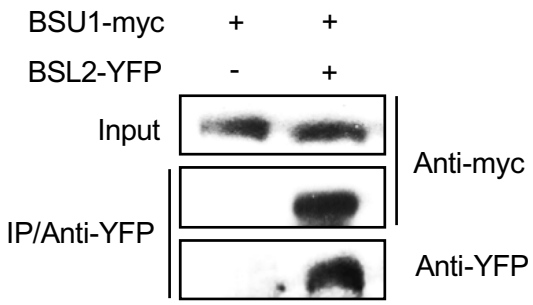
A

Protein	Gene	Peptide numbers	Sequence coverage
BSU1-YFP	At1g03445	51(51)	76.5%
BSL1	At4g03080	44(38)	64.2%
BSL2	At1g08420	49(11)	60.2%
BSL3	At2g27210	48(10)	59.1%

B



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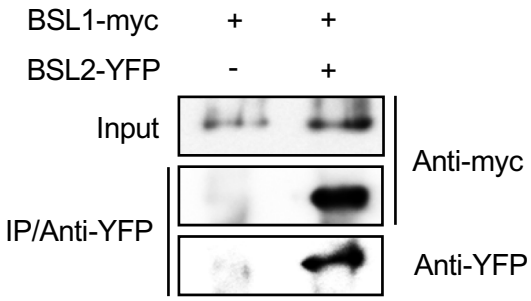
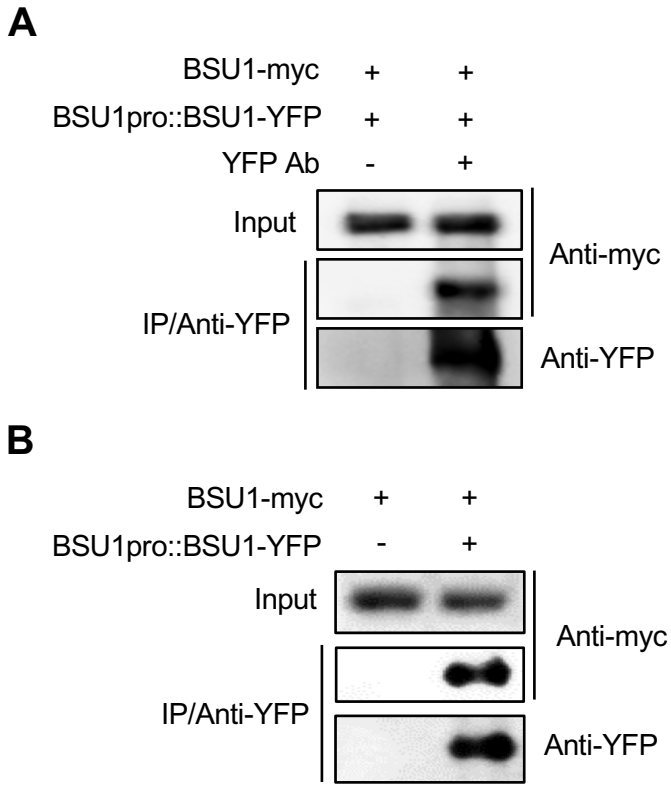
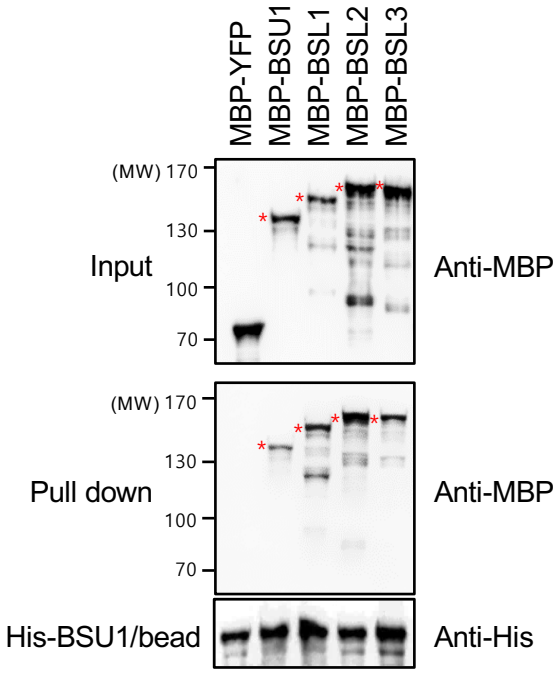


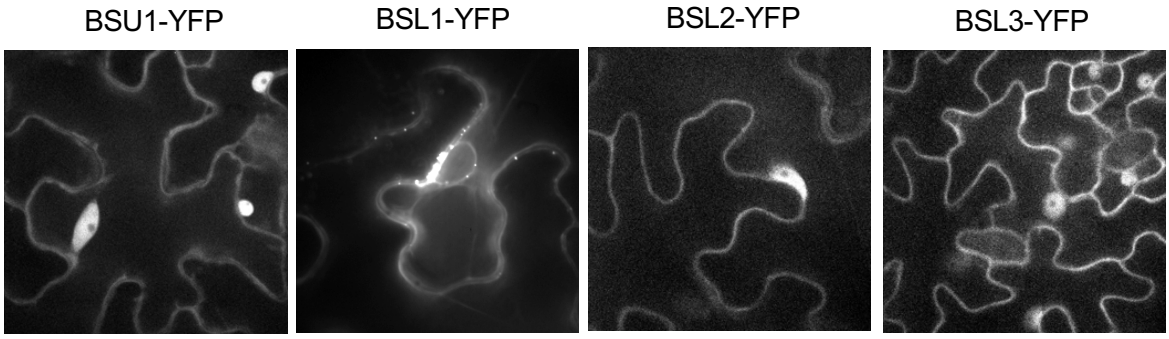
Supplemental Figure 1. BSU1 Family Members Exist in Complexes *in vivo.* **(A)** LC-MS/MS analyses of proteins co-immunoprecipitated with BSU1-YFP. BSU1-YFP was immunoprecipitated from protein extracts of BSU1-YFP transgenic *Arabidopsis* plants with anti-YFP antibodies. Associated proteins were trypsin-digested and analyzed by LC-MS/MS using an LTQ-FT mass spectrometer. The numbers of unique peptides that distinguished each protein from other BSUf members are indicated in parentheses. **(B-C)** Co-immunoprecipitation assays from double transgenic *Arabidopsis* plants co-expressing the indicated constructs. Myc-tagged and YFP-tagged constructs were under the control of the 35S and native promoter, respectively. Immunoprecipitations were performed from protein extracts of double transgenic *Arabidopsis* plants with anti-YFP antibodies. Associated proteins were immunoblotted with anti-myc and anti-YFP antibodies.



