Supplementary Figure Legends

Supplementary Figure 1. Allele plots of therapeutic editing at IVS1-110G>A and IVS2-654C>T alleles.
Consensus splice acceptor and donor sites[1] are illustrated above the aberrant splice sites. a) Enumeration of indel type following sgIVS1-110A SpCas9 RNP editing of $\beta^{+}\beta^{0}_{\#1}$ aligned to IVS1-110A reference. b) Enumeration of indel type following crIVS2-654T LbCas12a RNP editing of $\beta^{+}\beta^{0}_{\#4}$ aligned to IVS2-654T/rs1609812-T reference.

Supplementary Figure 2. Hemoglobin HPLC traces following therapeutic editing at IVS1-110G>A and IVS2-654C>T alleles.
a) Top shows hemoglobin HPLC traces in erythroid progeny after sgAAVS1 SpCas9 RNP editing and bottom after sgIVS1-110A SpCas9 RNP editing. b) Top shows hemoglobin HPLC traces in erythroid progeny after crAAVS1 LbCas12a RNP editing and bottom after crIVS2-654T LbCas12a RNP editing. HbA2, HbE, and HbLepore co-migrate.

Supplementary Figure 3. Edited hematopoietic populations.
a) Representative gating strategy indicating live singlets with CD34+ CD38+ (HPC) and CD34+ CD38- CD90+ CD45RA- (HSC) immunophenotype for sorting populations following editing.
b) Imaging flow cytometry to quantify the circularity of enucleated erythroid progeny following sgAAVS1 or sgIVS1-110A SpCas9 RNP editing.

Supplementary Tables

Supplementary Table 1. β-thalassemia patient HSPC donor genotypes.

Donor ID	β-globin mutation #1	β-globin mutation #2
$\beta^{+}\beta^{0}_{\#1}$	IVS1-110 G>A	Codon 39 (C>T; CAG>TAG)
$\beta^{+}\beta^{0}_{\#2}$	IVS1-110 G>A	Codon 39 (C>T; CAG>TAG)
$\beta^{+}\beta^{0}_{\#3}$	IVS1-110 G>A	Codon 5 (-CT; CCT->C--)
$\beta^{+}\beta^{+}$	IVS1-110 G>A	IVS1-110 G>A
$\beta^{+}\beta^{Lepore}$	IVS1-110 G>A	Lepore-Boston-Washington deletion
$\beta^{+}\beta^{0}_{\#4}$	IVS2-654 C>T	Codon 43 (G>T; GAG>TAG)
$\beta^{+}\beta^{0}_{\#5}$	IVS2-654 C>T	Codon 41/42 (--CTTT)
$\beta^{+}\beta^{E}_{\#1}$	IVS2-654 C>T	Codon 26 (G->A; GAG->AAG; HbE Glu26Lys)
$\beta^{+}\beta^{E}_{\#2}$	IVS2-654 C>T	Codon 26 (G->A; GAG->AAG; HbE Glu26Lys)

Supplementary Table 2. Linkage between IVS2-654C>T and rs1609812-T.

Singletons (no.)	IVS2-654C>T	Genotype at rs1609812
3	Homozygous	T / T
19	Heterozygous	T / T
11	Heterozygous	T / C

Family	Relationship	IVS2-654C>T	Other mutation	Genotype at rs1609812
#1	Father	Heterozygous	No	**T** / T
	Mother	No	Codons 41/42	T / T
	Daughter	Heterozygous	Codons 41/42	**T** / T
#2	Father	No	Codon 43	
	Mother	Heterozygous	No	**T** / T
	Son	Heterozygous	Codon 43	**T** / C
#3	Mother	Heterozygous	No	**T** / T
	Offspring	Heterozygous	No	**T** / C

#4	Father	No	Codons 41/42	T / C
	Mother	Heterozygous	No	**T** / C
	Offspring	No	No	C / C
#5	Father	Heterozygous	No	**T** / C
	Mother	No	Codon 26	T / C
	Offspring	No	Codon 26	C / C
#6	Sibling #1	Heterozygous	No	**T** / C
	Sibling #2	No	No	T / T
	Sibling #3	Heterozygous	No	**T** / T
#7	Father	No	Codons 41/42	T / C
	Mother	Heterozygous	No	**T** / C
	Offspring #1	No	No	C / C
	Offspring #2	No	Codons 41/42	T / C

Supplementary Table 3. Genomic mismatches to sgIVS1-110A and crIVS2-654T.

