Supplementary material

Arabidopsis heterotrimeric G protein β subunit, AGB1, regulates brassinosteroid signaling independently of BZR1

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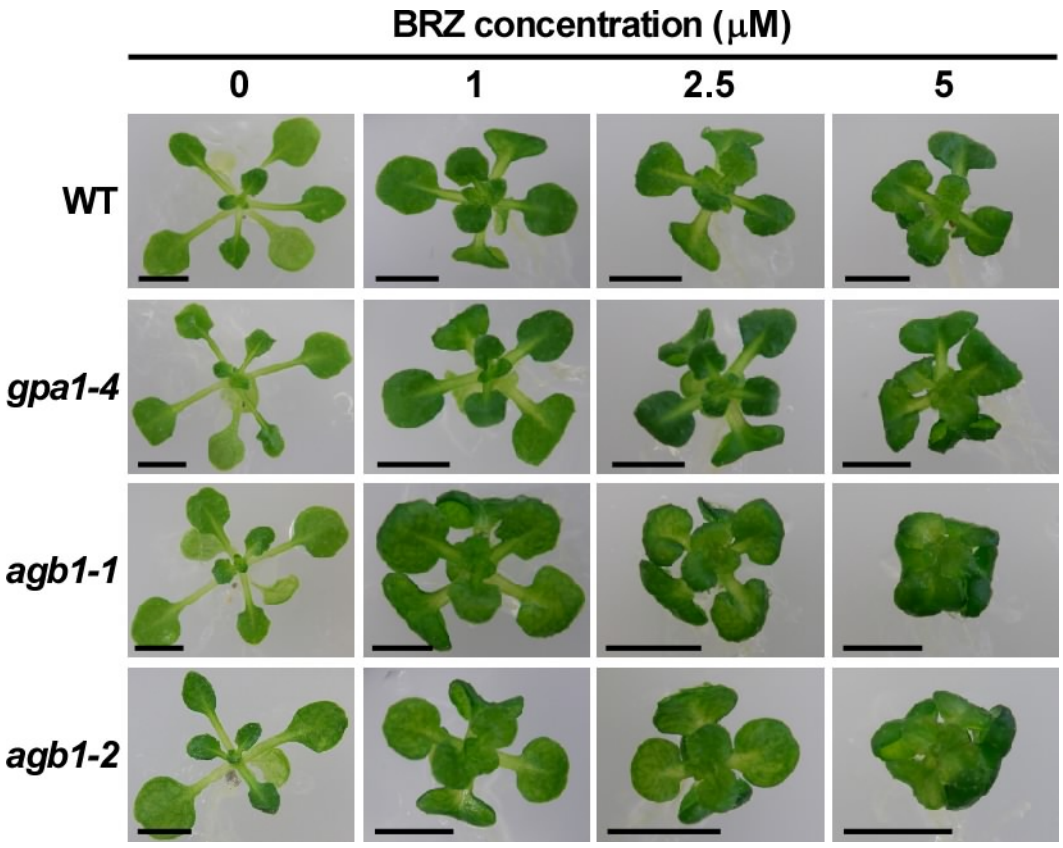
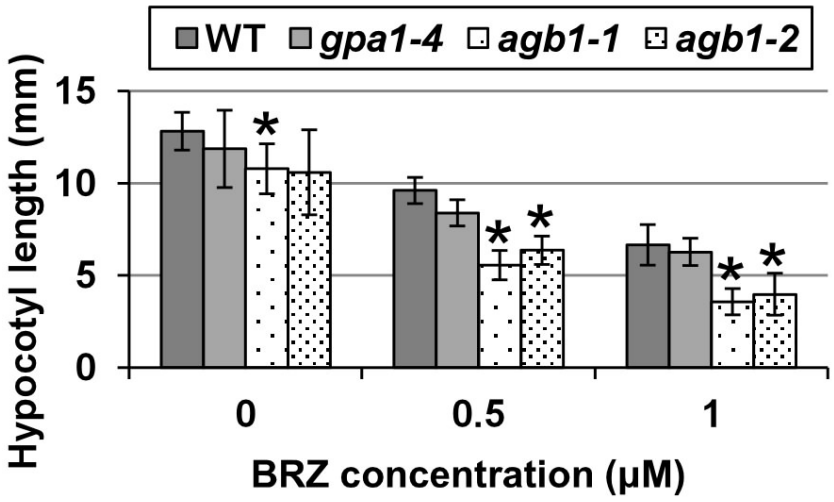
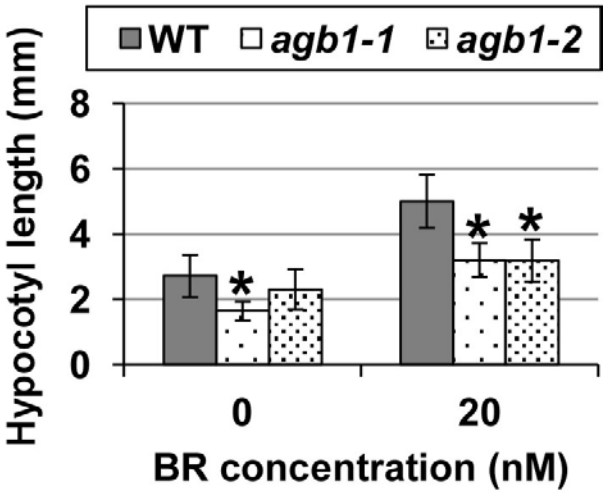
Supplementary figures



