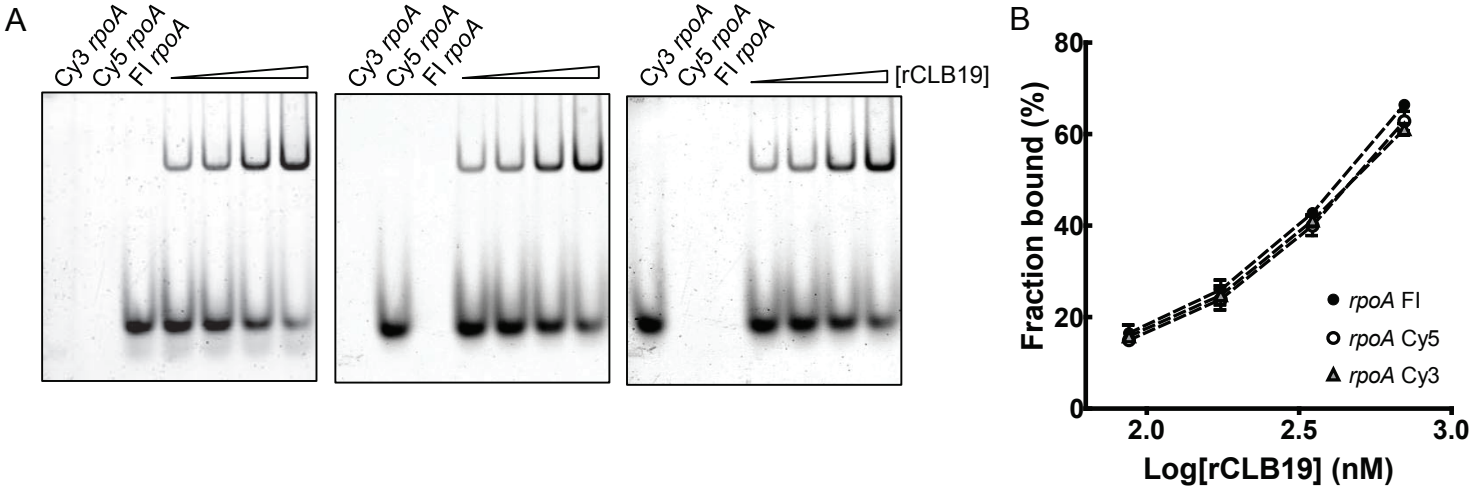
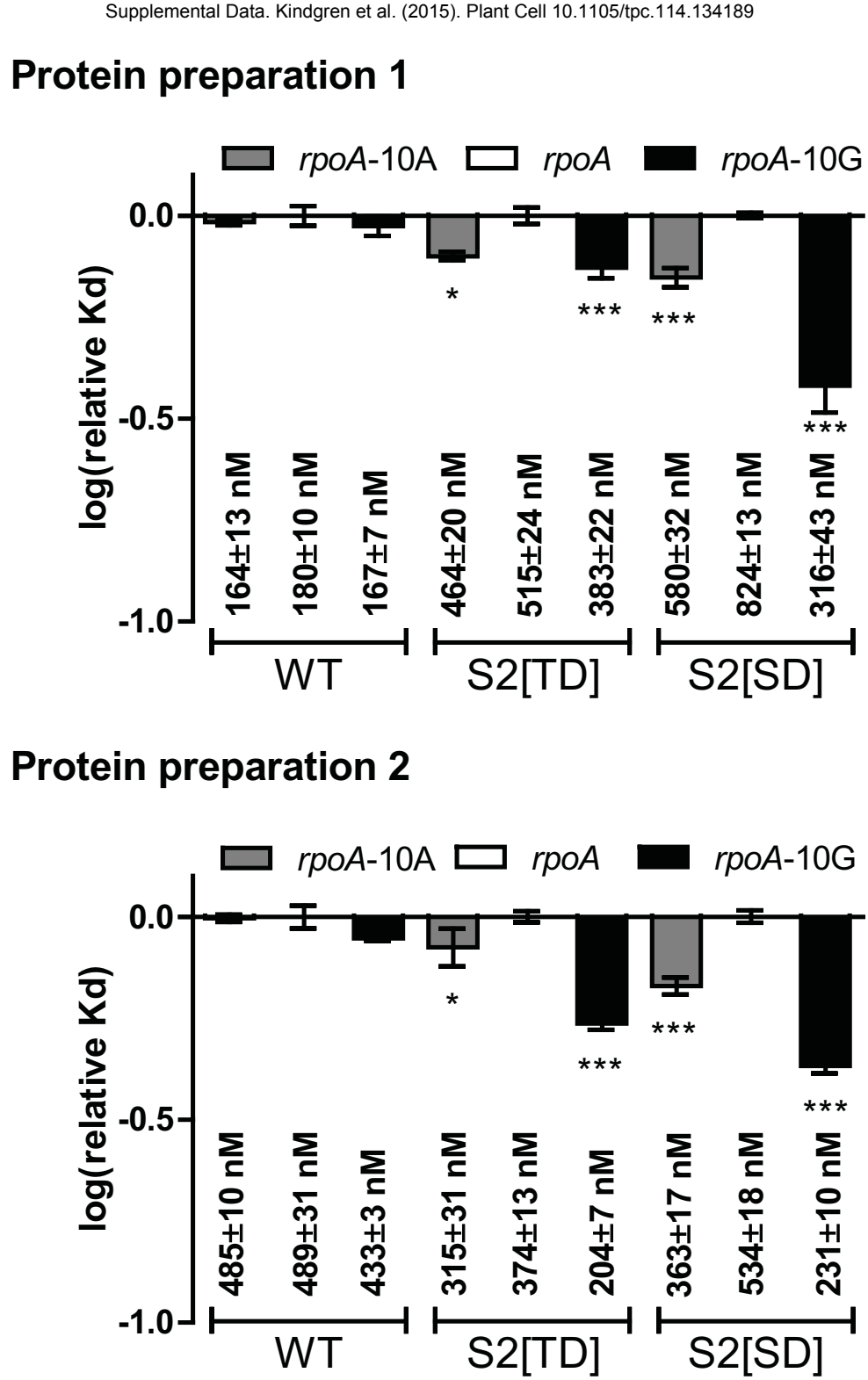
