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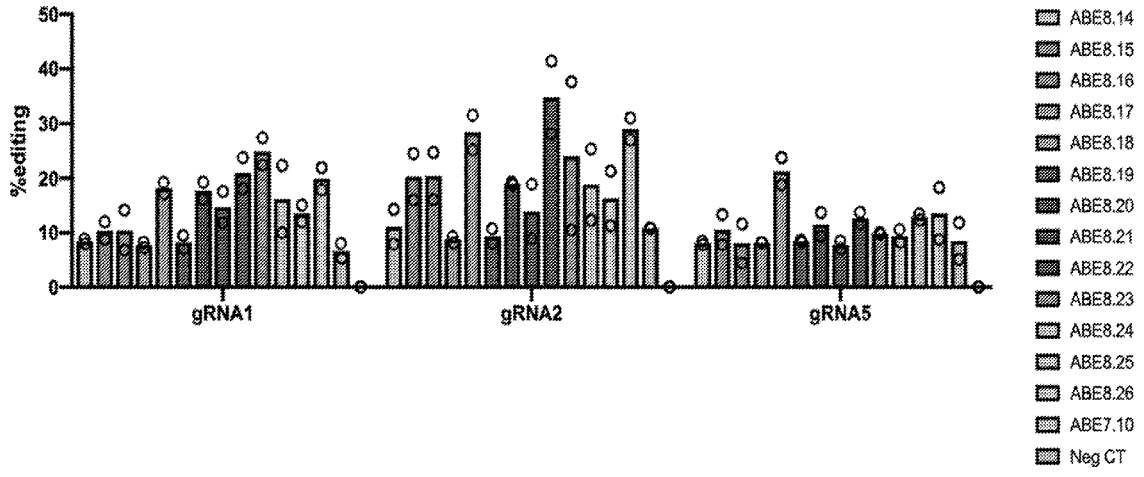
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(54) Title: METHODS OF EDITING A SINGLE NUCLEOTIDE POLYMORPHISM USING PROGRAMMABLE BASE EDITOR SYSTEMS

FIG. 3



(57) Abstract: Described are compositions and methods for altering mutations associated with Rett Syndrome (RETT). Provided herein are compositions and methods of using base editors (e.g., ABE8) comprising a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain in conjunction with a guide polynucleotide. Also provided herein are base editor systems for editing nucleobases of target nucleotide sequences.

[Continued on next page]

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METHODS OF EDITING A SINGLE NUCLEOTIDE POLYMORPHISM USING PROGRAMMABLE BASE EDITOR SYSTEMS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This International PCT application claims priority to and benefit of Provisional Patent Application No. 62/850,919, filed on May 21, 2019, the entire contents of which are incorporated by reference herein in their entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Rett Syndrome (RTT or RETT) is caused by a heterogeneous group of mutations in the methyl-CpG-binding protein 2 (*Mecp2)* gene that impair or abrogate the encoded protein's ability to modify chromatin and transcriptional states in the central nervous system (CNS). Gene therapy to deliver functional *Mecp2* or using RNA editing to repair the endogenous *Mecp2* (*MECP2)* mRNA transcripts are promising approaches to therapeutic interventions when delivered broadly throughout the CNS. However, both approaches must overcome significant challenges to achieve therapeutic efficacy. *Mecp2* gene therapy must tightly control the dosage of the delivered gene on a per-cell basis or risk mimicking the phenotype of *Mecp2* duplication syndrome. RNA editing platforms are unable to precisely correct the most prevalent *Mecp2* mutations accounting for more than 45% of RTT diagnoses and also induce efficient, unguided off-target editing.

[0003] The genetic mutations in *Mecp2* that cause Rett Syndrome (RTT) are highly heterogeneous. As a consequence, the favored strategy for therapy has been to deliver wild-type *Mecp2* carried by recombinant adeno-associated virus (rAAV). Because this strategy is agnostic to the causal mutation in each individual, a successful gene therapy approach would provide a therapeutic option to a large portion of the RTT patient population. To date, however, this strategy has been met with limited success. RTT patients are nearly always heterozygotic females, resulting in characteristic wild-type and mutant X- linked MeCP2 mosaic expression within the central nervous system (CNS) due to random X- chromosome inactivation. Thus, rAAV delivery and expression of wild-type MeCP2 in neurons already expressing wild-type MeCP2 is likely to partially mimic the phenotype of MeCP2 duplication syndrome. Consistent with this, high transduction efficiency in the CNS of RTT-model mice resulted in approximately 2-fold greater MeCP2 expression than found in wild-type mice.

[0004] Therefore, there is a need for novel compositions and methods for treating patients with Rett Syndrome.

INCORPORATION BY REFERENCE

[0005] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. Absent any indication otherwise, publications, patents, and patent applications mentioned in this specification are incorporated herein by reference in their entireties.

SUMMARY OF THE DISCLOSURE

[0006] As described below, the present invention features compositions and methods for the precise correction of pathogenic amino acids using a programmable nucleobase editor. In particular, the compositions and methods of the invention are useful for the treatment of Rett Syndrome (RTT or RETT). Thus, the invention provides compositions and methods for treating Rett Syndrome using an adenosine (A) base editor (ABE) (*e.g.*, ABE8) to precisely correct a single nucleotide polymorphism in the endogenous *Mecp2* gene to correct a deleterious mutation (*e.g.*, R106W, R133C, T158M, R255*, R270*, R306C).

[0007] In an aspect, a method of editing a methyl CpG binding protein 2 (*MECP2*) gene or regulatory element thereof in a subject is provided, in which the method comprising administering to a subject in need thereof (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 relative to a TadA reference sequence, or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the *MECP2* gene or a regulatory element thereof, which comprises a SNP associated with Rett syndrome (RETT); wherein the A-to-G nucleobase alteration is at the SNP associated with RETT, which results in an R133C or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene. In an embodiment, the TadA reference sequence comprises amino acid sequence

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH

AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD. In an embodiment, the A-to-G nucleobase alteration changes the SNP associated with RETT to a wild type nucleobase. In an embodiment, the A-to-G nucleobase alteration changes the SNP associated with RETT to a non-wild type nucleobase that results in one or more ameliorated symptoms of RETT. In an embodiment, the A-to-G nucleobase alteration at the SNP associated with RETT changes a cysteine to an arginine or stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide. In an embodiment, the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT. In an embodiment, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

[0008] In an aspect, a base editor system is provided, in which the base editor system comprises (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 relative to a TadA reference sequence or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (*MECP2*) gene or regulatory element thereof, which comprises a SNP associated with Rett syndrome (RETT); wherein the A-to-G nucleobase alteration is at the SNP associated with RETT, which results in an R133C or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene. In an embodiment, the A-to-G nucleobase alteration changes the SNP associated with RETT to a wild type nucleobase. In an embodiment, the A-to-G nucleobase alteration changes the SNP associated with RETT to a non-

wild type nucleobase that results in one or more ameliorated symptoms of RETT. In an embodiment, the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT. In an embodiment, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence selected from 5′-AGAGCAAAAGGCUUUUCCCU-3′, 5′-UAGAGCAAAAGGCUUUUCCC-3′, 5′-UAGAGCAAAAGGCUUUUCCCU-3′, 5′-UUUAGAGCAAAAGGCUUUUCCCU-3′, 5′-UCUUGCACUUCUUGAUGGGG-3′, 5′-CUUGCACUUCUUGAUGGGGAG-3′, or 5′-GUCUUGCACUUCUUGAUGGGGAG-3′.

[0009] In another aspect, a method of editing an *MECP2* polynucleotide comprising a single nucleotide polymorphism (SNP) associated with Rett Syndrome (RETT) is provided, in which the method comprises contacting the *MECP2* polynucleotide with an Adenosine Deaminase Base Editor 8 (ABE8) in a complex with one or more guide polynucleotides, wherein the ABE8 comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain, and wherein one or more of said guide polynucleotides target said base editor to effect an A•T to G•C alteration of the SNP in the *MECP2* polynucleotide associated with RETT, wherein the alteration is one or both of R133C or R306C. In an embodiment, the contacting is in a cell, a eukaryotic cell, a mammalian cell, or human cell. In an embodiment, the cell is *in vivo* or *ex vivo*. In an embodiment, the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, or stop codon to arginine in the methyl CpG binding protein 2 (Mecp2) polypeptide. In an embodiment, the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306. In an embodiment, the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof. In an embodiment, the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM). In an embodiment, the modified SpCas9 binds to a PAM comprising a nucleic acid sequence selected from 5′-NGT-3′ or 5′-NGG-3′. In an embodiment, the modified SpCas9 binds to a NGT PAM variant. In an embodiment, the NGT PAM variant comprises amino acid substitutions at one or more residues

1335, 1337, 1135, 1136, 1218, and/or 1219 of the modified SpCas9, or corresponding amino acid substitutions thereof. In an embodiment, the modified SpCas9 comprises the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, , T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof. In an embodiment, the modified SpCas9 comprises the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, G1218R, E1219F, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, T1337L, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof. In an embodiment, the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant. In an embodiment, the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof. In an embodiment, the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA). In an embodiment, the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD. In an embodiment, the adenosine deaminase domain comprises alterations at amino acid position 82 and 166. In an embodiment, the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration. In an embodiment, the adenosine deaminase domain further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R. In an embodiment, the adenosine deaminase domain comprises an alteration selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant. In an

embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*8 domain and wild-type TadA domain. In an embodiment, the adenosine deaminase monomer further comprises an alteration selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 base editor comprises a heterodimer comprising a wild-type TadA domain and an adenosine deaminase variant comprising a combination of alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence selected from AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'. In an embodiment, the adenosine deaminase is a TadA deaminase. In an embodiment, the TadA deaminase is a TadA*8 variant. In an embodiment, the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24. In an embodiment, the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24. In an embodiment, the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

In an embodiment, the ABE8 base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT. In an embodiment, the ABE8 base editor comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

[0010] In another aspect is provided a cell produced by introducing into the cell, or a progenitor thereof: (i) an ABE8 base editor, or a polynucleotide encoding said base editor, wherein said ABE8 base editor comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain; and (ii) one or more guide polynucleotides that target the base editor to effect an A•T to G•C alteration of the SNP in an *MECP2* polynucleotide associated with RETT syndrome (RETT), wherein the alteration is one or both of R133C or R306C. In an embodiment, the cell is a neuron. In an embodiment, the neuron expresses an MECP2 polypeptide. In an embodiment, the cell is from a subject having RETT. In an embodiment, the cell is a mammalian cell or a human cell. In an embodiment, the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide. In an embodiment, the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306. In an embodiment, the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof. In an embodiment, the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM). In an embodiment, the modified SpCas9 binds to a PAM comprising a nucleic acid sequence selected from 5′-NGT-3′ or 5′-NGG-3′. In an embodiment, the modified SpCas9 binds to a NGT PAM variant. In an embodiment, the NGT PAM variant comprises amino acid substitutions at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219 of the modified SpCas9, or corresponding amino acid substitutions thereof. In an embodiment, the modified SpCas9 comprises the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L, T1337Q, T1337I,

T1337V, T1337F, T1337S, T1337N, T1337K, , T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof. In an embodiment, the modified SpCas9 comprises the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, G1218R, E1219F, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, T1337L, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof. In an embodiment, the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant. In an embodiment, the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof. In an embodiment, the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA). In an embodiment, the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD. In an embodiment, the adenosine deaminase further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R. In an embodiment, the adenosine deaminase domain comprises a combination of alterations selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant. In an embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*7.10 domain and TadA*8 domain. In an embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 base editor comprises a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant comprising

alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'. In an embodiment, the adenosine deaminase is a TadA deaminase. In an embodiment, the TadA deaminase is a TadA*8 variant. In an embodiment, the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24. In an embodiment, the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24. In an embodiment, the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT. In an embodiment, the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT. In an embodiment, the ABE8 base editor comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD. In an embodiment, the gRNA comprises

a scaffold having the following sequence:
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC
CGAGTCGGTGCTTTTTTT.

