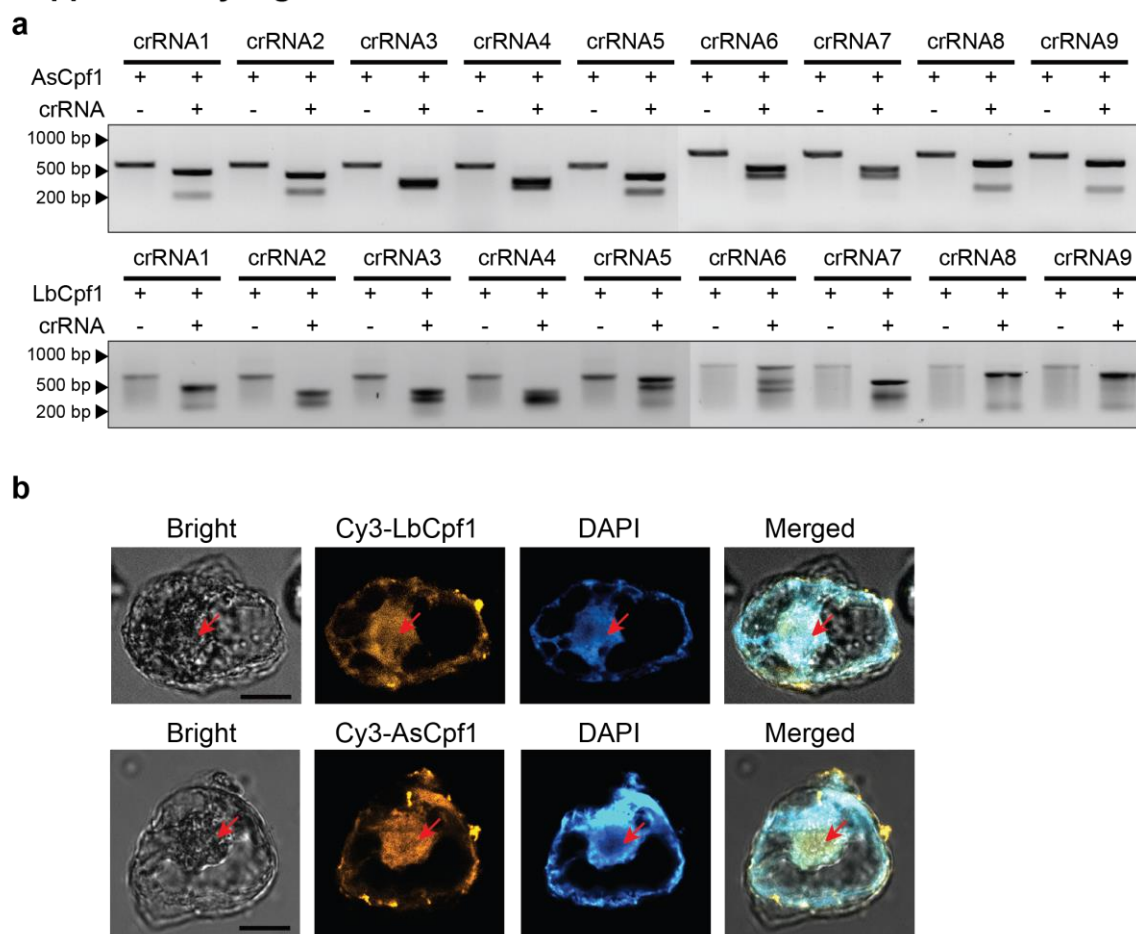


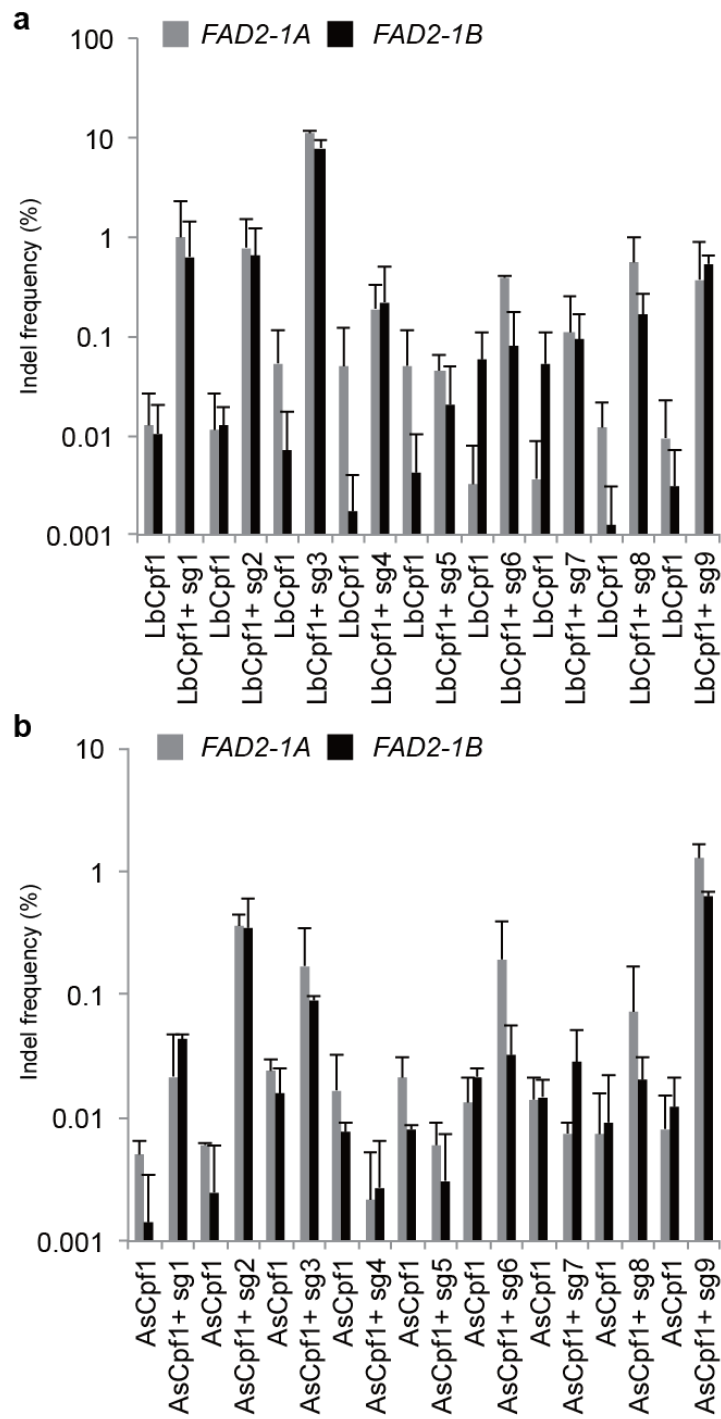
## Supplementary Information

### Supplementary Figure 1



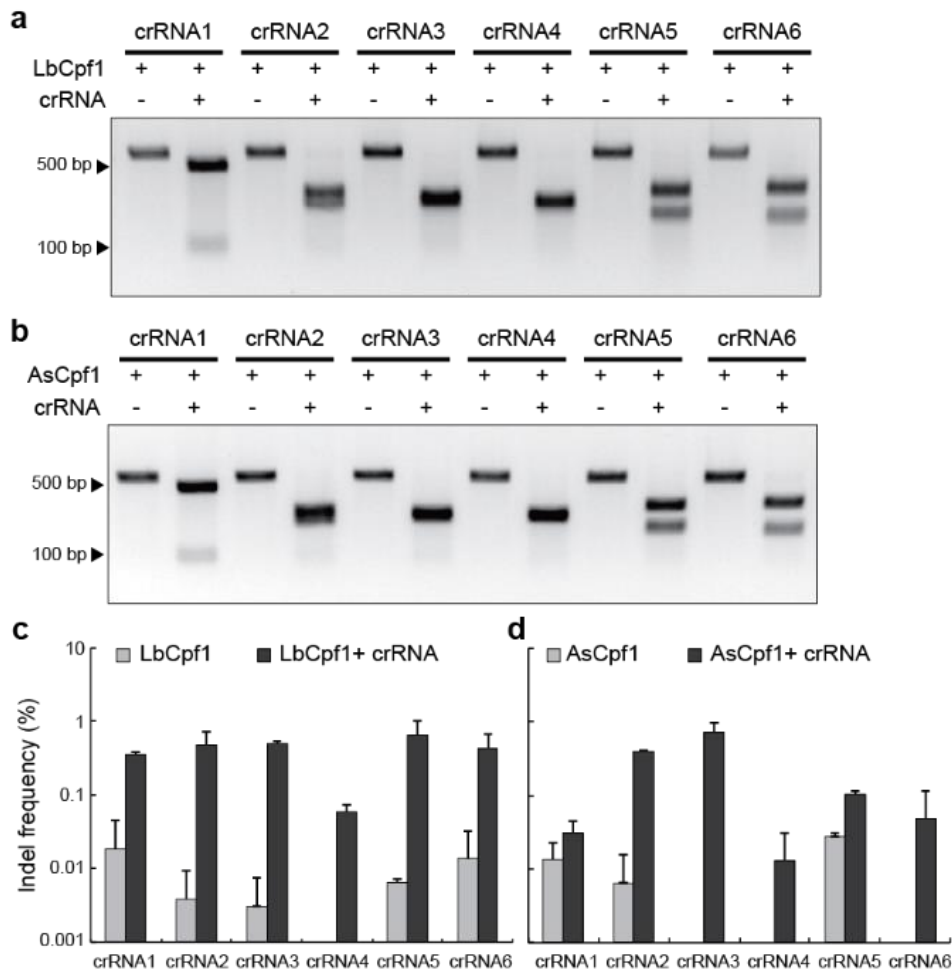
**Supplementary Figure 1.** *In vitro* cleavage assay for 9 crRNA-LbCpf1 complexes and localization