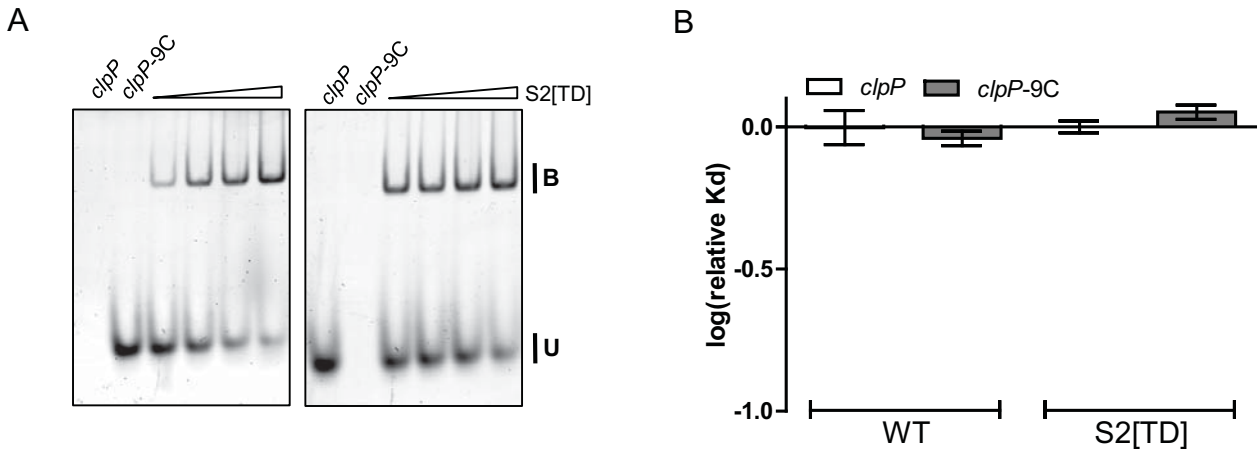


