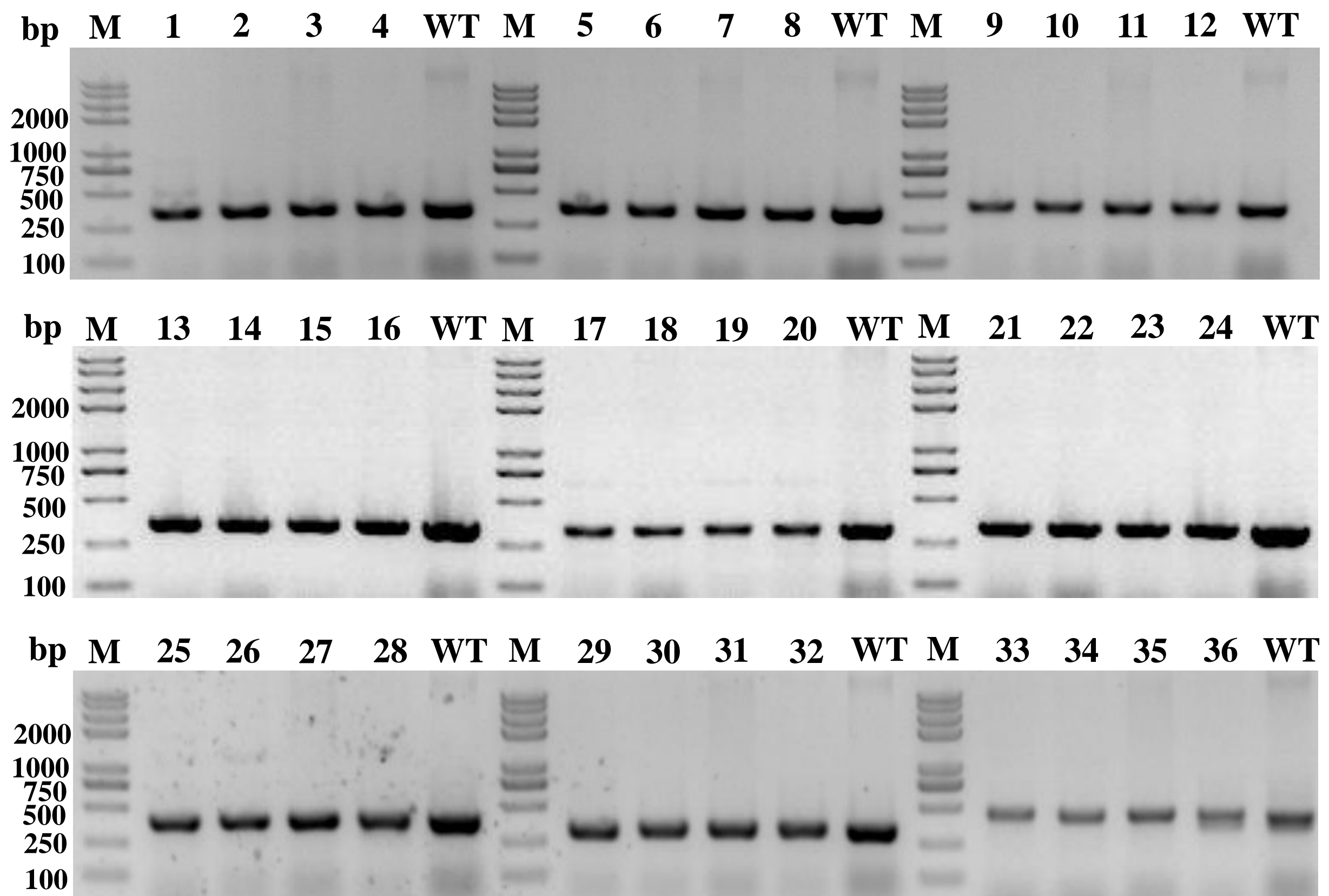


**A****B****gRNA1 off-target sites****Vigun05g271100**

gRNA1 off-target site 1

WT 5'—CTGGCTTACTGCTAC **ATTA**ACTAAATATTACACAAGG**TAG**AGCGCTTCCTGA—3'

Line1 CTGGCTTACTGCTAC **ATTA**ACTAAATATTACACAAGG**TAG**AGCGCTTCCTGA

Line3 CTGGCTTACTGCTAC **ATTA**ACTAAATATTACACAAGG**TAG**AGCGCTTCCTGA

Line18 CTGGCTTACTGCTAC **ATTA**ACTAAATATTACACAAGG**TAG**AGCGCTTCCTGA

Line29 CTGGCTTACTGCTAC **ATTA**ACTAAATATTACACAAGG**TAG**AGCGCTTCCTGA

**Vigun11g100700**

gRNA1 off-target site 2

WT 5'—AAAGGAAAAGGGAAA **ATATCAA**ACACATTACACATGG**CTT**CCACTTGATGAA—3'

