## Supplementary file

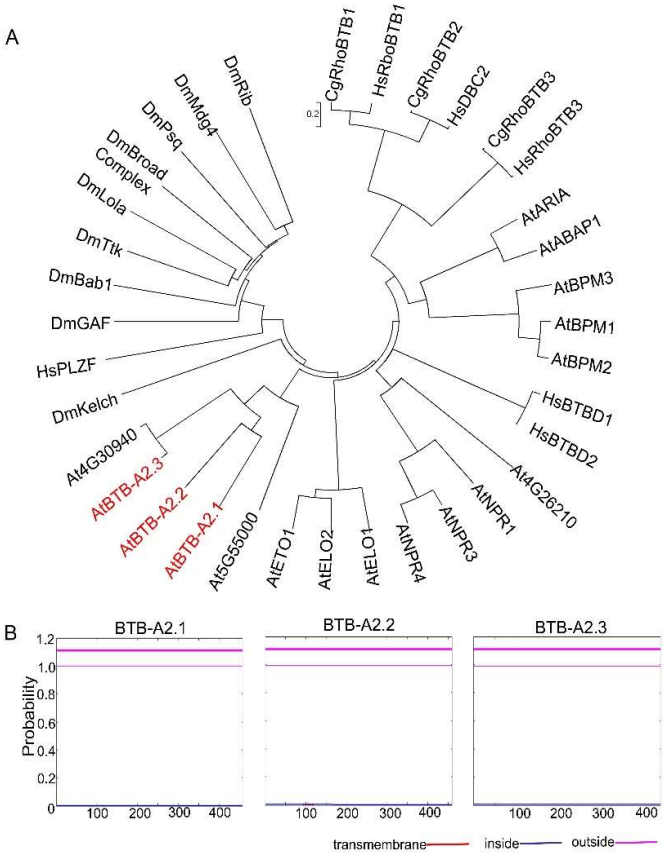


**Figure S1.** Sequence alignment of representative BTB proteins in animals and plants and transmembrane prediction of *Arabidopsis* BTB-A2.1, BTB-A2.2, and BTB-A2.3. (**A**) Phylogenetic relationship of representative BTB-A2 proteins in animals and plants. DmRib (NP_001261084), DmMdg4 (NP_788698), DmPsq (NP_523686), DmBroad Complex (NP_726750), DmLola (NP_788312), DmTtk (P17789), DmBab1 (NP_728565), DmGAF (Q08605), HsPLZF (NP_001018011), DmKelch (NP_724095), AtETO1 (At3g51770), AtEOL2 (At5g58550), AtEOL1 (At4g02680), AtNPR1 (At1g64280), AtNPR2 (At4g26120), AtNPR3 (At5g45110), AtNPR4 (At4g19660), AtARIA (At5g19330), AtABAP1 (At5g13060), AtBTB-A2.1 (At5g41330), AtBTB-A2.2 (At3g09030), AtBTB-A2.3 (At2g24240), AtBPM1 ( AT5G19000 ), AtBPM2 (At3g06190), AtBPM3 (At2g39760), HsRhoBTB3 (NP055714), HsDBC2 (NP003400), HsRboBTB1 (AAH41791), HsBTBD1 (NP_079514), HsBTBD2 (NP_001011885), CgRhoBTB1 (RLQ76268), CgRhoBTB2 (RLQ55967), CgRhoBTB3 (RLQ73570). (**B**) The transmembrane prediction of *Arabidopsis* BTB-A2.1, BTB-A2.2, and BTB-A2.3.

