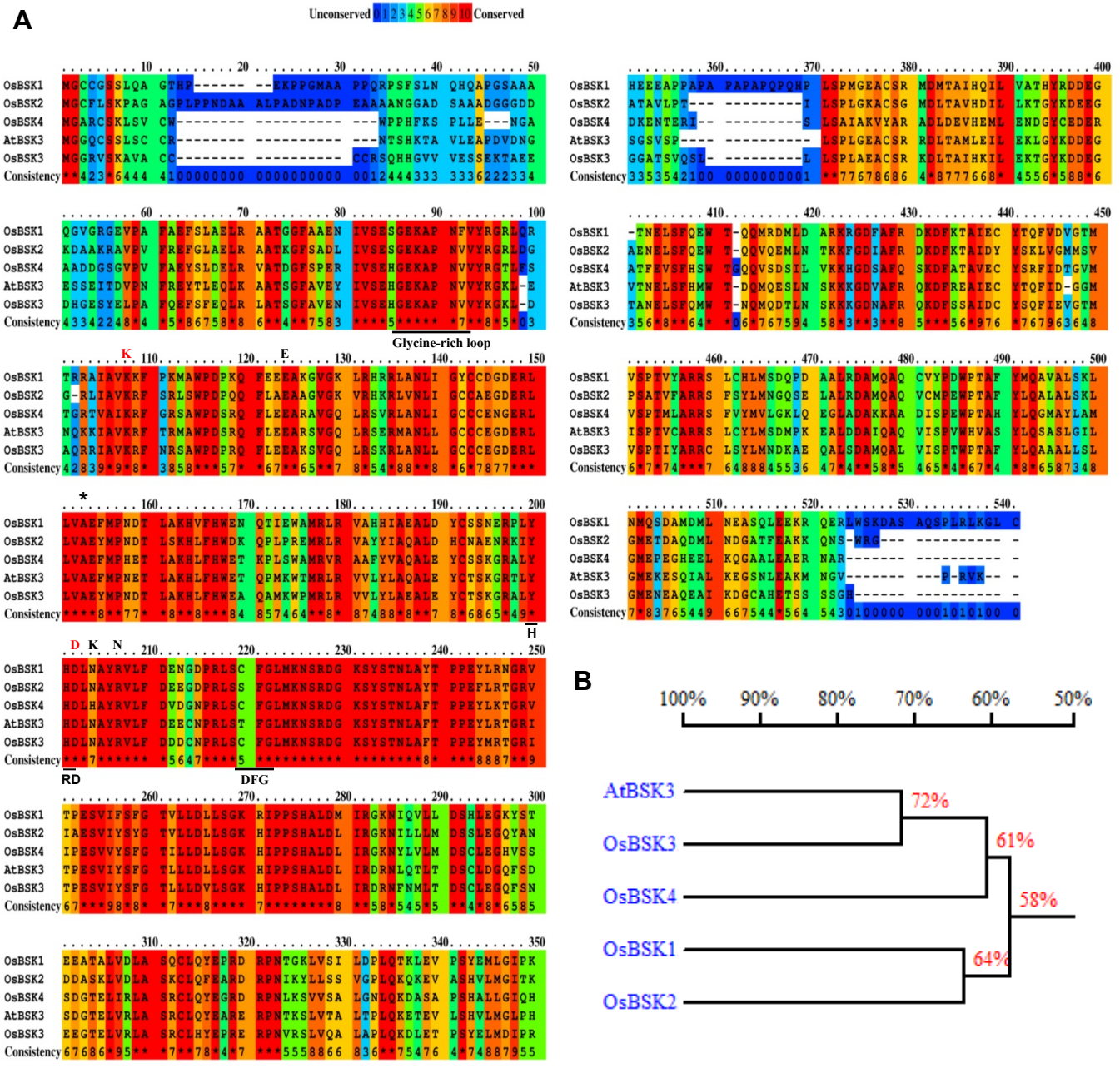

Figure S1. Four BSK orthologs are present in the rice genome. A. An alignment of the amino acid sequences of the OsBSKs with that of AtBSK3. All protein sequences were retrieved from an online database (http://rice.plantbiology.msu.edu/). The sequences were aligned using the multiple sequence alignment tool PRALINE (http://www.ibi.vu.nl/programs/pralinewww/), with a standard progressive strategy. Conserved amino acids known to be critical for kinase activity are marked by letters. K89 and D183 (colored in red) indicate the sites targeted for mutagenesis. The alanine gatekeeper residue is marked by an asterisk. **B**. Phylogenetic tree generated using DNAMAN based on the amino