

Supplementary Information for

CsBRC1 inhibits axillary bud outgrowth by directly repressing the auxin efflux carrier *CsPIN3* in cucumber

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Datasets S1

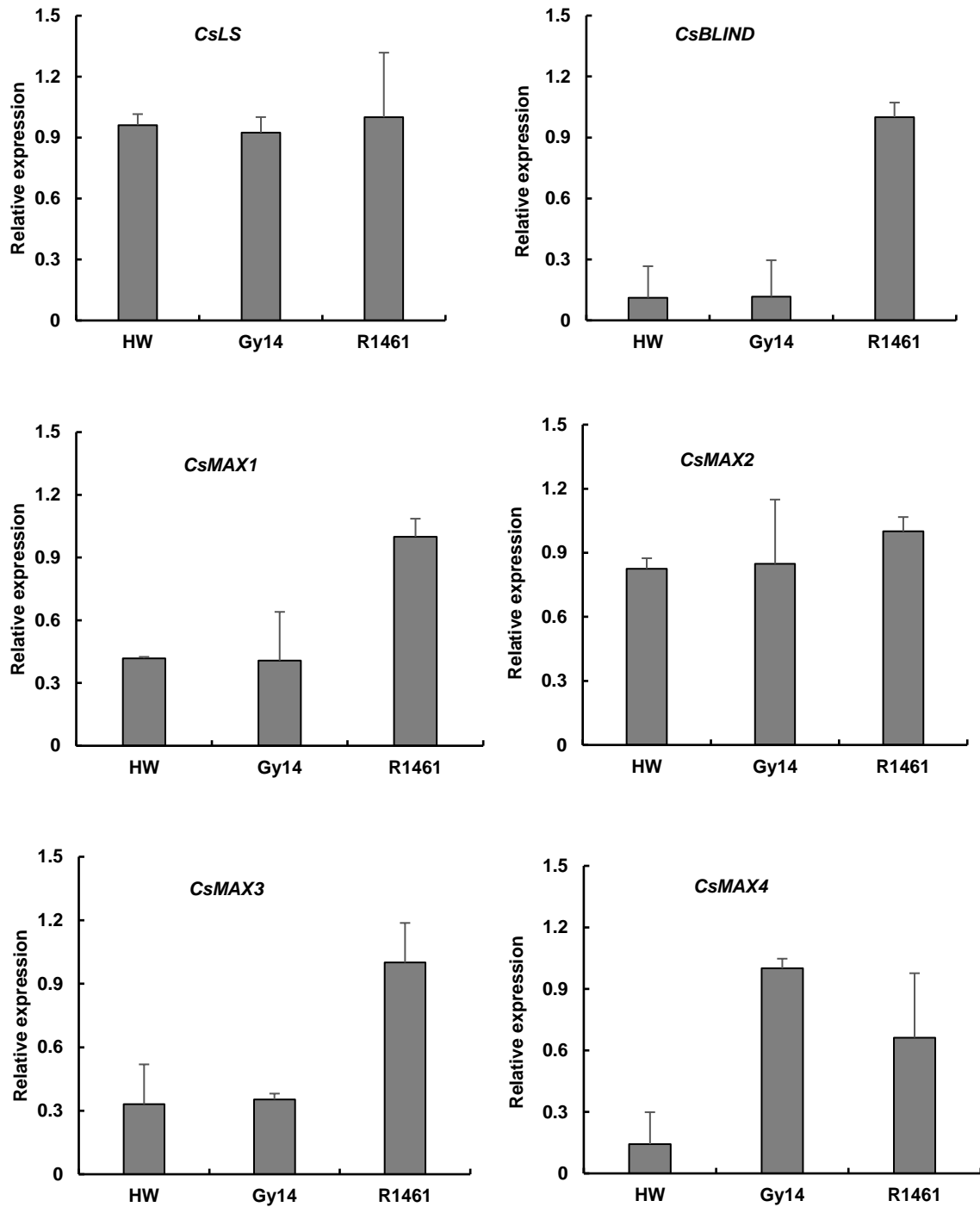


Fig. S1. Expression analyses of *CsLS* (*Csa3G039300*), *CsBLIND* (*Csa1G109320*), *CsMAX1* (*Csa4G646170*), *CsMAX2* (*Csa7G048640*), *CsMAX3* (*Csa6G106700*) and *CsMAX4* (*Csa2G373590*) by qRT-PCR in three cucumber lines. Values are means of three biological replicates of lateral buds from independent plants. Error bars represent \pm SD. HW, *hardwickii*.

		Basic	Helix I	Loop	Helix II	
Monocots	ZmTB1	TDRHSKIRTAQGV	RRMRLSVGVARE	FFALQDR	LGFDKASKTVN	WLLTQSKPAIDRLVD
	OsTB1	KDRHSKIS	TAGGMRDRR	MRLSLDVARK	FFALQDML	GFDKASKTVQWLLNMSKAAIREIMS
	SbTB1	KDRHSKICTAG	GMRDRR	MRLSLDVARK	FFALQDML	GFDKASKTVQWLLNTSKAAIQEIMA
	TaTB1	TDRHSKIRTAQGV	RRMRLSLDVAR	DFALQDQL	GFDKASKTVDWLLTQSKPAIDRLSE	
	CaTB1	KDRHSKICTAG	GMRDRR	MRLSLDVARK	FFALQDML	GFDKASKTVQWLLNTSKSAIQEIMT
Eudicots	AtBRC1	TDRHSKIKTAKG	TRDRR	MRLSLDVAKEL	FFGLQDML	GFDKASKTVEWLLTQAKPEIKIAT
	*CsBRC1	RDRHSKINTLHG	PRDRR	MRLSLPVAKE	FFGLQDML	GVDKASKTVEWLLFQARHAIKKLS-
	CmBRC1	RDRHSKINTLHG	PRDRR	MRLSLPVAKE	FFGLQDML	GVDKASKTVEWLLFQARHAIKKLS-
	NtBRC1	KDRHSKINTAHG	PRDRR	MRLSLDVARK	FFNLQDIL	LGFDKASKTVEWLLTKSKSAINELV-
	SlBRC1	KDRHSKINTARG	PRDRR	MRLSLDAARK	FFRLQDIL	LGFDKASKTVEWLLTQSDSAIEELVA
	StBRC1	KDRHSKINTAHG	PRDRR	MRLSLEIARK	FFNLQDIL	LGFDKASKTVEWLLTKSKSAVNDLV-
	PsBRC1	RDRHSKIKTAKG	LDRDR	MRLSLEVAKR	FFGLQDML	GFDKASKTVDWLLNQSKDGIKQLAG
	GhBRC1	TDRHSKINTANG	PRDRR	MRLSLDVARE	FFGLQDML	GVDKASKTVEWLLVQAKPEITKLM-
	PeBRC1	RDRHSKINTAQ	GPRDRR	MRLSLKVAARE	FFDLQDKL	RFDKASKTVEWLLIQAKNEIKKLS-

Fig. S2. Amino acid alignment of BRC1 and homologs from monocots and eudicots. Zm, *Zea mays*; Os, *Oryza sativa*; Sb, *Sorghum bicolor*; Ta, *Triticum aestivum*; Ca, *Cenchrus americanus*; At, *Arabidopsis thaliana*; Cs, *Cucumis sativus*; Cm, *Cucumis melo*; Nt, *Nicotiana tabacum*; Sl, *Solanum lycopersicum*; St, *Solanum tuberosum*; Ps, *Pisum sativum*; Gh, *Gossypium hirsutum*; Pe, *Populus euphratica*.

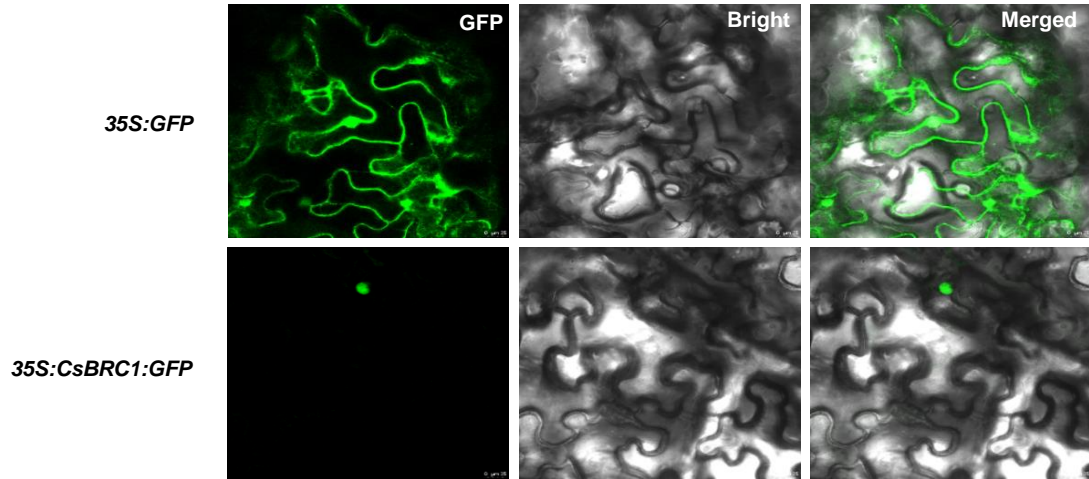


Fig. S3. Subcellular localization of CsBRC1 in tobacco leaves. GFP driven by the 35S promoter was used as a control. GFP is shown in green. The left, middle and right panels represent pictures taken under dark field, bright field and merge views, respectively.

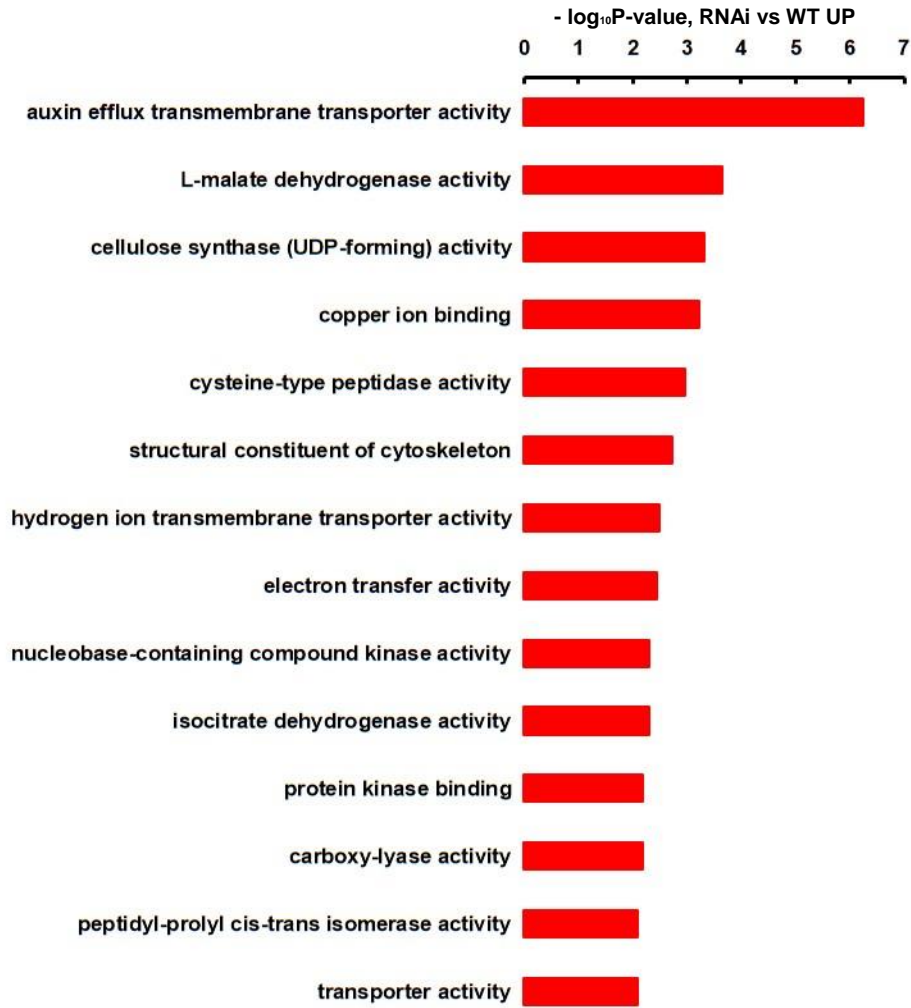


Fig. S4. Gene category enrichment of the up-regulated DEGs in axillary buds of *CsBRC1*-RNAi transgenic plants vs WT.

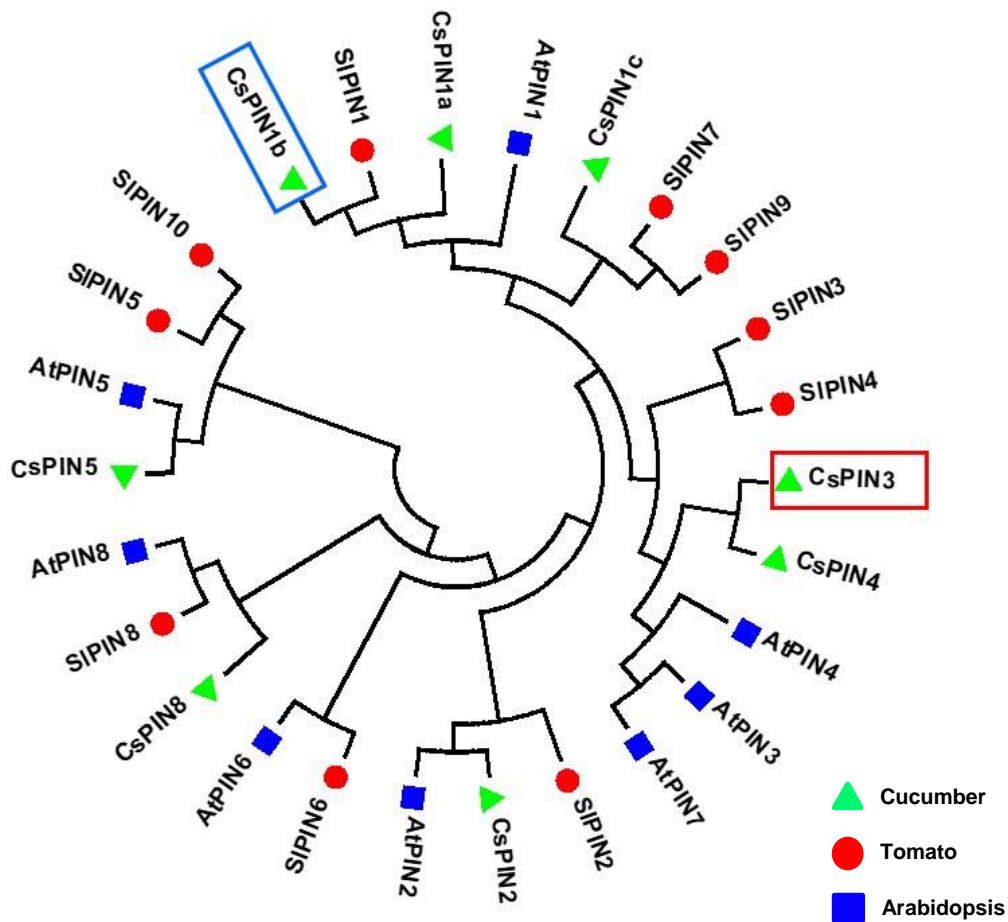


Fig. S5. Phylogenetic analysis of PIN family genes in cucumber, *Arabidopsis* and tomato. There are 8 PIN genes in *Arabidopsis* (blue square), 8 in cucumber (green triangle) and 10 in tomato (red circle). Blue and red squares indicate *CsPIN1b* and *CsPIN3*, respectively.

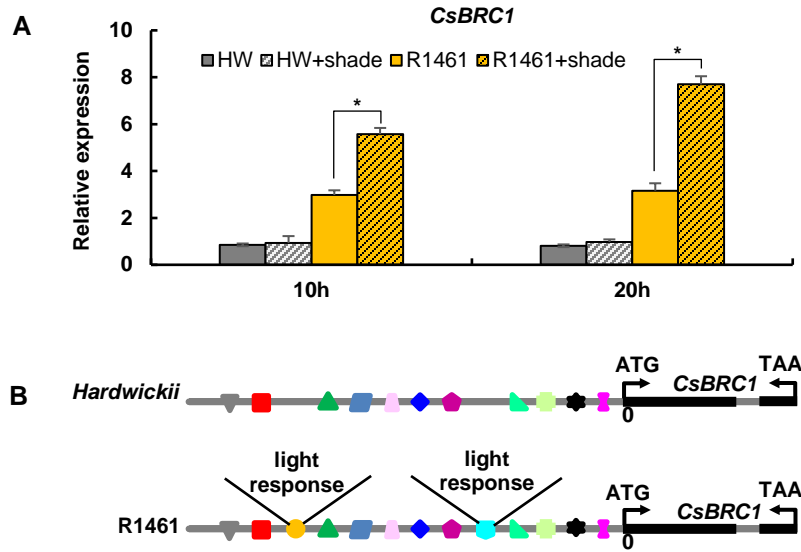


Fig. S6. Responses of *CsBRC1* expression to reduced light intensity in cucumber. (A) The relative expression level of *CsBRC1* in lateral buds upon shade treatment in *hardwickii* and R1461. Values are means of three biological replicates of lateral buds from independent plants. Error bars represent \pm SD. Significance analysis was conducted with the two-tailed Student's t-tests (* $P < 0.05$). (B) Schematic diagram of *cis*-acting regulatory elements on *CsBRC1* promoter in *hardwickii* and R1461. From left to right in *hardwickii*: AAGAA-motif, ABRE, ARE, CAAT-box, CAT-box, ERE, G-box, MYB, P-box, TATA-box and TCA-element. There are two additional light response elements in *CsBRC1* of R1461: AE-box and GATA-motif.

Table S1. Summary of transcriptome sequencing data.

Sample id	Clean reads ratio	Clean bases ratio	Unique mapped reads ratio	Total mapped ratio
WT-1	96.66%	94.24%	88.92%	95.12%
WT-2	96.09%	93.45%	87.77%	93.70%
RNAi-1	96.00%	93.51%	87.05%	92.59%
RNAi-2	95.79%	93.23%	87.46%	93.60%

Table S2. Gene information used in this study.

Gene name	Species	Accession number
<i>CsBRC1</i>	<i>Cucumis sativus</i>	Csa1G020890
<i>CsLS</i>	<i>Cucumis sativus</i>	Csa3G039300
<i>CsBLIND</i>	<i>Cucumis sativus</i>	Csa1G109320
<i>CsMAX1</i>	<i>Cucumis sativus</i>	Csa4G646170
<i>CsMAX2</i>	<i>Cucumis sativus</i>	Csa7G048640
<i>CsMAX3</i>	<i>Cucumis sativus</i>	Csa6G106700
<i>CsMAX4</i>	<i>Cucumis sativus</i>	Csa2G373590
<i>CsPIN1a</i>	<i>Cucumis sativus</i>	Csa1G042820
<i>CsPIN1b</i>	<i>Cucumis sativus</i>	Csa1G025070
<i>CsPIN1c</i>	<i>Cucumis sativus</i>	Csa4G430820
<i>CsPIN2</i>	<i>Cucumis sativus</i>	Csa1G427480
<i>CsPIN3</i>	<i>Cucumis sativus</i>	Csa5G576590
<i>CSPIN4</i>	<i>Cucumis sativus</i>	Csa5G284520
<i>CSPIN5</i>	<i>Cucumis sativus</i>	Csa2G074170
<i>CSPIN8</i>	<i>Cucumis sativus</i>	Csa3G827360
<i>AtPIN1</i>	<i>Arabidopsis thaliana</i>	At1G73590
<i>AtPIN2</i>	<i>Arabidopsis thaliana</i>	At5G57090
<i>AtPIN3</i>	<i>Arabidopsis thaliana</i>	At1G70940
<i>AtPIN4</i>	<i>Arabidopsis thaliana</i>	At2G01420
<i>AtPIN5</i>	<i>Arabidopsis thaliana</i>	At5G16530
<i>AtPIN6</i>	<i>Arabidopsis thaliana</i>	At1G77110
<i>AtPIN7</i>	<i>Arabidopsis thaliana</i>	At1G23080
<i>AtPIN8</i>	<i>Arabidopsis thaliana</i>	At5G15100
<i>SIPIN1</i>	<i>Solanum lycopersicum</i>	NM_001247234
<i>SIPIN2</i>	<i>Solanum lycopersicum</i>	NM_001247241
<i>SIPIN3</i>	<i>Solanum lycopersicum</i>	NM_001247248
<i>SIPIN4</i>	<i>Solanum lycopersicum</i>	NM_001247255
<i>SIPIN5</i>	<i>Solanum lycopersicum</i>	NM_001247263
<i>SIPIN6</i>	<i>Solanum lycopersicum</i>	NM_001247270
<i>SIPIN7</i>	<i>Solanum lycopersicum</i>	NM_001247275
<i>SIPIN8</i>	<i>Solanum lycopersicum</i>	NM_001247275
<i>SIPIN9</i>	<i>Solanum lycopersicum</i>	NM_001247291
<i>SIPIN10</i>	<i>Solanum lycopersicum</i>	NM_001247301
<i>AtBRC1</i>	<i>Arabidopsis thaliana</i>	AT3G18550
<i>CmBRC1</i>	<i>Cucumis melo</i>	XP_008453366
<i>NtTB1</i>	<i>Nicotiana tabacum</i>	XP_016506815
<i>SIBRC1</i>	<i>Solanum lycopersicum</i>	NP_001234572
<i>StBRC1</i>	<i>Solanum tuberosum</i>	XP_006341644
<i>PsBRC1</i>	<i>Pisum sativum</i>	AEL12230
<i>GhBRC1</i>	<i>Gossypium hirsutum</i>	AVA17437
<i>PeBRC1</i>	<i>Populus euphratica</i>	XP_011012754
<i>ZmTB1</i>	<i>Zea mays</i>	NP_001152465
<i>OsTB1</i>	<i>Oryza sativa</i>	XP_015630237
<i>SbTB1</i>	<i>Sorghum bicolor</i>	AAL75986
<i>TaTB1</i>	<i>Triticum aestivum</i>	XP_020173868
<i>CaTB1</i>	<i>Cenchrus americanus</i>	AAU89660

Table S3. Primer information used in this study.

Primers for <i>CsBRC1</i> amplification and vector construction	
<i>CsBRC1-clone-F</i>	ATGTTTGTGTTCAATAGTTGTAGTAAC
<i>CsBRC1-clone-R</i>	TTAAGTTTGTGATGTTTGGAAATTAC
<i>ProCsBRC1-clone-F</i>	ATACACACTATACATGTAAGTCGGAC
<i>ProCsBRC1-clone-R</i>	ATCTCTGATCTTTTAATCTCTCTTTT
<i>CsBRC1-Sense-F</i>	TTGGCGCGCCCTCTTTCAAGCTAGACATGCAA
<i>CsBRC1-Sense -R</i>	ATTTAAATAGTTTGTGATGTTTGGAAATTA
<i>CsBRC1-Antisense-F</i>	GGACTAGTCTCTTTCAAGCTAGACATGCAA
<i>CsBRC1-Antisense-R</i>	CGGGATCCAGTTTGTGATGTTTGGAAATTA
<i>CsPIN1b-clone-F</i>	ATGATTACATTATTAGACTTCTAC
<i>CsPIN1b-clone-R</i>	TCATAATCCCAACAAAATGTAGTA
<i>CsPIN1b-OV-F</i>	GCTCTAGAATGATTACATTATTAGACTTCTAC
<i>CsPIN1b-OV-R</i>	CGGGATCCTCATAATCCCAACAAAATGTAGTA
<i>CsPIN3-clone-F</i>	ATGATTTTCATGGAAGGATCTTTACACCG
<i>CsPIN3-clone-R</i>	TTACAGACCCAGCAGAACATAGTAGAGC
<i>CsPIN3-OV-F</i>	GCTCTAGAATGATTTTCATGGAAGGATCTTTACACCG
<i>CsPIN3-OV-R</i>	CGGGATCCTTACAGACCCAGCAGAACATAGTAGAGC
<i>CsBRC1-1300-F</i>	GCTCTAGAATGTTTGTGTTCAATAGTTGTAGTAAC
<i>CsBRC1-1300-R</i>	ACGCGTCGACAGTTTGTGATGTTTGGAAATTTACCTC
Primers for qRT-PCR	
<i>CsBRC1-Q-F</i>	TCACGAGGGAGTGTAGGGAGAA
<i>CsBRC1-Q-R</i>	GCAATAAATAGAGTTGGAGGGGC
<i>CsLS-Q-F</i>	GGTTTCAGTTTCACCCTTTG
<i>CsLS-Q-R</i>	CAATACTCGAACATCGTCCT
<i>CsBLIND-Q-F</i>	AATAACATTAGCCCTAGCCC
<i>CsBLIND-Q-R</i>	TCCAAACCCTAATAAGCAGC
<i>CsMAX1-Q-F</i>	CATATCTACTCCACAACGCA
<i>CsMAX1-Q-R</i>	CAGGACTTGTCTAAATGGCT
<i>CsMAX2-Q-F</i>	GATGAAGTTGGATTGCAGTG
<i>CsMAX2-Q-R</i>	GCCAATAATCGAGCTCTGTA
<i>CsMAX3-Q-F</i>	AGCCTGTGCTATATGGAGTA
<i>CsMAX3-Q-R</i>	GGTAGAAGCATATCCTCAGC
<i>CsMAX4-Q-F</i>	GAAGCTGCATTATTTACCC
<i>CsMAX4-Q-R</i>	CTTCTGATGGTGAAGTTGGA
<i>CsPIN1b-Q-F</i>	GATTTTTACTCCATGATCGGC
<i>CsPIN1b-Q-R</i>	ATGTTCCGGTGTAGGGTAATG
<i>CsPIN3-Q-F</i>	CGAACACGTATTCAAGTCTG
<i>CsPIN3-Q-R</i>	GCTAAACATAGCCATTCCAAG
<i>CsUBIeq-Q-F</i>	CACCAAGCCCAAGAAGATC
<i>CsUBI-eq-Q-R</i>	TAAACCTAATCACCACCAGC
Primers for <i>in situ</i> probes	
<i>CsBRC1-SP6</i>	GATTTAGGTGACACTATAGAATGCTATCAAGAACTCTCAAAGGATCAAC
<i>CsBRC1-T7</i>	TGTAATACGACTCACTATAGGGGCAATAAATAGAGTTGGAGGGGC
<i>CsPIN3-SP6</i>	GATTTAGGTGACACTATAGAATGCTATGATTTTCATGGAAGGATCTTTACACC
<i>CsPIN3-T7</i>	TGTAATACGACTCACTATAGGGCGGGGAGGAGGGCGGCGGTGGCTGAGG
Primers for yeast one-hybrid, LUC activity measure assay and Electrophoretic mobility shift assay	
<i>ProCsPIN1b-P1-F</i>	AGCTTATTTGGGGTCCATTATTTGGGGTCCATTATTTGGGGTCCATTG
<i>ProCsPIN1b-P1-R</i>	TCGACAATGGACCCCAATAATGGACCCCAATAATGGACCCCAATA
<i>ProCsPIN3-P3A-F</i>	AGCTTGTAGGGGCCACCATGTAGGGGCCACCATGTAGGGGCCACCATG
<i>ProCsPIN3-P3A-R</i>	TCGACTAGGTGGGCCCTACATGGTGGGCCCTACATGGTGGGCCCTACA
<i>ProCsPIN3-P3B-F</i>	AGCTTAGAGGTCCCCTCCAGAGGTCCCCTCCAGAGGTCCCCTCCACTCCG
<i>ProCsPIN3-P3B-R</i>	TCGACGGAGTGGGACCTCTGGAGTGGGACCTCTGGAGTGGGACCTCTA
<i>ProCsPIN3-P3C-F</i>	AGCTTGAAGGACCCACAGGGAAGGACCCACAGGGAAGGACCCACAGGG

