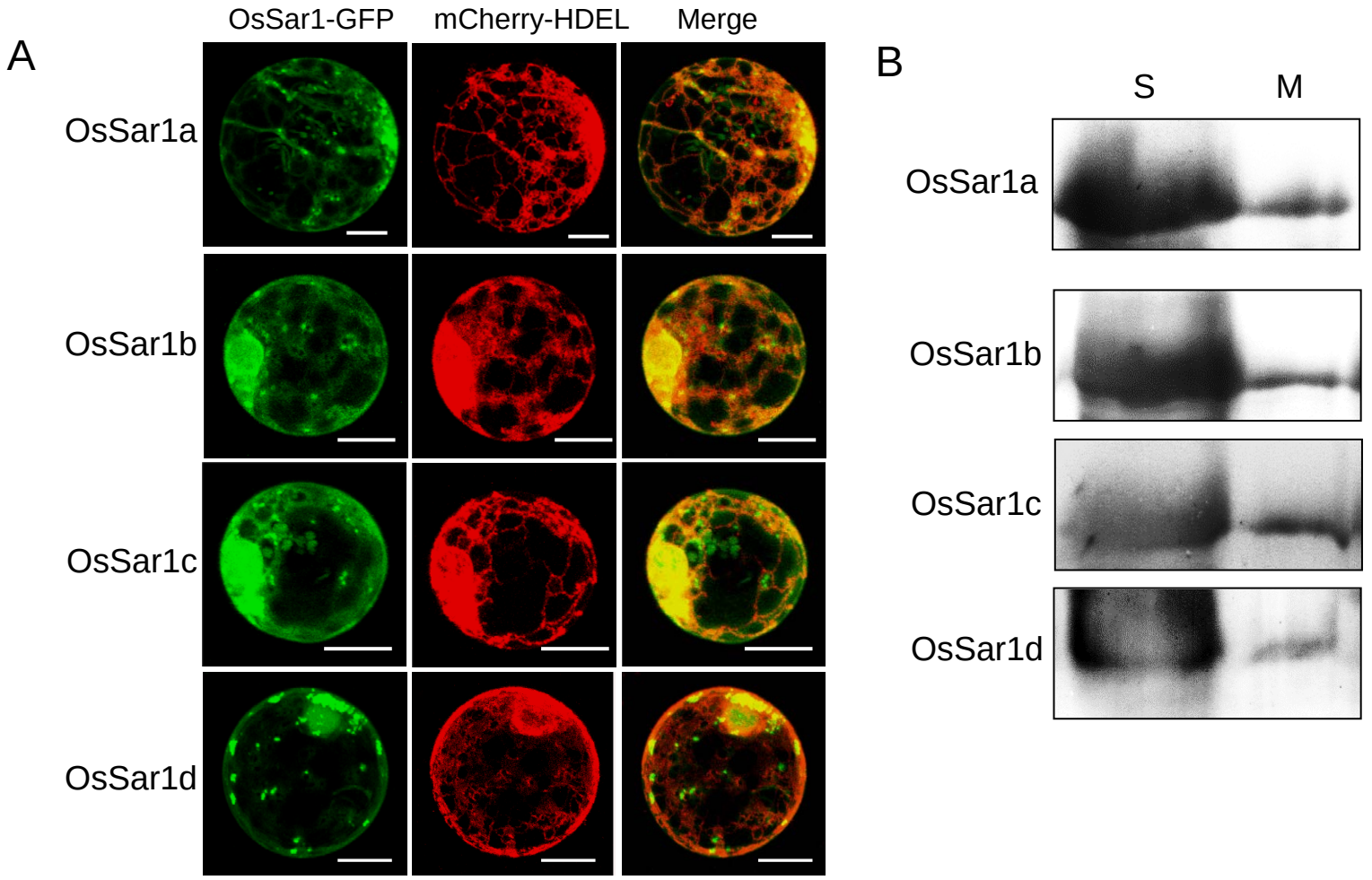
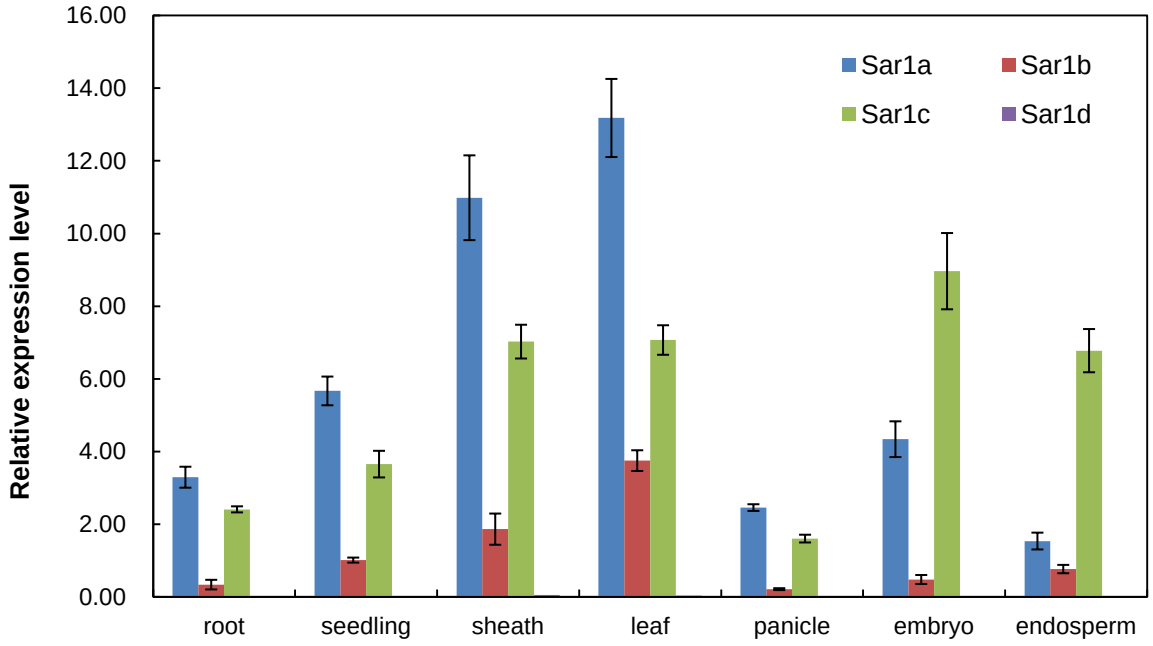
**Small GTPase Sar1 is crucial for proglutelin and α-globulin export from endoplasmic reticulum in rice endosperm**

