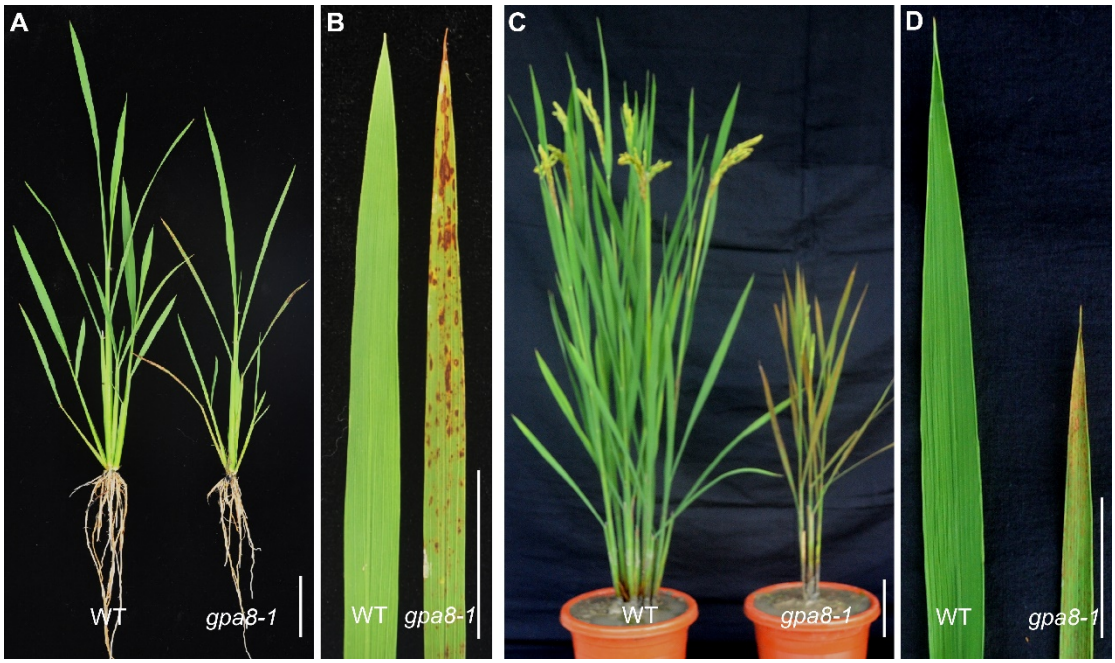
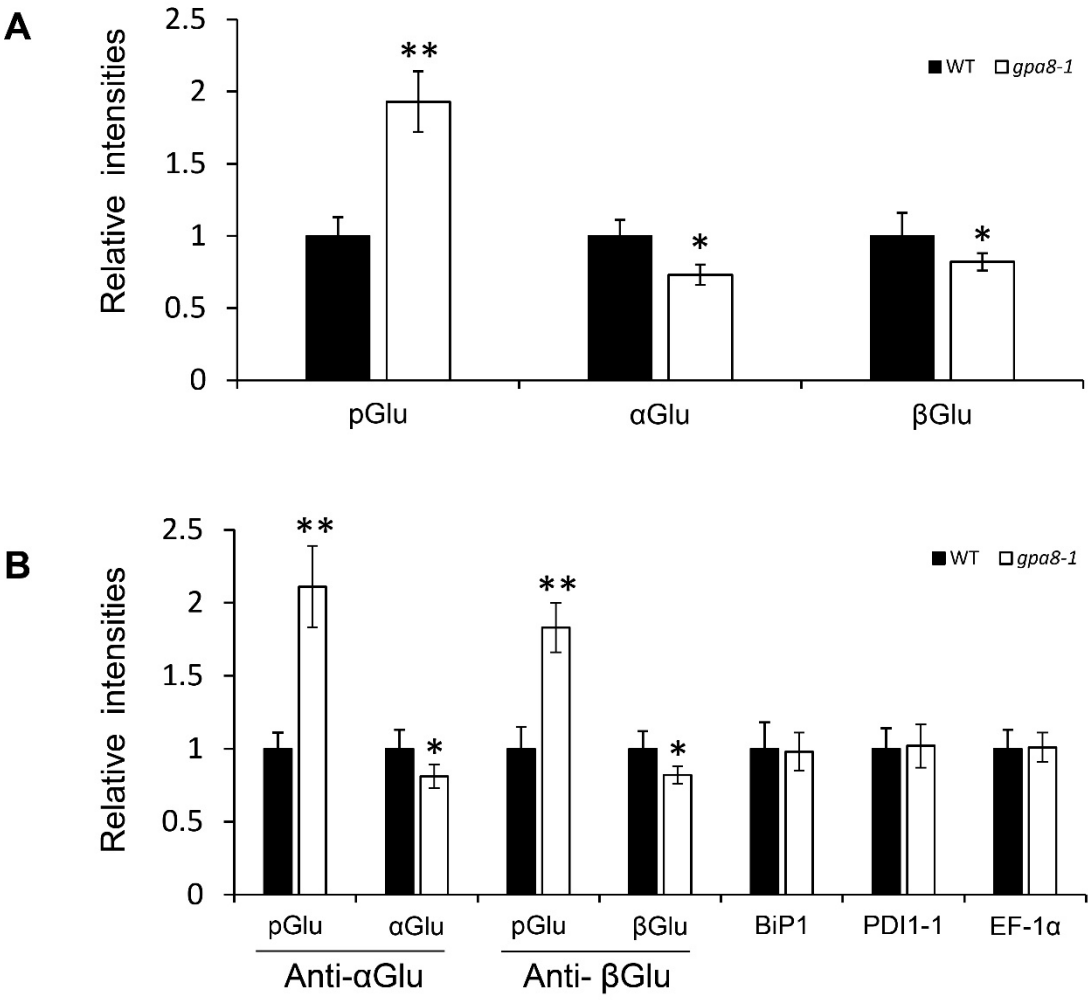


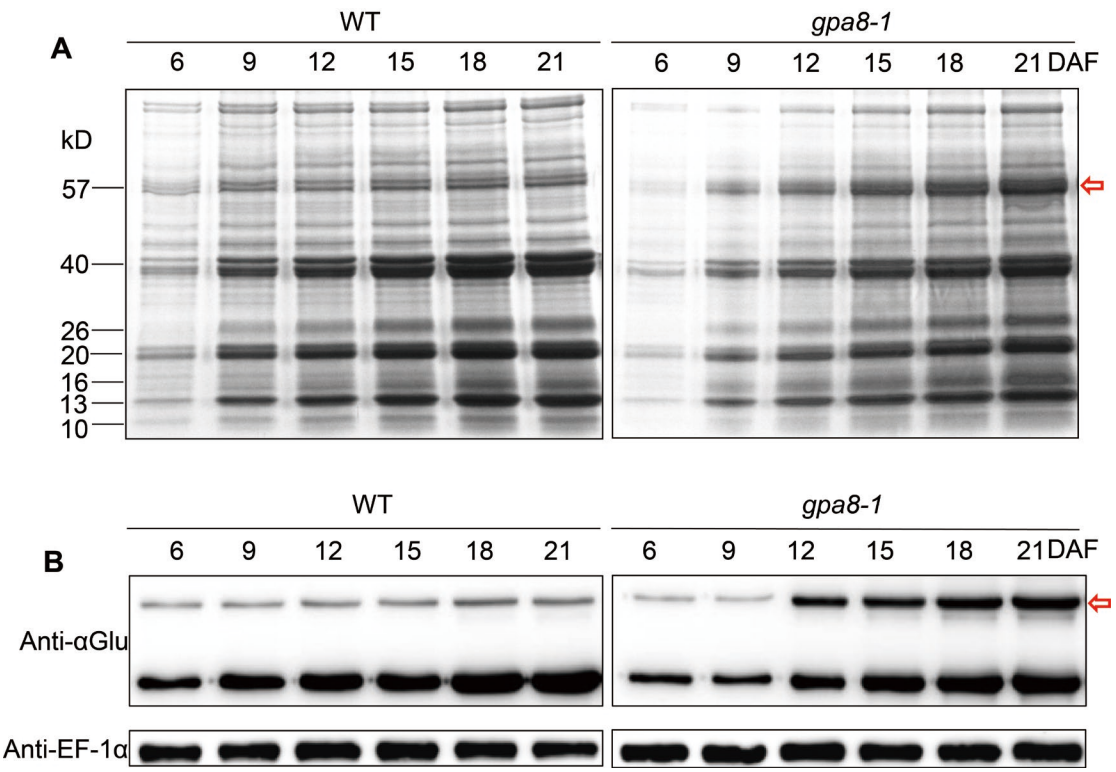
Supplemental Figure S1. Phenotypic characterization of wild type and *gpa8-1*.