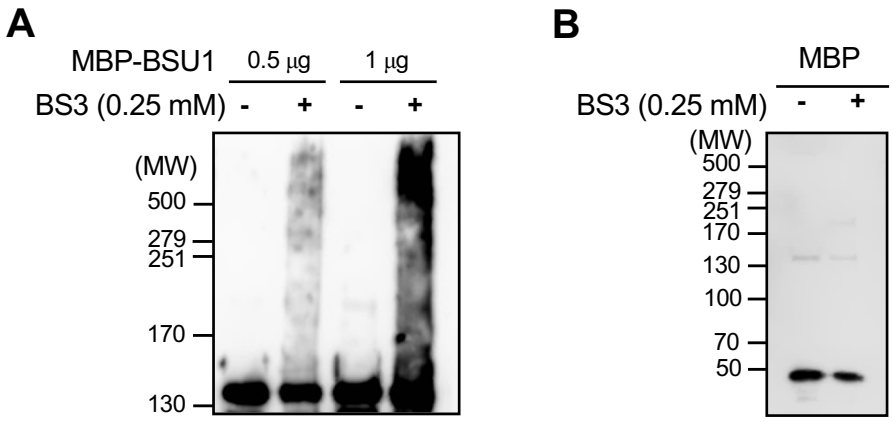
Supplemental Figure 2. Homo-oligomerization of BSU1. (A) BSU1-YFP interaction with BSU1-myc in plant extracts. BSU1-YFP immunoprecipitated by anti-YFP antibody from BSU1pro::BSU1-YFP plant was incubated with protein extracts of BSU1-myc plants. (B) Co-immunoprecipitation assay from double transgenic *Arabidopsis* plants co-expressing BSU1-YFP and BSU1-myc. Associated proteins were immunoblotted with anti-myc and anti-YFP antibodies. BSU1-YFP and BSU1-myc constructs were under the control of its native promoter and 35S promoter, respectively.



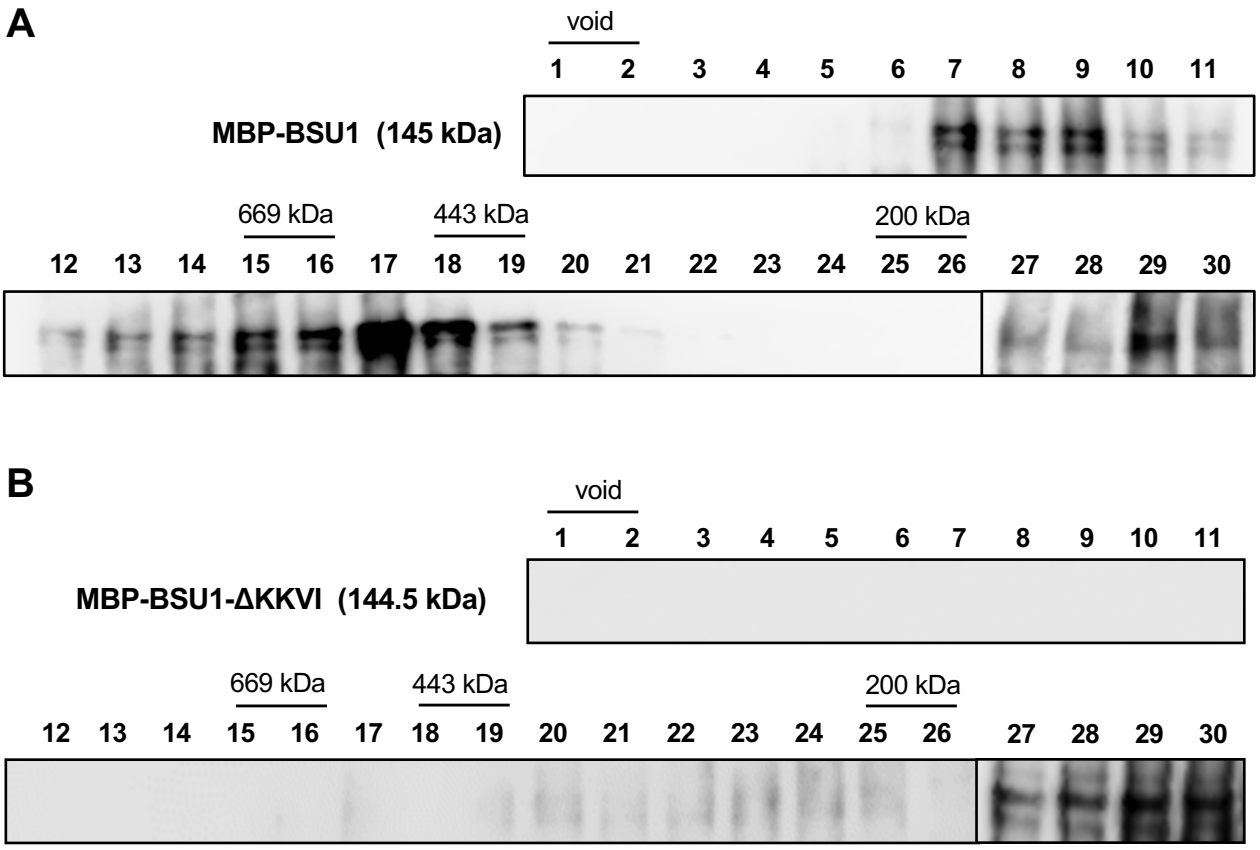
Supplemental Figure 3. BSU1 interacts directly with each member of the BSU1 family *in vitro*. *In vitro* pull down assay using BSU1 as bait and all four members of the BSU1 family as preys. MBP-fusion proteins of the BSU1 family members were pulled down with His-BSU1 immobilized on nickel agarose beads. Bound proteins were detected with anti-MBP antibodies. An asterisk indicates full length of MBP-BSUf proteins. The calculated molecular masses for MPB-YFP, MPB-BSU1, MBP-BSL1, MBP-BSL2, and MBP-BSL3 are 145 KDa, 153 KDa, 165 KDa, and 163 KDa, respectively.



