Supplementary Information

Supplementary Tables :

Supplementary Figures :

Table S1 : Sequences of pegRNAs used for *PpAPT* (pegRNA#1-8) and *StALS Prime Editing*.
For each pegRNA, target sequence (without PAM), RT template with edition written in lower case and PBS are indicated in 5'→3' orientation. Their respective sgRNA used for PE3 is precised (sequences in Table S2).

pegRNA				sgRNA used for PE3
Name	**Target sequence**	**RT template sequence**	**PBS sequence**	**Name**
pegRNA#1	GATGTCTTAGGCCCTGTGATT	TACTAcACTCTTagCTAAT	CACAGGGCCTAAG	sgRNA#PE3-1
pegRNA#2	GGTGAAGATGTCGGCCTCCA	GTCGCCATCCTTGGtatg	AGGCCGACATCTTC	sgRNA#PE3-2
pegRNA#3	TGTCTTAGGCCCTGTGATTA	TATACTCTTCtCaAA	TCACAGGGCCTAAG	sgRNA#PE3-1
pegRNA#4	CCACCCATTGCTCTTGCCAT	AAACTTCGCtCaGATG	GCAAGAGCAATGGG	sgRNA#PE3-3
pegRNA#5	CCACCCATTGCTCTTGCCAT	AAACTTCGCACGATG	GCAAGAGCAATGGG	sgRNA#PE3-3
pegRNA#6	GATGACTTAATTGCCACCGG	TCCAAGAGTtCaTCaG	GTGGCAATTAAGT	sgRNA#PE3-1
pegRNA#7	GCGTTACCGGGACCAGAAGG	ACAATGACcTaCACCTaCT	GGTCCCGGTA	sgRNA#PE3-3
pegRNA#8	GCAGCTCCAAGAGTGCCTCCGG	GACTTAATaGCACCG	GAGGCACTCTTGGA	sgRNA#PE3-4
pegRNA-StALS	CTATTACGGGTCAAGTGCCG	AATCATCCTtCTgGa	CACTTGACCCGTA	sgRNA-StALS

Table S2 : Sequences of sgRNAs used for *PpAPT* and *StALS* targeting.

Name	5' -> 3' sequence
APTgRNA-PE3#1	ACGTCAGCACAAAATTGTCAAGG
APTgRNA-PE3#2	GGATGCTGTCCGAGATATACTGG
APTgRNA-PE3#3	GAGTGCCTGTCAACCCTTACCTGG
APTgRNA-PE3#4	GAAGAGTATAGTCTAGAGTATGG
APTgRNA#C2	GTGAAGATGTCGGCCTCCAAGG
APTgRNA#C3	TGTCTTAGGCCCTGTGATTAGGG
APTgRNA#C6	GATGACTTAATTGCCACCGGAGG
APTgRNA#C8	GCAGCTCCAAGAGTGCCTCCGGTGG
sgRNA-StALS	TGACCCGTAATAGCAACAATCGG

Table S3 : Primers used in this study.

***P. patens* primers**	
Name	**5'-> 3' sequence**
PpAPT#5	ACAAGGTGGTGTCAACTTTCAAGG
PpAPT#25	GTCGTCACTCTCGGTTTTG
PpAPT#60	ATGGTCAATGTGGCAGCAAG
PpAPT#61	CCTGTCAACCCTTACCTGGA
PpRad51-1#6	TGAGGAGGAAGTTCATCATGG
PpRad51-1#7	ACCGCCAATGGGTTTATGC
peg2-OT1F	GCACTCCAACGTCGTTGAGA
peg2-OT1R	TGCATAGATTGACCGTGCCA
peg2-OT2F	GCAAAACACTTAGCGCACCA
peg2-OT2R	AAAGCTCCCAGTGTTAGGGC
peg2-OT3F	TGAACACAGCTTCTGCCGAT
peg2-OT3R	GAGCAGGTGTTGGTACTGCT
peg2-OT4F	TGGGATGGTTGTTCCTCACG
peg2-OT4R	TGAAGTCAGCGTCGTCGAAA
peg2-OT5F	TGAAGTCAGCGTCGTCGAAA
peg2-OT4R	TGGGATGGTTGTTCCTCACG
peg2-OT6F	TCCACGTTTCAAAATCAGCCC
peg2-OT6R	ACAGTGAGAGTGTGTGCTCG
peg2-OT7F	TCCACGTTTCAAAATCAGCCC
peg2-OT7R	ACAGTGAGAGTGTGTGCTCG
peg2-OT8F	TGTTCTGATTGGGAGGCGAC
peg2-OT8R	CTCCTGTCTCGGCAATGGTT
peg2-OT9F	TCCGAGGCATCACAAAGCTT
peg2-OT9R	GCTCGGAGAAAGATCTGCCA
peg6-OT1F	GAAGCTTTCCGAATCCTTA
peg6-OT1R	CGCAATACACAACACCTAACA
***S. tuberosum* primers**	
Name	**5'-> 3' sequence**
ALS1-F1	CCAATGTCGTCATATCCACTACC
ALS1-R1	CAGCTCCTCACTTGATTGCAA
ALS1-F2	CGTACCCAGGAGGTGCTTCT
ALS1-R2	GGTACATCAATCAAAACCGGC
ALS2-F1	AAATGTCATCCTATCAACCACGA
ALS2-R1	TCAGCTCCTCACTCGATTGTGT
ALS2-F2	CATACCCAGGAGGTGCTTCC
ALS2-R2	GGAACATCAATCAGAACCGGT

