Table S1. Primers used for vectors construction, mutation detection, off-target analysis and qPCR.

Primer name	Sequence (5'-3')
F1	GTGTGGTGTTGTCTGCTCTATGTTT
R1	CCAGCGCCCAATCATTTACCCTC
F2	AGTTGTTTGAATACTATGACCAGGATCTCCC
R2	GCAGCATATACGCGGAAAGAATTATAAACGAT
crispr-gRNA1-F	GGTCTCTgattCAGGTCTGTCCCATCAAGATGTTTTAGAGCTAGAAATAG
crispr-gRNA2-R	GGTCTCTAAACCTAAGCCAGTATCAGACTCCAATCACTACTTCGTCTCTA
crispr-gRNA3-F	GGTCTCTgattACTCGATGGATGATGATATAGTTTTAGAGCTAGAAATAG
crispr-gRNA4-R	GGTCTCTAAACGTCTTTAACAGTTAAACCATAATCACTACTTCGTCTCTA
ptg-gRNA1-F	GGTCTCTTGCACAGGTCTGTCCCATCAAGATGTTTCAGAGCTATGCTGGA
ptg-gRNA2-R	GGTCTCTAAACCTAAGCCAGTATCAGACTCCTGCACCAGCCGGGAATCGA
ptg-gRNA3-F	GGTCTCTTGCAACTCGATGGATGATGATATAGTTTCAGAGCTATGCTGGA
ptg-gRNA4-R	GGTCTCTAAACGTCTTTAACAGTTAAACCATTGCACCAGCCGGGAATCGA
SP-DL	GTCGTGCTCCACATGTTGACCGG
SP-R	CCCGACATAGATGCAATAACTTC
GF	aGAGACCGGTCTCGGTTTCAGAGCTATGCTGGAAACAGC
GR	AGCTCGAGAGGCGCGAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGAT
U6-1-F	GACCGGTAAGGCGCGAGAAATCTCAAAATTCCGGCAGAACAA
U6-1-C	CGAGACCGGTCTCtAATCACTACTTCGTCTCTAACCATATAT
U6-1-R	CACTGGTGCTTTGTTAATCACTACTTCGTCTCTAACCATATAT
TF	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGT
TR	CGAGACCGGTCTCtTGCACCAGCCGGGAATCGAACCCGGGTCTGTACCGTGG
gRNA-R	GCACCGACTCGGTGCCAC
gRNA1-F	CAGGTCTGTCCCATCAAGATGTTTCAGAGC
gRNA2-F	GGAGTCTGATACTGGCTTAGGTTTCAGAGC
gRNA3-F	ACTCGATGGATGATGATATAGTTTCAGAGC
gRNA4-F	ATGGTTTAACTGTTAAAGACGTTTCAGAGC
Achn107181-F	TGAGAGATTCCGTTGCCCAGAAGT
Achn107181-R	TTCCTTACTCATGCGGTCTGCGAT
PP2A-F	GCAGCACATAATTCCACAGG
PP2A-R	TTTCTGAGCCCATAACAGGAG
T1-F	GTGTGGTGTTGTCTGCTCTATGTTT
T1-R	CTCTCTCACATTGGTGGGGTTGT
T2-F	ACAACCCCACCAATGTGAGAGAG
T2-R	CCAGCGCCCAATCATTTACCCTC
T3-F	AGTTGTTTGAATACTATGACCAGGATCTCCC
T3-R	CATATGGCTGTCCACCTAGCATTGC
T4-F	AGCAATGCTAGGTGGACAGCCATATG
T4-R	GCAGCATATACGCGGAAAGAATTATAAACGAT
OT1-F	TCTCCACCTTCTTTATGC
OT1-R	TGCTATTTGTCCGTCTC
OT2-F	ATAACCAAGGCAGAGC
OT2-R	AACCCATGACCACTAAC
OT3-F	TGGCAATGAACACCTC
OT3-R	AACCCATGACCACTAACT
OT4-F	TCACCAGGTTCAAAGC
OT4-R	GTTAGCGTCCCTCACA

Table S2. Summary of mutagenesis frequencies in positive clones selected.

