

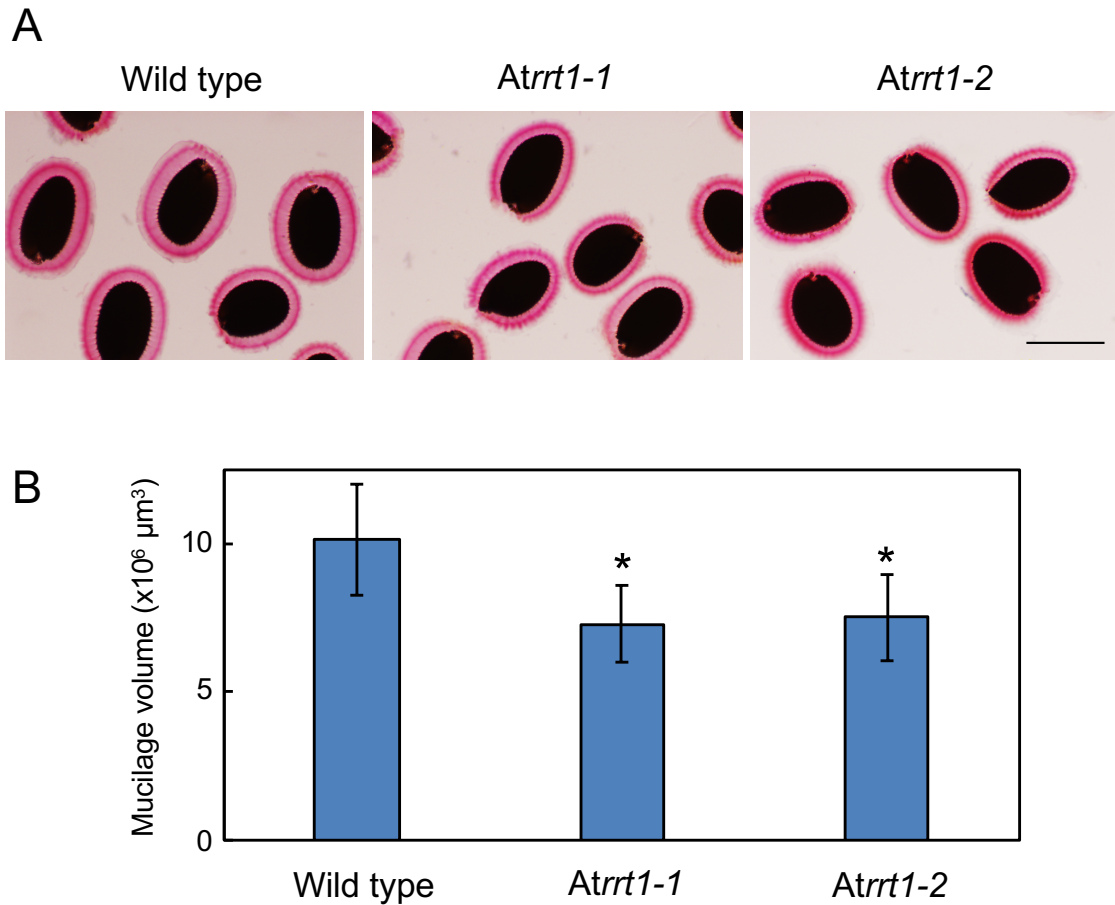
Supplementary Table 1. Nucleotide sequences of oligonucleotides used in this study.

