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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 August 2020 (06.08.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/160514 A1

(51) International Patent Classification:
C12N 15/62 (2006.01) *C12N 15/10* (2006.01)
C12N 15/11 (2006.01)

(21) International Application Number:
PCT/US2020/016285

(22) International Filing Date:
31 January 2020 (31.01.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/799,702 31 January 2019 (31.01.2019) US

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(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,

(54) Title: NUCLEOBASE EDITORS HAVING REDUCED NON-TARGET DEAMINATION AND ASSAYS FOR CHARACTERIZING NUCLEOBASE EDITORS

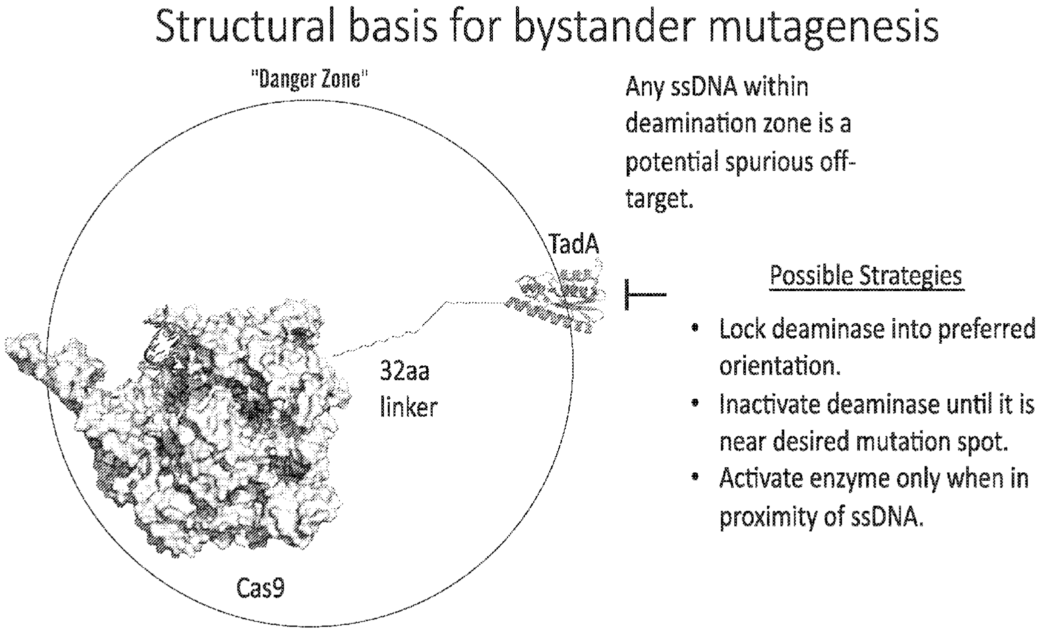

FIG. 1

(57) Abstract: The invention features base editors having reduced non-target deamination, methods of using the base editors, and assays for characterizing base editors as having decreased non-target deamination, e.g. compared to programmed, on-target deamination.

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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).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

NUCLEOBASE EDITORS HAVING REDUCED NON-TARGET DEAMINATION AND ASSAYS FOR CHARACTERIZING NUCLEOBASE EDITORS

CROSS-REFERENCE

This application claims the benefit of U.S. Provisional Patent Application No. 62/799,702, filed January 31, 2019, the contents of which are incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Deaminases combined with the precise targeting of CRISPR-Cas proteins, termed nucleobase editors, have the ability to introduce specific point mutations into target polynucleotides. Nucleobase editors induce base changes without introducing double-stranded DNA breaks, and include adenosine base editors that convert target A•T to G•C and cytidine base editors that convert target C•G to T•A. However, introduction of nucleobase editors in cells has the potential to generate undesired base editor-associated edits, including genome-wide spurious deamination, bystander mutation, and target proximal edits. Spurious deamination events may occur throughout the genome, catalyzed by the base editor deamination domain acting independently of targeted base editing via programming of CRISPR-Cas domain by a guide RNA. Without being bound by theory, genome-wide spurious deamination events have the potential to occur where a single stranded DNA substrate is formed, for example due to "DNA breathing" or at DNA replication forks. Target proximal edits are base editing events that occur outside the on-target sequence, but are within ~ 200bp upstream or downstream of the targeted region. Bystander mutations are mutations that occur within the on-target, Cas9/sgRNA guided, base editing window which are not the desired target nucleobase. Bystander mutation may result in either silent mutation (no amino acid change) or non-synonymous mutation (amino acid change). Thus, there is a need for base editors having reduced non-target deamination.

SUMMARY OF THE INVENTION

As described below, the present invention features nucleobase editor compositions and methods and assays for characterizing nucleobase editors as having decreased non-target deamination, e.g. compared to programmed, on-target deamination.

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.

In one aspect provided herein is a fusion protein comprising a deaminase inserted within a flexible loop of a Cas9 polypeptide, wherein the fusion protein comprises the structure:

NH2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker.

In one aspect provided herein is a fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

In some embodiments, the deaminase of the fusion protein deaminates a target nucleobase in a target polynucleotide sequence. In some embodiments, the flexible loop comprises an amino acid in proximity to the target nucleobase when the fusion protein deaminates the target nucleobase. In some embodiments, the flexible loop comprises a part of an alpha-helix structure of the Cas9 polypeptide. In some embodiments, the target nucleobase is deaminated with lower off-target deamination as compared to an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.

In some embodiments, the target nucleobase is 1-20 nucleobases away from a Protospacer Adjacent Motif (PAM) sequence in the target polynucleotide sequence. In some embodiments, the target nucleobase is 2-12 nucleobases upstream of the PAM sequence. In some embodiments, the flexible loop comprises a region selected from the group consisting of amino acid residues at positions 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 as numbered in SEQ ID NO: 1, or a corresponding region thereof. In some embodiments, the deaminase 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 SEQ ID NO: 1 or corresponding amino acid positions thereof.

In some embodiments, the deaminase is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase is inserted between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

In some embodiments, the N-terminal fragment comprises amino acid residues 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, and/or 1248-1297 of the Cas9 polypeptide as numbered in SEQ ID NO: 1, or corresponding residues thereof. In some embodiments, the C-terminal fragment comprises amino acid residues 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942, 580-685, and/or 538-568 of the Cas9 polypeptide as numbered SEQ ID NO: 1, or corresponding residues thereof. In some embodiments, the N terminal fragment or the C terminal fragment of the Cas9 polypeptide binds the target polynucleotide sequence.

In some embodiments, the N- terminal fragment or the C-terminal fragment of the Cas9 polypeptide comprises a DNA binding domain. In some embodiments, the N-terminal fragment or the C-terminal fragment comprises a RuvC domain. In some embodiments, the N-terminal fragment or the C terminal fragment comprises a HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a RuvC domain. In some embodiments, the Cas9 polypeptide comprises a partial or complete deletion in one or more structural domains. In some embodiments, the deaminase is inserted at the partial or complete deletion position of the Cas9 polypeptide.

In some embodiments, the deletion is within a RuvC domain. In some embodiments, the deletion is within an HNH domain. In some embodiments, the deletion bridges a RuvC domain and a C-terminal domain, a L-I domain and a HNH domain, or a RuvC domain and a L-I domain. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 1017-1069 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

In one aspect, provided herein is a fusion protein comprising a deaminase inserted within a Cas9 polypeptide, wherein the fusion protein comprises the structure:

NH2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, wherein the Cas9 polypeptide comprises a complete deletion of a HNH domain, and wherein the deaminase is inserted at the deletion position.

In some embodiments, the C terminal amino acid of the N terminal fragment is amino acid 791 as numbered in SEQ ID NO: 1. In some embodiments, the N terminal amino acid of the C terminal fragment is amino acid 907 as numbered in SEQ ID NO: 1. In some embodiments, the N terminal amino acid of the C terminal fragment is amino acid 873 as numbered in SEQ ID NO: 1.

In one aspect provided herein is a fusion protein comprising a deaminase inserted within a Cas9 polypeptide, wherein the fusion protein comprises the structure:

NH2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, and wherein the Cas9 comprises a complete deletion of a RuvC domain and wherein the deaminase is inserted at the deletion position.

In some embodiments, the deaminase is a cytidine deaminase or an adenosine deaminase. In some embodiments, the cytidine deaminase is an APOBEC cytidine deaminase, an activation induced cytidine deaminase (AID), or a CDA. In some embodiments, the APOBEC deaminase is APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3E, APOBEC3F, APOBEC3G, APOBEC3H, or APOBEC4. In some embodiments, the APOBEC deaminase is rAPOBEC1. In some embodiments, the fusion protein of any one of aspects above further comprises a UGI domain.

In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is a modified TadA. In some embodiments, the TadA deaminase is a TadA 7.10. In some embodiments, the adenosine deaminase is a TadA dimer. In some embodiments, the TadA dimer comprises a TadA 7.10 and a wild type TadA. In some embodiments, the optional linker comprises (SGGS)n, (GGGS)n, (GGGGS) n, (G)n, (EAAAK)n, (GGS)n, SGSETPGTSESATPES, or (XP)n motif, or a combination thereof, wherein n is independently an integer between 1 and 30.

In some embodiments, the N terminal fragment of the Cas9 polypeptide is fused to the deaminase without a linker. In some embodiments, the C terminal fragment of the Cas9 is fused to the deaminase without a linker. In some embodiments, the fusion protein of any one of aspects above, further comprises an additional catalytic domain.

In some embodiments, the additional catalytic domain is a second deaminase. In some embodiments, the second deaminase is fused to the N terminus or the C terminus of the fusion protein. In some embodiments, the deaminase is a cytidine deaminase or an adenosine deaminase. In some embodiments, the fusion protein of any one of aspects above further comprises a nuclear localization signal. In some embodiments, the nuclear localization signal is a bipartite nuclear localization signal. In some embodiments, the Cas9 polypeptide is a Streptococcus pyogenes Cas9 (SpCas9), Staphylococcus aureus Cas9 (SaCas9), Streptococcus thermophilus 1 Cas9 (St1Cas9), or variants thereof. In some embodiments, the Cas9 polypeptide is a modified Cas9 and has specificity for an altered PAM. In some embodiments, the Cas9 polypeptide is a nickase. In some embodiments, the Cas9 polypeptide is nuclease inactive. In some embodiments, the fusion protein of any one of aspects above in complex with a guide nucleic acid sequence to effect deamination of the target nucleobase. In some embodiments, the fusion protein is further complexed with the target polynucleotide.

Provided herein is a polynucleotide encoding the fusion protein of any one of aspects above.

Provided herein is an expression vector comprising the polynucleotide described above.

In some embodiments, the expression vector is a mammalian expression vector. In some embodiments, the vector is a viral vector selected from the group consisting of adeno-associated virus (AAV), retroviral vector, adenoviral vector, lentiviral vector, Sendai virus vector, and herpesvirus vector. In some embodiments, the vector comprises a promoter.

Provided herein is a cell comprising the fusion protein of any one of aspects above, the polynucleotide described above, or the vector described above.

In some embodiments, the cell is a bacterial cell, plant cell, insect cell, a human cell, or mammalian cell.

Provided herein is a kit comprising the fusion protein of any one of aspects above, the polynucleotide described above, or the vector described above.

Provided herein is a method for base editing comprising contacting a polynucleotide sequence with the fusion protein of any one of aspects above, wherein the deaminase of the fusion protein deaminates a nucleobase in the polynucleotide, thereby editing the polynucleotide sequence.

In some embodiments, the method further comprises contacting the target polynucleotide sequence with a guide nucleic acid sequence to effect deamination of the target nucleobase.

In one aspect, provided herein is a method for editing a target nucleobase in a target polynucleotide sequence, the method comprising: contacting the target polynucleotide sequence with a fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragments of a Cas9 polypeptide, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence, and wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

Provided herein is a method for editing a target nucleobase in a target polynucleotide sequence, the method comprising: contacting the target polynucleotide sequence with a fusion protein comprising a deaminase inserted within a flexible loop of a Cas9 polypeptide, wherein the fusion protein comprises the structure NH2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence.

In some embodiments, the method further comprises contacting the target polynucleotide sequence with a guide nucleic acid sequence to effect deamination of the target nucleobase. In some embodiments, the guide nucleic acid sequence comprises a spacer sequence complementary to a protospacer sequence of the target polynucleotide sequence, thereby forming a R-loop. In some embodiments, the target nucleobase is deaminated with lower off-target deamination as compared to an end terminus method comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1. In some embodiments, the deaminase of the fusion protein deaminates no more than two nucleobases within the range of the R-loop. In some embodiments, the target nucleobase is 1-20 nucleobases away from a PAM sequence in the target polynucleotide sequence. In some embodiments, the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.

In some embodiments, the flexible loop comprises an amino acid in proximity to the target nucleobase when the deaminase of the fusion protein deaminates the target nucleobase. In some embodiments, the flexible loop comprises a region selected from the group consisting of amino acid residues at positions 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 as numbered in SEQ ID NO: 1, or a corresponding region thereof. In some embodiments, the deaminase 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 SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase is inserted between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-

1070 or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

In some embodiments, the N-terminal fragment comprises amino acid residues 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, and/or 1248-1297 of the Cas9 polypeptide as numbered in SEQ ID NO: 1, or corresponding residues thereof. In some embodiments, the C-terminal fragment comprises amino acid residues 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942, 580-685, and/or 538-568 of the Cas9 polypeptide as numbered SEQ ID NO: 1, or corresponding residues thereof. In some embodiments, the N terminal fragment or the C terminal fragment of the Cas9 polypeptide binds the target polynucleotide sequence. In some embodiments, the N-terminal fragment or the C-terminal fragment comprises a RuvC domain. In some embodiments, the N-terminal fragment or the C-terminal fragment comprises a HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a RuvC domain.

In some embodiments, the Cas9 polypeptide comprises a partial or complete deletion in one or more structural domains. In some embodiments, the deaminase is inserted at the partial or complete deletion position of the Cas9 polypeptide. In some embodiments, the deletion is within a RuvC domain. In some embodiments, the deletion is within an HNH domain. In some embodiments, the deletion bridges a RuvC domain and a C-terminal domain, a L-I domain and a HNH domain, or a RuvC domain and a L-I domain. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 1017-1069 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof. In some embodiments, the deaminase is a cytidine deaminase. In some embodiments, the deaminase is an adenosine deaminase. In some embodiments, the Cas9 polypeptide is a modified Cas9 and has specificity for an altered protospacer-adjacent motif (PAM). In some embodiments, the Cas9 polypeptide is a nickase. In some embodiments, the Cas9 polypeptide is nuclease inactive.

In some embodiments, the contacting is performed in a cell. In some embodiments, the cell is a mammalian cell or a human cell. In some embodiments, the cell is a pluripotent cell. In some embodiments, the cell is in vivo or ex vivo. In some embodiments, the contacting is performed in a population of cells. In some embodiments, the population of cells are mammalian cells or human cells.

In one aspect provided herein is a method for treating a genetic condition in a subject, the method comprising: administering to the subject a fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide or a polynucleotide encoding the fusion protein, and a guide nucleic acid sequence or a polynucleotide encoding the guide nucleic acid sequence, wherein the guide nucleic acid sequence directs the fusion protein to deaminate a target nucleobase in a target polynucleotide sequence of the subject, thereby treating the genetic condition.

Provided herein is a method for treating a genetic condition in a subject, the method comprising: administering to the subject a fusion protein comprising a deaminase inserted within a flexible loop of a Cas9 polypeptide, wherein the fusion protein comprises the structure NH2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence of the subject, thereby treating the genetic condition.

In some embodiments, the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide. In some embodiments, the method further comprises administering to the subject a guide nucleic acid sequence to effect deamination of the target nucleobase. In some embodiments, the target nucleobase comprises a mutation associated with the genetic condition. In some embodiments, the deamination of the target nucleobase replaces the target nucleobase with a wild type nucleobase. In some embodiments, the deamination of the target nucleobase replaces the target nucleobase with a non-wild type nucleobase, and wherein the deamination of the target nucleobase ameliorates symptoms of the genetic condition.

In some embodiments, the target polynucleotide sequence comprises a mutation associated with the genetic condition at a nucleobase other than the target nucleobase. In some embodiments, the deamination of the target nucleobase ameliorates symptoms of the genetic condition. In some embodiments, the target nucleobase is 1-20 nucleobases away from a PAM sequence in the target polynucleotide sequence. In some embodiments, the target nucleobase is 2-12 nucleobases upstream of the PAM sequence. In some embodiments, the flexible loop comprises an amino acid in proximity to the target nucleobase when the deaminase of the fusion protein deaminates the target nucleobase.

In some embodiments, the flexible loop comprises a region selected from the group consisting of amino acid residues at positions 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 as numbered in SEQ ID NO: 1, or a corresponding region thereof.

In some embodiments, the deaminase 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 SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase is inserted between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

In some embodiments, the N-terminal fragment comprises amino acid residues 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, and/or 1248-1297 of the Cas9 polypeptide as numbered in SEQ ID NO: 1, or corresponding residues thereof. In some embodiments, the C-terminal fragment comprises amino acid residues 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942, 580-685, and/or 538-568 of the Cas9 polypeptide as numbered SEQ ID NO: 1, or corresponding residues thereof. In some embodiments, the N terminal fragment or the C terminal fragment of the Cas9 polypeptide binds the target polynucleotide sequence. In some embodiments, the N-terminal fragment or the C-terminal fragment comprises a RuvC domain. In some embodiments, the N-terminal fragment or the C-terminal fragment comprises a HNH domain.

In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a RuvC domain. In some embodiments, the Cas9 polypeptide comprises a partial or complete deletion in one or more structural domains. In some embodiments, the deaminase is inserted at the partial or complete deletion position of the Cas9 polypeptide. In some embodiments, the deletion is within a RuvC domain. In some embodiments, the deletion is within an HNH domain. In some embodiments, the deletion bridges a RuvC domain and a C-terminal domain, a L-I domain and a HNH domain, or a RuvC domain and a L-I domain. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 1017-1069 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof. In some embodiments, the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof. In some embodiments, the deaminase is a cytidine deaminase. In some embodiments, the deaminase is an adenosine deaminase. In some embodiments, the Cas9 polypeptide is a modified Cas9 and has specificity for an altered PAM. In some embodiments, the Cas9 polypeptide is a nickase. In some embodiments, the Cas9 polypeptide is nuclease inactive. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

Provided herein is a protein library for optimized base editing comprising a plurality of fusion proteins, wherein each one of the plurality of fusion proteins comprises a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the N-terminal fragment of each one of the fusion proteins differs from the N-terminal fragments of the rest of the plurality of fusion proteins or wherein the C-terminal fragment of each one of the fusion proteins differs from the C-terminal fragments of the rest of the plurality of fusion proteins, wherein the deaminase of each one of the fusion proteins deaminates a target nucleobase in proximity to a Protospacer Adjacent Motif (PAM) sequence in a target polynucleotide sequence, and wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide sequence.

In some embodiments, for each nucleobase from 1 to 20 nucleobases away of the PAM sequence, at least one of the plurality of fusion proteins deaminates the nucleobase. In some embodiments, the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment of the Cas9 polypeptide of each one of the plurality of fusion proteins comprises a part of a flexible loop of the Cas9 polypeptide. In some embodiments, at least one of the plurality of fusion proteins deaminates the target nucleobase with lower off-target deamination as compared to an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1. In some embodiments, at least one of the plurality of the fusion proteins deaminates a target nucleobase 2-12 nucleobases upstream of the PAM sequence. In some embodiments, the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment of a fusion protein of the plurality comprises an amino acid in proximity to the target nucleobase when the fusion protein deaminates the target nucleobase.

In some embodiments, the deaminase of at least one of the fusion proteins is 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 SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase of at least one of the fusion proteins is between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase of at least one of the fusion proteins is between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof. In some embodiments, the deaminase is an adenosine deaminase. In some embodiments, the deaminase is a cytidine deaminase.

In some embodiments, the Cas9 polypeptide is a Streptococcus pyogenes Cas9 (SpCas9), Staphylococcus aureus Cas9 (SaCas9), Streptococcus thermophilus 1 Cas9 (St1Cas9), or variants thereof. In some embodiments, the Cas9 polypeptide is a modified Cas9 and has specificity for an altered protospacer-adjacent motif (PAM). In some embodiments, the Cas9 polypeptide is a nickase. In some embodiments, the Cas9 polypeptide is nuclease inactive.

Definitions

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.

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Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

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. 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, the truncated ecTadA may be missing one or more N-terminal amino acids relative to a full-length ecTadA. In some embodiments, the truncated ecTadA may be 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 ecTadA. In some embodiments, the truncated ecTadA may be 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 ecTadA. In some embodiments, the ecTadA deaminase does not comprise an N-terminal methionine. In some embodiments, the TadA deaminase is an N-terminal truncated TadA. In particular embodiments, the TadA is any one of the TadA described in PCT/US2017/045381, which is incorporated herein by reference in its entirety.

In certain embodiments, the adenosine deaminase comprises the amino acid sequence:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPT
AHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKT

GAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD, which is termed "the TadA reference sequence".

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:

MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEG
WNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIG
RVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEI
KAQKKAQSSTD.

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 (AD AT). Exemplary AD AT homologs include, without limitation:

Staphylococcus aureus TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAH
AEHIAIERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCS
GS LMNLLQQS NFNHRAIVDKG VLKE AC S TLLTTFFKNLRANKKS TN

Bacillus subtilis TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEML
VIDEACKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGC S
GTLMN LLQEERFNHQAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEG
WNRPIGRHDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIG
RVVFGARDAKTGAAGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIK
ALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:

MDE YWMQVAMQM AEKAEAAGE VPVGA VLVKDGQQIATGYNLS IS QHDPT
AHAEI

LCLRSAGKKLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGT VVNLLQHPAFNHQVEVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

Haemophilus influenzae F3031 (*H. influenzae*) TadA:
MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQS DPT AH AEIIALRNG AKNIQN YRLLNS TLY VTLEPCTMC AG AILHS RIKRLVFG AS D YK
TGAIGSRFHFFDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSD K

Caulobacter crescentus (*C. crescentus*) TadA:
MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAH DPTAHAEIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADD PKGGAVVHGPKFFAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:
MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSN DPSAHAEMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDP KGGAAGSLYDLSADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALF IDERKVPPEP

TadA7.10
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPT AHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKT GAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

Exemplary sequences containing TadA7.10 or TadA7.10 variants include
GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG AVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLY VTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE ITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

TadA7.10 CP65
TAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVF

GVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQ
VFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTL
AKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDP

TadA7.10 CP83
YRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLH
YPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGS
ETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVL
NNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQN

TadA7.10 CP136
MNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETP
GTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNR
VIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM
CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG

TadA7.10 C-truncate
GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG
AVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLY
VTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE
ITEGILADECAALLCYFFRMPRQVFN

TadA7.10 C-truncate 2
GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG
AVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLY
VTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE
ITEGILADECAALLCYFFRMPRQ

TadA7.10 delta59-66+C-truncate
GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG
AVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM
CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD
ECAALLCYFFRMPRQVFN

TadA7.10 delta 59-66

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG
AVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM
CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD
ECAALLCYFFRMPRQVFNAQKKAQSSTD

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

By “alter a mutation”

By “alteration” is meant a change in the structure, 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 (e.g., increase or decrease) includes a 10% change in expression levels, a 25% change, a 40% change, and a 50% or greater change in expression levels.

By “analog” is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polynucleotide analog retains the biological activity of a corresponding naturally-occurring polynucleotide while having certain modifications that enhance the analog’s function relative to a naturally occurring polynucleotide. Such modifications could increase the polynucleotide’s affinity for DNA, half-life, and/or nuclease resistance. An analog may include an unnatural nucleotide or amino acid.

In this disclosure, “comprises,” “comprising,” “containing” and “having” and the like can have the meaning ascribed to them in U.S. Patent law and can mean “ includes,” “including,” and the like; “consisting essentially of” or “consists essentially” likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

By "base editor (BE)," or "nucleobase editor (NBE)" is meant an agent that binds a polynucleotide and has nucleobase modifying activity. In one embodiment, the agent is a fusion protein comprising a 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 domain having base editing activity is capable of deaminating a base within a nucleic acid molecule. In some embodiments, the base editor is capable of deaminating a base within a DNA molecule. In some embodiments, the base editor is capable of deaminating a cytosine (C) or an adenosine 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. 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 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 deaminase domain is a N-terminal or C-terminal fragment of the polynucletide programmable DNA binding domain. In some embodiments, the deaminase is flanked by an N-terminal and C-terminal fragment of a polynucleotide programmable DNA binding domain. In some embodiments, the deaminase domain is inserted into a site of the polynucleotide programmable DNA binding 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, the deaminase domain is inserted into regions of the polynucleotide programmable DNA binding domain. In some embodiments, the insertion site is determined by structural analysis of an napDNAbp. In some embodiments, the insertion site is a flexible loop. In some embodiments, the deaminase domain is inserted into a site in the polynucleotide programmable DNA binding domain, wherein the site is selected from at least one from a group of amino acid positions consisting of 1029, 1026, 1054, 1022, 1015, 1068, 1247, 1040, 1248, and 768. In some embodiments, the deaminase domain is inserted in place of a domain of polynucleotide programmable DNA binding domain. In some embodiments, the domain is selected from the group consisting of RuvC, Rec1, Rec2, and HNH. In some embodiments, the deaminase domain in inserted in place of a range of amino acid residues in the polynucleotide programmable DNA binding domain, wherein in the range of amino acid residues are selected from a group consisting of resdidues 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 of Cas9 as numbered in SEQ ID NO:1 or corresponding positions thereof. It would be apparent to the skilled artisan how to identify homologous regions in a different polynucleotide programmable DNA binding domain by comparing the Cas9 amino acid sequence. In some embodiments, the base editor comprises more than one deaminase domain inserted into more than one site of a polynucleotide programmable DNA binding domain, wherein the sites are described above.

In some embodiments, base editors are generated by cloning an adenosine deaminase variant (*e.g.*, TadA*7.10) 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*

The nucleobase components and the polynucleotide programmable nucleotide binding component of a base editor system may be associated with each other covalently or non-covalently. For example, in some embodiments, the deaminase domain can be targeted to a target nucleotide sequence by a polynucleotide programmable nucleotide binding domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to a deaminase domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can target a deaminase domain to a target nucleotide sequence by non-covalently interacting with or associating with the deaminase domain. For example, in some embodiments, the nucleobase editing component, e.g., the deaminase component can comprise an additional heterologous portion or domain that is capable of interacting with, associating with, or capable of forming a complex with an additional heterologous portion or domain that is part of a polynucleotide programmable nucleotide binding domain. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polypeptide. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion may be capable of binding to a polynucleotide linker. The additional heterologous portion may be a protein domain. In some embodiments, the additional heterologous portion may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat protein domain, a SfMu Com coat protein domain, a steril alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding motif and Sm7 protein, or a RNA recognition motif.

A base editor system may further comprise a guide polynucleotide component. It should be appreciated that components of the base editor system may be associated with each other via covalent bonds, noncovalent interactions, or any combination of associations and interactions thereof. In some embodiments, a deaminase domain can be targeted to a target nucleotide sequence by a guide polynucleotide. For example, in some embodiments, the nucleobase editing component of the base editor system, e.g., the deaminase component, can comprise an additional heterologous portion or domain (e.g., polynucleotide binding domain such as an RNA or DNA binding protein) that is capable of interacting with, associating with, or capable of forming a complex with a portion or segment (e.g., a polynucleotide motif) of a guide polynucleotide. In some embodiments, the additional heterologous portion or domain (e.g., polynucleotide binding domain such as an RNA or DNA binding protein) can be fused or linked to the deaminase domain. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polypeptide. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion may be capable of binding to a polynucleotide linker. The additional heterologous portion may be a protein domain. In some embodiments, the additional heterologous portion 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, a base editor system can further comprise an inhibitor of base excision repair (BER) component. It should be appreciated that components of the base editor system may be associated with each other via covalent bonds, noncovalent interactions, or any combination of associations and interactions thereof. The inhibitor of BER component may comprise a base excision repair inhibitor. In some embodiments, the inhibitor of base excision repair can be a uracil DNA glycosylase inhibitor (UGI). In some embodiments, the inhibitor of base excision repair can be an inosine base excision repair inhibitor. In some embodiments, the inhibitor of base excision repair can be targeted to the target nucleotide sequence by the polynucleotide programmable nucleotide binding domain. In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to an inhibitor of base excision repair. In some embodiments, a polynucleotide programmable nucleotide binding domain can be fused or linked to a deaminase domain and an inhibitor of base excision repair. In some embodiments, a polynucleotide programmable nucleotide binding domain can target an inhibitor of base excision repair to a target nucleotide sequence by non-covalently interacting with or associating with the inhibitor of base excision repair. For example, in some embodiments, the inhibitor of base excision repair component can comprise an additional heterologous portion or domain that is capable of interacting with, associating with, or capable of forming a complex with an additional heterologous portion or domain that is part of a polynucleotide programmable nucleotide binding domain. In some embodiments, the inhibitor of base excision repair can be targeted to the target nucleotide sequence by the guide polynucleotide. For example, in some embodiments, the inhibitor of base excision repair can comprise an additional heterologous portion or domain (e.g., polynucleotide binding domain such as an RNA or DNA binding protein) that is capable of interacting with, associating with, or capable of forming a complex with a portion or segment (e.g., a polynucleotide motif) of a guide polynucleotide. In some embodiments, the additional heterologous portion or domain of the guide polynucleotide (e.g., polynucleotide binding domain such as an RNA or DNA binding protein) can be fused or linked to the inhibitor of base excision repair. In some embodiments, the additional heterologous portion may be capable of binding to, interacting with, associating with, or forming a complex with a polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a guide polynucleotide. In some embodiments, the additional heterologous portion may be capable of binding to a polypeptide linker. In some embodiments, the additional heterologous portion may be capable of binding to a polynucleotide linker. The additional heterologous portion may be a protein domain. In some embodiments, the additional heterologous portion 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.

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 deaminase activity, e.g., converting A•T to G•C.

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 casn1 nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat)-associated nuclease. 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, 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. 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 *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.

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, 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). 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, 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
GACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGACCCCT
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
AGAAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGT
GATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCC
TACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGA
AGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCC
ATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAACTACC
AAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGC
TTCAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATTTAGCGTCC
CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTTTTGTTGAGCA
GCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGAGAGTCATCC
TAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATA
CGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGC
ATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGATTATATGAAACTCGGATAGATTTGTCACAG
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

REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD (SEQ ID NO: 1. 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.

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, 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.

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).

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.

Exemplary catalytically inactive Cas9 (dCas9):
DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ

HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Exemplary catalytically Cas9 nickase (nCas9):

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW
MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Exemplary catalytically active Cas9:

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE

KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD.

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, Cas9 refers to CasX or CasY, 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, Cas9 refers to CasX, or a variant of CasX. In some embodiments, Cas9 refers to a 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 napDNAbp is a naturally-occurring CasX or 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 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.

CasX (uniprot.org/uniprot/F0NN87; uniprot.org/uniprot/F0NH53)
>tr|F0NN87|F0NN87_SULIH CRISPR-associated Casx protein OS = *Sulfolobus islandicus* (strain HVE10/4) GN = SiH_0402 PE=4 SV=1
MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAE
RRGKAKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQV
KECEEVSAPSFVKPEFYEFGRSPGMVERTRRVKLEVEPHYLIIAAAGWVLTRLGKAK
VSEGDYVGVNVFTPTRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSV
VRIYTISDAVGQNPTTINGGFSIDLTKLLEKRYLLSERLEAIARNALSISSNMRERYIVL
ANYIYEYLTG SKRLEDLLYFANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG

>tr|F0NH53|F0NH53_SULIR CRISPR associated protein, Casx OS = *Sulfolobus islandicus* (strain REY15A) GN=SiRe_0771 PE=4 SV=1
MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAE
RRGKAKKKKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQV
KECEEVSAPSFVKPEFYKFGRSPGMVERTRRVKLEVEPHYLIMAAAGWVLTRLGKA
KVSEGDYVGVNVFTPTRGILYSLIQNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVS
VVSIYTISDAVGQNPTTINGGFSIDLTKLLEKRDLLSERLEAIARNALSISSNMRERYIV
LANYIYEYLTGSKRLEDLLYFANRDLIMNLNSDDGKVRDLKLISAYVNGELIRGEG

CasY (ncbi.nlm.nih.gov/protein/APG80656.1)

>APG80656.1 CRISPR-associated protein CasY [uncultured Parcubacteria group bacterium]

MSKRHPRISGVKGYRLHAQRLEYTGKSGAMRTIKYPLYSSPSGGRTVPREIVSAINDD
YVGLYGLSNFDDLYNAEKRNEEKVYSVLDFWYDCVQYGAVFSYTAPGLLKNVAEV
RGGSYELTKTLKGSHLYDELQIDKVIKFLNKKEISRANGSLDKLKKDIIDCFKAEYRE
RHKDQCNKLADDIKNAKKDAGASLGERQKKLFRDFFGISEQSENDKPSFTNPLNLTC
CLLPFDTVNNNRNRGEVLFNKLKEYAQKLDKNEGSLEMWEYIGIGNSGTAFSNFLG
EGFLGRLRENKITELKKAMMDITDAWRGQEQEEELEKRLRILAALTIKLREPKFDNH
WGGYRSDINGKLSSWLQNYINQTVKIKEDLKGHKKDLKKAKEMINRFGESDTKEEA
VVSSLLESIEKIVPDDSADDEKPDIPAIAIYRRFLSDGRLTLNRFVQREDVQEALIKERL
EAEKKKKPKKRKKKSDAEDEKETIDFKELFPHLAKPLKLVPNFYGDSKRELYKKYK
NAAIYTDALWKAVEKIYKSAFSSSLKNSFFDTDFDKDFFIKRLQKIFSVYRRFNTDKW
KPIVKNSFAPYCDIVSLAENEVLYKPKQSRSRKSAAIDKNRVRLPSTENIAKAGIALA
RELSVAGFDWKDLLKKEEHEEYIDLIELHKTALALLLAVTETQLDISALDFVENGTV
KDFMKTRDGNLVLEGRFLEMFSQSIVFSELRGLAGLMSRKEFITRSAIQTMNGKQAE
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QFLEWFLHRPKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSERVFVSQ
PFTIFPEKSAEEEGQRYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVK
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KIKKVYATLKKADVYSEIDADKNLQTTVWGKLAVASEISASYTSQFCGACKKLWRA
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KMRGNSCLFICPFCRANADADIQASQTIALLRYVKEEKKVEDYFERFRKLKN IKVLG
QMKKI

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 base sequence and amino acid sequence of PmCDA1 and the base sequence and amino acid sequence of CDS of human AID are shown herein below:

>tr|A5H718|A5H718_PETMA Cytosine deaminase OS=Petromyzon marinus OX=7757 PE=2 SV=1

MTDAEYVRIHEKLDIYTFKKQFFNNKKSVSHRCYVLFELKRRGERRACFWGYAVNKPQSG
TERGIHAEIFSIRKVEEYLRDNPGQFTINWYSSWSPCADCAEKILEWYNQELRGNGHTLK
IWACKLYYEKNARNQIGLWNLRDNGVGLNVMVSEHYQCCRKIFIQSSHNQLNENRWLEKT
LKRAEKRRSELSIMIQVKILHTTKSPAV

>EF094822.1 Petromyzon marinus isolate PmCDA.21 cytosine deaminase mRNA, complete cds

TGACACGACACAGCCGTGTATATGAGGAAGGGTAGCTGGATGGGGGGGGGGGAATACGTTCAGAGAGGA
CATTAGCGAGCGTCTTGTTGGTGGCCTTGAGTCTAGACACCTGCAGACATGACCGACGCTGAGTACGTGA
GAATCCATGAGAAGTTGGACATCTACACGTTTAAGAAACAGTTTTTCAACAACAAAAAATCCGTGTCGCA
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ATTCAGGTAAAAATACTCCACACCACTAAGAGTCCTGCTGTTTAAGAGGCTATGCGGATGGTTTTC

>tr|Q6QJ80|Q6QJ80_HUMAN Activation-induced cytidine deaminase OS=Homo sapiens OX=9606 GN=AICDA PE=2 SV=1

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHVELL
FLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRK
AEPEGLRRLHRAGVQIAIMTFKAPV

>NG_011588.1:5001-15681 Homo sapiens activation induced cytidine deaminase (AICDA), RefSeqGene (LRG_17) on chromosome 12

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CATAGTTCTAGCTGCTTGGGAGGTTGAGGAGGGAGGATGGCTTGAACACAGGTGTTCAAGGCCAGCCTGG
GCAACATAACAAGATCCTGTCTCTCAAAAAAAAAAAAAAAAAAAAAAGAAAGAGAGAGGGCCGGGCGTGGTG
GCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGCCGGGCGGATCACCTGTGGTCAGGAGTTTGAGA
CCAGCCTGGCCAACATGGCAAAACCCCGTCTGTACTCAAAATGCAAAAATTAGCCAGGCGTGGTAGCAGG
CACCTGTAATCCCAGCTACTTGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCAGGAGGTGGAGGTTGCA
GTAAGCTGAGATCGTGCCGTTGCACTCCAGCCTGGGCGACAAGAGCAAGACTCTGTCTCAGAAAAAAAAA
AAAAAAAGAGAGAGAGAGAAAGAGAACAATATTTGGGAGAGAAGGATGGGAAGCATTGCAAGGAAAT
TGTGCTTTATCCAACAAAATGTAAGGAGCCAATAAGGGATCCCTATTTGTCTCTTTTGGTGTCTATTTGT
CCCTAACAACTGTCTTTGACAGTGAGAAAAATATTCAGAATAACCATATCCCTGTGCCGTTATTACCTAG
CAACCCTTGCAATGAAGATGAGCAGATCCACAGGAAAACTTGAATGCACAACTGTCTTATTTTAATCTTA
TTGTACATAAGTTTGTAAAAGAGTTAAAAATTGTTACTTCATGTATTCATTTATATTTTATATTATTTTG
CGTCTAATGATTTTTTATTAACATGATTTCCTTTTCTGATATATTGAAATGGAGTCTCAAAGCTTCATAA
ATTTATAACTTTAGAAATGATTCTAATAACAACGTATGTAATTGTAACATTGCAGTAATGGTGCTACGAA
GCCATTTCTCTTGATTTTTAGTAAACTTTTATGACAGCAAATTTGCTTCTGGCTCACTTTCAATCAGTTA
AATAAATGATAAATAATTTTGGAAGCTGTGAAGATAAAATACCAAATAAAATAATATAAAAGTGATTTAT
ATGAAGTTAAAATAAAAAATCAGTATGATGGAATAAACTTG

Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (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. 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).

Other exemplary deaminases that can be fused to Cas9 according to aspects of this disclosure are provided below. 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:

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL (underline: nuclear localization sequence; double underline: nuclear export signal)

Mouse AID:

MDSLLMKQKKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSCSLDFGHLRNKSGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVAEFLRWNPNLSLRIFTARLYFCEDRKAEPEGLRRLHRAGVQIGIMTFKDYFYCWNTFVENRERTFKAWEGLHENSVRLTRQLRRILLPLYEVDDLRDAFRMLGF

(underline: nuclear localization sequence; double underline: nuclear export signal)

Dog AID:

MDSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGHLRNKSGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFAARLYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENREKTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL (underline: nuclear localization sequence; double underline: nuclear export signal)

Bovine AID:

MDSLLKKQRQFLYQFKNVRWAKGRHETYLCYVVKRRDSPTSFSLDFGHLRNKAGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFTARLYFCDKERKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL (underline: nuclear localization sequence; double underline: nuclear export signal)

Rat AID

MAVGSKPKAALVGPHWERERIWCFLCSTGLGTQQTGQTSRWLRPAATQDPVSPPRSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGYLRNKSGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLTGWGALPAGLMSPARPSDYF

YCWNTFVENHERTFKAWEGLHENSVRLSRRLRRILLPLYEVDDLRDAFRTLGL

(underline: nuclear localization sequence; double underline: nuclear export signal)

Mouse APOBEC-3

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)

Rhesus macaque APOBEC-3 G:

MVEPMDPRTFVSNFNNRPILSGLNTVWLCCEVKTKDPSGPPLDAKIFQGKVYSKAKYHPEMR
FLRWFHKWRQLHHDQEYKVTWYVSWSPCTRCANSVATFLAKDPKVTLTIFVARLYYFWKPDY
QQALRILCQKRGGPHATMKIMNYNEFQDCWNKFVDGRGKPFKPRNNLPKHYTLLQATLGELL
RHLMDPGTFTSNFNNKPWVSGQHETYLCYKVERLHNDTWVPLNQHRGFLRNQAPNIHGFPKG
RHAELCFLDLIPFWKLDGQQYRVTCFTSWSPCFSCAQEMAKFISNNEHVSLCIFAARIYDDQ
GRYQEGLRALHRDGAKIAMMNYSEFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Chimpanzee APOBEC-3 G:

MKPHFRNPVERMYQDTFSDNFYNRPILSHRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSKLKY*HPEM*
*RFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDVATFLAEDPKVTLTIFVARLYYFWDPDYQEALR
SLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTS
NFNNELWVRGRHETYLCYEVERLHNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLD*

*LHQDYRVTCFTSWSPCFSC*AQEMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLAKAGAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLEEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Green monkey APOBEC-3G:

MNPQIRNMVEQMEPDIFVYYFNNRPILSGRNTVWLCYEVKTKDPSGPPLDANIFQGKLYPEAKD*HPEMKFLHWFRKWRQLHRDQEYEVTWYVSWSPCTRC*ANSVATFLAEDPKVTLTIFVARLYYFWKPDYQQALRILCQERGGPHATMKIMNYNEFQHCWNEFVDGQGKPFKPRKNLPKHYTLLHATLGELLRHVMDPGTFTSNFNNKPWVSGQRETYLCYKVERSHNDTWVLLNQHRGFLRNQAPDRHGFPKGR*HAELCFLDLIPFWKLDDQQYRVTCFTSWSPCFSC*AQKMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLHRDGAKIAVMNYSEFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Human APOBEC-3G:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSELKY*HPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDMATFLAEDPKVTLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLDLDQDYRVTCFTSWSPCFSC*AQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN

(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Human APOBEC-3F:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQVYSQPEH*HAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKLAEFLAEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQVDPETH*CHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCWENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE

(italic: nucleic acid editing domain)

Human APOBEC-3B:

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGQVYFKPQY*HAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKLAEFLSEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVTIMDYEEFAYCWENFVYNEGQQFMPWYKFDENYAFLHRTLKEILRYLMDPDTFTFNFNN

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

Gorilla APOBEC3C

MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVFRNQVDSETH*CHAE*
*RCFLSWECDDILSPNTNYQVTWYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLYYFQDTDYQEGLR
SLSQEGVAVKIMDYKDFKYCWENFVYNDDEPFKPWKGLKYNFRFLKRRLQEILE

(italic: nucleic acid editing domain)

Human APOBEC-3 A:

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYG
R*HAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGC*AGEVRAFLQENTHVRLRIFAARIYDYDPLY
KEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3 A:

MDGSPASRPRHLMDPNTFTFNFNNDLSVRGRHQTYLCYEVERLDNGTWVPMDERRGFLCNKAKNVPCG
DYGC*HVELRFLCEVPSWQLDPAQTYRVTWFISWSPC*FRRGCAGQVRVFLQENKHVRLRIFAARIYDYD
PLYQEALRTLRDAGAQVSIMTYEEFKHCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Bovine APOBEC-3 A:

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
GLWNLRDNGVGLNVMVSEHYQCCRKIFIQSSHNQLNENRWLEKTLKRAEKRRSELSFMIQVKILHTTK
SPAV

Human APOBEC3G D316R D317R

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSELKYHPEM
RFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMATFLAEDPKVTLTIFVARLYYFWDPDYQEALR
SLCQKRDGPRATMKFNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHFMLGEILRHSMDPPTFTFN
FNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDL
DQDYRVTC
FTSWSPCFSCAQEMAKFISKKHVSLCIFTARIYRRQGRCQEGLRTLAEAGAKISFTYSEFKHCWDTFV
DHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN

Human APOBEC3G chain A

MDPPTFTFNFNNEPWWGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDV
IPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGA
KISF TYSEFKHCWDTFVDHQGCPFQPWDGLD EHSQDLSGRLRAILQ

Human APOBEC3G chain A D120R D121R

MDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLD
VIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYRRQGRCQEGLRTLAEAG
AKISFMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQ

The term "deaminase" or "deaminase domain" refers to a protein or fragment thereof that catalyzes a deamination reaction.

“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 ELISA), biotin, digoxigenin, or haptens.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, at least about 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.

"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 term "inhibitor of base repair" or "IBR" refers 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 IBR is an inhibitor of Endo V or hAAG. In some embodiments, the IBR is a catalytically inactive EndoV or a catalytically inactive hAAG.

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 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, refers to a bond (e.g., covalent bond), chemical group, or a molecule linking two molecules or moieties, e.g., two domains of a fusion protein. 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. Typically, 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. Longer or shorter linkers are also contemplated. In some embodiments, a linker comprises the amino acid sequence SGSETPGTSESATPES, which may also be referred to as the XTEN linker. In some embodiments, a linker comprises the amino acid sequence SGGS. In some embodiments, a linker comprises $(SGGS)_n$, $(GGGS)_n$, $(GGGGS)_n$, $(G)_n$, $(EAAAK)_n$, $(GGS)_n$, SGSETPGTSESATPES, or $(XP)_n$ motif, or a combination of any of these, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15.

In some embodiments, the domains of the nucleobase 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.

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* (4^{th} ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)).

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 “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 disclosure provides nucleic acid programable nucleic-acid (e.g., DNA or RNA) binding proteins. The nucleic acid programable nucleic-acid binding protein can be, for example, "nucleic acid programmable DNA binding protein" or "napDNAbp". The term "nucleic acid programmable DNA binding protein" or "napDNAbp" refers to a protein that associates with a nucleic acid (e.g., DNA or RNA), such as a guide nucleic acid, that guides the napDNAbp to a specific nucleic acid sequence. For example, 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). Examples of nucleic acid programmable DNA binding proteins include, without limitation, Cas9 (e.g., dCas9 and nCas9), CasX, CasY, Cpfl, Cas12b/C2c1, and Cas12c/C2c3. 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.

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

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). 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), though "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 ah, 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 U.S. Provisional Patent Application, U.S.S.N. 61/874,682, filed September 6, 2013, entitled "Switchable Cas9 Nucleases And Uses Thereof," and U.S. Provisional Patent Application, U.S.S.N. 61/874,746, filed September 6, 2013, 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." For example, an extended gRNA will, e.g., 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 said target site, providing the sequence specificity of the nuclease:RNA complex. In some embodiments, the RNA-programmable nuclease is the (CRIS PR-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).

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.

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, more at least about 25 amino acids, and even more preferably 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, and about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

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 one: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In another embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 .mu.g/ml denatured salmon sperm DNA (ssDNA). In another 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 preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably 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, more preferably of at least about 42° C, and even more preferably of 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 a more preferred 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 "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% or 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.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Subjects include livestock, domesticated animals raised to produce labor and to provide commodities, such as food, including without limitation, cattle, goats, chickens, horses, pigs, rabbits, and sheep.

The term "target site" refers to a sequence within a nucleic acid molecule that is modified by a nucleobase editor. In one embodiment, the target site is deaminated by a deaminase or a fusion protein comprising a deaminase (e.g., cytidine or adenine deaminase).

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).

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.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

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.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a model of an adenosine nucleobase editor and provides in part a structural basis for bystander mutagenesis.

FIG. 2 is a model depicting prediction of the location of target DNA for base editing.

FIG. 3 depicts a model showing positions of a deaminase domain in proximity to the locations of target DNA in FIG. 2.

FIG. 4 is a model of an adenosine nucleobase editor depicting regions identified where one or more deaminase domains may be inserted into Cas9. Loops (yellow) that are in proximity to where a deaminase domain may target single stranded DNA (magenta). Regions of interest include those marked A, B, C, D, E, F, G, and H.

FIG. 5 is a magnified view of the model in FIG. 4, showing residues in regions B, C, D, E, and F.

FIG. 6 is a magnified view of the model in FIG. 4, showing residues in regions F, G, and H.

FIG. 7 is a magnified view of the model in FIG. 4, showing residues in regions A, B, C, D, and E.

FIG. 8 depicts a high-throughput in vitro deamination assay. Spurious deamination of the probe can be distinguished from on-target deamination by comparing reactions containing nucleobase editor with an on-target probe containing a substrate for the base editor and a reaction in the absence of the base editor containing a probe for detecting off-target deamination.

FIG. 9 is a graph depicting results of a fluorescence assay for off-target deamination.

FIG. 10 is a graph depicting a comparison of adenosine base editor (ABE) v. an ABE system with TadA *in trans*.

FIG. 11 depicts potential substrates for spurious off-target base editing.

FIG. 12 depicts an assay to evaluate the activities of deaminases *in cis* and *in trans*.

FIG. 13 is a graph depicting the activities of rAPOBEC1 in the *in cis-in trans* assay.

FIG. 14 is a graph depicting the activities of TadA-TadA7.10 in the *in cis-in trans* assay.

FIG. 15 depicts that lower *in trans* activity was observed for TadA-TadA7.10 in base editor context (*in trans* ABE).

FIG. 16 is a graph depicting the results of dose-response for expression of GFP. Titration of pmaxGFP plasmid with empty vector resulted in decreased expression level of GFP.

FIG. 17 is a graph depicting dose-response for *in-cis* and *in-trans* activities of adenosine nucleobase editor ABE.

FIG. 18 is a graph depicting dose-response for *in-cis* and *in-trans* activities of cytidine nucleobase editor BE4.

FIG. 19 is a graph showing the results of screening of deaminases for reduced spurious deamination. The deaminases ppAPOBEC-2 (10), mAPOBEC-2 (8), mAPOBEC-3(12), and mfAPOBEC-4 (22) showed high *in cis/in trans* activity.

FIGs. 20A-Q depict base editing activity of the editors exmined. FIG. 20A is a schematic of ABE7.10, with TadA fused to Cas9 by an XTEN linker. FIG. 20B-Q show base editing activity of exemplary internal fusion base editors in percentage A to G deamination on the targeting strand within the range of the R-loop with target sequences GAACACAAAGCATAGACTGC (HEK2) and GGACAGCTTTTCCTAGACAG (T39). FIG. 20B, editing activity of ABE7.10. FIG. 20C, editing activity of ISLAY008. FIG. 20D, editing activity of ISLAY003. FIG. 20E, editing activity of ISLAY002. FIG. 20F, editing activity of ISLAY007. FIG. 20G, editing activity of ISLAY001. FIG. 20H, editing activity of ISLAY005. FIG. 20I, editing activity of ISLAY006. FIG. 20J, editing activity of ISLAY004. FIG. 20K, editing activity of ISLAY021. FIG. 20L, editing activity of ISLAY031. FIG. 20M, editing activity of ISLAY020. FIG. 20N, editing activity of ISLAY036.FIG. 20O, editing activity of ISLAY035. FIG. 20P, editing activity of ISLAY028. FIG. 20Q, editing activity of ISLAY009.

FIGs. 21A-B show schematics of exemplary base editors. FIG. 21A shows a schematic of exemplary base editor ABE7.10 and exemplary base editors (IBE002, IBE004, IBE005, IBE006, IBE008, IBE009, and IBE020). FIG. 21B shows a spatial cartoon representation of the above base editors, showing the location of deaminase insertion.

FIGs. 22A – D depict base editing efficiency of exemplary internal fusion base editors compared to ABE7.10 at 29 different genomic targets. FIG. 22A shows editing efficiency is normalized to ABE7.10 editing at the best position. FIG. 22B shows max editing efficiency of IBEs summarized and compared to ABE7.10. FIG. 22C shows a Gaussian smoothened representation of the peak editing position for each base editor. FIG. 22D shows a heatmap of normalized editing from the 29 tested targets.

FIG. 23 depicts spurious deamination measured by trans editing assay as 29 different targets normalized to ABE7.10 at each site.

FIGs. 24A-F show the percent editing of A-base editors at 6 genomic loci: HEK4 (FIG. 24A), FANCF (FIG. 24B), HEK-3 (FIG. 24C), HEK2-YY (FIG. 24D), EMX1 (FIG. 24E), HEK2 (FIG. 24F). X-axis: nucleobase positions with 1 being furthest from the PAM and 20 being PAM proximal (PAM being positions 21-23). Y axis: percentage of A to G editing measured by Illumina sequencing.

FIGs. 25A-F Percent editing of C-base editors at 6 genomic loci: HEK4 (FIG. 25A), FANCF (FIG. 25B), HEK-3 (FIG. 25C), HEK2-YY (FIG. 25D), EMX1 (FIG. 25E), HEK2 (FIG. 25F). X-axis: nucleobase positions with 1 being furthest from the PAM and 20 being PAM proximal (PAM being positions 21-23). Y axis: percentage of A to G editing measured by Illumina sequencing.

DETAILED DESCRIPTION OF THE INVENTION

As described below, the present invention features base editors having reduced non-target deamination, methods of using the base editors, and assays for characterizing base editors as having decreased non-target deamination, e.g. compared to programmed, on-target deamination.

Adenosine deaminases

In some embodiments, the nucleobase editors of the invention comprise an adenosine deaminase domain. 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. The adenosine deaminase may 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). 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*.

In one embodiment, a fusion protein of the invention comprises a wild-type TadA is linked to TadA7.10, which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA7.10 domain (e.g., provided as a monomer). In other embodiments, the ABE7.10 editor comprises TadA7.10 and TadA(wt), which are capable of forming heterodimers. The relevant sequences follow:

TadA(wt):

SEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIM
ALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVL
HHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

TadA7.10:

SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLH
YPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

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 identiy 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 adenosine deaminase comprises a D108X mutation in the 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 a D108G, D108N, D108V, D108A, or D108Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase. It should be appreciated, however, that additional deaminases may similarly be aligned to identify homologous amino acid residues that can be mutated as provided herein.

