Cytidine and adenine base editors with improved editing efficiencies in mammalian cells

Luke W. Koblan, Jordan L. Doman, Christopher Wilson, Jonathan M. Levy, Tristan Tay, Greg A. Newby, Juan Pablo Maianti, Aditya Raguram, David R. Liu

SUPPLEMENTARY INFORMATION

Supplementary Figure 16. Original western blots for BE4, BE4max, AncBE4max, ABE7.10, and ABEmax.

Supplementary Figure 17. 468-taxa unrooted phylogeny of APOBEC homologs.

Supplementary Note 1. ClinVar database analysis script.

Supplementary Sequences 1. APOBEC homologs.

Supplementary Sequences 2. Amino acid sequences of BE4, rAPOBEC, ancestral APOBECs, ABE7.10, and P2A–GFP.

Supplementary Sequences 3. Codon-optimized nucleotide sequences of BE4, rAPOBEC, ancestral APOBECs, ABE7.10, and P2A–GFP.

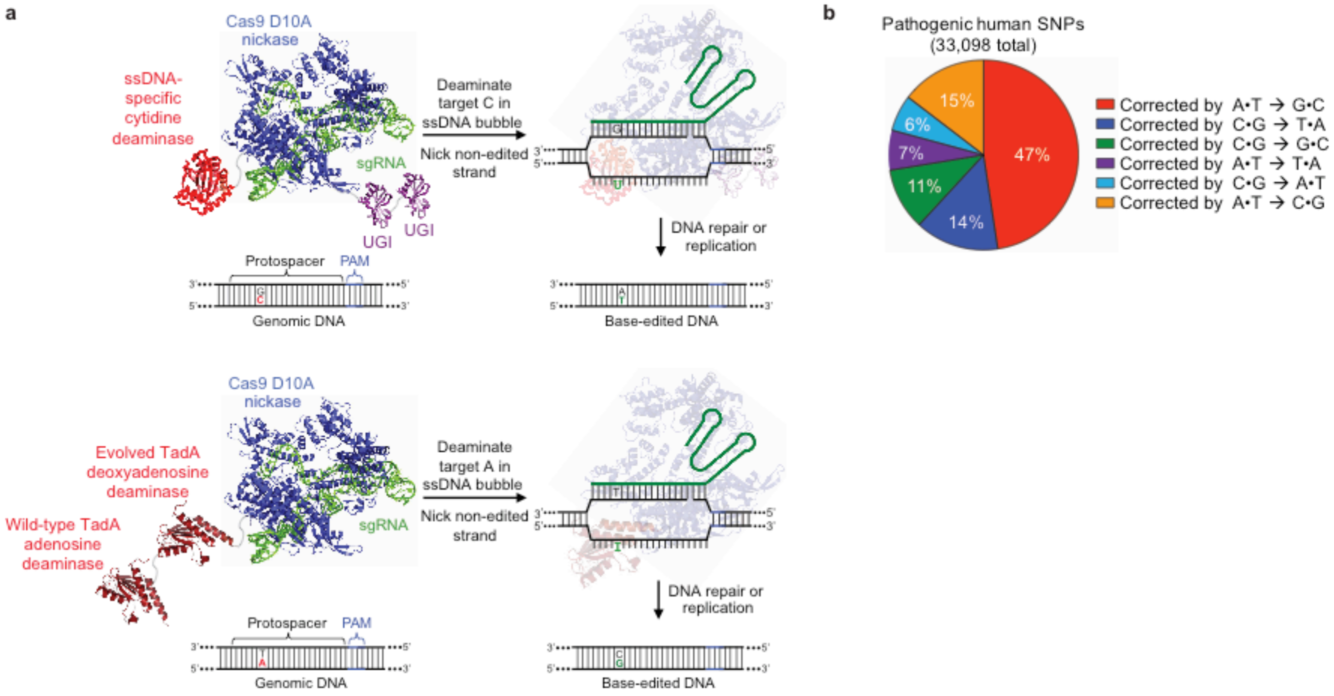
Supplementary Sequences 4. Target protospacer sequences analyzed in this study.

Supplementary Sequences 5. Primers used in this study.

Supplementary File 1. MAFFT alignment APOBEC homologs in FASTA format.

Supplementary File 2. Flow cytometry gating examples for all cell types used.

Supplementary File 3. APOBEC tree in Newick format.



Supplementary Figure 1

Cytidine and adenine base editing schematic and disease associated SNPs

(**a**), BE4 (top) and ABE (bottom) induce the deamination of target C or target A nucleotides, and nick the non-edited strand to direct DNA repair processes to replace that strand using the deaminated C (uracil, U) or the deaminated A (inosine, I) as a template. The result is BE4-mediated conversion of a target C•G base pair to a T•A base pair, and ABE-mediated conversion of a target A•T base pair to a G•C base pair. (**b**) Base pair changes required to correct pathogenic SNPs in the ClinVar database. The red wedge (47%) require conversion of the type mediated by ABE, while the dark blue wedge (14%) require conversion of the type mediated by BE4.

a

BE4 (IDT codons): SV40 vs. bis-bpNLS

Site	p-value	t value	Significance
HEK2 C4	0.0112	4.454	*
HEK2 C6	0.0072	5.053	**
HEK3 C4	0.2838	1.237	p>0.05
HEK3 C5	0.3363	1.092	p>0.05
HEK4 C5	0.0620	2.570	p>0.05
RNF2 C6	0.0217	3.655	*
EMX1 C5	0.042	2.948	*
EMX1 C6	0.0428	2.929	*

b

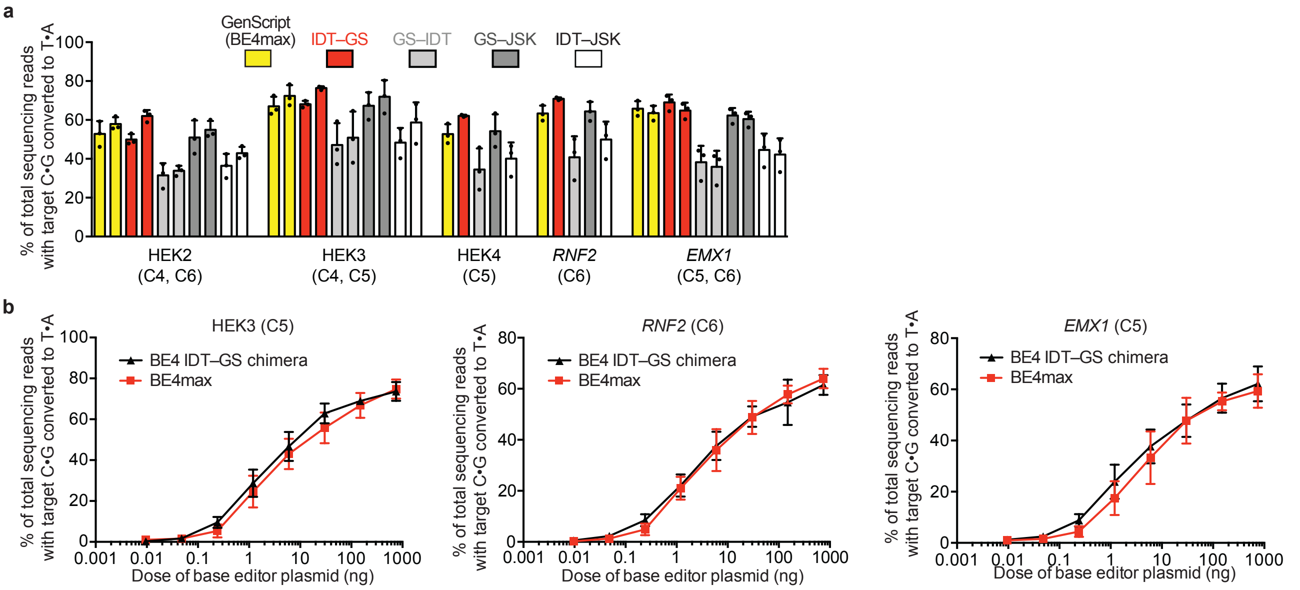
bis-bpNLS: IDT codons vs. GenScript codons

Site	p-value	t value	Significance
HEK2 C4	0.0119	4.376	*
HEK2 C6	0.0107	4.511	**
HEK3 C4	0.0194	3.782	*
HEK3 C5	0.0263	3.441	*
HEK4 C5	0.0176	3.896	*
RNF2 C6	0.0161	4.004	*
EMX1 C5	0.0057	5.398	*
EMX1 C6	0.0048	5.656	*

Supplementary Figure 2

Unpaired two-sided t-test p-values and t-values for NLS and codon optimizations

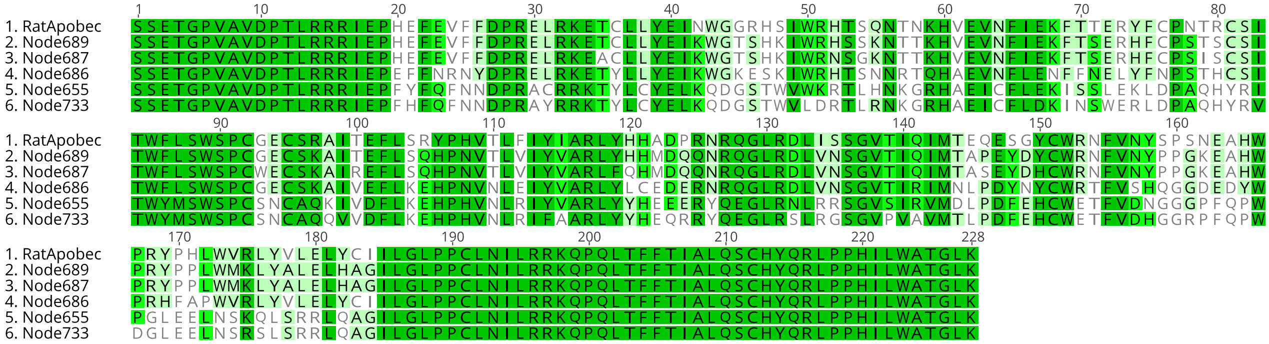
(**a**) Unpaired two-sided t-test p-values and t-values for previously reported BE4 (C-terminal SV40 NLS, IDT codons) compared to bis-bpNLS BE4 (IDT codons) at all Cs within the activity window across five endogenous genomic loci in HEK293T cells transfected with 750 ng of base editor plasmid and 250 ng of sgRNA plasmid. (**b**) Unpaired two-sided t-test p-values and t-values for BE4 bis-bpNLS using IDE codons compared to GenScript codons at all Cs within the activity window across five endogenous genomic loci in HEK293T cells transfected with 750 ng of base editor plasmid and 250 ng of sgRNA plasmid. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$. Values are from n=3 biologically independent experiments (dots) 3 days after transfection.



