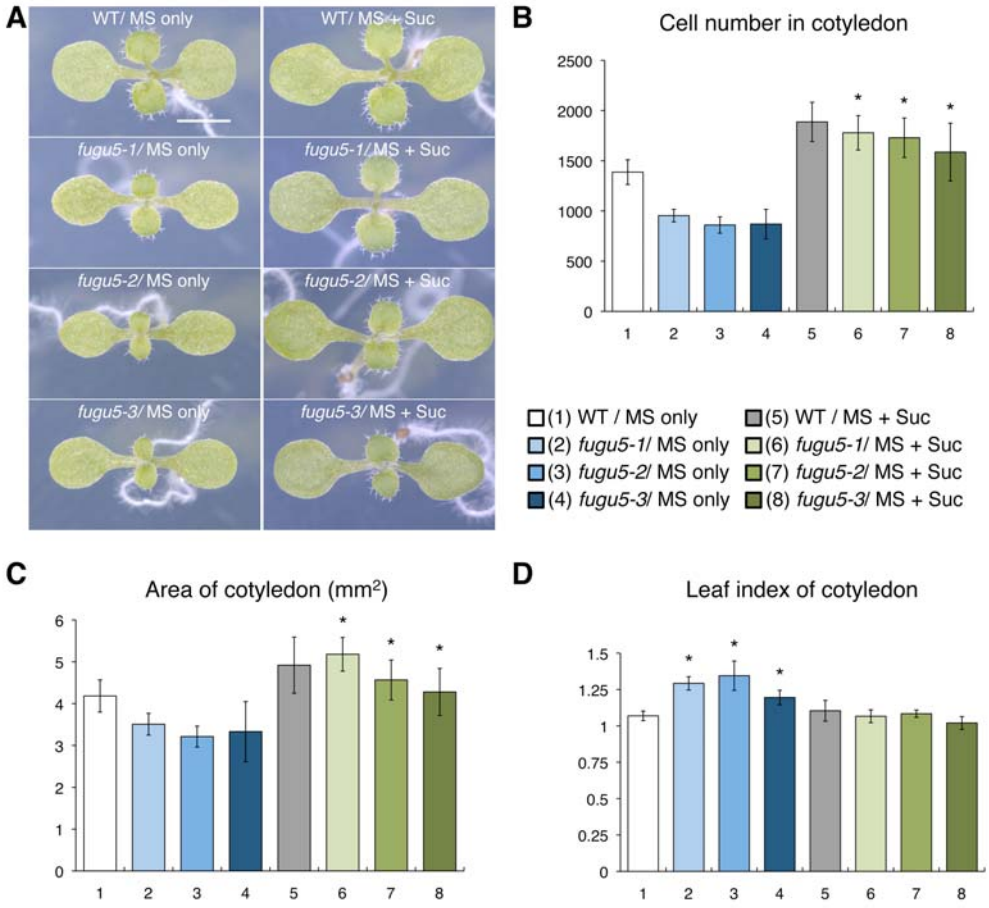
Supplemental Figure 2. Phenotype of WT and *fugu5* mutant young seedlings grown on MS media with different supplements.

Effect of Suc and vitamins on the gross phenotype of *fugu5* mutant cotyledons was investigated. WT and *fugu5-1* mutant seeds were grown on the indicated growth media for 8 DAS. Photos show seedlings of WT (left panels) and *fugu5-1* (central panels). Scale bar, 2 mm. Leaf index has been determined (right panels). Data are means and standard deviation (n = 8). NS, no significant difference between the two genotypes (WT and *fugu5-1*) under the indicated growth conditions. Asterisk, $P < 0.001$. Vit, Gamborg's B5 vitamins.



