

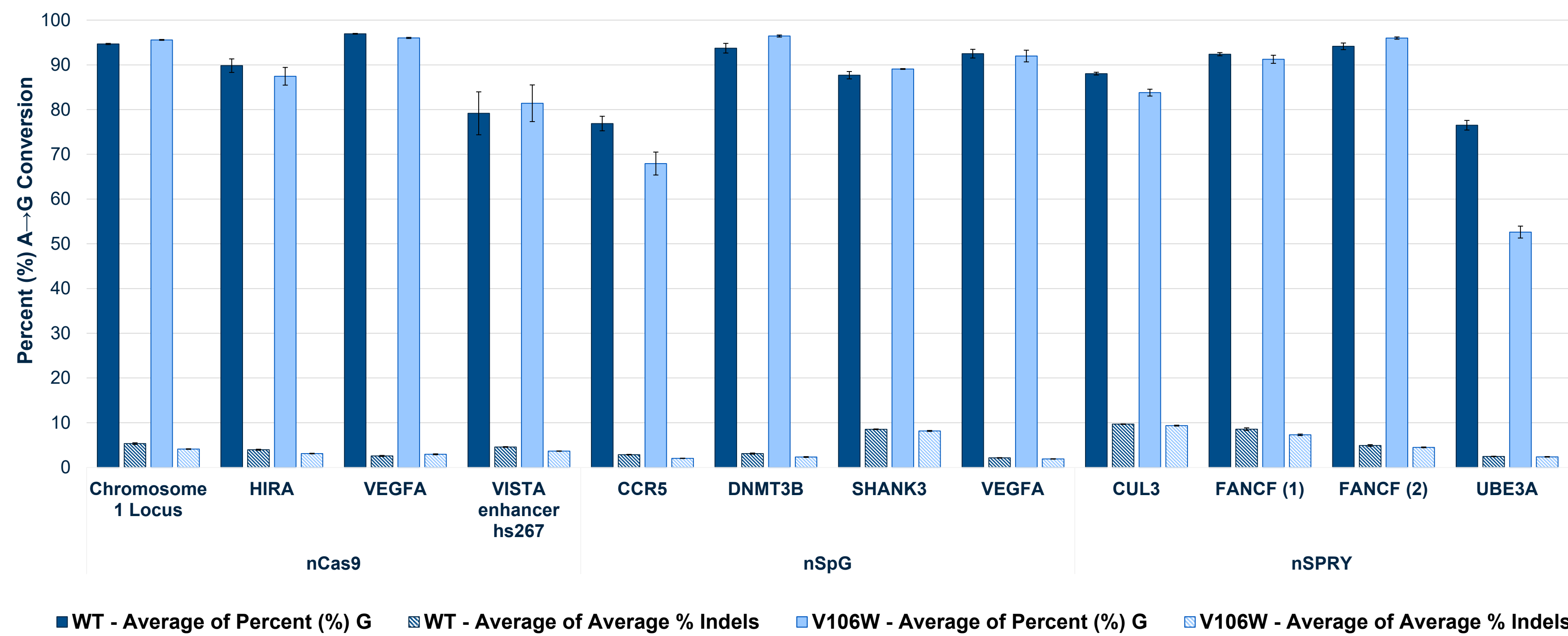
# A Comprehensive Adenine Base Editor mRNA Screening Platform for Optimizing Editing Specificity and PAM Flexibility

**Katie Keogh, Adam Brainard, Andrew Kristof, Karthik Murugan, Jessica Woodley, Michael Collingwood, and Christopher A. Vakulskas**  
Integrated DNA Technologies, Coralville, IA, USA