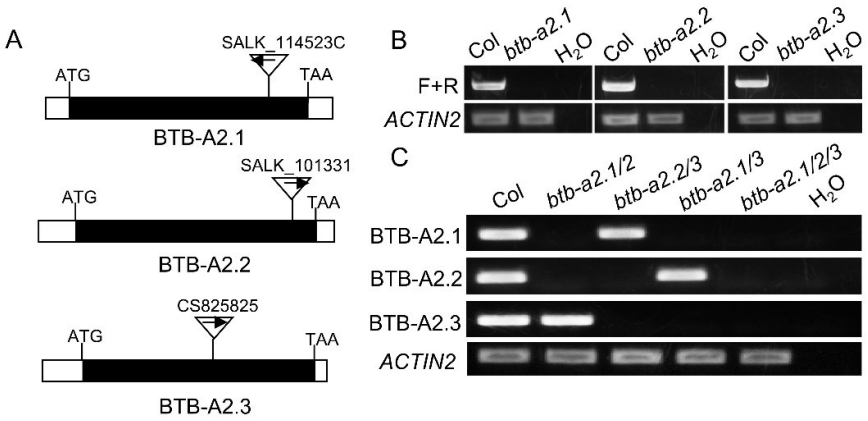

**Figure S2.** Identification of *btb-a2* single mutants, double mutants and triple mutant. (**A**) Schematic diagrams of gene structure and T-DNA insertion positions of *BTB-A2s*. The 5′ and 3′ non-translation regions were indicated by the hollow frame represents. The exons were indicated by the black solid frame. The position of T-DNA insertion in the mutant was indicated by the triangle. The direction of T-DNA insertions in the mutant was indicated by arrows. (**B**) RT-PCR analysis of the accumulation of *BTB-A2.1*, *BTB-A2.2*, and *BTB-A2.3* transcripts in Col, *btb-a2.1*, *btb-a2.2*, and *btb-a2.3*, respectively. (**C**) RT-PCR analysis of double and triple mutants. Expression of *ACTIN2* gene was used as internal reference.

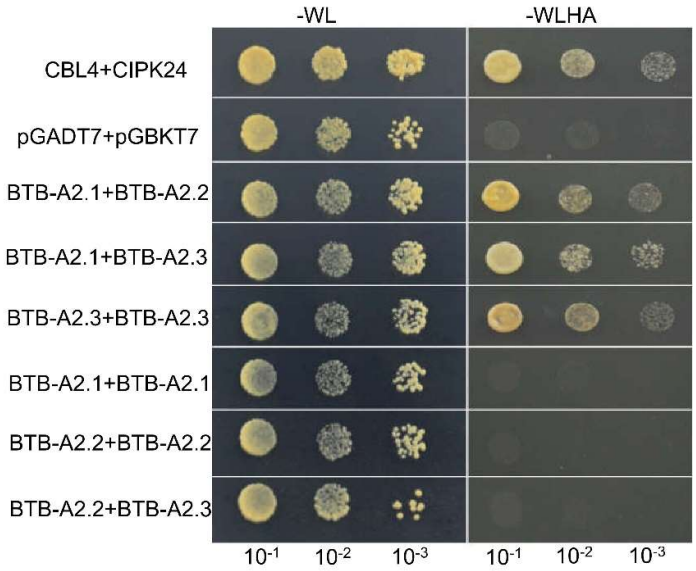


**Figure S3.** *Arabidopsis* BTB-A2s may function in polycomplex. The interaction among BTB-A2.1, BTB-A2.2 and BTB-A2.3 by yeast two-hybrid assays. Saturated cultures were spotted onto on SD-WL and SD-WLHA at different dilutions ($10^{-1}$, $10^{-2}$, and $10^{-3}$).

