Supporting Information

Ye et al. 10.1073/pnas.1205232109

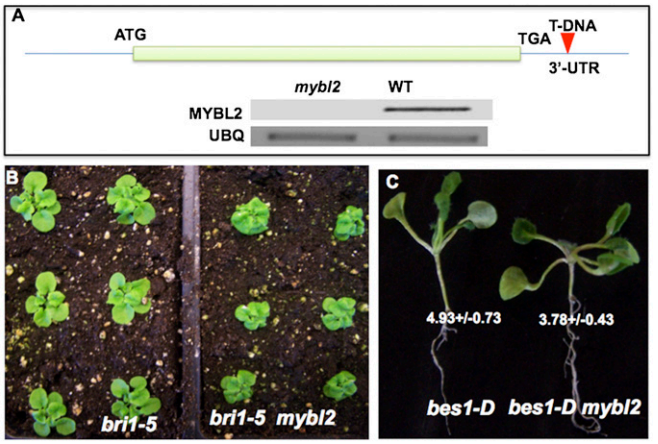

Fig. S1. MYBL2 T-DNA insertion mutant. (*A*) Schematic representation of T-DNA knockout allele of *MYBL2 gene*. *MYBL2* expression is not detected by RT-PCR in the T-DNA mutant. (*B*) *mybl2* suppressed *bes1-D* phenotype at seedling stage. (*C*) Two-week-old seedlings of *bes1-D* and *bes1-D mybl2* double mutants are shown. The average hypocotyl length and SDs are indicated (n = 10).

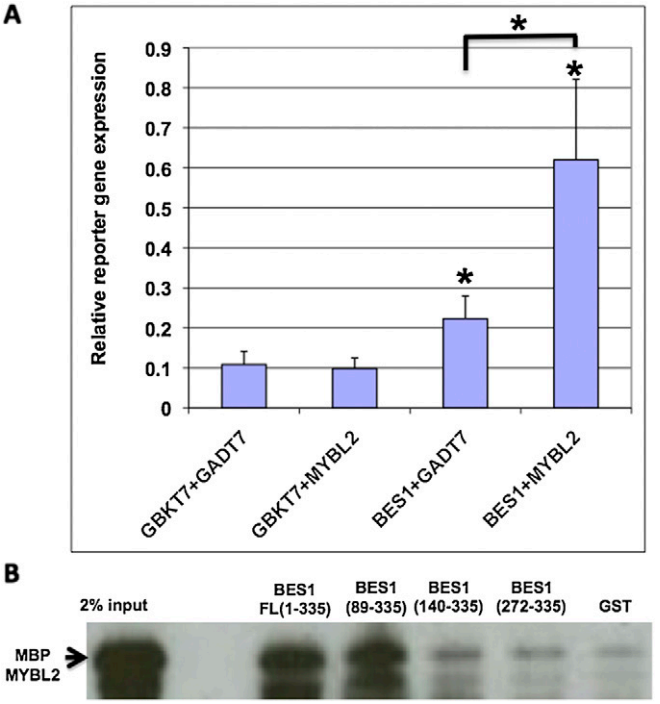

Fig. S2. BES1 interacts with MYBL2 in yeast and in vitro. (*A*) β-Galactosidase (LacZ) activity was detected with ortho-nitrophenyl-β-galactoside in a quantitative liquid-culture assay to test the protein–protein interaction in yeast two-hybrid experiment. (*B*) GST pull-down experiments using MBP-MYBL2 and GST-tagged full-length or different truncated BES1. MYBL2 was detected by Western blotting with anti-MBP antibody.

