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Supplemental Information

Increasing CRISPR Efficiency and Measuring Its Specificity in HSPCs Using a Clinically Relevant System

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Supplemental Information

Supplemental Figures and Legends

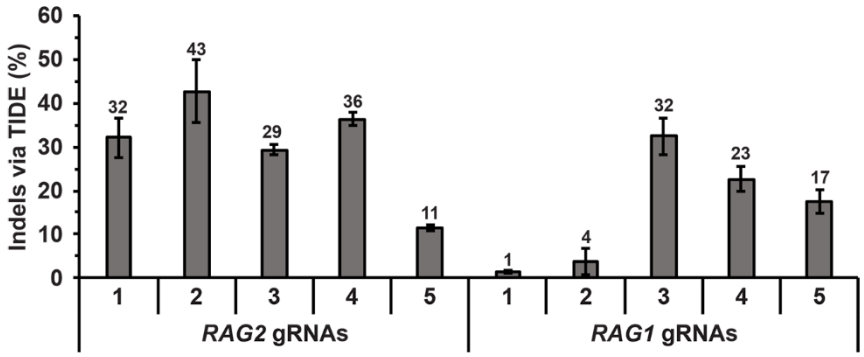

Figure S1. Screening for most active *RAG2* and *RAG1* gRNAs. Five potential gRNAs were identified by DESKGEN™ CRISPR software for *RAG2* (left) and *RAG1* (right) genes. The DNA sequence of each guide was cloned into the px330 plasmid and genome editing experiments were performed in the K562 cell line. The most active gRNAs (*RAG2* gRNA 2 and *RAG1* gRNA 3) were chosen for downstream experiments. Bars represent mean indel editing percentages analyzed by TIDE ± SEM (n=3). Editing percentages are designated above the bars.

