Supporting materials

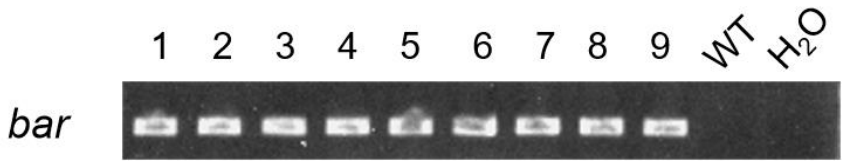

Figure S1. Identification of T0 transgenic lines generated by the UC system and the PSC system.

Gel images show PCR products amplified with bar-F/bar-R from T0 transgenic plants generated by the UC system (lanes 1, 2 and 3), the pZmPRO1 PSC system (lanes 4, 5 and 6) and the pZmPRO3 PSC system (lanes 7, 8 and 9). *bar*, Herbicide resistance *bar* gene. WT, H_2O, negative control.

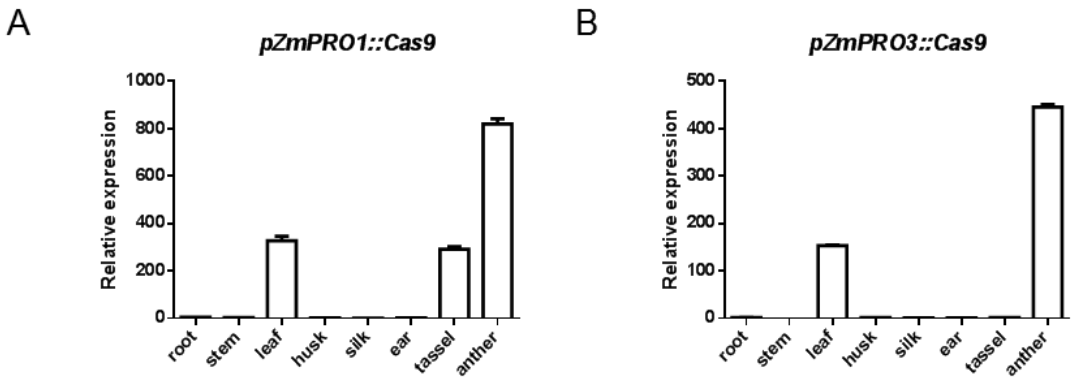

Figure S2. Expression patterns of *Cas9* in PSC system.

A) and B) RNA expression levels of *Cas9* in various tissues in T0 plants generated by the pZmPRO1 and pZmPRO3 PSC systems. For each RNA sample, three technical replicates were performed. Values are means with SE; n = 3 biological replicates.

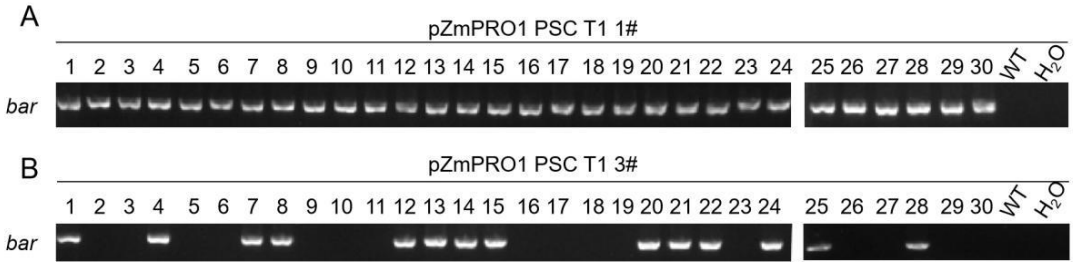

Figure S3. Identification of T1 transgenic lines generated by the pZmPRO1 PSC system.

Gel images show PCR products amplified with bar-F/bar-R from T1 plants generated by the ZmPRO1 system. *bar*, Herbicide resistance *bar* gene. WT, H_2O, negative control.

A) *bar* identification of 30 T1 plants of #1 generated by the pZmPRO1 PSC system;

B) *bar* identification of 30 T1 plants of #3 generated by the pZmPRO1 PSC system.