[0011] In another aspect, a method of treating RETT Syndrome (RETT) in a subject is provided, in which the method comprises administering to said subject an ABE8 base editor, or a polynucleotide encoding said base editor, wherein said ABE8 base editor comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain; and one or more guide polynucleotides that target the ABE8 base editor to effect an A•T to G•C alteration of the SNP in an MECP2 polynucleotide associated with RETT, wherein the alteration is one or both of R133C and/or R306C. In an embodiment, the subject is a mammal or a human. In an embodiment, the method comprises delivering the ABE8 base editor, or polynucleotide encoding said ABE8 base editor, and said one or more guide polynucleotides to a cell of the subject, optionally, wherein the cell is a neuron. In an embodiment of the method, the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, or stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide. In an embodiment, the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306. In an embodiment, the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof. In an embodiment, the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM). In an embodiment, the modified SpCas9 binds to a PAM comprising a nucleic acid sequence selected from 5′-NGT-3′ or 5′-NGG-3′. In an embodiment, the modified SpCas9 binds to a NGT PAM variant. In an embodiment, the NGT PAM variant comprises amino acid substitutions at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219, of the modified SpCas9, or corresponding amino acid substitutions thereof. In an embodiment, the modified SpCas9 comprises the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, and T1337M, or corresponding amino acid substitutions thereof. In an embodiment, the modified SpCas9 comprises the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L,

T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof. In an embodiment, the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant. In an embodiment, the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof. In an embodiment, the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA). In an embodiment, the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD. In an embodiment, the adenosine deaminase domain comprises alterations at amino acid position 82 and 166. In an embodiment, the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration. In an embodiment, the adenosine deaminase domain further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R. In an embodiment, the adenosine deaminase domain comprises an alteration selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant. In an embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*7.10 domain and TadA*8 domain. In an embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 base editor comprises a heterodimer comprising a TadA7.10 domain and an adenosine deaminase variant comprising alterations selected from the group consisting of Y147T + Q154R;

Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the guide polynucleotide has a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'. In an embodiment, the adenosine deaminase is a TadA deaminase. In an embodiment, the TadA deaminase is a TadA*8 variant. In an embodiment, the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24. In an embodiment, the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24. In an embodiment, the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT. In an embodiment, the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

[0012] In another aspect, a method of treating Rett syndrome (RETT) in a subject is provided, in which the method comprises administering to a subject in need thereof (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine

deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 relative to a TadA reference sequence, or a corresponding position thereof, wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (*MECP2*) gene or a regulatory element thereof comprising a SNP associated with RETT in the subject, thereby treating RETT in the subject, and wherein the SNP associated with RETT results in an R133C or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene. In an embodiment of the method, the administration ameliorates at least one symptom associated with RETT. In an embodiment of the method, the administration results in faster amelioration of at least one symptom related to RETT compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase. In an embodiment of the method, the A-to-G nucleobase alteration changes the SNP associated with RETT to a wild type nucleobase. In an embodiment, the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in ameliorated RETT symptoms. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT. In an embodiment, the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

[0013] In embodiments of the above-delineated aspects of methods of editing and the above-delineated aspect of the base editor system, and embodiments thereof, the guide polynucleotide comprises a nucleic acid sequence comprising at least 10 contiguous nucleotides that are complementary to the *MECP2* gene or a regulatory element thereof. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that are complementary to the *MECP2* gene ore a regulatory element thereof.

[0014] In embodiments of the above-delineated aspects of methods of editing or treating and the above-delineated aspect of the cell, and embodiments thereof, the guide polynucleotide comprises a nucleic acid sequence comprising at least 10 contiguous nucleotides that are

complementary to the *MECP2* polynucleotide. In an embodiment, the guide polynucleotide comprises a nucleic acid sequence comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that are complementary to the *MECP2* polynucleotide.

[0015] In embodiments of the above-delineated method of treating RETT and embodiments thereof, the SNP associated with RETT results in an R133C and/or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene. In an embodiment, the SNP associated with RETT results in an R133C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene. In an embodiment, the SNP associated with RETT results in an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

[0016] In embodiments of the above-delineated base editor system and embodiments thereof, the A-to-G nucleobase alteration at the SNP associated with RETT results in an R133C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene. In an embodiment of the base editor system, the A-to-G nucleobase alteration at the SNP associated with RETT results in an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

[0017] In an embodiment of the above-delineated method of editing an *MECP2* polynucleotide or method of treating RETT syndrome (RETT), and embodiments thereof, the alteration of the SNP associated with RETT comprises both R133C and R306C. In an embodiment, the alteration of the SNP associated with RETT is R133C. In an embodiment, the alteration of the SNP associated with RETT is R306C.

[0018] In an embodiment of the above-delineated cell and embodiments thereof, the alteration of the SNP associated with RETT syndrome (RETT) is R133C. In an embodiment, the alteration of the SNP associated with RETT syndrome (RETT) is R306C.

[0019] In another aspect, a guide polynucleotide or guide RNA is provided, in which the guide polynucleotide or guide RNA (gRNA) comprises 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an *MECP2* gene that encodes an MECP2 protein. In an embodiment, the guide polynucleotide or guide RNA comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'. In an

embodiment, the guide polynucleotide or guide RNA further comprises a scaffold sequence, wherein the scaffold sequence is optionally as follows: GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC CGAGTCGGTGCTTTTTTT.

[0020] In another aspect, a composition comprising an Adenosine Deaminase Base Editor 8 (ABE8) and a guide RNA is provided, wherein, in the composition, the ABE8 comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain, and wherein the guide RNA targets the base editor to effect an A•T to G•C alteration of the SNP in an *MECP2* polynucleotide associated with RETT syndrome, and wherein the alteration is one or both of R133C or R306C. In an embodiment of the composition, the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, or stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide. In an embodiment, the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306. In an embodiment, the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof. In an embodiment, the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM). In an embodiment, the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant. In an embodiment, the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof. In an embodiment, the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA). In an embodiment of the composition, the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD. In an embodiment, the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration. In an embodiment, the adenosine deaminase domain further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R. In an embodiment, the adenosine deaminase domain comprises an alteration selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y +

V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant. In an embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*8 domain and wild-type TadA domain. In an embodiment, the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. In an embodiment, the ABE8 base editor comprises a heterodimer comprising a wild-type TadA domain and an adenosine deaminase variant comprising a combination of alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In an embodiment, the guide RNA comprises a nucleic acid sequence selected from AGAGCAAAAGGCUUUUCCCU-3′, 5′-UAGAGCAAAAGGCUUUUCCC-3′, 5′-UAGAGCAAAAGGCUUUUCCCU-3′, 5′-UUUAGAGCAAAAGGCUUUUCCCU-3′, 5′-UCUUGCACUUCUUGAUGGGG-3′, 5′-CUUGCACUUCUUGAUGGGGAG-3′, or 5′-GUCUUGCACUUCUUGAUGGGGAG-3′. In an embodiment, the adenosine deaminase is a TadA deaminase. In an embodiment, the TadA deaminase is a TadA*8 variant. In an embodiment, the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24. In an embodiment, the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m,

ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24. In an embodiment of the composition, the guide RNA comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT. In an embodiment, the ABE8 base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT. In an embodiment, the ABE8 base editor comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD. In an embodiment, the composition further comprises a lipid, optionally wherein the lipid is a cationic lipid.

[0021] In an embodiment of the above-delineated aspects of the composition and its embodiments, the composition is a pharmaceutical composition comprising a pharmaceutically acceptable excipient or diluent. In an embodiment, the pharmaceutical composition is for the treatment of RETT syndrome. In an embodiment of the pharmaceutical composition, the gRNA and the ABE8 base editor are formulated together or separately. In an embodiment, the pharmaceutical composition further comprises a vector suitable for expression in a mammalian cell, wherein the vector comprises a polynucleotide encoding the ABE8 base editor. In an embodiment of the pharmaceutical composition, the vector is a viral vector. In an embodiment of the pharmaceutical composition, the viral vector is a retroviral vector, adenoviral vector, lentiviral vector, herpesvirus vector, or adeno-associated viral vector (AAV). In an embodiment, the pharmaceutical composition further comprises a ribonucleoparticle suitable for expression in a mammalian cell.

[0022] In another aspect, a method of treating RETT syndrome is provided in which the method comprises administering to a subject in need thereof the pharmaceutical composition as described in any of the above-delineated aspects and embodiments. In an embodiment of the method, the subject is a mammal or a human.

[0023] In another aspect, the use of the pharmaceutical composition as described in any of the above-delineated aspects and embodiments in the treatment of RETT syndrome in a subject is provided. In an embodiment of the use, the subject is a mammal or a human.

[0024] In an aspect, a composition comprising the cell as described in any of above-delineated aspects and embodiments is provided. In an embodiment the composition further comprises a pharmaceutically acceptable carrier or diluent.

[0025] In another aspect, a pharmaceutical composition comprising (i) a nucleic acid encoding an ABE8 base editor; and (ii) the guide polynucleotide or guide RNA as described in the above-delineated aspect and embodiments is provided. In an embodiment, the pharmaceutical composition further comprises a lipid. In an embodiment, the lipid is a cationic lipid. In an embodiment of the pharmaceutical composition, the nucleic acid encoding the base editor is an mRNA.

[0026] In another aspect, an ABE8 base editor is provided, in which the ABE8 base editor comprises (i) a modified SpCas9 comprising the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof; and (ii) a TadA*8 adenosine deaminase.

[0027] In another aspect, an ABE8 base editor is provided, in which the ABE8 base editor comprises (i) a modified SpCas9 comprising the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, G1218R, E1219F, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, T1337L, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof; and (ii) a TadA*8 deaminase.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0029] FIG. 1 is a graph depicting motor behavioral assessment based on RTT mutations.

[0030] FIG. 2 illustrates the functions of each domain of the MECP2 protein and the location of common RTT mutations.

[0031] FIG. 3 is a graph depicting the percentage of precise correction of R106W RTT mutation using ABE8 base editor variants using gRNA1, gRNA2, and gRNA5. The results for each base editor paired with each gRNA are shown from left to right ABE8.14 (farthest left) to Neg. Ct (farthest right).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0032] The present invention features compositions and methods for the precise correction of pathogenic amino acids associated with RTT using a programmable nucleobase editor (e.g., ABE8).

[0033] The invention is based, at least in part, on the discovery that a base editor featuring adenosine deaminase variants (termed Adenosine Base Editor 8 or "ABE8" herein) precisely corrects single nucleotide polymorphisms (SNPs) in the endogenous *Mecp2* gene (*e.g.*, R106W, R133C, T158M, R255*, R270*, R306C). In an embodiment, the SNP in the *Mecp2* gene is R133C. In an embodiment, the SNP in the *Mecp2* gene is R306C.

[0034] Described herein are compositions and methods providing base editing and base editing systems to precisely correct one or more mutations in the methyl-CpG-binding protein 2 (*Mecp2*) gene, which is causally related to the progressive neurodevelopmental disorder Rett Syndrome (RTT or RETT) and its symptoms. RTT is an X-linked dominant disorder that predominantly affects females, is associated in 96% of affected individuals with mutations in the *Mecp2* gene and is characterized by apparently normal early development followed by a regression with loss of fine motor skills and effective communication, stereotypic movements, and apraxia or complete absence of gait (*see* FIG. 1). Additional clinical features of afflicted individuals include abnormal postnatal deceleration in the rate of head growth, periodic breathing, gastrointestinal dysfunction, epilepsy, and scoliosis.

[0035] The most prevalent RTT-causing mutations are cytidine to thymidine (C→T) transition mutations, resulting in a C•G to T•A base pair substitution. This substitution may be reverted back to a wild-type, non-pathogenic genomic sequence with an adenosine base editor (ABE) which catalyzes A•T to G•C substitutions. By extension, highly prevalent RTT-causing mutations are potential targets for reversion to wild-type sequence using ABEs without the risks of inducing *Mecp2* gene overexpression, as may occur using gene therapy. Accordingly, A•T to

G•C DNA base editing has the potential to precisely correct one or more of the most prevalent RTT-causing mutations in the *Mecp2* gene.

[0036] The following description and examples illustrate embodiments of the present disclosure in detail. It is to be understood that this disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this disclosure, which are encompassed within its scope.

[0037] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0038] Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

DEFINITIONS

[0039] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0040] Unless defined otherwise, all technical and scientific terms as used in the present disclosure and the embodiments thereof have the meaning commonly understood by a person skilled in the pertinent art. The following references provide one of skill with a general definition of many of the terms used in this disclosure: 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).

[0041] In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise, and is understood to be inclusive.

Furthermore, use of the term "including" as well as other forms, such as "include," "includes," and "included," is not limiting.

[0042] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0043] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within one (1) or more than one (1) standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" means within an acceptable error range for the particular value should be assumed.

[0044] 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.

[0045] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

[0046] 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.

[0047] In some embodiments, the deaminase or deaminase domain is a variant of a naturally occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identical to a naturally occurring deaminase.

[0048] In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is TadA variant. In some embodiments, the TadA variant is a TadA*8. A wild type TadA(wt) adenosine deaminase has the following sequence (also termed TadA reference sequence):

[0049] MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAE IMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHP GMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD.

[0050] In some embodiments, the adenosine deaminase comprises an alteration in the following sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

(also termed TadA*7.10).

[0051] In some embodiments, TadA*7.10 comprises at least one alteration. In some embodiments, TadA*7.10 comprises an alteration at amino acid 82 and/or 166. In particular embodiments, a variant of the above-referenced sequence comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In other embodiments, the variant of the TadA*7.10 sequence comprises a combination of alterations selected from Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S +

Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; or I76Y + V82S + Y123H + Y147R + Q154R.

