SUPPLEMENTARY INFORMATION

Targeted mutagenesis in plants using Cas9 RNA-guided endonuclease

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Nature Biotechnology: doi:10.1038/nbt.2655

Material and methods

Construction of Cas9 and sgRNA expressing plasmids

Cas9 was PCR-amplified with primers Cas9GWF and Cas9STR using the clone described in Mali *et al*.[1] as a template. The resulting PCR product was cloned into the pENTR/D-TOPO vector (Life Technologies). The entry clone was subsequently recombined into the GW-compatible destination vector pK7WGF2[2] using the LR clonase (Life Technologies) to produce the 35Sp::GFP-Cas9 construct.

The Arabidopsis U6 promoter used in this study is the consensus sequence of the 3 U6 promoter variants present in the Arabidopsis genome[3]. The AtU6p clone was synthesised with Genescript (**Supplementary Fig. 2**). The promoter was PCR-amplified with primers U6p_GGAG_f and U6p_CAAT_r and cloned into a level 0 vector[4] via the BbsI cut-ligation using the Golden Gate (GG) cloning method. The sgRNA was PCR-amplified using primers PDS_gRNA1_BsaIf and gRNA_AGCG_BsaIr (*PDS* locus) or primers gRNA_T1_GFP_BsaIf and gRNA_AGCG_BsaIr (GFP) using the plasmid gRNA_GFP_T1 described in Mali *et al*.[1] as a template. The resulting PCR product was cloned into the pICH86966 vector (kindly provided by S. Marillonnet, based on the modular cloning system described in Weber *et al*.[4] under the AtU6p via the BsaI cut-ligation using the GG method. Both Cas9 and gRNA_GFP_T1 plasmids[1] were obtained from Addgene.

Transient gene expression in *N. benthamiana* and Cas9 intracellular localisation

Transient expression was performed using the AGL1 strain of *Agrobacterium tumefaciens* as described in Bos *et al*.[5]. GFP-Cas9 was localised in the leaf tissue of *N. benthamiana* 2 days post infiltration using the Leica SP5 confocal microscope in accordance with manufacturer's instructions.

Detection of Cas9-induced indels in plant genomic DNA

GFP-Cas9 and sgRNA carrying the guide sequence matching a site within the *PDS* gene of *N. benthamiana* were transiently co-expressed in the *N. benthamiana* leaf tissue. The tissue was harvested at 2 days post infiltration and the genomic DNA extracted using the DNeasy Plant Mini kit (Qiagen). 100 ng of the genomic DNA was then digested with the MlyI restriction enzyme. Upon digesting the reaction mix was desalted using Sepharose CL-6B (Sigma) and then added as a template into a PCR reaction performed with primers PDS_MlyIF and PDS_MlyIR and the Phusion DNA polymerase (New England Biolabs). The resulting PCR band was cloned into the pCR™-Blunt II-TOPO vector (Life Technologies). Plasmids from individual *Escherichia coli* colonies were sequenced using standard primers M13 forward and M13 reverse. Amplicons with mutated MlyI sites were recovered only when the Cas9 and sgRNA constructs were co-expressed (**Figure 1** and **Supplementary Fig. 3**) but weak bands can be occasionally observed in the negative controls probably due to incomplete digestion. To ensure that the amplicons indeed contain DNA fragments resistant to MlyI digestion, we performed a partial digestion of the genomic DNA with MlyI followed by a second MlyI digestion of the amplicons as shown in **Supplementary Fig. 4**.

Nature Biotechnology: doi:10.1038/nbt.2655

Transformation of *Nicotiana benthamiana*

Leaves of 3-4 weeks *N. benthamiana* plants were co-infiltrated with *Agrobacterium tumefaciens* AGL1 strains carrying GFP-Cas9 and the sgRNA as described above. The leaves were harvested 3 days later and surface sterilised by brief dipping into 70% ethanol and then immersing into 1% fresh sodium hypochlorite with a few drops of Tween 20 to act as a surfactant for 20-25 minutes. Leaves were then rinsed in sterile water and cut into 1-2 cm squares. Leaf squares were put on the selection medium (1X Murashige and Skoog basal salt mixture, 1X Gamborg's B5 vitamins, 3% Sucrose, 0.59g/L MES, 1.0mg/L BAP, 0.1mg/l NAA, 0.4% Agargel pH 5.7, 100 µg/ml kanamycin and 320 µg/ml timentin). Explants were subcultured onto fresh media every 7-10 days for around 1-2 months until the appearance of the first shoots. Shoots were removed with a sharp scalpel and planted into the rooting media (½ strength Murashige and Skoog medium, 0.5% Sucrose, 0.25% Gelrite pH 5.8, 100 µg/ml kanamycin and 320 µg/ml timentin).

Measurements of the mutation rate

In order to estimate the rate of Cas9/sgRNA-induced mutations in the *PDS* locus we used the method described by Qi *et al*.[6]. The PCR product amplified on the non-digested genomic DNA as a template was digested with MlyI and run on a gel. The intensity of non-saturated bands was quantified using the ImageJ software (http://rsb.info.nih.gov/ij). The mutation rate was calculated by dividing the intensity of the uncut band by the total intensity of all bands in a given lane.