(A) and (B) Plant phenotypes at seedling stage. Bars = 5 cm in (A) and 2 cm in (B). (C) and (D) Plant phenotypes at maturation stage. Bars = 10 cm in (C) and 4 cm in (D).



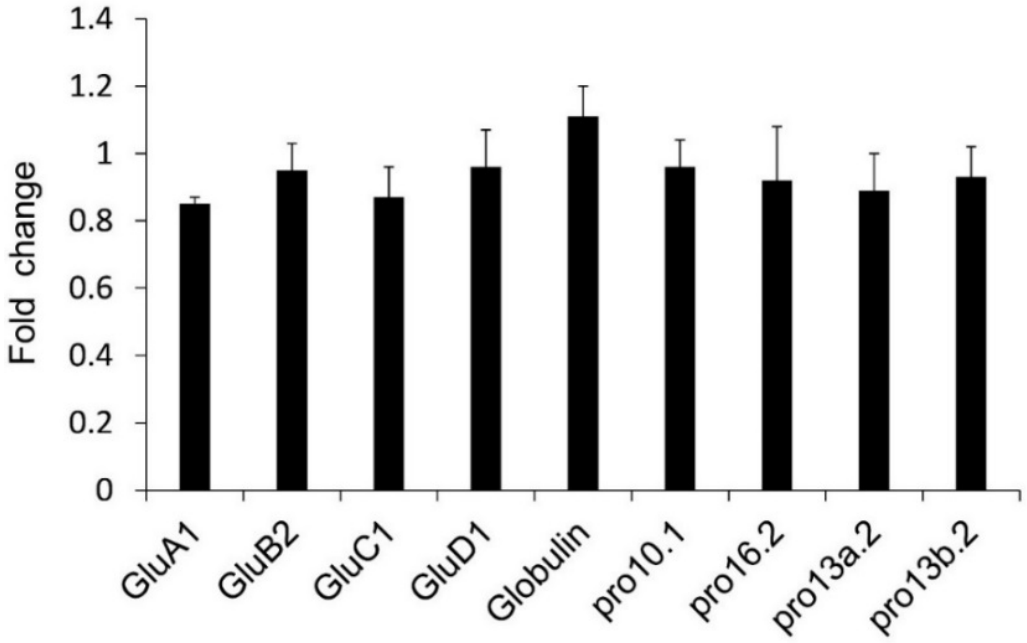
Supplemental Figure S2. Relative intensities of protein bands in SDS-PAGE and immunoblotting assays.

(A) Relative intensities of the protein bands of SDS-PAGE analysis in Fig. 1d. (B) Relative intensities of the immunostained bands in Fig. 1e. Using 1 as the intensity value for the WT. Three independent experiments were performed. Values are means ± SD. pGlu, 57 kD proglutelins; αGlu, 40 kD glutelin acidic subunits; βGlu, 20 kD glutelin basic subunits. $^{**}P < 0.01$, $^{*}P < 0.05$ (n = 3, Student's *t* test).

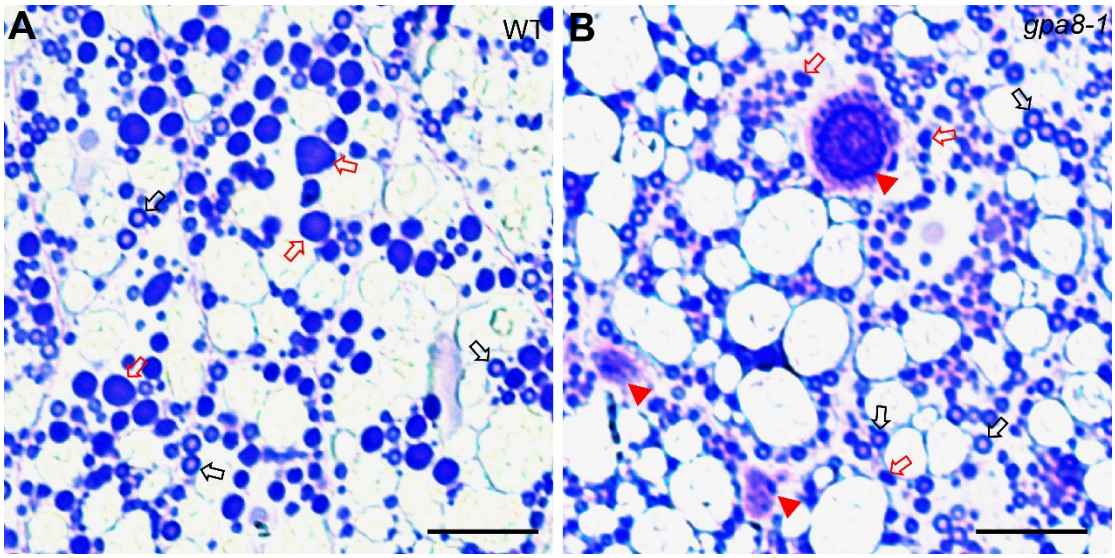


Supplemental Figure S3. Time-course analysis of storage protein accumulation during endosperm development of wild type Ninggeng 1 and the *gpa8-1* mutant.

(A) SDS-PAGE analyses of seed storage proteins during wild-type and *gpa8-1* endosperm development. DAF, days after flowering. (B) Immunoblot analysis of glutelins during wild-type and *gpa8-1* endosperm development. Red arrow indicates the 57-kD proglutelins.

