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Supplemental Information

Cytokinins initiate secondary growth in the *Arabidopsis* root through a set of LBD genes

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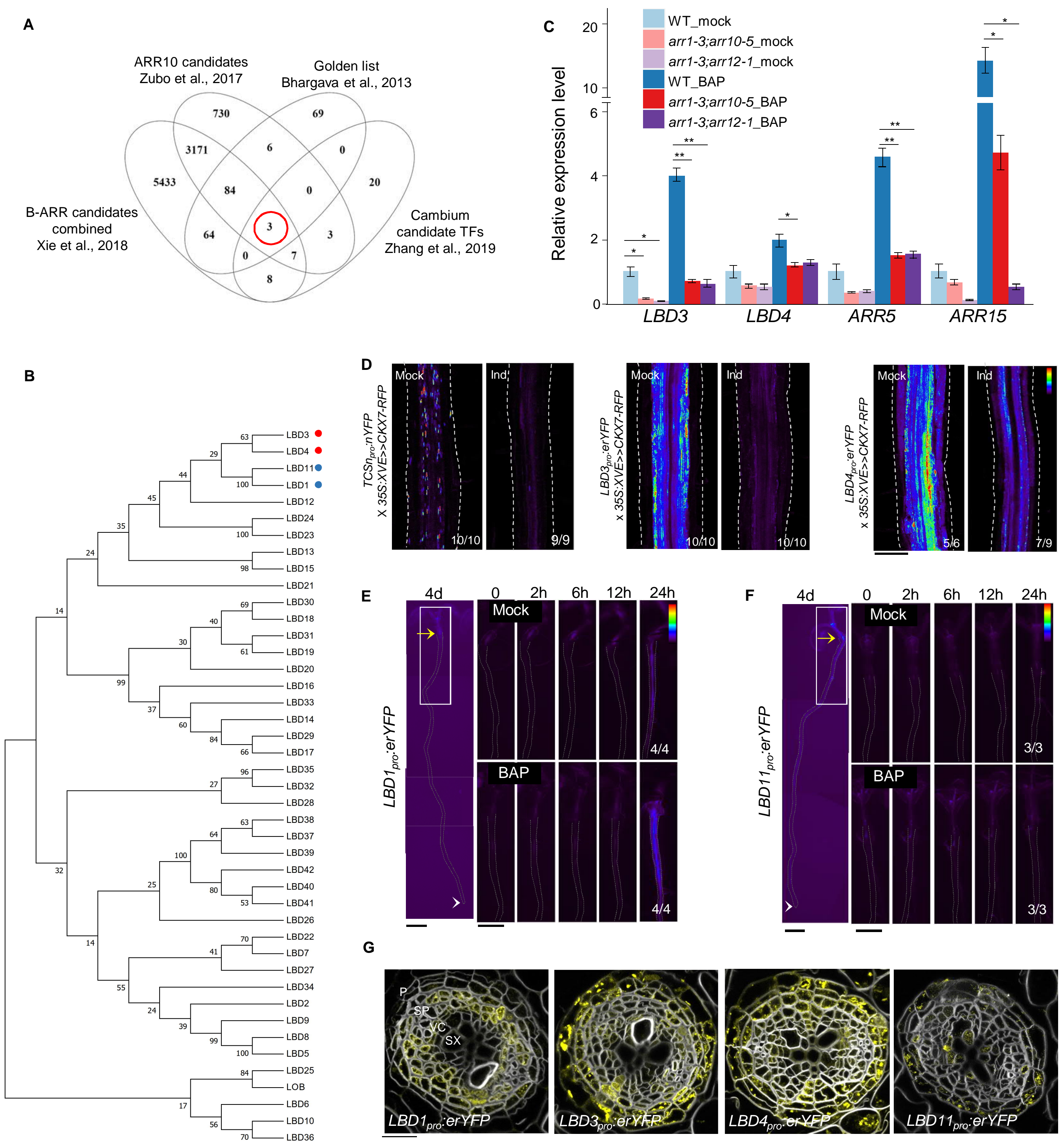

Figure S1 Identification of cambium transcription factors that are targets of type-B *ARRs*. Related to Figure 1 and Figure 4.

(**A**) Venn diagram comparing ARR10 Chromatin Immunoprecipitation-sequencing (ChIP-seq) candidates[S1], targets of ARR1, ARR10 or ARR12 ChIP-seq[S2], the golden list of cytokinins-regulated genes[S3] and cambium candidate transcription factors[S4]. Three genes overlapping in the four datasets are *SHORT HYPOCOTYL 2*, *LBD3* and *LBD4*. (**B**) Phylogeny of the LBD transcription factor gene family in *Arabidopsis thaliana*. Full-length protein sequences were aligned with Clustal X 2.1[S5]. A maximum-likelihood (ML) tree was generated using MEGA7[S6]. The JTT model was used as amino acid substitution model. Nearest neighbor interchange (NNI) was used as the ML heuristic method. Numbers next to nodes indicates the bootstrap value (1000 replicates). (**C**) qRT-PCR analysis of gene transcript levels in WT and mutants after 12 h mock or BAP treatment. Five-day-old roots (root tips discarded) were used for qRT-PCR. Data are presented as mean ± SE from three biological replicates. For each gene, expression over all mutants and treatments was normalized to WT_mock. Two-tailed t-test. $*$, $p < 0.05$; $**$, $p < 0.01$. (**D**) Induction of *CKX7* led to downregulation of *TCSn, LBD3* and *LBD4*. Confocal microscopy of fluorescent markers after 2-day *CKX7* induction in 6-day-old plants. Dashed lines represent root boundaries. (**E,F**) Stereo microscopy of *LBD1*(E) and *LBD11* (F) fluorescent markers in 4-day-old roots (left panel) and following a time-course of BAP treatment in 6-day-old roots (right panels). Numbers represent the frequency of the observed expression in independent roots. Yellow arrows indicate the root-hypocotyl junction. White arrowheads mark root tips. White boxes approximately represent the corresponding region visualized in the right-hand panels. (**G**) Confocal microscopy of LBD markers in root cross-sections. $LBD1_{pro}$*:erYFP* and $LBD11_{pro}$*:erYFP* are expressed in the secondary tissue but more weakly comparing to $LBD3_{pro}$*:erYFP* and $LBD4_{pro}$*:erYFP*. To better visualize the florescence signal of $LBD11_{pro}$*:erYFP*, laser power was adjusted to be stronger. Sections were collected from 5 mm below the root-hypocotyl junction of 9-day-old roots. P, periderm; SP, secondary phloem; VC, vascular cambium; SX, secondary xylem. Scale bars, 100 µm (D), 1 mm (E,F), 20 µm (G).

