Supplemental Data. Guo et al. (2015). Plant Cell 10.1105/tpc.15.00829



Supplemental Figure 1. The Mutant *exb1-D* Displayed Pleiotropic Phenotypes and Produced Branches in the Axils of Cotyledons.

- (A) Branches were developed in *exb1-D* but not in wild-type plants.
- (B) and (C) Deformed rosette leaves (B) and cauline leaves (C) were formed in *exb1-D*.
- (**D**) The *exb1-D* produced defective flowers with carpeloid sepals.
- (E) The exb1-D formed abnormal siliques with longer styles. Bars = 1 mm.



Supplemental Figure 2. *EXB1* Encoding WRKY71 Which Shares High Similarity with WRKY8, WRKY28, WRKY48 and WRKY57 in Amino Acid Sequence.

(A) The protein sequence of EXB1. The WRKY domain is indicated by pink color.

(B) The location of T-DNA insertion mutant *exb1* (*SALK_050011*). The exons are indicated by red boxes, and introns by blue lines.

(**C**) The relative expression level of *EXB1* in wild-type plants and *SALK_50011*. The expression level in the wild type was set to 1.0. The error bars represent the SD of three replicates.

(**D**) The alignments of WRKY domain of EXB1 with that of WRKY8, WRKY28, WRKY48 or WRKY57. Letters in red indicate the high conserved WRKYGQK peptide sequence and letters in orange and pink indicate the zinc finger motif. Asterisks indicate the same amino acid residues.

(E) Phylogenetic tree of EXB1, WRKY8, WRKY28, WRKY48 and WRKY57 were generated based on the full-length protein sequences using maximum likelihood (ML) method.



Supplemental Figure 3. EXB1 Had Highly Redundant Function With EXB2, EXB3, EXB4 and EXB5.

(A) Schematic representation of 4EnhEXB1p-WRKYs constructs.

(**B**) to (**F**) The phenotypes of 4EnhEXB1p-WRKY8 (**C**), 4EnhEXB1p-WRKY28 (**D**), 4EnhEXB1p-WRKY48 (**E**), and 4EnhEXB1p-WRKY57 (**F**) transgenic plants. The transgenic plants displayed excessive branching similar to that observed in *exb1-D* when compared to wild-type plants (**B**).

(G) to (J) The expression patterns of *EXB2* (G), *EXB3* (H), *EXB4* (I), and *EXB5* (J). The four genes had overlapping expression patterns with *EXB1*. Bars = 1 cm in (B) to (F), 1 mm in (G) to (J), and 100 μ m in the insets of (G) to (J).



Supplemental Figure 4. The Identification of *exb2-1*, *exb4-1*, and *exb5-1* Mutants.

(A) Schematic representation of T-DNA locations in the *exb2-1*, *exb4-1*, and *exb5-1* mutants.

(B) The relative expression level of *EXB2* or *EXB5* in the *exb2-1* or *exb5-1* mutants. *EXB2* was knocked down in *exb2-1* and *EXB5* was knocked out in *exb5-1*. The expression level of *EXB2* or *EXB5* in wild type was set to 1.0. The error bars represent the SD of three biological replicates.



Supplemental Figure 5. Disruption of EXB1 Caused Fewer Branches in pEXB1-EXB1-SRDX Transgenic Plants.

(A) The number of branches of 45-day-old wild type and pEXB1-EXB1-SRDX transgenic lines. Branch numbers were the average of more than ten plants. Two-tailed t-test was used to test the significance. Three asterisks represent p<0.001.

(**B**) The statistic analysis of AMs in each leaf axil of 50-day-old wild type and pEXB1-EXB1-SRDX-22 transgenic line in a short-day condition. Each column represents one independent plant and each row presents the position of the axils. The typical leaf axil morphologies represented by the two color grids were shown in (**C**) and (**D**). The white grids represent the absence of AMs; the yellow ones represent the AMs with leaf primordia. (**C**) and (**D**) The representative leaf axils of wild-plant and pEXB1-EXB1-SRDX-22. Bars = 100 μ m.



