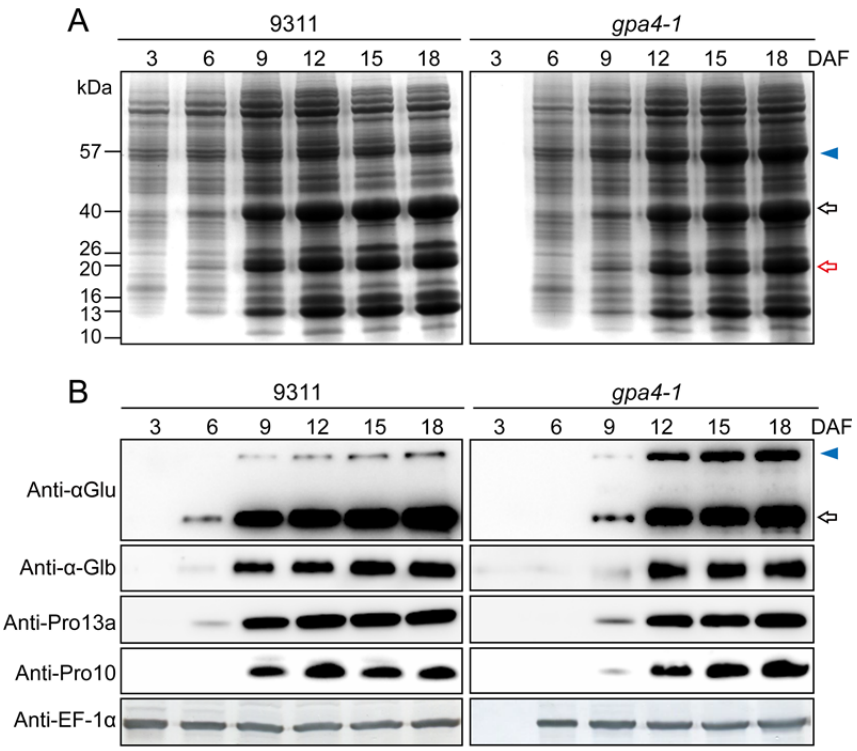
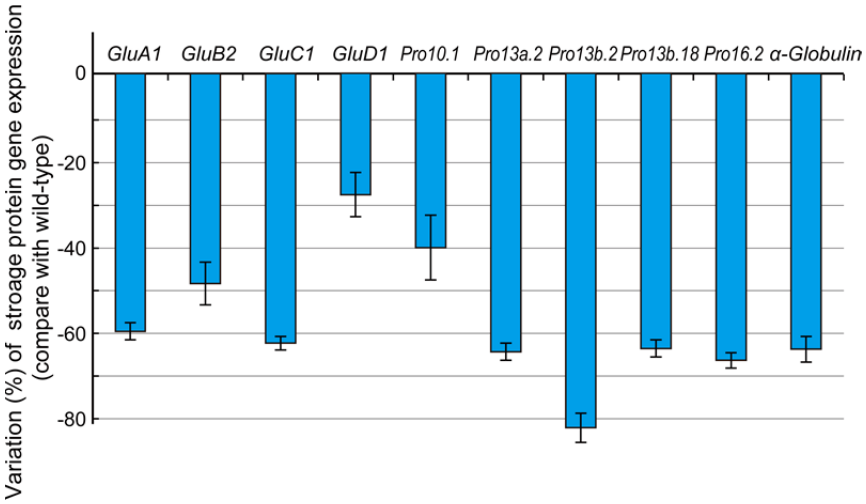



**Supplemental Figure 1.** Time-Course Analysis of Storage Proteins during Endosperm Development of the Wild-type 9311 and the *gpa4-1* Mutant.

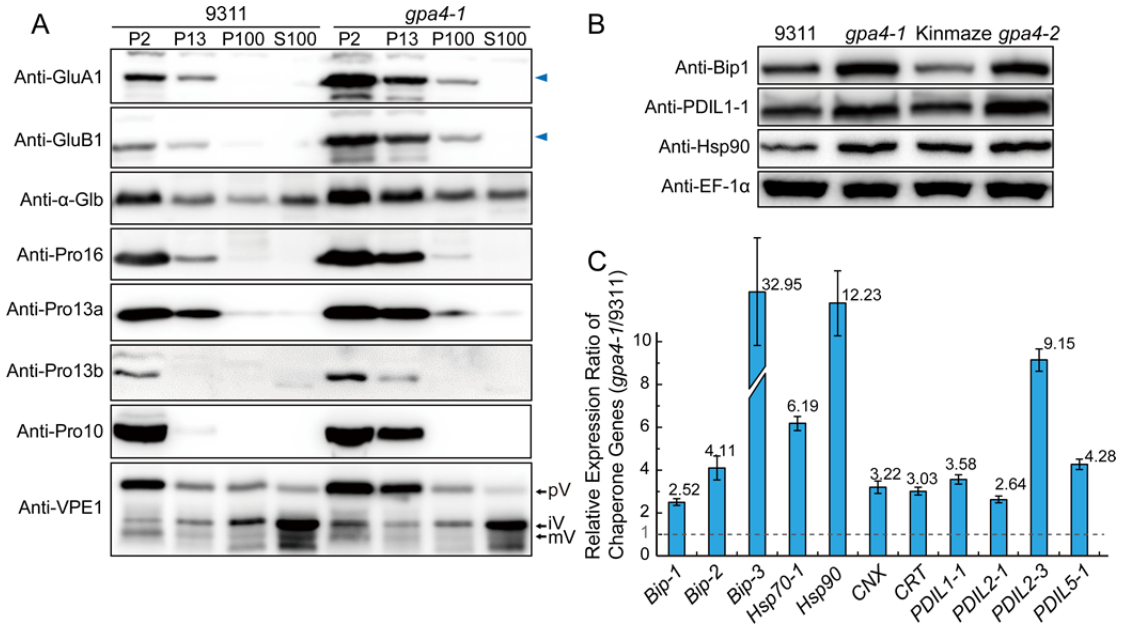
**(A)** SDS-PAGE analyses of seed storage proteins during wild-type and *gpa4-1* endosperm development. Blue triangles indicate the 57-kDa proglutelins. Black and red hollow arrows denote the acidic and basic subunit, respectively. DAF, days after flowering.