of Cpf1 proteins in soybean protoplasts. (a) Preassembled AsCpf1-crRNA and LbCpf1-crRNA complexes cleave their target DNA *in vitro*. (b) Both Cy3-conjugated Cpf1 proteins were targeted to the nucleus of soybean protoplasts. The red arrow indicates the nucleus. DAPI, a nuclear marker. Bars = 10  $\mu$ m.

**Supplementary Figure 2**



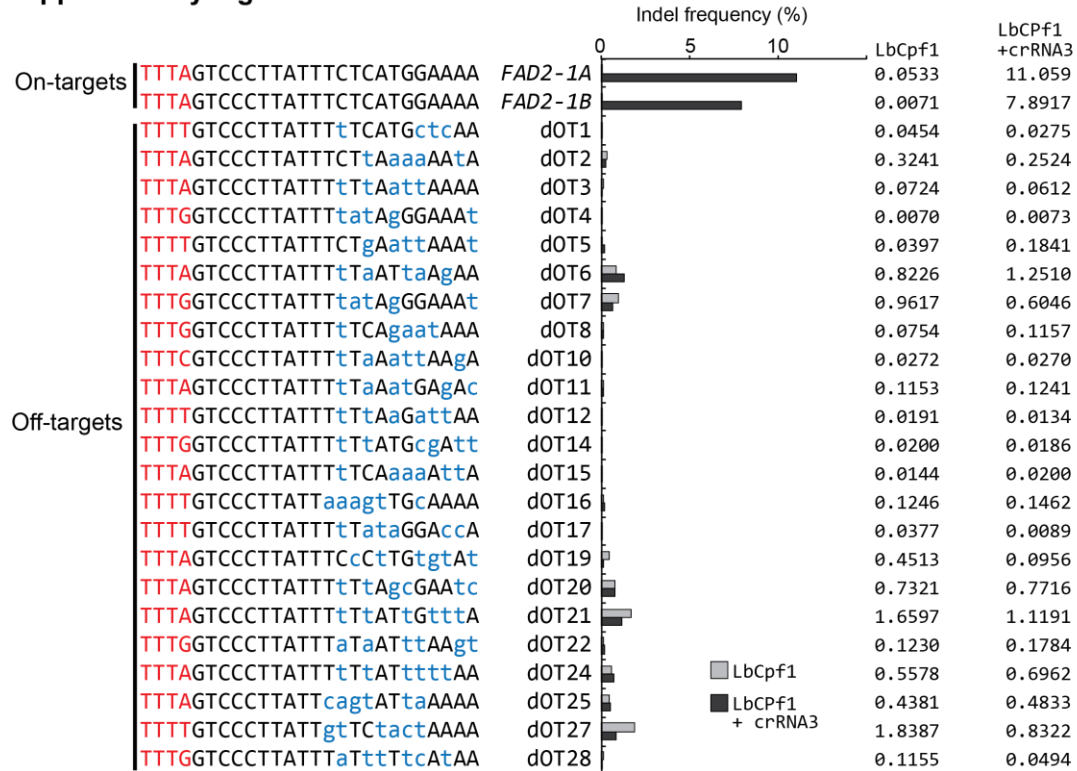
**Supplementary Figure 2.** Indel frequencies (% , Log<sub>10</sub> scale at Y-axis) for 9 crRNAs at target sites in *FAD2-1A* and *FAD2-1B*. Error bars represent standard deviation (n = 2).

