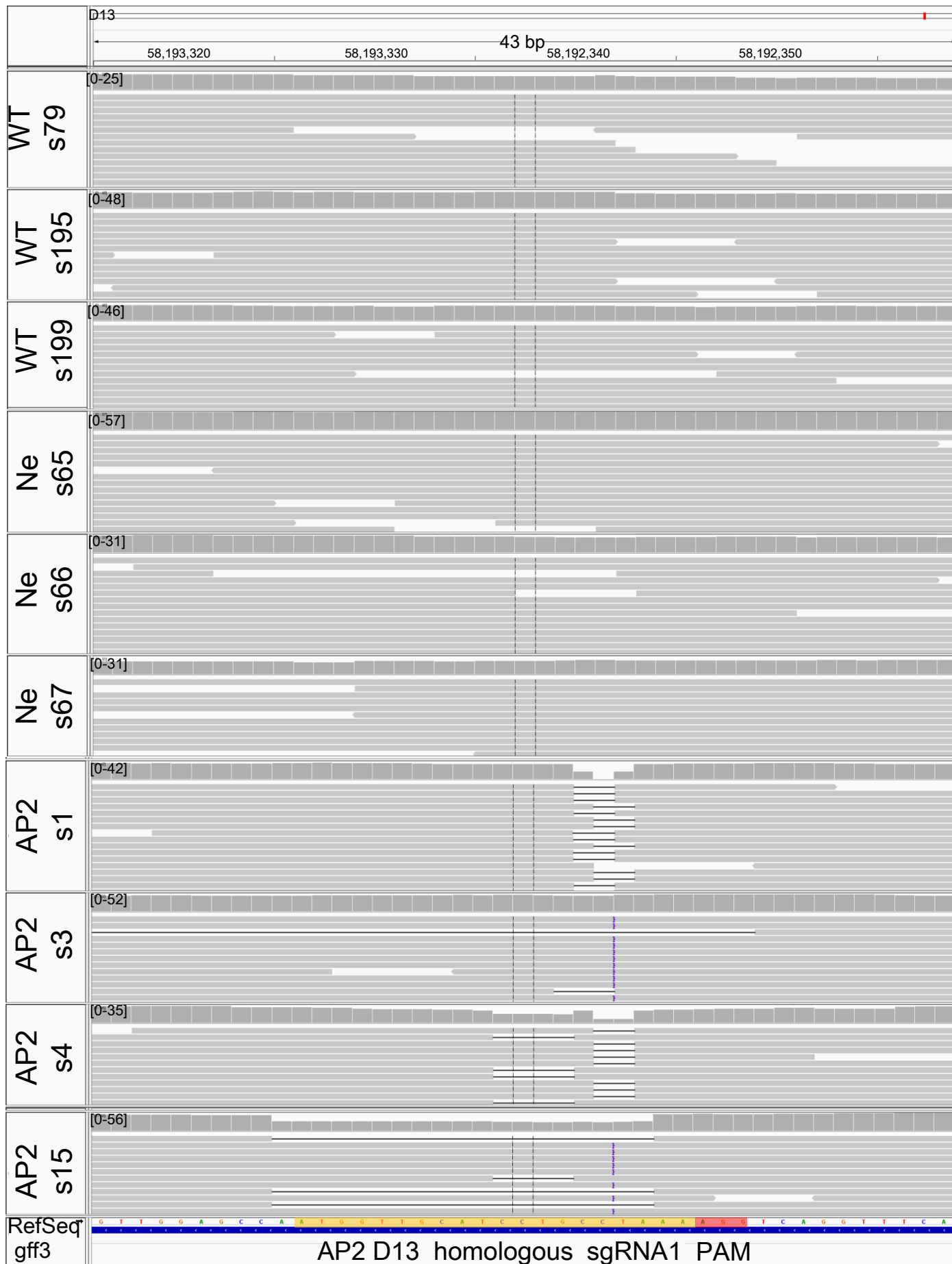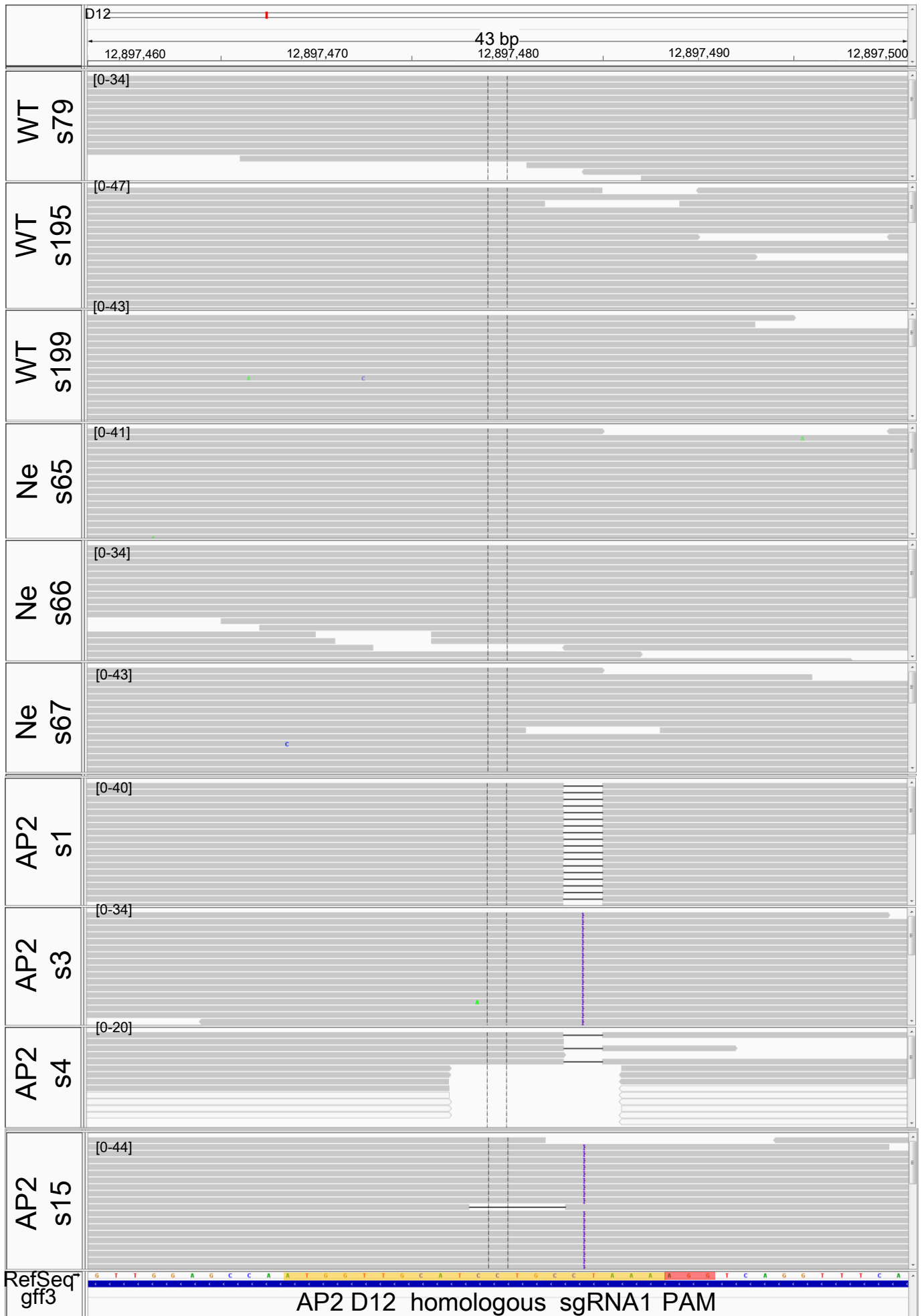**Figure S1** The compared genome editing efficiency in Cas9-edited plants. (a) The editing efficency in different Cas9-edited plants. (b) The compare genome editing efficiency of At and Dt-subgenome based on WGS data.
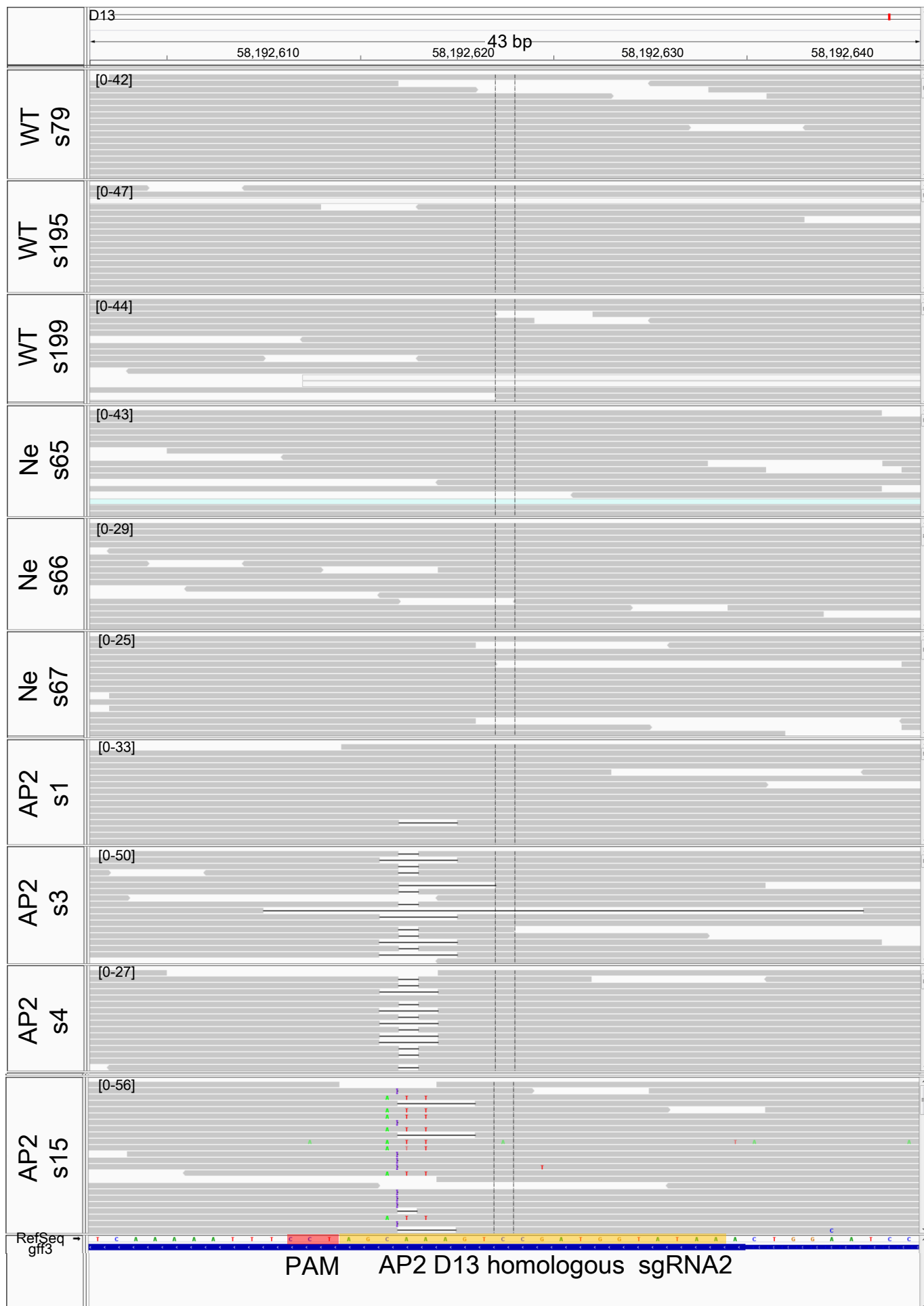
(a) AP2 D13 homologous sgRNA1