Table S4 : CRISPOR-predicted off-targets for pegRNA#2 and #6.

Name	mismatch	sequence	genomic location
pegRNA#2	-	AGTGAAGATGTCGGCCTCCA *AGG*	Chr08_10812966
peg2-OT1	4	A**a**T**a**AAGATGTCGGC**a**TC**a**A *AGG*	Chr03_16263830
peg2-OT2	4	AGTG**g**AGA**gt**TCGGCC**a**CCA *CGG*	Chr01_24568979
peg2-OT3	4	**g**GTGAA**a**ATG**g**C**t**GCCTCCA *CGG*	Chr02_10287524
peg2-OT4	4	AGTGAAGA**g**GT**gac**CCTCCA *GGG*	Chr15_8685405
peg2-OT5	4	AGTGAAGA**g**GT**gac**CCTCCA *GGG*	Chr15_8677239
peg2-OT6	4	A**t**TGAAGATGT**g**G**c**CC**a**CCA *CGG*	Chr01_15854114
peg2-OT7	4	A**t**TGAAGATGT**g**G**c**CC**a**CCA *CGG*	Chr01_13515936
peg2-OT8	4	AGTGA**t**GATG**g**C**t**GCCT**a**CA *TGG*	Chr07_17136251
peg2-OT9	2	AGTGAAGAT**t**TCGGCCTCC**t** *GGA*	Chr10_12172644
pegRNA#6	-	GATGACTTAATTGCCACCGG AGG	Chr08_10812019
peg6-OT1	4	G**t**TGACT**g**AATTGC**a**AC**t**GG *AGG*	Chr24_2863689

Table S5 : Genotyping of off-target loci in *P. patens apt* mutants

PE3/pegRNA#2							
Plant #	OffTarget1	OffTarget2	OffTarget3	OffTarget4	OffTarget6	OffTarget8	OffTarget9
1	WT	WT	WT	WT	WT	WT	WT
2	WT	WT	WT	WT	WT	WT	WT
3	WT	WT	WT	WT	WT	WT	WT
4	WT	WT	WT	WT	WT	WT	WT
5	WT	WT	WT	WT	WT	WT	WT
6	WT	WT	WT	WT	WT	WT	WT
7	WT	WT	WT	WT	WT	WT	WT
8	WT	WT	WT	WT	WT	WT	WT
10	WT	WT	WT	WT	WT	WT	WT
11	WT	WT	WT	WT	WT	WT	WT
12	WT	WT	WT	WT	WT	WT	WT
13	WT	WT	WT	WT	WT	WT	WT
14	WT	WT	WT	WT	WT	WT	WT
15	WT	WT	WT	WT	WT	WT	WT
16	WT	WT	WT	WT	WT	WT	WT
20	WT	WT	WT	WT	WT	WT	WT
21	WT	WT	WT	WT	WT	WT	WT
22	WT	WT	WT	WT	WT	WT	WT
23	WT	WT	WT	WT	WT	WT	WT
24	WT	WT	WT	WT	WT	WT	WT
25	WT	WT	WT	WT	WT	WT	WT
26	WT	WT	WT	WT	WT	WT	WT
27	WT	WT	WT	WT	WT	WT	WT
28	WT	WT	WT	WT	WT	WT	WT
29	WT	WT	WT	WT	WT	WT	WT
30	WT	WT	WT	WT	WT	WT	WT
31	WT	WT	WT	WT	WT	WT	WT
32	WT	WT	WT	WT	WT	WT	WT
33	WT	WT	WT	WT	WT	WT	WT
34	WT	WT	WT	WT	WT	WT	WT
35	WT	WT	WT	WT	WT	WT	WT
36	WT	WT	WT	WT	WT	WT	WT
38	WT	WT	WT	WT	WT	WT	WT
39	WT	WT	WT	WT	WT	WT	WT
40	WT	WT	WT	WT	WT	WT	WT
41	WT	WT	WT	WT	WT	WT	WT
42	WT	WT	WT	WT	WT	WT	WT
43	WT	WT	WT	WT	WT	WT	WT
46	WT	WT	WT	WT	WT	WT	WT
47	WT	WT	WT	WT	WT	WT	WT
48	WT	WT	WT	WT	WT	WT	WT
49	WT	WT	WT	WT	WT	WT	WT

