

## Supporting information

>Solyc03g044330 *SIALS1*

ATGCGGGCTGCTGCCACCATCTCCATGTTCTAAAACCCCTACCTCCATCTTCCCAAATCTCCACCATTCTACCTAG  
ATCTACCTCTCTTCCACAATCACCCACAAAAGCCTCACCCCTCATCTCATCCACGCTCAACATAATCGCTGGTTTG  
CCGTTGCCAATGTCGTATCCACTACCACCCATAACGACGTTCTGAACCTGAAACATTCTGTTCCGTTCGCCCCCTGAC  
GAACCCAGAAAGGGTTGTGATGTTCTGTGGAGGCACCTGAAAGGGAAAGGTGTTACGGATGTATTGCTACCCAGGAGGTGC  
TTCTATGGAGATTCAAGCTTGACACGTTGAATATTTCGTAATGTGCTACCCACGTATGAGCAAGGTGGTGTGTTTG  
CTGCAGAGGGTTACGCACGGCTACTGGGTTCCCTGGTGTGATTGCTACCTCTGGTCCCGAGCTACAAATCTGTTAGT  
GGTCTTGCAGATGCTTGTAGATAGTATTCCGATTGTTGCTATTACAGTCAGTCTGAGGATGATTCAGTGC  
GTTCCAGGAAACGCTATTGTTGAGGTAACGAGATCTATTACGAAGCATAATTATCTGTTATGGATGTAGAAGATATTCCCTA  
GGGTTGTTCTGAAAGCATTCTTCTGCAAATCGGGACGCCCTGGCCAGTTTGATTGATGACCTAAGGATATTCAAGCAA  
CAATTGGTGTACCTAATTGGATCAGCAATGAGGTTGCCCTGGTACATGCTAGGTTACCTAAATTGCTTAATGAAATGCT  
TTTGGAACAAATTGTTAGGCTGATTCGAGTCGAAGAAGCCTGTTGTATGTTGAGGTTGGGTGGTGGTGTGCAATCAAGTGAGG  
AGCTGAGACGATTGAGCTTACAGGTATTCTGTAGCGAGTACTTGATGGGTCTGGAGCTTTCAACTGGGATGAG  
CTTTCACCTCAAATGTTGGGTATGCTGGAACTGTGTTGCTAATTATGCTGTGGATAGTAGTGATTGTTGCTTGCTGCATTGG  
GGTAGGTTGATGATGAGTTACTGGTAAATTGGAAGCTTGTAGTCGAGCGAAAATTGTCACATTGATATTGATTGATTGG  
CAGAGATTGGAAAAAAACAAGCAACCTCATGTTCCATTGTCAGATATCAAGTGGCATTACAGGGTTGAATTCCATATTG  
GAGGGTAAAGAAGGTAAAGATGAAGTTAGATTCTGCCTGGAGGCAGGAGTTACGGAGCAGAAGATGAAGTACCCACTGAA  
TTTAAAGACTTTGGTATGCCATCCCTCACAATATGCTATTAGGTTCTGATGAGTTAACTAACGGAAATGCCATTATTA  
GTACTGGTGTGGGCAACACCAGATGTTGGCTGCCAATACATAAGTACAAAAGCCACGCCATGGTGACATCTGGTGG  
TTAGGAGCAATGGGATTGGTTGCCGCTGCTATAGGTGGCTGTTGGAGACGGGGTGGAGATTGTTGACATTGACGG  
TGATGGGAGTTTATCATGAATGTGCAAGAGTTAGCAACAAATTAGGTGGAGAATCTCCAGTTAAGATTATGTTGCTGAATA  
ATCAACACTTGGGATGGGTTCAATGGGAGGATGCTATAAGCTAACAGAGCACACACTTACTTGGGTGACCTCT  
AACGAGGAAGAGATCTCCCTAATATGTTGAAATTGTCAGAGGTTGTGGCTACCTGCTGCAAGAGTGTACACAGGGATGA  
TCTTAGAGCTGCCATTCAAAGATGTTAGACACTGGCCATACCTGTTGGATGTGATTGACCTCATCAGGAGCACGTT  
TACCTATGATTCCAGCGGTGGTGTCAAGATGTGATCACGGAGGGCGATGGGAGATGTTCTATTGAA