Supplementary Figure 3

C•G-to-T•A base editing outcomes for BE4 variants

(**a**) C•G-to-T•A base editing efficiency of bis-bpNLS BE4 variants using full-length GenScript codon optimization (BE4max) compared to chimeric constructs in which the APOBEC1 and Cas9 nickase components are constructed with different codon usages in HEK293T cells. Chimeras include (APOBEC1–Cas9 nickase): GenScript–IDT, GenScript–Jin Soo Kim, IDT–Jin Soo Kim (Kim, S., Bae, T., Hwang, J. & Kim, J.S. *Genome biology* **18**, 218 (2017)). (**b**) Comparison of BE4max and bis-bpNLS BE4 with chimeric IDT–GenScript codon usage at three endogenous genomic loci in HEK293T cells across eight different plasmid doses. Values represent the mean and standard deviation of n=3 biologically independent experiments (dots) 3 days after transfection.



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1. RatApobec  SSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCPNTRCSI
2. Node689    SSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEIKWGTSHKIWRHSSKNTTKHVEVNFIEKFTSERHFCPSTSCSI
3. Node687    SSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKEACLLYEIKWGTSHKIWRNSGKNTTKHVEVNFIEKFTSERHFCPSISCSI
4. Node686    SSETGPVAVDPTLRRRIEPEFFNRNYDPRELRKETYLLYEIKWGKESKIWRHTSNNRTQHAEVNFLENFFNELYFNPSTHCSI
5. Node655    SSETGPVAVDPTLRRRIEPFYFQFNNDPRACRRKTYLCYELKQDGSTWVWKRTLHNKGRHAEICFLEKISSLEKLDPAQHYRI
6. Node733    SSETGPVAVDPTLRRRIEPFHFQFNNDPRAYRRKTYLCYELKQDGSTWVLDRTLRNKGRHAEICFLDKINSWERLDPAQHYRV

1. RatApobec  TWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHW
2. Node689    TWFLSWSPCGECSKAITEFLSQHPNVTLVIYVARLYHHMDQQNRQGLRDLVNSGVTIQIMTAPEYDYCWRNFVNYPPGKEAHW
3. Node687    TWFLSWSPCWECSKAIREFLSQHPNVTLVIYVARLFQHMDQQNRQGLRDLVNSGVTIQIMTASEYDHCWRNFVNYPPGKEAHW
4. Node686    TWFLSWSPCGECSKAIVEFLKEHPNVNLEIYVARLYLCEDERNRQGLRDLVNSGVTIRIMNLPDYNYCWRTFVSHQGGDEDYW
5. Node655    TWYMSWSPCSNCAQKIVDFLKEHPHVNLRIYVARLYYHEEERYQEGLRNLRRSGVSIRVMDLPDFEHCWETFVDNGGGPFQPW
6. Node733    TWYMSWSPCSNCAQQVVDFLKEHPHVNLRIFAARLYYHEQRRYQEGLRSLRGSGVPVAVMTLPDFEHCWETFVDHGGRPFQPW

1. RatApobec  PRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK
2. Node689    PRYPPLWMKLYALELHAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK
3. Node687    PRYPPLWMKLYALELHAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK
4. Node686    PRHFAPWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK
5. Node655    PGLEELNSKQLSRRLQAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK
6. Node733    DGLEELNSRSLSRRLQAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK
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Supplementary Figure 4

Multiple sequence alignment of rat APOBEC1 and reconstructed ancestral cytidine deaminases.

Residues are colored based on the degree of conservation.

a

HEK3 (C5), BE4 vs. BE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.008	4.908	**
150	0.0531	2.718	p>0.05
30	0.0097	4.643	**
6	0.0013	7.975	**
1.2	0.0081	4.892	**
0.24	0.0849	2.279	p>0.05
0.048	0.1434	1.817	p>0.05
0.0096	0.5981	0.5718	p>0.05

RNF2 (C6), BE4 vs. BE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.0005	10.08	***
150	0.0126	4.301	*
30	0.0029	6.467	**
6	0.0031	6.380	**
1.2	0.002	7.186	**
0.24	0.0283	3.360	*
0.048	0.0277	3.382	*
0.0096	0.3329	1.100	p>0.05

EMX1 (C5), BE4 vs. BE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.0013	7.978	**
150	0.0006	9.785	***
30	0.0024	6.834	**
6	0.004	5.967	**
1.2	0.0063	5.252	**
0.24	0.0119	4.372	*
0.048	0.0019	7.225	**
0.0096	0.1188	1.980	p>0.05

b

HEK3 (C5), BE4 vs. AncBE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.0013	7.990	**
150	0.0125	4.316	*
30	0.001	8.704	***
6	< 0.0001	18.20	****
1.2	0.0002	13.10	***
0.24	0.0019	7.263	**
0.048	0.0156	4.039	*
0.0096	0.2015	1.527	p>0.05

RNF2 (C6), BE4 vs. AncBE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.0187	3.826	*
150	0.0173	3.916	*
30	0.0012	8.277	**
6	0.0005	10.340	***
1.2	0.0019	7.315	**
0.24	0.0039	5.995	**
0.048	0.004	5.944	**
0.0096	0.0115	4.421	*

EMX1 (C5), BE4 vs. AncBE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.0013	7.989	**
150	0.0006	9.980	***
30	0.0037	6.062	**
6	0.0007	9.521	***
1.2	0.0026	6.675	**
0.24	0.0026	6.704	**
0.048	0.0004	10.93	***
0.0096	0.0124	4.330	*

c

HEK3 (C5), BE4max vs. AncBE4max

Plasmid dose (ng)	p-value	t value	Significance	
750	0.0274	3.396	*	
150	0.0387	3.031	*	
30	0.0194	3.779	*	
6	0.0268	3.421	*	
1.2	0.0446	2.888	*	
0.24	0.0161	3.999	*	
0.048	0.0504	2.768	p>0.05	
0.0096	0.0454	2.872	*	Max > Anc

RNF2 (C6), BE4max vs AncBE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.8193	0.244	p>0.05
150	0.9993	0.001	p>0.05
30	0.5972	0.573	p>0.05
6	0.5895	0.586	p>0.05
1.2	0.4079	0.924	p>0.05
0.24	0.0777	2.359	p>0.05
0.048	0.0615	2.578	p>0.05
0.0096	0.015	4.084	*

EMX1 (C5), BE4max vs AncBE4max

Plasmid dose (ng)	p-value	t value	Significance
750	0.0608	2.589	p>0.05
150	0.0551	2.682	p>0.05
30	0.7657	0.319	p>0.05
6	0.4013	0.938	p>0.05
1.2	0.1746	1.649	p>0.05
0.24	0.0314	3.250	*
0.048	0.0033	6.287	**
0.0096	0.0216	3.657	*

Supplementary Figure 5

Unpaired two-sided t-test p-values and t-values for BE4, BE4max, and AncBE4max editing at three genomic loci

(**a**) Unpaired two-sided t-test p-values and t-values for BE4 compared to BE4max at HEK3 (C5), *RNF2* (C6), and *EMX1* (C5) across eight different base editor plasmid doses in HEK293T cells. (**b**) Unpaired two-sided t-test p-values and t-values for BE4 compared to AncBE4max at HEK3 (C5), *RNF2* (C6), and *EMX1* (C5) across eight different base editor plasmid doses in HEK293T cells. (**c**) Unpaired two-sided t-test p-values and t-values for BE4max compared to AncBE4max at HEK3 (C5), *RNF2* (C6), and *EMX1* (C5) across eight different base editor plasmid doses in HEK293T cells. *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. Values are from n=3 biologically independent experiments (dots) 3 days after transfection.

a

HEK3, BE4

Plasmid dose (ng)	% Indels	Stdev
750	3.25	0.51
150	3.65	0.44
30	3.54	0.50
6	1.76	0.51
1.2	1.13	0.09
0.24	0.51	0.15
0.048	0.28	0.23
0.0096	0.47	0.48

RNF2, BE4

Plasmid dose (ng)	% Indels	Stdev
750	0.03	0.01
150	0.02	0.00
30	0.03	0.01
6	0.03	0.01
1.2	0.02	0.01
0.24	0.03	0.00
0.048	0.02	0.01
0.0096	0.02	0.00

EMX1, BE4

Plasmid dose (ng)	% Indels	Stdev
750	0.10	0.02
150	0.08	0.01
30	0.09	0.02
6	0.08	0.02
1.2	0.08	0.02
0.24	0.08	0.01
0.048	0.08	0.00
0.0096	0.07	0.02

b

HEK3, BE4max

Plasmid dose (ng)	% Indels	Stdev
750	2.50	0.65
150	2.51	0.46
30	2.53	0.30
6	2.87	0.37
1.2	2.57	0.40
0.24	1.10	0.39
0.048	0.45	0.22
0.0096	0.17	0.11

RNF2, BE4max

Plasmid dose (ng)	% Indels	Stdev
750	0.03	0.01
150	0.03	0.01
30	0.03	0.01
6	0.04	0.01
1.2	0.03	0.01
0.24	0.03	0.00
0.048	0.02	0.00
0.0096	0.03	0.00

EMX1, BE4max

Plasmid dose (ng)	% Indels	Stdev
750	0.11	0.01
150	0.09	0.01
30	0.09	0.02
6	0.08	0.00
1.2	0.09	0.01
0.24	0.07	0.01
0.048	0.09	0.01
0.0096	0.10	0.02

c

HEK3, AncBE4max

Plasmid dose (ng)	% Indels	Stdev
750	2.23	0.14
150	2.85	0.24
30	2.92	0.56
6	3.73	0.60
1.2	3.57	0.84
0.24	2.55	0.28
0.048	0.82	0.04
0.0096	0.13	0.03

RNF2, AncBE4max

Plasmid dose (ng)	% Indels	Stdev
750	0.03	0.00
150	0.05	0.01
30	0.04	0.00
6	0.03	0.01
1.2	0.03	0.01
0.24	0.03	0.00
0.048	0.03	0.00
0.0096	0.03	0.01

