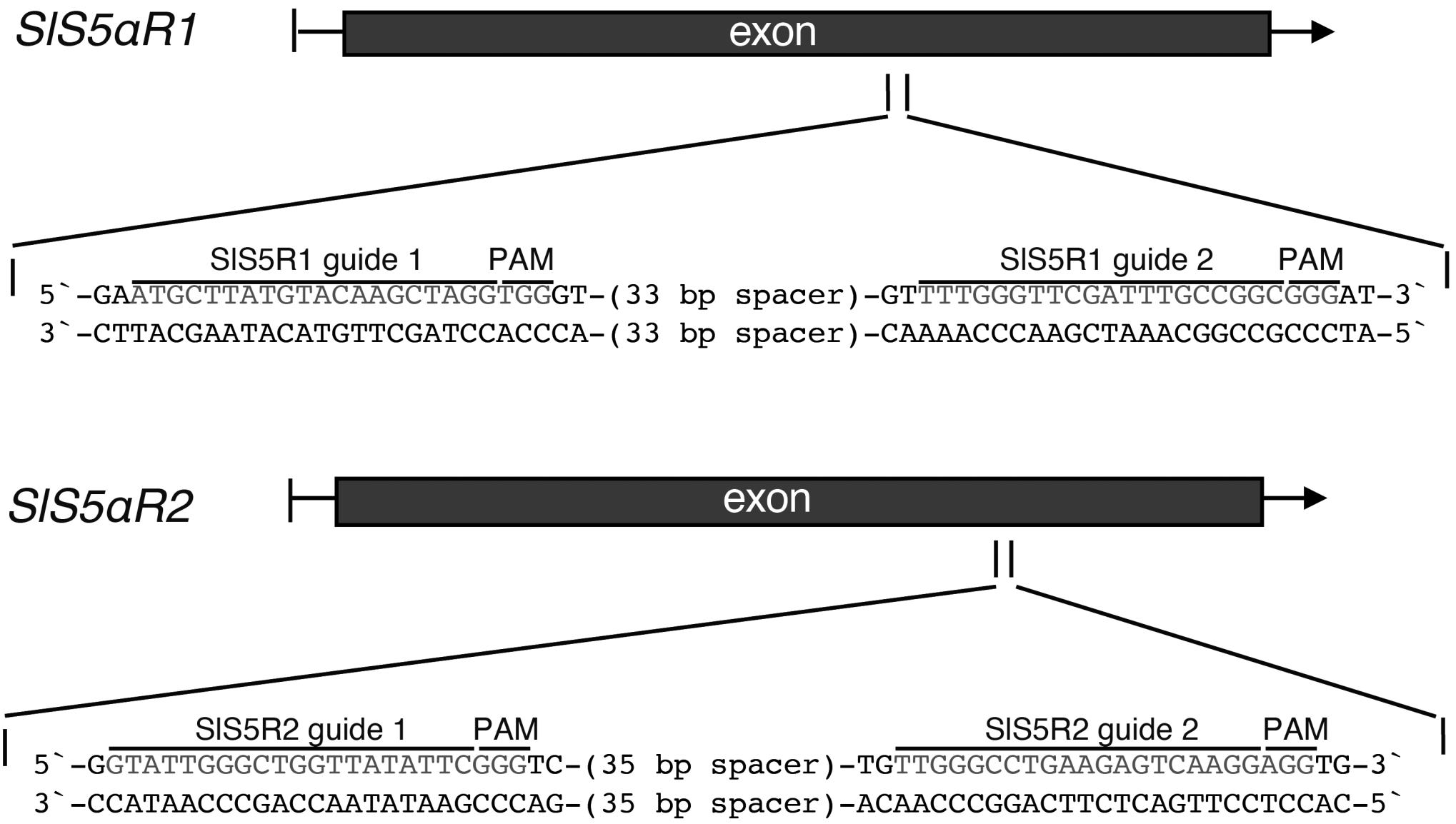