### Supplementary Figure 3



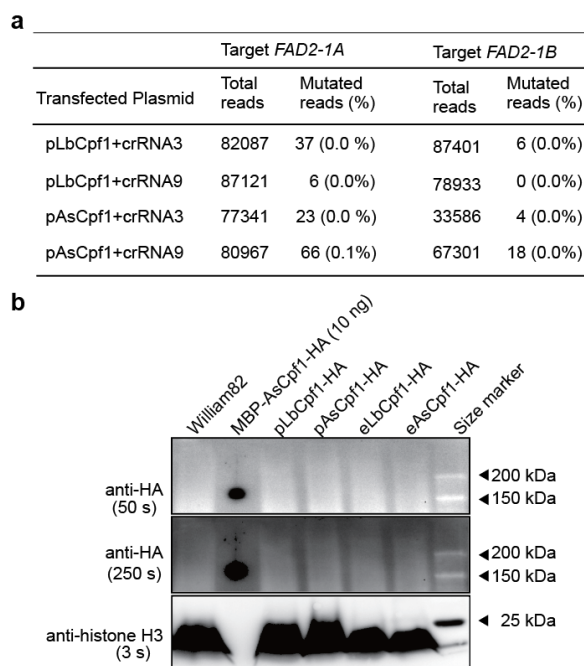
**Supplementary Figure 3.** CRISPR/Cpf1-mediated editing of the *AOC* gene in *Nicotiana attenuata* protoplasts. *In vitro* cleavage assay for 6 crRNAs complexed with (a) LbCpf1 and (b) AsCpf1 proteins. Indel frequencies (%; Log<sub>10</sub> scale at Y-axis) at the target sites in protoplasts for 6 crRNAs complexed with (c) LbCpf1 and (d) AsCpf1 proteins. *AOC*, *ALLEN OXIDE CYCLASE*. Error bars represent standard deviation (n = 2).

### Supplementary Figure 4



**Supplementary Figure 4.** *In vivo* evaluation of LbCpf1-crRNA3 activity at potential off-target sites in the genome. The indel frequencies (%) at 23 candidate distal off-target (dOT) sites (with up to 6 bp mismatches relative to the crRNAs) were measured and validated in LbCpf1-crRNA3-delivered soybean protoplasts by targeted deep sequencing. No mutations were detected at any of the 23 candidate loci. Red, PAM sequences; blue, mismatched nucleotide bases. Note, dOT21 and dOT27 have relatively high frequency of indel in some control samples, which we have frequently seen in AT-rich and A- or T-repeat regions<sup>12</sup>

### Supplementary Figure 5



**Supplementary Figure 5.** Editing activity of plasmid-expressed Cpf1 and Cpf1 expression in soybean protoplasts. (a) Indel frequencies (%) at two *FAD2* loci after transformation with the plasmid harboring plant-codon optimized LbCpf1-HA or AsCpf1-HA. (b) Protein expression from a plant codon (pLbCpf1/pAsCpf1) or *E. coli* codon (eLbCpf1/eAsCpf1) optimized Cpf1 plasmid in soybean protoplasts. The anti-HA antibody detects the Cpf1 protein and the anti-histone H3 antibody detects an endogenous soybean protein.