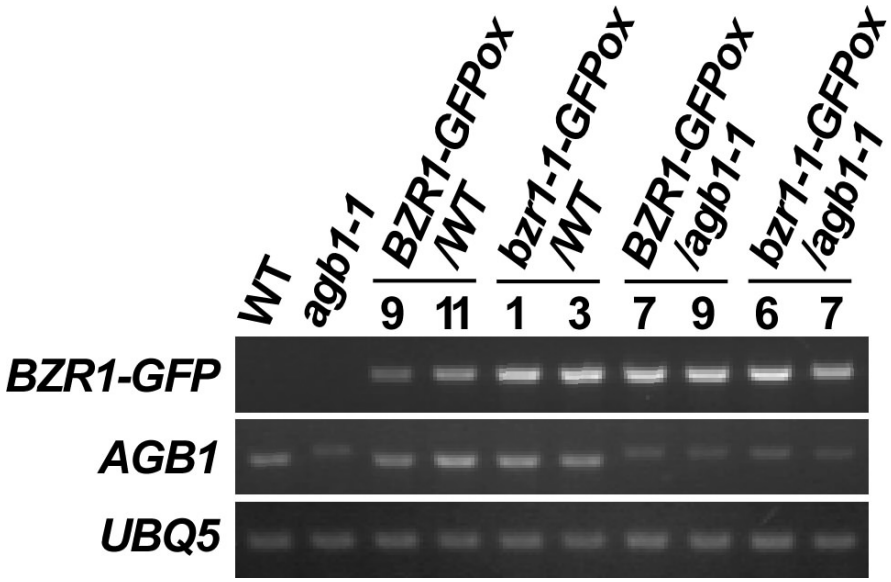
Supplementary Fig. S1. Phenotypes of *agb1* grown in different concentrations of BRZ. Plants were grown for 20 days in the presence of the indicated concentrations of BRZ. Scale bars = 1 cm.