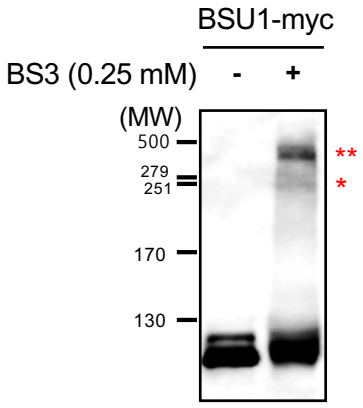
Supplemental Figure 4. Subcellular localization of BSUf members in *Arabidopsis*. Confocal microscope images of leaf epidermal cells of transgenic *Arabidopsis* plants expressing BSUf member fused to YFP are shown. All BSUf members were expressed by 35S-promoter.



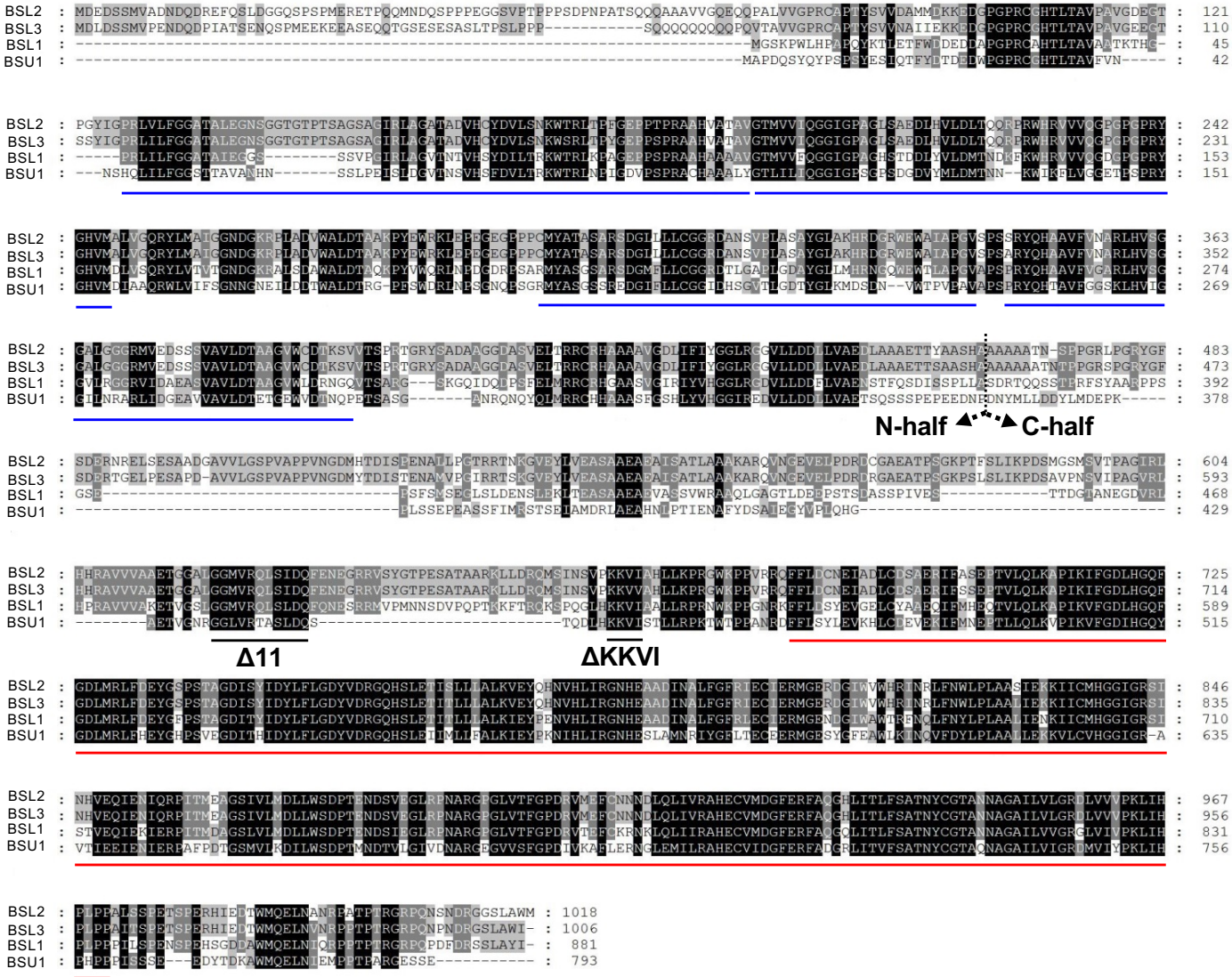
Supplemental Figure 5. Oligomeric Status of BSU1 protein *in vitro.* (A) Oligomerization of MBP-BSU1 after chemical cross-linking. MBP-BSU1 was cross-linked by BS3 treatment, separated by 5% SDS-PAGE gel, and detected by anti-MBP antibodies. (B) Chemical cross-linking of MBP. Proteins were separated by 7.5% SDS-PAGE gel, and detected by anti-MBP antibodies.



