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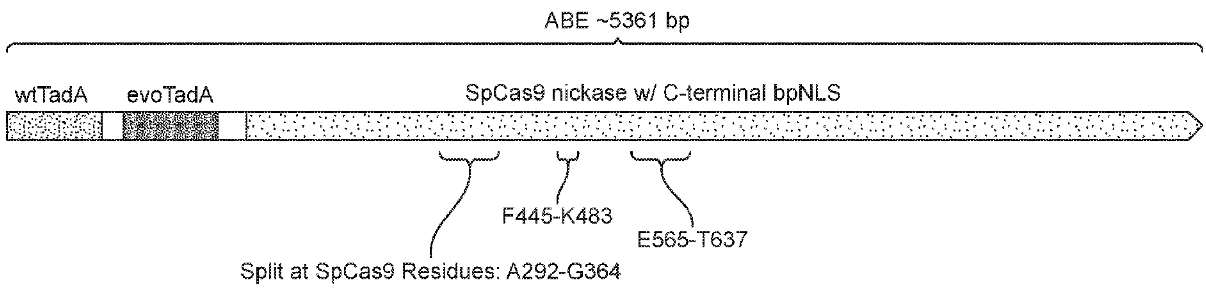

FIG. 1

(57) Abstract: The invention provides compositions and methods for delivering first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases (e.g., adenosine deaminases) and nCas9, wherein the first polynucleotide encodes an N-terminal fragment of nCas9 fused to an intein-N of a split intein pair and the second polynucleotide encodes a C-terminal fragment of nCas9 fused to an intein-C of a split intein pair, and methods for delivering these fragments together with an sgRNA to a cell (e.g., AAV delivery), where the fragments are spliced together by a split intein system, thereby reconstituting a functional base editing system in the cell.

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COMPOSITIONS AND METHODS FOR DELIVERING A NUCLEOBASE EDITING SYSTEM

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application Number 62/728,703, filed on September 7, 2018, and U.S. Provisional Patent Application Number 62/779,404, filed on December 13, 2018, the entire contents of which are hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

Discovery of the Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) has revolutionized the field of molecular biology. Much of this enthusiasm centers on the clinical potential of CRISPR/Cas9 for treating human disease and editing the human genome. Disease-causing mutations could potentially be repaired using CRISPR or CRISPR-based systems. One challenge to accomplishing this goal is delivery of the elements needed for genome editing. For example, with regard to CRISPR/Cas9, SpCas9 and sgRNA can be encoded in a DNA plasmid vector and delivered via adeno-associated virus (AAV). However due to the small packaging capacity of AAVs, it is difficult to include other elements (such as polypeptide domains, promoters, reporters, fluorescent tags, multiple sgRNAs, or DNA templates for HDR) to help achieve delivery of CRISPR/Cas9 components to cells and/or to meet desired gene editing objectives.

SUMMARY OF THE INVENTION

In some aspects, provided herein is a composition comprising (a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and (b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some aspects, provided herein is a composition comprising (a) a first polynucleotide encoding an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and (b) a second polynucleotide encoding fusion protein comprising a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some aspects, provided herein is a composition comprising (a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and (b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some aspects, provided herein is a composition comprising (a) a first polynucleotide encoding an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and (b) a second polynucleotide encoding a fusion protein comprising a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some embodiments, the N-terminal fragment of the Cas9 comprises up to amino acid 302, 309, 312, 354, 455, 459, 462, 465, 471, 473, 576, 588, or 589 as numbered in SEQ ID NO: 2. In some embodiments, the C-terminal fragment of Cas9 or the N-terminal fragment of Cas9 comprises an Ala/Cys, Ser/Cys, or Thr/Cys mutation at a residue corresponding to amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590 as numbered in SEQ ID NO: 2. In some embodiments, the composition further comprises a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA. In some embodiments, the first and the second polynucleotides are joined. In some embodiments, the first and the second polynucleotides are expressed separately. In some embodiments, the deaminase is an adenosine deaminase. In some embodiments, the deaminase is a wild-type TadA or TadA7.10. In some embodiments, the deaminase is a TadA dimer. In some embodiments, the TadA dimer comprises a wild-type TadA and a TadA 7.10. In some embodiments, the fusion protein comprises a nucleus localization signal (NLS). In some embodiments, the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 is joined with a NLS. In some embodiments, the NLS is a bipartite NLS. In some embodiments, the N-terminal fragment of Cas9 and the fusion protein are joined to form a base editor protein comprising a deaminase and a SpCas9. In some embodiments, the C-terminal fragment of Cas9 and the fusion protein are joined to form a base editor protein comprising a deaminase and a SpCas9. In some embodiments, the SpCas9 has nickase activity or is catalytically inactive.

In some aspects, provided herein is a composition comprising the fusion protein and the N-terminal fragment of Cas9 disclosed herein. In some aspects, provided herein is a composition comprising the fusion protein and the C-terminal fragment of Cas9 disclosed herein. In some embodiments, the N terminal fragment of Cas9 or the C terminal fragment of Cas9 and the deaminase are joined by a linker. In some embodiments, the linker is a peptide linker.

In some aspects, provided herein is a vector comprising the first and the second polynucleotide disclosed herein. In some embodiments, the vector comprises a promoter. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the constitutive promoter is a CMV or CAG promoter. In some embodiments, the vector is selected from the group consisting of retroviral vectors, adenoviral vectors, lentiviral vectors, herpesvirus vectors, and adeno-associated viral vectors. In some embodiments, the vector is an adeno-associated viral vector.

In some aspects, provided herein is a cell comprising the composition disclosed herein, or the vector disclosed herein. In some embodiments, the cell is a mammalian cell.

In some aspects, provided herein is a reconstituted A-to-G base editor protein comprising a Cas9 domain comprising an Ala/Cys, Ser/Cys, or Thr/Cys mutation. In some embodiments, the mutation is at a residue corresponding to SpCas9 amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590.

In some aspects, provided herein is a composition comprising one or more polynucleotides encoding (a) an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and (b) a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some aspects, provided herein is a composition comprising one or more polynucleotides encoding (a) an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at amino acid 302, 309, 312, 354, 455, 459, 462, 465, 471, 473, 576, 588, or 589 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and (b) a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at 303, 310, 313, 355, 456, 460, 463, 466, 472, 474, 577, 589, or 590 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some embodiments, the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 is joined with a nucleus localization signal (NLS). In some embodiments, the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 are both joined with a NLS. In some embodiments, the NLS is a bipartite NLS. In some embodiments, the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 are joined to form a SpCa9. In some embodiments, the SpCas9 has nickase activity or is catalytically inactive.

In some aspects, provided herein is a composition comprising the N-terminal fragment of Cas9 in (a) and the C-terminal fragment of Cas9 in (b) disclosed herein.

In some aspects, provided herein is a vector comprising the one or more polynucleotides disclosed herein. In some embodiments, the vector comprises a promoter. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the constitutive promoter is a CMV or CAG promoter. In some embodiments, the vector is selected from the group consisting of retroviral vectors, adenoviral vectors, lentiviral vectors, herpesvirus vectors, and adeno-associated viral vectors. In some embodiments, the vector is an adeno-associated viral vector.

In some aspects, provided herein is a cell comprising the composition disclosed herein, or the vector disclosed herein. In some embodiments, the cell is a mammalian cell.

In some aspects, provided herein is a Cas9 variant polypeptide comprising a Ala/Cys, Ser/Cys, or Thr/Cys mutation. In some aspects, provided herein is a Cas9 variant polypeptide comprising a Cys residue at amino acid 303, 310, 313, 355, 456, 460, 463, 466, 472, or 474.

In some aspects, provided herein is a method for delivering a base editor system to a cell, the method comprising contacting the cell with (a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, (b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and (c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

In some aspects, provided herein is a method for delivering a base editor system to a cell, the method comprising contacting the cell with (a) a first polynucleotide encoding a fusion protein comprising an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, (b) a second polynucleotide encoding a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and (c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

In some aspects, provided herein is a method for delivering a Base Editor System to a cell, the method comprising contacting the cell with (a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, (b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the

C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and (c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

In some aspects, provided herein is a method for delivering a Base Editor System to a cell, the method comprising contacting the cell with (a) a first polynucleotide encoding a fusion protein comprising an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, (b) a second polynucleotide encoding a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and (c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

In some embodiments, the sgRNA is complementary to a target polynucleotide. In some embodiments, the target polynucleotide is present in the genome of an organism. In some embodiments, the organism is an animal, plant, or bacteria. In some embodiments, first polynucleotides, the second polynucleotide, and/or the polynucleotide encoding the are contacted with the cell via a vector. In some embodiments, the vector is selected from the group consisting of retroviral vectors, adenoviral vectors, lentiviral vectors, herpesvirus vectors, and adeno-associated viral vectors. In some embodiments, the vector is an adeno-associated viral vector. In some embodiments, the C-terminal fragment of Cas9 or the N-terminal fragment of Cas9 comprises an Ala/Cys, Ser/Cys, or Thr/Cys mutation at a residue corresponding to amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590 as numbered in SEQ ID NO: 2. In some embodiments, the deaminase is an adenosine deaminase. In some embodiments, the deaminase is a TadA or a variant thereof. In some embodiments, the deaminase is a wild-type TadA or Tad7.10. In some embodiments, the deaminase is a TadA dimer. In some embodiments, the TadA dimer comprises a wild type TadA and a TadA7.10. In some embodiments, the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 comprises an NLS. In some embodiments, the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 both comprise an NLS. In some embodiments, the NLS is a bipartite NLS. In some embodiments, the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 are joined to form a SpCa9. In some embodiments, the SpCas9 has nickase activity or is catalytically inactive.

In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N. In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between F445-K483 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N. In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between E565-T637 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N. In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C. In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between F445-K483 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C. In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C. In some aspects, provided herein is a polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

In some embodiments, the C-terminal fragment of Cas9 or the N-terminal fragment of Cas9 comprises an Ala/Cys, Ser/Cys, or Thr/Cys mutation. In some embodiments, the mutation is at a residue corresponding to amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590 as numbered in SEQ ID NO: 2. In some embodiments, the deaminase is an adenosine deaminase. In some embodiments, the deaminase is a TadA or a variant thereof. In some embodiments, the deaminase is a wild-type TadA, or Tad7.10. In some embodiments, the fusion protein comprises two deaminases linked to each other. In some embodiments, the fusion protein comprises both a wild type TadA and a TadA7.10. In some embodiments, the fusion protein comprises an NLS. In some embodiments, the NLS is a bipartite NLS. In some embodiments, the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 comprises amino acid sequence of SpCas9. In some embodiments, the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 comprises one or more amino acid substitutions associated with reduced nuclease activity.

In some aspects, provided herein is an N-terminal fragment of a Cas9 protein comprising up to amino acid 302, 309, 312, 354, 455, 459, 462, 465, 471, or 473 fused to a split intein-N. In some aspects, provided herein is a C-terminal protein fragment of a Cas9 protein, wherein the N-terminus amino acid of the C-terminal fragment is a Cys substitution at amino acid 303, 310, 313, 355, 456, 460, 463, 466, 472, or 474 and is fused to a split intein-C. In some aspects, provided herein is a polynucleotide encoding a fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and an N-terminal fragment of Cas9, wherein the N-terminal fragment is fused to a split intein-N. In some aspects, provided herein is a polynucleotide encoding a fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and a C-terminal fragment of Cas9, wherein the C-terminal fragment is fused to a split intein-C. In some aspects, provided herein is a protein fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and an N-terminal fragment of Cas9, wherein the N-terminal fragment is fused to a split intein-N. In some aspects, provided herein is a protein fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and a C-terminal fragment of Cas9, wherein the C-terminal fragment is fused to a split intein-C.

In some aspects, provided herein is a composition comprising first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases and Cas9, wherein the first polynucleotide encodes an N-terminal fragment of Cas9 fused to a split intein-N and the second polynucleotide encodes a C-terminal fragment of Cas9 fused to a split intein-C. In some aspects, provided herein is a composition comprising N- and C terminal fragments of an A-to-G Base Editor fusion protein comprising one or more deaminases and SpCas9, wherein the N-terminal fragment comprises a fragment of SpCas9 fused to a split intein-N and the C-terminal fragment comprises the remainder of SpCas9 fused to a split intein-C.

In some aspects, provided herein is a method for delivering a Base Editor System to a cell, the method comprising contacting a cell with first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases and Cas9, wherein the first polynucleotide encodes an N-terminal fragment of Cas9 fused to a split intein-N and the second polynucleotide encodes a C-terminal fragment of Cas9 fused to a split intein-C, and either the first or the second polynucleotide encodes a single guide RNA. In some aspects, provided herein is a method for delivering a Base Editor System to a cell, the method comprising contacting a cell with N- and C terminal fragments of an A-to-G Base Editor fusion protein comprising one or more deaminases and SpCas9, wherein the N-terminal fragment comprises a fragment of SpCas9 fused to a split intein-N and the C-terminal fragment comprises the remainder of SpCas9 fused to a split intein-C, and a guide RNA. In some aspects, provided herein is a method for editing a target polynucleotide in a cell, the method comprising contacting a cell with first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases and Cas9, wherein the first polynucleotide encodes an N-terminal fragment of Cas9 fused to a split intein-N and the second polynucleotide encodes a C-terminal fragment of Cas9 fused to a split intein-C, and either the first or the second polynucleotide encodes a single guide RNA, and expressing the encoded proteins and single guide RNA in the cell.

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

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.

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

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

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 polypeptide analog retains at least some of the biological activity of a corresponding naturally-occurring polypeptide, while having certain sequence modifications that enhance the analog’s function relative to a naturally occurring polypeptide. Such modifications could increase the analog’s protease resistance, membrane permeability, or half-life, without altering, for example, polynucleotide binding activity. In another 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.

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 capable of deaminating a cytosine (C) and an adenosine (A) within DNA. In some embodiments, the base editor is a cytidine base editor (CBE). In some embodiments, the base editor is an adenosine base editor (ABE). In some embodiments, the base editor is an adenosine base editor (ABE) and a cytidine base editor (CBE). In some embodiments, the base editor is a nuclease-inactive Cas9 (dCas9) fused to an adenosine deaminase. In some embodiments, the Cas9 is a circular permutant Cas9 (e.g., spCas9 or saCas9). Circular permutant Cas9s are known in the art and described, for example, in Oakes et al., Cell 176, 254–267, 2019. In some embodiments, the base editor is fused to an inhibitor of base excision repair, for example, a UGI domain. 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.

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 may comprise one or more proteins, fusion proteins, polypeptides, or encoding polynucleotides thereof. In some embodiments, the base editor system may comprise a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of a napDNAbp and a second polynucleotide encoding a C-terminal fragment of a napDNAbp. For example, in particular embodiments, the N-terminal fragment of the napDNAbp may be fused to a intein-N and the C-terminal fragment of the napDNAbp may be fused to a intein-C, such that the N-terminal fragment and the C-terminal fragment of the napDNAbp may be reconstituted to form a base editor protein.

In some embodiments, a base editor system can further comprise an inhibitor of base excision repair (BER) component. 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 another embodiment, a base is excised from a polynucleotide. 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. 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 (SEQ ID NO:1)

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

EIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD
KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASH
YEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIR
EQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD (SEQ ID NO:2)

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

GGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACC
AACTTTGACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTA
TTTCACAGTGTACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCG
CCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAA
GTGACAGTTAAGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGA
GATCTCCGGGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGA
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TTGACTCTTACCCTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAAAAACATACGCTCA
CCTGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGAT
TGTCGCGGAAACTTATCAACGGGATAAGAGACAAGCAAAGTGGTAAAACTATTCTCGATTTT
CTAAAGAGCGACGGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGATGACTCTTTAAC
CTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTCATTGCACGAACATA
TTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCAGACAGTCAAAGTAGTG
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CAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATGTTGATCAGGA
ACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGTACCCCAATCCTTTTTGA
AGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAGTGAC
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GAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTG
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GGAAGTCAAAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAAT
TCTATAAAGTTAGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTAATGCCGTC
GTAGGGACCGCACTCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTA
CAAAGTTTATGACGTCCGTAAGATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAG
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TACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGA
AGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACCCC

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AAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGC
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TAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAACCCATA
CGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGCCGC
ATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAG
CTTGGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGA
CGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
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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
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CGTCTCAAACGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACA
GGAGATTTTTTCAAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGT
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GAAGTTGCTTATCATGAGAAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTC
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CAGTTGGTACAAACCTACAATCAATTATTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGA
TGCTAAAGCGATTCTTTCTGCACGATTGAGTAAATCAAGACGATTAGAAAATCTCATTGCTC
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ACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAATTACAGCTTTCAAAAGA
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CAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAAT
GGAGAGATTCGCAAACGCCCTCTAATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGA
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CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTTTGTGGAGCA
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CGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGC
TTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAG
ATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAG
CTAGGAGGTGACTGA

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

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

Prevotella intermedia (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquisI* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1), *Listeria innocua* (NCBI Ref: NP_472073.1), *Campylobacter jejuni* (NCBI Ref: YP_002344900.1) or *Neisseria. meningitidis* (NCBI Ref: YP_002342100.1) or to a Cas9 from any other organism.