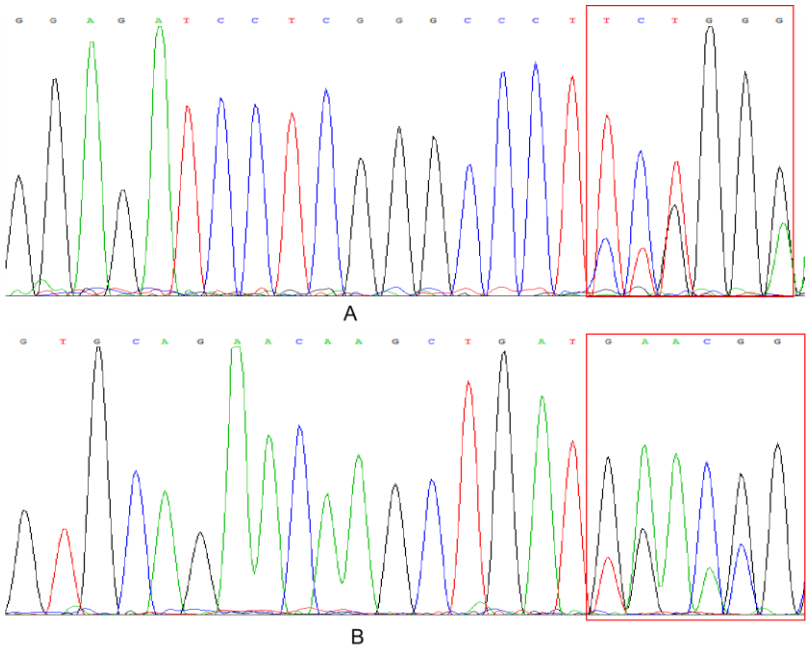

Figure S4. The sequencing chromatographs of PCR products from the leaf of #1 T1 plants generated by the pZmPRO1 PSC system.

A) Sequencing chromatograph of PCR product at target1. The 18th base "T" at target1 was deleted in only one chromosome that resulted in frame shift;

B) Sequencing chromatograph of PCR product at target4. The base "T" was inserted between the 17th and 18th bases at target4 in only one chromosome that resulted in frame shift.

Table S1. Primers used in this study

Purpose	Name	Sequences (5'-3')
Quantitative RT-PCR	qZmPRO1-F	ATTTGCTTGAGCGGCTCTTGTC
	qZmPRO1-R	AGCTGAATATTCCCAGCGCAAC
	qZmPRO3-F	CCAAGTACATGGTCATCCAAGGC
	qZmPRO1-R	TTATGCCTCCAGATCCCTTCTTCC
	qCas9-F	CCAGTCCAAGAACGGCTACG
	qCas9-R	GATGGTCTCCTCGGACTTGC
Vector Construction	ZmPRO1-F	AAGCTT CTATAGCGTGGGGTAAATGCG
	ZmPRO1-R	GGTACC CTTCGCTCGGCGCGTTGGTC
	ZmPRO3-F	GCACTTGTGTTGACACTCAATC
	ZmPRO1-R	TGCTCAACTACGACCTGCAAT
	pU6-F	AAGCTTCAGCAAATAATGGCATCCGA
	pU6-R	GCGCGCGATGCGGTGCTTCCGGTTTG
	tU6-F	GCGCGCAATTTTTTTGCGGATTTGCG
	tU6-R	AAGCTTGGGCTAAAGGAAAAAAATGT
	pU6-F	GGGATCCTCTAGAGTCGACCTGCAGCAAATA ATGGCATCCGATG
	tU6-R	CGCTGCAAAGCTTGCATGCCTGGGGCTAAA GGAAAAAAATGTTAG
Mutation identification for Sanger sequencing	O2-F	CTCCCCTTGCACTGCATCTC
	O2-R	GGGTTCAAGATGCCTACCGA
	O2-F1-3	AGCATCCAAGCGTACTCCTC
	O2-R1-3	TTTCAGTTATTGCACCCCCA
	O2-F4	ATATACCCATCCACAGGCGG
	O2-R4	CCGTCCATGTCTTCGTCTGA
bar identification	bar-F	GAAGTCCAGCTGCCAGAAAC
	bar-R	GCACCATCGTCAACCACTAC
Targets amplification for Hi-TOM	$O2\text{-}F_{1\text{-}2}$	GGAGTGAGTACGGTGTGCTATTGGGCATGG AGCACGTC
	$O2\text{-}R_{1\text{-}2}$	GAGTTGGATGCTGGATGGCGGTGTGCTTGT CGTCAG
	$O2\text{-}F_3$	GGAGTGAGTACGGTGTGCTCAGGCGCCCTA AATGTTGA
	$O2\text{-}R_3$	GAGTTGGATGCTGGATGGCGCCGAAGATAT AGCGAGGG

