

Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage

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Untreated																						Indel%			
Site 17	G ₁	A ₂	C ₃	A ₄	A ₅	A ₆	G ₇	A ₈	G ₉	G ₁₀	A ₁₁	A ₁₂	G ₁₃	A ₁₄	G ₁₅	A ₁₆	G ₇	A ₁₈	C ₁₉	G ₂₀	G	G	G		
A	0.0	100.0	0.0	99.9	99.6	99.6	0.0	99.9	0.0	0.0	100.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.077
C	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	
G	100.0	0.0	0.0	0.1	0.4	0.4	100.0	0.1	100.0	100.0	0.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	99.9	100.0	99.9	100.0	
T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
ABE6.3																						Indel%			
Site 17	G ₁	A ₂	C ₃	A ₄	A ₅	A ₆	G ₇	A ₈	G ₉	G ₁₀	A ₁₁	A ₁₂	G ₁₃	A ₁₄	G ₁₅	A ₁₆	G ₇	A ₁₈	C ₁₉	G ₂₀	G	G	G		
A	0.0	99.3	0.0	96.9	83.8	86.7	0.1	97.4	0.0	0.0	99.7	99.8	0.0	100.0	0.0	100.0	0.0	100.0	0.0	0.1	0.0	0.0	0.0	0.0	0.072
C	0.0	0.0	99.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	
G	100.0	0.7	0.1	3.1	16.1	13.3	99.9	2.6	100.0	100.0	0.2	0.2	99.9	0.0	100.0	0.0	100.0	0.0	0.0	99.8	100.0	100.0	100.0	0.0	
T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	
ABE7.8																						Indel%			
Site 17	G ₁	A ₂	C ₃	A ₄	A ₅	A ₆	G ₇	A ₈	G ₉	G ₁₀	A ₁₁	A ₁₂	G ₁₃	A ₁₄	G ₁₅	A ₁₆	G ₇	A ₁₈	C ₁₉	G ₂₀	G	G	G		
A	0.0	99.0	0.0	96.8	88.0	88.5	0.1	92.9	0.0	0.0	99.8	99.9	0.0	100.0	0.0	100.0	0.0	100.0	0.0	0.1	0.0	0.0	0.0	0.0	0.06
C	0.0	0.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	
G	100.0	0.9	0.1	3.2	12.0	11.5	99.9	7.0	100.0	100.0	0.2	0.1	99.9	0.0	100.0	0.0	100.0	0.0	0.0	99.9	100.0	100.0	100.0	0.0	
T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
ABE7.9																						Indel%			
Site 17	G ₁	A ₂	C ₃	A ₄	A ₅	A ₆	G ₇	A ₈	G ₉	G ₁₀	A ₁₁	A ₁₂	G ₁₃	A ₁₄	G ₁₅	A ₁₆	G ₇	A ₁₈	C ₁₉	G ₂₀	G	G	G		
A	0.0	99.5	0.0	97.8	84.1	77.9	0.1	90.4	0.0	0.0	99.7	99.9	0.0	100.0	0.0	100.0	0.0	100.0	0.0	0.1	0.0	0.0	0.0	0.0	0.053
C	0.0	0.0	99.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	
G	100.0	0.5	0.1	2.2	15.8	22.1	99.9	9.6	100.0	100.0	0.3	0.1	99.9	0.0	100.0	0.0	100.0	0.0	0.0	99.9	100.0	100.0	100.0	0.0	
T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
ABE7.10																						Indel%			
Site 17	G ₁	A ₂	C ₃	A ₄	A ₅	A ₆	G ₇	A ₈	G ₉	G ₁₀	A ₁₁	A ₁₂	G ₁₃	A ₁₄	G ₁₅	A ₁₆	G ₇	A ₁₈	C ₁₉	G ₂₀	G	G	G		
A	0.0	99.7	0.0	83.1	45.6	45.3	0.1	95.6	0.0	0.1	99.6	99.9	0.0	100.0	0.0	100.0	0.0	100.0	0.0	0.1	0.0	0.1	0.0	0.0	0.130
C	0.0	0.0	99.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	99.9	0.0	0.0	0.0	0.0	
G	100.0	0.2	0.1	16.8	54.3	54.7	99.9	4.4	100.0	99.9	0.4	0.1	99.9	0.0	100.0	0.0	100.0	0.0	0.0	99.8	99.9	99.9	100.0	0.0	
T	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	

Supplementary Table 2. Activities of ABE7.8, ABE7.9, and ABE7.10 at the HEK2 on-target and off-target sites previously characterized for *S. pyogenes* Cas9 nuclease.¹

		% of total sequencing reads with target A•T base pair converted to G•C																					
HEK2 (on-target site)		G1	A2	A3	C4	A5	C6	A7	A8	A9	G10	C11	A12	T13	A14	G15	A16	C17	T18	G19	C20	Indel%	
ABE 7.8		0.2	1.8			77.2		2.7	1.0	0.8			0.1		0.0		0.0						0.2
ABE 7.9			0.1	0.5		79.4		2.5	0.5	0.5			0.0		0.0		0.0						0.1
ABE 7.10			0.0	0.5		87.6		23.0	1.0	1.0			0.1		0.0		0.0						0.3
Cas9 nuclease			0.0	0.7				0.0	0.0	0.0			0.3		0.1		0.1						54.5
D10A Cas9 nickase			0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.2
H840A Cas9 nickase			0.0	0.1		0.0		0.0	0.0	0.0			0.2		0.0		0.0						5.3
dCas9 (D10A + H840A)			0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
no treatment			0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0

		% of total sequencing reads with target A•T base pair converted to G•C																					
HEK2 off-target site 1		G1	A2	A3	C4	A5	C6	A7	A8	T9	G10	C11	A12	T13	A14	G15	A16	T17	T18	G19	C20	Indel%	
ABE 7.8			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.0
ABE 7.9			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.0
ABE 7.10			0.0	0.0		0.3		0.1	0.0				0.0		0.0		0.0						0.0
Cas9 nuclease			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.5
D10A Cas9 nickase			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.0
H840A Cas9 nickase			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.0
dCas9 (D10A + H840A)			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.0
no treatment			0.0	0.0		0.0		0.0	0.0				0.0		0.0		0.0						0.0

		% of total sequencing reads with target A•T base pair converted to G•C																					
HEK2 off-target site 2		A1	A2	A3	C4	A5	T6	A7	A8	A9	G10	C11	A12	T13	A14	G15	A16	C17	T18	G19	C20	Indel%	
ABE 7.8		0.0	0.0	0.0		0.0		0.1	0.0	0.0			0.0		0.0		0.0						0.0
ABE 7.9		0.0	0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
ABE 7.10		0.0	0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
Cas9 nuclease		0.0	0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
D10A Cas9 nickase		0.0	0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
H840A Cas9 nickase		0.0	0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
dCas9 (D10A + H840A)		0.0	0.0	0.0		0.0		0.0	0.0	0.0			0.0		0.0		0.0						0.0
no treatment		0.0	0.0	0.0		0.0		0.1	0.0	0.0			0.0		0.0		0.0						0.0

Supplementary Table 3. Activities of ABE7.8, ABE7.9, and ABE7.10 at the HEK3 site previously characterized for on-target and off-target modification by *S. pyogenes* Cas9 nuclease.¹

		% of total sequencing reads with target A•T base pair converted to G•C																						
HEK3 (on-target site)		G1	G2	C3	C4	C5	A6	G7	A8	C9	T10	G11	A12	G13	C14	A15	C16	G17	T18	G19	A20	Indel%		
ABE 7.8							8.4		14.7				0.1			0.0					0.0	0.1		
ABE 7.9							12.2		9.4				0.2			0.0					0.0	0.2		
ABE 7.10							62.7		18.6				0.2			0.0					0.0	0.2		
Cas9 nuclease							0.0		0.0				0.0			0.2					0.2	64.8		
D10A Cas9 nickase							0.0		0.0				0.0			0.0					0.0	2.0		
H840A Cas9 nickase							0.0		0.0				0.0			0.0					0.0	0.4		
dCas9 (D10A + H840A)							0.0		0.0				0.0			0.0					0.0	0.0		
no treatment							0.0		0.0				0.0			0.0					0.0	0.0		

		% of total sequencing reads with target A•T base pair converted to G•C																						
HEK3 off-target site 1		C1	A2	C3	C4	C5	A6	G7	A8	C9	T10	G11	A12	G13	C14	A15	C16	G17	T18	G19	C20	Indel%		
ABE 7.8			0.0				0.0		0.0				0.0			0.0						0.0		0.0
ABE 7.9			0.0				0.0		0.0				0.0			0.0								0.0
ABE 7.10			0.0				0.0		0.0				0.0			0.0								0.0
Cas9 nuclease			0.0				0.0		0.0				0.0			0.0								1.0
D10A Cas9 nickase			0.0				0.0		0.0				0.0			0.0								0.0
H840A Cas9 nickase			0.0				0.0		0.0				0.0			0.0								0.0
dCas9 (D10A + H840A)			0.0				0.0		0.0				0.0			0.0								0.0
no treatment			0.0				0.0		0.0				0.0			0.0								0.0

		% of total sequencing reads with target A•T base pair converted to G•C																						
HEK3 off-target site 2		G1	A2	C3	A4	C5	A6	G7	A8	C9	C10	G11	G12	G13	C14	A15	C16	G17	T18	G19	A20	Indel%		
ABE 7.8			0.0				0.0		0.1							0.0						0.0		0.0
ABE 7.9			0.0		0.0		0.0		0.0							0.0						0.0		0.0
ABE 7.10			0.0		0.0		0.0		0.0							0.0						0.0		0.0
Cas9 nuclease			0.0		0.0		0.0		0.0							0.0						0.0		1.6
D10A Cas9 nickase			0.0		0.0		0.0		0.0							0.0						0.0		0.0
H840A Cas9 nickase			0.0		0.0		0.0		0.0							0.0						0.0		0.0
dCas9 (D10A + H840A)			0.0		0.0		0.0		0.0							0.0						0.0		0.0
no treatment			0.0		0.0		0.0		0.0							0.0						0.0		0.0

		% of total sequencing reads with target A•T base pair converted to G•C																						
HEK3 off-target site 3		A1	G2	C3	T4	C5	A6	G7	A8	C9	T10	G11	A12	G13	C14	A15	A16	G17	T18	G19	A20	Indel%		
ABE 7.8			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0
ABE 7.9			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0
ABE 7.10			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0
Cas9 nuclease			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.2
D10A Cas9 nickase			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0
H840A Cas9 nickase			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0
dCas9 (D10A + H840A)			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0
no treatment			0.0				0.0		0.0				0.0			0.0	0.0					0.0		0.0

		% of total sequencing reads with target A•T base pair converted to G•C																						
HEK3 off-target site 4		A1	G2	A3	C4	C5	A6	G7	A8	C9	T10	G11	A12	G13	C14	A15	A16	G17	A18	G19	A20	Indel%		
ABE 7.8			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
ABE 7.9			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
ABE 7.10			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
Cas9 nuclease			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
D10A Cas9 nickase			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
H840A Cas9 nickase			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
dCas9 (D10A + H840A)			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0
no treatment			0.0		0.0		0.0		0.0				0.0			0.0	0.0		0.0			0.0		0.0

		% of total sequencing reads with target A•T base pair converted to G•C																						
HEK3 off-target site 5		G1	A2	G3	C4	C5	A6	G7	A8	A9	T10	G11	A12	G13	C14	A15	C16	G17	T18	G19	A20	Indel%		
ABE 7.8			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.1
ABE 7.9			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.1
ABE 7.10			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.0
Cas9 nuclease			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.1
D10A Cas9 nickase			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.0
H840A Cas9 nickase			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.1
dCas9 (D10A + H840A)			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.1
no treatment			0.0				0.0		0.0	0.0			0.0			0.0						0.0		0.0

Supplementary Table 4. Activities of ABE7.8, ABE7.9, and ABE7.10 at the HEK4 site previously characterized for on-target and off-target modification by *S. pyogenes* Cas9 nuclease.¹ Although HEK4 off-target site 3 showed appreciable indel formation upon ABE treatment, this locus also showed unusually high (89%) indel formation by Cas9 nuclease and was the only tested off-target site exhibiting indel formation upon treatment with Cas9 nickases. We speculate that this locus is unusually fragile, and that indel formation here arises from simply nicking the site, rather than from ABE-mediated adenine deamination.

