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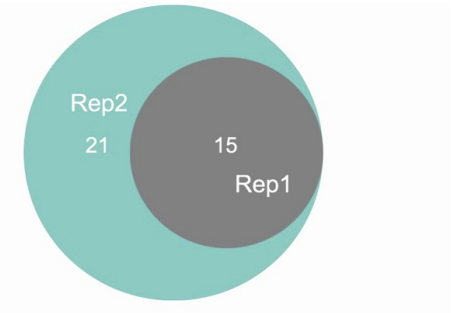
Supplemental Information

Prediction and validation of hematopoietic stem and progenitor cell off-target editing in transplanted rhesus macaques

Aisha A. AlJanahi, Cicera R. Lazzarotto, Shirley Chen, Tae-Hoon Shin, Stefan Cordes, Xing Fan, Isabel Jabara, Yifan Zhou, David J. Young, Byung-Chul Lee, Kyung-Rok Yu, Yuesheng Li, Bradley Toms, Ilker Tunc, So Gun Hong, Lauren L. Truitt, Julia Klermund, Geoffroy Andrieux, Miriam Y. Kim, Toni Cathomen, Saar Gill, Shengdar Q. Tsai, and Cynthia E. Dunbar

Figure S1. Overlap between CIRCLE-seq technical replicates for *TET2* and *CD33* gRNAs.

A.



B.

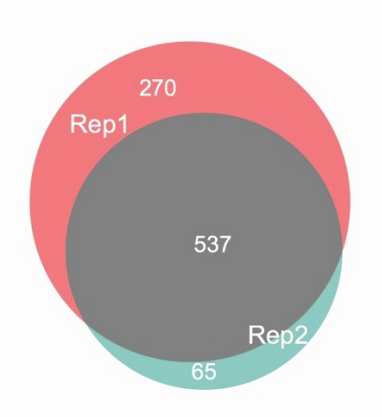


Figure S1. A) Overlap between the off-target sites predicted by the *TET2* CIRCLE-Seq technical replicates performed on animal ZL26. B) Overlap between the off-target sites predicted by the *CD33* CIRCLE-seq technical replicates performed on animal ZJ52.

Figure S2. Schematic of autologous HSPC editing in the rhesus macaque model.

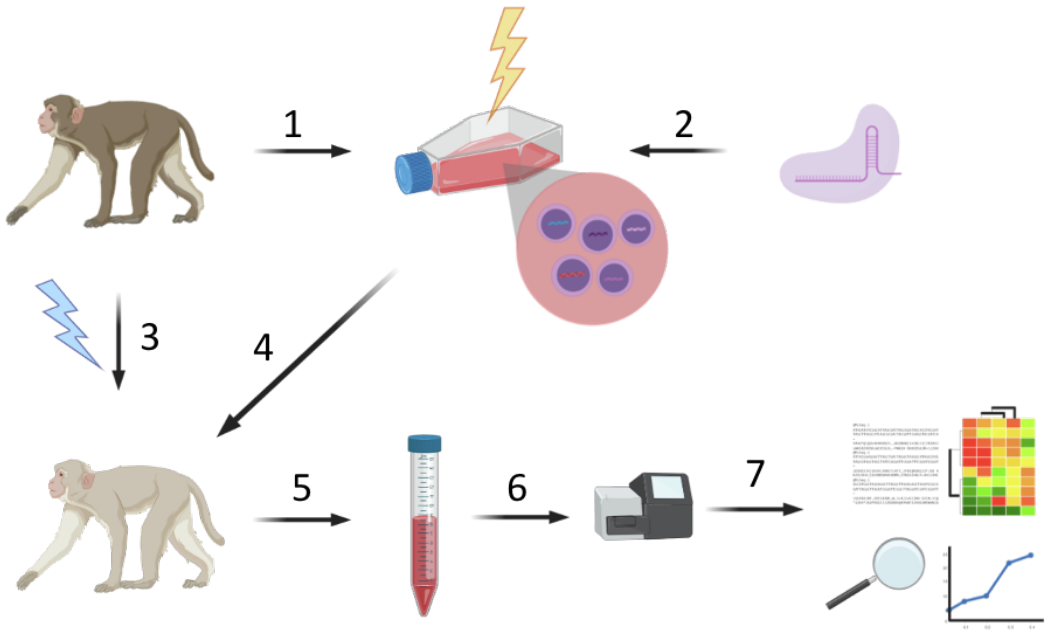


Figure S2. CD34+ cells mobilized into the blood with G-CSF and plerixafor are collected from the animal via apheresis and purified via immunoabsorption. 2- the collected cells are electroporated with Cas9+gRNA RNP complexes to create the infusion product. 3- the animal is given total body irradiation (500 rads X 2) to empty bone marrow niches facilitating engraftment of the edited HSPCs. 4- the edited cells are transplanted into the animal via intravenous infusion. 5- the infused HSPCs home to the bone marrow and begin producing daughter cells that differentiate and are released into the peripheral blood, which can be sampled and lineage-purified at different time points. 6- DNA from cells collected at different time points can be sequenced to search for editing at on-target and off-target sites. 7- sequencing output is analyzed to look for valid on-target and off-target editing.