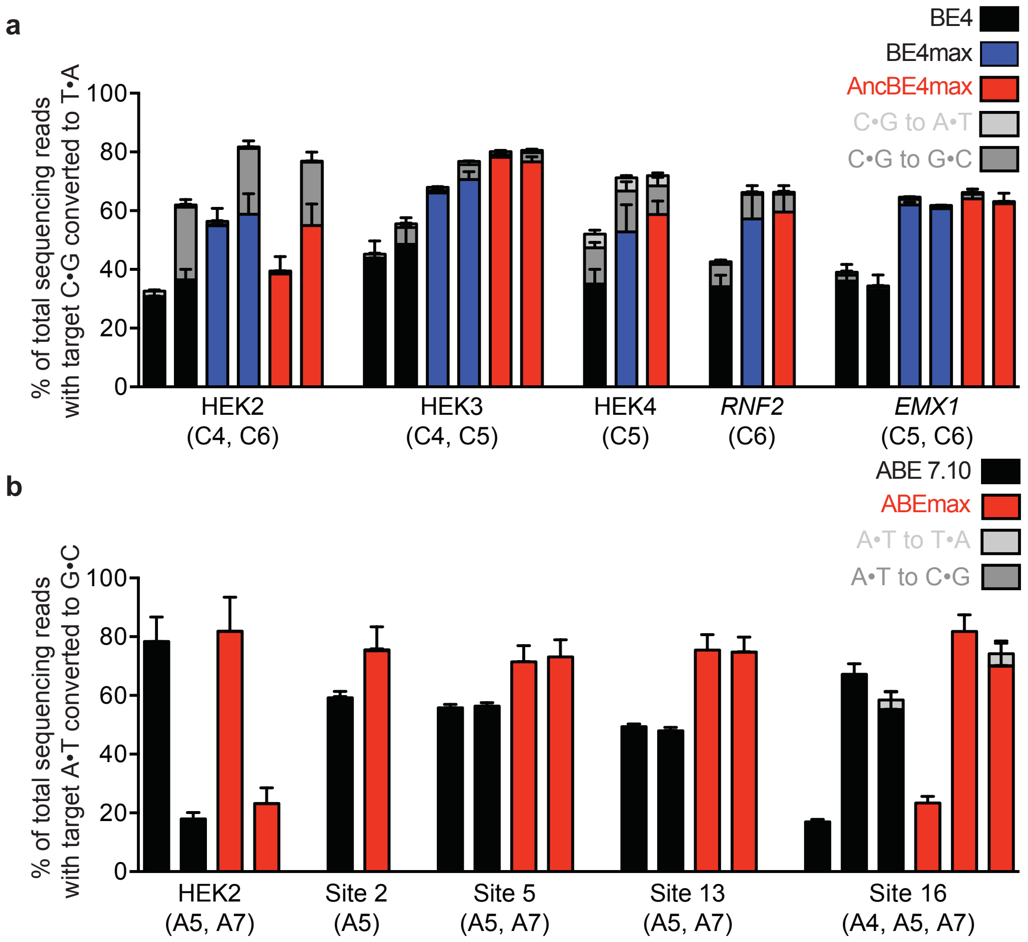
EMX1, AncBE4max

Plasmid dose (ng)	% Indels	Stdev
750	0.09	0.01
150	0.10	0.02
30	0.09	0.01
6	0.09	0.00
1.2	0.09	0.01
0.24	0.09	0.03
0.048	0.09	0.01
0.0096	0.09	0.00

Supplementary Figure 6

Indel frequencies for BE4, BE4max, and AncBE4 at three genomic loci

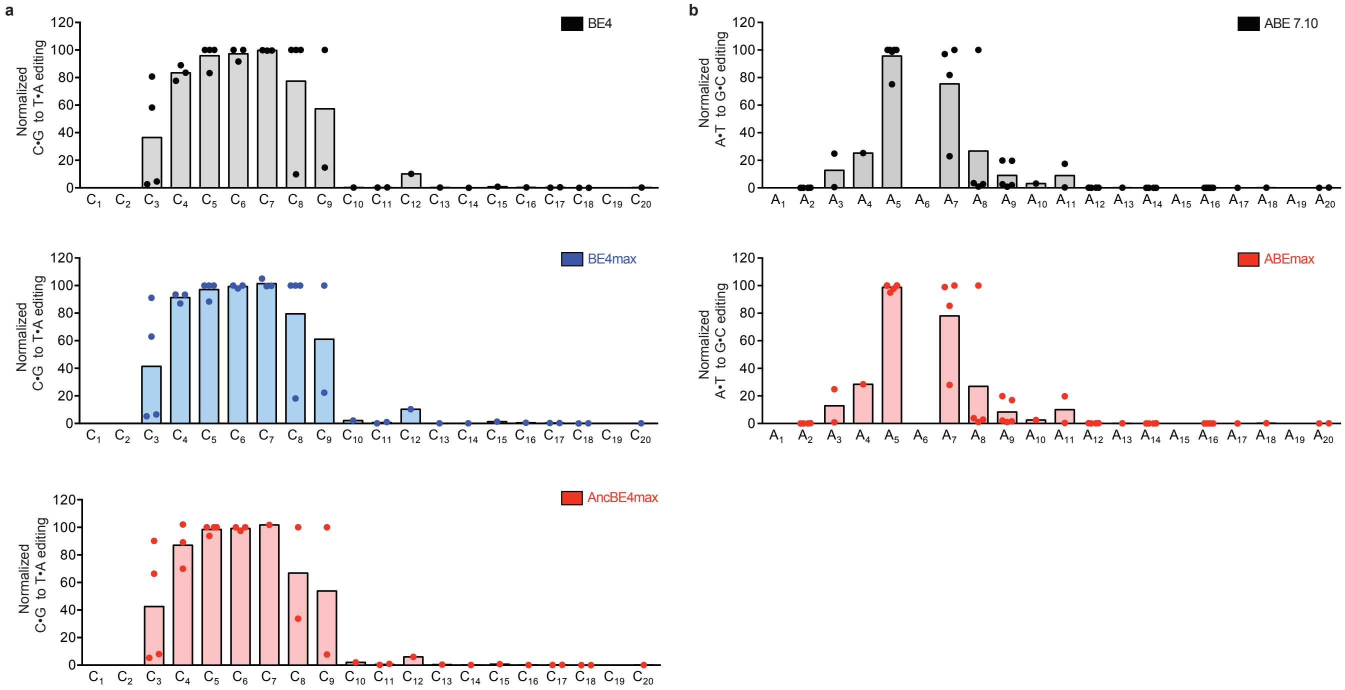
Indel frequencies are shown for BE4, BE4max, and AncBE4max at HEK3, *RNF2*, and *EMX1* sites across eight different plasmid doses in HEK293T cells. % Indel and Stdev values represent the mean and standard deviation of n=3 biologically independent experiments 3 days after transfection.



Supplementary Figure 7

Base editing product purity for cytidine and adenine base editors

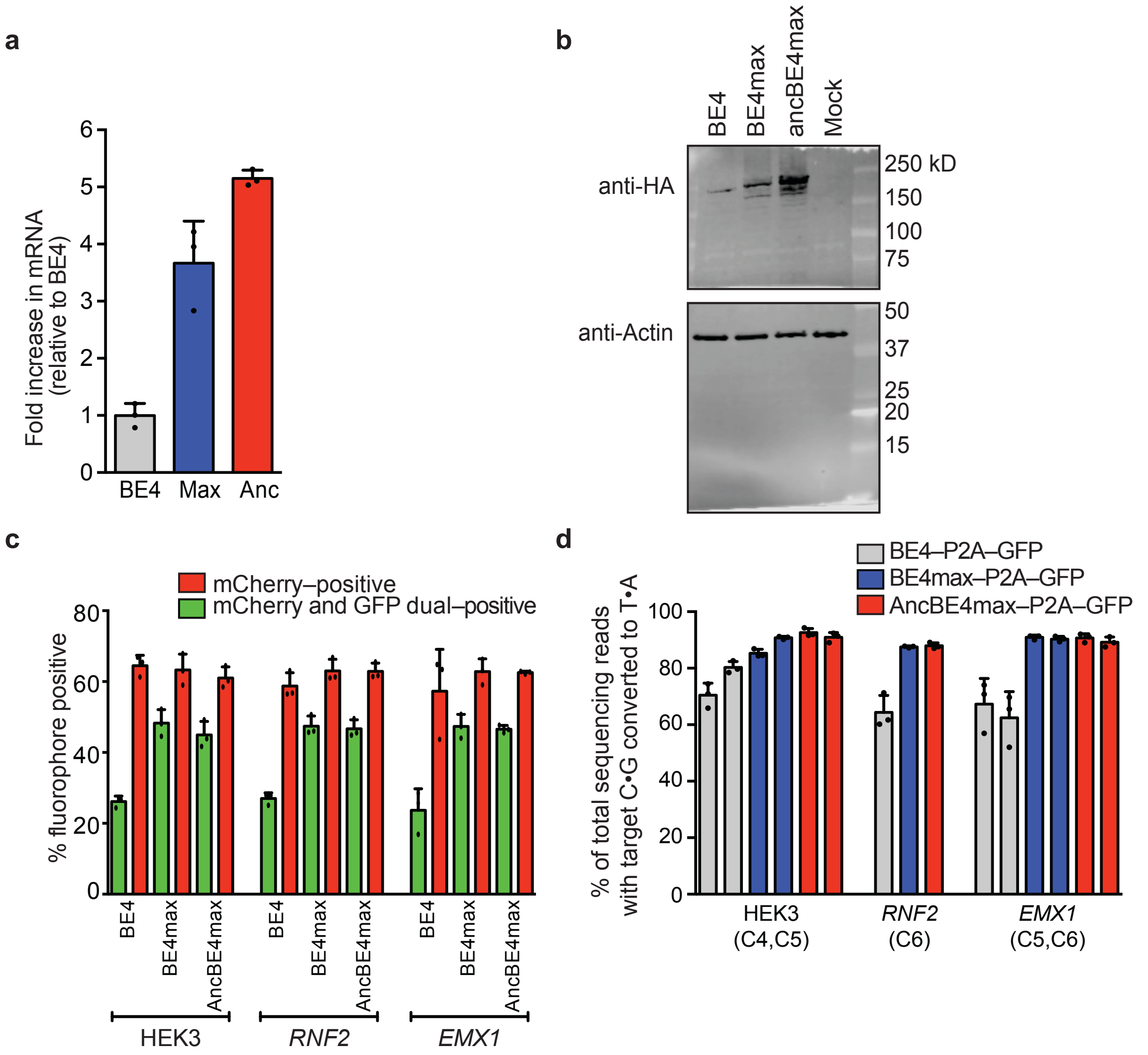
(**a**) Editing outcomes for BE4, BE4max, and AncBE4max at HEK2, HEK3, HEK4, *RNF2*, and *EMX1* sites. (**b**) Editing outcomes for ABE7.10 and ABEmax at HEK2, Site 2, Site 5, Site 13, and Site 16. Values represent the mean and standard deviation of n=3 biologically independent experiments 3 days after transfection.