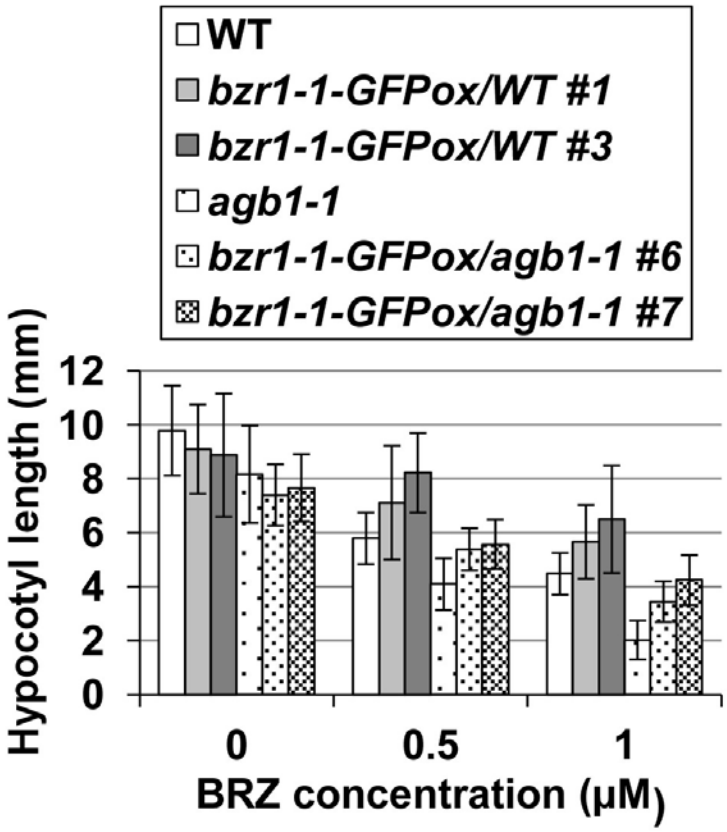
Supplementary Fig. S2. Absolute hypocotyl lengths of plants grown in the presence of BRZ. Hypocotyl lengths for calculating relative hypocotyl lengths in Fig. 1B are shown. Values are means ± SE (n = 11-18). *: $P < 0.05$ vs. the WT by Student's *t*-test.



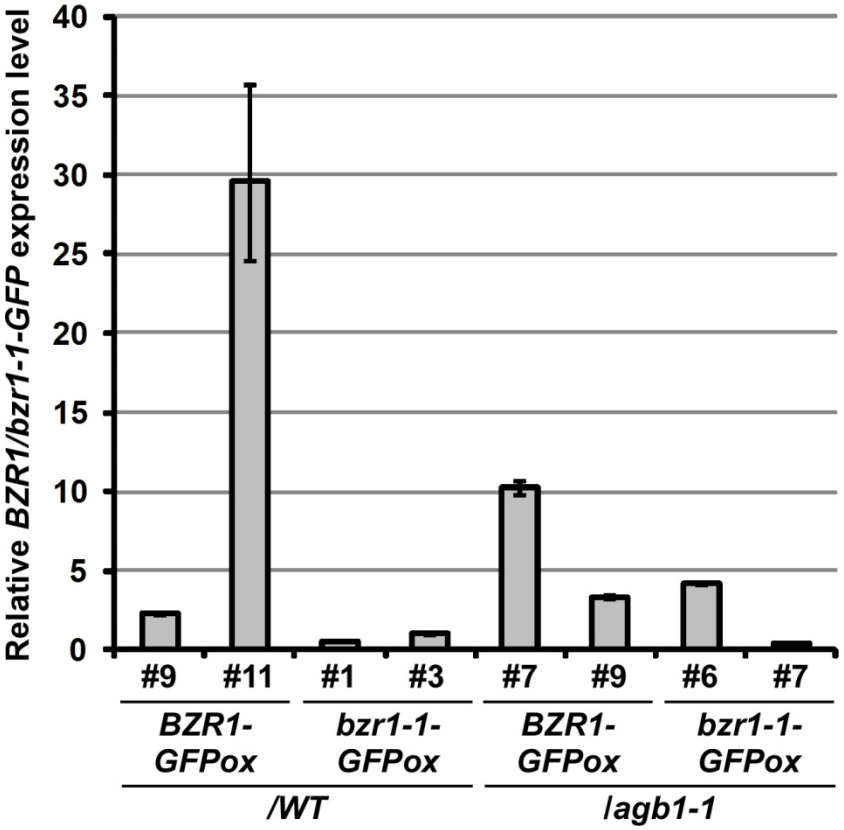
Supplementary Fig. S3. BR-induced hypocotyl elongations in *agb1*. Plants were grown under a 16-h light/8-h dark photoperiod for 15 days in the presence of 0 or 20 nM BR, and their hypocotyl lengths were measured. Values are means ± SE (n = 20-24). *: $P < 0.05$ vs. WT by Student's *t*-test.



