

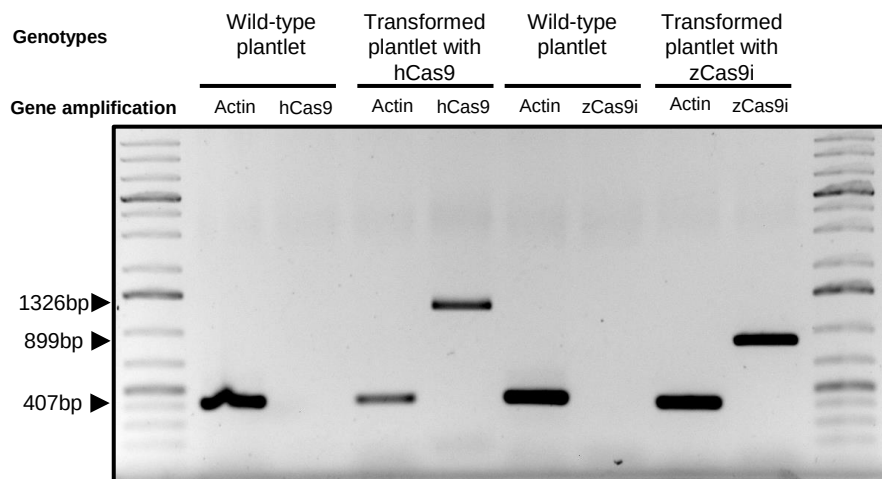


Figure S1. Specificity of Cas9 gene amplification on genomic DNA. PCR amplification of actin for wild-type and selected plantlets as positive control with a PCR product size of 407bp. PCR amplification of two different Cas9 gene on wild-type and corresponding transformed plantlets. PCR product size are 1326bp and 899bp for hCas9 and zCas9i gene respectively.

