

Figure S1

	E1	E4
(a) <i>AccEN4</i>	CCTAGGGTTGAAGTTCATGGAGG.....CCACAGGTTTGTGTTTGTGCTCT	
<i>AcCEN</i>	CCAAGGGTTGAAGTTCATGGAGG.....CCACAGGTTTGTGTTTGTGCTCT	
<i>AccEN1</i>	CCTAGGGTTGAAATCCAAGGAGG.....CCACAGGTTTGTGTTTGTCTCT	
<i>AcCEN2</i>	CCTAGAGTTGAAGTCCATGGAGG.....CCACAGGTTTGTGTTTGTCTGT	
<i>AcCEN3</i>	CCTAGAGTTGAAGTCCATGGAGG.....CCACAGGTTTGTGTTTGTCTGT	
<i>AcBFT1</i>	GCTCGCGTAGAGATCGGTGGAGA.....CCACCGGTACGTGTTTCATCTGT	
<i>AcBFT2</i>	CCTCGCGTAGAGATCGGCGGTGA.....CCACCGATACGTGTTCTTCTGT	
<i>AcBFT3</i>	CCTCGCGTAGAGATCGGTGGTGA.....CCACCGGTACGTGTTCTCTTGT	
<i>AcFT</i>	CCGAGGGTTGATATTGGTGGCGA.....CCATCGCTTTGTCTGGTGCTAT	
<i>AcFT1</i>	CCTAGAGTTGATGTTGGAGGTGA.....TCATCGCTTTGTGTTCTGTTGT	
<i>AcFT2</i>	CCTAGGGTTGATATTGGAGGTGA.....TCATCGCTTTGTGTTCTGTTGT	
<i>AcMFT1</i>	CCTAGGGTCACCATCTCTGGTCA.....CCACAGGTATACTAGGTCTCT	
<i>AcMFT2</i>	CCTAGGGTCACAATCACTGGCCA.....ACATCGCTGCATCCTGCTTCTGT	

(b) U6-CEN4 construct (1150 nt):

attB1-GCT-pU6-26 (387 bp)-

GATTGAGAGCACAAACACAAACCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA

ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTCAAGAGCTTGGAGTGGATGGAATCGGCAGCAAAGGA-

pU6-29 (319 bp)-

GATTGCCTAGGGTTGAAGTTCATGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA

ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTACCc-attL2

U3-CEN4 construct (868 nt):