Line1 AAAGGAAAAGGGAAA **ATATCAA**ACACATTACACATGG**CTT**CCACTTGATGAA

Line3 AAAGGAAAAGGGAAA **ATATCAA**ACACATTACACATGG**CTT**CCACTTGATGAA

Line18 AAAGGAAAAGGGAAA **ATATCAA**ACACATTACACATGG**CTT**CCACTTGATGAA

Line29 AAAGGAAAAGGGAAA **ATATCAA**ACACATTACACATGG**CTT**CCACTTGATGAA

**Vigun07g201000**

gRNA1 off-target site 3

WT 5'—CATGCAAATAAATAC **ATTCATGA**ACAGATTACACAAGG**TAA**ATAAACAGTACC—3'

Line1 CATGCAAATAAATAC **ATTCATGA**ACAGATTACACAAGG**TAA**ATAAACAGTACC

Line3 CATGCAAATAAATAC **ATTCATGA**ACAGATTACACAAGG**TAA**ATAAACAGTACC

Line18 CATGCAAATAAATAC **ATTCATGA**ACAGATTACACAAGG**TAA**ATAAACAGTACC

Line29 CATGCAAATAAATAC **ATTCATGA**ACAGATTACACAAGG**TAA**ATAAACAGTACC

## gRNA2 off-target sites

### Vigun03g327900

gRNA2 off-target site 1

WT 5'—CAGACCTCCGCATCG**AATGGCATTCTCCAAGTCCTTGG**ACTCCGCAACGAAAA—3'  
Line1 CAGACCTCCGCATCG**AATGGCATTCTCCAAGTCCTTGG**ACTCCGCAACGAAAA  
Line3 CAGACCTCCGCATCG**AATGGCATTCTCCAAGTCCTTGG**ACTCCGCAACGAAAA  
Line18 CAGACCTCCGCATCG**AATGGCATTCTCCAAGTCCTTGG**ACTCCGCAACGAAAA  
Line29 CAGACCTCCGCATCG**AATGGCATTCTCCAAGTCCTTGG**ACTCCGCAACGAAAA

### Vigun08g093300

gRNA2 off-target site 2

WT 5'—ACGAAAGTGCTCATC**AATTTTAATTTCCAAGTTCTGAG**GAACAAAAGGTAATT—3'  
Line1 ACGAAAGTGCTCATC**AATTTTAATTTCCAAGTTCTGAG**GAACAAAAGGTAATT  
Line3 ACGAAAGTGCTCATC**AATTTTAATTTCCAAGTTCTGAG**GAACAAAAGGTAATT  
Line18 ACGAAAGTGCTCATC**AATTTTAATTTCCAAGTTCTGAG**GAACAAAAGGTAATT  
Line29 ACGAAAGTGCTCATC**AATTTTAATTTCCAAGTTCTGAG**GAACAAAAGGTAATT

### Vigun09g252400

gRNA2 off-target site 3

WT 5'—AATTTGCAGACGGTT**CATCTCGGTTTCCATGTCCTGGG**CTCTTGAAACACGTG—3'  
Line1 AATTTGCAGACGGTT**CATCTCGGTTTCCATGTCCTGGG**CTCTTGAAACACGTG  
Line3 AATTTGCAGACGGTT**CATCTCGGTTTCCATGTCCTGGG**CTCTTGAAACACGTG  
Line18 AATTTGCAGACGGTT**CATCTCGGTTTCCATGTCCTGGG**CTCTTGAAACACGTG  
Line29 AATTTGCAGACGGTT**CATCTCGGTTTCCATGTCCTGGG**CTCTTGAAACACGTG

## gRNA3 off-target sites

### Vigun03g042200

gRNA3 off-target site 1

WT 5'—AGGAAAATGATGTGC**AAAAGCAGAAGCAGTCTCTGAAG**AACAAGGCGTGGGAA—3'  
Line1 AGGAAAATGATGTGC**AAAAGCAGAAGCAGTCTCTGAAG**AACAAGGCGTGGGAA  
Line3 AGGAAAATGATGTGC**AAAAGCAGAAGCAGTCTCTGAAG**AACAAGGCGTGGGAA  
Line18 AGGAAAATGATGTGC**AAAAGCAGAAGCAGTCTCTGAAG**AACAAGGCGTGGGAA  
Line29 AGGAAAATGATGTGC**AAAAGCAGAAGCAGTCTCTGAAG**AACAAGGCGTGGGAA

### Vigun03g308200

gRNA3 off-target site 2

WT 5'—TACTGTGACATGGAA**ATAAGTAGTAATAATCACTGGGG**GTGATCTATATTTTT—3'  
Line1 TACTGTGACATGGAA**ATAAGTAGTAATAATCACTGGGG**GTGATCTATATTTTT  
Line3 TACTGTGACATGGAA**ATAAGTAGTAATAATCACTGGGG**GTGATCTATATTTTT  
Line18 TACTGTGACATGGAA**ATAAGTAGTAATAATCACTGGGG**GTGATCTATATTTTT  
Line29 TACTGTGACATGGAA**ATAAGTAGTAATAATCACTGGGG**GTGATCTATATTTTT

