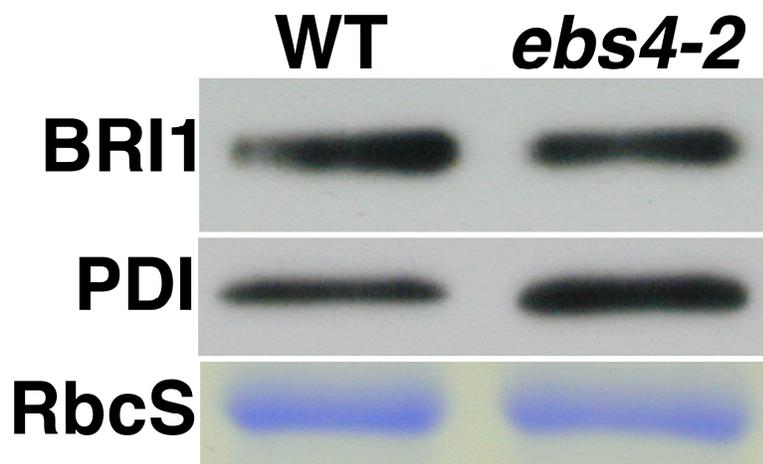


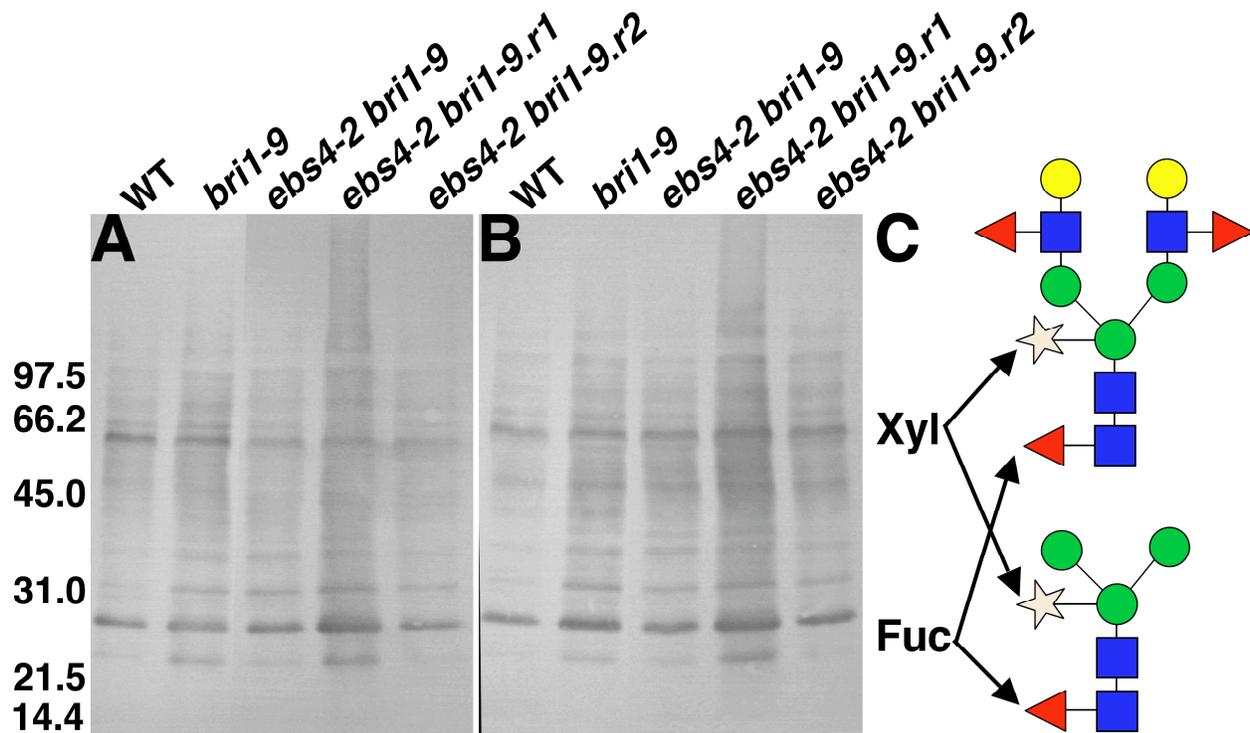
Supplemental Data, Hong et al., (2009) Mutations of an  $\alpha$ 1,6 mannosyltransferase inhibit endoplasmic reticulum-associated degradation of defective brassinosteroid receptors.



