Optimization of the modular Pin-point™ base editing platform for an engineered Type V CRISPR-Cas effector

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1 Abstract

The recent appreciation of the variable function of the natural diversity of CRISPR-associated nucleases has led to an interest in expanding the gene editing toolbox. The type V CRISPR-Cas system is particularly attractive for clinical development due to its smaller size than SpCas9 and its unique PAM specificities, permitting a greater variety of delivery vehicles and allowing for enhanced accessibility of editing sites not always attainable with type II CRISPR-Cas enzymes, such as Cas9.

Revvity's modular Pin-point™ base editing platform employs the delivery of either a nickase or deactivated Cas enzyme, a deaminase fused to an aptamer binding protein, and an aptameric guide RNA (gRNA) that assemble in-vivo and act in conjunction to facilitate precise single nucleotide conversions. While originally developed using Cas9 nickase, the modularity inherent to the Pin-point platform permits swapping of components to achieve optimal editing results.

Here, we demonstrate robust site-specific editing at different target loci using a deactivated CasONYX (dCasONYX) version of the Pin-point base editing platform. dCasONYX is an engineered version of dCasMINI developed by Epic Bio. It has an improved off-target profile compared to SpCas9, exhibits very low immunogenicity compared to Cas9 and is less than 1.5 Kb in size. Further utilizing the modularity of the Pin-point base editing platform, we show that additional components of the system such as different deaminases and aptameric gRNA scaffold configurations can be leveraged in combination with alternative Cas enzymes to adjust the base editing window; this is of particular advantage for applications including SNP correction and gene knockout at sites that are not usually accessible with the nCas9 configuration.

Finally, we have significantly improved the design and optimized the delivery parameters of the dCasONYX version of the Pin-point platform through generation of IVT mRNA and achieving robust chemical synthesis of gRNA over 150nt in length, making this configuration suitable for application to therapeutically relevant cell types such as T cells and induced pluripotent stem cells (iPSCs).

