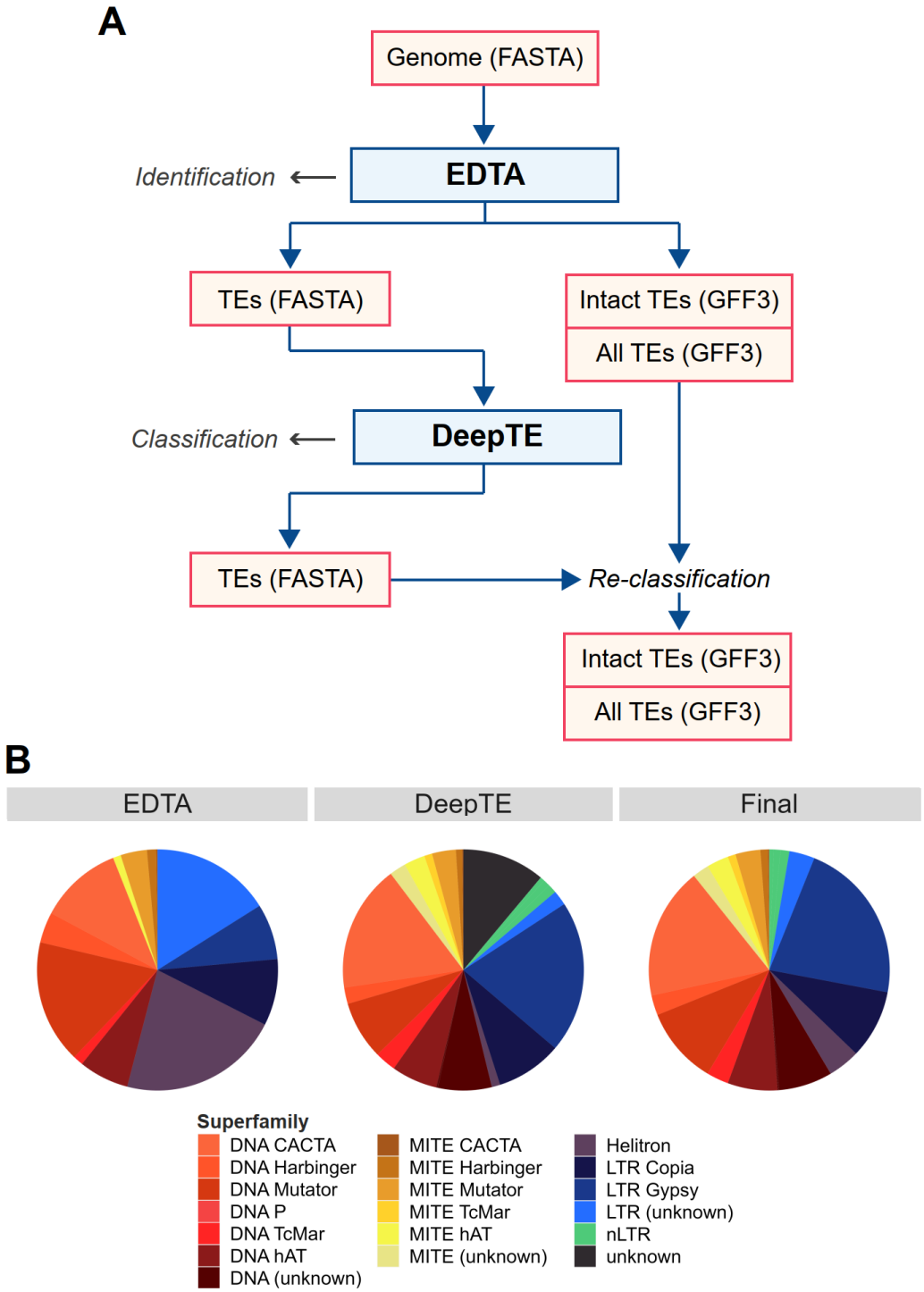

Figure S1. Annotation and classification of transposable elements (TEs) in the *Fragaria vesca* v4.0.a2 reference genome. (A) Representation of the pipeline used in this work for transposon annotation. Software employed are highlighted in blue boxes, while input and output files are shown in pink boxes, with their formats in parentheses. **(B)** Distribution of TE superfamilies as annotated by EDTA (left), by DeepTE (center), and by their combined pipeline (right) for the *F. vesca* v4.0 genome.

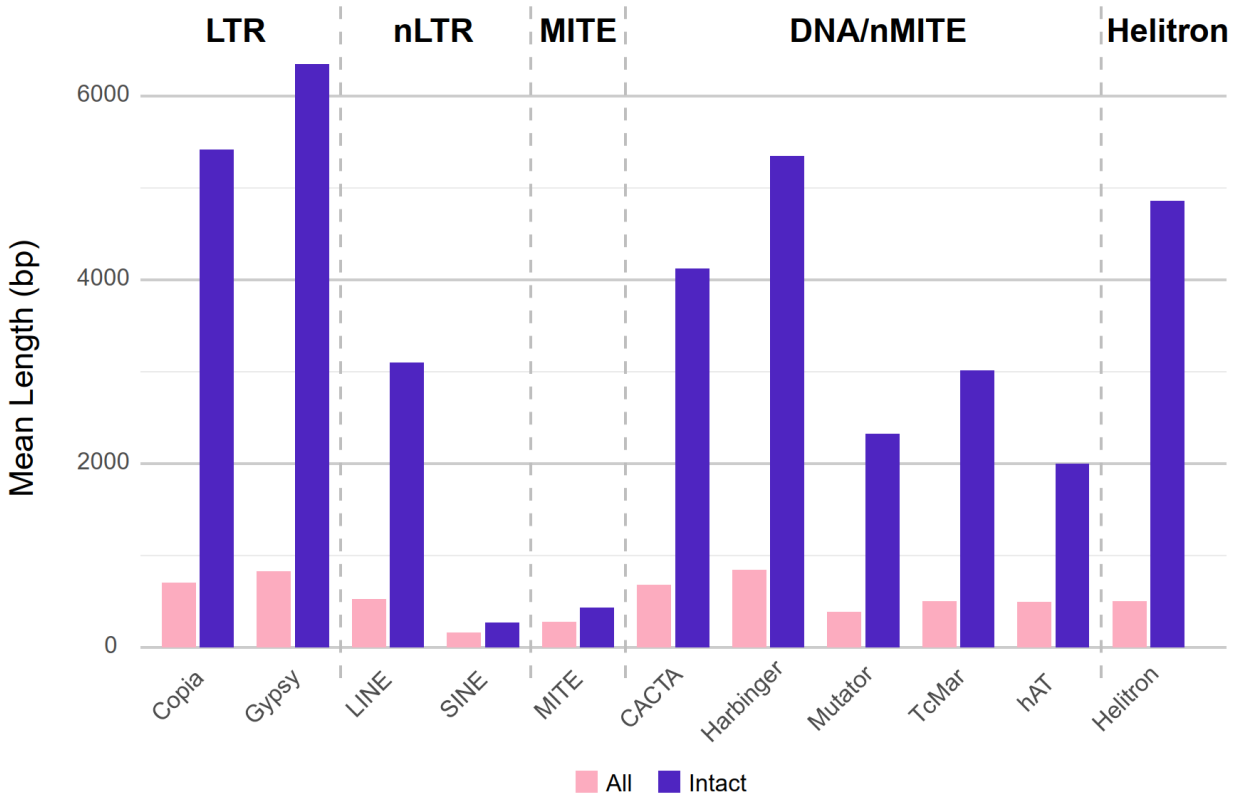

Figure S2. The average length of intact TEs and all annotated TEs in *F. vesca* genome (including fragmented TEs).