Figure S3. Selection of the *CD33* off-target sites for the custom AmpliSeq HD sequencing panel.

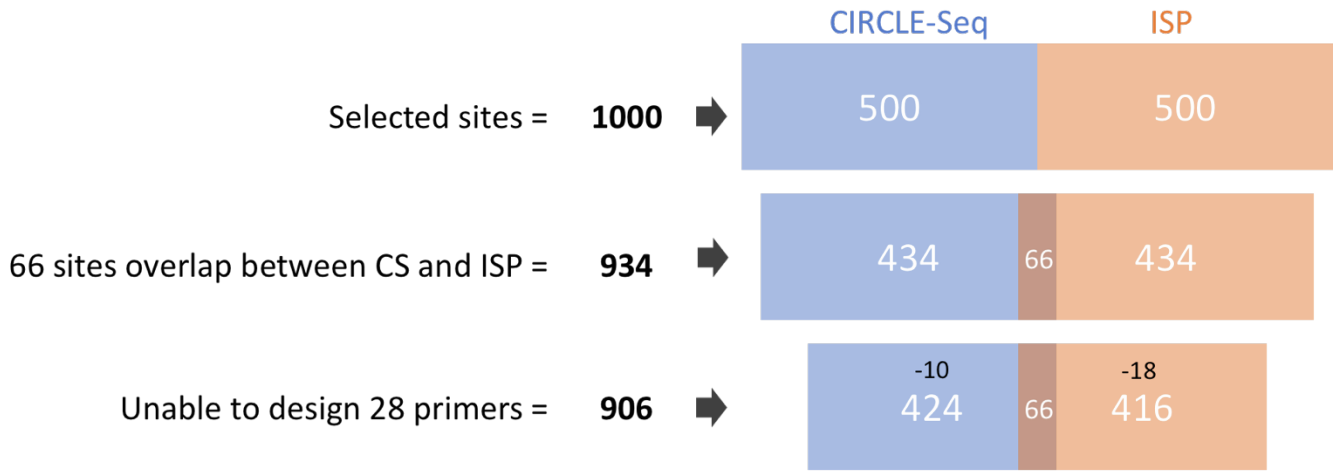


Figure S3. Distribution of the *CD33* off-target sites selected for AmpliSeq HD sequencing.

Figure S4. Editing patterns in the 19 *bona fide* CD33 off-target sites.