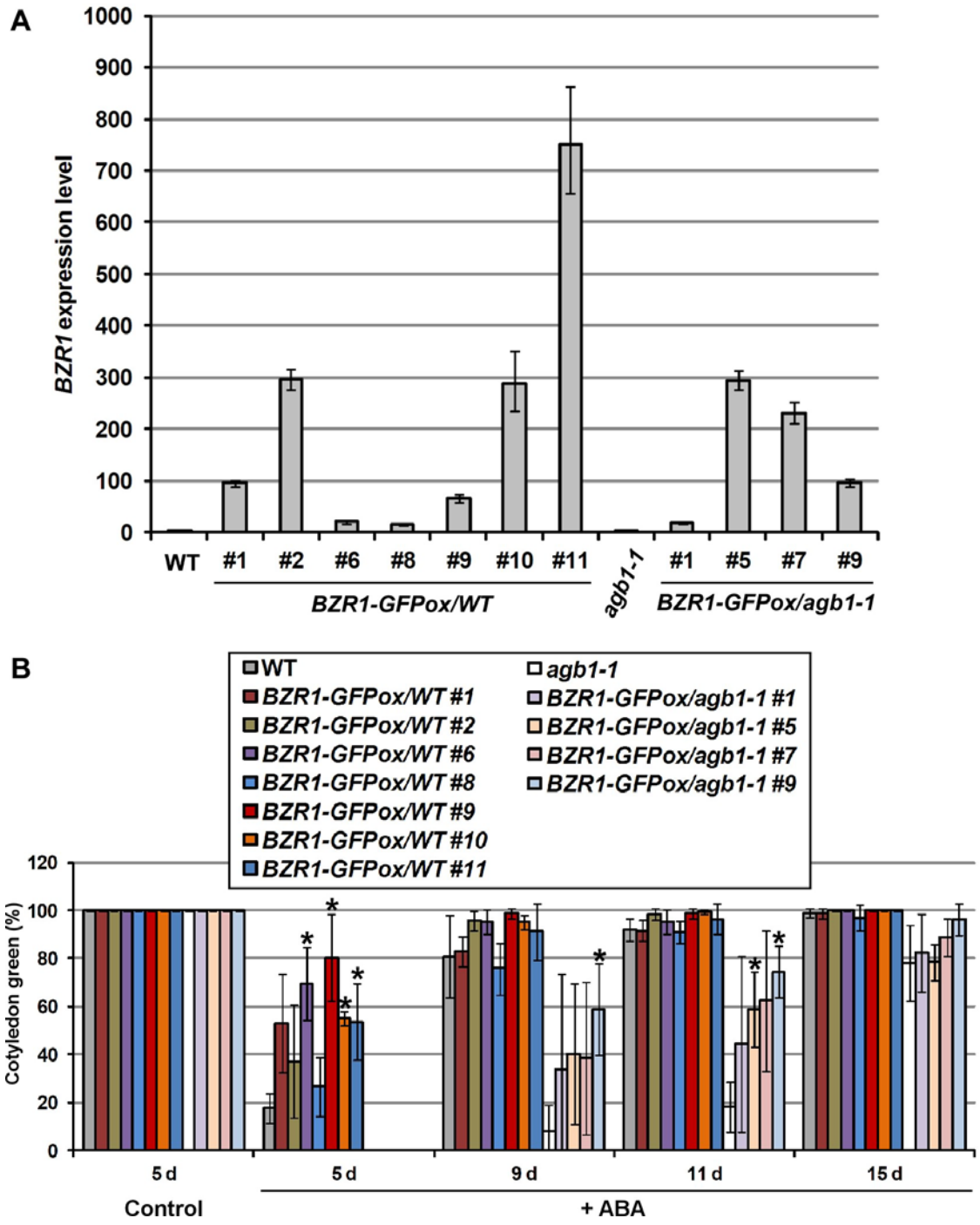
Supplementary Fig. S4. Semi-quantitative RT-PCR analysis of *BZR1-GFP* and *bzr1-1-GFP* expressions. Plants were grown for 20 days and sampled for RNA preparation. Transgenic lines used for the analysis are shown as numbers. The same primer pair was used to detect both *BZR1-GFP* expression and *bzr1-1-GFP* expression, thus the expressions of *BZR1-GFP* and *bzr1-1-GFP* are both shown as *BZR1-GFP*. *AGB1* transcripts were larger in size and lower in level in *agb1-1*-background lines than in WT-background lines, which is consistent with a previous report (Lease *et al*., 2001).