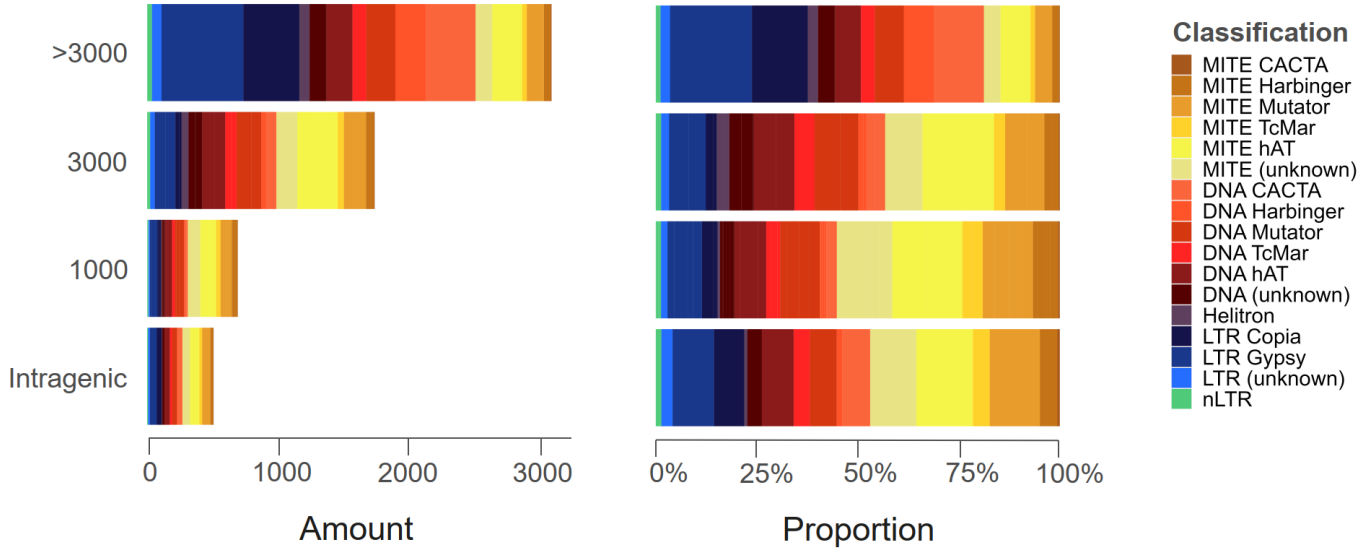

Figure S3. Distribution of intact TE superfamilies in the *Fragaria vesca* v4.0.a2 annotation based on their distance from a gene. Categories include: within a gene ('intragenic'), within 1 kb ('1000'), between 1 and 3 kb ('3000'), and more than 3 kb ('>3000'). Left: Total counts by superfamily. Right: Proportion by superfamily.

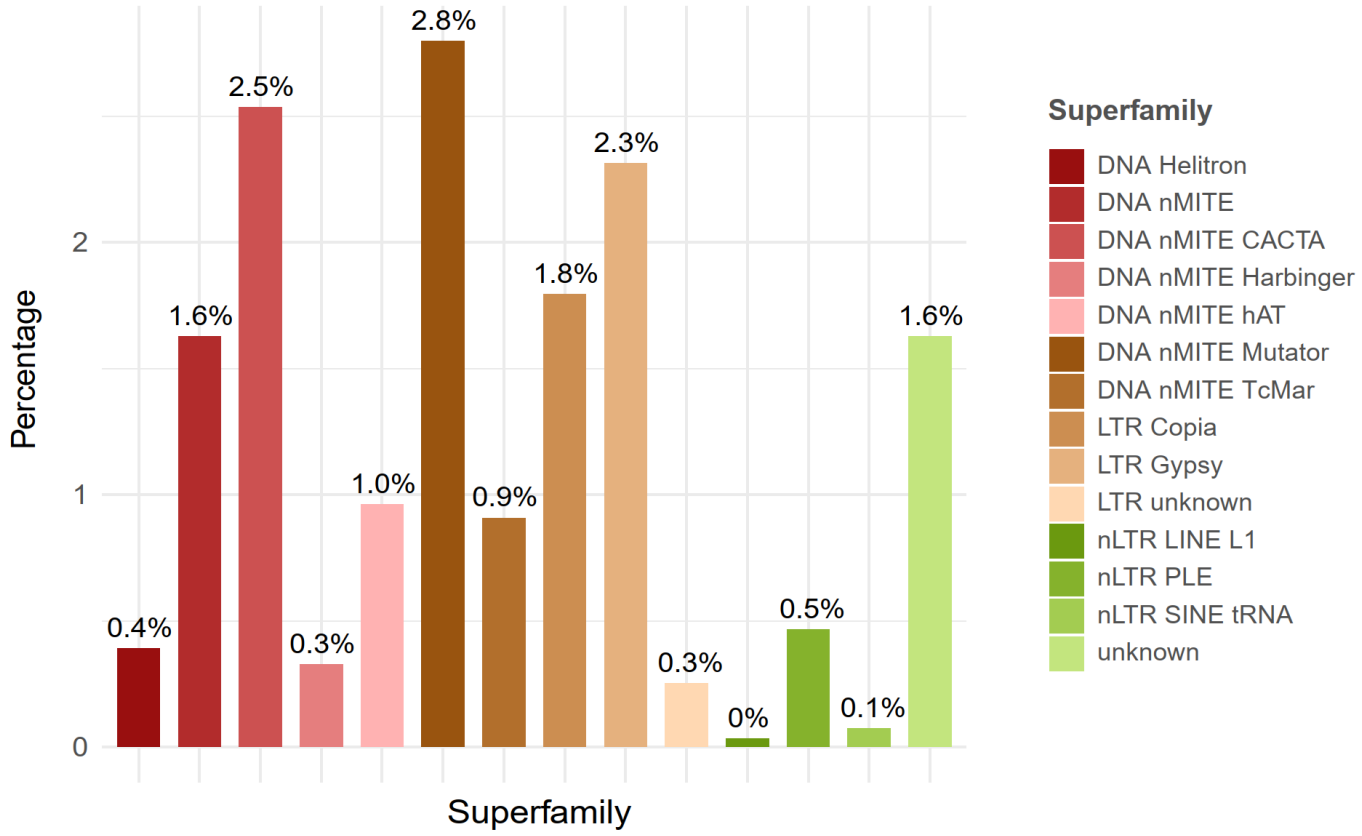

Figure S4. Proportion of IRs classified as 'Other', excluding MITE-type elements. Values are shown as percentages relative to the total number of IRs in the 'Other' category (16.1%).