Lihong Tian, Ling Ling Dai, Zhi Jie Yin, Masako Fukuda, Toshihiro Kumamaru, Xiang Bai Dong, Xiu Ping Xu, and Le Qing Qu

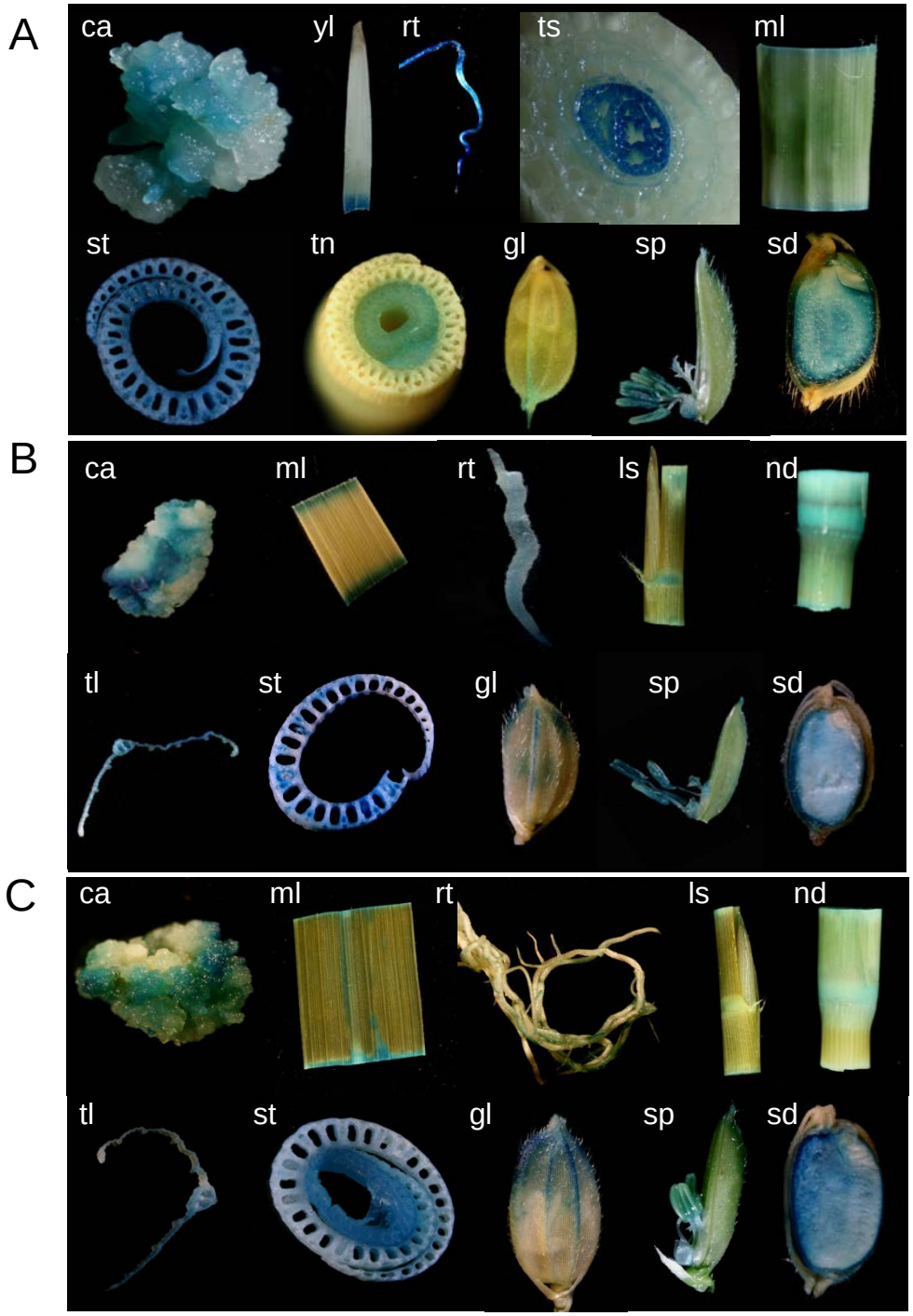
*Journal of Experimental Botany* 2013



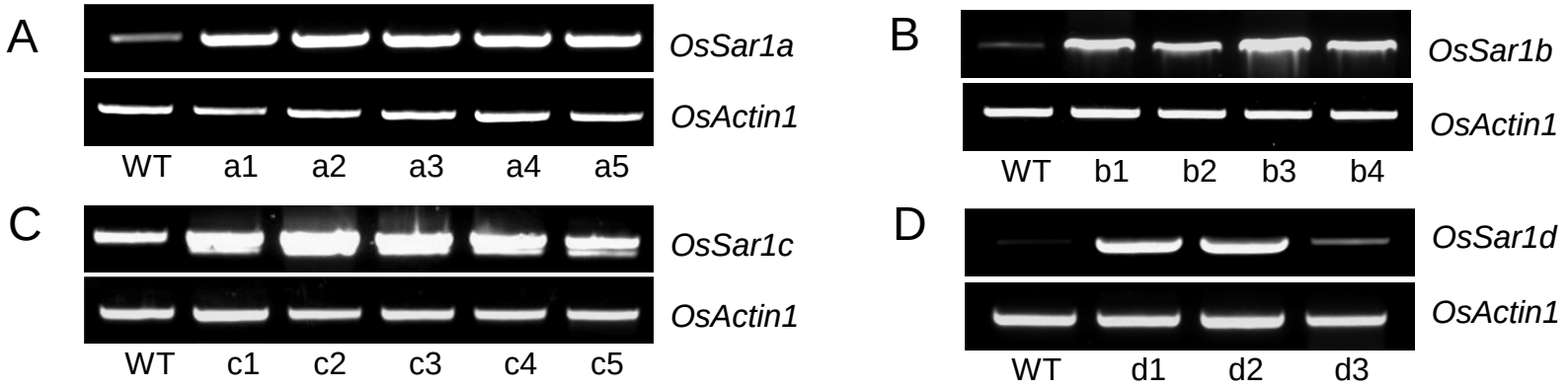

Supplementary Figure S1. Distribution of OsSar1 in rice protoplasts. A, Rice protoplasts were co-transformed OsSar1a/b/c/d-GFP with ER marker protein mCherry-HDEL. B, The soluble (S) and cell membranes (M) proteins were extracted from the OsSar1a/b/c/d-GFP