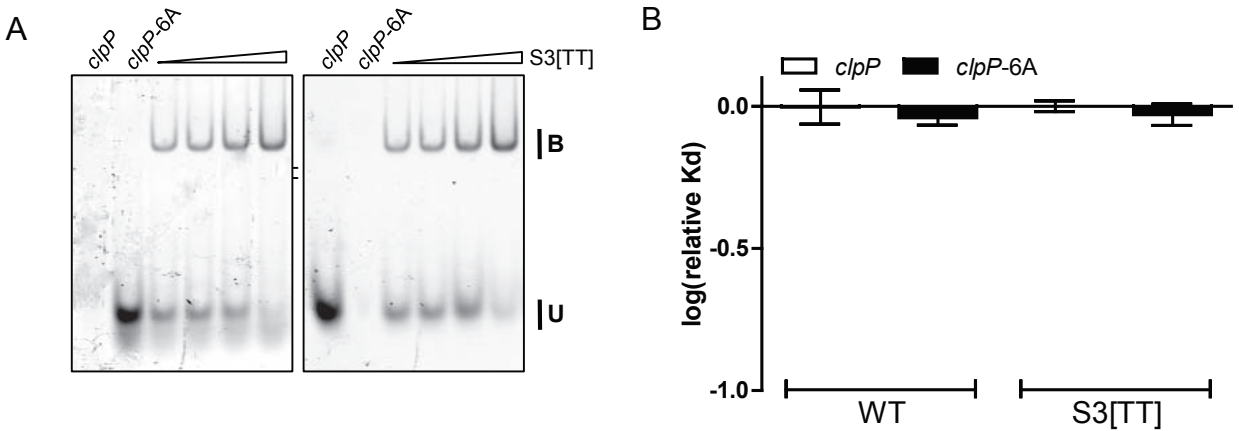
Supplemental Figure 1. CLB19 shows no label preference.
(A) RNA electrophoretic mobility shift assay of different concentrations of CLB19-MBP (75, 150, 300 and 600 nM) incubated with 700 pM each of oligonucleotides labelled with fluorescein, Cy3 or Cy5. The three panels show the same gel visualised through filters specific for each of the labels.
(B) Binding curve for the different oligonucleotides indicated in (A). The graphs show mean value ±SD (n=3).