	O2-F_4	GGAGTGAGTACGGTGTGCCTGACTCTCGATCTGGCTCAC
	O2-R_4	GAGTTGGATGCTGGATGGGGTAGGCATCTTGAACCCCA
Barcoding PCR for Hi-TOM	F-1	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGCGTTGGAGTGAGTACGGTGTGC
	F-2	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGTAGTGGAGTGAGTACGGTGTGC
	F-3	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTACGCTGGAGTGAGTACGGTGTGC
	F-4	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTCTCGTGGAGTGAGTACGGTGTGC
	F-5	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGCTCTGGAGTGAGTACGGTGTGC
	F-6	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTAGTCTGGAGTGAGTACGGTGTGC
	F-7	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTCGACTGGAGTGAGTACGGTGTGC
	F-8	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGATGTGGAGTGAGTACGGTGTGC
	F-9	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTATACTGGAGTGAGTACGGTGTGC
	F-10	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTCACATGGAGTGAGTACGGTGTGC
	F-11	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGTGCTGGAGTGAGTACGGTGTGC
	F-12	ACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTACTATGGAGTGAGTACGGTGTGC
	2P-F	AATGATACGGCGACCACCGAGATCTACACACCGACAAACACTCTTTCCCTACACGACGCTCTT
	R-A	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGCGTTGAGTTGGATGCTGGATGG
	R-B	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGTAGTGAGTTGGATGCTGGATGG
	R-C	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTACGCTGAGTTGGATGCTGGATGG

	R-D	GACTGGAGTTCAGACGTGTGCTCTTCCGATC TCTGTCTCGTGAGTTGGATGCTGGATGG
	R-E	GACTGGAGTTCAGACGTGTGCTCTTCCGATC TCTGTGCTCTGAGTTGGATGCTGGATGG
	R-F	GACTGGAGTTCAGACGTGTGCTCTTCCGATC TCTGTAGTCTGAGTTGGATGCTGGATGG
	R-G	GACTGGAGTTCAGACGTGTGCTCTTCCGATC TCTGTCGACTGAGTTGGATGCTGGATGG
	R-H	GACTGGAGTTCAGACGTGTGCTCTTCCGATC TCTGTGATGTGAGTTGGATGCTGGATGG

Table S2. Targets in this study (Hi-Ⅱ).

Targets	Sequences (5'-3')
Target1	GGAGATCCTCGGGCCCTTCTGGG
Target2	GTGGACCTTTGAGAGGTTACTGG
Target3	GGTAATGATGGCGCCTGCGGCGG
Target4	GTGCAGAACAAGCTGATGAACGG

Table S3. Target gene mutations caused by the pZmPRO1 PSC system in the T1 generation.

Construct	T1 lines	Target1	Target2	Target3	Target4
pZmPRO1::Cas9	#1-1	$D^{18}T$	N	N	N
	#1-2	$D^{18}T$	N	N	N
	#1-3	$I^{17\text{-}18}$ CCATTATTTATTATT GATAAATAATAAGCC CAGGAGACAGTCA (43bp)	N	N	N
	#1-4	$D^{18}T$	N	N	N
	#1-5	N	N	N	$I^{17\text{-}18}T$
	#1-6	$D^{18}T$	N	N	N
	#1-7	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-8	$D^{18}T$	N	N	N
	#1-9	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-10	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-12	$D^{18}T$	N	N	N
	#1-13	$D^{18}T$	N	N	N
	#1-14	$D^{18}T$	N	N	N
	#1-15	N	N	N	$I^{17\text{-}18}T$
	#1-16	$D^{18}T$	N	N	N
	#1-17	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-18	$D^{18}T$	N	N	N
	#1-19	N	N	N	$I^{17\text{-}18}T$
	#1-20	$D^{18}T$	N	N	N
	#1-21	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-22	$D^{18}T$	N	N	N
	#1-24	$D^{18}T$	N	N	N
	#1-25	$D^{18}T$	N	N	N
	#1-27	$D^{18}T$	N	N	N
	#1-28	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-29	$D^{18}T$	N	N	N
	#1-30	$D^{18}T$	N	N	N
	#3-13	$D^{16\text{-}20}$	$D^{1\text{-}16}$	N	N
	#3-15	$D^{18}T$	N	N	N
	#3-21	$D^{15\text{-}19}$ CCTTC	N	N	N

	#3-24	$D^{18}T$	N	N	N
	#3-25	$D^{18}T$	N	N	N
	#3-28	$D^{18}T$	N	N	N

D and I refer to deletion and insertion, respectively; D^{X}, I^{X}, 'x' represents the position of the base; For example, $D^{18}T$ indicates the eighteenth base 'T' was deleted. N represents no mutation.