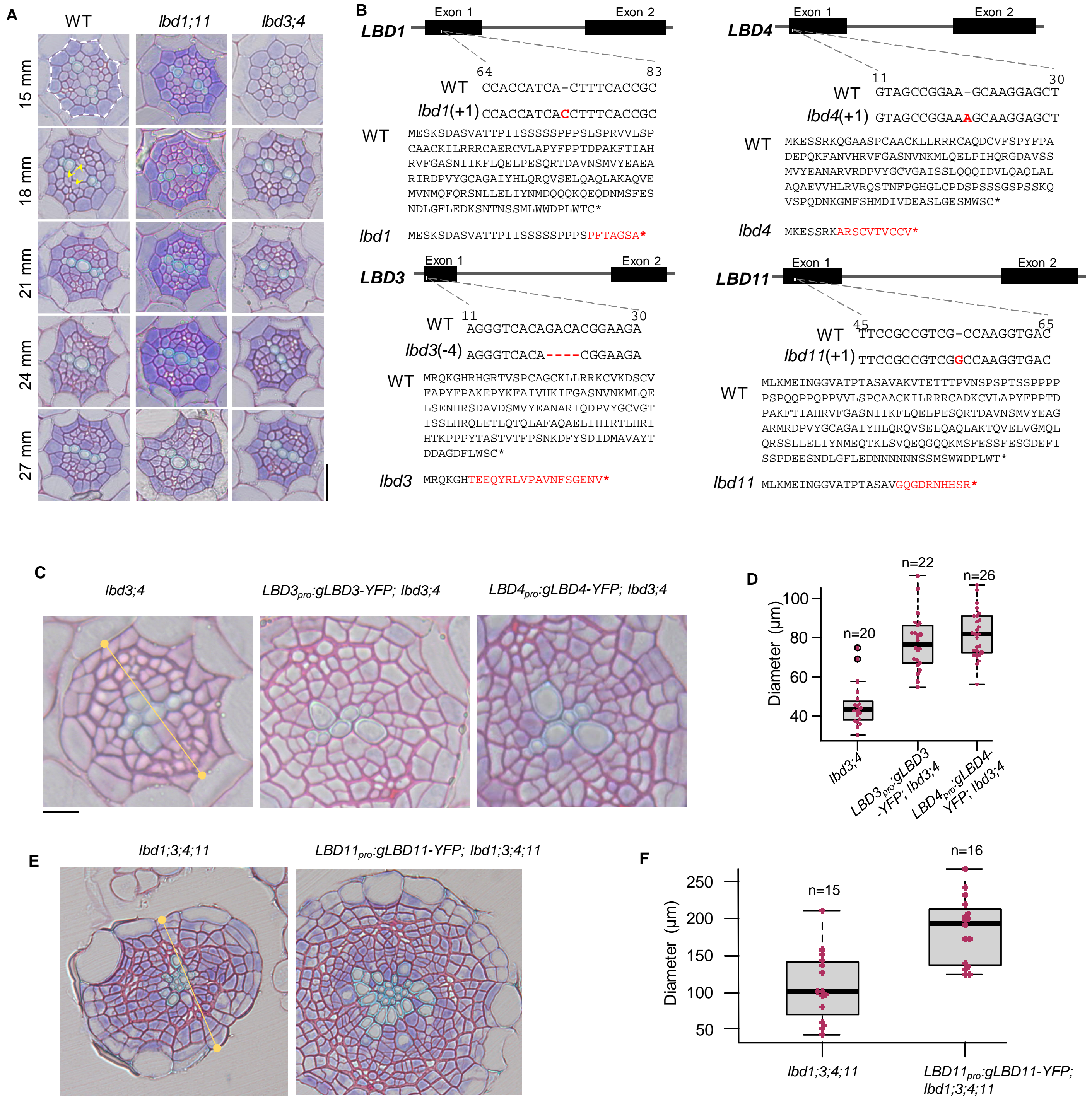

Figure S2 Characterization of LBD knock-out mutants generated by *CRISPR/Cas9* method. Related to Figure 1 and STAR Methods.