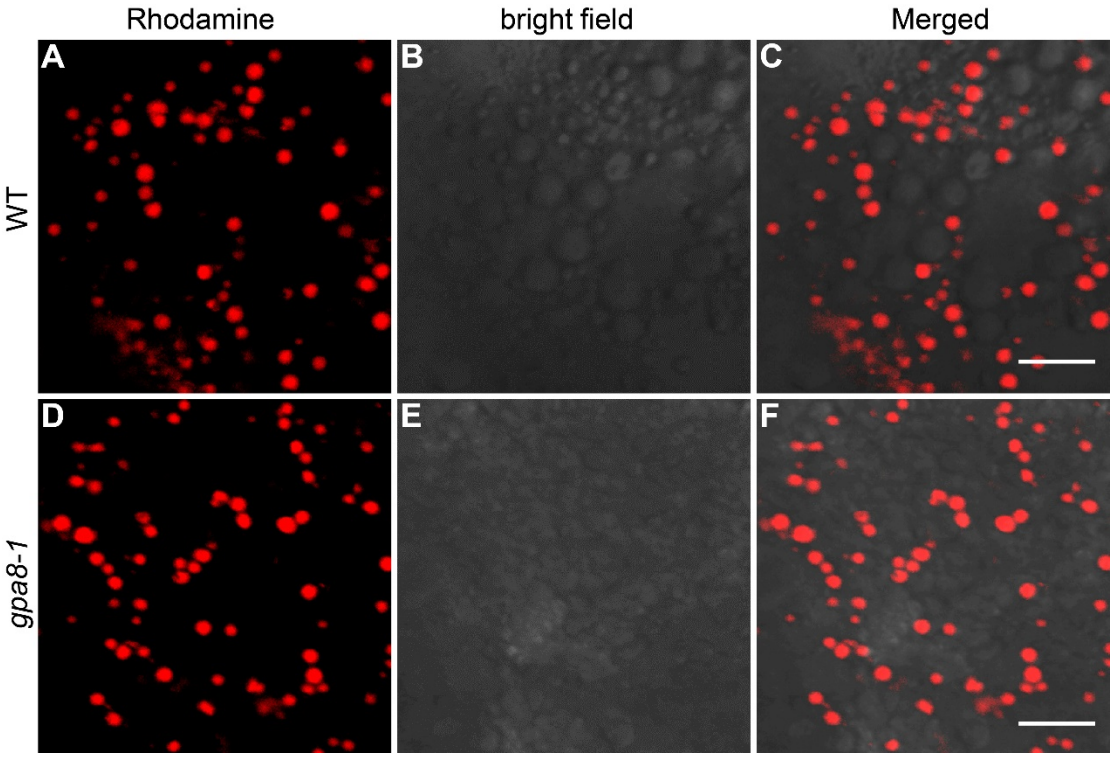


Supplemental Figure S4. RT-qPCR assay of the expression of representative genes coding for storage proteins in 12 DAF endosperm. Glutelin genes: *GluA1* (*LOC_Os01g55690*), *GluB2* (*LOC_Os02g15150*), *GluC1* (*LOC_Os02g25640*), *GluD1* (*LOC_Os02g15090*); prolamin genes: *pro10.1* (*LOC_Os05g41970*), *pro16.2* (*LOC_Os06g31070*), *pro13a.2* (*LOC_Os07g10580*), *pro13b.2* (*LOC_Os07g11910*). Using 1 as the gene expression value for the WT. Values are means ± SD (n = 3).

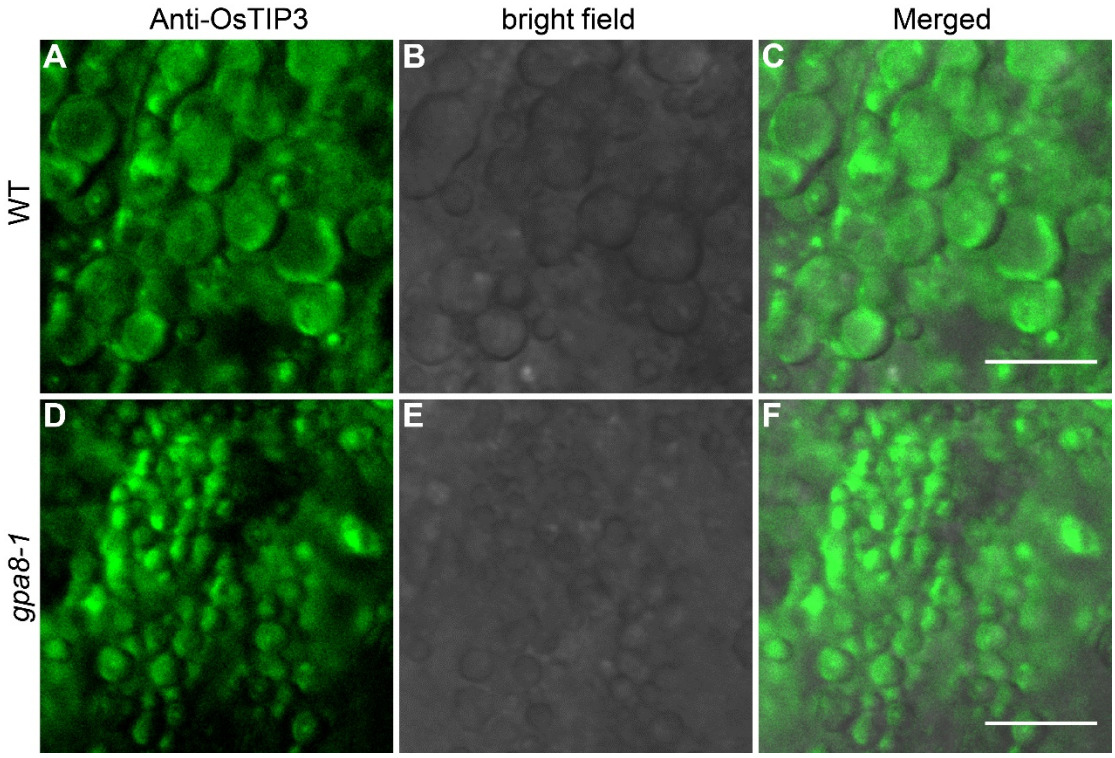


Supplemental Figure S5. Light microscopy of protein bodies in subaleurone cells of wild type and the *gpa8-1* mutant.

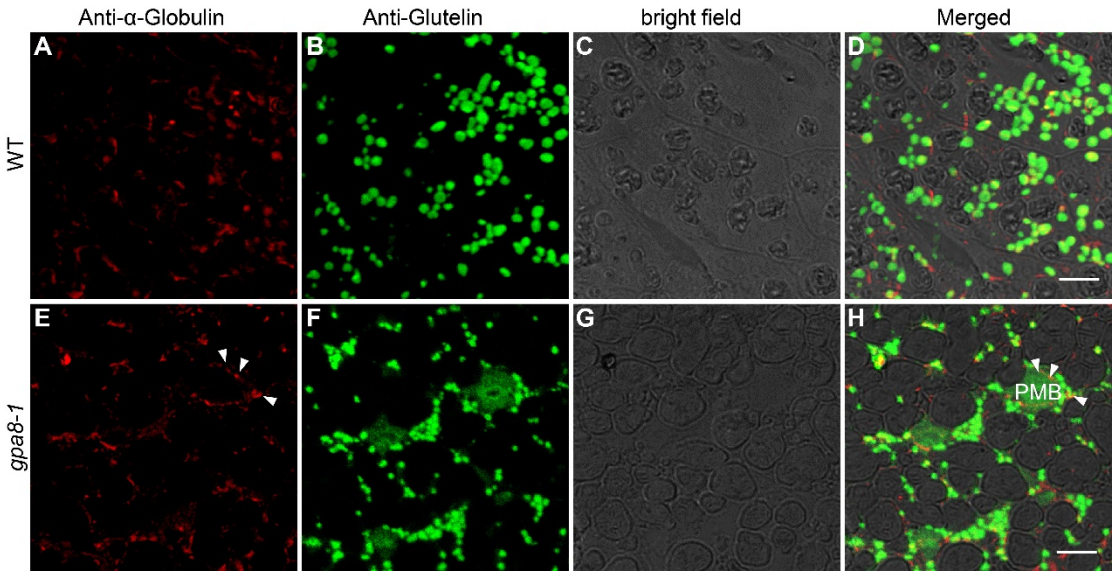
(A) and (B) Endosperm sections at 12 DAF in wild type (A) and the *gpa8-1* mutant (B) stained with Coomassie blue. Black and red arrows indicate PBIIs and PBIs, respectively. Red arrowheads denote the PMB structures in the *gpa8-1* mutant. Bars = 10 μm.



Supplemental Figure S6. Size of PBI in wild type and the *gpa8-1* mutant. (A) to (F) Confocal microscopy images of Rhodamine-labeled PBIs in WT (A-C) and *gpa8-1* (D-F) sub-aleurone cells. Bars = 10 μm.