% of total sequencing reads with target A•T base pair converted to G•C																							
HEK4 (on-target site)	G1	G2	C3	A4	C5	T6	G7	C8	G9	G10	C11	T12	G13	G14	A15	G16	G17	T18	G19	G20	Indel%		
ABE 7.8				4.8											0.0						0.2		
ABE 7.9				1.5											0.0						0.1		
ABE 7.10				16.0											0.0						0.2		
Cas9 nuclease				0.1											0.0						36.5		
D10A Cas9 nickase				0.0											0.0						0.8		
H840A Cas9 nickase				0.0											0.0						0.7		
dCas9 (D10A + H840A)				0.0											0.0						0.0		
no treatment				0.0											0.0						0.0		

% of total sequencing reads with target A•T base pair converted to G•C																							
HEK4 off-target site 1	T1	G2	C3	A4	C5	T6	G7	C8	G9	G10	C11	C12	G13	G14	A15	G16	G17	A18	G19	G20	Indel%		
ABE 7.8				0.6											0.0			0.0			0.1		
ABE 7.9				0.2											0.0			0.0			0.0		
ABE 7.10				1.2											0.0			0.0			0.0		
Cas9 nuclease				0.0											0.0			0.2			12.3		
D10A Cas9 nickase				0.0											0.0			0.0			0.0		
H840A Cas9 nickase				0.0											0.0			0.0			0.0		
dCas9 (D10A + H840A)				0.0											0.0			0.0			0.0		
no treatment				0.0											0.0			0.0			0.0		

% of total sequencing reads with target A•T base pair converted to G•C																							
HEK4 off-target site 2	G1	G2	C3	T4	C5	T6	G7	C8	G9	G10	C11	T12	G13	G14	A15	G16	G17	G18	G19	G20	Indel%		
ABE 7.8															0.0						0.0		
ABE 7.9															0.0						0.0		
ABE 7.10															0.0						0.0		
Cas9 nuclease															0.0						4.8		
D10A Cas9 nickase															0.0						0.0		
H840A Cas9 nickase															0.0						0.0		
dCas9 (D10A + H840A)															0.0						0.0		
no treatment															0.0						0.0		

% of total sequencing reads with target A•T base pair converted to G•C																							
HEK4 off-target site 3	G1	G2	C3	A4	C5	G6	A7	C8	G9	G10	C11	T12	G13	G14	A15	G16	G17	T18	G19	G20	Indel%		
ABE 7.8				0.5			16.8								0.0						1.1		
ABE 7.9				0.2			21.9								0.0						1.7		
ABE 7.10				1.4			7.8								0.0						3.7		
Cas9 nuclease				0.0			0.3								0.1						89.1		
D10A Cas9 nickase				0.0			0.0								0.0						0.6		
H840A Cas9 nickase				0.0			0.0								0.0						1.4		
dCas9 (D10A + H840A)				0.0			0.0								0.0						0.0		
no treatment				0.0			0.0								0.0						0.0		

% of total sequencing reads with target A•T base pair converted to G•C																							
HEK4 off-target site 4	G1	G2	C3	A4	T5	C6	A7	C8	G9	G10	C11	T12	G13	G14	A15	G16	G17	T18	G19	G20	Indel%		
ABE 7.8				0.3			0.5								0.0						0.0		
ABE 7.9				0.0			0.2								0.0						0.0		
ABE 7.10				0.4			1.8								0.0						0.0		
Cas9 nuclease				0.0			0.0								0.0						2.5		
D10A Cas9 nickase				0.0			0.0								0.0						0.0		
H840A Cas9 nickase				0.0			0.0								0.0						0.0		
dCas9 (D10A + H840A)				0.0			0.0								0.0						0.0		
no treatment				0.0			0.0								0.0						0.0		

% of total sequencing reads with target A•T base pair converted to G•C																							
HEK4 off-target site 5	G1	G2	C3	G4	C5	T6	G7	C8	G9	G10	C11	G12	G13	G14	A15	G16	G17	T18	G19	G20	Indel%		
ABE 7.8															0.0						0.0		
ABE 7.9															0.0						0.0		
ABE 7.10															0.0						0.0		
Cas9 nuclease															0.0						9.8		
D10A Cas9 nickase															0.0						0.0		
H840A Cas9 nickase															0.0						0.0		
dCas9 (D10A + H840A)															0.0						0.0		
no treatment															0.0						0.0		

Supplementary Table 5. Primers used for generating sgRNA plasmids. The 20-nt target protospacer is shown in red. When a target DNA sequence did not start with a 'G', a 'G' was added to the 5' end of the primer since the human U6 promoter prefers a 'G' at the transcription start site²⁻⁴. The pFYF sgRNA plasmid described previously⁵ was used as a template for PCR amplification.

Primer	Sequence
R-sgRNA	5'-GGTGTTCGTCCTTTCCACAAG-3'
F-site 1	5'-GAACACAAAGCATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 2	5'-GAGTATGAGGCATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 3	5'-GTCAAGAAAGCAGAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 4	5'-GAGCAAAGAGAATAGACTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 5	5'-GATGAGATAATGATGAGTCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 6	5'-GGATTGACCCAGGCCAGGGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 7	5'-GAATACTAAGCATAGACTCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 8	5'-GTAACAAAGCATAGACTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 9	5'-GAAGACCAAGGATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 10	5'-GAACATAAAGAATAGAATGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 11	5'-GGACAGGCAGCATAGACTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 12	5'-GTAGAAAAGTATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 13	5'-GAAGATAGAGAATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 14	5'-GGCTAAGACCATAGACTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 15	5'-GTCTAGAAAGCTTAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 16	5'-GGGAATAAATCATAGAATCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 17	5'-GACAAAGAGGAAGAGAGACGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 18	5'-GACACACACACTTAGAATCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-site 19	5'-GCACACACACTTAGAATCTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F- hbg1/2	5'-GTGGGGGAAGGGGCCCAAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'
F-HFE	5'-GACGTACCAGGTGGAGCACCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC-3'

Supplementary Table 6. Primers used for generating bacterial TadA* libraries.

Primer	Sequence
NMG-799	AGTTGTACGCG/ideoxyU/CCAAAAAACGGG
NMG-822	AGATTAGCGGATCCTACCTGAC
NMG-823	GCGGTCTGTATTTCCAGAAC
NMG-824	ACCGGGGACTTCAGAA/ideoxyU/CGGC
NMG-825	ATTCTGAAGTCCCCGG/ideoxyU/GTTTCG
NMG-826	ACGCGTACAAC/ideoxyU/CAAAGGAGGAAAAAAAAATG
NMG-1197	ACGCTGGCGAAACG/ideoxyU/GCCTGGGATNNKNNKGAAGTGCCGGTCGGCGC
NMG-1198	ACGTTTCGCCAGCG/ideoxyU/CAGCGCGTGACG
NMG-1199	ACGCGAAAACGGCGC/ideoxyU/GCG
NMG-1200	AGCGCCAGTTTTCGCG/ideoxyU/TMNNCACACCAAAGACCACGCGACC
NMG-1201	ACTGGCGGATGAG/ideoxyU/GCNNKNNKTTGCTCAGTTACTTCTTTTCGCATGCG
NMG-1202	ACTCATCCGCCAG/ideoxyU/ATTCCTTCCG

Supplementary Table 7. Starting constructs used for each round of TadA* mutagenesis and selection in *E. coli*. All plasmids contain an SC101 origin of replication, a β -lactamase gene for plasmid maintenance with carbenicillin, a P_{BAD} promoter driving TadA*–dCas9 expression, and a lac promoter driving sgRNA transcription. The architecture of the base editors used during bacterial selection is: TadA*–linker(16 aa)–dCas9.

Round	Template used for mutagenesis	TadA mutations	Guide RNA protospacer 1	Guide RNA protospacer 2
1	pNMG-104	wild-type	TACGGCGTAGTGACCTGGA	n/a
2	pNMG-128	H8Y, D108N, N127S	TACGGCGTAGTGACCTGGA	n/a
3	pNMG-288	A106V, D108N, D147Y, E155V	ATCTTATTCGATCATGCGAA	GCTTAGGTGGAGCGCCTATT
4	pNMG-343	A106V, D108N, D147Y, E155V	CAATGATGACTTCTACAGCG	n/a
5	pNMG-381	L84F, A106V, D108N, H123Y, D147Y, E155V, I156F	CAATGATGACTTCTACAGCG	TACGGCGTAGTGACCTGGA
6	round 1-5 plasmids + pNMG-104	all mutations accumulated	CAATGATGACTTCTACAGCG	TACGGCGTAGTGACCTGGA
7	multiple round 6 plasmids	H36L, P48S, L84P, S97C, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, K157N, K161T	TTCATTAAGTGTGGCCGGCT	ATCTTATTCGATCATGCGAA

Supplementary Table 8. Antibiotic selection plasmids and their corresponding *E. coli* antibiotic minimum inhibitory concentrations (MICs).

