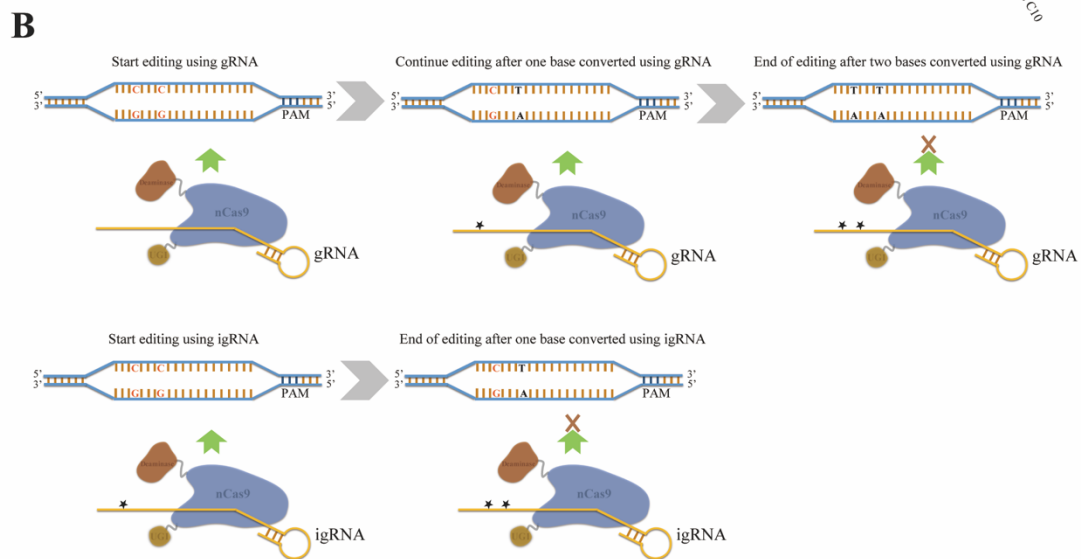
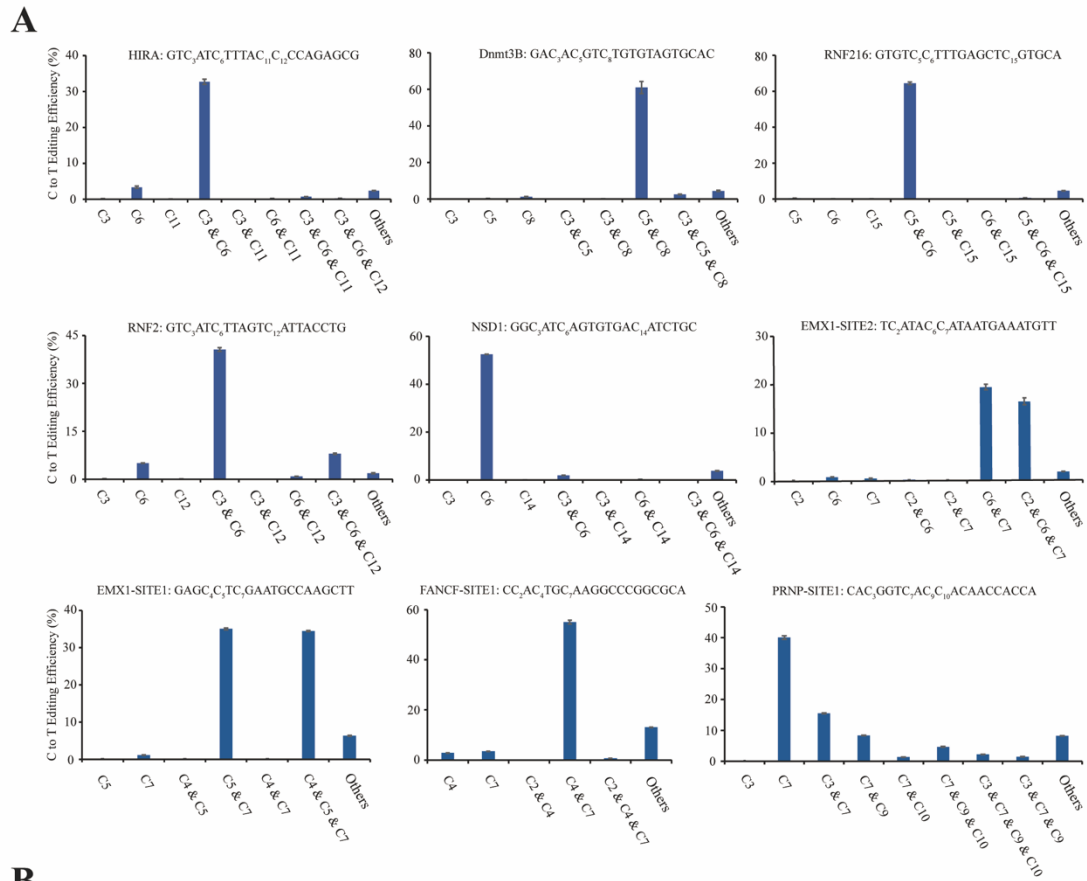