expressed protoplasts. Equal volumes of each extract were separated by SDS-PAGE gels and subjected to western-blot using GFP antibody. Bars =10 μm.



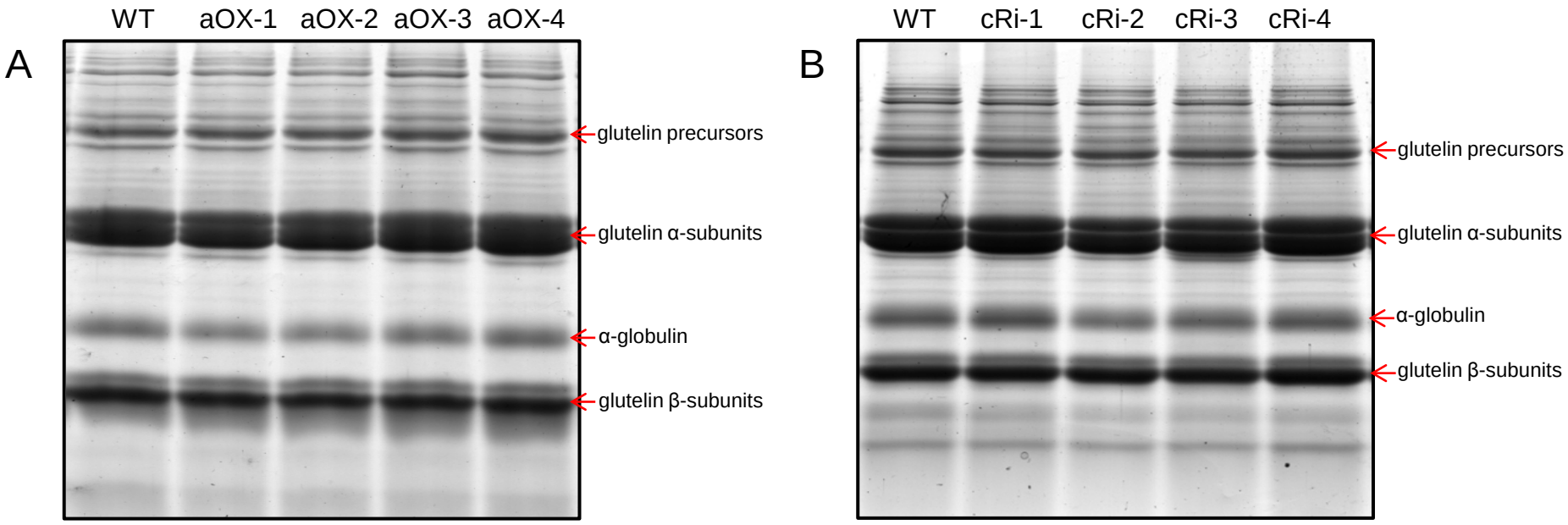

Supplementary Figure S2. The qRT-PCR analysis of relative mRNA expression of *OsSar1a*, *b*, *c*, *d* in rice tissue. The RNA were extracted from root, seedling, leaf sheath, mature leaves, panicles, embryo and endosperm of 15 DAF. The rice *Actin-1* gene was used as the endogenous control.