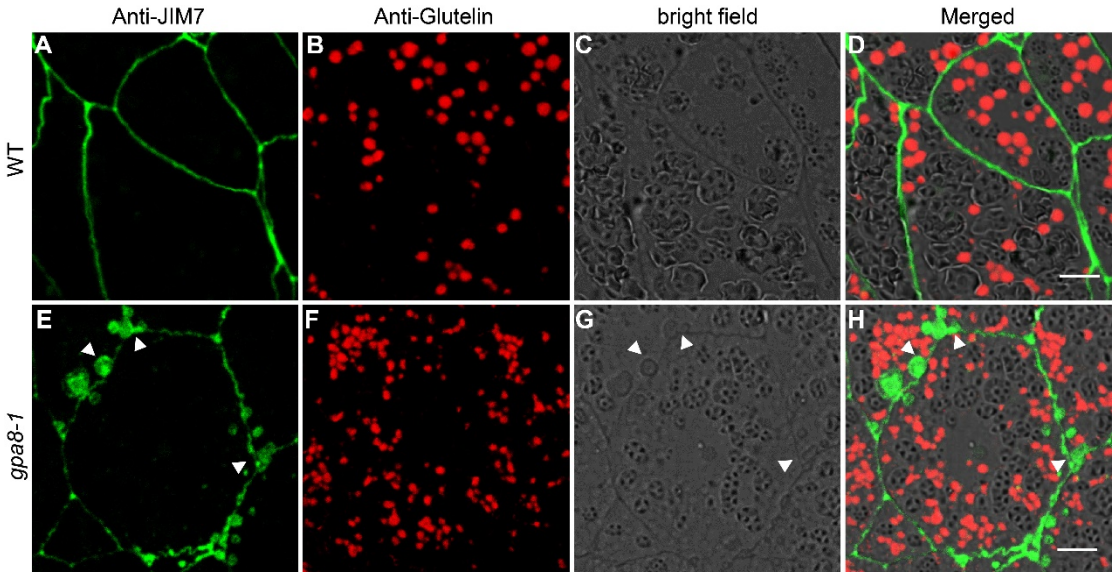


Supplemental Figure S7. Size of PSV/PBII in wild type and the *gpa8-1* mutant. (A) to (F) Confocal microscopy images of TIP3-marked PSVs/PBIIs in WT (A-C) and *gpa8-1* (D-F) sub-aleurone cells. Bars = 10 μm.



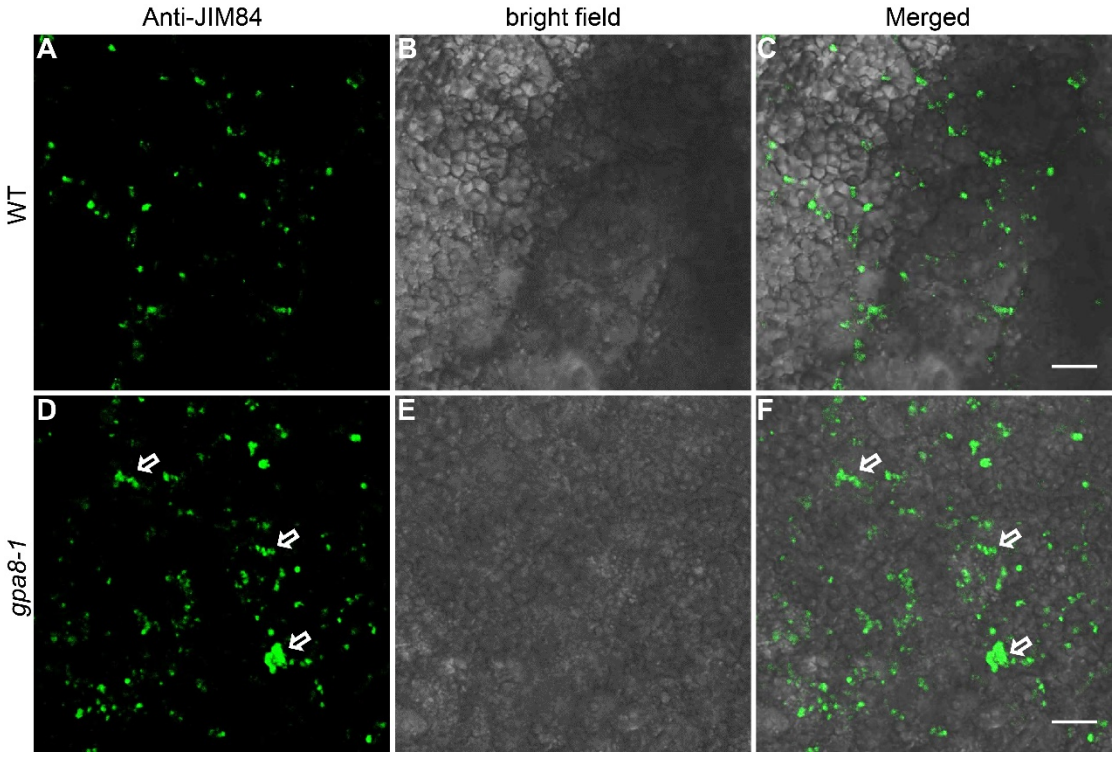
Supplemental Figure S8. Immunofluorescence microscopy of protein bodies in the sub-aleurone cells of wild type and the *gpa8-1* mutant.

(A) to (H) Immunofluorescence microscopy images of storage proteins in wild-type (A-D) and *gpa8-1* (E-F) 12 DAF seeds. (A, E) Secondary antibodies conjugated with Alexa fluor 555 (red) were used to trace the antigens recognized by anti-α-globulin antibodies. (B, F) Secondary antibodies conjugated with Alexa fluor 488 (green) were used to trace the antigens recognized by the anti-glutelin antibodies. (D, H) Merged images. White arrowheads in (H) indicate the mis-sorted α-globulin in the PMB. Bars = 10 μm (A-H).



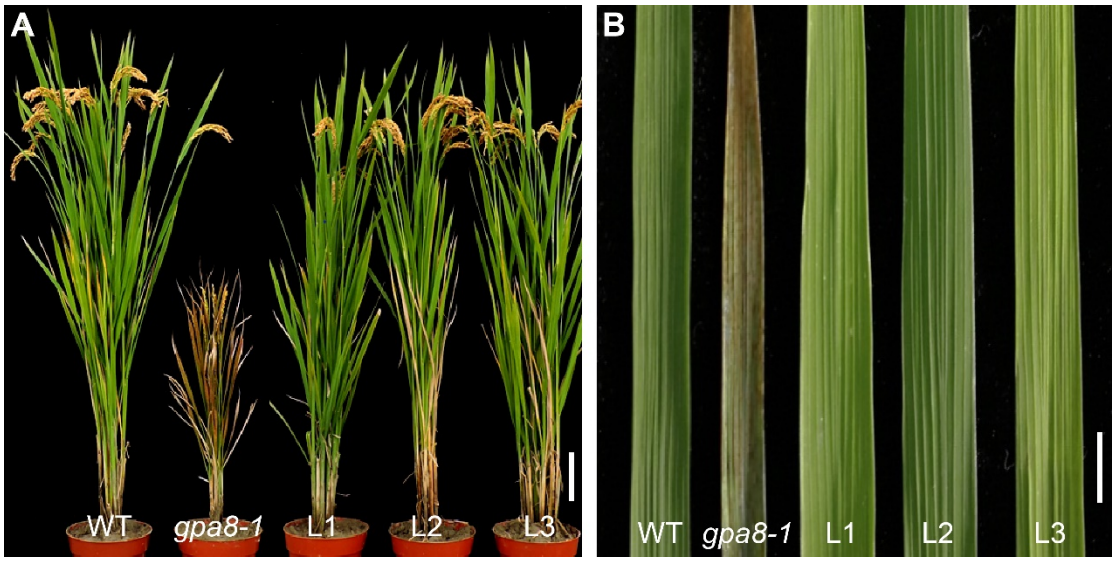
Supplemental Figure S9. Distribution of cell wall materials in 12 DAF endosperm cells.