Primer name	Sequence
MpRRT1-FLAG_F	ACGGGGGACTCTAGAATGCTCAAGGGGGGAATAC
MpRRT1-FLAG_R	GGGAAATTCGAGCTCTTACTTGTCGTCATCGTCCTTGTAGTCCTTGAAGGAAAAA TTCCG
MpRRT3-FLAG_F	ACACGGGGGACTCTAGAATGGACAGGGGGCGAC
MpRRT3-FLAG_R	ATCGGGGAAATTCGAGCTCTTACTTGTCGTCATCGTCCTTGTAGTCTTGCGTGGTG GTGC
MpRRT1_F	GCTAAGACCCTCGAGATGCTCAAGGGGGGAATAC
MpRRT1_R	AACTCTAGAACTAGTTCACTTGAAGGAAAAATTCCGCGAG
MpRRT1_CR1_F	CTCGATGCCAGGATGGTAGCCGAG
MpRRT1_CR1_R	AAACCTCGGCTACCATCCTGGCAT
MpRRT1_CR_seqF1	TCACCTTACTCCCCTTCCAGTA
MpRRT1_CR_seqF2	GTGCGTGAGAAGCAGTAATGTC
MpRRT1_CR_seqR	GCACCAATCTTCGACCTAATTC
MpRRT1-4217_F	CACCCAATTGGGCCTTGAGGTGTAAGCGC
MpRRT1_0_R	GGCTCCTGTCTTCCGAGTTCGGGTA
MpRRT1_1_F	CACCATGCTCAAGGGGGGAATACCGAGTC
MpRRT1+3107_nosto p_R	CTTGAAGGAAAAATTCCGCGAGTAG
AtRRT8-FLAG_F	ACGGGGGACTCTAGAATGTCAGTCGGCGTTCCAG
AtRRT8-FLAG_R	GGGAAATTCGAGCTCTTACTTGTCGTCATCGTCCTTGTAGTCTCTCAGAGATTGTG CTCGTAG

Supplementary Table 2. Abbreviations and structures of RG-I oligosaccharides used in this study.

Abbreviation	Structure
RG ₃ -PA	 PA
GR ₄ -PA	 PA
RG ₅ -PA	 PA
GR ₆ -PA	 PA
GR ₈ -PA	 PA
GR ₁₀ -PA	 PA
GR ₁₂ -PA	 PA
GR ₁₄ -PA	 PA

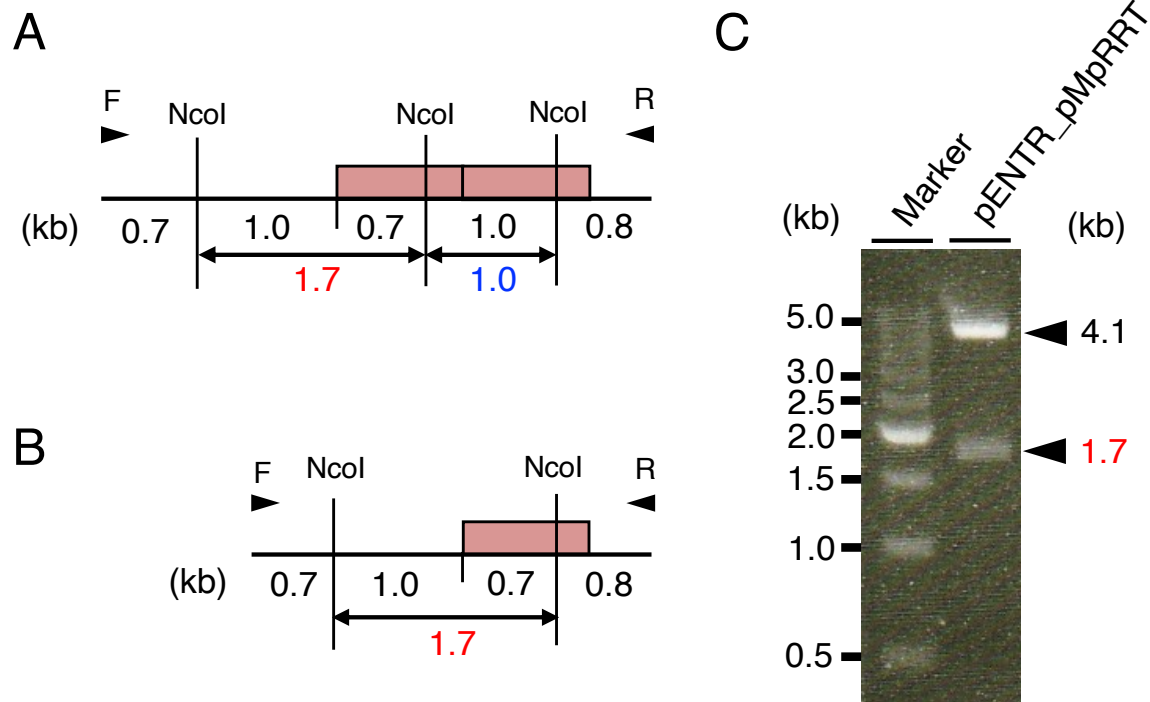
Green triangles, yellow divided diamonds, and PA represent rhamnose residue, galacturonic acid residue, and fluorescent pyridylamino group, respectively.



