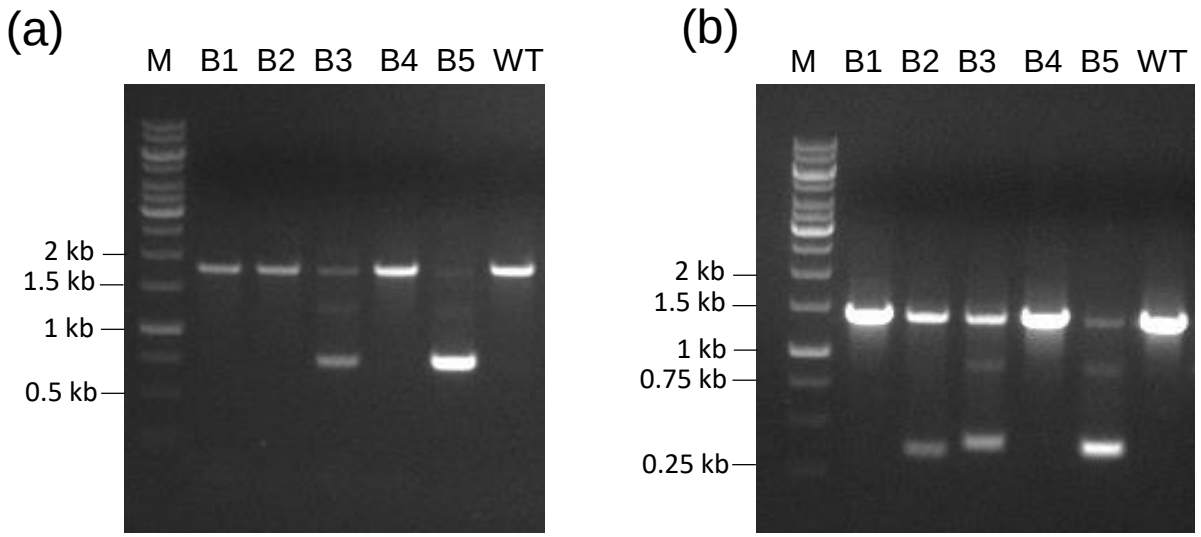

Figure S1. Identification of CRISPR-edited *GmFAD2* gene deletions in soybean hairy roots using PCR-based genotyping. a PCR-gel electrophoresis using specific primers for *GmFAD2-1A*. b PCR-gel electrophoresis using specific primers for *GmFAD2-1B*. Lanes B1-B5, DNA samples from pooled hairy roots of infected Plants; WT, DNA sample from uninfected roots of Williams 82; M, molecular weight markers.