(**A**) Serial cross-sections of wild-type (WT) and *lbd* mutants along 7-day-old roots. Cells and area inside of dotted line were used in cell file (Figure 1C) and total radial area (Figure 1D) quantification, respectively. X-axis indicate the distance of cross-sections from root tips. Arrowheads indicate cell divisions in xylem-procambial cells. (**B**) A schema of the LBD mutant alleles created with the *CRISPR/Cas9* system. Chromatograms show the sequencing results from both WT and homozygote mutant alleles. Red letters represent frameshift and premature terminations during translation. (**C**) Functional complementation of *lbd* alleles by introducing genomic LBD (*gLBD*) constructs translationally fused with *YFP*. Cross-sections were collected in 9-day-old roots. (**D**) Quantification of diameter (yellow line) in the experiment presented in (C). Red dots indicate diameters in individual roots. n= number of independent roots analyzed. (**E**) Functional complementation of *lbd* alleles by introducing genomic *LBD11* (*gLBD11*) constructs translationally fused with *YFP*. Cross-sections were collected in 14-day-old roots. (**F**) Quantification of diameter (yellow line) in the experiment presented in (E). Red dots indicate diameters in individual roots. n= number of independent roots analyzed. Scale bars, 20 µm.

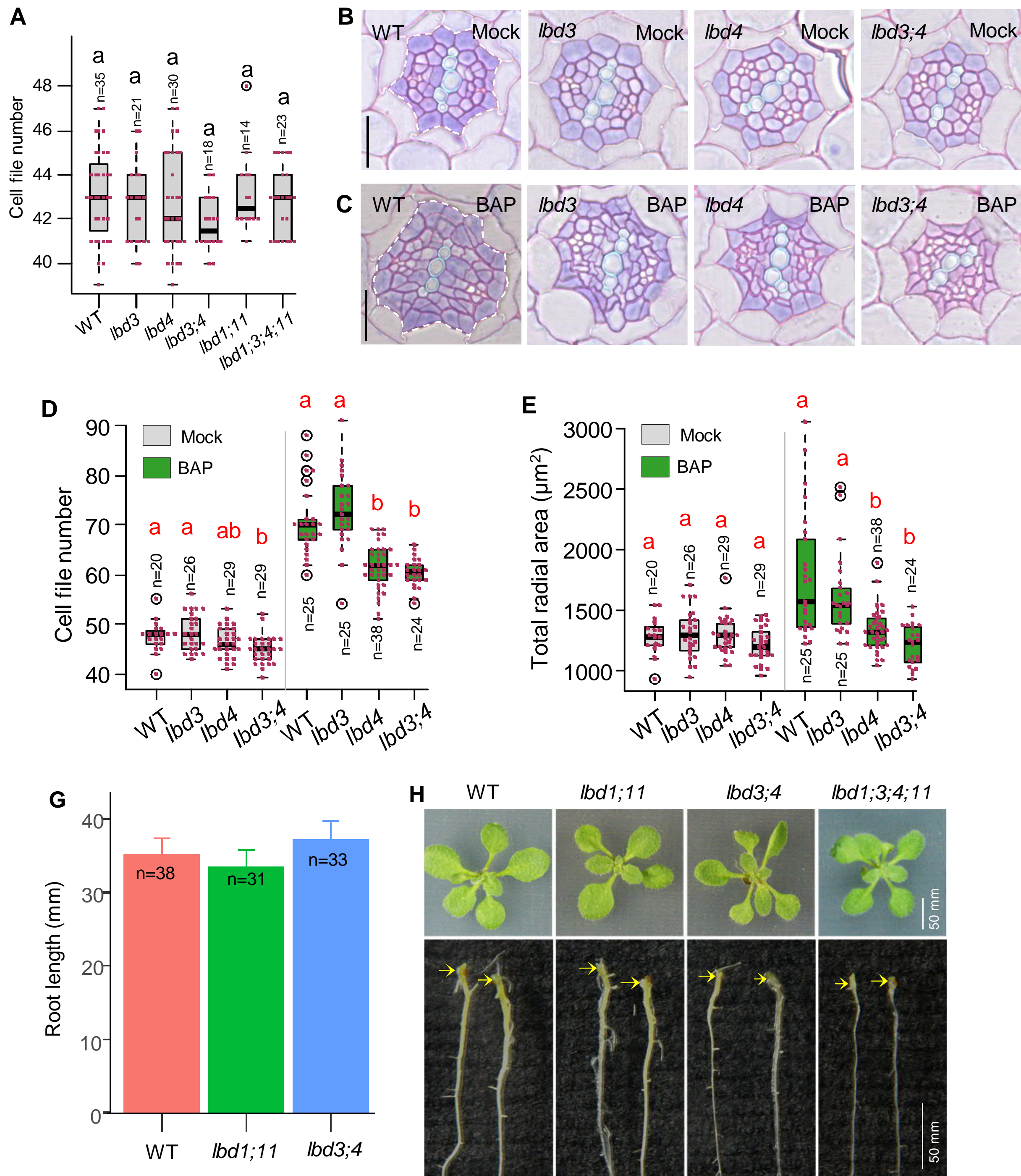

Figure S3 Phenotypic characterization of *lbd* mutants. Related to Figure 2, 3 and Data S2.

(**A**) Quantification of the cell file number in 3-day-old roots. One-way ANOVA with Tamhane's post-test at significance level alpha=0.05. All ANOVA results can be found in Data S2J. Red dots indicate cell file number in individual roots. n= number of independent roots analyzed. (**B, C**) Cross-sections of 5-day-old WT, *lbd3*, *lbd4* and *lbd3;4* roots. Three-day-old roots were treated for 2 days with mock (B) or 1 µM BAP (C). Cells inside of dotted line were used in cell file quantification (D). Scale bars, 20 µm. (**D, E**) Quantification of cell file number (D) and total radial area (E) in the experiment presented in (B) and (C). A separate ANOVA test was performed for mock and BAP treatment. Different letters indicate significant difference at level alpha=0.05, as determined by one-way ANOVA with Tamhane's post-test or Tukey post-hoc test. All ANOVA results can be found in Data S2K,L. Red dots indicate cell file number in individual roots. n= number of independent roots analyzed. (G) Root length quantifications of 7-day-old WT, *lbd1;11* and *lbd3;4* plants. Bars show mean ± SD. n= number of independent roots analyzed. (H) Rosettes and main roots of 14-day-old WT, *lbd1;11*, *lbd3;4* and *lbd1;3;4;11* plants. Arrows indicate the root-hypocotyl junction.

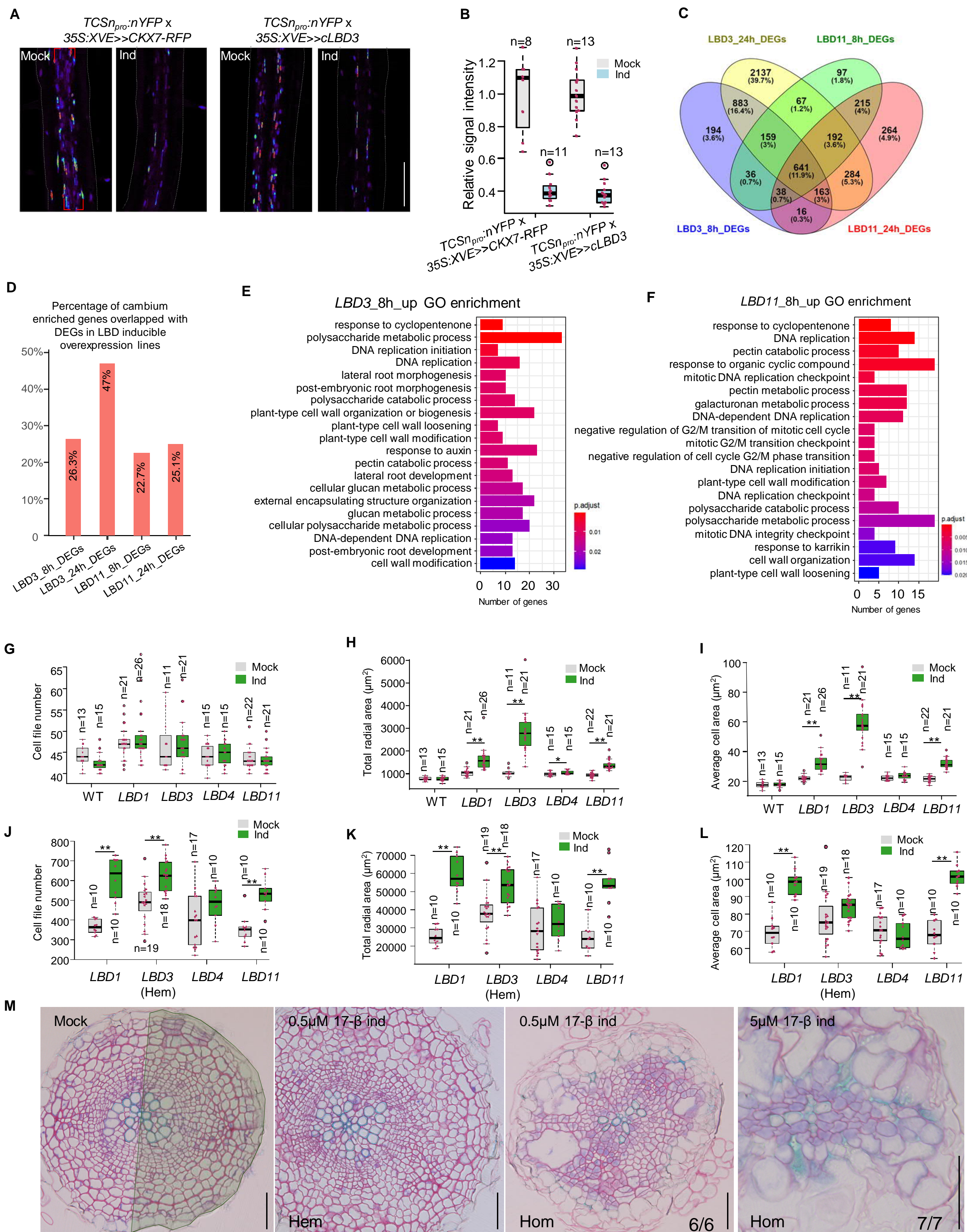

Figure S4 Analysis of RNA-seq datasets and phenotypic characterization of LBD inducible overexpression lines. Related to Figure 4, Data S1, S3 and STAR Methods.

