Supplementary Information

Expanding the genome-targeting scope and the site selectivity of high-precision base editors

Tan et al.

Supplementary Table 1 Target protospacer sequences analyzed in this study. Target Cs are shown in red, with the subscript numbers indicating their position relative to the PAM. The PAM sequence is shown in blue.

Target	Sequence (5' → 3')	Analysis method
Can1-1	ATACTAATC$_{-12}$C$_{-11}$ATGCCGCCAGTGG	canavanine selection
Can1-2	GCAAATTC$_{-13}$AAATATTTACGTTGG	canavanine selection
Can1-3	ACGTC$_{-16}$C$_{-15}$AAAATTGAATGACTTGG	canavanine selection
Can1-4	TTTC$_{-17}$AAGGTACTGAACTAGTTGG	canavanine selection
Can1-5	TC$_{-19}$C$_{-18}$AATAACGGAATCCAACTGGG	canavanine selection
PolyC-1-NGA	AC$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$C$_{-13}$C$_{-12}$TCATCTTTGAGTGA	NGS
PolyC-2-NGA	C$_{-20}$C$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$TGAGGCTTATGAGAGA	NGS
PolyC-3-NGCG	TC$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$TTCTGCCCAATTAGGCG	NGS
PolyC-4-NGCG	TTC$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$C$_{-13}$ACTCACAGGAAGAGCG	NGS
PolyC-5-NGC	AC$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$AAC$_{-12}$C$_{-11}$C$_{-10}$C$_{-9}$C$_{-8}$C$_{-7}$C$_{-6}$ATCAGTGC	NGS
PolyC-6-NGT	C$_{-20}$C$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$C$_{-13}$C$_{-12}$TTGATACTTCCTGT	NGS
PolyC-7	C$_{-21}$C$_{-20}$C$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$C$_{-13}$ATGTTC$_{-7}$C$_{-6}$GA GATCGG	NGS
PolyC-8	TATAC$_{-20}$C$_{-19}$C$_{-18}$C$_{-17}$C$_{-16}$C$_{-15}$C$_{-14}$C$_{-13}$TATATGGTAAAAAGG	NGS
PSEN1-L166P	GCC$_{-18}$TATTATATCATCTCTATTGT	-
TYR-Y327C	ATTCAC$_{-15}$ATTGGGTCAAACTCAGG	-

Supplementary Table 2 Width of the editing windows of A3A-derived BEs in two polycytidine motifs (polyC-7 and polyC-8; cf. Fig. 2). The approximate width of the editing window was defined as the number of nucleotides within which editing efficiency exceeds the half-maximal value (21).

Base editor	PolyC-7	PolyC-8
A3A-BE3	5	6
A3A-NL-BE3	9	7
A3AΔ194-BE3	4	4
A3AΔ190-BE3	6	3
A3AΔ186-BE3	4	2
A3AΔ182-BE3	3	2
A3AΔ178-BE3	3	2

Supplementary Table 3 Oligonucleotides used in this study.

Primers for construction of sgRNA plasmids

Primer name	Sequence (5' → 3')
sgRNA-Can1-1	AAAGATAAATGATCGATACTAATCCATGCCGCCAGGTTTTAGAGCTAGAAATAGCAAGT
sgRNA-Can1-2	AAAGATAAATGATCGGCAAATTCAAATATTTACGTGTTTTAGAGCTAGAAATAGCAAGT
sgRNA-Can1-3	AAAGATAAATGATCGACGTCCAAAATTGAATGACTGTTTTAGAGCTAGAAATAGCAAGT
sgRNA-Can1-4	AAAGATAAATGATCGTTTCAAGGTACTGAACTAGTGTTTTAGAGCTAGAAATAGCAAGT
sgRNA-Can1-5	AAAGATAAATGATCGTCCAATAACGGAATCCAACTGTTTTAGAGCTAGAAATAGCAAGT
sgRNA-PolyC-1	AAAGATAAATGATCGACCCCCCCCTCATCTTTGAGGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-2	AAAGATAAATGATCGCCCCCCCTGAGGCTTATGAGGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-3	AAAGATAAATGATCGTCCCCCCTTCTGCCCAATTAGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-4	AAAGATAAATGATCGTTCCCCCCACTCACAGGAAGGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-5	AAAGATAAATGATCGACCCCCAACCCCCCCATCAGGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-6	AAAGATAAATGATCGCCCCCCCCCTTGATACTTCCGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-7	AAAGATAAATGATCGCCCCCCCCCATGTTCCGAGATGTTTTAGAGCTAGAAATAGCAAGT
sgRNA- PolyC-8	AAAGATAAATGATCGCCCCCCCCCTATATGGTAAAAGTTTTAGAGCTAGAAATAGCAAGT
sgRNA-Rev	TATAGGGCGAATTGGGTACCGGCCGCAAATTAAAG

Primers for construction of base editors

Primer name	Sequence (5' → 3')	Constructs
VQR-BE3-1F	GGACAAGGGTAGGGATTTCG	VQR-BE3
VQR-BE3-1R	GAATCCGCCGTATTTCTTGG	
VQR-BE3-2F	CCAAGAAATACGGCGGATTCGTTTCTCCTACAGTCGCTTACAG	
VQR-BE3-2R	CAGGACCTCCTTTGTAGATCTGTATTGCTTTCTGTCTATGGTGGTGT	
VQR-BE3-3F	GATCTACAAAGGAGGTCCTG	
VQR-BE3-3R	TTGAATAACTAAAGCCCATG	
VRER-BE3-1F	GGACAAGGGTAGGGATTTCG	VRER-BE3
VRER-BE3-1R	ACCTTTCTGCAGCTCGCGCGCACTAGCGAGCAT	
VRER-BE3-2F	GAGCTGCAGAAAGGTAACGA	
VRER-BE3-2R	CTTTGTAGATCTGTATTCCTTTCTGTCTATGGT	
VRER-BE3-3F	TACAGATCTACAAAGGAGGT	
VRER-BE3-3R	ATGTTACATGCGTACACGCG	

Cas9NG-BE3-1F	GGACAAGGGTAGGGATTTCG	Cas9-NG-BE3
Cas9NG-BE3-1R	GCTGTTCCTTTTCGGGCGGATACTTTCCTTGGA	
Cas9NG-BE3-2F	CCGAAAAGGAACAGCGACAA	
Cas9NG-BE3-2R	GTTACCTTTCTGCAGGAAGCGCGCACTAGCGAGCAT	
Cas9NG-BE3-3F	CTGCAGAAAGGTAACGAGCT	
Cas9NG-BE3-3R	AAGTACTTGAAGGCTCGAGGCGCGCCCAAGTT	
Cas9NG-BE3-4F	GCCTTCAAGTACTTCGACACCACCATAGACAGAAAGGTATACAGATCTACAAAGGAGG	
Cas9NG-BE3-4R	ATGTTACATGCGTACACGCG	
cCDA1v-1F	GGACAAGGGTAGGGATTTCG	cCDA1-VQR/VRER/NG-BE3
cCDA1v-1R	CAGATTCAGAAGTACCTGGAGTTTCAGAACCAGAGTCTCCACCGAGCTGAGAGA	
cCDA1v-2F	TCCAGGTACTTCTGAATCTGCTACTCCAGAATCTATGACCGACGCTGAGTACGTGAG	
cCDA1v-2R	ATTTGCAGGCATTTGCTCGGCATGCCGGTAGAGGTGTGGT	
A3A-NL-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAGCCAGCCCAGCATC	A3A-NL-BE3
A3A-NL-1R	TCTTGTCCATGTTTCCCTGATTCTGGAGAA	
A3A-NL-2F	TCAGGGAAACATGGACAAGAAGTACTCCAT	
A3A-NL-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3B-NL-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGAATCCACAGATCAGAAA	A3B-NL-BE3
A3B-NL-1R	TCTTGTCCATGTTTCCCTGATTCTGGAGAA	
A3B-NL-2F	TCAGGGAAACATGGACAAGAAGTACTCCAT	
A3B-NL-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
hAID-NL-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGACAGCCTCTTGATGAA	hAID-NL-BE3
hAID -NL-1R	TCTTGTCCATAAGTCCCAAAGTACGAAATG	
hAID -NL-2F	TTTGGGACTTATGGACAAGAAGTACTCCAT	
hAID -NL-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
mAID-NL-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGACAGCCTTCTGATGAA	mAID-NL-BE3
mAID -NL-1R	TCTTGTCCATAAATCCCAACATACGAAATG	
mAID -NL-2F	GTTGGGATTTATGGACAAGAAGTACTCCAT	

mAID -NL-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
cAICDA -NL-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGAGCAAGCTGGACAGTGT	cAICDA-NL-BE3
cAICDA -NL-1R	TCTTGTCCATAAGGCCCAGCAGAGCGAAGC	
cAICDA -NL-2F	GCTGGGCCTTATGGACAAGAAGTACTCCAT	
cAICDA -NL-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3G -NL-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGAAACCGCATTTTCGCAA	A3G-NL-BE3
A3G -NL-1R	TCTTGTCCATATTTTCCTGGTTTTGCAGGA	
A3G -NL-2F	CCAGGAAAATATGGACAAGAAGTACTCCAT	
A3G -NL-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAGCCAGCCCAGCATC	A3A-BE3
A3A -1R	CAGATTCAGAAGTACCTGGAGTTTCAGAACCAGAGTTTCCCTGATTCTGGAGAA	
A3A-2F	TCCAGGTACTTCTGAATCTGCTACTCCAGAATCTATGGACAAGAAGTACTCC	
A3A-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A194-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAGCCAGCCCAGCATC	A3AΔ194-BE3
A3A194 -1R	TCTTGTCCATGAGAATGGCCCGCAGCCTCC	
A3A194-2F	GGCCATTCTCATGGACAAGAAGTACTCCAT	
A3A194-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A190-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAGCCAGCCCAGCATC	A3AΔ190-BE3
A3A190 -1R	TCTTGTCCATCAGCCTCCCACTCAGGGCTT	
A3A190-2F	TGGGAGGCTGATGGACAAGAAGTACTCCAT	
A3A190-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A186-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAGCCAGCCCAGCATC	A3AΔ186-BE3
A3A186 -1R	TCTTGTCCATCAGGGCTTGGCTGTGCTCAT	
A3A186-2F	CCAAGCCCTGATGGACAAGAAGTACTCCAT	
A3A186-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A182-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAGCCAGCCCAGCATC	A3AΔ182-BE3
A3A182 -1R	TCTTGTCCATGTGCTCATCTAGTCCATCCC	