(b) AP2 D12 homologous sgRNA1

(c) AP2 D13 homologous sgRNA2

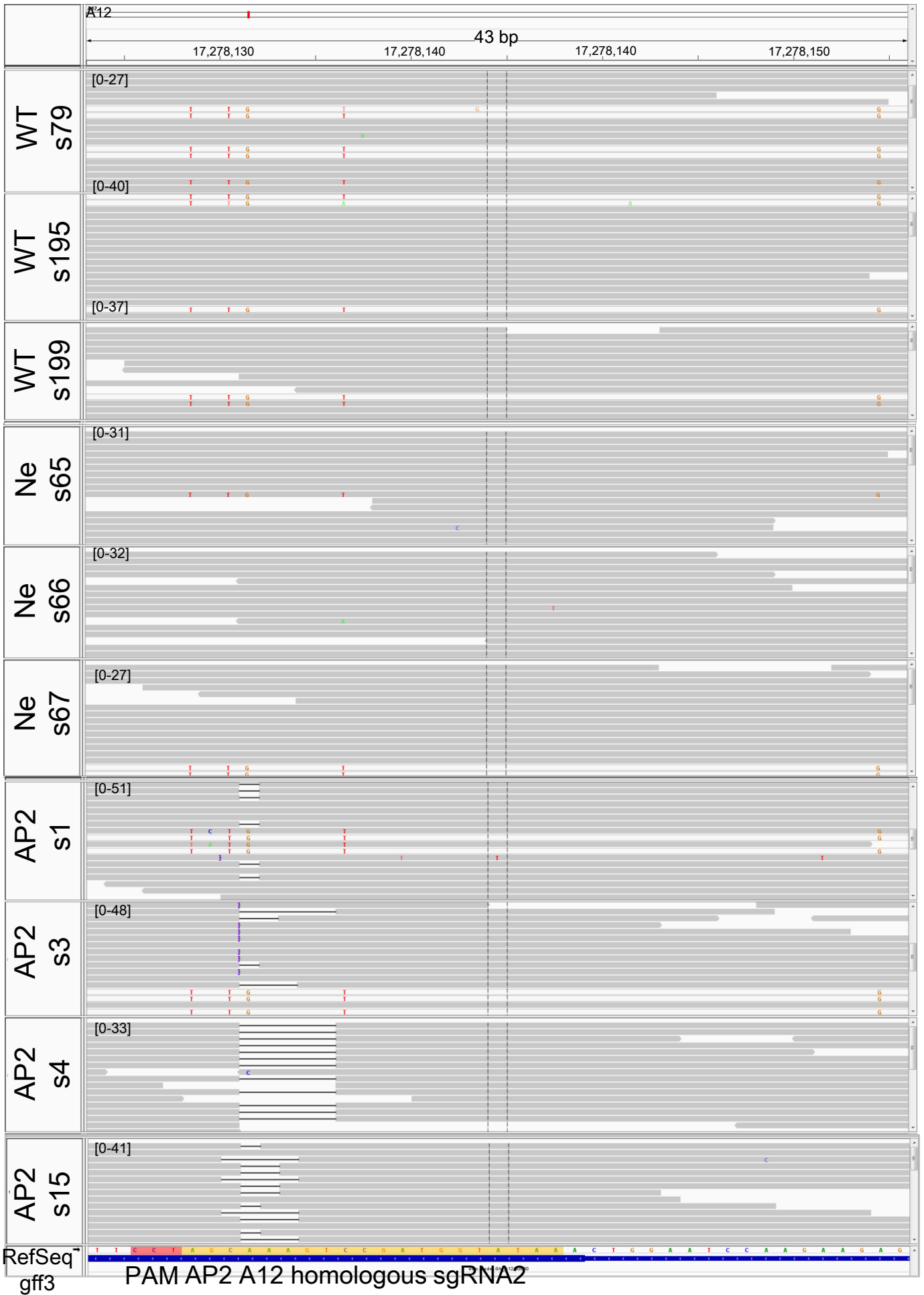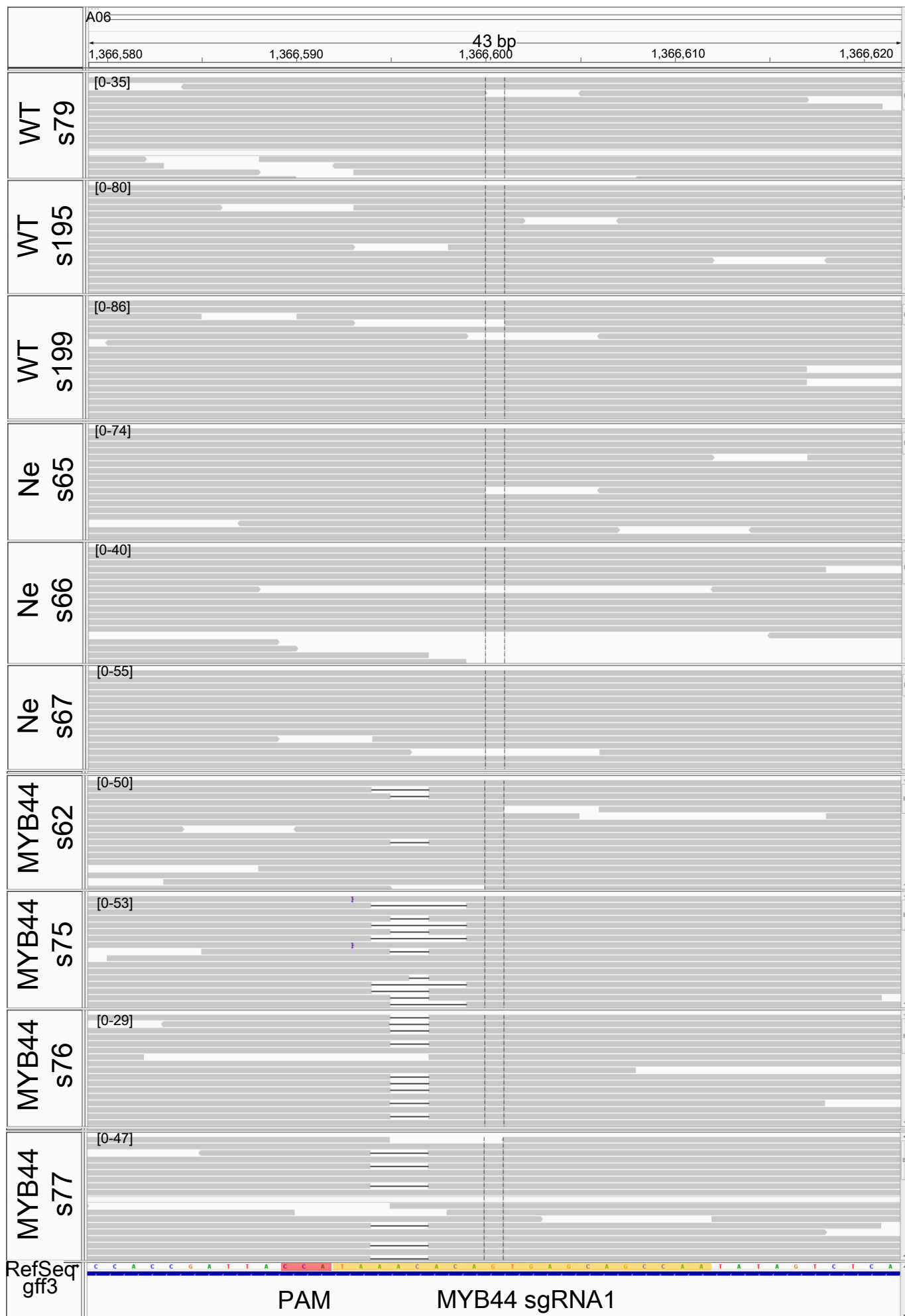## (d) AP2 A12 homologous sgRNA1