In some embodiments, the adenosine deaminase comprises an A106X 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 A106V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises a E155X mutation in TadA reference sequence, or a corresponding mutation 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 a E155D, E155G, or E155V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises a D147X mutation in TadA reference sequence, or a corresponding mutation 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 a D147Y, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

It should be appreciated that any of the mutations provided herein (e.g., based on the ecTadA amino acid sequence of TadA reference sequence) may be introduced into other adenosine deaminases, such as S. aureus TadA (saTadA), or other adenosine deaminases (e.g., bacterial adenosine deaminases). It would be apparent to the skilled artisan how to are homologous to the mutated residues in ecTadA. Thus, any of the mutations identified in ecTadA may be made in other adenosine deaminases that have homologous amino acid residues. It should also be appreciated that any of the mutations provided herein may be made individually or in any combination in ecTadA or another adenosine deaminase. For example, an adenosine deaminase may contain a D108N, a A106V, a E155V, and/or a D147Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase. 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: D108N and A106V; D108N and E155V; D108N and D147Y; A106V and E155V; A106V and D147Y; E155V and D147Y; D108N, A106V, and E55V; D108N, A106V, and D147Y; D108N, E55V, and D147Y; A106V, E55V, and D 147Y; and D108N, A106V, E55V, and D147Y. It should be appreciated, however, that any combination of corresponding mutations provided herein may 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, Kl lOX, 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, 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, 1951, V102A, F104L, A106V, R107C, or R107H, or R107P, D108G, or D108N, or D108V, or D108A, or D108Y, Kl 101, Ml 18K, 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.

In some embodiments, the adenosine deaminase comprises one or more of H8X, D108X, and/or N127X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase, 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.

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, 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.

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, 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, 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, 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, R126X, L68X, D108X, N127X, D147X, and E155X in TadA reference sequence, 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, 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. 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. 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. 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. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, R126W, L68Q, D108N, N127S, D147Y, and E155V in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase. 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.

In some embodiments, the adenosine deaminase comprises one or more of the or one or more corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108N, D108G, or D108V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A106V and D108N mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises R107C and D108N mutations in TadA reference sequence, or corresponding mutations in another adenosine deaminase. 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. In some embodiments, the adenosine deaminase comprises a H8Y, R24W, D108N, N127S, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108N, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises a H8Y, D108N, and S 127S mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase. 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.

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.

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.

In some embodiments, the adenosine deaminase comprises an H123X 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 H123Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an I157X 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 I157F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

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, 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, 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, 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. 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.

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, 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, R07K, 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. 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.

In some embodiments, the adenosine deaminase comprises an E25X 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 E25M, E25D, E25A, E25R, E25V, E25S, or E25Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an R26X 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 R26G, R26N, R26Q, R26C, R26L, or R26K mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an R107X 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 R107P, R07K, R107A, R107N, R107W, R107H, or R107S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an A142X 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 A142N, A142D, A142G, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an A143X 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 A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises one or more of a H36X, N37X, P48X, I49X, R51X, M70X, N72X, D77X, E134X, S 146X, Q154X, K157X, and/or K161X 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 H36L, N37T, N37S, P48T, P48L, I49V, R51H, R51L, M70L, N72S, D77G, E134G, S 146R, S 146C, Q154H, K157N, and/or K161T mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an H36X 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 H36L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an N37X 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 N37T, or N37S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an P48X 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 P48T, or P48L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

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.

In some embodiments, the adenosine deaminase comprises an S146X 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 S 146R, or S 146C mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an K157X 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 a K157N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an P48X 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 a P48S, P48T, or P48A mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an A142X 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 a A142N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an W23X 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 a W23R, or W23L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In some embodiments, the adenosine deaminase comprises an R152X 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 a R152P, or

R52H mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase.

In one embodiment, the adenosine deaminase may comprise the mutations H36L, R51L, L84F, A106V, D108N, H123Y, S 146C, 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_S 127S_D 147Y_Q154H), (H8Y_R24W_D108N_N127S_D147Y_E155V), (D108N_D147Y_E155V), (H8Y_D108N_S 127S), (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_D014N), (G22P_D 103 A_D 104N), (G22P_D 103 A_D 104N_S 138 A) , (D 103 A_D 104N_S 138A), (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_S 146C_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_S 146R_D147Y_E155V_I156F), (H36L_P48L_L84F_A106V_D108N_H123Y_E134G_D147Y_E155V_I156F), 57N), (H36L_L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F), (L84F_A106V_D108N_H123Y_S 146R_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_S 146T_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_S 146C_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_S 146C_D147Y_E155V_I156F_K157N_K161T), (L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F_K161T), (L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F_K157N_K160E_K161T), (L84F_A106V_D108N_H123Y_S 146C_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_S 146C_D147Y_E155V_I156F _K157N),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S 146C_A142N_D147Y_E155V_I156F
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F _K157N),
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_A142N_S 146C_D147Y_E155V_ I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S 146C_D147Y_E155V_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_A142N_D147Y_E155V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F _K157N),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_E155V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146R_D147Y_E155V_I156F _K161T),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_R152H_E155V_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_R152P_E155V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_R152P_E155V _I156F _K157N),

(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S 146C_D147Y_E155 V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S 146C_D147Y_R152P _E155V_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146R_D147Y_E155V_I156F _K161T),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S 146C_D147Y_R152P_E155V _I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S 146C_D147Y_R152P_E155 V_I156F _K157N).

Cytidine deaminase

In one embodiment, a fusion protein of the invention comprises a cytidine deaminase. In some embodiments, the cytidine deaminases provided herein are capable of deaminating cytosine or 5-methylcytosine to uracil or thymine. In some embodiments, the cytosine 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 deaminase domains with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the cytidine 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 cytidine deaminases provided herein. In some embodiments, the cytidine 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.

A fusion protein of the invention comprises a nucleic acid editing domain. In some embodiments, the nucleic acid editing domain can catalyze a C to U base change. In some embodiments, the nucleic acid editing domain is a deaminase domain. In some embodiments, the deaminase is a cytidine deaminase or an adenosine deaminase. In some embodiments, the deaminase is an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase. In some embodiments, the deaminase is an APOBECl deaminase. In some embodiments, the deaminase is an APOBEC2 deaminase. In some embodiments, the deaminase is an APOBEC3 deaminase. In some embodiments, the deaminase is an APOBEC3 A deaminase. In some embodiments, the deaminase is an APOBEC3B deaminase. In some embodiments, the deaminase is an APOBEC3C deaminase. In some embodiments, the deaminase is an APOBEC3D deaminase. In some embodiments, the deaminase is an APOBEC3E deaminase. In some embodiments, the deaminase is an APOBEC3F deaminase. In some embodiments, the deaminase is an APOBEC3G deaminase. In some embodiments, the deaminase is an APOBEC3H deaminase. In some embodiments, the deaminase is an APOBEC4 deaminase. In some embodiments, the deaminase is an activation-induced deaminase (AID). In some embodiments, the deaminase is a vertebrate deaminase. In some embodiments, the deaminase is an invertebrate deaminase. In some embodiments, the deaminase is a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse deaminase. In some embodiments, the deaminase is a human deaminase. In some embodiments, the deaminase is a rat deaminase, e.g., rAPOBECl . In some embodiments, the deaminase is a Petromyzon marinus cytidine deaminase 1 (pmCDAl). In some embodiments, the deminase is a human APOBEC3G. In some embodiments, the deaminase is a fragment of the human APOBEC3G. In some embodiments, the deaminase is a human APOBEC3G variant comprising a D316R D317R mutation. In some embodiments, the deaminase is a fragment of the human APOBEC3G and comprising mutations corresponding to the D316R D317R mutations. In some embodiments, the nucleic acid editing domain is at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%), or at least 99.5% identical to the deaminase domain of any deaminase described herein.

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).

Cas9 domains of Nucleobase Editors

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 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, 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).

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD (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).

Additional suitable nuclease-inactive dCas9 domains 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. 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). 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, 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.

Cas9 Domains with Reduced PAM 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); the entire contents of each are hereby incorporated by reference. Several PAM variants are described at Table 1 below:

Table 1. Cas9 proteins and corresponding PAM sequences

Variant	**PAM**
spCas9	NGG
spCas9-VRQR	NGA
spCas9-VRER	NGCG
xCas9 (sp)	NGN

saCas9	NNGRRT
saCas9-KKH	NNNRRT
spCas9-MQKSER	NGCG
spCas9-MQKSER	NGCN
spCas9-LRKIQK	NGTN
spCas9-LRVSQK	NGTN
spCas9-LRVSQL	NGTN
Cpf1	5' (TTTV)

In some embodiments, the Cas9 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 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 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.

Exemplary SaCas9 sequence

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRR
RRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRR

GVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKT
SDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEW
YEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN
VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENA
ELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDE
LWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIK
KYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIK
LHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEE**N**SKK
GNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFI
NRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKG
YKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIF
ITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNGLYDK
DNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTK
YSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVY
KFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYR
VIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLY
EVKSKKHPQIIKKG

Residue N579 above, which is underlined and in bold, may be mutated (*e.g.*, to a A579) to yield a SaCas9 nickase.

Exemplary SaCas9n sequence

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRR
RRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRR
GVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKT
SDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEW
YEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN
VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENA
ELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDE
LWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIK
KYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIK
LHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEE**A**SKK
GNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFI
NRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKG
YKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIF

ITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIVNNLNGLYDK
DNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTK
YSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVY
KFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKINGELYR
VIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLY
EVKSKKHPQIIKKG

Residue A579 above, which can be mutated from N579 to yield a SaCas9 nickase, is underlined and in bold.

Exemplary SaKKH Cas9

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRR
RRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRR
GVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKT
SDYVKEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEW
YEMLMGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN
VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENA
ELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDE
LWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIK
KYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIK
LHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEE**A**SKK
GNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFI
NRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKG
YKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIF
ITPHQIKHIKDFKDYKYSHRVDKKPNR*K*LINDTLYSTRKDDKGNTLIVNNLNGLYDK
DNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTK
YSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKPYRFDVYLDNGVY
KFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFY*K*NDLIKINGELYRV
IGVNNDLLNRIEVNMIDITYREYLENMNDKRPP*H*IIKTIASKTQSIKKYSTDILGNLYE
VKSKKHPQIIKKG.

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.

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 D1134X, a R1334X, and a T1336X 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 D1134E, R1334Q, and T1336R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1134E, a R1334Q, and a T1336R 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 D1134X, a R1334X, and a T1336X 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 D1134V, a R1334Q, and a T1336R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1134V, a R1334Q, and a T1336R 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 D1134X, a G1217X, a R1334X, and a T1336X 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 D1134V, a G1217R, a R1334Q, and a T1336R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1134V, a G1217R, a R1334Q, and a T1336R 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.

Exemplary SpCas9

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW
MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Exemplary SpCas9n

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH

PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Exemplary SpEQR Cas9

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS

VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF**E**SPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQLGGD

Residues E1134, Q1334, and R1336 above, which can be mutated from D1134, R1334, and T1336 to yield a SpEQR Cas9, are underlined and in bold.

Exemplary SpVQR Cas9

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW
MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK

RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF**<u>V</u>**SPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRK**<u>Q</u>**Y**<u>R</u>**STKEVLDATLIHQSITGLYETRIDLSQLGGD

Residues V1134, Q1334, and R1336 above, which can be mutated from D1134, R1334, and T1336 to yield a SpVQR Cas9, are underlined and in bold.

Exemplary SpVRER Cas9

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW
MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF**<u>V</u>**SPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASA**<u>R</u>**ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ

HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA PAAFKYFDTTIDRK**E**Y**R**STKEVLDATLIHQSITGLYETRIDLSQLGGD.

Residues V1134, R1217, Q1334, and R1336 above, which can be mutated from D1134, G1217, R1334, and T1336 to yield a SpVRER Cas9, are underlined and in bold. 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.*,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.

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.

Fusion proteins comprising two napDNAbp, a Deaminase Domain

Some aspects of the disclosure provide fusion proteins comprising a napDNAbp domain having nickase activity (e.g., nCas domain) and a catalytically inactive napDNAbp (e.g., dCas domain) and a nucleobase editor (e.g., adenosine deaminase domain, cytidine deaminase domain), where at least the napDNAbp domains are joined by a linker. It should be appreciated that the Cas domains may be any of the Cas domains or Cas proteins (*e.g.*, dCas9 and nCas9) provided herein. In some embodiments, any of the Cas domains, DNA binding protein domains, or Cas proteins include, without limitation, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpf1, 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. For example and without limitation, in some embodiments, the fusion protein comprises the structure, where the deaminase is adenosine deaminase or cytidine deaminase:

NH_2-[deaminase]-[nCas domain]-[dCas domain]-COOH;

NH_2-[deaminase]-[dCas domain]-[nCas domain]-COOH;
NH_2-[nCas domain]-[dCas domain]-[deaminase]-COOH;
NH_2-[dCas domain]-[nCas domain]-[deaminase]-COOH;
NH_2-[nCas domain]-[deaminase]-[dCas domain]-COOH;
NH_2-[dCas domain]-[deaminase]-[nCas domain]-COOH;

In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker. In some embodiments, the deaminase and a napDNAbp (e.g., Cas domain) are not joined by a linker sequence, but are directly fused. In some embodiments, a linker is present between the deaminase domain and the napDNAbp. In some embodiments, the deaminase or other nucleobase editor is directly fused to dCas and a linker joins dCas and nCas9. In some embodiments, the deaminase and the napDNAbps are fused via any of the linkers provided herein. For example, in some embodiments the deaminase and the napDNAbp are fused via any of the linkers provided below in the section entitled "Linkers". In some embodiments, the dCas domain and the deaminase are immediately adjacent and the nCas domain is joined to these domains (either 5' or 3') via a linker.

Fusion proteins with Internal Insertions

The disclosure provides 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 polypeptide. The deaminase in a fusion protein can be a cytidine deaminase. The deaminase in a fusion protein can be an adenosine deaminase.

The deaminase can be a circular permutant deaminase. For example, the deaminase can be a circular permutant adenosine deaminase or a circular permutant cytidine 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 in SEQ ID NO: 1.

The heterologous polypeptide (e.g., deaminase) can be inserted in the napDNAbp (e.g., Cas9) 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 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 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 can be inserted in the napDNAbp in a flexible loop region or a solvent-exposed region. In some embodiments, the deaminase is inserted in a flexible loop of the Cas9 polypeptide.

In some embodiments, the insertion location of a deaminase is determined by B-factor analysis of the crystal structure of Cas9 polypeptide. In some embodiments, the 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 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 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 SEQ ID No:1. 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 SEQ ID No:1.

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 SEQ ID NO: 1, 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 SEQ ID NO: 1 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 SEQ ID NO: 1 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. It should be understood that the reference to SEQ ID NO:1 with respect to insertion positions is for illustrative purpose. The insertions as discussed herein are not limited to the Cas9 polypeptide sequence of SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1 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 SEQ ID NO: 1 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 SEQ ID NO: 1, 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 shown in Fig. 4, Fig. 5, Fig. 6, or Fig. 7, 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 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. The deaminase can be inserted at the N-terminus or the C-terminus of the residue or replace the residue. In some embodiments, the deaminase is inserted at the C-terminus of the residue.

In some embodiments, an ABE (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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, an ABE (e.g., TadA) is inserted in place of residues 792-872, 792-906, or 2-791 as numbered in SEQ ID NO: 1, 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: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in SEQ ID NO: 1, 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: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in SEQ ID NO: 1, 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: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 768 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 768 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 768 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 768 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 791 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 791 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 791 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 791 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 792 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 792 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 792 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 792 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1016 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1016 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1016 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1016 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1022 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1022 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1022 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1022 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1023 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1023 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1023 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1023 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1026 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1026 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1026 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1026 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1029 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1029 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1029 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1029 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1040 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 140 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1040 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1040 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1052 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1052 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1052 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1052 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1054 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1054 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1054 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1054 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1067 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1067 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1067 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1067 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1068 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1068 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1068 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1068 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1069 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1069 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1069 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1069 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1246 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1246 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1246 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1246 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1247 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1247 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1247 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1247 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

In some embodiments, the deaminase is inserted at amino acid residue 1248 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of amino acid 1248 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of amino acid 1248 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace amino acid 1248 as numbered in SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (e.g., 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide.

A heterologous polypeptide (e.g., 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 792-906 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 2-791 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region correspond to residues 1017-1069 as numbered in SEQ ID NO: 1, or corresponding amino acid residues thereof.

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 a 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 a HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises a 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 SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. A suitable insertion position of a CBE can be an amino acid residue selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in SEQ ID NO: 1, or a corresponding amino acid residue in another Cas9 polypeptide. In certain embodiments, the insertion of the ABE can be inserted to the N terminus or the C terminus of any one of the above listed amino acid residues. In some embodiemnts, the insertion of the ABE can be inserted to replace any one of the above listed amino acid residues.

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 SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1, 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 SEQ ID NO: 1.

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 of the fusion protein deaminates no more than two nucleobases within the range of a 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. A R-loop is a three-stranded nucleic acid structure including a DNA:RNA hybrid, a DNA:DNA or a RNA: RNA complementary structure and the associated with single-stranded DNA. As used herein, a R-loop may be formed when a 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, a R-loop comprises a hybridized region of a spacer sequence and a target DNA complementary sequence. A 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 nuclebase 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, a R-loop region is not limited to the target DNA strand that hybridizes with the gudie polynucleotide. For example, editing of a target nucleobase within a 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 paris, 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 paris, about 13 to 17 base paris, 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 or upstream of the PAM sequence. In some embodiemtns, 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.

Accordingly, also provided herein are fusion protein libraries and method for using same to optimize base editing that allow for alternative preferred base editing windows compared to canonical base editors, e.g. BE4. In some embodiments, the disclosure provides a protein library for optimized base editing comprising a plurality of fusion proteins, wherein each one of the plurality of fusion proteins comprises a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the N-terminal fragment of each one of the fusion proteins differs from the N-terminal fragments of the rest of the plurality of fusion proteins or wherein the C-terminal fragment of each one of the fusion proteins differs from the C-terminal fragments of the rest of the plurality of fusion proteins, wherein the deaminase of each one of the fusion proteins deaminates a target nucleobase in proximity to a Protospacer Adjacent Motif (PAM) sequence in a target polynucleotide sequence, and wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide sequence. In some embodiments, for each nucleobase within a CRISPR R-loop, at least one of the plurality of fusion proteins deaminates the nucleobase. In some embodiments, for each nucleobase within of a target polynucleotide from 1 to 20 base pairs away of a PAM sequence, at least one of the plurality of fusion proteins deaminates the nucleobase. In some embodiments, provided herein is a kit comprising the fusion protein library that allows for optimized base editing.

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. Exemplary TadA or TadA7.10 sequence set forth below:

SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGL
HDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGR
VVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRM
PRQVFNAQKKAQSSTD

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG
AVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLY
VTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE
ITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

TAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVF
GVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQ
VFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTL
AKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDP

YRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLH
YPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGS
ETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVL

NNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQN

MNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETP GTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNR VIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG AVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLY VTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE ITEGILADECAALLCYFFRMPRQVFN

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG AVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLY VTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVE ITEGILADECAALLCYFFRMPRQ

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG AVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD ECAALLCYFFRMPRQVFN

GSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVG AVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD ECAALLCYFFRMPRQVFNAQKKAQSSTD

101 Cas9 TadAins 1015

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR

LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

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

RQVFNAQKKAQSSTDGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

103 Cas9 TadAins 1040

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK

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

VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGEGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDTGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

106 Cas9 TadAins 1247

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI

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

IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPGSSGSSGSETPGTSESATPESGSSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

110.6 Cas9 TadAins 1251

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

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

NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

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

VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

127 TadA CP65ins 1016

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK

YPKLESEFVYGDYKVTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

128 TadA CP65ins 1022

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP

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

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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

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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

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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

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.

In some embodiments, the SpCas9 has specificity for PAM nucleic acid sequence 5’-NGC-3’ or 5’-NGG-3’. In various embodiments of the above aspects, the SpCas9 is a Cas9 or Cas9 variant listed in Table 1. In various embodiments of the above aspects, the modified SpCas9 is spCas9-MQKFRAER. In some embodiments, the variant Cas protein can be spCas9, spCas9-VRQR, spCas9-VRER, xCas9 (sp), saCas9, saCas9-KKH, SpCas9-MQKFRAER, spCas9-MQKSER, spCas9-LRKIQK, or spCas9-LRVSQL. In one specific embodiment, 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’ is used.

In some embodiments, the PAM is NGT. In some embodiments, the NGT PAM is a variant. In some embodiments, the NGT PAM variant is created 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	
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 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 may be fused with any of the cytidine deaminases or adenosine deaminases provided herein

In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1336X 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 T1336R 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 T1336R 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 T1336X 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 T1336R 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 T1336R 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 G1217X, a R1335X, and a T1336X 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 G1217R, a R1335Q, and a T1336R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a G1217R, a R1335Q, and a T1336R 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 4 kilobase (kb) 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
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK

VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD
NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH
VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGF**E**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ
LGGD. 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 R1336, which can be mutated from D1135, R1335, and T1336 to yield a SpVQR Cas9, are underlined and in bold.

The amino acid sequence of an exemplary PAM-binding SpVRER 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 IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNFLYLAS HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**E**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ LGGD.

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.*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 a sugar-phosphate backbone of a DNA, as compared to a corresponding wild-type Cas9 domain. Without wishing to be bound by any particular theory, high fidelity Cas9 domains that have decreased electrostatic interactions with a sugar-phosphate backbone of DNA may have less off-target effects. In some embodiments, a Cas9 domain (*e.g.*, a wild type Cas9 domain) comprises one or more mutations that decreases the association between the Cas9 domain and a 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 a sugar-phosphate backbone of a DNA by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least10%, 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.

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

DKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW
MTRKSEETITPWNFEEVVDKGASAQSFIERMT**A**FDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWG**A**LSRKLINGIRDKQSGKTILDFLKSDGFANRN
FM**A**LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK

RQLVETR**<u>A</u>**ITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

Nucleic acid programmable DNA binding proteins

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), CasX, CasY, Cpf1, Cas12b/C2c1, and Cas12c/C2c3. One example of a nucleic acid programmable DNA-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 DNA-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 DNA binding protein (napDNAbp) 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.

Wild type *Francisella novicida* Cpf1 (D917, E1006, and D1255 are bolded and underlined)
MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYH
QFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSE
KFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWT
TYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIK
KDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGEN
TKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTM
QSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDY
SVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDI
DKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIK
DLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYI

TQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A (A917, E1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESV

SKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKN
HNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQM
RNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRI
KNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 E1006A (D917, A1006, and D1255 are bolded and underlined)
MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYH
QFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSE
KFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWT
TYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIK
KDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGEN
TKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTM
QSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDY
SVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDI
DKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIK
DLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYI
TQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFD
DKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKN
GSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVE
NQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDER
NLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKR
FTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**D**RGERHLAYYTLVDG
KGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQV
VHEIAKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEF
DKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESV
SKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKN
HNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQM
RNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRI
KNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D1255A (D917, E1006, and A1255 are bolded and underlined)
MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYH
QFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSE
KFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWT

TYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**<u>D</u>**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**<u>E</u>**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**<u>A</u>**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A/E1006A (A917, A1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKN

GSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A/D1255A (A917, E1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKN

HNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQM
RNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**<u>A</u>**ANGAYHIGLKGLMLLGRI
KNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 E1006A/D1255A (D917, A1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYH
QFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSE
KFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWT
TYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIK
KDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGEN
TKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTM
QSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDY
SVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDI
DKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIK
DLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYI
TQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFD
DKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKN
GSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVE
NQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDER
NLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKR
FTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSI**<u>D</u>**RGERHLAYYTLVDG
KGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQV
VHEIAKLVIEYNAIVVF**<u>A</u>**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEF
DKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESV
SKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKN
HNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQM
RNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDA**<u>A</u>**ANGAYHIGLKGLMLLGRI
KNNQEGKKLNLVIKNEEYFEFVQNRNN

Francisella novicida Cpf1 D917A/E1006A/D1255A (A917, A1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYH
QFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSE

KFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWT
TYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIK
KDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGEN
TKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTM
QSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDY
SVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDI
DKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIK
DLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNYI
TQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFD
DKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKN
GSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVE
NQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDER
NLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIKDKR
FTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSIARGERHLAYYTLVDG
KGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKINNIKEMKEGYLSQV
VHEIAKLVIEYNAIVVFADLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVFKDNEF
DKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESV
SKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKN
HNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQM
RNSKTGTELDYLISPVADVNGNFFDSRQAPKNMPQDAAANGAYHIGLKGLMLLGRI
KNNQEGKKLNLVIKNEEYFEFVQNRNN

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 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/C2c1depends 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/C2c1ternary 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.

Cas12b/C2c1 (uniprot.org/uniprot/T0D7A2#2)

sp|T0D7A2|C2C1_ALIAG CRISPR-associated endo- nuclease C2c1 OS = *Alicyclobacillus acido- terrestris* (strain ATCC 49025 / DSM 3922/ CIP 106132 / NCIMB 13137/GD3B) GN=c2c1 PE=1 SV=1
MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYRRSPNG DGEQECDKTAEECKAELLERLRARQVENGHRGPAGSDDELLQLARQLYELLVPQAI GAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKEKA ETRKSADRTADVLRALADFGLKPLMRVYTDSEMSSVEWKPLRKGQAVRTWDRDM FQQAIERMMSWESWNQRVGQEYAKLVEQKNRFEQKNFVGQEHLVHLVNQLQQDM KEASPGLESKEQTAHYVTGRALRGSDKVFEKWGKLAPDAPFDLYDAEIKNVQRRNT RRFGSHDLFAKLAEPEYQALWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDAT AHPIWTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDDVTVPISM SEQLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRGARD VYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKL GSEGLLSGLRVMSVDLGLRTSASISVFRVARKDELKPNSKGRVPFFFPIKGNDNLVAV HERSQLLKLPGETESKDLRAIREERQRTLRQLRTQLAYLRLLVRCGSEDVGRRERSW AKLIEQPVDAANHMTPDWREAFENELQKLKSLHGICSDKEWMDAVYESVRRVWRH MGKQVRDWRKDVRSGERPKIRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVS GQVIRAEKGSRFAITLREHIDHAKEDRLKKLADRIIMEALGYVYALDERGKGKWVA KYPPCQLILLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELINQAQVHDLLVGTM YAAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPWWLNKFVVEHTLDACPLRAD DLIPTGEGEIFVSPFSAEEGDFHQIHADLNAAQNLQQRLWSDFDISQIRLRCDWGEVD GELVLIPRLTGKRTADSYSNKVFYTNTGVTYYERERGKKRRKVFAQEKLSEEEAELL VEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMV NQRIEGYLVKQIRSRVPLQD SACENTGDI

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 PKKKRKVEGADKRTADGSEFES PKKKRKV, 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:

PKKKRKVEGADKRTADGSEFES PKKKRKV

In some embodiments, the fusion proteins of the invention do not comprise a linker sequence. In some embodiments, linker sequences between one or more of the domains or proteins 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.

Linkers

In certain embodiments, linkers may be used to link any of the peptides or peptide domains of the invention. The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (*e.g.*, a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, *etc.*). In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, the linker is polymeric (e.g., polyethylene, polyethylene glycol, polyamide, polyester, *etc.*). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (*e.g.*, glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, *etc.*). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, the linker is based on a carbocyclic moiety (e.g., cyclopentane, cyclohexane). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In other embodiments, the linker comprises amino acids. In certain embodiments, the linker comprises a peptide. In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. The linker may include functionalized moieties to facilitate attachment of a nucleophile (e.g., thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated amides, Michael acceptors, alkyl halides, aryl halides, acyl halides, and isothiocyanates.

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 a bond (e.g., a covalent bond), an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is about 3 to about 104 (e.g., 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, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100) amino acids in length.

Cas9 complexes with guide RNAs

Some aspects of this disclosure provide complexes comprising any of the fusion proteins provided herein, and a guide RNA. Any method for linking the fusion protein domains can be employed (*e.g.*, ranging from very flexible linkers of the form $(GGGS)_n$, $(GGGGS)_n$, and $(G)_n$ to more rigid linkers of the form $(EAAAK)_n$, $(SGGS)_n$, SGSETPGTSESATPES (see, e.g., Guilinger JP, Thompson DB, Liu DR. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 2014; 32(6): 577-82; the entire contents are incorporated herein by reference) and $(XP)_n$) in order to achieve the optimal length for activity for the nucleobase editor. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the linker comprises a $(GGS)_n$ motif, wherein n is 1, 3, or 7. In some embodiments, the Cas9 domain of the fusion proteins provided herein are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES:

In some embodiments, the guide nucleic acid (e.g., guide RNA) is from 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the guide RNA is 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 nucleotides long. In some embodiments, the guide RNA comprises a sequence of 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 is complementary to a target sequence. In some embodiments, the target sequence is a DNA sequence. In some embodiments, the target sequence is a sequence in the genome of a bacteria, yeast, fungi, insect, plant, or animal. In some embodiments, the target sequence is a sequence in the genome of a human. In some embodiments, the 3' end of the target sequence is immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the 3' end of the target sequence is immediately adjacent to a non-canonical PAM sequence (e.g., a sequence listed in Table 1).

Some aspects of this disclosure provide methods of using the fusion proteins, or complexes provided herein. For example, some aspects of this disclosure provide methods comprising contacting a DNA molecule with any of the fusion proteins provided herein, and with at least one guide RNA, wherein the guide RNA is about 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the 3' end of the target sequence is immediately adjacent to an AGC, GAG, TTT, GTG, or CAA sequence. In some embodiments, the 3' end of the target sequence is immediately adjacent to an NGA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, NGTN, NGTN, or 5' (TTTV) sequence.

It will be understood that the numbering of the specific positions or residues in the respective sequences depends on the particular protein and numbering scheme used. Numbering might be different, e.g., in precursors of a mature protein and the mature protein itself, and differences in sequences from species to species may affect numbering. One of skill in the art will be able to identify the respective residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment and determination of homologous residues.

It will be apparent to those of skill in the art that in order to target any of the fusion proteins disclosed herein, to a target site, *e.g.*, a site comprising a mutation to be edited, it is typically necessary to co-express the fusion protein together with a guide RNA. As explained in more detail elsewhere herein, a guide RNA typically comprises a tracrRNA framework allowing for Cas9 binding, and a guide sequence, which confers sequence specificity to the Cas9:nucleic acid editing enzyme/domain fusion protein. Alternatively, the guide RNA and tracrRNA may be provided separately, as two nucleic acid molecules. In some embodiments, the guide RNA comprises a structure, wherein the guide sequence comprises a sequence that is complementary to the target sequence. The guide sequence is typically 20 nucleotides long. The sequences of suitable guide RNAs for targeting Cas9:nucleic acid editing enzyme/domain fusion proteins to specific genomic target sites will be apparent to those of skill in the art based on the instant disclosure. Such suitable guide RNA sequences typically comprise guide sequences that are complementary to a nucleic sequence within 50 nucleotides upstream or downstream of the target nucleotide to be edited. Some exemplary guide RNA sequences suitable for targeting any of the provided fusion proteins to specific target sequences are provided herein.

Methods of using fusion proteins comprising a cytidine deaminase, adenosine deaminase and a Cas9 domain

Some aspects of this disclosure provide methods of using the fusion proteins, or complexes provided herein. For example, some aspects of this disclosure provide methods comprising contacting a DNA molecule encoding a mutation with any of the fusion proteins provided herein, and with at least one guide RNA, wherein the guide RNA is about 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the 3' end of the target sequence is immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the 3' end of the target sequence is not immediately adjacent to a canonical PAM sequence (NGG). In some embodiments, the 3' end of the target sequence is immediately adjacent to an AGC, GAG, TTT, GTG, or CAA sequence. In some embodiments, the 3' end of the target sequence is immediately adjacent to an NGA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, NGTN, NGTN, or 5' (TTTV) sequence.

It will be understood that the numbering of the specific positions or residues in the respective sequences depends on the particular protein and numbering scheme used. Numbering might be different, e.g., in precursors of a mature protein and the mature protein itself, and differences in sequences from species to species may affect numbering. One of skill in the art will be able to identify the respective residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment and determination of homologous residues.

It will be apparent to those of skill in the art that in order to target any of the fusion proteins comprising a Cas9 domain and a cytidine deaminase or an adenosine deaminase, as disclosed herein, to a target site, *e.g.*, a site comprising a mutation to be edited, it is typically necessary to co-express the fusion protein together with a guide RNA, *e.g.*, an sgRNA. As explained in more detail elsewhere herein, a guide RNA typically comprises a tracrRNA framework allowing for Cas9 binding, and a guide sequence, which confers sequence specificity to the Cas9:nucleic acid editing enzyme/domain fusion protein. Alternatively, the guide RNA and tracrRNA may be provided separately, as two nucleic acid molecules. In some embodiments, the guide RNA comprises a structure, wherein the guide sequence comprises a sequence that is complementary to the target sequence. The guide sequence is typically 20 nucleotides long. The sequences of suitable guide RNAs for targeting Cas9:nucleic acid editing enzyme/domain fusion proteins to specific genomic target sites will be apparent to those of skill in the art based on the instant disclosure. Such suitable guide RNA sequences typically comprise guide sequences that are complementary to a nucleic sequence within 50 nucleotides upstream or downstream of the target nucleotide to be edited.

Some exemplary guide RNA sequences suitable for targeting any of the provided fusion proteins to specific target sequences are provided herein.

Base Editor Efficiency

The fusion proteins of the invention advantageously modify a specific nucleotide base comprising a mutation without generating a significant proportion of indels. An "indel", as used herein, refers to the insertion or deletion of a nucleotide base within a nucleic acid. Such insertions or deletions can lead to frame shift mutations within a coding region of a gene. In some embodiments, it is desirable to generate base editors that efficiently modify (*e.g.* mutate) a specific nucleotide within a nucleic acid, without generating a large number of insertions or deletions (*i.e.*, indels) in the nucleic acid. In certain embodiments, any of the base editors provided herein are capable of generating a greater proportion of intended modifications (*e.g.*, mutations) versus indels. In some embodiments, the base editors provided herein are capable of generating a ratio of intended mutation to indels that is greater than 1:1. In some embodiments, the base editors provided herein are capable of generating a ratio of intended mutations to indels that is at least 1.5:1, at least 2:1, at least 2.5:1, at least 3:1, at least 3.5:1, at least 4:1, at least 4.5:1, at least 5:1, at least 5.5:1, at least 6:1, at least 6.5:1, at least 7:1, at least 7.5:1, at least 8:1, at least 10:1, at least 12:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, at least 40:1, at least 50:1, at least 100:1, at least 200:1, at least 300:1, at least 400:1, at least 500:1, at least 600:1, at least 700:1, at least 800:1, at least 900:1, or at least 1000:1, or more. The number of intended mutations and indels may be determined using any suitable method.

In some embodiments, the base editors provided herein are capable of limiting formation of indels in a region of a nucleic acid. In some embodiments, the region is at a nucleotide targeted by a base editor or a region within 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of a nucleotide targeted by a base editor. In some embodiments, any of the base editors provided herein are capable of limiting the formation of indels at a region of a nucleic acid to less than 1%, less than 1.5%, less than 2%, less than 2.5%, less than 3%, less than 3.5%, less than 4%, less than 4.5%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, less than 10%, less than 12%, less than 15%, or less than 20%. The number of indels formed at a nucleic acid region may depend on the amount of time a nucleic acid (*e.g.*, a nucleic acid within the genome of a cell) is exposed to a base editor. In some embodiments, an number or proportion of indels is determined after at least 1 hour, at least 2 hours, at least 6 hours, at least 12 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 3 days, at least 4 days, at least 5 days, at least 7 days, at least 10 days, or at least 14 days of exposing a nucleic acid (*e.g.*, a nucleic acid within the genome of a cell) to a base editor.

Some aspects of the disclosure are based on the recognition that any of the base editors provided herein are capable of efficiently generating an intended mutation in a nucleic acid (*e.g.* a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor bound to a gRNA, specifically designed to alter or correct a mutation. In some embodiments, any of the base editors provided herein are capable of generating a ratio of intended mutations to unintended mutations (*e.g.*, intended mutations:unintended mutations) that is greater than 1:1. In some embodiments, any of the base editors provided herein are capable of generating a ratio of intended mutations to unintended mutations that is at least 1.5:1, at least 2:1, at least 2.5:1, at least 3:1, at least 3.5:1, at least 4:1, at least 4.5:1, at least 5:1, at least 5.5:1, at least 6:1, at least 6.5:1, at least 7:1, at least 7.5:1, at least 8:1, at least 10:1, at least 12:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, at least 40:1, at least 50:1, at least 100:1, at least 150:1, at least 200:1, at least 250:1, at least 500:1, or at least 1000:1, or more. It should be appreciated that the characteristics of the base editors described in the "*Base Editor Efficiency*" section, herein, may be applied to any of the fusion proteins, or methods of using the fusion proteins provided herein.

Methods for Editing Nucleic Acids

Some aspects of the disclosure provide methods for editing a nucleic acid. In some embodiments, the method is a method for editing a nucleobase of a nucleic acid molecule encoding a polypeptide of interest (*e.g.*, the expression product of a disease gene). In some embodiments, the method comprises the steps of: a) contacting a target region of a nucleic acid (*e.g.*, a double-stranded DNA sequence) with a complex comprising a base editor and a guide nucleic acid (*e.g.*, gRNA), b) inducing strand separation of said target region, c) converting a first nucleobase of said target nucleobase pair in a single strand of the target region to a second nucleobase, and d) cutting no more than one strand of said target region using the nCas9, where a third nucleobase complementary to the first nucleobase base is replaced by a fourth nucleobase complementary to the second nucleobase. In some embodiments, the method results in less than 20% indel formation in the nucleic acid. It should be appreciated that in some embodiments, step b is omitted. In some embodiments, the method results in less than 19%, 18%, 16%, 14%, 12%, 10%, 8%, 6%, 4%, 2%, 1%, 0.5%, 0.2%, or less than 0.1% indel formation. In some embodiments, the method further comprises replacing the second nucleobase with a fifth nucleobase that is complementary to the fourth nucleobase, thereby generating an intended edited base pair (*e.g.*, G•C to A•T). In some embodiments, at least 5% of the intended base pairs are edited. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the intended base pairs are edited.

In some embodiments, the ratio of intended products to unintended products in the target nucleotide is at least 2:1, 5:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, 100:1, or 200:1, or more. In some embodiments, the ratio of intended mutation to indel formation is greater than 1:1, 10:1, 50:1, 100:1, 500:1, or 1000:1, or more. In some embodiments, the cut single strand (nicked strand) is hybridized to the guide nucleic acid. In some embodiments, the cut single strand is opposite to the strand comprising the first nucleobase. In some embodiments, the base editor comprises a dCas9 domain. In some embodiments, the base editor protects or binds the non-edited strand. In some embodiments, the intended edited base pair is upstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides upstream of the PAM site. In some embodiments, the intended edited base pair is downstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides downstream stream of the PAM site. In some embodiments, the method does not require a canonical (*e.g.*, NGG) PAM site. In some embodiments, the nucleobase editor comprises a linker. In some embodiments, the linker is 1-25 amino acids in length. In some embodiments, the linker is 5-20 amino acids in length. In some embodiments, linker is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In one embodiment, the linker is 32 amino acids in length. In another embodiment, a "long linker" is at least about 60 amino acids in length. In other embodiments, the linker is between about 3-100 amino acids in length. In some embodiments, the target region comprises a target window, wherein the target window comprises the target nucleobase pair. In some embodiments, the target window comprises 1-10 nucleotides. In some embodiments, the target window is 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotides in length. In some embodiments, the target window is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, the intended edited base pair is within the target window. In some embodiments, the target window comprises the intended edited base pair. In some embodiments, the method is performed using any of the base editors provided herein.

In some embodiments, the disclosure provides methods for editing a nucleotide (e.g., a SNP). In some embodiments, the disclosure provides a method for editing a nucleobase pair of a double-stranded DNA sequence. In some embodiments, the method comprises a) contacting a target region of the double-stranded DNA sequence with a complex comprising a base editor and a guide nucleic acid (*e.g.*, gRNA), where the target region comprises a target nucleobase pair, b) inducing strand separation of said target region, c) converting a first nucleobase of said target nucleobase pair in a single strand of the target region to a second nucleobase, d) cutting no more than one strand of said target region, wherein a third nucleobase complementary to the first nucleobase base is replaced by a fourth nucleobase complementary to the second nucleobase, and the second nucleobase is replaced with a fifth nucleobase that is complementary to the fourth nucleobase, thereby generating an intended edited base pair, wherein the efficiency of generating the intended edited base pair is at least 5%. It should be appreciated that in some embodiments, step b is omitted. In some embodiments, at least 5% of the intended base pairs are edited. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the intended base pairs are edited. In some embodiments, the method causes less than 19%, 18%, 16%, 14%, 12%, 10%, 8%, 6%, 4%, 2%, 1%, 0.5%, 0.2%, or less than 0.1% indel formation. In some embodiments, the ratio of intended product to unintended products at the target nucleotide is at least 2:1, 5:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, 100:1, or 200:1, or more. In some embodiments, the ratio of intended mutation to indel formation is greater than 1:1, 10:1, 50:1, 100:1, 500:1, or 1000:1, or more. In some embodiments, the cut single strand is hybridized to the guide nucleic acid. In some embodiments, the cut single strand is opposite to the strand comprising the first nucleobase. In some embodiments, the intended edited base pair is upstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides upstream of the PAM site. In some embodiments, the intended edited base pair is downstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides downstream stream of the PAM site. In some embodiments, the method does not require a canonical (e.g., NGG) PAM site. In some embodiments, the linker is 1-25 amino acids in length. In some embodiments, the linker is 5-20 amino acids in length. In some embodiments, the linker is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In some embodiments, the target region comprises a target window, wherein the target window comprises the target nucleobase pair. In some embodiments, the target window comprises 1-10 nucleotides. In some embodiments, the target window is 1-9, 1-8, 1-

7, 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotides in length. In some embodiments, the target window is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, the intended edited base pair occurs within the target window. In some embodiments, the target window comprises the intended edited base pair. In some embodiments, the nucleobase editor is any one of the base editors provided herein.

Multiplex Editing

In some embodiments, the base editor system provided herein is capable of multiplex editing of a plurality of nucleobase pairs in one or more genes. In some embodiments, the plurality of nucleobase pairs is located in the same gene. In some embodiments, the plurality of nucleobase pairs is located in one or more gene, wherein at least one gene is located in a different locus. In some embodiments, the multiplex editing can comprise one or more guide polynucleotides. In some embodiments, the multiplex editing can comprise one or more base editor system. In some embodiments, the multiplex editing can comprise one or more base editor systems with a single guide polynucleotide. In some embodiments, the multiplex editing can comprise one or more base editor system with a plurality of guide polynucleotides. In some embodiments, the multiplex editing can comprise one or more guide polynucleotide with a single base editor system. In some embodiments, the multiplex editing can comprise at least one guide polynucleotide that does not require a PAM sequence to target binding to a target polynucleotide sequence. In some embodiments, the multiplex editing can comprise at least one guide polynucleotide that requires a PAM sequence to target binding to a target polynucleotide sequence. In some embodiments, the multiplex editing can comprise a mix of at least one guide polynucleotide that does not require a PAM sequence to target binding to a target polynucleotide sequence and at least one guide polynucleotide that require a PAM sequence to target binding to a target polynucleotide sequence. It should be appreciated that the characteristics of the multiplex editing using any of the base editors as described herein can be applied to any of combination of the methods of using any of the base editor provided herein. It should also be appreciated that the multiplex editing using any of the base editors as described herein can comprise a sequential editing of a plurality of nucleobase pairs.

In some embodiments, the plurality of nucleobase pairs are in one more genes. In some embodiments, the plurality of nucleobase pairs is in the same gene. In some embodiments, at least one gene in the one more genes is located in a different locus.

In some embodiments, the editing is editing of the plurality of nucleobase pairs in at least one protein coding region. In some embodiments, the editing is editing of the plurality of nucleobase pairs in at least one protein non-coding region. In some embodiments, the editing is editing of the plurality of nucleobase pairs in at least one protein coding region and at least one protein non-coding region.

In some embodiments, the editing is in conjunction with one or more guide polynucleotides. In some embodiments, the base editor system can comprise one or more base editor system. In some embodiments, the base editor system can comprise one or more base editor systems in conjunction with a single guide polynucleotide. In some embodiments, the base editor system can comprise one or more base editor system in conjunction with a plurality of guide polynucleotides. In some embodiments, the editing is in conjunction with one or more guide polynucleotide with a single base editor system. In some embodiments, the editing is in conjunction with at least one guide polynucleotide that does not require a PAM sequence to target binding to a target polynucleotide sequence. In some embodiments, the editing is in conjunction with at least one guide polynucleotide that require a PAM sequence to target binding to a target polynucleotide sequence. In some embodiments, the editing is in conjunction with a mix of at least one guide polynucleotide that does not require a PAM sequence to target binding to a target polynucleotide sequence and at least one guide polynucleotide that require a PAM sequence to target binding to a target polynucleotide sequence. It should be appreciated that the characteristics of the multiplex editing using any of the base editors as described herein can be applied to any of combination of the methods of using any of the base editors provided herein. It should also be appreciated that the editing can comprise a sequential editing of a plurality of nucleobase pairs.

Expression of Fusion Proteins in a Host Cell

Fusion proteins of the invention may be expressed in virtually any host cell of interest, including but not limited to bacteria, yeast, fungi, insects, plants, and animal cells using routine methods known to the skilled artisan. For example, a DNA encoding a fusion protein of the invention can be cloned by designing suitable primers for the upstream and downstream of CDS based on the cDNA sequence. The cloned DNA may be directly, or after digestion with a restriction enzyme when desired, or after addition of a suitable linker and/or a nuclear localization signal ligated with a DNA encoding one or more additional components of a base editing system. The base editing system is translated in a host cell to form a complex.

Fusion proteins are generated by operably linking one or more polynucleotides encoding one or more domains having nucleobase modifying activity (*e.g.*, an adenosine deaminase, cytidine deaminase, DNA glycosylase) to a polynucleotide encoding a napDNAbp to prepare a polynucleotide that encodes a fusion protein of the invention. In some embodiments, a polynucleotide encoding a napDNAbp, and a DNA encoding a domain having nucleobase modifying activity may each be fused with a DNA encoding a binding domain or a binding partner thereof, or both DNAs may be fused with a DNA encoding a separation intein, whereby the nucleic acid sequence-recognizing conversion module and the nucleic acid base converting enzyme are translated in a host cell to form a complex. In these cases, a linker and/or a nuclear localization signal can be linked to a suitable position of one of or both DNAs when desired.

A DNA encoding a protein domain described herein can be obtained by chemically synthesizing the DNA, or by connecting synthesized partly overlapping oligoDNA short chains by utilizing the PCR method and the Gibson Assembly method to construct a DNA encoding the full length thereof. The advantage of constructing a full-length DNA by chemical synthesis or a combination of PCR method or Gibson Assembly method is that the codon to be used can be designed in CDS full-length according to the host into which the DNA is introduced. In the expression of a heterologous DNA, the protein expression level is expected to increase by converting the DNA sequence thereof to a codon highly frequently used in the host organism. As the data of codon use frequency in host to be used, for example, the genetic code use frequency database (http://www.kazusa.or.jp/codon/index.html) disclosed in the home page of Kazusa DNA Research Institute can be used, or documents showing the codon use frequency in each host may be referred to. By reference to the obtained data and the DNA sequence to be introduced, codons showing low use frequency in the host from among those used for the DNA sequence may be converted to a codon coding the same amino acid and showing high use frequency.

An expression vector containing a DNA encoding a nucleic acid sequence-recognizing module and/or a nucleic acid base converting enzyme can be produced, for example, by linking the DNA to the downstream of a promoter in a suitable expression vector.

As the expression vector, *Escherichia coli*-derived plasmids (*e.g.*, pBR322, pBR325, pUC12, pUC13); *Bacillus subtilis*-derived plasmids (*e.g.*, pUB110, pTP5, pC194); yeast-derived plasmids (*e.g.*, pSH19, pSH15); insect cell expression plasmids (*e.g.*, pFast-Bac); animal cell expression plasmids (*e.g.*, pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo); bacteriophages such as .lamda.phage and the like; insect virus vectors such as baculovirus and the like (*e.g.*, BmNPV, AcNPV); animal virus vectors such as retrovirus, vaccinia virus, adenovirus and the like, and the like are used.

As the promoter, any promoter appropriate for a host to be used for gene expression can be used. In a conventional method using DSB, since the survival rate of the host cell sometimes decreases markedly due to the toxicity, it is desirable to increase the number of cells by the start of the induction by using an inductive promoter. However, since sufficient cell proliferation can also be afforded by expressing the nucleic acid-modifying enzyme complex of the present invention, a constitution promoter can also be used without limitation.

For example, when the host is an animal cell, SR.alpha. promoter, SV40 promoter, LTR promoter, CMV (cytomegalovirus) promoter, RSV (*Rous sarcoma* virus) promoter, MoMuLV (Moloney mouse leukemia virus) LTR, HSV-TK (simple herpes virus thymidine kinase) promoter and the like are used. Of these, CMV promoter, SR.alpha promoter and the like are preferable. In one embodiment, the promoter is CMV promoter or SR alpha promoter. When the host cell is *Escherichia coli*, any of the following promoters may be used: trp promoter, lac promoter, recA promoter, lamda.P.sub.L promoter, lpp promoter, T7 promoter and the like. When the host is genus *Bacillus*, any of the following promoters may be used: SPO1 promoter, SPO2 promoter, penP promoter and the like. When the host is a yeast, any of the following promoters may be used: Gal1/10 promoter, PHO5 promoter, PGK promoter, GAP promoter, ADH promoter and the like. When the host is an insect cell, any of the following promoters may be used polyhedrin promoter, P10 promoter and the like. When the host is a plant cell, any of the following promoters may be used: CaMV35S promoter, CaMV19S promoter, NOS promoter and the like.

In some embodiments, the expression vector may contain an enhancer, splicing signal, terminator, polyA addition signal, a selection marker such as drug resistance gene, auxotrophic complementary gene and the like, replication origin and the like on demand.

An RNA encoding a protein domain described herein can be prepared by, for example, transcription to mRNA in a vitro transcription system known per se by using a vector encoding DNA encoding the above-mentioned nucleic acid sequence-recognizing module and/or a nucleic acid base converting enzyme as a template.

A fusion protein of the invention can be expressed by introducing an expression vector encoding a fusion protein into a host cell, and culturing the host cell. Host cells useful in the invention include bacterial cells, yeast, insect cells, mammalian cells and the like.

The genus *Escherichia* includes *Escherichia coli* K12.cndot.DH1 (Proc. Natl. Acad. Sci. USA, 60, 160 (1968)], *Escherichia coli* JM103 (Nucleic Acids Research, 9, 309 (1981)], *Escherichia coli* JA221 (Journal of Molecular Biology, 120, 517 (1978)], *Escherichia coli* HB101 (Journal of Molecular Biology, 41, 459 (1969)], *Escherichia coli* C600 (Genetics, 39, 440 (1954)] and the like.

The genus *Bacillus* includes *Bacillus subtilis* M1114 (Gene, 24, 255 (1983)], *Bacillus subtilis* 207-21 (Journal of Biochemistry, 95, 87 (1984)] and the like.

Yeast useful for expressing fusion proteins of the invention include *Saccharomyces cerevisiae* AH22, AH22R.sup.-, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, *Pichia pastoris* KM71 and the like.

Fusion proteins are expressed in insect cells using, for example, viral vectors, such as AcNPV. Insect host cells include any of the following cell lines: cabbage armyworm larva-derived established line (*Spodoptera frugiperda* cell; Sf cell), MG1 cells derived from the mid-intestine of *Trichoplusia ni*, High Five.TM. cells derived from an egg of *Trichoplusia ni*, *Mamestra brassicae*-derived cells, *Estigmena acrea*-derived cells and the like are used. When the virus is BmNPV, cells of *Bombyx mori*-derived established line (*Bombyx mori* N cell; BmN cell) and the like are used as insect cells. As the Sf cell, for example, Sf9 cell (ATCC CRL1711), Sf21 cell (all above, In Vivo, 13, 213-217 (1977)] and the like.

As the insect, for example, larva of *Bombyx mori*, *Drosophila*, cricket and the like are used to express fusion proteins (Nature, 315, 592 (1985)).

Mammalian cell lines may be used to express fusion proteins. Such cell lines include monkey COS-7 cell, monkey Vero cell, Chinese hamster ovary (CHO) cell, dhfr gene-deficient CHO cell, mouse L cell, mouse AtT-20 cell, mouse myeloma cell, rat GH3 cell, human FL cell and the like, pluripotent stem cells such as iPS cell, ES cell and the like of human and other mammals, and primary cultured cells prepared from various tissues are used. Furthermore, zebrafish embryo, *Xenopus* oocyte and the like can also be used.

Plant cells may be maintained in culture using methods well known to the skilled artisan. Plant cell culture involves suspending cultured cells, callus, protoplast, leaf segment, root segment and the like prepared from various plants (*e.g.*, grain such as rice, wheat, corn and the like, product crops such as tomato, cucumber, eggplant, carnations, *Eustoma russellianum*, tobacco, *Arabidopsis thaliana*).

All the above-mentioned host cells may be haploid (monoploid), or polyploid (*e.g.*, diploid, triploid, tetraploid and the like). In the conventional mutation introduction methods, mutation is, in principle, introduced into only one homologous chromosome to produce a hetero gene type. Therefore, desired phenotype is not expressed unless dominant mutation occurs, and homozygousness inconveniently requires labor and time. In contrast, according to the present invention, since mutation can be introduced into any allele on the homologous chromosome in the genome, desired phenotype can be expressed in a single generation even in the case of recessive mutation, which is extremely useful since the problem of the conventional method can be solved.