Analysis of potential off-targets

In order to select potential off-targets, we searched the *N. benthamiana* genome database using the NCBI BLASTN online tool against the sgRNA target site within the *PDS* gene (GCCGTTAATTTGAGAGTCCA). The search returned 98 potential off-target loci. We then picked 44 out of 98 off-targets with varying numbers of bases (10-18) matching the *PDS* target sequence (**Supplementary Table 1**). The 44 off-target sequences were selected based on the following two criteria: 1) presence of a MlyI site within a sequence and 2) absence of additional MlyI sites in close proximity (<50 bp) to the off-target site. Primers were designed to amplify the potential off-target loci across the off-target site. Out of the 44 loci amplified, 18 gave a PCR band of expected size without non-specific bands. We then proceeded further with these 18 loci (**Supplementary Table 2**). First, we tested if we could amplify the loci using MlyI-digested genomic DNA extracted from the leaf tissue expressing Cas9+sgRNA(PDS) or Cas9 alone. For 12 out of 18 loci it was possible to amplify a PCR product using the MlyI-digested genomic DNA and for the 1 out of 12 loci a weak band was observed only in the case of the Cas9+sgRNA(PDS) sample indicating potential off-target activity (**Supplementary Fig. 7c**). Nevertheless, in all 18 cases the PCR products could be digested by MlyI (**Supplementary Fig. 7a-c**), while the PCR product from the positive control (*PDS*) showed clear resistance to MlyI digestion (**Supplementary Fig. 7d**).

Nature Biotechnology: doi:10.1038/nbt.2655

References

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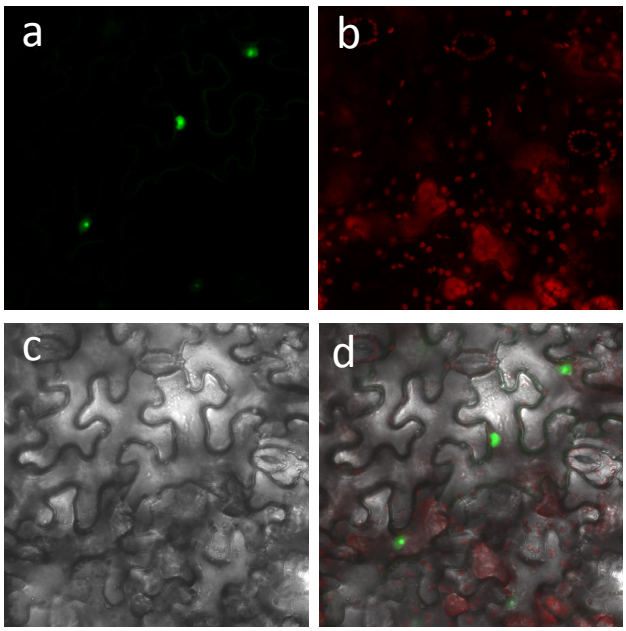
3. Waibel, F., Filipowicz, W. *Nucleic Acids Res* **18**, 3451-3458 (1990).
4. Weber, E. *et al* PLoS One **6**:e16765 (2011).
5. Bos, J. *et al*. Plant J **48**, 165-176 (2006).
6. Qi, Y. *et al*. *Genome Res* **23**, 547-554 (2013).

Nature Biotechnology: doi:10.1038/nbt.2655



Supplementary Figure 1. Cas9 localises to plant nuclei.
GFP-Cas9 was expressed in *N. benthamiana* leaf tissue and visualised 2 days post infiltration. (a) GFP. (b) Plastids autofluorescence. (c) Bright field. (d) Overlay of (a), (b) and (c).

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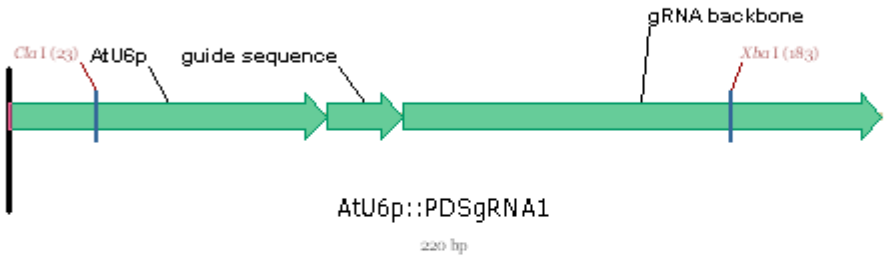
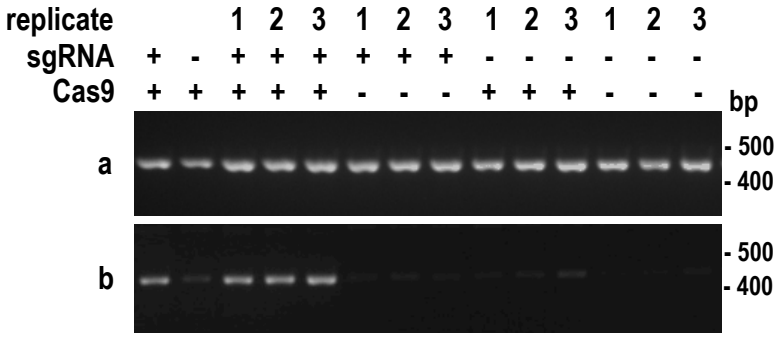
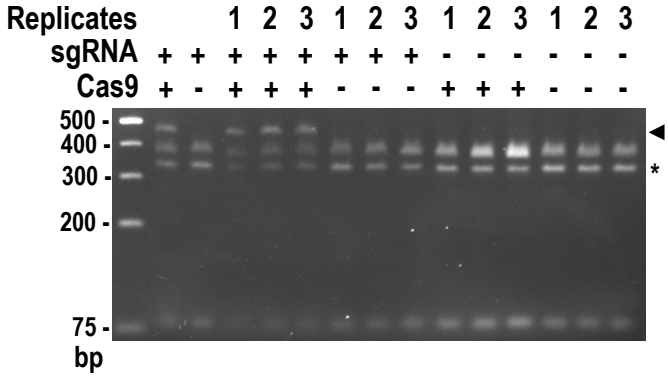