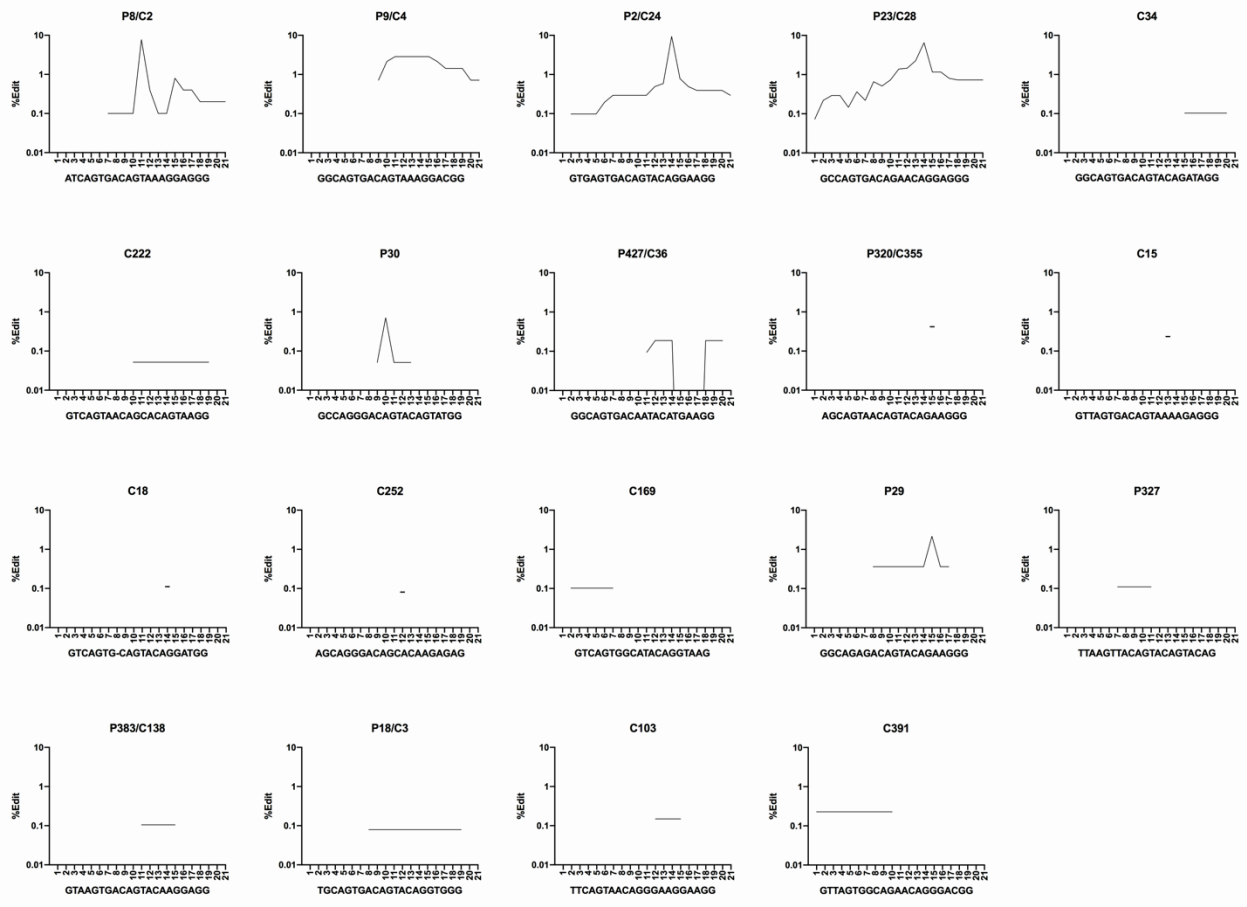


Figure S4. Percent editing in each position of the 21 bps complimentary to the *CD33* gRNA shown on a log scale. The editing percent is shown only for the highest edited sample for each of the 19 edited off-target sites. If the INDEL was an insertion, then the nucleotide previous to the insertion site is represented. If the INDEL was a deletion, then all deleted nucleotides are represented. All 19 sites showed no detectable variation from the reference in the un-edited samples.

Figure S5. Average % edit for each *bona fide* off-target site in the 40 sequenced samples.

ISP1,CS1 = Target
Found by CS and ISP
Found by CS only
Found by ISP only

CS rank	ISP rank	Highest % edit in any timepoint	average edit in the 40 sequenced samples
1	1	95.632	26.234975
2	8	8.601	1.486675
4	9	2.856	0.16685
24	2	9.42	1.439075
28	23	6.78	1.098725
34		0.103	0.00264103
222		0.052	0.0013
	30	0.708	0.077075
36	427	0.375	0.0181
355	320	0.417	0.028475
15		0.235	0.009025
18		0.111	0.002775
252		0.081	0.002025
169		0.102	0.00255
1567	29	2.166	0.15085
	327	0.11	0.00275
138	383	0.105	0.00445
3	18	0.079	0.001975
103		0.149	0.0067
391		0.228	0.0057

