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## Supplemental information

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### **Materials and methods**

#### **Plant growth and generation of transgenic plants**

The *Arabidopsis* plants were grown in growth rooms under long-day conditions (16h light/8h dark) at 22°C. *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* Columbia-0 (Col-0) was performed using the floral dipping method as previously described (Clough and Bent, 1998). Seeds collected from the transformed plants were sterilized with 2% sodium hypochlorite for 15 min and plated on Murashige and Skoog (MS) medium containing 30 mg/L hygromycin (for pCAMBIA1300-based constructs) or 50 mg/L kanamycin (for pCAMBIA2301-based constructs) plus 50 mg/L carbenicillin to inhibit *Agrobacterium* growth. The resistant transformants were transplanted to soil 2 weeks later. The rice *Nipponbare* cultivar was used for *Agrobacterium*-mediated transformation as described previously (Hiei et al., 1994). Transgenic rice plants were grown under standard greenhouse conditions (16h light at 30°C /8h dark at 22°C).

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## Vector construction

For transient expression of the reporter and endonuclease genes in *Arabidopsis* protoplasts, the pTOE vector was engineered based on the pMD18T vector (TAKARA, Japan) by inserting a 2×35S promoter from CaMV into the HindIII/PstI site and a polyadenylation signal from nopaline synthase (NOS) into the SacI/EcoRI site. The YFP CDS gene was inserted into the BamHI/SacI site of pTOE to produce the pTOE-YFP construct as a positive control. The reporter construct, pTOE-YFFP, was obtained by replacing the *YFP* gene in pTOE-YFP with two partially overlapped YFP fragments, YF (YFP CDS 1-289 bp region) and FP (YFP CDS 68-721 bp region), separated by a 55 bp MRS sequence containing one I-SceI cleavage site and two 13 bp TALEN recognition sites (EBE) in opposite orientations as indicated in Supplemental Figure 1. The endonuclease construct, pTOE-SceI, contains the 944bp *I-SceI* CDS (GI JF714900) at the Sall/SacI site of pTOE, while the pTOE-RFP-gdEBE expresses an *RFP* CDS, a PTV-2A peptide (Szymczak-Workman et al., 2012) for translational skipping and a GoldyTALEN array recognizing the AvrBs3 sequence (Bedell et al., 2012) in one reading frame between the KpnI/SacI site of pTOE.

To optimize the expression of the CRISPR-Cas system in plants, the human U6 promoter, the CAG promoter and the bGH-PA terminator in the px330 vector (Cong et al., 2013) were replaced with the corresponding plant promoters and terminators in *Arabidopsis* and rice as illustrated in Supplemental Figure 1. The 85 bp chimeric RNA with two BbsI sites and the 4101 bp 3×Flag-NLS-Cas9-NLS ORF was cloned from px330 by primers listed in the Supplemental Table 1. The AtU6 promoter (Wang et al., 2008), the AtUBQ1 promoter and terminator were cloned from the *Arabidopsis* genomic DNA. The obtained cassettes were integrated into the HindIII/EcoRI sites of the pMD18T vector generating the psgR-Cas9-At backbone. The psgR-Cas9 backbone was digested by BbsI and ligated with the synthesized sgRNA oligos (sgR-MRS-S/A and sgR-MRS\*-S/A) according to the protocol at <http://www.genome-engineering.org/crispr/>.

The GUUS reporter was produced by placing an MRS sequence between the GU

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(GUS CDS1-710 bp region) and the US (GUS CDS 155-1863 bp region) fragments to replace the original GUS gene in pCAMBIA2301. Then, the HindIII/EcoRI fragment from psgR-MRS was put into the same vector containing the GUUS reporter. The p2×sgR9-Cas9 vector containing two sgRNA cassettes in one construct was generated to target two sites simultaneously. Based on the psgR-Cas9 vector, the second pAtU6-sgR cassette was amplified by PCR after the insertion of target oligos and put into to the KpnI/EcoRI site of the above psgR-Cas9 vector, followed by subcloning into the HindIII/EcoRI site of the pCAMBIA1300 binary vector for stable transformation into *Arabidopsis*. For rice, the psgR-Cas9-Os backbone was generated in a similar way by using the OsU3 promoter and OsUBQ1 promoter for sgRNA and Cas9 expression, respectively. All primer sequences are listed in Supplemental Table 1.

### **Transient assays in *Arabidopsis* protoplasts**

Isolation and preparation of *Arabidopsis* mesophyll protoplasts was performed by the Tape-*Arabidopsis* Sandwich method (Wu et al., 2009). The reporter plasmids and the nuclease plasmids were mixed in equal quantities for PEG transformation as described (Yoo et al., 2007). The transformed *Arabidopsis* mesophyll protoplasts were incubated in the dark at room temperature for 12-24 h before examination. YFP positive cells were measured by flow cytometry (BECKMAN COULTER MoFlo™ XDP, USA). Photographs were taken by a fluorescence microscope (IX71, Olympus, Japan) under the RFP and YFP channels.

### **Histochemical GUS staining**

Cotyledons of 10-day-old T1 transgenic *Arabidopsis* plants were subjected to the histochemical GUS staining at 37°C for 12h as previously described (Jefferson et al., 1987) and decolorized in 70% EtOH before photographing with a BX51 microscope (BX51, Olympus, Japan).

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### **Detection of genome modifications**

DNA was extracted from transgenic plants by the CTAB methods (Rowland and Nguyen, 1993). Genomic region surrounding the CRISPR target site for each gene was amplified by PCR. The PCR products were subjected to SURVEYOR assay according to the manufacturer's instructions (SURVEYOR, Transgenomics, USA). Putative mutations were further confirmed by Sanger sequencing after cloning the PCR fragments into the pMD18T (TAKARA, Japan) vector.

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## Supplemental note

### Sequences of sgRNA and Cas9 cassettes

#### >pAtU6-sgRNA

AAGCTTCATTCGGAGTTTTTGTATCTTGTTTCATAGTTTGTCCCAGGATTAGAATGATTAGGCAT  
CGAACCTTCAAGAATTTGATTGAATAAAACATCTTCATTCTTAAGATATGAAGATAATCTTCAA  
AGGCCCTGGGAATCTGAAAGAAGAGAAGCAGGCCCATTTATATGGGAAAGAACAATAGTAT  
TTCTTATATAGGCCATTTAAGTTGAAAACAATCTTCAAAGTCCCACATCGCTTAGATAAGAA  
AACGAAGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGGGTCTTCGAGAAGACCTGT  
TTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC  
CGAGTCGGTGCTTTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTAGC  
GCGTGCGCCAATTCTGCAGACAAATGGCCCCGGG

#### >pOsU3-sgRNA

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AAAAGAGTTGTGCAGATGATCCGTGGCGGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAAT  
AGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT  
TTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTAGCGCGTGCGCCAATTCT  
GCAGACAAATGGCCCCGGG

#### >pAtUBQ-Cas9-tUBQ

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## Supplemental figure legends

**Supplemental Figure 1.** Schematic diagrams for the constructs used in this study. The restriction sites used for cloning were indicated with arrows. p35s: 2×35s promoter from CAMV; NOS: polyadenylation signal of nopaline synthase; I-SceI: CDS of homing endonuclease I-SceI; RFP: CDS of the monomeric red fluorescent protein; 2A: 2A peptide from porcine teschovirus-1; NLS: nuclear localization signal; FLAG: 3xFLAG tag for protein recognition; pAtUBQ: the *Arabidopsis* UBQ1 promoter; tUBQ: the *Arabidopsis* UBQ terminator; YF: the 5' YFP fragment of 289 bp; FP the 3' YFP fragment lacking the first 67bp. MRS: multiple recognition site; GU: the 5' GUS fragment of 710 bp; US: the 3' GUS fragment lacking the first 155 bp.

