

## Supporting information

>Solyc03g044330 *SIALS1*

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ATGGCGGCTGCTGCCTCACCATCTCCATGTTTCTCCAAAACCTACCTCCATCTTCTCCAAATCTTCCACCATTCTACCTAG
ATCTACCTTCTCTTCCACAATCACCACAAAAAGCCTCACCCCTTCATCTCATCCACGCTCAACATAATCGTTCGTGGTTTTG
CCGTTGCCAATGTCGTCATATCCACTACCACCATAACGACGTTTCTGAACCTGAAACATTCGTTTTCCCGTTTTCGCCCCTGAC
GAACCCAGAAAGGGTTGTGATGTTCTTGTGGAGGCACCTTGAAAGGGAAGGTGTTACGGATGTATTTGCATACCCAGGAGGTGC
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CTGCAGAGGGTTACGCACGGGCTACTGGGTTCCCTGGTGTGTTGCATTGCTACCTCTGGTCCCAGGACTACAAATCTTGTAGT
GGTCTTGC GGATGCTTGTAGATAGTATTCCGATTGTTGCTATTACAGGTCAAGTGC AAGGAGGATGATGGTACTGATGC
GTTCCAGGAAACGCTATTGTTGAGGTAACGAGATCTATTACGAAGCATAAATTATCTTGTATGGATGTAGAAGATATTCCTA
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CAATTGGTGATACCTAATTGGGATCAGCCAATGAGGTTGCCTGGTTACATGTCTAGGTTACCTAAATGCCTAATGAAATGCT
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TTAGGAGCAATGGGATTTGGTTTGCCTGCTGCTATAGGTGCGGCTGTTGGGAGACCGGGTGAGATTGTGGTTGACATTGACGG
TGATGGGAGTTTTATCATGAATGTGCAAGAGTTAGCAACAATTAAGTTGGAGAATCTCCAGTTAAGATATGTTGCTGAATA
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AACGAGGAAGAGATCTCCCTAATATGTTGAAATTTGCAGAGGCTTGTGGCGTACCTGCTGCAAGAGTGTACACAGGGATGA
TCTTAGAGCTGCCATTCAAAAGATGTTAGACACTCTGGGCCATACTTGTGGATGTGATTGTACCTCATCAGGAGCACGTTCT
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### Figure S1. *SIALS1* wild-type gene sequence

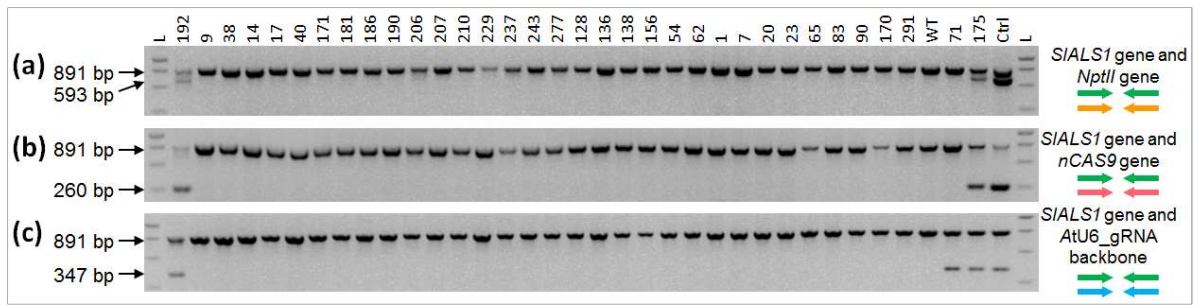
The start codon is represented with red letters and the stop codon is represented with bold letters. The target sequence is highlighted in blue. The three targeting cytidines are highlighted in red. The PAM site is highlighted in green.