Supplementary Figure 8

Base editing activity windows for cytidine and adenine base editors are unchanged after optimization

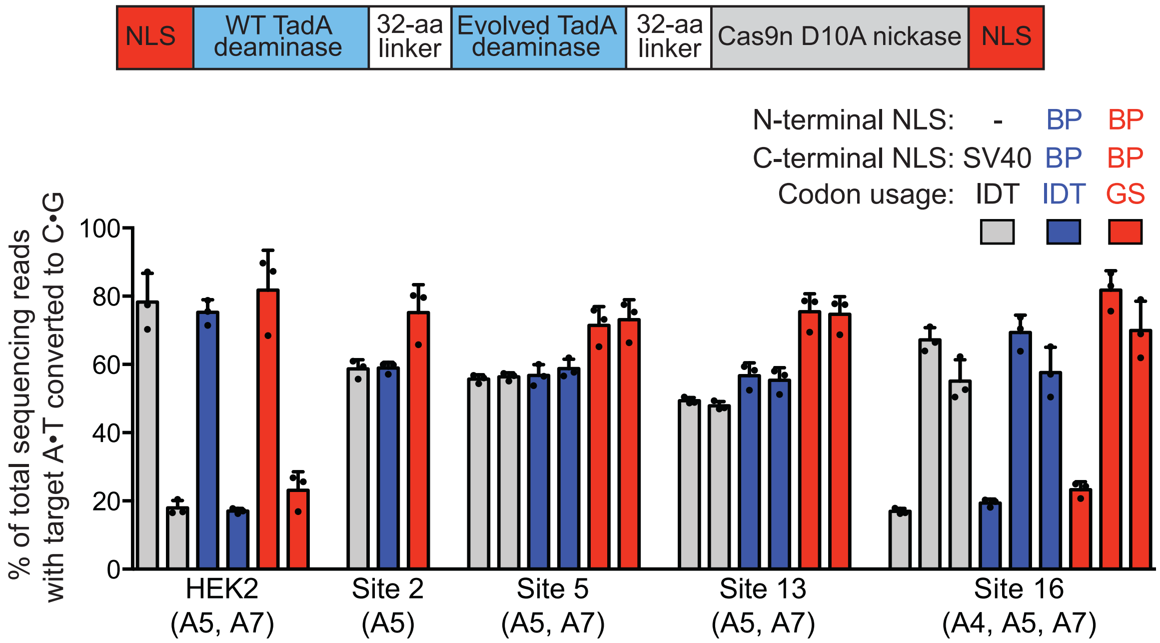
(**a**) Normalized C•G-to-T•A editing efficiency across the protospacer of HEK2, HEK3, HEK4, *RNF2*, *EMX1*, *MPDU1*, and *SCN9a* sites for BE4, BE4max, and AncBE4max. Protospacer bases are numbered such that the PAM is at positions 21-23. (**b**) Normalized A•T-to-G•C editing efficiency across the protospacer of HEK2, Site 2, Site 5, Site 13, Site 16, *HBG1*, and *HBG2* sites for ABE7.10 and ABEmax. Each point represents the average of n=3 biologically independent experiments for each position of each site sampled 3 days after transfection for each position at which a C or A was present in the editing window. No ABE loci tested in this study contained an A at protospacer position 6.



Supplementary Figure 9

Improved mRNA levels, protein levels, and base editing from BE4max and AncBE4max compared with BE4

(**a**) BE4max and AncBE4max result in 3.7- and 5.2-fold higher mRNA levels, respectively, than BE4 in HEK293T cells 3 days after base editor and guide RNA plasmid transfection as determined by RT-qPCR. Base editor mRNA levels were normalized to β−actin levels by ΔΔCt. Normalized values were adjusted for transfection efficiency as determined by qPCR amplification of the bGH terminator sequence present on BE4 plasmids. (**b**) Western blot of C-terminal HA-tagged BE4, BE4max, and AncBE4max in HEK293T cells 3 days after plasmid transfection, visualizing with anti-HA (top) or anti-actin (bottom) antibodies. The mock sample is non-transfected cells. This blot was performed once. (**c**) BE4max–P2A–GFP and AncBE4max–P2A–GFP show higher GFP and mCherry double-positive cell populations compared to BE4–P2A–GFP for three genomic loci 3 days after transfection. (**d**) C•G-to-T•A base editing outcomes for GFP and mCherry dual-positive cells expressing BE4–P2A–GFP, BE4max–P2A–GFP, and AncBE4max–P2A–GFP at three genomic loci tested. Values and error bars in (a), (c), and (d) represent the mean and standard deviation of n=3 biologically independent experiments (dots) 3 days after transfection.



Supplementary Figure 10

Optimization of the ABE7.10 A•T-to-G•C base editor by improving nuclear localization and improving codon usage

ABE architecture, effects of NLS configuration (SV40 versus bis-bpNLS), and effects of codon usage (IDT versus GenScript) on ABE base editing efficiency at five endogenous genomic loci in HEK293T cells. BP=bis-bpNLS; GS=GenScript codon usage. Values and error bars represent the mean and standard deviation of n=3 biologically independent experiments (dots) 3 days after transfection.

a

	ABE7.10	vs ABEmax	
Site	p-value	t value	Significance
HEK2 A5	0.6933	0.4241	p>0.05
HEK2 A7	0.2012	1.528	p>0.05
Site 2 A5	0.0288	3.343	*
Site 5 A5	0.0089	4.768	**
Site 5 A7	0.0086	4.800	**
Site 13 A5	0.001	8.519	***
Site 13 A7	0.001	8.694	***
Site 16 A4	0.0105	4.539	*
Site 16 A5	0.0194	3.780	*
Site 16 A7	0.0723	2.425	p>0.05

b

Site 5 (A7), ABE7.10 vs. ABEmax

Plasmid dose (ng)	p-value	t value	Significance
750	0.0019	7.276	**
150	0.0002	13.80	***
30	<.0001	17.45	****
6	<.0001	17.74	****
1.2	0.0005	10.59	***
0.24	0.0577	2.638	p>0.05
0.048	0.1378	1.851	p>0.05
0.0096	0.093	2.196	p>0.05

Site 13 (A5), ABE7.10 vs. ABEmax

Plasmid dose (ng)	p-value	t value	Significance
750	0.0024	6.811	**
150	0.0003	11.62	***
30	0.0001	14.28	***
6	<.0001	20.47	****
1.2	0.0033	6.260	**
0.24	0.0623	2.566	p>0.05
0.048	0.2684	1.284	p>0.05
0.0096	0.8167	0.2474	p>0.05

Site 16 (A5), ABE7.10 vs ABEmax

Plasmid dose (ng)	p-value	t value	Significance
750	0.0077	4.958	**
150	<.0001	18.92	****
30	<.0001	38.54	****
6	<.0001	21.96	****
1.2	0.0008	9.274	***
0.24	0.0289	3.339	*
0.048	0.1048	2.091	p>0.05
0.0096	0.3917	0.9593	p>0.05

Supplementary Figure 11
Unpaired two-sided t-test p-values and t-values for editing by ABE7.10 and ABEmax

(a) Unpaired two-sided t-test p-values comparing ABE7.10 and ABEmax at five genomic loci tested with 750ng of ABE editor and 250ng gRNA in HEK293T cells. (**b)** Unpaired two-sided t-test p-values for ABE7.10 and ABEmax at Site 5 (A5), Site 13 (A5), and Site 16 (A5) across eight different plasmid doses in HEK293T cells. *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$. Values are from n=3 biologically independent experiments (dots) 3 days after transfection.

Site 5, ABE7.10

Plasmid dose (ng)	Indel %	Stdev
750	0.20	0.14
150	0.15	0.08
30	0.07	0.02
6	0.05	0.01
1.2	0.04	0.00
0.24	0.03	0.01
0.048	0.03	0.00
0.0096	0.03	0.01

Site 5, ABEmax

Plasmid dose (ng)	Indel %	Stdev
750	1.64	1.01
150	0.96	0.03
30	0.85	0.34
6	0.31	0.12
1.2	0.13	0.01
0.24	0.05	0.01
0.048	0.05	0.01
0.0096	0.04	0.01

Site 13, ABE7.10

Plasmid dose (ng)	Indel %	Stdev
750	0.25	0.15
150	0.15	0.07
30	0.10	0.03
6	0.04	0.02
1.2	0.02	0.00
0.24	0.02	0.00
0.048	0.02	0.01
0.0096	0.02	0.01

Site 13, ABEmax

Plasmid dose (ng)	Indel %	Stdev
750	1.52	0.65
150	0.73	0.13
30	0.55	0.23
6	0.28	0.11
1.2	0.12	0.03
0.24	0.05	0.03
0.048	0.02	0.01
0.0096	0.02	0.01

Site 16, ABE7.10

Plasmid dose (ng)	Indel %	Stdev
750	0.15	0.03
150	0.22	0.03
30	0.10	0.03
6	0.06	0.01
1.2	0.06	0.02
0.24	0.03	0.02
0.048	0.03	0.01
0.0096	0.04	0.01

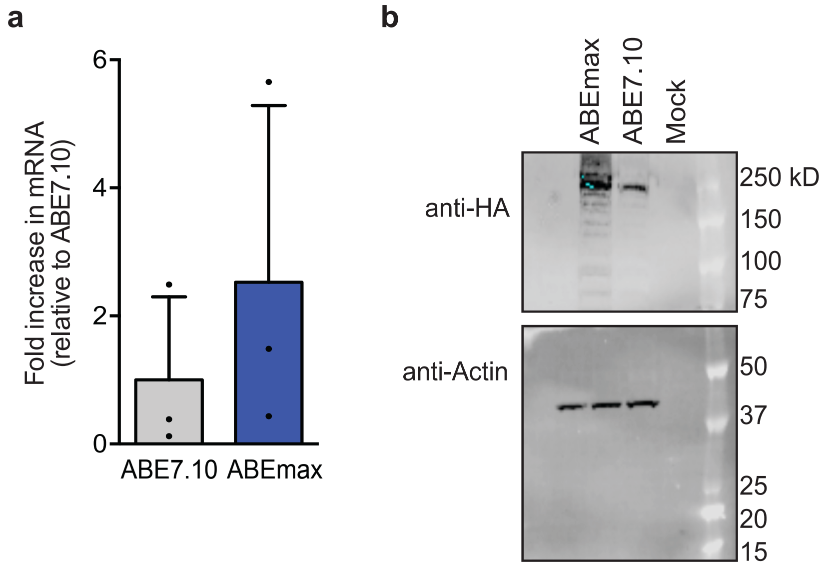
Site 16, ABEmax

Plasmid dose (ng)	Indel %	Stdev
750	0.47	0.12
150	0.41	0.09
30	0.36	0.11
6	0.23	0.05
1.2	0.17	0.04
0.24	0.08	0.02
0.048	0.04	0.01
0.0096	0.04	0.01

Supplementary Figure 12

Indel frequencies for ABE7.10 versus ABEmax at three genomic loci

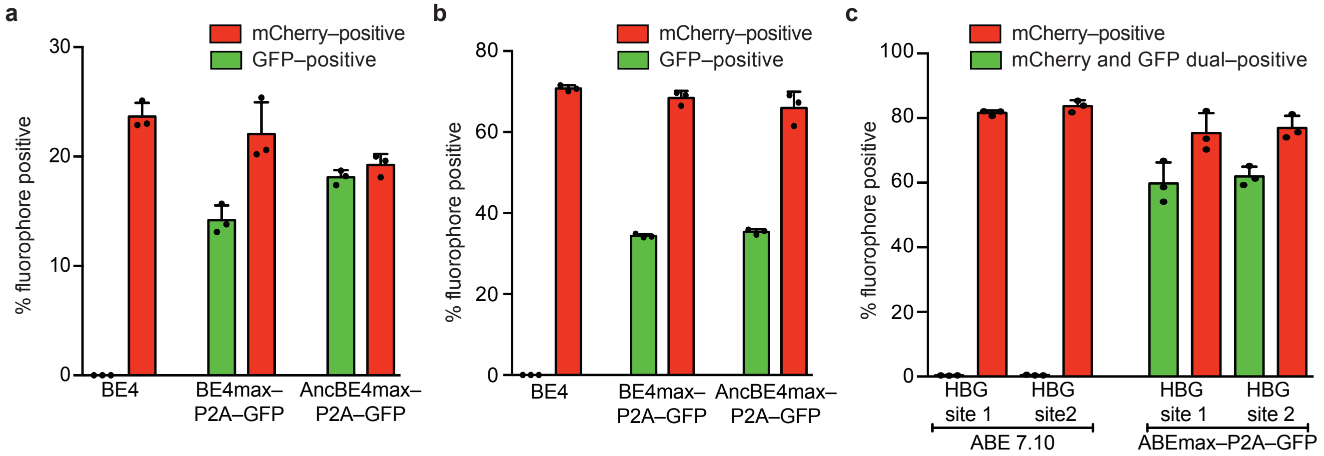
Indel frequencies are shown for ABE7.10 and ABEmax at Site 5, Site 13, and Site 16 across eight different plasmid doses in HEK293T cells. % Indel and Stdev values represent the mean and standard deviation of n=3 biologically independent experiments 3 days after transfection.