**Supplemental Figure 2.** The engineered CRISPR/Cas can generate targeted DSBs in *Arabidopsis* protoplasts. (A) The target sequences in the MRS of YFFP for binding of goldy TALEN, I-SceI and CRISPR-Cas. The binding sites for gdTALEN and I-SceI were colored in blue and pink, respectively and the CRISPR-Cas binding sites were labeled by orange lines,. (B) Transient assay for the activity of the CRISPR-Cas system in *Arabidopsis* protoplasts. Cells were examined under YFP and RFP channels for YFP signal and chloroplast autofluorescence or RFP signal, respectively. The proportion of YFP-positive cells in each sample was quantified by flow cytometry.

**Supplemental Figure 3.** Schematic diagrams to illustrate the procedure of DSB induced DNA repair in the pGUUS-sgRNA-MRS transgenic seedlings. The sgRNA and Cas9 protein were encoded by the sgR-MRS and Cas9 cassette respectively. A cleavage complex was supposedly formed at the presence of their target DNA. As an endonuclease, Cas9 makes a DSB at the MRS site in the nonfunctional *GUUS* gene. The free ends of DSB recruit some repair proteins to the damage site to perform DNA repairing either through the error prone NHEJ pathway or the error free HR pathway.

**Supplemental Figure 4.** Targeted indel mutations induced by CRISPR-Cas in the *GUUS* reporter gene of *Arabidopsis* pGUUS-sgR-EBE T1 transgenic plants. Alleles shown were amplified from genomic DNA isolated from 3 independent T1 transgenic plants

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showing no detectable GUS activity and sequenced after cloned into vectors. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The nucleotide changes were to the right of each sequence (+, insertion; -, deletion).

**Supplemental Figure 5.** Targeted indel mutations induced by CRISPR-Cas in the *CHL1* gene in *Arabidopsis*. Alleles shown were amplified from genomic DNA of 3 independent T1 transgenic plants of the p2× sgR-CHL1&2 vector. They correspond to the three plants in Figure 1E. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The type and frequency of nucleotide changes were on the right of each sequence (+, insertion; -, deletion).

**Supplemental Figure 6.** Targeted indel mutations induced by CRISPR-Cas in the *CHL2* gene in *Arabidopsis*. Alleles shown were amplified from DNA samples isolated from 3 independent T1 transgenic plants of the p2× sgR-CHL1&2 vector. They correspond to the three plants in Fig 1E. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The type and frequency of nucleotide changes were on the right of each sequence (+, insertion; -, deletion).

**Supplemental Figure 7.** CRISPR-Cas facilitated multiplex genome modification by targeting two sites in the *Arabidopsis TT4* gene simultaneously. Alleles shown were amplified from DNA samples isolated from 11 independent T1 transgenic plants of the p2×sgR-TT4 vector. The wild type sequence was shown at the top with the arrowhead indicating the target site. The arrows indicated the putative cleavage sites. The type and frequency of nucleotide changes at both sites were on the right of each sequence, separated by “;” (+, insertion; -, deletion).

**Supplemental Figure 8.** Multiplex genome editing at the *TT4* loci caused DNA inversions at the cleavage sites. Three types of DNA inversions were found from 11 T1 transformants. M23 contains a 499 bp DNA insertion before the first cleavage site.

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This insertion comes from a gene fragment located 1010 bp downstream of the second cleavage site. In M24, a 360 bp DNA sequence flanking the cleavage sites was duplicated, the upstream one had a 238bp fragment excision between the cleavage sites and the downstream one contained a deletion (< 50 bp) at each cleavage site. For M25, the 255 bp DNA fragment was just inverted between the two cleavage sites. The green arrowheads below the DNA sequence indicate the target sites and the yellow ones indicate the primers for PCR. The frequency of each mutation was on the right.

**Supplemental Figure 9.** Targeted indel mutations induced by CRISPR-Cas in the *OsMYB* (LOC\_Os1g12700) gene in rice. Alleles shown were amplified from genomic DNA isolated from 10 independent T1 transgenic plants of the p2×sgR-*OsMYB* vector. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The nucleotide changes were on the right of each sequence (+, insertion; -, deletion).

### **Supplemental Tables**

**Supplemental Table 1.** Target and PAM sequences used for gene editing in *Arabidopsis* and rice

**Supplemental Table 2.** The frequency of CRISPR-Cas induced gene editing in *Arabidopsis* and rice

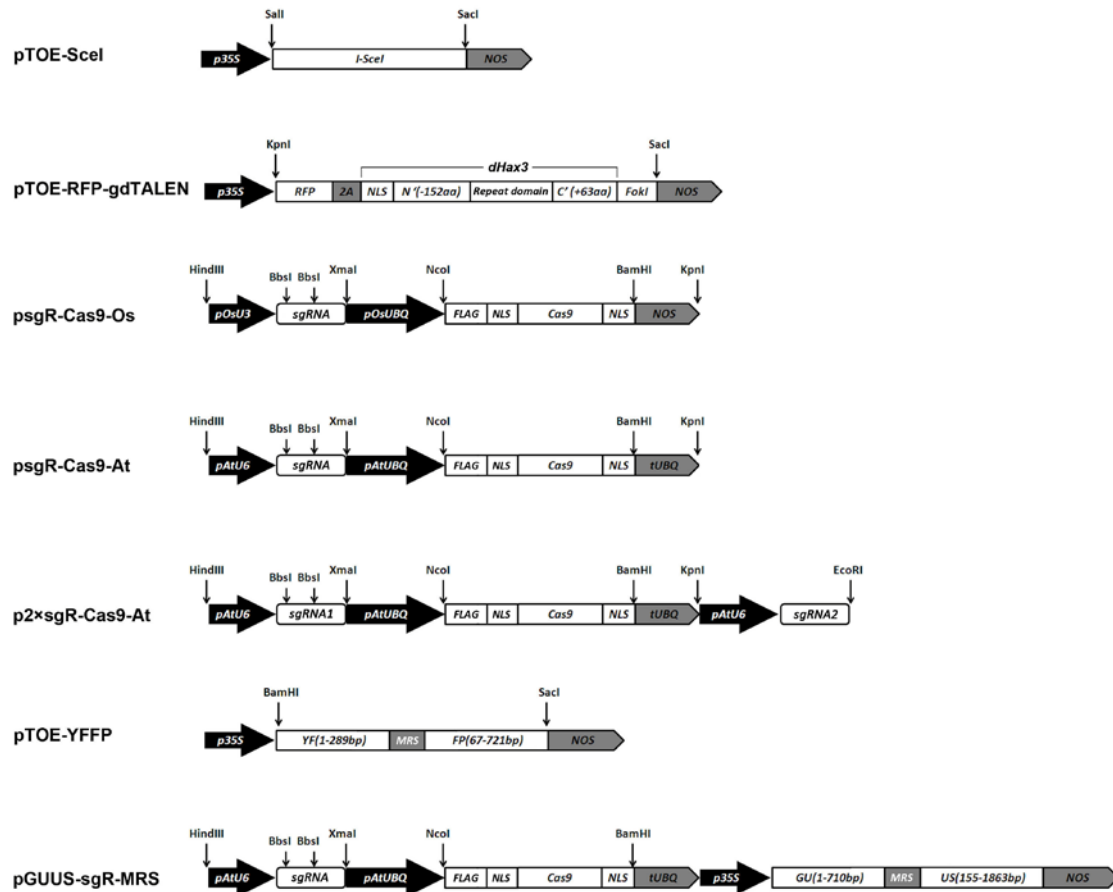
**Supplemental Table 3.** Summary of mutations detected from *Arabidopsis* T1 and rice T0 transformants of CRISPR-Cas

