Science Advances
AAAS

# Supplementary Materials for

## Precise CRISPR-Cas–mediated gene repair with minimal off-target and unintended on-target mutations in human hematopoietic stem cells

Ngoc Tung Tran *et al.*

Corresponding author: Klaus Rajewsky, klaus.rajewsky@mdc-berlin.de;
Van Trung Chu, vantrung.chu@mdc-berlin.de

*Sci. Adv.* **8**, eabm9106 (2022)
DOI: 10.1126/sciadv.abm9106

**This PDF file includes:**

Figs. S1 to S11
Tables S1 to S3

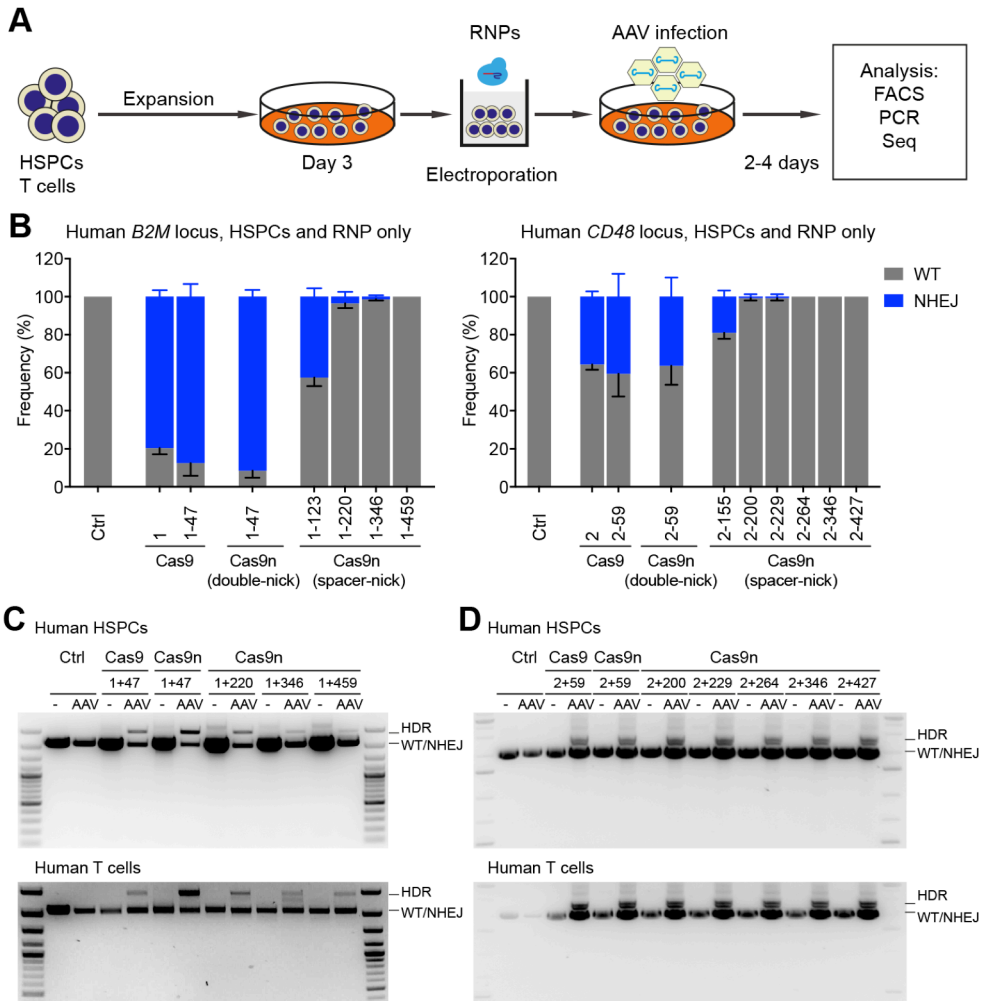


**Fig. S1. Gene targeting at the *B2M* and *CD48* loci in human HPSCs and T cells.**

(**A**) Experimental scheme of gene insertion in human HSPCs and T cells which were electroporated with RNPs and subsequently infected with AAV donor vectors. (**B**) Frequencies of NHEJ (blue) and wild-type (WT, grey) sequences at the targeted *B2M* (left) and *CD48* (right) loci in human HSPCs treated with control (Ctrl, no RNPs), Cas9, double-nick or spacer-nick RNPs. Data are shown as means ± SD from three independent experiments. Correct integration PCR showing HDR and WT/NHEJ sequences at the targeted *B2M* (**C**) and *CD48* (**D**) loci in human HSPCs (upper) and T cells (below) that were treated as indicated.

