

Expanded View Figures

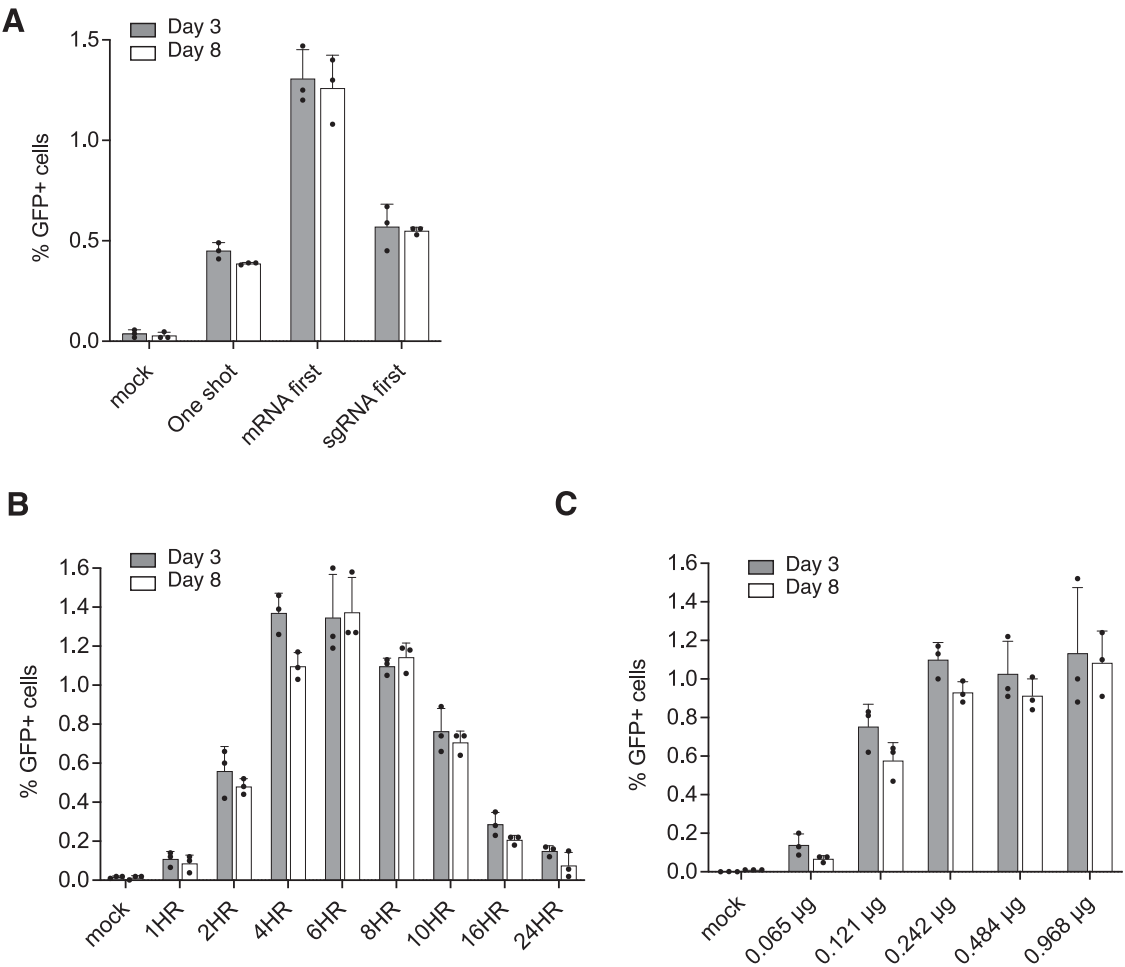