### Figure S1. *SIALS1* wild-type gene sequence

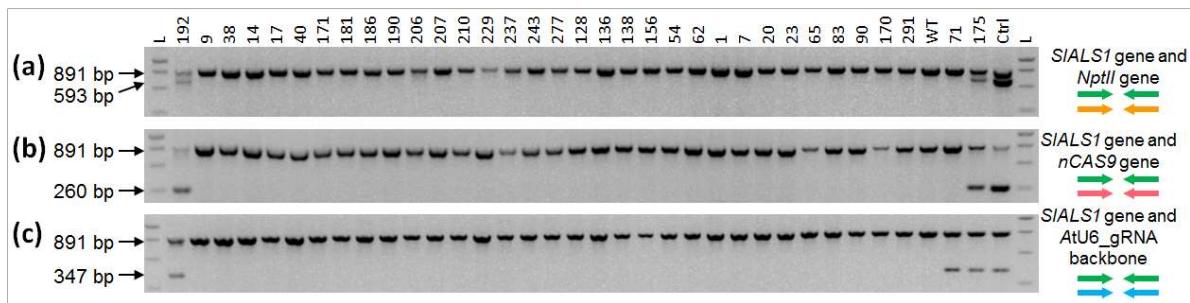
The start codon is represented with red letters and the stop codon is represented with bold letters. The target sequence is highlighted in blue. The three targeting cytidines are highlighted in red. The PAM site is highlighted in green.

>Solyc03g044330 protein SIALS1

MAAAASPSPCFSKTLPPSSKSSTILPRSTFSFHNPQKASPLHLIHAQHNRRGFAVANVISTTHNDVSEPETFVSRAFPD  
EPRKGCDVLVEALEREQVTDVFAYPGGASMEIHQLTRSNIIRNVLPRHEQGGVFAAEGYARATGPGVCIATSGPGATNLVS  
GLADALLDSIPIVAITGCV<sup>P</sup>RRMIGTDAFQETPIVEVTRSITKHNYLVMVEDIPRVVREAFFLAKSGRPGPVLIDVPKDIQQ  
QLVIPNWDQPMRLPGYMSRLPKLPNEMLLEQIVRLISESKKPVLVGGGCSQSSEELRRFVELTGIPVASTLMGLGAFPTGDE  
LSIQMLGMHGTVYANYAVDSSDLLLAFGVRFDDRVTKLEAFASRAKIVHIDSAEIGKNKQPHVSICADIKLALQGLNSIL  
EGKEGKMKLDFSARQELTEQKMYPLNFKTFGDAIPPPQYAIQVLDELTNGNAIISTGVGQHQMWAAQYYKKPRQWLTSGG  
LGAMGFGLPAIAVGAVGRGEIVVDIDGDGSFIMNVQELATIKVENLPVKIMLLNNQHLMVVQWEDRFYKANRAHTYLGDPS  
NEEEIFPNMLKFAEACGVPAARVSHRDDLRAAIQKMLDTPGPYLLDVIPHQEHLVPMIPSGGAFKDVITEGDGRCSY\*

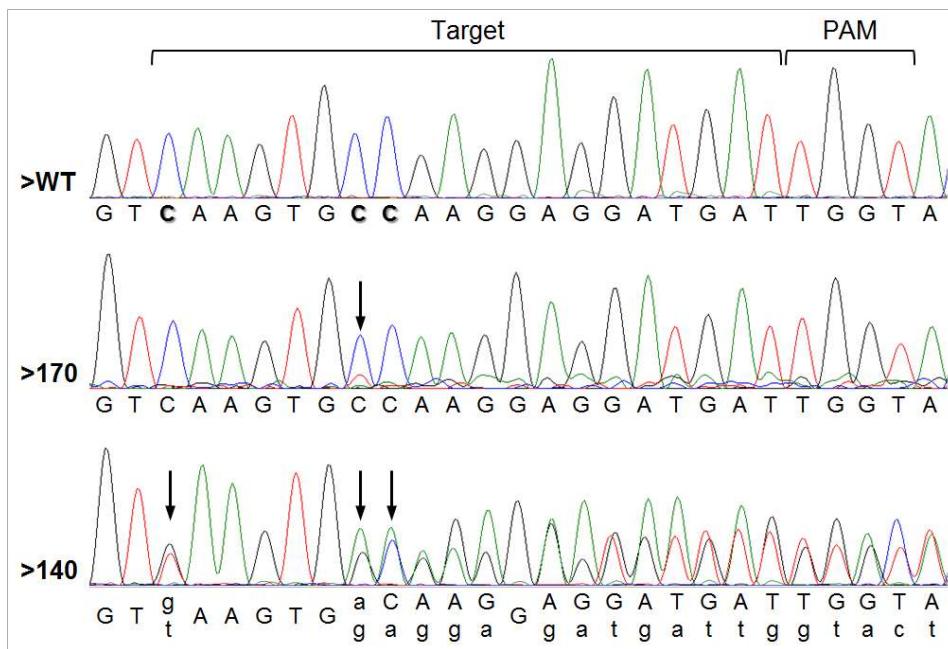
### Figure S2. *SIALS1* wild-type protein sequence

The SIALS1 Q184 and P186 are highlighted in purple.



