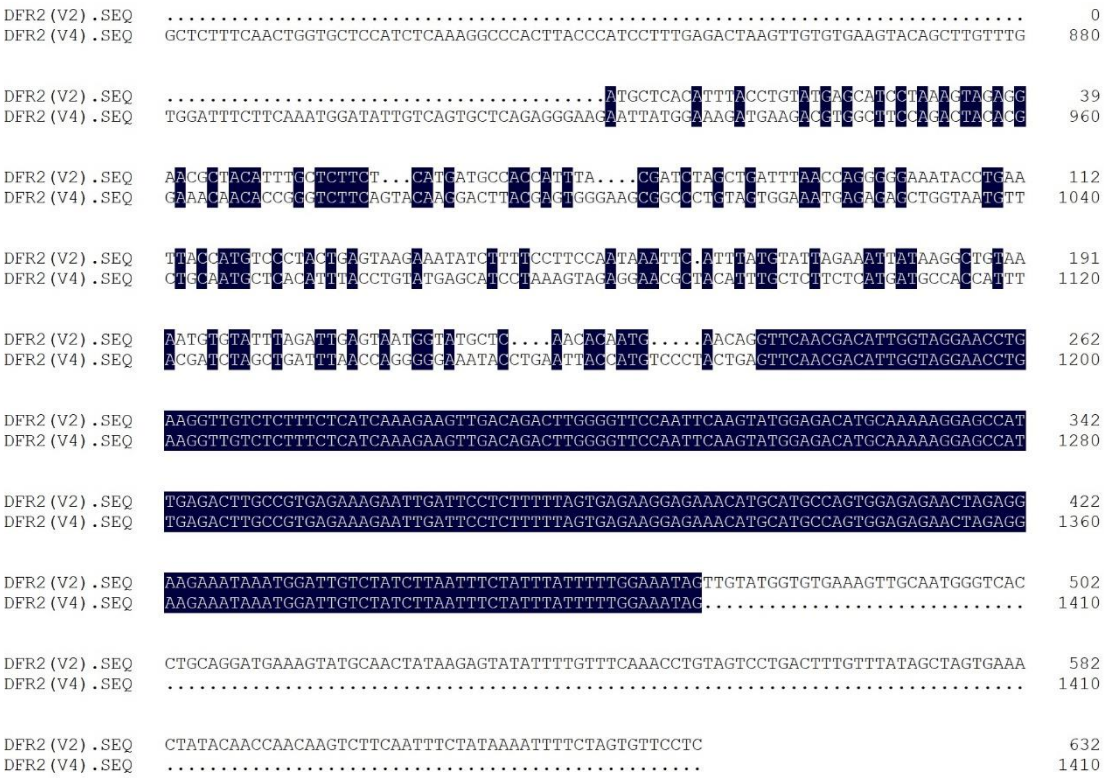


Fig. S1 Sequence alignment of the coding sequence of the *DFR2* gene in different genome annotations. The gene sequences from commonly used version 2 (V2) and newly annotated version 4 (V4) grape genomes were used for alignment.