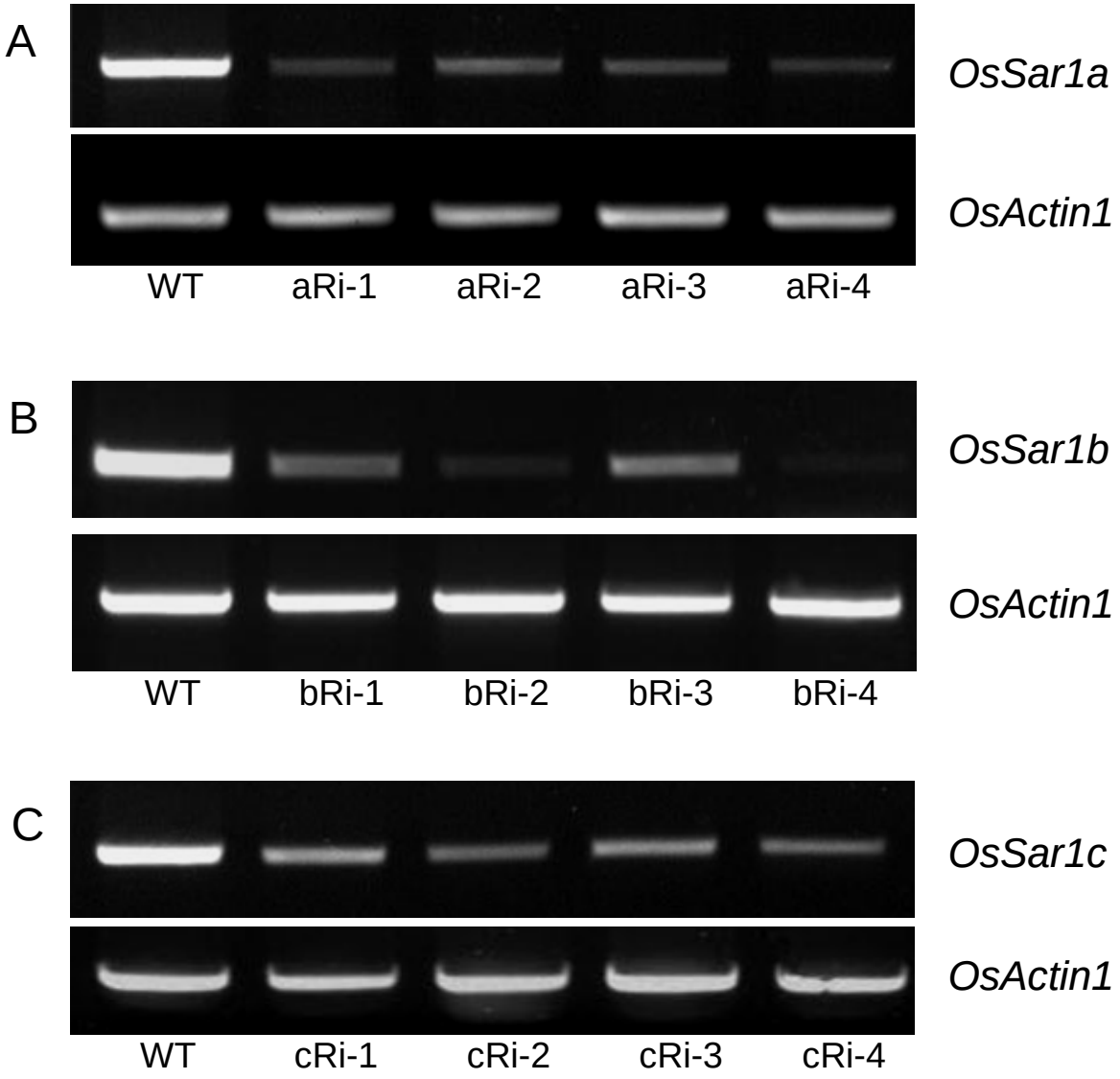

Supplementary Figure S3. Expression pattern of OsSar1 in rice.
GUS staining of tissue from (A) *OsSar1apro::GUS*, (B) *OsSar1bpro::GUS* and (C) *OsSar1cpro::GUS* transgenic lines.
ca, callus; rt, root; yl, young leaf; ml, mature leaf; tl, transverse section of mature leaf; ls, leaf sheath; ts, transverse section of sheath; nd, node; tn, transverse section of a node; st, stem; gl, glume; sp, stamen and pistil; sd, seed.