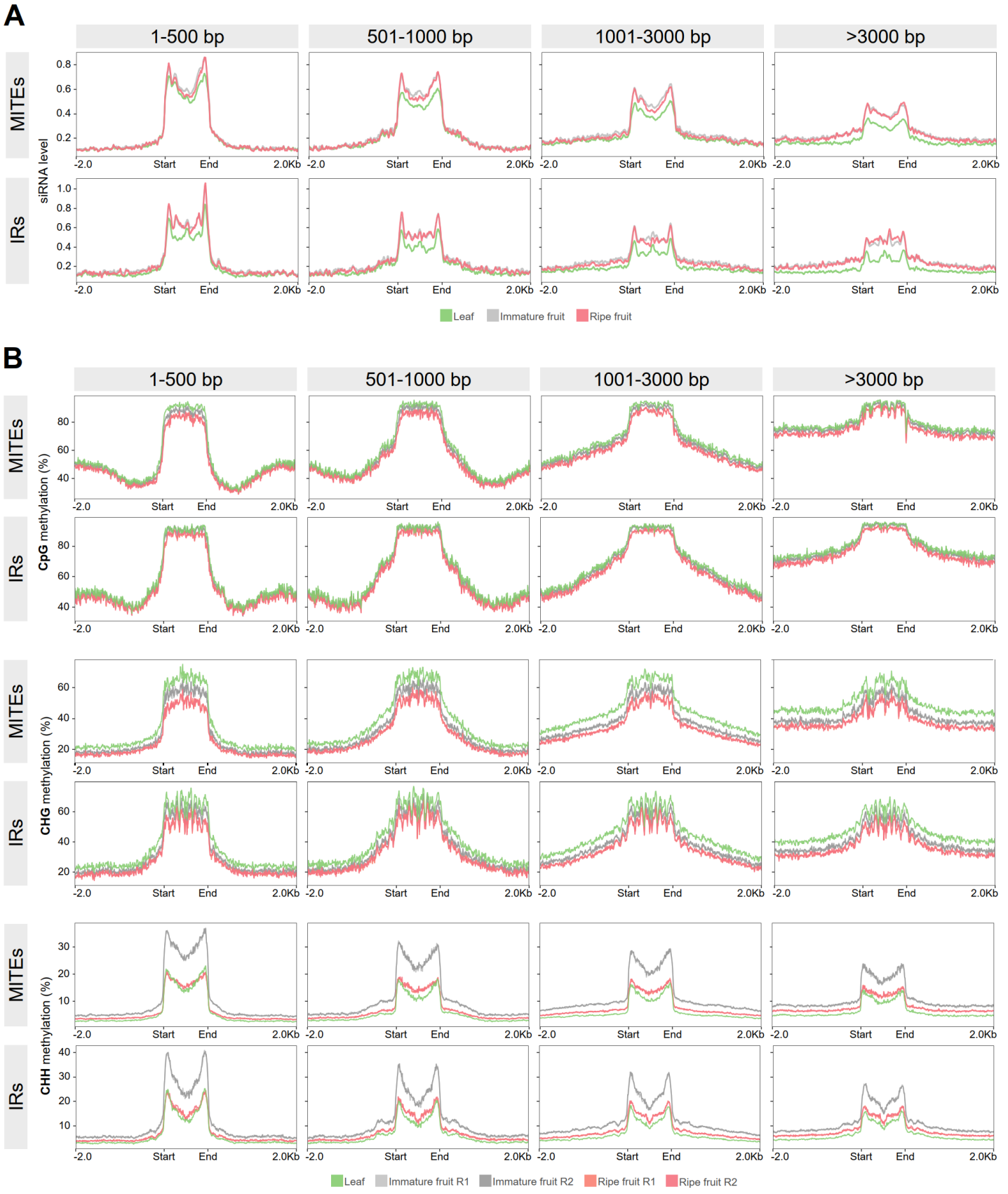

Figure S5. (A) Metagene profile of 24-nt siRNAs mapping to MITEs and IRs located at different ranges of distance to the closest protein-coding gene, in leaf (green), and immature (gray) and ripe fruits (pink). Plots show MITEs/IRs scaled from the start to the end plus 2,000 bp to each side. sRNA-seq replicates are plotted together. **(B)** Metagene profile of CpG, CHG, and CHH DNA methylation at MITEs/IRs located at different ranges of distance to the closest protein-coding gene, in leaf (green), and immature (gray) and ripe fruits (pink). Plots show MITEs/IRs scaled from the start to the end plus 2,000 bp to each side. Individual BS-seq replicates are plotted.