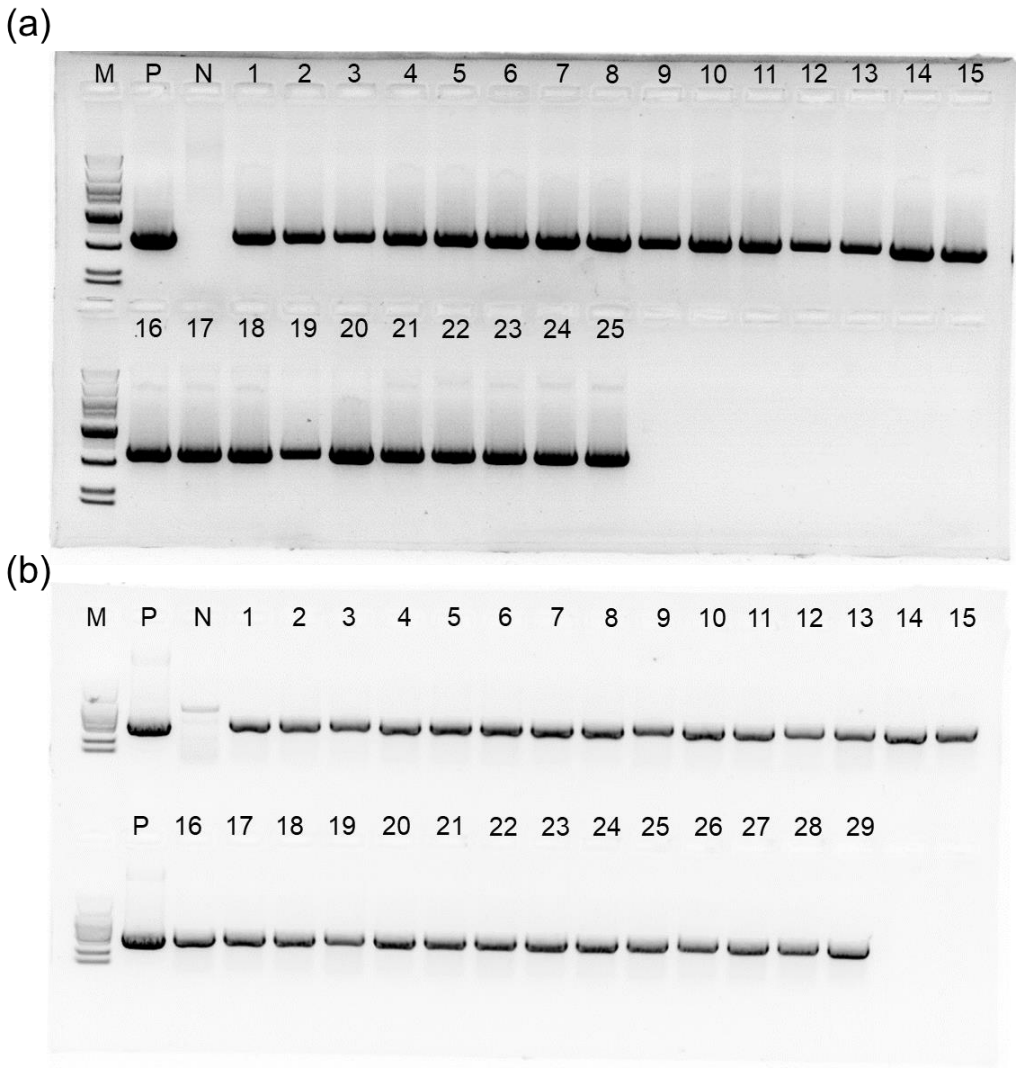
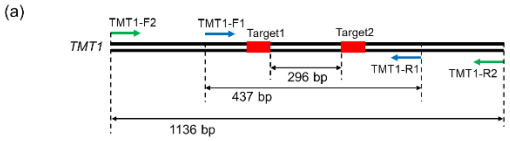
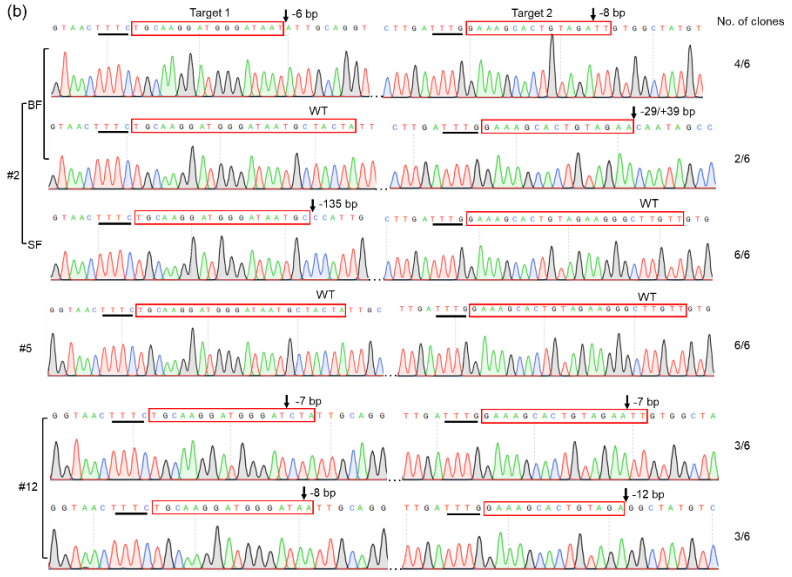


Fig. S2 PCR identification of transgenic plants regenerated from 41B embryogenic grape cells transformed with LbCas12a-TMT1 without (a) or with (b) heat treatment. The constructed plasmid and genomic DNA from wile-type plant were used as positive (P) and negative (N) controls, respectively. Lanes 1-25 in (a) and Lanes 1-29 in (b) represent independent regenerated plants.