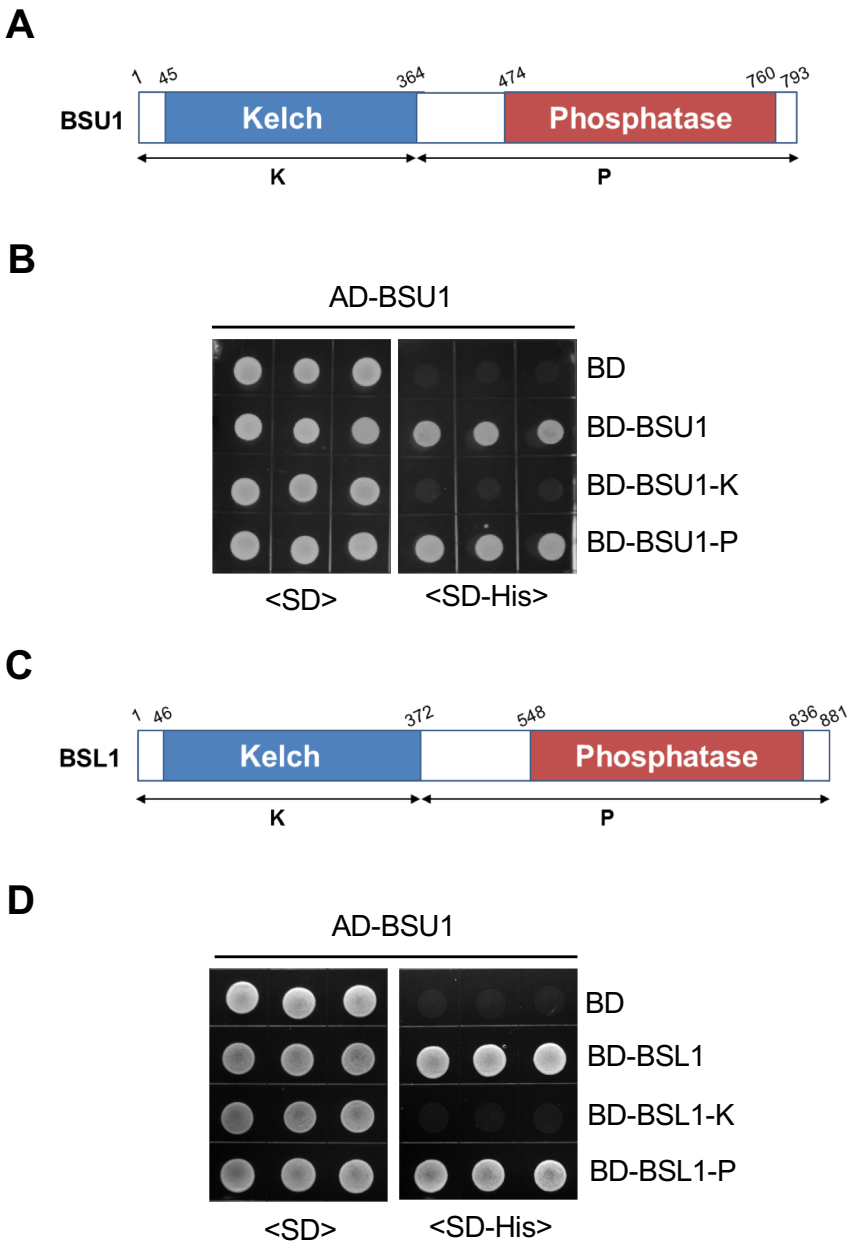
Supplemental Figure 6. Gel filtration chromatography of MBP-BSU1 and MBP-BSU1-ΔKKVI. MBP-BSU1 **(A)** or MBP-BSU1-ΔKKVI **(B)** was separated on a Superose 6 column and eluted fractions were collected. The proteins in each fraction were precipitated with trichloroacetic acid and analyzed by immunoblotting using anti-MBP antibodies. Dashed lines indicate fractions in which marker proteins with known molecular weights eluted.



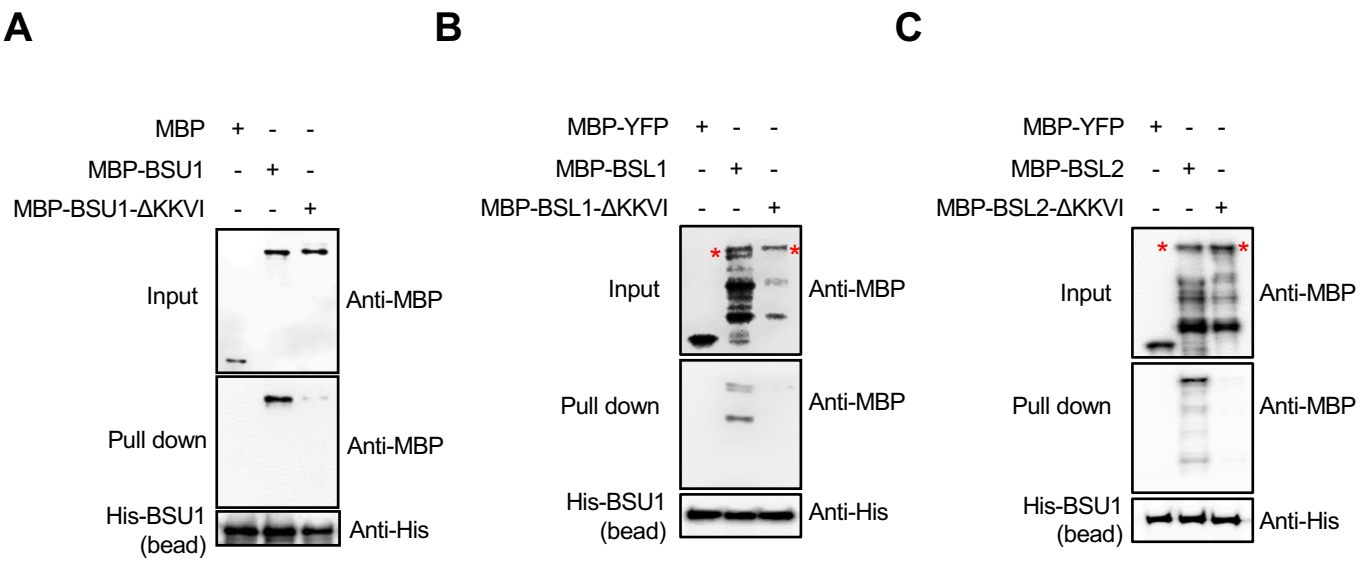
Supplemental Figure 7. Oligomeric Status of BSU1 protein *in vivo.* Oligomerization of BSU1-myc extracted from the transgenic plant overexpressing BSU1-myc. Single and double asterisk indicate dimeric and tetrameric forms, respectively.



