
An adenine base editor variant expands context compatibility

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Supplementary Notes

Supplementary Note 1: TadA-RAs do not function on *N*⁶-methyldeoxyadenosine.

To test whether TadA-RAs acquired activity on *N*⁶-methyldeoxyadenosine during *de novo* evolution, we co-delivered ABE-RA2.0, a sgRNA targeting a G^mATC site, and a plasmid bearing the target site (prepped from *E. coli* with G^mATC fully methylated, **Supplementary Fig. 1**) into HEK293T cells. While robust editing was observed at a genomic GATC site where A was unmethylated, ABE-RA2.0 failed to edit *N*⁶-methyldeoxyadenosine in the plasmid (**Supplementary Fig. 9**), confirming that ABE-RA editors did not acquire activity on *N*⁶-methyldeoxyadenosine.

Supplementary Note 2: TadA8r-dCas9 delivers clean A:T-to-G:C editing with minimal indels.

Indels are frequent byproducts of base editing when a highly active deaminase is fused to a Cas9 nickase, as simultaneous deamination and nicking may result in double-stranded breaks, likely through abasic site formation^{1, 2}. To reduce incidents of indels, we constructed an ABE8r variant in which nCas9 was replaced with dCas9 (TadA8r-dCas9, **Supplementary Fig. 17**). Editing activity remained high even when the target strand was not nicked, suggesting that the superior deamination efficiency of TadA8r may overrule preferences of cellular repair machinery. Importantly, with dCas9 serving as the DNA engaging module, indel formation was reduced to background level ($0.05 \pm 0.04\%$ across 7 sites). The resulting ABE, TadA8r-dCas9, may be particularly desired for applications with low tolerance of indels.

Supplementary Note 3: TadA8r is broadly compatible with DNA-targeting CRISPR systems.

We demonstrate that TadA8r is broadly compatible with DNA-targeting CRISPR proteins, including *Staphylococcus aureus* Cas9 (SaCas9)³, KKH-SaCas9⁴, *Lachnospiraceae bacterium* Cas12a (LbCas12a)⁵, and engineered *Acidaminococcus* Cas12a (enAsCas12a)⁶. Note that no nickase mutations are known for Cas12a. We therefore generated LbABE8r and enAsABE8r using nuclease-deficient Cas12a (dCas12a).

We characterized each ABE system using 4-6 target sites. SaABE8r, KKH-SaABE8r, LbABE8r, and enAsABE8r edited A to $60.4 \pm 15.3\%$, $44.1 \pm 6.6\%$, $32.0 \pm 16.4\%$, and $20.9 \pm 18.6\%$, respectively, considering positions best edited at individual target sites (**Fig. 3d**, **Supplementary Fig. 18** and **19**). The editing levels are comparable with those delivered by TadA8.20- ($59.7 \pm 13.6\%$, $54.2 \pm 12.0\%$, $19.3 \pm 11.8\%$, and $12.4 \pm 8.3\%$) and TadA8e-derived ($61.1 \pm 11.9\%$, $64.1 \pm 10.7\%$, $38.6 \pm 12.4\%$, and $36.1 \pm 15.2\%$) editors, and are higher than ABEs derived from TadA7.10 ($22.7 \pm 14.9\%$, $27.8 \pm 11.6\%$, $6.0 \pm 7.7\%$, and $1.9 \pm 1.9\%$). Note that TadA7.10 was previously reported to be less compatible with non-SpCas9 CRISPR systems⁶. As CRISPR proteins present R loops differently, the corresponding editors show varied editing windows, e.g., protospacer positions 3-16 for SaABE8r and KKH-SaABE8r, and protospacer positions 7-15 for LbABE8r and enAsABE8r. These results are generally consistent with editing windows proposed for corresponding cytosine base editors^{7, 8} and ABE8e².

Finally, we analyzed 45 As edited to >5% by at least one SaCas9-derived editor across 10 sites, wherein TadA8r outperformed TadA7.10, TadA8.20, and TadA8e at 14 of the 20 RA sites (**Supplementary Fig. 18**). Collectively, as a highly active deoxyadenosine deaminase, TadA8r is broadly compatible with CRISPR systems.

Supplementary Note 4: Targeting the same adenine using ABEs of different PAM specificities.

PAM-relaxed NG-Cas9⁹ and near PAM-less SpRY-Cas9¹⁰ can support base editing through non-NGG PAMs. To further compare the performance of ABE8e and ABE8r, we strategically placed the target As at more accessible protospacer positions using non-canonical PAMs and accessed them with ABEs derived from NG-Cas9 and SpRY-Cas9. We designed an additional three sgRNAs for A₃ of site 58 in *PCSK9* (**Supplementary Fig. 37**). Among these, A₆ of site 62 (PAM: AGC) can be accessed by both NG-ABE and SpRY-ABE, while A₅ of site 61 (PAM: GCT) and A₇ of site 63 (PAM: AAG) are only accessible through SpRY-ABE. SpRY-ABE8e and SpRY-ABE8r edited A₅ of site 61 to 66.0% and 68.4%, respectively, exceeding the 41.4% editing achieved by ABE8r via the canonical PAM.

Nevertheless, both NG-ABE and SpRY-ABE showed deteriorated performance with alternative sgRNA designs for A₃ of site 59 in *PCSK9*—A₅ of site 64 (PAM: CGA), A₆ of site 65 (PAM: GCG), and A₇ of site 66 (PAM: TGC)—despite the target A being placed at more accessible positions in the protospacers. Importantly, we observed significant fluctuations in editing efficiency with NG-ABE and SpRY-ABE. At sites 63, 65, and 66, SpRY-ABE7.10, SpRY-ABE8.20, SpRY-ABE8e, and SpRY-ABE8r all generated less than 10% A:T-to-G:C editing. Our findings are corroborated by previous studies reporting that base editors derived from PAM-relaxed Cas9 variants showed large performance variations in editing different genomic loci¹¹.

Further exploring this point, we designed 1-4 alternative sgRNAs for A₃ of site 1 (77.1% vs. 24.4%), A₂ of site 2 (42.0% vs. 6.2%), A₃ of site 8 (49.6% vs. 10.9%), and A₂ (43.9% vs. 4.1%) and A₈ (66.6% vs. 24.3%) of site 17—where ABE8r outperformed ABE8e—and accessed them using NG-ABE and SpRY-ABE (**Supplementary Fig. 38**). While comparable or higher editing levels were observed with some of the alternative designs for A₃ of site 1 (A₆ of site 36), A₂ of site 2 (A₇ of site 28), A₂ (A₇ of site 38) and A₈ (A₇ of site 31 and A₅ of site 39) of site 17, ABE8e and ABE8r derivatives performed at similar levels in all cases. Moreover, neither NG-ABE nor SpRY-ABE could efficiently edit A₃ of site 8 (A₆ of site 29, 5.9-10.4%). In conclusion, while PAM-relaxed Cas9 variants broaden the accessibility of base editors, their performance is less consistent than that of SpCas9. Spacer screening is often required to achieve, but does not always lead to, desired editing levels. Therefore, the expanded editing window and increased context compatibility of ABE8r provide indispensable advantages for editing some target A sites.

Supplementary Table 1 | ABE targets of clinical relevance.

Target site	Context	Aimed disease	Model system	Delivery method	Clinical relevance
CCCGC <u>A</u> CCCTGGCGCAGCGG TGG	C <u>A</u>	Heterozygous familial hypercholesterolemia (HeFH)	Liver; non-human primates	Lipid nanoparticle carrying ABE-encoding mRNA	Phase I clinical trial
GTGGGG <u>A</u> AGGGGCCCAAG AGG	G <u>A</u>	Sickle cell disease; beta thalassemia	Human hematopoietic stem cells (HSCs)	Electroporation / <i>Ex vivo</i>	Phase I/II clinical trial, NCT05456880
CTTCTCC <u>A</u> CAGGAGTCAGGTG CACC	C <u>A</u>	Sickle cell disease	Human hematopoietic stem cells (HSCs)	Electroporation / <i>Ex vivo</i>	IND enabling studies
CCAGTATGGAC <u>A</u> CTGTCCAAA GAGAAT	C <u>A</u>	Glycogen storage disease type Ia (GSDIa)	Transgenic mice harboring human <i>G6PC-p.Arg83Cys</i> transgene in place of mouse G6PC	Lipid nanoparticle carrying ABE-encoding mRNA	Preclinical
GCCCAGGACCT <u>A</u> GGCGAGGCAG TAG	T <u>A</u>	Glycogen storage disease type Ia (GSDIa)	Transgenic mice carrying heterozygous <i>huG6PC-Q347X</i> ; also tested in primary human hepatocytes (~40% editing)	Lipid nanoparticle carrying ABE-encoding mRNA	Preclinical
ATCGAC <u>A</u> AGAAAGGGACTGA AGC	C <u>A</u>	Alpha-1 antitrypsin deficiency (AATD)	the NSG-PiZ transgenic mouse model	Lipid nanoparticle carrying ABE-encoding mRNA	Preclinical
GGTCC <u>A</u> CCCACTGGGCTCC TGA	C <u>A</u>	Hutchinson–Gilford progeria syndrome (HGPS or progeria)	Cultured fibroblasts derived from patients; Mouse model of HGPS	<i>In vivo</i> AAV	Basic research
AACT <u>A</u> GCTTTTAATTGCTGT TGG	T <u>A</u>	Duchenne muscular dystrophy (DMD)	<i>Dmd</i> knockout mice	<i>In vivo</i> AAV	Basic research
GTT <u>A</u> TCTCCTGTTCTGCAGC TGT	T <u>A</u>	Duchenne muscular dystrophy (DMD)	mdx4cv mice	<i>In vivo</i> AAV	Basic research
GTACTT <u>A</u> CAGGCTCCAGTAG TG	T <u>A</u>	Duchenne muscular dystrophy (DMD)	ΔE51 mice	<i>In vivo</i> AAV	Basic research
CAACTC <u>A</u> CTTCAGCTCCTCA TGG	C <u>A</u>	Recessive dystrophic epidermolysis bullosa (RDEB)	Primary fibroblasts	Electroporation of mRNA	Basic research
GAACTC <u>A</u> CACCCGCTGCCC GGG	C <u>A</u>	Recessive dystrophic epidermolysis bullosa (RDEB)	Primary fibroblasts	Electroporation of mRNA	Basic research
CAGATTCT <u>A</u> CCCACTGGCCT TG	T <u>A</u>	Autosomal recessive congenital ichthyosis (ARCI)	Human embryos with heterozygous mutants	Micro-injection of mRNA into cytoplasm	Basic research
ACTGGAGC <u>A</u> GTAATGCCTGG TGG	C <u>A</u>	Hereditary tyrosinemia type 1 (HT1)	Mouse model of hereditary tyrosinemia type 1 (HT1)	Hydrodynamic tail-vein injection with plasmids	Basic research
ACATC <u>A</u> GAGGAGACTGCCAG TGA	C <u>A</u>	Leber congenital amaurosis (LCA)	Retinal degeneration 12 (rd12) mice harboring <i>Rpe65-R44X</i>	<i>In vivo</i> AAV	Basic research
CATC <u>A</u> GAGGAGACTGCCAGT GAG	C <u>A</u>	Leber congenital amaurosis (LCA)	Retinal degeneration 12 (rd12) mice harboring <i>Rpe65-R44X</i>	Lentivirus	Basic research

ACTCT <u>A</u> GGCAGAGGTCTCAA AGG	<u>T</u> <u>A</u>	Mucopolysaccharidosis type I (MPS I), Hurler syndrome	Newborn murine model harboring <i>Idua-W392X</i>	<i>In vivo</i> AAV	Basic research
CCGTGGC <u>A</u> CCAGTTGTGGTA GG	<u>C</u> <u>A</u>	Retinitis pigmentosa (RP)	Retinal degeneration 10 (rd10) mouse model harboring <i>Pde6b-R560C</i>	<i>In vivo</i> AAV	Basic research
CTTTGT <u>A</u> GGATCAAGCCTCA AGG	<u>T</u> <u>A</u>	Fanconi Anemia (FA)	FA patient lymphoblastoid cell lines (LCLs), FA patient HSPCs	Electroporation of mRNA	Basic research
GGCCC <u>A</u> ACAGCCTCTTTCTGAG G	<u>C</u> <u>A</u>	Fanconi Anemia (FA)	FA patient lymphoblastoid cell lines (LCLs)	Electroporation of mRNA	Basic research
ATTTCC <u>A</u> GGATCTGAATCAC AGG	<u>C</u> <u>A</u>	STAT3-hyper IgE syndrome (STAT3- HIES)	Patient fibroblasts	Electroporation of RNPs	Basic research
ACGT <u>A</u> CCAGGTGGAGCACCC AGG	<u>T</u> <u>A</u>	Haemochromatosis (HHC)	lymphoblastoid cell line (LCL) harbouring <i>HFE- C282Y</i> ; 129- <i>Hfe</i> ^{tm.1.1Nca} mouse model	Electroporation of plasmids; <i>In vivo</i> AAV for mouse model	Basic research
CCTC <u>A</u> GGTGAAGGTGGGGAA CGA	<u>C</u> <u>A</u>	Hypertrophic cardiomyopathy (HCM)	Mouse model for HCM	In utero injection of AAV9	Basic research
GCCGC <u>A</u> GTCTCGTAGTCCGG TGA	<u>C</u> <u>A</u>	Dilated cardiomyopathy (DCM)	Human isogenic induced pluripotent stem cells (iPSCs); mouse model	<i>In vivo</i> AAV	Basic research
GGCCCGGA <u>A</u> GGGGCTGGGCC GGGGACC	<u>A</u> <u>A</u>	Cancer	Mouse injected with <i>TERT</i> promoter- mutated PDX GBM cells	<i>In vivo</i> AAV	Basic research
CTT <u>A</u> CCCCACTTAACATCT TGG	<u>T</u> <u>A</u>	CAR-T therapy	primary human T cells	electroporation of mRNA	Basic research
CCCT <u>A</u> CCGTGCACAGGACC AGG	<u>T</u> <u>A</u>	CAR-T therapy	primary human T cells	electroporation of mRNA	Basic research
CACCT <u>A</u> CCTAAGAACCATCC TGG	<u>T</u> <u>A</u>	CAR-T therapy	primary human T cells	electroporation of mRNA	Basic research
CACTC <u>A</u> CCTTAGCCTGAGCA GGG	<u>C</u> <u>A</u>	CAR-T therapy	primary human T cells	electroporation of mRNA	Basic research
CTT <u>A</u> CCTGGGCTGGGGAAGA AGG	<u>T</u> <u>A</u>	CAR-T therapy	primary human T cells	electroporation of mRNA	Basic research
ATT <u>A</u> TACCTGCCATGCCGTA AGG	<u>T</u> <u>A</u>	CAR-T therapy	primary human T cells	electroporation of mRNA	Basic research
CCTC <u>A</u> GGTGAAAGTGGGCAA TGA	<u>C</u> <u>A</u>	Hypertrophic cardiomyopathy (HCM)	Humanized mouse model <i>Myh6</i> ^{h403/+}	<i>In vivo</i> AAV	Basic research
TTTGTCT <u>A</u> AAACCCTGTAAG GAA	<u>T</u> <u>A</u>	Spinalmuscular atrophy (SMA)	Δ 7SMA mouse	<i>In vivo</i> AAV	Basic research
TCC <u>A</u> TG <u>A</u> TGCACAGACAGGA GAC	<u>C</u> <u>A</u> , <u>G</u> <u>A</u>	Cardiovascular disease	Mouse	<i>In vivo</i> AAV	Basic research
TGTCG <u>A</u> AGTTCCGCCCTGGAG AGG	<u>G</u> <u>A</u>	Stargardt disease	<i>Abca4</i> ^{hu1961E/ms19 61G(KO)} mouse; cynomolgus macaques	<i>In vivo</i> AAV	Basic research

Supplementary Table 2 | Protospacer and PAM sequences used in the study.

Site	Protospacer	PAM	CRISPR protein
site 1 (<i>HEK2</i>)	GAACACAAAGCATAGACTGC	GGG	nSpCas9
site 2	AAGTGTGATCACTTGGGTGG	TGG	nSpCas9
site 3	CAGGACGGTCACCTTTGGGG	TGG	nSpCas9
site 4	AGGATGCAAGTTTGTCTTGG	GGG	nSpCas9
site 5	GGTACCTATCGATTGTCAGG	AGG	nSpCas9
site 6	GAGTATGAGGCATAGACTGC	AGG	nSpCas9
site 7	GGATTGACCCAGGCCAGGGC	TGG	nSpCas9
site 8	GAAGACCAAGGATAGACTGC	TGG	nSpCas9
site 9	AGCCAGGACGGTCACCTTTG	GGG	nSpCas9
site 10	GACAAGTGTGATCACTTGGG	TGG	nSpCas9
site 11	CCAAGGATTGACCCAGGCCA	GGG	nSpCas9
site 12	CCACCGCTGCGCCAAGGTGC	GGG	nSpCas9
site 13	GATGAGATAATGATGAGTCA	GGG	nSpCas9
site 14	AAGCAATTGTTATGATTA	TGG	nSpCas9
site 15	AATACATTCCAAAAGAAATG	GGG	nSpCas9
site 16	GAATACATTCCAAAAGAAAT	GGG	nSpCas9
site 17 (<i>EMX1</i>)	GAGTCCGAGCAGAAGAAGAA	GGG	nSpCas9
site 18	ATATTTGCATTGAGATAGTG	TGG	nSpCas9
site 19	CCAAGTACTTCAGGTAGCTG	AGG	nSpCas9
site 20	TAAGGCCCAAGGGGCAAGC	TGG	nSpCas9
site 21	GCAGGTGACCGTGGCCTGCG	AGG	nSpCas9
site 22	GGTTAACAAGGCCAAACTCC	AGAT	NG/VRQR-nSpCas9
site 23	GGGTCCAGTTCCGGGATTAG	CGAA	NG/VRQR-nSpCas9
site 24	CAAGGATAGACTGCTGGGCT	TGA	NG/VRQR-nSpCas9
site 25	GAGGACAAAGTACAAACGGC	AGA	NG/VRQR-nSpCas9
site 26	GATGACCCGTATTATCTGGC	AGTT	NG-nSpCas9
site 27	GGAGACATCAAACCATGACT	TGC	NG-nSpCas9
site 28	GTGACAAGTGTGATCACTTG	GGT	NG-nSpCas9/nSpRYCas9
site 29	AGTGAAGACCAAGGATAGAC	TGC	NG-nSpCas9/nSpRYCas9
site 30	GCCTGAGTCCGAGCAGAAGA	AGA	NG-nSpCas9/nSpRYCas9
site 31	AGTCCGAGCAGAAGAAGAAG	GGC	NG-nSpCas9/nSpRYCas9
site 32	CAAGTGATCACACTTGTAC	CACC	NRCH-nSpCas9
site 33	ATAGACTGCAGGAGACATCA	AACC	NRCH-nSpCas9
site 34	ATGACTTGCAGATGAAGAAG	CATT	NRTH-nSpCas9
site 35	GATTCAAAGCCATTTTCCA	GATA	NRTH-nSpCas9
site 36	CTGGAACACAAAGCATAGAC	TGC	nSpRYCas9
site 37	GTATGAGGCATAGACTGCAG	GAG	nSpRYCas9
site 38	GGCCTGAGTCCGAGCAGAAG	AAG	nSpRYCas9
site 39	TCCGAGCAGAAGAAGAAGGG	CTC	nSpRYCas9
site 40	GTGGTAGACAGCATGTGTCCTA	AAGGGT	nSaCas9

site 41	ATTTACAGCCTGGCCTTTGGGG	TCGGGT	nSaCas9
site 42	GTGTCAGGTAATGTGCTAAACA	GAGAGT	nSaCas9
site 43	GGTGGAGGAGGGTGCATGGGGT	CAGAAT	nSaCas9
site 44	ACTAGTGTGCGAAGTATCATAA	AGGAGT	nSaCas9
site 45	TACAGAGGGACAGAGGCCTGAC	CTGGGT	nSaCas9
site 46	ATGAGAAGTATGACAACAGCCT	CAAGAT	KKH-nSaCas9
site 47	GGCAGTCATCTTAGTCATTACC	TGAGGT	KKH-nSaCas9
site 48	GGACTAGAGTAGGATTGTACCC	CTCAGT	KKH-nSaCas9
site 49	GGCTGAGCTAACTGTGACAGCA	TGTGGT	KKH-nSaCas9
site 50	TGCTGCAAGTAAGCATGCATTTG	TTTC	dLbCas12a/denAsCas12a
site 51	CTAGACAGGGGCTAGTATGTGCA	TTTC	dLbCas12a/denAsCas12a
site 52	CAGCTATTCAAGGCTGGCCCGCCC	TTTG	dLbCas12a/denAsCas12a
site 53	GAAGCACATCAAGGACATTCTAA	TTTA	dLbCas12a/denAsCas12a
site 54	GGATAAGCACAGTTTTAAATAGT	TTTG	dLbCas12a/denAsCas12a
site 55	GTTTAAACACACCGGGTTAATAA	TTTG	dLbCas12a/denAsCas12a
site 56 (<i>HEK3</i>)	GGCCCAGACTGAGCACGTGA	TGG	nSpCas9
site 57 (<i>VEGFA3</i>)	GGTGAGTGAGTGTGTGCGTG	TGG	nSpCas9
site 58 (<i>PCSK9</i>)	TAAGGCCCAAGGGGGCAAGC	TGG	nSpCas9
site 59 (<i>PCSK9</i>)	GCAGGTGACCGTGGCCTGCG	AGG	nSpCas9
site 60 (<i>ABCA4-p.Gly1961Glu</i>)	CGAAGTTCGCCCTGGAGAGG	TGG	nSpCas9
site 61	TCTAAGGCCCAAGGGGGCAA	GCT	nSpRYCas9
site 62	CTCTAAGGCCCAAGGGGGCA	AGC	nSpRYCas9/NG-nSpCas9
site 63	ACTCTAAGGCCCAAGGGGGC	AAG	nSpRYCas9
site 64	TGGCAGGTGACCGTGGCCTG	CGA	nSpRYCas9/NG-nSpCas9
site 65	CTGGCAGGTGACCGTGGCCT	GCG	nSpRYCas9
site 66	TCTGGCAGGTGACCGTGGCC	TGC	nSpRYCas9/NG-nSpCas9
R-loop 1	GTGGTAGACAGCATGTGTCCTA	AAGGGT	dSaCas9/nSaCas9
R-loop 2	GATTTACAGCCTGGCCTTTGGGG	TCGGGT	dSaCas9/nSaCas9
R-loop 3	GTGTCAGGTAATGTGCTAAACA	GAGAGT	dSaCas9/nSaCas9
R-loop 4	GGTGGAGGAGGGTGCATGGGGT	CAGAAT	dSaCas9/nSaCas9
R-loop 5	GGCAAGAGGATTGATTGAGCCA	GAGAGT	dSaCas9/nSaCas9
R-loop 6	ACTAGTGTGCGAAGTATCATAA	AGGAGT	dSaCas9/nSaCas9
plasmid G ^m ATC site	GCTCTG ^m ATCTGAATACCACG	AGG	nSpCas9
genomic GATC site	AAGTGTGATCACTTGGGTGG	TGG	nSpCas9
<i>HEK2_OT1</i>	AGGCACAATGCATAGATTGC	CGG	nSpCas9
<i>HEK3_OT1</i>	CACCCAGACTGAGCACGTGC	TGG	nSpCas9
<i>HEK3_OT2</i>	GACACAGACCGGGCACGTGA	GGG	nSpCas9
<i>HEK3_OT3</i>	AGCTCAGACTGAGCAAGTGA	GGG	nSpCas9
<i>EMX1_OT1</i>	GAGTTAGAGCAGAAGAAGAA	AGG	nSpCas9
<i>EMX1_OT2</i>	GAGTCTAAGCAGAAGAAGAA	GAG	nSpCas9
<i>EMX1_OT3</i>	GAGGCCGAGCAGAAGAAGAA	CGG	nSpCas9
<i>VEGFA3_OT4</i>	GAGTGAGTGAGTGTGTGTGTG	GGG	nSpCas9
<i>VEGFA3_OT6</i>	GTGTGAGTAAGTGTGTGTGTG	TGG	nSpCas9

VEGFA3_OT12	GGTGAGTGTGTGTGTCATG	TGG	nSpCas9
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Supplementary Table 3 | Primers used to construct sgRNA plasmids

