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Supplemental Information

Efficient polymer nanoparticle-mediated delivery of gene editing reagents into human hematopoietic stem and progenitor cells

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Figure S1

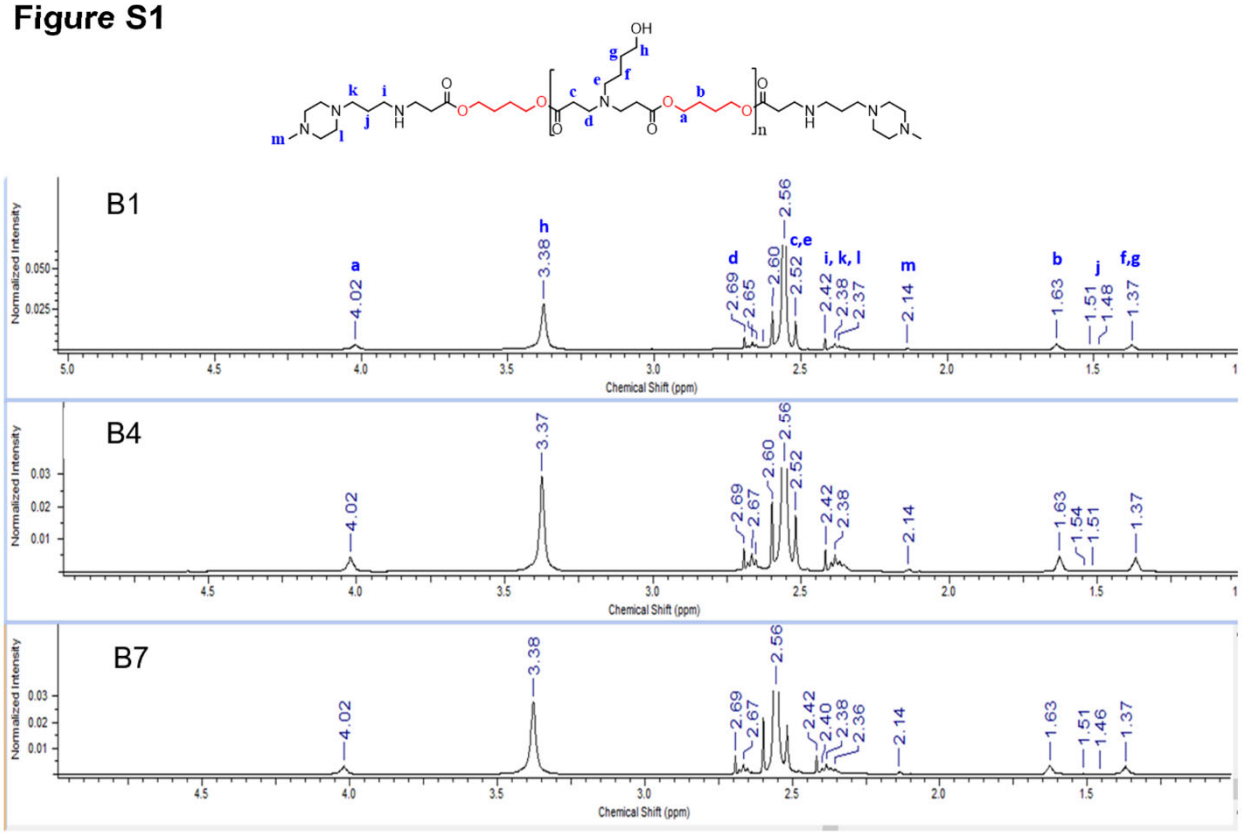

Figure S1. H1-NMR spectra of PBAE polymers

Representative H1-NMR spectra of PBAE polymers prepared with different molar ratios of 1.05:1, 1.1:1, and 1.2:1 (diacrylate to amine). Proton peaks are labeled with letters as indicated in the structure of PBAE at the very top.