A3A182-2F	AGATGAGCACATGGACAAGAAGTACTCCAT	
A3A182-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A178-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAG CCAGCCCAGCATC	A3AΔ178-BE3
A3A178 -1R	TCTTGTCCATTCCATCCCAGGGCTGGAAGG	
A3A178-2F	CTGGGATGGAATGGACAAGAAGTACTCCAT	
A3A178-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
A3A154-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAG CCAGCCCAGCATC	A3AΔ154-BE3
A3A154 -1R	TCTTGTCCATGGTCATGATGGAGACTTGGG	
A3A154-2F	CATCATGACCATGGACAAGAAGTACTCCAT	
A3A154-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
eA3A-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGGAAG CCAGCCCAGCATC	eA3A-BE3
eA3A-1R	AAGATTCTTAGCCTGGCCGTGTAGAAAGCCCCTGTGC	
eA3A-2F	CAGGCTAAGAATCTTCTCTG	
eA3A-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
R128A-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGAGTT CCGAGACAGGC	A3A(R128A)-BE3
R128A-1R	TCGTAATCATAGATGGCGGCAGCGAAGATACG	
R128A-2F	CATCTATGATTACGACCCCC	
R128A-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	
Y130F-1F	AGAAAAAACCCCGGATTCTAGAACTAGTGGATCCCCCGGGAAAAAAATGAGTT CCGAGACAGGC	A3A(Y130F)-BE3
Y130F-1R	AGGGGGTCGTAATCAAAGATGCGGGCAGCGA	
Y130F-2F	TGATTACGACCCCCTATATA	
Y130F-2R	ATTACTAAAGATCTCCTGCAGGTAGCAGATCCGATTCTTTC	

Primers for amplification of target regions for high-throughput sequencing (HTS)

Primer name	**Sequence (5' → 3')**
PolyC-1-HTS-index1-F	ATCACGGGAAAGACTGGCTCATCAAAACC
PolyC-1-HTS-index1-R	GCCTAATTTTATAAATTTTGAGGAAGCG
PolyC-1-HTS-index2-F	CGATGTGGAAAGACTGGCTCATCAAAACC
PolyC-1-HTS-index2-R	TGGTCATTTTATAAATTTTGAGGAAGCG
PolyC-1-HTS-index3-F	AGTTCCGGAAAGACTGGCTCATCAAAACC

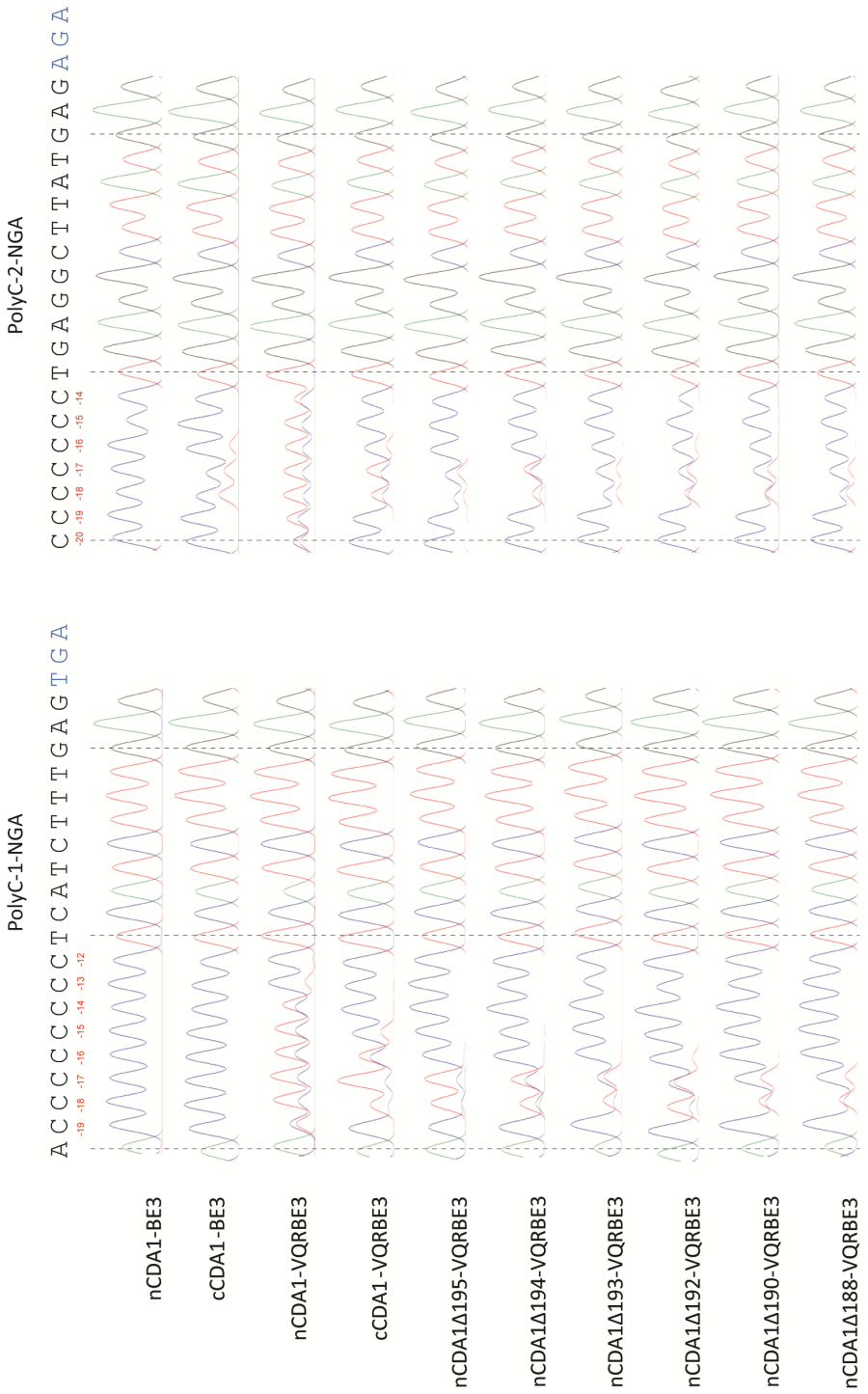
PolyC-1-HTS-index3-R	CTCTACTTTTATAAATTTTGAGGAAGCG
PolyC-1-HTS-index4-F	CACTCAGGAAAGACTGGCTCATCAAAACC
PolyC-1-HTS-index4-R	TGTTGGTTTTATAAATTTTGAGGAAGCG
PolyC-1-HTS-index5-F	GTGGCCGGAAAGACTGGCTCATCAAAACC
PolyC-1-HTS-index5-R	CGAAACTTTTATAAATTTTGAGGAAGCG
PolyC-1-HTS-index6-F	CGTACGGGAAAGACTGGCTCATCAAAACC
PolyC-1-HTS-index6-R	CCACTCTTTTATAAATTTTGAGGAAGCG
PolyC-1-HTS-index7-F	GGTAGCGGAAAGACTGGCTCATCAAAACC
PolyC-1-HTS-index7-R	ATCAGTTTTTATAAATTTTGAGGAAGCG
PolyC-2-HTS-index1-F	CACCGGTGCGCACATCAATCATTTTC
PolyC-2-HTS-index1-R	ATCGTGTGGCTTACTGTAAGCTACAGG
PolyC-2-HTS-index2-F	ATGAGCTGCGCACATCAATCATTTTC
PolyC-2-HTS-index2-R	AGGAATTGGCTTACTGTAAGCTACAGG
PolyC-2-HTS-index3-F	CAAAAGTGCGCACATCAATCATTTTC
PolyC-2-HTS-index3-R	TAGTTGTGGCTTACTGTAAGCTACAGG
PolyC-2-HTS-index4-F	TCGGCATGCGCACATCAATCATTTTC
PolyC-2-HTS-index4-R	GAATGATGGCTTACTGTAAGCTACAGG
PolyC-2-HTS-index5-F	TCCCGATGCGCACATCAATCATTTTC
PolyC-2-HTS-index5-R	CTTCGATGGCTTACTGTAAGCTACAGG
PolyC-2-HTS-index6-F	CTATACTGCGCACATCAATCATTTTC
PolyC-2-HTS-index6-R	TCTGAGTGGCTTACTGTAAGCTACAGG
PolyC-2-HTS-index7-F	TTAGGCTGCGCACATCAATCATTTTC
PolyC-2-HTS-index7-R	TGACCATGGCTTACTGTAAGCTACAGG
PolyC-3-HTS-index1-F	ACATGTTCCGCCATGTCCAACACG
PolyC-3-HTS-index1-R	CAGATCCTTTAATAATTTCCGAAATAGG
PolyC-3-HTS-index2-F	ACTTGATCCGCCATGTCCAACACG
PolyC-3-HTS-index2-R	GATCAGCTTTAATAATTTCCGAAATAGG
PolyC-3-HTS-index3-F	TAGCTTTCCGCCATGTCCAACACG
PolyC-3-HTS-index3-R	GGCTACCTTTAATAATTTCCGAAATAGG
PolyC-3-HTS-index4-F	CCGTCCTCCGCCATGTCCAACACG
PolyC-3-HTS-index4-R	GTAGAGCTTTAATAATTTCCGAAATAGG
PolyC-3-HTS-index5-F	GTCCGCTCCGCCATGTCCAACACG
PolyC-3-HTS-index5-R	GTGAAACTTTAATAATTTCCGAAATAGG
PolyC-3-HTS-index6-F	GTTTCGTCCGCCATGTCCAACACG
PolyC-3-HTS-index6-R	GAGTGGCTTTAATAATTTCCGAAATAGG
PolyC-3-HTS-index7-F	ACTGATTCCGCCATGTCCAACACG
PolyC-3-HTS-index7-R	ATTCCTCTTTAATAATTTCCGAAATAGG
PolyC-4-HTS-index1-F	CAACTAGGGAAAATAAAGGGAAAGACC
PolyC-4-HTS-index1-R	CACGATTGACCCAAAGGGAACATAAGAC
PolyC-4-HTS-index2-F	CAGGCGGGGAAAATAAAGGGAAAGACC