Site	Primer	Sequence
site 1-39, 56-66, GATC sites	Fwd primer	AGAGCUAGAAATAGCAAGTTAAAATAAGG
site 1	Rev primer	AGCTCUAAAACGCAGTCTATGCTTTGTGTTTCGGTGTTCGTCCTTTCCACAAG
site 2	Rev primer	AGCTCUAAAACCCACCCAAGTGATCACACTTCGGTGTTCGTCCTTTCCACAAG
site 3	Rev primer	AGCTCUAAAACCCCAAGGTGACCGTCCTGCGGTGTTCGTCCTTTCCACAAG
site 4	Rev primer	AGCTCUAAAACCCAAGACAAACTTGATCCTCGGTGTTCGTCCTTTCCACAAG
site 5	Rev primer	AGCTCUAAAACCTGACAATCGATAGGTACCGGTGTTCGTCCTTTCCACAAG
site 6	Rev primer	AGCTCUAAAACGCAGTCTATGCCTCATACTCGGTGTTCGTCCTTTCCACAAG
site 7	Rev primer	AGCTCUAAAACGCCTGGCCTGGGTCAATCCGGTGTTCGTCCTTTCCACAAG
site 8	Rev primer	AGCTCUAAAACGCAGTCTATCCTTGGTCTTCGGTGTTCGTCCTTTCCACAAG
site 9	Rev primer	AGCTCUAAAACCAAGGTGACCGTCCTGGCTCGGTGTTCGTCCTTTCCACAAG
site 10	Rev primer	AGCTCUAAAACCCAAGTGATCACACTTGTCGGTGTTCGTCCTTTCCACAAG
site 11	Rev primer	AGCTCUAAAACCTGGCCTGGGTCAATCCTTGGCGGTGTTCGTCCTTTCCACAAG
site 12	Rev primer	AGCTCUAAAACGCACCTTGGCGCAGCGGTGGCGGTGTTCGTCCTTTCCACAAG
site 13	Rev primer	AGCTCUAAAACCTGACTCATCATTATCTCATCGGTGTTCGTCCTTTCCACAAG
site 14	Rev primer	AGCTCUAAAACCTTAATCATAACAATTGCTTCGGTGTTCGTCCTTTCCACAAG
site 15	Rev primer	AGCTCUAAAACCATTTCTTTTGAATGTATTCGGTGTTCGTCCTTTCCACAAG
site 16	Rev primer	AGCTCUAAAACATTTCTTTTGAATGTATTCGGTGTTCGTCCTTTCCACAAG
site 17	Rev primer	AGCTCUAAAACCTTCTTCTCTGCTCGGACTCGGTGTTCGTCCTTTCCACAAG
site 18	Rev primer	AGCTCUAAAACCACTATCTCAATGCAAAATATCGGTGTTCGTCCTTTCCACAAG
site 19	Rev primer	AGCTCUAAAACCACTGACCTGAAGTACTTGGCGGTGTTCGTCCTTTCCACAAG
site 20	Rev primer	AGCTCUAAAACGCTTGCCCCCTTGGGCCTTACGGTGTTCGTCCTTTCCACAAG
site 21	Rev primer	AGCTCUAAAACCGCAGGCCACGGTCACTGCGGTGTTCGTCCTTTCCACAAG
site 22	Rev primer	AGCTCUAAAACGGAGTTTGGCCTTGTTAACCAGGTGTTCGTCCTTTCCACAAG
site 23	Rev primer	AGCTCUAAAACCTAATCCCGGAAGTGGACCCGGTGTTCGTCCTTTCCACAAG
site 24	Rev primer	AGCTCUAAAACAGCCCAGCAGTCTATCCTTGGGTGTTCGTCCTTTCCACAAG
site 25	Rev primer	AGCTCUAAAACGCCGTTTGTACTTTGTCTCGGTGTTCGTCCTTTCCACAAG
site 26	Rev primer	AGCTCUAAAACGCCAGATAATACGGGTATCGGTGTTCGTCCTTTCCACAAG
site 27	Rev primer	AGCTCUAAAACAGTCATGGTTTGATGTCTCCGGTGTTCGTCCTTTCCACAAG
site 28	Rev primer	AGCTCUAAAACCAAGTGATCACACTTGTCACGGTGTTCGTCCTTTCCACAAG
site 29	Rev primer	AGCTCUAAAACGTCTATCCTTGGTCTTCACTCGGTGTTCGTCCTTTCCACAAG
site 30	Rev primer	AGCTCUAAAACCTTCTGCTCGGACTCAGGCGGTGTTCGTCCTTTCCACAAG
site 31	Rev primer	AGCTCUAAAACCTTCTTCTTCTGCTCGGACTCGGTGTTCGTCCTTTCCACAAG
site 32	Rev primer	AGCTCUAAAACGTGACAAGTGTGATCACTTGCGGTGTTCGTCCTTTCCACAAG
site 33	Rev primer	AGCTCUAAAACGTGATGTCTCCTGCAGTCTATCGGTGTTCGTCCTTTCCACAAG
site 34	Rev primer	AGCTCUAAAACCTTCTCATCTGCAAGTCATCGGTGTTCGTCCTTTCCACAAG
site 35	Rev primer	AGCTCUAAAACCTGAAAAATGGCTTTGAATCGGTGTTCGTCCTTTCCACAAG
site 36	Rev primer	AGCTCUAAAACGTCTATGCTTTGTGTTCCAGCGGTGTTCGTCCTTTCCACAAG

site 37	Rev primer	AGCTCUAAAACCTGCAGTCTATGCCTCATACGGTGTTCGTCCTTTCCACAAG
site 38	Rev primer	AGCTCUAAAACCTTCTGCTCGGACTCAGGCCGGTGTTCGTCCTTTCCACAAG
site 39	Rev primer	AGCTCUAAAACCCCTTCTTCTTCTGCTCGGACGGTGTTCGTCCTTTCCACAAG
site 56	Rev primer	AGCTCUAAAACCTCACGTGCTCAGTCTGGGCCGGTGTTCGTCCTTTCCACAAG
site 57	Rev primer	AGCTCUAAAACCCAGCACACACTCACTCACC GG TGTTCGTCCTTTCCACAAG
site 58_ <i>PCSK9</i>	Rev primer	AGCTCUAAAACGCTTGCCCCCTGGGCCCTACGGTGTTCGTCCTTTCCACAAG
site 59_ <i>PCSK9</i>	Rev primer	AGCTCUAAAACCGCAGGCCACGGTCACCTGCGGTGTTCGTCCTTTCCACAAG
site 60_ <i>ABCA4</i>	Rev primer	AGCTCUAAAACCTCTCCAGGGCGAACTTCGCGGTGTTCGTCCTTTCCACAAG
site 61	Rev primer	AGCTCUAAAACCTTGCCCCCTGGGCCCTTAGACGGTGTTCGTCCTTTCCACAAG
site 62	Rev primer	AGCTCUAAAACCTGCCCCCTGGGCCCTTAGAGCGGTGTTCGTCCTTTCCACAAG
site 63	Rev primer	AGCTCUAAAACGCCCCCTGGGCCCTTAGAGTCGGTGTTCGTCCTTTCCACAAG
site 64	Rev primer	AGCTCUAAAACAGGCCACGGTCACCTGCCACGGTGTTCGTCCTTTCCACAAG
site 65	Rev primer	AGCTCUAAAACAGGCCACGGTCACCTGCCAGCGGTGTTCGTCCTTTCCACAAG
site 66	Rev primer	AGCTCUAAAACGCGCCACGGTCACCTGCCAGACGGTGTTCGTCCTTTCCACAAG
plasmid G ^m ATC site	Rev primer	AGCTCUAAAACCGTGGTATTGAGATCAGAGCGGTGTTCGTCCTTTCCACAAG
genomic GATC site	Rev primer	AGCTCUAAAACCCACCCAAGTGATCACACTTCGGTGTTCGTCCTTTCCACAAG
site 40-49	Fwd primer	AGTACTCUGGAAACAGAATCTACTAAACAAGGC
site 40	Rev primer	AGAGTACUAAAACCTAGGACACATGCTGTCTACCACGGTGTTCGTCCTTTCCACAAG
site 41	Rev primer	AGAGTACUAAAACCCCCAAAGGCCAGGCTGTAATCGGTGTTCGTCCTTTCCACAAG
site 42	Rev primer	AGAGTACUAAAACCTGTTTAGCACATTACCTGACACGGTGTTCGTCCTTTCCACAAG
site 43	Rev primer	AGAGTACUAAAACACCCCATGCACCTCCTCCACGGTGTTCGTCCTTTCCACAAG
site 44	Rev primer	AGAGTACUAAAACCTTATGATACTTCGCACACTAGTCGGTGTTCGTCCTTTCCACAAG
site 45	Rev primer	AGAGTACUAAAACGTCAGGCCTCTGTCCCTCTGTACGGTGTTCGTCCTTTCCACAAG
site 46	Rev primer	AGAGTACUAAAACAGGCTGTTGTCATACTTCTCATCGGTGTTCGTCCTTTCCACAAG
site 47	Rev primer	AGAGTACUAAAACGTAATGACTAAGATGACTGCCGGTGTTCGTCCTTTCCACAAG
site 48	Rev primer	AGAGTACUAAAACGGGTACAATCCTACTCTAGTCCGGTGTTCGTCCTTTCCACAAG
site 49	Rev primer	AGAGTACUAAAACCTGCTGTCACAGTTAGCTCAGCCGGTGTTCGTCCTTTCCACAAG
site 50-55_LbABE	Rev primer	ATCTACACUTAGTAGAAATTCGGTGTTCGTCCTTTCCACAAG
site 50_LbABE	Fwd primer	AGTGTAGAUTGCTGCAAGTAAGCATGCATTTGTTTTTAAAGCTTGGGCCGCTCGAG
site 51_LbABE	Fwd primer	AGTGTAGAUCTAGACAGGGGCTAGTATGTGCATTTTTTTAAAGCTTGGGCCGCTCGAG
site 52_LbABE	Fwd primer	AGTGTAGAUCAAGCTATTGAGGCTGGCCCGCCCTTTTTTAAAGCTTGGGCCGCTCGAG
site 53_LbABE	Fwd primer	AGTGTAGAUAGAAGCACATCAAGGACATTCTAATTTTTTAAAGCTTGGGCCGCTCGAG
site 54_LbABE	Fwd primer	AGTGTAGAUAGGATAAGCACAGTTTTAAATAGTTTTTTTTAAAGCTTGGGCCGCTCGAG
site 55_LbABE	Fwd primer	AGTGTAGAUGTTTAAACACACCGGGTTAATAATTTTTTAAAGCTTGGGCCGCTCGAG
site 50-55_enAsABE	Rev primer	ACAAGAGUAGAAATTCGGTGTTCGTCCTTTCCACAAG
site 50_enAsABE	Fwd primer	ACTCTTGUAGATTGCTGCAAGTAAGCATGCATTTGTTTTTAAAGCTTGGGCCGCTCGAG
site 51_enAsABE	Fwd primer	ACTCTTGUAGATCTAGACAGGGGCTAGTATGTGCATTTTTTTAAAGCTTGGGCCGCTCGAG
site 52_enAsABE	Fwd primer	ACTCTTGUAGATCAGCTATTGAGGCTGGCCCGCCCTTTTTTAAAGCTTGGGCCGCTCGAG
site 53_enAsABE	Fwd primer	ACTCTTGUAGATGAAGCACATCAAGGACATTCTAATTTTTTAAAGCTTGGGCCGCTCGAG
site 54_enAsABE	Fwd primer	ACTCTTGUAGATGGATAAGCACAGTTTTAAATAGTTTTTTTTAAAGCTTGGGCCGCTCGAG
site 55_enAsABE	Fwd primer	ACTCTTGUAGATGTTTAAACACACCGGGTTAATAATTTTTTAAAGCTTGGGCCGCTCGAG
site R loop 1-6	Fwd primer	AGTACTCUGGAAACAGAATCTACTAAACAAGGC
R loop 1	Rev primer	AGAGTACUAAAACCTAGGACACATGCTGTCTACCACGGTGTTCGTCCTTTCCACAAG

R loop 2	Rev primer	AGAGTACUAAAACCCCCAAAGGCCAGGCTGTAAATCGGTGTTTCGTCCTTTCCACAAG
R loop 3	Rev primer	AGAGTACUAAAACCTGTTTAGCACATTACCTGACACGGTGTTCGTCCTTTCCACAAG
R loop 4	Rev primer	AGAGTACUAAAACACCCCATGCACCCTCCTCCACCGGTGTTTCGTCCTTTCCACAAG
R loop 5	Rev primer	AGAGTACUAAAACCTGGCTCAATCAATCCTCTTGCCGGTGTTCGTCCTTTCCACAAG
R loop 6	Rev primer	AGAGTACUAAAACCTTATGATACTTCGCACACTAGTCGGTGTTCGTCCTTTCCACAAG

Supplementary Table 4 | DNA templates used for error prone PCR in evolution rounds 1-3

Round	Template	TadA mutations
1	<i>WT TadA</i>	/
2	<i>TadA-RA1.0</i> or <i>TadA-RA1.1</i>	D108G, K161N
3	<i>TadA-RA2.0</i> , <i>TadA-RA2.1</i> or <i>TadA-RA2.2</i>	P48A, R51H, I76F, D108G, K110R, M126I, N127K, H122R, K161N

Supplementary Table 5 | DNA shuffling fragments generated by overlapping PCR

Entry	Fwd primer	Rev primer	DNA template	Shuffled amino acids
1A	YX209	WT1681	<i>TadA_P48A/TadA_P48A_R51H</i> (1:1)	R51(R/H); I76(I/F); with P48A fixed
1B	WT1679/ WT1680 (1:1)	WT1682	DNA ultramer WT1675/WT1676 (1:1)	L84(L/F); A106(A/V); K110(K/R); T111(T/R); D119(D/N); H122(H/R); H123(H/Y); M126(M/I); N127(N/K); with D108G fixed
1C	WT1683	YX210	DNA ultramer WT1677/WT1678 (1:1)	S146(S/C); D147(D/R); F149(F/Y); R152(R/P); Q154(Q/R); E155(E/V); I156(I/F); K157(K/N); K161(K/N); T166(T/I); D167(D/N)
2A	YX209	YX443	<i>TadA8.20</i>	W23(W/R); E27(E/D)
2B	YX444	YX445	/	H36(H/L); R47(R/K); P48(P/A); H51(H/L)
2C	YX446	YX447/ YX448 (1:1)	<i>TadA8.20</i>	I76(F/Y); V82(V/S); L84(L/F);
2D	YX458	YX450/ YX451 (1:1)	/	M94(M/V); D108(G/N); A109(A/S); H111(H/R); A114(A/V); with A106V, K110R, D119N fixed
2E	YX452	YX210	<i>TadA_S146S/TadA_S146C</i> (1:1) carrying H123Y; M126I; N127K; D147R; R152P; Q154R; E155V; I156F	H122(H/N); S146(S/C); with H123Y; M126I; N127K; D147R; R152P; Q154R; E155V; I156F fixed

Supplementary Table 6 | DNA oligos used for generating, amplifying, and sequencing *TadA* libraries/variants. W = A, T; Y = C, T; R = A, G; S = C, G; K = G, T; M = A, C.

Primer	Sequence
YX209	GATTGGTCTCAACCTGCAGGTGCAGTAAGGAGGAAAAAAAAAATG
YX210	GATTGGTCTCAGTCCCCGGTGTTCGCTACCGGA
WT1679	CCACCCTGTATGTGACATTCGAGCCATGCGTGATGTG
WT1680	CCACCCTGTATGTGACACTGGAGCCATGCGTGATGTG
WT1681	TGTCACATACAGGGTGGCATCGAWCAGGCGGTAATTCTGCATG
WT1682	CGTCTGCCAGGATTCCCTCTGTGATCTCCACCCGGTG
WT1683	GGAATCCTGGCAGACGAGTGCGCCGCCCTGCTG
WT1675	GAGCCATGCGTGATGTGCGCAGGAGCAATGATCCACAGCAGGATCGGAAGAGTGGTGTTTCGGAGYGCGGGGCGCC ARGCGCGGCGCAGCAGGCTCCCTGATGRATGTGCTGCRCYACCCCGGCATRAASCACCGGGTGGAGATCACAG
WT1676	GAGCCATGCGTGATGTGCGCAGGAGCAATGATCCACAGCAGGATCGGAAGAGTGGTGTTTCGGAGYGCGGGGCGCC ARGACCGGCGCAGCAGGCTCCCTGATGRATGTGCTGCRCYACCCCGGCATRAASCACCGGGTGGAGATCACAG
WT1677	GTGCGCCGCCCTGCTGWGCCGTTTCTWTAGAATGCSGAGACRGGWGWTC AAKGCCAGAGAAGASGCACAGAGCT CCAYCRACTCCGGTAGCGAAACACCG
WT1678	GTGCGCCGCCCTGCTGWGCCGATTCTWTAGAATGCSGAGACRGGWGWTC AAKGCCAGAGAAGASGCACAGAGCT CCAYCRACTCCGGTAGCGAAACACCG
YX443	GGCGCCACGGGGACWTCTTTTCATCCCRTGCTCGCTTTGC
YX444	CCCCGTGGGCGCCGTGCTGGTGCWCAACAATAGAGTGATCGGAGAGGG
YX445	GCGGTAGGGTCGTGGWGGCCGATTGSCYTGTTCATCCCTCTCCGATCACTCT
YX446	CACGACCCTACCGCACACG
YX447	ACATCACGCATGGCTCGARTGTCACATACAGGGTGGCATCGWACAGGCGGTAATTCTGCA
YX448	ACATCACGCATGGCTCGARTGTCGAATACAGGGTGGCATCGWACAGGCGGTAATTCTGCA
YX458	GCCATGCGTGATGTGCGCAGGAGCARTGATCCACAGCAGGATCGGAAGAGTGGTGTTTCGG
YX450	CATTCATCAGGGAGCCTRCTGCGCCGYGCCTGGMGCCCCGCACTCCGAACACCACTCTTC
YX451	CATTCATCAGGGAGCCTRCTGCGCCGYGCCTGGMGTCCGCACTCCGAACACCACTCTTC
YX452	GGCTCCCTGATGAATGTGCTGMACTACCCGGC
WT022	CATTTTTCGCTTCAGCCAT
YX140	CAGTGATCACCGCCCATCC

Supplementary Table 7 | Selection plasmids used in the study and minimum inhibitory concentrations (MICs) for *E. coli* carrying these selection plasmids.

Round	Antibiotic resistance	Target sequence	Inactivating mutation	Position of target A in protospacer	MIC in S1030 (µg/mL)	Concentrations of antibiotics for selection (µg /mL)
1	<i>Cam^R</i>	GCTCTG <u>A</u> TCTGAATACCACG	<i>W106*</i>	7	8	8, 16, 32, 64
2	<i>Kan^R</i>	GCTTG <u>A</u> TCGGAGAGGCTATT	<i>W15*-W24*</i>	6, 6	4	12.5, 25, 50
		GACTG <u>A</u> TCGCAACAGACAAT				
3	<i>Kan^R</i>	GCTTG <u>A</u> TCGGAGAGGCTATT	<i>W15*-W24*</i>	6, 6	4	50, 100, 200
		GACTG <u>A</u> TCGCAACAGACAAT				
4	<i>Kan^R</i>	GCTTG <u>A</u> TCGGAGAGGCTATT	<i>W15*-W24*</i>	6, 6	4	100, 200, 400
		GACTG <u>A</u> TCGCAACAGACAAT				
5	<i>Cam^R</i>	TTCTTTTC <u>A</u> GTGCCATTGGG	<i>R18*-R65*-H193Y</i>	9, 4, 9	1	16, 32, 64, 128
		GTC <u>A</u> GGCTGCAATGTGAATA				
		TACGGCGT <u>A</u> GTGCACCTGGA				

Supplementary Table 8 | Sequences of DNA or RNA oligos for *in vitro* deamination

Oligo	Sequence
<i>E. coli</i> tRNA	rGrCrArUrCrCrGrUrArGrCrUrCrArGrCrUrGrGrArUrArGrArGrUrArCrUrCrGrGrCrUrArCrGrArArCrCrGrArGrCrGrGrUrCrGrGrArGrGrUrUrCrGrArArUrCrCrUrCrCrGrGrArUrGrCrArCrCrA
RT primer	TCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAATGATTGCCCCAAATGGTGCATCCG
Fwd primer for RT-PCR	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCATCCGTAGCTCAGCTGG
Rev primer for RT-PCR	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCGAATAGCGCCCTTCC

Supplementary Table 9 | Illumina primers used for next generation sequencing

Site	Primer	Sequence
site 1_Fwd	YX220	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGCCCATCTGTCAAACCT
site 1_Rev	YX221	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCCCTGGAAACAATGA
site 2_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 2_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 3_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 3_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 4_Fwd	YX327	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCGACAGCCAGTGGTTAAGT
site 4_Rev	YX328	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTTTCACCGACTGCACAG
site 5_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 5_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 6_Fwd	YX325	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
site 6_Rev	YX326	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
site 7_Fwd	YX939	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGCATGCATTTGTAGGCTTGATG
site 7_Rev	YX334	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTC AAC
site 8_Fwd	YX516	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTGCTTATTGCTGAGGGGCA
site 8_Rev	YX517	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTCTCCTCCAGCTGAG
site 9_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 9_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 10_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 10_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 11_Fwd	YX939	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGCATGCATTTGTAGGCTTGATG
site 11_Rev	YX334	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTC AAC
site 12_Fwd	YX799	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTAGCCTTGCGTCCGAGG
site 12_Rev	YX800	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGCAGTCCCAAGATCG
site 13_Fwd	YX331	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCTGAGGTCACACAGTGGG
site 13_Rev	YX332	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGAGCAGGGACCACATC
site 14_Fwd	YX766	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTACACCAATTCTCACTGATGC
site 14_Rev	YX767	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAACAAACGTTATGACAAACCTCC
site 15_Fwd	YX775	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGTGATTCAAAGGGTATCAGGCC
site 15_Rev	YX776	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCACTCATAAACAGAAGGTTCTACC
site 16_Fwd	YX775	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGTGATTCAAAGGGTATCAGGCC
site 16_Rev	YX776	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCACTCATAAACAGAAGGTTCTACC
site 17_Fwd	YX561	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTTGA
site 17_Rev	YX941	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCTTCGTGGCAATGCG
site 18_Fwd	YX797	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGGCCTCACTGGATACTC
site 18_Rev	YX940	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAATGACTGAATCGGAACAAGGC
site 19_Fwd	YX829	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGCTTATGAAGGCAGAGACTGAG
site 19_Rev	YX830	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTTACCTCTCCTTTCCAAGGCAC

site 20_Fwd	YX803	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTGCTTGAGTTGATCCTG
site 20_Rev	YX804	TGGAGTTCAGACGTGTGCTCTTCCGATCTATGCTGGCCTCAGCTGGTG
site 21_Fwd	YX805	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCACAGAAGGATGTCGGAG
site 21_Rev	YX806	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTAGTGCTGACGTC
site 22_Fwd	YX701	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCTTTAAACATTGTCTGTGCG
site 22_Rev	YX702	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTTCTGTCCCTCCCTCAGTA
site 23_Fwd	YX705	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGAGAGAGCAGGACGTCACA
site 23_Rev	YX706	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGCACTACCTACGTCAGCACCT
site 24_Fwd	YX516	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGCTTATTGCTGAGGGGCA
site 24_Rev	YX517	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTCTCTCCAGCTGAG
site 25_Fwd	YX925	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTCTGCTCGGACTCAGGCC
site 25_Rev	YX926	TGGAGTTCAGACGTGTGCTCTTCCGATCTAACCTATGTAGCCTCAGTCTCC
site 26_Fwd	YX709	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGACAGAGGGAGAGAAACAGAGC
site 26_Rev	YX710	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTAGATGCCGACAAAAGGAT
site 27_Fwd	YX325	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
site 27_Rev	YX326	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
site 28_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 28_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 29_Fwd	YX516	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGCTTATTGCTGAGGGGCA
site 29_Rev	YX517	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTCTCTCCAGCTGAG
site 30_Fwd	YX561	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTGA
site 30_Rev	YX941	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCTTCGTGGCAATGCG
site 31_Fwd	YX561	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTGA
site 31_Rev	YX941	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCTTCGTGGCAATGCG
site 32_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
site 32_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC
site 33_Fwd	YX325	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
site 33_Rev	YX326	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
site 34_Fwd	YX325	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
site 34_Rev	YX326	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
site 35_Fwd	YX707	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACTGCTGAACCAGTCAAACCTC
site 35_Rev	YX708	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATGGGGAAATATAAACTTG
site 36_Fwd	YX220	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGCCCCATCTGTCAAACCT
site 36_Rev	YX221	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCTTGAAACAATGA
site 37_Fwd	YX325	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
site 37_Rev	YX326	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
site 38_Fwd	YX561	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTGA
site 38_Rev	YX941	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCTTCGTGGCAATGCG
site 39_Fwd	YX561	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTGA
site 39_Rev	YX941	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCTTCGTGGCAATGCG
site 40_Fwd	YX101 7	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCTCCTGCTTCTCTGTGATAGG

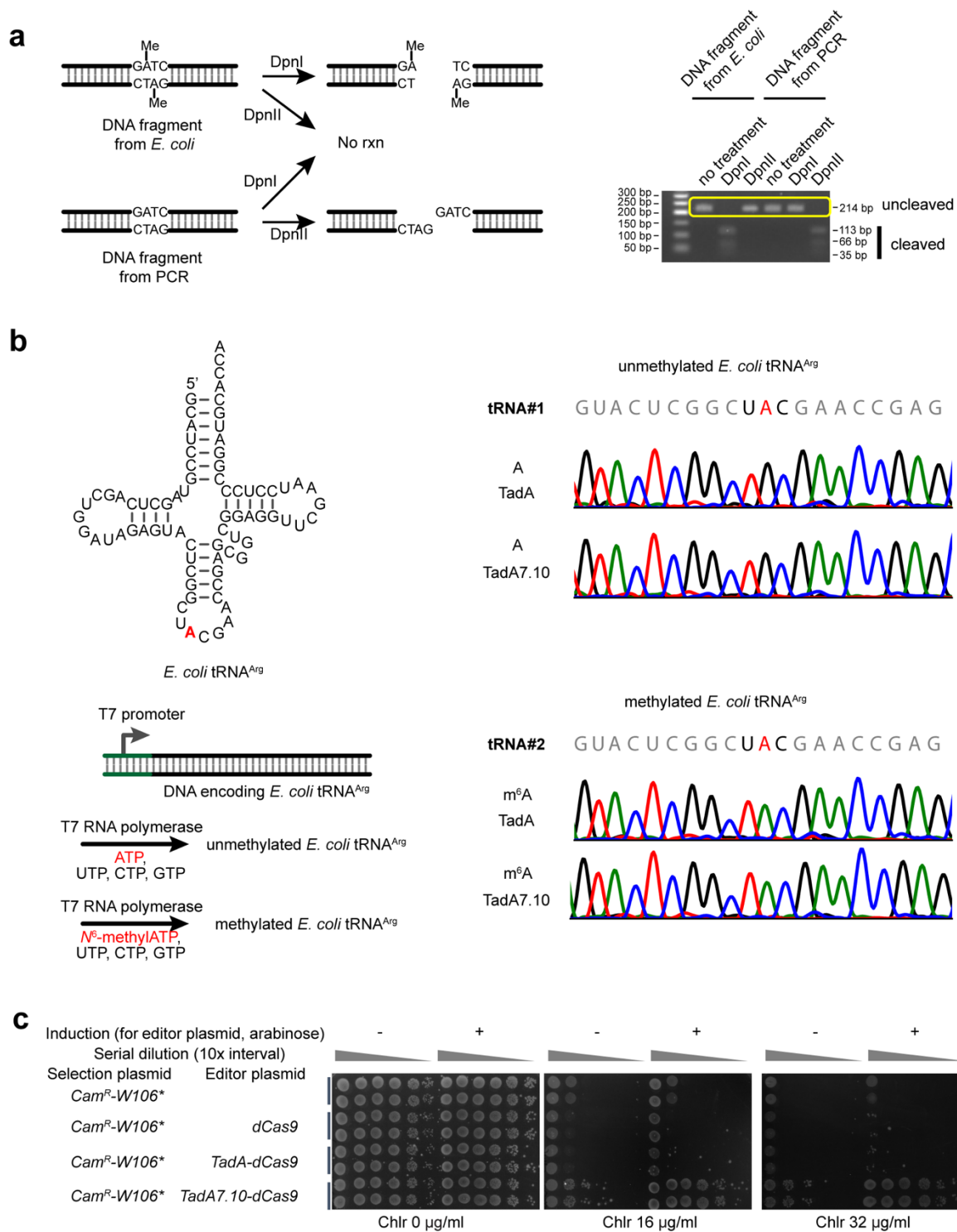
site 40_Rev	YX101 8	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAACATACATGCCCTTTGC
site 41_Fwd	YX587	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGACATTTCCACCGCAAAATG
site 41_Rev	YX588	TGGAGTTCAGACGTGTGCTCTTCCGATGCTACAGAAAGGTCAGCAGC
site 42_Fwd	YX745	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCTGTGGCATCCAGAGACATGG
site 42_Rev	YX945	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTTTGCTCCAGATTTCCTTC
site 43_Fwd	YX946	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAATCCTGGACAAGGTTGAAGG
site 43_Rev	YX592	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGAGGTCTAGGAACCCG
site 44_Fwd	YX845	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACTGGCCTTTATTCAGTCCCTC
site 44_Rev	YX846	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCACTGAGCATAGACCAAG
site 45_Fwd	YX847	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGAGTCTAGAGGGCAGTGGTG
site 45_Rev	YX848	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCACACACATTGAATCTCCTG
site 46_Fwd	YX715	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGACTCAGCCCTGCAAAAGG
site 46_Rev	YX716	TGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGTCAGGGGAGCGTGTC
site 47_Fwd	YX717	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGCTCATATGCCCTTGG
site 47_Rev	YX718	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGTAGGAATTTTGGTGGGACA
site 48_Fwd	YX721	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCCTGTTCCTAAAGCCACC
site 48_Rev	YX722	TGGAGTTCAGACGTGTGCTCTTCCGATCTACTGGTTCTGTTTGTGGCCA
site 49_Fwd	YX220	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGCCCCATCTGTCAAACCT
site 49_Rev	YX221	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCTTGGAACAATGA
site 50_Fwd	YX951	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGGGAAACGCCCATGC
site 50_Rev	YX654	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTAACC
site 51_Fwd	YX951	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGGGAAACGCCCATGC
site 51_Rev	YX654	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTAACC
site 52_Fwd	YX220	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGCCCCATCTGTCAAACCT
site 52_Rev	YX221	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCTTGGAACAATGA
site 53_Fwd	YX659	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAAAAGGGGCAAGCTTCAGAT
site 53_Rev	YX660	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGAGGAGAAGGCAGGAGG
site 54_Fwd	YX661	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTGTCTGCCCTACAGAGGT
site 54_Rev	YX662	TGGAGTTCAGACGTGTGCTCTTCCGATCCCAAAGGACATACGGGGAG
site 55_Fwd	YX663	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGCGTGCTTCTTACATGCC
site 55_Rev	YX664	TGGAGTTCAGACGTGTGCTCTTCCGATCCAAGTATGCCTTAAGCAGAAACAA
site 56_Fwd	YX942	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNTGTGCAAGTAAGCATGCATTG
site 56_Rev	YX629	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTAACC
site 57_Fwd	YX520	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGCAGACGGCAGTCACTAGG
site 57_Rev	YX521	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTATTGGAATCCTGGAGTGACCC
site 58_PCSK9_Fwd	YX803	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTGCTTGAGTTGATCCTG
site 58_PCSK9_Rev	YX804	TGGAGTTCAGACGTGTGCTCTTCCGATCTATGCTGGCCTCAGCTGGTG
site 59_PCSK9_Fwd	YX805	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTCACAGAAGGATGTCCGGAG
site 59_PCSK9_Rev	YX806	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTAGTGCTGACGTC
site 60_ABCA4_Fwd	YX109 5	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTGTCTCAGTTCTCAGTCCGG
site 60_ABCA4_Rev	YX109 6	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGCTCTGCCTTATGGGGAGG

site 61_Fwd	YX803	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTGCTTGAGTTGATCCTG
site 61_Rev	YX804	TGGAGTTCAGACGTGTGCTCTTCCGATCTATGCTGGCCTCAGCTGGTG
site 62_Fwd	YX803	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTGCTTGAGTTGATCCTG
site 62_Rev	YX804	TGGAGTTCAGACGTGTGCTCTTCCGATCTATGCTGGCCTCAGCTGGTG
site 63_Fwd	YX803	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTGCTTGAGTTGATCCTG
site 63_Rev	YX804	TGGAGTTCAGACGTGTGCTCTTCCGATCTATGCTGGCCTCAGCTGGTG
site 64_Fwd	YX805	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTCACAGAAGGATGTCGGAG
site 64_Rev	YX806	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTAGTGCTGACGTC
site 65_Fwd	YX805	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTCACAGAAGGATGTCGGAG
site 65_Rev	YX806	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTAGTGCTGACGTC
site 66_Fwd	YX805	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTCACAGAAGGATGTCGGAG
site 66_Rev	YX806	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTAGTGCTGACGTC
R loop 1_Fwd	YX1017	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCTCCTGCTTCTGTGATAGG
R loop 1_Rev	YX1018	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAACATACATGCCCTTTGC
R loop 2_Fwd	YX587	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGACATTTCCACCGAAAAATG
R loop 2_Rev	YX588	TGGAGTTCAGACGTGTGCTCTTCCGATGCTACAGAAAGGTCAGCAGC
R loop 3_Fwd	YX745	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCTGTGGCATCCAGAGACATGG
R loop 3_Rev	YX945	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTTTGCTCCAGATTCCCTTC
R loop 4_Fwd	YX946	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAATCCTGGACAAGGTTGAAGG
R loop 4_Rev	YX592	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGAGGTCTAGGAACCCG
R loop 5_Fwd	YX835	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCATGAACTGTAGCCCCAGCTAC
R loop 5_Rev	YX836	TGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGAACCAACCCAAATATTCTCT
R loop 6_Fwd	YX845	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACTGGCCTTTATTCAAGTCCCTC
R loop 6_Rev	YX846	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCACTGAGCATAGACCAAG
HEK2_OT1_Fwd	YX581	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGTGGAGAGTGAGTAAGCCA
HEK2_OT1_Rev	YX582	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGGTAGGATGATTCAGGCA
HEK3_OT1_Fwd	YX787	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCCTGTTGACCTGGAGAA
HEK3_OT1_Rev	YX788	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGTACTTGCCCTGACCA
HEK3_OT2_Fwd	YX789	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGTGTTGACAGGGAGCAA
HEK3_OT2_Rev	YX790	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGATGTGGGCAGAAGGG
HEK3_OT3_Fwd	YX791	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGAGGGAACAGAAGGGCT
HEK3_OT3_Rev	YX792	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCAAAGGCCCAAGAACCT
EMX1_OT1_Fwd	YX563	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGGAGATTGTCATCTGTGGAGG
EMX1_OT1_Rev	YX564	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTTTATACCATCTTGGGGTTACAG
EMX1_OT2_Fwd	YX565	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAATGTGCTTCAACCCATCACGG
EMX1_OT2_Rev	YX566	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGAATTTGTGATGGATGCAGTCTG
EMX1_OT3_Fwd	YX943	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGAGGTGCAGGAGCTAGAC
EMX1_OT3_Rev	YX944	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCGTCCTGCTCTCACTTAG
VEGFA3_OT4_Fwd	YX575	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCTCATTTCTACGGCCAG
VEGFA3_OT4_Rev	YX576	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCAGTGAGGAGGTGGTTC
VEGFA3_OT6_Fwd	YX577	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCCACAGGCACTAACTCTTCA

<i>VEGFA3_OT6_Rev</i>	YX578	TGGAGTTCAGACGTGTGCTCTTCCGATCTGATGAAGCTGCCTTTCTAAGC
<i>VEGFA3_OT12_Fwd</i>	YX579	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCGCTTTCCCTTTGCTAGAATC
<i>VEGFA3_OT12_Rev</i>	YX580	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAGCAATGCCTATATTACTGGC
plasmid G ^m ATC site_Fwd	YX126	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCAAACTGAAACGTTTTCATCGC
plasmid G ^m ATC site_Rev	YX127	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAACTGCCGGAAATCCTCG
genomic GATC site_Fwd	YX473	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAATGTGTCAACTCTTGACAGGGC
genomic GATC site_Rev	YX474	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCAGGTGTAATGAAGACC

Supplementary Table 10 | DNA oligos used for amplifying paired sgRNA-target library.

Primer	Sequence
YX1323	CCATAGCGTACAGGTCTCAACCG
YX1324	GATTGGTCTCAGCAGACGACTGGACCTAGAACC
YX1339	AAAACGTCTCAAAGGTCGGGCAGGAAGAGGGC
YX1340	AAAACGTCTCAGCAGACGACTGGACCTAGAACC
YX1451	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNATCTTGTGGAAAGGACGAAACACC
YX1452	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATCTTGTGGAAAGGACGAAACACC
YX1453	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNATCTTGTGGAAAGGACGAAACACC
YX1454	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNATCTTGTGGAAAGGACGAAACACC
YX1381	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGACGACTGGACCTAGAACC



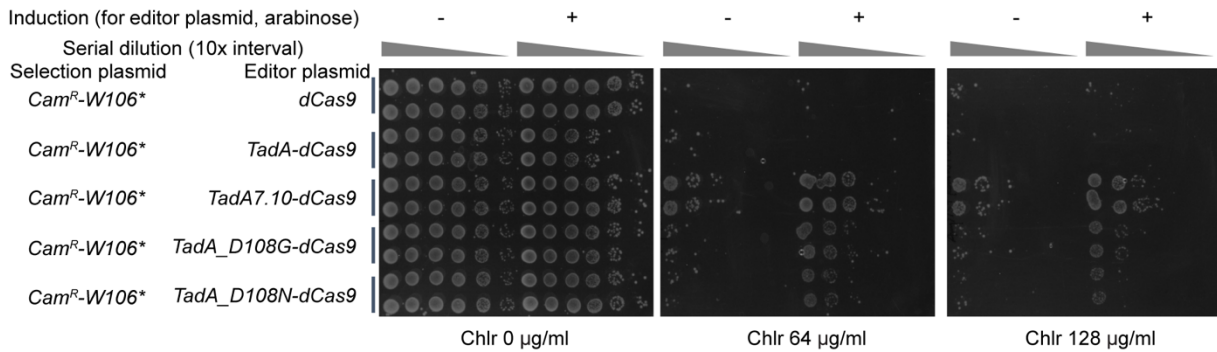
Supplementary Figure 1 | Validation of the directed evolution design for TadA to function on deoxyadenosine in RA sequences. **a**. Methylation of GATC sequences in *E. coli*. Two restriction enzymes, DpnI and DpnII, were employed to confirm methylation of the target GATC in the chloramphenicol acetyl transferase gene. A single experiment was performed. **b**. Unmethylated and methylated *E. coli* tRNA^{Arg}(ACG) treated with wildtype TadA and TadA7.10. Unmethylated

and methylated tRNA were prepared through *in vitro* transcription using ATP and *N*⁶-methyl-ATP as starting materials, respectively. Treated RNA was reverse transcribed, amplified by PCR, and subjected to Sanger sequencing. **c.** Serial dilutions of *E. coli* transformed with the selection plasmid and denoted editor plasmids plated on agar plates supplemented with 0, 16, or 32 µg/mL chloramphenicol. Expression of dCas9 and TadA-dCas9 is regulated by an arabinose-responsive promoter. Two individual colonies from each transformation were assayed. Chlr: Chloramphenicol.

a

Position	20	27	49	51	61	76	82	108	110	127	134	156	161
WT TadA	K	E	I	R	M	I	V	D	K	N	E	I	K
colony 1								G					
colony 2	R							G			G		
colony 3								G					
colony 4				H		F		G					
colony 5				H		T		G					N
colony 6		D		H				G					
colony 7								G					
colony 8								G					
colony 9							A	G	R	S			
colony 10					L			G				N	
colony 11								G					N
colony 12			N					H					
colony 13	R							G					

Position	108	161
WT TadA	D	K
TadA-RA1.0	G	
TadA-RA1.1	G	N

b

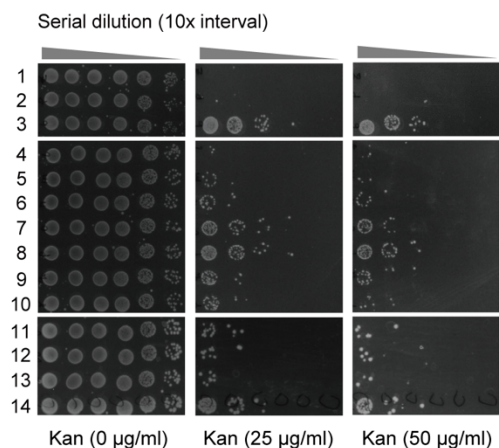
Supplementary Figure 2 | Initial-round directed evolution for TadA. **a**. Mutations identified in colonies that passed selection and validation. **b**. Serial dilutions of *E. coli* transformed with the selection plasmid encoding *Cam^R-W106** and denoted editor plasmids plated on agar plates supplemented with 0, 64, or 128 µg/mL chloramphenicol. Expression of dCas9 and TadA-dCas9 is regulated by an arabinose-responsive promoter. Two individual colonies from each transformation were assayed. Chlr: Chloramphenicol.

a

Position	34	47	48	50	51	72	73	76	80	94	96	108	110	119	122	126	127	128	133	134	136	142	161
WT TadA	L	R	P	G	R	N	Y	I	L	M	H	D	K	D	H	M	N	H	T	E	I	A	K
TadA_D108G												G											
colony 1												G	R				K						
colony 2												G	R										
colony 3			A									G	R										
colony 4			A									G	R										
colony 5		K			C		H					G	R										
colony 6			S	S							N	G											
colony 7			A					F				G									T		
colony 8					H			F				G											
colony 9					H			F				G											
colony 10					H			F				G											
colony 11								F				G											
colony 12						H						G										G	
TadA_D108G_K161N												G											N
colony 1			A					F				G											N
colony 2	M							F	M			G											N
colony 3					H			F				G											N
colony 4												G						R					N
colony 5												G						R					N
colony 6			A									G				I							N
colony 7			A									G				I							N
colony 8			A									G		N		I							N
colony 9			A									G											N
colony 10			A			S						G					K						N
colony 11												G					K						N
colony 12												G					K						N
colony 13												G					K						N
colony 14												G	R				K						N
colony 15												G											N
colony 16										V		G							A	G			N
colony 17												G			R								N
colony 18												G	M		R		S						N

Position	48	51	76	108	110	122	126	127	161
WT TadA	P	R	I	D	K	H	M	N	K
TadA-RA2.0	A	H	F	G	R	R	I	K	N
TadA-RA2.1	A	H		G	R	R	I	K	

b



Lane	Editor plasmid
1	dCas9
2	TadA_D108G_K161N-dCas9
3	TadA7.10-dCas9
4	TadA_D108G_K161N_H122R-dCas9
5	TadA_D108G_K161N_P48A-dCas9
6	TadA_D108G_K161N_N127K-dCas9
7	TadA_D108G_K161N_P48A_I76F-dCas9
8	TadA_D108G_K161N_P48A_N72S_N127K-dCas9
9	TadA_D108G_K161N_P48A_M126I-dCas9
10	TadA_D108G_K161N_P48A_K110R-dCas9
11	TadA_D108G_K161N_M126I-dCas9
12	TadA_D108G_K110R-dCas9
13	TadA_D108G_K161N_R51H-dCas9
14	TadA_D108G_R51H_I76F-dCas9

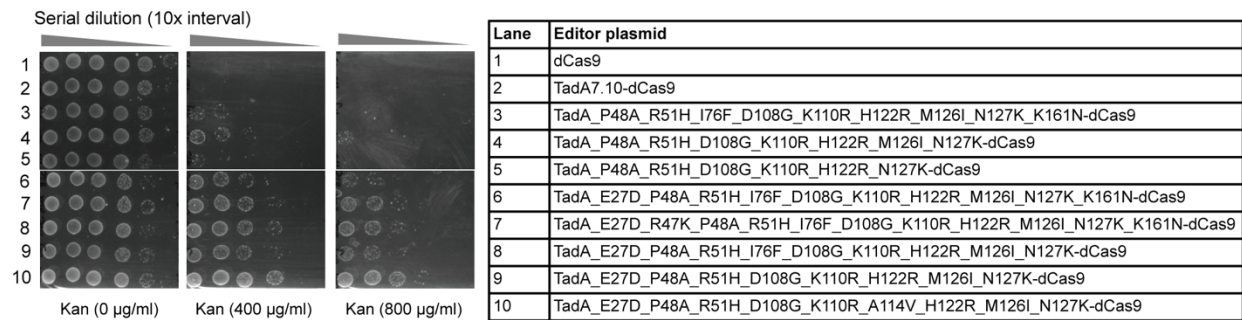
Supplementary Figure 3 | Second-round directed evolution for TadA. **a.** Mutations identified in colonies that passed selection and validation. **b.** Serial dilutions of *E. coli* transformed with the selection plasmid encoding *Kan^R-W15*-W24** and denoted editor plasmids. Bacteria were grown overnight in the presence of 1 mM arabinose to induce editor expression prior to serial dilution and plating on agar plates supplemented with 0, 25, or 50 µg/mL kanamycin. Kan: kanamycin.

a

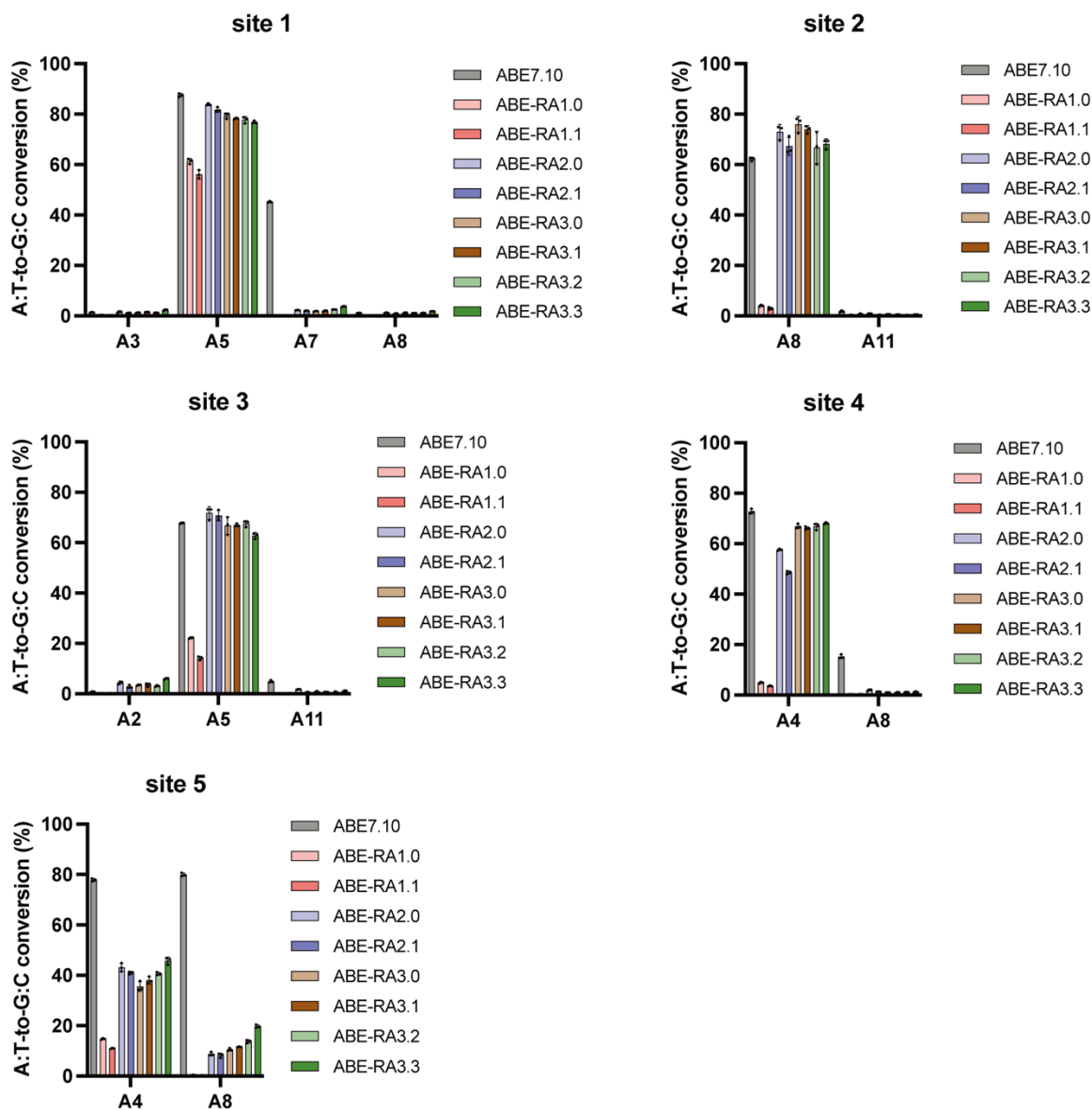
Position	27	37	47	48	51	55	71	76	94	108	110	114	122	126	127	142	161
WT TadA	E	N	R	P	R	T	Q	I	M	D	K	A	H	M	N	A	K
Starting template 1				A	H			F		G	R		R	I	K		N
colony 1				A	H			F		G	R		R	I	K		N
colony 2				A	H			F		G	R		R	I	K		N
colony 3				A	H			F		G	R		R	I	K		N
colony 4				A	H	I		F		G	R		R	I	K		N
colony 5	D		K	A	H			F		G	R		R	I	K		N
colony 6	D			A	H			F		G	R		R	I	K		N
colony 7	D			A	H			F		G	R		R	I	K		N
colony 8	D			A	H			F		G	R		R	I	K		N
Starting template 2				A	H					G	R		R	I	K		
colony 1	D			A	H					G	R	V	R	I	K		
colony 2	D			A	H					G	R		R	I	K		
colony 3				A	H					G	R		R	I	K		
colony 4				A	H					G	R		R	I	K		
colony 5	D	H		A	H					G	R		R	I	K		
colony 6	D			A	H					G	R	V	R	I	K		
colony 7	D			A	H					G	R	V	R	I	K		
Starting template 3				A	H					G	R		R		K		
colony 1	D			A	H					G	R		R		K		
colony 2				A	H					G	R		R		K		
colony 3	D			A	H					G	R		R		K		
colony 4	D			A	H		R		V	G	R		R		K		
colony 5	D			A	H		R		V	G	R		R		K		
colony 6	D			A	H					G	R		R		K		
colony 7				A	H					G	R		R		K	T	
colony 8	D			A	H					G	R		R		K		

Position	27	47	48	51	76	108	110	114	122	126	127	161
WT TadA	E	R	P	R	I	D	K	A	H	M	N	K
TadA-RA3.0	D		A	H	F	G	R		R	I	K	N
TadA-RA3.1	D	K	A	H	F	G	R		R	I	K	N
TadA-RA3.2	D		A	H		G	R	V	R	I	K	
TadA-RA3.3	D	K	A	H	F	G	R	V	R	I	K	

b



Supplementary Figure 4 | Third-round directed evolution for TadA. **a.** Mutations identified in colonies that passed selection and validation. **b.** Serial dilutions of *E. coli* transformed with the selection plasmid encoding *Kan^R-W15*-W24** and denoted editor plasmids. Bacteria were plated on agar plates supplemented with 0, 400, or 800 µg/mL kanamycin. Editor expression was induced with 1 mM arabinose overnight prior to serial dilution.