[0052] In other embodiments, adenosine deaminase variants are provided that include deletions comprising a deletion of the C terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, or 157, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a TadA (*e.g.*, TadA*8) monomer comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a TadA (*e.g.*, TadA*8) monomer comprising a combination of alterations selected from Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; or I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

[0053] In still other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains (*e.g.*, TadA*8) each having one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a homodimer comprising two adenosine deaminase domains (*e.g.*, TadA*8) each having a combination of alterations selected from Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; or I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

[0054] In other embodiments, the adenosine deaminase variant is a heterodimer comprising a wild-type TadA adenosine deaminase domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a

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

[0055] In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising one or more of the following alterations Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant is a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant domain (*e.g.*, TadA*8) comprising a combination of the following alterations: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

[0056] In one embodiment, the adenosine deaminase is a TadA*8 that comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

[0057] In some embodiments, the TadA*8 is truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

[0058] In particular embodiments, an adenosine deaminase heterodimer comprises an TadA*8 domain and an adenosine deaminase domain selected from one of the following:

Staphylococcus aureus (*S. aureus*) TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAH
AEHIAIERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGS
LMNLLQQSNFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

Bacillus subtilis (*B. subtilis*) TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEML
VIDEACKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGCSGTLMN
LLQEERFNHQAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEG
WNRPIGRHDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIG
RVVFGARDAKTGAAGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIK
ALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:

MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEI
LCLRSAGKKLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGT
VVNLLQHPAFNHQVEVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

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

MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDPTAH
AEIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYK
TGAIGSRFHFFDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSDK

Caulobacter crescentus (*C. crescentus*) TadA:

MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAH
DPTAHAEIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADD
PKGGAVVHGPKFFAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:

MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSN
DPSAHAEMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDP
KGGAAGSLYDLSADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALF
IDERKVPPEP

TadA*7.10

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

[0059] By "Adenosine Deaminase Base Editor 8 (ABE8) polypeptide" or "ABE8" is meant a base editor as defined herein comprising an adenosine deaminase variant comprising an alteration at amino acid position 82 and/or 166 of the following reference sequence:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

[0060] In some embodiments, ABE8 comprises further alterations, as described herein, relative to the reference sequence.

[0061] By "Adenosine Deaminase Base Editor 8 (ABE8) polynucleotide" is meant a polynucleotide encoding an ABE8.

[0062] "Administering" is referred to herein as providing one or more compositions described herein to a patient or a subject. By way of example and without limitation, composition administration, *e.g.*, injection, can be performed by intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or intramuscular (i.m.) injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. In some embodiments, parenteral administration includes infusing or injecting intravascularly, intravenously, intramuscularly, intraarterially, intrathecally, intratumorally, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally. Alternatively, or concurrently, administration can be by the oral route.

[0063] By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

[0064] By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

[0065] By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a

corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, and/or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

[0066] By "methyl CpG binding protein 2 (Mecp2) protein" is meant a polypeptide or fragment thereof having at least about 95% amino acid sequence identity to NCBI Accession No. NP_004983. In particular embodiments, an Mecp2 protein comprises one or more alterations relative to the following reference sequence. In particular embodiments, an Mecp2 protein associated with RTT comprises one or more mutations selected from R106W, R168*, R133C, T158M, R255*, R270*, and R306C. An exemplary Mecp2 amino acid sequence is provided below.

```
  1 mvagmlglre eksedqdlqg lkdkplkfkk vkkdkkeeke gkhepvqpsa hhsaepaeag
 61 kaetsegsgs apavpeasas pkqrrsiird rgpmyddptl pegwtrklkq rksgrsagky
121 dvylinpqgk afrskvelia yfekvgdtsl dpndfdftvt grgspsrreq kppkkpkspk
181 apgtgrgrgr pkgsgttrpk aatsegvqvk rvlekspgkl lvkmpfqtsp ggkaegggat
241 tstqvmvikr pgrkrkaead pqaipkkrgr kpgsvvaaaa aeakkkavke ssirsvqetv
301 lpikkrktre tvsievkevv kpllvstlge ksgkglktck spgrkskess pkgrsssass
361 ppkkehhhhh hhsespkapv pllpplpppp pepessedpt sppepqdlss svckeekmpr
421 ggslesdgcp kepaktqpav ataataaeky khrgegerkd ivsssmprpn reepvdsrtp
481 vtervs
```

[0067] By "*Mecp2* polynucleotide" is meant a nucleic acid molecule encoding an Mecp2 protein or fragment thereof. The sequence of an exemplary *Mecp2* polynucleotide, which is available at NCBI Accession No. NM_004992, is provided below. In particular embodiments, an *Mecp2* polynucleotide comprises one or more alterations relative to the following reference sequence. In particular embodiments, an *Mecp2* polynucleotide associated with RTT comprises one or more mutations selected from 316C>T, 397C>T, 473C>T, 763C>T, 808C>T and 916C>T.

```
  1 ccggcgtcgg cggcgcgcgc gctcctcct ctcggagaga gggctgtggt aaaagccgtc
 61 cggaaaatgg ccgccgccgc cgccgccgcg ccgagcggag gaggaggagg aggcgaggag
121 gagagactgc tccataaaaa tacagactca ccagttcctg ctttgatgtg acatgtgact
181 ccccagaata caccttgctt ctgtagacca gctccaacag gattccatgg tagctgggat
241 gttagggctc agggaagaaa agtcagaaga ccaggacctc cagggcctca aggacaaacc
301 cctcaagttt aaaaaggtga agaaagataa gaaagaagag aaagagggca agcatgagcc
361 cgtgcagcca tcagcccacc actctgctga gcccgcagag gcaggcaaag cagagacatc
421 agaagggtca ggctccgccc cggctgtgcc ggaagcttct gcctccccca aacagcggcg
```

481 ctccatcatc cgtgaccggg gacccatgta tgatgacccc accctgcctg aaggctggac
541 acggaagctt aagcaaagga aatctggccg ctctgctggg aagtatgatg tgtatttgat
601 caatccccag ggaaaagcct ttcgctctaa agtggagttg attgcgtact tcgaaaaggt
661 aggcgacaca tccctggacc ctaatgattt tgacttcacg gtaactggga gagggagccc
721 ctcccggcga gagcagaaac cacctaagaa gcccaaatct cccaaagctc caggaactgg
781 cagaggccgg ggacgcccca aagggagcgg caccacgaga cccaaggcgg ccacgtcaga
841 gggtgtgcag gtgaaaaggg tcctggagaa aagtcctggg aagctccttg tcaagatgcc
901 ttttcaaact tcgccagggg gcaaggctga ggggggtggg gccaccacat ccacccaggt
961 catggtgatc aaacgccccg gcaggaagcg aaaagctgag gccgaccctc aggccattcc
1021 caagaaacgg ggccgaaagc cggggagtgt ggtggcagcc gctgccgccg aggccaaaaa
1081 gaaagccgtg aaggagtctt ctatccgatc tgtgcaggag accgtactcc ccatcaagaa
1141 gcgcaagacc cgggagacgg tcagcatcga ggtcaaggaa gtggtgaagc ccctgctggt
1201 gtccaccctc ggtgagaaga gcgggaaagg actgaagacc tgtaagagcc ctgggcggaa
1261 aagcaaggag agcagcccca aggggcgcag cagcagcgcc tcctcacccc ccaagaagga
1321 gcaccaccac catcaccacc actcagagtc cccaaaggcc cccgtgccac tgctcccacc
1381 cctgccccca cctccacctg agcccgagag ctccgaggac cccaccagcc cccctgagcc
1441 ccaggacttg agcagcagcg tctgcaaaga ggagaagatg cccagaggag gctcactgga
1501 gagcgacggc tgccccaagg agccagctaa gactcagccc gcggttgcca ccgccgccac
1561 ggccgcagaa aagtacaaac accgagggga gggagagcgc aaagacattg tttcatcctc
1621 catgccaagg ccaaacagag aggagcctgt ggacagccgg acgcccgtga ccgagagagt
1681 tagctgactt tacacggagc ggattgcaaa gcaaaccaac aagaataaag gcagctgttg
1741 tctcttctcc ttatgggtag ggctctgaca aagcttcccg attaactgaa ataaaaaata
1801 ttttttttc tttcagtaaa cttagagttt cgtggcttca gggtgggagt agttggagca
1861 ttggggatgt ttttcttacc gacaagcaca gtcaggttga agacctaacc agggccagaa
1921 gtagctttgc acttttctaa actaggctcc ttcaacaagg cttgctgcag atactactga
1981 ccagacaagc tgttgaccag gcacctcccc tcccgcccaa acctttcccc catgtggtcg
2041 ttagagacag agcgacagag cagttgagag gacactcccg ttttcggtgc catcagtgcc
2101 ccgtctacag ctcccccagc tccccccacc tcccccactc ccaaccacgt tgggacaggg
2161 aggtgtgagg caggagagac agttggattc tttagagaag atggatatga ccagtggcta
2221 tggcctgtgc gatcccaccc gtggtggctc aagtctggcc ccacaccagc cccaatccaa
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2701 acagatgctc tgagagcaaa ctggcttgaa ttggtgacat ttagtccctc aagccaccag
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10021 ttattgattg ctttttttgta gtgttcagaa aaaaggttct ttgtgtatag ccaaatgact

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10081 gaaagcactg atatatttaa aaacaaaagg caatttatta aggaaatttg taccatttca
10141 gtaaacctgt ctgaatgtac ctgtatacgt ttcaaaaaca ccccccccc actgaatccc
10201 tgtaacctat ttattatata aagagtttgc cttataaatt t
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[0068] By "base editor (BE)" or "nucleobase editor (NBE)" is meant an agent that binds a polynucleotide and has nucleobase modifying activity. In various embodiments, the base editor comprises a nucleobase modifying polypeptide (*e.g.*, a deaminase) and a nucleic acid programmable nucleotide binding domain in conjunction with a guide polynucleotide (*e.g.*, guide RNA). In various embodiments, the agent is a biomolecular complex comprising a protein domain having base editing activity, *i.e.*, a domain capable of modifying a base (*e.g.*, A, T, C, G, or U) within a nucleic acid molecule (*e.g.*, DNA). In some embodiments, the polynucleotide programmable DNA binding domain is fused or linked to a deaminase domain. In one embodiment, the agent is a fusion protein comprising a domain having base editing activity. In another embodiment, the protein domain having base editing activity is linked to the guide RNA (*e.g.*, via an RNA binding motif on the guide RNA and an RNA binding domain fused to the deaminase). In some embodiments, the 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 one or more bases within a DNA molecule. In some embodiments, the base editor is capable of deaminating an adenosine (A) within DNA. In some embodiments, the base editor is an adenosine base editor (ABE).

[0069] In some embodiments, base editors are generated (*e.g.*, ABE8) by cloning an adenosine deaminase variant (*e.g.*, TadA*8) into a scaffold that includes a circular permutant Cas9 (*e.g.*, spCAS9 or saCAS9) and a bipartite nuclear localization sequence. Circular permutant Cas9's are known in the art and described, for example, in Oakes *et al.*, Cell 176, 254–267, 2019. An exemplary circular permutant follows where the bold sequence indicates sequence derived from Cas9, the italics sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence,

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

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQV
NIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEKGKSKKL
KSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAKFLQK
GNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL
DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYRSTKEVLDATLIHQSI
TGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM***DKKYSIGLAIGTNSVGWAVITDEYKVPS**

KKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVD
DSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA
HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLEN
LIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENED
ILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTI
LDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNE
KLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEE
VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRM
NTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKL
ESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

[0070] In some embodiments, the base editor is an Adenosine Deaminase Base Editor 8 (ABE8). In some embodiments, the ABE8 is selected from a base editor from **Table 9** *infra*. In some embodiments, ABE8 contains an adenosine deaminase variant evolved from TadA. In some embodiments, the adenosine deaminase variant of ABE8 is a TadA*8 variant as described in **Table 9** *infra*. In some embodiments, the adenosine deaminase variant is TadA*7.10 variant (*e.g.*, TadA*8) comprising one or more of an alteration selected from the group of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R. In various embodiments, ABE8 comprises TadA*7.10 varient (*e.g.*, TadA*8) with a combination of alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In some embodiments ABE8 is a monomeric construct. In some embodiments, ABE8 is a heterodimeric construct. In some embodiments the ABE8 base editor comprises the sequence:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

[0071] In some embodiments, the polynucleotide programmable DNA binding domain is a CRISPR associated (*e.g.*, Cas or Cpf1) enzyme. In some embodiments, the base editor is a catalytically dead Cas9 (dCas9) fused to a deaminase domain. In some embodiments, the base editor is a Cas9 nickase (nCas9) fused to a deaminase domain. In some embodiments, the base editor is fused to an inhibitor of base excision repair (BER). In some embodiments, the inhibitor of base excision repair is a uracil DNA glycosylase inhibitor (UGI). In some embodiments, the inhibitor of base excision repair is an inosine base excision repair inhibitor. Details of base editors are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1, the entire contents of which are hereby incorporated by reference.