**Supplementary Table 1.** List of crRNAs used in this study.

Target gene	Name	<sup>a</sup> Sequence
<i>Glycin max</i> <i>FAD2-1A/B</i>	crRNA1	TTTCACATTGCCACCACCTACTTCC
	crRNA2	TTTCCTCATTGCATGGCCAATCTAT
	crRNA3	TTTAGTCCCTTATTTCTCATGGAAAA
	crRNA4	TTTCATGGAAAATAAGCCATCGCC
	crRNA5	TTTGTCCTCCAAAACAAAATCCAAAGT
	crRNA6	TTTGCTGCTATGTGTTTATGGGGTG
	crRNA7	TTTGGCAACTATGGACAGAGATTATG
	crRNA8	TTTGATGACACACCATTTTACAAGGC
	crRNA9	TTTACAAGGCACTGTGGAGAGAAGC
<i>Nicotiana attenuata</i> <i>AOC</i>	crRNA1	TTTGTTGCTAAAGGGGACAAGATCTCC
	crRNA2	TTTGATCAGAATGCAGAGTCCAGCCGT
	crRNA3	TTTCCTCTTCGTGTTTGATCAGAATGC
	crRNA4	TTTCTTTTCCTCTTCGTGTTTGATCAG
	crRNA5	TTTACAGCTTCTATTTTCGGCGATTATG
	crRNA6	TTTCGGCGATTATGGTCACATCGCCGT

<sup>a</sup>Red indicates the PAM sequence.

**Supplementary Table 2.** List of putative off-target sequences of FAD2-targeted crRNA3 in the soybean genome.

<sup>a</sup> Off-target sequence	Chr.	Position	Direction	Mismatches	Name
TTTGGTCaCTaATTTCTCATGGATcA	chr5	7021658	+	4	<sup>b</sup> OT1
TTTTGTCTCTTTTTTTTCATcGAAAA	chr6	14339586	+	4	OT2
TTTGGaCCCTTATTTacCATGGAcAA	chr7	9126069	-	4	OT3
TTTTGCaCTcATTTTCATGGAAAA	chr7	29877437	-	4	OT4
TTTTGTttCTTATTTCTCATtGAAAg	chr8	6892768	-	4	OT5
TTTCGTCCgTTATTTtATGAAAA	chr8	20703738	-	4	OT6
TTTTGTCCCTTTTTTTTCATttAAAA	chr9	18027312	+	4	OT7
TTTTGTCaCaaATTTTCATGGAAAA	chr10	2521140	+	4	OT8
TTTAGTCTcTTATTTCTaATaaAAAA	chr10	48025754	+	4	OT9
TTTGGTCCtTTATTTCCcATtGAtAA	chr11	28094966	+	4	OT10
TTTctTCCCTTTTTTCTCATaaAAAA	chr12	20572847	-	4	OT11
TTTTGTCCCTTATTTTCATGctcAA	chr17	2674940	+	4	OT12