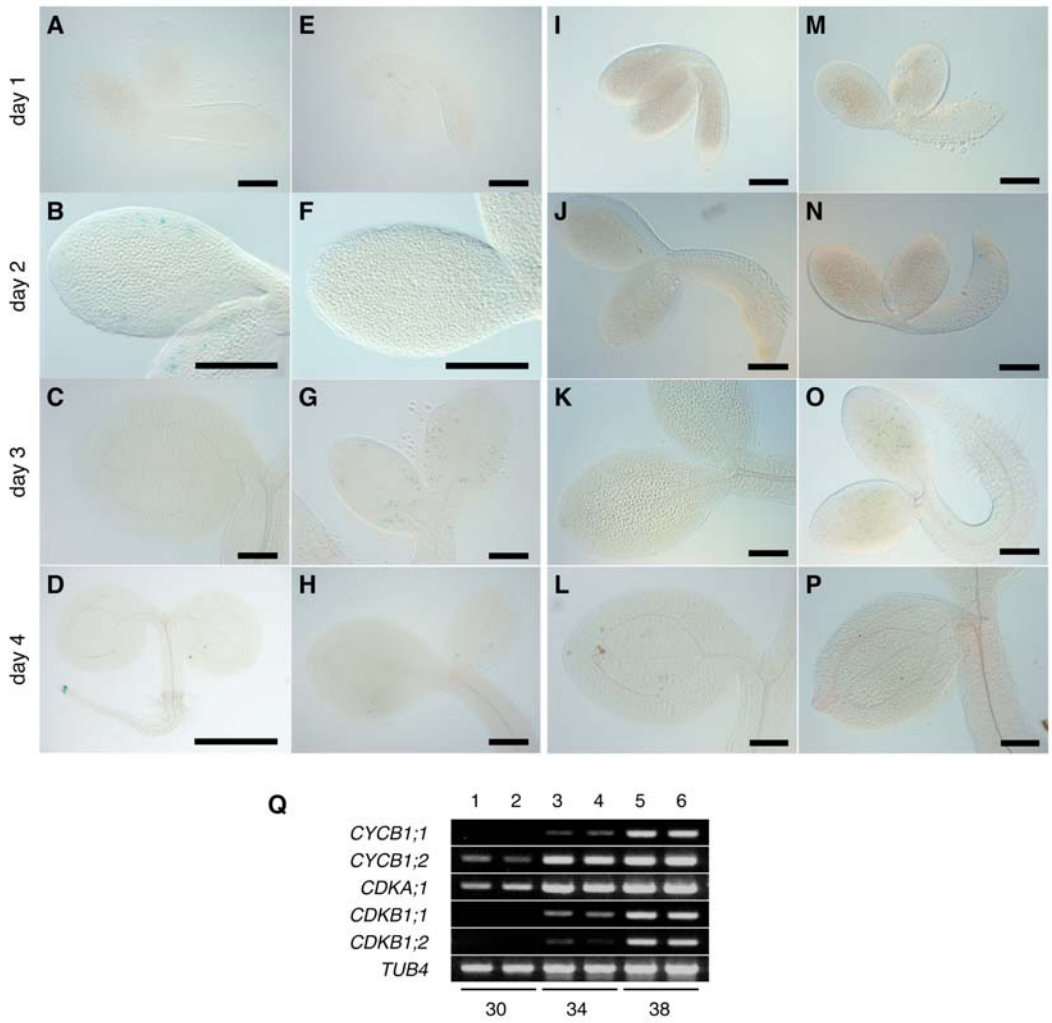
Supplemental Figure 3. Effect of a glucose analog and osmotic stress on the *fugu5* phenotype.

WT and *fugu5-1* mutants were grown on MS-only medium supplemented with either 58 mM of 3-*O*-methylglucose (top panels) or sorbitol (lower panels). Photos of 8-day-old seedlings are shown. Scale bar, 2 mm.