Supplementary Fig. S5. Absolute hypocotyl lengths of plants grown in the presence of BRZ. Hypocotyl lengths for calculating relative hypocotyl lengths in Fig. 2A are shown. Values are means ± SE (n = 15-24). *: $P < 0.05$ vs. the WT by Student's *t*-test.

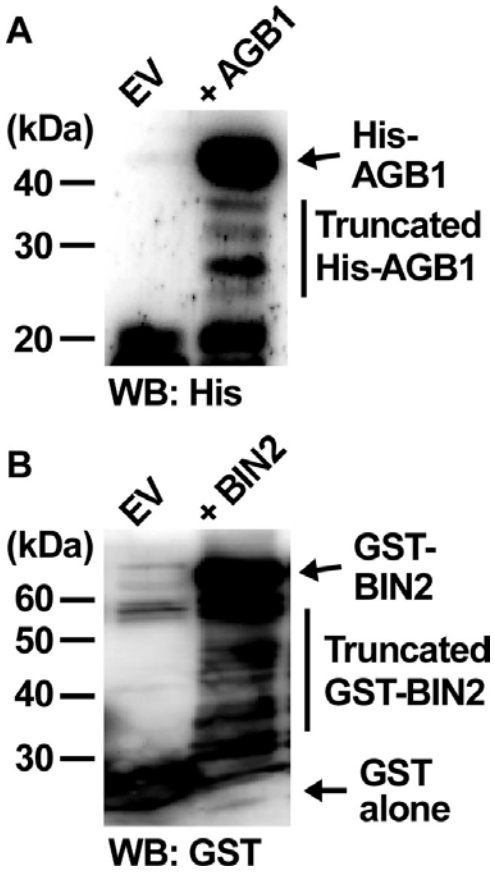


Supplementary Fig. S6. Expression levels of *BZR1-GFP* and *bzr1-1-GFP* (*BZR1/bzr1-1-GFP*) in transgenic plants. Relative expression levels of were calculated by the comparative C_T method using *UBQ5* as an internal control gene and *bzr1-1-GFPox/WT* #3 sample as a reference sample. Experiments were performed in triplicate. Values are means ± SE.

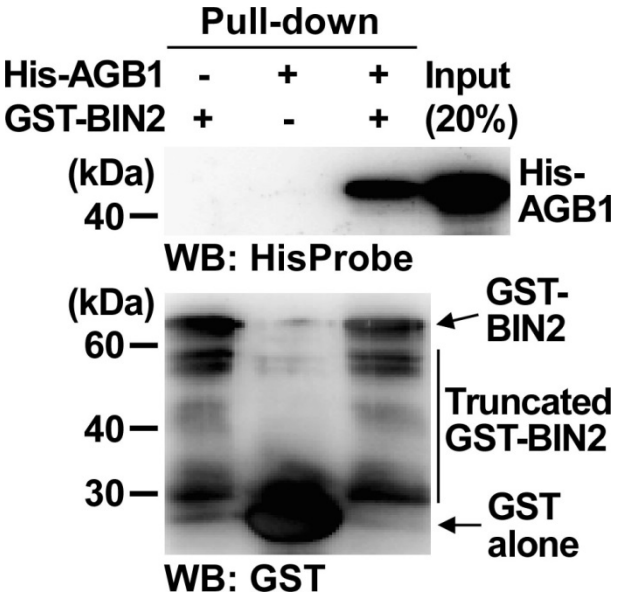


Supplementary Fig. S7. *BZR1* expression levels in *BZR1-GFP*-overexpressing lines and their responses to ABA. (A) Quantitative RT-PCR analysis of *BZR1* expression levels in *BZR1*-overexpressing lines. Relative *BZR1* expression levels were calculated by the comparative C_T method using *UBQ5* as an internal control gene and WT sample