PE2/pegRNA#6	
Plant #	OffTarget1
4	WT
5	WT
6	WT
7	WT
8	WT
9	WT
10	WT
11	WT
12	WT
13	WT
14	WT
15	WT
16	WT
17	WT
18	WT
19	WT
21	WT
22	WT
23	WT
24	WT
25	WT
26	WT
28	WT
29	WT
30	WT
31	WT
32	WT
33	WT
34	WT
35	WT
37	WT
38	WT
39	WT
40	WT
41	WT
42	WT
43	WT

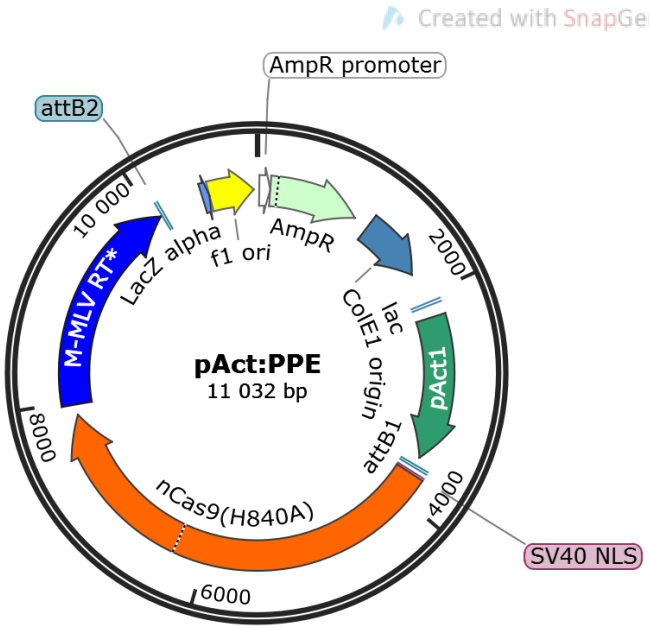
Table S6 : Allele cloning performed on WT (Desiree) and *StALS* prime edited plant.

***StALS1* allele cloning**	Desiree	Prime edited n°1
Nb of reads	11	10
Nb of prime edited reads		1

Table S7 : Molecular analysis of *StALS* loci after *Prime Editing*.

	pDeCas9-StALS	pDePPE2-StALS		pDePPE3-StALS	
Plant selection	Kanamycin	Kanamycin	Chlorsulfuron	Kanamycin	Chlorsulfuron
Nb of transgenic plants	12	20	2	6	2
Nb of mutated plants	11 (92%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)

a



b

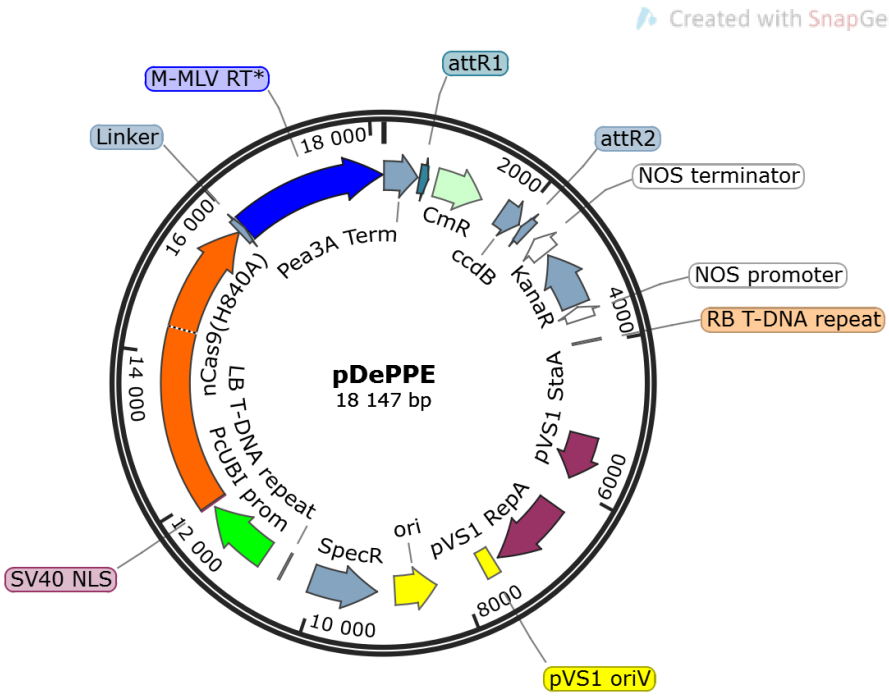


Fig. S1 : Plasmid maps of Prime Editors used in *P. patens* and potato.