PAM AP2 A12 homologous sgRNA2

Figure S2 Confirmation of the *AP2* on-target mutation in two sgRNAs from WT, negative and CRISPR/Cas9 plants based on the WGS data. The orange box represents the target sites, and the red box represents the PAM sequence. Whole genome sequencing analysis at sgRNA2 target region of *MYB44* in 3 wild-type, 3 negative plants and 4 CRISPR/Cas9-edited *AP2* plants by Integrative Genomics Viewer (IGV). The number in the square brackets e.g. [0-56] represents the WGS supporting sequence reads with on-target sites and the pileup strip represent Cas9-edited *MYB44* plants a heterozygous deletion of 43-bp of *AP2*. The *AP2* sgRNA1 and sgRNA2 homologous loci reads were showed in (a-b) and (c-d) , respectively.
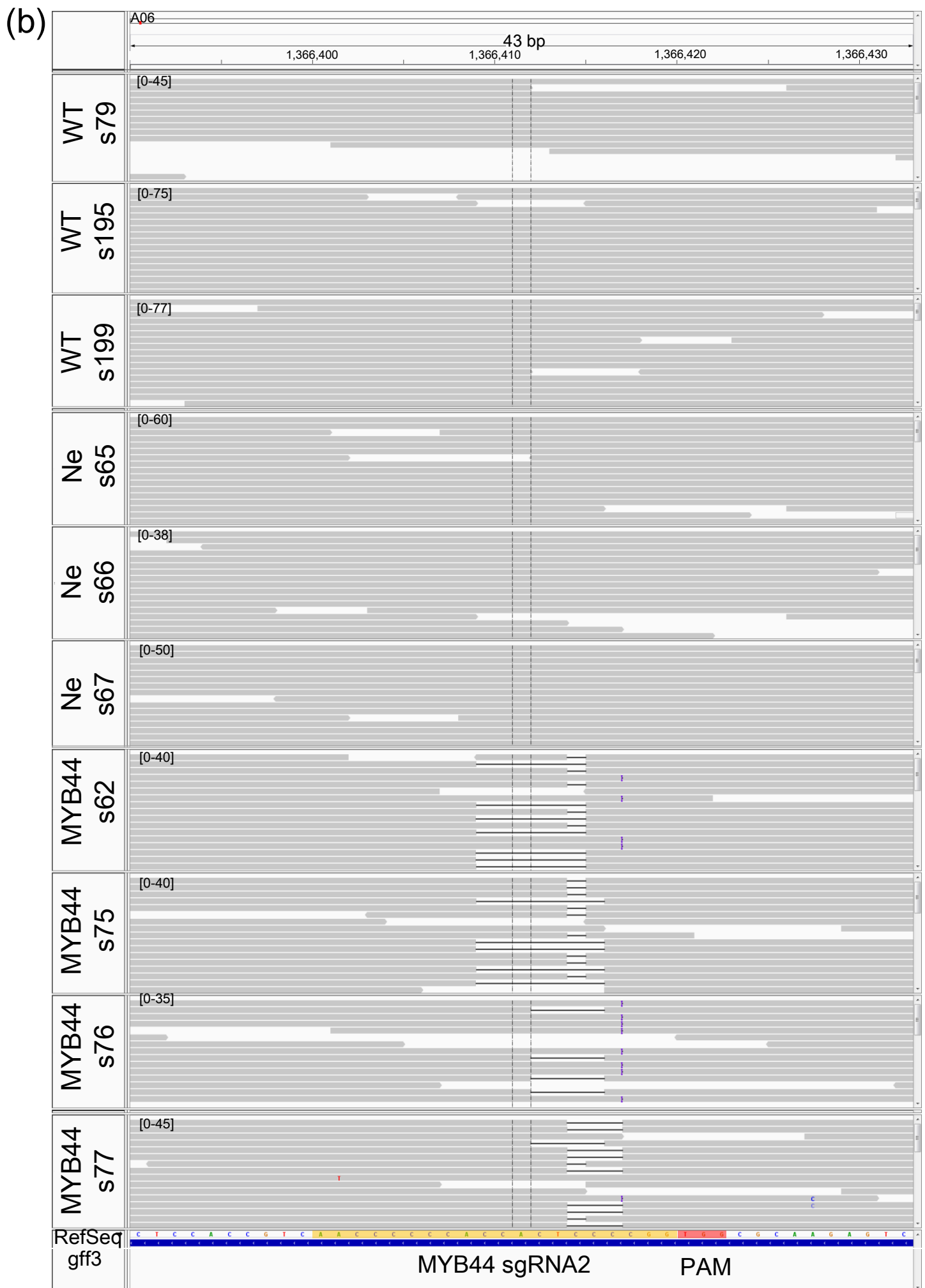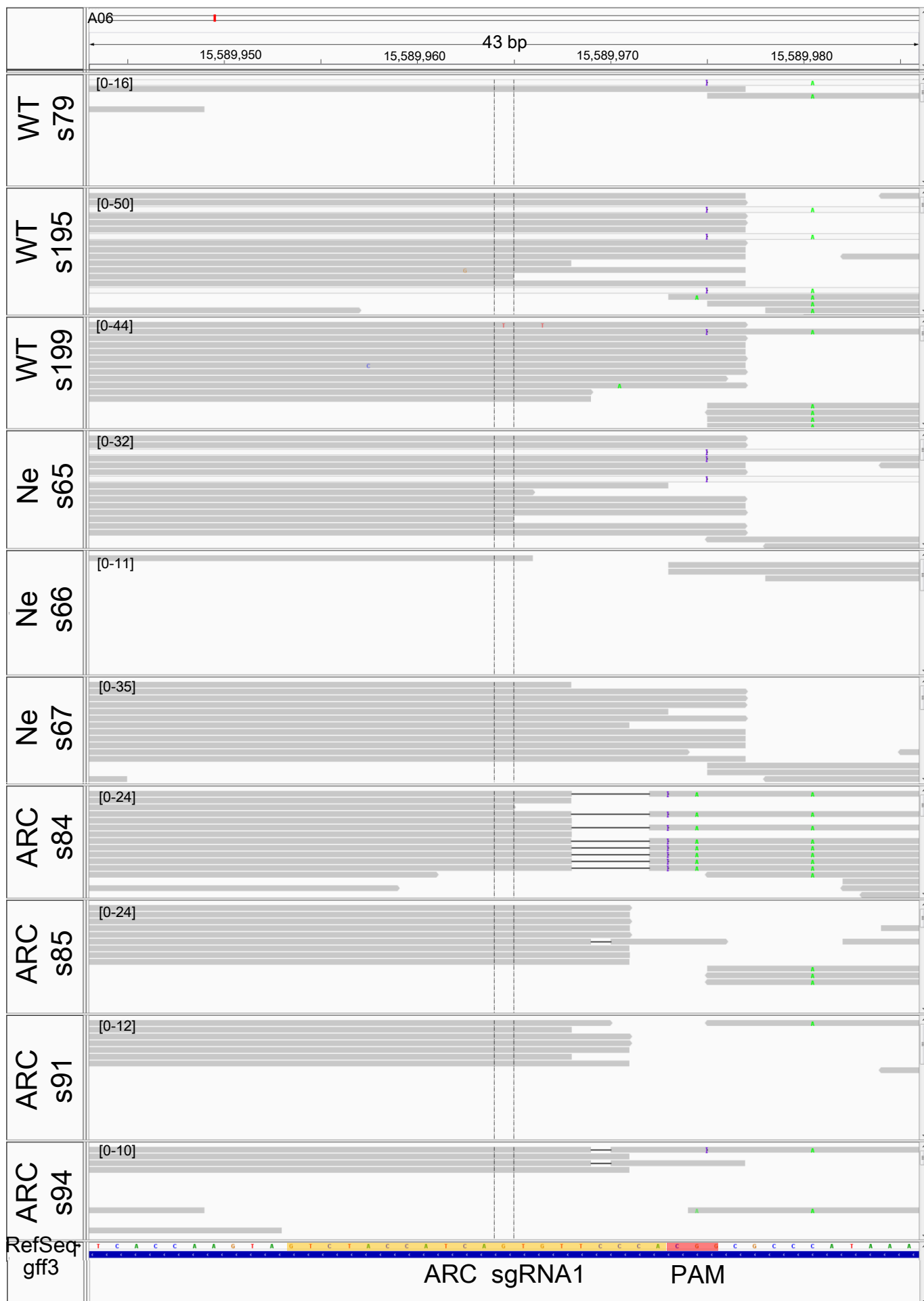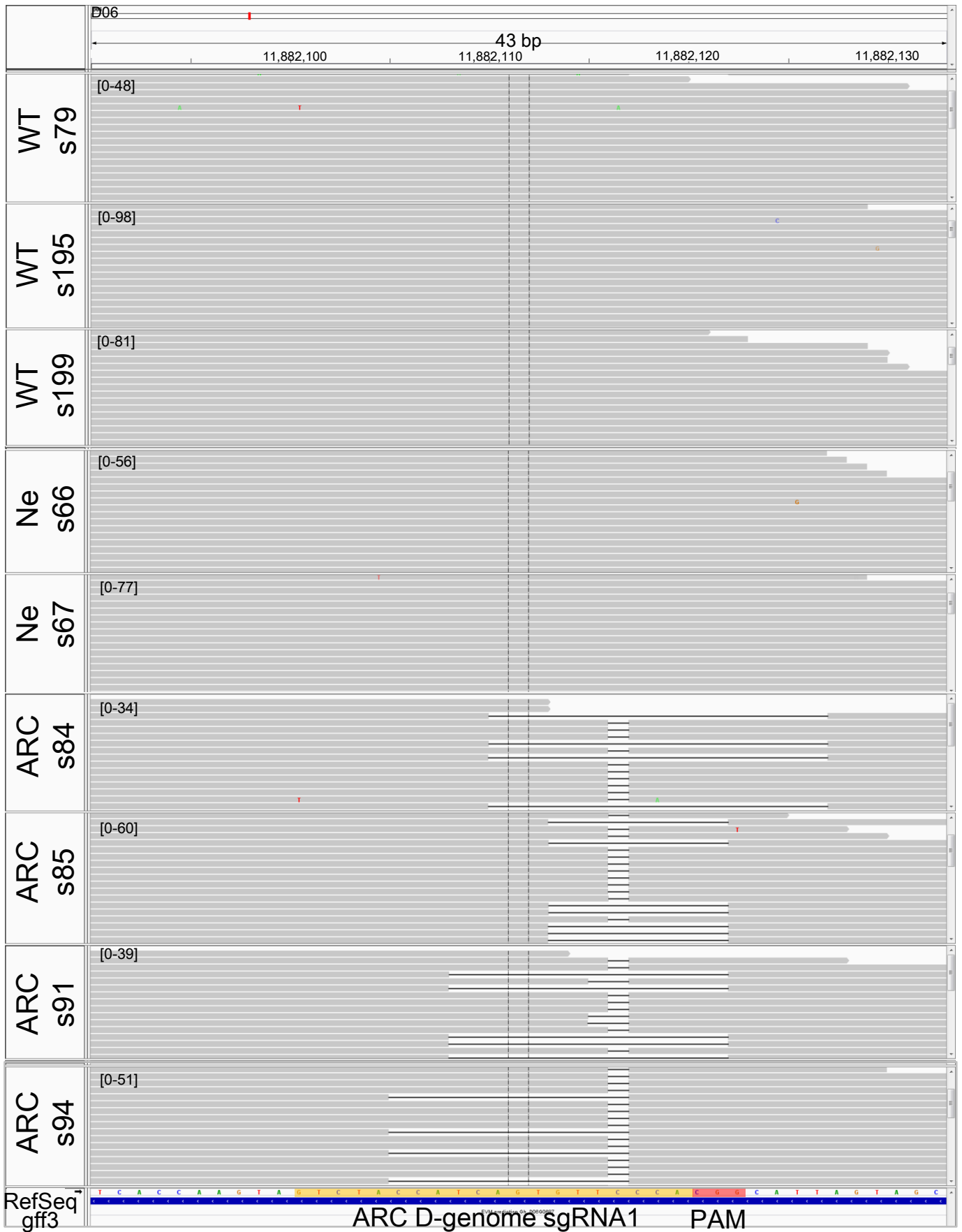
(a)

Figure S3 Confirmation of the *MYB44* on-target mutation in two sgRNA loci from WT, negative and CRISPR/Cas9 plants based on the WGS data.
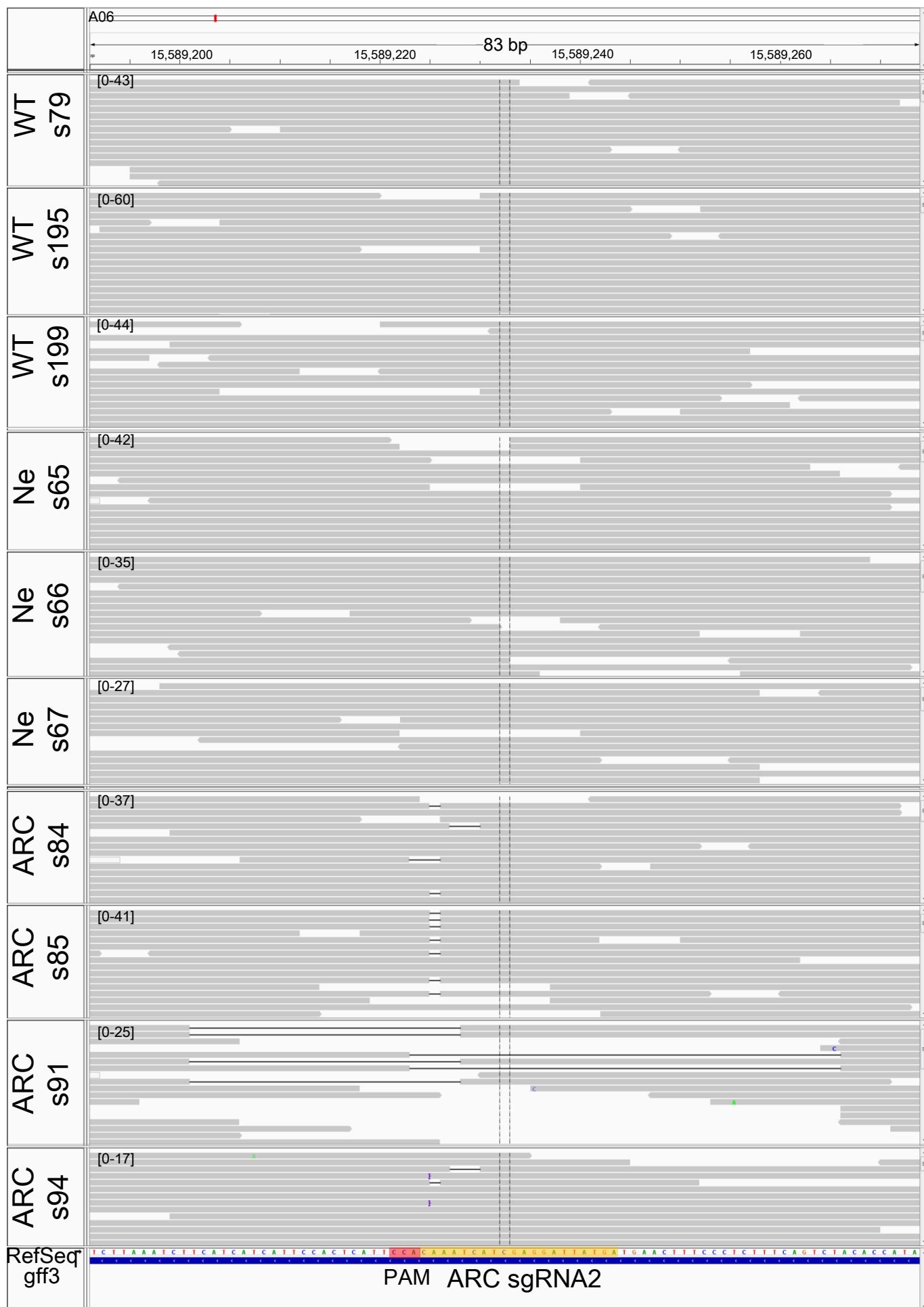
(a) ARC A-genome sgRNA1