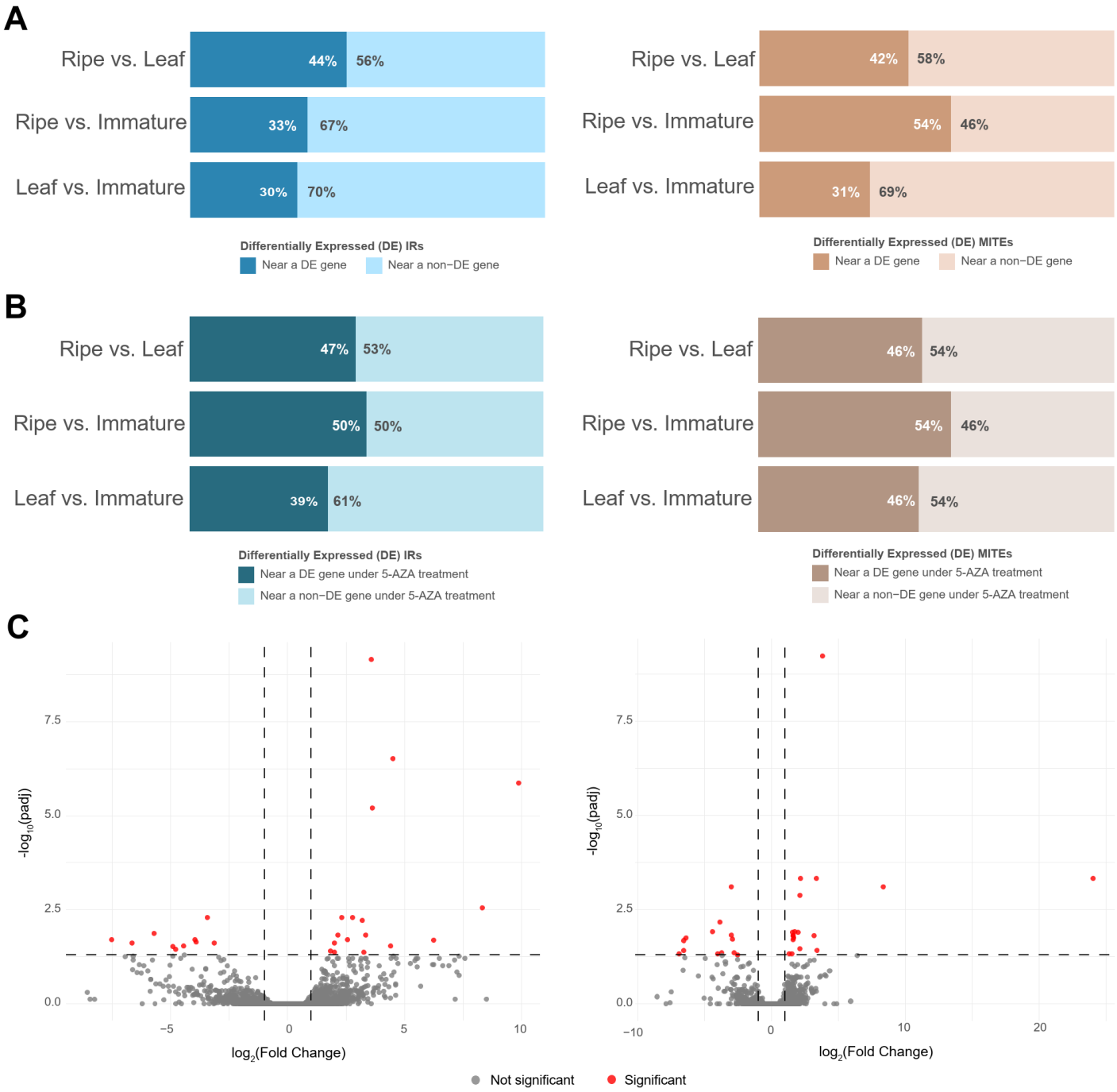

Figure S6. (A) Differential accumulation analysis of 24-nt siRNAs derived from IRs (left) and MITEs (right) elements across leaf tissue, immature and ripe fruits, using thresholds of log2-fold change (FC) ≥0.5 and adjusted p-value (padj) ≤0.05. The percentages of elements located within 3000 bp of either differentially expressed genes (DE) or non-DE genes are shown. **(B)** Volcano plots showing the log2FC of the differential expression analysis for IRs (left) and MITEs (right) between immature and ripe stages. Red points represent elements with an adjusted p-value ≤0.05. One element from each analysis, which had an extremely low adjusted p-value, was removed only for clarity.

A

```
F.vesca           1 GAGAAATTTTTAAATGCTACGGGAGGACCATGTGGCAGTCTAACGTGGTC    50
                    ||||||||||||||||.|||||||||||||.|||||.|||.||||||||
F.chiloensis      1 GAGAAATTTTTAAATGTTACGGGAGGACCACGTGGCAATCTGACGTGGTC    50

F.vesca          51 CTACGATCCAATCAAATTTAGACATGTGGATTTTTTACAACTAAAATATA   100
                    |||||.|.||||||||||||||||||||||||||||||||||||||||||
F.chiloensis     51 CTACGTTTCAATCAAATTTAGACATGTGGATTTTTTACAACTAAAATATA   100

F.vesca         101 AACAAATATTTTCTATTTTTTGTGAAATGACATTCATGGGTATTTAGCAA   150
                    ||||||||||||||||||||||||||||||||||||||||||||||||||
F.chiloensis    101 AACAAATATTTTCTATTTTTTGTGAAATGACATTCATGGGTATTTAGCAA   150

F.vesca         151 GTTCAAATTAGGGTCTAGGGTATAGGGTTTAAAGTTTAGGGTTAGAGTTT   200
                    |||||||.|||||||||||||||.|||||||||||||||||||||||||||
F.chiloensis    151 GTTCAAACTAGGGTCTAGGGTATAAGGTTTAAAGTTTAGGGTTAGAGTTT   200

F.vesca         201 AGGGTATAGGGTTTAGGGTTTAGGGTTTAGGGTTTGGGGTTTAGGGTTTA   250
                    ||||||||                              |||||||||||||.
F.chiloensis    201 AGGGTATA----------------------------GGGTTTAGGGTTTG   222

F.vesca         251 GGGCTTAGGGTTTAGTATTAAAAAAACAACAAAAAATCTAAAATGGTCTT   300
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
F.chiloensis    223 GGGCTTAGGGTTTAGTATTAAAAAAACAACAAAAAATCTAAAATGGTCTT   272

F.vesca         301 TGACAAAATAGCTGAAATAATTTTTCGTAAAGAAAATCTACATGTCTAAA   350
                    ||||||||||||||||||||||||||||||||||||||||.|||||||||
F.chiloensis    273 TGACAAAATAGCTGAAATAATTTTTCGTAAAGAAAATCTATATGTCTAAA   322

F.vesca         351 TTTGATTGGAAAGAAGGCCCACGTCAGACTGCCACGTGGTCCTCCCGTAG   400
                    ||||||.||||||.||||.|||||||||||||.||.|||||||||||||.