(A) to (H) 12 DAF sections of wild type (A-D) and the *gpa8-1* mutant (E-H) were incubated with anti-pectin (JIM7) or glutelin antibodies, followed by secondary antibodies conjugated to Alexa-555 or Alexa-488. White arrowheads in (E, G, H) indicate the mis-sorted pectin in the PMB. Bars = 10 μm (A-H).



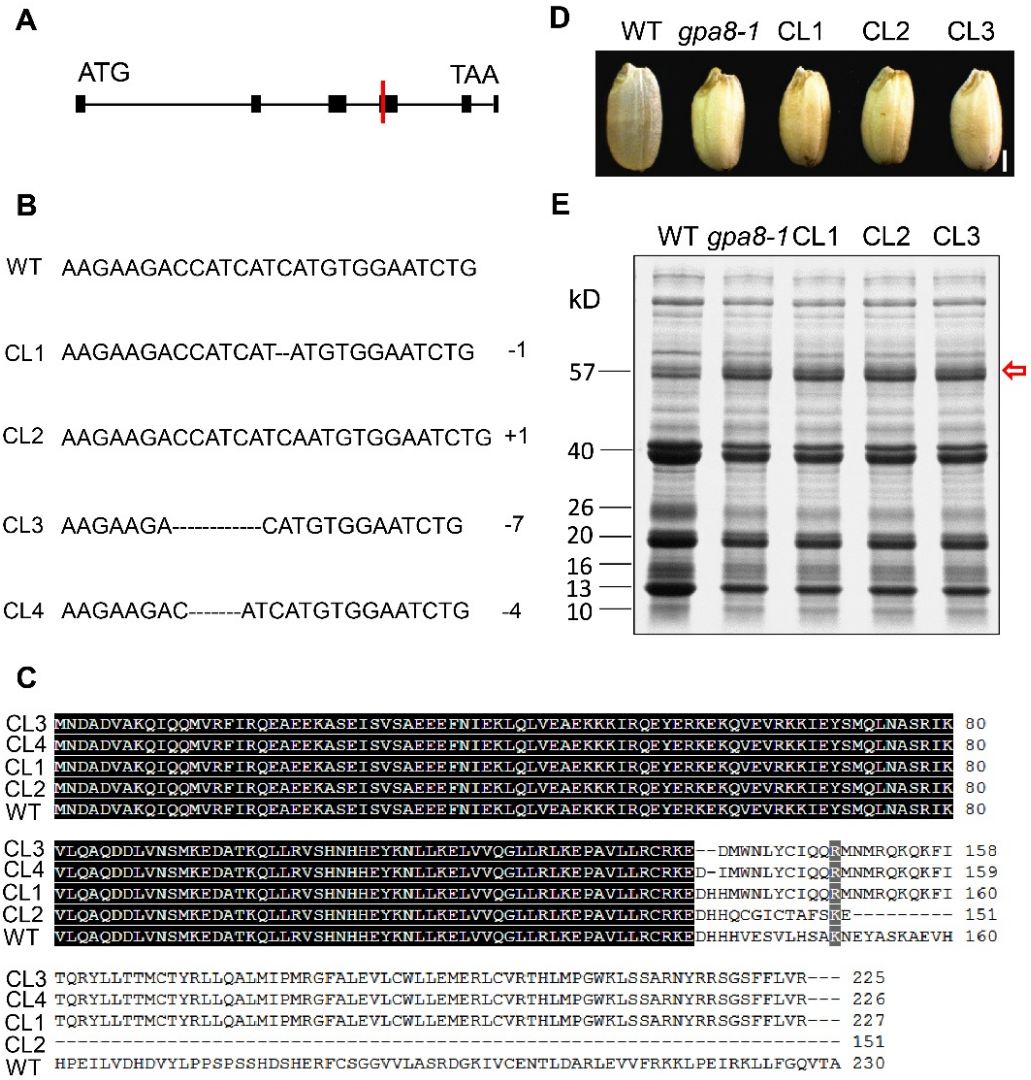
Supplemental Figure S10. Distribution of Golgi stacks in 12 DAF endosperm cells.

(A) to (F) Sections of 12 DAF endosperms from wild type (A-C) and the *gpa8-1* mutant (D-F) were incubated with JIM84 antibody, followed by secondary antibodies conjugated to Alexa-555. Golgi stacks were marked by JIM84 antibody. Arrowheads indicate clusters of Golgi stacks. Bars = 10 μm.



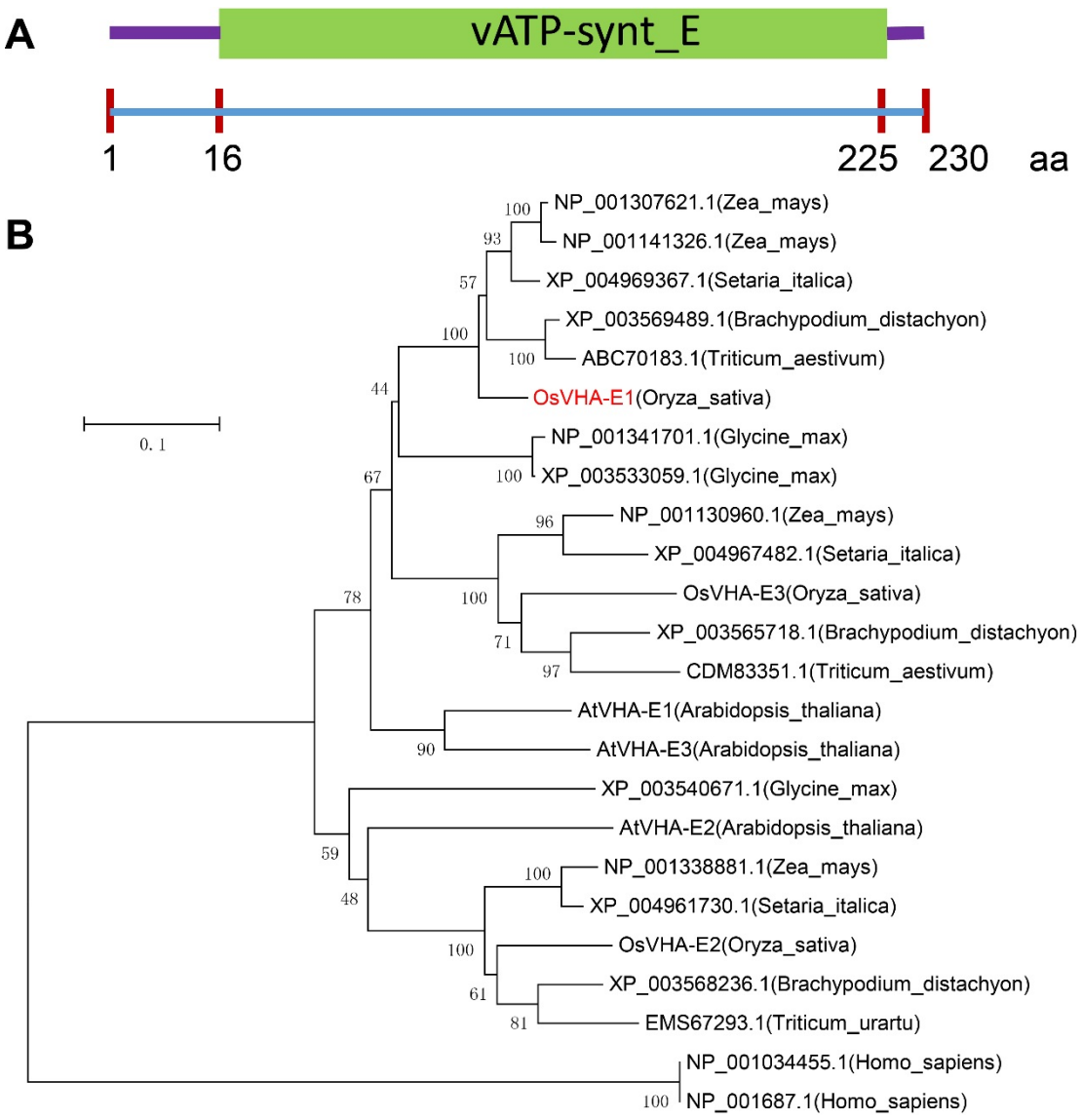
Supplemental Figure S11. Genetic complementation.