Figure S2

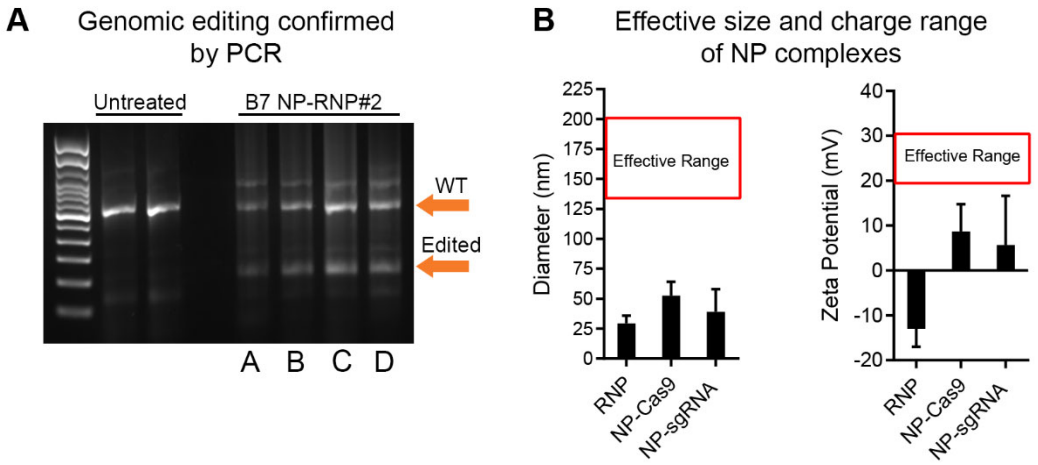

Figure S2. PCR-based quantification of genomic editing and assessment of the physiochemical properties of individual RNP components

(A) PCR analysis of untreated and NP-RNP treated human CD34^{+} cells from four independent experiments. **(B)** Size and zeta potential of the RNP without polymer, Cas9 with polymer (NP-Cas9), and sgRNA with polymer (NP-sgRNA). Statistics: (B) mean ± SD.

Figure S3

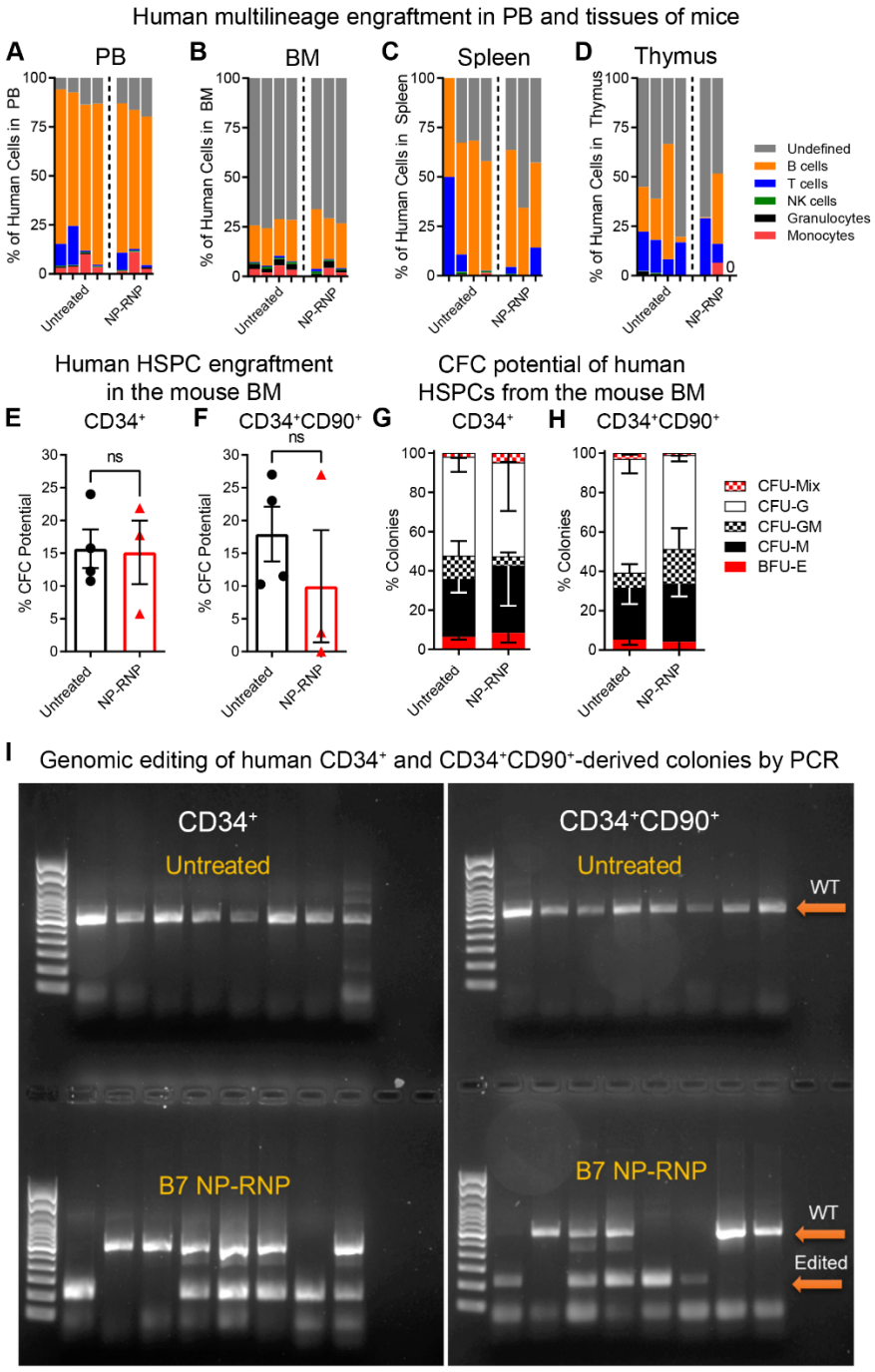

Figure S3. Multilineage differentiation potential of NP-RNP-edited human CD34$^+$ cells in NSG mice
(A-D) Human multilineage engraftment in the (A) PB, (B) BM, (C) spleen, and (D) thymus of mice 20 weeks post-transplant. **(E-F)** CFC potential of human (E) CD34$^+$ and (F) CD34$^+$CD90$^+$ cells engrafted in the murine BM. **(G-H)** Quantification of erythroid, myeloid, and erythro-myeloid colonies derived from (G) CD34$^+$ and (H) CD34$^+$CD90$^+$ cells. Abbreviations: CFU: colony-forming unit; CFU-M: CFU monocyte/macrophage; CFU-G: CFU granulocyte; CFU-GM: CFU, granulocyte/monocyte/macrophage; BFU-E: burst forming unit erythrocyte; CFU-MIX: CFU containing a mix of erythroid and myeloid cells. **(I)** PCR analysis to determine the CD33 genotype of individual human CD34$^+$- and CD34$^+$CD90$^+$-derived colonies. Statistics: (E), (F), (G), and (H) mean ± SD.

Figure S4

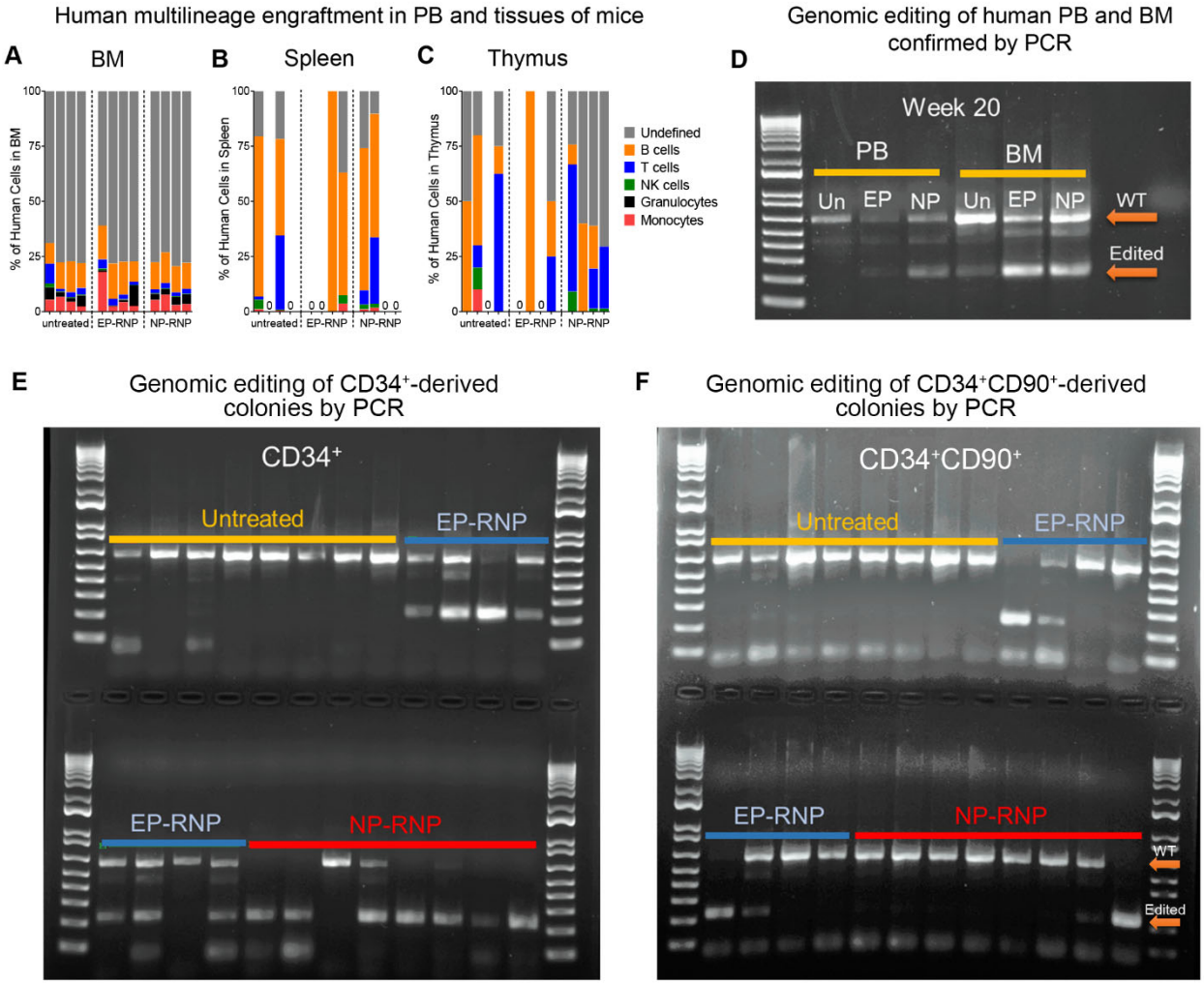

Figure S4. Multilineage differentiation potential of NP- vs EP-edited human CD34⁺ cells in NSG mice
(A-C) Human multilineage engraftment in the (A) BM, (B) spleen, and (C) thymus of mice 20 weeks post-transplant. **(D)** PCR validation of CD33 editing in human cells in the PB and BM at week 20 post-transplant. **(E)** PCR analysis to determine the CD33 genotype of individual human CD34⁺- and CD34⁺CD90⁺-derived colonies.

Table S1. Monomer molar ratios used to prepare PBAE polymer.

PBAE Polymer Batch #	**Molar ratio Diacrylate: amine**
B1	1.05:1
B2	1.05:1
B3	1.05:1
B4	1.1:1
B5	1.1:1
B6	1.1:1
B7	1.2:1
B8	1.2:1
B9	1.2:1

Table S2. List of sgRNA and primers used.

Name	Sequence
CD33 5'-sgRNA	5'-TCCATAGCCAGGGCCCCTGT-3'
CD33 3'-sgRNA	5'-GCATGTGACAGGTGAGGCAC-3'
CD33 Exon 2	F: 5'-CTGCTCACACAGGAAGCCCTG-3'
	R: 5'-CTCCCAGTACCAGGGTCCCATC-3'

Of note, sgRNAs for CD33 used in this study recognize an off-target binding site in SINGLEC22P,[1,2] potentially leading to chromosomal rearrangements that were not further investigated and are not expected to impact the outcome of this proof-of-concept work. Alternative sgRNAs without off-target recognition can be found in Borot et al.[3]

Table S3. Antibodies.

Antigen	Provider	Catalog Number	Clone Name	Lot Number	Fluorochrome	Application
CD3	BioLegend	300468	UCHT1	B312505	Brilliant Violet 650	Mouse BM, PB, Spleen, Thymus
CD4	Caprico	1120196	SK3	120A6F1	iFluor700	Mouse BM, PB, Spleen, Thymus
CD8	Caprico	1109156	SK1	109SBF1	mFluor500	Mouse BM, PB, Spleen, Thymus
CD14	Caprico	103486	26ic	34A2T2	PE-Cyanine7	Mouse BM, PB, Spleen, Thymus
CD15	Caprico	105026	FUT4/815	50AE3	PE	Mouse BM, PB, Spleen, Thymus
CD16	Caprico	101496	3GB	14A4T2	APC-Cyanine7	Mouse BM, PB, Spleen, Thymus
CD19	Caprico	102966	4G7	29AT1	PerCP-Cyanine5.5	Mouse BM, PB, Spleen, Thymus
CD20	Caprico	103766	2H7	37AT1	PerCP-Cyanine5.5	Mouse BM, PB, Spleen, Thymus
CD33	Miltenyi Biotec	130-113-345	AC104.3E3	5201201244 5210310027 5200506236	APC	Mouse BM, PB, Spleen, Thymus
CD34	BD	550761	563	0009121 0073826 8220707 9102676	PE	Human HSPCs, Mouse BM
CD38	BioLegend	303522	HIT2	B301400	PerCP/Cyanine5.5	Human HSPCs, Mouse BM
CD45 (hu)	BD Biosciences	560367	HI30	9289771	V450	Human HSPCs, Mouse BM, PB, Spleen, Thymus
CD45 (hu)	Caprico	1016146	F10-89-4	16A4F2 16A4F3	mFluor450	Human HSPCs, Mouse BM, PB, Spleen, Thymus
CD45(mu)	BD Biosciences	562383	30-F11	9351589 0013784 1018574	PE-CF594	Mouse BM, PB, Spleen, Thymus
CD45RA	BD Biosciences	561212	5H9	66660	APC-H7	Human HSPCs, Mouse BM
CD56	Caprico	101696	MY31	106A4T3	APC-Cyanine7	Mouse BM, PB, Spleen, Thymus
CD90	BioLegend	328124	5E10	B277256	PE/Cy7	Human HSPCs, Mouse BM
CD90	BD Biosciences	561971	5E10	5247689	APC	Human HSPCs, Mouse BM

SUPPLEMENTAL REFERENCES

1. Shaw, B.C., and Estus, S. (2021). Pseudogene-mediated gene conversion after CRISPR-Cas9 editing demonstrated by partial CD33 conversion with SIGLEC22P. *CRISPR J* **4**: 699-709.
2. Kim, M.Y., Yu, K.R., Kenderian, S.S., Ruella, M., Chen, S., Shin, T.H., Aljanahi, A.A., Schreeder, D., Klichinsky, M., Shestova, O*., et al.* (2018). Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. *Cell* **173**: 1439-1453 e1419.
3. Borot, F., Wang, H., Ma, Y., Jafarov, T., Raza, A., Ali, A.M., and Mukherjee, S. (2019). Gene-edited stem cells enable CD33-directed immune therapy for myeloid malignancies. *Proc Natl Acad Sci U S A* **116**: 11978-11987.