Expression vectors encoding a fusion protein of the invention are introduced into host cells using any transfection method (*e.g.*, lysozyme method, competent method, PEG method, $CaCl_2$ coprecipitation method, electroporation method, the microinjection method, the particle gun method, lipofection method, Agrobacterium method and the like). The transfection method is selected based on the host cell to be transfected.

Escherichia coli can be transformed according to the methods described in, for example, Proc. Natl. Acad. Sci. USA, 69, 2110 (1972), Gene, 17, 107 (1982) and the like. The genus *Bacillus* can be introduced into a vector according to the methods described in, for example, Molecular & General Genetics, 168, 111 (1979) and the like. Yeast cells can be introduced into a vector according to the methods described in, for example, Methods in Enzymology, 194, 182-187 (1991), Proc. Natl. Acad. Sci. USA, 75, 1929 (1978) and the like. Insect cells can be introduced into a vector according to the methods described in, for example, Bio/Technology, 6, 47-55 (1988) and the like. Mammalian cells can be introduced into a vector according to the methods described in, for example, Cell Engineering additional volume 8, New Cell Engineering Experiment Protocol, 263-267 (1995) (published by Shujunsha), and Virology, 52, 456 (1973).

Cells comprising expression vectors of the invention are cultured according to known methods, which vary depending on the host. For example, when *Escherichia coli* or genus *Bacillus* are cultured, a liquid medium is preferable as a medium to be used for the culture. The medium preferably contains a carbon source, nitrogen source, inorganic substance and the like necessary for the growth of the transformant. Examples of the carbon source include glucose, dextrin, soluble starch, sucrose and the like; examples of the nitrogen source include inorganic or organic substances such as ammonium salts, nitrate salts, corn steep liquor, peptone, casein, meat extract, soybean cake, potato extract and the like; and examples of the inorganic substance include calcium chloride, sodium dihydrogen phosphate, magnesium chloride and the like. The medium may contain yeast extract, vitamins, growth promoting factor and the like. The pH of the medium is preferably about 5- about 8.

As a medium for culturing *Escherichia coli*, for example, M9 medium containing glucose, casamino acid (Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York 1972] is preferable. Where necessary, for example, agents such as 3.beta.-indolylacrylic acid may be added to the medium to ensure an efficient function of a promoter. *Escherichia coli* is cultured at generally about 15- about 43°C. Where necessary, aeration and stirring may be performed.

The genus *Bacillus* is cultured at generally about 30- about 40°C. Where necessary, aeration and stirring may be performed.

Examples of the medium for culturing yeast include Burkholder minimum medium (Proc. Natl. Acad. Sci. USA, 77, 4505 (1980)], SD medium containing 0.5% casamino acid (Proc. Natl. Acad. Sci. USA, 81, 5330 (1984)] and the like. The pH of the medium is preferably about 5- about 8. The culture is performed at generally about 20°C.-about 35°C. Where necessary, aeration and stirring may be performed.

As a medium for culturing an insect cell or insect, for example, Grace's Insect Medium (Nature, 195, 788 (1962)] containing an additive such as inactivated 10% bovine serum and the like as appropriate and the like are used. The pH of the medium is preferably about 6.2 to about 6.4. The culture is performed at generally about 27°C. Where necessary, aeration and stirring may be performed.

As a medium for culturing an animal cell, for example, minimum essential medium (MEM) containing about 5- about 20% of fetal bovine serum (Science, 122, 501 (1952)], Dulbecco's modified Eagle medium (DMEM) (Virology, 8, 396 (1959)], RPMI 1640 medium (The Journal of the American Medical Association, 199, 519 (1967)], 199 medium (Proceeding of the Society for the Biological Medicine, 73, 1 (1950)] and the like are used. The pH of the medium is preferably about 6- about 8. The culture is performed at generally about 30°C to about 40°C. Where necessary, aeration and stirring may be performed.

As a medium for culturing a plant cell, for example, MS medium, LS medium, B5 medium and the like are used. The pH of the medium is preferably about 5- about 8. The culture is performed at generally about 20°C-about 30°C. Where necessary, aeration and stirring may be performed.

When a higher eukaryotic cell, such as animal cell, insect cell, plant cell and the like is used as a host cell, a DNA encoding a base editing system of the present invention is introduced into a host cell under the regulation of an inducible promoter (*e.g.*, metallothionein promoter (induced by heavy metal ion), heat shock protein promoter (induced by heat shock), Tet-ON/Tet-OFF system promoter (induced by addition or removal of tetracycline or a derivative thereof), steroid-responsive promoter (induced by steroid hormone or a derivative thereof) etc.), the induction substance is added to the medium (or removed from the medium) at an appropriate stage to induce expression of the nucleic acid-modifying enzyme complex, culture is performed for a given period to carry out a base editing and, introduction of a mutation into a target gene, transient expression of the base editing system can be realized.

Prokaryotic cells such as *Escherichia coli* and the like can utilize an inducible promoter. Examples of the inducible promoter include, but are not limited to, lac promoter (induced by IPTG), cspA promoter (induced by cold shock), araBAD promoter (induced by arabinose) and the like.

Alternatively, the above-mentioned inductive promoter can also be utilized as a vector removal mechanism when higher eukaryotic cells, such as animal cell, insect cell, plant cell and the like are used as a host cell. That is, a vector is mounted with a replication origin that functions in a host cell, and a nucleic acid encoding a protein necessary for replication (*e.g.*, SV40 on and large T antigen, oriP and EBNA-1 etc. for animal cells), of the expression of the nucleic acid encoding the protein is regulated by the above-mentioned inducible promoter. As a result, while the vector is autonomously replicatable in the presence of an induction substance, when the induction substance is removed, autonomous replication is not available, and the vector naturally falls off along with cell division (autonomous replication is not possible by the addition of tetracycline and doxycycline in Tet-OFF system vector).

DELIVERY SYSTEM

Nucleic Acid-Based Delivery of a Nucleobase Editors and gRNAs

Nucleic acids encoding base editing systems (*e.g.*, multi-effector nucleobase editor) according to the present disclosure can be administered to subjects or delivered into cells *in vitro* or *in vivo* by art-known methods or as described herein. In one embodiment, nucleobase editors or multi-effector nucleobase editors can be delivered by, *e.g.*, vectors (*e.g.*, viral or non-viral vectors), non-vector based methods (*e.g.*, using naked DNA, DNA complexes, lipid nanoparticles), or a combination thereof.

Nucleic acids encoding nucleobase editors or multi-effector nucleobase editors can be delivered directly to cells (*e.g.*, hematopoietic cells or their progenitors, hematopoietic stem cells, and/or induced pluripotent stem cells) as naked DNA or RNA, for instance by means of transfection or electroporation, or can be conjugated to molecules (*e.g.*, N-acetylgalactosamine) promoting uptake by the target cells. Nucleic acid vectors, such as the vectors described herein can also be used.

Nucleic acid vectors can comprise one or more sequences encoding a domain of a fusion protein described herein. A vector can also comprise a sequence encoding a signal peptide (*e.g.*, for nuclear localization, nucleolar localization, or mitochondrial localization), associated with (*e.g.*, inserted into or fused to) a sequence coding for a protein. As one example, a nucleic acid vectors can include a Cas9 coding sequence that includes one or more nuclear localization sequences (*e.g.*, a nuclear localization sequence from SV40), and deaminase (*e.g.*, an adenosine deaminase and/or cytidine deaminase).

The nucleic acid vector can also include any suitable number of regulatory/control elements, *e.g.*, promoters, enhancers, introns, polyadenylation signals, Kozak consensus sequences, or internal ribosome entry sites (IRES). These elements are well known in the art. For hematopoietic cells suitable promoters can include IFNbeta or CD45.

Nucleic acid vectors according to this disclosure include recombinant viral vectors. Exemplary viral vectors are set forth herein. Other viral vectors known in the art can also be used. In addition, viral particles can be used to deliver base editing system components in nucleic acid and/or peptide form. For example, "empty" viral particles can be assembled to contain any suitable cargo. Viral vectors and viral particles can also be engineered to incorporate targeting ligands to alter target tissue specificity.

In addition to viral vectors, non-viral vectors can be used to deliver nucleic acids encoding genome editing systems according to the present disclosure. One important category of non-viral nucleic acid vectors are nanoparticles, which can be organic or inorganic. Nanoparticles are well known in the art. Any suitable nanoparticle design can be used to deliver genome editing system components or nucleic acids encoding such components. For instance, organic (*e.g.* lipid and/or polymer) nanoparticles can be suitable for use as delivery vehicles in certain embodiments of this disclosure. Exemplary lipids for use in nanoparticle formulations, and/or gene transfer are shown in **Table 6** (below).

Table 6

Lipids Used for Gene Transfer

Lipid	Abbreviation	Feature
1,2-Dioleoyl-sn-glycero-3-phosphatidylcholine	DOPC	Helper
1,2-Dioleoyl-sn-glycero-3-phosphatidylethanolamine	DOPE	Helper
Cholesterol		Helper
N-[1-(2,3-Dioleyloxy)prophyl]N,N,N-trimethylammonium chloride	DOTMA	Cationic
1,2-Dioleoyloxy-3-trimethylammonium-propane	DOTAP	Cationic
Dioctadecylamidoglycylspermine	DOGS	Cationic
N-(3-Aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide	GAP-DLRIE	Cationic
Cetyltrimethylammonium bromide	CTAB	Cationic
6-Lauroxyhexyl ornithinate	LHON	Cationic
1-(2,3-Dioleoyloxypropyl)-2,4,6-trimethylpyridinium	2Oc	Cationic
2,3-Dioleyloxy-N-[2(sperminecarboxamido-ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate	DOSPA	Cationic
1,2-Dioleyl-3-trimethylammonium-propane	DOPA	Cationic
N-(2-Hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide	MDRIE	Cationic
Dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide	DMRI	Cationic
3β-[N-(N',N'-Dimethylaminoethane)-carbamoyl]cholesterol	DC-Chol	Cationic
Bis-guanidium-tren-cholesterol	BGTC	Cationic
1,3-Diodeoxy-2-(6-carboxy-spermyl)-propylamide	DOSPER	Cationic
Dimethyloctadecylammonium bromide	DDAB	Cationic
Dioctadecylamidoglicylspermidin	DSL	Cationic
rac-[(2,3-Dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride	CLIP-1	Cationic
rac-[2(2,3-Dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammoniun bromide	CLIP-6	Cationic
Ethyldimyristoylphosphatidylcholine	EDMPC	Cationic
1,2-Distearyloxy-N,N-dimethyl-3-aminopropane	DSDMA	Cationic
1,2-Dimyristoyl-trimethylammonium propane	DMTAP	Cationic
O,O'-Dimyristyl-N-lysyl aspartate	DMKE	Cationic
1,2-Distearoyl-sn-glycero-3-ethylpho sphocholine	DSEPC	Cationic

Lipids Used for Gene Transfer		
Lipid	Abbreviation	Feature
N-Palmitoyl D-erythro-sphingosyl carbamoyl-spermine	CCS	Cationic
N-t-Butyl-N0-tetradecyl-3-tetradecylaminopropionamidine	diC14-amidine	Cationic
Octadecenolyoxy[ethyl-2-heptadecenyl-3 hydroxyethyl] imidazolinium chloride	DOTIM	Cationic
N1 -Cholesteryloxycarbonyl-3,7-diazanonane-1,9-diamine	CDAN	Cationic
2-(3-[Bis(3-amino-propyl)-amino]propylamino)-N-ditetradecylcarbamoylme-ethyl-acetamide	RPR209120	Cationic
1,2-dilinoleyloxy-3-dimethylaminopropane	DLinDMA	Cationic
2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane	DLin-KC2-DMA	Cationic
dilinoleyl-methyl-4-dimethylaminobutyrate	DLin-MC3-DMA	Cationic

Table 7 lists exemplary polymers for use in gene transfer and/or nanoparticle formulations.

Table 7

Polymers Used for Gene Transfer	
Polymer	Abbreviation
Poly(ethylene)glycol	PEG
Polyethylenimine	PEI
Dithiobis (succinimidylpropionate)	DSP
Dimethyl-3,3'-dithiobispropionimidate	DTBP
Poly(ethylene imine)biscarbamate	PEIC
Poly(L-lysine)	PLL
Histidine modified PLL	
Poly(N-vinylpyrrolidone)	PVP
Poly(propylenimine)	PPI
Poly(amidoamine)	PAMAM
Poly(amidoethylenimine)	SS-PAEI
Triethylenetetramine	TETA
Poly(β-aminoester)	

Polymers Used for Gene Transfer	
Polymer	Abbreviation
Poly(4-hydroxy-L-proline ester)	PHP
Poly(allylamine)	
Poly(α-[4-aminobutyl]-L-glycolic acid)	PAGA
Poly(D,L-lactic-co-glycolic acid)	PLGA
Poly(N-ethyl-4-vinylpyridinium bromide)	
Poly(phosphazene)s	PPZ
Poly(phosphoester)s	PPE
Poly(phosphoramidate)s	PPA
Poly(N-2-hydroxypropylmethacrylamide)	pHPMA
Poly (2-(dimethylamino)ethyl methacrylate)	pDMAEMA
Poly(2-aminoethyl propylene phosphate)	PPE-EA
Chitosan	
Galactosylated chitosan	
N-Dodacylated chitosan	
Histone	
Collagen	
Dextran-spermine	D-SPM

Table 8 summarizes delivery methods for a polynucleotide encoding a fusion protein described herein.

Table 8

Delivery	Vector/Mode	Delivery into Non-Dividing Cells	Duration of Expression	Genome Integration	Type of Molecule Delivered
Physical	(*e.g.*, electroporation, particle gun, Calcium Phosphate transfection	YES	Transient	NO	Nucleic Acids and Proteins
Viral	Retrovirus	NO	Stable	YES	RNA

Delivery	Vector/Mode	Delivery into Non-Dividing Cells	Duration of Expression	Genome Integration	Type of Molecule Delivered
	Lentivirus	YES	Stable	YES/NO with modification	RNA
	Adenovirus	YES	Transient	NO	DNA
	Adeno-Associated Virus (AAV)	YES	Stable	NO	DNA
	Vaccinia Virus	YES	Very Transient	NO	DNA
	Herpes Simplex Virus	YES	Stable	NO	DNA
Non-Viral	Cationic Liposomes	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
	Polymeric Nanoparticles	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
Biological Non-Viral Delivery Vehicles	Attenuated Bacteria	YES	Transient	NO	Nucleic Acids
	Engineered Bacteriophages	YES	Transient	NO	Nucleic Acids
	Mammalian Virus-like Particles	YES	Transient	NO	Nucleic Acids
	Biological liposomes: Erythrocyte Ghosts and Exosomes	YES	Transient	NO	Nucleic Acids

In another aspect, the delivery of genome editing system components or nucleic acids encoding such components, for example, a nucleic acid binding protein such as, for example, Cas9 or variants thereof, and a gRNA targeting a genomic nucleic acid sequence of interest, may be accomplished by delivering a ribonucleoprotein (RNP) to cells. The RNP comprises the nucleic acid binding protein, *e.g.*, Cas9, in complex with the targeting gRNA. RNPs may be delivered to cells using known methods, such as electroporation, nucleofection, or cationic lipid-mediated methods, for example, as reported by Zuris, J.A. *et al.*, 2015, *Nat. Biotechnology*, 33(1):73-80. RNPs are advantageous for use in CRISPR base editing systems, particularly for cells that are difficult to transfect, such as primary cells. In addition, RNPs can also alleviate difficulties that may occur with protein expression in cells, especially when eukaryotic promoters, *e.g.*, CMV or EF1A, which may be used in CRISPR plasmids, are not well-expressed. Advantageously, the use of RNPs does not require the delivery of foreign DNA into cells. Moreover, because an RNP comprising a nucleic acid binding protein and gRNA complex is degraded over time, the use of RNPs has the potential to limit off-target effects. In a manner similar to that for plasmid based techniques, RNPs can be used to deliver binding protein (*e.g.*, Cas9 variants) and to direct homology directed repair (HDR).

A promoter used to drive base editor coding nucleic acid molecule expression can include AAV ITR. This can be advantageous for eliminating the need for an additional promoter element, which can take up space in the vector. The additional space freed up can be used to drive the expression of additional elements, such as a guide nucleic acid or a selectable marker. ITR activity is relatively weak, so it can be used to reduce potential toxicity due to over expression of the chosen nuclease.

Any suitable promoter can be used to drive expression of the base editor and, where appropriate, the guide nucleic acid. For ubiquitous expression, promoters that can be used include CMV, CAG, CBh, PGK, SV40, Ferritin heavy or light chains, etc. For brain or other CNS cell expression, suitable promoters can include: SynapsinI for all neurons, CaMKIIalpha for excitatory neurons, GAD67 or GAD65 or VGAT for GABAergic neurons, etc. For liver cell expression, suitable promoters include the Albumin promoter. For lung cell expression, suitable promoters can include SP-B. For endothelial cells, suitable promoters can include ICAM. For hematopoietic cells suitable promoters can include IFNbeta or CD45. For Osteoblasts suitable promoters can include OG-2.

In some embodiments, a base editor of the present disclosure is of small enough size to allow separate promoters to drive expression of the base editor and a compatible guide nucleic acid within the same nucleic acid molecule. For instance, a vector or viral vector can comprise a first promoter operably linked to a nucleic acid encoding the base editor and a second promoter operably linked to the guide nucleic acid.

The promoter used to drive expression of a guide nucleic acid can include: Pol III promoters such as U6 or H1 Use of Pol II promoter and intronic cassettes to express gRNA Adeno Associated Virus (AAV).

Viral Vectors

A base editor described herein can therefore be delivered with viral vectors. In some embodiments, a base editor disclosed herein can be encoded on a nucleic acid that is contained in a viral vector. In some embodiments, one or more components of the base editor system can be encoded on one or more viral vectors. For example, a base editor and guide nucleic acid can be encoded on a single viral vector. In other embodiments, the base editor and guide nucleic acid are encoded on different viral vectors. In either case, the base editor and guide nucleic acid can each be operably linked to a promoter and terminator. The combination of components encoded on a viral vector can be determined by the cargo size constraints of the chosen viral vector.

The use of RNA or DNA viral based systems for the delivery of a base editor takes advantage of highly evolved processes for targeting a virus to specific cells in culture or in the host and trafficking the viral payload to the nucleus or host cell genome. Viral vectors can be administered directly to cells in culture, patients (*in vivo*), or they can be used to treat cells *in vitro*, and the modified cells can optionally be administered to patients (*ex vivo*). Conventional viral based systems could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

Viral vectors can include lentivirus (*e.g.*, HIV and FIV-based vectors), Adenovirus (*e.g.*, AD100), Retrovirus (*e.g.*, Maloney murine leukemia virus, MML-V), herpesvirus vectors (*e.g.*, HSV-2), and Adeno-associated viruses (AAVs), or other plasmid or viral vector types, in particular, using formulations and doses from, for example, U.S. Patent No. 8,454,972 (formulations, doses for adenovirus), U.S. Patent No. 8,404,658 (formulations, doses for AAV) and U.S. Patent No. 5,846,946 (formulations, doses for DNA plasmids) and from clinical trials and publications regarding the clinical trials involving lentivirus, AAV and adenovirus. For example, for AAV, the route of administration, formulation and dose can be as in U.S. Patent No. 8,454,972 and as in clinical trials involving AAV. For Adenovirus, the route of administration, formulation and dose can be as in U.S. Patent No. 8,404,658 and as in clinical trials involving adenovirus. For plasmid delivery, the route of administration, formulation and dose can be as in U.S. Patent No. 5,846,946 and as in clinical studies involving plasmids. Doses can be based on or extrapolated to an average 70 kg individual (*e.g.* a male adult human), and can be adjusted for patients, subjects, mammals of different weight and species. Frequency of administration is within the ambit of the medical or veterinary practitioner (*e.g.*, physician, veterinarian), depending on usual factors including the age, sex, general health, other conditions of the patient or subject and the particular condition or symptoms being addressed. The viral vectors can be injected into the tissue of interest. For cell-type specific base editing, the expression of the base editor and optional guide nucleic acid can be driven by a cell-type specific promoter.

The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof (*See, e.g.*, Buchscher *et al.*, J. Virol. 66:2731-2739 (1992); Johann *et al.*, J. Virol. 66:1635-1640 (1992); Sommnerfelt *et al.*, Virol. 176:58-59 (1990); Wilson *et al.*, J. Virol. 63:2374-2378 (1989); Miller *et al.*, J. Virol. 65:2220-2224 (1991); PCT/US94/05700).

Retroviral vectors, especially lentiviral vectors, can require polynucleotide sequences smaller than a given length for efficient integration into a target cell. For example, retroviral vectors of length greater than 9 kb can result in low viral titers compared with those of smaller size. In some embodiments, a base editor of the present disclosure is of sufficient size so as to enable efficient packaging and delivery into a target cell via a retroviral vector. In some embodiments, a base editor is of a size so as to allow efficient packing and delivery even when expressed together with a guide nucleic acid and/or other components of a targetable nuclease system.

In applications where transient expression is preferred, adenoviral based systems can be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors can also be used to transduce cells with target nucleic acids, *e.g.*, in the *in vitro* production of nucleic acids and peptides, and for *in vivo* and *ex vivo* gene therapy procedures (*See, e.g.*, West *et al.*, Virology 160:38-47 (1987); U.S. Patent No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994). The construction of recombinant AAV vectors is described in a number of publications, including U.S. Patent No. 5,173,414; Tratschin *et al.*, Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, *et al.*, Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski *et al.*, J. Virol. 63:03822-3828 (1989).

AAV is a small, single-stranded DNA dependent virus belonging to the parvovirus family. The 4.7 kb wild-type (wt) AAV genome is made up of two genes that encode four replication proteins and three capsid proteins, respectively, and is flanked on either side by 145-bp inverted terminal repeats (ITRs). The virion is composed of three capsid proteins, Vp1, Vp2, and Vp3, produced in a 1:1:10 ratio from the same open reading frame but from differential splicing (Vp1) and alternative translational start sites (Vp2 and Vp3, respectively). Vp3 is the most abundant subunit in the virion and participates in receptor recognition at the cell surface defining the tropism of the virus. A phospholipase domain, which functions in viral infectivity, has been identified in the unique N terminus of Vp1.

Similar to wt AAV, recombinant AAV (rAAV) utilizes the *cis*-acting 145-bp ITRs to flank vector transgene cassettes, providing up to 4.5 kb for packaging of foreign DNA. Subsequent to infection, rAAV can express a fusion protein of the invention and persist without integration into the host genome by existing episomally in circular head-to-tail concatemers. Although there are numerous examples of rAAV success using this system, *in vitro* and *in vivo*, the limited packaging capacity has limited the use of AAV-mediated gene delivery when the length of the coding sequence of the gene is equal or greater in size than the wt AAV genome.

Viral vectors can be selected based on the application. For example, for *in vivo* gene delivery, AAV can be advantageous over other viral vectors. In some embodiments, AAV allows low toxicity, which can be due to the purification method not requiring ultra-centrifugation of cell particles that can activate the immune response. In some embodiments, AAV allows low probability of causing insertional mutagenesis because it doesn't integrate into the host genome. Adenoviruses are commonly used as vaccines because of the strong immunogenic response they induce. Packaging capacity of the viral vectors can limit the size of the base editor that can be packaged into the vector.

AAV has a packaging capacity of about 4.5 Kb or 4.75 Kb including two 145 base inverted terminal repeats (ITRs). This means disclosed base editor as well as a promoter and transcription terminator can fit into a single viral vector. Constructs larger than 4.5 or 4.75 Kb can lead to significantly reduced virus production. For example, SpCas9 is quite large, the gene itself is over 4.1 Kb, which makes it difficult for packing into AAV. Therefore, embodiments of the present disclosure include utilizing a disclosed base editor which is shorter in length than conventional base editors. In some examples, the base editors are less than 4 kb. Disclosed base editors can be less than 4.5 kb, 4.4 kb, 4.3 kb, 4.2 kb, 4.1 kb, 4 kb, 3.9 kb, 3.8 kb, 3.7 kb, 3.6 kb, 3.5 kb, 3.4 kb, 3.3 kb, 3.2 kb, 3.1 kb, 3 kb, 2.9 kb, 2.8 kb, 2.7 kb, 2.6 kb, 2.5 kb, 2 kb, or 1.5 kb. In some embodiments, the disclosed base editors are 4.5 kb or less in length.

An AAV can be AAV1, AAV2, AAV5 or any combination thereof. One can select the type of AAV with regard to the cells to be targeted; *e.g.*, one can select AAV serotypes 1, 2, 5 or a hybrid capsid AAV1, AAV2, AAV5 or any combination thereof for targeting brain or neuronal cells; and one can select AAV4 for targeting cardiac tissue. AAV8 is useful for delivery to the liver. A tabulation of certain AAV serotypes as to these cells can be found in Grimm, D. *et al*, J. Virol. 82: 5887-5911 (2008)).

Lentiviruses are complex retroviruses that have the ability to infect and express their genes in both mitotic and post-mitotic cells. The most commonly known lentivirus is the human immunodeficiency virus (HIV), which uses the envelope glycoproteins of other viruses to target a broad range of cell types.

Lentiviruses can be prepared as follows. After cloning pCasES10 (which contains a lentiviral transfer plasmid backbone), HEK293FT at low passage (p=5) were seeded in a T-75 flask to 50% confluence the day before transfection in DMEM with 10% fetal bovine serum and without antibiotics. After 20 hours, media is changed to OptiMEM (serum-free) media and transfection was done 4 hours later. Cells are transfected with 10 µg of lentiviral transfer plasmid (pCasES10) and the following packaging plasmids: 5 µg of pMD2.G (VSV-g pseudotype), and 7.5 µg of psPAX2 (gag/pol/rev/tat). Transfection can be done in 4 mL OptiMEM with a cationic lipid delivery agent (50 µl Lipofectamine 2000 and 100 ul Plus reagent). After 6 hours, the media is changed to antibiotic-free DMEM with 10% fetal bovine serum. These methods use serum during cell culture, but serum-free methods are preferred.

Lentivirus can be purified as follows. Viral supernatants are harvested after 48 hours. Supernatants are first cleared of debris and filtered through a 0.45 μm low protein binding (PVDF) filter. They are then spun in an ultracentrifuge for 2 hours at 24,000 rpm. Viral pellets are resuspended in 50 μl of DMEM overnight at 4° C. They are then aliquoted and immediately frozen at -80°C.

In another embodiment, minimal non-primate lentiviral vectors based on the equine infectious anemia virus (EIAV) are also contemplated. In another embodiment, RetinoStat.RTM., an equine infectious anemia virus-based lentiviral gene therapy vector that expresses angiostatic proteins endostatin and angiostatin that is contemplated to be delivered via a subretinal injection. In another embodiment, use of self-inactivating lentiviral vectors are contemplated.

Any RNA of the systems, for example a guide RNA or a base editor-encoding mRNA, can be delivered in the form of RNA. Base editor-encoding mRNA can be generated using *in vitro* transcription. For example, nuclease mRNA can be synthesized using a PCR cassette containing the following elements: T7 promoter, optional kozak sequence (GCCACC), nuclease sequence, and 3' UTR such as a 3' UTR from beta globin-polyA tail. The cassette can be used for transcription by T7 polymerase. Guide polynucleotides (*e.g.*, gRNA) can also be transcribed using *in vitro* transcription from a cassette containing a T7 promoter, followed by the sequence "GG", and guide polynucleotide sequence.

To enhance expression and reduce possible toxicity, the base editor-coding sequence and/or the guide nucleic acid can be modified to include one or more modified nucleoside *e.g.* using pseudo-U or 5-Methyl-C.

The small packaging capacity of AAV vectors makes the delivery of a number of genes that exceed this size and/or the use of large physiological regulatory elements challenging. These challenges can be addressed, for example, by dividing the protein(s) to be delivered into two or more fragments, wherein the N-terminal fragment is fused to a split intein-N and the C-terminal fragment is fused to a split intein-C. These fragments are then packaged into two or more AAV vectors. In one embodiment, inteins are utilized to join fragments or portions of a multi-effector base editor protein that is grafted onto an AAV capsid protein. As used herein, "intein" refers to a self-splicing protein intron (*e.g.*, peptide) that ligates flanking N-terminal and C-terminal exteins (*e.g.*, fragments to be joined). The use of certain inteins for joining heterologous protein fragments is described, for example, in

Wood *et al.*, J. Biol. Chem. 289(21); 14512-9 (2014). For example, when fused to separate protein fragments, the inteins IntN and IntC recognize each other, splice themselves out and simultaneously ligate the flanking N- and C-terminal exteins of the protein fragments to which they were fused, thereby reconstituting a full-length protein from the two protein fragments. Other suitable inteins will be apparent to a person of skill in the art.

A fragment of a fusion protein of the invention can vary in length. In some embodiments, a protein fragment ranges from 2 amino acids to about 1000 amino acids in length. In some embodiments, a protein fragment ranges from about 5 amino acids to about 500 amino acids in length. In some embodiments, a protein fragment ranges from about 20 amino acids to about 200 amino acids in length. In some embodiments, a protein fragment ranges from about 10 amino acids to about 100 amino acids in length. Suitable protein fragments of other lengths will be apparent to a person of skill in the art.

In one embodiment, dual AAV vectors are generated by splitting a large transgene expression cassette in two separate halves (5′ and 3′ ends, or head and tail), where each half of the cassette is packaged in a single AAV vector (of <5 kb). The re-assembly of the full-length transgene expression cassette is then achieved upon co-infection of the same cell by both dual AAV vectors followed by: (1) homologous recombination (HR) between 5′ and 3′ genomes (dual AAV overlapping vectors); (2) ITR-mediated tail-to-head concatemerization of 5′ and 3′ genomes (dual AAV *trans*-splicing vectors); or (3) a combination of these two mechanisms (dual AAV hybrid vectors). The use of dual AAV vectors *in vivo* results in the expression of full-length proteins. The use of the dual AAV vector platform represents an efficient and viable gene transfer strategy for transgenes of >4.7 kb in size.

Inteins

In some embodiments, a portion or fragment of a nuclease (*e.g.*, Cas9) is fused to an intein. The nuclease can be fused to the N-terminus or the C-terminus of the intein. In some embodiments, a portion or fragment of a fusion protein is fused to an intein and fused to an AAV capsid protein. The intein, nuclease and capsid protein can be fused together in any arrangement (*e.g.*, nuclease-intein-capsid, intein-nuclease-capsid, capsid-intein-nuclease, etc.). In some embodiments, the N-terminus of an intein is fused to the C-terminus of a fusion protein and the C-terminus of the intein is fused to the N-terminus of an AAV capsid protein.

Inteins (intervening protein) are auto-processing domains found in a variety of diverse organisms, which carry out a process known as protein splicing. Protein splicing is a multi-step biochemical reaction comprised of both the cleavage and formation of peptide bonds. While the endogenous substrates of protein splicing are proteins found in intein-containing organisms, inteins can also be used to chemically manipulate virtually any polypeptide backbone.

In protein splicing, the intein excises itself out of a precursor polypeptide by cleaving two peptide bonds, thereby ligating the flanking extein (external protein) sequences via the formation of a new peptide bond. This rearrangement occurs post-translationally (or possibly co-translationally). Intein-mediated protein splicing occurs spontaneously, requiring only the folding of the intein domain.

About 5% of inteins are split inteins, which are transcribed and translated as two separate polypeptides, the N-intein and C-intein, each fused to one extein. Upon translation, the intein fragments spontaneously and non-covalently assemble into the canonical intein structure to carry out protein splicing in trans. The mechanism of protein splicing entails a series of acyl-transfer reactions that result in the cleavage of two peptide bonds at the intein-extein junctions and the formation of a new peptide bond between the N- and C-exteins. This process is initiated by activation of the peptide bond joining the N-extein and the N-terminus of the intein. Virtually all inteins have a cysteine or serine at their N-terminus that attacks the carbonyl carbon of the C-terminal N-extein residue. This N to O/S acyl-shift is facilitated by a conserved threonine and histidine (referred to as the TXXH motif), along with a commonly found aspartate, which results in the formation of a linear (thio)ester intermediate. Next, this intermediate is subject to trans-(thio)esterification by nucleophilic attack of the first C-extein residue (+1), which is a cysteine, serine, or threonine. The resulting branched (thio)ester intermediate is resolved through a unique transformation: cyclization of the highly conserved C-terminal asparagine of the intein. This process is facilitated by the histidine (found in a highly conserved HNF motif) and the penultimate histidine and may also involve the aspartate. This succinimide formation reaction excises the intein from the reactive complex and leaves behind the exteins attached through a non-peptidic linkage. This structure rapidly rearranges into a stable peptide bond in an intein-independent fashion.

In some embodiments, an N-terminal fragment of a base editor (*e.g.*, ABE, CBE) is fused to a split intein-N and a C-terminal fragment is fused to a split intein-C. These fragments are then packaged into two or more AAV vectors. The use of certain inteins for joining heterologous protein fragments is described, for example, in Wood *et al.*, J. Biol. Chem. 289(21); 14512-9 (2014). For example, when fused to separate protein fragments, the inteins IntN and IntC recognize each other, splice themselves out and simultaneously ligate the flanking N- and C-terminal exteins of the protein fragments to which they were fused, thereby reconstituting a full-length protein from the two protein fragments. Other suitable inteins will be apparent to a person of skill in the art.

In some embodiments, an ABE was split into N- and C- terminal fragments at Ala, Ser, Thr, or Cys residues within selected regions of SpCas9. These regions correspond to loop regions identified by Cas9 crystal structure analysis. The N-terminus of each fragment is fused to an intein-N and the C- terminus of each fragment is fused to an intein C at amino acid positions S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, and S590, which are indicated in Bold Capitals in the sequence below.

1 mdkkysigld igtnsvgwav itdeykvpsk kfkvlgntdr hsikknliga llfdsgetae
61 atrlkrtarr rytrrknric ylqeifsnem akvddsffhr leesflveed kkherhpifg
121 nivdevayhe kyptiyhlrk klvdstdkad lrliylalah mikfrghfli egdlnpdnsd
181 vdklfiqlvq tynqlfeenp inasgvdaka ilsarlsksr rlenliaqlp gekknglfgn
241 lialslgltp nfksnfdlae daklqlskdt ydddldnlla qigdqyadlf laaknlsdai
301 ll**S**dilrvn**T** ei**T**kaplsas mikrydehhq dltllkalvr qqlpekykei ffdq**S**kngya
361 gyidggasqe efykfikpil ekmdgteell vklnredllr kqrtfdngsi phqihlgelh
421 ailrrqedfy pflkdnreki ekiltfripy yvgpl**A**rgn**S** rf**A**wm**T**rk**S**e e**T**i**T**pwnfee
481 vvdkgasaqs fiermtnfdk nlpnekvlpk hsllyeyftv yneltkvkyv tegmrkpafl
541 sgeqkkaivd llfktnrkvt vkqlkedyfk kie**C**fd**S**vei sgvedrfn**AS** lgtyhdllki
601 ikdkdfldne enediledīv ltltlfedre mieerlktya hlfddkvmkq lkrrrytgwg
661 rlsrklingi rdkqsgktil dflksdgfan rnfmqlihdd sltfkediqk aqvsgqgdsl
721 hehianlags paikkgilqt vkvvdelvkv mgrhkpeniv iemarenqtt qkgqknsrer
781 mkrieegike lgsqilkehp ventqlqnek lylyylqngr dmyvdqeldi nrlsdydvdh
841 ivpqsflkdd sidnkvltrs dknrgksdnv pseevvkkmk nywrqllnak litqrkfdnl
901 tkaergglse ldkagfikrq lvetrqitkh vaqildsrmn tkydendkli revkvitlks
961 klvsdfrkdf qfykvreinn yhhahdayln avvgtalikk ypklesefvy gdykvydvrk
1021 miakseqeig katakyffys nimnffktei tlangeirkr plietngetg eivwdkgrdf
1081 atvrkvlsmp qvnivkktev qtggfskesi lpkrnsdkli arkkdwdpkk yggfdsptva
1141 ysvlvvakve kgkskklksv kellgitime rssfeknpid fleakgykev kkdliiklpk
1201 yslfelengr krmlasagel qkgnelalps kyvnflylas hyeklkgspe dneqkqlfve
1261 qhkhyldeii eqisefskrv iladanldkv lsaynkhrdk pireqaenii hlftltnlga
1321 paafkyfdtt idrkrytstk evldatlihq sitglyetri dlsqlggd

Use of Nucleobase Editors to Target Mutations

The suitability of nucleobase editors or multi-effector nucleobase editors that target one or more mutations is evaluated as described herein. In one embodiment, a single cell of interest is transduced with a base editing system together with a small amount of a vector encoding a reporter (*e.g.*, GFP). These cells can be any cell line known in the art, including immortalized human cell lines, such as 293T, K562 or U20S. Alternatively, primary cells (*e.g.*, human) may be used. Such cells may be relevant to the eventual cell target.

Delivery may be performed using a viral vector. In one embodiment, transfection may be performed using lipid transfection (such as Lipofectamine or Fugene) or by electroporation. Following transfection, expression of GFP can be determined either by fluorescence microscopy or by flow cytometry to confirm consistent and high levels of transfection. These preliminary transfections can comprise different nucleobase editors to determine which combinations of editors give the greatest activity.

The activity of the nucleobase editor is assessed as described herein, *i.e.*, by sequencing the genome of the cells to detect alterations in a target sequence. For Sanger sequencing, purified PCR amplicons are cloned into a plasmid backbone, transformed, miniprepped and sequenced with a single primer. Sequencing may also be performed using next generation sequencing techniques. When using next generation sequencing, amplicons may be 300-500 bp with the intended cut site placed asymmetrically. Following PCR, next generation sequencing adapters and barcodes (for example Illumina multiplex adapters and indexes) may be added to the ends of the amplicon, *e.g.*, for use in high throughput sequencing (for example on an Illumina MiSeq).

The fusion proteins that induce the greatest levels of target specific alterations in initial tests can be selected for further evaluation.

In particular embodiments, the nucleobase editors or multi-effector base editors are used to target polynucleotides of interest. In one embodiment, a nucleobase editor or multi-effector base editor of the invention is delivered to cells (*e.g.*, hematopoietic cells or their progenitors, hematopoietic stem cells, and/or induced pluripotent stem cells) in conjunction with a guide RNA that is used to target a mutation of interest within the genome of a cell, thereby altering the mutation. In some embodiments, a base editor is targeted by a guide RNA to introduce one or more edits to the sequence of a gene of interest.

In one embodiment, a nucleobase editor or multi-effector nucleobase editor is used to target a regulatory sequence, including but not limited to splice sites, enhancers, and transcriptional regulatory elements. The effect of the alteration on the expression of a gene controlled by the regulatory element is then assayed using any method known in the art.

In other embodiments, a nucleobase editor or multi-effector nucleobase editor of the invention is used to target a polynucleotide encoding a Complementarity Determining Region (CDR), thereby creating alterations in the expressed CDR. The effect of these alterations on CDR function is then assayed, for example, by measuring the specific binding of the CDR to its antigen.

In still other embodiments, a multi-effector nucleobase editor of the invention is used to target polynucleotides of interest within the genome of an organism. In one embodiment, a multi-effector nucleobase editor of the invention is delivered to cells in conjunction with a library of guide RNAs that are used to tile a variety of sequences within the genome of a cell, thereby systematically altering sequences throughout the genome.

The system can comprise one or more different vectors. In an aspect, the base editor is codon optimized for expression the desired cell type, preferentially a eukaryotic cell, preferably a mammalian cell or a human cell.

In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon (*e.g.* about or more than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are readily available, for example, at the "Codon Usage Database" available at www.kazusa.orjp/codon/ (visited Jul. 9, 2002), and these tables can be adapted in a number of ways. See, Nakamura, Y., *et al.* "Codon usage tabulated from the international DNA sequence databases: status for the year 2000" Nucl. Acids Res. 28:292 (2000). Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge (Aptagen; Jacobus, Pa.), are also available. In some embodiments, one or more codons (*e.g.* 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more, or all codons) in a sequence encoding an engineered nuclease correspond to the most frequently used codon for a particular amino acid.

Packaging cells are typically used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and psi.2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producing a cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the polynucleotide(s) to be expressed. The missing viral functions are typically supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA can be packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line can also be infected with adenovirus as a helper. The helper virus can promote replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid in some cases is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, *e.g.*, heat treatment to which adenovirus is more sensitive than AAV.

Applications for Multi-Effector Nucleobase Editors

The multi-effector nucleobase editors can be used to target polynucleotides of interest to create alterations that modify protein expression. In one embodiment, a multi-effector nucleobase editor is used to modify a non-coding or regulatory sequence, including but not limited to splice sites, enhancers, and transcriptional regulatory elements. The effect of the alteration on the expression of a gene controlled by the regulatory element is then assayed using any method known in the art. In a particular embodiment, a multi-effector nucleobase editor is able to substantially alter a regulatory sequence, thereby abolishing its ability to regulate gene expression. Advantageously, this can be done without generating double-stranded breaks in the genomic target sequence, in contrast to other RNA-programmable nucleases.

The multi-effector nucleobase editors can be used to target polynucleotides of interest to create alterations that modify protein activity. In the context of mutagenesis, for example, multi-effector nucleobase editors have a number of advantages over error-prone PCR and other polymerase-based methods. Because multi-effector nucleobase editors of the invention create alterations at multiple bases in a target region, such mutations are more likely to be expressed at the protein level relative to mutations introduced by error-prone PCR, which are less likely to be expressed at the protein level given that a single nucleotide change in a codon may still encode the same amino acid (*e.g.*, codon degeneracy). Unlike error-prone PCR, which induces random alterations throughout a polynucleotide, multi-effector nucleobase editors of the invention can be used to target specific amino acids within a small or defined region of a protein of interest.

In other embodiments, a multi-effector nucleobase editor of the invention is used to target a polynucleotide of interest within the genome of an organism. In one embodiment, the organism is a bacteria of the microbiome (*e.g.*, *Bacteriodetes, Verrucomicrobia, Firmicutes; Gammaproteobacteria, Alphaproteobacteria, Bacteriodetes, Clostridia, Erysipelotrichia, Bacilli; Enterobacteriales, Bacteriodales, Verrucomicrobiales, Clostridiales, Erysiopelotrichales, Lactobacillales; Enterobacteriaceae, Bacteroidaceae, Erysiopelotrichaceae, Prevotellaceae, Coriobacteriaceae, and Alcaligenaceae, Escherichia, Bacteroides, Alistipes, Akkermansia, Clostridium, Lactobacillus*). In another embodiment, the organism is an agriculturally important animal (*e.g.*, cow, sheep, goat, horse, chicken, turkey) or plant (*e.g.*, soybeans, wheat, corn, rice, tobacco, apples, grapes, peaches, plums, cherries). In one embodiment, a multi-effector nucleobase editor of the invention is delivered to cells in conjunction with a library of guide RNAs that are used to tile a variety of sequences within the genome of a cell, thereby systematically altering sequences throughout the genome.

Mutations may be made in any of a variety of proteins to facilitate structure function analysis or to alter the endogenous activity of the protein. Mutations may be made, for example, in an enzyme (*e.g.*, kinase, phosphatase, carboxylase, phosphodiesterase) or in an enzyme substrate, in a receptor or in its ligand, and in an antibody and its antigen. In one embodiment, a multi-effector nucleobase editor targets a nucleic acid molecule encoding the active site of the enzyme, the ligand binding site of a receptor, or a complementarity determining region (CDR) of an antibody. In the case of an enzyme, inducing mutations in the active site could increase, decrease, or abolish the enzyme's activity. The effect of mutations on the enzyme is characterized in an enzyme activity assay, including any of a number of assays known in the art and/or that would be apparent to the skilled artisan. In the case of a receptor, mutations made at the ligand binding site could increase, decrease or abolish the receptors affinity for its ligand. The effect of such mutations is assayed in a receptor/ligand binding assay, including any of a number of assays known in the art and/or that would be apparent to the skilled artisan. In the case of a CDR, mutations made within the CDR could increase, decrease or abolish binding to the antigen. Alternatively, mutations made within the CDR could alter the specificity of the antibody for the antigen. The effect of these alterations on CDR function is then assayed, for example, by measuring the specific binding of the CDR to its antigen or in any other type of immunoassay.

Pharmaceutical Compositions

Other aspects of the present disclosure relate to pharmaceutical compositions comprising any of the base editors, fusion proteins, or the fusion protein-guide polynucleotide complexes described herein. In some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises additional agents (*e.g.*, for specific delivery, increasing half-life, or other therapeutic compounds).

Suitable pharmaceutically acceptable carriers generally comprise inert substances that aid in administering the pharmaceutical composition to a subject, aid in processing the pharmaceutical compositions into deliverable preparations, or aid in storing the pharmaceutical composition prior to administration. Pharmaceutically acceptable carriers can include agents that can stabilize, optimize or otherwise alter the form, consistency, viscosity, pH, pharmacokinetics, solubility of the formulation.

Some nonlimiting examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Buffering agents, wetting agents, emulsifying agents, diluents, encapsulating agents, skin penetration enhancers, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. For example, carriers can include, but are not limited to, saline, buffered saline, dextrose, arginine, sucrose, water, glycerol, ethanol, sorbitol, dextran, sodium carboxymethyl cellulose, and combinations thereof.

Pharmaceutical compositions can comprise one or more pH buffering compounds to maintain the pH of the formulation at a predetermined level that reflects physiological pH, such as in the range of about 5.0 to about 8.0. The pH buffering compound used in the aqueous liquid formulation can be an amino acid or mixture of amino acids, such as histidine or a mixture of amino acids such as histidine and glycine. Alternatively, the pH buffering compound is preferably an agent which maintains the pH of the formulation at a predetermined level, such as in the range of about 5.0 to about 8.0, and which does not chelate calcium ions. Illustrative examples of such pH buffering compounds include, but are not limited to, imidazole and acetate ions. The pH buffering compound may be present in any amount suitable to maintain the pH of the formulation at a predetermined level.

Pharmaceutical compositions can also contain one or more osmotic modulating agents, *i.e.*, a compound that modulates the osmotic properties (*e.g.*, tonicity, osmolality, and/or osmotic pressure) of the formulation to a level that is acceptable to the blood stream and blood cells of recipient individuals. The osmotic modulating agent can be an agent that does not chelate calcium ions. The osmotic modulating agent can be any compound known or available to those skilled in the art that modulates the osmotic properties of the formulation. One skilled in the art may empirically determine the suitability of a given osmotic modulating agent for use in the inventive formulation. Illustrative examples of suitable types of osmotic modulating agents include, but are not limited to: salts, such as sodium chloride and sodium acetate; sugars, such as sucrose, dextrose, and mannitol; amino acids, such as glycine; and mixtures of one or more of these agents and/or types of agents. The osmotic modulating agent(s) may be present in any concentration sufficient to modulate the osmotic properties of the formulation.

In some embodiments, the pharmaceutical composition is formulated for delivery to a subject, *e.g.*, for gene editing. In some embodiments, administration of the pharmaceutical compositions contemplated herein may be carried out using conventional techniques including, but not limited to, infusion, transfusion, or parenterally. In some embodiments, parenteral administration includes infusing or injecting intravascularly, intravenously, intramuscularly, intraarterially, intrathecally, intratumorally, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally. In some embodiments, suitable routes of administrating the pharmaceutical composition described herein include, without limitation: topical, subcutaneous, transdermal, intradermal, intralesional, intraarticular, intraperitoneal, intravesical, transmucosal, gingival, intradental, intracochlear, transtympanic, intraorgan, epidural, intrathecal, intramuscular, intravenous, intravascular, intraosseus, periocular, intratumoral, intracerebral, and intracerebroventricular administration.

In some embodiments, the pharmaceutical composition described herein is administered locally to a diseased site (*e.g.*, tumor site). In some embodiments, the pharmaceutical composition described herein is administered to a subject by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber.

In other embodiments, the pharmaceutical composition described herein is delivered in a controlled release system. In one embodiment, a pump can be used (see, *e.g.*, Langer, 1990, Science 249: 1527-1533; Sefton, 1989, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald *et al.*, 1980, Surgery 88:507; Saudek *et al*, 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used. (See, *e.g.*, Medical Applications of Controlled Release (Langer and Wise eds., CRC Press, Boca Raton, Fla., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., Wiley, New York, 1984); Ranger and Peppas, 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61. See also Levy *et al.*, 1985, Science 228: 190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard et ah, 1989, J. Neurosurg. 71: 105.) Other controlled release systems are discussed, for example, in Langer, supra.

In some embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a composition adapted for intravenous or subcutaneous administration to a subject, *e.g.*, a human. In some embodiments, pharmaceutical composition for administration by injection are solutions in sterile isotonic use as solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

A pharmaceutical composition for systemic administration can be a liquid, *e.g.*, sterile saline, lactated Ringer's or Hank's solution. In addition, the pharmaceutical composition can be in solid forms and re-dissolved or suspended immediately prior to use. Lyophilized forms are also contemplated. The pharmaceutical composition can be contained within a lipid particle or vesicle, such as a liposome or microcrystal, which is also suitable for parenteral administration. The particles can be of any suitable structure, such as unilamellar or plurilamellar, so long as compositions are contained therein. Compounds can be entrapped in “stabilized plasmid-lipid particles” (SPLP) containing the fusogenic lipid dioleoylphosphatidylethanolamine (DOPE), low levels (5-10 mol%) of cationic lipid, and stabilized by a polyethyleneglycol (PEG) coating (Zhang Y. P. et ah, Gene Ther. 1999, 6: 1438-47). Positively charged lipids such as N-[l-(2,3-dioleoyloxi)propyl]-N,N,N-trimethyl-amoniummethylsulfate, or “DOTAP,” are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, *e.g.*, U.S. Patent Nos. 4,880,635; 4,906,477; 4,911,928; 4,917,951; 4,920,016; and 4,921,757; each of which is incorporated herein by reference.

The pharmaceutical composition described herein can be administered or packaged as a unit dose, for example. The term “unit dose” when used in reference to a pharmaceutical composition of the present disclosure refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing a compound of the invention in lyophilized form and (b) a second container containing a pharmaceutically acceptable diluent (*e.g.*, sterile used for reconstitution or dilution of the lyophilized compound of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

In another aspect, an article of manufacture containing materials useful for the treatment of the diseases described above is included. In some embodiments, the article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers can be formed from a variety of materials such as glass or plastic. In some embodiments, the container holds a composition that is effective for treating a disease described herein and can have a sterile access port. For example, the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle. The active agent in the composition is a compound of the invention. In some embodiments, the label on or associated with the container indicates that the composition is used for treating the disease of choice. The article of manufacture can further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, or dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

In some embodiments, any of the fusion proteins, gRNAs, and/or complexes described herein are provided as part of a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises any of the fusion proteins provided herein. In some embodiments, the pharmaceutical composition comprises any of the complexes provided herein. In some embodiments, the pharmaceutical composition comprises a ribonucleoprotein complex comprising an RNA-guided nuclease (*e.g.*, Cas9) that forms a complex with a gRNA and a cationic lipid. In some embodiments pharmaceutical composition comprises a gRNA, a nucleic acid programmable DNA binding protein, a cationic lipid, and a pharmaceutically acceptable excipient. Pharmaceutical compositions can optionally comprise one or more additional therapeutically active substances.

In some embodiments, compositions provided herein are administered to a subject, for example, to a human subject, in order to effect a targeted genomic modification within the subject. In some embodiments, cells are obtained from the subject and contacted with any of the pharmaceutical compositions provided herein. In some embodiments, cells removed from a subject and contacted ex vivo with a pharmaceutical composition are re-introduced into the subject, optionally after the desired genomic modification has been effected or detected in the cells. Methods of delivering pharmaceutical compositions comprising nucleases are known, and are described, for example, in U.S. Patent Nos. 6,453,242; 6,503,717; 6,534,261; 6,599,692; 6,607,882; 6,689,558; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, the disclosures of which are incorporated by reference herein in their entireties. Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals or organisms of all sorts, for example, for veterinary use.

Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, domesticated animals, pets, and commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as chickens, ducks, geese, and/or turkeys.

Formulations of the pharmaceutical compositions described herein can be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient(s) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit. Pharmaceutical formulations can additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated in its entirety herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. See also PCT application PCT/US2010/055131 (Publication number WO2011/053982 A8, filed Nov. 2, 2010), incorporated in its entirety herein by reference, for additional suitable methods, reagents, excipients and solvents for producing pharmaceutical compositions comprising a nuclease.

Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this disclosure.

The compositions, as described above, can be administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being treated, and the desired outcome. It may also depend upon the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well-known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result.

In some embodiments, compositions in accordance with the present disclosure can be used for treatment of any of a variety of diseases, disorders, and/or conditions.

Kits, Vectors, Cells

Various aspects of this disclosure provide kits comprising a base editor system. In one embodiment, the kit comprises a nucleic acid construct comprising a nucleotide sequence encoding a nucleobase editor fusion protein. The fusion protein comprises one or more deaminase domains (*e.g.*, cytidine deaminase and/or adenine deaminase) and a nucleic acid programmable DNA binding protein (napDNAbp). In some embodiments, the kit comprises at least one guide RNA capable of targeting a nucleic acid molecule of interest. In some embodiments, the kit comprises a nucleic acid construct comprising a nucleotide sequence encoding at least one guide RNA. In some embodiments, the kit comprises a nucleic acid construct, comprising a nucleotide sequence encoding (a) a Cas9 domain fused to an adenosine deaminase and/or a cytidine deaminase as provided herein; and (b) a heterologous promoter that drives expression of the sequence of (a).