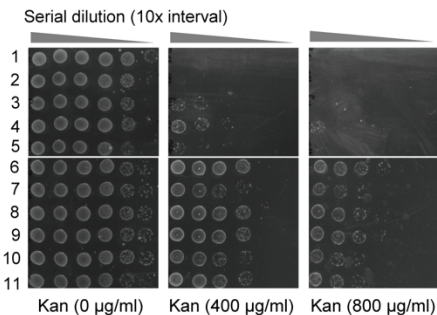
Supplementary Figure 5 | A:T-to-G:C editing mediated by ABE-RA1.0-1.1, 2.0-2.1, and 3.0-3.3 in HEK293T cells. Five target sites were assayed, with ABE7.10 as a control. Data are presented as mean $\pm$ s.d. (n = 3 independent experiments).

a

Position	51	76	84	106	110	111	119	122	123	126	127	146	147	149	152	154	155	156	157	161	166	167
WT TadA	R	I	L	A	K	T	D	H	H	M	N	S	D	F	R	Q	E	I	K	K	T	D
TadA7.10	L	I	F	V	K	T	D	H	Y	M	N	C	Y	F	P	Q	V	F	N	K	T	D
TadA8.20	L	Y	F	V	K	T	D	H	H	M	N	C	R	F	P	R	V	F	N	K	T	D
TadA8e	L	I	F	V	K	R	N	N	Y	M	N	C	D	Y	P	Q	V	F	N	K	I	N
TadA-RA2.0	H	F	L	A	R	T	D	R	H	I	K	S	D	F	R	Q	E	I	K	N	T	D
4th round lib	R/H	I/F	L/F	A/V	K/R	T/R	D/N	H/R	H/Y	M/I	N/K	S/C	D/R	F/Y	R/P	Q/R	E/V	I/F	K/N	K/N	T/I	D/N
colony 1	H	F		V	R	H	N		Y	I	K				P	R	V		N			
colony 2	H				R	H	N		Y	I	K				P	R	V					
colony 3	H	F		V	R	H	N	R	Y	I	K		R		P	R	V	F		N	I	
colony 4	H	F		V	R		N		Y		K	C			P	R	V	F				
colony 5	H	F		V	R		N	R	Y		K		R		P	R		F			I	
colony 6	H	F		V				R	Y		K	C	R		P	R	V		N		I	
colony 7				V	R	H	N			I	K	C	R		P	R	V	F	N		I	N
colony 8	H	F		V	R	H	N			I	K	C	R		P		V			N	I	
colony 9	H				R			R	Y		K	C	R		P	R		F			I	
colony 10		F		V	R	H	N			I	K	C	R		P	R	V		N			N
colony 11	H	F		V	R	H	N	R	Y	I	K		R		P	R	V	F		N	I	
colony 12	H				R			R	Y		K	C	R		P	R		F			I	
colony 13	H			V			N		Y	I	K		R		P	R	V	F		N	I	
colony 14				V	R		N		Y	I	K	C	R		P		V	F	N		I	
colony 15	H	F		V	R		N		Y	I	K	C	R		P	R	V	F	N	N		
colony 16	H	F			R	H		R	Y		K	C	R		P		V		N			N
colony 17	H	F		V	R	H	N	R	Y	I		C	R		P	R	V	F			I	
colony 18		F		V	R		N			I	K	C	R		P	R	V	F		N	I	
colony 19	H	F			R		N	R		I			R		P	R	V				I	
colony 20	H				R		N		Y	I		C	R		P	R	V	F	N			
colony 21	H	F		V	R	H	N		Y		K	C	R		P		V	F		N		
colony 22	H	F		V	R	H	N			I	K		R		P	R		F	N	N	I	
colony 23	H	F			R	H	N	R	Y	I		C	R		P	R	V		N	N	I	N
colony 24	H	F		V		H	N		Y		K		R		P	R	V					N
colony 25	H				R		N		Y	I	K				P	R	V	F				
colony 26					R		N			I	K	C				R		F	N		I	
colony 27		F		V	R	H	N			I	K	C	R		P	R	V		N			N
colony 28	H	F					N			K	C	R		P		V	F			I	N	
colony 29	H	F					N	R		I	K	C	R		P	R	V	F		N	I	N
colony 30	H			V				R	Y		K		R			R		F	N	N	I	N
colony 31	H	F			R		N		Y		K	C	R		P	R	V	F	N			
colony 32	H				R	H	N		Y		K		R		P	R	V	F		N	I	
Ratio	6/26	11/21	32/0	13/19	6/26	17/0/15	5/27	20/12	10/22	12/20	4/28	12/20	5/27	32/0	2/30	5/27	6/26	10/22	18/14	20/12	12/20	23/9

Position	48	51	76	106	108	110	111	119	122	123	126	127	146	147	152	154	155	156	157	161	166	167
WTTadA	P	R	I	A	D	K	T	D	H	H	M	N	S	D	R	Q	E	I	K	K	T	D
TadA-RA4.0	A	H	F	V	G	R	H	N		Y	I	K		R	P	R	V	F				
TadA-RA4.1	A	H	F	V	G	R	H	N	R	Y	I	K		R	P	R	V	F				
TadA-RA4.2	A	H	F	V	G	R	H	N		Y	I	K	C	R	P	R	V	F				
TadA-RA4.3	A	H	F	V	G	R	H	N		Y	I	K		R	P	R	V	F	N			
TadA-RA4.4	A	H	F	V	G	R	H	N		Y	I	K		R	P	R	V	F		N		
TadA-RA4.5	A	H	F	V	G	R	H	N		Y	I	K		R	P	R	V	F			I	
TadA-RA4.6	A	H	F	V	G	R	H	N		Y	I	K		R	P	R	V	F				N

b



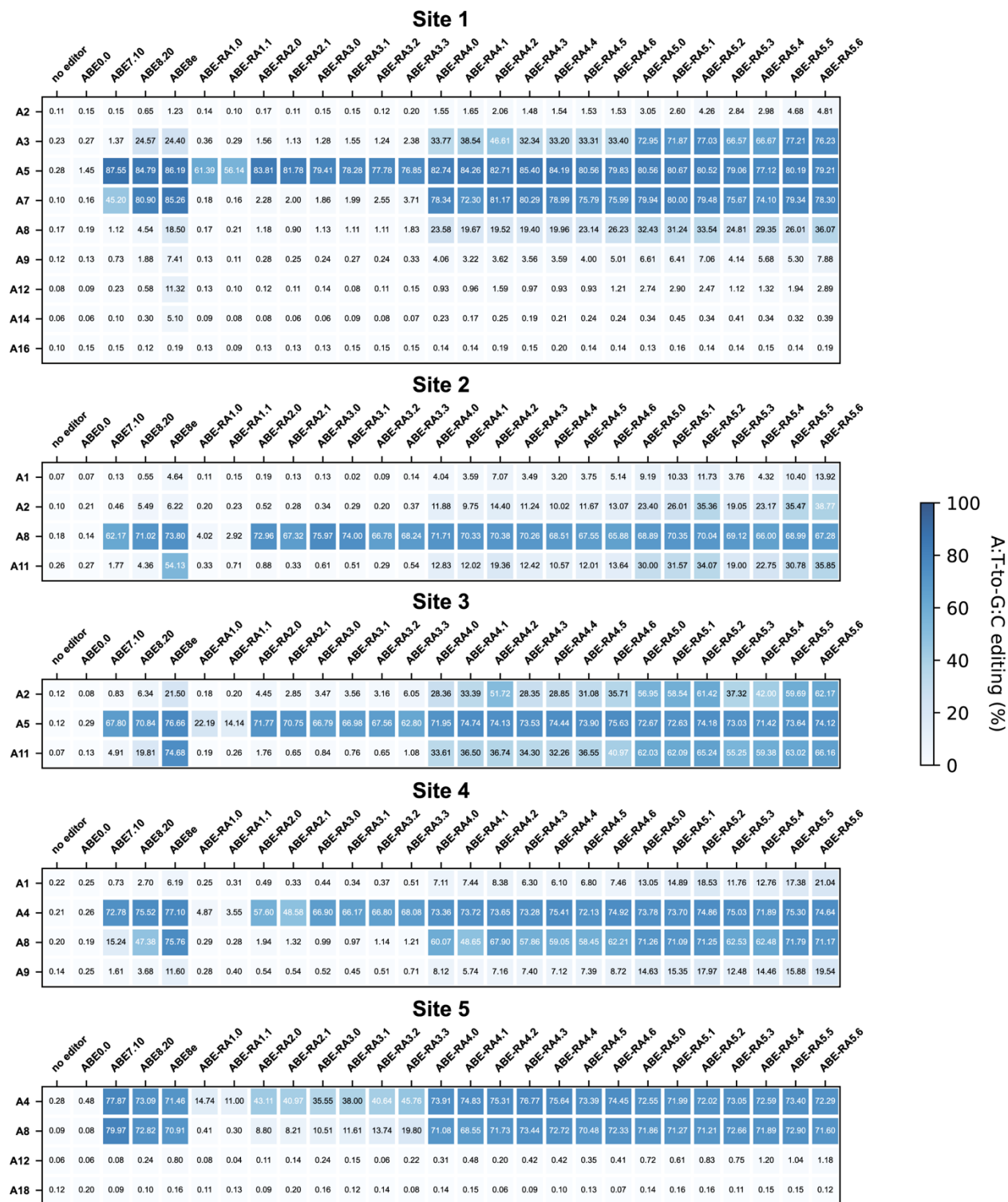
Lane	Editor plasmid
1	dCas9
2	TadA7.10-dCas9
3	TadA-RA2.0-dCas9
4	TadA-RA2.1-dCas9
5	TadA_P48A_R51H_D108G_K110R_H122R_N127K-dCas9
6	TadA-RA4.0-dCas9
7	TadA-RA4.1-dCas9
8	TadA-RA4.2-dCas9
9	TadA-RA4.3-dCas9
10	TadA-RA4.4-dCas9
11	TadA-RA4.6-dCas9

Supplementary Figure 6 | Fourth-round directed evolution for TadA. **a.** Mutations identified in colonies that passed selection and validation. P48A and D108G were fixed in the initial library. Mutations in TadA7.10 are colored in grey; additional mutations reported in TadA8.20, TadA8e, and the current study are colored in orange, purple, and green, respectively. **b.** Serial dilutions of *E. coli* transformed with the selection plasmid encoding *Kan^R-W15*-W24** and denoted editor plasmids. Editor expression was induced with 1 mM arabinose overnight prior to serial dilution and plating on agar plates supplemented with 0, 400, or 800 µg/mL kanamycin.

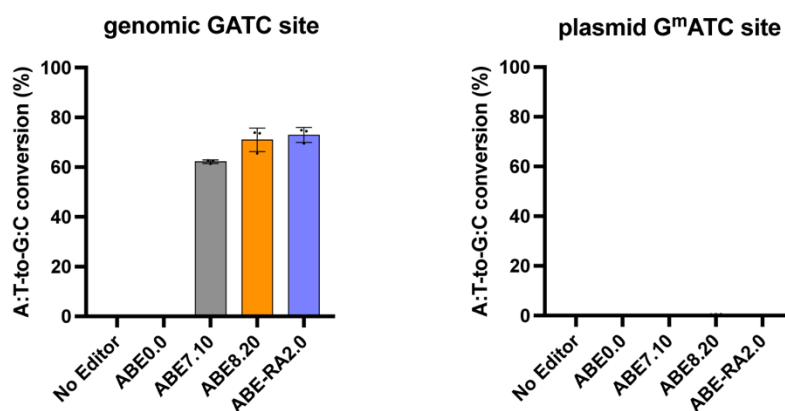
Position	27	23	36	47	48	51	76	82	84	94	108	109	111	114	122	146
WT TadA	E	W	H	R	P	R	I	V	L	M	D	A	T	A	H	S
TadA7.10	E	R	L	R	A	L	I	V	F	M	N	A	T	A	H	C
TadA8.20	E	R	L	R	A	L	Y	S	F	M	N	A	T	A	H	C
TadA8e	E	R	L	R	A	L	I	V	F	M	N	S	R	A	N	C
TadA-RA4.0	E	W	H	R	A	H	F	V	L	M	G	A	H	A	H	S
5th round lib	E/D	W/R	H/L	R/K	P/A	H/L	F/Y	V/S	L/F	M/V	N/G	A/S	R/H	A/V	H/N	S/C
colony 1		R	L	K	A	L					G		H		N	C
colony 2		R	L	K	A	L	Y	S			G		H	V		C
colony 3		R		K	A	L	Y	S			G	S	H	V		
colony 4		R	L	K	A	L	Y	S			G		H	V	N	
colony 5					A	L					G		H	V		
colony 6		R		K	A	L	Y				G		H			C
colony 7		R	L	K	A		Y	S			G	S	H			C
colony 8		R			A	L		S	F		G	S	H	V	N	
colony 9		R	L		A	L		S			G		H	V	N	C
colony 10		R		K	A	L	Y				G	S	H			C
colony 11		R		K	A	L	Y	S	F		G		H	V		
colony 12		R		K	A	L	Y	S	F		G		H	V		
colony 13			L	K	A	L	Y	S			G	S	H			C
colony 14		R	L	K	A	L		S	F		G		H	V	N	C
colony 15		R	L	K	A	L		S			G		H		N	C
colony 16		R	L		A	L		S			G		H		N	C
colony 17			L	K	A		Y	S			G	S	H	V	N	
colony 18		R	L	K	A	L		S			G	S	H	V		
colony 19		R		K	A		Y	S			G		H	V		C
colony 20		R	L	K	A		Y	S			G		H	V		
colony 21		R	L		A	L		S			G		H	V		C
colony 22		R	L	K	A		Y				G	S	H			C
colony 23		R	L		A	L	Y	S			G	S	H	V		
colony 24		R	L	K	A	L					G		H			C
colony 25		R		K	A						G	S	H		N	
colony 26		R			A		Y	S			G	S	H	V	N	
Ratio	26/0	3/23	10/16	7/19	0/26	7/19	11/15	7/19	22/4	26/0	0/26	15/11	0/26	10/16	16/10	12/14

Position	23	36	47	48	51	76	82	106	108	109	110	111	114	119	122	123	126	127	146	147	152	154	155	156	157	161	166	167
WT TadA	W	H	R	P	R	I	V	A	D	A	K	T	A	D	H	H	M	N	S	D	R	Q	E	I	K	K	T	D
TadA-RA5.0	R	L	K	A	L	F	S	V	G		R	H		N		Y	I	K	C	R	P	R	V	F				
TadA-RA5.1	R	L	K	A	L	F	S	V	G		R	H		N	N	Y	I	K	C	R	P	R	V	F				
TadA-RA5.2	R	L	K	A	L	F	S	V	G		R	H	V	N		Y	I	K	C	R	P	R	V	F				
TadA-RA5.3	R	L	K	A	L	F	S	V	G	S	R	H	V	N		Y	I	K		R	P	R	V	F				
TadA-RA5.4	R		K	A	L	Y	S	V	G	S	R	H	V	N		Y	I	K		R	P	R	V	F				
TadA-RA5.5	R	L	K	A	L	Y	S	V	G		R	H	V	N		Y	I	K	C	R	P	R	V	F	N	N	I	N
TadA-RA5.6	R	L	K	A	L	Y	S	V	G		R	H	V	N		Y	I	K	C	R	P	R	V	F			I	N

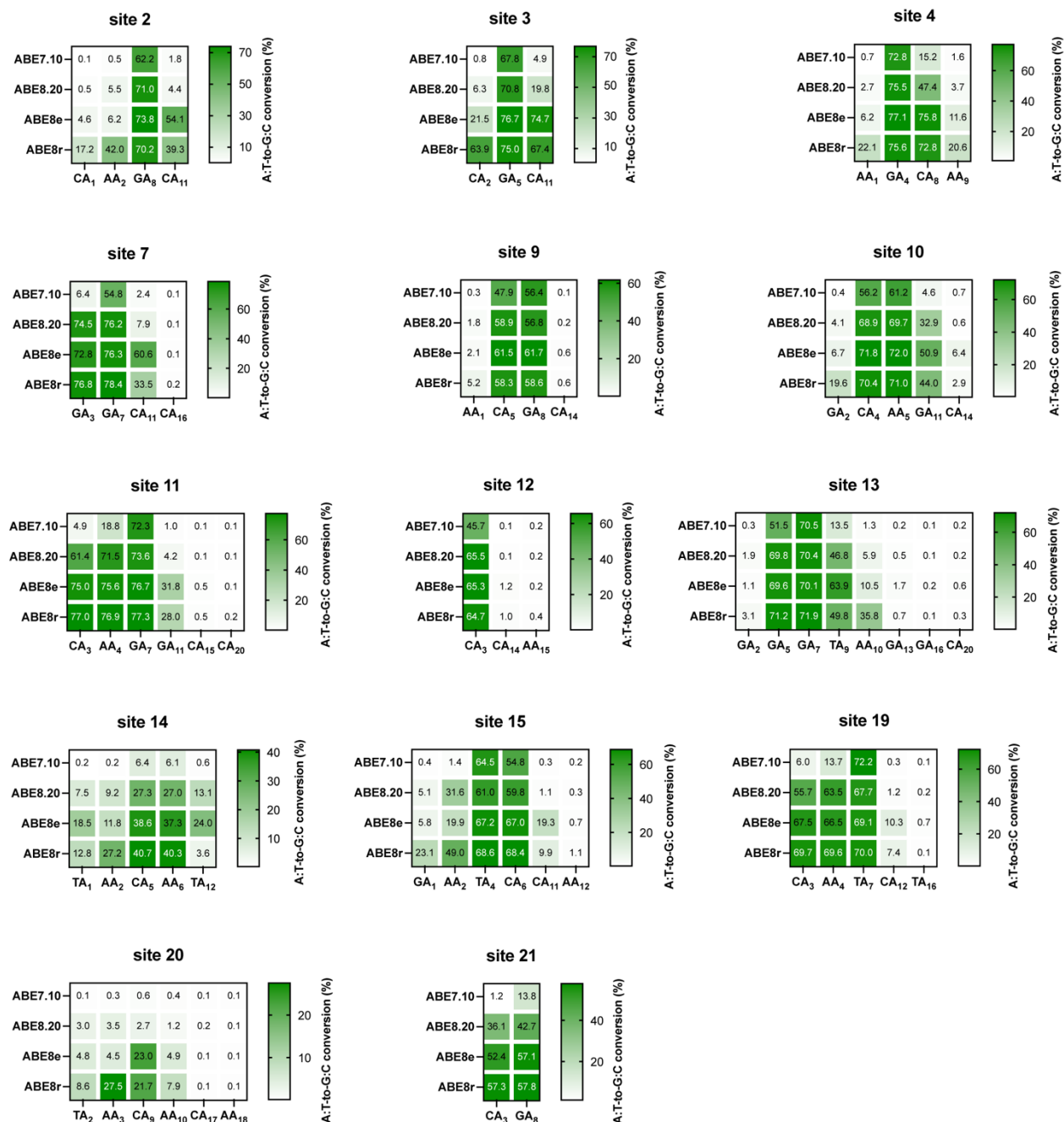
Supplementary Figure 7 | Mutations in colonies harvested in fifth-round directed evolution. A106V, K110R, D119N, H123Y, M126I, N127K, D147R, R152P, Q154R, E155V, and I156F were fixed in the initial library. Mutations in TadA7.10 are colored in grey; additional mutations reported in TadA8.20, TadA8e, and the current study are colored in orange, purple, and green, respectively.



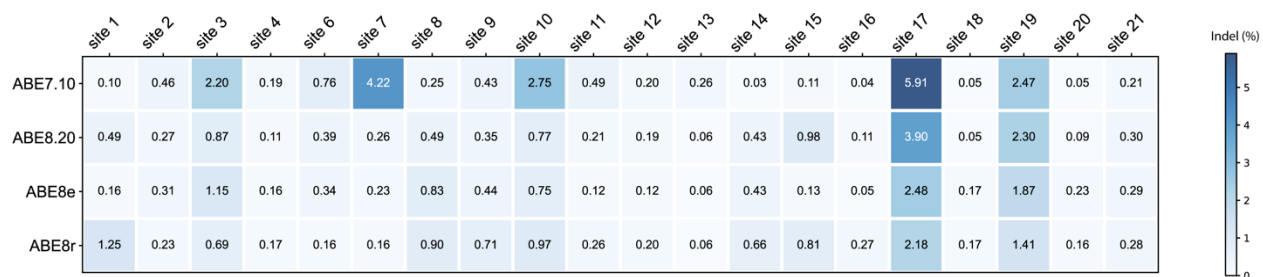
Supplementary Figure 8 | A:T-to-G:C editing in HEK293T cells enabled by ABE-RA editors isolated from five rounds of directed evolution. Five target sites were assayed, with ABE7.10, ABE8.20, and ABE8e included as controls. ABE0.0 was constructed by fusing wildtype TadA to Cas9 nickase. Mean editing rates (n = 3 independent experiments) are presented.