GGAGTGATCAAAAGTCCCACATCGATCAGGTGATATATAGCAGCTTAGTTTATATAATGATAG
AGTCGACATAGCGATTGCCGTTAATTTGAGAGTCCAGTTTTAGAGCTAGAAATAGCAAGTTAA
AATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTCTAGACC
CAGCTTTCTTGTACAAAGTTGGCATTACGCT

Supplementary Figure 2. Description of the U6p::sgRNA construct.
Arabidopsis U6 promoter sequence is shown in blue; the guide sequence is shown in red; the sgRNA backbone sequence is shown in green

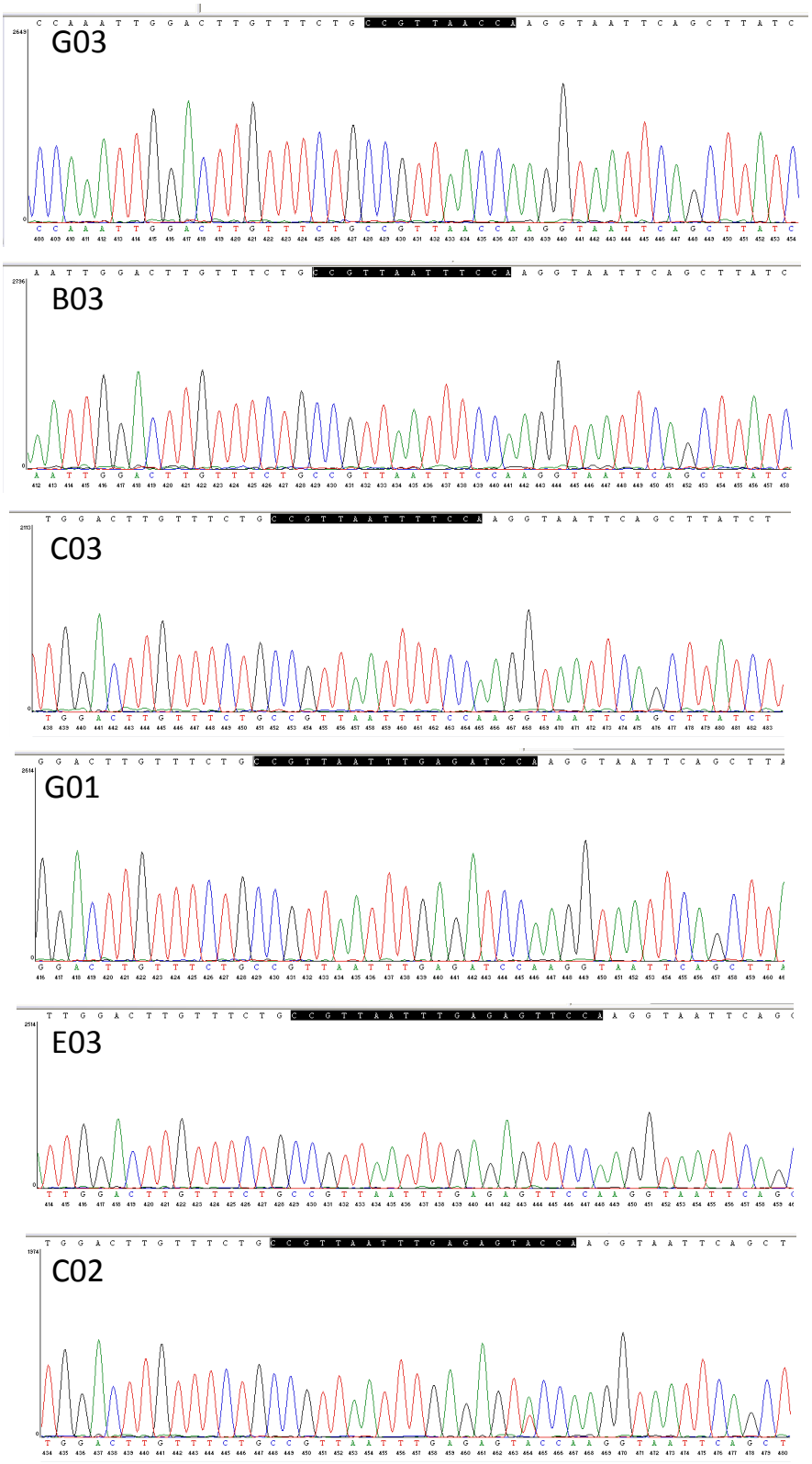


Supplementary Figure 3. Replicates of the experiment presented in Figure 1. The PCR was done with undigested genomic DNA (a) or MlyI-digested genomic DNA (b). In the case of the pre-digested genomic DNA, co-expression of both Cas9 and sgRNA results in markedly increased levels of the amplicon. A total of three independent replicates (1-3) are shown.