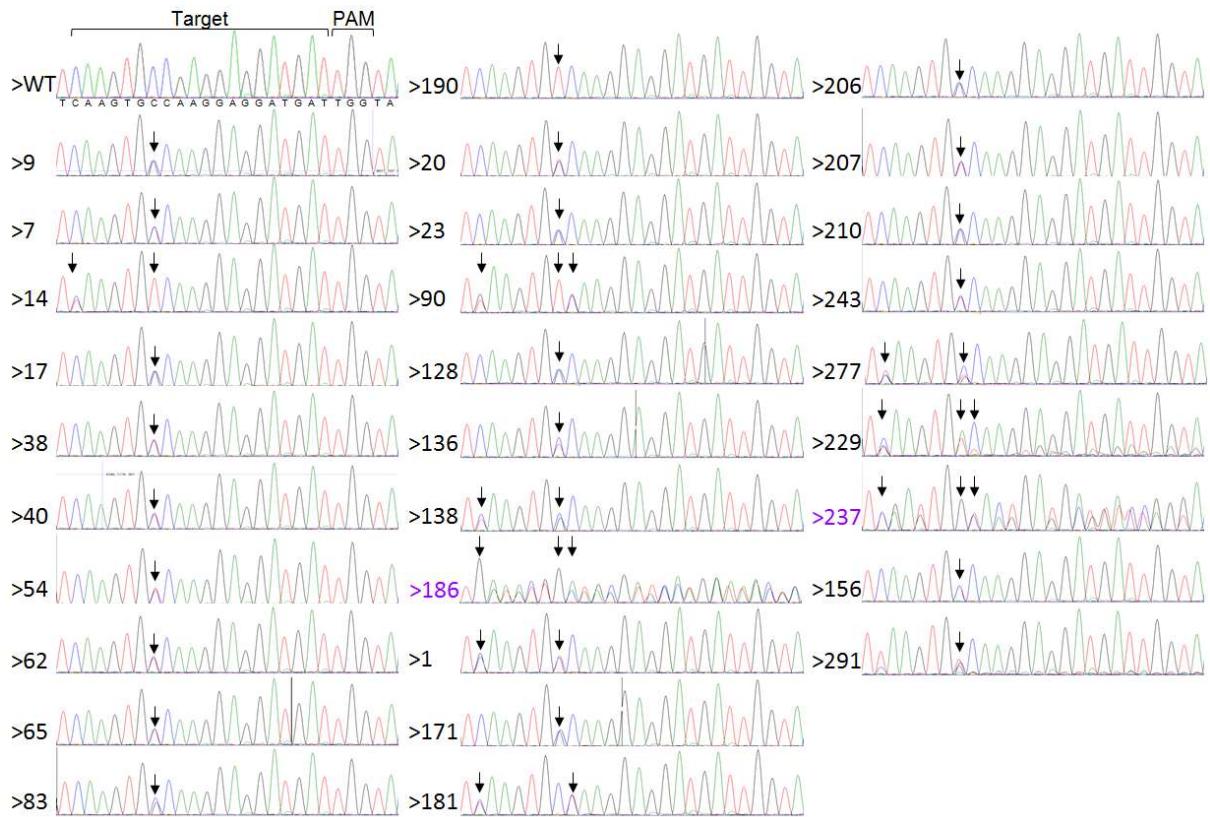
**Figure S3. Molecular analysis of thirty plants without T-DNA insertion**

Detection of the T-DNA was performed by multiplex PCR analysis on the thirty *SIALS1* 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 *SIALS1* edited plant containing a fragment of T-DNA (71) and two *SIALS1* edited plant containing a T-DNA (192, 175). L: molecular marker. (a) Amplification of the *SIALS1* gene and the *NptII* gene. (b) Amplification of the *SIALS1* gene and the *Cas9* gene. (c) Amplification of the *SIALS1* gene and the *AtU6*-gRNA backbone.