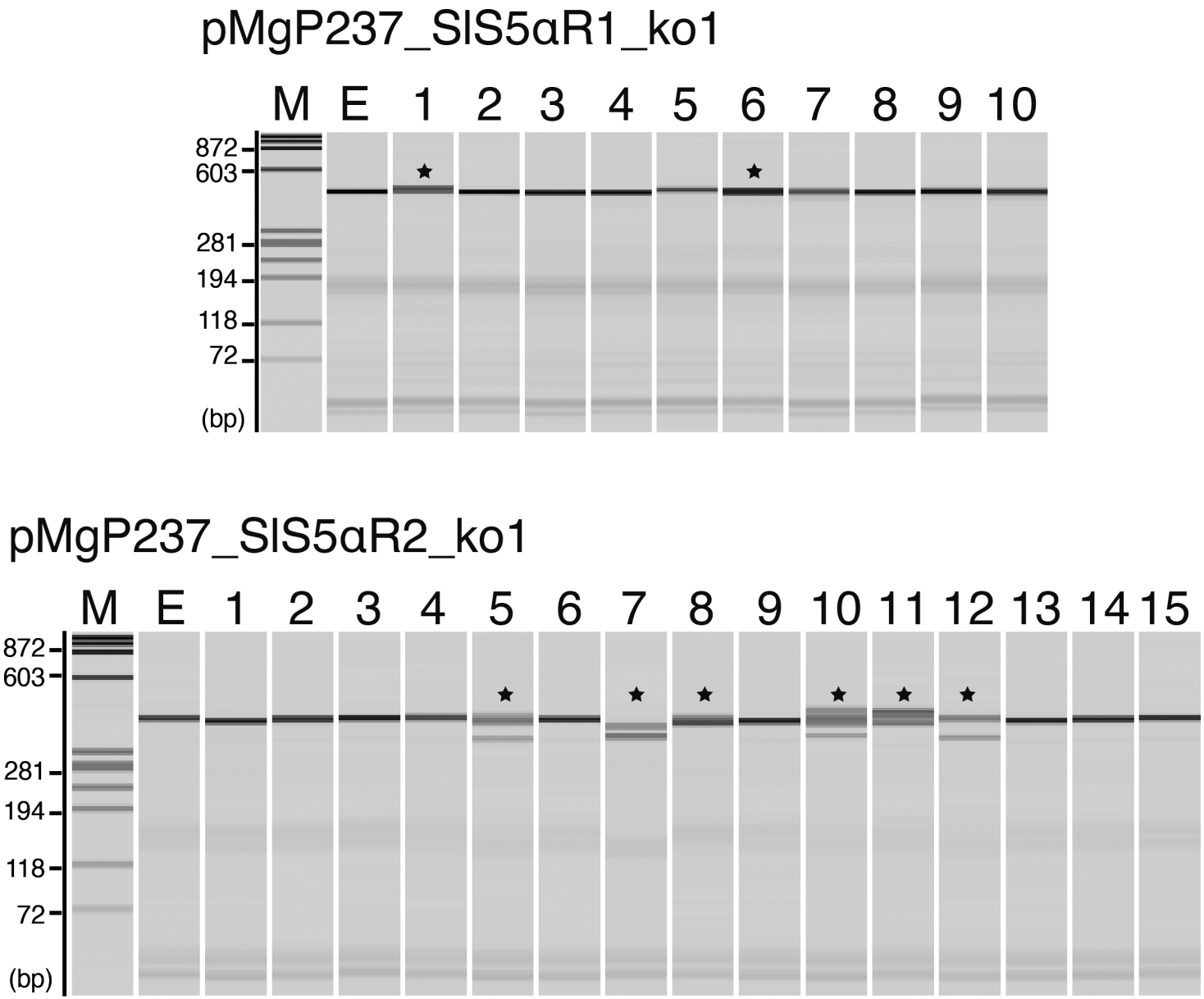