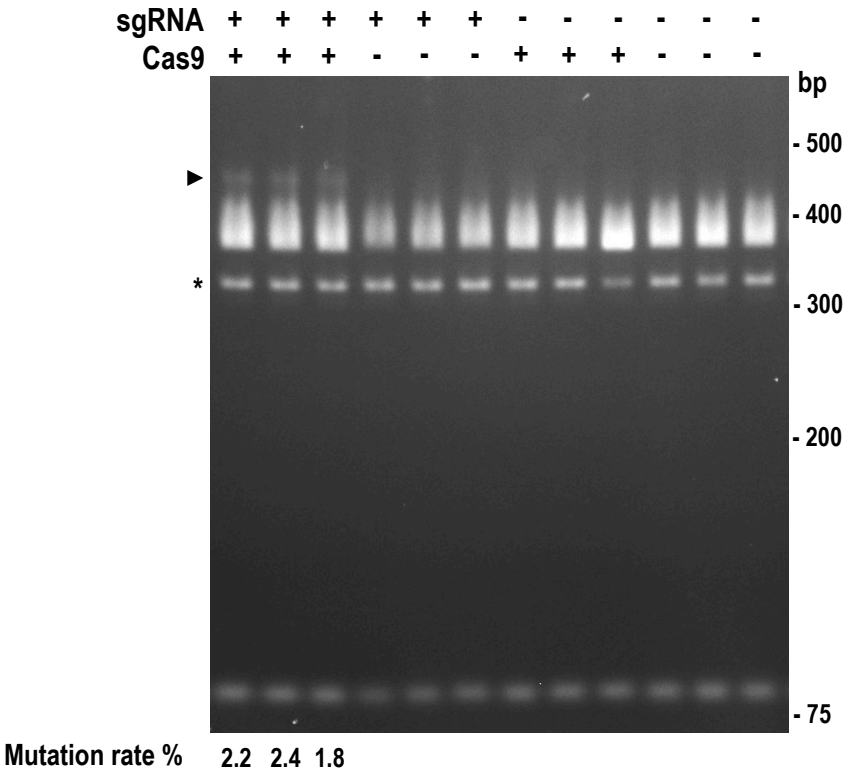


Supplementary Figure 4. PCR products amplified on genomic DNA partially digested with MlyI are resistant to MlyI digestion only when Cas9 and sgRNA are co-expressed in plant tissue.

The arrow head indicates the MlyI- resistant band; the asterisk indicates the band resulting from MlyI star activity. A total of three independent replicates (1-3) are shown.

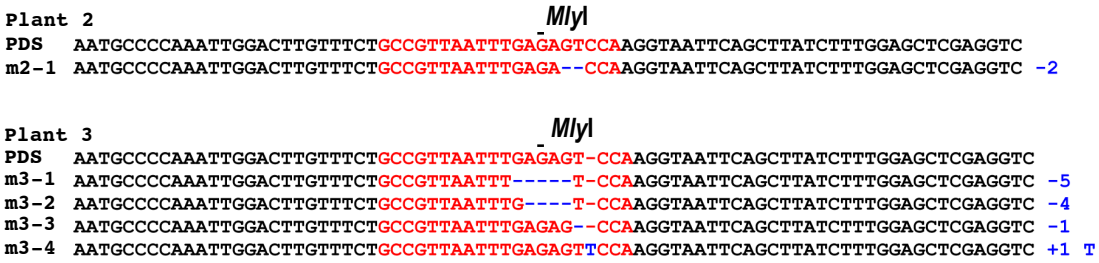


Supplementary Figure 5. Representative sequence chromatograms of Cas9-induced indels in the *Nicotiana benthamiana* genome.
Regions highlighted in black belong to the target site within the *PDS* gene.