**Supplemental Table 4.** Primers used in this study.

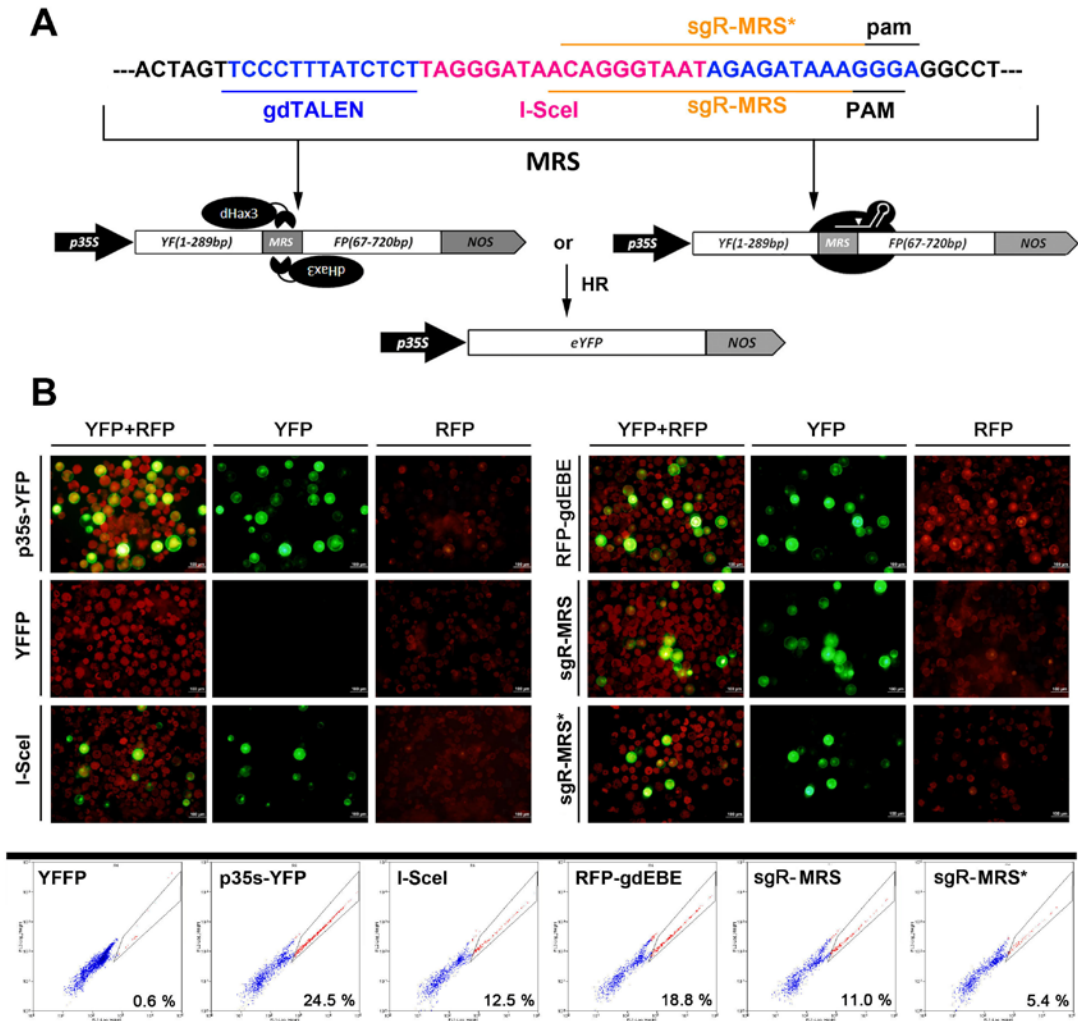
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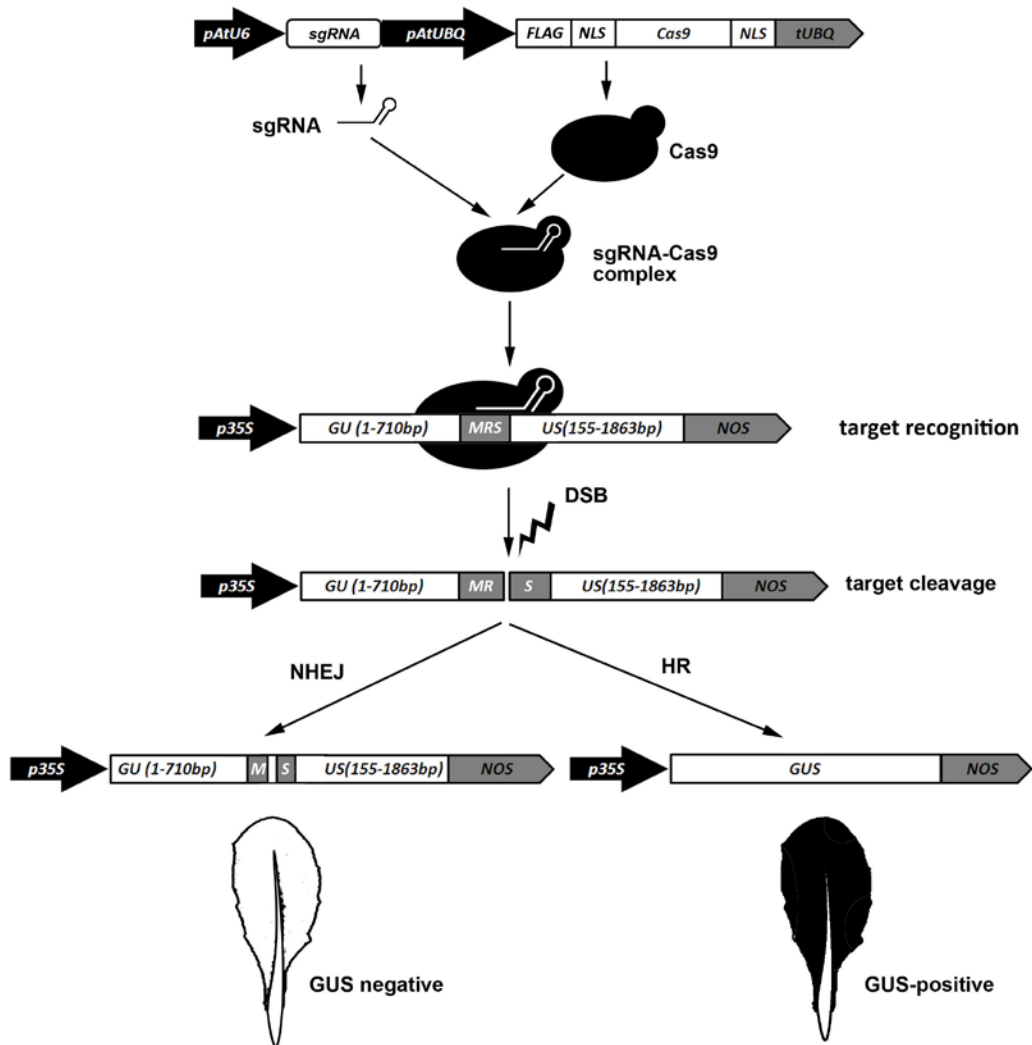
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**Supplemental Figure 1.** Schematic diagrams for the constructs used in this study. The restriction sites used for cloning were indicated with arrows. p35s: 2×35s promoter from CAMV; NOS: polyadenylation signal of nopaline synthase; I-SceI: CDS of homing endonuclease I-SceI; RFP: CDS of the monomeric red fluorescent protein; 2A: 2A peptide from porcine teschovirus-1; NLS: nuclear localization signal; FLAG: 3xFLAG tag for protein recognition; pAtUBQ: the *Arabidopsis* UBQ1 promoter; tUBQ: the *Arabidopsis* UBQ terminator; YF: the 5' YFP fragment of 289 bp; FP the 3' YFP fragment lacking the first 67bp. MRS: multiple recognition site; GU: the 5' GUS fragment of 710 bp; US: the 3' GUS fragment lacking the first 155 bp.