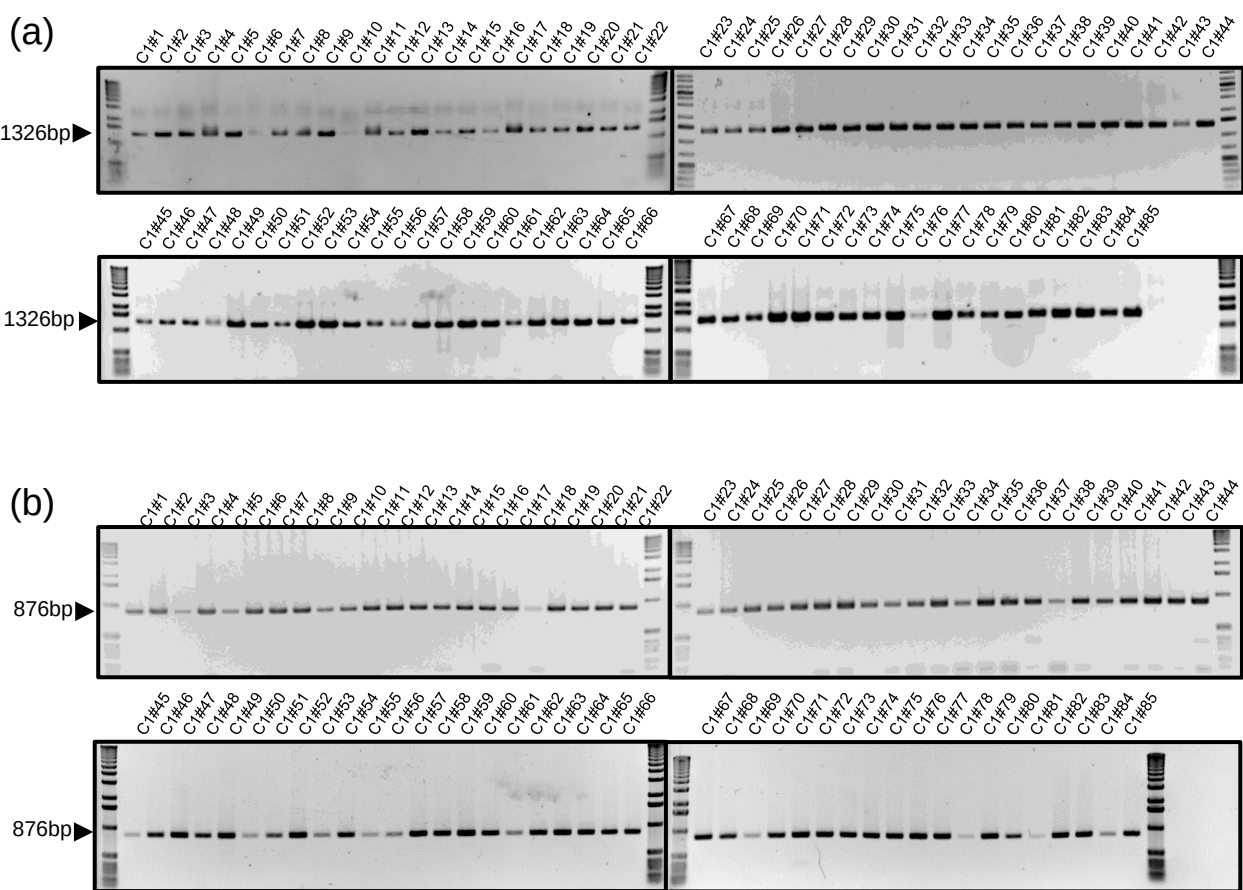


Figure S2. Genotyping of 85 selected plantlets transformed with the construct 1 (*p35S::hCas9*) for mutation rate analysis. (a) PCR amplification of a part of the *Cas9* gene (1326bp) in 85 independent plantlets showing the presence of T-DNA insertion. (b) PCR amplification of the target gene (876bp) on the same 85 plantlets for sequencing analysis and study of target gene editing efficiency.