acid sequence similarities between the various BSKs. Similarities between the OsBSKs and AtBSK3 are shown in red.

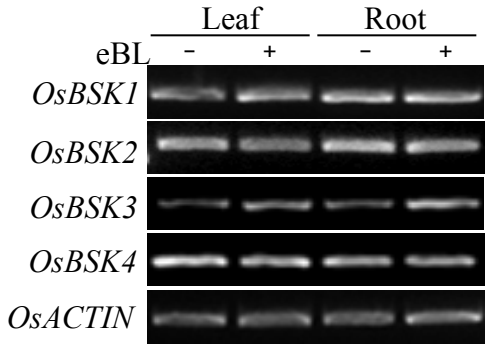

Figure S2. Semi-quantitative RT-PCR analysis of the expression of the OsBSKs in rice seedlings. Three-day-old dark-germinated rice seedlings were allowed to grow for 4 days in the light under LD conditions before being treated with 10 nM eBL for 3 days. Leaves and roots were harvested separately for analysis.

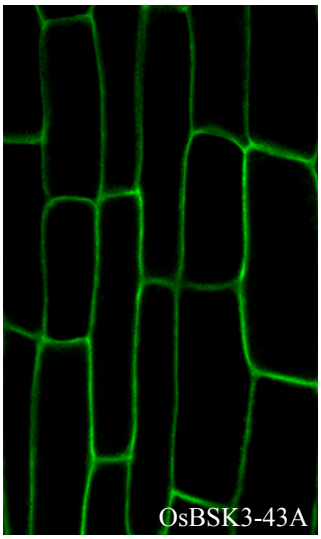

Figure S3. Subcellular localization of OsBSK3 in rice root. Confocal images indicate the localization of OsBSK3-GFP in the roots of 4-day-old dark-grown seedlings overexpressing OsBSK3 with a GFP tag at the C-terminus.