Supplementary Figure 13

Improved mRNA and protein levels for ABEmax compared to ABE7.10

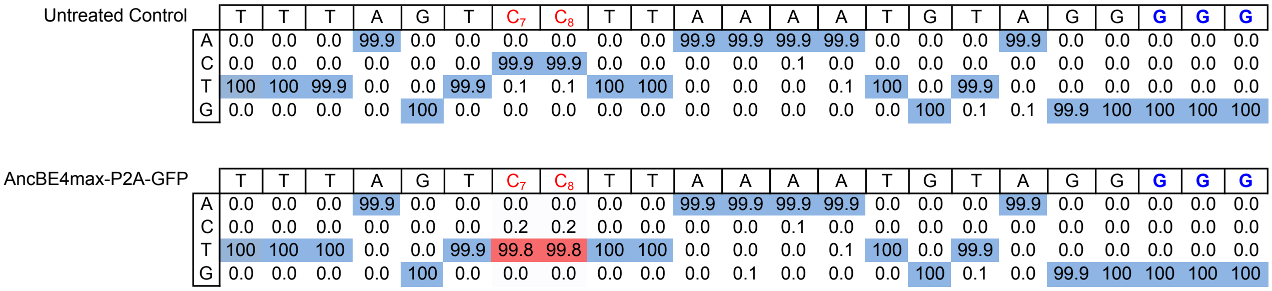
(**a**) ABEmax results in higher mRNA levels than ABE7.10 for all three replicates in HEK293T cells 3 days after base editor and sgRNA plasmid transfection as determined by RT-qPCR. mRNA and transfection levels were normalizations as described in Supplementary Fig. 9a. (**b**) Western blot of C-terminal HA-tagged ABE7.10 and ABEmax in HEK293T cells 3 days after plasmid transfection, visualizing with anti-HA (top) or anti-actin (bottom) antibodies. The mock sample is non-transfected cells. This blow was performed once. Values and error bars in (a) represent the mean and standard deviation of n=3 biologically independent experiments (dots) 3 days after transfection.



Supplementary Figure 14

Nucleofection and transfection efficiencies in type 1f fibroblasts, N2a cells, and HEK293T cells for disease-associated targets

(**a**) Congenital disorder of glycosylation type 1f fibroblasts were nucleofected with a plasmid encoding mCherry, a plasmid encoding the targeting sgRNA, and a plasmid encoding BE4, BE4max–P2A–GFP, or AncBE4max–P2A–GFP, then sorted after 3 days. (**b**) N2a cells were transfected with a plasmid encoding mCherry, a plasmid encoding the targeting sgRNA, and a plasmid encoding BE4, BE4max–P2A–GFP, or AncBE4max–P2A–GFP, then sorted after 3 days. (**c**) HEK293T cells were transfected with a plasmid encoding mCherry, a plasmid encoding the targeting sgRNA, and a plasmid encoding ABE7.10 or ABEmax–P2A–GFP, then sorted after 3 days. Values and error bars represent the mean and standard deviation of n=3 biologically independent experiments (dots) 3 days after transfection.



Untreated Control

	T	T	T	A	G	T	C_7	C_8	T	T	A	A	A	A	T	G	T	A	G	G	G	G	G
A	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0	99.9	99.9	99.9	99.9	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0	99.9	99.9	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T	100	100	99.9	0.0	0.0	99.9	0.1	0.1	100	100	0.0	0.0	0.0	0.1	100	0.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0
G	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100	0.1	0.1	99.9	100	100	100	100

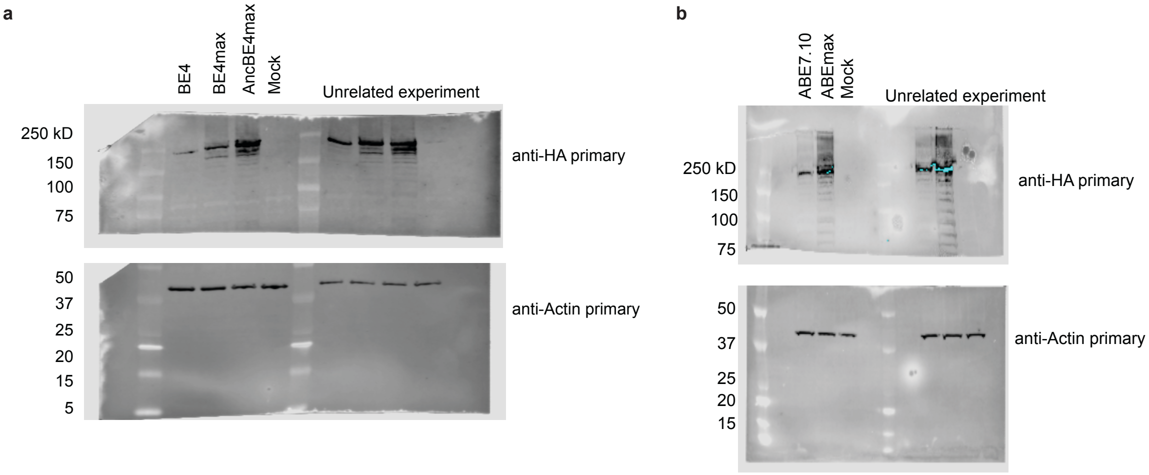
AncBE4max-P2A-GFP

	T	T	T	A	G	T	C_7	C_8	T	T	A	A	A	A	T	G	T	A	G	G	G	G	G
A	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0	99.9	99.9	99.9	99.9	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T	100	100	100	0.0	0.0	99.9	99.8	99.8	100	100	0.0	0.0	0.0	0.1	100	0.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0
G	0.0	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	100	0.1	0.0	99.9	100	100	100	100

Supplementary Figure 15

C•G-to-T•A base editing of the 3’ splice acceptor of SCN9a intron 6 in sorted N2a cells

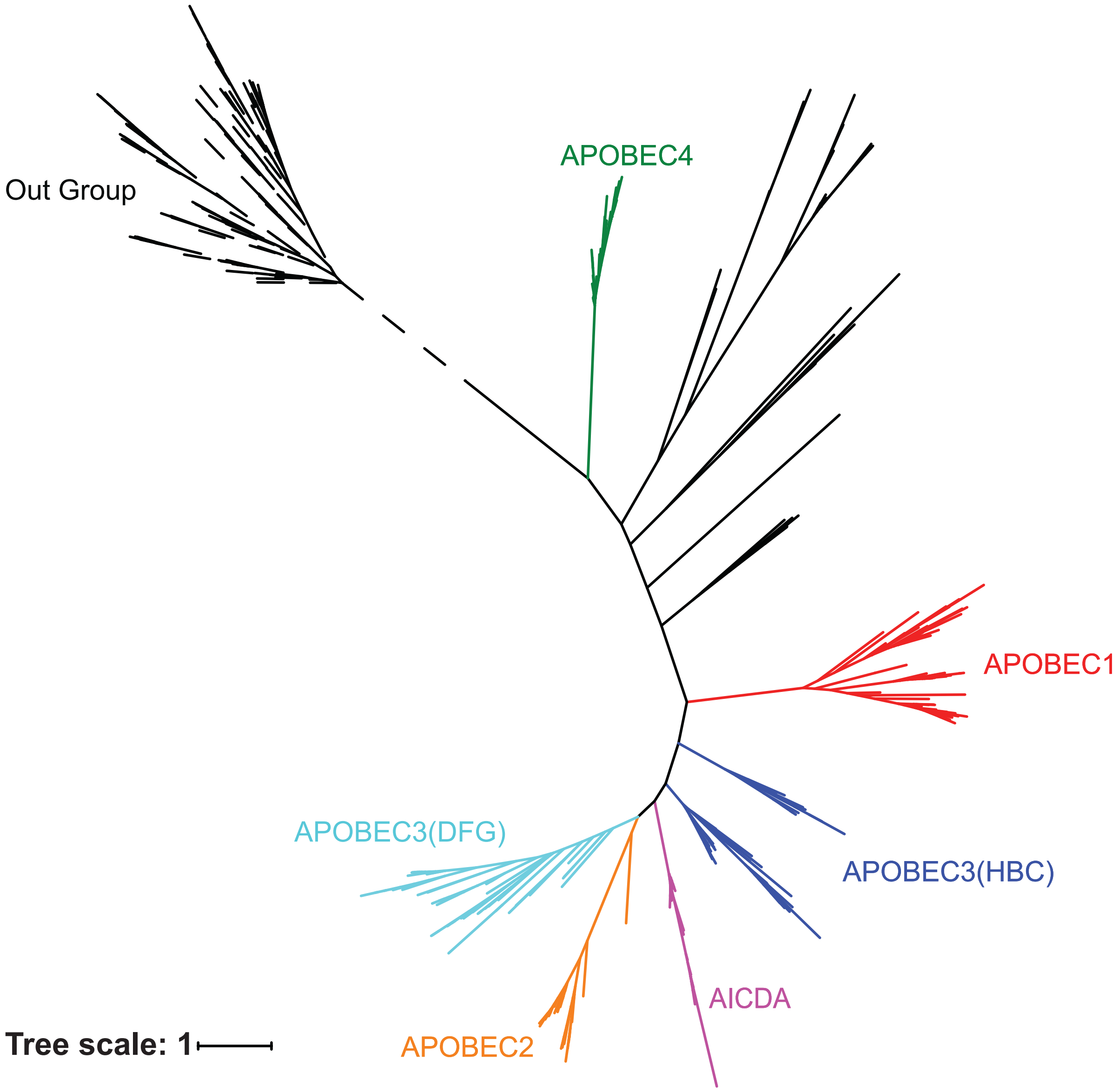
N2a cells were nucleofected with plasmids encoding AncBE4max–P2A–GFP and the targeting sgRNA. Following a 3 day incubation, GFP–positive cells were isolated by FACS and analyzed by HTS. The protospacer of the non-transcribed strand is shown here; C_7 corresponds to +1 G and C_8 corresponds to -1 G of the 3’ splice acceptor. The PAM is shown in blue.



Supplementary Figure 16

Original western blots for BE4, BE4max, AncBE4max, ABE7.10, and ABEmax

Unrelated experiments are of samples not discussed in this work.



Supplementary Figure 17

468-taxa unrooted phylogeny of APOBEC homologs

The tree is colored according to the UNIPROT annotation of sequences within each clade. Dotted lines indicate sequences used as an outgroup to root the tree in Figure 1f.