**(B)** Immunoblot analysis showing the delayed accumulation of glutelins, 26-kDa α-globulins, 13-kDa and 10-kDa prolamins in the *gpa4-1* endosperm.




**Supplemental Figure 2.** RT-qPCR Assay of the Expression of Representative Genes Coding for Storage Proteins in 12 DAF Endosperm.

All of the genes analyzed were down-regulated in the *gpa4-1* mutant compared with wild type. DAF, days after flowering. Values are mean ± SD. *n* = 3.

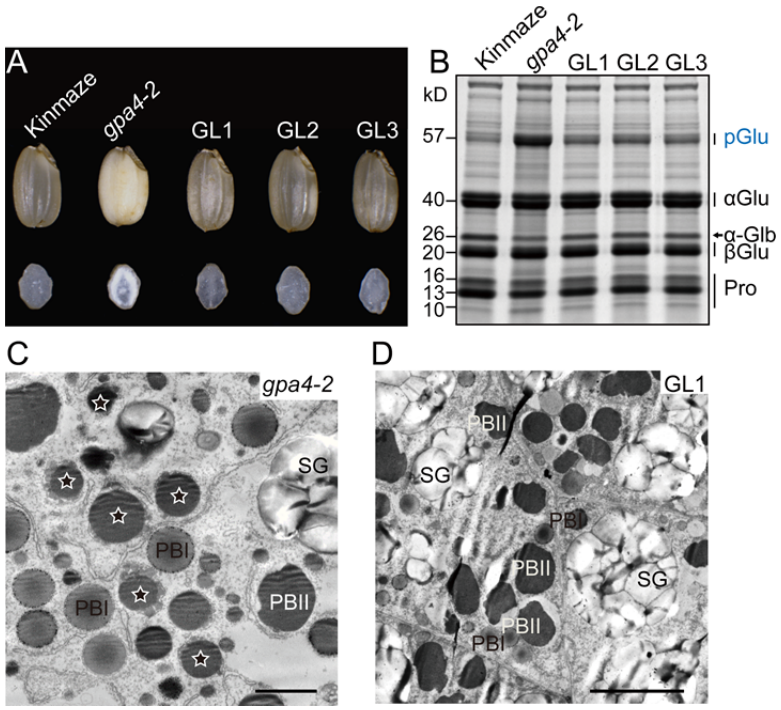



**Supplemental Figure 3.** Immunoblot Analyses Showing Defective Export of Storage Proteins from the ER in *gpa4-1*.

**(A)** Immunoblot analysis of GluA1, GluB1, α-globulin, 16-kD prolamin (Pro16), 13-kD prolamin-a (Pro13a), 13-kD prolamin-b (Pro13b), 10-kD prolamin (Pro10), and VPE1 showing their distribution in various differential centrifugation fractions using their specific antibodies. P2, pellet obtained following centrifugation at 2000*g*; P13, pellet obtained following centrifugation at 13,000*g*; P100, pellet obtained following centrifugation at 100,000*g*; S100, supernatant obtained following centrifugation at 100,000*g*. Blue triangles indicate the 57-kD proglutelins. Arrows indicate different forms of VPE1. pV, VPE1 precursor; iV, intermediate VPE1; mV, mature VPE1.

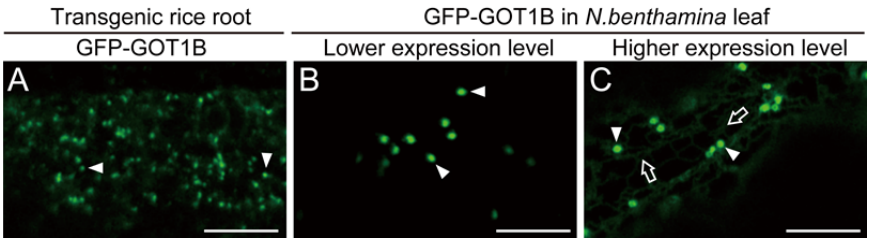
**(B)** Immunoblot analysis using specific antibodies for chaperone proteins. The accumulation of Bip1, PDIL1-1, and Hsp90 proteins is increased in the *gpa4* mutants compared with the corresponding wild types.

**(C)** Expression analysis of ER stress response-related genes in 12 DAF endosperm. The transcript levels of these genes are highly elevated in the *gpa4-1* mutant. DAF: days after flowering. Values are mean ± SD. *n* = 3.