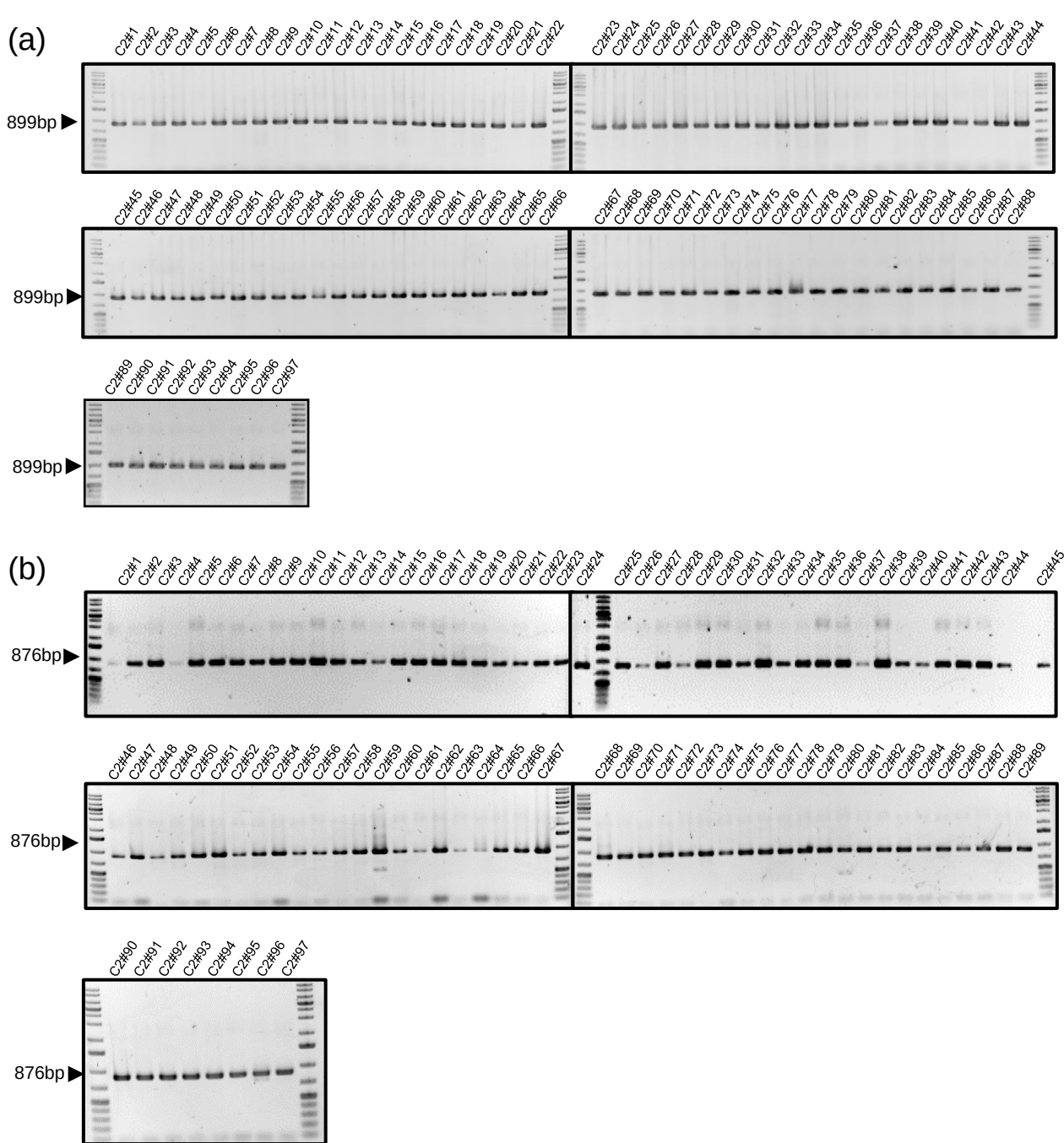


Figure S3. Genotyping of 97 selected plantlets transformed with the construct 2 (*p35S::zCas9*) for mutation rate analysis. (a) PCR amplification of a part of the *Cas9* gene (899bp) in 97 independent plantlets showing the presence of T-DNA insertion. (b) PCR amplification of the target gene (876bp) of the same 97 plantlets for sequencing analysis and study of target gene editing efficiency.