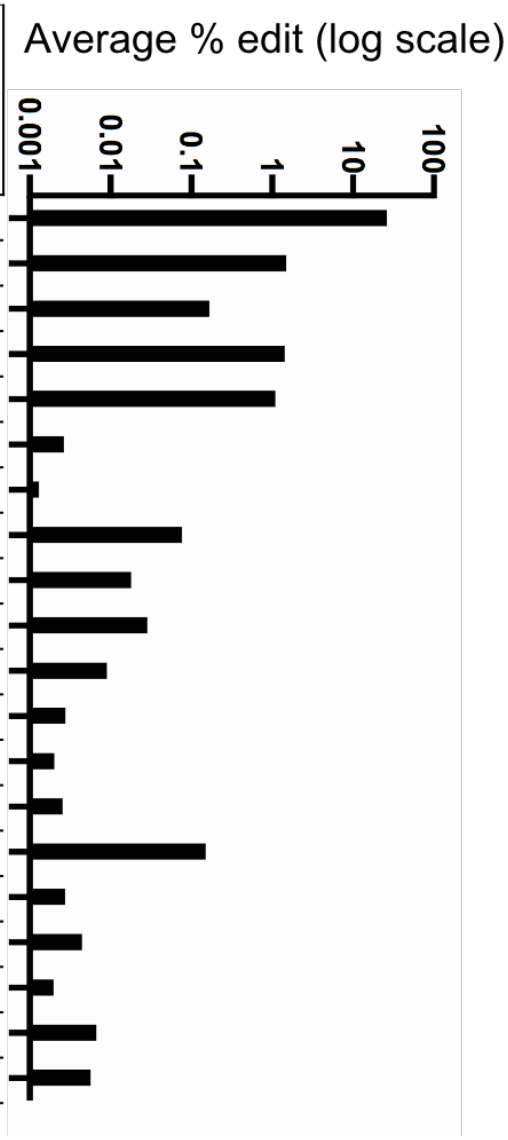


Figure S6. Rank of validated off-target sites via CIRCLE-Seq and ISP.

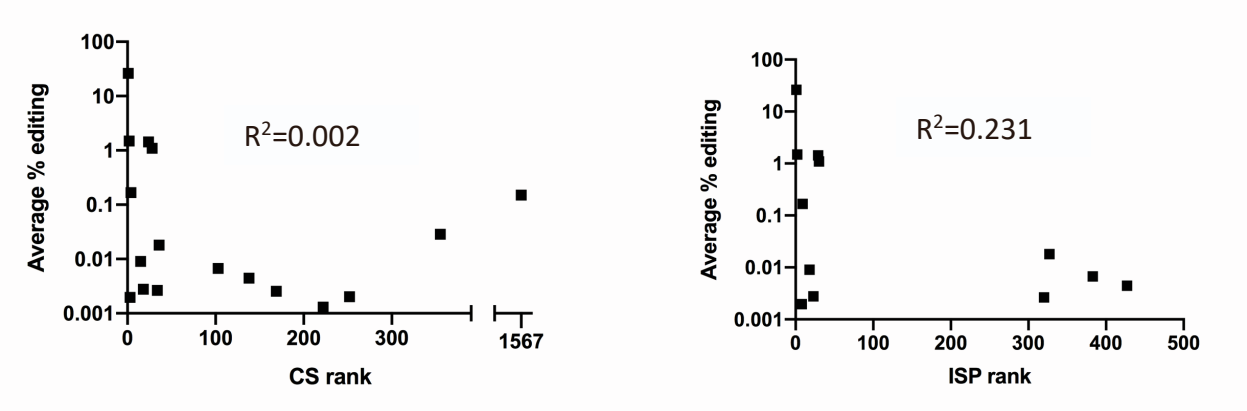


Figure S6. Dot plots showing the rank of each of the valid off-target sites predicted by each method on the X axis, and the average percent edit (log scale) for each of *the* off-target sites in all 40 sequenced samples on the Y axis.

Table S1. Top 20 edited reads in the infusion product of ZL52 at site ZJ52-CS2/ISP8.

ZJ52-CS2/ISP8

overall edit rate = 5.1

	Aligned sequence	%Reads
Unedited	**CGCCTCTTCTGTTTCCCTCCTTTACTGTCACTGATTGCAC**	**92.958**
1	CGCCTCTTCTGTTTCCCTCCTTTACTGTCACTGATTGCAC	2.438
2	CGCCTCTTCTGTTTCCCTCC-TTACTGTCACTGATTGCAC	0.557
3	CGCCTCTTCTGTTTCCCTC----CTGTCACTGATTGCAC	0.146
4	CGCCTCTTCTGTTTCCCTCC--TACTGTCACTGATTGCAC	0.124
5	CGCCTCTTCTGTTTCCCT----CTGTCACTGATTGCAC	0.122
6	CGCCTCTTCTGTTTCC----CTGTCACTGATTGCAC	0.082
7	CGCCTCTTCTGTTTCCCT-CTTTACTGTCACTGATTGCAC	0.071
8	CGCCTCTTCTG---------TTTACTGTCACTGATTGCAC	0.068
9	CGCCTCTTCTGTTTCC----------CTGATTGCAC	0.060
10	CGCCTCTTCTG----------TTACTGTCACTGATTGCAC	0.055
11	CGCCTCTTCTGTTTCCCTC----------CTGATTGCAC	0.053
12	CGCCTCTTCTGTTTCCCTCCTGTCACTGTCACTGATTGCAC	0.047
13	CGCCTCTT----------CTGTCACTGATTGCAC	0.046
14	CGCCTCTTCTGT--------TTTACTGTCACTGATTGCAC	0.043
15	CGCCTC----------TTACTGTCACTGATTGCAC	0.035
16	CGCCTCTTCTGTTTCCC---TTACTGTCACTGATTGCAC	0.033
17	CGCCT----------CTGTCACTGATTGCAC	0.028
18	CGCCTCTTCTGTTTCCC-----TACTGTCACTGATTGCAC	0.028
19	CGCCTCTTCTGTTTCCCTCCTTATACTGTCACTGATTGCAC	0.027
20	CGCCTCTTCTGTTTCC--CCTTTACTGTCACTGATTGCAC	0.025

Table S2. Top 20 edited reads in the infusion product of ZL52 at site ZJ52-CS4/ISP9.

ZJ52-CS4/ISP9

overall edit rate = 1.1

	Aligned sequence	%Reads
Unedited	**GCTACAGCCAAATGCCGTCCTTTACTGTCACTGCCATGGC**	**94.861**
1	GCTACAGCCAAATGCCGTCCTTTTACTGTCACTGCCATGGC	0.671
2	GCTACAGCCAAATGCCGTCC--TACTGTCACTGCCATGGC	0.055
3	GCTACAGCCAAATGCCGTCC-TTACTGTCACTGCCATGGC	0.055
4	GCTACAGCCAAATGCCGT-CTTTACTGTCACTGCCATGGC	0.036
5	GCTACAGCCAAATGCCGTCACAAATACTGTCACTGCCATGGC	0.035
6	GCTACAGCCAAATGCCGTCCT--------CCTGCCATGGC	0.034
7	GCT----------TTTACTGTCACTGCCATGGC	0.030
8	GCTACAGCCAAATGCCG---TTTACTGTCACTGCCATGGC	0.021
9	GCTACAGCC-AATGCCGTCCTTTACTGTCACTGCCATGGC	0.019
10	GCTACAGCCAAATGCC----------GTCACTGCCATGGC	0.018
11	GCTACAGCCAAATGCCGTCCTTTTACTGTCACTGCCGTGGC	0.011
12	GCTACAGCCAAATGCCGTCCTTTACTGTCACTGCCAT-GC	0.005
13	GCTACAGCCAAATGCCGTCCTTTACTGTCACTG-CATGGC	0.004
14	GCTACAGCCAAAATGCCGTCCTTTACTGTCACTGCCATGGC	0.003
15	-CTACAGCCAAATGCCGTCCTTTACTGTCACTGCCATGGC	0.002
16	GCTACAGCCAAATGCC-TCCTTTACTGTCACTGCCATGGC	0.002
17	GCTACAGCCAAATGCCGTCCTTT-CTGTCACTGCCATGGC	0.002
18	GCTACAGCCAAATGCCGTCCTTTTACTGTCACTGCCATGA	0.002
19	CTACAGCCAAATGCCGTCCCTTTACTGTCACTGCCATGGC	0.002
20	GCTACAGTCAAATGCCGTCCTTTTACTGTCACTGCCATGG	0.001

Expected cut site

Table S3. Top 20 edited reads in the infusion product of ZL52 at site ZJ52-CS26/ISP2.

ZJ52-CS26/ISP2

overall edit rate = 8.3

	Aligned sequence	%Reads
Unedited	**AAATCTCACTCTCTCCTTCCTGTACTGTCACTCACTGTAC**	**90.412**
1	AAATCTCACTCTCTCCTTCCTTGTACTGTCACTCACTGTAC	5.459
2	AAATCTCACTCTCTCCTTC----CTGTCACTCACTGTAC	0.224
3	AAATCTCACTCTCTCCTTC----------CTCACTGTAC	0.156
4	AAATCTCACTCTCTCCTTCC--TACTGTCACTCACTGTAC	0.151
5	AAATC---------------------TCACTCACTGTAC	0.127
6	AAATCTCACTCTCTCCTTCCT---CTGTCACTCACTGTAC	0.118
7	AAATCTCACTCTCTCCTT-CTGTACTGTCACTCACTGTAC	0.091
8	AAATCTCACTCTCTCCTTCCT----------TCACTGTAC	0.081
9	AAATCTCACTCTCTCCTTCCT--------------------	0.076
10	AAATCTCACTCTCTCC-----TACTGTCACTCACTGTAC	0.070
11	AAATCTCACTCTCTC------ACTGTCACTCACTGTAC	0.064
12	AAATCTCACTCTCTC-----CTGTCACTCACTGTAC	0.064
13	AAATCTCACTCTCTCCTTCCT----TGTCACTCACTGTAC	0.063
14	AAATCTCACTCTCTCCTTCCT------------ACTGTAC	0.060
15	AAATCTCACTCTCTCCTTCCT-TACTGTCACTCACTGTAC	0.055
16	AAATCTCACTCTC-------------------TAC	0.053
17	AA-----------------ACTGTCACTCACT-TAC	0.050
18	AAATCTCACTCTCTCCTTCC-------TCACTCACTGTAC	0.049
19	AAATCTCACTCTC-------------TCACTCACTGTAC	0.047
20	AAATCTCACTCTCTC-----------CACTGTAC	0.046

Expected cut site

Table S4. Top 20 edited reads in the infusion product of ZL52 at site ZJ52-CS10.