## Introduction

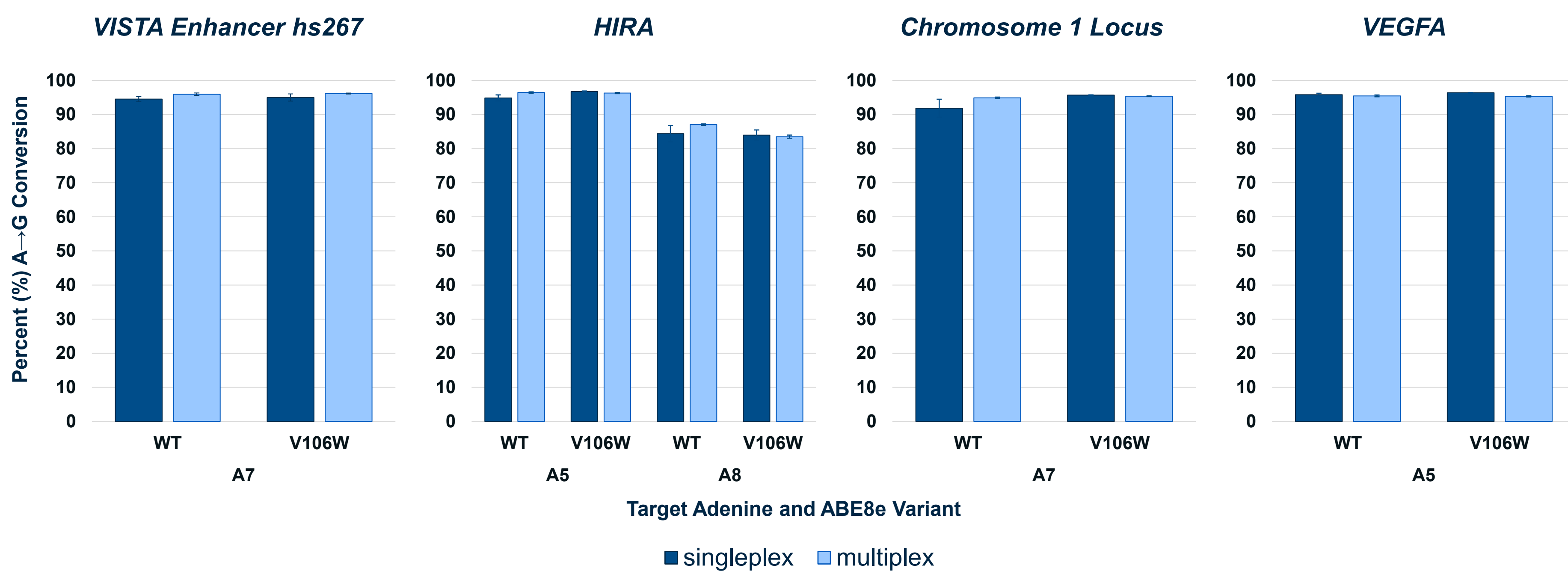
Adenine base editors (ABEs) have expanded the genome editing landscape by enabling precise A•T-to-G•C conversions without introducing double-strand DNA breaks. Among these systems, ABE8e is a highly active editor that achieves robust conversion efficiencies across diverse genomic targets. Optimal base editing requires balancing target specificity, bystander editing, and protospacer adjacent motif (PAM) recognition, as these factors directly influence target accessibility and outcomes. Delivery modality is another critical determinant of editing success. As RNA-based genome editing technologies advance, mRNA has become an attractive platform for transient editor expression providing temporal control while minimizing prolonged nuclease exposure. Furthermore, compatibility with delivery approaches such as lipid nanoparticles (LNPs) supports broad application across genome editing workflows. Here, we establish a comprehensive mRNA-based adenine base editor screening platform comprising a diverse panel of ABE8e-derived enzymes with enhanced specificity and expanded PAM recognition. Across multiple genomic loci and cell types, including human iPSCs, primary T cells, and immortalized cell lines, ABE8e WT and V106W achieved comparable on-target editing following both nucleofection and LNP-mediated delivery. We evaluate multiplex editing capability and bystander activity across ABE8e variants. Finally, we broaden targetable site coverage by demonstrating efficient editing with PAM-flexible Cas variants paired with both ABE8e backgrounds.