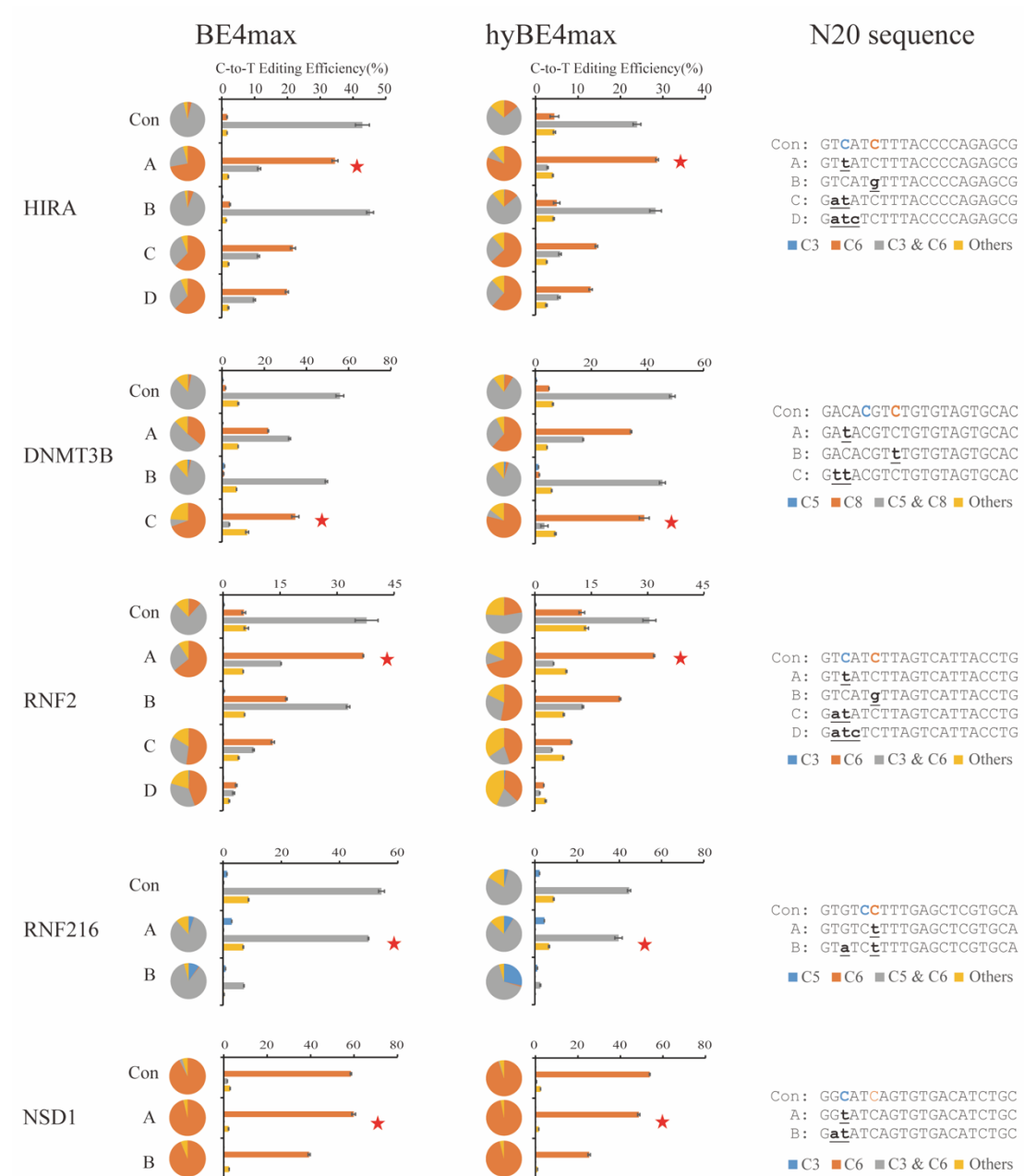
Supplementary Information

Imperfect guide-RNA (igRNA) enables CRISPR single-base editing with ABE and CBE

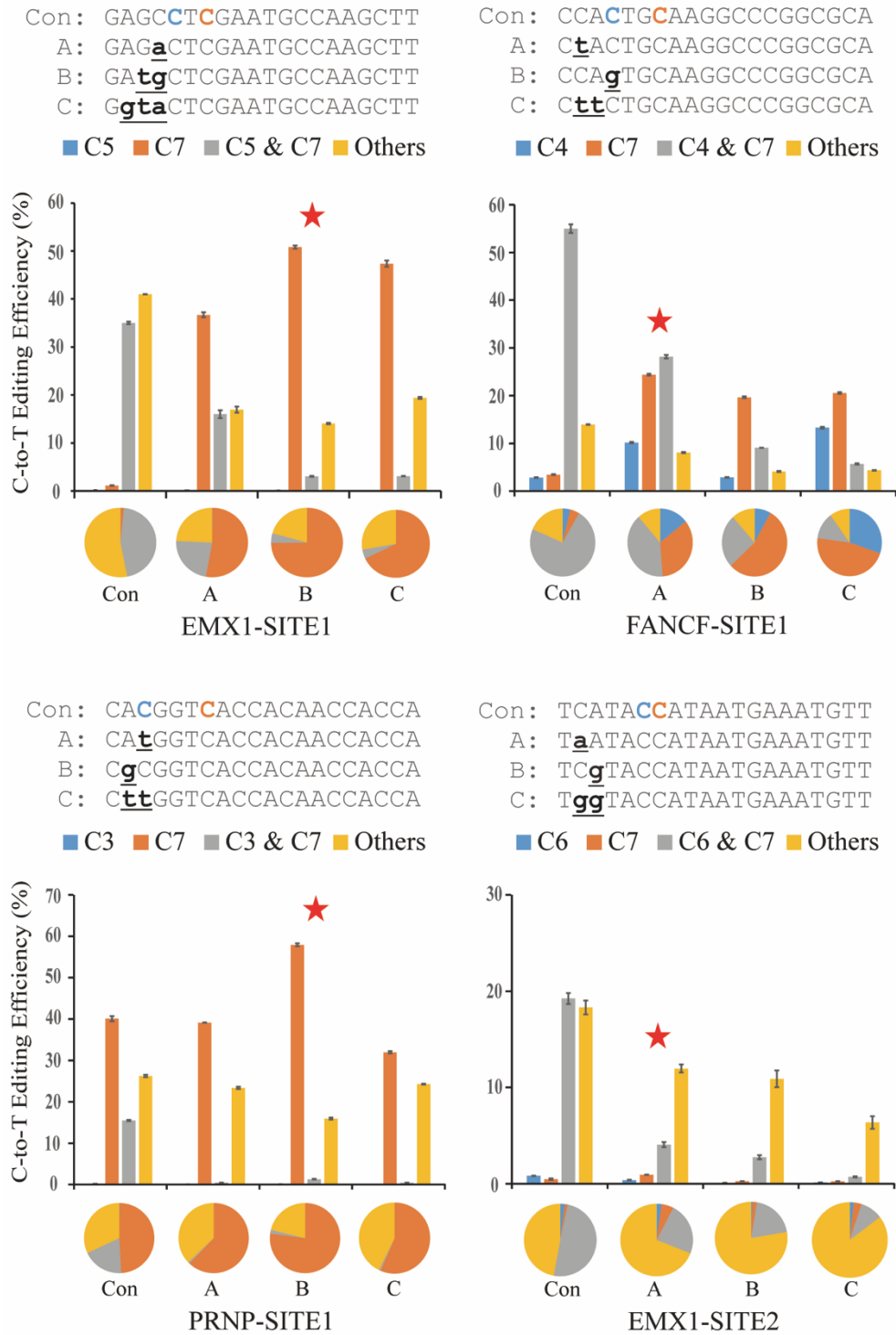
Dongdong Zhao, Guo Jiang, Ju Li, Xuxu Chen, Siwei Li, Jie Wang, Zuping Zhou,
Shiming Pu, Zhubo Dai, Yanhe Ma, Changhao Bi & Xueli Zhang



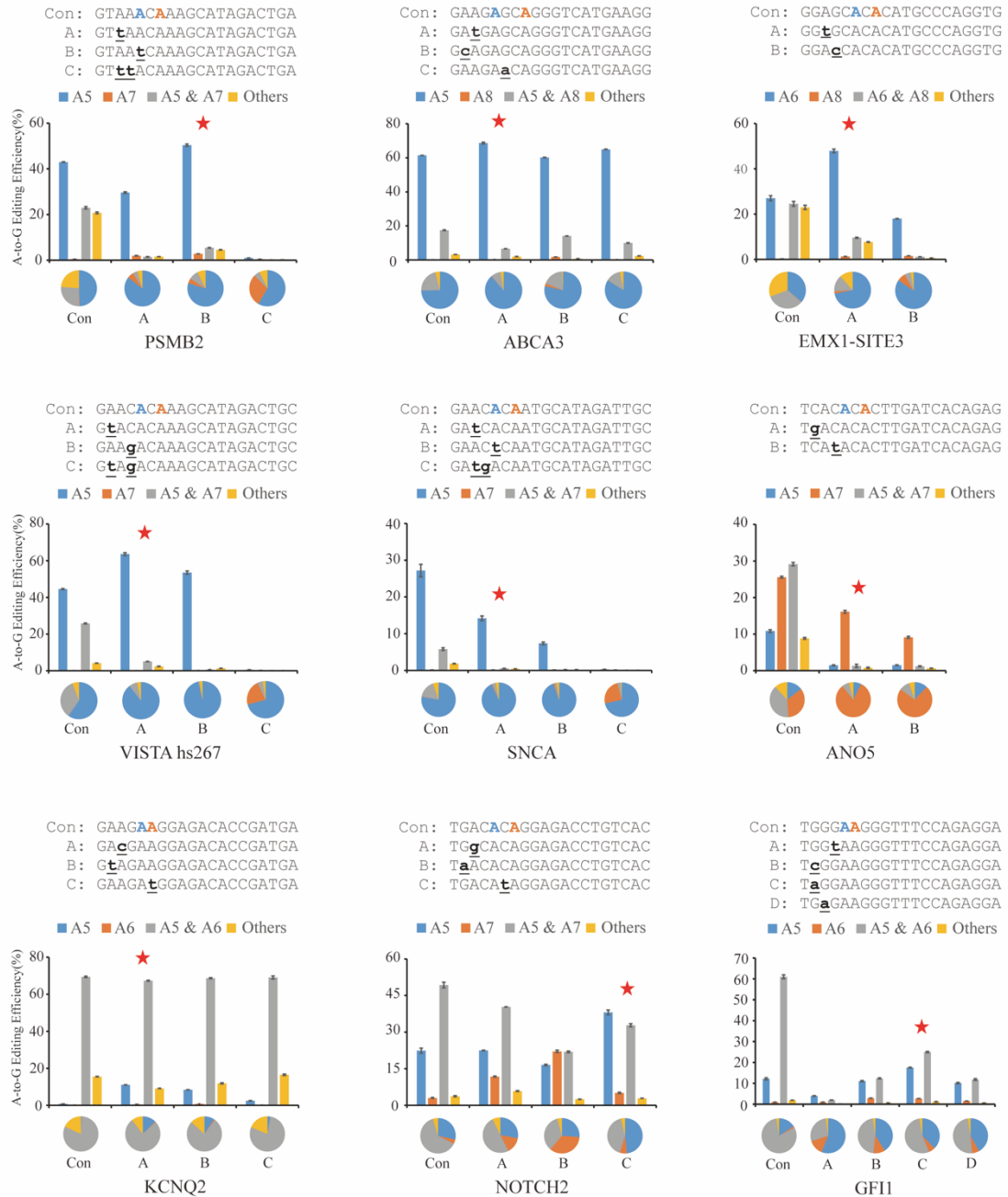
Supplementary Figure S1. Distribution of the various cytosine base editing products and the schematic diagram of the working principle of igRNA. **A.** The editing efficiencies of various base editing products demonstrating the distribution of the editing patterns of BE4max at 9 loci in HEK293T cells. The editing results were calculated from the deep sequencing, which provided the sequence of each single DNA strand. **B.** The schematic diagram of the hypothesized working principle of igRNA. The bars represent the average editing specificity, and the error bars represent the s.d. of three independent biological replicates.



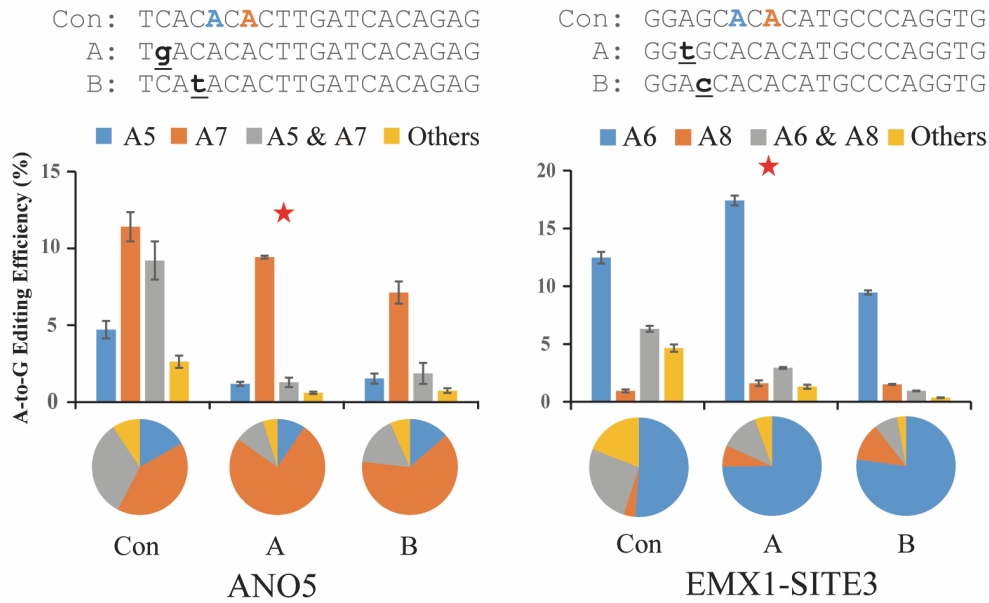
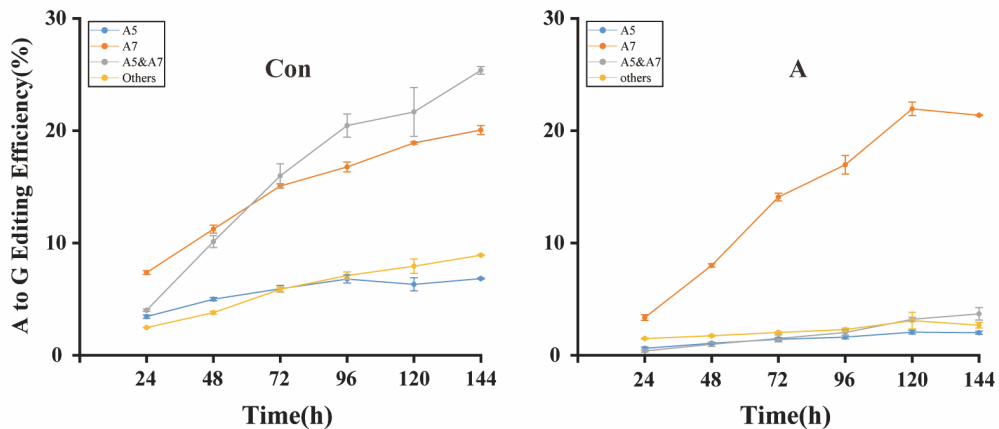
Supplementary Figure S2. Editing efficiencies and editing product fractions of cytosine base editing with igRNA in HeLa cells. The base editing efficiency and fractions of editing products were obtained from 5 loci using gRNAs and igRNAs with the BE4max and hyBE4max editors. Con, the control gRNAs that exactly matched the target sequence. Capital A to D, different igRNAs. The red asterisk represents the igRNA with the best performance. The bars represent mean values, and the error bars represent the s.d. of three independent biological replicates. The pie charts represent the fractions of various types of editing products.