F.chiloensis    323 TTTGATCGGAAAAAAGGACCACGTCAGACTGTCATGTGGTCCTCCCGTAA   372

F.vesca         401 CACTGAAAAATTTCTC    416
                    ||||||||||||||||
F.chiloensis    373 CACTGAAAAATTTCTC    388
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F.vesca           1 GAGAAATTTTTAAATGCTACGGGAGGACCATGTGGCAGTCTAACGTGGTC    50
                    ||||||||||||||||.||.||||||||||.||||||||||.||||||||
F.ananassa        1 GAGAAATTTTTAAATGTTATGGGAGGACCACGTGGCAGTCTGACGTGGTC    50

F.vesca          51 CTACGATCCAATCAAATTTAGACATGTGGATTTTTTACAACTAAAATATA   100
                    |||.|.||||||||||||||||||||||||||||||||||||||||||||
F.ananassa       51 CTAGGTTCCAATCAAATTTAGACATGTGGATTTTTTACAACTAAAATATA   100

F.vesca         101 AACAAATATTTTCTATTTTTTGTGAAATGACATTCATGGGTATTTAGCAA   150
                    ||||||||.||||||||||||||||||||||||||||||||||||||||||
F.ananassa      101 AACAAATGTTTTCTATTTTTTGTGAAATGACATTCATGGGTATTTAGCAA   150

F.vesca         151 GTTCAAATTAGGGTCTAGGGTATAGGGTTTAAAGTTTAGGGTTAGAGTTT   200
                    ||||||||.||||||||||||||||||||||||||||||||||||||||||
F.ananassa      151 GTTCAAACTAGGGTCTAGGGTATAGGGTTTAAAGTTTAGGGTTAGAGTTT   200

F.vesca         201 AGGGTATAGGGTTTAGGGTTTAGGGTTTAGGGTTTGGGGTTTAGGGTTTA   250
                    |||||||||||||||||||||||||||||||||||||||       |||||||
F.ananassa      201 AGGGTATAGGGTTTAGGGTTTAGGGTTTAGGGTTTG-------GGGTTTA   243

F.vesca         251 GGGCTTAGGGTTTAGTATTAAAAAAACAACAAAAAATCTAAAATGGTCTT   300
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
F.ananassa      244 GGGCTTAGGGTTTAGTATTAAAAAAACAACAAAAAATCTAAAATGGTCTT   293

F.vesca         301 TGACAAAATAGCTGAAATAATTTTTCGTAAAGAAAATCTACATGTCTAAA   350
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||
F.ananassa      294 TGACAAAATAGCTGAAATAATTTTTCGTAAAGAAAATCTACATGTCTAAA   343

F.vesca         351 TTTGATTGGAAAGAAGGCCCACGTCAGACTGCCACGTGGTCCTCCCGTAG   400
                    ||||||.|||||||.|.||||||||||||||||||||||||||||||||||
F.ananassa      344 TTTGATCGGAAAGAAAGACCACGTCAGACTGCCACGTGGTCCTCCCGTAG   393

F.vesca         401 CACTGAAAAATTTCTC    416
                    ||||||||||||||||
F.ananassa      394 CACTGAAAAATTTCTC    409
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B

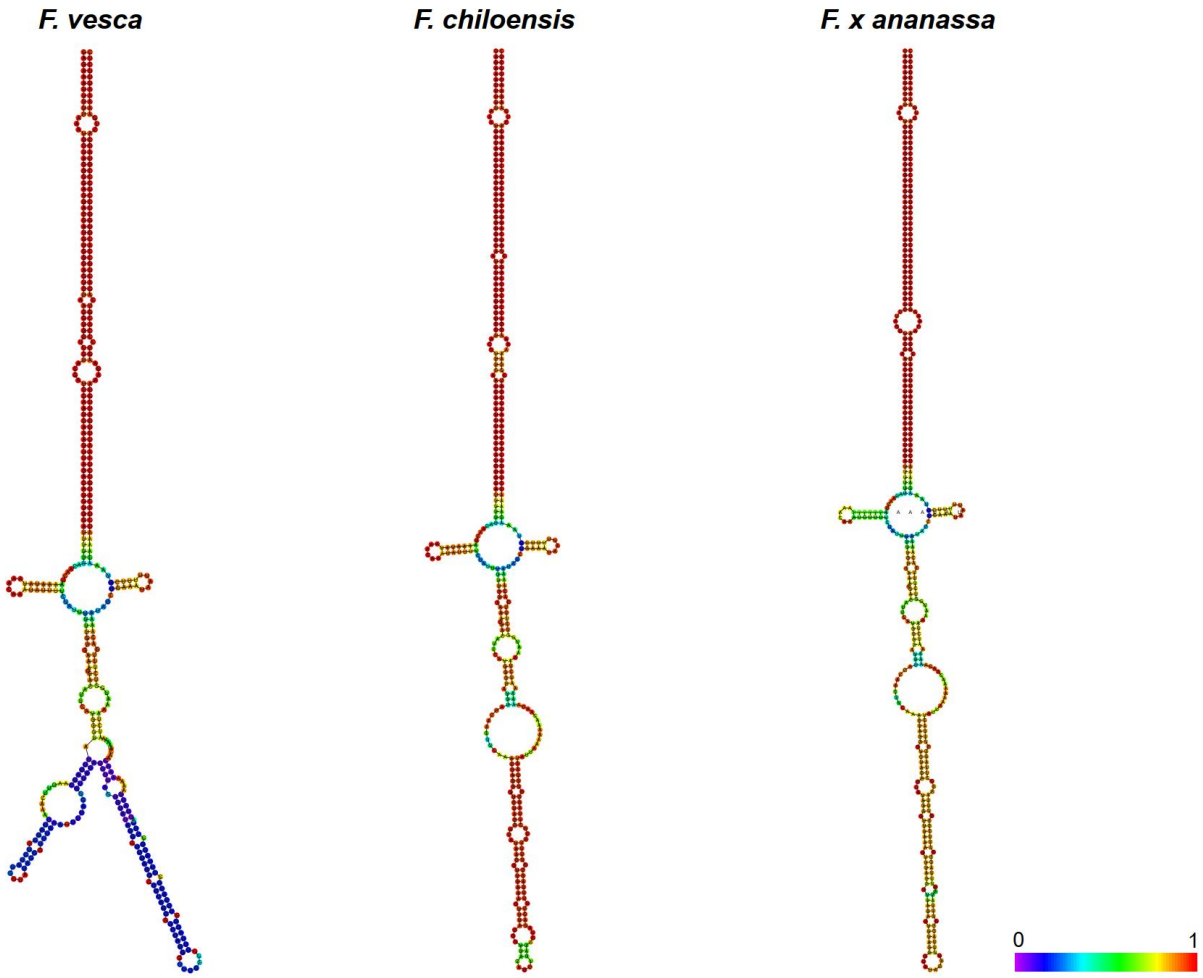

Figure S7. Conservation of IR_9195 from *Fragaria vesca* in the orthologous genomic regions of commercial strawberry species *Fragaria chiloensis* and *Fragaria* × *ananassa*. (A) Local alignment of *F. vesca IR_9195* with the IR located near the FvH4_7g18570 ortholog in *F. chiloensis* (left) and *F.* × *ananassa* (right). **(B)** Minimum free energy (MFE) RNA secondary structure predicted by RNAfold server of each IR. The color scale shows base-pairing probability 0 (purple) to 1 (red).