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):

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MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
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EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF
LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
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QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSD
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VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN
GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS
HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI
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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):
DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDL
NPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE
KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTF
DNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAW
MTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS
VEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL
KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRN
FMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDK
NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIK
RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
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, Cas protein 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 napDNAbp is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9). Non-limiting examples of nucleic acid programmable DNA binding proteins include, Cas9 (*e.g.*, dCas9 and nCas9), Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues thereof, or modified or engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova *et al.* "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" *CRISPR J.* 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan *et al.*, "Functionally diverse type V CRISPR-Cas systems" *Science.* 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

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
LLYIPHEFQSAKITTPKEMSRAFLDLAPAEFATSLEPESLSEKSLLKLKQMRYYPHYFG
YELTRTGQGIDGGVAENALRLEKSPVKKREIKCKQYKTLGRGQNKIVLYVRSSYYQT
QFLEWFLHRPKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSERVFVSQ
PFTIFPEKSAEEEGQRYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVK
GLKLDQRRGTFAMPSTKIARIRESLVHSLRNRIHHLALKHKAKIVYELEVSRFEEGKQ
KIKKVYATLKKADVYSEIDADKNLQTTVWGKLAVASEISASYTSQFCGACKKLWRA
EMQVDETITTQELIGTVRVIKGGTLIDAIKDFMRPPIFDENDTPFPKYRDFCDKHHISK
KMRGNSCLFICPFCRANADADIQASQTIALLRYVKEEKKVEDYFERFRKLKN IKVLG
QMKKI

The term “CRISPR-Cas domain” or “CRISPR-Cas DNA binding domain” refers to an RNA-guided protein comprising a CRISPR associated (Cas) protein, or a fragment thereof (*e.g.*, a protein comprising an active, inactive, or partially active DNA cleavage domain of a Cas protein, and/or the gRNA binding domain of Cas protein). CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In some CRISPR systems, correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA and/or tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. In nature, DNA-binding and cleavage may require 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. Cas proteins recognize a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. CRISPR-Cas proteins include without limitation Cas9, CasX, CasY, Cpf1, C2c1, and C2c3 or active fragments thereof. Additional suitable CRISPR-Cas proteins and sequences will be apparent to those of skill in the art based on this disclosure.

A nuclease-inactivated CRISPR-Cas protein may interchangeably be referred to as a “dCas” protein (for nuclease-“dead” Cas) or catalytically inactive Cas. Methods for generating a Cas 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 Cas nuclease has an inactive (e.g., an inactivated) DNA cleavage domain, that is, the Cas is a nickase, referred to as an “nCas” protein (for “nickase” Cas). A Cas variant shares homology to a CRISPR-Cas protein, or a fragment thereof. For example, a Cas 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 a wild type CRISPR-Cas protein. In some embodiments, the Cas 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 a wild type CRISPR-Cas protein. In some embodiments, the Cas variant comprises a fragment of a CRISPR-Cas protein (*e.g.*, a gRNA programmable DNA 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 a wild type CRISPR-Cas protein. 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 CRISPR-Cas protein. 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 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 "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

TGACACGACACAGCCGTGTATATGAGGAAGGGTAGCTGGATGGGGGGGGGGGGAATACGTTCAGAGAGGA
CATTAGCGAGCGTCTTGTTGGTGGCCTTGAGTCTAGACACCTGCAGACATGACCGACGCTGAGTACGTGA
GAATCCATGAGAAGTTGGACATCTACACGTTTAAGAAACAGTTTTTCAACAACAAAAAATCCGTGTCGCA
TAGATGCTACGTTCTCTTTGAATTAAAACGACGGGGTGAACGTAGAGCGTGTTTTTGGGGCTATGCTGTG
AATAAACCACAGAGCGGGACAGAACGTGGAATTCACGCCGAAATCTTTAGCATTAGAAAAGTCGAAGAAT
ACCTGCGCGACAACCCCGGACAATTCACGATAAATTGGTACTCATCCTGGAGTCCTTGTGCAGATTGCGC
TGAAAAGATCTTAGAATGGTATAACCAGGAGCTGCGGGGGAACGGCCACACTTTGAAAATCTGGGCTTGC
AAACTCTATTACGAGAAAAATGCGAGGAATCAAATTGGGCTGTGGAACCTCAGAGATAACGGGGTTGGGT
TGAATGTAATGGTAAGTGAACACTACCAATGTTGCAGGAAAATATTCATCCAATCGTCGCACAATCAATT
GAATGAGAATAGATGGCTTGAGAAGACTTTGAAGCGAGCTGAAAAACGACGGAGCGAGTTGTCCATTATG
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

AGAGAACCATCATTAATTGAAGTGAGATTTTTCTGGCCTGAGACTTGCAGGGAGGCAAGAAGACACTCTG
GACACCACTATGGACAGGTAAAGAGGCAGTCTTCTCGTGGGTGATTGCACTGGCCTTCCTCTCAGAGCAA
ATCTGAGTAATGAGACTGGTAGCTATCCCTTTCTCTCATGTAACTGTCTGACTGATAAGATCAGCTTGAT
CAATATGCATATATATTTTTTGATCTGTCTCCTTTTCTTCTATTCAGATCTTATACGCTGTCAGCCCAAT
TCTTTCTGTTTCAGACTTCTCTTGATTTCCCTCTTTTTCATGTGGCAAAAGAAGTAGTGCGTACAATGTA
CTGATTCGTCCTGAGATTTGTACCATGGTTGAAACTAATTTATGGTAATAATATTAACATAGCAAATCTT

TAGAGACTCAAATCATGAAAAGGTAATAGCAGTACTGTACTAAAAACGGTAGTGCTAATTTTCGTAATAA
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GTGCTGGAATTATAGACATGAGCCATCACATCCAATATACAGAATAAAGATTTTTAATGGAGGATTTAAT
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GCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGCCGGGCGGATCACCTGTGGTCAGGAGTTTGAGA
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CAACCCTTGCAATGAAGATGAGCAGATCCACAGGAAAACTTGAATGCACAACTGTCTTATTTTAATCTTA
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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)

Canine 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

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

Mouse APOBEC-3

MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRKDCDSPVSLHHGVFKNKDNI*HAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFEC*AEQIVRFLATHHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKRLLTNFRYQDSKLQEILRPCYIPVPSSSSSTLSNICLTKGLPETRFCVEGRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKPYLCYQLEQFNGQAPLKGCLLSEKGKQ*HAEILFLDKIRSMELSQVTITCYLTWSPCPNC*AWQLAAFKRDRPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEIISRRTQRRLRRIKESWGLQDLVNDFGNLQLGPPMS (italic: nucleic acid editing domain)

Rat APOBEC-3 :

MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNRLRYAIDRKDTFLCYEVTRKDCDSPVSLHHGVFKNKDNI*HAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFEC*AEQVLRFLATHHNLSLDIFSSRLYNIRDPENQQNLCRLVQEGAQVAAMDLYEFKKCWKKFVDNGGRRFRPWKKLLTNFRYQDSKLQEILRPCYIPVPSSSSSTLSNICLTKGLPETRFCVERRRVHLLSEEEFYSQFYNQRVKHLCYYHGVKPYLCYQLEQFNGQAPLKGCLLSEKGKQ*HAEILFLDKIRSMELSQVIITCYLTWSPCPNC*AWQLAAFKRDRPDLILHIYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEIISRRTQRRLHRIKESWGLQDLVNDFGNLQLGPPMS (italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3 G:

MVEPMDPRTFVSNFNNRPILSGLNTVWLCCEVKTKDPSGPPLDAKIFQGKVYSKAKYHPEMRFLRWFHKWRQLHHDQEYKVTWYVSWSPCTRCANSVATFLAKDPKVTLTIFVARLYYFWKPDYQQALRILCQKRGGPHATMKIMNYNEFQDCWNKFVDGRGKPFKPRNNLPKHYTLLQATLGELLRHLMDPGTFTSNFNNKPWVSGQHETYLCYKVERLHNDTWVPLNQHRGFLRNQAPNIHGFPKGRHAELCFLDLIPFWKLDGQQYRVTCFTSWSPCFSCAQEMAKFISNNEHVSLCIFAARIYDDQGRYQEGLRALHRDGAKIAMMNYSEFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI

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

Chimpanzee APOBEC-3 G:

MKPHFRNPVERMYQDTFSDNFYNRPILSHRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSKLKY*HPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDVATFLAEDPKVTLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTSNFNNELWVRGRHETYLCYEVERLHNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLDLHQDYRVTCFTSWSPCFSC*AQEMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLAKAGAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLEEHSQALSGRLRAILQNQGN

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

Green monkey APOBEC-3G:

MNPQIRNMVEQMEPDIFVYYFNNRPILSGRNTVWLCYEVKTKDPSGPPLDANIFQGKLYPEAKD*HPEMKFLHWFRKWRQLHRDQEYEVTWYVSWSPCTRC*ANSVATFLAEDPKVTLTIFVARLYYFWKPDYQQALRILCQERGGPHATMKIMNYNEFQHCWNEFVDGQGKPFKPRKNLPKHYTLLHATLGELLRHVMDPGTFTS

NFNNKPWVSGQRETYLCYKVERSHNDTWVLLNQHRGFLRNQAPDRHGFPKGR*HAELCFLDLIPFWKLD*
*DQQYRVTCFTSWSPCFSC*AQKMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLHRDGAKIAVMNYS
EFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI

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

Human APOBEC-3G:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGQVYSELKY*HPEM*
*RFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDMATFLAEDPKVTLTIFVARLYYFWDPDYQEALR
SLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTF
NFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLD*
*LDQDYRVTCFTSWSPCFSC*AQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTY
SEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN

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

Human APOBEC-3F:

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQVYSQPEH*HAEM*
*CFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKLAEFLAEHPNVTLTISAARLYYYWERDYRRALCR
LSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPWYKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHF
KNLRKAYGRNESWLCFTMEVVKHHSPVSWKRGVFRNQVDPETH*CHAERCFLSWFCDDILSPNTNYEVT*
*WYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCW
ENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE

(italic: nucleic acid editing domain)

Human APOBEC-3B:

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGQVYFKPQY*HAE*
*MCFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKLAEFLSEHPNVTLTISAARLYYYWERDYRRALC
RLSQAGARVTIMDYEEFAYCWENFVYNEGQQFMPWYKFDENYAFLHRTLKEILRYLMDPDTFTFNFNN
DPLVLRRRQTYLCYEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFY*GRHAELRFLDLVPSLQLDPAQI*
*YRVTWFISWSPCFSWGC*AGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDE
FEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Rat APOBEC-3B:

MQPQGLGPNAGMGPVCLGCSHRRPYSPIRNPLKKLYQQTFYFHFKNVRYAWGRKNNFLCYEVNGMDCA
LPVPLRQGVFRKQGHIHAELCFIYWFHDKVLRVLSPMEEFKVTWYMSWSPCSKCAEQVARFLAAHRNL
SLAIFSSRLYYYLRNPNYQQKLCRLIQEGVHVAAMDLPEFKKCWNKFVDNDGQPFRPWMRLRINFSFY
DCKLQEIFSRMNLLREDVFYLQFNNSHRVKPVQNRYYRRKSYLCYQLERANGQEPLKGYLLYKKGEQH

VEILFLEKMRSMELSQVRITCYLTWSPCPNCARQLAAFKKDHPDLILRIYTSRLYFWRKKFQKGLCTLWRSGIHVDVMDLPQFADCWTNFVNPQRPFRPWNELEKNSWRIQRRLRRIKESWGL

Bovine APOBEC-3B:

DGWEVAFRSGTVLKAGVLGVSMTEGWAGSGHPGQGACVWTPGTRNTMNLLREVLFKQQFGNQPRVPAPYYRRKTYLCYQLKQRNDLTLDRGCFRNKKQRHAERFIDKINSLDLNPSQSYKIICYITWSPCPNCANELVNFITRNNHLKLEIFASRLYFHWIKSFKMGLQDLQNAGISVAVMTHTEFEDCWEQFVDNQSRPFQPWDKLEQYSASIRRRLQRILTAPI

Chimpanzee APOBEC-3B:

MNPQIRNPMEWMYQRTFYYNFENEPILYGRSYTWLCYEVKIRRGHSNLLWDTGVFRGQMYSQPEHHAEMCFLSWFCGNQLSAYKCFQITWFVSWTPCPDCVAKLAKFLAEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYNEGQPFMPWYKFDDNYAFLHRTLKEIIRHLMDPDTFTFNFNNDPLVLRRHQTYLCYEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCAGQVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQVRASSLCMVPHRPPPPPQSPGPCLPLCSEPPLGSLLPTGRPAPSLPFLLTASFSFPPPASLPPLPSLSLSPGHLPVPSFHSLTSCSIQPPCSSRIRETEGWASVSKEGRDLG

Human APOBEC-3C:

MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVFRNQVDSETH*CHAERCFLSWFCDDILSPNTKYQVTWYTSWSPCPDC*AGEVAEFLARHSNVNLTIFTARLYYFQYPCYQEGLRSLSQEGVAVEIMDYEDFKYCWENFVYNDNEPFKPWKGLKTNFRLLKRRLRESLQ

(italic: nucleic acid editing domain)

Gorilla APOBEC3C

MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVFRNQVDSETH*CHAERCFLSWECDDILSPNTNYQVTWYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLYYFQDTDYQEGLRSLSQEGVAVKIMDYKDFKYCWENFVYNDDEPFKPWKGLKYNFRFLKRRLQEILE

(italic: nucleic acid editing domain)

Human APOBEC-3A:

MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQHRGFLHNQAKNLLCGFYGR*HAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGC*AGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWDTFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3A:

MDGSPASRPRHLMDPNTFTFNFNNDLSVRGRHQTYLCYEVERLDNGTWVPMDERRGFLCNKAKNVPCGDYGC*HVELRFLCEVPSWQLDPAQTYRVTWFISWSPC*FRRGCAGQVRVFLQENKHVRLRIFAARIYDYDPLYQEALRTLRDAGAQVSIMTYEEFKHCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAILQNQGN

(italic: nucleic acid editing domain)

Bovine APOBEC-3A:

MDEYTFTENFNNQGWPSKTYLCYEMERLDGDATIPLDEYKGFVRNKGLDQPEKPC*HAELYFLGKIHSWNLDRNQHYRLTCFISWSPC*YDCAQKLTTFLKENHHISLHILASRIYTHNRFGCHQSGLCELQAAGARITIMTFEDFKHCWETFVDHKGKPFQPWEGLNVKSQALCTELQAILKTQQN

(italic: nucleic acid editing domain)

Human APOBEC-3H:

MALLTAETFRLQFNNKRRLRRPYYPRKALLCYQLTPQNGSTPTRGYFENKKKC*HAEICFINEIKSMGLDETQCYQVTCYLTWSPCSSC*AWELVDFIKAHDHLNLGIFASRLYYHWCKPQQKGLRLLCGSQVPVEVMGFPKFADCWENFVDHEKPLSFNPYKMLEELDKNSRAIKRRLERIKIPGVRAQGRYMDILCDAEV

(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3H:

MALLTAKTFSLQFNNKRRVNKPYYPRKALLCYQLTPQNGSTPTRGHLKNKKKDHAEIRFINKIKSMGLDETQCYQVTCYLTWSPCPSCAGELVDFIKAHRHLNLRIFASRLYYHWRPNYQEGLLLLCGSQVPVEVMGLPEFTDCWENFVDHKEPPSFNPSEKLEELDKNSQAIKRRLERIKSRSVDVLENGLRSLQLGPVTPSSSIRNSR

Human APOBEC-3D:

MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLWDTGVFRGPVLPKRQSNHRQEVYFRFEN*HAEMCFLSWFCGNRLPANRRFQITWFVSWNPC*LPCVVKVTKFLAEHPNVTLTISAARLYYYRDRDWRWVLLRLHKAGARVKIMDYEDFAYCWENFVCNEGQPFMPWYKFDDNYASLHRTLKEILRNPMEAMYPHIFYFHFKNLLKACGRNESWLCFTMEVTKHHSAVFRKRGVFRNQVDPETHC*HAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPEC*AGEVAEFLARHSNVNLTIFTARLCYFWDTDYQEGLCSLSQEGASVKIMGYKDFVSCWKNFVYSDDEPFKPWKGLQTNFRLLKRRLREILQ

(italic: nucleic acid editing domain)

Human APOBEC-1 :

MTSEKGPSTGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIWRSSGKNTTNHVEVNFIKKFTSERDFHPSMSCSITWFLSWSPCWECSQAIREFLSRHPGVTLVIYVARLFWHMDQQNRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDEAHWPQYPPLWMMLYALELHCIILSLPPCLKISRRWQNHLTFFRLHLQNCHYQTIPPHILLATGLIHPSVAWR

Mouse APOBEC-1 :

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSVWRHTSQNTSNHVEVNFLEKFTTERYFRPNTRCSITWFLSWSPCGECSRAITEFLSRHPYVTLFIYIARLYHHTDQRNRQGLRDLISSGVTIQIMTEQEYCYCWRNFVNYPPSNEAYWPRYPHLWVKLYVLELYCIILGLPPCLKILRRKQPQLTFFTITLQTCHYQRIPPHLLWATGLK

Rat APOBEC-1 :

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWRHTSQNTNKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVTLFIYIARLYHHADPRNRQGLRDLIS

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 “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. In particular embodiments, a disease amenable to treatment with compositions of the invention is associated with a point mutation, a splicing event, a premature stop codon, or a misfolding event.

By “DNA binding protein domain” is meant a polypeptide or fragment thereof that binds DNA. In some embodiments, the DNA binding protein domain is a Zinc Finger or TALE domain having sequence specific DNA binding activity. In other embodiments, the DNA binding protein domain is a domain of a CRISPR-Cas protein (e.g., Cas9) that binds DNA, including, for example, that binds a protospacer adjacent motif (PAM). In some embodiments, the DNA binding protein domain forms a complex with a polynucleotide (e.g., single-guide RNA), and the complex binds DNA sequences specified by a gRNA and a protospacer adjacent motif. In some embodiments, the DNA binding protein domain comprises nickase activity (e.g., nCas9) or is catalytically inactive (e.g., dCas9, Zinc finger domain, TALE). In still other embodiments, the DNA binding protein domain is a catalytically inactive variant of the homing endonuclease I-SceI or the DNA-binding domain of the TALE protein AvrBs4. See, for example, Gabsalilow et al., Nucleic Acids Research, Volume 41, Issue 7, 1 April 2013, Pages e83. In some embodiments, a DNA binding protein domain is fused to a domain having catalytic activity (e.g., FokI, MutH). In particular embodiments, a Zinc finger domain is fused to a catalytic domain of the endonuclease FokI. In other embodiments, a TALE is fused to MutH, which comprises site-specific DNA nicking activity.

The term “effective amount,” as used herein, refers to an amount of a biologically active agent that is sufficient to elicit a desired biological response. In particular embodiments, an effective amount is the amount of two or more plasmids comprising portions of a base editor system that are sufficient to express an active base editing system in a cell transfected with the plasmids. As will be appreciated by the skilled artisan, the effective amount of an agent, e.g., a fusion protein may vary depending on various factors as, for example, on the desired biological response, e.g., on the specific allele, genome, or target site to be edited, on the cell or tissue being targeted, and on the agent being used.

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.

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

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

Exemplary nucleotide and amino acid sequences of inteins are provided.

DnaE Intein-N DNA:
TGCCTGTCATACGAAACCGAGATACTGACAGTAGAATATGGCCTTCTGCC
AATCGGGAAGATTGTGGAGAAACGGATAGAATGCACAGTTTACTCTGTCG
ATAACAATGGTAACATTTATACTCAGCCAGTTGCCCAGTGGCACGACCGG
GGAGAGCAGGAAGTATTCGAATACTGTCTGGAGGATGGAAGTCTCATTAG
GGCCACTAAGGACCACAAATTTATGACAGTCGATGGCCAGATGCTGCCTA
TAGACGAAATCTTTGAGCGAGAGTTGGACCTCATGCGAGTTGACAACCTT
CCTAAT

DnaE Intein-N Protein:
CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDR
GEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNL PN

DnaE Intein-C DNA:
ATGATCAAGATAGCTACAAGGAAGTATCTTGGCAAACAAAACGTTTATGA
TATTGGAGTCGAAAGAGATCACAACTTTGCTCTGAAGAACGGATTCATAG
CTTCTAAT

Intein-C: MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN

Cfa-N DNA:

TGCCTGTCTTATGATACCGAGATACTTACCGTTGAATATGGCTTCTTGCC
TATTGGAAAGATTGTCGAAGAGAGAATTGAATGCACAGTATATACTGTAG
ACAAGAATGGTTTCGTTTACACACAGCCCATTGCTCAATGGCACAATCGC
GGCGAACAAGAAGTATTTGAGTACTGTCTCGAGGATGGAAGCATCATACG
AGCAACTAAAGATCATAAATTCATGACCACTGACGGGCAGATGTTGCCAA
TAGATGAGATATTCGAGCGGGGCTTGGATCTCAAACAAGTGGATGGATTG CCA

Cfa-N Protein:

CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNR
GEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGL P

Cfa-C DNA:

ATGAAGAGGACTGCCGATGGATCAGAGTTTGAATCTCCCAAGAAGAAGAG
GAAAGTAAAGATAATATCTCGAAAAAGTCTTGGTACCCAAAATGTCTATG
ATATTGGAGTGGAGAAAGATCACAACTTCCTTCTCAAGAACGGTCTCGTA
GCCAGCAAC

Cfa-C Protein:

MKRTADGSEFESPKKKRKVKIISRKSLGTQNVYDIGVEKDHNFLLKNGLV ASN

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

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. 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.

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

The term "mutation," as used herein, refers to a substitution of a residue within a sequence, *e.g.*, a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (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 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, Cpf1, C2c1, and 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.

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

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. In one embodiment, a reference is the activity of a full length nucleobase editor expressed in a single plasmid in the same cells and under the same conditions as a nucleobase editor expressed in fragments comprising inteins for intein-dependent re-assembly.

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.

The term “single nucleotide polymorphism (SNP)” is a variation in a single nucleotide that occurs at a specific position in the genome, where each variation is present to some appreciable degree within a population (*e.g.,* > 1%). For example, at a specific base position in the human genome, the C nucleotide can appear in most individuals, but in a minority of individuals, the position is occupied by an A. This means that there is a SNP at this specific position, and the two possible nucleotide variations, C or A, are said to be alleles for this position. SNPs underlie differences in susceptibility to disease. The severity of illness and the way our body responds to treatments are also manifestations of genetic variations. SNPs can fall within coding regions of genes, non-coding regions of genes, or in the intergenic regions (regions between genes). In some embodiments, SNPs within a coding sequence do not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code. SNPs in the coding region are of two types: synonymous and nonsynonymous SNPs. Synonymous SNPs do not affect the protein sequence, while nonsynonymous SNPs change the amino acid sequence of protein. The nonsynonymous SNPs are of two types: missense and nonsense. SNPs that are not in protein-coding regions can still affect gene splicing, transcription factor binding, messenger RNA degradation, or the sequence of noncoding RNA. Gene expression affected by this type of SNP is referred to as an eSNP (expression SNP) and can be upstream or downstream from the gene. A single nucleotide variant (SNV) is a variation in a single nucleotide without any limitations of frequency and can arise in somatic cells. A somatic single nucleotide variation can also be called a single-nucleotide alteration.

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

A "split Cas9 protein" or "split Cas9" refers to a Cas9 protein that is provided as an N-terminal fragment and a C-terminal fragment encoded by two separate nucleotide sequences. The polypeptides corresponding to the N-terminal portion and the C-terminal portion of the Cas9 protein may be spliced to form a “reconstituted” Cas9 protein. In particular embodiments, the Cas9 protein is divided into two fragments within a disordered region of the protein, e.g., as described in Nishimasu et al., Cell, Volume 156, Issue 5, pp. 935-949, 2014, or as described in Jiang et al. (2016) Science 351: 867-871. PDB file: 5F9R, each of which is incorporated herein by reference. A disordered region may be determined by one or more protein structure determination techniques known in the art, including without limitation, X-ray crystallography, NMR spectroscopy, electron microscopy (e.g., cryoEM), and/or in silico protein modeling. In some embodiments, the protein is divided into two fragments at any C, T, A, or S within a region of SpCas9 between about amino acids A292-G364, F445-K483, or E565-T637, or at corresponding positions in any other Cas9, Cas9 variant (e.g., nCas9, dCas9), or other napDNAbp. In some embodiments, protein is divided into two fragments at SpCas9 T310, T313, A456, S469, or C574. In some embodiments, the process of dividing the protein into two fragments is referred to as “splitting” the protein.

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.

As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

The term "uracil glycosylase inhibitor" or "UGI," as used herein, refers to a protein that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a modified version thereof. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. For example, in some embodiments, a UGI domain comprises a fragment of the amino acid sequence set forth herein below. In some embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of an exemplary UGI sequence provided herein. In some embodiments, a UGI comprises an amino acid sequence homologous to the amino acid sequence set forth herein below, or an amino acid sequence homologous to a fragment of the amino acid sequence set forth herein below. In some embodiments, proteins comprising UGI or fragments of UGI or homologs of UGI or UGI fragments are referred to as "UGI variants." A UGI variant shares homology to UGI, or a fragment thereof. For example a UGI variant is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to a wild type UGI or a UGI as set forth herein. In some embodiments, the UGI variant comprises a fragment of UGI, such that the fragment is at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% to the corresponding fragment of wild-type UGI or a UGI as set forth below. In some embodiments, the UGI comprises the following amino acid sequence:

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>splP14739IUNGI_BPPB2 Uracil-DNA glycosylase inhibitor
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLT S
D APE YKPW ALVIQDS NGENKIKML
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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 is a schematic diagram of an A-to-G Base Editor (ABE) fusion protein nucleobase editor comprising two adenosine deaminase domains, wild-type (wt) TadA and an evolved (evo) version of TadA fused to *S. pyogenes* (Sp) Cas9 nickase (nCas9) with a C-terminal bipartite Nuclear Localization Signal (NLS). The schematic also identifies three regions of the Cas9 protein that are unstructured where the fusion protein may be split into N- and C- terminal fragments that can be reconstituted using a split intein system (i.e., fusing an intein-N and an intein-C to the N- and C-terminal fragments, respectively).

FIG. 2 reproduces the fusion protein described in FIG. 1 and provides three graphs, which quantitate the base editing activity of a base editing system that includes a nucleobase editor fusion protein (i.e., ABE) comprising a spliced nCas9. ABE was split at the indicated amino acid positions (e.g., T310, T313, A456, S469, and C574 with reference to SpCas9 amino acid sequence). The N- and C- terminal fragments of ABE were fused to an intein-N and intein-C, respectively. These fragments and the indicated guide RNA, were each expressed on separate plasmids in cultured HEK 293 cells that express the protein having an

ABCA4 gene with a 5882G>A mutation. The base editing activity of the reconstituted ABE on the ABCA4 5882A>G target was compared to the activity of a control ABE. Base editing activity was dependent on the presence of both the N- and C- terminal fragments of ABE. When only one of the N- or C- terminal fragments of ABE was expressed no base editing activity was observed.

FIG. 3 is a graph confirming base editing activity with the 21-nt guide as described in FIG. 2. Note the experiments in FIG. 3 were performed in a different format from those in FIG. 2. All of the reconstituted ABEs showed good base editing activity. This activity was dependent on the presence of both the N- and C- terminal fragments of nCas9. When only the N- or C- terminal fragment of nCas9 was expressed, no activity was observed. The activity of the reconstituted ABE was compared to the activity of control ABE7.09 and ABE7.10 fusion proteins.

FIG. 4 is a graph quantitating base editing activity as described in FIG. 2. In FIG. 4, a 20-nucleotide (nt) guide RNA was used, which included a hammer ribozyme (HRz). The activity of the various reconstituted ABEs was compared to the activity of control ABE7.09 and ABE7.10 fusion proteins.

FIGS. 5A-5D show determination of multiplicity of infection (MOI) for AAV2 co-infection of ARPE-19 cells. FIG. 5A are graphs showing 1:1 coinfections of AAV2/ CMV-mCherry and AAV2/ CMV-EmGFP at various viral loads (vg/cell). FIG. 5B depicts fluorescence images detecting EmGFP (left) and mCherry (center); and a merged image showing EmGFP and mCherry co-localization (right). FIG. 5C is a graph depicting percentage of cells expressing mCherry at various viral loads (vg/cell), when infected with AAV2/ CMV-mCherry. FIG. 5D is a graph depicting percentage of cells expressing EmGFP at various viral loads (vg/cell), when infected with AAV2/ EmGFP.

FIG. 6 is a series of graphs showing delivery of split editor to ARPE-19 cells via dual AAV2 infection yields high A>G conversion at ABCA4 5882A. Multipicity of infection (MOI) of dual infection at 20,000 vg/cell (top, left); 30,000 vg/cell (top, right); 40,000 vg/cell (bottom, left); and 60,000 vg/cell (bottom, right) are shown.

DETAILED DESCRIPTION OF THE INVENTION

As described below, the present invention provides compositions and methods for delivering a base editing system. The invention is based, at least in part, on the discovery that an A-to-G nucleobase editor (ABE) can be "split" and reconstituted using split inteins. Polynucleotides encoding N- and C-terminal fragments of ABE fused respectively to intein-N and intein-C of a split intein pair and delivered to a cell on separate vectors, together with a single guide RNA. The encoded ABE fragments were spliced together to reconstitute a functional nucleobase editor fusion protein that is useful *inter alia* for targeted editing of nucleic acid sequences.

Inteins

Inteins (*in*tervening pro*tein*) 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 (*ex*ternal pro*tein*) 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.

Adenosine deaminases

In some embodiments, the fusion proteins 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 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 TadA (e.g., having double-stranded substrate activity) or TadA7.10 is provided as a homodimer or as a monomer.

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

In some embodiments, the 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 S 146X 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., rAPOBEC1 . In some embodiments, the deaminase is a Petromyzon marinus cytidine deaminase 1 (pmCDAl). In some embodiments, the deaminase 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).

Other Nucleobase Editors

The invention provides for a nucleobase editor fusion protein where virtually any nucleobase editor known in the art can be substituted for a cytidine deaminase or adenosine deaminase domain in a fusion protein of the invention.

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).

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGE
TAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE

RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEG DLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLP GEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYA DLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQ RTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRF AWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFT VYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECF DSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN RNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDEL VKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENT QLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRS DKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAG FIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVL VVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYS LFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTN LGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD (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

In one particular embodiment, the invention features a nucleobase editor comprising a Cas9 domain split into two fragments, each having terminal inteins, i.e., the N-terminal fragment fused to one member of the intein system at its C-terminal, and the C-terminal fragment having a member of the intein system at its N-terminal:

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
SpCas9-MQKFRAER	NGC
xCas9 (sp)	NGN
saCas9	NNGRRT
saCas9-KKH	NNNRRT
spCas9-MQKSER	NGCG
spCas9-MQKSER	NGCN
spCas9-LRKIQK	NGTN
spCas9-LRVSQK	NGTN

Variant	PAM
spCas9-LRVSQL	NGTN
SpyMacCas9	NAA
Cpf1	5' (TTTV)

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

In some embodiments, the 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**V**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**<u>E</u>**Y**<u>R</u>**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.

In particular embodiments, a fusion protein of the invention comprises a dCas9 domain that binds a canonical PAM sequence and an nCas9 domain that binds a non-canonical PAM sequence (e.g., a non-canonical PAM identified in Table 1). In another embodiment, a fusion protein of the invention comprises an nCas9 domain that binds a canonical PAM sequence and an dCas9 domain that binds a non-canonical PAM sequence (e.g., a non-canonical PAM identified in Table 1).

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**A**ITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGK
ATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM
PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
AKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

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: $(1-(1-(b+c)/(a+b+c))^{1/2})\times100$, 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.

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, wherein X is any amino acid except for D. In some embodiments, the SpCas9 comprises a D9A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having an NGG, a NGA, or a NGCG PAM sequence.

In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a 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 RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFVEEDKKHERHPIFGNIVDE

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

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

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFI
QLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGL
TPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF
YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK
DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT
NFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRK
VTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIV
LTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDF

LKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSD NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKH VAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS DKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPI REQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQ LGGD. **In this sequence, residues V1135, Q1335, and 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.

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. The linker may be a peptide linker or a non-peptide linker. In certain embodiments, the linker may be a UV-cleavable linker. In some embodiments, the linker may be a polynucleotide linker, e.g. a RNA linker. 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, 61, 62, 63, 64, 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 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). In some embodiments, the guide nucleic acid (e.g., guide RNA) is complementary to a sequence associated with a disease or disorder.

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.

In some embodiments, a fusion protein of the invention is used for mutagenizing a target of interest. These mutations may affect the function of the target. For example, when a nucleobase editor is used to target a regulatory region the function of the regulatory region is altered and the expression of the downstream protein is reduced.

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 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.

In some embodiments, a fusion protein of the invention is used for mutagenizing a target of interest. In particular, a multi-effector nucleobase editor described herein is capable of making multiple mutations within a target sequence. These mutations may affect the function of the target. For example, when a multi-effector nucleobase editor is used to target a regulatory region the function of the regulatory region is altered and the expression of the downstream protein is reduced.

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 improve base editor efficiency by modifying a specific nucleotide base 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 generate the intended mutation. In some embodiments, the intended mutation is a mutation that generates a stop codon, for example, a premature stop codon within the coding region of a gene. In some embodiments, the intended mutation is a mutation that eliminates a stop codon. In some embodiments, the intended mutation is a mutation that alters the splicing of a gene. In some embodiments, the intended mutation is a mutation that alters the regulatory sequence of a gene (*e.g.*, a gene promotor or gene repressor). 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 (*e.g.*, a base pair of a double-stranded DNA sequence). 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. 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.

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. 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 or cytidine deaminase) to a polynucleotide encoding a napDNAbp to prepare a polynucleotide that encodes a fusion protein of the invention

A DNA encoding a protein domain described herein can be obtained by any method known in the art, such as by chemically synthesizing the DNA chain, by PCR, or by the Gibson Assembly method. The advantage of constructing a full-length DNA by chemical synthesis or a combination of PCR method or Gibson Assembly method is that the codons may be optimized to ensure that the fusion protein is expressed at a high level in a host cell. Optimized codons may be selected using the genetic code use frequency database (http://www.kazusa.or.jp/codon/index.html), which is disclosed in the home page of Kazusa DNA Research Institute. Once obtained polynucleotides encoding fusion proteins are incorporated into suitable expression vectors.

Suitable expression vectors include *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); plasmids suitable for expression in insect cells (e.g., pFast-Bac); plasmids suitable for expression in mammalian cells (e.g., pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo); also bacteriophages, such as lamda phage and the like; other vectors that may be used include insect viral vectors, such as baculovirus and the like (e.g., BmNPV, AcNPV); and viral vectors suitable for expression in a mammalian cell, such as retrovirus, vaccinia virus, adenovirus and the like.

Fusion protein encoding polynucleotides are typically expressed under the control of a suitable promoter that is useful for expression in a desired host cell. For example, when the host is an animal cell, any one of the following promoters are used 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. 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.

If desired, the expression vector also includes any one or more of an enhancer, splicing signal, terminator, polyA addition signal, a selection marker (e.g., a drug resistance gene, auxotrophic complementary gene and the like), or a replication origin.

An RNA encoding a protein domain described herein can be prepared by, for example, by transcribing an mRNA in an *in vitro transcription* system.

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.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 are used.

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 *Trichoplusiani*, High Five, cells derived from an egg of *Trichoplusiani*, *Mamestra brassicae*-derived cells, *Estigmena acrea*-derived cells and the like are used. When the virus is BmNPV, cells of a *Bombyx mori*-derived line (Bombyx mori N cell; BmN cell) and the like are used. Sf cells include, for example, Sf9 cell (ATCC CRL1711), Sf21 cell [all above, In Vivo, 13, 213-217 (1977)] and the like.

With regard to insects, 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, which are prepared from various plants (e.g., s rice, wheat, corn, tomato, cucumber, eggplant, carnations, Eustoma russellianum, tobacco, *Arabidopsis thaliana* a.

All the above-mentioned host cells may be haploid (monoploid), or polyploid (e.g., diploid, triploid, tetraploid and the like.

Expression vectors encoding a fusion protein of the invention are introduced into host cells using any transfection method (e.g., using lysozyme, PEG, CaCl2 coprecipitation, electroporation, microinjection, particle gun, lipofection, Agrobacterium 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. Methods for transducing the genus Bacillus are described in, for example, Molecular & General Genetics, 168, 111 (1979).

Yeast cells are transduced using 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 are transfected using methods described in, for example, Bio/Technology, 6, 47-55 (1988) and the like.

Mammalian cells are transfected using 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 cells are cultured, a liquid medium is used. The medium preferably contains a carbon source, nitrogen source, inorganic substance and other components 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 also contain yeast extract, vitamins, growth promoting factors and the like. The pH of the medium is preferably between about 5 to 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 used. 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 to about 40°C. Where necessary, aeration and stirring is performed.

Examples of medium suitable 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 to about 35°C. Where necessary, aeration and stirring may be performed.

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

Mammalian cells are cultured, for example, in any one of minimum essential medium (MEM) containing about 5 to 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. The pH of the medium is preferably about 6 to about 8. The culture is performed at 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 to about 8. The culture is performed at generally about 20°C to about 30°C. Where necessary, aeration and stirring may be performed.

Fusion protein expression may be regulated using 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 inducing agent is added to the medium (or removed from the medium) at an appropriate stage to induce expression of the fusion protein.

Prokaryotic cells such as Escherichia coli and the like can utilize an inductive promoter. Examples of the inducible promoters 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.

Nucleic Acid-Based Delivery of a Nucleobase Editor

Nucleic acids encoding nucleobase editors according to the present disclosure can be administered to subjects or delivered into cells (e.g., bacteria, yeast, fungi, insects, plants, and animal cells) by art-known methods or as described herein. For example, 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 or DNA complexes), or a combination thereof.

Nucleic acids encoding nucleobase editors can be delivered directly to cells (e.g., bacteria, yeast, fungi, insects, plants, and animal 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 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 one or more deaminases.

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.

Nucleic acid vectors according to this disclosure include recombinant viral vectors. Exemplary viral vectors are set forth herein above. Other viral vectors known in the art can also be used. In addition, viral particles can be used to deliver genome 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
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)	
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
	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 some aspects, the disclosure relates to the viral delivery of a fusion protein using, for example, a viral vector. Exemplary viral vectors include retroviral vectors (e.g. Maloney murine leukemia virus, MML-V), adenoviral vectors (e.g. AD100), lentiviral vectors (HIV and FIV-based vectors), herpesvirus vectors (e.g. HSV-2), and adeno-associated viral vectors.

Adeno-Associated Viral Vectors

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.

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 nucleobase 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 some embodiments, a portion or fragment of a nuclease (e.g., a fragment of a deaminase, such as cytidine deaminase, adenosine deaminase, or a fragment of *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.

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.

Screening of Nucleobase Editors

The suitability of nucleobase editors fusion proteins comprising "split" and reassembled Cas9 can be evaluated in various screening approaches as described herein. Each fragment of the fusion protein to be tested is delivered to a single cell of interest (e.g., a bacteria, yeast, fungi, insect, plant, or animal cell) together with a small amount of a vector encoding a reporter (e.g., GFP). These cells can be immortalized in human cell lines such as

293T, K562 or U20S. Alternatively, primary human cells 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 are used to target polynucleotides of interest. In one embodiment, a 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 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 nucleobase editor of the invention is used to target polynucleotides of interest within the genome of an organism (e.g., bacteria, yeast, fungi, insect, plant, and animal). In one embodiment, a 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.

Applications for Nucleobase Editors

The nucleobase editors can be used to target polynucleotides of interest to create alterations that modify protein expression. In one embodiment, a 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 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 nucleobase editors can be used to target polynucleotides of interest to create alterations that modify protein activity. In the context of mutagenesis, for example, nucleobase editors have a number of advantages over error-prone PCR and other polymerase-based methods. Unlike error-prone PCR, which induces random alterations throughout a polynucleotide, nucleobase editors of the invention can be used to target specific amino acids within a defined region of a protein of interest.

In other embodiments, 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., soybean, wheat, corn, cotton, canola, rice, tobacco, apple, grape, peach, plum, cherry). In one embodiment, a 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 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.

The present disclosure provides methods for the treatment of a subject diagnosed with diseases associated with or caused by gene mutations, including gene conversion, point mutations that affect splicing (e.g., alter a splice donor or acceptor site), abberrent or mis-folded proteins due to point mutations that can be corrected by a base editor system provided herein. For example, in some embodiments, a method is provided that comprises administering to a subject having such a disease, *e.g.*, a disease caused by a gene conversion or other genetic mutation, an effective amount of a nucleobase editor (*e.g.*, an adenosine deaminase base editor or a cytidine deaminase base editor, including one or more than one DNA binding protein domains) that edits the nucleoside base directly or indirectly associated with the mutation in the disease associatedgene. In a certain aspect, methods are provided for the treatment of additional diseases or disorders, *e.g.*, diseases or disorders that are associated or caused by a point mutation that can be corrected or ameliorated by deaminase mediated gene editing. Some such diseases are described herein, and additional suitable diseases that can be treated with the strategies and fusion proteins provided herein will be apparent to those of skill in the art based on the instant disclosure.

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. The term "pharmaceutical composition", as used herein, refers to a composition formulated for pharmaceutical use. 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).

As used here, the term "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the compound from one site (*e.g.*, the delivery site) of the body, to another site (*e.g.*, organ, tissue or portion of the body). A pharmaceutically acceptable carrier is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the tissue of the subject (*e.g.*, physiologically compatible, sterile, physiologic pH, etc.).

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. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient," "carrier," "pharmaceutically acceptable carrier," "vehicle," or the like are used interchangeably herein.

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. 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, any of the fusion proteins, gRNAs, systems, 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 systems or complexes provided herein. In some embodiments, the pharmaceutical composition comprises a ribonucleoprotein complex comprising an RNA-guided nuclease (e.g., Cas9) or a fragment thereof that forms a complex with a gRNA and a cationic lipid. In some embodiments, the pharmaceutical composition comprises a ribonucleoprotein complex comprising multiple programmable DNA binding proteins (e.g. Cas9, Zinc Finger, TALE, TALE-N proteins or fragments thereof). The programmable DNA binding proteins may comprise nuclease activity, nickase activity, or no nuclease activity. In some embodiments, the pharmaceutical composition comprises a gRNA, a nucleic acid programmable DNA binding protein, a cationic lipid, and a pharmaceutically acceptable excipient. Pharmaceutical compositions as described herein may 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. Pat. 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 all 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. 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 may 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 may 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 WO2011053982 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. In some embodiments, compositions in accordance with the present invention may 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 nucleic acid construct comprising a nucleotide sequence encoding a nucleobase editor fusion protein comprising a deaminase, a SpnCas9 domain that is "split" into two fragments, the N-terminal fragment comprising one member of an intein system, and the C-terminal fragment comprising another member of an intein system, and an NLS; wherein the nucleotide sequence is under the control of a heterologous promoter that drives expression of the fusion protein.

Some aspects of this disclosure provide cells (e.g., bacteria, yeast, fungi, insects, plants, and animal cells) comprising any of the nucleobase editor/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: Split and reassembled Cas9 retains function and activity in a base editing system

Viral vectors provide an attractive delivery system, but present technologies have limited capacity to deliver larger polynucleotides. To explore these challenges as they relate to base editing systems, a fusion protein was generated comprising, from N- to C-terminus, wild-type adenosine deaminase, TadA, fused to an evolved version of TadA, fused to nCas9 fused to a bipartite NLS. The fusion protein was split into N- and C-terminal fragments within unstructured regions of SpCas9 and delivered in two vectors together with a third plasmid encoding the guide RNA. The N and C-terminal fragments of nCas9 were fused to an intein-N and intein-C, respectively. The amino acid sequences encoding these inteins follow:

Cfa-N intein in fusions:
CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEY
CLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

Cfa(GEP)-C intein in fusions:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNC

The polynucleotide sequences encoding these inteins follow:

Cfa-N intein in fusions:
TGCCTGAGCTACGATACCGAGATCCTGACCGTGGAATACGGCTTCCTGCCTAT
CGGCAAGATCGTCGAGGAACGGATCGAGTGCACAGTGTACACCGTGGATAAGA
ATGGCTTCGTGTACACCCAGCCTATCGCTCAGTGGCACAACAGAGGCGAGCAA
GAGGTGTTCGAGTACTGCCTGGAAGATGGCAGCATCATCCGGGCCACCAAGGA
CCACAAGTTTATGACCACCGACGGCCAGATGCTGCCCATCGACGAGATCTTTG
AGAGAGGCCTGGACCTGAAACAGGTGGACGGACTGCCT

Cfa(GEP)-C intein in fusions:
ATGGTCAAGATCATCAGCAGAAAGAGCCTGGGCACCCAGAACGTGTACGATAT
CGGAGTGGGCGAGCCCCACAACTTTCTGCTCAAGAATGGCCTGGTGGCCAGC
AACTGC

Three regions of spCas9 were selected where the ABE fusion protein 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 was fused to an intein-N and the C- terminus of each fragment was fused to an intein C at the amino acid positions indicated in FIGS. 2-4, i.e., 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 enedilediv 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 tkaerggls e 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

HEK 293 cells were transfected with the plasmids. The fusion protein was reconstituted using the split intein system when the N- and C- terminal fragments were expressed and spliced together in the cultured cells where they associated with the guide RNA, thereby generating a functional nucleobase editing system. The sequences of the guide RNAs used follows:

20-nt guide protospacer (PAM: AGG)

5'-TGTCGAAGTTCGCCCTGGAG-3'

The hammerhead ribozyme (sequence below) is fused to the 5'-end of the protospacer above:

5'-GTCGACACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC-3'

21-nt ABCA4 guide protospacer (PAM: AGG)

5'-GTGTCGAAGTTCGCCCTGGAG-3'

HEK2 guide protospacer (PAM: GGG)

5'-GAACACAAAGCATAGACTGC-3'

Full guide sequence is the protospacer sequence appended directly to the 5'-side of the following sequence:

5'-
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAAC
TTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT-3'

Full guide transcripts are the following prior to hammerhead cleavage in the case of the 20-nt guide:

20-nt guide

5'-
GTCGACACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCTGTCGA
AGTTCGCCCTGGAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCT
AGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT-3'

21-nt guide

5'-
GTGTCGAAGTTCGCCCTGGAGGTTTTAGAGCTAGAAATAGCAAGTTAAAA
TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTT
TTT-3'

HEK2 guide

5'-
GAACACAAAGCATAGACTGCGTTTTAGAGCTAGAAATAGCAAGTTAAAAT
AAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT
TT-3'

Base-editing activity was assayed by detecting alterations in the ABCA4 gene. Sequence changes following base editing were detected using high throughput sequencing (HTS). The activity of the reconstituted ABE was compared to the activity of ABE7.10, which was expressed in the same cell type, but delivered using a single plasmid vector. The results of these experiments are shown in FIG. 2-4.

FIG. 2 shows the effect of splitting ABE into two fragments within the first region of Cas9, amino acids 292-365, on base editing activity of the reconstituted fusion protein. Surprisingly, the reconstituted base editor showed comparable activity to a control base editor expressed in a single vector in the presence of 21-nucleotide, 20-nucleotide and a HEK2 guide RNA.

The effect of splitting the ABE into two fragments at amino acids within the following regions: F445–K483 and E565–T637 of SpCas9 on base editing activity of the reconstituted fusion protein are shown at FIG. 3. Regardless of the position where the ABE was split, when the fusion protein was reconstituted using the intein system, it showed comparable activity to ABE7.10. When only the N- or C-terminal portion of the fragmented fusion protein was expressed, no base editing activity was observed. Thus, editing was dependent on the presence of both the N- and C- terminal fragments of Cas9. As shown in FIG. 4, fusion proteins reconstituted using the split intein system also showed activity when the 20-nucleotide guide RNA included a hammer head ribozyme. The experiments in FIG. 2 were performed in a different format from those in FIGS. 3 and 4.

The results reported herein were carried out using the following methods and materials.

A base editing system was transfected into cultured HEK 293T cells that contain a lentiviral integrated ABCA4 polynucleotide containing a 5882G>A mutation (HEK/ABCA4/ 5882G>A cells). Transfection was carried out using 1.5 μL of Lipofectamine 2000 per μg of DNA transfected. The base editing system includes a fusion protein, ABE7.10, comprising wild-type adenosine deaminase, TadA, fused to an evolved version of TadA, fused to SpCas9 fused to a C-terminal bipartite NLS.

N- and C- terminal fragments of ABE7.10 were each cloned into pCAG plasmids where their expression was driven by the CAG promoter. ABE7.10 under the control of the CMV promoter was expressed in HEK 293T cells as a reference. Each of the aforementioned nucleic acid sequences was codon optimized. Cells were transfected with three plasmids, a first plasmid encoding the N-terminal fragment of ABE7.10 fused to an intein-N, a second plasmid encoding the C-terminal fragment of ABE7.10 fused to an intein-C, and a plasmid expressing the guide RNA. The two plasmids encoding the base editor were transfected in equimolar ratios (9.05 x 10^{-}14 mol of each half; 863 ng total editor DNA). With regard to the guide, 127 ng of guide plasmid (9.05 x 10^-14 mol) was used in each transfection.

Control: 490 ng (9.05 x 10^{-}14 mol) of pCMV-ABE7.10 with bipartite NLS (C-terminal) and GeneArt codon optimization + pNMG-B8 (a non-relevant plasmid that does not express in mammalian cells used to normalize amount of DNA transfected).