Round	Antibiotic resistance	Target sequence	Inactivating mutation	Position of target A in protospacer	MIC in S1030 cells (µg/mL)	Selection antibiotic concentration (µg/mL)	Library c.f.u. after USER assembly
1	Cam ^R	TACGGCGT A GTGCACCTGGA	H193Y	9	1	2, 4, 8, 16	2 x 10 ⁶
2	Cam ^R	TACGGCGT A GTGCACCTGGA	H193Y	9	1	16, 32, 64, 128	2 x 10 ⁶
3	Kan ^R	ATCTT A TTTCGATCATGCGAA GCTT A GGTGGAGCGCCTATT	Q4* and W15*	6, 5	8	16, 32, 64, 128	5 x 10 ⁶
4	Spect ^R	CAATG A TGACTTCTACAGCG	T89I	6	32	64, 128, 256, 512	5 x 10 ⁶
5	Spect ^R Cam ^R	CAATG A TGACTTCTACAGCG TACGGCGT A GTGCACCTGGA	T89I (spect) H193Y (chlor)	6, 9	32(spect) 1 (chlor)	64, 128, 256, 512 (spect) 16, 32, 64, 128 (chlor)	5 x 10 ⁶
6	Spect ^R	CAATG A TGACTTCTACAGCG	T89I	6, 9	32	128, 256, 384, 512	5 x 10 ⁶
7	Kan ^R	ATCTT A TTTCGATCATGCGAA TTCATT A ACTGTGGCCGGCT	Q4* and D208N	6, 7	8	64, 128, 256, 384	8 x 10 ⁶

Supplementary Table 9. Primers used for mammalian cell genomic DNA and RNA amplification.

Primer name	Sequence
fwd_site 1_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNCCAGCCCCATCTGTCAAAC
rev_site 1_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCCTTGGAAACAATGA
fwd_site 2_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNAGAGACTGATTGCGTGGAGT
rev_site 2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCCAGCCTAGGCAACAA
fwd_site 3_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCCGACAGCCAGTGGTTAAGT
rev_site 3_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTTTCACCGACTGCACAG
fwd_site 4_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCTGCACCTAGCCTCCATGTC
rev_site 4_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTGCACTGAGACCGTGAA
fwd_site 5_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGTCTGAGGTCACACAGTGGG
rev_site 5_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGAGCAGGGACCACATC
fwd_site 6_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
rev_site 6_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTCAACC
fwd_site 7_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGATGCCCTCCATCTTCTCCG
rev_site 7_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGGTTTGCATAGACCTGCC
fwd_site 8_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCCCTGTTCTAAAGCCCACC
rev_site 8_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTACTGGTTCTGTTTGTGGCCA
fwd_site 9_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTTGCTTATTGCTGAGGGGCA
rev_site 9_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTCTCCTCCAGCTGAG
fwd_site 10_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTCCACCTCCCCACTTCTCTT
rev_site 10_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGAAATGAGCAAGGCACA
fwd_site 11_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCCCTAAACCACCTGCAGAGG
rev_site 11_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCCCAGCCACATTCTAT
fwd_site 12_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNACCCATGTGCCTGACATAGG
rev_site 12_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTGATTATGGTTACACAGCG
fwd_site 13_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTCACTTCCAGCCAGGAGTAT
rev_site 13_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTTTCTCTCCCCACCC
fwd_site 14_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGAACCTGAAGCCTTCCCCA
rev_site 14_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTAACCTGTGTGACACTTGGCA
fwd_site 15_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGCAGACACCCACAACCTGTCT
rev_site 15_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCACTCAGCTAGACTTAACTCCC
fwd_site 16_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGGGAGGTGGAGAGAGGATGT
rev_site 16_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGAGGTCTAGGAACCCG
fwd_site 17_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCGCGGGCTGAAGTAGATCAA

rev_site 17_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTCTCTGCTCCTTTGTCCCC
fwd_site 18/19_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGCATTACCTGGGAGCCTGTT
rev_site 18/19_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTAACTTCAGCGGGCATCAGAA
fwd_site HGB1/2_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGTGGAGTTTAGCCAGGGACC
rev_site HGB1/2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTACAGGCCTCACTGGAGCTA
fwd_site HFE_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGGCTGGATAACCTTGGCTGT
rev_site HFE_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCTCAGGCACTCCTCTCAA
fwd_HEK2_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGCCCCATCTGTCAAAT
rev_HEK2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTGAATGGATTCTTGGAAACAATGA
fwd_HEK3_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
rev_HEK3_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAAACTTGTCAACC
fwd_HEK4_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGAACCCAGGTAGCCAGAGAC
rev_HEK4_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCTTCAACCCGAACGGAG
fwd_HEK2_off1_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGTGTGGAGAGTGAGTAAGCCA
rev_HEK2_off1_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGGTAGGATGATTTCAAGCA
fwd_HEK2_off2_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCACAAAGCAGTGTAGCTCAGG
rev_HEK2_off2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTTTTTTGGTACTCGAGTGTATTTCAG
fwd_HEK3_off1_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTCCCCTGTTGACCTGGAGAA
rev_HEK3_off1_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGTACTTGCCTGACCA
fwd_HEK3_off2_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGTGTGACAGGGAGCAA
rev_HEK3_off2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGATGTGGGCAGAAGGG
fwd_HEK3_off3_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGAGGGAACAGAAGGGCT
rev_HEK3_off3_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCAAAGGCCCAAGAACCT
fwd_HEK3_off4_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNCTAGCACTTTGGAAGGTCCG
rev_HEK3_off4_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCATCTTAATCTGCTCAGCC
fwd_HEK3_off5_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNAAGGAGCAGCTCTTCCCTGG
rev_HEK3_off5_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTGCACCATCTCCCACAA
fwd_HEK4_off1_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGGCATGGCTTCTGAGACTCA
rev_HEK4_off1_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCCTTGCCTCCCTGTCTTT
fwd_HEK4_off2_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGCAATGGAGGCATTGG
rev_HEK4_off2_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGAGGCTGCCCATGAGAG
fwd_HEK4_off3_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNGGTCTGAGGCTCGAATCCTG
rev_HEK4_off3_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGGCCTCCATATCCCTG
fwd_HEK4_off4_HTS	ACACTCTTCCCTACACGACGCTCTTCCGATCTNNNNTTCCACCAGAACTCAGCCC
rev_HEK4_off4_HTS	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCGGTTCCCTCCACAACAC

fwd_HEK4_off5_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNCACGGGAAGGACAGGAGAAG
rev_HEK4_off5_HTS	TGGAGTTCAGACGTGTGCTCTCCGATCTGCAGGGGAGGGATAAAGCAG
fwd_site 1_HDR_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNCCAGCCCCATCTGTCAAAC
rev_site 1_HDR_HTS	TGGAGTTCAGACGTGTGCTCTCCGATCTTGAATGGATTCTTGAAACAATGA
fwd_site 2_HDR_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNCCCTGAGATACAGTCACGAGGT
rev_site 2_HDR_HTS	TGGAGTTCAGACGTGTGCTCTCCGATCTCCTGAAATGCTGTGCGTGTCTA
fwd_site 3_HDR_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNGCCACATTACCTTGGTGCATA
rev_site 3_HDR_HTS	TGGAGTTCAGACGTGTGCTCTCCGATCTGGCAGGCAGATTATCATCCCA
fwd_site 4_HDR_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNAAAGTGCTGCGATTACAGGC
rev_site 4_HDR_HTS	TGGAGTTCAGACGTGTGCTCTCCGATCTGTGGCATCCAGAGACATGGT
fwd_site 6_HDR_HTS	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
rev_site 6_HDR_HTS	TGGAGTTCAGACGTGTGCTCTCCGATCTCCCAGCCAAACTTGTCAACC
B_Catenin_mRNA_fwd	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNATTTGATGGAGTTGGACATGGCC
B_Catenin_mRNA_rev	TGGAGTTCAGACGTGTGCTCTCCAGCTACTTGTCTTGAGTGAAGG
B_Actin_mRNA_fwd	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNGACAAAACCTAACTTGCAGAAAACAAGATG
B_Actin_mRNA_rev	TGGAGTTCAGACGTGTGCTCTGCTTTTAGGATGGCAAGGGACTTCCTG
GAPDH_mRNA_fwd	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNGGCTACAGCAACAGGGTGGTGGAC
GAPDH_mRNA_rev	TGGAGTTCAGACGTGTGCTCTCCATCAATAAAGTACCCTGTGCTCAACC
RB1_mRNA_fwd	ACACTCTTCCCTACACGACGCTCTCCGATCTNNNNNGAAGGATTATGATAGGGACAAGG
RB1_mRNA_rev	TGGAGTTCAGACGTGTGCTCTCCACAATTCCTTCATATGTTCAAAC

Supplementary Table 10. 100-mer single-stranded oligonucleotide donor templates (ssODNs) used in HDR experiments.

Target site	Sequence
1	5'-TTTTCCAGCCCGCTGGCCCTGTAAAGGAACTGGAACGCAAAGCATAGACTGCGCGGCGG GCCAGCCTGAATAGCTGCAAACAAGTGCAGAATATCTGAT-3'
2	5'-CATGAAAAAGAGACTGATTGCGTGGAGTTCATGGAGTGTGAGGCATAGACTGCACGAGACA TAAACCATGACTTGCAGATGAAGAAGCATTTTAAAAGT-3'
3	5'-GACAGCCAGTGGTTAAGTCAGAACCCGACTCAGGTCAGGAAAGCAGAGACTGCCCGGGGT TGGGAAGGCGGTGAACTCAGAGATAGAAACAGGGTGGGTG-3'
4	5'-ATTTTAAGCTGTAGTATTATGAAGGGAAATCTGGAGCGAAGAGAATAGACTGTACGGAAACC AGTTAAGAAATAGGACATGGAGGCTAGGTGCAGTGGCT-3'
6	5'-CCTCTGCCATCACGTGCTCAGTCTGGGCCCAAGGATTGGCCCAGGCCAGGGCTCGAGAA GCAGAAAAAAGCATCAAGCCTACAAATGCATGCTTACTT-3'

Supplementary Sequences 1. DNA sequences of adenine deaminases used in this study.