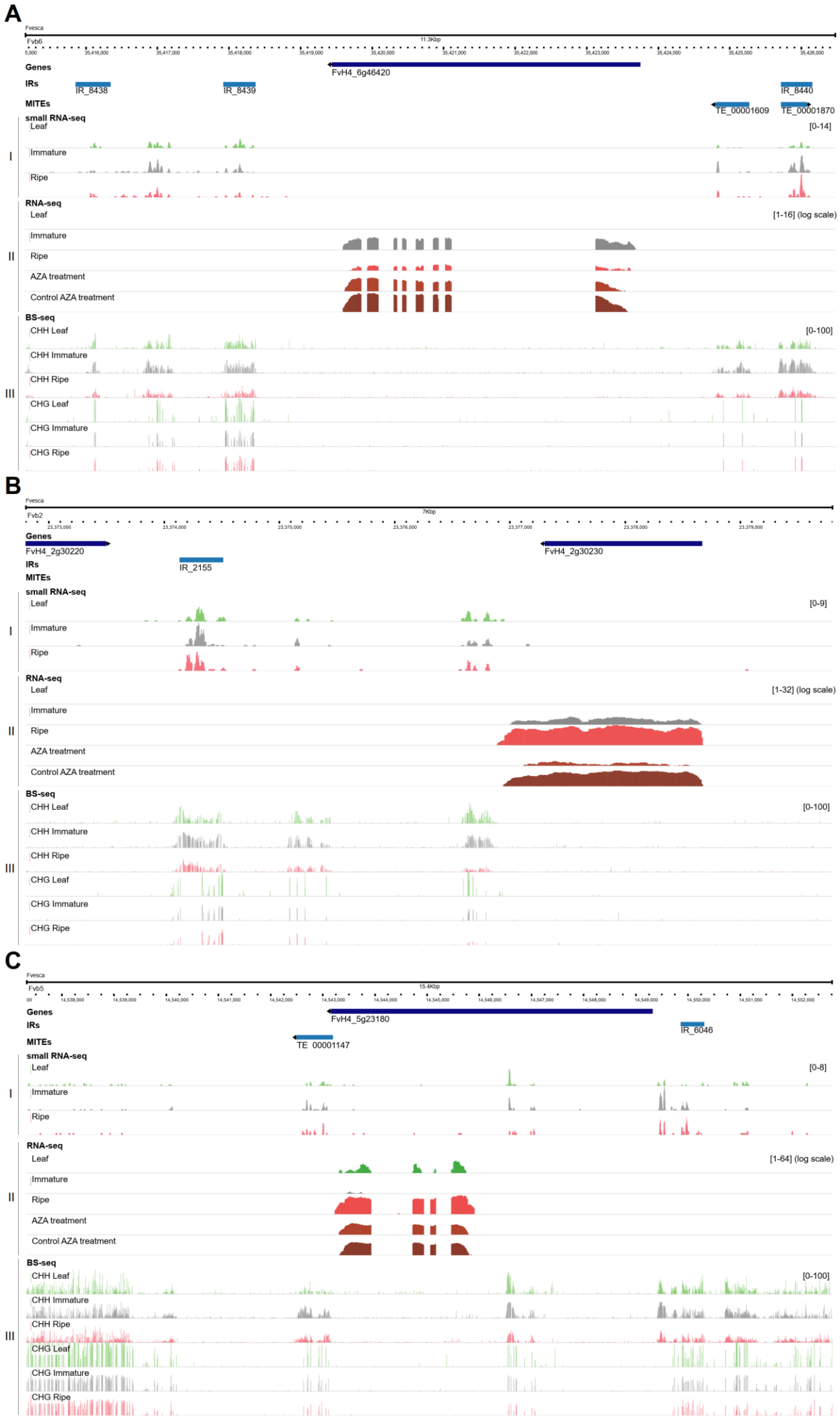
A
Fvesca
Fvb6
11.3Kbp
Genes
FvH4_6g46420
IRs
IR_8438
IR_8439
IR_8440
MITEs
TE_00001609
TE_00001870
small RNA-seq
Leaf
[0-14]
Immature
Ripe
I
RNA-seq
Leaf
[1-16] (log scale)
Immature
Ripe
AZA treatment
Control AZA treatment
II
BS-seq
CHH Leaf
[0-100]
CHH Immature
CHH Ripe
CHG Leaf
CHG Immature
CHG Ripe
III
B
Fvesca
Fvb2
7Kbp
Genes
FvH4_2g30220
FvH4_2g30230
IRs
IR_2155
MITEs
small RNA-seq
Leaf
[0-9]
Immature
Ripe
I
RNA-seq
Leaf
[1-32] (log scale)
Immature
Ripe
AZA treatment
Control AZA treatment
II
BS-seq
CHH Leaf
[0-100]
CHH Immature
CHH Ripe
CHG Leaf
CHG Immature
CHG Ripe
III
C
Fvesca
Fvb5
15.4Kbp
Genes
FvH4_5g23180
IRs
IR_6046
MITEs
TE_00001147
small RNA-seq
Leaf
[0-8]
Immature
Ripe
I
RNA-seq
Leaf
[1-64] (log scale)
Immature
Ripe
AZA treatment
Control AZA treatment
II
BS-seq
CHH Leaf
[0-100]
CHH Immature
CHH Ripe
CHG Leaf
CHG Immature
CHG Ripe
III

Figure S8. Region of the F. vesca v4.0.a2 reference genome containing the FvH4_6g46420 **(A)**, FvH4_2g30230 **(B),** and FvH4_5g23180 **(C)** loci displaying the IR and MITE elements annotated in the region and expression and epigenetic profiles. (I) 24-nt siRNAs mapping to the genomic regions as determined by sRNA sequencing of leaf (green), immature (gray) and ripe (pink) tissues. Replicates are plotted together. (II) Expression of each gene in leaf (green), immature (gray) and ripe (pink) tissues as well as strawberries treated with 5-azacytidine (5-AZA) and its control, measured by RNA-seq. (III) Cytosine DNA methylation in CG, CHG, and CHH contexts in leaf (green), immature (gray) and ripe (pink) fruits. The average of individual BS-seq replicates is plotted for each condition.

