PNAS

Supplementary Information for

The CsHEC1-CsOVATE module contributes to fruit neck length variation via modulating auxin biosynthesis in cucumber

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Materials and Methods

Plant materials and growth conditions

Cucumber (*Cucumis sativus* L.) inbred line XTMC (a North China type) and GFC (a South China type) were used for genetic transformation. Cucumber inbred lines (199, 20, 75, 144, 64, XTMC, 63, 67) with different fruit neck length were used for expression analysis. Cucumber seeds were germinated at 28°C in the dark, and then grown in a chamber with 16 h light at 25°C and 8 h darkness at 18°C until the two true-leaf stage. Seedlings were then transferred to the greenhouse in China Agricultural University, Beijing. Water and fertilizer management and pest control were performed using the standard procedures. Cotyledons of cucumber seedlings growing for 7-12 days in growth chamber were selected for protoplast isolation and transformation. *Nicotiana benthamiana* plants were grown in a growth chamber under a long-day condition (16-h-light/8-h-dark) at 24°C.

Phylogenetic analysis

To identify HEC homologous proteins from *Cucurbitaceous* species, *Oryza sativa Japonica Group*, and *Solanum lycopersicum*, the amino acid sequences of *Arabidopsis* HEC1/2/3 were used as queries for BLASTp searches. HEC sequences were aligned by ClustalW in MEGA6.0, and this alignment was used to generate a phylogenetic tree using the Neighbor-joining method with 1000 bootstrap replications (1). Accession numbers of all sequences used for phylogenetic analysis are provided in *SI Appendix*, Table S1.

CRISPR/Cas9-mediated mutations in cucumber

To generate mutations in *CsHEC1*, *CsOVATE* and *CsYUC4* using the CRISPR/Cas9 system, specific target sites were obtained from the website (http://crispr.hzau.edu.cn/cgi-bin/CRISPR2/CRISPR), and amplified from pCBC-DT1T2 template using four primers DT1-BsF/DT1-F0 and DT2-R0/DT2-BsR (2, 3). Then, corresponding double guide RNAs were cloned into a CRISPR/Cas9 vector pKSE401G (same as the pHSE401G vector except that plant Hygromycin resistance cassette was replaced by Kanamycin) (4) and transformed into cucumber inbred lines using the optimized cotyledon transformation method as previously described (5). To identify CRISPR/Cas9-mediated mutant genotypes, total DNA was extracted from T1 transgenic plants, and fragments containing the target sites or potential off-target sites

were amplified and sequenced using gene-specific primers. The primer information is listed in *SI Appendix*, Table S4.

Phenotypic characterization

For fruit morphology, fruits at 0 DPP, 10 DPP and 40 DPP were assessed for each transgenic line. At least ten plants and each plant with only one pollinated fruit from similar internode (7 to 10) were used for phenotypical analysis. For fruit neck measurement, the mature cucumber fruits were cut longitudinally, and the distance between the proximal end of seed cavity and the distal end of peduncle was defined as the fruit neck length, which usually has no spines/tubercules on the surface and no placenta inside.

Generation of *Pro35S:CsHEC1*, *ProCsHEC1:GUS* and *ProCsHEC1:CsYUC4* transgenic plants

The full-length coding sequence (CDS) without the termination codon of *CsHEC1* was amplified from cDNA and cloned into pCAMBIA1300-Flag vector to generate the *Pro35S:CsHEC1-Flag* construct (6). A 2184 bp promoter sequence was amplified from cucumber genomic DNA and cloned into PBI121 vector (7) which harbors a GUS gene to generate the *ProCsHEC1:GUS* reporter construct. The CDS of *CsYUC4* was cloned and replaced into the *ProCsHEC1:GUS* vector to construct the *ProCsHEC1:CsYUC4* vector. All the recombinant constructs were delivered into *Agrobacterium tumefaciens* strain EHA105 and then transformed into cucumber inbred lines using the optimized cotyledon transformation method as described above (5). The primer information is listed in *SI Appendix*, Table S4.

Subcellular localization

The full-length CDS without the termination codon of *CsHEC1* or *CsOVATE* was cloned into the pCAMBIA1300-GFP or pCAMBIA1300-mCherry vector (pCAMBIA1300 vector harboring a Super promoter, which consists of three copies of the octopine synthase upstream activating sequence preceded by the mannopine synthase promoter) (8, 9) to generate the CsHEC1-GFP, CsHEC1-mCherry or CsOVATE-GFP construct. Subcellular localization assay was performed in fully flattened young leaves of about six-week-old *Nicotiana benthamiana* as previously described (6). The empty pCAMBIA1300-GFP vector was used as a positive control. The images were taken using a confocal laser-scanning microscope (Olympus FV3000, Japan)

at the excitation/emission wavelength of 488/510nm (GFP) and 561/610nm (mCherry). The primer information is listed in *SI Appendix*, Table S4.

RNA extraction and expression analysis

Total RNA was isolated using the Eastep® Super Total RNA Extraction Kit (Promega, Madison, USA) and reverse transcribed into cDNA with the FastKing gDNA Dispelling RT SuperMix Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The RT-qPCR was performed on the CFX384 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) using the TB Green® *Premix Ex Taq*™ II reagent (Takara, Shiga, Japan). Three biological and three technical replicates were performed for each gene. The *CsUBI* (CsaV3_5G031430) was used as an internal control. The primer sequences are listed in *SI Appendix*, Table S4.

For analysis of *CsHEC1* expression pattern, different tissues including roots, stems, leaves, male buds, female buds, female flowers at 3 DBA, female flowers on anthesis and ovary at 3 DBA were harvested for RNA extraction. For determination of *CsHEC1*, *CsYUC4* and *CsOVATE* expression levels in different transgenic lines, the proximal part containing the fruit neck of ovaries at 3 days before anthesis was collected for gene expression analysis. The fruit necks of ovaries at anthesis were used for expression analyses of *CsHEC1*, *CsYUC4* and *CsOVATE* in different cucumber germplasms.

For GUS histochemical assay, tissues from *ProCsHEC1:GUS* transgenic plants were vacuumed in the X-Gluc solution (Obiolab, Beijing, China) for 15 min and then incubated at 37 ℃ for GUS staining. Images was taken using a stereomicroscope (Leica S8 APO, Germany).

RNA *in situ* hybridization

The cucumber shoot apexes of 10-, 17-, 21-day-old seedlings, male flower buds and female flower buds were fixed in 3.7% formal-acetic-alcohol solution and *in situ* hybridization was performed as previously described (10, 11). The *CsHEC1*, *CsHEC2* and *CsOVATE* probes were designed according to their specific region of the corresponding CDS, and sense and antisense probes were synthesized by PCR amplification and *in vitro* transcription were performed using DIG RNA Labeling Kit with SP6 and T7 polymerases (Roche, Basel,

Switzerland) following the description of manufacturer's instructions. The primer sequences are listed in *SI Appendix*, Table S4.

Yeast one-hybrid assay

Two antiparallel oligonucleotides of each CsHEC1-binding site from *CsYUC4* promoter (-1199, -889) with three repeats separated by a 7-bp flanking sequence were designed and synthesized, and then inserted into the pAbAi vector (used as the reporter plasmid). The linearized pAbAi vectors containing the binding sites were transformed into the yeast Y1H Gold strain following the Matchmaker® Gold Yeast One-Hybrid Library Screening System manufacturer's manual (Clontech, CA, USA). Positive colonies were screened with optimal Aureobasidin A (AbA) concentration on SD/-Ura (Synthetic Dropout Medium/-Uracil) medium. The full length *CsHEC1* coding sequence was amplified and inserted into the pGADT7 vector (used as the effector plasmid). The resultant pGADT7-CsHEC1 was then transformed into the yeast strain Y1HGold containing pE-box-AbAi colonies and selected on SD/-Leu (Synthetic Dropout Medium/-Leucine) medium with optimal AbA concentration. The primer sequences are listed in *SI Appendix*, Table S4.

Dual-Luciferase reporter (DLR) assay

For transcriptional activity test, the full-length CDS of *CsHEC1* or *VP16* sequence was cloned into the effector GAL4DB vector to generate the GAL4DB-CsHEC1 or GAL4DB-VP16 (positive control) vectors. The resultant fusion effector plasmids and reporter plasmid (GAL4-LUC) were introduced into *Agrobacterium* strain GV3101 (pSoup19), respectively. After co-injection in *N. benthamiana* leaves, activity was determined as described previously (12, 13).

The *CsYUC4* promoter of 1,365 bp in length was amplified and introduced into pGreen II 0800-LUC vector as the reporter construct. The full-length CDS of *CsHEC1*, *CsOVATE* or *Csovate* mutant version was cloned into pGreen II 62-SK as the effector (14). The reporter and effectors were co-expressed in *N. benthamiana* leaves by *Agrobacterium*-mediated transient expression. The Firefly LUC and REN activities were determined by using the Dual-Luciferase® reporter assay reagents (Promega, Madison, USA). The primer sequences were listed in *SI Appendix*, Table S4.