[0072] By way of example, the adenine base editor ABE as used in the base editing compositions, systems and methods described herein has the nucleic acid sequence (8877 base pairs), (Addgene, Watertown, MA.; Gaudelli NM, *et al.*, Nature. 2017 Nov 23;551(7681):464-471. doi: 10.1038/nature24644; Koblan LW, *et al.*, Nat Biotechnol. 2018 Oct;36(9):843-846. doi: 10.1038/nbt.4172.) as provided below. Polynucleotide sequences having at least 95% or greater identity to the ABE nucleic acid sequence are also encompassed.

ATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACAT
GACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTG
ACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCC
ATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGT
CAGATCCGCTAGAGATCCGCGGCCGCTAATACGACTCACTATAGGGAGAGCCGCCACCATGAAACGGACA
GCCGACGGAAGCGAGTTCGAGTCACCAAAGAAGAAGCGGAAAGTCTCTGAAGTCGAGTTTAGCCACGAGT

ATTGGATGAGGCACGCACTGACCCTGGCAAAGCGAGCATGGGATGAAAGAGAAGTCCCCGTGGGCGCCGT
GCTGGTGCACAACAATAGAGTGATCGGAGAGGGATGGAACAGGCCAATCGGCCGCCACGACCCTACCGCA
CACGCAGAGATCATGGCACTGAGGCAGGGAGGCCTGGTCATGCAGAATTACCGCCTGATCGATGCCACCC
TGTATGTGACACTGGAGCCATGCGTGATGTGCGCAGGAGCAATGATCCACAGCAGGATCGGAAGAGTGGT
GTTCGGAGCACGGGACGCCAAGACCGGCGCAGCAGGCTCCCTGATGGATGTGCTGCACCACCCCGGCATG
AACCACCGGGTGGAGATCACAGAGGGAATCCTGGCAGACGAGTGCGCCGCCCTGCTGAGCGATTTCTTTA
GAATGCGGAGACAGGAGATCAAGGCCCAGAAGAAGGCACAGAGCTCCACCGACTCTGGAGGATCTAGCGG
AGGATCCTCTGGAAGCGAGACACCAGGCACAAGCGAGTCCGCCACACCAGAGAGCTCCGGCGGCTCCTCC
GGAGGATCCTCTGAGGTGGAGTTTTCCCACGAGTACTGGATGAGACATGCCCTGACCCTGGCCAAGAGGG
CACGCGATGAGAGGGAGGTGCCTGTGGGAGCCGTGCTGGTGCTGAACAATAGAGTGATCGGCGAGGGCTG
GAACAGAGCCATCGGCCTGCACGACCCAACAGCCCATGCCGAAATTATGGCCCTGAGACAGGGCGGCCTG
GTCATGCAGAACTACAGACTGATTGACGCCACCCTGTACGTGACATTCGAGCCTTGCGTGATGTGCGCCG
GCGCCATGATCCACTCTAGGATCGGCCGCGTGGTGTTTGGCGTGAGGAACGCAAAAACCGGCGCCGCAGG
CTCCCTGATGGACGTGCTGCACTACCCCGGCATGAATCACCGCGTCGAAATTACCGAGGGAATCCTGGCA
GATGAATGTGCCGCCCTGCTGTGCTATTTCTTTCGGATGCCTAGACAGGTGTTCAATGCTCAGAAGAAGG
CCCAGAGCTCCACCGACTCCGGAGGATCTAGCGGAGGCTCCTCTGGCTCTGAGACACCTGGCACAAGCGA
GAGCGCAACACCTGAAAGCAGCGGGGGCAGCAGCGGGGGGTCAGACAAGAAGTACAGCATCGGCCTGGCC
ATCGGCACCAACTCTGTGGGCTGGGCCGTGATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGG
TGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGA
AACAGCCGAGGCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGC
TATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGACTGGAAGAGT
CCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGC
CTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAGAAACTGGTGGACAGCACCGACAAGGCCGAC
CTGCGGCTGATCTATCTGGCCCTGGCCCACATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACC
TGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGA
GGAAAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGA
CGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGAAACCTGATTGCCC
TGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGATGCCAAACTGCAGCTGAG
CAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTT
CTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCA
AGGCCCCCCTGAGCGCCTCTATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGC
TCTCGTGCGGCAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCC
GGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTGGAAAAGATGG
ACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAA
CGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCACGCCATTCTGCGGCGGCAGGAAGATTTTTAC
CCATTCCTGAAGGACAACCGGGAAAAGATCGAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCC
CTCTGGCCAGGGGAAACAGCAGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAA
CTTCGAGGAAGTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAG
AACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTATAACGAGC
TGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTGAGCGGCGAGCAGAAAAAGGC

CATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACTTCAAG
AAAATCGAGTGCTTCGACTCCGTGGAAATCTCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACAT
ACCACGATCTGCTGAAAATTATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGA
AGATATCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCC
CACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGCAGGCTGAGCC
GGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTGGATTTCCTGAAGTCCGACGG
CTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTTAAAGAGGACATCCAGAAA
GCCCAGGTGTCCGGCCAGGGCGATAGCCTGCACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTA
AGAAGGGCATCCTGCAGACAGTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGA
GAACATCGTGATCGAAATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGA
ATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCCGTGGAAAACA
CCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGGGATATGTACGTGGACCAGGA
ACTGGACATCAACCGGCTGTCCGACTACGATGTGGACCATATCGTGCCTCAGAGCTTTCTGAAGGACGAC
TCCATCGACAACAAGGTGCTGACCAGAAGCGACAAGAACCGGGGCAAGAGCGACAACGTGCCCTCCGAAG
AGGTCGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTT
CGACAATCTGACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAG
CTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAACACTAAGTACG
ACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCCAAGCTGGTGTCCGATTTCCG
GAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAACTACCACCACGCCCACGACGCCTACCTGAAC
GCCGTCGTGGGAACCGCCCTGATCAAAAAGTACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACA
AGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTT
CTTCTACAGCAACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGG
CCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTTGCCACCGTGC
GGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTGCAGACAGGCGGCTTCAGCAA
AGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATCGCCAGAAAGAAGGACTGGGACCCTAAGAAG
TACGGCGGCTTCGACAGCCCCACCGTGGCCTATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGT
CCAAGAAACTGAAGAGTGTGAAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAA
TCCCATCGACTTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAG
TACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTGCAGAAGGGAA
ACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGCCACTATGAGAAGCTGAAGGG
CTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAACAGCACAAGCACTACCTGGACGAGATCATC
GAGCAGATCAGCGAGTTCTCCAAGAGAGTGATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCT
ACAACAAGCACCGGGATAAGCCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAA
TCTGGGAGCCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAA
GAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATCGACCTGTCTC
AGCTGGGAGGTGACTCTGGCGGCTCAAAAAGAACCGCCGACGGCAGCGAATTCGAGCCCAAGAAGAAGAG
GAAAGTCTAACCGGTCATCATCACCATCACCATTGAGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC
TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGT
GGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCT

CTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCGATACCGTCGACCTCTAGCTAGAGCTTGGCGTA
ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGA
AGCATAAAGTGTAAAGCCTAGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGC
CCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGG
TTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGA
GCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACA
TGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCT
CCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAA
AGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGAT
ACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC
GGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA
TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTA
ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTA
CACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGC
TCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCA
GAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACACTCAGTGGAACGAAAACTC
ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGA
AGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGG
CACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTAC
GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCA
GATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCT
CCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGT
TGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCC
CAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGA
TCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTAC
TGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGT
ATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAA
AAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG
TTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGA
GCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATAC
TCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATG
TATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCGACGGA
TCGGGAGATCGATCTCCCGATCCCCTAGGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA
GCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAAC
AAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGAT
GTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT
TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC
CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATC

[0073] By "base editing activity" is meant acting to chemically alter a base within a polynucleotide. In one embodiment, a first base is converted to a second base. In one embodiment, *e.g.*, the base editing activity is adenosine or adenine deaminase activity, *e.g.*, converting A•T to G•C.

[0074] In some embodiments, base editing activity is assessed by efficiency of editing. Base editing efficiency may be measured by any suitable means, for example, by sanger sequencing or next generation sequencing. In some embodiments, base editing efficiency is measured by percentage of total sequencing reads with nucleobase conversion effected by the base editor, for example, percentage of total sequencing reads with target A•T base pair converted to a G•C base pair. In some embodiments, base editing efficiency is measured by percentage of total cells with nucleobase conversion effected by the base editor, when base editing is performed in a population of cells.

[0075] The term "base editor system" refers to a system for editing a nucleobase of a target nucleotide sequence. In various embodiments, the base editor (BE) system comprises (1) a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9); (2) a deaminase domain for deaminating said nucleobase (*e.g.* an adenosine deaminase); and (3) one or more guide polynucleotides (*e.g.*, guide RNA). In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the base editor is an adenine or adenosine base editor (ABE). In some embodiments, the base editor system is and Adenosine Deaminase Base Editor (ABE8). In some embodiments, the ABE8 is a monomeric construct. In some embodiments, the ABE8 is ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m. In some embodiments, the ABE8 is a heteromeric construct. In some embodiments, the ABE8 is ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, or ABE8.24-d.

[0076] In some embodiments, a nucleobase editor system may comprise more than one base editing component. For example, a base editor system may include one or more adenosine deaminases. In some embodiments, a single guide polynucleotide may be utilized to target different deaminases to a target nucleic acid sequence. In some embodiments, a single pair of

guide polynucleotides may be utilized to target different deaminases to a target nucleic acid sequence.

[0077] The deaminase domain and the polynucleotide programmable nucleotide binding component of a base editor system may be associated with each other covalently or non-covalently, or any combination of associations and interactions thereof. For example, in some embodiments, a 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 deaminase domain 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.

[0078] 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 deaminase domain 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.

[0079] 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 (BER) inhibitor. In some embodiments, the inhibitor of base excision repair (BER) can be a uracil DNA glycosylase inhibitor (UGI). In some embodiments, the inhibitor of base excision repair can be an inosine base excision repair (BER) inhibitor. In some embodiments, the inhibitor of base excision repair (BER) 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 (BER). 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 (BER). 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 (BER). For example, in some embodiments, the inhibitor of base excision repair (BER) 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 (BER) can be targeted to the target nucleotide sequence by the guide polynucleotide. For example, in some embodiments, the inhibitor of base excision repair (BER) 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 (BER). 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.

[0080] The term "Cas9" or "Cas9 domain" refers to an RNA guided nuclease comprising a Cas9 protein, or a fragment thereof (*e.g.*, a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A Cas9 nuclease is also referred to sometimes as a Casnl nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat) associated nuclease. CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut

endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA," or simply "gRNA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. *See, e.g.*, Jinek M., *et al.*, *Science* 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. Cas9 nuclease sequences and structures are well known to those of skill in the art (*see, e.g.*, "Complete genome sequence of an M1 strain of Streptococcus pyogenes." Ferretti *et al.*, 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., *et al.*, Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., *et al.*, *Science* 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences 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.

[0081] An exemplary Cas9, is *Streptococcus pyogenes* Cas9 (spCas9), the amino acid sequence of which is provided below:

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQIYNQL
FEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNSEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDRGMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGHSLHEQIANLAGSPAIKKGILQTVKIVDELVKVMGHKPENIVIEMARENQTTQKGQKNSRERM

KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF
IKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP
IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD (single underline: HNH domain; double underline: RuvC domain)

[0082] 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.

[0083] 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.

[0084] 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).