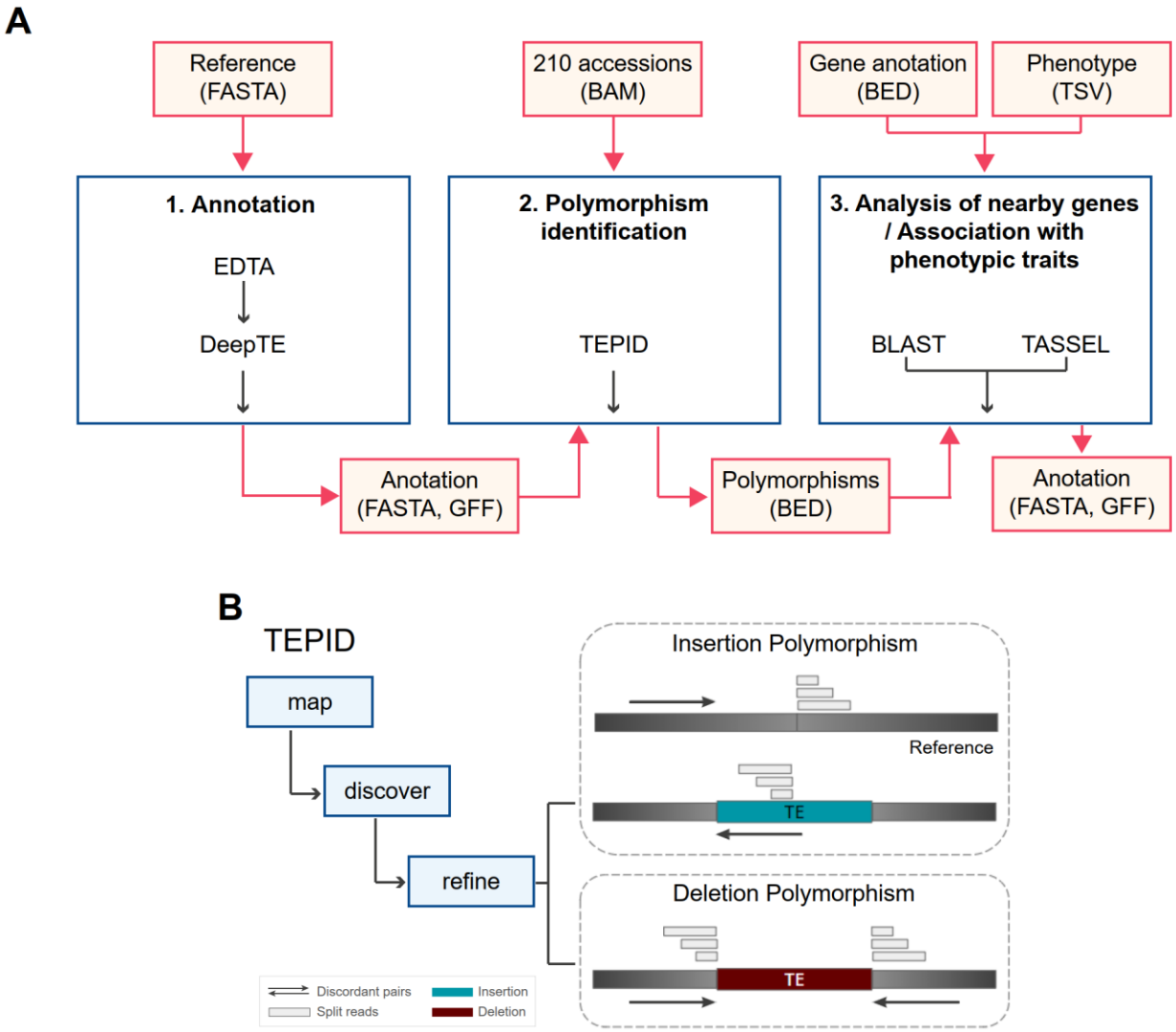

Figure S9. (A) Representation of the pipeline used in this study. The main inputs are shown at the top, while the main outputs are placed at the bottom. File formats are indicated in parentheses. **(B)** Representation of the TEPID pipeline used for identifying transposable element insertion polymorphisms (TIPs) and absence polymorphisms (TAPs).

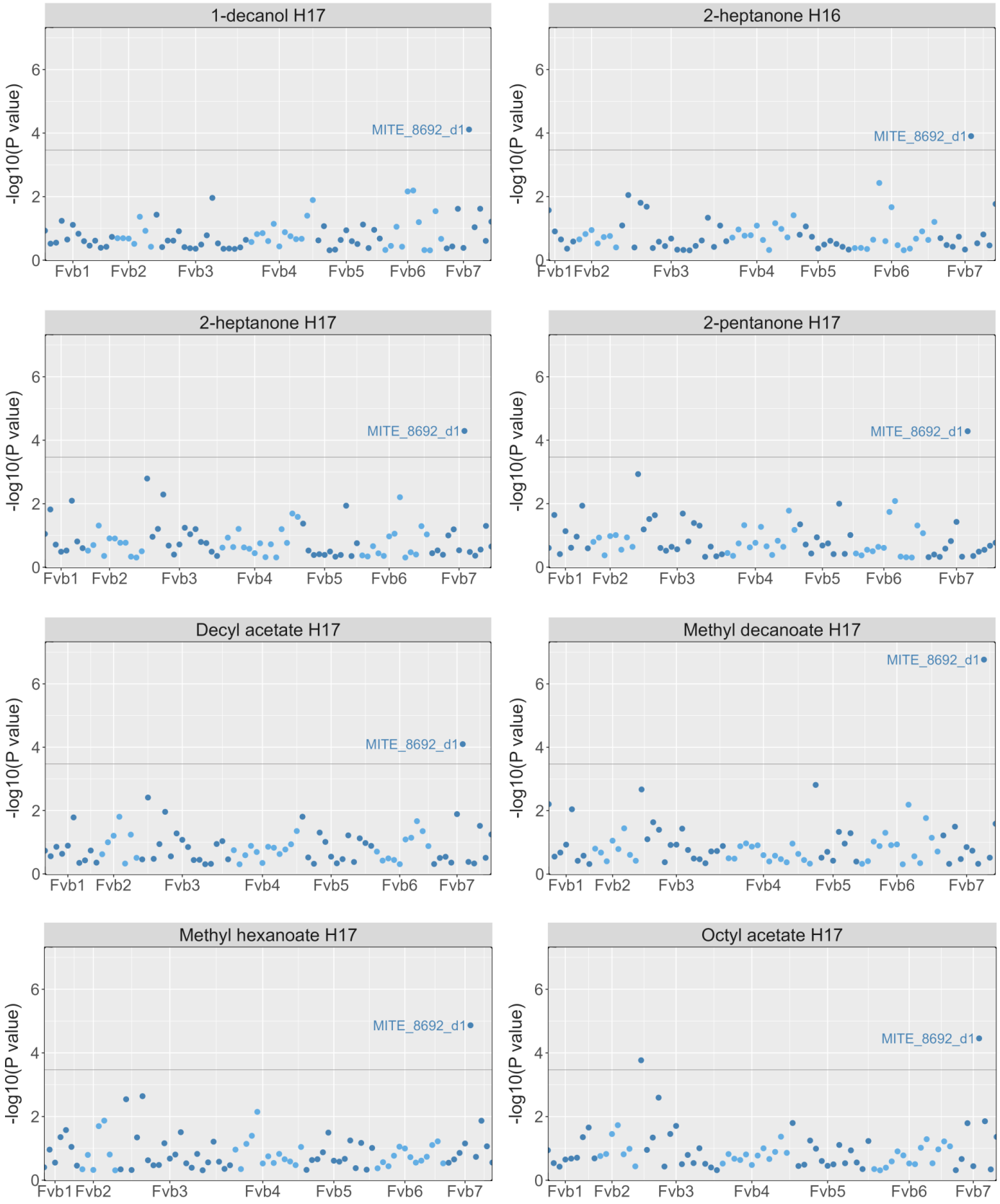

Figure S10. Association between a MITE deletion polymorphism and the relative amounts of volatile compounds in the H16 and H17 harvests. Manhattan plots illustrate the association between the deletion polymorphism 'MITE_8692_d1' and the relative amount of 1-decanol, 2-heptanone, 2-pentanone, decyl acetate, methyl decanoate, methyl hexanoate and octyl acetate, for the H16 and H17 harvests as indicated. A gray line indicates the significance threshold determined via the Bonferroni test (threshold = 3.47), and the polymorphism with a significant association is labeled.