Supplementary Figure S1. Target sites for *SlS5αR1* and *SlS5αR2*. The 20-bp target sequences and protospacer adjacent motif (PAM) sequences are indicated in gray.



Supplementary Figure S2. Heteroduplex mobility assay with the MultiNA electrophoresis system. Multiple heteroduplex peaks were detected in PCR amplicons from the CRISPR/Cas9 transgenic tomato hairy roots. Black stars indicate the potential mutated lines. E: hairy roots transformed by empty pMgP237 vector. M: internal marker.

Supplementary Table S1. Candidate S5αRs from tomatoes were selected based on the BLASTP search in the Sol Genomics Network (http://solgenomics.net). RPKM values were obtained from the Tomato Functional Genomics Database (http://ted.bti.cornell.edu).

Clone ID	Bud	Flower	Leaf	Root	1cm_Fruit	2cm_Fruit	3cm_Fruit	Mature green	Breaker
SlS5αR1	34.5	21.4	16.4	15.0	41.9	27.5	38.0	55.3	43.5
SlS5αR2	74.8	12.8	30.2	22.9	105.3	32.3	7.4	3.8	1.2

Supplementary Table S2. Oligonucleotides used in the present study.

Primer No.	Descripton	Sequences
1	SlS5αR1qPCR Fw	5`-TGTGGTTTTGGGTTCGATTTGCCG-3`
2	SlS5αR1qPCR Rv	5`-TCACTCTTCAGGCCCACTAACACC-3`
3	SlS5αR2 qPCR Fw	5`-TTCACTTCTCCTTACGGCAAACACTACA-3`
4	SlS5αR2 qPCR Rv	5-`CGAAATACGATAATTGTGAGCCAAAGCGT-3`
5	ubiquitin qPCR Fw	5`-CACCAAGCCAAAGAAGATCAAGC-3`
6	ubiquitin qPCR Rv	5`-TCAGCATTAGGGCATCCCTTACG-3`
7	SlS5αR1 ORF BamHI Fw	5`-GGATCCATGTTTTCAGATCAAAACTTGTATCAC-3`
8	SlS5αR1 ORF SalI Rv	5`-GTCGACTCAATACAAAAATGGAATAACAGCTTTT-3`
9	SlS5αR2 ORF BamHI Fw	5`-GGATCCATGTTCTCTTCAGATGAAAATCTCTTC-3`
10	SlS5αR2 ORF SalI Rv	5`-GTCGACTCAATACAAAAAGGGGAAAACAGC-3`
11	Sl3βHSD1 ORF BamHI-NdeI Fw	5`-GAATTCCATATGGCAAATAAGCTCAGGTTGGAGGGC-3`
12	Sl3βHSD2 ORF SalI Rv	5`-GTCGACTTATTGTAGCTTCAAAATAGAACTTAGTCCACC-3`.
13	F2_tgRNA_SlS5R1_492-511	5`-TTGGGTCTCGTGCAGATGCTTATGTACAAGCTAGGGTTTTAGAGCTAGAAATAGCA-3`
14	R2_tgRNA_SlS5R1_552-571	5`-TTGGGTCTCCAAACGCCGGCAAATCGAACCCAAACTGCACCAGCCGGGAATCGAA-3`
15	F2_tgRNA_SlS5R1_449-468	5`-TTGGGTCTCGTGCAGTATTGGGCTGGTTATATTCGTTTTAGAGCTAGAAATAGCA-3‘
16	R2_tgRNA_SlS5R1_511-530	5`-TTGGGTCTCCAAACCCTTGACTCTTCAGGCCCAACTGCACCAGCCGGGAATCGAA-3`
17	AtU6-pMgP237 Fw	5`-GGCCCCTGGGAATCTGAAAG-3`
18	AtU6-pMgP237 Rv	5`-GGAAGAAGAAATCGATCTGGAAAATTTTGC-3`
19	SlS5αR1_g43 Fw	5`-CTCTTCCTCATCGGACCACC-3`
20	SlS5αR1_g501 Rv	5`-CCAAACATTCACCGCCATCC-3`
21	SlS5αR2_g296 Fw	5`-TTCGCCTCCGTAGCAGAAAC-3`
22	SlS5αR2_g678 Rv	5`-TCGAAACAGCTCGAGGAACC-3`