(b) ARC D-genome sgRNA1

(c) ARC A-genome sgRNA2
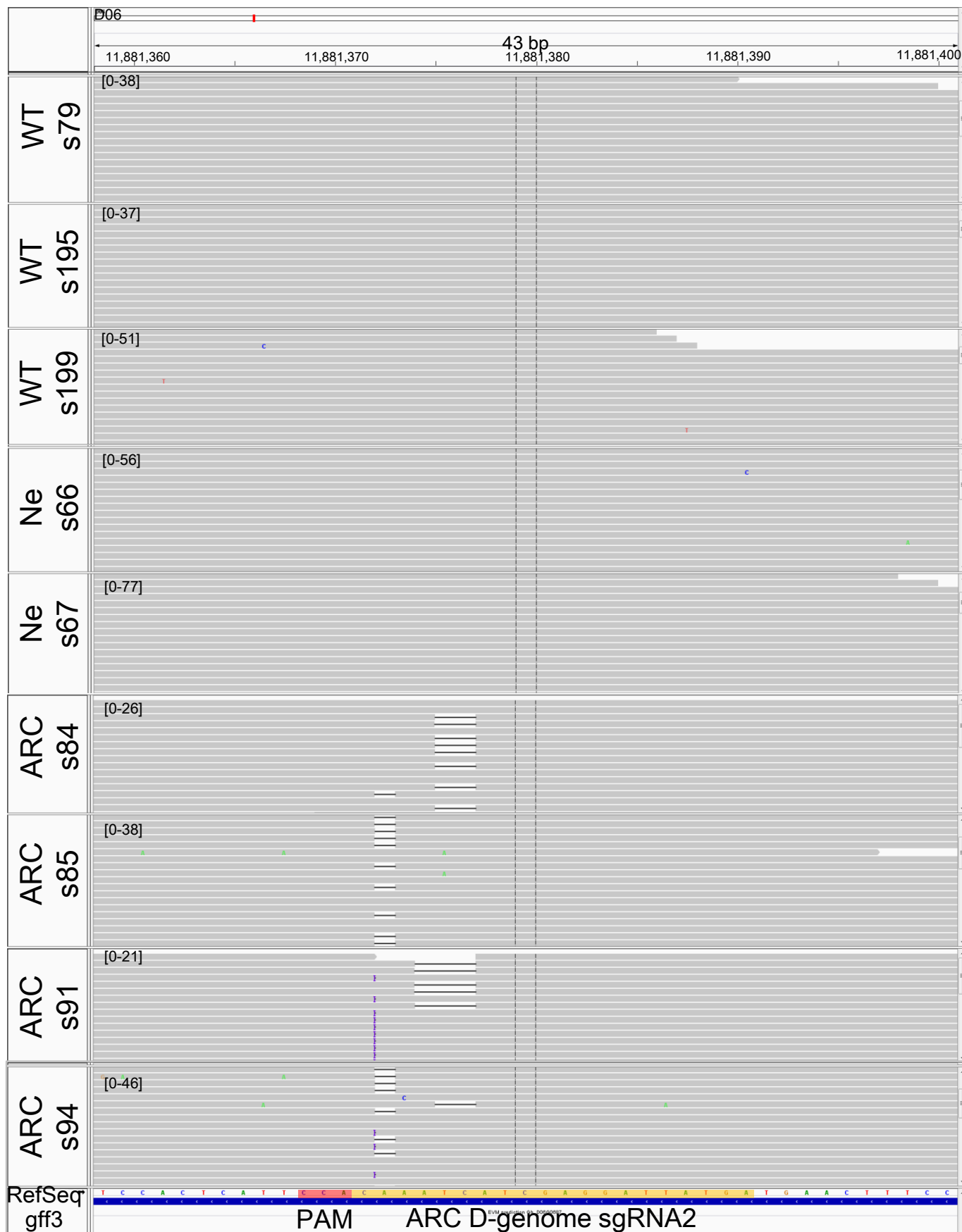
(d) ARC D-genome sgRNA2