Table S4. Target gene mutations caused by the UC system in the T1 generation.

Construct	T1 lines	Target1	Target2	Target3	Target4
pZmUbi::Cas9	#1-1	D^{1-20}	D^{1-20}	N	$I^{17-18}T$
	#1-2	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-3	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-4	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-5	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-6	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-7	N	N	N	N
	#1-8	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-9	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-10	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-11	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-12	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-13	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-14	$I^{17-18}T$	N	N	$I^{17-18}T$
	#1-15	$I^{17-18}T$	N	N	$I^{17-18}T$
	#2-1	N	N	N	$I^{17-18}T$
	#2-2	$D^{18}T$	N	N	$I^{17-18}T$
	#2-3	$D^{18}T$	$I^{17-18}T$	N	$I^{17-18}T$
	#2-4	N	N	N	$I^{17-18}T$
	#2-5	$D^{18}T$	N	N	$I^{17-18}T$
	#2-6	N	N	N	$I^{17-18}T$
	#2-7	$D^{16-17}CT$	N	$D^{16-18}TGC$	$I^{17-18}T$
	#2-8	N	N	N	$I^{17-18}T$
	#2-9	$D^{15-16}CC$	N	N	$I^{17-18}T$
	#2-10	N	N	N	$I^{17-18}T$
	#2-11	N	N	N	$I^{17-18}T$

	#2-12	N	N	N	$I^{17-18}T$
	#2-13	N	N	N	$I^{17-18}T$
	#2-14	$I^{17-18}T$	N	N	$I^{17-18}T$
	#2-15	N	N	N	$I^{17-18}T$
	#3-1	$D^{18}T$	N	N	$I^{17-18}T$
	#3-2	$D^{18}T$	N	N	$I^{17-18}T$
	#3-3	N	N	N	N
	#3-4	$D^{14-17}CCCT$	N	N	$I^{17-18}T$
	#3-5	$D^{16}C$	N	N	$I^{17-18}T$
	#3-6	$D^{18}T$	N	N	$I^{17-18}T$
	#3-7	N	N	N	N
	#3-8	$D^{18}T$	N	N	$I^{17-18}T$
	#3-9	N	N	N	$I^{17-18}T$
	#3-10	$D^{18-20}TTC$	$I^{17-18}T$	D^{1-20}	$I^{17-18}T$
	#3-11	N	N	N	N
	#3-12	D^{1-20}	$I^{17-18}T$	N	$I^{17-18}T$
	#3-13	N	N	N	$I^{17-18}T$
	#3-14	$D^{18}T$	N	N	$I^{17-18}T$
	#3-15	$D^{18}T$	N	N	$I^{17-18}T$

D and I refer to deletion and insertion, respectively; D^{X}, I^{X}, 'x' represents the position of the base; For example, $D^{18}T$ indicates the eighteenth base 'T' was deleted. N represents no mutation.

Table S5. Target gene mutations caused by the pZmPRO1 PSC system in the T2 generation.

Construct	T2 plants	Target1	Target2	Target3	Target4
pZmPRO1::Cas9	#1-1-1	$D^{18}T$	N	N	N
	#1-1-2	$D^{18}T$	N	N	N
	#1-1-3	N	N	N	N
	#1-2-1	N	N	N	N
	#1-2-2	$D^{18}T$	N	N	N
	#1-2-3	$D^{18}T$	N	N	N
	#1-3-1	I^{17-18} CCATTATTTATTATTG ATAAATAATAAGCCC AGGAGACAGTCA (43bp)	N	N	N