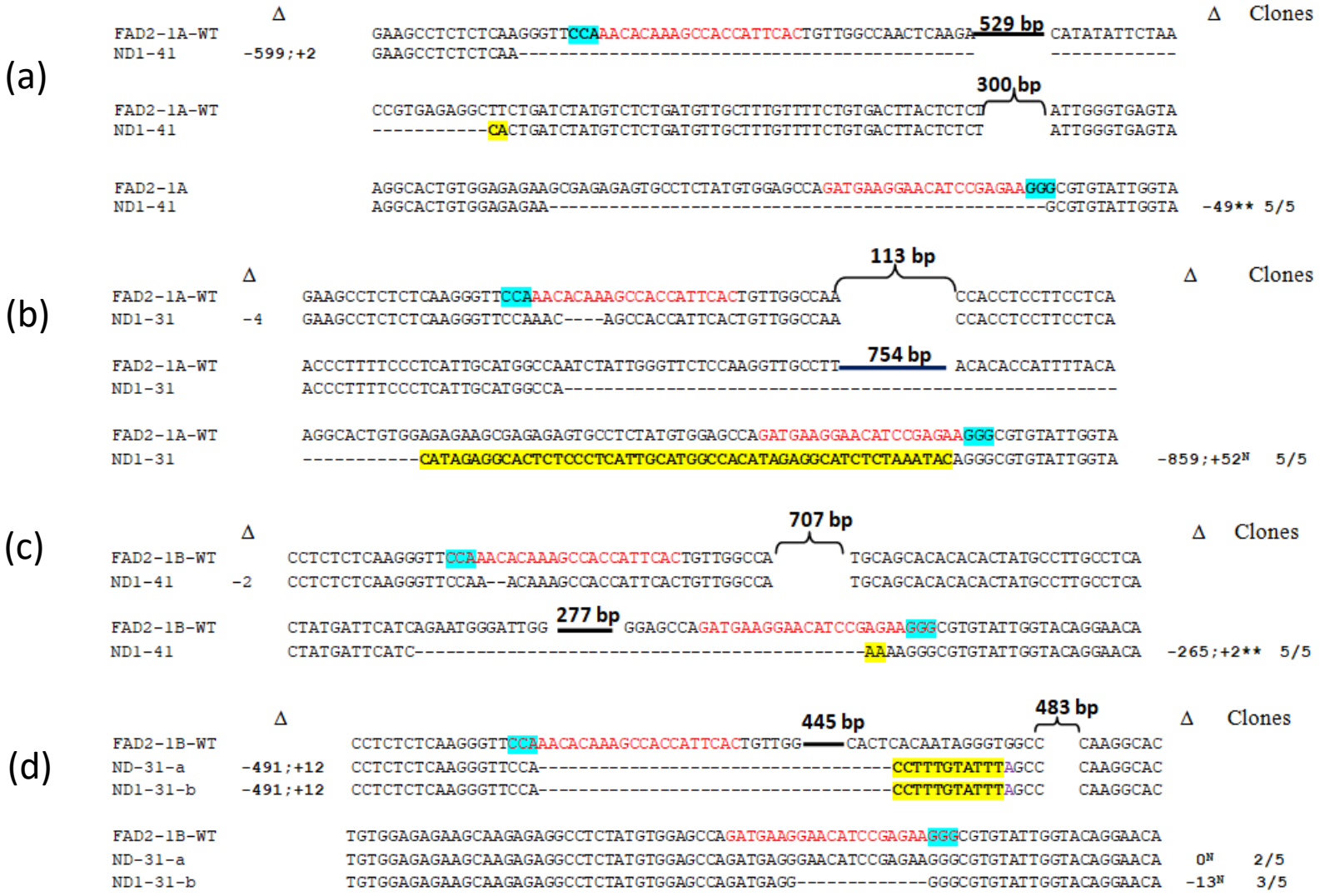

Figure S2. Partial deletions and insertions detected in *GmFAD2* genes. a,c Deletions in *GmFAD2-1A* and *GmFAD2-1B* in T0 event ND1-41, respectively. b,d Deletions in *GmFAD2-1A* and *GmFAD2-1B* in T0 event ND1-31, respectively. (Δ) indels in target gene sequences (negative: deleted nucleotides; positive: inserted nucleotides; 0: no deletion or insertion); (Clones) number of mutated amplicons out of total sequenced clones; small letters (a, b…) different alleles detected in each event ; ** the inheritance was confirmed in T1 and/or T2 progenies; N, non-inherited alleles.