>Solyc03g044330 protein SIALS1

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MAAAASPSPCFSKTLPSSSKSSTILPRSTFSFHNHPQKASPLHLIHAQHNRRGFVANVVI STTTHNDVSEPETFVSRFAPD
EPRKGC DVLVEALERE GVTDFVAYPGGASMEIHQALTRSNIIRNVLPRHEQGGVFAAEGYARATGFPGVCIATSGPGATNLVS
GLADALLDSIPIVATGQVPRRMIGTDAFQETPIVEVTRSITKHNLYLMDVEDIPRVVREAFFLAKSGRPGPVLIDVPKDIQQ
QLVIPNWDQPMRLPGYMSRLPKLPNEMLLEQIVRLISESKKPVLYVGGGCSQSSEELRRFVELTGIPVASTLMGLGAFPTGDE
LSLQMLGMHGTVYANYAVDSSDLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHVSIKADIKLALQGLNSIL
EGKEGKMKLDFSAWRQELTEQKMKYPLNFKTFGDAIPPQYAIQVLDLDELNGNAIISTGVGQHQMWAAQYKYKKPRQWLTSGG
LGAMGFGLPAAIGAAVGRPEIVVDIDGDGSFIMNVQELATIKVENLPVKIMLLNNQHLGMVVQWEDRFYKANRAHTYLGDPQ
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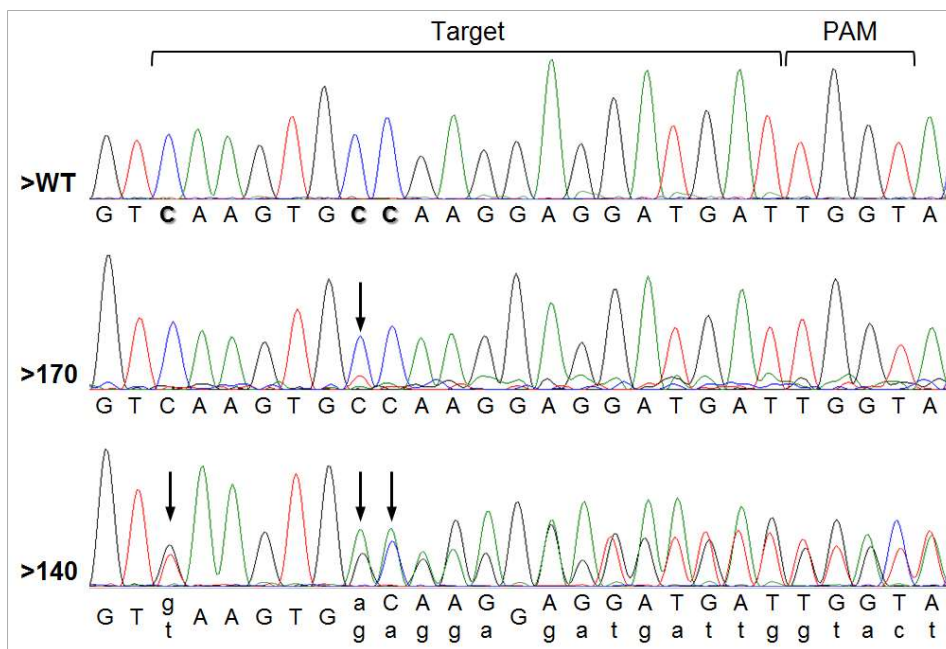
### Figure S2. *SIALS1* wild-type protein sequence

The SIALS1 Q184 and P186 are highlighted in purple.