Supplemental Figure 2. Relative Kd, but not absolute Kd, is reproducible between protein preparations. Log2(relative Kd) normalised to the Kd of *rpoA* for each variant of CLB19 ±SD (n=3). The Kd for each oligo is indicated below the respective bar. Two independent protein preparations of CLB19 and the variants S2[TD] and S2[SD] were prepared and incubated with indicated oligos. Significant preferences within each graph (One-way ANOVA, Tukey's comparison test) are indicated by asterisks: $p<0.05$ (*), $p<0.001$ (***).

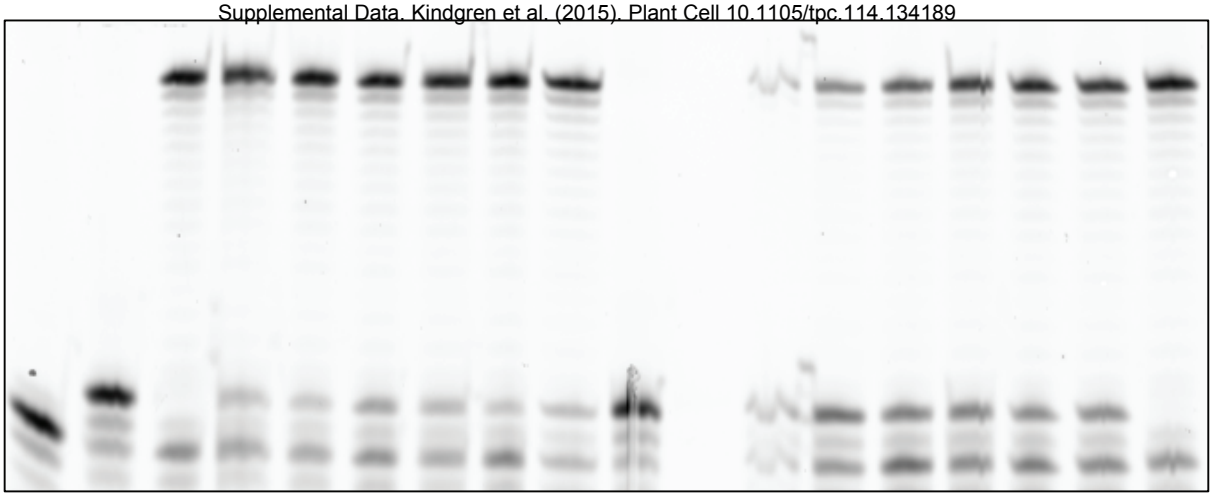


Supplemental Figure 3. Alignment control for S2[TD].
(A) RNA electromobility shift assay of different concentrations of CLB19 and S2[TD] (87.5, 175, 350 and 700 nM) that were incubated with 750 pM each of a fluorescein- and a Cy5-labelled oligo. The changes in the oligos are in a position that is not predicted to align with S2. Each reaction was done at least 3 times with the same expressed protein.
(B) Log(relative Kd) normalised to the Kd of *clpP* for CLB19 and the variant S2[TD] ±SD (n=3). No significant differences were found.

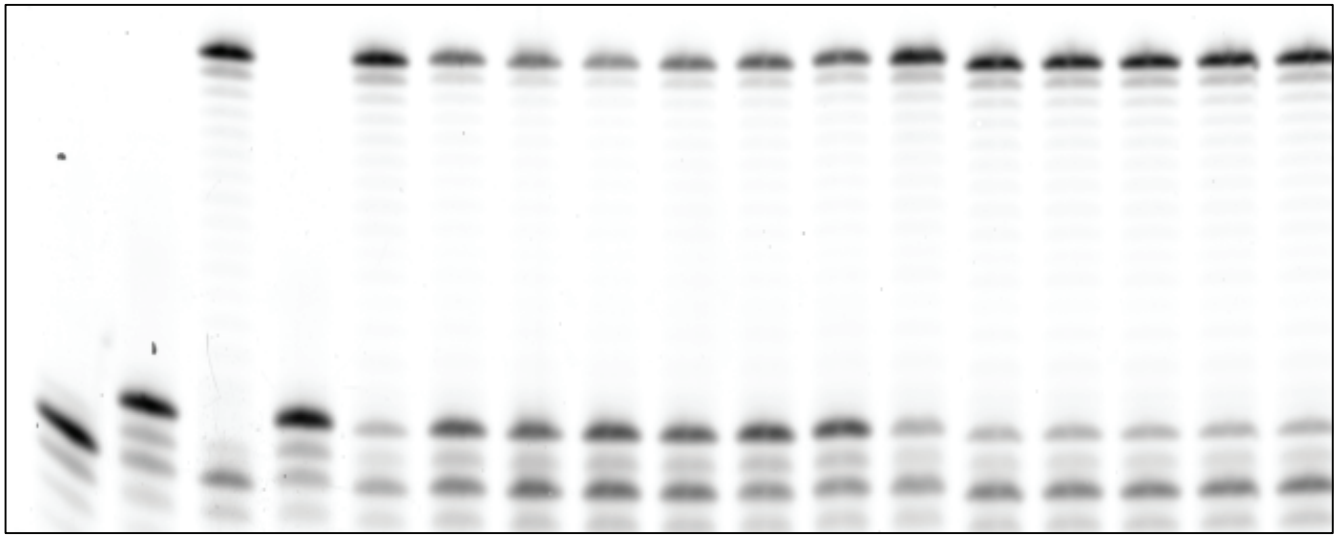


Supplemental Figure 4. Alignment control for S3[TT].
(A) RNA electrophoretic mobility shift assay of different concentrations of CLB19 and S3[TT] and (87.5, 175, 350 and 700 nM) that were incubated with 750 pM each of fluorescein-labeled *clpP* and Cy5-labeled *clpP*-6A. The -6A position is not predicted to align with S3. Each reaction was repeated at least 3 times with the same expressed protein.
(B) Log(relative Kd) normalised to the Kd of *clpP* for CLB19 and the variant S3[TT] ±SD (n=3). No significant differences were found.