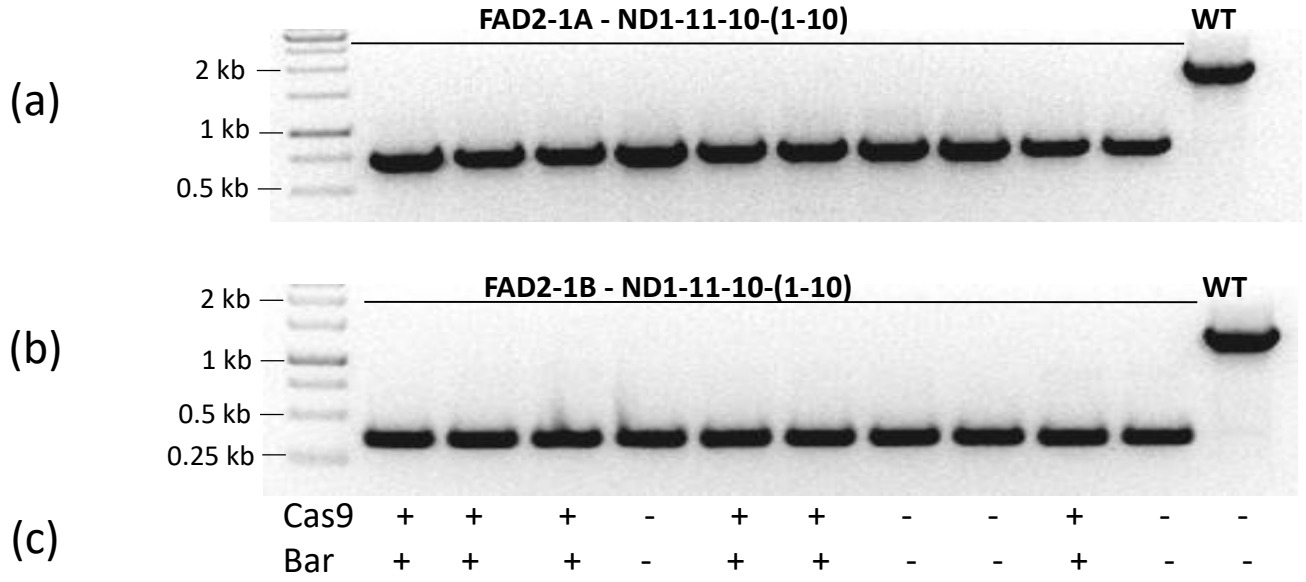

Figure S3 . Genotyping of homozygous expected deletions and transgenes in T2 generation of event ND1-11. a PCR-based genotyping of *GmFAD2-1A*. b PCR-based genotyping of *GmFAD2-1b*. c Genotyping results for bar and Cas9 genes; (+) positive; (-) negative.