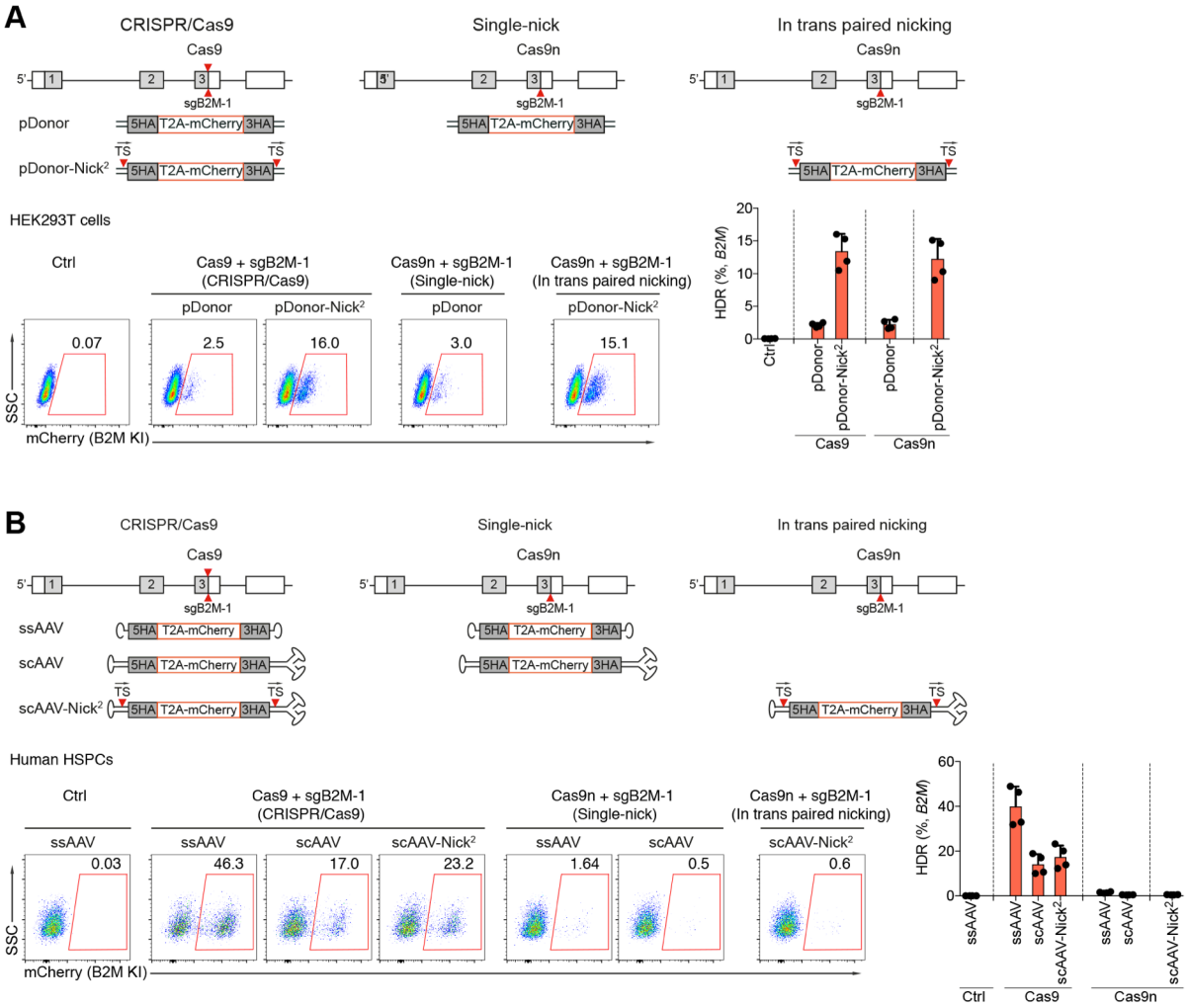


**Fig. S2. In trans paired nicking approach leads to inefficient HDR in human HSPCs.**

(**A**) Targeting strategy to insert T2A-mCherry into the human *B2M* locus using CRISPR/Cas9, single-nick or in trans paired nicking approach in HEK293T cells. Donor plasmids without (pDonor) or with 2 target sequences (TS) (pDonor-Nick$^2$) were used. The common sgRNA targeting the *B2M* is indicated in red. FACS analysis of the percentages of mCherry$^+$ HEK293T cells six days post targeting the *B2M* locus with the indicated methods. Graph summarizes frequencies of mCherry$^+$ (HDR) HEK293T cells as measured by FACS. (**B**) Insertion of T2A-mCherry into the human *B2M* locus using CRISPR/Cas9, single-nick or in trans paired nicking approach in human HSPCs. Single-stranded (ss) AAV and self-complementary (sc) AAV without (scAAV) or with 2 TS (scAAV-Nick$^2$) were used as donor templates. FACS analysis showing frequencies of mCherry$^+$ HSPCs three days post targeting. Bar graph showing HDR (mCherry$^+$) efficiencies. Data are shown as means ± SD from four independent experiments.

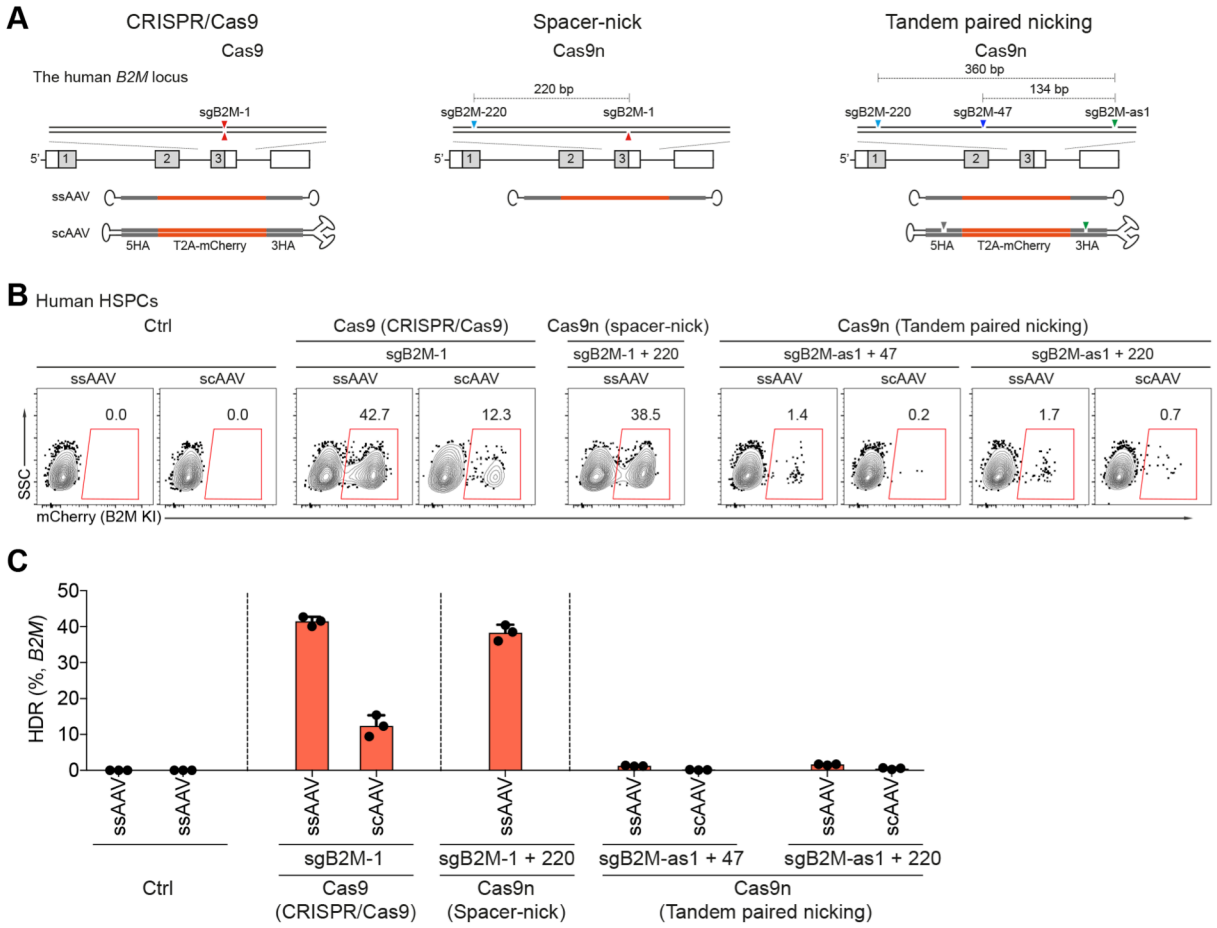


**Fig. S3. Tandem paired nicking approach leads to low HDR efficiency in human HSPCs.**

(**A**) Experimental scheme to insert T2A-mCherry into the human *B2M* locus in human HSPCs using CRISPR/Cas9, spacer-nick, or tandem paired nicking approach. SsAAV and scAAV donor vectors with the indicated HAs flanking T2A-mCherry fragments were depicted. Common sgB2M-1, sgB2M-47, sgB2M-220, and sgB2M-as1 are indicated as red, dark blue, light blue, and green arrows, respectively. (**B**) FACS analysis of the percentages of mCherry$^{+}$ HSPCs three days post targeting. (**C**) Bar graph showing HDR efficiencies in (**B**). Data are shown as means ± SD from three independent experiments.