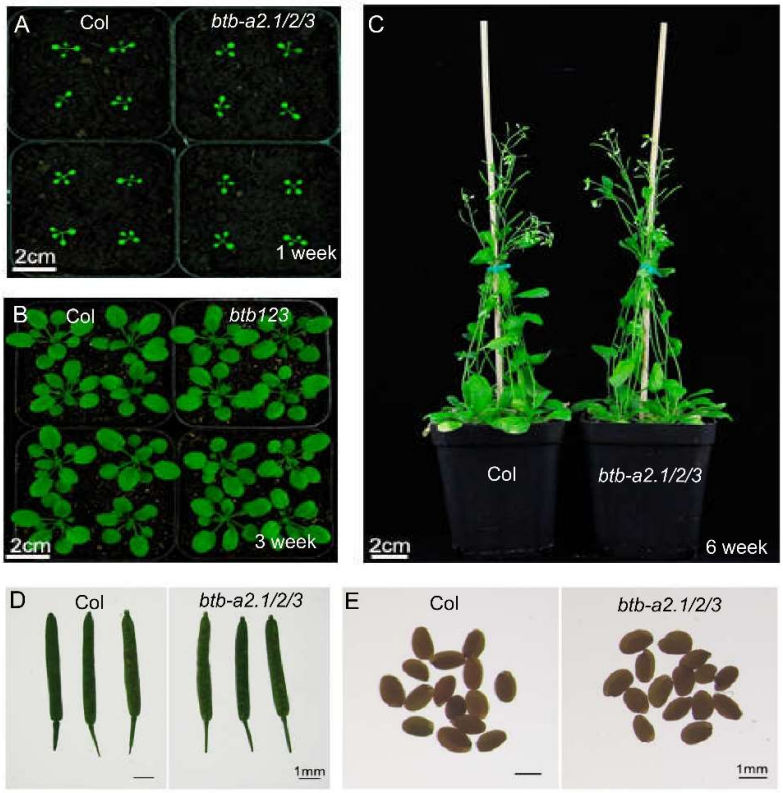


**Figure S4.** The growth situation of *Arabidopsis btb-a2.1/2/3* in normal condition at each growth stage. (**A-C**) The growth of WT and *Arabidopsis* triple mutant *btb-a2.1/2/3* at one week (**A**), three weeks (**B**), six weeks (**C**), Bar=2cm. (**D**, **E**) The size of siliques and seeds of WT and *Arabidopsis* triple mutant *btb-a2.1/2/3*. Bar=1mm.

**Figure S5.** *Arabidopsis btb-a2.1/2/3* displayed no different performance compared with WT in SA and ethylene conditions. (**A**) The phenotypic analysis of WT and triple mutant *btb-a2.1/2/3* growing in 1/2 MS medium containing SA, BA, pHBA (10 μM, 30 μM, and 50 μM) for 10 days. (**B**) The root length of WT and triple mutant btb-a2.1/2/3 growing in 1/2 MS medium containing SA, BA, *p*HBA (10 μM, 30 μM, and 50 μM) for 10 days. (**C**, **E**) The phenotypic analysis of WT and triple mutant *btb-a2.1/2/3* growing in1/2 MS medium containing 10 μM ACC, 50 μM ACC and 10 μM AgNO3 in dark for 4 days (**C**) and in light for 7 days (**E**). (**D**) The statistics of the hypocotyl length in dark. (**F**) The statistics of the root length analysis in light. Data are mean ± SD. Values labeled with different letters are significantly different ($p<0.05$).

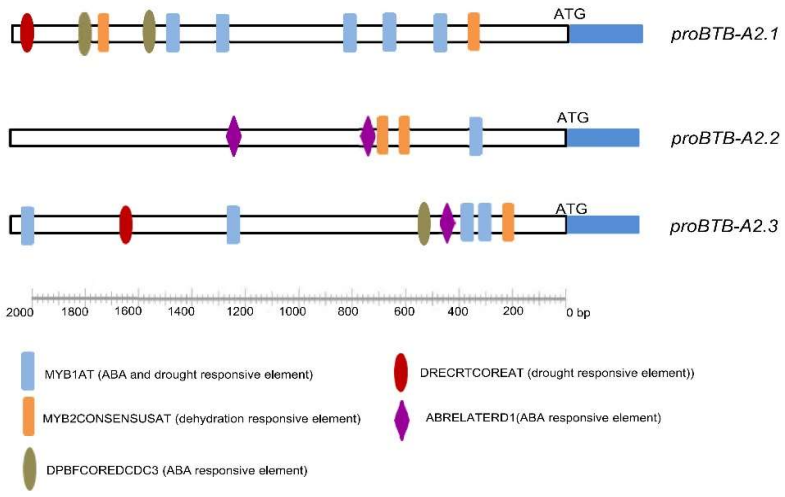


**Figure S6.** Analysis of *cis*-elements in the promoter of *AtBTB-A2s*. About 2000bp promoter of the BTB-A2s were respectively analyzed using *PlantCARE*.