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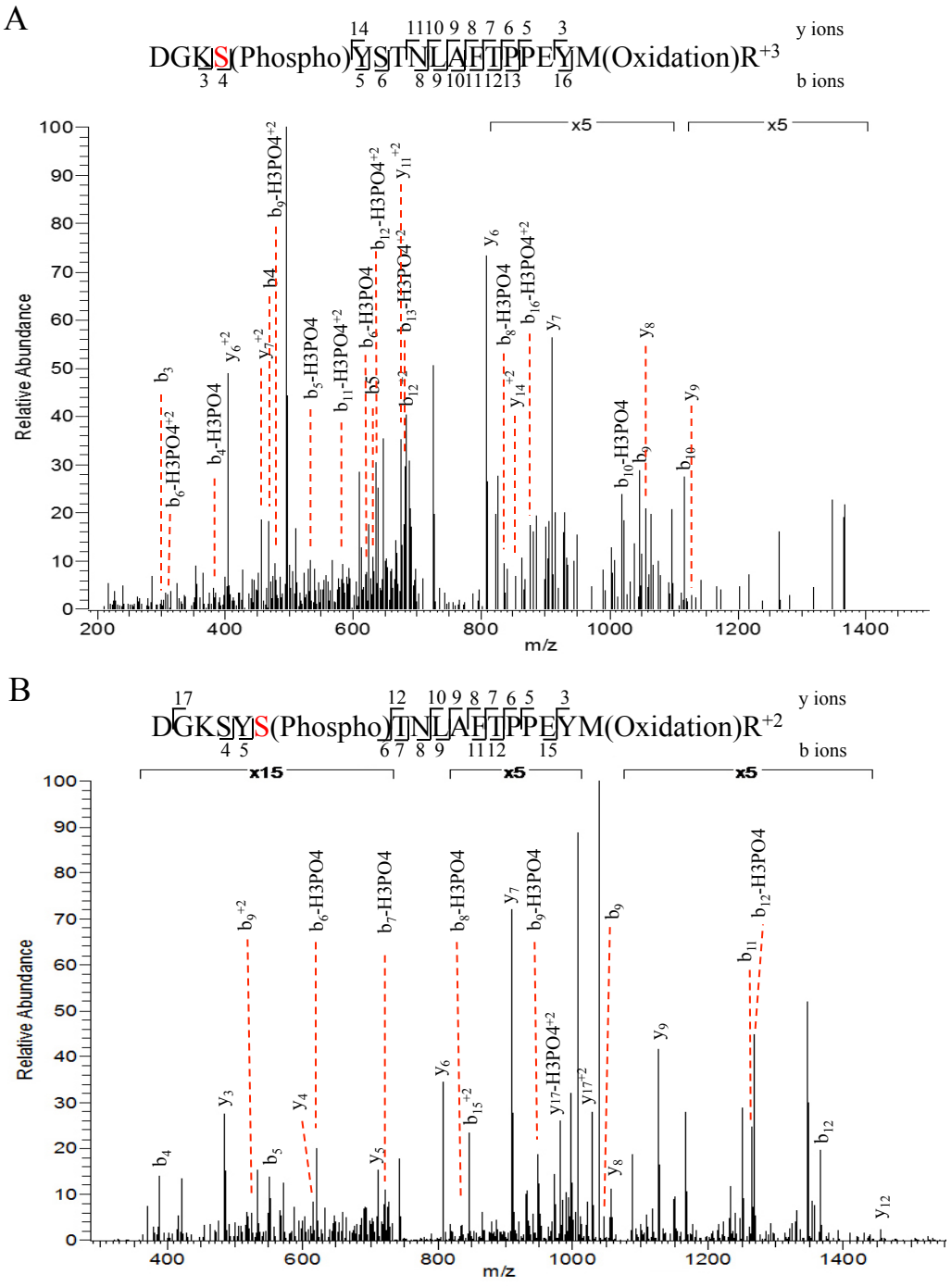

Figure S4. The *in vitro* OsBRI1-phosphorylated peptides identified by mass spectrometry from OsBSK3. A and **B**. Representative tandem mass spectra obtained from precursor ions with monoisotopic *m/z* values of 724.9864 (A) and 1086.9731 (B), corresponding to the unique sequence DGKSYSTNLAFTPPEYMR (spanning residues D210–R227) in OsBSK3, are shown. The observed sequence ions are displayed. M226 is an oxidized methionine. Phosphorylated residues are shown in red.