Figure S4 Confirmation of the *ARC* on-target mutation in two sgRNA loci from WT, negative and CRISPR/Cas9 plants based on the WGS data. The *ARC* sgRNA1 and sgRNA2 homologous loci reads were showed in (a-b) and (c-d), respectively.
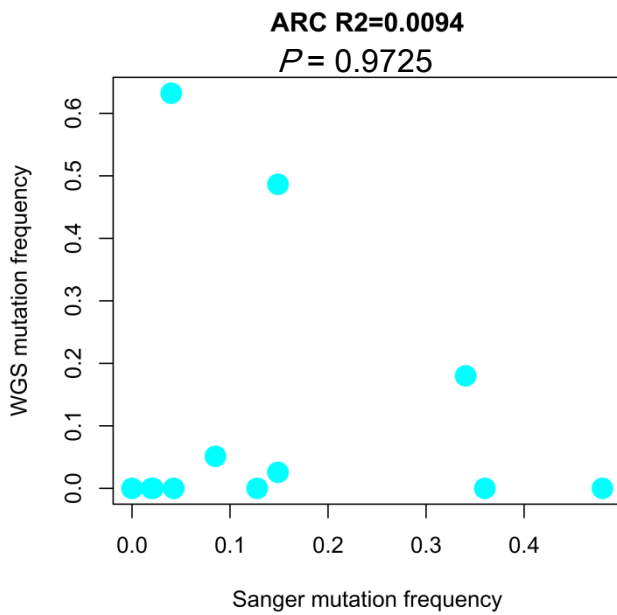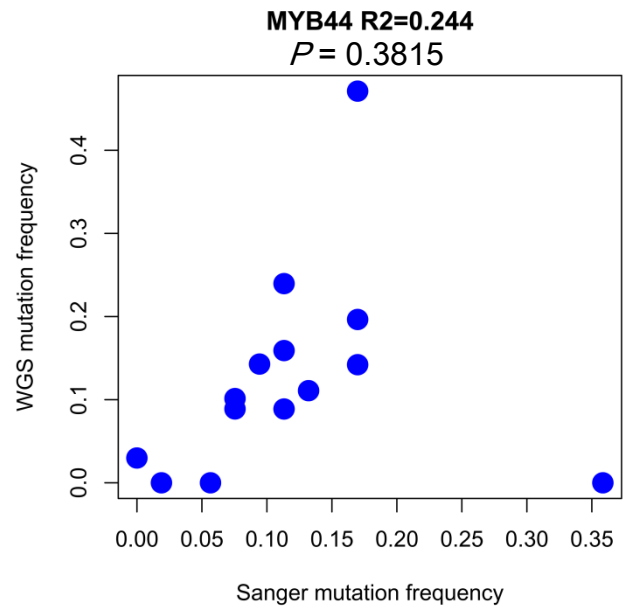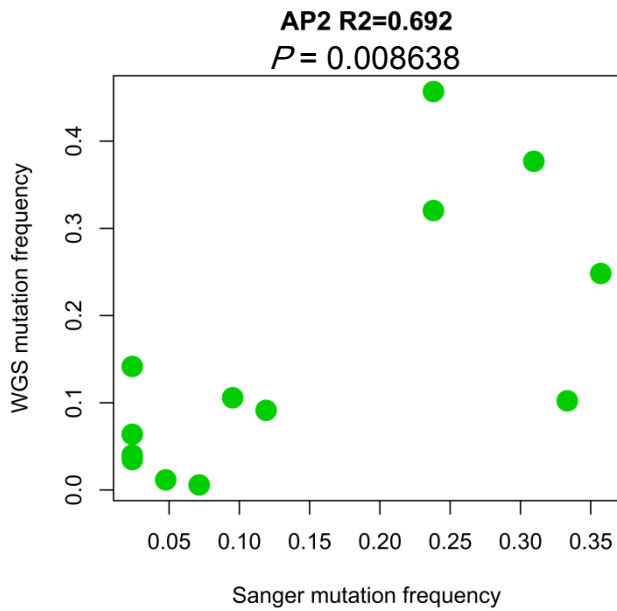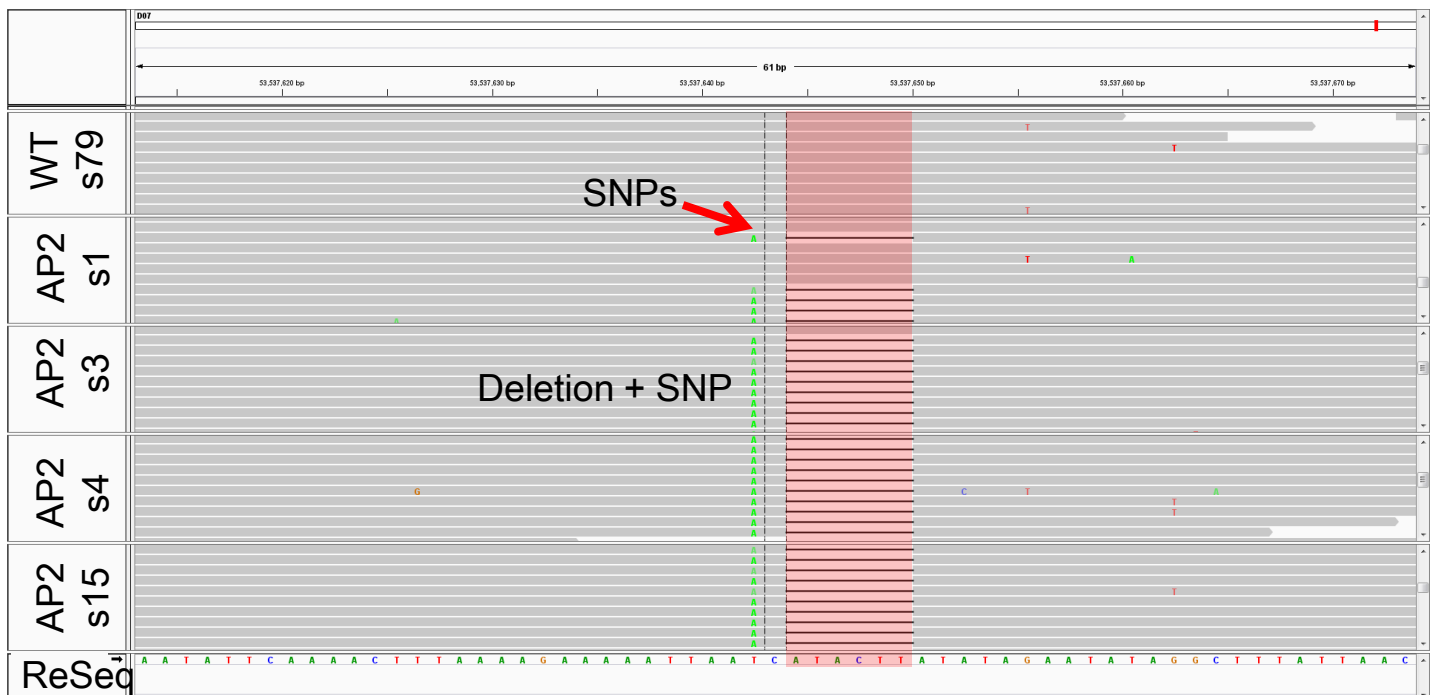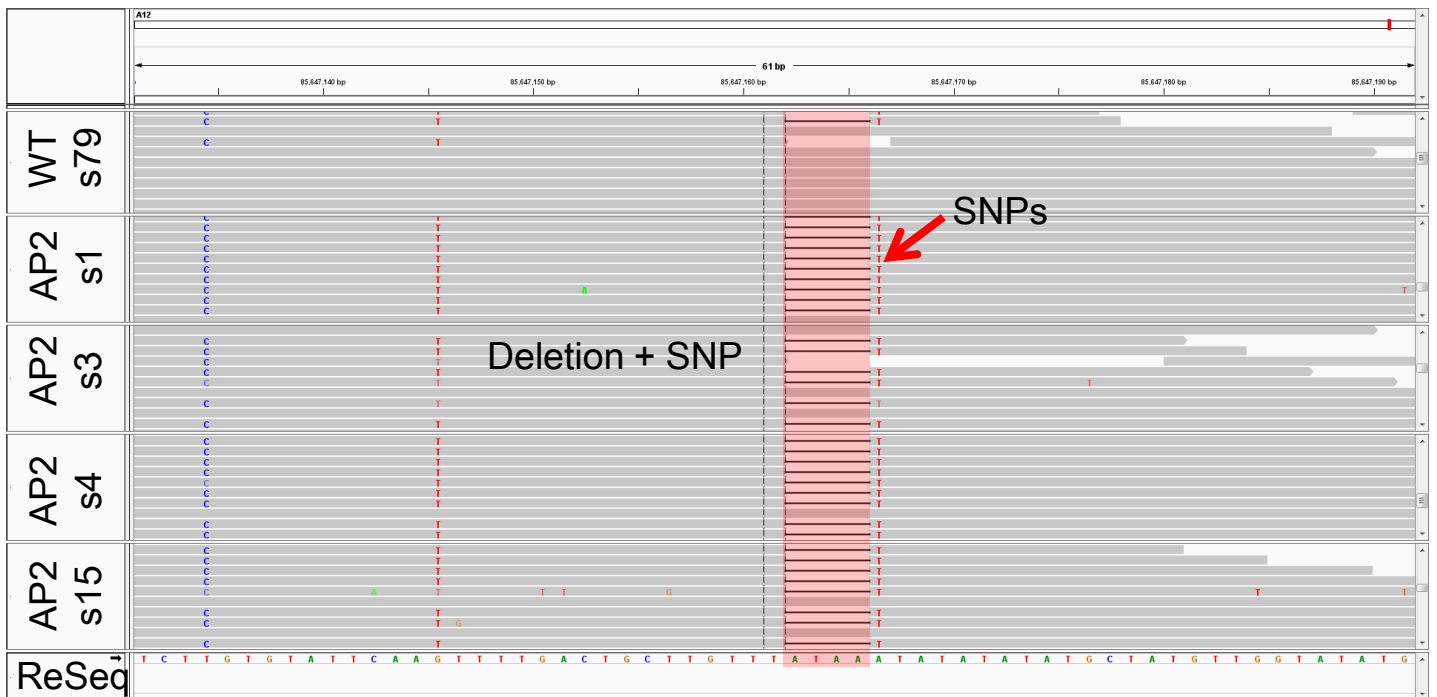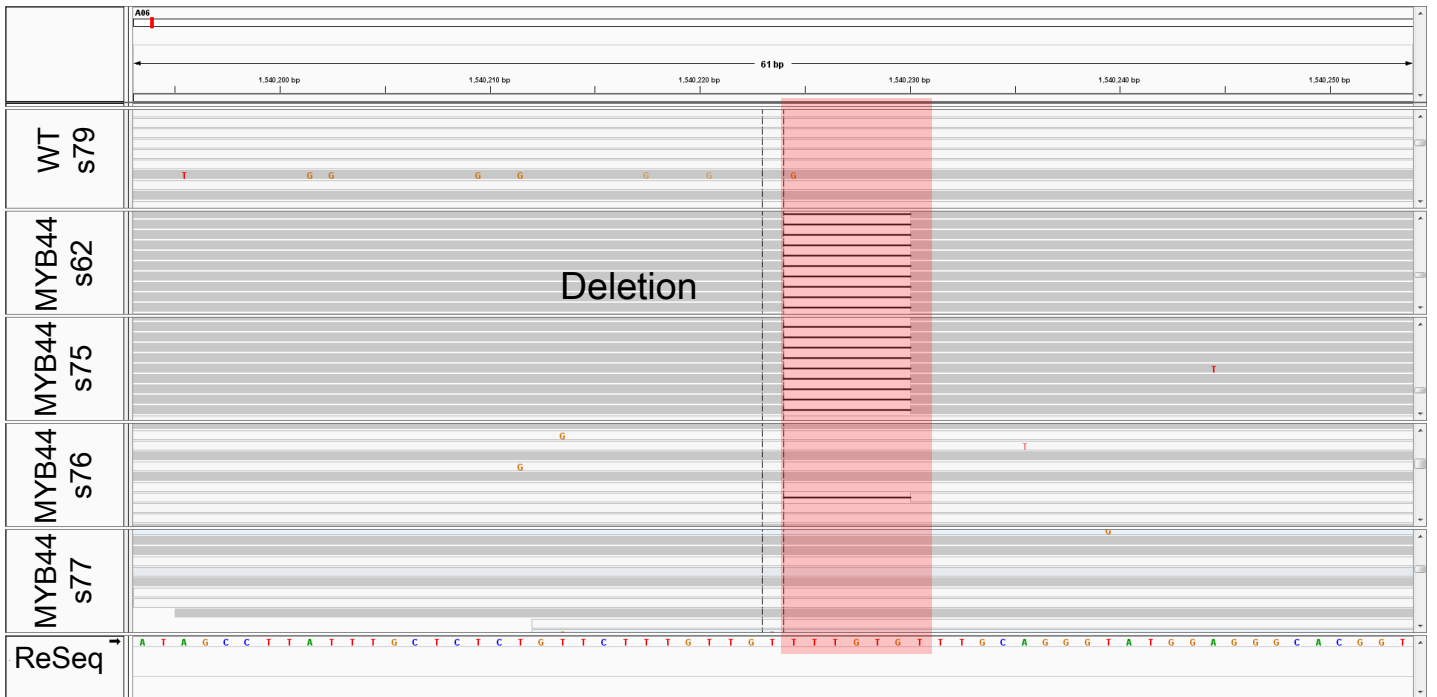
Figure S5 Scatterplot of on-target site correlation of sanger sequencing (x axis) and Whole genome sequencing (y axis). The Correlation Coefficient was calculated based on Pearson's method (Pearson's correlation coefficient).

Tissue culture process (TSP) mutations in AP2 Cas9-edited plants

Tissue culture process (TSP) mutations in MYB44 Cas9-edited plants

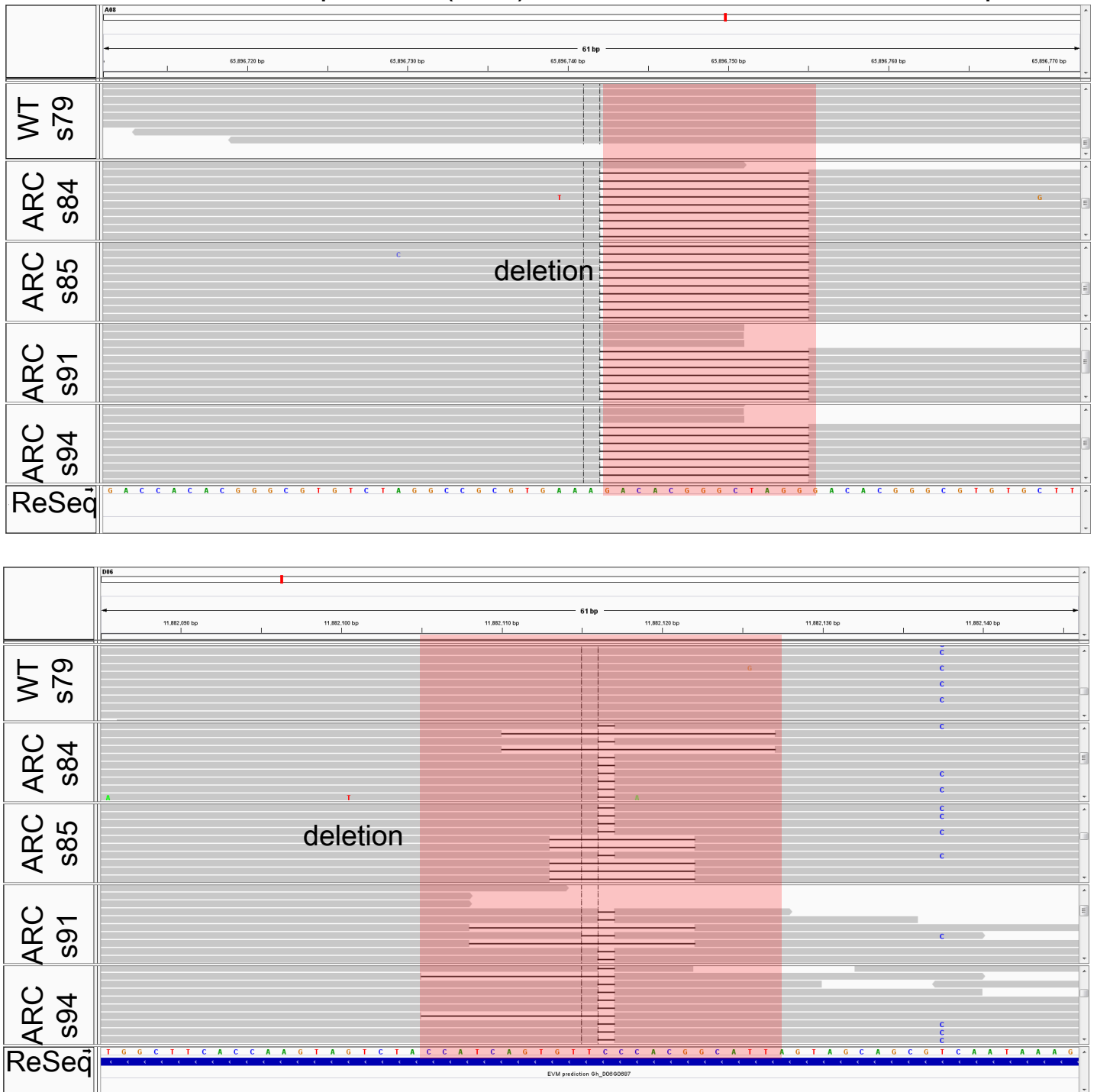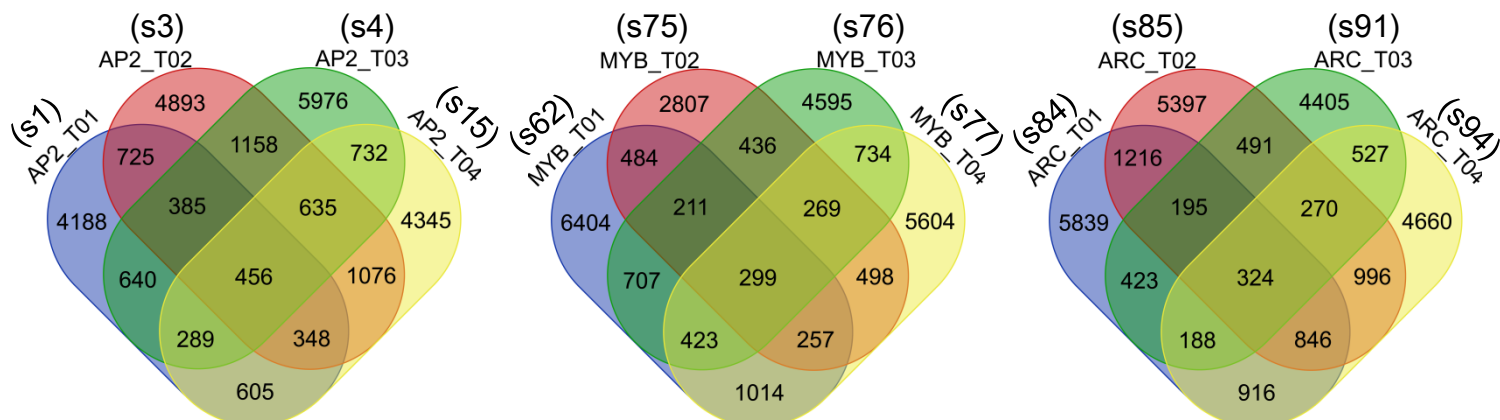# Tissue culture process (TSP) mutations in ARC Cas9-edited plants