Bacterial codon-optimized ecTadA (wild-type):

ATGTCTGAAGTCAATTTAGCCACGAATACTGGATGCGTCACGCGCTGACGCTGGCGAAACGTGCCTGGGATGAGC
 GGGAAGTGCCGGTCGGCGCGGTATTAGTGCATAACAATCGGGTAATCGGCGAAGGCTGGAACCGCCCGATTGGTC
 GCCATGATCCCACCGCACATGCAGAAATCATGGCCCTGCGGCAGGGTGGTCTGGTATGCAAATTATCGTCTGATC
 GACGCCACGTTGTATGTCACGCTTGAACCATGTGTAATGTGTGCCGGAGCGATGATCCACAGTCGCATTGGTCGCGT
 GGTCTTTGGTGC GCGTGACGCGAAAACCTGGCGCTGCGGGATCTTTAATGGATGTGCTGCATCATCCGGGTATGAATC
 ACCGAGTGGAATTACGGAAGGAATACTGGCGGATGAGTGCGCGGCGTTGCTCAGTGACTTCTTTTCGCATGCGCCG
 CCAGGAAATTAAGCGCAGAAAAAAGCGCAATCCTCGACGGAT

Mammalian codon-optimized ecTadA (wild-type):

ATGTCCGAAGTTCGAGTTTTCCCATGAGTACTGGATGAGACACGCATTGACTCTCGCAAAGAGGGCTTGGGATGAACG
 CGAGGTGCCCGTGGGGGCGAGTACTCGTGCATAACAATCGCGTAATCGGCGAAGGTTGGAATAGGCCGATCGGACG
 CCACGACCCCACTGCACATGCGGAAATCATGGCCCTTCGACAGGGAGGGCTTGTGATGCAGAATTATCGACTTATCG
 ATGCGACGCTGTACGTCACGCTTGAACCTTGCGTAATGTGCGCGGGAGCTATGATCACTCCCGCATTGGACGAGTT
 GTATTCGGTGGCCGCGACGCCAAGACGGGTGCCGACGTTCACTGATGGACGTGCTGCATCACCCAGGCATGAACC
 ACCGGGTAGAAATCACAGAAGGCATATTGGCGGACGAATGTGCGGCGCTGTTGTCCGACTTTTTTCGCATGCGGAG
 GCAGGAGATCAAGGCCCGAGAAAAAAGCACAATCCTCTACTGAC

Mammalian codon-optimized mADA:

ATGGCCCAGACACCCGCATTCAACAAACCCAAAGTAGAGTTACACGTCCACCTGGATGGAGCCATCAAGCCAGAAAC
 CATCTTATACTTTGGCAAGAAGAGAGGCATCGCCCTCCCGGCAGATACAGTGGAGGAGCTGCGCAACATTATCGGCA
 TGGACAAGCCCTCTCGCTCCCAGGCTTCTGGCCAAGTTTACTACTACATGCCTGTGATTGCGGGCTGCAGAGAG
 GCCATCAAGAGGATCGCCTACGAGTTTGTGGAGATGAAGGCAAAGGAGGGCGTGGTCTATGTGGAAGTGC GCTATA
 GCCACACCTGCTGGCCAATTCCAAGGTGGACCCAATGCCCTGGAACCAGACTGAAGGGGACGTCACCCCTGATGA
 CGTTGTGGATCTTGTGAACCAGGGCCTGCAGGAGGGAGAGCAAGCATTGGCATCAAGGTCCGGTCCATTCTGTGC
 TGCATGCGCCACCAGCCAGCTGGTCCCTTGGAGTGTGGAGCTGTGTAAGAAGTACAATCAGAAGACCGTGGTGG
 CTATGGACTTGGCTGGGGATGAGACCATTGAAGGAAGTAGCCTCTTCCAGGCCACGTGGAAGCCTATGAGGGCGC
 AGTAAAGAATGGCATTTCATCGGACCGTCCACGCTGGCGAGGTGGGCTCTCCTGAGGTTGTGCGTGAGGCTGTGGAC
 ATCCTCAAGACAGAGAGGGTGGGACATGGTTATCACACCATCGAGGATGAAGCTCTCTACAACAGACTACTGAAAGA
 AAACATGCACTTTGAGGTCTGCCCTGTCCAGCTACCTCACAGGCGCCTGGGATCCCAAAACGACGCATGCGGTT
 GTTCGCTTCAAGAATGATAAGGCCAACTACTCACTCAACACAGACGACCCCTCATCTTCAAGTCCACCCTAGACACT
 GACTACCAGATGACCAAGAAAGACATGGGCTTCACTGAGGAGGAGTTCAAGCGACTGAACATCAACGCAGCGAAGT
 CAAGCTTCTCCAGAGGAAGAGAAGAAGGAACCTTCTGGAACGGCTCTACAGAGAATACCAA

Mammalian codon optimized hADAR2 (catalytic domain):

ATGCATCTCGATCAAACCCCGAGCCGCAACCAATCCCGAGTGAAGGCCTGCAACTGCATCTGCCACAAGTTCTGGC
 GGATGCCGTTAGCCGCTGGTCTTGGGTAAGTTCCGGTATCTGACAGACAACTTTTCTAGTCCACATGCTCGCCGTA
 AGGTGCTGGCTGGCGTTGTGATGACCACAGGTACAGACGTCAAAGATGCTAAAGTGATTTCTGTGTCTACTGGCACG
 AAGTGCATTAACGGCGAATATATGTCTGACCGTGGCTTAGCGCTTAACGATTGTCATGCCGAAATCATCTCCCGTCGT
 TCATTGCTTCGCTTCTGTACACGCAGTTGGAAGTGTATCTGAATAACAAGACGATCAGAAGCGTTCTATTTTCCAG
 AAGTCTGAGCGCGGCGGGTTCGGTCTTAAAGAGAATGTGCAGTTTACCTTTATATTTCAACCTCTCCTTGTGGTGT
 GCCCGTATTTTTTACCACACGAACCTATTTTAGAGGAACCGGCCGATCGTCATCCGAACCGCAAAGCCCGTGGGCA
 GCTGCGTACGAAAATCGAATCAGGTGAAGGCACCATTCCCGTCCGCTCCAATGCGAGCATTCAAACGTGGGACGGT
 GTTTACAGGGCGAACGCCTGTTAACCATGAGCTGCTCAGACAAAATTGCACGTTGGAACGTGGTAGGCATCCAGG
 GCTCGTTATTGAGCATTTCGTGGAGCCGATTTATTTTAGTTCCATCATTTCGGGCTCACTCTACCACGGCGATCACCT
 TAGCCGCGGATGTACCAGCGCATTAGTAACATCGAAGATTTACCGCCCTGTATACCCTGAACAAACCACTGTAA
 GCGGTATTTCTAACCGGAGGCGCGTACGCTGGTAAAGCCCCGAACTTCAGTGTGAACTGGACTGTGGGTGATTC
 TGCAATTGAGGTAATTAACGCGACGACGGGTAAAGATGAACTGGGCCGTGCCTCTCGTCTGTGTAACACGCGCTGT
 ACTGTCGTTGGATGCGCGTGCACGGTAAAGTTCCAGTCATCTGTTACGTAGCAAGATCACCAGCCAAATGTCTAC
 CACGAATCGAAGCTGGCCGCGAAAAGAATACCAAGCGGCTAAGGCGCGTCTGTTACCGCCTTTATTAAGGCTGGCTT
 AGGGGCCTGGGTGGAAAAACCAACCGAGCAAGATCAATTCAGTCTGACCCCG

Mammalian codon optimized hADAT2:

ATGGAGGCGAAGGCGGCACCCAAGCCAGCTGCAAGCGGCGCGTGTCTGGTGTGCGCAGAGGAGACCGAAAAGTG
 GATGGAGGAGGCGATGCACATGGCCAAAGAAGCCCTCGAAAATACTGAAGTTCCTGTTGGCTGTCTTATGGTCTACA
 ACAATGAAGTTGTAGGGAAGGGGAGAAATGAAGTTAACCAAAACCAAAAATGCTACTCGACATGCAGAAATGGTGGCC
 ATCGATCAGGTCCTCGATTGGTGTCTGTCAAAGTGGCAAGAGTCCCTCTGAAGTATTTGAACACACTGTGTTGTATGTC
 ACTGTGGAGCCGTGCATTATGTGTGCAGCTGCTCTCCGCTGATGAAAATCCCGCTGGTTGTATATGGCTGTCAGAA
 TGAACGATTTGGTGGTGTGGCTCTGTTCTAAATATTGCCTCTGCTGACCTACCAAACTGGGAGACCATTTCAGTG

TATCCCTGGATATCGGGCTGAGGAAGCAGTGGAAATGTTAAAGACCTTCTACAAACAAGAAAATCCAAATGCACCAA
ATCGAAAGTTCGGAAAAAGGAATGTCAGAAATCT

Supplementary Sequences 2. DNA sequences of antibiotic resistance genes used in this study. Inactivating mutations are shown in red.

Chloramphenicol resistance gene (Cam^R) H193Y:

ATGAGAGAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGT
CAGTTGCTCAATGTACCTATAACCAGACCGTTTACGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGC
ACAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCTGATGAATGCTCATCCGGAGTTCCGTATGGCAATGAAAG
ACGGTGAGCTGGTATATGGGATAGTGTTCACCCTTGTACACCGTTTTCCATGAGCAAACGAAACGTTTTTCATCGC
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTTCGCAAGATGTGGCGTGTACGGTGAAAAC
TGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTTGA
TTTTAACGTGGCCAATATGGACAACCTTTCGCCCCCGTTTTCACTATGGGCAAATATTATACGCAAGGCGACAAGGT
GCTGATGCCGCTGGCCATCCAGGTGCAC^TACGCCGATGCGACGGCTTCCATGTCGGCAGAATGCTTAATGAATTA
CAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAA

Kanamycin resistance gene (Kan^R) Q4STOP and W15STOP:

ATGATCGAA^TAAGATGGATTGCACGCAGGTTCTCCGGCCGCTT^AGGTGGAGCGCCTATTCGGCTATGACTGGGCAC
AACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGTTCTTTTTGTCAAGAC
CGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCC
TTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGA
TCTCCTGTCATCTCACCTTGTCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTG
ATCCGGCTACCTGCCATTTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTA^TCGGATGGAAGCCGGTCT
TGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGA^ACTGTTCCGCCAGGCTCAAGGCGCG
CATGCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGC
TTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATAT
TGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGACGCGC
ATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTAA

Spectinomycin resistance gene (Spect^R) T89I:

ATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAACTATCAGAGGTAGTTGGCGTCATCGAGCGCCATCTCGAAC
CGACGTTGCTGGCCGTACATTTGTACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGATATTGATTTGCTG
GTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTTTGATCAACGACCTTTTGGAAACTTCGGCTTCCCC
TGGAGAGAGCGAGATTCTCCGCGCTGTAGAAGTCA^TCATTGTTGTGCACGACGACATCATTCCGTGGCGTTATCCAG
CTAAGCGCAACTGCAATTTGGAGAATGGCAGCGCAATGACATTCTTGAGGTATCTTCGAGCCAGCCACGATCGAC
ATTGATCTGGCTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGA^ACTCTT
TGATCCGGTTCCTGAACAGGATCTATTTGAGGCGCTAAATGAAACCTAACGCTATGGA^ACTCGCCGCCGACTGGG
CTGGCGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTGGTACAGCGCAGTAACCGGCAAATCGCGCCGAA
GGATGTGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGCT
TATCTTGGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAAAGGCGA
GATACCAAGGTAGTCGGCAAATAA

Kanamycin resistance gene (Kan^R) Q4STOP and D208N:

ATGATCGAA^TAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCAC
AACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGTTCTTTTTGTCAAGAC
CGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCC
TTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTAT AGCCGGCCACAGTTAATGAA
TGGGCGAAGTGCCGGGGCAGGATCTCCTGTATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCA
ATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTTCGACCACCAAGCGAAACATCGCATCGAGCGAGCAC
GTA^TCTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGA^ACT
GTTCCGCCAGGCTCAAGGCGCGCATGCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGGCGAA
TATCATGGTGGAAAATGGCCGCTTTTTCTGGATTCATT^AACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGAC
ATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTAT
CGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTAA

Supplementary Sequences 3. Amino acid sequences of late-stage ABEs developed in this study.