ZJ52-CS10

overall edit rate = 1.4

	Aligned sequence	%Reads
Unedited	**CTCCACTCACTGACAGTAAAGGTCGGTATTATCTTTCCTA**	**94.860**
1	CTCCACTCACTGACAGT-AAGGTCGGTATTATCTTTCCTA	0.480
2	CTCCACTCACTGACAGTAAAGGTCGGTATTATC-TTCCTA	0.197
3	CT-CACTCACTGACAGTAAAGGTCGGTATTATCTTTCCTA	0.164
4	CTCCACTCACTGACAGTAAAGGTCGGTATT-TCTTTCCTA	0.099
5	CTCCACTCACTGACAGTAAAGGTCGGTA-TATCTTTCCTA	0.097
6	-TCCACTCACTGACAGTAAAGGTCGGTATTATCTTTCCTA	0.062
7	CTCCACTCACTGACAGTAAAGGTCGGTATTATCTTTCC-A	0.042
8	C-CCACTCACTGACAGTAAAGGTCGGTATTATCTTTCCTA	0.030
9	CTCCACTCACTGACAGTAAAGGT-GGTATTATCTTTCCTA	0.026
10	CTCCACTCACTGACAGTAAAGGTCGGTATTATCTTT-CTA	0.019
11	CTCCACTCACT-ACAGTAAAGGTCGGTATTATCTTTCCTA	0.019
12	CTCCACTCACTGACAGTAAAGGTC-GTATTATCTTTCCTA	0.016
13	CTCCACTCACTGACAGTAAAGGTCGGTATTATCTTTC--A	0.014
14	CTC--CTCACTGACAGTAAAGGTCGGTATTATCTTTCCTA	0.014
15	CTCCACTCACTGACAGTAAAGGTCGGTATTATC--TCCTA	0.014
16	CTCCACTCACTGACAG-AAAGGTCGGTATTATCTTTCCTA	0.011
17	CTCCACTCACTGACAGT--AGGTCGGTATTATCTTTCCTA	0.009
18	CTCCACTCACTG--AGTAAAGGTCGGTATTATCTTTCCTA	0.009
19	CTCCACTCACTGACAGTAAA-GTCGGTATTATCTTTCCTA	0.009
20	CTCCACTCCCTGACAGT-AAGGTCGGTATTATCTTTCCTA	0.007

Expected cut site

Table S5. Top 20 edited reads in the infusion product of ZL52 at site ZJ52-CS14.

ZJ52-CS14

overall edit rate = 1.6

	Aligned sequence	%Reads
Unedited	**GACAGATCAAGCAACCCTCTTTTACTGTCACTAACTCACC**	**94.996**
1	GACA-ATCAAGCAACCCTCTTTTACTGTCACTAACTCACC	1.005
2	GACAG-TCAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.152
3	GACAGATCAAGCAACCCTC-TTTACTGTCACTAACTCACC	0.061
4	GACAGA-CAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.050
5	GACAGGATCAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.046
6	GACAGATCAAGCAACCCTC--TTACTGTCACTAACTCACC	0.041
7	GACAGATCAAGCAACCCTCTTTTTACTGTCACTAACTCACC	0.025
8	GAC--ATCAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.017
9	GACAGATCAAGCAACCCTC---TACTGTCACTAACTCACC	0.015
10	GACAGATCAAGCAACCCT-----CTGTCACTAACTCACC	0.014
11	GACAGATCAAGCAACCCTCT----------CC	0.012
12	GACAGATCAAGCAACCCT----------CTAACTCACC	0.011
13	GACAGAATCAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.010
14	GACAGATCAAGCAACCCTCTTTTACTGTCACT-ACTCACC	0.009
15	GACAGATCAAGCAACCCTCTTGATCTGTTACTGTCACTAACTCACC	0.009
16	G-CA-ATCAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.009
17	GACAGATCAAGCAACCCTCTT---CTGTCACTAACTCACC	0.007
18	-ACA-ATCAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.005
19	GACAGATCAAGCAACCCTCTTTTACTGTCACTAACTCA-C	0.005
20	GACAG--CAAGCAACCCTCTTTTACTGTCACTAACTCACC	0.004

Table S6. Details of the transplantation and editing parameters for each edited rhesus.