Supplemental Figure 6. 4EnhEXB1p-EXB1GR Transgenic Plants Produced More Branches after DEX Treatment and the RAX Downstream Gene *ROX* and *CUC2* Were Up-regulated Significantly in *exb1-D*.

(A) The phenotypes of 16-day-old 4EnhEXB1p-EXB1GR transgenic plants treated with DMSO or DEX for one week. The red arrow indicates one branch in a leaf axil. Bars = 1 mm.

(B) The expression level of *ROX* and *CUC2* was increased in *exb1-D*. The expression level of *ROX* or *CUC2* in wild type was set to 1.0. The error bars represent the SD of three biological replicates.

Α

AACACCAATGAGTCAATACTGTTTTCCCTCTTATGCTAATTATTCCATACTATTTTCTACATATGCATTGCAACATTACAACTCAAGGGCCAGGCGTAGGAGAAA GGGATTGATGCAATCGTAAAT<mark>TGAC</mark>AAAAGAATTAAAAAGCCAAAGATCACCTGCAGTTCAAATTCTTTATTATTATCATTAACCACTCAATAAATTACGTGTT TTTAATTAATTTTACCTAGAAAAAAATATTGT**TGAC**ATTATTTGCCTCGACAAAGCTTCACCCAAAATCCAGTCCCTAGTACTTCACATGATAACACGATTTAC TCGAGTCAAAAATCCGACGTATAAGACTACAAATATAGACCATCAACCATTTGACTAAAGACACCTTTTCTTATATAGAAAAATAAAAATTAAAAATAAAGATATT AATATGCATATTTTTAACTACAACATCACATATATATATTTAGAATATTGTTTTGATTATATTTTACATTAAAAGAGTAGAATATTGAGGATACTAATTTGACATTCAAATATTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGAATATTGAGGATACTAATTTGAGAATATTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGAATATTGAGAATATTGAGAATATTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGGATACTAATTTGAGAATATTGAGAATATTGAGGATACTAATTTGAGGATACTAATTTGAGAATATTGAGAATATTGAGGATACTAATTTGAGATATTGAGAATATTGAGAATATTGAGGATACTAATTTGAGGATACTAATTTGAGAATATTGAGAATATTGAG TTAATTTTTTTTAATAATTACTTATCAACCTAATATCCTATATGTTTGGGGCTGTAGTGAATTTGCAGCCCAATTTGGAGATTTTAAACCCCTTAATTTATCTTAC CACATTTGTGGAAATGAGTGTGTGACTATATCTGCATGCGTAAATCAAACACATGTACCTTAAATGATGTAAAACAGTAGATCTATCAATTGTACACACATATAT ATGGATACATCGATGG<mark>GTCA</mark>TTATAGCTCTGAAGTTGATTAGTCGGGCCAAAAATACACCTGATCATCATGATATTCATATATGTATAGAATATAGAGCACA GAAAATACATTTGGACGAATGATCAAATCAAGAAACCTGCTGATAATTTTAGTTAA<mark>TGAC</mark>TTTTTTTT<mark>GTCA</mark>ACTCATTACAAAGTTCTGGATTTTAAATATT TGCATTTCTCTTGATATTAATGATTCAATTTTGATTGTTTGGAGTTTGGACCATGCGTATGCTGACAACCAAGTTACTCTGGAATTACTCTGGAATTTGGTAG ATTAAAAGGGCACATATTCAACTACTCCCTCCATAAACACAAAAAGTCCATCCTAAAAAAGCAAGTAAGAGATAACAATTTCTCTCTTTTTCTTTTCTCTTTT CAAACTTCAATTCACTAACGAGAGAAATG