2 The Pin-point base editing platform

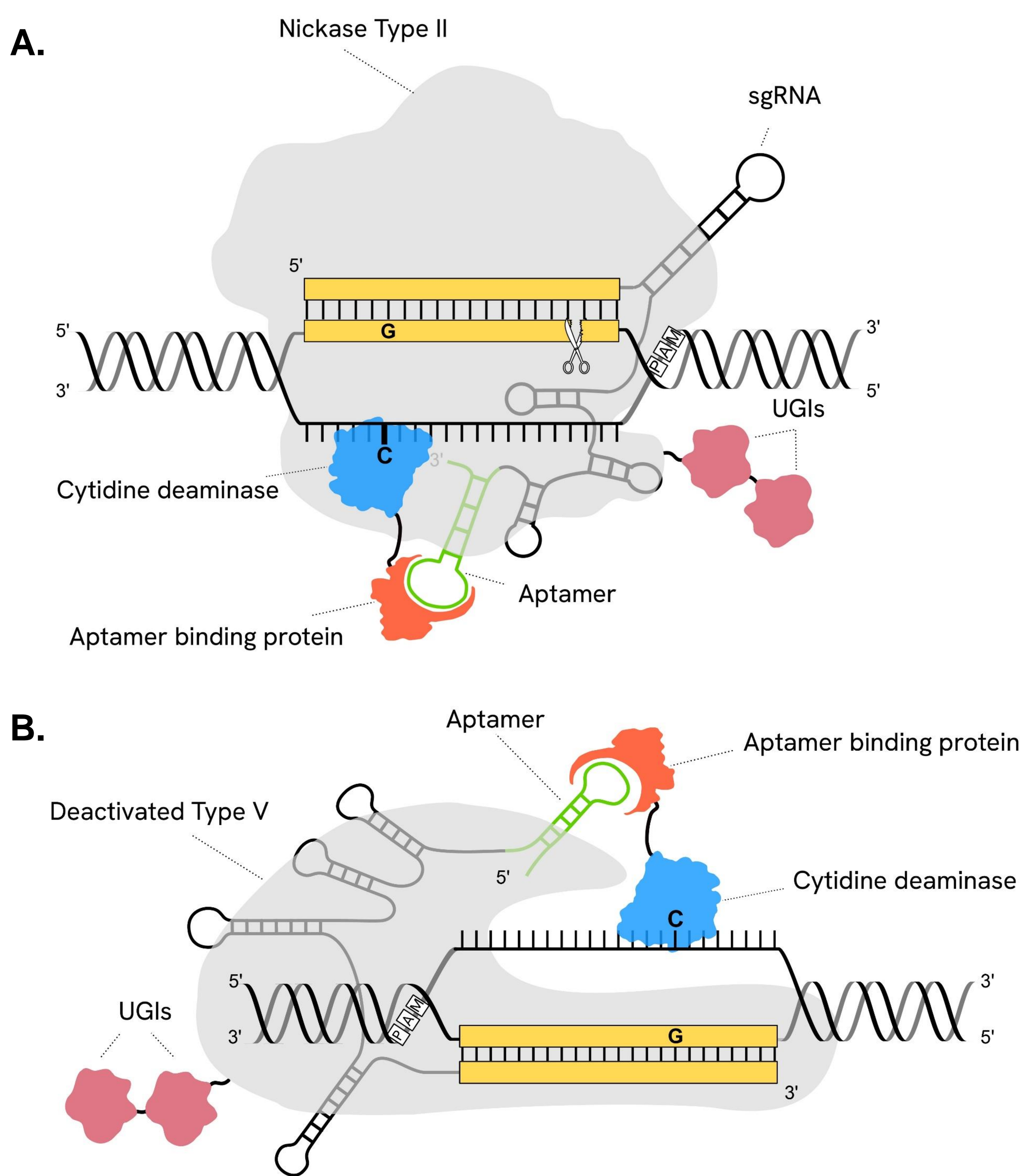

Figure 1: The Pin-point base editing platform allows precise genome modification by single nucleotide conversion. [1-2] A Type II CRISPR nickase (A) or a deactivated (d) Type V CRISPR effector (B) is guided to a DNA target site via a single guide RNA (sgRNA) with an aptamer engineered into the scaffold. The aptamer recruits a deaminase via fusion with an aptamer binding protein. The combination of these components enable efficient base editing at a DNA target of interest.

3 dCasONYX: an evolved Type V effector

Figure 2: (A) dCasONYX is a compact Type V protein less than half the size of SpCas9. (B) PAM recognition sequences are represented from the central circle reading outward. Percentages indicate relative binding efficiency. The most active PAM of dCasONYX is TTTR, but wider targeting is possible. Figures and data courtesy of Epicrispr Biotechnologies (Epic Bio). [3]