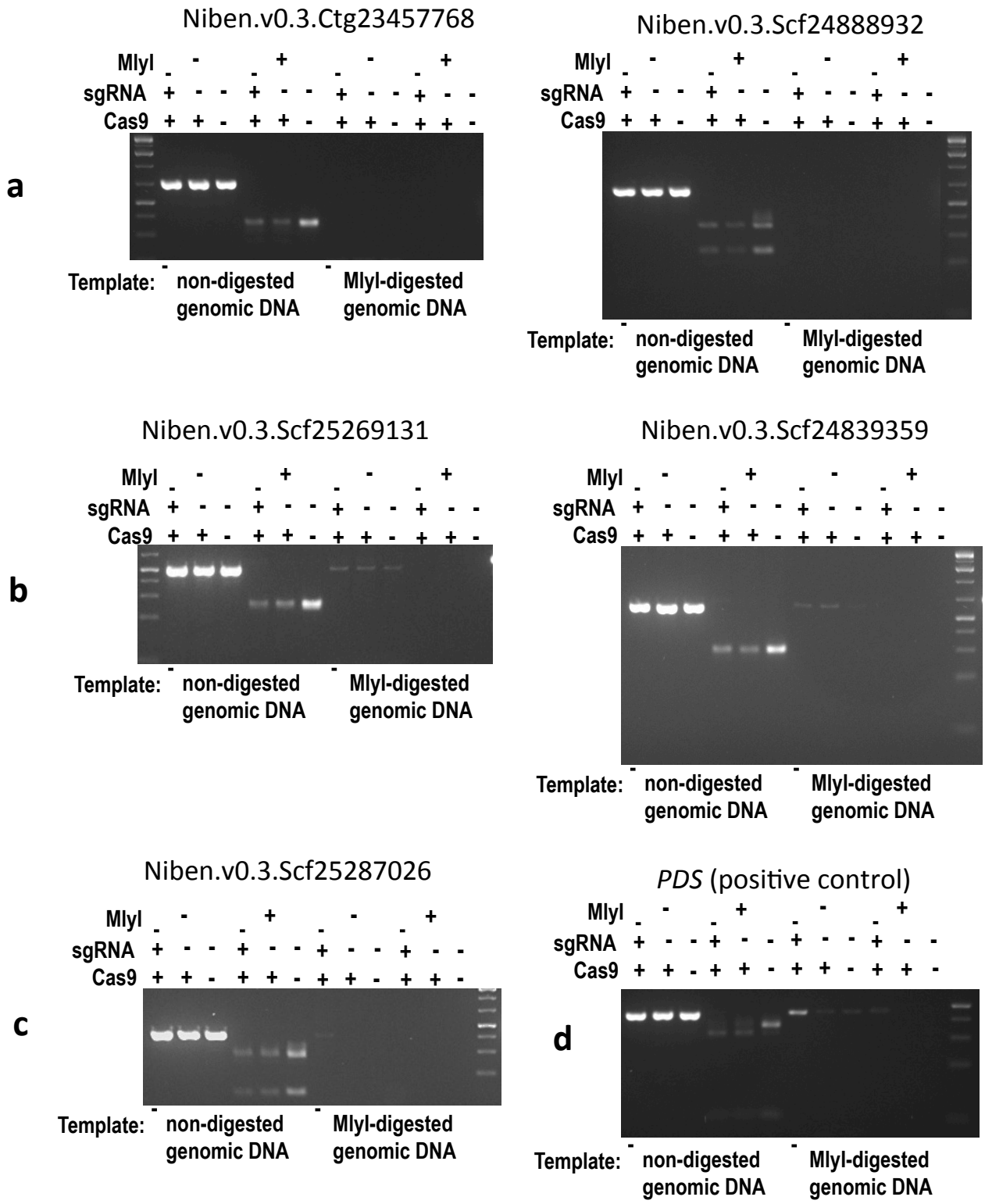
Supplementary Figure 6. Mutation rate replicates.
The PCR was done with the undigested genomic DNA as a template. Resulting amplicons were then digested with MlyI. The band intensity was quantified with the ImageJ software. The arrowhead indicates the MlyI- resistant band; the asterisk indicates the band resulting from the star activity of MlyI.

Nature Biotechnology: doi:10.1038/nbt.2655



Supplementary Figure 7. Mutations detected in the *PDS* locus of transgenic *Nicotiana benthamiana* plants.

DNA fragments produced by PCR amplification using the MlyI-digetsed genomic DNA from transgenic *N. benthamiana* plants 2 and 3 (**Fig. 2b**) were cloned into the pCR™-Blunt II-TOPO vector and sequenced. In the case of the plant 2, only one mutant allele (m2-1) has been found in 5 out of 5 sequenced clones. In the case of the plant 3, four different mutant alleles have been found: m3-1 (1 out of 8 sequenced), m3-2 (1 out of 8 sequenced), m3-3(2 out of 8 sequenced) and m3-4 (4 out 8 sequenced). The sequence within *PDS* targeted by the sgRNA is shown in red whereas the mutations are shown in blue.



Supplementary Figure 8. Examples of restriction-site loss assays with potential off-targets.

Analysis examples of potential off-targets from groups 1 (a), 2 (b) and 3 (c) (Supplementary Table 1). The *PDS* positive control is shown in (d).

Supplementary Table 1. Complete list of off-sites identified for the *PDS* target sequence in the *Nicotiana benthamiana* genome.

The MlyI restriction site is shown in blue; mismatching bases are shown in red.

5'