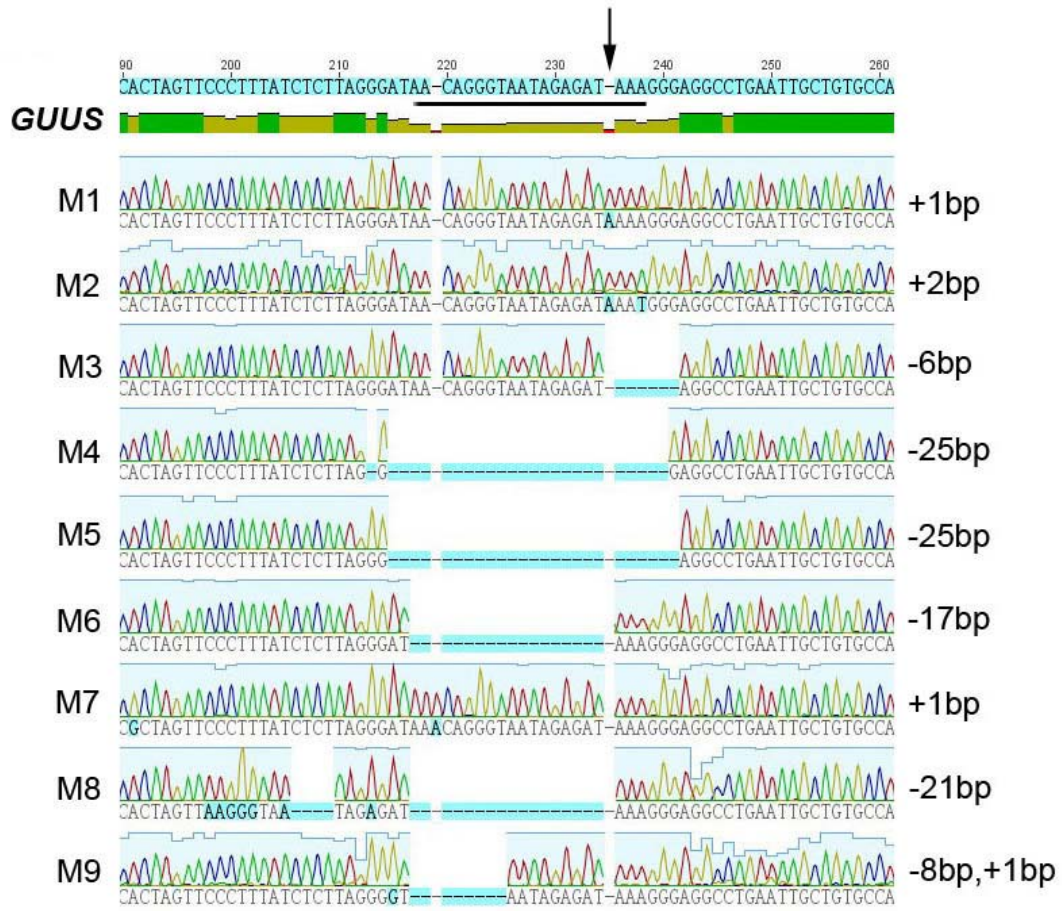


**Supplemental Figure 2.** The engineered CRISPR/Cas can generate targeted DSBs in *Arabidopsis* protoplasts. (A) The target sequences in the MRS of YFP for binding of goldy TALEN, I-SceI and CRISPR-Cas. The binding sites for gdTALEN and I-SceI were colored in blue and pink, respectively and the CRISPR-Cas binding sites were labeled by orange lines,. (B) Transient assay for the activity of the CRISPR-Cas system in *Arabidopsis* protoplasts. Cells were examined under YFP and RFP channels for YFP signal and chloroplast autofluorescence or RFP signal, respectively. The proportion of YFP-positive cells in each sample was quantified by flow cytometry.

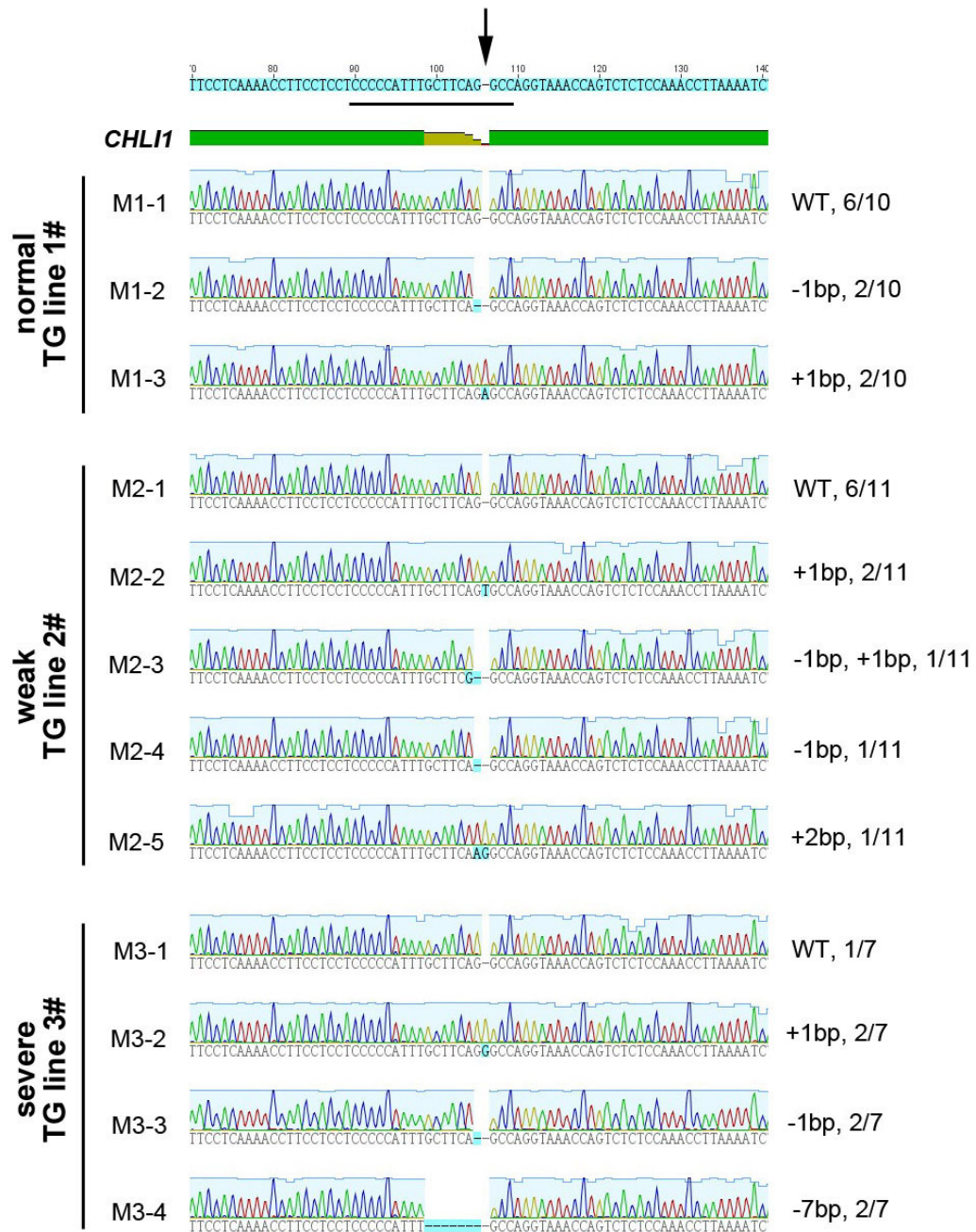


Supplemental Figure 3. Schematic diagrams to illustrate the procedure of DSB induced DNA repair in the pGUUS-sgRNA-MRS transgenic seedlings. The sgRNA and Cas9 protein were encoded by the sgR-MRS and Cas9 cassette respectively. A cleavage complex was supposedly formed at the presence of their target DNA. As an endonuclease, Cas9 makes a DSB at the MRS site in the nonfunctional *GUUS* gene. The free ends of DSB recruit some repair proteins to the damage site to perform DNA repairing either through the error prone NHEJ pathway or the error free HR pathway.