	Target 1	Target 2	Mutation	No. of clones
#3	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAA----------		-6/-13 bp	2/6
	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-6 bp/WT	4/6
#4	TAACTTTCTGCAAGGATGGGATAATG---CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGC---TTGTG		-3/-3 bp	3/6
	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGC----TGTG		-6/-4 bp	3/6
#6	TAACTTTCTGCAAGGATGGGATAAT--------/290bp/ATTTGGAAAGCACTGTAGAAG------TTGTG		-8/-6 bp	1/6
	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT---TGTG		-6/-3 bp	3/6
	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT----GTG		-5/-4 bp	2/6
#7	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGC----TGTG		-5/-4 bp	5/6
	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT---TGTG		-3/-3 bp	1/6
#8	TAACTTTCTGCAAGGATGGGATAAT-------T/290bp/ATTTGGAAAGCACTGTAGAAGGGC----TGTG		-7/-4 bp	3/6
	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		-3/-5 bp	3/6
#9	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT---TGTG		-5/-3 bp	2/6
	TAACTTTCTGCAAGGATGGGATA----TACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGG-----TGTG		-4/-5 bp	4/6
#10	TAACTTTCTGCAAGGATGGGATA---------T/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-9 bp/WT	2/6
	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAG------TTGTG		-3/-6 bp	4/6
#11	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT----GTG		-3/-4 bp	1/6
	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-6 bp/WT	2/6
	TAACTTTCTGCAAGGATGGGATAA----ACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTT---GTG		-4/-3 bp	2/6
#13	TAACTTTCTGCAAGGATGGGATAATGCTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		WT/WT	6/6
#14	TAACTTTCTGCAAGGATGGGATAAT--------/290bp/ATTTGGAAAGCACTGTAGAAG------TTGTG		-8/-6 bp	2/6
	TAACTTTCTGCAAGGATGGGATAAT-------T/290bp/ATTTGGAAAGCACTGTAGAAGGG-----TGTG		-7/-5 bp	4/6
#15	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-6 bp/WT	5/6
	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		-3/-5 bp	1/6
#16	TAACTTTCTGCAAGGATGGGATAA----ACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGC---TTGTG		-4/-3bp	4/6
	TAACTTTCTGCAAGGATGGGATAATG-----AT/290bp/ATTTGGAAAGCACTGTAGAA------GTTGTG		-5/-6bp	1/6
	TAACTTTCTGCAAGGATGGGATAATG---CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-3 bp/WT	1/6
#17	TAACTTTCTGCAAGGATGGGATAAT-------T/290bp/ATTTGGAAAGCACTGTAG------TTGTTGTG		-7/-6bp	3/6
	TAACTTTCTGCAAGGATGGGA--------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGG----TTGTG		-8/-4 bp	3/6
#18	TAACTTTCTGCAAGGATGGGAT---GCTACTAT/290bp/ATTTGGAAAGCACTGTAGAAG------TTGTG		-3/-6bp	5/6
	TAACTTTCTGCAAGGATGGGATAATGCTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		WT/-5 bp	1/6
#19	TAACTTTCTGCAAGGATGGGATAATGCTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		WT/WT	1/6
	TAACTTTCTGCAAGGATGGGATAAT-------T/290bp/ATTTGGAAAGCACTGTAG------TTGTTGTG		-7/-6bp	4/6
	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		-3/-5 bp	1/6
#20	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT---TGTG		-5/-3 bp	3/6
	TAACTTTCTGCAAGGATGGGATA----TACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGG-----TGTG		-4/-5 bp	3/6
#21	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		-5/-5 bp	1/6
	TAACTTTCTGCAAGGATGGGATA-------TAT/290bp/ATTTGGAAAGCACTGTAGAAGGG-----TGTG		-7/-5 bp	5/6
#22	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGC----TGTG		-6/-4 bp	3/6
	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCT---TGTG		-5/-3 bp	3/6
#23	TAACTTTCTGCAAGGATGGGATA------CTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGC----TGTG		-6/-4 bp	4/6
	TAACTTTCTGCAAGGATGGGATAATG-----AT/290bp/ATTTGGAAAGCACTGTAG------TTGTTGTG		-5/-6 bp	2/6
#24	TAACTTTCTGCAAGGATGGGATA---------T/290bp/ATTTGGAAAGCACTGTAGAA-------TTGTG		-9/-7 bp	2/6
	TAACTTTCTGCAAGGATGGGATAATG----TAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-4 bp/WT	4/6
#26	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-3 bp/WT	2/6
	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAGAAGG----GTTGTG		-5/-4 bp	2/6
	TAACTTTCTGCAAGGATGGGATA---------T/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		-9/-5 bp	1/6
	TAACTTTCTGCAAGGATGGGAT--------TAT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-8 bp/WT	1/6
#27	TAACTTTCTGCAAGGATGGGATAAT--------/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-8 bp/WT	1/6
	TAACTTTCTGCAAGGATGGGATA-------TAT/290bp/ATTTGGAAAGCACTGTAGAAGGG----TTGTG		-7/-4 bp	1/6
	TAACTTTCTGCAAGGATGGGATAAT------AT/290bp/ATTTGGAAAGCACTGTAGAAGGGC-----GTG		-6/-5 bp	3/6
	TAACTTTCTGCAAGGATGGGAT---------AT/290bp/ATTTGGAAAGCACTGTAGAAGGGCTTGTTGTG		-9 bp/WT	1/6
#28	TAACTTTCTGCAAGGATGGGATA---CTACTAT/290bp/ATTTGGAAAGCACTGTAGAAGG-----TTGTG		-3/-5bp	5/6
	TAACTTTCTGCAAGGATGGGATAA----ACTAT/290bp/ATTTGGAAAGCACTGTAGAAGGGTCCGCCTCA		-4/-14,+1 bp	1/6
#29	TAACTTTCTGCAAGGATGGGATAATG--ACTAT/290bp/ATTTGGAAAGCACTGTAGAA--------TGTG		-2/-8 bp	2/6
	TAACTTTCTGCAAGGATGGGAT-----------/290bp/ATTTGGAAAGCACTGTAGAAGGG----TTGTG		-11/-4 bp	1/6
	TAACTTTCTGCAAGGATGGGATAA-----CTAT/290bp/ATTTGGAAAGCACTGTAG----------TGTG		-5/-10 bp	2/6
	TAACTTTCTGCAAGGATGGGATAA----ACTAT/290bp/ATTTGGAAAGCACTGTAGAAG-------TGTG		-4/-7 bp	1/6