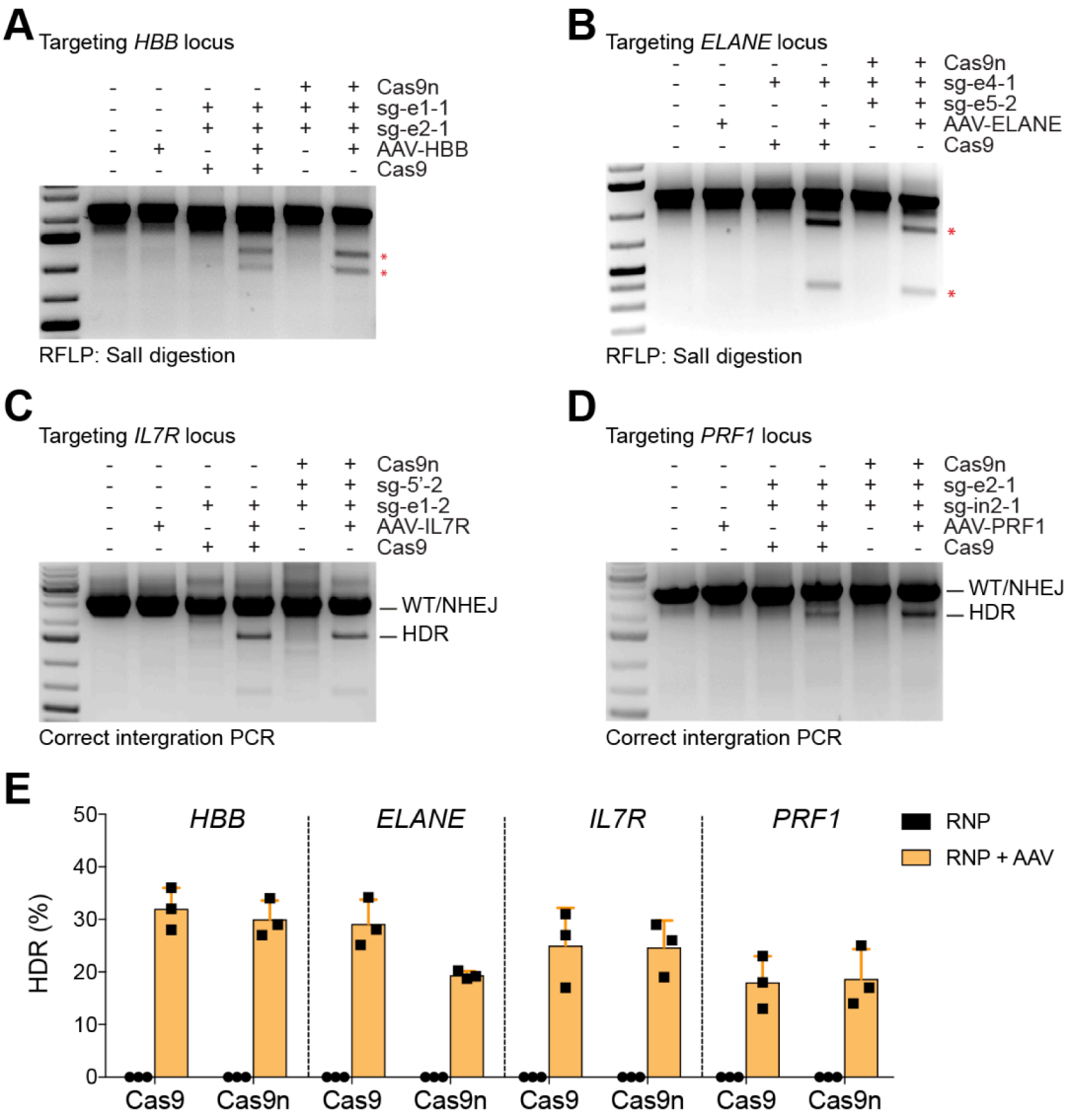


**Fig. S4. Gene correction efficiencies in human HSPCs.**

Sall-mediated RFLP assays showing the gene correction efficiency of the targeted *HBB* (**A**) and *ELANE* (**B**) loci in the HSPCs treated with indicated approaches. Asterisks depict the Sall-cleaved bands. Correct integration PCR showing WT/NHEJ and HDR events at the targeted *IL7R* (**C**) and *PRF1* (**D**) loci in the HSPCs treated as indicated. (**E**) Quantification of gene correction efficiencies (HDR) at the targeted loci in the HSPCs treated with RNP only (black) or RNP and AAV donor vectors (yellow).

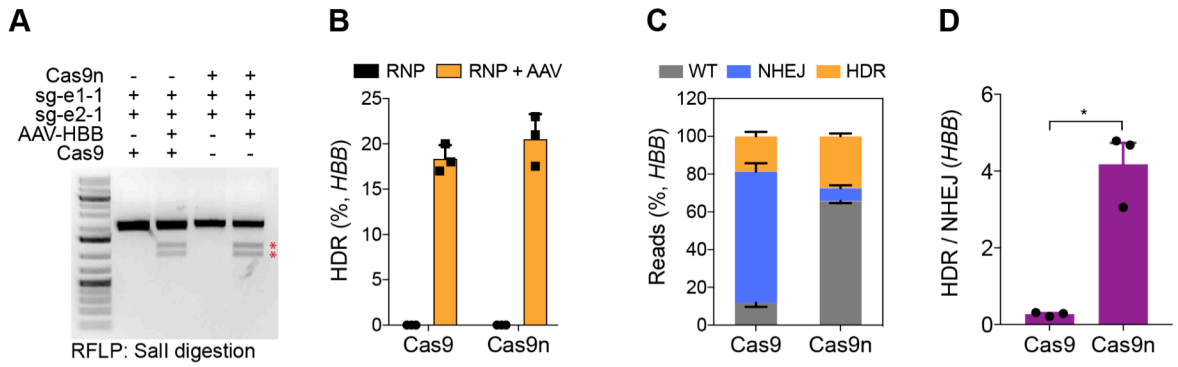


**Fig. S5. Spacer-nick leads to efficient *HBB* gene correction in human T cells.**

**(A)** SalI-mediated RFLP assay showing efficiency of *HBB* gene correction in human T cells treated with RNPs only or RNPs and AAV donor vectors. Asterisks indicate the SalI-cleaved bands. (**B**) Quantification of HDR efficiency in T cells shown in (**A**) from three independent experiments. (**C**) Frequencies of WT (grey), NHEJ (blue), and HDR (orange) sequences at the targeted *HBB* locus in human T cells treated as indicated. (**D**) The ratio of HDR:NHEJ at the targeted *HBB* locus. Data represent means ± SD from three independent experiments, * $P<0.05$.