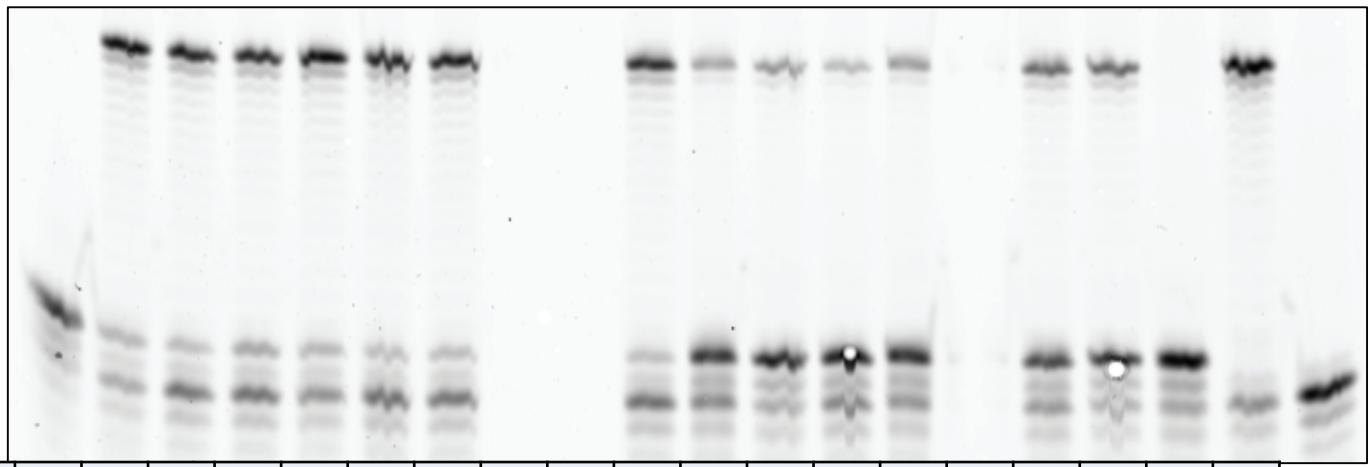
clpP



%E	0	100	88	94	84	89	94	91	0			36	59	67	76	72	100
	PU	PE	CLB19wt					WT	c19			S2[SD]					PE



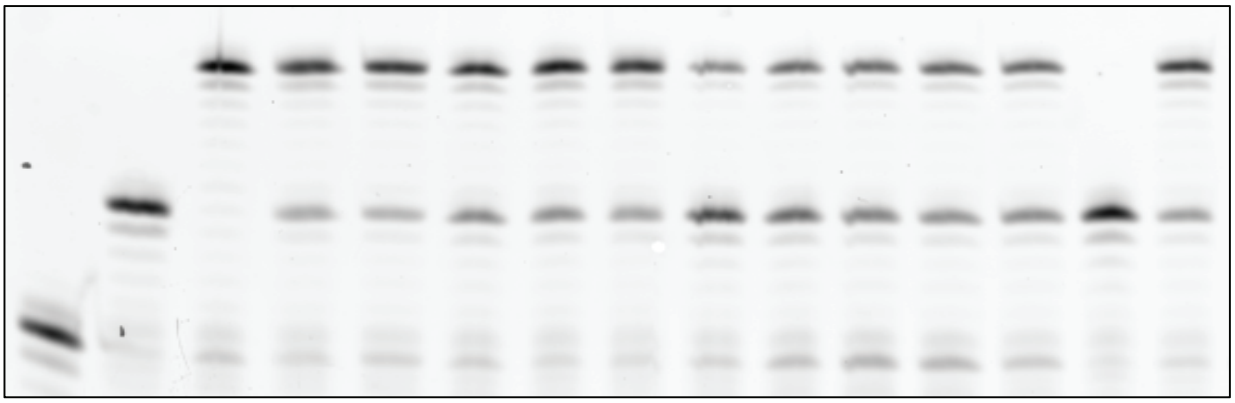
%E	0	100	0	89	43	42	28	41	42	45	86	90	86	87	87	87
	PU	PE	c19	WT	S3[TT]						S2[TN]					