Fig. S3 Sequencing results of *TMT1* editing in LbCas12a-TMT1 grapevine plants. The results obtained with transgenic plants after HT are shown as examples. (a) Schematic illustration of primers design for amplification of target sequences of *TMT1* gene. (b) Sanger sequencing results of the two targets in grapevine plants. Representative sequencing chromatograms as well as identified sequences are shown. The PAM sequences are underlined and the targets are shown in red box in chromatograms. For each plant, 6 clones of amplicons were randomly selected and analyzed by Sanger sequencing. Mutation types and corresponding number of clones are shown on the right.

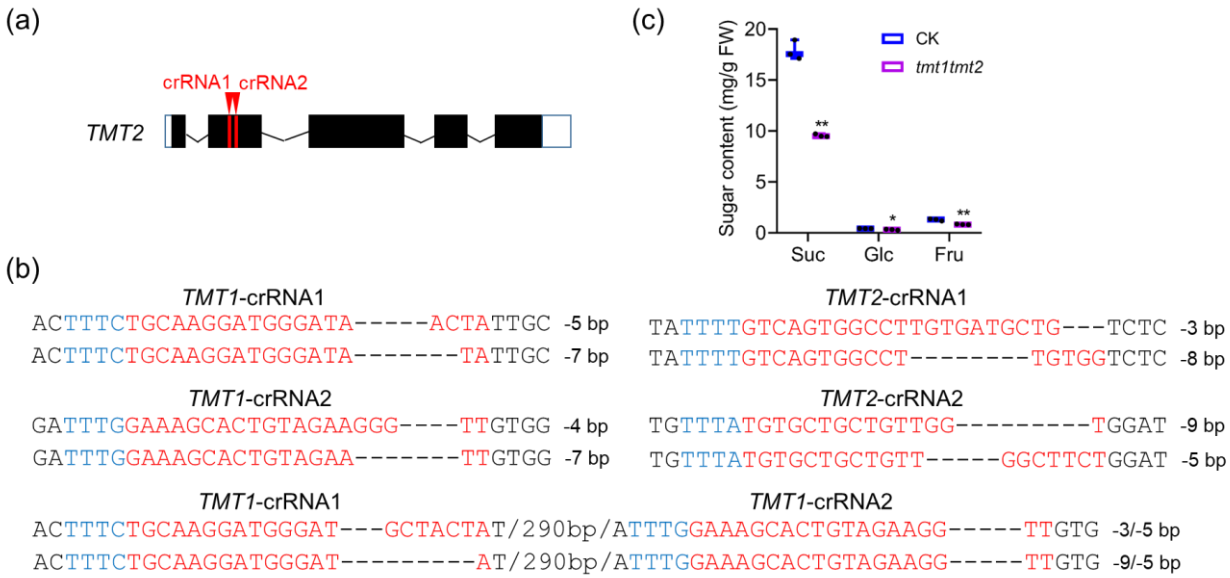


Fig. S4 Knockout of *TMT1* and *TMT2* genes in grape. (a) Schematic illustration of the targets design for *TMT2*. (b) Representative mutated sequences detected at the four targets in *TMT1* and *TMT2*. (c) Sugar content in *tmt1tmt2* cells. The cells transformed with empty vector were used as the control (CK). The significant differences are determined by Student's *t*-test. * $P < 0.05$; ** $P < 0.01$. Suc, sucrose; Glc, glucose, Fru, fructose.