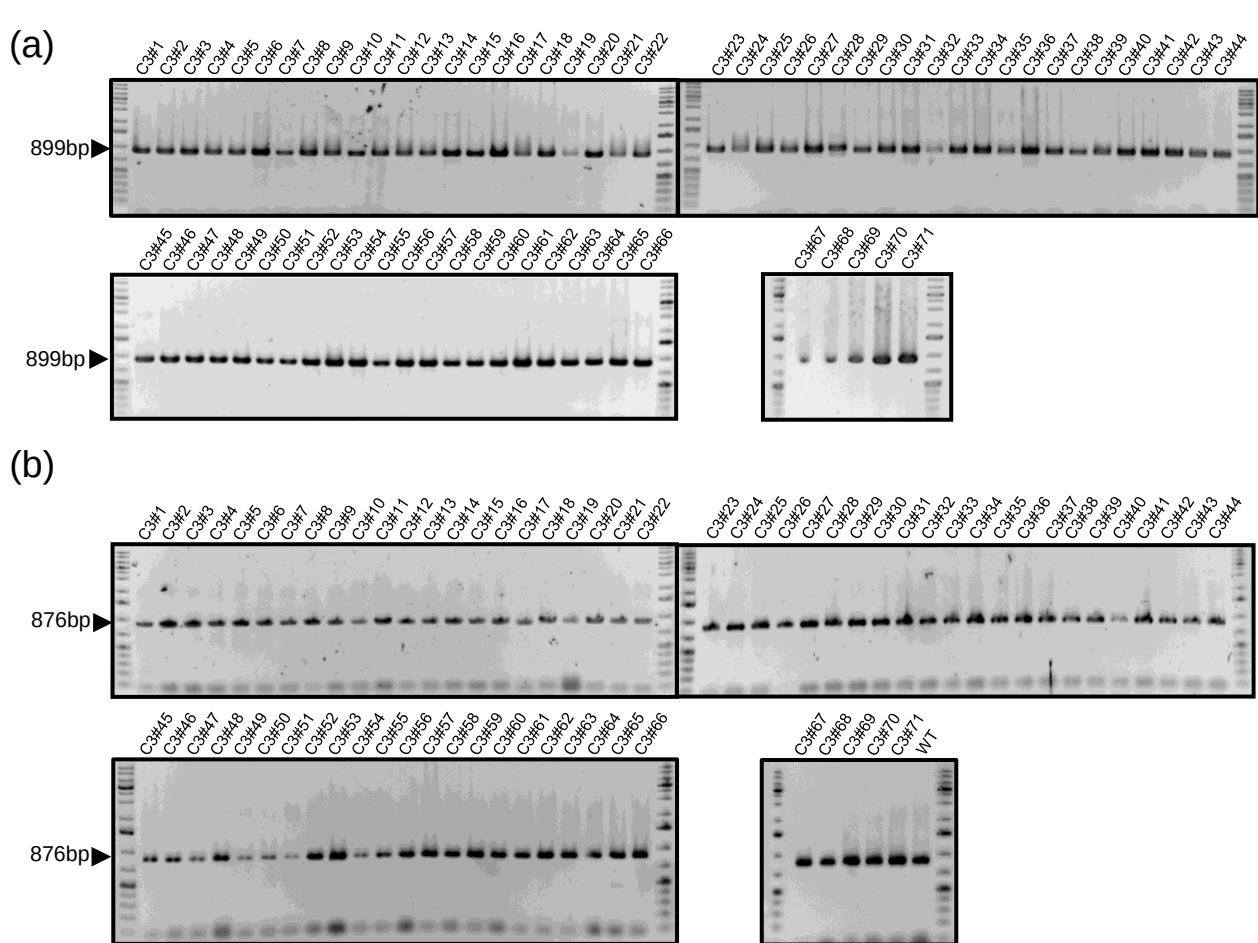


Figure S4. Genotyping of 71 selected plantlets transformed with the construct 3 (*pRPS5a::zCas9i*) for mutation rate analysis. (a) PCR amplification of a part of the Cas9 gene (899bp) in 71 independent plantlets showing the presence of T-DNA insertion. (b) PCR amplification of the target gene (876bp) of the same 71 plantlets for sequencing analysis and study of target gene editing efficiency.

[illegible]

[illegible]

Figure S5. Alignment of the 85 PCR products sequenced against wild-type sequence in the region of the 3 sgRNA for transgenic lines transformed with construct 1 (*p35S::hCas9*). Wild-type sgRNA sequence is highlighted in grey and all insertions or deletions are highlighted in red. Each allele is represented for all plantlets to highlight monoallelic or biallelic mutations.

[illegible]

WT	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
C2_85	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	300..709	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	739..1016	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1047
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	300..709	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	739..1016	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
C2_86	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1043
C2_87	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1046
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
C2_88	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1043
C2_89	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1043
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1043
C2_90	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1043
C2_91	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1046
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
C2_92	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1046
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
C2_93	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	300..709	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	740..1017	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1048
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	300..709	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	739..1016	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
C2_94	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	300..709	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	739..1016	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1047
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	300..709	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	739..1016	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
C2_95	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1046
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
C2_96	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1046
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	299..708	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	738..1015	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1044
C2_97	Allele	1	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1045
	Allele	2	268	AACCCCTT	CG	TCGCCGGTCAAAGCCTCTTCG	298..707	CACAGCCTAATGTGACCGCTT-CGC	CGGCGAC	737..1014	GTTT	TAGTGAGCGTTCCCTGAT--CCA	AGGGTCT	1043

Figure S6. Alignment of the 97 PCR products sequenced against wild-type sequence in the region of the 3 sgRNA for transgenic lines transformed with construct 2 (*p35S::zCas9*). Wild-type sgRNA sequence is highlighted in grey and all insertions or deletions are highlighted in red. Each allele is represented for all plantlets to highlight monoallelic or biallelic mutations.