PolyC-4-HTS-index2-R	CATGGCTGACCCAAAGGGAACATAAGAC
PolyC-4-HTS-index3-F	ACAGTGGGGAAAATAAAGGGAAAGACC
PolyC-4-HTS-index3-R	GCCAATTGACCCAAAGGGAACATAAGAC
PolyC-4-HTS-index4-F	CTTGTAGGGAAAATAAAGGGAAAGACC
PolyC-4-HTS-index4-R	AGTCAATGACCCAAAGGGAACATAAGAC
PolyC-4-HTS-index5-F	ATGTCAGGGAAAATAAAGGGAAAGACC
PolyC-4-HTS-index5-R	CATTTTTGACCCAAAGGGAACATAAGAC
PolyC-4-HTS-index6-F	CCAACAGGGAAAATAAAGGGAAAGACC
PolyC-4-HTS-index6-R	CGGAATTGACCCAAAGGGAACATAAGAC
PolyC-4-HTS-index7-F	TCATTCGGGAAAATAAAGGGAAAGACC
PolyC-4-HTS-index7-R	CTAGCTTGACCCAAAGGGAACATAAGAC
PolyC-5-HTS-index1-F	CACCGGGTCACACCACGCTCAAACGG
PolyC-5-HTS-index1-R	ATCGTGAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index2-F	ATGAGCGTCACACCACGCTCAAACGG
PolyC-5-HTS-index2-R	AGGAATAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index3-F	CAAAAGGTCACACCACGCTCAAACGG
PolyC-5-HTS-index3-R	TAGTTGAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index4-F	TCGGCAGTCACACCACGCTCAAACGG
PolyC-5-HTS-index4-R	GAATGAAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index5-F	TCCCGAGTCACACCACGCTCAAACGG
PolyC-5-HTS-index5-R	CTTCGAAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index6-F	CTATACGTCACACCACGCTCAAACGG
PolyC-5-HTS-index6-R	TCTGAGAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index7-F	TTAGGCGTCACACCACGCTCAAACGG
PolyC-5-HTS-index7-R	TGACCAAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index8-F	ACATGTGTCACACCACGCTCAAACGG
PolyC-5-HTS-index8-R	CAGATCAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index9-F	ACTTGAGTCACACCACGCTCAAACGG
PolyC-5-HTS-index9-R	GATCAGAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index10-F	TAGCTTGTCACACCACGCTCAAACGG
PolyC-5-HTS-index10-R	GGCTACAGATCTGGTCCACGGCCTGG
PolyC-5-HTS-index11-F	CCGTCCGTCACACCACGCTCAAACGG
PolyC-5-HTS-index11-R	GTAGAGAGATCTGGTCCACGGCCTGG
PolyC-6-HTS-index1-F	GTCCGCTTCGGGGTTATTTATTTTTCG
PolyC-6-HTS-index1-R	GTGAAAGTTTGATTGGTCAAGTTGGC
PolyC-6-HTS-index2-F	GTTTCGTTCGGGGTTATTTATTTTTCG
PolyC-6-HTS-index2-R	GAGTGGGTTTGATTGGTCAAGTTGGC
PolyC-6-HTS-index3-F	ACTGATTTCGGGGTTATTTATTTTTCG
PolyC-6-HTS-index3-R	ATTCCTGTTTGATTGGTCAAGTTGGC
PolyC-6-HTS-index4-F	CAACTATTCGGGGTTATTTATTTTTCG

PolyC-6-HTS-index4-R	CACGATGTTTGATTGGTCAAGTTGGC
PolyC-6-HTS-index5-F	CAGGCGTTCGGGGTTATTTATTTTTCG
PolyC-6-HTS-index5-R	CATGGCGTTTGATTGGTCAAGTTGGC
PolyC-6-HTS-index6-F	ACAGTGTTCGGGGTTATTTATTTTTCG
PolyC-6-HTS-index6-R	GCCAATGTTTGATTGGTCAAGTTGGC
PolyC-6-HTS-index7-F	CTTGTATTCGGGGTTATTTATTTTTCG
PolyC-6-HTS-index7-R	AGTCAAGTTTGATTGGTCAAGTTGGC
PolyC-7-HTS-index1-F	CGATGTACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index1-R	TGGTCATATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index2-F	ATCACGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index2-R	GCCTAATATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index3-F	AGTTCCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index3-R	CTCTACTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index4-F	CACTCAACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index4-R	TGTTGGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index5-F	GTGGCCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index5-R	CGAAACTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index6-F	CGTACGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index6-R	CCACTCTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index7-F	GGTAGCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index7-R	ATCAGTTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index8-F	CACCGGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index8-R	ATCGTGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index9-F	ATGAGCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index9-R	AGGAATTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index10-F	CAAAAGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index10-R	TAGTTGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index11-F	TCGGCAACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index11-R	GAATGATATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index12-F	TCCCGAACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index12-R	CTTCGATATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index13-F	CTATACACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index13-R	TCTGAGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index14-F	TTAGGCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index14-R	TGACCATATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index15-F	ACATGTACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index15-F	CAGATCTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index16-F	ACTTGAACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index16-R	GATCAGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index17-F	TAGCTTACTGCGGAAGTGAGGGGAGC

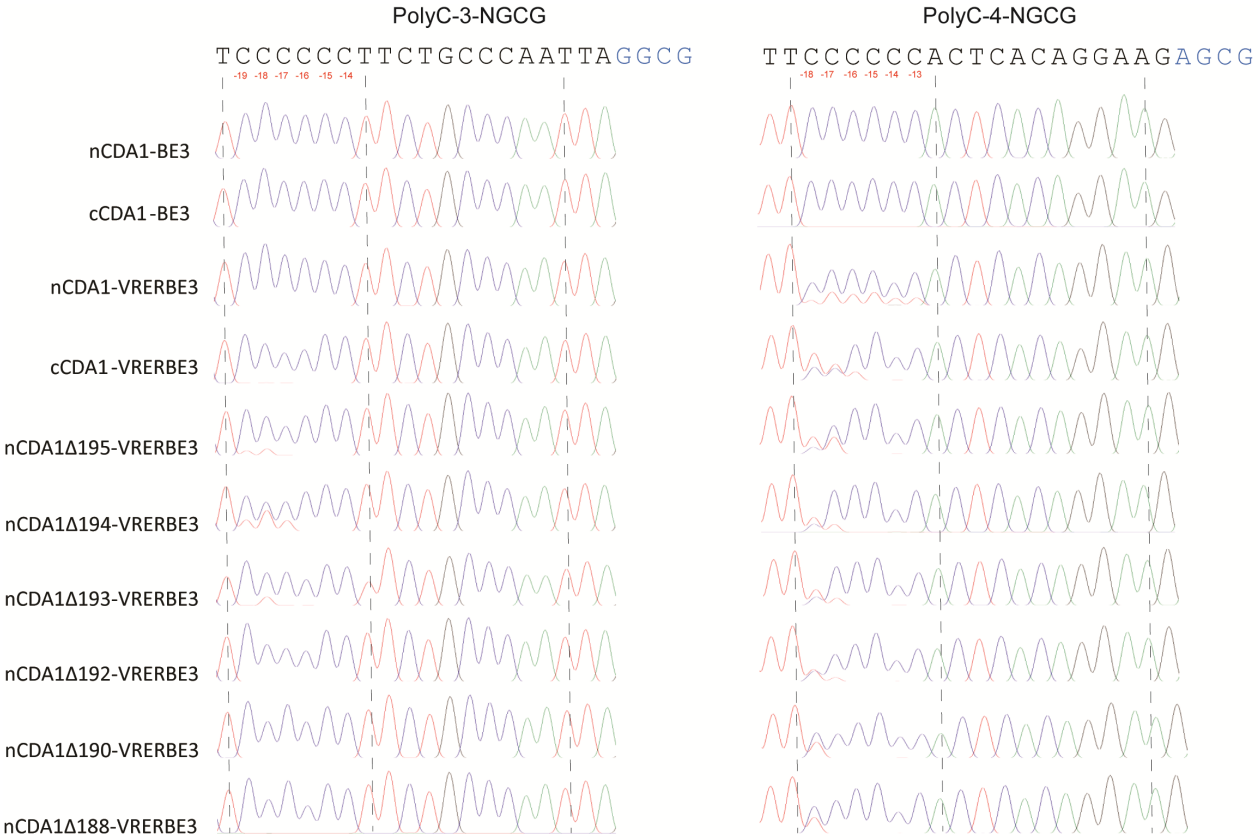
PolyC-7-HTS-index17-R	GGCTACTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index18-F	CCGTCCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index18-R	GTAGAGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index19-F	GTCCGCACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index19-R	GTGAAATATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index20-F	GTTTCGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index20-R	GAGTGGTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index21-F	ACTGATACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index21-R	ATTCCTTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index22-F	CAACTAACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index22-R	CACGATTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index23-F	CAGGCGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index23-R	CATGGCTATCCGTGCGCGTAATCCTTCT
PolyC-7-HTS-index24-F	ACAGTGACTGCGGAAGTGAGGGGAGC
PolyC-7-HTS-index24-R	GCCAATTATCCGTGCGCGTAATCCTTCT
PolyC-8-HTS-index1-F	ATCACGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index1-R	CGATGTATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index2-F	TTAGGCTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index2-R	TGACCAATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index3-F	ACAGTGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index3-R	GCCAATATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index4-F	CAGATCTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index4-R	ACTTGAATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index5-F	GATCAGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index5-R	TAGCTTATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index6-F	GGCTACTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index6-R	CTTGTAATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index7-F	AGTCAATTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index7-R	AGTTCCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index8-F	ATGTCATTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index8-R	CCGTCCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index9-F	GTAGAGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index9-R	GTCCGCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index10-F	GTGAAATTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index10-R	GTGGCCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index11-F	GTTTCGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index11-R	CGTACGATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index12-F	GAGTGGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index12-R	GGTAGCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index13-F	ACTGATTTCATCTATAAGGATATGGGTCG

PolyC-8-HTS-index13-R	ATGAGCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index14-F	ATTCCTTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index14-R	CAAAAGATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index15-F	CAACTATTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index15-R	CACCGGATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index16-F	CACGATTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index16-R	CACTCAATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index17-F	CAGGCGTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index17-R	CATGGCATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index18-F	CATTTTTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index18-R	CCAACAATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index19-F	CGGAATTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index19-R	CTAGCTATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index20-F	CTATACTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index20-R	CTCAGAATAGCATATAATAAAAGTGGAG
PolyC-8-HTS-index21-F	GACGACTTCATCTATAAGGATATGGGTCG
PolyC-8-HTS-index21-R	TAATCGATAGCATATAATAAAAGTGGAG