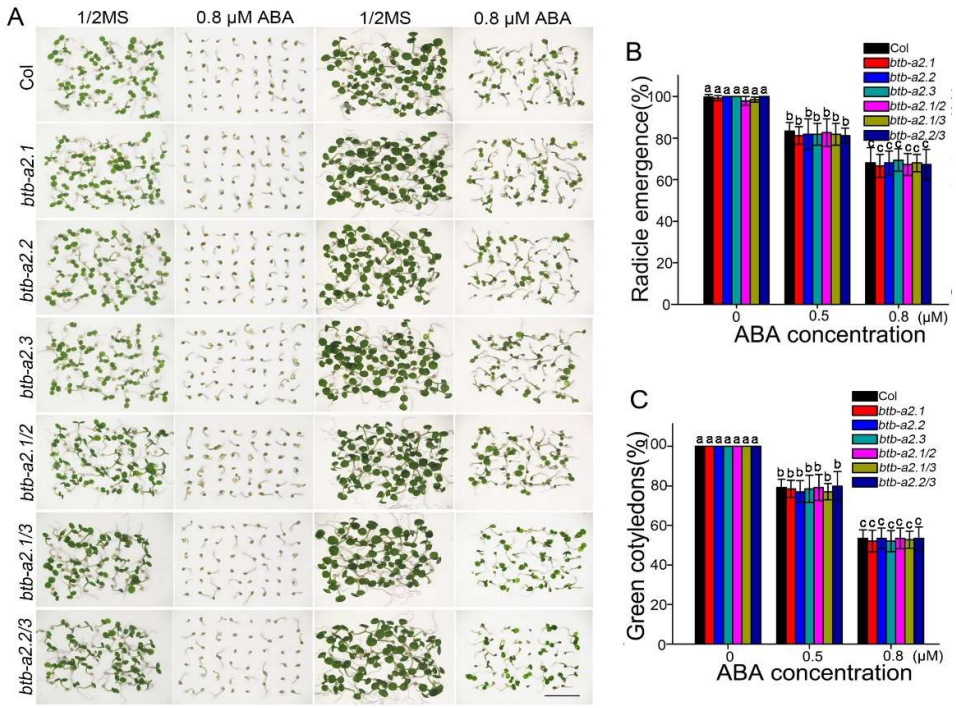


**Figure S7.** *Arabidopsis btb-a2* single and double mutant display no sensitivity to ABA in germination. (**A**) Germination of WT and *btb-a2.1*, *btb-a2.2*, *btb-a2.3* single and double mutants in normal and 0.8 μM ABA 1/2 MS medium. The images were taken after 3 days (first two columns) and 5 days (last two columns) of stratification, respectively. (**B**) Germination rate statistics. (**C**) Green cotyledon statistics. About 150 seeds of each line were used in each experiment. Values labeled with different letters are significantly different ($p<0.05$).

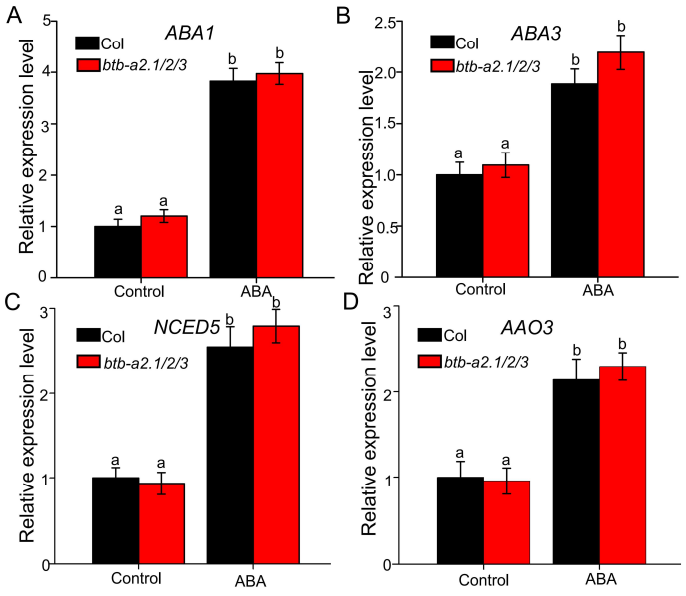


**Figure S8.** Expression levels of ABA synthesis related genes in WT and triple mutant *btb-a2.1/2/3*. Total RNA was isolated from 7-day-old wild-type and *btb-a2.1/2/3* seedlings growing under normal and 0.5 μM ABA conditions. *ACTIN2* gene was used as internal reference, and the results were shown by mean standard deviation. Data are mean ±SD. n=3. Values labeled with different letters are significantly different ($p<0.05$).