TTTGGTCaCTaATTTCTCATGGATcA	chr17	12553219	+	4	OT13
TTTTGTCCCTTATTTTCATGctcAA	chr17	2674940	+	4	<sup>c</sup> dOT1
TTTAGTCCCTTATTTCTtAaaaAAAtA	chr1	1189388	+	5	dOT2
TTTAGTCCCTTATTTtTAattAAAA	chr9	3561633	-	5	dOT3
TTTGGTCCCTTATTTtatAgGGAAAt	chr11	15389742	+	5	dOT4
TTTTGTCCCTTATTTCTgAattAAAt	chr15	43359905	-	5	dOT5
TTTAGTCCCTTATTTtAAttaAgAA	chr17	11701652	-	5	dOT6
TTTGGTCCCTTATTTtatAgGGAAAt	chr18	4987048	+	5	dOT7
TTTGGTCCCTTATTTtTCagaatAAA	chr18	11727316	+	5	dOT8
TTTTGTCCCTTATTTCTgAattAAAt	chr18	44803139	-	5	dOT9
TTTCGTCCCTTATTTtAaattAAGa	chr2	39113938	+	6	dOT10
TTTAGTCCCTTATTTtAaAtGAGAc	chr3	43408144	+	6	dOT11
TTTTGTCCCTTATTTtTtAaGattAA	chr3	43679365	+	6	dOT12
TTTAGTCCCTTATTTttagcGGAttA	chr4	8732797	+	6	dOT13
TTTGGTCCCTTATTTtTtATGcgAtt	chr5	37988161	+	6	dOT14
TTTAGTCCCTTATTTtTCAaaaAttA	chr6	45888996	+	6	dOT15
TTTTGTCCCTTATTaaagtTGcAAAA	chr7	17897295	+	6	dOT16
TTTTGTCCCTTATTTtTataGGAccA	chr11	25098470	-	6	dOT17
TTTAGTCCCTTATTTtaaAaaaaAAAA	chr13	40317930	-	6	dOT18
TTTAGTCCCTTATTTcCtTgtgtAt	chr13	44326839	-	6	dOT19
TTTAGTCCCTTATTTtTtAgcGAAtc	chr14	45961325	-	6	dOT20
TTTAGTCCCTTATTTtTtATtGtttA	chr15	9547205	-	6	dOT21
TTTGGTCCCTTATTTtAaAtttAAgt	chr16	7599366	-	6	dOT22
TTTAGTCCCTTATTTtaaATatAAAc	chr16	29724887	+	6	dOT23
TTTAGTCCCTTATTTtTtATttttAA	chr17	38497391	+	6	dOT24
TTTAGTCCCTTATTcagtATtaAAAA	chr18	5202244	+	6	dOT25
TTTTGTCCCTTATTTcAtATtAttA	chr19	2704492	-	6	dOT26
TTTTGTCCCTTATTgtTctactAAAA	chr19	45846421	+	6	dOT27
TTTGGTCCCTTATTTaTttTtcAtAA	chr19	49944362	-	6	dOT28
TTTTGTCCCTTATTTtaaATtGgAgA	chr20	26326125	+	6	dOT29