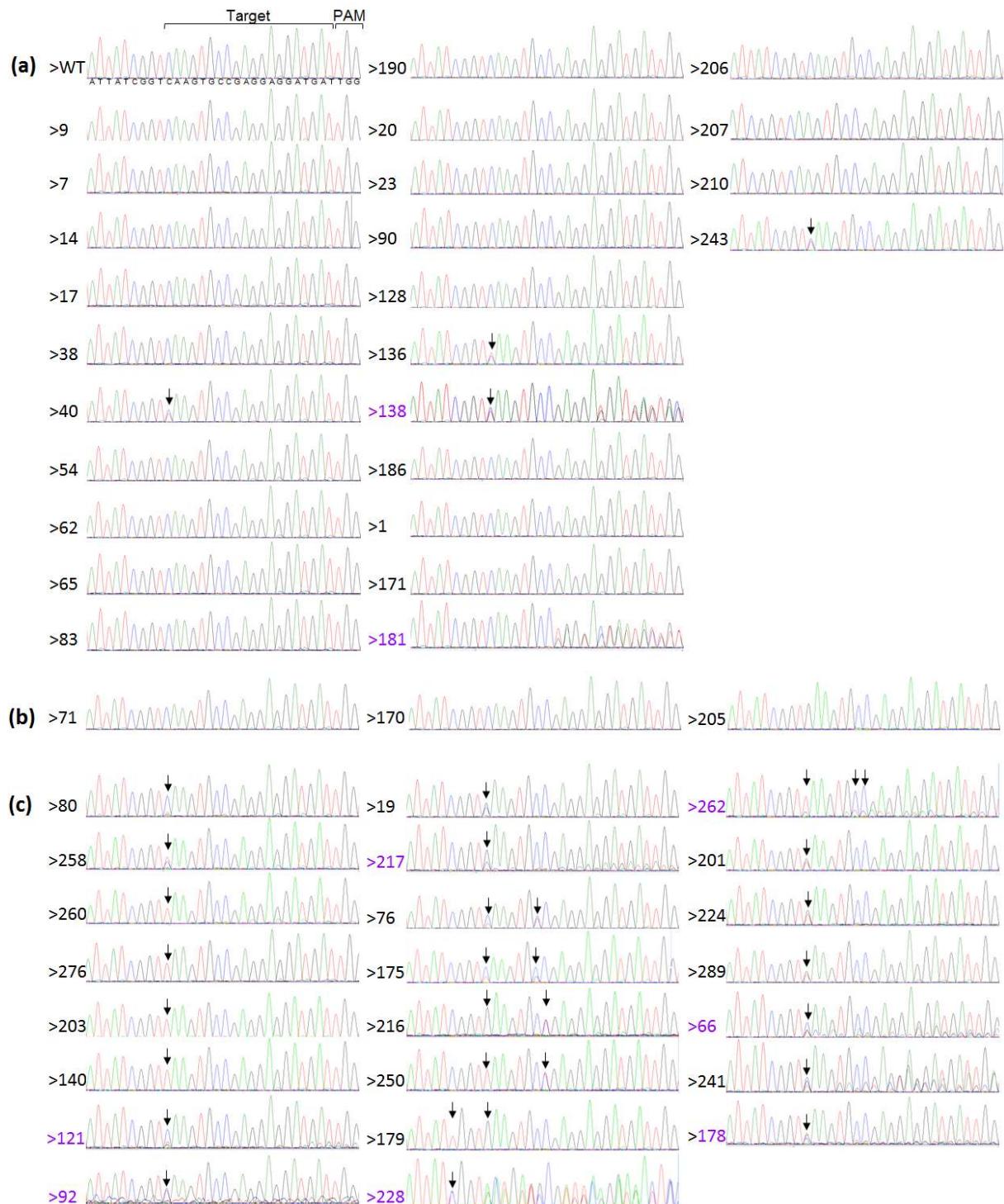
**Figure S4. Sequencing chromatograms of the targeted *SIALS1* region of the wild-type (WT), a potential chimerical event (170) and an indel event (140).**

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 SIALS1.F and SIALS1.R.