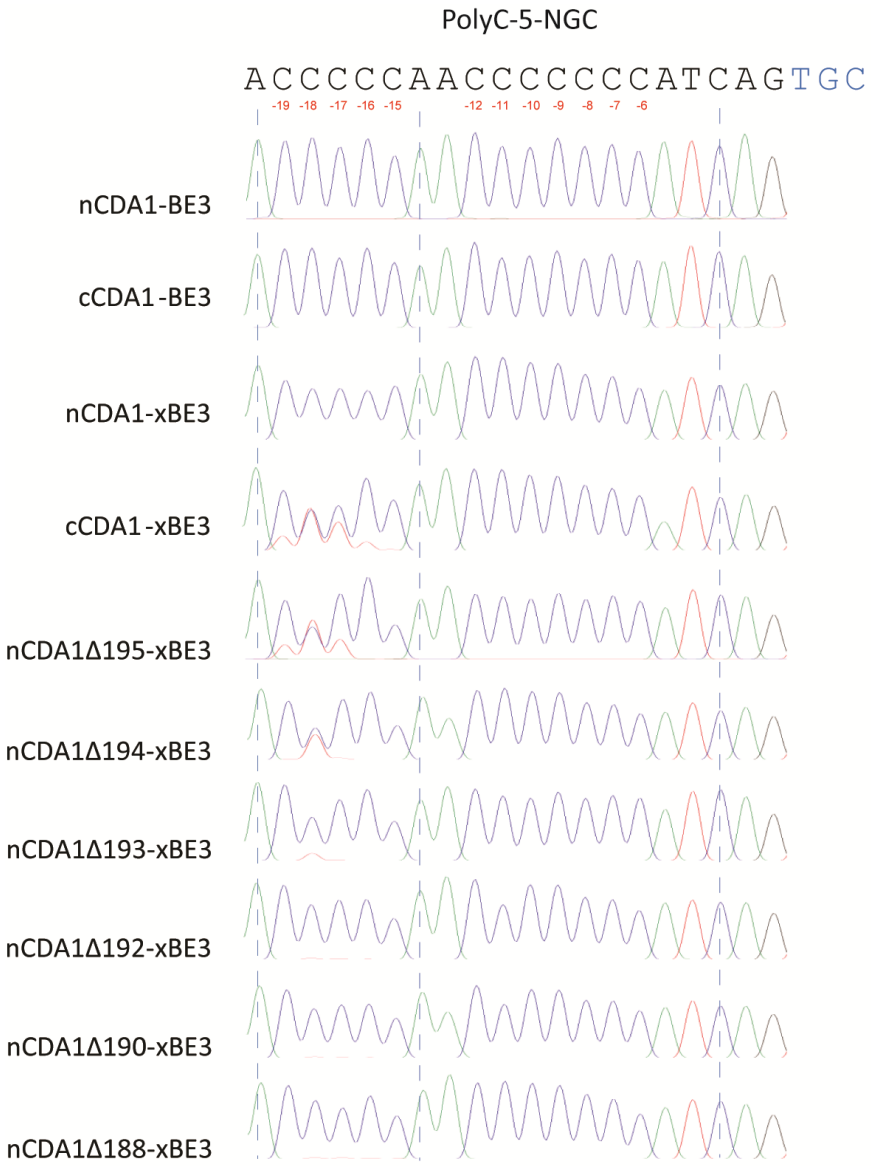


Supplementary Figure 1 Analysis of base editing of a set of VQR-Cas9 BEs fused to the full-length CDA1 (nCDA1-VQRBE3) or C-terminally truncated versions of CDA1 (nCDA1Δ195-VQRBE3; nCDA1Δ194-VQRBE3; nCDA1Δ193-VQRBE3; nCDA1Δ192-VQRBE3; nCDA1Δ190-VQRBE3; nCDA1Δ188-VQRBE3; Fig. 1a,c). The two tested target sequences (PolyC-1-NGA and PolyC-2-NGA) both contain a polyC stretch upstream of the

PAM sequence NGA that is recognized by VQR-Cas9 (Fig. 1b). The amplified target sequences were directly sequenced by the dideoxy chain termination method (see Methods), and the sequence chromatograms are shown for each BE. The nucleotide sequences are shown above the chromatograms, with the PAM indicated in blue and the positions of the cytidines in the polyC stretch given in red (relative to the PAM). The reciprocal full-length fusion, cCDA1-VQRBE3, was also analyzed. nCDA1-BE3 and cCDA1-BE3 have no PAM in proper distance from the polyC stretch and served as negative controls.

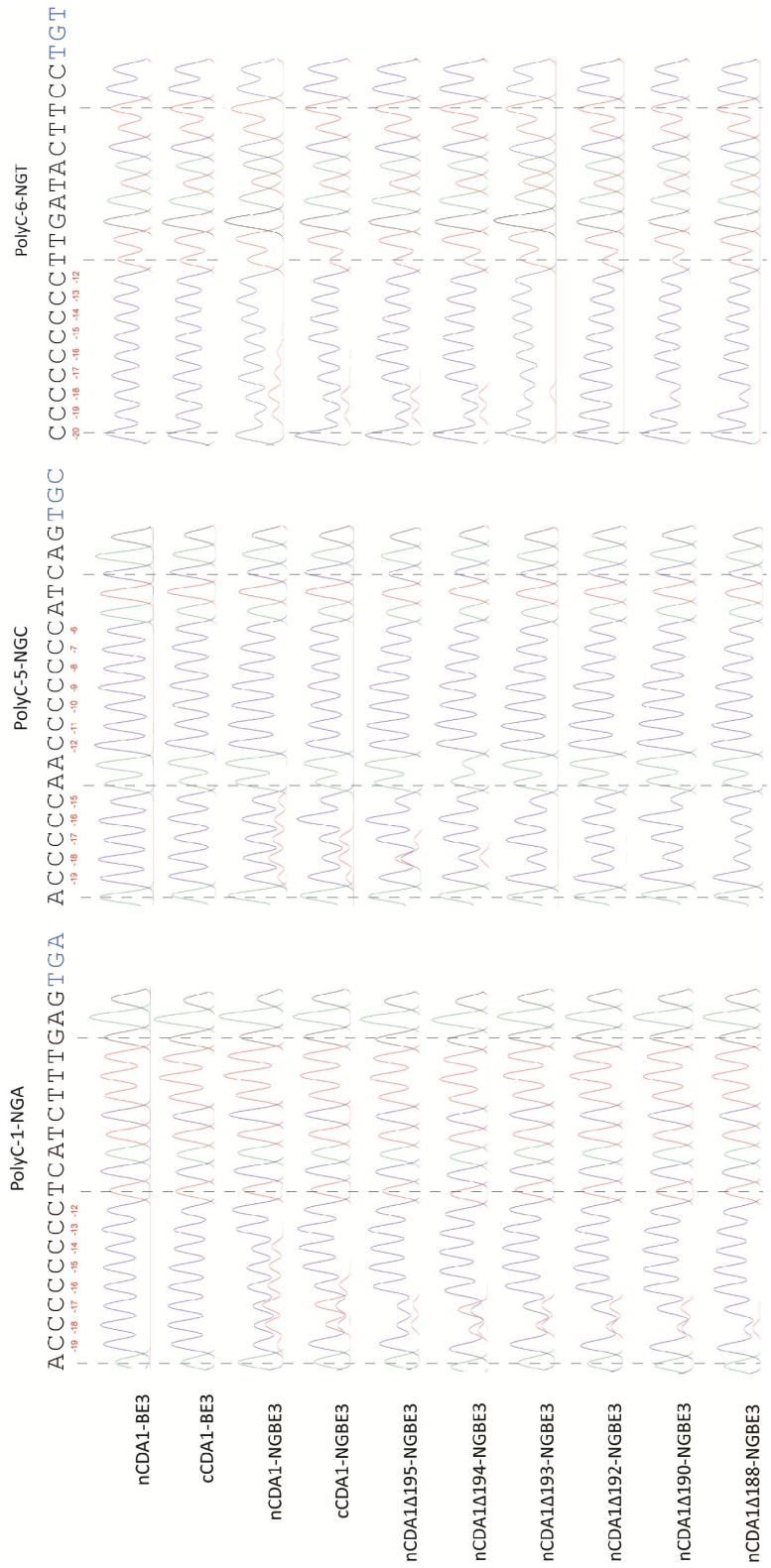


Supplementary Figure 2 Analysis of base editing of a set of VRER-Cas9 BEs fused to the full-length CDA1 (nCDA1-VRERBE3) or C-terminally truncated versions of CDA1 (nCDA1Δ195-VRERBE3; nCDA1Δ194-VRERBE3; nCDA1Δ193-VRERBE3; nCDA1Δ192-VRERBE3; nCDA1Δ190-VRERBE3; nCDA1Δ188-VRERBE3; Fig. 1a,d). The two tested target sequences (PolyC-3-NGCG and PolyC-4-NGCG) both contain a polyC stretch upstream of the PAM sequence NGCG that is recognized by VRER-Cas9 (Fig. 1b). The amplified target sequences were directly sequenced by the dideoxy chain termination method (see Methods), and the sequence chromatograms are shown for each BE. The nucleotide sequences are shown above the chromatograms, with the PAM indicated in blue and the positions of the cytidines in the polyC stretch given in red (relative to the PAM). The reciprocal full-length fusion, cCDA1-VRERBE3, was also analyzed. nCDA1-BE3 and cCDA1-BE3 have no PAM in proper distance from the polyC stretch and served as negative controls.



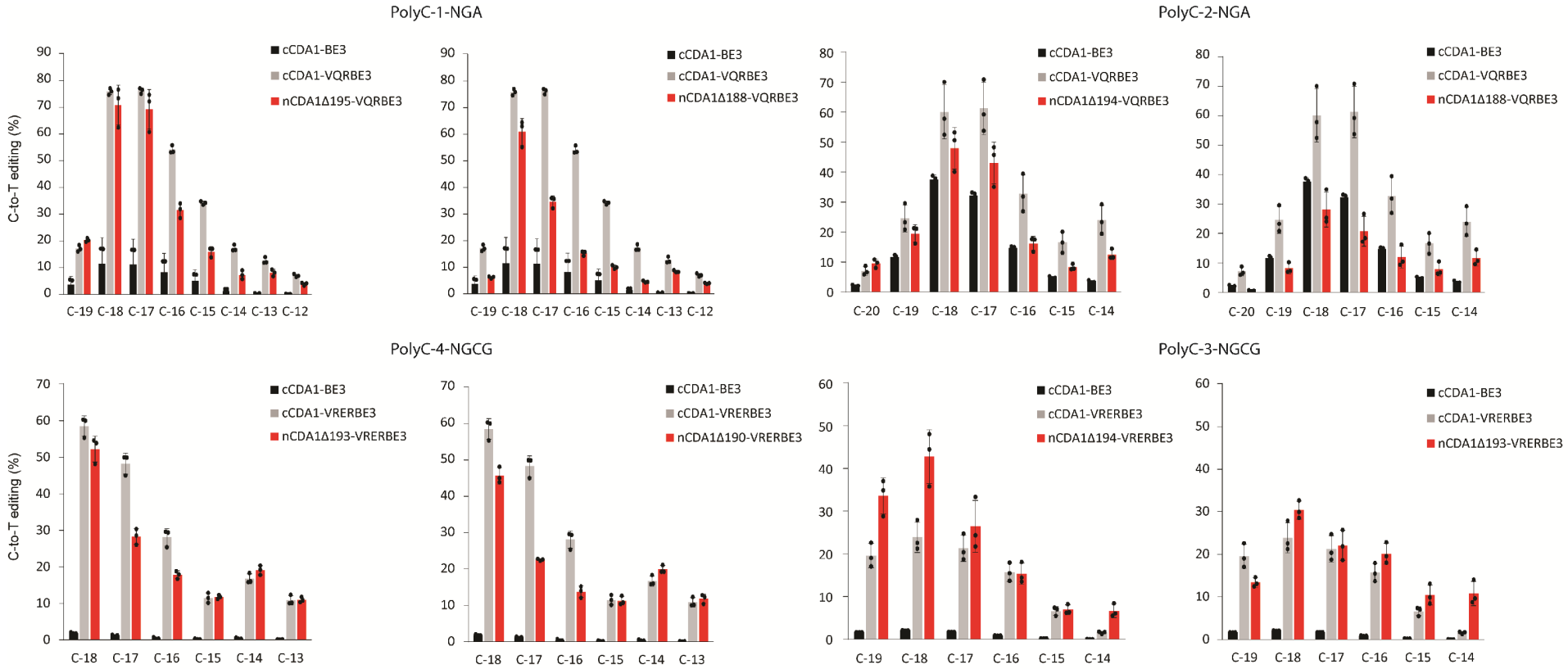
Supplementary Figure 3 Analysis of base editing of a set of xCas9 BEs fused to the full-length CDA1 (nCDA1-xBE3) or C-terminally truncated versions of CDA1 (nCDA1Δ195-xBE3; nCDA1Δ194-xBE3; nCDA1Δ193-xBE3; nCDA1Δ192-xBE3; nCDA1Δ190-xBE3; nCDA1Δ188-xBE3; Fig. 1a,e). The tested target sequence (PolyC-5-NGC) contains a polyC stretch upstream of the PAM sequence NG that is recognized by xCas9 (Fig. 1b). The amplified target sequences were directly sequenced by the dideoxy chain termination method (see Methods), and the sequence chromatograms are shown for each BE. The nucleotide sequence is shown above the chromatograms, with the PAM indicated in blue and the positions of the cytidines in the polyC stretch given in red (relative to the PAM). The

reciprocal full-length fusion, cCDA1-xBE3, was also analyzed. nCDA1-BE3 and cCDA1-BE3 have no PAM in proper distance from the polyC stretch and served as negative controls.