(**a**) Plasmid map of pAct-PPE expression vector. (**b**) Plasmid map pDe-PPE expression vector.

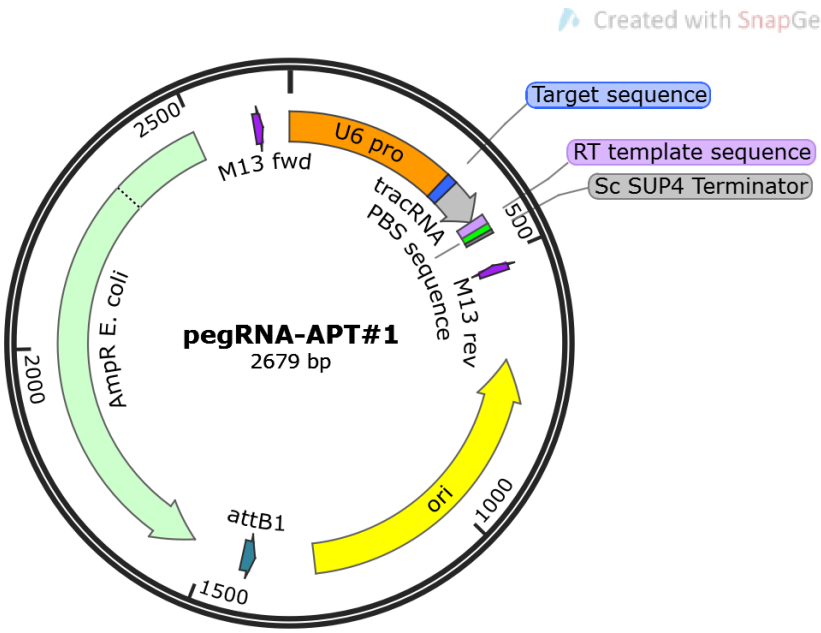
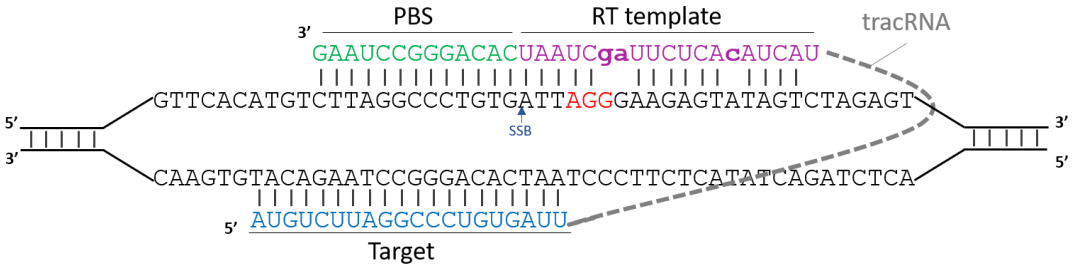


Fig. S2 : Plasmid map model for pegRNA expression vector in *P. patens*.
Plasmid map model of pegRNA expression vector. Example given for pegRNA#1. Target sequence, RT template sequence and PBS sequence are specific to each pegRNA.

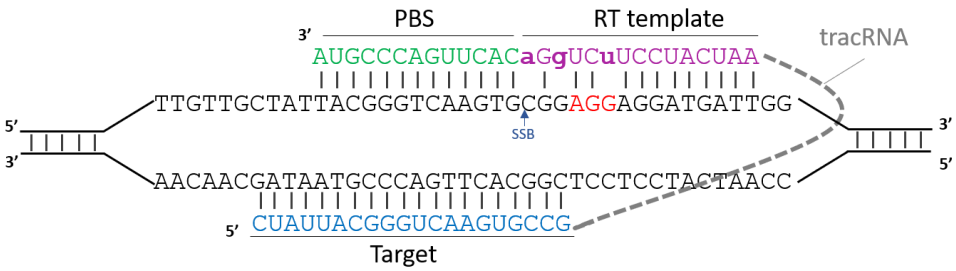
a



b

```
ATTGAATGTCCATTGAAGCAGACGTGTTGCGACAGGTTAGCGACGATGGGTGTAGATG
TGATGTGATGTGATGGTGTGGTTCTTCCACGGCGGCGTCCTTGCGGTGGCGGAGAAGG
GGATATCCCGAAGGAGCGGCAGCGGGAGAGCACAAGCAGAAAGGGTGCAGTGAGTGAG
TGGGTCCAGCTGGGTGGCTGGCCGAGTGGACGCGACCGGGTTTCGAGGGGGcGGGGGA
GAAAAGGGATGGAGCGAGGGATATAACCCACATGGAATGGAGGTGGGTGTGAAGGCGG
GTATATAGGAAGGTGGAGGACTTACAACCCATGATGTCTTAGGCCCTGTGATTGTTTT
AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC
ACCGAGTCGGTGCTACTAcACTCTTagCTAATCACAGGGCCTAAGTTTTTTT
```