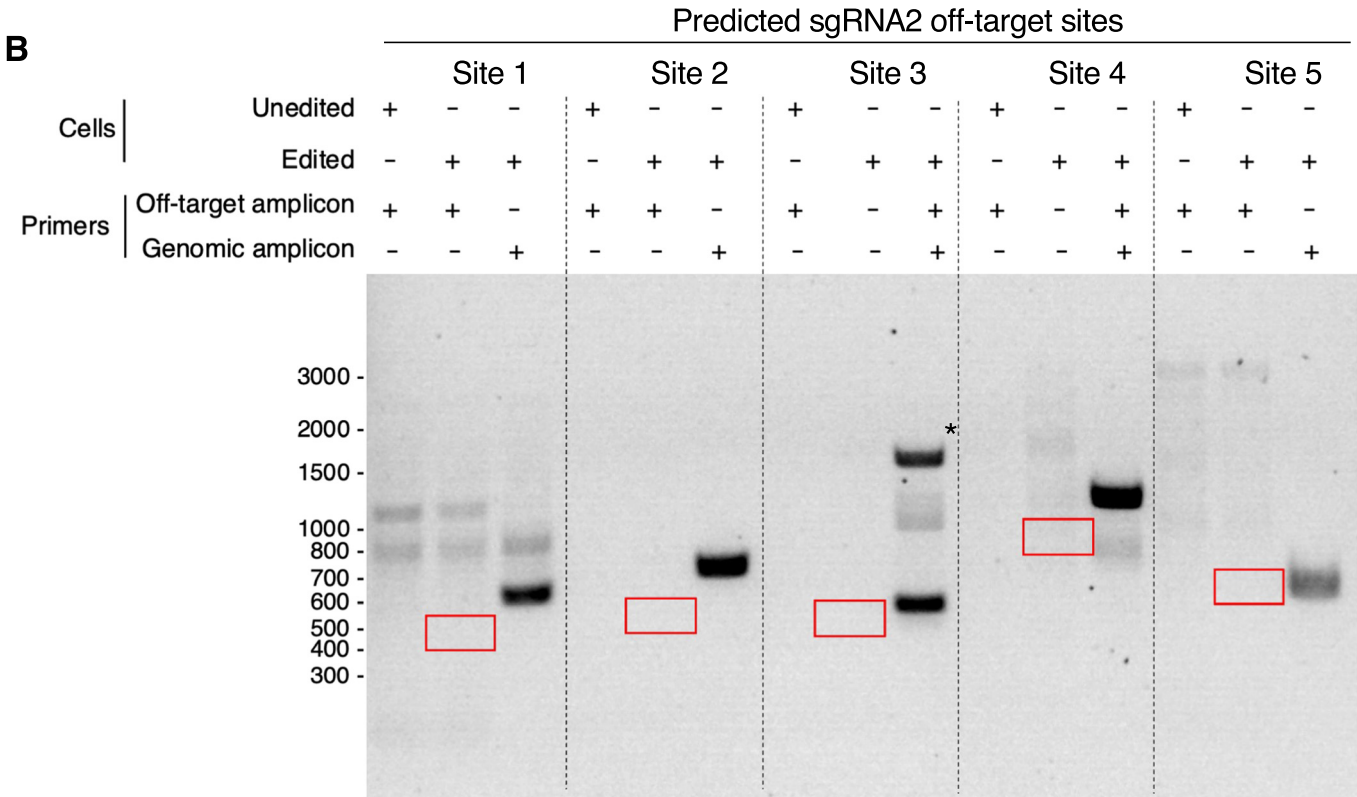
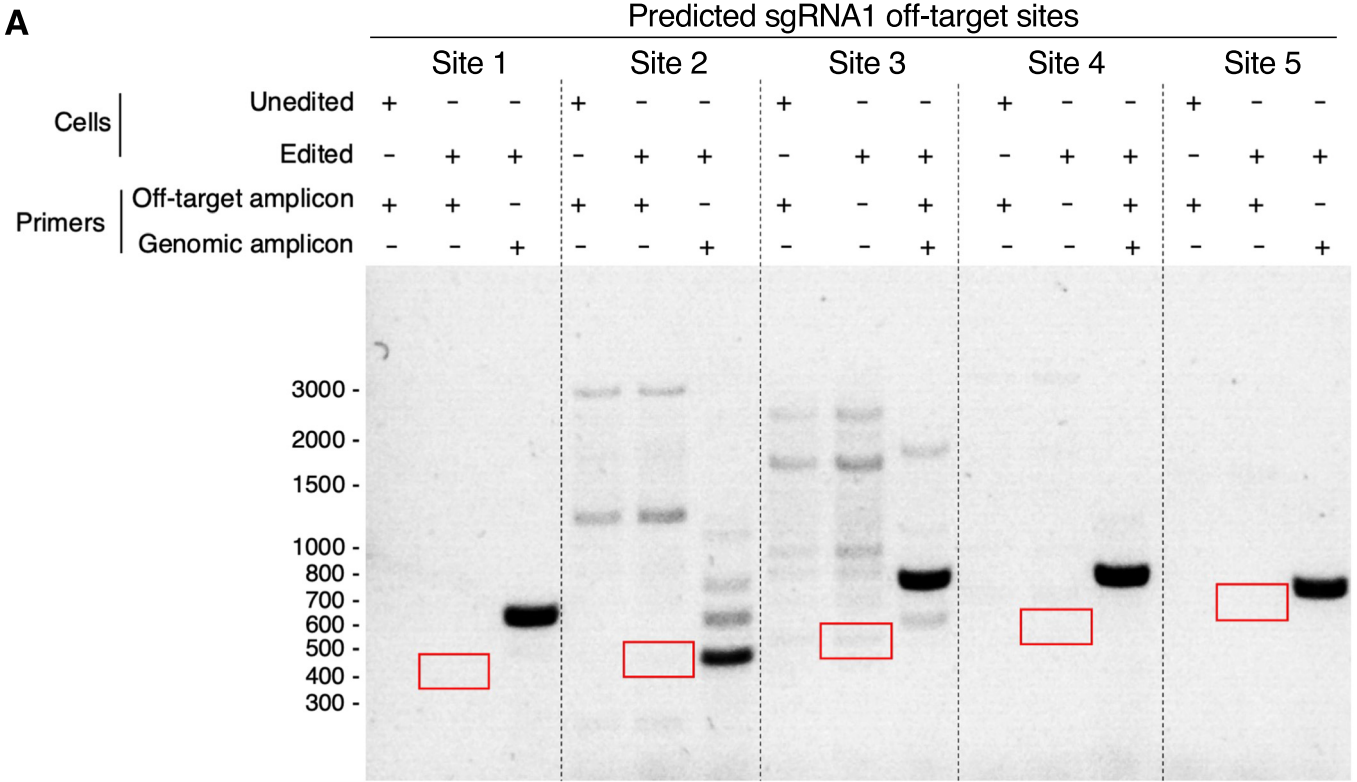