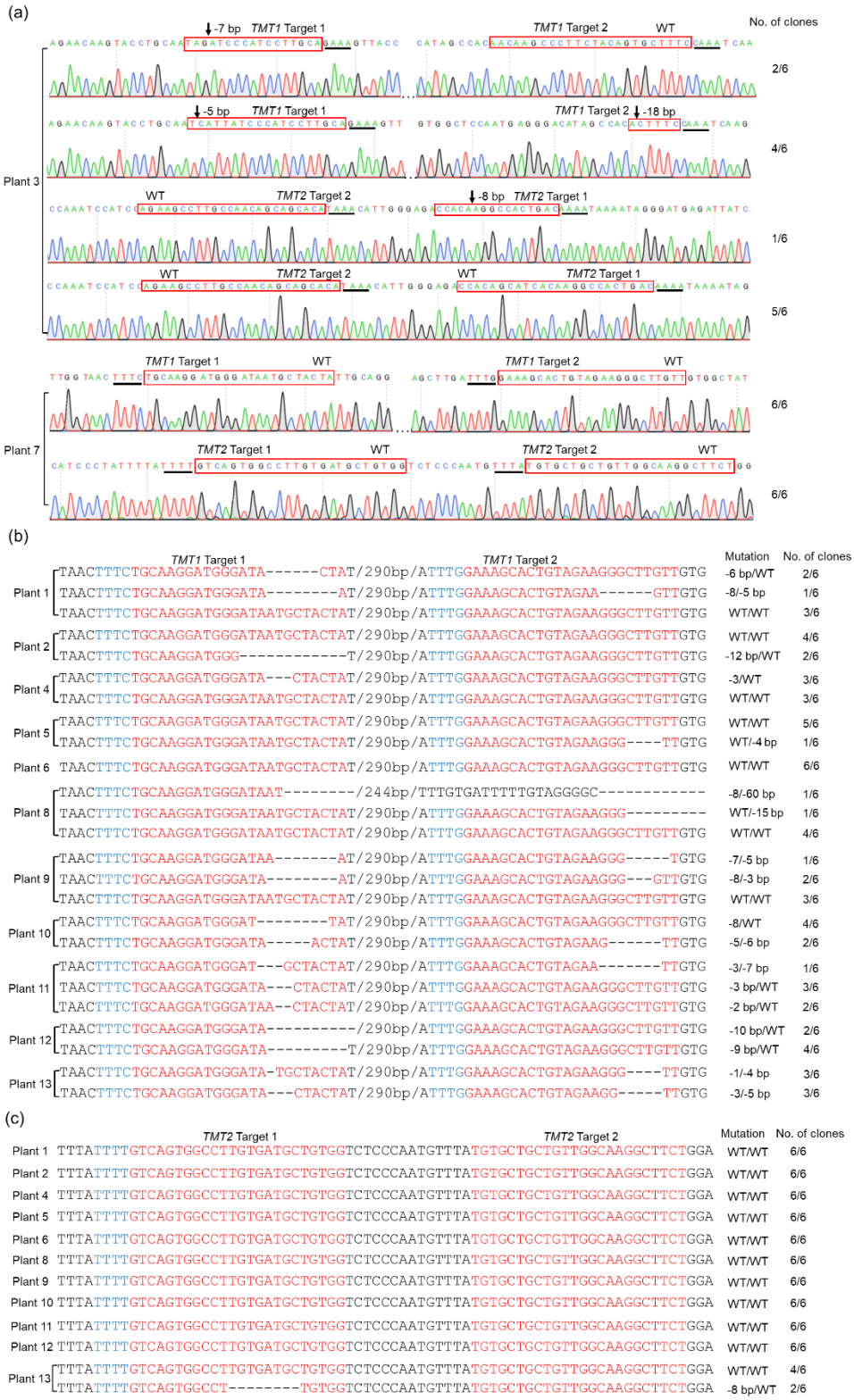


Fig. S5 Sequencing results of *TMT1* and *TMT2* editing in LbCas12a-TMTs plants. Representative sequencing chromatograms (a) and identified sequences for each plant (b and c) are shown. The PAM sequences are underlined and the targets are shown in red box in chromatograms. For each plant, 6 clones of amplicons were randomly selected and analyzed by Sanger sequencing. Mutation types and corresponding number of clones are shown on the right.

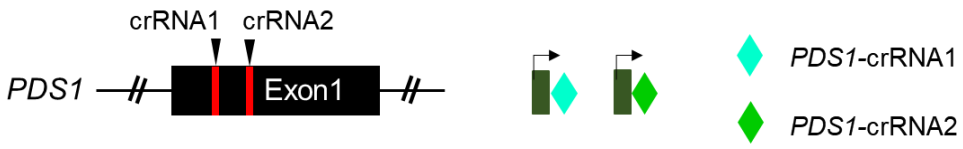


Fig. S6 Schematic illustration of the targets design for *PDS1*.