c



d

```
CTTTTTTTCTTCTTCTTCGTTCATACAGTTTTTTTTTGTTTATCAGCTTACATTTTC
TTGAACCGTAGCTTTCGTTTTCTTCTTTTTAACTTTCCATTCGGAGTTTTTGTATCT
TGTTTCATAGTTTGTCCCAGGATTAGAATGATTAGGCATCGAACCTTCAAGAATTTG
ATTGAATAAAACATCTTCATTCTTAAGATATGAAGATAATCTTCAAAAGGCCCCTGG
GAATCTGAAAGAAGAGAAGCAGGCCCATTTATATGGGAAAGAACAATAGTATTTCTT
ATATAGGCCCATTTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAAG
AAAACGAAGCTGAGTTTATATACAGCTAGAGTCGAAGTAGTGATTGCTATTACGGGT
CAAGTGCCGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCGAGTCGGTGCAATCATCCTtCTgGaCACTTGACCCGTAT
TTTTTT
```

Fig. S3 : Schematic representation of DNA/RNA interaction at the targeted loci using the PE2 strategy and sequence of pegRNA expression module.
(**a**) *PpAPT* : example given for pegRNA#1 (**b**) Sequence of pegRNA#1 expression module driven by PpU6 promoter and ScSUP4 terminator (**c**) *StALS* : pegRNA-StALS (**d**) Sequence of pegRNA-StALS expression module driven by AtU6-26 promoter and ScSUP4 terminator Target sequence in blue, RT template in pink with edition written in lower case and PBS sequence in green. Promoter and terminator sequences in black, sgRNA scaffold (tracRNA) in gray.