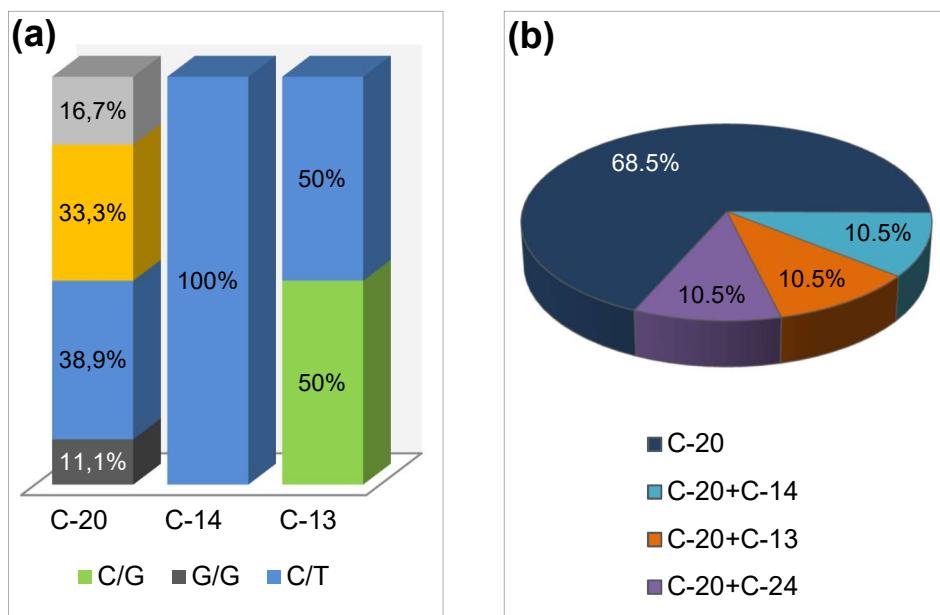
**Figure S5. Sequencing chromatograms of the targeted *SIALS1* region of the wild-type (WT) and the thirty *SIALS1* edited plants without T-DNA insertion**

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 *SIALS1.F* and *SIALS1.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 *St4LS2* locus**

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

> StALS1 gene sequence (PGSC0003DMG400034102)

**ATGGCGGCTGCTGCCCTACCATCTCCATGTTCTCAAACCCATCTCCATCTCCTCCAAATCTCCACCATTCTCTAGATCTACCTCC  
CTTTCACAAATCACCCCTCAAAAGCCTCACCCCTCATCTACCCACACCCATCATCGTCGTGGTTCGCCGTTCCAATGTGTCATATC  
CACTACCAACCCATAACGGAGCTTCTGAACCTGAAACATCTCGTCTCCCGTTCGCCCTGACGAACCCAGAAAGGGTTGTGATGTTCTGTGGAG  
GCACATGGAAGGGAGGGGGTACGGATGATATTGCGTACCCAGGGAGGTCTCATGGGATTCAGCAGGCTTGCACAGCTGAATTATTATTC  
GTAATGTCGCTGCCACGTCATGAGCAAGGTGCTGTTGCTGAGAGGGTTACGCACGGGACTGGTTCCCTGGTTGCTGATTGTCACCT  
TGGTCGGGGAGCTACGAATTCTGTTAGTGGTCTGGGATGCTTGTGGATAGTATTCCGATTGTCATTACGGGAAAGTGCCTGAGGGAG  
**ATGATTCG**TACTGATGCCCTTCAAGAACCCATTGTTGAGGTAAACGAGATCTATTACGAAGCATAATTATCTGTTAATGGATGAGGATA  
TTCTCTAGGGTTGTCGTGAAGCGTTTTCTAGCGAAATCGGGACGGCTGGCCGGTTTGATTGATGTCACCTAAGGATATTCAAGCAACAATT  
GGTGTACCTAATTGGATCAGCCAATGAGGTGCTGGTTACATGTCAGGTTACCTAAATTGCTTAATGAGATGCTTGGAAACAATTATT  
AGGCTGATTTCGGAGTCGAAGAACCTGTTTGATGTTGGTGTGGGTGTTGCAATCAAGTGAGGAGCTGAGACGATTGTCGGAGCTTACGG  
GTATTCTCTGTCGAGTACTTGTGATGGGCTTGGAGCTTCCAACTGGGAGTGGATCAGCTTCCCTCAAATGTTGGTATGTCATGGACTGTGA  
TGCTAAATTATGTCGTTGAGTGGTAGTGTGATTGTTGCTGCTGAGATTGGGAGAACAAGCAACCTCATGTTCCATTGTCGAGATTAATGGAAAGCTTGGCTAGC  
CGGGCGAAATTGTCATATTGGAGGGTAAGAAGGTAAGCTGAAGTTGAGCTTCTGCTTGGAGACAGGGAGTTAACGGAACAGAAGGTGA  
TACAGGTTGAAATTCCATTGGAGGGTAAGAAGGTAAGCTGAAGTTGAGCTTCTGCTTGGAGACAGGGAGTTAACGGAACAGAAGGTGA  
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TGTGCAAGGAGTTAGCAACAATTAGGTGAGAATCTCCAGTTAGATTGTCGAAATAACACTTGGGAATGGTGTCAATGGGAG  
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CTTGTGGCGTACCTGTCGAAGAGTGTACACAGGGATGATCTTAGAGCTGCCATTCAAAGATGTTAGACACTCCTGGGCCATACTGTTGGA  
TGTGATTGTCACCTCATCAGGAGCACGTTCTACCTATGATTCCCAGCAGGGCGGTGCTTCAAAAGATGTCGATCACAGAGGGTGA  
TGGGAGACGTTCC**