Color coding is as follows:

green = ecTadA (wt), monomer 1 of 2

orange = linker

black + red = evolved ecTadA* internal monomer 2 of 2, with mutations highlighted in red

blue = Cas9 nickase (D10A mutation underlined)

purple = NLS

ABE6.3 (ecTadA(wt)–linker(32 aa)–ecTadA*(6.3)–linker(32 aa)–Cas9 nickase–NLS):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDAT
 LYVTLPEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAKQ
 KQSSSTDSSGSSGGSSGSETPGTSESATPESSGGSSGGSSSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVNNRV
 IGEGWNR**SIGL**HDPTAHAEIMALRQGGLVMQNYRLIDATLYVT**F**EPCVMCAGAMIHSRIGRVVFG**V**RNAKTGAAGSLMDV
 LH**Y**PGMNHRVEITEGILADECAALL**CY**FFRMRRQ**V**FNAQKKAQSSSTD**SSGSSGGSSGSETPGTSESATPESSGGSSGGSS**
 DKKYSIGLAIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQE
 IFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGH
 FLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPN
 FKSNDLAEDAQLQSKDQYDADDLNDLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASMIKRYDEHHQDLTLL
 KALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
 ELHAILRRQEDFYPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMWTRKSEETITPWNFEVVVDKGASAQSFIERMTNFD
 KNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
 VEDRFNASLGTYHLLKIKDKDFLDNEENEDILEDIVLTLTFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRK
 LINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELV
 KVMGRHKPENIVEMARENQTTQKGQKNSRERMKRIEIGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
 DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLS
 ELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKREINNYHHAHDAYLNAV
 VGTALIKKYPKLESEFVYGDYKVDYVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDK
 GRDFATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKPYGGFDSPTVAYSVLVAKVEKGKSKKL
 KSVKELLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLASH
 YEKLKGPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHRDKPIREQAENIIHLFTLNLGAPAAFK
 YFDTTIDRKRYTSTKEVLDATLIHQISITGLYETRIDLSQLGGD**SSGSSPKKKRKV***

ABE7.8 (ecTadA(wt)–linker(32 aa)–ecTadA*(7.8)–linker(32 aa)–Cas9 nickase–NLS):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDAT
 LYVTLPEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAKQ
 KQSSSTDSSGSSGGSSGSETPGTSESATPESSGGSSGGSSSEVEFSHEYWMRHALTLAKRALDEREVPVGAVLVNNRVI
 GEGWNR**AIGL**HDPTAHAEIMALRQGGLVMQNYRLIDATLYVT**F**EPCVMCAGAMIHSRIGRVVFG**V**RNAKTGAAGSLMDVL
 H**Y**PGMNHRVEITEGILADECA**N**ALL**CY**FFRMRRQ**V**FNAQKKAQSSSTD**SSGSSGGSSGSETPGTSESATPESSGGSSGGSSD**
 KKYSIGLAIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI
 FSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHF
 LIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNF
 KSNFDLAEDAQLQSKDQYDADDLNDLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLK
 ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
 HAILRRQEDFYPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMWTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKN
 LPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE
 DRFNASLGTYHLLKIKDKDFLDNEENEDILEDIVLTLTFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLI
 NGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKV
 MGRHKPENIVEMARENQTTQKGQKNSRERMKRIEIGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSYDVIDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELD
 KAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKREINNYHHAHDAYLNAVVT
 ALIKKYPKLESEFVYGDYKVDYVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD
 FATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKPYGGFDSPTVAYSVLVAKVEKGKSKKLKSV
 KELLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLASHYEK
 LKGPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHRDKPIREQAENIIHLFTLNLGAPAAFKYFD
 TTIDRKRYTSTKEVLDATLIHQISITGLYETRIDLSQLGGD**SSGSSPKKKRKV***

ABE7.9 (ecTadA(wt)–linker(32 aa)–ecTadA*(7.9)–linker(32 aa)–Cas9 nickase–NLS):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDAT
 LYVTLPEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAKQ
 KQSSSTDSSGSSGGSSGSETPGTSESATPESSGGSSGGSSSEVEFSHEYWMRHALTLAKRALDEREVPVGAVLVNNRVI

GEGWNR**AIGL**HDPTAHAEIMALRQGGLVMQNYRLIDATLYVT**F**EPCVMCAGAMIHSRIGRVVFG**V**RNAKTGAAGSLMDVL
 HYPGMNHRVEITEGILADECN**ALL**CYFFRM**PRQ**V**FNA**QKKAQSSTD**SGGSSGGSSGSETPGTSESATP**ESSGGSSGGSD
 KKYSIGL**A**IGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI
 FSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHF
 LIEGDLNPDNSDVDFKLIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNF
 KSNFDLAEDAKLQLSKDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLK
 ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
 HAILRRQEDFYFPLKDNREKIEKILTRIPYVVGPLARGNSRFAMWTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDFKN
 LPNEKVLPHKSHLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE
 DRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLI
 NGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKV
 MGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDIN
 RLSYDQVDHIVPQSFLKDDSIDNKVLTRSDKNRKGSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLELD
 KAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVINNYHHAHDAYLNAVVG
 ALIKKYPKLESEFVYGDYKVDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKPLIETNGETGEIVWDKGRD
 FATVRKVL SMPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGSKLLKSV
 KELLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIILPKYSLFELENRKRMLASAGELQKGNELALPSKYVNFYLYASHYEK
 LKGSPEDEQKQLFVEQHKHYLDEIEQISEFSKRVILADANLDKVL SAYNKHRDKPIREQAENIIHLFTLNLGAPAAFKYFD
 TTIDRKRYTSTKEVLDATLIHQSIITGLYETRIDLSQLGGD**SGGSPKKRKV***

ABE7.10 (ecTadA(wt)–linker(32 aa)–ecTadA*(7.10)–linker(32 aa)–Cas9 nickase–NLS):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGA^LLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDAT
 LYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAKQ
 KAQSSTD**SGGSSGGSSGSETPGTSESATP**ESSGGSSGGSSSEVEFSHEYWMRHALTLAKR**R**DEREVPVGA^LLV**L**NNRVI
 GEGWNR**AIGL**HDPTAHAEIMALRQGGLVMQNYRLIDATLYVT**F**EPCVMCAGAMIHSRIGRVVFG**V**RNAKTGAAGSLMDVL
 HYPGMNHRVEITEGILADECA**ALL**CYFFRM**PRQ**V**FNA**QKKAQSSTD**SGGSSGGSSGSETPGTSESATP**ESSGGSSGGSD
 KKYSIGL**A**IGTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI
 FSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHF
 LIEGDLNPDNSDVDFKLIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNF
 KSNFDLAEDAKLQLSKDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLK
 ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
 HAILRRQEDFYFPLKDNREKIEKILTRIPYVVGPLARGNSRFAMWTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDFKN
 LPNEKVLPHKSHLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE
 DRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLI
 NGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKV
 MGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDIN
 RLSYDQVDHIVPQSFLKDDSIDNKVLTRSDKNRKGSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLELD
 KAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVINNYHHAHDAYLNAVVG
 ALIKKYPKLESEFVYGDYKVDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKPLIETNGETGEIVWDKGRD
 FATVRKVL SMPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGSKLLKSV
 KELLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIILPKYSLFELENRKRMLASAGELQKGNELALPSKYVNFYLYASHYEK
 LKGSPEDEQKQLFVEQHKHYLDEIEQISEFSKRVILADANLDKVL SAYNKHRDKPIREQAENIIHLFTLNLGAPAAFKYFD
 TTIDRKRYTSTKEVLDATLIHQSIITGLYETRIDLSQLGGD**SGGSPKKRKV***

Supplementary Note 1. Matlab script for base calling.

```

function basecall(WTnuc, directory)
%cycle through fastq files for different samples
cd directory
files=dir('*.fastq');
for d=1:2
    filename=files(d).name;
    %read fastq file
    [header,seqs,qscore] = fastqread(filename);
    seqsLength = length(seqs);           % number of sequences
    seqsFile = strrep(filename, '.fastq', ''); % trims off .fastq
    %create a directory with the same name as fastq file
    if exist(seqsFile, 'dir');
        error('Directory already exists. Please rename or move it before moving on.');
```

end

```

mkdir(seqsFile); % make directory
wtLength = length(WTnuc); % length of wildtype sequence
%% aligning back to the wildtype nucleotide sequence
%
% ALN is a matrix of the nucleotide alignment
window=1:wtLength;
sBLength = length(seqs); % number of sequences
% counts number of skips
nSkips = 0;
ALN= repmat(' ', [sBLength wtLength]);
% iterate through each sequencing read
for i = 1:sBLength
    %If you only have forward read fastq files leave as is
    %If you have R1 forward and R2 is reverse fastq files uncomment the
    %next four lines of code and the subsequent end statement
    %
    if mod(d,2)==0;
    %
        reverse = seqrcomplement(seqs{i});
    %
        [score,alignment,start] = swalign(reverse,WTnuc, 'Alphabet', 'NT');
```

else

```

    [score,alignment,start] = swalign(seqs{i},WTnuc, 'Alphabet', 'NT');
```

end

```

% length of the sequencing read
len = length(alignment(3,:));
% if there is a gap in the alignment , skip = 1 and we will
% throw away the entire read
skip = 0;
for j = 1:len
    if (alignment(3,j) == '-' || alignment(1,j) == '-')
        skip = 1;
        break;
    end
    %in addition if the qscore for any given base in the read is
    %below 31 the nucleotide is turned into an N (fastq qscores that are not
letters)
    if isletter(qscore{i}(start(1)+j-1))
    else
        alignment(1,j) = 'N';
    end
end
end
if skip == 0 && len>10
    ALN(i, start(2):(start(2)+length(alignment)-1))=alignment(1,:);
end
end
% with the alignment matrices we can simply tally up the occurrences of
```

```

% each nucleotide at each column in the alignment these
% tallies ignore bases annotated as N
% due to low qscores
TallyNTD=zeros(5,wtLength);
FreqNTD=zeros(4,wtLength);
SUM=zeros(1,wtLength);
for i=1:wtLength

TallyNTD(:,i)=[sum(ALN(:,i)=='A'),sum(ALN(:,i)=='C'),sum(ALN(:,i)=='G'),sum(ALN(:,i)=='T'
),sum(ALN(:,i)=='N')];
end

for i=1:wtLength
    FreqNTD(:,i)=100*TallyNTD(1:4,i)/sum(TallyNTD(1:4,i));
end
for i=1:wtLength
    SUM(:,i)=sum(TallyNTD(1:4,i));
end

% we then save these tally matrices in the respective folder for
% further processing

save(strcat(seqsFile, '/TallyNTD'), 'TallyNTD');
dlmwrite(strcat(seqsFile, '/TallyNTD.csv'), TallyNTD, 'precision', '%.3f', 'newline',
'pc');
save(strcat(seqsFile, '/FreqNTD'), 'FreqNTD');
dlmwrite(strcat(seqsFile, '/FreqNTD.csv'), FreqNTD, 'precision', '%.3f', 'newline',
'pc');
fid = fopen('FrequencySummary.csv', 'a');
fprintf(fid, '\n \n');
fprintf(fid, filename);
fprintf(fid, '\n \n');
dlmwrite('FrequencySummary.csv', FreqNTD, 'precision', '%.3f', 'newline', 'pc', '-
append');
dlmwrite('FrequencySummary.csv', SUM, 'precision', '%.3f', 'newline', 'pc', '-
append');
end

% set up queue of basecalling runs

% change directory to folder of fastq files for a given target site
cd('/Users/michaelpacker/Documents/MATLAB/BaseCallingWithSummary')
cd PUTFOLDERNAMEHERE
% call upon the basecall program
basecall(PUTWTSEQUENCEHERE)
% and repeat
cd('/Users/michaelpacker/Documents/MATLAB/BaseCallingWithSummary')
cd PUTFOLDERNAMEHERE
basecall(PUTWTSEQUENCEHERE)
% and repeat...