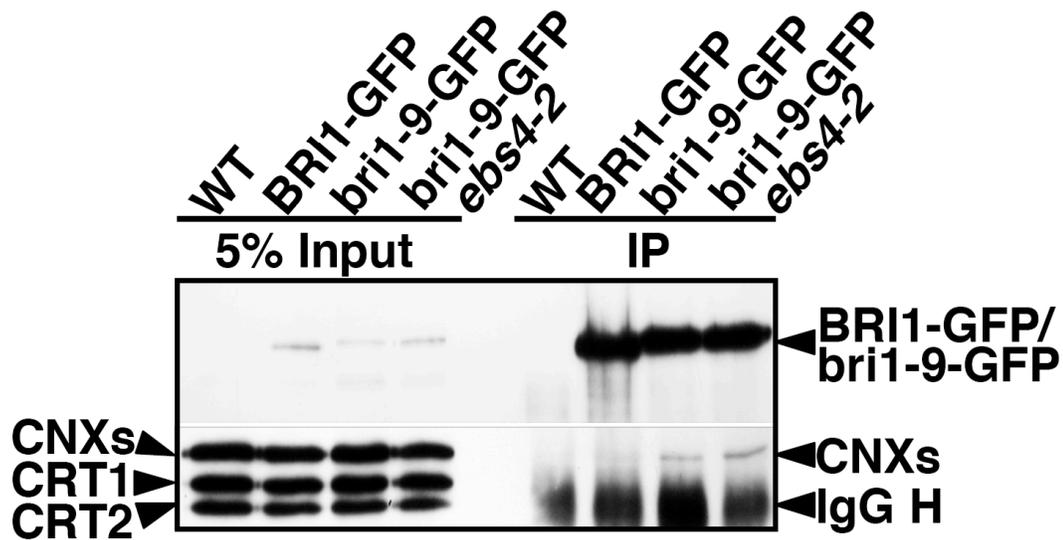
**Supplemental Figure 1. Overexpression of *bri1-9*:GFP can Suppress the Dwarf Phenotype of *bri1-9*.** Shown here from left to right are 4-week-old soil-grown plants of wild-type, *bri1-9*, and 4 independent transgenic *bri1-9* lines expressing the *pBRI1:bri1-9-GFP* transgene.



**Supplemental Figure 2. The *ebs4-2* Mutation has Little Effect on the Molecular Weight of the Wild-Type BRI1.** Total protein extracts of 3-week-old seedlings of wild-type and *ebs4-2* were extracted in 2 X SDS buffer, separated by 7% SDS-PAGE, and analyzed by immunoblotting with an anti-BRI1 or anti-PDI antibody. Coomassie staining of RbcS serves as the loading control.



**Supplemental Figure 3. The *ebs4-2* Mutation Has No Effect on the C-Type N-Glycan Biosynthesis.** Total proteins were extracted from lyophilized leaf samples of 4-week-old soil-grown plants of wild-type, *bri1-9*, *ebs4-2 bri1-9*, and two independent *gEBS4*-rescued *ebs4-2 bri1-9* lines using the phenol extraction method (Fitchette et al., 1999), resolved in 1 X SDS sample buffer, separated by SDS-PAGE, and analyzed by immunoblotting with antibodies made against  $\beta$ 1,2-xylose (**A**) or  $\alpha$ 1,3-fucose (**B**). Equal amounts of total proteins were loaded onto each lane for both experiments. (**D**) Structure of plant complex-type glycans containing  $\beta$ 1,2-xylose and  $\alpha$ 1,3-fucose residues. Blue square indicates N-acetylglucosamine, green circle represents mannose, red triangle denotes  $\alpha$ 1,3-fucose, star symbolizes  $\beta$ 1,2-xylose, and yellow circle designates  $\beta$ 1,4-galactose.



**Supplemental Figure 4. The *ebs4-2* Mutation Does not Inhibit the *bri1-9*-CNX Interaction.**

0.4 g of 3-week-old seedlings of wild-type, *pBRI1:BRI1-GFP*, *pBRI1:bri1-9-GFP*, and *pBRI1:bri1-9-GFP ebs4-2* transgenic lines were ground in liquid N<sub>2</sub> and extracted with the extraction buffer (50 mM Tris pH 8.0, 100 mM NaCl, 5 mM EDTA, 0.2% Triton X-100, 10% glycerol, and protease inhibitors). After centrifugation, the clear supernatant was incubated for 1 h with a polyclonal anti-GFP antibody (TP401, Torrey Pines Biolabs) and protein A-agarose for 1 additional h. The resulting immunoprecipitates were washed with the extraction buffer, separated by SDS-PAGE, and analyzed by immunoblotting using a monoclonal anti-GFP antibody (MMS-118P, Covance) (upper panel) or anti-maize-CRT antibody that detects Arabidopsis CNXs, CRT1, and CRT2 (indicated by arrows on the left) (lower panel). The left 4 lanes contain 5% of total protein extracts used for the coimmunoprecipitation experiment while the right 4 lanes contain 50% of total immunoprecipitates. The positions of BRI1-GFP, bri1-9-GFP, CNXs, and the heavy chain of IgG molecules were also indicated by arrows.