## Editing Efficiency and Indel Frequency



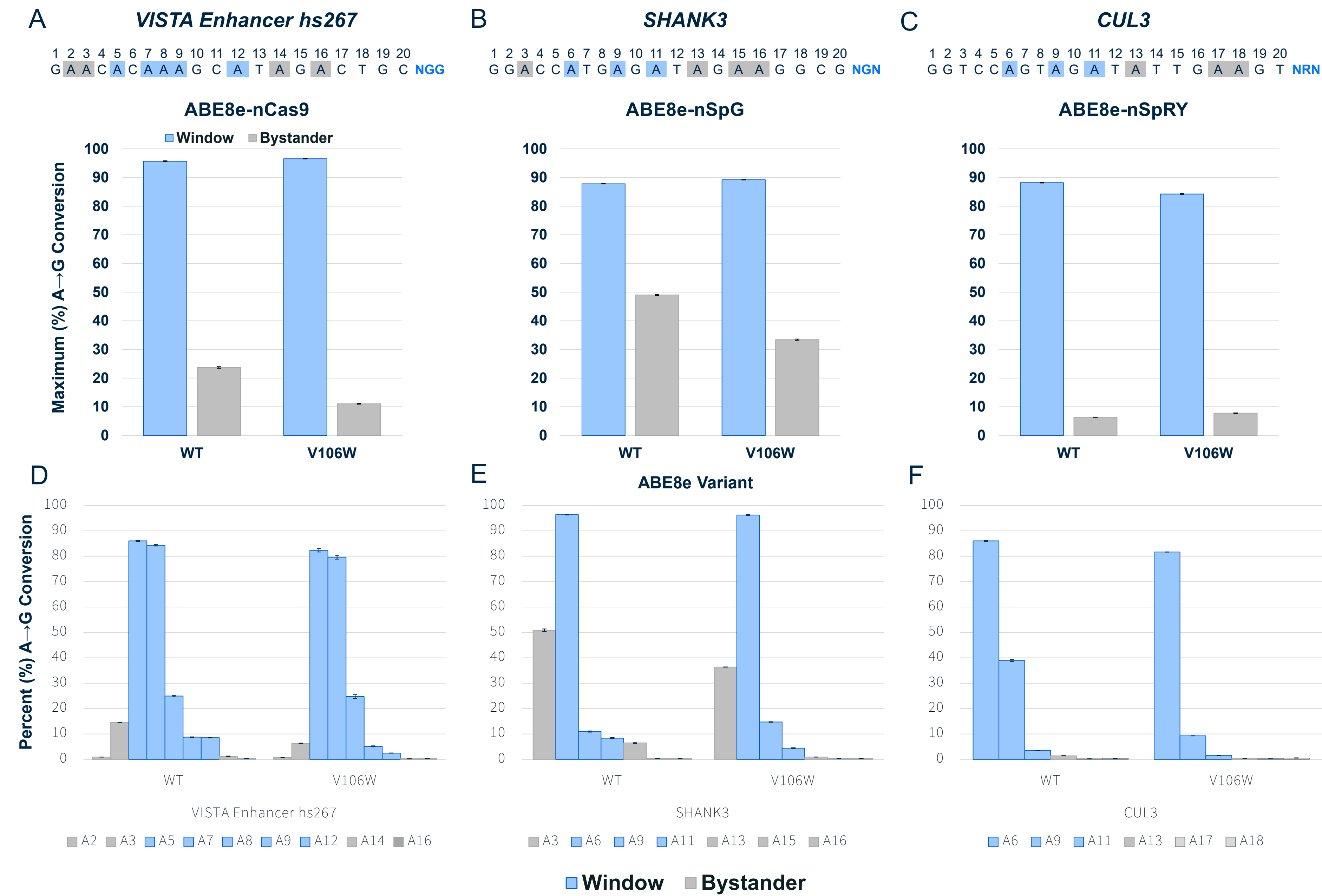
**Figure 1. Comparison of editing efficiency and indel formation between ABE8e-WT and ABE8e-V106W base editors across multiple target sites.** Percent A→G conversion (within bases 4-8 of the protospacer) and indel frequencies were assessed at four genomic target sites spanning nCas9 (NGG), nSpG (NGN), and nSPRY (NRN) PAM extended variants. Solid bars indicate A→G conversion efficiency, while hatched bars indicate indel frequency. HEK293 cells were nucleofected with 1 µg base editor mRNA and 2 µM gRNA using the Lonza nucleofection system. Bars represent mean ± SEM. (N = 3)