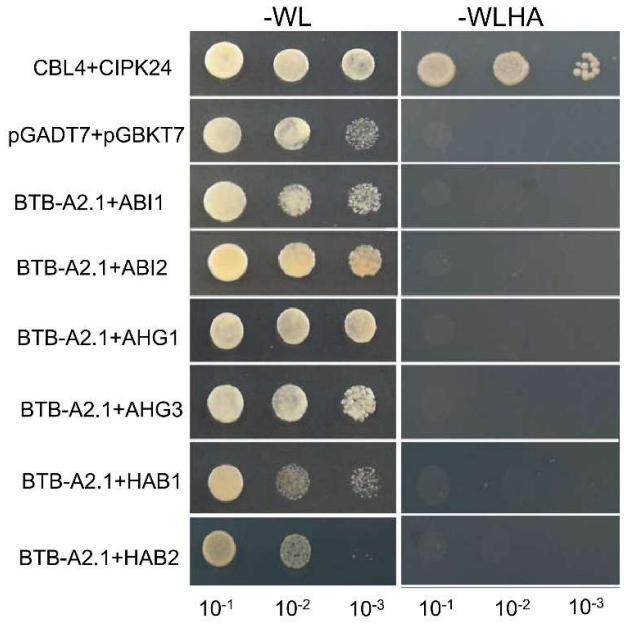


**Figure S9.** BTB-A2.1 may do not interact with PP2Cs. The interaction of BTB-A2.1 with ABI1, ABI2, AHG1, AHG3, HAB1, and HAB2 was performed by yeast two-hybrid assays. Saturated cultures were spotted onto on SD-WL and SD-WLHA at different dilutions ($10^{-1}$, $10^{-2}$, and $10^{-3}$).

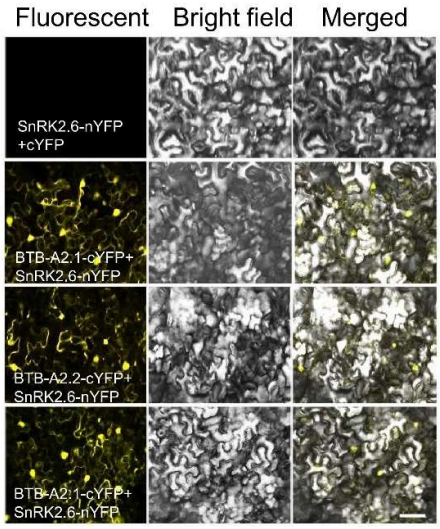


**Figure S10.** The interactions between BTB-A2.1, BTB-A2.2, and BTB-A2.3 with SnRK2.6 by BiFC assays in *N. benthamiana* leaves. Columns from left to right were fluorescent signal, bright field images, and merged images, respectively. Bar=50 μm.