<sup>a</sup>Red indicates the mismatch sequence.

<sup>b</sup>OT indicates off-target sites with up to 4 nucleotide mismatches.

<sup>c</sup>dOT indicates off-target sites with up to 6 nucleotide mismatches in the distal region.

**Supplementary Table 3. List of primers used for targeted deep sequencing.**

Name	Sequence	Name	Sequence
FAD2-1A nested F	ATTGATAGCCCTCCGTTC	FAD2-1A nested R	AGTCATTACGCGGCAATCC
FAD2-1A F1 crRNA1,2	CACCATTCACTGTTGGCCAA	FAD2-1A R1 crRNA1,2	CCCACAACATCATCAACCCA
FAD2-1A F2 crRNA3-5	CTGGTGTGGGTGATTGC	FAD2-1A R2 crRNA3-5	ACGAGAAGAGAAACAGCCCT
FAD2-1A F3 crRNA6,7	ACTCTCTACCGTGTGCA	FAD2-1A R3 crRNA6,7	TGTAGAGAAGAGATGGTGAAGCC
FAD2-1A F4 crRNA8,9	CAACCAATGCAATCAAGCCA	FAD2-1A R4 crRNA8,9	AGTCATTACGCGGCAATCC
FAD2-1B nested F	GGCACCTACAATATCAGCACT	FAD2-1B nested R	TGACAAAACCTCCATAACTCCCA
FAD2-1B F1 crRNA1,2	CACCATTCACTGTTGGCCAA	FAD2-1B R1 crRNA1,2	CCCATGGGTACTGTGTA
FAD2-1B F2 crRNA3-5	CTTTCAGCAAGTACCAATGG	FAD2-1B R2 crRNA3-5	CTGCCAGAGACATTGAAGGC
FAD2-1B F3 crRNA6,7	TTGCTCTACCGTGTGCAAC	FAD2-1B R3 crRNA6,7	GCCTCCGTGTGATGGTAAT
FAD2-1B F4 crRNA8,9	ATAACTGATACTCATGTGGCTCA	FAD2-1B R4 crRNA8,9	TGACAAAACCTCCATAACTCCCA
FAD2-1A 2nd F1	ACACTCTTCCCTACACGACGCTCTCCGATCTCACCATTCACTGTTGGCCAA		
FAD2-1A 2nd R1	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTCCACAACATCATCAACCCA		
FAD2-1A 2nd F2	ACACTCTTCCCTACACGACGCTCTCCGATCTCTGGTGTGGGTGATTGC		
FAD2-1A 2nd R2	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTACGAGAAGAGAAACAGCCCT		
FAD2-1A 2nd F3	ACACTCTTCCCTACACGACGCTCTCCGATCTACTCTCTACTCCGTGTGCA		
FAD2-1A 2nd R3	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTGTAGAGAAGAGATGGTGAAGCC		
FAD2-1A 2nd F4	ACACTCTTCCCTACACGACGCTCTCCGATCTCAACCAATGCAATCAAGCCA		
FAD2-1A 2nd R4	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTAGTCAATACGCGGCAAAATCC		
FAD2-1B 2nd F1	ACACTCTTCCCTACACGACGCTCTCCGATCTCACCATTCACTGTTGGCCAA		
FAD2-1B 2nd R1	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTCCCAATGGGTACTTGTGAA		
FAD2-1B 2nd F2	ACACTCTTCCCTACACGACGCTCTCCGATCTCCTTCAGCAAGTACCAATGG		
FAD2-1B 2nd R2	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTCCGATCTGCGAGAGACATTGAAGGC		
FAD2-1B 2nd F3	ACACTCTTCCCTACACGACGCTCTCCGATCTTGTGCTTACCGTGTGCAAC		
FAD2-1B 2nd R3	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTGCTCCGTTGATGGTAAT		
FAD2-1B 2nd F4	ACACTCTTCCCTACACGACGCTCTCCGATCTATAAATGATACTCATGTGGCTCA		
FAD2-1B 2nd R4	GTGACTGGAGTTCAGACGTGTGCTCTCCGATCTTGACAAAACCTCCATAACTCCCA		
crRNA3 OT4MM-1 F	CCCAAACTTTTATGCTCGAAATTT	crRNA3 OT4MM-1 R	AAAACGTTCAATACACAACATTTAGGT
crRNA3 OT4MM-2 F	GCACGATGCGGAAAGATT	crRNA3 OT4MM-2 R	TTGACCTCCATTGTGTGA
crRNA3 OT4MM-3 F	CCAACCTCAATATGGGTGCTG	crRNA3 OT4MM-3 R	CGCATTATGCTCATGTGACAA
crRNA3 OT4MM-4 F	ACACATGCGGATCCACAATTTTT	crRNA3 OT4MM-4 R	ATTGACATGCATGTATATATGTGTG