Figure S6 The somaclonal variation or/and inherent genetic variation were detected in *AP2*, *MYB44*, *ARC* Cas9-edited plants and WT by IGV. The red box and arrow represent the SNPs and indels, respectively.

Common and private SNPs in different Cas9-edited lines

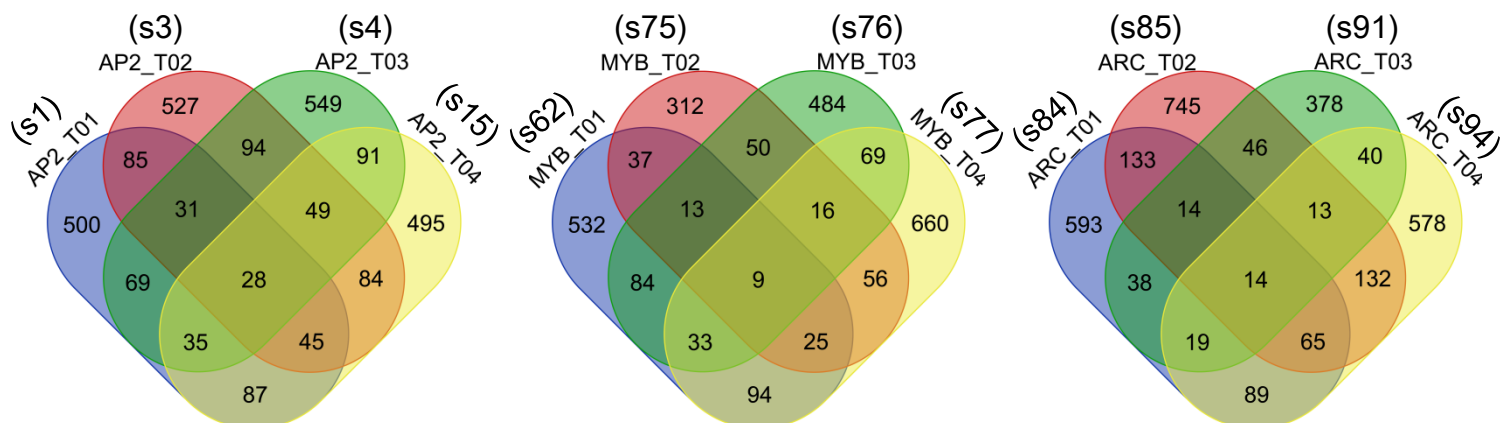# Common and private Indels in different Cas9-edited lines