Vector ID	sgRNA	No. of clones analyzed	No. of mutated clones	Mutation frequency (%)
A1	sgRNA1	120	9	7.50%
	sgRNA2		9	7.50%
	sgRNA1&sgRNA2		9	7.50%
A2	sgRNA3	280	0	0.00%
	sgRNA4		19	6.78%
	sgRNA3&sgRNA4		0	0.00%
B1	sgRNA1	120	109	90.83%
	sgRNA2		98	81.67%
	sgRNA1&sgRNA2		98	81.67%
B2	sgRNA3	260	190	73.07%
	sgRNA4		158	65.38%
	sgRNA3&sgRNA4		156	60.00%

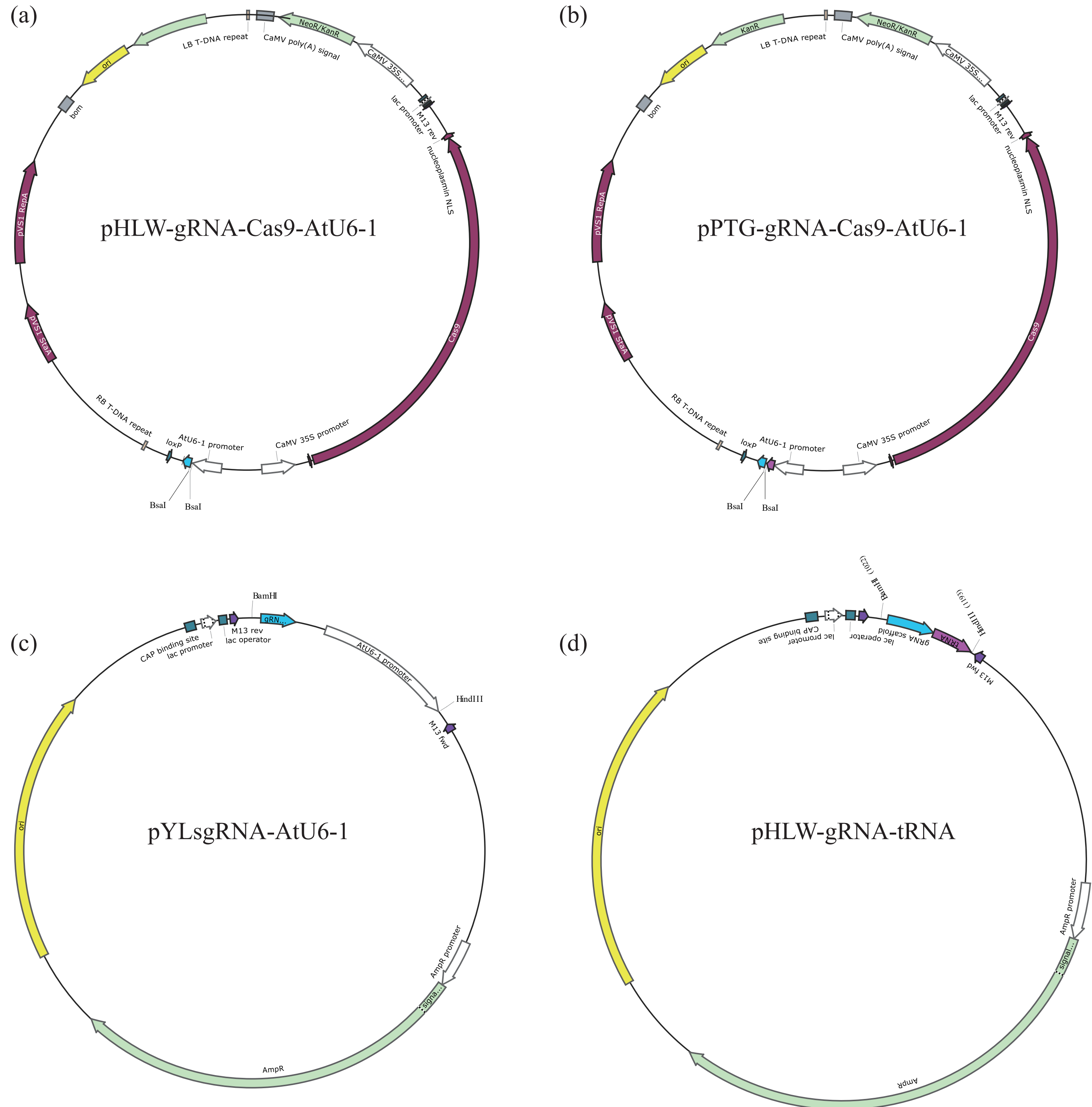

Figure S1. Overall structure of vectors for CRISPR/Cas system. (a) Overall structure of the Cas9 binary expression vector for CRISPR/Cas9 system. (b) Overall structure of the Cas9 binary expression vector for PTG/Cas9 system. (c) Overall structure of the sgRNA intermediate vector for CRISPR/Cas9 system. (d) Overall structure of the sgRNA intermediate vector for PTG/Cas9 system.