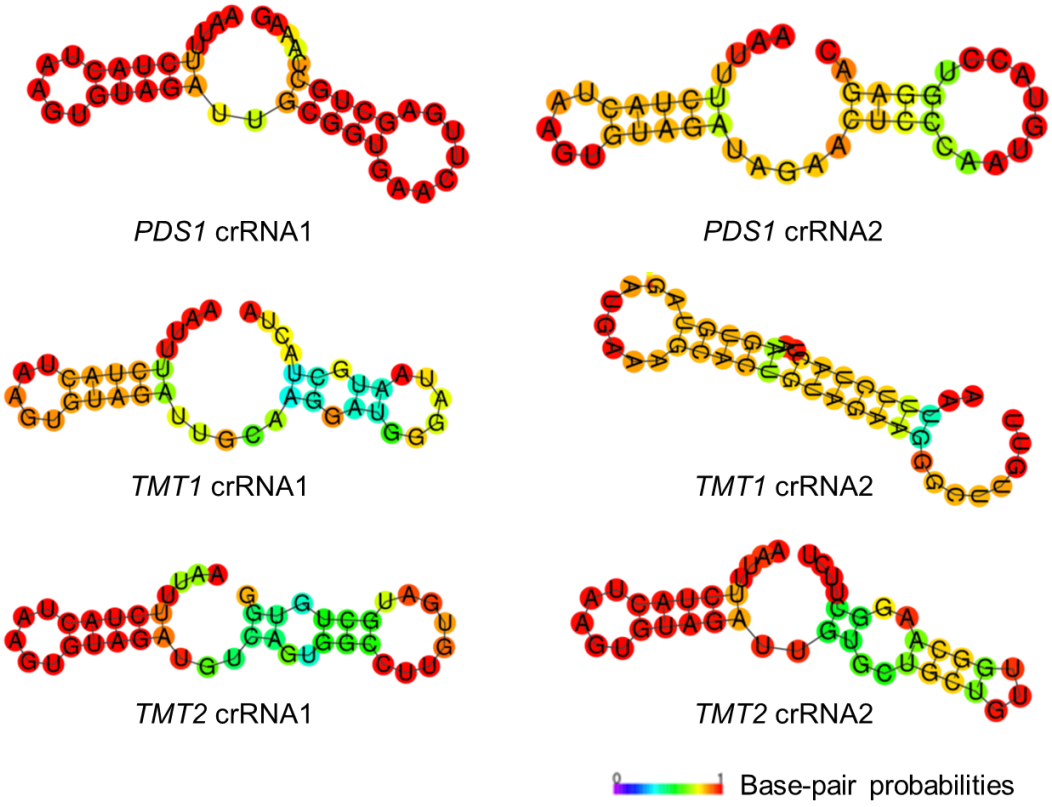


Fig. S7 Predication of the secondary structures of *PDS1* and *TMTs* crRNAs. The RNA secondary structures were predicted using *RNAfold* web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) with default parameters.