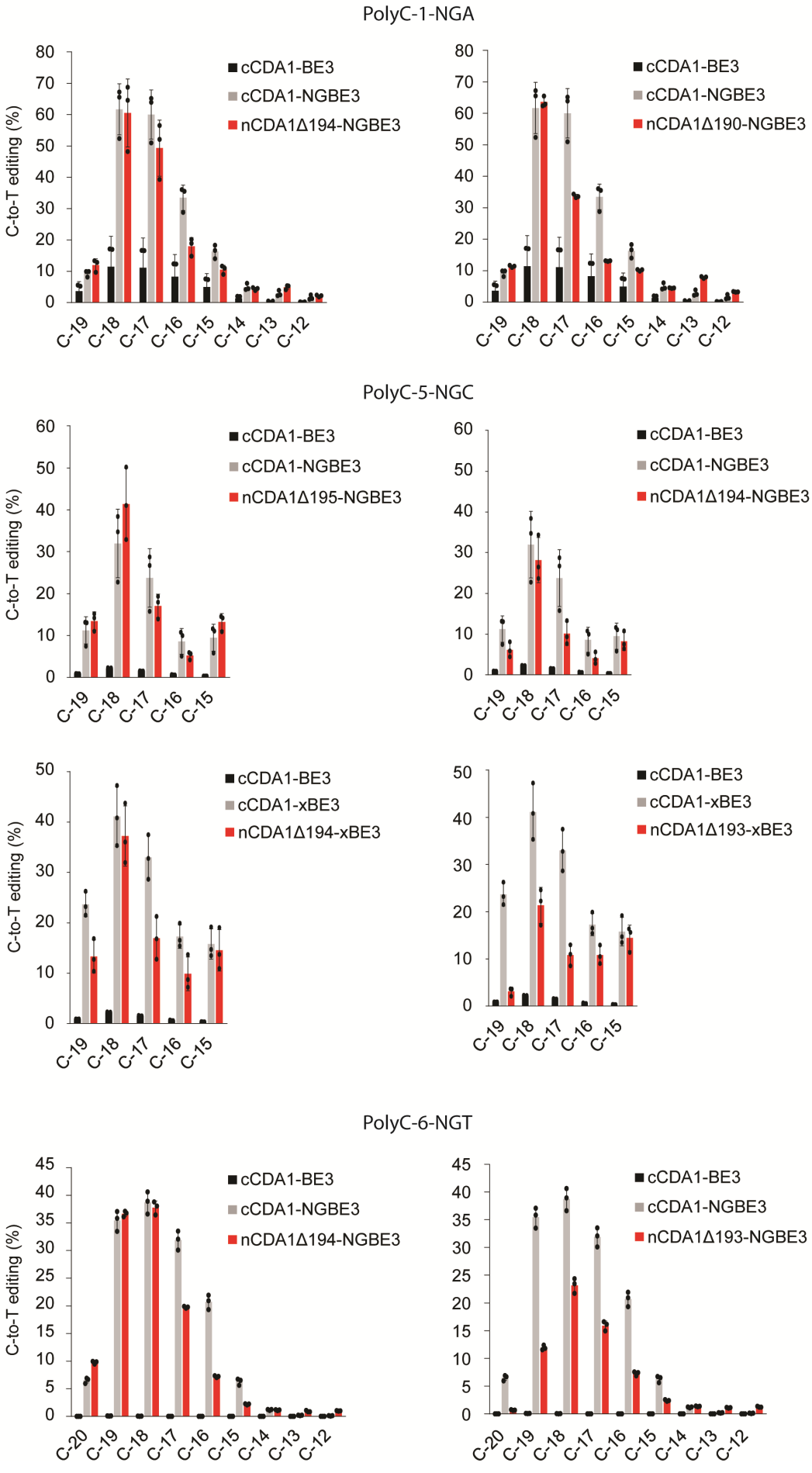


Supplementary Figure 4 Analysis of base editing of a set of SpCas9-NG BEs fused to the full-length CDA1 (nCDA1-NGBE3) or C-terminally truncated versions of CDA1 (nCDA1Δ195-NGBE3; nCDA1Δ194-NGBE3; nCDA1Δ193-NGBE3; nCDA1Δ192-NGBE3;

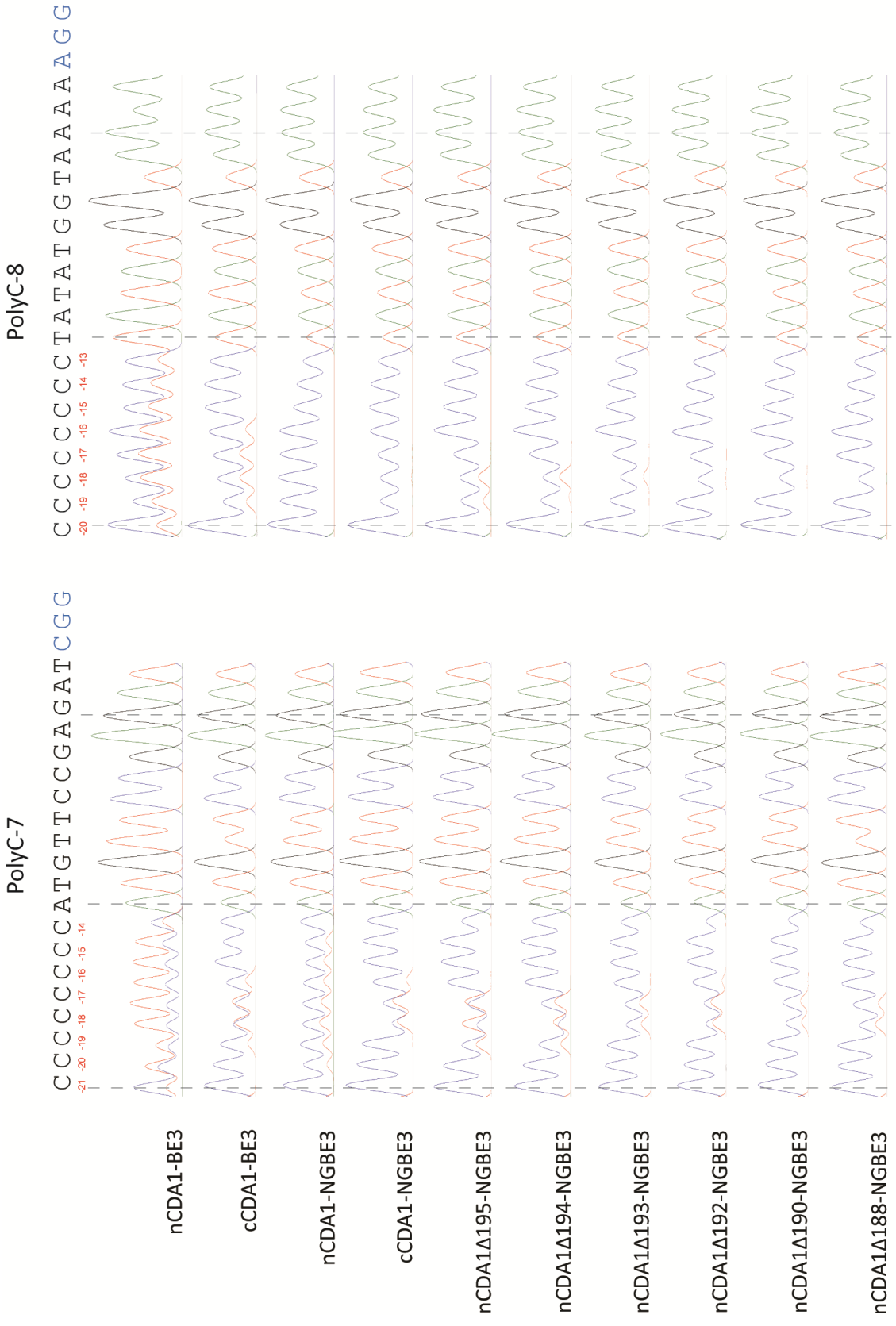
nCDA1Δ190-NGBE3; nCDA1Δ188-NGBE3; Fig. 1a,f,g). The three tested target sequences (PolyC-1-NGA, PolyC-5-NGC and PolyC-6-NGT) all contain a polyC stretch upstream of the PAM sequence NG that is recognized by SpCas9-NG (Fig. 1b). The amplified target sequences were directly sequenced by the dideoxy chain termination method (see Methods), and the sequence chromatograms are shown for each BE. The nucleotide sequences are shown above the chromatograms, with the PAM indicated in blue and the positions of the cytidines in the polyC stretch given in red (relative to the PAM). The reciprocal full-length fusion, cCDA1-NGBE3, was also analyzed. nCDA1-BE3 and cCDA1-BE3 have no PAM in proper distance from the polyC stretch and served as negative controls.


PolyC-1-NGA
C-to-T editing (%)
cCDA1-BE3
cCDA1-VQRBE3
nCDA1Δ195-VQRBE3
C-19 C-18 C-17 C-16 C-15 C-14 C-13 C-12
cCDA1-BE3
cCDA1-VQRBE3
nCDA1Δ188-VQRBE3
C-19 C-18 C-17 C-16 C-15 C-14 C-13 C-12
PolyC-2-NGA
cCDA1-BE3
cCDA1-VQRBE3
nCDA1Δ194-VQRBE3
C-20 C-19 C-18 C-17 C-16 C-15 C-14
cCDA1-BE3
cCDA1-VQRBE3
nCDA1Δ188-VQRBE3
C-20 C-19 C-18 C-17 C-16 C-15 C-14
PolyC-4-NGCG
C-to-T editing (%)
cCDA1-BE3
cCDA1-VRERBE3
nCDA1Δ193-VRERBE3
C-18 C-17 C-16 C-15 C-14 C-13
cCDA1-BE3
cCDA1-VRERBE3
nCDA1Δ190-VRERBE3
C-18 C-17 C-16 C-15 C-14 C-13
PolyC-3-NGCG
cCDA1-BE3
cCDA1-VRERBE3
nCDA1Δ194-VRERBE3
C-19 C-18 C-17 C-16 C-15 C-14
cCDA1-BE3
cCDA1-VRERBE3
nCDA1Δ193-VRERBE3
C-19 C-18 C-17 C-16 C-15 C-14

Supplementary Figure 5 Base editing outcomes of cCDA1-VQRBE3, cCDA1-VRERBE3 and BE variants with SpCas9-VQR or SpCas9-VRER fusions to C-terminally truncated CDA1 variants. Two NGA target sites were tested (PolyC-1-NGA, PolyC-2-NGA; for sequences see Supplementary Table 1; for sequence chromatograms, see Supplementary Figure 1) for BEs with SpCas9-VQR and two NGCG target sites were tested (PolyC-3-NGCG, PolyC-4-NGCG; for sequences see Supplementary Table 1; for sequence chromatograms, see Supplementary Figure 2) for BEs with SpCas9-VRER. For comparison, the cCDA1-BE3 editor (recognizing the PAM sequence NGG) was also included. % of C-to-T editing represents the percentage of total sequencing reads with the target C converted to T. Values and error bars represent the mean and standard deviation of three independent biological replicates. Source data are provided as a Source Data file.