4 Improved editing efficiency by engineering the aptameric sgRNA

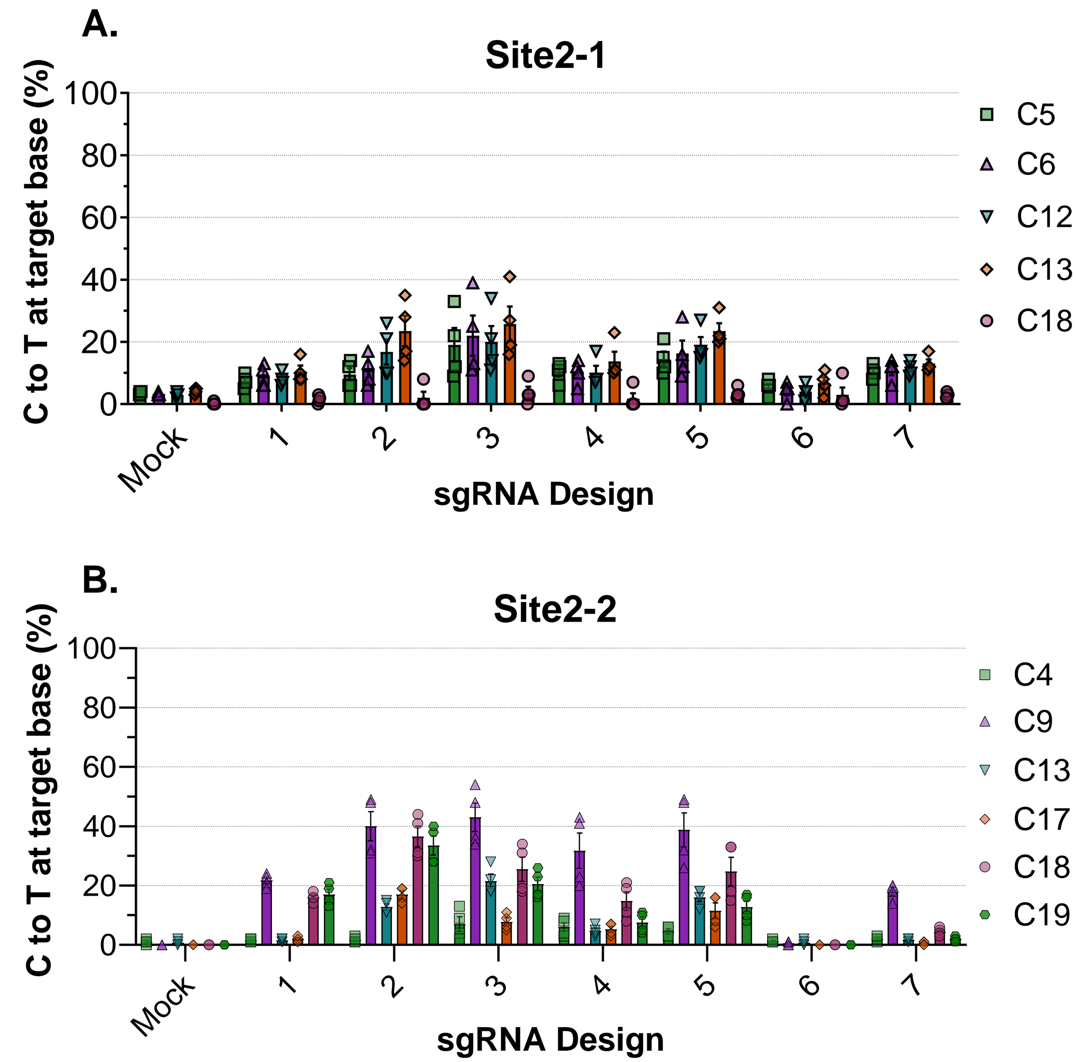

Figure 3: HEK293T cells were transfected with either no DNA (mock) or with plasmids encoding dCasONYX, Anolis APOBEC1, and one of seven engineered versions of the aptameric sgRNA targeting one of two genomic loci (A: Site2-1; B: Site2-2). Cells were lysed after 72h, genomic DNA was amplified by PCR, and editing efficiency at the indicated cytosine was measured by Sanger sequencing. n=4; error bars ±SEM.

