SUPPLEMENTARY MATERIAL *for*

Targeted homology-directed repair in blood stem and progenitor cells with CRISPR nanoformulations

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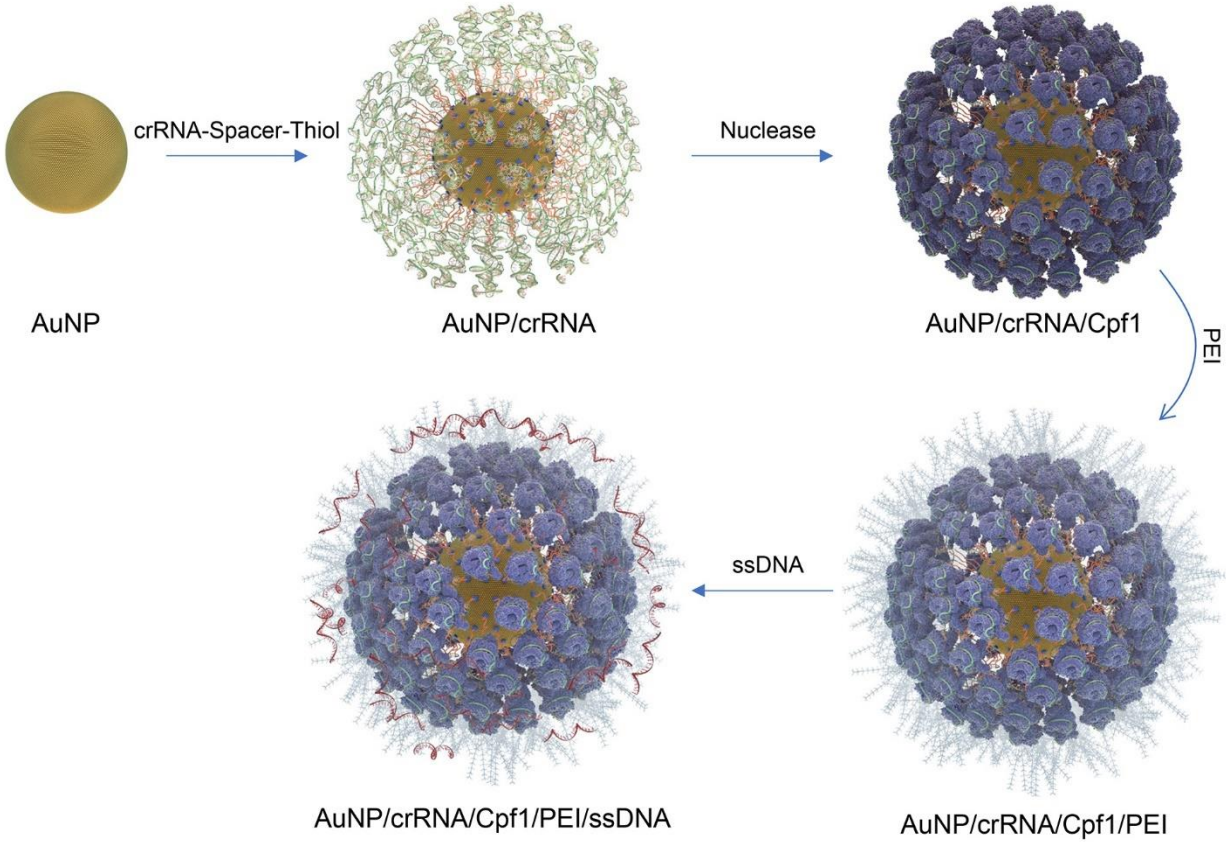
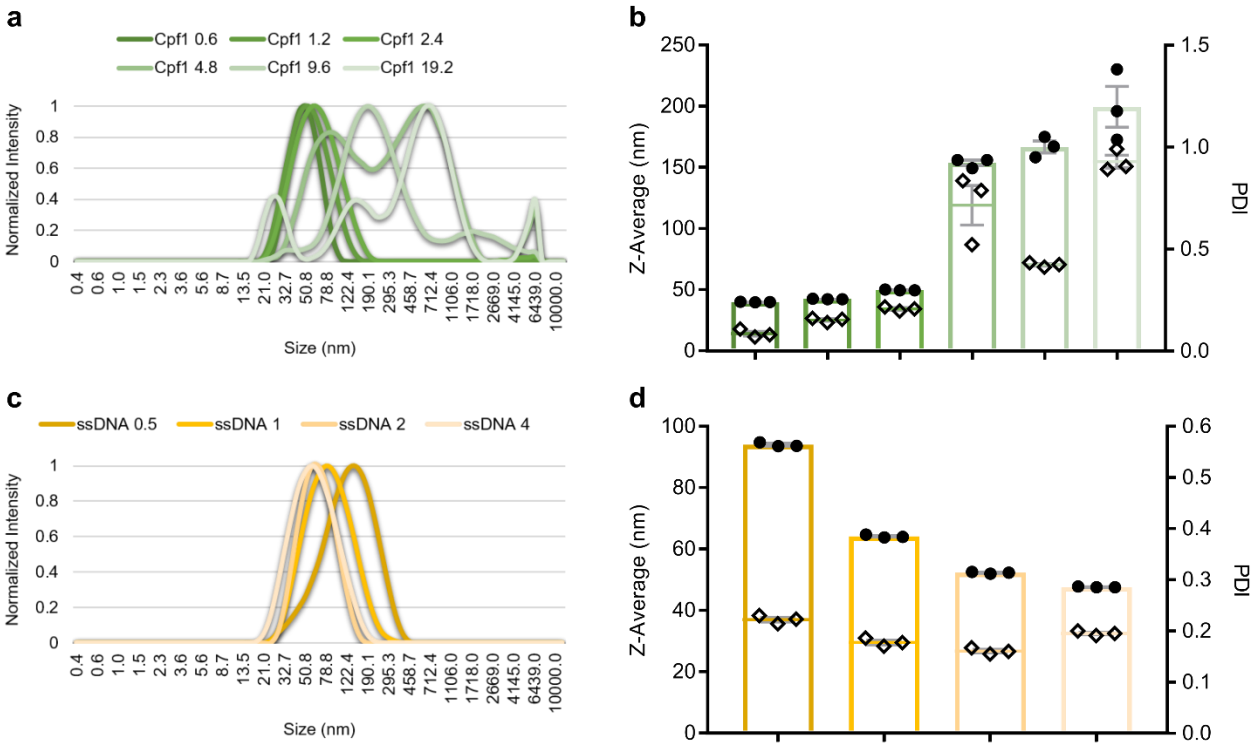
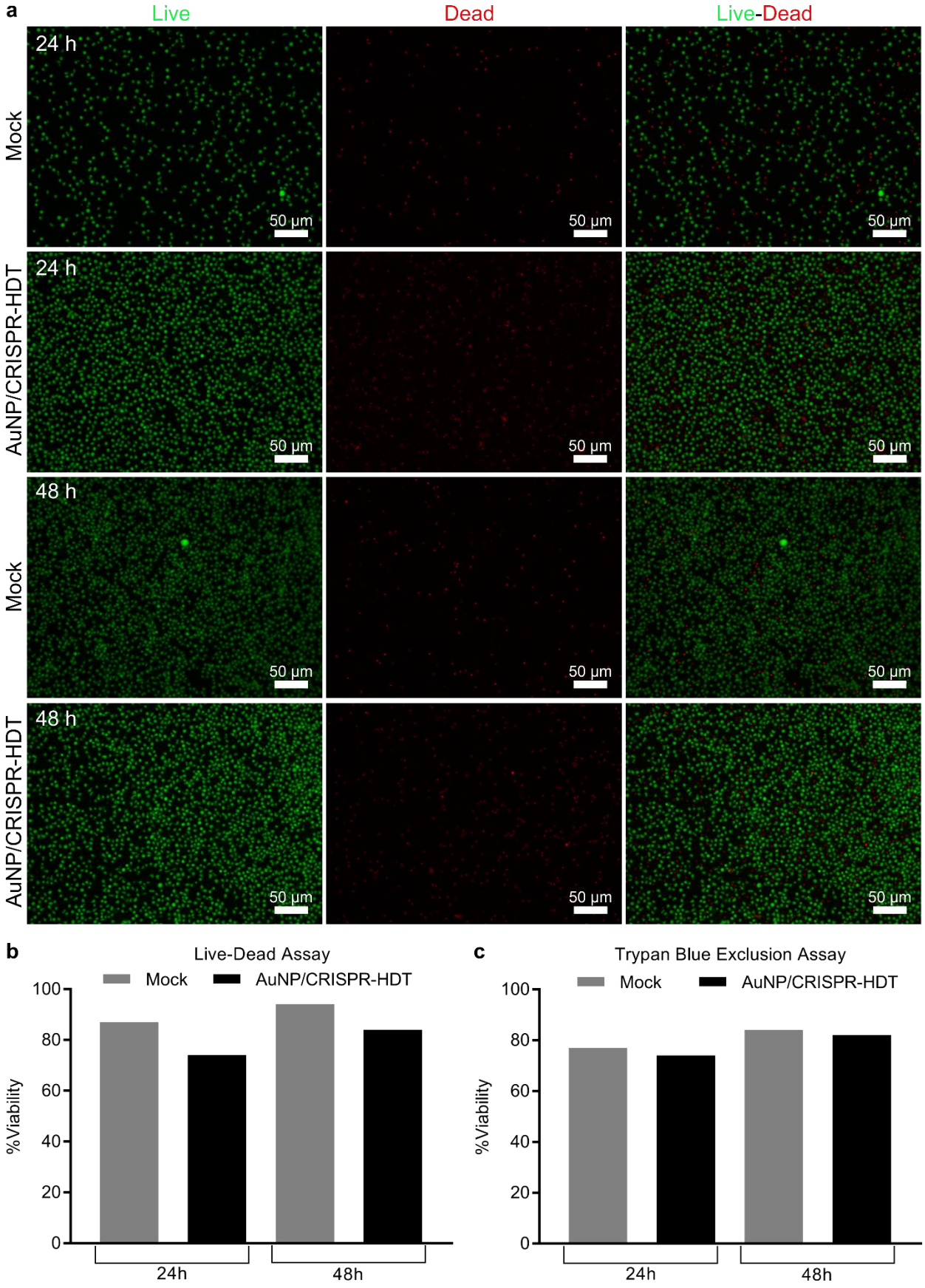
SUPPLEMENTAL FIGURES