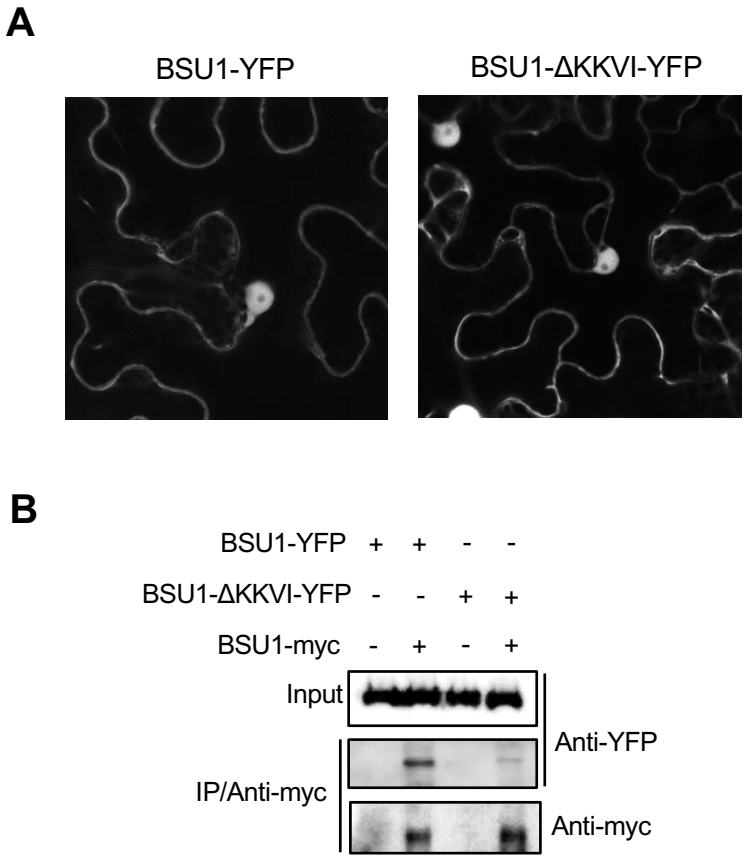
Supplemental Figure 8. Alignment of amino acid sequences of BSUf members. Kelch-repeat motif and phosphatase motif are underlined with blue and red color, respectively. Deletion constructs, N-terminal, and C-terminal half used in yeast two-hybrid assays are indicated.



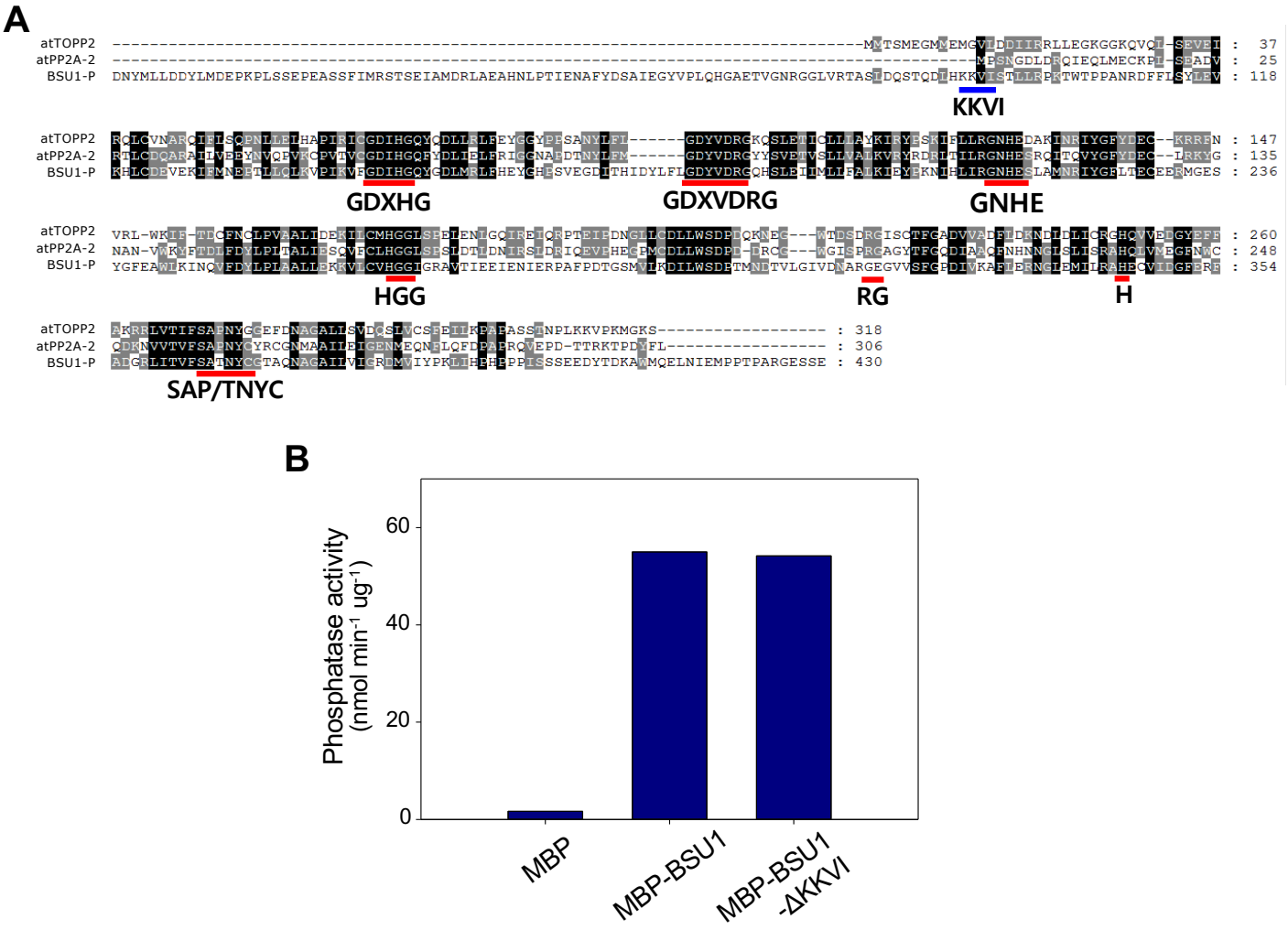
Supplemental Figure 9. C-terminal region including phosphatase domain is essential for BSU1 interaction with BSU1 or BSL1. (A) Schematic diagram of BSU1 structure. **(B)** Yeast two-hybrid assays between BSU1 and deletion constructs of BSU1. BD, BD-BSU1, BD-BSU1-K, and BD-BSU1-P plasmids were transformed into yeast cells containing AD-BSU1 constructs. **(C)** Schematic diagram of BSL1 structure. **(D)** Yeast two-hybrid assays between BSU1 and deletion constructs of BSL1. BD, BD-BSL1, BD-BSL1-K, and BD-BSL1-P plasmids were transformed into yeast cells containing AD-BSU1 constructs. Yeast cells were grown on synthetic dropout (SD) or SD-Histidine (His) medium.