Supplementary Figure S3. Editing efficiencies and editing product fractions of cytosine base editing with igRNA in HEK293T cells. The base editing efficiencies and fractions of editing products were obtained from 4 loci using gRNAs and igRNAs with the BE4max editors. Con, the control gRNAs that exactly matched the target sequence. Capital A to C, different igRNAs. The red asterisk represents the igRNA with the best performance. The bars represent mean values, and the error bars represent the s.d. of three independent biological replicates. The pie charts represent the fractions of various types of editing products.



Supplementary Figure S4. Editing efficiencies and editing product fractions of adenine base editing with igRNA in HeLa cells. Base editing efficiency and fractions of editing products obtained from 9 loci by the NG-ABEmax editor using gRNAs and igRNAs. Con, the control gRNAs that exactly matched the target sequence. Capital A to D, different igRNAs. The red asterisk represents the igRNA with the best performance. The bars represent mean values, and the error bars represent the s.d. of three independent biological replicates. The pie charts represent the fractions of various types of editing products.

A**B**

Supplementary Figure S5. Editing results of adenine base editing at different time points or without selection pressure using igRNA in HEK293T cells. A. Base editing efficiency and fractions at 2 loci using NG-ABEmax with the corresponding parental gRNA and igRNAs in the absence of puromycin selection. **B.** At the ANO5 loci, the editing efficiency of NG-ABEmax with corresponding parent gRNA and igRNAs at different time points. Con, the control gRNAs that exactly matched the target sequence. Capital A and B, different igRNAs. The red asterisk represents the igRNA with the best performance. The bars represent mean values, and the error bars represent the s.d. of three independent biological replicates. The pie charts represent the fractions of various types of editing products.

Supplementary Table S1. C to T editing fractions of CBEs in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing fractions of BE4max				Editing fractions of hyBE4max			
		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
HIRA	Con	0.32±0.01	8.40±0.66	82.17±0.54	9.11±0.14	0.21±0.03	14.22±0.39	51.91±0.33	33.66±0.09
	A	0.20±0.01	55.75±0.62***	34.17±0.76	9.88±0.36	0.11±0.01	54.90±0.50***	12.08±0.37	32.91±0.70
	B	0.39±0.05	41.15±13.25	49.00±13.42	9.47±0.39	0.32±0.02	18.53±0.86	48.47±0.51	32.68±0.42
	C	0.27±0.08	57.08±0.95	31.35±0.24	11.3±0.70	0.16±0.02	52.69±0.93	20.61±0.50	26.53±0.49
	D	0.45±0.07	58.27±0.80	28.75±0.40	12.53±0.45	0.36±0.11	50.97±1.95	21.55±2.71	27.12±0.88
			C5	C8	C5 & C8	Others	C5	C8	C5 & C8
DNMT3B	Con	0.46±0.02	1.89±0.07	87.57±0.12	10.08±0.04	0.43±0.04	4.13±0.14	86.17±0.23	9.27±0.09
	A	0.44±0.01	31.00±0.26	58.82±0.28	9.74±0.12	0.24±0.01	55.61±0.17	37.91±0.16	6.25±0.07
	B	1.61±0.01	0.97±0.03	87.26±0.41	10.16±0.43	1.43±0.07	1.43±0.03	87.88±0.20	9.26±0.16
	C	0.11±0.01	74.66±0.44***	10.65±0.16	14.57±0.50	0.07±0.00	84.98±0.14***	4.62±0.04	10.33±0.12
RNF2	Con	0.21±0.06	8.89±0.17	71.82±0.55	19.08±0.35	0.23±0.02	17.63±0.19	49.37±0.30	32.76±0.23
	A	0.09±0.03	54.52±0.27***	30.24±0.06	15.15±0.32	0.09±0.04	56.45±0.55***	13.93±0.27	29.52±0.50
	B	0.26±0.06	15.82±0.18	65.18±0.22	18.75±0.22	0.22±0.04	24.61±0.42	40.07±0.39	35.10±0.16
	C	0.14±0.04	31.31±0.18	34.33±0.43	34.22±0.29	0.23±0.14	25.17±0.59	21.48±0.28	53.12±0.45
	D	0.36±0.05	17.58±0.62	33.20±0.54	48.86±0.52	0.43±0.07	16.87±1.31	16.67±0.74	66.03±1.62
			C5	C6	C5 & C6	Others	C5	C6	C5 & C6
RNF216	Con	0.70±0.03	0.23±0.01	91.49±0.18	7.59±0.18	0.90±0.20	0.22±0.03	68.69±13.2	30.19±13.42
	A	1.81±0.09***	0.21±0.01	90.02±0.16	7.96±0.24	3.95±0.23***	0.24±0.01	84.86±0.30	10.95±0.10
	B	2.64±0.24	0.56±0.02	88.73±0.08	8.07±0.26	6.94±0.17	0.73±0.00	73.90±0.33	18.43±0.16
NSD1	Con	0.04±0.01	89.58±0.27	3.31±0.06	7.07±0.22	0.04±0.01	92.35±0.43	0.92±0.09	6.69±0.35
	A	0.02±0.01	95.06±0.11***	0.21±0.03	4.71±0.07	0.06±0.01	94.94±0.16***	0.11±0.01	4.89±0.16
	B	0.19±0.04	89.14±0.35	0.53±0.03	10.15±0.29	0.19±0.04	92.56±0.30	0.12±0.10	7.14±0.22
			C3	C6	C3 & C6	Others	C3	C6	C3 & C6

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S2. C to T editing efficiency of CBEs in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing efficiency of BE4max				Editing efficiency of hyBE4max			
		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
HIRA	Con	0.13±0.01	3.34±0.29	32.70±0.71	3.62±0.07	0.08±0.01	5.35±0.34	19.51±0.57	12.65±0.42
	A	0.07±0.00	19.72±0.81***	12.09±0.59	3.49±0.04	0.04±0.01	18.57±0.26***	4.09±0.07	11.13±0.35
	B	0.11±0.01	12.00±4.11	14.04±3.38	2.73±0.16	0.1±0.01	5.75±0.02	15.07±0.92	10.16±0.64
	C	0.05±0.02	10.79±0.54	5.92±0.22	2.13±0.09	0.04±0.00	11.93±0.55	4.67±0.32	6.02±0.45
	D	0.06±0.00	7.65±0.94	3.77±0.43	1.65±0.22	0.07±0.02	9.63±0.79	4.04±0.35	5.12±0.38
			C5	C8	C5 & C8	Others	C5	C8	C5 & C8
DNMT3B	Con	0.32±0.01	1.32±0.08	61.01±3.32	7.03±0.39	0.29±0.02	2.85±0.07	59.48±0.92	6.40±0.07
	A	0.31±0.01	22.10±0.42	41.94±0.38	6.95±0.07	0.15±0.01	35.44±0.31	24.16±0.16	3.98±0.03
	B	1.09±0.04	0.66±0.04	59.38±1.91	6.91±0.41	0.91±0.04	0.92±0.03	56.27±0.80	5.93±0.04
	C	0.07±0.01	42.92±0.55***	6.13±0.17	8.38±0.29	0.04±0.00	49.69±1.03***	2.70±0.05	6.04±0.20
RNF2	Con	0.12±0.03	5.03±0.08	40.62±0.58	10.79±0.24	0.11±0.01	8.60±0.14	24.07±0.14	15.97±0.27
	A	0.04±0.01	26.03±0.62***	14.44±0.32	7.23±0.17	0.03±0.01	19.37±0.05***	4.78±0.12	10.14±0.27
	B	0.10±0.02	6.29±0.46	25.90±1.55	7.45±0.48	0.08±0.01	9.38±0.19	15.27±0.43	13.37±0.24
	C	0.04±0.01	7.99±0.24	8.75±0.20	8.73±0.22	0.04±0.02	4.63±0.28	3.95±0.20	9.76±0.51
	D	0.04±0.01	1.74±0.10	3.29±0.28	4.85±0.44	0.04±0.01	1.69±0.12	1.66±0.01	6.61±0.43
			C5	C6	C5 & C6	Others	C5	C6	C5 & C6
RNF216	Con	0.49±0.02	0.16±0.01	64.46±0.72	5.35±0.09	0.55±0.12	0.14±0.02	41.98±7.15	18.69±8.47
	A	1.28±0.06***	0.15±0.01	63.69±0.52	5.63±0.19	2.49±0.13***	0.15±0.01	53.5±0.64	6.90±0.02
	B	0.32±0.02	0.07±0.00	10.63±0.27	0.97±0.05	0.52±0.01	0.05±0.00	5.57±0.08	1.39±0.00
NSD1	Con	0.02±0.01	52.49±0.06	1.94±0.04	4.14±0.14	0.03±0.01	54.19±1.44	0.54±0.07	3.93±0.32
	A	0.01±0.01	59.34±0.18***	0.13±0.02	2.94±0.06	0.03±0.00	47.03±0.60	0.05±0.00	2.42±0.10
	B	0.04±0.01	20.56±0.07	0.12±0.01	2.34±0.08	0.04±0.01	21.59±0.86	0.03±0.03	1.67±0.11
			C3	C6	C3 & C6	Others	C3	C6	C3 & C6