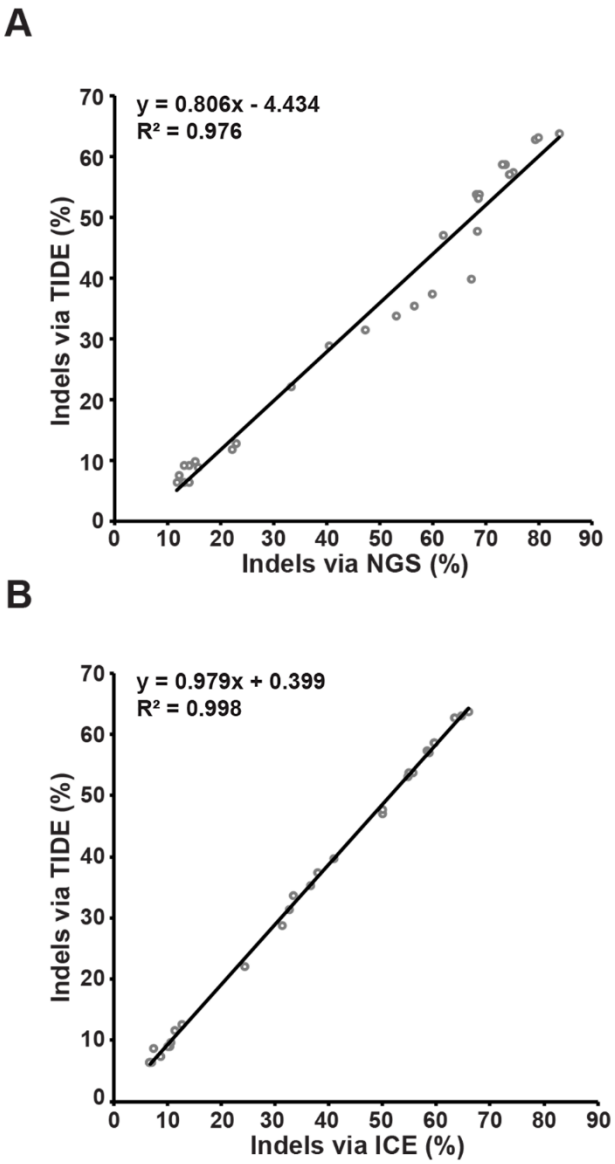

Figure S2. On-target editing detection in CD34+ HSPCs by NGS compared to TIDE and ICE chromatogram decomposition algorithms. (**A**) Amplicons of *RAG2* and *RAG1* on-target sites from CD34+ HSPCs (**Figure 1**) were Sanger sequenced, and chromatograms were analyzed by decomposition with TIDE software. Indel-editing frequencies quantified by NGS (**Figure 1**) were plotted against the TIDE quantification values. (**B**) Chromatograms were analyzed by decomposition with ICE software, and indel-editing frequencies were plotted against the TIDE analysis.

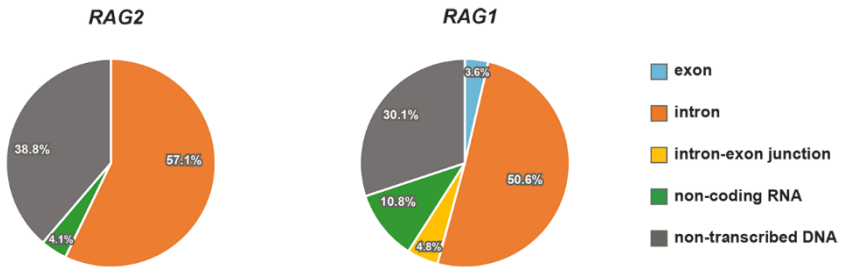

Figure S3: *RAG2* and *RAG1* gRNAs off-target sites were found dispersed throughout the genome. Off-target sites identified by GUIDE-seq in HEK293-Cas9 cells using Alt-R 2-part XT gRNA were categorized according to their genomic location either in exons, introns, intron-exon junction, non-coding RNA, or non-transcribed DNA.

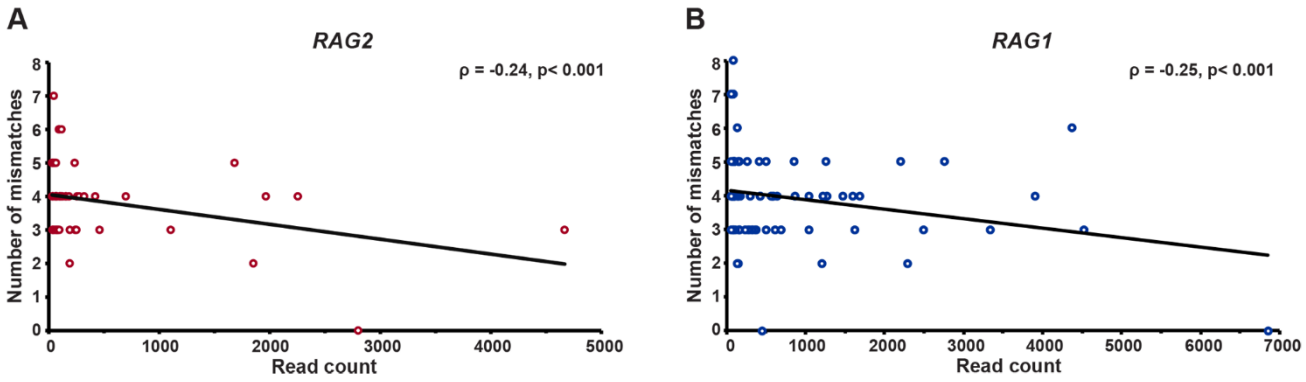

Figure S4: Correlation between the GUIDE-seq read counts and the number of mismatches. GUIDE-seq read counts identified in HEK293-Cas9 cells, following editing with *RAG2* (**A**) and *RAG1* (**B**) Alt-R 2-part XT gRNAs were plotted against the number of mismatches in each site (Spearman correlation).