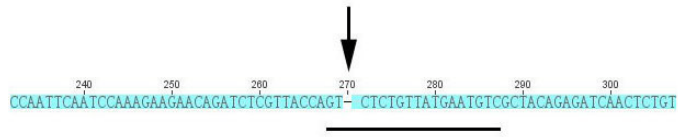




**Supplemental Figure 4.** Targeted indel mutations induced by CRISPR-Cas in the *GUUS* reporter gene of *Arabidopsis* pGUUS-sgR-EBE T1 transgenic plants. Alleles shown were amplified from genomic DNA isolated from 3 independent T1 transgenic plants showing no detectable GUS activity and sequenced after cloned into vectors. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The nucleotide changes were to the right of each sequence (+, insertion; -, deletion).

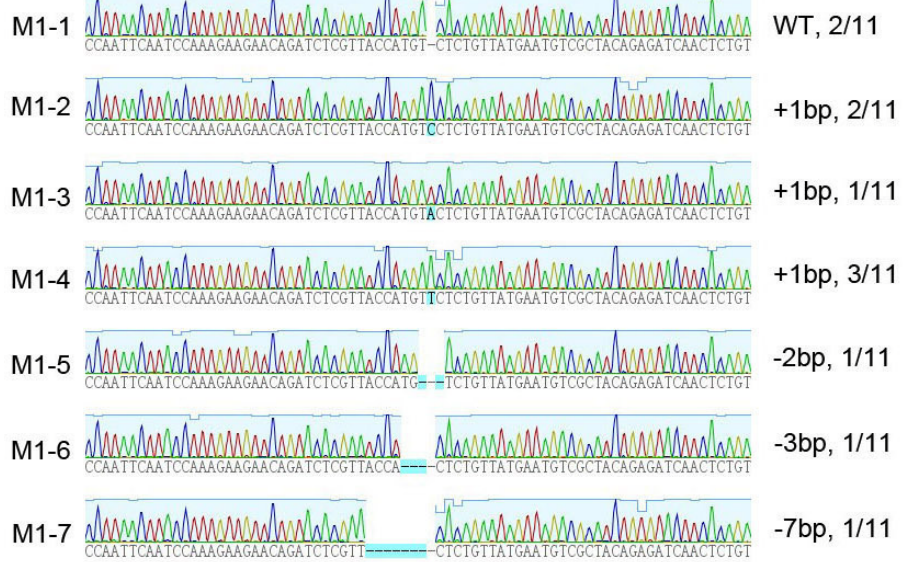


**Supplemental Figure 5.** Targeted indel mutations induced by CRISPR-Cas in the *CHL11* gene in *Arabidopsis*. Alleles shown were amplified from genomic DNA of 3 independent T1 transgenic plants of the p2× sgR-CHL11&2 vector. They correspond to the three plants in Figure 1E. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The type and frequency of nucleotide changes were on the right of each sequence (+, insertion; -, deletion).