ChIP-PCR assay

The full-length CDS of *CsHEC1* or *CsHEC2* without the termination codon was cloned into the transient expression vector pUC19-35S-FLAG-RBS (15). Cucumber protoplasts were transfected with the indicated plasmids, incubated overnight, and then treated with the final concentration of 1% (v/v) formaldehyde to crosslink protein-DNA complexes and stop the cross-linking by adding the final concentration of 0.125 M glycine. Another approach was to harvest and crosslink tissues from WT or Flag-fusion overexpressed transgenic lines. Subsequently, the ChIP-PCR assay was performed as previous described with minor modifications (16, 17). ChIP reaction was performed using 50 μl agarose-conjugated anti-FLAG antibody (Sigma-Aldrich, USA, Catalog No. A2220), and the final precipitated DNA was purified using QIAquick PCR Purification Kit (QIAGEN, Germany) for RT-qPCR analysis. The cucumber *TUBULIN* gene (GenBank: AJ715498) was used as an internal control (18), and three biological replicates and three technical replicates were performed. The primers for ChIP-PCR are listed in *SI Appendix*, Table S4.

Yeast two-hybrid assay

The full-length CDSs of *CsHEC1* and *CsOVATE* were amplified from cDNA and inserted into the bait vector pGADT7 (Takara Bio USA, Cat. Nos. 630442) and prey vector pGBKT7 (Takara Bio USA, Cat. Nos. 630443), respectively. Positive control (pGADT7-T + pGBKT7-53) from Matchmaker® Gold Yeast Two-Hybrid System (Takara Bio USA, Cat. Nos. 630489) (19, 20). The resulting plasmids were then co-transformed into the yeast strain AH109 following the instructions for the yeast transformation. The yeast transformants were selected on synthetic defined SD/-Trp/-Leu agar medium and protein interactions were detected on selective medium SD/-Trp/-Leu/-His/-Ade with X-α-Gal according to Matchmaker® Gold Yeast Two-Hybrid System User Manual. The primers are provided in *SI Appendix*, Table S4.

Protein purification and pull-down assay

GST and GST-CsHEC1 were expressed in *E. coli* strain BL21 (DE3) and purified using BeyoGold™ GST-tag Purification Resin and His-CsOVATE was purified using BeyoGold™ His-tag Purification Resin (Beyotime Biotech, Shanghai, China) according to the manufacturer's protocols. The primers for pull-down constructs are listed in *SI Appendix*, Table S4.

For pull-down assay, His-CsOVATE (10 μg) was incubated with GST-CsHEC1 (10 μg) or GST (10 μg) with 25 μl of GST resin in a buffer (50 mM Tris-HCl, 0.2% glycerol, 0.25% Triton X-100, 0.1 M NaCl, and 1 mM PMSF) for 2 h at 4 ℃. After incubation, the resin was washed two times with buffer I (50 mM Tris-HCl, pH 7.5, 150 mM NaCl), buffer II (50 mM Tris-HCl, pH 7.5, 200 mM NaCl), and buffer III (50 mM Tris-HCl, pH 7.5, 300 mM NaCl). The bound proteins were eluted and detected by anti-GST and anti-His antibodies immunoblot.

Firefly luciferase complementation imaging (LCI) assay

The full-length CDSs of *CsHEC1*, *CsHEC2*, and *CsOVATE* were cloned into pCAMBIA1300-nLUC or pCAMBIA1300-cLUC vector by In-Fusion cloning and then transformed into *Agrobacterium tumefaciens* strain GV3101, respectively. The *Agrobacterium tumefaciens* containing the indicated plasmids were infiltrated in *N. benthamiana* leaves by *Agrobacterium*-mediated transient expression and incubated for 48 h before the LUC activity imaging and measurement. The abaxial sides of leaves were sprayed with 1 mM D-Luciferin, Potassium Salt (Biovision, CA, USA), and the firefly luciferase imaging were captured using a CCD camera system (1300B, Roper Scientific, USA) and performed as previously described (21). The primers for all constructs are listed in *SI Appendix*, Table S4.

Co-IP assay

The CDS of *CsHEC1* was cloned into pCAMBIA1300-GFP vector, and the CDS of *CsOVATE* was cloned into pCAMBIA1300-FLAG vector. Confirmed vectors were transferred into the *Agrobacterium* strain GV3101 and then co-infiltrated into *N. benthamiana* leaves. After 48h of infiltration, total protein was extracted and immunoprecipitation was carried out, according to the previous procedure (6). The eluted proteins were separated by SDS-PAGE and detected by immunoblot with anti-GFP (TransGen Biotech, China, Catalog No. HT801) and anti-FLAG (Sigma-Aldrich, USA, Catalog No. F3165) antibodies. The primer information is listed in *SI Appendix*, Table S4.

Extraction and quantification of endogenous auxin

To measure the auxin (IAA) levels in WT and transgenic plants, about 0.1 g fresh fruit neck from ovaries at 3 DBA were collected as a biological repeat for IAA extraction, and three biological repeats were performed for each genotype. The extraction, purification and

quantification of endogenous auxin were performed using enzyme-linked immunosorbent assay according to the method described previously (22).

Histology observation

Fruit neck samples from 40 DAA fruits were fixed in a 3.7% (v/v) FAA solution (ethanol: acetic acid: glycerol: 37% formaldehyde: ddH_2O = 50 mL: 5 mL: 5 mL: 10 mL: 30 mL), overnight. After dehydration, embedding and trimming, the samples were sectioned 6 μm thick in the longitudinal direction for aniline blue staining. The sections were imaged using a light microscope (Olympus D72, Japan), and cell length assessments were made using the ImageJ software. All measurements were made on three fields of each tissue section, and three sections from each line.

Fig. S1

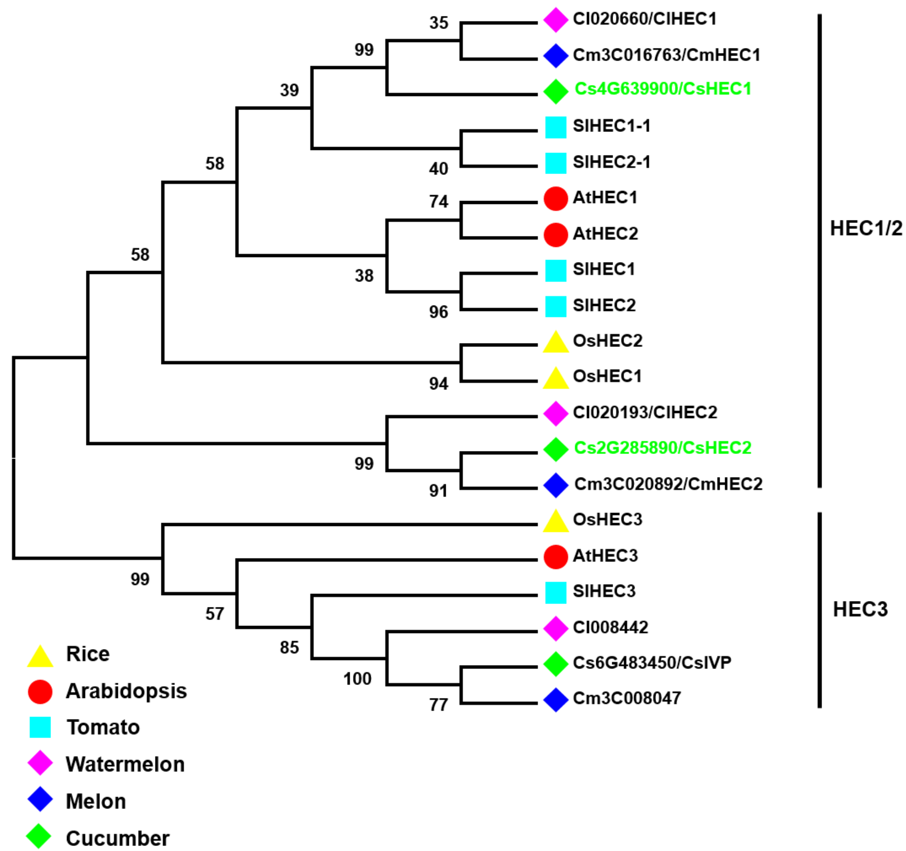

Fig. S1. Phylogenetic tree analysis of HEC homologs. The phylogenetic tree was generated using the neighbor-joining method with 1000 bootstrap replications by MEGA 6.0 software.