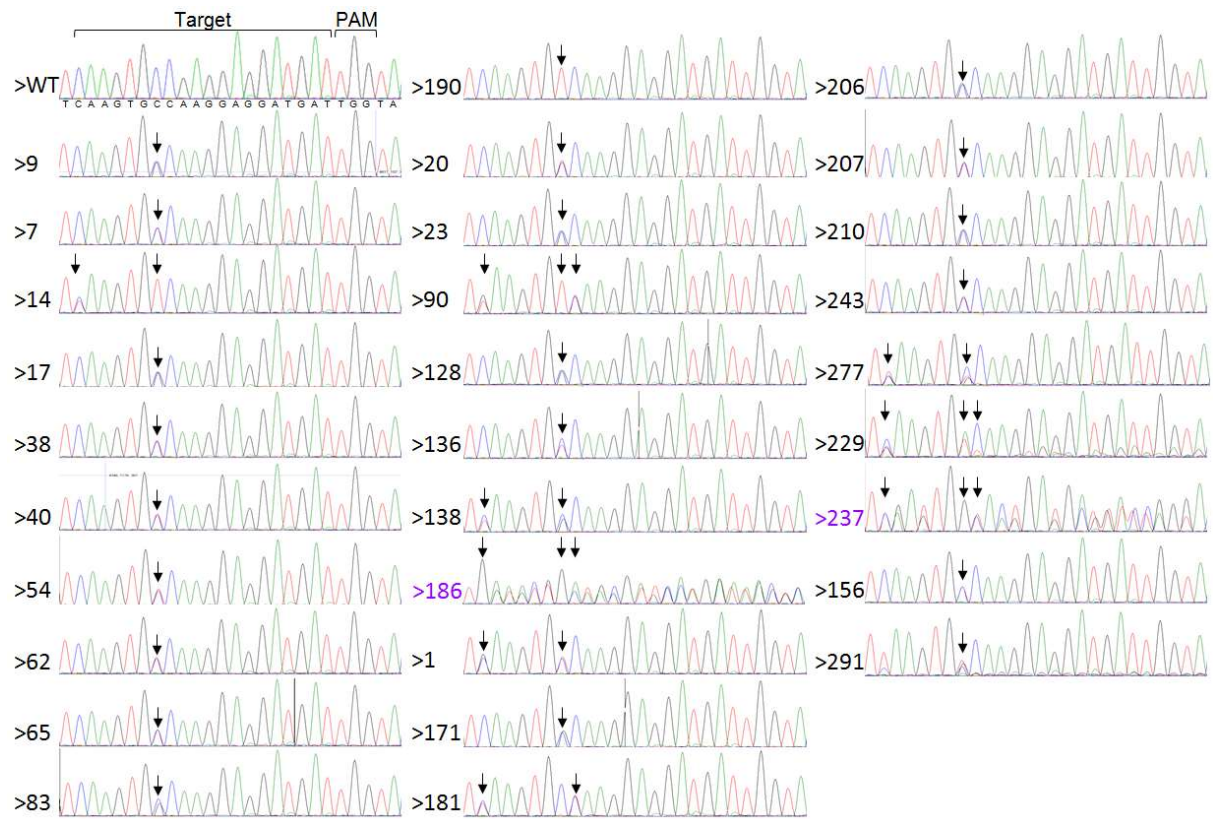
**Figure S3. Molecular analysis of thirty plants without T-DNA insertion**

Detection of the T-DNA was performed by multiplex PCR analysis on the thirty *SIALLS1* edited plants without T-DNA (9, 38, 14, 17, 40, 171, 181, 186, 190, 206, 207, 210, 229, 237, 243, 277, 128, 136, 138, 156, 54, 62, 1, 7, 20, 23, 65, 83, 90, 291), the wild-type (WT), a plant carrying a potentially chimerical editing event (170), the PCR positive control (Ctrl), one *SIALLS1* edited plant containing a fragment of T-DNA (71) and two *SIALLS1* edited plant containing a T-DNA (192, 175). L: molecular marker. (a) Amplification of the *SIALLS1* gene and the *NptII* gene. (b) Amplification of the *SIALLS1* gene and the *Cas9* gene. (c) Amplification of the *SIALLS1* gene and the *AtU6*-gRNA backbone.



**Figure S4. Sequencing chromatograms of the targeted *SIALLS1* region of the wild-type (WT), a potential chimerical event (170) and an indel event (140).**

The three targeting cytidines are bolded in the wild-type sequence. The two independent plantlets (170, 140) are chlorsulfuron resistant. The black arrows indicate potential modifications. Primers used for this sequencing are *SIALLS1.F* and *SIALLS1.R*.