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S3. C to T editing fractions of CBEs in HeLa cells (%)

Target genes	gRNA & igRNA	Editing fractions of BE4max				Editing fractions of hyBE4max			
		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
HIRA	Con	0.42±0.04	3.47±0.04	92.58±0.13	3.52±0.12	0.41±0.03	13.45±3.18	72.63±2.58	13.51±0.73
	A	0.11±0.02	72.00±0.39***	23.73±0.36	4.17±0.04	0.11±0.00	80.51±0.51***	7.93±0.34	11.45±0.20
	B	0.59±0.06	5.02±0.27	91.62±0.28	2.76±0.04	0.67±0.03	13.14±1.43	74.86±0.94	11.33±0.47
	C	0.16±0.03	62.03±0.16	31.95±0.13	5.86±0.14	0.22±0.03	62.96±0.83	25.25±0.77	11.57±0.08
	D	0.30±0.02	62.22±0.76	31.17±0.55	6.31±0.27	0.31±0.05	61.57±0.57	26.06±0.26	12.06±0.33
	Con	0.73±0.03	2.51±0.07	85.04±0.28	11.71±0.25	0.74±0.06	8.07±0.12	80.83±0.30	10.37±0.28
DNMT3B	A	0.58±0.03	35.36±0.16	51.76±0.25	12.30±0.12	0.36±0.01	61.43±0.07	30.67±0.06	7.53±0.13
	B	1.97±0.03	1.35±0.07	84.93±0.19	11.75±0.14	2.04±0.12	2.63±0.16	84.53±0.51	10.80±0.25
	C	0.15±0.02	69.24±0.09***	6.88±0.17	23.73±0.16	0.18±0.04	78.73±2.50***	6.59±2.68	14.50±0.25
	Con	0.54±0.06	11.01±0.09	76.09±0.22	12.36±0.18	0.40±0.06	21.93±0.19	53.52±0.27	24.15±0.37
RNF2	A	0.11±0.01	64.15±0.10***	26.52±0.12	9.22±0.03	0.12±0.00	70.30±0.13***	11.01±0.12	18.56±0.22
	B	0.55±0.05	30.01±0.02	59.23±0.11	10.22±0.04	0.47±0.01	52.34±0.38	29.43±0.23	17.76±0.16
	C	0.29±0.02	51.77±0.61	31.71±0.25	16.23±0.37	0.41±0.03	44.38±0.30	20.55±0.12	34.65±0.23
	D	0.98±0.07	43.56±1.37	35.06±1.70	20.40±0.39	1.30±0.08	35.91±1.05	19.62±0.50	43.17±0.87
	Con	2.12±0.05	0.33±0.01	83.17±0.18	0.00±0.00	3.88±0.12	0.36±0.02	79.82±0.07	15.94±0.07
	A	4.91±0.03***	0.26±0.02	82.29±0.18	0.00±0.00	8.78±0.28***	0.25±0.03	77.84±0.37	13.14±0.17
RNF216	B	9.62±0.21	0.63±0.16	82.91±0.64	0.00±0.00	28.55±0.49	1.06±0.11	65.8±0.74	4.59±0.40
	Con	0.00±0.00	92.51±0.07	2.73±0.01	4.76±0.07	0.02±0.00	94.94±0.11	0.81±0.05	4.23±0.06
	A	0.00±0.00	96.23±0.08***	0.14±0.02	3.63±0.07	0.05±0.01	96.84±0.01***	0.10±0.01	3.02±0.02
NSD1	B	0.09±0.01	93.72±0.05	0.18±0.00	6.01±0.04	0.19±0.02	95.98±0.09	0.13±0.01	3.70±0.10

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S4. C to T editing efficiency of CBEs in HeLa cells (%)

Target genes	gRNA & igRNA	Editing efficiency of BE4max				Editing efficiency of hyBE4max			
		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
HIRA		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
	Con	0.20±0.02	1.61±0.07	42.87±2.11	1.63±0.04	0.13±0.01	4.42±1.03	23.9±0.91	4.45±0.27
	A	0.05±0.01	34.52±0.89***	11.38±0.46	2.00±0.07	0.04±0.00	28.64±0.31***	2.82±0.14	4.07±0.07
	B	0.29±0.03	2.48±0.12	45.22±1.05	1.36±0.05	0.25±0.01	4.98±0.76	28.27±1.37	4.27±0.15
	C	0.06±0.01	21.70±0.75	11.17±0.32	2.05±0.10	0.05±0.00	14.34±0.28	5.76±0.29	2.64±0.07
	D	0.10±0.01	19.85±0.47	9.95±0.31	2.02±0.13	0.07±0.01	12.97±0.37	5.50±0.26	2.55±0.17
DNMT3B		C5	C8	C5 & C8	Others	C5	C8	C5 & C8	Others
	Con	0.48±0.02	1.65±0.03	55.86±1.85	7.69±0.21	0.45±0.03	4.88±0.04	48.87±0.98	6.27±0.17
	A	0.36±0.02	21.84±0.17	31.98±0.47	7.60±0.02	0.20±0.01	34.16±0.23	17.06±0.14	4.19±0.05
	B	1.15±0.03	0.79±0.03	49.43±0.55	6.84±0.04	1.09±0.05	1.41±0.07	45.33±1.05	5.79±0.11
	C	0.08±0.01	34.7±1.68***	3.45±0.12	11.90±0.66	0.09±0.02	38.85±1.77***	3.24±1.31	7.16±0.27
RNF2		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
	Con	0.27±0.01	5.47±0.48	37.77±3.02	6.14±0.57	0.23±0.02	12.5±0.74	30.48±1.72	13.74±0.51
	A	0.06±0.00	36.9±0.09***	15.26±0.06	5.30±0.02	0.05±0.00	31.74±0.15***	4.97±0.05	8.38±0.11
	B	0.30±0.02	16.67±0.18	32.89±0.44	5.68±0.05	0.20±0.00	22.65±0.22	12.74±0.21	7.69±0.15
	C	0.07±0.00	13.08±0.42	8.01±0.22	4.10±0.11	0.09±0.01	9.73±0.10	4.48±0.09	7.53±0.10
	D	0.08±0.00	3.45±0.19	2.78±0.24	1.62±0.10	0.08±0.00	2.36±0.04	1.24±0.01	2.75±0.12
RNF216		C5	C6	C5 & C6	Others	C5	C6	C5 & C6	Others
	Con	1.38±0.01	0.22±0.01	54.41±1.00	8.78±0.09	2.17±0.05	0.20±0.01	44.62±0.76	8.91±0.18
	A	2.98±0.02***	0.15±0.01	49.93±0.12	6.96±0.09	4.46±0.03***	0.13±0.02	39.61±1.72	6.68±0.26
	B	0.83±0.02	0.05±0.01	7.18±0.07	0.34±0.05	1.1±0.09	0.04±0.00	2.54±0.23	0.18±0.00
NSD1		C3	C6	C3 & C6	Others	C3	C6	C3 & C6	Others
	Con	0.00±0.00	58.56±0.33	1.73±0.00	2.97±0.07	0.01±0.00	53.79±0.16	0.46±0.03	2.39±0.04
	A	0.00±0.00	59.84±0.85	0.09±0.01	2.26±0.06	0.02±0.00	48.62±0.50	0.05±0.00	1.52±0.02
	B	0.04±0.01	39.32±0.48	0.08±0.00	2.52±0.05	0.05±0.01	25.27±0.56	0.04±0.00	0.97±0.02

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S5. C to T editing fractions and efficiency of BE4max in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing fractions of BE4max				Editing efficiency of BE4max			
		C5	C7	C5 & C7	Others	C5	C7	C5 & C7	Others
EMX1-SITE1	Con	0.20±0.01	1.44±0.08	45.31±0.18	53.05±0.26	0.15±0.00	1.12±0.07	35.02±0.27	41.00±0.05
	A	0.18±0.01	52.66±0.77	22.88±0.55	24.28±0.23	0.13±0.01	36.70±0.51	15.96±0.83	16.93±0.64
	B	0.09±0.02	74.78±0.31***	4.46±0.10	20.67±0.25	0.06±0.01	50.83±0.33***	3.03±0.08	14.05±0.17
	C	0.13±0.03	67.75±0.17	4.38±0.09	27.74±0.14	0.09±0.02	47.37±0.65	3.06±0.05	19.40±0.21
PRNP-SITE1	Con	0.21±0.03	48.89±0.57	18.89±0.23	32.01±0.37	0.17±0.02	40.08±0.61	15.48±0.15	26.24±0.27
	A	0.10±0.00	62.07±0.25	0.76±0.01	37.07±0.25	0.06±0.00	39.13±0.04	0.48±0.01	23.37±0.27
	B	0.10±0.01	76.96±0.40***	1.73±0.08	21.21±0.34	0.08±0.01	57.92±0.36***	1.30±0.06	15.96±0.24
	C	0.09±0.01	56.34±0.26	0.78±0.03	42.78±0.22	0.05±0.00	31.97±0.27	0.44±0.02	24.28±0.10
FANCF-SITE1	Con	3.78±0.17	4.61±0.18	73.07±0.48	18.54±0.16	2.84±0.10	3.47±0.11	54.98±0.89	13.95±0.07
	A	14.36±0.11	34.47±0.24***	39.77±0.44	11.40±0.14	10.17±0.14	24.41±0.20	28.17±0.38	8.07±0.11
	B	8.07±0.23	55.00±0.29	25.39±0.13	11.53±0.21	2.89±0.08	19.66±0.18	9.08±0.03	4.12±0.11
	C	30.27±0.32	46.83±0.18	12.95±0.17	9.95±0.08	13.29±0.18	20.56±0.18	5.69±0.13	4.37±0.08
EMX1-SITE2	Con	2.14±0.09	1.25±0.23	49.50±0.37	47.11±0.55	0.83±0.03	0.48±0.08	19.24±0.57	18.32±0.72
	A	2.24±0.12	5.39±0.13***	23.40±0.63	68.97±0.58	0.39±0.04	0.94±0.03***	4.07±0.27	11.97±0.42
	B	0.61±0.15	1.98±0.17	19.76±0.23	77.65±0.39	0.09±0.02	0.28±0.00	2.77±0.21	10.90±0.87
	C	1.73±0.19	3.32±0.02	9.64±0.29	85.3±0.25	0.13±0.02	0.25±0.02	0.72±0.05	6.37±0.65