Fig. S2

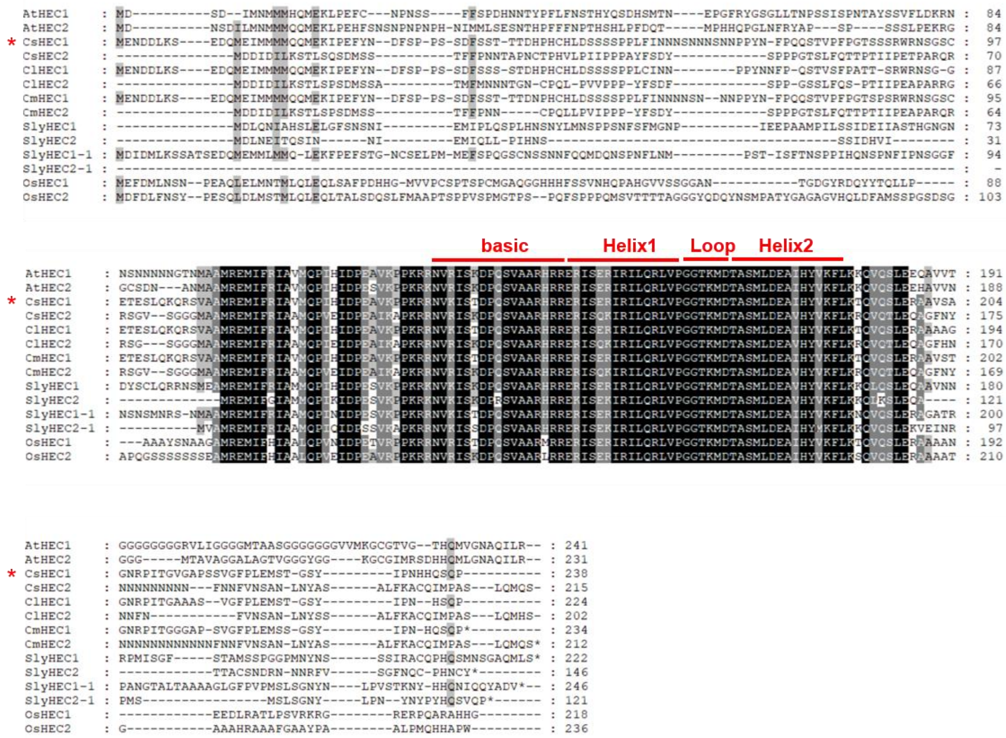

Fig. S2. Protein sequence alignment of HEC1/2 homologs. At, *Arabidopsis thaliana*; Cs, *Cucumis sativus*; Cl, *Citrullus lanatus*; Cm, *Cucumis melo*; Sl, *Solanum lycopersicum*; Os, *Oryza sativa*. The red lines represent the conserved bHLH domain.

Fig. S3

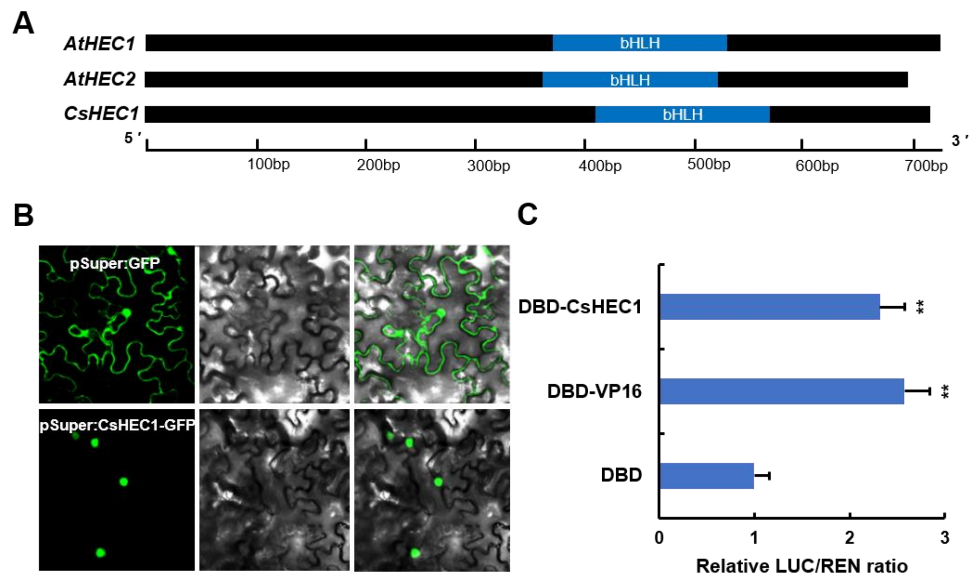

Fig. S3. Characterization of the putative transcription factor CsHEC1. (*A*) Gene structure of *CsHEC1.* Blue boxes represent the bHLH domain. (*B*) Subcellular localization of CsHEC1 in *N. benthamiana* leaves. The empty pSuper:GFP vector was used as a control. (*C*) Transactivation activity analysis of CsHEC1 protein in *N. benthamiana* leaves using a GAL4/UAS-based system. DBD and DBD-VP16 were used as a negative and positive control, respectively. The mean values ± SD from six biological replicates. Significance analysis compared to DBD control was performed with the two-tailed Student's *t* test (** $p < 0.01$).

Fig. S4

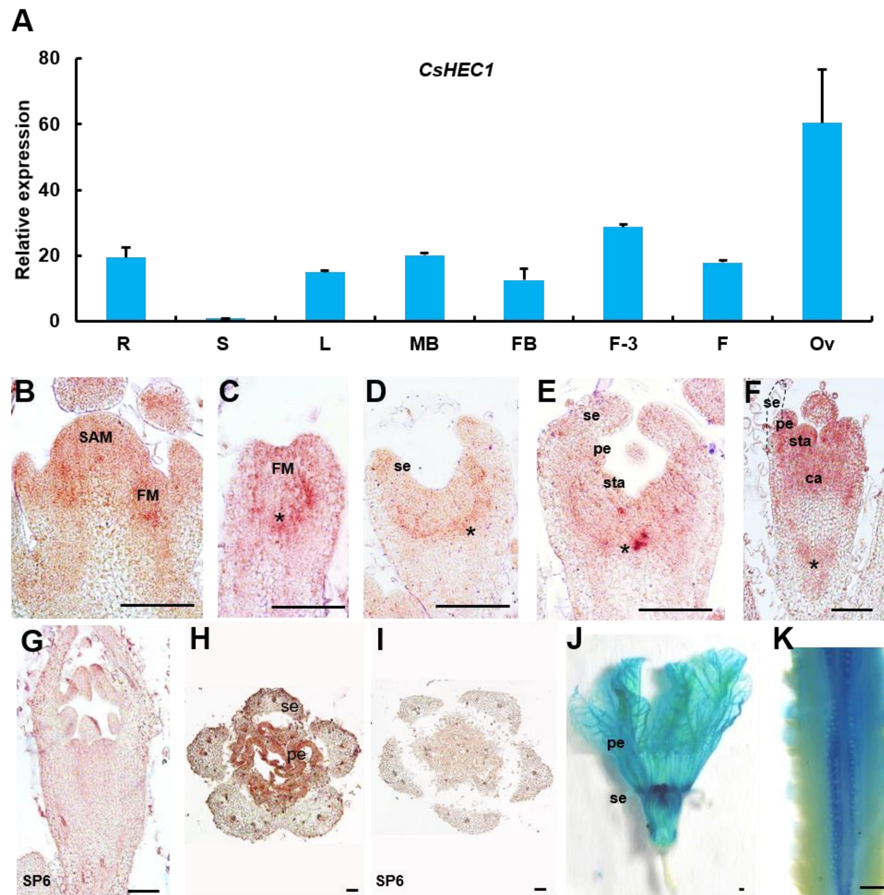

Fig. S4. Gene expression analysis of *CsHEC1* in cucumber. (*A*) The expression pattern of *CsHEC1* in different organs. R, root; S, stems; L, leaves; MB, male buds; FB, female buds; F-3, female flowers at 3 days before anthesis (DBA); F, female flowers at anthesis; Ov, ovary at 3 DBA. *CsHEC1* transcripts were quantified by RT-qPCR using *CsUBI* as the internal standard. Values are means ± SD (n = 3). (*B-I*) *In situ* hybridization analysis of *CsHEC1*. *CsHEC1* is expressed in the shoot apical meristem (SAM) and floral meristem (FM) (*B*). Longitudinal sections of developing flower primordium at stage 1 (*C*), stage 3 (*D*), stage 4 (*E*) and stage 7 (*F*). Cross sections of the female flower corolla at stage 8 (*H*, *I*). The sense *CsHEC1* probe was hybridized as a negative control (*G*, *I*). Asterisks indicate the developing fruit neck in (*C-F*). Scale bar, 100 μm. (*J and K*) *CsHEC1* expression was examined using the *ProCsHEC1:GUS* reporter system. High expression of *CsHEC1* was found in sepal and petal at anthesis (*J*), as well as in developing ovules in the female flower (*K*). Scale bar, 1 mm. se, sepal; pe, petal; sta, stamen; ca, carpel.