The polynucleotide and amino acid sequences encoding full length ABE7.10 and the ABE7.10 "split" Cas9s depicted in FIGS. 2-4 follow:

Intact ABE7.10:

ATGAGCGAGGTGGAATTCAGCCACGAGTACTGGATGCGGCACGCCCTGACACT
GGCCAAAAGAGCTTGGGACGAGAGGGAAGTGCCTGTGGGAGCTGTGCTGGTG
CACAACAACAGAGTGATCGGCGAAGGCTGGAACAGACCCATCGGCAGACACG
ATCCTACAGCTCACGCCGAGATCATGGCCCTGAGACAAGGCGGACTGGTCATG
CAGAACTACCGGCTGATCGACGCCACACTGTACGTGACCCTGGAACCTTGCGT
GATGTGTGCCGGCGCTATGATCCACAGCAGAATCGGCAGAGTGGTGTTCGGC
GCCAGAGATGCCAAAACAGGCGCTGCCGGAAGCCTGATGGATGTGCTGCATCA
CCCCGGCATGAACCACAGAGTGGAAATCACCGAGGGCATCCTGGCCGATGAAT
GTGCCGCTCTGCTGAGCGACTTCTTCCGGATGCGGCGGCAAGAGATCAAGGC
CCAGAAGAAGGCCCAGTCCAGCACAGATAGCGGCGGATCTAGCGGAGGCAGC
TCTGGATCTGAGACACCTGGCACAAGCGAGAGCGCCACACCTGAAAGTTCTGG
CGGTTCTTCTGGCGGCAGCAGCGAGGTCGAGTTCTCTCACGAATATTGGATGA
GACACGCTCTCACCCTGGCTAAGAGAGCCAGGGACGAAAGAGAGGTGCCAGT
TGGCGCTGTCCTGGTGTTGAACAATCGCGTCATCGGAGAAGGATGGAATCGCG
CCATTGGCCTGCACGATCCAACCGCACATGCCGAAATTATGGCTCTGCGGCAA
GGCGGCCTCGTGATGCAAAATTACAGACTGATCGATGCTACCCTCTACGTCAC
CTTCGAGCCCTGTGTCATGTGTGCTGGGGCAATGATTCACTCCCGGATTGGCC
GCGTGGTGTTTGGAGTGCGGAATGCCAAGACTGGCGCCGCTGGATCTCTGATG
GACGTCCTGCACTATCCTGGGATGAACCACCGGGTCGAGATCACAGAGGGAAT
TCTGGCTGACGAGTGCGCTGCCCTGCTGTGCTACTTCTTTAGAATGCCCAGAC
AGGTGTTCAACGCCCAGAAAAAAGCTCAGAGCAGCACCGATTCCGGCGGAAGC
AGCGGAGGATCTTCTGGAAGCGAAACCCCAGGCACCAGCGAGTCTGCCACAC
CAGAATCATCTGGCGGTAGCTCCGGCGGCAGCGACAAGAAGTATTCTATCGGA

CTGGCCATCGGCACCAACTCTGTTGGATGGGCCGTGATCACCGACGAGTACAA GGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACAGGCACAGCATCA AGAAGAACCTGATCGGCGCACTGCTGTTCGACTCTGGCGAAACAGCCGAGGCC ACCAGACTGAAGAGAACAGCCCGCAGACGGTACACCAGAAGAAAGAACCGGAT CTGCTACCTCCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCT TCTTCCACAGACTGGAAGAGTCCTTCCTGGTGGAAGAGGACAAGAAGCACGAG AGACACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTA CCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCG ACCTGAGACTGATCTATCTGGCCCTGGCTCACATGATCAAGTTCCGGGGCCAC TTCCTGATCGAGGGCGACCTGAATCCTGACAACAGCGACGTGGACAAGCTGTT CATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCCATCAACG CCAGCGGAGTGGATGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAG ACGGCTGGAAAATCTGATCGCCCAGCTGCCTGGCGAGAAGAAGAATGGCCTGT TCGGCAACCTGATTGCCCTGAGCCTGGGCCTGACACCTAACTTCAAGAGCAAC TTCGACCTGGCCGAGGACGCCAAACTGCAGCTGAGCAAGGACACCTACGACG ACGACCTGGACAATCTGCTGGCCCAGATCGGCGATCAGTACGCCGACTTGTTT CTGGCCGCCAAGAATCTGAGCGACGCCATCCTGCTGTCCGACATCCTGAGAGT GAACACCGAGATCACCAAGGCACCTCTGAGCGCCTCTATGATCAAGAGATACG ACGAGCACCACCAGGATCTGACCCTGCTGAAGGCCCTCGTTAGACAGCAGCTG CCAGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCCGG CTACATTGATGGCGGAGCCAGCCAAGAGGAATTCTACAAGTTCATCAAGCCCAT CCTCGAGAAGATGGACGGCACCGAGGAACTGCTGGTCAAGCTGAACAGAGAG GACCTGCTGAGAAAGCAGAGAACCTTCGACAACGGCAGCATCCCTCACCAGAT CCACCTGGGAGAACTGCACGCCATTCTGCGGAGACAAGAGGACTTTTACCCAT TCCTGAAGGACAACCGGGAAAAGATCGAGAAAATCCTGACCTTCAGGATCCCC TACTACGTGGGACCACTGGCCAGAGGCAATAGCAGATTCGCCTGGATGACCAG AAAGAGCGAGGAAACCATCACTCCCTGGAACTTCGAGGAAGTGGTGGACAAGG GCGCCAGCGCTCAGTCCTTCATCGAGCGGATGACCAACTTCGATAAGAACCTG CCTAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGT GTACAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCG CCTTTCTGAGCGGCGAGCAGAAAAAGGCCATCGTGGATCTGCTGTTCAAGACC AACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGA GTGCTTCGACAGCGTCGAGATCTCCGGCGTGGAAGATCGGTTCAATGCCAGCC TGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACA

ACGAAGAGAACGAGGACATCCTTGAGGACATCGTGCTGACACTGACCCTGTTT
GAGGACAGAGAGATGATCGAGGAACGGCTGAAAACATACGCCCACCTGTTCGA
CGACAAAGTGATGAAGCAACTGAAGCGGCGGAGATACACCGGCTGGGGCAGA
CTGTCTCGGAAGCTGATCAACGGCATCCGGGATAAGCAGTCCGGCAAGACCAT
CCTGGACTTTCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGAT
TCACGACGACAGCCTCACCTTCAAAGAGGATATCCAGAAAGCCCAGGTGTCCG
GCCAGGGCGATTCTCTGCATGAGCACATTGCCAACCTGGCCGGCTCTCCCGCC
ATTAAGAAAGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTTGTGAAAGT
GATGGGCAGACACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACC
AGACCACACAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGA
AGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAA
ACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGACGG
GATATGTACGTGGACCAAGAGCTGGACATCAACAGACTGTCCGACTACGATGT
GGACCATATCGTGCCCCAGTCTTTTCTGAAGGACGACTCCATCGACAACAAGGT
CCTGACCAGATCCGACAAGAATCGGGGCAAGAGCGACAACGTGCCCTCCGAA
GAGGTGGTCAAGAAGATGAAGAACTACTGGCGACAGCTGCTGAACGCCAAGCT
GATTACCCAGCGGAAGTTCGACAATCTGACCAAGGCCGAAAGAGGCGGCCTGA
GCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCA
GATCACAAAGCACGTGGCACAGATTCTGGACTCTCGGATGAACACTAAGTACG
ACGAGAACGACAAACTGATCCGCGAAGTGAAAGTCATCACCCTGAAGTCCAAG
CTGGTGTCCGATTTCCGGAAGGATTTCCAGTTCTACAAAGTGCGCGAGATCAAC
AACTACCATCACGCCCACGACGCCTACCTGAATGCCGTTGTTGGAACAGCCCT
GATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGG
TGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAAGAGATTGGCAAGGC
AACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACAGAGATC
ACCCTCGCCAACGGCGAGATCAGAAAGCGGCCTCTGATCGAGACAAACGGCG
AAACCGGCGAGATTGTGTGGGATAAGGGCAGAGACTTTGCCACAGTGCGGAAA
GTGCTGAGCATGCCCCAAGTGAATATCGTGAAGAAAACCGAGGTGCAGACAGG
CGGCTTCAGCAAAGAGTCTATCCTGCCTAAGCGGAACTCCGACAAGCTGATCG
CCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGATTCTCCTACC
GTGGCCTATAGCGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAA
ACTCAAGAGCGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCT
TCGAGAAGAATCCGATCGATTTCCTCGAGGCCAAGGGCTACAAAGAAGTGAAA
AAGGACCTGATCATCAAGCTCCCCAAGTACTCCCTGTTCGAGCTGGAAAACGG

CCGGAAGAGAATGCTGGCCTCTGCTGGCGAACTGCAGAAGGGAAACGAACTG
GCCCTGCCTAGCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAG
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ABE7.10_Cfa(GEP)-C_Split_S577C:
ATGGTCAAGATCATCAGCAGAAAGAGCCTGGGCACCCAGAACGTGTACGATATCGGAGTGGGCGAGCCCCACAACTTTCTGCTCAAGAATGGCCTGGTGGCCAGCAACTGCGTCGAGATCTCCGGCGTGGAAGATCGGTTCAATGCCAGCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAACGAAGAGAACGAGGACATCCTTGAGGACATCGTGCTGACACTGACCCTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACATACGCCCACCTGTTCGACGACAAAGTGATGAAGCAACTGAAGCGGCGGAGATACACCGGCTGGGGCAGACTGTCTCGGAAGCTGATCAACGGCATCCGGGATAAGCAGTCCGGCAAGACCATCCTGGACTTTCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATTCACGACGACAGCCTCACCTTCAAAGAGGATATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATTCTCTGCATGAGCACATTGCCAACCTGGCCGGCTCTCCCGCCATTAAGAAAGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTTGTGAAAGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACACAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGACGGGATATGTACGTGGACCAAGAGCTGGACATCAACAGACTGTCCGACTACGATGTGGACCATATCGTGCCCCAGTCTTTTCTGAAGGACGACTCCATCGACAACAAGGTCCTGACCAGATCCGACAAGAATCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTGGTCAAGAAGATGAAGAACTACTGGCGACAGCTGCTGAACGCCAAGCTGATTACCCAGCGGAAGTTCGACAATCTGACCAAGGCCGAAAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATTCTGGACTCTCGGATGAACACTAAGTACGACGAGAACGACAAACTGATCCGCGAAGTGAAAGTCATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTCTACAAAGTGCGCGAGATCAACAACTACCATCACGCCCACGACGCCTACCTGAATGCCGTTGTTGGAACAGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGA

CGTGCGGAAGATGATCGCCAAGAGCGAGCAAGAGATTGGCAAGGCAACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACAGAGATCACCCTCGCCAACGGCGAGATCAGAAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGCGAGATTGTGTGGGATAAGGGCAGAGACTTTGCCACAGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAGAAAACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCTAAGCGGAACTCCGACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGATTCTCCTACCGTGGCCTATAGCGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTCAAGAGCGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCGATCGATTTCCTCGAGGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTCCCCAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCTGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCTAGCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCAGCCCCGAGGACAATGAGCAAAAGCAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTTAGCAAGAGAGTGATTCTGGCCGACGCCAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCTATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAACCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAAGAGGTGCTGGACGCCACTCTGATCCACCAGTCTATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAACTCGGAGGCGACGAAGGCGCCGATAAGAGAACCGCCGATGGCTCTGAGTTCGAGAGCCCCAAGAAAAAGCGCAAAGTGTGA

ABE7.10_Cfa(GEP)-C_Split_A589C:

ATGGTCAAGATCATCAGCAGAAAGAGCCTGGGCACCCAGAACGTGTACGATATCGGAGTGGGCGAGCCCCACAACTTTCTGCTCAAGAATGGCCTGGTGGCCAGCAACTGCAGCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAACGAAGAGAACGAGGACATCCTTGAGGACATCGTGCTGACACTGACCCTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACATACGCCCACCTGTTCGACGACAAAGTGATGAAGCAACTGAAGCGGCGGAGATACACCGGCTGGGGCAGACTGTCTCGGAAGCTGATCAACGGCATCCGGGATAAGCAGTCCGGCAAGACCATCCTGGACTTTCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATTCACGACGACAGCCTCACCTTCAAAGAGGATATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATTCTCTGCATGAGCACATTGCCAACCTGGCC

GGCTCTCCCGCCATTAAGAAAGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTTGTGAAAGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACACAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGACGGGATATGTACGTGGACCAAGAGCTGGACATCAACAGACTGTCCGACTACGATGTGGACCATATCGTGCCCCAGTCTTTTCTGAAGGACGACTCCATCGACAACAAGGTCCTGACCAGATCCGACAAGAATCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTGGTCAAGAAGATGAAGAACTACTGGCGACAGCTGCTGAACGCCAAGCTGATTACCCAGCGGAAGTTCGACAATCTGACCAAGGCCGAAAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATTCTGGACTCTCGGATGAACACTAAGTACGACGAGAACGACAAACTGATCCGCGAAGTGAAAGTCATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTCTACAAAGTGCGCGAGATCAACAACTACCATCACGCCCACGACGCCTACCTGAATGCCGTTGTTGGAACAGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAAGAGATTGGCAAGGCAACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGACAGAGATCACCCTCGCCAACGGCGAGATCAGAAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGCGAGATTGTGTGGGATAAGGGCAGAGACTTTGCCACAGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAGAAAACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCTAAGCGGAACTCCGACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGATTCTCCTACCGTGGCCTATAGCGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTCAAGAGCGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCGATCGATTTCCTCGAGGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTCCCCAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCTGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCTAGCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCAGCCCCGAGGACAATGAGCAAAAGCAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTTAGCAAGAGAGTGATTCTGGCCGACGCCAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCTATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAACCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACAC

CACCATCGACCGGAAGCGGTACACCTCCACCAAAGAGGTGCTGGACGCCACTCTGATCCACCAGTCTATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAACTCGGAGGCGACGAAGGCGCCGATAAGAGAACCGCCGATGGCTCTGAGTTCGAGAGCCCCAAGAAAAAGCGCAAAGTGTGA

ABE7.10_Cfa(GEP)-C_Split_S590C:

ATGGTCAAGATCATCAGCAGAAAGAGCCTGGGCACCCAGAACGTGTACGATATCGGAGTGGGCGAGCCCCACAACTTTCTGCTCAAGAATGGCCTGGTGGCCAGCAACTGCCTGGGCACATACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAACGAAGAGAACGAGGACATCCTTGAGGACATCGTGCTGACACTGACCCTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACATACGCCCACCTGTTCGACGACAAAGTGATGAAGCAACTGAAGCGGCGGAGATACACCGGCTGGGGCAGACTGTCTCGGAAGCTGATCAACGGCATCCGGGATAAGCAGTCCGGCAAGACCATCCTGGACTTTCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATTCACGACGACAGCCTCACCTTCAAAGAGGATATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATTCTCTGCATGAGCACATTGCCAACCTGGCCGGCTCTCCCGCCATTAAGAAAGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTTGTGAAAGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACACAGAAGGGACAGAAGAACAGCCGCGAGAGAATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGACGGGATATGTACGTGGACCAAGAGCTGGACATCAACAGACTGTCCGACTACGATGTGGACCATATCGTGCCCCAGTCTTTTCTGAAGGACGACTCCATCGACAACAAGGTCCTGACCAGATCCGACAAGAATCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTGGTCAAGAAGATGAAGAACTACTGGCGACAGCTGCTGAACGCCAAGCTGATTACCCAGCGGAAGTTCGACAATCTGACCAAGGCCGAAAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAGCTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATTCTGGACTCTCGGATGAACACTAAGTACGACGAGAACGACAAACTGATCCGCGAAGTGAAAGTCATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTCTACAAAGTGCGCGAGATCAACAACTACCATCACGCCCACGACGCCTACCTGAATGCCGTTGTTGGAACAGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAAGAGATTGGCAAGGCAACCGCCAAGTACTTCTTCTACAGCAACATCATGAACTTTTTCAAGAC

AGAGATCACCCTCGCCAACGGCGAGATCAGAAAGCGGCCTCTGATCGAGACAAACGGCGAAACCGGCGAGATTGTGTGGGATAAGGGCAGAGACTTTGCCACAGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAGAAAACCGAGGTGCAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCTAAGCGGAACTCCGACAAGCTGATCGCCAGAAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGATTCTCCTACCGTGGCCTATAGCGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTCAAGAGCGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCGATCGATTTCCTCGAGGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTCCCCAAGTACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCTGGCGAACTGCAGAAGGGAAACGAACTGGCCCTGCCTAGCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGGCAGCCCCGAGGACAATGAGCAAAAGCAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTTAGCAAGAGAGTGATTCTGGCCGACGCCAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGACAAGCCTATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAACCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGCGGTACACCTCCACCAAAGAGGTGCTGGACGCCACTCTGATCCACCAGTCTATCACCGGCCTGTACGAGACACGGATCGACCTGTCTCAACTCGGAGGCGACGAAGGCGCCGATAAGAGAACCGCCGATGGCTCTGAGTTCGAGAGCCCCAAGAAAAAGCGCAAAGTGTGA

Intact ABE7.10:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA

QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa-N_Split_S303C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA

QIGDQYADLFLAAKNLSDAILLCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_T310C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_T313C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA

QIGDQYADLFLAAKNLSDAILLSDILRVNTEICLSYDTEILTVEYGFLPIGKIVEERIECT
VYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPID
EIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_S355C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK
TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST
DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA
RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP
ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF
LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL
ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA
QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV
RQQLPEKYKEIFFDQCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPI
AQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGL
P

ABE7.10_Cfa-N_Split_A456C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK
TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST
DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA
RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP
ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF
LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL

ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEY CLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_S460C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL ARGNCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQ EVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_A463C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP

ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF
LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL
ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA
QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV
RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE
DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNR
GEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_T466C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK
TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST
DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA
RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP
ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF
LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL
ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA
QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV
RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE
DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQW
HNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_S469C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK

TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL ARGNSRFAWMTRKCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIA QWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_T472C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL ARGNSRFAWMTRKSEECLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYT QPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQV

DGLP

ABE7.10_Cfa-N_Split_T474C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETICLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_C574C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL

ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA
QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV
RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE
DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKED
YFKKIECLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGE
QEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_S577C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK
TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST
DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRA
RDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITE
GILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATP
ESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESF
LVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKF
RGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRL
ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLA
QIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALV
RQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE
DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKED
YFKKIECFDCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHN
RGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_A589C:
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAK
TGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSST

DSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNCLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

ABE7.10_Cfa-N_Split_S590C:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL

ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK
HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKED
YFKKIECFDSVEISGVEDRFNACLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNG
FVYTQPIAQWHNRGEQEVFEYCLEDGSIIRATKDHKFMTTDGQMLPIDEIFERGLDL
KQVDGLP

ABE7.10_Cfa(GEP)-C_Split_S303C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCDILRVNTEITKAPLSASMIKR
YDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILE
KMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNRE
KIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERM
TNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLL
FKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNE
ENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLIN
GIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIA
NLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDV
DHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFV
YGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNG
ETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKK
DWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF
LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYL
ASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLY
ETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_T310C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCEITKAPLSASMIKRYDEHHQ
DLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTF
RIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNL
PNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKV

TVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_T313C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG

SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQ
AENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_S355C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCKNGYAGYIDGGASQEEFYK
FIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFL
KDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQS
FIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKD
FLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRL
SRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDS
LHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKN
SRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRL
SDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLN
AKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDE
NDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPK
LESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP
LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDK
LIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFE
KNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYV
NFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
SAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_A456C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCRGNSRFAWMTRKSEETITP
WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN
ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLT
FKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVI
EMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQ
NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSE

EVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_S460C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_A463C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRR

RYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_T446C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_T469C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCEETITPWNFEEVVDKGASAQ SFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQK KAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDK DFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGR LSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGD SLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQK NSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN RLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQL LNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKY DENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKY PKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRK RPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSK YVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDK VLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLI HQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_T472C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCITPWNFEEVVDKGASAQSFI ERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAI VDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDF LDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSL HEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLS DYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNA KLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEN DKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKL ESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLI ARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEK NPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVN FLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLS

AYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQS
ITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_T474C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCPWNFEEVVDKGASAQSFIE
RMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFL
DNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLH
EHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSR
ERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD
YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKL
ITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLES
EFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIET
NGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR
KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY
NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSIT
GLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_C574C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCFDSVEISGVEDRFNASLGTY
HDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKR
RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQK
AQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM
KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILD
SRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVV
GTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEIT
LANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKE
SILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLG

ITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_S577C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_A589C:

MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCSLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF

LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYL
ASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLY
ETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

ABE7.10_Cfa(GEP)-C_Split_S590C:
MVKIISRKSLGTQNVYDIGVGEPHNFLLKNGLVASNCLGTYHDLLKIIKDKDFLDNEE
NEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLIN
GIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIA
NLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDV
DHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
EVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFV
YGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNG
ETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKK
DWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF
LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYL
ASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLY
ETRIDLSQLGGDEGADKRTADGSEFESPKKKRKV

Example 2: Delivery of Split A-to-G Base Editor (ABE) via dual AAV infection yields high A>G conversion.

AAV delivery of a split A-to-G base editor fused to intein-N and intein-C was evaluated, including the ability of split ABE to reassemble and reconstitute base editing activity in co-infected cells. Initial studies were performed using fluorescent reporter constructs to determine optimal multiplicity of infection (MOI) for AAV2 co-infection in retinal cells. AAV2 expression vectors containing CMV-mCherry and CMV-EmGFP were used to monitor co-infections. Flow cytometry revealed optimal MOI for AAV2 co-infection of ARPE-19 cells is 60,000 – 100,000 vg/cell (FIG. 5A). Fluorescence microscopy at day 3 post co-infection at 60,000 vg/cell showed EmGFP and mCherry co-localization (FIG. 5B). Single infections also showed high numbers of cells (>95%) with fluorescent reporter expression at 50,000 vg/cell for both CMV-mCherry (FIG. 5C) and CMV-EmGFP (FIG. 5D). Percentage of fluorescent cells was dependent on total viral load.

Delivery of split editor to ARPE-19 retinal cells via dual AAV2 infection yielded high A>G conversion at ABCA4 5882A (FIG. 6). In this experiment, ABE split at Cas9 T310 (N-terminus fused to intein-N at Cas9 amino acid position N309 and the C- terminus fused to intein C at Cas9 amino acid position T310) was evaluated. The following constructs were packaged into AAV2 vectors:

AAV2-N: AAV2/ P_{CMV} Split_ABE7.10$^{N(T310)}$-Int^{N}–rGpA | P_{U6}sgRNA

AAV2-C: AAV2/ P_{CMV} Int^{C}-Split_ABE7.10$^{C(T310)}$–rGpA

according to routine methods for AAV packaging (Viral Vector Core, University of Massachusetts Medical School). Retinal ARPE-19 cells were co-infected with both AAV vectors; infected with each AAV vector singly; or untreated. As shown at various MOIs (dual infection) from 20,000 – 60,000 vg/cell, A>G conversion was observed at ABCA4 5882A. A>G conversion increased with increasing MOI of dual infection (~13% at 20,000 vg/cell - ~30% at 60,000 vg/cell). A>G conversion was accompanied by low levels of (< .1%) indel formation. No A>G conversion at the target site was observed in cells infected with N- or C-terminal fragments alone nor in untreated cells.

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.

What is claimed is:

1. A composition comprising

(a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and

(b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

2. A composition comprising

(a) a first polynucleotide encoding an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and

(b) a second polynucleotide encoding fusion protein comprising a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

3. A composition comprising

(a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and

(b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

4. A composition comprising

(a) a first polynucleotide encoding an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and

(b) a second polynucleotide encoding a fusion protein comprising a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

5. The composition of any one of the preceding claims, wherein the N-terminal fragment of the Cas9 comprises up to amino acid 302, 309, 312, 354, 455, 459, 462, 465, 471, 473, 576, 588, or 589 as numbered in SEQ ID NO: 2.

6. The composition of any one of claims 1-4, wherein the C-terminal fragment of Cas9 or the N-terminal fragment of Cas9 comprises an Ala/Cys, Ser/Cys, or Thr/Cys mutation at a residue corresponding to amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590 as numbered in SEQ ID NO: 2.

7. The composition of claim 1 or 2, further comprising a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

8. The composition of any one of the preceding claims wherein the first and the second polynucleotides are joined.

9. The composition of any one of claims 1-7 wherein the wherein the first and the second polynucleotides are expressed separately.

10. The composition of any one of the preceding claims wherein the deaminase is an adenosine deaminase.

11. The composition of claim 10, where the deaminase is a wild-type TadA or TadA7.10.

12. The composition of any one of the preceding claims, wherein the deaminase is a TadA dimer.

13. The composition of claim 12, wherein the TadA dimer comprises a wild-type TadA and a TadA 7.10.

14. The composition of any one of the preceding claims, wherein the fusion protein comprises a nucleus localization signal (NLS).

15. The composition of any one of claims 1-13, wherein the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 is joined with a NLS.

16. The composition of claim 14 or 15, wherein the NLS is a bipartite NLS.

17. The composition of any one of the preceding claims, wherein the N-terminal fragment of Cas9 and the fusion protein are joined to form a base editor protein comprising a deaminase and a SpCas9.

18. The composition of any one of claims 1-16, wherein the C-terminal fragment of Cas9 and the fusion protein are joined to form a base editor protein comprising a deaminase and a SpCas9.

19. The composition of claim 17 or 18, wherein the SpCas9 has nickase activity or is catalytically inactive.

20. A composition comprising the fusion protein and the N-terminal fragment of Cas9 of any one of the preceding claims.

21. A composition comprising the fusion protein and the C-terminal fragment of Cas9 of any one of the preceding claims.

22. The composition of any one of the preceding claims, wherein the N terminal fragment of Cas9 or the C terminal fragment of Cas9 and the deaminase are joined by a linker.

23. The composition of claim 22, wherein the linker is a peptide linker.

24. A vector comprising the first and the second polynucleotide of any one of the preceding claims.

25. The vector of claim 24, wherein the vector comprises a promoter.

26. The vector of claim 25, wherein the promoter is a constitutive promoter.

27. The vector of claim 26, wherein the constitutive promoter is a CMV or CAG promoter.

28. The vector of any one of claims 24-27, wherein the vector is selected from the group consisting of retroviral vectors, adenoviral vectors, lentiviral vectors, herpesvirus vectors, and adeno-associated viral vectors.

29. The vector of claim 28, wherein the vector is an adeno-associated viral vector.

30. A cell comprising the composition of any one of claims 1-13, or the vector of any one of claims 24-30.

31. The cell of claim 30, wherein the cell is a mammalian cell.

32. A reconstituted A-to-G base editor protein comprising a Cas9 domain comprising an Ala/Cys, Ser/Cys, or Thr/Cys mutation.

33. The reconstituted base editor protein of claim 32, wherein the mutation is at a residue corresponding to SpCas9 amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590.

34. A composition comprising one or more polynucleotides encoding

(a) an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and

(b) a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

35. A composition comprising one or more polynucleotides encoding

(a) an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at amino acid 302, 309, 312, 354, 455, 459, 462, 465, 471, 473, 576, 588, or 589 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N, and

(b) a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at 303, 310, 313, 355, 456, 460, 463, 466, 472, 474, 577, 589, or 590 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

36. The composition of claim 34 or 35, wherein the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 is joined with a nucleus localization signal (NLS).

37. The composition of claim 36 wherein the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 are both joined with a NLS.

38. The composition of claim 36 or 37, wherein the NLS is a bipartite NLS.

39. The composition of any one of claims 34-38, wherein the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 are joined to form a SpCa9.

40. The composition of claim 39, wherein the SpCas9 has nickase activity or is catalytically inactive.

41. A composition comprising the N-terminal fragment of Cas9 in (a) and the C-terminal fragment of Cas9 in (b) in any one of claims 34-40.

42. A vector comprising the one or more polynucleotides of any one of the claims 34-40.

43. The vector of claim 42, wherein the vector comprises a promoter.

44. The vector of claim 43, wherein the promoter is a constitutive promoter.

45. The vector of claim 44, wherein the constitutive promoter is a CMV or CAG promoter.

46. The vector of any one of claims 42-45, wherein the vector is selected from the group consisting of retroviral vectors, adenoviral vectors, lentiviral vectors, herpesvirus vectors, and adeno-associated viral vectors.

47. The vector of claim 46, wherein the vector is an adeno-associated viral vector.

48. A cell comprising the composition of any one of claims 34-41, or the vector of any one of claims 42-47.

49. The cell of claim 48, wherein the cell is a mammalian cell.

50. A Cas9 variant polypeptide comprising a Ala/Cys, Ser/Cys, or Thr/Cys mutation.

51. A Cas9 variant polypeptide comprising a Cys residue at amino acid 303, 310, 313, 355, 456, 460, 463, 466, 472, or 474.

52. A method for delivering a base editor system to a cell, the method comprising contacting the cell with

(a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N,

(b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and

(c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

53. A method for delivering a base editor system to a cell, the method comprising contacting the cell with

(a) a first polynucleotide encoding a fusion protein comprising an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N,

(b) a second polynucleotide encoding a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and

(c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

54. A method for delivering a Base Editor System to a cell, the method comprising contacting the cell with

(a) a first polynucleotide encoding a fusion protein comprising a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N,

(b) a second polynucleotide encoding a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and

(c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

55. A method for delivering a Base Editor System to a cell, the method comprising contacting the cell with

(a) a first polynucleotide encoding a fusion protein comprising an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2, wherein the N-terminal fragment of Cas9 is fused to a split intein-N,

(b) a second polynucleotide encoding a C-terminal fragment of Cas9 and a deaminase, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C, and

(c) a single guide RNA (sgRNA) or a polynucleotide encoding the sgRNA.

56. The method of any one of claims 52-55, wherein the sgRNA is complementary to a target polynucleotide.

57. The method of claim 56, wherein the target polynucleotide is present in the genome of an organism.

58. The method of claim 57, wherein the organism is an animal, plant, or bacteria.

59. The method of any one of claims 52-58, wherein the first polynucleotides, the second polynucleotide, and/or the polynucleotide encoding the are contacted with the cell via a vector.

60. The method of claim 59, wherein the vector is selected from the group consisting of retroviral vectors, adenoviral vectors, lentiviral vectors, herpesvirus vectors, and adeno-associated viral vectors.

61. The method of claim 60, wherein the vector is an adeno-associated viral vector.

62. The method of any one of claims 52-61, wherein the C-terminal fragment of Cas9 or the N-terminal fragment of Cas9 comprises an Ala/Cys, Ser/Cys, or Thr/Cys mutation at a residue corresponding to amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590 as numbered in SEQ ID NO: 2.

63. The method of any one of claims 52-62, wherein the deaminase is an adenosine deaminase.

64. The method of claim 63, wherein the deaminase is a TadA or a variant thereof.

65. The method of claim 64, where the deaminase is a wild-type TadA or Tad7.10.

66. The method of any one of claims 52-65, wherein deaminase is a TadA dimer.

67. The method of claim 66, wherein the TadA dimer comprises a wild type TadA and a TadA7.10.

68. The method of any one of claims 52-67, wherein the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 comprises an NLS.

69. The method of claim 68, wherein the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 both comprise an NLS.

70. The method of claim 68 or 69, wherein the NLS is a bipartite NLS.

71. The method of any one of claims 52-70, wherein the N-terminal fragment of Cas9 and the C-terminal fragment of Cas9 are joined to form a SpCa9.

72. The method of claim 71, wherein the SpCas9 has nickase activity or is catalytically inactive.

73. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position

between A292-G364 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N.

74. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between F445-K483 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N.

75. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and an N-terminal fragment of Cas9, wherein the N-terminal fragment of Cas9 starts at the N-terminus of Cas9 and is a contiguous sequence that terminates at a position between E565-T637 of Cas9 as numbered in SEQ ID NO: 2, and wherein the N-terminal fragment of Cas9 is fused to a split intein-N.

76. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

77. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between F445-K483 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

78. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

79. A polynucleotide encoding a fusion protein, wherein the fusion protein comprises a deaminase and a C-terminal fragment of Cas9, wherein the C-terminal fragment of Cas9 starts at a position between A292-G364, F445-K483, or E565-T637 of Cas9 as numbered in SEQ ID NO: 2 and is a contiguous sequence that terminates at the C-terminus of Cas9, wherein the N-terminus residue of the C-terminal fragment of Cas9 is a Cys substituted for an Ala, Ser, or Thr, and wherein the C-terminal fragment of Cas9 is fused to a split intein-C.

80. The polynucleotide of any one of claims 73-79, wherein the C-terminal fragment of Cas9 or the N-terminal fragment of Cas9 comprises an Ala/Cys, Ser/Cys, or Thr/Cys mutation.

81. The polynucleotide of claim 80, wherein the mutation is at a residue corresponding to amino acid S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, or S590 as numbered in SEQ ID NO: 2.

82. The polynucleotide of any one of claims 73-81, wherein the deaminase is an adenosine deaminase.

83. The polynucleotide of claim 82, wherein the deaminase is a TadA or a variant thereof.

84. The polynucleotide of claim 83, where the deaminase is a wild-type TadA, or Tad7.10.

85. The polynucleotide of any one of claims 73-84, wherein the fusion protein comprises two deaminases linked to each other.

86. The polynucleotide of claim 85, wherein the fusion protein comprises both a wild type TadA and a TadA7.10.

87. The polynucleotide of any one of claims 73-86, wherein the fusion protein comprises an NLS.

88. The polynucleotide of claim 87, wherein the NLS is a bipartite NLS.

89. The polynucleotide of any one of claims 73-88, wherein the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 comprises amino acid sequence of SpCas9.

90. The polynucleotide of any one of claims 73-89, wherein the N-terminal fragment of Cas9 or the C-terminal fragment of Cas9 comprises one or more amino acid substitutions associated with reduced nuclease activity.

91. An N-terminal fragment of a Cas9 protein comprising up to amino acid 302, 309, 312, 354, 455, 459, 462, 465, 471, or 473 fused to a split intein-N.

92. A C-terminal protein fragment of a Cas9 protein, wherein the N-terminus amino acid of the C-terminal fragment is a Cys substitution at amino acid 303, 310, 313, 355, 456, 460, 463, 466, 472, or 474 and is fused to a split intein-C.

93. A polynucleotide encoding a fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and an N-terminal fragment of Cas9, wherein the N-terminal fragment is fused to a split intein-N.

94. A polynucleotide encoding a fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and a C-terminal fragment of Cas9, wherein the C-terminal fragment is fused to a split intein-C.

95. A protein fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and an N-terminal fragment of Cas9, wherein the N-terminal fragment is fused to a split intein-N.

96. A protein fragment of an A-to-G Base Editor fusion protein, the fusion protein comprising one or more deaminases and a C-terminal fragment of Cas9, wherein the C-terminal fragment is fused to a split intein-C.

97. A composition comprising first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases and Cas9, wherein the first polynucleotide encodes an N-terminal fragment of Cas9 fused to a split intein-N and the second polynucleotide encodes a C-terminal fragment of Cas9 fused to a split intein-C.

98. A composition comprising N- and C terminal fragments of an A-to-G Base Editor fusion protein comprising one or more deaminases and SpCas9, wherein the N-terminal fragment comprises a fragment of SpCas9 fused to a split intein-N and the C-terminal fragment comprises the remainder of SpCas9 fused to a split intein-C.

99. A method for delivering a Base Editor System to a cell, the method comprising contacting a cell with first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases and Cas9, wherein the first polynucleotide encodes an N-terminal fragment of Cas9 fused to a split intein-N and the second polynucleotide encodes a C-terminal fragment of Cas9 fused to a split intein-C, and either the first or the second polynucleotide encodes a single guide RNA.

100. A method for delivering a Base Editor System to a cell, the method comprising contacting a cell with N- and C terminal fragments of an A-to-G Base Editor fusion protein comprising one or more deaminases and SpCas9, wherein the N-terminal fragment comprises a fragment of SpCas9 fused to a split intein-N and the C-terminal fragment comprises the remainder of SpCas9 fused to a split intein-C, and a guide RNA.

101. A method for editing a target polynucleotide in a cell, the method comprising contacting a cell with first and second polynucleotides each encoding a fragment of an A-to-G Base Editor fusion protein comprising one or more deaminases and Cas9, wherein the first polynucleotide encodes an N-terminal fragment of Cas9 fused to a split intein-N and the second polynucleotide encodes a C-terminal fragment of Cas9 fused to a split intein-C, and either the first or the second polynucleotide encodes a single guide RNA, and expressing the encoded proteins and single guide RNA in the cell.

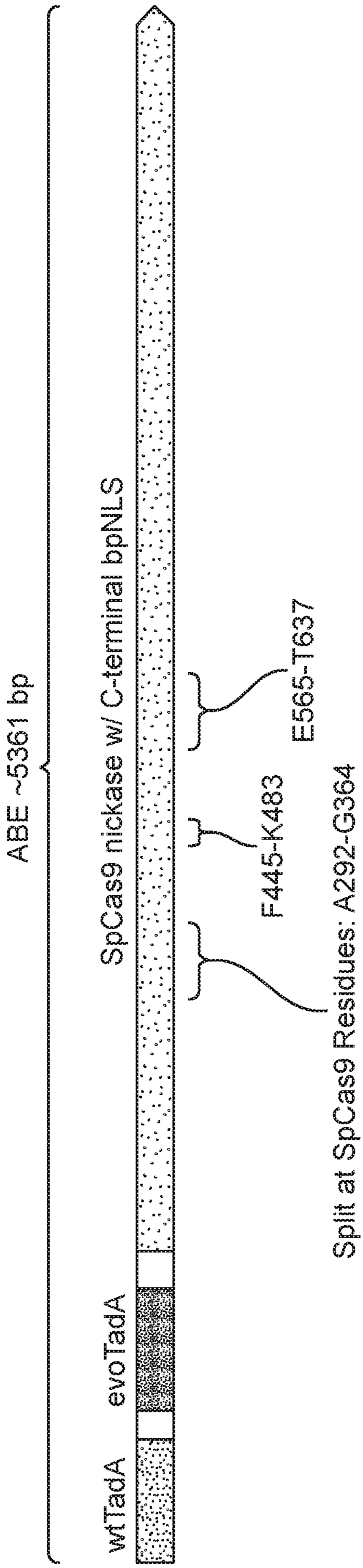

ABE ~5361 bp
wtTadA
evoTadA
SpCas9 nickase w/ C-terminal bpNLS
Split at SpCas9 Residues: A292-G364
F445-K483
E565-T637

FIG. 1

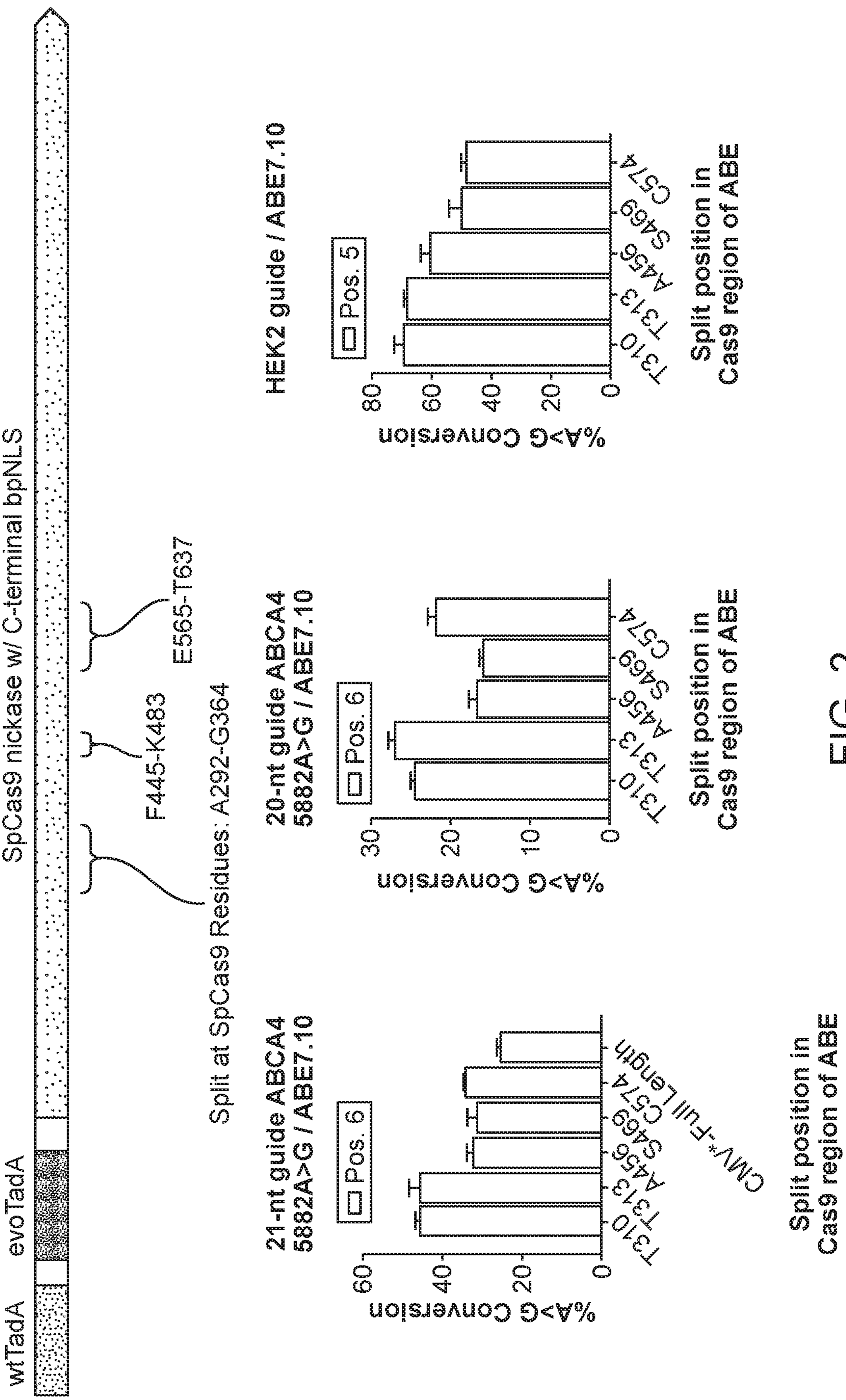

wtTadA
evoTadA
SpCas9 nickase w/ C-terminal bpNLS
Split at SpCas9 Residues: A292-G364
F445-K483
E565-T637
21-nt guide ABCA4
5882A>G / ABE7.10
Pos. 6
%A>G Conversion
60
40
20
0
T310
T313
A456
S469
C574
CMV-Full Length
Split position in
Cas9 region of ABE
20-nt guide ABCA4
5882A>G / ABE7.10
Pos. 6
%A>G Conversion
30
20
10
0
T310
T313
A456
S469
C574
Split position in
Cas9 region of ABE
HEK2 guide / ABE7.10
Pos. 5
%A>G Conversion
80
60
40
20
0
T310
T313
A456
S469
C574
Split position in
Cas9 region of ABE

FIG. 2

FIG. 3

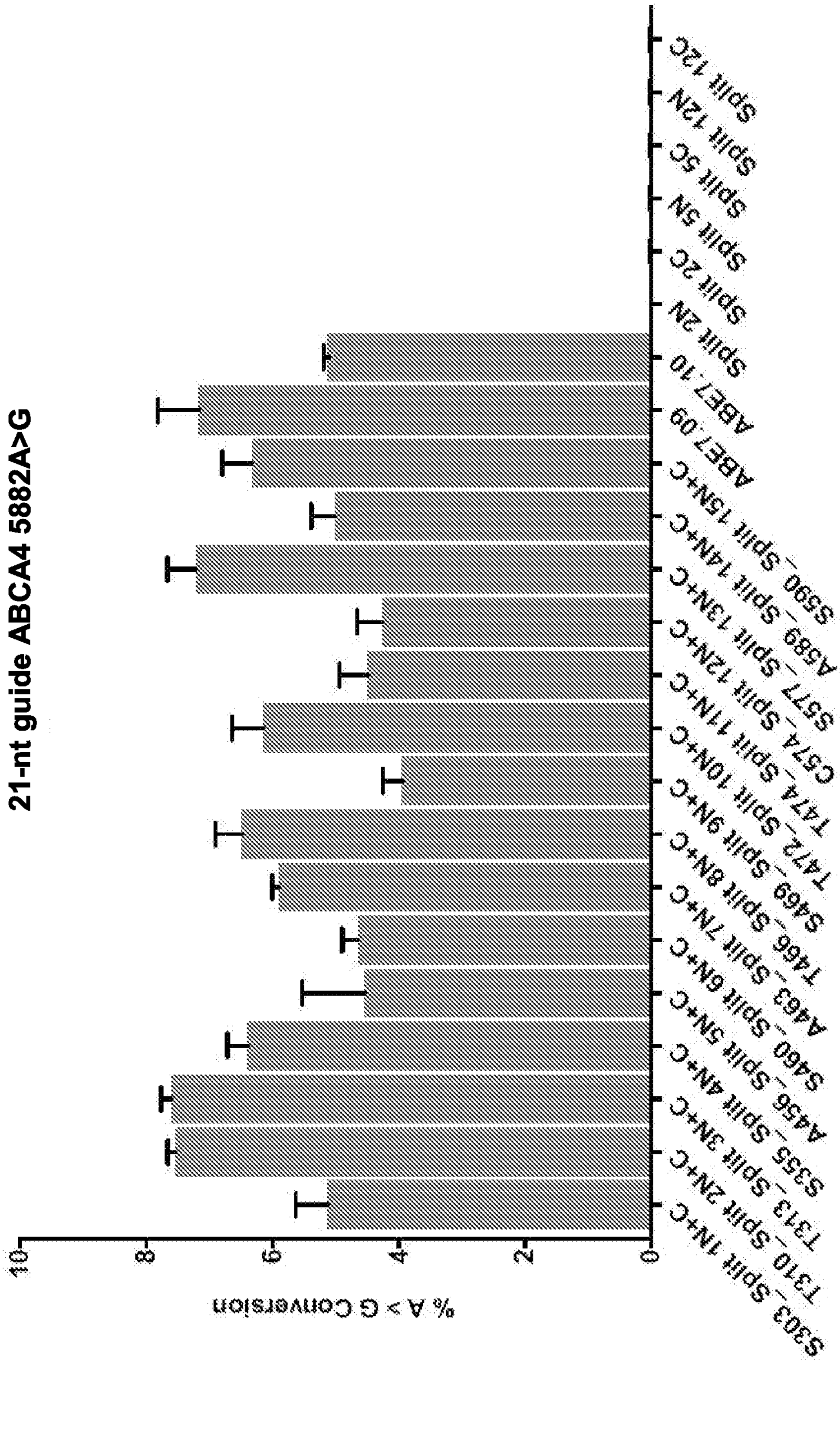
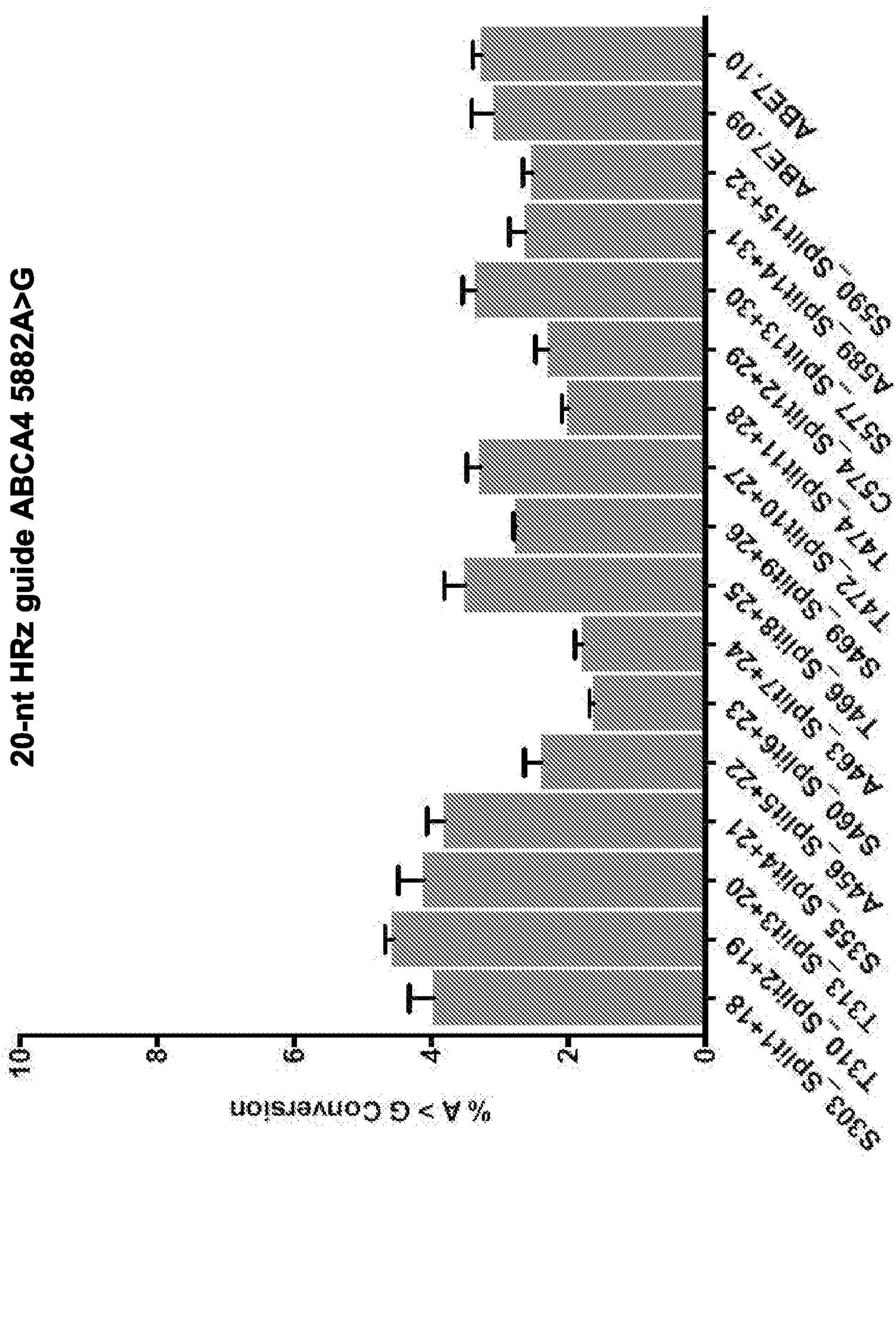