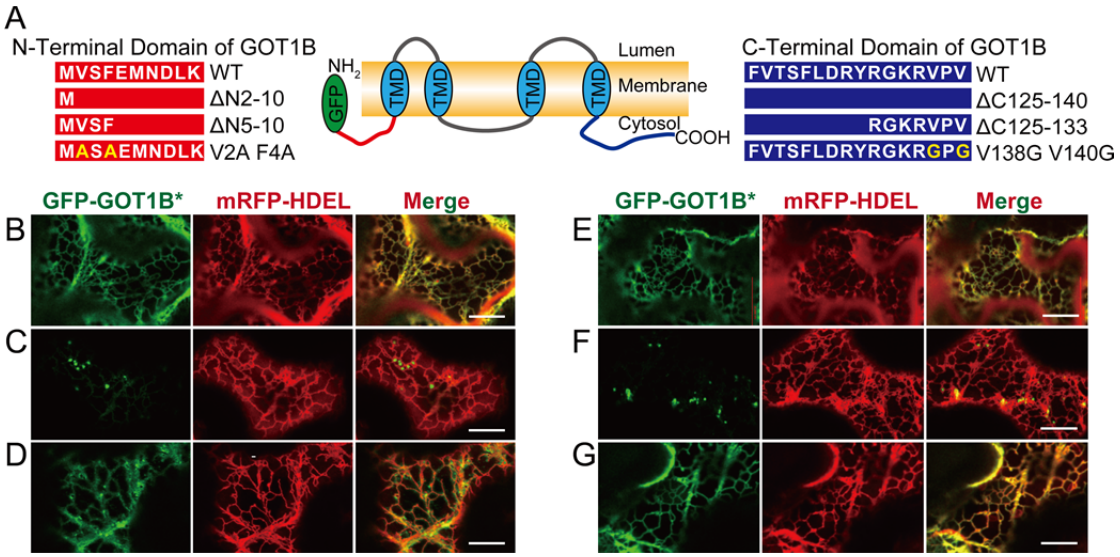



**Supplemental Figure 4.** Rescue of the *gpa4-2* Mutant Phenotype by the *GFP-GOT1B* Fusion Construct.

**(A)** to **(D)** The fusion construct *GFP-GOT1B* under the control of *GOT1B* endogenous promoter rescues the grain appearance **(A)**, the storage protein composition pattern **(B)**, and the storage protein trafficking defects (**C** and **D**) of the *gpa4-2* mutant. GL1 to GL3 denote the grains from three independent T1 transgenic lines. Stars represent the first type of novel structure with a glutelin core and a prolamin periphery. Bars = 2 μm in **(C)**, and 5 μm in **(D)**.