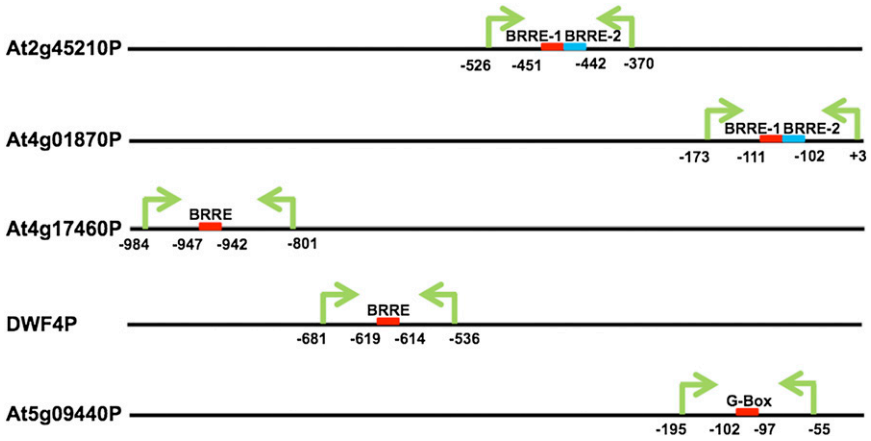

Fig. S3. BRRE or G-box in BR-repressed BES1 target gene promoters. The BRRE and G-box (both are enriched in BR-repressed BES1/BZR1 target genes) are shown in the promoters used for ChIP-PCR analysis with BES1 and MYBL2. The numbers indicate nucleotide positions relative to transcription start sites.

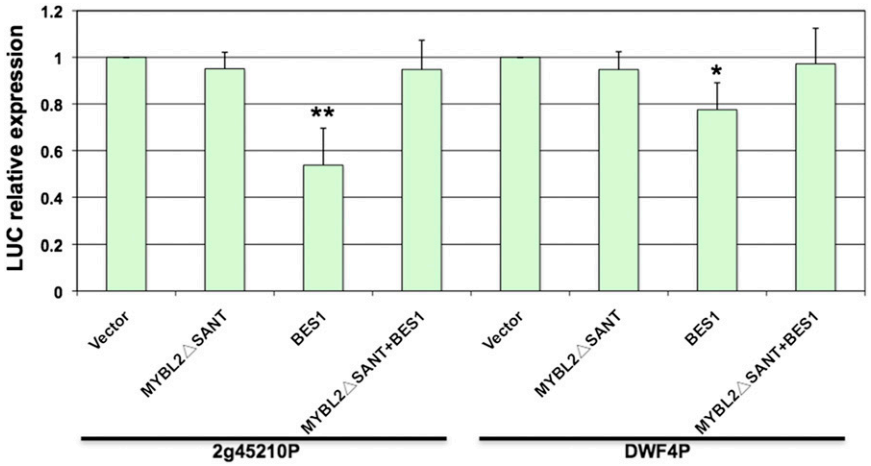

Fig. S4. BES1 and MYBL2△SANT did not have synergistic repression effect on BR-repressed genes. Transient gene expression assays were performed in tobacco leaves with At2g45210-LUC and DWF4-LUC reporter genes cotransfected with BES1 and/or MYBL2△SANT via *Agrobacterium*. The relative expression levels were normalized with total protein. The average and SDs were from three biological repeats. The significant difference was analyzed by Student's *t* test (*<0.05, **<0.01).

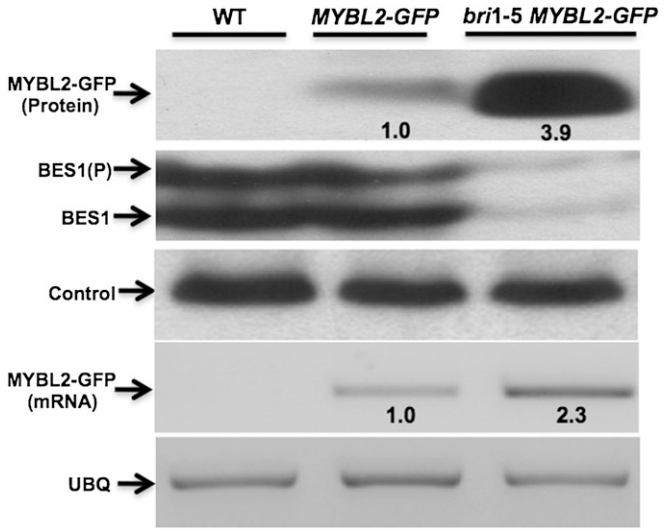

Fig. S5. MYBL2 protein accumulated in *bri1-5* background. MYBL2-GFP protein and mRNA level were examined in WT and *bri1-5* background. The numbers indicated the amount of MYBL2-GFP protein or transcripts quantified using Alphalmager 3400.