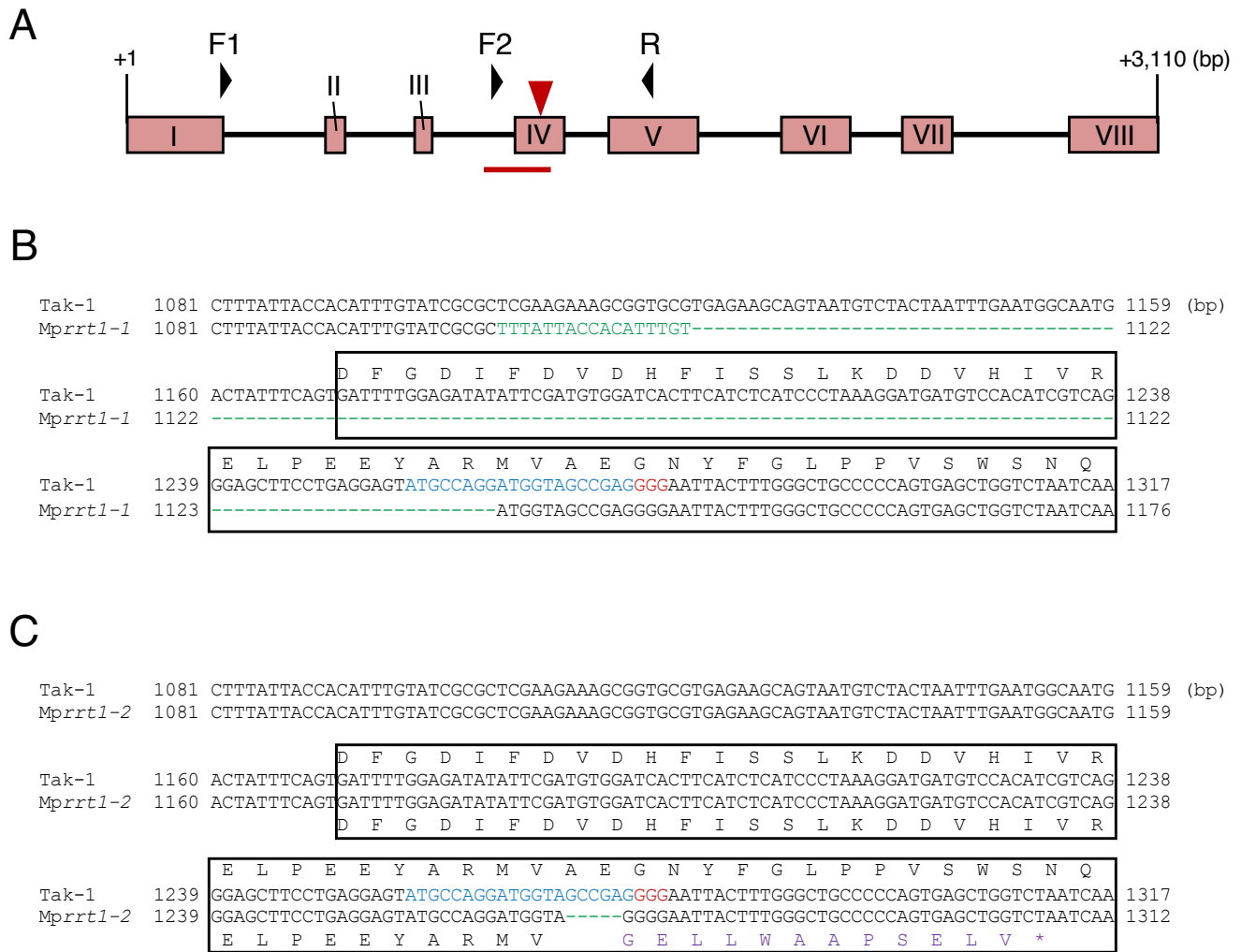
Supplementary Figure 1. Seed coat mucilage phenotype of *Atrrt1*. (A) *Arabidopsis* seed coat mucilage of the wild-type, *Atrrt1-1* (SALK_022924), and *Atrrt1-2* (SALK_042968) strains, stained with ruthenium red. Scale bar, 250 μm. (B) Mucilage volumes of seed coats in the wild-type, *Atrrt1-1*, and *Atrrt1-2* strains. The mucilage volumes are presented as the mean values of 20 seeds with SD. Asterisk indicates that the value was significantly different from those of the wild type and two *Atrrt1* mutant strains by Student's t-test: *P < 0.05.