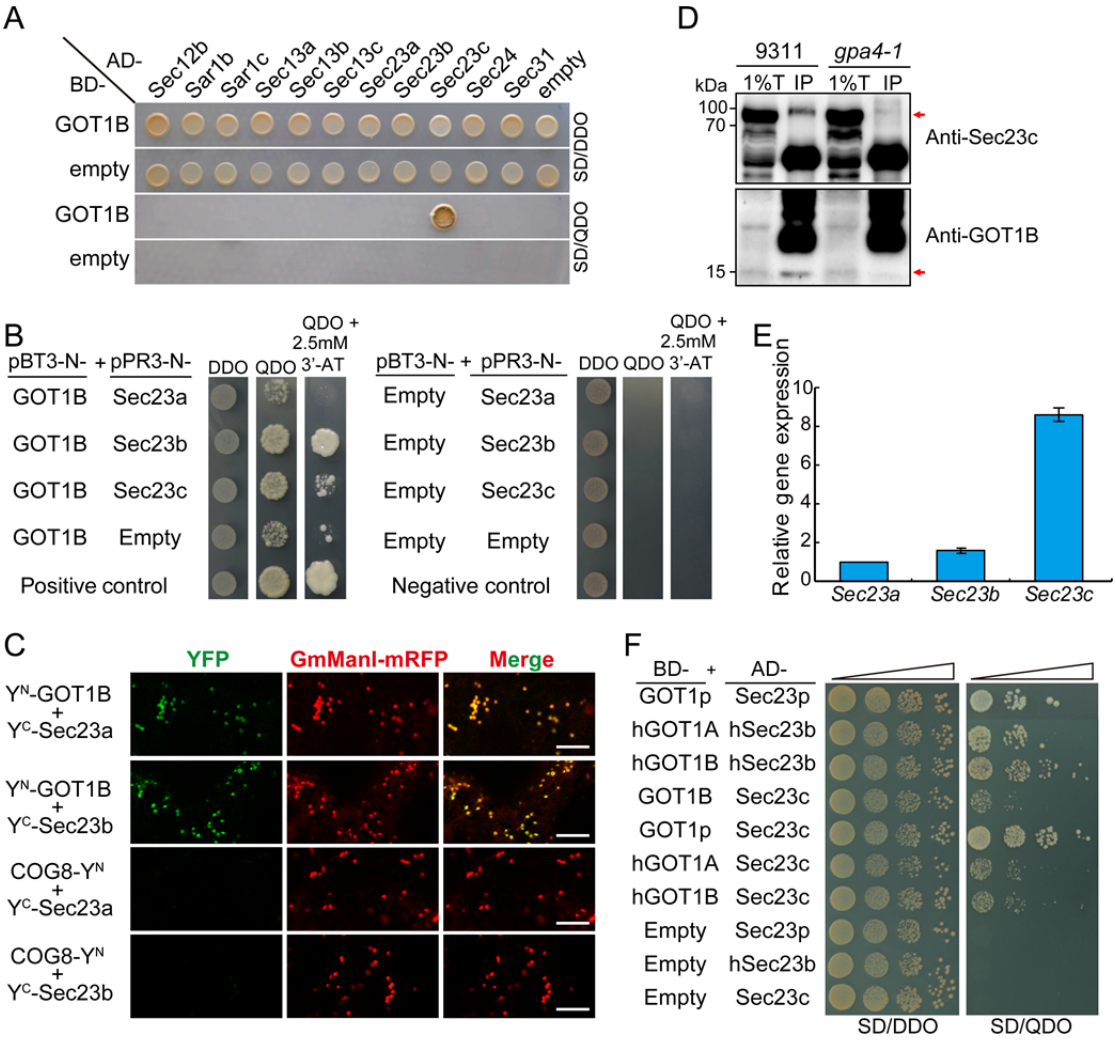

**Supplemental Figure 5.** Localization Pattern of GFP-GOT1B Fusion Protein.
**(A)** GFP-GOT1B is localized in the punctate structures in the root cells of *GFP-GOT1B* transgenic lines.
**(B)** and **(C)** Localization of GFP-GOT1B in *N. benthamiana* leaf epidermal cells. **(B)** GFP-GOT1B shows a typical punctate pattern in cells with lower expression. **(C)** Faint signals in ER networks are observed in cells with higher expression. Triangles indicate the punctate structures of GFP-GOT1B. Arrows indicate the faint signals of GFP-GOT1B in ER networks. Bars = 10 μm.




**Supplemental Figure 6.** Both the N- and C-termini Are Essential for the Proper Localization of GOT1B.

(**A**) Scheme of GOT1B N- and C- terminus mutation constructs for expression in the leaf epidermal cells of *N. benthamiana*. TMD, transmembrane domain.

(**B**) to (**G**) Typical subcellular localization patterns of the various GFP-fused GOT1B mutant forms co-expressed with a known ER marker (mRFP-HDEL) in *N. benthamiana* leaf cells. Bars = 10 μm in (**B** to **G**). GFP-GOT1B* indicates different mutant isoforms of GFP-GOT1B as shown in **(A)**.




**Supplemental Figure 7.** Interaction between GOT1B and Sec23.

**(A)** Y2H assay showing that GOT1B specifically interacts with the COPII component Sec23c.

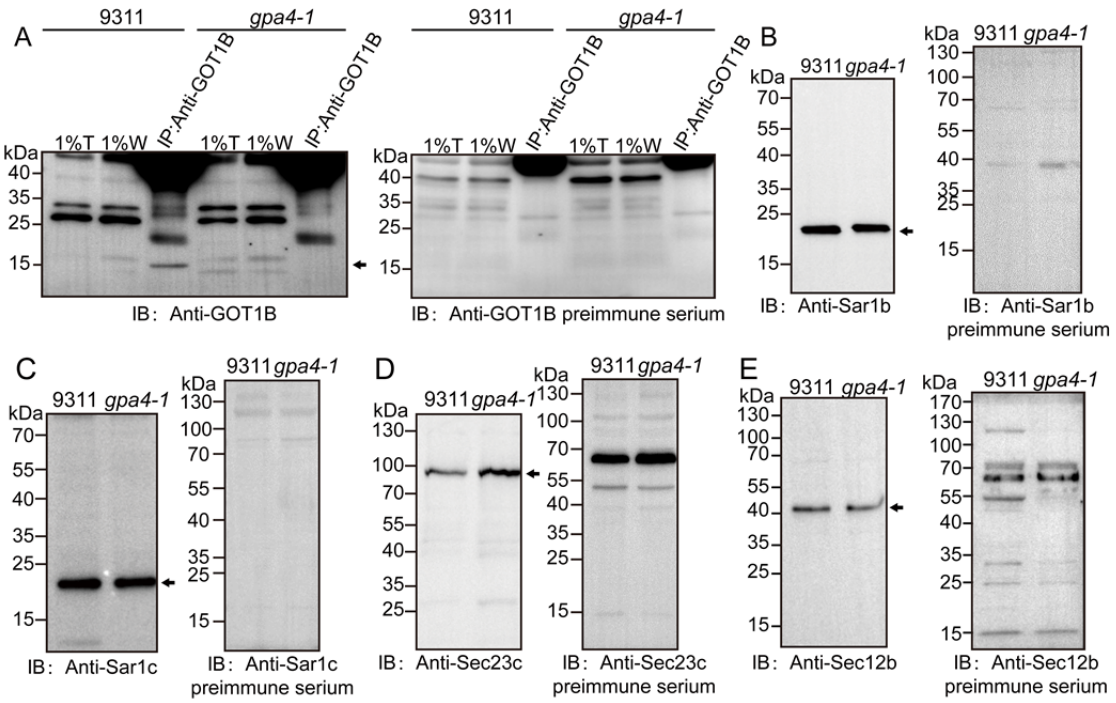
**(B)** GOT1B interacts with Sec23b and Sec23c in the split-ubiquitin based Y2H assay.

**(C)** BiFC assay showing that GOT1B can interact with both Sec23b and Sec23c in leaf epidermal cells of *N. benthamiana*. The Golgi-localized membrane proteins COG3 and COG8 (a pair of interacting proteins, Tan et al., 2016) were used as the negative control. Bars = 10 μm.

**(D)** No detectable Sec23c protein was immunoprecipitated by anti-Sar1b antibodies in the *gpa4-1* mutant.

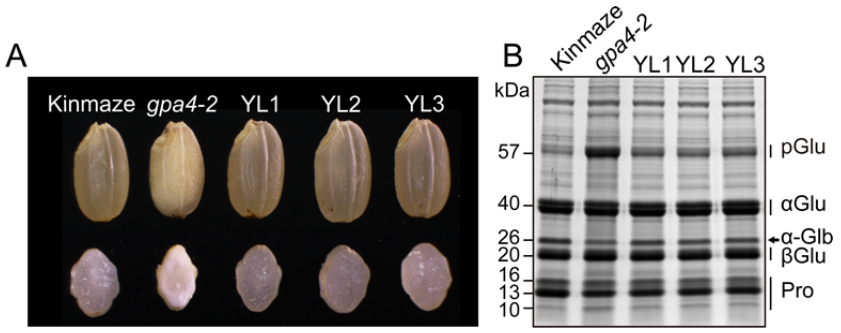
**(E)** RT-qPCR assay showing that *Sec23c* has the highest expression in 12 DAF endosperm of the wild type.

**(F)** Y2H assay showing that Got1p and human Got1 (hGot1a and hGot1b) not only interact with their corresponding Sec23, but also cross interact with rice Sec23c.



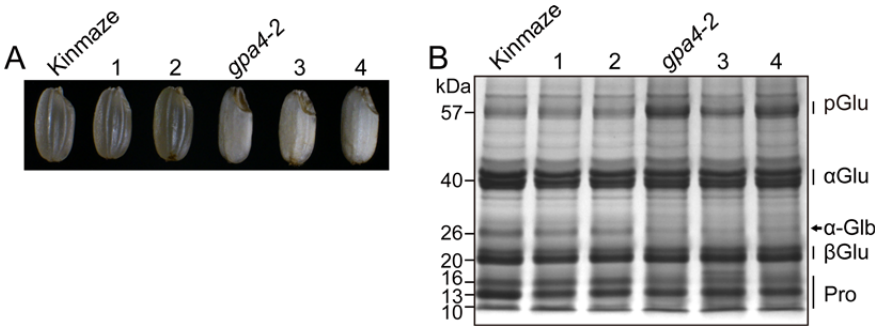

**Supplemental Figure 8.** Immunoblot Analyses Showing the Specificity of Antibodies.

**(A)** to **(E)** Immunoblot analyses showing the specificity of antibodies for GOT1B **(A)**, Sar1b **(B)**, Sar1c **(C)**, Sec23c **(D),** and Sec12b **(E)**. **(A)** GOT1B protein can be detected only in IP sample precipitated by anti-GOT1B antibodies. Total extracts were loaded for immunoblot assays in **(B)** to **(E).** T, total extract; W, wash-off solution; IP, immunoprecipitation. Arrows indicate the corresponding protein bands.




**Supplemental Figure 9.** Rescue of the *gpa4-2* Mutant Phenotypes by Yeast *GOT1*.

**(A)** and **(B)** Yeast *Got1* rescues the grain appearance **(A)**, and the storage protein composition pattern **(B)** of the *gpa4-2* mutant. YL1 to YL3 denote the grains from three independent T1 transgenic lines.




**Supplemental Figure 10.** The Seeds of Transgenic Rice Lines Expressing AtSar1b-GFP Show No Difference from Their Corresponding Recipient Plants. **(A)** and **(B)** The grain appearance **(A)** and the storage protein composition pattern **(B)** of Kinmaze and *gpa4-2* transformed with *AtSar1b-GFP*. 1 to 4 are transgenic lines using Kinmaze (1 and 2) or *gpa4-2* (3 and 4) as the recipients, respectively.

**Supplemental Table 1**. Comparison of Important Agronomic Traits between Wild Type and *gpa4* Mutants

| Characteristic | 9311 | *gpa4-1* | Kinmaze | *gpa4-2* |
|---|---|---|---|---|
| Plant height (cm) | 114.1 ± 2.4 | 95.2 ± 4.7** | 95.7 ± 2.8 | 94.3 ±1.6 |
| Panicle length (cm) | 23.26 ± 1.38 | 23.19 ± 0.84 | 19.11 ± 1.33 | 19.20 ± 1.20 |
| Tiller number | 6.58 ± 1.57 | 5.94 ± 1.58 | 6.86 ± 2.25 | 6.29 ± 1.76 |
| 1,000-grain weight (g) | 30.42 ± 0.01 | 21.29 ± 0.03** | 22.71 ± 0.02 | 19.04 ± 0.13** |
| Protein content (mg/grain) | 2.88 ± 0.05 | 1.96 ± 0.02** | 2.05 ± 0.01 | 1.62 ± 0.13** |
| Amylose content (mg/grain) | 4.56 ± 0.14 | 2.83 ± 0.05** | 3.02 ± 0.02 | 2.52 ± 0.10** |

The asterisks indicate statistical significance between the *gpa4* mutants and the corresponding wild type, as determined by a Student's *t*-test (**$P < 0.01$).

**Supplemental Table 2**. Segregation of Mutant Phenotype in Reciprocal Crosses between the Wild Type and the *gpa4-1* Mutant.

| Cross | Normal | 57H/Floury | $X^2_{3:1}$ |
|---|---|---|---|
| *gpa4-1*/9311 | 175 | 64 | 0.31 |
| 9311/*gpa4-1* | 135 | 51 | 0.46 |

**Supplemental Table 3**. The Distribution of COPII-Related Proteins in the Endomembrane System

| | | P2 | P13 | P100 | S100 |
|---|---|---|---|---|---|
| Sar1b | 9311 | 5.53 ± 0.25 | 20.17 ± 1.32 | 40.43 ± 1.83 | 33.88 ± 1.72 |
| | *gpa4-1* | 13.92 ± 0.61** | 27.07 ± 1.56** | 25.09 ± 1.21** | 33.91 ± 1.84 |
| Sar1c | 9311 | 43.15 ± 2.11 | 10.26 ± 0.53 | 30.95 ± 1.75 | 15.64 ± 0.94 |
| | *gpa4-1* | 33.96 ± 1.52** | 17.12 ± 0.68** | 30.09 ± 1.59 | 18.83 ± 1.32 |
| Sec23c | 9311 | 15.07 ± 0.65 | 14.16 ± 0.89 | 16.16 ± 1.18 | 54.60 ± 2.73 |
| | *gpa4-1* | 14.85 ± 0.71 | 13.54 ± 0.56 | 13.64 ± 0.94 | 57.96 ± 3.15 |
| Sec12b | 9311 | 17.23 ± 0.75 | 34.91 ± 1.12 | 43.17 ± 1.56 | 4.68 ± 0.23 |
| | *gpa4-1* | 18.31 ± 0.83 | 34.24 ± 1.24 | 41.50 ± 1.63 | 4.93 ± 0.25 |

The asterisks indicate statistical significance between the wild type and the *gpa4-1* mutant, as determined by a Student's *t*-test (**$P < 0.01$).

**Supplemental Table 4**. Comparison of Important Agronomic Traits between AtSar1b-GFP Transgenic Rice Lines and Their Corresponding Recipient Plants.

| | Kinmaze | *AtSar1b-GFP* /Kinmaze | *gpa4-2* | *AtSar1b-GFP* /*gpa4-2* |
|---|---|---|---|---|
| Plant height (cm) | 93.8 ± 1.4 | 92.8 ± 2.6 | 92.0 ± 1.4 | 91.5 ±2.3 |
| Panicle length (cm) | 19.10 ± 1.26 | 19.23 ± 1.34 | 19.45 ± 1.19 | 19.28 ± 1.23 |
| Tiller number | 6.87 ± 1.20 | 6.56 ± 1.65 | 6.13 ± 1.20 | 6.35 ± 1.56 |
| 1000-grain weight (g) | 22.69 ± 0.11 | 22.55 ± 0.16 | 18.89 ± 0.15 | 18.76 ± 0.13 |

**Supplemental Table 5**. Primers Used in This Study.

| Usage | Primer name | Sequence |
|---|---|---|
| Primers for fine mapping | G1-F | 5' TTCCCACCACCTCCTCCG 3' |
| | G1-R | 5' GCCACTCCTCCACGCACA 3' |
| | G3-F | 5' CTGCCCCCTTGCCCCTTC 3' |
| | G3-R | 5' CTCACCACGCCACCCTCG 3' |
| | G4-F | 5' TGCTGCTCCGCTACACAAA 3' |
| | G4-R | 5' CCTCCTTCCCCTCCTCACT 3' |
| | G9-F | 5' TAGCAGCTACCTCACCGC 3' |
| | G9-R | 5' ACCAGGACCTCCCGACCC 3' |
| | G11-F | 5' ACCTGCGAACCACCACGGAC 3' |
| | G11-R | 5' GCGAGAGGCGAAGGAAATAG 3' |
| | G15-F | 5' GTGCTGCTTTAGCCGTTTAC 3' |
| | G15-R | 5' TCAGGGTGTGGGAATGGTAG 3' |
| RT-qPCR primers for storage protein genes | GluA1-F | 5' TGATGGTGAAGTGCCAGTTGTTGC 3' |
| | GluA1-R | 5' ACGCCTGTATGCTTGAGGGTTTCT 3' |
| | GluB2-F | 5' TGTCCTTCGCCCTGGACAACTATT 3' |
| | GluB2-R | 5' TGGTAAGGCGCGGAATACTGAGTT 3' |
| | GluC1-F | 5' ATGTAAAGTTCAACCGCGGCGATG 3' |
| | GluC1-R | 5' TCTCGTTGATCTGCCACTCAGCAT 3' |
| | GluD1-F | 5' AAATGAGCAGTTTCGATGCGCTGG 3' |
| | GluD1-R | 5' TGTTCGAGTATCGAGGCACCACAA 3' |
| | Pro10.1-F | 5' GTTGCATGCAGCTACAAGGCATGA 3' |
| | Pro10.1-R | 5' TCTGCATCGCCATCTTCACCATCT 3' |
| | Pro13a.2-F | 5' TTCAGGCGATTGTGCAGCAACTAC 3' |
| | Pro13a.2-R | 5' GGATGGCAAGTTTAAGGCCAGCAA 3' |
| | Pro13b.2-F | 5' GTATAGCATTGCGGCAAGCACCTT 3' |
| | Pro13b.2-R | 5' ATGGCCTGGACAACGTTAATGTCC 3' |
| | Pro13b.18-F | 5' ACATTGTTCAGGCCATAGTGCAGC 3' |
| | Pro13b.18-R | 5' ACAGAGCTTGAGCTTGAGCCAGAT 3' |
| | Pro16.2-F | 5' GCTCTCAATTTGCCCTCCATGTGT 3' |
| | Pro16.2-R | 5' TGGTACACACTACCAAGAACCGCA 3' |
| | α-Globulin-F | 5' GATCAGTTCATCACCAACAAAACA 3' |
| | α-Globulin-R | 5' TCCAGAACGCACAAAATCAT 3' |
| RT-qPCR primers for internal control | Ubiquitin-F | 5' TCTTCCCCTTCAGGACGTGT 3' |
| | Ubiquitin-R | 5' GGACCAAAGGTCACCACCAT 3' |