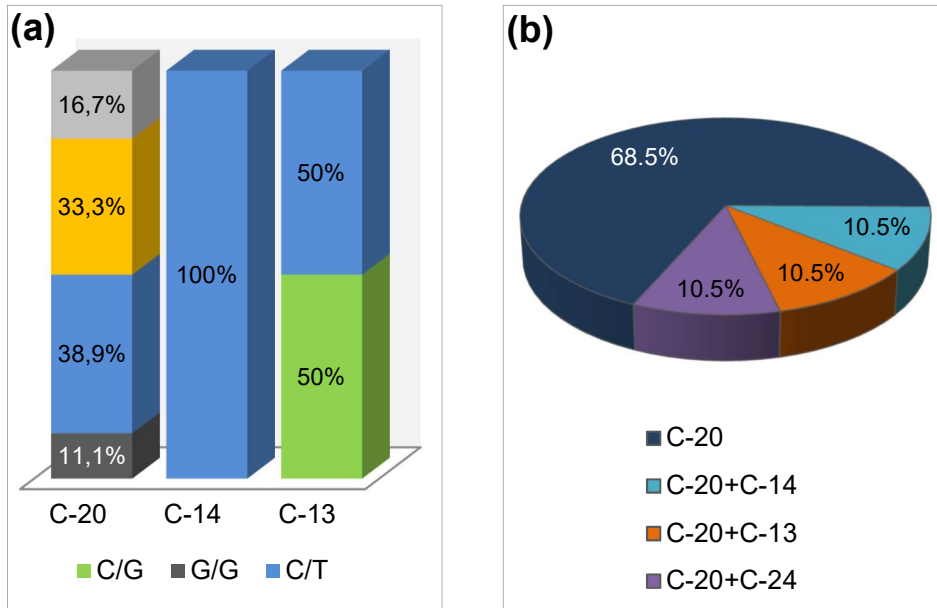
**Figure S5. Sequencing chromatograms of the targeted *SIALLS1* region of the wild-type (WT) and the thirty *SIALLS1* edited plants without T-DNA insertion**

The black arrows indicate modifications on C-20, C-14 or C-13. Purple writing indicates the presence of indels in the sequence. Primers used for this sequencing are SIALLS1.F and SIALLS1.R.