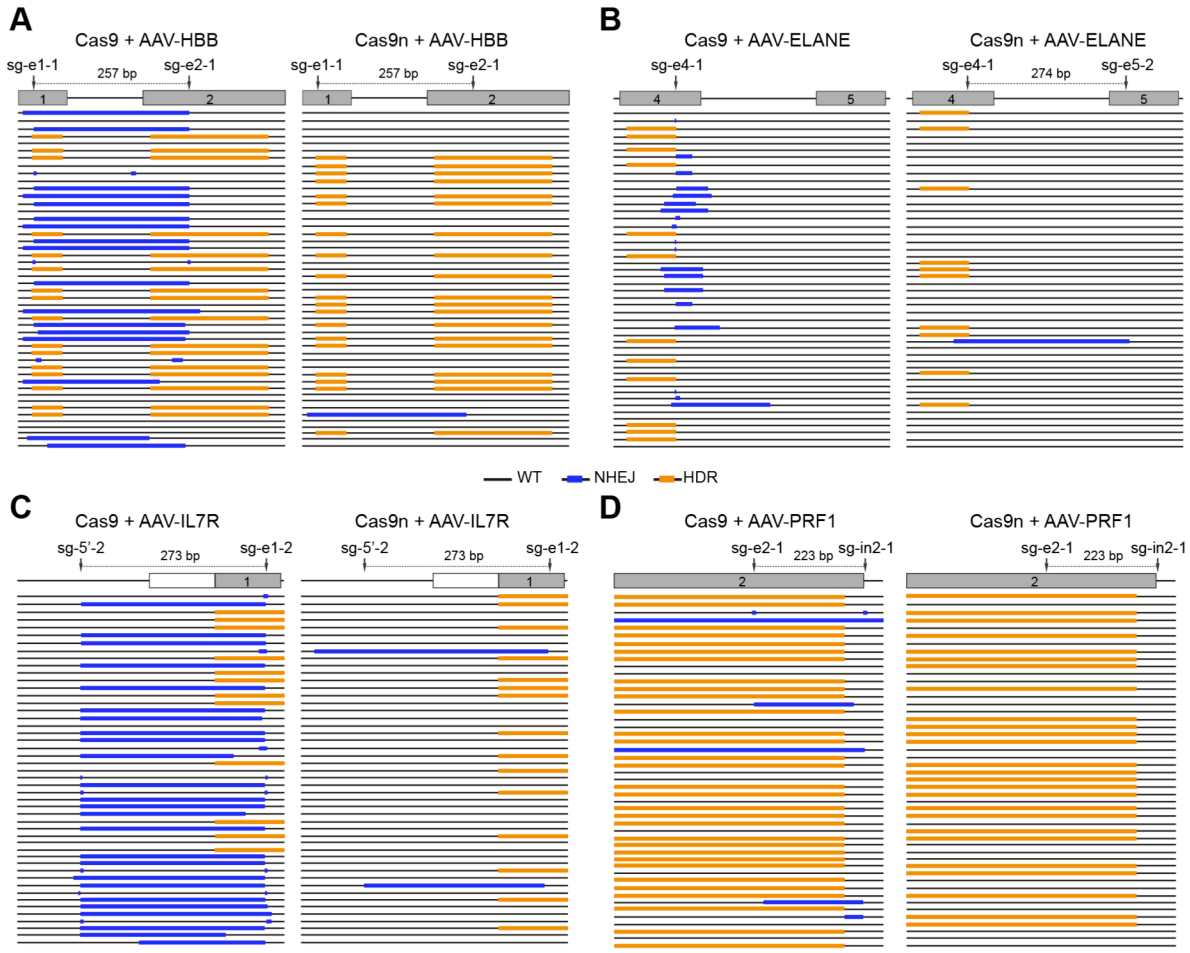


**Fig. S6. Sanger sequencing analysis at the targeted loci in human HSPCs.**

Representative sequences of WT (black), NHEJ (blue) and HDR (orange) at the targeted *HBB* (**A**), *ELANE* (**B**), *IL7R* (**C**) and *PRF1* (**D**) loci in human HSPCs that received either Cas9 or Cas9n RNPs, and AAV donor vectors.

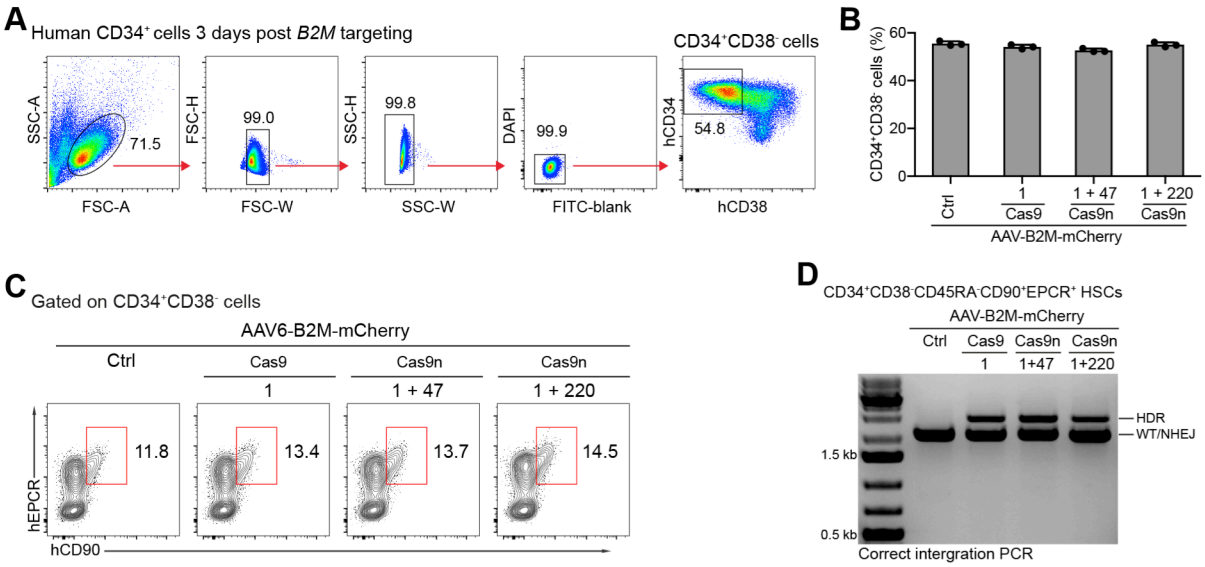


**Fig. S7. Gene insertion into the *B2M* locus in long-term HSCs.**

(**A**) Gating strategy for $CD34^+CD38^-$ population after three days targeting the *B2M* locus in human $CD34^+$ cells. (**B**) Frequencies of $CD34^+CD38^-$ HSPCs treated with control (Ctrl, no RNPs), Cas9, double-nick or spacer-nick RNPs, and AAV donor vectors. Data are shown as means ± SD from three independent experiments. (**C**) FACS analysis, pre-gated on $CD34^+CD38^-$ cells, showing the co-expression of CD90 and EPCR markers in long-term HSCs. (**D**) Correct integration PCR showing WT/NHEJ and HDR events at the targeted *B2M* locus in the sorted $CD34^+CD38^-CD45RA^-CD90^+EPCR^+$ HSCs.