(A) and (B) *LOC_Os01g46980* coding sequence under the control of maize *ubiquitin* promoter rescues lesion-mimics and early senescence phenotypes of *gpa8-1*. Bars =10 cm in (A) and 1 cm in (B).



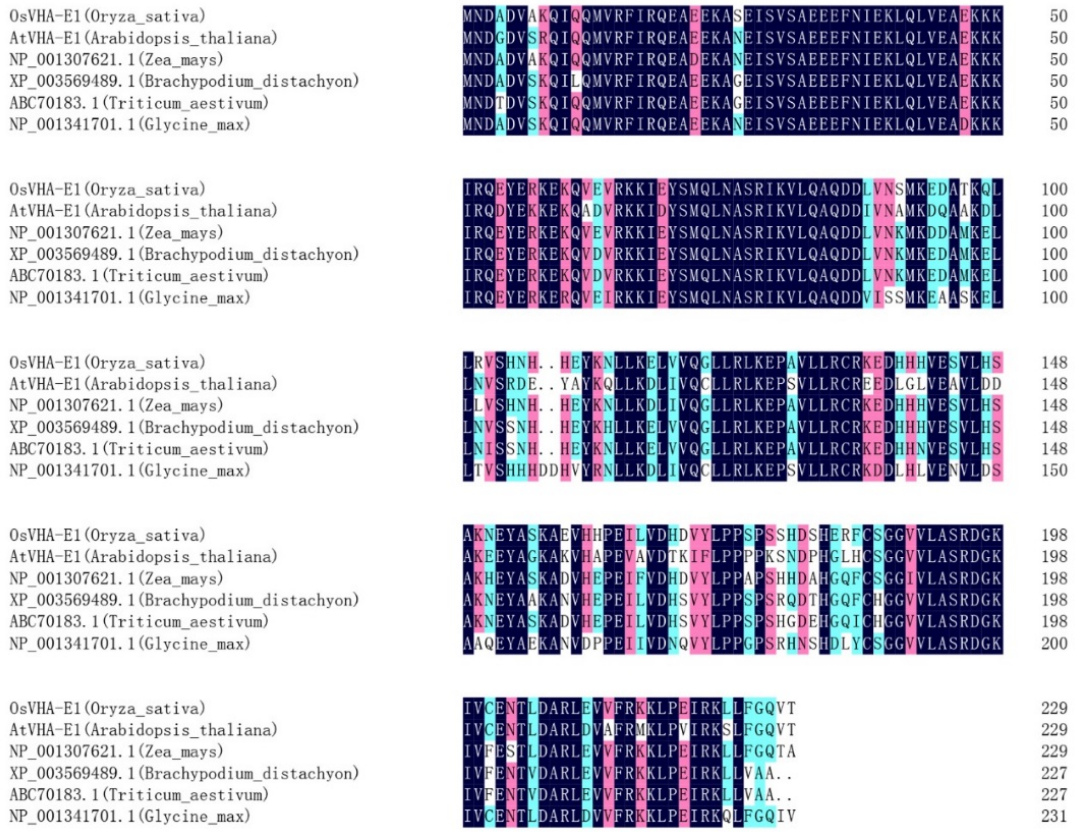
Supplemental Figure S12. CRISPR/Cas9 lines of *OsVHA-E1*.

(A) Schematic diagram of the CRISPR target site on *OsVHA-E1*. (B) Mutations at the target site in four representative lines generated by CRISPR/Cas9. (C) The predicted truncated proteins encoded by the CRISPR alleles. (D) Grain appearance of WT and three CRISPR/Cas9 lines (CL1-CL3). Bar = 1 mm. (E) SDS-PAGE profiles of total seed storage proteins of the wild type and CRISPR/Cas9 lines. Red arrow indicates the 57 kD proglutelins.

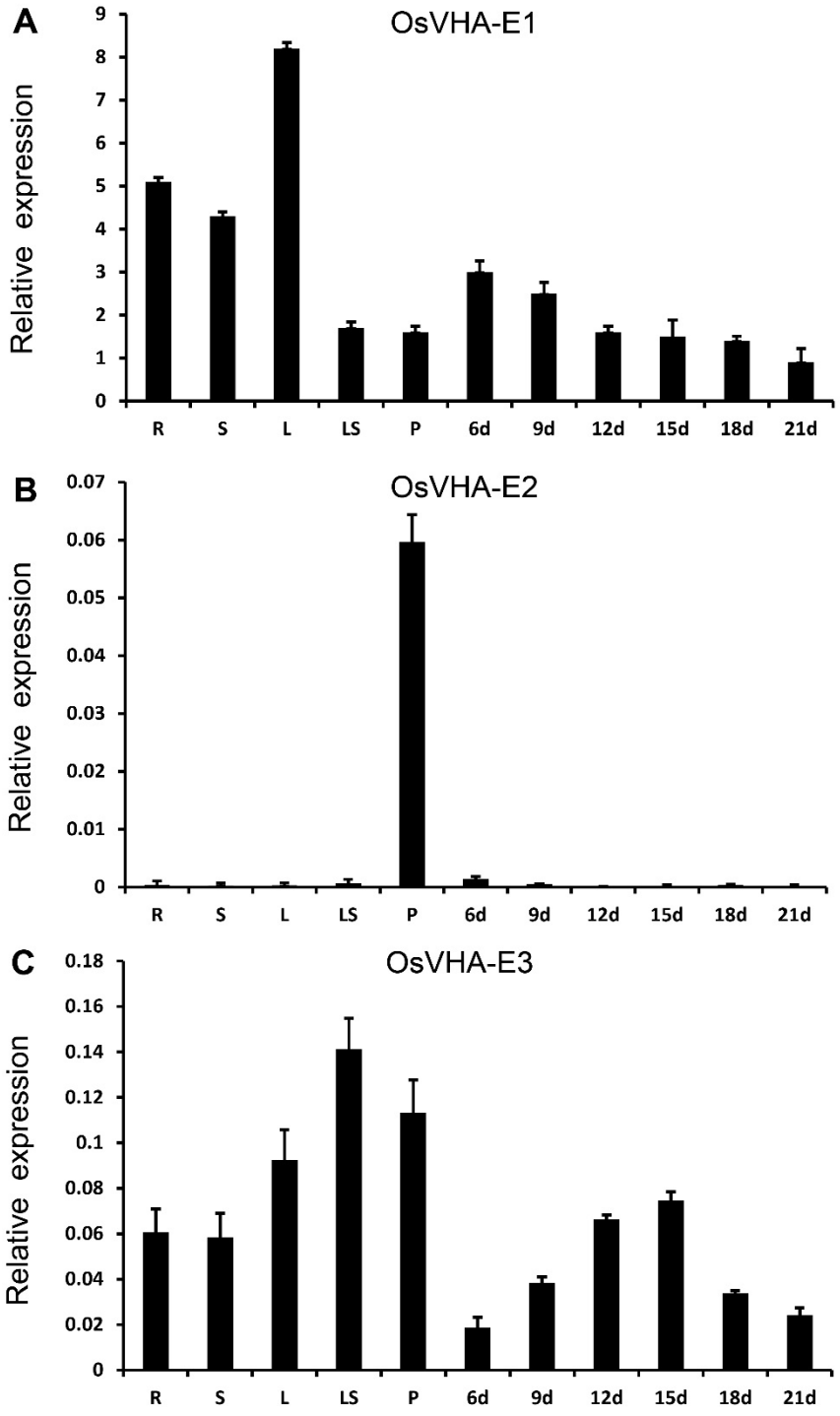


Supplemental Figure S13. Sequence and phylogenetic analyses of OsVHA-E1.