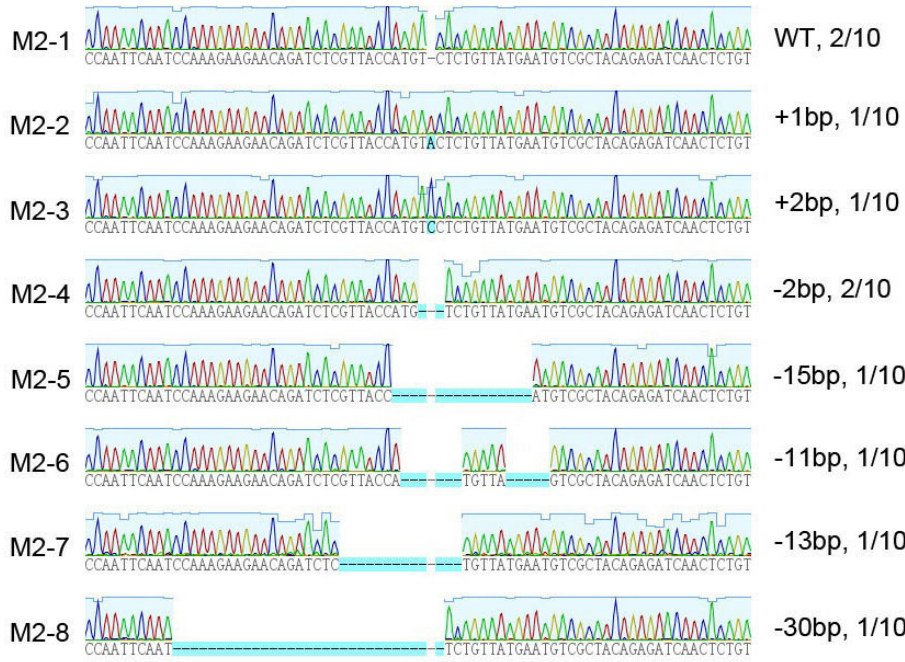


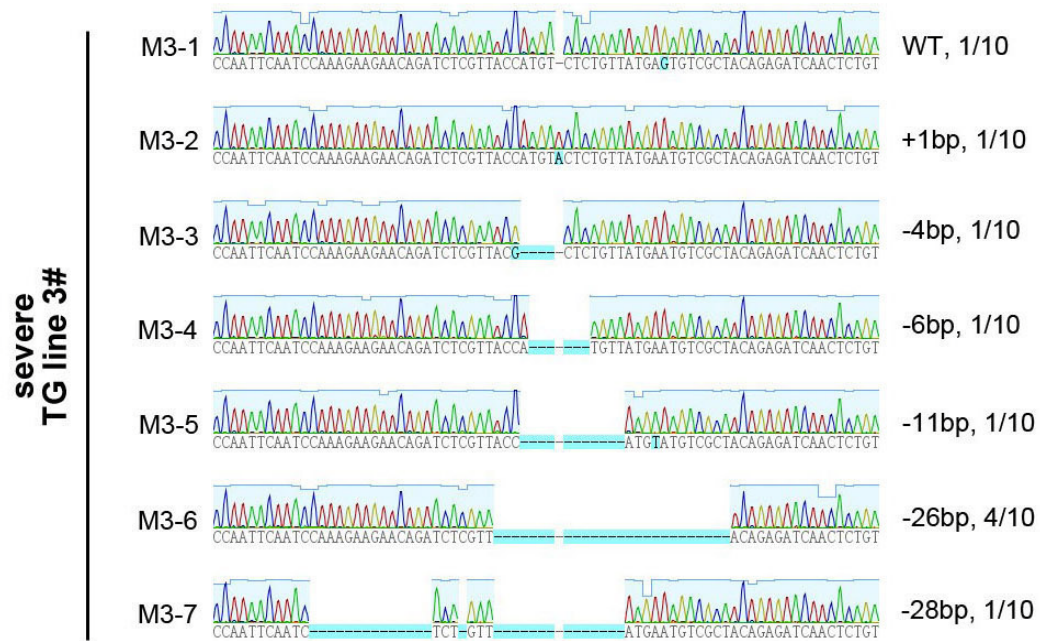
**CHL12**

normal  
TG line 1#

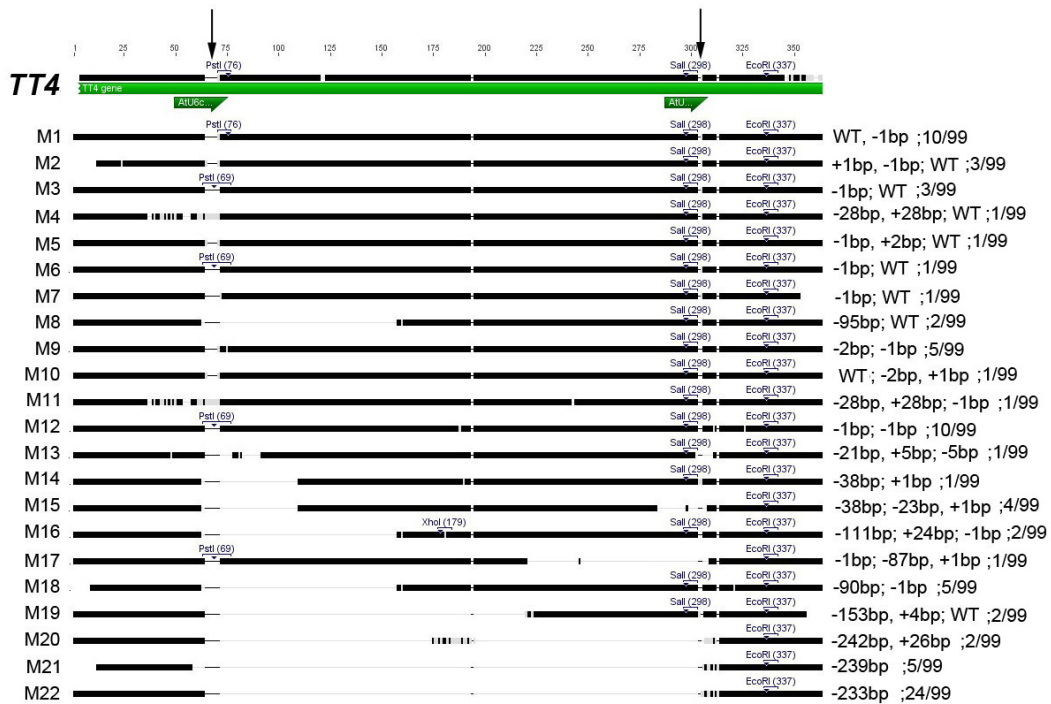


weak  
TG line 2#

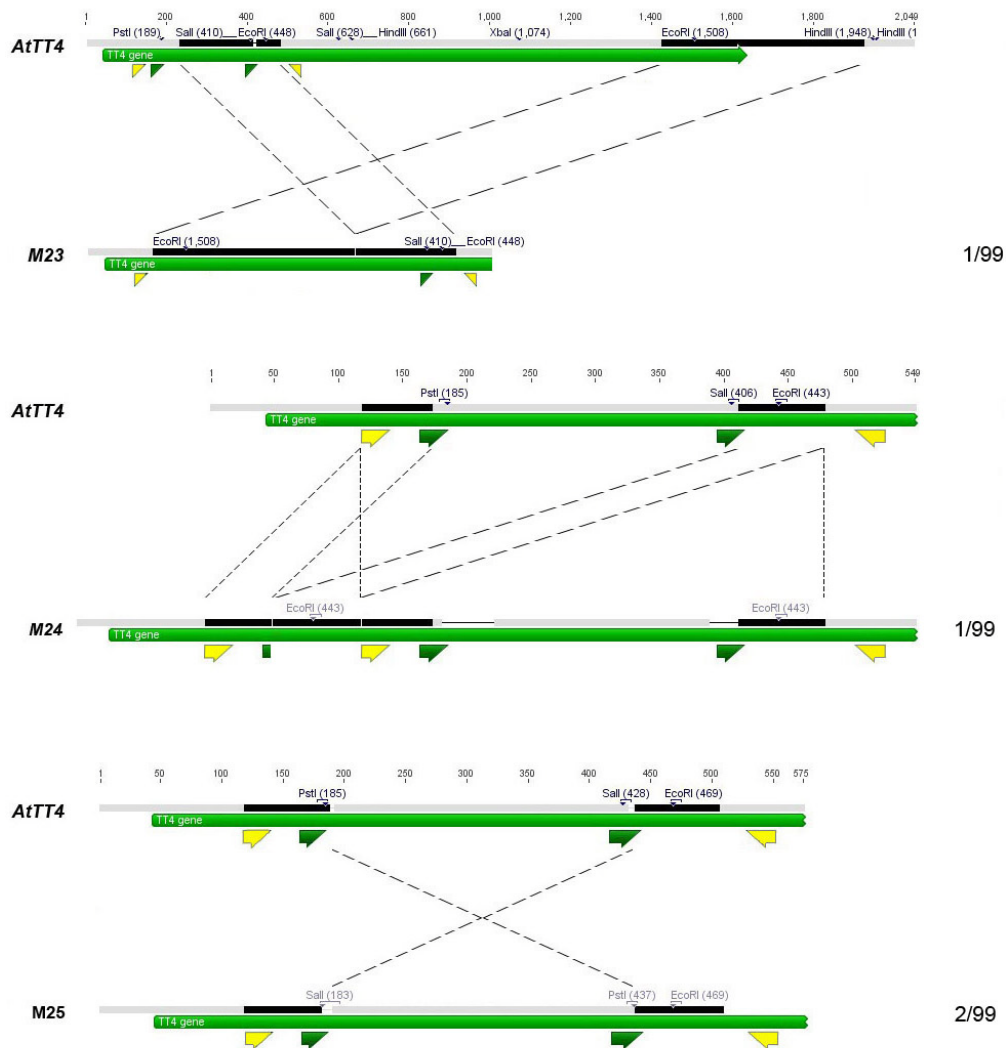




**Supplemental Figure 6.** Targeted indel mutations induced by CRISPR-Cas in the *CHL12* gene in *Arabidopsis*. Alleles shown were amplified from DNA samples isolated from 3 independent T1 transgenic plants of the p2× sgR-CHL1&2 vector. They correspond to the three plants in Fig 1E. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The type and frequency of nucleotide changes were on the right of each sequence (+, insertion; -, deletion).