В

С

TTCTTTTAGCCTTTTGTTTTTTCAATATTCATACTAATGTAAACTACAATCATTGTATCATTCTATAATCATATGAATTTTTT<mark>GTCA</mark>ACACCATGCATTATGCTA CTTTAAAGTTCAAAAAACTTTATTTACTTTTTCAGTGATAATTCTGTATAAAAATAACATATAACAATGAGGAAAAAACTCAATAATTTGAATCGCTTA**TGAC**TTT TATACAGTAAATTATTAGTACTTTTATTTTATTTTAACCACAGTTCATTGGCTGTATGAGATTTCTGTTCAAGAAAACATATAAATCGAAAACCAAAAGCAAAAG ACATTATTTGCTTCGGTTTCTTCTACTCAAGCTCATTTTTAGTAAAACTAAATTGTATTTCATTTTCTTAAAAAGTTAATTGTTTTTTGTAAAAATCAGTTTTA ATAGAACTTACCGTTATAGAATCATTTGTAGATTGTTCATCGTGATTGTTAAGATTAGATTCCCATTTGTAATATAACAGAAACTCGAAAAGTACTTATATTAA ATATCACTCTTTAGAGTAATGTATTATCTTTCTTATTGTTTTACCCTGGGATTAATAAGTTTACCGGATCCGGAGTAGAATTTCTTCGGTTTGGCAATTTTGTT CACCTACCATAACGAACTTTTACCCTTATACTTAGTTTCAATCTACTTTTGGTGCATCCTTTCAATTTCAACTGCAAAGAAGTAGTTAAAAGATCCAATACATA CACATATCAATAATATTAGCTAGGAAAAAACTTGTGAGAAATTTCTTTGACGACTGCGTGTGCAATAGTCTTCCTTATCTTCTCACCATTTCTTAATTCCAC TATTGTATAGTATTTACTTATGTTGAATTAAAATAAAAATTAAAACATTTAAATTTCCCTAAAGTTACTTTTTGATTTTTGATTTTCCTATTTTTTATTACAACTTTCCTACT TTACACAAACATCATCACCAACATATATAATCTTGATCATAGAGAGAACAAGAGGCCGCTATCAAGAACAAGACTAAGAACAAGACTTCACTAGGAGT ACAAGTATG

Supplemental Figure 7. The Possible W-box *Cis*-elements in the Promoters of *RAX* Genes.

(A) The possible W-box *cis*-elements in the promoter of *RAX1*.

(B) The possible W-box *cis*-elements in the promoter of *RAX2*.

(**C**) The possible W-box *cis*-elements in the promoter of *RAX3*. Red bold letters represent possible W-box *cis*-elements. Purple bold letters represent start codons of *RAX* genes.



Supplemental Figure 8. The Auxin Homeostasis Was Compromised by *EXB1* Overexpression .

(A) The hypocotyl elongation of *exb1-D* was compromised in the treatment under 29 °C. (B) and (C) Auxin reporter DR5-GUS analysis showed that auxin pathway was affected by *EXB1* overexpression. Bars = 1 mm.

Gene ID	Gene	Value of	Value of	Log2(fold	<i>p</i> _value
	product	mock-	DEX-	change)	
		treated	treated		
		plants	plants		
AT1G70560	TAA1	6.130240	1.939510	-1.7	0.000378
AT4G13260	YUC2	0.878797	0.455587	-0.9	0.158667
AT1G04610	YUC3	0.077682	0.039684	-1.0	0.469674
AT5G16530	PIN5	5.950950	1.099360	-2.4	0.000043
AT5G15100	PIN8	0.494479	0.249029	-1.0	0.250873
AT2G46530	ARF11	3.966420	1.745610	-1.2	0.007063
AT3G61830	ARF18	13.58180	5.873470	-1.2	0.000174
AT1G34410	ARF21	0.029921	0.000405	-6.2	0.578522
AT1G52830	IAA6	1.556270	0.613108	-1.3	0.064486
AT4G14550	IAA14	2.936580	1.232950	-1.3	0.0274252
AT5G57420	IAA33	0.407542	0.078609	-2.4	0.1609220
AT4G28720	YUC8	0.102336	0.551910	2.4	0.0045511
AT3G23050	IAA7	104.1030	222.0350	1.1	0.0002182
AT3G17600	IAA31	0.059568	0.188590	1.7	0.2857590

Supplemental Table 1. The transcript alteration of genes in auxin pathway by EXB1 induction.