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGATCAC
TGATGATTATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACAGTATCA
AAAAAAATCTTATAGGGGCTCTTTTATTTGGCAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAA
CGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTC
AAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTTGGTGGAAG
AAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAG
AAATATCCAACTATCTATCATCTGCGAAAAAAATTGGCAGATTCTACTGATAAAGCGGATTTGCG
CTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGTCATTTTTTGATTGAGGGAGATT
TAAATCCTGATAATAGTGATGTGGACAAACTATTTATCCAGTTGGTACAAATCTACAATCAATTA
TTTGAAGAAAACCCTATTAACGCAAGTAGAGTAGATGCTAAAGCGATTCTTTCTGCACGATTGAG
TAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAGAAATGGCTTGTTTG
GGAATCTCATTGCTTTGTCATTGGGATTGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAA
GATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGCAAAT
TGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAG
ATATCCTAAGAGTAAATAGTGAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAGCGCTAC
GATGAACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTA

TAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCC
AAGAAGAATTTTATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTG
GTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCA
TCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAA
AAGACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATTG
GCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGAA
TTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACAAACTTTG
ATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTATTTTACGGTT
TATAACGAATTGACAAAGGTCAAATATGTTACTGAGGGAATGCGAAAACCAGCATTTCTTTCAGG
TGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAAGTAACCGTTAAGCAAT
TAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAGTTGAAGAT
AGATTTAATGCTTCATTAGGCGCCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTT
GGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAAGATA
GGGGGATGATTGAGGAAAGACTTAAAACATATGCTCACCTCTTTGATGATAAGGTGATGAAACAG
CTTAAACGTCGCCGTTATACTGGTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGA
TAAGCAATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGCAATTTTA
TGCAGCTGATCCATGATGATAGTTTGACATTTAAAGAAGATATTCAAAAAGCACAGGTGTCTGGA
CAAGGCCATAGTTTACATGAACAGATTGCTAACTTAGCTGGCAGTCCTGCTATTAAAAAAGGTAT
TTTACAGACTGTAAAAATTGTTGATGAACTGGTCAAAGTAATGGGGCATAAGCCAGAAAATATCG
TTATTGAAATGGCACGTGAAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATG
AAACGAATCGAAGAAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAA
TACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTACAAAATGGAAGAGACATGTATGTGG
ACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTC
ATTAAAGACGATTCAATAGACAATAAGGTACTAACGCGTTCTGATAAAAATCGTGGTAAATCGGA
TAACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGCCA
AGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAACTT
GATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTGGCACA
AATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGAGGTTAAAG
TGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCTATAAAGTACGT
GAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTTGAT
TAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTATGATGTTCGTA
AAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTAAT
ATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCTAAT
CGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTTTGCCACAGTGCGCA

AAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGAAAACAGAAGTACAGACAGGCGGATTCTCC
AAGGAGTCAATTTTACCAAAAAGAAATTCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGATCC
AAAAAAATATGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGG
AAAAAGGGAAATCGAAGAAGTTAAAATCCGTTAAAGAGTTACTAGGGATCACAATTATGGAAAGA
AGTTCCTTTGAAAAAAATCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGA
CTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGG
CTAGTGCCGGAGAATTACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTA
TATTTAGCTAGTCATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTT
TGTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTG
TTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCA
ATACGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGC
TTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATG
CCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTAGGA
GGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQIYNQL
FEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNSEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDRGMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGHSLHEQIANLAGSPAIKKGILQTVKIVDELVKVMGHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF
IKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL

YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP
IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD

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

[0085] In some embodiments, wild-type Cas9 corresponds to, or comprises the following nucleotide and/or amino acid sequences:

ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCACTAATTCCGTTGGATGGGCTGTCATAAC
CGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCATTCGATTA
AAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCGACTCGCCTGAAA
CGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTACTTACAAGAAATTTTTAG
CAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGTCCTTCCTTGTCGAAG
AGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGATGAGGTGGCATATCATGAA
AAGTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTCAACTGATAAAGCGGACCTGAG
GTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATC
TAAATCCGGACAACTCGGATGTCGACAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTG
TTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTC
TAAATCCCGACGGCTAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCG
GTAACCTTATAGCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAA
GATGCCAAATTGCAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAAAT
TGGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTG
ACATACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTAC
GATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATA
TAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTC
AAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTT
GTAAAACTCAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACA
TCAAATCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCA
AAGACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCTG
GCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAA
TTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTG
ACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTG
TACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGG
AGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTAAGCAAT
TGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCGGGGTAGAAGAT

CGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTAAAGATAAGGACTTCCT
GGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGACTCTTACCCTCTTTGAAGATC
GGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACCTGTTCGACGATAAGGTTATGAAACAG
TTAAAGAGGCGTCGCTATACGGGCTGGGGACGATTGTCGCGGAAACTTATCAACGGGATAAGAGA
CAAGCAAAGTGGTAAAACTATTCTCGATTTTCTAAAGAGCGACGGCTTCGCCAATAGGAACTTTA
TGCAGCTGATCCATGATGACTCTTTAACCTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGA
CAAGGGGACTCATTGCACGAACATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCAT
ACTCCAGACAGTCAAAGTAGTGGATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACA
TTGTAATCGAGATGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGG
ATGAAGAGAATAGAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGA
AAATACCCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATG
TTGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGTACCCCAATCC
TTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAG
TGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATG
CGAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTGAA
CTTGACAAGGCCGGATTTATTAAACGTCAGCTCGTGGAAACCCGCCAAATCACAAAGCATGTTGC
ACAGATACTAGATTCCCGAATGAATACGAAATACGACGAGAACGATAAGCTGATTCGGGAAGTCA
AAGTAATCACTTTAAAGTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAATTCTATAAAGTT
AGGGAGATAAATAACTACCACCATGCGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACT
CATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACGTCC
GTAAGATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTCTTTTATTCT
AACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATACGCAAACGACCTTT
AATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTCGCGACGGTGA
GAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAACTGAGGTGCAGACCGGAGGGTTT
TCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCTCGTAAAAAGGACTGGGA
CCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAG
TTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAG
CGCTCGTCTTTTGAAAAGAACCCCATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAA
GGATCTCATAATTAAACTACCAAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGT
TGGCTAGCGCCGGAGAGCTTCAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTC
CTGTATTTAGCGTCCCATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACT
TTTTGTTGAGCAGCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTAAGA
GAGTCATCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAA
CCCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGC

CGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAGCTT
GGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGACGGTGA
TTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

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

[0086] 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).

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGATCAC
TGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACAGTATCA

AAAAAAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAA
CGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTC
AAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTTGGTGGAAG
AAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAG
AAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGGATTTGCG
CTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGTCATTTTTTGATTGAGGGAGATT
TAAATCCTGATAATAGTGATGTGGACAAACTATTTATCCAGTTGGTACAAACCTACAATCAATTA
TTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTGCACGATTGAG
TAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTATTTG
GGAATCTCATTGCTTTGTCATTGGGTTTGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAA
GATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGCAAAT
TGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAG
ATATCCTAAGAGTAAATACTGAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTAC
GATGAACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTA
TAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCC
AAGAAGAATTTTATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTG
GTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCA
TCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAA
AAGACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATTG
GCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGAA
TTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACAAACTTTG
ATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTATTTTACGGTT
TATAACGAATTGACAAAGGTCAAATATGTTACTGAAGGAATGCGAAAACCAGCATTTCTTTCAGG
TGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAAGTAACCGTTAAGCAAT
TAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAGTTGAAGAT
AGATTTAATGCTTCATTAGGTACCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTT
GGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAAGATA
GGGAGATGATTGAGGAAAGACTTAAAACATATGCTCACCTCTTTGATGATAAGGTGATGAAACAG
CTTAAACGTCGCCGTTATACTGGTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGA
TAAGCAATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGCAATTTTA
TGCAGCTGATCCATGATGATAGTTTGACATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGA
CAAGGCGATAGTTTACATGAACATATTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTAT
TTTACAGACTGTAAAAGTTGTTGATGAATTGGTCAAAGTAATGGGGCGGCATAAGCCAGAAAATA
TCGTTATTGAAATGGCACGTGAAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGT

ATGAAACGAATCGAAGAAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGA
AAATACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGACATGTATG
TGGACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGT
TTCCTTAAAGACGATTCAATAGACAATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAAATC
GGATAACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACG
CCAAGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAA
CTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTGGC
ACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGAGGTTA
AAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCTATAAAGTA
CGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTT
GATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTATGATGTTC
GTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCT
AATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCT
AATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTTTGCCACAGTGC
GCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGAAAACAGAAGTACAGACAGGCGGATTC
TCCAAGGAGTCAATTTTACCAAAAAGAAATTCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGA
TCCAAAAAAATATGGTGGTTTTGATAGTCCAACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGG
TGGAAAAAGGGAAATCGAAGAAGTTAAAATCCGTTAAAGAGTTACTAGGGATCACAATTATGGAA
AGAAGTTCCTTTGAAAAAAATCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAA
AGACTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGC
TGGCTAGTGCCGGAGAATTACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTT
TTATATTTAGCTAGTCATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATT
GTTTGTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGC
GTGTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAA
CCAATACGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGC
TGCTTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAG
ATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTA
GGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY

DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD (single underline: HNH domain; double underline: RuvC domain)

[0087] 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.

[0088] In some embodiments, the Cas9 is from *Neisseria* meningitidis (Nme). In some embodiments, the Cas9 is Nme1, Nme2 or Nme3. In some embodiments, the PAM-interacting domains for Nme1, Nme2 or Nme3 are N_4GAT, N_4CC, and N_4CAAA, respectively (*see e.g.*, Edraki, A., *et al.*, A Compact, High-Accuracy Cas9 with a Dinucleotide PAM for *In Vivo* Genome Editing, Molecular Cell (2018)). An exemplary *Neisseria meningitidis* Cas9 protein, Nme1Cas9, (NCBI Reference: WP_002235162.1; type II CRISPR RNA-guided endonuclease Cas9) has the following amino acid sequence:

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   1 maafkpnpin yilgldigia svgwamveid edenpiclid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvadnahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlspelqd eigtafslfk tdeditgrlk driqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlgrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkernlndt ryvnrflcqf vadrmrltgk gkkrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgevlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 qghmetvksa krldegvsvl rvpltqlklk dlekmvnrer epklyealka rleahkddpa
 901 kafaepfyky dkagnrtqqv kavrveqvqk tgvwvrnhng iadnatmvrv dvfekgdkyy
 961 lvpiyswqva kgilpdravv qgkdeedwql iddsfnfkfs lhpndlvevi tkkarmfgyf
1021 aschrgtgni nirihdldhk igkngilegi gvktalsfqk yqidelgkei rpcrlkkrpp
1081 vr
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[0089] Another exemplary *Neisseria meningitidis* Cas9 protein, Nme2Cas9, (NCBI Reference: WP_002230835; type II CRISPR RNA-guided endonuclease Cas9) has the following amino acid sequence:

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   1 maafkpnpin yilgldigia svgwamveid eeenpirlid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvannahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlsselqd eigtafslfk tdeditgrlk drvqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlvrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkecnlndt ryvnrflcqf vadhilltgk gkrrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgkvlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 ahkdtlrsak rfvkhnekis vkrvwlteik ladlenmvny kngreielye alkarleayg
 901 gnakqafdpk dnpfykkggq lvkavrvekt qesgvllnkk naytiadngd mvrvdvfckv
 961 dkkgknqyfi vpiyawqvae nilpdidckg yriddsytfc fslhkydlia fqkdekskve
1021 fayyincdss ngrfylawhd kgskeqqfri stqnlvliqk yqvnelgkei rpcrlkkrpp
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1081 vr

[0090] 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):

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

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

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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.

[0095] Exemplary catalytically inactive Cas9 (dCas9):

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR
TARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK
YPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLF
EENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYD
EHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLV
KLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLA
RGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDR
FNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQL

KRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSF
LKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP
IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD

[0096] Exemplary catalytically Cas9 nickase (nCas9):

DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR
TARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK
YPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLF
EENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYD
EHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLV
KLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLA
RGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDR
FNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQL
KRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF
LKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP

IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD

[0097] Exemplary catalytically active Cas9:

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR
TARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEK
YPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLF
EENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYD
EHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLV
KLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLA
RGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVY
NELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDR
FNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQL
KRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF
LKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP
IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD.

[0098] In some embodiments, Cas9 refers to a Cas9 from archaea (*e.g.* nanoarchaea), which constitute a domain and kingdom of single-celled prokaryotic microbes. In some embodiments, Cas9 refers to CasX or CasY, which have been described in, for example, Burstein *et al.*, "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little- studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously

unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered. In some embodiments, Cas9 refers to CasX, or a variant of CasX. In some embodiments, Cas9 refers to a CasY, or a variant of CasY. It should be appreciated that other RNA-guided DNA binding proteins may be used as a nucleic acid programmable DNA binding protein (napDNAbp), and are within the scope of this disclosure.

[0099] In particular embodiments, napDNAbps useful in the methods of the disclosuer include circular permutants, which are known in the art and described, for example, by Oakes *et al.*, Cell 176, 254–267, 2019. An exemplary circular permutant follows where the bold sequence indicates sequence derived from Cas9, the italics sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

[0100] **CP5** (with MSP "NGC=Pam Variant with mutations Regular Cas9 likes NGG" PID=Protein Interacting Domain and "D10A" nickase):

EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQV
NIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEKGKSKKL
KSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAKFLQK
GNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL
DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYRSTKEVLDATLIHQSI
TGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM***DKKYSIGLAIGTNSVGWAVITDEYKVPS**
KKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVD
DSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA
HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLEN
LIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL
FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDK
GASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENED
ILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTI
LDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVV
DELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNE
KLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEE
VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRM

NTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKL
ESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

[0101] Non-limiting examples of a polynucleotide programmable nucleotide binding domain which can be incorporated into a base editor include a CRISPR protein-derived domain, a restriction nuclease, a meganuclease, TAL nuclease (TALEN), and a zinc finger nuclease (ZFN).

[0102] 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 Cas12b/C2c1, CasX and CasY from other bacterial species may also be used in accordance with the present disclosure.