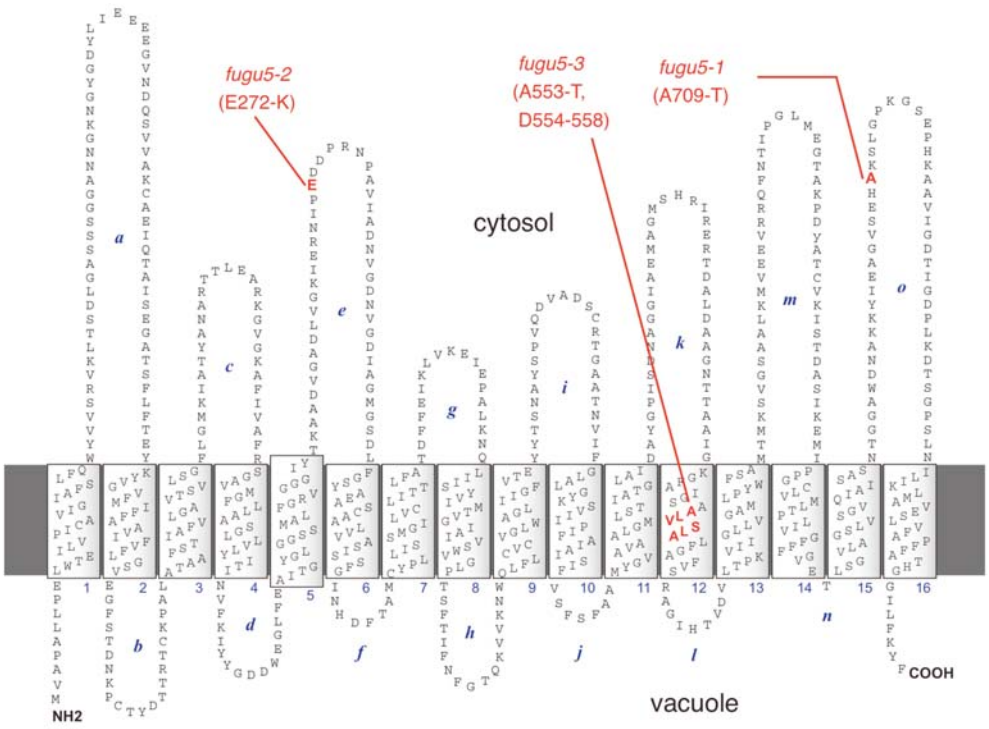
Supplemental Figure 4. Effect of Suc on the cotyledon phenotype of different alleles of the *fugu5* mutant.

(A) Effect of exogenous Suc on the gross phenotype of different alleles of the *fugu5* mutant. Photos show WT and three alleles of *fugu5* mutant seedlings grown for 8 DAS either on MS-only or MS medium supplemented with 2% Suc. Scale bar, 2 mm. (B-D) Cotyledon cell number, area and shape of different alleles of the *fugu5* mutant recover upon Suc supply. (B) Cotyledons average cell numbers, (C) areas, (D) leaf indices were determined. (B-D) Data are means and standard deviation ($n = 8$). (B) and (C), Asterisk, $P < 0.01$ compared with no Suc addition. (D), Asterisk, $P < 0.01$ compared with Suc addition.



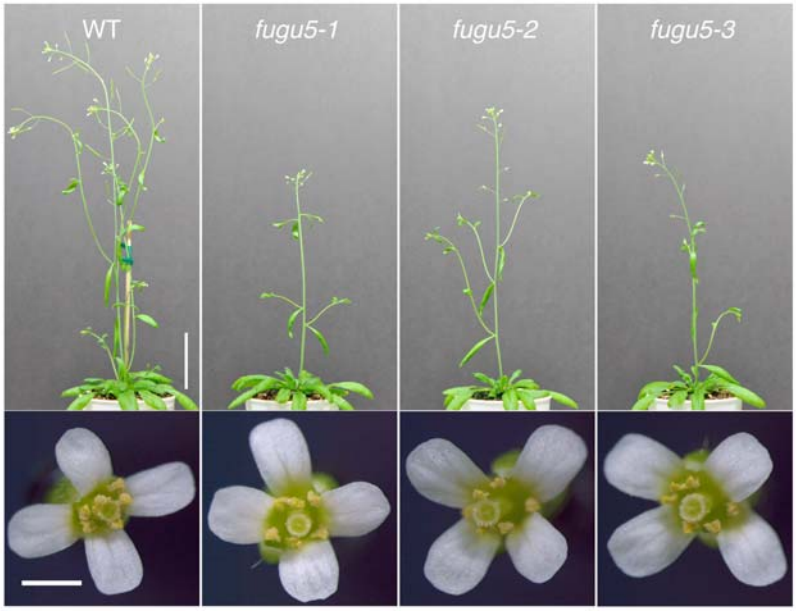
Supplemental Figure 5. Effect of Suc on the expression of the *CYCB1;1*$_{pro}$*:GUS* reporter and several core cell cycle genes.