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S6. A to G editing fractions of NG-ABEmax in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing fractions of NG-ABEmax				Target genes	gRNA & igRNA	Editing fractions of NG-ABEmax			
PSMB2		A5	A7	A5 & A7	Others	ANO5		A5	A7	A5 & A7	Others
	Con	63.49±1.74	0.68±0.08	18.57±0.63	17.26±1.14		Con	10.26±0.09	27.75±0.03	48.56±0.23	13.43±0.33
	A	80.44±0.23	5.72±0.29	4.26±0.24	9.57±0.15		A	5.64±0.33	84.23±0.98***	3.94±0.47	6.19±0.54
	B	77.40±0.21***	4.23±0.06	9.01±0.05	9.36±0.18		B	9.81±0.33	69.18±1.12	9.63±0.69	11.37±0.44
	C	22.88±0.46	26.33±2.16	1.22±0.21	49.57±1.87			A5	A6	A5 & A6	Others
ABCA3		A5	A8	A5 & A8	Others	KCNQ2	Con	1.39±0.18	0.22±0.03	82.36±0.49	16.03±0.51
	Con	73.33±0.32	0.14±0.01	22.13±0.33	4.40±0.01		A	15.5±0.54***	1.25±0.04	75.22±0.39	8.03±0.22
	A	84.86±0.45***	0.30±0.02	11.08±0.28	3.75±0.24		B	12.31±0.46	1.60±0.10	75.38±0.49	10.70±0.08
	B	70.39±0.83	1.99±0.16	23.62±0.58	4.00±0.22		C	4.20±0.26	0.33±0.01	80.83±0.34	14.64±0.20
	C	81.45±0.55	0.18±0.02	13.09±2.06	5.28±1.71			A5	A7	A5 & A7	Others
EMX1-SITE3		A6	A8	A6 & A8	Others	NOTCH2	Con	25.36±0.72	4.95±0.14	61.18±0.86	8.51±0.06
	Con	48.20±0.31	0.90±0.01	25.92±0.38	24.98±0.30		A	22.77±0.50	13.35±0.19	55.28±0.50	8.60±0.44
	A	72.37±0.40***	3.18±0.16	10.62±0.13	13.82±0.24		B	25.84±0.20	34.21±0.48	32.42±0.58	7.53±0.42
	B	70.71±0.27	7.31±0.27	9.06±0.35	12.93±0.15		C	41.91±0.70***	10.58±0.24	40.98±1.00	6.53±0.22
VISTA hs267		A5	A7	A5 & A7	Others	GF11		A5	A6	A5 & A6	Others
	Con	61.85±0.55	0.08±0.02	31.32±0.42	6.74±0.18		Con	22.76±0.15	2.70±0.10	71.38±0.39	3.16±0.22
	A	78.14±0.23	0.08±0.00	16.60±0.10	5.18±0.14		A	49.87±0.81	15.14±0.22	25.63±0.46	9.36±0.52
	B	87.04±0.41***	0.12±0.01	6.55±0.30	6.28±0.13		B	39.75±1.25	12.58±0.36	42.49±0.35	5.18±0.65
	C	40.59±1.72	7.88±0.23	1.35±0.31	50.18±2.10		C	48.25±0.81***	10.86±0.42	37.42±0.35	3.47±0.38
SNCA		A5	A7	A5 & A7	Others	AGXT	D	46.33±0.20	11.57±0.17	35.62±0.25	6.48±0.32
	Con	79.73±0.41	0.19±0.01	14.54±0.33	5.54±0.21			A5	A7	A5 & A7	Others
	A	82.63±0.37**	0.45±0.07	10.50±0.13	6.43±0.24		Con	15.89±0.27	1.72±0.08	76.22±0.67	6.17±0.34
	B	80.78±0.03	0.59±0.03	10.88±0.18	7.75±0.21		A	47.15±0.12	8.03±0.05	36.95±0.15	7.86±0.03
	C	18.91±0.59	10.22±1.94	1.04±0.74	69.83±2.14		B	28.32±0.41	8.17±0.38	56.27±0.83	7.24±0.35
EMX1-SITE4		A5	A8	A5 & A8	Others	EMX1-SITE8	C	32.78±0.33***	5.12±0.16	56.43±0.45	5.67±0.32
	Con	42.70±0.27	0.53±0.04	48.6±0.19	8.17±0.13		D	40.71±0.60	11.18±0.03	42.02±0.34	6.09±0.32
	A	62.96±0.48	14.48±0.05	11.02±0.17	11.53±0.44		E	45.11±0.47	9.79±0.15	40.17±0.32	4.94±0.27
	B	66.12±0.52***	3.78±0.23	22.76±0.32	7.34±0.11		F	43.69±0.45	9.68±0.10	40.90±0.30	5.73±0.24
	C	73.87±0.38	1.85±0.10	16.19±0.26	8.09±0.12			A4	A7	A4 & A7	Others
EMX1-SITE5		A5	A7	A5 & A7	Others	PRNP-SITE2	Con	39.15±0.29	12.00±0.09	43.32±0.13	5.52±0.17
	Con	17.25±0.13	4.77±0.21	54.58±0.34	23.40±0.07		A	16.08±0.33	46.21±0.34	20.12±0.42	17.59±0.45
	A	15.29±0.06	40.46±0.50***	20.58±0.16	23.67±0.45		B	14.45±0.14	29.73±0.70***	48.81±0.49	7.01±0.17
	B	29.13±0.21	16.98±0.24	37.09±0.26	16.80±0.27		C	35.11±0.91	12.86±0.90	16.29±1.76	35.74±1.96
	C	10.77±0.73	53.65±1.26	7.46±0.81	28.13±0.26			A4	A7	A4 & A7	Others
EMX1-SITE6		A5	A7	A5 & A7	Others	Con	31.67±0.24	2.81±0.17	57.85±0.34	7.66±0.03	
	Con	8.24±0.80	1.08±0.13	35.82±3.04	54.86±3.97	A	38.09±0.65	47.33±0.51***	9.20±0.48	5.38±0.07	
	A	7.66±0.68	50.97±3.88	11.17±1.08	30.20±5.63	B	12.32±0.66	52.17±1.85	2.75±0.37	32.75±1.62	

	B	53.83±1.06***	8.84±0.40	26.84±0.92	10.49±0.47		A5	A8	A5 & A8	Others	
EMX1- SITE7		A5	A6	A5 & A6	Others	SLC22A5- SITE1	Con	73.56±0.33	8.53±0.23	15.33±0.34	2.58±0.23
	Con	6.79±0.11	3.26±0.09	79.77±0.88	10.18±0.86	A	81.50±0.81***	11.13±0.86	3.61±0.13	3.75±0.11	
	A	28.67±0.34	33.61±0.23***	12.75±0.46	24.97±0.65	B	76.4±0.53	13.67±0.63	4.79±0.34	5.15±0.41	
	B	20.72±1.94	25.55±0.80	9.32±0.77	44.41±0.53						

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001, **P < 0.01).