(**A**) Induction of LBD3 led to downregulation of *TCSn* equally rapidly with induction of CKX7. Confocal microscopy of $TCSn_{pro}$*:nYFP* after 8 h CKX7 induction or LBD3 induction in 6-day-old plants. Dashed lines represent root boundaries. Expression intensity within the vascular area marked with brackets was quantified and is shown in (B). (**B**) Quantification of average fluorescent signal intensity in (A). Red dots indicate average fluorescent signal intensity in individual roots. n= number of independent roots analyzed. (**C**) Venn diagram comparing DEGs in the *LBD3* inducible overexpression line and the *LBD11* inducible overexpression line. DEGs for all samples can be found in Data S1. (**D**) A bar plot showing the percentage of cambium enriched genes[S4] overlapped with DEGs in LBD inducible overexpression lines. The lists of overlapped genes can be found in Data S1. (**E**, **F**) Gene ontology (GO) enrichment analysis of differentially upregulated genes in the *LBD3* line (E) and the *LBD11* line (F) with 8 h induction. The top twenty enriched biological process GO terms are presented. The full list of GO terms is listed in Data S3. (**G**-**I**) Quantification of cell file number (G), total radial area (H) and average cell area (I) in the experiment presented in Figure 4H. Three-day-old roots were treated for 2 days with mock or 5 μM 17-β, except in the case of *LBD3* which was treated with 0.5 μM 17-β. WT was used as control. Red dots indicate individual roots. n= number of independent roots analyzed. (**J**-**L**) Quantification of cell file number (J), total radial area (K) and average cell area (L) in the experiment presented in Figure 4I. Eight-day-old roots were treated for 6 days with mock or 5 μM 17-β, except 0.5 μM 17-β was used for the *LBD3* hemizygous (Hem) line. Two-tailed t-test. ∗, $p < 0.05$; ∗∗, $p < 0.01$. Red dots indicate individual roots. n= number of independent roots analyzed. (**M**) LBD3 functions in a dose-dependent manner. The severity of the *LBD3* overexpression phenotype depends on the copy number of the construct (Hem or Hom) and the concentration of the inducer (17-β). Eight-day-old roots were treat with mock or 17-β induction for 6 days. The numbers in the bottom right corner represent the frequency of the observed phenotype. Hem, hemizygous. Hom, Homozygous. Half of the cross section area (marked in green) was used for quantification in (J-L). Scale bars, 100 μm (A), 50 μm (M).

Purpose	Sequence (5'-3')	Note
Cloning primers		
attB4-pLBD1-3152bp-F	ATAGAAAAGTTGAACCCGAGAGTCATGGCTGTTT	*LBD1* promoter cloning
attB1-pLBD1-R	TGTACAAACTTGCACCAACGCAAAAACTGTGAATTATGG	
attB4-pLBD4-4434bp-F	ATAGAAAAGTTGAATGCCTTGAGGAACAAAGTGTTG	*LBD4* promoter cloning
attB1-pLBD4-R	TGTACAAACTTGCCTCTCGCCGCCACGTGTC	
attB4-pLBD11-4943bp-F	ATAGAAAAGTTGAAAAAGGTCCACGCGCATTAGA	*LBD11* promoter cloning
attB1-pLBD11-R	TGTACAAACTTGCGATTCTCAAAATATTTTGGGGGCTT	
attB4-pTCS–F	ATAGAAAAGTTGCCGACGCGTAAGCTTGACTAGTC	*TCS* promoter cloning
attB1-pTCS–R	TGTACAAACTTGCCAAACTTGTTGATGGGTCCTC	
attB1-LBD1-F	AAAAAGCAGGCTCGATGGAGAGTAAAAGTGACGCTTC	*LBD1* coding sequence cloning
attB2-LBD1-R	AGAAAGCTGGGTGTCAACATGTCCAAAGAGGATCCC	
attB1-LBD3-F	AAAAAGCAGGCTCGATGAGACAAAAGGGTCACAGAC	*LBD3* genomic sequence cloning
attB2-LBD3-R	AGAAAGCTGGGTGGCAAGACCAAAGGAAGTCTCC	
attB1-LBD4-F	AAAAAGCAGGCTCGATGAAAGAAAGTAGCCGGAAGC	*LBD4* genomic sequence cloning
attB2-LBD4-R	AGAAAGCTGGGTGGCAAGACCACATAGACTCTCCC	
attB1-LBD11-F	AAAAAGCAGGCTCGATGCTAAAGATGGAGATTAACGG	*LBD11* cloning
attB2-LBD11-R1	AGAAAGCTGGGTGTCATGTCCAAAGAGGATCCCACCACG	*LBD11* coding sequencing cloning
attB2-LBD11-R2	AGAAAGCTGGGTGTGTCCAAAGAGGATCCCACCACG	*LBD11* genomic sequencing cloning
attB1-CKX7-F	AAAAAGCAGGCTAAATGATAGCTTACATAGAACC	*CKX7* genomic sequence cloning
attB2-CKX7-R	AGAAAGCTGGGTTAAGAGACCTATTGAAAATCT	
adapter-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT	full length BP adaptors
adapter-attB2--R	GGGGACCACTTTGTACAAGAAAGCTGGGT	
adapter-attB4-F	GGGGACAACTTTGTATAGAAAAGTTG	
adapter-attB1-R	GGGGACTGCTTTTTTGTACAAACTTG	
***CRISPR-Cas9* primers**		
attB4-pEC1.2en EC1.1p-F	ATAGAAAAGTTGAAGAATAAAAGCATTTGCGTTTGGTT	Egg cell specific promoter cloning
attB1-pEC1.2en EC1.1p-R	TGTACAAACTTGCTTCTCAACAGATTGATAAGGTCGA	
attB1-zCAS9-rbcS-F	AAAAAGCAGGCTCGATGGATTACAAGGACCACGACG	*Cas9* cloning
attB2-zCAS9-rbcS-R	AGAAAGCTGGGTGGTTGTCAATCAATTGGCAAGTCATA	
LBD1-gRT#+	ACTACCCGCGGTGAAAGTGAGTTTTAGAGCTAGAAAT	guide RNA cloning
LBD3-gRT#+	AGACAAAAGGGTCACAGACAGTTTTAGAGCTAGAAAT	
LBD4-gRT#+	GTGACGCAGCTCCTTGCTTCGTTTTAGAGCTAGAAAT	
LBD11-gRT#+	GGTTTCTGTCACCTTGGCGAGTTTTAGAGCTAGAAAT	
LBD1-AtU3bT#-	TCACTTTCACCGCGGGTAGTTGACCAATGTTGCTCC	
LBD3-AtU3dT#-	TGTCTGTGACCCTTTTGTCTTGACCAATGGTGCTTTG	
LBD4-AtU6-1T#-	GAAGCAAGGAGCTGCGTCACCAATCACTACTTCGTCT	
LBD11-AtU6-29T#-	TCGCCAAGGTGACAGAAACCCAATCTCTTAGTCGACT	
LBD4-AtU3bT#-	GAAGCAAGGAGCTGCGTCACTGACCAATGTTGCTCC	
Genotyping primers		
1) dCAPs primers		
lbd1-c1-dcaps-F	CATATCTTCTTCCTCTTCTCCTCCACCAGTAC	After ScaI digestion: mut :179 bp; WT: 147bp and 33bp
lbd1-dcaps-R	GTTGCTGGCTCCAAAGACGC	
lbd3-c1-dcaps-F	CTCTCTCTTCTAAATGAGACAAAAGGGTCTCA	After DdeI digestion: mut :197 bp; WT: 170bp and 31bp
lbd3-c1-dcaps-R	CCTGCAACATCTTATTGACATTACTAGCACC	
lbd11-c1-dcaps-F	CGCTACACCTACTGCTTCCGCGGCCG	After NotI digestion: mut :136 bp; WT: 115bp and 21bp
lbd11-c1-dcaps-R	GGGCTTAGTACAACCGGTGGTTGTGGC	
2) sequencing primers		
LBD3-CRISPR-F	CTCTTCTTGTTTTGCTCTCAATTC	amplify *LBD3* for sequencing
LBD3-CRISPR-R	CGGTGGTTCTCCGACAGCTC	
LBD3-CRISPR-R-seq	CTGCAACATCTTATTGACATT	*LBD3* sequecing primer
LBD1-CRISPR-R-seq	CTGCAAGAACTTAATGATGTT	*LBD1* sequecing primer, *attB1-LBD1-F/attB2-LBD1-R* used to amplify *LBD1*
LBD4-CRISPR-F	GACAATCCAACGGTTGAGATT	amplify *LBD4* together with *attB2-LBD4-R*
LBD4-CRISPR-R-seq	CTGAAGCATCTTGTTGACGTT	*LBD4* sequecing primer