[illegible][illegible]

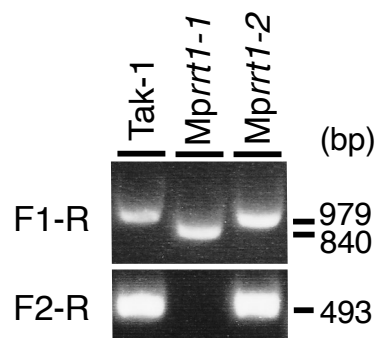
Supplementary Figure 2. MpRRT1 promoter sequence in the Tak-1 genome. *proMpRRT1_MarpolBase* (4,217 bp) is the sequence extracted from the MarpolBase {scaffold_33: 1,349,919.. 1,354,136 (- strand)}. *proMpRRT1* (3,217 bp) is the sequence confirmed by Sanger sequencing and used in reporter analyses in this study (−3,217 to −1). Duplicated 1,000-bp sequences are highlighted in yellow and sky blue. Red sequences indicate the 5'UTR region based on the MarpolBase. The *NcoI* sites used in Supplementary Figure 3 are indicated with rectangles.



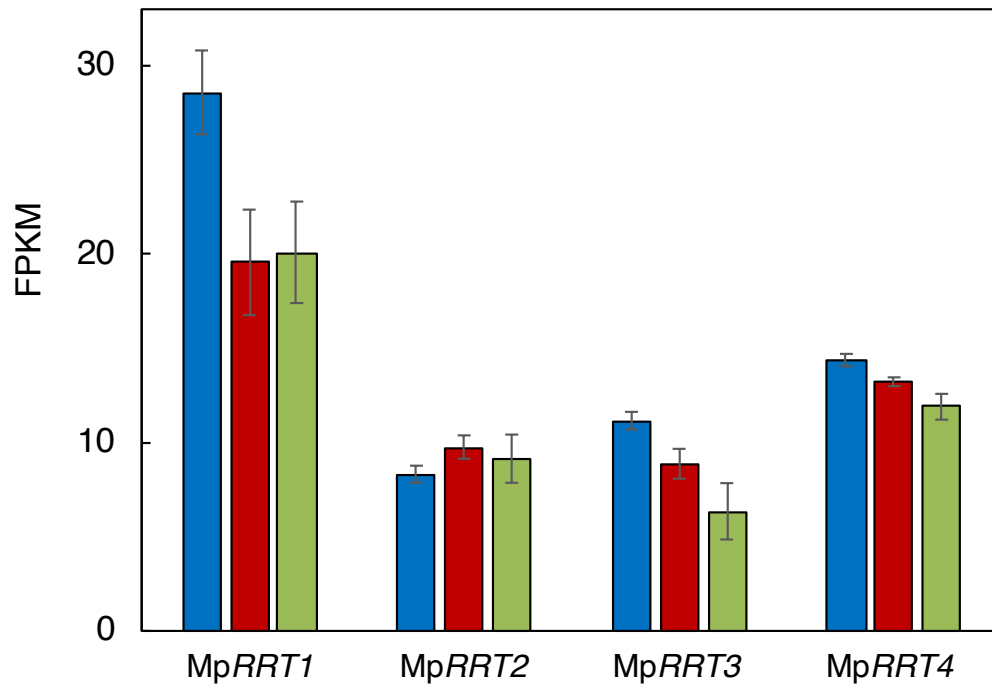
Supplementary Figure 3. The 1.0-kbp sequence of the *MpRRT1* promoter is not tandemly duplicated in Tak-1. (A, B) Schematic representation of the *MpRRT1* promoter based on MarpolBase (A) and this study (B). The expected fragment sequences amplified with primers F (*MpRRT1*-4217_F) and R (*MpRRT1*_0_R) (Supplementary Table 1) are shown as arrowheads. *MpRRT1* promoter sequence of MarpolBase is derived from scaffold_33: 1,349,919..1,354,136 (- strand). Orange rectangles indicate the 1.0-kbp sequence tandemly duplicated in MarpolBase. The positions of recognition sites for *NcoI* restriction enzyme are shown. Expected fragment sizes after digestion with *NcoI* are shown by bidirectional arrows. (C) DNA fragments of pENTR_pMpRRT1 digested with restriction enzyme *NcoI*. Based on the length of the pENTR/D_TOPO plasmid (2.6 kb), expected fragments for (A) and (B) are 4.1/1.7/1.0 kb and 4.1/1.7 kb, respectively. No 1.0-kb fragment indicates the absence of the 1.0-kbp duplication.