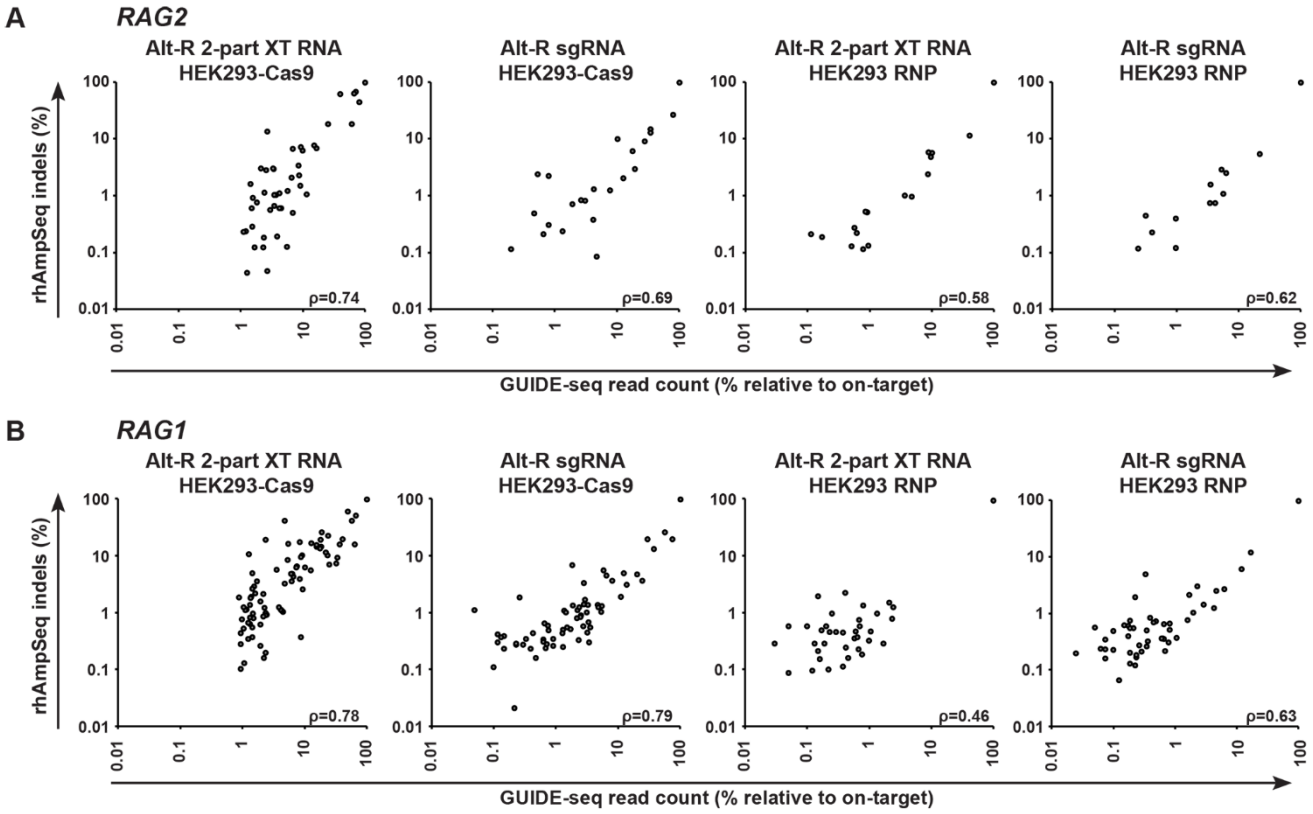

Figure S5: Correlation between rhAmpSeq and GUIDE-seq. Genomic DNA (gDNA) samples of the HEK293-Cas9 and HEK293 cells used in the GUIDE-seq experiments were subjected to rhAmpSeq analysis. The rhAmpSeq indel-editing percentages of *RAG2* (**A**) and *RAG1* (**B**) sites are plotted against the GUIDE-seq measurements, presented as a percentage normalized to 100% of the on-target site (Spearman correlation). P< 0.01.