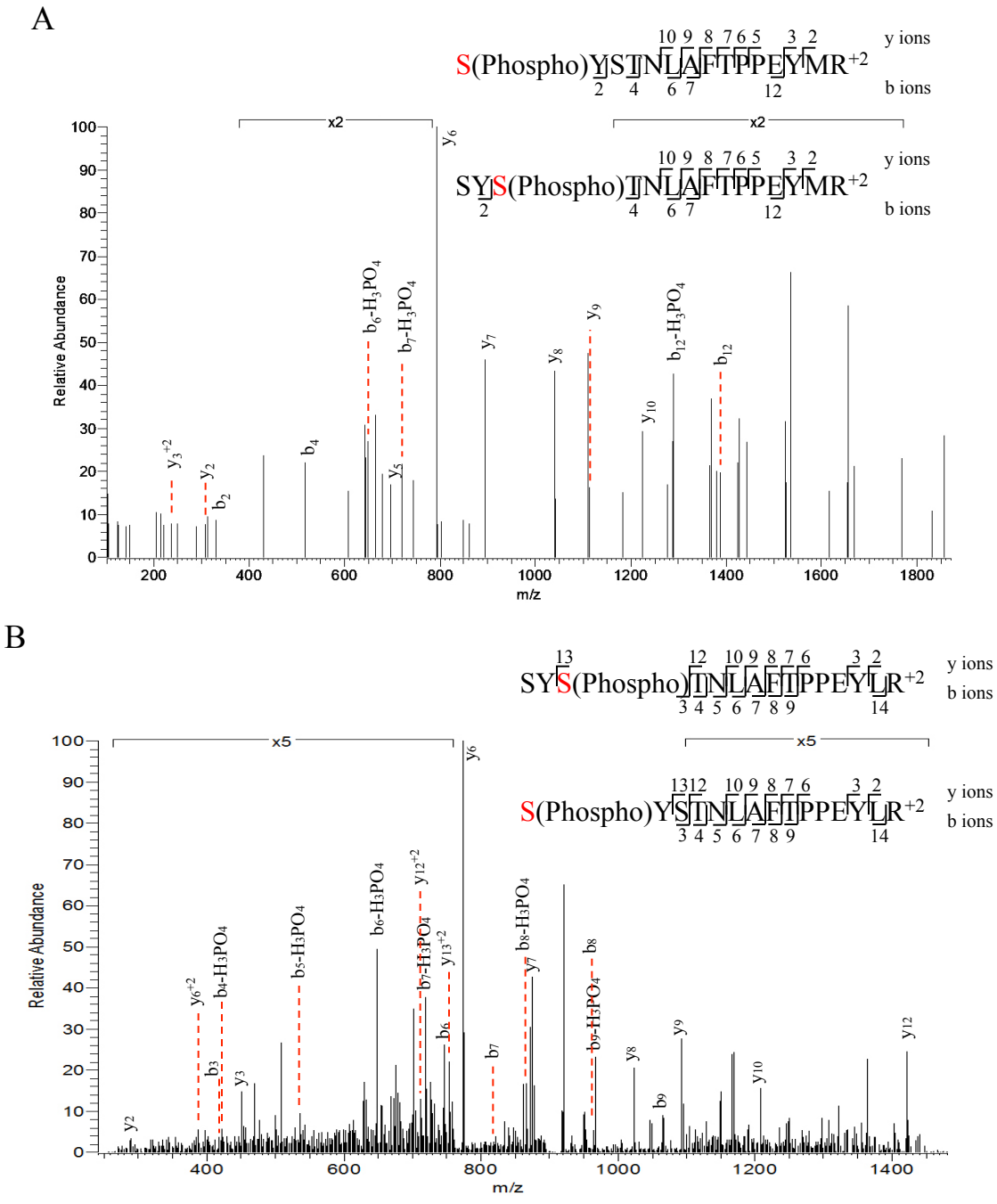

Figure S5. *In vivo*-phosphorylated peptides identified by mass spectrometry from immunoprecipitated OsBSK3 or AtBSK3. A and **B**. Representative tandem mass spectra obtained from precursor ions with monoisotopic *m/z* values of 928.9009 (A) and 919.9195 (B), corresponding to the unique sequences SYSTNLAFTPPEYMR (spanning residues S213–R227 of OsBSK3) and SYSTNLAFTPPEYLR (spanning residues S210–R224 of AtBSK3), respectively, are shown. The observed sequence ions are displayed. Potentially phosphorylated residues are shown in red.