**Figure S6. Sequencing chromatograms of the *SIALS2* locus of the wild-type (WT) and fifty one *SIALS1* edited plants**

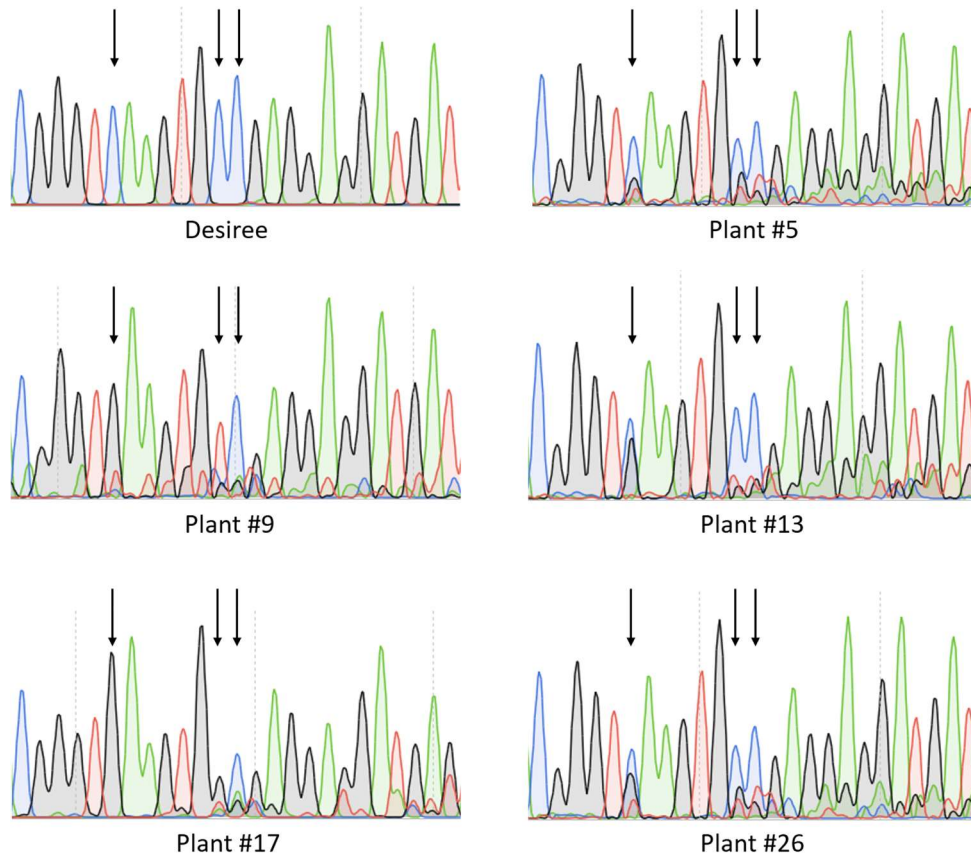
The black arrows indicate modifications on targeted cytidines. Purple writing indicates the presence of indels in the sequence. (a) Sequencing chromatograms of 25 *SIALS1* edited plants without T-DNA insertion. Four plantlets are edited on *SIALS2* locus (>40, >136, >138, >243). (b) Sequencing chromatograms of 3 *SIALS1* edited plants with T-DNA insertion, and without editing on *SIALS2* locus. (c) Sequencing chromatograms of 23 *SIALS1* edited plants with T-DNA insertion, and with editing on *SIALS2* locus. Primers used for this sequencing are *SIALS2.F* and *SIALS2.R*.



**Figure S7. Base editing outcomes at the *StALS2* locus**

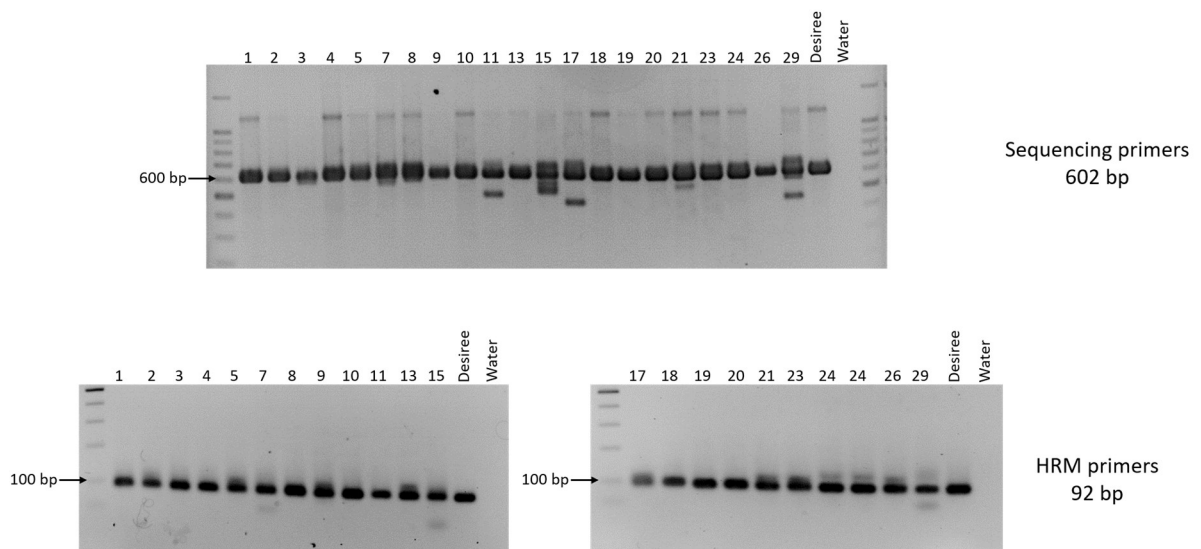
(a) Percentage of each type of nucleotide changes found on cytidines C-20, C-14 or C-13. The total number of edited plants used in the study was 18 for C-20, 2 for C-14, 2 for C-13. (b) Percentage of single editing event: C-20 in dark blue, double editing events: C-20+C-14 in clear blue, and C-20+C-13 in orange and C-20+C-24 in purple. 19 edited plants were used in the study.