attB1-GCT-pU3-b(324 bp)-

GGTCAGAGCACAAACACAAACCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA

CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTCAAGAGCTTGGAGTGGATGGAATCGGCAGCAAAGGA-

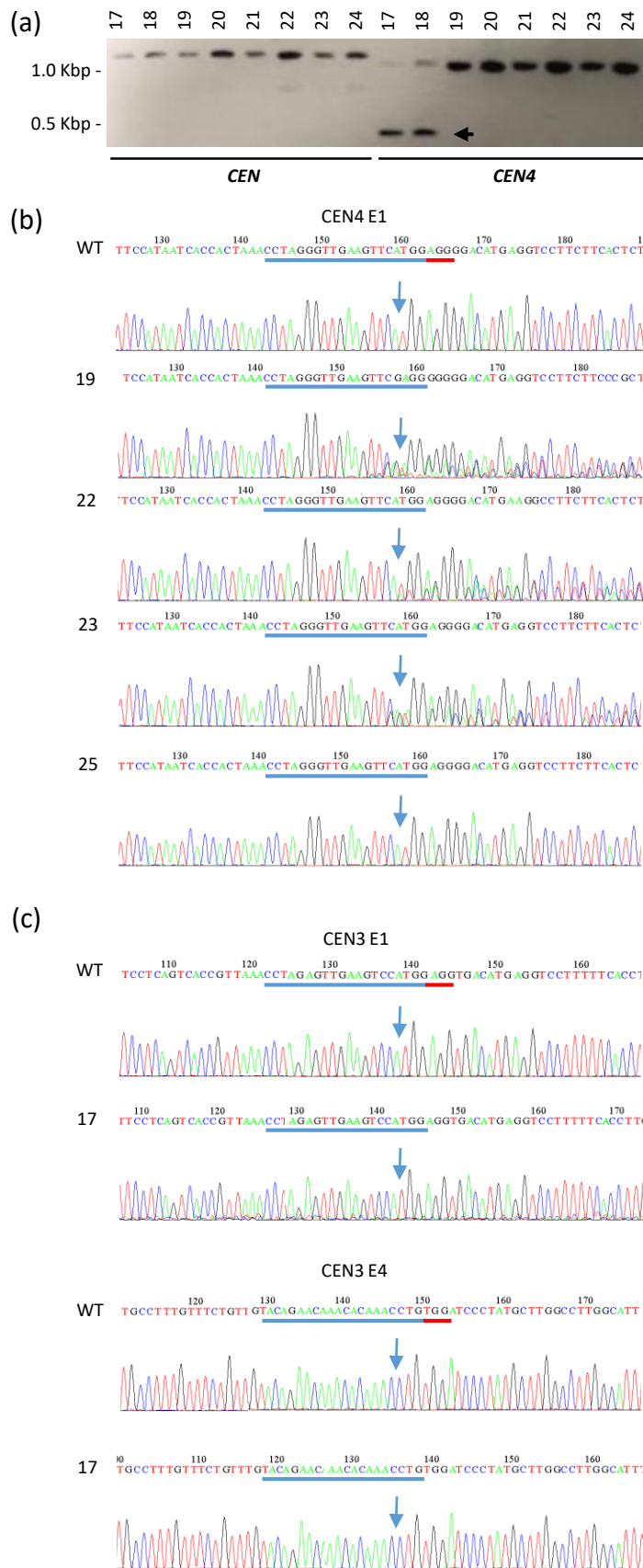
pU3-d (101 bp)-

GGTCACCTAGGGTTGAAGTTCATGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA

ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTACC-attL2

Supplementary Figure S1. Selection of CRISPR/Cas9 target sequences and construct design. (a) Alignment of the corresponding target E1 and E4 regions in *AccEN4*, *AcCEN* and other *A. chinensis* *CEN*, *BROTHER OF FT (BFT)*, *MOTHER OF FT (MFT)* and *FT* genes. PAM sequence is in red and mismatches are highlighted in yellow. (b) The sequence of constructs used for CRISPR/Cas9-mediated editing of *AccEN4* and *AcCEN* genes. Green, gene-specific target sequence (protospacer); red, guide RNA (crRNA, linker loop and tracrRNA); blue, end of the promoter sequence; underlined, +1 position (appropriate nucleotide added where necessary, G and A for pU6 and pU3, respectively).

Figure S2



Supplementary Figure S2. Evaluation of CRISPR/Cas9-mediated gene editing in transgenic kiwifruit. (a) Example of amplification using gene-specific primers, which identified the E1-E4 deletion in *AccEN4* in lines 17 and 18 (arrow). (b) Examples of wild-type (top) and edited *AccEN4* E1 regions identified by sequencing of the PCR fragment. (c) Wild-type *AccEN3* E1 and E4 regions identified by sequencing of the PCR fragment in early flowering line 17.

Figure S3

(a)

	<i>AccEN4</i>	E1	E4		<i>AccEN</i>	E1	E4
	WT	...CCTAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...		WT	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
U6-CEN4#14 *	14.DEL	...CCTAGGGTTGAAGTTCA-----	-----GTTTGTGTTTGTGCTCT...		CEN.1	...CCAAGGGTTGAAGTTCATGG.....	...CAG (156nt) GTTTGTGTTTGTGCTCT...
	CEN4.1	...CCTAGGGTTGAAGTTCAATGG.....	...CAG-----TGTTTGTGCTCT...		CEN.2	...CCAAGGGTTGAAGTTCATGG.....	...CAG (156nt) GTTTGTGTTTGTGCTCT...
	CEN4.2	...CCTAGGGTTGAAGTTCAATGG.....	...CAG-----TGTTTGTGCTCT...		CEN.3	...CCAAGGGTTGAAGTTCATGG.....	...CAG (156nt) GTTTGTGTTTGTGCTCT...
	CEN4.3	...CCTAGGGTTGAAGTTCAATGG.....	...CAG-TTT-----		CEN.4	...CCAAGGGTTGAAGTTCATGG.....	...CAG (156nt) GTTTGTGTTTGTGCTCT...
	CEN4.4	...CCTAGGGTTGAAG--ATGG.....	...CAG-TTT-----				
U6-CEN4#18 **	18.DEL	...CCTAGGGTTGAAGTTCA-----	-----GTTTGTGTTTGTGCTCT...		CEN.1	...CCAAGGGTTGAAGTT-ATGG.....	...CAGAGTTTGTGTTTGTGCTCT...
	CEN4.1	...CCTAGGGTTGAAG--ATGG.....	...CAG-TTT-----		CEN.2	...CCAAGGGTTGAAGTTCATGG.....	...CAG-----GTGTTTGTGCTCT...
	CEN4.2	...CCTAGGGTTGAAGTTCAATGG.....	...CAG-----TGTTTGTGCTCT...		CEN.3	...CCAAGGGTTGAAGTTCATGG.....	...CAG-----GTGTTTGTGCTCT...
	CEN4.3	...CCTAGGGTTGAAG--ATGG.....	...CAG-TTT-----		CEN.4	...CCAAGGGTTGAAGTT-ATGG.....	...CAGAGTTTGTGTTTGTGCTCT...
	CEN4.4	...CCTAGGGTTGAAG--ATGG.....	...CAG-TTT-----				
U6-CEN4#17 **	17.DEL	...CCTAGGGTTGAAGTTCA-----	-----GTTTGTGTTTGTGCTCT...		CEN.1	...CCAAGGGTTGAAGTT-ATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.1	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.2	...CCAAGGGTTGAAGTT-ATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.2	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.3	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.3	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.4	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.4	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...				
U6-CEN4#7 ***	CEN4.1	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.1	...CCAAGGGTTGAAGTTCATGG.....	...CAGAGTTTGTGTTTGTGCTCT...
	CEN4.2	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.2	...CCAAGGGTTGAAGTTCATGG.....	...CAGAGTTTGTGTTTGTGCTCT...
	CEN4.3	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.3	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.4	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.4	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
U6-CEN4#22 ****	CEN4.1	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.1	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.2	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.2	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.3	...CCTAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.3	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.4	...CCTAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.4	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...
U6-CEN4#19 ****	CEN4.1	...CCTAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.1	...CCAAGGGTTGAAGTTCATGG.....	...CAG--TGTTTGTGCTCT...
	CEN4.2	...CCTAGGGTTGAAGTT--GG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.2	...CCAAGGGTTGAAGTTCAATGG.....	...CAGGTTTGTGTTTGTGCTCT...
	CEN4.3	...CCTAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.3	...CCAAGGGTTGAAG--GG.....	...CAG--TGTTTGTGCTCT...
	CEN4.4	...CCTAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...		CEN.4	...CCAAGGGTTGAAGTTCATGG.....	...CAGGTTTGTGTTTGTGCTCT...