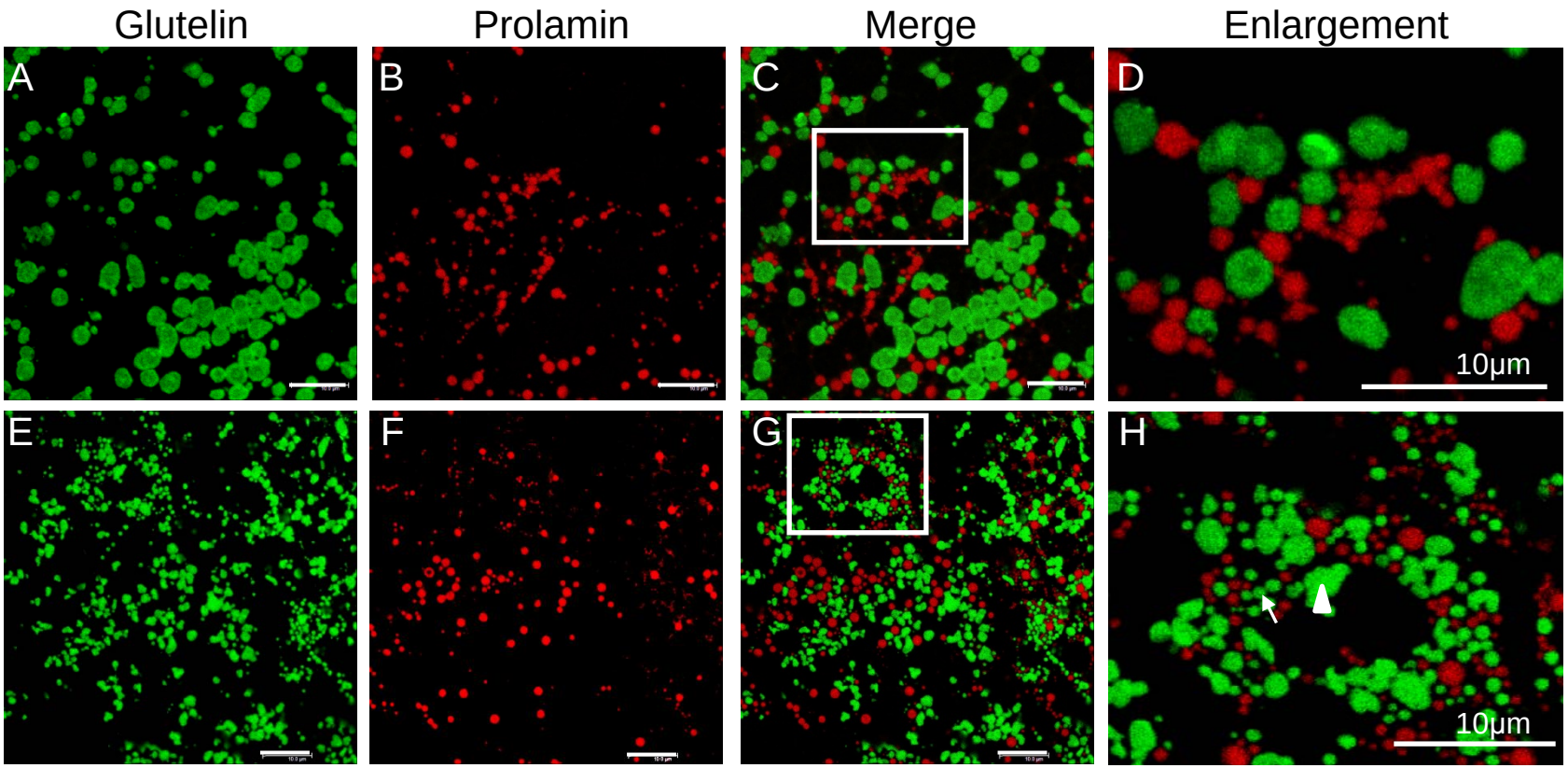

Supplementary Figure S4. RT-PCR analysis of mRNA expression of *OsSar1* in wild type (WT) and overexpression transgenic seeds. A, B, C, D is the detection *OsSar1a*, *OsSar1b*, *OsSar1c and OsSar1d* over-expressed lines respectively. *OsActin1* is used as a control.



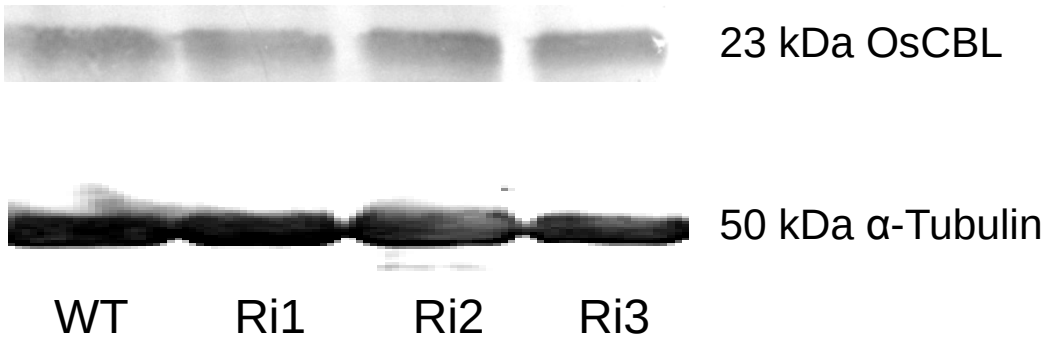

Supplementary Figure S5. SDS-PAGE of total seed protein level in *OsSar1a* overexpression lines (A) and *OsSar1c* RNAi lines (B).




Supplementary Figure S6. RT-PCR analysis of mRNA expression of *OsSar1a* (A), *OsSar1b* (B) and *OsSar1c* (C) in seeds of relative RNAi transgenic lines. *OsActin1* is used as a control.




Supplementary Figure S7. Immunofluorescence analysis of glutelin and prolamin in rice endosperm.
Secondary antibodies labeled with FITC (green, A and E) and rhodamine (red, B and F) were used to visualize glutelin and prolamin, respectively, in wild type (A-D) and OsSar1abc RNAi (E-H). C, G are merged images, (D, H) are enlarged images of the boxed areas in (C, G). The white arrowhead indicates the normal protein body II (PB-II) and and white arrow indicates the small novel protein body. Bars =10 μm.




Supplementary Figure S8. Western blot analysis of protein levels of OsCBL in *OsSar1abc* RNAi transformant seeds. The α-tubulin was used as a loading control.

**Supplementary Table S1** Primer sequences used in this study

| Experiment | Primer name | Sequence |
|---|---|---|
| Subcellular Localization | Sar1a-GFP-F | 5’-GCTCTAGAATGTTCCTGTGGGACTGGTT-3’ |
| | Sar1a-GFP-R | 5’-CTTGATGTACTGGGAGACCC-3’ |
| | Sar1b-GFP-F | 5’-GCTCTAGAATGTTCCTGGTGGACTGGTT-3’ |
| | Sar1b-GFP-R | 5’-CTTGATGTACTGTGACACCC-3’ |
| | Sar1c-GFP-F | 5’-GCTCTAGAATGTTCCTGGTTGACTGGTT-3’ |
| | Sar1c-GFP-R | 5’-TTTGATGTACTGGGACATCC-3’ |
| | Sar1d-GFP-F | 5’-GCTCTAGAATGTCGTTTCTGCTGGATTG-3’ |
| | Sar1d-GFP-R | 5’-CTTGATGTACTGTGACATCCACC-3’ |
| Expression pattern | Sar1a-GUS-F | 5’-CCCAAGCTTTCGGCTGTCTCTCGCTTTAG-3’ |
| | Sar1a-GUS-R | 5’-ACGCGTCGACCTTCTCTCCCCACGCCTCTT-3’ |
| | Sar1b-GUS-F | 5’-ACGCGTCGACCCAGCAAGTGAAAAGAGAAGC-3’ |
| | Sar1b-GUS-R | 5’-ACGCGTCGAC GGAGGAGAGGAAGAAGAAGGGT-3’ |
| | Sar1c-GUS-F | 5’-CCCAAGCTTGACGGCCAGAGTCACCTAACGAGG-3’ |
| | Sar1c-GUS-R | 5’-TCCCCCGGGCTCCGGCGATCCCCCTCCCCG-3’ |
| | Sar1d-GUS-F | 5’-CCCAAGCTTGCGGTTCCGCTATTTTTTCTAC-3’ |
| | Sar1d-GUS-R | 5’-ACGCGTCGACCTCCAGGTATGTTATGTTCTTCTTC-3’ |
| Over-expression | Sar1a-OX-F | 5’-ACGCGTCGACATGTTCCTGTGGGACTGGTT-3’ |
| | Sar1a-OX-R | 5’-CTACTTGATGTACTGGGAGAC-3’ |
| | Sar1b-OX-F | 5’-TCCCCCCGGGATGTTCCTGGTGGACTGGTT-3’ |
| | Sar1b-OX-R | 5’-CTACTTGATGTACTGTGACACCC-3’ |
| | Sar1c-OX-F | 5’-GCGTCGACATGTTCCTGGTTGACTGGTT-3’ |
| | Sar1c-OX-R | 5’-CGAGCTCTCATTTGATGTACTGGGACA-3’ |
| | Sar1d-OX-F | 5’-GCGTCGACATGTCGTTTCTGCTGGATTG-3’ |
| | Sar1d-OX-R | 5’-CGAGCTCCTACTTGATGTACTGTGACATCC-3’ |
| Knock-down (RNAi) | Sar1a-RNAi-F | 5’-GGGGTACCACTAGTATCCCTTGTCAAGACATACTTGC-3’ |
| | Sar1a-RNAi-R | 5’-CGGGATCCGAGCTCGCAGCAATCCATAAGCAAA-3’ |
| | Sar1b-RNAi-F | 5’-GGGGTACCACTAGTGGTGTTGTTAGTTTTTGCTG-3’ |
| | Sar1b-RNAi-R | 5’-CGGGATCCGAGCTCGATGTTTCTATATCTTTAAAAGTCAC-3’ |
| | Sar1c-RNAi-F | 5’-GGGGTACCACTAGTGGGGCTTCTGCTCCTCAATT-3’ |
| | Sar1c-RNAi-R | 5’-CGGGATCCGAGCTCAGCAGCCAAGAGACCGAAG-3’ |
| | Sar1abc-RNAi-F | 5’-GGGGTACCACTAGTCAGAAGGAGGCCAAGATCC-3’ |
| | Sar1abc-RNAi-R | 5’-CGGGATCCGAGCTCGGTTCACGTTGCCCTTACC-3’ |
| qRT-PCR | Sar1a-Q-F | 5’-AGTGTTGTCCGCAAGATGGG-3’ |
| | Sar1a-Q-R | 5’-CGCTGGGCAGAGTATGCAAG-3’ |
| | Sar1b-Q-F | 5’-GCAAGATGGGCTATGGGGA-3’ |
| | Sar1b-Q-R | 5’-TGGTAAGGTGAAACAGGAGTATGAAC-3’ |
| | Sar1c-Q-F | 5’-GCGTCGTCCGCAAGATG-3’ |
| | Sar1c-Q-R | 5’-AGGAGAGTTGATAAACAGAACCAGAG-3’ |
| | Sar1d-Q-F | 5’-GCTACGGCGATGGCTTCA-3’ |
| | Sar1d-Q-R | 5’-CATGCTCTGAGTCTCTACCATGTTC-3’ |
| | Actin-Q-F | 5’-ACCATTGGTGCTGAGCGTTT-3’ |
| | Actin-Q-R | 5’-CGCAGCTTCCATTCCTATGAA-3’ |