5 Robust editing at loci across the human genome

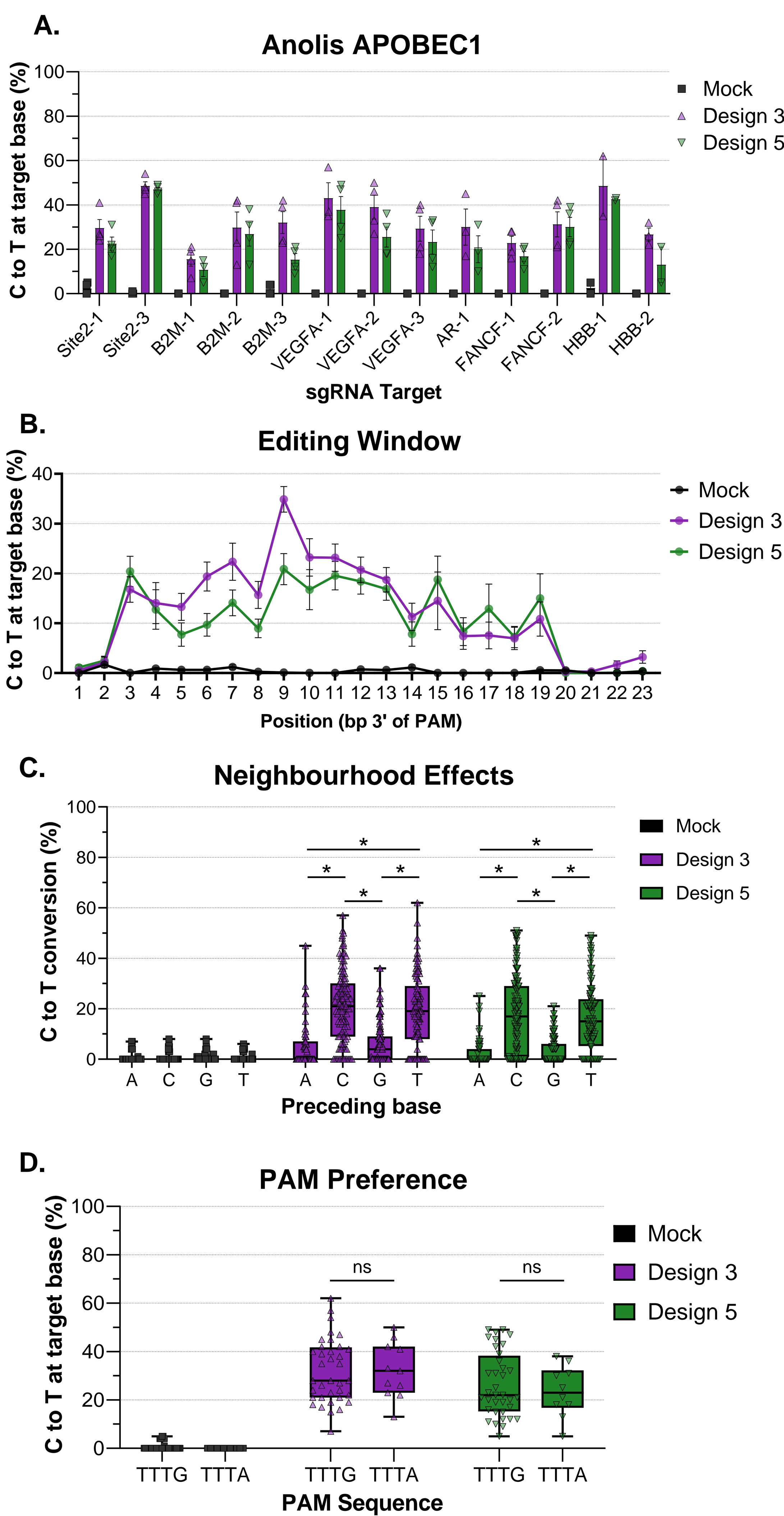

Figure 4: Aptamer-sgRNA designs 3 and 5 were further tested across a variety of genomic targets in HEK293T cells. (A) Thirteen sgRNA targets with TTTR PAM sites were designed for seven different genomic loci. Percentages of target C to T conversion in samples analysed by Sanger sequencing are reported across target sites. n≥3; error bars ±SEM. (B) The C to T editing efficiency at each position of the 23bp target was averaged over each replicate of each sgRNA tested. n≥7 for each position; error bars ±SEM. (C) The C to T editing efficiencies from each C in each sgRNA were grouped by the preceding base. n≥58 for each group; box 25th-75th percentile with line at median, whiskers to min & max; *p<0.05 for the indicated groups by two-tailed unpaired t-tests. (D) The editing efficiency of the target C for each sgRNA was grouped by its PAM sequence. n≥10 for each group; box 25th-75th percentile with line at median, whiskers to min & max; no significant differences were detected between TTTG and TTTA for either sgRNA design by two-tailed unpaired t-tests.