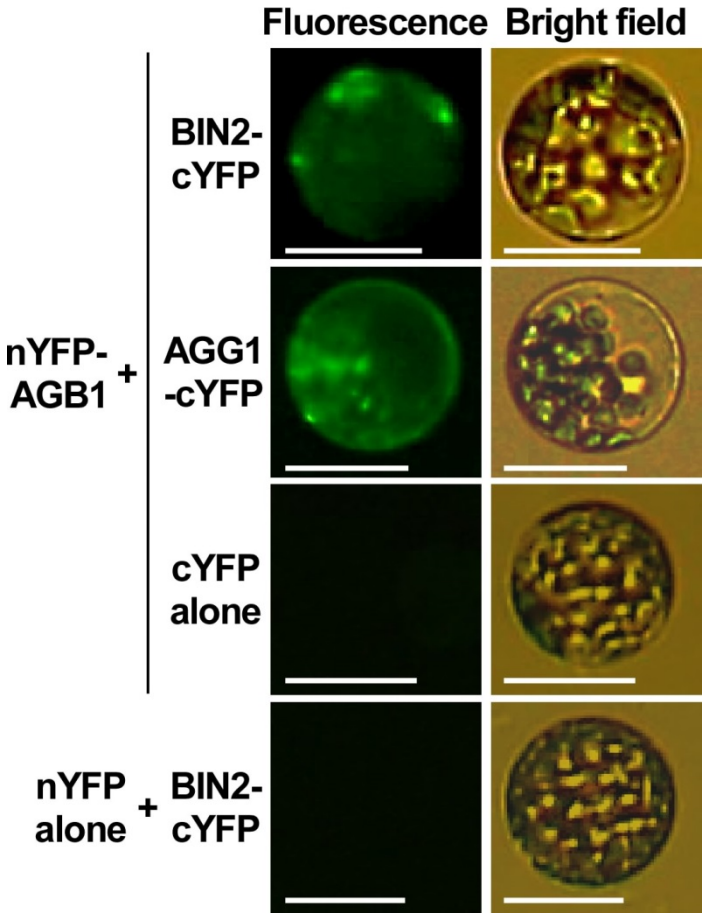
as a reference sample. Experiments were performed in triplicate. Values are means ± SE. (B) Plants were grown in the presence of 0 (Control) or 1 μM ABA (+ ABA). Green cotyledons were scored at the indicated time points. More than 30 plants were used for scoring green cotyledons in each genotype. Experiments were performed in triplicate. Values are means ± SE. *: $P < 0.05$ vs. non-transgenic lines by Student's *t*-test.



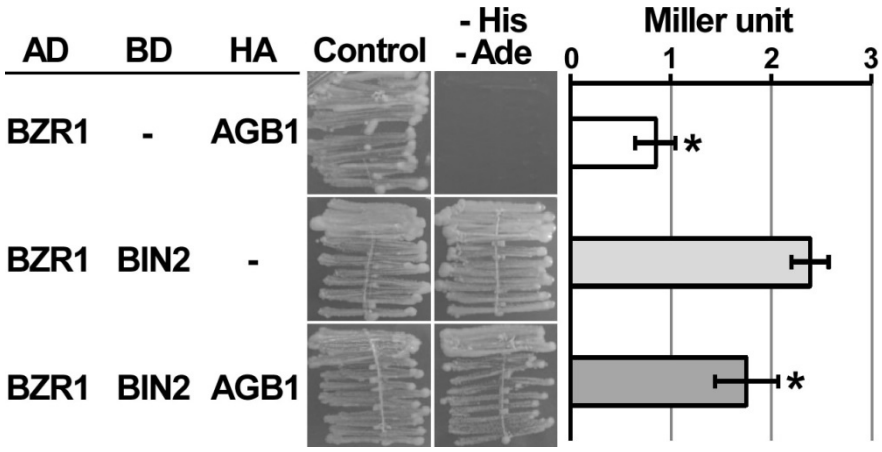
Supplementary Fig. S8. Expressions of His-AGB1 and GST-BIN2 in *E. coli*. (A) Crude extracts of *E. coli* transformed with empty vector (EV) or a construct to express hexahistidine-tagged AGB1 (+ AGB1) were subjected to Western blotting using a polyhistidine probe, HisProbe-HRP (WB: His). (B) Crude extracts of *E. coli* transformed with empty vector (EV) or a construct to express GST-fused BIN2 (+ BIN2) were subjected to Western blotting using an anti-GST antibody (WB: GST).