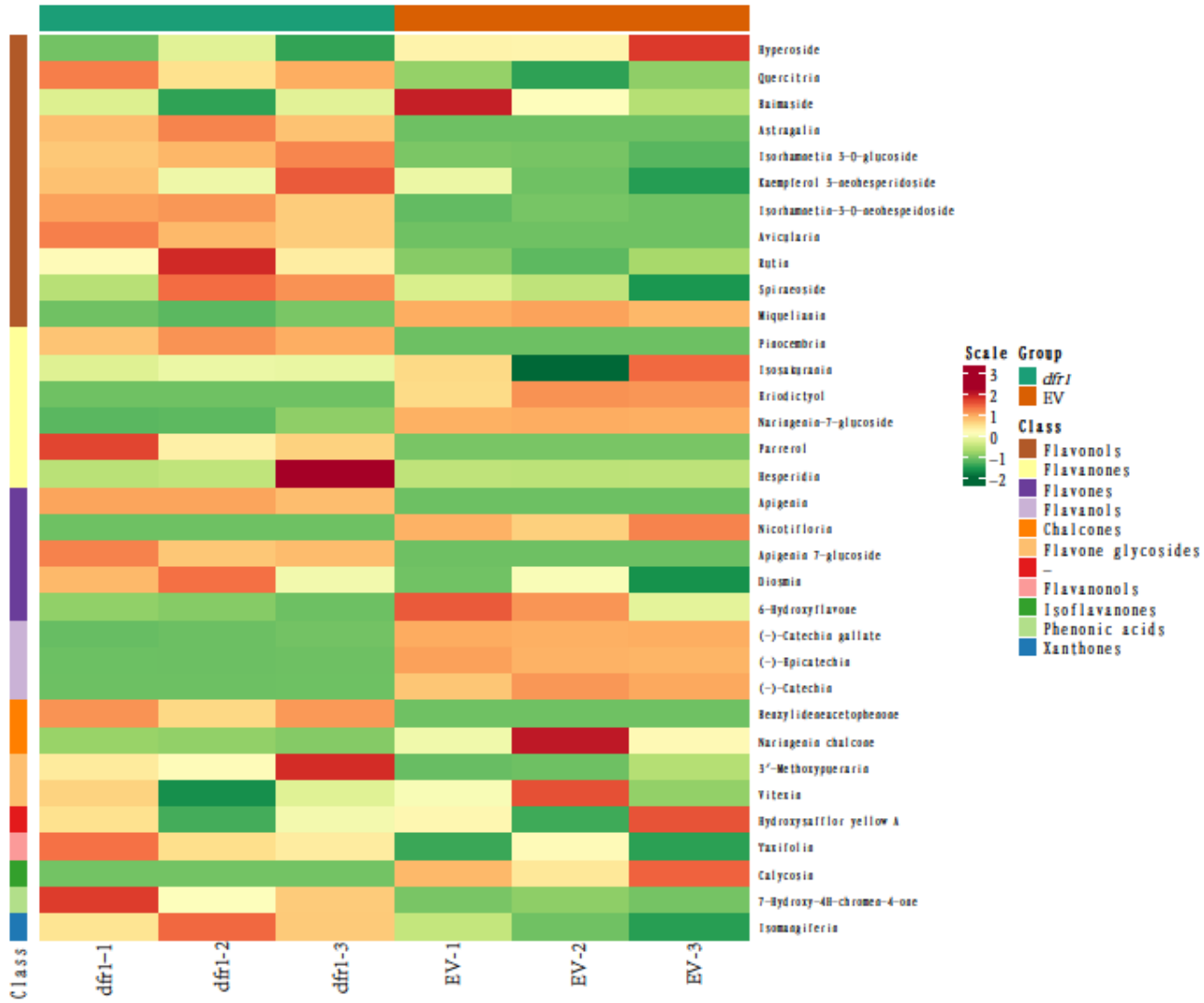


Fig. S8 Heatmap of the determined compounds in *dfr1* and EV cells. The log2-transformed values were visualized as the heatmap using *R*.

VvU3.1-*TMT1* crRNAs:

AAGCTTAGTACTTTCATAGGAATAGGTTTCAAATGAACCTTTGTGATACACTTCGATCCTGACTCTCTCTAAAAGCAAA
AATCATTAATATTTTTTCAATTATATTTTAATTTTTACAAATACTAACAAATATTAATAATTCTAATATCTTTCTTGTT
TTGAAAAATAAAAGAAAAATAATAATGTTTGGTATATTCCGTATATATTATTTTAAATGAATCCAGAAGTTTCCAAGAA
TTTCACTGGCAATCAATCGTGCATCAGCTGTCAATCGTTGTTCCCAGGAAGGCTCATTGGAAGTCTATAACCAATGAGA
ACACGCGGTGACTAGCCGTCCCACATCGAAAATGCAGGAAACATTTAATAACTATATAACAAAGGATAGGAGATTCACA
TGCCAATTTCTACTAAGTGTAGATTGCAAGGATGGGATAATGCTACTAAATTTCTACTAAGTGTAGATGAAAGCACTGT
AGAAGGGCTTGTTTTTTTTAAGCTT

VvU3.1-*TMTs* crRNAs:

AAGCTTAGTACTTTCATAGGAATAGGTTTCAAATGAACCTTTGTGATACACTTCGATCCTGACTCTCTCTAAAAGCAAA
AATCATTAATATTTTTTCAATTATATTTTAATTTTTACAAATACTAACAAATATTAATAATTCTAATATCTTTCTTGTT
TTGAAAAATAAAAGAAAAATAATAATGTTTGGTATATTCCGTATATATTATTTTAAATGAATCCAGAAGTTTCCAAGAA
TTTCACTGGCAATCAATCGTGCATCAGCTGTCAATCGTTGTTCCCAGGAAGGCTCATTGGAAGTCTATAACCAATGAGA
ACACGCGGTGACTAGCCGTCCCACATCGAAAATGCAGGAAACATTTAATAACTATATAACAAAGGATAGGAGATTCACA
TGCCAATTTCTACTAAGTGTAGATTGCAAGGATGGGATAATGCTACTAAATTTCTACTAAGTGTAGATGAAAGCACTGT
AGAAGGGCTTGTTAATTTCTACTAAGTGTAGATGTCAGTGGCCTTGTGATGCTGTGGAATTTCTACTAAGTGTAGATTG
TGCTGCTGTTGGCAAGGCTTCTTTTTTTAAGCTT

The reverse *TMTs*-crRNAs:

AAGCTTAGTACTTTCATAGGAATAGGTTTCAAATGAACCTTTGTGATACACTTCGATCCTGACTCTCTCTAAAAGCAAA
AATCATTAATATTTTTTCAATTATATTTTAATTTTTACAAATACTAACAAATATTAATAATTCTAATATCTTTCTTGTT
TTGAAAAATAAAAGAAAAATAATAATGTTTGGTATATTCCGTATATATTATTTTAAATGAATCCAGAAGTTTCCAAGAA
TTTCACTGGCAATCAATCGTGCATCAGCTGTCAATCGTTGTTCCCAGGAAGGCTCATTGGAAGTCTATAACCAATGAGA
ACACGCGGTGACTAGCCGTCCCACATCGAAAATGCAGGAAACATTTAATAACTATATAACAAAGGATAGGAGATTCACA
TGCCAATTTCTACTAAGTGTAGATTGTGCTGCTGTTGGCAAGGCTTCTAATTTCTACTAAGTGTAGATGTCAGTGGCCT
TGTGATGCTGTGGAATTTCTACTAAGTGTAGATGAAAGCACTGTAGAAGGGCTTGTTAATTTCTACTAAGTGTAGATTG
CAAGGATGGGATAATGCTACTA
TTTTTTAAGCTT

The reverse *PDS1-TMTs*-crRNAs:

AAGCTTAGTACTTTCATAGGAATAGGTTTCAAATGAACCTTTGTGATACACTTCGATCCTGACTCTCTCTAAAAGCAAA
AATCATTAATATTTTTTCAATTATATTTTAATTTTTACAAATACTAACAAATATTAATAATTCTAATATCTTTCTTGTT
TTGAAAAATAAAAGAAAAATAATAATGTTTGGTATATTCCGTATATATTATTTTAAATGAATCCAGAAGTTTCCAAGAA
TTTCACTGGCAATCAATCGTGCATCAGCTGTCAATCGTTGTTCCCAGGAAGGCTCATTGGAAGTCTATAACCAATGAGA
ACACGCGGTGACTAGCCGTCCCACATCGAAAATGCAGGAAACATTTAATAACTATATAACAAAGGATAGGAGATTCACA
TGCCAATTTCTACTAAGTGTAGATTGCGGTGAACTTGAGCTGCCAAAGAATTTCTACTAAGTGTAGATAGAACTCCCAA
TGTACCTGGAGACAATTTCTACTAAGTGTAGATTGTGCTGCTGTTGGCAAGGCTTCTAATTTCTACTAAGTGTAGATGT
CAGTGGCCTTGTGATGCTGTGGAATTTCTACTAAGTGTAGATGAAAGCACTGTAGAAGGGCTTGTTAATTTCTACTAAG
TGTAGATTGCAAGGATGGGATAATGCTACTATTTTTTAAGCTT

Fig. S9 Sequences of *TMT1*, *TMTs* (*TMT1* and *TMT2*), reverse *TMTs* and reverse *PDS1-TMTs* crRNAs expression cassettes. The VvU3.1 promoter is indicated in blue and DR sequences are denoted in red. The two designed *TMT1* crRNA targets are indicated in green and purple, and the two crRNA targets for *TMT2* are indicated in orange and light

blue, respectively. The two crRNA targets for *PDS1* are indicated in black and pink, respectively. The *Hin*dIII recognition sites are highlighted in yellow.

VvU3.1-*DFR1* crRNAs:

AAGCTTAGTACTTTCATAGGAATAGGTTTCAAATGAACCTTTGTGATACACTTCGATCCTGACTCTCTCTAAAAGCAAA
AATCATTAATATTTTTTCAATTATATTTTAATTTTTACAAATACTAACAAATATTAATAATTCTAATATCTTTCTTGTT
TTGAAAAATAAAAGAAAAATAATAATGTTTGGTATATTCCGTATATATTATTTTAAATGAATCCAGAAGTTTCCAAGAA
TTTCACTGGCAATCAATCGTGCATCAGCTGTCAATCGTTGTTCCCAGGAAGGCTCATTGGAAGTCTATAACCAATGAGA
ACACGCGGTGACTAGCCGTCCCACATCGAAAATGCAGGAAACATTTAATAACTATATAACAAAGGATAGGAGATTCACA
TGCCAATTTCTACTAAGTGTAGATTTGCTTCCTCGCCGATTCCAGGATAATTTCTACTAAGTGTAGATATCGGTTCATG
GCTGGTCATGAGGTTTTTTAAGCTT

Fig. S10 Sequence of *DFR1* crRNAs expression cassette. The VvU3.1 promoter is indicated in blue and DR sequences are denoted in red. The two designed crRNA targets for *DFR1* are underlined. The *Hin*dIII recognition sites are highlighted in yellow.