See attached.

Supplementary Table 4. Aberrant splice site targeting in the thalassemias and other blood disorders.

See attached.

Supplementary Table 5. Oligonucleotides used in this study.

Primers for Sanger analysis.

IVS1-110_Sanger_F	TGGATGAAGTTGGTGGTGAG
IVS1-110_Sanger_R	AAACATCAAGCGTCCCATAGA
IVS2-654_Sanger_F	TGACCAAATCAGGGTAATTTTGC
IVS2-654_Sanger_R	CAGGAGCTGTGGGAGGAAGA
AAVS1_1F	CACCTTATATTCCCAGGGCCG
AAVS1_1R	CCTAGGACGCACCATTCTCAC

AAVS1_2F	ATTGGGTCTAACCCCCACCT
AAVS1_2R	TCAGTGAAACGCACCAGACA

Primers for deep sequencing.

IVS1-110_deep_3F-HBBsp	CTCCTGAGGAGAAGTCTGCCGTTAC
IVS1-110_deep_3R-HBBsp	GCAGCTCACTCAGTGTGGC
IVS1-110_deep_1F	TGGGCAGGTTGGTATCAAGG
IVS1-110_deep_1R	GCACTTTCTTGCCATGAGCC
IVS2-654_deep_2F	CTCTTTCTTTCAGGGCAATAATGATAC
IVS2-654_deep_2R	CCAGCCTTATCCCAACCATAAA

Primers for RT-PCR.

HBB-exon1_F	GCAAGGTGAACGTGGATGAAGTT
HBB-exon2_R	GGACAGATCCCCAAAGGACTCAA
HBB-S_qPCR	TGAGGAGAAGTCTGCCGTTAC
HBB_exon3_R	CACCAGCCACCACTTTCTGA

Primers for RT-qPCR.

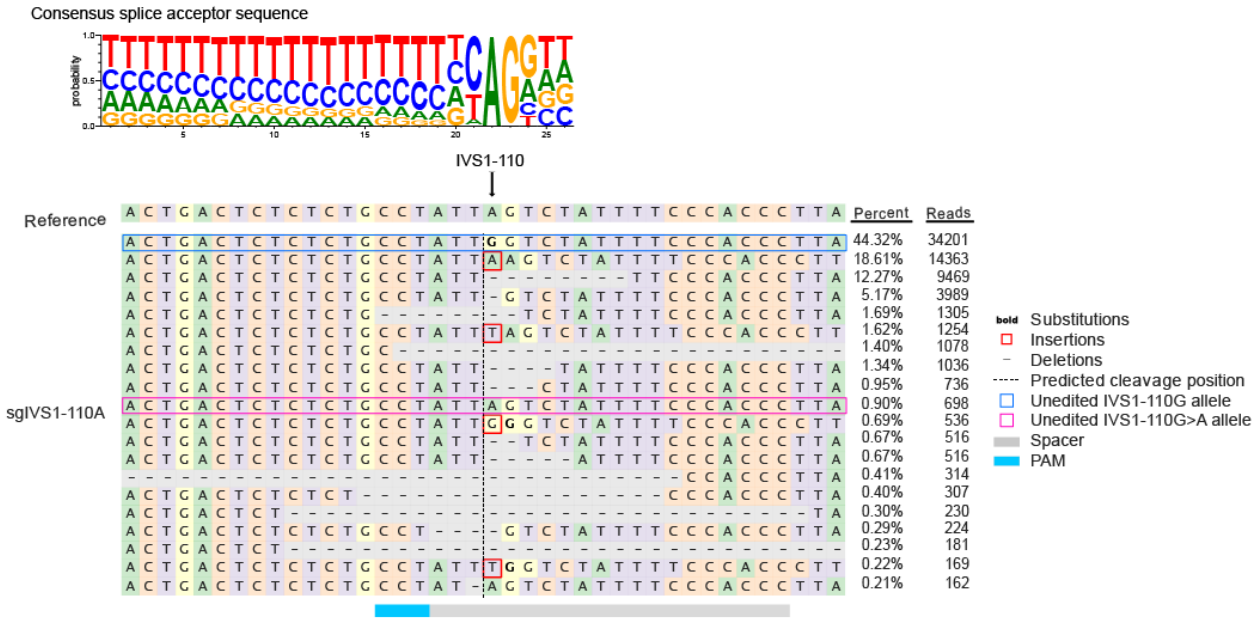
HBB-S_qPCR	TGAGGAGAAGTCTGCCGTTAC
HBB-AS_qPCR	ACCACCAGCAGCCTGCCCA
HBB_e2-e3	TTCAGGCTCCTGGGCAAC
R_HBB_exon3	CACCAGCCACCACTTTCTGA
HBA-S_qPCR	GCCCTGGAGAGGATGTTC
HBA-A_qPCR	TTCTTGCCGTGGCCCTTA
HBG-S_qPCR	GGTTATCAATAAGCTCCTAGTCC
HBG-AS_qPCR	ACAACCAGGAGCCTTCCCA
HBD_RT93_e1_F	GAGGAGAAGACTGCTGTCAATG
HBD_RT93_e2_R	AGGGTAGACCACCAGTAATCTG