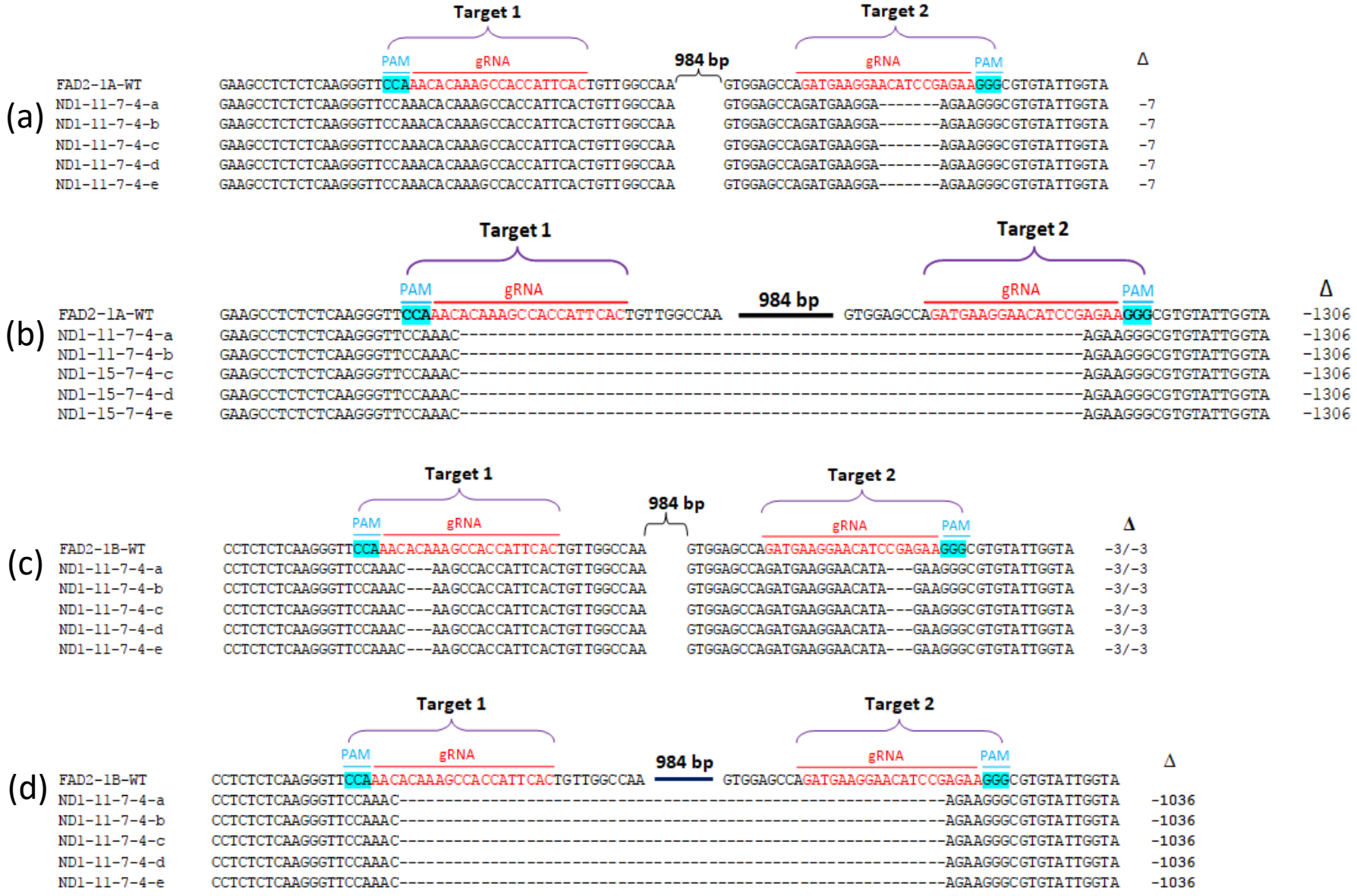

Figure S4. Inheritance of *GmFAD2* mutations in T2 progenies of event ND1-11. a,b Small deletions in *GmFAD2-1A* and *GmFAD2-1B*, respectively. c,d Expected deletions in GmFAD2-1A and GmFAD2-1B. Δ, Indels in target gene sequences; -, deleted nucleotides; numerator, Indels in target1; denominator, Indels in target2; small letters (a,b…) sequenced clones.

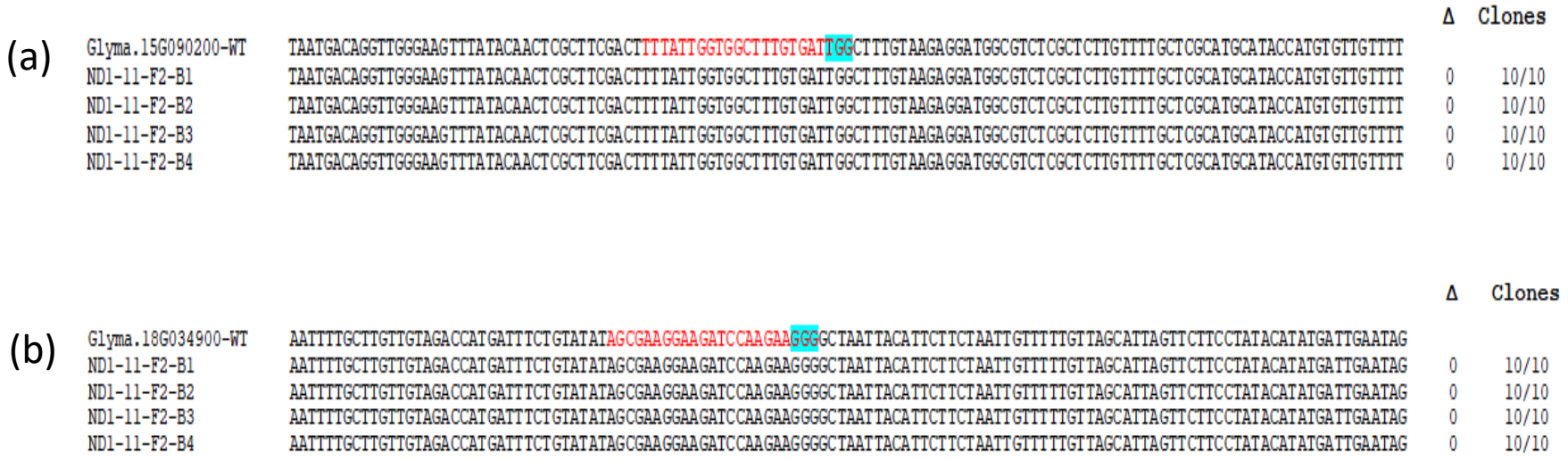

Figure S5. Sequencing results of off-target and flanking regions. a Off-target of gRNA1 (target 1) in chromosome15. b Off-target of gRNA2 (target 2) in chromosome18. Δ, Indels in target gene sequences (0: no deletion or insertion); Clones, number of mutated amplicons out of total sequenced clones.