6 Efficient editing with multiple deaminases

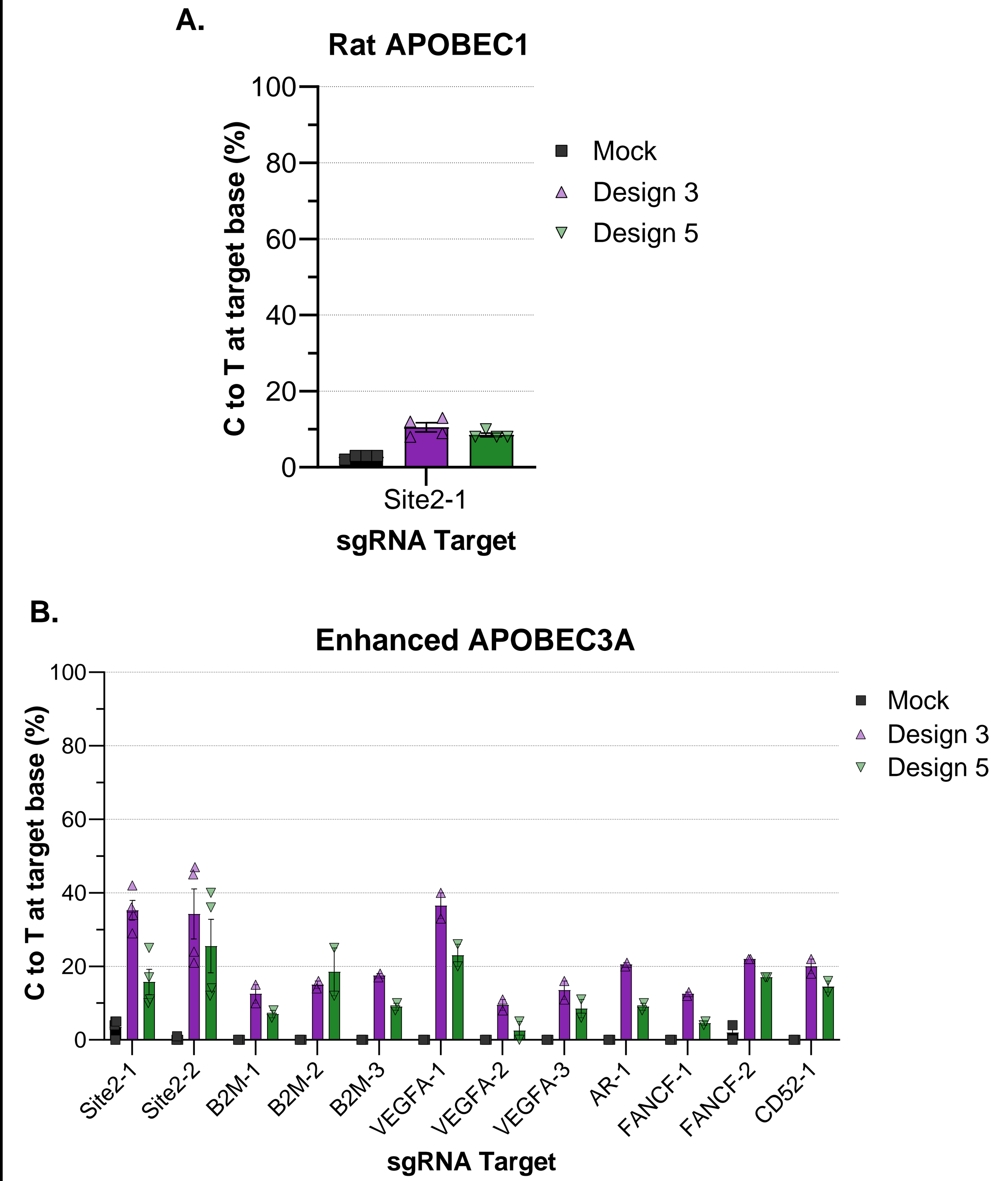

Figure 5: HEK293T cells were transfected with either no DNA (mock) or plasmids encoding dCasONYX, either (A) rat APOBEC1 or (B) enhanced APOBEC3A (eApo3A) [4], and one of the sgRNAs from Fig. 4A. Cells were lysed after 72h, genomic DNA was amplified by PCR, and editing efficiency at the target C was measured by Sanger sequencing. n=4 for Site 2-1 and Site 2-2; n=2 for other sgRNAs; error bars ±SEM.

7 Demonstrated compatibility of the Pin-point platform with multiple nucleases

	Type II			Type V						
	A	B	C	D	E	F	G	H	I	J
Enzyme activity	nickase	nickase	nickase	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated
Demonstrated nuclease activity in mammalian cells	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Demonstrated with the Pin-point system	✓	✓	In progress	✓	In progress	✓	In progress	✓	✓	In progress
sgRNA optimized	✓	✓		In progress		✓		✓	✓	
Confirmed at multiple targets (2+)	✓	✓				✓		✓	✓	
Demonstrated in multiple cell types (2+)	✓	In progress				✓		✓		
Demonstrated with multiple deaminases (2+)	✓							✓	✓	

Chart 1: The Pin-point base editing system has been preliminarily evaluated with a variety of Type II and Type V nucleases, as well as with multiple deaminases, sgRNA designs, and in multiple cell types.

8 Summary

- The Pin-point base editing system is inherently modular, enabling swapping of components.
- dCasONYX is a novel evolved Type V nuclease generated by Epic Bio, with unique characteristics when used in the Pin-point system.
- We have engineered, validated, and characterized two aptameric sgRNA molecules for dCasONYX that allow efficient base editing at multiple target sites across the human genome.
- Three common cytidine deaminases were shown to be compatible with dCasONYX in the Pin-point system.

9 References

[1] Collantes, J. C., *et al.* (2021). Development and characterization of a modular CRISPR and RNA aptamer mediated base editing system. *The CRISPR Journal* 4(1).
[2] Porreca, I., *et al.* (2024). An aptamer-mediated base editing platform for simultaneous knock-in and multiple gene knockout for allogeneic CAR-T cells generation. *Molecular Therapy* 32(8) p2692-2710.
[3] Silvis, M. R., *et al.* (2023, October 24-27). Design and characterization of compact and precise Cas molecules for treating diseases in patients [Poster presentation]. ESGCT, Brussels.
[4] Wang, X., *et al.* (2020). Cas12a base editors induce efficient and specific editing with low DNA damage response. *Cell Reports, 31*(9).

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