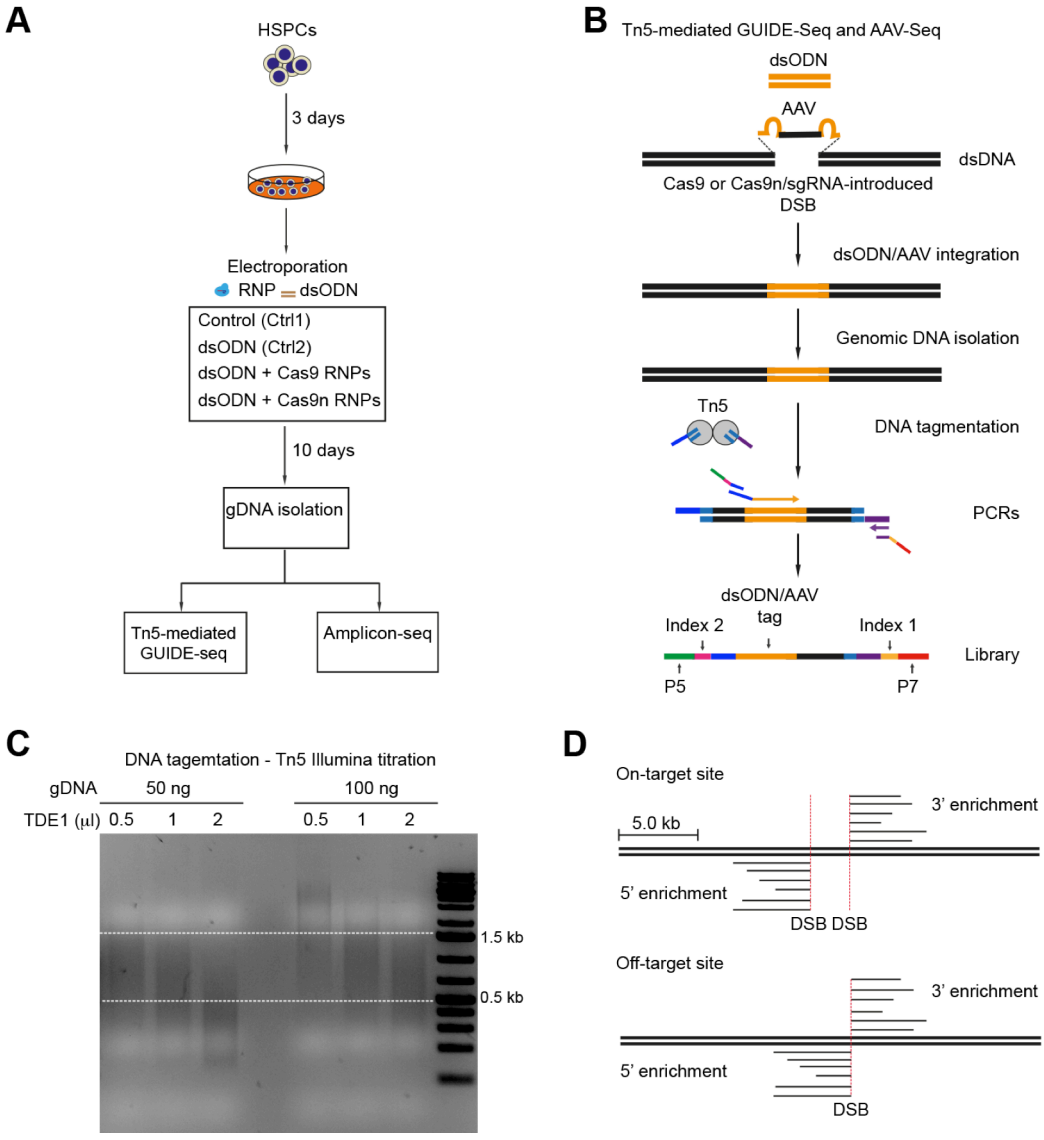


**Fig. S8. Nextera Tn5-mediated GUIDE-seq and AAV-seq.**

**(A)** Experimental scheme of GUIDE-seq and Amplicon-seq in the targeted HSPCs. (**B**) Workflow of Tn5-mediated GUIDE-seq and AAV-seq methods. (**C**) Optimization of DNA tagmentation with different amounts of Nextera Tn5 transposase (TDE1) in 20 μl reaction with 50 or 100 ng of genomic DNA. The expected size of tagmented DNA fragments is 0.5-1.5 kb (white lines). (**D**) By mapping of reads to human genome within 5.0 kb distance on both sides (5' and 3'), on- and off-target DSB positions were identified.

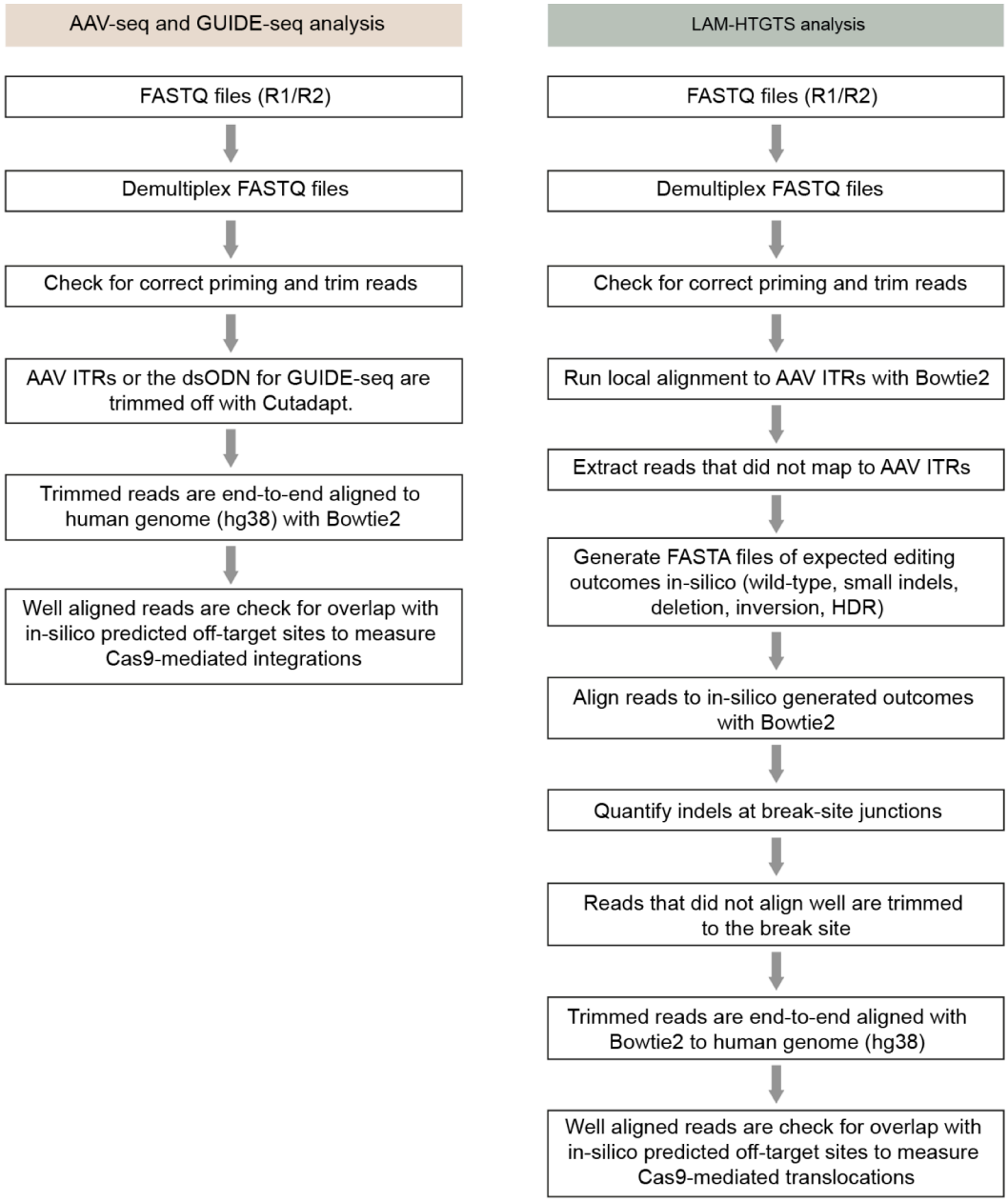


**Fig. S9. Pipelines analyze GUIDE-seq, AAV-seq and LAM-HTGTS data.**

For GUIDE-seq and AAV-seq analysis, samples were demultiplexed, reads were checked for correct priming and the sequences of AAV inverted terminal repeats (ITRs) and dsODN were trimmed to adjacent genomic sequences that are globally mapped to human genome (hg38) using Bowtie2. Mapped reads that were overlapped the off- predicted target sites, were quantified. For LAM-HTGTS analysis, samples were demultiplexed, checked for correct priming and trimmed to genome interface. The trimmed reads were aligned to sequences of the AAV ITRs for quantifying AAV integrations. The reads that were not aligned to AAV ITRs, were globally aligned to human genome for quantifying wild-type, indel/and deletion, inversion, HDR and translocation events.

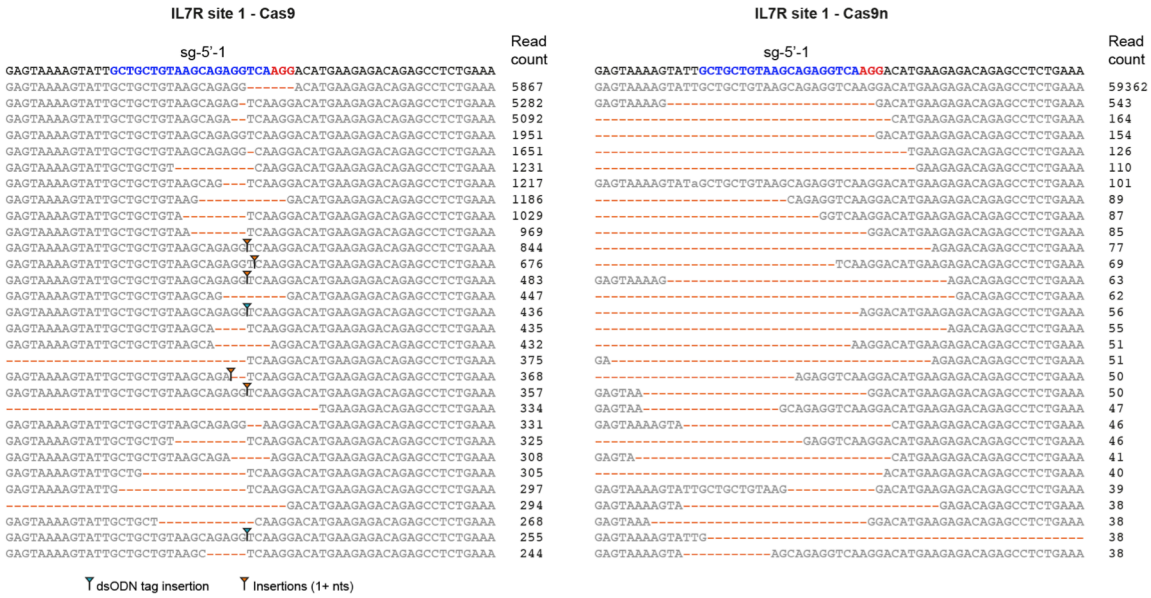


**Fig. S10. Amplicon-seq analysis.**

On- and off-target sites were amplified from genomic DNA of the targeted HSPCs treated with dsODN tag and either Cas9 or Cas9n RNPs. PCR products were indexed, pooled and sequenced using Miniseq. Indel profiles showing number of intact, deletion/ or insertion (1+ nts) and dsODN tag integration reads at the *IL7R* on-target site 1.

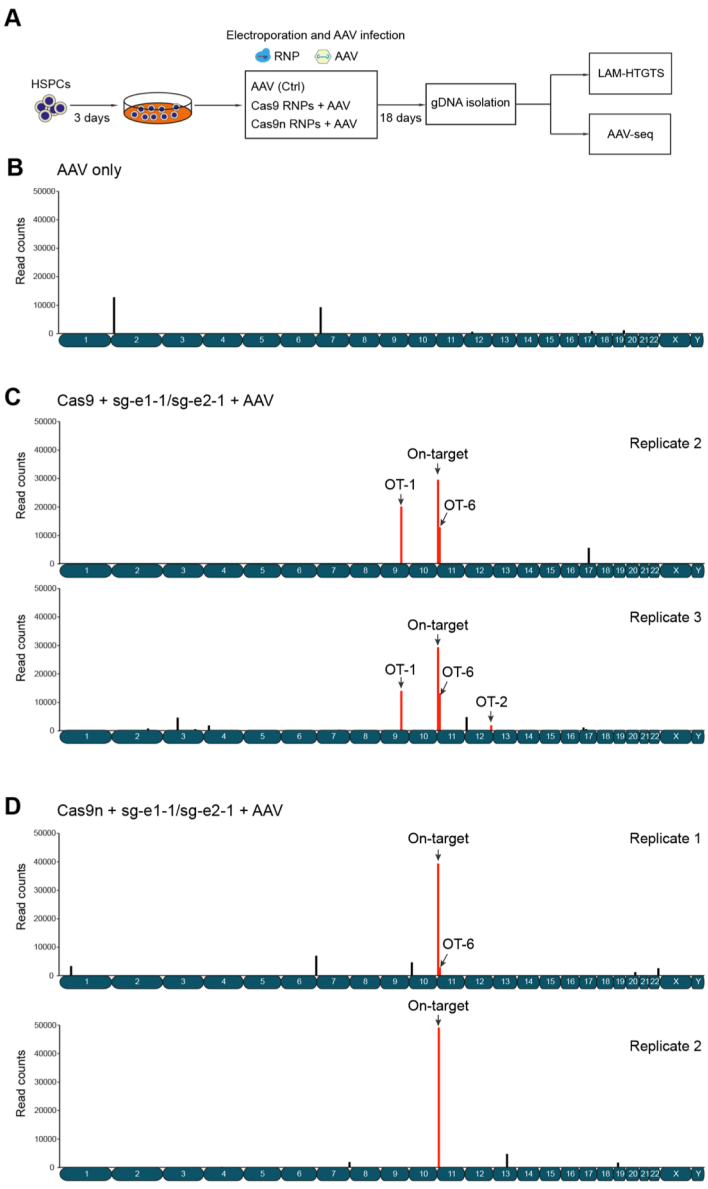


**Fig. S11. AAV integration sites.**

(**A**) Experimental scheme of AAV-seq and LAM-HTGTS methods. Bar graphs showing number of AAV integration sites mapped on human chromosomes of *HBB*-targeted HSPCs treated with AAV donor vectors only (**B**), sgRNAs/Cas9 (**C**) or sgRNAs/Cas9n RNPs (**D**), and AAV donor vectors. The top hits of AAV integrations are indicated in red including the on-target site and 3 high-risk off-target sites (OT-1, OT-2 and OT-6), identified by GUIDE-seq.

**Table S1. List of sgRNAs and gene editing efficiencies.**

| Name | Sequence (5' to 3') | Gene editing (indels) | |
|---|---|---|---|
| | | ICE (%) | T7EI (%) |
| ***B2M*** | | | |
| sgB2M-1 | GAGACATGTAAGCAGCATCA | 92 | 84 |
| sgB2M-47 | AAAGACAGTGGAGAAAAAAA | 95 | 83 |
| sgB2M-123 | AGAAATAAGGCTGGCAGAAT | 92 | 80 |
| sgB2M-220 | CCAATCCAGCCAGAAAGTAC | 90 | 72 |
| sgB2M-346 | AAAAGCTAGAGGAAGCCAGT | 94 | 73 |
| sgB2M-459 | CTGTGCATCAGTATCTCAGC | 83 | 69 |
| sgB2M-as1 | CAATGTTCTCCACATAGTGA | 87 | 85 |
| ***CD48*** | | | |
| sgCD48-2 | GGCCAGAAGATCTTGCCTGT | 90 | 94.1 |
| sgCD48-59 | GCTCATCTCAGGTAAGTAAC | 94 | 73.5 |
| sgCD48-155 | CAAGAAAACCCTATATGCTT | 87 | 82.0 |
| sgCD48-200 | TTGAGAAGGGGCAAGTGTAC | 90 | 89.0 |
| sgCD48-229 | GTGTGGGAGCTCACAGAGCA | 94 | 83.9 |
| sgCD48-264 | TCCCTGAATCCCATCCTATT | 84 | 89.7 |
| sgCD48-346 | TTAAATATCACTGGCTTACC | 86 | 87.2 |
| sgCD48-427 | CTCTGGACATCAACGAGGTA | 92 | 84.6 |
| ***HBB*** | | | |
| sgHBB-ex1-1 | CTTGCCCCACAGGGCAGTAA | (*5*) | |
| sgHBB-Ex1-2 | GTAACGGCAGACTTCTCCTC | (*45*) | |
| sgHBB-Ex1-3 | CACGTTCACCTTGCCCCACA | 50 | 44 |
| sgHBB-Ex1-4 | CCTTGATACCAACCTGCCCA | 42 | 35 |
| sgHBB-5'UTR-1 | CTCAGGAGTCAGATGCACCA | 30 | 25 |
| sgHBB-5'UTR-2 | TGCACCATGGTGTCTGTTTG | 10 | 5 |
| sgHBB-5'UTR-3 | AAGCAAATGTAAGCAATAGA | 65 | 52 |
| sgHBB-Ex2-1 | TCCACTCCTGATGCTGTTAT | 57 | 50 |
| sgHBB-Ex2-2 | TCCCACCCTTAGGCTGCTGG | 47 | 53 |
| sgHBB-Ex2-3 | GCTCATGGCAAGAAAGTGCT | 26 | 17 |
| sgHBB-Ex2-4 | TGCTGTTATGGGCAACCCTA | 29 | 20 |
| sgHBB-Ex2-5 | TATGGGCAACCCTAAGGTGA | 59 | 53 |
| ***ELANE*** | | | |
| sgELANE-Ex4-1 | GAGTGCAGACGTTGCTGCGA | (*44*) | |
| sgELANE-Ex4-2 | GACGTTGCTGCGACGGCAGA | 49 | 37 |
| sgELANE-Ex4-3 | GCAGGACGCTGGCGATCCCA | 52 | 30 |
| sgELANE-Ex4-4 | CGAAACAGACGCCGGCCTGC | 7 | ND |
| sgELANE-Ex4-5 | GGCACGTACGAAACAGACGC | 5 | ND |
| sgELANE-Ex4-6 | CCGTCACGTTGAGCTCCTGC | ND | ND |
| sgELANE-Ex4-7 | GTTGAGCTCCTGCAGGACGC | ND | ND |
| sgELANE-Ex5-1 | GGAATTGCCTCCTTCGTCCG | 50 | 41 |
| sgELANE-Ex5-2 | ATTGCCTCCTTCGTCCGGGG | 81 | 72 |
| sgELANE-Ex5-3 | ACCTTGTCTGCCTCCACAGG | 49 | 80 |
| sgELANE-Ex5-4 | CTGCCTCCACAGGGGGACTC | 53 | 85 |
| sgELANE-Ex5-5 | GGCAGCCCCTTGGTCTGCAA | 6 | 10 |
| ***IL7R*** | | | |
| sgIL7R-5'UTR-1 | GCTGCTGTAAGCAGAGGTCA | 54 | 23.0 |
| sgIL7R-5'UTR-2 | AGTATTGCTGCTGTAAGCAG | 52 | 43.9 |
| sgIL7R-5'UTR-3 | AGAAGGCAAGTTCCAGAAAC | 27 | 22.8 |