LBD11-CRISPR-R	GTTCTTTGCGATTCTGGAAGTTC	amplify *LBD11* together with *attB1-LBD11-F*
LBD11-CRISPR-R-seq	CCAAAGACACGGTGAGCGATT	*LBD11* sequecing primer
qPCR primers		References
TIP41	GTGAAAACTGTTGGAGAGAAGCAA	S7
	TCAACTGGATACCCTTTCGCA	
ACT2	ACATTGTGCTCAGTGGTGGA	S8
	CTGAGGGAAGCAAGAATGGA	
UBQ10	CGTCTTCGTGGTGGTTTCTAAA	S8
	ACAAGGCCCCAAAACACAAA	
TDR	TCAAACCGACGAATCCATGTC	S9
	CACGACCTACCCATGCTTTTG	
WOX4	GACAAGAACATCATCGTCACTAGACA	S10
	TTCTCCACCATTGGTTCTCTCA	
LBD1	ACGCTTCTGTCGCCACCACT	this study
	TCTCTGCACAACGTCGCCTCA	
LBD3	ATGTTGCAGGAGCTGTCGG	S4
	CATCGAATCCACGGCGTC	
LBD4	CGTCAACAAGATGCTTCAGG	S4
	CCTCGTAAACCATGCTGCTC	
LBD11	CCTACTGCTTCCGCCGTCGC	this study
	GCAAGCCGCACAAGGGCTTA	
ARR15	TGGGACTAGGGCTCTGCAGT	this study
	GAAGATCCATTGTCTCCATCTAAACC	
ARR5	TCAGAGAACATCTTGCCTCGT	S11
	ATTTCACAGGCTTCAATAAGAAATC	

Table S1 Primers used in this study. Related to STAR Methods.

Induction \ Hormone		Abscisic acid		Auxin		Cytokinin	
		Hormone metabolism; proportion of genes	Signaling transduction	Hormone metabolism; proportion of genes	Signaling transduction	Hormone metabolism; proportion of genes	Signaling transduction
LBD3_8h Induction	Up-regulated DEGs	NCED9, ABA1; 2/15 (13.3%)	HAI3; 1/44 (2.3%)	TAA1; 1/32 (3.1%)	SAUR54, GH3.17, LAX3, GH3.1, SAUR8, SAUR38, SAUR45, AUX1, IAA14, IAA1, IAA11, IAA29, SAUR50, SAUR9, SAUR30; 15/120 (12.5%)	AtCKX1; 1/39 (2.6%)	
	Down-regulated DEGs	-	HAI2, ABI5, PYL7, SNRK2.7; 4/44 (9.1%)	AAO1, CYP79B2, CYP79B3, SUR2, SUR1, TAR3, YUCCA6, YUCCA8; 8/32 (25%)	AT1G48670, AXR3, BRU6, GH3.3, GH3.9, IAA16, IAA28, IAA30, IAA5, SAUR33, SAUR4, SAUR40, SAUR51, SAUR55, SAUR6, SHY2; 16/120 (13.3%)	APT2, APT3, APT4, AtCKX2, AtCKX3, AtCKX4, AtCKX6, AtIPT3, AtLOG3, AtLOG6, CYP735A1, CYP735A2, UGT76C2; 13/39 (33.3%)	ARR15, ARR4, ARR5, ARR6, ARR7, ARR9, WOL; 7/33 (21.2%)
LBD3_24h Induction	Up-regulated DEGs	NCED5, NCED9, ABA1; 3/15 (20%)	PYL2, HAI3, EEL; 3/44 (6.8%)	TAA1; 1/32 (3.1%)	SAUR54, GH3.17, SAUR71, SAUR52, LAX3, GH3.1, SAUR8, SAUR38, SAUR45, AUX1, TIR1, IAA14, IAA1, IAA29, SAUR50, SAUR9, SAUR30; 17/120 (14.2%)	AtCKX1; 1/39 (2.6%)	ARR10; 1/33 (3%)
	Down-regulated DEGs	BG1, NCED3, NCED2; 3/15 (20%)	HAI2, PYL7, SNRK2.7; 3/44 (6.8%)	AAO1, CYP79B2, CYP79B3, CYP83B1, NIT1, SUR1, TAR3, TSA1, TSB2, YUCCA2, YUCCA6; 11/32 (34.4%)	AT1G48670, AXR3, BRU6, GH3.3, GH3.9, IAA13, IAA16, IAA18, IAA28, IAA30, IAA31, IAA32, IAA5, IAA7, IAA9, PAP2, PBS3, SAUR14, SAUR29, SAUR31, SAUR32, SAUR33, SAUR34, SAUR36, SAUR40, SAUR51, SAUR53, SAUR55, SAUR6, SAUR4, SHY2, WES1; 32/120 (26.7%)	APT2, APT3, APT4, AtCKX2, AtCKX3, AtCKX4, AtCKX5, AtCKX6, AtIPT3, AtIPT5, AtLOG1, AtLOG3, AtLOG5, AtLOG6, CYP735A1, CYP735A2, UGT76C2; 14/39 (35.9%)	ARR11, ARR15, ARR16, ARR3, ARR4, ARR5, ARR6, ARR7, ARR9, WOL; 10/33 (30.3%)
LBD11_8h Induction	Up-regulated DEGs	NCED9; 1/15 (6.7%)	RCAR1; 1/44 (2.3%)	NIT4; 1/32 (3.1%)	IAA10, SAUR71, SAUR8, SAUR45, SAUR34, IAA29; 6/120 (5%)	AtLOG5; 1/39 (2.6%)	AHP4; 1/33 (3%)
	Down-regulated DEGs	-	PYL7, PYL12; 2/44 (4.5%)	AMI1, CYP79B3, NIT1; 3/32 (9.4%)	AT1G48660, AXR3, DFL1, GH3.9, IAA28, LAX1, PAP2, SAUR29, SAUR51, SAUR6; 10/120 (8.3%)	APT2, APT3, APT4, AtCKX2, AtCKX4, AtCKX6, AtIPT3, AtLOG3, AtLOG6, CYP735A1; 10/39 (25.6%)	ARR15, ARR4, ARR5, ARR6, ARR7, WOL; 6/33 (18.2%)
LBD11_24h Induction	Up-regulated DEGs	NCED9; 1/15 (6.7%)	-	NIT4; 1/32 (3.1%)	GH3.3, IAA10, IAA29, SAUR30, SAUR45, SAUR5, SAUR71, SAUR8; 8/120 (6.7%)	AtCKX1, AtLOG5; 2/39 (5.1%)	AHP4, ARR18; 2/33 (6%)
	Down-regulated DEGs	-	HAI2, ABI5, SNRK2.7, PYL12; 4/44 (9.1%)	AMI1, YUCCA6; 2/32 (6.3%)	AT1G48660, AT1G48670, AXR3, DFL1, GH3.9, IAA28, IAA5, LAX1, PAP2, SAUR14, SAUR32, SAUR33, SAUR51, SAUR59, SAUR6; 15/120 (12.5%)	APT2, APT3, APT4, AtCKX3, AtCKX4, AtCKX5, AtCKX6, AtLOG3, AtLOG6, CYP735A1, CYP735A2, UGT73C5; 12/39 (30.8%)	ARR15, ARR4, ARR5, ARR6, ARR7, ARR9, WOL; 7/33 (21.2%)

Induction \ Hormone		Brassinosteroid		Ethylene		Gibberellin		Jasmonic acid		Salicylic acid	
		Hormone metabolism; proportion of genes	Signaling transduction	Hormone metabolism; proportion of genes	Signaling transduction	Hormone metabolism; proportion of genes	Signaling transduction	Hormone metabolism; proportion of genes	Signaling transduction	Hormone metabolism; proportion of genes	Signaling transduction
LBD3_8h Induction	Up-regulated DEGs	BAS1, BR6ox1; 2/13 (15.4%)	-	-	-	AtGA2ox6; 1/24 (4.2%)	RGL2; 1/12 (8.3%)	-	-	-	CAP, PR-1-LIKE; 2/24 (8.3%)
	Down-regulated DEGs	CYP90D1, BR6ox2, DWF4; 3/13 (23.1%)	-	-	-	AtGA3ox1,AtGA2ox7; 2/24 (8.3%)	-	AOC2; 1/17 (5.9%)	JAZ4, JAZ6; 2/13 (15.4%)	ICS2, ICS1, AtMES1, AtMES9; 4/9 (44.4%)	TGA4, TGA1; 2/24 (8.4%)
LBD3_24h Induction	Up-regulated DEGs	BAS1, ROT3, BR6ox1; 3/13 (23.1%)	BEH2, BRI1, BKI1; 3/22 (13.6%)	-	-	AtGA2ox6; 1/24 (4.2%)	RGL2; 1/12 (8.3%)	-	-	-	CAP, PR-1-LIKE, BOP1; 3/24 (12.5%)
	Down-regulated DEGs	CYP90D1, BR6ox2, DWF4; 3/13 (23.2%)	BES1, BEH1; 2/22 (9.1%)	ACO2; 1/12 (8%)	ERF1; 1/21 (4.8%)	AtGA2ox1, AtGA2ox4, AtGA2ox7, AtGA2ox8, AtGA3ox1/GA4, AtGA3ox2, AtKAO2; 7/24 (29.2%)	GID1C, PIF3, PIF4, PIL6, RGL1; 5/12 (41.7%)	AOC2, AOS, AtST2a, OPR3; 4/17 (23.5%)	JAZ10, JAZ3, JAZ4, JAZ6, MYC2, TIFY10B, TIFY7; 7/13 (53.8%)	AtMES1, AtMES2, AtMES9, ICS1, ICS2; 5/9 (55.6%)	CAP, TGA1, TGA4; 3/24 (12.5%)
LBD11_8h Induction	Up-regulated DEGs	BAS1, BR6ox1; 2/13 (15.4%)	-	-	-	-	-	-	-	-	-
	Down-regulated DEGs	CYP90D1, BR6ox2; 2/13 (15.5%)	-	-	-	AtGA2ox7, AtGA20ox2; 2/24 (8.3%)	-	-	TIFY7; 1/13 (7.7%)	AtMES9, ICS1, ICS2; 3/9 (33.3%)	CAP; 1/24 (4.2%)
LBD11_24h Induction	Up-regulated DEGs	BAS1, BR6ox1; 2/13 (15.6%)	-	ACS2; 1/12 (8%)	-	-	-	-	-	-	-
	Down-regulated DEGs	UGT73C5; 1/13 (7.7%)	-	-	-	AtGA2ox2; 1/24 (4.2%)	-	-	JAZ4; 1/13 (7.7%)	ICS1, ICS2; 2/9 (22.2%)	PAN, CAP, TGA4; 3/24 (12.5%)

Table S2 Hormone-related genes differentially expressed in *LBD3* or *LBD11* inducible overexpression RNA-seq data. Related to Figure 4 and STAR Methods.

SUPPLEMENTAL REFERENCES

S1. Zubo, Y.O., Blakley, I.C., Yamburenko, M.V., Worthen, J.M., Street, I.H., Franco-Zorrilla, J.M., Zhang, W., Hill, K., Raines, T., Solano, R., et al. (2017). Cytokinin induces genome-wide binding of the type-B response regulator ARR10 to regulate growth and development in Arabidopsis. Proc. Natl. Acad. Sci. USA *114*, E5995-E6004.

S2. Xie, M., Chen, H., Huang, L., O'Neil, R.C., Shokhirev, M.N., and Ecker, J.R. (2018). A B-ARR-mediated cytokinin transcriptional network directs hormone cross-regulation and shoot development. Nat. Commun. *9*, 1604.

S3. Bhargava, A., Clabaugh, I., To, J.P., Maxwell, B.B., Chiang, Y.H., Schaller, G.E., Loraine, A., and Kieber, J.J. (2013). Identification of cytokinin-responsive genes using microarray meta-analysis and RNA-Seq in Arabidopsis. Plant Physiol. *162*, 272-294.

S4. Zhang, J., Eswaran, G., Alonso-Serra, J., Kucukoglu, M., Xiang, J., Yang, W., Elo, A., Nieminen, K., Damen, T., Joung, J.G., et al. (2019). Transcriptional regulatory framework for vascular cambium development in Arabidopsis roots. Nat. Plants *5*, 1033-1042.

S5. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., et al. (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947-2948.

S6. Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. *33*, 1870-1874.

S7. Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., and Scheible, W.R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiol. *139*, 5-17.

S8. Randall, R.S., Miyashima, S., Blomster, T., Zhang, J., Elo, A., Karlberg, A., Immanen, J., Nieminen, K., Lee, J.Y., Kakimoto, T., et al. (2015). AINTEGUMENTA and the D-type cyclin CYCD3;1 regulate root secondary growth and respond to cytokinins. Biol. Open *4*, 1229-1236.

S9. Wang, J., Kucukoglu, M., Zhang, L., Chen, P., Decker, D., Nilsson, O., Jones, B., Sandberg, G., and Zheng, B. (2013). The Arabidopsis LRR-RLK, PXC1, is a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway. BMC Plant Biol. *13*, 94.

S10. Smetana, O., Makila, R., Lyu, M., Amiryousefi, A., Sanchez Rodriguez, F., Wu, M.F., Sole-Gil, A., Leal Gavarron, M., Siligato, R., Miyashima, S., et al. (2019). High levels of auxin signalling define the stem-cell organizer of the vascular cambium. Nature *565*, 485-489.

S11. Gordon, S.P., Chickarmane, V.S., Ohno, C., and Meyerowitz, E.M. (2009). Multiple feedback loops through cytokinin signaling control stem cell number within the Arabidopsis shoot meristem. Proc. Natl. Acad. Sci. USA *106*, 16529-16534.