> StALS2 gene sequence (PGSC0003DMG400007078)

**AT**GGCGGCTGCATCTCCATCTCCTTGTCCCCAAAACCTACCTCATCTTCAAAATCTTCCATCCTCTTCCAAATCTACCTTACTT  
TCCACAATCCCCAAAAAATACCTCACCCCTCACCTTACCCACCCAAACATCATAGCGTTTCACTGTCTCAAATGTCTATCAACCAC  
GACCCATAACGACGTTCTGAACCCGAAATCTCGTTCACGTTGCCCTGACGAACCCAGAAAGGGTTGTATGTTCTGTGGAGGACTT  
GAAAGGGAGGGGTTAACGGATGTATTGCACTACCCAGGAGGTCTTCATCAGGGCTTGTGACACGTTCAATTATTTCGTAATG  
TGCCTGCCACGTCAAGACAGGGTGTGTTGCGAGGGTACCGCAGGGCACTGGGTTCTGTGTTGCACTACCTGGTCC  
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ATTTCGGAGTCGAAGAACGCTGTTGTATCTGGTGGGTGACACAATCGAGTGAGGAGCTGAGACGATTGTTGGAGCTTACGGTATT  
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TTATGCGGGTAGTAGTGTAGTTGCTGCTGGGGTAGGGTTGAGTAGTGATGAGTTACTGTGTTAATTGGAAAGCTTGTGAGCCAGCG  
AAAATTGTCACATGATTGATTGTCGGCTGAGATTGGAAAGAACAGAACCATGTTGCTGGAGATATCAAGTGGCATTACAGG  
GTTGAATTCCATATTGGAGGTTAACAGCTGAAGTGGACTTTCTGCTTGGAGGCGAGGACTAACGGAGCAAGGGTGAAGTACCC  
ATTGAATTATAAGACTTGGTGAAGCCATCCCTCCACAATATGCTATTAGGTTCTGTAGGTTAACTAACGGAAATGCCATTATTAGTACT  
GGTGTGGGCAACACCAAAATGTGGGCTGCCAATACTATAAGTACAAAAGCCACGCCATGGTTGACATCTGGTGGATTAGGAGCAATGGGAT  
TTGGTTTGCTGCTGCTATAGGTGGCGCTGTGGAAAGACGGGTGAGATTGTGGTTGACATTGATGGTACGGGAGTTTATCATGAATGTGCA  
GGAGTTAGCAACAAATTAGGTGGAAATCTCCAGTTAACATGTTACTGGTAAGATTATGTTACTGGTAATACACATTGGGAATGGTGGTCACTGG  
TCTATAAGGCTAACAGAGCACACACTTACGGGTAATCTCTCTAAATGAGGAGAAATCTCCCTAAATTGCTGAAATCTGGAGGCTTGTG  
GGCTACCTGCTGCAAGAGTGTACACAGGGATGATCTTAGAGCTGCCATTCAAAAGATGTTAGACACTCTGGGCAACTTGTGAGGAGTGTGAT  
TGTACCTCATCAGGAGCACGGTCTACCTATGATTCCAGTGGTGGCGCTTCAAGATGTTAGACGGAGGGTGTGGAGACGTTCTATTG**GA**

>StALS1 protein sequence (XP\_006361740)