Table S1. Primer sequences for genotyping *GmFAD2* gene mutations

Primer pairs	Sequences	PCR products of WT	PCR products of mutants/Transgenic lines	Positions	
				Chromosome	Start
FAD2-1A-F	5'-AGGCTTAGGTGTAGGCACCTAGC-3'	1796 bp	≤1796 bp	10	50013799
FAD2-1A-R	5'- GTAATCCTCTAGATGAACCTCTGC-3'				50015594
FAD2-1B-F	5'-CTCTCTAATCTGTCACTTCCCTCC-3'	1413 bp	≤1413 bp	20	35317536
FAD2-1B-R	5'-CACACAAGTCATTACGCGGC-3'				35318948
D22-F	5'-AGCCAGATGAAGGAACATCCG-3'	396 bp		10	50015199
FAD2-1A-R	5'- GTAATCCTCTAGATGAACCTCTGC-3'				50015594
D22-F	5'-AGCCAGATGAAGGAACATCCG-3'	162 bp		20	35318787
FAD2-1B-R	5'-CACACAAGTCATTACGCGGC-3'				35318948
FAD2-1A-F	5'-AGGCTTAGGTGTAGGCACCTAGC-3'	407 bp		10	50013799
D22-R	5'-CAACAGTGAATGGTGGCTTTGT-3'				50014205
FAD2-1B-F	5'-CTCTCTAATCTGTCACTTCCCTCC-3'	258 bp		20	35317536
D22-R	5'-CAACAGTGAATGGTGGCTTTGT-3'				35317793
G18-off2-F2	5'-CGCATGATCATATTTGCTGGCT-3'	245 bp	245 bp	18	2713153
G18-off2-R	5'-CTGATGCTGGTTCATAGCAGTG-3'				2713397
G15-off1-F1	5'-GTGTCATGGTCCTGCACGTA-3'	769	769 bp	15	6943532
G15-off1-R1	5'-GCAGAAGTGCTCCCATTCCT-3'				6942764
Bar-F	5'-TACCATGAGCCCAGAACGACGCCC-3'	0	336 bp	-	-
Bar-R	5'-CTTCAGCAGGTGGGTGTAGAGCG-3'				
Cas-F	5'-GCCCAAGAGGAACAGCGATAAGC-3'	0	328 bp	-	-
Cas-R	5'-CAGTTCGCCGGCAGAGGCCAGC-3'				
F-FAD2-Crispr1	5'-GATTGATGAAGGAACATCCGAGAA-3'	-	-	-	-
R-FAD2-Crispr1	5'-AAACTTCTCGGATGTTCCTTCATC-3'				
F-FAD2-cirspr2	5'-GATTGTGAATGGTGGCTTTGTGTT-3'	-	-	-	-
R-FAD2-Crispr2	5'-AAACAACACAAAGCCACCATTCAC-3'				

Table S2. Segregation of *Bar* and *Cas9* in T2 progenies derived from event ND1-11. Cas9 was detected by PCR while Bar was detected by both PCR and herbicide leaf painting**.**

