Precise and Efficient Immune Cell Engineering via Pin-point™ Base Editing Platform: Insights from TotalSeq™ Single-Cell Multiomic Analysis

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Abstract

Many gene editing platforms, such as CRISPR-Cas9, rely on nuclease activity to introduce a targeted protein knockout through the introduction of DNA double-strand breaks (DSBs). While this revolutionary platform has been used in a wide array of cell and gene therapy applications and at varying stages of pre-clinical research up through recent FDA approvals, the technology does not come without risks. DSBs introduced by nucleases can lead to unwanted genomic alterations and cytotoxicity. These effects are magnified when more complex genomic perturbations, such as editing at multiple genomic sites, are introduced.

Here, we demonstrate efficient multiplex gene editing using the Pin-point base editing platform as a suitable technology for the creation of CAR-T cell therapies. The Pin-point platform can efficiently knock out four or more protein targets simultaneously with limited impact on cell viability in T cells and can also be utilized for a number of iPSC and HSPC therapeutic engineering applications. Through single-cell multiomic approaches, we can characterize the effect of editing with the Pin-point platform at the RNA and protein level. We see that the platform can efficiently edit multiple subpopulations of cells, for example, CD4, CD8, and dual CD4/CD8 positive T-cells, with high on-target efficacy and with no detectable off-target phenotypic impacts identified via interrogation of the whole transcriptome and a highly-multiplexed protein panel.

From discovery to pre-clinical development, the Pin-point platform and TotalSeq platform are well suited for engineering and characterizing next generation cell and gene therapies.

1 Pin-point base editing platform

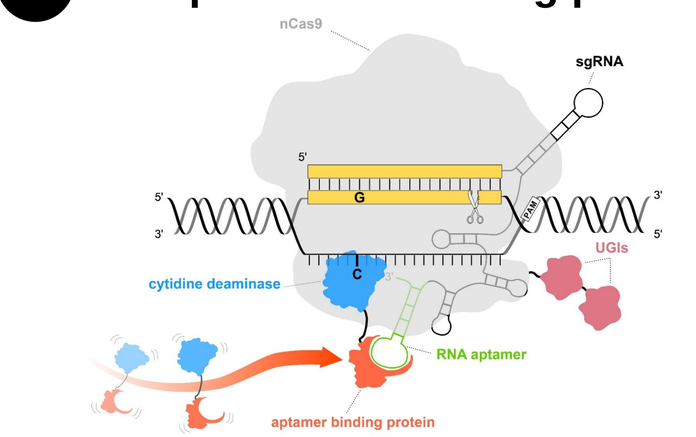

Figure 1: Schematic of a modular Pin-point base editor system.
In one possible configuration of the platform, a Cas nickase (e.g.nCas9) is recruited to the DNA target site via a single guide RNA (sgRNA) encoding an aptamer in the scaffold region. The aptamer recruits a DNA-modifying deaminase to the DNA target site via an aptamer binding protein. The three independent components of the system can be configured according to editing requirements and delivered to cells either as mRNA and synthetic sgRNA or packaged in viral particles.