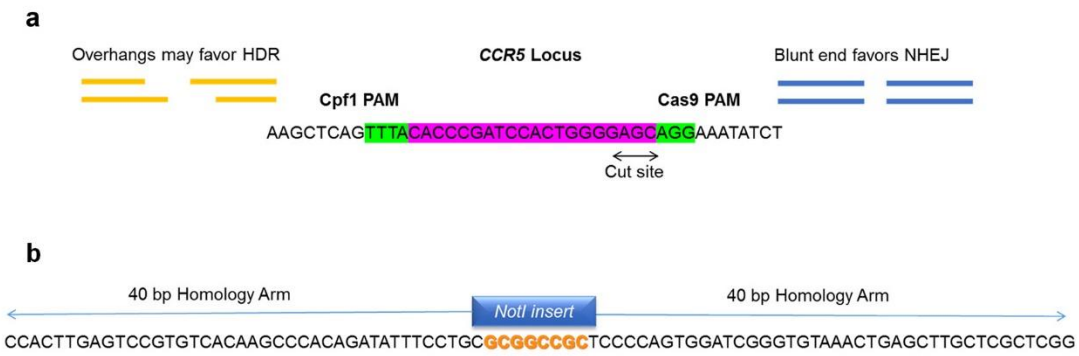
Supplementary Figure 1. Schematic representation of the AuNP/CRISPR assembly process. 1) Gold nanoparticle (AuNP) cores are synthesized and purified. 2) Clustered regularly-interspaced palindromic repeat (CRISPR) RNA guides (crRNAs) with a oligoethylene glycol (OEG) spacer arm and thiol group are conjugated to the surface of gold cores. 3) Ribonucleoprotein (RNP) complex is formed on the surface by the interaction of the CRISPR nuclease with bound crRNA. 4) RNP complexes on the AuNP cores are coated with polyethylenimine (PEI) of 2K MW. 5) A single-stranded homology-directed repair template (ssDNA) is captured on the surface of loaded AuNPs by electrostatic interaction with PEI.