The kit provides, in some embodiments, instructions for using the kit to edit one or more mutations. The instructions will generally include information about the use of the kit for editing nucleic acid molecules. In other embodiments, the instructions include at least one of the following: precautions; warnings; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container. In a further embodiment, a kit can comprise instructions in the form of a label or separate insert (package insert) for suitable operational parameters. In yet another embodiment, the kit can comprise one or more containers with appropriate positive and negative controls or control samples, to be used as standard(s) for detection, calibration, or normalization. The kit can further comprise a second container comprising a pharmaceutically-acceptable buffer, such as (sterile) phosphate-buffered saline, Ringer's solution, or dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

Some aspects of this disclosure provide cells comprising any of the nucleobase editors or multi-effector nucleobase editors or fusion proteins provided herein. In some embodiments, the cells comprise any of the nucleotides or vectors provided herein.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 1989); “Oligonucleotide Synthesis” (Gait, 1984); “Animal Cell Culture” (Freshney, 1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis, 1994); “Current Protocols in Immunology” (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1: Construction of nucleobase editors having reduced non-target deamination

Nucleobase editors (e.g., fusion proteins of a CRISPR-Cas protein and a deaminase joined by a linker) can be used to introduce specific point mutations into target polynucleotides. However, nucleobase editors carry with them the potential for unintended genome-wide spurious deamination, bystander mutation, and target proximal edits. Without being bound by theory, shortening or removing the linker from base editors would reduce the potential for unintended deamination events and/or promote desired target deamination (FIG. 1). This may be due in part to reducing the effective radius of activity for the deaminase domain of the nucleobase editor. Although the structure of Cas9 bound to DNA has been determined by X-ray crystallography, no structural information exist for the portion of DNA where base editing occurs. Modeling of Cas9 predicts that the DNA where base editing occurs could be at 2 positions in proximity to Cas9 (FIG. 2). Based on these predictions, positioning a deaminase or fragment thereof at one or more of these positions has the potential to promote on-target base editing while reducing undesired deamination events (FIG. 3). Several regions were identified in the adenosine base editor (e.g., Cas9 fused to TadA) that were amenable to insertion of the TadA deaminase or fragment thereof (FIGS. 4-7). Accordingly, adenosine deaminase base editors were generated to insert TadA or variants thereof into the Cas9 polypeptide at the identified positions.

Example 2: High-throughput in vitro assays for measuring on-target and off-target deamination

An in vitro assay was developed to assess nucleobase editors and for characterizing candidate constructs that measures on-target deamination vs. non-target deamination, including spurious deamination. A FRET-based version of the assay uses a fluorescent reporter for detection, although the assay can be adapted for gel-based readout (FIG. 8). Probes for the in vitro deamination assay include substrates for deamination, and in particular substrates for nucleobase editors (FIG. 8). In addition to containing a nucleotide that can be deaminated, probes may include PAM sequences, target specific sequences, and the like, or even random sequences. Deamination reactions using sets of probes can be performed in parallel (e.g., high throughput format). Deamination of the substrate (C→U or A→I) renders the substrate cleavable by a deamination specific endonuclease (USER/EndonucleaseV, respectively) (FIG. 8). Cleavage of the substrate uncouples the fluorescent reporter from the quencher molecule, thereby generating a fluorescent signal (FIG. 8). A high on-target to off-target fluorescence ratio for indicates that a base editor is effect. Any interacting fluorophore and quencher pair or FRET donor-acceptor pair known in the art can be used. In certain embodiments, the fluorophore is one or more or FAM, TET, HEX, TAMRA, JOE, or ROX. In various embodiments, the quencher is one or more of dabcyl, dabsyl, a Black Hole Quencher dye, including 5 ' Iowa Black® RQ (5IabRQ). In general, the quenching dye is an excitation matched quenching dye. Fluorophore-quencher pairs and their selection are described for example in Marras, Selection of Fluorophore and Quencher Pairs for Fluorescent Nucleic Acid Hybridization Probes in Methods in Molecular Biology: Fluorescent Energy Transfer Nucleic Acid Probes: Designs and Protocols. Edited by: V.V. Didenko © Humana Press Inc., Totowa, NJ.

As a demonstration of the assay, an adenosine base editor was assayed for the potential to generate off-target deamination by comparing the on-target deamination of the adenosine base editor to deamination occurring in the presence of SpCas9 (no deamination domain) or no protein (FIG. 9). The adenosine base editor reaction generated fluorescent signal above that of SpCas9 and no protein reactions, indicating that ABE was effective at on-target base editing. In another example, adenosine base editor was compared to an adenosine base editing in trans (ABE -TadA) where SpCas9 is present with TadA in trans (FIG. 10). ABE generated increased fluorescence compared to ABE -TadA, SpCas9, and no protein reaction and was effective at on-target base editing. Potential substrates for spurious off-target base editing can be tested in this assay, including single-stranded structures and branched structures, which may reflect other structures in the genome (e.g., DNA "breathing," replication forks, transcriptional active DNA, etc.) (FIG. 11).

Example 3: Assays to evaluate the activities of deaminases *in cis* and *in trans*.

An assay was developed to distinguish between the activities of deaminases *in cis* (deamination domain covalently bound to CRISPR-Cas) and *in trans* (CRISPR-Cas protein with deamination domain provided *in trans*) (FIG. 12). Deamination occuring *in cis* indicates deamination by targeted base editing whereas deamination *in trans* indicates spurious deamination. A high ratio of *in cis* to *in trans* activity indicates that a deaminase has reduced spurious deamination is effective as a base editor.

Rat APOBEC1 was tested in the *in cis-in trans* assay. Briefly, HEK293T cells were transfected with construct expressing the base editor BE4 (rAPOBEC1-nCas9-UGI-UGI), rAPOBEC1 and nCas9, nCas9 and a guide RNA, or rAPOBEC1 and guide RNA. Genomic DNA was isolated from the cells and sequencing was obtained for 4 genomic target sites. At all sites, rAPOBEC1 showed higher *in cis* deaminase activity, compared to *in trans* deaminase activity, as well as the other control reactions lacking at least one of the components for targeted base editing (FIG. 13). Likewise, TadA7.10 also showed higher *in cis* deaminase activity, compared to *in trans* deaminase activity and other deamination events (FIG. 14). To understand the effect of the adenosine base editor *in trans* separate from the guide, an SaCas9-ABE and SaCas9 guide were tested in combination with SpCas9-ABE and an SaCas9 guide, and sterically hindered ABE variants and SaCas9 guides (FIG. 15). In this context, SpCas9-ABE showed lower *in trans* activity for TadA-TadA7.10 in base editor context. The ratio of *in cis/in trans* activity for ABE and sterically hindered ABE variants was estimated using the *in trans* measurements from the SaCas9 guide assay and the activity of ABE and sterically hindered ABE variants. The estimated ratios for ABE and sterically hindered ABE variants was relatively high. Dose response studies for *in cis* and *in trans* activities were also conducted to determine if high *in cis* to *in trans* activity could be modulated by dose (e.g., where *in cis* activity increases more quickly that *in trans* activity with increasing dose). Under the conditions tested, a dose response of *in cis* to *in trans* activity was not observed (FIGS. 16-18).

The *in cis-in trans* assay was used to evaluate a variety of deaminases for reduced spurious deamination listed in Table 9 below:

Table 9. Deaminases Screened using *in cis-in trans* assay

1	rAPOBEC-1	9	hAPOBEC-2	17	hAPOBEC-3F	25	btAID
2	mAPOBEC-1	10	ppAPOBEC-2	18	hAPOBEC-3G	26	mAID
3	maAPOBEC-1	11	btAPOBEC-2	19	hAPOBEC-4	27	pmCDA-1
4	hAPOBEC-1	12	mAPOBEC-3	20	mAPOBEC-4	28	pmCDA-2
5	ppAPOBEC-1	13	hAPOBEC-3A	21	rAPOBEC-4	29	pmCDA-5
6	ocAPOBEC1	14	hAPOBEC-3B	22	mfAPOBEC-4	30	yCD
7	mdAPOBEC-1	15	hAPOBEC-3C	23	hAID	31	rAPOBEC-1-delta 177-186
8	mAPOBEC-2	16	hAPOBEC-3D	24	clAID	32	rAPOBEC-1-delta 202-213

Interestingly, several deaminases showed high *in cis/in trans* activity, including ppAPOBEC-2, mAPOBEC-2, mAPOBEC-3, and mfAPOBEC-4.

rAPOBEC-1 *Rattus norvegicus*

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNT
NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIAR
LYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLW
VRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK

mAPOBEC-1 *Mus musculus*

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSVWRHTSQN
TSN
HVEVNFLEKFTTERYFRPNTRCSITWFLSWSPCGECSRAITEFLSRHPYVTLFIYIARL
Y
HHTDQRNRQGLRDLISSGVTIQIMTEQEYCYCWRNFVNYPPSNEAYWPRYPHLWVK
LYVLELYCIILGLPPCLKILRRKQPQLTFFTITLQTCHYQRIPPHLLWATGLK

maAPOBEC-1 *Mesocricetus auratus*

MSSETGPVVVDPTLRRRIEPHEFDAFFDQGELRKETCLLYEIRWGGRHNIWRHTGQN TSRHVEINFIEKFTSERYFYPSTRCSIVWFLSWSPCGECSKAITEFLSGHPNVTLFIYAA RLY HHTDQRNRQGLRDLISRGVTIRIMTEQEYCYCWRNFVNYPPSNEVYWPRYPNLWMR LYALELYCIHLGLPPCLKIKRRHQYPLTFFRLNLQSCHYQRIPPHILWATGFI

hAPOBEC-1 *Homo sapiens*

MTSEKGPSTGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKN TTNHVEVNFIKKFTSERDFHPSMSCSITWFLSWSPCWECSQAIREFLSRHPGVTLVIYV ARLF WHMDQQNRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLW MMLYALELHCIILSLPPCLKISRRWQNHLTFFRLHLQNCHYQTIPPHILLATGLIHPSV AWR

ppAPOBEC-1 *Pongo pygmaeus*

MTSEKGPSTGDPTLRRRIESWEFDVFYDPRELRKETCLLYEIKWGMSRKIWRSSGKN TTNHVEVNFIKKFTSERRFHSSISCSITWFLSWSPCWECSQAIREFLSQHPGVTLVIYV ARLF WHMDQRNRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLW MMLYALELHCIILSLPPCLKISRRWQNHLAFFRLHLQNCHYQTIPPHILLATGLIHPSV TWR

ocAPOBEC1 *Oryctolagus cuniculus*

MASEKGPSNKDYTLRRRIEPWEFEVFFDPQELRKEACLLYEIKWGASSKTWRSSGKN TTNHVEVNFLEKLTSEGRLGPSTCCSITWFLSWSPCWECSMAIREFLSQHPGVTLIIFV ARLF QHMDRRNRQGLKDLVTSGVTVRVMSVSEYCYCWENFVNYPPGKAAQWPRYPPRW MLMYALELYCIILGLPPCLKISRRHQKQLTFFSLTPQYCHYKMIPPYILLATGLLQPSV PWR

mdAPOBEC-1 *Monodelphis domestica*

MNSKTGPSVGDATLRRRIKPWEFVAFFNPQELRKETCLLYEIKWGNQNIWRHSNQN TSQHAEINFMEKFTAERHFNSSVRCSITWFLSWSPCWECSKAIRKFLDHYPNVTLAIFI

SRLYWHMDQQHRQGLKELVHSGVTIQIMSYSEYHYCWRNFVDYPQGEEDYWPKYP
YLWIMLYVLELHCIILGLPPCLKISGSHSNQLALFSLDLQDCHYQKIPYNVLVATGLV
QPFVTWR

mAPOBEC-2 *Mus musculus*
MAQKEEAAEAAAPASQNGDDLENLEDPEKLKELIDLPPFEIVTGVRLPVNFFKFQFR
NVEYSSGRNKTFLCYVVEVQSKGGQAQATQGYLEDEHAGAHAEEAFFNTILPAFDP
ALKYNVTWYVSSSPCAACADRILKTLSKTKNLRLLILVSRLFMWEEPEVQAALKKL
KEAGCKLRIMKPQDFEYIWQNFVEQEEGESKAFEPWEDIQENFLYYEEKLADILK

hAPOBEC-2 *Homo sapiens*
MAQKEEAAVATEAASQNGEDLENLDDPEKLKELIELPPFEIVTGERLPANFFKFQFRN
VE
YSSGRNKTFLCYVVEAQGKGGQVQASRGYLEDEHAAAHAEEAFFNTILPAFDPALR
YNVTWYVSSSPCAACADRIIKTLSKTKNLRLLILVGRLFMWEEPEIQAALKKLKEAG
CKLRIMKPQDFEYVWQNFVEQEEGESKAFQPWEDIQENFLYYEEKLADILK

ppAPOBEC-2 *Pongo pygmaeus*
MAQKEEAAAATEAASQNGEDLENLDDPEKLKELIELPPFEIVTGERLPANFFKFQFRN
VE
YSSGRNKTFLCYVVEAQGKGGQVQASRGYLEDEHAAAHAEEAFFNTILPAFDPALR
YNVTWYVSSSPCAACADRIIKTLSKTKNLRLLILVGRLFMWEELEIQDALKKLKEAG
CKLRIMKPQDFEYVWQNFVEQEEGESKAFQPWEDIQENFLYYEEKLADILK

btAPOBEC-2 *Bos Taurus*
MAQKEEAAAAAEPASQNGEEVENLEDPEKLKELIELPPFEIVTGERLPAHYFKFQFRN
VE
YSSGRNKTFLCYVVEAQSKGGQVQASRGYLEDEHATNHAEEAFFNSIMPTFDPALR
YMVTWYVSSSPCAACADRIVKTLNKTKNLRLLILVGRLFMWEEPEIQAALRKLKEA
GCRLRIMKPQDFEYIWQNFVEQEEGESKAFEPWEDIQENFLYYEEKLADILK

mAPOBEC-3 *Mus musculus*
MQPQRLGPRAGMGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCY
EVTRKDCDSPVSLHHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSW

SPCFECAEQIVRFLATHHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKRLLTNFRYQDSKLQEILRPCYISVPSSSSSTLSNICLTKGLPETRFWVEGRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKPYLCYQLEQFNGQAPLKGCLLSEKGKQHAEILFLDKIRSMELSQVTITCYLTWSPCPNCAWQLAAFKRDRPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEIISRRTQRRLRRIKESWGLQDLVNDFGNLQLGPPMS

hAPOBEC-3A *Homo sapiens*

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGN

hAPOBEC-3B *Homo sapiens*

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGQVYFKPQYHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEFLSEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVTIMDYEEFAYCWENFVYNEGQQFMPWYKFDENYAFLHRTLKEILRYLMDPDTFTFNFNNDPLVLRRRQTYLCYEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQNQGN

hAPOBEC-3C *Homo sapiens*

MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVFRNQVDSETHCHAERCFLSWFCDDILSPNTKYQVTWYTSWSPCPDCAGEVAEFLARHSNVNLTIFTARLYYFQYPCYQEGLRSLSQEGVAVEIMDYEDFKYCWENFVYNDNEPFKPWKGLKTNFRLLKRRLRESLQ

hAPOBEC-3D *Homo sapiens*

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGPVLPKRQSNHRQEVYFRFENHAEMCFLSWFCGNRLPANRRFQITWFVSWNPCLPCVVKVTKFLAEHPNVTLTISAARLYYYRDRDWRWVLLRLHKAGARVKIMDYEDFAYCWENFVCNEGQPFMPWYKFDDNYASLHRTLKEILRNPMEAMYPHIFYFHFKNLLKACGRNESWLCFTMEVTKHHSAVFRKRGVFRNQVDPETHCHAERCFLSWFCDDILSPN

TNYEVTWYTSWSPCPECAGEVAEFLARHSNVNLTIFTARLCYFWDTDYQEGLCSLS
QEGASVKIMGYKDFVSCWKNFVYSDDEPFKPWKGLQTNFRLLKRRLREILQ

hAPOBEC-3F *Homo sapiens*
MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQ
VYSQPEHHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEFLAEHPNV
TLTISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYSEGQPFMP
WYKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEV
VKHHSPVSWKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWYTSWSPC
PECAGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFK
YCWENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE

hAPOBEC-3G *Homo sapiens*
MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQ
VYSELKYHPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMATFLAEDP
KVTLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYS
QRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETYLCYEV
ERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLDQDYRV
TCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISI
MTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN
hAPOBEC-4 *Homo sapiens*
MEPIYEEYLANHGTIVKPYYWLSFSLDCSNCPYHIRTGEEARVSLTEFCQIFGFPYGTT
F
PQTKHLTFYELKTSSGSLVQKGHASSCTGNYIHPESMLFEMNGYLDSAIYNNDSIRHII
L
YSNNSPCNEANHCCISKMYNFLITYPGITLSIYFSQLYHTEMDFPASAWNREALRSLA
SL
WPRVVLSPISGGIWHSVLHSFISGVSGSHVFQPILTGRALADRHNAYEINAITGVKPYF
T
DVLLQTKRNPNTKAQEALESYPLNNAFPGQFFQMPSGQLQPNLPPDLRAPVVFVLVP
LRDLPPMHMGQNPNKPRNIVRHLNMPQMSFQETKDLGRLPTGRSVEIVEITEQFASS
KEADEKKKKKGKK

mAPOBEC-4 *Mus musculus*

MDSLLMKQKKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSCSLDFGHLRNKSGC
HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVAEFLRWNPNLSLRIFTAR
LYFCEDRKAEPEGLRRLHRAGVQIGIMTFKDYFYCWNTFVENRERTFKAWEGLHEN
SVRLTRQLRRILLPLYEVDDLRDAFRMLGF

rAPOBEC-4 *Rattus norvegicus*
MEPLYEEYLTHSGTIVKPYYWLSVSLNCTNCPYHIRTGEEARVPYTEFHQTFGFPWS
TYP
QTKHLTFYELRSSSGNLIQKGLASNCTGSHTHPESMLFERDGYLDSLIFHDSNIRHIIL
Y
SNNSPCDEANHCCISKMYNFLMNYPEVTLSVFFSQLYHTENQFPTSAWNREALRGLA
SLWPQVTLSAISGGIWQSILETFVSGISEGLTAVRPFTAGRTLTDRYNAYEINCITEVK
PYFT
DALHSWQKENQDQKVWAASENQPLHNTTPAQWQPDMSQDCRTPAVFMLVPYRDL
PPIHVNPSPQKPRTVVRHLNTLQLSASKVKALRKSPSGRPVKKEEARKGSTRSQEAN
ETNKSKWKKQTLFIKSNICHLLEREQKKIGILSSWSV

mfAPOBEC-4*Macaca fascicularis*
MEPTYEEYLANHGTIVKPYYWLSFSLDCSNCPYHIRTGEEARVSLTEFCQIFGFPYGT
TY
PQTKHLTFYELKTSSGSLVQKGHASSCTGNYIHPESMLFEMNGYLDSAIYNNDSIRHII
L
YCNNSPCNEANHCCISKVYNFLITYPGITLSIYFSQLYHTEMDFPASAWNREALRSLA
SL
WPRVVLSPISGGIWHSVLHSFVSGVSGSHVFQPILTGRALTDRYNAYEINAITGVKPFF
T
DVLLHTKRNPNTKAQMALESYPLNNAFPGQSFQMTSGIPPDLRAPVVFVLLPLRDLP
PMHMGQDPNKPRNIIRHLNMPQMSFQETKDLERLPTRRSVETVEITERFASSKQAEE
KTKKKKGKK

hAID *Homo sapiens*
MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGC
HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTAR

LYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHEN
SVRLSRQLRRILLPLYEVDDLRDAFRTLGL

clAID *Canis lupus familiaris*

MDSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGHLRNKSGC
HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFAAR
LYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENREKTFKAWEGLHEN
SVRLSRQLRRILLPLYEVDDLRDAFRTLGL

btAID *Bos Taurus*

MDSLLKKQRQFLYQFKNVRWAKGRHETYLCYVVKRRDSPTSFSLDFGHLRNKAGC
HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFTAR
LYFCDKERKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHE
NSVRLSRQLRRILLPLYEVDDLRDAFRTLGL

mAID *Mus musculus*

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGC
HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTAR
LYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHEN
SVRLSRQLRRILLPLYEVDDLRDAFRTLGL

pmCDA-1 *Petromyzon marinus*

MAGYECVRVSEKLDFDTFEFQFENLHYATERHRTYVIFDVKPQSAGGRSRRLWGYII
NNPNVCHAELILMSMIDRHLESNPGVYAMTWYMSWSPCANCSSKLNPWLKNLLEE
QGHTLTMHFSRIYDRDREGDHRGLRGLKHVSNSFRMGVVGRAEVKECLAEYVEAS
RRTLTWLDTTESMAAKMRRKLFCILVRCAGMRESGIPLHLFTLQTPLLSGRVVWWR
V

pmCDA-2 *Petromyzon marinus*

MELREVVDCALASCVRHEPLSRVAFLRCFAAPSQKPRGTVILFYVEGAGRGVTGGH
AVNYNKQGTSIHAEVLLLSAVRAALLRRRRCEDGEEATRGCTLHCYSTYSPCRDCV
EYIQEFGASTGVRVVIHCCRLYELDVNRRRSEAEGVLRSLSRLGRDFRLMGPRDAIA
LLLGGRLANTADGESGASGNAWVTETNVVEPLVDMTGFGDEDLHAQVQRNKQIRE

AYANYASAVSLMLGELHVDPDKFPFLAEFLAQTSVEPSGTPRETRGRPRGASSRGPEI
GRQRPADFERALGAYGLFLHPRIVSREADREEIKRDLIVVMRKHNYQGP

pmCDA-5 *Petromyzon marinus*
MAGDENVRVSEKLDFDTFEFQFENLHYATERHRTYVIFDVKPQSAGGRSRRLWGYII
NNPNVCHAELILMSMIDRHLESNPGVYAMTWYMSWSPCANCSSKLNPWLKNLLEE
QGHTLMMHFSRIYDRDREGDHRGLRGLKHVSNSFRMGVVGRAEVKECLAEYVEAS
RRTLTWLDTTESMAAKMRRKLFCILVRCAGMRESGMPLHLFT

yCD *Saccharomyces cerevisiae*
MVTGGMASKWDQKGMDIAYEEAALGYKEGGVPIGGCLINNKDGSVLGRGHNMRF
QKGSATLHGEISTLENCGRLEGKVYKDTTLYTTLSPCDMCTGAIIMYGIPRCVVGEN
VNFKSKGEKYLQTRGHEVVVVDDERCKKIMKQFIDERPQDWFEDIGE

rAPOBEC-1 (delta 177-186)
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNT
NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIAR
LYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLW
VRGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK

rAPOBEC-1 (delta 202-213)
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNT
NKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIAR
LYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLW
VRLYVLELYCIILGLPPCLNILRRKQPQHYQRLPPHILWATGLK

Example 4: Construction of CBE and ABE internal fusions

CBE and ABE internal fusion constructs were generated by cloning deaminases into a high b-factor position within SpCas9 or SpCas9 nickase with a D10A mutation. In some cases, a structural or functional domain of the Cas9 was partially or deleted and replaced with a TadA domain (IBE020). CBEs were inserted in the same manner and were modified on the C-terminal end with a uracil DNA glycosylase inhibitor (UGI) domain.

Exemplary internal fusions base editors are provided in Table 10 below:

Table 10:

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
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
HR001	GGS-rAPOBEC1-XTEN-ins-site1_Y1016-D10A-UGIx2	pHRB-043
HR002	GGS-rAPOBEC1-XTEN ins-site2_A1023-D10A-UGIx2	pHRB-044
HR003	GGS-rAPOBEC1-XTEN ins-site3_E1029-D10A-UGIx2	pHRB-045
	GGS-rAPOBEC1-XTEN ins-site4_N1040-D10A-UGIx2	pHRB-046
HR004	GGS-rAPOBEC1-XTEN ins-site5-T1069-D10A-UGIx2	pHRB-047
HR005	GGS-rAPOBEC1-XTEN ins-site6-G1247-D10A-UGIx2	pHRB-048

Sequences of the constructs are provided below.

Cas9 TadAins 1015
ATGGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACTCTGTGGGCTGG
GCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGC
AACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGAC
AGCGGCGAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATA
CACCAGACGGAAGAACCGGATCTGCTATCTGCAAGAGATCTTCAGCAACGAGAT
GGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGA
AGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGT
GGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGA
CAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCACATGATC
AAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGAC
GTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAA
AACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTG
AGCAAGAGCAGACGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAA
GAATGGCCTGTTCGGAAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTC
AAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAGCAAGGACACC
TACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGAC

CTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGA GAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCTCTATGATCAAGAGAT ACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGC TGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCG GCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCA TCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAG GACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATC CACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCC TGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACT ACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGA GCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTT CCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACG AGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACG AGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGA GCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAG TGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACT CCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACC ACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACG AGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGA TGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGA AGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTG ATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAG TCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTG ACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTG CACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTG CAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCC GAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACA GAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGG GCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAG AAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAA CTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCT TTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACC GGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAAC TACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAAT CTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATC AAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTG GACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTG AAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGT TTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGA ACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGT TCGTGTACGGCGACTACAAGGTGGGTTCTAGCGGCAGCGAGACTCCCGGGACCT CAGAGTCCGCCACACCCGAAAGTTCTGGTTCCGAAGTCGAGTTTTCCCATGAGTA CTGGATGAGACACGCATTGACTCTCGCAAAGAGGGCTCGAGATGAACGCGAGGT GCCCGTGGGGGCAGTACTCGTGCTCAACAATCGCGTAATCGGCGAAGGTTGGAA TAGGGCAATCGGACTCCACGACCCCACTGCACATGCGGAAATCATGGCCCTTCG ACAGGGAGGGCTTGTGATGCAGAATTATCGACTTATCGATGCGACGCTGTACGTC

ACGTTTGAACCTTGCGTAATGTGCGCGGGAGCTATGATTCACTCCCGCATTGGAC GAGTTGTATTCGGTGTTCGCAACGCCAAGACGGGTGCCGCAGGTTCACTGATGG ACGTGCTGCATTACCCAGGCATGAACCACCGGGTAGAAATCACAGAAGGCATAT TGGCGGACGAATGTGCGGCGCTGTTGTGTTACTTTTTCGCATGCCCAGGCAGGT CTTTAACGCCCAGAAAAAAGCACAATCCTCTACTGACTACGACGTGCGGAAGAT GATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTA CAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGAT CCGGAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGG ATAAGGGCCGGGATTTTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGA ATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCC TGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCT AAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGG CCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTGAAAGAGCTGCTG GGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGACTTTCTG GAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAA GTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGG CGAACTGCAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCT GTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCA GAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCA GATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGT GCTGTCCGCCTACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAA TATCATCCACCTGTTTACCCTGACCAATCTGGGAGCCCCTGCCGCCTTCAAGTAC TTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAAGAGGTGCTGGAC GCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTG TCTCAGCTGGGAGGTGAC

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Cas9 TadAins 768
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IBE021_N-te: N-term fusion single TadA helix truncated 165-end

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IBE036_ISLA: delta 792-906 TadAins

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IBE036_ISLA : delta 792-906 TadAins

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IBE043_ISLA: TadA CP65ins 1246

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IBE044_ISLA: TadAins C-term truncate2 791
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pHRB-047_GGS-rAP : rAPOBEC1-XTEN ins-site5-T1069-D10A-UGIx2

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pHRB-048_GGS-rAP: rAPOBEC1-XTEN ins-site6-G1247-D10A-UGIx2

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ACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGGCAGCAGC TGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCG GCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCA TCCTGGAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAG GACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATC CACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCC TGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACT ACGTGGGCCCTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGA GCGAGGAAACCATCACCCCCTGGAACTTCGAGGAAGTGGTGGACAAGGGCGCTT CCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTGCCCAACG AGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACG AGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGA GCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAG TGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACT CCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACC ACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACG AGGACATTCTGGAAGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGA TGATCGAGGAACGGCTGAAAACCTATGCCCACCTGTTCGACGACAAAGTGATGA AGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCCGGAAGCTG ATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAG TCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTG ACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTG CACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTG CAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCC GAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACA GAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGG GCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAG AAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGAA CTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCT TTCTGAAGGACGACTCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACC GGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAGAAC TACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAAT CTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATC AAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTG GACTCCCGGATGAACACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTG AAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGT TTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGA ACGCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGT TCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCG AGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGCAACATCATGA ACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGGCCTCT GATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATT TTGCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGA CCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACA GCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCT TCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGG

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AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTC GGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGG CGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGG TCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATC CACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAA AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGAC AGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCT GTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGT GGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCT CCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATC CGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCA GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCT GCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGG CAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACG CGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACG CTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAA GGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAG TATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCT ATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTA GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACC GCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGG AAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATT AATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACG TTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCA TTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCA AAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGC AGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATA GTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCG CCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGA AAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTG CACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAA AACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTT GAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGT CTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTC CGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCGACGGATCGGGAGATCGAT CTCCCGATCCCCTAGGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTT AAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGC AAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTG CTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTT GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGC TGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAG TAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAAC

TGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC
GTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATG
CGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC
CAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACG
GGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAG
GCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGAT
CCGCTAGAGATCCGCGGCCGCTAATACGACTCACTATAGGGAGAGCCGCCACC

pHRB-048_GGS-rAP : rAPOBEC1-XTEN ins-site6-G1247-D10A-UGIx2

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGE
TAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE
RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG
DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLP
GEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYA
DLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE
KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQ
RTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRF
AWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFT
VYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECF
DSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE
RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN
RNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDEL
VKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENT
QLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRS
DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAG
FIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY
KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEI
GKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL
SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVL
VVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYS
LFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGGGSGGSSSETG
PVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEV
NFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHAD
PRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPRYPHLWVRLYVLE
LYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLKGSSGSETPGTSE
SATPESSGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLY
ETRIDLSQLGGDSGGSGGSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPES
DILVHTAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKMLSGGSGGSGGST
NLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSD
APEYKPWALVIQDSNGENKIKMLPKKKRKVEGADKRTADGSEFESPKKKRKV*

Example 5: ABE Internal Fusion Base Editors

To assess the base editing in cells, HEK293T cells were co-transfected with 100 ng of a sgRNA-encoding plasmid and a base editor encoding plasmid using Lipofectamine 2000 (Life Technologies). After 4 days, genomic DNA was isolated, and the targeted genomic region was amplified by PCR. Sequencing adaptors were added to generate a library of PCR products. The prepared PCR library containing the base-edited region was sequenced on an Illumina MiSeq.

IBE003, IBE008, IBE007, IBE002, IBE001, IBE005, IBE006, IBE004, IBE021, IBE031, IBE020, IBE036, IBE035, IBE028, and IBE009 was used to determine the percent editing in two different target sequences, HEK2 (GAACACAAAGCATAGACTGC) and T39 (GGACAGCTTTTCCTAGACAG) **(FIG. 20C-Q)**.

Example 6: Internal Fusion Base Editor Efficiency.

HEK293T were co-transfected with 100 ng of a sgRNA-encoding plasmid and a base editor encoding plasmid using Lipofectamine 2000 (Life Technologies) After 4 days, genomic DNA was isolated, and the targeted genomic region was amplified by PCR. Sequencing adaptors were added to generate a library of PCR products. The prepared PCR library containing the base-edited region was sequenced on an Illumina MiSeq. Target sites used for this experiment are shown in Table 11 below.

Table 11:

Name	Sequence
Target 1	GGCCTCCGTATCACTCTCTGACTGGGGT
Target 2	GTAACTGAACCCCTGCAATCAATGGGAT
Target 3	GCCACAGACTTTTCCATTTGCAGGAGT
Target 4	GCGAAAGGCTCGCGGCGAAGGAAGGAAT
Target 5	GCCTGGCAGATGAGAACCAGGAGGAAT
Target 6	GTATTACTATTATTATCTGAGATGGGGT
Target 7	GCCACAGTGGGAGGGGACATGGGGAAT
Target 8	GCCCTGATCTGCACTGAACAGAGGGGT
Target 9	GCCTCAAGTCTGGTTATTTTAGGGGGAT
Target 10	GGTCGACCCTTGGTATCCATGGGGGAT
Target 11	GAAAGAGACAGAGAAGGGGCAGGGGGT
Target 12	GAGTGGGAACTTTCTGATGCCATGGAAT
Target 13	GTGGGACTGATCCCTTAATGTGTGGGGT
Target 14	GCCCAGCTCCAGCCTCTGATGAGGGGT
Target 15	GAAGGCTTTACTGTATTACAGAAGGGGT
Target 16	GGAGCCAGAGACCAGTGGGCAGGGGGT
Target 17	GCTTTCCTTAGCTGTAAAAGAAAGGGAT

Target 18	GAGAAGAAACCAGGGAACAGGTAGGAGT
Target 19	GGCCTCCGTATCACTCTCTGACTGGGGT
Target 20	GATGTGTCTACTGTTACTTACAAGGAAT
Target 21	GACCAGGTCAGCAAACATGTTTGGAAT
Target 22	GCACCCAGGGGTTCTGCAGAGCAGGGAT
Target 23	GCCCAGCAATTCACTGTGAAGAGGGAT
Target 24	GACCAAAACGAGGGACATTTAGGGGAT
Target 25	GCTCCTCTCACCCTTATGACTCAGGGAT
Target 26	GACTCAGCGCCCCTGCCGGGCCTGGGAT
Target 27	GGTCGTAGCCAGTCCGAACCCCGGAGT
Target 28	GCATTCCACTCCGTCCGCCTCCGGAGT
Target 29	GGGTACCTGAGTGGGGTGCATTTGGGGT

Sequencing reads were aligned to the original target sequence and the percent editing was measured. Referring to FIG. 22A, efficiency of internal fusions compared to ABE7.10 at 29 genomic targets was examined. Editing efficiency was normalized to ABE7.10 editing at the best position (position 14, with 20 being furthest from the PAM and 1 being closest to the PAM in this graph). The maximum editing efficiency of the internal base editors across all sites and is normalized to the maximum editing efficiency of ABE7.10 (FIG. 22B). Effective base editing window based on max editing efficiency normalized to ABE7.10 indicates altered max editing windows in internal fusion A base editors compared to ABE7.10 (FIG. 22C, D).

Example 7: Evaluation of Spurious Deamination of Internal Fusion Base Editors

Base editors with guide were transfected together with SaCas9 and guide targeting different loci. SaCas9 generates ssDNA which is subject to deamination by spurious base editing (spurious = not guide-targeted). The SaCas9 target loci was sequenced to measure spurious deamination. Trans-editing was normalized to ABE7.10 trans-editing at each site. Spurious deamination across 29 different IBE target was measured by trans-editing assay normalized to ABE7.10 trans-editing sites at each site for comparison. Total trans-editing was summed per site before normalizing to ABE7.10 trans-editing at that site. The tested internal base editors (IBE002, IBE004, IBE005, IBE006, IBE008, IBE009, IBE020) showed a reduced average spurious deamination compared to ABE7.10.

Example 8: Evaluation of Base editing of internal fusion A Base Editors

Base editing using ABE internal fusions was evaluated using HEK293T cells as described in Example 6 using high-throughput sequencing. In this assay, guides were designed to target 6 different sites HEK4, GGCACTGCGGCTGGAGGTGG (FIG. 24A);

FANCF, GTAGGGCCTTCGCGCACCTCA (FIG. 24B); HEK-3, GGCCCAGACTGAGCACGTGA (FIG. 24C); HEK2-YY, GGAAACCTTGAATAAGAATGGA (FIG. 24D); EMX1, GAGTCCGAGCAGAAGAAGAA (FIG. 24E), and HEK2, GAACACAAAGCATAGACTGC (FIG. 24F).

10,000 – 20,000 HEK293T cells were seeded per well. 75 ng of sgRNA and 175 ng of base editor or Cas9 plasmid was transfected with 1 µl of Lipofectamine 2000. Four days after transfection, genomic DNA was isolated, and the target site was PCR amplified and sequenced on an Illumina MiSeq. Percent editing was calculated by the percent of 40,000 Illumina sequencing reads that have an A mutations to a G at a noted position. Internal fusion adenosine base editors exhibit different max editing window and reduced off-target editing compared to ABE7.10. (FIG.s 24A-F).

Example 9: Evaluation of Base editing of internal fusion C Base Editors.

Base editing using CBE internal fusions was evaluated using HEK293T cells as described in Example 6 using high-throughput sequencing. In this assay the following CBE base editors were used, BE4, HR001, HR002, HR003, HR004, HR005. In this assay, guides were designed to target 6 different sites HEK4, GGCACTGCGGCTGGAGGTGG (FIG. 25A); FANCF, GTAGGGCCTTCGCGCACCTCA (FIG. 25B); HEK-3, GGCCCAGACTGAGCACGTGA (FIG. 25C); HEK2-YY, GGAAACCTTGAATAAGAATGGA (FIG. 25D); EMX1, GAGTCCGAGCAGAAGAAGAA (FIG. 25E), and HEK2, GAACACAAAGCATAGACTGC (FIG. 25F). Percent editing was calculated by the percent of 40,000 Illumina sequencing reads that have an C mutations to a T at a noted position. Internal fusion cytidine base editors exhibit different max editing window and reduced off-target editing compared to ABE7.10. (FIG.s 24A-F).

The following numbered additional embodiments encompassing the methods and compositions of the base editor systems and uses are envisioned herein:

1. A fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the deaminase of the fusion protein deaminates a target nucleobase in a target polynucleotide sequence, wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide

sequence, and wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

2. The fusion protein of embodiment 1, wherein the target nucleobase is deaminated with lower off-target deamination as compared to an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.
3. The fusion protein of embodiment 1 or 2, wherein the target nucleobase is 1-20 nucleobases away from a Protospacer Adjacent Motif (PAM) sequence in the target polynucleotide sequence.
4. The fusion protein of embodiment 3, wherein the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.
5. The fusion protein of any one of embodiments 1-4, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises an amino acid in proximity to the target nucleobase when the fusion protein deaminates the target nucleobase.
6. The fusion protein of any one of embodiments 1-4, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of an alpha-helix structure of the Cas9 polypeptide.
7. The fusion protein of any one of embodiments 1-4, wherein the N- terminal fragment or the C-terminal fragment comprises a DNA binding domain.
8. The fusion protein of any one of embodiments 1-4, wherein the N-terminal fragment or the C-terminal fragment comprises a RuvC domain.
9. The fusion protein of any one of embodiments 1-4, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain.
10. The fusion protein of any one of embodiments 1-4, wherein the flexible loop of the Cas9 polypeptide comprises an amino acid at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof.
11. The fusion protein of embodiment 10, wherein the N-terminal fragment starts at the N-terminus of the Cas9 polypeptide and is a contiguous sequence that terminates at a position between 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, or 1248-1297 as numbered in SEQ ID NO: 1 or corresponding positions thereof.
12. The fusion protein of embodiment 10, wherein the C-terminal fragment starts at a position between 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942,

580-685, or 538-568 as numbered in SEQ ID NO: 1 and is a contiguous sequence that terminates at the C-terminus of the Cas9 polypeptide.

13. The fusion protein of embodiment 10, wherein the C-terminal amino acid of the N-terminal fragment is amino acid 1016, 1023, 1029, 1040, 1069, or 1247 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

14. The fusion protein of embodiment 10, wherein the N-terminal amino acid of the C-terminal fragment is amino acid 1017, 1024, 1030, 1041, 1070, 1248 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

15. The fusion protein of any one of embodiments 11-14, wherein the deaminase is a cytidine deaminase.

16. The fusion protein of embodiment 10, wherein the N-terminal fragment starts at the N-terminus of the Cas9 polypeptide and is a contiguous sequence that terminates at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof.

17. The fusion protein of embodiment 10, wherein the C-terminal fragment starts at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 and is a contiguous sequence that terminates at the C-terminus of the Cas9 polypeptide.

18. The fusion protein of embodiment 10, wherein the C-terminal amino acid of the N-terminal fragment is amino acid 1022, 1029, 1040, 1068, 1069, 1247, 1054, 1026, 768, 791, 792, 1248, 1052, or 1246 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

19. The fusion protein of embodiment 10, wherein the N-terminal amino acid of the C-terminal fragment is amino acid 1023, 1030, 1041, 1069, 1070, 1248, 1055, 1026, 769, 792, 793, 873, 907, 1249, 1053, or 1247 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

20. The fusion protein of any one of embodiments 16-19, wherein the deaminase is an adenosine deaminase.

21. The fusion protein of any one of embodiments 1-20, further comprising an additional catalytic domain.

22. The fusion protein of embodiment 21, wherein the additional catalytic domain is a cytidine deaminase or an adenosine deaminase.

23. The fusion protein of any one of embodiments 1-22 further comprising a linker between the N-terminal fragment and the deaminase.

24. The fusion protein of any one of embodiments 1-22 further comprising a linker between the C-terminal fragment and the deaminase.

25. The fusion protein of any one of embodiments 1-22 further comprising a nuclear localization signal.

26. The fusion protein of embodiment 25, wherein the nuclear localization signal is a bipartite nuclear localization signal.

27. The fusion protein of any one of embodiments 1-26, wherein the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), or variants thereof.

28. The fusion protein of any one of embodiments 1-27, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered PAM.

29. The fusion protein of any one of embodiments 1-28, wherein the Cas9 polypeptide is a nickase.

30. The fusion protein of any one of embodiments 1-28, wherein the Cas9 polypeptide is nuclease inactive.

31. The fusion protein of any one of embodiments 1-30 in complex with a guide nucleic acid sequence to effect deamination of the target nucleobase.

32. A protein library for optimized base editing comprising a plurality of fusion proteins, wherein each one of the plurality of fusion proteins comprises a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the N-terminal fragment of each one of the fusion proteins differs from the N-terminal fragments of the rest of the plurality of fusion proteins or wherein the C-terminal fragment of each one of the fusion proteins differs from the C-terminal fragments of the rest of the plurality of fusion proteins, wherein the deaminase of each one of the fusion proteins deaminates a target nucleobase in proximity to a Protospacer Adjacent Motif (PAM) sequence in a target polynucleotide sequence, and wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide sequence.

33. The protein library of embodiment 32, wherein for each nucleobase from 1 to 20 nucleobases away of the PAM sequence, at least one of the plurality of fusion proteins deaminates the nucleobase.

34. The protein library of embodiment 32, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment of the Cas9 polypeptide of each one of the plurality of fusion proteins comprises a part of a flexible loop of the Cas9 polypeptide.

35. The protein library of any one of embodiments 32-34, wherein at least one of the plurality of fusion proteins deaminates the target nucleobase with lower off-target deamination as compared to an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.

36. The protein library of anyone of embodiments 32-35, wherein at least one of the plurality of the fusion proteins deaminates a target nucleobase 2-12 nucleobases upstream of the PAM sequence.

37. The protein library of any one of embodiments 32-36, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment of a fusion protein of the plurality comprises an amino acid in proximity to the target nucleobase when the fusion protein deaminates the target nucleobase.

38. The protein library of any one of embodiments 34-36, wherein the flexible loop of the Cas9 polypeptide comprises an amino acid at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof

39. The protein library of embodiment 38, wherein the plurality of fusion proteins comprise a fusion protein that comprises a N-terminal fragment starting at the N-terminus of the Cas9 polypeptide and is a contiguous sequence that terminates at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof

40. The protein library of embodiment 38, wherein the plurality of fusion proteins comprise a fusion protein that comprises a C-terminal fragment starting at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof and is a contiguous sequence that terminates at the C-terminus of the Cas9 polypeptide.

41. The protein library of any one of embodiments 38-40, wherein the C-terminal amino acid of the N-terminal fragment is amino acid 1022, 1029, 1040, 1068, 1069, 1247,

1054, 1026, 768, 791, 792, 1248, 1052, or 1246 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

42. The protein library of any one of embodiments 38-40, wherein the N-terminal amino acid of the C-terminal fragment is amino acid 1023, 1030, 1041, 1069, 1070, 1248, 1055, 1026, 769, 792, 793, 873, 907, 1249, 1053, or 1247 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

43. The protein library of any one of embodiments 32-42, wherein the deaminase is an adenosine deaminase.

44. The protein library of any one of embodiments 32-42, wherein the deaminase is a cytidine deaminase.

45. The protein library of any one of embodiments 32-44, wherein the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), or variants thereof.

46. The protein library of any one of embodiments 32-45, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered protospacer-adjacent motif (PAM).

47. The protein library of any one of embodiments 32-46, wherein the Cas9 polypeptide is a nickase.

48. The protein library of any one of embodiments 32-46, wherein the Cas9 polypeptide is nuclease inactive.

49. A cell comprising the fusion protein of any one of embodiments 1-31.

50. The cell of embodiment 49, wherein the cell is a mammalian cell or a human cell.

51. A method for editing a target nucleobase in a target polynucleotide sequence, the method comprising: contacting the target polynucleotide with a fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragments of a Cas9 polypeptide, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence, wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide sequence, and wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

52. The method of embodiment 51, further comprising contacting the target polynucleotide sequence with a guide nucleic acid sequence to effect deamination of the target nucleobase.

53. The method of embodiment 52, wherein the guide nucleic acid sequence comprises a spacer sequence complementary to a protospacer sequence of the target polynucleotide sequence, thereby forming a R-loop.
54. The method of embodiment 53, wherein the target nucleobase is deaminated with lower off-target deamination as compared to an end terminus method comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.
55. The method of embodiment 54, wherein the deaminase of the fusion protein deaminates no more than two nucleobases within the range of the R-loop.
56. The method of any one of embodiments 51-55, wherein the target nucleobase is 1-20 nucleobases away from a PAM sequence in the target polynucleotide sequence.
57. The method of embodiment 55, wherein the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.
58. The method of any one of embodiments 51-57, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises an amino acid in proximity to the target nucleobase when the deaminase of the fusion protein deaminates the target nucleobase.
59. The method of any one of embodiments 51-57, wherein the N-terminal fragment or the C-terminal fragment comprises a RuvC domain.
60. The method of any one of embodiments 51-57, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain.
61. The method of any one of embodiments 51-57, wherein the flexible loop of the Cas9 polypeptide comprises an amino acid at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof.
62. The method of embodiment 61, wherein the N-terminal fragment starts at the N-terminus of the Cas9 polypeptide and is a contiguous sequence that terminates at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof.
63. The method of embodiment 61, wherein the C-terminal fragment starts at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 as numbered in SEQ ID NO: 1 or corresponding positions thereof and is a contiguous sequence that terminates at the C-terminus of the Cas9 polypeptide.

64. The method of embodiment 61, wherein the C-terminal amino acid of the N-terminal fragment is amino acid 1016, 1023, 1029, 1040, 1069, or 1247 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

65. The method of embodiment 61, wherein the N-terminal amino acid of the C-terminal fragment is amino acid 1017, 1024, 1030, 1041, 1070, 1248 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

66. The method of any one of embodiments 62-65, wherein the deaminase is a cytidine deaminase.

67. The method of embodiment 61, wherein the N-terminal fragment starts at the N-terminus of the Cas9 polypeptide and is a contiguous sequence that terminates at a position between 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, or 1248-1297 as numbered in SEQ ID NO: 1 or corresponding positions thereof.

68. The method of embodiment 61, wherein the C-terminal fragment starts at a position between 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, or 1248-1297 as numbered in SEQ ID NO: 1 or corresponding positions thereof and is a contiguous sequence that terminates at the C-terminus of the Cas9 polypeptide.

69. The method of embodiment 61, wherein the C-terminal amino acid of the N-terminal fragment is is amino acid 1022, 1029, 1040, 1068, 1069, 1247, 1054, 1026, 768, 791, 792, 1248, 1052, or 1246 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

70. The method of embodiment 61, wherein the N-terminal amino acid of the C-terminal fragment is amino acid 1023, 1030, 1041, 1069, 1070, 1248, 1055, 1026, 769, 792, 793, 873, 907, 1249, 1053, or 1247 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

71. The method of any one of embodiments 67-70, wherein the deaminase is an adenosine deaminase.

72. The method of any one of embodiments 51-71, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered protospacer-adjacent motif (PAM).

73. The method of any one of embodiments 51-72, wherein the Cas9 polypeptide is a nickase.

74. The method of any one of embodiments 51-72, wherein the Cas9 polypeptide is nuclease inactive.

75. The method of any one of embodiments 51-74, wherein the contacting is performed in a cell.

76. The method of embodiment 75, wherein the cell is a mammalian cell or a human cell.

77. The method of embodiment 76, wherein the cell is a pluripotent cell.

78. The method of embodiment any one of embodiments 75-77, wherein the cell is *in vivo* or *ex vivo*.

79. The method of any one of embodiments 51-74, wherein the contacting is performed in a population of cells.

80. The method of embodiment 79, wherein the population of cells are mammalian cells or human cells.

81. A method for treating a genetic condition in a subject, the method comprising: administering to the subject a fusion protein comprising a deaminase flanked by a N-terminal fragment and a C-terminal fragment of a Cas9 polypeptide or a polynucleotide encoding the fusion protein, and a guide nucleic acid sequence or a polynucleotide encoding the guide nucleic acid sequence, wherein the guide nucleic acid sequence directs the fusion protein to deaminate a target nucleobase in a target polynucleotide sequence of the subject, wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide sequence, thereby treating the genetic condition.

82. The method of embodiment 81, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

83. The method of embodiment 81 or 82, further comprising administering to the subject a guide nucleic acid sequence to effect deamination of the target nucleobase.

84. The method of any one of embodiments 81-83, wherein the target nucleobase comprises a mutation associated with the genetic condition.

85. The method of embodiment 84, wherein the deamination of the target nucleobase replaces the target nucleobase with a wild type nucleobase.

86. The method of embodiment 84, wherein the deamination of the target nucleobase replaces the target nucleobase with a non-wild type nucleobase, and wherein the deamination of the target nucleobase ameliorates symptoms of the genetic condition.

87. The method of any one of embodiments 81-83, wherein the target polynucleotide sequence comprises a mutation associated with the genetic condition at a nucleobase other than the target nucleobase.

88. The method of embodiment 87, wherein the deamination of the target nucleobase ameliorates symptoms of the genetic condition.

89. The method of any one of embodiments 81-88, wherein the target nucleobase is 1-20 nucleobases away from a PAM sequence in the target polynucleotide sequence.

90. The method of embodiment 89, wherein the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.

91. The method of any one of embodiments 81-90, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises an amino acid in proximity to the target nucleobase when the deaminase of the fusion protein deaminates the target nucleobase.

92. The method of any one of embodiments 81-90, wherein the N-terminal fragment or the C-terminal fragment comprises a RuvC domain.

93. The method of any one of embodiments 81-90, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain.

94. The method of any one of embodiments 81-90, wherein the flexible loop of the Cas9 polypeptide comprises an amino acid at a position between between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 or corresponding positions thereof.

95. The method of embodiment 94, wherein the N-terminal fragment starts at the N-terminus of the Cas9 polypeptide and is a contiguous sequence that terminates at a position between V530-P537, F569-E579, D686-R691, Y943-D947, L1052-E1077, P1002-S1025, Y1232-G1247,or R1298-K1300 as numbered in SEQ ID NO: 1.

96. The method of embodiment 94, wherein the C-terminal fragment starts at a position between 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, or 1298-1300 as numbered in SEQ ID NO: 1 and is a contiguous sequence that terminates at the C-terminus of the Cas9 polypeptide.

97. The method of embodiment 94, wherein the C-terminal amino acid of the N-terminal fragment is amino acid 1016, 1023, 1029, 1040, 1069, 1022, 1029, 1040, 1068, 1069, 1247, 1054, 1026, 768, 791, 792, 1246, 1247, 1248, or 1052 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

98. The method of embodiment 94, wherein the N-terminal amino acid of the C-terminal fragment is amino acid 1017, 1023, 1024, 1030, 1041, 1069, 1070, 1247, 1248, 1249, 1055, 1026, 769, 792, 793, 873, 907, or 1053 as numbered in SEQ ID NO: 1 or a corresponding amino acid thereof.

99. The method of any one of embodiments 81-98, wherein the deaminase is a cytidine deaminase.
100. The method of any one of embodiments 81-98, wherein the deaminase is an adenosine deaminase.
101. The method of any one of embodiments 81-100, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered PAM.
102. The method of any one of embodiments 81-101, wherein the Cas9 polypeptide is a nickase.
103. The method of any one of embodiments 81-101, wherein the Cas9 polypeptide is nuclease inactive.
104. The method of any one of embodiments 81-103, wherein the subject is a mammal.
105. The method of any one of embodiment 81-104, wherein the subject is a human.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. A fusion protein comprising a deaminase inserted within a flexible loop of a Cas9 polypeptide, wherein the fusion protein comprises the structure:

   NH_2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH,

   wherein each instance of "]-[" is an optional linker.

2. A fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

3. The fusion protein of claim 1 or 2 wherein the deaminase of the fusion protein deaminates a target nucleobase in a target polynucleotide sequence.

4. The fusion protein of claim 3, wherein the flexible loop comprises an amino acid in proximity to the target nucleobase when the fusion protein deaminates the target nucleobase.

5. The fusion protein of claim 4, wherein the flexible loop comprises a part of an alpha-helix structure of the Cas9 polypeptide.

6. The fusion protein of claim 4 or 5, wherein the target nucleobase is deaminated with lower off-target deamination as compared to an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.

7. The fusion protein of claim 4 or 5, wherein the target nucleobase is 1-20 nucleobases away from a Protospacer Adjacent Motif (PAM) sequence in the target polynucleotide sequence.

8. The fusion protein of claim 7, wherein the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.

9. The fusion protein of any one of claims 1-8, wherein the flexible loop comprises a region selected from the group consisting of amino acid residues at positions 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 as numbered in SEQ ID NO: 1, or a corresponding region thereof.

10. The fusion protein of any one of claims 1-8 wherein the deaminase 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 SEQ ID NO: 1 or corresponding amino acid positions thereof.

11. The fusion protein of claim 10 wherein the deaminase is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

12. The fusion protein of any one of claims 1-8, wherein the deaminase is inserted between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

13. The fusion protein of any one of claims 1-8, wherein the N-terminal fragment comprises amino acid residues 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, and/or 1248-1297 of the Cas9 polypeptide as numbered in SEQ ID NO: 1, or corresponding residues thereof.