Supplementary Note 1
Python script to analyze pathogenic SNPs within the ClinVar database

```
import numpy as np
import pandas as pd

#download latest ClinVar from ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/tab_delimited/
#convert to csv
ClinVar = pd.read_csv('2018-04-23-variant_summary.csv')

#restrict to SNPs
ClinVar = ClinVar[ClinVar.Type == 'single nucleotide variant']

#restrict to pathogenic
ClinVar = ClinVar[ClinVar.ClinicalSignificance == 'Pathogenic']

#remove nans
ClinVar = ClinVar[ClinVar.ReferenceAllele != 'na']
ClinVar = ClinVar[ClinVar.AlternateAllele != 'na']

#drop duplicates of AlleleID
ClinVar = ClinVar.drop_duplicates('#AlleleID')

#total SNPs in ClinVar
total_SNPs = len(ClinVar)

def SNP_count(cv, ref, alt):
        ClinVar_ref = cv[cv.ReferenceAllele == ref]
        ClinVar_ref_alt = ClinVar_ref[ClinVar_ref.AlternateAllele == alt]
        return len(ClinVar_ref_alt)

counts = np.array([['', 'A', 'T', 'G', 'C'],
        ['A', 0, SNP_count(ClinVar, 'A', 'T'), SNP_count(ClinVar, 'A', 'G'), SNP_count(ClinVar, 'A', 'C')],
        ['T', SNP_count(ClinVar, 'T', 'A'), 0, SNP_count(ClinVar, 'T', 'G'), SNP_count(ClinVar, 'T', 'C')],
        ['G', SNP_count(ClinVar, 'G', 'A'), SNP_count(ClinVar, 'G', 'T'), 0, SNP_count(ClinVar, 'G', 'C')],
        ['C', SNP_count(ClinVar, 'C', 'A'), SNP_count(ClinVar, 'C', 'T'), SNP_count(ClinVar, 'C', 'G'), 0]
        ])

np.savetxt('ClinVar_SNPs.csv', counts, fmt='%5s', delimiter=',')
```