Figure S7 The common and private SNPs/indels in four Cas9-edited lines of *AP2, MYB44, ARC*.
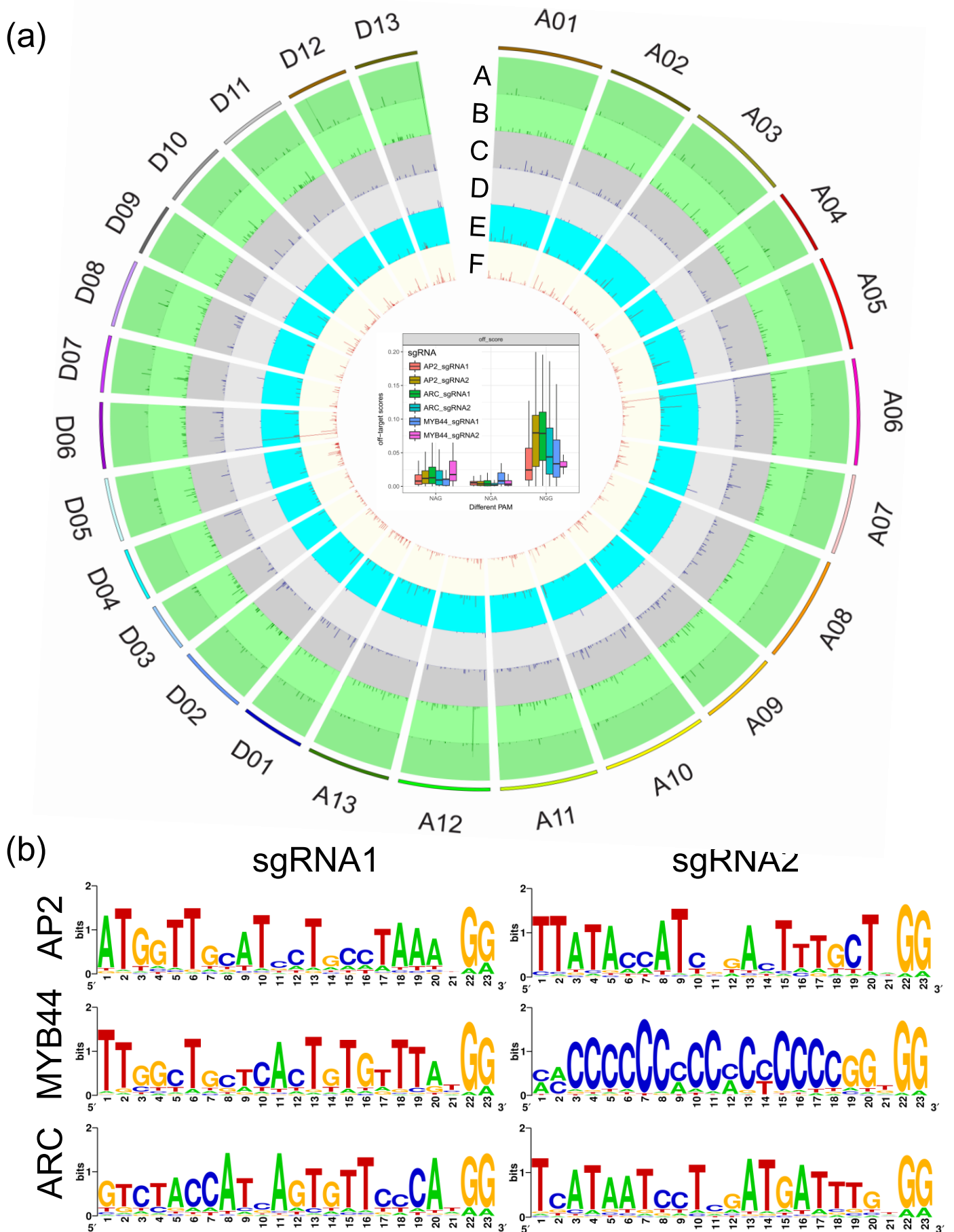
Figure S8 Genome-wide prediction off-target sites. (a) Genome-wide circos plot representing off-target site scores for AP2 sgRNA1 (A) and sgRNA2 (B), MYB44 sgRNA1 (C), and sgRNA2 (D), ARC sgRNA1 (E), and sgRNA2 (F). The different off-target PAM scores were highlighted circos center. (b) The off-target sequences logos were showed.
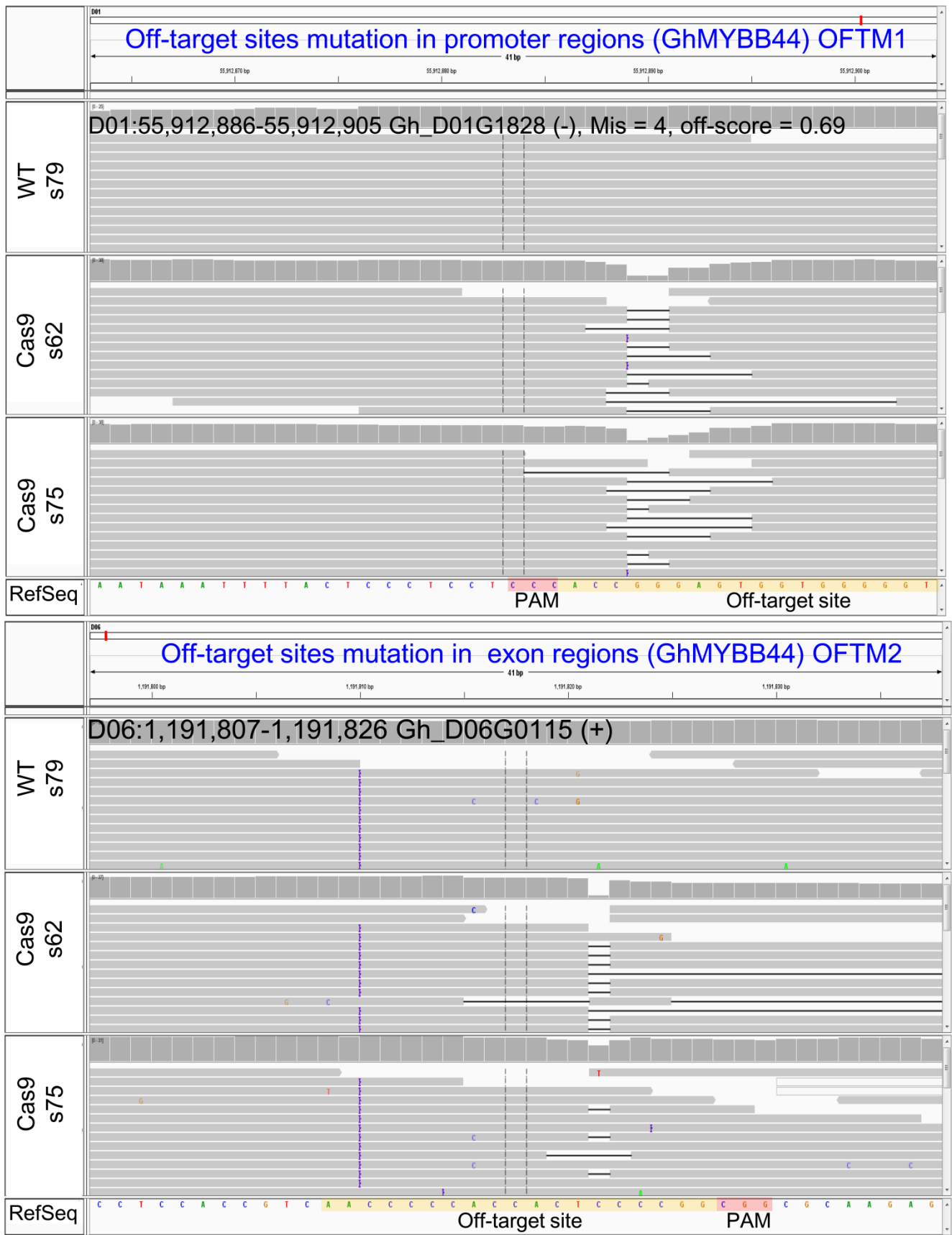
Figure S9 The potential off-target mutations in *MYB44* Cas9-edited cotton plants. The data show the variations in Cas9-s62, Cas9-s75 and WT plants.
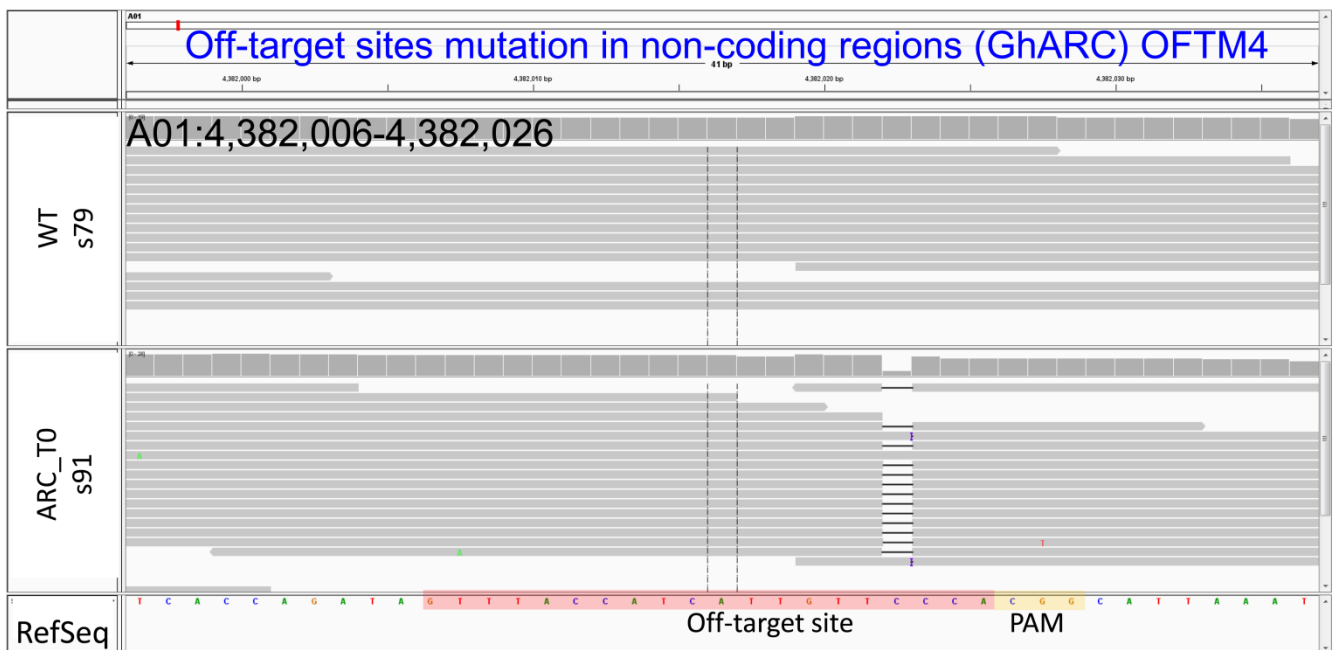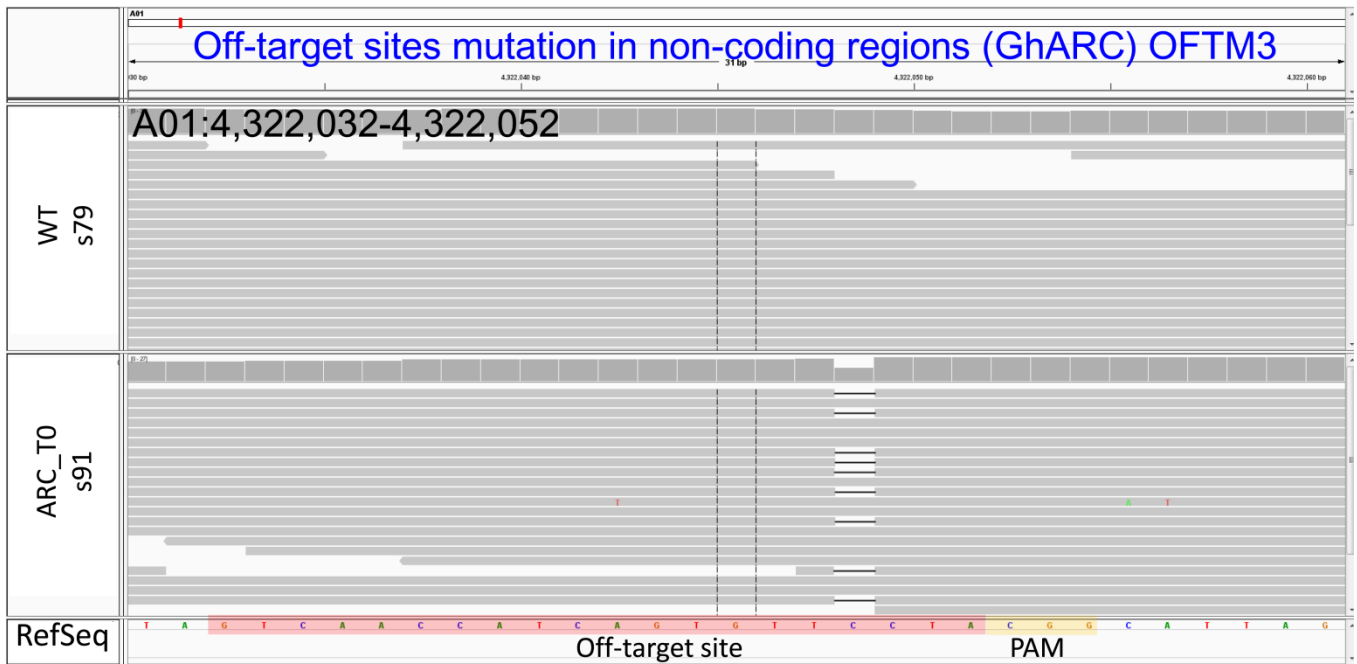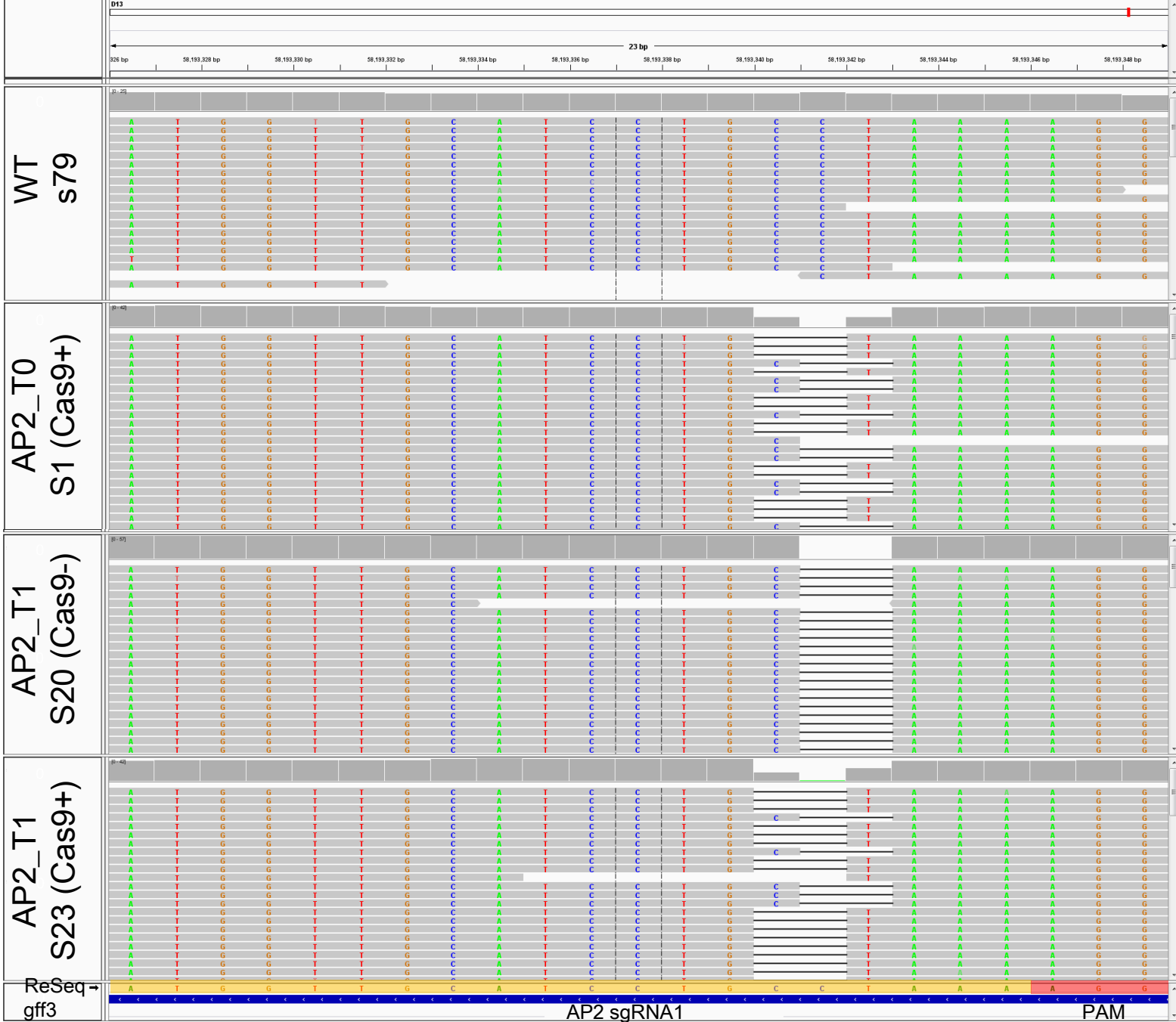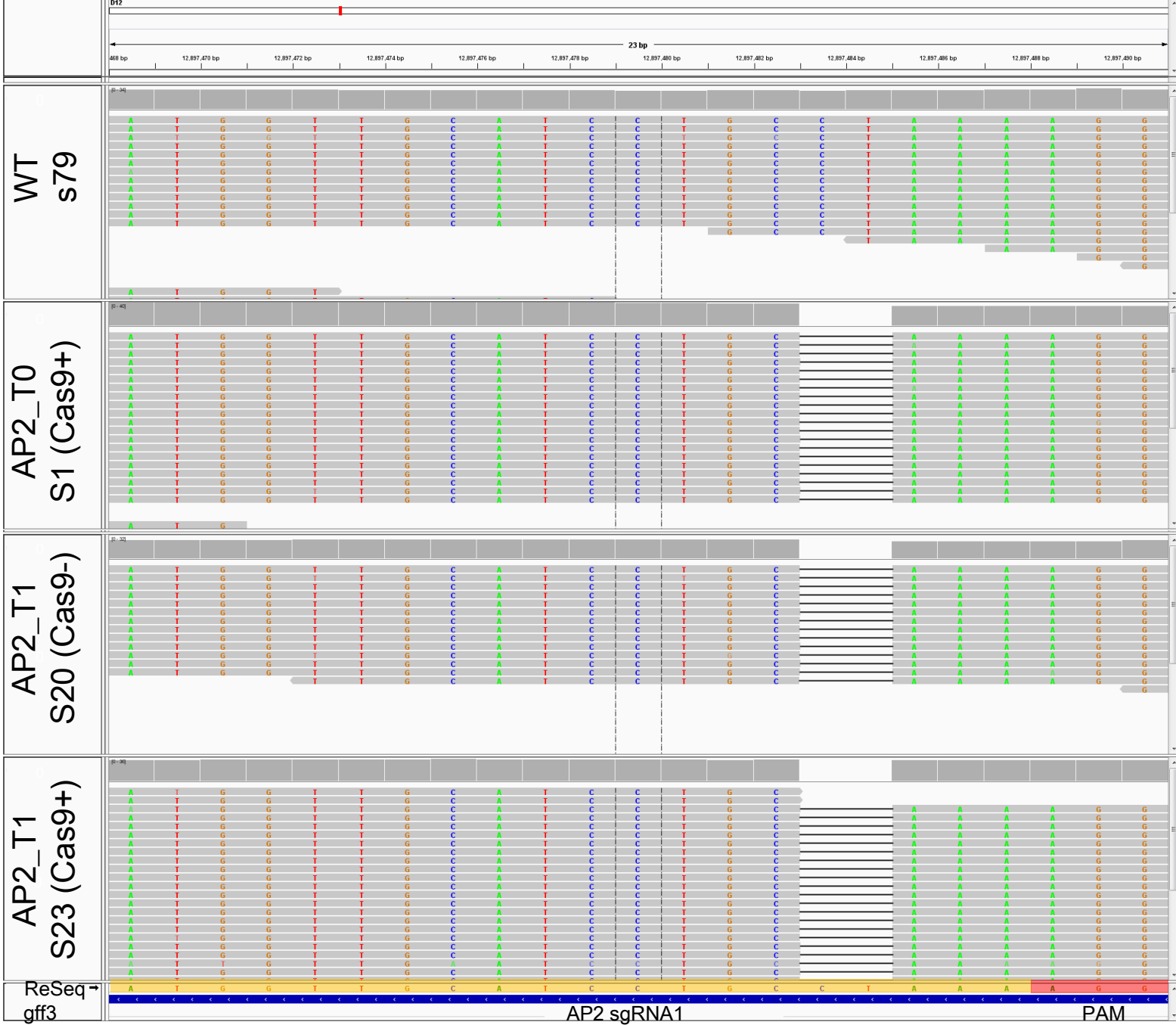
Figure S10 The potential off-target mutations in ARC Cas9-edited cotton plants. The data show the variations in Cas9-s91 and WT Plants.
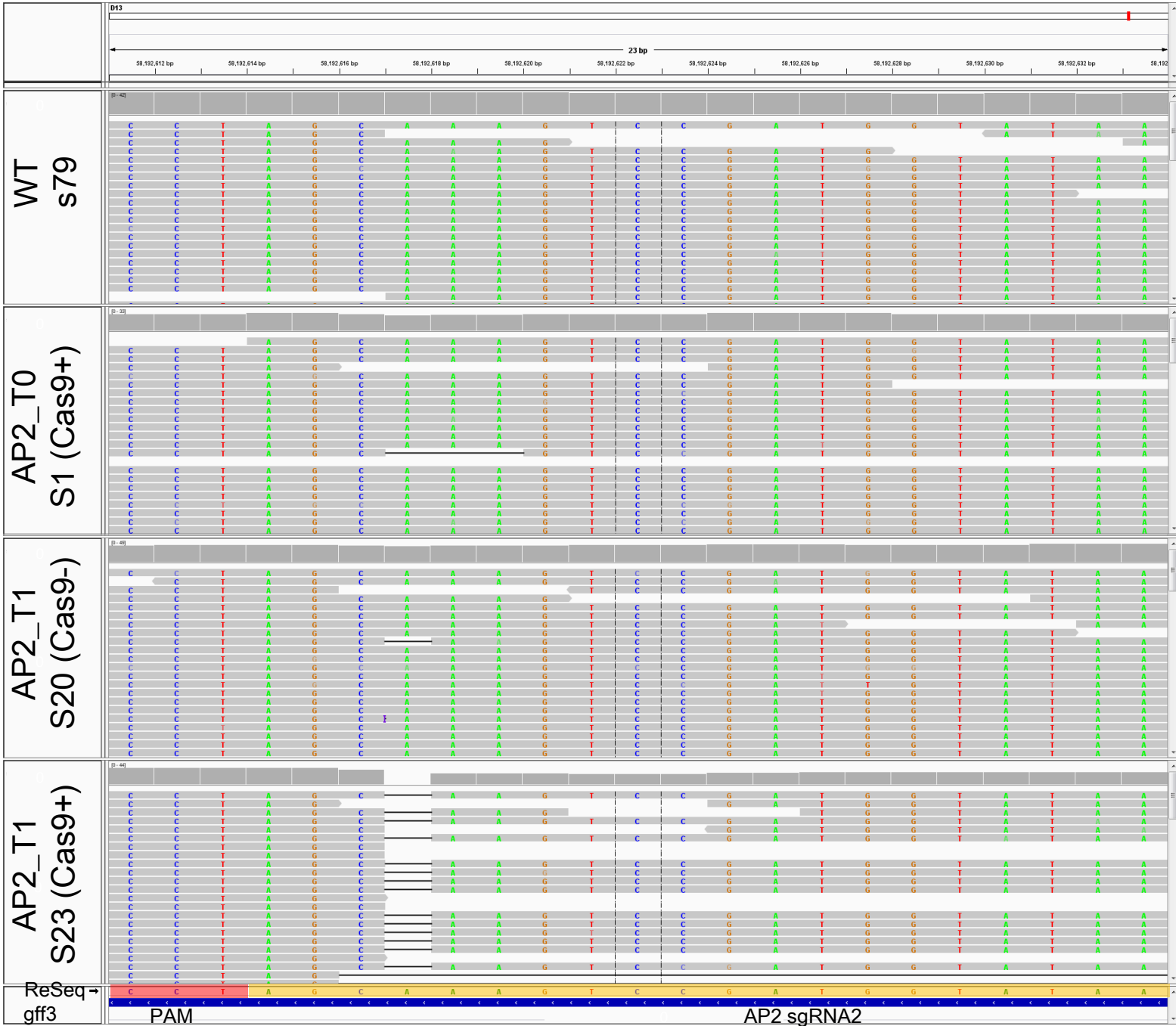
AP2 sgRNA1 site1   D13: 58,193,326-58,193,348 (Gh_A12G0630)