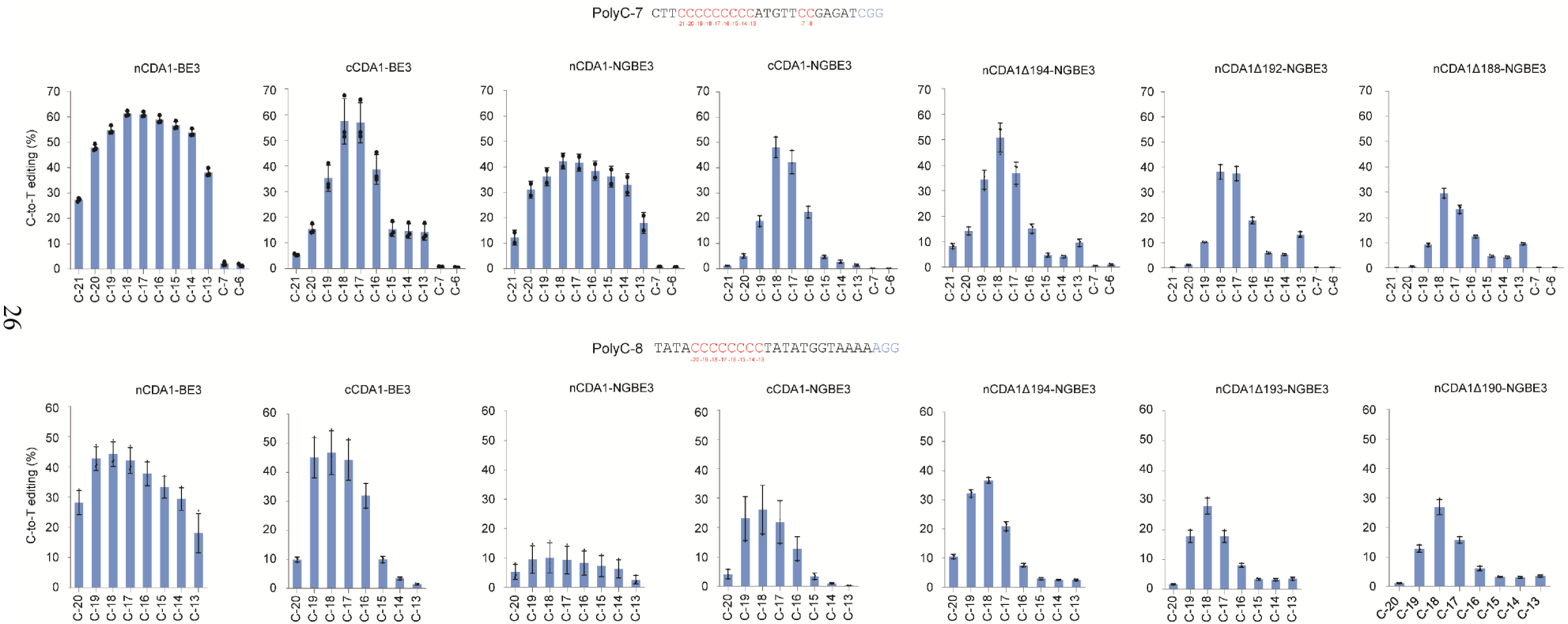

PolyC-1-NGA
C-to-T editing (%)
cCDA1-BE3
cCDA1-NGBE3
nCDA1Δ194-NGBE3
nCDA1Δ190-NGBE3
C-19 C-18 C-17 C-16 C-15 C-14 C-13 C-12
PolyC-5-NGC
cCDA1-BE3
cCDA1-NGBE3
nCDA1Δ195-NGBE3
nCDA1Δ194-NGBE3
C-19 C-18 C-17 C-16 C-15
cCDA1-BE3
cCDA1-xBE3
nCDA1Δ194-xBE3
nCDA1Δ193-xBE3
C-19 C-18 C-17 C-16 C-15
PolyC-6-NGT
cCDA1-BE3
cCDA1-NGBE3
nCDA1Δ194-NGBE3
nCDA1Δ193-NGBE3
C-20 C-19 C-18 C-17 C-16 C-15 C-14 C-13 C-12

Supplementary Figure 6 Base editing outcomes of cCDA1-NGBE3, cCDA1-xBE3 and BE variants with SpCas9-NG or xCas9 fusions to C-terminally truncated CDA1 variants. Three non-NGG target sites were tested (PolyC-1-NGA, PolyC-5-NGC and PolyC-6-NGT; for sequences see Supplementary Table 1; for sequence chromatograms, see Supplementary Figures 3 and 4). For comparison the cCDA1-BE3 editor (recognizing the PAM sequence NGG) was also included. % of C-to-T editing represents the percentage of total sequencing reads with the target C converted to T. Values and error bars represent the mean and standard deviation of three independent biological replicates. Source data are provided as a Source Data file.

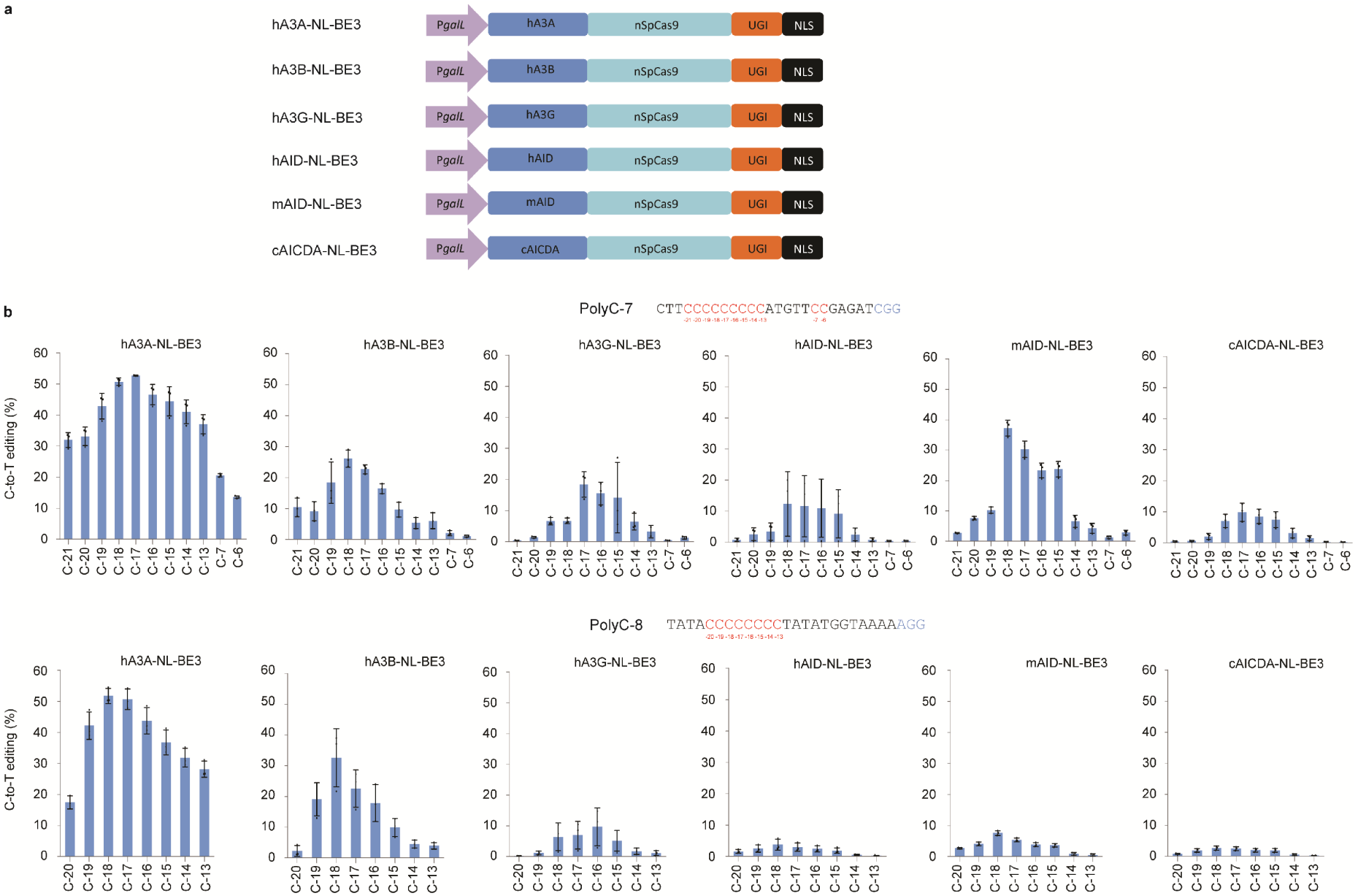


Supplementary Figure 7 Analysis of base editing of a set of SpCas9-NG BEs fused to the full-length CDA1 (nCDA1-NGBE3) or C-terminally truncated versions of CDA1