(A) OsVHA-E1 contains a vATP-synt_E domain predicted by SMART (http://smart.embl-heidelberg.de). (B) Neighbor-joining tree of OsVHA-E1 and its homologs. The tree was constructed using MEGA and bootstrapped with 1,000 replicates.

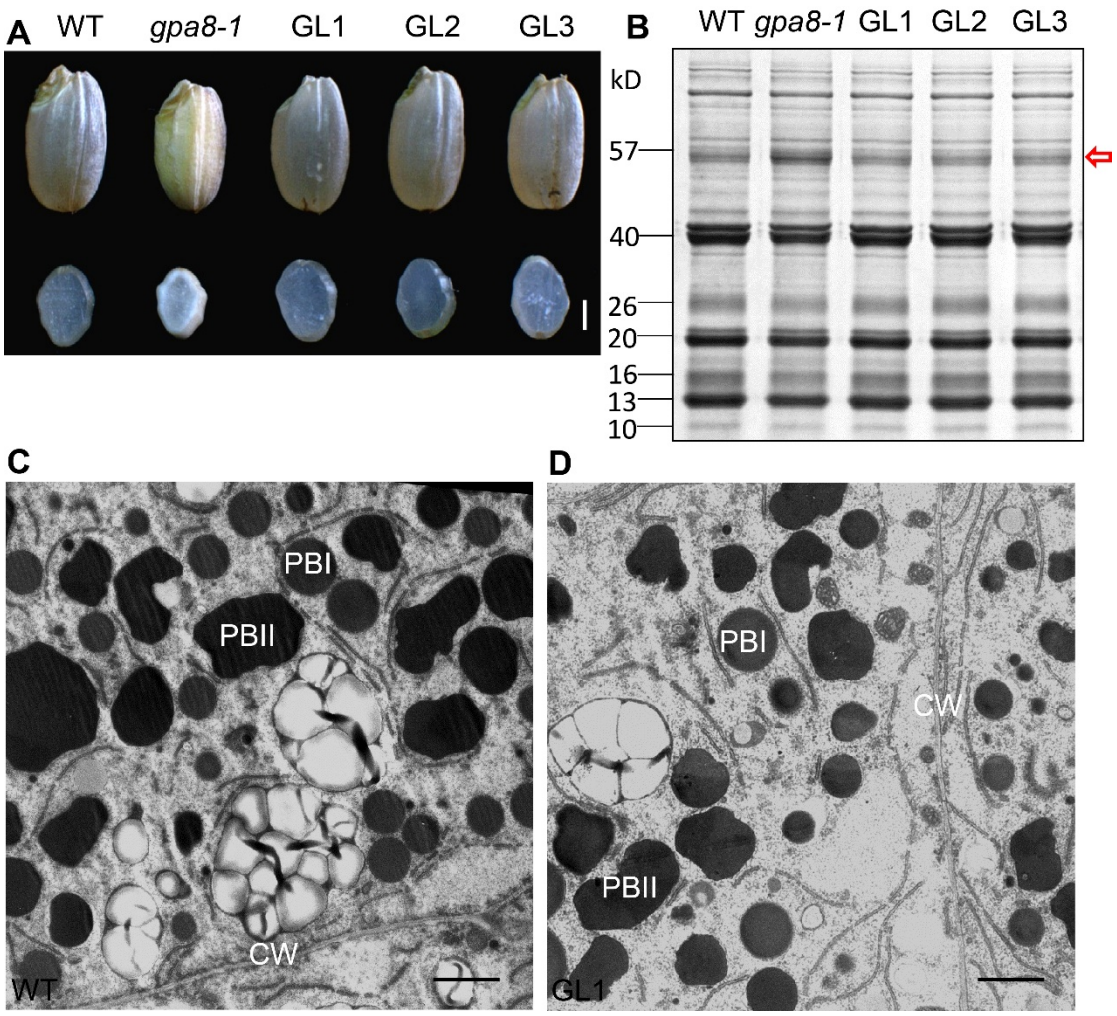


Supplemental Figure S14. Amino acid sequence alignment of OsVHA-E1 and its homologs. Different colors indicate percentage sequence similarity (Deep blue, 100%; Pink, 75% to 100%; Light blue, 50% to 75%). GenBank protein accession numbers: *Oryza_sativa*, XP_015621799; *Arabidopsis_thaliana*, NP_192853; *Zea_mays*, NP_001307621; *Brachypodium_distachyon*, XP_003569489; *Triticum_aestivum*, ABC70183; Glycine_max, NP_001341701.



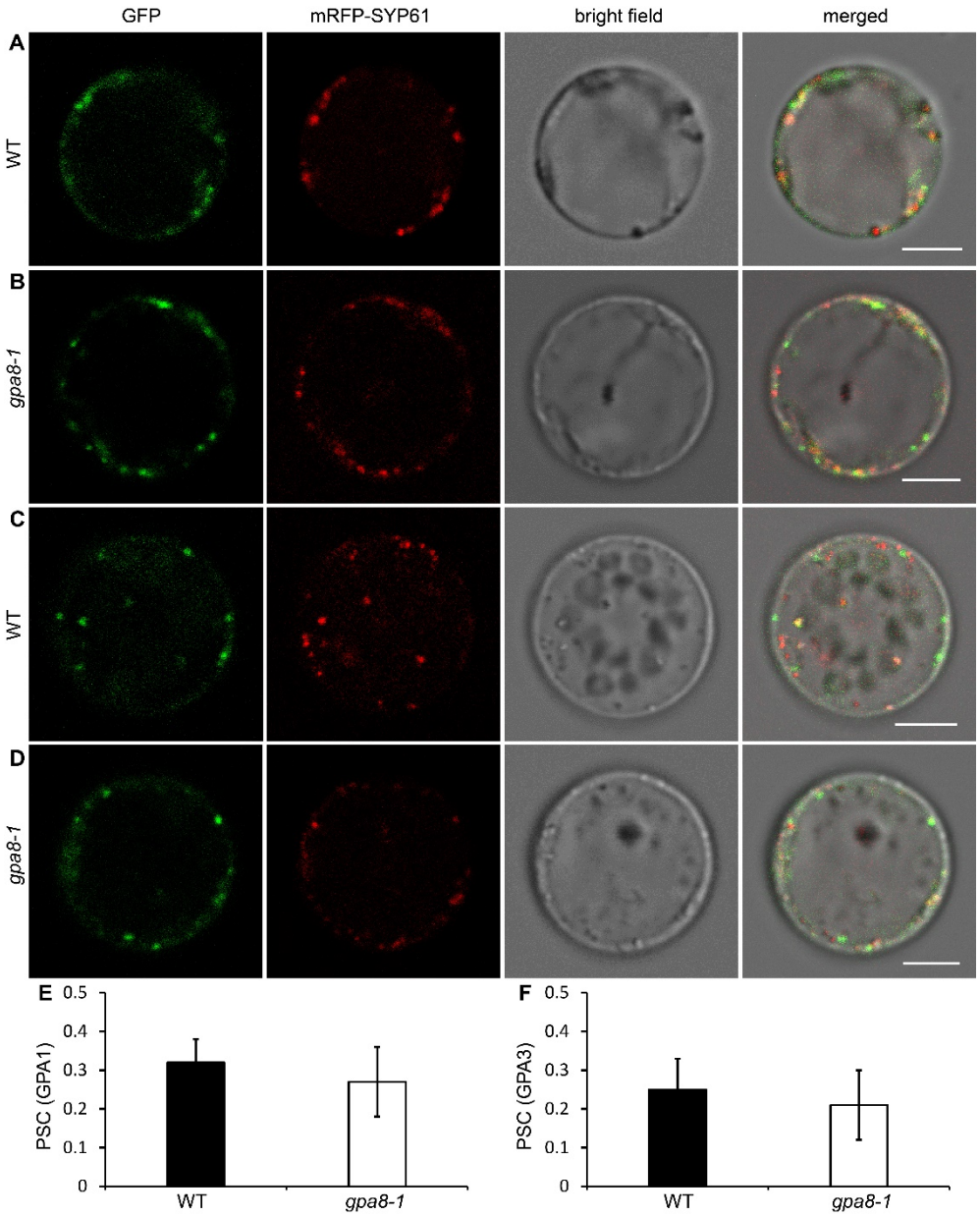
Supplemental Figure S15. Spatial expression patterns of *OsVHA-E*.