Supplementary References

1. Ma, S. L. *et al.* Whole Exome Sequencing Reveals Novel PHEX Splice Site Mutations in Patients with Hypophosphatemic Rickets. 1–12 (2015). doi:10.1371/journal.pone.0130729

Supplementary Figure 1

a



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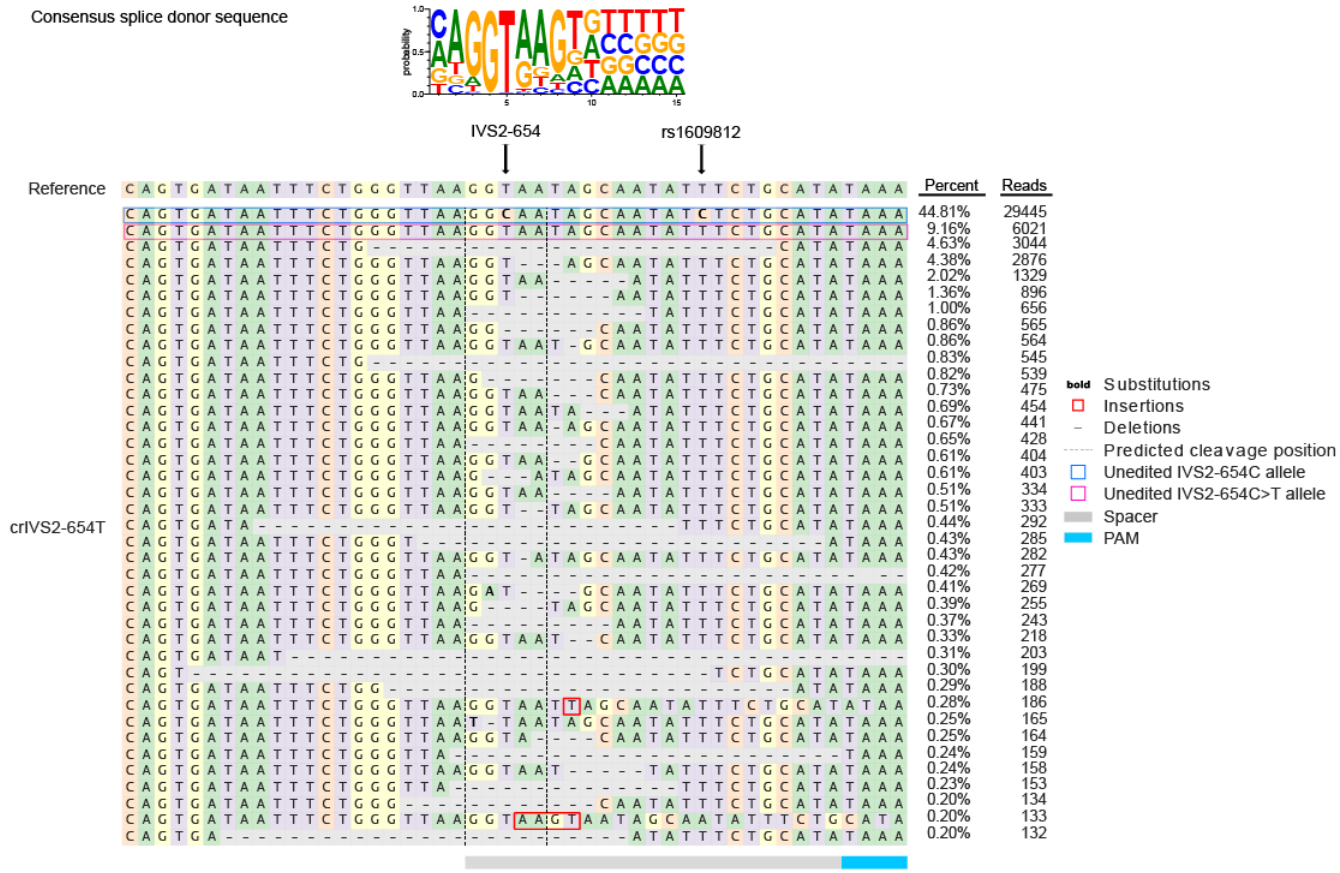
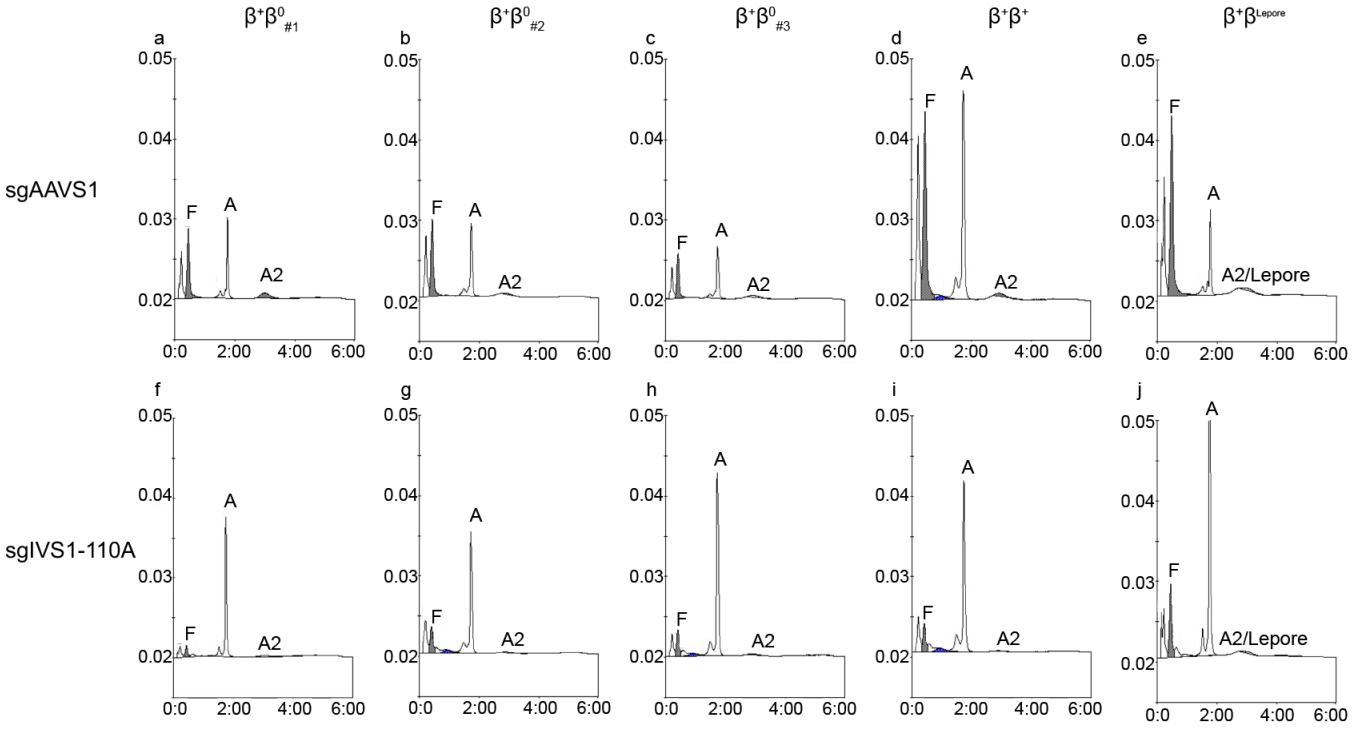


Supplementary Figure 2

a



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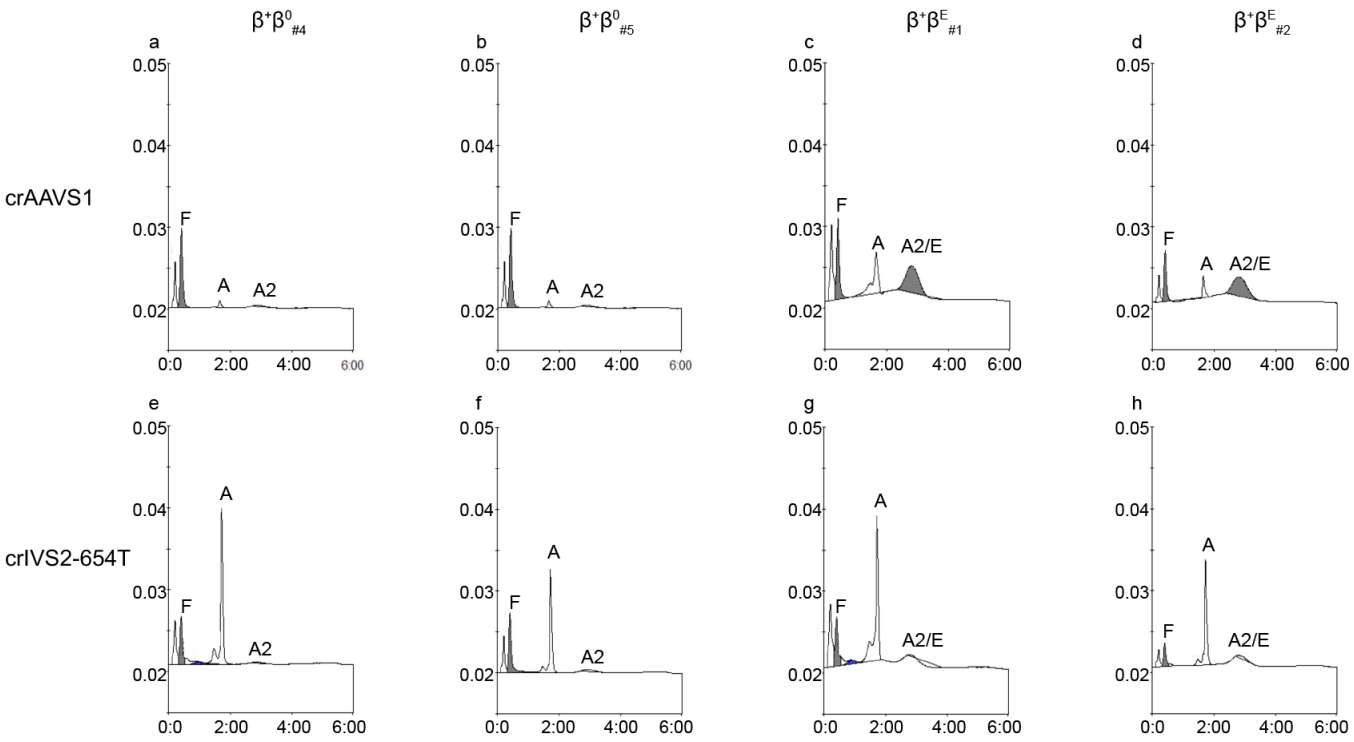
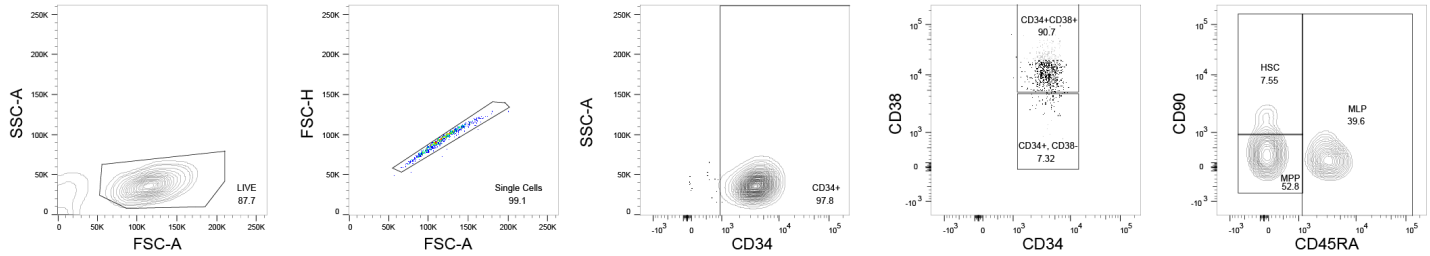


Supplementary Figure 3

a



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