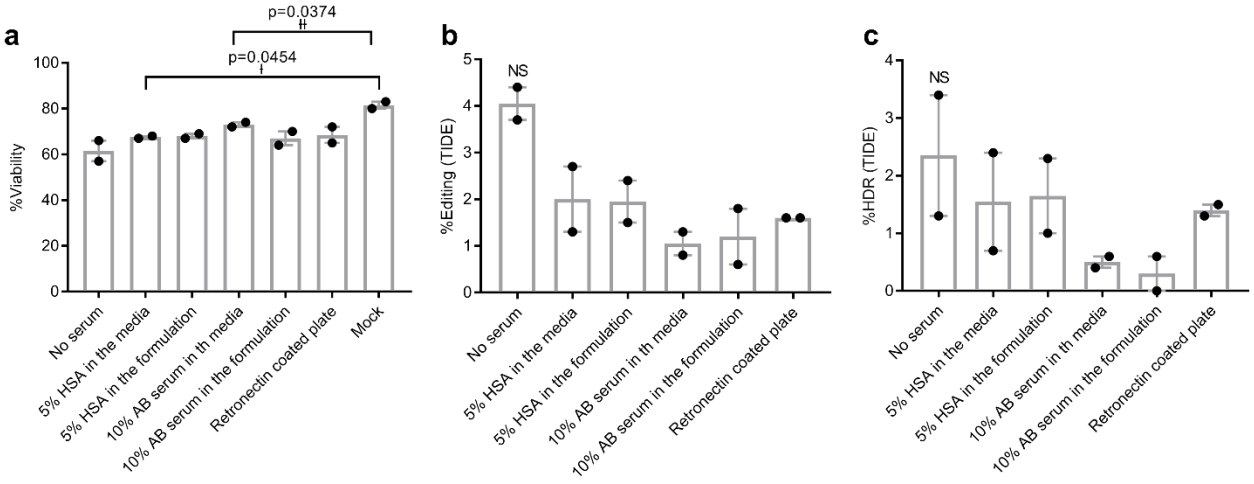
Supplementary Figure 2. Characterization of the optimal amounts of Cpf1 and ssDNA. **a,** Size analysis of nanoformulations with different AuNP/Cpf1 weight-to-weight (w/w) ratios. Measurements were taken in triplicate (n=3 technical replicates). Data have been normalized. **b,** Z-average (dots) and polydispersity index (PDI; diamonds) values in different AuNP/Cpf1 w/w ratios. AuNP/Cpf1 w/w ratio of 0.6 was found to be optimal in terms of size and PDI. Measurements were taken in triplicate (n=3 technical replicates). Error bars represent means ± standard error of the mean (SEM). **c,** Size analysis of nanoformulation in different AuNP/ssDNA w/w ratios. Measurements were taken in triplicate (n=3 technical replicates). Data have been normalized. **d,** Z-average (dots) and PDI (diamonds) values in different AuNP/ssDNA w/w ratios. The AuNP/ssDNA w/w ratio of 1 was found to be optimal in terms of size and PDI. Measurements were taken in triplicate (n=3 technical replicates). Error bars represent means ± SEM.


a
Live
Dead
Live-Dead
24 h
Mock
AuNP/CRISPR-HDT
48 h
50 μm
b
Live-Dead Assay
Mock
AuNP/CRISPR-HDT
%Viability
100
80
60
40
20
0
24h
48h
c
Trypan Blue Exclusion Assay

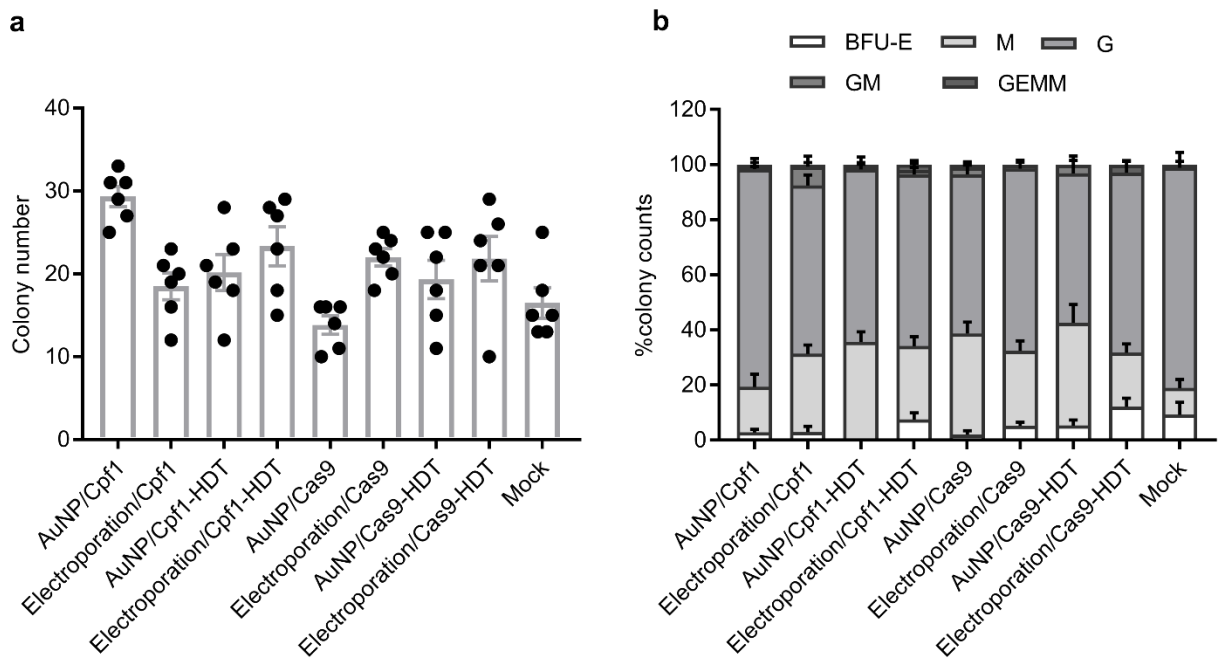
Supplementary Figure 3. AuNP/CRISPR nanoformulations are non-toxic to human HSPC. **a,b** Live-Dead viability assay results after 24h and 48 h. **c,** Cell viabilities by trypan blue dye exclusion assay. Data are representative of a single human donor different from all other donors reported in this study. Experiment has been repeated once with a different biological donor with similar results.