Fig. S5

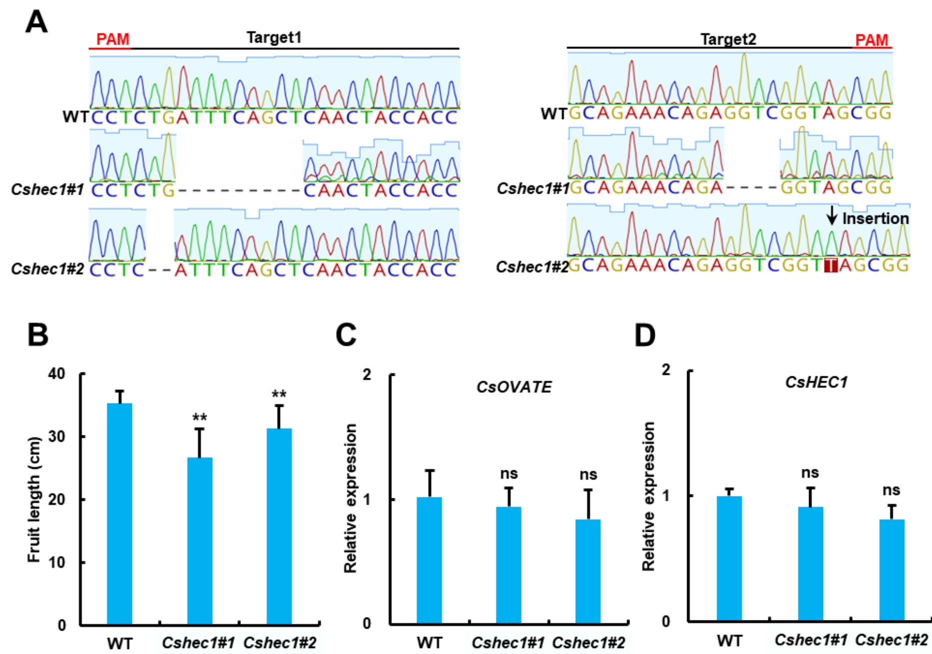

Fig. S5. Genotyping and quantitative analysis of *Cshec1* mutants. (*A*) Sequencing chromatogram analysis of edited sites in *Cshec1* mutants. Black underlines indicate targeted sequences and red underlines represent the protospacer-adjacent motif (PAM) sequences. Nucleotide deletions are indicated by dashes and nucleotide insertion by arrow. (*B*) Quantitative analysis of fruit lengths from fruits at 40 DPP. (*C* and *D*) Expression analysis of *CsOVATE* (*C*) and *CsHEC1* (*D*) in *Cshec1* mutants. Values are means ± SD (Student's *t* test, two-tailed; $^{**}P < 0.01$; ns, no significant difference).

Fig. S6

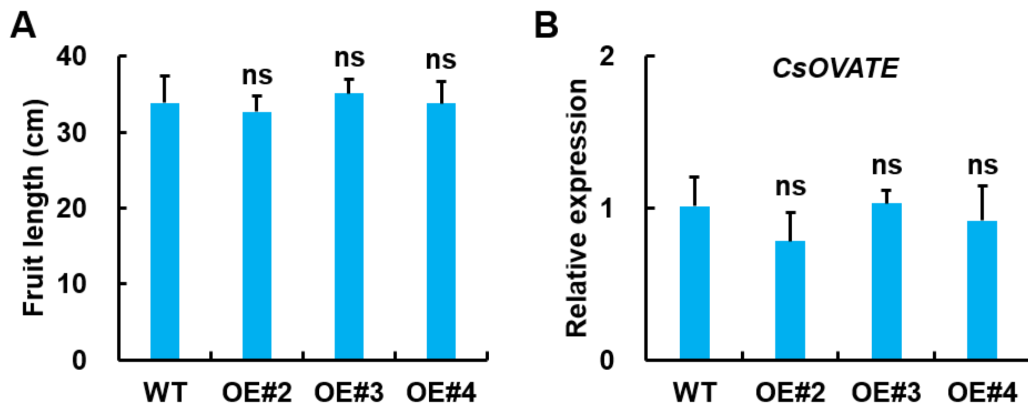

Fig. S6. Quantitative analysis of *CsHEC1*-OE lines. (*A*) Quantitative analysis of fruit lengths from fruits at 40 DPP. (*B*) Expression analysis of *CsOVATE* in *CsHEC1*-OE lines. Values are means ± SD (n = 6) Significance analysis compared to WT was performed with the two-tailed Student's t test (ns, no significant difference).

Fig. S7

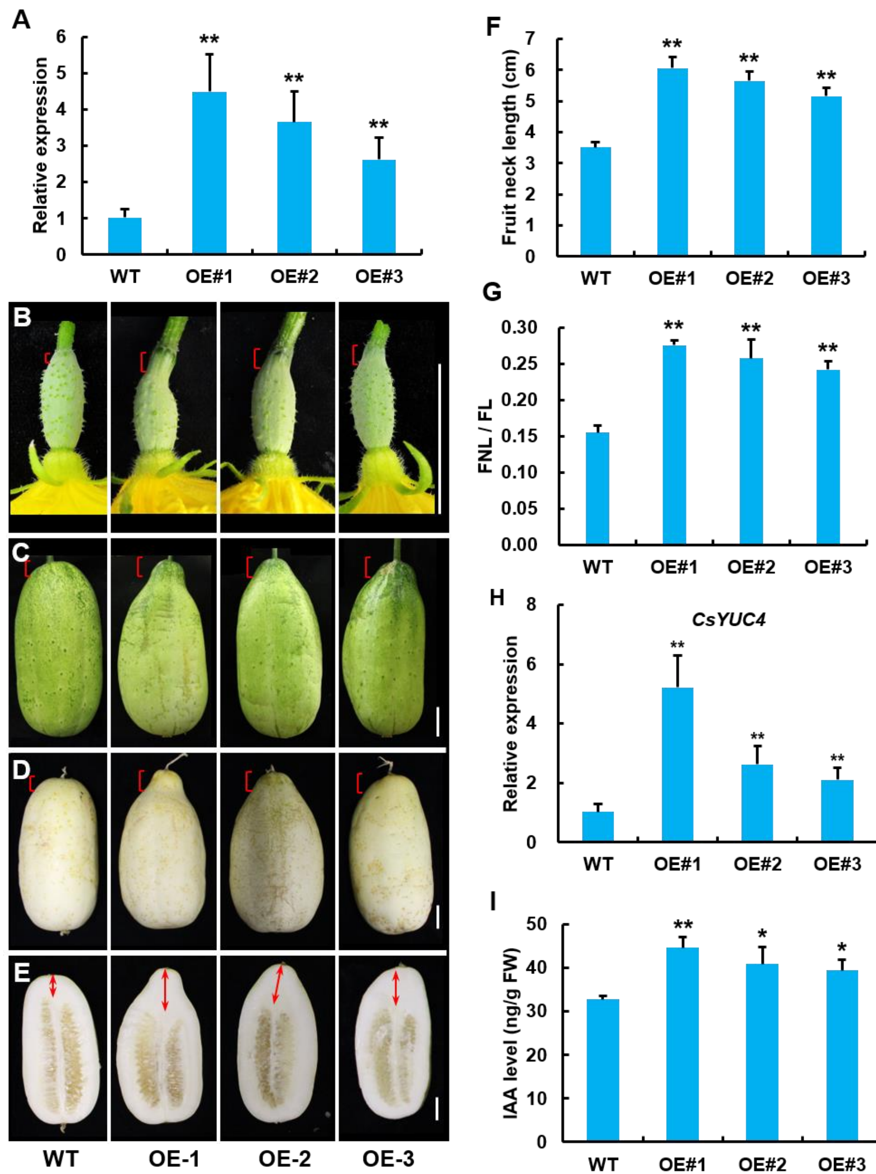

Fig. S7. Overexpression of *CsHEC1* resulted in elongated fruit neck and increased auxin content in South China type cucumber GFC. (*A*) *CsHEC1* expression in the WT (GFC) and three overexpression transgenic lines. (*B-E*) Fruit phenotype at 0 DPP (*B*), 15 DPP (*C*) and 40 DPP (*D*, *E*) in *35S:CsHEC1* transgenic plants. The red brackets indicate the fruit neck and the double arrows represent the measured length of fruit neck. Scale bars, 3 cm. (*F and G*) Quantification of fruit neck length (*F*) and ratio of FNL/FL (*G*) in WT and *CsHEC1*-OE fruits at 40 DPP. (*H*) *CsYUC4* expression analysis. (*I*) IAA content in the fruit necks. Values are means ± SD (n = 5 in *F* and *G*; n = 3 *H* and *I*) (**$P < 0.01$; *$P < 0.05$; Student's *t* test, two-tailed).