crRNA3 OT4MM-5 F	CAACAGCGATGTTAAAATGTACAGATAG	crRNA3 OT4MM-5 R	CATGAGATTCAAAAATGCACCGT
crRNA3 OT4MM-6 F	AAATATGTAATAATGCTGACAAATTCATCTC	crRNA3 OT4MM-6 R	GGACATCTTCTCAGCATAGC
crRNA3 OT4MM-7 F	TGACCATTTGGAGTGTGACACA	crRNA3 OT4MM-7 R	GACGAAAGACAGCGATTGGT
crRNA3 OT4MM-8 F	AGGAAATTTAATCAGCGGACCTTTTA	crRNA3 OT4MM-8 R	TCAAAAATTTGGACGAAAAATCCA
crRNA3 OT4MM-9 F	TAAGGATAGATTGAAAGTATTAGTCA	crRNA3 OT4MM-9 R	GCAATTAGCATCGCATCAAAAAT
crRNA3 OT4MM-10 F	GCGGCTTTCATCTCTTTGAG	crRNA3 OT4MM-10 R	GCTGTATTAATAATTAAGCATTCCC
crRNA3 OT4MM-11 F	CAAAGTATGGCTTATAATGGAGAAC	crRNA3 OT4MM-11 R	GTTCCTTCAGTGGCACAA
crRNA3 OT4MM-12 F	GCGGTACGGTTCAGATAGCA	crRNA3 OT4MM-12 R	TCTACCGTAGAACTACACAGGTG
crRNA3 OT4MM-13 F	GGGCGCATTGTAAGACTAAA	crRNA3 OT4MM-13 R	TTTGGTATCTTTTTCGATTTCTATCAG
LbCpf1crRNA	GAAATTAATACGACTCACTATAGGG AATTTCTACTAAGTGTAGAT		
LbCpf1 FAD2 sg1	GGAAGTAGGTGGGCAATGTAATCTACACTTAGTAGAAATT	LbCpf1 FAD2 sg2	ATAGATTGCCATGCAATGAGGATCTACACTTAGTAGAAATT
LbCpf1 FAD2 sg3	TTTTCCATGAGAAAATAAGGACATCTACACTTAGTAGAAATT	LbCpf1 FAD2 sg4	GGCGATGGCTTATTTCCATGAATCTACACTTAGTAGAAATT
LbCpf1 FAD2 sg5	ACTTTGGATTTGGTTTTGGGAATCTACACTTAGTAGAAATT	LbCpf1 FAD2 sg6	CACCCATAAACACATAGCAGCATCTACACTTAGTAGAAATT
LbCpf1 FAD2 sg7	CATAATCTCTGTCCATAGTGTGATCTACACTTAGTAGAAATT	LbCpf1 FAD2 sg8	GCCTTGTAATAAGTGTGTGATATCTACACTTAGTAGAAATT
LbCpf1 FAD2 sg9	GCTTCTCTCCACAGTGCCTTGATCTACACTTAGTAGAAATT		
AsCpf1crRNA	GAAATTAATACGACTCACTATAGGG TAATTTCTACTCTTGTAGAT		
AsCpf1 FAD2 sg1	GGAAGTAGGTGGGCAATGTAATCTACAAGAGTAGAAATTA	AsCpf1 FAD2 sg2	ATAGATTGCCATGCAATGAGGATCTACAAGAGTAGAAATTA
AsCpf1 FAD2 sg3	TTTTCCATGAGAAAATAAGGACATCTACAAGAGTAGAAATTA	AsCpf1 FAD2 sg4	GGCGATGGCTTATTTCCATGAATCTACAAGAGTAGAAATTA
AsCpf1 FAD2 sg5	ACTTTGGATTTGGTTTTGGGAATCTACAAGAGTAGAAATTA	AsCpf1 FAD2 sg6	CACCCATAAACACATAGCAGCATCTACAAGAGTAGAAATTA
AsCpf1 FAD2 sg7	CATAATCTCTGTCCATAGTGTGATCTACAAGAGTAGAAATTA	AsCpf1 FAD2 sg8	GCCTTGTAATAAGTGTGTGATATCTACAAGAGTAGAAATTA
AsCpf1 FAD2 sg9	GCTTCTCTCCACAGTGCCTTGATCTACAAGAGTAGAAATTA		



Supplementary Note 1. Sequence information of Cpf1-harboring vectors.

p2GW7-eAsCpf1-HA, *E. coli*-codon optimized AsCpf1.

Amp-R35s promoter 5' NLS eAsCpf1 3' NLS HA Stop codon 35s terminator

```
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p2GW7-eLbCpf1-HA, *E. coli*-codon optimized LbCpf1.

Amp-R35s promoter 5' NLS eLbCpf1 3' NLS HA Stop codon 35s terminator

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p2GW7-AsCpf1-HA, plant-codon-optimized AsCpf1.

Amp-R35s promoter<sup>5'</sup> NLS pAsCpf1<sup>3'</sup> NLS HA Stop codon<sup>35s</sup> terminator

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p2GW7-LbCpf1-HA, plant-codon-optimized LbCpf1.

Amp-R35s promoter 5' NLS pLbCpf1 3' NLS HA Stop codon 35s terminator

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