| | | | |
|---|---|---|---|
| sgIL7R-5'UTR-4 | TATAAACACAGCAAAAAAGA | 35 | 27.8 |
| sgIL7R-5'UTR-5 | ACTTATCTAACCACAGACAA | 58 | 49.5 |
| sgIL7R-Ex1-1 | TCTAGGTACAACTTTTGGCA | 10 | 5.3 |
| sgIL7R-Ex1-2 | CAAGTCGTTTCTGGAGAAAG | 56 | 18.0 |
| sgIL7R-Ex1-3 | GAAAGTGGCTATGCTCAAAA | 55 | 38.4 |
| ***PRF1*** | | | |
| sgPRF1-ex2-1 | TGGCCCTGGTTACATGGCGC | 83 | ND |
| sgPRF1-ex2-2 | GCGCTTGCACTCTGAGCGTG | 48 | ND |
| sgPRF1-ex2-3 | CTGGGAAGGAGCCCGAGCGG | 63 | ND |
| sgPRF1-in2-1 | CGGTGGAGTGCCGCTTCTAC | 80 | ND |
| sgPRF1-in2-2 | CCGCTTCTACAGGTGAGAGC | 10 | ND |
| sgPRF1-in2-3 | TAGGGGTGGGGGGCTGGAAA | 7 | ND |
| sgPRF1-in2-4 | GAAAAGGCGCGGGAAACTCT | 8 | ND |

Gene editing efficiencies of sgRNAs were measured by ICE analysis and T7EI assay. ND: not determined.

**Table S2. Potential combination of spacer-nick sgRNAs for gene correction.**

| sgRNA-1 | sgRNA-2 | Distance (bp) | HDR (%) | NHEJ (%) |
|---|---|---|---|---|
| **Targeting *HBB* locus** | | | | |
| sgHBB-Ex1-1 | sgHBB-Ex2-1 | 257 | 35 | 1.3 |
| sgHBB-Ex1-1 | sgHBB-Ex2-4 | 268 | 27 | 0.5 |
| sgHBB-Ex1-1 | sgHBB-Ex2-5 | 274 | 26 | 0.8 |
| sgHBB-Ex1-2 | sgHBB-Ex2-1 | 273 | 27 | 1.8 |
| sgHBB-Ex1-2 | sgHBB-Ex2-4 | 284 | 22 | 1.0 |
| sgHBB-Ex1-2 | sgHBB-Ex2-5 | 290 | 19 | 0.7 |
| sgHBB-Ex1-3 | sgHBB-Ex2-1 | 248 | 23 | 1.4 |
| sgHBB-Ex1-4 | sgHBB-Ex2-1 | 206 | 31 | 4.6 |
| sgHBB-5'UTR-1 | sgHBB-Ex2-2 | 223 | 10 | 0.9 |
| sgHBB-5'UTR-2 | sgHBB-Ex2-2 | 236 | 18 | 1.3 |
| sgHBB-5'UTR-3 | sgHBB-Ex2-2 | 284 | ND | ND |
| **Targeting *ELANE* locus** | | | | |
| sgELANE-Ex4-1 | sgELANE-Ex5-1 | 271 | 28 | 2.2 |
| sgELANE-Ex4-1 | sgELANE-Ex5-2 | 274 | 29 | 1.6 |
| sgELANE-Ex4-1 | sgELANE-Ex5-3 | 210 | 30 | 3.5 |
| sgELANE-Ex4-1 | sgELANE-Ex5-4 | 217 | 29 | 2.9 |
| sgELANE-Ex4-2 | sgELANE-Ex5-1 | 278 | 19 | 1.6 |
| sgELANE-Ex4-2 | sgELANE-Ex5-2 | 281 | 13 | 1.0 |
| sgELANE-Ex4-2 | sgELANE-Ex5-3 | 217 | 17 | 2.9 |
| sgELANE-Ex4-2 | sgELANE-Ex5-4 | 224 | 16 | 2.1 |
| sgELANE-Ex4-3 | sgELANE-Ex5-3 | 267 | ND | ND |
| sgELANE-Ex4-3 | sgELANE-Ex5-4 | 274 | ND | ND |
| **Targeting *IL7R* locus** | | | | |
| sgIL7R-5'UTR-1 | sgIL7R-Ex1-2 | 279 | 10.0 | 1.7 |
| sgIL7R-5'UTR-1 | sgIL7R-Ex1-3 | 294 | 15 | 1.2 |
| sgIL7R-5'UTR-2 | sgIL7R-Ex1-2 | 273 | 20 | 1.4 |
| sgIL7R-5'UTR-2 | sgIL7R-Ex1-3 | 288 | 25 | 2.0 |
| sgIL7R-5'UTR-5 | sgIL7R-Ex1-2 | 194 | 20 | 14 |
| sgIL7R-5'UTR-5 | sgIL7R-Ex1-3 | 209 | 17 | 5.2 |
| **Targeting *PRF1* locus** | | | | |
| sgPRF1-ex2-1 | sgPRF1-in2-1 | 223 | 55 | 3.1 |
| sgPRF1-ex2-2 | sgPRF1-in2-1 | 452 | 30 | 1.0 |
| sgPRF1-ex2-3 | sgPRF1-in2-1 | 367 | 20 | 1.3 |

This table shows additional pairs of spacer-nick sgRNAs for spacer-nick-mediated gene correction. Percentages of HDR and NHEJ sequences were measured by TOPO cloning and Sanger sequencing. ND: not determined.