| | | |
|---|---|---|
| RT-qPCR primers for *GPA4* and its homologous genes | GPA4-F | 5' TGTTCTGCTCTTCACTGGTGTGGT 3' |
| | GPA4-R | 5' AGCCTAGCCCAATCTTCTTGAGGT 3' |
| | Os10g0337600-F | 5' ATTGGGATAGGGCTGACAGGCTTT 3' |
| | Os10g0337600-R | 5' GCCTGACAGGAAGAGAATATTGCCCA 3' |
| | Os02g0602800-F | 5' TGCTTGGTTGGCAATCTATGTGGC 3' |
| | Os02g0602800-R | 5' ACAGGCCATCGGACAAAGAGAAGA 3' |
| *GPA4* cDNA cloning | GPA4-cDNA-F | 5' GAAAACGCCGCTACGCTACCAC 3' |
| | GPA4-cDNA-R | 5' GCTCAAGCATAAGCCGACAAGG 3' |
| GPA4 gDNA cloning | GPA4-gDNA-F | 5' GCACCTAACTGTTTCACTTTCTG 3' |
| | GPA4-gDNA-R | 5' CCTCTACTGCTACCTTGTCCC 3' |
| Membrane protein verification | 1305GOT1B-F | 5' TCTAGAATGGTTTCCTTCGAGATGAA 3' |
| | 1305GOT1B-R | 5' AGATCTGCTCAAGCATAAGCCGACAA 3' |
| | 1305OsTip3-1-F | 5' TCTAGAATGAGCACGGCGGCGGCGA 3' |
| | 1305OsTip3-1-R | 5' AGATCTCTAGTAGTCCTCCGGCGC 3' |
| Subcellular localization | 1305GFP-GOT1B-F | 5' TGGACGAGCTGTACAGATCTATGGTTTCCTTCGAGATG 3' |
| | 1305GFP-GOT1B-R | 5' GGGCGGCCGCTTTAAGATCTCTACACTGGAACCCGTTT 3' |
| | 1305-GOT1B-GFP-F | 5' TCTAGAATGGTTTCCTTCGAGATG 3' |
| | 1305-GOT1B-GFP-R | 5' GGATCCCACTGGAACCCGTTTTCC 3' |
| Y2H assay | pGBT9-GOT1B-F | 5' GAATTCATGGTTTCCTTCGAGATGA 3' |
| | pGBT9-GOT1B-R | 5' GGATCCGCTCAAGCATAAGCCGACAA 3' |
| | pGADSec12b-F | 5' GGAGGCCAGTGAATTCATGGCGCGGCGCGGCGGC 3' |
| | pGADSec12b-R | 5' CACCCGGGTGGAATTCTCAATGTAGAAACCTTGC 3' |
| | pGADSar1b-F | 5’ GAATTCATGTTCCTGTGGGACTGG 3’ |
| | PGADSar1b-R | 5’ GGATCCCTACTTGATGTACTGGGA 3’ |
| | pGADSar1c-F | 5' GAATTCATGTCGTTTCTGCTGGAT 3' |
| | pGADSar1c-F | 5' GGATCCCTACTTGATGTACTGTGA 3' |
| | pGADSec13a-F | 5' GGAGGCCAGTGAATTCATGCCTCCCCATAAGATA 3' |
| | pGADSec13a-R | 5' CACCCGGGTGGAATTCCTAAGCCTCAACAGTGGT 3' |
| | pGADSec13b-F | 5' GGAGGCCAGTGAATTCATGTCGTCAAACAAGATA 3' |
| | pGADSec13b-R | 5' CACCCGGGTGGAATTCCTATGGCTCCACCTTGGT 3' |
| | pGADSec13c-F | 5' GGAGGCCAGTGAATTCATGTCATCGAAGAAAATA 3' |
| | pGADSec13c-R | 5' CACCCGGGTGGAATTCCTACTGCTCAGCCTTCTT 3' |
| | pGADSec23a-F | 5' GGAGGCCAGTGAATTCATGGAGGAGACGTCGACG 3' |
| | pGADSec23a-R | 5' CACCCGGGTGGAATTCTTAGCTTGGTTCAGGTGG 3' |