### Vigun02g034200

gRNA3 off-target site 3

WT 5'—ACCATAAAAAATTAC**ATAATTAGAAATATTTTCTGGAG**CATTATTGATTAAGA—3'  
Line1 ACCATAAAAAATTAC**ATAATTAGAAATATTTTCTGGAG**CATTATTGATTAAGA  
Line3 ACCATAAAAAATTAC**ATAATTAGAAATATTTTCTGGAG**CATTATTGATTAAGA  
Line18 ACCATAAAAAATTAC**ATAATTAGAAATATTTTCTGGAG**CATTATTGATTAAGA  
Line29 ACCATAAAAAATTAC**ATAATTAGAAATATTTTCTGGAG**CATTATTGATTAAGA

**Figure S1.** Analysis of potential off-target sites of VuSYMRK gRNAs. **(A)** PCR detection of potential off-target sites of the designed three gRNAs. Lanes 1-4, gRNA1 off-target site 1 (Vigun05g271100) of lines 1, 3, 18, 29; Lanes 5-8, gRNA1 off-target site 2 (Vigun11g100700) of corresponding lines; Lanes 9-12, gRNA1 off-target site 3 (Vigun07g201000) of corresponding lines; Lanes 13-16, gRNA2 off-target site 1 (Vigun03g327900); Lanes 17-20, gRNA2 off-target site 2 (Vigun08g093300); Lanes 21-24, gRNA2 off-target site 3 (Vigun09g252400); Lanes 25-28, gRNA3 off-target site 1 (Vigun03g042200); Lanes 29-32, gRNA3 off-target site 2 (Vigun03g308200); Lanes 33-36, gRNA3 off-target site 3 (Vigun02g034200). **(B)** Sequencing results of potential off-target sites in transgenic roots compared to the wild-type root (WT).

**Table S1. Primers used in this study**

<b>Primer Name</b>	<b>Sequence (5' to 3')</b>
VuSYMRK-exon1-F	ATGATGGAGTTACCAGAAATTTGGG
VuSYMRK-exon3-R	CATCTTTTTGGTTGGTCTCTTC
gRNA1	ATTACACAACAGATTACACATGG
gRNA2	AATCTCAATTTCCAAGTCCTGGGG
gRNA3	ATAAGCAGAAATAGTCTCTGGGG
VuSYMRK-gRNA1-BbsI-F	GTTTCGATTACACAACAGATTACACA
VuSYMRK-gRNA1-BbsI-R	AAACTGTGTAATCTGTTGTGTAATC
VuSYMRK-gRNA2-BbsI-F	GTTTCGATCTCAATTTCCAAGTCCTG
VuSYMRK-gRNA2-BbsI-R	AAACCAGGACTTGGAATTTGAGATC
VuSYMRK-gRNA3-BbsI-F	GTTTCGATAAGCAGAAATAGTCTCTG
VuSYMRK-gRNA3-BbsI-R	AAACCAGAGACTATTTCTGCTTATC
SYMRK-gDNA-F	GGGAACATCCCTTTTAGTAC
SYMRK-gDNA-R	CCTAAGCCTTG TAGATTTC
symrk-gRNA1-off-target-1-F	GAGCGATCAAACAGCTGC
symrk-gRNA1-off-target-1-R	ACCTATTCCGGCAAAC
symrk-gRNA1-off-target-2-F	GCAACTTGGATGTTCTTTC
symrk-gRNA1-off-target-2-R	CAACATCATGGCCTAAGTG
symrk-gRNA1-off-target-3-F	GATTATTTCCCACTGTGC
symrk-gRNA1-off-target-3-R	TGTGAACATTCTGCAGTG
symrk-gRNA2-off-target-1-F	CACCACCGAGACCAATTC
symrk-gRNA2-off-target-1-R	CTGTTTTGGGACCAATTCC
symrk-gRNA2-off-target-2-F	CGTCTCAGTCAAACCTTCTC
symrk-gRNA2-off-target-2-R	GA CTCAA AATTTCTCCACTC
symrk-gRNA2-off-target-3-F	CCAAGTCTCAGTTGCTAGAG
symrk-gRNA2-off-target-3-R	GGGTTGCAACGCATGATTTC
symrk-gRNA3-off-target-1-F	CAATGCTCAACAGCAGCAAC
symrk-gRNA3-off-target-1-R	CGGAAGATATTTCTTCCC
symrk-gRNA3-off-target-2-F	CTTCCAGTATGTTGCGGG
symrk-gRNA3-off-target-2-R	GCCATTACTACCGCTAGC
symrk-gRNA3-off-target-3-F	GTAGTGCAGTGCAGTTTGG
symrk-gRNA3-off-target-3-R	GA ACTCGTCCATGTCTGAG