(A-P) The expression of *CYCB1;1*$_{pro}$*:GUS* was monitored in embryos or young seedlings grown on MS-media with or without 2% Suc. Time-course analysis of *CYCB1;1*$_{pro}$*:GUS* was carried out every day after the beginning of seed imbibition for 4 days. (A-D) and (E-H) *CYCB1;1*$_{pro}$*:GUS* (WT) grown on either MS-only or MS (+) 2% Suc, respectively. (I-L) and (M-P) *fugu5-1 CYCB1;1*$_{pro}$*:GUS* line grown on MS-only or MS with 2% Suc, respectively. All scale bars except (D), 200 μm. Scale bar in (D), 1 mm. (Q) Expression of several core cell cycle genes is not affected in *fugu5* mutant. Total RNA was extracted from seeds that had been imbibed for 48 h in MS medium alone. The expression levels of the indicated genes were evaluated by RT-PCR analyses. Lane numbers 1, 3, and 5 indicate WT samples. Lane numbers 2, 4, and 6 indicate *fugu5-1* mutant samples. The numbers of PCR cycles are indicated at the bottom.



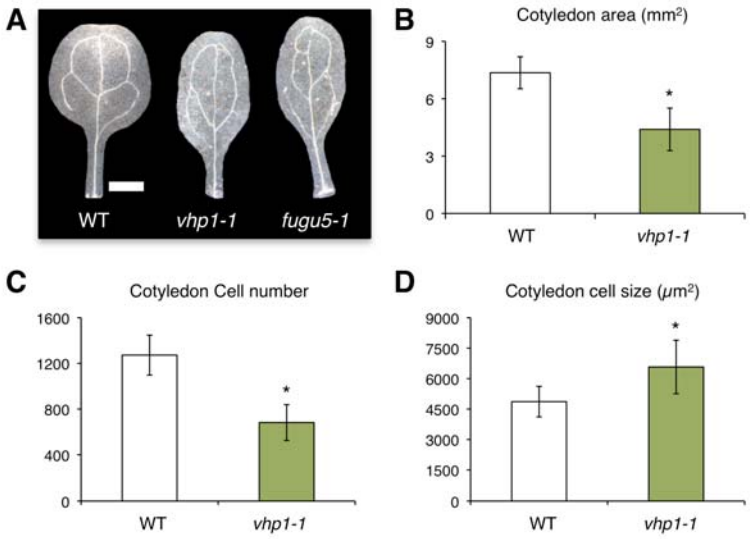
Supplemental Figure 6. Mutation sites and membrane topology of V-PPase.

The molecular lesions in each of the three loss-of-function *fugu5* alleles are highlighted in red. The topological arrangement of the mutation sites identified in the V-PPase protein in three different *fugu5* alleles are shown. The membrane topology model of *A. thaliana* V-PPase is based on a previous report on *Streptomyces coelicolor* (Mimura et al., 2004).