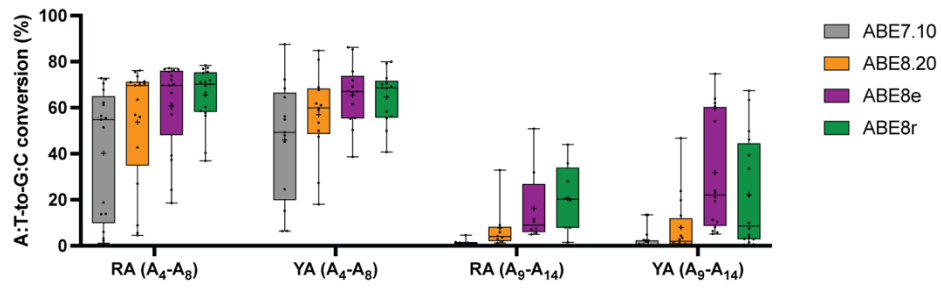
Supplementary Figure 9 | Editing of deoxyadenosine and *N*⁶-methyldeoxyadenosine by ABE0.0, ABE7.10, ABE8.20, and ABE-RA2.0. The target GATC sites are either an unmethylated genomic site or a methylated site in plasmid DNA. Editing assays were carried out in HEK293T cells with data presented as mean $\pm$ s.d. (n = 3 independent experiments).



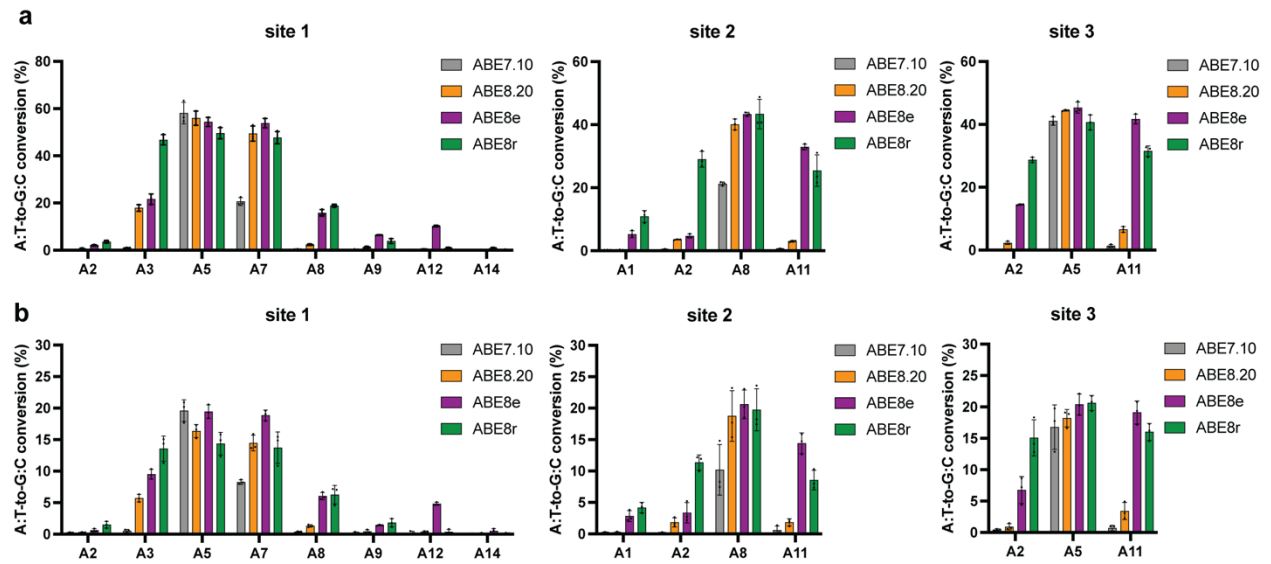
Supplementary Figure 10 | A:T-to-G:C editing mediated by ABE7.10, ABE8.20, ABE8e, and ABE8r at 14 genomic loci in HEK293T cells. Heatmaps are generated using editing rates averaged from 3 independent experiments. Six additional target sites are presented in **Fig. 3a**.



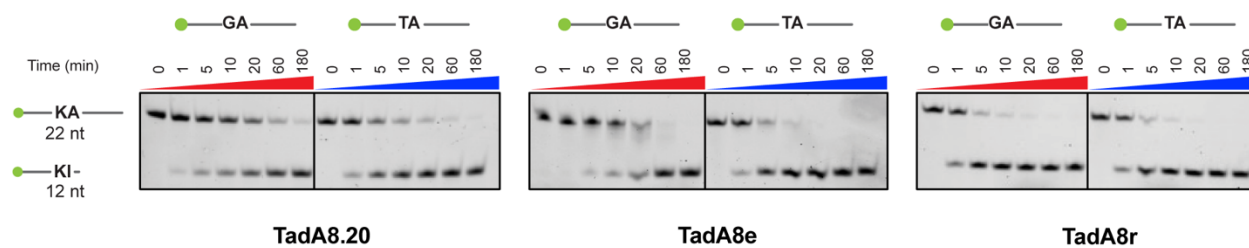
Supplementary Figure 11 | Indel frequencies observed with ABE7.10, ABE8.20, ABE8e, and ABE8r at 20 genomic loci in HEK293T cells. Mean indel frequencies are derived from the same dataset used to plot **Fig. 3a** and **Supplementary Fig. 10** (n = 3 independent experiments).



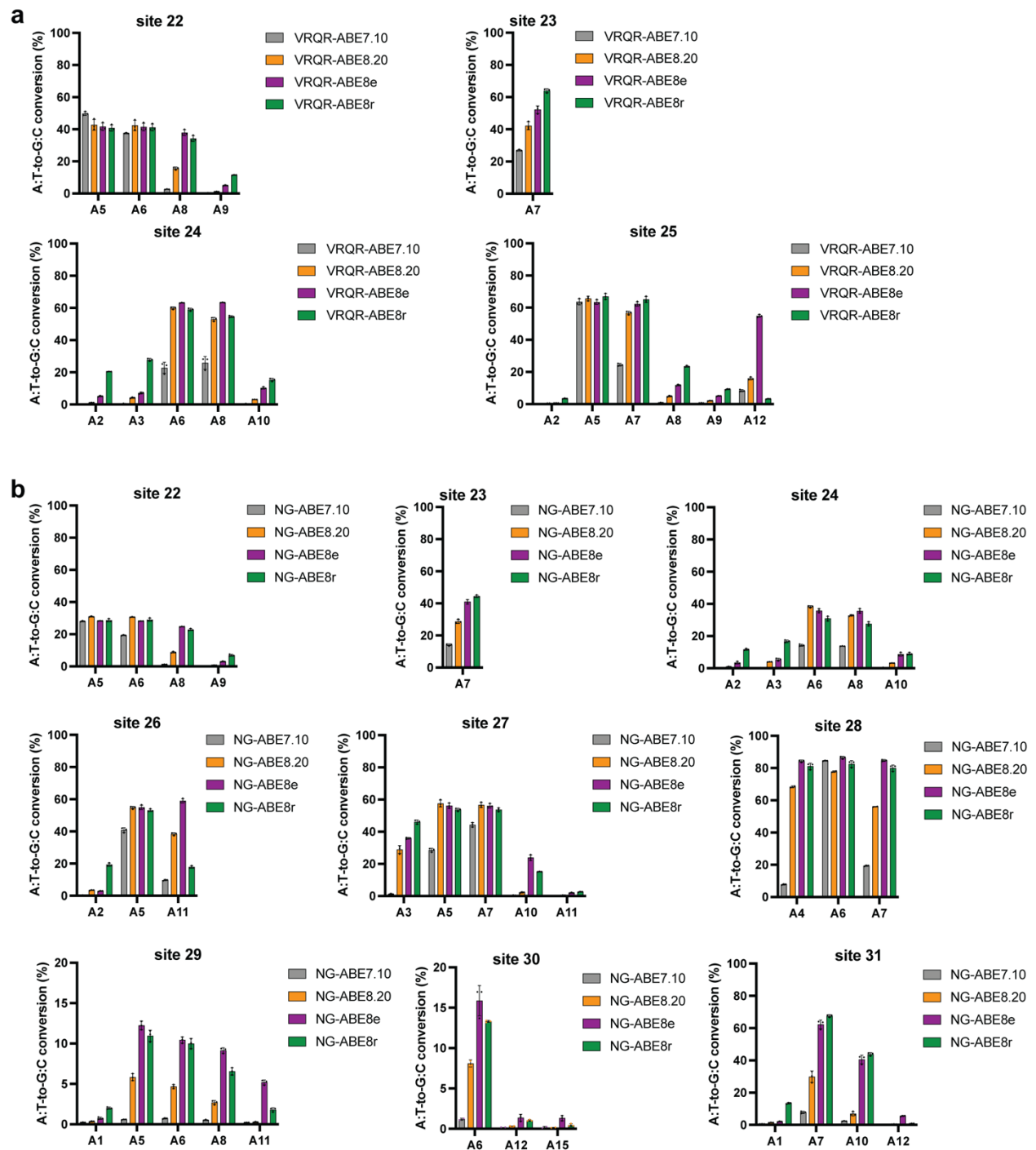
Supplementary Figure 12 | Box plot of editing rates observed at A₄-A₈ and A₉-A₁₄ across 20 genomic loci in HEK293T cells. Plots were grouped by sequence contexts and protospacer positions. RA (A₄-A₈): n = 17; YA (A₄-A₈): n = 13; RA (A₉-A₁₄): n = 8; YA (A₉-A₁₄): n = 16; n refers to the number of As included in each box. Lower and upper hinges denote first and third quartiles. The central line and plus sign represent the median and mean, respectively. Similar analysis for A₁-A₃ is provided in **Fig. 3b**. Plots are derived from the same dataset used in **Fig. 3a** and **Supplementary Fig. 10** (n = 3 independent experiments).



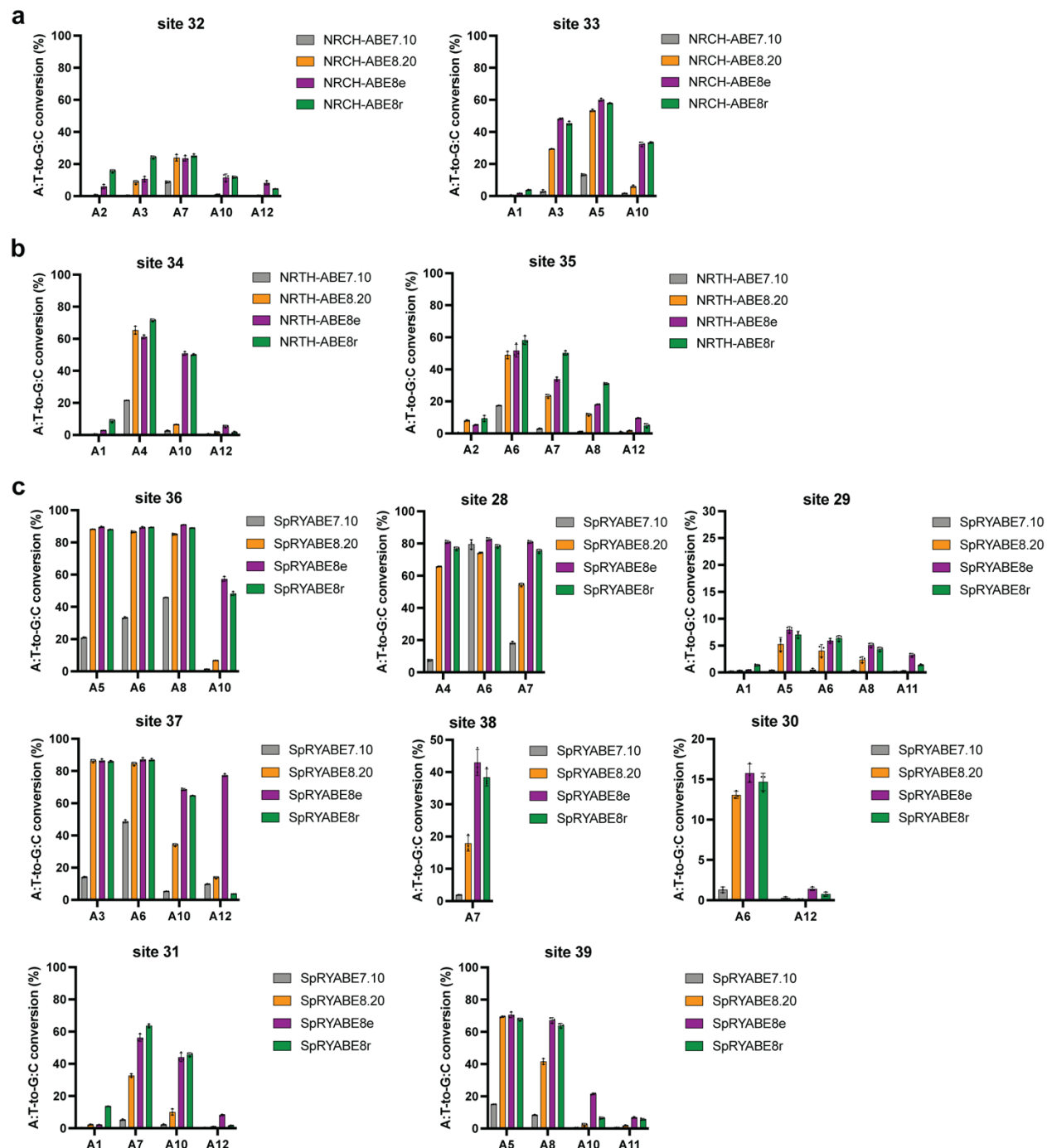
Supplementary Figure 13 | A:T-to-G:C editing delivered by ABE7.10, ABE8.20, ABE8e, and ABE8r in HeLa (a) and A549 (b) cells. Data are presented as mean $\pm$ s.d. (n = 3 independent experiments).



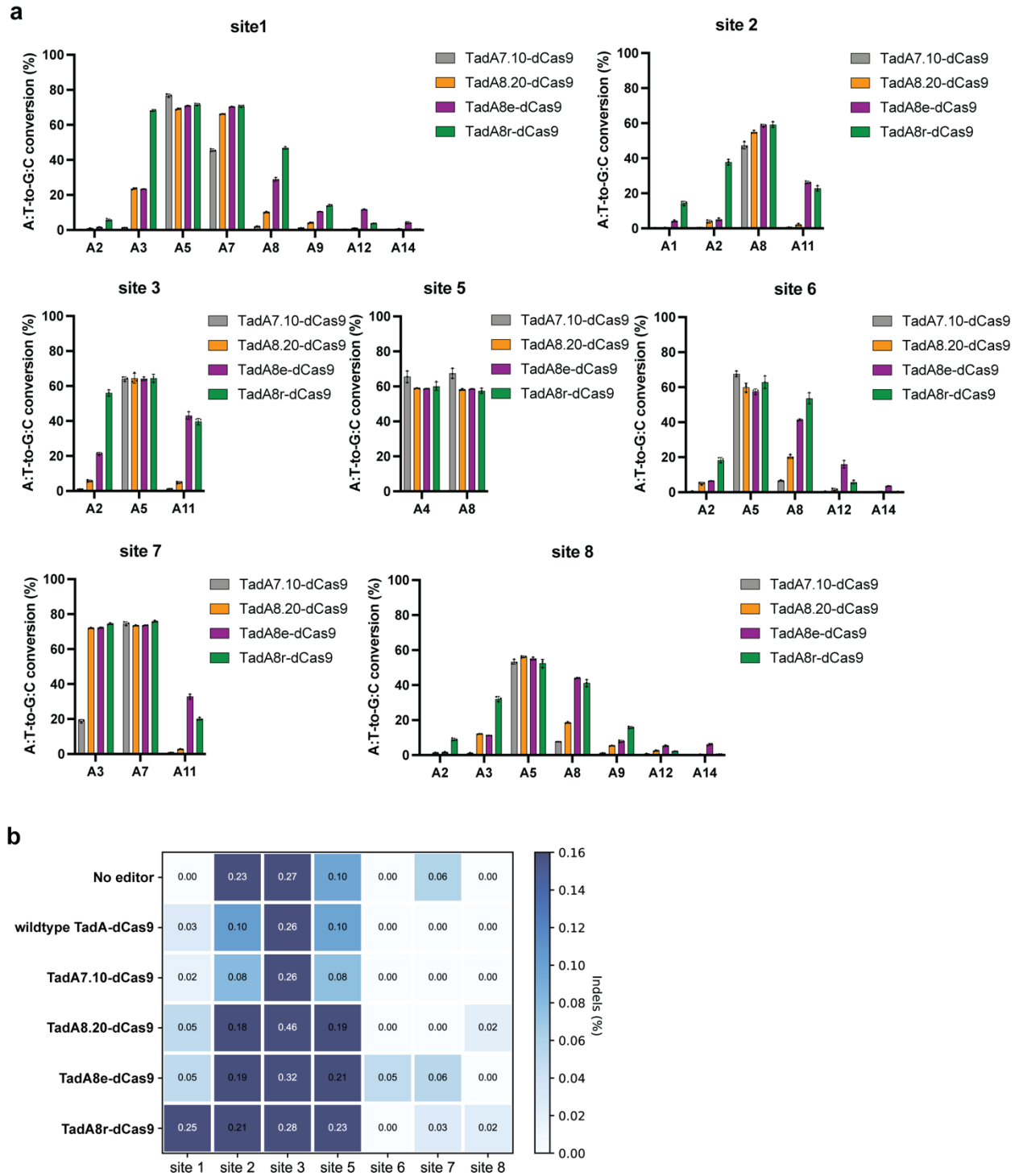
Supplementary Figure 14 | Replicate of the *in vitro* deamination assay. PAGE gel images of ssDNA treated sequentially by the deaminase and Endonuclease V are presented. Two independent experiments were carried out; PAGE images of replicate 1 are shown in **Fig. 3c**.



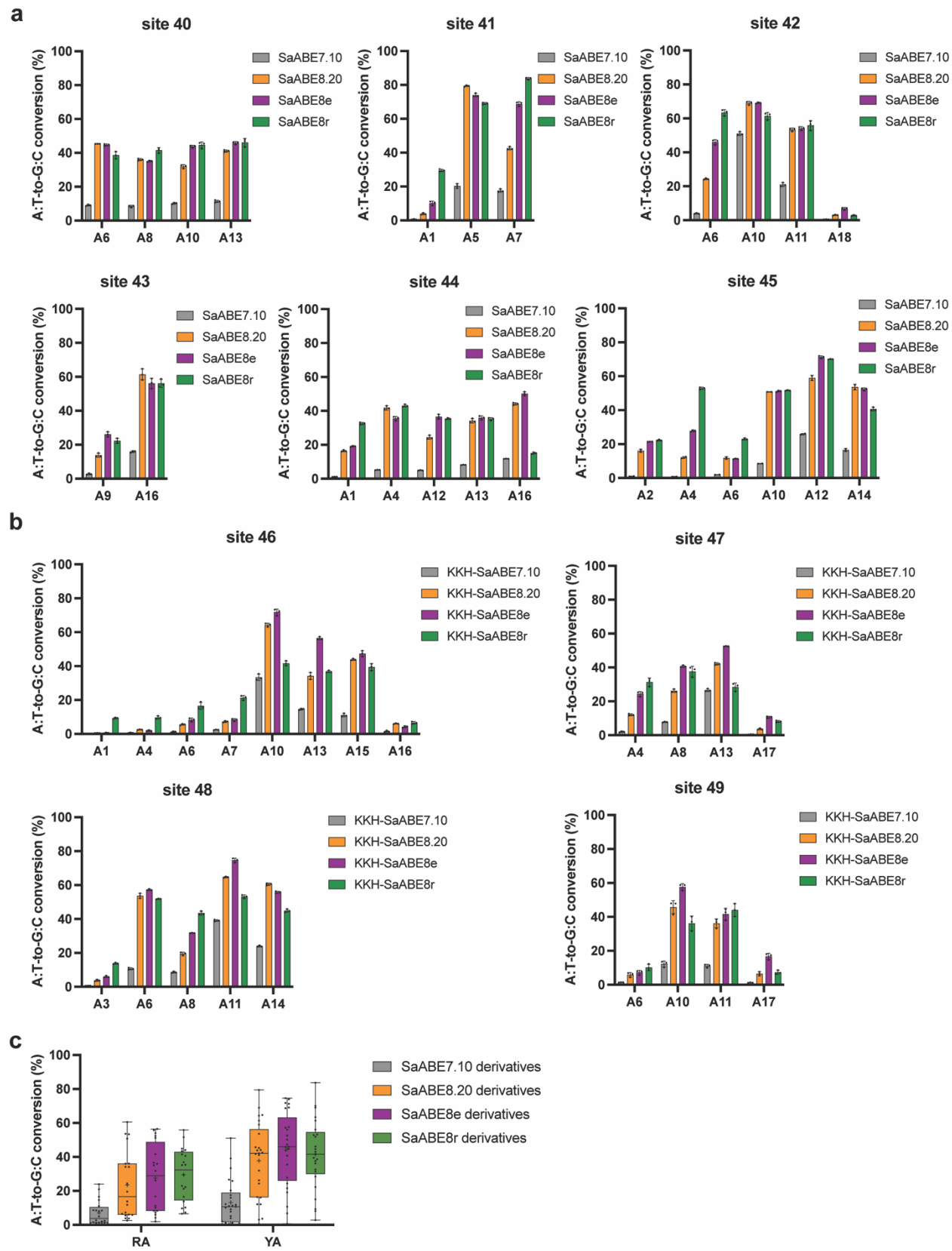
Supplementary Figure 15 | A:T-to-G:C editing at sites of non-NGG PAMs mediated by VRQR-ABEs (a) and NG-ABEs (b). All experiments were carried out in HEK293T cells with editing rates presented as mean $\pm$ s.d. (n = 3 independent experiments).



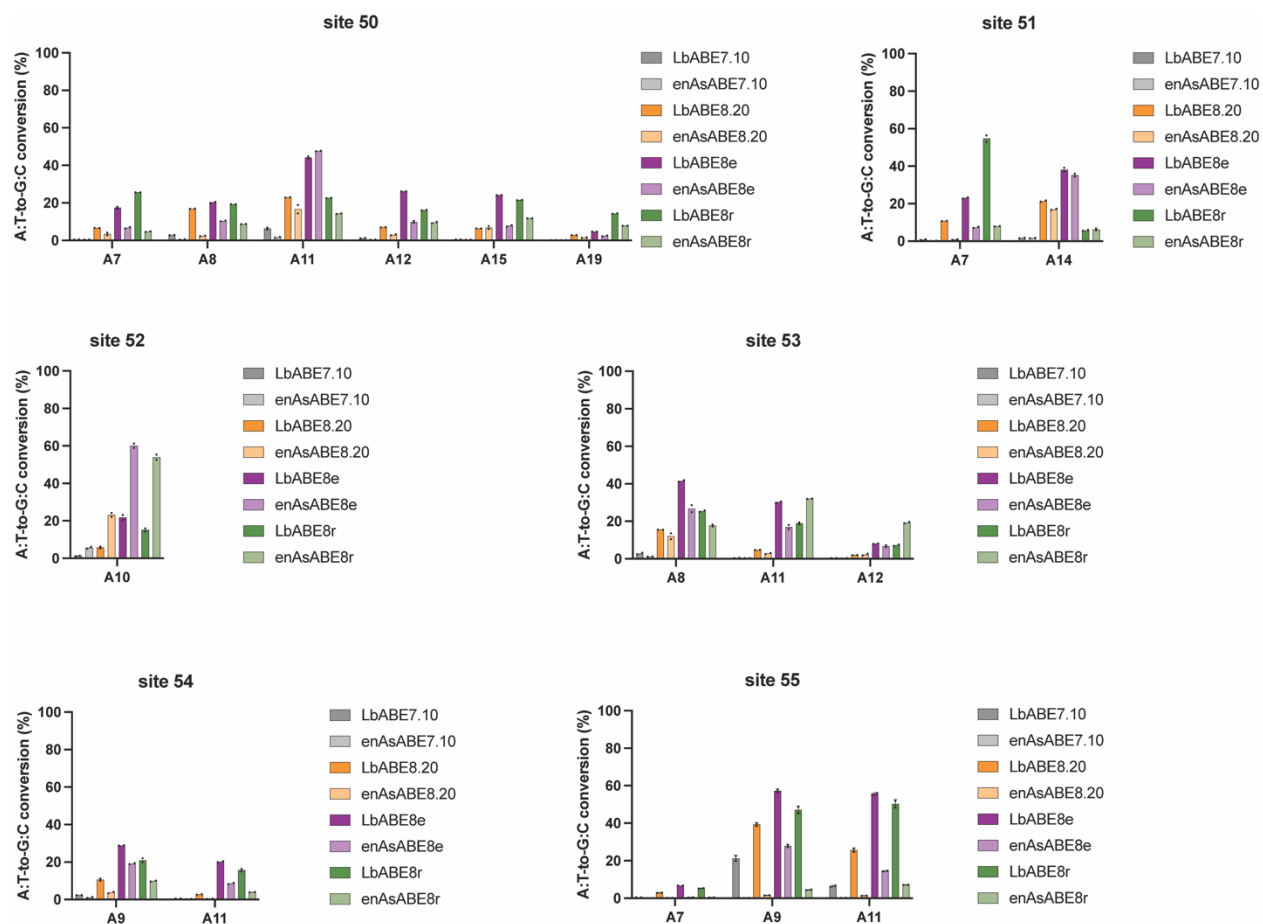
Supplementary Figure 16 | A:T-to-G:C editing at sites of non-NGG PAMs mediated by NRCH-ABEs (a), NRTH-ABEs (b), and SpRY-ABEs (c). All experiments were carried out in HEK293T cells with editing rates presented as mean $\pm$ s.d. (n = 3 independent experiments).