[0103] Cas12b/C2c1 (uniprot.org/uniprot/T0D7A2#2)

sp|T0D7A2|C2C1_ALIAG CRISPR-associated endo- nuclease C2c1 OS = *Alicyclobacillus acido- terrestris* (strain ATCC 49025 / DSM 3922/ CIP 106132 / NCIMB 13137/GD3B) GN=c2c1 PE=1 SV=1

MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYRRSPNGDGEQECDKTA
EECKAELLERLRARQVENGHRGPAGSDDELLQLARQLYELLVPQAIGAKGDAQQIARKFLSPLAD
KDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKEKAETRKSADRTADVLRALADFGLKPLMRVYT
DSEMSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKLVEQKNRFEQKNFVGQ
EHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGRALRGSDKVFEKWGKLAPDAPFDLYDAEIKNV
QRRNTRRFGSHDLFAKLAEPEYQALWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDATAHPI
WTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDDVTVPISMSEQLDNLLPRDP
NEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRGARDVYLNVSVRVQSQSEARGERRP
PYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVA
RKDELKPNSKGRVPFFFPIKGNDNLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQLRTQLA
YLRLLVRCGSEDVGRRERSWAKLIEQPVDAANHMTPDWREAFENELQKLKSLHGICSDKEWMDAV
YESVRRVWRHMGKQVRDWRKDVRSGERPKIRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKV

SGQVIRAEKGSRFAITLREHIDHAKEDRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLI
LLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELINQAQVHDLLVGTMYAAFSSRFDARTGAPGI
RCRRVPARCTQEHNPEPFPWWLNKFVVEHTLDACPLRADDLIPTGEGEIFVSPFSAEEGDFHQIH
ADLNAAQNLQQRLWSDFDISQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYE
RERGKKRRKVFAQEKLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMV NQRI
EGYLVKQIRSRVPLQDSACENTGDI

[0104] 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
MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGKAKK
KKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAPSFVKPE
FYEFGRSPGMVERTRRVKLEVEPHYLIIAAAGWVLTRLGKAKVSEGDYVGVNVFTPTRGILYSLI
QNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVRIYTISDAVGQNPTTINGGFSIDLTKLLE
KRYLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTG SKRLEDLLYFANRDLIMNLNSDDG
KVRDLKLISAYVNGELIRGEG

[0105] >tr|F0NH53|F0NH53_SULIR CRISPR associated protein, Casx OS = *Sulfolobus islandicus* (strain REY15A) GN=SiRe_0771 PE=4 SV=1
MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGKAKK
KKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAPSFVKPE
FYKFGRSPGMVERTRRVKLEVEPHYLIMAAAGWVLTRLGKAKVSEGDYVGVNVFTPTRGILYSLI
QNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVSIYTISDAVGQNPTTINGGFSIDLTKLLE
KRDLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTGSKRLEDLLYFANRDLIMNLNSDDGK
VRDLKLISAYVNGELIRGEG

[0106] Deltaproteobacteria CasX
MEKRINKIRKKLSADNATKPVSRSGPMKTLLVRVMTDDLKKRLEKRRKKPEVMPQVISNNAANNL
RMLLDDYTKMKEAILQVYWQEFKDDHVGLMCKFAQPASKKIDQNKLKPEMDEKGNLTTAGFACSQ
CGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPVKDSDEAVTYSLGKFGQRALDF
YSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQDIIIEHQKVVKGNQKR
LESLRELAGKENLEYPSVTLPPQPHTKEGVDAYNEVIARVRMWVNLNLWQKLKLSRDDAKPLLRL
KGFPSFPVVERRENEVDWWNTINEVKKLIDAKRDMGRVFWSGVTAEKRNTILEGYNYLPNENDHK
KREGSLENPKKPAKRQFGDLLLYLEKKYAGDWGKVFDEAWERIDKKIAGLTSHIEREEARNAEDA
QSKAVLTDWLRAKASFVLERLKEMDEKEFYACEIQLQKWYGDLRGNPFAVEAENRVVDISGFSIG
SDGHSIQYRNLLAWKYLENGKREFYLLMNYGKKGRIRFTDGTDIKKSGKWQGLLYGGGKAKVIDL

TFDPDDEQLIILPLAFGTRQGREFIWNDLLSLETGLIKLANGRVIEKTIYNKKIGRDEPALFVAL
TFERREVVDPSNIKPVNLIGVARGENIPAVIALTDPEGCPLPEFKDSSGGPTDILRIGEGYKEKQ
RAIQAAKEVEQRRAGGYSRKFASKSRNLADDMVRNSARDLFYHAVTHDAVLVFANLSRGFGRQGK
RTFMTERQYTKMEDWLTAKLAYEGLTSKTYLSKTLAQYTSKTCSNCGFTITYADMDVMLVRLKKT
SDGWATTLNNKELKAEYQITYYNRYKRQTVEKELSAELDRLSEESGNNDISKWTKGRRDEALFLL
KKRFSHRPVQEQFVCLDCGHEVHAAEQAALNIARSWLFLNSNSTEFKSYKSGKQPFVGAWQAFYK
RRLKEVWKPNA

[0107] CasY (ncbi.nlm.nih.gov/protein/APG80656.1)

>APG80656.1 CRISPR-associated protein CasY (uncultured *Parcubacteria* group bacterium)

MSKRHPRISGVKGYRLHAQRLEYTGKSGAMRTIKYPLYSSPSGGRTVPREIVSAINDDYVGLYGL
SNFDDLYNAEKRNEEKVYSVLDFWYDCVQYGAVFSYTAPGLLKNVAEVRGGSYELTKTLKGSHLY
DELQIDKVIKFLNKKEISRANGSLDKLKKDIIDCFKAEYRERHKDQCNKLADDIKNAKKDAGASL
GERQKKLFRDFFGISEQSENDKPSFTNPLNLTCCLLPFDTVNNNRNRGEVLFNKLKEYAQKLDKN
EGSLEMWEYIGIGNSGTAFSNFLGEGFLGRLRENKITELKKAMMDITDAWRGQEQEEELEKRLRI
LAALTIKLREPKFDNHWGGYRSDINGKLSSWLQNYINQTVKIKEDLKGHKKDLKKAKEMINRFGE
SDTKEEAVVSSLLESIEKIVPDDSADDEKPDIPAIAIYRRFLSDGRLTLNRFVQREDVQEALIKE
RLEAEKKKKPKKRKKKSDAEDEKETIDFKELFPHLAKPLKLVPNFYGDSKRELYKKYKNAAIYTD
ALWKAVEKIYKSAFSSSLKNSFFDTDFDKDFFIKRLQKIFSVYRRFNTDKWKPIVKNSFAPYCDI
VSLAENEVLYKPKQSRSRKSAAIDKNRVRLPSTENIAKAGIALARELSVAGFDWKDLLKKEEHEE
YIDLIELHKTALALLLAVTETQLDISALDFVENGTVKDFMKTRDGNLVLEGRFLEMFSQSIVFSE
LRGLAGLMSRKEFITRSAIQTMNGKQAELLYIPHEFQSAKITTPKEMSRAFLDLAPAEFATSLEP
ESLSEKSLLKLKQMRYYPHYFGYELTRTGQGIDGGVAENALRLEKSPVKKREIKCKQYKTLGRGQ
NKIVLYVRSSYYQTQFLEWFLHRPKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSER
VFVSQPFTIFPEKSAEEEGQRYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVK
GLKLDQRRGTFAMPSTKIARIRESLVHSLRNRIHHLALKHKAKIVYELEVSRFEEGKQKIKKVYA
TLKKADVYSEIDADKNLQTTVWGKLAVASEISASYTSQFCGACKKLWRAEMQVDETITTQELIGT
VRVIKGGTLIDAIKDFMRPPIFDENDTPFPKYRDFCDKHHISKKMRGNSCLFICPFCRANADADI
QASQTIALLRYVKEEKKVEDYFERFRKLKNIKVLGQMKKI

[0108] The term "Cas12" or "Cas12 domain" refers to an RNA guided nuclease comprising a Cas12 protein or a fragment thereof (*e.g.*, a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas12, and/or the gRNA binding domain of Cas12). Cas12 belongs to the class 2, Type V CRISPR/Cas system. A Cas12 nuclease is also referred to

sometimes as a CRISPR (clustered regularly interspaced short palindromic repeat) associated nuclease. The sequence of an exemplary Bacillus hisashii Cas 12b (BhCas12b) Cas 12 domain is provided below:

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAIYEH
HEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRELYEELVPSSVEKKGEAN
QLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDPLAKILGKLA
EYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNLKVKEEYEKV
EKEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLRGWREIIQKWLKMDEN
EPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPYLYATFCEIDKKKKDAKQQ
ATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKLTVQLDRLIYPTESGGWEEKGK
VDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGTLGGARVQFDRDHLRRYPHKVESGN
VGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKPKELTEWIKDSKGKKLKSGIESLEIGLR
VMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIKGTELYAVHRASFNIKLPGETLVKSREVLRK
AREDNLKLMNQKLNFLRNVLHFQQFEDITEREKRVTKWISRQENSDVPLVYQDELIQIRELMYKP
YKDWVAFLKQLHKRLEVEIGKEVKHWRKSLSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEP
GEVRRLEPGQRFAIDQLNHLNALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFED
LSNYNPYEERSRFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSV
VTKEKLQDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADINAAQNL
QKRFWTRTHGFYKVYCKAYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIK
KGSSKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISK
LTNQYSISTIEDDSSKQSMKRPAATKKAGQAKKKK.

Amino acid sequences having at least 85% or greater identity to the BhCas12b amino acid sequence are also useful in the methods of the disclosure.

[0109] The term "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., supra). Non-limiting examples of conservative mutations include amino acid substitutions of amino acids, for example, lysine for arginine and vice versa such that a positive charge can be maintained;

glutamic acid for aspartic acid and vice versa such that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free $–NH_2$ can be maintained.

[0110] The term "coding sequence" or "protein coding sequence" as used interchangeably herein refers to a segment of a polynucleotide that codes for a protein. The region or sequence is bounded nearer the 5′ end by a start codon and nearer the 3′ end with a stop codon. Coding sequences can also be referred to as open reading frames.

[0111] The term "deaminase" or "deaminase domain," as used herein, refers to a protein or enzyme that catalyzes a deamination reaction. In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenine to hypoxanthine. In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenosine or adenine (A) to inosine (I). In some embodiments, the deaminase or deaminase domain is an adenosine deaminase, catalyzing the hydrolytic deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (*e.g.*, engineered adenosine deaminases, evolved adenosine deaminases) provided herein can be from any organism, such as a bacterium. In some embodiments, the adenosine deaminase is from a bacterium, such as *E. coli*, *S. aureus*, *S. typhi*, *S. putrefaciens*, *H. influenzae*, or *C. crescentus*.

[0112] In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is TadA*7.10 variant. In some embodiments, the TadA*7.10 variant is a TadA*8. In some embodiments, the TadA*8 is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24. In some embodiments, the deaminase or deaminase domain is a variant of a naturally occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identical to a naturally occurring

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

[0113] By "detectable label" is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an enzyme-linked immunosorbent assay (ELISA)), biotin, digoxigenin, or haptens.

[0114] By "disease" is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. An example of a disease includes Rett Syndrome.

[0115] The term "effective amount," as used herein, refers to an amount of a biologically active agent that is sufficient to elicit a biological response. In some embodiements, an effect amount is an amount required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active agent(s) used in the practice of the methods and uses described herein for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount. In one embodiment, an effective amount is the amount of a base editor as described herein (*e.g.*, a fusion protein comprising a programable DNA binding protein, a nucleobase editor and gRNA) sufficient to introduce an alteration in a gene of interest (*e.g.*, Mecp2) in a cell (*e.g.*, a cell in vitro or in vivo). In one embodiment, an effective amount is the amount of a base editor required to achieve a therapeutic effect (*e.g.*, to reduce or control Rett Syndrome or a symptom or condition thereof). Such therapeutic effect need not be sufficient to alter Mecp2 in all cells of a subject, tissue or organ, but only to alter Mecp2 in about 1%, 5%,

10%, 25%, 50%, 75% or more of the cells present in a subject, tissue or organ. In one embodiment, an effective amount is sufficient to ameliorate one or more symptoms of Rett Syndrome.

[0116] In some embodiments, an effective amount of a fusion protein provided herein, *e.g.*, of a nucleobase editor comprising a nCas9 domain and a deaminase domain (*e.g.*, adenosine deaminase) refers to the amount of the fusion protein that is sufficient to induce editing of a target site specifically bound and edited by the nucleobase editors described herein. As will be appreciated by the skilled artisan, the effective amount of an agent, *e.g.*, a fusion protein, a nuclease, a hybrid protein, a protein dimer, a complex of a protein (or protein dimer) and a polynucleotide, or a polynucleotide, 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/or on the agent being used.

[0117] By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, 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.

[0118] By "guide RNA" or "gRNA" is meant a polynucleotide which can be specific for a target sequence and can form a complex with a polynucleotide programmable nucleotide binding domain protein (*e.g.*, Cas9 or Cas12). In an embodiment, the guide polynucleotide is a guide RNA (gRNA). gRNAs can exist as a complex of two or more RNAs, or as a single RNA molecule. gRNAs that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), 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 or Cas12 complex to the target); and (2) a domain that binds a Cas9 or Cas12 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA, and comprises a stem-loop structure. For example, in some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek *et al.*, Science 337:816-821(2012), the entire contents of which is incorporated herein by reference. Other examples of gRNAs (*e.g.*, those including domain 2) can be found in 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." An extended gRNA will bind two or more Cas9 or Cas12 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.

[0119] By "heterodimer" is meant a fusion protein comprising two domains, such as a wild type TadA domain and a variant of TadA domain (*e.g.*, TadA*8) or two variant TadA domains (*e.g.*, TadA*7.10 and TadA*8 or two TadA*8 domains).

[0120] "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.

[0121] By "increases" is meant a positive alteration of at least 10%, 25%, 50%, 75%, or 100%.

[0122] 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 (BER). 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. In some embodiments, the base repair inhibitor is an inhibitor of Endo V or hAAG. In some embodiments, the base repair inhibitor is a catalytically inactive EndoV or a catalytically inactive hAAG.

[0123] In some embodiments, the base repair inhibitor is uracil glycosylase inhibitor (UGI). UGI refers to a protein that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a fragment of a wild-type UGI. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. In some embodiments, the base repair inhibitor is an inhibitor of inosine base excision repair. In some embodiments, the base repair inhibitor is a "catalytically inactive inosine specific nuclease" or "dead inosine specific nuclease. Without wishing to be bound by any particular theory, catalytically inactive inosine glycosylases (*e.g.*, alkyl adenine glycosylase (AAG)) can bind inosine, but cannot create an

abasic site or remove the inosine, thereby sterically blocking the newly formed inosine moiety from DNA damage/repair mechanisms. In some embodiments, the catalytically inactive inosine specific nuclease can be capable of binding an inosine in a nucleic acid but does not cleave the nucleic acid. Non-limiting exemplary catalytically inactive inosine specific nucleases include catalytically inactive alkyl adenosine glycosylase (AAG nuclease), for example, from a human, and catalytically inactive endonuclease V (EndoV nuclease), for example, from *E. coli*. In some embodiments, the catalytically inactive AAG nuclease comprises an E125Q mutation or a corresponding mutation in another AAG nuclease.

[0124] 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."

[0125] Other intein systems may also be used. For example, a synthetic intein based on the dnaE intein, the Cfa-N (*e.g.*, split intein-N) and Cfa-C (*e.g.*, split intein-C) intein pair, has been described (*e.g.*, in Stevens *et al.*, J Am Chem Soc. 2016 Feb. 24; 138(7):2162-5, incorporated herein by reference). Non-limiting examples of intein pairs that may be used in accordance with the present disclosure include: Cfa DnaE intein, Ssp GyrB intein, Ssp DnaX intein, Ter DnaE3 intein, Ter ThyX intein, Rma DnaB intein and Cne Prp8 intein (*e.g.*, as described in U.S. Patent No. 8,394,604, incorporated herein by reference.

[0126] Exemplary nucleotide and amino acid sequences of inteins are provided.

DnaE Intein-N DNA:

TGCCTGTCATACGAAACCGAGATACTGACAGTAGAATATGGCCTTCTGCCAATCGGGAAGATTGT
GGAGAAACGGATAGAATGCACAGTTTACTCTGTCGATAACAATGGTAACATTTATACTCAGCCAG
TTGCCCAGTGGCACGACCGGGGAGAGCAGGAAGTATTCGAATACTGTCTGGAGGATGGAAGTCTC
ATTAGGGCCACTAAGGACCACAAATTTATGACAGTCGATGGCCAGATGCTGCCTATAGACGAAAT
CTTTGAGCGAGAGTTGGACCTCATGCGAGTTGACAACCTTCCTAAT

DnaE Intein-N Protein:

CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWHDR

GEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN

DnaE Intein-C DNA:

ATGATCAAGATAGCTACAAGGAAGTATCTTGGCAAACAAAACGTTTATGA

TATTGGAGTCGAAAGAGATCACAACTTTGCTCTGAAGAACGGATTCATAGCTTCTAAT

Intein-C: MIKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN

Cfa-N DNA:

TGCCTGTCTTATGATACCGAGATACTTACCGTTGAATATGGCTTCTTGCCTATTGGAAAGATTGT

CGAAGAGAGAATTGAATGCACAGTATATACTGTAGACAAGAATGGTTTCGTTTACACACAGCCCA

TTGCTCAATGGCACAATCGCGGCGAACAAGAAGTATTTGAGTACTGTCTCGAGGATGGAAGCATC

ATACGAGCAACTAAAGATCATAAATTCATGACCACTGACGGGCAGATGTTGCCAATAGATGAGAT

ATTCGAGCGGGGCTTGGATCTCAAACAAGTGGATGGATTGCCA

Cfa-N Protein:

CLSYDTEILTVEYGFLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCLEDGSI

IRATKDHKFMTTDGQMLPIDEIFERGLDLKQVDGLP

Cfa-C DNA:

ATGAAGAGGACTGCCGATGGATCAGAGTTTGAATCTCCCAAGAAGAAGAGGAAAGTAAAGATAAT

ATCTCGAAAAAGTCTTGGTACCCAAAATGTCTATGATATTGGAGTGGAGAAAGATCACAACTTCC

TTCTCAAGAACGGTCTCGTAGCCAGCAAC

Cfa-C Protein: MKRTADGSEFESPKKKRKVKIISRKSLGTQNVYDIGVEKDHNFLLKNGLVASN

[0127] 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.

[0128] 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 as described herein 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.

[0129] 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 as described herein 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.

[0130] By an "isolated polypeptide" is meant a polypeptide as described herein 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, of a polypeptide as described herein. An isolated polypeptide of the disclosure 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.

[0131] The term "linker", as used herein, can refer to a covalent linker (*e.g.*, covalent bond), a non-covalent linker, a chemical group, or a molecule linking two molecules or moieties, *e.g.*, two components of a protein complex or a ribonucleocomplex, or two domains of a fusion protein, such as, for example, a polynucleotide programmable DNA binding domain (*e.g.*, dCas9) and a deaminase domain (*e.g.*, an adenosine deaminase). A linker can join different components of, or different portions of components of, a base editor system. For example, in some embodiments, a linker can join a guide polynucleotide binding domain of a polynucleotide programmable nucleotide binding domain and a catalytic domain of a deaminase. In some embodiments, a linker can join a CRISPR polypeptide and a deaminase. In some embodiments, a linker can join a Cas9 and a deaminase. In some embodiments, a linker can join a dCas9 and a deaminase. In some embodiments, a linker can join a nCas9 and a deaminase. In some embodiments, a linker can join a guide polynucleotide and a deaminase. In some embodiments, a linker can join a deaminating component and a polynucleotide programmable nucleotide binding component of a base editor system. In some embodiments, a linker can join a RNA-binding portion of a deaminating component and a polynucleotide programmable nucleotide binding component of a base editor system. In some embodiments, a linker can join a RNA-binding portion of a deaminating component and a RNA-binding portion of a polynucleotide programmable nucleotide binding component of a base editor system. A linker can be positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond or non-covalent interaction, thus connecting the two. In some embodiments, the linker can be an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker can be a polynucleotide. In some embodiments, the linker can be a DNA linker. In some embodiments, the linker can be a RNA linker. In some embodiments, a linker can comprise an aptamer capable of binding to a ligand. In some embodiments, the ligand may be carbohydrate, a peptide, a protein, or a nucleic acid. In some embodiments, the linker may comprise an aptamer may be derived from a riboswitch. The riboswitch from which the aptamer is derived may be selected from a theophylline riboswitch, a thiamine pyrophosphate (TPP) riboswitch, an adenosine cobalamin (AdoCbl) riboswitch, an S-adenosyl methionine (SAM) riboswitch, an SAH riboswitch, a flavin mononucleotide (FMN) riboswitch, a tetrahydrofolate riboswitch, a lysine riboswitch, a glycine riboswitch, a purine riboswitch, a GlmS riboswitch, or a pre-queosine1 (PreQ1) riboswitch. In some embodiments, a linker may comprise an aptamer bound to a polypeptide or a protein domain, such as a polypeptide ligand. In some embodiments, the polypeptide ligand may be a K Homology (KH) domain, a MS2 coat protein domain, a PP7 coat

protein domain, a SfMu Com coat protein domain, a sterile alpha motif, a telomerase Ku binding motif and Ku protein, a telomerase Sm7 binding motif and Sm7 protein, or a RNA recognition motif. In some embodiments, the polypeptide ligand may be a portion of a base editor system component. For example, a nucleobase editing component may comprise a deaminase domain and a RNA recognition motif.

[0132] In some embodiments, the linker can be an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, the linker can be about 5-100 amino acids in length, for example, about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 amino acids in length. In some embodiments, the linker can be about 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, or 450-500 amino acids in length. Longer or shorter linkers can be also contemplated.

[0133] In some embodiments, a linker joins a gRNA binding domain of an RNA-programmable nuclease, including a Cas9 nuclease domain, and the catalytic domain of a nucleic acid editing protein (*e.g.*, adenosine deaminase). In some embodiments, a linker joins a dCas9 and a nucleic acid editing protein. For example, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two. In some embodiments, the linker is an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-200 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 35, 45, 50, 55, 60, 60, 65, 70, 70, 75, 80, 85, 90, 90, 95, 100, 101, 102, 103, 104, 105, 110, 120, 130, 140, 150, 160, 175, 180, 190, or 200 amino acids in length. 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, where n is independently an integer between 1 and 30, and where 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, a linker comprises a plurality of proline residues and is 5-21, 5-14, 5-9, 5-7 amino acids in length, *e.g.*, PAPAP, PAPAPA, PAPAPAP, PAPAPAPA, $P(AP)_4$, $P(AP)_7$, $P(AP)_{10}$. Such proline-rich linkers are also termed "rigid" linkers.

[0134] In some embodiments, the domains of a base editor are fused via a linker that comprises the amino acid sequence of:

SGGSSGSETPGTSESATPESSGGS,

SGGSSGGSSGSETPGTSESATPESSGGSSGGS, or

GGSGGSPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTE

PSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGGSGGS.

[0135] In some embodiments, domains of the base 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

SGGSSGGSSGSETPGTSESATPESSGGSSGGSSGGSSGGSSGSETPGTSESATPESSGGS

SGGS.

[0136] In some embodiments, the linker is 92 amino acids in length. In some embodiments, the linker comprises the amino acid sequence

PGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAP

GTSTEPSEGSAPGTSESATPESGPGSEPATS.

[0137] The term "mutation," as used herein, refers to a substitution of a residue within a sequence, *e.g.*, a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)). In some embodiments, the presently disclosed base editors can efficiently generate an "intended mutation," such as a point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor (*e.g.*, adenosine base editor) bound to a guide polynucleotide (*e.g.*, gRNA), specifically designed to generate the intended mutation. In general, mutations made or identified in a sequence (*e.g.*, an amino acid sequence as described herein) are numbered in relation to a reference (or wild type)

sequence, *i.e.*, a sequence that does not contain the mutations. The skilled practitioner in the art would readily understand how to determine the position of mutations in amino acid and nucleic acid sequences relative to a reference sequence.

[0138] The term "non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the wild-type protein.

[0139] 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.

[0140] 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 (*e.g.*, 2′-fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5′-*N*-phosphoramidite linkages).

[0141] The term "nucleobase," "nitrogenous base," or "base," used interchangeably herein, refers to a nitrogen-containing biological compound that forms a nucleoside, which in turn is a component of a nucleotide. The ability of nucleobases to form base pairs and to stack one upon another leads directly to long-chain helical structures such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Five nucleobases – adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U) – are called primary or canonical. Adenine and guanine are derived from purine, and cytosine, uracil, and thymine are derived from pyrimidine. DNA and RNA can also contain other (non-primary) bases that are modified. Non-limiting exemplary modified nucleobases can include hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-

methylcytosine (m5C), and 5-hydromethylcytosine. Hypoxanthine and xanthine can be created through mutagen presence, both of them through deamination (replacement of the amine group with a carbonyl group). Hypoxanthine can be modified from adenine. Xanthine can be modified from guanine. Uracil can result from deamination of cytosine. A "nucleoside" consists of a nucleobase and a five-carbon sugar (either ribose or deoxyribose). Examples of a nucleoside include adenosine, guanosine, uridine, cytidine, 5-methyluridine (m5U), deoxyadenosine, deoxyguanosine, thymidine, deoxyuridine, and deoxycytidine. Examples of a nucleoside with a modified nucleobase includes inosine (I), xanthosine (X), 7-methylguanosine (m7G), dihydrouridine (D), 5-methylcytidine (m5C), and pseudouridine (Ψ). A "nucleotide" consists of a nucleobase, a five-carbon sugar (either ribose or deoxyribose), and at least one phosphate group.