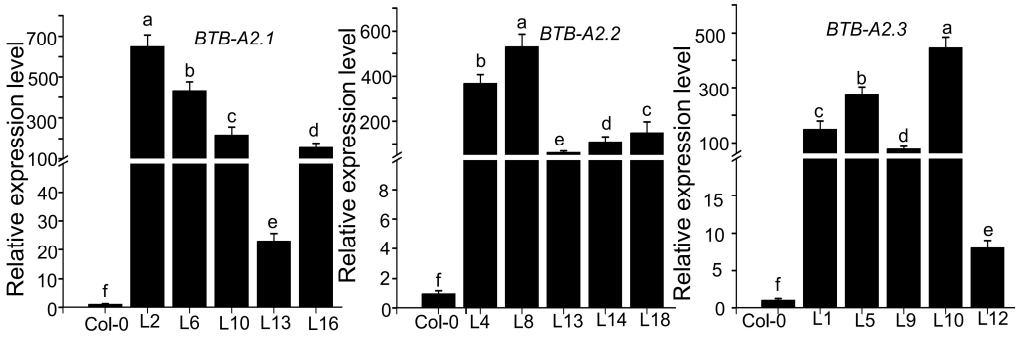


**Figure S11.** Expression levels of *BTB-A2.1*, *BTB-A2.2* and *BTB-A2.3* in transformed *Arabidopsis* plants by qPCR. Total RNA was isolated from 2-week-old hydroponic culture seedlings. *ACTIN2* gene was used as internal reference. Data are mean ±SD. n=3. Values labeled with different letters are significantly different ($p<0.05$).

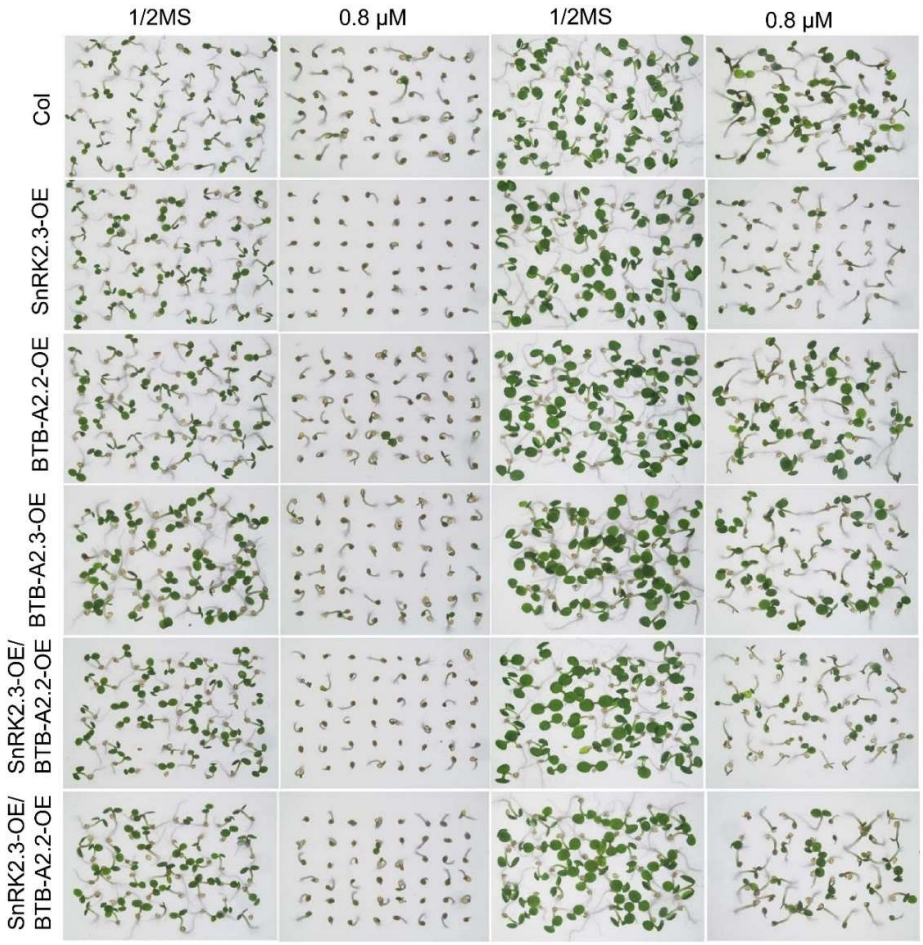


**Figure S12.** Overexpression of *BTB-A2.2*, *BTB-A2.3* inhibits the ABA hypersensitive phenotypes of lines overexpressing *SnRK2.3*. The seeds of WT, *SnRK2.3*-OE lines, *BTB-A2.2*-OE lines, *BTB-A2.3*-OE lines, *SnRK2.3*-OE lines in *BTB-A2.2-OE* background and *SnRK2.3*-OE lines in *BTB-A2.3*-OE background were germinated in 1/2 MS medium containing 0 and 0.8 μM ABA. The images of first two columns and last two columns were taken after 3 and 5 days of seed stratification, respectively. About 150 seeds of each line were used in each experiment, and each assay repeated 3 times.