Target	TET2 exon2	CD33			
Guide+PAM	GGAAGGCCGTCCATTCTCAG**GGG**	GTCAGTGACAGTACAGGA**GGG**			
Rhesus	***ZL26***	***ZL38***	***ZL33***	***ZJ52***	***ZM36***
Date of birth	5/1/13	5/10/13	5/9/13	7/21/11	8/18/14
Transplant date	6/30/16	9/29/16	2/16/17	5/24/18	1/25/19
Cells edited (million)	47.5	36	37	55	32
Cells re-transfused (million)	40	20	36.9	40	45
Type of gRNA used	In vitro transcribed	In vitro transcribed	In vitro transcribed	Chemically modified	Chemically modified
Guide pool	20% AAVS1 pool, 80% DNMT3A exon3/19+TET2 exon1/2+ASXL1 exon1/2 pool	100% CD33 exon2 pool	100% CD33 exon2 #1 only	100% CD33 exon2 #1 only	100% CD33 exon2 #1 only
Sex	Male	Male	Male	Female	Male
Comments				Euthanized 10/24/2018 due to radiation pneumonitis	

Table S7. Coordinates of the *bona fide* off-target sites and the genes perturbed by off-target editing using the CD33 guide.

ISP rank	CS rank	Loci	Gene	Position	Gene name	Genes within 200kbp
ISP1	CS1	19:46496828-46496849	CD33	exon	On-target	9
ISP2	CS24	2:41549567-41549588	EPS8	exon	epidermal growth factor receptor kinase substrate 8	5
ISP8	CS2	11:16366413-16366434	SUSD3	intron	promoter - sushi domain containing 3 (SUSD3) is a promoter of estrogen-	2
ISP23	CS28	3:10674602-10674623				6
ISP9	CS4	15:43200241-43200262	SETD4	intron	SET domain containing 4	5
ISP29	CS1567	5:182408689-182408692				1
ISP30		5:61556537-61556540	CCDC88A	intron	coiled-coil domain containing 88A	4
ISP320	CS355	1:97591083-97591104	ANKRD17	exon	ankyrin repeat domain 17	2
ISP427	CS36	8:26411695-26411716	BNIP3L	intron	BCL2 interacting protein 3 like	4
	CS15	15:39058484-39058505				2
	CS18	6:55417537-55417557	TMEFF1	intron	Transmembrane Protein With EGF Like And Two Follistatin Like Domains 1	2
ISP327		7:121963117-121963120				1
ISP383	CS138	3:158401396-158401417				1
	CS34	11:79245055-79245076	GRM8	intron	glutamate metabotropic receptor 8	2
	CS169	3:152775429-152775449				1
	CS252	8:130583758-130583779	LRRC9	intron	leucine rich repeat containing 9	6
ISP18	CS3	9:118739404-118739425	PLXNA4	intron	Plexin-A4	6
	CS222	13:56343337-56343358	Cdcp3	intron	CUB domain-containing protein 3	13
	103	9:36140774-36140795				9
	391	3:177315835-177315856	AGAP3	intron	ArfGAP With GTPase Domain, Ankyrin Repeat And PH Domain 3	5

ISP1,CS1 = Target

Found by CS and ISP

Found by CS only

Found by ISP only

Table S8. The number of off-target sites predicted via 5 different ISP algorithms for the CD33 gRNA.

Method	Total number of predicted sites	Number of predicted sites without duplicates	Notes
Cas-OFFinder	21,091	14,447	No ranking
CCtop	2,777	2,777	No ranking
COSMID	12,11	963	
CRISPOR	252	252	
E-CRISP	115	115	

Since some algorithms call the same site multiple times (often due to the site meeting both the mismatch criteria and the gap criteria) we removed duplicates from each of these counts. A duplicate was defined as sites with the exact same start sites on the same chromosome.

Table S9. Prediction of the valid 19 off-target sites via 5 different ISP algorithms. Red = predicted.