Fig. S8

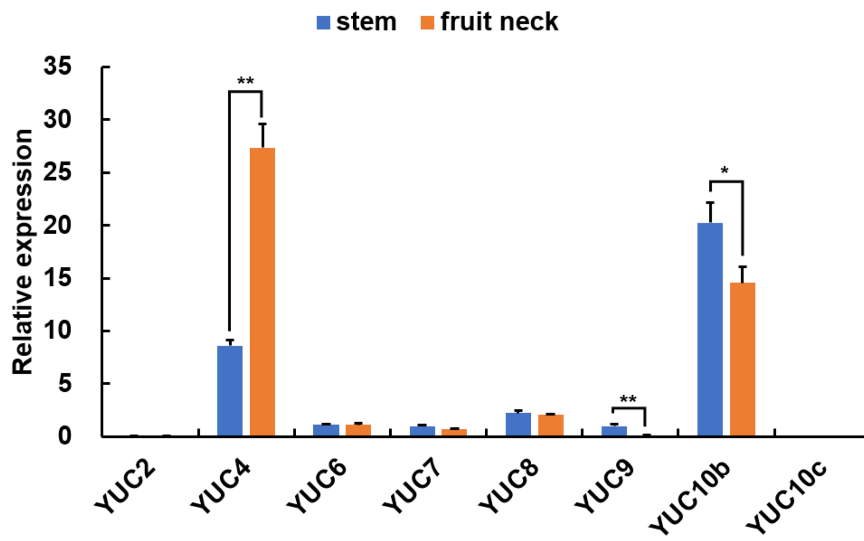

Fig. S8. Gene expression analysis of *CsYUCs* in young stems and fruit necks at 3 DBA. Values are means ± SD of 3 independent biological replicates. Asterisks indicate significant differences according to a Student's *t* test (two-tailed, *$P < 0.05$, **$P < 0.01$).

Fig. S9

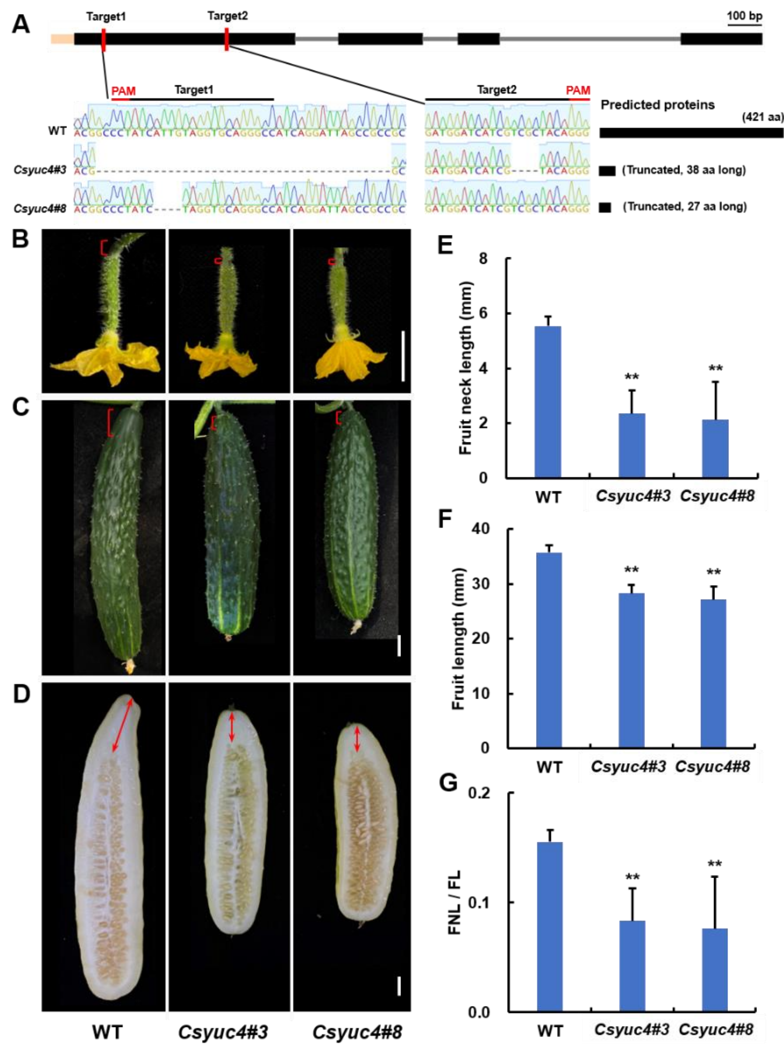

Fig. S9. Knockout of *CsYUC4* resulted in reduced fruit neck length in cucumber. (*A*) Genotype identification of *Csyuc4* knockout lines indicated the *Csyuc4#3* allele with 41-bp and 4-bp deletions and the *Csyuc4#8* allele with a 4-bp deletion, both generating a premature stop codon and resulting in truncated proteins of 33 amino acids and 27 amino acids in length, respectively. (*B-D*) Fruit morphology of WT and *Csyuc4* mutants at 0 DPP (*B*), 10 DPP (*C*) and 40 DPP (*D*). The brackets indicated the fruit necks and the double arrows represent the measured fruit neck length. Scale bars, 2 cm. (*E-G*) Quantification of fruit neck length (*E*), fruit length (*F*) and ratio of FNL/FL (*G*) in WT and *Csyuc4* mutants at anthesis. Values are means ± SD (n = 7). Significance analysis compared to WT was performed with the two-tailed Student's t test (**P < 0.01).

Fig. S10

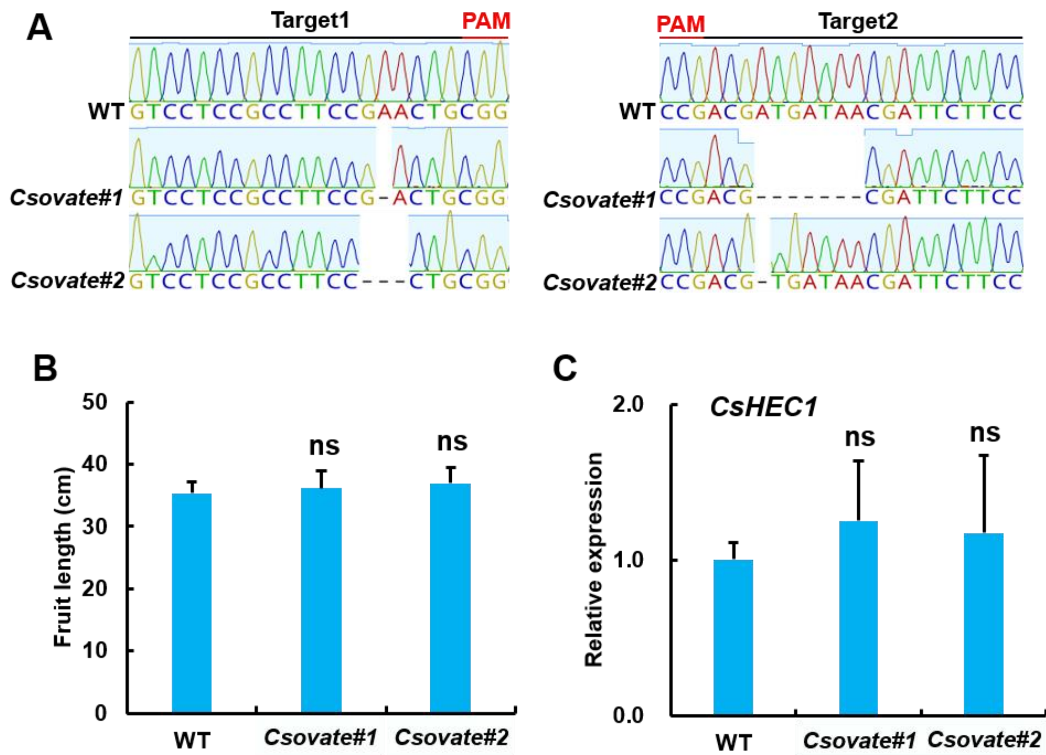

Fig. S10. Genotyping and quantitative analysis of *Csovate* mutants. (*A*) Sequencing chromatogram analysis of edited sites in *Csovate* mutants. Black underlines indicate targeted sequences and red underlines represent the PAM sequences. Nucleotide deletions are indicated by dashes. (*B*) Quantitative analysis of fruit lengths from fruits at 40 DPP. (*C*) Expression analysis of *CsHEC1* in *Csovate* mutants. Values are means ± SD (Student's *t* test, two-tailed; ns, no significant difference).