Off-target locus	Sequence of the off-target site	Number of matching bases	*Mly*I site:	Tested?
Niben.v0.3.Scf25001368	GGTGTTAATTTGAGAGTCAT	16	Yes	Yes
Niben.v0.3.Scf24984291	TTGGTTAATTTGAGAGTCTA	16	Yes	Yes
Niben.v0.3.Scf25296596	GCCGTCAATTTGAGAGTCTG	17	Yes	Yes
Niben.v0.3.Scf25265859	GCCGTTAATTTAAGAGTCTG	17	Yes	Yes
Niben.v0.3.Scf24888932	ACTTGAAATTTGAGAGTCCA	15	Yes	Yes
Niben.v0.3.Ctg23457768	TGCTTTAATTTGAGAGTCGG	15	Yes	Yes
Niben.v0.3.Scf25215920	TCTTTTAATTTGAGAGTCCT	16	Yes	Yes
Niben.v0.3.Scf25013025	TTAAATAATTTGAGAGTCCA	15	Yes	Yes
Niben.v0.3.Scf25296544	CTTTTTAATTTGAGAGTCTT	14	Yes	Yes
Niben.v0.3.Scf25295436	ATAAAAAATTTGAGAGTCCA	14	Yes	Yes
Niben.v0.3.Scf25293111	GAGCTTAATTTGAGAGTCAT	15	Yes	Yes
Niben.v0.3.Scf25269131	AATTTTAATTTGAGAGTCGT	14	Yes	Yes
Niben.v0.3.Scf25264778	TCAATAAATTTGAGAGTCCA	16	Yes	Yes
Niben.v0.3.Scf25249221	AATTTTAATTTGAGAGTCTA	15	Yes	Yes
Niben.v0.3.Scf25179005	AATTTTAATTTGAGAGTCTA	15	Yes	Yes
Niben.v0.3.Scf25159671	GTAATAAATTTGAGAGTCCA	16	Yes	Yes
Niben.v0.3.Scf24839359	GGTTTTAATTTGAGAGTCAG	15	Yes	Yes
Niben.v0.3.Scf25287026	ATCAAAAATTTGAGAGTCCA	15	Yes	Yes
Niben.v0.3.Scf25193839	AGGGTTAATTTGAGAGTCAA	16	Yes	No
Niben.v0.3.Scf25145763	GGAGTTAATTTGAGAGTCAT	16	Yes	No
Niben.v0.3.Scf25118108	AAGCTTAATTTGAGAGTCCT	15	Yes	No
Niben.v0.3.Scf25101348	TAGATTAATTTGAGAGTCCC	15	Yes	No
Niben.v0.3.Scf25000492	ATCCTTAATTTGAGAGTCCC	16	Yes	No
Niben.v0.3.Scf24897441	CTCATTCTAATATGAGTCTA	10	Yes	No
Niben.v0.3.Scf24749765	TCCGTTAGTTTGAGAGTCCA	18	Yes	No
Niben.v0.3.Ctg7562303	TAAGTTAATTTGAGAGTCAA	16	Yes	No
Niben.v0.3.Scf25299987	GGCTGAAATTTGAGAGTCCA	16	Yes	No
Niben.v0.3.Scf25293323	TTTCTTAATTTGAGAGTCAA	15	Yes	No
Niben.v0.3.Scf25290546	TACTTGAATTTGAGAGTCCA	16	Yes	No
Niben.v0.3.Scf25284967	AGTTGAAATTTGAGAGTCCA	14	Yes	No
Niben.v0.3.Scf25279608	CTGGGTAATTTGAGAGTCCT	15	Yes	No
Niben.v0.3.Scf25270807	ATTCTTAATTTGAGAGTCAT	14	Yes	No
Niben.v0.3.Scf25268991	AGATTTAATTTGAGAGTCGT	14	Yes	No
Niben.v0.3.Scf25249034	AACTAAAATTTGAGAGTCCA	15	Yes	No
Niben.v0.3.Scf25109337	AATATAAATTTGAGAGTCCA	15	Yes	No
Niben.v0.3.Scf25105922	TGCTTAAATTTGAGAGTCCA	16	Yes	No
Niben.v0.3.Scf25043710	ACTCGTAATTTGAGAGTCCT	15	Yes	No
Niben.v0.3.Scf24913622	TCTCTTAATTTGAGAGTCAA	16	Yes	No

Supplementary Table 1. Complete list of off-sites identified for the *PDS* target sequence in the *Nicotiana benthamiana* genome (continued).
The MlyI restriction site is shown in blue; mismatching bases are shown in red.

Off-target locus	Sequence of the off-target site	Number of matching bases	*Mly*I site:	Tested?
Niben.v0.3.Scf24912538	TCTCCAAATTTGAGAGTCCA	15	Yes	No
Niben.v0.3.Scf24901695	AAAGAAAATTTGAGAGTCCA	15	Yes	No
Niben.v0.3.Scf24858152	GGATTTAATTTGAGAGTCAA	16	Yes	No
Niben.v0.3.Scf24828564	TAATTTAATTTGAGAGTCTT	14	Yes	No
Niben.v0.3.Ctg22385253	GAGCTTAATTTGAGAGTCAT	15	Yes	No
Niben.v0.3.Ctg21326560	CAACTTAATTTGAGAGTCAT	14	Yes	No
Niben.v0.3.Scf25104628	GCCGTTAATTTGAGAGATCA	18	No	No
Niben.v0.3.Scf25042327	GTTGTTAATTTGAGAGTAAG	15	No	No
Niben.v0.3.Scf24806379	GGTGTTAATTTGAGAGTAGG	15	No	No
Niben.v0.3.Scf25041266	GGTGTTAATTTGAGAGTGAT	15	No	No
Niben.v0.3.Scf24855208	CCTGTTAATTTGAGAGTGGT	15	No	No
Niben.v0.3.Scf25028504	AGTGTTAATTTGAGAGTTGG	14	No	No
Niben.v0.3.Scf24593869	CTTGTTAATTTGAGAGTTGC	14	No	No
Niben.v0.3.Scf25297445	ATAGTTAATTTGAGAGTTAT	14	No	No
Niben.v0.3.Scf24977871	ATAGTTAATTTGAGAGTTTT	14	No	No
Niben.v0.3.Scf25284598	ATGGTTAATTTGAGAGTTGT	14	No	No
Niben.v0.3.Scf25241386	ATGGTTAATTTGAGAGTTGT	14	No	No
Niben.v0.3.Scf25208465	ATGGTTAATTTGAGAGTTGT	14	No	No
Niben.v0.3.Scf25164462	ATGGTTAATTTGAGAGTTGT	14	No	No
Niben.v0.3.Scf25055666	ATGGTTAATTTGAGAGTTGT	14	No	No
Niben.v0.3.Scf25201582	ATGGTTAATTTGAGAGTTAT	14	No	No
Niben.v0.3.Scf25221420	TTCGTTAATTTGAGAGTTGT	15	No	No
Niben.v0.3.Scf24906680	TTGGTTAATTTGAGAGTGAA	15	No	No
Niben.v0.3.Scf25228086	CACGTTAATTTGAGAGTATT	15	No	No
Niben.v0.3.Scf25129185	AATGTTAATTTGAGAGTTTT	14	No	No
Niben.v0.3.Ctg13658950	ATCGTTAATTTGAGAGTTTA	16	No	No
Niben.v0.3.Scf24888659	ATTGTTAATTTGAGAGTTTA	15	No	No
Niben.v0.3.Ctg24082496	GGGGTTAATTTGAGAGTTTA	16	No	No
Niben.v0.3.Scf25300957	GCCGTTAATTTGAGTGTTTG	16	No	No
Niben.v0.3.Scf25237042	ACCGTTAATTTGAGAATTTA	16	No	No
Niben.v0.3.Scf25274465	ACAGTTAATTTGAGAGTTAT	15	No	No
Niben.v0.3.Scf24895145	TCAGTTAATTTGAGAGTTAC	15	No	No
Niben.v0.3.Scf25298151	CAAGTTAATTTGAGAGTTAA	15	No	No
Niben.v0.3.Scf25288085	GTCGTTAATTTGAGAGATGT	15	No	No
Niben.v0.3.Scf25285360	GTCGTTAATTTGAGAGACGC	16	No	No
Niben.v0.3.Scf25202251	TTCGTTAATTTGAGAGAAGA	15	No	No
Niben.v0.3.Scf25238086	GTCGTTAATTTGAGAGATAC	15	No	No
Niben.v0.3.Scf25203527	GTCGTTAATTTGAGAGATAC	15	No	No
Niben.v0.3.Scf24814834	GTCGTTAATTTGAGAGATAC	15	No	No
Niben.v0.3.Scf25268972	GCCGTTAATTTGAGAACATT	15	No	No