2 Efficient multi-gene editing in T cells and iPSCs

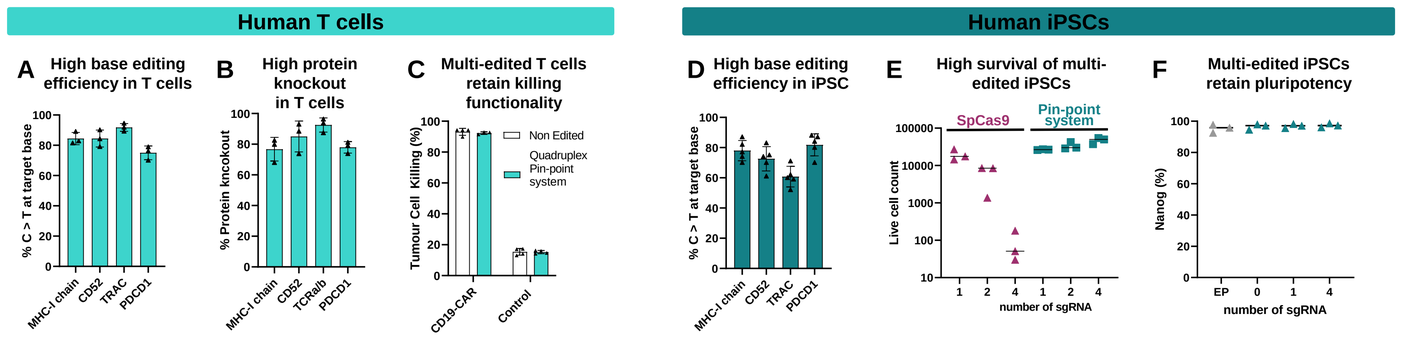

Figure 2: Pin-point platform facilitates robust, high efficiency multi-gene editing in human T cells and iPSCs.
A) Four genes are edited simultaneously in T cells after delivery of Pin-point mRNAs (nSpCas9 and rAPOBEC1) and synthetic aptameric sgRNAs. Percentages of C to T conversion are reported. **B)** Protein knockout of 4 targets analyzed by flow cytometry. **C)** In vitro tumor cell killing assay performed with either empty (control) or CD19-CAR expressing lentiviral vector transduced cells and mock electroporated (non-edited) or T cells edited with the Pin-point platform (Quadruplex Pin-point system). n = 3 T cell donors for A-B, n = 2 T cell donors for C. **D)** Four genes are edited simultaneously in iPSCs after delivery of Pin-point mRNAs (nSpCas9 and rAPOBEC1) and synthetic aptameric sgRNAs. Percentages of C to T conversion are reported. **E)** Cell counts of iPSCs edited with single or multiple sgRNAs using the Pin-point system or Cas9 analyzed by flow cytometry 48hrs post electroporation. Cell counts were normalized to a no sgRNA control. **F**) Expression of the pluripotency marker Nanog in cells edited with the Pin-point base editing system analyzed by flow cytometry 4 days post electroporation. Data shown in D, E, F are from two independent experiments with 2 iPSC lines.

3 Overview of a Pin-point base editing experiment and CITE-seq analysis

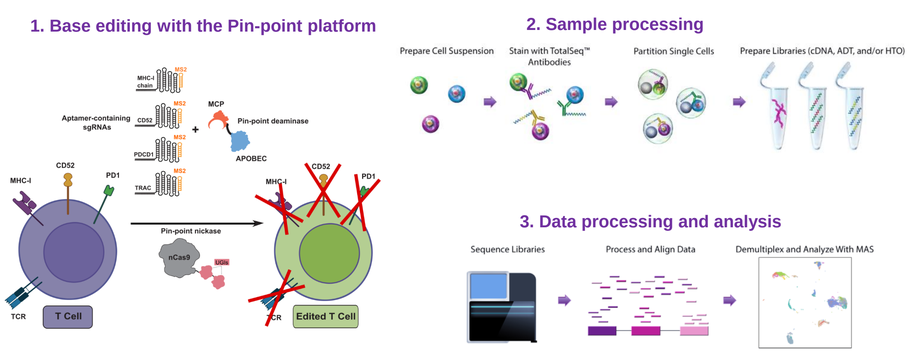

Figure 3: Overview of base editing with the Pin-point system and CITE-seq workflow using TotalSeq antibodies.
1. T cells were edited with the Pin-point system (quadruplex knockout or no template control (NTC)) and electroporation control (EP) as described in Figure 2. Edited cells were expanded for 6 days and then CITE-Seq analysis was performed. 2. Briefly, cells were stained with the TotalSeq-B Human Universal Cocktail v1.0, which contains 134 unique antibodies to cell surface antigens. The stained cells were then subjected to single cell analysis using Chromium Single Cell Gene Expression v3.1 kit from 10X Genomics to analyze both RNA and protein.
3. Raw FASTQ data was processed with 10x Cell Ranger. Downstream analysis was performed with Multiomics Analysis Software (MAS).