14. The fusion protein of any one of claims 1-8, wherein the C-terminal fragment comprises amino acid residues 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942, 580-685, and/or 538-568 of the Cas9 polypeptide as numbered SEQ ID NO: 1, or corresponding residues thereof.

15. The fusion protein of any one of claims 3-8 wherein the N terminal fragment or the C terminal fragment of the Cas9 polypeptide binds the target polynucleotide sequence.

16. The fusion protein of any one of claims 1-15, wherein the N- terminal fragment or the C-terminal fragment of the Cas9 polypeptide comprises a DNA binding domain.

17. The fusion protein of any one of claims 1-16, wherein the N-terminal fragment or the C-terminal fragment comprises a RuvC domain.

18. The fusion protein of any one of claims 1-17, wherein the N-terminal fragment or the C terminal fragment comprises a HNH domain.

19. The fusion protein of any one of claims 1-8, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain.

20. The fusion protein of any one of claims 1-8, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a RuvC domain.

21. The fusion protein of any one of claims 1-8, wherein the Cas9 polypeptide comprises a partial or complete deletion in one or more structural domains.

22. The fusion protein of claim 21, wherein the deaminase is inserted at the partial or complete deletion position of the Cas9 polypeptide.

23. The fusion protein of claim 21 or 22, wherein the deletion is within a RuvC domain.

24. The fusion protein of claim 21 or 22, wherein the deletion is within an HNH domain.

25. The fusion protein of 21 or 22, wherein the deletion bridges a RuvC domain and a C-terminal domain, a L-I domain and a HNH domain, or a RuvC domain and a L-I domain.

26. The fusion protein of claim 21 or 22, wherein the Cas9 polypeptide comprises a deletion of amino acids 1017-1069 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

27. The fusion protein of claim 21 or 22, wherein the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

28. The fusion protein of claim 21 or 22, wherein the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

29. A fusion protein comprising a deaminase inserted within a Cas9 polypeptide, wherein the fusion protein comprises the structure:
NH_2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker,
wherein the Cas9 polypeptide comprises a complete deletion of a HNH domain, and wherein the deaminase is inserted at the deletion position.

30. The fusion protein of claim 29, wherein the C terminal amino acid of the N terminal fragment is amino acid 791 as numbered in SEQ ID NO: 1.

31. The fusion protein of claim 30, wherein the N terminal amino acid of the C terminal fragment is amino acid 907 as numbered in SEQ ID NO: 1.

32. The fusion protein of claim 30, wherein the N terminal amino acid of the C terminal fragment is amino acid 873 as numbered in SEQ ID NO: 1.

33. A fusion protein comprising a deaminase inserted within a Cas9 polypeptide, wherein the fusion protein comprises the structure:
NH_2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, and wherein the Cas9 comprises a complete deletion of a RuvC domain and wherein the deaminase is inserted at the deletion position.

34. The fusion protein of any one of claims 1-33, wherein the deaminase is a cytidine deaminase or an adenosine deaminase.

35. The fusion protein of claim 34, wherein the cytidine deaminase is an APOBEC cytidine deaminase, an activation induced cytidine deaminase (AID), or a CDA.

36. The fusion protein of claim 35, wherein the APOBEC deaminase is APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3E, APOBEC3F, APOBEC3G, APOBEC3H, or APOBEC4.

37. The fusion protein of claim 36, wherein the APOBEC deaminase is rAPOBEC1.

38. The fusion protein of any one of claims 34-37 further comprising a UGI domain.

39. The fusion protein of claim 34, wherein the adenosine deaminase is a TadA deaminase.

40. The fusion protein of claim 39, wherein the TadA deaminase is a modified TadA.

41. The fusion protein of claim 40, wherein the TadA deaminase is a TadA 7.10.

42. The fusion protein 41, wherein the adenosine deaminase is a TadA dimer.

43. The fusion protein 42, wherein the TadA dimer comprises a TadA 7.10 and a wild type TadA.

44. The fusion protein of any one of claims 3-43, wherein the optional linker comprises (SGGS)n, (GGGS)n, (GGGGS) n, (G)n, (EAAAK)n, (GGS)n, SGSETPGTSESATPES, or (XP)n motif, or a combination thereof, wherein n is independently an integer between 1 and 30.

45. The fusion protein of any one of claims 1-43, wherein the N terminal fragment of the Cas9 polypeptide is fused to the deaminase without a linker.

46. The fusion protein of any one of claims 1-43 wherein the C terminal fragment of the Cas9 is fused to the deaminase without a linker.

47. The fusion protein of any one of claims 1-46, further comprising an additional catalytic domain.

48. The fusion protein of claim 47, wherein the additional catalytic domain is a second deaminase.

49. The fusion protein of claim 48, wherein the second deaminase is fused to the N terminus or the C terminus of the fusion protein.

50. The fusion protein of claim 48 or 49, wherein the deaminase is a cytidine deaminase or an adenosine deaminase.

51. The fusion protein of any one of claims 1-50 further comprising a nuclear localization signal.

52. The fusion protein of claim 51, wherein the nuclear localization signal is a bipartite nuclear localization signal.

53. The fusion protein of any one of claims 1-52, wherein the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), or variants thereof.

54. The fusion protein of any one of claims 1-53, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered PAM.

55. The fusion protein of any one of claims 1-54, wherein the Cas9 polypeptide is a nickase.

56. The fusion protein of any one of claims 1-54, wherein the Cas9 polypeptide is nuclease inactive.

57. The fusion protein of any one of claims 3-56 in complex with a guide nucleic acid sequence to effect deamination of the target nucleobase.

58. The fusion protein of claim 57 further complexed with the target polynucleotide.

59. A polynucleotide encoding the fusion protein of any one of claims 1-58.

60. An expression vector comprising the polynucleotide of claim 59.

61. The expression vector of claim 60, wherein the expression vector is a mammalian expression vector.

62. The expression vector of claim 61, wherein the vector is a viral vector selected from the group consisting of adeno-associated virus (AAV), retroviral vector, adenoviral vector, lentiviral vector, Sendai virus vector, and herpesvirus vector.

63. The expression vector of any one of claims 60-62, wherein the vector comprises a promoter.

64. A cell comprising the fusion protein of any one of claims 1-58, the polynucleotide of claim 59, or the vector of any one of claims 60-63.

65. The cell of claim 64, wherein the cell is a bacterial cell, plant cell, insect cell, a human cell, or mammalian cell.

66. A kit comprising the fusion protein of any one of claims 1-58, the polynucleotide of claim 59, or the vector of any one of claims 60-63.

67. A method for base editing comprising contacting a polynucleotide sequence with the fusion protein of any one of claims 1-58, wherein the deaminase of the fusion protein deaminates a nucleobase in the polynucleotide, thereby editing the polynucleotide sequence.

68. The method of claim 67, further comprising contacting the target polynucleotide sequence with a guide nucleic acid sequence to effect deamination of the target nucleobase.

69. A method for editing a target nucleobase in a target polynucleotide sequence, the method comprising: contacting the target polynucleotide sequence with a fusion protein comprising a deaminase flanked by a N- terminal fragment and a C-terminal fragments of a Cas9 polypeptide, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence, and wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

70. A method for editing a target nucleobase in a target polynucleotide sequence, the method comprising: contacting the target polynucleotide sequence with a fusion protein comprising a deaminase inserted within a flexible loop of a Cas9 polypeptide, wherein the fusion protein comprises the structure NH_2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence.

71. The method of claim 69 or 70, further comprising contacting the target polynucleotide sequence with a guide nucleic acid sequence to effect deamination of the target nucleobase.

72. The method of claim 71, wherein the guide nucleic acid sequence comprises a spacer sequence complementary to a protospacer sequence of the target polynucleotide sequence, thereby forming a R-loop.

73. The method of any one of claims 69-72, wherein the target nucleobase is deaminated with lower off-target deamination as compared to an end terminus method comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.

74. The method of claim 72, wherein the deaminase of the fusion protein deaminates no more than two nucleobases within the range of the R-loop.

75. The method of any one of claims 69-74, wherein the target nucleobase is 1-20 nucleobases away from a PAM sequence in the target polynucleotide sequence.

76. The method of claim 75, wherein the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.

77. The method of any one of claims 69-76, wherein the flexible loop comprises an amino acid in proximity to the target nucleobase when the deaminase of the fusion protein deaminates the target nucleobase.

78. The method of any one of claims 69-77, wherein the flexible loop comprises a region selected from the group consisting of amino acid residues at positions 530-537, 569-

579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 as numbered in SEQ ID NO: 1, or a corresponding region thereof.

79. The method of any one of claims 69-77, wherein the deaminase 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 SEQ ID NO: 1 or corresponding amino acid positions thereof.

80. The method of claim 79 wherein the deaminase is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

81. The method of any one of claims 69-77, wherein the deaminase is inserted between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

82. The method of any one of claims 69-77, wherein the N-terminal fragment comprises amino acid residues 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, and/or 1248-1297 of the Cas9 polypeptide as numbered in SEQ ID NO: 1, or corresponding residues thereof.

83. The method of any one of claims 69-77, wherein the C-terminal fragment comprises amino acid residues 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942, 580-685, and/or 538-568 of the Cas9 polypeptide as numbered SEQ ID NO: 1, or corresponding residues thereof.

84. The method of any one of claims 69-77, wherein the N terminal fragment or the C terminal fragment of the Cas9 polypeptide binds the target polynucleotide sequence.

85. The method of any one of claims 69-84, wherein the N-terminal fragment or the C-terminal fragment comprises a RuvC domain.

86. The method of any one of claims 69-85, wherein the N-terminal fragment or the C-terminal fragment comprises a HNH domain.

87. The method of any one of claims 69-77, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain.

88. The method of any one of claims 69-77, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a RuvC domain.

89. The method of any one of claims 69-77, wherein the Cas9 polypeptide comprises a partial or complete deletion in one or more structural domains.

90. The method of claim 89, wherein the deaminase is inserted at the partial or complete deletion position of the Cas9 polypeptide.

91. The method of claim 89 or 90, wherein the deletion is within a RuvC domain.

92. The method of claim 89 or 90, wherein the deletion is within an HNH domain.

93. The method of 89 or 90, wherein the deletion bridges a RuvC domain and a C-terminal domain, a L-I domain and a HNH domain, or a RuvC domain and a L-I domain.

94. The method of claim 90, wherein the Cas9 polypeptide comprises a deletion of amino acids 1017-1069 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

95. The method of claim 90, wherein the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

96. The method of claim 90, wherein the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

97. The method of any one of claims 69-96, wherein the deaminase is a cytidine deaminase.

98. The method of any one of claims 69-96, wherein the deaminase is an adenosine deaminase.

99. The method of any one of claims 69-98, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered protospacer-adjacent motif (PAM).

100. The method of any one of claims 69-99, wherein the Cas9 polypeptide is a nickase.

101. The method of any one of claims 69-99, wherein the Cas9 polypeptide is nuclease inactive.

102. The method of any one of claims 67-101, wherein the contacting is performed in a cell.

103. The method of claim 102, wherein the cell is a mammalian cell or a human cell.

104. The method of claim 103, wherein the cell is a pluripotent cell.

105. The method of claim any one of claims 102-104, wherein the cell is *in vivo* or *ex vivo*.

106. The method of any one of claims 67-101, wherein the contacting is performed in a population of cells.

107. The method of claim 1-6, wherein the population of cells are mammalian cells or human cells.

108. A method for treating a genetic condition in a subject, the method comprising: administering to the subject a fusion protein comprising a deaminase flanked by a N-terminal fragment and a C-terminal fragment of a Cas9 polypeptide or a

polynucleotide encoding the fusion protein, and a guide nucleic acid sequence or a polynucleotide encoding the guide nucleic acid sequence, wherein the guide nucleic acid sequence directs the fusion protein to deaminate a target nucleobase in a target polynucleotide sequence of the subject, thereby treating the genetic condition.

109. A method for treating a genetic condition in a subject, the method comprising: administering to the subject a fusion protein comprising a deaminase inserted within a flexible loop of a Cas9 polypeptide, wherein the fusion protein comprises the structure NH_2-[N-terminal fragment of a Cas9]-[deaminase]-[C-terminal fragment of a Cas9]-COOH, wherein each instance of "]-[" is an optional linker, wherein the deaminase of the fusion protein deaminates the target nucleobase in the target polynucleotide sequence of the subject, thereby treating the genetic condition.

110. The method of claim 108 or 109, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment comprises a part of a flexible loop of the Cas9 polypeptide.

111. The method of any one of claims 108-110, further comprising administering to the subject a guide nucleic acid sequence to effect deamination of the target nucleobase.

112. The method of any one of claims 108-111, wherein the target nucleobase comprises a mutation associated with the genetic condition.

113. The method of claim 112, wherein the deamination of the target nucleobase replaces the target nucleobase with a wild type nucleobase.

114. The method of claim 112, wherein the deamination of the target nucleobase replaces the target nucleobase with a non-wild type nucleobase, and wherein the deamination of the target nucleobase ameliorates symptoms of the genetic condition.

115. The method of any one of claims 108-111, wherein the target polynucleotide sequence comprises a mutation associated with the genetic condition at a nucleobase other than the target nucleobase.

116. The method of claim 115, wherein the deamination of the target nucleobase ameliorates symptoms of the genetic condition.

117. The method of any one of claims 108-116, wherein the target nucleobase is 1-20 nucleobases away from a PAM sequence in the target polynucleotide sequence.

118. The method of claim 117, wherein the target nucleobase is 2-12 nucleobases upstream of the PAM sequence.

119. The method of any one of claims 108-118, wherein the flexible loop comprises an amino acid in proximity to the target nucleobase when the deaminase of the fusion protein deaminates the target nucleobase.

120. The method of any one of claims 108-119, wherein the flexible loop comprises a region selected from the group consisting of amino acid residues at positions 530-537, 569-579, 686-691, 768-793, 943-947, 1002-1040, 1052-1077, 1232-1248, and 1298-1300 as numbered in SEQ ID NO: 1, or a corresponding region thereof.

121. The method of any one of claims 108-119, wherein the deaminase 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 SEQ ID NO: 1 or corresponding amino acid positions thereof.

122. The method of claim 121 wherein the deaminase is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

123. The method of any one of claims 108-119, wherein the deaminase is inserted between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

124. The method of any one of claims 108-119, wherein the N-terminal fragment comprises amino acid residues 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, and/or 1248-1297 of the Cas9 polypeptide as numbered in SEQ ID NO: 1, or corresponding residues thereof.

125. The method of any one of claims 108-119, wherein the C-terminal fragment comprises amino acid residues 1301-1368, 1248-1297, 1078-1231, 1026-1051, 948-1001, 692-942, 580-685, and/or 538-568 of the Cas9 polypeptide as numbered SEQ ID NO: 1, or corresponding residues thereof.

126. The method of any one of claims 108-119, wherein the N terminal fragment or the C terminal fragment of the Cas9 polypeptide binds the target polynucleotide sequence.

127. The method of any one of claims 108-119, wherein the N-terminal fragment or the C-terminal fragment comprises a RuvC domain.

128. The method of any one of claims 108-119, wherein the N-terminal fragment or the C-terminal fragment comprises a HNH domain.

129. The method of any one of claims 108-119, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a HNH domain.

130. The method of any one of claims 108-119, wherein neither of the N-terminal fragment and the C-terminal fragment comprises a RuvC domain.

131. The method of any one of claims 108-119, wherein the Cas9 polypeptide comprises a partial or complete deletion in one or more structural domains.

132. The method of claim 131, wherein the deaminase is inserted at the partial or complete deletion position of the Cas9 polypeptide.

133. The method of claim 131 or 132, wherein the deletion is within a RuvC domain.

134. The method of claim 131 or 132, wherein the deletion is within an HNH domain.

135. The method of 131 or 132, wherein the deletion bridges a RuvC domain and a C-terminal domain, a L-I domain and a HNH domain, or a RuvC domain and a L-I domain.

136. The method of claim 131 or 132, wherein the Cas9 polypeptide comprises a deletion of amino acids 1017-1069 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

137. The method of claim 131 or 132, wherein the Cas9 polypeptide comprises a deletion of amino acids 792-872 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

138. The method of claim 131 or 132, wherein the Cas9 polypeptide comprises a deletion of amino acids 792-906 as numbered in SEQ ID NO: 1 or corresponding amino acids thereof.

139. The method of any one of claims 108-138, wherein the deaminase is a cytidine deaminase.

140. The method of any one of claims 108-138, wherein the deaminase is an adenosine deaminase.

141. The method of any one of claims 108-140, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered PAM.

142. The method of any one of claims 108-141, wherein the Cas9 polypeptide is a nickase.

143. The method of any one of claims 108-142, wherein the Cas9 polypeptide is nuclease inactive.

144. The method of any one of claims 108-143, wherein the subject is a mammal.

145. The method of any one of claim 108-144, wherein the subject is a human.

146. A protein library for optimized base editing comprising a plurality of fusion proteins, wherein each one of the plurality of fusion proteins comprises a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide, wherein the N-terminal fragment of each one of the fusion proteins differs from the N-terminal fragments of the rest of the plurality of fusion proteins or wherein the C-terminal fragment of each one of the fusion proteins differs from the C-terminal fragments of the rest of the plurality of fusion proteins, wherein the deaminase of each one of the fusion proteins deaminates a target nucleobase in proximity to a Protospacer Adjacent Motif (PAM) sequence in a target polynucleotide sequence, and wherein the N terminal fragment or the C terminal fragment binds the target polynucleotide sequence.

147. The protein library of claim 146, wherein for each nucleobase from 1 to 20 nucleobases away of the PAM sequence, at least one of the plurality of fusion proteins deaminates the nucleobase.

148. The protein library of claim 147, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment of the Cas9 polypeptide of each one of the plurality of fusion proteins comprises a part of a flexible loop of the Cas9 polypeptide.

149. The protein library of any one of claims 146-148, wherein at least one of the plurality of fusion proteins deaminates the target nucleobase with lower off-target deamination as compared to an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of SEQ ID NO: 1.

150. The protein library of anyone of claims 146-149, wherein at least one of the plurality of the fusion proteins deaminates a target nucleobase 2-12 nucleobases upstream of the PAM sequence.

151. The protein library of any one of claims 146-150, wherein the C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment of a fusion protein of the plurality comprises an amino acid in proximity to the target nucleobase when the fusion protein deaminates the target nucleobase.

152. The protein library of any one of claims 146-150, wherein the deaminase of at least one of the fusion proteins is 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 SEQ ID NO: 1 or corresponding amino acid positions thereof.

153. The protein library of claim 152, wherein the deaminase of at least one of the fusion proteins is between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1040-1041, 1068-1069, or 1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

154. The method of any one of claims 146-150, wherein the deaminase of at least one of the fusion proteins is between amino acid positions 1016-1017, 1023-1024, 1029-1030, 1040-1041, 1069-1070 or1247-1248 as numbered in SEQ ID NO: 1 or corresponding amino acid positions thereof.

155. The protein library of any one of claims 146-154, wherein the deaminase is an adenosine deaminase.

156. The protein library of any one of claims 146-154, wherein the deaminase is a cytidine deaminase.

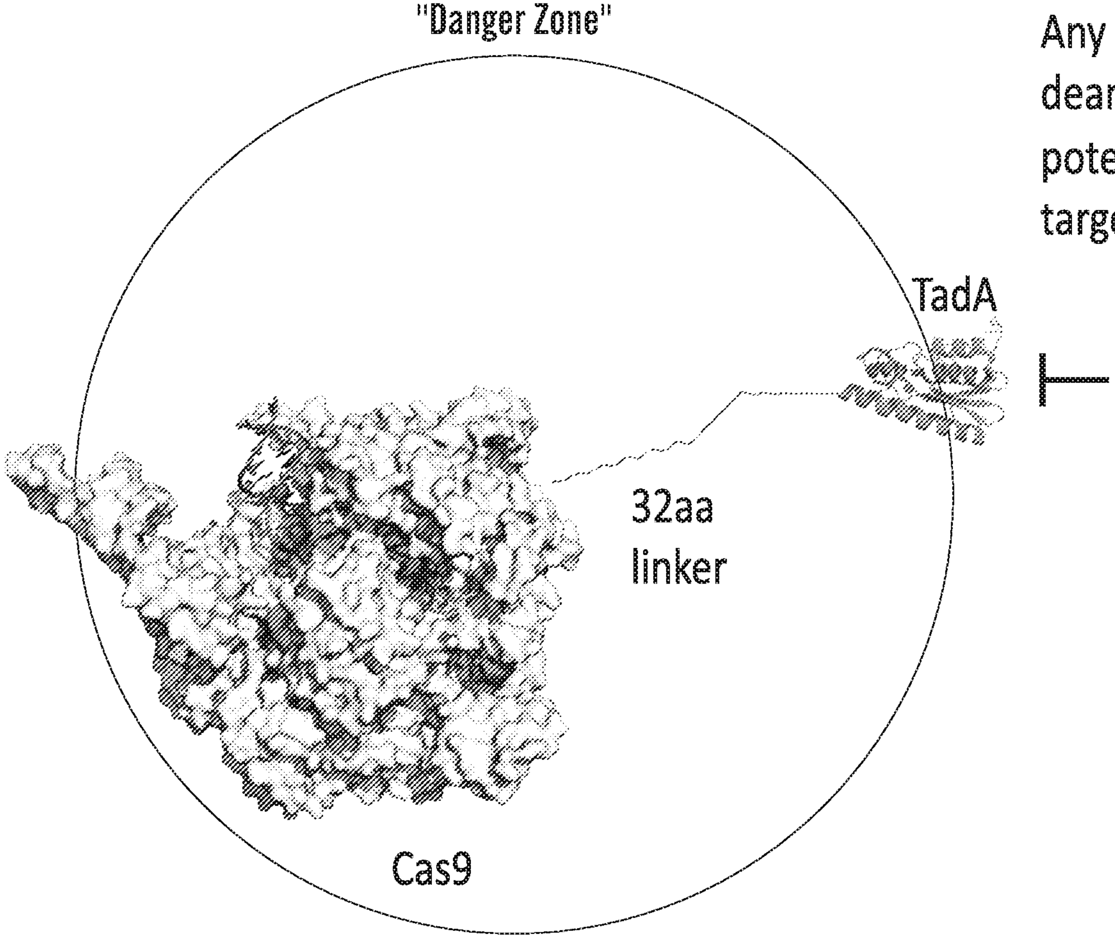
157. The protein library of any one of claims 146-156, wherein the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), or variants thereof.

158. The protein library of any one of claims 146-157, wherein the Cas9 polypeptide is a modified Cas9 and has specificity for an altered protospacer-adjacent motif (PAM).

159. The protein library of any one of claims 146-157, wherein the Cas9 polypeptide is a nickase.

160. The protein library of any one of claims 146-157, wherein the Cas9 polypeptide is nuclease inactive.

Structural basis for bystander mutagenesis



Any ssDNA within deamination zone is a potential spurious off-target.

<u>Possible Strategies</u>

- Lock deaminase into preferred orientation.
- Inactivate deaminase until it is near desired mutation spot.
- Activate enzyme only when in proximity of ssDNA.

FIG. 1

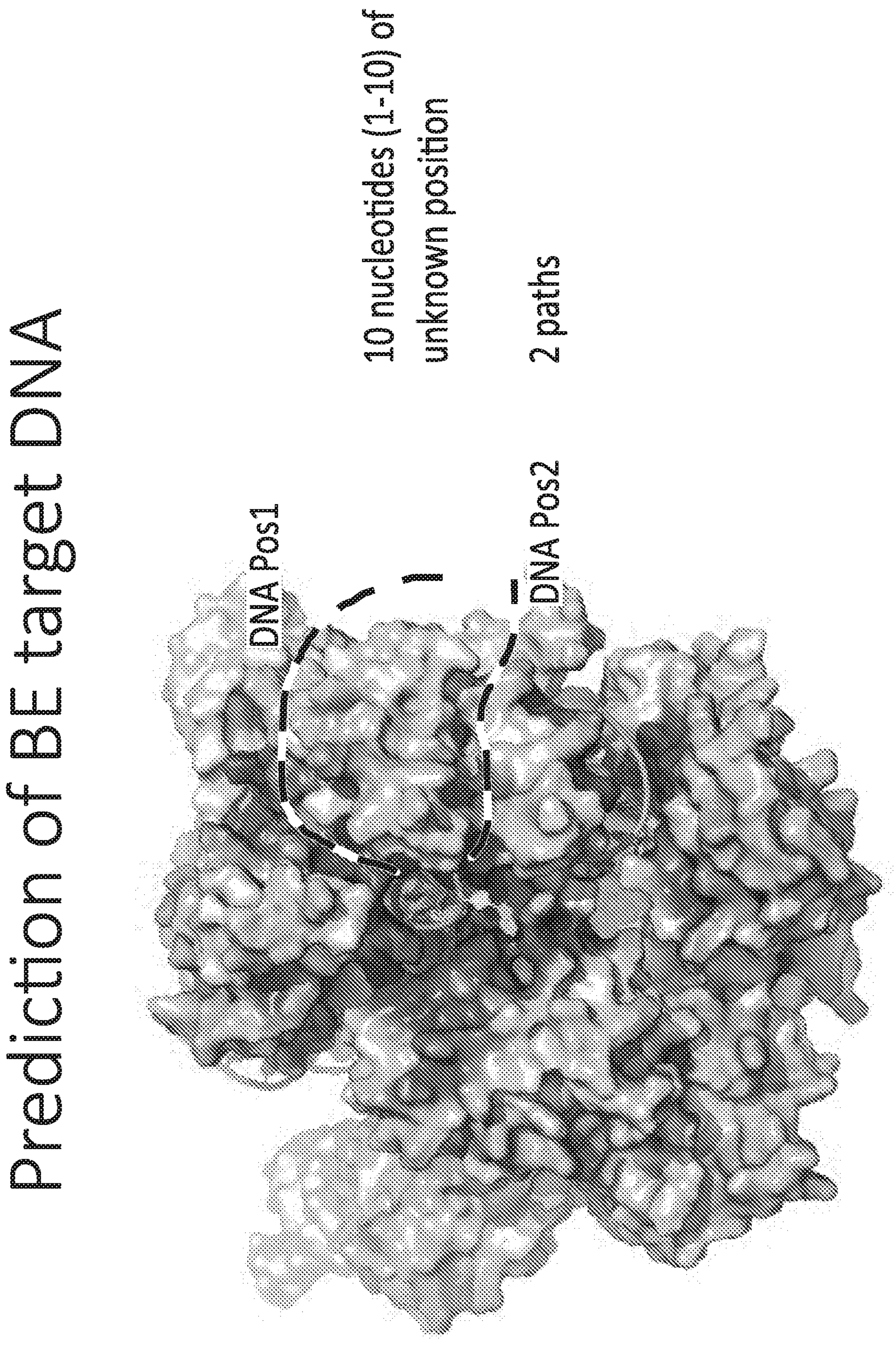

Prediction of BE target DNA
10 nucleotides (1-10) of
unknown position
2 paths
DNA Pos1
DNA Pos2

FIG. 2

Engineer preferred orientation

- Position deaminase in proximity to BE target DNA by insertion into Cas9
- Linker conformation

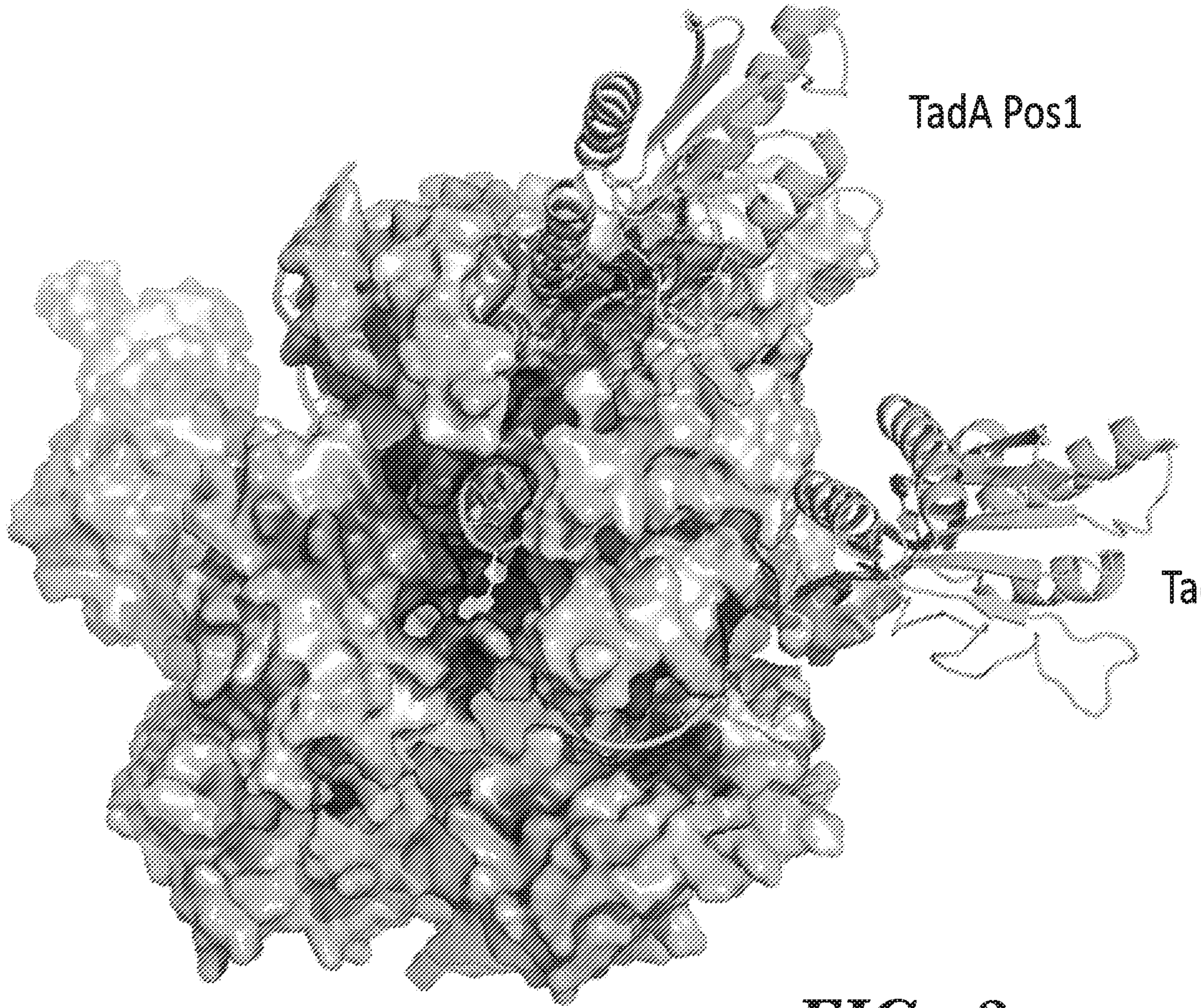


FIG. 3

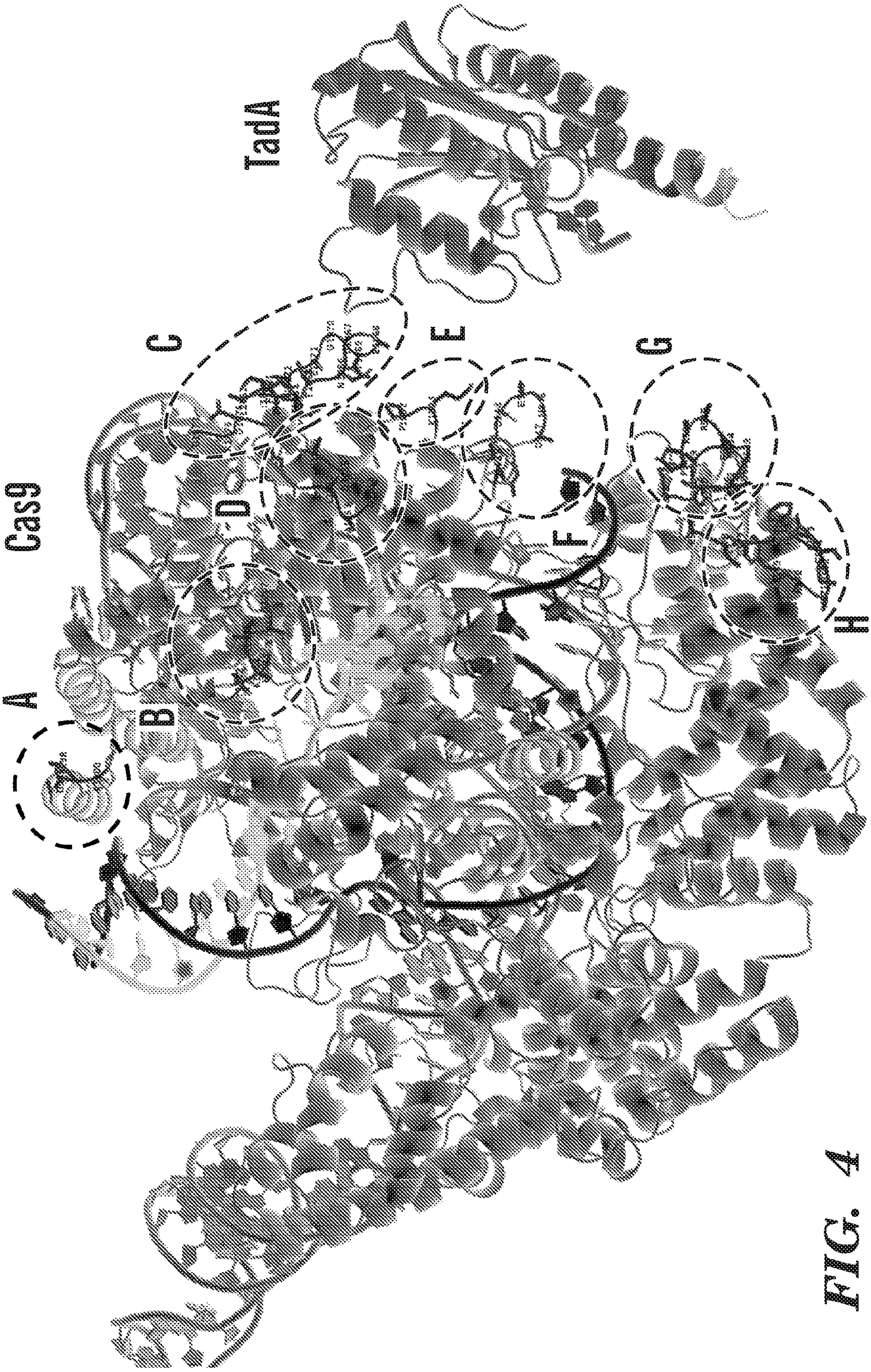


FIG. 4

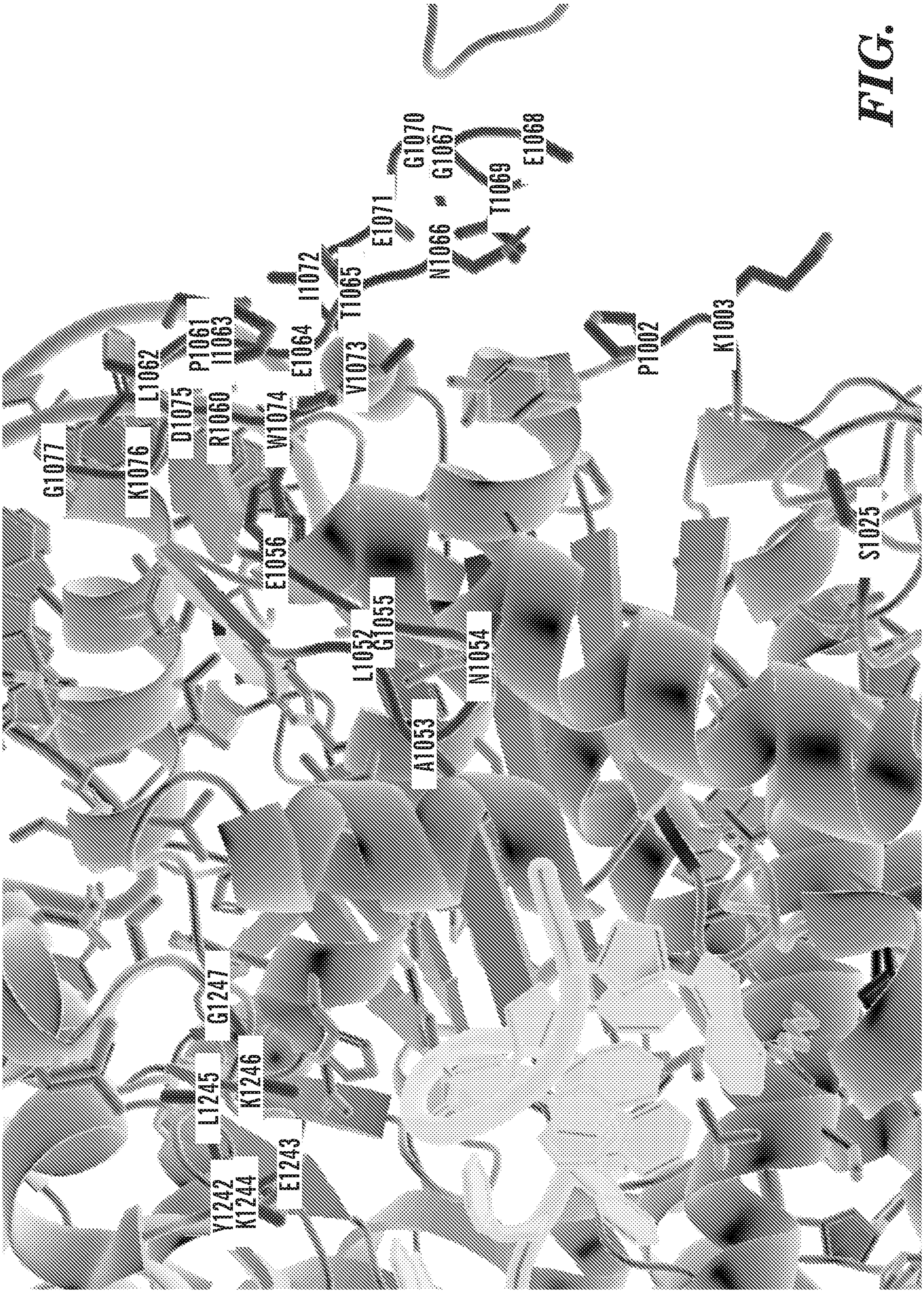
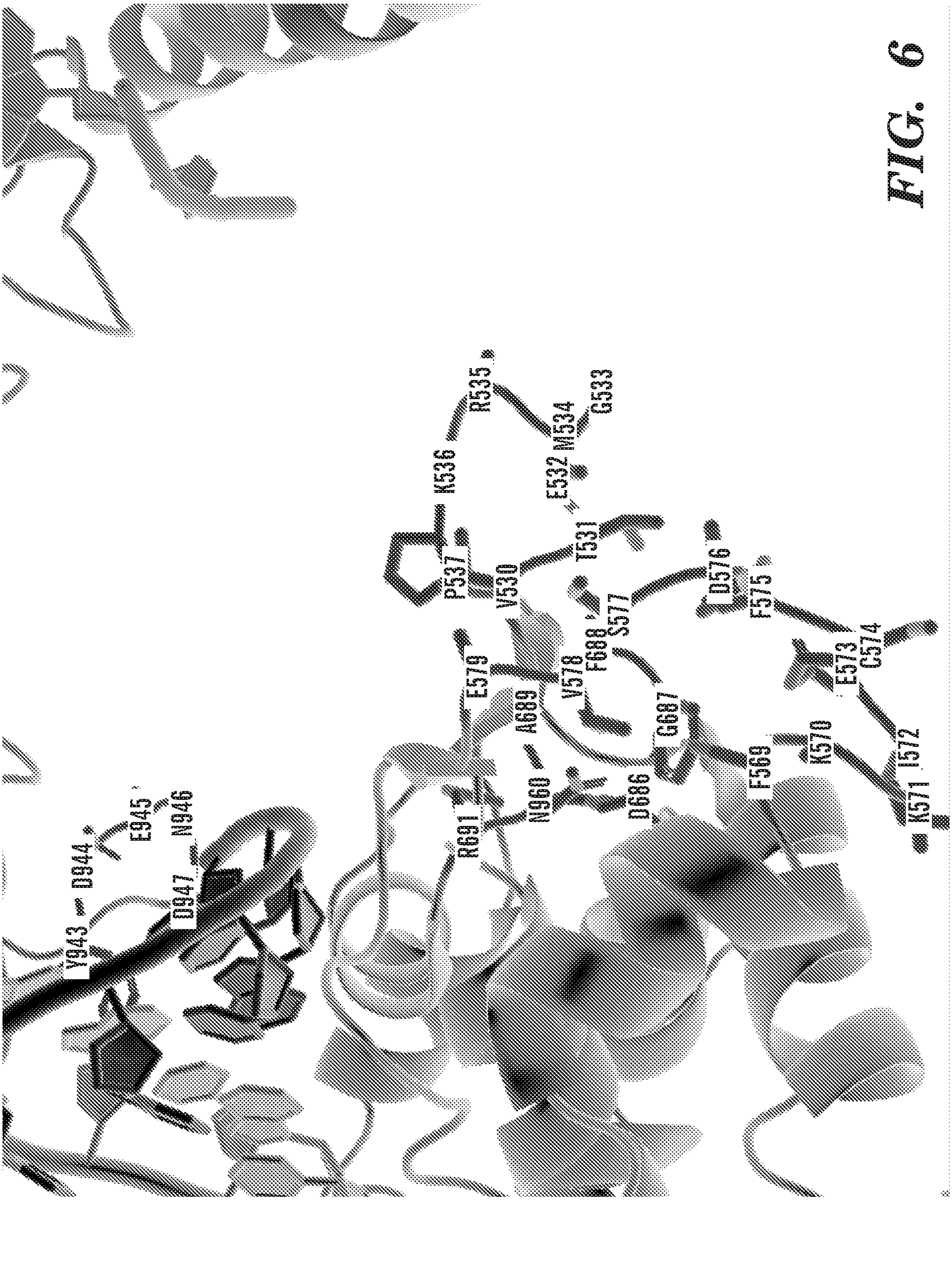

G1070
E1068
G1067
T1069
E1071
N1066
I1072
T1065
P1061
I1063
L1062
E1064
V1073
P1002
K1003
D1075
R1060
W1074
G1077
K1076
E1056
S1025
L1052
G1055
N1054
A1053
G1247
L1245
K1246
E1243
Y1242
K1244

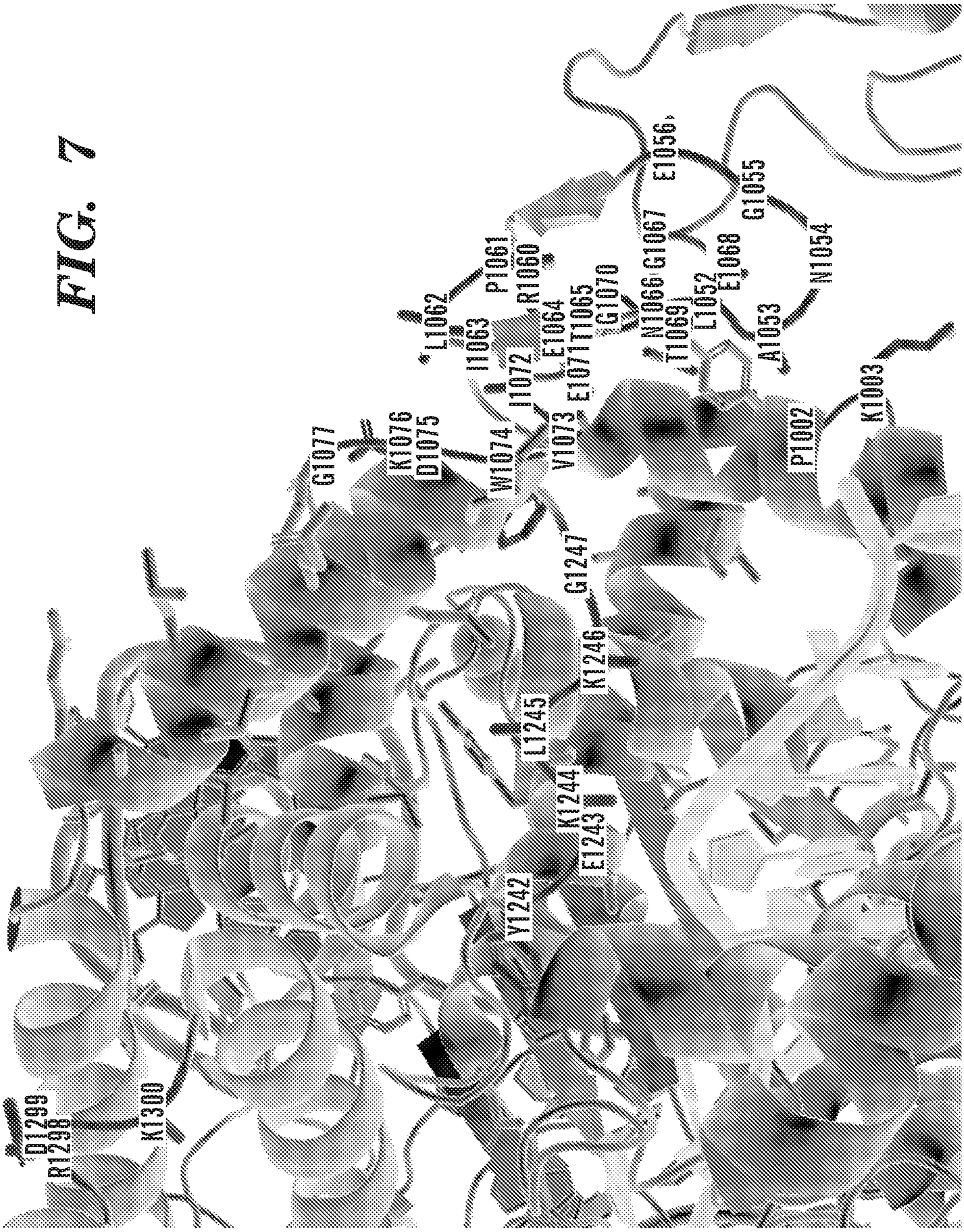
FIG. 5



Y943
D944
E945
N946
D947
R691
N960
D686
F569
K571
I572
K570
E573
C574
F575
D576
S577
F688
G687
A689
V578
E579
V530
P537
K536
T531
E532
M534
G533
R535

FIG. 6

FIG. 7


E1056
G1055
N1054
G1067
E1068
A1053
L1052
G1070
T1069
N1066
T1065
E1071
E1064
R1060
P1061
L1062
I1063
I1072
K1003
P1002
V1073
W1074
D1075
K1076
G1077
G1247
K1246
L1245
K1244
E1243
Y1242
D1299
R1298
K1300

A, B, C, D, E

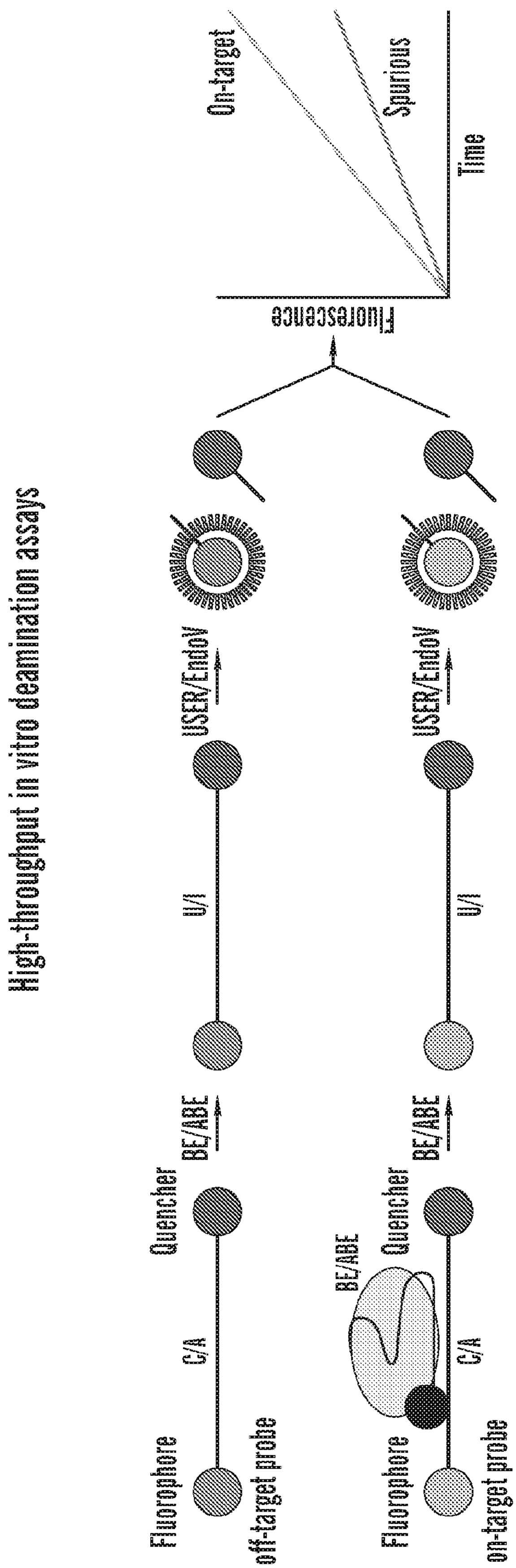

High-throughput in vitro deamination assays
Fluorophore
C/A
Quencher
off-target probe
BE/ABE
U/I
USER/EndoV
BE/ABE
Fluorophore
C/A
Quencher
on-target probe
BE/ABE
U/I
USER/EndoV
Fluorescence
Time
On-target
Spurious

FIG. 8

Fluorescence assay for off-targets.

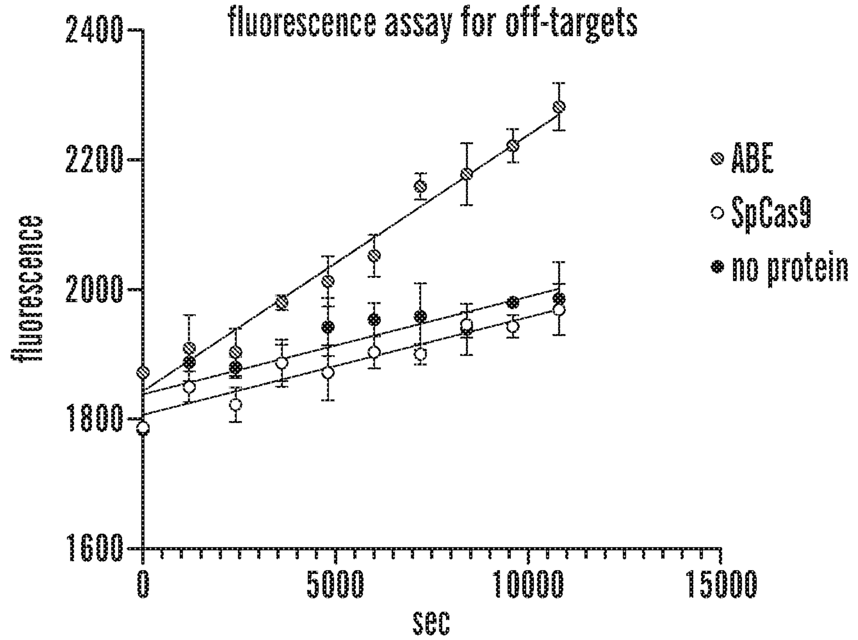


FIG. 9

Comparison of ABE v. ABE system with TadA in Trans.

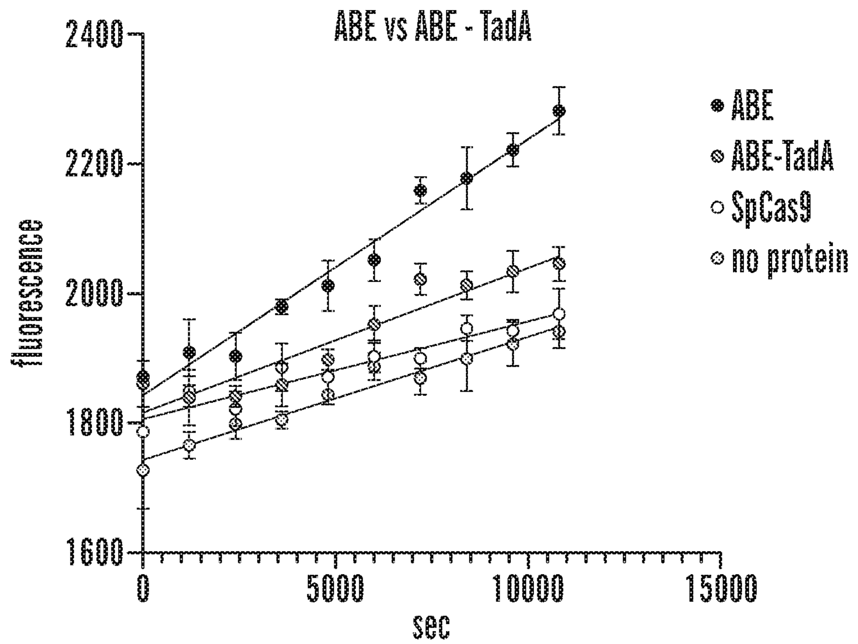


FIG. 10

Potential substrates for spurious off-target base editing can be tested.