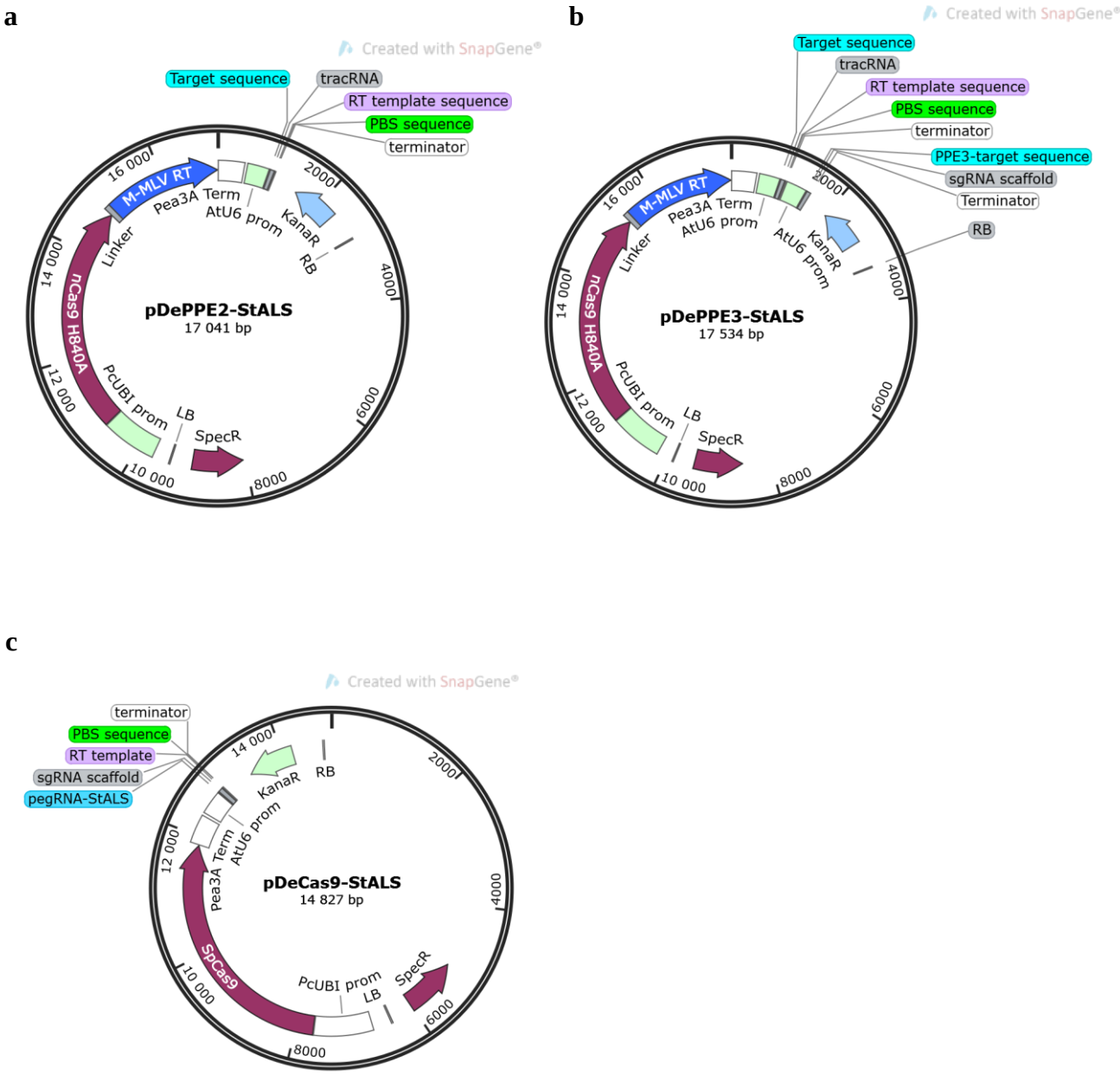


Fig. S4 : Plasmids used for *StALS Prime Editing* in potato.
(**a**) Plasmid map of pegRNA-StALS expression vector used for PE2. (**b**) Plasmid map of pegRNA-StALS and sgRNA-StALS expression vector used for PE3. (**c**) Plasmid map of sgRNA expression vector used for control with Cas9.