[illegible]

[illegible]

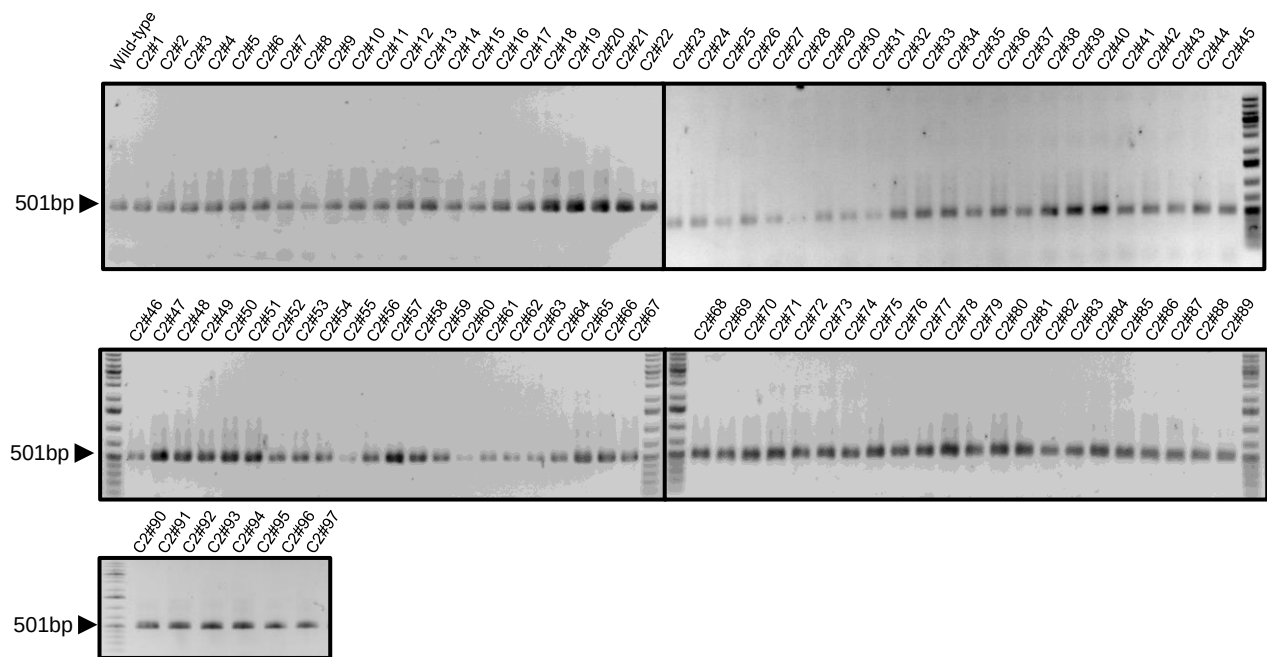


Figure S8. Genotyping of 97 selected plantlets for off-target 1 analysis with transgenic plantlets expressing construct 2 (*p35S::zCas9*). PCR amplification of a region including off-target 1 sequence (501bp) in 97 independent plantlets showing the presence of T-DNA insertion.