```

Supplementary Note 2. Matlab script for indel analysis.

```

%WTnuc='CGGTGGGAGGTCTATATAAGCAGAGCTGGTTTTAGTGAACCGTCAGATCCGCTAGAGATCCGCGGCCGCTAATACGACTCAC
CCTAGGGAGAGCCGCCACCGTGGTGAGCAAGGGCGAGGAGCTGTTTACCAGGGGTGGTGCCCATCCTGGTTCGAGCTGGACGGCGACGTAA
ACGGCCACAAGTTTACGCGTGTCCGGCGAG';
%cycle through fastq files for different samples
files=dir('*.fastq');
indelstart=55;
width=30;
flank=10;

for d=1:2
    filename=files(d).name;
    %read fastq file
    [header,seqs,qscore] = fastqread(filename);
    seqsLength = length(seqs);           % number of sequences
    seqsFile = strcat(strrep(filename, '.fastq', ''), '_INDELS');           % trims off .fastq
    %create a directory with the same name as fastq file+_INDELS
    if exist(seqsFile, 'dir');
        error('Directory already exists. Please rename or move it before moving on.');
```

```

    end
    mkdir(seqsFile);                    % make directory
    wtLength = length(WTnuc);           % length of wildtype sequence
    sBLength = length(seqs);            % number of sequences

    % initialize counters and cell arrays
    nSkips = 0;
    notINDEL=0;
    ins={};
    dels={};
    NumIns=0;
    NumDels=0;
    % iterate through each sequencing read
    for i = 1:sBLength
        %search for 10BP sequences that should flank both sides of the "INDEL WINDOW"
        windowstart=strfind(seqs{i},WTnuc(indelstart-flank:indelstart));
        windowend=strfind(seqs{i},WTnuc(indelstart+width:indelstart+width+flank));
        %if these flanks are found and more than half of base calls
        %are above Q31 THEN proceed OTHERWISE save as a skip
        if length(windowstart)==1 && length(windowend)==1 &&
            (sum(isletter(qscore{i}))/length(qscore{i}))>=0.5
            %if the sequence length matches the INDEL window length save as
            %not INDEL
            if windowend-windowstart==width+flank
                notINDEL=notINDEL+1;
            %if the sequence is ONE or more bases longer than the INDEL
            %window length save as an Insertion
            elseif windowend-windowstart>=width+flank+1
                NumIns=NumIns+1;
                ins{NumIns}=seqs{i};
            %if the sequence is ONE or more bases shorter than the INDEL
            %window length save as a Deletion
            elseif windowend-windowstart<=width+flank-1
                NumDels=NumDels+1;
                dels{NumDels}=seqs{i};
            end
            %keep track of skipped sequences that do not possess matching flank
            %sequences and do not pass quality cutoff
            else
                nSkips=nSkips+1;
            end
        end
    end
end

```

```
end
INDELrate=(NumIns+NumDels)/(NumIns+NumDels+notINDEL)*100.;
FID = fopen('INDELSummary.csv', 'a');
fprintf(FID, '\n \n');
fprintf(FID, filename);
fprintf(FID, '\n');
fprintf(FID, num2str(INDELrate));

fid=fopen(strcat(seqsFile, '/summary.txt'), 'wt');
fprintf(fid, 'Skipped reads %i\n not INDEL %i\n Insertions %i\n Deletions %i\n INDEL
percent %e\n', [nSkips, notINDEL, NumIns, NumDels, INDELrate]);
fclose(fid);
save(strcat(seqsFile, '/nSkips'), 'nSkips');
save(strcat(seqsFile, '/notINDEL'), 'notINDEL');
save(strcat(seqsFile, '/NumIns'), 'NumIns');
save(strcat(seqsFile, '/NumDels'), 'NumDels');
save(strcat(seqsFile, '/INDELrate'), 'INDELrate');
save(strcat(seqsFile, '/dels'), 'dels');
C = dels;
fid = fopen(strcat(seqsFile, '/dels.txt'), 'wt');
fprintf(fid, "%s\n", C{:});
fclose(fid);
save(strcat(seqsFile, '/ins'), 'ins');
C = ins;
fid = fopen(strcat(seqsFile, '/ins.txt'), 'wt');
fprintf(fid, "%s\n", C{:});
fclose(fid);
```

Supplementary Note 3. Python script for analysis of *HBG1* and *HBG2* base editing and indels.

```

%matplotlib inline
import numpy as np
import scipy as sp
import matplotlib as mpl
import matplotlib.cm as cm
import matplotlib.pyplot as plt
import pandas as pd
pd.set_option('display.width', 500)
pd.set_option('display.max_columns', 100)
pd.set_option('display.notebook_repr_html', True)
import seaborn as sns
sns.set_style("whitegrid")
sns.set_context("poster")
import requests
import time
from bs4 import BeautifulSoup
import regex
import re
import os
from Bio import SeqIO
import Bio
from Bio import motifs
from Bio import pairwise2
from Bio.pairwise2 import format_alignment
from Bio.Alphabet import IUPAC
from sklearn import preprocessing

basecall analysis with 50% Q31 cutoff on protospacer region (as defined by flanks)
#includes a check for match with two HBG1 SNPs
#inputs:
#directory, working directory folder containing all fastq files
#site, genomic site name as it appears in the fastq filenames
#orientation, 'FWD' if you want output in the same direction as the sequencing read or
'REV' if you want reverse complement output,
#flank1, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#flank2, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#width, expected bp length of basecalling window
#
#outputs:
#'_counts.csv', all base editing product sequences with corresponding number of
occurrences
#'_rawsummary.csv', summarizes base call counts for all samples
#'_normalizedsummary.csv', summarizes base call percentages for all samples
def basecallhbg1(directory, site, orientation, flank1, flank2, width):
    indir=directory
    outdir=directory
    filenames=os.listdir(indir)
    for i in range(len(filenames)):
        seqs={}
        if (filenames[i][-5:]=='fastq') and (site in filenames[i]):
            for record in SeqIO.parse(indir+filenames[i], "fastq") :
                recordqual=[x>31 for x in record.letter_annotations['phred_quality']]
                #only process reads that have more than half of basecalls >Q31 and
                contain two HBG1 specific SNPs at 3' end of read
                if (record.seq.find('GTTTTTCTCTAATTTATTCTTCCCTTTAGCTAGTTTC')>0) and
                (float(sum(recordqual))/float(len(recordqual))>=.5):
                    recordseq="".join([y if x else 'N' for (x,y) in zip(recordqual,
                    record.seq)])

```



```

recordseq="" .join([y if x else 'N' for (x,y) in zip(recordqual,
record.seq)])

#split prior to spacer window
split1=recordseq.split(flank1)
if len(split1)==2:
    #take second item in first split
    #split again at the sequence right after the protospacer and take
first item
    split2=split1[1].split(flank2)[0]
    #keep only entries with exact width
    if (len(split2)==width):
        if orientation=='FWD':
            seqs[record.id]=split2
        elif orientation=='REV':
            seqs[record.id]=Bio.Seq.reverse_complement(split2)
    frame=pd.DataFrame({'Spacer':seqs.values()}, index=seqs.keys())
    Motif=motifs.create(frame.Spacer.values, alphabet=IUPAC.IUPACAmbiguousDNA())
    raw=pd.DataFrame(Motif.counts, index=[str(s+1) for s in
range(width)])[['A','C','G','T','N']].transpose()
    normalized=pd.DataFrame(Motif.counts, index=[str(s+1) for s in
range(width)])[['A','C','G','T']].transpose()
    normalized=normalized/normalized.sum(axis=0)*100.
    normalized=normalized.round(2)
    Counts=pd.DataFrame(seqs.items(), columns=['ID','Window'])
    Counts=Counts[['N' not in x for x in Counts.Window]]
    Counts=Counts.groupby('Window').count().sort_values('ID', ascending=False)
    Counts.to_csv(outdir+filenames[i].strip('.fastq')+'_hbgl.csv')
    fd=open(directory+site+'_normalizedsummary_hbgl.csv','a')
    fd.write('\n'+filenames[i]+'\n')
    normalized.to_csv(fd)
    fd.close()
    fd=open(directory+site+'_rawsummary_hbgl.csv','a')
    fd.write('\n'+filenames[i]+'\n')
    raw.to_csv(fd)
    fd.close()

return

#basecall analysis with 50% Q31 cutoff on protospacer region (as defined by flanks)
#includes a check for match with two HBG2 SNPs
#inputs:
#directory, working directory folder containing all fastq files
#site, genomic site name as it appears in the fastq filenames
#orientation, 'FWD' if you want output in the same direction as the sequencing read or
'REV' if you want reverse complement output,
#flank1, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#flank2, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#width, expected bp length of basecalling window
#
#outputs:
#'_counts.csv', all base editing product sequences with corresponding number of
occurrences
#'_rawsummary.csv', summarizes base call counts for all samples
#'_normalizedsummary.csv', summarizes base call percentages for all samples
def basecallhb2(directory, site, orientation, flank1, flank2, width):
    indir=directory
    outdir=directory
    filenames=os.listdir(indir)
    for i in range(len(filenames)):
        seqs={}
        if (filenames[i][-5:]=='fastq') and (site in filenames[i]):