**Figure S9: Sequencing chromatograms of the targeted region (*StALS1* and *StALS2*) of Desiree and some mutants harbouring indels.**

The black arrows indicate the targeted cytidines.



**Figure S10: PCR genotyping of the targeted region (*StALS1* and *StALS2*).** PCR products were run on an agarose gel for the detection of band-shifts, indicating the presence/absence of indels in the targeted region.











**Table S1****List of primers used in tomato**

| Target   | Primer ID   | Sequence (5'-3')        | Size (bp) |
|--|---|-------------------------|-----------|
| PCR detection/sequencing of the <i>SIALS1</i> gene |  | GGCACTTGAAAGGGAAGG      | 891       |
|  |  | CCAATCTCTGCCGAATCAAT    |           |
| PCR detection of the <i>nCAS9</i> gene             |  | TCCCTTACTACGTGGGACCTC   | 260       |
|  |  | CATTCCCTCGGTCACGTACT    |           |
| PCR detection of the <i>NptII</i> gene             |  | AGACAATCGGCTGCTCTGAT    | 593       |
|  |  | AGCCAACGCTATGTCTGAT     |           |
| PCR detection of the <i>AtU6</i> -gRNA backbone    |  | TCCCAGGATTAGAATGATTAGG  | 347       |
|  |  | CGGTGCCACTTTTTCAAGTT    |           |
| HRM detection of the <i>SIALS1</i> gene            |  | CTTGTTAGTGGTCTTGCGGATG  | 104       |
|  |  | GTTTCCTGGAACGCATCAGTACC |           |
| Sequencing of the <i>SIALS1</i> gene               | SIALS1.F  | GTGGTTTTGCCGTTGCCAAT    | 761       |
|  | SIALS1.R  | CAGCTCCTCACTTGATTGCG    |           |
| Sequencing of the <i>SIALS2</i> gene               | SIALS2.F  | GCCGTTTCACTGTTTCAAAT    | 761       |
|  | SIALS2.R  | CAGCTCCTCACTCGACTGTG    |           |



**Table S2**

**List of primers used in potato**

| Target  | Primer ID   | Sequence (5'-3')         | Size (bp) |
|---|---|--------------------------|-----------|
| Sequencing of the <i>StALS1</i> and <i>StALS2</i> genes |    | GATGTTCTTGTGGAGGCACTTG   | 602       |
|   |    | TTCTTCGACTCCGAAATCAGC    |           |
| PCR detection of the <i>nCAS9</i> gene                  |    | ACTTTTCGTTGAGCAGCACAA    | 350       |
|   |    | GATCAGCCCTTGAATCACCA     |           |
| PCR detection of the <i>NptII</i> gene                  |    | GATGGATTGCACGCAGGTTTC    | 406       |
|   |    | GATGTTTCGCTTGGTGGTCG     |           |
| PCR detection of the <i>AtU6</i> -gRNA backbone         |    | AAACATCATCCTCCTCGGCACTTG | 207       |
|   |    | GATAATCTTCAAAAGGCCCTGG   |           |
| PCR detection of the control gene ( <i>StGBSSI</i> )    |   | AGACCACACATCACATGGCAA    | 352       |
|   |   | CTTACTGCAAGGGCTGGTGG     |           |
| HRM of the <i>StALS1</i> and <i>StALS2</i> genes        |  | CTTGCGGATGCTTTGTTGG      | 92        |
|   |  | GTTTCCTGAAACGCATCAGTACC  |           |