Supplementary Table 1. Complete list of off-sites identified for the *PDS* target sequence in the *Nicotiana benthamiana* genome (continued).
The MlyI restriction site is shown in blue; mismatching bases are shown in red.

Off-target locus	Sequence of the off-target site	Number of matching bases	*Mly*I site:	Tested?
Niben.v0.3.Scf25214375	GCCGTTAATTTGAGAACATT	15	No	No
Niben.v0.3.Scf25261529	GCCGTTAATTTGAGTATCGA	17	No	No
Niben.v0.3.Scf25115392	ACCGTTAATTTGAGAACCGC	15	No	No
Niben.v0.3.Scf24711774	TCCGTTAATTTGAGAGCGGC	15	No	No
Niben.v0.3.Scf25074954	ACCGTTAATTTGAGATAGGT	14	No	No
Niben.v0.3.Scf25294133	GCCGTTAATTTGAGGTATAA	15	No	No
Niben.v0.3.Scf24884557	GCCGTTAATTTGAGTTGTTA	15	No	No
Niben.v0.3.Scf25092660	CCCGTTAATTTGAGATGTAC	14	No	No
Niben.v0.3.Scf25051318	ACCGTTAATTTGAGAGGTAA	16	No	No
Niben.v0.3.Scf24814356	TCCGTTAATTTGAGAGGAAT	15	No	No
Niben.v0.3.Scf25293162	TGCATTAATTTGAGAGTCGG	15	Yes	No
Niben.v0.3.Scf25205360	TGCTTTAATTTGAGAGTCGG	15	Yes	No
Niben.v0.3.Scf24809183	GCCTTTAATTTGAGAGTCGC	17	Yes	No
Niben.v0.3.Scf25282254	ACCGTTAATTTGATAGTCCG	17	No	No
Niben.v0.3.Scf25117869	ACCCCGAATTTGAGAGTCCA	16	Yes	No
Niben.v0.3.Scf24847789	AAGCCCAATTTGAGAGTCCA	14	Yes	No
Niben.v0.3.Scf25017618	CGACAAAATTTGAGAGTCCA	14	Yes	No
Niben.v0.3.Scf24999762	TTTTGAAATTTGAGAGTCCA	14	Yes	No
Niben.v0.3.Scf25023216	GCCGAAAATTTGAGAGTCCA	18	Yes	No
Niben.v0.3.Scf24878949	ATAGTGAATTTGAGAGTCCA	16	Yes	No

Supplementary Table 2. Summary of the off-target analysis.
The MlyI restriction site is shown in blue; mismatching bases are shown in red.