Primer	Primer sequences (5' - 3')			
name				
EXB1-1	atgGATGATCATGTTGAGCACAATT			
EXB1-2	tcaAGACTCGTTCTTGGAGAACATT			
EXB1-2N	AGACTCGTTCTTGGAGAACATT			
EXB1-3	caccATGGATGATCATGTTGAGCACAATT			
Enh-1	CTAGAGCTAGTAGATCCCCAACATG			
Enh-2	AACACTGATAGTTTCGGATCTAG			
EXB1-	ctaAGCGAAACCCAAACGGAGTTCTAGATCCAGATCCAGAGACTCGTTC			
SRDX	TTGGAGAACA			
EXB1P-1	GGGGACAACTTTGTATAGAAAAGTTGCTGAAGGTTTTTTGGCTCCCTAA			
EXB1P-2	GGGGACTGCTTTTTTGTACAAACTTGCTGAAGAAGAGGAGAAAGA			
EXB1P-3	CACCGAAGGTTTTTTGGCTCCCTAA			
EXB1P-4	TGAAGAAGAGGAGAAAGA			
GR-1	atgATTCAGCAAGCCACTGCAGGAG			
GR-2	TGCTCAACATGATCATCCATTCATTTTGATGAAACAGAA			
GR-3	TTCTGTTTCATCAAAAATGAATGGATGATCATGTTGAGCA			
RAX1P-1	AACACCAATGAGTCAATACTGTTTT			
RAX1P-2	TTCTCTCGTTAGTGAATTGAAGTTT			
RAX2P-1	TCTTCCCATAAGAATATATGTATGT			
RAX2P-2	CTCTCTATCTGTCTCTTGGTCTA			
RAX3P-1	TCTTTTAGCCTTTTGTTTTTTCAATA			
RAX3P-2	ACTTGTACTCCTAGTGAAGTCTTGTTCT			
FLUC-1	caccATGGAAGACGCCAAAAACATAAAGA			
FLUC-2	ttaCAATTTGGACTTTCCGCCCTTC			
35S-1	GCCAACATGGTGGAGCACGACACTC			
35S-2	AAGCTCGAGAGAGATAGATTTGTAG			
VP16-1	GCCCCCCGACCGATGTCAGCCTGG			
VP16-2	ctaCCCACCGTACTCGTCAATTCCA			
VP16-3	caccGCCCCCCGACCGATGTCAGCCTGG			
G4DBD-1	caccATGAAGCTACTGTCTTCTATCGAAC			
G4DBD-2	ttaTTGATTCGACCTCGACGATACA			
EXB1 RT-1	CCATGGGACCTCTAGTTACAATTTT			
EXB1 RT-2	TAATGCAACCATAACGATACGATCT			
EXB2 RT-1	CATCTCCCTCCATTACCA			
EXB2 RT-2	TCAAGGGTTGCGCATAGTTT			
EXB5 RT-1	CTCGAAGACGGCTATCGTTGGAGAA			
EXB5 RT-2	CGGATGGTTGTGTTGACTCTCGTAGG			
Actin8 RT-1	TCCAGGCATTGTCCACAGAA			
Actin8 RT-2	ACCTGCTCCTTAGACAT			