Supplementary Table S7. A to G editing efficiency of NG-ABEmax in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing efficiency of NG-ABEmax				Target genes	gRNA & igRNA	Editing efficiency of NG-ABEmax			
		A5	A7	A5 & A7	Others			A5	A7	A5 & A7	Others
PSMB2	Con	44.22±0.97	0.47±0.05	12.94±0.55	12.03±0.92	ANO5	Con	5.72±0.24	15.46±0.66	27.05±1.07	7.49±0.48
	A	27.42±0.91	1.95±0.05	1.46±0.13	3.26±0.12		A	1.24±0.07	18.56±0.35**	0.87±0.11	1.36±0.12
	B	40.66±4.04	2.23±0.25	4.73±0.46	4.92±0.54		B	1.25±0.04	8.80±0.14	1.23±0.09	1.45±0.06
	C	0.86±0.02	0.99±0.07	0.05±0.01	1.87±0.09			A5	A6	A5 & A6	Others
							Con	0.79±0.11	0.13±0.01	47.12±2.69	9.15±0.28
ABCA3	Con	47.77±0.68	0.09±0.01	14.42±0.46	2.86±0.06	KCNQ2	A	10.41±0.50***	0.84±0.04	50.47±0.57	5.39±0.14
	A	56.72±1.64**	0.20±0.01	7.40±0.24	2.51±0.21		B	7.99±0.33	1.04±0.09	48.95±1.17	6.95±0.22
	B	47.22±1.81	1.33±0.08	15.83±0.16	2.68±0.12		C	2.36±0.21	0.18±0.01	45.33±1.64	8.21±0.43
	C	52.14±0.93	0.12±0.01	8.39±1.38	3.37±1.06			A5	A7	A5 & A7	Others
							Con	13.93±0.76	2.72±0.18	33.64±2.06	4.67±0.23
EMX1-SITE3	Con	19.54±0.44	0.37±0.00	10.51±0.23	10.12±0.11	NOTCH2	A	15.90±0.77	9.32±0.39	38.57±0.98	5.99±0.16
	A	27.15±0.29***	1.19±0.06	3.99±0.07	5.19±0.06		B	10.19±1.09	13.49±1.45	12.80±1.48	2.95±0.20
	B	13.71±0.44	1.42±0.10	1.76±0.08	2.51±0.12		C	22.74±0.69***	5.74±0.10	22.24±1.10	3.54±0.02
								A5	A6	A5 & A6	Others
VISTA hs267	Con	38.10±3.82	0.05±0.02	19.28±1.74	4.16±0.44	GFII	Con	9.57±0.87	1.13±0.10	30.03±3.06	1.32±0.07
	A	57.45±0.59	0.06±0.00	12.20±0.09	3.81±0.09		A	3.35±0.15	1.02±0.03	1.72±0.02	0.63±0.02
	B	64.71±0.85***	0.09±0.01	4.87±0.25	4.67±0.14		B	6.67±1.46	2.09±0.37	7.08±1.33	0.84±0.07
	C	1.62±0.06	0.31±0.01	0.05±0.01	2.00±0.10		C	13.24±1.49*	2.96±0.20	10.25±0.99	0.94±0.03
							D	4.92±0.36	1.23±0.10	3.78±0.26	0.69±0.03
SNCA	Con	34.72±1.27	0.08±0.00	6.33±0.12	2.41±0.07	AGXT	Con	7.8±0.98	0.84±0.08	37.55±5.42	3.01±0.27
	A	22.61±1.36	0.12±0.02	2.87±0.14	1.76±0.11		A	9.10±0.17	1.55±0.02	7.13±0.15	1.52±0.02
	B	13.39±0.16	0.1±0.00	1.80±0.03	1.28±0.04		B	7.20±0.44	2.08±0.18	14.29±0.73	1.84±0.05
	C	0.20±0.02	0.11±0.02	0.01±0.01	0.73±0.06		C	15.30±0.61***	2.39±0.14	26.33±0.84	2.64±0.08
							D	10.82±1.29	2.97±0.32	11.15±1.15	1.61±0.10
EMX1-SITE4	Con	36.06±0.63	0.44±0.03	41.04±0.68	6.9±0.07	EMX1-SITE8	E	12.82±0.87	2.78±0.12	11.40±0.63	1.40±0.07
	A	17.82±0.27	4.10±0.10	3.12±0.10	3.27±0.19		F	13.25±1.33	2.93±0.28	12.39±1.04	1.73±0.13
	B	42.36±1.07**	2.42±0.16	14.58±0.30	4.70±0.05			A4	A7	A4 & A7	Others
	C	32.31±0.83	0.81±0.05	7.08±0.33	3.54±0.16		Con	27.01±0.43	8.28±0.20	29.89±0.63	3.81±0.12
							A	1.44±0.04	4.14±0.06	1.80±0.04	1.58±0.07
EMX1-SITE5	Con	11.03±0.28	3.05±0.19	34.91±0.72	14.97±0.40	PRNP-SITE2	B	7.31±0.09	15.04±0.36***	24.70±0.27	3.55±0.08
	A	8.57±0.21	22.67±0.47***	11.53±0.17	13.26±0.46		C	1.17±0.07	0.43±0.04	0.54±0.04	1.19±0.10
	B	11.23±0.23	6.55±0.20	14.30±0.39	6.48±0.28			A4	A7	A4 & A7	Others
	C	1.87±0.14	9.31±0.18	1.30±0.15	4.88±0.07		Con	14.30±0.35	1.27±0.07	26.11±0.42	3.46±0.07
							A	12.28±0.78	15.27±1.02***	2.96±0.10	1.73±0.08
EMX1-SITE6	Con	6.18±0.58	0.81±0.09	26.86±2.20	41.16±3.11	B	0.36±0.01	1.53±0.11	0.08±0.01	0.96±0.01	
	A	1.32±0.20	8.76±1.21	1.92±0.30	5.10±0.58						

	B	14.95±0.14***	2.46±0.11	7.45±0.32	2.91±0.17			A5	A8	A5 & A8	Others
EMX1- SITE7		A5	A6	A5 & A6	Others	SLC22A5-	Con	35.30±1.04	4.09±0.10	7.35±0.21	1.24±0.13
	Con	2.15±0.23	1.04±0.16	25.33±3.47	3.20±0.12	SITE1	A	11.32±0.49	1.55±0.20	0.50±0.01	0.52±0.04
	A	1.37±0.19	1.61±0.21*	0.61±0.10	1.19±0.13		B	6.08±0.16	1.09±0.04	0.38±0.03	0.41±0.04
	B	0.67±0.12	0.82±0.05	0.30±0.03	1.43±0.12						

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001, **P < 0.01, *P < 0.05).

Supplementary Table S8. A to G editing fractions of NG-ABEmax in HeLa cells (%)

Target genes	gRNA & igRNA	Editing fractions of NG-ABEmax				Target genes	gRNA & igRNA	Editing fractions of NG-ABEmax				
		A5	A7	A5 & A7	Others			A5	A7	A5 & A7	Others	
PSMB2	Con	49.36±0.47	0.49±0.02	26.36±0.29	23.80±0.22	ANO5	Con	14.58±0.26	34.37±0.38	39.17±0.25	11.88±0.14	
	A	85.6±0.59	5.65±0.23	4.24±0.32	4.52±0.09		A	7.50±0.34	81.86±2.59***	6.66±1.76	3.99±0.53	
	B	79.79±0.23***	4.40±0.05	8.59±0.10	7.21±0.16		B	12.07±0.65	73.09±1.84	9.64±0.93	5.20±0.48	
	C	58.61±2.73	28.76±1.31	5.58±1.25	7.05±1.84							
ABCA3	Con	74.78±0.25	0.09±0.01	21.27±0.22	3.87±0.03	KCNQ2	Con	0.93±0.02	0.12±0.01	80.86±0.06	18.09±0.09	
	A	88.61±0.13***	0.32±0.02	8.48±0.06	2.59±0.15		A	12.55±0.07***	0.60±0.02	76.43±0.10	10.42±0.13	
	B	78.47±0.14	2.25±0.04	18.38±0.09	0.90±0.10		B	9.39±0.07	0.85±0.02	76.53±0.28	13.22±0.30	
	C	83.9±0.23	0.20±0.02	12.86±0.17	3.04±0.06		C	2.80±0.18	0.10±0.01	78.36±0.37	18.74±0.50	
EMX1-SITE3	Con	36.16±0.06	0.26±0.04	32.84±0.08	30.74±0.10	NOTCH2	Con	28.57±0.41	3.83±0.15	62.90±0.63	4.70±0.11	
	A	72.06±0.13***	1.94±0.04	14.43±0.09	11.57±0.09		A	27.96±0.21	14.63±0.22	50.16±0.09	7.24±0.15	
	B	84.68±0.41	7.34±0.18	5.40±0.17	2.58±0.41		B	26.22±0.48	35.04±0.80	34.78±0.49	3.96±0.13	
							C	48.32±0.31***	6.40±0.23	41.65±0.51	3.63±0.14	
VISTA hs267	Con	59.74±0.39	0.10±0.01	34.59±0.30	5.57±0.08	GF11	Con	16.00±0.75	1.28±0.08	80.19±0.79	2.53±0.04	
	A	89.52±0.18***	0.06±0.01	7.10±0.17	3.33±0.17		A	56.07±0.79	13.87±0.78	28.10±0.01	1.96±0.10	
	B	96.38±0.07	0.15±0.01	1.01±0.06	2.45±0.09		B	40.99±0.34	10.80±0.26	45.94±0.07	2.27±0.12	
	C	70.72±2.40	22.14±2.30	4.74±0.64	2.41±0.48		C	37.77±0.48***	5.88±0.07	53.80±0.36	2.56±0.17	
							D	41.95±0.20	6.53±0.18	49.07±0.42	2.45±0.04	
SNCA	Con	77.79±0.24	0.30±0.02	16.64±0.05	5.28±0.27							
	A	92.87±0.17***	0.76±0.06	3.43±0.23	2.94±0.14							
	B	94.37±0.33	1.14±0.09	2.16±0.27	2.32±0.14							
	IC	71.47±3.79	24.47±5.65	3.22±1.62	0.85±0.79							

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S9. A to G editing efficiency of NG-ABEmax in HeLa cells (%)