```

```

for record in SeqIO.parse(indir+filenames[i], "fastq") :
    recordqual=[x>31 for x in record.letter_annotations['phred_quality']]
    #only process reads that have more than half of basecalls >Q31 and
contain two HBG2 specific SNPs at 3' end of read
    if (record.seq.find('ATTTTCTCTAATTTATTCTTCCCTTTAGCTAGTTTT')>0) and
(float(sum(recordqual))/float(len(recordqual))>=.5):
        recordseq="".join([y if x else 'N' for (x,y) in zip(recordqual,
record.seq)])

        #split prior to spacer window
        split1=recordseq.split(flank1)
        if len(split1)==2:
            #take second item in first split
            #split again at the sequence right after the protospacer and take
first item

            split2=split1[1].split(flank2)[0]
            #keep only entries with exact width
            if (len(split2)==width):
                if orientation=='FWD':
                    seqs[record.id]=split2
                elif orientation=='REV':
                    seqs[record.id]=Bio.Seq.reverse_complement(split2)
            frame=pd.DataFrame({'Spacer':seqs.values()}, index=seqs.keys())
            Motif=motifs.create(frame.Spacer.values, alphabet=IUPAC.IUPACAmbiguousDNA())
            raw=pd.DataFrame(Motif.counts, index=[str(s+1) for s in
range(width)])[['A','C','G','T','N']].transpose()
            normalized=pd.DataFrame(Motif.counts, index=[str(s+1) for s in
range(width)])[['A','C','G','T']].transpose()
            normalized=normalized/normalized.sum(axis=0)*100.
            normalized=normalized.round(2)
            Counts=pd.DataFrame(seqs.items(), columns=['ID','Window'])
            Counts=Counts[['N' not in x for x in Counts.Window]]
            Counts=Counts.groupby('Window').count().sort_values('ID', ascending=False)
            Counts.to_csv(outdir+filenames[i].strip('.fastq')+'_hbg2.csv')
            fd=open(directory+site+'_normalizedsummary_hbg2.csv','a')
            fd.write('\n'+filenames[i]+'\n')
            normalized.to_csv(fd)
            fd.close()
            fd=open(directory+site+'_rawsummary_hbg2.csv','a')
            fd.write('\n'+filenames[i]+'\n')
            raw.to_csv(fd)
            fd.close()

        return

#indel analysis
#includes a check for match with two HBG1 SNPs
#inputs:
#directory, working directory folder containing all fastq files
#site, genomic site name as it appears in the fastq filenames
#orientation, 'FWD' if you want output in the same direction as the sequencing read or
'REV' if you want reverse complement output,
#flank1, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#flank2, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#width, expected bp length of basecalling window
#ouputs:
#"_Insertions_hbg1.csv", sequences of all insertion reads
#"_deletions_hbg1.csv", sequences of all deletion reads
#'indelsummary_hbg1.csv', contains all indel stats for all fastq files
def indelshbg1(directory, site, flank1, flank2, width):
    indir=directory
    outdir=directory

```

```

filenames=os.listdir(indir)
for i in range(len(filenames)):
    seqs={}
    if (filenames[i][-5:]=='fastq') and (site in filenames[i]):
        skips=0
        ins=0
        insertions=[]
        dels=0
        deletions=[]
        notindel=0
        for record in SeqIO.parse(indir+filenames[i], "fastq") :
            recordqual=[x>31 for x in record.letter_annotations['phred_quality']]
            #only process reads that have more than half of basecalls >Q31 and
            contain two HBG1 specific SNPs at 3' end of read
            if (record.seq.find('GTTTTTCTCTAATTTATTCTTCCCTTTAGCTAGTTTC')>0) and
            (float(sum(recordqual))/float(len(recordqual))>=.5):
                #split prior to indel window
                split1=record.seq.split(flank1)
                if len(split1)==2:
                    #take second item in first split
                    #split again at the sequence right after the indel window
                    if len(split1[1].split(flank2))==2:
                        split2=split1[1].split(flank2)[0]
                    #if INDEL window is +1 add to Insertions
                    if (len(split2)>=width+1):
                        ins=ins+1
                        insertions.append(split2)
                    #if INDEL window is -1 add to Deletions
                    if (len(split2)<=width-1):
                        dels=dels+1
                        deletions.append(split2)
                    if len(split2)==width:
                        notindel=notindel+1
                else:
                    skips=skips+1
            else:
                skips=skips+1
        else:
            skips=skips+1
        fd=open(directory+'indelsummary_hbg1.csv','a')
        fd.write('\n'+filenames[i]+'\n')
        fd.write('skipped reads: '+str(skips)+'\n')
        fd.write('insertions: '+str(ins)+'\n')
        fd.write('deletions: '+str(dels)+'\n')
        fd.write('notindels: '+str(notindel)+'\n')
        fd.write('indel rate:
'+str(float((ins+dels)/float((ins+dels+notindel))*100.)+'%\n')
        fd.close()
        pd.DataFrame(insertions).to_csv(directory+filenames[i]+'Insertions_hbg1.csv')
        pd.DataFrame(deletions).to_csv(directory+filenames[i]+'Deletions_hbg1.csv')
    return

```

```

#indel analysis
#includes a check for match with two HBG2 SNPs
#inputs:
#directory, working directory folder containing all fastq files
#site, genomic site name as it appears in the fastq filenames
#orientation, 'FWD' if you want output in the same direction as the sequencing read or
'REV' if you want reverse complement output,

```

```

#flank1, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#flank2, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#width, expected bp length of basecalling window
#ouputs:
#"_Insertions_hbg2.csv", sequences of all insertion reads
#"_deletions_hbg2.csv", sequences of all deletion reads
#'indelsummary_hbg2.csv', contains all indel stats for all fastq files
def indelshbg2(directory, site, flank1, flank2, width):
    indir=directory
    outdir=directory
    filenames=os.listdir(indir)
    for i in range(len(filenames)):
        seqs={}
        if (filenames[i][-5:]=='fastq') and (site in filenames[i]):
            skips=0
            ins=0
            insertions=[]
            dels=0
            deletions=[]
            notindel=0
            for record in SeqIO.parse(indir+filenames[i], "fastq") :
                recordqual=[x>31 for x in record.letter_annotations['phred_quality']]
                #only process reads that have more than half of basecalls >Q31 and
                contain two HBG2 specific SNPs at 3' end of read
                if (record.seq.find('ATTTTCTCTAATTTATTCTTCCCTTTAGCTAGTTTT')>0) and
                (float(sum(recordqual))/float(len(recordqual))>=.5):
                    #split prior to indel window
                    split1=record.seq.split(flank1)
                    if len(split1)==2:
                        #take second item in first split
                        #split again at the sequence right after the indel window
                        if len(split1[1].split(flank2))==2:
                            split2=split1[1].split(flank2)[0]
                            #if INDEL window is +1 add to Insertions
                            if (len(split2)>=width+1):
                                ins=ins+1
                                insertions.append(split2)
                            #if INDEL window is -1 add to Deletions
                            if (len(split2)<=width-1):
                                dels=dels+1
                                deletions.append(split2)
                            if len(split2)==width:
                                notindel=notindel+1
                        else:
                            skips=skips+1
                    else:
                        skips=skips+1
                else:
                    skips=skips+1
            fd=open(directory+'indelsummary_hbg2.csv', 'a')
            fd.write('\n'+filenames[i]+'\n')
            fd.write('skipped reads: '+str(skips)+'\n')
            fd.write('insertions: '+str(ins)+'\n')
            fd.write('deletions: '+str(dels)+'\n')
            fd.write('notindels: '+str(notindel)+'\n')
            fd.write('indel rate:
'+str(float((ins+dels)/float(ins+dels+notindel)*100.))+'%\n')
            fd.close()
            pd.DataFrame(insertions).to_csv(directory+filenames[i]+'Insertions_hbg2.csv')
            pd.DataFrame(deletions).to_csv(directory+filenames[i]+'Deletions_hbg2.csv')
    return

```

```
directory1='/Users/michaelpacker/Desktop/Liu_Lab/MiSeqData/y-globin_632/'  
basecallhbg1(directory1, '632', 'FWD', 'ATTTGCA', 'TTAATTTTTT', 43)  
basecallhbg2(directory1, '632', 'FWD', 'ATTTGCA', 'TTAATTTTTT', 43)  
indelshbg1(directory1, '632', 'ATTTGCA', 'TTAATTTTTT', 43)  
indelshbg2(directory1, '632', 'ATTTGCA', 'TTAATTTTTT', 43)
```

Supplementary Note 4. Python script for analysis of base editing linkage disequilibrium.

```

%matplotlib inline
import numpy as np
import scipy as sp
import matplotlib as mpl
import matplotlib.cm as cm
import matplotlib.pyplot as plt
import pandas as pd
pd.set_option('display.width', 500)
pd.set_option('display.max_columns', 100)
pd.set_option('display.notebook_repr_html', True)
import seaborn as sns
sns.set_style("whitegrid")
sns.set_context("poster")
import requests
import time
from bs4 import BeautifulSoup
import regex
import re
import os
from Bio import SeqIO
import Bio
from Bio import motifs

#ABE processivity analysis
#inputs:
#directory, working directory folder containing all fastq files for a single ABE
#site, genomic site name as it appears in the fastq filenames
#orientation, 'FWD' if you want output in the same direction as the sequencing read or
'REV' if you want reverse complement output,
#flank1, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#flank2, sequence that is used to define the 5' end of protospacer in the sequencing read
direction,
#primaryposition, site of primary target A in protospacer with the position furthest from
the PAM as 0
#secondaryposition, site of secondary target A in protospacer with the position furthest
from the PAM as 0
#outputs:
#'_counts.csv', all base editing product sequences with corresponding number of
occurrences
#'_RawMotifs.csv', unnormalized nucleotide counts at all 20 positions of protospacer as
well as counts conditional on the identity of the primary target position
#'_NormalizedMotifs.csv' normalized nucleotide frequencies at all 20 positions of
protospacer as well as frequencies conditional on the identity of the primary target
position
#'_probability.csv', summary containing editing probabilities at both positions as well
as observed probability of double editing
def processivity(directory, site, orientation, flank1, flank2, primaryposition,
secondaryposition):
    indir=directory
    outdir=directory
    filenames=os.listdir(indir)
    probabilities=pd.DataFrame({'P1':[], 'P2':[], 'P21':[], 'P2P1':[]})
    for i in range(len(filenames)):
        seqs={}
        if (filenames[i][-5:]=='fastq') and (site in filenames[i]):
            for record in SeqIO.parse(indir+filenames[i], "fastq") :
                #split prior to spacer window
                split1=record.seq.tostring().split(flank1)
                if len(split1)==2:
                    #take second item in first split