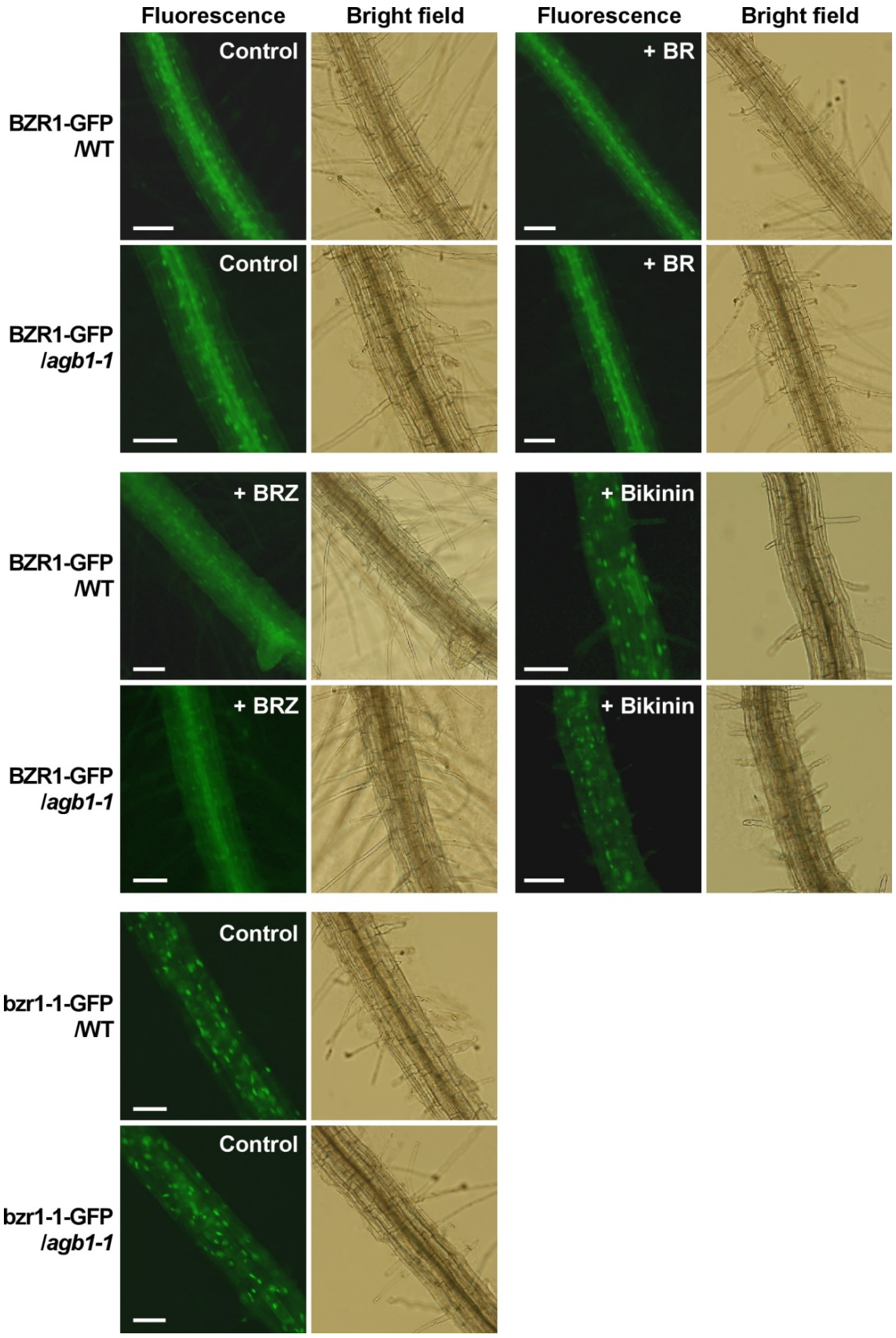
Supplementary Fig. S9. In vitro GST pull-down assay. Hexahistidine-tagged AGB1 (His-AGB1) and GST-fused BIN2 (GST-BIN2) were expressed in *E. coli* and used for the analysis. For His-AGB1-, 250 mM imidazole was used instead of a solution containing purified His-AGB1. For GST-BIN2-, GST alone was use instead of GST-BIN2. His-AGB1 was analyzed by Western blotting using a polyhistidine probe, HisProbe-HRP (WB: HisProbe). Input His-AGB1 (20% of the amount used for the pull-down assay) is shown as Input (20%) for control. GST-BIN2 in the same protein samples was analyzed by Western blotting using an anti-GST antibody (WB: GST) for control. Some truncated forms of GST-BIN2 were detected, probably due to protein degradation and/or unexpected translational termination. Experiments were performed in triplicate, and a representative result is shown.