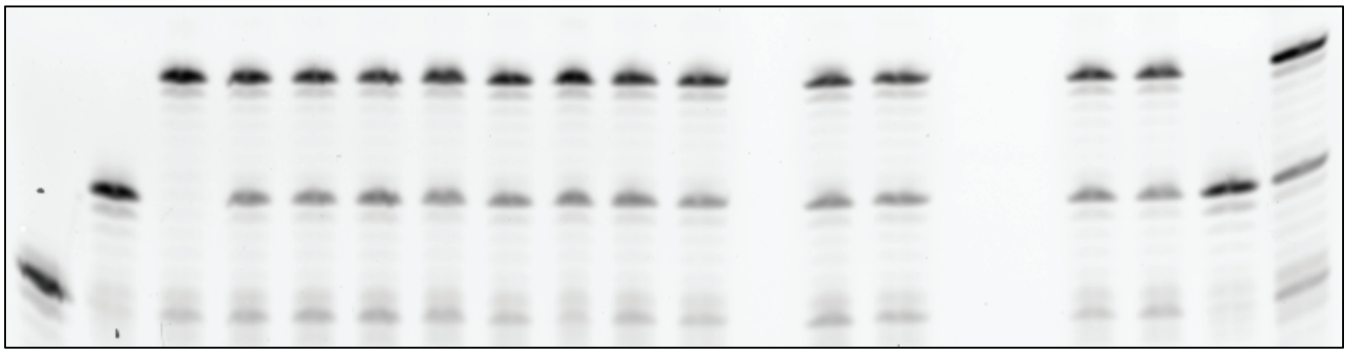
%E	0	88	90	82	90	91	91			90	14	18	7	15		35	35	0	100
	c19	WT	S2[TD]								S3[TD]							PU	PE

Supplemental Figure 5. *clpP* editing induced by variants of CLB19.
Poisoned primer extension analysis of *clpP* in the different CLB19 genotypes. RNA was extracted from 3-week old plants and converted to cDNA. Each reaction comes from a biological replicate. PU and PE indicate an unedited and edited control, respectively. WT indicates Colombia wild-type, c19 indicates *clb19* and CLB19wt indicates plants transformed with wild-type *CLB19* driven by the same promoter as used to drive expression of the variants. % editing was calculated by measuring the intensity of the edited band.

rpoA



%E	0	100	81	84	72	77	80	27	46	54	63	59	0	76
	PU	PE	CLB19wt					S2[SD]					c19	Col