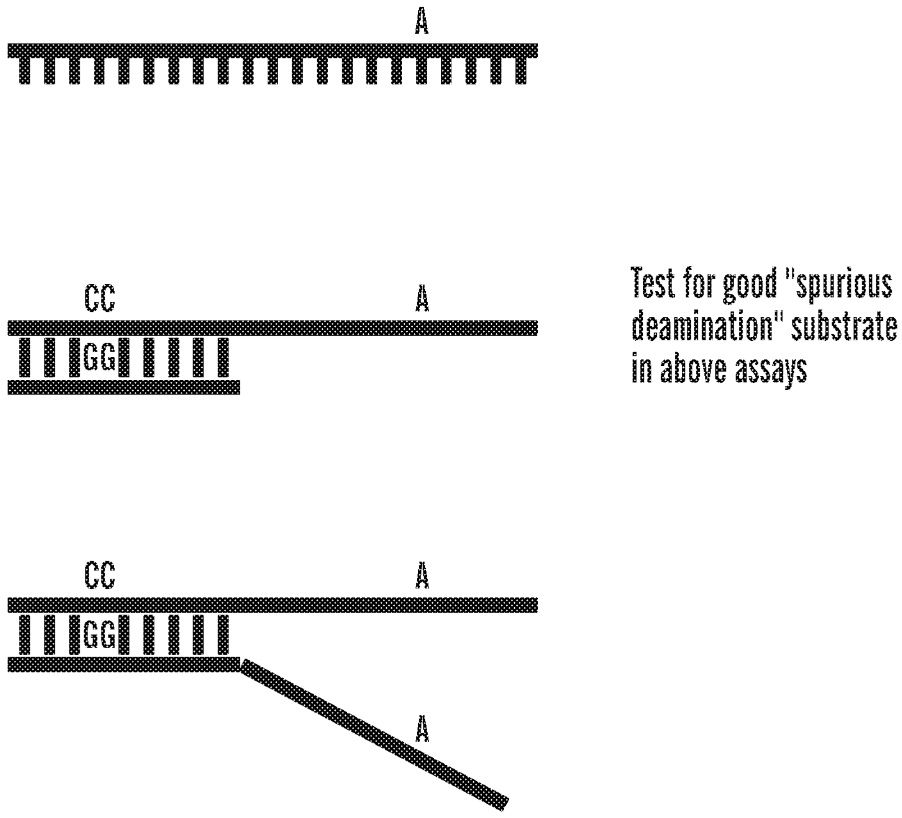


FIG. 11

Assay to evaluate the activities of deaminases in the *in cis* - *in trans* assay

Experimental design: evaluate base editing with various deaminases *in cis* (covalent, base editor context) vs. *in trans* (spurious deamination mimic)

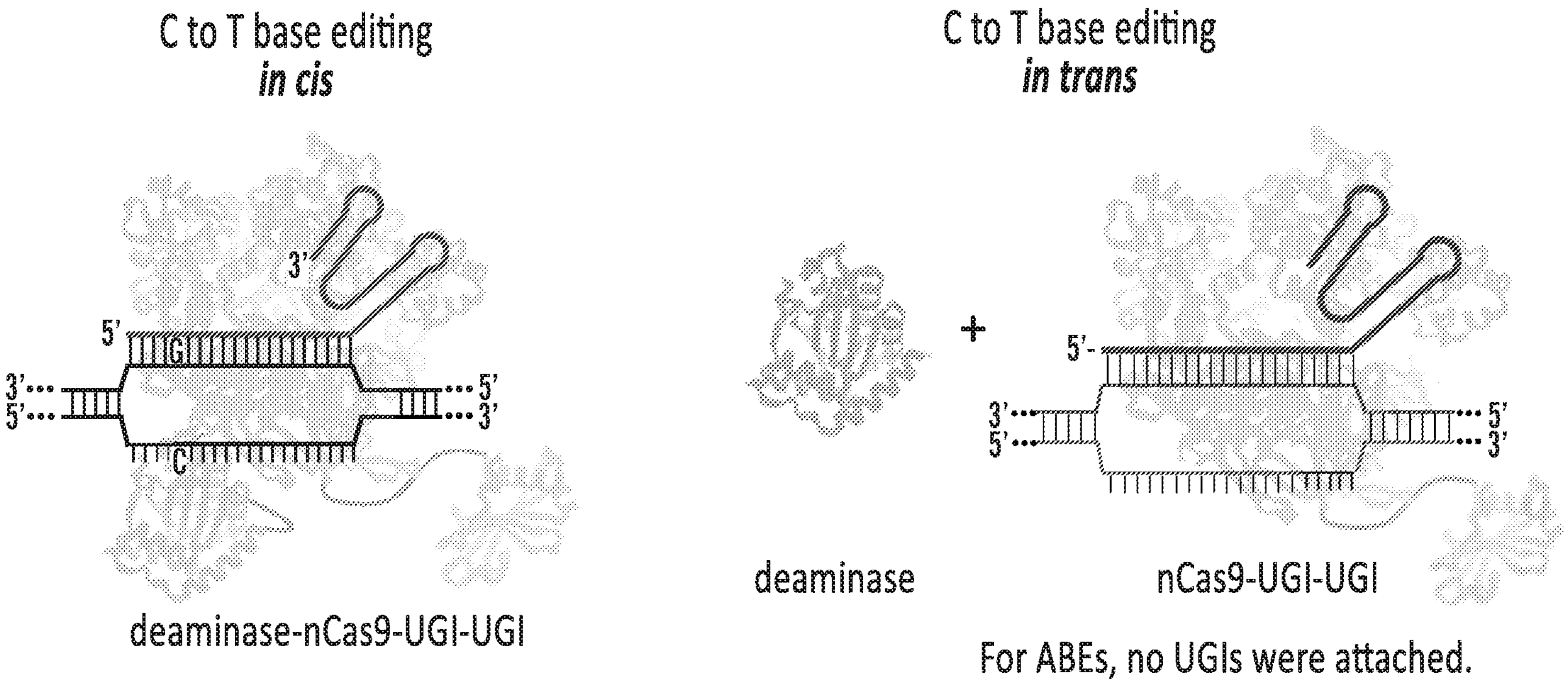


FIG. 12

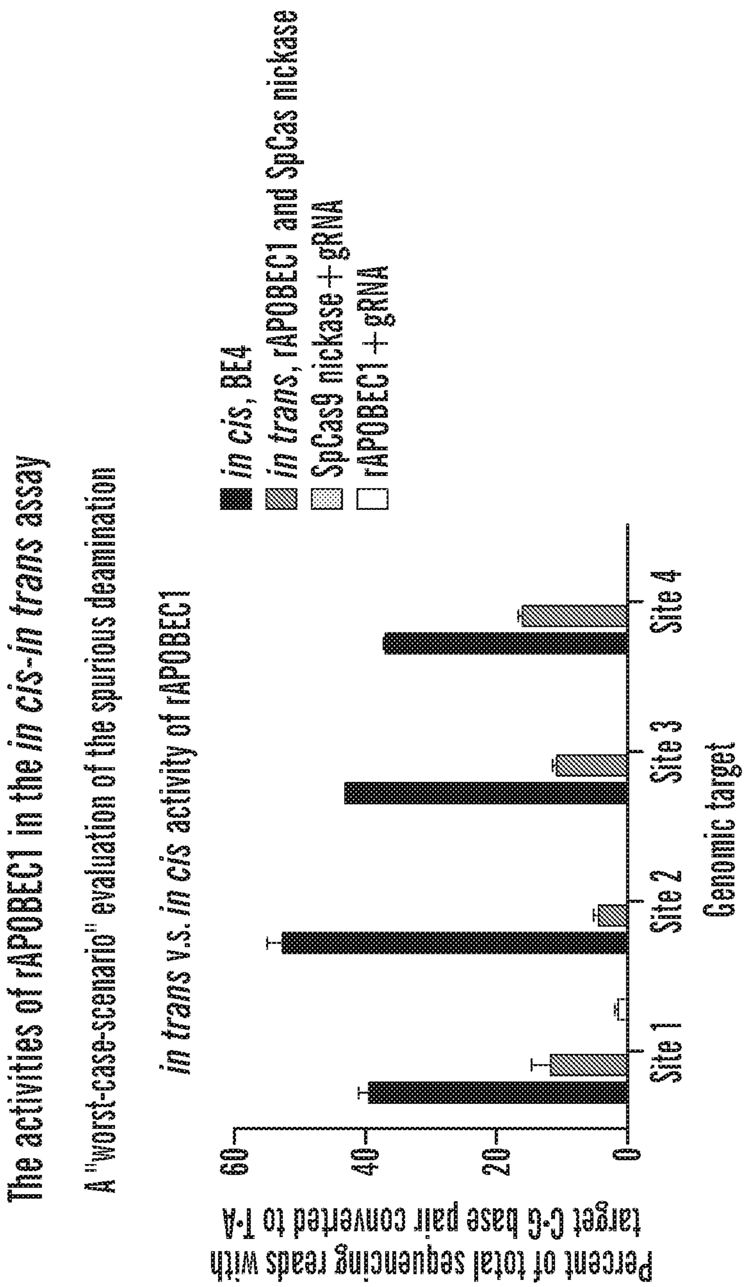
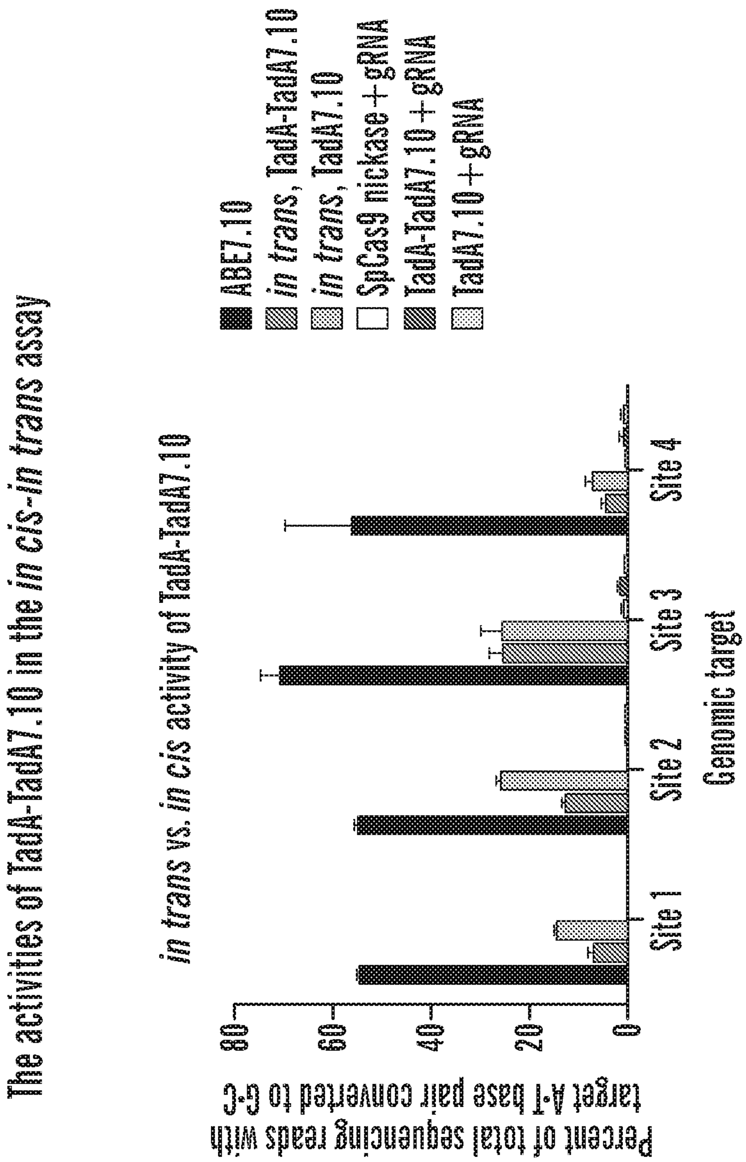

The activities of rAPOBEC1 in the *in cis-in trans* assay
A "worst-case-scenario" evaluation of the spurious deamination
in trans v.s. *in cis* activity of rAPOBEC1
Percent of total sequencing reads with target C·G base pair converted to T·A
60
40
20
0
Site 1
Site 2
Site 3
Site 4
Genomic target
in cis, BE4
in trans, rAPOBEC1 and SpCas nickase
SpCas9 nickase + gRNA
rAPOBEC1 + gRNA

FIG. 13



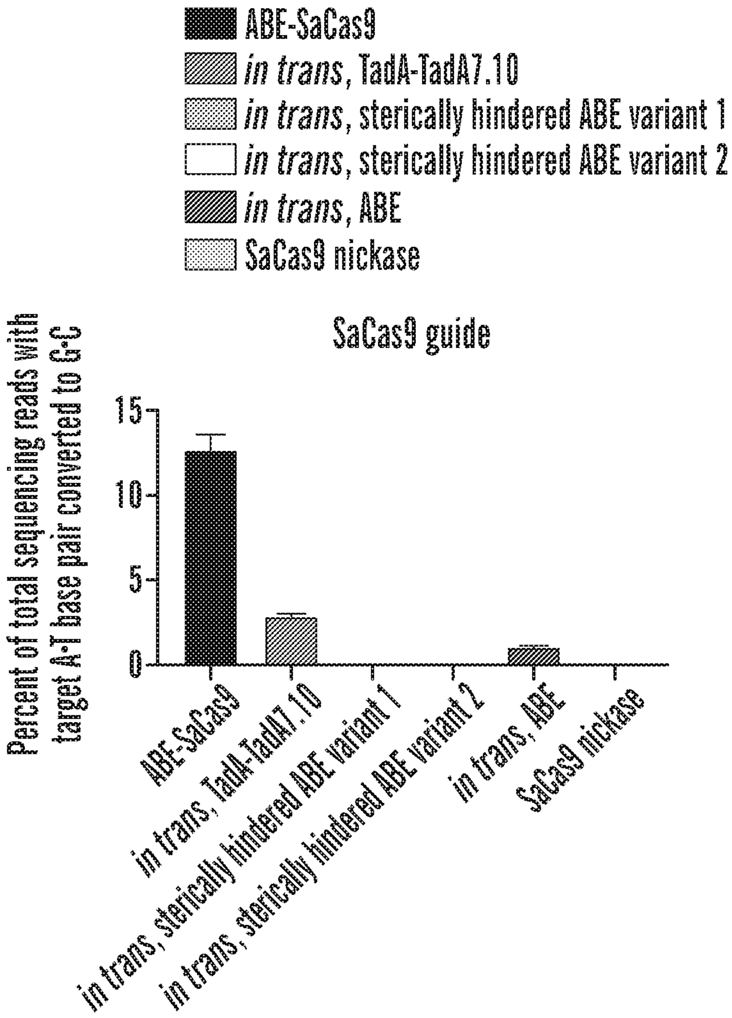
The activities of TadA-TadA7.10 in the in cis-in trans assay
in trans vs. in cis activity of TadA-TadA7.10
Percent of total sequencing reads with target A-T base pair converted to G-C
80
60
40
20
0
Site 1
Site 2
Site 3
Site 4
Genomic target
ABE7.10
in trans, TadA-TadA7.10
in trans, TadA7.10
SpCas9 nickase + gRNA
TadA-TadA7.10 + gRNA
TadA7.10 + gRNA

FIG. 14

Lower *in trans* activity observed for TadA-TadA7.10 in base editor context

in cis/in trans activity

sterically hindered ABE variant 1	sterically hindered ABE variant 2	ABE
111.76	146.35	72.45



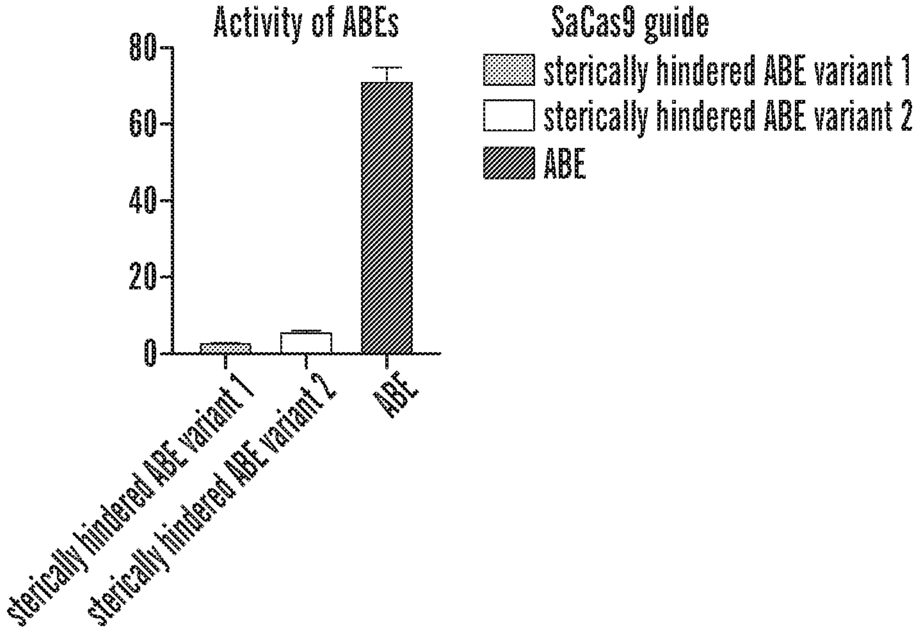


FIG. 15

Dose-response for the *in-cis* and *in-trans* activities

Titration of pmaxGFP plasmid with empty vector resulted in decreased expression level of GFP

Titration of pmaxGFP plasmid used in transfection

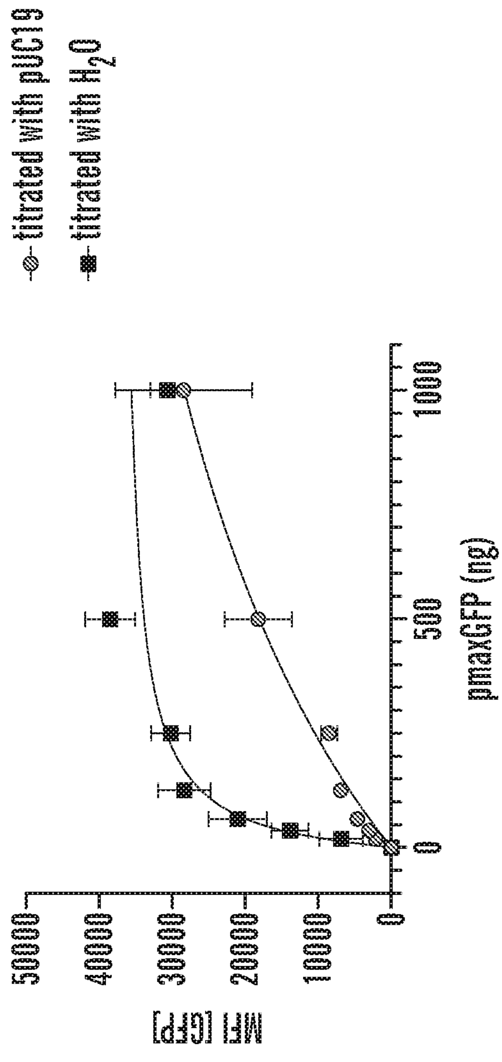


FIG. 16

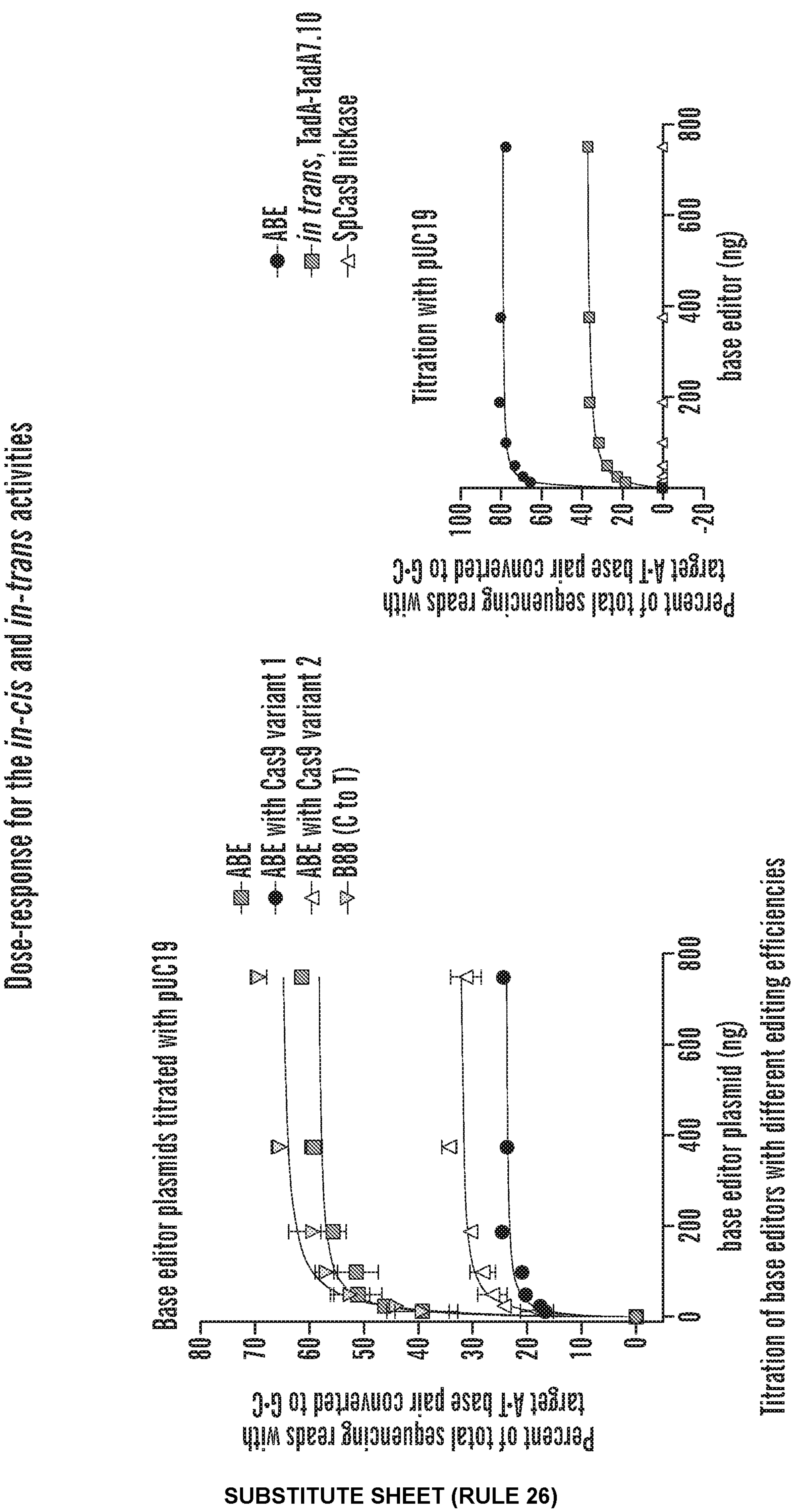
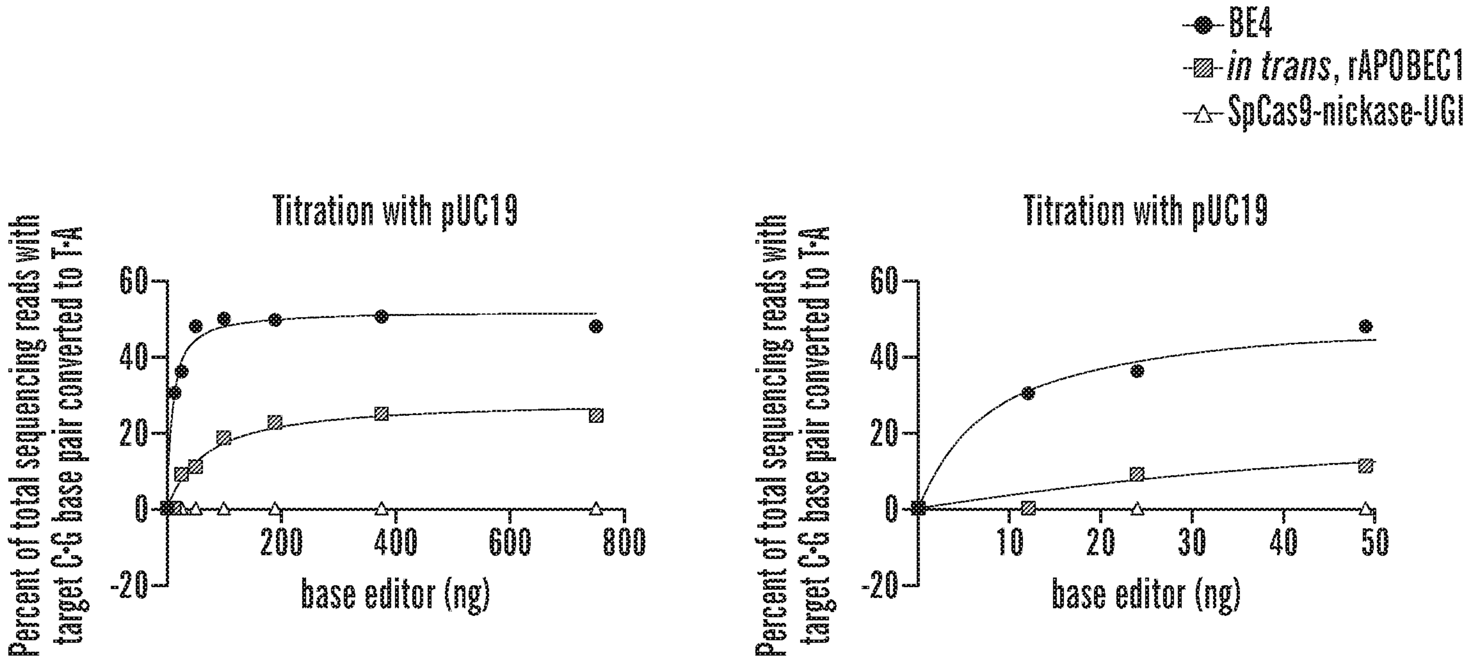

Dose-response for the *in-cis* and *in-trans* activities
Base editor plasmids titrated with pUC19
Percent of total sequencing reads with target A•T base pair converted to G•C
base editor plasmid (ng)
ABE
ABE with Cas9 variant 1
ABE with Cas9 variant 2
B88 (C to T)
Titration of base editors with different editing efficiencies
Titration with pUC19
Percent of total sequencing reads with target A•T base pair converted to G•C
base editor (ng)
ABE
in trans, TadA-TadA7.10
SpCas9 nickase

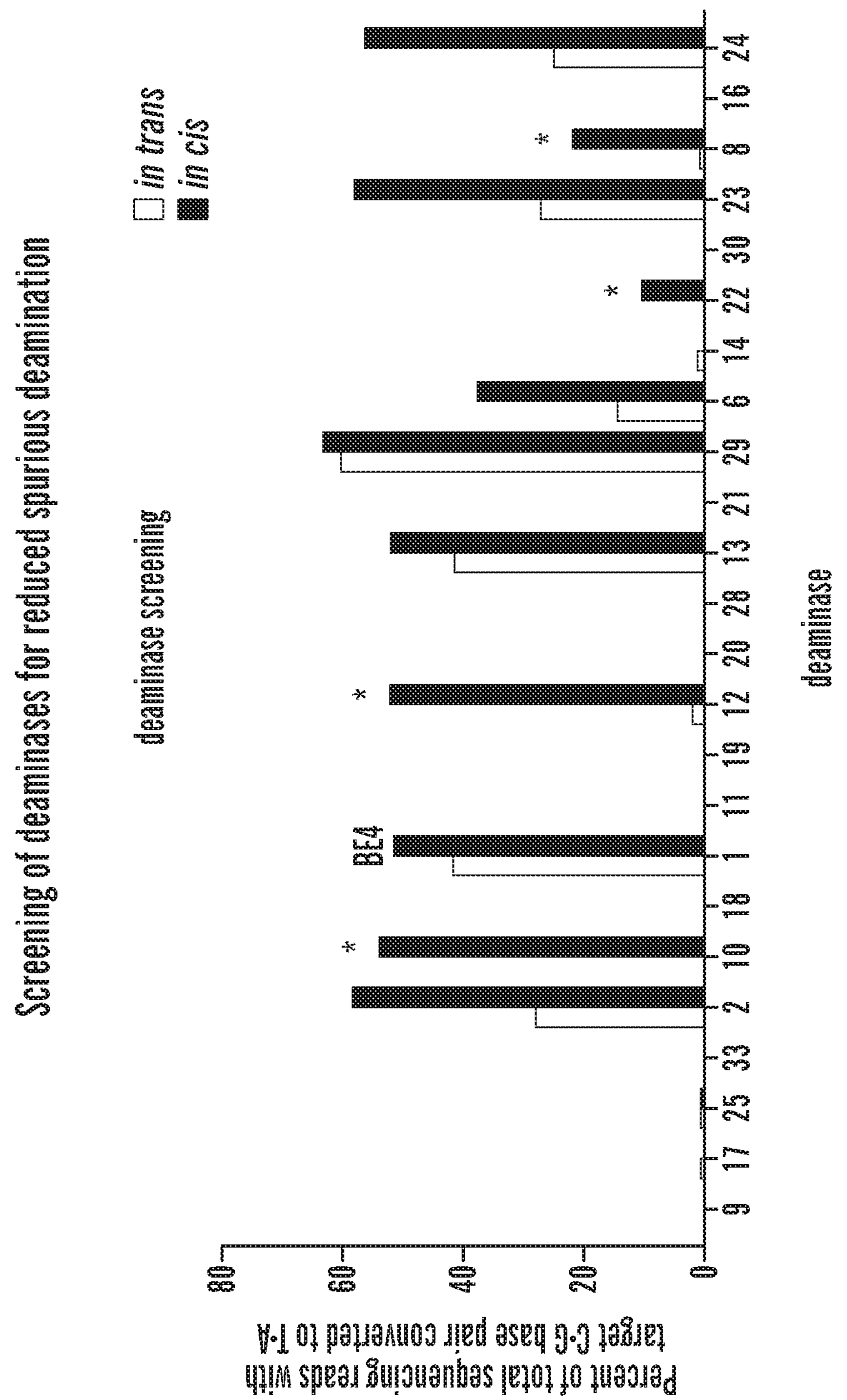
FIG. 17

Dose-response for the *in-cis* and *in-trans* activities



A careful exam of the curve at low concentration is being performed.

FIG. 18



Screening of deaminases for reduced spurious deamination
deaminase screening
in trans
in cis
Percent of total sequencing reads with target C·G base pair converted to T·A
0
20
40
60
80
9 17 25 33 2 10 18 1 11 19 12 20 28 13 21 29 6 14 22 30 23 8 16 24
deaminase
BE4
*

FIG. 19

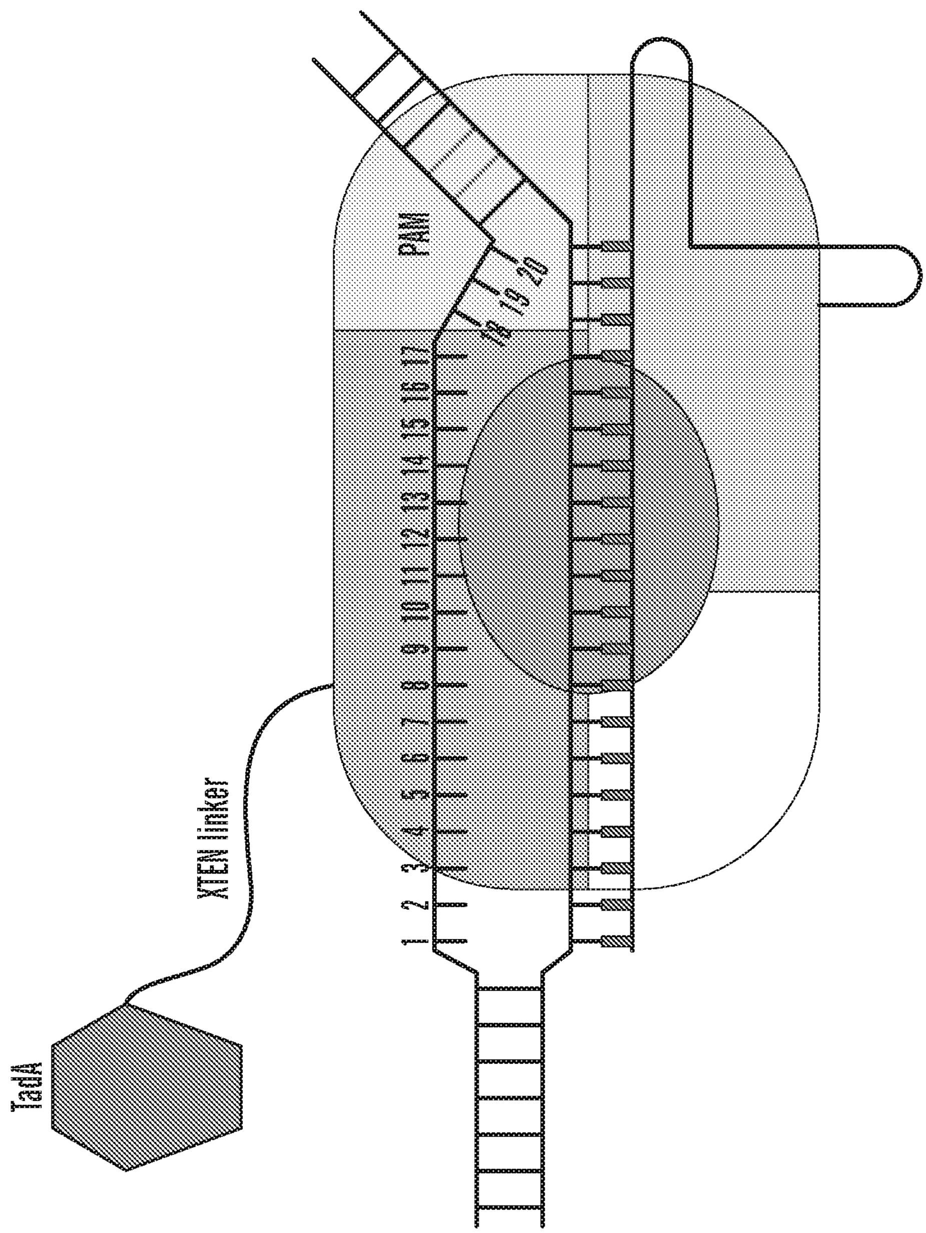


FIG. 20A

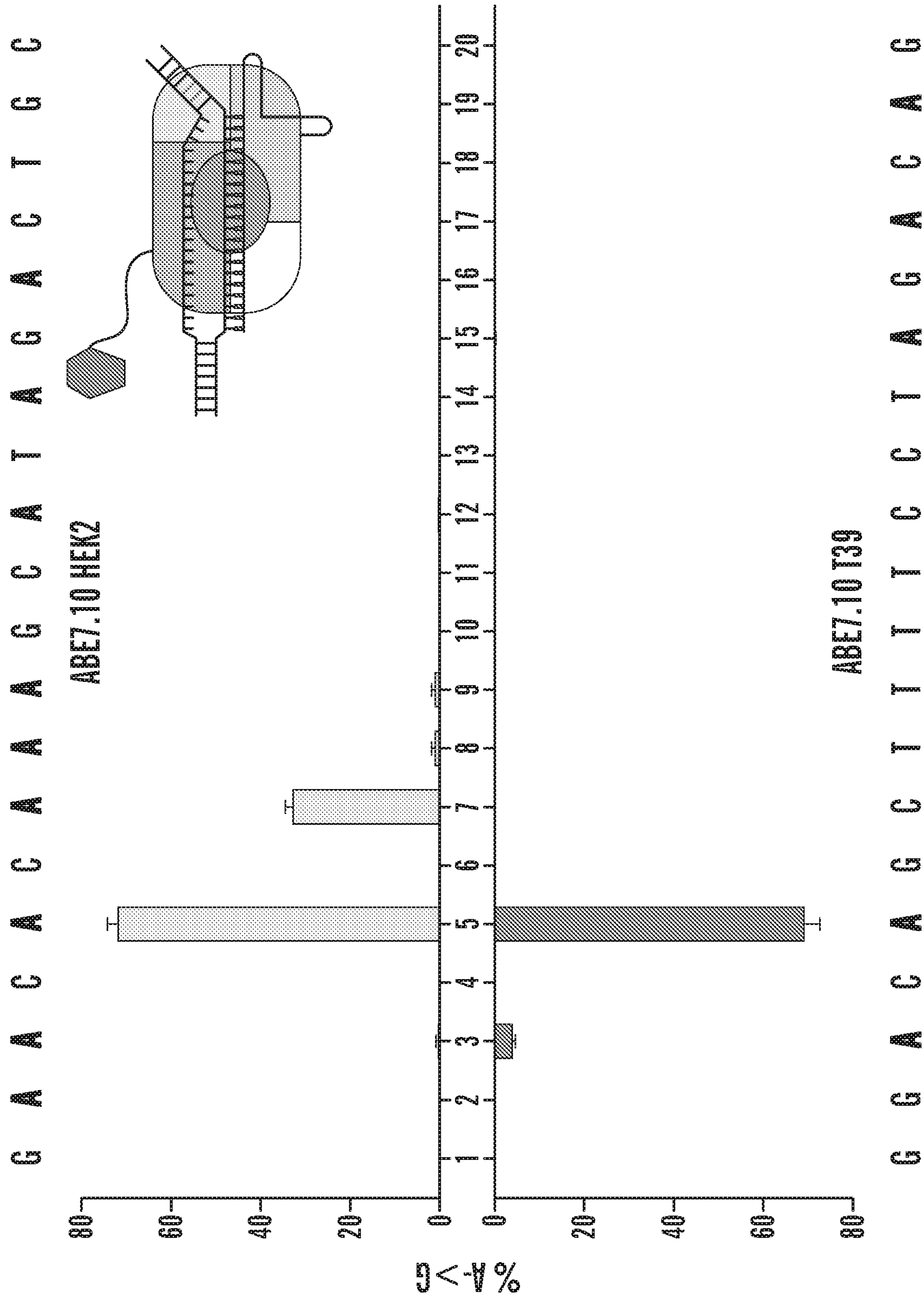

G A A C A C A A A G C A T A G A C T G C
ABE7.10 HEK2
ABE7.10 T39
G G A C A G C T T T T C C T A G A C A G
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
80 60 40 20 0
0 20 40 60 80
%A->G

FIG. 20B

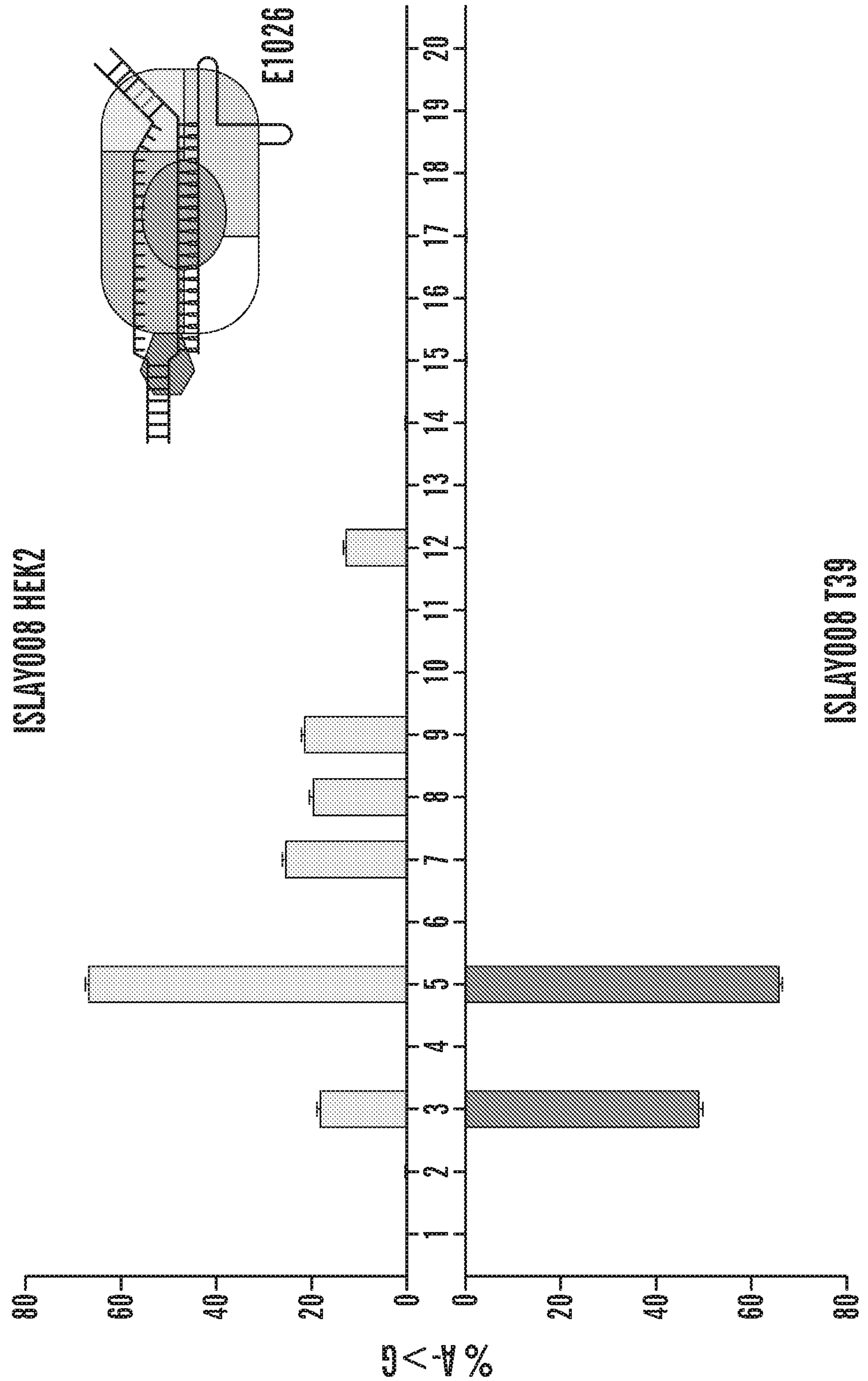

ISLAY008 HEK2
ISLAY008 T39
E1026
%A->G
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20C

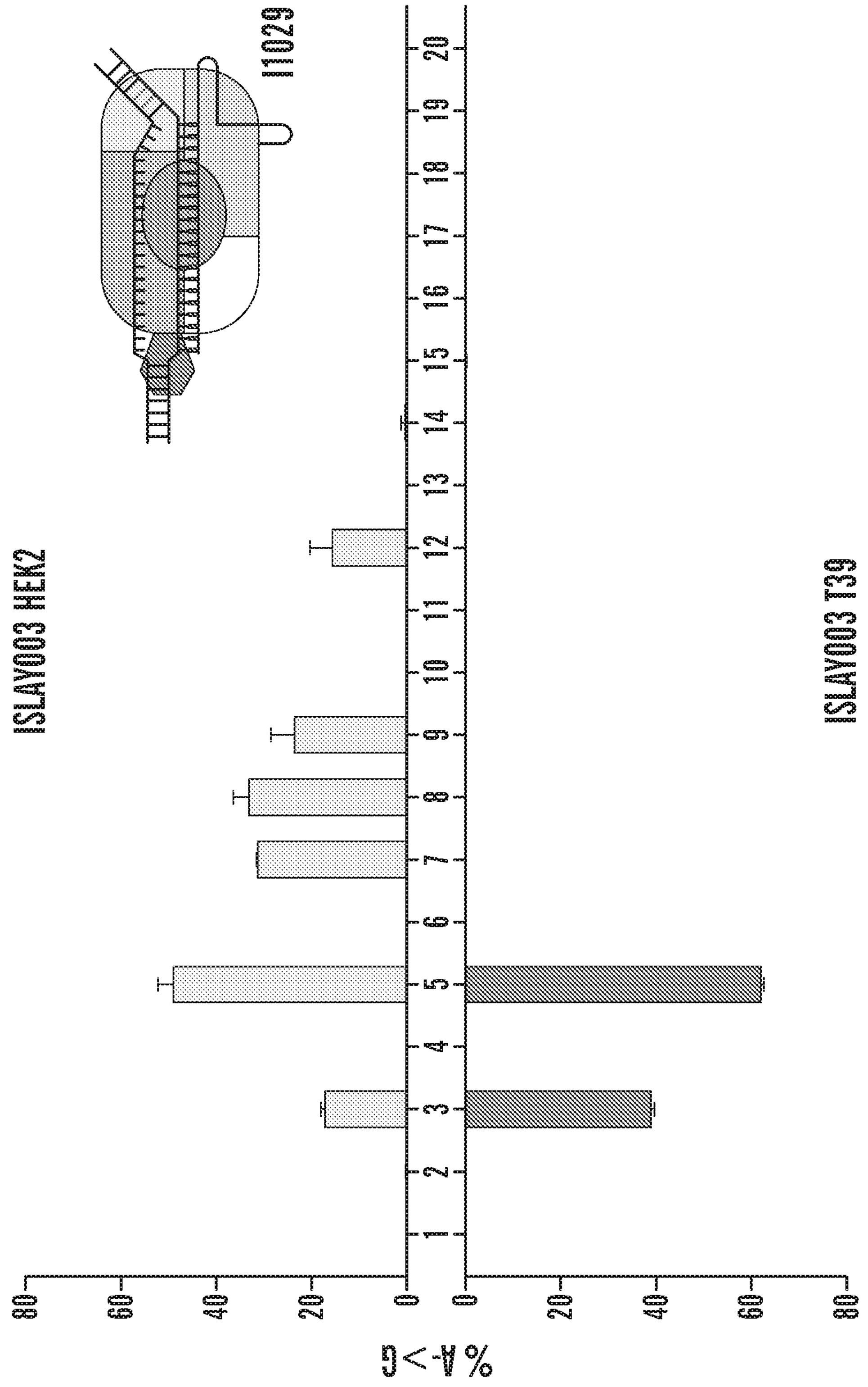

ISLAV003 HEK2
ISLAV003 T39
I1029
% A->G
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20D

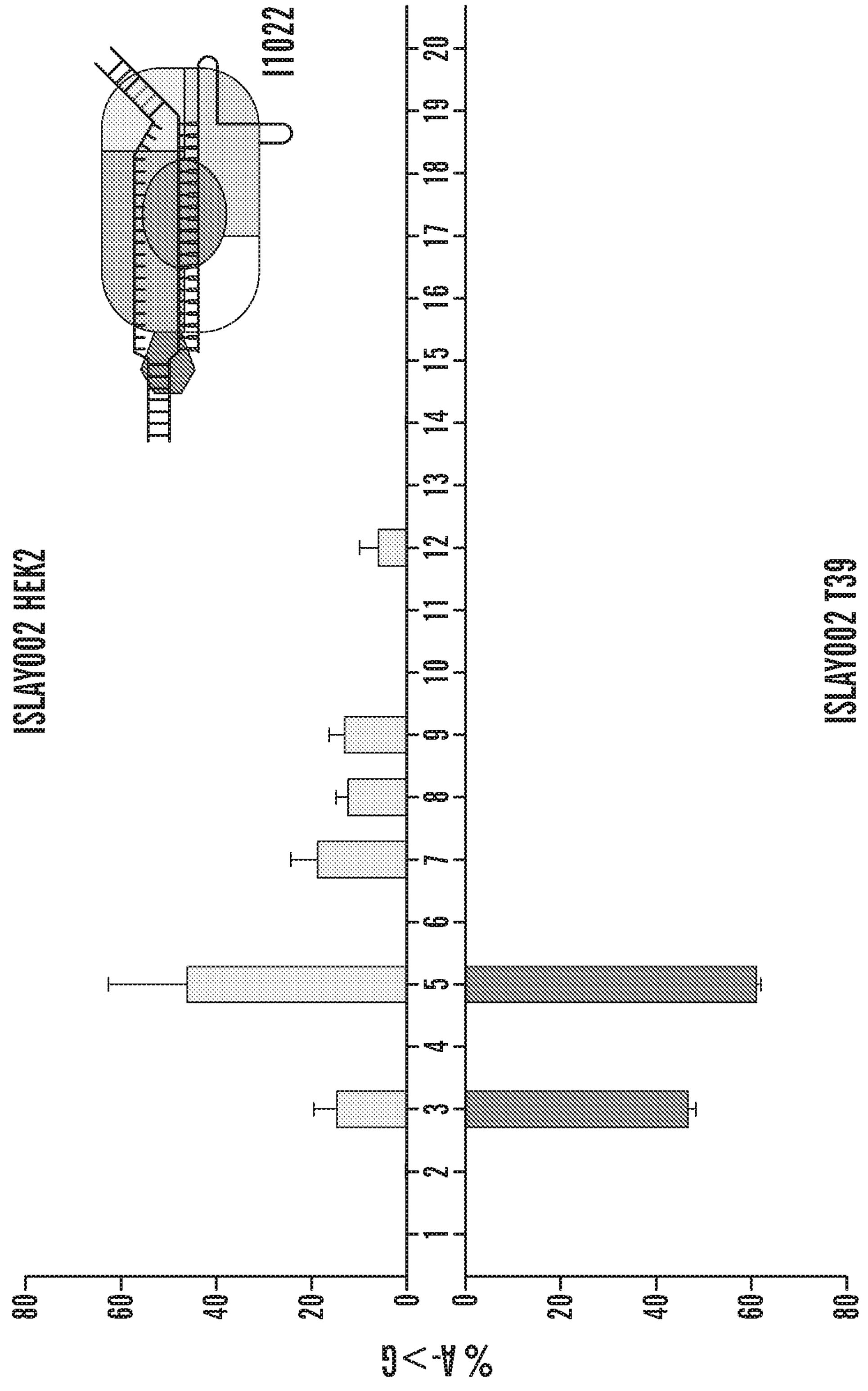

ISLAV002 HEK2
I1022
ISLAV002 T39
%A->G
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20E

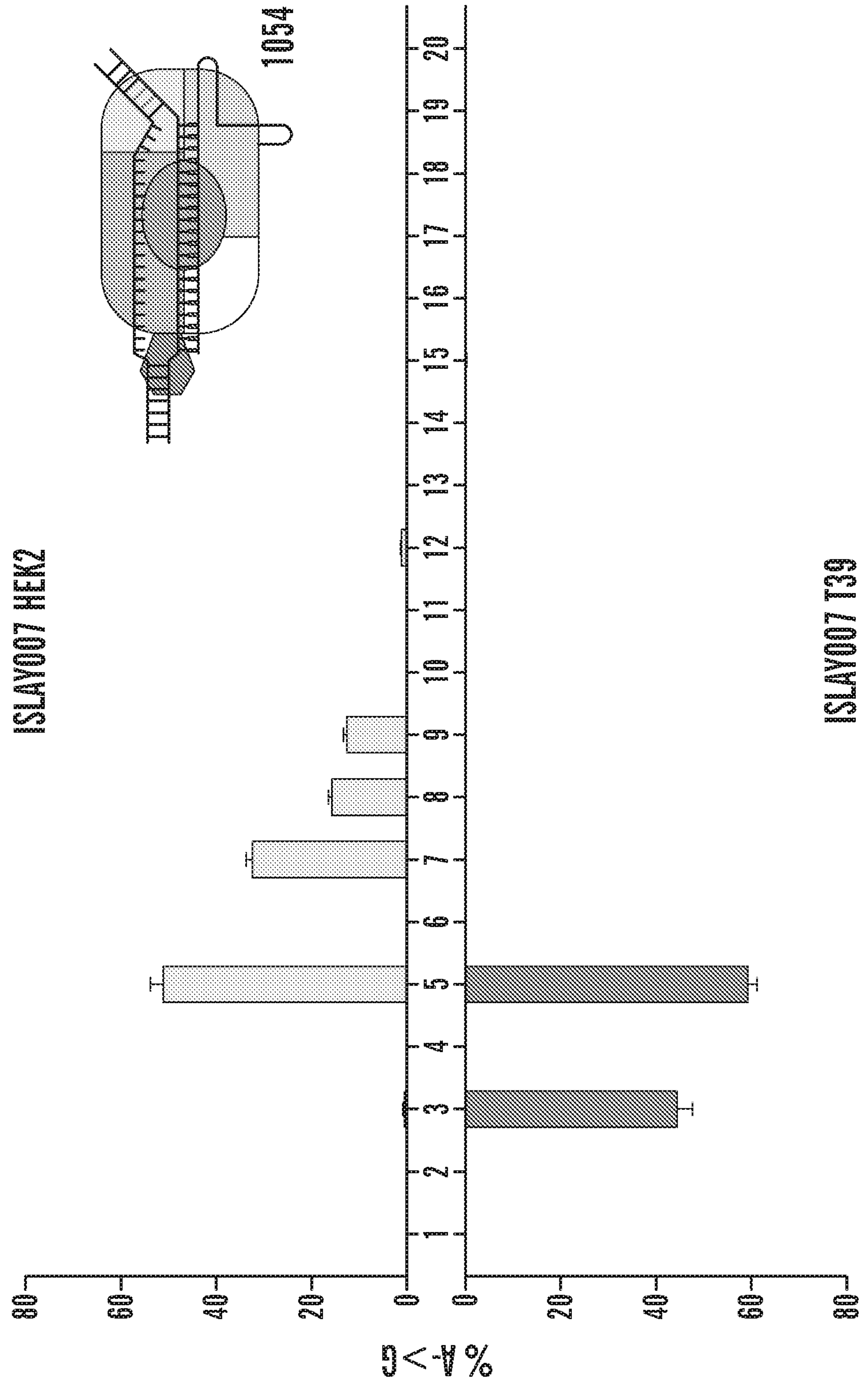

ISLAY007 HEK2
ISLAY007 T39
1054
%A->G
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 20F