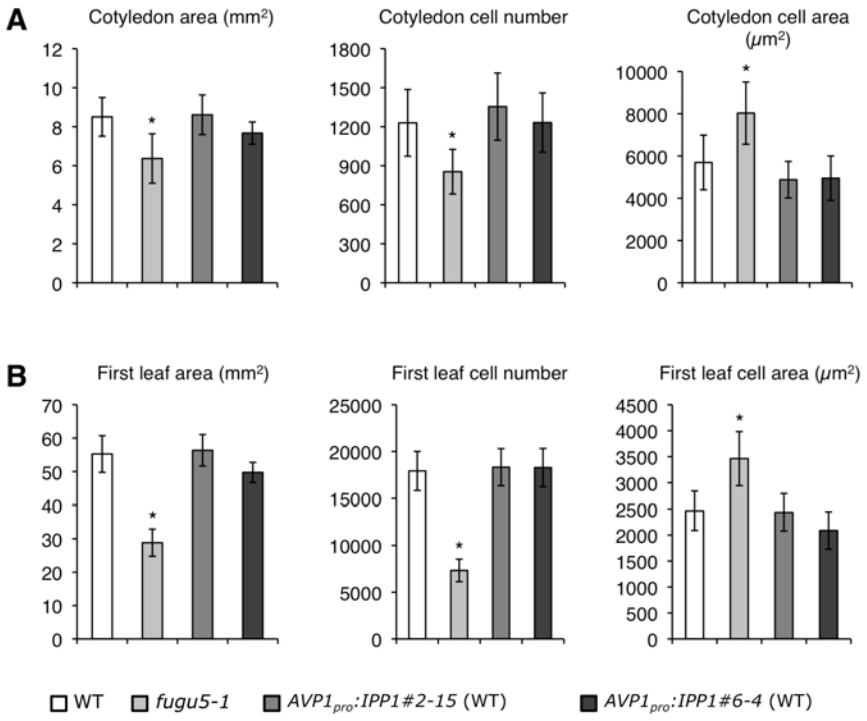
Supplemental Figure 7. Gross morphology of WT and *fugu5* mutant allele plants.

WT and *fugu5* mutant alleles were grown on rockwool until flowering. Gross morphology of plants 39 DAS (upper four panels). Scale bar, 4 cm. Flowers of the indicated plants (lower four panels). Scale bar, 1 mm.



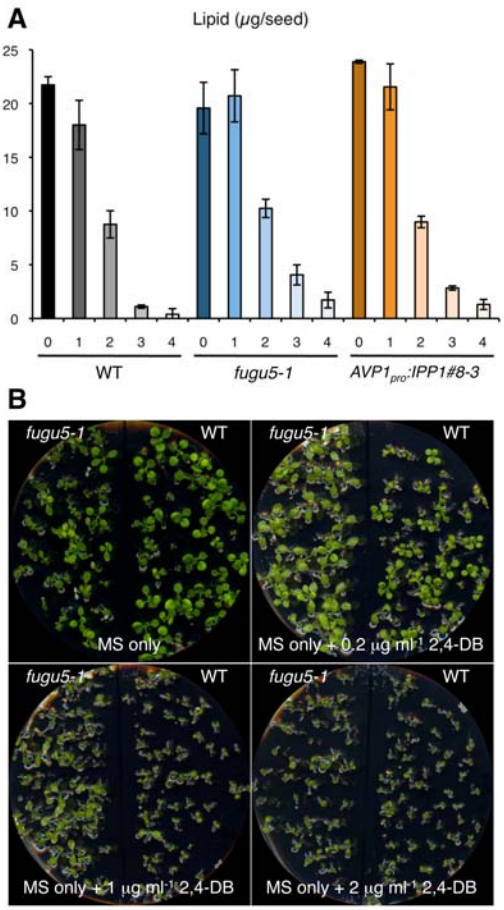
Supplemental Figure 8. The *vhp1-1* mutant, a T-DNA insertion line allele of *fugu5*, exhibits compensation.