21-nt guide ABCA4 5882A>G
% A > G Conversion
0
2
4
6
8
10
S303_Split 1N+C
T310_Split 2N+C
T313_Split 3N+C
S355_Split 4N+C
A456_Split 5N+C
S460_Split 6N+C
A463_Split 7N+C
T466_Split 8N+C
S469_Split 9N+C
T472_Split 10N+C
T474_Split 11N+C
C574_Split 12N+C
S577_Split 13N+C
A589_Split 14N+C
S590_Split 15N+C
ABE7.09
ABE7.10
Split 2N
Split 2C
Split 5N
Split 5C
Split 12N
Split 12C

FIG. 4


20-nt HRz guide ABCA4 5882A>G
% A > G Conversion
0
2
4
6
8
10
S303_Split1+18
T310_Split2+19
T313_Split3+20
S355_Split4+21
A456_Split5+22
S460_Split6+23
A463_Split7+24
T466_Split8+25
S469_Split9+26
T472_Split10+27
T474_Split11+28
C574_Split12+29
S577_Split13+30
A589_Split14+31
S590_Split15+32
ABE7.09
ABE7.10

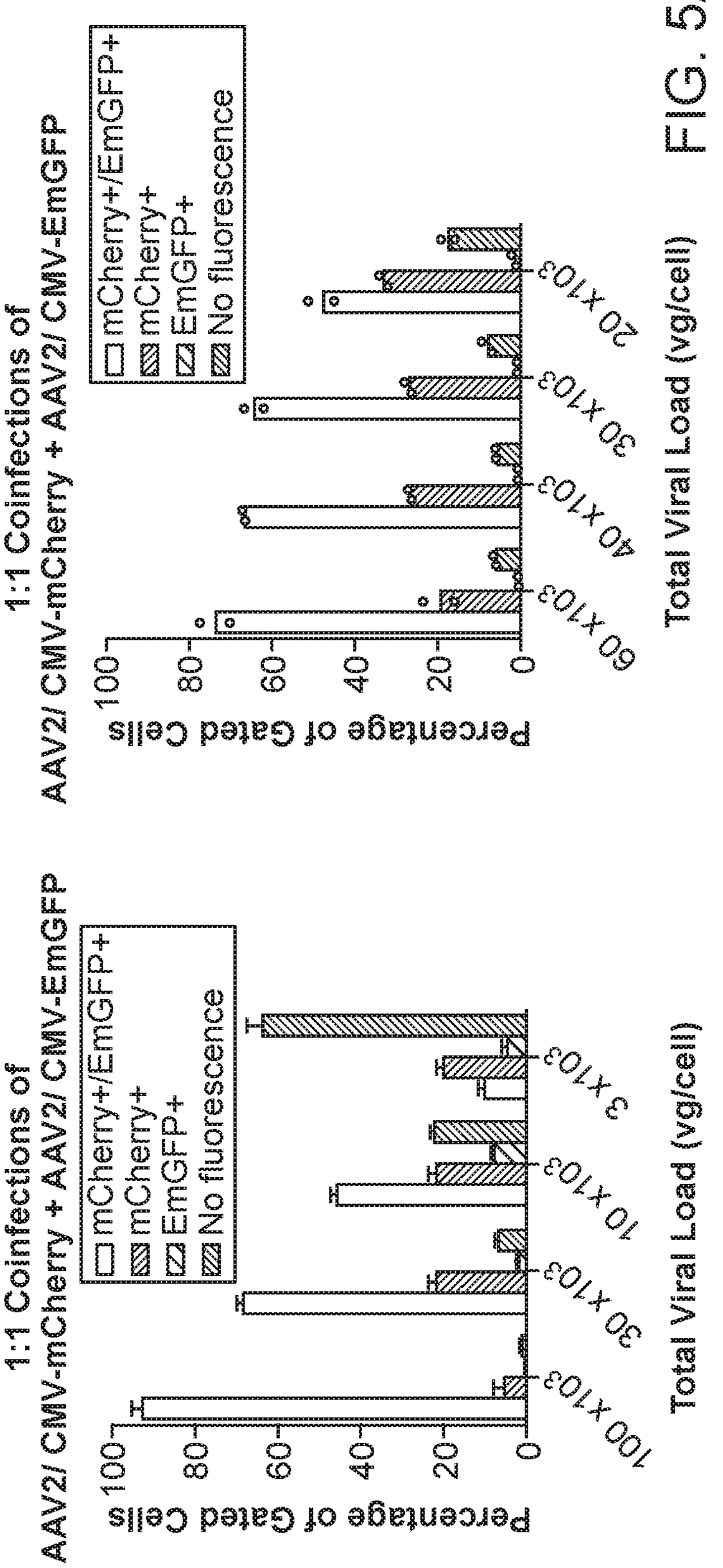

1:1 Coinfections of
AAV2/ CMV-mCherry + AAV2/ CMV-EmGFP
mCherry+/EmGFP+
mCherry+
EmGFP+
No fluorescence
Percentage of Gated Cells
100
80
60
40
20
0
100 x10³
30 x10³
10 x10³
3 x10³
Total Viral Load (vg/cell)
1:1 Coinfections of
AAV2/ CMV-mCherry + AAV2/ CMV-EmGFP
mCherry+/EmGFP+
mCherry+
EmGFP+
No fluorescence
Percentage of Gated Cells
60 x10³
40 x10³
30 x10³
20 x10³
Total Viral Load (vg/cell)

FIG. 5A

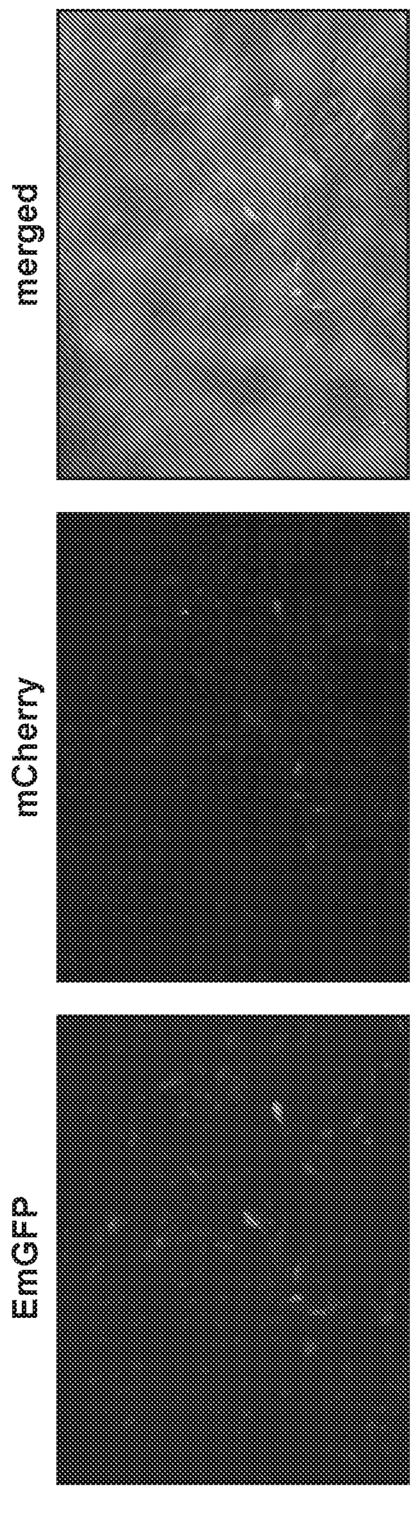

EmGFP
mCherry
merged

FIG. 5B

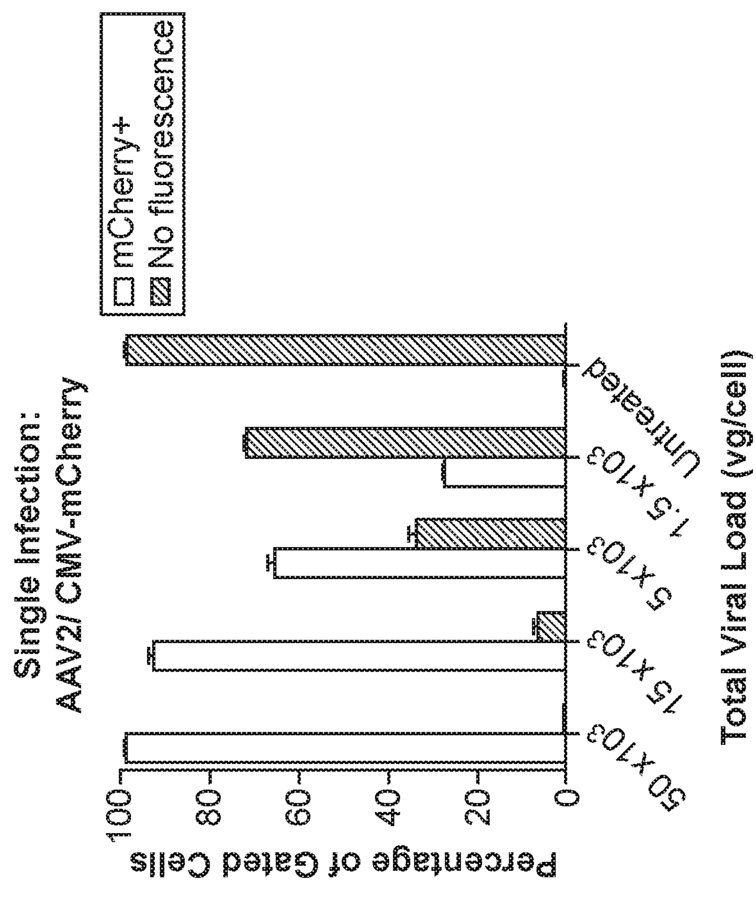

Single Infection:
AAV2/ CMV-mCherry
mCherry+
No fluorescence
Percentage of Gated Cells
100
80
60
40
20
0
50 x10³
15 x10³
5 x10³
1.5 x10³
Untreated
Total Viral Load (vg/cell)

FIG. 5C

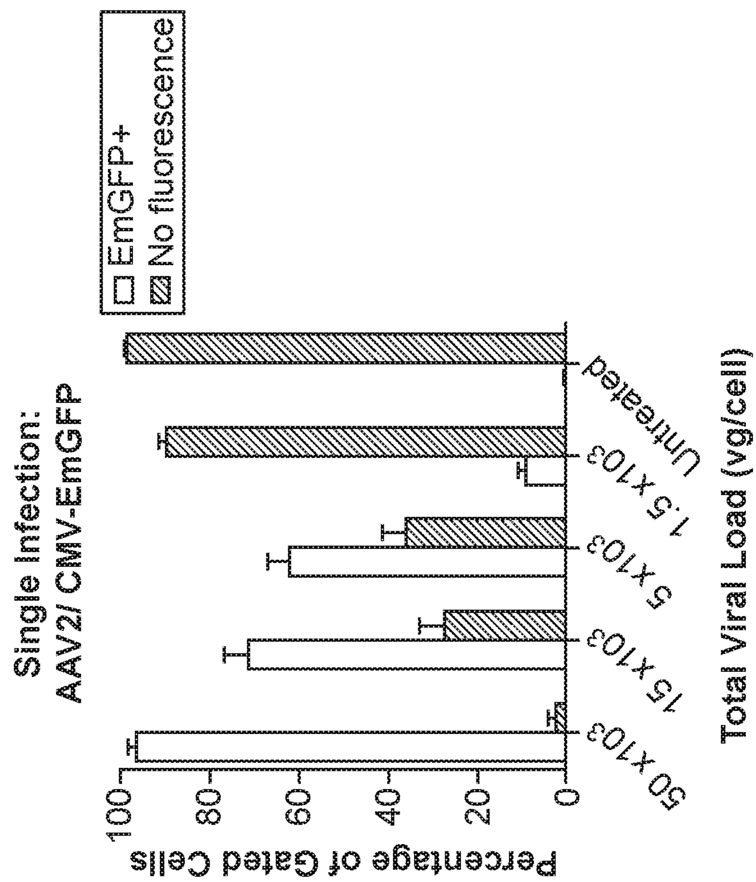

Single Infection:
AAV2/ CMV-EmGFP
EmGFP+
No fluorescence
Percentage of Gated Cells
100
80
60
40
20
0
50 x10³
15 x10³
5 x10³
1.5 x10³
Untreated
Total Viral Load (vg/cell)

FIG. 5D

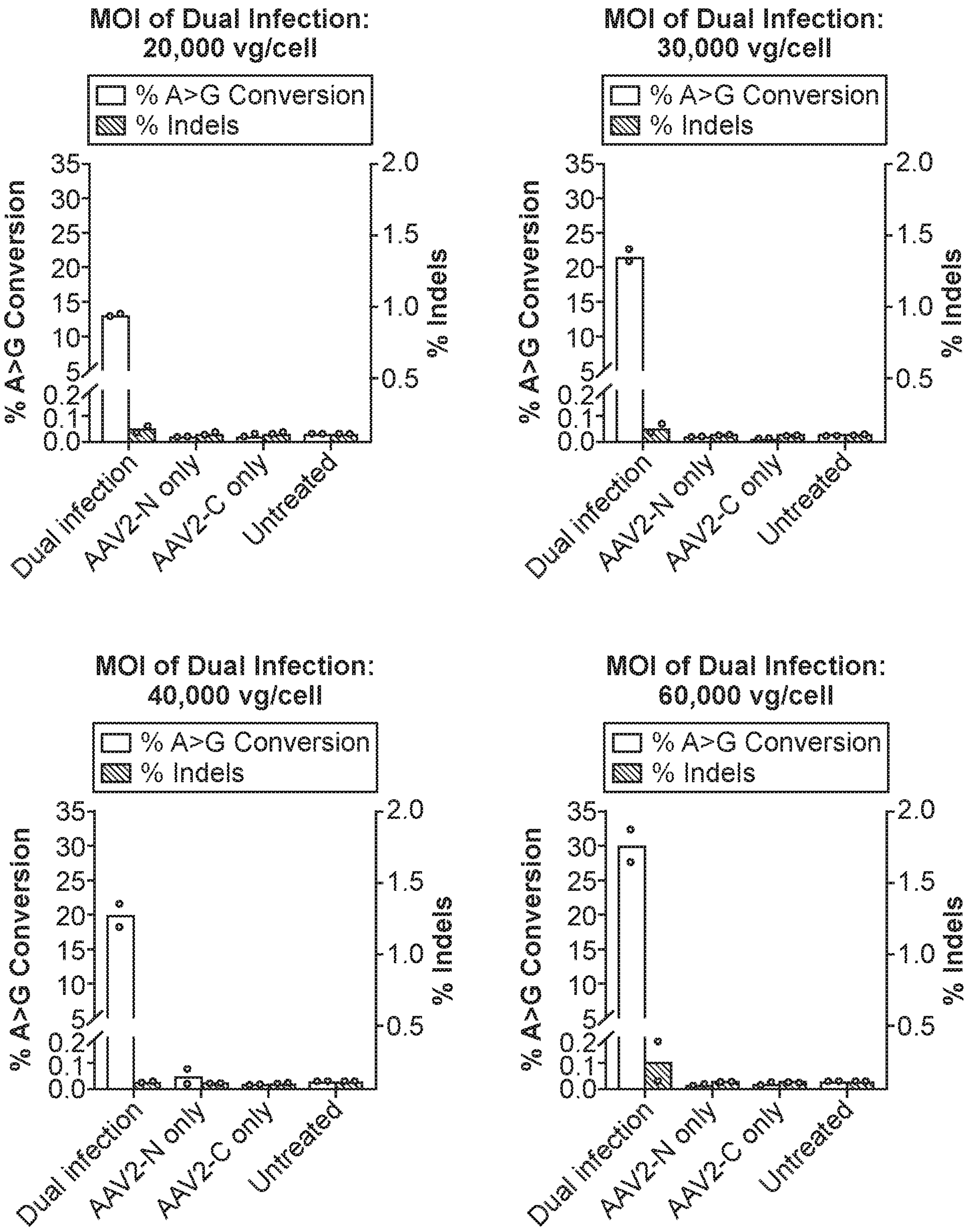

MOI of Dual Infection:
20,000 vg/cell
MOI of Dual Infection:
30,000 vg/cell
MOI of Dual Infection:
40,000 vg/cell
MOI of Dual Infection:
60,000 vg/cell
% A>G Conversion
% Indels
Dual infection
AAV2-N only
AAV2-C only
Untreated

FIG. 6