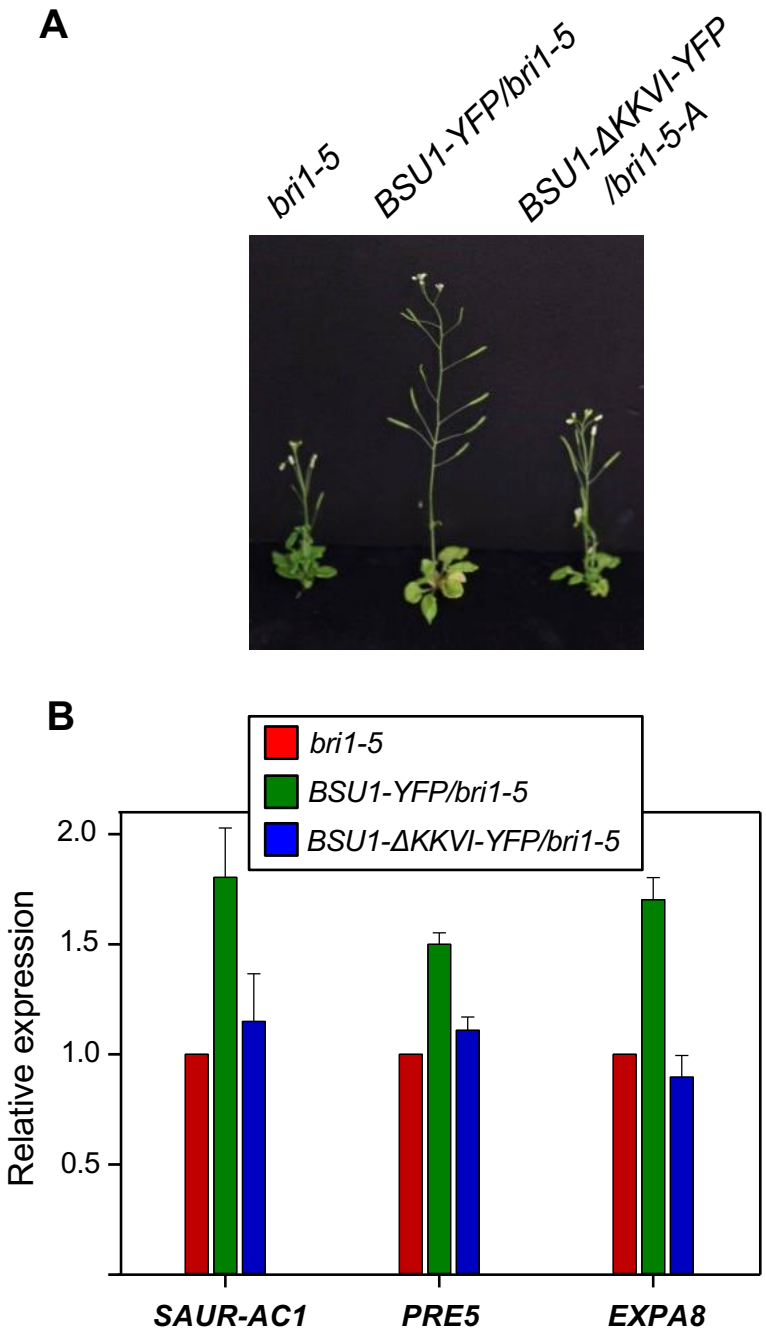
Supplemental Figure 10. Identification of the Four Amino Acids that Mediate BSUf Oligomerization. *In vitro* pull down assays to test the interaction between BSU1 and BSUf members containing the ΔKKVI mutation. His-BSU1 was immobilized on nickel agarose beads and then incubated with MBP-BSU1 or MBP-BSU1-ΔKKVI **(A)**, MBP-BSL1 or MBP-BSL1-ΔKKVI **(B)**, and MBP-BSL2 or MBP-BSL2-ΔKKVI **(C)**, respectively. Captured proteins were analyzed by immunoblotting with anti-MBP antibodies. Asterisk indicates full length of protein.