## Multiplex Editing



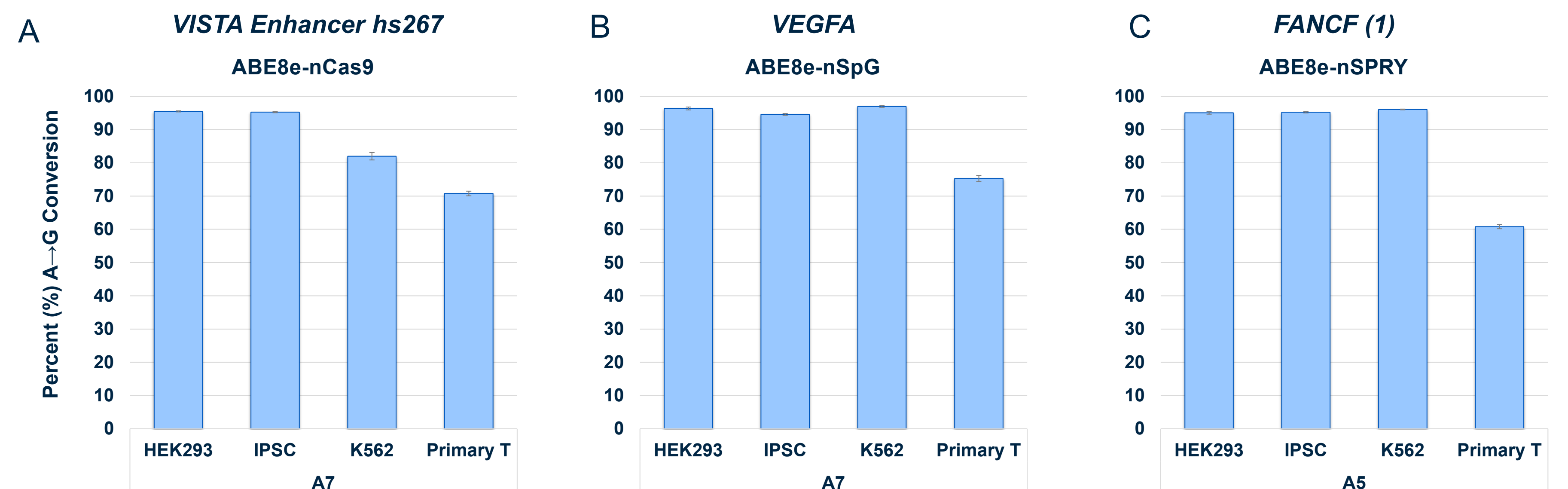
**Figure 2. Target adenine conversion under singleplex and multiplex editing conditions.** Target adenine conversion was evaluated at four separate genomic sites in HEK293 cells following delivery of ABE8e-WT or ABE8e-V106W paired with nCas9 under singleplex or multiplex pooled gRNA conditions. Cells were nucleofected using the Lonza nucleofection system with 1 µg base editor mRNA and 2 µM gRNA for singleplex editing or 2 µM of each gRNA for multiplex editing. Bars represent mean ± SEM. (N = 3)