(A) to (C) *OsVHA-E1* (A) and *OsVHA-E3* (C) are expressed in various tissues and in developing endosperm at 6, 9, 12, 15, 18, 21 DAF. *OsVHA-E2* (B) is mainly expressed in panicles. R, roots; S, stems; L, leaves; LS, leaf sheaths; P, panicles. *Actin1* was used as an internal control. For each RNA sample, three technical replicates were performed. Values are means ± SD.



Supplemental Figure S16. Complementation of *gpa8-1* mutant phenotypes by *p35S*::*OsVHA-E1-GFP*.

(A-D) *p35S*::*OsVHA-E1-GFP* transgene rescues the grain appearance (A), storage protein composition pattern (B), and the storage protein trafficking defects (C and D) of the *gpa8-1* mutant. . GL1 to GL3 are three independent T_1 transgenic lines. Bars = 1 mm in (A). Bars = 2 μm in (C) and (D).



Supplemental Figure S17. Intracellular acidification increased degree of colocalization of GPA1 and GPA3 with mRFP-SYP61 in *gpa8-1*.

(A) and (B) Localization of GFP-GPA1 in WT (A) and *gpa8-1* (B) protoplasts. (C) and (D) Localization of GPA3-GFP in WT (C) and *gpa8-1* (D) protoplasts. *gpa8-1* protoplasts were incubated for 5 min in acidic equilibration buffer, pH 6.2. pH was equilibrated with 10 µM nigericin, 60 mM KCl, and 10 mM MES/HEPES Bis-Tris-propane. mRFP-SYP61 serves as TGN marker. Bars = 5 µm in (A-D). (E) and (F) Pearson's correlation coefficient (PSC) of GPA1 (E) and GPA3 (F) with mRFP-SYP61. Values are means ± SD. **P* < 0.05 (n = 37 protoplasts, Student's *t* test).

Supplemental Table S1 Properties of wild type and *gpa8-1* in maturation stage.

	WT	*gpa8-1*
Plant height (cm)	97.5 ± 2.3	51.2 ± 1.5**
Tiller number	10.3 ± 1.7	5.2 ± 1.1**
1,000-grain weight (g)	27.6 ± 0.8	19.8 ± 0.6**
Protein content (%)	8.11 ± 0.04	8.24 ± 0.07
Amylose content (%)	17.42 ± 0.37	13.52 ± 0.28**

Values are means ± SD. ** $P < 0.01$ (Student's *t* test).

Supplemental Table S2 Segregation of mutant phenotypes in reciprocal crosses between wild type and the *gpa8-1* mutant.

Cross	Normal	57H/Floury	$X^2_{3:1}$
gpa8-1/wild type F_2	171	62	0.24
wild type/*gpa8-1* F_2	167	60	0.18

Supplemental Table S4 Primers used for mapping.

Primer name	Forward sequence	Reverse sequence
D28	ATGGAACTGCAGATTTGATGGA	ACTTCATATTCCACTGGGCGTC
D31	CCCTTATCCTTATCCCCTCCCA	ACAACCTGCCCGTGCATCGCCGCCTC
D13	AGCCTGGATAAGATGGTTCGTC	GCTGTAGTTGCTGTTTGCCTGT
D12	TAGCCTCATGGCTCGGTCACTC	GTGCTGCCTAACTTGGCGGAAT
D38	ATGTCAGTAAGCCACATCAGCACC	GTGCCACCTCCTGTGCAAGAGC
Indel1-11	GGATGATTTTGAGGAACATGG	ATCTTTGCCTGCAGAGTGCT

Supplemental Table S5 Primers used for RT-qPCR analysis.

Primer name	Forward sequence	Reverse sequence
GluA1	TGATGGTGAAGTGCCAGTTGTTGC	ACGCCTGTATGCTTGAGGGTTTCT
GluB2	TGTCCTTCGCCCTGGACAACTATT	TGGTAAGGCGCGGAATACTGAGTT
GluC1	ATGTAAAGTTCAACCGCGGCGATG	TCTCGTTGATCTGCCACTCAGCAT
GluD1	AAATGAGCAGTTTCGATGCGCTGG	TGTTCGAGTATCGAGGCACCACAA
Pro10.1	GTTGCATGCAGCTACAAGGCATGA	TCTGCATCGCCATCTTCACCATCT
Pro13a.2	TTCAGGCGATTGTGCAGCAACTAC	GGATGGCAAGTTTAAGGCCAGCAA
Pro13b.2	GTATAGCATTGCGGCAAGCACCTT	ATGGCCTGGACAACGTTAATGTCC
Pro16.2	GCTCTCAATTTGCCCTCCATGTGT	TGGTACACACTACCAAGAACCGCA
Globulin	GATCAGTTCATCACCAACAAAACA	TCCAGAACGCACAAAATCAT
OsVHA-E1	AAAGCAACTCCTGCGTGTCAGC	TTTCAACCGAAGCAAACCCTGAAC
Actin1	TGGTCGTACCACAGGTATTG	CCACATCTGCTGGAATGTGCTG

Supplemental Table S6 Primers used for vector construction.

Use	Primer name	Sequence
Ubi-OsVHA-E1	1390-OsVHA-E1-F	CCGGCGCGCCAAGCTTATGAACGACGCCGATGTCG
	1390-OsVHA-E1-R	GAATTCCCGGGGATCCTTATGCCGTCACCTGACCAA
Subcellular localization	1305-OsVHA-E1-GFP-F	CGGAGCTAGCTCTAGAATGAACGACGCCGATGTCG
	1305-OsVHA-E1-GFP-R	TGCTCACCATGGATCCTGCCGTCACCTGACCAAAA
CRISPR	CRISPR- OsVHA-E1-F	AGATGATCCGTGGCAAAGAAGACCATCATCATGGTTTTAGAGCTATGC
	CRISPR-OsVHA-E1-R	GCATAGCTCTAAAACCATGATGATGGTCTTCTTTGCCACGGATCATCT