Off-target		Sequence	PAM	CCtop	COSMID	CRISPOR	E-CRISP	Cas-OFFinder
ISP8	CS2	ATCAGTGACAGTAAAGGA	GGG					
ISP9	CS4	GGCAGTGACAGTAAAGGA	CGG					
ISP2	CS24	GTGAGTGACAGTACAGGA	AGG					
ISP23	CS28	GCCAGTGACAGAACAGGA	GGG					
	CS34	GGCAGTGACAGTACAGAT	AGG					
	CS222	GTCAGTAACAGCACAGTA	AGG					
ISP30		GCCAGGGACAGTACAGTA	TGG					
ISP427	CS36	GGCAGTGACAATACATGA	AGG					
ISP320	CS355	AGCAGTAACAGTACAGAA	GGG					
	CS15	GTTAGTGACAGTAAAAGA	GGG					
	CS18	GTCAGTG-CAGTACAGGA	TGG					
	CS252	AGCAGGGACAGCACAAGA	GAG					
	CS169	GTCAGTGGCA-TACAGGT	AAG					
ISP29	CS1567	GGCAGAGACAGTACAGAA	GGG					
ISP327		TTAAGTTACAGTACAGTA	CAG					
ISP383	CS138	GTAAGTGACAGTACAAGG	AGG					
ISP18	CS3	TGCAGTGACAGTACAGGT	GGG					
	CS103	TTCAGTAACAGGGAAGGA	AGG					
	CS391	GTTAGTGGCAGAACAGGGA	CGG					
			Sum =	**12**	**13**	**10**	**5**	**14**

Table S10. Bona fide off-target sites within open chromatin.

ISP1,CS1 = Target
Found by CS and ISP
Found by CS only
Found by ISP only

Site	Gene	Position	ATAC-Seq
ISP1,CS1	CD33	exon	
ISP8,CS2	EPS8	exon	
ISP9,CS4	SUSD3	intron	Open
ISP2,CS24			
ISP23,CS28	SETD4	intron	
CS34			
CS222	CCDC88A	intron	
ISP30	ANKRD17	exon	
ISP427,CS36	BNIP3L	intron	
ISP320,CS355			
CS15	TMEFF1	intron	
CS18			
CS252			
CS169	GRM8	intron	
ISP29			Open
ISP327	LRRC9	intron	
ISP383,CS138	PLXNA4	intron	Open
ISP18,CS3	Cdcp3	intron	
CS103			
CS391			Open

Table S11. Sequences of the primers used for translocation confirmation and the sequenced translocation.

PRIMERS:	
On-Target forward primer	TGTGGGCAGGTGAGTGACTG
Off-target CS28/ISP23 reverse primer	CTACAAGTGGCGTGGAGGTTTG
SEQUENCED AMPLICON:	
TGTGGGCAGGTGAGTGACTGCTGGGAGGGGGGTTGTCGGGCTGGGC CAAGCTGACCCTCATTTCCCACAGGGGCCCTGGCTATGGATCCAAGAG TCA**GGCTGG**GGGCAGGACTTCATTCACTTATGGGTCACACAGGCTTGT CATGATGATAACATTCAGTCCTTGCCTGCAGAGAGCACCCACCCCTCGT GCTTTTTCTTCTCAGCTCCTCCTCCTCCTTTCTTATTACCTGGTCGGCTTC CACACAGGAGCTACAAGTGGCGTGGAGGTTTG	

On-target site/Chromosome 19: Green. Off-target site/Chromosome3: Red. Shared: Grey.

Table S12. Monoclonal antibody clones used for flow-cytometry sorting.

Cell	Marker	Fluorochrome	Clone	Cat no.
NK	CD16	BV605	3G8	BioLegend (302040)
NK	CD159a (NKG2A)	APC	Z199	Beckman Coulter (A60797)
T	CD3	BV786	SP34-2	BD Biosciences (563918)
B	CD20	APC-Cy7	L27	BD Biosciences (335794)

Table S13. **Adaptor sequences for primers of targeted Illumina sequencing library prep.**

Adaptor sequence for the forward primers	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
Adaptor sequence for the reverse primers	GACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Table S14. Sequences of the oligos used for CAST-Seq on ZJ52.

Adaptor	
Positive strand linker oligo	GTAATACGACTCACTATAGGGCTCCGCTTAAGGGACT
Negative strand linker oligo	P-GTCCCTTAAGCGGAGC-NH3
PCR I	
Initial linker prey primers	GTAATACGACTCACTATAGGGC
Initial CD33 bait	CAAGCTGACCCTCATTTCC
Initial CD33 decoy forward	CAGTTCATGGTTACTGGTTCC
Initial CD33 decoy reverse	GGTACGGGATGGAAGAAAG
PCR II	
Nested linker prey primers	ACACTCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGGGCTCCGCTTAAGGGAC
Nested CD33 bait nested	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATCCAAGAGTCAGGCTGG