Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3139324 A1 2020/11/19

(21) 3 139 324

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2020/05/08
(87) Date publication PCT/PCT Publication Date: 2020/11/19
(85) Entrée phase nationale/National Entry: 2021/11/04
(86) N° demande PCT/PCT Application No.: US 2020/032226
(87) N° publication PCT/PCT Publication No.: 2020/231863
(30) Priorités/Priorities: 2019/05/10 (US62/846,422); 2019/10/29 (US62/927,585)

(51) Cl.Int./Int.Cl. *A61K 48/00* (2006.01), *C12N 15/10* (2006.01), *C12N 15/113* (2010.01), *C12N 15/85* (2006.01), *C12N 15/90* (2006.01), *C12N 9/22* (2006.01)

(71) Demandeur/Applicant:
BEAM THERAPEUTICS INC., US

(72) Inventeurs/Inventors:
SMEKALOVA, ELENA, US;
PACKER, MICHAEL, US;
GREGOIRE, FRANCINE, US;
BARRERA, LUIS, US;
CIARAMELLA, GIUSEPPE, US

(74) Agent: NORTON ROSE FULBRIGHT CANADA LLP/S.E.N.C.R.L., S.R.L.

(54) Titre : COMPOSITIONS ET METHODES DE TRAITEMENT DE L'HEPATITE B
(54) Title: COMPOSITIONS AND METHODS FOR TREATING HEPATITIS B

(57) Abrégé/Abstract:
The invention features compositions and methods for introducing mutations into the hepatitis B virus (HBV) genome.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 November 2020 (19.11.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/231863 A1

(51) International Patent Classification:
A61K 48/00 (2006.01) *C12N 15/90* (2006.01)
C12N 15/113 (2010.01) *C12N 15/10* (2006.01)
C12N 15/85 (2006.01) *C12N 9/22* (2006.01)

(21) International Application Number:
PCT/US2020/032226

(22) International Filing Date:
08 May 2020 (08.05.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/846,422 10 May 2019 (10.05.2019) US
62/927,585 29 October 2019 (29.10.2019) US

(71) Applicant: **BEAM THERAPEUTICS INC.** [US/US]; 26 Landsdowne St., 2nd Floor, Cambridge, Massachusetts 02139 (US).

(72) Inventors: **SMEKALOVA, Elena**; c/o Beam Therapeutics Inc., 26 Landsdowne St., 2nd. Floor, Cambridge, Massachusetts 02129 (US). **PACKER, Michael**; c/o Beam Therapeutics Inc., 26 Landsdowne St., 2nd Floor, Cambridge, Massachusetts 02139 (US). **GREGOIRE, Francine**; c/o Beam Therapeutics Inc., 26 Landsdowne St., 2nd Floor, Cambridge, Massachusetts 02139 (US). **BARRERA, Luis**; c/o Beam Therapeutics Inc., 26 Landsdowne St., 2nd Floor, Cambridge, Massachusetts 02139 (US). **CIARAMELLA, Giuseppe**; c/o Beam Therapeutics Inc., 26 Landsdowne St., 2nd Floor, Cambridge, Massachusetts 02139 (US).

(74) Agent: **HUNTER-ENSOR, Melissa**; c/o Greenberg Traurig, LLP, One International Place, Boston, Massachusetts 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2020/231863 A1

(54) Title: COMPOSITIONS AND METHODS FOR TREATING HEPATITIS B

(57) Abstract: The invention features compositions and methods for introducing mutations into the hepatitis B virus (HBV) genome.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 263

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 263

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

COMPOSITIONS AND METHODS FOR TREATING HEPATITIS B

CROSS REFERENCE TO RELATED APPLICATIONS

This International PCT Application claims priority to and benefit of U.S. Provisional Application No. 62/846,422, filed on May 10, 2019 and U.S. Provisional Application No. 62/927,585, filed on October 29, 2019, the contents of each of which are incorporated by reference herein in their entireties.

BACKGROUND OF THE INVENTION

Hepatitis B is a serious liver infection caused by the hepatitis B virus (HBV). HBV is a small DNA hepadnavirus that replicates through an RNA intermediate and can persist in infected cells by integrating into a host's genome. Approximately 257 million people worldwide, including between 850,000 and 2.2 million people in the United States, are chronically infected with HBV. Chronic HBV infection manifests as chronic hepatitis, cirrhosis, and/or hepatocellular carcinoma. Between 20% and 30% of adults who have chronic HBV infection develop hepatocellular carcinoma or cirrhosis. HBV infection is responsible for between 600,000 and 1,000,000 deaths per year.

Current therapeutic approaches to HBV infection have severe limitations. Antiviral medications, e.g., tenofovir, a nucleotide reverse transcriptase inhibitor, can decrease viral replication but do not cure HBV infected patients. These antiviral therapies can cost patients as much as $500 to $1500 monthly. Due to the extent of liver damage caused by HBV, a transplant becomes necessary in some cases. In addition to the risks inherent in organ transplants, the cost can be prohibitive. Therefore, improved methods for treating HBV infection are urgently required.

SUMMARY OF THE INVENTION

As described below, the present invention features compositions and methods for treating hepatitis B virus (HBV) infection by introducing alterations into the HBV genome. In particular embodiments, the invention provides a base editor system (e.g., a fusion protein comprising a programable DNA binding protein, a nucleobase editor and gRNA) for modifying the HBV genome to introduce changes, such as premature stop codons or in the coding sequence of HBV or deamination of nucleobases in HBV covalently closed circular DNA (cccDNA).

Provided herein are methods and compositions for editing hepatitis B (HBV) genome and related treatment and uses thereof. In one aspect, provided herein is a method of editing a nucleobase of a hepatitis B virus (HBV) genome, in which the method comprises contacting the HBV genome with one or more guide RNAs and a base editor comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase or cytidine deaminase domain, wherein said guide RNA targets said base editor to effect an alteration of the nucleobase of the HBV genome. In some embodiments, the nucleobase of the HBV genome in a polynucleotide encoding an HBV protein. In some embodiments, the contacting is in a eukaryotic cell, a mammalian cell, or a human cell. In some embodiments, the contacting is in a cell *in vivo* or *ex vivo*. In some embodiments, the cytidine deaminase converts a target C to U in the HBV genome. In some embodiments, the cytidine deaminase converts a target C•G to T•A in the polynucleotide encoding the HBV protein. In some embodiments, the adenosine deaminase converts a target A•T to G•C in the polynucleotide encoding the HBV protein.

In some embodiments of the above-delineated method, the alteration of the nucleobase in the HBV genome in the polynucleotide encoding the HBV protein results in a premature termination codon. In some embodiments, the alteration of the nucleobase results in an R87* or W120* termination in an HBV X protein. In some embodiments, the alteration of the nucleobase results in an W35* or W36* in an HBV S protein. In some embodiments, the alteration of the HBV polynucleotide is a missense mutation. In some embodiments, the missense mutation is in an HBV pol gene. In some embodiments, the missense mutation results in a E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P, R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P in an HBV polymerase protein encoded by the HBV pol gene. In some embodiments, the missense mutation is in an HBV core gene. In some embodiments, the missense mutation results in a T160A, T160A, P161F, S162L, C183R, or *184Q in an HBV core protein encoded by the HBV core gene. In some embodiments, the missense mutation is in an HBV X gene. In some embodiments, the missense mutation results in a H86R, W120R, E122K, E121K, or L141P in an HBV X protein encoded by the HBV X gene. In certain embodiments, the missense mutation results in a S38F, L39F, W35R, W36R, T37I, T37A, R78Q, S34L, F80P, or D33G in an HBV S protein encoded by the HBV S gene.

In some embodiments of the above-delineated method, the polynucleotide programmable DNA binding domain provided herein is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof. In some embodiments, the Cas9 has protospacer-adjacent motif (PAM) specificity for a nucleic acid sequence selected from 5′-NGG-3′, 5′-NAG-3′, 5′-NGA-3′, 5′-NAA-3′, 5′-NNAGGA-3′, or 5′-NNACCA-3′. In some embodiments, the polynucleotide programmable DNA binding domain comprises a modified Cas9 having an altered protospacer-adjacent motif (PAM) specificity. In some embodiments, the altered PAM is selected from 5′-NNNRRT-3′, NGA-3′, 5′-NGCG-3′, 5′-NGN-3′, NGCN-3′, 5′-NGTN-3′, or 5′-NAA-3′. In some embodiments, the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant. In some embodiments, the nuclease inactive or nickase variant is a nuclease inactivated Cas9 (dCas9) which comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

In some embodiments of the above-delineated method, the adenosine deaminase domain is capable of deaminating adenine in deoxyribonucleic acid (DNA). In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is TadA*7.10, TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24. In some embodiments, the cytidine deaminase domain is capable of deaminating cytidine in DNA. In some embodiments, the cytidine deaminase is APOBEC or a derivative thereof. In some embodiments, the base editor further comprises a uracil glycosylase inhibitor (UGI). In some embodiments, the base editor does not comprise a uracil glycosylase inhibitor (UGI).

In some embodiments of the above-delineated method, the one or more guide RNAs for editing a nucleobase in the HBV genome comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to an HBV nucleic acid sequence. In some embodiments, the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an HBV nucleic acid sequence. In some embodiments, the HBV protein is the HBV S, polymerase (pol), core, or X protein.

In some aspects, the above-delineated method for editing a nucleobase of a hepatitis B virus (HBV) genome comprises editing one or more nucleobases. In some embodiments, the method comprises two or more guide RNAs that target two or more HBV nucleic acid sequences. In some embodiments, the guide RNAs comprise a sequence, from 5′ to 3′, or a 1, 2, 3, 4, or 5 nucleotide 5′ truncation fragment thereof, selected from one or more of UCAAUCCCAACAAGGACACC;
GGGAACAAGAUCUACAGCAU;AAGCCCAGGAUGAUGGGAUG;
CUGCCAACUGGAUCCUGCGC;GACACAUCCAGCGAUAACCA;
GCUGCCAACUGGAUCCUGCG;UAUGGAUGAUGUGGUAUUGG;
CCAUGCCCCAAAGCCACCCA;AAGCCACCCAAGGCACAGCU;
GAGAAGUCCACCACGAGUCU;CUUCUCUCAAUUUUCUAGGG;
GACGACGAGGCAGGUCCCCU;CCCAACAAGGACACCUGGCC;
UGCCAACUGGAUCCUGCGCG;AGGAGUUCCGCAGUAUGGAU;
CCGCAGUAUGGAUCGGCAGA;CCUCUGCCGAUCCAUACUGC;
CGCCCACCGAAUGUUGCCCA;GACUUCUCUCAAUUUUCUAG;
GUUCCGCAGUAUGGAUCGGC;UACUAACAUUGAGGUUCCCG;
UCCGCAGUAUGGAUCGGCAG;UCCUCUGCCGAUCCAUACUG;
GUAGCUCCAAAUUCUUUAUA; or AAUCCACACUCCGAAAGACA.

In another aspect, a method of treating hepatitis B virus (HBV) infection in a subject is provided, in which the method comprises administering to a subject in need thereof a fusion protein or polynucleotide encoding said fusion protein, the fusion protein comprising a polynucleotide programmable DNA binding domain and a base editor domain that is an adenosine deaminase or a cytidine deaminase domain and one or more guide polynucleotides that target the base editor domain to effect an A•T to G•C, C•G to T•A, or C•G to U•A alteration of the nucleic acid sequence encoding an HBV polypeptide.

In another aspect, a method of treating hepatitis B virus (HBV) infection in a subject is provided, in which the method comprises administering to a subject in need thereof one or more polynucleotides encoding a polynucleotide programmable DNA binding domain and a base editor domain that is an adenosine deaminase or a cytidine deaminase domain, and one or more guide polynucleotides that target the base editor domain to effect an A•T to G•C, C•G to T•A, or C•G to U•A alteration of the nucleic acid sequence encoding an HBV polypeptide.

In some embodiments of the above-delineated treatment methods, the subject is a mammal or a human. In some embodiments, the methods comprise delivering the fusion protein, the polynucleotide encoding said fusion protein, or the one or more polynucleotides encoding a polynucleotide programmable DNA binding domain and the base editor domain, and said one or more guide polynucleotides to a cell of the subject. In some embodiments, the cell is a hepatocyte. In some embodiments, the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or a variant thereof. In some embodiments, the Cas9 has protospacer-adjacent motif (PAM) specificity for a nucleic acid sequence selected from 5′-NGG-3′, 5′-NAG-3′, 5′-NGA-3′, 5′-NAA-3′, 5′-NNAGGA-3′, or 5′-NNACCA-3′. In some embodiments, the polynucleotide programmable DNA binding domain comprises a modified Cas9 having an altered protospacer-adjacent motif (PAM) specificity. In some embodiments, the nucleic acid sequence of the altered PAM is selected from 5′-NNNRRT-3′, NGA-3′, 5′-NGCG-3′, 5′-NGN-3′, NGCN-3′, 5′-NGTN-3′, or 5′-NAA-3′. In some embodiments, the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant. In some embodiments, the nuclease inactive or nickase variant is a nuclease inactivated Cas9 (dCas9) which comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof. In some embodiments, the adenosine deaminase domain is capable of deaminating adenine in deoxyribonucleic acid (DNA). In some embodiments, adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is TadA*7.10, TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24. In some embodiments, the cytidine deaminase domain is capable of deaminating cytidine in DNA. In some embodiments, the cytidine deaminase is APOBEC or a derivative thereof. In some embodiments, the base editor further comprises one or more uracil glycosylase inhibitors (UGIs). In some embodiments, the base editor does not comprise a uracil glycosylase inhibitor (UGI). In some embodiments, the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to an HBV nucleic acid sequence. In some embodiments, the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an HBV nucleic acid

sequence. In some embodiment, the sgRNA comprises a nucleic acid sequence comprising at least 10 contiguous nucleotides that are complementary to the HBV nucleic acid sequence. In some embodiments, the sgRNA comprises a nucleic acid sequence comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that are complementary to the HBV nucleic acid sequence.

In some embodiments, the above-delineated methods comprise editing one or more nucleobases. In some embodiments, the above described methods comprise two or more guide RNAs that target two or more HBV nucleic acid sequences. In some embodiments, the above-delineated methods comprise two or more guide RNAs that target three, four, or five HBV nucleic acid sequences. In some embodiments of the above-delineated methods, the HBV nucleic acid sequences encode one or more HBV proteins selected from HBV polymerase, HBV core protein, HBV S protein, HBV X protein, or a combination thereof. In some embodiments of the above-delineated methods, the one or more guide RNAs comprise a sequence, from 5′ to 3′, or a 1, 2, 3, 4, or 5 nucleotide 5′ truncation fragment thereof, selected from one or more of UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU;AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC;GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG;UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA;AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU;CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU;CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG;AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA;CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA;GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC;UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG;UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; or AAUCCACACUCCGAAAGACA. In some embodiments, the alteration of the polynucleotide encoding the HBV protein is a premature termination codon. In some embodiments, the alteration of the nucleic acid sequence results in an R87* or W120* in an HBV X protein encoded by the nucleic acid. In some embodiments, the alteration of the nucleic acid sequence results in a W35* or W36* in an HBV S protein encoded by the nucleic acid. In some embodiments, the alteration of the polynucleotide encoding the HBV protein is a missense mutation. In some embodiments, the

missense mutation is in an HBV pol gene. In some embodiments, the missense mutation in the HBV pol gene results in a E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P, R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P in an HBV polymerase encoded by the HBV pol gene. In some embodiments, the missense mutation is in an HBV core gene. In some embodiments, the missense mutation in the HBV core gene results in a T160A, T160A, P161F, S162L, C183R, or *184Q in an HBV core protein encoded by the HBV core gene. In some embodiments, the missense mutation is in an HBV X gene. In some embodiments, the missense mutation results in a H86R, W120R, E122K, E121K, or L141P in an HBV X protein ncoded by the HBV X gene. In some embodiments, the missense mutation is in an HBV S gene. In some embodiments, the missense mutation results in a S38F, L39F, W35R, W36R, T37I, T37A, R78Q, S34L, F80P, or D33G in an HBV S protein encoded by the HBV S gene. In some embodiments, the base editor is a BE4 or a variant of BE4 where APOBEC-1 is replaced with the sequence of APOBEC-3A, and/or Cas9 is replaced with a Cas9 variant comprising V1134, R1217, Q1334, and R1336 (termed SpCas9-VRQR).

In an aspect, compositions are provided, e.g., for treatment of HBV infection. In one aspect, composition is provided, in which the composition comprises a base editor bound to a guide RNA, wherein the guide RNA comprises a nucleic acid sequence that is complementary to an HBV gene. In an embodiment, the base editor is an adenosine deaminase or a cytidine deaminase. In an embodiment, the adenosine deaminase is capable of deaminating adenine in deoxyribonucleic acid (DNA). In an embodiment, the adenosine deaminase is a TadA deaminase. In an embodiment, the TadA deaminase is TadA*7.10, TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24. In an embodiment, the cytidine deaminase domain is capable of deaminating cytidine in DNA. In an embodiment, the cytidine deaminase is APOBEC or a derivative thereof. In an embodiment, the base editor further comprises one or more uracil glycosylase inhibitors (UGIs). In an embodiment, the base editor does not comprise a uracil glycosylase inhibitor (UGI). In an embodiment, the base editor (i) comprises a Cas9 nickase;

(ii) comprises a nuclease inactive Cas9;

(iii) does not comprise a UGI domain;

(iv) comprises an APOBEC-1 or APOBEC-3A cytidine deaminase;

(v) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQN
TNKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARL
YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLE
LYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETP
GTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKK
NLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLV
EEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFL
IEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPG
EKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA
KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS
KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
ELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE
EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS
GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD
KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRK
LINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL
AGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIK
ELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDS
IDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDK
AGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVK
ELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATL
IHQSITGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE;or

(vi) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI

RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE.

In an embodiment, the guide RNA of the composition comprises a nucleic acid sequence that is complementary to an HBV gene encoding an HBV polymerase, HBV core protein, HBV S protein, or HBV X protein. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV X protein. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV S protein. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV polymerase. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV core protein. In an embodiment, the guide RNA comprises a 1, 2, 3, 4, or 5 nucleic acid truncation from the 5′ end of a nucleic acid selected from the group consisting of, from 5′ to 3′,

UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU;
AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC;
GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG;
UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA;
AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU;
CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU;
CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG;
AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA;
CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA;
GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC;
UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG;

UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA. In an embodiment, the guide RNA comprises a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU; AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC; GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG; UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA; AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU; CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU; CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA. In an embodiment, the above-delineated composition further comprises a lipid. In an embodiment, the lipid is a cationic lipid. In an embodiment, the composition further comprises a pharmaceutically acceptable excipient.

In another aspect, a pharmaceutical composition is provided, in which the pharmaceutical composition comprises a base editor, or a nucleic acid encoding the base editor, and one or more guide RNAs (gRNAs) comprising a nucleic acid sequence complementary to an HBV gene in a pharmaceutically acceptable excipient. In an embodiment, the base editor (i) comprises a Cas9 nickase;

(ii) comprises a nuclease inactive Cas9;

(iii) does not comprise a UGI domain;

(iv) comprises an APOBEC-1 or APOBEC-3A cytidine deaminase;

(v) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL

NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE;or

(vi) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE.

In an embodiment, the base editor comprises a Cas9, or a Cas9 variant comprising V1134, R1217, Q1334, and R1336 (SpCas9-VRQR). In an embodiment, the gRNA and the base editor are formulated together or separately. In an embodiment, the gRNA comprises a nucleic acid sequence, from 5′ to 3′, or a 1, 2, 3, 4, or 5 nucleotide 5′ truncation fragment thereof, selected from one or more of UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; or AAUCCACACUCCGAAAGACA. In an embodiment, the pharmaceutical composition further comprises a vector suitable for expression in a mammalian cell, wherein the vector comprises a polynucleotide encoding the base editor. In an embodiment, the vector is a viral vector. In an embodiment, the viral vector is a retroviral vector, adenoviral vector, lentiviral vector, herpesvirus vector, or adeno-associated viral vector (AAV). In an embodiment, the pharmaceutical composition further comprises a ribonucleoparticle suitable for expression in a mammalian cell.

In another aspect, a method of treating HBV infection is provided, in which the method comprises administering to a subject in need thereof the above-delineated composition or pharmaceutical composition.

Another aspect provides an HBV genome comprising an alteration selected from the group consisting of:

a premature termination codon introducing a R87STOP or W120STOP in the X gene;

a premature termination codon introducing a W35STOP or W36STOP in the S gene;

a missense mutation in the HBV pol gene that introduces a E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P,

R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P in HBV polymerase;

a missense mutation is in the HBV core gene that introduces a T160A, T160A, P161F, S162L, C183R, or STOP184Q in the HBV Core polypeptide;

a missense mutation is in the X gene that introduces a H86R, W120R, E122K, E121K, or L141P in the HBV X polypeptide; and

a missense mutation in the S gene that introduces a S38F, L39F, W35R, W36R, T37I, T37A, R78Q, S34L, F80P, or D33G in the HBV S polypeptide.

In an embodiment, the HBV genome comprises two or more of the above described alterations.

In embodiments of the above-delineated methods, or the above-delineated HBV genome, the HBV is of genotype C or genotype D.

Provided in another aspect is a use of the composition of any of the above-delineated aspects and embodiments in the treatment of HBV infection in a subject.

Provided in another aspect is a use of the pharmaceutical composition of any of the above-delineated aspects and embodiments in the treatment of HBV infection in a subject.

In an embodiment of the above-delineated uses, the subject is a mammal. In an embodiment of the above-delineated uses, the subject is a human.

In an embodiment of the above-delineated methods or pharmaceutical compositions, the one or more guide RNAs are as listed in Table 26.

In another aspect, a guide RNA (gRNA) is provided which comprises a nucleic acid sequence that is complementary to an HBV gene. In an embodiment, the guide RNA comprises a nucleic acid sequence that is complementary to an HBV gene encoding an HBV polymerase, HBV core protein, HBV S protein, or HBV X protein. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV X protein. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV S protein. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV polymerase. In an embodiment, the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that

encodes an HBV core protein. In an embodiment, the guide RNA comprises a 1, 2, 3, 4, or 5 nucleic acid truncation from the 5′ end of a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU; AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC; GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG; UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA; AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU; CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU; CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA. In an embodiment, the guide RNA comprises a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU; AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC; GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG; UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA; AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU; CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU; CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA.

In another aspect, a pharmaceutical composition is provided, in which the pharmaceutical composition comprises (i) a nucleic acid encoding a base editor; and (ii) the guide RNA of any of the above-delineated aspects and embodiments. In an embodiment, the

pharmaceutical composition further comprises a lipid. In an embodiment of the tpharmaceutical composition, the nucleic acid encoding the base editor is an mRNA.

Compositions and articles defined by the invention were isolated or otherwise manufactured in connection with the examples provided below. Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive

or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, e.g., within 5-fold, within 2-fold of a value. Where particular values are described in the application and claims, unless otherwise stated, the term "about" means within an acceptable error range for the particular value should be assumed.

Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

By "adenosine deaminase" is meant a polypeptide or fragment thereof capable of catalyzing the hydrolytic deamination of adenine or adenosine. In some embodiments, the deaminase or deaminase domain is an adenosine deaminase catalyzing the hydrolytic deamination of adenosine to inosine or deoxy adenosine to deoxyinosine. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (*e.g.*, engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism, such as a bacterium.

In some embodiments, the deaminase or deaminase domain is a variant of a naturally-occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%,

or at least 99.5% identical to a naturally-occurring deaminase. In some embodiments, the adenosine deaminase is from a bacterium, such as, E. coli, S. aureus, S. typhi, S. putrefaciens, H. influenzae, or C. crescentus. In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is an E. coli TadA (ecTadA) deaminase or a fragment thereof.

For example, deaminase domains are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also, see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017)), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1, the entire contents of which are hereby incorporated by reference.

A wild type TadA(wt) adenosine deaminase has the following sequence (also termed TadA reference sequence):

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD.

In some embodiments, the adenosine deaminase comprises an alteration in the following sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

(also termed TadA*7.10).

In some embodiments, TadA*7.10 comprises at least one alteration. In some embodiments, TadA*7.10 comprises an alteration at amino acid 82 and/or 166. In particular embodiments, a variant of the above-referenced sequence comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. The alteration Y123H refers to the alteration H123Y in TadA*7.10 reverted back to Y123H

TadA(wt). In other embodiments, a variant of the TadA*7.10 sequence comprises a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In other embodiments, the invention provides adenosine deaminase variants that include deletions, *e.g.*, TadA*8, comprising a deletion of the C-terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, or 157, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a TadA (*e.g.,* TadA*8) monomer comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a TadA (*e.g.*, TadA*8) monomer comprising the following alterations: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

In still other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains each having one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains (*e.g.*, TadA*8) each having a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y;

V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a wild-type TadA adenosine deaminase domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a wild-type TadA adenosine deaminase domain and an adenosine deaminase variant domain (*e.g.* TadA*8) comprising a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.* TadA*8) comprising a combination of the following alterations: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; or I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In one embodiment, the adenosine deaminase is a TadA*8 that comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

In some embodiments, the TadA*8 is truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8. In particular embodiments, an adenosine deaminase heterodimer comprises a TadA*8 domain and an adenosine deaminase domain selected from one of the following:

Staphylococcus aureus (S. aureus) TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAH
AEHIAIERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGS
LMNLLQQSNFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

Bacillus subtilis (B. subtilis) TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEML
VIDEACKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGCSGTLMN
LLQEERFNHQAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEG
WNRPIGRHDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIG
RVVFGARDAKTGAAGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIK
ALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:

MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEI
LCLRSAGKKLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGT
VVNLLQHPAFNHQVEVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

Haemophilus influenzae F3031 (*H. influenzae*) TadA:

MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDPTAH
AEIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYK
TGAIGSRFHFFDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSDK

Caulobacter crescentus (*C. crescentus*) TadA:

MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAH
DPTAHAEIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADD
PKGGAVVHGPKFFAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:

MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSN
DPSAHAEMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDP
KGGAAGSLYDLSADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALF
IDERKVPPEP

TadA*7.10

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

By "Adenosine Deaminase Base Editor 8 (ABE8) polypeptide" or "ABE8" is meant a base editor as defined herein comprising an adenosine deaminase variant comprising an alteration at amino acid position 82 and/or 166 of the following reference sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

In some embodiments, ABE8 comprises further alterations, as described herein, relative to the reference sequence.

By "Adenosine Deaminase Base Editor 8 (ABE8) polynucleotide" is meant a polynucleotide encoding an ABE8.

"Administering" is referred to herein as providing one or more compositions described herein to a patient or a subject. By way of example and without limitation, composition administration, *e.g.*, injection, can be performed by intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or intramuscular (i.m.) injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. Alternatively, or concurrently, administration can be by an oral route.

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those

described herein. As used herein, an alteration includes a 10% change in expression levels, a 25% change, a 40% change, and a 50% or greater change in expression levels.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By “analog” is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

By "base editor (BE)," or "nucleobase editor (NBE)" is meant an agent that binds a polynucleotide and has nucleobase modifying activity. In various embodiments, the base editor comprises a nucleobase modifying polypeptide (*e.g.*, a deaminase) and a polynucleotide programmable nucleotide binding domain in conjunction with a guide polynucleotide (*e.g.*, guide RNA). In various embodiments, the agent is a biomolecular complex comprising a protein domain having base editing activity, *i.e.*, a domain capable of modifying a base (*e.g.*, A, T, C, G, or U) within a nucleic acid molecule (*e.g.*, DNA). In some embodiments, the polynucleotide programmable DNA binding domain is fused or linked to a deaminase domain. In one embodiment, the agent is a fusion protein comprising one or more domains having base editing activity. In another embodiment, the protein domains having base editing activity are linked to the guide RNA (*e.g.*, via an RNA binding motif on the guide RNA and an RNA binding domain fused to the deaminase). In some embodiments, the domains having base editing activity are capable of deaminating a base within a nucleic acid molecule. In some embodiments, the base editor is capable of deaminating one or more bases within a DNA molecule. In some embodiments, the base editor is capable of deaminating a cytosine (C) or an adenosine (A) within DNA. In some embodiments, the base editor is capable of deaminating a cytosine (C) and an adenosine (A) within DNA. In some embodiments, the base editor is a cytidine base editor (CBE). In some embodiments, the base editor is an adenosine base editor (ABE). In some embodiments, the base editor is an adenosine base editor (ABE) and a cytidine base editor (CBE). In some embodiments, the base editor is a nuclease-inactive Cas9 (dCas9) fused to an adenosine deaminase. In some embodiments, the Cas9 is a circular permutant Cas9 (e.g., spCas9 or

saCas9). Circular permutant Cas9s are known in the art and described, for example, in Oakes et al., Cell 176, 254–267, 2019. In some embodiments, the base editor is fused to an inhibitor of base excision repair, for example, a UGI domain, or a dISN domain. In some embodiments, the fusion protein comprises a Cas9 nickase fused to a deaminase and an inhibitor of base excision repair, such as a UGI or dISN domain. In other embodiments the base editor is an abasic base editor.

In some embodiments, an adenosine deaminase is evolved from TadA. In some embodiments, the polynucleotide programmable DNA binding domain is a CRISPR associated (*e.g.*, Cas or Cpf1) enzyme. In some embodiments, the base editor is a catalytically dead Cas9 (dCas9) fused to a deaminase domain. In some embodiments, the base editor is a Cas9 nickase (nCas9) fused to a deaminase domain. In some embodiments, the base editor is fused to an inhibitor of base excision repair (BER). In some embodiments, the inhibitor of base excision repair is a uracil DNA glycosylase inhibitor (UGI). In some embodiments, the inhibitor of base excision repair is an inosine base excision repair inhibitor. Details of base editors are described in International PCT Application Nos. PCT/2017/045381 (WO2018/027078) and PCT/US2016/058344 (WO2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1, the entire contents of which are hereby incorporated by reference.

In some embodiments, base editors are generated (*e.g.,* ABE8) by cloning an adenosine deaminase variant (*e.g.*, TadA*8) into a scaffold that includes a circular permutant Cas9 (*e.g.*, spCAS9) and a bipartite nuclear localization sequence. Circular permutant Cas9s are known in the art and described, for example, in Oakes *et al.*, Cell 176, 254–267, 2019. Exemplary circular permutant sequences are set forth below, in which the bold sequence indicates sequence derived from Cas9, the italics sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

CP5 (with MSP "NGC=Pam Variant with mutations Regular Cas9 likes NGG" PID=Protein Interacting Domain and "D10A" nickase):

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYR
STKEVLDATLIHQSITGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM*DKKYSIGLAI
GTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTI
YHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE
ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLA
EDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILT
FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF
KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHK
PENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ
NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM
KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNT
KYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPK
LESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

In some embodiments, the ABE8 is selected from a base editor from Table 8 *infra*. In some embodiments, ABE8 contains an adenosine deaminase variant evolved from TadA. In some embodiments, the adenosine deaminase variant of ABE8 is a TadA*8 variant as described in Table 8 *infra*. In some embodiments, the adenosine deaminase variant is the TadA*7.10 variant (*e.g.*, TadA*8) comprising one or more of an alteration selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In various embodiments, ABE8 comprises TadA*7.10 variant (*e.g.* TadA*8) with a combination of alterations selected from the group of Y147T + Q154R; Y147T + Q154S; Y147R +

Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

In some embodiments ABE8 is a monomeric construct. In some embodiments, ABE8 is a heterodimeric construct. In some embodiments the ABE8 base editor comprises the sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD

By way of example, the adenine base editor ABE to be used in the base editing compositions, systems and methods described herein has the nucleic acid sequence (8877 base pairs), (Addgene, Watertown, MA.; Gaudelli NM, *et al.*, Nature. 2017 Nov 23;551(7681):464-471. doi: 10.1038/nature24644; Koblan LW, *et al.*, Nat Biotechnol. 2018 Oct;36(9):843-846. doi: 10.1038/nbt.4172.) as provided below. Polynucleotide sequences having at least 95% or greater identity to the ABE nucleic acid sequence are also encompassed.

ATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT
GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG
ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCC
ATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGT
CAGATCCGCTAGAGATCCGCGGCCGCTAATACGACTCACTATAGGGAGAGCCGCCACCATGAAACGGACA
GCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTCTCTGAAGTCGAGTTTAGCCACGAGT
ATTGGATGAGGCACGCACTGACCCTGGCAAAGCGAGCATGGGATGAAAGAGAAGTCCCCGTGGGCGCCGT
GCTGGTGCACAACAATAGAGTGATCGGAGAGGGATGGAACAGGCCAATCGGCCGCCACGACCCTACCGCA
CACGCAGAGATCATGGCACTGAGGCAGGGAGGCCTGGTCATGCAGAATTACCGCCTGATCGATGCCACCC
TGTATGTGACACTGGAGCCATGCGTGATGTGCGCAGGAGCAATGATCCACAGCAGGATCGGAAGAGTGGT
GTTCGGAGCACGGGACGCCAAGACCGGCGCAGCAGGCTCCCTGATGGATGTGCTGCACCACCCCGGCATG
AACCACCGGGTGGAGATCACAGAGGGAATCCTGGCAGACGAGTGCGCCGCCCTGCTGAGCGATTTCTTTA
GAATGCGGAGACAGGAGATCAAGGCCCAGAAGAAGGCACAGAGCTCCACCGACTCTGGAGGATCTAGCGG
AGGATCCTCTGGAAGCGAGACACCAGGCACAAGCGAGTCCGCCACACCAGAGAGCTCCGGCGGCTCCTCC
GGAGGATCCTCTGAGGTGGAGTTTTCCCACGAGTACTGGATGAGACATGCCCTGACCCTGGCCAAGAGGG
CACGCGATGAGAGGGAGGTGCCTGTGGGAGCCGTGCTGGTGCTGAACAATAGAGTGATCGGCGAGGGCTG
GAACAGAGCCATCGGCCTGCACGACCCAACAGCCCATGCCGAAATTATGGCCCTGAGACAGGGCGGCCTG

GTCATGCAGAACTACAGACTGATTGACGCCACCCTGTACGTGACATTCGAGCCTTGCGTGATGTGCGCCG
GCGCCATGATCCACTCTAGGATCGGCCGCGTGGTGTTTGGCGTGAGGAACGCAAAAACCGGCGCCGCAGG
CTCCCTGATGGACGTGCTGCACTACCCCGGCATGAATCACCGCGTCGAAATTACCGAGGGAATCCTGGCA
GATGAATGTGCCGCCCTGCTGTGCTATTTCTTTCGGATGCCTAGACAGGTGTTCAATGCTCAGAAGAAGG
CCCAGAGCTCCACCGACTCCGGAGGATCTAGCGGAGGCTCCTCTGGCTCTGAGACACCTGGCACAAGCGA
GAGCGCAACACCTGAAAGCAGCGGGGGCAGCAGCGGGGGGTCAGACAAGAAGTACAGCATCGGCCTGGCC
ATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGG
TGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGA
AACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGC
TATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGT
CCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGC
CTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGAC
CTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACC
TGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGA
GGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGA
CGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCC
TGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAG
CAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTT
CTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCA
AGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGC
TCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCC
GGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGG
ACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAA
CGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTAC
CCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCC
CTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAA
CTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAG
AACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGC
TGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGC
CATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAG
AAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACAT
ACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGA
AGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCC
CACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCC
GGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGG
CTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAA
GCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTA
AGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGA
GAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGA
ATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACA

CCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGA
ACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGAC
TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAG
AGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTT
CGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAG
CTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACG
ACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCG
GAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAAC
GCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACA
AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTT
CTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGG
CCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGC
GGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAA
AGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAG
TACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGT
CCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAA
TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAG
TACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAA
ACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGG
CTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATC
GAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCT
ACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAA
TCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAA
GAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTC
AGCTGGGAGGTGACTCTGGCGGCTCAAAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAAGAG
GAAAGTCTAACCGGTCATCATCACCATCACCATTGAGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC
TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT
GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCT
CTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCGATACCGTCGACCTCTAGCTAGAGCTTGGCGTA
ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGA
AGCATAAAGTGTAAAGCCTAGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGC
CCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGG
TTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGA
GCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACA
TGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT
CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAA
AGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGAT
ACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC
GGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA

TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTA
ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC
TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCA
GAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACACTCAGTGGAACGAAAACTC
ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGA
AGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGG
CACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTAC
GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCA
GATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCT
CCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGT
TGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCC
CAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGA
TCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTAC
TGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGT
ATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAA
AAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG
TTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGA
GCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATAC
TCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATG
TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCGACGGA
TCGGGAGATCGATCTCCCGATCCCCTAGGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA
GCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAAC
AAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGAT
GTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT
TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATC

By way of example, a cytidine base editor (CBE) as used in the base editing compositions, systems and methods described herein has the following nucleic acid sequence (8877 base pairs), (Addgene, Watertown, MA.; Komor AC, *et al.*, 2017, Sci Adv., 30;3(8):eaao4774. doi: 10.1126/sciadv.aao4774) as provided below. Polynucleotide sequences having at least 95% or greater identity to the BE4 nucleic acid sequence are also encompassed.

1 ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA ATGGCCCGCC TGGCATTATG
61 CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG
121 CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG GCGTGGATAG CGGTTTGACT
181 CACGGGGATT TCCAAGTCTC CACCCCATTG ACGTCAATGG GAGTTTGTTT TGGCACCAAA
241 ATCAACGGGA CTTTCCAAAA TGTCGTAACA ACTCCGCCCC ATTGACGCAA ATGGGCGGTA

301 GGCGTGTACG GTGGGAGGTC TATATAAGCA GAGCTGGTTT AGTGAACCGT CAGATCCGCT
361 AGAGATCCGC GGCCGCTAAT ACGACTCACT ATAGGGAGAG CCGCCACCAT GAGCTCAGAG
421 ACTGGCCCAG TGGCTGTGGA CCCCACATTG AGACGGCGGA TCGAGCCCCA TGAGTTTGAG
481 GTATTCTTCG ATCCGAGAGA GCTCCGCAAG GAGACCTGCC TGCTTTACGA AATTAATTGG
541 GGGGGCCGGC ACTCCATTTG GCGACATACA TCACAGAACA CTAACAAGCA CGTCGAAGTC
601 AACTTCATCG AGAAGTTCAC GACAGAAAGA TATTTCTGTC CGAACACAAG GTGCAGCATT
661 ACCTGGTTTC TCAGCTGGAG CCCATGCGGC GAATGTAGTA GGGCCATCAC TGAATTCCTG
721 TCAAGGTATC CCCACGTCAC TCTGTTTATT TACATCGCAA GGCTGTACCA CCACGCTGAC
781 CCCCGCAATC GACAAGGCCT GCGGGATTTG ATCTCTTCAG GTGTGACTAT CCAAATTATG
841 ACTGAGCAGG AGTCAGGATA CTGCTGGAGA AACTTTGTGA ATTATAGCCC GAGTAATGAA
901 GCCCACTGGC CTAGGTATCC CCATCTGTGG GTACGACTGT ACGTTCTTGA ACTGTACTGC
961 ATCATACTGG GCCTGCCTCC TTGTCTCAAC ATTCTGAGAA GGAAGCAGCC ACAGCTGACA
1021 TTCTTTACCA TCGCTCTTCA GTCTTGTCAT TACCAGCGAC TGCCCCCACA CATTCTCTGG
1081 GCCACCGGGT TGAAATCTGG TGGTTCTTCT GGTGGTTCTA GCGGCAGCGA GACTCCCGGG
1141 ACCTCAGAGT CCGCCACACC CGAAAGTTCT GGTGGTTCTT CTGGTGGTTC TGATAAAAAG
1201 TATTCTATTG GTTTAGCCAT CGGCACTAAT TCCGTTGGAT GGGCTGTCAT AACCGATGAA
1261 TACAAAGTAC CTTCAAAGAA ATTTAAGGTG TTGGGGAACA CAGACCGTCA TTCGATTAAA
1321 AAGAATCTTA TCGGTGCCCT CCTATTCGAT AGTGGCGAAA CGGCAGAGGC GACTCGCCTG
1381 AAACGAACCG CTCGGAGAAG GTATACACGT CGCAAGAACC GAATATGTTA CTTACAAGAA
1441 ATTTTTAGCA ATGAGATGGC CAAAGTTGAC GATTCTTTCT TTCACCGTTT GGAAGAGTCC
1501 TTCCTTGTCG AAGAGGACAA GAAACATGAA CGGCACCCCA TCTTTGGAAA CATAGTAGAT
1561 GAGGTGGCAT ATCATGAAAA GTACCCAACG ATTTATCACC TCAGAAAAAA GCTAGTTGAC
1621 TCAACTGATA AAGCGGACCT GAGGTTAATC TACTTGGCTC TTGCCCATAT GATAAAGTTC
1681 CGTGGGCACT TTCTCATTGA GGGTGATCTA AATCCGGACA ACTCGGATGT CGACAAACTG
1741 TTCATCCAGT TAGTACAAAC CTATAATCAG TTGTTTGAAG AGAACCCTAT AAATGCAAGT
1801 GGCGTGGATG CGAAGGCTAT TCTTAGCGCC CGCCTCTCTA AATCCCGACG GCTAGAAAAC
1861 CTGATCGCAC AATTACCCGG AGAGAAGAAA AATGGGTTGT TCGGTAACCT TATAGCGCTC
1921 TCACTAGGCC TGACACCAAA TTTTAAGTCG AACTTCGACT TAGCTGAAGA TGCCAAATTG
1981 CAGCTTAGTA AGGACACGTA CGATGACGAT CTCGACAATC TACTGGCACA AATTGGAGAT
2041 CAGTATGCGG ACTTATTTTT GGCTGCCAAA AACCTTAGCG ATGCAATCCT CCTATCTGAC
2101 ATACTGAGAG TTAATACTGA GATTACCAAG GCGCCGTTAT CCGCTTCAAT GATCAAAAGG
2161 TACGATGAAC ATCACCAAGA CTTGACACTT CTCAAGGCCC TAGTCCGTCA GCAACTGCCT
2221 GAGAAATATA AGGAAATATT CTTTGATCAG TCGAAAAACG GGTACGCAGG TTATATTGAC
2281 GGCGGAGCGA GTCAAGAGGA ATTCTACAAG TTTATCAAAC CCATATTAGA GAAGATGGAT
2341 GGGACGGAAG AGTTGCTTGT AAAACTCAAT CGCGAAGATC TACTGCGAAA GCAGCGGACT
2401 TTCGACAACG GTAGCATTCC ACATCAAATC CACTTAGGCG AATTGCATGC TATACTTAGA
2461 AGGCAGGAGG ATTTTTATCC GTTCCTCAAA GACAATCGTG AAAAGATTGA GAAAATCCTA
2521 ACCTTTCGCA TACCTTACTA TGTGGGACCC CTGGCCCGAG GGAACTCTCG GTTCGCATGG
2581 ATGACAAGAA AGTCCGAAGA AACGATTACT CCATGGAATT TTGAGGAAGT TGTCGATAAA
2641 GGTGCGTCAG CTCAATCGTT CATCGAGAGG ATGACCAACT TTGACAAGAA TTTACCGAAC

2701 GAAAAAGTAT TGCCTAAGCA CAGTTTACTT TACGAGTATT TCACAGTGTA CAATGAACTC
2761 ACGAAAGTTA AGTATGTCAC TGAGGGCATG CGTAAACCCG CCTTTCTAAG CGGAGAACAG
2821 AAGAAAGCAA TAGTAGATCT GTTATTCAAG ACCAACCGCA AAGTGACAGT TAAGCAATTG
2881 AAAGAGGACT ACTTTAAGAA AATTGAATGC TTCGATTCTG TCGAGATCTC CGGGGTAGAA
2941 GATCGATTTA ATGCGTCACT TGGTACGTAT CATGACCTCC TAAAGATAAT TAAAGATAAG
3001 GACTTCCTGG ATAACGAAGA GAATGAAGAT ATCTTAGAAG ATATAGTGTT GACTCTTACC
3061 CTCTTTGAAG ATCGGGAAAT GATTGAGGAA AGACTAAAAA CATACGCTCA CCTGTTCGAC
3121 GATAAGGTTA TGAAACAGTT AAAGAGGCGT CGCTATACGG GCTGGGGACG ATTGTCGCGG
3181 AAACTTATCA ACGGGATAAG AGACAAGCAA AGTGGTAAAA CTATTCTCGA TTTTCTAAAG
3241 AGCGACGGCT TCGCCAATAG GAACTTTATG CAGCTGATCC ATGATGACTC TTTAACCTTC
3301 AAAGAGGATA TACAAAAGGC ACAGGTTTCC GGACAAGGGG ACTCATTGCA CGAACATATT
3361 GCGAATCTTG CTGGTTCGCC AGCCATCAAA AAGGGCATAC TCCAGACAGT CAAAGTAGTG
3421 GATGAGCTAG TTAAGGTCAT GGGACGTCAC AAACCGGAAA ACATTGTAAT CGAGATGGCA
3481 CGCGAAAATC AAACGACTCA GAAGGGGCAA AAAAACAGTC GAGAGCGGAT GAAGAGAATA
3541 GAAGAGGGTA TTAAAGAACT GGGCAGCCAG ATCTTAAAGG AGCATCCTGT GGAAAATACC
3601 CAATTGCAGA ACGAGAAACT TTACCTCTAT TACCTACAAA ATGGAAGGGA CATGTATGTT
3661 GATCAGGAAC TGGACATAAA CCGTTTATCT GATTACGACG TCGATCACAT TGTACCCCAA
3721 TCCTTTTTGA AGGACGATTC AATCGACAAT AAAGTGCTTA CACGCTCGGA TAAGAACCGA
3781 GGGAAAAGTG ACAATGTTCC AAGCGAGGAA GTCGTAAAGA AAATGAAGAA CTATTGGCGG
3841 CAGCTCCTAA ATGCGAAACT GATAACGCAA AGAAAGTTCG ATAACTTAAC TAAAGCTGAG
3901 AGGGGTGGCT TGTCTGAACT TGACAAGGCC GGATTTATTA AACGTCAGCT CGTGGAAACC
3961 CGCCAAATCA CAAAGCATGT TGCACAGATA CTAGATTCCC GAATGAATAC GAAATACGAC
4021 GAGAACGATA AGCTGATTCG GGAAGTCAAA GTAATCACTT TAAAGTCAAA ATTGGTGTCG
4081 GACTTCAGAA AGGATTTTCA ATTCTATAAA GTTAGGGAGA TAAATAACTA CCACCATGCG
4141 CACGACGCTT ATCTTAATGC CGTCGTAGGG ACCGCACTCA TTAAGAAATA CCCGAAGCTA
4201 GAAAGTGAGT TTGTGTATGG TGATTACAAA GTTTATGACG TCCGTAAGAT GATCGCGAAA
4261 AGCGAACAGG AGATAGGCAA GGCTACAGCC AAATACTTCT TTTATTCTAA CATTATGAAT
4321 TTCTTTAAGA CGGAAATCAC TCTGGCAAAC GGAGAGATAC GCAAACGACC TTTAATTGAA
4381 ACCAATGGGG AGACAGGTGA AATCGTATGG GATAAGGGCC GGGACTTCGC GACGGTGAGA
4441 AAAGTTTTGT CCATGCCCCA AGTCAACATA GTAAAGAAAA CTGAGGTGCA GACCGGAGGG
4501 TTTTCAAAGG AATCGATTCT TCCAAAAAGG AATAGTGATA AGCTCATCGC TCGTAAAAAG
4561 GACTGGGACC CGAAAAAGTA CGGTGGCTTC GATAGCCCTA CAGTTGCCTA TTCTGTCCTA
4621 GTAGTGGCAA AAGTTGAGAA GGGAAAATCC AAGAAACTGA AGTCAGTCAA AGAATTATTG
4681 GGGATAACGA TTATGGAGCG CTCGTCTTTT GAAAAGAACC CCATCGACTT CCTTGAGGCG
4741 AAAGGTTACA AGGAAGTAAA AAAGGATCTC ATAATTAAAC TACCAAAGTA TAGTCTGTTT
4801 GAGTTAGAAA ATGGCCGAAA ACGGATGTTG GCTAGCGCCG GAGAGCTTCA AAAGGGGAAC
4861 GAACTCGCAC TACCGTCTAA ATACGTGAAT TTCCTGTATT TAGCGTCCCA TTACGAGAAG
4921 TTGAAAGGTT CACCTGAAGA TAACGAACAG AAGCAACTTT TTGTTGAGCA GCACAAACAT
4981 TATCTCGACG AAATCATAGA GCAAATTTCG GAATTCAGTA AGAGAGTCAT CCTAGCTGAT
5041 GCCAATCTGG ACAAAGTATT AAGCGCATAC AACAAGCACA GGGATAAACC CATACGTGAG

5101 CAGGCGGAAA ATATTATCCA TTTGTTTACT CTTACCAACC TCGGCGCTCC AGCCGCATTC
5161 AAGTATTTTG ACACAACGAT AGATCGCAAA CGATACACTT CTACCAAGGA GGTGCTAGAC
5221 GCGACACTGA TTCACCAATC CATCACGGGA TTATATGAAA CTCGGATAGA TTTGTCACAG
5281 CTTGGGGGTG ACTCTGGTGG TTCTGGAGGA TCTGGTGGTT CTACTAATCT GTCAGATATT
5341 ATTGAAAAGG AGACCGGTAA GCAACTGGTT ATCCAGGAAT CCATCCTCAT GCTCCCAGAG
5401 GAGGTGGAAG AAGTCATTGG GAACAAGCCG GAAAGCGATA TACTCGTGCA CACCGCCTAC
5461 GACGAGAGCA CCGACGAGAA TGTCATGCTT CTGACTAGCG ACGCCCCTGA ATACAAGCCT
5521 TGGGCTCTGG TCATACAGGA TAGCAACGGT GAGAACAAGA TTAAGATGCT CTCTGGTGGT
5581 TCTGGAGGAT CTGGTGGTTC TACTAATCTG TCAGATATTA TTGAAAAGGA GACCGGTAAG
5641 CAACTGGTTA TCCAGGAATC CATCCTCATG CTCCCAGAGG AGGTGGAAGA AGTCATTGGG
5701 AACAAGCCGG AAAGCGATAT ACTCGTGCAC ACCGCCTACG ACGAGAGCAC CGACGAGAAT
5761 GTCATGCTTC TGACTAGCGA CGCCCCTGAA TACAAGCCTT GGGCTCTGGT CATACAGGAT
5821 AGCAACGGTG AGAACAAGAT TAAGATGCTC TCTGGTGGTT CTCCCAAGAA GAAGAGGAAA
5881 GTCTAACCGG TCATCATCAC CATCACCATT GAGTTTAAAC CCGCTGATCA GCCTCGACTG
5941 TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT GCCCCTCCCC CGTGCCTTCC TTGACCCTGG
6001 AAGGTGCCAC TCCCACTGTC CTTTCCTAAT AAAATGAGGA AATTGCATCG CATTGTCTGA
6061 GTAGGTGTCA TTCTATTCTG GGGGGTGGGG TGGGGCAGGA CAGCAAGGGG GAGGATTGGG
6121 AAGACAATAG CAGGCATGCT GGGGATGCGG TGGGCTCTAT GGCTTCTGAG GCGGAAAGAA
6181 CCAGCTGGGG CTCGATACCG TCGACCTCTA GCTAGAGCTT GGCGTAATCA TGGTCATAGC
6241 TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA
6301 TAAAGTGTAA AGCCTAGGGT GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT
6361 CACTGCCCGC TTTCCAGTCG GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC
6421 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC
6481 TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT
6541 TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG
6601 CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG
6661 AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT
6721 ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA
6781 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT
6841 GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC
6901 CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA
6961 GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG
7021 TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGAACAG
7081 TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT
7141 GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA
7201 CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC
7261 AGTGGAACGA AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA
7321 CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA
7381 CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT
7441 TTCGTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT

7501 TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT
7561 TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT
7621 CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA
7681 ATAGTTTGCG CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG
7741 GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT
7801 TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG
7861 CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG
7921 TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC
7981 GGCGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA
8041 CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC
8101 CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT
8161 TTACTTTCAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG
8221 GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA
8281 GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA
8341 AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC GACGGATCGG
8401 GAGATCGATC TCCCGATCCC CTAGGGTCGA CTCTCAGTAC AATCTGCTCT GATGCCGCAT
8461 AGTTAAGCCA GTATCTGCTC CCTGCTTGTG TGTTGGAGGT CGCTGAGTAG TGCGCGAGCA
8521 AAATTTAAGC TACAACAAGG CAAGGCTTGA CCGACAATTG CATGAAGAAT CTGCTTAGGG
8581 TTAGGCGTTT TGCGCTGCTT CGCGATGTAC GGGCCAGATA TACGCGTTGA CATTGATTAT
8641 TGACTAGTTA TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA TATATGGAGT
8701 TCCGCGTTAC ATAACTTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC
8761 CATTGACGTC AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC
8821 GTCAATGGGT GGAGTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATC

In some embodiments, the cytidine base editor is BE4 having a nucleic acid sequence selected from one of the following:

Original BE4 nucleic acid sequence:

ATGagctcagagactggcccagtggctgtggaccccacattgagacggcggatcgagccccatgagtt
tgaggtattcttcgatccgagagagctccgcaaggagacctgcctgctttacgaaattaattgggggg
gccggcactccatttggcgacatacatcacagaacactaacaagcacgtcgaagtcaacttcatcgag
aagttcacgacagaaagatatttctgtccgaacacaaggtgcagcattacctggtttctcagctggag
ccgcgaatgtagtagggccatcactgaattcctgtcaaggtatccccacgtcactctgtttatttaca
tcgcaaggctgtaccaccacgctgacccccgcaatcgacaaggcctgcgggatttgatctcttcaggt
gtgactatccaaattatgactgagcaggagtcaggatactgctggagaaactttgtgaattatagccc
gagtaatgaagcccactggcctaggtatccccatctgtgggtacgactgtacgttcttgaactgtact
gcatcatactgggcctgcctccttgtctcaacattctgagaaggaagcagccacagctgacattcttt
accatcgctcttcagtcttgtcattaccagcgactgcccccacacattctctgggccaccgggttgaa
atctggtggttcttctggtggttctagcggcagcgagactcccgggacctcagagtccgccacacccg
aaagttctggtggttcttctggtggttctgataaaaagtattctattggtttagccatcggcactaat

tccgttggatgggctgtcataaccgatgaatacaaagtaccttcaaagaaatttaaggtgttggggaa
cacagaccgtcattcgattaaaaagaatcttatcggtgccctcctattcgatagtggcgaaacggcag
aggcgactcgcctgaaacgaaccgctcggagaaggtatacacgtcgcaagaaccgaatatgttactta
caagaaatttttagcaatgagatggccaaagttgacgattctttctttcaccgtttggaagagtcctt
ccttgtcgaagaggacaagaaacatgaacggcaccccatctttggaaacatagtagatgaggtggcat
atcatgaaaagtacccaacgatttatcacctcagaaaaaagctagttgactcaactgataaagcggac
ctgaggttaatctacttggctcttgcccatatgataaagttccgtgggcactttctcattgagggtga
tctaaatccggacaactcggatgtcgacaaactgttcatccagttagtacaaacctataatcagttgt
ttgaagagaaccctataaatgcaagtggcgtggatgcgaaggctattcttagcgcccgcctctctaaa
tcccgacggctagaaaacctgatcgcacaattacccggagagaagaaaaatgggttgttcggtaacct
tatagcgctctcactaggcctgacaccaaattttaagtcgaacttcgacttagctgaagatgccaaat
tgcagcttagtaaggacacgtacgatgacgatctcgacaatctactggcacaaattggagatcagtat
gcggacttatttttggctgccaaaaaccttagcgatgcaatcctcctatctgacatactgagagttaa
tactgagattaccaaggcgccgttatccgcttcaatgatcaaaaggtacgatgaacatcaccaagact
tgacacttctcaaggccctagtccgtcagcaactgcctgagaaatataaggaaatattctttgatcag
tcgaaaaacgggtacgcaggttatattgacggcggagcgagtcaagaggaattctacaagtttatcaa
acccatattagagaagatggatgggacggaagagttgcttgtaaaactcaatcgcgaagatctactgc
gaaagcagcggactttcgacaacggtagcattccacatcaaatccacttaggcgaattgcatgctata
cttagaaggcaggaggatttttatccgttcctcaaagacaatcgtgaaaagattgagaaaatcctaac
ctttcgcataccttactatgtgggacccctggcccgagggaactctcggttcgcatggatgacaagaa
agtccgaagaaacgattactccatggaattttgaggaagttgtcgataaaggtgcgtcagctcaatcg
ttcatcgagaggatgaccaactttgacaagaatttaccgaacgaaaaagtattgcctaagcacagttt
actttacgagtatttcacagtgtacaatgaactcacgaaagttaagtatgtcactgagggcatgcgta
aacccgcctttctaagcggagaacagaagaaagcaatagtagatctgttattcaagaccaaccgcaaa
gtgacagttaagcaattgaaagaggactactttaagaaaattgaatgcttcgattctgtcgagatctc
cggggtagaagatcgatttaatgcgtcacttggtacgtatcatgacctcctaaagataattaaagata
aggacttcctggataacgaagagaatgaagatatcttagaagatatagtgttgactcttaccctcttt
gaagatcgggaaatgattgaggaaagactaaaaacatacgctcacctgttcgacgataaggttatgaa
acagttaaagaggcgtcgctatacgggctggggacgattgtcgcggaaacttatcaacgggataagag
acaagcaaagtggtaaaactattctcgattttctaaagagcgacggcttcgccaataggaactttatg
cagctgatccatgatgactctttaaccttcaaagaggatatacaaaaggcacaggtttccggacaagg
ggactcattgcacgaacatattgcgaatcttgctggttcgccagccatcaaaaagggcatactccaga
cagtcaaagtagtggatgagctagttaaggtcatgggacgtcacaaaccggaaaacattgtaatcgag
atggcacgcgaaaatcaaacgactcagaaggggcaaaaaaacagtcgagagcggatgaagagaataga
agagggtattaaagaactgggcagccagatcttaaaggagcatcctgtggaaaatacccaattgcaga
acgagaaactttacctctattacctacaaaatggaagggacatgtatgttgatcaggaactggacata
aaccgtttatctgattacgacgtcgatcacattgtaccccaatccttttgaaggacgattcaatcga

caataaagtgcttacacgctcggataagaaccgagggaaaagtgacaatgttccaagcgaggaagtcg
taaagaaaatgaagaactattggcggcagctcctaaatgcgaaactgataacgcaaagaaagttcgat
aacttaactaaagctgagaggggtggcttgtctgaacttgacaaggccggatttattaaacgtcagct
cgtggaaacccgccaaatcacaaagcatgttgcacagatactagattcccgaatgaatacgaaatacg
acgagaacgataagctgattcgggaagtcaaagtaatcactttaaagtcaaaattggtgtcggacttc
agaaaggattttcaattctataaagttagggagataaataactaccaccatgcgcacgacgcttatct
taatgccgtcgtagggaccgcactcattaagaaatacccgaagctagaaagtgagtttgtgtatggtg
attacaaagtttatgacgtccgtaagatgatcgcgaaaagcgaacaggagataggcaaggctacagcc
aaatacttcttttattctaacattatgaatttctttaagacggaaatcactctggcaaacggagagat
acgcaaacgacctttaattgaaaccaatggggagacaggtgaaatcgtatgggataagggccgggact
tcgcgacggtgagaaaagttttgtccatgccccaagtcaacatagtaaagaaaactgaggtgcagacc
ggagggttttcaaaggaatcgattcttccaaaaaggaatagtgataagctcatcgctcgtaaaaagga
ctgggacccgaaaaagtacggtggcttcgatagccctacagttgcctattctgtcctagtagtggcaa
aagttgagaagggaaaatccaagaaactgaagtcagtcaaagaattattggggataacgattatggag
cgctcgtcttttgaaaagaaccccatcgacttccttgaggcgaaaggttacaaggaagtaaaaaagga
tctcataattaaactaccaaagtatagtctgtttgagttagaaaatggccgaaaacggatgttggcta
gcgccggagagcttcaaaaggggaacgaactcgcactaccgtctaaatacgtgaatttcctgtattta
gcgtcccattacgagaagttgaaaggttcacctgaagataacgaacagaagcaacttttttgttgagca
gcacaaacattatctcgacgaaatcatagagcaaatttcggaattcagtaagagagtcatcctagctg
atgccaatctggacaaagtattaagcgcatacaacaagcacagggataaacccatacgtgagcaggcg
gaaaatattatccatttgtttactcttaccaacctcggcgctccagccgcattcaagtattttgacac
aacgatagatcgcaaacgatacacttctaccaaggaggtgctagacgcgacactgattcaccaatcca
tcacgggattatatgaaactcggatagatttgtcacagcttgggggtgactctggtggttctggagga
tctggtggttctactaatctgtcagatattattgaaaaggagaccggtaagcaactggttatccagga
atccatcctcatgctcccagaggaggtggaagaagtcattgggaacaagccggaaagcgatatactcg
tgcacaccgcctacgacgagagcaccgacgagaatgtcatgcttctgactagcgacgcccctgaatac
aagccttgggctctggtcatacaggatagcaacggtgagaacaagattaagatgctctctggtggttc
tggaggatctggtggttctactaatctgtcagatattattgaaaaggagaccggtaagcaactggtta
tccaggaatccatcctcatgctcccagaggaggtggaagaagtcattgggaacaagccggaaagcgat
atactcgtgcacaccgcctacgacgagagcaccgacgagaatgtcatgcttctgactagcgacgcccc
tgaatacaagccttgggctctggtcatacaggatagcaacggtgagaacaagattaagatgctctctg
gtggttctAAAAGGACGGCGGACGGATCAGAGTTCGAGAGTCCGAAAAAAAAACGAAAGGTCGAAtaa

BE4 Codon Optimization 1 nucleic acid sequence:

ATGTCATCCGAAACCGGGCCAGTGGCCGTAGACCCAACACTCAGGAGGCGGATAGAACCCCATGAGTT
TGAAGTGTTCTTCGACCCCAGAGAGCTGCGCAAAGAGACTTGCCTCCTGTATGAAATAAATTGGGGGG
GTCGCCATTCAATTTGGAGGCACACTAGCCAGAATACTAACAAACACGTGGAGGTAAATTTTATCGAG
AAGTTTACCACCGAAAGATACTTTTGCCCCAATACACGGTGTTCAATTACCTGGTTTCTGTCATGGAG

TCCATGTGGAGAATGTAGTAGAGCGATAACTGAGTTCCTGTCTCGATATCCTCACGTCACGTTGTTTA
TATACATCGCTCGGCTTTATCACCATGCGGACCCGCGGAACAGGCAAGGTCTTCGGGACCTCATATCC
TCTGGGGTGACCATCCAGATAATGACGGAGCAAGAGAGCGGATACTGCTGGCGAAACTTTGTTAACTA
CAGCCCAAGCAATGAGGCACACTGGCCTAGATATCCGCATCTCTGGGTTCGACTGTATGTCCTTGAAC
TGTACTGCATAATTCTGGGACTTCCGCCATGCTTGAACATTCTGCGGCGGAAACAACCACAGCTGACC
TTTTTCACGATTGCTCTCCAAAGTTGTCACTACCAGCGATTGCCACCCCACATCTTGTGGGCTACTGG
ACTCAAGTCTGGAGGAAGTTCAGGCGGAAGCAGCGGGTCTGAAACGCCCGGAACCTCAGAGAGCGCAA
CGCCCGAAAGCTCTGGAGGGTCAAGTGGTGGTAGTGATAAGAAATACTCCATCGGCCTCGCCATCGGT
ACGAATTCTGTCGGTTGGGCCGTTATCACCGATGAGTACAAGGTCCCTTCTAAGAAATTCAAGGTTTT
GGGCAATACAGACCGCCATTCTATAAAAAAAAACCTGATCGGCGCCCTTTTGTTTGACAGTGGTGAGA
CTGCTGAAGCGACTCGCCTGAAGCGAACTGCCAGGAGGCGGTATACGAGGCGAAAAAACCGAATTTGT
TACCTCCAGGAGATTTTCTCAAATGAAATGGCCAAGGTAGATGATAGTTTTTTTCACCGCTTGGAAGA
AAGTTTTCTCGTTGAGGAGGACAAAAAGCACGAGAGGCACCCAATCTTTGGCAACATAGTCGATGAGG
TCGCATACCATGAGAAATATCCTACGATCTATCATCTCCGCAAGAAGCTGGTCGATAGCACGGATAAA
GCTGACCTCCGGCTGATCTACCTTGCTCTTGCTCACATGATTAAATTCAGGGGCCATTTCCTGATAGA
AGGAGACCTCAATCCCGACAATTCTGATGTCGACAAACTGTTTATTCAGCTCGTTCAGACCTATAATC
AACTCTTTGAGGAGAACCCCATCAATGCTTCAGGGGTGGACGCAAAGGCCATTTTGTCCGCGCGCTTG
AGTAAATCACGACGCCTCGAGAATTTGATAGCTCAACTGCCGGGTGAGAAGAAAAACGGGTTGTTTGG
GAATCTCATAGCGTTGAGTTTGGGACTTACGCCAAACTTTAAGTCTAACTTTGATTTGGCCGAAGATG
CCAAATTGCAGCTGTCCAAAGATACCTATGATGACGACTTGGATAACCTTCTTGCGCAGATTGGTGAC
CAATACGCGGATCTGTTTCTTGCCGCAAAAAATCTGTCCGACGCCATACTCTTGTCCGATATACTGCG
CGTCAATACTGAGATAACTAAGGCTCCCCTCAGCGCGTCCATGATTAAAAGATACGATGAGCACCACC
AAGATCTCACTCTGTTGAAAGCCCTGGTTCGCCAGCAGCTTCCAGAGAAGTATAAGGAGATATTTTTC
GACCAATCTAAAAACGGCTATGCGGGTTACATTGACGGTGGCGCCTCTCAAGAAGAATTCTACAAGTT
TATAAAGCCGATACTTGAGAAAATGGACGGTACAGAGGAATTGTTGGTTAAGCTCAATCGCGAGGACT
TGTTGAGAAAGCAGCGCACATTTGACAATGGTAGTATTCCACACCAGATTCATCTGGGCGAGTTGCAT
GCCATTCTTAGAAGACAAGAAGATTTTTATCCGTTTCTGAAAGATAACAGAGAAAAGATTGAAAAGAT
ACTTACCTTTCGCATACCGTATTATGTAGGTCCCCTGGCTAGAGGGAACAGTCGCTTCGCTTGGATGA
CTCGAAAATCAGAAGAAACAATAACCCCCTGGAATTTTGAAGAAGTGGTAGATAAAGGTGCGAGTGCC
CAATCTTTTATTGAGCGGATGACAAATTTTGACAAGAATCTGCCTAACGAAAAGGTGCTTCCCAAGCA
TTCCCTTTTGTATGAATACTTTACAGTATATAATGAACTGACTAAAGTGAAGTACGTTACCGAGGGGA
TGCGAAAGCCAGCTTTTCTCAGTGGCGAGCAGAAAAAAGCAATAGTTGACCTGCTGTTCAAGACGAAT
AGGAAGGTTACCGTCAAACAGCTCAAAGAAGATTACTTTAAAAAGATCGAATGTTTTGATTCAGTTGA
GATAAGCGGAGTAGAGGATAGATTTAACGCAAGTCTTGGAACTTATCATGACCTTTTGAAGATCATCA
AGGATAAAGATTTTTTGGACAACGAGGAGAATGAAGATATCCTGGAAGATATAGTACTTACCTTGACG
CTTTTTGAAGATCGAGAGATGATCGAGGAGCGACTTAAGACGTACGCACATCTCTTTGACGATAAGGT
TATGAAACAATTGAAACGCCGGCGGTATACTGGCTGGGGCAGGCTTTCTCGAAAGCTGATTAATGGTA

TCCGCGATAAGCAGTCTGGAAAGACAATCCTTGACTTTCTGAAAAGTGATGGATTTGCAAATAGAAAC
TTTATGCAGCTTATACATGATGACTCTTTGACGTTCAAGGAAGACATCCAGAAGGCACAGGTATCCGG
CCAAGGGGATAGCCTCCATGAACACATAGCCAACCTGGCCGGCTCACCAGCTATTAAAAAGGGAATAT
TGCAAACCGTTAAGGTTGTTGACGAACTCGTTAAGGTTATGGGCCGACACAAACCAGAGAATATCGTG
ATTGAGATGGCTAGGGAGAATCAGACCACTCAAAAAGGTCAGAAAAATTCTCGCGAAAGGATGAAGCG
AATTGAAGAGGGAATCAAAGAACTTGGCTCTCAAATTTTGAAAGAGCACCCGGTAGAAAACACTCAGC
TGCAGAATGAAAAGCTGTATCTGTATTATCTGCAGAATGGTCGAGATATGTACGTTGATCAGGAGCTG
GATATCAATAGGCTCAGTGACTACGATGTCGACCACATCGTTCCTCAATCTTTCCTGAAAGATGACTC
TATCGACAACAAAGTGTTGACGCGATCAGATAAGAACCGGGGAAAATCCGACAATGTACCCTCAGAAG
AAGTTGTCAAGAAGATGAAAAACTATTGGAGACAATTGCTGAACGCCAAGCTCATAACACAACGCAAG
TTCGATAACTTGACGAAAGCCGAAAGAGGTGGGTTGTCAGAATTGGACAAAGCTGGCTTTATTAAGCG
CCAATTGGTGGAGACCCGGCAGATTACGAAACACGTAGCACAAATTTTGGATTCACGAATGAATACCA
AATACGACGAAAACGACAAATTGATACGCGAGGTGAAAGTGATTACGCTTAAGAGTAAGTTGGTTTCC
GATTTCAGGAAGGATTTTCAGTTTTACAAAGTAAGAGAAATAAACAACTACCACCACGCCCATGATGC
TTACCTCAACGCGGTAGTTGGCACAGCTCTTATCAAAAAATATCCAAAGCTGGAAAGCGAGTTCGTTT
ACGGTGACTATAAAGTATACGACGTTCGGAAGATGATAGCCAAATCAGAGCAGGAAATTGGGAAGGCA
ACCGCAAAATACTTCTTCTATTCAAACATCATGAACTTCTTTAAGACGGAGATTACGCTCGCGAACGG
CGAAATACGCAAGAGGCCCCTCATAGAGACTAACGGCGAAACCGGGGAGATCGTATGGGACAAAGGAC
GGGACTTTGCGACCGTTAGAAAAGTACTTTCAATGCCACAAGTGAATATTGTTAAAAAGACAGAAGTA
CAAACAGGGGGGTTCAGTAAGGAATCCATTTTGCCCAAGCGGAACAGTGATAAATTGATAGCAAGGAA
AAAAGATTGGGACCCTAAGAAGTACGGTGGTTTCGACTCTCCTACCGTTGCATATTCAGTCCTTGTAG
TTGCGAAAGTGGAAAAGGGGAAAAGTAAGAAGCTTAAGAGTGTTAAAGAGCTTCTGGGCATAACCATA
ATGGAACGGTCTAGCTTCGAGAAAAATCCAATTGACTTTCTCGAGGCTAAAGGTTACAAGGAGGTAAA
AAAGGACCTGATAATTAAACTCCCAAAGTACAGTCTCTTCGAGTTGGAGAATGGGAGGAAGAGAATGT
TGGCATCTGCAGGGGAGCTCCAAAAGGGGAACGAGCTGGCTCTGCCTTCAAAATACGTGAACTTTCTG
TACCTGGCCAGCCACTACGAGAAACTCAAGGGTTCTCCTGAGGATAACGAGCAGAAACAGCTGTTTGT
AGAGCAGCACAAGCATTACCTGGACGAGATAATTGAGCAAATTAGTGAGTTCTCAAAAAGAGTAATCC
TTGCAGACGCGAATCTGGATAAAGTTCTTTCCGCCTATAATAAGCACCGGGACAAGCCTATACGAGAA
CAAGCCGAGAACATCATTCACCTCTTTACCCTTACTAATCTGGGCGCGCCGGCCGCCTTCAAATACTT
CGACACCACGATAGACAGGAAAAGGTATACGAGTACCAAAGAAGTACTTGACGCCACTCTCATCCACC
AGTCTATAACAGGGTTGTACGAAACGAGGATAGATTTGTCCCAGCTCGGCGGCGACTCAGGAGGGTCA
GGCGGCTCCGGTGGATCAACGAATCTTTCCGACATAATCGAGAAAGAAACCGGCAAACAGTTGGTGAT
CCAAGAATCAATCCTGATGCTGCCTGAAGAAGTAGAAGAGGTGATTGGCAACAAACCTGAGTCTGACA
TTCTTGTCCACACCGCGTATGACGAGAGCACGGACGAGAACGTTATGCTTCTCACTAGCGACGCCCCT
GAGTATAAACCATGGGCGCTGGTCATCCAAGATTCCAATGGGGAAAACAAGATTAAGATGCTTAGTGG
TGGGTCTGGAGGGAGCGGTGGGTCCACGAACCTCAGCGACATTATTGAAAAAGAGACTGGTAAACAAC
TTGTAATACAAGAGTCTATTCTGATGTTGCCTGAAGAGGTGGAGGAGGTGATTGGGAACAAACCGGAG

TCTGATATACTTGTTCATACCGCCTATGACGAATCTACTGATGAGAATGTGATGCTTTTaACGTCAGA
CGCTCCCGAGTACAAACCCTGGGCTCTGGTGATTCAGGACAGCAATGGTGAGAATAAGATTAAAATGT
TGAGTGGGGGCTCAAAGCGCACGGCTGACGGTAGCGAATTTGAGAGCCCCAAAAAAAAACGAAAGGTC
GAAtaa

BE4 Codon Optimization 2 nucleic acid sequence:

ATGAGCAGCGAGACAGGCCCTGTGGCTGTGGATCCTACACTGCGGAGAAGAATCGAGCCCCACGAGTT
CGAGGTGTTCTTCGACCCCAGAGAGCTGCGGAAAGAGACATGCCTGCTGTACGAGATCAACTGGGGCG
GCAGACACTCTATCTGGCGGCACACAAGCCAGAACACCAACAAGCACGTGGAAGTGAACTTTATCGAG
AAGTTTACGACCGAGCGGTACTTCTGCCCCAACACCAGATGCAGCATCACCTGGTTTCTGAGCTGGTC
CCCTTGCGGCGAGTGCAGCAGAGCCATCACCGAGTTTCTGTCCAGATATCCCCACGTGACCCTGTTCA
TCTATATCGCCCGGCTGTACCACCACGCCGATCCTAGAAATAGACAGGGACTGCGCGACCTGATCAGC
AGCGGAGTGACCATCCAGATCATGACCGAGCAAGAGAGCGGCTACTGCTGGCGGAACTTCGTGAACTA
CAGCCCCAGCAACGAAGCCCACTGGCCTAGATATCCTCACCTGTGGGTCCGACTGTACGTGCTGGAAC
TGTACTGCATCATCCTGGGCCTGCCTCCATGCCTGAACATCCTGAGAAGAAAGCAGCCTCAGCTGACC
TTCTTCACAATCGCCCTGCAGAGCTGCCACTACCAGAGACTGCCTCCACACATCCTGTGGGCCACCGG
ACTTAAGAGCGGAGGATCTAGCGGCGGCTCTAGCGGATCTGAGACACCTGGCACAAGCGAGTCTGCCA
CACCTGAGAGTAGCGGCGGATCTTCTGGCGGCTCCGACAAGAAGTACTCTATCGGACTGGCCATCGGC
ACCAACTCTGTTGGATGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCT
GGGCAACACCGACCGGCACAGCATCAAGAAGAATCTGATCGGCGCCCTGCTGTTCGACTCTGGCGAAA
CAGCCGAAGCCACCAGACTGAAGAGAACCGCCAGGCGGAGATACACCCGGCGGAAGAACCGGATCTGC
TACCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGA
GTCCTTCCTGGTGGAAGAGGACAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGATGAGG
TGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAG
GCCGACCTGAGACTGATCTACCTGGCTCTGGCCCACATGATCAAGTTCCGGGGCCACTTTCTGATCGA
GGGCGATCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACC
AGCTGTTCGAGGAAAACCCCATCAACGCCTCTGGCGTGGACGCCAAGGCTATCCTGTCTGCCAGACTG
AGCAAGAGCAGAAGGCTGGAAAACCTGATCGCCCAGCTGCCTGGCGAGAAGAAGAATGGCCTGTTCGG
CAACCTGATTGCCCTGAGCCTGGGACTGACCCCTAACTTCAAGAGCAACTTCGACCTGGCCGAGGATG
CCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAATCTGCTGGCCCAGATCGGCGAT
CAGTACGCCGACTTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGATATCCTGAG
AGTGAACACCGAGATCACAAAGGCCCCTCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACC
AGGATCTGACCCTGCTGAAGGCCCTCGTTAGACAGCAGCTGCCAGAGAAGTACAAAGAGATTTTCTTC
GATCAGTCCAAGAACGGCTACGCCGGCTACATTGATGGCGGAGCCAGCCAAGAGGAATTCTACAAGTT
CATCAAGCCCATCCTGGAAAAGATGGACGGCACCGAGGAACTGCTGGTCAAGCTGAACAGAGAGGACC
TGCTGCGGAAGCAGCGGACCTTCGACAATGGCTCTATCCCTCACCAGATCCACCTGGGAGAGCTGCAC
GCCATTCTGCGGAGACAAGAGGACTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGAT
CCTGACCTTCAGGATCCCCTACTACGTGGGACCACTGGCCAGAGGCAATAGCAGATTCGCCTGGATGA

CCAGAAAGAGCGAGGAAACCATCACACCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCCAGCGCT
CAGTCCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCTAACGAGAAGGTGCTGCCCAAGCA
CTCCCTGCTGTATGAGTACTTCACCGTGTACAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAA
TGAGAAAGCCCGCCTTTCTGAGCGGCGAGCAGAAAAAGGCCATTGTGGATCTGCTGTTCAAGACCAAC
CGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACAGCGTGGA
AATCAGCGGCGTGGAAGATCGGTTCAATGCCAGCCTGGGCACATACCACGACCTGCTGAAAATTATCA
AGGACAAGGACTTCCTGGACAACGAAGAGAACGAGGACATTCTCGAGGACATCGTGCTGACCCTGACA
CTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACATACGCCCACCTGTTCGACGACAAAGT
GATGAAGCAACTGAAGCGGAGGCGGTACACAGGCTGGGGCAGACTGTCTCGGAAGCTGATCAACGGCA
TCCGGGATAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAAC
TTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGG
CCAAGGCGATTCTCTGCACGAGCACATTGCCAACCTGGCCGGATCTCCCGCCATTAAGAAGGGCATCC
TGCAGACAGTGAAGGTGGTGGACGAGCTTGTGAAAGTGATGGGCAGACACAAGCCCGAGAACATCGTG
ATCGAAATGGCCAGAGAGAACCAGACCACACAGAAGGGCCAGAAGAACAGCCGCGAGAGAATGAAGCG
GATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGC
TGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGACGGGATATGTACGTGGACCAAGAGCTG
GACATCAACCGGCTGAGCGACTACGATGTGGACCATATCGTGCCCCAGAGCTTTCTGAAGGACGACTC
CATCGATAACAAGGTCCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGATAACGTGCCCTCCGAAG
AGGTGGTCAAGAAGATGAAGAACTACTGGCGACAGCTGCTGAACGCCAAGCTGATTACCCAGCGGAAG
TTCGATAACCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTTGATAAGGCCGGCTTCATTAAGCG
GCAGCTGGTGGAAACCCGGCAGATCACCAAACACGTGGCACAGATTCTGGACTCCCGGATGAACACTA
AGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTCATCACCCTGAAGTCTAAGCTGGTGTCC
GATTTCCGGAAGGATTTCCAGTTCTACAAAGTGCGGGAAATCAACAACTACCATCACGCCCACGACGC
CTACCTGAATGCCGTTGTTGGAACAGCCCTGATCAAGAAGTATCCCAAGCTGGAAAGCGAGTTCGTGT
ACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAACAAGAGATCGGCAAGGCT
ACCGCCAAGTACTTTTTCTACAGCAACATCATGAACTTTTTCAAGACAGAGATCACCCTGGCCAACGG
CGAGATCCGGAAAAGACCCCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCA
GAGATTTTGCCACAGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAGAAAACCGAGGTG
CAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCTAAGCGGAACAGCGATAAGCTGATCGCCAGAAA
GAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGATAGCCCTACCGTGGCCTATTCTGTGCTGGTGG
TGGCCAAAGTGGAAAAGGGCAAGTCCAAAAAGCTCAAGAGCGTGAAAGAGCTGCTGGGGATCACCATC
ATGGAAAGAAGCAGCTTTGAGAAGAACCCGATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTCAA
GAAGGACCTCATCATCAAGCTCCCCAAGTACAGCCTGTTCGAGCTGGAAAATGGCCGGAAGCGGATGC
TGGCCTCAGCAGGCGAACTGCAGAAAGGCAATGAACTGGCCCTGCCTAGCAAATACGTCAACTTCCTG
TACCTGGCCAGCCACTATGAGAAGCTGAAGGGCAGCCCCGAGGACAATGAGCAAAAGCAGCTGTTTGT
GGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCC
TGGCCGACGCTAACCTGGATAAGGTGCTGTCTGCCTATAACAAGCACCGGGACAAGCCTATCAGAGAG

CAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAACCTGGGAGCCCCTGCCGCCTTCAAGTACTT
CGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGACGCCACACTGATCCACC
AGTCTATCACCGGCCTGTACGAAACCCGGATCGACCTGTCTCAGCTCGGCGGCGATTCTGGTGGTTCT
GGCGGAAGTGGCGGATCCACCAATCTGAGCGACATCATCGAAAAAGAGACAGGCAAGCAGCTCGTGAT
CCAAGAATCCATCCTGATGCTGCCTGAAGAGGTTGAGGAAGTGATCGGCAACAAGCCTGAGTCCGACA
TCCTGGTGCACACCGCCTACGATGAGAGCACCGATGAGAACGTCATGCTGCTGACAAGCGACGCCCCT
GAGTACAAGCCTTGGGCTCTCGTGATTCAGGACAGCAATGGGGAGAACAAGATCAAGATGCTGAGCGG
AGGTAGCGGAGGCAGTGGCGGAAGCACAAACCTGTCTGATATCATTGAAAAAGAAACCGGGAAGCAAC
TGGTCATTCAAGAGTCCATTCTCATGCTCCCGGAAGAAGTCGAGGAAGTCATTGGAAACAAACCCGAG
AGCGATATTCTGGTCCACACAGCCTATGACGAGTCTACAGACGAAAACGTGATGCTCCTGACCTCTGA
CGCTCCCGAGTATAAGCCCTGGGCACTTGTTATCCAGGACTCTAACGGGGAAAACAAAATCAAAATGT
TGTCCGGCGGCAGCAAGCGGACAGCCGATGGATCTGAGTTCGAGAGCCCCAAGAAGAAACGGAAGGTg
GAGtaa

By "base editing activity" is meant acting to chemically alter a base within a polynucleotide. In one embodiment, a first base is converted to a second base. In one embodiment, the base editing activity is cytidine deaminase activity, e.g., converting target C•G to T•A. In another embodiment, the base editing activity is adenosine or adenine deaminase activity, *e.g.*, converting A•T to G•C. In another embodiment, the base editing activity is cytidine deaminase activity, *e.g.*, converting target C•G to T•A and adenosine or adenine deaminase activity, e.g., converting A•T to G•C.

The term "base editor system" refers to a system for editing a nucleobase of a target nucleotide sequence. In various embodiments, the base editor (BE) system comprises (1) a polynucleotide programmable nucleotide binding domain, a deaminase domain and a cytidine deaminase domain for deaminating nucleobases in the target nucleotide sequence; and (2) one or more guide polynucleotides (*e.g.*, guide RNA) in conjunction with the polynucleotide programmable nucleotide binding domain. In various embodiments, the base editor (BE) system comprises a nucleobase editor domains selected from an adenosine deaminase or a cytidine deaminase, and a domain having nucleic acid sequence specific binding activity. In some embodiments, the base editor system comprises (1) a base editor (BE) comprising a polynucleotide programmable DNA binding domain and a deaminase domain for deaminating one or more nucleobases in a target nucleotide sequence; and (2) one or more guide RNAs in conjunction with the polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the base editor

is a cytidine base editor (CBE). In some embodiments, the base editor is an adenine or adenosine base editor (ABE). In some embodiments, the base editor is an adenine or adenosine base editor (ABE) or a cytidine base editor (CBE).

The term "Cas9" or "Cas9 domain" refers to an RNA guided nuclease comprising a Cas9 protein, or a fragment thereof (*e.g.*, a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A Cas9 nuclease is also referred to sometimes as a casnl nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat) associated nuclease. An exemplary Cas9, is *Streptococcus pyogenes* Cas9 (spCas9), the amino acid sequence of which is provided below:

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNS
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSLHEQIANLAGSPAIKKGILQTVKIV
DELVKVMGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD (single underline: HNH domain; double underline: RuvC domain)

The term "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., *supra*). Non-limiting examples of conservative mutations include amino acid substitutions of amino acids, for example, lysine for arginine and vice versa such that a positive charge can be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free $–NH_2$ can be maintained.

The term "coding sequence" or "protein coding sequence" as used interchangeably herein refers to a segment of a polynucleotide that codes for a protein. The region or sequence is bounded nearer the 5′ end by a start codon and nearer the 3′ end with a stop codon. Stop codons useful with the base editors described herein include the following:

Glutamine	**C**AG → **T**AG	Stop codon
	CAA → **T**AA	
Arginine	CGA → **T**GA	
Tryptophan	TG**G** → TG**A**	
	T**G**G → T**A**G	
	T**G**G → T**AA**	

Coding sequences can also be referred to as open reading frames.

By "cytidine deaminase" is meant a polypeptide or fragment thereof capable of catalyzing a deamination reaction that converts an amino group to a carbonyl group. In one embodiment, the cytidine deaminase converts cytosine to uracil or 5-methylcytosine to thymine. PmCDA1, which is derived from Petromyzon marinus (Petromyzon marinus cytosine deaminase 1, "PmCDA1"), AID (Activation-induced cytidine deaminase; AICDA), which is derived from a mammal (e.g., human, swine, bovine, horse, monkey etc.), and APOBEC are exemplary cytidine deaminases.

The term "deaminase" or "deaminase domain," as used herein, refers to a protein or enzyme that catalyzes a deamination reaction. In some embodiments, the deaminase or

deaminase domain is a cytidine deaminase, catalyzing the hydrolytic deamination of cytidine or deoxycytidine to uridine or deoxyuridine, respectively. In some embodiments, the deaminase or deaminase domain is a cytosine deaminase, catalyzing the hydrolytic deamination of cytosine to uracil. In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenine to hypoxanthine. In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenosine or adenine (A) to inosine (I). In some embodiments, the deaminase or deaminase domain is an adenosine deaminase, catalyzing the hydrolytic deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenosine in deoxyribonucleic acid (DNA). The adenosine deaminase (*e.g.*, engineered adenosine deaminase, evolved adenosine deaminase) provided herein can be from any organism, such as a bacterium. In some embodiments, the adenosine deaminase is from a bacterium, such as *E. coli*, *S. aureus*, *S. typhi*, *S. putrefaciens*, *H. influenzae*, or *C. crescentus*. In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the deaminase or deaminase domain is a variant of a naturally occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identical to a naturally occurring deaminase.

"Detect" refers to identifying the presence, absence or amount of the analyte to be detected. In one embodiment, a sequence alteration in a polynucleotide or polypeptide is detected. In another embodiment, the presence of indels is detected.

By "detectable label" is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an enzyme linked immunosorbent assay (ELISA), biotin, digoxigenin, or haptens.

By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include HBV infection, as well as related diseases and disorders, including cirrhosis, hepatocellular carcinoma (HCC), and any other disease associated with or resulting from HBV infection.

By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount. In one embodiment, an effective amount is the amount of a base editor of the invention sufficient to introduce an alteration in an HBV genome in a cell (e.g., a cell in vitro or in vivo). In one embodiment, an effective amount is the amount of a base editor required to achieve a therapeutic effect (e.g., to reduce or control an HBV infection). Such therapeutic effect need not be sufficient to alter an HBV genome in all cells of a subject, tissue or organ, but only to alter an HBV genome in about 1%, 5%, 10%, 25%, 50%, 75% or more of the cells present in a subject, tissue or organ. In one embodiment, an effective amount is sufficient to ameliorate one or more symptoms of HBV.

In some embodiments, an effective amount of a fusion protein provided herein, e.g., of a nucleobase editor comprising a nCas9 domain and a deaminase domain (e.g., adenosine deaminase, cytidine deaminase) refers to the amount that is sufficient to induce editing of a target site specifically bound and edited by the nucleobase editors described herein. As will be appreciated by the skilled artisan, the effective amount of an agent, e.g., a fusion protein, may vary depending on various factors as, for example, on the desired biological response, e.g., on the specific genome or target site to be edited, on the cell or tissue being targeted, and/or on the agent being used.

In some embodiments, an effective amount of a fusion protein provided herein, e.g., of a fusion protein comprising a nCas9 domain and a deaminase domain may refer to the amount of the fusion protein that is sufficient to induce editing of a target site specifically bound and edited by the fusion protein. As will be appreciated by the skilled artisan, the effective amount of an agent, e.g., a fusion protein, may vary depending on various factors as, for example, on the desired biological response, e.g., on the specific genome or target site to be edited, on the cell or tissue being targeted, and/or on the agent being used.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

By "guide RNA" or "gRNA" is meant a polynucleotide which is specific for a target sequence and can form a complex with a polynucleotide programmable nucleotide binding domain protein (e.g., Cas9 or Cpf1). In an embodiment, the guide polynucleotide is a guide RNA (gRNA). gRNAs can exist as a complex of two or more RNAs, or as a single RNA molecule. gRNAs that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), although "gRNA" is used interchangeably to refer to guide RNAs that exist as either single molecules or as a complex of two or more molecules. Typically, gRNAs that exist as single RNA species comprise two domains: (1) a domain that shares homology to a target nucleic acid (e.g., and directs binding of a Cas9 complex to the target); and (2) a domain that binds a Cas9 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA, and comprises a stem-loop structure. For example, in some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek et al., Science 337:816-821(2012), the entire contents of which is incorporated herein by reference. Other examples of gRNAs (e.g., those including domain 2) can be found in US20160208288, entitled "Switchable Cas9 Nucleases and Uses Thereof," and US 9,737,604, entitled "Delivery System For Functional Nucleases," the entire contents of each are hereby incorporated by reference in their entirety. In some embodiments, a gRNA comprises two or more of domains (1) and (2), and may be referred to as an "extended gRNA." An extended gRNA will bind two or more Cas9 proteins and bind a target nucleic acid at two or more distinct regions, as described herein. The gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to the target site, providing the sequence specificity of the nuclease:RNA complex.

By "HBV polymerase protein" is meant a polypeptide having at least about 95% identity to a wild-type HBV polymerase amino acid sequence or fragment thereof that functions in a hepatitis B viral infection. In one embodiment, the HBV polymerase is encoded by an HBV A, B, C, D, E, F, G, or H genotype. In one embodiment, the HBV polymerase amino acid sequence is provided at UniPro Accession No. Q8B5R0-1, which is reproduced below.

```
        10         20         30         40         50
MPLSYQHFRR LLLLDDEAGP LEEELPRLAD EGLNRRVAED LNLGNLNVSI
        60         70         80         90        100
PWTHKVGNFT GLYSSTVPVF NPHWKTPSFP NIHLHQDIIK KCEQFVGPLT
       110        120        130        140        150
VNEKRRLQLI MPARFYPKVT KYLPLDKGIK PYYPEHLVNH YFQTRHYLHT
       160        170        180        190
LWKAGILYKR ETTHSASFCG SPYSWEQDLQ HGAESFHQQS
```

Mutations in HBV polymerase include: E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P, R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P.

Other exemplary HBV DNA polymerases include, for example, NCBI Accession No. AAB59972.1, which has the following sequence.

```
MPLSYQHFRKLLLLDDEAGPLEEELPRLADEGLNRRVAEDLNLG
NLNVSIPWTHKVGNFTGLYSSTVPVFNPHWKTPSFPNIHLHQDIIKKCEQFVGPLTVN
EKRRLQLIMPARFYPKVTKYLPLDKGIKPYYPEHLVNHYFQTRHYLHTLWKAGILYKR
ETTHSASFCGSPYSWEQDLQHGAESFHQQSSGILSRPPVGSSLQSKHSKSRLGLQSQQ
GHLARRQQGRSWSIRAGFHPTARRPFGVEPSGSGHTTNFASKSASCLHQSPDRKAAYP
AVSTFEKHSSSGHAVEFHNLSPNSARSQSERPVFPCWWLQFRSSKPCSDYCLSLIVNL
LEDWGPCAEHGEHHIRIPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFSRGNYRVS
WPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLSRYVARL
SSNSRILNHQHGTMPNLHDYCSRNLYVSLLLLYQTFGRKLHLYSHPIILGFRKIPMGV
GLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHLESLFTAVTNFLLSL
GIHLNPNKTKRWGYSLNFMGYVIGSYGSLPQEHIIQKIKECFRKLPINRPIDWKVCQR
IVGLLGFAAPFTQCGYPALMPLYACIQSKQAFTFSPTYKAFLCKQYLNLYPVARQRPG
LCQVFADATPTGWGLVMGHQRVRGTFSAPLPIHTAELLAACFARSRSGANIIGTDNSV
VLSRKYTSYPWLLGCAANWILRGTSFVYVPSALNPADDPSRGRLGLSRPLLRLPFRPT
TGRTSLYADSPSVPSHLPDRVHFASPLHVAWRPP
```

By “HBV polymerase gene” is meant a polynucleotide encoding an HBV polymerase.

By “Hepatitis B surface antigen (HBsAg) polypeptide” is meant an antigenic protein or fragment thereof having at least about 85% identity to NCBI Accession No. AAB59969.1, which functions in an HBV viral infection. An exemplary HBsAg amino acid sequence is provided below:

```
MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTSNHSPTSCPPT
CPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLIPGSSTTSTGPCRTCMTTAQGTSMYPS
```

CCCTKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPS
LYSILSPFLPLLPIFFCLWVYI

By "HbsAg polynucleotide" is meant a polynucleotide encoding an HBsAg protein.

By "HBV X-protein" is meant a polypeptide or fragment thereof having at least about 85% identity to NCBI Accession No. AAB59970.1, which functions in an HBV viral infection. An exemplary amino acid sequence is provided below:

1 maarlccqld pardvlclrp vgaescgrpf sgslgtlssp spsavptdhg ahlslrglpv
61 cafssagpca lrftsarrme ttvnahrmlp kvlhkrtlgl samsttdlea yfkdclfkdw
121 eelgeeirlk vfvlggcrhk lvcapapcnf ftsa

By "core antigen precursor" is meant a polypeptide or fragment thereof having at least about 85% identity to NCBI Accession No. AAB59971.1, which functions in an HBV viral infection.

By "HBV core protein" is meant a polypeptide having at least about 95% identity to a wild-type HBV core protein amino acid sequence or fragment thereof. In an embodiment, the HBV core protein functions in a hepatitis B viral infection. In one embodiment, the HBV core protein is encoded by an HBV A, B, C, D, E, F, G, or H genotype. In one embodiment, the HBV core protein amino acid sequence is provided at NCBI GenBank Accession No. AXG50928.1, provided below:

1 mdidpykefg asvellsflp sdffpsirdl ldtasalyre alespehcsp hhtalrqail
61 cwgelmnlat wvgsnledpa srelvvsyvn vnmglkirql lwfhiscltf gretvleylv
121 sfgvwirtpp ayrppnapil stlpettvvr rrgrsprrrt psprrrrsqs prrrrsqsre
181 sqc

By "HBV X protein" is meant a polynucleotide encoding an HBV X-protein.

By "HBV X protein (genotype B)" is meant a polypeptide having at least about 95% identity to a wild-type HBV genotype B X protein amino acid sequence or fragment thereof. In an embodiment, the HBV X protein functions in a hepatitis B viral infection. In one embodiment, the HBV genotype B X protein amino acid sequence is provided at NCBI GenBank Accession No. BAQ95575.1, provided below:

1 maarlccqld pardvlclrp vgaesrgrpl pgplgalppa sppvvpsdhg ahlslrglpv
61 cafssxgpca lrftsarrme ttvnahrnlp kvlhkrtlgl samsttdlea yfkdcvfxew
121 eelgeexrlk vfvlggcrhk lvcspapcnf ftsa

By "HBV X protein (genotype C)" is meant a polypeptide having at least about 95% identity to a wild-type HBV genotype C X protein amino acid sequence or fragment thereof.

In an embodiment, the HBV X protein functions in a hepatitis B viral infection. In one embodiment, the HBV genotype C X protein amino acid sequence is provided at NCBI GenBank Accession No. BAQ95563.1, provided below:

```
1 maarvccqld pardvlclrp vgaesrgrpv sgpfgplpsp sssavpadyg ahlslrglpv
61 cafssagpca lrftsarrme ttvnahqvlp kllhkrtlgl samsttdlea yfkdclfkdw
121 eelgeeirlk vfvlggcrhk lvcspapcnf ftsa
```

By "HBV S protein" is meant a polypeptide having at least about 95% identity to a wild-type HBV S protein amino acid sequence or fragment thereof. In an embodiment, the HBV S protein functions in a hepatitis B viral infection. In one embodiment, the HBV S protein is encoded by an HBV A, B, C, D, E, F, G, or H genotype. In one embodiment, the HBV S protein amino acid sequence is provided at NCBI GenBank Accession No. ABV02793.1, provided below:

```
1 menttsgflg pllvlqagff lltrnltipq sldswwtsln flggaptcpg qnsqsptsnh
61 sptscppicp gyrwmclrrf iiflfilllc lifllvlldy qgmlpvcpll pgtsttstgp
121 cktctipaqg tsmfpsccct kpsdgnctci pipsswafar flwewasvrf swlslvpfv
181 qwfvglsptv wlsviwmmwy wgpslynils pflpllpiff clwvyi
```

The complete genome of Hepatitis B virus subtype ayw, complete genome, which includes polynucleotides encoding HBV polymerase, HBsAg protein, HBV X protein, and the core antigen precursor, is provided at GenBank Accession No. U95551.1, which is reproduced below:

```
   1 aattccacaa cctttcacca aactctgcaa gatcccagag tgagaggcct gtatttccct
  61 gctggtggct ccagttcagg agcagtaaac cctgttccga ctactgcctc tcccttatcg
 121 tcaatcttct cgaggattgg ggaccctgcg ctgaacatgg agaacatcac atcaggattc
 181 ctaggacccc ttctcgtgtt acaggcgggg tttttcttgt tgacaagaat cctcacaata
 241 ccgcagagtc tagactcgtg gtggacttct ctcaattttc tagggggaac taccgtgtgt
 301 cttggccaaa attcgcagtc cccaacctcc aatcactcac caacctcctg tcctccaact
 361 tgtcctggtt atcgctggat gtgtctgcgg cgttttatca tcttcctctt catcctgctg
 421 ctatgcctca tcttcttgtt ggttcttctg gactatcaag gtatgttgcc cgtttgtcct
 481 ctaattccag gatcctcaac caccagcacg ggaccatgcc gaacctgcat gactactgct
 541 caaggaacct ctatgtatcc ctcctgttgc tgtaccaaac cttcggacgg aaattgcacc
 601 tgtattccca tcccatcatc ctgggctttc ggaaaattcc tatgggagtg ggcctcagcc
 661 cgtttctcct ggctcagttt actagtgcca tttgttcagt ggttcgtagg gctttccccc
 721 actgtttggc tttcagttat atggatgatg tggtattggg ggccaagtct gtacagcatc
 781 ttgagtccct ttttaccgct gttaccaatt ttcttttgtc tttgggtata catttaaacc
 841 ctaacaaaac aaagagatgg ggttactctc tgaattttat gggttatgtc attggaagtt
 901 atgggtcctt gccacaagaa cacatcatac aaaaaatcaa agaatgtttt agaaaacttc
 961 ctattaacag gcctattgat tggaaagtat gtcaacgaat tgtgggtctt ttgggttttg
1021 ctgccccatt tacacaatgt ggttatcctg cgttaatgcc cttgtatgca tgtattcaat
```

```
1081 ctaagcaggc tttcactttc tcgccaactt acaaggcctt tctgtgtaaa caatacctga
1141 acctttaccc cgttgcccgg caacggccag gtctgtgcca agtgtttgct gacgcaaccc
1201 ccactggctg gggcttggtc atgggccatc agcgcgtgcg tggaaccttt tcggctcctc
1261 tgccgatcca tactgcggaa ctcctagccg cttgttttgc tcgcagcagg tctggagcaa
1321 acattatcgg gactgataac tctgttgtcc tctcccgcaa atatacatcg tatccatggc
1381 tgctaggctg tgctgccaac tggatcctgc gcgggacgtc ctttgtttac gtcccgtcgg
1441 cgctgaatcc tgcggacgac ccttctcggg gtcgcttggg actctctcgt ccccttctcc
1501 gtctgccgtt ccgaccgacc acggggcgca cctctcttta cgcggactcc ccgtctgtgc
1561 cttctcatct gccggaccgt gtgcacttcg cttcacctct gcacgtcgca tggagaccac
1621 cgtgaacgcc caccgaatgt tgcccaaggt cttacataag aggactcttg gactctctgc
1681 aatgtcaacg accgaccttg aggcatactt caaagactgt ttgtttaaag actgggagga
1741 gttgggggag gagattagat taaaggtctt tgtactagga ggctgtaggc ataaattggt
1801 ctgcgcacca gcaccatgca acttttcac ctctgcctaa tcatctcttg ttcatgtcct
1861 actgttcaag cctccaagct gtgccttggg tggctttggg gcatggacat cgacccttat
1921 aaagaatttg gagctactgt ggagttactc tcgtttttgc cttctgactt ctttccttca
1981 gtacgagatc ttctagatac cgcctcagct ctgtatcggg aagccttaga gtctcctgag
2041 cattgttcac ctcaccatac tgcactcagg caagcaattc tttgctgggg ggaactaatg
2101 actctagcta cctgggtggg tgttaatttg gaagatccag catctagaga cctagtagtc
2161 agttatgtca acactaatat gggcctaaag ttcaggcaac tcttgtggtt tcacatttct
2221 tgtctcactt ttggaagaga aaccgttata gagtatttgg tgtctttcgg agtgtggatt
2281 cgcactcctc cagcttatag accaccaaat gcccctatcc tatcaacact tccggaaact
2341 actgttgtta gacgacgagg caggtcccct agaagaagaa ctccctcgcc tcgcagacga
2401 aggtctcaat cgccgcgtcg cagaagatct caatctcggg aacctcaatg ttagtattcc
2461 ttggactcat aaggtgggga actttactgg tctttattct tctactgtac ctgtctttaa
2521 tcctcattgg aaaacaccat cttttcctaa tatacattta caccaagaca ttatcaaaaa
2581 atgtgaacag tttgtaggcc cacttacagt taatgagaaa agaagattgc aattgattat
2641 gcctgctagg ttttatccaa aggttaccaa atatttacca ttggataagg gtattaaacc
2701 ttattatcca gaacatctag ttaatcatta cttccaaact agacactatt tacacactct
2761 atggaaggcg ggtatattat ataagagaga aacaacacat agcgcctcat tttgtgggtc
2821 accatattct tgggaacaag atctacagca tggggcagaa tctttccacc agcaatcctc
2881 tgggattctt tcccgaccac cagttggatc cagccttcag agcaaacaca gcaaatccag
2941 attgggactt caatcccaac aaggacacct ggccagacgc caacaaggta ggagctggag
3001 cattcgggct gggtttcacc ccaccgcacg gaggcctttt ggggtggagc cctcaggctc
3061 agggcatact acaaactttg ccagcaaatc cgcctcctgc ctccaccaat cgccagacag
3121 gaaggcagcc taccccgctg tctccacctt tgagaaacac tcatcctcag gccatgcagt
3181 gg
```

By "heterodimer" is meant a fusion protein comprising two domains, such as a wild type TadA domain and a variant of TadA domain (*e.g.*, TadA*8) or two variant TadA domains (*e.g.*, TadA*7.10 and TadA*8 or two TadA*8 domains).

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For

example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify" denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high-performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By "increases" is meant a positive alteration of at least 10%, 25%, 50%, 75%, or 100%.

The terms "inhibitor of base repair", "base repair inhibitor", "IBR" or their grammatical equivalents refer to a protein that is capable in inhibiting the activity of a nucleic acid repair enzyme, for example a base excision repair enzyme. In some embodiments, the IBR is an inhibitor of inosine base excision repair. Exemplary inhibitors of base repair

include inhibitors of APE1, Endo III, Endo IV, Endo V, Endo VIII, Fpg, hOGGl, hNEILl, T7 Endol, T4PDG, UDG, hSMUGl, and hAAG. In some embodiments, the base repair inhibitor is an inhibitor of Endo V or hAAG. In some embodiments, the IBR is an inhibitor of Endo V or hAAG. In some embodiments, the IBR is a catalytically inactive EndoV or a catalytically inactive hAAG. In some embodiments, the base repair inhibitor is a catalytically inactive EndoV or a catalytically inactive hAAG. In some embodiments, the base repair inhibitor is uracil glycosylase inhibitor (UGI). UGI refers to a protein that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a fragment of a wild-type UGI. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. In some embodiments, the base repair inhibitor is an inhibitor of inosine base excision repair. In some embodiments, the base repair inhibitor is a "catalytically inactive inosine specific nuclease" or "dead inosine specific nuclease." Without wishing to be bound by any particular theory, catalytically inactive inosine glycosylases (*e.g.*, alkyl adenine glycosylase (AAG)) can bind inosine, but cannot create an abasic site or remove the inosine, thereby sterically blocking the newly formed inosine moiety from DNA damage/repair mechanisms. In some embodiments, the catalytically inactive inosine specific nuclease can be capable of binding an inosine in a nucleic acid but does not cleave the nucleic acid. Non-limiting exemplary catalytically inactive inosine specific nucleases include catalytically inactive alkyl adenosine glycosylase (AAG nuclease), for example, from a human, and catalytically inactive endonuclease V (EndoV nuclease), for example, from *E. coli*. In some embodiments, the catalytically inactive AAG nuclease comprises an E125Q mutation or a corresponding mutation in another AAG nuclease.

An "intein" is a fragment of a protein that is able to excise itself and join the remaining fragments (the exteins) with a peptide bond in a process known as protein splicing. Inteins are also referred to as "protein introns." The process of an intein excising itself and joining the remaining portions of the protein is herein termed "protein splicing" or "intein-mediated protein splicing." In some embodiments, an intein of a precursor protein (an intein containing protein prior to intein-mediated protein splicing) comes from two genes. Such intein is referred to herein as a split intein (e.g., split intein-N and split intein-C). For example, in cyanobacteria, DnaE, the catalytic subunit a of DNA polymerase III, is encoded by two separate genes, dnaE-n and dnaE-c. The intein encoded by the dnaE-n gene may be

herein referred as "intein-N." The intein encoded by the dnaE-c gene may be herein referred as "intein-C."

Other intein systems may also be used. For example, a synthetic intein based on the dnaE intein, the Cfa-N (e.g., split intein-N) and Cfa-C (e.g., split intein-C) intein pair, has been described (e.g., in Stevens et al., J Am Chem Soc. 2016 Feb. 24; 138(7):2162-5, incorporated herein by reference). Non-limiting examples of intein pairs that may be used in accordance with the present disclosure include: Cfa DnaE intein, Ssp GyrB intein, Ssp DnaX intein, Ter DnaE3 intein, Ter ThyX intein, Rma DnaB intein and Cne Prp8 intein (e.g., as described in U.S. Patent No. 8,394,604, incorporated herein by reference.

Exemplary nucleotide and amino acid sequences of inteins are provided.

DnaE Intein-N DNA:

TGCCTGTCATACGAAACCGAGATACTGACAGTAGAATATGGCCTTCTGCCAATCGGGAAGAT
TGTGGAGAAACGGATAGAATGCACAGTTTACTCTGTCGATAACAATGGTAACATTTATACTC
AGCCAGTTGCCCAGTGGCACGACCGGGGAGAGCAGGAAGTATTCGAATACTGTCTGGAGGAT
GGAAGTCTCATTAGGGCCACTAAGGACCACAAATTTATGACAGTCGATGGCCAGATGCTGCC
TATAGACGAAATCTTTGAGCGAGAGTTGGACCTCATGCGAGTTGACAACCTTCCTAAT

DnaE Intein-N Protein:

CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDR
GEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNL PN

DnaE Intein-C DNA:

ATGATCAAGATAGCTACAAGGAAGTATCTTGGCAAACAAAACGTTTATGA
TATTGGAGTCGAAAGAGATCACAACTTTGCTCTGAAGAACGGATTCATAG CTTCTAAT

Intein-C: MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN

Cfa-N DNA:

TGCCTGTCTTATGATACCGAGATACTTACCGTTGAATATGGCTTCTTGCCTATTGGAAAGAT
TGTCGAAGAGAGAATTGAATGCACAGTATATACTGTAGACAAGAATGGTTTCGTTTACACAC
AGCCCATTGCTCAATGGCACAATCGCGGCGAACAAGAAGTATTTGAGTACTGTCTCGAGGAT
GGAAGCATCATACGAGCAACTAAAGATCATAAATTCATGACCACTGACGGGCAGATGTTGCC
AATAGATGAGATATTCGAGCGGGGCTTGGATCTCAAACAAGTGGATGGATTGCCA

Cfa-N Protein:

CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLED
GSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

Cfa-C DNA:

ATGAAGAGGACTGCCGATGGATCAGAGTTTGAATCTCCCAAGAAGAAGAGGAAAGTAAAGAT
AATATCTCGAAAAAGTCTTGGTACCCAAAATGTCTATGATATTGGAGTGGAGAAAGATCACA
ACTTCCTTCTCAAGAACGGTCTCGTAGCCAGCAAC

Cfa-C Protein:

MKRTADGSEFESPKKKRKVKIISRKSLGTQNVYDIGVEKDHNFLLKNGLVASN

Intein-N and intein-C may be fused to the N-terminal portion of the split Cas9 and the C-terminal portion of the split Cas9, respectively, for the joining of the N-terminal portion of the split Cas9 and the C-terminal portion of the split Cas9. For example, in some embodiments, an intein-N is fused to the C-terminus of the N-terminal portion of the split Cas9, i.e., to form a structure of N--[N-terminal portion of the split Cas9]-[intein-N]--C. In some embodiments, an intein-C is fused to the N-terminus of the C-terminal portion of the split Cas9, i.e., to form a structure of N-[intein-C]--[C-terminal portion of the split Cas9]-C. The mechanism of intein-mediated protein splicing for joining the proteins the inteins are fused to (e.g., split Cas9) is known in the art, e.g., as described in Shah et al., *Chem Sci.* 2014; 5(1):446-461, incorporated herein by reference. Methods for designing and using inteins are known in the art and described, for example by WO2014004336, WO2017132580, US20150344549, and US20180127780, each of which is incorporated herein by reference in their entirety.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

The term "linker", as used herein, can refer to a covalent linker (*e.g.*, covalent bond), a non-covalent linker, a chemical group, or a molecule linking two molecules or moieties, *e.g.*, two components of a protein complex or a ribonucleocomplex, or two domains of a fusion protein, such as, for example, a polynucleotide programmable DNA binding domain

(*e.g.*, dCas9) and a deaminase domain ((*e.g.*, an adenosine deaminase, a cytidine deaminase, or an adenosine deaminase and a cytidine deaminase). A linker can join different components of, or different portions of components of, a base editor system. For example, in some embodiments, a linker can join a guide polynucleotide binding domain of a polynucleotide programmable nucleotide binding domain and a catalytic domain of a deaminase. In some embodiments, a linker can join a CRISPR polypeptide and a deaminase. In some embodiments, a linker can join a Cas9 and a deaminase. In some embodiments, a linker can join a dCas9 and a deaminase. In some embodiments, a linker can join a nCas9 and a deaminase. In some embodiments, a linker can join a guide polynucleotide and a deaminase. In some embodiments, a linker can join a deaminating component and a polynucleotide programmable nucleotide binding component of a base editor system. In some embodiments, a linker can join a RNA-binding portion of a deaminating component and a polynucleotide programmable nucleotide binding component of a base editor system. In some embodiments, a linker can join a RNA-binding portion of a deaminating component and a RNA-binding portion of a polynucleotide programmable nucleotide binding component of a base editor system. A linker can be positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond or non-covalent interaction, thus connecting the two. In some embodiments, the linker can be an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker can be a polynucleotide. In some embodiments, the linker can be a DNA linker. In some embodiments, the linker can be a RNA linker. In some embodiments, a linker can comprise an aptamer capable of binding to a ligand. In some embodiments, the ligand may be carbohydrate, a peptide, a protein, or a nucleic acid. In some embodiments, the linker may comprise an aptamer may be derived from a riboswitch. The riboswitch from which the aptamer is derived may be selected from a theophylline riboswitch, a thiamine pyrophosphate (TPP) riboswitch, an adenosine cobalamin (AdoCbl) riboswitch, an S-adenosyl methionine (SAM) riboswitch, an SAH riboswitch, a flavin mononucleotide (FMN) riboswitch, a tetrahydrofolate riboswitch, a lysine riboswitch, a glycine riboswitch, a purine riboswitch, a GlmS riboswitch, or a pre-queosine1 (PreQ1) riboswitch. In some embodiments, a linker may comprise an aptamer bound to a polypeptide or a protein domain, such as a polypeptide ligand. In some embodiments, the polypeptide ligand may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat protein domain, a SfMu Com coat protein domain, a sterile alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding

motif and Sm7 protein, or a RNA recognition motif. In some embodiments, the polypeptide ligand may be a portion of a base editor system component. For example, a nucleobase editing component may comprise a deaminase domain and a RNA recognition motif.

In some embodiments, the linker can be an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, the linker can be about 5-100 amino acids in length, for example, about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 amino acids in length. In some embodiments, the linker can be about 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, or 450-500 amino acids in length. Longer or shorter linkers can be also contemplated.

In some embodiments, a linker joins a gRNA binding domain of an RNA-programmable nuclease, including a Cas9 nuclease domain, and the catalytic domain of a nucleic-acid editing protein (e.g., cytidine or adenosine deaminase). In some embodiments, a linker joins a dCas9 and a nucleic-acid editing protein. For example, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two. In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein). In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-200 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 35, 45, 50, 55, 60, 60, 65, 70, 70, 75, 80, 85, 90, 90, 95, 100, 101, 102, 103, 104, 105, 110, 120, 130, 140, 150, 160, 175, 180, 190, or 200 amino acids in length.

In some embodiments, the domains of a base editor are fused via a linker that comprises the amino acid sequence of SGGSSGSETPGTSESATPESSGGS, SGGSSGGSSGSETPGTSESATPESSGGSSGGS, or GGSGGSPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGGSGGS. In some embodiments, domains of the nucleobase editor are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES, which may also be referred to as the XTEN linker. In some embodiments, the linker is 24 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPES. In some embodiments, the linker is 40 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGS. In some embodiments, the

linker is 64 amino acids in length. In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGSSGGS. In some embodiments, the linker is 92 amino acids in length. In some embodiments, the linker comprises the amino acid sequence PGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATS.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

The term "mutation," as used herein, refers to a substitution of a residue within a sequence, *e.g.*, a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)). In some embodiments, the presently disclosed base editors can efficiently generate an "intended mutation", such as a point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor (*e.g.*, cytidine base editor or adenosine base editor) bound to a guide polynucleotide (*e.g.*, gRNA), specifically designed to generate the intended mutation.

In general, mutations made or identified in a sequence (e.g., an amino acid sequence as described herein) are numbered in relation to a reference (or wild type) sequence, i.e., a sequence that does not contain the mutations. The skilled practitioner in the art would readily understand how to determine the position of mutations in amino acid and nucleic acid sequences relative to a reference sequence.

The term "non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid

substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the wild-type protein.

In some embodiments, the presently disclosed base editors can efficiently generate an "intended mutation", such as a point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor (*e.g.*, cytidine base editor or adenosine base editor) bound to a guide polynucleotide (*e.g.*, gRNA), specifically designed to generate the intended mutation.

In general, mutations made or identified in a sequence (e.g., an amino acid sequence as described herein) are numbered in relation to a reference (or wild type) sequence, i.e., a sequence that does not contain the mutations. The skilled practitioner in the art would readily understand how to determine the position of mutations in amino acid and nucleic acid sequences relative to a reference sequence.

The term "non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the wild-type protein.

The term "nuclear localization sequence," "nuclear localization signal," or "NLS" refers to an amino acid sequence that promotes import of a protein into the cell nucleus. Nuclear localization sequences are known in the art and described, for example, in Plank *et al.*, International PCT application, PCT/EP2000/011690, filed November 23, 2000, published as WO/2001/038547 on May 31, 2001, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In other embodiments, the NLS is an optimized NLS described, for example, by Koblan *et al.*, Nature Biotech. 2018 doi:10.1038/nbt.4172. In some embodiments, an NLS comprises the amino acid sequence KRTADGSEFESPKKKRKV, KRPAATKKAGQAKKKK, KKTELQTTNAENKTKKL, KRGINDRNFWRGENGRKTR, RKSGKIAAIVVKRPRK, PKKKRKV, or MDSLLMNRRKFLYQFKNVRWAKGRRETYLC.

The term "nucleobase," "nitrogenous base," or "base," used interchangeably herein, refers to a nitrogen-containing biological compound that forms a nucleoside, which in turn is

a component of a nucleotide. The ability of nucleobases to form base pairs and to stack one upon another leads directly to long-chain helical structures such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Five nucleobases – adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U) – are called primary or canonical. Adenine and guanine are derived from purine, and cytosine, uracil, and thymine are derived from pyrimidine. DNA and RNA can also contain other (non-primary) bases that are modified. Non-limiting exemplary modified nucleobases can include hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine (m5C), and 5-hydromethylcytosine. Hypoxanthine and xanthine can be created through mutagen presence, both of them through deamination (replacement of the amine group with a carbonyl group). Hypoxanthine can be modified from adenine. Xanthine can be modified from guanine. Uracil can result from deamination of cytosine. A "nucleoside" consists of a nucleobase and a five carbon sugar (either ribose or deoxyribose). Examples of a nucleoside include adenosine, guanosine, uridine, cytidine, 5-methyluridine (m5U), deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine, and deoxycytidine. Examples of a nucleoside with a modified nucleobase includes inosine (I), xanthosine (X), 7-methylguanosine (m7G), dihydrouridine (D), 5-methylcytidine (m5C), and pseudouridine (Ψ). A "nucleotide" consists of a nucleobase, a five carbon sugar (either ribose or deoxyribose), and at least one phosphate group.

The terms "nucleic acid" and "nucleic acid molecule," as used herein, refer to a compound comprising a nucleobase and an acidic moiety, e.g., a nucleoside, a nucleotide, or a polymer of nucleotides. Typically, polymeric nucleic acids, e.g., nucleic acid molecules comprising three or more nucleotides are linear molecules, in which adjacent nucleotides are linked to each other via a phosphodiester linkage. In some embodiments, "nucleic acid" refers to individual nucleic acid residues (*e.g.* nucleotides and/or nucleosides). In some embodiments, "nucleic acid" refers to an oligonucleotide chain comprising three or more individual nucleotide residues. As used herein, the terms "oligonucleotide" and "polynucleotide" can be used interchangeably to refer to a polymer of nucleotides (e.g., a string of at least three nucleotides). In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA. Nucleic acids may be naturally occurring, for example, in the context of a genome, a transcript, an mRNA, tRNA, rRNA, siRNA, snRNA, a plasmid, cosmid, chromosome, chromatid, or other naturally occurring nucleic acid molecule. On the other hand, a nucleic acid molecule may be a non-naturally occurring molecule, *e.g.*, a recombinant DNA or RNA, an artificial chromosome, an engineered

genome, or fragment thereof, or a synthetic DNA, RNA, DNA/RNA hybrid, or including non-naturally occurring nucleotides or nucleosides. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, *e.g.,* analogs having other than a phosphodiester backbone. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, e.g., in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and backbone modifications. A nucleic acid sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (*e.g.* adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (2′-e.g.,fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5′-*N*-phosphoramidite linkages).

The term "nucleic acid programmable DNA binding protein" or "napDNAbp" may be used interchangeably with "polynucleotide programmable nucleotide binding domain" to refer to a protein that associates with a nucleic acid (e.g., DNA or RNA), such as a guide nucleic acid or guide polynucleotide (*e.g.,* gRNA), that guides the napDNAbp to a specific nucleic acid sequence. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable RNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas9 protein. A Cas9 protein can associate with a guide RNA that guides the Cas9 protein to a specific DNA sequence that is complementary to the guide RNA. In some embodiments, the napDNAbp is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9). Non-limiting examples of nucleic acid programmable DNA binding proteins include, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3,

Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. Non-limiting examples of Cas enzymes include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues thereof, or modified or engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova *et al.* "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" *CRISPR J.* 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan *et al.*, "Functionally diverse type V CRISPR-Cas systems" *Science*. 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

The terms "nucleobase editing domain" or "nucleobase editing protein," as used herein, refers to a protein or enzyme that can catalyze a nucleobase modification in RNA or DNA, such as cytosine (or cytidine) to uracil (or uridine) or thymine (or thymidine), and adenine (or adenosine) to hypoxanthine (or inosine) deaminations, as well as non-templated nucleotide additions and insertions. In some embodiments, the nucleobase editing domain is a deaminase domain (*e.g.*, an adenine deaminase or an adenosine deaminase; or a cytidine deaminase or a cytosine deaminase). In some embodiments, the nucleobase editing domain is more than one deaminase domain (*e.g.*, an adenine deaminase or an adenosine deaminase and a cytidine or a cytosine deaminase). In some embodiments, the nucleobase editing domain can be a naturally occurring nucleobase editing domain. In some embodiments, the nucleobase editing domain can be an engineered or evolved nucleobase editing domain from the naturally occurring nucleobase editing domain. The nucleobase editing domain can be from any organism, such as a bacterium, human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse.

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

A "patient" or "subject" as used herein refers to a mammalian subject or individual diagnosed with, at risk of having or developing, or suspected of having or developing a disease or a disorder. In some embodiments, the term "patient" refers to a mammalian subject with a higher than average likelihood of developing a disease or a disorder. Exemplary patients can be humans, non-human primates, cats, dogs, pigs, cattle, cats, horses, camels, llamas, goats, sheep, rodents (*e.g.*, mice, rabbits, rats, or guinea pigs) and other mammalians that can benefit from the therapies disclosed herein. Exemplary human patients can be male and/or female.

"Patient in need thereof" or "subject in need thereof" is referred to herein as a patient diagnosed with, at risk or having, predetermined to have, or suspected of having a disease or disorder.

The terms "protein," "peptide," "polypeptide," and their grammatical equivalents are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. The terms refer to a protein, peptide, or polypeptide of any size, structure, or function. Typically, a protein, peptide, or polypeptide will be at least three amino acids long. A protein, peptide, or polypeptide can refer to an individual protein or a collection of proteins. One or more of the amino acids in a protein, peptide, or polypeptide can be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modifications, etc. A protein, peptide, or polypeptide can also be a single molecule or can be a multi-molecular complex. A protein, peptide, or polypeptide can be just a fragment of a naturally occurring protein or peptide. A protein, peptide, or polypeptide can be naturally occurring, recombinant, or synthetic, or any combination thereof. The term "fusion protein" as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein can be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an amino-terminal fusion protein or a carboxy-terminal fusion protein, respectively. A protein can comprise different domains, for example, a nucleic acid binding domain (*e.g.*, the gRNA binding domain of Cas9 that directs the binding of the protein to a target site) and a nucleic acid cleavage domain, or a catalytic domain of a nucleic acid editing protein. In some embodiments, a protein comprises a proteinaceous part, *e.g.*, an amino acid sequence constituting a nucleic acid binding domain, and an organic compound, *e.g.*, a compound that can act as a nucleic acid cleavage agent. In

some embodiments, a protein is in a complex with, or is in association with, a nucleic acid, *e.g.*, RNA or DNA. Any of the proteins provided herein can be produced by any method known in the art. For example, the proteins provided herein can be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

Polypeptides and proteins disclosed herein (including functional portions and functional variants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine. The polypeptides and proteins can be associated with post-translational modifications of one or more amino acids of the polypeptide constructs. Non-limiting examples of post-translational modifications include phosphorylation, acylation including acetylation and formylation, glycosylation (including N-linked and O-linked), amidation, hydroxylation, alkylation including methylation and ethylation, ubiquitylation, addition of pyrrolidone carboxylic acid, formation of disulfide bridges, sulfation, myristoylation, palmitoylation, isoprenylation, farnesylation, geranylation, glypiation, lipoylation and iodination.

The term "recombinant" as used herein in the context of proteins or nucleic acids refers to proteins or nucleic acids that do not occur in nature, but are the product of human engineering. For example, in some embodiments, a recombinant protein or nucleic acid molecule comprises an amino acid or nucleotide sequence that comprises at least one, at least

two, at least three, at least four, at least five, at least six, or at least seven mutations as compared to any naturally occurring sequence.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition. In one embodiment, the viral load present in a cell treated with a base editor system described herein is compared to the level of HBV infection present in an untreated control cell, which control serves as a reference. In another embodiment, the sequence of an HBV genome present in cell contacted with a base editor system described herein is compared to the sequence of an HBV genome present in an untreated control cell.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, at least about 20 amino acids, at least about 25 amino acids, about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, at least about 60 nucleotides, at least about 75 nucleotides, about 100 nucleotides, or about 300 nucleotides or any integer thereabout or therebetween. In some embodiments, a reference sequence is a wild-type sequence of a protein of interest. In other embodiments, a reference sequence is a polynucleotide sequence encoding a wild-type protein.

The term "RNA-programmable nuclease," and "RNA-guided nuclease" are used with (e.g., binds or associates with) one or more RNA(s) that is not a target for cleavage. In some embodiments, an RNA-programmable nuclease, when in a complex with an RNA, may be referred to as a nuclease:RNA complex. Typically, the bound RNA(s) is referred to as a guide RNA (gRNA). In some embodiments, the RNA-programmable nuclease is the (CRISPR-associated system) Cas9 endonuclease, for example, Cas9 (Csnl) from *Streptococcus pyogenes* (*See*, e.g., "Complete genome sequence of an Ml strain of Streptococcus pyogenes." Ferretti J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C, Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and

host factor RNase III." Deltcheva E., Chylinski K., Sharma CM., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607(2011).

By "specifically binds" is meant a nucleic acid molecule, polypeptide, or complex thereof (e.g., a nucleic acid programmable DNA binding domain and guide nucleic acid), compound, or molecule that recognizes and binds a polypeptide and/or nucleic acid molecule of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these

various conditions as needed. In a preferred: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will be less than about 30 mM NaCl and 3 mM trisodium citrate or less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, at least about 42° C or even at least about 68° C. In an embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In another embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By “split” is meant divided into two or more fragments.

A "split Cas9 protein" or "split Cas9" refers to a Cas9 protein that is provided as an N-terminal fragment and a C-terminal fragment encoded by two separate nucleotide sequences. The polypeptides corresponding to the N-terminal portion and the C-terminal portion of the Cas9 protein may be spliced to form a “reconstituted” Cas9 protein. In particular embodiments, the Cas9 protein is divided into two fragments within a disordered region of the protein, e.g., as described in Nishimasu et al., Cell, Volume 156, Issue 5, pp. 935-949, 2014, or as described in Jiang et al. (2016) Science 351: 867-871. PDB file: 5F9R, each of

which is incorporated herein by reference. In some embodiments, the protein is divided into two fragments at any C, T, A, or S within a region of SpCas9 between about amino acids A292-G364, F445-K483, or E565-T637, or at corresponding positions in any other Cas9, Cas9 variant (e.g., nCas9, dCas9), or other napDNAbp. In some embodiments, protein is divided into two fragments at SpCas9 T310, T313, A456, S469, or C574. In some embodiments, the process of dividing the protein into two fragments is referred to as "splitting" the protein.

In other embodiments, the N-terminal portion of the Cas9 protein comprises amino acids 1-573 or 1-637 S. pyogenes Cas9 wild-type (SpCas9) (NCBI Reference Sequence: NC_002737.2, Uniprot Reference Sequence: Q99ZW2) and the C-terminal portion of the Cas9 protein comprises a portion of amino acids 574-1368 or 638-1368 of SpCas9 wild-type.

The C-terminal portion of the split Cas9 can be joined with the N-terminal portion of the split Cas9 to form a complete Cas9 protein. In some embodiments, the C-terminal portion of the Cas9 protein starts from where the N-terminal portion of the Cas9 protein ends. As such, in some embodiments, the C-terminal portion of the split Cas9 comprises a portion of amino acids (551-651)-1368 of spCas9. "(551-651)-1368" means starting at an amino acid between amino acids 551-651 (inclusive) and ending at amino acid 1368. For example, the C-terminal portion of the split Cas9 may comprise a portion of any one of amino acid 551-1368, 552-1368, 553-1368, 554-1368, 555-1368, 556-1368, 557-1368, 558-1368, 559-1368, 560-1368, 561-1368, 562-1368, 563-1368, 564-1368, 565-1368, 566-1368, 567-1368, 568-1368, 569-1368, 570-1368, 571-1368, 572-1368, 573-1368, 574-1368, 575-1368, 576-1368, 577-1368, 578-1368, 579-1368, 580-1368, 581-1368, 582-1368, 583-1368, 584-1368, 585-1368, 586-1368, 587-1368, 588-1368, 589-1368, 590-1368, 591-1368, 592-1368, 593-1368, 594-1368, 595-1368, 596-1368, 597-1368, 598-1368, 599-1368, 600-1368, 601-1368, 602-1368, 603-1368, 604-1368, 605-1368, 606-1368, 607-1368, 608-1368, 609-1368, 610-1368, 611-1368, 612-1368, 613-1368, 614-1368, 615-1368, 616-1368, 617-1368, 618-1368, 619-1368, 620-1368, 621-1368, 622-1368, 623-1368, 624-1368, 625-1368, 626-1368, 627-1368, 628-1368, 629-1368, 630-1368, 631-1368, 632-1368, 633-1368, 634-1368, 635-1368, 636-1368, 637-1368, 638-1368, 639-1368, 640-1368, 641-1368, 642-1368, 643-1368, 644-1368, 645-1368, 646-1368, 647-1368, 648-1368, 649-1368, 650-1368, or 651-1368 of spCas9. In some embodiments, the C-terminal portion of the split Cas9 protein comprises a portion of amino acids 574-1368 or 638-1368 of SpCas9.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a non-human primate (monkey), bovine, equine, canine, ovine, or feline. In some embodiments, a subject described herein is infected with HBV or has a propensity to develop HBV.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In one embodiment, such a sequence is at least 60%, 80%, 85%, 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. COBALT is used, for example, with the following parameters:

a) alignment parameters: Gap penalties-11,-1 and End-Gap penalties-5,-1,
b) CDD Parameters: Use RPS BLAST on; Blast E-value 0.003; Find Conserved columns and Recompute on, and
c) Query Clustering Parameters: Use query clusters on; Word Size 4; Max cluster distance 0.8; Alphabet Regular.

EMBOSS Needle is used, for example, with the following parameters:

a) Matrix: BLOSUM62;
b) GAP OPEN: 10;
c) GAP EXTEND: 0.5;
d) OUTPUT FORMAT: pair;
e) END GAP PENALTY: false;
f) END GAP OPEN: 10; and

g) END GAP EXTEND: 0.5.

The term "target site" refers to a sequence within a nucleic acid molecule that is deaminated by a deaminase or a fusion protein comprising a deaminase (e.g., cytidine or adenine deaminase) fusion protein or a base editor disclosed herein).

Because RNA-programmable nucleases (e.g., Cas9) use RNA:DNA hybridization to target DNA cleavage sites, these proteins are able to be targeted, in principle, to any sequence specified by the guide RNA. Methods of using RNA-programmable nucleases, such as Cas9, for site-specific cleavage (e.g., to modify a genome) are known in the art (see e.g., Cong, L. et ah, Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819-823 (2013); Mali, P. et ah, RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013); Hwang, W.Y. et ah, Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature biotechnology 31, 227-229 (2013); Jinek, M. et ah, RNA-programmed genome editing in human cells. eLife 2, e00471 (2013); Dicarlo, J.E. et ah, Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic acids research (2013); Jiang, W. et ah RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature biotechnology 31, 233-239 (2013); the entire contents of each of which are incorporated herein by reference).

As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith or obtaining a desired pharmacologic and/or physiologic effect. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated. In some embodiments, the effect is therapeutic, *i.e.*, without limitation, the effect partially or completely reduces, diminishes, abrogates, abates, alleviates, decreases the intensity of, or cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the effect is preventative, *i.e.*, the effect protects or prevents an occurrence or reoccurrence of a disease or condition. To this end, the presently disclosed methods comprise administering a therapeutically effective amount of a compositions as described herein. In one embodiment, the invention provides for the treatment of HBV infection.

By “uracil glycosylase inhibitor” or “UGI” is meant an agent that inhibits the uracil-excision repair system. In one embodiment, the agent is a protein or fragment thereof that binds a host uracil-DNA glycosylase and prevents removal of uracil residues from DNA. In an embodiment, a UGI is a protein, a fragment thereof, or a domain that is capable of

inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a modified version thereof. In some embodiments, a UGI domain comprises a fragment of the exemplary amino acid sequence set forth below. In some embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the exemplary UGI sequence provided below. In some embodiments, a UGI comprises an amino acid sequence that is homologous to the exemplary UGI amino acid sequence or fragment thereof, as set forth below. In some embodiments, the UGI, or a portion thereof, is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% identical to a wild type UGI or a UGI sequence, or portion thereof, as set forth below. An exemplary UGI comprises an amino acid sequence as follows:

>splP14739IUNGI_BPPB2 Uracil-DNA glycosylase inhibitor

MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLT S D APE YKPW ALVIQDS NGENKIKML.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

The description and examples herein illustrate embodiments of the present disclosure in detail. It is to be understood that this disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this disclosure, which are encompassed within its scope.

All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

The practice of some embodiments disclosed herein employ, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See for example Sambrook and Green, Molecular Cloning: A Laboratory Manual, 4th Edition (2012); the series Current Protocols in Molecular Biology (F. M. Ausubel, *et al.* eds.); the series Methods In Enzymology (Academic Press, Inc.), PCR 2: A Practical Approach (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications, 6th Edition (R.I. Freshney, ed. (2010)).

Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and in view of the accompanying drawings as described hereinbelow.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an illustration showing the partially double-stranded and the overlapping open reading frames (ORFs) for the hepatitis B surface antigen (HBsAg) gene, the polymerase gene, the protein X gene, and the core gene. The HBsAg gene comprises ORF PreS1, ORF PreS2, and ORF S, which encode the large, middle, and small surface proteins, respectively. ORF core and Pre C encode capsid proteins.

FIG. 2 is an illustration depicting the HBV life cycle. The term "ER" denotes endoplasmic reticulum. The term "HBsAg" denotes hepatitis B surface antigen. "HBx transcriptional activator" is an HBV-specific transcriptional activator of polymerase II and III promoters.

FIG. 3A is a map of the geographic distribution of hepatitis B virus genotypes worldwide.

FIG. 3B provides a summary of a base editing strategies for introducing stop codons in viral genes and for generating abasic sites to treat chronic HBV.

FIG. 3C provides a summary of guide RNA screening strategies adapted for introducing stop codons and for generating abasic via base editing.

FIG. 3D is a diagram illustrating conserved gRNA design for generating abasic sites in cccDNA.

FIG. 3E is a diagram of the HBV cccDNA showing the relative position of 16 guide RNAs (depicted as triangles) that are expected to generate an amino acid that occurs in less than 0.05% of HBV genomes.

FIG. 3F is a graph showing the highest percentage of base editing generated by gRNA candidates.

FIG. 3G is a chart summarizing information relating to gRNA candidates.

FIGS. 4A and 4B depict base editors. FIG. 4A is a depiction of a base editor having an APOBEC cytidine deaminase domain, a Cas9 domain, and two uracil glycosylase inhibitor (UGI) domains. FIG. 4B provides a diagram of BE4.

FIG. 5 is an illustration showing where guide RNAs of the present disclosure map to the HBV genome. Each triangle represents a unique guide RNA.

FIG. 6 is a schematic illustration summarizing the screen for guide RNA molecules that target an HBV gene and a subset of observed results from the screen. "PAM" refers to protospacer adjacent motif. "Pol" refers to the HBV polymerase gene; "S" refers to the HBV surface protein; "X" refers to the HBV protein X gene; and "Core" refers to the HBV core protein. MSPbeam52, 50, …, etc. refer to guide RNA, which are also termed M52, M50, …, etc., in the application. The screen identified 12 gRNAs that exhibited greater than 20% on-target base editing.

FIG. 7 comprises graphs comparing the BE4 and A3ABE4 base editors. The graphs show the percent editing observed for different guide RNAs used with each base editor. MSPbeam39, 40, …, etc. are also termed M39, M40, …, etc., in the application.

FIG. 8 is a graph illustrating functional base editing observed in a HepG2-NTCP cell line transduced with an HBV lentiviral vector and transfected with a nucleic acid construct encoding a base editor. The nucleic acid constructs tested were DNA molecules, wild type RNA molecules, or RNA molecules comprising pseudo-uridine (PsU) modified at the N1 residue. "NTCP" refers to sodium taurocholate co-transporting polypeptide. MSPbeam39, 40, ..., etc. are also termed M39, M40, ..., etc., in the application.

FIG. 9 is a graph illustrating functional base editing observed in a HepG2-NTCP cell line transduced with an HBV lentiviral vector and transfected with a nucleic acid construct encoding a base editor. The nucleic acid constructs tested were DNA format transfection (two plasmids one encoding the base editor and one encoding the gRNA) or RNA format (PsU-modified in-house mRNA encoding the base editor where the RNA is modified at the N1 residue and a synthetic gRNA). Up to 80% editing in HepG2-NTCP lenti HBV cell lines was observed when using base editors and lead Stop/Functional Change ("FC") gRNAs in an RNA format. "NTCP" refers to sodium taurocholate co-transporting polypeptide. MSPbeam39, 40, ..., etc. are also termed M39, M40, ..., etc., in the application.

FIG. 10 is a graph illustrating functional base editing observed in a HepG2-NTCP cell line transduced with an HBV lentiviral vector and transfected with one of three nucleic acid constructs encoding a BE4, BE4-VRQR, or ABE base editor. MSPbeam39, 40, ..., etc. are also termed M39, M40, ..., etc., in the application.

FIG. 11 is an illustration depicting guide RNAs that map to conserved regions of the HBV genome.

FIG. 12A is a schematic illustrating long-term primary hepatocyte co-cultures. FIG. 12B provides an experimental timeline for hepatocyte monolayers or hepatocyte co-cultures. FIG. 12C shows images of transduced primary hepatocytes from donors (RSE, TVR) used in the co-culture system.

FIGS. 13A-13F characterize an HBV-infected primary human hepatocyte (PHH) system. FIG. 13A is a timeline showing the infection and treatment schedule for the 13 days from plating to study end-point. FIG. 13B is a graph showing the amount of extracellular HBV DNA present in a PHH culture after no treatment of HBV infected PHH cells, treatment with interferon, or treatment with tenofovir. As a negative control, PHH cells were exposed to the HBV virus without polyethylene glycol. FIG. 13C is a graph showing the amount of HBV surface antigen (HBsAg) present in PHH cultures exposed to the experimental conditions described in the legend for FIGS. 13B and 13C. FIG. 13D is a graph showing the

amount of intracellular HBV DNA present in PHH cultures exposed to the experimental conditions described in the legend for FIGS. 13B and 13C. FIG. 13E is a graph showing the amount of total HBV RNA present in PHH cultures exposed to the experimental conditions described in the legend for FIGS. 13B and 13C. FIG. 13F is a graph showing the amount of pregenomic RNA (pgRNA) present in PHH cultures exposed to the experimental conditions described in the legend for FIGS. 13B and 13C.

FIG. 14 is a graph showing that transfection with a BE4 and gRNAs leads to a decrease in HBV marker levels in HBV infected PHH. Guide RNAs 52 and 190, which target the BE4 base editor to the Pol and X gene regions of the HBV genome, respectively, were used. BE4 was tested with and without a Uridine Glycosylase Inhibitor (UGI) domain.

FIG. 15 is a graph showing the identification of functional guide RNAs in a screen in HBV-infected PHH cells, where decreased levels of HBsAg, which is a surrogate of cccDNA, are indicative of a functional gRNA. Guide RNAs introducing stop codons are noted as Stop-39, etc. . . ., Guide RNAs introducing changes at conserved amino acids are indicated as Conserved-4, etc. . . . gRNAs (Stop-191, Conserved-12) selected for further analysis are indicated with boxes.

FIGS. 16A and 16B illustrate mechanistic aspects of base editing action on HBV. FIG. 16A is a graph showing the levels of HBsAg in HBV infected PHH cells transfected with mRNA encoding either a BE4 base editor with a UGI domain (BE4), a BE4 base editor with no UGI domain (BE4_noUGI), Cas9, a catalytically dead (i.e., having no nickase activity) BE4 base editor with no UGI domain (dBE4_noUGI), or a dead Cas9 (dCas9). The cells were transfected with mRNA encoding the base editor only, or were also transfected with either gRNA191 or gRNA12. FIG. 16B is a graph showing the levels of extracellular HBV DNA in HBV infected PHH cells transfected as described for FIG. 16A.

FIGS. 17A and 17B compare base editing in HepG2-NTCP Lenti-HBV and HBV infected PHH. FIG. 17A is a graph showing the editing efficiencies observed in HepG2-NTCP Lenti-HBV transfected with BE4 and UGI versus BE4 without UGI. FIG. 17B is a graph showing the editing efficiencies observed in HBV infected PHH transfected with BE4 and UGI versus BE4 without UGI.

FIG. 18 is a graph comparing the base editing, indel rates, and transversion rates (i.e., C to A or G) using gRNA190 in HBV-Lenti-HepG2 versus HBV infected PHH.

FIG. 19 shows a schematic timeline related to the use of primary hepatocyte co-culture (PHH) infected with HBV virus as a clinically relevant system for assessing anti-viral

activity of the base editing reagents described herein. In some embodiments, PHH co-cultures infected with HBV were used in the experiments described herein to assay and assess the antiviral efficacy of the base editors. In brief, the base editing reagents (base editor mRNA and synthetic gRNA) were transfected into PHH co-cultures via lipofection twice over the course of two weeks. The first transfection was performed 3 days after infection with HBV to ensure that the cccDNA was completely formed at the time of virus transfection. Extracellular parameters (HBsAg, HBeAg, and HBV DNA) were monitored over the course of the described experiments, and intracellular parameters (HBV DNA, viral RNA, and editing) were monitored at the end of the described experiments. HbsAg refers to the surface protein antigen of HBV. Its detection indicates HBV infection in an individual. HBeAg refers to the hepatitis B e-antigen, a HBV protein antigen that circulates in infected blood when the virus is actively replicating. The presence of HBeAg suggests that an individual is infectious and is able to spread the virus to others.

FIG. 20 shows a bar graph presenting the results of a 14-day experiment employing HBV-infected primary hepatocyte co-cultures (PHH) and gRNA12, which targets a polynucleotide sequence in the intersection of the HBV Polymerase and S gene sequences. The antiviral drug entecavir was used as a control to assess the efficacy of the base editors (BE4 and BE4-noUGI). As observed, the BE4-noUGI base editor and the gRNA12 resulted in a reduction of all 4 viral marker parameters tested, namely, a reduction in the amounts or levels of the HBV DNA, HBsAg, HBeAg and pgRNA marker parameters. In addition, the BE4-noUGI base editor and the gRNA12 showed an overall superior performance in reducing all 4 HBV parameters tested compared with entecavir. Accordingly, the base editing approach described herein was demonstrated to be more efficient in reducing the viral (HBV) parameters tested compared with the HBV antiviral drug entecavir.

FIG. 21 shows a bar graph presenting the results of employing multiple gRNAs (gRNA multiplexing) in conjunction with BE4. The HBV parameters assessed included pgRNA, HBsAg, HBeAg and HBV total DNA. The results indicate a gRNA-specific reduction in particular HBV parameters, with gRNA19 demonstrating an improved HBV inhibition activity compared with other gRNAs tested. In addition, a measurable improvement in HBV inhibition was observed using gRNA multiplexing, particularly with the combination of gRNA19 + gRNA190, and with a combination of gRNA190, gRNA12, gRNA40 and gRNA52, which showed optimal HBV inhibition activities.

FIG. 22 shows a bar graph presenting the results of base editing using the HBV-infected PHH culture system and the BE4 base editor. NGS sequencing was performed on the total DNA purified from HBV-infected PHH cultures and on the same samples enriched for cccDNA. The results demonstrated significantly increased base editing (% base editing) in cccDNA enriched samples, thus indicating the successful base editing of HBV cccDNA by the BE4 base editor and gRNAs. The finding of reduced base editing in total genomic DNA purified from HBV-PHH suggests the inability of edited cccDNA to propagate into a replication-competent viral particle.

FIG. 23 shows a bar graph presenting the results of employing multiple gRNAs (gRNA multiplexing) with BE4 and noUGI (BE4_noUGI), e.g., as described in Example 10, *infra*. The HBV parameters assessed included HBsAg, HBeAg, pgRNA and HBV total DNA. The results indicate a significant gRNA-specific inhibition of HBV parameters, with gRNA12 and gRNA19 demonstrating increased inhibition activities. In addition, the HBV-inhibition activity of gRNA19 with BE_noUGI was found to be equally effective as combinations of other gRNAs tested.

FIG. 24 shows a bar graph presenting the results of base editing using the HBV-infected PHH culture system and the BE4_noUGI base editor. NGS sequencing was performed on the total DNA purified from HBV-infected PHH cultures and on the same samples enriched for cccDNA. The results demonstrated significantly increased base editing (% base editing) in cccDNA enriched samples, thus indicating the successful base editing of HBV cccDNA by BE_noUGI and gRNAs. The finding of robust base editing activity in total genomic DNA purified from HBV-PHH suggests the inability of edited cccDNA to propagate into a replication-competent viral particle.

FIGS. 25A-25D show graphs and bar graphs related to the use of the base editor dBE4_noUGI (H840A) without nickase activity and the HBV-infected PHH system in a long term (e.g., 25-day) experiment to assess the efficacy of the base editor on HBV viral parameters HBsAg (FIG. 25A), extracellular HBV DNA (FIG. 25B), HBeAg (FIG. 25C), and albumin (cell viability/metabolic rate), (FIG. 25D). The results of this experiment showed that dBE4_noUGI (D10A_H840A) and gRNA12 reduced viral parameters in HBV-infected PHH. In addition, while both interferon and the base editing components (dBE4_noUGI+gRNA12) decreased HBV viral parameters, interferon treatment was found to be more toxic compared to the use of the base editor and base editing system described herein. Base editor dBE4_noUGI (H840A) comprises the amino acid sequence

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE

FIGS. 26A-26C present graphs and bar graphs showing the results of long-term (e.g., 25 day) experiments involving PHH cultures infected with HBV of genotypes D and C to assess the base editor (e.g., dBE4_no UGI) and BE system (e.g., dBE4_no UGI + gRNA, e.g., gRNA12) as described herein in reducing or inhibiting HBV by assessing HBV parameters, namely, HBsAg (FIG. 26A), HBeAg (FIG. 26B) and extracellular HBV DNA (FIG. 26C). The experiments demonstrated that HBV of genotype C infected cells more aggressively, as the viral load was higher at the termination of the experiment. In addition, transfection of HBV-infected PHH cultures with dBE4_no UGI and gRNA12 led to a reduction of viral parameters compared to controls for both HBV of genotype D and HBV of genotype C.

FIGS. 27A and 27B present bar graphs demonstrating the results of transfection of HBV-infected PHH cultures with the adenine base editor ABE7.10 and an HBV-specific gRNA, e.g., gRNA94, which targets HBV polymerase active site. As demonstrated, ABE7.10 + gRNA94 showed significant gRNA-specific HBV inhibition and reduction of the

HBV markers HBsAg, HBeAg, pgRNA and HBV total DNA in the assayed PHH cultures relative to controls (no treatment of PHH and ABE7.10-only treatment of PHH). (FIG. 27A). In addition, ABE7.10 + gRNA94 in HBV-infected PHH resulted in robust HBV cccDNA editing. (FIG. 27B). The lack of base editing observed in total HBV genomic DNA suggests an inability of edited HBV cccDNA to propagate into a replication-competent viral particle.

DETAILED DESCRIPTION OF THE INVENTION

The invention features compositions and methods for editing the HBV genome. For example, the compositions contemplated herein can, in some embodiments, include a base editor a guide nucleic acid that targets a particular nucleotide in an HBV gene. In some embodiments, the editing introduces a premature stop codon in the coding sequence of one of the viral proteins. In another embodiment, the editing introduces one or more functional substitutions in the coding sequence of one or more HBV proteins.

The HBV genome comprises 3.2 kb of partially double-stranded DNA and open read frames (ORFs) encoding seven proteins. Referring to FIG. 1, the open reading frame (ORF) P encodes the viral polymerase. The ORF C/PreC encodes capsid proteins. ORF PreS1, ORF PreS2, and ORF S encode large (L), middle (M) and small (S) surface proteins, respectively. ORF X encodes the secretary X protein.

The partially double-stranded HBV genome is converted by host factors to covalently closed circular DNA (cccDNA). The cccDNA is transcribed by a host RNA polymerase to produce viral mRNA including pre-genomic RNA (pgRNA). pgRNA is reversed transcribed by the HBV polymerase into genomic HBV DNA that can be converted into cccDNA, packaged into virions, or integrated into the host cell's genome (FIG. 2). cccDNA, a key component of the HBV life cycle, is a stable molecule responsible for chronic HBV infection. Editing of the HBV genome can disrupt the formation of cccDNA, thereby reducing the pathogenicity of the virus.

There are ten different HBV genotypes (A-J) (FIG. 3A). A "genotype" is characterized by < 92% sequence identity with any other genome, and a sub-genotype is characterized by < 96 to 92% sequence identity. HBV of genotype D is the most prevalent in the United States (FIG. 3A). Research models of HBV genotype D are available including viral stocks (e.g., genotype D, subgenotype ayw (Imquest)) and mouse models (e.g., humanized mouse model (Phoenixbio). Thus, in some embodiments, methods and compositions are provided that target HBV ORFs for editing. These compositions can

comprise a nucleobase editor having a Cas9 or other nucleic acid programmable DNA binding protein domain and an adenosine or cytosine deaminase domain. In some embodiments, the base editor introduces one or more alterations into an HBV ORF. In some embodiments, the alteration results in a mutation in a conserved portion of an HBV protein. In particular embodiments, the alteration introduces one or more stop codons. Throughout the specification, the introduction of a stop codon, resulting in the premature termination of the protein is represented by the amino acid symbol, the amino acid position, and the term STOP (e.g., R87STOP indicates that the codon encoding Arginine at amino acid position 87 is replaced by a Stop codon). Advantageously, the methods of the present invention do not introduce double stranded breaks in the HBV genome.

The invention provides strategies for using base editing to treat chronic HBV (FIG. 3B). Described herein are screens for identifying guide RNAs that introduce stop codons or functional mutations into HBV genes or that identify gRNAs that generate abasic sites in superconserved regions of the HBV genome (FIG. 3C). Introducing stop condons into viral genes using the methods and compositions described herein can be accomplished without generating double strand breaks, thereby eliminating or reducing the risk of cutting host genetic material after HBV integrates into the host's genome. Additionally, the compositions employ a deaminase that is a natural HBV antiviral restriction factor. For example, inducing APOBEC cytodine deaminases with interferon alpha or Lymphotoxin β receptor (LTBR) promotes abasic site formation and cccDNA degradation (FIG. 3B). Furthermore, using a base editor without uracil glycosilase inhibitor domains can target cellular uracil glycosylase to cccDNA and promote its degradation.

Another screen provided identifies conserved gRNAs that can be used to generate abasic sites in cccDNA. Referring to FIG. 3D, 7 guide RNAs were identified that had greater than 20% editing efficiency when a lentivirus was used to introduce a base editor and gRNA (Lenti-HBV). The gRNAs targeting conserved regions are shown at FIG. 3E. Several gRNAs had at least 45% editing efficiency (FIGS. 3F and 3G).

In some aspects, methods and compositions are provided for editing HBV cccDNA with a base editor comprising a cytidine deaminase or adenosine deaminase domain. In one embodiment, a base editor comprises an APOBEC cytidine deaminase domain, a Cas9 domain, and, optionally, one or more uracil glycosylase inhibitor (UGI) domains (FIGS. 4A, 4B).

NUCLEOBASE EDITOR

Disclosed herein is a base editor or a nucleobase editor for editing, modifying or altering a target nucleotide sequence of a polynucleotide. Described herein is a nucleobase editor or a base editor comprising a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (e.g., adenosine deaminase, cytidine deaminase). A polynucleotide programmable nucleotide binding domain, when in conjunction with a bound guide polynucleotide (*e.g.*, gRNA), can specifically bind to a target polynucleotide sequence (*i.e.*, via complementary base pairing between bases of the bound guide nucleic acid and bases of the target polynucleotide sequence) and thereby localize the base editor to the target nucleic acid sequence desired to be edited. In some embodiments, the target polynucleotide sequence comprises single-stranded DNA or double-stranded DNA. In some embodiments, the target polynucleotide sequence comprises RNA. In some embodiments, the target polynucleotide sequence comprises a DNA-RNA hybrid.

Polynucleotide Programmable Nucleotide Binding Domain

It should be appreciated that polynucleotide programmable nucleotide binding domains can also include nucleic acid programmable proteins that bind RNA. For example, the polynucleotide programmable nucleotide binding domain can be associated with a nucleic acid that guides the polynucleotide programmable nucleotide binding domain to an RNA. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, though they are not specifically listed in this disclosure.

A polynucleotide programmable nucleotide binding domain of a base editor can itself comprise one or more domains. For example, a polynucleotide programmable nucleotide binding domain can comprise one or more nuclease domains. In some embodiments, the nuclease domain of a polynucleotide programmable nucleotide binding domain can comprise an endonuclease or an exonuclease. Herein the term "exonuclease" refers to a protein or polypeptide capable of digesting a nucleic acid (*e.g.*, RNA or DNA) from free ends, and the term "endonuclease" refers to a protein or polypeptide capable of catalyzing (*e.g.*, cleaving) internal regions in a nucleic acid (*e.g.*, DNA or RNA). In some embodiments, an endonuclease can cleave a single strand of a double-stranded nucleic acid. In some embodiments, an endonuclease can cleave both strands of a double-stranded nucleic acid molecule. In some embodiments a polynucleotide programmable nucleotide binding domain

can be a deoxyribonuclease. In some embodiments a polynucleotide programmable nucleotide binding domain can be a ribonuclease.

In some embodiments, a nuclease domain of a polynucleotide programmable nucleotide binding domain can cut zero, one, or two strands of a target polynucleotide. In some cases, the polynucleotide programmable nucleotide binding domain can comprise a nickase domain. Herein the term "nickase" refers to a polynucleotide programmable nucleotide binding domain comprising a nuclease domain that is capable of cleaving only one strand of the two strands in a duplexed nucleic acid molecule (*e.g.,* DNA). In some embodiments, a nickase can be derived from a fully catalytically active (*e.g.,* natural) form of a polynucleotide programmable nucleotide binding domain by introducing one or more mutations into the active polynucleotide programmable nucleotide binding domain. For example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can include a D10A mutation and a histidine at position 840. In such cases, the residue H840 retains catalytic activity and can thereby cleave a single strand of the nucleic acid duplex. In another example, a Cas9-derived nickase domain can comprise an H840A mutation, while the amino acid residue at position 10 remains a D. In some embodiments, a nickase can be derived from a fully catalytically active (*e.g.,* natural) form of a polynucleotide programmable nucleotide binding domain by removing all or a portion of a nuclease domain that is not required for the nickase activity. For example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can comprise a deletion of all or a portion of the RuvC domain or the HNH domain.

The amino acid sequence of an exemplary catalytically active Cas9 is as follows:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK

VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD.

A base editor comprising a polynucleotide programmable nucleotide binding domain comprising a nickase domain is thus able to generate a single-strand DNA break (nick) at a specific polynucleotide target sequence (*e.g.,* determined by the complementary sequence of a bound guide nucleic acid). In some embodiments, the strand of a nucleic acid duplex target polynucleotide sequence that is cleaved by a base editor comprising a nickase domain (*e.g.,* Cas9-derived nickase domain) is the strand that is not edited by the base editor (*i.e.*, the strand that is cleaved by the base editor is opposite to a strand comprising a base to be edited). In other embodiments, a base editor comprising a nickase domain (*e.g.,* Cas9-derived nickase domain) can cleave the strand of a DNA molecule which is being targeted for editing. In such cases, the non-targeted strand is not cleaved.

Also provided herein are base editors comprising a polynucleotide programmable nucleotide binding domain which is catalytically dead (*i.e.*, incapable of cleaving a target polynucleotide sequence). Herein the terms "catalytically dead" and "nuclease dead" are used interchangeably to refer to a polynucleotide programmable nucleotide binding domain which has one or more mutations and/or deletions resulting in its inability to cleave a strand of a nucleic acid. In some embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain base editor can lack nuclease activity as a result of specific point mutations in one or more nuclease domains. For example, in the case of a base editor comprising a Cas9 domain, the Cas9 can comprise both a D10A mutation and an H840A

mutation. Such mutations inactivate both nuclease domains, thereby resulting in the loss of nuclease activity. In other embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain can comprise one or more deletions of all or a portion of a catalytic domain (*e.g.*, RuvC1 and/or HNH domains). In further embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain comprises a point mutation (*e.g.*, D10A or H840A) as well as a deletion of all or a portion of a nuclease domain.

Also contemplated herein are mutations capable of generating a catalytically dead polynucleotide programmable nucleotide binding domain from a previously functional version of the polynucleotide programmable nucleotide binding domain. For example, in the case of catalytically dead Cas9 ("dCas9"), variants having mutations other than D10A and H840A are provided, which result in nuclease inactivated Cas9. Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain). Additional suitable nuclease-inactive dCas9 domains can be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure. Such additional exemplary suitable nuclease-inactive Cas9 domains include, but are not limited to, D10A/H840A, D10A/D839A/H840A, and D10A/D839A/H840A/N863A mutant domains (*See*, *e.g.*, Prashant *et al.*, CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature Biotechnology*. 2013; 31(9): 833-838, the entire contents of which are incorporated herein by reference).

Non-limiting examples of a polynucleotide programmable nucleotide binding domain which can be incorporated into a base editor include a CRISPR protein-derived domain, a restriction nuclease, a meganuclease, TAL nuclease (TALEN), and a zinc finger nuclease (ZFN). In some cases, a base editor comprises a polynucleotide programmable nucleotide binding domain comprising a natural or modified protein or portion thereof which via a bound guide nucleic acid is capable of binding to a nucleic acid sequence during CRISPR (*i.e.*, Clustered Regularly Interspaced Short Palindromic Repeats)-mediated modification of a nucleic acid. Such a protein is referred to herein as a "CRISPR protein". Accordingly, disclosed herein is a base editor comprising a polynucleotide programmable nucleotide binding domain comprising all or a portion of a CRISPR protein (*i.e.* a base editor comprising as a domain all or a portion of a CRISPR protein, also referred to as a "CRISPR protein-derived domain" of the base editor). A CRISPR protein-derived domain incorporated

into a base editor can be modified compared to a wild-type or natural version of the CRISPR protein. For example, as described below a CRISPR protein-derived domain can comprise one or more mutations, insertions, deletions, rearrangements and/or recombinations relative to a wild-type or natural version of the CRISPR protein.

CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems, correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, and then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. *See, e.g.*, Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J. A., Charpentier E. Science 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self-versus-non-self.

In some embodiments, the methods described herein can utilize an engineered Cas protein. A guide RNA (gRNA) is a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding and a user-defined ~20 nucleotide spacer that defines the genomic (or polynucleotide, e.g., DNA or RNA) target to be modified. Thus, a skilled artisan can change the genomic or polynucleotide target of the Cas protein by changing the target sequence present in the gRNA. The specificity of the Cas protein is partially determined by how specific the gRNA targeting sequence is for the genomic polynucleotide target sequence compared to the rest of the genome.

In some embodiments, the gRNA scaffold sequence is as follows: GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUUUU.

In an embodiment, the RNA scaffold comprises a stem loop. In an embodiment, the RNA scaffold comprises the nucleic acid sequence: GUUUUUGUACUCUCAAGAUUUAAGUAACUGUACAACGAAACUUACACAGUUACUUAAAUCUUGCAGAAGCUACAAAGAUAAGGCUUCAUGCCGAAAUCAACACCCUGUCAUUUUAUGGCAGGGUG. In an embodiment, the RNA scaffold comprises the nucleic acid sequence: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU.

In an embodiment, an *S. pyrogenes* sgRNA scaffold polynucleotide sequence is as follows: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC.

In an embodiment, an *S. aureus* sgRNA scaffold polynucleotide sequence is as follows: GUUUUAGUACUCUGUAAUGAAAAUUACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUUUAUCUCGUCAACUUGUUGGCGAGA.

In an embodiment, a BhCas12b sgRNA scaffold has the following polynucleotide sequence: GUUCUGTCUUUUGGUCAGGACAACCGUCUAGCUAUAAGUGCUGCAGGGUGUGAGAAACUCCUAUUGCUGGACGAUGUCUCUUACGAGGCAUUAGCAC.

In an embodiment, a BvCas12b sgRNA scaffold has the following polynucleotide sequence: GACCUAUAGGGUCAAUGAAUCUGUGCGUGUGCCAUAAGUAAUUAAAAAUUACCCACCACAGGAGCACCUGAAAACAGGUGCUUGGCAC.

In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is an endonuclease (*e.g.*, deoxyribonuclease or ribonuclease) capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a nickase capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a catalytically dead domain capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a target polynucleotide bound by a CRISPR protein derived domain of a base editor is DNA. In some embodiments, a target polynucleotide bound by a CRISPR protein-derived domain of a base editor is RNA.

Cas proteins that can be used herein include class 1 and class 2. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 or Csx12), Cas10, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i, CARF, DinG, homologues thereof, or modified versions thereof. An unmodified CRISPR enzyme can have DNA cleavage activity, such as Cas9, which has two functional endonuclease domains: RuvC and HNH. A CRISPR enzyme can direct cleavage of one or both strands at a target sequence, such as within a target sequence and/or within a complement of a target sequence. For example, a CRISPR enzyme can direct cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence.

A vector that encodes a CRISPR enzyme that is mutated to with respect, to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence can be used. Cas9 can refer to a polypeptide with at least or at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild type exemplary Cas9 polypeptide (*e.g.*, Cas9 from *S. pyogenes*). Cas9 can refer to a polypeptide with at most or at most about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild type exemplary Cas9 polypeptide (*e.g.*, from *S. pyogenes*). Cas9 can refer to the wild type or a modified form of the Cas9 protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation, fusion, chimera, or any combination thereof.

In some embodiments, a CRISPR protein-derived domain of a base editor can include all or a portion of Cas9 from *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma syrphidicola* (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus*

torquis (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1); *Listeria innocua* (NCBI Ref: NP_472073.1); *Campylobacter jejuni* (NCBI Ref: YP_002344900.1); *Neisseria meningitidis* (NCBI Ref: YP_002342100.1), *Streptococcus pyogenes*, or *Staphylococcus aureus*.

Cas9 domains of Nucleobase Editors

Cas9 nuclease sequences and structures are well known to those of skill in the art (*See, e.g.*, "Complete genome sequence of an Ml strain of *Streptococcus pyogenes*." Ferretti *et al.*, J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C, Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C.M., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference.

In some aspects, a nucleic acid programmable DNA binding protein (napDNAbp) is a Cas9 domain. Non-limiting, exemplary Cas9 domains are provided herein. The Cas9 domain may be a nuclease active Cas9 domain, a nuclease inactive Cas9 domain, or a Cas9 nickase. In some embodiments, the Cas9 domain is a nuclease active domain. For example, the Cas9 domain may be a Cas9 domain that cuts both strands of a duplexed nucleic acid (*e.g.*, both strands of a duplexed DNA molecule). In some embodiments, the Cas9 domain comprises any one of the amino acid sequences as set forth herein. In some embodiments the Cas9 domain comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, or at least 1200 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth herein.

In some embodiments, proteins comprising fragments of Cas9 are provided. For example, in some embodiments, a protein comprises one of two Cas9 domains: (1) the gRNA binding domain of Cas9; or (2) the DNA cleavage domain of Cas9. In some embodiments, proteins comprising Cas9 or fragments thereof are referred to as "Cas9 variants." A Cas9 variant shares homology to Cas9, or a fragment thereof. For example, a Cas9 variant is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to wild type Cas9. In some embodiments, the Cas9 variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acid changes compared to wild type Cas9. In some embodiments, the Cas9 variant comprises a fragment of Cas9 (*e.g.*, a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild type Cas9. In some embodiments, the fragment is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild type Cas9. In some embodiments, the fragment is at least 100 amino acids in length. In some

embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, or at least 1300 amino acids in length.

In some embodiments, Cas9 fusion proteins as provided herein comprise the full-length amino acid sequence of a Cas9 protein, *e.g.*, one of the Cas9 sequences provided herein. In other embodiments, however, fusion proteins as provided herein do not comprise a full-length Cas9 sequence, but only one or more fragments thereof. Exemplary amino acid sequences of suitable Cas9 domains and Cas9 fragments are provided herein, and additional suitable sequences of Cas9 domains and fragments will be apparent to those of skill in the art.

A Cas9 protein can associate with a guide RNA that guides the Cas9 protein to a specific DNA sequence that has complementary to the guide RNA. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9). Examples of nucleic acid programmable DNA binding proteins include, without limitation, Cas9 (*e.g.*, dCas9 and nCas9), CasX, CasY, Cpf1, Cas12b/C2C1, and Cas12c/C2C3.

In some embodiments, wild type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_017053.1, nucleotide and amino acid sequences as follows).

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGAT
CACTGATGATTATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACA
GTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGGCAGTGGAGAGACAGCGGAAGCGACT
CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA
GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
CTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGAT
GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGCAGATTC
TACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATC
CAGTTGGTACAAATCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTAGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
AGCTCCCCGGTGAGAAGAGAAATGGCTTGTTTGGGAATCTCATTGCTTTGTCATTGGGATTG
ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
TACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGT
TTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATAGT

GAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAGCGCTACGATGAACATCATCAAGA
CTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT
TTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTT
TATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACT
AAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA
TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAA
GACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATT
GGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACA
AACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTA
TTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAGGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAA
GTAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGA
AATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGCGCCTACCATGATTTGCTAAAAA
TTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTT
TTAACATTGACCTTATTTGAAGATAGGGGGATGATTGAGGAAAGACTTAAAACATATGCTCA
CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTT
TGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTT
TTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGAC
ATTTAAAGAAGATATTCAAAAAGCACAGGTGTCTGGACAAGGCCATAGTTTACATGAACAGA
TTGCTAACTTAGCTGGCAGTCCTGCTATTAAAAAAGGTATTTTACAGACTGTAAAAATTGTT
GATGAACTGGTCAAAGTAATGGGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACGTGA
AAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAG
GTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTGCAA
AATGAAAAGCTCTATCTCTATTATCTACAAAATGGAAGAGACATGTATGTGGACCAAGAATT
AGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTCATTAAAG
ACGATTCAATAGACAATAAGGTACTAACGCGTTCTGATAAAAATCGTGGTAAATCGGATAAC
GTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGCCAA
GTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAAC
TTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTG
GCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGA
GGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCT
ATAAAGTACGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTT
GGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAA

AGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAA
AATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGA
GAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAA
AGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGA
AAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCGGAC
AAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAGTCCAAC
GGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAAAAT
CCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCGATT
GACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACCTAA
ATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAATTAC
AAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGTCAT
TATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCAGCA
TAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTGTTATTTTAG
CAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCAATACGT
GAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTT
TAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATG
CCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTA
GGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNS
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSLHEQIANLAGSPAIKKGILQTVKIV
DELVKVMGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV

AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

(single underline: HNH domain; double underline: RuvC domain)

In some embodiments, wild type Cas9 corresponds to, or comprises the following nucleotide and/or amino acid sequences:

ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCACTAATTCCGTTGGATGGGCTGTCAT
AACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCATT
CGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCGACT
CGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTACTTACA
AGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGT
CCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGAT
GAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTC
AACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTG
GGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTCGGATGTCGACAAACTGTTCATC
CAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGA
TGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAACCTGATCGCAC
AATTACCCGGAGAGAAGAAAAATGGGTTGTTCGGTAACCTTATAGCGCTCTCACTAGGCCTG
ACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGCCAAATTGCAGCTTAGTAAGGA
CACGTACGATGACGATCTCGACAATCTACTGGCACAAATTGGAGATCAGTATGCGGACTTAT
TTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTGACATACTGAGAGTTAATACT
GAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTACGATGAACATCACCAAGA
CTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATATAAGGAAATATTCT
TTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTCAAGAGGAATTC
TACAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTTGTAAAACT
CAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACATCAAA
TCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCAAA
GACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCT
GGCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCAT

GGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACC
AACTTTGACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTA
TTTCACAGTGTACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCG
CCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAA
GTGACAGTTAAGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGA
GATCTCCGGGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGA
TAATTAAAGATAAGGACTTCCTGGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTG
TTGACTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCA
CCTGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGAT
TGTCGCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTT
CTAAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAAC
CTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAACATA
TTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAGTAGTG
GATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACATTGTAATCGAGATGGCACG
CGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGGATGAAGAGAATAGAAG
AGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGAAAATACCCAATTG
CAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATGTTGATCAGGA
ACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGTACCCCAATCCTTTTTGA
AGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAGTGAC
AATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATGC
GAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTG
AACTTGACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAACCCGCCAAATCACAAAGCAT
GTTGCACAGATACTAGATTCCCGAATGAATACGAAATACGACGAGAACGATAAGCTGATTCG
GGAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAAT
TCTATAAAGTTAGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTAATGCCGTC
GTAGGGACCGCACTCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTA
CAAAGTTTATGACGTCCGTAAGATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAG
CCAAATACTTCTTTTATTCTAACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAAC
GGAGAGATACGCAAACGACCTTTAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGA
TAAGGGCCGGGACTTCGCGACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAA
AGAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGT
GATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCC
TACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGA
AGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCC

ATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACC
AAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGC
TTCAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCC
CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGCA
GCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCATCC
TAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATA
CGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGC
ATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAG
CTTGGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGA
CGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI

REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

(single underline: HNH domain; double underline: RuvC domain).

In some embodiments, wild type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_002737.2 (nucleotide sequence as follows); and Uniprot Reference Sequence: Q99ZW2 (amino acid sequence as follows):

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGAT
CACTGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACA
GTATCAAAAAAAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACT
CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA
GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
CTTTTTTGGTGGAAGAAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGAT
GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTC
TACTGATAAAGCGGATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTG
GTCATTTTTTGATTGAGGGAGATTTAAATCCTGATAATAGTGATGTGGACAAACTATTTATC
CAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
AGCTCCCCGGTGAGAAGAAAAATGGCTTATTTGGGAATCTCATTGCTTTGTCATTGGGTTTG
ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
TACTTACGATGATGATTTAGATAATTTATTGGCGCAAATTGGAGATCAATATGCTGATTTGT
TTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAGATATCCTAAGAGTAAATACT
GAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATGAACATCATCAAGA
CTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT
TTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCCAAGAAGAATTT
TATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTGGTGAAACT
AAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA
TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAAAA
GACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATT
GGCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCAT
GGAATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACA
AACTTTGATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTA
TTTTACGGTTTATAACGAATTGACAAAGGTCAAATATGTTACTGAAGGAATGCGAAAACCAG
CATTTCTTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAA
GTAACCGTTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGA

AATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGTACCTACCATGATTTGCTAAAAA
TTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTT
TTAACATTGACCTTATTTGAAGATAGGGAGATGATTGAGGAAAGACTTAAAACATATGCTCA
CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTT
TGTCTCGAAAATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTT
TTGAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGAC
ATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAAGGCGATAGTTTACATGAACATA
TTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTATTTTACAGACTGTAAAAGTTGTT
GATGAATTGGTCAAAGTAATGGGGCGGCATAAGCCAGAAAATATCGTTATTGAAATGGCACG
TGAAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAG
AAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTCAATTG
CAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGACATGTATGTGGACCAAGA
ATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTCCTTA
AAGACGATTCAATAGACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATCGGAT
AACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGC
CAAGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTG
AACTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCAT
GTGGCACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCG
AGAGGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAAT
TCTATAAAGTACGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTC
GTTGGAACTGCTTTGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTA
TAAAGTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCG
CAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAAT
GGAGAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGA
TAAAGGGCGAGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCA
AGAAAACAGAAGTACAGACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCG
GACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAGTCC
AACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAATCGAAGAAGTTAA
AATCCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCCG
ATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGACTTAATCATTAAACTACC
TAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAAT
TACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGT
CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCA
GCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTGTTATTT

TAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCAATA
CGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGC
TTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAG
ATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAG
CTAGGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD (single underline: HNH domain; double underline: RuvC domain)

In some embodiments, Cas9 refers to Cas9 from: *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma syrphidicola* (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref:

NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquisI* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1), *Listeria innocua* (NCBI Ref: NP_472073.1), *Campylobacter jejuni* (NCBI Ref: YP_002344900.1) or *Neisseria meningitidis* (NCBI Ref: YP_002342100.1) or to a Cas9 from any other organism.

It should be appreciated that additional Cas9 proteins (*e.g.,* a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9), including variants and homologs thereof, are within the scope of this disclosure. Exemplary Cas9 proteins include, without limitation, those provided below. In some embodiments, the Cas9 protein is a nuclease dead Cas9 (dCas9). In some embodiments, the Cas9 protein is a Cas9 nickase (nCas9). In some embodiments, the Cas9 protein is a nuclease active Cas9.

In some embodiments, the Cas9 domain is a nuclease-inactive Cas9 domain (dCas9). For example, the dCas9 domain may bind to a duplexed nucleic acid molecule (*e.g.,* via a gRNA molecule) without cleaving either strand of the duplexed nucleic acid molecule. In some embodiments, the nuclease-inactive dCas9 domain comprises a D10X mutation and a H840X mutation of the amino acid sequence set forth herein, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid change. In some embodiments, the nuclease-inactive dCas9 domain comprises a D10A mutation and a H840A mutation of the amino acid sequence set forth herein, or a corresponding mutation in any of the amino acid sequences provided herein. As one example, a nuclease-inactive Cas9 domain comprises the amino acid sequence set forth in Cloning vector pPlatTET-gRNA2 (Accession No. BAV54124).

The amino acid sequence of an exemplary catalytically inactive Cas9 (dCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK

VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

(see, *e.g.*, Qi *et al.*, "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression." *Cell.* 2013; 152(5):1173-83, the entire contents of which are incorporated herein by reference).

In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase, referred to as an "nCas9" protein (for "nickase" Cas9). A nuclease-inactivated Cas9 protein may interchangeably be referred to as a "dCas9" protein (for nuclease-"dead" Cas9) or catalytically inactive Cas9. Methods for generating a Cas9 protein (or a fragment thereof) having an inactive DNA cleavage domain are known (*See, e.g.*, Jinek *et al., Science.* 337:816-821(2012); Qi *et al.,* "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression" (2013) *Cell.* 28;152(5):1173-83, the entire contents of each of which are incorporated herein by reference). For example, the DNA cleavage domain of Cas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of Cas9. For example, the mutations D10A and H840A completely inactivate the nuclease activity of *S. pyogenes* Cas9 (Jinek *et al., Science.* 337:816-821(2012); Qi *et al., Cell.* 28;152(5):1173-83 (2013)).

In some embodiments, the dCas9 domain comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the dCas9 domains provided herein. In some embodiments, the Cas9 domain comprises an amino acid sequences that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, or at least 1200 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth herein.

In some embodiments, dCas9 corresponds to, or comprises in part or in whole, a Cas9 amino acid sequence having one or more mutations that inactivate the Cas9 nuclease activity. For example, in some embodiments, a dCas9 domain comprises D10A and an H840A mutation or corresponding mutations in another Cas9.

In some embodiments, the dCas9 comprises the amino acid sequence of dCas9 (D10A and H840A):

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD

NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD (single underline: HNH domain; double underline: RuvC domain).

In some embodiments, the Cas9 domain comprises a D10A mutation, while the residue at position 840 remains a histidine in the amino acid sequence provided above, or at corresponding positions in any of the amino acid sequences provided herein.

In other embodiments, dCas9 variants having mutations other than D10A and H840A are provided, which, *e.g.*, result in nuclease inactivated Cas9 (dCas9). Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain). In some embodiments, variants or homologues of dCas9 are provided which are at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical. In some embodiments, variants of dCas9 are provided having amino acid sequences which are shorter, or longer, by about 5 amino acids, by about 10 amino acids, by about 15 amino acids, by about 20 amino acids, by about 25 amino acids, by about 30 amino acids, by about 40 amino acids, by about 50 amino acids, by about 75 amino acids, by about 100 amino acids or more.

In some embodiments, the Cas9 domain is a Cas9 nickase. The Cas9 nickase may be a Cas9 protein that is capable of cleaving only one strand of a duplexed nucleic acid molecule (*e.g.,* a duplexed DNA molecule). In some embodiments, the Cas9 nickase cleaves the target strand of a duplexed nucleic acid molecule, meaning that the Cas9 nickase cleaves the strand that is base paired to (complementary to) a gRNA (*e.g.,* an sgRNA) that is bound to the Cas9. In some embodiments, a Cas9 nickase comprises a D10A mutation and has a histidine at position 840. In some embodiments, the Cas9 nickase cleaves the non-target, non-base-edited strand of a duplexed nucleic acid molecule, meaning that the Cas9 nickase cleaves the

strand that is not base paired to a gRNA (*e.g.*, an sgRNA) that is bound to the Cas9. In some embodiments, a Cas9 nickase comprises an H840A mutation and has an aspartic acid residue at position 10, or a corresponding mutation. In some embodiments, the Cas9 nickase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the Cas9 nickases provided herein. Additional suitable Cas9 nickases will be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure.

The amino acid sequence of an exemplary catalytically Cas9 nickase (nCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

In some embodiments, Cas9 refers to a Cas9 from archaea (*e.g.*, nanoarchaea), which constitute a domain and kingdom of single-celled prokaryotic microbes. In some embodiments, the programmable nucleotide binding protein may be a CasX or CasY protein, which have been described in, for example, Burstein *et al.*, "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little-studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered. In some embodiments, in a base editor system described herein Cas9 is replaced by CasX, or a variant of CasX. In some embodiments, in a base editor system described herein Cas9 is replaced by CasY, or a variant of CasY. It should be appreciated that other RNA-guided DNA binding proteins may be used as a nucleic acid programmable DNA binding protein (napDNAbp), and are within the scope of this disclosure.

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a CasX or CasY protein. In some embodiments, the napDNAbp is a CasX protein. In some embodiments, the napDNAbp is a CasY protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a naturally-occurring CasX or CasY protein. In some embodiments, the programmable nucleotide binding protein is a naturally-occurring CasX or CasY protein. In some embodiments, the programmable nucleotide binding protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any CasX or CasY protein described herein. It should be appreciated that CasX and CasY from other bacterial species may also be used in accordance with the present disclosure.

An exemplary CasX ((uniprot.org/uniprot/F0NN87; uniprot.org/uniprot/F0NH53) tr|F0NN87|F0NN87_SULIHCRISPR-associatedCasx protein OS = Sulfolobus islandicus (strain HVE10/4) GN = SiH_0402 PE=4 SV=1) amino acid sequence is as follows:

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGK
AKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAP
SFVKPEFYEFGRSPGMVERTRRVKLEVEPHYLIIAAAGWVLTRLGKAKVSEGDYVGVNVFTP
TRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVRIYTISDAVGQNPTTIN
GGFSIDLTKLLEKRYLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTG SKRLEDLLY
FANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG.

An exemplary CasX (>tr|F0NH53|F0NH53_SULIR CRISPR associated protein, Casx OS = *Sulfolobus islandicus* (strain REY15A) GN=SiRe_0771 PE=4 SV=1) amino acid sequence is as follows:

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGK
AKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAP
SFVKPEFYKFGRSPGMVERTRRVKLEVEPHYLIMAAAGWVLTRLGKAKVSEGDYVGVNVFTP
TRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVSIYTISDAVGQNPTTIN
GGFSIDLTKLLEKRDLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTGSKRLEDLLYF
ANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG.

Deltaproteobacteria CasX

MEKRINKIRKKLSADNATKPVSRSGPMKTLLVRVMTDDLKKRLEKRRKKPEVMPQVISNNAA
NNLRMLLDDYTKMKEAILQVYWQEFKDDHVGLMCKFAQPASKKIDQNKLKPEMDEKGNLTTA
GFACSQCGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPVKDSDEAVTYSLG
KFGQRALDFYSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQDIII
EHQKVVKGNQKRLESLRELAGKENLEYPSVTLPPQPHTKEGVDfAYNEVIARVRMWVNLNLW
QKLKLSRDDAKPLLRLKGFPSFPVVERRENEVDWWNTINEVKKLIDAKRDMGRVFWSGVTAE
KRNTILEGYNYLPNENDHKKREGSLENPKKPAKRQFGDLLLYLEKKYAGDWGKVFDEAWERI
DKKIAGLTSHIEREEARNAEDAQSKAVLTDWLRAKASFVLERLKEMDEKEFYACEIQLQKWY
GDLRGNPFAVEAENRVVDISGFSIGSDGHSIQYRNLLAWKYLENGKREFYLLMNYGKKGRIR
FTDGTDIKKSGKWQGLLYGGGKAKVIDLTFDPDDEQLIILPLAFGTRQGREFIWNDLLSLET
GLIKLANGRVIEKTIYNKKIGRDEPALFVALTFERREVVDPSNIKPVNLIGVARGENIPAVI
ALTDPEGCPLPEFKDSSGGPTDILRIGEGYKEKQRAIQAAKEVEQRRAGGYSRKFASKSRNL
ADDMVRNSARDLFYHAVTHDAVLVFANLSRGFGRQGKRTFMTERQYTKMEDWLTAKLAYEGL
TSKTYLSKTLAQYTSKTCSNCGFTITYADMDVMLVRLKKTSDGWATTLNNKELKAEYQITYY
NRYKRQTVEKELSAELDRLSEESGNNDISKWTKGRRDEALFLLKKRFSHRPVQEQFVCLDCG
HEVHAAEQAALNIARSWLFLNSNSTEFKSYKSGKQPFVGAWQAFYKRRLKEVWKPNA

An exemplary CasY ((ncbi.nlm.nih.gov/protein/APG80656.1) >APG80656.1 CRISPR-associated protein CasY [uncultured Parcubacteria group bacterium]) amino acid sequence is as follows:

MSKRHPRISGVKGYRLHAQRLEYTGKSGAMRTIKYPLYSSPSGGRTVPREIVSAINDDYVGL
YGLSNFDDLYNAEKRNEEKVYSVLDFWYDCVQYGAVFSYTAPGLLKNVAEVRGGSYELTKTL
KGSHLYDELQIDKVIKFLNKKEISRANGSLDKLKKDIIDCFKAEYRERHKDQCNKLADDIKN
AKKDAGASLGERQKKLFRDFFGISEQSENDKPSFTNPLNLTCCLLPFDTVNNNRNRGEVLFN
KLKEYAQKLDKNEGSLEMWEYIGIGNSGTAFSNFLGEGFLGRLRENKITELKKAMMDITDAW
RGQEQEEELEKRLRILAALTIKLREPKFDNHWGGYRSDINGKLSSWLQNYINQTVKIKEDLK
GHKKDLKKAKEMINRFGESDTKEEAVVSSLLESIEKIVPDDSADDEKPDIPAIAIYRRFLSD
GRLTLNRFVQREDVQEALIKERLEAEKKKKPKKRKKKSDAEDEKETIDFKELFPHLAKPLKL
VPNFYGDSKRELYKKYKNAAIYTDALWKAVEKIYKSAFSSSLKNSFFDTDFDKDFFIKRLQK
IFSVYRRFNTDKWKPIVKNSFAPYCDIVSLAENEVLYKPKQSRSRKSAAIDKNRVRLPSTEN
IAKAGIALARELSVAGFDWKDLLKKEEHEEYIDLIELHKTALALLLAVTETQLDISALDFVE
NGTVKDFMKTRDGNLVLEGRFLEMFSQSIVFSELRGLAGLMSRKEFITRSAIQTMNGKQAEL
LYIPHEFQSAKITTPKEMSRAFLDLAPAEFATSLEPESLSEKSLLKLKQMRYYPHYFGYELT
RTGQGIDGGVAENALRLEKSPVKKREIKCKQYKTLGRGQNKIVLYVRSSYYQTQFLEWFLHR
PKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSERVFVSQPFTIFPEKSAEEEGQ
RYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVKGLKLDQRRGTFAMPSTK
IARIRESLVHSLRNRIHHLALKHKAKIVYELEVSRFEEGKQKIKKVYATLKKADVYSEIDAD
KNLQTTVWGKLAVASEISASYTSQFCGACKKLWRAEMQVDETITTQELIGTVRVIKGGTLID
AIKDFMRPPIFDENDTPFPKYRDFCDKHHISKKMRGNSCLFICPFCRANADADIQASQTIAL
LRYVKEEKKVEDYFERFRKLKNIKVLGQMKKI.

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) is a single effector of a microbial CRISPR-Cas system. Single effectors of microbial CRISPR-Cas systems include, without limitation, Cas9, Cpf1, Cas12b/C2c1, and Cas12c/C2c3. Typically, microbial CRISPR-Cas systems are divided into Class 1 and Class 2 systems. Class 1 systems have multisubunit effector complexes, while Class 2 systems have a single protein effector. For example, Cas9 and Cpf1 are Class 2 effectors. In addition to Cas9 and Cpf1, three distinct Class 2 CRISPR-Cas systems (Cas12b/C2c1, and Cas12c/C2c3) have been described by Shmakov *et al.*, "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems", *Mol. Cell,* 2015 Nov. 5; 60(3): 385-397, the entire contents of which is hereby incorporated by reference. Effectors of two of the systems, Cas12b/C2c1,

and Cas12c/C2c3, contain RuvC-like endonuclease domains related to Cpf1. A third system contains an effector with two predicated HEPN RNase domains. Production of mature CRISPR RNA is tracrRNA-independent, unlike production of CRISPR RNA by Cas12b/C2c1. Cas12b/C2c1 depends on both CRISPR RNA and tracrRNA for DNA cleavage.

The crystal structure of *Alicyclobaccillus acidoterrastris* Cas12b/C2c1 (AacC2c1) has been reported in complex with a chimeric single-molecule guide RNA (sgRNA). See *e.g.*, Liu *et al.*, "C2c1-sgRNA Complex Structure Reveals RNA-Guided DNA Cleavage Mechanism", *Mol. Cell,* 2017 Jan. 19; 65(2):310-322, the entire contents of which are hereby incorporated by reference. The crystal structure has also been reported in *Alicyclobacillus acidoterrestris* C2c1 bound to target DNAs as ternary complexes. See *e.g.*, Yang *et al.*, "PAM-dependent Target DNA Recognition and Cleavage by C2C1 CRISPR-Cas endonuclease", *Cell,* 2016 Dec. 15; 167(7):1814-1828, the entire contents of which are hereby incorporated by reference. Catalytically competent conformations of AacC2c1, both with target and non-target DNA strands, have been captured independently positioned within a single RuvC catalytic pocket, with Cas12b/C2c1-mediated cleavage resulting in a staggered seven-nucleotide break of target DNA. Structural comparisons between Cas12b/C2c1 ternary complexes and previously identified Cas9 and Cpf1 counterparts demonstrate the diversity of mechanisms used by CRISPR-Cas9 systems.

In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a Cas12b/C2c1, or a Cas12c/C2c3 protein. In some embodiments, the napDNAbp is a Cas12b/C2c1 protein. In some embodiments, the napDNAbp is a Cas12c/C2c3 protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a naturally-occurring Cas12b/C2c1 or Cas12c/C2c3 protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12b/C2c1 or Cas12c/C2c3 protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any one of the napDNAbp sequences provided herein. It should be appreciated that Cas12b/C2c1 or Cas12c/C2c3 from other bacterial species may also be used in accordance with the present disclosure.

A Cas12b/C2c1 ((uniprot.org/uniprot/T0D7A2#2) sp|T0D7A2|C2C1_ALIAG CRISPR-associated endonuclease C2c1 OS = *Alicyclobacillus acido-terrestris* (strain ATCC 49025 / DSM 3922/ CIP 106132 / NCIMB 13137/GD3B) GN=c2c1 PE=1 SV=1) amino acid sequence is as follows:

MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYRRSPNGDGEQECD
KTAEECKAELLERLRARQVENGHRGPAGSDDELLQLARQLYELLVPQAIGAKGDAQQIARKF
LSPLADKDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKEKAETRKSADRTADVLRALADFG
LKPLMRVYTDSEMSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKLVEQ
KNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGRALRGSDKVFEKWGKLA
PDAPFDLYDAEIKNVQRRNTRRFGSHDLFAKLAEPEYQALWREDASFLTRYAVYNSILRKLN
HAKMFATFTLPDATAHPIWTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREV
DDVTVPISMSEQLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRG
ARDVYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSE
GLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSKGRVPFFFPIKGNDNLVAVHERSQLL
KLPGETESKDLRAIREERQRTLRQLRTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPVDAAN
HMTPDWREAFENELQKLKSLHGICSDKEWMDAVYESVRRVWRHMGKQVRDWRKDVRSGERPK
IRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSGQVIRAEKGSRFAITLREHIDHAKE
DRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLILLEELSEYQFNNDRPPSENNQLM
QWSHRGVFQELINQAQVHDLLVGTMYAAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPW
WLNKFVVEHTLDACPLRADDLIPTGEGEIFVSPFSAEEGDFHQIHADLNAAQNLQQRLWSDF
DISQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYERERGKKRRKVFAQE
KLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMV NQRIEGYLVKQIRSR
VPLQDSACENTGDI

BhCas12b (*Bacillus hisashii*) NCBI Reference Sequence: WP_095142515

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAI
YEHHEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRELYEELVPSSVE
KKGEANQLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDP
LAKILGKLAEYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWES
WNLKVKEEYEKVEKEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLR
GWREIIQKWLKMDENEPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPY
LYATFCEIDKKKKDAKQQATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKL
TVQLDRLIYPTESGGWEEKGKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGT

LGGARVQFDRDHLRRYPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKP
KELTEWIKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIK
GTELYAVHRASFNIKLPGETLVKSREVLRKAREDNLKLMNQKLNFLRNVLHFQQFEDITERE
KRVTKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAFLKQLHKRLEVEIGKEVKHWRKS
LSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQLNHLNALKED
RLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPYEERSRFENSKLMKWSR
REIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVTKEKLQDNRFFKNLQREGR
LTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADINAAQNLQKRFWTRTHGFYKVYCK
AYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIKKGSSKQSSSELVDS
DILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISKLTNQYSISTIE
DDSSKQSMKRPAATKKAGQAKKKK

In some embodiments, the Cas12b is BvCas12B, which is a variant of BhCas12b and comprises the following changes relative to BhCas12B: S893R, K846R, and E837G.

BvCas12b (Bacillus sp. V3-13) NCBI Reference Sequence: WP_101661451.1

MAIRSIKLKMKTNSGTDSIYLRKALWRTHQLINEGIAYYMNLLTLYRQEAIGDKTKEAYQAE
LINIIRNQQRNNGSSEEHGSDQEILALLRQLYELIIPSSIGESGDANQLGNKFLYPLVDPNS
QSGKGTSNAGRKPRWKRLKEEGNPDWELEKKKDEERKAKDPTVKIFDNLNKYGLLPLFPLFT
NIQKDIEWLPLGKRQSVRKWDKDMFIQAIERLLSWESWNRRVADEYKQLKEKTESYYKEHLT
GGEEWIEKIRKFEKERNMELEKNAFAPNDGYFITSRQIRGWDRVYEKWSKLPESASPEELWK
VVAEQQNKMSEGFGDPKVFSFLANRENRDIWRGHSERIYHIAAYNGLQKKLSRTKEQATFTL
PDAIEHPLWIRYESPGGTNLNLFKLEEKQKKNYYVTLSKIIWPSEEKWIEKENIEIPLAPSI
QFNRQIKLKQHVKGKQEISFSDYSSRISLDGVLGGSRIQFNRKYIKNHKELLGEGDIGPVFF
NLVVDVAPLQETRNGRLQSPIGKALKVISSDFSKVIDYKPKELMDWMNTGSASNSFGVASLL
EGMRVMSIDMGQRTSASVSIFEVVKELPKDQEQKLFYSINDTELFAIHKRSFLLNLPGEVVT
KNNKQQRQERRKKRQFVRSQIRMLANVLRLETKKTPDERKKAIHKLMEIVQSYDSWTASQKE
VWEKELNLLTNMAAFNDEIWKESLVELHHRIEPYVGQIVSKWRKGLSEGRKNLAGISMWNID
ELEDTRRLLISWSKRSRTPGEANRIETDEPFGSSLLQHIQNVKDDRLKQMANLIIMTALGFK
YDKEEKDRYKRWKETYPACQIILFENLNRYLFNLDRSRRENSRLMKWAHRSIPRTVSMQGEM
FGLQVGDVRSEYSSRFHAKTGAPGIRCHALTEEDLKAGSNTLKRLIEDGFINESELAYLKKG
DIIPSQGGELFVTLSKRYKKDSDNNELTVIHADINAAQNLQKRFWQQNSEVYRVPCQLARMG
EDKLYIPKSQTETIKKYFGKGSFVKNNTEQEVYKWEKSEKMKIKTDTTFDLQDLDGFEDISK
TIELAQEQQKKYLTMFRDPSGYFFNNETWRPQKEYWSIVNNIIKSCLKKKILSNKVEL

The Cas9 nuclease has two functional endonuclease domains: RuvC and HNH. Cas9 undergoes a conformational change upon target binding that positions the nuclease domains to cleave opposite strands of the target DNA. The end result of Cas9-mediated DNA cleavage is a double-strand break (DSB) within the target DNA (~3-4 nucleotides upstream of the PAM sequence). The resulting DSB is then repaired by one of two general repair pathways: (1) the efficient but error-prone non-homologous end joining (NHEJ) pathway; or (2) the less efficient but high-fidelity homology directed repair (HDR) pathway.

The "efficiency" of non-homologous end joining (NHEJ) and/or homology directed repair (HDR) can be calculated by any convenient method. For example, in some cases, efficiency can be expressed in terms of percentage of successful HDR. For example, a surveyor nuclease assay can be used to generate cleavage products and the ratio of products to substrate can be used to calculate the percentage. For example, a surveyor nuclease enzyme can be used that directly cleaves DNA containing a newly integrated restriction sequence as the result of successful HDR. More cleaved substrate indicates a greater percent HDR (a greater efficiency of HDR). As an illustrative example, a fraction (percentage) of HDR can be calculated using the following equation [(cleavage products)/(substrate plus cleavage products)] (*e.g.*, (b+c)/(a+b+c), where "a" is the band intensity of DNA substrate and "b" and "c" are the cleavage products).

In some cases, efficiency can be expressed in terms of percentage of successful NHEJ. For example, a T7 endonuclease I assay can be used to generate cleavage products and the ratio of products to substrate can be used to calculate the percentage NHEJ. T7 endonuclease Icleaves mismatched heteroduplex DNA which arises from hybridization of wild-type and mutant DNA strands (NHEJ generates small random insertions or deletions (indels) at the site of the original break). More cleavage indicates a greater percent NHEJ (a greater efficiency of NHEJ). As an illustrative example, a fraction (percentage) of NHEJ can be calculated using the following equation: $(1-(1-(b+c)/(a+b+c))^{1/2})\times 100$, where "a" is the band intensity of DNA substrate and "b" and "c" are the cleavage products (Ran *et. al.*, Cell. 2013 Sep. 12; 154(6):1380-9; and Ran *et al.*, Nat Protoc. 2013 Nov.; 8(11): 2281–2308).

The NHEJ repair pathway is the most active repair mechanism, and it frequently causes small nucleotide insertions or deletions (indels) at the DSB site. The randomness of NHEJ-mediated DSB repair has important practical implications, because a population of cells expressing Cas9 and a gRNA or a guide polynucleotide can result in a diverse array of mutations. In most cases, NHEJ gives rise to small indels in the target DNA that result in

amino acid deletions, insertions, or frameshift mutations leading to premature stop codons within the open reading frame (ORF) of the targeted gene. The ideal end result is a loss-of-function mutation within the targeted gene.

While NHEJ-mediated DSB repair often disrupts the open reading frame of the gene, homology directed repair (HDR) can be used to generate specific nucleotide changes ranging from a single nucleotide change to large insertions like the addition of a fluorophore or tag. In order to utilize HDR for gene editing, a DNA repair template containing the desired sequence can be delivered into the cell type of interest with the gRNA(s) and Cas9 or Cas9 nickase. The repair template can contain the desired edit as well as additional homologous sequence immediately upstream and downstream of the target (termed left & right homology arms). The length of each homology arm can be dependent on the size of the change being introduced, with larger insertions requiring longer homology arms. The repair template can be a single-stranded oligonucleotide, double-stranded oligonucleotide, or a double-stranded DNA plasmid. The efficiency of HDR is generally low (<10% of modified alleles) even in cells that express Cas9, gRNA and an exogenous repair template. The efficiency of HDR can be enhanced by synchronizing the cells, since HDR takes place during the S and G2 phases of the cell cycle. Chemically or genetically inhibiting genes involved in NHEJ can also increase HDR frequency.

In some embodiments, Cas9 is a modified Cas9. A given gRNA targeting sequence can have additional sites throughout the genome where partial homology exists. These sites are called off-targets and need to be considered when designing a gRNA. In addition to optimizing gRNA design, CRISPR specificity can also be increased through modifications to Cas9. Cas9 generates double-strand breaks (DSBs) through the combined activity of two nuclease domains, RuvC and HNH. Cas9 nickase, a D10A mutant of SpCas9, retains one nuclease domain and generates a DNA nick rather than a DSB. The nickase system can also be combined with HDR-mediated gene editing for specific gene edits.

In some cases, Cas9 is a variant Cas9 protein. A variant Cas9 polypeptide has an amino acid sequence that is different by one amino acid (*e.g.*, has a deletion, insertion, substitution, fusion) when compared to the amino acid sequence of a wild type Cas9 protein. In some instances, the variant Cas9 polypeptide has an amino acid change (*e.g.*, deletion, insertion, or substitution) that reduces the nuclease activity of the Cas9 polypeptide. For example, in some instances, the variant Cas9 polypeptide has less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1% of the nuclease

activity of the corresponding wild-type Cas9 protein. In some cases, the variant Cas9 protein has no substantial nuclease activity. When a subject Cas9 protein is a variant Cas9 protein that has no substantial nuclease activity, it can be referred to as "dCas9."

In some cases, a variant Cas9 protein has reduced nuclease activity. For example, a variant Cas9 protein exhibits less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or less than about 0.1%, of the endonuclease activity of a wild-type Cas9 protein, *e.g.*, a wild-type Cas9 protein.

In some cases, a variant Cas9 protein can cleave the complementary strand of a guide target sequence but has reduced ability to cleave the non-complementary strand of a double stranded guide target sequence. For example, the variant Cas9 protein can have a mutation (amino acid substitution) that reduces the function of the RuvC domain. As a non-limiting example, in some embodiments, a variant Cas9 protein has a D10A (aspartate to alanine at amino acid position 10) and can therefore cleave the complementary strand of a double stranded guide target sequence but has reduced ability to cleave the non-complementary strand of a double stranded guide target sequence (thus resulting in a single strand break (SSB) instead of a double strand break (DSB) when the variant Cas9 protein cleaves a double stranded target nucleic acid) (see, for example, Jinek *et al.*, Science. 2012 Aug. 17; 337(6096):816-21).

In some cases, a variant Cas9 protein can cleave the non-complementary strand of a double stranded guide target sequence but has reduced ability to cleave the complementary strand of the guide target sequence. For example, the variant Cas9 protein can have a mutation (amino acid substitution) that reduces the function of the HNH domain (RuvC/HNH/RuvC domain motifs). As a non-limiting example, in some embodiments, the variant Cas9 protein has an H840A (histidine to alanine at amino acid position 840) mutation and can therefore cleave the non-complementary strand of the guide target sequence but has reduced ability to cleave the complementary strand of the guide target sequence (thus resulting in a SSB instead of a DSB when the variant Cas9 protein cleaves a double stranded guide target sequence). Such a Cas9 protein has a reduced ability to cleave a guide target sequence (*e.g.*, a single stranded guide target sequence) but retains the ability to bind a guide target sequence (*e.g.*, a single stranded guide target sequence).

In some cases, a variant Cas9 protein has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target DNA. As a non-limiting example, in some cases, the variant Cas9 protein harbors both the D10A and the

H840A mutations such that the polypeptide has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA).

As another non-limiting example, in some cases, the variant Cas9 protein harbors W476A and W1126A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA).

As another non-limiting example, in some cases, the variant Cas9 protein harbors P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA).

As another non-limiting example, in some cases, the variant Cas9 protein harbors H840A, W476A, and W1126A, mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). As another non-limiting example, in some cases, the variant Cas9 protein harbors H840A, D10A, W476A, and W1126A, mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). In some embodiments, the variant Cas9 has restored catalytic His residue at position 840 in the Cas9 HNH domain (A840H).

As another non-limiting example, in some cases, the variant Cas9 protein harbors, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). As another non-limiting example, in some cases, the variant Cas9 protein harbors D10A, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single

stranded target DNA). In some cases, when a variant Cas9 protein harbors W476A and W1126A mutations or when the variant Cas9 protein harbors P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations, the variant Cas9 protein does not bind efficiently to a PAM sequence. Thus, in some such cases, when such a variant Cas9 protein is used in a method of binding, the method does not require a PAM sequence. In other words, in some cases, when such a variant Cas9 protein is used in a method of binding, the method can include a guide RNA, but the method can be performed in the absence of a PAM sequence (and the specificity of binding is therefore provided by the targeting segment of the guide RNA). Other residues can be mutated to achieve the above effects (*i.e.*, inactivate one or the other nuclease portions). As non-limiting examples, residues D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or A987 can be altered (*i.e.*, substituted). Also, mutations other than alanine substitutions are suitable.

In some embodiments, a variant Cas9 protein that has reduced catalytic activity (*e.g.*, when a Cas9 protein has a D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or a A987 mutation, *e.g.*, D10A, G12A, G17A, E762A, H840A, N854A, N863A, H982A, H983A, A984A, and/or D986A), the variant Cas9 protein can still bind to target DNA in a site-specific manner (because it is still guided to a target DNA sequence by a guide RNA) as long as it retains the ability to interact with the guide RNA.

In some embodiments, the variant Cas protein can be spCas9, spCas9-VRQR, spCas9-VRER, xCas9 (sp), saCas9, saCas9-KKH, spCas9-MQKSER, spCas9-LRKIQK, or spCas9-LRVSQL.

In some embodiments, a modified SpCas9 including amino acid substitutions D1135M, S1136Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R (SpCas9-MQKFRAER) and having specificity for the altered PAM 5′-NGC-3′ was used.

Alternatives to *S. pyogenes* Cas9 can include RNA-guided endonucleases from the Cpf1 family that display cleavage activity in mammalian cells. CRISPR from *Prevotella* and *Francisella 1* (CRISPR/Cpf1) is a DNA-editing technology analogous to the CRISPR/Cas9 system. Cpf1 is an RNA-guided endonuclease of a class II CRISPR/Cas system. This acquired immune mechanism is found in *Prevotella* and *Francisella* bacteria. Cpf1 genes are associated with the CRISPR locus, coding for an endonuclease that use a guide RNA to find and cleave viral DNA. Cpf1 is a smaller and simpler endonuclease than Cas9, overcoming some of the CRISPR/Cas9 system limitations. Unlike Cas9 nucleases, the result of Cpf1-mediated DNA cleavage is a double-strand break with a short 3′ overhang. Cpf1's staggered

cleavage pattern can open up the possibility of directional gene transfer, analogous to traditional restriction enzyme cloning, which can increase the efficiency of gene editing. Like the Cas9 variants and orthologues described above, Cpf1 can also expand the number of sites that can be targeted by CRISPR to AT-rich regions or AT-rich genomes that lack the NGG PAM sites favored by SpCas9. The Cpf1 locus contains a mixed alpha/beta domain, a RuvC-I followed by a helical region, a RuvC-II and a zinc finger-like domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9. Furthermore, Cpf1 does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alpha-helical recognition lobe of Cas9. Cpf1 CRISPR-Cas domain architecture shows that Cpf1 is functionally unique, being classified as Class 2, type V CRISPR system. The Cpf1 loci encode Cas1, Cas2 and Cas4 proteins more similar to types I and III than from type II systems. Functional Cpf1 doesn't need the trans-activating CRISPR RNA (tracrRNA), therefore, only CRISPR (crRNA) is required. This benefits genome editing because Cpf1 is not only smaller than Cas9, but also it has a smaller sgRNA molecule (proximately half as many nucleotides as Cas9). The Cpf1-crRNA complex cleaves target DNA or RNA by identification of a protospacer adjacent motif 5′-YTN-3′ in contrast to the G-rich PAM targeted by Cas9. After identification of PAM, Cpf1 introduces a sticky-end-like DNA double- stranded break of 4 or 5 nucleotides overhang.

Some aspects of the disclosure provide fusion proteins comprising domains that act as nucleic acid programmable DNA binding proteins, which may be used to guide a protein, such as a base editor, to a specific nucleic acid (*e.g.*, DNA or RNA) sequence. In particular embodiments, a fusion protein comprises a nucleic acid programmable DNA binding protein domain and a deaminase domain. DNA binding proteins include, without limitation, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. One example of a programmable polynucleotide-binding protein that has different PAM specificity than Cas9 is Clustered Regularly Interspaced Short Palindromic Repeats from *Prevotella* and *Francisella*1 (Cpf1). Similar to Cas9, Cpf1 is also a class 2 CRISPR effector. It has been shown that Cpf1 mediates robust DNA interference with features distinct from Cas9. Cpf1 is a single RNA-guided endonuclease lacking tracrRNA, and it utilizes a T-rich protospacer-adjacent motif (TTN, TTTN, or YTN). Moreover, Cpf1 cleaves DNA via a staggered DNA double-stranded break. Out of 16 Cpf1-family proteins, two enzymes from *Acidaminococcus* and *Lachnospiraceae* are shown to have efficient genome-editing activity in human cells. Cpf1

proteins are known in the art and have been described previously, for example Yamano *et al.*, "Crystal structure of Cpf1 in complex with guide RNA and target DNA." *Cell* (165) 2016, p. 949-962; the entire contents of which is hereby incorporated by reference.

Also useful in the present compositions and methods are nuclease-inactive Cpf1 (dCpf1) variants that may be used as a guide nucleotide sequence-programmable polynucleotide-binding protein domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9 but does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alfa-helical recognition lobe of Cas9. It was shown in Zetsche *et al.*, *Cell,* 163, 759-771, 2015 (which is incorporated herein by reference) that, the RuvC-like domain of Cpf1 is responsible for cleaving both DNA strands and inactivation of the RuvC-like domain inactivates Cpf1 nuclease activity. For example, mutations corresponding to D917A, E1006A, or D1255A in *Francisella novicida* Cpf1 inactivate Cpf1 nuclease activity. In some embodiments, the dCpf1 of the present disclosure comprises mutations corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/E1006A/D1255A. It is to be understood that any mutations, *e.g.*, substitution mutations, deletions, or insertions that inactivate the RuvC domain of Cpf1, may be used in accordance with the present disclosure.

In some embodiments, the nucleic acid programmable nucleotide binding protein of any of the fusion proteins provided herein may be a Cpf1 protein. In some embodiments, the Cpf1 protein is a Cpf1 nickase (nCpf1). In some embodiments, the Cpf1 protein is a nuclease inactive Cpf1 (dCpf1). In some embodiments, the Cpf1, the nCpf1, or the dCpf1 comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a Cpf1 sequence disclosed herein. In some embodiments, the dCpf1comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a Cpf1 sequence disclosed herein, and comprises mutations corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/E1006A/D1255A. It should be appreciated that Cpf1 from other bacterial species may also be used in accordance with the present disclosure.

The amino acid sequence of wild type *Francisella novicida* Cpf1 follows. D917, E1006, and D1255 are bolded and underlined.

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

The amino acid sequence of *Francisella novicida* Cpf1 D917A follows. (A917, E1006, and D1255 are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY

NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

The amino acid sequence of *Francisella novicida* Cpf1 E1006A follows. (D917, A1006, and D1255 are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

The amino acid sequence of *Francisella novicida* Cpf1 D1255A follows. (D917, E1006, and A1255 mutation positions are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

The amino acid sequence of *Francisella novicida* Cpf1 D917A/E1006A follows. (A917, A1006, and D1255 are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD

EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

The amino acid sequence of *Francisella novicida* Cpf1 D917A/D1255A follows. (A917, E1006, and A1255 are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL

KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

The amino acid sequence of *Francisella novicida* Cpf1 E1006A/D1255A follows. (D917, A1006, and A1255 are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS
KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

The amino acid sequence of *Francisella novicida* Cpf1 D917A/E1006A/D1255A follows. (A917, A1006, and A1255 are bolded and underlined).

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFI
EEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFN
QNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENR
KNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDY
KTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQ
INDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKE
TLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPS

KKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNK
DNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKD
EHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAI
LFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFY
NPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDT
QRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTL
YWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLI
KDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSIARGERHLAYYTLVD
GKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEI
AKLVIEYNAIVVFADLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRA
YQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKIC
YNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLL
KDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNF
FDSRQAPKNMPQDAAANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN.

In some embodiments, one of the Cas9 domains present in the fusion protein may be replaced with a guide nucleotide sequence-programmable DNA-binding protein domain that has no requirements for a PAM sequence.

In some embodiments, the Cas domain is a Cas9 domain from *Staphylococcus aureus* (SaCas9). In some embodiments, the SaCas9 domain is a nuclease active SaCas9, a nuclease inactive SaCas9 (SaCas9d), or a SaCas9 nickase (SaCas9n). In some embodiments, the SaCas9 domain comprises a N579A mutation, or a corresponding mutation in any of the amino acid sequences provided herein.

In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a NNGRRT or a NNGRRT PAM sequence. In some embodiments, the SaCas9 domain comprises one or more of a E781X, a N967X, and a R1014X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SaCas9 domain comprises one or more of a E781K, a N967K, and a R1014H mutation, or one or more corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SaCas9 domain comprises a E781K, a N967K, or a R1014H mutation, or corresponding mutations in any of the amino acid sequences provided herein.

The amino acid sequence of an exemplary SaCas9 is as follows:

MKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRH
RIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEV
EEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKV
QKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVK
YAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDI
KGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLN
SELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQK
EIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQK
RNRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHI
IPRSVSFDNSFNNKVLVKQEE**N**SKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISK
TKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTS
FLRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEI
ETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLN
GLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKY
SKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKN
LDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRI
EVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG.

In this sequence, residue N579, which is underlined and in bold, may be mutated (*e.g.*, to a A579) to yield a SaCas9 nickase.

The amino acid sequence of an exemplary SaCas9n is as follows:

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHR
IQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVE
EDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQ
KAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKY
AYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIK
GYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNS
ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKE
IPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKR
NRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII
PRSVSFDNSFNNKVLVKQEE**A**SKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKT
KKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSF
LRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIE

TEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNG
LYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYS
KKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNL
DVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIE
VNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG.

In this sequence, residue A579, which can be mutated from N579 to yield a SaCas9 nickase, is underlined and in bold.

The amino acid sequences of an exemplary SaKKH Cas9 is as follows:

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHR
IQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVE
EDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQ
KAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKY
AYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIK
GYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNS
ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKE
IPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKR
NRQTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII
PRSVSFDNSFNNKVLVKQEE**A**SKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKT
KKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSF
LRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIE
TEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNR*K*LINDTLYSTRKDDKGNTLIVNNLNG
LYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYS
KKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNL
DVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFY*K*NDLIKINGELYRVIGVNNDLLNRIE
VNMIDITYREYLENMNDKRPP*H*IIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG.

Residue A579 above, which can be mutated from N579 to yield a SaCas9 nickase, is underlined and in bold. Residues K781, K967, and H1014 above, which can be mutated from E781, N967, and R1014 to yield a SaKKH Cas9 are underlined and in italics.

High fidelity Cas9 domains

Some aspects of the disclosure provide high fidelity Cas9 domains. In some embodiments, high fidelity Cas9 domains are engineered Cas9 domains comprising one or more mutations that decrease electrostatic interactions between the Cas9 domain and the

sugar-phosphate backbone of a DNA, relative to a corresponding wild-type Cas9 domain. High fidelity Cas9 domains that have decreased electrostatic interactions with the sugar-phosphate backbone of DNA can have less off-target effects. In some embodiments, the Cas9 domain (*e.g.*, a wild type Cas9 domain) comprises one or more mutations that decrease the association between the Cas9 domain and the sugar-phosphate backbone of a DNA. In some embodiments, a Cas9 domain comprises one or more mutations that decreases the association between the Cas9 domain and the sugar-phosphate backbone of DNA by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70%.

In some embodiments, any of the Cas9 fusion proteins provided herein comprise one or more of a N497X, a R661X, a Q695X, and/or a Q926X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, any of the Cas9 fusion proteins provided herein comprise one or more of a N497A, a R661A, a Q695A, and/or a Q926A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the Cas9 domain comprises a D10A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. Cas9 domains with high fidelity are known in the art and would be apparent to the skilled artisan. For example, Cas9 domains with high fidelity have been described in Kleinstiver, B.P., *et al.* "High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects." *Nature* 529, 490-495 (2016); and Slaymaker, I.M., *et al.* "Rationally engineered Cas9 nucleases with improved specificity." *Science* 351, 84-88 (2015); the entire contents of each are incorporated herein by reference.

In some embodiments, the modified Cas9 is a high fidelity Cas9 enzyme. In some embodiments, the high fidelity Cas9 enzyme is SpCas9(K855A), eSpCas9(1.1), SpCas9-HF1, or hyper accurate Cas9 variant (HypaCas9). The modified Cas9 eSpCas9(1.1) contains alanine substitutions that weaken the interactions between the HNH/RuvC groove and the non-target DNA strand, preventing strand separation and cutting at off-target sites. Similarly, SpCas9-HF1 lowers off-target editing through alanine substitutions that disrupt Cas9's interactions with the DNA phosphate backbone. HypaCas9 contains mutations (SpCas9 N692A/M694A/Q695A/H698A) in the REC3 domain that increase Cas9 proofreading and target discrimination. All three high fidelity enzymes generate less off-target editing than wildtype Cas9.

An exemplary high fidelity Cas9 is provided below.

High Fidelity Cas9 domain mutations relative to Cas9 are shown in bold and underline

MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
AFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWG**A**LSRKLINGIRDKQSGKTILDF
LKSDGFANRNFM**A**LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETR**A**ITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD.

Guide Polynucleotides

In an embodiment, the guide polynucleotide is a guide RNA. An RNA/Cas complex can assist in "guiding" Cas protein to a target DNA. Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. *See,*

e.g., Jinek M. *et al.*, Science 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self-versus-non self. Cas9 nuclease sequences and structures are well known to those of skill in the art (see *e.g.*, "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti, J.J. *et al.*, Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E. *et al.*, Nature 471:602-607(2011); and "Programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M.*et al*, Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences can be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase.

In some embodiments, the guide polynucleotide is at least one single guide RNA ("sgRNA" or "gNRA"). In some embodiments, the guide polynucleotide is at least one tracrRNA. In some embodiments, the guide polynucleotide does not require PAM sequence to guide the polynucleotide-programmable DNA-binding domain (*e.g.*, Cas9 or Cpf1) to the target nucleotide sequence.

The polynucleotide programmable nucleotide binding domain (*e.g.*, a CRISPR-derived domain) of the base editors disclosed herein can recognize a target polynucleotide sequence by associating with a guide polynucleotide. A guide polynucleotide (*e.g.*, gRNA) is typically single-stranded and can be programmed to site-specifically bind (*i.e.*, via complementary base pairing) to a target sequence of a polynucleotide, thereby directing a base editor that is in conjunction with the guide nucleic acid to the target sequence. A guide polynucleotide can be DNA. A guide polynucleotide can be RNA. In some cases, the guide polynucleotide comprises natural nucleotides (*e.g.*, adenosine). In some cases, the guide polynucleotide comprises non-natural (or unnatural) nucleotides (*e.g.*, peptide nucleic acid or nucleotide analogs). In some cases, the targeting region of a guide nucleic acid sequence can be at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in

length. A targeting region of a guide nucleic acid can be between 10-30 nucleotides in length, or between 15-25 nucleotides in length, or between 15-20 nucleotides in length.

In some embodiments, a guide polynucleotide comprises two or more individual polynucleotides, which can interact with one another via for example complementary base pairing (*e.g.*, a dual guide polynucleotide). For example, a guide polynucleotide can comprise a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA). For example, a guide polynucleotide can comprise one or more trans-activating CRISPR RNA (tracrRNA).

In type II CRISPR systems, targeting of a nucleic acid by a CRISPR protein (*e.g.*, Cas9) typically requires complementary base pairing between a first RNA molecule (crRNA) comprising a sequence that recognizes the target sequence and a second RNA molecule (trRNA) comprising repeat sequences which forms a scaffold region that stabilizes the guide RNA-CRISPR protein complex. Such dual guide RNA systems can be employed as a guide polynucleotide to direct the base editors disclosed herein to a target polynucleotide sequence.

In some embodiments, the base editor provided herein utilizes a single guide polynucleotide (*e.g.*, gRNA). In some embodiments, the base editor provided herein utilizes a dual guide polynucleotide (*e.g.*, dual gRNAs). In some embodiments, the base editor provided herein utilizes one or more guide polynucleotide (*e.g.*, multiple gRNA). In some embodiments, a single guide polynucleotide is utilized for different base editors described herein. For example, a single guide polynucleotide can be utilized for a cytidine base editor and an adenosine base editor.

In other embodiments, a guide polynucleotide can comprise both the polynucleotide targeting portion of the nucleic acid and the scaffold portion of the nucleic acid in a single molecule (*i.e.*, a single-molecule guide nucleic acid). For example, a single-molecule guide polynucleotide can be a single guide RNA (sgRNA or gRNA). Herein the term guide polynucleotide sequence contemplates any single, dual, or multi-molecule nucleic acid capable of interacting with and directing a base editor to a target polynucleotide sequence.

Typically, a guide polynucleotide (*e.g.*, crRNA/trRNA complex or a gRNA) comprises a "polynucleotide-targeting segment" that includes a sequence capable of recognizing and binding to a target polynucleotide sequence, and a "protein-binding segment" that stabilizes the guide polynucleotide within a polynucleotide programmable nucleotide binding domain component of a base editor. In some embodiments, the polynucleotide targeting segment of the guide polynucleotide recognizes and binds to a DNA

polynucleotide, thereby facilitating the editing of a base in DNA. In other cases, the polynucleotide targeting segment of the guide polynucleotide recognizes and binds to an RNA polynucleotide, thereby facilitating the editing of a base in RNA. Herein a "segment" refers to a section or region of a molecule, *e.g.*, a contiguous stretch of nucleotides in the guide polynucleotide. A segment can also refer to a region/section of a complex such that a segment can comprise regions of more than one molecule. For example, where a guide polynucleotide comprises multiple nucleic acid molecules, the protein-binding segment of can include all or a portion of multiple separate molecules that are for instance hybridized along a region of complementarity. In some embodiments, a protein-binding segment of a DNA-targeting RNA that comprises two separate molecules can comprise (i) base pairs 40-75 of a first RNA molecule that is 100 base pairs in length; and (ii) base pairs 10-25 of a second RNA molecule that is 50 base pairs in length. The definition of "segment," unless otherwise specifically defined in a particular context, is not limited to a specific number of total base pairs, is not limited to any particular number of base pairs from a given RNA molecule, is not limited to a particular number of separate molecules within a complex, and can include regions of RNA molecules that are of any total length and can include regions with complementarity to other molecules.

A guide RNA or a guide polynucleotide can comprise two or more RNAs, *e.g.*, CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA). A guide RNA or a guide polynucleotide can sometimes comprise a single-chain RNA, or single guide RNA (sgRNA) formed by fusion of a portion (*e.g.*, a functional portion) of crRNA and tracrRNA. A guide RNA or a guide polynucleotide can also be a dual RNA comprising a crRNA and a tracrRNA. Furthermore, a crRNA can hybridize with a target DNA.

As discussed above, a guide RNA or a guide polynucleotide can be an expression product. For example, a DNA that encodes a guide RNA can be a vector comprising a sequence coding for the guide RNA. A guide RNA or a guide polynucleotide can be transferred into a cell by transfecting the cell with an isolated guide RNA or plasmid DNA comprising a sequence coding for the guide RNA and a promoter. A guide RNA or a guide polynucleotide can also be transferred into a cell in other way, such as using virus-mediated gene delivery.

A guide RNA or a guide polynucleotide can be isolated. For example, a guide RNA can be transfected in the form of an isolated RNA into a cell or organism. A guide RNA can be prepared by *in vitro* transcription using any *in vitro* transcription system known in the art.

A guide RNA can be transferred to a cell in the form of isolated RNA rather than in the form of plasmid comprising encoding sequence for a guide RNA.

A guide RNA or a guide polynucleotide can comprise three regions: a first region at the 5′ end that can be complementary to a target site in a chromosomal sequence, a second internal region that can form a stem loop structure, and a third 3′ region that can be single-stranded. A first region of each guide RNA can also be different such that each guide RNA guides a fusion protein to a specific target site. Further, second and third regions of each guide RNA can be identical in all guide RNAs.

A first region of a guide RNA or a guide polynucleotide can be complementary to sequence at a target site in a chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In some cases, a first region of a guide RNA can comprise from or from about 10 nucleotides to 25 nucleotides (*i.e.*, from 10 nucleotides to nucleotides; or from about 10 nucleotides to about 25 nucleotides; or from 10 nucleotides to about 25 nucleotides; or from about 10 nucleotides to 25 nucleotides) or more. For example, a region of base pairing between a first region of a guide RNA and a target site in a chromosomal sequence can be or can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more nucleotides in length. Sometimes, a first region of a guide RNA can be or can be about 19, 20, or 21 nucleotides in length.

A guide RNA or a guide polynucleotide can also comprise a second region that forms a secondary structure. For example, a secondary structure formed by a guide RNA can comprise a stem (or hairpin) and a loop. A length of a loop and a stem can vary. For example, a loop can range from or from about 3 to 10 nucleotides in length, and a stem can range from or from about 6 to 20 base pairs in length. A stem can comprise one or more bulges of 1 to 10 or about 10 nucleotides. The overall length of a second region can range from or from about 16 to 60 nucleotides in length. For example, a loop can be or can be about 4 nucleotides in length and a stem can be or can be about 12 base pairs.

A guide RNA or a guide polynucleotide can also comprise a third region at the 3′ end that can be essentially single-stranded. For example, a third region is sometimes not complementarity to any chromosomal sequence in a cell of interest and is sometimes not complementarity to the rest of a guide RNA. Further, the length of a third region can vary. A third region can be more than or more than about 4 nucleotides in length. For example, the length of a third region can range from or from about 5 to 60 nucleotides in length.

A guide RNA or a guide polynucleotide can target any exon or intron of a gene target. In some cases, a guide can target exon 1 or 2 of a gene, in other cases; a guide can target exon 3 or 4 of a gene. A composition can comprise multiple guide RNAs that all target the same exon or in some cases, multiple guide RNAs that can target different exons. An exon and an intron of a gene can be targeted.

A guide RNA or a guide polynucleotide can target a nucleic acid sequence of or of about 20 nucleotides. A target nucleic acid can be less than or less than about 20 nucleotides. A target nucleic acid can be at least or at least about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, or anywhere between 1-100 nucleotides in length. A target nucleic acid can be at most or at most about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, or anywhere between 1-100 nucleotides in length. A target nucleic acid sequence can be or can be about 20 bases immediately 5′ of the first nucleotide of the PAM. A guide RNA can target a nucleic acid sequence. A target nucleic acid can be at least or at least about 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, or 1-100 nucleotides.

A guide polynucleotide, for example, a guide RNA, can refer to a nucleic acid that can hybridize to another nucleic acid, for example, the target nucleic acid or protospacer in a genome of a cell. A guide polynucleotide can be RNA. A guide polynucleotide can be DNA. The guide polynucleotide can be programmed or designed to bind to a sequence of nucleic acid site-specifically. A guide polynucleotide can comprise a polynucleotide chain and can be called a single guide polynucleotide. A guide polynucleotide can comprise two polynucleotide chains and can be called a double guide polynucleotide. A guide RNA can be introduced into a cell or embryo as an RNA molecule. For example, a RNA molecule can be transcribed *in vitro* and/or can be chemically synthesized. An RNA can be transcribed from a synthetic DNA molecule, *e.g.*, a gBlocks® gene fragment. A guide RNA can then be introduced into a cell or embryo as an RNA molecule. A guide RNA can also be introduced into a cell or embryo in the form of a non-RNA nucleic acid molecule, *e.g.*, DNA molecule. For example, a DNA encoding a guide RNA can be operably linked to promoter control sequence for expression of the guide RNA in a cell or embryo of interest. A RNA coding sequence can be operably linked to a promoter sequence that is recognized by RNA polymerase III (Pol III). Plasmid vectors that can be used to express guide RNA include, but are not limited to, px330 vectors and px333 vectors. In some cases, a plasmid vector (*e.g.*, px333 vector) can comprise at least two guide RNA-encoding DNA sequences.

Methods for selecting, designing, and validating guide polynucleotides, e.g., guide RNAs and targeting sequences are described herein and known to those skilled in the art. For example, to minimize the impact of potential substrate promiscuity of a deaminase domain in the nucleobase editor system (e.g., an AID domain), the number of residues that could unintentionally be targeted for deamination (e.g., off-target C residues that could potentially reside on ssDNA within the target nucleic acid locus) may be minimized. In addition, software tools can be used to optimize the gRNAs corresponding to a target nucleic acid sequence, e.g., to minimize total off-target activity across the genome. For example, for each possible targeting domain choice using S. pyogenes Cas9, all off-target sequences (preceding selected PAMs, e.g., NAG or NGG) may be identified across the genome that contain up to certain number (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of mismatched base-pairs. First regions of gRNAs complementary to a target site can be identified, and all first regions (e.g., crRNAs) can be ranked according to its total predicted off-target score; the top-ranked targeting domains represent those that are likely to have the greatest on-target and the least off-target activity. Candidate targeting gRNAs can be functionally evaluated by using methods known in the art and/or as set forth herein.

As a non-limiting example, target DNA hybridizing sequences in crRNAs of a guide RNA for use with Cas9s may be identified using a DNA sequence searching algorithm. gRNA design may be carried out using custom gRNA design software based on the public tool cas-offinder as described in Bae S., Park J., & Kim J.-S. Cas-OFFinder: A fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics 30, 1473-1475 (2014). This software scores guides after calculating their genome-wide off-target propensity. Typically matches ranging from perfect matches to 7 mismatches are considered for guides ranging in length from 17 to 24. Once the off-target sites are computationally-determined, an aggregate score is calculated for each guide and summarized in a tabular output using a web-interface. In addition to identifying potential target sites adjacent to PAM sequences, the software also identifies all PAM adjacent sequences that differ by 1, 2, 3 or more than 3 nucleotides from the selected target sites. Genomic DNA sequences for a target nucleic acid sequence, e.g., a target gene may be obtained and repeat elements may be screened using publicly available tools, for example, the RepeatMasker program. RepeatMasker searches input DNA sequences for repeated elements and regions of low complexity. The output is a detailed annotation of the repeats present in a given query sequence.

Following identification, first regions of guide RNAs, e.g., crRNAs, may be ranked into tiers based on their distance to the target site, their orthogonality and presence of 5′ nucleotides for close matches with relevant PAM sequences (for example, a 5′ G based on identification of close matches in the human genome containing a relevant PAM e.g., NGG PAM for S. pyogenes, NNGRRT or NNGRRV PAM for S. aureus). As used herein, orthogonality refers to the number of sequences in the human genome that contain a minimum number of mismatches to the target sequence. A "high level of orthogonality" or "good orthogonality" may, for example, refer to 20-mer targeting domains that have no identical sequences in the human genome besides the intended target, nor any sequences that contain one or two mismatches in the target sequence. Targeting domains with good orthogonality may be selected to minimize off-target DNA cleavage.

In some embodiments, a reporter system may be used for detecting base-editing activity and testing candidate guide polynucleotides. In some embodiments, a reporter system may comprise a reporter gene based assay where base editing activity leads to expression of the reporter gene. For example, a reporter system may include a reporter gene comprising a deactivated start codon, e.g., a mutation on the template strand from 3′-TAC-5′ to 3′-CAC-5′. Upon successful deamination of the target C, the corresponding mRNA will be transcribed as 5′-AUG-3′ instead of 5′-GUG-3′, enabling the translation of the reporter gene. Suitable reporter genes will be apparent to those of skill in the art. Non-limiting examples of reporter genes include gene encoding green fluorescence protein (GFP), red fluorescence protein (RFP), luciferase, secreted alkaline phosphatase (SEAP), or any other gene whose expression are detectable and apparent to those skilled in the art. The reporter system can be used to test many different gRNAs, e.g., in order to determine which residue(s) with respect to the target DNA sequence the respective deaminase will target. sgRNAs that target non-template strand can also be tested in order to assess off-target effects of a specific base editing protein, e.g., a Cas9 deaminase fusion protein. In some embodiments, such gRNAs can be designed such that the mutated start codon will not be base-paired with the gRNA. The guide polynucleotides can comprise standard ribonucleotides, modified ribonucleotides (e.g., pseudouridine), ribonucleotide isomers, and/or ribonucleotide analogs. In some embodiments, the guide polynucleotide can comprise at least one detectable label. The detectable label can be a fluorophore (e.g., FAM, TMR, Cy3, Cy5, Texas Red, Oregon Green, Alexa Fluors, Halo tags, or suitable fluorescent dye), a detection tag (e.g., biotin, digoxigenin, and the like), quantum dots, or gold particles.

The guide polynucleotides can be synthesized chemically, synthesized enzymatically, or a combination thereof. For example, the guide RNA can be synthesized using standard phosphoramidite-based solid-phase synthesis methods. Alternatively, the guide RNA can be synthesized in vitro by operably linking DNA encoding the guide RNA to a promoter control sequence that is recognized by a phage RNA polymerase. Examples of suitable phage promoter sequences include T7, T3, SP6 promoter sequences, or variations thereof. In embodiments in which the guide RNA comprises two separate molecules (e.g.., crRNA and tracrRNA), the crRNA can be chemically synthesized and the tracrRNA can be enzymatically synthesized.

In some embodiments, a base editor system may comprise multiple guide polynucleotides, e.g., gRNAs. For example, the gRNAs may target to one or more target loci (e.g., at least 1 gRNA, at least 2 gRNA, at least 5 gRNA, at least 10 gRNA, at least 20 gRNA, at least 30 g RNA, at least 50 gRNA) comprised in a base editor system. The multiple gRNA sequences can be tandemly arranged and are preferably separated by a direct repeat.

A DNA sequence encoding a guide RNA (gRNA) or a guide polynucleotide can also be part of a vector. Further, a vector can comprise additional expression control sequences (*e.g.*, enhancer sequences, Kozak sequences, polyadenylation sequences, transcriptional termination sequences, etc.), selectable marker sequences (*e.g.*, GFP or antibiotic resistance genes such as puromycin), origins of replication, and the like. A DNA molecule encoding a guide RNA can also be linear. A DNA molecule encoding a guide RNA (gRNA) or a guide polynucleotide can also be circular.

In some embodiments, one or more components of a base editor system may be encoded by DNA sequences. Such DNA sequences may be introduced into an expression system, e.g., a cell, together or separately. For example, DNA sequences encoding a polynucleotide programmable nucleotide binding domain and a guide RNA may be introduced into a cell, each DNA sequence can be part of a separate molecule (*e.g.*, one vector containing the polynucleotide programmable nucleotide binding domain coding sequence and a second vector containing the guide RNA coding sequence) or both can be part of a same molecule (*e.g.*, one vector containing coding (and regulatory) sequence for both the polynucleotide programmable nucleotide binding domain and the guide RNA).

A guide polynucleotide can comprise one or more modifications to provide a nucleic acid with a new or enhanced feature. A guide polynucleotide can comprise a nucleic acid

affinity tag. A guide polynucleotide can comprise synthetic nucleotide, synthetic nucleotide analog, nucleotide derivatives, and/or modified nucleotides.

In some cases, a gRNA or a guide polynucleotide can comprise modifications. A modification can be made at any location of a gRNA or a guide polynucleotide. More than one modification can be made to a single gRNA or a guide polynucleotide. A gRNA or a guide polynucleotide can undergo quality control after a modification. In some cases, quality control can include PAGE, HPLC, MS, or any combination thereof.

A modification of a gRNA or a guide polynucleotide can be a substitution, insertion, deletion, chemical modification, physical modification, stabilization, purification, or any combination thereof.

A gRNA or a guide polynucleotide can also be modified by 5′adenylate, 5′ guanosine-triphosphate cap, 5′N7-Methylguanosine-triphosphate cap, 5′triphosphate cap, 3′phosphate, 3′thiophosphate, 5′phosphate, 5′thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC spacer, rSpacer, Spacer 18, Spacer 9,3′-3′ modifications, 5′-5′ modifications, abasic, acridine, azobenzene, biotin, biotin BB, biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3′DABCYL, black hole quencher 1, black hole quencer 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2’-deoxyribonucleoside analog purine, 2’-deoxyribonucleoside analog pyrimidine, ribonucleoside analog, 2’-O-methyl ribonucleoside analog, sugar modified analogs, wobble/universal bases, fluorescent dye label, 2’-fluoro RNA, 2’-O-methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5′-triphosphate, 5′-methylcytidine-5′-triphosphate, or any combination thereof.

In some cases, a modification is permanent. In other cases, a modification is transient. In some cases, multiple modifications are made to a gRNA or a guide polynucleotide. A gRNA or a guide polynucleotide modification can alter physiochemical properties of a nucleotide, such as their conformation, polarity, hydrophobicity, chemical reactivity, base-pairing interactions, or any combination thereof.

The PAM sequence can be any PAM sequence known in the art. Suitable PAM sequences include, but are not limited to, NGG, NGA, NGC, NGN, NGT, NGCG, NGAG, NGAN, NGNG, NGCN, NGCG, NGTN, NNGRRT, NNNRRT, NNGRR(N), TTTV, TYCV,

TYCV, TATV, NNNNGATT, NNAGAAW, or NAAAAC. Y is a pyrimidine; N is any nucleotide base; W is A or T.

A modification can also be a phosphorothioate substitute. In some cases, a natural phosphodiester bond can be susceptible to rapid degradation by cellular nucleases and; a modification of internucleotide linkage using phosphorothioate (PS) bond substitutes can be more stable towards hydrolysis by cellular degradation. A modification can increase stability in a gRNA or a guide polynucleotide. A modification can also enhance biological activity. In some cases, a phosphorothioate enhanced RNA gRNA can inhibit RNase A, RNase T1, calf serum nucleases, or any combinations thereof. These properties can allow the use of PS-RNA gRNAs to be used in applications where exposure to nucleases is of high probability *in vivo* or *in vitro*. For example, phosphorothioate (PS) bonds can be introduced between the last 3-5 nucleotides at the 5′- or ‘'-end of a gRNA which can inhibit exonuclease degradation. In some cases, phosphorothioate bonds can be added throughout an entire gRNA to reduce attack by endonucleases.

Protospacer Adjacent Motif

The term “protospacer adjacent motif (PAM)” or PAM-like motif refers to a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease in the CRISPR bacterial adaptive immune system. In some embodiments, the PAM can be a 5′ PAM (*i.e.*, located upstream of the 5′ end of the protospacer). In other embodiments, the PAM can be a 3′ PAM (*i.e.*, located downstream of the 5′ end of the protospacer).

The PAM sequence is essential for target binding, but the exact sequence depends on a type of Cas protein.

A base editor provided herein can comprise a CRISPR protein-derived domain that is capable of binding a nucleotide sequence that contains a canonical or non-canonical protospacer adjacent motif (PAM) sequence. A PAM site is a nucleotide sequence in proximity to a target polynucleotide sequence. Some aspects of the disclosure provide for base editors comprising all or a portion of CRISPR proteins that have different PAM specificities. For example, typically Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a canonical NGG PAM sequence to bind a particular nucleic acid region, where the “N” in “NGG” is adenine (A), thymine (T), guanine (G), or cytosine (C), and the G is guanine. A PAM can be CRISPR protein-specific and can be different between different base editors comprising different CRISPR protein-derived domains. A PAM can be 5′ or 3′

of a target sequence. A PAM can be upstream or downstream of a target sequence. A PAM can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides in length. Often, a PAM is between 2-6 nucleotides in length. Several PAM variants are described in Table 1 below.

Table 1. Cas9 proteins and corresponding PAM sequences

Variant	PAM
spCas9	NGG
spCas9-VRQR	NGA
spCas9-VRER	NGCG
spCas9-MQKFRAER	NGC
xCas9 (sp)	NGN
saCas9	NNGRRT
saCas9-KKH	NNNRRT
spCas9-MQKSER	NGCG
spCas9-MQKSER	NGCN
spCas9-LRKIQK	NGTN
spCas9-LRVSQK	NGTN
spCas9-LRVSQL	NGTN
SpyMacCas9	NAA
Cpf1	5′ (TTTV)

In some embodiments, the PAM is NGC. In some embodiments, the NGC PAM is recognized by a Cas9 variant. In some embodiments, the NGC PAM variant includes one or more amino acid substitutions selected from D1135M, S1136Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R (collectively termed “MQKFRAER”).

In some embodiments, the PAM is NGT. In some embodiments, the NGT PAM is recognized by a Cas9 variant. In some embodiments, the NGT PAM variant is generated through targeted mutations at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219. In some embodiments, the NGT PAM variant is created through targeted mutations at one or more residues 1219, 1335, 1337, 1218. In some embodiments, the NGT PAM variant is created through targeted mutations at one or more residues 1135, 1136, 1218, 1219, and 1335. In some embodiments, the NGT PAM variant is selected from the set of targeted mutations provided in Tables 2 and 3 below.

Table 2: NGT PAM Variant Mutations at residues 1219, 1335, 1337, 1218

Variant	E1219V	R1335Q	T1337	G1218
1	F	V	T	
2	F	V	R	
3	F	V	Q	
4	F	V	L	
5	F	V	T	R
6	F	V	R	R
7	F	V	Q	R
8	F	V	L	R
9	L	L	T	
10	L	L	R	
11	L	L	Q	
12	L	L	L	
13	F	I	T	
14	F	I	R	
15	F	I	Q	
16	F	I	L	
17	F	G	C	
18	H	L	N	
19	F	G	C	A
20	H	L	N	V
21	L	A	W	
22	L	A	F	
23	L	A	Y	
24	I	A	W	
25	I	A	F	
26	I	A	Y	

Table 3: NGT PAM Variant Mutations at residues 1135, 1136, 1218, 1219, and 1335

Variant	D1135L	S1136R	G1218S	E1219V	R1335Q
27	G				
28	V				
29	I				
30		A			
31		W			
32		H			
33		K			
34			K		
35			R		
36			Q		
37			T		
38			N		
39				I	
40				A	
41				N	

Variant	D1135L	S1136R	G1218S	E1219V	R1335Q
42				Q	
43				G	
44				L	
45				S	
46				T	
47					L
48					I
49					V
50					N
51					S
52					T
53					F
54					Y
55	N1286Q	I1331F			

In some embodiments, the NGT PAM variant is selected from variant 5, 7, 28, 31, or 36 in Tables 2 and 3. In some embodiments, the variants have improved NGT PAM recognition.

In some embodiments, the NGT PAM variants have mutations at residues 1219, 1335, 1337, and/or 1218. In some embodiments, the NGT PAM variant is selected with mutations for improved recognition from the variants provided in Table 4 below.

Table 4: NGT PAM Variant Mutations at residues 1219, 1335, 1337, and 1218

Variant	E1219V	R1335Q	T1337	G1218
1	F	V	T	
2	F	V	R	
3	F	V	Q	
4	F	V	L	
5	F	V	T	R
6	F	V	R	R
7	F	V	Q	R
8	F	V	L	R

In some embodiments, the NGT PAM is selected from the variants provided in Table 5 below.

Table 5. NGT PAM variants

	NGTN variant	D1135	S1136	G1218	E1219	A1322R	R1335	T1337
Variant 1	LRKIQK	L	R	K	I	-	Q	K
Variant 2	LRSVQK	L	R	S	V	-	Q	K
Variant 3	LRSVQL	L	R	S	V	-	Q	L
Variant 4	LRKIRQK	L	R	K	I	R	Q	K
Variant 5	LRSVRQK	L	R	S	V	R	Q	K

Variant 6	LRSVRQL	L	R	S	V	R	Q	L

In some embodiments the NGTN variant is variant 1. In some embodiments, the NGTN variant is variant 2. In some embodiments, the NGTN variant is variant 3. In some embodiments, the NGTN variant is variant 4. In some embodiments, the NGTN variant is variant 5. In some embodiments, the NGTN variant is variant 6.

In some embodiments, the Cas9 domain is a Cas9 domain from *Streptococcus pyogenes* (SpCas9). In some embodiments, the SpCas9 domain is a nuclease active SpCas9, a nuclease inactive SpCas9 (SpCas9d), or a SpCas9 nickase (SpCas9n). In some embodiments, the SpCas9 comprises a D9X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid except for D. In some embodiments, the SpCas9 comprises a D9A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having an NGG, a NGA, or a NGCG PAM sequence.

In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135E, R1335Q, and T1337R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135E, a R1335Q, and a T1337R mutation, or corresponding mutations in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135V, a R1335Q, and a T1337R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a R1335Q, and a T1337R mutation, or corresponding mutations in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a G1218X, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or

more of a D1135V, a G1218R, a R1335Q, and a T1337R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a G1218R, a R1335Q, and a T1337R mutation, or corresponding mutations in any of the amino acid sequences provided herein.

In some embodiments, the Cas9 domains of any of the fusion proteins provided herein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a Cas9 polypeptide described herein. In some embodiments, the Cas9 domains of any of the fusion proteins provided herein comprises the amino acid sequence of any Cas9 polypeptide described herein. In some embodiments, the Cas9 domains of any of the fusion proteins provided herein consists of the amino acid sequence of any Cas9 polypeptide described herein.

In some examples, a PAM recognized by a CRISPR protein-derived domain of a base editor disclosed herein can be provided to a cell on a separate oligonucleotide to an insert (*e.g.*, an AAV insert) encoding the base editor. In such embodiments, providing PAM on a separate oligonucleotide can allow cleavage of a target sequence that otherwise would not be able to be cleaved, because no adjacent PAM is present on the same polynucleotide as the target sequence.

In an embodiment, *S. pyogenes* Cas9 (SpCas9) can be used as a CRISPR endonuclease for genome engineering. However, others can be used. In some embodiments, a different endonuclease can be used to target certain genomic targets. In some embodiments, synthetic SpCas9-derived variants with non-NGG PAM sequences can be used. Additionally, other Cas9 orthologues from various species have been identified and these "non-SpCas9s" can bind a variety of PAM sequences that can also be useful for the present disclosure. For example, the relatively large size of SpCas9 (approximately 4kb coding sequence) can lead to plasmids carrying the SpCas9 cDNA that cannot be efficiently expressed in a cell. Conversely, the coding sequence for *Staphylococcus aureus* Cas9 (SaCas9) is approximately 1 kilobase shorter than SpCas9, possibly allowing it to be efficiently expressed in a cell. Similar to SpCas9, the SaCas9 endonuclease is capable of modifying target genes in mammalian cells *in vitro* and in mice *in vivo*. In some embodiments, a Cas protein can target a different PAM sequence. In some embodiments, a target gene can be adjacent to a Cas9 PAM, 5′-NGG, for example. In other embodiments, other Cas9 orthologs can have different PAM requirements. For example, other PAMs such

as those of *S. thermophilus* (5′-NNAGAA for CRISPR1 and 5′-NGGNG for CRISPR3) and *Neisseria meningiditis* (5′-NNNNGATT) can also be found adjacent to a target gene.

In some embodiments, for a *S. pyogenes* system, a target gene sequence can precede (*i.e.*, be 5′ to) a 5′-NGG PAM, and a 20-nt guide RNA sequence can base pair with an opposite strand to mediate a Cas9 cleavage adjacent to a PAM. In some embodiments, an adjacent cut can be or can be about 3 base pairs upstream of a PAM. In some embodiments, an adjacent cut can be or can be about 10 base pairs upstream of a PAM. In some embodiments, an adjacent cut can be or can be about 0-20 base pairs upstream of a PAM. For example, an adjacent cut can be next to, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs upstream of a PAM. An adjacent cut can also be downstream of a PAM by 1 to 30 base pairs. The sequences of exemplary SpCas9 proteins capable of binding a PAM sequence follow:

The amino acid sequence of an exemplary PAM-binding SpCas9 is as follows:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS

HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD.

The amino acid sequence of an exemplary PAM-binding SpCas9n is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD.

The amino acid sequence of an exemplary PAM-binding SpEQR Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQ
LVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLT
PNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY

KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKD
NREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN
FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKV
TVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL
TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFL
KSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD
ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGF**E**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQL
GGD. In this sequence, residues E1135, Q1335 and R1337, which can be mutated from D1135, R1335, and T1337 to yield a SpEQR Cas9, are underlined and in bold.

The amino acid sequence of an exemplary PAM-binding SpVQR Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD

NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ
LGGD. In this sequence, residues V1135, Q1335, and R1337, which can be mutated from D1135, R1335, and T1337 to yield a SpVQR Cas9, are underlined and in bold.

The amino acid sequence of an exemplary PAM-binding SpVRER Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGE
TAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF
GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDV
DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIA
LSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDI
LRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGA
SQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDF
YPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSF
IERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF
KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDI
LEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGK
TILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQ
TVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV
ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKN
RGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETR
QITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDA
YLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTE
ITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL
PKRNSDKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS
FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**E**Y**R**STKEVLDATLIHQSITGLYETR

IDLSQLGGD. In the above sequence, residues V1135, R1218, Q1335, and R1337, which can be mutated from D1134, G1217, R1335, and T1337 to yield a SpVRER Cas9, are underlined and in bold.

In some embodiments, the Cas9 domain is a recombinant Cas9 domain. In some embodiments, the recombinant Cas9 domain is a SpyMacCas9 domain. In some embodiments, the SpyMacCas9 domain is a nuclease active SpyMacCas9, a nuclease inactive SpyMacCas9 (SpyMacCas9d), or a SpyMacCas9 nickase (SpyMacCas9n). In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SpyMacCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having a NAA PAM sequence.

Exemplary SpyMacCas9

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQIYNQLFEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNS
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDRGMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGHSLHEQIANLAGSPAIKKGILQTVKIV
DELVKVMGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQ
NEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANG
EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEIQTVGQNGGLFDDNPKSP
LEVTPSKLVPLKKELNPKKYGGYQKPTTAYPVLLITDTKQLIPISVMNKKQFEQNPVKFLRD
RGYQQVGKNDFIKLPKYTLVDIGDGIKRLWASSKEIHKGNQLVVSKKSQILLYHAHHLDSDL

SNDYLQNHNQQFDVLFNEIISFSKKCKLGKEHIQKIENVYSNKKNSASIEELAESFIKLLGF
TQLGATSPFNFLGVKLNQKQYKGKKDYILPCTEGTLIRQSITGLYETRVDLSKIGED.

In some cases, a variant Cas9 protein harbors, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1218A mutations such that the polypeptide has a reduced ability to cleave a target DNA or RNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). As another non-limiting example, in some cases, the variant Cas9 protein harbors D10A, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1218A mutations such that the polypeptide has a reduced ability to cleave a target DNA. Such a Cas9 protein has a reduced ability to cleave a target DNA (*e.g.*, a single stranded target DNA) but retains the ability to bind a target DNA (*e.g.*, a single stranded target DNA). In some cases, when a variant Cas9 protein harbors W476A and W1126A mutations or when the variant Cas9 protein harbors P475A, W476A, N477A, D1125A, W1126A, and D1218A mutations, the variant Cas9 protein does not bind efficiently to a PAM sequence. Thus, in some such cases, when such a variant Cas9 protein is used in a method of binding, the method does not require a PAM sequence. In other words, in some cases, when such a variant Cas9 protein is used in a method of binding, the method can include a guide RNA, but the method can be performed in the absence of a PAM sequence (and the specificity of binding is therefore provided by the targeting segment of the guide RNA). Other residues can be mutated to achieve the above effects (*i.e.*, inactivate one or the other nuclease portions). As non-limiting examples, residues D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or A987 can be altered (*i.e.*, substituted). Also, mutations other than alanine substitutions are suitable.

In some embodiments, a CRISPR protein-derived domain of a base editor can comprise all or a portion of a Cas9 protein with a canonical PAM sequence (NGG). In other embodiments, a Cas9-derived domain of a base editor can employ a non-canonical PAM sequence. Such sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" Nature 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition" Nature Biotechnology 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference.

Cas9 Domains with Reduced Exclusivity

Typically, Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a canonical NGG PAM sequence to bind a particular nucleic acid region, where the "N" in "NGG" is adenosine (A), thymidine (T), or cytosine (C), and the G is guanosine. This may limit the ability to edit desired bases within a genome. In some embodiments, the base editing fusion proteins provided herein may need to be placed at a precise location, for example a region comprising a target base that is upstream of the PAM. See *e.g.*, Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" *Nature* 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference. Accordingly, in some embodiments, any of the fusion proteins provided herein may contain a Cas9 domain that is capable of binding a nucleotide sequence that does not contain a canonical (*e.g.,* NGG) PAM sequence. Cas9 domains that bind to non-canonical PAM sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" *Nature* 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition" *Nature Biotechnology* 33, 1293-1298 (2015); Nishimasu, H., *et al.*, "Engineered CRISPR-Cas9 nuclease with expanded targeting space" *Science*. 2018 Sep 21;361(6408):1259-1262, Chatterjee, P., *et al.*, Minimal PAM specificity of a highly similar SpCas9 ortholog" *Sci Adv.* 2018 Oct 24;4(10):eaau0766. doi: 10.1126/sciadv.aau0766, the entire contents of each are hereby incorporated by reference.

Fusion proteins comprising a Cas9 domain and a Cytidine Deaminase and/or Adenosine Deaminase

Some aspects of the disclosure provide fusion proteins comprising a Cas9 domain or other nucleic acid programmable DNA binding protein and one or more adenosine deaminase domain, cytidine deaminase domain, and/or DNA glycosylase domains. It should be appreciated that the Cas9 domain may be any of the Cas9 domains or Cas9 proteins (*e.g.*, dCas9 or nCas9) provided herein. In some embodiments, any of the Cas9 domains or Cas9 proteins (*e.g.*, dCas9 or nCas9) provided herein may be fused with any of the cytidine

deaminases and adenosine deaminases provided herein. The domains of the base editors disclosed herein can be arranged in any order.

For example, and without limitation, in some embodiments, the fusion protein comprises the structure:

NH_2-[cytidine deaminase]-[Cas9 domain]-[adenosine deaminase]-COOH;
NH_2-[adenosine deaminase]-[Cas9 domain]-[cytidine deaminase]-COOH;
NH_2-[adenosine deaminase]-[cytidine deaminase]-[Cas9 domain]-COOH;
NH_2-[cytidine deaminase]-[adenosine deaminase]-[Cas9 domain]-COOH;
NH_2-[Cas9 domain]-[adenosine deaminase]-[cytidine deaminase]-COOH; or
NH_2-[Cas9 domain]-[cytidine deaminase]-[adenosine deaminase]-COOH.

In some embodiments, the adenosine deaminase of the fusion protein comprises a TadA*8 and a cytidine deaminase. In some embodiments, the TadA*8 is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

Exemplary fusion protein structures include the following:

NH_2-[adenosine deaminase]-[Cas9]-[cytidine deaminase]-COOH;
NH_2-[cytidine deaminase]-[Cas9]-[adenosine deaminase]-COOH;
NH_2-[TadA*8]-[Cas9]-[cytidine deaminase]-COOH; or
NH_2-[cytidine deaminase]-[Cas9]-[TadA*8]-COOH.

In some embodiments, the fusion proteins comprising a cytidine deaminase, abasic editor, and adenosine deaminase and a napDNAbp (e.g., Cas9 domain) do not include a linker sequence. In some embodiments, a linker is present between the cytidine deaminase and adenosine deaminase domains and the napDNAbp. In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker. In some embodiments, the cytidine deaminase and adenosine deaminase and the napDNAbp are fused via any of the linkers provided herein. For example, in some embodiments the cytidine deaminase and adenosine deaminase and the napDNAbp are fused via any of the linkers provided below in the section entitled "Linkers".

In some embodiments, the general architecture of exemplary Cas9 or Cas12 fusion proteins with a cytidine deaminase, adenosine deaminase and a Cas9 or Cas12 domain comprises any one of the following structures, where NLS is a nuclear localization sequence

(*e.g.*, any NLS provided herein), NH_2 is the N-terminus of the fusion protein, and COOH is the C-terminus of the fusion protein.

NH_2-NLS-[cytidine deaminase]-[Cas9 domain]-[adenosine deaminase]-COOH;
NH_2-NLS-[adenosine deaminase]-[Cas9 domain]-[cytidine deaminase]-COOH;
NH_2-NLS-[adenosine deaminase] [cytidine deaminase]-[Cas9 domain]-COOH;
NH_2-NLS-[cytidine deaminase]-[adenosine deaminase]-[Cas9 domain]-COOH;
NH_2-NLS-[Cas9 domain]-[adenosine deaminase]-[cytidine deaminase]-COOH;
NH_2-NLS-[Cas9 domain]-[cytidine deaminase]-[adenosine deaminase]-COOH;
NH_2-[cytidine deaminase]-[Cas9 domain]-[adenosine deaminase]-NLS-COOH;
NH_2-[adenosine deaminase]-[Cas9 domain]-[cytidine deaminase]-NL2-COOH;
NH_2-[adenosine deaminase] [cytidine deaminase]-[Cas9 domain]-NLS-COOH;
NH_2-[cytidine deaminase]-[adenosine deaminase]-[Cas9 domain]-NLS-COOH;
NH_2-[Cas9 domain]-[adenosine deaminase]-[cytidine deaminase]-NLS-COOH; or
NH_2-[Cas9 domain]-[cytidine deaminase]-[adenosine deaminase]-NLS-COOH.

In some embodiments, the NLS is present in a linker or the NLS is flanked by linkers, for example described herein. In some embodiments, the N-terminus or C-terminus NLS is a bipartite NLS. A bipartite NLS comprises two basic amino acid clusters, which are separated by a relatively short spacer sequence (hence bipartite - 2 parts, while monopartite NLSs are not). The NLS of nucleoplasmin, KR[PAATKKAGQA]KKKK, is the prototype of the ubiquitous bipartite signal: two clusters of basic amino acids, separated by a spacer of about 10 amino acids. The sequence of an exemplary bipartite NLS follows: PKKKRKVEGADKRTADGSEFESPKKKRKV.

In some embodiments, the fusion proteins comprising a cytidine deaminase, adenosine deaminase, a Cas9 domain and an NLS do not comprise a linker sequence. In some embodiments, linker sequences between one or more of the domains or proteins (*e.g.*, cytidine deaminase, adenosine deaminase, Cas9 domain or NLS) are present.

It should be appreciated that the fusion proteins of the present disclosure may comprise one or more additional features. For example, in some embodiments, the fusion protein may comprise inhibitors, cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine tags,

also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (e.g., Softag 1, Softag 3), strep-tags , biotin ligase tags, FlAsH tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein comprises one or more His tags.

Exemplary, yet nonlimiting, fusion proteins are described in International PCT Application Nos. PCT/2017/044935 and PCT/US2020/016288, each of which is incorporated herein by reference for its entirety.

Fusion proteins comprising a nuclear localization sequence (NLS)

In some embodiments, the fusion proteins provided herein further comprise one or more (*e.g.*, 2, 3, 4, 5) nuclear targeting sequences, for example a nuclear localization sequence (NLS). In one embodiment, a bipartite NLS is used. In some embodiments, a NLS comprises an amino acid sequence that facilitates the importation of a protein, that comprises an NLS, into the cell nucleus (*e.g.*, by nuclear transport). In some embodiments, any of the fusion proteins provided herein further comprise a nuclear localization sequence (NLS). In some embodiments, the NLS is fused to the N-terminus of the fusion protein. In some embodiments, the NLS is fused to the C-terminus of the fusion protein. In some embodiments, the NLS is fused to the N-terminus of the Cas9 domain. In some embodiments, the NLS is fused to the C-terminus of an nCas9 domain or a dCas9 domain. In some embodiments, the NLS is fused to the N-terminus of the deaminase. In some embodiments, the NLS is fused to the C-terminus of the deaminase. In some embodiments, the NLS is fused to the fusion protein via one or more linkers. In some embodiments, the NLS is fused to the fusion protein without a linker. In some embodiments, the NLS comprises an amino acid sequence of any one of the NLS sequences provided or referenced herein. Additional nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank *et al.*, PCT/EP2000/011690, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In some embodiments, an NLS comprises the amino acid sequence PKKKRKVEGADKRTADGSEFESPKKKRKV, KRTADGSEFESPKKKRKV, KRPAATKKAGQAKKKK, KKTELQTTNAENKTKKL, KRGINDRNFWRGENGRKTR, RKSGKIAAIVVKRPRKPKKKRKV, or MDSLLMNRRKFLYQFKNVRWAKGRRETYLC.

In some embodiments, the NLS is present in a linker or the NLS is flanked by linkers, for example, the linkers described herein. In some embodiments, the N-terminus or C-terminus NLS is a bipartite NLS. A bipartite NLS comprises two basic amino acid clusters, which are separated by a relatively short spacer sequence (hence bipartite - 2 parts, while monopartite NLSs are not). The NLS of nucleoplasmin, KR[PAATKKAGQA]KKKK, is the prototype of the ubiquitous bipartite signal: two clusters of basic amino acids, separated by a spacer of about 10 amino acids. The sequence of an exemplary bipartite NLS follows: PKKKRKVEGADKRTADGSEFESPKKKRKV

In some embodiments, the fusion proteins do not comprise a linker sequence. In some embodiments, linker sequences between one or more of the domains or proteins are present. In some embodiments, the general architecture of exemplary Cas9 fusion proteins with an adenosine deaminase or a cytidine deaminase and a Cas9 domain comprises any one of the following structures, where NLS is a nuclear localization sequence (*e.g.*, any NLS provided herein), NH_2 is the N-terminus of the fusion protein, and COOH is the C-terminus of the fusion protein:

NH_2-NLS-[adenosine deaminase]-[Cas9 domain]-COOH;

NH_2-NLS [Cas9 domain]-[adenosine deaminase]-COOH;

NH_2-[adenosine deaminase]-[Cas9 domain]-NLS-COOH;

NH_2-[Cas9 domain]-[adenosine deaminase]-NLS-COOH;

NH_2-NLS-[cytidine deaminase]-[Cas9 domain]-COOH;

NH_2-NLS [Cas9 domain]-[cytidine deaminase]-COOH;

NH_2-[cytidine deaminase]-[Cas9 domain]-NLS-COOH; or

NH_2-[Cas9 domain]-[cytidine deaminase]-NLS-COOH.

It should be appreciated that the fusion proteins of the present disclosure may comprise one or more additional features. For example, in some embodiments, the fusion protein may comprise inhibitors, cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (*e.g.*, Softag 1, Softag 3), strep-tags , biotin ligase tags, FlAsH tags, V5 tags,

and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein comprises one or more His tags.

A vector that encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLSs) can be used. For example, there can be or be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 NLSs used. A CRISPR enzyme can comprise the NLSs at or near the ammo-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 NLSs at or near the carboxy-terminus, or any combination of these (*e.g.*, one or more NLS at the ammo-terminus and one or more NLS at the carboxy terminus). When more than one NLS is present, each can be selected independently of others, such that a single NLS can be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies.

CRISPR enzymes used in the methods can comprise about 6 NLSs. An NLS is considered near the N- or C-terminus when the nearest amino acid to the NLS is within about 50 amino acids along a polypeptide chain from the N- or C-terminus, *e.g.*, within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, or 50 amino acids.

Fusion proteins with Internal Insertions

Provided herein are fusion proteins comprising a heterologous polypeptide fused to a nucleic acid programmable nucleic acid binding protein, for example, a napDNAbp. A heterologous polypeptide can be a polypeptide that is not found in the native or wild-type napDNAbp polypeptide sequence. The heterologous polypeptide can be fused to the napDNAbp at a C-terminal end of the napDNAbp, an N-terminal end of the napDNAbp, or inserted at an internal location of the napDNAbp. In some embodiments, the heterologous polypeptide is inserted at an internal location of the napDNAbp.

In some embodiments, the heterologous polypeptide is a deaminase or a functional fragment thereof. For example, a fusion protein can comprise a deaminase flanked by an N-terminal fragment and a C-terminal fragment of a Cas9 or Cas12 (*e.g.*, Cas12b/C2c1), polypeptide. The deaminase in a fusion protein can be an adenosine deaminase. In some embodiments, the adenosine deaminase is a TadA (*e.g.*, TadA7.10 or TadA*8). In some embodiments, the TadA is a TadA*8. TadA sequences (*e.g.*, TadA7.10 or TadA*8) as described herein are suitable deaminases for the above-described fusion proteins.

The deaminase can be a circular permutant deaminase. For example, the deaminase can be a circular permutant adenosine deaminase. In some embodiments, the deaminase is a circular permutant TadA, circularly permutated at amino acid residue 116 as numbered in the

TadA reference sequence. In some embodiments, the deaminase is a circular permutant TadA, circularly permutated at amino acid residue 136 as numbered in the TadA reference sequence. In some embodiments, the deaminase is a circular permutant TadA, circularly permutated at amino acid residue 65 as numbered in the TadA reference sequence.

The fusion protein can comprise more than one deaminase. The fusion protein can comprise, for example, 1, 2, 3, 4, 5 or more deaminases. In some embodiments, the fusion protein comprises one deaminase. In some embodiments, the fusion protein comprises two deaminases. The two or more deaminases in a fusion protein can be an adenosine deaminase. cytidine deaminase, or a combination thereof. The two or more deaminases can be homodimers. The two or more deaminases can be heterodimers. The two or more deaminases can be inserted in tandem in the napDNAbp. In some embodiments, the two or more deaminases may not be in tandem in the napDNAbp.

In some embodiments, the napDNAbp in the fusion protein is a Cas9 polypeptide or a fragment thereof. The Cas9 polypeptide can be a variant Cas9 polypeptide. In some embodiments, the Cas9 polypeptide is a Cas9 nickase (nCas9) polypeptide or a fragment thereof. In some embodiments, the Cas9 polypeptide is a nuclease dead Cas9 (dCas9) polypeptide or a fragment thereof. The Cas9 polypeptide in a fusion protein can be a full-length Cas9 polypeptide. In some cases, the Cas9 polypeptide in a fusion protein may not be a full length Cas9 polypeptide. The Cas9 polypeptide can be truncated, for example, at a N-terminal or C-terminal end relative to a naturally-occurring Cas9 protein. The Cas9 polypeptide can be a circularly permuted Cas9 protein. The Cas9 polypeptide can be a fragment, a portion, or a domain of a Cas9 polypeptide, that is still capable of binding the target polynucleotide and a guide nucleic acid sequence.

In some embodiments, the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), or fragments or variants thereof.

The Cas9 polypeptide of a fusion protein can comprise an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring Cas9 polypeptide.

The Cas9 polypeptide of a fusion protein can comprise an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%,

at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the Cas9 amino acid sequence set forth below (called the "Cas9 reference sequence" below):

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD (single underline: HNH domain; double underline: RuvC domain).

Fusion proteins comprising a heterologous catalytic domain flanked by N- and C-terminal fragments of a Cas9 polypeptide are also useful for base editing in the methods as described herein. Fusion proteins comprising Cas9 and one or more deaminase domains, *e.g.*, adenosine deaminase, or comprising an adenosine deaminase domain flanked by Cas9 sequences are also useful for highly specific and efficient base editing of target sequences. In an embodiment, a chimeric Cas9 fusion protein contains a heterologous catalytic domain (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) inserted within a Cas9 polypeptide. In some embodiments, the fusion protein

comprises an adenosine deaminase domain and a cytidine deaminase domain inserted within a Cas9. In some embodiments, an adenosine deaminase is fused within a Cas9 and a cytidine deaminase is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas9 and a cytidine deaminase fused to the N-terminus. In some embodiments, a cytidine deaminase is fused within Cas9 and an adenosine deaminase is fused to the C-terminus. In some embodiments, a cytidine deaminase is fused within Cas9 and an adenosine deaminase fused to the N-terminus.

Exemplary structures of a fusion protein with an adenosine deaminase and a cytidine deaminase and a Cas9 are provided as follows:

NH_2-[Cas9(adenosine deaminase)]-[cytidine deaminase]-COOH;

NH_2-[cytidine deaminase]-[Cas9(adenosine deaminase)]-COOH;

NH_2-[Cas9(cytidine deaminase)]-[adenosine deaminase]-COOH; or

NH_2-[adenosine deaminase]-[Cas9(cytidine deaminase)]-COOH.

In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

In various embodiments, the catalytic domain has DNA modifying activity (*e.g.*, deaminase activity), such as adenosine deaminase activity. In some embodiments, the adenosine deaminase is a TadA (*e.g.*, TadA7.10). In some embodiments, the TadA is a TadA*8. In some embodiments, a TadA*8 is fused within Cas9 and a cytidine deaminase is fused to the C-terminus. In some embodiments, a TadA*8 is fused within Cas9 and a cytidine deaminase fused to the N-terminus. In some embodiments, a cytidine deaminase is fused within Cas9 and a TadA*8 is fused to the C-terminus. In some embodiments, a cytidine deaminase is fused within Cas9 and a TadA*8 fused to the N-terminus. Exemplary structures of a fusion protein with a TadA*8 and a cytidine deaminase and a Cas9 are provided as follows:

NH_2-[Cas9(TadA*8)]-[cytidine deaminase]-COOH;

NH_2-[cytidine deaminase]-[Cas9(TadA*8)]-COOH;

NH_2-[Cas9(cytidine deaminase)]-[TadA*8]-COOH; or

NH_2-[TadA*8]-[Cas9(cytidine deaminase)]-COOH.

In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

The heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp (*e.g.*, Cas9 or Cas12 (*e.g.*, Cas12b/C2c1)) at a suitable location, for example, such that the

napDNAbp retains its ability to bind the target polynucleotide and a guide nucleic acid. A deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) can be inserted into a napDNAbp without compromising function of the deaminase (*e.g.*, base editing activity) or the napDNAbp (*e.g.*, ability to bind to target nucleic acid and guide nucleic acid). A deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) can be inserted in the napDNAbp at, for example, a disordered region or a region comprising a high temperature factor or B-factor as shown by crystallographic studies. Regions of a protein that are less ordered, disordered, or unstructured, for example solvent exposed regions and loops, can be used for insertion without compromising structure or function. A deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase)can be inserted in the napDNAbp in a flexible loop region or a solvent-exposed region. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted in a flexible loop of the Cas9 or the Cas12b/C2c1 polypeptide.

In some embodiments, the insertion location of a deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is determined by B-factor analysis of the crystal structure of Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted in regions of the Cas9 polypeptide comprising higher than average B-factors (*e.g.*, higher B factors compared to the total protein or the protein domain comprising the disordered region). B-factor or temperature factor can indicate the fluctuation of atoms from their average position (for example, as a result of temperature-dependent atomic vibrations or static disorder in a crystal lattice). A high B-factor (*e.g.*, higher than average B-factor) for backbone atoms can be indicative of a region with relatively high local mobility. Such a region can be used for inserting a deaminase without compromising structure or function. A deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) can be inserted at a location with a residue having a Cα atom with a B-factor that is 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, or greater than 200% more than the average B-factor for the total protein. A deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) can be inserted at a location with a residue having a Cα atom with a B-factor that is 50%, 60%, 70%,

80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% or greater than 200% more than the average B-factor for a Cas9 protein domain comprising the residue. Cas9 polypeptide positions comprising a higher than average B-factor can include, for example, residues 768, 792, 1052, 1015, 1022, 1026, 1029, 1067, 1040, 1054, 1068, 1246, 1247, and 1248 as numbered in the above Cas9 reference sequence. Cas9 polypeptide regions comprising a higher than average B-factor can include, for example, residues 792-872, 792-906, and 2-791 as numbered in the above Cas9 reference sequence.

A heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue selected from the group consisting of: 768, 791, 792, 1015, 1016, 1022, 1023, 1026, 1029, 1040, 1052, 1054, 1067, 1068, 1069, 1246, 1247, and 1248 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the heterologous polypeptide is inserted between amino acid positions 768-769, 791-792, 792-793, 1015-1016, 1022-1023, 1026-1027, 1029-1030, 1040-1041, 1052-1053, 1054-1055, 1067-1068, 1068-1069, 1247-1248, or 1248-1249 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the heterologous polypeptide is inserted between amino acid positions 769-770, 792-793, 793-794, 1016-1017, 1023-1024, 1027-1028, 1030-1031, 1041-1042, 1053-1054, 1055-1056, 1068-1069, 1069-1070, 1248-1249, or 1249-1250 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the heterologous polypeptide replaces an amino acid residue selected from the group consisting of: 768, 791, 792, 1015, 1016, 1022, 1023, 1026, 1029, 1040, 1052, 1054, 1067, 1068, 1069, 1246, 1247, and 1248 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. It should be understood that the reference to the above Cas9 reference sequence with respect to insertion positions is for illustrative purposes. The insertions as discussed herein are not limited to the Cas9 polypeptide sequence of the above Cas9 reference sequence, but include insertion at corresponding locations in variant Cas9 polypeptides, for example a Cas9 nickase (nCas9), nuclease dead Cas9 (dCas9), a Cas9 variant lacking a nuclease domain, a truncated Cas9, or a Cas9 domain lacking partial or complete HNH domain.

A heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue selected from the group consisting of: 768, 792, 1022, 1026, 1040, 1068, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the heterologous polypeptide is

inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1029-1030, 1040-1041, 1068-1069, or 1247-1248 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the heterologous polypeptide is inserted between amino acid positions 769-770, 793-794, 1023-1024, 1027-1028, 1030-1031, 1041-1042, 1069-1070, or 1248-1249 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the heterologous polypeptide replaces an amino acid residue selected from the group consisting of: 768, 792, 1022, 1026, 1040, 1068, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue as described herein, or a corresponding amino acid residue in another Cas9 polypeptide. In an embodiment, a heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue selected from the group consisting of: 1002, 1003, 1025, 1052-1056, 1242-1247, 1061-1077, 943-947, 686-691, 569-578, 530-539, and 1060-1077 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) can be inserted at the N-terminus or the C-terminus of the residue or replace the residue. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of the residue.

In some embodiments, an adenosine deaminase (*e.g.*, TadA) is inserted at an amino acid residue selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, an adenosine deaminase (*e.g.*, TadA) is inserted in place of residues 792-872, 792-906, or 2-791 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the adenosine deaminase is inserted at the N-terminus of an amino acid selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the adenosine deaminase is inserted at the C-terminus of an amino acid selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9

reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the adenosine deaminase is inserted to replace an amino acid selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, a CBE (*e.g.*, APOBEC1) is inserted at an amino acid residue selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of an amino acid selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of an amino acid selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace an amino acid selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 768 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 768 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 768 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 768 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 791 or is inserted at amino acid residue 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 791 or is inserted at the N-terminus of amino acid 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid 791 or is inserted at the N-terminus of amino acid 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid 791, or is inserted to replace amino acid 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1022, or is inserted at amino acid residue 1023, as numbered in the above Cas9 reference sequence, or a

corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1022 or is inserted at the N-terminus of amino acid residue 1023, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1022 or is inserted at the C-terminus of amino acid residue 1023, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1022, or is inserted to replace amino acid residue 1023, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1026, or is inserted at amino acid residue 1029, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1026 or is inserted at the N-terminus of amino acid residue 1029, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1026 or is inserted at the C-terminus of amino acid residue 1029, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1026, or is inserted to replace amino acid residue 1029, as numbered in the above Cas9 reference sequence, or corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase,

cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1052, or is inserted at amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1052 or is inserted at the N-terminus of amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1052 or is inserted at the C-terminus of amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1052, or is inserted to replace amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1067, or is inserted at amino acid residue 1068, or is inserted at amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1067 or is inserted at the N-terminus of amino acid residue 1068 or is

inserted at the N-terminus of amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1067 or is inserted at the C-terminus of amino acid residue 1068 or is inserted at the C-terminus of amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1067, or is inserted to replace amino acid residue 1068, or is inserted to replace amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at amino acid residue 1246, or is inserted at amino acid residue 1247, or is inserted at amino acid residue 1248, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the N-terminus of amino acid residue 1246 or is inserted at the N-terminus of amino acid residue 1247 or is inserted at the N-terminus of amino acid residue 1248, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted at the C-terminus of amino acid residue 1246 or is inserted at the C-terminus of amino acid residue 1247 or is inserted at the C-terminus of amino acid residue 1248, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) is inserted to replace amino acid residue 1246, or is inserted to replace amino acid residue 1247, or is inserted to replace amino acid residue 1248, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, a heterologous polypeptide (*e.g.*, deaminase) is inserted in a flexible loop of a Cas9 polypeptide. The flexible loop portions can be selected from the

group consisting of 530-537, 569-570, 686-691, 943-947, 1002-1025, 1052-1077, 1232-1247, or 1298-1300 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The flexible loop portions can be selected from the group consisting of: 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, or 1248-1297 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (*e.g.*, adenine deaminase) can be inserted into a Cas9 polypeptide region corresponding to amino acid residues: 1017-1069, 1242-1247, 1052-1056, 1060-1077, 1002 – 1003, 943-947, 530-537, 568-579, 686-691, 1242-1247, 1298 – 1300, 1066-1077, 1052-1056, or 1060-1077 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (*e.g.*, adenine deaminase) can be inserted in place of a deleted region of a Cas9 polypeptide. The deleted region can correspond to an N-terminal or C-terminal portion of the Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 792-872 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 792-906 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 2-791 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 1017-1069 as numbered in the above Cas9 reference sequence, or corresponding amino acid residues thereof.

Exemplary internal fusions base editors are provided in **Table 5A** below:

Table 5A: Insertion loci in Cas9 proteins

BE ID	Modification	Other ID
IBE001	Cas9 TadA ins 1015	ISLAY01
IBE002	Cas9 TadA ins 1022	ISLAY02
IBE003	Cas9 TadA ins 1029	ISLAY03
IBE004	Cas9 TadA ins 1040	ISLAY04
IBE005	Cas9 TadA ins 1068	ISLAY05
IBE006	Cas9 TadA ins 1247	ISLAY06
IBE007	Cas9 TadA ins 1054	ISLAY07
IBE008	Cas9 TadA ins 1026	ISLAY08
IBE009	Cas9 TadA ins 768	ISLAY09
IBE020	delta HNH TadA 792	ISLAY20
IBE021	N-term fusion single TadA helix truncated 165-end	ISLAY21
IBE029	TadA-Circular Permutant116 ins1067	ISLAY29

BE ID	Modification	Other ID
IBE031	TadA- Circular Permutant 136 ins1248	ISLAY31
IBE032	TadA- Circular Permutant 136ins 1052	ISLAY32
IBE035	delta 792-872 TadA ins	ISLAY35
IBE036	delta 792-906 TadA ins	ISLAY36
IBE043	TadA-Circular Permutant 65 ins1246	ISLAY43
IBE044	TadA ins C-term truncate2 791	ISLAY44

A heterologous polypeptide (*e.g.*, deaminase) can be inserted within a structural or functional domain of a Cas9 polypeptide. A heterologous polypeptide (*e.g.*, deaminase) can be inserted between two structural or functional domains of a Cas9 polypeptide. A heterologous polypeptide (*e.g.*, deaminase) can be inserted in place of a structural or functional domain of a Cas9 polypeptide, for example, after deleting the domain from the Cas9 polypeptide. The structural or functional domains of a Cas9 polypeptide can include, for example, RuvC I, RuvC II, RuvC III, Rec1, Rec2, PI, or HNH.

In some embodiments, the Cas9 polypeptide lacks one or more domains selected from the group consisting of: RuvC I, RuvC II, RuvC III, Rec1, Rec2, PI, or HNH domain. In some embodiments, the Cas9 polypeptide lacks a nuclease domain. In some embodiments, the Cas9 polypeptide lacks an HNH domain. In some embodiments, the Cas9 polypeptide lacks a portion of the HNH domain such that the Cas9 polypeptide has reduced or abolished HNH activity. In some embodiments, the Cas9 polypeptide comprises a deletion of the nuclease domain, and the deaminase is inserted to replace the nuclease domain. In some embodiments, the HNH domain is deleted and the deaminase is inserted in its place. In some embodiments, one or more of the RuvC domains is deleted and the deaminase is inserted in its place.

A fusion protein comprising a heterologous polypeptide can be flanked by a N-terminal and a C-terminal fragment of a napDNAbp. In some embodiments, the fusion protein comprises a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide. The N terminal fragment or the C terminal fragment can bind the target polynucleotide sequence. The C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment can comprise a part of a flexible loop of a Cas9 polypeptide. The C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment can comprise a part of an alpha-helix structure of the Cas9 polypeptide. The N-terminal fragment or the C-terminal fragment can comprise a DNA binding domain. The N-terminal fragment or the C-terminal fragment can comprise a RuvC domain. The N-terminal

fragment or the C-terminal fragment can comprise an HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises an HNH domain.

In some embodiments, the C-terminus of the N terminal Cas9 fragment comprises an amino acid that is in proximity to a target nucleobase when the fusion protein deaminates the target nucleobase. In some embodiments, the N-terminus of the C terminal Cas9 fragment comprises an amino acid that is in proximity to a target nucleobase when the fusion protein deaminates the target nucleobase. The insertion location of different deaminases can be different in order to have proximity between the target nucleobase and an amino acid in the C-terminus of the N terminal Cas9 fragment or the N-terminus of the C terminal Cas9 fragment. For example, the insertion position of an ABE can be at an amino acid residue selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

The N-terminal Cas9 fragment of a fusion protein (*i.e.* the N-terminal Cas9 fragment flanking the deaminase in a fusion protein) can comprise the N-terminus of a Cas9 polypeptide. The N-terminal Cas9 fragment of a fusion protein can comprise a length of at least about: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 amino acids. The N-terminal Cas9 fragment of a fusion protein can comprise a sequence corresponding to amino acid residues: 1-56, 1-95, 1-200, 1-300, 1-400, 1-500, 1-600, 1-700, 1-718, 1-765, 1-780, 1-906, 1-918, or 1-1100 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The N-terminal Cas9 fragment can comprise a sequence comprising at least: 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to amino acid residues: 1-56, 1-95, 1-200, 1-300, 1-400, 1-500, 1-600, 1-700, 1-718, 1-765, 1-780, 1-906, 1-918, or 1-1100 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

The C-terminal Cas9 fragment of a fusion protein (*i.e.* the C-terminal Cas9 fragment flanking the deaminase in a fusion protein) can comprise the C-terminus of a Cas9 polypeptide. The C-terminal Cas9 fragment of a fusion protein can comprise a length of at least about: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 amino acids. The C-terminal Cas9 fragment of a fusion protein can comprise a sequence corresponding to amino acid residues: 1099-1368, 918-1368, 906-1368, 780-1368, 765-1368,

718-1368, 94-1368, or 56-1368 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The N-terminal Cas9 fragment can comprise a sequence comprising at least: 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to amino acid residues: 1099-1368, 918-1368, 906-1368, 780-1368, 765-1368, 718-1368, 94-1368, or 56-1368 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

The N-terminal Cas9 fragment and C-terminal Cas9 fragment of a fusion protein taken together may not correspond to a full-length naturally occurring Cas9 polypeptide sequence, for example, as set forth in the above Cas9 reference sequence.

The fusion protein described herein can effect targeted deamination with reduced deamination at non-target sites (*e.g.*, off-target sites), such as reduced genome wide spurious deamination. The fusion protein described herein can effect targeted deamination with reduced bystander deamination at non-target sites. The undesired deamination or off-target deamination can be reduced by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% compared with, for example, an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of a Cas9 polypeptide. The undesired deamination or off-target deamination can be reduced by at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least tenfold, at least fifteen fold, at least twenty fold, at least thirty fold, at least forty fold, at least fifty fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, or at least hundred fold, compared with, for example, an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of a Cas9 polypeptide.

In some embodiments, the deaminase (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) of the fusion protein deaminates no more than two nucleobases within the range of an R-loop. In some embodiments, the deaminase of the fusion protein deaminates no more than three nucleobases within the range of the R-loop. In some embodiments, the deaminase of the fusion protein deaminates no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleobases within the range of the R-loop. An R-loop is a three-stranded nucleic acid structure including a DNA:RNA hybrid, a DNA:DNA or an RNA: RNA complementary structure and the associated with single-stranded DNA. As used herein, an R-loop may be formed when a target polynucleotide is contacted with a CRISPR complex or a base editing complex, wherein a portion of a guide polynucleotide, *e.g.* a guide RNA,

hybridizes with and displaces with a portion of a target polynucleotide, *e.g.* a target DNA. In some embodiments, an R-loop comprises a hybridized region of a spacer sequence and a target DNA complementary sequence. An R-loop region may be of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleobase pairs in length. In some embodiments, the R-loop region is about 20 nucleobase pairs in length. It should be understood that, as used herein, an R-loop region is not limited to the target DNA strand that hybridizes with the guide polynucleotide. For example, editing of a target nucleobase within an R-loop region may be to a DNA strand that comprises the complementary strand to a guide RNA, or may be to a DNA strand that is the opposing strand of the strand complementary to the guide RNA. In some embodiments, editing in the region of the R-loop comprises editing a nucleobase on non-complementary strand (protospacer strand) to a guide RNA in a target DNA sequence.

The fusion protein described herein can effect target deamination in an editing window different from canonical base editing. In some embodiments, a target nucleobase is from about 1 to about 20 bases upstream of a PAM sequence in the target polynucleotide sequence. In some embodiments, a target nucleobase is from about 2 to about 12 bases upstream of a PAM sequence in the target polynucleotide sequence. In some embodiments, a target nucleobase is from about 1 to 9 base pairs, about 2 to 10 base pairs, about 3 to 11 base pairs, about 4 to 12 base pairs, about 5 to 13 base pairs, about 6 to 14 base pairs, about 7 to 15 base pairs, about 8 to 16 base pairs, about 9 to 17 base pairs, about 10 to 18 base pairs, about 11 to 19 base pairs, about 12 to 20 base pairs, about 1 to 7 base pairs, about 2 to 8 base pairs, about 3 to 9 base pairs, about 4 to 10 base pairs, about 5 to 11 base pairs, about 6 to 12 base pairs, about 7 to 13 base pairs, about 8 to 14 base pairs, about 9 to 15 base pairs, about 10 to 16 base pairs, about 11 to 17 base pairs, about 12 to 18 base pairs, about 13 to 19 base pairs, about 14 to 20 base pairs, about 1 to 5 base pairs, about 2 to 6 base pairs, about 3 to 7 base pairs, about 4 to 8 base pairs, about 5 to 9 base pairs, about 6 to 10 base pairs, about 7 to 11 base pairs, about 8 to 12 base pairs, about 9 to 13 base pairs, about 10 to 14 base pairs, about 11 to 15 base pairs, about 12 to 16 base pairs, about 13 to 17 base pairs, about 14 to 18 base pairs, about 15 to 19 base pairs, about 16 to 20 base pairs, about 1 to 3 base pairs, about 2 to 4 base pairs, about 3 to 5 base pairs, about 4 to 6 base pairs, about 5 to 7 base pairs, about 6 to 8 base pairs, about 7 to 9 base pairs, about 8 to 10 base pairs, about 9 to 11 base pairs, about 10 to 12 base pairs, about 11 to 13 base pairs, about 12 to 14 base pairs, about 13 to 15 base

pairs, about 14 to 16 base pairs, about 15 to 17 base pairs, about 16 to 18 base pairs, about 17 to 19 base pairs, about 18 to 20 base pairs away or upstream of the PAM sequence. In some embodiments, a target nucleobase is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more base pairs away from or upstream of the PAM sequence. In some embodiments, a target nucleobase is about 1, 2, 3, 4, 5, 6, 7, 8, or 9 base pairs upstream of the PAM sequence. In some embodiments, a target nucleobase is about 2, 3, 4, or 6 base pairs upstream of the PAM sequence.

The fusion protein can comprise more than one heterologous polypeptide. For example, the fusion protein can additionally comprise one or more UGI domains and/or one or more nuclear localization signals. The two or more heterologous domains can be inserted in tandem. The two or more heterologous domains can be inserted at locations such that they are not in tandem in the NapDNAbp.

A fusion protein can comprise a linker between the deaminase and the napDNAbp polypeptide. The linker can be a peptide or a non-peptide linker. For example, the linker can be an XTEN, (GGGS)n, (GGGGS)n, (G)n, (EAAAK)n, (GGS)n, SGSETPGTSESATPES. In some embodiments, the fusion protein comprises a linker between the N-terminal Cas9 fragment and the deaminase. In some embodiments, the fusion protein comprises a linker between the C-terminal Cas9 fragment and the deaminase. In some embodiments, the N-terminal and C-terminal fragments of napDNAbp are connected to the deaminase with a linker. In some embodiments, the N-terminal and C-terminal fragments are joined to the deaminase domain without a linker. In some embodiments, the fusion protein comprises a linker between the N-terminal Cas9 fragment and the deaminase, but does not comprise a linker between the C-terminal Cas9 fragment and the deaminase. In some embodiments, the fusion protein comprises a linker between the C-terminal Cas9 fragment and the deaminase, but does not comprise a linker between the N-terminal Cas9 fragment and the deaminase.

In some embodiments, the napDNAbp in the fusion protein is a Cas12 polypeptide, *e.g.*, Cas12b/C2c1, or a fragment thereof. The Cas12 polypeptide can be a variant Cas12 polypeptide. In other embodiments, the N- or C-terminal fragments of the Cas12 polypeptide comprise a nucleic acid programmable DNA binding domain or a RuvC domain. In other embodiments, the fusion protein contains a linker between the Cas12 polypeptide and the catalytic domain. In other embodiments, the amino acid sequence of the linker is GGSGGS or GSSGSETPGTSESATPESSG. In other embodiments, the linker is a rigid linker. In other

embodiments of the above aspects, the linker is encoded by GGAGGCTCTGGAGGAAGC or GGCTCTTCTGGATCTGAAACACCTGGCACAAGCGAGAGCGCCACCCCTGAGAGCTCTGGC.

Fusion proteins comprising a heterologous catalytic domain flanked by N- and C-terminal fragments of a Cas12 polypeptide are also useful for base editing in the methods as described herein. Fusion proteins comprising Cas12 and one or more deaminase domains, *e.g.*, adenosine deaminase, or comprising an adenosine deaminase domain flanked by Cas12 sequences are also useful for highly specific and efficient base editing of target sequences. In an embodiment, a chimeric Cas12 fusion protein contains a heterologous catalytic domain (*e.g.*, adenosine deaminase, cytidine deaminase, or adenosine deaminase and cytidine deaminase) inserted within a Cas12 polypeptide. In some embodiments, the fusion protein comprises an adenosine deaminase domain and a cytidine deaminase domain inserted within a Cas12. In some embodiments, an adenosine deaminase is fused within Cas12 and a cytidine deaminase is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas12 and a cytidine deaminase fused to the N-terminus. In some embodiments, a cytidine deaminase is fused within Cas12 and an adenosine deaminase is fused to the C-terminus. In some embodiments, a cytidine deaminase is fused within Cas12 and an adenosine deaminase fused to the N-terminus. Exemplary structures of a fusion protein with an adenosine deaminase and a cytidine deaminase and a Cas12 are provided as follows:

NH_2-[Cas12(adenosine deaminase)]-[cytidine deaminase]-COOH;
NH_2-[cytidine deaminase]-[Cas12(adenosine deaminase)]-COOH;
NH_2-[Cas12(cytidine deaminase)]-[adenosine deaminase]-COOH; or
NH_2-[adenosine deaminase]-[Cas12(cytidine deaminase)]-COOH;

In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

In various embodiments, the catalytic domain has DNA modifying activity (*e.g.*, deaminase activity), such as adenosine deaminase activity. In some embodiments, the adenosine deaminase is a TadA (*e.g.*, TadA7.10). In some embodiments, the TadA is a TadA*8. In some embodiments, a TadA*8 is fused within Cas12 and a cytidine deaminase is fused to the C-terminus. In some embodiments, a TadA*8 is fused within Cas12 and a cytidine deaminase fused to the N-terminus. In some embodiments, a cytidine deaminase is fused within Cas12 and a TadA*8 is fused to the C-terminus. In some embodiments, a cytidine deaminase is fused within Cas12 and a TadA*8 fused to the N-terminus. Exemplary

structures of a fusion protein with a TadA*8 and a cytidine deaminase and a Cas12 are provided as follows:

N-[Cas12(TadA*8)]-[cytidine deaminase]-C;

N-[cytidine deaminase]-[Cas12(TadA*8)]-C;

N-[Cas12(cytidine deaminase)]-[TadA*8]-C; or

N-[TadA*8]-[Cas12(cytidine deaminase)]-C.

In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

In other embodiments, the fusion protein contains one or more catalytic domains. In other embodiments, at least one of the one or more catalytic domains is inserted within the Cas12 polypeptide or is fused at the Cas12 N- terminus or C-terminus. In other embodiments, at least one of the one or more catalytic domains is inserted within a loop, an alpha helix region, an unstructured portion, or a solvent accessible portion of the Cas12 polypeptide. In other embodiments, the Cas12 polypeptide is Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the Cas12 polypeptide has at least about 85% amino acid sequence identity to *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b. In other embodiments, the Cas12 polypeptide has at least about 90% amino acid sequence identity to *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b. In other embodiments, the Cas12 polypeptide has at least about 95% amino acid sequence identity to *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b. In other embodiments, the Cas12 polypeptide contains or consists essentially of a fragment of *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b.

In other embodiments, the catalytic domain is inserted between amino acid positions 153-154, 255-256, 306-307, 980-981, 1019-1020, 534-535, 604-605, or 344-345 of BhCas12b or a corresponding amino acid residue of Cas12a, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the catalytic domain is inserted between amino acids P153 and S154 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids K255 and E256 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids D980 and G981 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids K1019 and L1020 of

BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids F534 and P535 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids K604 and G605 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids H344 and F345 of BhCas12b. In other embodiments, catalytic domain is inserted between amino acid positions 147 and 148, 248 and 249, 299 and 300, 991 and 992, or 1031 and 1032 of BvCas12b or a corresponding amino acid residue of Cas12a, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the catalytic domain is inserted between amino acids P147 and D148 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids G248 and G249 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids P299 and E300 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids G991 and E992 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids K1031 and M1032 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acid positions 157 and 158, 258 and 259, 310 and 311, 1008 and 1009, or 1044 and 1045 of AaCas12b or a corresponding amino acid residue of Cas12a, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the catalytic domain is inserted between amino acids P157 and G158 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids V258 and G259 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids D310 and P311 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids G1008 and E1009 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids G1044 and K1045 at of AaCas12b.

In other embodiments, the fusion protein contains a nuclear localization signal (*e.g.*, a bipartite nuclear localization signal). In other embodiments, the amino acid sequence of the nuclear localization signal is MAPKKKRKVGIHGVPAA. In other embodiments of the above aspects, the nuclear localization signal is encoded by the following sequence: ATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCC. In other embodiments, the Cas12b polypeptide contains a mutation that silences the catalytic activity of a RuvC domain. In other embodiments, the Cas12b polypeptide contains D574A, D829A and/or D952A mutations. In other embodiments, the fusion protein further contains a tag (*e.g.*, an influenza hemagglutinin tag).

In some embodiments, the fusion protein comprises a napDNAbp domain (*e.g.*, Cas12-derived domain) with an internally fused nucleobase editing domain (*e.g.*, all or a

portion of a deaminase domain, *e.g.*, an adenosine deaminase domain). In some embodiments, the napDNAbp is a Cas12b. In some embodiments, the base editor comprises a BhCas12b domain with an internally fused TadA*8 domain inserted at the loci provided in **Table 6** below.

Table 6: Insertion loci in Cas12b proteins

BhCas12b	**Insertion site**	**Inserted between aa**
position 1	153	PS
position 2	255	KE
position 3	306	DE
position 4	980	DG
position 5	1019	KL
position 6	534	FP
position 7	604	KG
position 8	344	HF
BvCas12b	**Insertion site**	**Inserted between aa**
position 1	147	PD
position 2	248	GG
position 3	299	PE
position 4	991	GE
position 5	1031	KM
AaCas12b	**Insertion site**	**Inserted between aa**
position 1	157	PG
position 2	258	VG
position 3	310	DP
position 4	1008	GE
position 5	1044	GK

By way of nonlimiting example, an adenosine deaminase (*e.g.*, ABE8.13) may be inserted into a BhCas12b to produce a fusion protein (*e.g.*, ABE8.13-BhCas12b) that effectively edits a nucleic acid sequence.

In some embodiments, the base editing system described herein comprises an ABE with TadA inserted into a Cas9. Sequences of relevant ABEs with TadA inserted into a Cas9 are provided.

101 Cas9 TadAins 1015

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVGSSGSETPGTSESATPESSGSEVEFSHEYWMRHAL
TLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQG
GLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGS
LMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSST
DYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

102 Cas9 TadAins 1022

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIGSSGSETPGTSESATPESSGSEVEFSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

103 Cas9 TadAins 1029

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGSSGSETPGTSESATPESSGS
EVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLH
DPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRV
VFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMP
RQVFNAQKKAQSSTDGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

103 Cas9 TadAins 1040

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADEC
AALLCYFFRMPRQVFNAQKKAQSSTDNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

105 Cas9 TadAins 1068

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGEGSSGSETPGTSESATPESSGSEVEFSHEYWMR
HALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMAL
RQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQ
SSTDTGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

106 Cas9 TadAins 1247

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGGSS
GSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVL
VLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTF
EPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

107 Cas9 TadAins 1054

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

108 Cas9 TadAins 1026

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEGSSGSETPGTSESATPESSGSEVE
FSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG
VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQV
FNAQKKAQSSTDQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

109 Cas9 TadAins 768

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQGSSGSETPGTSESATPESSGSEVEFSHEYWMR
HALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMAL
RQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRTTQKGQKNSR
ERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK
MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQIT
KHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREI
NNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQE
IGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGR
DFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDP
KKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSK
RVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.1 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR

LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYV
TFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEI
TEGILADECAALLCYFFRMPREDNEQKQLFVEQHKHYLDEIIEQISEFSK
RVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.2 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD

LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVP
VGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDAT
LYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHR
VEITEGILADECAALLCYFFRMPREDNEQKQLFVEQHKHYLDEIIEQISE
FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFK
YFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.3 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP

INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMPREDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.4 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP

NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMRREDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.5 Cas9 TadAins 1249

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI

LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSGS
SGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMRRPEDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.5 Cas9 TadAins delta 59-66 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI

FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFE
PCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEG
ILADECAALLCYFFRMPRQVFNAQKKAQSSTDEDNEQKQLFVEQHKHYLD
EIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTN
LGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGG
D

110.6 Cas9 TadAins 1251

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI

FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
GSSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDE
REVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRL
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG
MNHRVEITEGILADECAALLCYFFRMRRDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.7 Cas9 TadAins 1252

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
DGSSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARD
EREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYR
LIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMRRNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.8 Cas9 TadAins delta 59-66 C-truncate 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY

YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMC
AGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADE
CAALLCYFFRMPRQEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
YTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

111.1 Cas9 TadAins 997

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK

NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGSSGSETPGTSESATPESSGIKKYPKLESEFVYGDYKVYDVR
KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGET
GEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKL
IARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM
ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGE
LQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEI
IEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLG
APAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

111.2 Cas9 TadAins 997

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD

LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGSSGSSGSETPGTSESATPESSGGSSIKKYPKLESEFVYGDY
KVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI
ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPK
RNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKEL
LGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK
HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLF
TLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS
QLGGD

112 delta HNH TadA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD

LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSEVEFSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND
KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAL
IKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFK
TEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKK
TEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVA
KVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIK
LPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG
SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATL
IHQSITGLYETRIDLSQLGGD

113 N-term single TadA helix trunc 165-end

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIG
LHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIG
RVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFR
MPRSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSV
GWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR
TARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRG
HFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARL
SKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQL
SKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAP
LSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG
ASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT
RKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE

YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKE
DYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL
EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKL
INGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARE
NQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL
QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGK
SDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGF
IKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDF
RKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVY
DVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETN
GETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLAS
AGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYL
DEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT
NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD

114 N-term single TadA helix trunc 165-end delta 59-65

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVR
NAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRSGGS
SGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITD
EYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIV
DEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGD
LNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLE
NLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDD
DLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIK
RYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL
RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETI
TPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNE

LTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE
CFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTL
TLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDK
QSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEH
IANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKG
QKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSE
EVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVE
TRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY
KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA
KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK
KDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS
FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQI
SEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAA
FKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

115.1 Cas9 TadAins1004

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV

MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREV
PVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH
RVEITEGILADECAALLCYFFRMPRQLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

115.2 Cas9 TadAins1005

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP

VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMPRQESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

115.3 Cas9 TadAins1006

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD

SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLEGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMPRQSEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

115.4 Cas9 TadAins1007

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL

TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDE
REVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRL
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG
MNHRVEITEGILADECAALLCYFFRMPRQEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

116.1 Cas9 TadAins C-term truncate2 792

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGGSSGSETP
GTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNR
VIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM
CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD

ECAALLCYFFRMPRQSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE
LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI
TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVRE
INNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

116.2 Cas9 TadAins C-term truncate2 791

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSSGSETPG
TSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRV
IGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMC
AGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADE
CAALLCYFFRMPRQGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE

LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI
TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVRE
INNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

116.3 Cas9 TadAins C-term truncate2 790

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADEC
AALLCYFFRMPRQLGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE
LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK

KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI
TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVRE
INNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

117 Cas9 delta 1017-1069

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYSSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYV

TFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEI
TEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGEIVWDKGRDFATVR
KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF
DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEA
KGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVN
FLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILAD
ANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK
RYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

118 Cas9 TadA-CP116ins 1067

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRAR
DEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNY

RLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHY
PGGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

119 Cas9 TadAins 701

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPV
GAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATL
YVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDLTFKEDIQKAQVS
GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMA
RENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLY
YLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNR
GKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKA
GFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVS
DFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK

VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

120 Cas9 TadACP136ins 1248

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK

YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSMN
HRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

121 Cas9 TadACP136ins 1052

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLAMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGS
ETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVL
NNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEP

CVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGNGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

122 Cas9 TadACP136ins 1041

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSMNHRVEITEG
ILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPES
SGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI
GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRI

GRVVFGVRNAKTGAAGSLMDVLHYPGNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

123 Cas9 TadACP139ins 1299

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK

YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
DNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRMN
HRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

124 Cas9 delta 792-872 TadAins

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSEVEFSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKA
GFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVS
DFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK
VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR

NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

125 Cas9 delta 792-906 TadAins

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSEVEFSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALI
KKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKT
EITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKT
EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAK
VEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKL
PKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS

PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLI
HQSITGLYETRIDLSQLGGD

126 TadA CP65ins 1003

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGR
VVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRM
PRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHA
LTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPLESEFVYGDYK
VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

127 TadA CP65ins 1016

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM
CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD
ECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSGSE
VEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHD
PYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

128 TadA CP65ins 1022

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMITAHAEIMALRQGGLVMQNYRLIDATLYV
TFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEI
TEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESAT
PESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWN
RAIGLHDPAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

129 TadA CP65ins 1029

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEITAHAEIMALRQGGLVMQNYRL
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG
MNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETP
GTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNR
VIGEGWNRAIGLHDPGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

130 TadA CP65ins 1041

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSTAHAEIMALR
QGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAA
GSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQS
STDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREV
PVGAVLVLNNRVIGEGWNRAIGLHDPNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

131 TadA CP65ins 1054

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIG
RVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFR
MPRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRH
ALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

132 TadA CP65ins 1246

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVR
NAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFN
AQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKR
ARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPSPEDNEQKQLFVEQHKH

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

In some embodiments, adenosine deaminase base editors were generated to insert TadA or variants thereof into the Cas9 polypeptide at the identified positions.

Exemplary, yet nonlimiting, fusion proteins are described in International PCT Application Nos. PCT/US2020/016285 and U.S. Provisional Application Nos. 62/852,228 and 62/852,224, the contents of which are incorporated by reference herein in their entireties.

Nucleobase Editing Domain

Described herein are base editors comprising a fusion protein that includes a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (*e.g.*, a deaminase domain). The base editor can be programmed to edit one or more bases in a target polynucleotide sequence by interacting with a guide polynucleotide capable of recognizing the target sequence. Once the target sequence has been recognized, the base editor is anchored on the polynucleotide where editing is to occur and the deaminase domain components of the base editor can then edit a target base.

In some embodiments, the nucleobase editing domain includes a deaminase domain. As particularly described herein, the deaminase domain includes a cytosine deaminase or an adenosine deaminase. In some embodiments, the terms "cytosine deaminase" and "cytidine deaminase" can be used interchangeably. In some embodiments, the terms "adenine deaminase" and "adenosine deaminase" can be used interchangeably. Details of nucleobase editing proteins are described in International PCT Application Nos. PCT/2017/045381 (WO2018/027078) and PCT/US2016/058344 (WO2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

A to G Editing

In some embodiments, a base editor described herein can comprise a deaminase domain which includes an adenosine deaminase. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. Adenosine deaminase is capable of deaminating (*i.e.*, removing an amine group) adenine of a deoxyadenosine residue in deoxyribonucleic acid (DNA).

In some embodiments, the nucleobase editors provided herein can be made by fusing together one or more protein domains, thereby generating a fusion protein. In certain embodiments, the fusion proteins provided herein comprise one or more features that improve the base editing activity (*e.g.*, efficiency, selectivity, and specificity) of the fusion proteins. For example, the fusion proteins provided herein can comprise a Cas9 domain that has reduced nuclease activity. In some embodiments, the fusion proteins provided herein can have a Cas9 domain that does not have nuclease activity (dCas9), or a Cas9 domain that cuts one strand of a duplexed DNA molecule, referred to as a Cas9 nickase (nCas9). Without wishing to be bound by any particular theory, the presence of the catalytic residue (*e.g.*, H840) maintains the activity of the Cas9 to cleave the non-edited (*e.g.*, non-deaminated) strand containing a T opposite the targeted A. Mutation of the catalytic residue (*e.g.*, D10 to A10) of Cas9 prevents cleavage of the edited strand containing the targeted A residue. Such Cas9 variants are able to generate a single-strand DNA break (nick) at a specific location based on the gRNA-defined target sequence, leading to repair of the non-edited strand, ultimately resulting in a T to C change on the non-edited strand. In some embodiments, an A-to-G base editor further comprises an inhibitor of inosine base excision repair, for example, a uracil glycosylase inhibitor (UGI) domain or a catalytically inactive inosine specific nuclease. Without wishing to be bound by any particular theory, the UGI domain or catalytically inactive inosine specific nuclease can inhibit or prevent base excision repair of a deaminated adenosine residue (*e.g.*, inosine), which can improve the activity or efficiency of the base editor.

A base editor comprising an adenosine deaminase can act on any polynucleotide, including DNA, RNA and DNA-RNA hybrids. In certain embodiments, a base editor comprising an adenosine deaminase can deaminate a target A of a polynucleotide comprising RNA. For example, the base editor can comprise an adenosine deaminase domain capable of deaminating a target A of an RNA polynucleotide and/or a DNA-RNA hybrid

polynucleotide. In an embodiment, an adenosine deaminase incorporated into a base editor comprises all or a portion of adenosine deaminase acting on RNA (ADAR, *e.g.*, ADAR1 or ADAR2). In another embodiment, an adenosine deaminase incorporated into a base editor comprises all or a portion of adenosine deaminase acting on tRNA (ADAT). A base editor comprising an adenosine deaminase domain can also be capable of deaminating an A nucleobase of a DNA polynucleotide. In an embodiment an adenosine deaminase domain of a base editor comprises all or a portion of an ADAT comprising one or more mutations which permit the ADAT to deaminate a target A in DNA. For example, the base editor can comprise all or a portion of an ADAT from *Escherichia coli* (EcTadA) comprising one or more of the following mutations: D108N, A106V, D147Y, E155V, L84F, H123Y, I156F, or a corresponding mutation in another adenosine deaminase.

The adenosine deaminase can be derived from any suitable organism (*e.g.*, *E. coli*). In some embodiments, the adenine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). The corresponding residue in any homologous protein can be identified by *e.g.*, sequence alignment and determination of homologous residues. The mutations in any naturally-occurring adenosine deaminase (*e.g.*, having homology to ecTadA) that corresponds to any of the mutations described herein (*e.g.*, any of the mutations identified in ecTadA) can be generated accordingly.

Adenosine deaminases

In some embodiments, fusion proteins described herein can comprise a deaminase domain which includes an adenosine deaminase. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. Adenosine deaminase is capable of deaminating (*i.e.*, removing an amine group) adenine of a deoxyadenosine residue in deoxyribonucleic acid (DNA).

In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine. In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine in a deoxyadenosine residue of DNA. In some embodiments, the adenine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). One of skill in the art will be able to identify the corresponding residue in any

homologous protein, *e.g.*, by sequence alignment and determination of homologous residues. Accordingly, one of skill in the art would be able to generate mutations in any naturally-occurring adenosine deaminase (*e.g.*, having homology to ecTadA) that corresponds to any of the mutations described herein, *e.g.*, any of the mutations identified in ecTadA. In some embodiments, the adenosine deaminase is from a prokaryote. In some embodiments, the adenosine deaminase is from a bacterium. In some embodiments, the adenosine deaminase is from *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shewanella putrefaciens, Haemophilus influenzae, Caulobacter crescentus, or Bacillus subtilis*. In some embodiments, the adenosine deaminase is from *E. coli*.

The disclosure provides adenosine deaminase variants that have increased efficiency (>50-60%) and specificity. In particular, the adenosine deaminase variants described herein are more likely to edit a desired base within a polynucleotide, and are less likely to edit bases that are not intended to be altered (*i.e.*, "bystanders").

In particular embodiments, the TadA is any one of the TadA described in PCT/US2017/045381 (WO 2018/027078), which is incorporated herein by reference in its entirety.

In some embodiments, the nucleobase editors of the disclosure are adenosine deaminase variants comprising an alteration in the following sequence: MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (also termed TadA*7.10).

In particular embodiments, the fusion proteins comprise a single (*e.g.*, provided as a monomer) TadA*8 variant. In some embodiments, the TadA*8 is linked to a Cas9 nickase. In some embodiments, the fusion proteins of the disclosure comprise as a heterodimer of a wild-type TadA (TadA(wt)) linked to a TadA*8 variant. In other embodiments, the fusion proteins of the disclosure comprise as a heterodimer of a TadA*7.10 linked to a TadA*8 variant. In some embodiments, the base editor is ABE8 comprising a TadA*8 variant monomer. In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant and a TadA(wt). In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant and TadA*7.10. In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant. In some embodiments, the TadA*8 variant is selected from Table 8. In some embodiments, the ABE8 is selected from Table 8, 9, or 10. The relevant sequences follow:

Wild-type TadA (TadA(wt)) or "the TadA reference sequence"

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

TadA*7.10:

MSEVEFSHEYW MRHALTLAKR ARDEREVPVG AVLVLNNRVI GEGWNRAIGL
HDPTAHAEIM ALRQGGLVMQ NYRLIDATLY VTFEPCVMCA GAMIHSRIGR
VVFGVRNAKT GAAGSLMDVL HYPGMNHRVE ITEGILADEC AALLCYFFRM
PRQVFNAQKK AQSSTD

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences known in the art or described herein.

In some embodiments the TadA deaminase is a full-length *E. coli* TadA deaminase. For example, in certain embodiments, the adenosine deaminase comprises the amino acid sequence:

MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGR
HDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGA
AGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD.

It should be appreciated, however, that additional adenosine deaminases useful in the present application would be apparent to the skilled artisan and are within the scope of this disclosure. For example, the adenosine deaminase may be a homolog of adenosine deaminase acting on tRNA (ADAT). Without limitation, the amino acid sequences of exemplary AD AT homologs include the following:

Staphylococcus aureus TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAHAEHIA
IERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGS
LMNLLQQSNFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

Bacillus subtilis TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEMLVIDEA
CKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGCSGTLMNLLQEERFNH
QAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEGWNRPIGR
HDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIGRVVFGARDAKTGA
AGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIKALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:

MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEILCLRSAGK
KLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGTVVNLLQHPAFNHQV
EVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

Haemophilus influenzae F3031 (*H. influenzae*) TadA:

MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDPTAHA
EIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYKTGAIGSRFHF
FDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSDK

Caulobacter crescentus (*C. crescentus*) TadA:

MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAHDPTAHA
EIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADDPKGGAVVHGPKF
FAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:

MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSNDPSAHA
EMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDPKGGAAGSLYDL
SADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALFIDERKVPPEP

An embodiment of *E. Coli* TadA (ecTadA) includes the following:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

In some embodiments, the adenosine deaminase is from a prokaryote. In some embodiments, the adenosine deaminase is from a bacterium. In some embodiments, the adenosine deaminase is from *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shewanella putrefaciens*, *Haemophilus influenzae*, *Caulobacter crescentus*, or *Bacillus subtilis*. In some embodiments, the adenosine deaminase is from *E. coli*.

In one embodiment, a fusion protein of the disclosure comprises a wild-type TadA linked to TadA*7.10, which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*7.10 domain (*e.g.*, provided as a monomer). In other embodiments, the ABE7.10 editor comprises TadA*7.10 and TadA(wt), which are capable of forming heterodimers.

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at

least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences known in the art or described herein.

It should be appreciated that any of the mutations provided herein (*e.g.*, based on the TadA reference sequence) can be introduced into other adenosine deaminases, such as *E. coli* TadA (ecTadA), *S. aureus* TadA (saTadA), or other adenosine deaminases (*e.g.*, bacterial adenosine deaminases). It would be apparent to the skilled artisan that additional deaminases may similarly be aligned to identify homologous amino acid residues that can be mutated as provided herein. Thus, any of the mutations identified in the TadA reference sequence can be made in other adenosine deaminases (*e.g.*, ecTada) that have homologous amino acid residues. It should also be appreciated that any of the mutations provided herein can be made individually or in any combination in the TadA reference sequence or another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises a D108X mutation in the TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108G, D108N, D108V, D108A, or D108Y mutation, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an A106X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A106V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, wild-type TadA or ecTadA).

In some embodiments, the adenosine deaminase comprises a E155X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a E155D, E155G, or E155V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises a D147X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding

amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D147Y, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A106X, E155X, or D147X, mutation in the TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E155D, E155G, or E155V mutation. In some embodiments, the adenosine deaminase comprises a D147Y.

For example, an adenosine deaminase can contain a D108N, a A106V, a E155V, and/or a D147Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, an adenosine deaminase comprises the following group of mutations (groups of mutations are separated by a ";") in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA): D108N and A106V; D108N and E155V; D108N and D147Y; A106V and E155V; A106V and D147Y; E155V and D147Y; D108N, A106V, and E155V; D108N, A106V, and D147Y; D108N, E155V, and D147Y; A106V, E155V, and D147Y; and D108N, A106V, E155V, and D147Y. It should be appreciated, however, that any combination of corresponding mutations provided herein can be made in an adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a H8X, T17X, L18X, W23X, L34X, W45X, R51X, A56X, E59X, E85X, M94X, I95X, V102X, F104X, A106X, R107X, D108X, K110X, M118X, N127X, A138X, F149X, M151X, R153X, Q154X, I156X, and/or K157X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, T17S, L18E, W23L, L34S, W45L, R51H, A56E, or A56S, E59G, E85K, or E85G, M94L, I95L, V102A, F104L, A106V, R107C, or R107H, or R107P, D108G, or D108N, or D108V, or D108A, or D108Y, K110I, M118K, N127S, A138V, F149Y, M151V, R153C, Q154L, I156D, and/or K157R mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a H8X, D108X, and/or N127X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid. In some embodiments, the adenosine deaminase comprises one or more of a H8Y, D108N, and/or N127S mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of H8X, R26X, M61X, L68X, M70X, A106X, D108X, A109X, N127X, D147X, R152X, Q154X, E155X, K161X, Q163X, and/or T166X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, R26W, M61I, L68Q, M70V, A106T, D108N, A109T, N127S, D147Y, R152C, Q154H or Q154R, E155G or E155V or E155D, K161Q, Q163H, and/or T166P mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, D108X, N127X, D147X, R152X, and Q154X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, M61X, M70X, D108X, N127X, Q154X, E155X, and Q163X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, D108X, N127X, E155X, and T166X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, A106X, D108X, mutation or

mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, R26X, L68X, D108X, N127X, D147X, and E155X, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, D108X, A109X, N127X, and E155X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, D108N, N127S, D147Y, R152C, and Q154H in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, M61I, M70V, D108N, N127S, Q154R, E155G and Q163H in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, D108N, N127S, E155V, and T166P in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, A106T, D108N, N127S, E155D, and K161Q in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, R26W, L68Q, D108N, N127S, D147Y, and E155V in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, D108N, A109T, N127S, and E155G in TadA

reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA).

Any of the mutations provided herein and any additional mutations (*e.g.*, based on the ecTadA amino acid sequence) can be introduced into any other adenosine deaminases. Any of the mutations provided herein can be made individually or in any combination in TadA reference sequence or another adenosine deaminase (*e.g.*, ecTadA).

Details of A to G nucleobase editing proteins are described in International PCT Application No. PCT/2017/045381 (WO2018/027078) and Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature, 551, 464-471 (2017), the entire contents of which are hereby incorporated by reference.

In some embodiments, the adenosine deaminase comprises one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a D108N, D108G, or D108V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a A106V and D108N mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises R107C and D108N mutations in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and Q154H mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a D108N, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, and N127S mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a A106V, D108N, D147Y and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a S2X, H8X, I49X, L84X, H123X, N127X, I156X and/or K160X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of S2A, H8Y, I49F, L84F, H123Y, N127S, I156F and/or K160S mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.,* ecTadA).

In some embodiments, the adenosine deaminase comprises an L84X mutation adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an L84F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

In some embodiments, the adenosine deaminase comprises an H123X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H123Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

In some embodiments, the adenosine deaminase comprises an I156X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an I156F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84X, A106X, D108X, H123X, D147X, E155X, and I156X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2X, I49X, A106X, D108X, D147X, and E155X in TadA reference sequence, or a corresponding mutation or mutations in

another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, A106X, D108X, N127X, and K160X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84F, A106V, D108N, H123Y, D147Y, E155V, and I156F in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2A, I49F, A106V, D108N, D147Y, and E155V in TadA reference sequence.

In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, A106T, D108N, N127S, and K160S in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a E25X, R26X, R107X, A142X, and/or A143X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of E25M, E25D, E25A, E25R, E25V, E25S, E25Y, R26G, R26N, R26Q, R26C, R26L, R26K, R107P, R107K, R107A, R107N, R107W, R107H, R107S, A142N, A142D, A142G, A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one or more of the mutations described herein corresponding to TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an E25X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the

wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E25M, E25D, E25A, E25R, E25V, E25S, or E25Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R26X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises R26G, R26N, R26Q, R26C, R26L, or R26K mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R107X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R107P, R107K, R107A, R107N, R107W, R107H, or R107S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A142X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A142N, A142D, A142G, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A143X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises one or more of a H36X, N37X, P48X, I49X, R51X, M70X, N72X, D77X, E134X, S146X, Q154X, K157X, and/or K161X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some

embodiments, the adenosine deaminase comprises one or more of H36L, N37T, N37S, P48T, P48L, I49V, R51H, R51L, M70L, N72S, D77G, E134G, S146R, S146C, Q154H, K157N, and/or K161T mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an H36X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H36L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an N37X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an N37T, or N37S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an P48X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an P48T, or P48L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R51X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R51H, or R51L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an S146X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises

an S146R, or S146C mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an K157X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a K157N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an P48X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a P48S, P48T, or P48A mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an A142X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A142N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an W23X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a W23R, or W23L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In some embodiments, the adenosine deaminase comprises an R152X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a R152P, or R52H mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

In one embodiment, the adenosine deaminase may comprise the mutations H36L, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, and K157N. In some embodiments, the adenosine deaminase comprises the following combination of mutations relative to TadA reference sequence, where each mutation of a combination is separated by a "_" and each combination of mutations is between parentheses:

(A106V_D108N),
(R107C_D108N),
(H8Y_D108N_N127S_D147Y_Q154H),
(H8Y_D108N_N127S_D147Y_E155V),
(D108N_D147Y_E155V),
(H8Y_D108N_N127S),
(H8Y_D108N_N127S_D147Y_Q154H),
(A106V_D108N_D147Y_E155V),
(D108Q_D147Y_E155V),
(D108M_D147Y_E155V),
(D108L_D147Y_E155V),
(D108K_D147Y_E155V),
(D108I_D147Y_E155V),
(D108F_D147Y_E155V),
(A106V_D108N_D147Y),
(A106V_D108M_D147Y_E155V),
(E59A_A106V_D108N_D147Y_E155V),
(E59A cat dead_A106V_D108N_D147Y_E155V),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156Y),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(D103A_D104N),
(G22P_D103A_D104N),
(D103A_D104N_S138A),
(R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(E25G_R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(E25D_R26G_L84F_A106V_R107K_D108N_H123Y_A142N_A143G_D147Y_E155V_I156F),

(R26Q_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(E25M_R26G_L84F_A106V_R107P_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(R26C_L84F_A106V_R107H_D108N_H123Y_A142N_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_A142N_A143L_D147Y_E155V_I156F),
(R26G_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(E25A_R26G_L84F_A106V_R107N_D108N_H123Y_A142N_A143E_D147Y_E155V_I156F),
(R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(A106V_D108N_A142N_D147Y_E155V),
(R26G_A106V_D108N_A142N_D147Y_E155V),
(E25D_R26G_A106V_R107K_D108N_A142N_A143G_D147Y_E155V),
(R26G_A106V_D108N_R107H_A142N_A143D_D147Y_E155V),
(E25D_R26G_A106V_D108N_A142N_D147Y_E155V),
(A106V_R107K_D108N_A142N_D147Y_E155V),
(A106V_D108N_A142N_A143G_D147Y_E155V),
(A106V_D108N_A142N_A143L_D147Y_E155V),
(H36L_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F _K157N),
(N37T_P48T_M70L_L84F_A106V_D108N_H123Y_D147Y_I49V_E155V_I156F),
(N37S_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K161T),
(H36L_L84F_A106V_D108N_H123Y_D147Y_Q154H_E155V_I156F),
(N72S_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F),
(H36L_P48L_L84F_A106V_D108N_H123Y_E134G_D147Y_E155V_I156F),
(H36L_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K157N),
(H36L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(N37S_R51H_D77G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R51L_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K157N),
(D24G_Q71R_L84F_H96L_A106V_D108N_H123Y_D147Y_E155V_I156F_K160E),
(H36L_G67V_L84F_A106V_D108N_H123Y_S146T_D147Y_E155V_I156F),
(Q71L_L84F_A106V_D108N_H123Y_L137M_A143E_D147Y_E155V_I156F),
(E25G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_Q159L),
(L84F_A91T_F104I_A106V_D108N_H123Y_D147Y_E155V_I156F),

(N72D_L84F_A106V_D108N_H123Y_G125A_D147Y_E155V_I156F),
(P48S_L84F_S97C_A106V_D108N_H123Y_D147Y_E155V_I156F),
(W23G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(D24G_P48L_Q71R_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_Q159L),
(L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(H36L_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_I156F _K157N), (N37S_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F_K161T),
(L84F_A106V_D108N_D147Y_E155V_I156F),
(R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K160E_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K160E),
(R74Q_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R74A_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R74Q_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_R98Q_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_R129Q_D147Y_E155V_I156F),
(P48S_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(P48S_A142N),
(P48T_I49V_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F_L157N),
(P48T_I49V_A142N),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S146C_A142N_D147Y_E155V_I156F
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F _K157N),
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V _ I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_I156F _K157N),

(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_A142N_D147Y_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152H_E155V_I156F_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S146C_D147Y_E155V
_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S146C_D147Y_R152P _E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_R152P_E155V
_I156F _K157N).

In certain embodiments, the fusion proteins provided herein comprise one or more features that improve the base editing activity of the fusion proteins. For example, any of the fusion proteins provided herein may comprise a Cas9 domain that has reduced nuclease activity. In some embodiments, any of the fusion proteins provided herein may have a Cas9 domain that does not have nuclease activity (dCas9), or a Cas9 domain that cuts one strand of a duplexed DNA molecule, referred to as a Cas9 nickase (nCas9).

In some embodiments, the adenosine deaminase is TadA*7.10. In some embodiments, TadA*7.10 comprises at least one alteration. In particular embodiments, TadA*7.10 comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. The alteration Y123H is also referred to herein as H123H (the alteration H123Y in TadA*7.10 reverted back to Y123H (wt)). In other embodiments, the TadA*7.10 comprises a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In particular embodiments, an adenosine deaminase variant comprises a deletion of the C terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, and 157, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

In other embodiments, a base editor of the disclosure is a monomer comprising an adenosine deaminase variant (*e.g.*, TadA*8) comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant (TadA*8) is a monomer comprising a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, a base editor is a heterodimer comprising a wild-type adenosine deaminase and an adenosine deaminase variant (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the base editor is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S +

Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

In one embodiment, an adenosine deaminase is a TadA*8 that comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD

In some embodiments, the TadA*8 is a truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

In some embodiments the TadA*8 is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

In one embodiment, a fusion protein of the disclosure comprises a wild-type TadA is linked to an adenosine deaminase variant described herein (*e.g.*, TadA*8), which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*8 domain (*e.g.*, provided as a monomer). In other embodiments, the base editor comprises TadA*8 and TadA(wt), which are capable of forming heterodimers. Exemplary sequences follow:

TadA(wt) or "the TadA reference sequence":

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP
GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

TadA*7.10:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

TadA*8:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to a reference sequence, or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences known in the art or described herein.

In particular embodiments, a TadA*8 comprises one or more mutations at any of the following positions shown in bold. In other embodiments, a TadA*8 comprises one or more mutations at any of the positions shown with underlining:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG 50
LHDPTAHAEI MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG 100
RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCYFFR 150
MPRQVFNAQK KAQSSTD

For example, the TadA*8 comprises alterations at amino acid position 82 and/or 166 (*e.g.*, V82S, T166R) alone or in combination with any one or more of the following Y147T,

Y147R, Q154S, Y123H, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In particular embodiments, a combination of alterations is selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

In some embodiments, the adenosine deaminase is TadA*8, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCTFFR MPRQVFNAQK KAQSSTD

In some embodiments, the TadA*8 is truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

In one embodiment, a fusion protein of the disclosure comprises a wild-type TadA is linked to an adenosine deaminase variant described herein (*e.g.*, TadA*8), which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*8 domain (*e.g.*, provided as a monomer). In other embodiments, the base editor comprises TadA*8 and TadA(wt), which are capable of forming heterodimers.

C to T Editing

In some embodiments, a base editor disclosed herein comprises a fusion protein comprising cytidine deaminase capable of deaminating a target cytidine (C) base of a polynucleotide to produce uridine (U), which has the base pairing properties of thymine. In some embodiments, for example where the polynucleotide is double-stranded (*e.g.*, DNA),

the uridine base can then be substituted with a thymidine base (*e.g.*, by cellular repair machinery) to give rise to a C:G to a T:A transition. In other embodiments, deamination of a C to U in a nucleic acid by a base editor cannot be accompanied by substitution of the U to a T.

The deamination of a target C in a polynucleotide to give rise to a U is a non-limiting example of a type of base editing that can be executed by a base editor described herein. In another example, a base editor comprising a cytidine deaminase domain can mediate conversion of a cytosine (C) base to a guanine (G) base. For example, a U of a polynucleotide produced by deamination of a cytidine by a cytidine deaminase domain of a base editor can be excised from the polynucleotide by a base excision repair mechanism (*e.g.*, by a uracil DNA glycosylase (UDG) domain), producing an abasic site. The nucleobase opposite the abasic site can then be substituted (*e.g.*, by base repair machinery) with another base, such as a C, by for example a translesion polymerase. Although it is typical for a nucleobase opposite an abasic site to be replaced with a C, other substitutions (*e.g.*, A, G or T) can also occur.

Accordingly, in some embodiments a base editor described herein comprises a deamination domain (*e.g.*, cytidine deaminase domain) capable of deaminating a target C to a U in a polynucleotide. Further, as described below, the base editor can comprise additional domains which facilitate conversion of the U resulting from deamination to, in some embodiments, a T or a G. For example, a base editor comprising a cytidine deaminase domain can further comprise a uracil glycosylase inhibitor (UGI) domain to mediate substitution of a U by a T, completing a C-to-T base editing event. In another example, a base editor can incorporate a translesion polymerase to improve the efficiency of C-to-G base editing, since a translesion polymerase can facilitate incorporation of a C opposite an abasic site (*i.e.*, resulting in incorporation of a G at the abasic site, completing the C-to-G base editing event).

A base editor comprising a cytidine deaminase as a domain can deaminate a target C in any polynucleotide, including DNA, RNA and DNA-RNA hybrids. Typically, a cytidine deaminase catalyzes a C nucleobase that is positioned in the context of a single-stranded portion of a polynucleotide. In some embodiments, the entire polynucleotide comprising a target C can be single-stranded. For example, a cytidine deaminase incorporated into the base editor can deaminate a target C in a single-stranded RNA polynucleotide. In other embodiments, a base editor comprising a cytidine deaminase domain can act on a double-

stranded polynucleotide, but the target C can be positioned in a portion of the polynucleotide which at the time of the deamination reaction is in a single-stranded state. For example, in embodiments where the NAGPB domain comprises a Cas9 domain, several nucleotides can be left unpaired during formation of the Cas9-gRNA-target DNA complex, resulting in formation of a Cas9 "R-loop complex". These unpaired nucleotides can form a bubble of single-stranded DNA that can serve as a substrate for a single-strand specific nucleotide deaminase enzyme (*e.g.*, cytidine deaminase).

In some embodiments, a cytidine deaminase of a base editor can comprise all or a portion of an apolipoprotein B mRNA editing complex (APOBEC) family deaminase. APOBEC is a family of evolutionarily conserved cytidine deaminases. Members of this family are C-to-U editing enzymes. The N-terminal domain of APOBEC like proteins is the catalytic domain, while the C-terminal domain is a pseudocatalytic domain. More specifically, the catalytic domain is a zinc dependent cytidine deaminase domain and is important for cytidine deamination. APOBEC family members include APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D ("APOBEC3E" now refers to this), APOBEC3F, APOBEC3G, APOBEC3H, APOBEC4, and Activation-induced (cytidine) deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of an APOBEC1 deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC2 deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of is an APOBEC3 deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of an APOBEC3A deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3B deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3C deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3D deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3E deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3F deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3G deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC3H deaminase. In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of APOBEC4 deaminase. In some embodiments, a deaminase incorporated into a base editor

comprises all or a portion of activation-induced deaminase (AID). In some embodiments a deaminase incorporated into a base editor comprises all or a portion of cytidine deaminase 1 (CDA1). It should be appreciated that a base editor can comprise a deaminase from any suitable organism (*e.g.*, a human or a rat). In some embodiments, a deaminase domain of a base editor is from a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase domain of the base editor is derived from rat (*e.g.*, rat APOBEC1). In some embodiments, the deaminase domain of the base editor is human APOBEC1. In some embodiments, the deaminase domain of the base editor is pmCDA1.

The amino acid and nucleic acid sequences of PmCDA1 are shown herein below.

>tr|A5H718|A5H718_PETMA Cytosine deaminase OS=Petromyzon marinus OX=7757 PE=2 SV=1 amino acid sequence:

MTDAEYVRIHEKLDIYTFKKQFFNNKKSVSHRCYVLFELKRRGERRACFWGYAVNKPQSGTE
RGIHAEIFSIRKVEEYLRDNPGQFTINWYSSWSPCADCAEKILEWYNQELRGNGHTLKIWAC
KLYYEKNARNQIGLWNLRDNGVGLNVMVSEHYQCCRKIFIQSSHNQLNENRWLEKTLKRAEK
RRSELSIMIQVKILHTTKSPAV

Nucleic acid sequence: >EF094822.1 Petromyzon marinus isolate PmCDA.21 cytosine deaminase mRNA, complete cds:

TGACACGACACAGCCGTGTATATGAGGAAGGGTAGCTGGATGGGGGGGGGGGGAATACGTTC
AGAGAGGACATTAGCGAGCGTCTTGTTGGTGGCCTTGAGTCTAGACACCTGCAGACATGACC
GACGCTGAGTACGTGAGAATCCATGAGAAGTTGGACATCTACACGTTTAAGAAACAGTTTTT
CAACAACAAAAAATCCGTGTCGCATAGATGCTACGTTCTCTTTGAATTAAAACGACGGGGTG
AACGTAGAGCGTGTTTTTGGGGCTATGCTGTGAATAAACCACAGAGCGGGACAGAACGTGGA
ATTCACGCCGAAATCTTTAGCATTAGAAAAGTCGAAGAATACCTGCGCGACAACCCCGGACA
ATTCACGATAAATTGGTACTCATCCTGGAGTCCTTGTGCAGATTGCGCTGAAAAGATCTTAG
AATGGTATAACCAGGAGCTGCGGGGGAACGGCCACACTTTGAAAATCTGGGCTTGCAAACTC
TATTACGAGAAAAATGCGAGGAATCAAATTGGGCTGTGGAACCTCAGAGATAACGGGGTTGG
GTTGAATGTAATGGTAAGTGAACACTACCAATGTTGCAGGAAAATATTCATCCAATCGTCGC
ACAATCAATTGAATGAGAATAGATGGCTTGAGAAGACTTTGAAGCGAGCTGAAAAACGACGG
AGCGAGTTGTCCATTATGATTCAGGTAAAAATACTCCACACCACTAAGAGTCCTGCTGTTTA
AGAGGCTATGCGGATGGTTTTC

The amino acid and nucleic acid sequences of the coding sequence (CDS) of human activation-induced cytidine deaminase (AID) are shown below.

>tr|Q6QJ80|Q6QJ80_HUMAN Activation-induced cytidine deaminase OS=Homo sapiens OX=9606 GN=AICDA PE=2 SV=1 amino acid sequence:

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHVELL
FLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRK
AEPEGLRRLHRAGVQIAIMTFKAPV

The amino acid and nucleic acid sequences of the coding sequence (CDS) of human activation-induced cytidine deaminase (AID) are shown below.

>tr|Q6QJ80|Q6QJ80_HUMAN Activation-induced cytidine deaminase OS=Homo sapiens OX=9606 GN=AICDA PE=2 SV=1 amino acid sequence:

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHVELL
FLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRK
AEPEGLRRLHRAGVQIAIMTFKAPV

Nucleic acid sequence: >NG_011588.1:5001-15681 Homo sapiens activation induced cytidine deaminase (AICDA), RefSeqGene (LRG_17) on chromosome 12:

AGAGAACCATCATTAATTGAAGTGAGATTTTTCTGGCCTGAGACTTGCAGGGAGGCAAGAAG
ACACTCTGGACACCACTATGGACAGGTAAAGAGGCAGTCTTCTCGTGGGTGATTGCACTGGC
CTTCCTCTCAGAGCAAATCTGAGTAATGAGACTGGTAGCTATCCCTTTCTCTCATGTAACTG
TCTGACTGATAAGATCAGCTTGATCAATATGCATATATATTTTTTGATCTGTCTCCTTTTCT
TCTATTCAGATCTTATACGCTGTCAGCCCAATTCTTTCTGTTTCAGACTTCTCTTGATTTCC
CTCTTTTTCATGTGGCAAAAGAAGTAGTGCGTACAATGTACTGATTCGTCCTGAGATTTGTA
CCATGGTTGAAACTAATTTATGGTAATAATATTAACATAGCAAATCTTTAGAGACTCAAATC
ATGAAAAGGTAATAGCAGTACTGTACTAAAAACGGTAGTGCTAATTTTCGTAATAATTTTGT
AAATATTCAACAGTAAAACAACTTGAAGACACACTTTCCTAGGGAGGCGTTACTGAAATAAT
TTAGCTATAGTAAGAAAATTTGTAATTTTAGAAATGCCAAGCATTCTAAATTAATTGCTTGA
AAGTCACTATGATTGTGTCCATTATAAGGAGACAAATTCATTCAAGCAAGTTATTTAATGTT
AAAGGCCCAATTGTTAGGCAGTTAATGGCACTTTTACTATTAACTAATCTTTCCATTTGTTC
AGACGTAGCTTAACTTACCTCTTAGGTGTGAATTTGGTTAAGGTCCTCATAATGTCTTTATG
TGCAGTTTTTGATAGGTTATTGTCATAGAACTTATTCTATTCCTACATTTATGATTACTATG
GATGTATGAGAATAACACCTAATCCTTATACTTTACCTCAATTTAACTCCTTTATAAAGAAC
TTACATTACAGAATAAAGATTTTTTAAAAATATATTTTTTTGTAGAGACAGGGTCTTAGCCC
AGCCGAGGCTGGTCTCTAAGTCCTGGCCCAAGCGATCCTCCTGCCTGGGCCTCCTAAAGTGC
TGGAATTATAGACATGAGCCATCACATCCAATATACAGAATAAAGATTTTTAATGGAGGATT
TAATGTTCTTCAGAAAATTTTCTTGAGGTCAGACAATGTCAAATGTCTCCTCAGTTTACACT

GAGATTTTGAAAACAAGTCTGAGCTATAGGTCCTTGTGAAGGGTCCATTGGAAATACTTGTT
CAAAGTAAAATGGAAAGCAAAGGTAAAATCAGCAGTTGAAATTCAGAGAAAGACAGAAAAGG
AGAAAAGATGAAATTCAACAGGACAGAAGGGAAATATATTATCATTAAGGAGGACAGTATCT
GTAGAGCTCATTAGTGATGGCAAAATGACTTGGTCAGGATTATTTTTAACCCGCTTGTTTCT
GGTTTGCACGGCTGGGGATGCAGCTAGGGTTCTGCCTCAGGGAGCACAGCTGTCCAGAGCAG
CTGTCAGCCTGCAAGCCTGAAACACTCCCTCGGTAAAGTCCTTCCTACTCAGGACAGAAATG
ACGAGAACAGGGAGCTGGAAACAGGCCCCTAACCAGAGAAGGGAAGTAATGGATCAACAAAG
TTAACTAGCAGGTCAGGATCACGCAATTCATTTCACTCTGACTGGTAACATGTGACAGAAAC
AGTGTAGGCTTATTGTATTTTCATGTAGAGTAGGACCCAAAAATCCACCCAAAGTCCTTTAT
CTATGCCACATCCTTCTTATCTATACTTCCAGGACACTTTTTCTTCCTTATGATAAGGCTCT
CTCTCTCTCCACACACACACACACACACACACACACACACACACACACACACACACACAAACACAC
ACCCCGCCAACCAAGGTGCATGTAAAAAGATGTAGATTCCTCTGCCTTTCTCATCTACACAG
CCCAGGAGGGTAAGTTAATATAAGAGGGATTTATTGGTAAGAGATGATGCTTAATCTGTTTA
ACACTGGGCCTCAAAGAGAGAATTTCTTTTCTTCTGTACTTATTAAGCACCTATTATGTGTT
GAGCTTATATATACAAAGGGTTATTATATGCTAATATAGTAATAGTAATGGTGGTTGGTACT
ATGGTAATTACCATAAAAATTATTATCCTTTTAAAATAAAGCTAATTATTATTGGATCTTTT
TTAGTATTCATTTTATGTTTTTTATGTTTTTGATTTTTTAAAAGACAATCTCACCCTGTTAC
CCAGGCTGGAGTGCAGTGGTGCAATCATAGCTTTCTGCAGTCTTGAACTCCTGGGCTCAAGC
AATCCTCCTGCCTTGGCCTCCCAAAGTGTTGGGATACAGTCATGAGCCACTGCATCTGGCCT
AGGATCCATTTAGATTAAAATATGCATTTTAAATTTTAAAATAATATGGCTAATTTTTACCT
TATGTAATGTGTATACTGGCAATAAATCTAGTTTGCTGCCTAAAGTTTAAAGTGCTTTCCAG
TAAGCTTCATGTACGTGAGGGGAGACATTTAAAGTGAAACAGACAGCCAGGTGTGGTGGCTC
ACGCCTGTAATCCCAGCACTCTGGGAGGCTGAGGTGGGTGGATCGCTTGAGCCCTGGAGTTC
AAGACCAGCCTGAGCAACATGGCAAAACGCTGTTTCTATAACAAAAATTAGCCGGGCATGGT
GGCATGTGCCTGTGGTCCCAGCTACTAGGGGGCTGAGGCAGGAGAATCGTTGGAGCCCAGGA
GGTCAAGGCTGCACTGAGCAGTGCTTGCGCCACTGCACTCCAGCCTGGGTGACAGGACCAGA
CCTTGCCTCAAAAAAATAAGAAGAAAAATTAAAAATAAATGGAAACAACTACAAAGAGCTGT
TGTCCTAGATGAGCTACTTAGTTAGGCTGATATTTTGGTATTTAACTTTTAAAGTCAGGGTC
TGTCACCTGCACTACATTATTAAAATATCAATTCTCAATGTATATCCACACAAAGACTGGTA
CGTGAATGTTCATAGTACCTTTATTCACAAAACCCCAAAGTAGAGACTATCCAAATATCCAT
CAACAAGTGAACAAATAAACAAAATGTGCTATATCCATGCAATGGAATACCACCCTGCAGTA
CAAAGAAGCTACTTGGGGATGAATCCCAAAGTCATGACGCTAAATGAAAGAGTCAGACATGA
AGGAGGAGATAATGTATGCCATACGAAATTCTAGAAAATGAAAGTAACTTATAGTTACAGAA
AGCAAATCAGGGCAGGCATAGAGGCTCACACCTGTAATCCCAGCACTTTGAGAGGCCACGTG

GGAAGATTGCTAGAACTCAGGAGTTCAAGACCAGCCTGGGCAACACAGTGAAACTCCATTCT
CCACAAAAATGGGAAAAAAAGAAAGCAAATCAGTGGTTGTCCTGTGGGGAGGGGAAGGACTG
CAAAGAGGGAAGAAGCTCTGGTGGGGTGAGGGTGGTGATTCAGGTTCTGTATCCTGACTGTG
GTAGCAGTTTGGGGTGTTTACATCCAAAAATATTCGTAGAATTATGCATCTTAAATGGGTGG
AGTTTACTGTATGTAAATTATACCTCAATGTAAGAAAAAATAATGTGTAAGAAAACTTTCAA
TTCTCTTGCCAGCAAACGTTATTCAAATTCCTGAGCCCTTTACTTCGCAAATTCTCTGCACT
TCTGCCCCGTACCATTAGGTGACAGCACTAGCTCCACAAATTGGATAAATGCATTTCTGGAA
AAGACTAGGGACAAAATCCAGGCATCACTTGTGCTTTCATATCAACCATGCTGTACAGCTTG
TGTTGCTGTCTGCAGCTGCAATGGGGACTCTTGATTTCTTTAAGGAAACTTGGGTTACCAGA
GTATTTCCACAAATGCTATTCAAATTAGTGCTTATGATATGCAAGACACTGTGCTAGGAGCC
AGAAAACAAAGAGGAGGAGAAATCAGTCATTATGTGGGAACAACATAGCAAGATATTTAGAT
CATTTTGACTAGTTAAAAAAGCAGCAGAGTACAAAATCACACATGCAATCAGTATAATCCAA
ATCATGTAAATATGTGCCTGTAGAAAGACTAGAGGAATAAACACAAGAATCTTAACAGTCAT
TGTCATTAGACACTAAGTCTAATTATTATTATTAGACACTATGATATTTGAGATTTAAAAAA
TCTTTAATATTTTAAAATTTAGAGCTCTTCTATTTTTCCATAGTATTCAAGTTTGACAATGA
TCAAGTATTACTCTTTCTTTTTTTTTTTTTTTTTTTTTTTTTGAGATGGAGTTTTGGTCTTG
TTGCCCATGCTGGAGTGGAATGGCATGACCATAGCTCACTGCAACCTCCACCTCCTGGGTTC
AAGCAAAGCTGTCGCCTCAGCCTCCCGGGTAGATGGGATTACAGGCGCCCACCACCACACTC
GGCTAATGTTTGTATTTTTAGTAGAGATGGGGTTTCACCATGTTGGCCAGGCTGGTCTCAAA
CTCCTGACCTCAGAGGATCCACCTGCCTCAGCCTCCCAAAGTGCTGGGATTACAGATGTAGG
CCACTGCGCCCGGCCAAGTATTGCTCTTATACATTAAAAAACAGGTGTGAGCCACTGCGCCC
AGCCAGGTATTGCTCTTATACATTAAAAAATAGGCCGGTGCAGTGGCTCACGCCTGTAATCC
CAGCACTTTGGGAAGCCAAGGCGGGCAGAACACCCGAGGTCAGGAGTCCAAGGCCAGCCTGG
CCAAGATGGTGAAACCCCGTCTCTATTAAAAATACAAACATTACCTGGGCATGATGGTGGGC
GCCTGTAATCCCAGCTACTCAGGAGGCTGAGGCAGGAGGATCCGCGGAGCCTGGCAGATCTG
CCTGAGCCTGGGAGGTTGAGGCTACAGTAAGCCAAGATCATGCCAGTATACTTCAGCCTGGG
CGACAAAGTGAGACCGTAACAAAAAAAAAAAAATTTAAAAAAAGAAATTTAGATCAAGATCC
AACTGTAAAAAGTGGCCTAAACACCACATTAAAGAGTTTGGAGTTTATTCTGCAGGCAGAAG
AGAACCATCAGGGGGTCTTCAGCATGGGAATGGCATGGTGCACCTGGTTTTTGTGAGATCAT
GGTGGTGACAGTGTGGGGAATGTTATTTTGGAGGGACTGGAGGCAGACAGACCGGTTAAAAG
GCCAGCACAACAGATAAGGAGGAAGAAGATGAGGGCTTGGACCGAAGCAGAGAAGAGCAAAC
AGGGAAGGTACAAATTCAAGAAATATTGGGGGGTTTGAATCAACACATTTAGATGATTAATT
AAATATGAGGACTGAGGAATAAGAAATGAGTCAAGGATGGTTCCAGGCTGCTAGGCTGCTTA
CCTGAGGTGGCAAAGTCGGGAGGAGTGGCAGTTTAGGACAGGGGGCAGTTGAGGAATATTGT

TTTGATCATTTTGAGTTTGAGGTACAAGTTGGACACTTAGGTAAAGACTGGAGGGGAAATCT
GAATATACAATTATGGGACTGAGGAACAAGTTTATTTTATTTTTTGTTTCGTTTTCTTGTTG
AAGAACAAATTTAATTGTAATCCCAAGTCATCAGCATCTAGAAGACAGTGGCAGGAGGTGAC
TGTCTTGTGGGTAAGGGTTTGGGGTCCTTGATGAGTATCTCTCAATTGGCCTTAAATATAAG
CAGGAAAAGGAGTTTATGATGGATTCCAGGCTCAGCAGGGCTCAGGAGGGCTCAGGCAGCCA
GCAGAGGAAGTCAGAGCATCTTCTTTGGTTTAGCCCAAGTAATGACTTCCTTAAAAAGCTGA
AGGAAAATCCAGAGTGACCAGATTATAAACTGTACTCTTGCATTTTCTCTCCCTCCTCTCAC
CCACAGCCTCTTGATGAACCGGAGGAAGTTTCTTTACCAATTCAAAAATGTCCGCTGGGCTA
AGGGTCGGCGTGAGACCTACCTGTGCTACGTAGTGAAGAGGCGTGACAGTGCTACATCCTTT
TCACTGGACTTTGGTTATCTTCGCAATAAGGTATCAATTAAAGTCGGCTTTGCAAGCAGTTT
AATGGTCAACTGTGAGTGCTTTTAGAGCCACCTGCTGATGGTATTACTTCCATCCTTTTTTG
GCATTTGTGTCTCTATCACATTCCTCAAATCCTTTTTTTTTATTTCTTTTTCCATGTCCATGC
ACCCATATTAGACATGGCCCAAAATATGTGATTTAATTCCTCCCCAGTAATGCTGGGCACCC
TAATACCACTCCTTCCTTCAGTGCCAAGAACAACTGCTCCCAAACTGTTTACCAGCTTTCCT
CAGCATCTGAATTGCCTTTGAGATTAATTAAGCTAAAAGCATTTTTATATGGGAGAATATTA
TCAGCTTGTCCAAGCAAAAATTTTAAATGTGAAAAACAAATTGTGTCTTAAGCATTTTTGAA
AATTAAGGAAGAAGAATTTGGGAAAAAATTAACGGTGGCTCAATTCTGTCTTCCAAATGATT
TCTTTTCCCTCCTACTCACATGGGTCGTAGGCCAGTGAATACATTCAACATGGTGATCCCCA
GAAAACTCAGAGAAGCCTCGGCTGATGATTAATTAAATTGATCTTTCGGCTACCCGAGAGAA
TTACATTTCCAAGAGACTTCTTCACCAAAATCCAGATGGGTTTACATAAACTTCTGCCCACG
GGTATCTCCTCTCTCCTAACACGCTGTGACGTCTGGGCTTGGTGGAATCTCAGGGAAGCATC
CGTGGGGTGGAAGGTCATCGTCTGGCTCGTTGTTTGATGGTTATATTACCATGCAATTTTCT
TTGCCTACATTTGTATTGAATACATCCCAATCTCCTTCCTATTCGGTGACATGACACATTCT
ATTTCAGAAGGCTTTGATTTTATCAAGCACTTTCATTTACTTCTCATGGCAGTGCCTATTAC
TTCTCTTACAATACCCATCTGTCTGCTTTACCAAAATCTATTTCCCCTTTTCAGATCCTCCC
AAATGGTCCTCATAAACTGTCCTGCCTCCACCTAGTGGTCCAGGTATATTTCCACAATGTTA
CATCAACAGGCACTTCTAGCCATTTTCCTTCTCAAAAGGTGCAAAAAGCAACTTCATAAACA
CAAATTAAATCTTCGGTGAGGTAGTGTGATGCTGCTTCCTCCCAACTCAGCGCACTTCGTCT
TCCTCATTCCACAAAAACCCATAGCCTTCCTTCACTCTGCAGGACTAGTGCTGCCAAGGGTT
CAGCTCTACCTACTGGTGTGCTCTTTTGAGCAAGTTGCTTAGCCTCTCTGTAACACAAGGAC
AATAGCTGCAAGCATCCCCAAAGATCATTGCAGGAGACAATGACTAAGGCTACCAGAGCCGC
AATAAAAGTCAGTGAATTTTAGCGTGGTCCTCTCTGTCTCTCCAGAACGGCTGCCACGTGGA
ATTGCTCTTCCTCCGCTACATCTCGGACTGGGACCTAGACCCTGGCCGCTGCTACCGCGTCA
CCTGGTTCACCTCCTGGAGCCCCTGCTACGACTGTGCCCGACATGTGGCCGACTTTCTGCGA

GGGAACCCCAACCTCAGTCTGAGGATCTTCACCGCGCGCCTCTACTTCTGTGAGGACCGCAA
GGCTGAGCCCGAGGGGCTGCGGCGGCTGCACCGCGCCGGGGTGCAAATAGCCATCATGACCT
TCAAAGGTGCGAAAGGGCCTTCCGCGCAGGCGCAGTGCAGCAGCCCGCATTCGGGATTGCGA
TGCGGAATGAATGAGTTAGTGGGGAAGCTCGAGGGGAAGAAGTGGGCGGGGATTCTGGTTCA
CCTCTGGAGCCGAAATTAAAGATTAGAAGCAGAGAAAAGAGTGAATGGCTCAGAGACAAGGC
CCCGAGGAAATGAGAAAATGGGGCCAGGGTTGCTTCTTTCCCCTCGATTTGGAACCTGAACT
GTCTTCTACCCCCATATCCCCGCCTTTTTTTCCTTTTTTTTTTTTTTGAAGATTATTTTTACT
GCTGGAATACTTTTGTAGAAAACCACGAAAGAACTTTCAAAGCCTGGGAAGGGCTGCATGAA
AATTCAGTTCGTCTCTCCAGACAGCTTCGGCGCATCCTTTTGGTAAGGGGCTTCCTCGCTTT
TTAAATTTTCTTTCTTTCTCTACAGTCTTTTTTGGAGTTTCGTATATTTCTTATATTTTCTT
ATTGTTCAATCACTCTCAGTTTTCATCTGATGAAAACTTTATTTCTCCTCCACATCAGCTTT
TTCTTCTGCTGTTTCACCATTCAGAGCCCTCTGCTAAGGTTCCTTTTCCCTCCCTTTTCTTT
CTTTTGTTGTTTCACATCTTTAAATTTCTGTCTCTCCCCAGGGTTGCGTTTCCTTCCTGGTC
AGAATTCTTTTCTCCTTTTTTTTTTTTTTTTTTTTTTTTTTTAAACAAACAAACAAAAAACC
CAAAAAAACTCTTTCCCAATTTACTTTCTTCCAACATGTTACAAAGCCATCCACTCAGTTTA
GAAGACTCTCCGGCCCCACCGACCCCCAACCTCGTTTTGAAGCCATTCACTCAATTTGCTTC
TCTCTTTCTCTACAGCCCCTGTATGAGGTTGATGACTTACGAGACGCATTTCGTACTTTGGG
ACTTTGATAGCAACTTCCAGGAATGTCACACACGATGAAATATCTCTGCTGAAGACAGTGGA
TAAAAAACAGTCCTTCAAGTCTTCTCTGTTTTTATTCTTCAACTCTCACTTTCTTAGAGTTT
ACAGAAAAAATATTTATATACGACTCTTTAAAAAGATCTATGTCTTGAAAATAGAGAAGGAA
CACAGGTCTGGCCAGGGACGTGCTGCAATTGGTGCAGTTTTGAATGCAACATTGTCCCCTAC
TGGGAATAACAGAACTGCAGGACCTGGGAGCATCCTAAAGTGTCAACGTTTTTCTATGACTT
TTAGGTAGGATGAGAGCAGAAGGTAGATCCTAAAAAGCATGGTGAGAGGATCAAATGTTTTT
ATATCAACATCCTTTATTATTTGATTCATTTGAGTTAACAGTGGTGTTAGTGATAGATTTTT
CTATTCTTTTCCCTTGACGTTTACTTTCAAGTAACACAAACTCTTCCATCAGGCCATGATCT
ATAGGACCTCCTAATGAGAGTATCTGGGTGATTGTGACCCCAAACCATCTCTCCAAAGCATT
AATATCCAATCATGCGCTGTATGTTTTAATCAGCAGAAGCATGTTTTTATGTTTGTACAAAA
GAAGATTGTTATGGGTGGGGATGGAGGTATAGACCATGCATGGTCACCTTCAAGCTACTTTA
ATAAAGGATCTTAAAATGGGCAGGAGGACTGTGAACAAGACACCCTAATAATGGGTTGATGT
CTGAAGTAGCAAATCTTCTGGAAACGCAAACTCTTTTAAGGAAGTCCCTAATTTAGAAACAC
CCACAAACTTCACATATCATAATTAGCAAACAATTGGAAGGAAGTTGCTTGAATGTTGGGGA
GAGGAAAATCTATTGGCTCTCGTGGGTCTCTTCATCTCAGAAATGCCAATCAGGTCAAGGTT
TGCTACATTTTGTATGTGTGTGATGCTTCTCCCAAAGGTATATTAACTATATAAGAGAGTTG
TGACAAAACAGAATGATAAAGCTGCGAACCGTGGCACACGCTCATAGTTCTAGCTGCTTGGG

AGGTTGAGGAGGGAGGATGGCTTGAACACAGGTGTTCAAGGCCAGCCTGGGCAACATAACAA
GATCCTGTCTCTCAAAAAAAAAAAAAAAAAAAGAAAGAGAGAGGGCCGGGCGTGGTGGCTC
ACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGCCGGGCGGATCACCTGTGGTCAGGAGTTT
GAGACCAGCCTGGCCAACATGGCAAAACCCCGTCTGTACTCAAAATGCAAAAATTAGCCAGG
CGTGGTAGCAGGCACCTGTAATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCGCTTGAA
CCCAGGAGGTGGAGGTTGCAGTAAGCTGAGATCGTGCCGTTGCACTCCAGCCTGGGCGACAA
GAGCAAGACTCTGTCTCAGAAAAAAAAAAAAAAAAGAGAGAGAGAGAGAGAAAGAGAACAATAT
TTGGGAGAGAAGGATGGGGAAGCATTGCAAGGAAATTGTGCTTTATCCAACAAAATGTAAGG
AGCCAATAAGGGATCCCTATTTGTCTCTTTTGGTGTCTATTTGTCCCTAACAACTGTCTTTG
ACAGTGAGAAAAATATTCAGAATAACCATATCCCTGTGCCGTTATTACCTAGCAACCCTTGC
AATGAAGATGAGCAGATCCACAGGAAAACTTGAATGCACAACTGTCTTATTTTAATCTTATT
GTACATAAGTTTGTAAAAGAGTTAAAAATTGTTACTTCATGTATTCATTTATATTTTATATT
ATTTTGCGTCTAATGATTTTTTATTAACATGATTTCCTTTTCTGATATATTGAAATGGAGTC
TCAAAGCTTCATAAATTTATAACTTTAGAAATGATTCTAATAACAACGTATGTAATTGTAAC
ATTGCAGTAATGGTGCTACGAAGCCATTTCTCTTGATTTTTAGTAAACTTTTATGACAGCAA
ATTTGCTTCTGGCTCACTTTCAATCAGTTAAATAAATGATAAATAATTTTGGAAGCTGTGAA
GATAAAATACCAAATAAAATAATATAAAAGTGATTTATATGAAGTTAAAATAAAAAATCAGT
ATGATGGAATAAACTTG

Other exemplary deaminases that can be fused to Cas9 according to aspects of this disclosure are provided below. In embodiments, the deaminases are activation-induced deaminases (AID). It should be understood that, in some embodiments, the active domain of the respective sequence can be used, *e.g.*, the domain without a localizing signal (nuclear localization sequence, without nuclear export signal, cytoplasmic localizing signal).

Human AID:

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHVELLFL
RYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPE
GLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYEV
DDLRDAFRTLGL (underline: nuclear localization sequence; double underline: nuclear export signal)

Mouse AID:

MDSLLMKQKKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSCSLDFGHLRNKSGCHVELLFL
RYISDWDLDPGRCYRVTWFTSWSPCYDCARHVAEFLRWNPNLSLRIFTARLYFCEDRKAEPE
GLRRLHRAGVQIGIMTFKDYFYCWNTFVENRERTFKAWEGLHENSVRLTRQLRRILLPLYEV

DDLRDAFRMLGF (underline: nuclear localization sequence; double underline: nuclear export signal)

Canine AID:

MDSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGHLRNKSGCHVELLFL
RYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFAARLYFCEDRKAEPE
GLRRLHRAGVQIAIMTFKDYFYCWNTFVENREKTFKAWEGLHENSVRLSRQLRRILLPLYEV
DDLRDAFRTLGL (underline: nuclear localization sequence; double underline: nuclear export signal)

Bovine AID:

MDSLLKKQRQFLYQFKNVRWAKGRHETYLCYVVKRRDSPTSFSLDFGHLRNKAGCHVELLFL
RYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFTARLYFCDKERKAEP
EGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYE
VDDLRDAFRTLGL (underline: nuclear localization sequence; double underline: nuclear export signal)

Rat AID:

MAVGSKPKAALVGPHWERERIWCFLCSTGLGTQQTGQTSRWLRPAATQDPVSPPRSLLMKQR
KFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGYLRNKSGCHVELLFLRYISDWDLD
PGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLTGWGALPAGLMSPARPSDYF
YCWNTFVENHERTFKAWEGLHENSVRLSRRLRRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence; double underline: nuclear export signal)

clAID (*Canis lupus familiaris*):

MDSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGHLRNKSGCHVELLFLRYISDW
DLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFAARLYFCEDRKAEPEGLRRLHRAGVQI
AIMTFKDYFYCWNTFVENREKTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL

btAID (*Bos Taurus*):

MDSLLKKQRQFLYQFKNVRWAKGRHETYLCYVVKRRDSPTSFSLDFGHLRNKAGCHVELLFLRYISDW
DLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFTARLYFCDKERKAEPEGLRRLHRAGVQ
IAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL

mAID (*Mus musculus*):

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHVELLFLRYISDW
DLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPEGLRRLHRAGVQI
AIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL

rAPOBEC-1 (*Rattus norvegicus*):

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIE
KFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLIS
SGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLT
FFTIALQSCHYQRLPPHILWATGLK

maAPOBEC-1 (*Mesocricetus auratus*):

MSSETGPVVVDPTLRRRIEPHEFDAFFDQGELRKETCLLYEIRWGGRHNIWRHTGQNTSRHVEINFIE
KFTSERYFYPSTRCSIVWFLSWSPCGECSKAITEFLSGHPNVTLFIYAARLYHHTDQRNRQGLRDLIS
RGVTIRIMTEQEYCYCWRNFVNYPPSNEVYWPRYPNLWMRLYALELYCIHLGLPPCLKIKRRHQYPLT
FFRLNLQSCHYQRIPPHILWATGFI

ppAPOBEC-1 (*Pongo pygmaeus*):

MTSEKGPSTGDPTLRRRIESWEFDVFYDPRELRKETCLLYEIKWGMSRKIWRSSGKNTTNHVEVNFIK
KFTSERRFHSSISCSITWFLSWSPCWECSQAIREFLSQHPGVTLVIYVARLFWHMDQRNRQGLRDLVN
SGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMMLYALELHCIILSLPPCLKISRRWQNHLA
FFRLHLQNCHYQTIPPHILLATGLIHPSVTWR

ocAPOBEC1 (*Oryctolagus cuniculus*):

MASEKGPSNKDYTLRRRIEPWEFEVFFDPQELRKEACLLYEIKWGASSKTWRSSGKNTTNHVEVNFLE
KLTSEGRLGPSTCCSITWFLSWSPCWECSMAIREFLSQHPGVTLIIFVARLFQHMDRRNRQGLKDLVT
SGVTVRVMSVSEYCYCWENFVNYPPGKAAQWPRYPPRWMLMYALELYCIILGLPPCLKISRRHQKQLT
FFSLTPQYCHYKMIPPYILLATGLLQPSVPWR

mdAPOBEC-1 (*Monodelphis domestica):*

MNSKTGPSVGDATLRRRIKPWEFVAFFNPQELRKETCLLYEIKWGNQNIWRHSNQNTSQHAEINFMEK
FTAERHFNSSVRCSITWFLSWSPCWECSKAIRKFLDHYPNVTLAIFISRLYWHMDQQHRQGLKELVHS
GVTIQIMSYSEYHYCWRNFVDYPQGEEDYWPKYPYLWIMLYVLELHCIILGLPPCLKISGSHSNQLAL
FSLDLQDCHYQKIPYNVLVATGLVQPFVTWR

ppAPOBEC-2 (*Pongo pygmaeus*):

MAQKEEAAAATEAASQNGEDLENLDDPEKLKELIELPPFEIVTGERLPANFFKFQFRNVEYSSGRNKT
FLCYVVEAQGKGGQVQASRGYLEDEHAAAHAEEAFFNTILPAFDPALRYNVTWYVSSSPCAACADRII
KTLSKTKNLRLLILVGRLFMWEELEIQDALKKLKEAGCKLRIMKPQDFEYVWQNFVEQEEGESKAFQP
WEDIQENFLYYEEKLADILK

btAPOBEC-2 (*Bos Taurus*):

MAQKEEAAAAAEPASQNGEEVENLEDPEKLKELIELPPFEIVTGERLPAHYFKFQFRNVEYSSGRNKT
FLCYVVEAQSKGGQVQASRGYLEDEHATNHAEEAFFNSIMPTFDPALRYMVTWYVSSSPCAACADRIV
KTLNKTKNLRLLILVGRLFMWEEPEIQAALRKLKEAGCRLRIMKPQDFEYIWQNFVEQEEGESKAFEP
WEDIQENFLYYEEKLADILK

mAPOBEC-3-(1) (*Mus musculus*):

MQPQRLGPRAGMGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPV
SLHHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECAEQIVRFLATHHNLSLD
IFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKRLLTNFRYQDSKL
QEILRPCYISVPSSSSSTLSNICLTKGLPETRFWVEGRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKP
YLCYQLEQFNGQAPLKGCLLSEKGKQHAEILFLDKIRSMELSQVTITCYLTWSPCPNCAWQLAAFKRD
RPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEIISR
RTQRRLRRIKESWGLQDLVNDFGNLQLGPPMS

Mouse APOBEC-3-(2):

MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSLHHGVFKNKD
NI*HAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFEC*AEQIVRFLATHHNLSLDIFSSRLYNVQD
PETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKRLLTNFRYQDSKLQEILRPCYIPV
PSSSSSTLSNICLTKGLPETRFCVEGRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKPYLCYQLEQFNG
QAPLKGCLLSEKGKQ*HAEILFLDKIRSMELSQVTITCYLTWSPCPNC*AWQLAAFKRDRPDLILHIYTS
RLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEIISRRTQRRLRRIKE
SWGLQDLVNDFGNLQLGPPMS (italic: nucleic acid editing domain)

Rat APOBEC-3:

MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNRLRYAIDRKDTFLCYEVTRKDCDSPVSLHHGVFKNK
DNI*HAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFEC*AEQVLRFLATHHNLSLDIFSSRLYNIR
DPENQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKKLLTNFRYQDSKLQEILRPCYIP
VPSSSSSTLSNICLTKGLPETRFCVERRRVHLLSEEEFYSQFYNQRVKHLCYYHGVKPYLCYQLEQFN
GQAPLKGCLLSEKGKQ*HAEILFLDKIRSMELSQVIITCYLTWSPCPNC*AWQLAAFKRDRPDLILHIYT
SRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEIISRRTQRRLHRIK
ESWGLQDLVNDFGNLQLGPPMS (italic: nucleic acid editing domain)

hAPOBEC-3A (*Homo sapiens*):

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYG
RHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLY
KEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGN

hAPOBEC-3F (*Homo sapiens*):

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQVYSQPEHHAEM
CFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEFLAEHPNVTLTISAARLYYYWERDYRRALCR
LSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHF
KNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVT
WYTSWSPCPECAGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCW
ENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE

Rhesus macaque APOBEC-3G:

MVEPMDPRTFVSNFNNRPILSGLNTVWLCCEVKTKDPSGPPLDAKIFQGKVYSKAKYHPEMRFLRWFH
KWRQLHHDQEYKVTWYVSWSPCTRCANSVATFLAKDPKVTLTIFVARLYYFWKPDYQQALRILCQKRG
GPHATMKIMNYNEFQDCWNKFVDGRGKPFKPRNNLPKHYTLLQATLGELLRHLMDPGTFTSNFNNKPW
VSGQHETYLCYKVERLHNDTWVPLNQHRGFLRNQAPNIHGFPKGRHAELCFLDLIPFWKLDGQQYRVT
CFTSWSPCFSCAQEMAKFISNNEHVSLCIFAARIYDDQGRYQEGLRALHRDGAKIAMMNYSEFEYCWD
TFVDRQGRPFQPWDGLDEHSQALSGRLRAI (italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Chimpanzee APOBEC-3G:

MKPHFRNPVERMYQDTFSDNFYNRPILSHRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSKLKY*HPEM*
*RFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDVATFLAEDPKVTLTIFVARLYYFWDPDYQEALR
SLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTS
NFNNELWVRGRHETYLCYEVERLHNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLD*
*LHQDYRVTCFTSWSPCFSC*AQEMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLAKAGAKISIMTY
SEFKHCWDTFVDHQGCPFQPWDGLEEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Green monkey APOBEC-3G:

MNPQIRNMVEQMEPDIFVYYFNNRPILSGRNTVWLCYEVKTKDPSGPPLDANIFQGKLYPEAKD*HPEM*
*KFLHWFRKWRQLHRDQEYEVTWYVSWSPCTRC*ANSVATFLAEDPKVTLTIFVARLYYFWKPDYQQALR
ILCQERGGPHATMKIMNYNEFQHCWNEFVDGQGKPFKPRKNLPKHYTLLHATLGELLRHVMDPGTFTS
NFNNKPWVSGQRETYLCYKVERSHNDTWVLLNQHRGFLRNQAPDRHGFPKGR*HAELCFLDLIPFWKLD*
*DQQYRVTCFTSWSPCFSC*AQKMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLHRDGAKIAVMNYS
EFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Human APOBEC-3G:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSELKY*HPEM*
*RFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDMATFLAEDPKVTLTIFVARLYYFWDPDYQEALR
SLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTF
NFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLD*
*LDQDYRVTCFTSWSPCFSC*AQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTY
SEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Human APOBEC-3F:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQVYSQPEH*HAEM*
*CFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKLAEFLAEHPNVTLTISAARLYYYWERDYRRALCR
LSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHF
KNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQVDPETH*CHAERCFLSWFCDDILSPNTNYEVT*

*WYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCW
ENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE

(italic: nucleic acid editing domain)

Human APOBEC-3B:

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGQVYFKPQY*HAE*
*MCFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKLAEFLSEHPNVTLTISAARLYYYWERDYRRALC
RLSQAGARVTIMDYEEFAYCWENFVYNEGQQFMPWYKFDENYAFLHRTLKEILRYLMDPDTFTFNFNN
DPLVLRRRQTYLCYEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFY*GRHAELRFLDLVPSLQLDPAQI*
*YRVTWFISWSPCFSWGC*AGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDE
FEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Rat APOBEC-3B:

MQPQGLGPNAGMGPVCLGCSHRRPYSPIRNPLKKLYQQTFYFHFKNVRYAWGRKNNFLCYEVNGMDCA
LPVPLRQGVFRKQGHIHAELCFIYWFHDKVLRVLSPMEEFKVTWYMSWSPCSKCAEQVARFLAAHRNL
SLAIFSSRLYYYLRNPNYQQKLCRLIQEGVHVAAMDLPEFKKCWNKFVDNDGQPFRPWMRLRINFSFY
DCKLQEIFSRMNLLREDVFYLQFNNSHRVKPVQNRYYRRKSYLCYQLERANGQEPLKGYLLYKKGEQH
VEILFLEKMRSMELSQVRITCYLTWSPCPNCARQLAAFKKDHPDLILRIYTSRLYFWRKKFQKGLCTL
WRSGIHVDVMDLPQFADCWTNFVNPQRPFRPWNELEKNSWRIQRRLRRIKESWGL

Bovine APOBEC-3B:

DGWEVAFRSGTVLKAGVLGVSMTEGWAGSGHPGQGACVWTPGTRNTMNLLREVLFKQQFGNQPRVPAP
YYRRKTYLCYQLKQRNDLTLDRGCFRNKKQRHAERFIDKINSLDLNPSQSYKIICYITWSPCPNCANE
LVNFITRNNHLKLEIFASRLYFHWIKSFKMGLQDLQNAGISVAVMTHTEFEDCWEQFVDNQSRPFQPW
DKLEQYSASIRRRLQRILTAPI

Chimpanzee APOBEC-3B:

MNPQIRNPMEWMYQRTFYYNFENEPILYGRSYTWLCYEVKIRRGHSNLLWDTGVFRGQMYSQPEHHAE
MCFLSWFCGNQLSAYKCFQITWFVSWTPCPDCVAKLAKFLAEHPNVTLTISAARLYYYWERDYRRALC
RLSQAGARVKIMDDEEFAYCWENFVYNEGQPFMPWYKFDDNYAFLHRTLKEIIRHLMDPDTFTFNFNN
DPLVLRRHQTYLCYEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFYGRHAELRFLDLVPSLQLDPAQI
YRVTWFISWSPCFSWGCAGQVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDE
FEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQVRASSLCMVPHRPPPPPQSPGPCLPLCSEP
PLGSLLPTGRPAPSLPFLLTASFSFPPPASLPPLPSLSLSPGHLPVPSFHSLTSCSIQPPCSSRIRET
EGWASVSKEGRDLG

Human APOBEC-3C:

MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVFRNQVDSETH*CHAE*
*RCFLSWFCDDILSPNTKYQVTWYTSWSPCPDC*AGEVAEFLARHSNVNLTIFTARLYYFQYPCYQEGLR
SLSQEGVAVEIMDYEDFKYCWENFVYNDNEPFKPWKGLKTNFRLLKRRLRESLQ

(italic: nucleic acid editing domain)

Gorilla APOBEC-3C

MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVFRNQVDSETH*CHAE*
*RCFLSWECDDILSPNTNYQVTWYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLYYFQDTDYQEGLR
SLSQEGVAVKIMDYKDFKYCWENFVYNDDEPFKPWKGLKYNFRFLKRRLQEILE

(italic: nucleic acid editing domain)

Human APOBEC-3A:

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYG
R*HAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGC*AGEVRAFLQENTHVRLRIFAARIYDYDPLY
KEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3A:

MDGSPASRPRHLMDPNTFTFNFNNDLSVRGRHQTYLCYEVERLDNGTWVPMDERRGFLCNKAKNVPCG
DYGC*HVELRFLCEVPSWQLDPAQTYRVTWFISWSPC*FRRGCAGQVRVFLQENKHVRLRIFAARIYDYD
PLYQEALRTLRDAGAQVSIMTYEEFKHCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Bovine APOBEC-3A:

MDEYTFTENFNNQGWPSKTYLCYEMERLDGDATIPLDEYKGFVRNKGLDQPEKPC*HAELYFLGKIHSW*
*NLDRNQHYRLTCFISWSPC*YDCAQKLTTFLKENHHISLHILASRIYTHNRFGCHQSGLCELQAAGARI
TIMTFEDFKHCWETFVDHKGKPFQPWEGLNVKSQALCTELQAILKTQQN

(italic: nucleic acid editing domain)

Human APOBEC-3H:

MALLTAETFRLQFNNKRRLRRPYYPRKALLCYQLTPQNGSTPTRGYFENKKKC*HAEICFINEIKSMGL*
*DETQCYQVTCYLTWSPCSSC*AWELVDFIKAHDHLNLGIFASRLYYHWCKPQQKGLRLLCGSQVPVEVM
GFPKFADCWENFVDHEKPLSFNPYKMLEELDKNSRAIKRRLERIKIPGVRAQGRYMDILCDAEV

(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3H:

MALLTAKTFSLQFNNKRRVNKPYYPRKALLCYQLTPQNGSTPTRGHLKNKKKDHAEIRFINKIKSMGL
DETQCYQVTCYLTWSPCPSCAGELVDFIKAHRHLNLRIFASRLYYHWRPNYQEGLLLLCGSQVPVEVM
GLPEFTDCWENFVDHKEPPSFNPSEKLEELDKNSQAIKRRLERIKSRSVDVLENGLRSLQLGPVTPSS
SIRNSR

Human APOBEC-3D:

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGPVLPKRQSNHR
QEVYFRFEN*HAEMCFLSWFCGNRLPANRRFQITWFVSWNPC*LPCVVKVTKFLAEHPNVTLTISAARLY
YYRDRDWRWVLLRLHKAGARVKIMDYEDFAYCWENFVCNEGQPFMPWYKFDDNYASLHRTLKEILRNP

MEAMYPHIFYFHFKNLLKACGRNESWLCFTMEVTKHHSAVFRKRGVFRNQVDPETHC*HAERCFLSWFC*
*DDILSPNTNYEVTWYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLCYFWDTDYQEGLCSLSQEGAS
VKIMGYKDFVSCWKNFVYSDDEPFKPWKGLQTNFRLLKRRLREILQ

(italic: nucleic acid editing domain)

Human APOBEC-1:

MTSEKGPSTGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKNTTNHVEVNFIK
KFTSERDFHPSMSCSITWFLSWSPCWECSQAIREFLSRHPGVTLVIYVARLFWHMDQQNRQGLRDLVN
SGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMMLYALELHCIILSLPPCLKISRRWQNHLT
FFRLHLQNCHYQTIPPHILLATGLIHPSVAWR

Mouse APOBEC-1:

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSVWRHTSQNTSNHVEVNFLE
KFTTERYFRPNTRCSITWFLSWSPCGECSRAITEFLSRHPYVTLFIYIARLYHHTDQRNRQGLRDLIS
SGVTIQIMTEQEYCYCWRNFVNYPPSNEAYWPRYPHLWVKLYVLELYCIILGLPPCLKILRRKQPQLT
FFTITLQTCHYQRIPPHLLWATGLK

Rat APOBEC-1:

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIE
KFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLIS
SGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLT
FFTIALQSCHYQRLPPHILWATGLK

Human APOBEC-2:

MAQKEEAAVATEAASQNGEDLENLDDPEKLKELIELPPFEIVTGERLPANFFKFQFRNVEYSSGRNKT
FLCYVVEAQGKGGQVQASRGYLEDEHAAAHAEEAFFNTILPAFDPALRYNVTWYVSSSPCAACADRII
KTLSKTKNLRLLILVGRLFMWEEPEIQAALKKLKEAGCKLRIMKPQDFEYVWQNFVEQEEGESKAFQP
WEDIQENFLYYEEKLADILK

Mouse APOBEC-2:

MAQKEEAAEAAAPASQNGDDLENLEDPEKLKELIDLPPFEIVTGVRLPVNFFKFQFRNVEYSSGRNKT
FLCYVVEVQSKGGQAQATQGYLEDEHAGAHAEEAFFNTILPAFDPALKYNVTWYVSSSPCAACADRIL
KTLSKTKNLRLLILVSRLFMWEEPEVQAALKKLKEAGCKLRIMKPQDFEYIWQNFVEQEEGESKAFEP
WEDIQENFLYYEEKLADILK

Rat APOBEC-2:

MAQKEEAAEAAAPASQNGDDLENLEDPEKLKELIDLPPFEIVTGVRLPVNFFKFQFRNVEYSSGRNKT
FLCYVVEAQSKGGQVQATQGYLEDEHAGAHAEEAFFNTILPAFDPALKYNVTWYVSSSPCAACADRIL
KTLSKTKNLRLLILVSRLFMWEEPEVQAALKKLKEAGCKLRIMKPQDFEYLWQNFVEQEEGESKAFEP
WEDIQENFLYYEEKLADILK

Bovine APOBEC-2:

MAQKEEAAAAAEPASQNGEEVENLEDPEKLKELIELPPFEIVTGERLPAHYFKFQFRNVEYSSGRNKT
FLCYVVEAQSKGGQVQASRGYLEDEHATNHAEEAFFNSIMPTFDPALRYMVTWYVSSSPCAACADRIV
KTLNKTKNLRLLILVGRLFMWEEPEIQAALRKLKEAGCRLRIMKPQDFEYIWQNFVEQEEGESKAFEP
WEDIQENFLYYEEKLADILK

Petromyzon marinus CDA1 (pmCDAl):

MTDAEYVRIHEKLDIYTFKKQFFNNKKSVSHRCYVLFELKRRGERRACFWGYAVNKPQSGTERGIHAE
IFSIRKVEEYLRDNPGQFTINWYSSWSPCADCAEKILEWYNQELRGNGHTLKIWACKLYYEKNARNQI
GLWNLRDNGVGLNVMVSEHYQCCRKIFIQSSHNQ
LNENRWLEKTLKRAEKRRSELSFMIQVKILHTTKSPAV

Human APOBEC3G D316R D317R:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSELKYHPEM
RFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMATFLAEDPKVTLTIFVARLYYFWDPDYQEALR
SLCQKRDGPRATMKFNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHFMLGEILRHSMDPPTFTFN
FNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDL
DQDYRVTCFTSWSPCFSCAQEMAKFISKKHVSLCIFTARIYRRQGRCQEGLRTLAEAGAKISFTYSEF
KHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN

Human APOBEC3G chain A:

MDPPTFTFNFNNEPWWGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDV
IPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGA
KISFTYSEFKHCWDTFVDHQGCPFQPWDGLD EHSQDLSGRLRAILQ

Human APOBEC3G chain A D120R D121R:

MDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLD
VIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYRRQGRCQEGLRTLAEAG
AKISFMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQ

hAPOBEC-4 (*Homo sapiens*):

MEPIYEEYLANHGTIVKPYYWLSFSLDCSNCPYHIRTGEEARVSLTEFCQIFGFPYGTTFPQTKHLTF
YELKTSSGSLVQKGHASSCTGNYIHPESMLFEMNGYLDSAIYNNDSIRHIILYSNNSPCNEANHCCIS
KMYNFLITYPGITLSIYFSQLYHTEMDFPASAWNREALRSLASLWPRVVLSPISGGIWHSVLHSFISG
VSGSHVFQPILTGRALADRHNAYEINAITGVKPYFTDVLLQTKRNPNTKAQEALESYPLNNAFPGQFF
QMPSGQLQPNLPPDLRAPVVFVLVPLRDLPPMHMGQNPNKPRNIVRHLNMPQMSFQETKDLGRLPTGR
SVEIVEITEQFASSKEADEKKKKKGKK

mAPOBEC-4 (*Mus musculus*):

MDSLLMKQKKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSCSLDFGHLRNKSGCHVELLFLRYISDW
DLDPGRCYRVTWFTSWSPCYDCARHVAEFLRWNPNLSLRIFTARLYFCEDRKAEPEGLRRLHRAGVQI
GIMTFKDYFYCWNTFVENRERTFKAWEGLHENSVRLTRQLRRILLPLYEVDDLRDAFRMLGF

rAPOBEC-4 (*Rattus norvegicus*):

MEPLYEEYLTHSGTIVKPYYWLSVSLNCTNCPYHIRTGEEARVPYTEFHQTFGFPWSTYPQTKHLTFY
ELRSSSGNLIQKGLASNCTGSHTHPESMLFERDGYLDSLIFHDSNIRHIILYSNNSPCDEANHCCISK
MYNFLMNYPEVTLSVFFSQLYHTENQFPTSAWNREALRGLASLWPQVTLSAISGGIWQSILETFVSGI
SEGLTAVRPFTAGRTLTDRYNAYEINCITEVKPYFTDALHSWQKENQDQKVWAASENQPLHNTTPAQW
QPDMSQDCRTPAVFMLVPYRDLPPIHVNPSPQKPRTVVRHLNTLQLSASKVKALRKSPSGRPVKKEEA
RKGSTRSQEANETNKSKWKKQTLFIKSNICHLLEREQKKIGILSSWSV

mfAPOBEC-4 (*Macaca fascicularis*):

MEPTYEEYLANHGTIVKPYYWLSFSLDCSNCPYHIRTGEEARVSLTEFCQIFGFPYGTTYPQTKHLTF
YELKTSSGSLVQKGHASSCTGNYIHPESMLFEMNGYLDSAIYNNDSIRHIILYCNNSPCNEANHCCIS
KVYNFLITYPGITLSIYFSQLYHTEMDFPASAWNREALRSLASLWPRVVLSPISGGIWHSVLHSFVSG
VSGSHVFQPILTGRALTDRYNAYEINAITGVKPFFTDVLLHTKRNPNTKAQMALESYPLNNAFPGQSF
QMTSGIPPDLRAPVVFVLLPLRDLPPMHMGQDPNKPRNIIRHLNMPQMSFQETKDLERLPTRRSVETV
EITERFASSKQAEEKTKKKKGKK

pmCDA-1 (*Petromyzon marinus*):

MAGYECVRVSEKLDFDTFEFQFENLHYATERHRTYVIFDVKPQSAGGRSRRLWGYIINNPNVCHAELI
LMSMIDRHLESNPGVYAMTWYMSWSPCANCSSKLNPWLKNLLEEQGHTLTMHFSRIYDRDREGDHRGL
RGLKHVSNSFRMGVVGRAEVKECLAEYVEASRRTLTWLDTTESMAAKMRRKLFCILVRCAGMRESGIP
LHLFTLQTPLLSGRVVWWRV

pmCDA-2 (*Petromyzon marinus*):

MELREVVDCALASCVRHEPLSRVAFLRCFAAPSQKPRGTVILFYVEGAGRGVTGGHAVNYNKQGTSIH
AEVLLLSAVRAALLRRRRCEDGEEATRGCTLHCYSTYSPCRDCVEYIQEFGASTGVRVVIHCCRLYEL
DVNRRRSEAEGVLRSLSRLGRDFRLMGPRDAIALLLGGRLANTADGESGASGNAWVTETNVVEPLVDM
TGFGDEDLHAQVQRNKQIREAYANYASAVSLMLGELHVDPDKFPFLAEFLAQTSVEPSGTPRETRGRP
RGASSRGPEIGRQRPADFERALGAYGLFLHPRIVSREADREEIKRDLIVVMRKHNYQGP

pmCDA-5 (*Petromyzon marinus*):

MAGDENVRVSEKLDFDTFEFQFENLHYATERHRTYVIFDVKPQSAGGRSRRLWGYIINNPNVCHAELI
LMSMIDRHLESNPGVYAMTWYMSWSPCANCSSKLNPWLKNLLEEQGHTLMMHFSRIYDRDREGDHRGL
RGLKHVSNSFRMGVVGRAEVKECLAEYVEASRRTLTWLDTTESMAAKMRRKLFCILVRCAGMRESGMP
LHLFT

yCD (*Saccharomyces cerevisiae*):

MVTGGMASKWDQKGMDIAYEEAALGYKEGGVPIGGCLINNKDGSVLGRGHNMRFQKGSATLHGEISTL
ENCGRLEGKVYKDTTLYTTLSPCDMCTGAIIMYGIPRCVVGENVNFKSKGEKYLQTRGHEVVVVDDER
CKKIMKQFIDERPQDWFEDIGE

rAPOBEC-1 (delta 177-186):

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIE
KFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLIS

SGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRGLPPCLNILRRKQPQLTFFTIALQSCHY
QRLPPHILWATGLK

rAPOBEC-1 (delta 202-213):

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIE
KFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLIS
SGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCIILGLPPCLNILRRKQPQHY
QRLPPHILWATGLK

Mouse APOBEC-3:

MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSLHHG
VFKNKDNI*HAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFEC*AEQIVRFLATHHNLSL
DIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKRLLTNF
RYQDSKLQEILRPCYIPVPSSSSSTLSNICLTKGLPETRFCVEGRRMDPLSEEEFYSQFYNQ
RVKHLCYYHRMKPYLCYQLEQFNGQAPLKGCLLSEKGKQ*HAEILFLDKIRSMELSQVTITCY*
*LTWSPCPNC*AWQLAAFKRDRPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQF
TDCWTNFVNPKRPFWPWKGLEIISRRTQRRLRRIKESWGLQDLVNDFGNLQLGPPMS

(italic: nucleic acid editing domain)

Some aspects of the present disclosure are based on the recognition that modulating the deaminase domain catalytic activity of any of the fusion proteins described herein, for example by making point mutations in the deaminase domain, affect the processivity of the fusion proteins (*e.g.*, base editors). For example, mutations that reduce, but do not eliminate, the catalytic activity of a deaminase domain within a base editing fusion protein can make it less likely that the deaminase domain will catalyze the deamination of a residue adjacent to a target residue, thereby narrowing the deamination window. The ability to narrow the deamination window can prevent unwanted deamination of residues adjacent to specific target residues, which can decrease or prevent off-target effects.

For example, in some embodiments, an APOBEC deaminase incorporated into a base editor can comprise one or more mutations selected from the group consisting of H121X, H122X, R126X, R126X, R118X, W90X, W90X, and R132X of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase, wherein X is any amino acid. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise one or more mutations selected from the group consisting of H121R, H122R, R126A, R126E, R118A, W90A, W90Y, and R132E of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase.

In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise one or more mutations selected from the group consisting of D316X, D317X, R320X, R320X, R313X, W285X, W285X, R326X of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase, wherein X is any amino acid. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising one or more mutations selected from the group consisting of D316R, D317R, R320A, R320E, R313A, W285A, W285Y, R326E of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase.

In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise a H121R and a H122R mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R126A mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R126E mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R118A mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W90A mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W90Y mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R132E mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W90Y and a R126E mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R126E and a R132E mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an

APOBEC deaminase comprising a W90Y and a R132E mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W90Y, R126E, and R132E mutation of rAPOBEC1, or one or more corresponding mutations in another APOBEC deaminase.

In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a D316R and a D317R mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R320A mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R320E mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R313A mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W285A mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W285Y mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R326E mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W285Y and a R320E mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a R320E and a R326E mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor can comprise an APOBEC deaminase comprising a W285Y and a R326E mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, an APOBEC deaminase incorporated into a base editor

can comprise an APOBEC deaminase comprising a W285Y, R320E, and R326E mutation of hAPOBEC3G, or one or more corresponding mutations in another APOBEC deaminase.

A number of modified cytidine deaminases are commercially available, including, but not limited to, SaBE3, SaKKH-BE3, VQR-BE3, EQR-BE3, VRER-BE3, YE1-BE3, EE-BE3, YE2-BE3, and YEE-BE3, which are available from Addgene (plasmids 85169, 85170, 85171, 85172, 85173, 85174, 85175, 85176, 85177). In some embodiments, a deaminase incorporated into a base editor comprises all or a portion of an APOBEC1 deaminase.

Details of C to T nucleobase editing proteins are described in International PCT Application No. PCT/US2016/058344 (WO2017/070632) and Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference.

Cytidine Deaminases

The fusion proteins provided herein comprise one or more cytidine deaminases. In some embodiments, the cytidine deaminases provided herein are capable of deaminating cytosine or 5-methylcytosine to uracil or thymine. In some embodiments, the cytidine deaminases provided herein are capable of deaminating cytosine in DNA. The cytidine deaminase may be derived from any suitable organism. In some embodiments, the cytidine deaminase is a naturally-occurring cytidine deaminase that includes one or more mutations corresponding to any of the mutations provided herein. One of skill in the art will be able to identify the corresponding residue in any homologous protein, e.g., by sequence alignment and determination of homologous residues. Accordingly, one of skill in the art would be able to generate mutations in any naturally-occurring cytidine deaminase that corresponds to any of the mutations described herein. In some embodiments, the cytidine deaminase is from a prokaryote. In some embodiments, the cytidine deaminase is from a bacterium. In some embodiments, the cytidine deaminase is from a mammal (e.g., human).

In some embodiments, the cytidine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the cytidine deaminase amino acid sequences set forth herein. It should be appreciated that cytidine deaminases provided herein may include one or more mutations (*e.g.*, any of the mutations provided herein). The disclosure provides any

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 263

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 263

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

What is claimed is:

1. A method of editing a nucleobase of a hepatitis B virus (HBV) genome, the method comprising contacting the HBV genome with one or more guide RNAs and a base editor comprising a polynucleotide programmable DNA binding domain and an adenosine deaminase or cytidine deaminase domain, wherein said guide RNA targets said base editor to effect an alteration of the nucleobase of the HBV genome.

2. The method of claim 1, wherein the nucleobase is in a polynucleotide encoding an HBV protein.

3. The method of claim 1 or 2, wherein the contacting is in a eukaryotic cell, a mammalian cell, or a human cell.

4. The method of any one of claims 1 to 3, wherein the cell is *in vivo* or *ex vivo*.

5. The method of any one of claims 1 to 4, wherein the cytidine deaminase converts a target C to U in the HBV genome.

6. The method of any one of claims 1 to 4, wherein the cytidine deaminase converts a target C•G to T•A in the polynucleotide encoding the HBV protein.

7. The method of any one of claims 1 to 4, wherein the adenosine deaminase converts a target A•T to G•C in the polynucleotide encoding the HBV protein.

8. The method of any one of claims 2-7, wherein alteration of the nucleobase in the polynucleotide encoding the HBV protein results in a premature termination codon.

9. The method of claim 8, wherein the alteration of the nucleobase results in an R87* or W120* termination in an HBV X protein.

10. The method of claim 8, wherein the alteration of the nucleobase results in an W35* or W36* in an HBV S protein.

11. The method of claim of any one of claims 2-7, wherein the alteration of the HBV polynucleotide is a missense mutation.

12. The method of claim 11, wherein the missense mutation is in an HBV pol gene.

13. The method of claim 12, wherein the missense mutation results in an E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P, R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P in an HBV polymerase protein encoded by the HBV pol gene.

14. The method of claim 11, wherein the missense mutation is in an HBV core gene.

15. The method of claim 14, wherein the missense mutation results in a T160A, T160A, P161F, S162L, C183R, or *184Q in an HBV core protein encoded by the HBV core gene.

16. The method of claim 11, wherein the missense mutation is in an HBV X gene.

17. The method of claim 16, wherein the missense mutation results in a H86R, W120R, E122K, E121K, or L141P in an HBV X protein encoded by the HBV X gene.

18. The method of claim 11, wherein the missense mutation is in an HBV S gene.

19. The method of claim 18, wherein the missense mutation results in a S38F, L39F, W35R, W36R, T37I, T37A, R78Q, S34L, F80P, or D33G in an HBV S protein encoded by the HBV S gene.

20. The method of any one of claims 1-19, wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof.

21. The method of claim 18, wherein the Cas9 has protospacer-adjacent motif (PAM) specificity for a nucleic acid sequence selected from 5′-NGG-3′, 5′-NAG-3′, 5′-NGA-3′, 5′-NAA-3′, 5′-NNAGGA-3′, or 5′-NNACCA-3′.

22. The method of any one of claims 1-20, wherein the polynucleotide programmable DNA binding domain comprises a modified Cas9 having an altered protospacer-adjacent motif (PAM) specificity.

23. The method of claim 19, wherein the nucleic acid sequence of the altered PAM is selected from 5′-NNNRRT-3′, NGA-3′, 5′-NGCG-3′, 5′-NGN-3′, NGCN-3′, 5′-NGTN-3′, or 5′-NAA-3′.

24. The method of any one of claims 1-23, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant.

25. The method of claim 24, wherein the nuclease inactive or nickase variant is a nuclease inactivated Cas9 (dCas9) which comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

26. The method of any one of claims 1-25, wherein the adenosine deaminase domain is capable of deaminating adenine in deoxyribonucleic acid (DNA).

27. The method of any one of claims 1-26, wherein the adenosine deaminase is a TadA deaminase.

28. The method of claim 27, wherein the TadA deaminase is TadA*7.10, TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

29. The method of any one of claims 1-25, wherein the cytidine deaminase domain is capable of deaminating cytidine in DNA.

30. The method of claim 29, wherein the cytidine deaminase is APOBEC or a derivative thereof.

31. The method of claim any one of claims 1-30, wherein the base editor further comprises one or more uracil glycosylase inhibitors (UGIs).

32. The method of any one of claims 1-31, wherein the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to an HBV nucleic acid sequence.

33. The method of any one of claims 1-32, wherein the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an HBV nucleic acid sequence.

34. The method of any one of claims 2-33, wherein the HBV protein is the S, pol, core or X protein.

35. The method of any one of claims 1-34, comprising editing one or more nucleobases.

36. The method of any one of claims 1-35, comprising two or more guide RNAs that target two or more HBV nucleic acid sequences.

37. The method of any one of claims 1-36, wherein the guide RNAs comprise a sequence, from 5′ to 3′, or a 1, 2, 3, 4, or 5 nucleotide 5′ truncation fragment thereof, selected from one or more of
UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU;
AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC;
GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG;
UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA;
AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU;
CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU;

CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; or AAUCCACACUCCGAAAGACA.

38. A method of treating hepatitis B virus (HBV) infection in a subject comprising administering to a subject in need thereof a fusion protein or polynucleotide encoding said fusion protein, the fusion protein comprising a polynucleotide programmable DNA binding domain and a base editor domain that is an adenosine deaminase or a cytidine deaminase domain, and one or more guide polynucleotides that target the base editor domain to effect an A•T to G•C, C•G to T•A, or C•G to U•A alteration of the nucleic acid sequence encoding an HBV polypeptide.

39. A method of treating hepatitis B virus (HBV) infection in a subject, comprising administering to a subject in need thereof one or more polynucleotides encoding a polynucleotide programmable DNA binding domain and a base editor domain that is an adenosine deaminase or a cytidine deaminase domain, and one or more guide polynucleotides that target the base editor domain to effect an A•T to G•C, C•G to T•A, or C•G to U•A alteration of the nucleic acid sequence encoding an HBV polypeptide.

40. The method of claim 38 or claim 39, wherein the subject is a mammal or a human.

41. The method of any one of claims 38-40, comprising delivering the fusion protein, the polynucleotide encoding said fusion protein, or the one or more polynucleotides encoding a polynucleotide programmable DNA binding domain and the base editor domain, and said one or more guide polynucleotides to a cell of the subject.

42. The method of any one of claims 38-41, wherein the cell is a hepatocyte.

43. The method of any one of claims 38-42, wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof.

44. The method of claim 43, wherein the Cas9 has protospacer-adjacent motif (PAM) specificity for a nucleic acid sequence selected from 5′-NGG-3′, 5′-NAG-3′, 5′-NGA-3′, 5′-NAA-3′, 5′-NNAGGA-3′, or 5′-NNACCA-3′.

45. The method of any one of claims 38-44, wherein the polynucleotide programmable DNA binding domain comprises a modified Cas9 having an altered protospacer-adjacent motif (PAM) specificity.

46. The method of claim 45, wherein the nucleic acid sequence of the altered PAM is selected from 5′-NNNRRT-3′, NGA-3′, 5′-NGCG-3′, 5′-NGN-3′, NGCN-3′, 5′-NGTN-3′, or 5′-NAA-3′.

47. The method of any one of claims 38-46, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant.

48. The method of claim 47, wherein the nuclease inactive or nickase variant is a nuclease inactivated Cas9 (dCas9) which comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

49. The method of any one of claims 38-48, wherein the adenosine deaminase domain is capable of deaminating adenine in deoxyribonucleic acid (DNA).

50. The method of claim 49, wherein the adenosine deaminase is a TadA deaminase.

51. The method of claim 50, wherein the TadA deaminase is TadA*7.10, TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16,

TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

52. The method of any one of claims 38-48, wherein the cytidine deaminase domain is capable of deaminating cytidine in DNA.

53. The method of claim 52, wherein the cytidine deaminase is APOBEC or a derivative thereof.

54. The method of any one of claims 52-54, wherein the base editor further comprises one or more uracil glycosylase inhibitors (UGIs).

55. The method of any one of claims 52-54, wherein the base editor does not comprise a uracil glycosylase inhibitor (UGI).

56. The method of any one of claims 38-55, wherein the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to an HBV nucleic acid sequence.

57. The method of any one of claims 38-56, wherein the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an HBV nucleic acid sequence.

58. The method of claim 57, wherein the sgRNA comprises a nucleic acid sequence comprising at least 10 contiguous nucleotides that are complementary to the HBV nucleic acid sequence.

59. The method of claim 58, wherein the sgRNA comprises a nucleic acid sequence comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that are complementary to the HBV nucleic acid sequence.

60. The method of any one of claims 38-59, comprising editing one or more nucleobases.

61. The method of any one of claims 38-60, comprising two or more guide RNAs that target two or more HBV nucleic acid sequences.

62. The method of claim 61, comprising two or more guide RNAs that target three, four, or five HBV nucleic acid sequences.

63. The method of claim 61 or claim 62, wherein the HBV nucleic acid sequences encode one or more HBV proteins selected from HBV polymerase, HBV core protein, HBV S protein, HBV X protein, or a combination thereof.

64. The method of any one of claims 38-63, wherein the one or more guide RNAs comprise a sequence, from 5′ to 3′, or a 1, 2, 3, 4, or 5 nucleotide 5′ truncation fragment thereof, selected from one or more of
UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU;
AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC;
GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG;
UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA;
AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU;
CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU;
CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG;
AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA;
CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA;
GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC;
UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG;
UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; or
AAUCCACACUCCGAAAGACA.

65. The method of any one of claims 38-64, wherein the alteration of the polynucleotide encoding the HBV protein is a premature termination codon.

66. The method of any only of claims 38-65, wherein the alteration of the nucleic acid sequence results in an R87* or W120* in an HBV X protein encoded by the nucleic acid.

67. The method of claim 66, wherein the alteration of the nucleic acid sequence results in a W35* or W36* in an HBV S protein encoded by the nucleic acid.

68. The method of claim of claim 38-64, wherein the alteration of the polynucleotide encoding the HBV protein is a missense mutation.

69. The method of claim 68, wherein the missense mutation is in an HBV pol gene.

70. The method of claim 69, wherein the missense mutation in the HBV pol gene results in a E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P, R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P in an HBV polymerase encoded by the HBV pol gene.

71. The method of claim 68, wherein the missense mutation is in an HBV core gene.

72. The method of claim 71, wherein the missense mutation in the HBV core gene results in a T160A, T160A, P161F, S162L, C183R, or *184Q in an HBV core protein encoded by the HBV core gene.

73. The method of claim 68, wherein the missense mutation is in an HBV X gene.

74. The method of claim 73, wherein the missense mutation in the HBV X gene results in an H86R, W120R, E122K, E121K, or L141P in an HBV X protein encoded by the HBV X gene.

75. The method of claim 68, wherein the missense mutation is in an HBV S gene.

76. The method of claim 75, wherein the missense mutation in the HBV S gene results in a S38F, L39F, W35R, W36R, T37I, T37A, R78Q, S34L, F80P, or D33G in an HBV S protein encoded by the HBV S gene.

77. The method of any one of claims 1-76, wherein the base editor is a BE4 or a variant of BE4 where APOBEC-1 is replaced with the sequence of APOBEC-3A, and/or Cas9 is replaced with a Cas9 variant comprising V1134, R1217, Q1334, and R1336 (termed SpCas9-VRQR).

78. A composition comprising a base editor bound to a guide RNA, wherein the guide RNA comprises a nucleic acid sequence that is complementary to an HBV gene.

79. The composition of claim 78, wherein the base editor comprises an adenosine deaminase or a cytidine deaminase.

80. The composition of claim 79, wherein the adenosine deaminase is capable of deaminating adenine in deoxyribonucleic acid (DNA).

81. The composition of claim 80, wherein the adenosine deaminase is a TadA deaminase.

82. The composition of claim 81, wherein the TadA deaminase is TadA*7.10, TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

83. The composition of claim 79, wherein the cytidine deaminase domain is capable of deaminating cytidine in DNA.

84. The composition of claim 83, wherein the cytidine deaminase is APOBEC or a derivative thereof.

85. The composition of claim 83 or claim 84, wherein the base editor further comprises one or more uracil glycosylase inhibitors (UGIs).

86. The composition of claim 83 or claim 84, wherein the base editor does not comprise a uracil glycosylase inhibitor (UGI).

87. The composition of any one of claims 78-86, wherein the base editor

(i) comprises a Cas9 nickase;

(ii) comprises a nuclease inactive Cas9;

(iii) does not comprise a UGI domain;

(iv) comprises an APOBEC-1 or APOBEC-3A cytidine deaminase;

(v) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQN
TNKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARL
YHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLE
LYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETP
GTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKK
NLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLV
EEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFL
IEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPG
EKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA
KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS
KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
ELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE
EVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS
GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKD
KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRK
LINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL
AGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIK
ELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDS
IDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDK
AGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVK
ELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATL
IHQSITGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE;or

(vi) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE.

88. The composition of any one of claims 78-87, wherein the guide RNA comprises a nucleic acid sequence that is complementary to an HBV gene encoding an HBV polymerase, HBV core protein, HBV S protein, or HBV X protein.

89. The composition of any one of claims 78-88, wherein the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV X protein.

90. The composition of any one of claims 78-88, wherein the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV S protein.

91. The composition of any one of claims 78-8888, wherein the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV polymerase.

92. The composition of any one of claims 78-88, wherein the guide RNA comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV core protein.

93. The composition of any one of claims 78-92, wherein the guide RNA comprises a 1, 2, 3, 4, or 5 nucleic acid truncation from the 5′ end of a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC;
GGGAACAAGAUCUACAGCAU;
AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC;
GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG;
UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA;
AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU;
CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU;
CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG;
AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA;
CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA;
GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC;
UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG;
UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and
AAUCCACACUCCGAAAGACA.

94. The composition of any one of claims 78-92, wherein the guide RNA comprises a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU;
AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC;
GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG;
UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA;
AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU;
CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU;

CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA.

95. The composition of any one of claims 78-94, further comprising a lipid, optionally wherein the lipid is a cationic lipid.

96. The composition of any one of claims 78-95, further comprising a pharmaceutically acceptable excipient.

97. A pharmaceutical composition for the treatment of HBV infection comprising

(i) a base editor, or a nucleic acid encoding the base editor, and one or more guide RNAs (gRNA) comprising a nucleic acid sequence complementary to an HBV gene in a pharmaceutically acceptable excipient.

98. The pharmaceutical composition of claim 97, wherein the base editor

(i) comprises a Cas9 nickase;

(ii) comprises a nuclease inactive Cas9;

(iii) does not comprise a UGI domain;

(iv) comprises an APOBEC-1 or APOBEC-3A cytidine deaminase;

(v) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI

LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE;or

(vi) comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHV
EVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLELYCII
LGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKSGGSSGGSSGSETPGTSES
ATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD
AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
LRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ
ILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKV
LTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINN
YHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNI
MNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSI
TGLYETRIDLSQLGGDSGGSKRTADGSEFESPKKKRKVE.

99. The pharmaceutical composition of claim 97 or claim 98, wherein the base editor comprises a Cas9, or a Cas9 variant comprising V1134, R1217, Q1334, and R1336 (SpCas9-VRQR).

100. The pharmaceutical composition of any one of claims 97-99, wherein the gRNA and the base editor are formulated together or separately.

101. The pharmaceutical composition of any one of claims 97-100, wherein the gRNA comprises a nucleic acid sequence, from 5′ to 3′, or a 1, 2, 3, 4, or 5 nucleotide 5′ truncation fragment thereof, selected from one or more of UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; or AAUCCACACUCCGAAAGACA.

102. The pharmaceutical composition of any one of claims 97-101, further comprising a vector suitable for expression in a mammalian cell, wherein the vector comprises a polynucleotide encoding the base editor.

103.. The pharmaceutical composition of claim 102, wherein the vector is a viral vector.

104. The pharmaceutical composition of claim 103, wherein the viral vector is a retroviral vector, adenoviral vector, lentiviral vector, herpesvirus vector, or adeno-associated viral vector (AAV).

105. The pharmaceutical composition of any one of claims 97-101, further comprising a ribonucleoparticle suitable for expression in a mammalian cell.

106. A method of treating HBV infection, the method comprising administering to a subject in need thereof the composition of claim 96.

107. A method of treating HBV infection, the method comprising administering to a subject in need thereof the pharmaceutical composition of any one of claims 97-105.

108. An HBV genome comprising an alteration selected from the group consisting of:

a premature termination codon introducing a R87STOP or W120STOP in the X gene;

a premature termination codon introducing a W35STOP or W36STOP in the S gene;

a missense mutation in the HBV pol gene that introduces a E24G, L25F, P26F, R27C, V48A, V48I, S382F, V378I, V378A, V379I, V379A, L377F, D380G, D380N, F381P, R376G, A422T, F423P, A432V, M433V, P434S, D540G, A688V, D689G, A717T, E718K, P713S, P713L, or L719P in HBV polymerase;

a missense mutation is in the HBV core gene that introduces a T160A, T160A, P161F, S162L, C183R, or STOP184Q in the HBV Core polypeptide;

a missense mutation is in the X gene that introduces a H86R, W120R, E122K, E121K, or L141P in the HBV X polypeptide; and

a missense mutation in the S gene that introduces a S38F, L39F, W35R, W36R, T37I, T37A, R78Q, S34L, F80P, or D33G in the HBV S polypeptide.

109. The HBV genome of claim 108, wherein the genome comprises two or more of said alterations.

110. The method of any one of claims 1-77, or the HBV genome of claim 108 or claim 109, wherein the HBV is of genotype C or genotype D.

111. Use of the composition of any one of claims 78-96 in the treatment of HBV infection in a subject.

112. Use of the pharmaceutical composition of any one of claims 97-105 in the treatment of HBV infection in a subject.

113. The use of claim 111 or claim 112, wherein the subject is a mammal.

114. The use of any one of claims 111-113, wherein the subject is a human.

115. The method of any one of claims 1-77, the pharmaceutical composition of any one of claims 97-100, wherein the one or more guide RNAs are as listed in Table 26.

116. A guide RNA comprising a nucleic acid sequence that is complementary to an HBV gene.

117. The guide RNA of claim 116 comprising a nucleic acid sequence that is complementary to an HBV gene encoding an HBV polymerase, HBV core protein, HBV S protein, or HBV X protein.

118. The guide RNA of claim 116 or 117 comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV X protein.

119. The guide RNA of claim 116 or 117 comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV S protein.

120. The guide RNA of claim 116 or 117 comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV polymerase.

121. The guide RNA of claim 116 or 117 comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an HBV gene that encodes an HBV core protein.

122. The guide RNA of claim 116 or 117 comprising a 1, 2, 3, 4, or 5 nucleic acid truncation from the 5′ end of a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU; AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC; GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG; UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA; AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU; CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU; CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA.

123. The guide RNA of claim 116 or 117 comprising a nucleic acid selected from the group consisting of, from 5′ to 3′, UCAAUCCCAACAAGGACACC; GGGAACAAGAUCUACAGCAU; AAGCCCAGGAUGAUGGGAUG; CUGCCAACUGGAUCCUGCGC; GACACAUCCAGCGAUAACCA; GCUGCCAACUGGAUCCUGCG; UAUGGAUGAUGUGGUAUUGG; CCAUGCCCCAAAGCCACCCA; AAGCCACCCAAGGCACAGCU; GAGAAGUCCACCACGAGUCU; CUUCUCUCAAUUUUCUAGGG; GACGACGAGGCAGGUCCCCU; CCCAACAAGGACACCUGGCC; UGCCAACUGGAUCCUGCGCG; AGGAGUUCCGCAGUAUGGAU; CCGCAGUAUGGAUCGGCAGA; CCUCUGCCGAUCCAUACUGC; CGCCCACCGAAUGUUGCCCA; GACUUCUCUCAAUUUUCUAG; GUUCCGCAGUAUGGAUCGGC; UACUAACAUUGAGGUUCCCG; UCCGCAGUAUGGAUCGGCAG; UCCUCUGCCGAUCCAUACUG; GUAGCUCCAAAUUCUUUAUA; and AAUCCACACUCCGAAAGACA.

124. A pharmaceutical composition comprising (i) a nucleic acid encoding a base editor; and (ii) the guide RNA of any one of claims 116-123.

125. The pharmaceutical composition of claim 124, further comprising a lipid.

126. The pharmaceutical composition of claim 124 or 125, wherein the nucleic acid encoding the base editor is an mRNA.

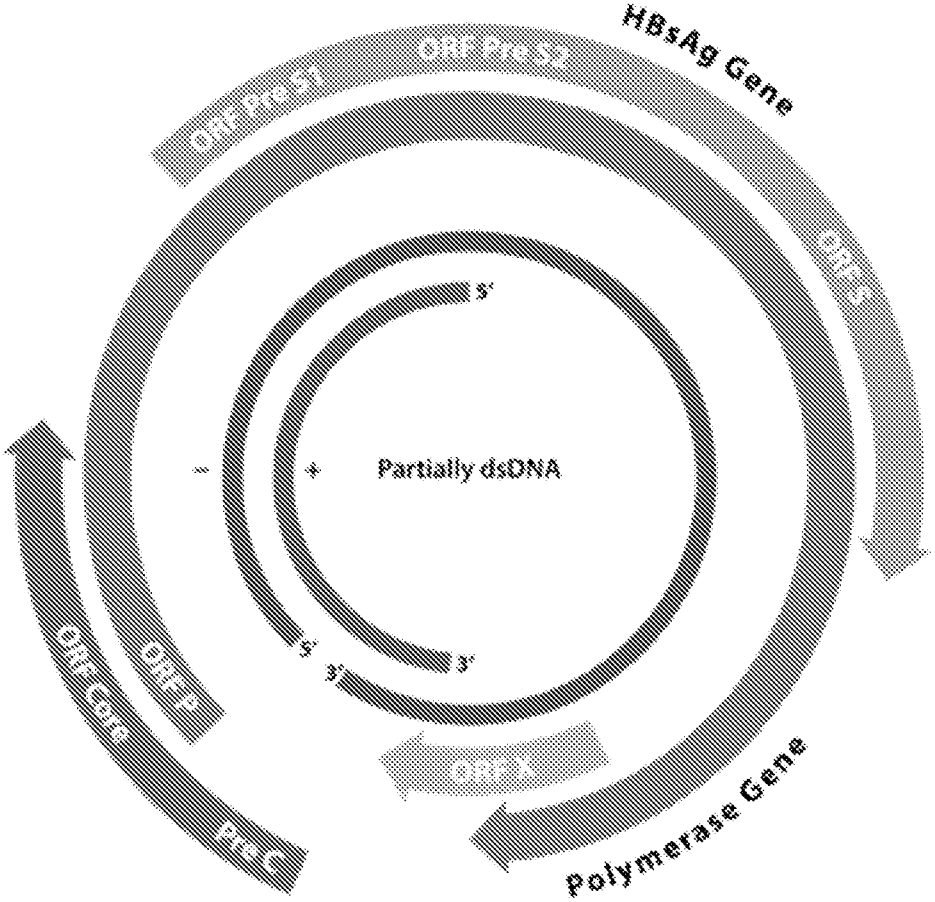

HBsAg Gene
ORF Pre-S1
ORF Pre-S2
ORF S
Partially dsDNA
5'
3'
ORF X
Polymerase Gene
ORF Pre C
ORF Core
ORF P

FIG. 1

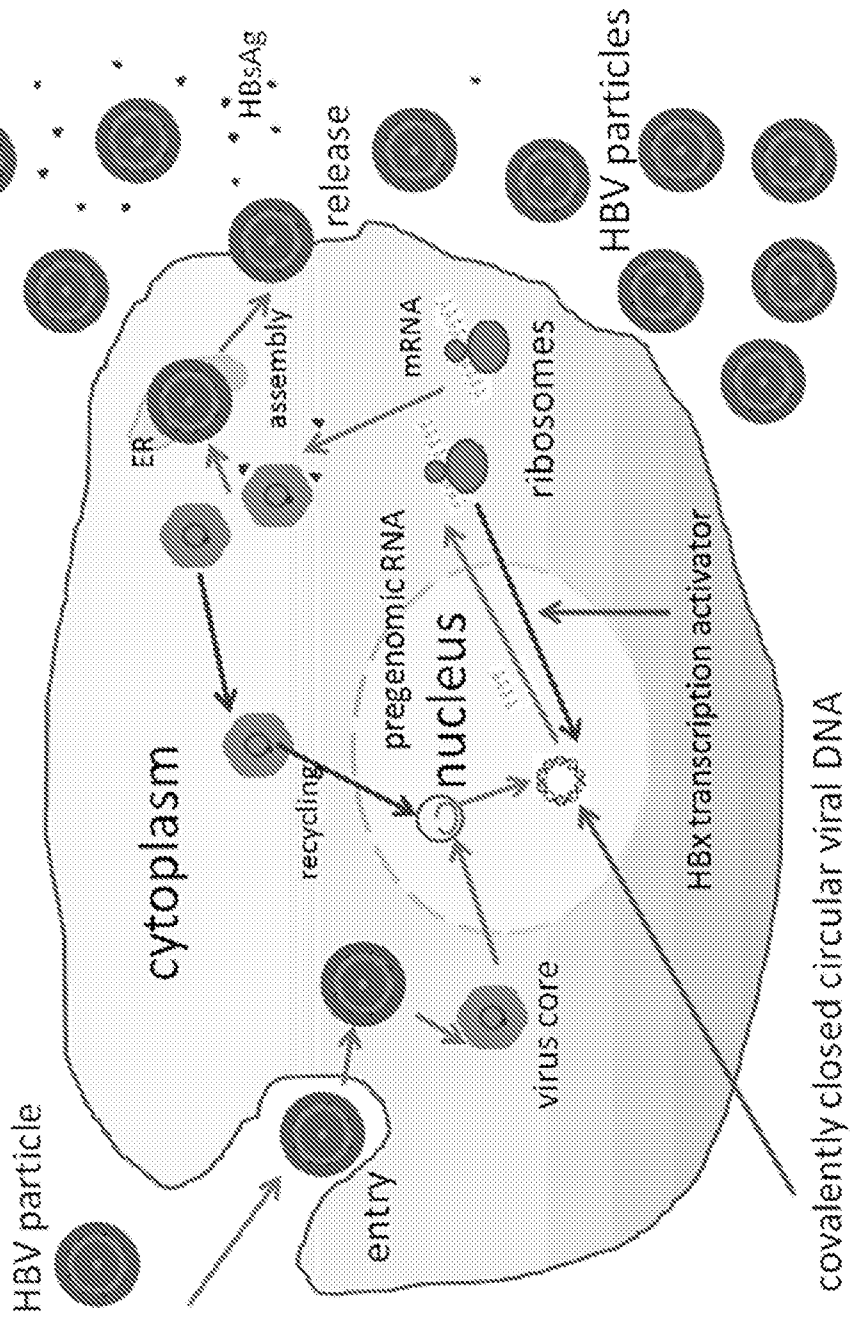
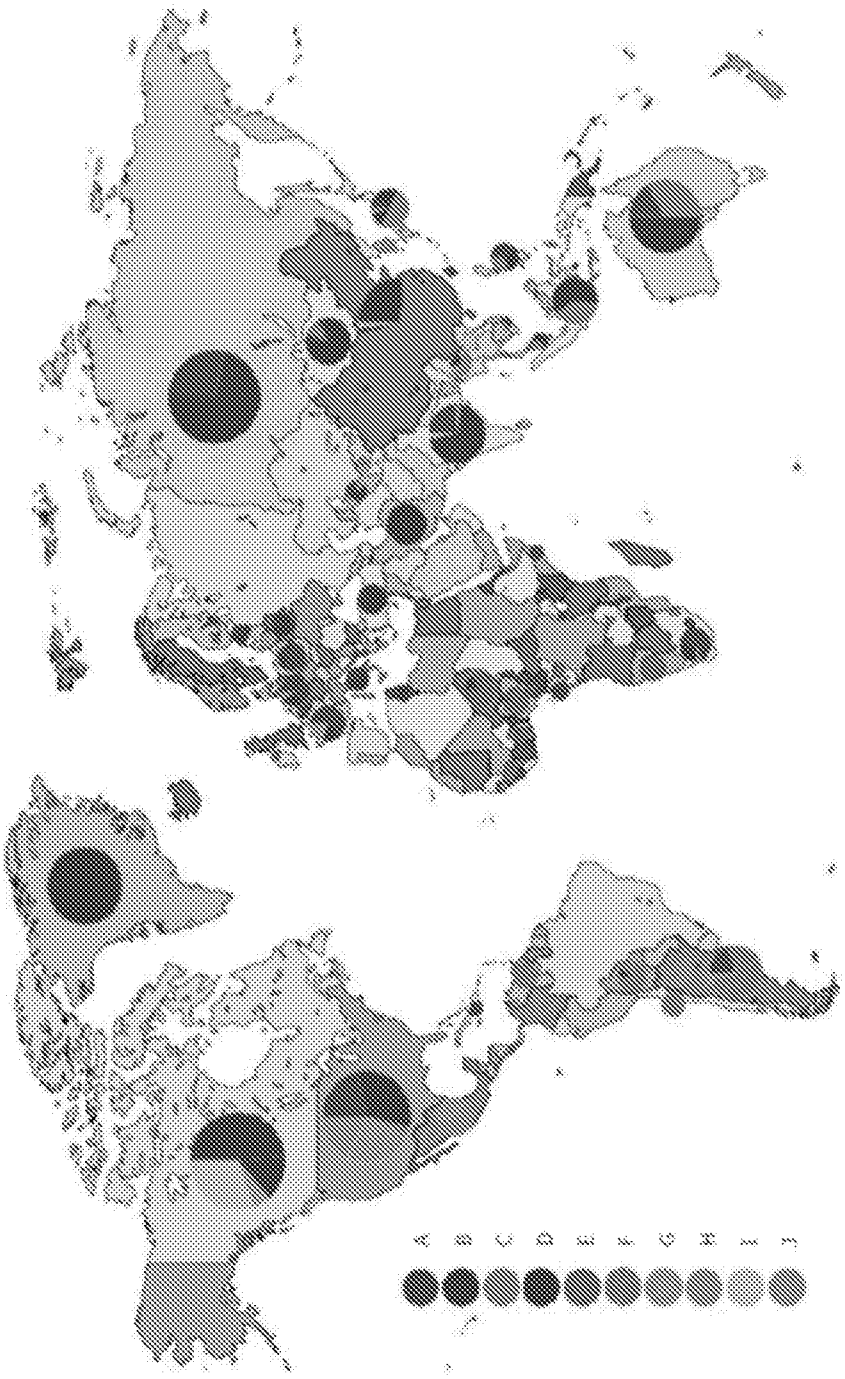

HBV particle
entry
cytoplasm
recycling
pregenomic RNA
nucleus
virus core
ER
assembly
HBsAg
release
mRNA
ribosomes
HBx transcription activator
HBV particles
covalently closed circular viral DNA

FIG. 2



World Gastroenterology Organisation Global Guideline, 2015)

FIG. 3A

Base Editing as a Strategy to Treat Chronic HBV
• Allows introducing stop codons in viral genes without generating double strand breaks
- Although Cas9 nucleases effectively mediate HBV cccDNA disruption, the risk of cutting integrated HBV genomes has raised serious concerns
• Employs deaminase, which is known to be a natural HBV antiviral restriction factor
Cellular deaminase expression
Uracil Glycosilase (UNG)
cccDNA degradation
BE4
APOBEC
Cas9
UGI
Using BE without UGI could target cellular Uracil glycosylase to cccDNA and thus promote its degradation

FIG. 3B

Guide RNA Screening Strategies

Identifying conserved HBV regions with a focus on *genotype D, subgenotype ayw*

- Most abundant in US
- Existence of cellular and animal models (Fabien Zoulim lab (INSERM), Humanized mouse model

	CRITERIA	
	Screen 1	**Screen 2**
gRNA design	• gRNAs introducing Stop codons or functional mutations in HBV genes	• Super conserved gRNAs • Main aim is to generate abasic sites in HBV genome • Will avoid known resistant mutants
gRNA conservation across HBV genotypes	No initial filter	>50% in all genotypes
Base Editor (BE4)	With or without UGI	
Screening strategy	Editing efficiency in Hek293-Lenti-HBV_DNA/ plasmid format	

FIG. 3C

WO 2020/231863 PCT/US2020/032226
CA 03139324 2021-11-04

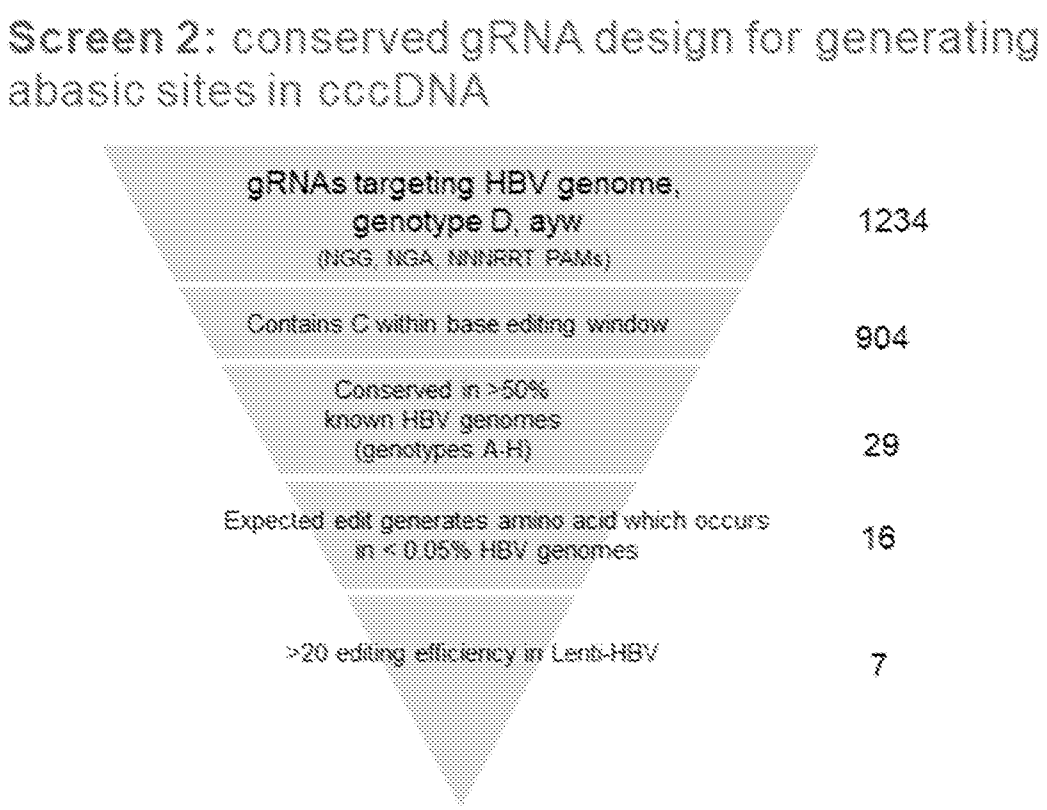

Screen 2: conserved gRNA design for generating abasic sites in cccDNA
gRNAs targeting HBV genome, genotype D, ayw
(NGG, NGA, NNNRRT PAMs)
1234
Contains C within base editing window
904
Conserved in >50% known HBV genomes (genotypes A-H)
29
Expected edit generates amino acid which occurs in < 0.05% HBV genomes
16
>20 editing efficiency in Lenti-HBV
7

FIG. 3D

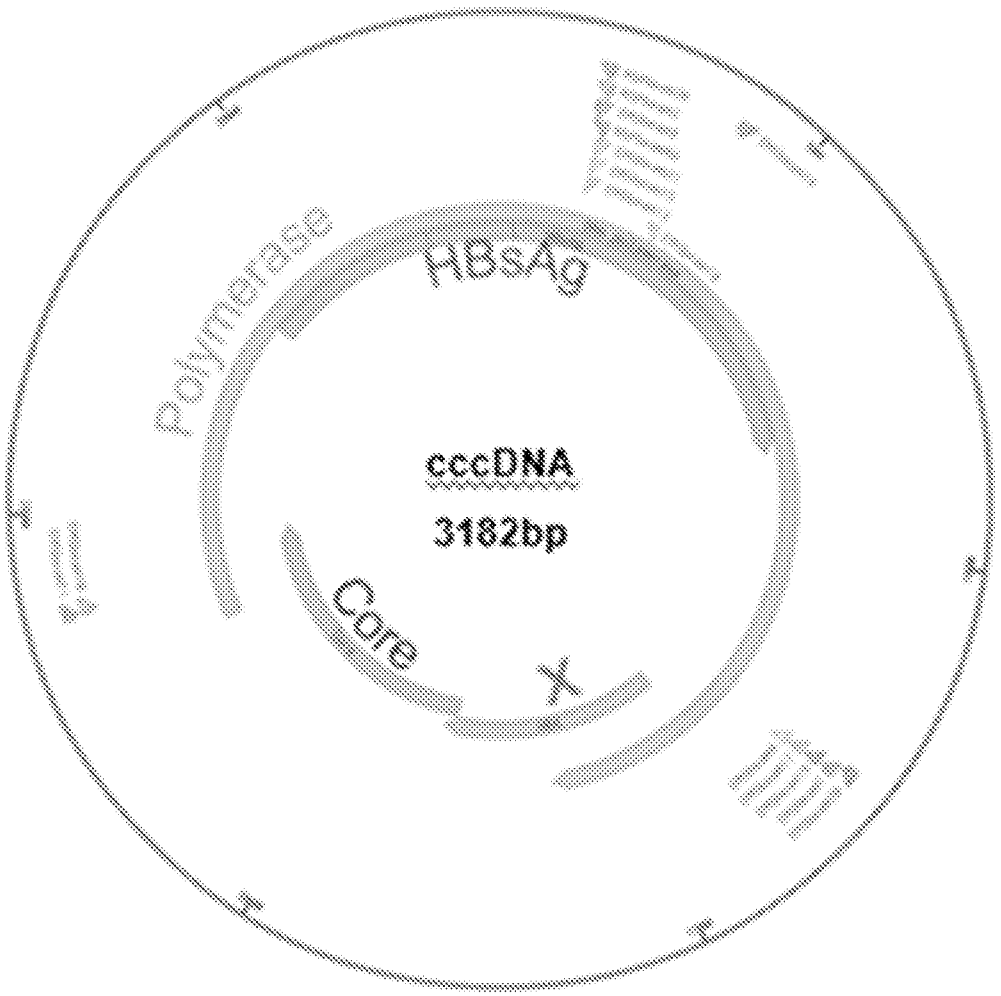


FIG. 3E

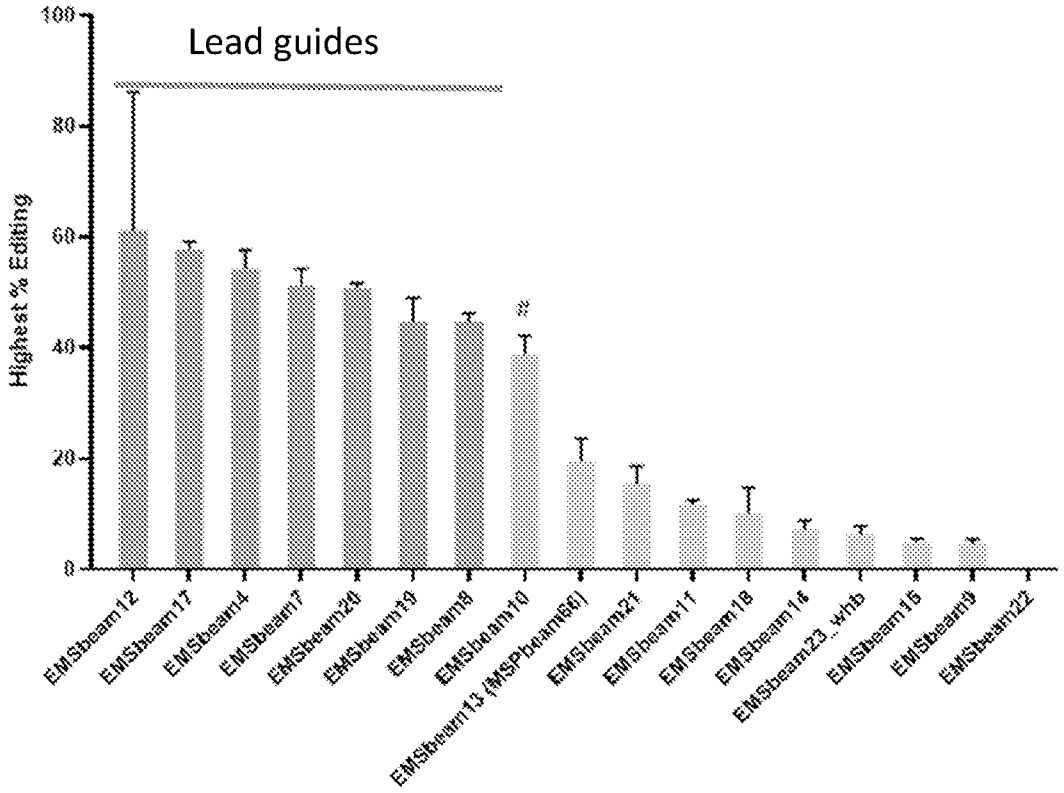


FIG. 3F

gRNA ID	Highest % Edit	Guide Strand	PAM	Gene	% Conservation All Genotypes
Cons-12	61.0	1	NGG	S/Pol	96
Cons-17	58.0	-1	NGA	Pol	93
Cons-19	45.0	-1	NGG	Pol	92
Cons-20	51.0	1	NGG	Pol	92
Cons-4	54.0	-1	NGG	Pol	95
Cons-7	51.0	-1	NGA	Pol	92
Cons-8	45.0	1	NGA	Pol	92

FIG. 3G


APOBEC
Cas9
UGI
UGI

FIG. 4A

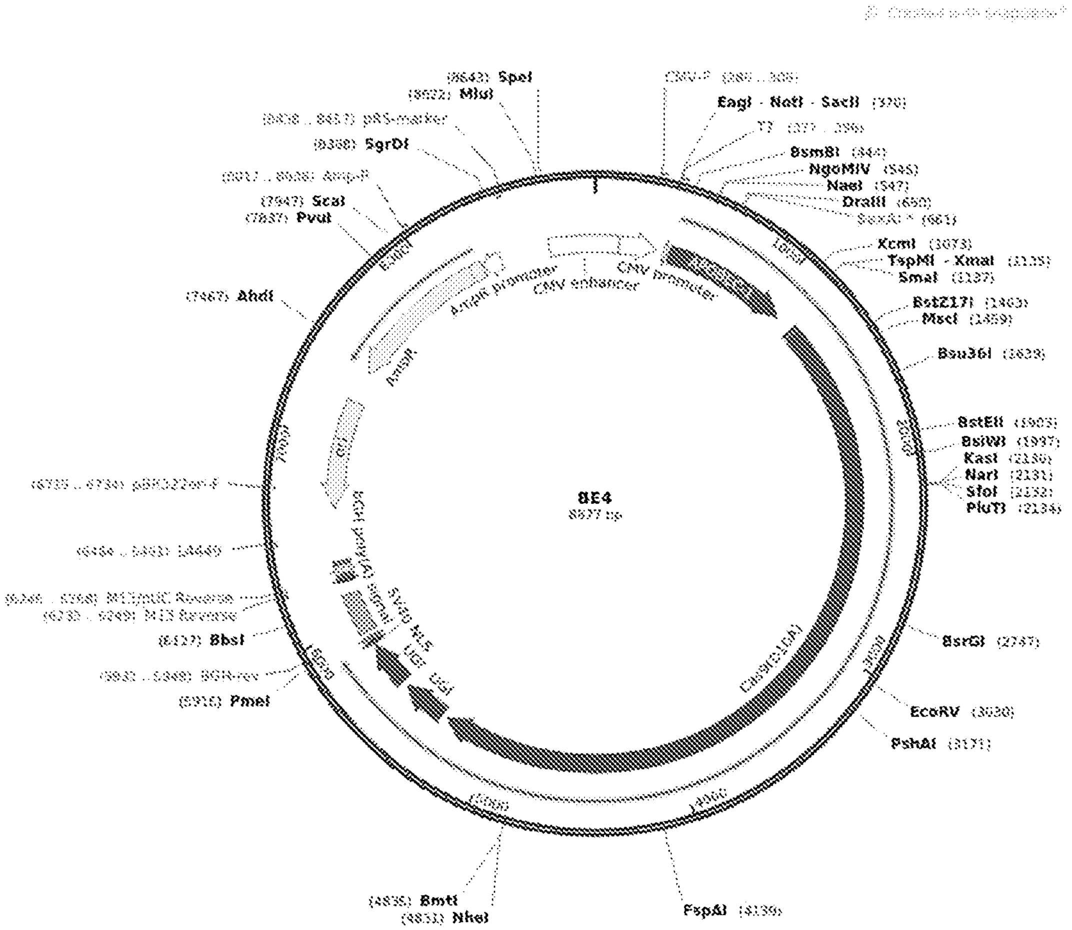

Spel
Mlul
SgrDI
Scal
Pvul
Ahdl
Bbsl
Pmel
Bmtl
Nhel
Eagl - NotI - SacII
BsmBI
NgoMIV
NaeI
DraIII
XcmI
TspMI - XmaI
SmaI
BstZ17I
MscI
Bsu36I
BstEII
BsiWI
KasI
NarI
SfoI
PluTI
BsrGI
EcoRV
PshAI
FspAI
AmpR promoter
CMV enhancer
CMV promoter
AmpR
SV40 NLS
UGI
UGI
BE4

FIG. 4B

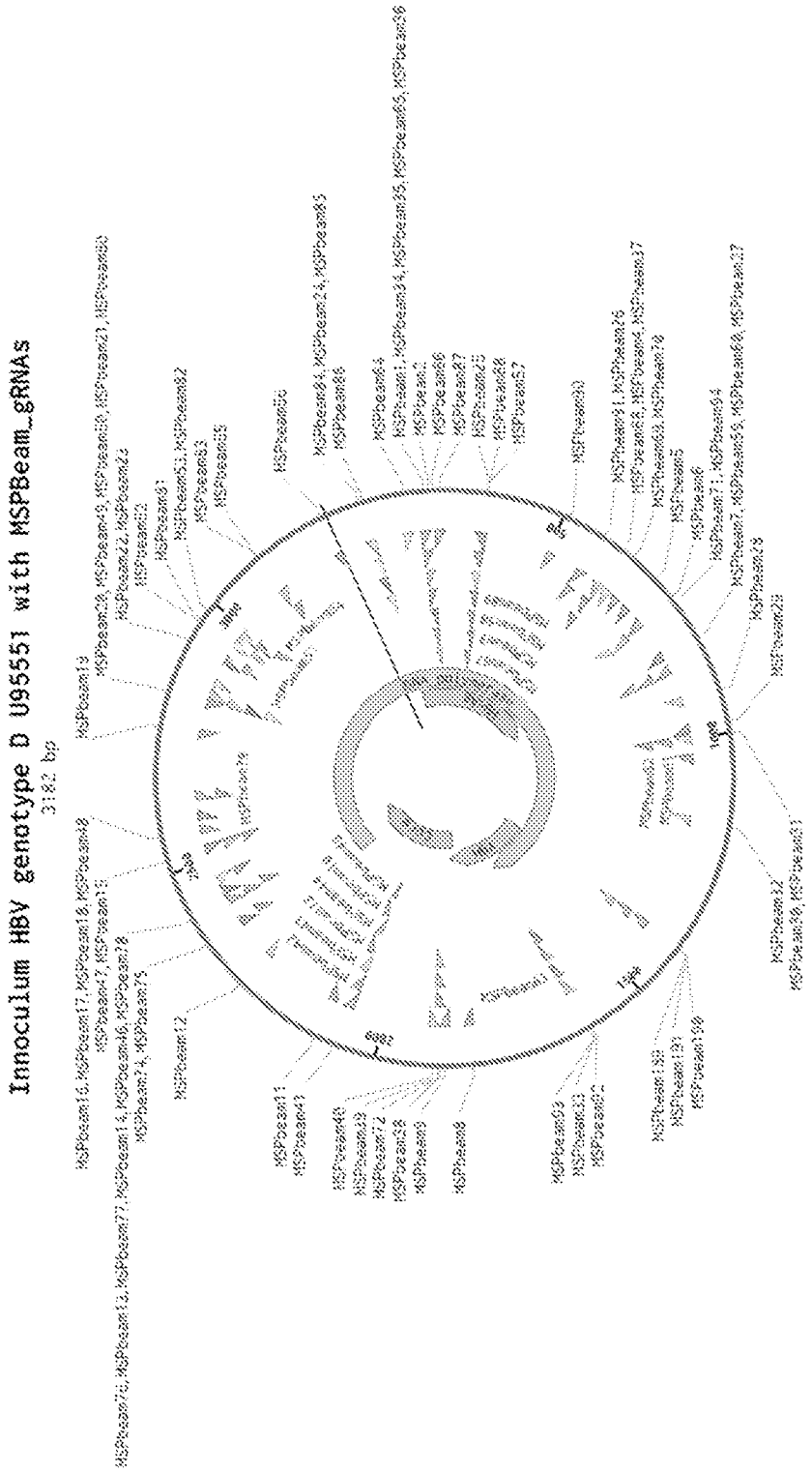


FIG. 5

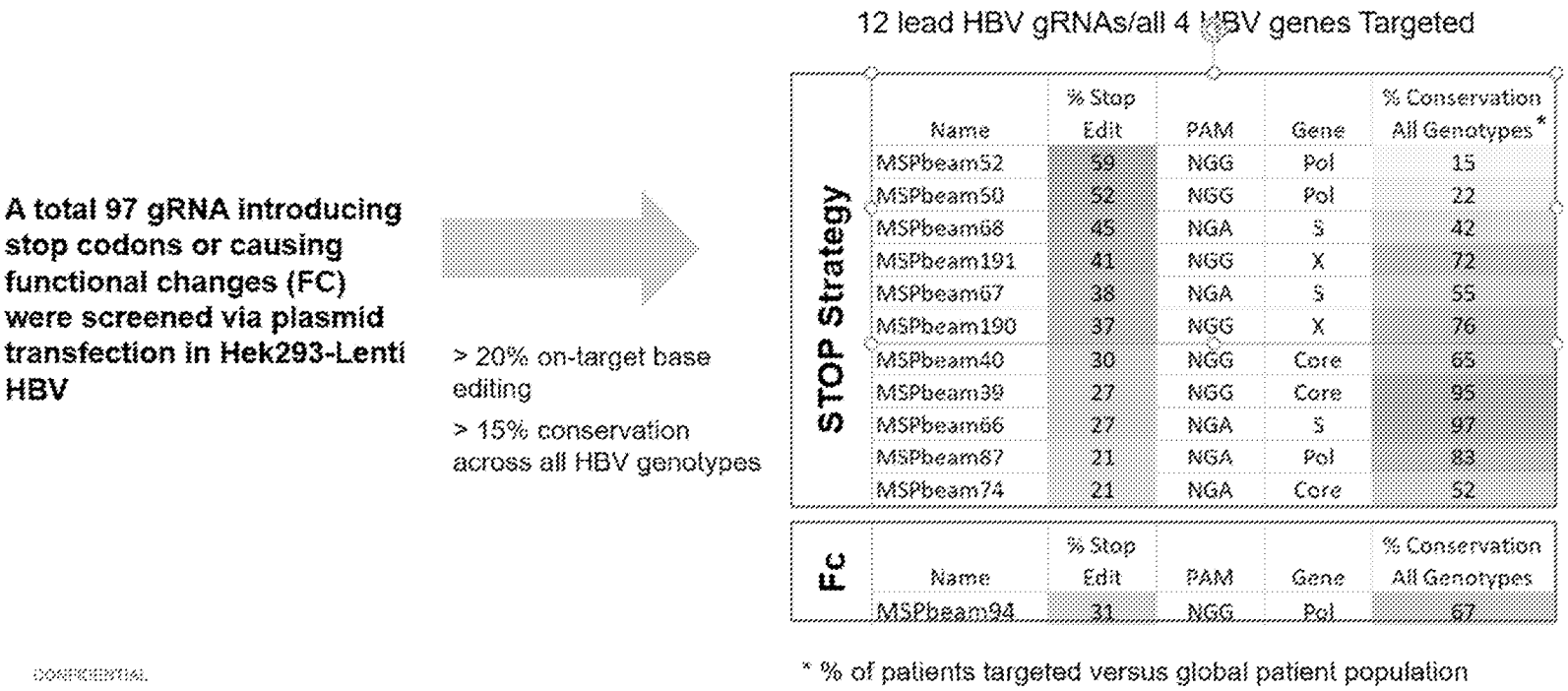


FIG. 6

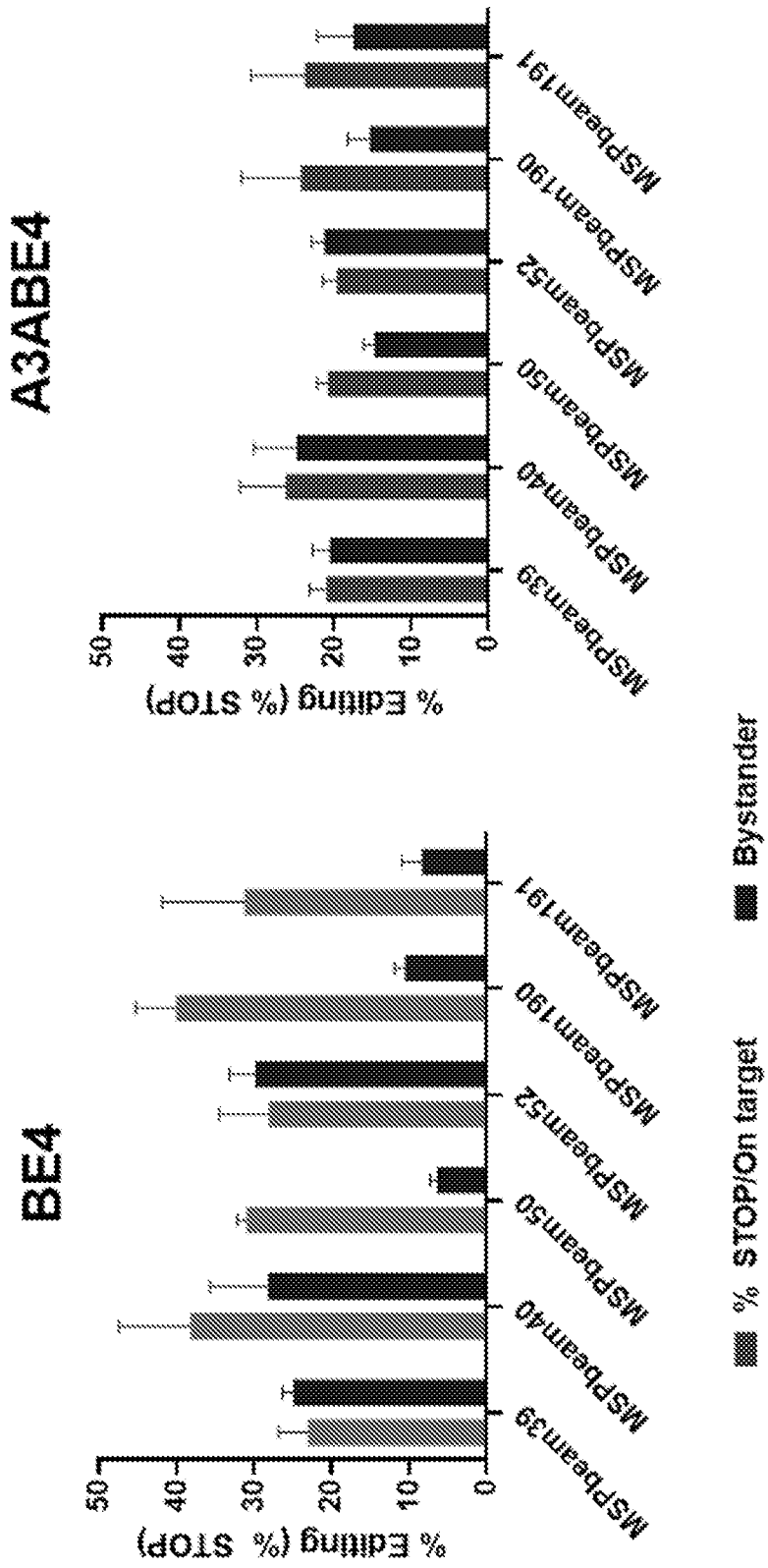

A3ABE4
% Editing (% STOP)
50
40
30
20
10
0
MSPbeam39
MSPbeam40
MSPbeam50
MSPbeam52
MSPbeam190
MSPbeam191
BE4
% Editing (% STOP)
50
40
30
20
10
0
MSPbeam39
MSPbeam40
MSPbeam50
MSPbeam52
MSPbeam190
MSPbeam191
% STOP/On target
Bystander

FIG. 7

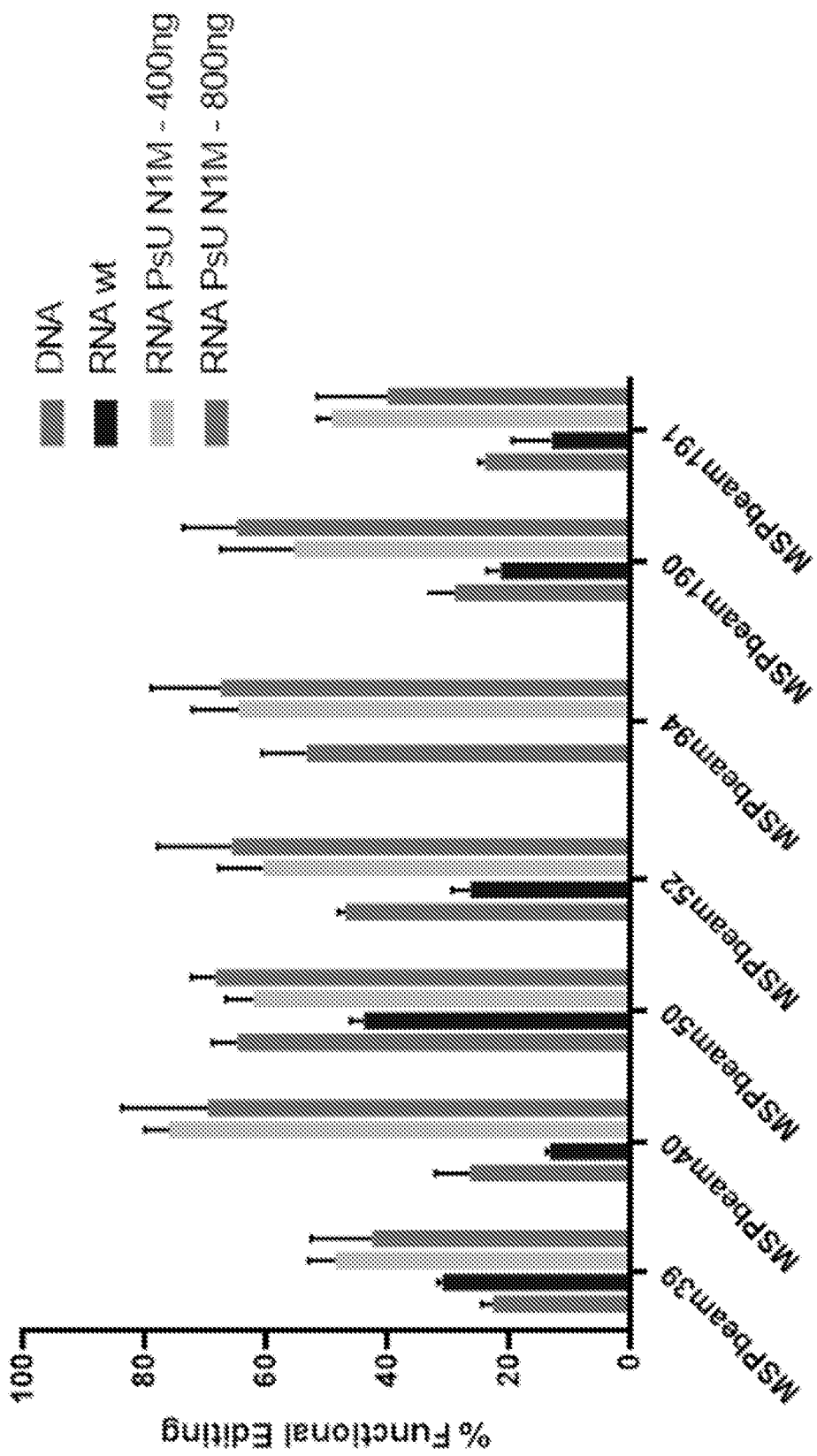

DNA
RNA wt
RNA PsU N1M - 400ng
RNA PsU N1M - 800ng
% Functional Editing
100
80
60
40
20
0
MSPbeam39
MSPbeam40
MSPbeam50
MSPbeam52
MSPbeam94
MSPbeam190
MSPbeam191

FIG. 8

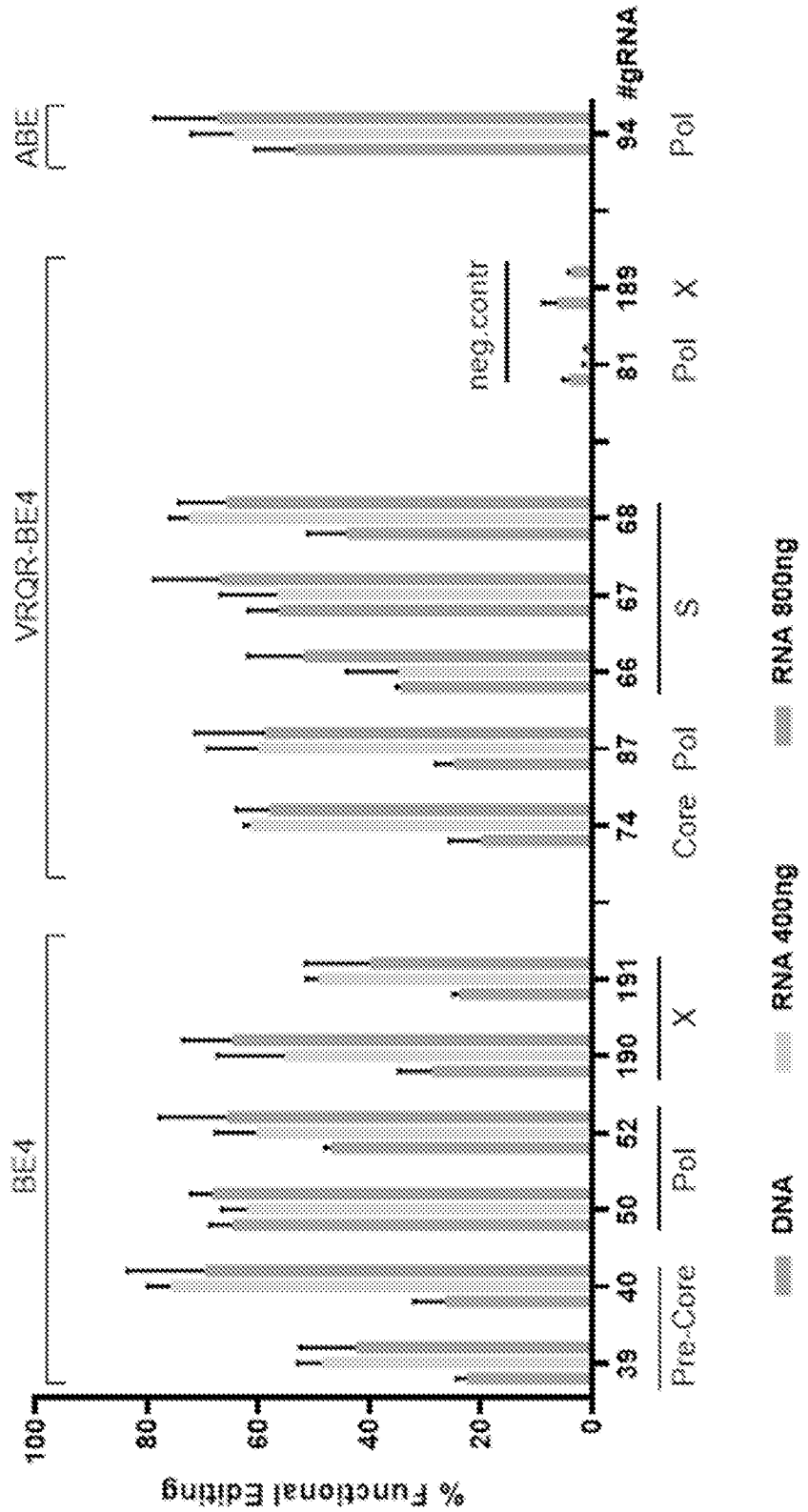

% Functional Editing
100
80
60
40
20
0
BE4
VRQR-BE4
ABE
Pre-Core
Pol
X
Core
Pol
S
Pol
X
Pol
39
40
50
52
190
191
74
87
66
67
68
81
189
94
#gRNA
neg.contr
DNA
RNA 400ng
RNA 800ng

FIG. 9

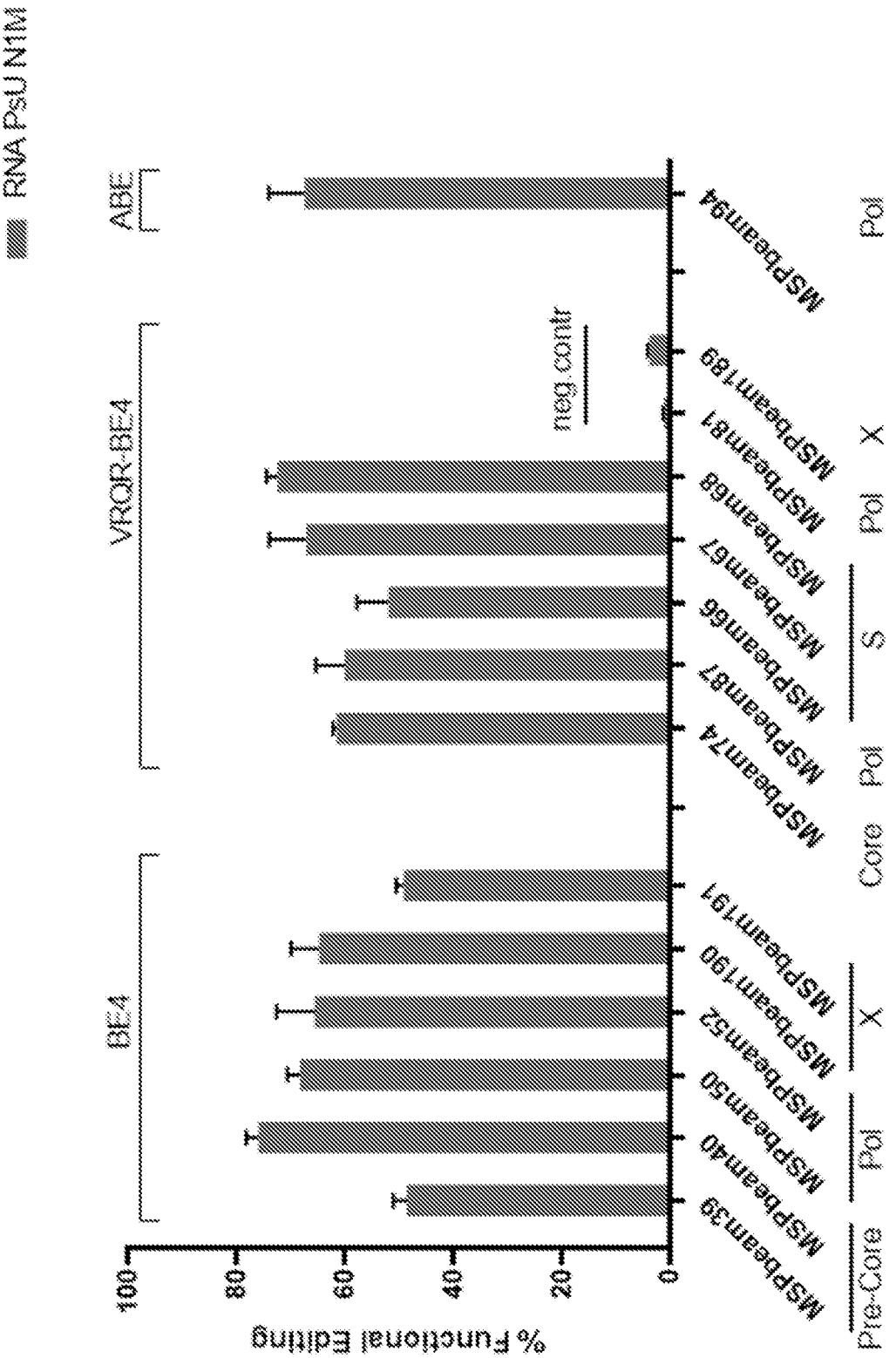

RNA PsU N1M
% Functional Editing
100
80
60
40
20
0
BE4
VRQR-BE4
ABE
neg contr
MSPbeam39
MSPbeam40
MSPbeam50
MSPbeam52
MSPbeam190
MSPbeam191
MSPbeam74
MSPbeam87
MSPbeam66
MSPbeam67
MSPbeam68
MSPbeam81
MSPbeam189
MSPbeam94
Pre-Core
Pol
X
Core
Pol
S
Pol
X
Pol

FIG. 10

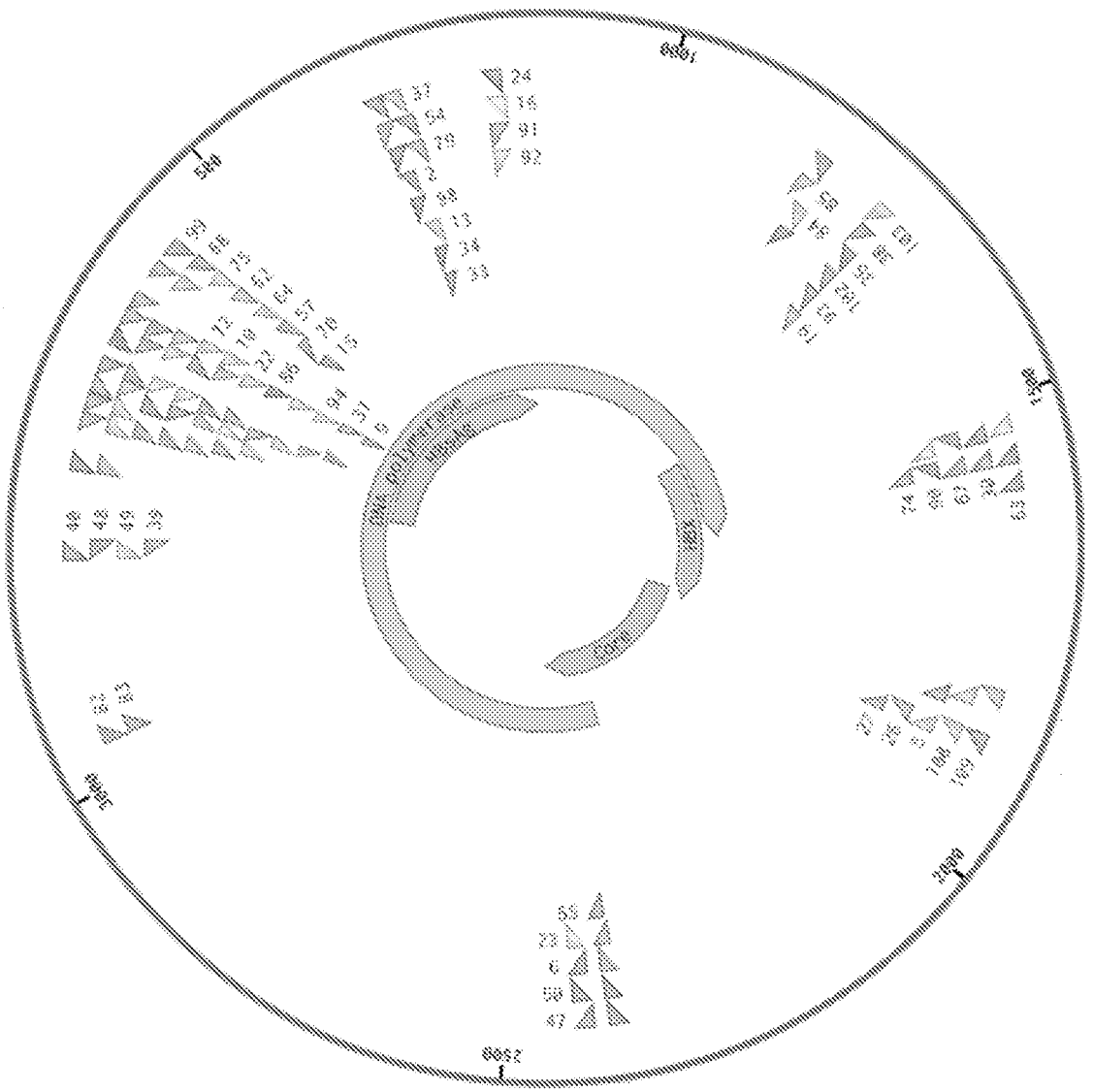


FIG. 11

Long-term primary hepatocyte co-cultures
Primary human hepatocytes
Mouse stromal cells
Winer et al., 2017
• Persistent infection for over 30 days in a self-assembling, primary hepatocyte co-culture system
• Mimics the physiological environment of primary hepatocytes
• High levels of base editing achievable

FIG. 12A

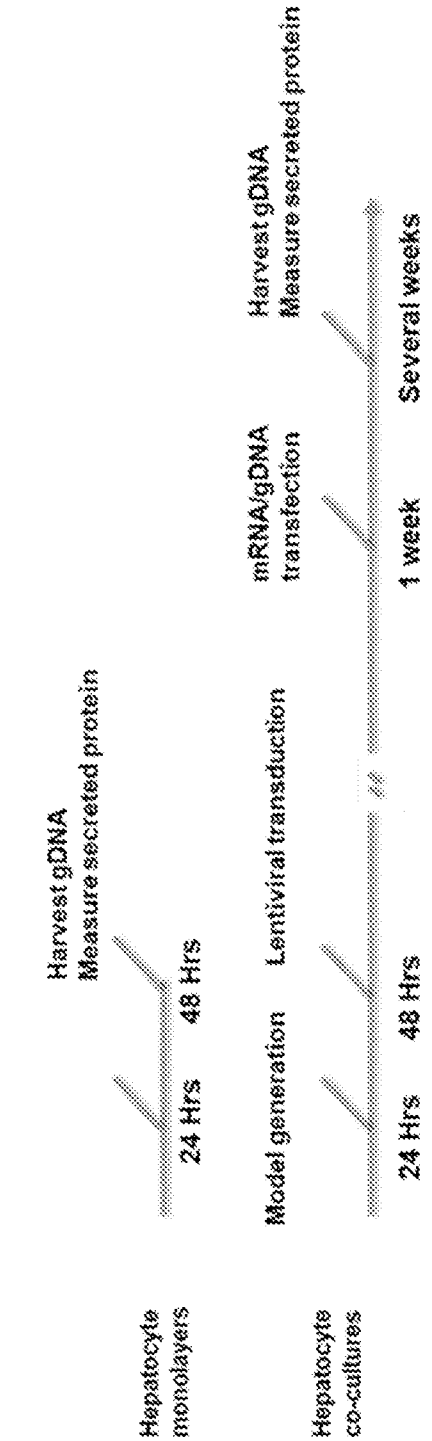

Hepatocyte monolayers
Harvest gDNA
Measure secreted protein
24 Hrs
48 Hrs
Hepatocyte co-cultures
Model generation
Lentiviral transduction
24 Hrs
48 Hrs
mRNA/gDNA transfection
1 week
Harvest gDNA
Measure secreted protein
Several weeks

FIG. 12B

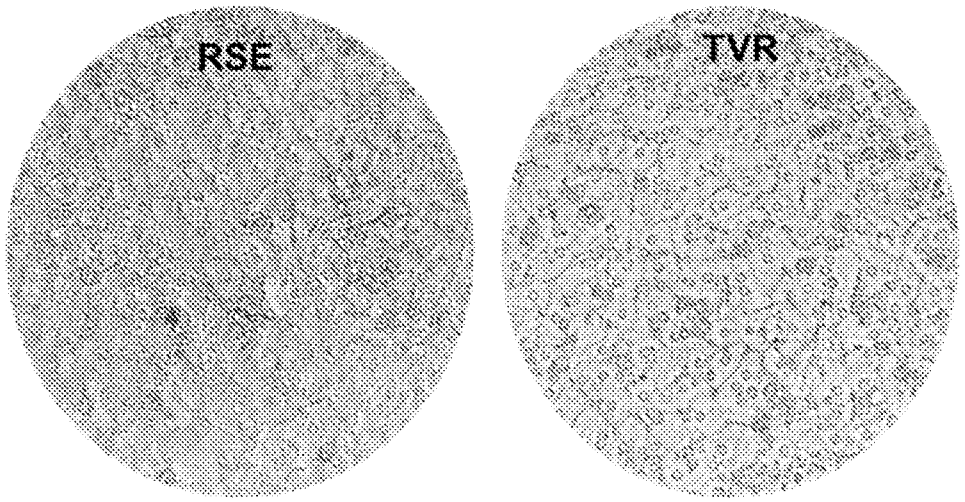


FIG. 12C

Establishment / validation of HBV-infected PHH
Interferon (IFN) – inhibits replication and reduces cccDNA
Tenofovir (TDF) – inhibits replication, doesn't influence cccDNA level
Cell lysis
Assessment of intracellular
• HBV DNA
• viral RNA
PHH plating
infection
0
1
5
7
9
11
13
day
Media change and collection
• qPCR for HBV extracellular DNA
• HBsAg ELISA
HBV markers respond to the treatment as expected
Physiologically relevant system to assess HBV replication / cccDNA activity is established

FIG. 13A

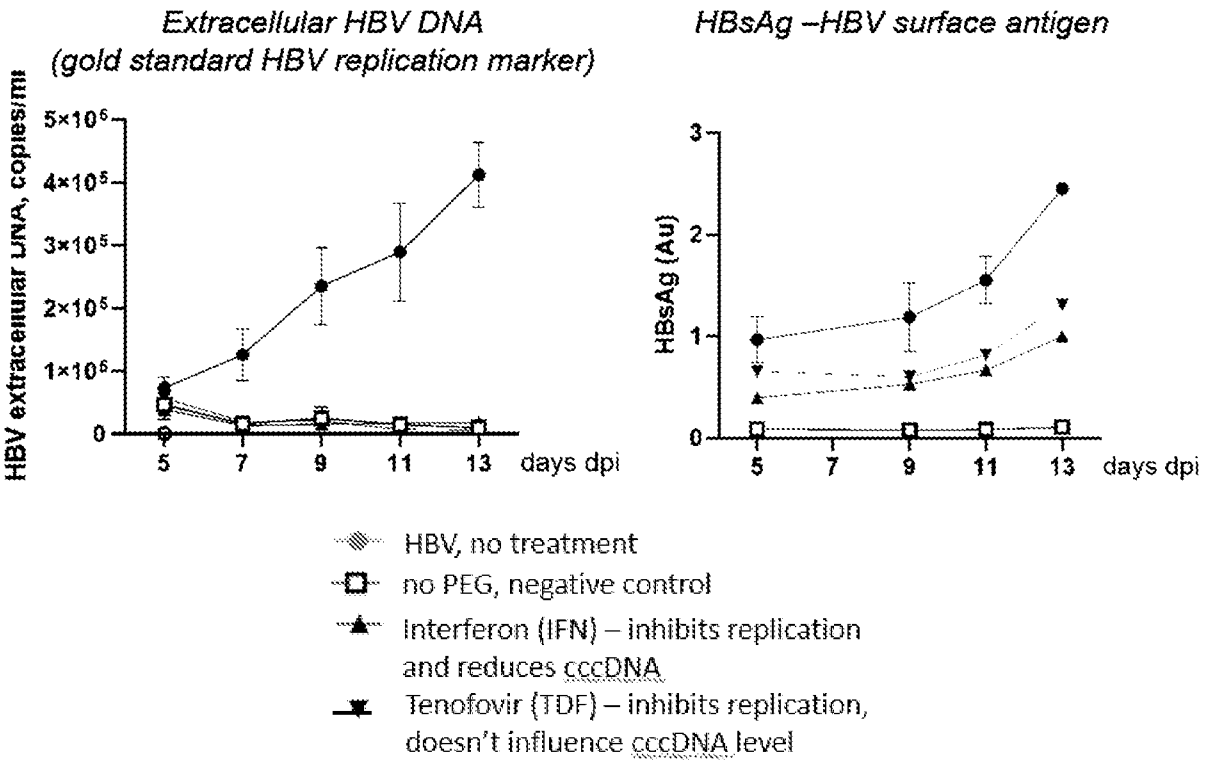


FIG. 13B

FIG. 13C

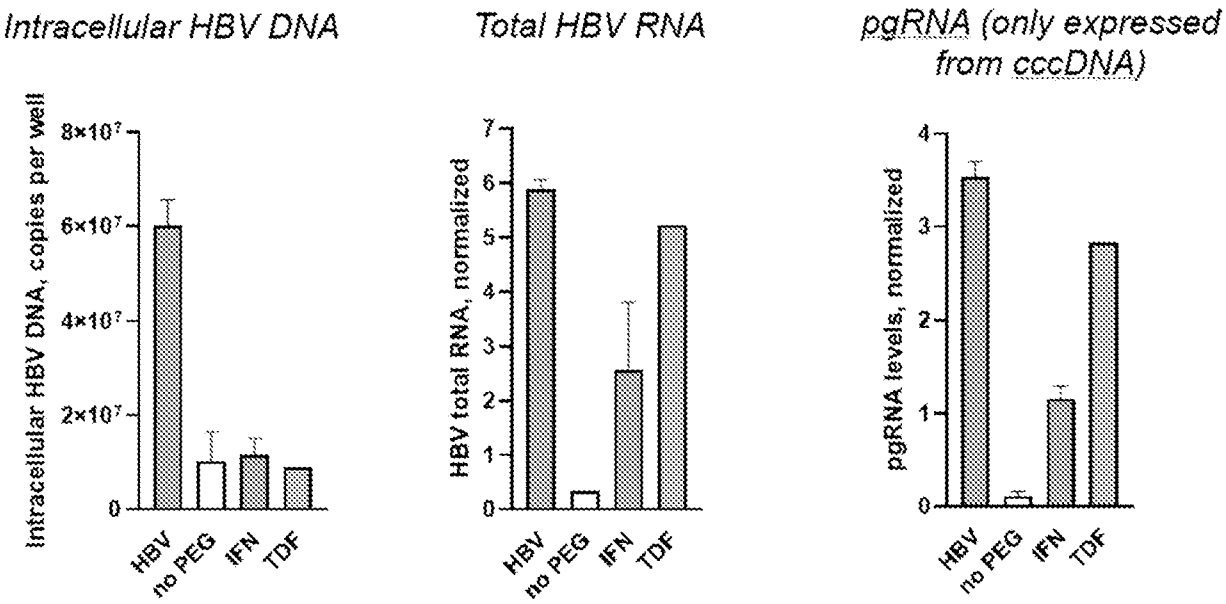


FIG. 13D

FIG. 13E

FIG. 13F

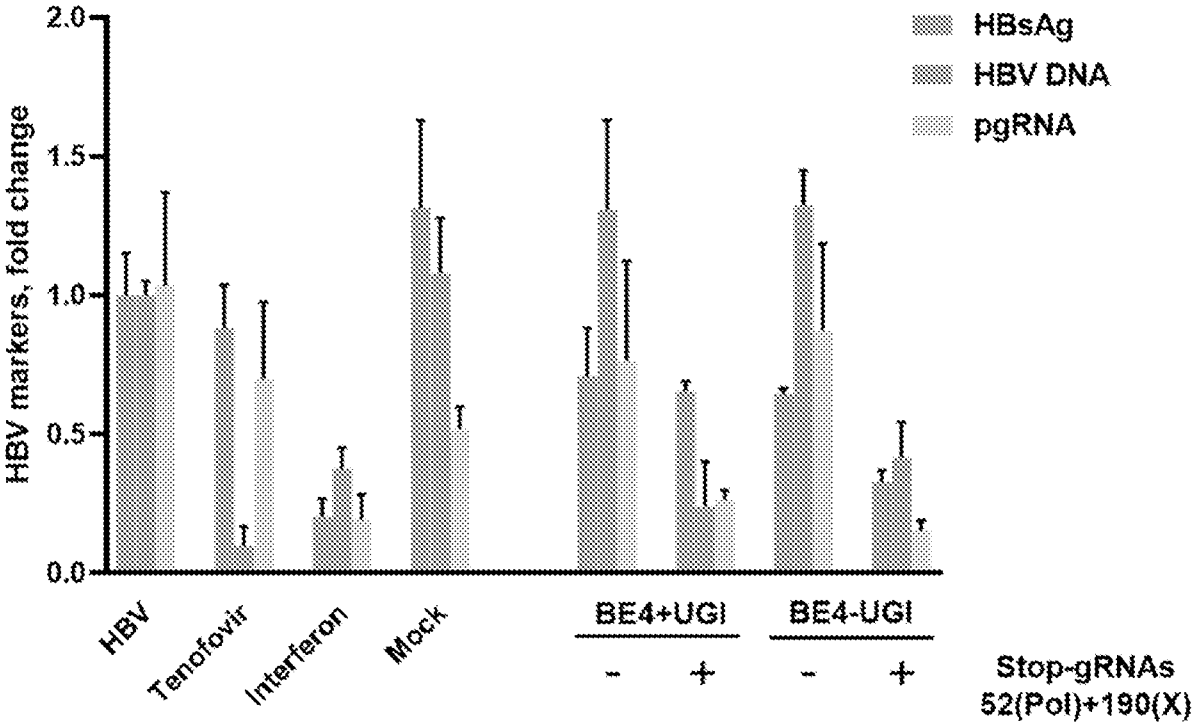

HBsAg
HBV DNA
pgRNA
HBV markers, fold change
2.0
1.5
1.0
0.5
0.0
HBV
Tenofovir
Interferon
Mock
BE4+UGI
BE4-UGI
-
+
-
+
Stop-gRNAs
52(Pol)+190(X)

FIG. 14

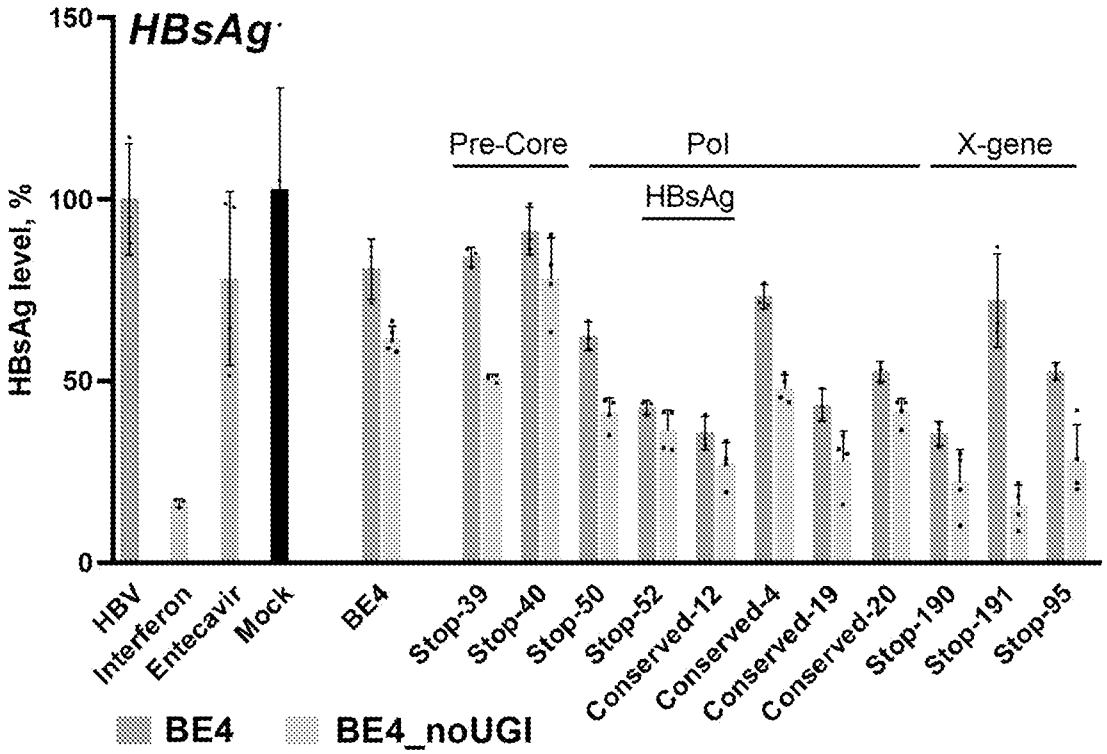

HBsAg
HBsAg level, %
150
100
50
0
Pre-Core
Pol
X-gene
HBsAg
HBV
Interferon
Entecavir
Mock
BE4
Stop-39
Stop-40
Stop-50
Stop-52
Conserved-12
Conserved-4
Conserved-19
Conserved-20
Stop-190
Stop-191
Stop-95
BE4
BE4_noUGI

FIG. 15

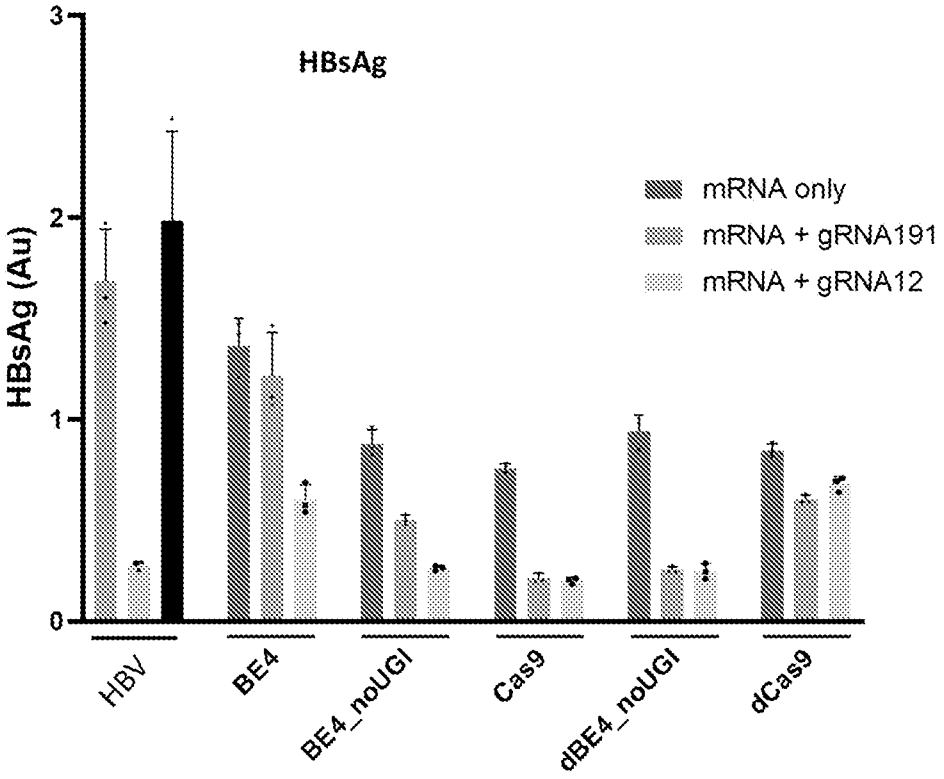

HBsAg
HBsAg (Au)
3
2
1
0
mRNA only
mRNA + gRNA191
mRNA + gRNA12
HBV
BE4
BE4_noUGI
Cas9
dBE4_noUGI
dCas9

FIG. 16A

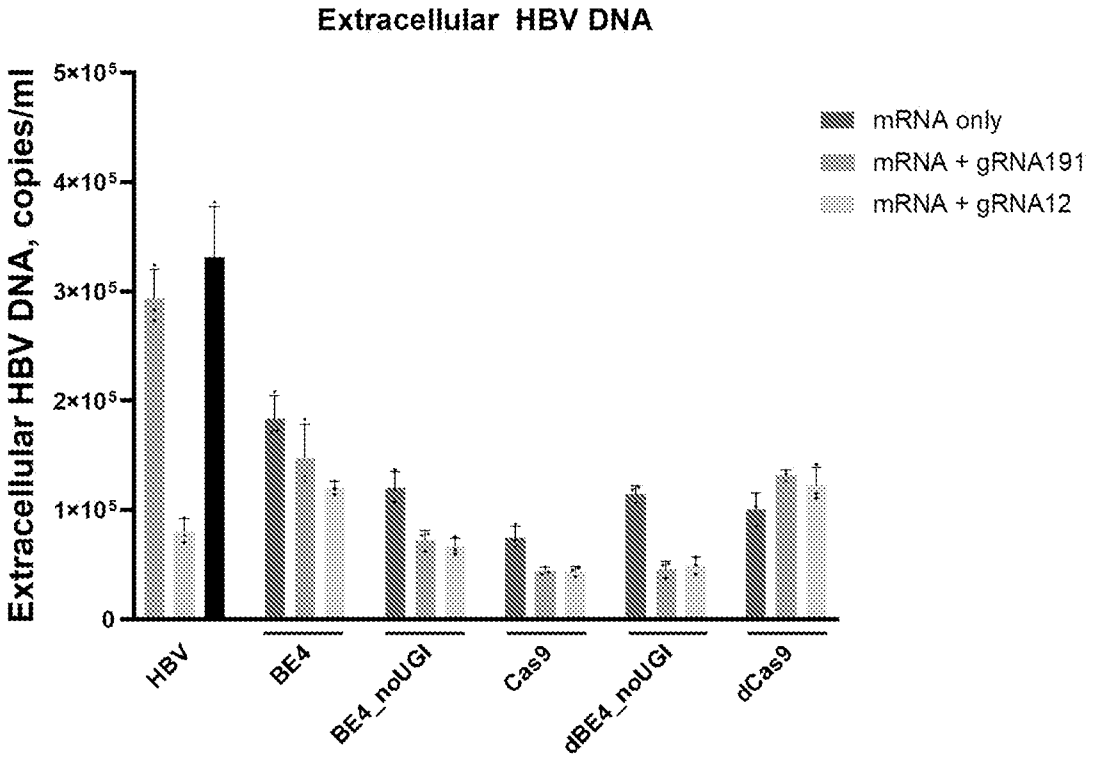

Extracellular HBV DNA
Extracellular HBV DNA, copies/ml
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
0
mRNA only
mRNA + gRNA191
mRNA + gRNA12
HBV
BE4
BE4_noUGI
Cas9
dBE4_noUGI
dCas9

FIG. 16B

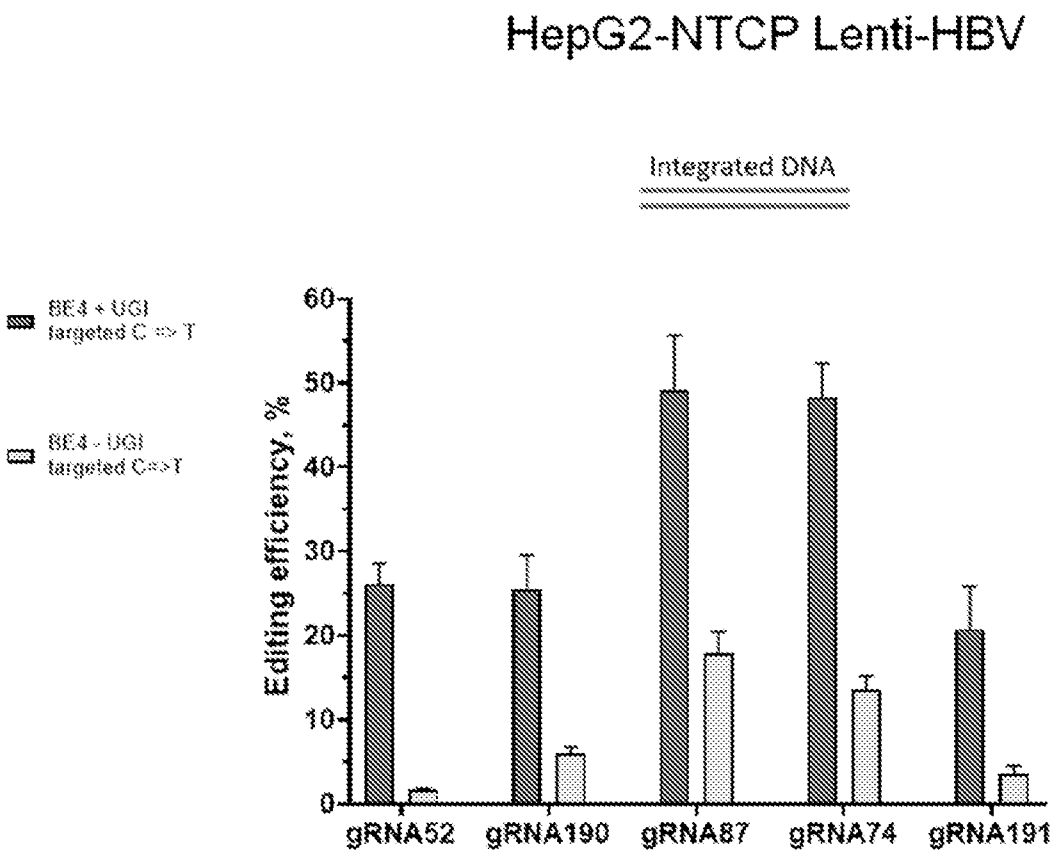

HepG2-NTCP Lenti-HBV
Integrated DNA
BE4 + UGI
targeted C => T
BE4 - UGI
targeted C=>T
Editing efficiency, %
60
50
40
30
20
10
0
gRNA52
gRNA190
gRNA87
gRNA74
gRNA191

FIG. 17A

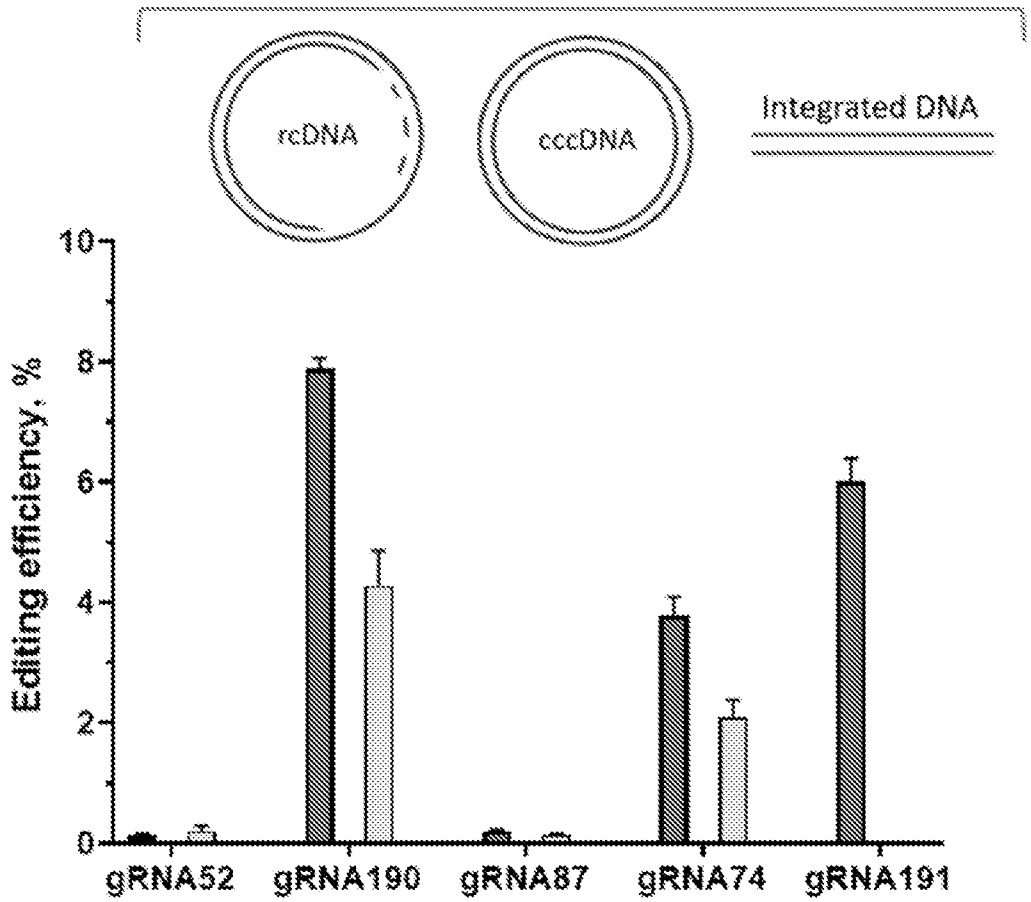

rcDNA
cccDNA
Integrated DNA
Editing efficiency, %
10
8
6
4
2
0
gRNA52
gRNA190
gRNA87
gRNA74
gRNA191

FIG. 17B

* BE4_noUGI: Editing, indel, and transversion rate (C=>A,G) for gRNA190

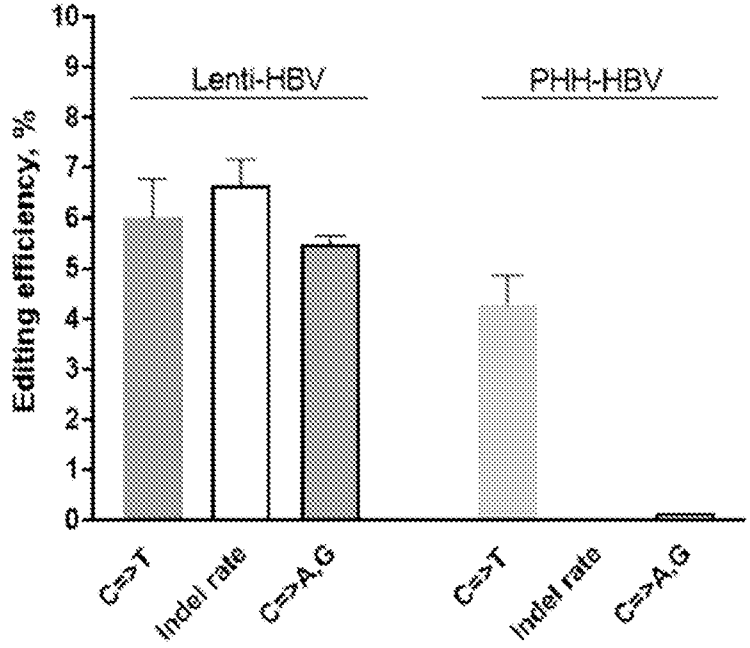


FIG. 18

FIG. 19

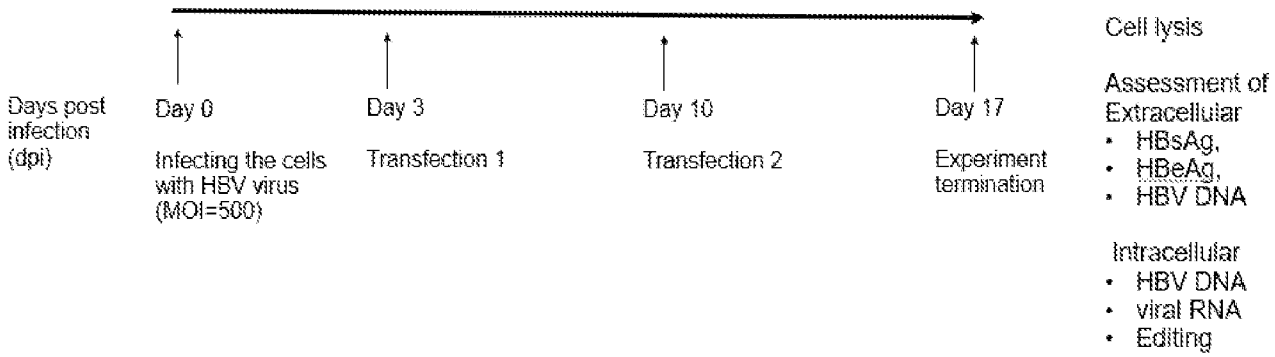

Days post infection (dpi)
Day 0
Infecting the cells with HBV virus (MOI=500)
Day 3
Transfection 1
Day 10
Transfection 2
Day 17
Experiment termination
Cell lysis
Assessment of Extracellular
• HBsAg,
• HBeAg,
• HBV DNA
Intracellular
• HBV DNA
• viral RNA
• Editing

FIG. 20

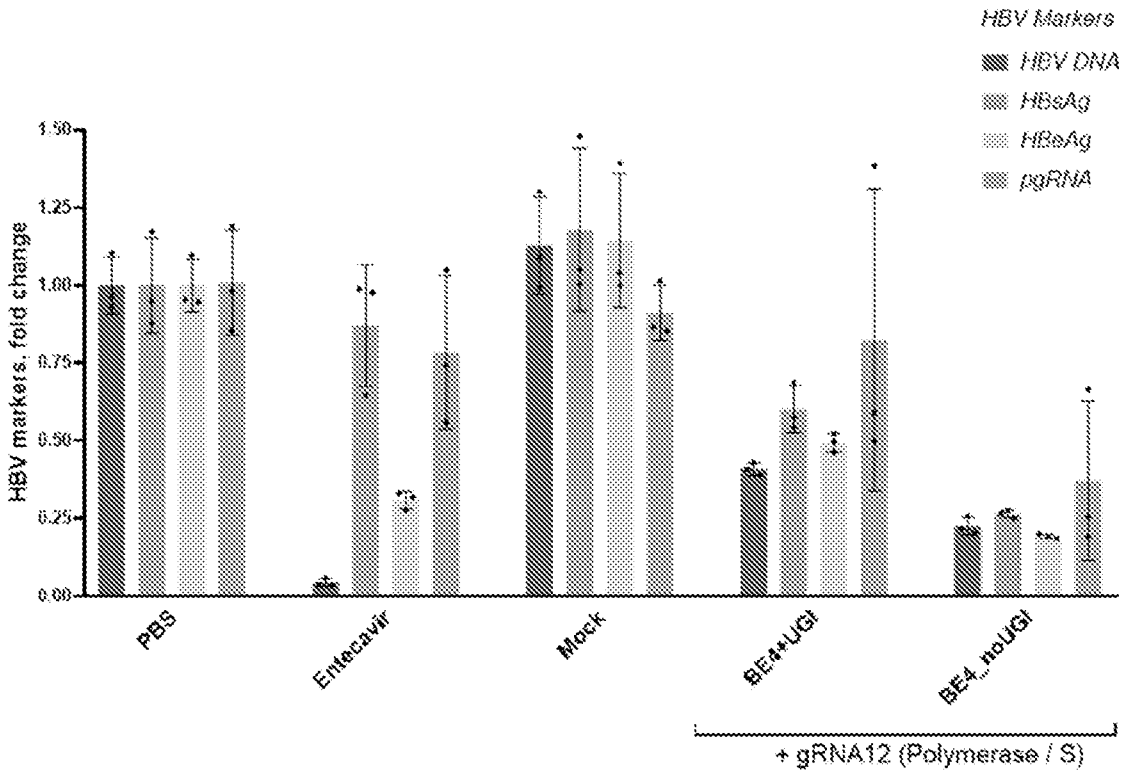

HBV Markers
HBV DNA
HBsAg
HBeAg
pgRNA
HBV markers, fold change
1.50
1.25
1.00
0.75
0.50
0.25
0.00
PBS
Entecavir
Mock
BE4+UGI
BE4_noUGI
+ gRNA12 (Polymerase / S)

FIG. 21

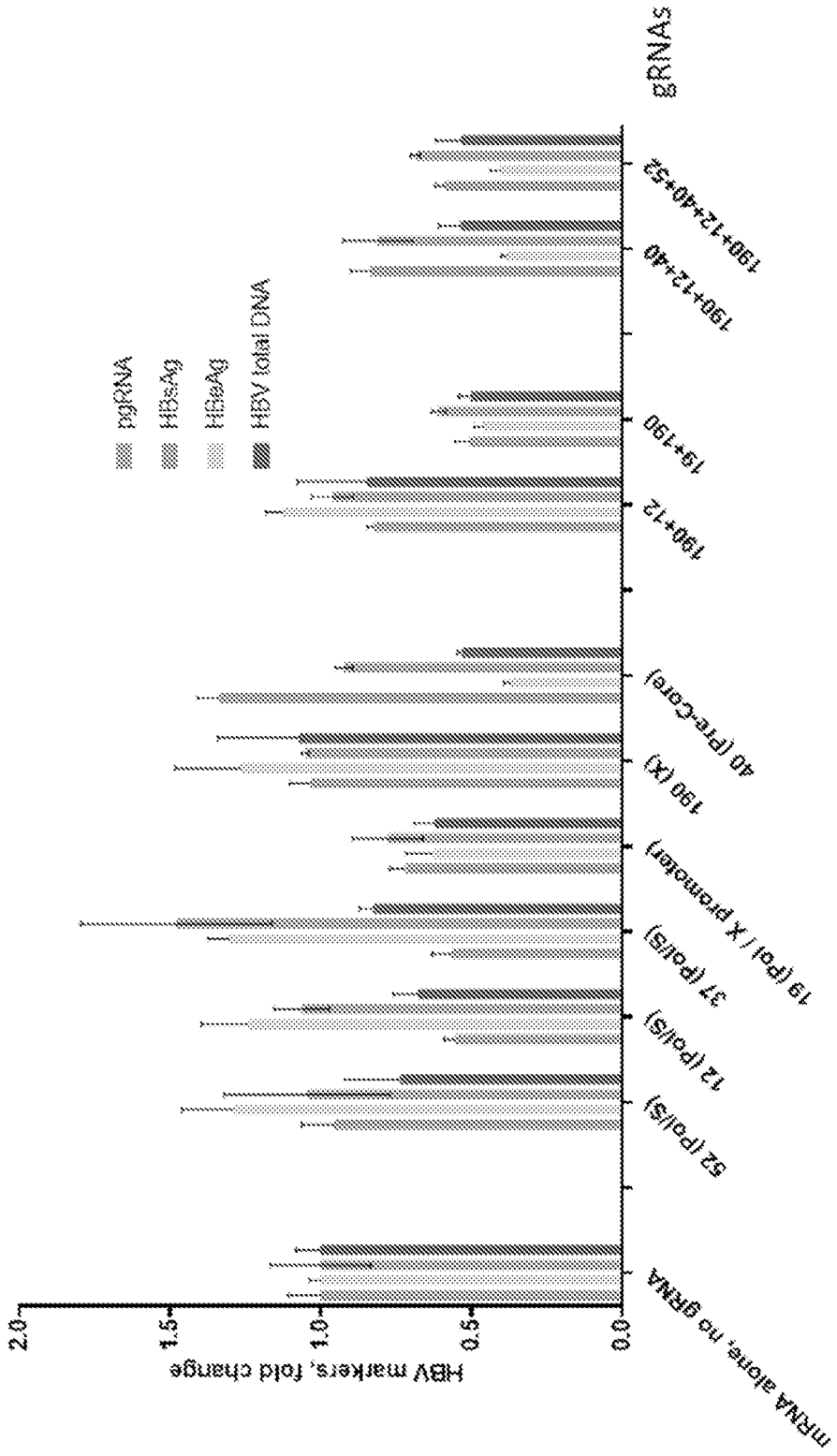

pgRNA
HBsAg
HBeAg
HBV total DNA
HBV markers, fold change
2.0
1.5
1.0
0.5
0.0
mRNA alone, no gRNA
52 (Pol/S)
12 (Pol/S)
37 (Pol/S)
19 (Pol / X promoter)
190 (X)
40 (Pre-Core)
190+12
19+190
190+12+40
190+12+40+52
gRNAs

FIG. 22

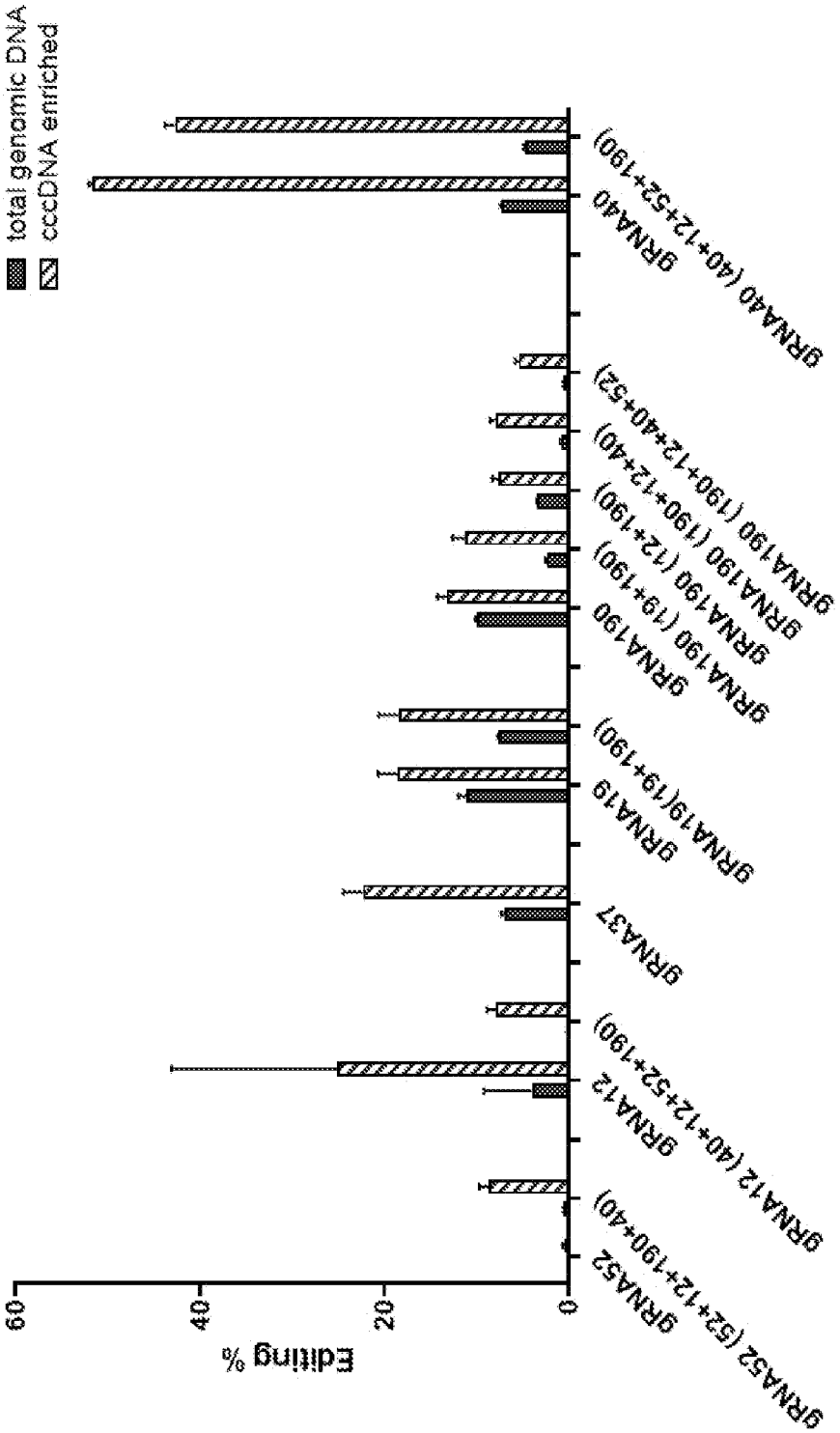

total genomic DNA
cccDNA enriched
Editing %
0
20
40
60
gRNA52
gRNA12
gRNA37
gRNA19
gRNA190
gRNA40
gRNA52 (52+12+190+40)
gRNA12 (40+12+52+190)
gRNA19(19+190)
gRNA190 (19+190)
gRNA190 (12+190)
gRNA190 (190+12+40)
gRNA40 (40+12+52+190)

FIG. 23

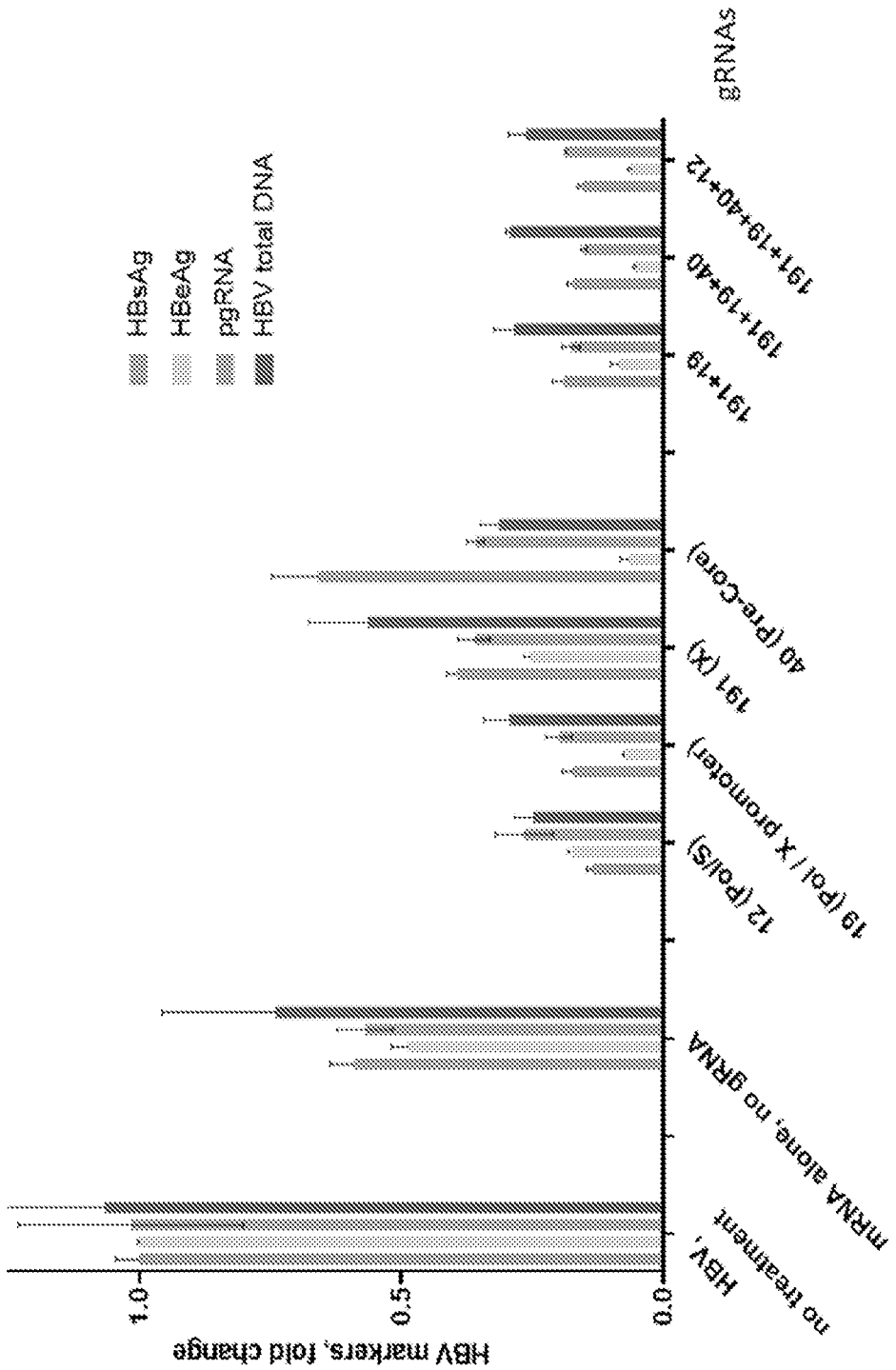

HBV markers, fold change
1.0
0.5
0.0
HBsAg
HBeAg
pgRNA
HBV total DNA
no treatment
HBV
mRNA alone, no gRNA
12 (Pol/S)
19 (Pol / X promoter)
191 (X)
40 (Pre-Core)
191+19
191+19+40
191+19+40+12
gRNAs

FIG. 24

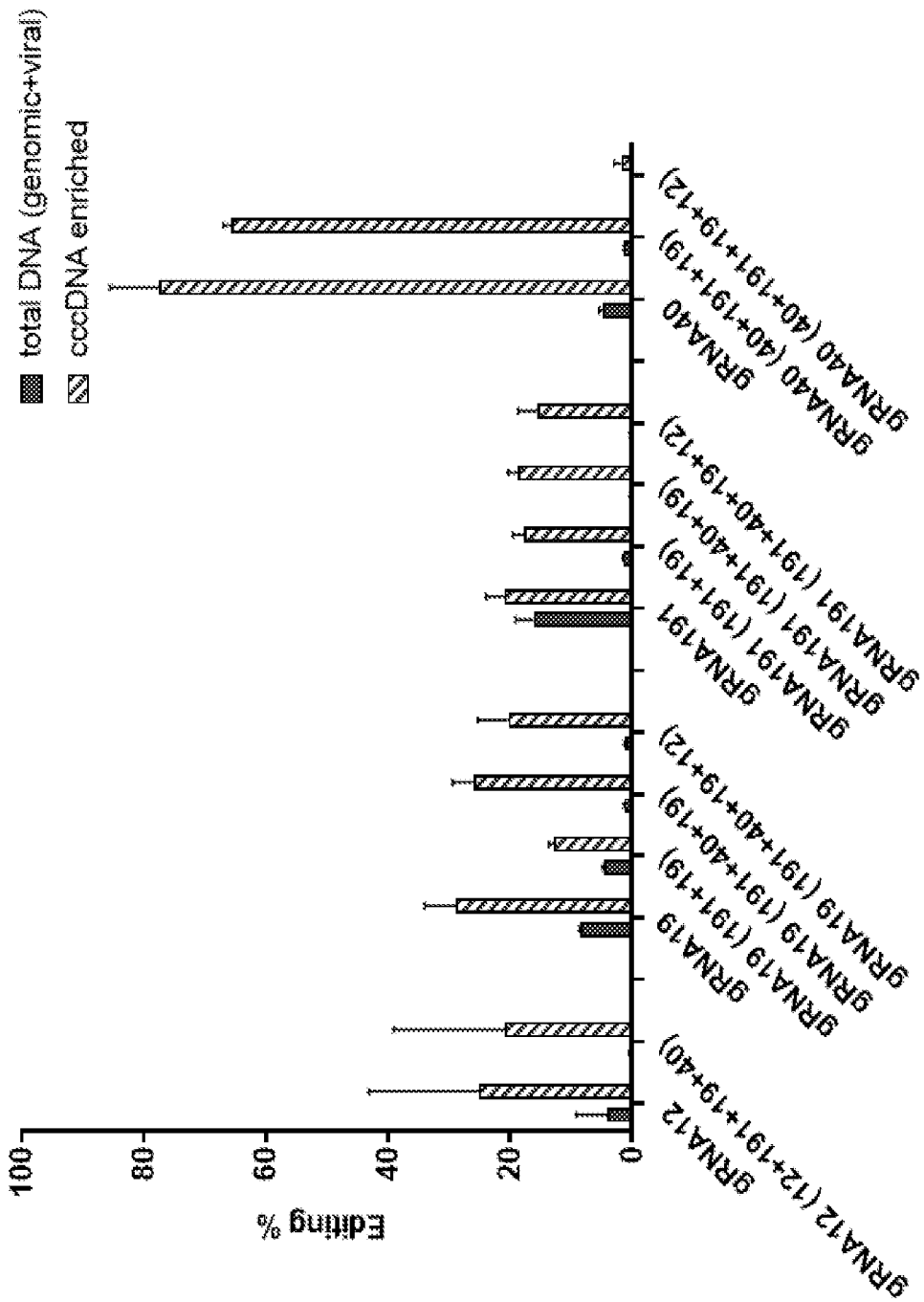

total DNA (genomic+viral)
cccDNA enriched
Editing %
100
80
60
40
20
0
gRNA12
gRNA12 (12+191+19+40)
gRNA19
gRNA19 (191+19)
gRNA19 (191+40+19)
gRNA19 (191+40+19+12)
gRNA191
gRNA191 (191+19)
gRNA191 (191+40+19)
gRNA191 (191+40+19+12)
gRNA40
gRNA40 (40+191+19)
gRNA40 (40+191+19+12)

FIG. 25A

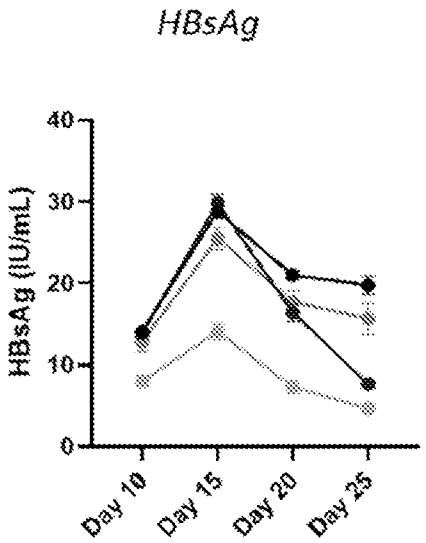


FIG. 25B

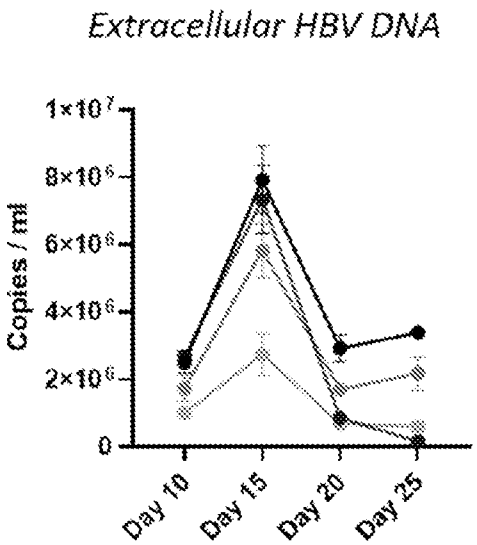


FIG. 25C

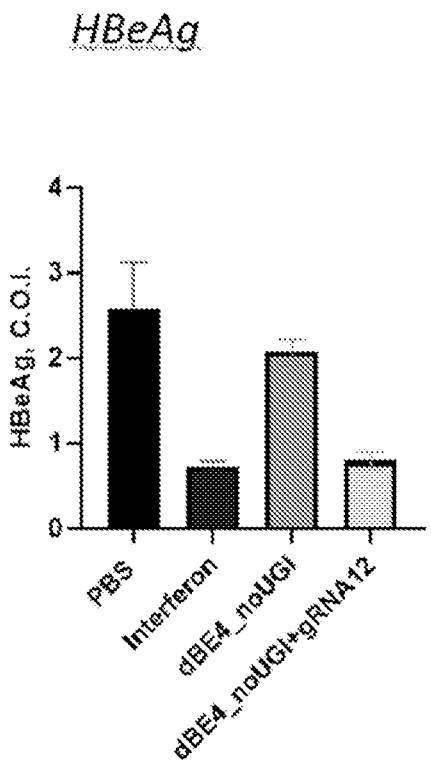


FIG. 25D

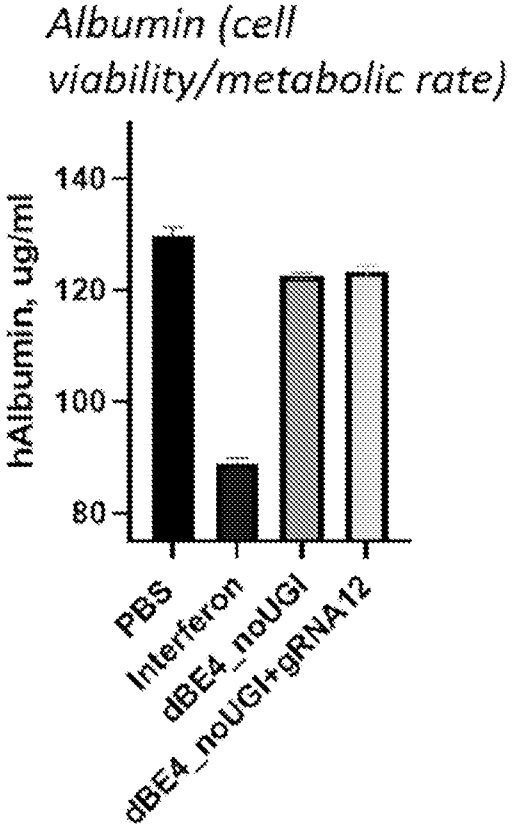


FIG. 26A

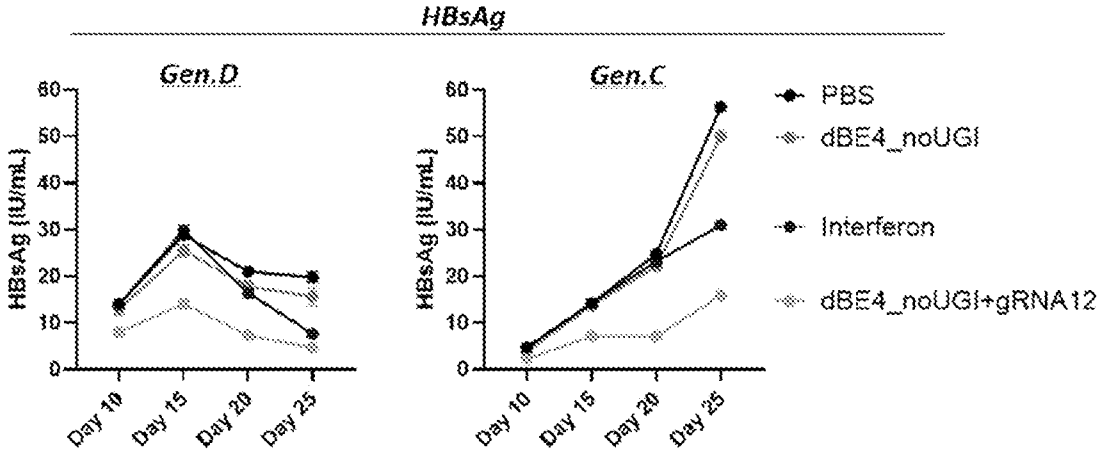


FIG. 26B

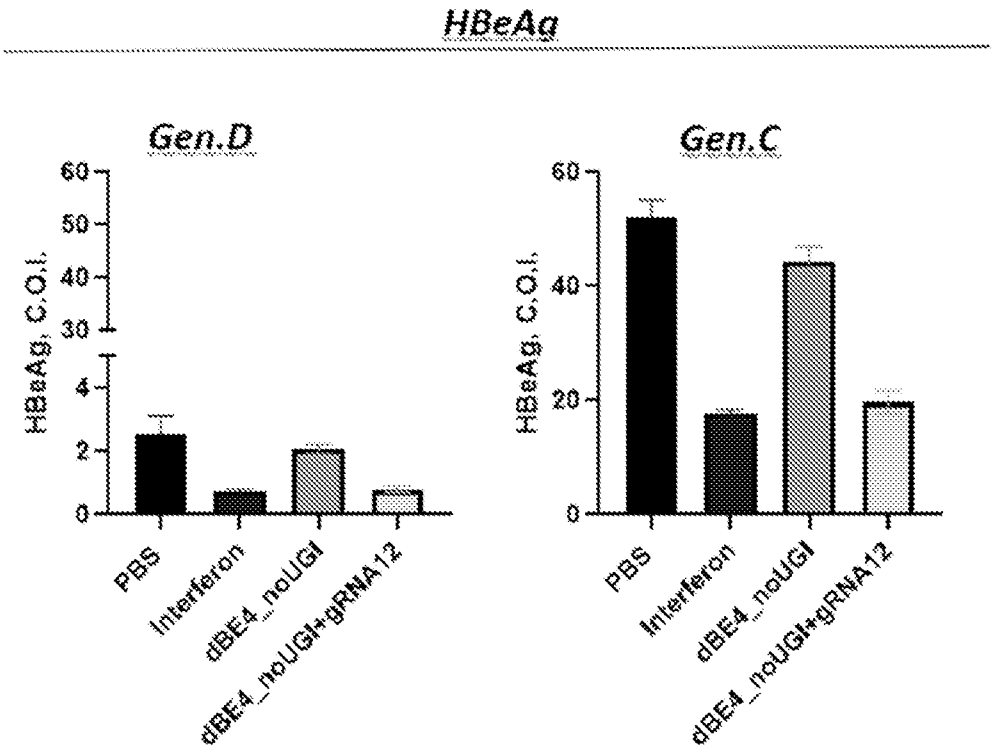


FIG. 26C

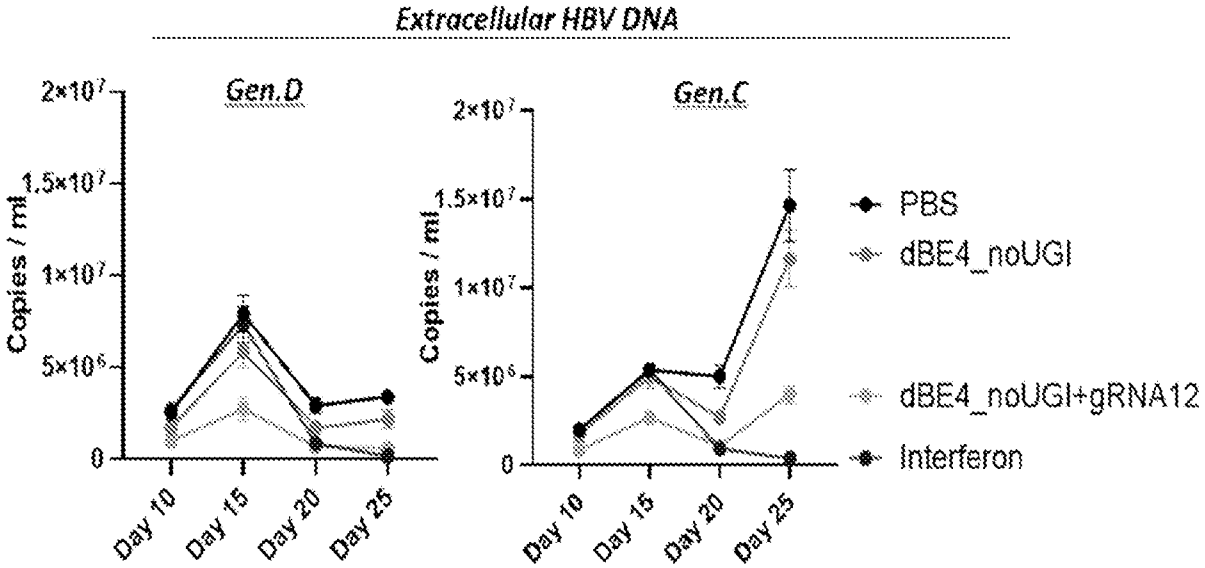


FIG. 27A

HBV viral parameters decrease after transfection with ABE7.10 & gRNA94 (targeting viral Polymerase active site)

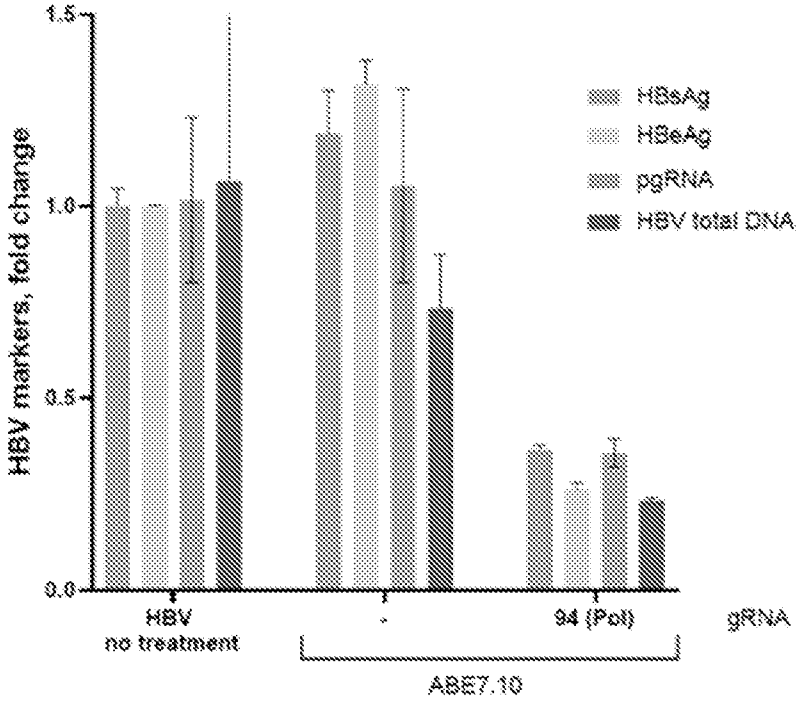
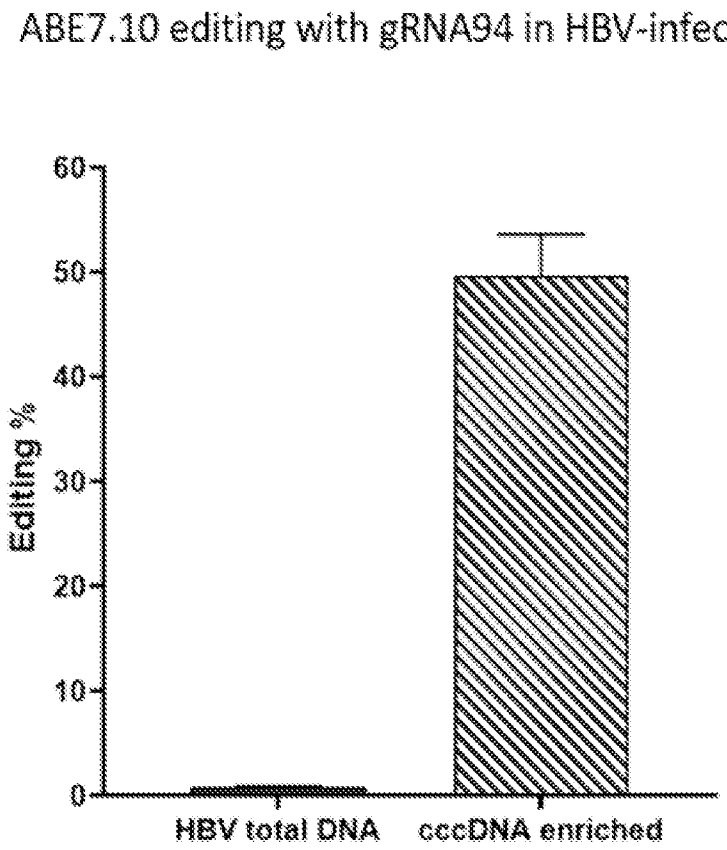


FIG. 27B


ABE7.10 editing with gRNA94 in HBV-infected PHH
Editing %
60
50
40
30
20
10
0
HBV total DNA
cccDNA enriched