Supplementary Sequences 1
APOBEC homologs used for ancestral reconstruction

```
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>E9IK04_SOLIN/50-395
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>F6QYL4_MACMU/258-573
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>W5M4T6_LEPOC/238-558
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>ADR2_CAEEL/167-486
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>F6VU78_ORNAN/48-231
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>W5KNC9_ASTMX/26-194
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>H2M4W2_ORYLA/70-234
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>H2TE47_TAKRU/80-244
FQFQFKNVEYSGRNKTFLCYLVDTGKADEGLQRGYLEDEGSHAEEAFFTQCLPHDPALKYSVTWYMSSSPCCACAAKMAEALKARRNIKLSIFAARLFEWEEAEIQAGLKALHIAGCKIRVMKPLDFSYTWDTFVENDQPLNLWADCKENYEYYHERLAD

>I3JAF9_ORENI/80-244
FKFQFKNVEYSGRNKTLLCYLVDKGNTSDGLLRGYLEDEGTHAEEAFFTQCLPHNPALKYTVTWYVSSSPCSACAAKIAELLKDQKNLKLNIFAARLFEWEEGEIQAGLRTLHAAGCKLRVMKPLDFSYTWDTFVENDQSLNLWEDCKENYEYYHEKLSD

>A0A0F8BFJ4_LARCR/500-664
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>G3PCV8_GASAC/21-185
FKFQFKNVEYSGRNKTFLCYLVDKGNTDDGLLRGYLEDEGSHAEEAFFIQCIPNDPSVRYTVTWYVSSSPCAACAAKIAEVLKARKNIKLSIFAARLFECEETDIQAGLKAMHTAGCKLRVMKPLDFSYTWDTFVENEEPLNLWEDCKENYEYYQEKLAD

>A0A151P4M1_ALLMI/14-181
FKFQFKNVEYSGRNKTFLCYIVETQGKESGTIRGYLEDEAAHAEDAFFNTILPEESSLRYNVTWYVSSSPCVACAERIAEILKKNKNLRLAILVSRLFMWEEPEMQAVLKKMKAAGCKLRIMKPQDFEYVWQNFVEQESKDFVPWEDIQENFLYYEEKLAE

>K7G457_PELSI/58-225
FKFQFRNVEYSGRNKTFLCYIVETQGKESGSFRGYLEDEAAHAEGAFFNNILPTEPSLRYNVTWYVSSSPCVPCADQIAQILQKNKNLRLTILVSRLFMWEEPEMQAALKKLKTAGCKLKIMKPQDFEYIWQNFVEQEAKAFEPWEDIQENFLYYEEKLAE

>M7B9Y3_CHEMY/50-217
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>V8P1W3_OPHHA/51-183
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>G1KSP1_ANOCA/17-185
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>R7VNA1_COLLI/52-220
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>H1A4Q1_TAEGU/51-219
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>R0L7B9_ANAPL/57-225
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>A0A0Q3TEK9_AMAAE/51-219
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>F1NNK5_CHICK/51-219
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>F6PKH9_ORNAN/19-179
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>F7EXQ1_MONDO/49-218
FKFQFRNVEYSGRNKTFLCYVVEVQGKQGQISRGYLEDEAAHAEEAFFKTILPTDPALRYNVTWYVSSSPCAACADRISSTLSKTKNLKMLLLVGRLFMWEEPEIKTALKKLKESGCKLRIMKPQDFEYVWQNFVEQESKAFVPWEDIQENFLYYEEKLAE

>G3WZU9_SARHA/50-219
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>I3N5I3_ICTTR/52-221
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>A0A0B4J2I1_PONAB/52-221
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>F6QZ00_CALJA/52-221
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>G3TH88_LOXAF/52-221
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>H0WL56_OTOGA/52-221
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>L9KUD0_TUPCH/52-221
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>G3I9V7_CRIGR/52-221
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>ABEC2_MOUSE/52-221
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>F7AZT6_HORSE/52-221
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>ABEC2_BOVIN/52-221
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>S9WK76_CAMFR/64-233
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>F1RVM1_PIG/52-221
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>H0V2E8_CAVPO/56-225
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>A0A091DJF3_FUKDA/59-228
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>G5BNG8_HETGA/56-225
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>G1PHZ8_MYOLU/58-227
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>G1U7Q3_RABIT/55-224
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>M3W1K8_FELCA/61-230
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>G1LCS0_AILME/80-249
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>M3YQH9_MUSPF/61-230
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>E2RDL7_CANLF/52-221
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>S7PKW6_MYOBR/115-291
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>S7NDM8_MYOBR/6-177
FNSNFKNLDGGCKSTFLCFEVEREDGSVLYQNGVFRNQHAELCFIEWFHEKVLCPDAQYHVTWYISWSPCFECAEQVADFLNENENVDLSISAARLYLCEDEDEQGLQDLVATGAKVAMMAPEDFKYCWDNFVYNGWQFTYWKNVRRNYGRLQEKLDE

>S7PKW6_MYOBR/281-406
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>T0NHJ8_CAMFR/406-573
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>F7DDE1_HORSE/12-179
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>F7B644_HORSE/190-346
FSFHFRNLKFAGRKCSYLCYRVEGLSGSPGSEQGVFLNERRHAELCFLDWFRVRLSPDEYYRVTWFISWSPCSYCAREVADFLKQYRNVKLSIFAARLYYCRDHAQGLRSLCSSGAQLAIMFFWDFRYCWDNFVHNGRDFIPW

>F1MP61_BOVIN/29-199
FYFQFCNLLYARRNCSYICYKVERKYHSRAFDWGVFHNQRCHTELRFLSWFHAEKLRPNERYHITWFMSWSPCMKCAKEVADFLGRHQNVTLSIFTARLYNFQEEGSRQGLLRLSDQGAHVDIMSYQEFKYCWKKFVNSRRPFRPWKKLYRNYQRLVEELED

>L5K8J0_PTEAL/256-426
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>M3W3R0_FELCA/20-189
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>G1LWD3_AILME/20-186
FFFQFPSLCYAGRKFCYLCFQVGRGHPSDWGVFRNKPYHAESCFLSWFRAQNLSPDEDYHVTWFSSWSPCHTCADEVVEFLGQYRHVTLSIFAARLYYFWDPPFQNGLRRLQSAGVRLDIMSFADYKRCWENFVDHGMRFQSRNLLRHRDLLASRLEN

>L9KTG4_TUPCH/9-177
FYFHFQNLLFAGRNTTFLCCRVDKERHGTVLVSGVFTHQYHAESHFLLWFQKNFLSLDKDFQVTWYLSWSPCPACAKQVADFLAVHRNVSLTIFSARLYYFWDPEFRDGLHRLFEKGARVAIMSPKDFENCWEGFVFNGRDFRPWDNMVENYQSLRITLQE

>I3M955_ICTTR/14-186
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>G3I2J2_CRIGR/40-210
FYFHFKNLRYALRKNTFLCYEVNRECNELVLCQGVFRKENLHAEVCFLYWFHTQVLPPDEKYKITWYVSWSPCNECAEKVASFLDTHRNLSLAIFSSRLYYFWDPDYQDKLRRLNQAGAQIAAMDFPEFEKCWNKFVDNGKSFRPWKRLKINFRFQDNKLRD

>F7EWS7_RAT/40-210
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>ABEC3_RAT/26-195
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>ABEC3_MOUSE/26-195
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>F6XHA6_MACMU/213-383
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>A0A096NK50_PAPAN/213-383
FYFHFKNLRTADRNETWLCFAVEIKQRSTVWRTGVFRNQHCHAERCFLSWFRDNPLSPKKNYQVTWYTSWSPCPECAGEVAEFLARYSNVQLTIYTARLYYFWDTDYQEGLRSLSEEGASVEIMGYEDFKYCWENFVCDGEPFKPWKGINTNFRFLERHLRK

>G3RD21_GORGO/200-370
FYFHFKNLRKAGRNESWLCFTMEVKHHSPVWKRGVFRNQHCHAERCFLSWFCDDILSPNTNYQVTWYTSWSPCPECAGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLNQEGASVKIMGYKDFKYCWENFVYNDEPFKPWKGLKYNFLFLDSKLQE

>A0A096NK49_PAPAN/167-338
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>A0A0D9R238_CHLSB/197-367
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>G3RRB4_GORGO/39-209
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>G3RUE8_GORGO/17-187
FYFQFKNLWEADRNETWLCFTVEGKRRSVVWKTGVFRNQHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGKVAEFLARHSNVNLTIFTARLCYFWDTDYQEGLRSLNQEGASVKIMGYKDFVSCWKNFVYSDEPFKPWKGLQTNFRLLKRRLRE

>Q6ICH2_HUMAN/17-199
FYDNFENEPILGRSYTWLCYEVKIRGRSNLWDTGVFRGEHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPECAGEVAEFLARHSNVNLTIFTARLCYFWDTDYQEGLCSLSQEGASVKIMGYKDFVSCWKNFVYSDEPFKPWKGLQTNFRLLKRRLRE

>ABC3D_HUMAN/213-383
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>ABC3C_GORGO/17-187
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>ABC3C_HUMAN/17-187
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>H2P4E8_PONAB/17-187
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>A0A096NK46_PAPAN/17-187
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>A0A0D9R255_CHLSB/17-187
FYFQFKNLREANRNETWLCFTVEGRHHLTVWKTGVFRNQHCHAEKCFLSWFCKNILSPNTDYQVTWYTSWSPCPECAREVAKFLARHNNVMLTIYTARLYYSQCPNYQQGLRSLSEKGVSVEIMDYEDFKYCWQNFVYDGEPFKPWKGLKTSFRFLKRCLRE

>F6XHB3_MACMU/37-207
FYFQFKNLREANRNETWLCFAVEIKQRSTVLKTGVFRNQHCHAERCFLSWFCEDILSPNTDYQVTWYTSWSPCLDCAGEVAKFLARHNNVMLTIYTARLYYSQYPNYQQGLRSLSEKGVSVKIMDYEDFKYCWEKFVYDGEPFKPWKGLKTSFRFLKRRLRE

>H0VE76_CAVPO/1-121
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>G5AYU4_HETGA/17-183
FYFHFENLPYAGRNKTFLCYEVKRRDNKLHKGVVQNQLSRTELSFISCFHATELCLDETYKVTWYISWSPCVECAEEIVKFLANHRNVFLTVFIARLYYYREHTFKEGLQALDNGGVQMHMMCLQDFKDCWSLFVSETFRPWKGLRKYYLFQNKTLKQ

>H0VZF7_CAVPO/17-183
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>G5AYU3_HETGA/12-181
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>G5AYU5_HETGA/20-189
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>A0A091EM42_FUKDA/42-211
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>A0A091EM42_FUKDA/225-394
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>H0XYD2_OTOGA/17-183
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>H0XYD2_OTOGA/192-348
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>A0A096N7U5_PAPAN/17-167
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>H9KW44_CALJA/2-132
QHHPEMRFLHWFRKWKLHSDQEYEVTWFVSWSPCPVCARNVAEFLTEDGKVTLTIFVARLYYFWIPHYREELRRLCQPRATMKIMSYGEFQHCWDKFVDNRLYKPWNKLPKHYTLLHITLGE

>ABC3G_PAPAN/17-191
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>G7PFK4_MACFA/13-187
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>A0A0D9R229_CHLSB/28-202
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>ABC3G_MACMU/10-184
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>H2P4E9_PONAB/31-202
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>ABC3G_HUMAN/17-190
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>ABC3G_GORGO/17-190
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>G3RD21_GORGO/17-186
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>ABC3F_HUMAN/17-186
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>A0A096NK45_PAPAN/17-187
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>F7FXJ2_MACMU/2-130
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>A0A0D9R238_CHLSB/17-183
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>A0A096NK50_PAPAN/17-199
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>F6XHA6_MACMU/17-199
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>A0A0D9R289_CHLSB/17-187
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>G1RYY7_NOMLE/17-187
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>H2P4E7_PONAB/17-187
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>H2QLP5_PANTR/17-187
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>ABC3D_HUMAN/17-199
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>ABC3B_HUMAN/17-187
FYDNFENEPILGRSYTWLCYEVKIRGRSNLWDTGVFRGQQYHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEFLSEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVTIMDYEEFAYCWENFVYNGQQFMPWYKFDENYAFLHRTLKE

>G3SFT2_GORGO/17-187
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Supplementary Sequences 2
Amino acid sequences of BE4, rAPOBEC1, ancestral APOBECs, ABE7.10, and P2A–GFP

Within base editor sequences, NLS sequences are purple, APOBEC sequences are blue, linkers are yellow, Cas9 nickase sequence is green, and UGI sequences are grey.

BE4max and AncBE4max
MKRTADGSEFESPKKKRKV_[APOBEC]_SGGSSGGSSGSETPGTSESATPESSGGSSGGS_DKKYSIGLAIGT
NSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFR
GHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI
ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNR
EDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEE
TITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTL
FEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQK
NSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVET
RQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKY
PKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE
QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD_SGGSGGSGGS_T
NLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGEN
KIKML_SGGSGGSGGS_TNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSD
APEYKPWALVIQDSNGENKIKML_SGGS_KRTADGSEFEPKKKRKV [optional P2A–GFP]

Rat APOBEC1
SSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYF
CPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYC
WRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATG
LK

Anc689 APOBEC
SSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEIKWGTSHKIWRHSSKNTTKHVEVNFIEKFTSERHF
CPSTSCSITWFLSWSPCGECSKAITEFLSQHPNVTLVIYVARLYHHMDQQNRQGLRDLVNSGVTIQIMTAPEYDY
CWRNFVNYPPGKEAHWPRYPPLWMKLYALELHAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWA
TGLK

Anc687 APOBEC
SSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKEACLLYEIKWGTSHKIWRNSGKNTTKHVEVNFIEKFTSERHF
CPSISCSITWFLSWSPCWECSKAIREFLSQHPNVTLVIYVARLFQHMDQQNRQGLRDLVNSGVTIQIMTASEYDH
CWRNFVNYPPGKEAHWPRYPPLWMKLYALELHAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWA
TGLK

Anc686 APOBEC
SSETGPVAVDPTLRRRIEPEFFNRNYDPRELRKETYLLYEIKWGKESKIWRHTSNNRTQHAEVNFLENFFNELYF
NPSTHCSITWFLSWSPCGECSKAIVEFLKEHPNVNLEIYVARLYLCEDERNRQGLRDLVNSGVTIRIMNLPDYNY

CWRTFVSHQGGDEDYWPRHFAPWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK

Anc655 APOBEC

SSETGPVAVDPTLRRRIEPFYFQFNNDPRACRRKTYLCYELKQDGSTWVWKRTLHNKGRHAEICFLEKISSLEKLDPAQHYRITWYMSWSPCSNCAQKIVDFLKEHPHVNLRIYVARLYYHEEERYQEGLRNLRRSGVSIRVMDLPDFEHCWETFVDNGGGPFQPWPGLEELNSKQLSRRLQAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK

Anc733 APOBEC

SSETGPVAVDPTLRRRIEPFHFQFNNDPRAYRRKTYLCYELKQDGSTWVLDRTLRNKGRHAEICFLDKINSWERLDPAQHYRVTWYMSWSPCSNCAQQVVDFLKEHPHVNLRIFAARLYYHEQRRYQEGLRSLRGSGVPVAVMTLPDFEHCWETFVDHGGRPFQPWDGLEELNSRSLSRRLQAGILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK

P2A–GFP

GSGATNFSLLKQAGDVEENPGP_MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKSGGSPKKKRKV

ABEmax

MKRTADGSEFESPKKKRKV_MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD_SGGSSGGSSGSETPGTSESATPESSGGSSGGSS_EVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD_KRTADGSEFEPKKKRKV[optional P2A–GFP]

Supplementary Sequences 3

Codon optimized nucleotide sequences of BE4, rAPOBEC, Ancestral APOBECs, ABE7.10, and P2A GFP

Within base editor sequences, NLS sequences are purple, APOBEC sequences are blue, linkers are yellow, Cas9 nickase sequence is green, and UGI sequences are grey.