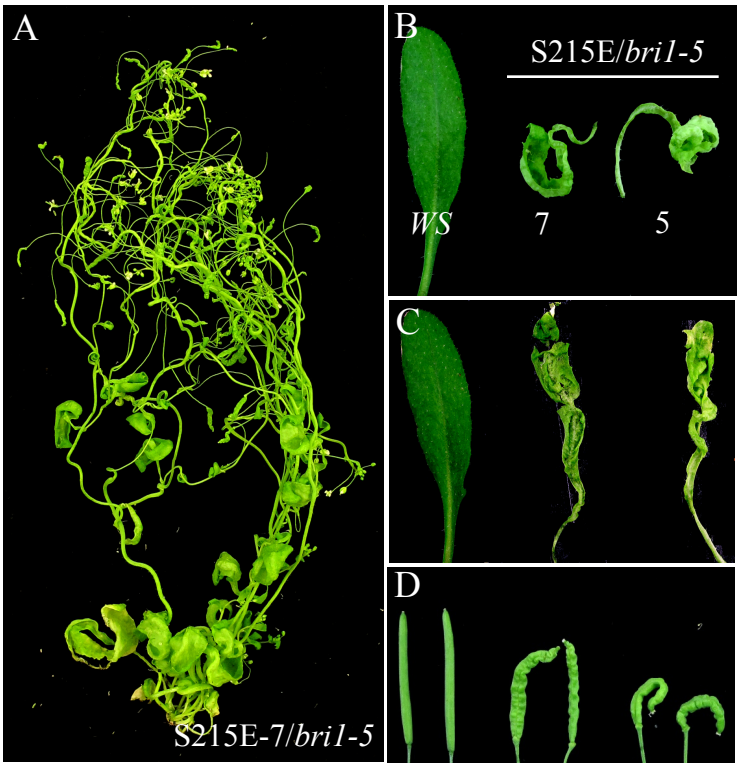

Figure S6. Phenotypes of S215E-overexpressing *bri1-5* mutant lines. A. Nine-week-old S215E-YFP-overexpressing *bri1-5* mutant plants grown under LD conditions are shown. **B**–**D**. Leaves and siliques from 8-week-old wild-type (*WS*) or *bri1-5* mutant plants overexpressing S215E-YFP are shown. Panel (C) shows the fully opened leaves of the plants shown in (B). #5 and #7 are two independent transgenic homozygous lines.

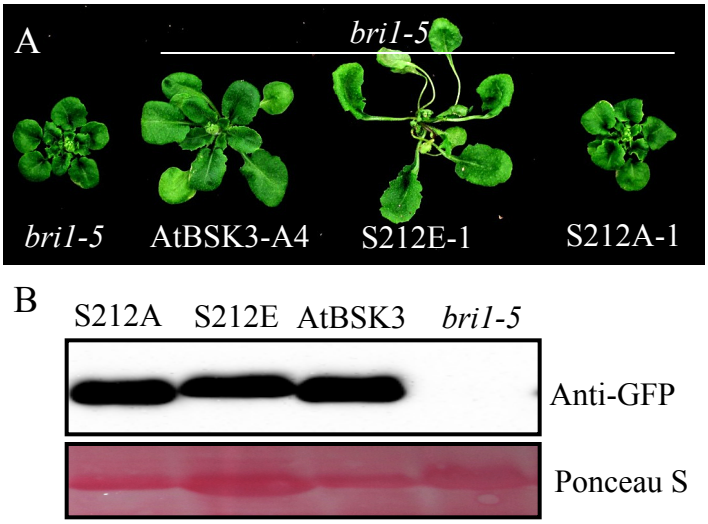

Figure S7. The phosphorylation of AtBSK3 at Ser212 activates BR signaling in *Arabidopsis*. **A**. Five-week-old *bri1-5* mutant plants or *bri1-5* plants overexpressing wild-type, S212E- or S212A-substituted AtBSK3-YFP are shown. **B**. Immunoblotting with anti-GFP antibodies revealed the expression levels of the various AtBSK3 forms in the seedlings shown in (A). Ponceau S staining of the rubisco large subunit was used as an equal loading control.