Fig. S11

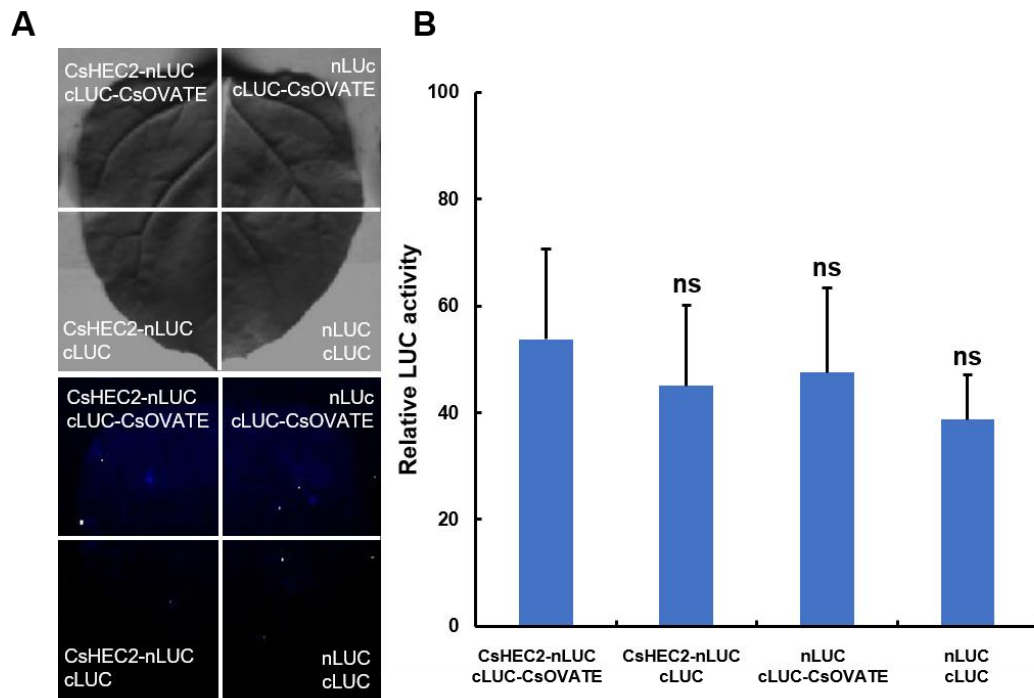

Fig. S11. CsHEC2 did not interact with CsOVATE in *N. benthamiana* leaves. The firefly luciferase complementation imaging assay was performed with the indicated constructs in the left panel (*A*), and relative luciferase activity was shown in the right panel (*B*). cLUC, C-terminal LUC; nLUC, N-terminal LUC.The mean values ± SD from six biological replicates (Student's *t* test, two-tailed, ns, no significant difference).

Fig. S12

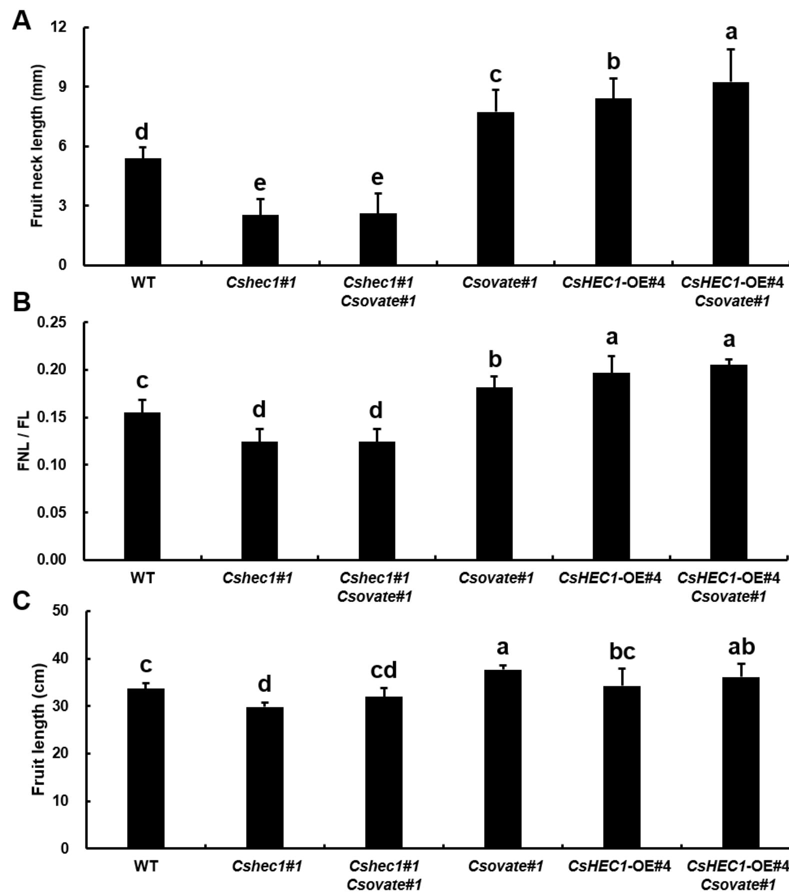

Fig. S12. Quantification of fruit neck length (*A*) at anthesis, ratio of FNL/FL (*B*) and fruit length (*C*) at maturity in the indicated lines. Values are means ± SD (n = 15 in *A*; n = 7 in *B* and *C*). The different lowercase letters indicate significant differences ($P < 0.05$) by one-way ANOVA analysis with Duncan's test.

Fig. S13

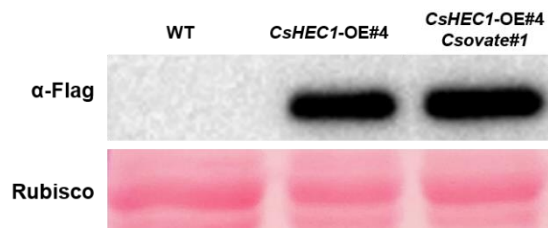

Fig. S13. Immunoblot analysis of CsHEC1 protein levels in *CsHEC1*-OE#4 and *CsHEC1*-OE#4/*Csovate#1* lines using anti-Flag antibody. Rubisco large subunit stained by Ponceau S was served as a loading control.

Table S1. Gene information used in this study.

Gene name	Species	Family	Accession
AtHEC1	*Arabidopsis thaliana*	Brassicaceae	AT5G67060.1
AtHEC2	*Arabidopsis thaliana*	Brassicaceae	AT3G50330.1
AtHEC3	*Arabidopsis thaliana*	Brassicaceae	AT5G09750.1
CsHEC1	*Cucumis sativus*	Cucurbitaceae	Csa4G639900
CsHEC2	*Cucumis sativus*	Cucurbitaceae	Csa2G285890
CsIVP	*Cucumis sativus*	Cucurbitaceae	Csa6G483450
ClHEC1	*Citrullus lanatus*	Cucurbitaceae	Cla020660
ClHEC2	*Citrullus lanatus*	Cucurbitaceae	Cla020193
ClHEC3	*Citrullus lanatus*	Cucurbitaceae	Cla008442
CmHEC1	*Cucumis melo*	Cucurbitaceae	MELO3C016763
CmHEC2	*Cucumis melo*	Cucurbitaceae	MELO3C020892
CmHEC3	*Cucumis melo*	Cucurbitaceae	MELO3C008047
OsHEC1	*Oryza sativa* Japonica Group	Poaceae	XP_015649315.1
OsHEC2	*Oryza sativa* Japonica Group	Poaceae	XP_015610996.1
OsHEC3	*Oryza sativa* Japonica Group	Poaceae	XP_015649611.1
SlHEC1	*Solanum lycopersicum*	Solanaceae	Solyc02g090950.1.1
SlHEC1-1	*Solanum lycopersicum*	Solanaceae	Solyc04g078790.2.1
SlHEC2	*Solanum lycopersicum*	Solanaceae	Solyc03g044460.1.1
SlHEC2-1	*Solanum lycopersicum*	Solanaceae	Solyc12g088790.1.1
SlHEC3	*Solanum lycopersicum*	Solanaceae	Solyc11g005780.1.1
CsOVATE	*Cucumis sativus*	Cucurbitaceae	Csa4G038760
CsYUC4	*Cucumis sativus*	Cucurbitaceae	Csa2G379350
CsYUC2	*Cucumis sativus*	Cucurbitaceae	Csa1G242600
CsYUC6	*Cucumis sativus*	Cucurbitaceae	Csa2G375750
CsYUC7	*Cucumis sativus*	Cucurbitaceae	Csa3G133910
CsYUC8	*Cucumis sativus*	Cucurbitaceae	Csa6G087870
CsYUC9	*Cucumis sativus*	Cucurbitaceae	Csa3G619930
CsYUC10a	*Cucumis sativus*	Cucurbitaceae	Csa2G302220
CsYUC10b	*Cucumis sativus*	Cucurbitaceae	Csa3G190380
CsYUC10c	*Cucumis sativus*	Cucurbitaceae	Csa7G390100
CsYUC11	*Cucumis sativus*	Cucurbitaceae	Csa6G396640

Table S2. Analysis of the potential off-target sites for *CsHEC1* sgRNA.