(A) Gross morphology of mature cotyledons. WT, *vhp1-1* and *fugu5-1* mutant cotyledons from plants grown on rockwool for 25 DAS. Scale bar, 1 mm. (B-D) The *vhp1-1* mutant, a T-DNA insertion line allele of *fugu5*, exhibits compensation. A T-DNA insertion mutant line *vhp1-1* (reference number, KG8420) was isolated from the T-DNA tag-line library composed of 32,640 lines, which was provided by the Kazusa DNA institute by our laboratory (Dr. Y. Nakanishi and Ms. M. Inagaki, Nagoya University). (B) Average areas, (C) cell numbers, and (D) cell sizes in WT and *vhp1-1* cotyledons. (B-D) Data are means and standard deviation (n = 8). Asterisk, $P < 0.01$.



Supplemental Figure 9. Phenotype of *AVP1*$_{pro}$*:IPP1* (WT) transgenic plants.

(A-B) The heterologous expression of *IPP1* gene in WT background does not affect its cellular phenotype. Average area, cell numbers and cell sizes of cotyledons (A) and first leaves (B) of WT, *fugu5-1* and two *AVP1*$_{Pro}$*:IPP1* (WT) representative lines were determined at 25 DAS. Data are means and standard deviation (n = 8). Asterisk, $P < 0.01$.



Supplemental Figure 10. Mobilization of seed lipid reserves and effect of 2,4-DB on *fugu5* mutant.

(A) The *fugu5-1* mutant and the *AVP1*$_{Pro}$*:IPP1#8-3* transgenic line exhibit normal degradation of seed lipid reserves during postgerminative growth. The amounts of reserved lipids in WT, *fugu5-1* mutant and the *AVP1*$_{Pro}$*:IPP1#8-3* transgenic line were determined during post-germinative growth. Samples were prepared from dry seeds (day zero) and etiolated seedlings after 1, 2, 3, and 4 d, as described in the experimental procedures. Data are means and standard deviation from three or six independent experiments. (B) Effects of 2,4-DB on the growth of the *fugu5-1* mutant. WT and *fugu5-1* mutant were grown for 10 DAS on MS-only medium either without or with the indicated concentrations of 2,4-dichlorophenoxybutyric acid (2,4-DB) under 16/8-h light/dark cycle.

Supplemental Table 1. List of primers used in this study.

Primers used for RT-PCR analyses			
Gene name	Primer name	Primer sequence (5' ->3') Forward	Reverse
CYCB1;1	CYCB1;1-FW/-RV	tcatcgtcct cgtacacgat ctca	cggacatgca catcaatcaa ccac
CYCB1;2	CYCB1;2-FW/-RV	tggccacctc aggttaacga tct	ccggtgtgga actgcaatgt atcag
CDKA;1	CDKA;1-FW/-RV	cgccggtgac attttataag tgtgg	gtcctgacag ggataccgaa tgc
CDKB1;1	CDKB1;1-FW/-RV	gtgttgcgca ttgtcatagt catgg	tctttcggct ggattgtact tgagc
CDKB1;2	CDKB1;2-FW/-RV	ctgcgtcgaa catgttattc aatcg	ccactttgga tagacatgcc agtca
IPP1	IPP1-P1/-P2	cacccagaaactaaggcagt	ctcaacagacctgggaagta
TUBULINß 4	TUB4-FW/-RV	aatacgtcggcgattctccg	cttaggagaaggaaacactg

Primers used for gateway system construction of *IPP1* transgenic plants	
Primer name	Primer sequence (5' ->3')
B4F-pAVP1-FW	GGGGACAACT TTGTATAGAA AAGTTGCCCA TTCTTTGCTT GTTCGTTT
B1R-pAVP1-RV	GGGGACTGCT TTTTTGTACA AACTTGCTTC TCTCCTCCGT ATAAGAGA
IPP1-FW	AAAAAGCAGG CTATGACCTA CACTACCAGA CAAA
IPP1-RV	AGAAAGCTGG GTTTAAACAG AACCGGAGAT GAAG
B1	GGGGACAAGTTTGTACAAAAAAGCAGGCT
B2	GGGGACCACTTTGTACAAGAAAGCTGGGT