**Table S1.** Primers used in this study.

| Primer Name | Primer Sequences (5'-3') |
|---|---|
| LBa1 | TGGTTCACGTAGTGGGCCATCG |
| SLLBb1 | GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC |
| A140-Oligod(T) | TTTTTTTTTTTTTTTTT |
| 35S | GACGCACAATCCCACTATCC |
| BTB-A2.1-LP | AATGCAAACATCCTTCACAGC |
| BTB-A2.1-RP | ATCACTTCGTTTGATTGGTCG |
| BTB-A2.2-LP | ACGATATCACATCGATCTGCC |
| BTB-A2.2-RP | TTTCCCGCAACAATTAGTGTC |
| BTB-A2.3-LP | CTCCGTACGGGAGACCTTAAC |
| BTB-A2.3-RP | ACGTGAACAAAAGCAAACCAG |
| BTB-A2.1-OE-F | ACGGGGGACTCTTGACCATGGATGAATTTTCCGACGATCC |
| BTB-A2.1-OE-R | GTCACCTGTAATTCACACGTGGTGGTGGTGGTGGTGCTCGACG GATACCACACCGG |
| BTB-A2.2-OE-F | ACGGGGGACTCTTGACCATGGATGGTGGTTTCCGATGGCG |
| BTB-A2.2-OE-R | GTCACCTGTAATTCACACGTGGTGGTGGTGGTGGTGCTCGACG GATACCACACCGG |
| BTB-A2.3-OE-F | ACGGGGGACTCTTGACCATGGATGGGTATCTCAAAAGACAG |