## Table S3. Primers were used in this study.

| Primer name | Sequence (5'>>3') |
|---|---|
| | **Primers for T7EI, RFLP and correct integration PCR** |
| B2M-T7-For | GATTCAGGTTTACTCACGTCATCCAGCAGA |
| B2M-T7-Rev | CCATACTGGCAGTTCCTTTGCCCTCTC |
| B2M-5HAextern-For | CATGTAGACTCTTGAGTGATGTGTTAAGGAATGCTATGA |
| B2M-3HAextern-Rev | ACTGGGCAGATCATCCACCTTCCTGATGCT |
| CD48-T7-For | GTGACAGTGTGGTATCTGACCATGAGAACA |
| CD48-T7-Rev | CTGTCTGACAGATTTGGGACAACTTCAGGA |
| CD48-5HAextern-For | CCAGCACTCTGGGAAATACCAGAGTCAGAT |
| CD48-3HAextern-Rev | CGCCTACCTGCATATAGTGGACAACTCTGG |
| HBB-T7-For | CCAGAAGAGCCAAGGACAGGTACGGCTG |
| HBB-T7-Rev | CGATCCTGAGACTTCCACACTGATGCAATC |
| HBB-5HAextern-For | ACTCTTGCAGATTAGTCCAGGCAGAAACAG |
| HBB-3HAextern-Rev | ATGAACATGATTAGCAAAAGGGCCTAGCTT |
| ELANE-T7-For | CTCAACGGGTCGGCCACCATCAACGCCA |
| ELANE-T7-Rev | TGTCCTCGGAGCGTTGGATGATAGAGTC |
| ELANE-5HAextern-For | CCAGGCTGGAGCGCAGTGGCACAATCTCAG |
| ELANE-3HA-Rev | CCTCGGAGCGTTGGATGATAGAGTCGATCC |
| IL7R-T7-For | GCATAGTGGCATTTGCCTGTAGTCCTAGCT |
| IL7R-T7-Rev | CCATAGTATTGATCCCCTGCCCCCTGTCAT |
| IL7R-5HAextern-For | CCATGGGAGCAATTCTACCTAATGACAGTGAATCCA |
| IL7R-3HAextern-Rev | CAACTTTCAACAAGCAGCAACTGAATGTGAATTCCT |
| IL7R-modicDNA-For | TTAAGTTGCATCATTTTGTCTGACTAGGTGTCCTTC |
| PRF1-T7-For | TATCTCAGCCCCTCCCCTTC |
| PRF1-T7-Rev | TGGGACCTCCTGGATGAGAG |
| PRF1-5HAextern-For | CCACCCTTCAAGTCACACCTTTGGG |
| PRF1-3HAextern-Rev | GGGGGAGTGTGTACCACATGGAAAc |
| PRF1-modicDNA-For | ACAGCCACAGATGCCTACGTGAAGC |
| | **Primers for Amplicon-seq** |
| ON-HBB-1-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCAGGGAGGGCAGGAGCCAGGGCTGGGCA |
| On-HBB-1-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTCTCCTTAAACCTGTCTTGTAACCTTGA |
| OT-1-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTTCCCGTTCTCCACCCAATAGCTATGGA |
| OT-1-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTACGGCCTAAGAAATTATAGTTTAGCAGT |
| OT-2-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGCTTCCATCTGATCAGGAGGTCTCCAGTA |
| OT-2-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTATCTGTGTCATCCCAGGAAAGCTTGTCT |
| OT-3-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGTGCTATGGGAAATAATGATTGTTGCTT |
| OT-3-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAGCCAGTAACTCTAAGCCCCTGTAATT |
| ON-HBB-2-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCACTGACTCTCTCTGCCTATTGGTCTAT |
| ON-HBB-2-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAAGAAAACATCAAGCGTCCCATAGACT |
| OT-4-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGAGAAGACTCTTGGGTTTCTGATAGGCA |
| OT-4-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCAGGCCATCACTAAAGGCACCTAGCACC |
| ON-ELANE-1-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACGGTGCCTGTTGCTGCAGTCCGGGCTGG |
| ON-ELANE-1-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTTCTGGGCAGGAACCGTGGGATCGCCAG |
| ON-ELANE-2-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGCCTCGCAGTCCAGCTTCCCCACCTTGT |
| ON-ELANE-2-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTTGTCCTCGGAGCGTTGGATGATAGAGT |
| OT-7-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCTGGAGAACGATCGTATTTGGATGGCCT |
| OT-7-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAGCGATTCCAGCTGGCAGCGCCGTGACA |
| OT-8-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCAACTCCCCTATTGTTGATATTGTCCCTG |
| OT-8-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATGCGAACGACAACGCACCAGCGTTTGCT |
| ON-IL7R-1-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACCACAGACAAGGGAAGTATAAACACAGC |
| ON-IL7R-1-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGCCTTACTCTGATGTAAGCACAGTAAGTGT |
| OT-9-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAACCCAACATGGTCAGACCTGGACTGAAG |
| OT-9-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCAACTTGCCCTTTTGGCCTCTACCTTGCT |
| OT-10-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCTCAGCTTTCTAAACCTTACACCAGTAATG |
| OT-10-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGAGCATAAAGCCTGGTAGGCAGAGGGACA |
| OT-11-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGCAGCTAATCCTCTGTTGTCAGCTTTCA |
| OT-11-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTTCCTGTGGCTGTGAGGTAATGCCCCAA |
| OT-12-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCCAGGCCATCAGTCGGGGATTGAATACA |
| OT-12-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCACAGCAGCCACAGTCATCTCTCCGTCA |
| OT-13-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACAGAGCCTGGAGAAATCACAAAATACTTGT |
| OT-13-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTCTAGGAAACACAGTCCTGTACCAGAGC |
| ON-IL7R-2-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCATTTCATACACACTGGCTCACACATCTAC |
| ON-IL7R-2-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGGAACTGAATAACCTGAAACCATGCTACA |
| OT-14-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAAGCCCTTGAGCTTTCAATTTGGGGCA |
| OT-14-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCCTTGGCATATATGAGGAGGTAAAGTA |
| ON-PRF1-1-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATTGCTGGTGGGCTTAGGAGTCACGTCCA |
| ON-PRF1-1-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCGACGGCACCTGCACCCTCTGTGAA |
| OT-15-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGCACTGCCGACCATCCACAGTGGACATT |
| OT-15-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCCTACTTTATGATTGGATCGGGAGGA |
| ON-PRF1-2-For | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCAGCAATGTGCATGTGTCTGTGGCCG |
| ON-PRF1-2-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCCAAGTTTGATTGGAGGACTCTGCCT |
| | **Primers for GUIDE-seq** |
| i5Nextera-3'-GSP (-) | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTTAATTGAGTTGTCATATGTTAATAACGGT |
| i5Nextera-5'-GSP (+) | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATACCGTTATTAACATATGACAACTCAATTAA |

| | |
|---|---|
| i7Nextera-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAG |
| | **Primers for AAV-seq** |
| AAV-+-1 | GGATCTCGACGCTCTCCCTGGAGTTGGCCACTCCCTCTCTG |
| i5Nextera-AAV-+-2 | TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCTCTGCGCGCTCGCTCG |
| i7Nextera-Rev | GTCTCGTGGGCTCGGAGATGTGTATAAG |
| | **Primers for LAM-HTGTS** |
| HBB-5'extern-For | ACTCTTGCAGATTAGTCCAGGCAGAAACAG |
| HBB-5'-For-nested | AGCCATCTATTGCTTACATTTGCTTCTGACAC |
| HBB-3'extern-Rev | ACATGATTAGCAAAAGGGCCTAGCTTGGAC |
| HBB-3'-Rev-nested | TGTCACAGTGCAGCTCACTCAG |
| ELANE-5'extern-For | CCAGGCTGGAGCGCAGTGGCACAATCTCAG |
| ELANE-5'-For-Nested | CTTCTGGGCAGGAACCGTG |
| ELANE-3'extern-Rev | CTTACTCCAGAGATGCCCAAGAGACTCTGGA |
| ELANE-3'-Rev-nested | CCTCGGAGCGTTGGATGATAGAGTCGATCC |
| PRF1-5'extern-For | GGCTCCAGCTATAATGGGGGCTC |
| PRF1-5'-For-nested | AAGTGCCCCCTGTCTCTGCAGC |
| PRF1-3'extern-Rev | CCCAGGGGAGTATTTCCCCCATT |
| PRF1-3'-Rev-nested | TTCCAGGGCTCCTAGACCAC |
| Adapter-upper | GCGACTATAGGGCACGCGTGGNNNNNN[AmC3] |
| Adapter-lower | [Phos]CCACGCGTGCCCTATAGTCGC[AmC3] |
| Adapter primer | GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG NNNNN |
| Adapter nested primer | TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG NNNNN |