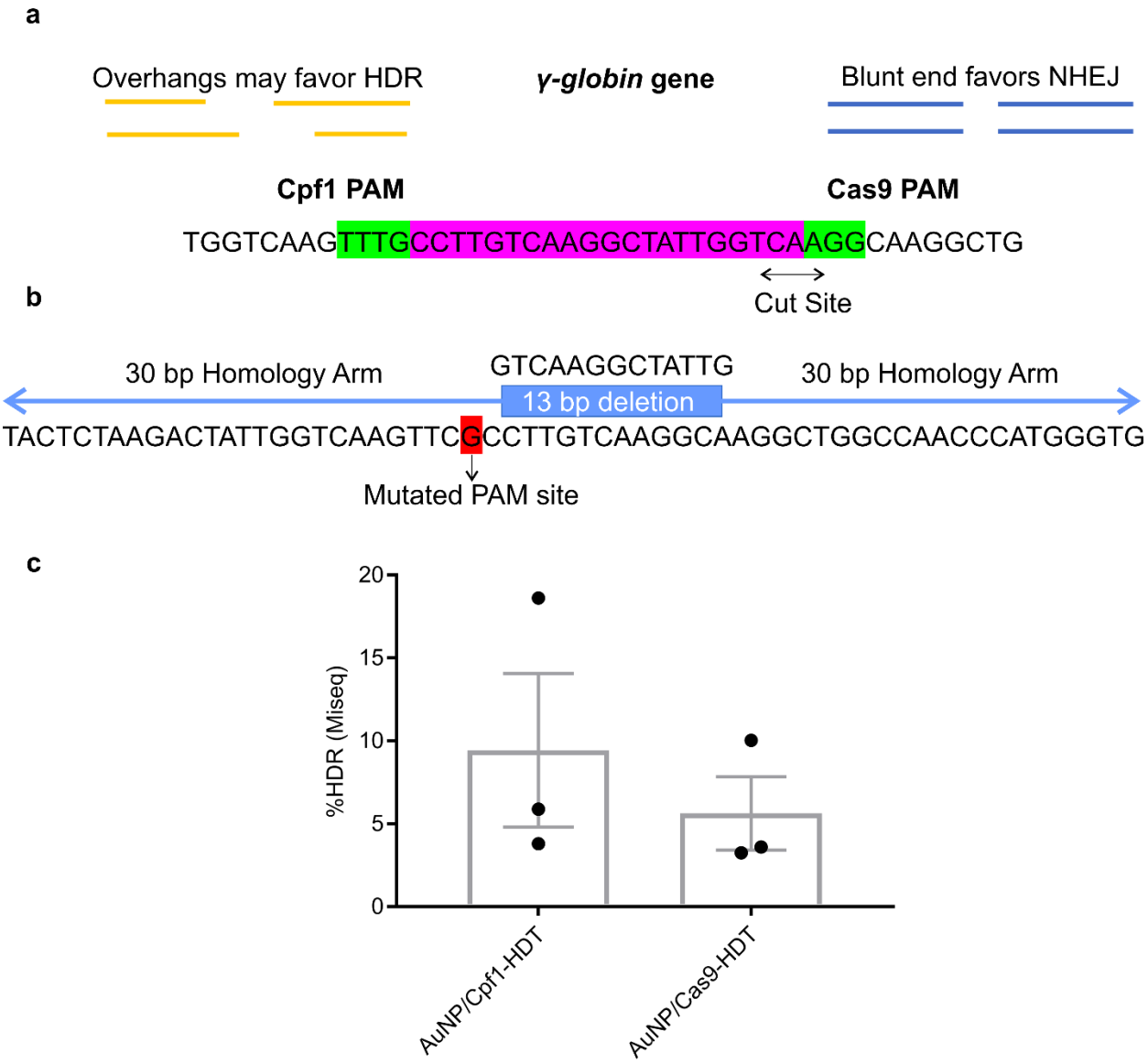
Supplementary Figure 4. Targeting locus within the human chemokine receptor 5 (*CCR5)* gene. a, The target genomic locus contained protospacer adjacent motif (PAM) sites both for Cpf1 and Cas9 with a 20 bp guide segment in the middle. **b,** Homology-directed repair template (HDT) was designed around the cut site with an 8 bp *NotI* restriction enzyme recognition sequence insert and symmetric homology arms of 40 bp in length.