Supplementary Figure 4. Construction of the MpRRT1 genome-edited lines. (A) Schematic representation indicating positions of guide RNAs for the genome editing of MpRRT1. Orange boxes and black lines represent predicted exons and introns, respectively. Roman numerals represent exon numbers. A red arrowhead indicates the position of the guide RNA for the genome editing of MpRRT1. F1, F2, and R represent primers used to confirm genome editing (Supplementary Figure 5). **(B, C)** Comparison of MpRRT1 genomic sequences in the Tak-1 and MpRRT1 genome-edited lines, Mprrt1-1 **(B)** and Mprrt1-2 **(C)**. Nucleotide sequences of the red line in A are shown. The squares indicate the sequences of the 4th exon. The Tak-1 sequence is shown together with the PAM sequence (red) and the guide RNA (blue). The amino acid sequences encoded by Tak-1 and MpRRT1 genome-edited lines are shown at the top and bottom of their nucleotide sequences, respectively. Mprrt1-1 had a 155-bp deletion and a 16-bp insertion at the 3rd intron and 4th exon, respectively (B; green) resulting in deletion of amino acids (Δ D158 to R188). Mprrt1-2 had a 5-bp deletion (C; green) resulting in a frameshift that introduced a premature stop codon (purple).



Supplementary Figure 5. Genotype of the Tak-1 and MpRRT1 genome-edited lines. Primer combinations are shown on the left. F1, F2, and R represent primers MpRRT1_CR_seqF1, MpRRT1_CR_seqF2, and MpRRT1_CR_seqR (Supplementary Table 1), respectively. The positions of each primer in MpRRT1 are shown in Supplementary Figure 4A. The sizes of the PCR products from Tak-1 (979 bp) and Mprrt1-1 (840 bp) are indicated on the right.



Supplementary Figure 6. Expression profiles of MpRRTs from composite RNA-Seq data. The expression levels of four MpRRTs in thallus (blue), gemma cup (red), and midrib (green) were estimated based on the relative abundances of transcripts with the unit fragments per kilobase million (FPKM). The values are presented as the mean values of three biological replicates with SD.