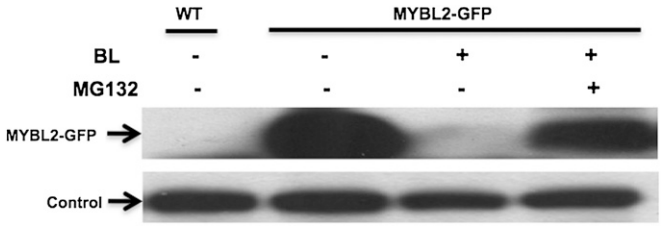

Fig. S6. The reduced MYBL2 protein level caused by BL was reversed by proteasome inhibitor MG132. The plant samples were treated without BL, with 1 μM BL, and with both BL and MG132 (30 μM) for 4 h and used to prepare protein to detect MYBL2 (*Top*) and a control protein (*Bottom*).

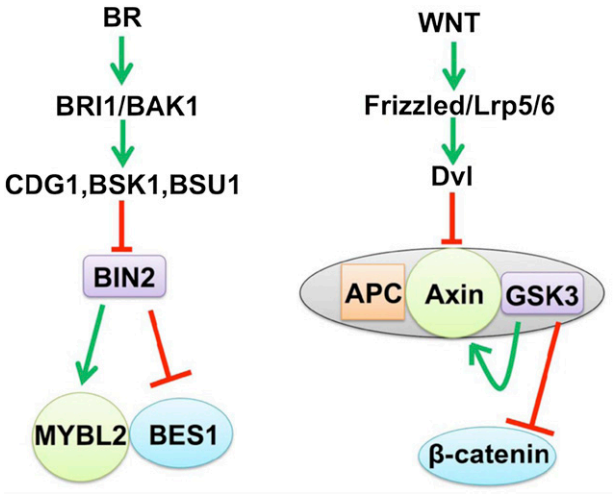

Fig. S7. BR and WNT signaling pathways. The regulation of BES1 and MYBL2 by BIN2 phosphorylation in the BR pathway is similar to the regulation of β-catenin and Axin by GSK3 kinase in the WNT pathway, despite the fact that the substrates in each pathway do not have any similarities in protein sequences.

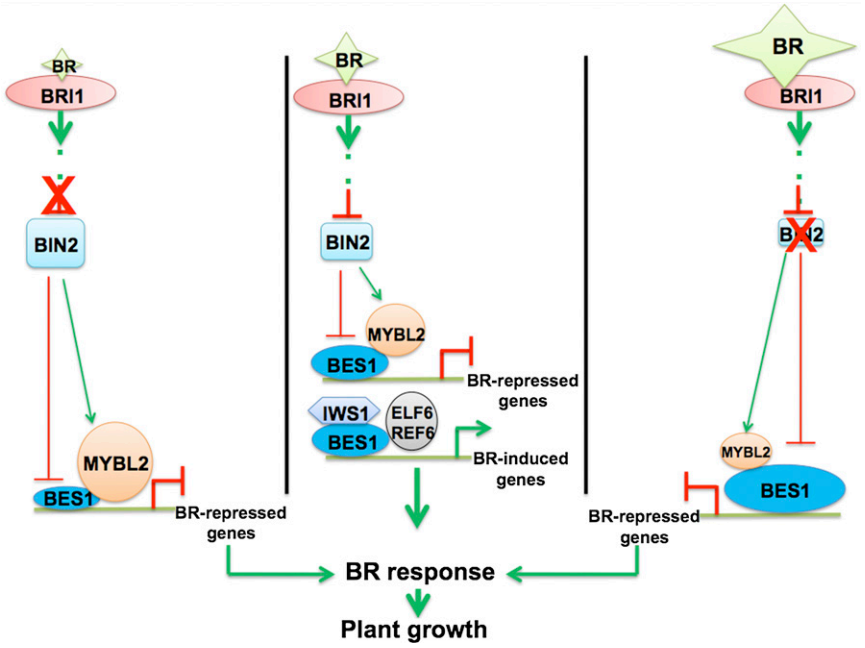

Fig. S8. A model for MYBL2 function in the BR pathway. Under optimal conditions, the plants respond to BR signaling and maintain balanced levels of BES1 and MYBL2 to regulate BR-repressed genes (*Center*). However, when BR level and signaling is reduced, BES1 function will be reduced by increased BIN2 phosphorylation (*Left*). In the meantime, MYBL2 protein level is increased due to the increased MYBL2 transcript by reduced BES1 and stabilization of MYBL2 by BIN2 phosphorylation. The increased MYBL2 protein likely compensates for the reduced BES1 in BR-repressed gene expression. On the other hand, if the concentration of BR is increased in plants (*Right*), BES1 protein will accumulate and MYBL2 will be reduced to balance the BR-repressed gene expression. The increased BES1 leads to decreased MYBL2 transcript level, and dephosphorylation of MYBL2 destabilizes the protein. The reduced MYBL2 protein level will alleviate the transcription repression caused by increased BES1.