Target genes	gRNA & igRNA	Editing efficiency of NG-ABEmax				Target genes	gRNA & igRNA	Editing efficiency of NG-ABEmax			
		A5	A7	A5 & A7	Others			A5	A7	A5 & A7	Others
PSMB2		A5	A7	A5 & A7	Others	ANOS		A5	A7	A5 & A7	Others
	Con	42.96±0.12	0.42±0.02	22.95±0.52	20.72±0.4		Con	10.85±0.36	25.57±0.27	29.15±0.47	8.84±0.24
	A	29.59±0.34	1.95±0.09	1.47±0.12	1.56±0.02		A	1.48±0.13	16.11±0.37	1.33±0.43	0.79±0.15
	B	50.35±0.50***	2.78±0.03	5.42±0.12	4.55±0.13		B	1.51±0.08	9.13±0.27	1.20±0.11	0.65±0.07
	C	0.91±0.05	0.45±0.01	0.09±0.02	0.11±0.03			A5	A6	A5 & A6	Others
ABCA3		A5	A8	A5 & A8	Others	KCNQ2		A5	A6	A5 & A6	Others
	Con	61.41±0.03	0.07±0.01	17.47±0.23	3.18±0.04		Con	0.80±0.01	0.10±0.01	69.41±0.36	15.53±0.16
	A	68.66±0.46***	0.24±0.01	6.57±0.08	2.01±0.11		A	11.06±0.08***	0.53±0.02	67.36±0.26	9.18±0.09
	B	60.15±0.20	1.72±0.03	14.09±0.06	0.69±0.07		B	8.43±0.09	0.77±0.02	68.7±0.29	11.87±0.28
	C	64.93±0.16	0.15±0.01	9.95±0.15	2.35±0.05		C	2.47±0.16	0.09±0.01	69.09±0.81	16.52±0.40
EMX1-SITE3		A6	A8	A6 & A8	Others	NOTCH2		A5	A7	A5 & A7	Others
	Con	27.04±1.07	0.20±0.02	24.56±1.06	22.98±0.95		Con	22.42±1.03	3.01±0.22	49.32±1.17	3.69±0.20
	A	47.91±0.80***	1.29±0.05	9.60±0.24	7.69±0.14		A	22.48±0.11	11.77±0.21	40.33±0.06	5.82±0.13
	B	18.01±0.09	1.56±0.03	1.15±0.04	0.55±0.09		B	16.53±0.29	22.09±0.49	21.92±0.34	2.50±0.08
VISTA hs267		A5	A7	A5 & A7	Others	GFI1		A5	A6	A5 & A6	Others
	Con	44.55±0.26	0.07±0.01	25.79±0.27	4.16±0.07		Con	12.17±0.52	0.98±0.06	61.02±0.89	1.93±0.03
	A	63.59±0.75	0.04±0.00	5.04±0.11	2.36±0.11		A	3.98±0.10	0.99±0.07	2.00±0.06	0.14±0.00
	B	53.55±0.88	0.09±0.01	0.56±0.04	1.36±0.06		B	11.06±0.29	2.91±0.02	12.39±0.24	0.61±0.04
	C	0.49±0.04	0.15±0.01	0.03±0.00	0.02±0.00		C	17.55±0.15***	2.73±0.02	24.99±0.29	1.19±0.08
SNCA		A5	A7	A5 & A7	Others						
	Con	27.18±1.69	0.10±0.00	5.81±0.35	1.84±0.08						
	A	14.18±0.63	0.12±0.01	0.52±0.04	0.45±0.02						
	B	7.36±0.34	0.09±0.01	0.17±0.02	0.18±0.02						
	C	0.27±0.02	0.09±0.02	0.01±0.01	0.00±0.00						

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001).

Supplementary Table S10. A to G editing fractions and efficiency of NG-ABEmax without puromycin selection in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing fractions of NG-ABEmax				Editing efficiency of NG-ABEmax			
		A5	A7	A5 & A7	Others	A5	A7	A5 & A7	Others
ANOS	Con	16.85±0.16	40.91±1.15	32.86±0.68	9.38±0.37	4.72±0.57	11.41±0.95	9.21±1.24	2.63±0.40
	A	9.51±0.63	75.42±3.01***	10.23±1.98	4.84±0.40	1.19±0.13	9.43±0.09	1.29±0.31	0.61±0.08
	B	13.53±0.72	63.75±3.87	16.08±3.04	6.65±0.26	1.54±0.33	7.13±0.72	1.87±0.68	0.76±0.16
EMX1-SITE3	Con	51.18±0.45	3.86±0.68	25.89±0.33	19.07±0.62	12.47±0.50	0.94±0.14	6.31±0.25	4.65±0.32
	A	74.86±0.59***	6.92±1.19	12.61±0.07	5.60±0.65	17.41±0.43***	1.60±0.25	2.93±0.07	1.31±0.17
	B	77.16±0.13	12.33±0.28	7.64±0.13	2.86±0.28	9.46±0.18	1.51±0.04	0.94±0.03	0.35±0.03

Note: All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***) ($P < 0.001$).

Supplementary Table S11. A to G editing fractions and efficiency of ABEs in HEK293T cells (%)

Target genes	gRNA & igRNA	Editing fractions of ABEs				Editing efficiency of ABEs			
		A ^I	A ^{II}	A ^I & A ^{II}	Others	A ^I	A ^{II}	A ^I & A ^{II}	Others
NOTCH2 (NG.ABE _{max})		A ^I	A ^{II}	A ^I & A ^{II}	Others	A ^I	A ^{II}	A ^I & A ^{II}	Others
	PAM1	25.36±0.72	4.95±0.14	61.18±0.86	8.51±0.06	13.93±0.76	2.72±0.18	33.64±2.06	4.67±0.23
	PAM1-C	41.91±0.70***	10.58±0.24	40.98±1.00	6.53±0.22	22.74±0.69***	5.74±0.10	22.24±1.10	3.54±0.02
NOTCH2 (SpRY-ABE _{max})	PAM2	0.23±0.01	29.59±0.41	44.3±0.21	25.87±0.39	0.16±0.01	19.89±0.68	29.77±0.83	17.38±0.25
	PAM2-A	0.19±0.03	64.28±0.27***	12.48±0.09	23.05±0.23	0.13±0.02	45.23±2.03***	8.78±0.38	16.22±0.58
	PAM2-B	9.58±2.94	58.97±0.04	23.23±0.75	8.22±3.50	0.15±0.02	0.96±0.15	0.38±0.05	0.14±0.08
PSMB2 (NG.ABE _{max})		A ^I	A ^{II}	A ^I & A ^{II}	Others	A ^I	A ^{II}	A ^I & A ^{II}	Others
	PAM1	63.49±1.74	0.68±0.08	18.57±0.63	17.26±1.14	44.22±0.97	0.47±0.05	12.94±0.55	12.03±0.92
	PAM1-B	77.40±0.21***	4.23±0.06	9.01±0.05	9.36±0.18	40.66±4.04	2.23±0.25	4.73±0.46	4.92±0.54
PSMB2 (SpRY-ABE _{max})	PAM2	0.77±0.04	39.32±0.46	0.78±0.05	59.13±0.51	0.49±0.02	25.30±0.25	0.50±0.03	38.06±1.13
	PAM2-A	7.19±0.16	73.14±0.80	1.67±0.11	18.00±0.66	1.69±0.05	17.17±0.13	0.39±0.03	4.23±0.18
	PAM2-B	12.89±0.60	64.90±1.89	1.38±0.12	20.83±2.46	1.79±0.10	9.00±0.47	0.19±0.01	2.92±0.60
	PAM2-C	8.11±0.38	67.99±0.42***	2.25±0.08	21.65±0.44	2.57±0.20	21.51±0.66	0.71±0.01	6.85±0.15
KCNQ2 (NG.ABE _{max})		A ^I	A ^{II}	A ^I & A ^{II}	Others	A ^I	A ^{II}	A ^I & A ^{II}	Others
	PAM1	1.39±0.18	0.22±0.03	82.36±0.49	16.03±0.51	0.79±0.11	0.13±0.01	47.12±2.69	9.15±0.28
	PAM1-A	15.5±0.54***	1.25±0.04	75.22±0.39	8.03±0.22	10.41±0.50***	0.84±0.04	50.47±0.57	5.39±0.14
KCNQ2 (SpRY-ABE _{max})	PAM2	2.36±0.10	1.86±0.09	40.34±0.18	55.45±0.31	1.80±0.10	1.41±0.05	30.73±0.54	42.25±0.79
	PAM2-A	5.59±0.24	28.77±0.23***	39.13±0.40	26.51±0.20	3.66±0.26	18.82±0.67***	25.58±0.56	17.33±0.40
	PAM2-B	3.33±0.18	76.62±3.46	9.75±2.95	10.3±0.40	0.37±0.03	8.52±0.15	1.10±0.35	1.15±0.07
GF11 (NG.ABE _{max})		A ^I	A ^{II}	A ^I & A ^{II}	Others	A ^I	A ^{II}	A ^I & A ^{II}	Others
	PAM1	22.76±0.15	2.70±0.10	71.38±0.39	3.16±0.22	9.57±0.87	1.13±0.10	30.03±3.06	1.32±0.07
	PAM1-C	48.25±0.81***	10.86±0.42	37.42±0.35	3.47±0.38	13.24±1.49*	2.96±0.20	10.25±0.99	0.94±0.03
GF11 (SpRY-ABE _{max})	PAM2	64.99±1.27	2.25±0.14	30.28±1.25	2.48±0.09	40.95±2.02	1.42±0.01	19.12±1.72	1.57±0.15
	PAM2-A	71.60±0.12**	5.32±0.06	21.04±0.09	2.05±0.09	28.04±0.52	2.08±0.04	8.24±0.14	0.80±0.02
	PAM2-B	74.34±0.16	7.60±0.06	15.93±0.17	2.13±0.03	23.34±0.20	2.39±0.01	5.00±0.10	0.67±0.01

Note: A^I represents primarily edited bases, and A^{II} represents the other bases that are also within the editing windows. PAM1, the control gRNAs for NG-ABE_{max} editor exactly matched the target sequence. PAM2, the gRNAs for SpRY-ABE_{max} editor also exactly matched the target sequence. Capital A to C, different igRNAs. All data are expressed as mean ± s.d. of three independent experiments. The bold represents single-base editing with the best performance, asterisks indicate a significant difference compared with the control (***P < 0.001, **P < 0.01, *P < 0.05).