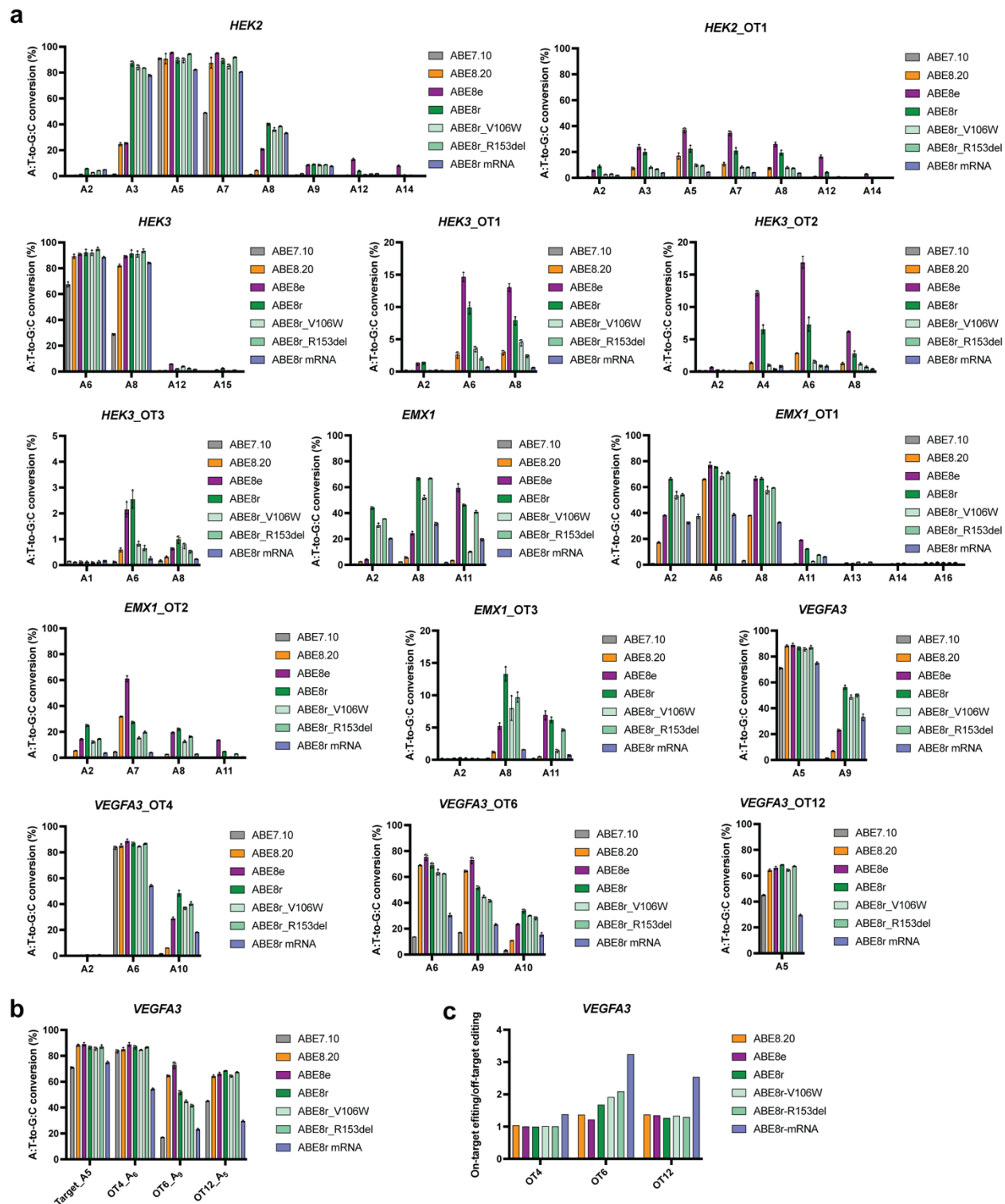
Supplementary Figure 17 | A:T-to-G:C editing (**a**) and indel frequencies (**b**) delivered by TadA7.10-dCas9, TadA8.20-dCas9, TadA8e-dCas9, and TadA8r-dCas9 at seven genomic loci. All experiments were carried out in HEK293T cells. Data are presented as mean $\pm$ s.d. in **a** and mean in **b** ($n = 3$ independent experiments).



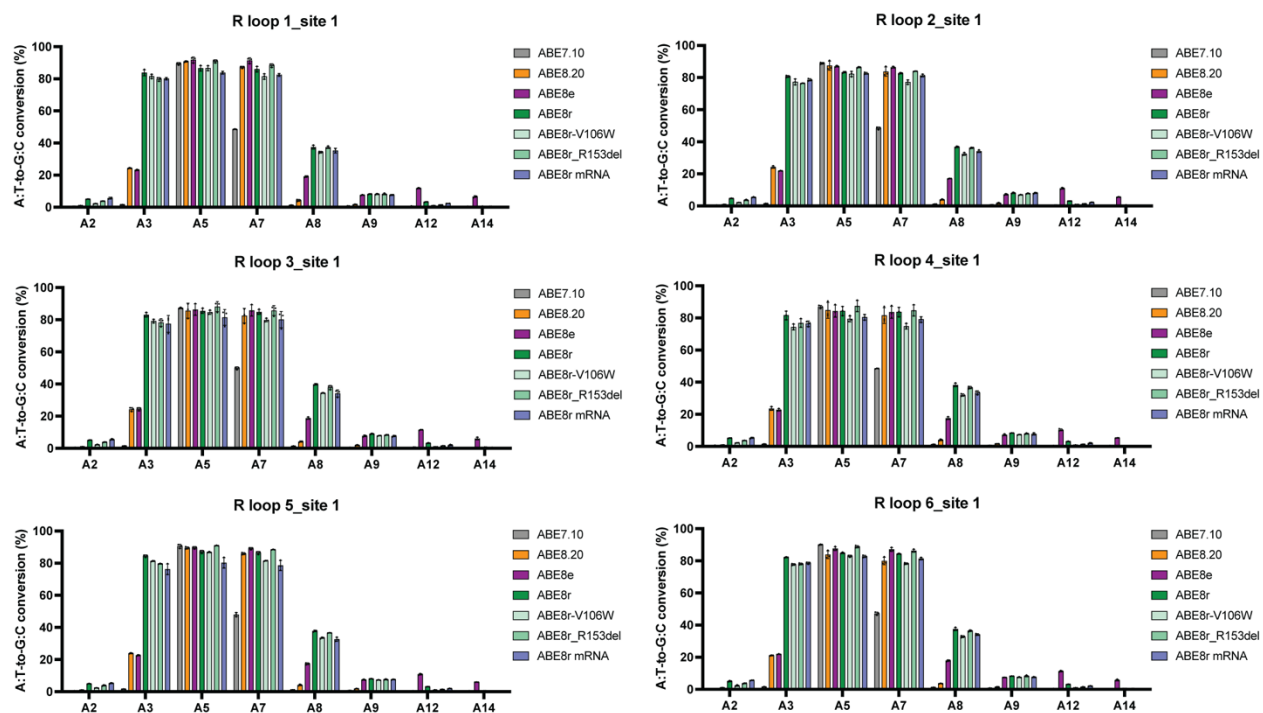
Supplementary Figure 18 | A:T-to-G:C editing mediated by SaCas9-derived (a) and KKH-SaCas9-derived (b) ABEs. (c) Box plot of editing rates by SaABEs and KKH-SaABEs at 10 genomic loci. Plots were grouped by sequence contexts. $R\text{A}$: $n = 20$; $Y\text{A}$: $n = 25$; n refers to the number of As included in each box. Lower and upper hinges denote first and third quartiles. The central line and plus sign represent the median and mean, respectively. All experiments were carried out in HEK293T cells with editing rates presented as mean $\pm$ s.d. ($n = 3$ independent experiments).



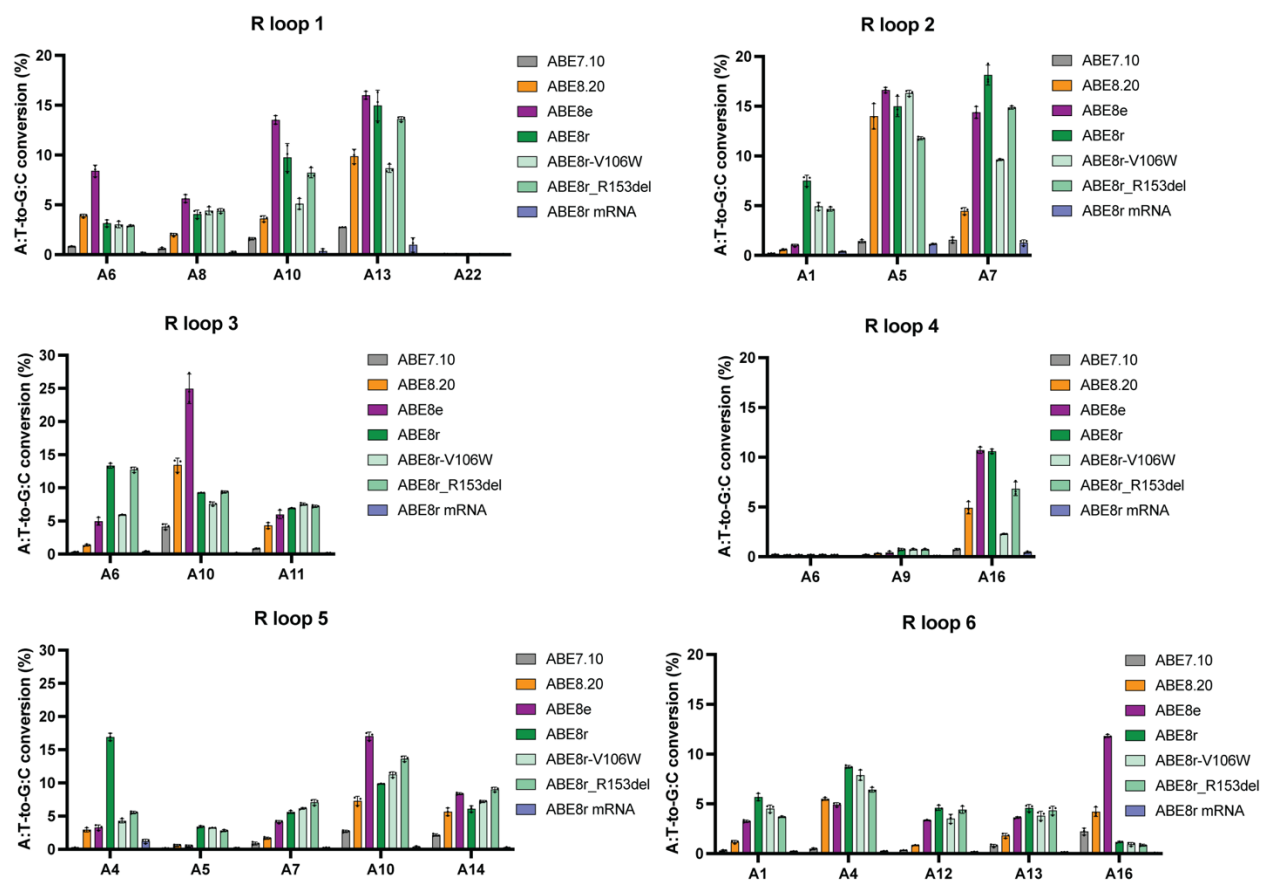
Supplementary Figure 19 | A:T-to-G:C editing mediated by dLbCas12a-derived and denAsCas12a-derived ABEs. All experiments were carried out in HEK293T cells with editing rates presented as mean $\pm$ s.d. (n = 2 independent experiments).



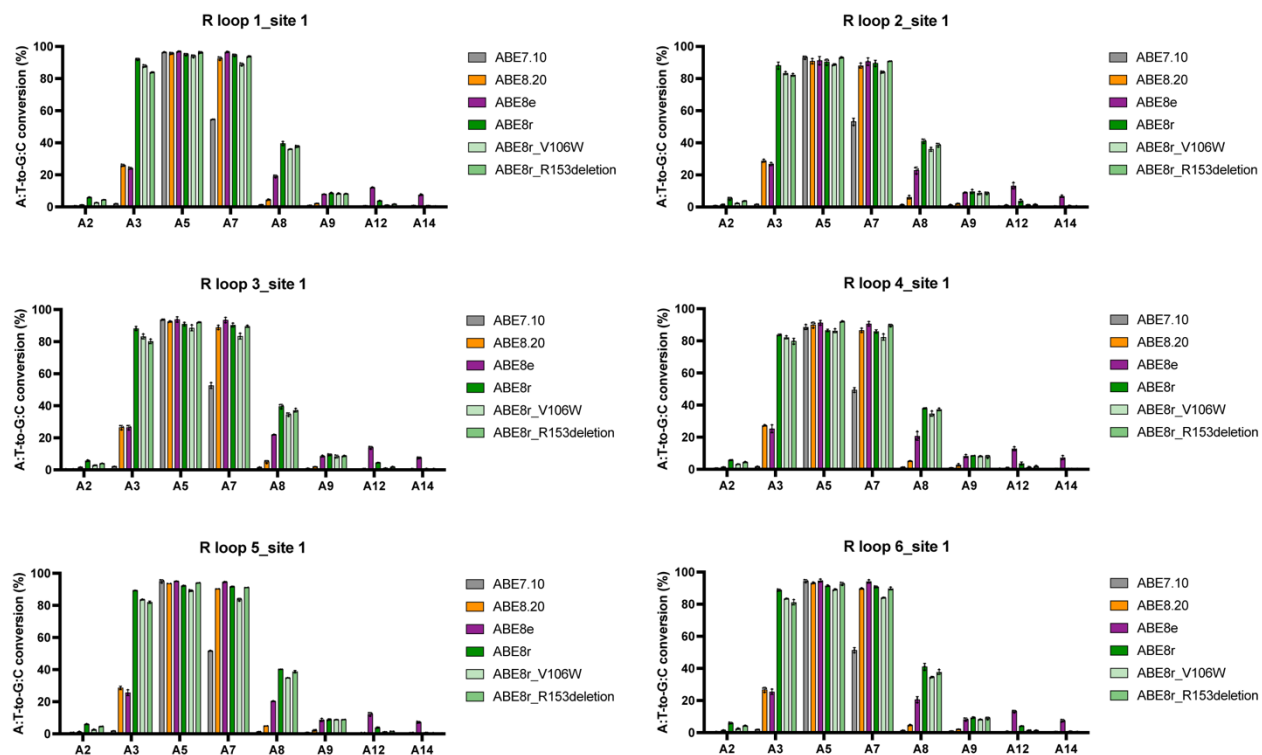
plotted for As most strongly edited by ABE7.10 at individual on- or off-target sites. **c.** On- and off-target editing ratios for three OTs of *VEGFA3*. All experiments were carried out in HEK293T cells with editing rates presented as mean $\pm$ s.d. (n = 3 independent experiments).



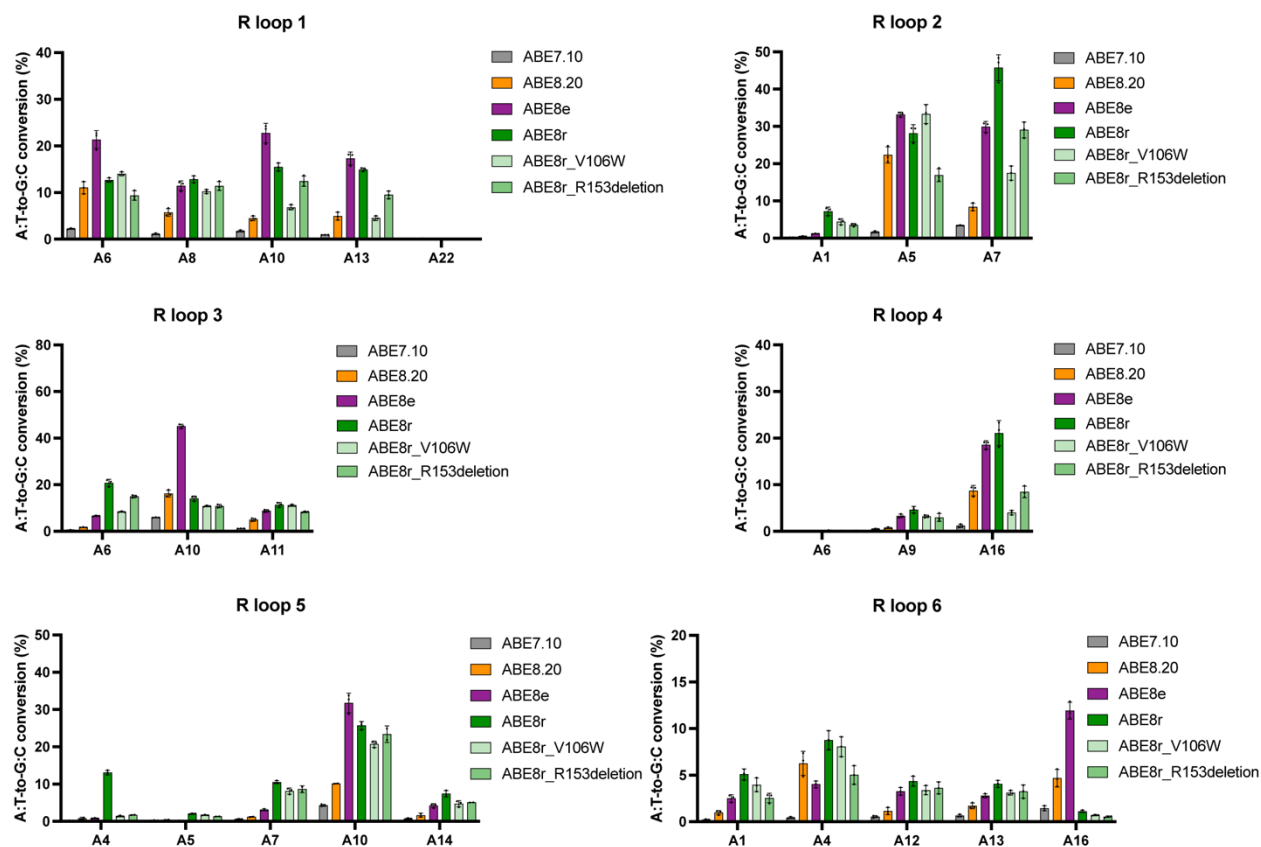
Supplementary Figure 21 | On-target editing rates delivered by ABEs in the dSaCas9-mediated orthogonal R-loop assay. Experiments were carried out in HEK293T cells with editing rates plotted as mean $\pm$ s.d. (n = 3 independent experiments).



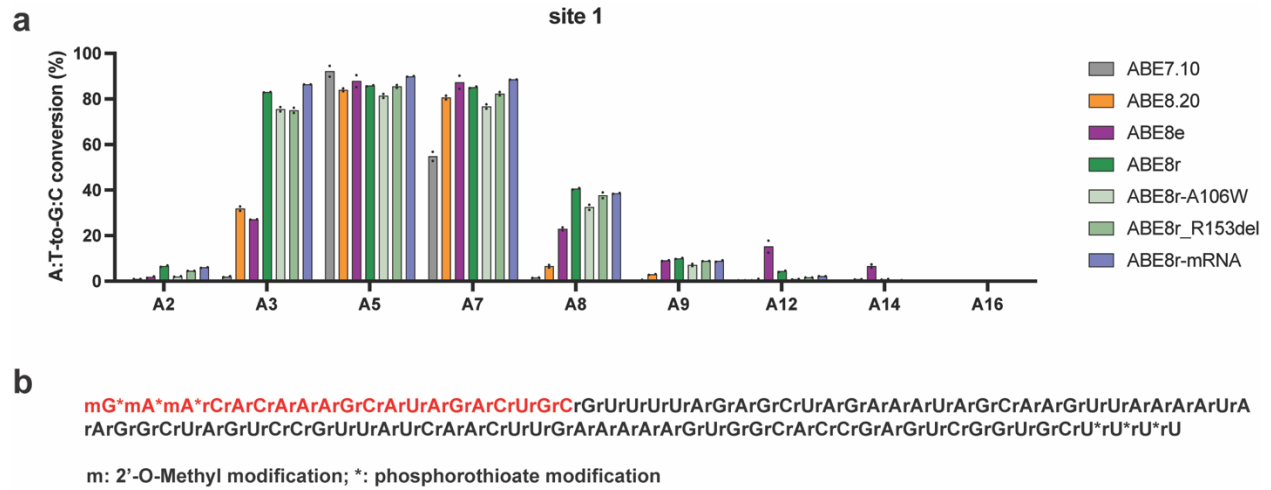
Supplementary Figure 22 | gRNA-independent A:T-to-G:C editing at R loops formed by dSaCas9. Experiments were carried out in HEK293T cells with editing rates plotted as mean $\pm$ s.d. ($n = 3$ independent experiments). **Supplementary Fig. 21** and **22** are derived from the same set of experiments.



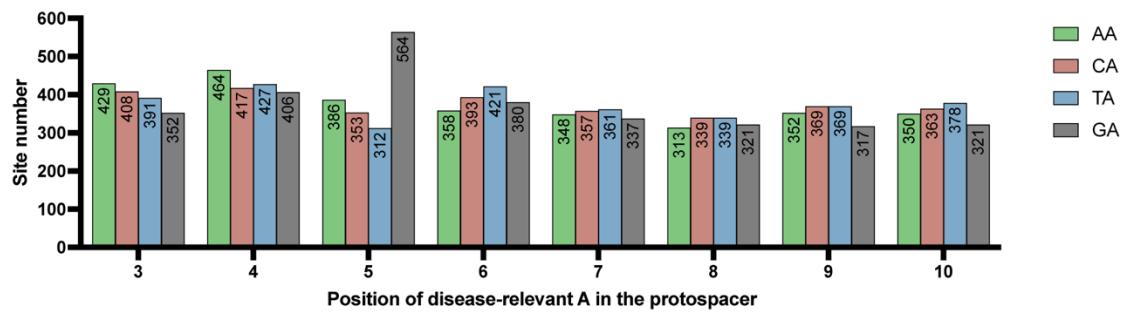
Supplementary Figure 23 | On-target editing rates delivered by ABEs in the nSaCas9-mediated orthogonal R-loop assay. Experiments were carried out in HEK293T cells with editing rates plotted as mean $\pm$ s.d. (n = 3 independent experiments).



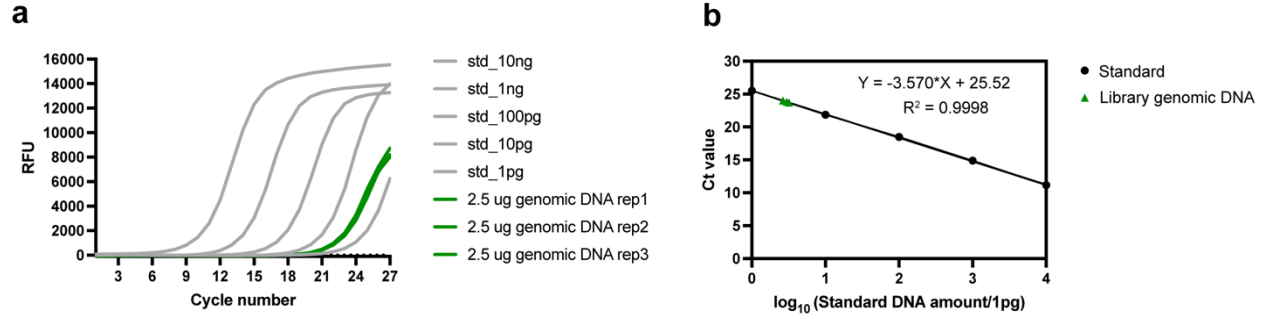
Supplementary Figure 24 | gRNA-independent A:T-to-G:C editing detected at R loops formed by nSaCas9. Experiments were carried out in HEK293T cells with editing rates plotted as mean $\pm$ s.d. ($n = 3$ independent experiments). **Supplementary Fig. 23** and **24** are derived from the same set of experiments.