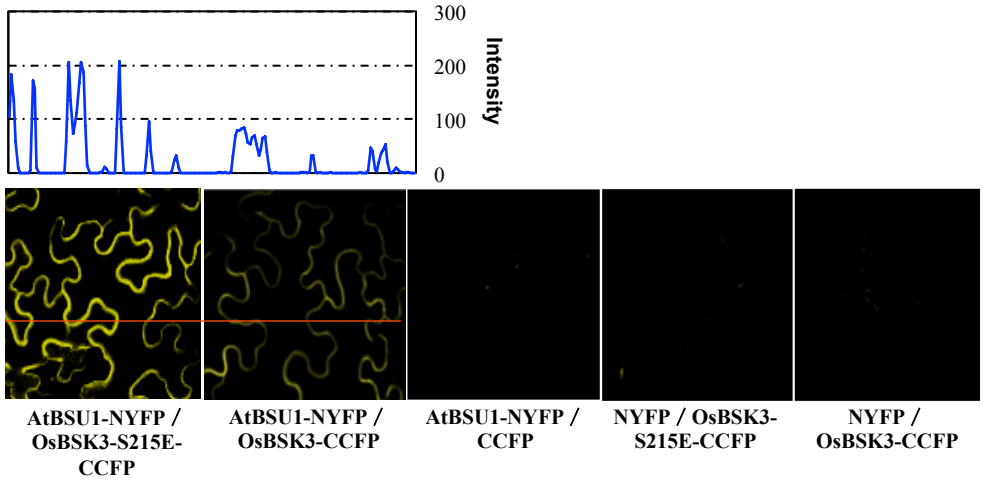

Figure S8. BiFC assays showed that AtBSU1 interacted more strongly with the S215E form of OsBSK3. Lower panel: Four-week-old *N. benthamiana* leaf epidermal cells co-infiltrated with *Agrobacterium* containing the indicated vector pairs. Images were collected 36–48 h after infiltration. Upper panel: The YFP signal was quantified using ZEN 2010 B SP1 software in the region marked by a red line in the lower panel.

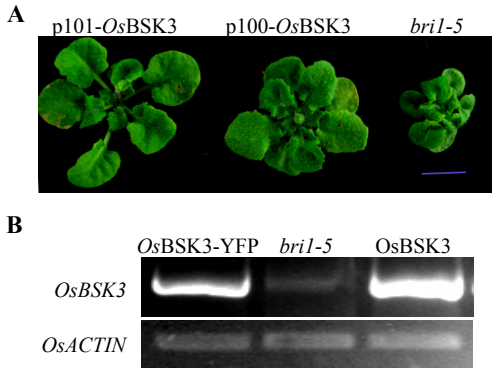

Figure S9. OsBSK3 with YFP fused to its C-terminus was more efficient at suppressing the dwarf phenotype of *bri1-5* mutant plants. **A**. Four-week-old *bri1-5* mutant plants and *bri1*-5 plants overexpressing OsBSK3 (p100-OsBSK3) or OsBSK3-YFP (p101-*Os*BSK3) are shown. **B**. Semi-quantitative RT-PCR analysis of the expression level of OsBSK3 in the plants shown in (A).

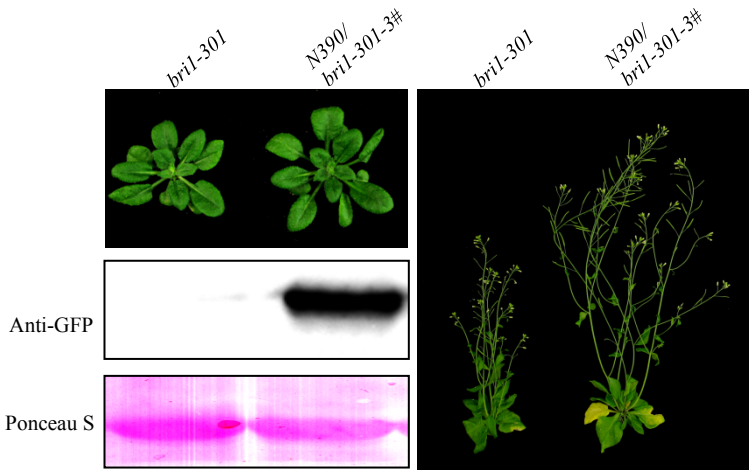

Figure S10. Overexpression of the kinase domain of OsBSK3 partially suppressed the semi-dwarf phenotype of *bri1-301* mutant plants. Four-week-old (upper left) or six-week-old (right) *bri1-301* mutant plants or *bri1*-301 plants overexpressing the kinase domain of OsBSK3 as YFP fusions are shown. Lower left: The expression level of N390-YFP based on immunoblotting using anti-GFP antibodies is shown.