Table S1. Primer sequences used in the study

	Sequence
Genotyping	
mybl2LP	GAGATGTCGATTGAGAGGTCG
mybl2RP	GCTGTATTAGCTATAATTTCTTACAG
Transgenic plant	
gMYBL2NAsp718	CGCGGTACCTCTTATATGATTTTGGAGTAGATGGTAAGTGAG
gMYBL2CSalI	CGCGTCGACTCGGAATAGAAGAAGCGTTTCTTGACCTG
Yeast two hybrid	
MYBL2AD7NEcoR1	GCGGAATTCATGAACAAAACCCGCCTTCGTGC
MYBL2AD7CXho1	GCGCTCGAGTCATCGGAATAGAAGAAGCGT
MYBL2AD7D1NEcoR1	GCGGAATTCAAGATTATTAGTGATCAATC
MYBL2AD7D2NEcoR1	GCGGAATTCAGTCATTTGCCTGACCTAAACA
MYBL2BDNEcoR1	CGCGAATTCAAACAACGCAACTTCTCAAAAGATG
MYBL2BDCSal1	CGCGTCGACTCACCTTTTTAGGTAAGTTTCCCAAT
Protein expression	
MYBL2MBPNEcoR1	GCCGAATTCGATGAACAAAACCCGCCTTCGTGC
MYBL2MBPMBPCAsp718	CGCGGTACCTCATCGGAATAGAAGAAGCGT
BiFC	
cMYBL2NAsp718	CGCGGTACCTCAACCCACCAGTCCAAGTCAAACCTCCTC
gMYBL2CSalI	CGCGTCGACTCGGAATAGAAGAAGCGTTTCTTGACCTG
Gene expression	
MYBL2RTF	ATAGTACTAGTACCGGACGAAGTC
MYBL2RTR	CAAACATCGTTATACCATCTCTCTAGTG
2G45210RTF	CTGTCATAGAGTTTTGGTACCCATC
2G45210RTR	CGAAATCTGAATACAGACAAGGAAT
4G01870RTF	CATGTGAGTTTCAATAAAGATGGTG
4G01870RTR	CGTCTAATTTCACAACGTACAAATC
4G17460RTF	AGAAGCTAGGTTTAACAGCAAGACA
4G17460RTR	CTTCCGTTAATTTCTCAACACATCT
5G09440RTF	ATTATAAACATCGCGACTCTTCTTG
5G09440RTR	CACTCGTCTTGTCTACGAGAACC
DWF4RTF	GAAATGTAGTTAGGTTTTTGCATCG
DWF4RTR	GAGATTAGGTTGGTCATAACGAGAA
ChIP qPCR	
MYBL2Chip1F	TGTGGGACCAATTAACAAGG
MYBL2Chip1R	GATGGCTTGAGGAGGTTTGA
MYBL2Chip2F	ATGGTAAGTGAGATAGGGAAGTGG
MYBL2Chip2R	GGTTCAGGAACAGATAAGGGAGA
MYBL2Chip3F	CGATAACCGCTGCTTATTTGA
MYBL2Chip3R	TGTAGTTTGGAGGAAAATGAACA
Transient expression	
2g45210PFBamH1	CGCGGATCCTCATCAACGTACACAAGTAACGCAACTAG
2g45210PRHind3	CGCAAGCTTCTTCTTATAGCTAACTTTAAAAAACAG
DWF4FBamH1	CGCGGATCCTGGAATGGAAGTAGTAATATACATTAAGC
DWF4REcoR1	CGCGAATTCGGAGCTAGTTTCTCTCTCTCTCTCACTCAC
MYBL2DENAsp	CGCGGTACCATGAAGATTATTAGTGATCAATC
MYBL2DECSal	CGCGTCGACTCATCGGAATAGAAGAAGCGT