| | |
|---|---|
| BTB-A2.3-OE-R | GTCACCTGTAATTCACACGTGGTGGTGGTGGTGGTGTATGATCG GACAAGGCGGAGT |
| BTB-A2.1-GFP-F | GGGGTACCATGAATTTTCCGACGATCCCTC |
| BTB-A2.1-GFP-R | TCCCCCCGGGGATAGATATTCCACGACTAGGAC |
| BTB-A2.2-GFP-F | GGGGTACCATGGTGGTTTCCGATGGCGGCAAAC |
| BTB-A2.2-GFP-R | TCCCCCCGGGGCTCGACGGATACCACACCGGAGAAAC |
| BTB-A2.3-GFP-F | CCCTCGAGATGGGTATCTCAAAAGACAG |
| BTB-A2.3-GFP-R | GGATCCCGTATGATCGGACAAGGCGGAGT |
| BTB-A2.1-GUS-F | GCTCTAGATTCGCCAAGCAAACGCAG |
| BTB-A2.1-GUS-R | CGGGATCCGTTCGATTCATGAGGGATCGTC |
| BTB-A2.2-GUS-F | TGCCTGCAGGTCGACTCTAGACAACTATATGAAAGTAAGCG |
| BTB-A2.2-GUS-R | ATAAGGGACTGACCACCCGGGGACACTCGTTTGCCGCCATC |
| BTB-A2.3-GUS-F | GCTCTAGAGACCTTGAGTCCTTGACGAT |
| BTB-A2.3-GUS-R | CGGGATCCTCCACCAACGTTGAATTTGAT |
| BTB-A2.1-EcoRI-Y2H-F | ATGGCCATGGAGGCCGAATTCATGAATTTTCCGACGATCCCT |
| BTB-A2.1-BamHI-Y2H-R | CCGCTGCAGGTCGACGGATCCTTAGATAGATATTCCACGAC |
| BTB-A2.2-EcoRI-F(YH) | CGGAATTCATGGTGGTTTCCGATGGCGGC |
| BTB-A2.2-XhoI-R(YH-AD) | CCCTCGAGTTACTCGACGGATACCACACCGGAG |
| BTB-A2.2-PstI-R(YH-BD) | AACTGCAGTTACTCGACGGATACCACACCGGAG |
| BTB-A2.3-EcoRI-Y2H-F | GGAATTCATGGGTATCTCAAAAGACAGGAT |
| BTB-A2.3-BamHI-Y2H-R | CGGGATCCTTATATGATCGGACAAGGCGGAGT |
| SnRK2.3-Flag-Nco-F | ATAAGATGGATCGAGCTCCGGTGAC |
| SnRK2.3-SpeI-R | GACTAGTTTAGAGAGCGTAAACTATCTCT |
| SnRK2.3-BIFC-BamHI-F | CGGGATCCATGGATCGAGCTCCGGTGACCAC |
| SnRK2.3-BIFC-KpnI-R | GGGGTACCGAGAGCGTAAACTATCTCT |
| SnRK2.3-YH-F | GGAATTCATGGATCGAGCTCCGGTGACCAC |
| SnRK2.3-YH-R | CGGGATCCTTAGAGAGCGTAAACTATCTCT |
| SnRK2.6-Flag-Nco-F | ATAAGATGGATCGACCAGCAGTGAGT |
| SnRK2.6-SpeI-R | GACTAGTTCACATTGCGTACACAATCTC |
| SnRK2.6-BIFC-BamHI-F | CGGGATCCATGGATCGACCAGCAGTGAGTGGTC |
| SnRK2.6-BIFC-KpnI-R | GGGGTACCCATTGCGTACACAATCTCTCCG |
| SnRK2.6-YH-F | GGAATTCATGGATCGACCAGCAGTGAGTGGTC |
| SnRK2.6-YH-R | CGGGATCCTCACATTGCGTACACAATCTCTCCG |
| SnRK2.2-YH-F | GGAATTCATGGATCCGGCGACTAATTCACCG |
| SnRK2.2-YH-R | CTGCAGTCAGAGAGCATAAACTATCTCTCCAC |
| BTB-A2.1-qRT-F | AGGGGAAGTTTTCTACGCCG |
| BTB-A2.1-qRT-R | GCTTGCATTCCCCACCAAAC |
| BTB-A2.2-qRT-F | GGAACCGTCCGTACACATCT |
| BTB-A2.2-qRT-R | TTCCGAATCAGCAACGGCG |
| BTB-A2.3-qRT-F | GCCAACATCCCTGAGCGTCT |
| BTB-A2.3-qRT-R | ATCAGGACCTGCCCTGATGGC |
| ABI3-qRT-F | CACAGCCAGAGTTCCTTCCTTTACT |
| ABI3-qRT-R | TAGTTGCTGAGGAACACAAACGG |
| ABI4-qRT-F | GGGCAGGAACAAGGAGGAAGTG |
| ABI4-qRT-R | TCTCCTCCAAAAGGCCAAATGGT |
| ABI5-qRT-F | ATGATCAAGAACCGCGAGTCTGC |
| ABI5-qRT-R | CGGTTGTGCCCTTGACTTCAAAC |
| RAB18-qRT-F | GGCTTGGGAGGAATGCTTCA |
| RAB18-qRT-R | CGCTTGAGCTTGACCAGACT |
| RD29A-qRT-F | GGAAGTGAAAGGAGGAGGAGGAA |
| RD29A-qRT-R | CACCACCAAACCAGCCAGATG |
| RD29B-qRT-F | GAATCAAAAGCTGGGATGGA |
| RD29B-qRT-R | TGCTCTGTGTAGGTGCTTGG |
| ABA1-qRT-F | CGTGCGGTTGGAGAAGATGTGAT |
| ABA1-qRT -R | TCTCAGAATGGCTTCCTCCTCAGT |
| ABA3-qPCR-F | AGTGGATATTGAAGAGGCAGC |
| ABA3-qPCR-R | CACCAGATCTAGATTAAACCTCAGG |
| AAO3-qRT -F | CAACCGCATGCGCACTAG |
| AAO3-qRT -R | GTCTTGCGGTTCAAAAACATCTT |

| | |
|---|---|
| NCED3-qPCR-F | GAGTGTCCTGTCTGAAATCCG |
| NCED3-qPCR-R | CGAATCCTGAGACTTTAGGCC |
| Actin2-F | ACTCTCCCGCTATGTATGTCGCC |
| Actin2-R | ATTTCCCGCTCTGCTGTTGTGGT |