## Maximum and Profile Bystander Comparison



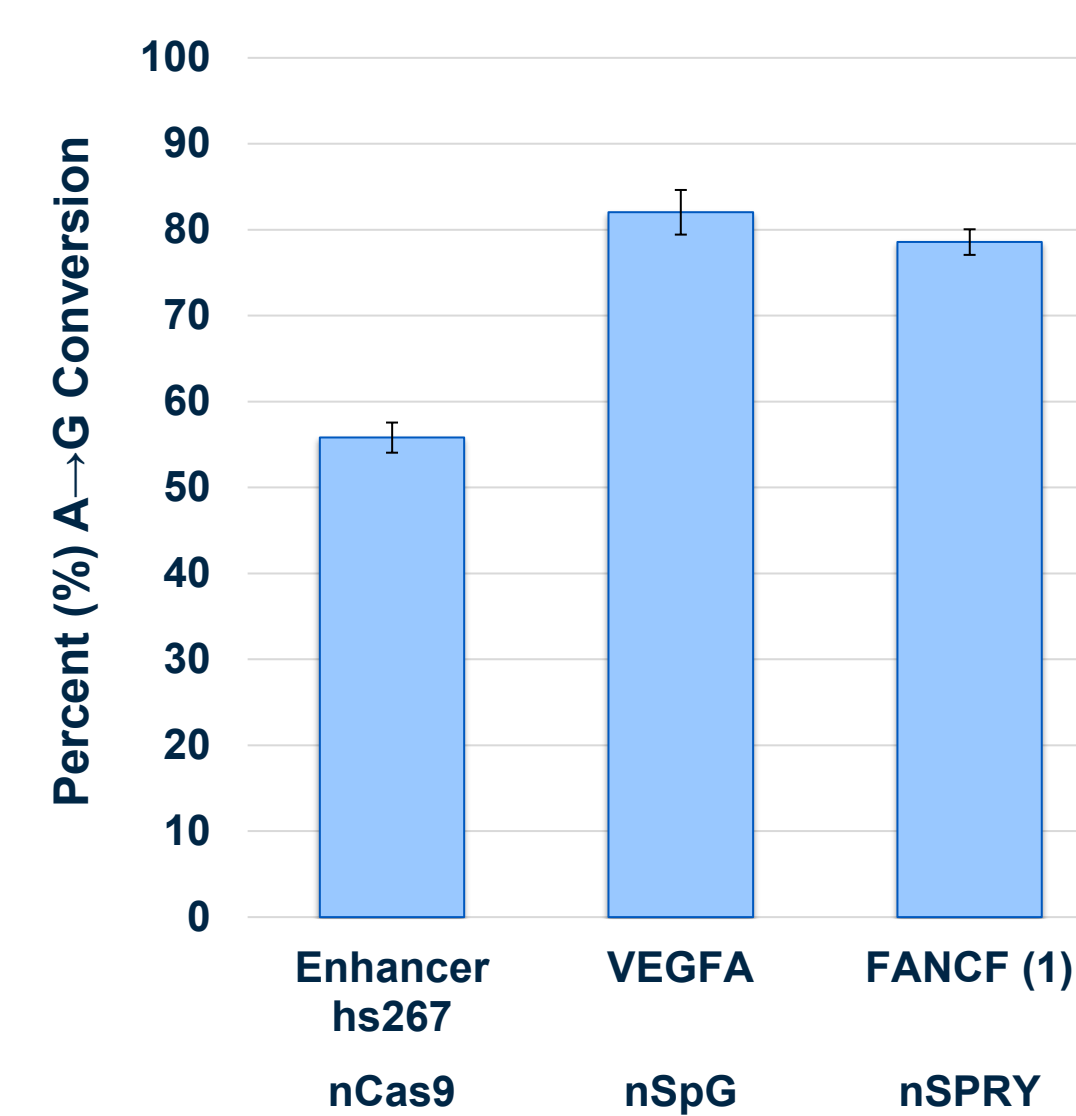
**Figure 1. Comparison of maximal window and bystander editing for ABE8e variants.** (A-C) Maximum ± SEM on-target A→G conversion at one base within the canonical ABE8e editing window (protospacer positions 4-12) was compared with the highest observed bystander edit outside the window for ABE8e-WT and ABE8e-V106W paired with nCas9 (A), nSpG (B), or nSPRY (C) across representative genomic loci in HEK293 cells. (D-E) Mean ± SEM percent A→G conversion across all adenines within the protospacer sequence. Cells were nucleofected with 1 µg base editor mRNA and 2 µM gRNA using the Lonza nucleofection system. (N = 3)

## Editing Efficiency Across Cell Types



**Figure 5. Target adenine editing efficiency across cell types for ABE8e-Cas variant combinations.** Target adenine conversion at the indicated genomic loci was measured in HEK293, iPSC, K562, and primary T cells using ABE8e editors paired with A) nCas9, B) nSpG, or C) nSPRY. Data from ABE8e-WT and ABE8e-V106W were combined due to comparable on-target editing efficiencies and are presented as mean ± SEM (N = 6 biological replicates; 3 WT and 3 V106W). Cells were nucleofected using the Lonza nucleofection system with 1 µg base editor mRNA in HEK293, K562, and primary T cells or 500 ng in iPSCs. gRNA was delivered at 2 µM in HEK293, K562, and iPSCs and 4 µM in primary T cells.

## Lipid Nanoparticle Delivery



**Figure 3. Target base conversion following LNP delivery of ABE8e editor systems.** Mean ± SEM target adenine editing at the indicated genomic loci was measured in primary T cells following lipid nanoparticle delivery of ABE8e-WT paired with nCas9, nSpG, or nSPRY. ABE8e mRNAs and Alt-R XT gRNAs were encapsulated into lipid nanoparticles (LNP) using the GenVoy-ILM™ T Cell Kit on the NanoAssemblr™ Spark instrument, following manufacturer's instructions. Briefly, RNA working solutions were prepared in the supplied formulation buffer with mRNA and sgRNA mixed at a 1:1 ratio by RNA weight. The lipid phase and RNA solution were then combined in the Spark instrument. Primary T cells were thawed and activated 72 hours prior to LNP addition. Cells were seeded at 0.1 x 10<sup>6</sup> cells/well, followed by direct addition of the LNPs at 4 µg total RNA per million cells. Genomic DNA (gDNA) was extracted 72 hours post delivery and editing was assessed by NGS. (N=3)