AP2 sgRNA1 site2   D12: 12,897,468-12,897,490 (Gh_D12G0647)

WT s79

AP2_T0 S1 (Cas9+)

AP2_T1 S20 (Cas9-)

AP2_T1 S23 (Cas9+)

ReSeq→ gff3

AP2 sgRNA1   PAM

AP2 sgRNA2 site1   D13: 58,192,611-58,192,633 (Gh_D13G2207)

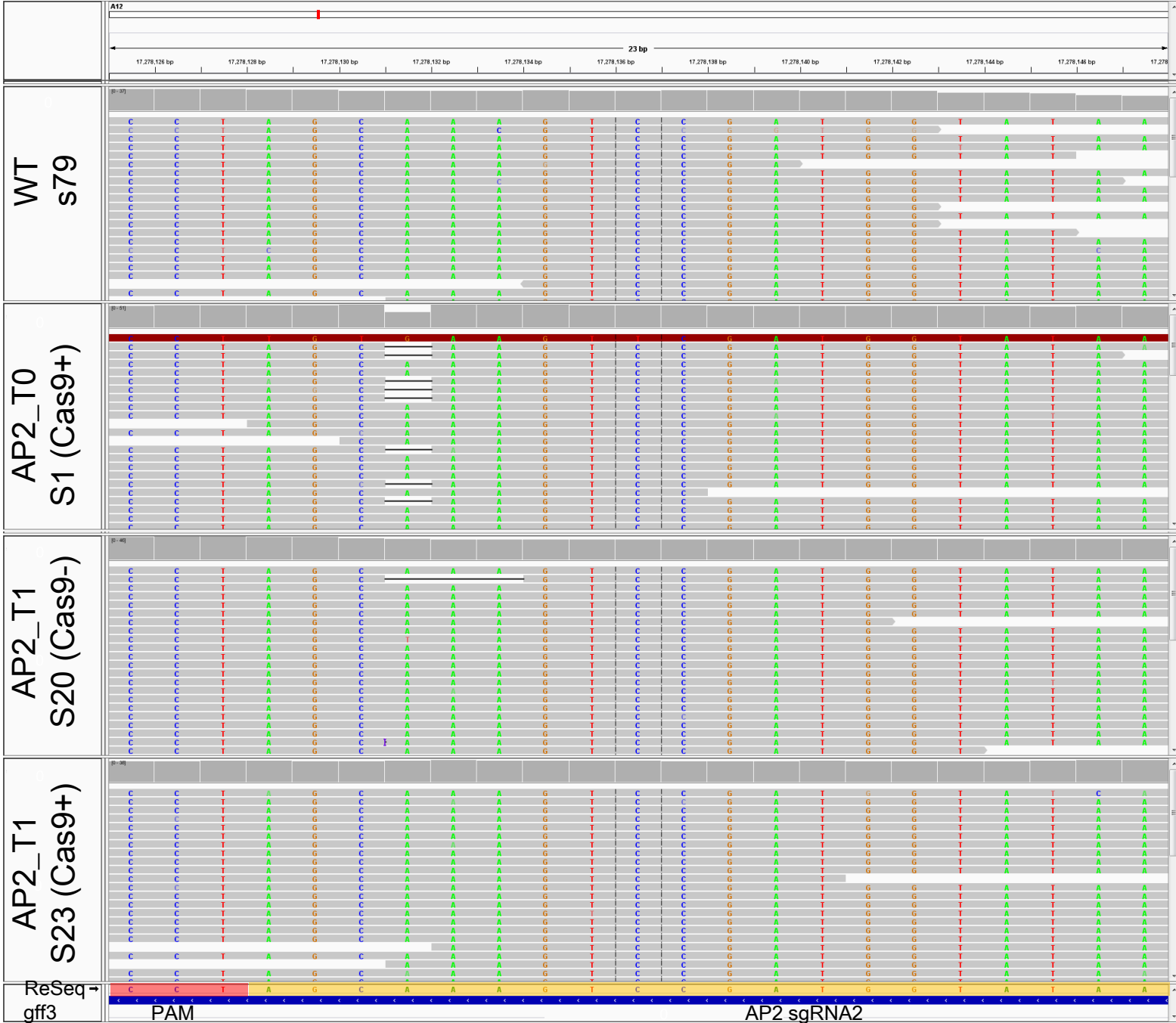AP2 sgRNA2 site2   A12: 17,278,125-17,278,147 (Gh_A12G0630)

Figure S11 Confirmation of the *AP2* mutations of inheritance from T0 to T1 plants in two sgRNA loci by WGS data. The orange box represents the sgRNA target sites, and the red box represents the PAM sequence.