	#1-3-2	N	N	N	N
	#1-3-3	N	N	N	N
	#1-4-1	$D^{18}T$	N	N	N
	#1-4-2	N	N	N	N
	#1-4-3	N	N	N	N
	#1-5-1	N	N	N	N
	#1-5-2	N	N	N	N
	#1-5-3	N	N	N	$I^{17\text{-}18}T$
	#1-6-1	N	N	N	N
	#1-6-2	$D^{18}T$	N	N	N
	#1-6-2	N	N	N	N
	#1-7-1	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-7-2	N	N	N	N
	#1-7-3	N	N	N	N
	#1-8-1	$D^{18}T$	N	N	N
	#1-8-2	$D^{18}T$	N	N	N
	#1-8-3	N	N	N	N
	#1-9-1	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-9-2	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-9-3	N	N	N	N
	#1-10-1	N	N	N	N
	#1-10-2	$D^{18\text{-}20}TCT$	N	N	$I^{17\text{-}18}T$
	#1-10-3	N	N	N	N
	#1-12-1	$D^{18}T$	N	N	N
	#1-12-2	$D^{18}T$	N	N	N
	#1-12-3	N	N	N	N
	#1-13-1	$D^{18}T$	N	N	N
	#1-13-2	N	N	N	N
	#1-13-3	N	N	N	N
	#1-14-1	$D^{18}T$	N	N	N
	#1-14-2	$D^{18}T$	N	N	N
	#1-14-3	N	N	N	N
	#1-15-1	N	N	N	N
	#1-15-2	N	N	N	$I^{17\text{-}18}T$
	#1-15-3	N	N	N	N
	#1-16-1	N	N	N	N
	#1-16-2	$D^{18}T$	N	N	N

	#1-16-3	$D^{18}T$	N	N	N
	#1-17-1	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-17-2	N	N	N	N
	#1-17-3	N	N	N	N
	#1-18-1	$D^{18}T$	N	N	N
	#1-18-2	N	N	N	N
	#1-18-3	$D^{18}T$	N	N	N
	#1-19-1	N	N	N	N
	#1-19-2	N	N	N	$I^{17\text{-}18}T$
	#1-19-3	N	N	N	N
	#1-20-1	N	N	N	N
	#1-20-2	$D^{18}T$	N	N	N
	#1-20-3	N	N	N	N
	#1-21-1	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-21-2	N	N	N	N
	#1-21-3	N	N	N	N
	#1-22-1	N	N	N	N
	#1-22-2	$D^{18}T$	N	N	N
	#1-22-3	$D^{18}T$	N	N	N
	#1-24-1	N	N	N	N
	#1-24-2	$D^{18}T$	N	N	N
	#1-24-3	N	N	N	N
	#1-25-1	$D^{18}T$	N	N	N
	#1-25-2	N	N	N	N
	#1-25-3	N	N	N	N
	#1-27-1	N	N	N	N
	#1-27-2	$D^{18}T$	N	N	N
	#1-27-3	N	N	N	N
	#1-28-1	$D^{18}T$	N	N	$I^{17\text{-}18}T$
	#1-28-2	$D^{18}T$	N	N	N
	#1-28-3	N	N	N	N
	#1-29-1	N	N	N	N
	#1-29-2	N	N	N	N
	#1-29-3	$D^{18}T$	N	N	N
	#1-30-1	N	N	N	N
	#1-30-2	N	N	N	N
	#1-30-3	$D^{18}T$	N	N	N

	#3-13-1	N	N	N	N
	#3-13-2	D^{19-20}	D^{1-18}	N	N
	#3-13-3	N	N	N	N
	#3-15-1	$D^{18}T$	N	N	N
	#3-15-2	$D^{18}T$	N	N	N
	#3-15-3	N	N	N	N
	#3-21-1	$D^{18}T$	N	N	N
	#3-21-2	N	N	N	N
	#3-21-3	N	N	N	N
	#3-24-1	$D^{18}T$	N	N	N
	#3-24-2	$D^{18}T$	N	N	N
	#3-24-3	N	N	N	N
	#3-25-1	$D^{18}T$	N	N	N
	#3-25-2	N	N	N	N
	#3-25-3	N	N	N	N
	#3-28-1	$D^{18}T$	N	N	N
	#3-28-2	N	N	N	N
	#3-28-3	$D^{18}T$	N	N	N

D and I refer to deletion and insertion, respectively; D^{X}, I^{X}, 'x' represents the position of the base; For example, $D^{18}T$ indicates the eighteenth base 'T' was deleted. N represents no mutation. New mutations were shown in red.