Supplementary Fig. S10. Bimolecular fluorescence complementation between AGB1 and BIN2. The ORF of *AGB1* was cloned in frame behind the coding sequence of the N-terminal region of YFP (nYFP) to express nYFP-fused AGB1 (nYFP-AGB1), and the ORFs of *AGG1* and *BIN2* were cloned in frame in front of the coding sequence of the C-terminal region of YFP (cYFP) to express cYFP-fused AGG1 and cYFP-fused BIN2 (AGG1-cYFP and BIN2-cYFP, respectively). The proteins were co-expressed in the indicated combinations in Arabidopsis mesophyll protoplasts. More than 20 cells were observed and a representative cell is shown for each combination. Scale bars = 20 μm.



Supplementary Fig. S11. Effects of AGB1 on the interaction between BIN2 and BZR1 in a yeast three-hybrid system. The yeast reporter strain, AH109, was transformed with pGADT7-Rec containing *BZR1* to express GAL4 activation domain (AD)-fused BZR1, and pBridge containing either or both of *BIN2* and *AGB1* to express GAL4 DNA-binding domain (BD)-fused BIN2 and HA-tagged AGB1, respectively. The combinations of the expressed proteins are shown in the left table. Transformed cells were grown on SD medium (Control) and SD medium lacking histidine and adenine (-His - Ade) (middle panels). For each transformed cell line, four individual colonies that had appeared after transformation were tested, and representative results are shown. The activities of β-galactosidase in the transformed lines were quantified and shown as Miller units (right panel). For each transformed cell line, 4-8 individual colonies were used for the β-galactosidase assay. Values are means ± SE. *: $P < 0.05$ vs. the cell line expressing BZR1, BIN2, and AGB1 by Student's *t*-test.


Fluorescence
Bright field
Fluorescence
Bright field
Control
+ BR
BZR1-GFP /WT
Control
+ BR
BZR1-GFP /agb1-1
+ BRZ
+ Bikinin
BZR1-GFP /WT
+ BRZ
+ Bikinin
BZR1-GFP /agb1-1
Control
bzr1-1-GFP /WT
Control
bzr1-1-GFP /agb1-1

Supplementary Fig. S12. Subcellular localizations of BZR1-GFP and bzr1-1-GFP in *agb1*. *BZR1-GFPox/WT* #9 and *BZR1-GFPox/agb1-1* #9 were grown under a 16-h light/8-h dark photoperiod for 15 days in the presence of 20 nM BR (+ BR), 2.5 μM BRZ (+ BRZ), or 40 μM bikinin (+ Bikinin), or in the absence of BR, BRZ, or bikinin (Control). *bzr1-1-GFPox/WT* #1 and *bzr1-1-GFPox/agb1-1* #6 were grown under a 16-h light/8-h dark photoperiod for 15 days under normal growth conditions (Control). GFP fluorescence in a root is shown in each panel. Scale bars = 100 μm.

Supplementary Table S1. Primer pairs used for RT-PCR analyses

Gene		Primer sequence (5' > 3')
BZR1-GFP	Fw	TCGCCACCAGTTTCATACCC
	Rv	GTAGGTGGCATCGCCCTCGC
BZR1	Fw	TCGCCACCAGTTTCATACCC
	Rv	TCCTCTAGAACCACGAGCCTTCCCATTTCC
AGB1	Fw	AGACGCCTCCAGCTCCTCGA
	Rv	GCACTTCCATCTGCTGACAACCCC
RAB18	Fw	TCCAGCTCTAGCTCGGAGGATGA
	Rv	GGATCCCATGCCGCCCATCG
RD29A	Fw	GCCGGAATCTGACGGCGGTT
	Rv	CCCGTCGGCACATCCTTGTCG
CPD	Fw	GGGCCAAGGCTATGTCCCGG
	Rv	ACGGCGCTTCACGAAGATCGG
DWF4	Fw	ACCGGTGATCTCAGCCGTACA
	Rv	TTGGCCCTCCTCCAAACGGC
UBQ5	Fw	GACGCTTCATCTCGTCC
	Rv	CCACAGGTTGCGTTAG