Supplementary Figure 25 | On-target DNA editing by ABEs in HEK293T cells (**a**) and the sequence of modified sgRNA (**b**). The same samples were used to evaluate transcriptome-wide off-target editing. Data are presented as mean $\pm$ s.d. (n = 2 independent experiments).



Supplementary Figure 26 | Distributions of disease-relevant As across different protospacer positions and sequence contexts in the paired sgRNA-target library.



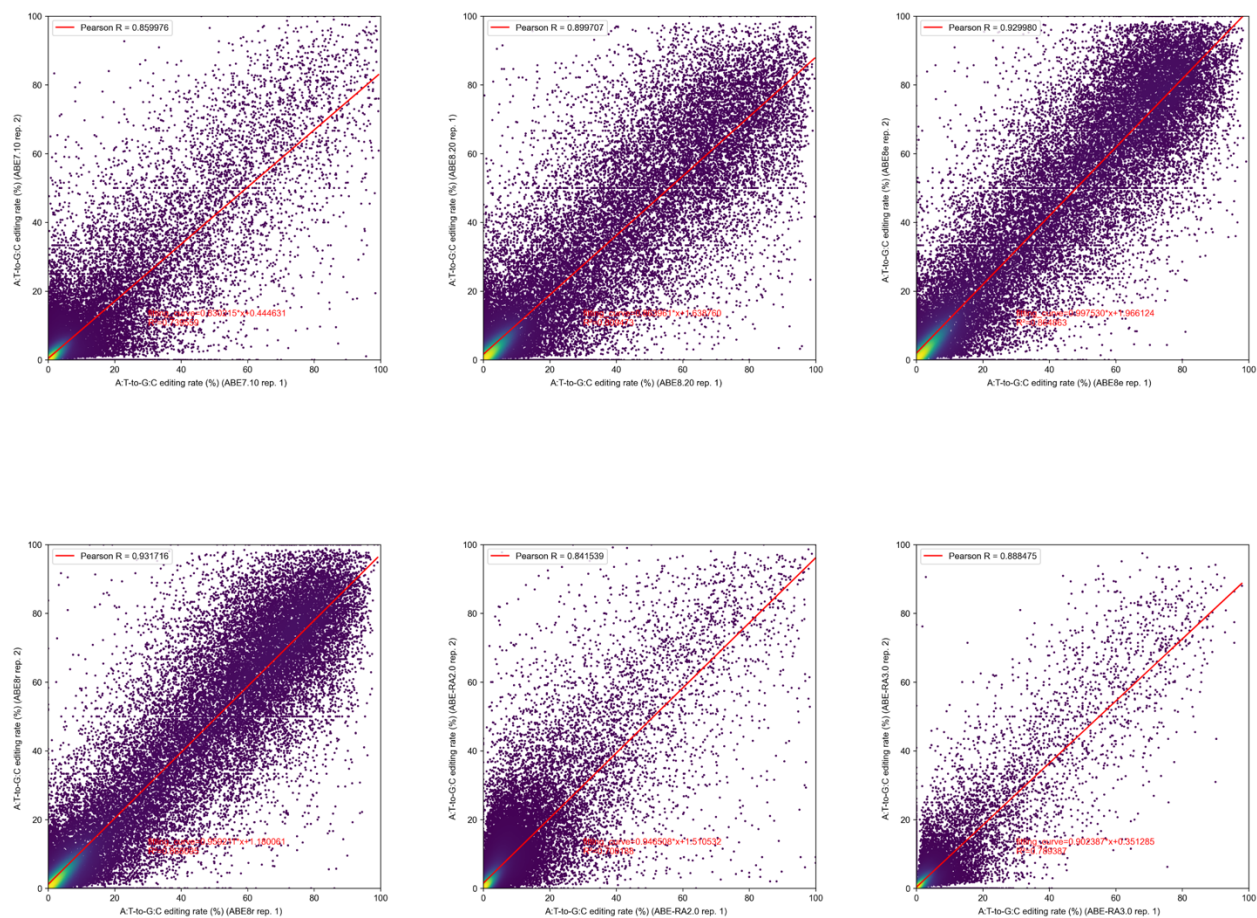
Supplementary Figure 27 | Copy number of the paired sgRNA-target library in HEK293T cells.

a. Amplification curves for the DNA standard and library genomic DNA. We used the lenti-vector library plasmid as the standard, with a pair of primers (YX 1452 and YX 1381, **Supplementary Table 10**) amplifying the entire sgRNA-target cassette. $n = 3$ for library genomic DNA. **b.** Standard curve for quantification. qPCR was set up using $0.5 \mu\text{M}$ of each primer and specified amounts of the DNA standard, or $2.5 \mu\text{g}$ of library genomic DNA, in $50 \mu\text{L}$ 1x Forget-Me-Not™ EvaGreen® qPCR Master Mix. The size of the plasmid DNA is $7.4 \times 10^3 \text{ bp}$, while the length of one copy of genomic DNA is $3.2 \times 10^9 \text{ bp}$. The relative amount of sgRNA-target DNA in $2.5 \mu\text{g}$ of genomic DNA is 2.93-fold to that in 1 pg of plasmid DNA, according to the standard curve. The copy number of the sgRNA-target cassette in haploid genomic DNA can be estimated using the following equation.

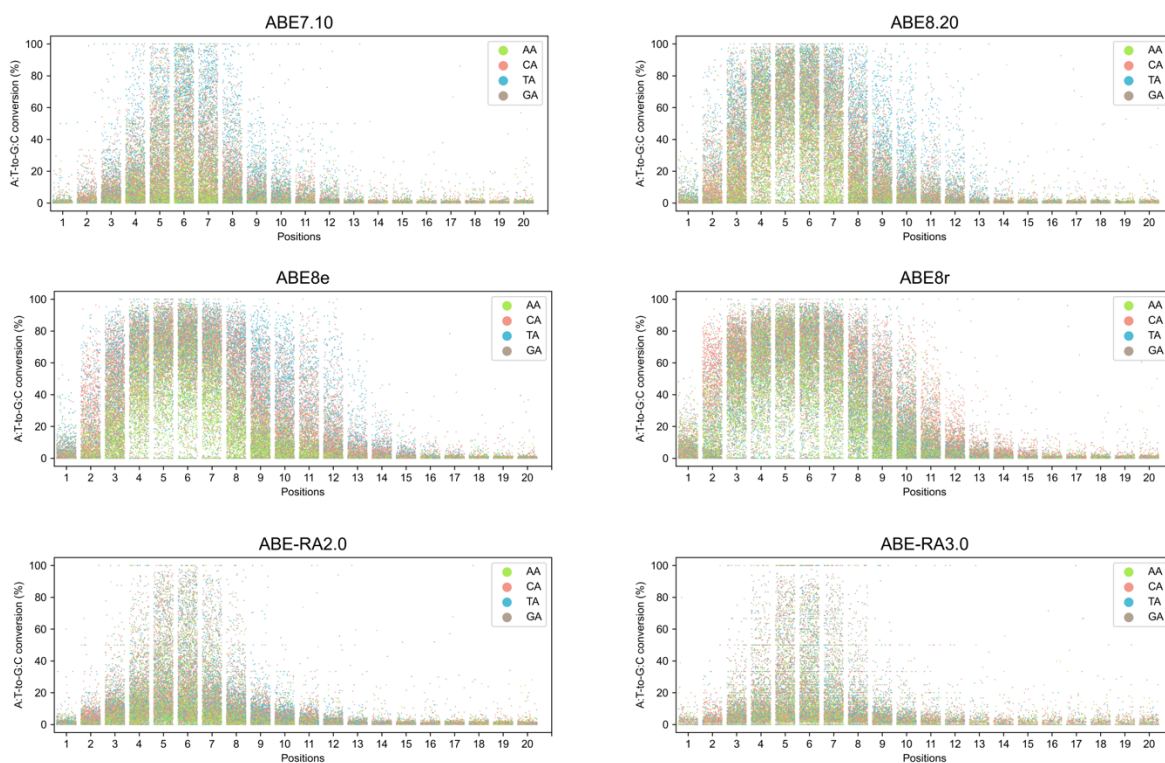
$$\frac{\frac{2.5 \text{ ug}}{3.2 \times 10^9 \text{ bp}} \times \text{Copy number in haploid genomic DNA}}{\frac{1 \text{ pg}}{7.4 \times 10^3 \text{ bp}} \times 1 \text{ Copy in standard plasmid DNA}} = \frac{2.93}{1}$$

$$\text{Copy number in haploid genomic DNA} = 0.5$$

$$\text{Copy number in HEK293T cells} = 1$$

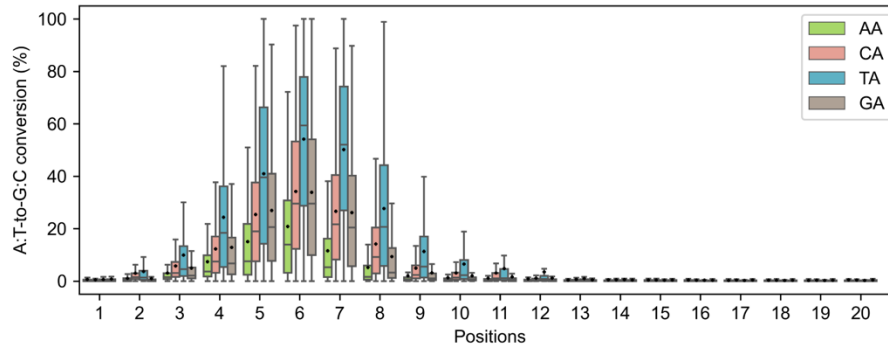


Supplementary Figure 28 | Correlations between biological replicates in the library editing experiments.



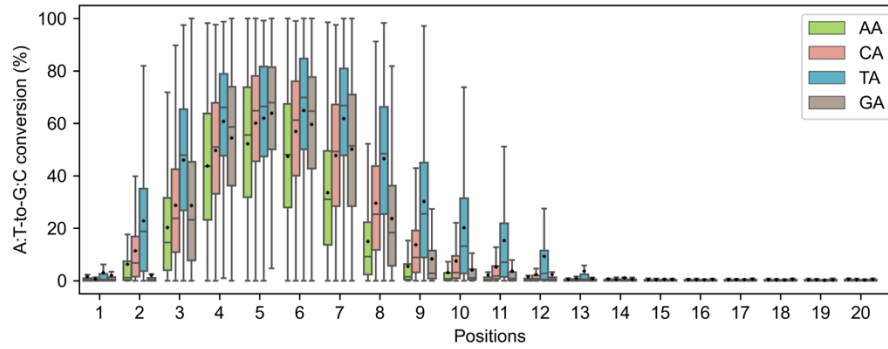
Supplementary Figure 29 | Scatter plot of A:T-to-G:C editing in the entire protospacer mediated by ABE7.10, ABE8.20, ABE8e, ABE8r, ABE-RA2.0, and ABE-RA3.0.

ABE7.10



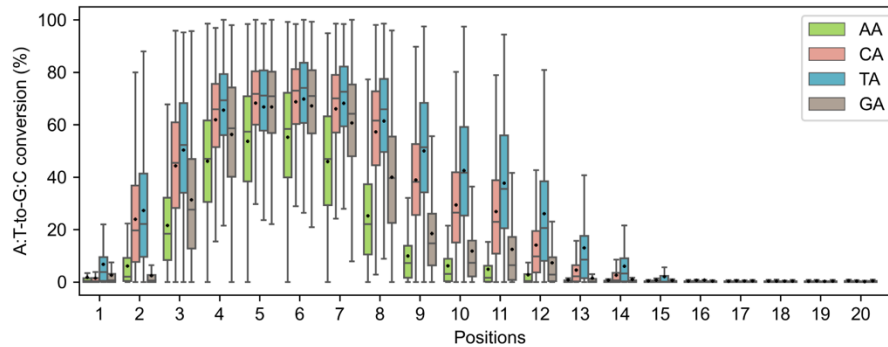
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AA	512	568	969	1065	953	1097	989	905	1123	1033	752	681	595	526	593	578	559	657	555	572
CA	846	1042	1274	1057	987	1052	964	956	1046	908	768	821	706	849	786	748	867	762	794	892
TA	345	371	685	662	604	732	652	658	734	701	363	427	352	309	314	369	288	346	331	286
GA	572	599	908	831	1043	1001	846	989	943	881	720	875	689	759	959	670	821	845	597	820

ABE8.20



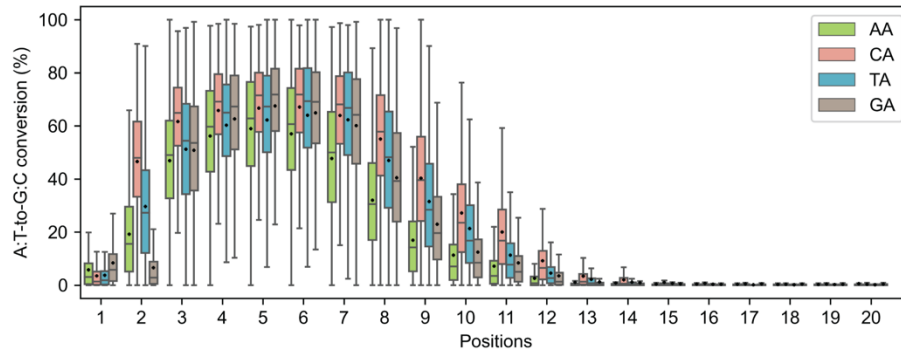
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AA	515	571	965	1062	944	1095	998	907	1127	1032	748	676	591	520	592	578	559	659	557	575
CA	843	1035	1274	1055	992	1051	960	958	1051	906	761	820	703	851	784	750	865	765	796	895
TA	344	374	682	661	607	734	650	652	736	706	359	430	349	312	314	367	286	349	330	287
GA	570	592	899	823	1047	996	840	990	937	875	718	869	681	757	959	669	824	838	598	818

ABE8e



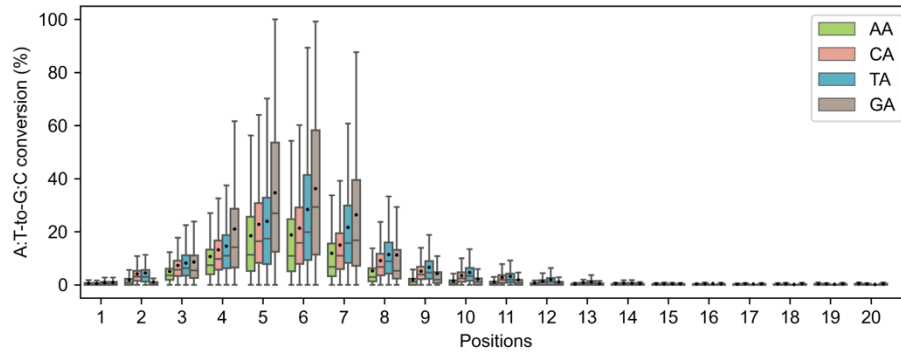
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AA	515	568	973	1070	953	1103	993	912	1129	1033	753	683	591	520	591	581	557	655	556	577
CA	842	1043	1274	1063	991	1055	966	967	1052	913	769	818	708	857	789	751	872	764	794	902
TA	350	373	689	662	608	738	649	656	742	706	363	429	351	311	321	368	292	348	330	287
GA	571	599	912	825	1051	998	843	992	938	880	729	870	690	761	965	676	826	855	601	824

ABE8r



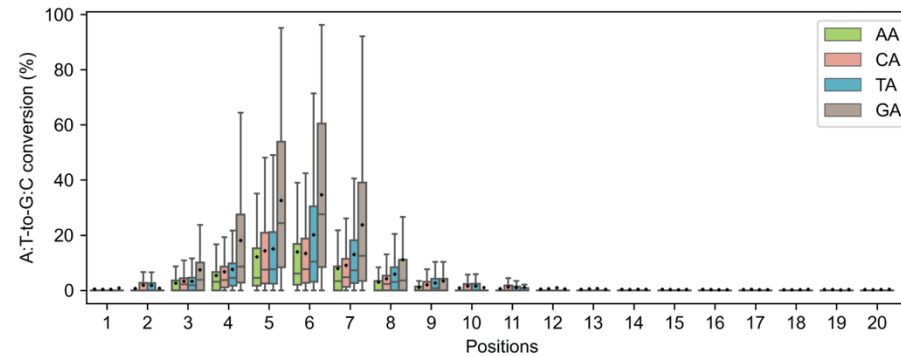
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AA	517	574	973	1072	955	1115	1005	917	1135	1039	755	685	596	521	593	582	563	665	560	578
CA	848	1044	1279	1061	995	1061	969	966	1057	912	775	826	709	853	794	752	874	766	799	905
TA	354	376	690	663	614	741	652	657	742	710	364	431	351	311	322	368	293	351	331	292
GA	573	598	916	830	1056	1001	851	996	945	885	728	874	692	771	969	680	831	857	605	825

ABE-RA2.0



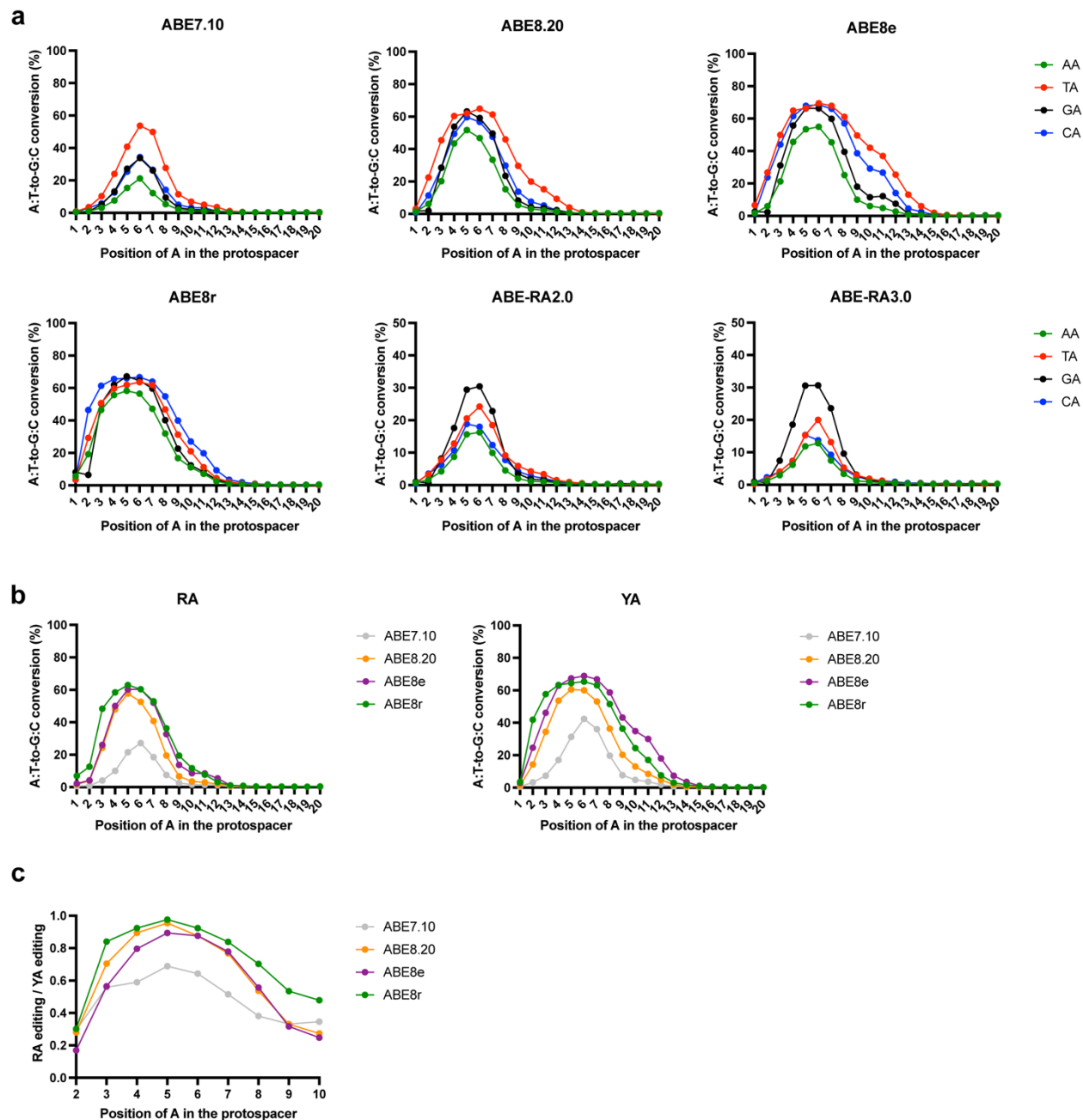
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AA	486	545	939	1036	925	1068	958	861	1084	985	717	658	570	505	567	562	538	633	539	559
CA	816	1020	1255	1038	970	1020	942	943	1022	892	754	804	692	824	769	734	844	748	773	875
TA	330	359	674	642	581	715	621	632	711	673	352	415	336	298	307	357	275	338	317	283
GA	551	576	858	791	1004	954	803	962	894	849	693	831	669	733	929	645	791	821	585	794

ABE-RA3.0

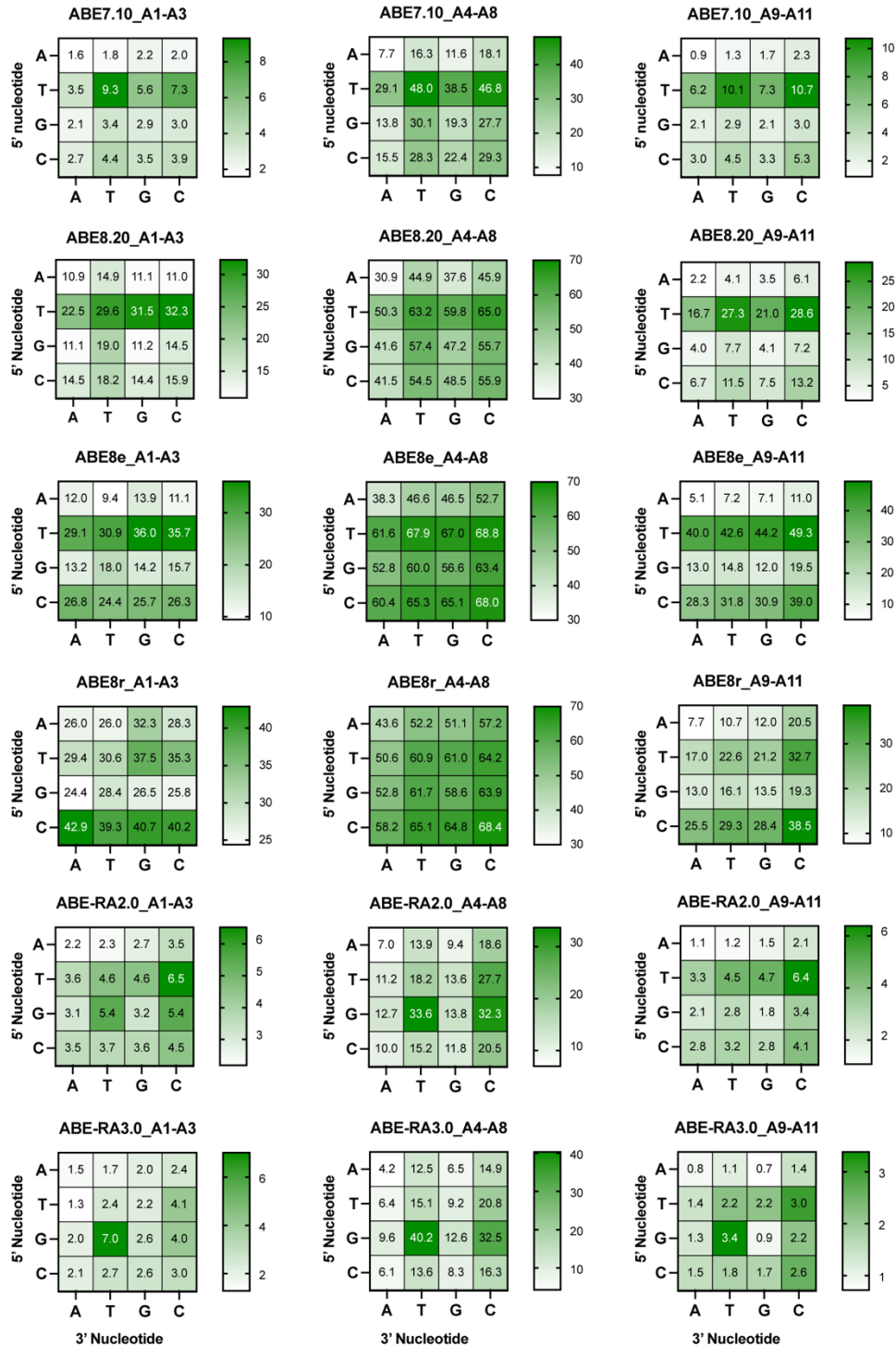


Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AA	253	274	514	569	526	622	533	482	617	559	379	340	316	272	310	306	289	337	276	302
CA	511	682	905	714	656	683	632	664	713	618	513	543	462	572	509	496	571	519	499	606
TA	187	226	451	414	346	419	388	345	418	414	203	255	168	164	186	194	145	206	199	160
GA	299	246	423	454	611	540	426	553	520	455	361	466	375	414	517	381	444	445	331	452

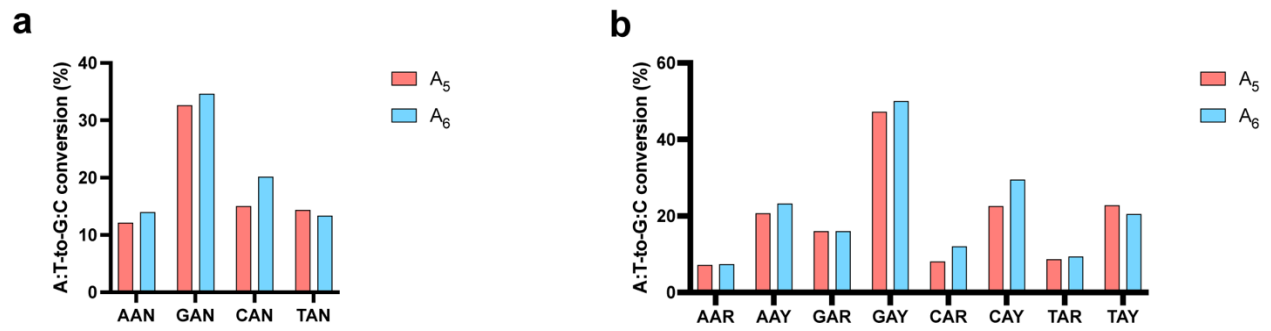
Supplementary Figure 30 | Box plot of A:T-to-G:C editing of the paired sgRNA-target library by ABE7.10, ABE8.20, ABE8e, ABE8r, ABE-RA2.0, and ABE-RA3.0. Data were grouped by both sequence contexts and protospacer positions. The number of A sites in each box (n) is provided in the table below each box plot. Lower and upper hinges denote first and third quartiles. The central line and plus sign represent the median and mean, respectively.