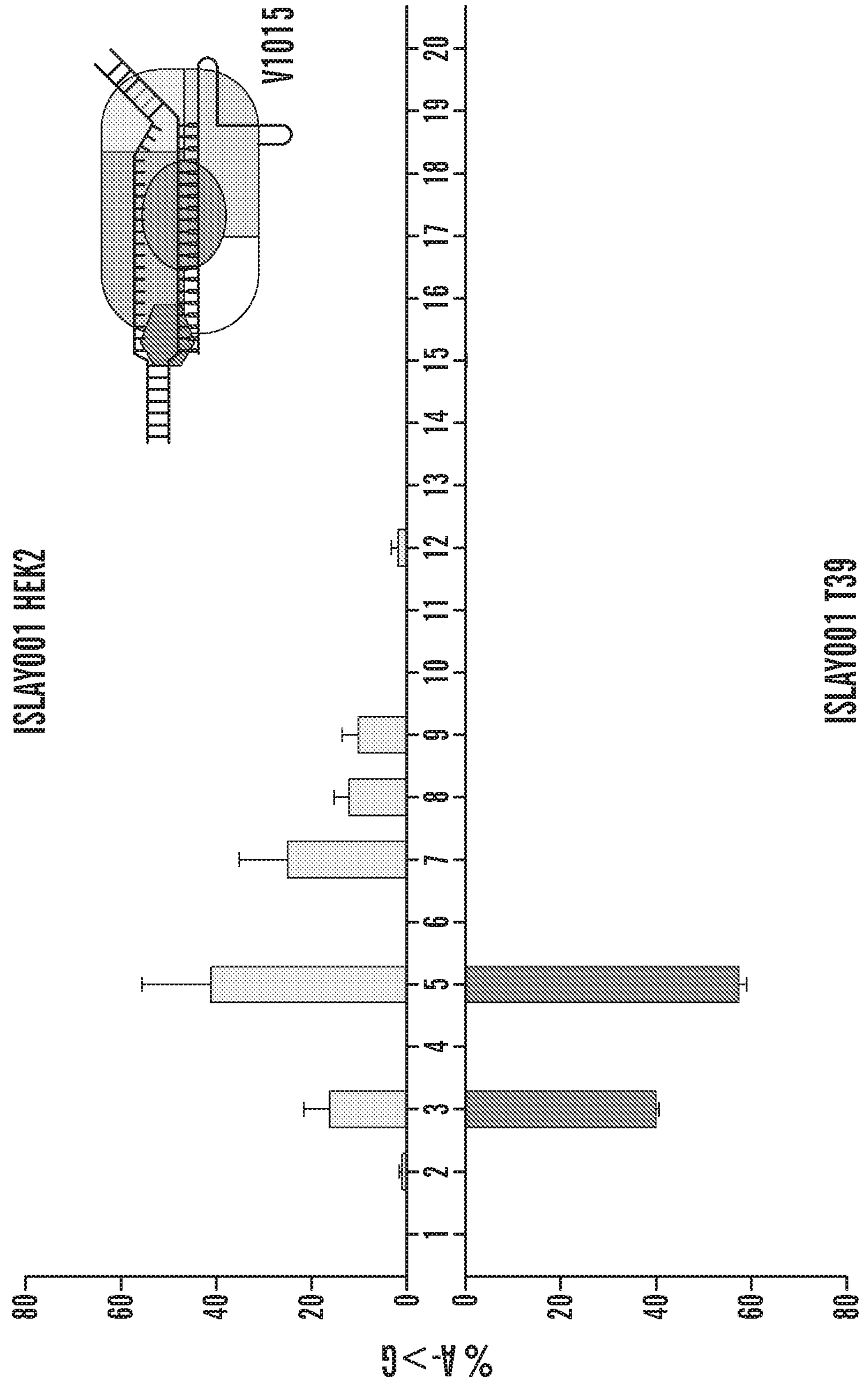

ISLAV001 HEK2
V1015
ISLAV001 T39
% A->G
80
60
40
20
0
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20G

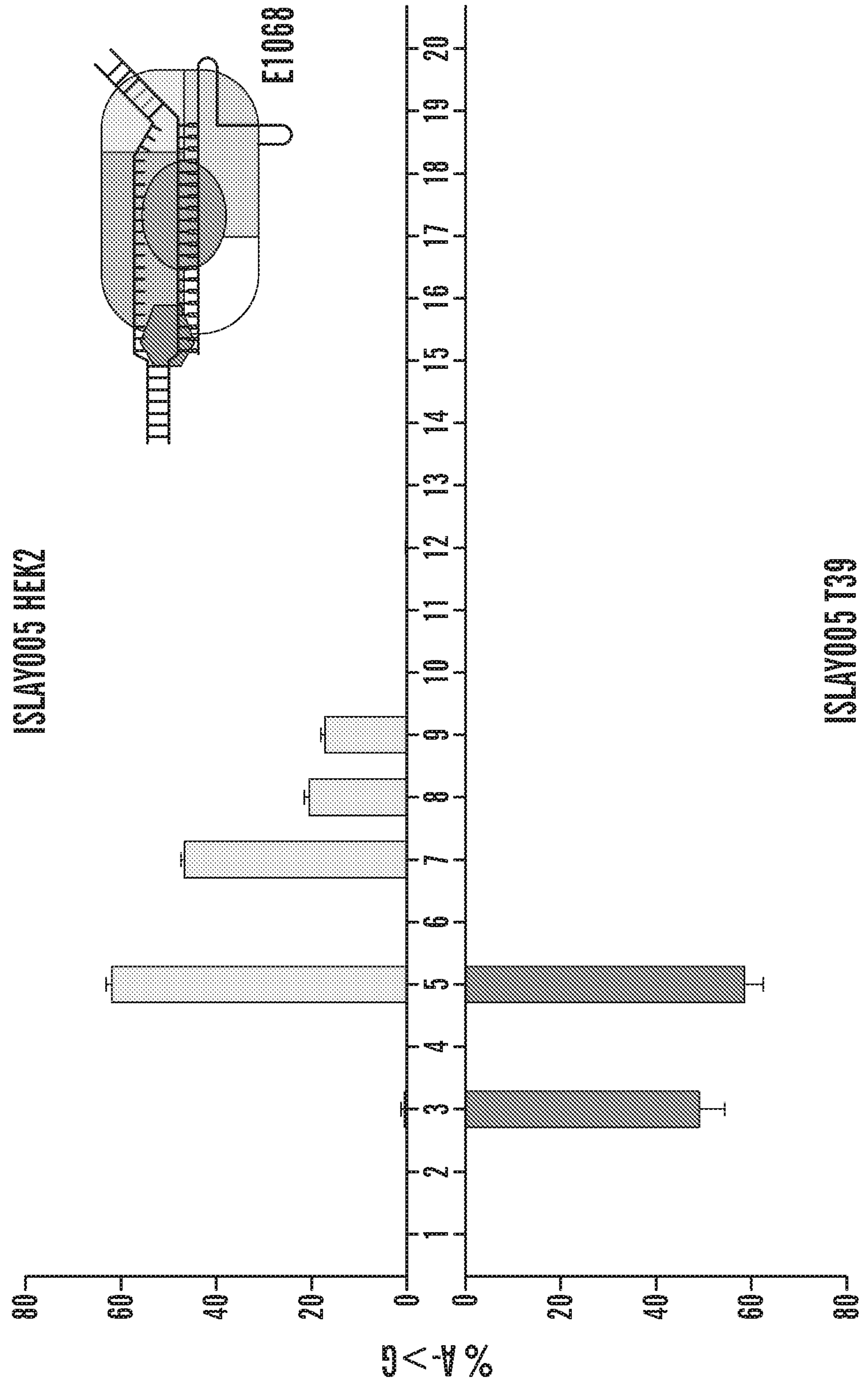

ISLAY005 HEK2
E1068
ISLAY005 T39
%A->G
80
60
40
20
0
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20H

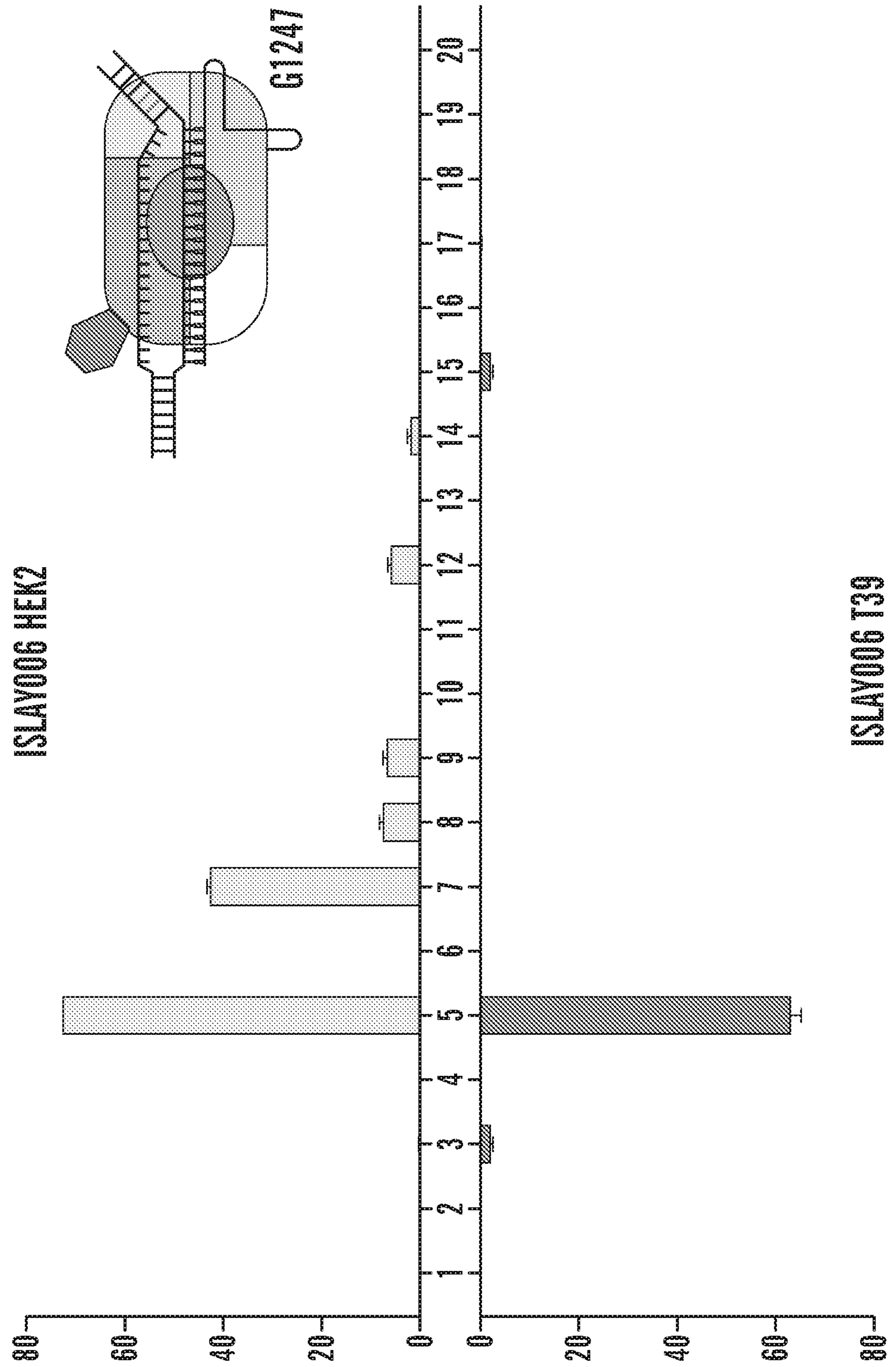

ISLAY006 HEK2
ISLAY006 T39
G1247
%A->G
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 20I

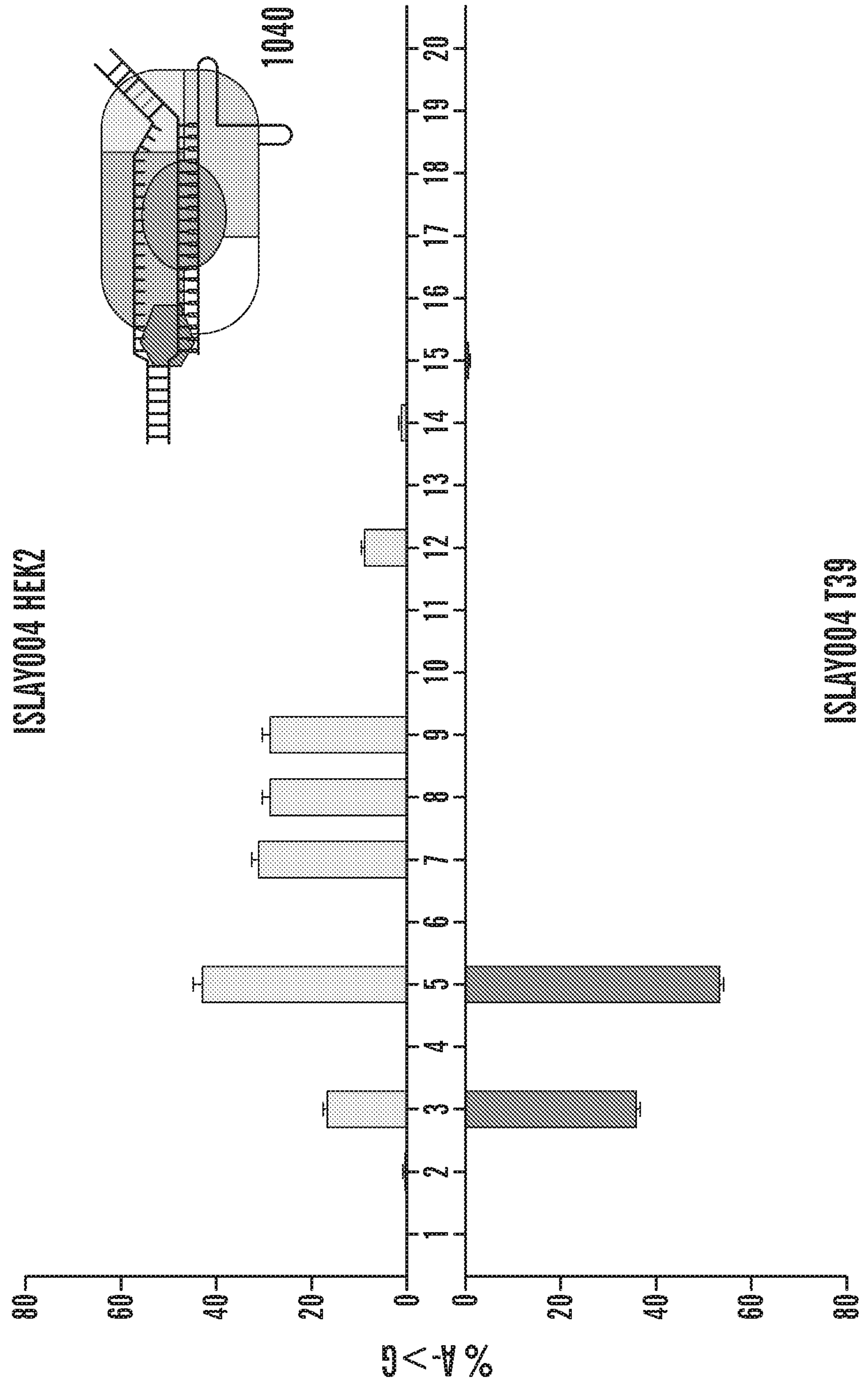

ISLAY004 HEK2
ISLAY004 T39
1040
%A->G
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20J

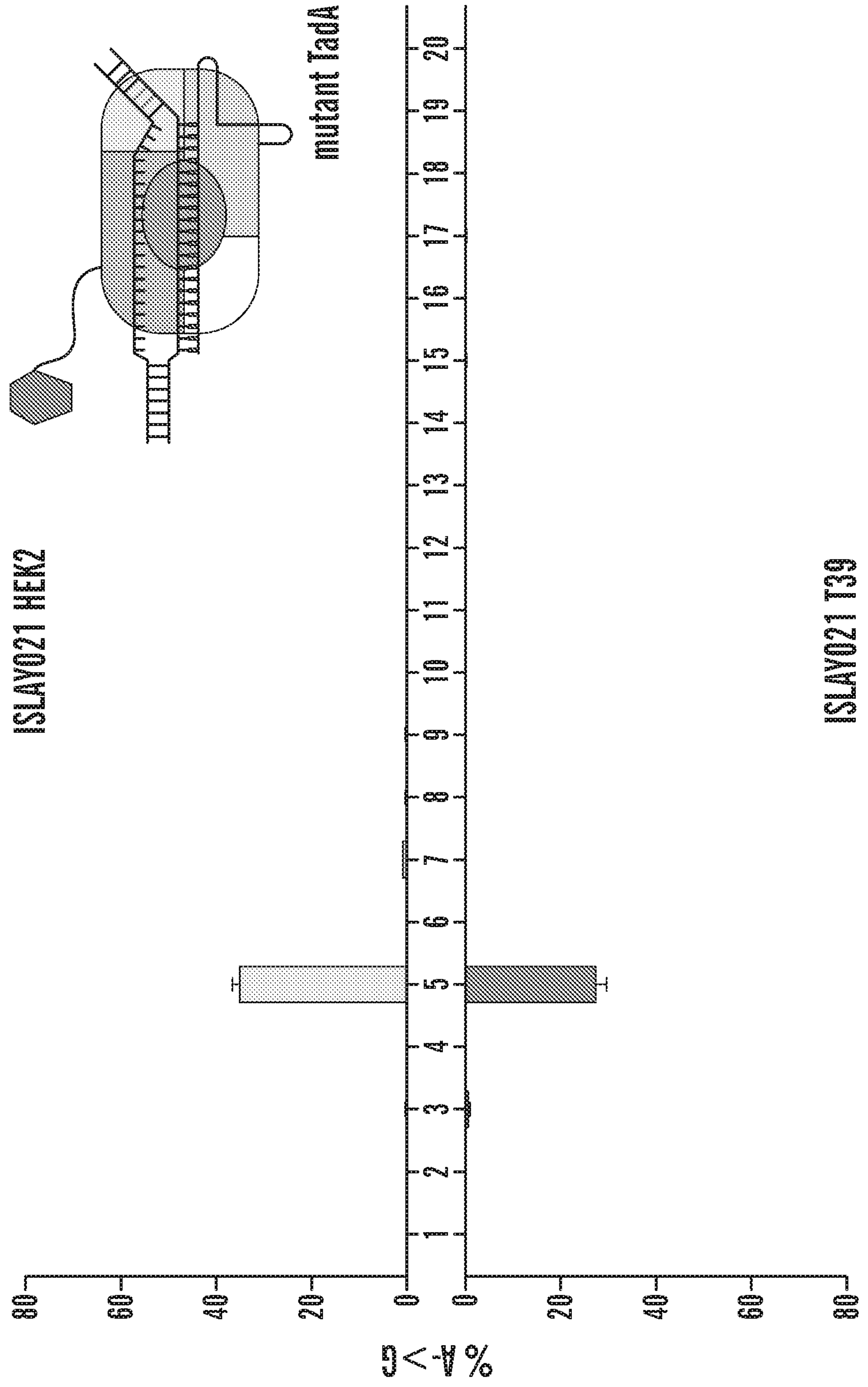

ISLAV021 HEK2
ISLAV021 T39
mutant TadA
% A>G
80
60
40
20
0
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20K

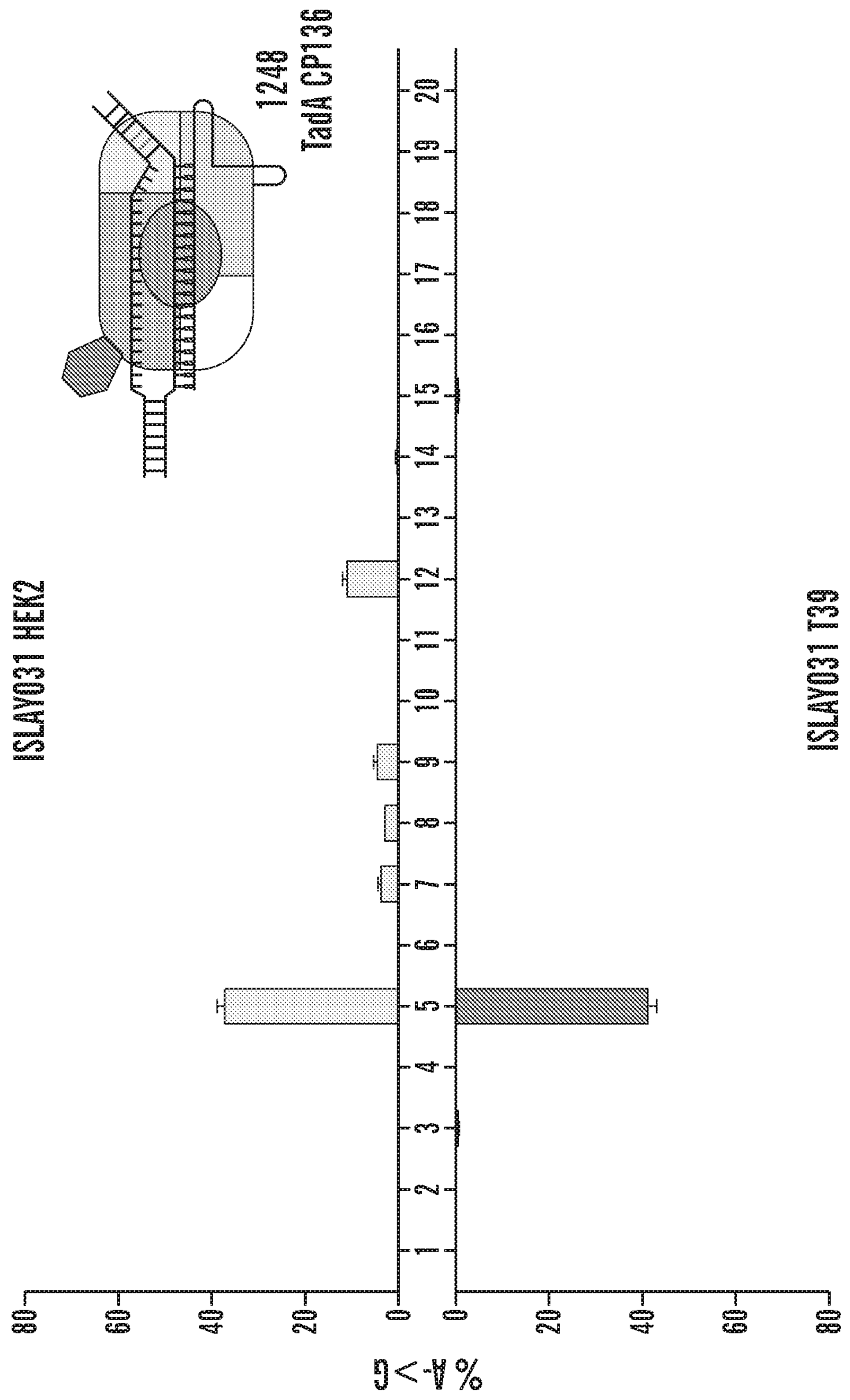

ISLAY031 HEK2
1248
TadA CP136
ISLAY031 T39
% A->G
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20L

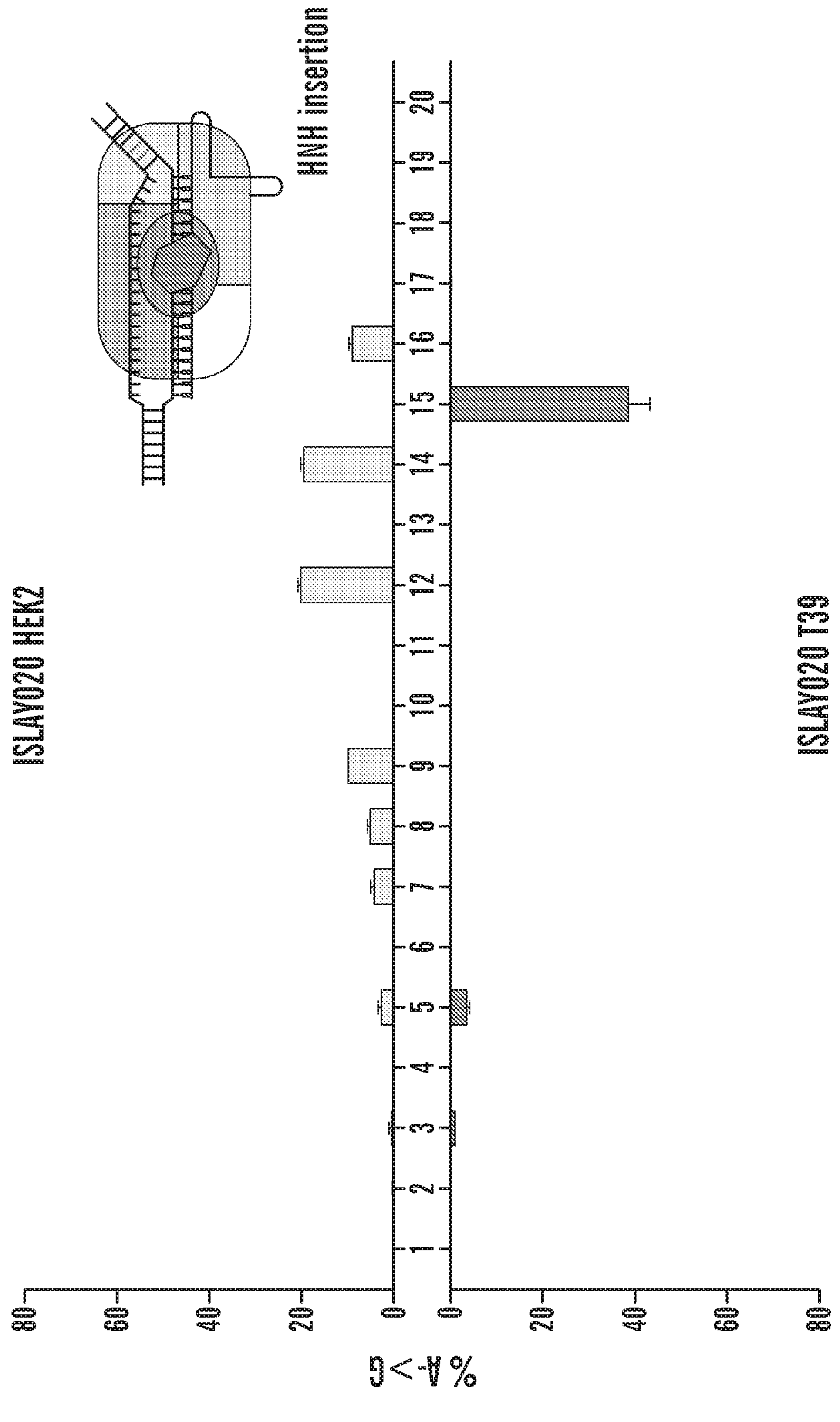

ISLAY020 HEK2
HNH insertion
ISLAY020 T39
%A->G
80
60
40
20
0
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20M

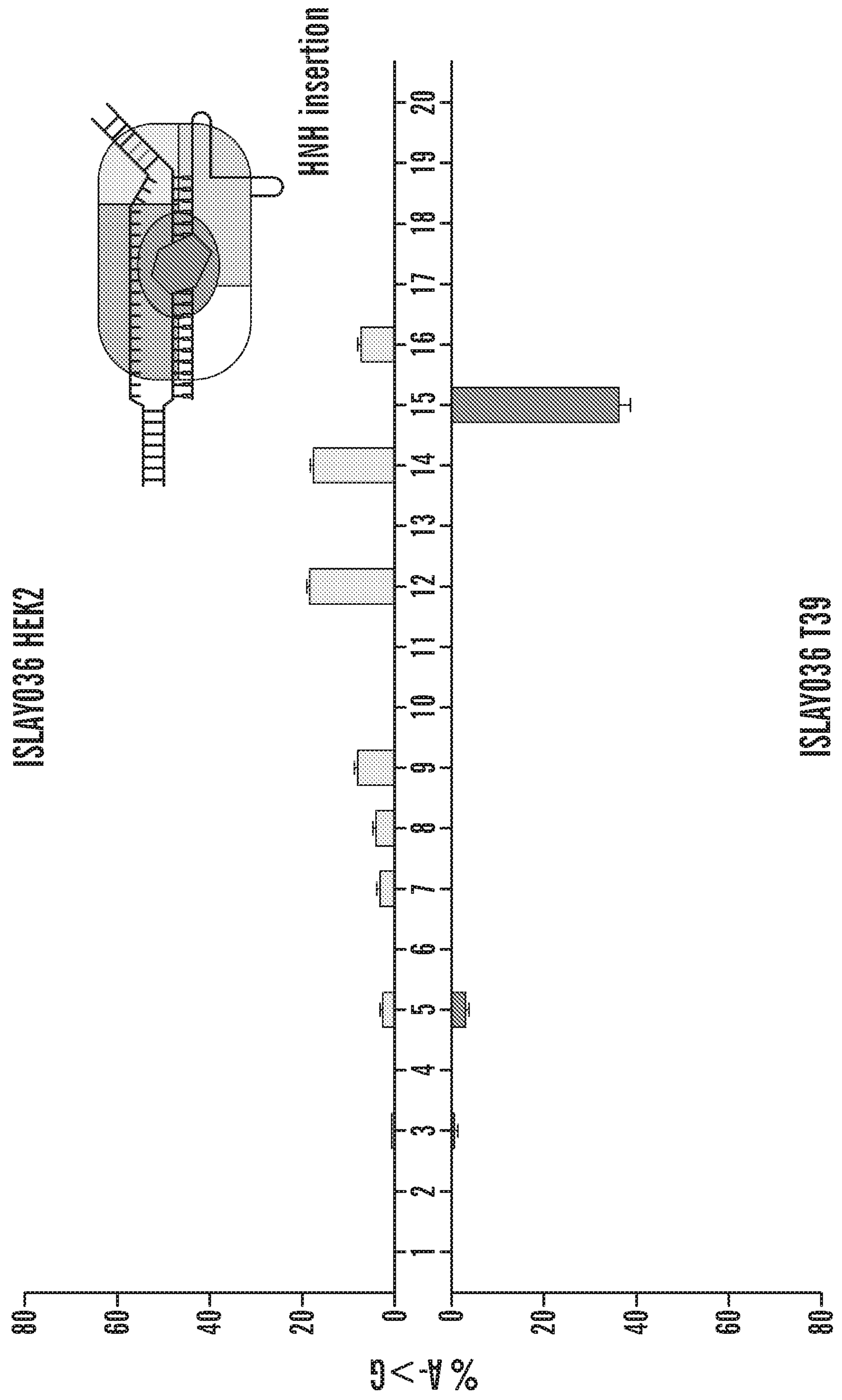

ISLAY036 HEK2
HNH insertion
ISLAY036 T39
%A->G
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20N

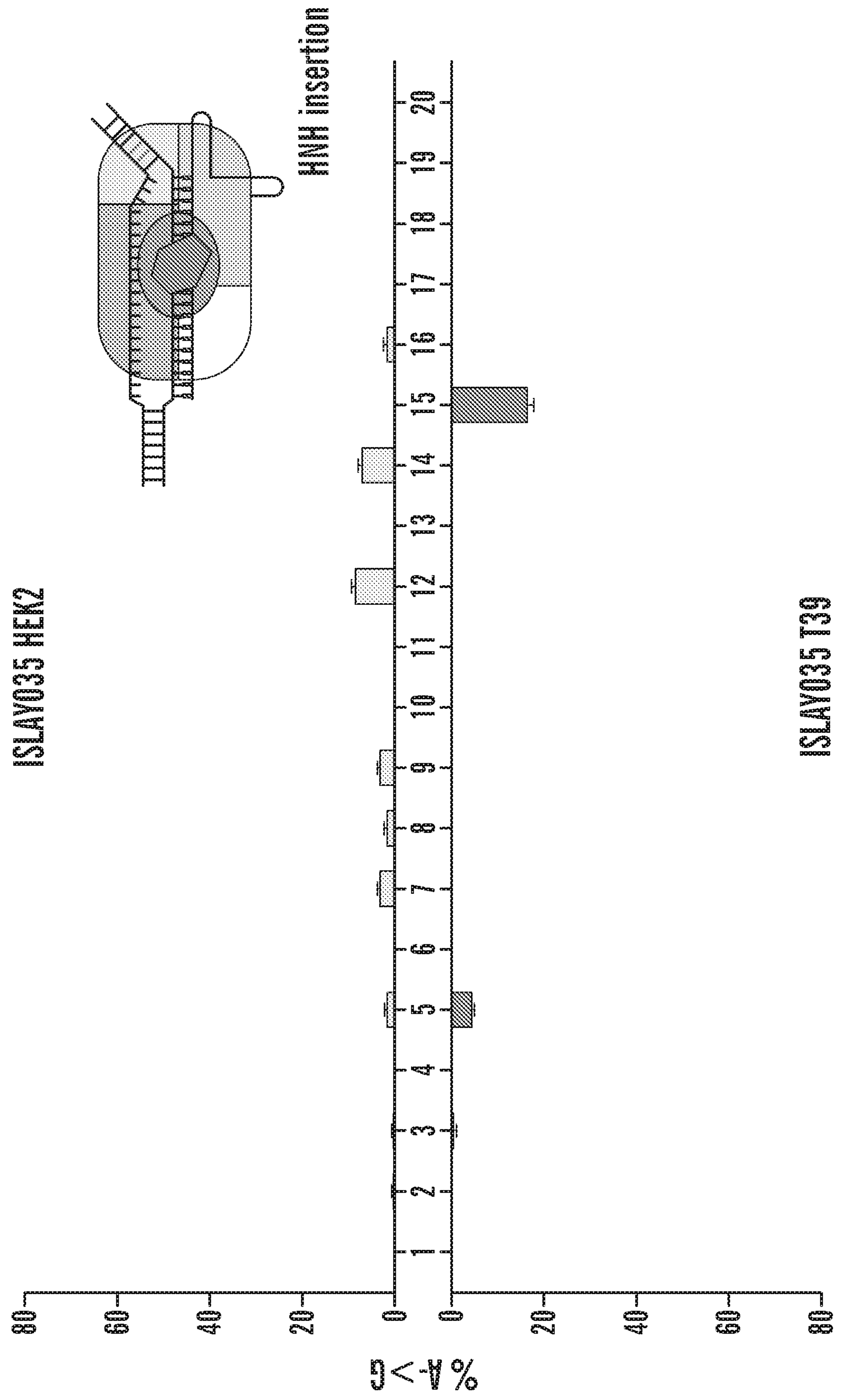
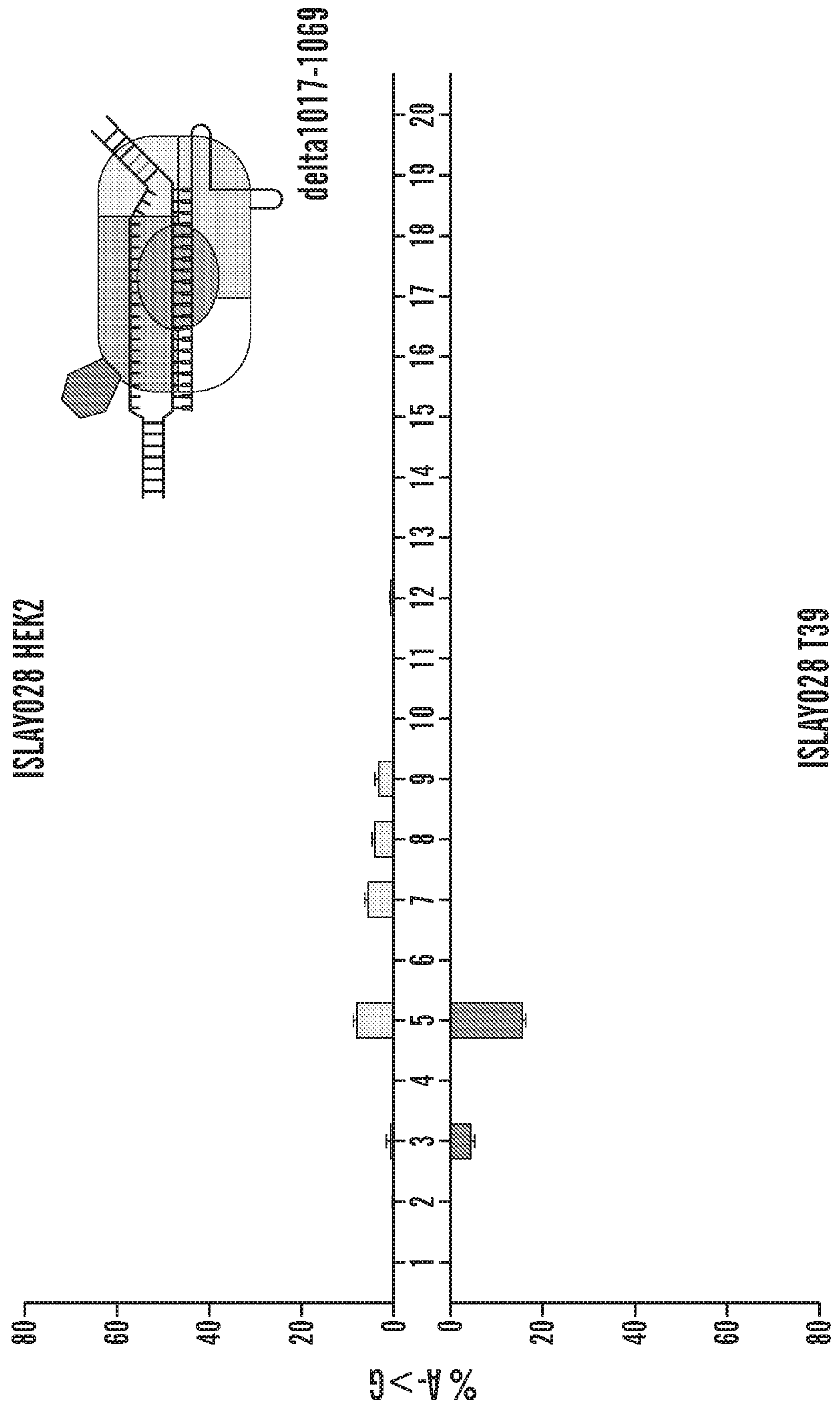

ISLAY035 HEK2
HNH insertion
ISLAY035 T39
%A->G
80
60
40
20
0
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 20O



ISLAV028 HEK2
ISLAV028 T39
delta1017-1069
% A->G
80 60 40 20 0 0 20 40 60 80
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 20P

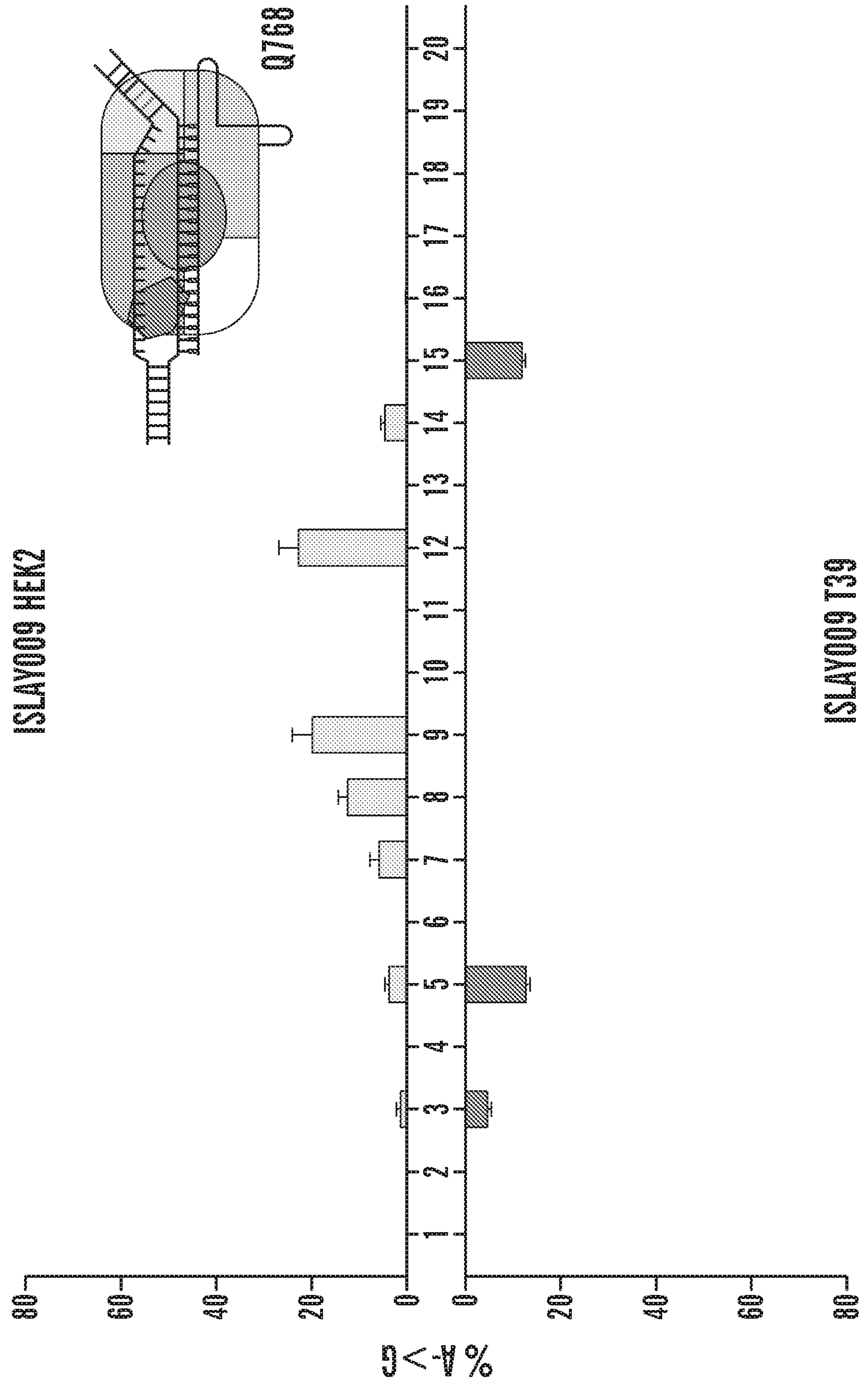

ISLAY009 HEK2
ISLAY009 T39
Q768
% A>G
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 20Q

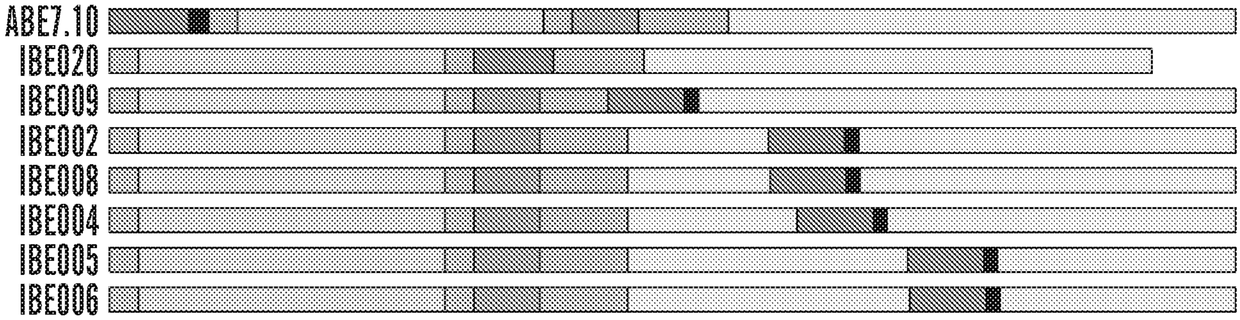


FIG. 21A

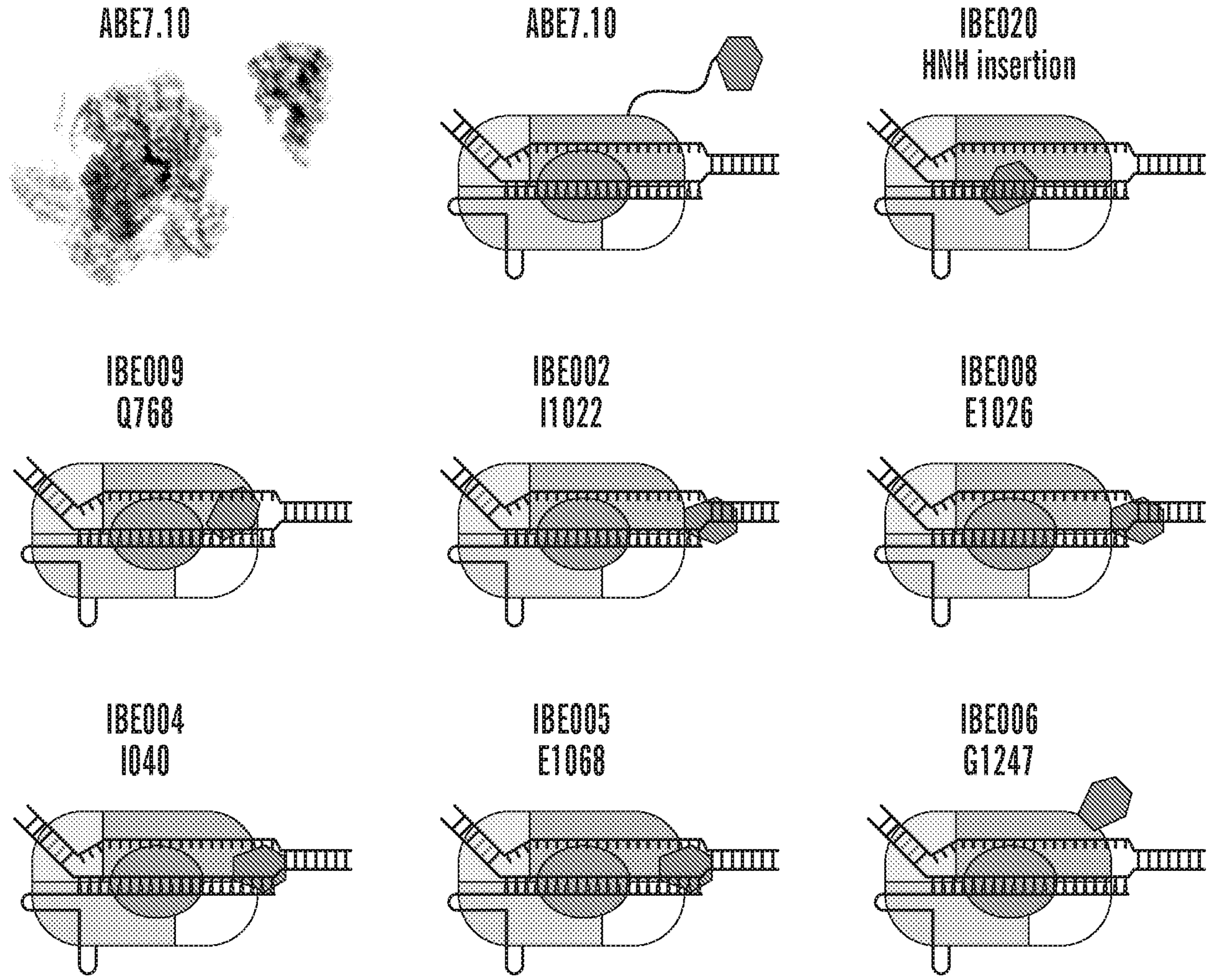


FIG. 21B

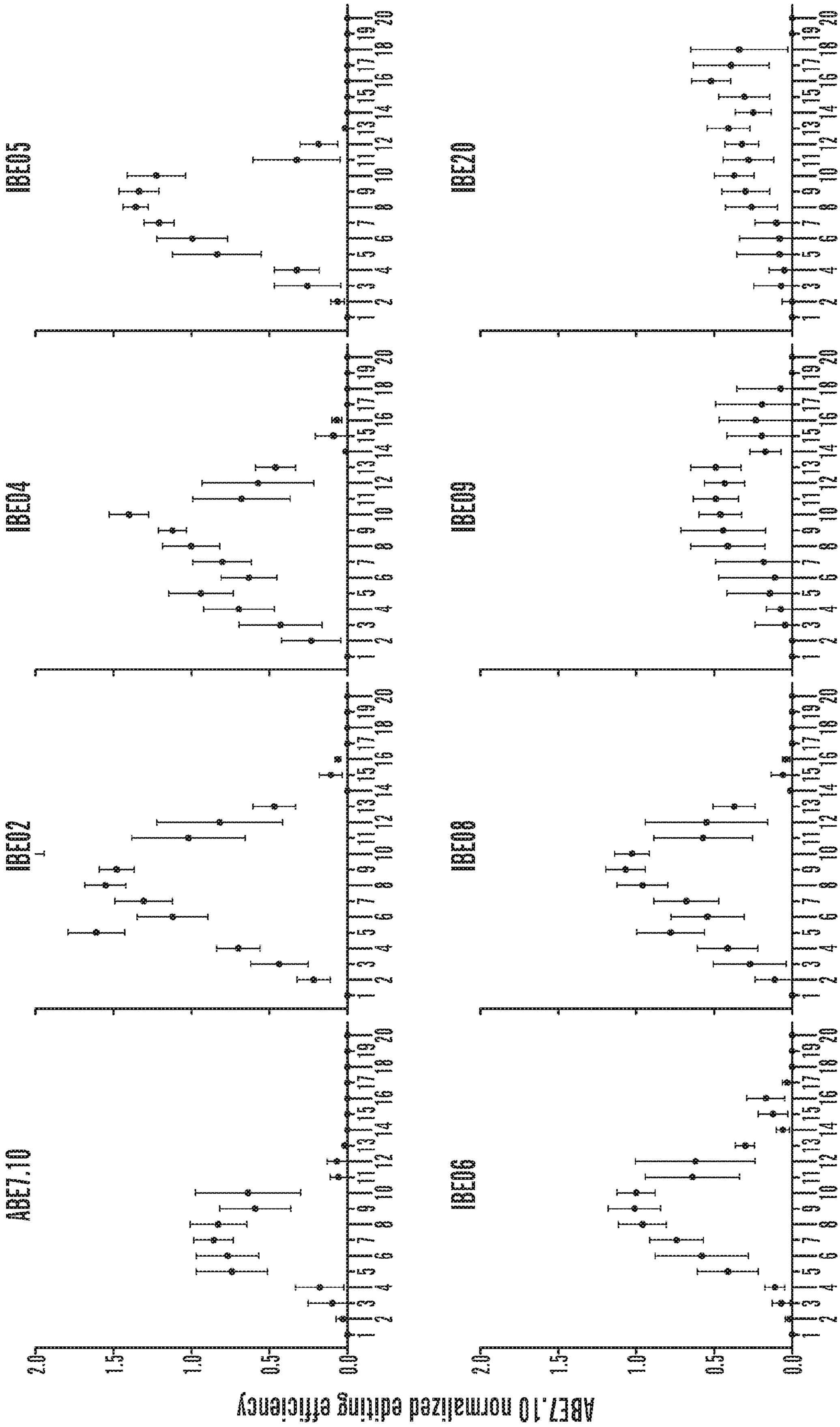

ABE7.10
IBE02
IBE04
IBE05
IBE06
IBE08
IBE09
IBE20
ABE7.10 normalized editing efficiency
2.0
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 22A

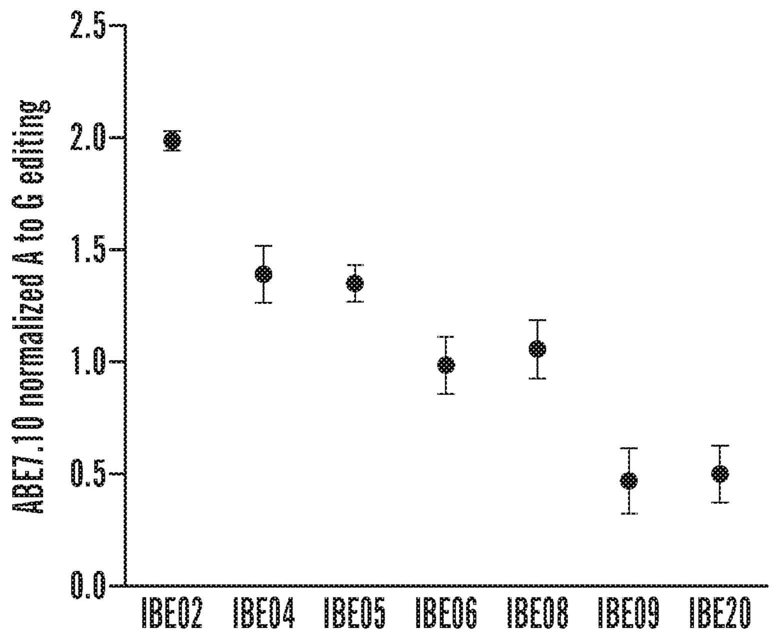


FIG. 22B

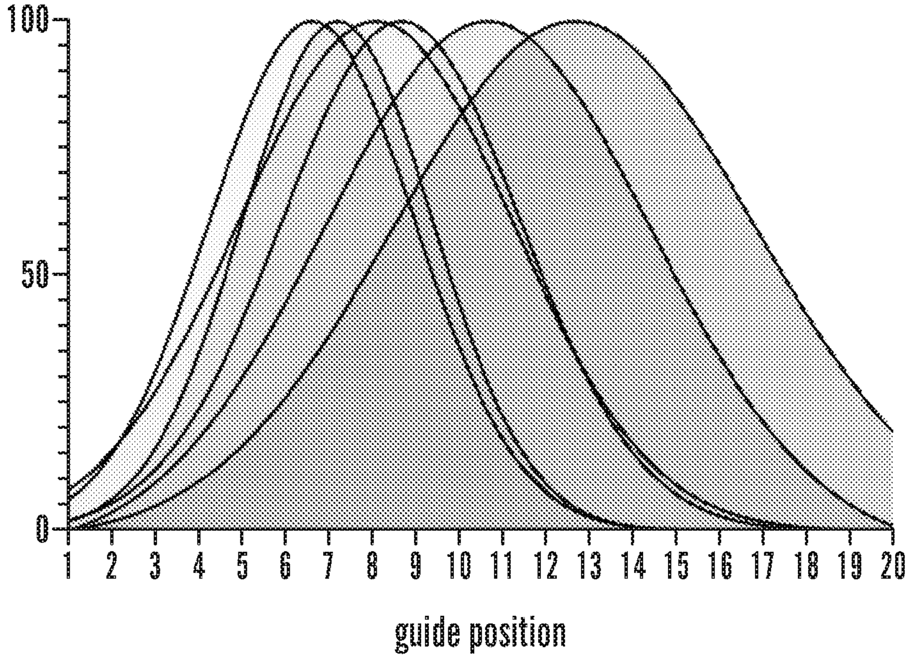


FIG. 22C

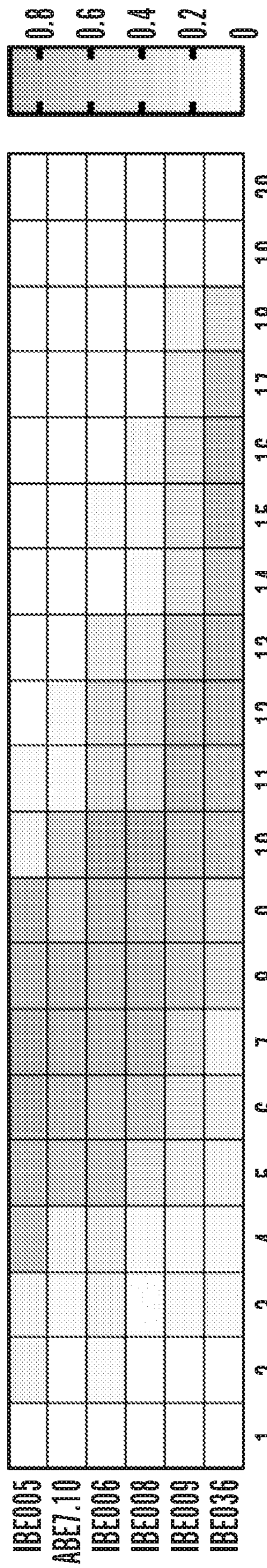

0.8
0.6
0.4
0.2
0
IBE005
ABE7.10
IBE006
IBE008
IBE009
IBE036
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 22D