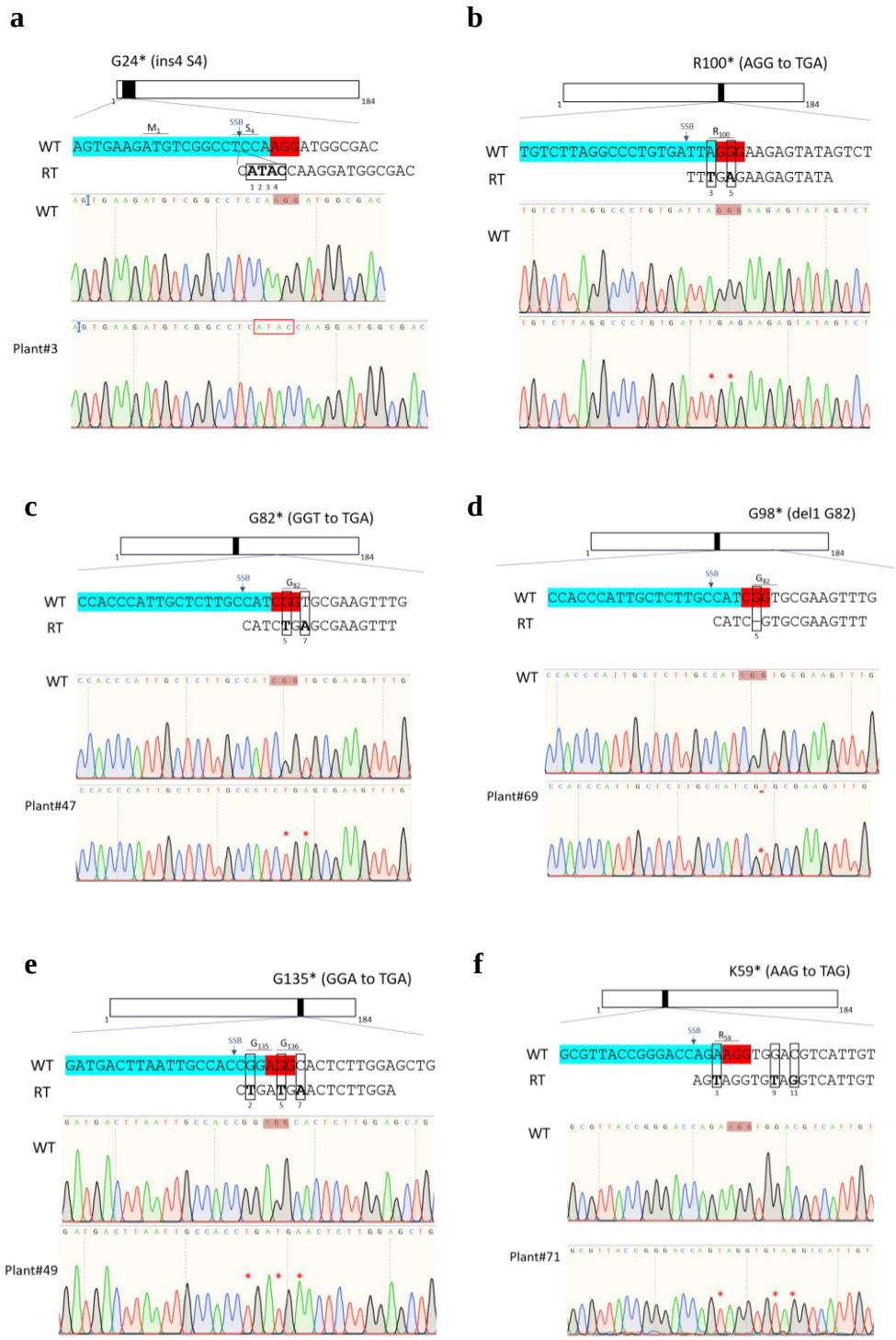


Fig. S5 : Examples of edited plants using *PpAPT Prime Editing*.
(**a-f**) : Schematic representation of the APRT protein with the wild-type genomic sequence (WT) and expected RT product (RT) using each pegRNA. (**a**) pegRNA#2. (**b**) pegRNA#3. (**c**) pegRNA#4. (**d**) pegRNA#5. (**e**) pegRNA#6. (**f**) pegRNA#7. Target sequence is highlighted in blue and PAM in red, blue arrow represents SSB site and relative position of mutations are precised below expected RT product sequence. A sequencing chromatogram of an edited plant using each pegRNA is shown below WT sequence, editions are indicated by red stars.

>WT
TCTGAGTTAGTTACTGACGAAGCTCTGTAAATTGTGTGTGGACAGGAAG**TGAAGATGTCGGCCTCCAAGG**A
TGGCGACCCTCGGAT**CCA**GTATATCTCGGACAGCATCCGTACCATTCCTGATTTTCCTCACAAAGGTACTA
TGCTCCACGCCATAGTCGTCACTCTCGGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTTTTTT
CACCTTTAGCGAAGGTTTTGTATGGAATTCTCTTTCGTAGCGCGAGTTTACGTGATGAATTTGGTGCAGGC
ATTATGTTCCGAGATGTGACGACGTTGCTGTTGGATCATAAGGCTTTCAAAGACACGATCGACATCTTTGT
TGAGCGTTACCGGGACCAGAAGGTGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCTTGACGTACCATA

>Plant#41- predicted editing (ins4)
TCTGAGTTAGTTACTGACGAAGCTCTGTAAATTGTGTGTGGACAGGAAG**TGAAGATGTCGGCCTC**ATAC**CA**
AGGATGGCGACCCTCGGAT**CCA**GTATATCTCGGACAGCATCCGTACCATTCCTGATTTTCCTCACAAAGGT
ACTATGCTCCACGCCATAGTCGTCACTCTCGGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTT
TTTTCACCTTTAGCGAAGGTTTTGTATGGAATTCTCTTTCGTAGCGCGAGTTTACGTGATGAATTTGGTGC
AGGCATTATGTTCCGAGATGTGACGACGTTGCTGTTGGATCATAAGGCTTTCAAAGACACGATCGACATCT
TTGTTGAGCGTTACCGGGACCAGAAGGTGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCTTGACGTAC

>Plant#46- editing (ins4) and additional SNP
TCTGAGTTAGTTACTGACGAAGCTCTGTAAATTGTGTGTGGACAGGAAG**TGAAGATGTCGGCCTC**ATAC**CA**
AGGATGGCGAC**G**CTCGGAT**CCA**GTATATCTCGGACAGCATCCGTACCATTCCTGATTTTCCTCACAAAGGT
ACTATGCTCCACGCCATAGTCGTCACTCTCGGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTT
TTTTCACCTTTAGCGAAGGTTTTGTATGGAATTCTCTTTCGTAGCGCGAGTTTACGTGATGAATTTGGTGC
AGGCATTATGTTCCGAGATGTGACGACGTTGCTGTTGGATCATAAGGCTTTCAAAGACACGATCGACATCT
TTGTTGAGCGTTACCGGGACCAGAAGGTGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCTTGACGTAC

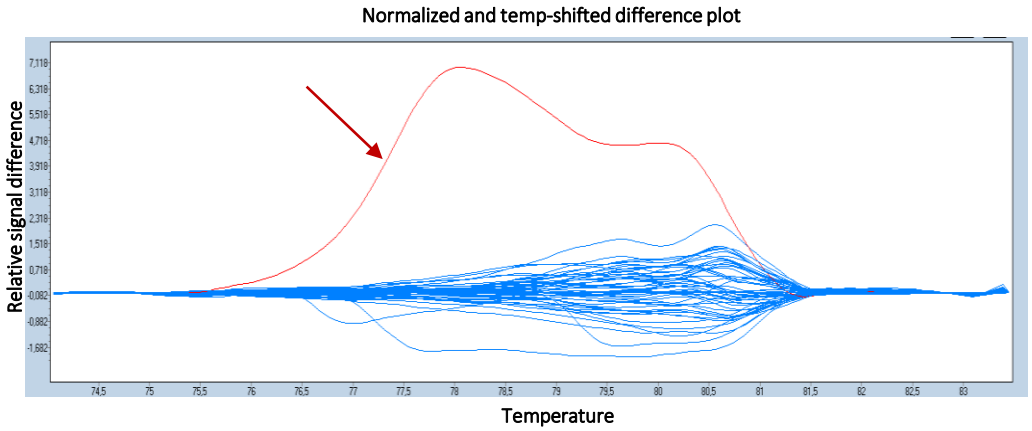
>Plant#17- 16pb-deletion
TCTGAGTTAGTTACTGACGAAGCTCTGTAAATTGTGTGTGGACAGGAAG**TGAAGATGTCGGCCTCCAAGG**A
TGGCGACCCTCGGA**CAGCATCC**GTACCATTCCTGATTTTCCTCACAAAGGTACTATGCTCCACGCCATAGT
CGTCACTCTCGGTTTTGTTTTGCAGTGTTCGTGACATGTGGTTTTTTTCTTTTTTCACCTTTAGCGAAGGT
TTTGTATGGAATTCTCTTTCGTAGCGCGAGTTTACGTGATGAATTTGGTGCAGGCATTATGTTCCGAGATG
TGACGACGTTGCTGTTGGATCATAAGGCTTTCAAAGACACGATCGACATCTTTGTTGAGCGTTACCGGGAC
CAGAAGGTGGACGTCATTGTGGGTGCGATGCCCTTGACTCCCTTGACGTACCATAACGGTTTTGTGTAATA

Fig. S6 : Observed deletions using the PE3 strategy for pegRNA#2.
Sequences of WT and edited plants of the targeted locus : target sequence of pegRNA#2 is written in dark blue, target sequence of sgRNA#PE3-2 in light blue, PAM in red and editing (ins4) is highlighted in yellow, additional SNP in orange.

a

Wt sequence	A	C	G	G	G	T	C	A	A	G	T	G	C	C	G	A	G	G	A	G	G	A	T	G
			Thr			Gly			Gln			Val			Pro186			Arg			Arg			Met
Expected sequence	A	C	G	G	G	T	C	A	A	G	T	G	T	C	C	A	G	A	A	G	G	A	T	G
			Thr			Gly			Gln			Val			Ser186			Arg			Arg			Met

b



c

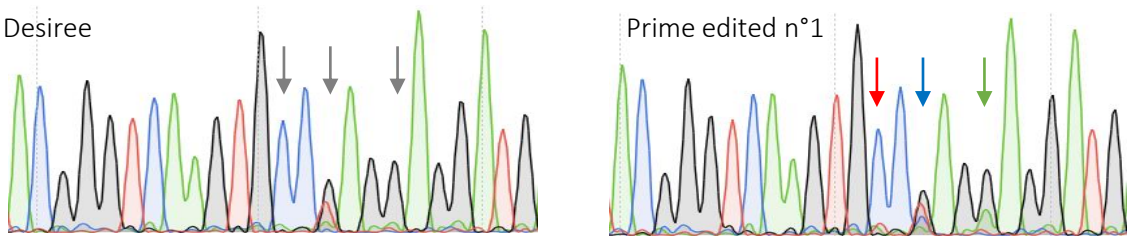


Fig. S7 : Genotyping of *StALS* loci using *Prime Editing*.
(**a**) Schematic representation of the WT and expected sequences, with amino acids indicated under the nucleotides. The Proline 186 is in red, and targeted nucleotides are underlined. (**b**) Screenshot of the HRM output for the pDePPE2-StALS condition is dispayed with blue curves representing wild-type profiles, while the red curve (indicated with a red arrow) represents the melting profile of a mutated plant (prime edited N°1). Both *StALS1* and *StALS2* genes were simultaneously analysed using primers matching all 8 alleles. (**c**) Sanger chromatograms of the targeted loci for Desiree and the prime edited plant n°1. The sequencing reaction has been performed with primers matching all the alleles of *StALS1* and *StALS2* genes. Targeted nucleotides are indicated with grey or color arrows on Desiree or mutated chromatograms, respectively. One natural SNP (G/T) is present, as evidenced by the double peak on the Desiree chromatogram. A: green; T: red; C: blue; G: black.