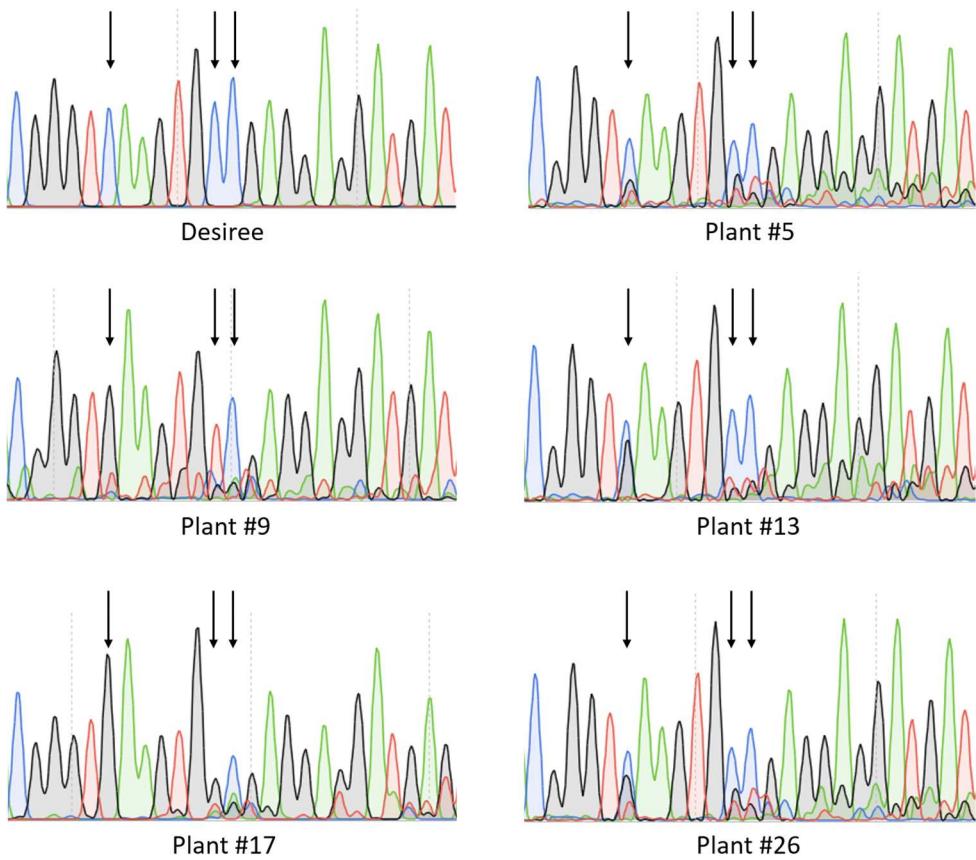
MAAASPSPCFSKTLPSSKSSTLPRSTPFFHNPQKASPLHLTHHHRRGFAVSNVIISTTHNDVSEPETFVSRFAPDEPRKGCDVILE  
ALEREGVTDFVFAYPGGASMIEHQALTRSNIIRNVLPRHEQGGVFAAEQYARATGFPGVCIATSGPGATNLVSGGLADALLDSIPIVAITGQVERR  
MIGTDAFQETPIVEVTRSIKHNLYLMDVEDIPRVVREAFFLAKSGRGPGVLIIDVPKDIIQQQLVIPNWDQPMRLPGYMSRLPKLPNEMLLEQII  
RLISESKKPVLVGGGLQSSEELRFRVELTGIPIVASTLMGLAFPTGDELSLQMLGMHGTVYANYAVDGSLLLAFGVRFDDRTVGKLEAFAS  
RAKIVHIDIDSIEGKKNQPHVSICADIKLALQGLNSILEGKEGKLKLDFAWRQELTEQKVKYPLSFKTFGEAIPPQYAIQLVDELTNNGNAII  
STGVGQHQMWAAQYYKYKKPRQWLTSGLGAMGFGPLPAAIAGAVERPGEIVVDDIDGDSFIMNVQELATIKVENLPVKIMLLNNQHLMGVVQWE  
Y

>StALS2 protein sequence (XP\_006348357)