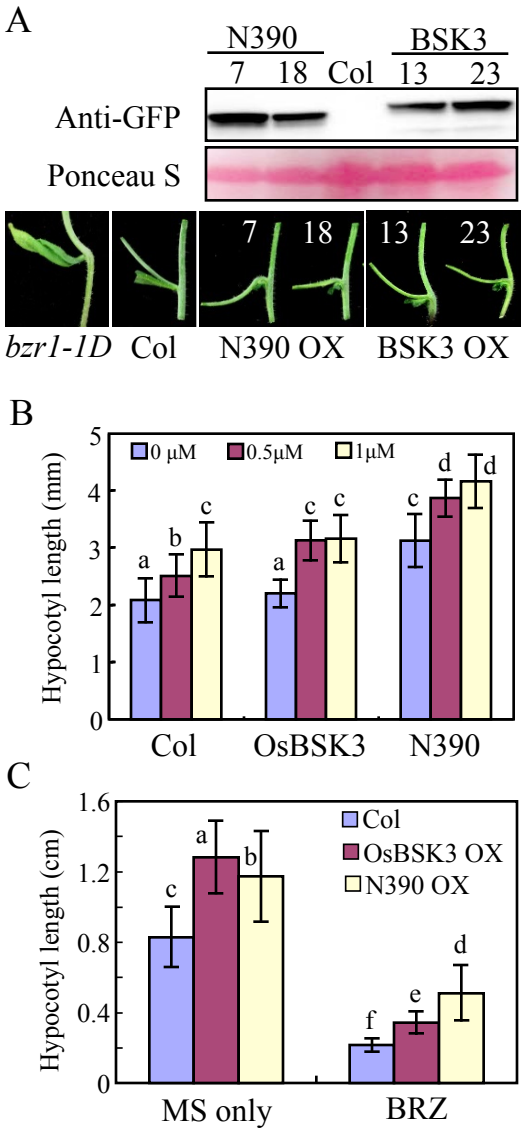

Figure S11. BR signaling was hyperactivated in N390-overexpressing *Arabidopsis* plants. A. The axillary branches of plants overexpressing N390-YFP or OsBSK3-YFP were kinked. Upper panel: The level of expressed protein as determined using anti-GFP antibodies. The numbers represent independent transgenic lines. **B** and **C**. Quantitative analysis of the hypocotyl of seedlings grown in the presence of eBL (B) and BRZ (C). Seedlings were grown on 1/2 MS medium with or without the indicated concentration of eBL in the light or 1 mM BRZ in the dark for 1 week. Each value represents the average of at least 45 individual seedlings from three biological repeats. Error bars represent the SE. A one-way ANOVA was performed. Statistically significant differences are indicated by different lowercase letters ($P<0.05$).