Supplemental Tables

Gene	gRNA name	gRNA sequence	Genomic position
RAG2	gRNA 1	AGAAACTATGTCTCTGCAGA	Chr11:36594156-36594175
	gRNA 2*	TGAGAAGCCTGGCTGAATTA	Chr11:36594109-36594128
	gRNA 3	AACATAGCCTTAATTCAGCC	Chr11:36594119-36594138
	gRNA 4	AAATTCATCAGTGAGAAGCC	Chr11:36594098-36594117
	gRNA 5	TTCTCACTGATGAATTTTGA	Chr11:36594077-36594096
RAG1	gRNA 1	TGGGAAAGAGGCTGCCATGC	Chr11:36573303-36573322
	gRNA 2	TCCCAAGGTGGGTGGGAAAG	Chr11:36568025-36579756
	gRNA 3*	AACTGAGTCCCAAGGTGGGT	Chr11:36573322-36573341
	gRNA 4	GAACTGAGTCCCAAGGTGGG	Chr11:36573323-36573342
	gRNA 5	AGCCTCTTTCCCACCCACCT	Chr11:36573310-36573329

Table S1: Five potential gRNA sequences for *RAG2* and *RAG1* genes, were identified by DESKGEN™ CRISPR software. Asterisks indicate the gRNAs used in all subsequent experiments.

Table S2 (Submitted separately as an Excel file): GUIDE-seq in HEK293-Cas9 cells edited with Alt-R 2-part XT gRNA. Full list of *RAG2* and *RAG1* off-target sites, as identified by GUIDE-Seq and their genomic details.

Supplemental Methods and Materials

gRNA Competition: Five 20 bp gRNAs, closest to the initiation start codon, were chosen for each target gene by DESKGEN™ CRISPR bioinformatics tool (see **Table S1** for sequences).[1] The chosen oligonucleotides were cloned, as previously described,[2] into px330 sgRNA expression plasmid vectors (Addgene plasmid #42230) containing a human codon-optimized SpCas9 expression cassette and a human U6 promoter driving the expression of the sgRNA. Human K562 cells were cultured in RPMI 1640, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 1% L-glutamine (Biological Industries Ltd). 1*10^6 cells were electroporated with 5 µg of each of the cloned expression plasmids. Electroporation was performed in 100 µl nucleofection buffer (containing 100 mM KH2PO4, 15 mM NaHCO3, 12 mM MgCl2 × 6H2O, 8 mM ATP, 2 mM glucose (pH 7.4)) using the Lonza 2b Nucleofector (program T-016). 72 h post-electroporation gDNA was extracted by QuickExtract (Lucigen Corporation, WI), and indel editing frequencies were determined by chromatogram decomposition (TIDE software[3]).

On-target indel-editing frequency quantification using decomposition analysis: The following specific primers were used to amplify the gDNA sequences flanking the on-target sites of the genes of interest: RAG2_Fw: 5'-ACGGATTCTTGGGAAATGTG-3', RAG2_Re: 5'-GATGGTGTCATTTTTGGCAAT-3', and RAG1_Fw: 5'- CCTTAAGGTTTTTGTGGAAGGA-3', RAG1_Re: 5'- GGGCTTTTAACAATGGCTGA-3'. Amplicons were Sanger sequenced, and indel-editing frequencies were quantified by chromatogram decomposition using the TIDE software compared to mock-electroporated controls.

Supplemental References

1. Hough, SH, Ajetunmobi, A, Brody, L, Humphryes-Kirilov, N, and Perello, E (2016). Desktop Genetics. *Per Med* **13**: 517-521.
2. Ran, FA, Hsu, PD, Wright, J, Agarwala, V, Scott, DA, and Zhang, F (2013). Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* **8**: 2281-2308.
3. Brinkman, EK, Chen, T, Amendola, M, and van Steensel, B (2014). Easy quantitative assessment of genome editing by sequence trace decomposition. *Nucleic Acids Res* **42**: e168.