MAAASPSPCFSKNLPSSSSKSSILLPKSTFTFHNPKNTPSLHLHTQHHSRFTVSNVILSTTHNDVSEPEIFVSRFAPDEPRKGCDVLVEALE  
EREGVKDVFAYPGGASMEIHQALTRSNIIRNVLPRHEQGGVFAAEGYARATGFPVGCIATSGPATNLVGLADALLDSIPIVAITGOPRRMI  
GTDAFQETPIVEVTRSIITKHNYLVMVEDIPRVVREAFFFLAKSGRGPGVLDVPKDIQQQLVIPNWDPQMRLLPGYISRLPKLPNEMLLEQIVRL  
ISESKKPVLVGGGCTQSSEELRRFVELTJGIPVASTLMLGLTFPCGDELSLQMLGMHGTVYANYAVDSDLLAFGVRFDRVTGKLEAFASRA  
KIVHIDIDSÆIGKNGKQPHVSICADIKLALQGLNSILEGKEGKLKDLSAWRQELETQEKVVKPLNYKTGEAPIPPQYAQLVWDELTINGNAIIST  
GVGQHQMWAAQYYKKKPRWLTSGLLGAMGFGPLAAGIAAVGRPGEIVVWDIDGDSFIMVNQELATKVENLPVKIMLNNQHGLMVQWEDR  
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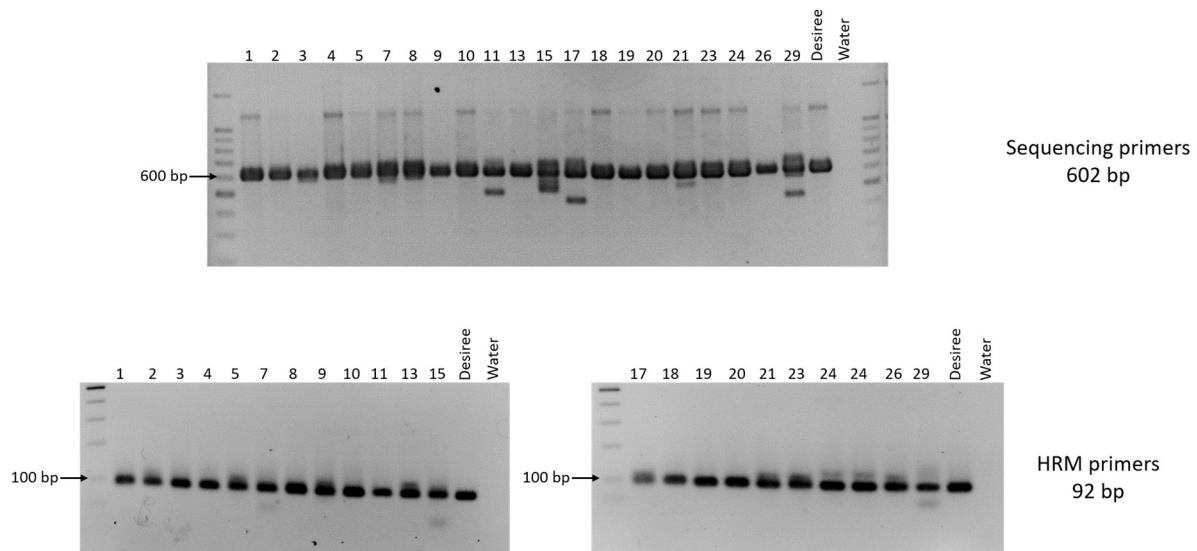
**Figure S8: Acetolactate synthase StALS1-2 gene and StALS1-2 protein sequences**

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. The StALS1 Q184 and P186 are highlighted in purple.



**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>SlALS1</i> gene	→	GGCACTTGAAAGGGAAAGG	891
	←	CCAATCTCTGCCGAATCAAT	
PCR detection of the <i>nCAS9</i> gene	→	TCCCTTACTACGTGGGACCTC	260
	←	CATTCCCTCGGTACGTACT	
PCR detection of the <i>NptII</i> gene	→	AGACAATCGGCTGCTCTGAT	593
	←	AGCCAACGCTATGTCCTGAT	
PCR detection of the <i>AtU6</i> -gRNA backbone	→	TCCCAGGATTAGAATGATTAGG	347
	←	CGGTGCCACTTTTCAAGTT	
HRM detection of the <i>SlALS1</i> gene	→	CTTGTAGTGGTCTTGCAGATG	104
	←	GTTTCCTGGAACGCATCAGTACC	
Sequencing of the <i>SlALS1</i> gene	SlALS1.F	GTGGTTTGCCGTTGCCAAT	761
	SlALS1.R	CAGCTCCTCACTTGATTGCG	
Sequencing of the <i>SlALS2</i> gene	SlALS2.F	GCCGTTCACTGTTCAAAT	761
	SlALS2.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	→	GATGTTCTTGTGGAGGCAC TTG	602
	←	TTCTTCGACTCCGAAATCAGC	
PCR detection of the <i>nCAS9</i> gene	→	ACTTTTCGTTGAGCAGCACAA	350
	←	GATCAGCCCTTGAATCACCA	
PCR detection of the <i>NptII</i> gene	→	GATGGATTGCACGCAGGTTC	406
	←	GATGTTTCGCTTGGTGGTCG	
PCR detection of the <i>AtU6</i> -gRNA backbone	→	AAACATCATCCTCCTCGGCAC TTG	207
	←	GATAATCTTCAAAAGGCCCTGG	
PCR detection of the control gene ( <i>StGBSS1</i> )		AGACCACACATCACATGGCAA	352
		CTTACTGCAAGGGCTGGTGG	
HRM of the <i>StALS1</i> and <i>StALS2</i> genes	→	CTTGC GGATGCTTGTGG	92
	←	GTTTCCTGAAACGCATCAGTACC	