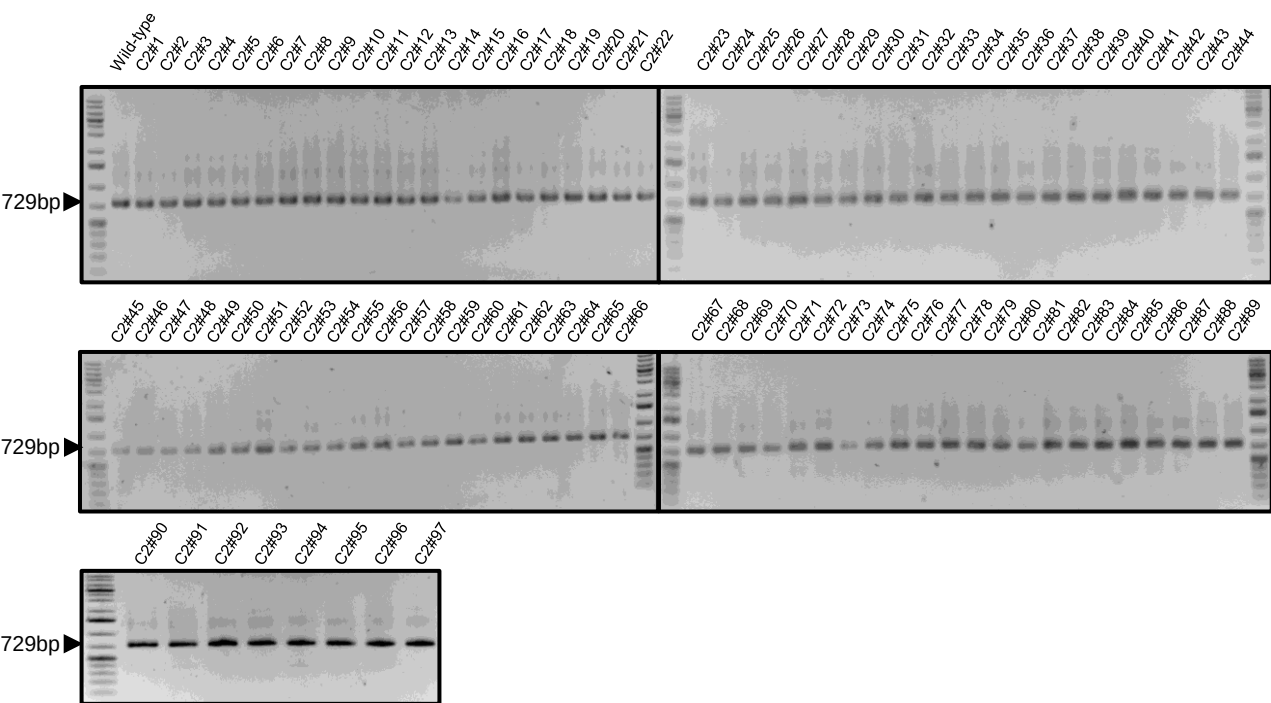


Figure S9. Genotyping of 97 selected plantlets for off-target 3 analysis with transgenic plantlets expressing construct 2 (*p35S::zCas9*). PCR amplification of a region including off-target 3 sequence (729bp) in 97 independent plantlets showing the presence of T-DNA insertion.

Figure S10. Alignment of sequenced Off-target1 and off-target3 sites from 97 independent transgenic lines expressing construct 2 (*p35S::zCas9i*). Wild-type sequence is highlighted in grey. In the off-target 1 sequence TGGCA(A/G)AAGCC, the base A or G in (A/G) is normally found as an heterozygous nucleotide in wild-type.

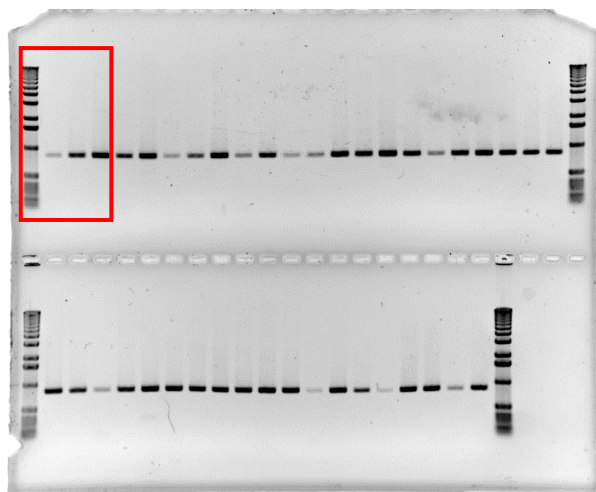


Figure S11. Original agarose gel illustrated Figure 2h. The surrounded area in red is the part illustrated figure 2h.