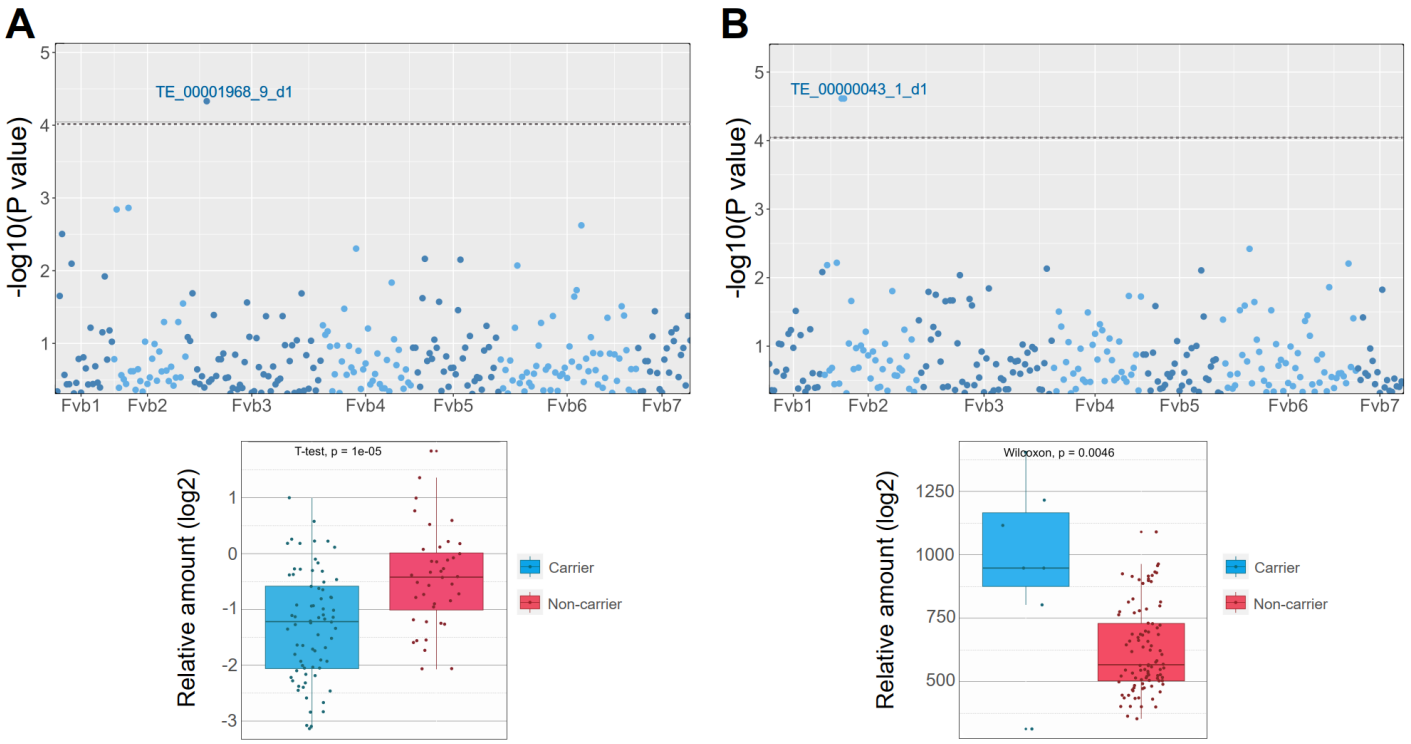

Figure S11. TE polymorphisms linked to phenotypic characteristics of woodland strawberry fruits. (A) Association between the TE deletion polymorphism 'TE_00001968_9_d1' and the relative quantity of (Z)-3-hexenyl acetate in the H16 harvest. Top: Manhattan plot showing the association between TE deletion polymorphisms and the relative quantity of (Z)-3-hexenyl acetate. A dotted gray line indicates the significance threshold calculated using the Bonferroni test (threshold = 4.05), and the polymorphism with a significant association is labeled. Bottom: Box plot of the relative quantity of (Z)-3-hexenyl acetate for accessions carrying the polymorphism (light blue) versus non-carriers (red). The p-value from t-test is shown. **(B)** Association between the TE deletion polymorphism 'TE_00000043_1_d1' and average fruit volume in the H16 harvest. Top: Manhattan plot showing the association between TE deletion polymorphisms and average strawberry fruit volume. The significance threshold calculated via the Bonferroni test (threshold = 4.05) is indicated by a dotted gray line, and the polymorphism with significant association is labeled. Bottom: Box plot of average fruit volume for accessions carrying the polymorphism (light blue) and non-carriers (red). The p-value from Wilcoxon test is shown.