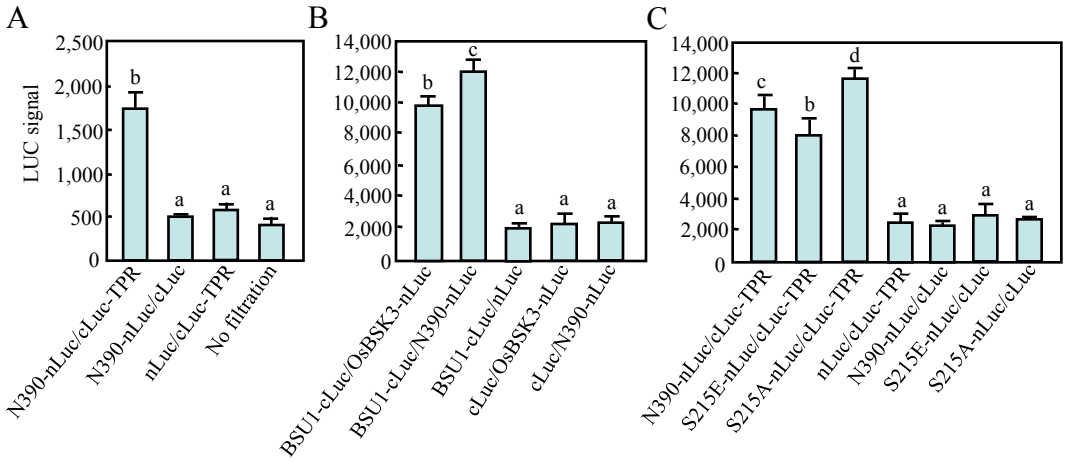

Figure S12. Quantitative analysis of the interaction between the kinase and TPR domains of OsBSK3 and AtBSU1. Agrobacteria transformed with the indicated split luciferase constructs were co-infiltrated into *N. benthamiana* leaves. Complemented luciferase activity was measured 36–48 h after infiltration. S215E and S215A represent the mutated kinase domain of OsBSK3 (N390). All experiments were repeated at least three times with consistent results. Representative results are shown. A one-way ANOVA was performed. Statistically significant differences are indicated by different lowercase letters ($P<0.05$).

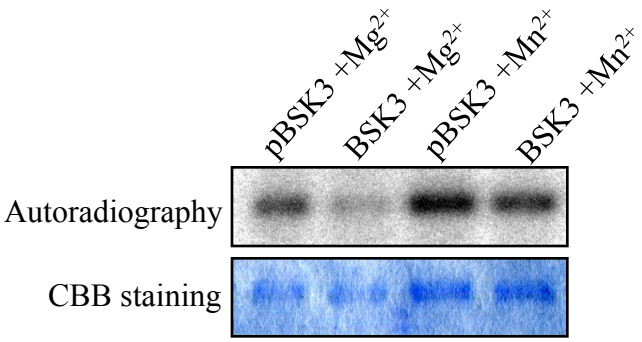

Figure S13. Assay for OsBSK3 autophosphorylation. Recombinant OsBSK3-6His protein was incubated with glutathione-bound GST-OsBRI1 in the presence (pBSK3) or absence (BSK3) of ATP. OsBSK3 was collected and used for *in vitro* autophosphorylation assays in the presence of 10 mM Mg^{2+} or Mn^{2+}. Total protein staining with Coomassie blue is shown as an equal loading

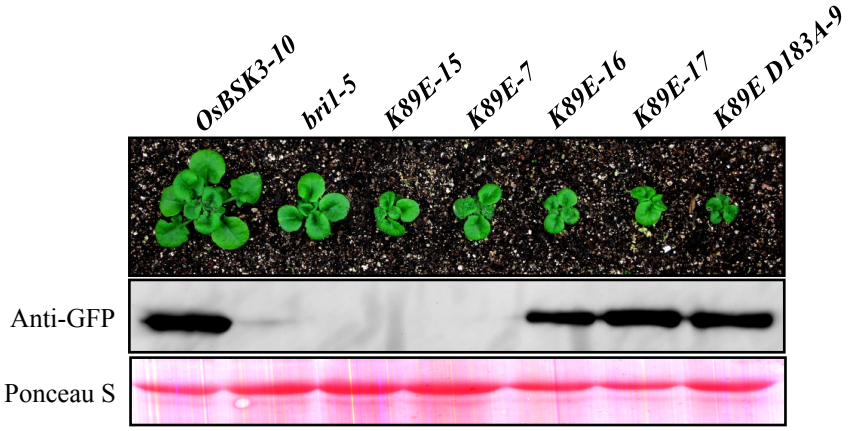

Figure S14. Overexpression of the K89E or K89E D183A forms of OsBSK3 could not rescue *bri1-5* mutant plants. Three-week-old *bri1-5* mutant plants or *bri1-5* plants overexpressing wild-type or the K89E or K89E D183A mutant forms of OsBSK3-YFP are shown (T_1 generation). OsBSK3 expression in the transgenic plants was detected using anti-GFP antibodies.