Figure EV1. Optimization of transfection protocol to improve CREATE editing efficiency.

(A) Optimization of transfection protocol improved editing efficiency. Protocol 1 (one shot), Protocol 2 (mRNA first) and Protocol 3 (sgRNA first). (B) Optimization of the incubation time between mRNA and sgRNA transfections. (C) Optimization of the total amount of sgRNAs transfected. Data are mean $\pm$ SD ($n = 3$ biological replicates).



**Figure EV2. PCR detection of potential off-target editing in AAVS1 loci edited cells.**

Top 5 predicted off-target sites for sgRNA1 and sgRNA2 were examined. Red box indicate the size of the expected PCR product if off-target integration occurred. * indicate non-specific band amplicon.

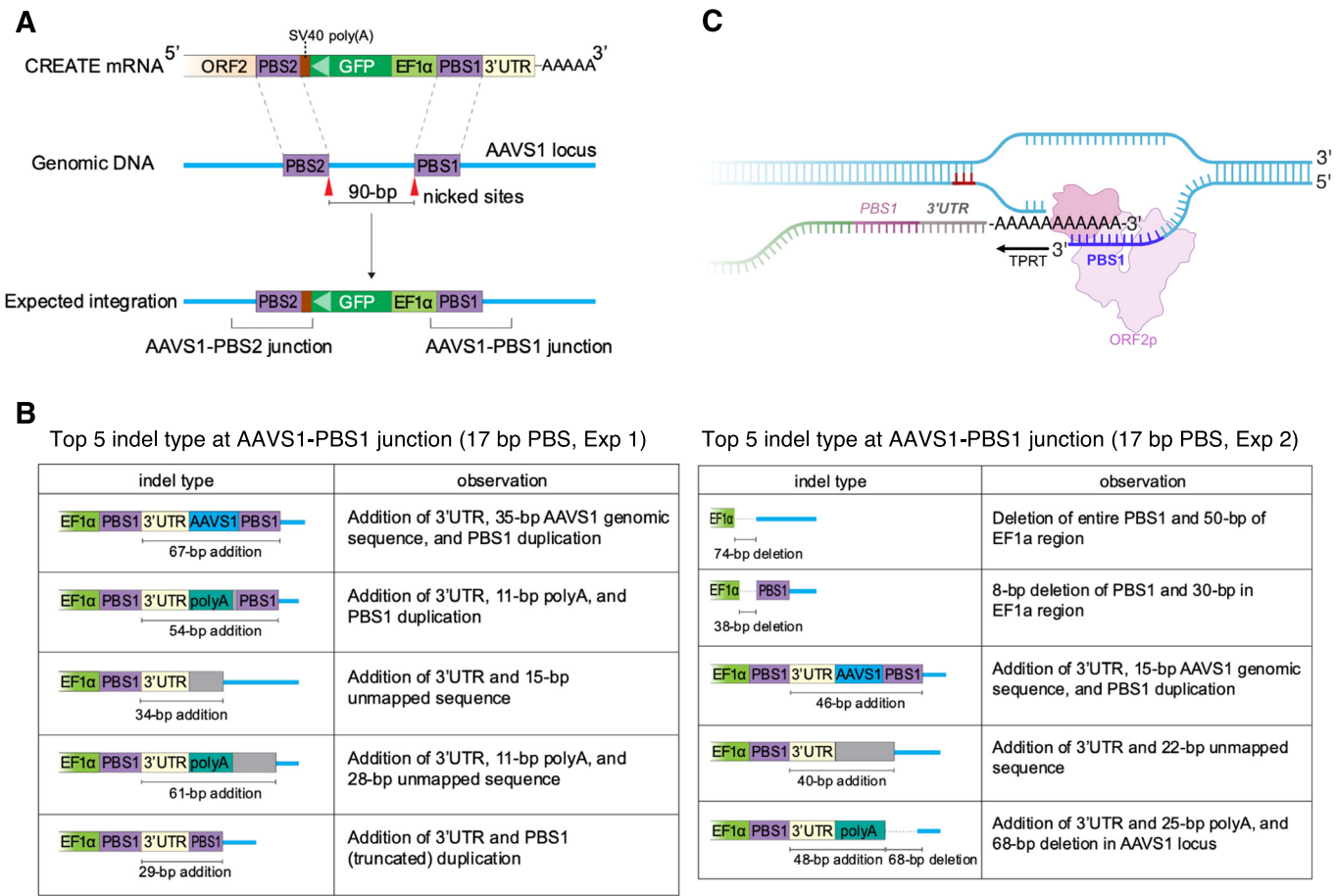


Figure EV3. Analysis of the most frequently observed indels at PBS1 and PBS2 junctions.

(A) A diagram showing expected transgene integration at AAVS1 locus. (B) Diagrams and descriptions of the most frequently observed indel patterns at PBS1 junctions in two independent experiments. (C) Potential mechanisms of alternative TPRT initiation that explain the observed indels.

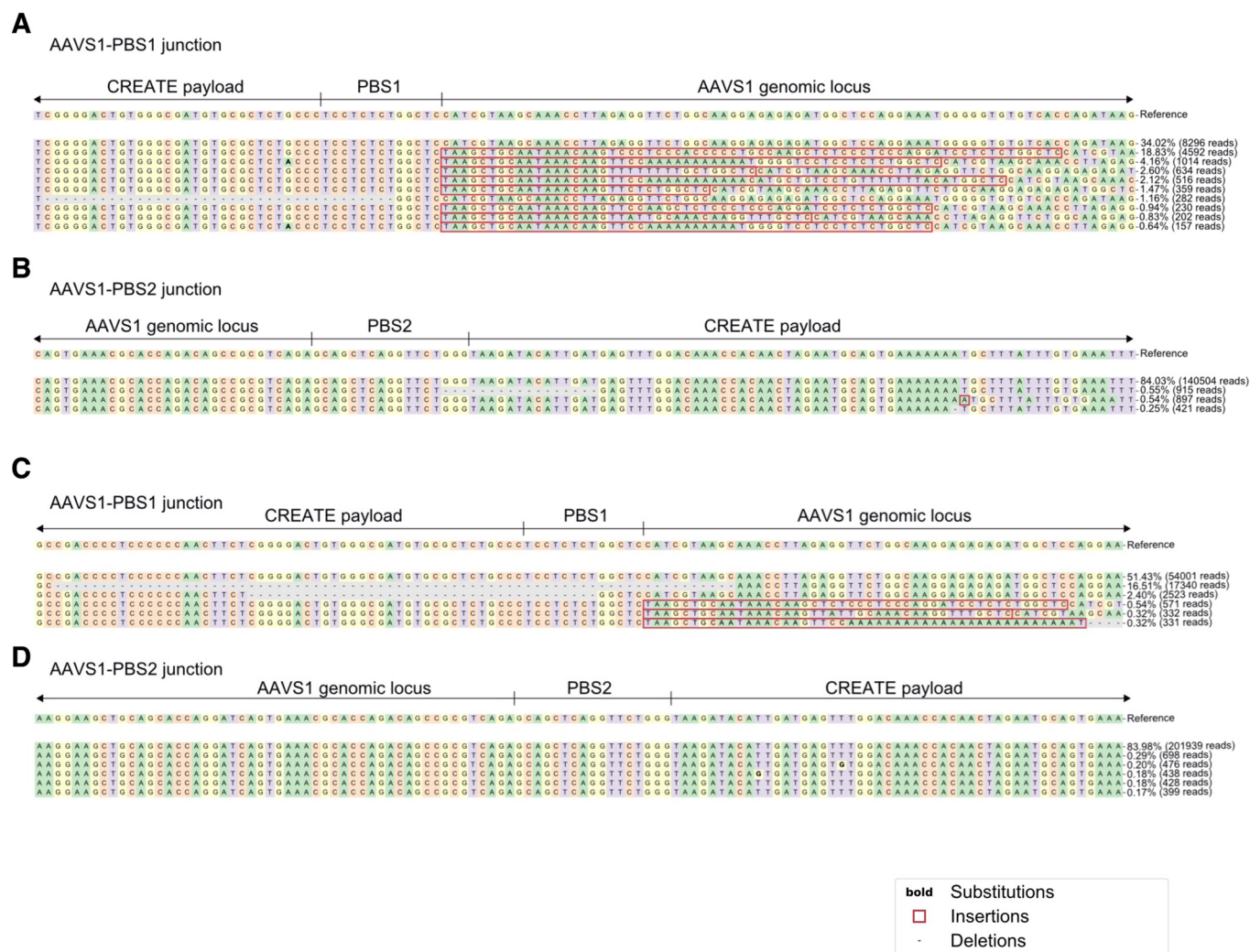


Figure EV4. Allele plots of PBS1 and PBS2 junctions from NGS analysis of AAVS1 locus edited cells.

(A) and (B) are from AAVS1 (17 bp PBS) Exp1 edited cells. (C) and (D) are from AAVS1 (17 bp PBS) Exp2 edited cells.

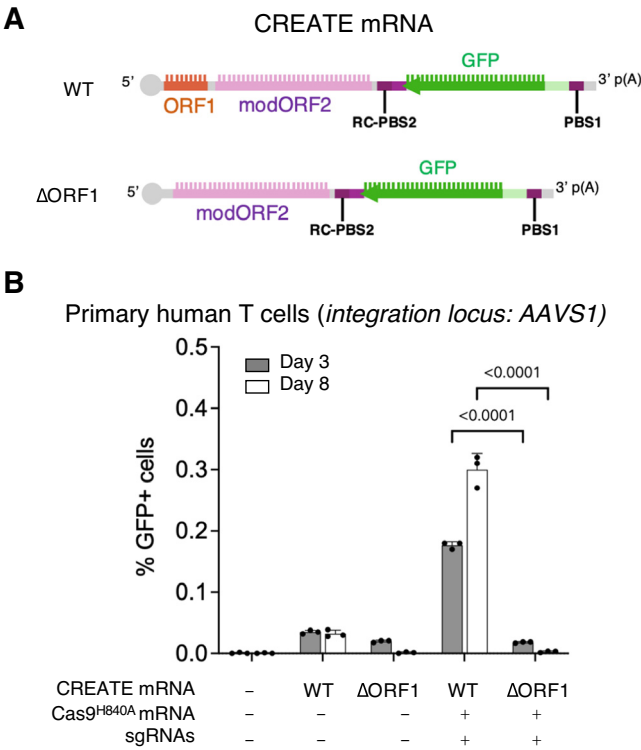


Figure EV5. ORF1 is necessary for CREATE editing in T cells.

(A) Diagrams of CREATE mRNA used in the experiment. (B) Deletion of ORF1 from CREATE mRNA (Δ ORF1) abolished CREATE-mediated integration of GFP payload in primary T cells. Statistical analysis was performed using two-way ANOVA with Dunnett's multiple comparisons test comparing each sample against the sample with WT CREATE mRNA, nCas9^{H840A} mRNA and sgRNAs. Data are mean $\pm$ SD ($n = 3$ biological replicates). Experiments were repeated twice. Exact p values: <0.0001 (WT vs Δ ORF1, Day 3), <0.0001 (WT vs Δ ORF1, Day 8).