Group	Off-target locus	Sequence of the off-target site	Number of matching bases	Results of the analysis for the off-target activity
1	Niben.v0.3.Scf25001368	GGTGTTAATTTGAGAGTCAT	16	No amplification on the MlyI-digested genomic DNA (Supplementary Fig. 6**a**)
	Niben.v0.3.Scf24984291	TTGGTTAATTTGAGAGTCTA	16	
	Niben.v0.3.Scf25296596	GCCGTCAATTTGAGAGTCTG	17	
	Niben.v0.3.Scf25265859	GCCGTTAATTTAAGAGTCTG	17	
	Niben.v0.3.Scf24888932	ACTTGAAATTTGAGAGTCCA	15	
	Niben.v0.3.Ctg23457768	TGCTTTAATTTGAGAGTCGG	15	
2	Niben.v0.3.Scf25215920	TCTTTTAATTTGAGAGTCCT	16	Amplification on the MlyI-digested genomic DNA but there is no increased level of the amplicon in the case of Cas9 + sgRNA as compared to the Cas9 negative control. The amplicon is digested by MlyI. (Supplementary Fig. 6**b**)
	Niben.v0.3.Scf25013025	TTAAATAATTTGAGAGTCCA	15	
	Niben.v0.3.Scf25296544	CTTTTTAATTTGAGAGTCTT	14	
	Niben.v0.3.Scf25295436	ATAAAAAATTTGAGAGTCCA	14	
	Niben.v0.3.Scf25293111	GAGCTTAATTTGAGAGTCAT	15	
	Niben.v0.3.Scf25269131	AATTTTAATTTGAGAGTCGT	14	
	Niben.v0.3.Scf25264778	TCAATAAATTTGAGAGTCCA	16	
	Niben.v0.3.Scf25249221	AATTTTAATTTGAGAGTCTA	15	
	Niben.v0.3.Scf25179005	AATTTTAATTTGAGAGTCTA	15	
	Niben.v0.3.Scf25159671	GTAATAAATTTGAGAGTCCA	16	
	Niben.v0.3.Scf24839359	GGTTTTAATTTGAGAGTCAG	15	
3	Niben.v0.3.Scf25287026	ATCAAAAATTTGAGAGTCCA	15	Amplification on the MlyI-digested genomic DNA and there is slightly increased level of the amplicon in the case of Cas9 + sgRNA as compared to the Cas9 negative control. The amplicon is digested by MlyI. (Supplementary Fig. 6**c**)

Supplementary Table 3. Primers used in this study.

Primer name	Primer sequence
U6p_GGAG_f	GAGGAAGACAA GGAG TGATCAAAAGTCCCAC
U6p_CAAT_r	GAGGAAGACAA CAAT CGCTATGTCGACTC
PDS_gRNA1_BsaIf	TGTGGTCTCAATTGCCGTTAATTTGAGAGTCCAGTTTTAGAG CTAGAAATAGCAAG
gRNA_T1_GFP_BsaIf	TGTGGTCTCAATTG TGAACCGCATCGAGCTGAA GTTTTAGAGCTAGAAATAGCAAG
gRNA_AGCG_BsaIr	TGTGGTCTCA AGCG TAATGCCAACTTTGTAC
Cas9GWF	CACCATGGACAAGAAGTACTCCATTG
Cas9STR	TCACACCTTCCTCTTCTTCTTG
PDS_MlyIF	GCTTTGCTTGAGAAAAGCTCTC
PDS_MlyIR	ACATAACAAATTCCTTTGCAAGC
M13 forward	GTTGTAAAACGACGGCCAGT
M13 reverse	CAGGAAACAGCTATGACC
25215920F	TTGGTCCTAAGTACAATAGCTGGT
25215920R1	TCGCTCCTTGTGTCAACTTCA
25013025F	GGGTTGGCAGTTACAGTTGAA
25013025R	GTGTTGAAGGCGTTGGAGAT
25001368F1	TTAGCATTGAATGGGTCCAGG
25001368R	GCCTCCAACCCACCATAAA
24984291F	TCTTTGCCAGGATGTAATTAAGA
24984291R	GCACATAGGAGTAATAGTGGGTGA

Supplementary Table 3. Primers used in this study (continued)

Primer name	Primer sequence
25296596F	GTATCACCCCAAGACCAAGC
25296596R	TGTTCAACTATGCTTGTTTATTCC
25296544F	AAGAGAGGTCCCCTCCATTT
25296544R	CAAAGGCGGAGCTACAAAAA
25295436F	TCAATGTTCTTGGGGGAAAG
25295436R	CCAGCCTTGGTCTGTCCATA
25293111F	GAGATCTTTATGTTTGGACATGTGA
25293111R1	TCATTATATCAGAGCTGGTATCT
25287026F	TGTGAAAAGAAACTAGCTAACTGGAA
25287026R1	CTCCATATGGCCCTCTTACAG
25269131F	CCATAGTGTTGAGCCCATGA
25269131R1	TTATGGTTTTCACTAATATTATGCA
25265859_1F	ATTTTCAATTATGCTTGTTTATTCC
25265859_1R	CCAATTCCAAGCTTTGTAACC
25264778_2F	CTTCCGGGATCGAATCAATA
25264778_2R	AATTCAAATCAAAGGTGGGAAT
25249221F	CTGTATGGCGCCAGCTTT
25249221R	TGTTGAGCTTAGCTTCCATTG
25179005F	CGCGACTTTCTGGATGATAA
25179005R	GCTTCGTCGTGTGGTAAGTTC

Supplementary Table 3. Primers used in this study (continued)

Primer name	Primer sequence
25159671F	CTTCCGGGATCGAATCAATA
25159671R	TTCAAATCATCGGTGGGAGT
24888932F	GCAACATGAAGTTAGTTTGAGCA
24888932R	GGGGCAGAAGGTAATTAGCAG
24839359F	ATCAGACCTTGGGGGTACAA
24839359R	GGTTAGTTCACTGATCGTCTAAGG
23457768F	TCCTGTAATTTTCTTTGTGATTCG
23457768R	TTCCCCTTTCTTGAGTTCCA