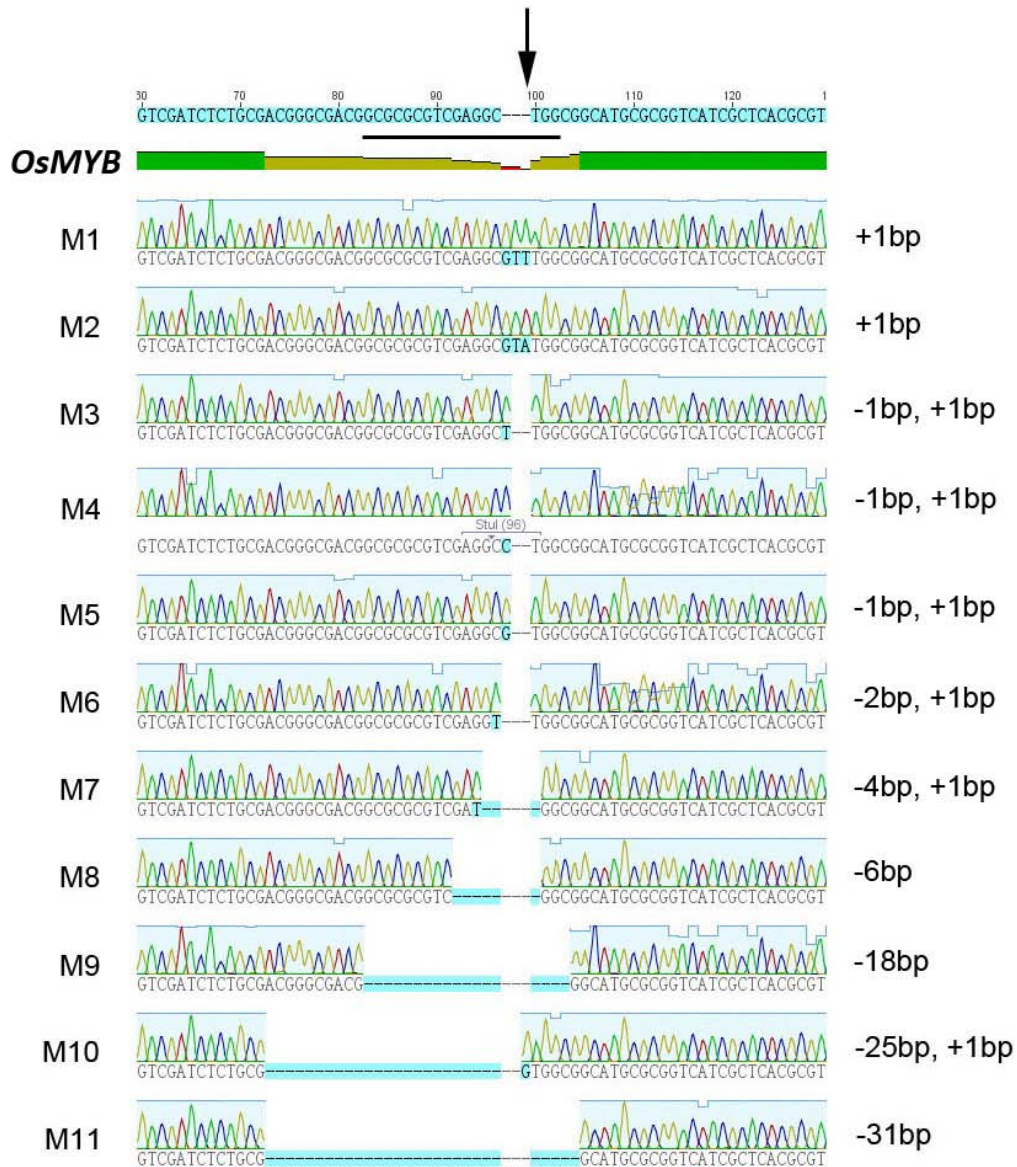


**Supplemental Figure 7.** CRISPR-Cas facilitated multiplex genome modification by targeting two sites in the *Arabidopsis TT4* gene simultaneously. Alleles shown were amplified from DNA samples isolated from 11 independent T1 transgenic plants of the p2×sgR-TT4 vector. The wild type sequence was shown at the top with the arrowhead indicating the target site. The arrows indicated the putative cleavage sites. The type and frequency of nucleotide changes at both sites were on the right of each sequence, separated by “;” (+, insertion; -, deletion).



**Supplemental Figure 8.** Multiplex genome editing at the *TT4* loci caused DNA inversions at the cleavage sites. Three types of DNA inversions were found from 11 T1 transformants. M23 contains a 499 bp DNA insertion before the first cleavage site. This insertion comes from a gene fragment located 1010 bp downstream of the second cleavage site. In M24, a 360 bp DNA sequence flanking the cleavage sites was duplicated, the upstream one had a 238bp fragment excision between the cleavage sites and the downstream one contained a deletion (< 50 bp) at each cleavage site. For M25, the 255 bp DNA fragment was just inverted between the two cleavage sites. The green arrowheads below the DNA sequence indicate the target sites and the

yellow ones indicate the primers for PCR. The frequency of each mutation was on the right.



**Supplemental Figure 9.** Targeted indel mutations induced by CRISPR-Cas in the *OsMYB* (LOC\_Os1g12700) gene in rice. Alleles shown were amplified from genomic DNA isolated from 10 independent T1 transgenic plants of the p2×sgR-*OsMYB* vector. The wild type sequence was shown at the top with the underline indicating the target site. The arrow indicates the putative cleavage site. The nucleotide changes were on the right of each sequence (+, insertion; -, deletion).

**Supplemental Table 1. Target and PAM sequences used for gene editing in *Arabidopsis* and rice**

Species	Target Gene	CRISPR ID	Protospacer	PAM	Target Strand
<i>Arabidopsis</i>	GUUS/YFFP	EBE	5'-ACAGGGTAATAGAGATAAA-3'	GGG	+
	GUUS/YFFP	EBE*	5'-CAGGGTAATAGAGATAAAAG-3'	GGA*	+
	AtCHLI1	AtCHLI1-101	5'-CCCCATTTGCTTCAGGCC-3'	AGG	+
	AtCHLI2	AtCHLI2-280	5'-GACATTCATAACAGAGACA-3'	TGG	-
	AtTT4	AtTT4-65	5'-AGAGAGCTGATGGACCTGC-3'	AGG	+
	AtTT4	AtTT4-296	5'-AGGCGACAAGTCGACAATT-3'	CGG	+
<i>Oryza sativa</i>	LOC_Os01g12700	OsMYB-354	5'-GCGCGCGTCGAGGCGTTGG-3'	CGG	+

\*\* indicate the improper PAM sequence used for gene targeting

**Supplemental Table 2. The frequency of CRISPR induced gene editing in *Arabidopsis* and rice**

Species	Target loci	Construct ID	Total number of TG lines	Number of NHEJ repair	Number of HR repair	Mutation frequency	HR Ratio	Number of double mutation	Double mutation frequency
<i>Arabidopsis</i>	GUUS-MRS	sgR-MRS	44	35	5	80%	11%	/	/
	CHLI1-101	2×sgR-CHLI1&2	37	28	/	76%	/	25	68%
	CHLI2-280	2×sgR-CHLI1&2	37	33	/	89%	/		
	TT4-65	2×sgR-TT4	58	49	/	84%	/	43	74%
	TT4-296	2×sgR-TT4	58	45	/	78%	/		
<i>Oryza sativa</i>	OsMYB-354	sgR-OsMYB	20	10	/	50%	/	/	/

The frequency of NHEJ repairment was determined by surveyor assay followed by subcloning of the PCR products for sequencing. The frequency of HR repairment at the GUUS Loci was identified by GUS staining.

**Supplemental Table 3. Summary of the mutations detected from *Arabidopsis* T1 and rice T0 transformants of CRISPR-Cas**

Species	Target loci	Transgenic line	No. of sequenced clones	No. of WT	No. of mutations	No. of unique mutations	
<i>Arabidopsis</i>	GUUS-MRS		8	0	8	4	
			19	1	7	4	
			44	3	5	2	
		total	24	4	20	9	
	CHLI1-101		1	10	6	4	2
			2	11	6	5	4
			3	7	1	6	3
		total	28	13	15	7	
	CHLI2-280		1	11	2	9	6
			2	10	2	8	7
			3	10	1	9	6
		total	31	5	26	15	
	AtTT4-65		14	10	0	10	2
			15	8	0	8	3
			19	4	0	4	1
			22	7	2	5	2
			23	13	0	13	4
			24	10	3	7	3
			29	8	2	6	2
			51	9	3	6	2
			54	10	3	7	4
			56	12	5	7	4
			58	8	2	6	3
		total	99	20	79	20	
		AtTT4-296		14	10	3	7
			15	8	4	4	2
			19	4	0	4	1
	22		7	1	6	2	
	23		13	0	13	3	
	24		10	6	4	3	
	29		8	0	8	4	
	51		9	2	7	3	
	54		10	5	5	2	
	56		12	0	12	2	
	58		8	0	8	2	
total	99		21	78	11		
<i>Oryza sativa</i>	OsMYB-354			1	8	0	8
			4	2	1	1	1
			5	7	0	7	3
			6	6	4	2	2
			7	6	0	6	3
			9	6	0	6	2
			11	7	0	7	2
			15	8	0	8	2
			17	10	7	3	2
			20	8	0	8	2
total	68	12	56	12			