4 Confirmation of successful knockout in T cells

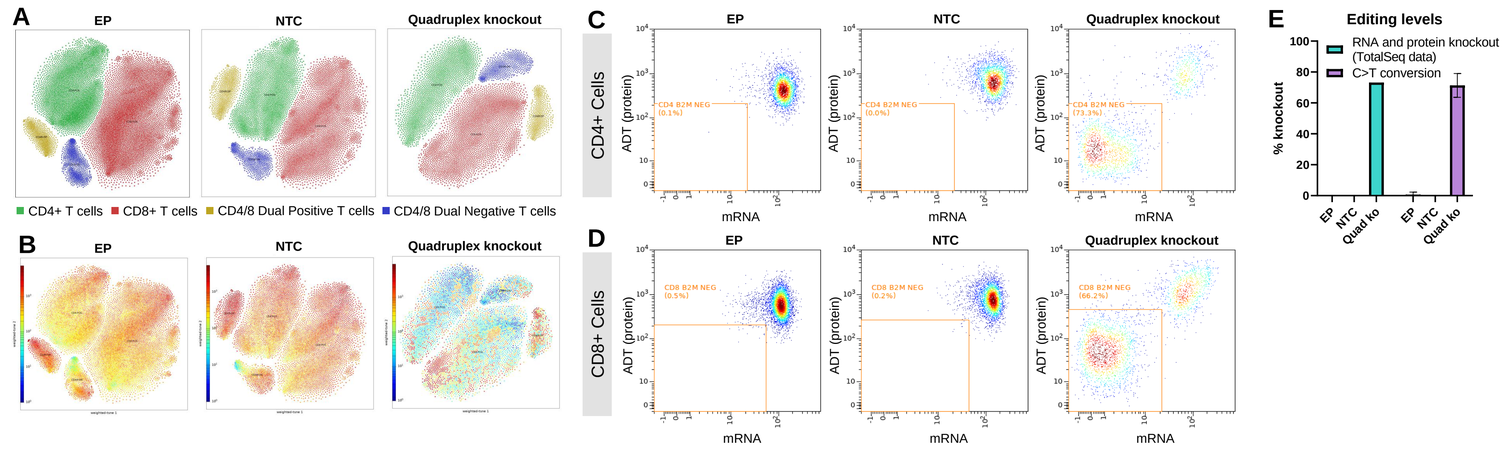

Figure 4: CITE-seq analysis of T cells edited with the Pin-point system using TotalSeq antibodies.
A) t-SNE plots of edited samples and controls show no differences in CD4 or CD8 T cell frequencies. **B)** TotalSeq analysis for characterization of T cells edited with the Pin-point system shows uniform knockout across the different T cell sub-populations, with only subtle differences between CD4+ and CD8+ T cell populations. Red = high expression, blue = low expression. **C,D)** Representative figures of RNA and protein (ADT) knockout for one of the four edited genes in CD4+ and CD8+ T cells, respectively. **E)** Quantification of TotalSeq data (levels of RNA and protein knockout) and comparison to Sanger sequencing data (C>T conversion) for one of the representative genes. Two methods show similar levels of knockout.

5 Differences between transcriptional and post-transcriptional signatures in T cells edited with the Pin-point base editing system