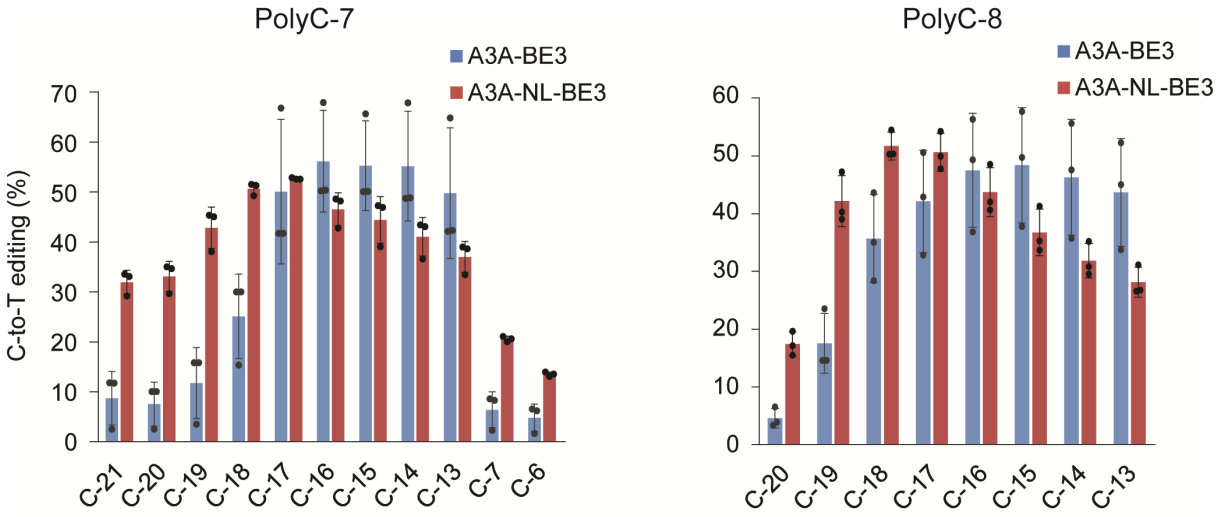
(nCDA1Δ195-NGBE3; nCDA1Δ194-NGBE3; nCDA1Δ193-NGBE3; nCDA1Δ192-NGBE3; nCDA1Δ190-NGBE3; nCDA1Δ188-NGBE3) in two target sequences containing the PAM sequence NGG (PolyC-7, PolyC-8). Both target sequences contain a polyC stretch upstream of the PAM. Note that the PAM sequence NGG can be recognized by both the wild-type Cas9 (as in nCDA1-BE3 and cCDA1-BE3) and the SpCas9-NG variants. The amplified target sequences were directly sequenced by the dideoxy chain termination method (see Methods), and the sequence chromatograms are shown for each BE. The nucleotide sequences are shown above the chromatograms, with the PAM indicated in blue and the positions of the cytidines in the polyC stretch given in red (relative to the PAM). The reciprocal full-length fusion, cCDA1-NGBE3, was also analyzed.


PolyC-7 CTTCCCCCCCCCATGTTCCGAGATCGG
-21 -20 -19 -18 -17 -16 -15 -14 -13
-7 -6
nCDA1-BE3
cCDA1-BE3
nCDA1-NGBE3
cCDA1-NGBE3
nCDA1Δ194-NGBE3
nCDA1Δ192-NGBE3
nCDA1Δ188-NGBE3
C-to-T editing (%)
C-21 C-20 C-19 C-18 C-17 C-16 C-15 C-14 C-13 C-7 C-6
PolyC-8 TATACCCCCCCCTATATGGTAAAAAGG
-20 -19 -18 -17 -16 -15 -14 -13
nCDA1-BE3
cCDA1-BE3
nCDA1-NGBE3
cCDA1-NGBE3
nCDA1Δ194-NGBE3
nCDA1Δ193-NGBE3
nCDA1Δ190-NGBE3
C-to-T editing (%)
C-20 C-19 C-18 C-17 C-16 C-15 C-14 C-13

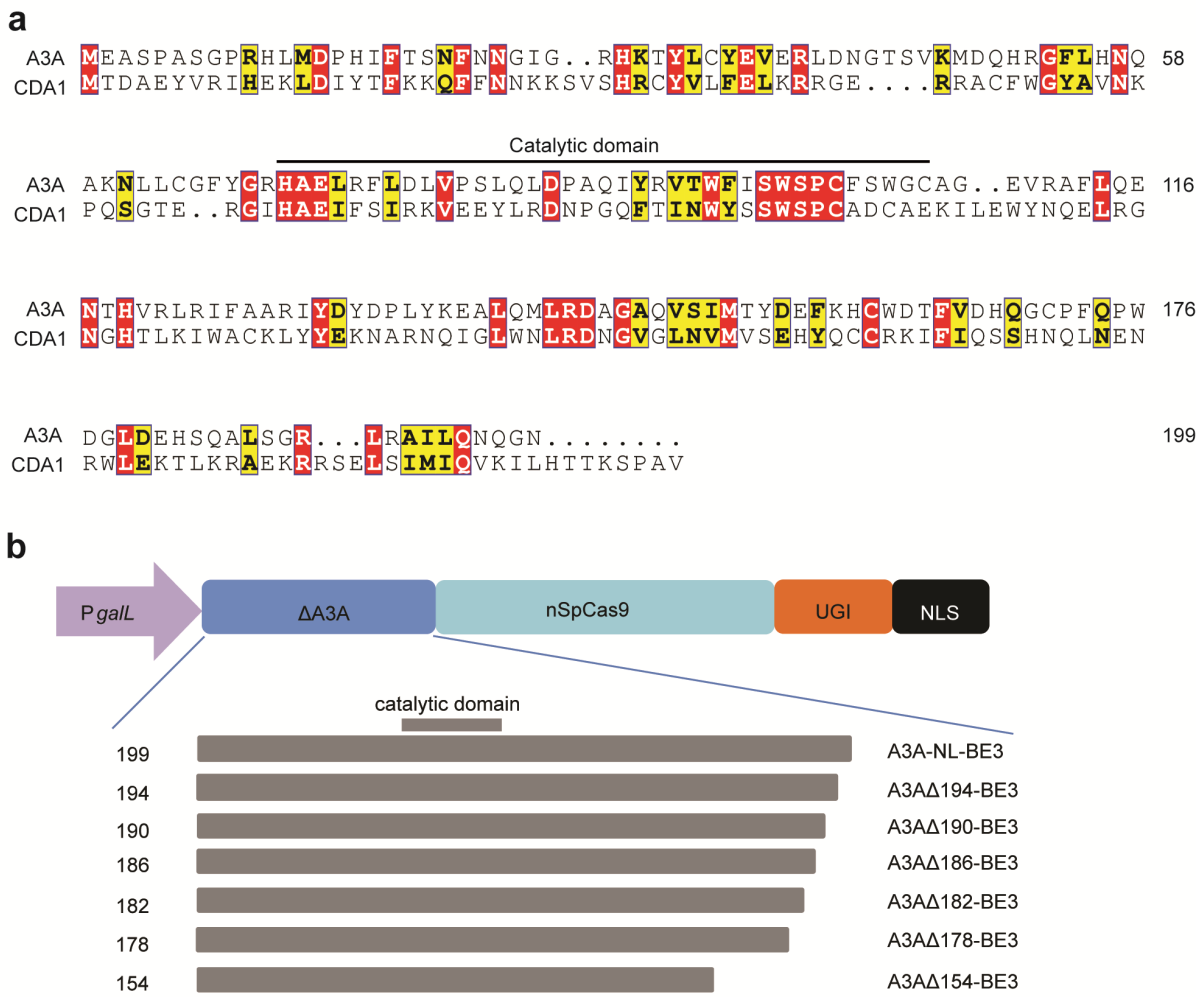
Supplementary Figure 8 Quantitative analysis of base editing outcomes obtained with a set of SpCas9-NG BEs fused to the full-length CDA1 (nCDA1-NGBE3) or C-terminally truncated versions of CDA1 (nCDA1Δ194-NGBE3; nCDA1Δ193-NGBE3; nCDA1Δ192-NGBE3; nCDA1Δ190-NGBE3; nCDA1Δ188-NGBE3) in comparison to the two full-length CDA1 fusions to the wild-type Cas9 (nCDA1-BE3; cCDA1-BE3). Both tested target sequences contain the PAM sequence NGG (PolyC-7, PolyC-8). The nucleotide sequences are shown above the diagrams, with the PAM indicated in blue and the positions of the cytidines in the polyC stretch given in red (relative to the PAM). % of C-to-T editing represents the percentage of total sequencing reads with the target C converted to T. Values and error bars represent the mean and standard deviation of three independent biological replicates. Source data are provided as a Source Data file.


a
hA3A-NL-BE3
hA3B-NL-BE3
hA3G-NL-BE3
hAID-NL-BE3
mAID-NL-BE3
cAICDA-NL-BE3
PgalL
hA3A
hA3B
hA3G
hAID
mAID
cAICDA
nSpCas9
UGI
NLS
b
PolyC-7 CTTCCCCCCCCCATGTTCCGAGATCGG
PolyC-8 TATACCCCCCCCTATATGGTAAAAAGG
C-to-T editing (%)
hA3A-NL-BE3
hA3B-NL-BE3
hA3G-NL-BE3
hAID-NL-BE3
mAID-NL-BE3
cAICDA-NL-BE3

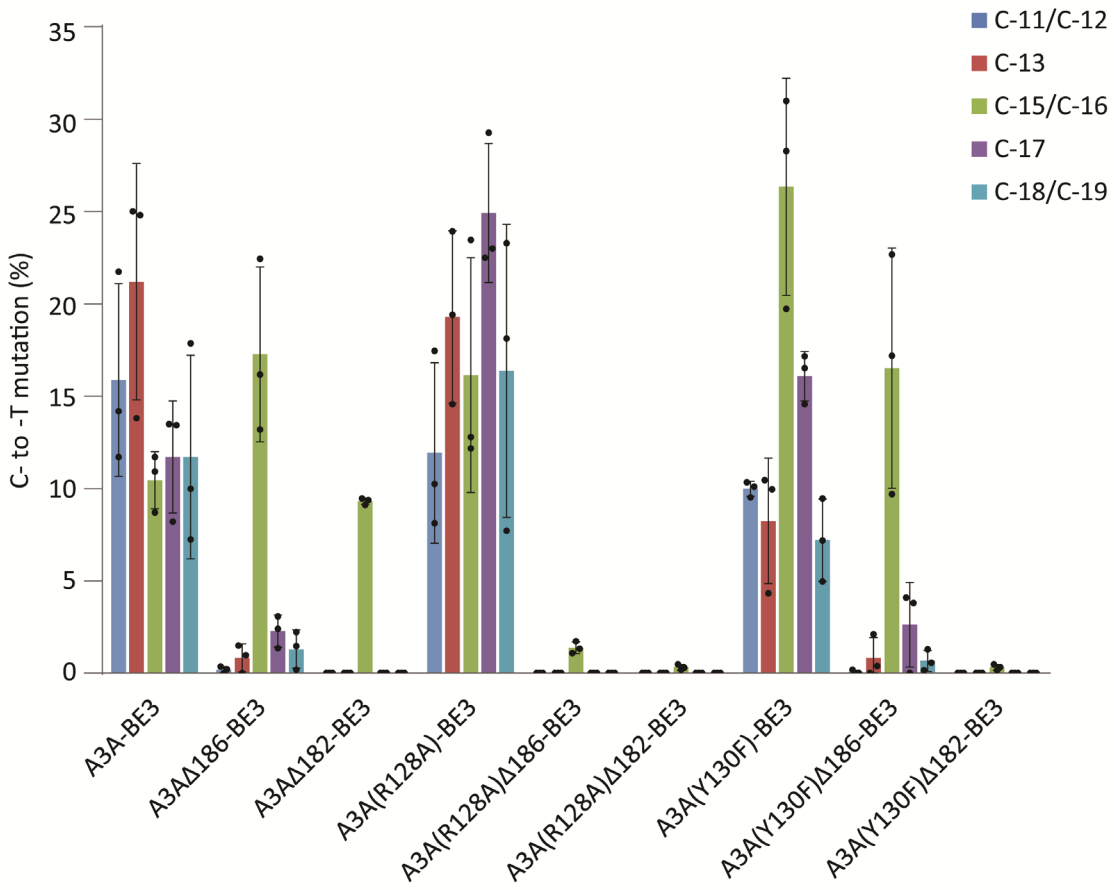
Supplementary Figure 9 Screening of deaminases directly fused to Cas9 (without linkers) for efficient base editing. **a** Series of new BEs constructed by directly fusing different APOBEC and AID deaminases with the Cas9 nickase (nCas9) and the uracil DNA glycosylase inhibitor (UGI) when targeting two polyC sites (polyC-7 and polyC-8). **b** Base editing outcomes of new BEs when targeting two polyC sites (polyC-7 and polyC-8). The sequence of each target site is shown with the numbers indicating the position of possible editing targets (red) relative to the PAM (blue). % of C-to-T editing represents the percentage of total sequencing reads with the target C converted to T. Values and error bars represent the mean and standard deviation of three independent biological replicates. hA3A: human *APOBEC3A* (GenBank accession number NM_145699); hA3B: human *APOBEC3B* (NM_004900); hA3G: human *APOBEC3G* (NM_021822); hAID: human *AID* (NM_020661); mAID: mouse *AID* (NM_009645); cAICDA: channel catfish *AICDA* (NM_001200185). Source data underlying panels **b** are provided as a Source Data file.