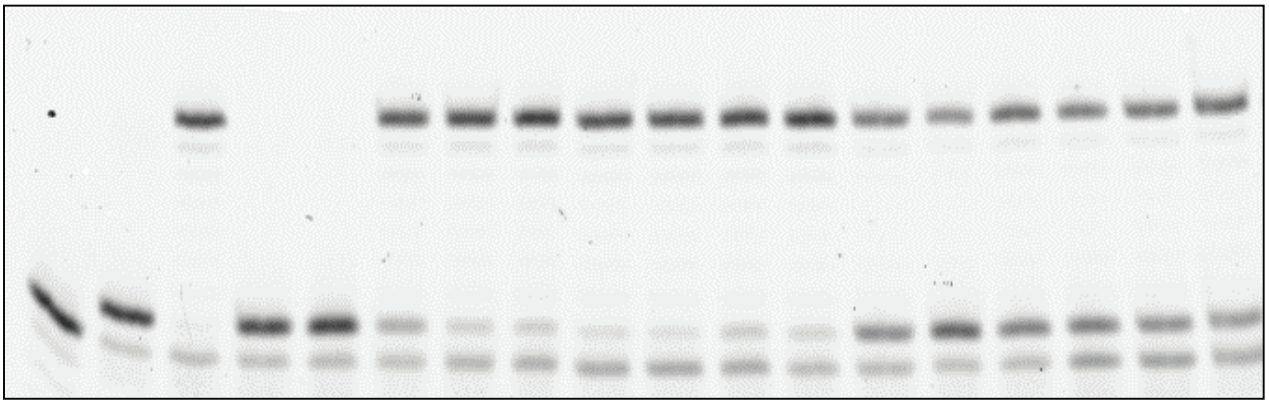


%E	0	100	76	73	65	75	75	75	71	77		71	72			71	74	0	75
	PU	PE	S3[TT]						S2[TN]									c19	Col

%E	0	100	1	76	68	62	72	73	70	70	76	79	67	77	84	85
	PU	PE	c19	WT	S2[TD]						S3[TD]					

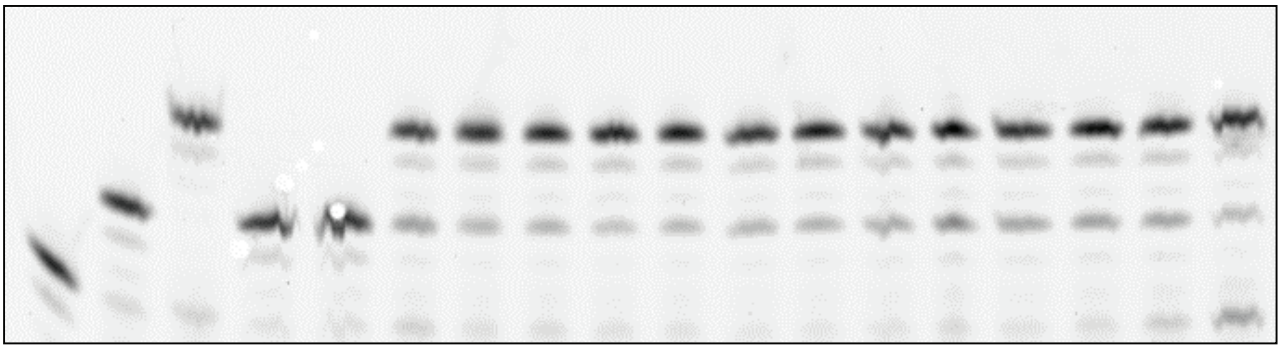
Supplemental Figure 6. *rpoA* editing induced by variants of CLB19.
Poisoned primer extension analysis of *rpoA* in the different CLB19 genotypes. RNA was extracted from 3-week old plants and converted to cDNA. Each reaction contains cDNA from a biological replicate. PU and PE indicate an unedited and edited control, respectively. WT indicates Colombia wild-type, c19 indicates *clb19* and CLB19wt indicates plants transformed with wild-type *CLB19* driven by the same promoter as used to drive expression of the variants. % editing was calculated by measuring the intensity of the edited band.

A *ndhG*



%e	0	100	1	2	72	87	87	92	94	86	87	51	39	53	47	55	61
	PU	PE	*otp82*		Col	OTP82wt						S3[TD]					

B *ndhB* 95644



%e	0	100	0	0	69	78	79	84	82	77	78	73	69	72	74	73	82
	PU	PE	*otp82*		Col	OTP82wt						S3[TD]					

Supplemental Figure 7. Editing induced by variants of OTP82.
Poisoned primer extension analysis of (A) *ndhG* and (B) *ndhB* of the different OTP82 genotypes. RNA was extracted from 3-week old plants and converted to cDNA. Each reaction comes from a biological replicate. PU and PE indicate an unedited and edited control, respectively. Col indicates Colombia wild-type and OTP82wt indicates *otp82* plants transformed with a construct that includes a wild-type copy of OTP82 driven by the same promoter as used to drive expression of the S3[TD] variant. % editing was calculated by measuring the intensity of the edited band.