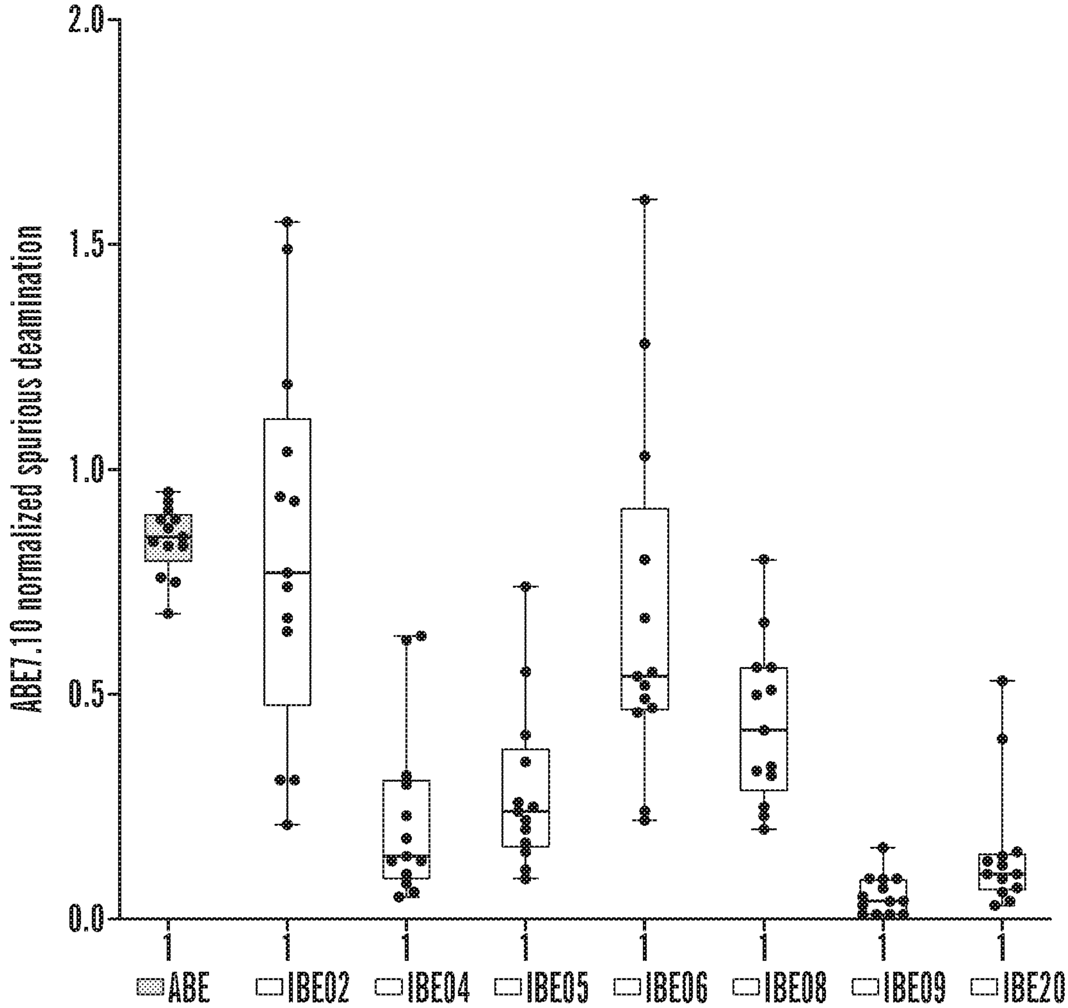

2.0
1.5
1.0
0.5
0.0
ABE7.10 normalized spurious deamination
1
ABE
IBE02
IBE04
IBE05
IBE06
IBE08
IBE09
IBE20

FIG. 23

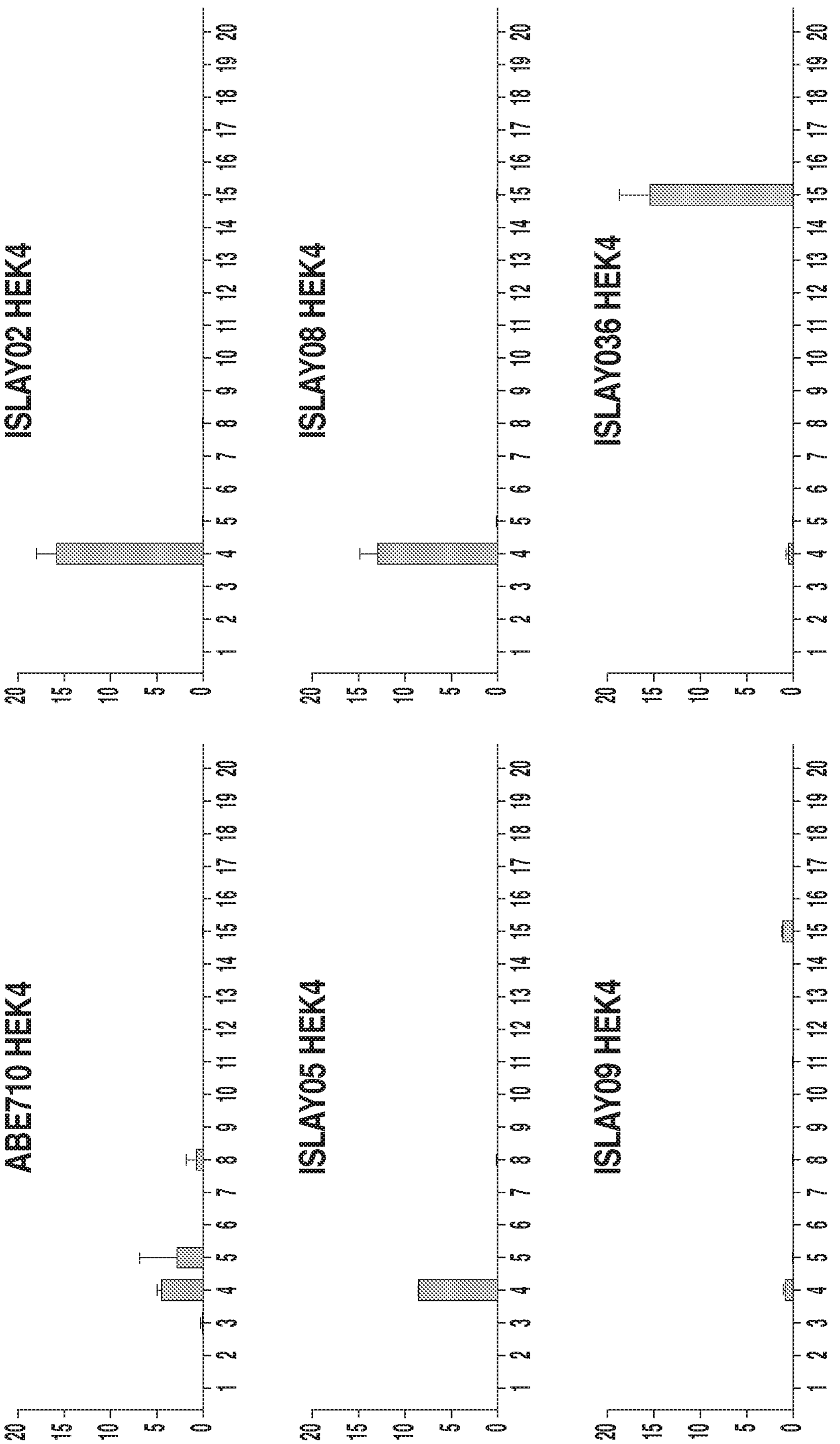

ABE710 HEK4
ISLAY02 HEK4
ISLAY05 HEK4
ISLAY08 HEK4
ISLAY09 HEK4
ISLAY036 HEK4

FIG. 24A

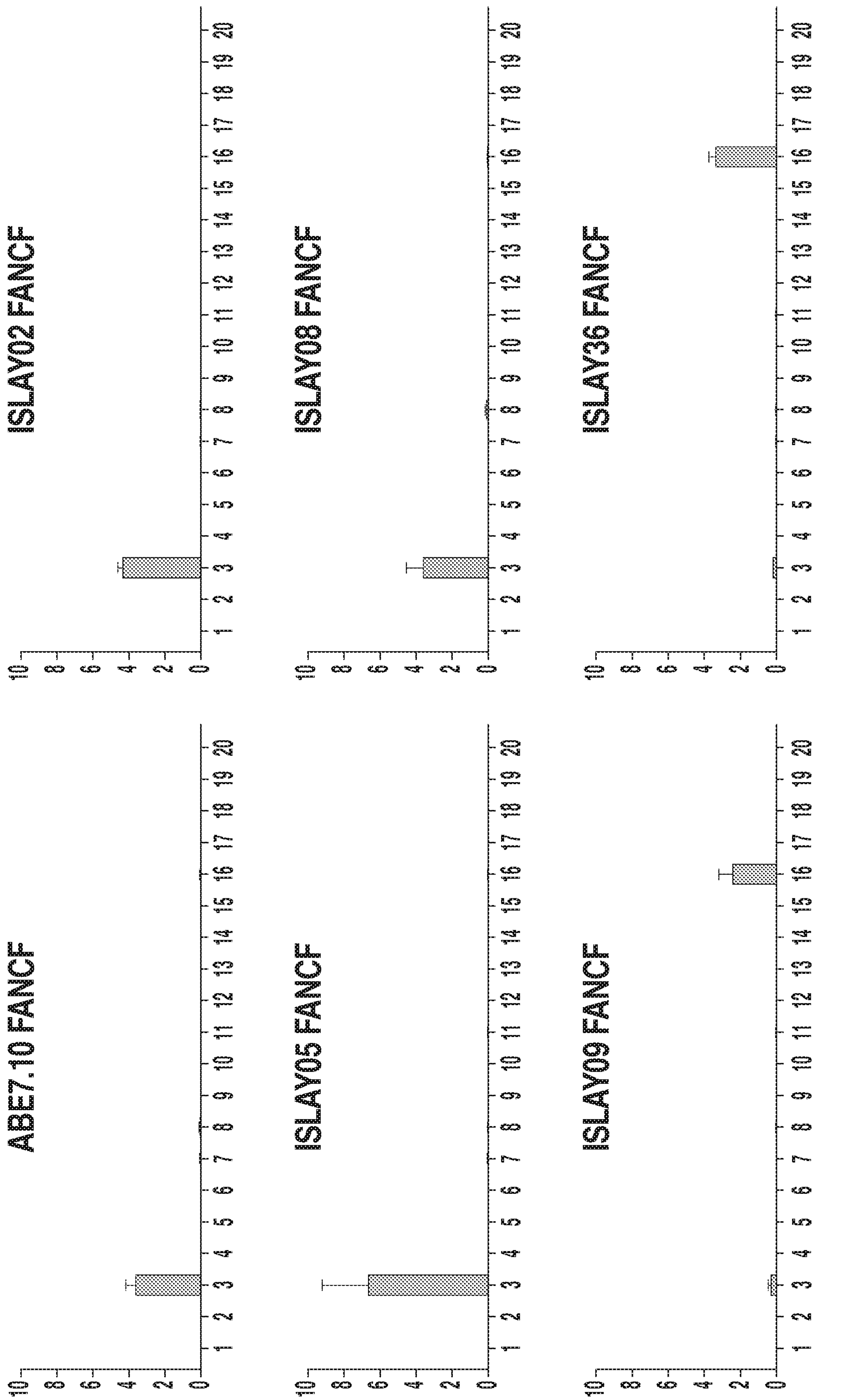

ISLAY02 FANCF
ISLAY08 FANCF
ISLAY36 FANCF
ABE7.10 FANCF
ISLAY05 FANCF
ISLAY09 FANCF

FIG. 24B

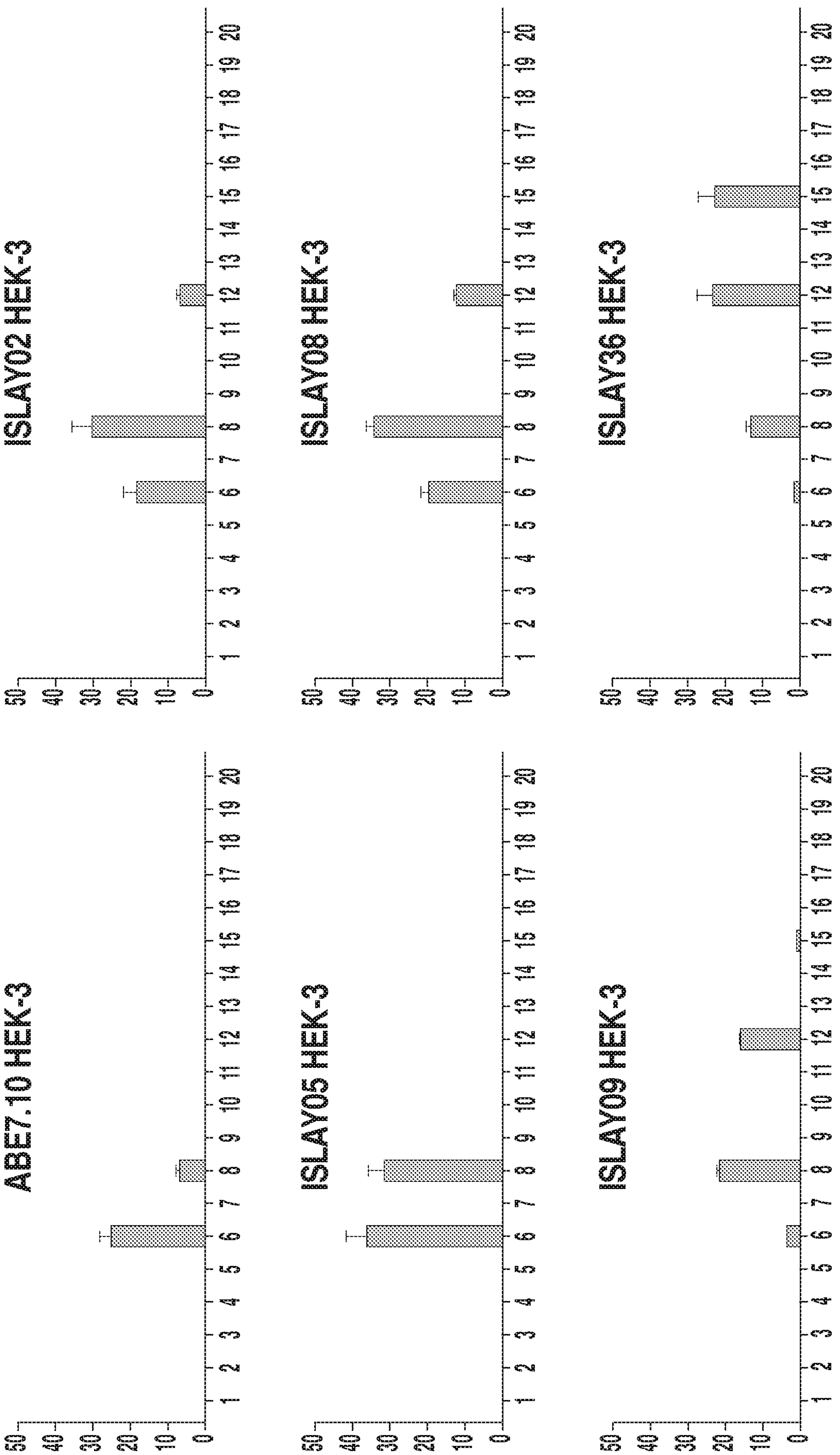

ABE7.10 HEK-3
ISLAY02 HEK-3
ISLAY05 HEK-3
ISLAY08 HEK-3
ISLAY09 HEK-3
ISLAY36 HEK-3
0 10 20 30 40 50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 24C

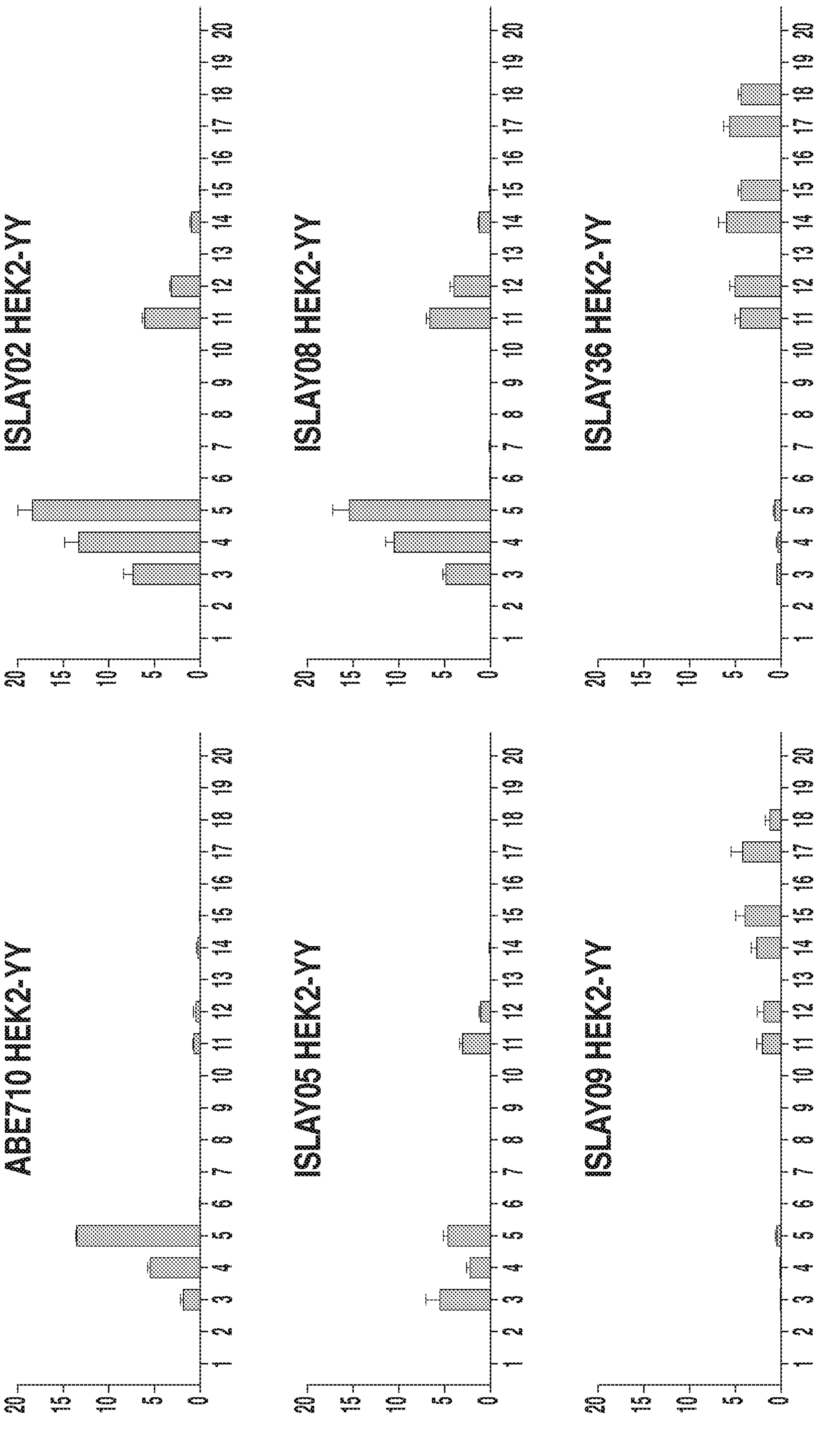

ABE710 HEK2-YY
ISLAY05 HEK2-YY
ISLAY09 HEK2-YY
ISLAY02 HEK2-YY
ISLAY08 HEK2-YY
ISLAY36 HEK2-YY

FIG. 24D

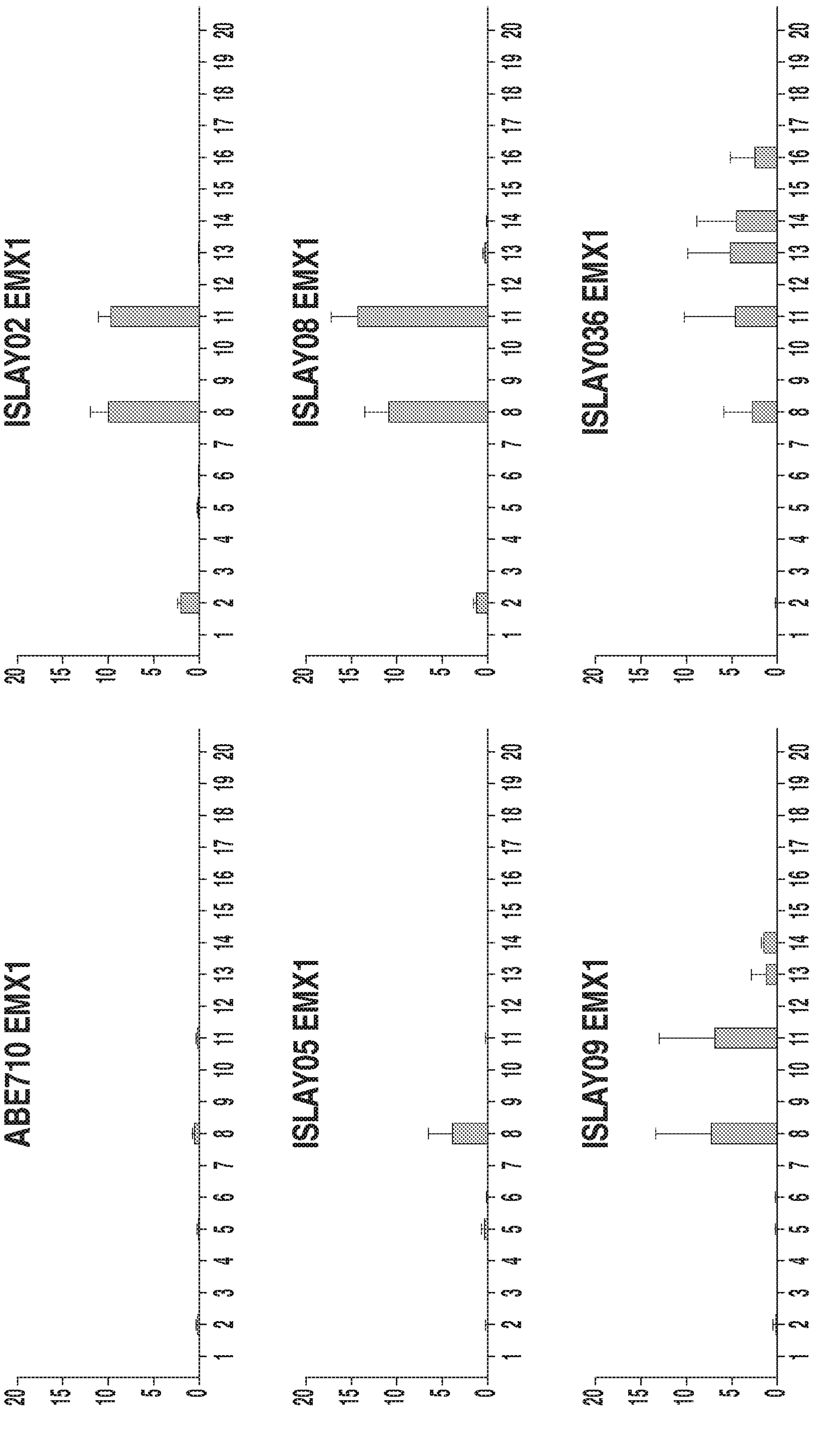

ABE710 EMX1
ISLAY02 EMX1
ISLAY05 EMX1
ISLAY08 EMX1
ISLAY09 EMX1
ISLAY036 EMX1
0
5
10
15
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 24E

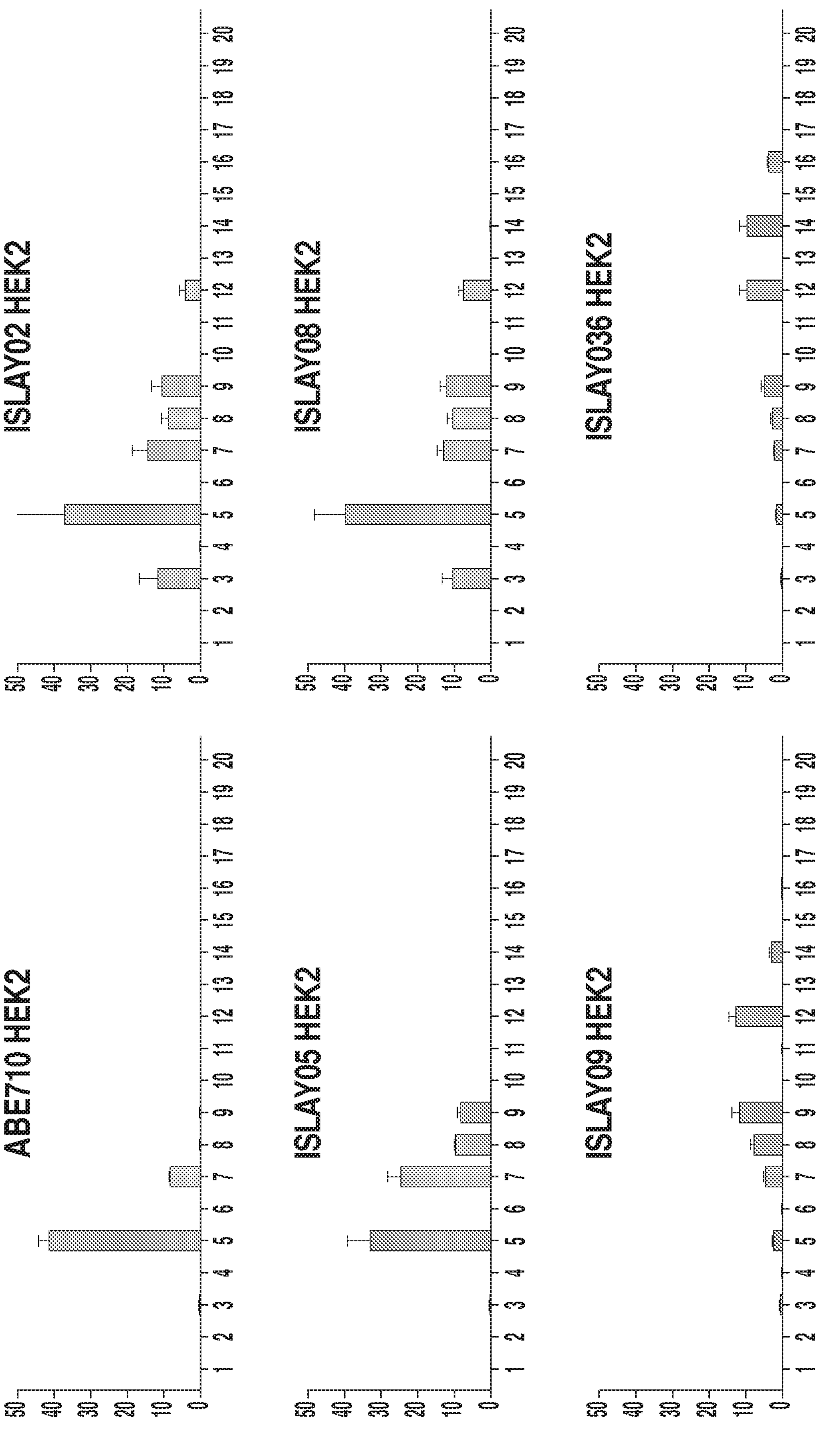

ABE710 HEK2
ISLAY02 HEK2
ISLAY05 HEK2
ISLAY08 HEK2
ISLAY09 HEK2
ISLAY036 HEK2
0 10 20 30 40 50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 24F

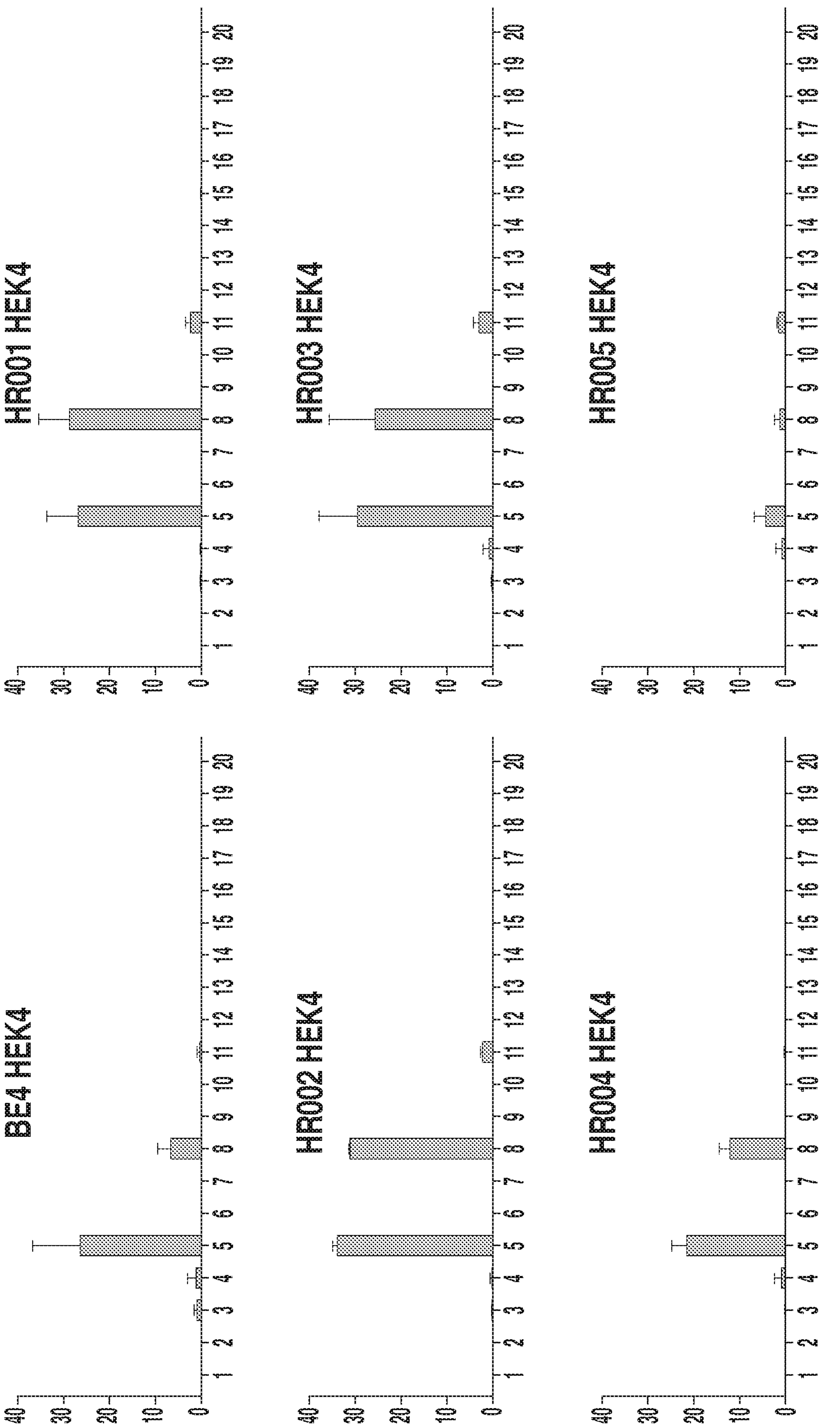

BE4 HEK4
HR001 HEK4
HR002 HEK4
HR003 HEK4
HR004 HEK4
HR005 HEK4
0
10
20
30
40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 25A

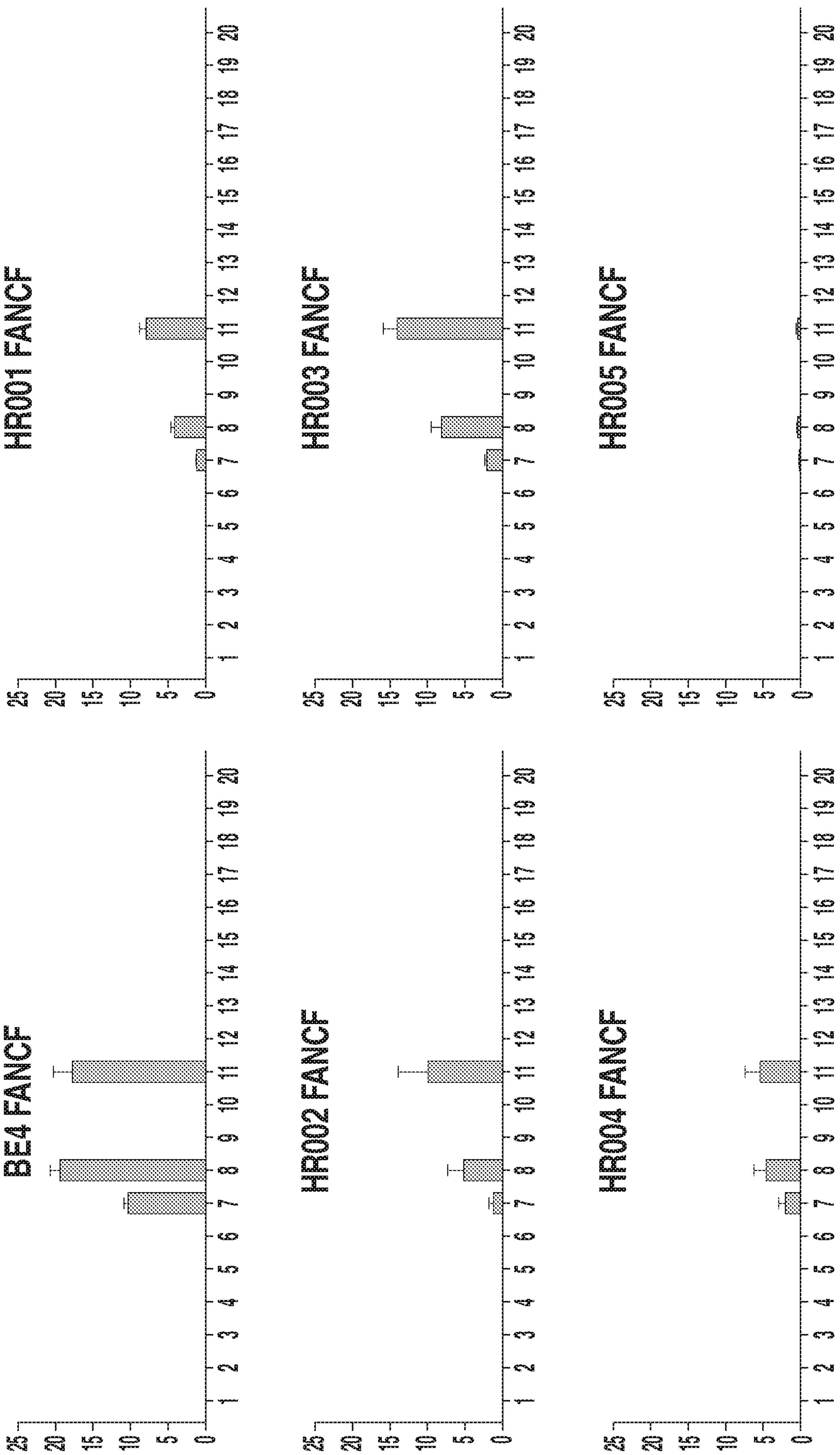

HR001 FANCF
HR003 FANCF
HR005 FANCF
BE4 FANCF
HR002 FANCF
HR004 FANCF
25 20 15 10 5 0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 25B

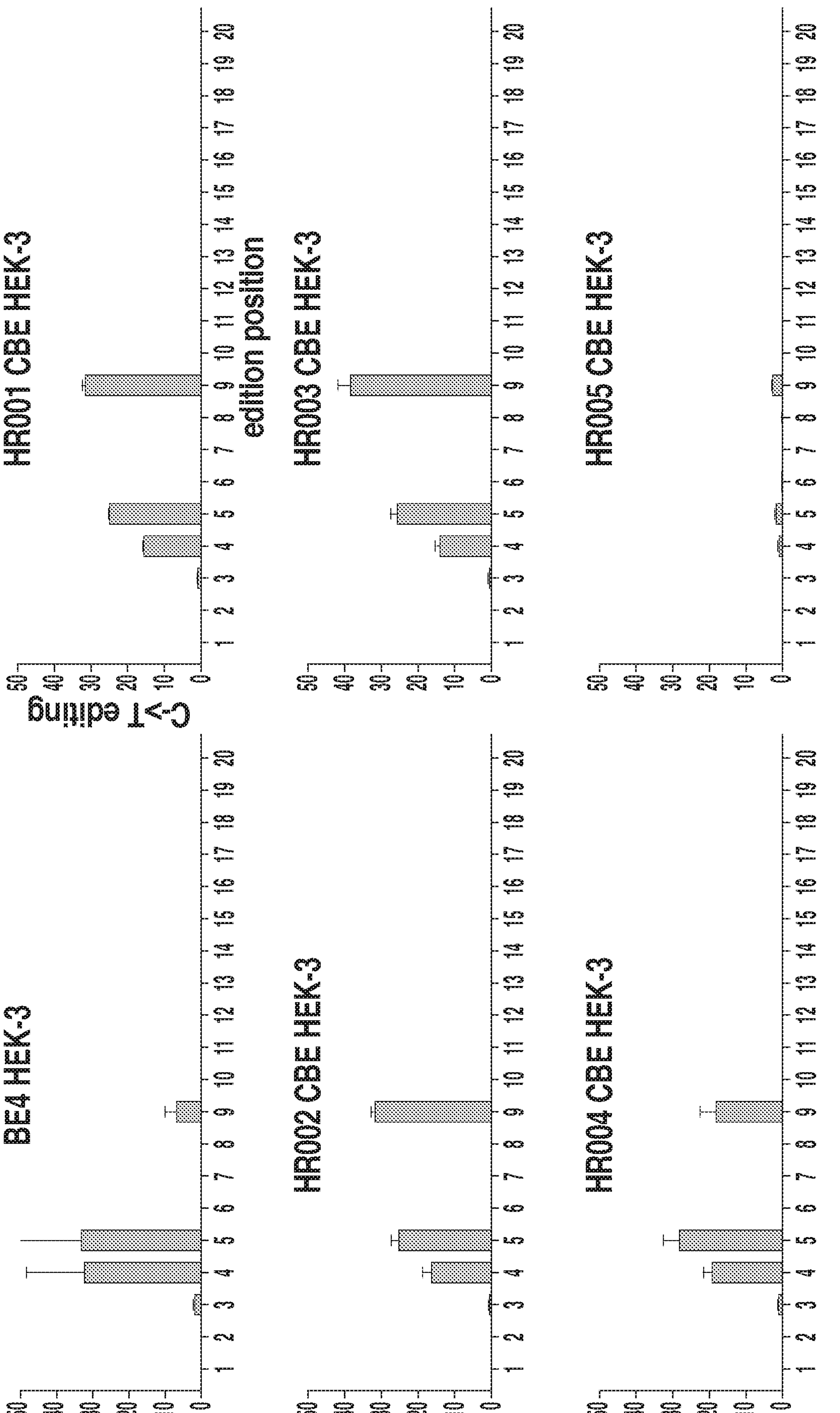

BE4 HEK-3
HR001 CBE HEK-3
HR002 CBE HEK-3
HR003 CBE HEK-3
HR004 CBE HEK-3
HR005 CBE HEK-3
C->T editing
edition position

FIG. 25C

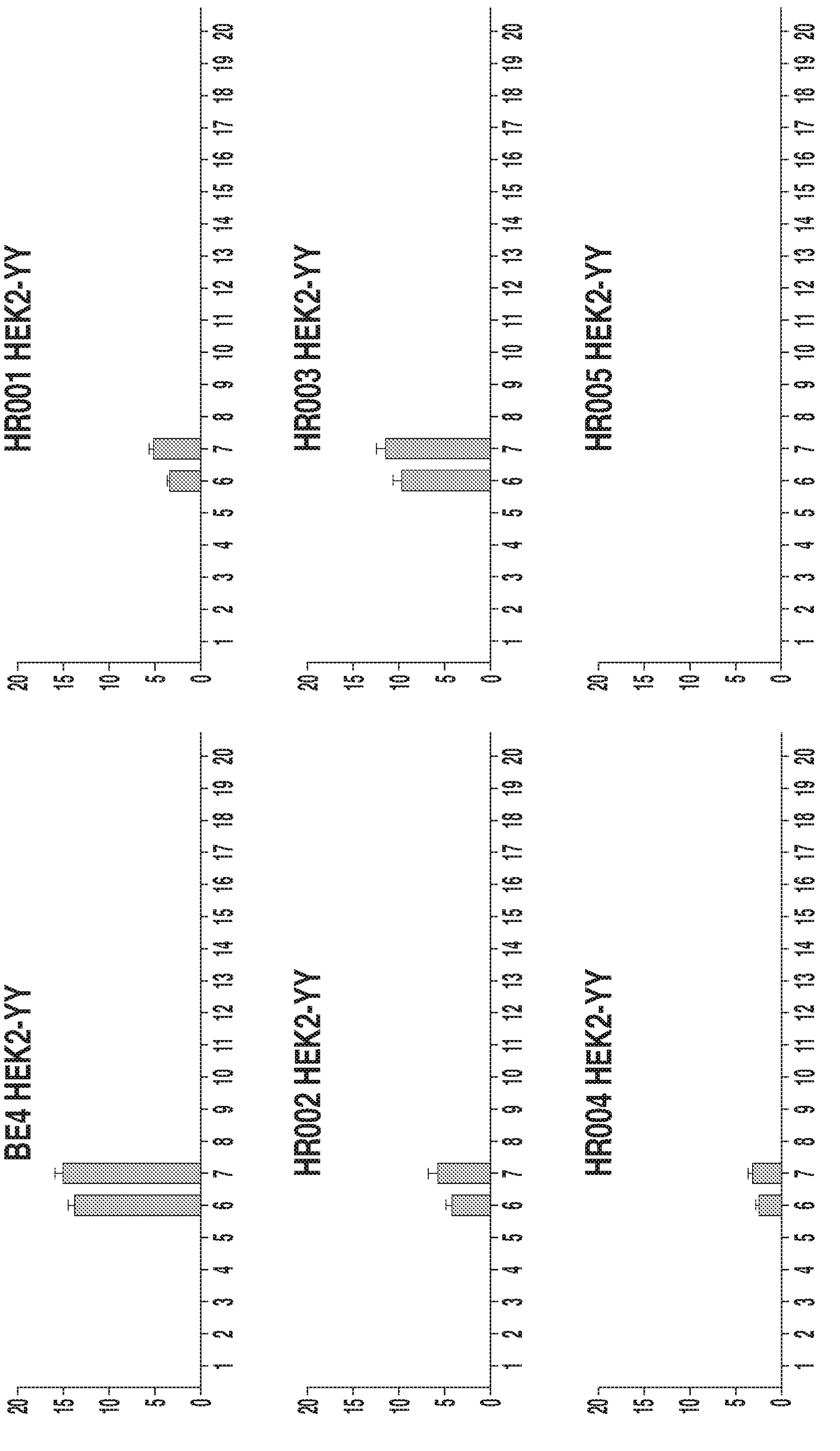

HR001 HEK2-YY
HR003 HEK2-YY
HR005 HEK2-YY
BE4 HEK2-YY
HR002 HEK2-YY
HR004 HEK2-YY

FIG. 25D

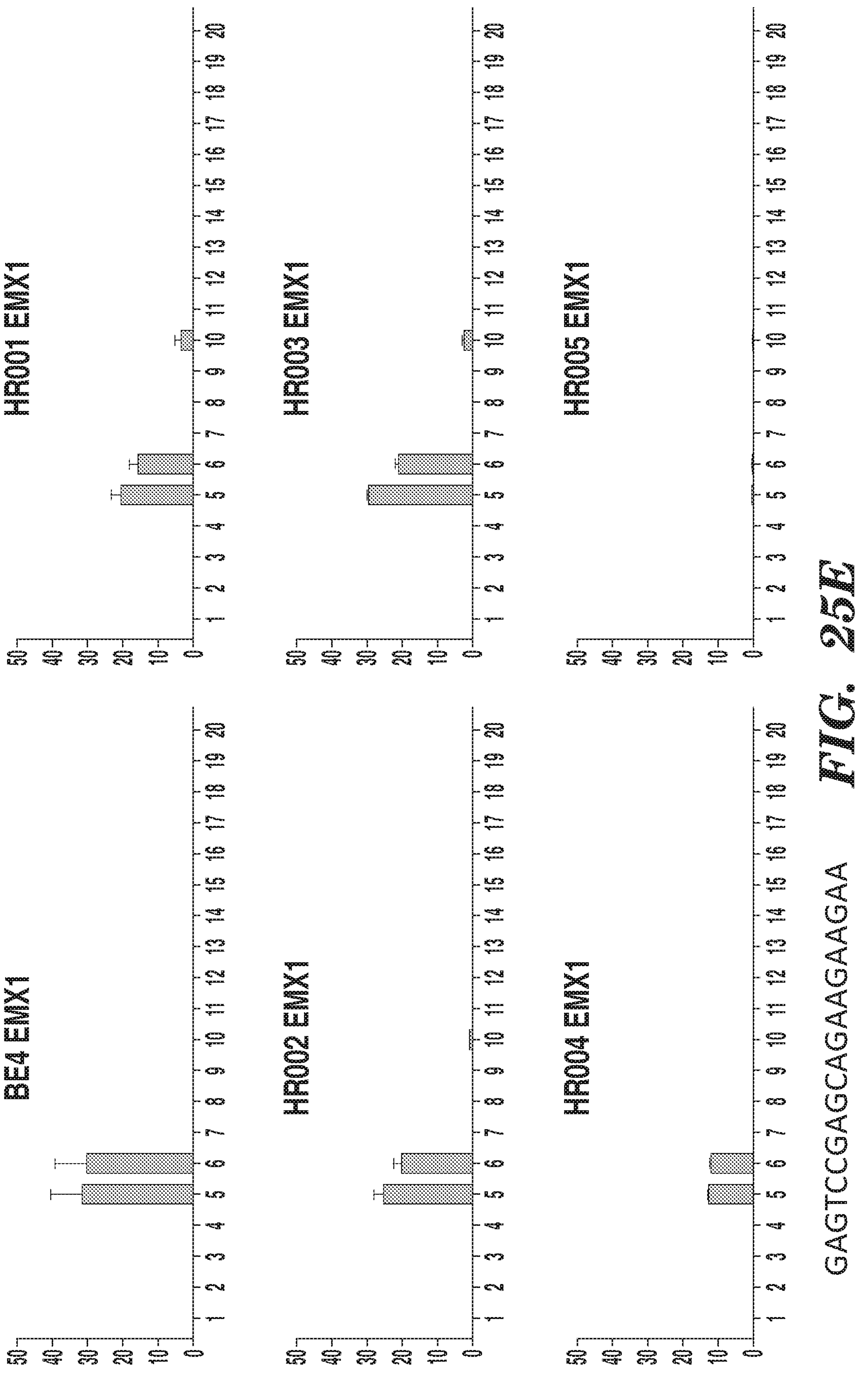

BE4 EMX1
HR001 EMX1
HR002 EMX1
HR003 EMX1
HR004 EMX1
HR005 EMX1
GAGTCCGAGCAGAAGAAGAA

FIG. 25E

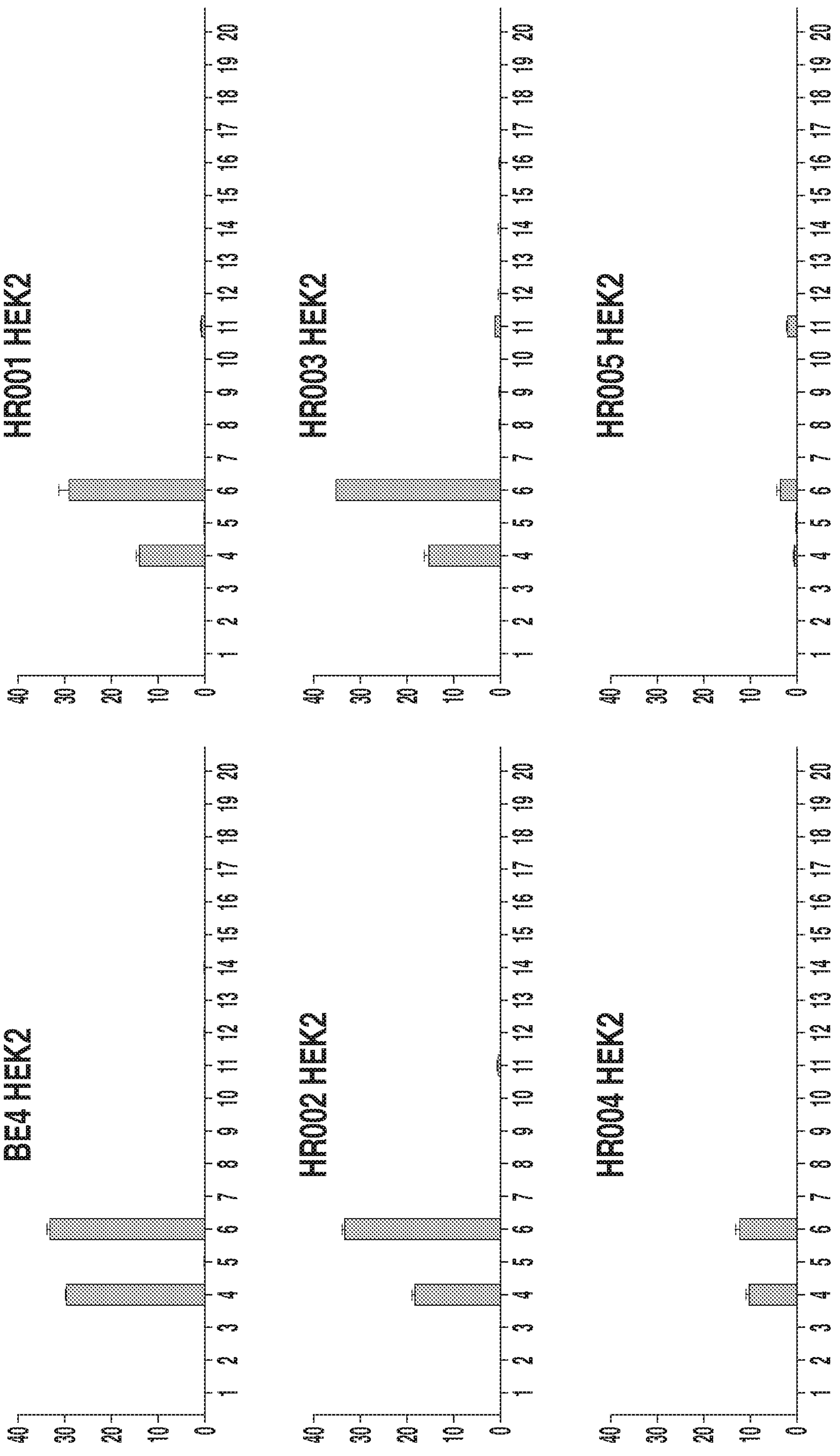

BE4 HEK2
HR001 HEK2
HR002 HEK2
HR003 HEK2
HR004 HEK2
HR005 HEK2
0 10 20 30 40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIG. 25F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/16285

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/62, 15/11, 15/10 (2020.01)

CPC - C12N 15/62, 15/111, 9/22, 15/102, 9/78, 15/11; A61K 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018/176009 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 27 September 2018; figure 4; paragraphs [0006], [0054], [00167], [00210], [00242], [00244], [00301], [00364], [00372], [00396], [00487]	1-2, 3/1-2, 4/3/1-2, 5/4/3/1-2, 29-33, 69-70, 71/69-70, 72/71/69-70, 108-109, 110/108-109, 146-148, 149/146-148
A	US 2018/0179503 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 28 June 2018; paragraphs [0124], [0233]	1-2, 3/1-2, 4/3/1-2, 5/4/3/1-2, 29-33, 69-70, 71/69-70, 72/71/69-70, 108-109, 110/108-109, 146-148, 149/146-148
A	US 2016/0304846 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 20 October 2016; paragraph [0047]	1-2, 3/1-2, 4/3/1-2, 5/4/3/1-2, 29-33, 69-70, 71/69-70, 72/71/69-70, 108-109, 110/108-109, 146-148, 149/146-148
A	US 2018/0298421 A1 (IDENTIFYGENOMICS, LLC) 18 October 2018; paragraph [0026]	146-148, 149/146-148

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2020 (23.04.2020)	04 MAY 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/16285

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-28, 34-68, 73-107, 111-145, 150-160
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.