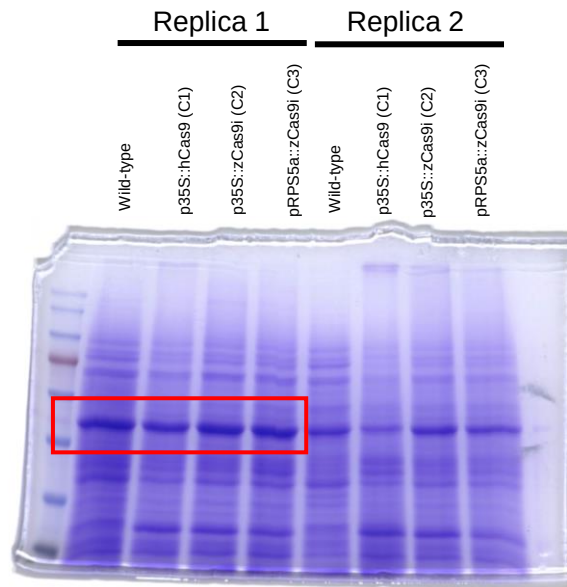


Figure S12. Original Coomassie blue-stained gel illustrated figure 5b. Coomassie blue-stained gel with surrounded area in red corresponding to Coomassie blue picture illustrated figure 5b.