Supplementary Table S12. A to G editing fractions and efficiency of NG-ABEmax with different time for ANO5 loci in HEK293T cells (%)

gRNA & igRNA	Time (h)	Editing fractions of NG-ABEmax				Editing efficiency of NG-ABEmax			
		A5	A7	A5 & A7	Others	A5	A7	A5 & A7	Others
Con	24	19.91±0.67	42.62±0.87	23.20±0.25	14.27±0.20	3.44±0.15	7.37±0.17	4.01±0.11	2.47±0.01
	48	16.57±0.38	37.26±0.23	33.56±0.65	12.61±0.06	5.00±0.15	11.24±0.35	10.13±0.53	3.80±0.13
	72	13.83±0.12	35.22±1.03	37.25±0.99	13.7±0.11	5.93±0.29	15.08±0.20	15.99±1.07	5.87±0.21
	96	13.28±0.32	32.83±0.51	40.00±0.53	13.89±0.22	6.79±0.36	16.78±0.44	20.46±1.04	7.10±0.32
	120	12.01±1.44	33.32±3.86	39.71±2.95	14.96±8.13	6.82±0.25	18.91±0.11	22.67±1.32	9.15±5.83
	144	11.17±0.06	32.78±0.20	41.47±0.01	14.57±0.13	6.83±0.06	20.06±0.40	25.37±0.34	8.91±0.04
A	24	10.42±1.28	57.47±1.34	6.52±1.84	25.6±1.99	0.62±0.13	3.36±0.25	0.39±0.14	1.49±0.05
	48	9.10±0.33	67.84±1.53	8.32±1.34	14.74±0.30	1.08±0.08	8.00±0.15	0.99±0.20	1.74±0.05
	72	7.47±0.69	73.99±2.16	7.87±1.45	10.67±0.06	1.43±0.15	14.10±0.35	1.51±0.31	2.03±0.07
	96	7.10±0.73	74.04±2.41	8.85±1.16	10.01±0.54	1.62±0.16	16.97±0.83	2.03±0.26	2.29±0.11
	120	6.80±0.48	72.45±2.91	10.58±0.51	10.17±2.24	2.06±0.17	21.95±0.60	3.21±0.17	3.09±0.74
	144	6.76±0.23	71.88±2.24	12.37±1.48	8.99±0.60	2.02±0.13	21.38±0.04	3.70±0.56	2.68±0.25

Note: All data are expressed as mean ± s.d. of three independent experiments.

Supplementary Table S13. The off-target site analysis of CBE targets*

Target genes	gRNA & igRNA	Protospacer	Number of off-target site	Target genes	gRNA & igRNA	Protospacer	Number of off-target site
HIRA	Con	GTCATCTTTACCCCAGAGCG	6	EMX1- site1	Con	GAGCCTCGAATGCCAAGCTT	43
	A	GTtATCTTTACCCCAGAGCG	5		A	GAGaCTCGAATGCCAAGCTT	11
	B	GTCATgTTTACCCCAGAGCG	3		B	GatgCTCGAATGCCAAGCTT	5
	C	GatATCTTTACCCCAGAGCG	1		C	GgtaCTCGAATGCCAAGCTT	7
	D	GatcTCTTTACCCCAGAGCG	2		FANCF- site1	Con	CCACTGCAAGGCCCGCGCA
DNMT3B	Con	GACACGTCTGTGTAGTGAC	3	A		CtACTGCAAGGCCCGCGCA	5
	A	GATACGTCTGTGTAGTGAC	2	B		CCAgTGCAGGCCCGCGCA	16
	B	GACACGTtTGTGTAGTGAC	3	C		CttTGCAGGCCCGCGCA	12
	C	GtACGTCTGTGTAGTGAC	6	Con		CACGGTCACCACAACCACCA	25
RNF2	Con	GTCATCTTAGTCATTACCTG	7	PRNP- site1	A	CAtGGTCACCACAACCACCA	34
	A	GTtATCTTAGTCATTACCTG	10		B	CgCGGTCACCACAACCACCA	4
	B	GTCATgTTAGTCATTACCTG	7		C	CttGGTCACCACAACCACCA	16
	C	GatATCTTAGTCATTACCTG	7	EMX1- site2	Con	TCATACCATAATGAAATGTT	19
	D	GatcTCTTAGTCATTACCTG	15		A	TaATACCATAATGAAATGTT	17
RNF216	Con	GTGTCCTTTGAGCTCGTGCA	11	B	TCgTACCATAATGAAATGTT	4	
	A	GTGTcTTTTGAGCTCGTGCA	8	C	TggTACCATAATGAAATGTT	15	
	B	GTaTcTTTTGAGCTCGTGCA	7				
NSD1	Con	GGCATCAGTGTGACATCTGC	24				
	A	GGtATCAGTGTGACATCTGC	22				
	B	GatATCAGTGTGACATCTGC	13				

* the prediction was performed with Cas-OFFinder (<http://www.rgenome.net/cas-offinder/>) with condition of up to 3 mismatches, no bulges.

Supplementary Table S14. The off-target analysis of ABE targets*

Target genes	gRNA & igRNA	Protospacer	Number of Mismatch (≤ 3)	Target genes	gRNA & igRNA	Protospacer	Number of Mismatch (≤ 3)	
SNCA	Con	GAACACAATGCATAGATTGC	11	ANO5	Con	TCACACACTTGATCACAGAG	7	
	A	GATCACAAATGCATAGATTGC	6		A	TgACACACTTGATCACAGAG	9	
	B	GAActCAATGCATAGATTGC	8		B	TCAtAGACTTGATCACAGAG	8	
	C	GAtgACAATGCATAGATTGC	13		Con	GGTGACCATCCGGATATTCC	6	
EMX1-site3	Con	GGAGCACACATGCCAGGTG	22	SLC22A5-site1	A	GcTGACCATCCGGATATTCC	3	
	A	GGtGCACACATGCCAGGTG	36		B)	GGaGACCATCCGGATATTCC	13	
	B	GGAcCACACATGCCAGGTG	13		Con (PAM1)	TGGGAAGGGTTTCCAGAGGA	48	
EMX1-site4	Con	GGTATCATAAGAGAATCAC	10	GF11	A	TGGtAAGGGTTTCCAGAGGA	21	
	A	GtCTATCATAAGAGAATCAC	4		B	TcGGAAGGGTTTCCAGAGGA	23	
	B	GaCTATCATAAGAGAATCAC	18		C	TaGGAAGGGTTTCCAGAGGA	43	
	C	GGCTcTATAAGAGAATCAC	15		D	TGaGAAGGGTTTCCAGAGGA	47	
EMX1-site5	Con	CGAGAGAAAGGAGGTATCAC	12		PAM2	TGTGGGAAGGGTTTCCAGAG	38	
	A	CGgGAGAAAGGAGGTATCAC	11		PAM2-A	TcTGGGAAGGGTTTCCAGAG	29	
	B	CGAgTAAAGGAGGTATCAC	12		PAM2-B	TGaGGGAAGGGTTTCCAGAG	54	
	C	CtAGAGAAAGGAGGTATCAC	22		Con	GAAGAGCAGGGTCATGAAGG	35	
EMX1-site6	Con	TGATACATGGAATGAGGGAA	24		ABCA3	A	GAtGAGCAGGGTCATGAAGG	39
	A	TtATACATGGAATGAGGGAA	51			B	GcAGAGCAGGGTCATGAAGG	42
	B	TGAtcCATGGAATGAGGGAA	23	C		GAAGAAcAGGGTCATGAAGG	53	
EMX1-site7	Con	ATGGAATGAGGGAAAGGGCTA	43	PSMB2	Con (PAM1)	GTAACAAGGCATAGACTGA	19	
	A	AaGGAATGAGGGAAAGGGCTA	98		A	GttAACAAAGCATAGACTGA	13	
	B	AttGAATGAGGGAAAGGGCTA	22		B	GTAAtCAAAGCATAGACTGA	12	
EMX1-site8	Con	CACAGCAGTGACCGAGAGAA	28	PSMB2	C	GtttACAAGGCATAGACTGA	20	
	A	CAtAGCAGTGACCGAGAGAA	8		PAM2	AAACAAGGCATAGACTGAGG	29	
	B	CACgCAGTGACCGAGAGAA	8		PAM2-A	AAtCAAAGGCATAGACTGAGG	14	
	C	CACAcCAGTGACCGAGAGAA	12		PAM2-B	AgACAAGGCATAGACTGAGG	16	
PRNP-site2	Con	GAGATCAGGAGGATCACAGG	27	NOTCH2	Con (PAM1)	TGACACAGGAGACCTGTCCAC	17	
	A	GtGATCAGGAGGATCACAGG	23		A	TGgCACAGGAGACCTGTCCAC	18	
	B	GAcATCAGGAGGATCACAGG	28		B	TaACACAGGAGACCTGTCCAC	12	
AGXT	Con	AAGGAGATGAGCGAGGTCCC	22		NOTCH2	C	TGACAtAGGAGACCTGTCCAC	8
	A	AAGcAGATGAGCGAGGTCCC	10			PAM2	ACACAGGAGACCTGTCCACAG	22
	B	AAGtAGATGAGCGAGGTCCC	6			PAM2-A	AtACAGGAGACCTGTCCACAG	19
	C	AAGaAGATGAGCGAGGTCCC	15			PAM2-B	ACgCAGGAGACCTGTCCACAG	10
	D	AAaGAGATGAGCGAGGTCCC	17			Con (PAM1)	GAAGAAGGAGACCCGATGA	17
	E	AAcGAGATGAGCGAGGTCCC	2			A	GAcGAAGGAGACCCGATGA	11
	F	AAtGAGATGAGCGAGGTCCC	9			B	GtAGAAGGAGACCCGATGA	10
VISTA-hs267	Con	GAACACAAAGCATAGACTGC	14	KCNQ2	C	GAAGAtGGAGACCCGATGA	15	
	A	GtACACAAAGCATAGACTGC	13		PAM2	AAGAAGGAGACCCGATGAG	19	
	B	GAAgACAAAGCATAGACTGC	34		PAM2-A	AtGAAGGAGACCCGATGAG	13	
	C	GtAgACAAAGCATAGACTGC	16		PAM2-B	AAcAAGGAGACCCGATGAG	3	

* the prediction was performed with Cas-OFFinder (<http://www.rgenome.net/cas-offinder/>) with condition of up to 3 mismatches, no bulges.