Targets	Potential off-target sites				Number of plants examined	Number of plants mutated
	Name	Sequence	Locus	Number of mismatches		
Target1	T1-off1	GAGTGTTGAGCAGAAATCAG**CGG**	scaffold01658: +330210	4	6	0
	T1-off2	GATAGTTTACCTGAAATCAT**CGG**	scaffold00919: -1650832	4	6	0
	T1-off3	GGCAGTGGAGTTGAAATCAG**TAG**	scaffold02219: +622668	3	6	0
	T1-off4	GGCAGTTGAGGAGAAAACAG**AAG**	scaffold00542: -958406	4	6	0
Target2	T2-off1	CCATAAAGAGAGGTCGGTAG**AGG**	scaffold01256: +37721	3	6	0
	T2-off2	GCAGAAACTGAGATAGATAG**AGG**	scaffold01416: -652177	4	6	0
	T2-off3	CCAGAAACAAAGGTCGATGG**CAG**	scaffold02951: -891739	4	6	0
	T2-off4	GCAGAAAAAGAGGACAGTAC**CAG**	scaffold00493: -111727	4	6	0
	T2-off5	GCAGAACCACAGGTCGGCGG**CGG**	scaffold03356: -2313772	4	6	0

Note: PAM sequences or potential PAM sequences are written in bold; Mismatch bases are written in red letters.

Table S3. Analysis of the potential off-target sites for *CsOVATE* sgRNA.

Targets	Potential off-target sites				Number of plants examined	Number of plants mutated
	Name	Sequence	Locus	Number of mismatches		
Target1	T1-off1	TTTCTCATCCTTCCGAACTG**CAG**	scaffold01933: -88576	4	6	0
	T1-off2	CTCCTCCGAGTTCCGAGCTG**AGG**	scaffold01079: +444457	4	6	0
Target2	T2-off1	GTAAGAAACTTTATCATCTT**CAG**	scaffold04100: +1247758	4	6	0
	T2-off2	GGAAGAATTGTTCCCATCTT**TGG**	scaffold01037: +1250068	4	6	0
	T2-off3	GGAAGAAGGGTTTTCGTCGT**CGG**	scaffold03746: +143195	4	6	0
	T2-off4	GGAAGAATCGTTGTGATGGT**TGG**	scaffold01989: -5216	3	6	0

Note: PAM sequences or potential PAM sequences are written in bold; Mismatch bases are written in red letters.

Table S4. Primers used in this study

Primers for gene amplification and vector construction	
CsHEC1-clone-F	ATGGAAAATGATGATTTAAAATCGG
CsHEC1-clone-R	TCAAGGTTGGGATTGATGATGAT
CsHEC2-clone-F	ATGGACGATATCGACATCCTCA
CsHEC2-clone-R	TCAAGACTGCATTTGCAAAGAA
CsOVATE-clone-F	ATGATGATGACACCAAAACG
CsOVATE-clone-R	TCAGTTACAGAACAGAGACTG
CsHEC1-OE-F	GCTCTAGAATGGAAAATGATGATTTAAAATCGG
CsHEC1-OE-R	TCCCCCGGGTCAAGGTTGGGATTGATGATGAT
ProCsHEC1-clone-F	AACGGTGACGTATTTGGATCAAAATCC
ProCsHEC1-clone-R	AGGATTAAAGAAAAAAGAATCTCCGACCTGATC
CsYUC4-clone-F	ATGGCTTCTTGCAAAGACCA
CsYUC4-clone-R	TCATTTTCGTATGGATTCTTTAAGGACT
CsYUC4-OE-F	GAGAACACGGGGGACTCTAGAGGATCCATGGCTTCTTGCAAAGACC
CsYUC4-OE-R	CGATCGGGGAAATTCGAGCTCTCATTTTCGTATGGATTCTTTAAGG
D35SGFP-Terminal-F	ATTTGGGTGATGGTTCACGTAGTGAAGAACATCGATTTTCCATGGC
D35SGFP-Terminal-R	TCAGGGCGATGGCCCACTACGTGTGTTTGACAGCTTATCATCGG
CsHEC1-GFP-F	TACACCAAATCGACTCTAGAATGGAAAATGATGATTTAAAATCGG
CsHEC1-GFP-R	TGGTGATGGTCGACCCCGGGAGGTTGGGATTGATGATGAT
CsHEC1-mCherry-F	TACACCAAATCGACTCTAGAATGGAAAATGATGATTTAAAATCGG
CsHEC1-mCherry-R	CTGGTACCGGATCCACTAGTAGGTTGGGATTGATGATGAT
CsOVATE-GFP-F	CACCAAATCGACTCTAGAATGATGATGACACCAAAACG
CsOVATE-GFP-R	GTGATGGTCGACCCCGGGGTTACAGAACAGAGACTGCC
CsHEC1-DT1-BsF	ATATATGGTCTCGATTGCAGAAACAGAGGTCGGTAGGTT
CsHEC1-DT1-F0	TGCAGAAACAGAGGTCGGTAGGTTTTAGAGCTAGAAATAGC
CsHEC1-DT2-R0	AACCTGATTTCAGCTCAACTACCAATCTCTTAGTCGACTCTAC
CsHEC1-DT2-BsR	ATTATTGGTCTCGAAACCTGATTTCAGCTCAACTACCAA
CsOVATE-DT1-BsF	ATATATGGTCTCGATTGTCCTCCGCCTTCCGAACTGGTT
CsOVATE-DT1-F0	TGTCCTCCGCCTTCCGAACTGGTTTTAGAGCTAGAAATAGC
CsOVATE-DT2-R0	AACACGATGATAACGATTCTTCCAATCTCTTAGTCGACTCTAC
CsOVATE-DT2-BsR	ATTATTGGTCTCGAAACACGATGATAACGATTCTTCCAA
CsYUC4-DT1-BsF	ATATATGGTCTCGATTGGCCCTGCACCTACAATGATGTT
CsYUC4-DT1-F0	TGGCCCTGCACCTACAATGATGTTTTAGAGCTAGAAATAGC
CsYUC4-DT2-R0	AACTGTAGCGACGATGATCCATCAATCTCTTAGTCGACTCTAC
CsYUC4-DT2-BsR	ATTATTGGTCTCGAAACTGTAGCGACGATGATCCATCAA

Primers for qRT-PCR	
CsHEC1-Q-F	TGGATTCCTCTTCATCGCCTC
CsHEC1-Q-R	GCTTCACCGCTTCTGGGTCTAT
CsHEC2-Q-F	CGGGAAAGGATTAGCCAGAAA
CsHEC2-Q-R	CTTGAAAAGGGCAGAAGCGTA
CsYUC4-Q-F	GGTATAGAAGCAATGTGCCAAG

CsYUC4-Q-R	AATGCTTGCCATCTTTATTCCC
CsYUC2-Q-F	TGCAAGGCAAAAGACTTCACG
CsYUC2-Q-R	GCTATACATTCGGCTCTTTCAAGGA
CsYUC6-Q-F	GAAGTATTTGGAGGATTACGCTG
CsYUC6-Q-R	TGTTTCCTCAGAACGACCGC
CsYUC7-Q-F	GGTGAGGCTTACCGTGGGAAAC
CsYUC7-Q-R	CTTGGCATCATGGTTACAAAGA
CsYUC8-Q-F	GGGCTATCTGGGGCTTCATC
CsYUC8-Q-R	AGCGTCTATGGCAAGCAGTT
CsYUC9-Q-F	CCGAGTCTTCCGTTTCTGGT
CsYUC9-Q-R	GGCATGACACACTCTGCATTTTC
CsYUC10a-Q-F	GTCCTTCTGGCTTGGCTACCT
CsYUC10a-Q-R	TGGCTAAGTGAAGGCATAAACG
CsYUC10b-Q-F	CCTTCTGGTCTTGCCACTGC
CsYUC10b-Q-R	CAAAACCGATTGGGGAGG
CsYUC10c-Q-F	CTATCCACCGCCGCATGTTTA
CsYUC10c-Q-R	CGCCAGCTCCGATGATTTCTT
CsYUC11-Q-F	CAATGCACCGACGTATATTTCG
CsYUC11-Q-R	CCTCTCTTGCTCACCACTACTTGT
CsUBI-Q-F	CACCAAGCCCAAGAAGATC
CsUBI-Q-R	TAAACCTAATCACCACCAGC
Primers for *in situ* probes	
CsHEC1-SP6	GATTTAGGTGACACTATAGAATGCTATGGAAAATGATGATTTAAA
CsHEC1-T7	TGTAATACGACTCACTATAGGGTCAAGGTTGGGATTGATGATG
CsOVATE-SP6	GATTTAGGTGACACTATAGAATGCTATGATGATGACACCAAAACG
CsOVATE-T7	TGTAATACGACTCACTATAGGGGAGATGATCGTTGTCCTCT
CsHEC1-SP6-2	GATTTAGGTGACACTATAGAATGCTATTCTTAAAAACCCAAGTTCAGT
CsHEC1-T7-2	TGTAATACGACTCACTATAGGGTCAAGGTTGGGATTGATGA
CsHEC2-SP6	GATTTAGGTGACACTATAGAATGCTATGGACGATATCGACATCCT
CsHEC2-T7	TGTAATACGACTCACTATAGGGTCAAGACTGCATTTGCAAAG
Primers for potential off-target sites analysis	
HEC1-T1-off1F	CAATTGTCAATGTGAAGTTCG
HEC1-T1-off1R	CTCCGACAACTTGCAAGA
HEC1-T1- off2F	GGAGCCATTATAGCCTTCAC
HEC1-T1- off2R	CTAAGTTGAGCATTATTGTGC
HEC1-T1-off3F	AACGGAAATCTGTTGTGCT
HEC1-T1-off3R	AAGGGATTTAACCAGATTCTGT
HEC1-T1-off4F	GTGAATGAAATGACACTTGTG
HEC1-T1-off4R	TCCCAGTAGTATTGCCAGAG
HEC1-T1-off1F	GTTGAGATGAACTGTGAAGG
HEC1-T2-off1R	ACCTTCCTTCAAAACCCTTC
HEC1-T2-off2F	TCGATAATCCACTCGATCTC
HEC1-T2-off2R	TCAGGTGGAGATATACTTCC
HEC1-T2-off3F	CTCACCGTGCAAGATTTGT