T2 lines	Bar -PCR	Leaf painting	Cas9-PCR	T2 lines	Bar -PCR	Leaf painting	Cas9-PCR
ND1-11-1-1	+	R	+	ND1-11-2-1	-	S	-
ND1-11-1-2	+	R	+	ND1-11-2-2	+	R	+
ND1-11-1-3	+	R	+	ND1-11-2-3	+	R	+
ND1-11-1-4	+	R	+	ND1-11-2-4	-	S	-
ND1-11-1-5	+	R	+	ND1-11-2-5	+	R	+
ND1-11-1-6	+	R	+	ND1-11-2-6	+	R	+
ND1-11-1-7	+	R	+	ND1-11-2-7	+	R	+
ND1-11-1-8	+	R	+	ND1-11-2-8	+	R	+
ND1-11-1-9	+	R	+	ND1-11-2-9	+	R	+
ND1-11-1-10	+	R	+	ND1-11-2-10	+	R	+
ND1-11-1-11	+	R	+	ND1-11-2-11	+	R	+
ND1-11-1-12	+	R	+	ND1-11-2-12	+	R	+
ND1-11-1-13	+	R	+	ND1-11-2-13	+	R	+
ND1-11-1-14	+	R	+	ND1-11-2-14	+	R	+
ND1-11-1-15	+	R	+	ND1-11-2-15	-	S	-
ND1-11-1-16	+	R	+	ND1-11-2-16	-	S	-
ND1-11-14-1	-	S	-	ND1-11-2-17	+	R	+
ND1-11-14-2	-	S	-	ND1-11-2-18	+	R	+
ND1-11-14-3	-	S	-	ND1-11-2-19	-	S	-
ND1-11-14-4	-	S	-	ND1-11-2-20	+	R	+
ND1-11-14-5	-	S	-	ND1-11-10-1	+	R	+
ND1-11-14-6	-	S	-	ND1-11-10-2	+	R	+
ND1-11-14-7	-	S	-	ND1-11-10-3	+	R	+
ND1-11-14-8	-	S	-	ND1-11-10-4	-	S	-
ND1-11-14-9	-	S	-	ND1-11-10-5	+	R	+
ND1-11-14-10	-	S	-	ND1-11-10-6	+	R	+
ND1-11-14-11	-	S	-	ND1-11-10-7	-	S	-
ND1-11-14-12	-	S	-	ND1-11-10-8	-	S	-
ND1-11-14-13	-	S	-	ND1-11-10-9	+	R	+
ND1-11-14-14	-	S	-	ND1-11-10-10	-	S	-
ND1-11-14-15	-	S	-				
ND1-11-14-16	-	S	-				

+. Possitive; -. Negative; R. Resistance; S. Susceptible

Table S3. Potential off-target mutations in transgenic T2 plants derived from event ND1-11.

Target sites	Potential off-targets				No. of Bulks [b]	Identified mutant
	Sequences	MMs [a]	Gene locus	Region		
FAD2-Target 1	**T**TT**T**A**T**TGGTGGCTTTGTG**A**TTGG	4	Glyma.15G090200	exon	4	0
FAD2-Target 2	AG**C**GAAGGAA**G**ATCC**A**AGAAGGG	5	Glyma.18G034900	intron	4	0

[a] Mismatched bases are shown in bold letters; [b] Each bulk contained equal DNA from 8-10 T2 plants

Table S4. Protein and oil content in ND1-11-14 and wild type (Williams 82 and Maverick) seeds. Measurements were performed over two years under greenhouse (2017) and field (2018) conditions.

Environment (Year)	Seed samples	Protein Dry basis %	Oil Dry basis %	Linoleic acid Dry basis %	Oleic acid Dry basis %	Palmitic acid Dry basis %	Stearic acid Dry basis %
Greenhouse (2017)	ND1-11-1	37.8±1.44 ^{a}	22.8±0.09 ^{a}	7.49±0.51 ^{b}	75.1±0.78 ^{a}	7.93±0.44 ^{c}	3.70±0.04 ^{b}
	Maverick	32.8±1.45 ^{b}	22.9±0.13 ^{a}	61.4±0.93 ^{a}	10.0±1.37 ^{b}	11.1±0.27 ^{b}	4.11±0.33 ^{ab}
	Williams 82	40.5±0.77 ^{a}	19.9±0.57 ^{b}	61.1±1.49 ^{a}	7.79±1.95 ^{b}	11.9±0.26 ^{a}	4.32±0.12 ^{a}
Field (2018)	ND1-11-1-1	42.5±0.64 ^{a}	21.3±0.20 ^{a}	8.16±1.30 ^{c}	71.9±1.67 ^{a}	7.71±0.43 ^{b}	3.95±0.19 ^{b}
	Maverick	42.5±1.35 ^{a}	20.9±0.71 ^{a}	43.3±1.58 ^{b}	29.3±2.31 ^{b}	10.8±0.18 ^{a}	4.46±0.09 ^{a}
	Williams 82	42.3±0.74 ^{a}	20.9±0.21 ^{a}	55.7±4.99 ^{a}	15.6±6.46 ^{c}	10.8±0.19 ^{a}	4.18±0.20 ^{ab}

Shown are means ± SD for n= 3. Different letters denote significant differences at $p<0.05$ using one-way ANOVA followed by a post-hoc Turkey's multiple range test.