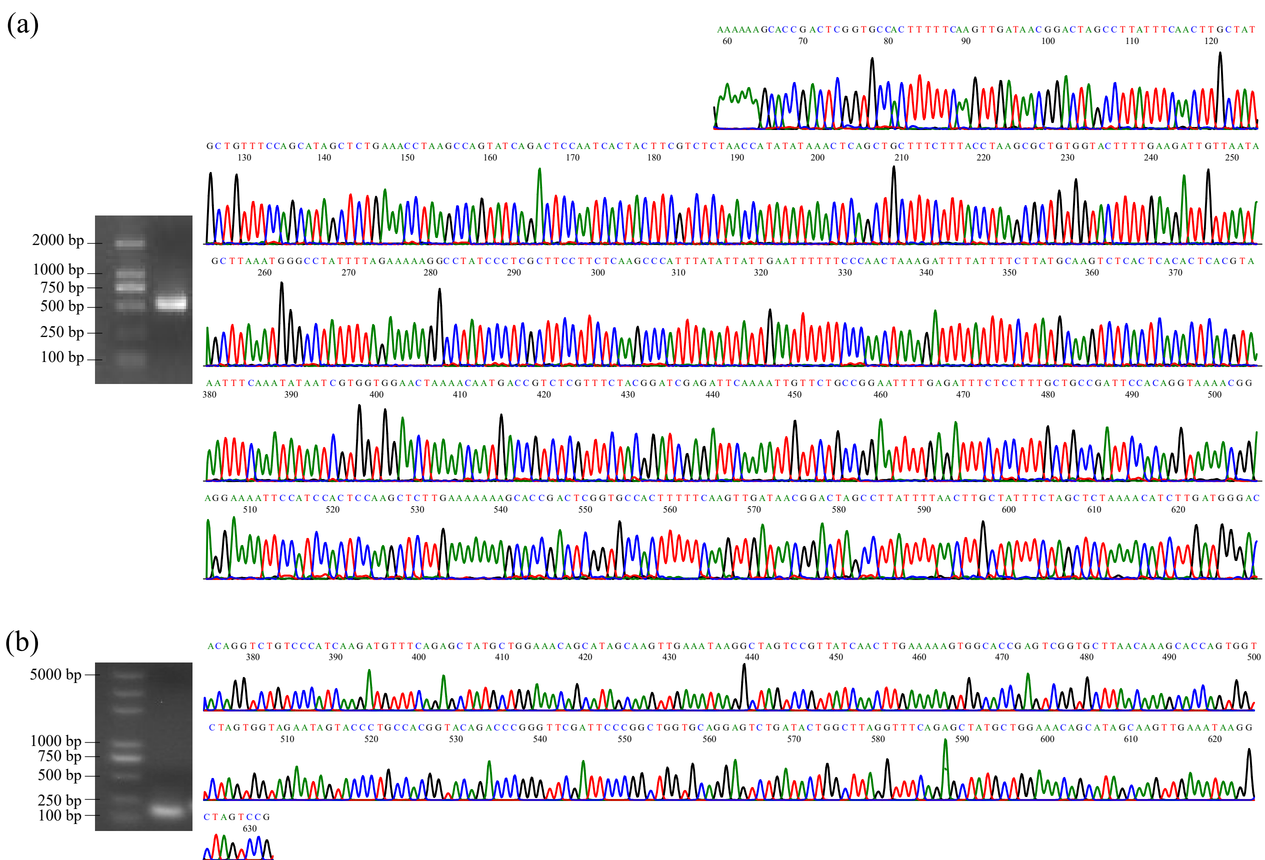

Figure S2. PCR validation and Sanger sequencing of PCR products amplified from paired-sgRNAs/Cas9 vectors. (a) Validation for CRISPR/Cas9 system. (b) Validation for PTG/Cas9 system.