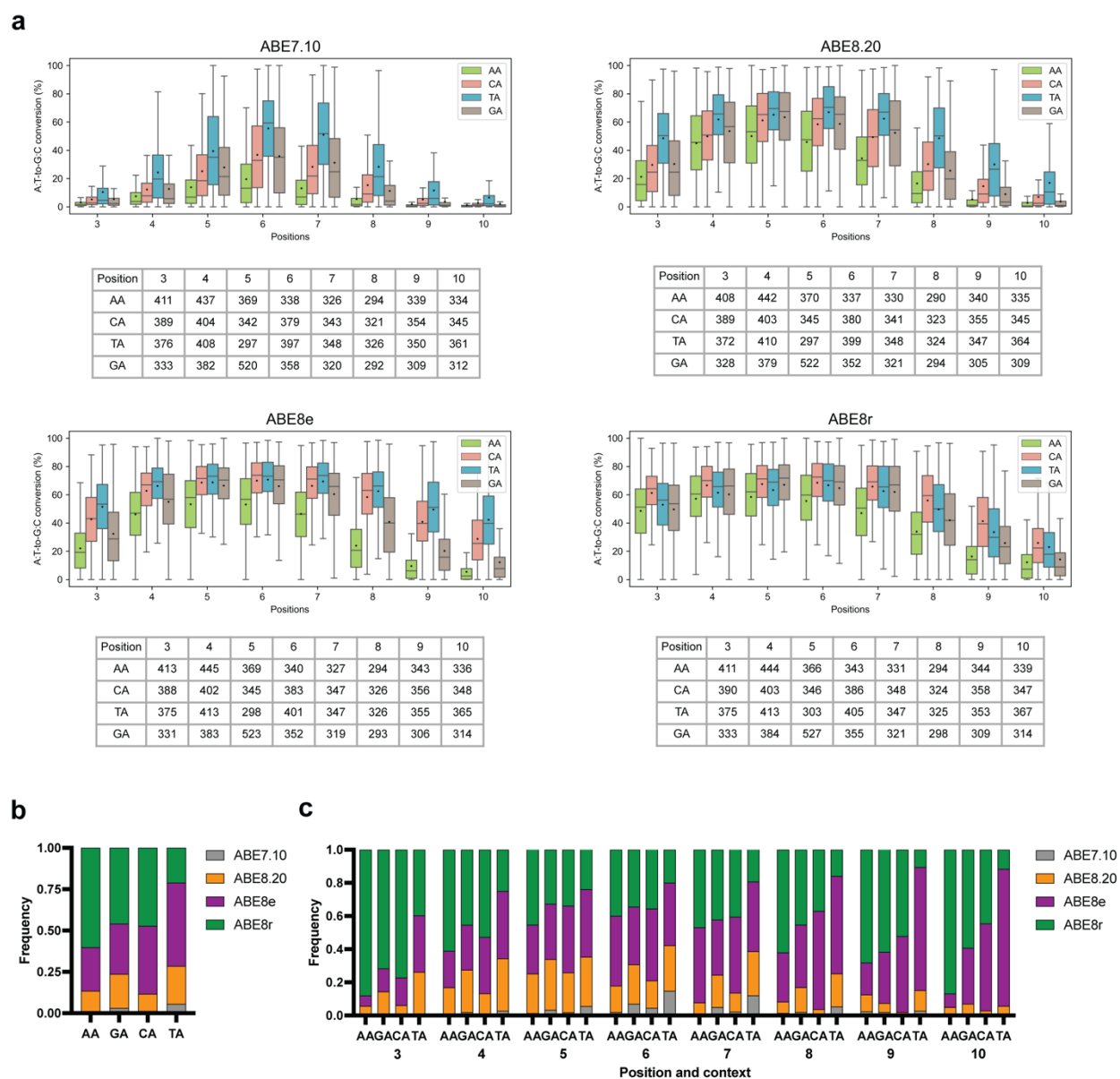
Supplementary Figure 31 | A:T-to-G:C editing of the paired sgRNA-target library dissected by sequence contexts. **a.** ABE7.10-, ABE8.20-, ABE8e-, ABE8r-, ABE-RA2.0-, and ABE-RA3.0-mediated context-specific editing across the protospacer. **b.** RA and YA editing generated by ABE7.10, ABE8.20, ABE8e, ABE8r. **c.** Ratio of RA editing to YA editing at protospacer positions 2-10. ABE8r shows a higher ratio of RA editing to YA editing than other ABEs across all positions, especially those outside the strong editing window. In other words, ABE8r exhibits improved RA context compatibility regardless of absolute editing levels.



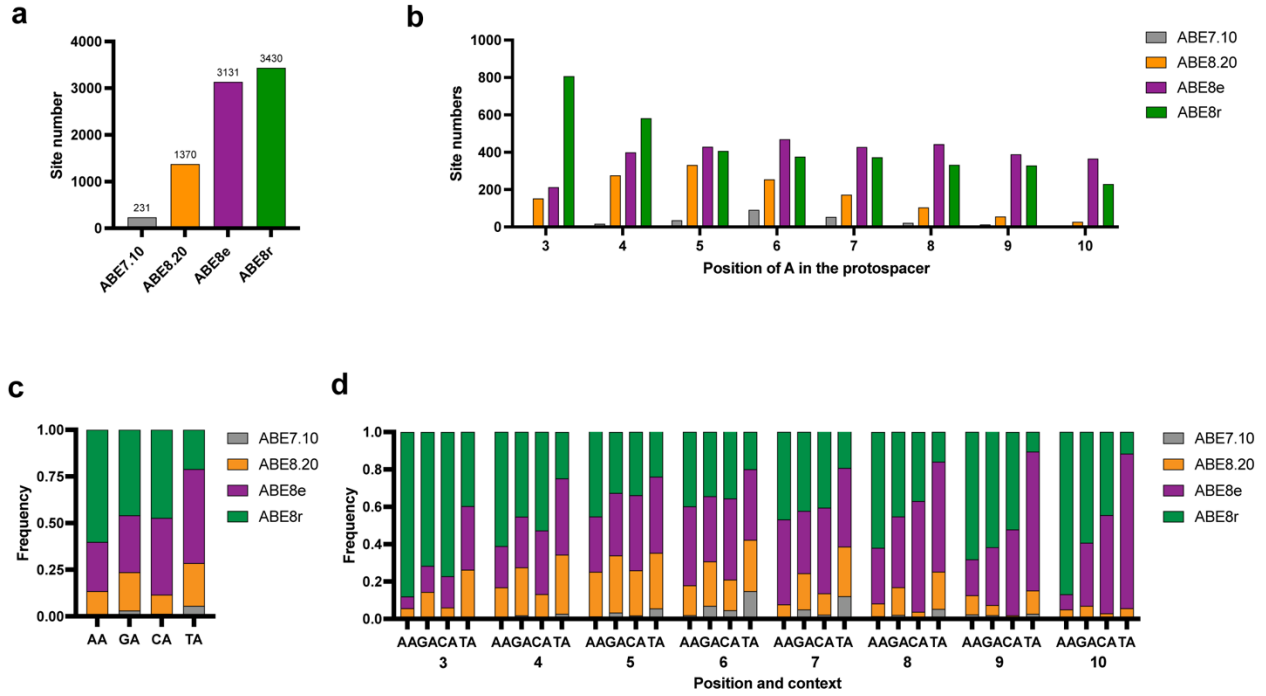
Supplementary Figure 32 | Heatmap representation of A:T-to-G:C editing in the paired sgRNA-target library mediated by ABE7.10, ABE8.20, ABE8e, ABE8r, ABE-RA-2.0, and ABE-RA3.0. Data were grouped by both sequence contexts and protospacer positions. The y axis refers to the nucleotide 5' to the target A, while the x axis refers to the nucleotide 3' to the target A. Average editing rates are presented.



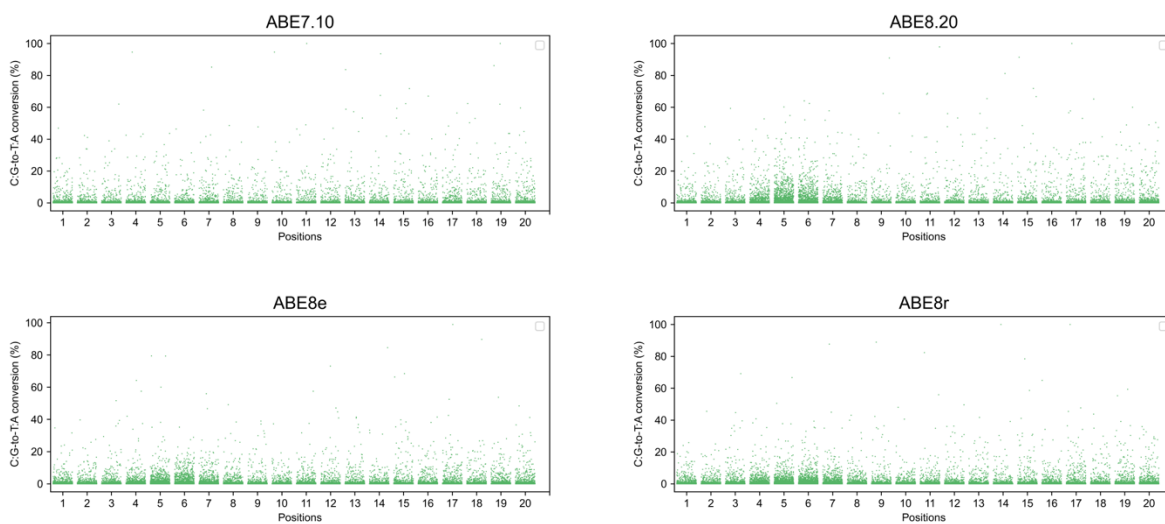
Supplementary Figure 33 | ABE-RA3.0-mediated A:T-to-G:C editing at A₅ and A₆ in the paired sgRNA-target library grouped by **(a)** AAN, GAN, CAN, and TAN contexts; and **(b)** AAR, AAY, GAR, GAY, CAR, CAY, TAR, and TAY contexts.



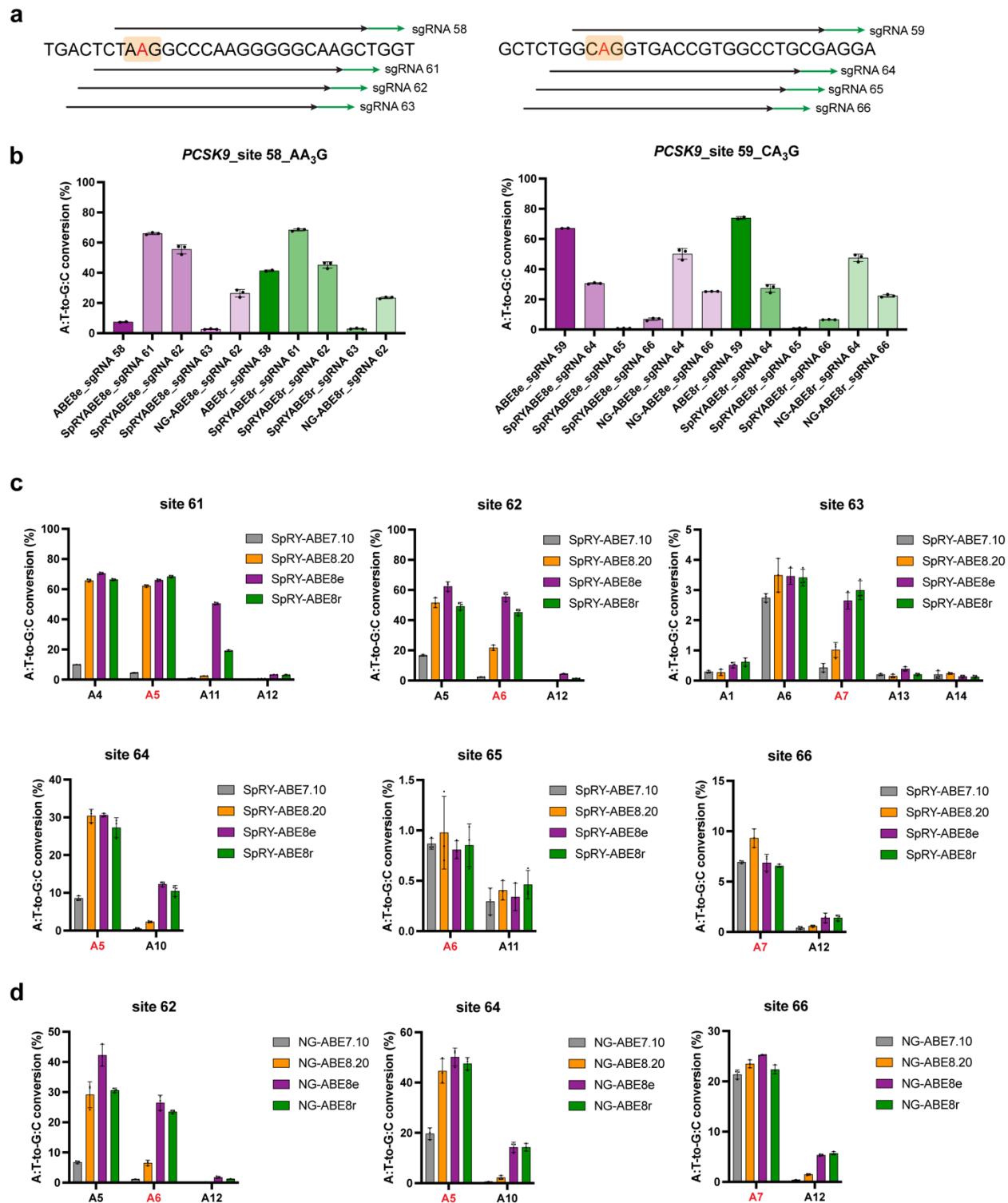
Supplementary Figure 34 | A:T-to-G:C editing at disease-relevant A sites. **a.** Box plot of AA, GA, TA, and CA editing generated by each editor. The number of A sites in each box (n) is provided in the table below each box plot. Lower and upper hinges denote first and third quartiles. The central line and plus sign represent the median and mean, respectively. **b.** Frequencies of each editor emerging as the editor of choice at different sequence contexts. **c.** Frequencies of each editor emerging as the editor of choice across different sequence contexts and protospacer positions.



Supplementary Figure 35 | Performance of editors at disease-relevant A sites with potential for clinical translation. **a.** Numbers of disease-relevant As most efficiently edited by ABE7.10, ABE8.20, ABE8e, or ABE8r. **b.** Editor of choice for individual disease-relevant As dissected by protospacer positions. **c.** Frequencies of each editor emerging as the editor of choice at different sequence contexts. **d.** Frequencies of each editor emerging as the editor of choice across different sequence contexts and protospacer positions. Target sites with sequencing depths of ≥ 20 in all four samples and edited to $\geq 20\%$ by at least one editor are included in the analysis ($n = 8,162$).

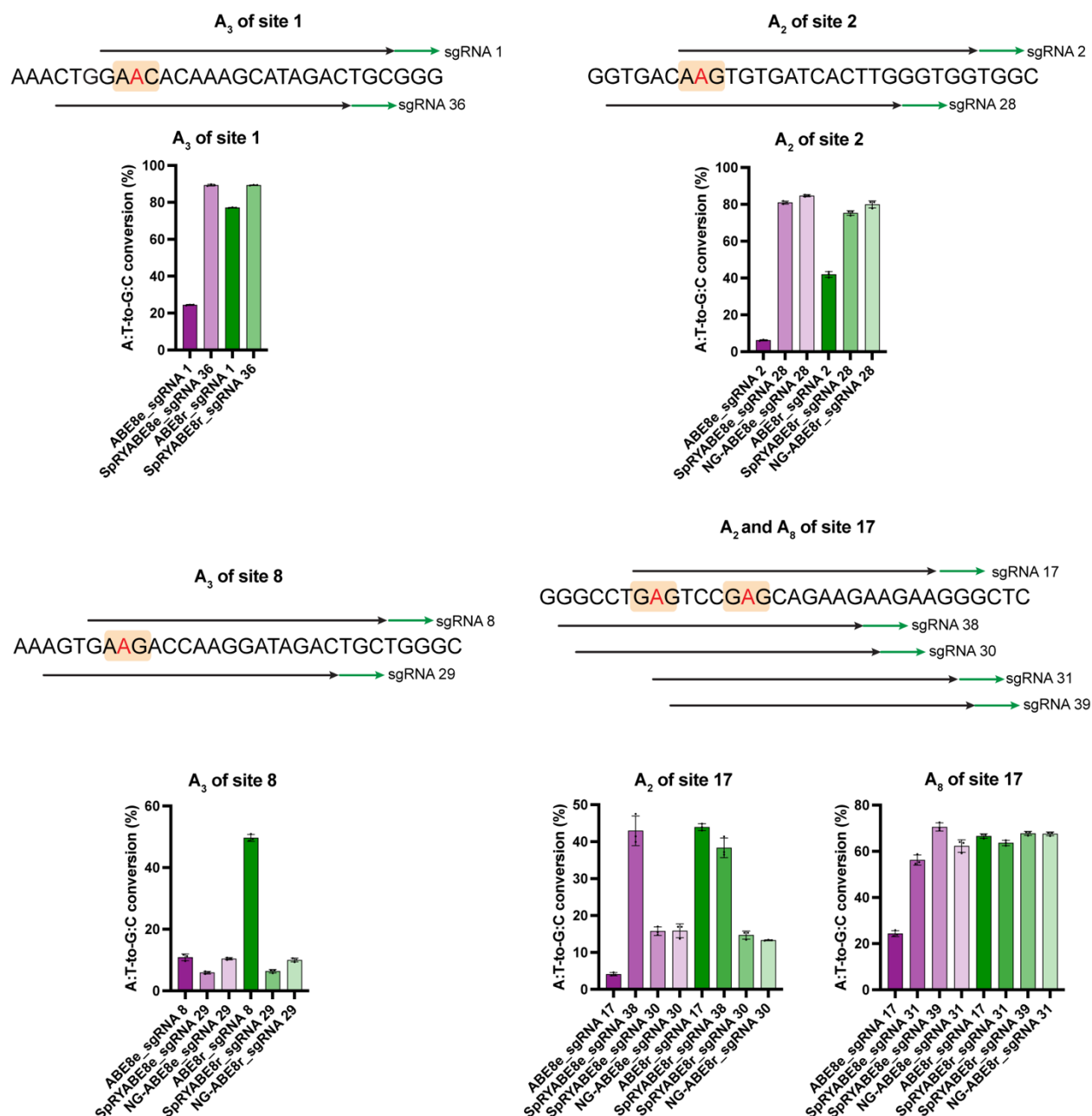


Supplementary Figure 36 | ABE7.10-, ABE8.20-, ABE8e-, and ABE8r-mediated bystander C:G-to-T:A editing in the paired sgRNA-target library.

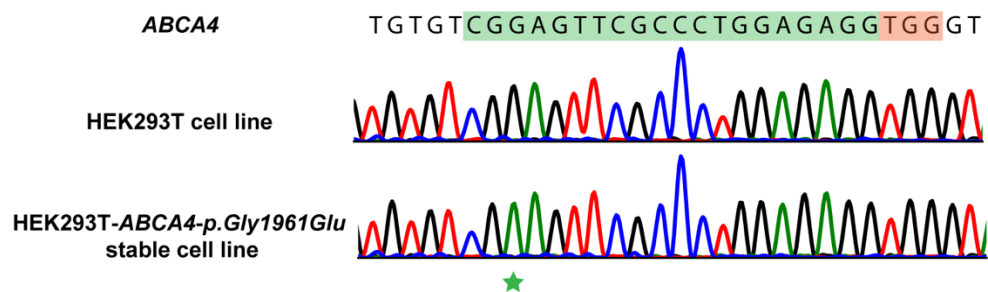


Supplementary Figure 37 | A:T-to-G:C editing at *PCSK9* splicing sites generated by ABEs, NG-ABEs, and SpRY-ABEs in HEK293T cells. **a.** Design of sgRNAs. **b.** ABE-, NG-ABE-, and SpRY-ABE-mediated editing of *PCSK9* splicing sites. n = 2 independent experiments for site 58 with sgRNA 58 and site 59 with sgRNA59 (mean); n = 3 independent experiments for site 58 with

sgRNA 61-63 and site 59 with sgRNA64-66 (mean $\pm$ s.d.). **c.** SpRY-ABE-mediated A:T-to-G:C editing in the entire protospacers. n = 3 independent experiments (mean $\pm$ s.d.). **d.** NG-ABE-mediated A:T-to-G:C editing in the entire protospacers. n = 3 independent experiments (mean $\pm$ s.d.).



Supplementary Figure 38 | ABE-, NG-ABE-, and SpRY-ABE-mediated editing at A₃ of site 1, A₂ of site 2, A₃ of site 8, and A₂ and A₈ of site 17 with alternative spacer designs. Top: design of sgRNAs. Bottom: ABE-, NG-ABE-, and SpRY-ABE-mediated editing of the target A sites. n = 3 independent experiments in HEK293T cells (mean ± s.d.).



Supplementary Figure 39 | Sanger traces covering *ABCA4* amplified from HEK293T and HEK293T-*ABCA4*-p.Gly1961Glu cells.

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