BE4max and AncBE4max

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTC_APOBEC_TCTGGAGGATCTAGCGGAGGATCCTCTGGCAGCGAGACACCAGGAACAAGCGAGTCAGCAACACCAGAGAGCAGTGGCGGCAGCAGCGGCGGCAGC_GACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGC

AGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCT
GATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTT
ACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGC
CCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGG
AAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCAT
GAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAAC
GGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATG
CCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCA
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CCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGT
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AGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTGG
AAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCT
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CAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTC
CAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAG
CCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTT
CAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTG
ATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGTGAC_AGCGGC
GGGAGCGGCGGGAGCGGGGGGAGC_ACTAATCTGAGCGACATCATTGAGAAGGAGACTGGGAAACAGCT
GGTCATTCAGGAGTCCATCCTGATGCTGCCTGAGGAGGTGGAGGAAGTGATCGGCAACAAGCCAGAGTCT
GACATCCTGGTGCACACCGCCTACGACGAGTCCACAGATGAGAATGTGATGCTGCTGACCTCTGACGCCC
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AGGATCCGGAGGATCTGGAGGCAGC_ACCAACCTGTCTGACATCATCGAGAAGGAGACAGGCAAGCAGCT
GGTCATCCAGGAGAGCATCCTGATGCTGCCCGAAGAAGTCGAAGAAGTGATCGGAAACAAGCCTGAGAGC
GATATCCTGGTCCATACCGCCTACGACGAGAGTACCGACGAAAATGTGATGCTGCTGACATCCGACGCCC
CAGAGTATAAGCCCTGGGCTCTGGTCATCCAGGATTCCAACGGAGAGAACAAAATCAAAATGCTG_TCTGG
CGGCTCA_AAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAAGAGGAAAGTC_ [optional P2A–
GFP]_TAA

Rat APOBEC1
TCCTCAGAGACTGGGCCTGTCGCCGTCGATCCAACCCTGCGCCGCCGGATTGAACCTCACGAGTTTGAAG
TGTTCTTTGACCCCCGGGAGCTGAGAAAGGAGACATGCCTGCTGTACGAGATCAACTGGGGAGGCAGGCA
CTCCATCTGGAGGCACACCTCTCAGAACACAAATAAGCACGTGGAGGTGAACTTCATCGAGAAGTTTACCA
CAGAGCGGTACTTCTGCCCCAATACCAGATGTAGCATCACATGGTTTCTGAGCTGGTCCCCTTGCGGAGAG
TGTAGCAGGGCCATCACCGAGTTCCTGTCCAGATATCCACACGTGACACTGTTTATCTACATCGCCAGGCT
GTATCACCACGCAGACCCAAGGAATAGGCAGGGCCTGCGCGATCTGATCAGCTCCGGCGTGACCATCCAG
ATCATGACAGAGCAGGAGTCCGGCTACTGCTGGCGGAACTTCGTGAATTATTCTCCTAGCAACGAGGCCCA
CTGGCCTAGGTACCCACACCTGTGGGTGCGCCTGTACGTGCTGGAGCTGTATTGCATCATCCTGGGCCTG
CCCCCTTGTCTGAATATCCTGCGGAGAAAGCAGCCCCAGCTGACCTTCTTTACAATCGCCCTGCAGTCTTG
TCACTATCAGAGGCTGCCACCCCACATCCTGTGGGCCACAGGCCTGAAG

Anc689 APOBEC
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CAAGATCTGGCGCCACAGCTCCAAGAACACCACAAAGCACGTGGAAGTGAATTTCATCGAGAAGTTTACCT
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GTATCACCACATGGACCAGCAGAACAGGCAGGGCCTGCGCGATCTGGTGAATTCTGGCGTGACCATCCAG
ATCATGACAGCCCCAGAGTACGACTATTGCTGGCGGAACTTCGTGAATTATCCACCTGGCAAGGAGGCACA
CTGGCCAAGATACCCACCCCTGTGGATGAAGCTGTATGCACTGGAGCTGCACGCAGGAATCCTGGGCCTG

CCTCCATGTCTGAATATCCTGCGGAGAAAGCAGCCCCAGCTGACATTTTTCACCATTGCTCTGCAGTCTTGT
CACTATCAGCGGCTGCCTCCTCATATTCTGTGGGCTACAGGCCTTAAA

Anc687 APOBEC
TCATCAGAAACAGGACCAGTCGCCGTGGACCCAACACTGAGGAGAAGGATTGAGCCCCATGAATTTGAAG
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CAAGATCTGGCGCAACAGCGGCAAGAACACCACAAAGCACGTGGAAGTGAATTTCATCGAGAAGTTTACCT
CCGAGCGGCACTTCTGCCCCTCTATCAGCTGTTCCATCACATGGTTTCTGTCTTGGAGCCCTTGCTGGGAG
TGTTCCAAGGCCATCCGCGAGTTCCTGTCTCAGCACCCTAACGTGACCCTGGTCATCTACGTGGCCCGGC
TGTTTCAACACATGGACCAGCAGAACAGGCAGGGCCTGCGCGATCTGGTGAATTCTGGCGTGACCATCCA
GATCATGACAGCCTCAGAGTACGACCATTGCTGGCGGAACTTCGTGAATTATCCACCTGGCAAGGAGGCA
CACTGGCCAAGATACCCACCCCTGTGGATGAAGCTGTATGCACTGGAGCTGCACGCAGGAATCCTGGGCC
TGCCTCCATGTCTGAATATCCTGCGGAGAAAGCAGCCCCAGCTGACATTTTTCACTATCGCACTGCAGAGC
TGTCATTACCAGAGACTGCCTCCTCATATCCTGTGGGCTACAGGCCTTAAA

Anc686 APOBEC
AGCAGCGAGACAGGACCCGTGGCAGTGGACCCTACACTGAGGAGGAGGATTGAGCCCGAATTTTTCAACA
GGAACTACGACCCCAGAGAGCTGCGGAAGGAGACATACCTGCTGTATGAGATCAAGTGGGGCAAGGAGTC
CAAGATCTGGCGGCACACCTCTAACAATAGAACACAGCACGCCGAGGTGAACTTCCTGGAGAACTTCTTTA
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TGTTCTAAGGCCATCGTGGAGTTTCTGAAGGAGCACCCCAACGTGAATCTGGAGATCTACGTGGCCAGGCT
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GCCCCCTTGTCTGAACATCCTGCGGAGAAAGCAGCCCCAGCTGACATTCTTCACCATCGCACTGCAGAGTT
GTCATTACCAGCGACTGCCTCCTCATATCCTGTGGGCTACAGGCCTTAAA

Anc655 APOBEC
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CAACAACGACCCAAGAGCCTGCCGGAGAAAGACCTACCTGTGCTATGAGCTGAAGCAGGACGGCTCTACC
TGGGTGTGGAAGCGGACACTGCACAACAAGGGCAGACACGCCGAGATCTGCTTCCTGGAGAAGATCAGCT
CCCTGGAGAAGCTGGACCCTGCCCAGCACTACAGGATCACATGGTATATGTCTTGGAGCCCATGCTCCAA
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CTGTACTATCACGAGGAGGAGAGGTATCAGGAGGGCCTGAGGAACCTGAGGCGCTCCGGCGTGTCTATCA
GAGTGATGGACCTGCCCGATTTCGAGCACTGCTGGGAGACATTCGTGGATAACGGAGGAGGACCTTTCCA
GCCATGGCCCGGCCTGGAGGAGCTGAATAGCAAGCAGCTGTCCCGGAGACTGCAGGCAGGAATCCTGGG
CCTGCCCCCTTGTCTGAATATCCTGAGGCGCAAGCAGCCCCAGCTGACATTTTTCACCATCGCACTGCAGA
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Anc733 APOBEC
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CCTGGGAGAGGCTGGATCCCGCCCAGCACTACCGCGTGACATGGTATATGAGCTGGTCCCCTTGCTCTAA
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CTGTACTATCACGAGCAGAGGCGCTATCAGGAGGGCCTGCGGAGCCTGAGGGGAAGCGGAGTGCCTGTG
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GCCTGCCCCCTTGTCTGAATATCCTGAGGCGCAAGCAGCCCCAGCTGACCTTTTTTACCATCGCACTGCAG
AGTTGTCACTACCAGAGACTGCCTCCTCATATCCTGTGGGCTACAGGCCTTAAA

P2A–GFP

TCTGGTGGTTCTCCCAAGAAGAAGAGGAAAGTCGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGG
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CCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCG
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TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTA
CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGA
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GCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCA
CTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG
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AGAGGAAAGTCTAA

ABEmax

ATGAAACGGACAGCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTC_TCTGAAGTCGAG
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CGAGGATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATC
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AGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCG
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AGGAAAACGAGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAG
GAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACA
CCGGCTGGGGCAGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCC
TGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACC
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TGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGG
GACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCC
TGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGG
GCGGGATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTG
CCTCAGAGCTTTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCA
AGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGC
CAAGCTGATTACCCAGAGAAAGTTCGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGAT
AAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGG
ACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAA
GTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCACC
ACGCCCACGACGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAG
CGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATC
GGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGC
CAACGGCGAGATCCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAA
GGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAG
GTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAA
AGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGT
GGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATG
GAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGA
CCTGATCATCAAGCTGCCTAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCT
GCCGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCA
GCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAA
GCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAAT
CTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCA
TCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGG
AAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACG
AGACACGGATCGACCTGTCTCAGCTGGGAGGTGAC_TCTGGCGGCTCAA_AAAGAACCGCCGACGGCAGC
GAATTCGAGCCCAAGAAGAAGAGGAAAGTC_[optional P2A–GFP]_TAA

Supplementary Sequences 4

Target protospacer sequences used in this study.

Target Cs and As are shown in red, with a subscripted number denoting spacer position. PAM sequences are shown in blue.

HEK293_site 2	GAAC$_4$AC$_6$AAAGCATAGACTGCGGG
HEK293_site 3	GGCC$_4$C$_5$AGACTGAGCACGTGATGG
HEK293_site 4	GGCAC$_5$TGCGGCTGGAGGTCCGGG
RNF2	GTCATC$_6$TTAGTCATTACCTGAGG
EMX1	GAGTC$_5$C$_6$GAGCAGAAGAAGAAGGG
SCN9a	GTTAGTC$_7$C$_8$TTAAAATGTAGGGGGG
MPDU1	GTTC$_4$C$_5$C$_6$GGTC$_{10}$ATGCACTACAGAGG
ABE_site 2	GAGTA$_5$TGA$_7$GGCATAGACTGCAGG
ABE_site 5	GATGA$_5$GA$_7$TAATGATGAGTCAGGG
ABE_site 13	GAAGA$_5$TA$_7$GAGAATAGACTGCTGG
ABE_site 16	GGGA$_4$A$_5$TA$_7$AATCATAGAATCCTGG
*HBG*_site 1	CTTGA$_5$CCA$_8$A$_9$TA$_{11}$GCCTTGACAAGG
*HBG*_site 2	A$_1$TA$_3$TTTGCA$_9$TTGA$_{13}$GATAGTGTGG

Supplementary Sequences 5
Primers used in this study

All oligonucleotides were purchased from Integrated DNA Technologies (IDT).

Primers used for generating sgRNA plasmids

The *MPDU1* guide plasmid was cloned by digesting a modified version of pFYF1320 (Fu, Y. et al. *Nature biotechnology* **31**, 822-826 (2013)) in which BsmBI restriction cut sites were installed via KLD cloning. The primers below were phosphorylated and annealed to enable ligation into BsmBI cut backbone. All guides were designed to include a 5'-G to enable transcription from the hU6 transcription. CCACC was included at the 5' end of the forward primer, and AAAC was included at the 5' end of the reverse primer to complement the overhands generated by restriction digest. The HEK2, HEK3, HEK4, *RNF2*, *EMX1*, ABE site 2, ABE site 5, ABE site 13, ABE site 16, *SCN9a*, *HBG* site 1, *HBG* site 2 sgRNAs were prepared by KLD cloning as previously described (Kim, Y.B. et al. *Nature biotechnology* **35**, 371-376 (2017)) using the primers listed below.

Primers for MPDU1 sgRNA

JLD 85	CACCGTTCCCGGTCATGCACTACAG
JLD 86	AAACCTGTAGTGCATGACCGGGAAC

Primers for SCN9a, HBG site 1, HBG site 2, and previously used sgRNAs

Universal reverse primer GGTGTTTCGTCCTTTCCACAAG

fwd_HEK293_site 2	GAACACAAAGCATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_HEK293_site 3	GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_HEK293_site 4	GGCACTGCGGCTGGAGGTGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_RNF2	GTCATCTTAGTCATTACCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_EMX1	GAGTCCGAGCAGAAGAAGAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_ABE_site 2	GAGTATGAGGCATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_ABE_site 5	GATGAGATAATGATGAGTCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_ABE_site 13	GAAGATAGAGAATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_ABE_site 16	GGGAATAAATCATAGAATCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_SCN9a	GTTAGTCCTTAAAATGTAGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_HBG_site 1	GCTTGACCAATAGCCTTGACAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
fwd_HBG_site 2	GATATTTGCATTGAGATAGTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC

Primers to amplify genomic loci for HTS of mammalian cell culture experiments

SCN9a HTS Fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCGAACACACTGAGACAGAAC
SCN9a HTS Rev	TGGAGTTCAGACGTGTGCTCTTCCGATCT GCACTCCTAGTTAGGCTTGTG
Type 1f HTS Fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCCCTGGATGGATGGGCTATGG
Type 1f HTS Rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTTTCCCAGACCTGAGTTCCC
HBG HTS Fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCTGGCCTCACTGGATACTC
HBG HTS Rev	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGACAAAAGAAGTCCTGGTATC
fwd_HEK293_site 2_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGCCCCATCTGTCAAACT
rev_HEK293_site 2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCCTTGGAAACAATGA
fwd_HEK293_site 3_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
rev_HEK293_site 3_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCCAAACTTGTCAACC
fwd_HEK293_site 4_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAACCCAGGTAGCCAGAGAC
rev_HEK293_site 4_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTTCAACCCGAACGGAG
fwd_RNF2_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGTCTCATATGCCCCTTGG

rev_RNF2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGTAGGAATTTTGGTGGGACA
fwd_EMX1_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTTGA
rev_EMX1_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGTGGGTTTGTGGTTGC
fwd_ABE_site 2_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
rev_ABE_site 2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
fwd_ABE_site 5_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCTGAGGTCACACAGTGGG
rev_ABE_site 5_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGAGCAGGGACCACATC
fwd_ABE_site 13_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCACTTCAGCCCAGGAGTAT
rev_ABE_site 13_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTTTCTCTCCCCCACCC
fwd_ABE_site 16_HTS	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGGAGGTGGAGAGAGGATGT
rev_ABE_site 16_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGAGGTCTAGGAACCCG