

Figure S1 Genotyping and phenotypic analysis of *Csexo70b* mutants. (a) Sequencing analysis of target sites in *Csexo70b* mutants, *Csexo70b-5[#]* and *Csexo70b-4[#]* mutants led to truncated proteins with 136 amino acid (aa) and 156 aa, respectively. (b) Morphology of male flowers in *Csexo70b* mutants at anthesis. Scale bar = 2 cm. (c) Morphology of cucumber seeds of WT and *Csexo70b* mutants. Scale bar = 1 cm. (d) Cross-sections indicated WT and *Csexo70b* mutants exhibited different carpel numbers. Scale bar = 2 cm. (e-h), Quantification analysis of diameter of male flower (e), seed length (f), carpel numbers (g), and plant height (h) of WT and *Csexo70b* mutants. Data are shown as means \pm SD, $n \geq 6$. Statistical significances were indicated by asterisks (**, $P < 0.01$, one-way ANOVA with Tukey's test).

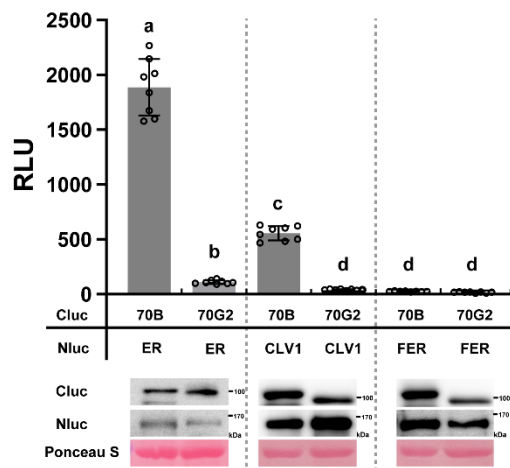


Figure S2 CsExo70B interacts with CsERECTA and CsCLV1 in *Nicotiana benthamiana* (*N. benthamiana*) plants. The indicated constructs were expressed in *N. benthamiana* leaves, and luciferase activity was recorded by a luminometer. Values are means \pm SD ($n = 8$). The different lowercase letters indicate significant differences ($P < 0.01$) by one-way ANOVA analysis with Tukey's test. These indicated fusion proteins were detected by anti-Cluc and anti-HA antibodies, respectively. 70B, CsExo70B; 70G2, CsExo70G2; ER, CsERECTA; FER, CsFERONIA.

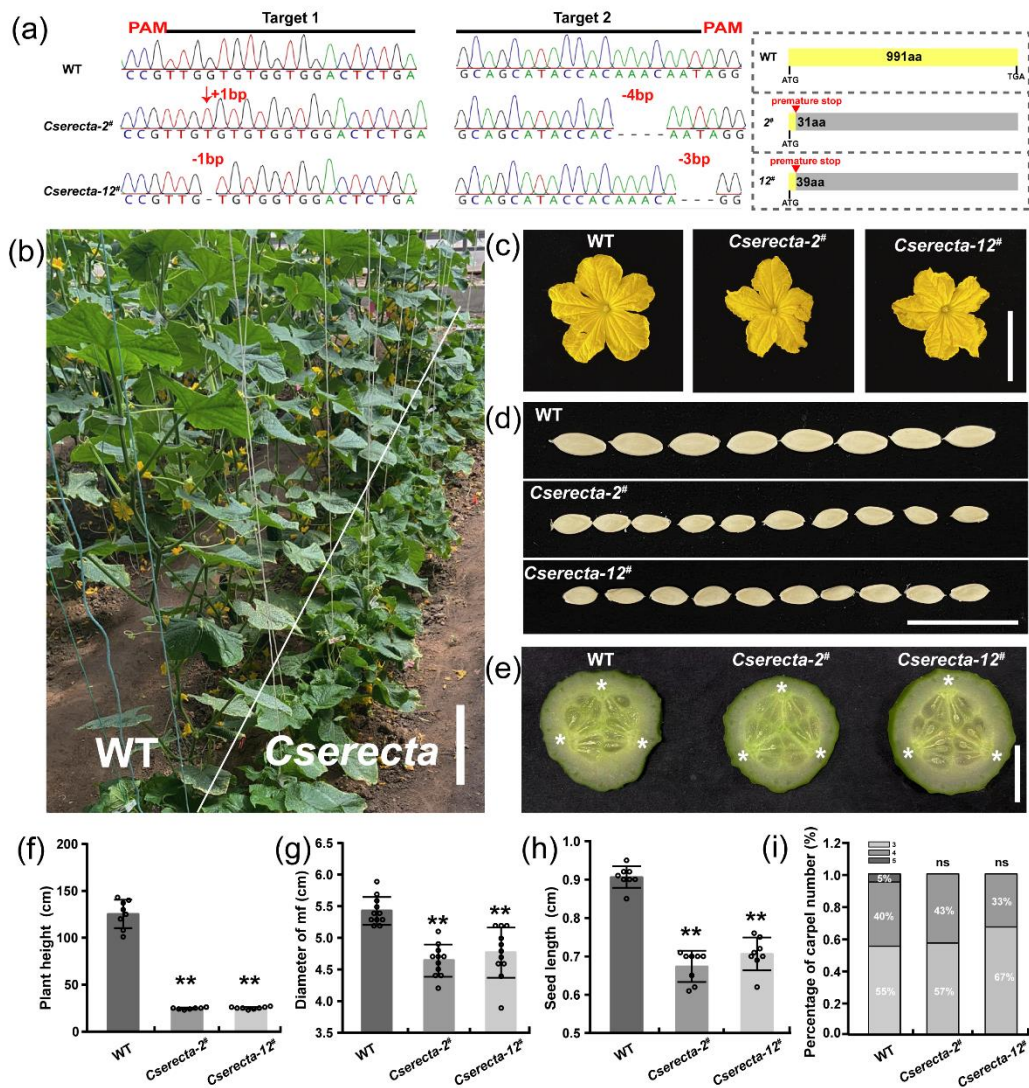


Figure S3 Knockout of *CsERECTA* resulted in dwarfed plants, smaller male flowers and seeds in cucumber. (a) Sequencing analysis of target sites in *Cserecta* mutants, both *Cserecta-2[#]* and *Cserecta-12[#]* mutants lead to truncated proteins with 31 aa and 39 aa in length, respectively. (b-e) Phenotypes of whole plant (b), male flower (c), seed length (d) and carpel number (e) in *Cserecta* mutants, scale bar = 20 cm (b) or 2 cm (c-e). (f-i) Quantification of plant height (f), diameter of male flower (g), seed length (h) and carpel numbers (i) in WT and *Cserecta* mutants, data are shown as means \pm SD ($n \geq 8$), statistical significances were determined by one-way ANOVA (**, $P < 0.01$; ns, no significant difference).

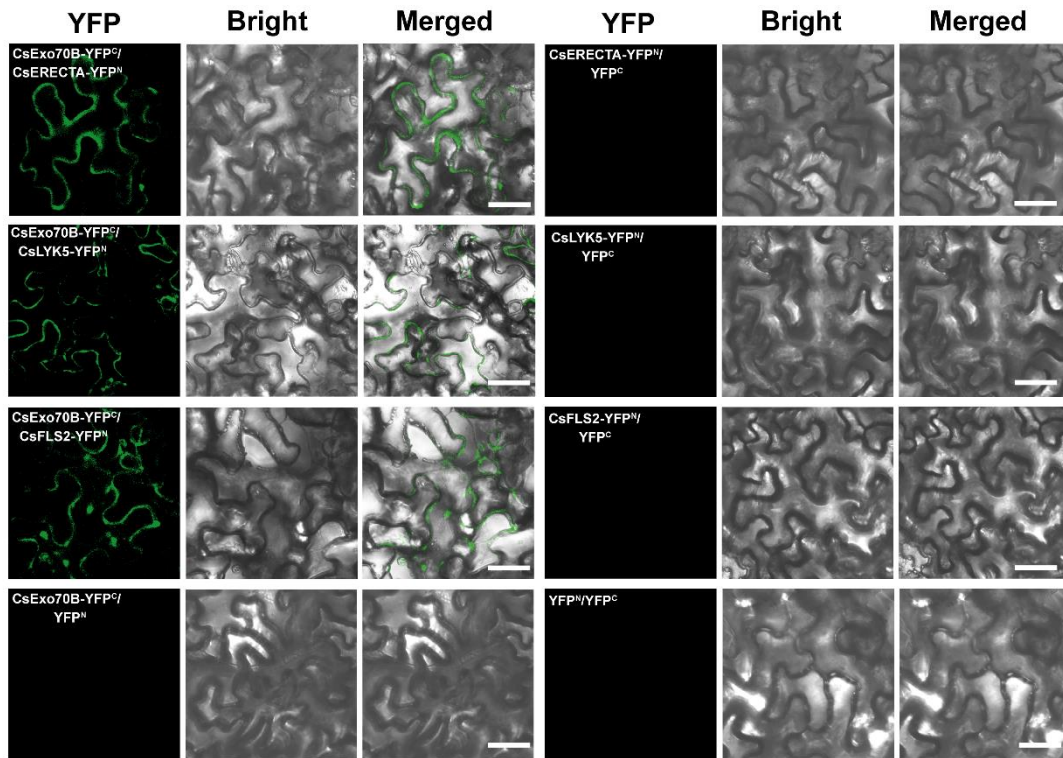


Figure S4 The bimolecular fluorescence complementation (BiFC) assays showing CsExo70B interacts with CsERECTA, CsFLS2 and CsLYK5. Agrobacteria containing the indicated constructs were infiltrated into *N. benthamiana* leaves, and YFP signals were captured by confocal microscopy after 72h post-infiltration, scale bar = 20 μ m.

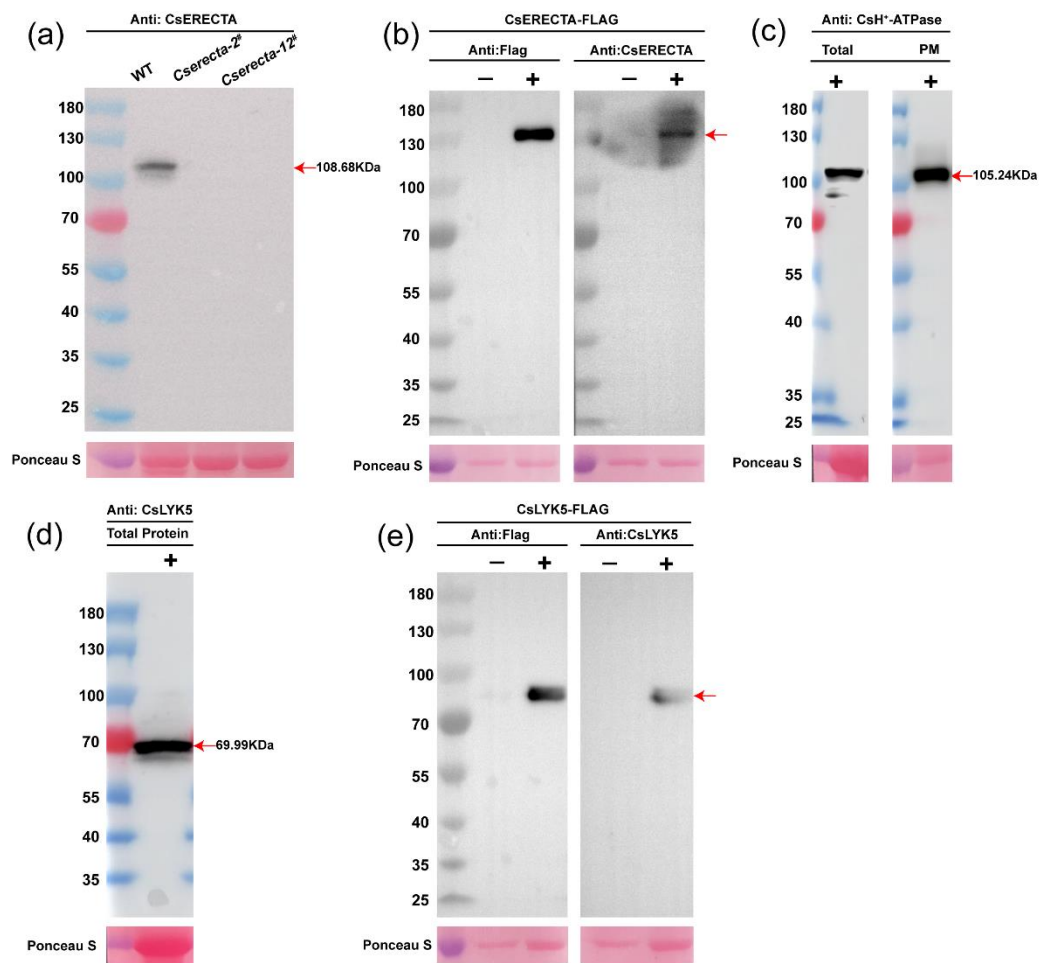


Figure S5 Detection the specificity of CsERECTA, CsLYK5, and CsH⁺-ATPase antibodies. (a) Total proteins were extracted from cucumber plants, and subjected to immunoblotting using anti-CsERECTA antibody. *Cserecta* mutants were used as the negative control. The predicted molecular weight of CsERECTA protein was 108.68kDa. (b) Total protein were extracted from *N. benthamiana* leaves expressing CsERECTA-FALG, and detected using anti-FALG and anti-CsERECTA antibodies, respectively. (c) Detection of CsH⁺-ATPase antibody in cucumber total and plasma membrane (PM) proteins, the predicted molecular weight of CsH⁺-ATPase was 105.24kDa. (d) Detection of CsLYK5 antibody in cucumber total protein, the predicted molecular weight of CsLYK5 protein was 69.99kDa. (e) Total protein were extracted from *N. benthamiana* leaves expressing CsLYK5-FALG, and detected by anti-FALG and anti-CsLYK5 antibodies, respectively.

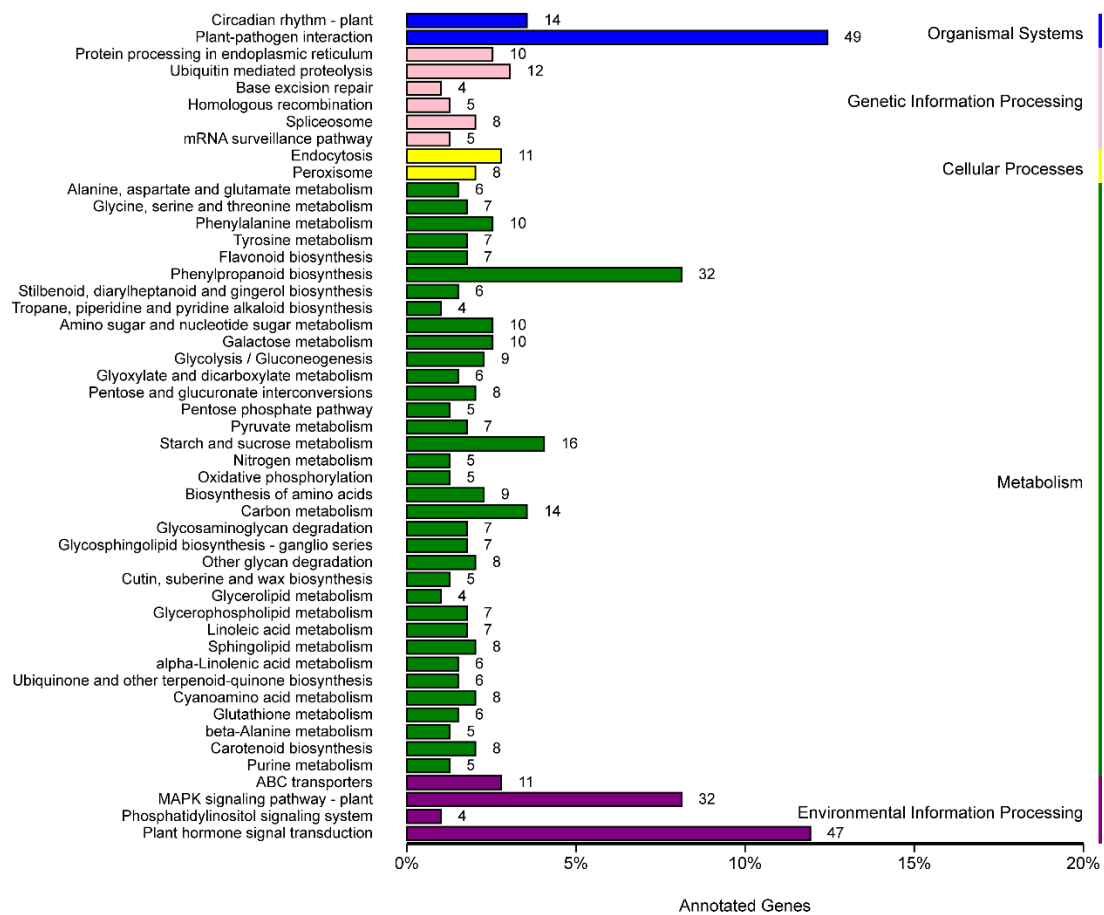


Figure S6 KEGG enrichment analyses of common up-regulated DEGs in *Csexo70b-5#* and *Cserecta-2#* mutants.

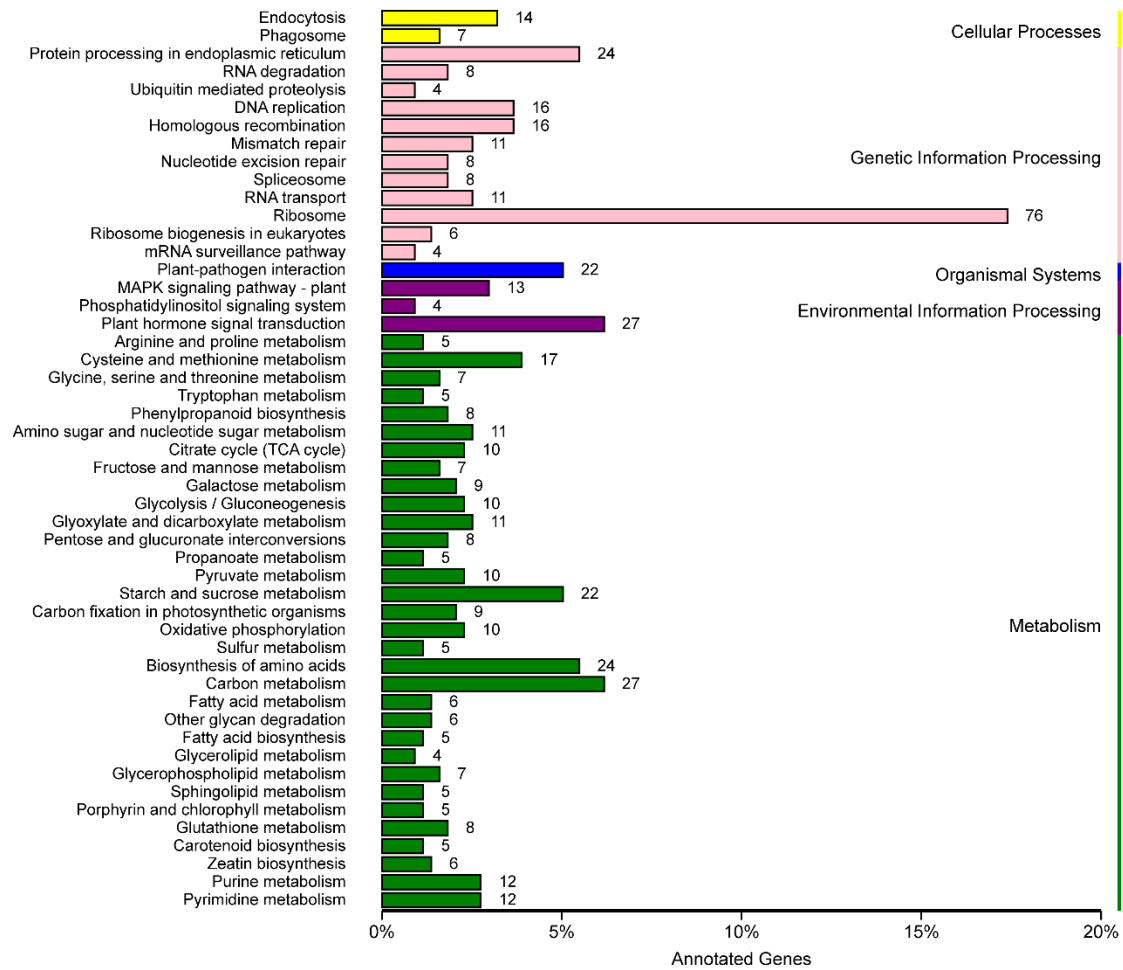


Figure S7 KEGG enrichment analyses of common down-regulated DEGs in *Csexo70b-5#* and *Cserecta-2#* mutants.

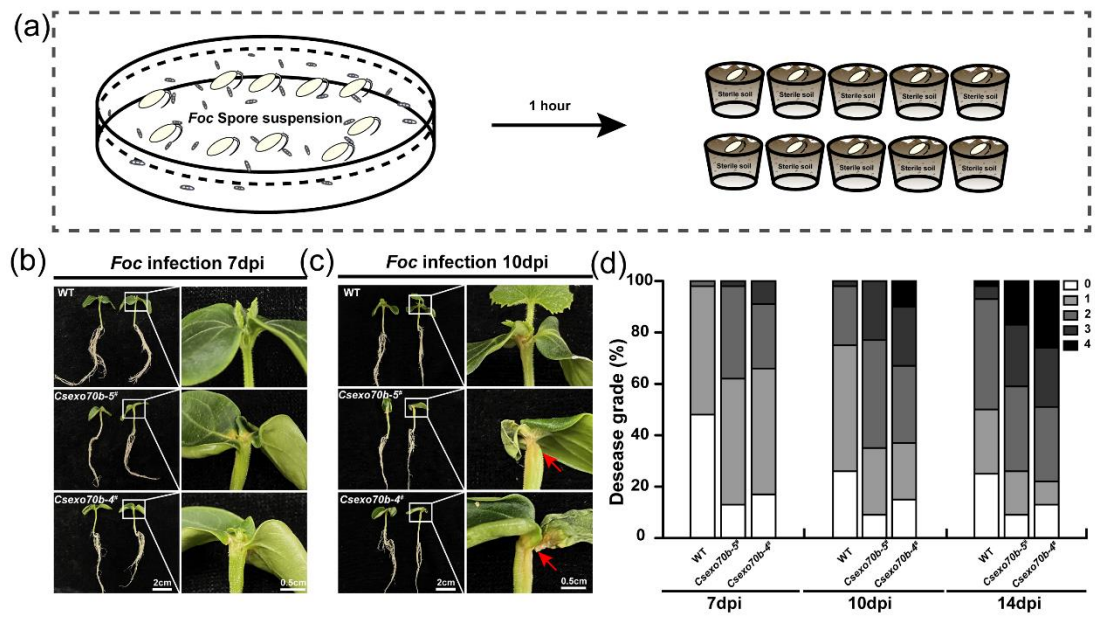


Figure S8 Knockout of *CsExo70B* promotes *Fusarium oxysporum* f. sp. *Cucumerinum* Owen (*Foc*) infection in cucumber. (a) The schematic diagram of radicle dipping infection method. The cucumber seedlings with 1cm radicles were inoculated in *Foc* spore suspension for 1 hour, and then planted in sterilized soil. Disease index (DI) was measured at indicated time points. (b and c) The morphology of *Csexo70b* mutants and WT at 7dpi (b) and 10 dpi (c). Red arrows mean wilted stems and leaves. (d) The disease grade was counted from 30 plantlets in WT and *Csexo70b* mutants, respectively.

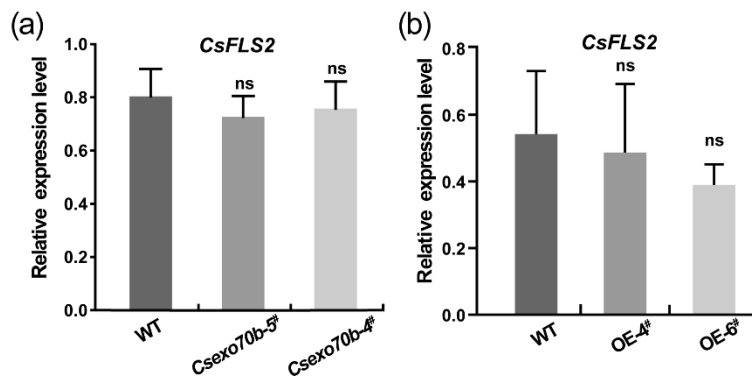


Figure S9 The expression level of *CsFLS2* was normal in *Csexo70b* mutants and *CsExo70B*-OE lines. (a and b) Transcript analysis of *CsFLS2* in *Csexo70b* mutants (a) and *CsExo70B*-OE lines (b). Data are means \pm SD, $n = 3$ (ns, no significant difference, one-way ANOVA analysis with Tukey's test).

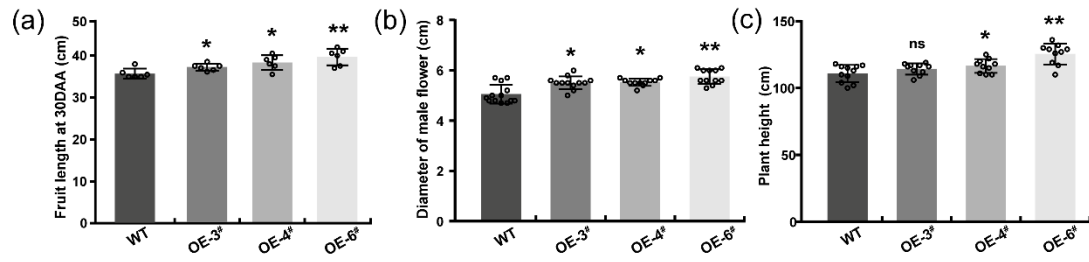


Figure S10 Phenotypic analysis of *CsExo70B* overexpression lines. (a) Quantification of fruit length at 30 DAA, $n = 6$. (b and c) Quantification of diameter of male flower (c), and plant height (d) in WT and *CsExo70B* overexpression lines, $n \geq 10$. Data are shown as means \pm SD (*, $P < 0.05$; **, $P < 0.01$; one-way ANOVA with Tukey's test).

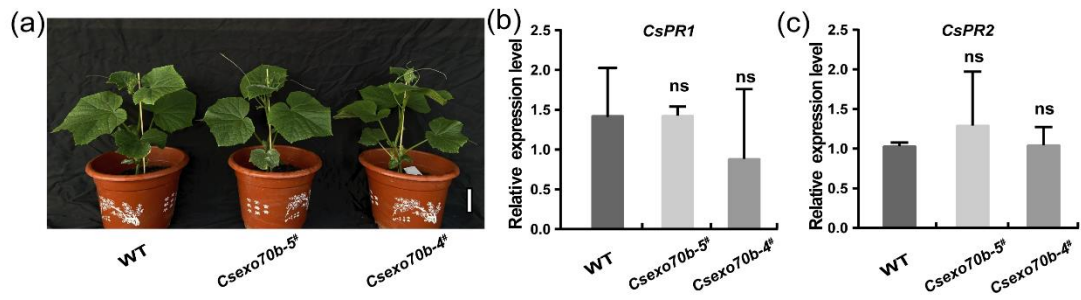


Figure S11 The phenotypes of *Csexo70b* mutants. (a) No cell death was observed in the *Csexo70b* mutants. Scale bar = 5cm. (b and c) The expression levels of *CsPR1* (b) and *CsPR2* (c) were normal in *Csexo70b* mutants. Data are means \pm SD, $n \geq 2$ (ns, no significant difference, one-way ANOVA).

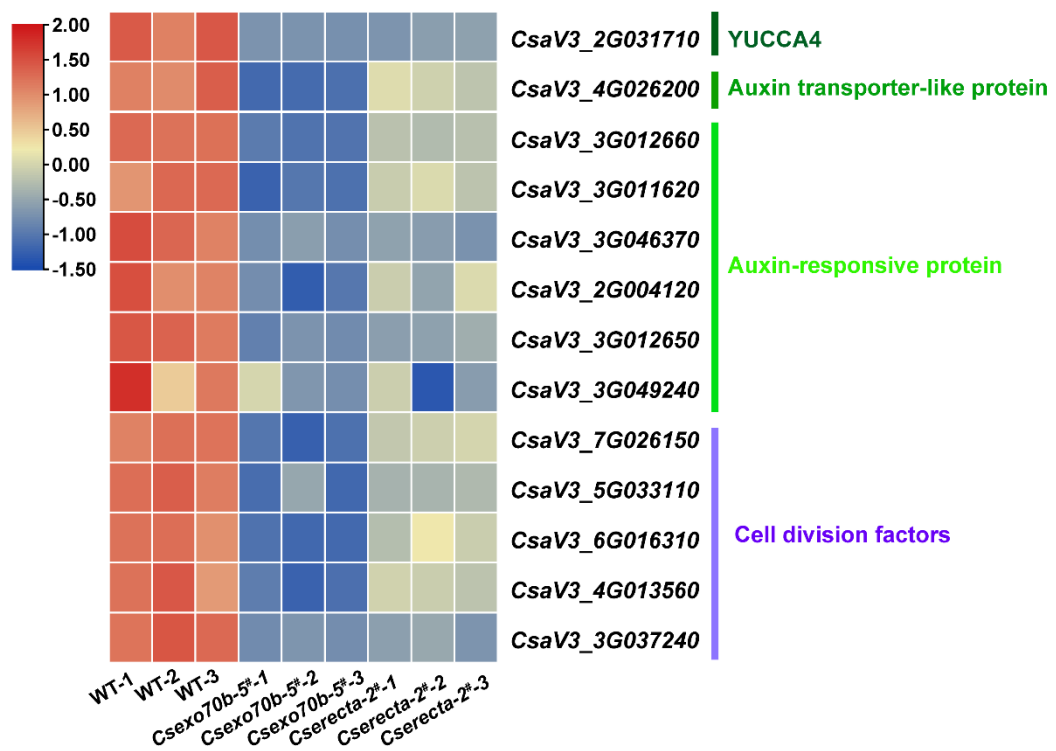


Figure S12 Heat map showing the expression pattern of genes involved in auxin-related and cell division-related pathway. Three biological replicates were performed and the colored bar on the left represents fold change (log2 value).

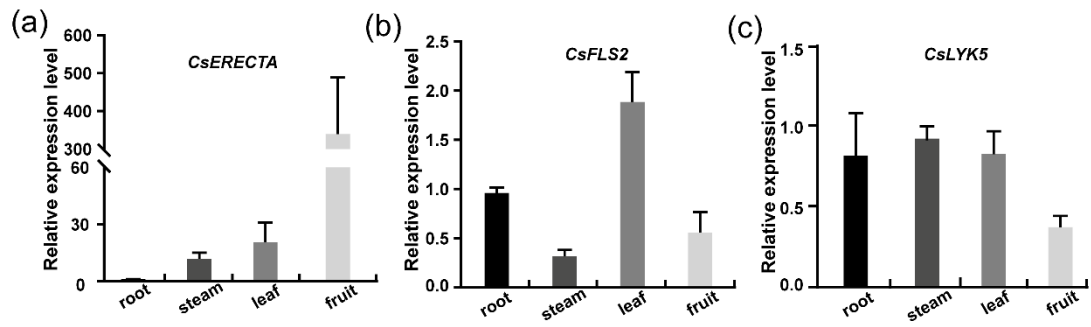


Figure S13 Expression patterns of *CsERECTA*, *CsFLS2*, and *CsLYK5* in cucumber different tissues. Data represent means \pm SD, n = 3.

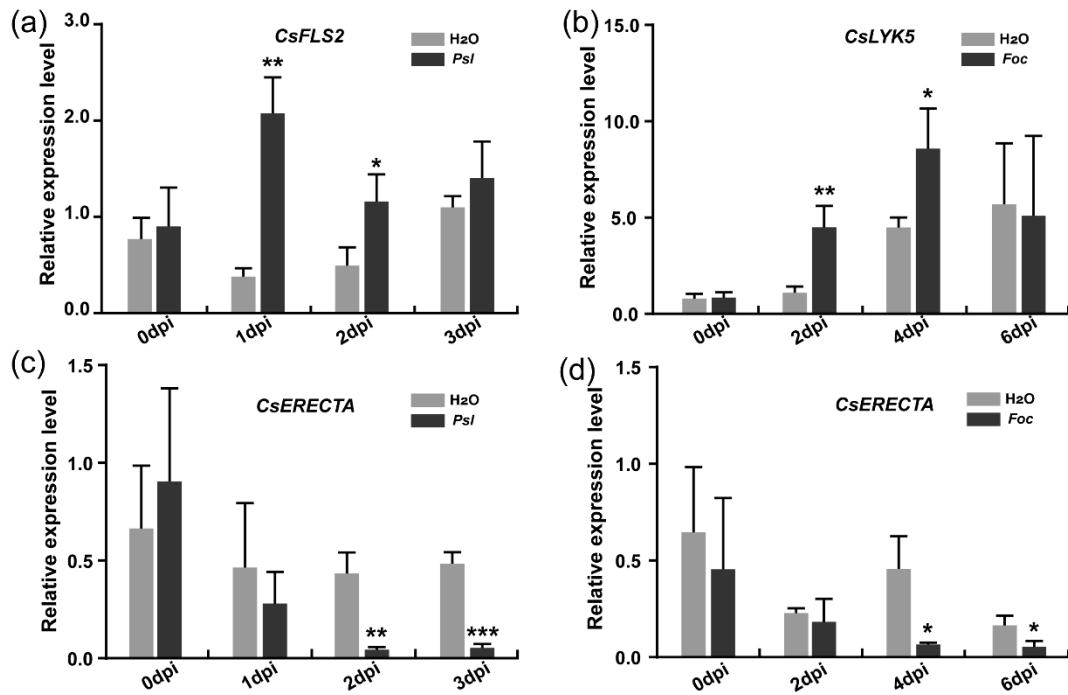


Figure S14 Pathogen-induced *RK* expression in cucumber. (a) *Psi*-induced *CsFLS2* expression. (b) *Foc*-induced *CsLYK5* expression. (c and d) The expression of *CsERECTA* was significantly down-regulated by *Psi* (c), and *Foc* (d) infection. The cucumber leaves were infected by *Psi*, and roots were inoculated by *Foc*, H₂O treatment as control. qRT-PCR data are means \pm SD, n = 3. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, Student's *t* test).

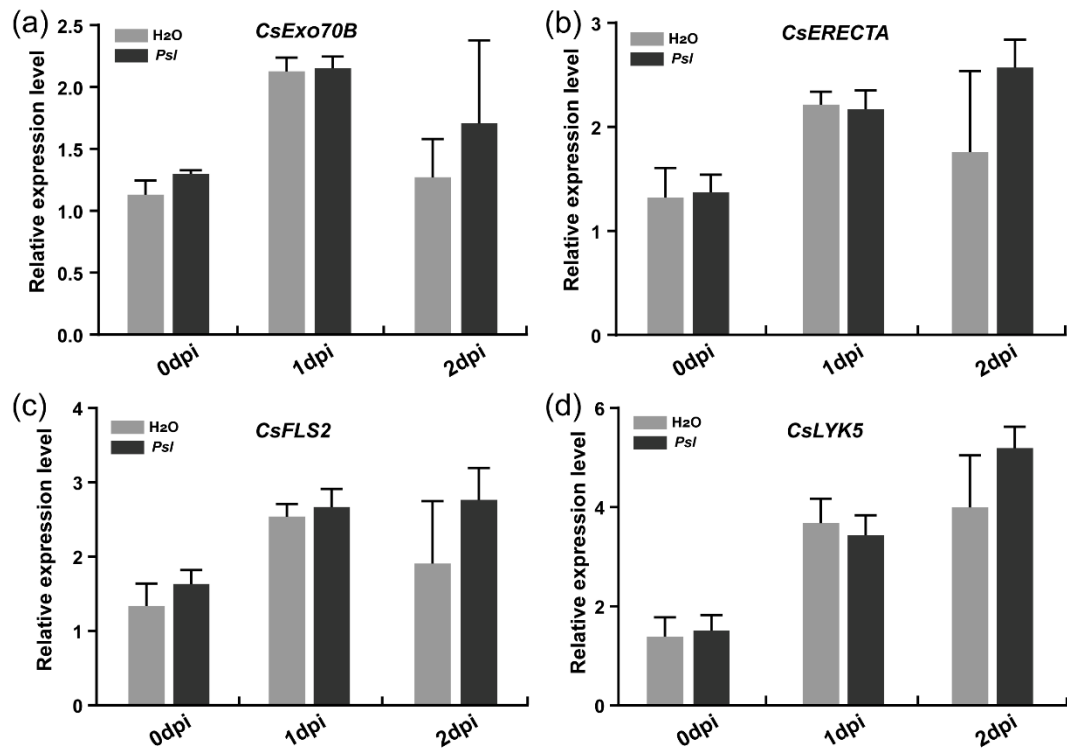


Figure S15 The expression of *CsExo70B* and *RKs* were normal in fruits infected by pathogen. The transcripts of *CsExo70B* (a), *CsERECTA* (b), *CsFLS2* (c), and *CsLYK5* (d) were analyzed in ovaries at anthesis after 0, 1, and 2dpi with *PsI* infection. Data are means \pm SD, n = 3.

Table S1. List of off-target analyses of CsExo70B and CsERECTA

Genes	Potential off-target sites				Examined plants	Presence of mutation
	Off-target	Sequence	Region	Number of mismatches		
<i>CsExo70B</i>	1	CTGTG ACGACGAAGAGTTCCGG	CDS	3	5 [#] &4 [#]	none
<i>CsExo70B</i>	1	CAGTGT CCAAGAAGAGTTCTGG	CDS	4	5 [#] &4 [#]	none
<i>CsExo70B</i>	1	CGCTGT CGACGAAGGTTCTGG	CDS	4	5 [#] &4 [#]	none
<i>CsExo70B</i>	2	GATGGG GCGGAGGGAATTCCGG	CDS	4	5 [#] &4 [#]	none
<i>CsExo70B</i>	2	GTAGT GCGGAGGCAATTCCGG	CDS	4	5 [#] &4 [#]	none
<i>CsExo70B</i>	2	GAAGTT GATGAAGCGATTCCGGG	CDS	4	5 [#] &4 [#]	none
<i>CsExo70B</i>	2	GAAGT AGCCGAAGAAATTGAGG	CDS	4	5 [#] &4 [#]	none
<i>CsERECTA</i>	1	TGAG CGTCGACCACCAACAG	CDS	3	2 [#] &12 [#]	none
<i>CsERECTA</i>	1	G CAGAGTTCATCACTCCAAAGG	CDS	3	2 [#] &12 [#]	none
<i>CsERECTA</i>	1	TCA AAATTCAGCACACCAACAG	CDS	4	2 [#] &12 [#]	none
<i>CsERECTA</i>	2	TTAGAATATCACAACAATAGG	intron	4	2 [#] &12 [#]	none
<i>CsERECTA</i>	2	CCAGCAAACCACAAAAAACGG	intron	4	2 [#] &12 [#]	none
<i>CsERECTA</i>	2	TCAGCAGACCAAAACCAATGGG	CDS	4	2 [#] &12 [#]	none
<i>CsERECTA</i>	2	TCAGCATCCCACAATAATTGG	intron	4	2 [#] &12 [#]	none

Note: Bold represents PAM sequences; Red represents mismatch.

Table S4. List of primers used in this study

Primers for cucumber transformation	
<i>CsExo70B-DT1-BSF</i>	5'- ATATATGGTCTCGATTGCCGTGTCGACCAAGAGTTCGTT -3'
<i>CsExo70B-DT1-F0</i>	5'- TGCCGTGTCGACCAAGAGTTTCGTTTTAGAGCTAGAAAATAGC -3'
<i>CsExo70B-DT1-BSR</i>	5'- AACGAATTCCTTCCGCAACTTCCAATCTCTTAGTCGACTCTAC -3'
<i>CsExo70B-DT1-R0</i>	5'- ATTATTGGTCTCGAAACGAATTCCTTCCGCAACTTCCAA -3'
<i>CsERECTA-DT1-BSF</i>	5'- ATATATGGTCTCGATTGTCAGAGTCCACCACACCAAGTT -3'
<i>CsERECTA -DT1-F0</i>	5'- TGTCAGAGTCCACCACACCAAGTTTTAGAGCTAGAAAATAGC-3'
<i>CsERECTA -DT1-BSR</i>	5'-ATTATTGGTCTCGAAACATTGTTTGTGGTATGCTGCCAA-3'
<i>CsERECTA -DT1-R0</i>	5'-AACATTGTTTGTGGTATGCTGCCAATCTCTTAGTCGACTCTAC-3'
<i>CsExo70B-PUC19-HA-F (KpnI)</i>	5'-cgggggacgagctc ggtacc ATGGTTGACAATGGCGAAGAAAAGT-3'
<i>CsExo70B-PUC19-HA-R (Sall)</i>	5'-acatcgatggta gtcgac TCAAGCGTAATCTGGAACGTC-3'
<i>CsExo70B-HA-1305.4-F (SmaI)</i>	5'-cgggggactctagaggatc cccggg ATGGTTGACAATGGCGAAGAAAAGT-3'
<i>CsExo70B-HA-1305.4-R (BstEI)</i>	5'-gaacgatcggggaaattcgagct ggtcacc TCAAGCGTAATCTGGAACGTC-3'
Primers for qRT-PCR	
<i>CsExo70B-Q-F</i>	5'-TCCGGGACTATCAACGATCT-3'
<i>CsExo70B-Q-R</i>	5'-TCCCTCCTGCAGCTACTATAA-3'
<i>CsERECTA-Q-F</i>	5'-GACGAGATTGGTGACTGTTCTT-3'
<i>CsERECTA-Q-R</i>	5'-CATTTCAGCTGCTTCAACTTC-3'
<i>CsLYK5-Q-F</i>	5'-CAATCAACTGACCGGACTCTAC-3'
<i>CsLYK5-Q-R</i>	5'-GGAAGGAACGGAATCGTAAGG-3'
<i>CsFLS2-Q-F</i>	5'-CTGCAATTGGTCTGGCATTATC-3'
<i>CsFLS2-Q-R</i>	5'-GAGCTGAGAGGTTCCCAATAAA-3'
<i>CsPR1-Q-F</i>	5'-TCACCTCCTATCGCTCAA-3'
<i>CsPR1-Q-R</i>	5'-AGTTCGCTACAGTTTCATCCC-3'
<i>CsPR2-Q-F</i>	5'-CCTTATGCCTTCTTGGTCTTCT-3'
<i>CsPR2-Q-R</i>	5'-AGAGAGTCACCACTTCTGTTTG-3'
Primers for off-target sites analysis of <i>CsExo70B</i> and <i>CsERECTA</i>	
<i>CsExo70B-T1-off1-F</i>	5'-ATGCGAGTCACGGTATCAG-3'
<i>CsExo70B-T1-off1-R</i>	5'-CGTCCGGATCGGGCACC-3'
<i>CsExo70B-T1-off2-F</i>	5'-CACTCTTAGAGGGAAGCTG-3'
<i>CsExo70B-T1-off2-R</i>	5'-CGGCATTGAGTACTAGTGTC-3'
<i>CsExo70B-T1-off3-F</i>	5'-ATGGCCTACGATTACGGATC-3'
<i>CsExo70B-T1-off3-R</i>	5'-CAACAACATTCTTTCCTGGTTC-3'
<i>CsExo70B-T2-off1-F</i>	5'-GAGATATTATCTTTAACGGGATC-3'
<i>CsExo70B-T2-off1-R</i>	5'-TCAATAAGATGCTCTCCC-3'
<i>CsExo70B-T2-off2-F</i>	5'-ATGCCTCAATCTTCTTTCAA-3'
<i>CsExo70B-T2-off2-R</i>	5'-CACTACCACTACCACTACCACC-3'
<i>CsExo70B-T2-off3-F</i>	5'-GAAACTTATGCAATGCTG-3'
<i>CsExo70B-T2-off3-R</i>	5'-CTAATCTCTACCAACAG-3'
<i>CsExo70B-T2-off4-F</i>	5'-GGGCATCCCGTACATGTTCCGGTT-3'
<i>CsExo70B-T2-off4-R</i>	5'-AGTATACCGATCACCCTCCCG-3'
<i>CsERECTA-T1-off1-F</i>	5'-TTTGAAGATGTTGCTGATAGTAAGG-3'

<i>CsERECTA-T1-off1-R</i>	5'-ATTGTTGCATTACTGGCTAAGATAC-3'
<i>CsERECTA-T1-off2-F</i>	5'-TGGTTGGAGATAAAGAGCATCC-3'
<i>CsERECTA-T1-off2-R</i>	5'-GCTGCATGTAACCCCTTCTAGT-3'
<i>CsERECTA-T1-off3-F</i>	5'-ACTGATCCAGCGACTATTCTAAC-3'
<i>CsERECTA-T1-off3-R</i>	5'-ACGGGAGAAGAGATAGGAAATTG-3'
<i>CsERECTA-T2-off1-F</i>	5'-TCCATTGGGATCTCCAGGGA-3'
<i>CsERECTA-T2-off1-R</i>	5'-CATGCATTGTAATCTAAGA-3'
<i>CsERECTA-T2-off2-F</i>	5'-CTACGAGTTACCTTGACACCTAAA-3'
<i>CsERECTA-T2-off2-R</i>	5'-ACGCATCTCAGTTACCTTATCC-3'
<i>CsERECTA-T2-off3-F</i>	5'-CTCATGTAGAGTTTGGGAAGGAG-3'
<i>CsERECTA-T2-off3-R</i>	5'-GGAGGCTAAACATGGCAAATG-3'
<i>CsERECTA-T2-off4-F</i>	5'-ACTAGTGGCTCCATAGCTGAA-3'
<i>CsERECTA-T2-off4-R</i>	5'-CCGTTGCACACGTCGATTA-3'

Primers for BiFC, Split-LUC and Co-IP

<i>CsExo70B-CE-F (BamHI)</i>	5'-cgcgccactagtgatccATGGTTGACAATGGCGAAGAA-3'
<i>CsExo70B-CE-R (BamHI)</i>	5'-agtactatcgatgatccCCTCCTTCCACTGCCTGA-3'
<i>CsERECTA-NE-F (BamHI)</i>	5'-cgcgccactagtgatccATGAAGTCTGTGAAGAGGGCAG-3'
<i>CsERECTA-NE-R (BamHI)</i>	5'-agtactatcgatgatccCTCACTGTTTTGTGATATTACC-3'
<i>CsFLS2-NE-F (BamHI)</i>	5'-cgcgccactagtgatccATGGCTTCTTACGTTAGTTTAGC-3'
<i>CsFLS2-NE-R (BamHI)</i>	5'-agtactatcgatgatccCAATTCATCCCTCTGCAGCT-3'
<i>CsLYK5-NE-F (BamHI)</i>	5'-cgcgccactagtgatccATGAAAATAATTTCAACTTTTTATTCTCT-3'
<i>CsLYK5-NE-R (BamHI)</i>	5'-agtactatcgatgatccTAATTCACATTTGTCTCTGCC-3'
<i>Cluc-CsExo70B-F (KpnI)</i>	5'-gtccccgggctgaccATGGTTGACAATGGCGAAGAA-3'
<i>Cluc-CsExo70B-R (Sall)</i>	5'-gaaagctctgcaggtcgacTTACCTCCTTCCACTGCCTGA-3'
<i>CsExo70G2-Cluc-F (KpnI)</i>	5'-acgggggacgagctcggtaccATGGACGAGATGGAAGCCACT -3'
<i>CsExo70G2-Cluc-R (Sall)</i>	5'-cgcgtagcagatctggtcgacCGAAGCAGTAATGGACGCG-3'
<i>CsERECTA-Nluc-F (KpnI)</i>	5'-cgggggacgagctcggtaccATGAAGTCTGTGAAGAGGGCAG-3'
<i>CsERECTA-Nluc-R (Sall)</i>	5'-acatcgatgggtagtcgacCTCACTGTTTTGTGATATTACC-3'
<i>CsFLS2-Nluc-F (KpnI)</i>	5'-cgggggacgagctcggtaccATGGCTTCTTACGTTAGTTTAGC-3'
<i>CsFLS2-Nluc-R (Sall)</i>	5'-acatcgatgggtagtcgacCAATTCATCCCTCTGCAGCT-3'
<i>CsLYK5-Nluc-F (KpnI)</i>	5'-cgggggacgagctcggtaccATGAAAATAATTTCAACTTTTTATTCTCT-3'
<i>CsLYK5-Nluc-R (Sall)</i>	5'-acatcgatgggtagtcgacTAATTCACATTTGTCTCTGCC-3'
<i>CsBAK1-Nluc-F (KpnI)</i>	5'-cgggggacgagctcggtaccATGTCGGTGATGTTTCAGTTCTTCG-3'
<i>CsBAK1-Nluc-R (Sall)</i>	5'-acatcgatgggtagtcgacTCTTGGACCAGACAATTCATCAGG-3'
<i>CsFER-Nluc-F (KpnI)</i>	5'-cgggggacgagctcggtaccATGAAGGAAATTCGTGGTCTTG-3'
<i>CsFER-Nluc-R (Sall)</i>	5'-acatcgatgggtagtcgacACGTCTTTTTGGTTTCATGATC-3'
<i>CsCLV1-Nluc-F (KpnI)</i>	5'-cgggggacgagctcggtaccATGAAGAGAAGACCGATTGATCCT-3'
<i>CsCLV1-Nluc-R (Sall)</i>	5'-acatcgatgggtagtcgacAGAAATAACTGGAGCGATGTAGCC-3'
<i>CsExo70B-HA-1300-F (KpnI)</i>	5'-ggacgagctcggtaccATGGTTGACAATGGCGAAGAAA-3'
<i>CsExo70B-HA-1300-R (PstI)</i>	5'-aacgaaagctcgagTCAAGCGTAATCTGGAACGTCA-3'
<i>CsERECTA-1300-FLAG-F (KpnI)</i>	5'-ggacgagctcggtaccATGAAGTCTGTGAAGAGGGCAG-3'
<i>CsERECTA-1300-FLAG-R (BstBI)</i>	5'-ggtcttttagtctcgaaCTCACTGTTTTGTGATATTACC-3'
<i>CsFLS2-1300-FLAG-F (KpnI)</i>	5'-ggacgagctcggtaccATGGCTTCTTACGTTAGTTTAGC-3'
<i>CsFLS2-1300-FLAG-R (BstBI)</i>	5'-ggtcttttagtctcgaaCAATTCATCCCTCTGCAGCT-3'

<i>CsLYK5-1300-FLAG-F (KpnI)</i>	5'-ggacgagctc ggta ccATGAAAATAATTTCAACTTTTTATTCTCT-3'
<i>CsLYK5-1300-FLAG-R (BstBI)</i>	5'-ggtctttgtagct tcgaa TAATTCCACATTTGTCTCTGCC-3'
<i>CsBAK1-1300-FLAG-F (KpnI)</i>	5'-acgggggacgagctc ggta ccATGTCGGTGATGTTTCAGTTCTTCG-3'
<i>CsBAK1-1300-FALG-R (BstBI)</i>	5'-atggtctttgtagct tcgaa TCTTGGACCAGACAATTCATCAGG-3'

Note: The lower letters represent the vector sequence and the red represents the restriction site of the vector.

Table S5. List of gene accession numbers used in this study

Gene name	Gene ID	Species	Family
<i>CsExo70B</i>	<i>CsaV3_1G036490.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsExo70G2</i>	<i>CsaV3_7G033810.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsERECTA</i>	<i>CsaV3_4G036080.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsFLS2</i>	<i>CsaV3_6G051540.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsLYK5</i>	<i>CsaV3_2G028380.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsFERONIA</i>	<i>CsaV3_4G027260.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsCLAVATA1</i>	<i>CsaV3_3G045960</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsBAK1</i>	<i>CsaV3_4G009310</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsPR1</i>	<i>CsaV3_7G007620.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsPR2</i>	<i>CsaV3_1G044780.1</i>	<i>Cucumis sativus</i>	Cucurbitaceae
<i>CsH⁺-ATPase</i>	<i>CsaV3_2G031420</i>	<i>Cucumis sativus</i>	Cucurbitaceae