**Supplemental Table 4. Primers used in this study**

Primer ID	Sequences
<b>for vector construction</b>	
psgR-Cas9-At	
pAtU6-F-HindIII	5'-GCCAAGCTTCATTCGGAGTTTTGTATCTTGTTTC-3'
pAtU6-R	5'-AATCACTACTTCGACTCTAGCTGTATATAAACTCAGCTTCG-3'
sgR-F-U6	5'-CGAAGTAGTGATTGGGTCTTCGAGAAGACCTGTTTAG-3'
sgR-R-Smal	5'TATCCCGGGGCCATTTGTCTGCAGAATTGGC-3'
pAtUBQ1-F-Smal	5'-TGGCCCCGGGATATTTCAAAAATTGAACATAGACTAC-3'
pAtUBQ1-R-Cas	5'-CCTTATAGTCCATGGTTTGTGTTTCGTCTCTCACGTAG-3'
Cas9-F-pUBQ	5'-CACAAACCATGGACTATAAGGACCACGACGGAG-3'
Cas9-R-BamHI	5'-TCTGGATCCTTACTTTTTCTTTTTGCTCGGCCGGCC-3'
tUBQ1-F-BamHI	5'-TAAGGATCCAGAGACTCTTATCAAGAATCCCATCTCTTGC-3'
tUBQ-R-KpnI	5'-ACGGTACCACATAAACGGTCATTATTTACGATACTTGTATAG-3'
psgR-Cas9-Os	
pOsU3-F-HindIII	5'-GCCAAGCTTGACGTTGGAAAACCACGTGATG-3'
pOsU3-R-sgR	5'-CCGCCACGGATCATCTGCACAACCTTTTAAATC-3'
sgR-F-OsU3	5'-GGCGGGTCTTCGAGAAGACCTGTTTAGAGC-3'
pOsUBQ-F-Smal	5'-AATCCCGGGCTGCAAGTTCAAATCTTGCAATTAG-3'
pOsUBQ-R-NcoI	5'-AGTCCATGGTCGATTGACTCGCTATGCG-3'
Nos-F-BamHI	5'-TAAGGATCCAGGCGTTCAAACATTTGGCAATAAAG-3'
Nos-R-KpnI	5'-TTACGGTACCTTGGCAATCCCGATCTAGTAACATAG-3'
p2×sgR-Cas9-At	
pAtU6-F-KpnI	5'-GTGGTACCCATTCGGAGTTTTGTATCTTGTTTC-3'
sgR-R-EcoRI	5'-ACGAATTCGCCATTTGTCTGCAGAATTGGC-3'
pTOE	
p35s-HindIII-F	5'-ACCAAGCTTCCATGGAGTCAAAGATTCA-3'
p35s-PstI-R	5'-AAACTGCAGTGTCTCTCCAAATGAAATG-3'
Nos-F-SacI	5'-CGGAGCTCAGGCGTTCAAACATTTGGCA-3'
Nos-R-EcoRI	5'-CGGAATTCCTGGCAATCCCGATCTAGT-3'
pTOE-YFP	
eYFP-N-F	5'-AATCGGATCCATGGTGAGCAAGGGCGAGGAGC-3'
eYFP-r1-R	5'-AAAGGGAAGTAGTGCTCCTGGACGTAGCCTTCGGGC-3'
MRS-Scel-F	5'-GAGCACTAGTCCCTTTATCTCTTAGGGATAACAGGGTAAT-3'
eYFP-Scel-R	5'-TTTACAGGCCTCCCTTTATCTCTATTACCCTGTTATCCCTA-3'
MRS-r2-F	5'-AAAGGGAGGCCTGTAACGGCCACAAGTTCAGCGTG-3'
eYFP-C-R	5'-AATGGAGCTCTTACTTGTACAGCTCGTCCATGCCGAGAGT-3'
pTOE-Scel	
Scel-Sall-F	5'-AATGTCGACGCCTCTGCTAACCATGTTTCATG-3'
Scel-SacI-R	5'-AATGAGCTCCTCGATCGACTTATTATTTACAGAAAGTTTCGG-3'
pGUUS	
p35s-MfeI-F	5'-GGGCAATTGAGACTTTTCAACAAAGGGTAATATCCGG-3'
p35s-EcoRI-F	5'-GGGGAATTCAGACTTTTCAACAAAGGGTAATATCCGG-3'
GUS-r1-R	5'-GGAAGTGTGCTTGTCCAGTTGCAACCACC-3'
MRS-Scel-F	5'-GAGCACTAGTCCCTTTATCTCTTAGGGATAACAGGGTAAT-3'
GUS-Scel-R	5'-CAGGAATTCAGGCCTCCCTTTATCTCTATTACCCTGTTATCCCT-3'

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**for surveyor assay**

GU-F2	5'-ATGCCGGAATCCATCGCAGCG-3'
US-R2	5'-CACCATTCCCGCGGGATAGTC-3'
CHLI1-3-F	5'-GGCGTCTCTTCTTGAACATC-3'
CHLI1-262-R	5'-CCGAAACATGGTAACGAGACC-3'
CHLI1-454-F	5'-CCATTGCAGCTATAGTAGGGC-3'
CHLI1-763-R	5'-GTTGCACCCAAAGGAAGATC-3'
CHLI2-3-F	5'-GGCGTCTCTTCTCGGAAGAT-3'
CHLI2-463-R	5'-CGGATAAACAGGTCTTGCAC-3'
TT4-1-F	5'-ATGGTGATGGCTGGTGCTTC-3'
TT4-362-R	5'-CATGTAAGCACACATGTGTGGG-3'
TT4-F-159	5'-CTGCCCGTCCATCTAACCTAC-3'
TT4-407-R	5'-GACTTCGACCACCACGATGT-3'
MYB-F-24	5'-TGATCCGTGATACAGTATGTGAGT-3'
MYB-467-R	5'-GGTGCGGATTCATAAATAAATAGCA-3'

**sgRNA oligos**

sgR-MRS-S	5'-GATTGACAGGGTAATAGAGATAAA-3'
sgR-MRS-A	5'-AAACTTTATCTCTATTACCCTGTC-3'
sgR-MRS*-S	5'-GATTGCAGGGTAATAGAGATAAAG-3'
sgR-MRS*-A	5'-AAACCTTTATCTCTATTACCCTGC-3'
sgR-CHLI1-S101	5'-GATTGCCCCCATTTGCTTCAGGCC-3'
sgR-CHLI1-A101	5'-AAACGGCCTGAAGCAAATGGGGGC-3'
sgR-CHLI1-S646	5'-GATTGTATCGGATCCGAGTTATAC-3'
sgR-CHLI1-A646	5'-AAACGTATAACTCGGATCCGATAC-3'
sgR-CHLI2-S101	5'-GATTGCCCATCTCTGCTTCGGACC-3'
sgR-CHLI2-A101	5'-AAACGGTCCGAAGCAGAGATGGGC-3'
sgR-CHLI2-S280	5'-GATTGGACATTCATAACAGAGACA-3'
sgR-CHLI2-A280	5'-AAACTGTCTCTGTTATGAATGTCC-3'
sgR-TT4-S65	5'-GATTGAGAGAGCTGATGGACCTGC-3'
sgR-TT4-A65	5'-AAACGCAGGTCCATCAGCTCTCTC-3'
sgR-TT4-S296	5'-GATTGAGGCGACAAGTCGACAATT-3'
sgR-TT4-A296	5'-AAACAATTGTCGACTTGTCGCCTC-3'
sgR-MYB-S354	5'-TGGCGGCGCGCGTTCGAGGCGTTGG-3'
sgR-MYB-A354	5'-AAACCCAACGCCTCGACGCGGCC-3'