```

```

#split again at the sequence right after the protospacer and take
first item
split2=split1[1].split(flank2)[0]
#keep only 20 basepair long protospacers
if (len(split2)==20) & (split2.find('N')== -1):
    if orientation=='FWD':
        seqs[record.id]=split2
    elif orientation=='REV':
        seqs[record.id]=Bio.Seq.reverse_complement(split2)
frame=pd.DataFrame({'Spacer':seqs.values(),
'Primary_Position':[x[primaryposition] for x in seqs.values()]}, index=seqs.keys())
MotifAll=motifs.create(frame.Spacer.values)
#in the event that no reads have a given base call at the primary position we
will save a dummy motif for a polyA sequence
if len(frame[frame.Primary_Position=='A'])>0:
    MotifA=motifs.create(frame[frame.Primary_Position=='A'].Spacer.values)
else:
    MotifA=motifs.create(['A'*20])
if len(frame[frame.Primary_Position=='C'])>0:
    MotifC=motifs.create(frame[frame.Primary_Position=='C'].Spacer.values)
else:
    MotifC=motifs.create(['A'*20])
if len(frame[frame.Primary_Position=='G'])>0:
    MotifG=motifs.create(frame[frame.Primary_Position=='G'].Spacer.values)
else:
    MotifG=motifs.create(['A'*20])
if len(frame[frame.Primary_Position=='T'])>0:
    MotifT=motifs.create(frame[frame.Primary_Position=='T'].Spacer.values)
else:
    MotifT=motifs.create(['A'*20])
#save motifs both raw and normalized conditional on the primary position
being each of the four bases
a=pd.DataFrame(MotifA.counts, index=['A'+str(s) for s in range(20)])
A=pd.DataFrame(MotifA.counts.normalize(), index=['A'+str(s) for s in
range(20)])
c=pd.DataFrame(MotifC.counts, index=['C'+str(s) for s in range(20)])
C=pd.DataFrame(MotifC.counts.normalize(), index=['C'+str(s) for s in
range(20)])
g=pd.DataFrame(MotifG.counts, index=['G'+str(s) for s in range(20)])
G=pd.DataFrame(MotifG.counts.normalize(), index=['G'+str(s) for s in
range(20)])
t=pd.DataFrame(MotifT.counts, index=['T'+str(s) for s in range(20)])
T=pd.DataFrame(MotifT.counts.normalize(), index=['T'+str(s) for s in
range(20)])
#save motifs both raw and normalized for all base editing products
All=pd.DataFrame(MotifAll.counts, index=['All'+str(s) for s in range(20)])
ALL=pd.DataFrame(MotifAll.counts.normalize(), index=['All'+str(s) for s in
range(20)])
#append all motifs and export, indices contain protospacer position as well
as an identifier for the primary position
All.append(a).append(c).append(g).append(t).to_csv(outdir+filenames[i].strip('.fastq')+'R
awMotifs.csv')
ALL.append(A).append(C).append(G).append(T).to_csv(outdir+filenames[i].strip('.fastq')+'N
ormalizedMotifs.csv')
#save the base editing product sequences and corresponding number of
occurrences
Counts=pd.DataFrame(seqs.items(),
columns=['ID', 'Window']).groupby('Window').count().sort_values('ID', ascending=False)
Counts.to_csv(outdir+filenames[i].strip('.fastq')+'.csv')
#evaluate editing probability at both primary and secondary positions
P1=ALL['G'].iloc[primaryposition]

```

```

P2=ALL['G'].iloc[secondaryposition]
#evaluate observed probability of joint editing as P(2|1)*P(1)
P21=G['G'].iloc[secondaryposition]*P1
#evaluate expected probability of joint editing given statistical
independence as P(1)*P(2)
P2P1=P1*P2
#export probabilities

probabilities=probabilities.append(pd.DataFrame({'P1':[P1],'P2':[P2],'P21':[P21],
'P2P1':[P2P1]}, index=[site]))
    probabilities.to_csv(outdir+site+'_probabilities.csv')
return

#ABE processivity analysis, for when flank1 needs to be short, we instead split on flank2
first and then find flank1
#program is otherwise identical to processivity
def processivity2(directory, site, orientation, flank1, flank2, primaryposition,
secondaryposition):
    indir=directory
    outdir=directory
    filenames=os.listdir(indir)
    probabilities=pd.DataFrame({'P1':[],'P2':[],'P21':[], 'P2P1':[]})
    for i in range(len(filenames)):
        seqs={}
        if (filenames[i][-5:]=='fastq') and (site in filenames[i]):
            for record in SeqIO.parse(indir+filenames[i], "fastq") :
                #split prior to spacer window
                split1=record.seq.tostring().split(flank2)
                if len(split1)==2:
                    #take second item in first split
                    #split again at the sequence right after the protospacer and take
first item
                    if len(split1[0].split(flank1))==2:
                        split2=split1[0].split(flank1)[1]
                        #keep only 20 basepair long protospacers
                        if (len(split2)==20) & (split2.find('N')== -1):
                            if orientation=='FWD':
                                seqs[record.id]=split2
                            elif orientation=='REV':
                                seqs[record.id]=Bio.Seq.reverse_complement(split2)
                    frame=pd.DataFrame({'Spacer':seqs.values(),
'Primary_Position':[x[primaryposition] for x in seqs.values()]}, index=seqs.keys())
                    MotifAll=motifs.create(frame.Spacer.values)
                    if len(frame[frame.Primary_Position=='A'])>0:
                        MotifA=motifs.create(frame[frame.Primary_Position=='A'].Spacer.values)
                    else:
                        MotifA=motifs.create(['A'*20])
                    if len(frame[frame.Primary_Position=='C'])>0:
                        MotifC=motifs.create(frame[frame.Primary_Position=='C'].Spacer.values)
                    else:
                        MotifC=motifs.create(['A'*20])
                    if len(frame[frame.Primary_Position=='G'])>0:
                        MotifG=motifs.create(frame[frame.Primary_Position=='G'].Spacer.values)
                    else:
                        MotifG=motifs.create(['A'*20])
                    if len(frame[frame.Primary_Position=='T'])>0:
                        MotifT=motifs.create(frame[frame.Primary_Position=='T'].Spacer.values)
                    else:
                        MotifT=motifs.create(['A'*20])
                    a=pd.DataFrame(MotifA.counts, index=['A'+str(s) for s in range(20)])
                    A=pd.DataFrame(MotifA.counts.normalize(),index=['A'+str(s) for s in
range(20)])

```



```

c=pd.DataFrame(MotifC.counts, index=['C'+str(s) for s in range(20)])
C=pd.DataFrame(MotifC.counts.normalize(), index=['C'+str(s) for s in
range(20)])
g=pd.DataFrame(MotifG.counts, index=['G'+str(s) for s in range(20)])
G=pd.DataFrame(MotifG.counts.normalize(), index=['G'+str(s) for s in
range(20)])
t=pd.DataFrame(MotifT.counts, index=['T'+str(s) for s in range(20)])
T=pd.DataFrame(MotifT.counts.normalize(), index=['T'+str(s) for s in
range(20)])
All=pd.DataFrame(MotifAll.counts, index=['All'+str(s) for s in range(20)])
ALL=pd.DataFrame(MotifAll.counts.normalize(),index=['All'+str(s) for s in
range(20)])

All.append(a).append(c).append(g).append(t).to_csv(outdir+filenames[i].strip('.fastq')+'R
awMotifs.csv')

ALL.append(A).append(C).append(G).append(T).to_csv(outdir+filenames[i].strip('.fastq')+'N
ormalizedMotifs.csv')
Counts=pd.DataFrame(seqs.items(),
columns=['ID', 'Window']).groupby('Window').count().sort_values('ID', ascending=False)
Counts.to_csv(outdir+filenames[i].strip('.fastq')+'.csv')
P1=ALL['G'].iloc[primaryposition]
P2=ALL['G'].iloc[secondaryposition]
P21=G['G'].iloc[secondaryposition]*P1
P2P1=P1*P2

probabilities=probabilities.append(pd.DataFrame({'P1':[P1], 'P2':[P2], 'P21':[P21],
'P2P1':[P2P1]}, index=[site]))
probabilities.to_csv(outdir+site+'_probabilities.csv')

return

directory1='/Users/michaelpacker/Desktop/Liu_Lab/MiSeqData/2017_0824_MSP/144/'
processivity(directory1, '299', 'REV', 'CCGCCCC', 'CAGTTTC', 5-1, 7-1)
processivity(directory1, '310', 'FWD', 'ATCGAAA', 'AGGATAA', 5-1, 8-1)
processivity(directory1, '311', 'FWD', 'ACTCAGA', 'GGGGTAC', 5-1, 8-1)
processivity2(directory1, '314', 'FWD', 'AAGT', 'TGGGCTTG', 5-1, 8-1)
processivity(directory1, '318', 'REV', 'GTAACCA', 'ATGAGTTCA', 5-1, 7-1)
processivity(directory1, '463', 'FWD', 'GATACAA', 'GGGT', 5-1, 3-1)
processivity(directory1, '464', 'FWD', 'ACCAGGA', 'AGGCAAA', 5-1, 6-1)
processivity(directory1, '466', 'FWD', 'ATCTCAT', 'TGGTTAC', 5-1, 7-1)
processivity(directory1, '467', 'FWD', 'GAGACTG', 'GGGAATG', 5-1, 6-1)
processivity(directory1, '468', 'FWD', 'AACGACT', 'TGGTATC', 5-1, 8-1)
processivity(directory1, '469', 'FWD', 'TCATG', 'AGGAGAC', 5-1, 8-1)
processivity(directory1, '470', 'FWD', 'GACTCAG', 'CGGGGGT', 5-1, 7-1)
processivity(directory1, '471', 'FWD', 'GCCTCAG', 'TGGACAA', 5-1, 7-1)
processivity(directory1, '472', 'REV', 'TGGTTCCCT', 'CAGATTT', 5-1, 6-1)
processivity(directory1, '501', 'FWD', 'CTGAGAG', 'GGGAGA', 5-1, 6-1)
processivity2(directory1, '505', 'FWD', 'AGT', 'GGGTCGCTGAAAA', 5-1, 8-1)
processivity(directory1, '508', 'FWD', 'GGTGAGG', 'GGGCTTC', 5-1, 7-1)
processivity(directory1, '536', 'REV', 'TTCTCCA', 'TTGGGGC', 7-1, 3-1)
processivity(directory1, '601', 'FWD', 'CACAGAC', 'TGGGAGT', 5-1, 7-1)
processivity(directory1, '602', 'FWD', 'ACAGACA', 'GGGAGTG', 6-1, 8-1)

#script to combine all sites into one summary file for each ABE
indir=directory1
filenames=os.listdir(indir)
summary=pd.DataFrame({'P1':[], 'P2':[], 'P21':[], 'P2P1':[]})
for i in range(len(filenames)):
    if 'probabilities' in filenames[i]:
        summary=summary.append(pd.read_csv(indir+filenames[i], index_col=0))
summary.to_csv(indir+'summary.csv')

```

Supplementary References

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- 3 Kunkel, G. R., Maser, R. L., Calvet, J. P. & Pederson, T. U6 small nuclear RNA is transcribed by RNA polymerase III. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 8575-8579 (1986).
- 4 Mussolino, C. & Cathomen, T. in *Nature Biotechnology* Vol. 31 208-209 (2013).
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