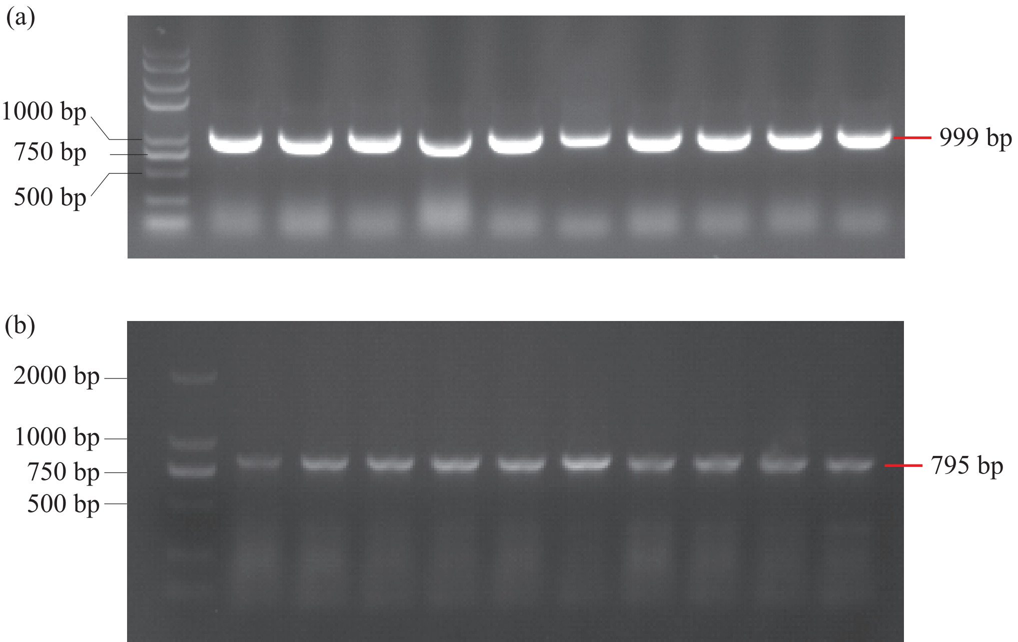

Figure S3. Transgenic positivity check in G418-resistance callus lines with Cas9 specific primers SP-DL/SP-R. (a) Representation of CRISPR/Cas9 system. (b) Representation of PTG/Cas9 system.

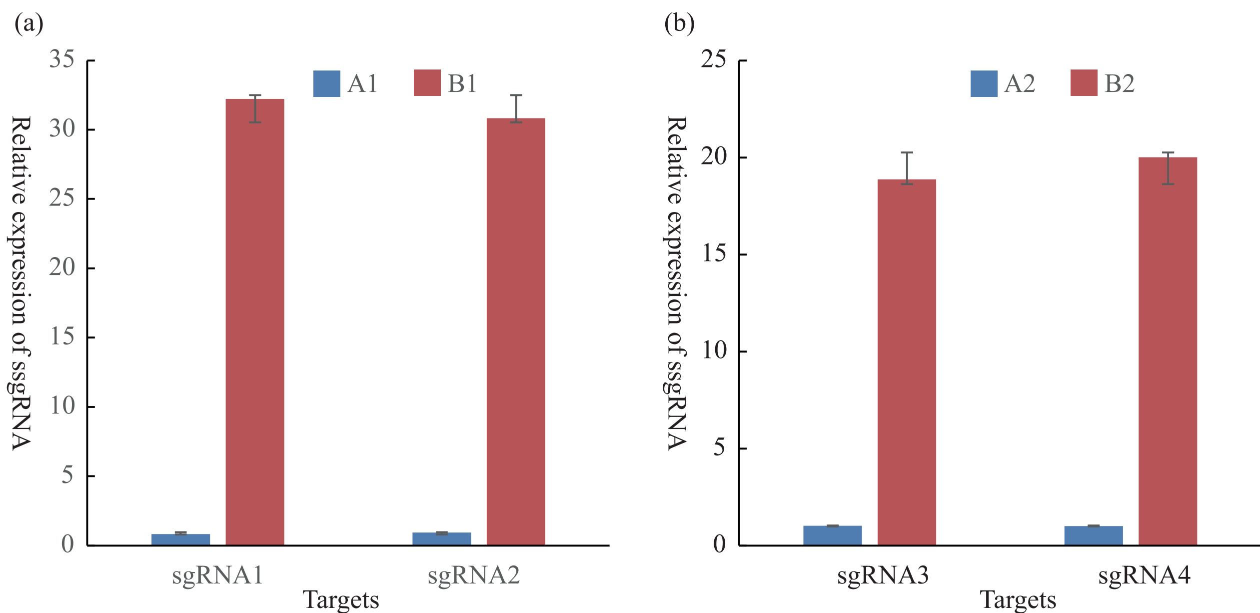

Figure S4. The relative expression of ssgRNAs. (a) The relative expression levels of sgRNA1 and sgRNA2 in kiwifruit G418-resistance callus lines transformed with A1/B1. (b) The relative expression levels sgRNA3 and sgRNA4 in kiwifruit G418-resistance callus lines transformed with A2/B2.