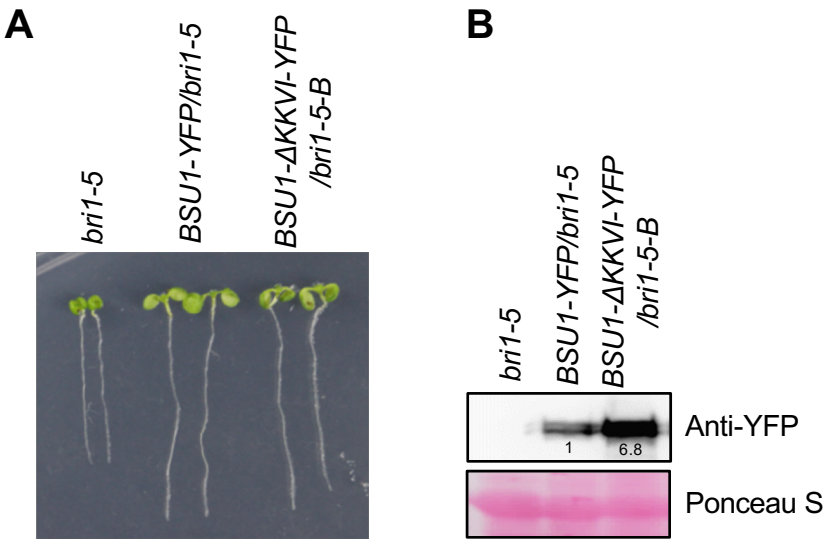
Supplemental Figure 11. Subcellular localization of BSU1-YFP and BSU1-ΔKKVI-YFP, and their interaction with BSU1-myc in tobacco leaves. (A) subcellular localization of BSU1-YFP and BSU1-ΔKKVI-YFP that were transiently expressed in tobacco leaf cells. **(B)** Co-immunoprecipitation of BSU1-YFP or BSU1-ΔKKVI-YFP with BSU1-myc in tobacco leaves. Immunoprecipitation was performed with anti-myc antibodies. Associated proteins were immunoblotted with anti-YFP and anti-myc antibodies.



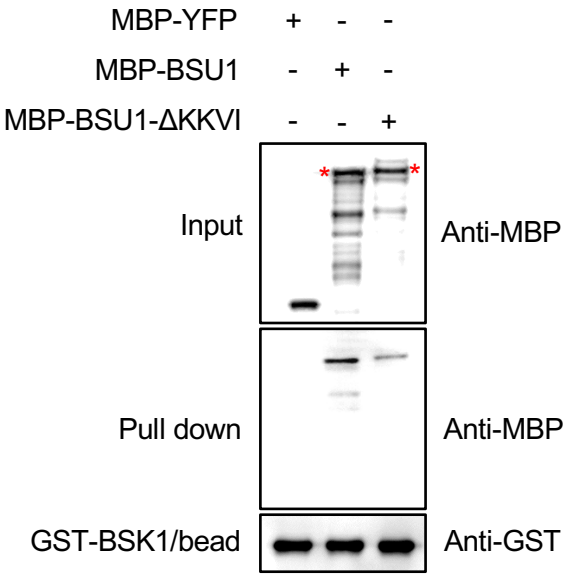
Supplemental Figure 12. The deletion of KKVI residues does not alter phosphatase activity of BSU1. (A) Alignment of amino acid sequences of BSU1 and other plant phosphatases. Amino acid sequences of full length of PP1 (atTOPPs; At5g19160), full length of PP2A catalytic subunit (atPP2A-2; At1g10430), and C-terminal region of BSU1 (365~793 residues) were compared. Core catalytic residues conserved in phosphatases are underlined with red color. KKVI residues of BSU1 are underlined with blue color. **(B)** Comparison of phosphatase activities of MBP-BSU1 and MBP-BSU1-ΔKKVI. pNPP was used as a substrate to determine phosphatase activity.



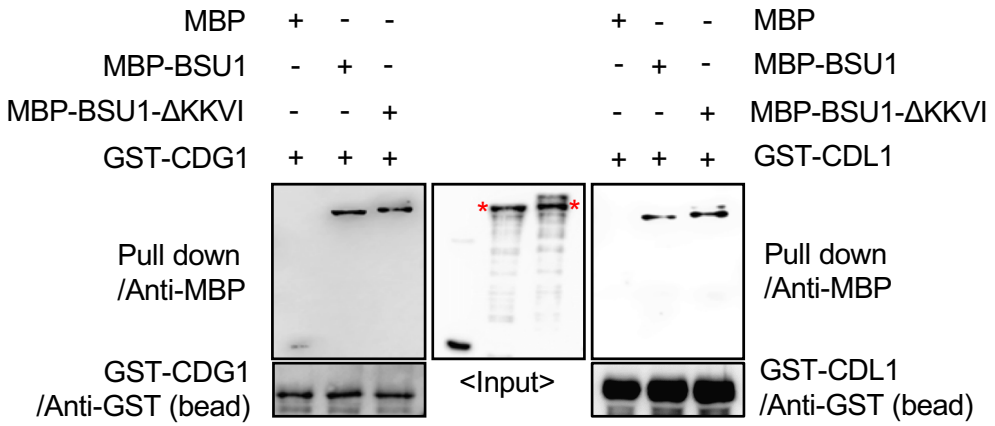
Supplemental Figure 13. Oligomerization of BSUf is Required for the Activation of BR Signaling. (A) Phenotypes of 4-week-old *bri1-5* mutants overexpressing either BSU1-YFP or BSU1-ΔKKVI-YFP. **(B)** BR-inducible gene expression in transgenic *bri1-5* mutants overexpressing BSU1 or BSU1-ΔKKVI. Quantitative RT-PCR analysis of expression levels of BR-regulated genes in the plants shown in Figure 1G.



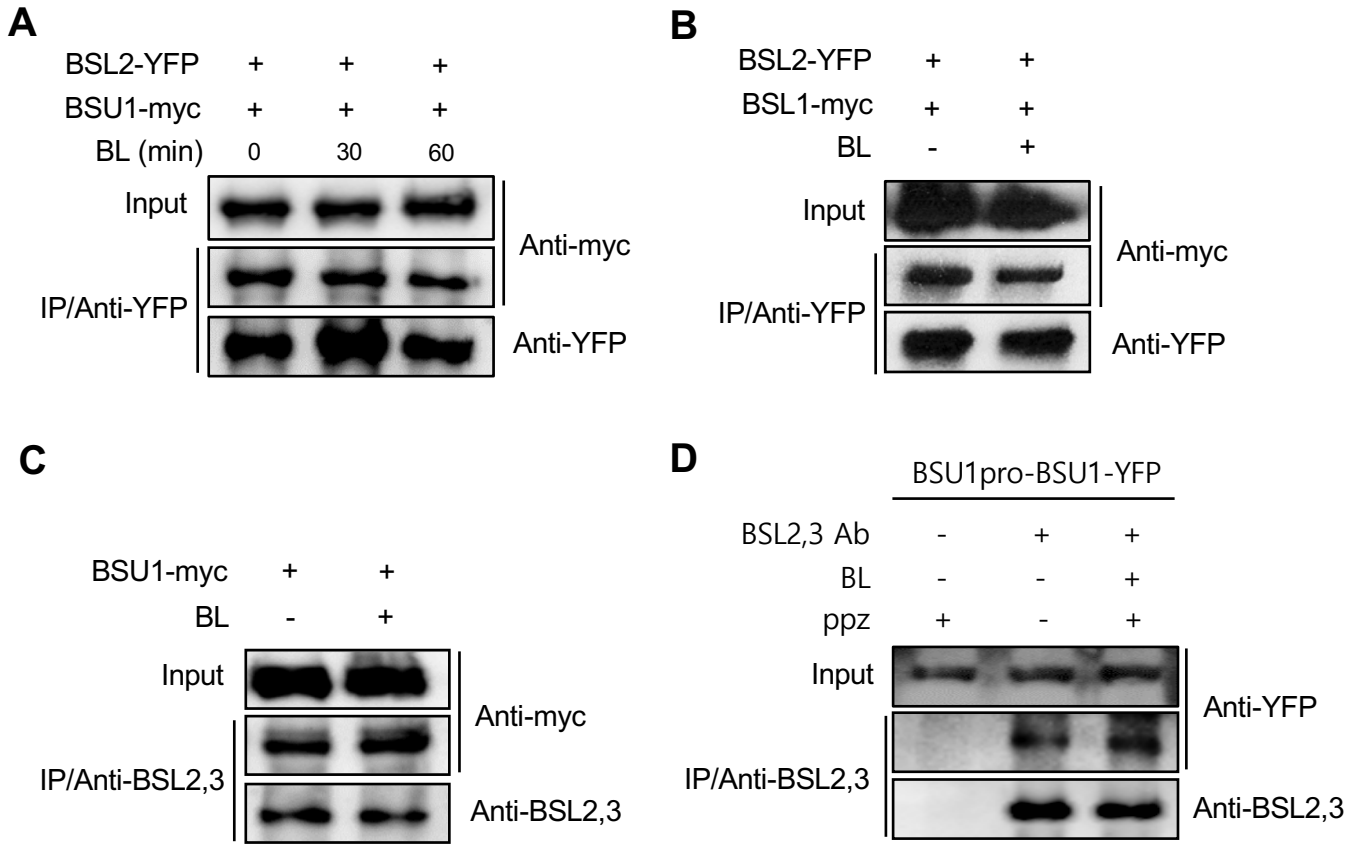
Supplemental Figure 14. Phenotypes and protein level of *bri1-5* expressing BSU1-YFP or BSU1-ΔKKVI-YFP. **(A)** Phenotype of 7-d-old *bri1-5* mutants expressing BSU1-YFP or BSU1-ΔKKVI-YFP. **(B)** Immunoblot analysis for protein expression level in *Arabidopsis* shown in **(A)**. Numbers indicate relative signal level.



Supplemental Figure 15. BSU1 oligomerization increases the binding affinity for BSK1. MBP-YFP, MBP-BSU1, and MBP-BSU1-ΔKKVI were pulled down with GST-BSK1 and detected by anti-MBP antibodies.



Supplemental Figure 16. Oligomerization of BSU1 does not alter BSU1 binding affinity to CDG1 or CDL1. MBP, MBP-BSU1 or MBP-BSU1-ΔKKVI was incubated with GST-CDG1 or GST-CDL1 immobilized on GST agarose beads, and immunoblot was detected by anti-MBP antibodies. An asterisk indicates full length of protein.



Supplemental Figure 17. BL effects on the oligomerization between BSUf members. (A) Co-immunoprecipitation of BSU1 and BSL2 upon brassinolide (BL) treatment. *Arabidopsis* seedlings co-expressing 35S-BSU1-myc and BSL2pro-BSL2-YFP were treated with BL for the indicated times. Immunoprecipitations were then performed with anti-YFP antibodies and associated proteins were detected with anti-myc antibodies. **(B)** Co-immunoprecipitation of BSL1 and BSL2. Transgenic *Arabidopsis* plants co-expressing BSL2-YFP driven its native promoter and 35S-BSL1-myc were treated by mock or 100 nM of BL for 30 min. BSL2-YFP was immunoprecipitated with anti-YFP antibodies and the immunoblot was probed by anti-myc and anti-YFP antibodies. **(C)** Co-immunoprecipitation of BSU1 and BSL2,3. *Arabidopsis* plants expressing 35S-BSU1-myc were treated by mock or 100 nM of BL for 30 min. BSL2 and BSL3 were immunoprecipitated with anti-BSL2,3 antibody and the immunoblot was probed by anti-myc and anti-BSL2,3 antibodies. **(D)** Oligomerization of BSU1 with BSL2,3 in endogenous expression level. BSU1promoter-BSU1-YFP plants grown on MS medium containing 1 uM propiconazole (ppz) were treated by mock or 100 nM of BL for 30 min.