<i>ProCsPIN3-P3C-R</i>	TCGACCCTGTGGGTCCTTCCCTGTGGGTCCTTCCCTGTGGGTCCTCA
<i>ProCsPIN3-P3D-F</i>	AGCTTTGAGGGCCAGTGGTGAGGGCCAGTGGTGAGGGCCAGTGGG
<i>ProCsPIN3-P3D-R</i>	TCGACCCTGTGGGTCCTTCCCTGTGGGTCCTTCCCTGTGGGTCCTCA
<i>ProCsPIN3-P3E-F</i>	AGCTTAGTGGGCCCCAAATGAGTGGGCCCCAAATGAGTGGGCCCCAAATGG
<i>ProCsPIN3-P3E-R</i>	TCGACCATTTGGGCCCCACTCATTTGGGCCCCACTCATTTGGGCCCCACTA
<i>CsBRC1-AD-F</i>	GGAATTCCATATGATGTTTGTGTTCAATAGTTGTAGTAA
<i>CsBRC1-AD-R</i>	CGGAATTCCTAAGTTTGTGATGTTTGGAAAT
<i>ProCsPIN1b-0800-F</i>	GGTATCGATAAGCTTTGACAAAATAATGCAAAGCTTCATC
<i>ProCsPIN1b-0800-R</i>	AGAAGTAGTGGATCCTTTGAAAAGAAGTTTGAATTCAGAGAG
<i>ProCsPIN3-0800-F</i>	GGTATCGATAAGCTTCTTTCAATATTCATGGGTTTTGACC
<i>ProCsPIN3-0800-R</i>	AGAAGTAGTGGATCCGGCGGTTTTTGTGGTGAGTGGCAG
<i>CsBRC1-62SK-F</i>	GCTCTAGAATGTTTGTGTTCAATAGTTGTAGTAA
<i>CsBRC1-62SK-R</i>	CGCGGATCCTTAAGTTTGTGATGTTTGGAAATTTACCTC
<i>CsBRC1-MBP-F</i>	CGGGATCCATGTTTGTGTTCAATAGTTGTAGT
<i>CsBRC1-MBP-R</i>	ACGCGTCGACTTAAGTTTGTGATGTTTGGAAAT
<i>P1-CHIP-F</i>	TTAAGAGAGAGTGTGGGAAAC
<i>P1-CHIP-R</i>	TAGTGGGAAGAGGAGAAAAGG
<i>P3A-CHIP-F</i>	GGATGTGAGTGAGAGAGGAT
<i>P3A-CHIP-R</i>	AACCGCCTACTATGGTTAGTT
<i>P3B-CHIP-F</i>	GGTCGATTGTCTAATTTCCG
<i>P3B-CHIP-R</i>	TTGAGGCTGAACGGCAGAGT
<i>P3C-CHIP-F</i>	TCAGCGGCAAAGAAGGGGAG
<i>P3C-CHIP-R</i>	TGCGCCGGCGGAAACTTTCC
<i>P3DE-CHIP-F</i>	AATCTTGGGCCTTGACCAGC
<i>P3DE-CHIP-R</i>	GGACTGTACAATGGATGGCA
<i>P1-BIOTIN-F</i>	GTGGGAAACAAAATTTGGGGTCCATTAATATATATAT
<i>P1-BIOTIN-R</i>	ATATATATATTTAATGGACCCCAAATTTTGTTCAC
<i>P3A-BIOTIN-F</i>	CACTTGTGCAAAGTAGGGCCACCATAACCCGAATTGG
<i>P3A-BIOTIN-R</i>	CCAATTCGGGTTATGGTGGGCCCTACTTTGCACAAGTG
<i>P3B-BIOTIN-F</i>	GTTTCAGTCCTCCAGAGGTCCCACTCCCCGGCCGTCAA
<i>P3B-BIOTIN-R</i>	TTTGACGGCCGGGAGTGGGACCTCTGGAGGACTGAAC
<i>P3C-BIOTIN-F</i>	GATGATCAGAAAGAAGGACCCACAGGCTCAACAGGAGA
<i>P3C-BIOTIN-R</i>	TCTCCTGTTGAGCCTGTGGGTCCTTCTTTCTGATCATC
<i>P1-M-F</i>	GTGGGAAACAAAATTTGGGGAAGATTAATATATATAT
<i>P1-M-R</i>	ATATATATATTTAATCTTCCCAAATTTTGTTCAC
<i>P3A-M-F</i>	CACTTGTGCAAAGTAGGGTGTACCATAACCCGAATTGG
<i>P3A-M-R</i>	CCAATTCGGGTTATGGTACACCCTACTTTGCACAAGTG
<i>P3B-M-F</i>	GTTTCAGTCCTCCAGAGGTGATACTCCCCGGCCGTCAA
<i>P3B-M-R</i>	TTTGACGGCCGGGAGTATCACCTCTGGAGGACTGAAC
<i>P3C-M-F</i>	GATGATCAGAAAGAAGGAAAGACAGGCTCAACAGGAGA
<i>P3C-M-R</i>	TCTCCTGTTGAGCCTGTCTTCCCTTCTTTCTGATCATC

Additional dataset S1 (separate file)

Dataset S1. List of genes that were differentially expressed in *CsBRC1*-RNAi plants.