(b)

		g1	g2	g3	g4
	WT <i>AcCEN4</i>	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCTTTGGTATGTATTAATTA.....	...CTTCAGTACCCGGAAATTG...
PTG-CEN4 ***	CEN4.1	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCT-----ATTA.....	...CTTCAGTACCCGGAAATTG...
	CEN4.2	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAA-CTA.....	...CCT-----ATTA.....	...CTTCAGTACCCGGAA-TTG...
	CEN4.3	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAA-CTA.....	...CCT-----ATTA.....	...CTTCAGTACCCGGAA-TTG...
	CEN4.4	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAA-CTA.....	...CCT-----ATTA.....	...CTTCAGTACCCGGAA-TTG...
	WT <i>AcCEN</i>	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATCCATATCTGA.....	...TCCTGTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCCAAA...
CEN.1	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATCCATATCTGA.....	...TCCTGTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCCAAA...	
CEN.2	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATCCATATCTGA.....	...TCCTGTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCCAAA...	
CEN.3	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATCCATATCTGA.....	...TCCTGTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCCAAA...	
CEN.4	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATCCATATCTGA.....	...TCCTGTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCCAAA...	

(c)

		g1	g2	g3	g4
	WT <i>AcCEN4</i>	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCTTTGGTATGTATTAATTA.....	...CTTCAGTACCCGGAAATTG...
PTG-CEN ***	CEN4.1	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCTTTGGTATGTATTAATTA.....	...CTTCAGTACCCGGAAATTG...
	CEN4.2	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCTTTGGTATGTATTAATTA.....	...CTTCAGTACCCGGAAATTG...
	CEN4.3	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCTTTGGTATGTATTAATTA.....	...CTTCAGTACCCGGAAATTG...
	CEN4.4	...CTAGTAGTTGGAAGAGTGAT.....	...CATAATCACCACCTAAACCTA.....	...CCTTTGGTATGTATTAATTA.....	...CTTCAGTACCCGGAAATTG...
	WT <i>AcCEN</i>	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATCCATATCTGA.....	...TCCTGTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCCAAA...
CEN.1	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATC-----TGA.....	...TCC--TTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGC--AAA...	
CEN.2	...TCCA--GTCACCACCTAAACC.....	...TCCTAGTGATCCATA--TGA.....	...TCC--TTGCTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGCC--AA...	
CEN.3	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATC-----TGA.....	...TCC--TTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGC--AAA...	
CEN.4	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATC-----TGA.....	...TCC--TTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGC--AAA...	
CEN.5	...TCCATAGTCACCACCTAAACC.....	...TCCTAGTGATC-----TGA.....	...TCC--TTGCTGCTGTGTCTCT.....	...TGTGTTCTTCAATGC--AAA...	

Supplementary Figure S3. Mutations (red font) identified in *AccEN4* and *AccEN* alleles in U6-CEN4 (a), PTG-CEN4 (b) and PTG-CEN (c) lines. At least four clones of gene-specific amplification products were subjected to sequence analysis. wt, wild-type; +, insertion; -, deletion.