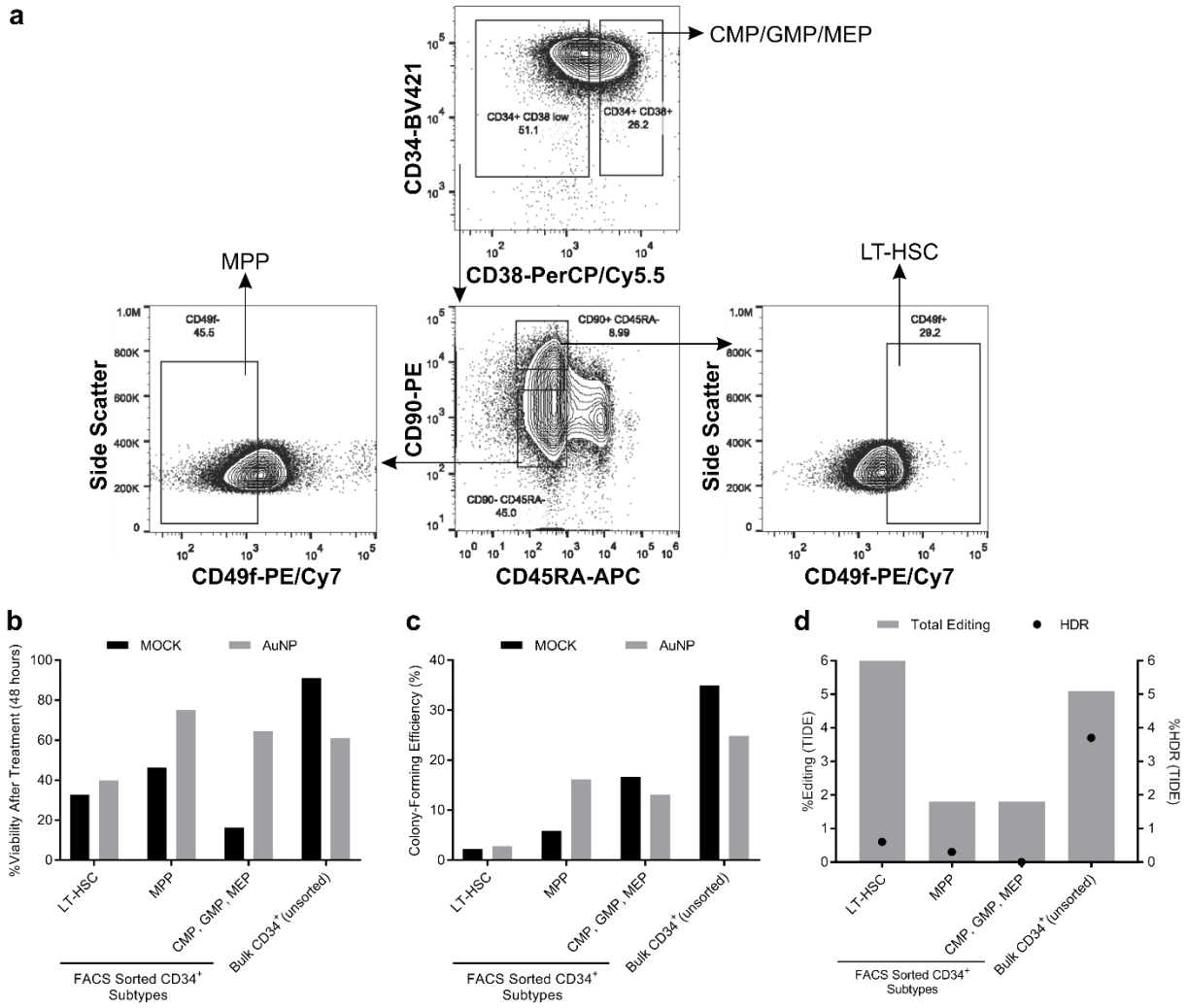
Supplementary Figure 5. Effect of serum conditions and retronectin on gene editing. a, Cell viability measured by trypan blue dye exclusion after 48 h treatment in different culture conditions. Data are means ± SEM (n=2 independent experiments). Statistical significance was determined by a two-sided t-test. †: t value=14, degrees of freedom (df)=1, effect size (d)=149; ††: t value=17, df=1, d=123.6. **b,** Total editing levels determined by tracking of indels by decomposition (TIDE) assay. Data are means ± SEM (n=2 independent experiments). Statistical significance was determined by a two-sided t-test. NS: not significant (p=0.3013; t value=1.952, df=1, d=17). **c,** Homology-directed repair (HDR) levels determined by TIDE assay. Data are means ± SEM (n=2 independent experiments). Statistical significance was determined by a two-sided t-test. NS: not significant (p=0.7463, t value=0.4211, df=1, d=4.96). Data are representative of a single human donor different from all other donors reported in this study.



Supplementary Figure 6. Colony forming cell (CFC) potential of long term progenitors. a, Replated CFC assay results showing total colony numbers. Data are means ± SEM (n=6 secondary replates from one pooled, primary experimental treatment group). **b,** CFC assay results showing the frequency of different colony morphologies. BFU-E: burst-forming unit-erythroid; M: macrophage; G: granulocyte; GM: granulocyte-macrophage; GEMM: granulocyte-erythrocyte-macrophage-monocyte. Data are means ± SEM (n=6 secondary replates from one pooled, primary experimental treatment group). Data are representative of a single human donor different from all other donors reported in this study. Experiment has been repeated once with an independent biological donor and a different target genomic locus with similar results.

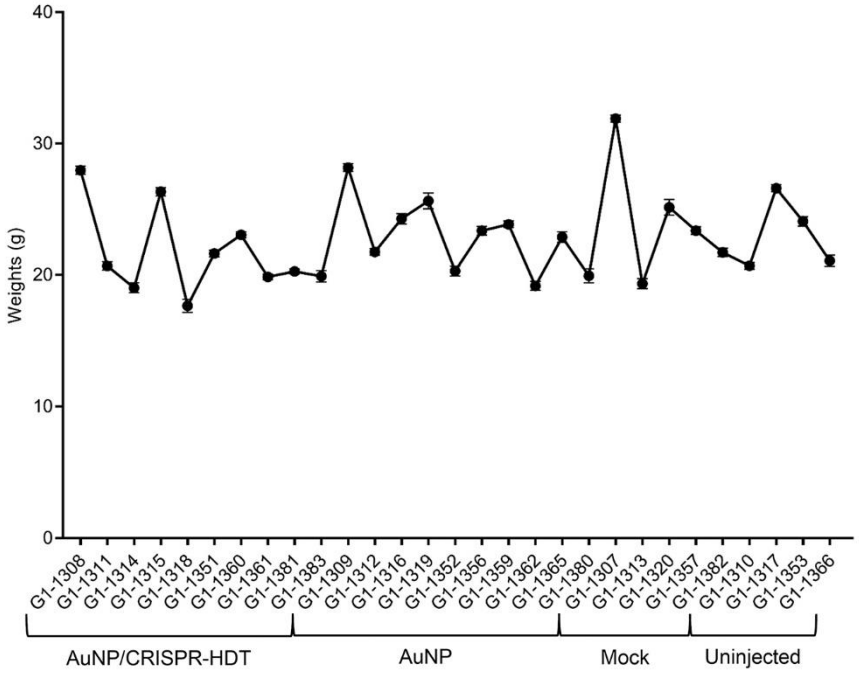


Supplementary Figure 7. Targeting locus within the human *γ-globin* promoter. a, The target locus had PAM sites both for Cpf1 and Cas9 with 21 bp guide segment in the middle. **b,** HDT was designed around the cut site to include a documented 13 bp deletion associated with hereditary persistence of fetal hemoglobin (HPFH) flanked by symmetrical homology arms of 30 bp in length. **c,** HDR results by next generation DNA sequencing (MiSeq) analysis. Data are means ± SEM (n=3 independent experiments). Data are representative of a single human donor different from all other donors reported in this study.

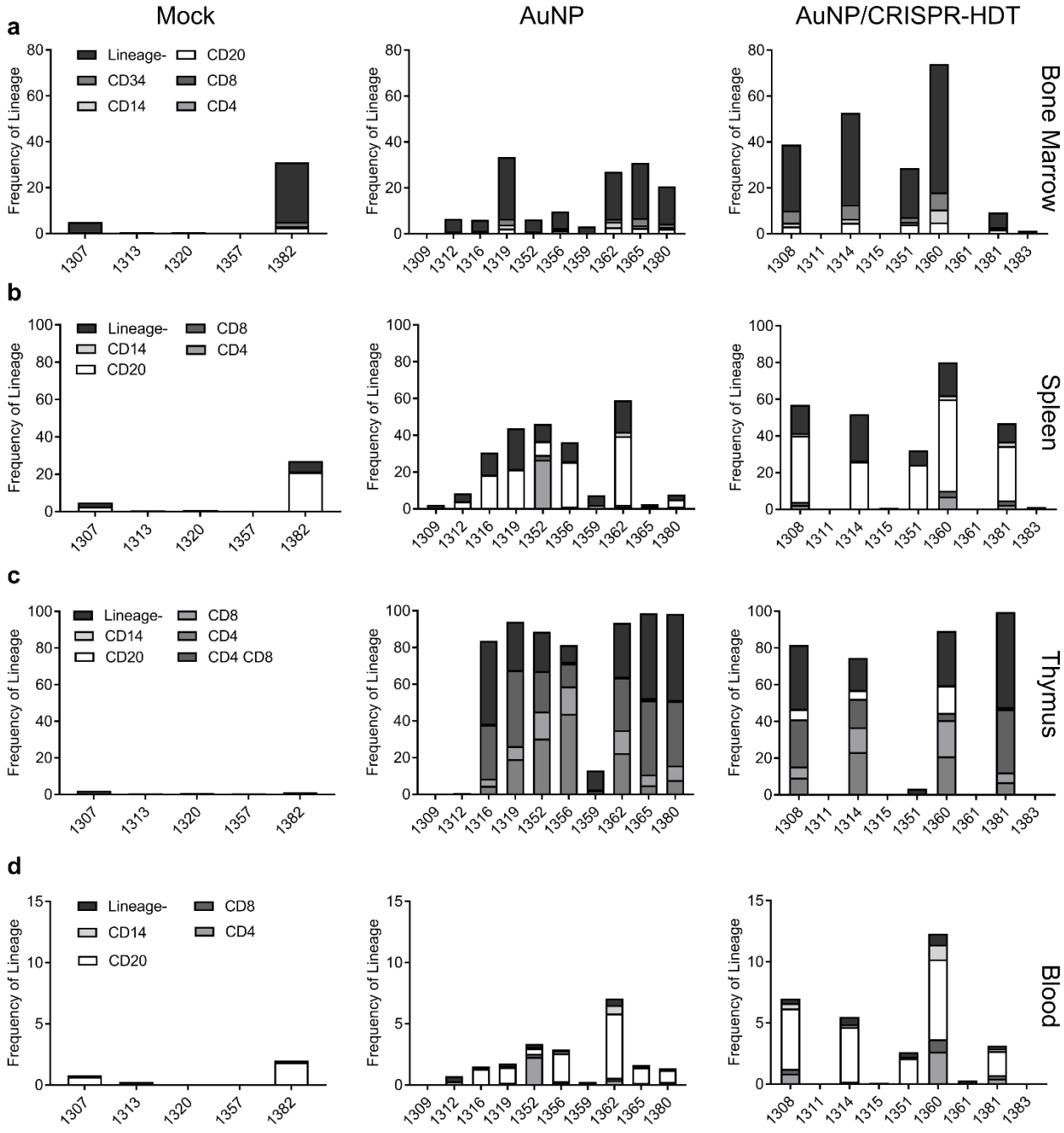


Supplementary Figure 8. Primitive CD34⁺ HSPC are successfully edited by AuNPs. a, Gating strategy for fluorescence activated cell sorting of bulk CD34⁺ hematopoietic cells from a granulocyte colony stimulating factor (G-CSF) mobilized healthy adult donor into fractions: LT-HSC (long-term hematopoietic stem cells); MPP (multipotent progenitor cells); and CMP/GMP/MEP (common myeloid progenitors/granulocyte-monocyte progenitors/megakaryocyte-erythroid progenitors). **b and c,** Graphs show viability by trypan blue dye exclusion and CFC capacity, respectively, for sorted and unsorted cells after treatment with either fully-loaded AuNPs targeting the *CCR5* locus (AuNP; gray bars), or molecular grade water (MOCK; black bars). **d,** Graph shows levels of total gene editing (primary y-axis; gray bars) and HDR (secondary y-axis; black dots) observed at the *CCR5* locus in each sorted and unsorted cell fraction after treatment. Editing was analyzed by TIDE analysis. Data are representative of a single

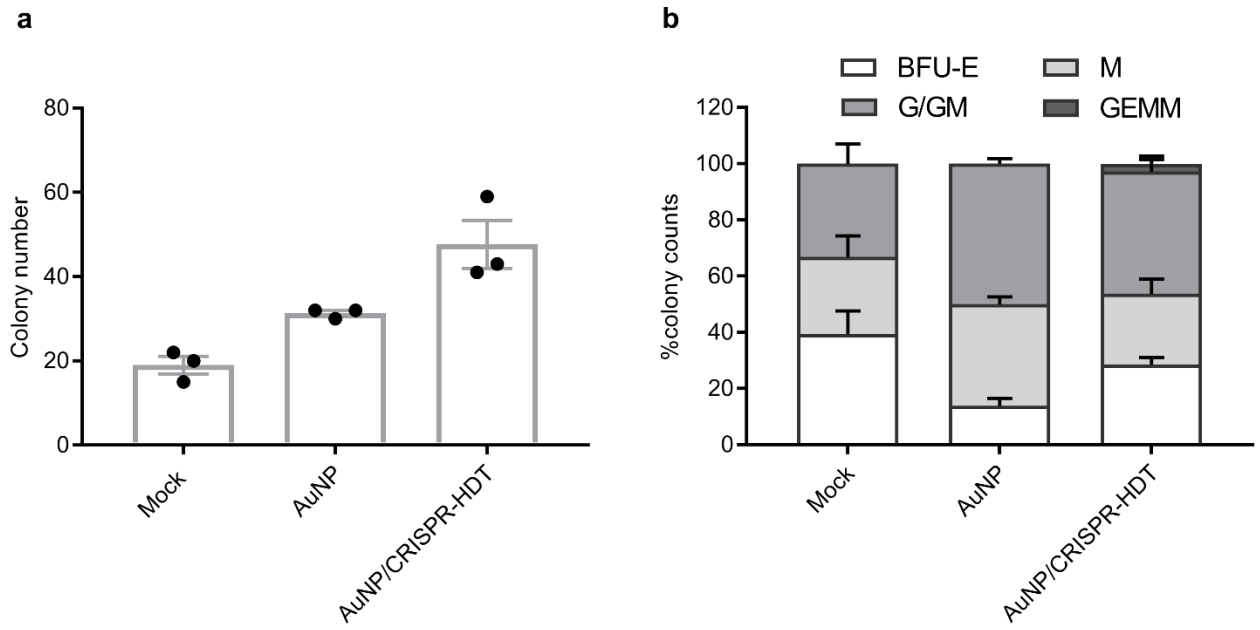
human donor different from all other donors reported in this study. Experiment was repeated once with a different donor as an independent biological replicate with similar results.



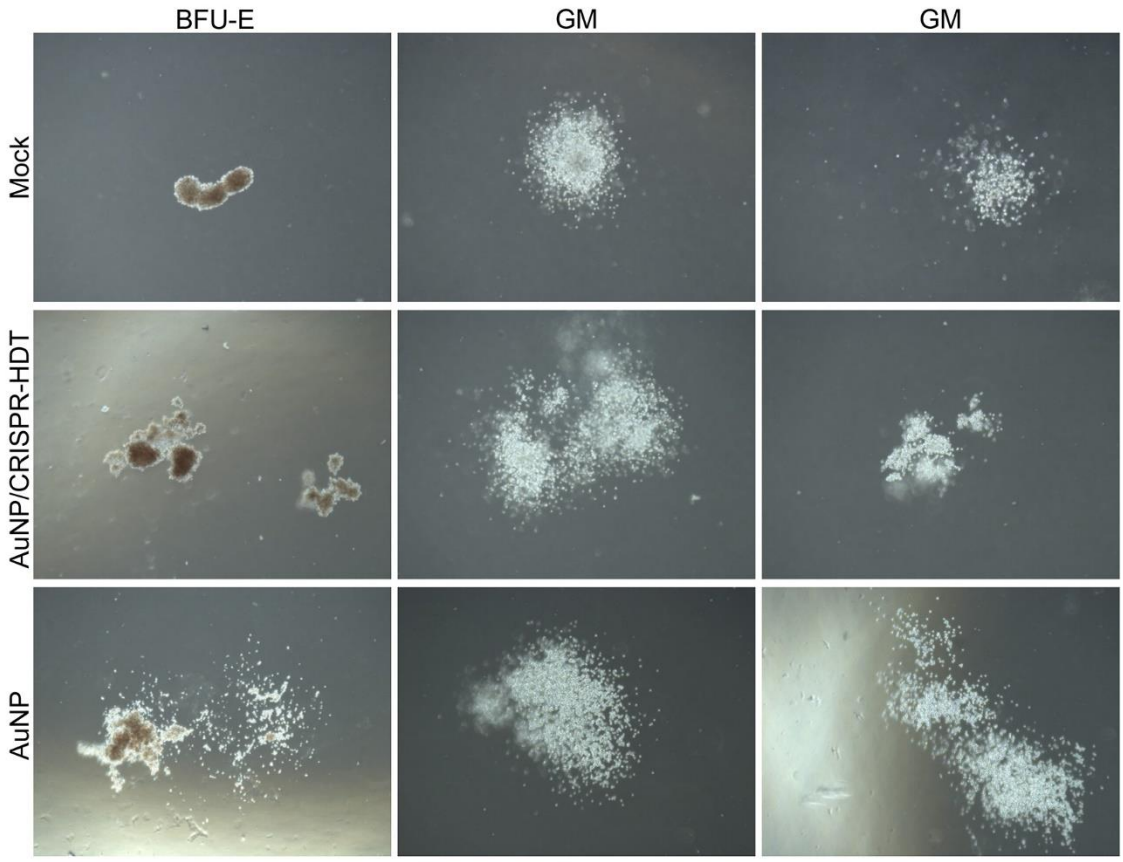
Supplementary Figure 9. Mice body weights were stable over the course of study. Data are means ± SEM (n=10 mice receiving AuNP/CRISPR-HDT treated HSPC, n=10 mice receiving AuNP-treated HSPC, n=5 mock treated mice, n=4 un-injected mice).



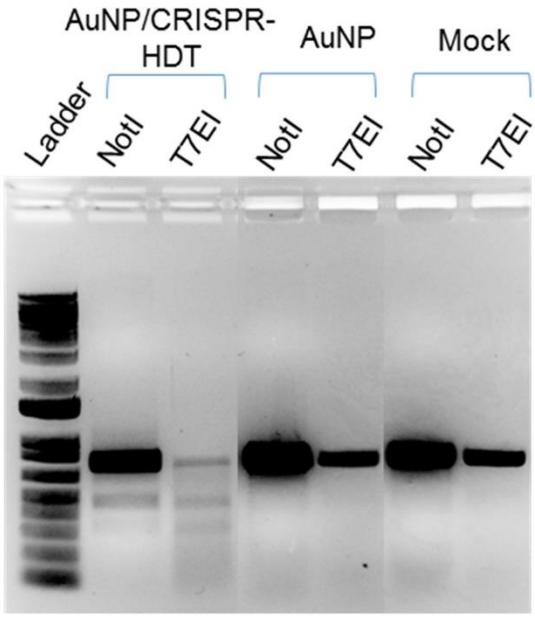
Supplementary Figure 10. Engraftment levels of AuNP/CRISPR treated cells at necropsy. Engraftment levels of human hematopoietic cells in the **a,** bone marrow, **b,** spleen, **c,** thymus and **d,** peripheral blood of immune-deficient mice at the time of necropsy as determined by flow cytometry. In all graphs the x-axis represents individual mouse identification numbers (independent biological replicates).



Supplementary Figure 11. CFC potential of AuNP/CRISPR treated cells before transplant. **a,** CFC assay showing the total colony numbers before reinfusion into sublethally irradiated mice. Data are means ± SEM (n=3 colony assay plates from one treated human HSPC sample). **b,** CFC assay results showing the percentage of different colony morphologies. Data are means ± SEM (n=3 colony assay plates from one treated human HSPC sample). Data are representative of a single human donor different from all other donors reported in this study. This experiment has been repeated twice as an independent biological replicate with similar results.



Supplementary Figure 12. Representative colony morphologies observed. Images were captured at 4x magnification of representative burst forming unit-erythroid (BFU-E), and granulocyte macrophage (GM) type colonies. This experiment has been repeated twice as an independent biological replicate with similar results.



Supplementary Figure 13. *NotI* and *T7EI* restriction enzyme digestion after treatment. Agarose gel image of PCR-amplified and enzyme-digested gDNA isolated from human HSPC treated with AuNP/CRISPR-HDT, AuNP alone or mock-treated with molecular grade water. The HSPC source for this experiment was the same as that represented in Supplementary Figure 11. This experiment has been repeated once with a similar result.

Supplementary Table 1. Sequences of crRNAs, HDT templates and primers.

Name	Sequence
CCR5/Cpf1 crRNA	5'/UAAUUUCUACUCUUGUAGAUCACCCGAUCCACUGGGGAGCA/iSp18//3ThioMC3-D/3'
CCR5/Fluorophore labeled Cpf1 crRNA	5'/5Alex488N/UAAUUUCUACUCUUGUAGAUCACCCGAUCCACUGGGGAGCA/iSp18//3ThioMC3-D/3'
CCR5/Cas9 crRNA	5'/5ThioMC6-D//iSp18/CACCCGAUCCACUGGGGAGCGUUUUAGAGCUAUGCU/3'
Cas9 tracrRNA	5'/AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUU/3'
CCR5/HDT template for target strand	5'/CCGAGCGAGCAAGCTCAGTTTACACCCGATCCACTGGGGAGCGGCCGCGCAGGAAATATCTGTGGGCTTGTGACACG GACTCAAGTGG/3'
CCR5/HDT template for non-target strand	5'/CCACTTGAGTCCGTGTCACAAGCCCACAGATATTTCCTGCGCGGCCGCTCCCCAGTGGATCGGGTGTAAACTGAGCTT GCTCGCTCGG/3'
CCR5/Fluorophore labeled HDT template	5'/5Alex660N/CCACTTGAGTCCGTGTCACAAGCCCACAGATATTTCCTGCGCGGCCGCTCCCCAGTGGATCGGGTGTAAAC TGAGCTTGCTCGCTCGG/3'
CCR5/Forward primer for TIDE analysis	5'/AGATAGTCATCTTGGGGCTGG/3'
CCR5/Reverse primer for TIDE analysis	5'/GGAGTGAAGGGAGAGTTTGTC/3'
CCR5/Forward primer for MiSeq analysis	5'/TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACATTGCCAAACGCTTCTGC/3'
CCR5/Reverse primer for MiSeq analysis	5'/GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCACAACTCTGACTGGGTC/3'
γ-globin/Cpf1 crRNA	5'/UAAUUUCUACUCUUGUAGAUCCUUGUCAAGGCUAUUGGUCA/iSp18//3ThioMC3-D/3’
γ-globin/Cas9 crRNA	/5ThioMC6-D//iSp18/CUUGUCAAGGCUAUUGGUCAGUUUUAGAGCUAUGCU
γ-globin/HDT template for non-target strand	5'/CACCCATGGGTTGGCCAGCCTTGCCTTGACAAGGCGAACTTGACCAATAGTCTTAGAGTA/3'
γ-globin/Forward primer for TIDE analysis	5'/CCTTCTTGCCATGTGCCTTG/3'
γ-globin/Reverse primer for TIDE analysis	5'/TCTATGGTGGGAGAAGAAAACTAGC/3'
γ-globin/Forward primer for MiSeq analysis	5'/TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCCCCTGGCCTCACT/3'
γ-globin/Reverse primer for MiSeq analysis	5'/GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCAATGCAAATATCTGTCTGAAACG/3'

Supplementary Table 2. Potential off target cutting sites in the human genome for Cpf1 and Cas9 guides designed against *CCR5* and *γ-globin* target sites.

	Target	Mismatches	Number of Found Targets
CCR5	Cpf1 crRNA: TTTNCACCCGATCCACTGGGGAGCA DNA: TTTACACCCGATCCACTGGGGAGCA	3	0
	Cas9 crRNA: CACCCGATCCACTGGGGAGCNGG DNA: CACCCGATCCACTGGGGAGCAGG	3	16
γ-globin	Cpf1 crRNA: TTTNCCTTGTCAAGGCTATTGGTCA DNA: TTTGCCTTGTCAAGGCTATTGGTCA	3	0
	Cas9 crRNA: CCTTGTCAAGGCTATTGGTCANGG DNA: CCTTGTCAgGGCTgTTGGTCgAGG	3	0