Supplemental Table 1.

Primers used in this study (mutated base indicated by lower case letters)

Primer name	Sequence	Comment
CLB19 in vivo F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGATGGGTCTCCTTCCCGTCGT	
CLB19 in vivo R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCCACCTCCGGATCAAGCATTGAGGAGATCAC	
CLB19 expr. F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGAGAATCTTTATTTTCAGGGCGCCAATCCAAAGATACAGAGA	
CLB19 expr. R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGCATTGAGGAGATCACC	
CLB19mutP1[T;D] F	GTAGAGCCTgACCACATCACTTTC	T;N to T;D mutation of P1
CLB19mutP1[T;D] R	GAAAGTGATGTGGTcAGGCTCTAC	
CLB19mutP1[N;N] F	CTGTTTCATGGAaTTCTCGCATC	T;N to N;N mutation of P1
CLB19mutP1[N;N] R	GATGCGAGAAtTCCATGAAACAG	
CLB19mutS1[N;N] F	TGTCATGGTTGGCAaCGCAATTAT	T;N to N;N mutation of S1
CLB19mutS1[N;N] R	CGATAATTGCGtTGCCAACCATGA	
CLB19mutS1[T;D] F	GAAGATAAAgATTCGGTTACTTGG	T;N to T;D mutation of S1
CLB19mutS1[T;D] R	AGTAACCGAATcTTTATCTTCCAT	
CLB19mutS2[T;D] F	CGGTTACTTGGAcTACAATGATCGA	N;D to T;D mutation of S2
CLB19mutS2[T;D] R	TCGATCATTGTAgTCCAAGTAACCG	
CLB19mutS2[S;D] F	CGGTTACTTGGAgTACAATGATCGA	N;D to S;D mutation of S2
CLB19mutS2[S;D] R	TCGATCATTGTATcCCAAGTAACCG	
CLB19mutS2[T;N] F	CCTGAACGAaACTTGATTTCTTGG	N;D to T;N mutation of S2
CLB19mutS2[T;N] R	CCAAGAAATCAAGTtTCGTTCAGG	
CLB19mutP2[N;D] F	ATTTCTTGGAatGCTATGATAAAT	T;D to N;D mutation of P2
CLB19mutP2[N;D] R	ATTTATCATAGCatTCCAAGAAAT	
CLB19mutP2[T;N] F	GTAAAACCAaATTACGTTGCTATT	T;D to T;N mutation of P2
CLB19mutP2[T;N] R	TAGCAACGTAATtTGGTTTTACTC	
CLB19mutS3[T;T] F	TGAGGGTGAGTAcTTCACTGATCGATT	N;T to T;T mutation of S3
CLB19mutS3[T;T] R	AATCGATCAGTGAAgTACTCACCCTCA	
CLB19mutS3[T;D] F	GAGAAACGAgaCGTAGTTTCGTGG	N;D to T;D mutation of S3
CLB19mutS3[T;D] R	GAAACTACGtcTCGTTTCTCCAT	
CLB19mutP3[T;D] F	GTTTCGTGGAcTTCAGTCATTGTT	N;D to T;D mutation of P3
CLB19mutP3[T;D] R	AATGACTGAAgTCCACGAAACTAC	
CLB19mutP3[T;N] F	TTTAAACCTaACGCAGTCACTTTC	N;D to T;N mutation of P3
CLB19mutP3[T;N] R	AGTGACTGCGTtAGGTTTAAAGCC	
CLB19mutL2[T;N] F	CGTTGCTATTAcTGCTGCTCT	I;N to T;N mutation of L2
CLB19mutL2[T;N] R	AGAGCAGCAgTAATAGCAACG	
OTP82 in vivo F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGATGCTCTCGTGTTCTCC	
OTP82 in vivo R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCAGTAGTCATTGCAGGAAC	
OTP82mutS3[T;D] F	CTCAAGATTGTTAcTGCTCTCATTGAT	N;D to T;D mutation of S3
OTP82mutS3[T;D] R	ATCAATGAGAGCAgTAACAATCTTGAG	