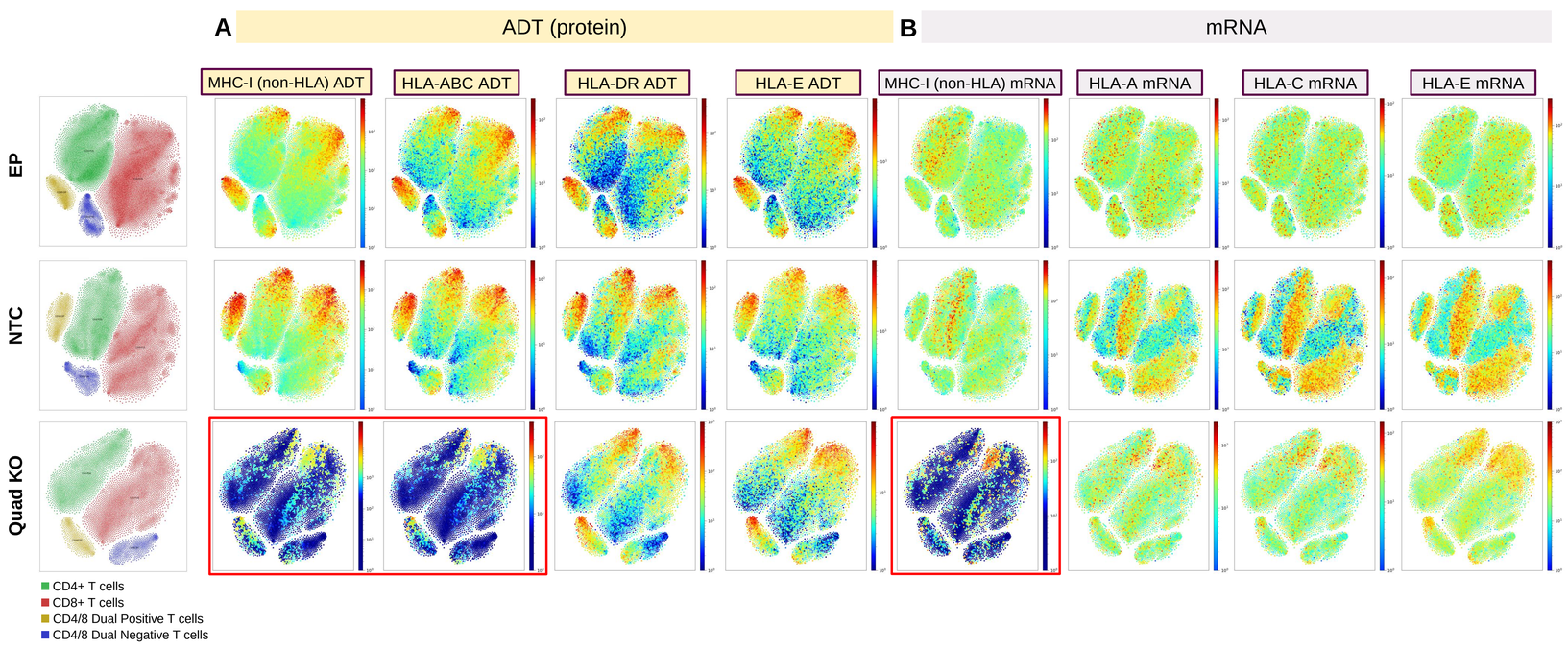

Figure 5: Comparison of transcriptional and post-transcriptional effects of base editing with the Pin-point system.
A) Knockout of MHC-I (non-HLA) chain after base editing with the Pin-point system results in a loss of protein staining, confirming successful editing. Additionally, we are able to detect a loss of staining with antibodies recognizing classical MHC class I complexes (HLA-A, B, and C; red box), while MHC class II (HLA-DR) staining and non-classical MHC class I molecule HLA-E staining remained unaffected. **B)** MHC-I (non-HLA) chain transcript was detected at lower levels in Pin-point quadruplex knockout condition compared to controls (red box), however the transcripts for classical MHC class I alleles (HLA-A and C) remained readily detectable in the knockout condition. This demonstrates the impact of MHC-I (non-HLA) acting on the assembled MHC class I complexes on the cell surface, not inhibiting transcription of MHC.

Summary

We applied multiplex base editing using the Pin-point system to the development of engineered CAR-T cells and hypoimmunogenic iPSCs.

The Pin-point base editing system achieved greater than 70% knockout efficiency without any selection or enrichment and high purity at therapeutically relevant target sites in T cells and iPSCs.

We subjected T cells edited with the Pin-point system to CITE-seq using TotalSeq antibodies and confirmed efficient knockout of target genes without any alteration in cell type distribution or T cell profile.

Notable differences in editing efficiencies were observed between DP and DN subsets of T cells.

Interestingly, there were differences in the RNA and protein expression levels of classical MHC class I alleles after base editing with the Pin-point system.

The application of CITE-Seq to T cells edited with the Pin-point base editing system can be used to gain a comprehensive view of gene editing outcomes.

By simultaneously examining transcriptomic and proteomic changes at the single-cell level, we can gain deeper insights into both the direct and indirect consequences of genetic modifications.

References

Collantes et al. The CRISPR Journal. Feb 2021.58–68
Porreca, Immacolata et al. Molecular Therapy, Volume 32, Issue 8, 2692 – 2710
App notes on Pin-point base editing platform (horizondiscovery.com/en/gene-editing/pin-point-base-editing-platform)

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