| | | |
|---|---|---|
| | pGADSec23b-F | 5' GGAGGCCAGTGAATTCATGTCTGAGTTCCTGGAT 3' |
| | pGADSec23c-R | 5' CACCCGGGTGGAATTCCTACTGAACTGCCAGCCG 3' |
| | pGADSec23c-F | 5' GGAGGCCAGTGAATTCATGGCGGCCGATCCCTCC 3' |
| | pGADSec23c-R | 5' CACCCGGGTGGAATTCTTAGGACTGAACGGCCAG 3' |
| | pGADSec24-F | 5' GGAGGCCAGTGAATTCATGCAACCACCGATGAGA 3' |
| | pGADSec24-R | 5' CACCCGGGTGGAATTCTCACGTCATCTTGCTCTG 3' |
| | pGADSec31-F | 5' GGAGGCCAGTGAATTCATGATCAACTCCGAGGGG 3' |
| | pGADSec31-R | 5' CACCCGGGTGGAATTCTTACATTCTGAAATTCTG 3' |
| | pGBT9-Got1p-F | 5' ACTGTATCGCCGGAATTCATGTGGCTCACAGAGGCT 3' |
| | pGBT9-Got1p-R | 5' ACGGATCCCCGGGAATTCTTATACTGGCAGAACCCT 3' |
| | pGBT9-hGOT1A-F | 5' ACTGTATCGCCGGAATTCATGATCTCCATCACCGAA 3' |
| | pGBT9-hGOT1A-R | 5' ACGGATCCCCGGGAATTCTCAGACCATCGAGCTAGT3' |
| | pGBT9-hGOT1B-F | 5' ACTGTATCGCCGGAATTCATGATCTCCTTAACGGAC 3' |
| | pGBT9-hGOT1B-R | 5' ACGGATCCCCGGGAATTCTTATACCATATTGTTGCT 3' |
| | pGADSec23p-F | 5' ATGGAGGCCAGTGAATTCATGGACTTCGAGACTAATGA3' |
| | pGADSec23p-R | 5' CCCACCCGGGTGGAATTCCTATGCCTGACCAGAGACGG 3' |
| | pGADhSec23b-F | 5' ATGGAGGCCAGTGAATTCATGGCGACATACCTGGAG 3' |
| | pGADhSec23b-R | 5' CCCACCCGGGTGGAATTCTTAACAGGCACTGGAGAC 3' |
| | pBT3N-GOT1B-F | 5’ GCCGCCTCGGCCCCATGGATGGTTTCCTTCGAGATG 3’ |
| | pBT3N-GOT1B-R | 5’ GTTAGCTACTTACCATGGCTACACTGGAACCCGTTT 3’ |
| | pPR3N-Sec23a-F | 5’ TTCCAGATTACGCTGGATCCATGGAGGAGACGTCGACG 3’ |
| | pPR3N-Sec23a-R | 5’ TGATACCACTGCTTGGATCCTTAGCTTGGTTCAGGTGG 3’ |
| | pPR3N-Sec23b-F | 5’ TTCCAGATTACGCTGGATCCATGTCTGAGTTCCTGGAT 3’ |
| | pPR3N-Sec23b-R | 5’ TGATACCACTGCTTGGATCCCTACTGAACTGCCAGCCG 3’ |
| | pPR3N-Sec23c-F | 5’ TTCCAGATTACGCTGGATCCATGGCGGCCGATCCCTCC 3’ |
| | pPR3N-Sec23c-R | 5’ TGATACCACTGCTTGGATCCTTAGGACTGAACGGCCAG 3’ |
| qPCR primers for *OsSec23* genes | OsSec23a-F | 5' TGGCAAGTGTGCAGGATGATGTTG 3' |
| | OsSec23a-R | 5' ATAGCATCTGAGGCAGTTCTGGCA 3' |
| | OsSec23b-F | 5' GATCAGCAAGGGCATGAGGCATTT 3' |

| | | |
|---|---|---|
| | OsSec23b-R | 5' TGATCACACACGACCAAACGAGGA 3' |
| | OsSec23c-F | 5' AGGACGATTTCCTGCTCCAAGACT 3' |
| | OsSec23c-R | 5' ACCTGGAGGAACATCATGTGCTGA 3' |
| BiFC assay | $Y^N$-GOT1B-F | 5' GAAGGGAGGTACTAGTATGGTTTCCTTCGAGATGAA 3' |
| | $Y^N$-GOT1B-R | 5' TAGAAGCCATACTAGTCTACACTGGAACCCGTTTT 3' |
| | $Y^C$-OsSec23c-F | 5' CAAGGGCGGTACTAGTATGGCGGCCGATCCCTCC 3' |
| | $Y^C$-OsSec23c-R | 5' TAGAAGCCATACTAGTTTAGGACTGAACGGCCAG 3' |
| | $Y^C$-OsSec23a-F | 5' CAAGGGCGGTACTAGTTTAGCTTGGTTCAGGTGG 3' |
| | $Y^C$-OsSec23a-R | 5' TAGAAGCCATACTAGTTTAGCTTGGTTCAGGTGG 3' |
| | $Y^C$-OsSec23b-F | 5' CAAGGGCGGTACTAGTATGTCTGAGTTCCTGGAT 3' |
| | $Y^C$-OsSec23b-R | 5' TAGAAGCCATACTAGTCTACTGAACTGCCAGCCG 3' |