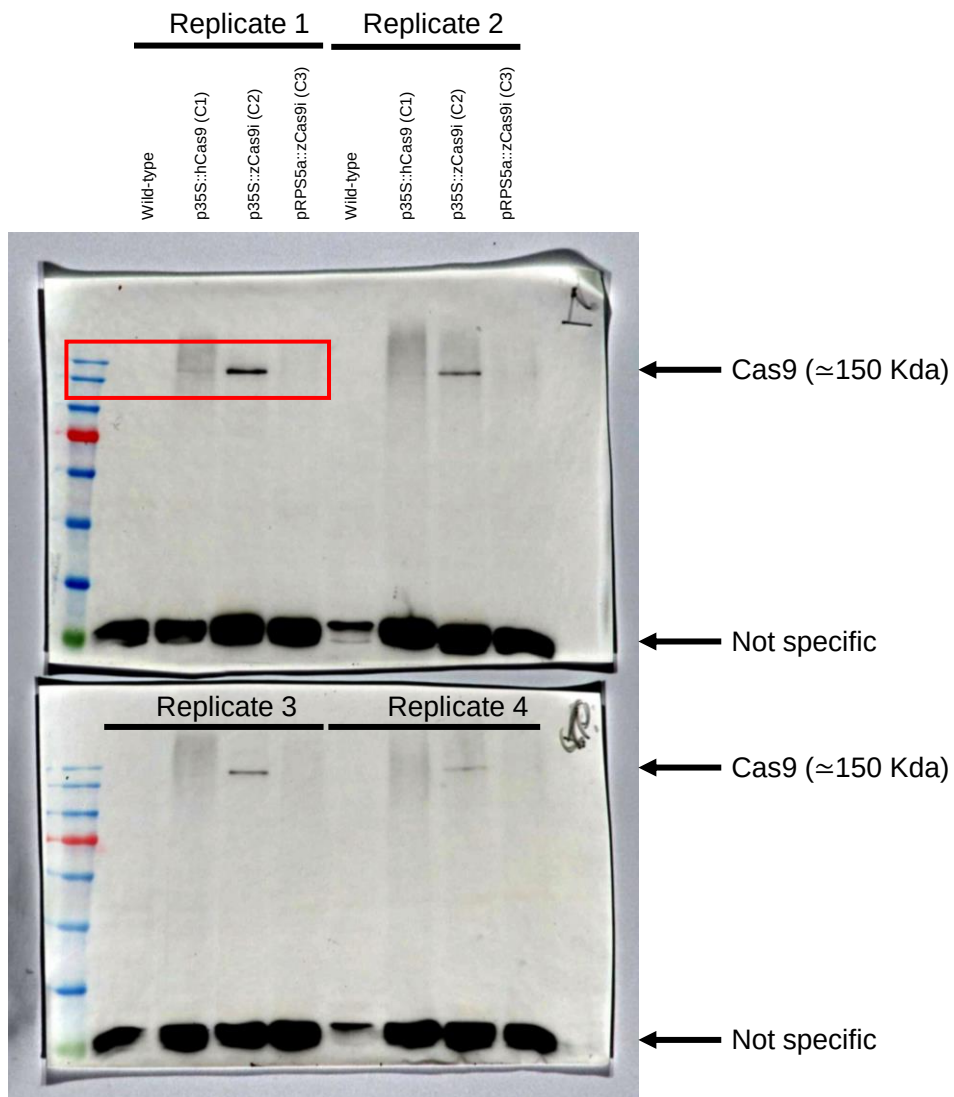


Figure S13. Original western blotting with Cas9 antibody illustrated figure 5b. The surrounded area in red is the part illustrated figure 5b.

primer name	5' -> 3' sequence
VvLYK6_cDNA_Full_F	ATGAAGCTCAAACCCCTAAACGA
VvLYK6_cDNA_Full_R	CTATCTAGCTATAACTGCAGCGGG
hCas9_genotyping_FP	GCCTGTTTGGTAATCTTATCGC
hCas9_genotyping_RP	TCTTTCCACTCTGCTTGTCTCG
Zcas9i_genotyping_FP	TTGGCACATACCATGACCTG
Zcas9i_genotyping_RP	ATCTGACTCCCAAGCTCCTT
VvLYK6_genotyping_FP	GAGCCCAGCAACATCTCCTC
VvLYK6_genotyping_RP	CAGCTCTTCATAGGCATTCCACT
hCas9_QPCR_FP2	AGACCGAAGTACAGACCGGA
hCas9_QPCR_RP2	ATCCGCCGTATTTCTTGGGG
zCas9i_QPCR_FP2	GCCAAGGCAATACTGTCTGC
zCas9i_QPCR_FP2	CCCAGAGACAGTGCAATCAGA
hcas9_Quant_FP	ATTCGGAAGCGACCACTTATC
hcas9_Quant_RP	CAGTTCCTTGACGCTTTTGAG
zCas9i_Quant_FP	GCTCGTCCAAACCTACAATCA
zCas9i_Quant_RP	GCGAGGTCAAAGTTGCTTTT
VvEF1alpha-QPCR_FP	TCTGCCTTCTTCCTTGGGTA
VvEF1alpha-QPCR_RP	GCACCTCGATCAAAAGAGGA
VvEF1alpha-Quant_FP	CTGTCATAGATCGTCCGCTTT
VvEF1alpha-Quant_RP	TCCGGAGTAAAAGACACAACAA
VATP16_QPCR_FP	GTTTTTCGGATTCTCCTCGGCG
VATP16_QPCR_RP	CGAGAGATGCGCATAGCCA
VATP16_Quant_FP	CGGCACCGTTTTTCGGATTCT
VATP16_Quant_RP	ATGCGCATAGCCATCGAAAAG
Off-target1_genotyping_FP	AGCAGCATCTTCCAATTTCCGG
Off-target1_genotyping_RP	CTTGGGTGGTGGCATCTACA
Off-target2_genotyping_FP	GTGGTCAACAATTTGGGTCCG
Off-target2_genotyping_RP	ATGCTCTCGGGGATTGCTTC

Table S1. Primers used in this study.