Supplemental Table 2. The primer list used in this work

ROX RT-1	TCCACCACTTCCTCATCCTC
ROX RT-2	ACCAGGGACCATGCTTTGTA
RAX1 RT-1	CTCTCGGACAGCAACAACAA
RAX1 RT-2	CCTCTGGTCAATGTGGTGGT
RAX2 RT-1	TGGAAGCAGGTGGTCAGTAA
RAX2 RT-2	GCAATGGCTAAGTGGTGATG
RAX3 RT-1	TCTATCTTGAGCGCCAACAC
RAX3 RT-2	GGAGGCTCCTGAGAACAAGT
CUC2 RT-1	AAGCTCCAAGGATGAATGGG
CUC2 RT-2	TTGTTGAGGGTACTTTCGTC
DL1	GACAACATGTCGAGGCTCAGCAGG
EXB1 Co-1	GTGTATGATGGTCGATGATCTGAGAT
EXB1 Co-2	TTGACGAGTAGAAAGAGTTGGAAGTA
SPL-1	caccATGGCGACTTCTCTCTTCTT
SPL-2	ttaAAGCTTCAAGGACAAAT
WRKY8-1	ATGTCTCATGAAATCAAAGA
WRKY8-2	TCAAGGCTCTTGTTTGAAGA
WRKY28-1	ATGTCTAATGAAACCAGAGA
WRKY28-2	TCAAGGCTCTTGCTTAAAGA
WRKY48-1	ATGGAGAAGAAAAAAGAAGA
WRKY48-2	TCATTTCTTATTCTCTTCAT
WRKY57-1	ATGAACGATCCTGATAATCC
WRKY57-2	TCAAGGGTTGCGCATAGTTT
EXB2p-1	caccTAATACACATTACAACATGATTGAA
EXB2p-2	GACGAAGAACAAAGAGAAAAAACTT
EXB3p-1	caccTCAATCCTCTGCAACTTTTTGTTTT
EXB3p-2	GGTGAAGAACAATGAAGAGAGAGGT
EXB4p-1	ccacACTTTTGAGACCCATGGTATGTATT
EXB4p-2	GTAATAGAGAAAAGATGGAATCTAT
EXB5p-1	caccCGTTGTGGAGAGAGAGAGAGAGAGATC
EXB5p-2	GATGATTGATCGGCGAGAGAAGTAG
LBb1.3	ATTTTGCCGATTTCGGAAC
050011LP	GTAGCAGTGCCAGTGTGTCAG
050011RP	AATGAAGGCGAGCCTAAAGAG
107668LP	TTGACTGCTTTTTGGCCATAC
107668RP	CTCGATCAAGAGAACGGTTTG
066438LP	TTACCGGTGACCAGTGTTTTC
066438RP	CTTTTTGGCCGTATTTTCTCC
006260LP	TTTCCGTGGTGTTTTGACTG
006260RP	CTGGCCGACATATCTATCGAG

Supplemental Methods

Plant Materials

Arabidopsis T-DNA insertion mutants were ordered from the *Arabidopsis* Biological Resource Center (ABRC, <u>http://abrc.osu.edu</u>). Homozygous mutants were genotyped by PCR using gene specific primers and T-DNA specific primer LBb1.3.

DR5:GUS line was crossed to *exb1-D* and the homozygous lines in the T3 generation were used for analysis.

The High Temperature-induced Hypocotyl Elongation Assay

To perform the high temperature-induced hypocotyl elongation assay, the 5day-old seedlings of wild type (Col-0) and *exb1-D* were transferred to new plates containing 1/2 MS (Murashige and Skoog) media. Then the plates were put vertically at 29°C or 22°C under long-day conditions for 7 days. The hypocotyl length of the seedlings was measured for statistical analysis. Twotailed t-test was used to test the significance.

Generation of Binary Constructs

To generate 4EnhEXB1p-WRKYs, the coding sequences of WRKY8, WRKY28, WRKY48 and WRKY57 were amplified from wild-type *Arabidopsis* genomic DNA using primer pairs: WRKY8-1 and WRKY8-2, WRKY28-1 and WRKY28-2, WRKY48-1 and WRKY48-2, and WRKY57-1 and WRKY57-2, respectively. The fragments were then cloned into pQDR2L3 vector to generate pQDR2L3-WRKYs. 4EnhEXB1p-WRKYs were generated by the LR reactions using the plasmids including pQDL4R1-4Enh, pENTR/D-pEXB1, pQDR2L3-WRKYs and pK7m34GW.

To analyze the expression patterns of *EXB2*, *EXB3*, *EXB4* and *EXB5*, the corresponding promoter regions were amplified from wild-type *Arabidopsis* genomic DNA using primer pairs EXB2p-1 and EXB2p-2, EXB3p-1 and EXB3p-2, EXB4p-1 and EXB4p-2, and EXB5p-1 and EXB5p-2, respectively. The fragments were cloned into pENTR/D-TOPO to generate pENTR/D-EXB2p, pENTR/D-EXB3p, pENTR/D- EXB4p and pENTR/D-EXB5p, respectively. pEXB2-GUS, pEXB3-GUS, pEXB4-GUS and pEXB5-GUS were generated by LR reactions between and pKGWFS7 and pENTR/D-EXB2p, pENTR/D-EXB3p, pENTR/D-EXB5p.