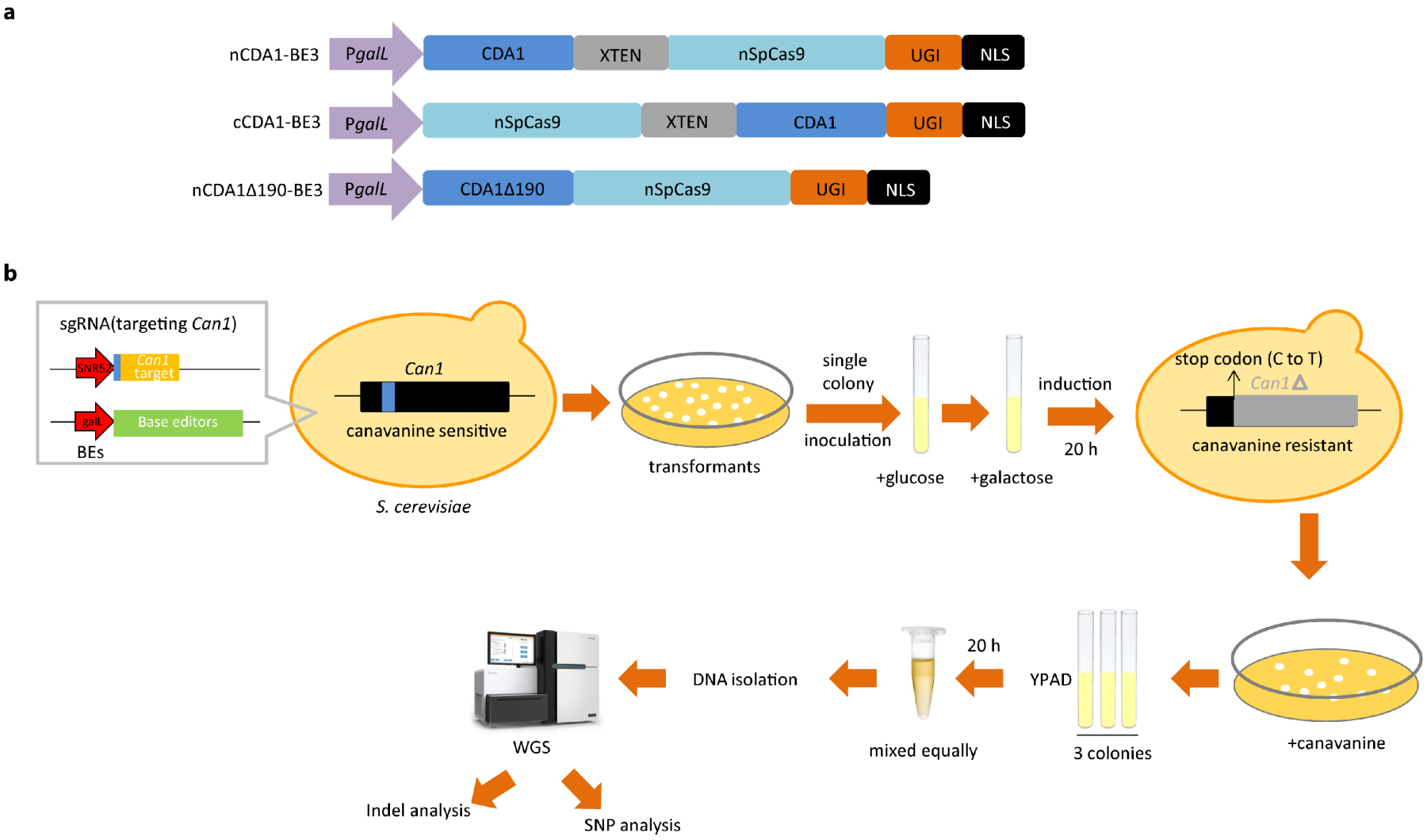
Supplementary Figure 10 Removal of the linker between A3A and nCas9 broadens the width of the editing window while not appreciably affecting editing efficiency. Two polyC target sequences (polyC-7 and polyC-8; see Supplementary Figure 9) were tested. The x-axis shows the target Cs and their positions relative to the PAM. % of C-to-T editing represents the percentage of total sequencing reads with the target C converted to T. Values and error bars represent the mean and standard deviation of three independent biological replicates. Source data are provided as a Source Data file.



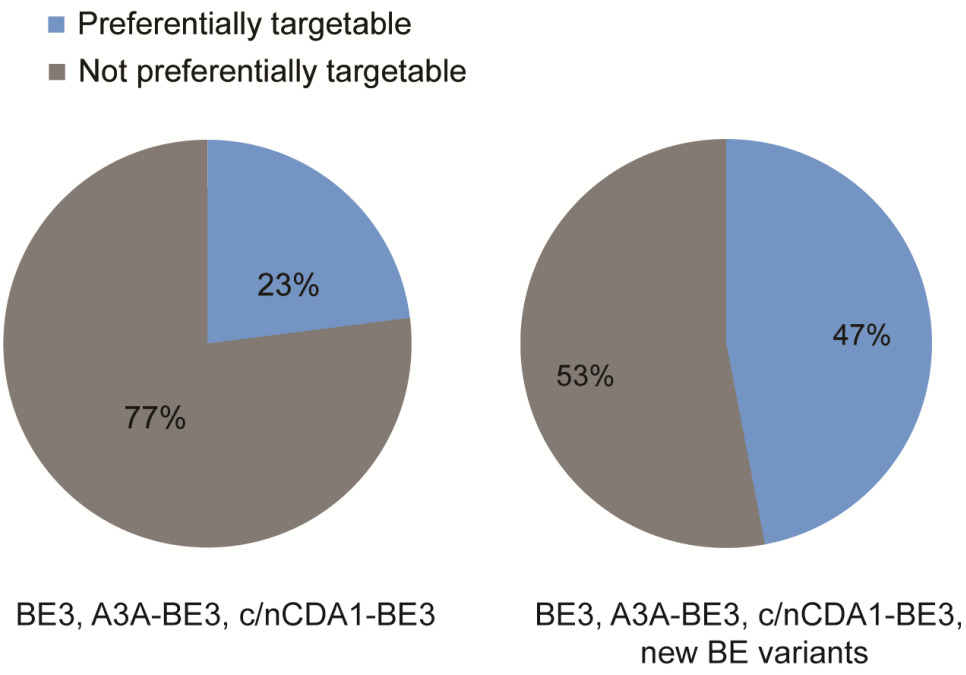
Supplementary Figure 11 Design of base editors with truncated A3A domains. **a** Amino acid sequence alignment of A3A and CDA1. The catalytic domain is indicated by the black horizontal line. The alignment was created by CLUSTAL W (ref. 43; https://www.genome.jp/tools-bin/clustalw) and graphically formatted with the help of the ESPript 3.0 server (ref. 44; http://espript.ibcp.fr/ESPript/ESPript/). Identical amino acid residues are marked in red, similar residues in yellow. **b** Schematic representation of BEs with C-terminal A3A truncations. The truncated variants are named after the last A3A residue included.



Supplementary Figure 12 Base editing outcomes of A3A-BE3, two narrow-window A3AΔ-BE3 variants, two recently reported mutant BE3 variants that reduce off-target RNA editing (A3A(R128A)-BE3, A3A(Y130F)-BE3; 33) and the combinations of these mutations with each of the two narrow-window A3AΔ-BE3 variants. Five sites in the yeast *Can1* gene (containing Cs at different positions) were targeted and their sequences are listed in Supplementary Table 1. Edited clones were identified by using the canavanine selection strategy (see Methods). The x-axis represents the target Cs within the protospacers. The y-axis shows their C-to-T editing frequencies (see Methods). Values and error bars represent the mean and standard deviation of three independent biological replicates. Source data are provided as a Source Data file.


a
nCDA1-BE3
PgalL
CDA1
XTEN
nSpCas9
UGI
NLS
cCDA1-BE3
PgalL
nSpCas9
XTEN
CDA1
UGI
NLS
nCDA1Δ190-BE3
PgalL
CDA1Δ190
nSpCas9
UGI
NLS
b
sgRNA(targeting Can1)
SNR52
Can1 target
gall
Base editors
BEs
Can1
canavanine sensitive
S. cerevisiae
transformants
single colony
inoculation
+glucose
+galactose
induction
20 h
stop codon (C to T)
Can1Δ
canavanine resistant
+canavanine
YPAD
3 colonies
20 h
mixed equally
DNA isolation
WGS
Indel analysis
SNP analysis

Supplementary Figure 13 Analysis of off-target editing induced by base editors nCDA1-BE3, cCDA1-BE3 and nCDA1Δ190-BE3 in yeast. **a** Schematic representation of the three base editors. **b** Experimental workflow.



Supplementary Figure 14 Fraction of pathogenic T-to-C and A-to-G SNPs in the ClinVar database (5074 mutations in total; ref. 8) that can be precisely addressed by the BEs available previously (left) and the expanded set of BEs that includes the high-precision BEs developed in the course of this work (right). The editing windows were assumed as follows: BE3 and A3A-BE3: C_{-13} to C_{-17}; cCDA1-BE3: C_{-16} to C_{-19}; nCDA1-BE3: C_{-14} to C_{-20} (refs. 15, 22, 23). The BE variants described in this work, including the A3AΔ-BE3 and nCDA1Δ-BE3 mutants, were assumed to have a narrowed window (C_{-15} to C_{-16}) recognizing the PAM NGG and an editing preference of $C_{-18} > C_{-17} > C_{-19}$ recognizing the PAM NG, respectively.