HEC1-T2-off3R	ATCAGATGAGGTTGGAAGC
HEC1-T2-off4F	GAGATTGAACATATACGTGACG
HEC1-T2-off4R	AGAAATGCAAGTGATGATTC
HEC1-T2-off5F	CACAATCGAGAATCACCAATC
HEC1-T2-off5R	TCAACCAATAGTTTGGGTCA
OVATE-T1-off1F	CTGTTGAAACAGTGACCAAC
OVATE -T1-off1R	CTCAATTCAGTGTACTGATGAG
OVATE-T1- off2F	GCAGTAGCTCAATCTGAATC
OVATE -T1- off2R	CCATAACCGATATAGAGTATGTG
OVATE-T2-off1F	CAGATACCAAATCTACAGTTGG
OVATE-T2-off1R	GAATCATGTGCCAATCTAAGC
OVATE-T2-off2F	ATGAGAGGGATATGTGCAAG
OVATE-T2-off2R	CATAGTGGACGGTAATATCTTC
OVATE-T2-off3F	GGTTCTGGGTCAATGACA
OVATE-T2-off3R	GAGTTATGCTCGTTAGGACA
OVATE-T2-off4F	TCAACACACGCAATTAGT
OVATE-T2-off4R	CATCGTTAGAGTCATTGATATG
Primers for yeast two-hybrid, Split-LUC, pull-down and Co-IP assay	
AtHAN-YTH-F	GGAATTCCATATGATGATGCAGACTCCGTACACT
AtHAN -YTH-R	CGGGATCCCTCTGGTAAAGTCATGGACAAGAC
CsHEC1-YTH-F	GGGAATTCCATATGATGGAAAATGATGATTTAAAATCGG
CsHEC1-YTH-R	CGCGGATCCTCAAGGTTGGGATTGATGATGAT
CsHEC2-YTH-F	GGGAATTCCATATGATGGACGATATCGACATCCTCAAA
CsHEC2-YTH-R	CGCGGATCCTCAAGACTGCATTTGCAAAGAAGC
CsOVATE-YTH-F	GATTACGCTCATATGATGATGATGACACCAAAACG
CsOVATE-YTH-R	GAGCTCGATGGATCCTCAGTTACAGAACAGAGACTG
CsHEC1-nLuc-F	GACGAGCTCGGTACCATGGAAAATGATGATTTAAAATCGG
CsHEC1-nLuc-R	CGAGATCTGGTCGACAGGTTGGGATTGATGATGATTAG
CsHEC1-cLuc-F	TCCCGGGGCGGTACCATGGAAAATGATGATTTAAAATCGG
CsHEC1-cuc-R	GCTCTGCAGGTCGACTCAAGGTTGGGATTGATGATG
CsOVATE-cLuc-F	TCCCGGGGCGGTACCATGATGATGACACCAAAACG
CsOVATE-cLuc-R	GCTCTGCAGGTCGACTCAGTTACAGAACAGAGACTG
CsHEC1-pGEX-4T-F	GGATCCCCGGAATTCATGGAAAATGATGATTTAAAATCGG
CsHEC1-pGEX-4T-R	TCGACCCGGGAATTCTCAAGGTTGGGATTGATGATG
CsOVATE-pET-28a-F	CGCGGATCCGAATTCATGATGATGACACCAAAACGAT
CsOVATE-pET-28a-R	ACGGAGCTCGAATTCTCAGTTACAGAACAGAGACTGC
CsOVATE-CoIP-F	CGACTCTAGAAAGCTTATGATGATGACACCAAAACG
CsOVATE-CoIP-R	TACCGGATCCACTAGTGTTACAGAACAGAGACTGC
Primers for yeast one-hybrid, LUC activity measure assay	
AtPID-Ebox-F	AGCTTTCTCACGCGTTGTCTCACGCGTTGTCTCACGCGTTGG
AtPID-Ebox-R	TCGACCAACGCGTGAGACAACGCGTGAGACAACGCGTGAGAA
CsYUC4-P1-F	AGCTTCAAGCATTTGAAAACAAGCATTTGAAAACAAGCATTTGAAAAG
CsYUC4-P2-R	TCGACTTTTCAAATGCTTGTTTTCAAATGCTTGTTTTCAAATGCTTGA
CsYUC4-P1-F	AGCTTAAGACAAATGACAAAAGACAAATGACAAAAGACAAATGACAAG

CsYUC4-P2-R	TCGACTTGTCATTTGTCTTTTGTCATTTGTCTTTTGTCATTTGTCTTA
CsHEC1-62SK-F	AGAACTAGTGGATCCATGGAAAATGATGATTTAAAATC
CsHEC1-62SK-R	GGTATCGATAAGCTTTCAAGGTTGGGATTGATG
CsHEC2-62SK-F	AGAACTAGTGGATCCATGGACGATATCGACATC
CsHEC2-62SK-R	GGTATCGATAAGCTTTCAAGACTGCATTTGCA
CsOVATE-62SK-F	AGAACTAGTGGATCCATGATGATGACACCAAAACGA
CsOVATE-62SK-R	GGTATCGATAAGCTTTCAGTTACAGAACAGAGACTGC
Csovate#1-62SK-R	TCGACGGTATCGATAAGCTTCTAGTTCGTCGATTTCATCGA
Csovate#2-62SK-R	TCGACGGTATCGATAAGCTTTTATTTTGTTTGGTTTCTCCCG
ProCsYUC4-0800F	TCGACGGTATCGATAAGCTTAGCAATGTTAATGCTGATGTC
ProCsYUC4-0800R	GCTCTAGAACTAGTGGATCCTAAAGTGAGTCTGTGTTTTGGT
Primers for ChIP-PCR and EMSA	
CsHEC1-puc19flag-F	CGGGGGACGAGCTCGGTACCATGGAAAATGATGATTTAAAATCGG
CsHEC1-puc19flag-R	ATGGTCTTTGTAGTCTTCGAAAGGTTGGGATTGATGATGAT
CsHEC2-puc19flag-F	CGGGGGACGAGCTCGGTACCATGGACGATATCGACATCCTCA
CsHEC2-puc19flag-R	ATGGTCTTTGTAGTCTTCGAAAGACTGCATTTGCAAAGAAGC
Chip-CsYUC4-P1F	ACAGTTAAAGAGAAGGCGGGAATG
Chip-CsYUC4-P1R	AGGAAAATAAGGAGAGTTAAATTGTTAATTCCT
Chip-CsYUC4-P2F	CGACTTGAATTTGGTTGATGAAC
Chip-CsYUC4-P2R	GTTAATATGATTCACACACTCACGA
Tubulin F	ACGCTGTTGGTGGTGGTAC
Tubulin R	GAGAGGGGTAAACAGTGAATC
YUC4-Biotin-P1F	Biotin-TGGAGCGTAACTTTCAAGCATTTGAAAATGCGTGGGTGGA
YUC4-Biotin-P1R	Biotin-TCCACCCACGCATTTTCAAATGCTTGAAAGTTACGCTCCA
YUC4-cold-P1F	TGGAGCGTAACTTTCAAGCATTTGAAAATGCGTGGGTGGA
YUC4-cold-P1R	TCCACCCACGCATTTTCAAATGCTTGAAAGTTACGCTCCA
YUC4-mutBiotin-P1F	Biotin-TGGAGCGTAACTTTCAAGAAAGGGAAAATGCGTGGGTGGA
YUC4-mutBiotin-P1R	Biotin-TCCACCCACGCATTTTCCCTTTCTTGAAAGTTACGCTCCA

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