[0142] The term "nucleic acid programmable DNA binding protein" or "napDNAbp" may be used interchangeably with "polynucleotide programmable nucleotide binding domain" to refer to a protein that associates with a nucleic acid (*e.g.*, DNA or RNA), such as a guide nucleic acid (*e.g.*, gRNA), that guides the napDNAbp to a specific nucleic acid sequence. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable RNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a Cas9 protein. A Cas9 protein can associate with a guide RNA that guides the Cas9 protein to a specific DNA sequence that is complementary to the guide RNA. In some embodiments, the napDNAbp is a Cas9 domain, for example a nuclease active Cas9, a Cas9 nickase (nCas9), or a nuclease inactive Cas9 (dCas9). Non-limiting examples of nucleic acid programmable DNA binding proteins include, without limitation, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. Non-limiting examples of Cas enzymes include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues

thereof, or modified or engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova *et al.* "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" *CRISPR J.* 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan *et al.*, "Functionally diverse type V CRISPR-Cas systems" *Science*. 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

[0143] The terms "nucleobase editing domain" or "nucleobase editing protein," as used herein, refers to a protein or enzyme that can catalyze a nucleobase modification in RNA or DNA, such as cytosine (or cytidine) to uracil (or uridine) or thymine (or thymidine), and adenine (or adenosine) to hypoxanthine (or inosine) deaminations, as well as non-templated nucleotide additions and insertions. In some embodiments, the nucleobase editing domain is a deaminase domain (*e.g.*, an adenine deaminase or an adenosine deaminase). In some embodiments, the nucleobase editing domain can be a naturally occurring nucleobase editing domain. In some embodiments, the nucleobase editing domain can be an engineered or evolved nucleobase editing domain from the naturally occurring nucleobase editing domain. The nucleobase editing domain can be from any organism, such as a bacterium, human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse.

[0144] As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

[0145] A "patient" or "subject" as used herein refers to a mammalian subject or individual diagnosed with, at risk of having or developing, or suspected of having or developing a disease or a disorder. In some embodiments, the term "patient" refers to a mammalian subject with a higher than average likelihood of developing a disease or a disorder. Exemplary patients can be humans, non-human primates, cats, dogs, pigs, cattle, cats, horses, camels, llamas, goats, sheep, rodents (*e.g.*, mice, rabbits, rats, or guinea pigs) and other mammalians that can benefit from the therapies disclosed herein. Exemplary human patients can be male and/or female.

[0146] "Patient in need thereof" or "subject in need thereof" is referred to herein as a patient diagnosed with, at risk or having, predetermined to have, or suspected of having a disease or disorder, for instance, but not restricted to Rett Syndrome (RTT).

[0147] The terms "pathogenic mutation," "pathogenic variant," "disease casing mutation," "disease causing variant," "deleterious mutation," or "predisposing mutation" refers to a genetic alteration or mutation that increases an individual's susceptibility or predisposition to a certain

disease or disorder. In some embodiments, the pathogenic mutation comprises at least one wild-type amino acid substituted by at least one pathogenic amino acid in a protein encoded by a gene.

[0148] 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.). The terms such as "excipient," "carrier," "pharmaceutically acceptable carrier," "vehicle," or the like are used interchangeably herein.

[0149] The term "pharmaceutical composition" means a composition formulated for pharmaceutical use.

[0150] The terms "protein," "peptide," "polypeptide," and their grammatical equivalents are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. The terms refer to a protein, peptide, or polypeptide of any size, structure, or function. Typically, a protein, peptide, or polypeptide will be at least three amino acids long. A protein, peptide, or polypeptide can refer to an individual protein or a collection of proteins. One or more of the amino acids in a protein, peptide, or polypeptide can be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modifications, etc. A protein, peptide, or polypeptide can also be a single molecule or can be a multi-molecular complex. A protein, peptide, or polypeptide can be just a fragment of a naturally occurring protein or peptide. A protein, peptide, or polypeptide can be naturally occurring, recombinant, or synthetic, or any combination thereof. The term "fusion protein" as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein can be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an amino-terminal fusion protein or a carboxy-terminal fusion protein, respectively. A protein can comprise different domains, for example, a nucleic acid binding domain (*e.g.*, the gRNA binding domain of Cas9 that directs the binding of the protein to a target site) and a nucleic acid cleavage domain, or a catalytic domain of a nucleic acid editing protein. In some

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

[0151] Polypeptides and proteins disclosed herein (including functional portions and functional variants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine. The polypeptides and proteins can be associated with post-translational modifications of one or more amino acids of the polypeptide constructs. Non-limiting examples of post-translational modifications include phosphorylation, acylation including acetylation and formylation, glycosylation (including N-linked and O-linked), amidation, hydroxylation, alkylation including methylation and ethylation, ubiquitylation, addition of pyrrolidone carboxylic acid, formation of disulfide bridges, sulfation, myristoylation, palmitoylation, isoprenylation, farnesylation, geranylation, glypiation, lipoylation and iodination.

[0152] 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.

[0153] By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

[0154] By "reference" is meant a standard or control condition. In one embodiment, the reference is a wild-type or healthy cell. In other embodiments and without limitation, a reference is an untreated cell that is not subjected to a test condition, or is subjected to placebo or normal saline, medium, buffer, and/or a control vector that does not harbor a polynucleotide of interest.

[0155] 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, preferably at least about 20 amino acids, more preferably 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, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween. In some embodiments, a reference sequence is a wild-type sequence of a protein of interest. In other embodiments, a reference sequence is a polynucleotide sequence encoding a wild-type protein.

[0156] 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 al.*, Science 337:816-821(2012), the entire contents of which is incorporated herein by reference. Other examples of gRNAs (*e.g.*,

those including domain 2) can be found in 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.

[0157] In some embodiments, the RNA-programmable nuclease is the (CRISPR-associated system) Cas9 endonuclease, for example, Cas9 (Casnl) from *Streptococcus pyogenes* (see, *e.g.*, "Complete genome sequence of an Ml strain of Streptococcus pyogenes." Ferretti J.J., *et al.*, 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., *et al.*, Nature 471:602-607(2011).

[0158] 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 al.*, Multiplex genome engineering using CRISPR/Cas systems. Science 339, 819-823 (2013); Mali, P., *et al.*, RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013); Hwang, W.Y. *et al.*, Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature biotechnology 31, 227-229 (2013); Jinek, M. *et al.*, RNA-programmed genome editing in human cells. eLife 2, e00471 (2013); Dicarlo, J.E. *et al.*, Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic acids research (2013); Jiang, W. *et al.* 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).

[0159] 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 (*e.g.*, associated with cancer) can also be called a single-nucleotide alteration.

[0160] 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 disclosure, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample.

[0161] Nucleic acid molecules useful in the methods as described herein include any nucleic acid molecule that encodes a polypeptide of the of the disclosure,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 described herein include any nucleic acid molecule that encodes a polypeptide of the disclosure, 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).

[0162] 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 certain embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

[0163] 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 a preferred 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.

[0164] By "split" is meant divided into two or more fragments.

[0165] A "split Cas9 protein" or "split Cas9" refers to a Cas9 protein that is provided as an N-terminal fragment and a C-terminal fragment encoded by two separate nucleotide sequences. The polypeptides corresponding to the N-terminal portion and the C-terminal portion of the Cas9 protein may be spliced to form a "reconstituted" Cas9 protein. In particular embodiments, the Cas9 protein is divided into two fragments within a disordered region of the protein, *e.g.*, as described in Nishimasu *et al.*, Cell, Volume 156, Issue 5, pp. 935-949, 2014, or as described in Jiang *et al.* (2016) Science 351: 867-871. PDB file: 5F9R, each of which is incorporated herein by reference. In some embodiments, the protein is divided into two fragments at any C, T, A, or S within a region of SpCas9 between about amino acids A292-G364, F445-K483, or E565-T637, or at corresponding positions in any other Cas9, Cas9 variant (*e.g.*, nCas9, dCas9), or other napDNAbp. In some embodiments, protein is divided into two fragments at SpCas9 T310, T313, A456, S469, or C574. In some embodiments, the process of dividing the protein into two fragments is referred to as "splitting" the protein.

[0166] In other embodiments, the N-terminal portion of the Cas9 protein comprises amino acids 1-573 or 1-637 S. pyogenes Cas9 wild-type (SpCas9) (NCBI Reference Sequence: NC_002737.2, Uniprot Reference Sequence: Q99ZW2) and the C-terminal portion of the Cas9 protein comprises a portion of amino acids 574-1368 or 638-1368 of SpCas9 wild-type, or a corresponding position thereof.

[0167] The C-terminal portion of the split Cas9 can be joined with the N-terminal portion of the split Cas9 to form a complete Cas9 protein. In some embodiments, the C-terminal portion of the Cas9 protein starts from where the N-terminal portion of the Cas9 protein ends. As such, in some embodiments, the C-terminal portion of the split Cas9 comprises a portion of amino acids (551-651)-1368 of spCas9. "(551-651)-1368" means starting at an amino acid between amino acids 551-651 (inclusive) and ending at amino acid 1368. For example, the C-terminal portion of the split Cas9 may comprise a portion of any one of amino acid 551-1368, 552-1368, 553-1368, 554-1368, 555-1368, 556-1368, 557-1368, 558-1368, 559-1368, 560-1368, 561-1368, 562-1368, 563-1368, 564-1368, 565-1368, 566-1368, 567-1368, 568-1368, 569-1368, 570-1368, 571-1368, 572-1368, 573-1368, 574-1368, 575-1368, 576-1368, 577-1368, 578-1368, 579-1368, 580-1368, 581-

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[0168] 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.

[0169] 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). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

[0170] 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, COBALT, EMBOSS Needle, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. COBALT is used, for example, with the following parameters:

a) alignment parameters: Gap penalties-11, -1 and End-Gap penalties-5, -1,

b) CDD Parameters: Use RPS BLAST on; Blast E-value 0.003; Find Conserved columns and Recompute on, and

c) Query Clustering Parameters: Use query clusters on; Word Size 4; Max cluster distance 0.8; Alphabet Regular.

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

a) Matrix: BLOSUM62;

b) GAP OPEN: 10;

c) GAP EXTEND: 0.5;

d) OUTPUT FORMAT: pair;

e) END GAP PENALTY: false;

f) END GAP OPEN: 10; and

g) END GAP EXTEND: 0.5.

[0171] 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.*, an adenine deaminase).

[0172] As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptom(s) associated therewith or obtaining a desired pharmacologic and/or physiologic effect. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated. In some embodiments, the effect is therapeutic, *i.e.*, without limitation, the effect partially or completely reduces, diminishes, abrogates, abates, alleviates, decreases the intensity of, or cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the effect is preventative, *i.e.,* the effect protects or prevents an occurrence or reoccurrence of a disease or condition. To this end, the presently disclosed methods comprise administering a therapeutically effective amount of a compositions as described herein.

[0173] By “uracil glycosylase inhibitor” of “UGI” is meant an agent that inhibits the uracil-excision repair system. In one embodiment, the agent is a protein or fragment thereof that binds a host uracil-DNA glycosylase and prevents removal of uracil residues from DNA. In an embodiment, a UGI is a protein, a fragment thereof, or a domain that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a modified version thereof. In some embodiments, a UGI domain comprises a fragment of the exemplary amino acid sequence set forth below. In some

embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the exemplary UGI sequence provided below. In some embodiments, a UGI comprises an amino acid sequence that is homologous to the exemplary UGI amino acid sequence or fragment thereof, as set forth below. In some embodiments, the UGI, or a portion thereof, is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or 100% identical to a wild-type UGI or a UGI sequence, or portion thereof, as set forth below. An exemplary UGI comprises an amino acid sequence as follows:
>splP14739IUNGI_BPPB2 Uracil-DNA glycosylase inhibitor
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLTSD
APEYKPWALVIQDSNGENKIKML.

[0174] The term "vector" refers to a means of introducing a nucleic acid sequence into a cell, resulting in a transformed cell. Vectors include plasmids, transposons, phages, viruses, liposomes, and episome. "Expression vectors" are nucleic acid sequences comprising the nucleotide sequence to be expressed in the recipient cell. Expression vectors may include additional nucleic acid sequences to promote and/or facilitate the expression of the of the introduced sequence such as start, stop, enhancer, promoter, and secretion sequences.

[0175] 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.

[0176] Any compositions or methods provided and described herein can be combined with one or more of any of the other compositions and methods provided and described herein.

[0177] DNA editing has emerged as a viable means to modify disease states by correcting pathogenic mutations at the genetic level. Until recently, all DNA editing platforms have functioned by inducing a DNA double strand break (DSB) at a specified genomic site and relying on endogenous DNA repair pathways to determine the product outcome in a semi-stochastic manner, resulting in complex populations of genetic products. Though precise, user-defined repair outcomes can be achieved through the homology directed repair (HDR) pathway, a number of challenges have prevented high efficiency repair using HDR in therapeutically-relevant cell types. In practice, this pathway is inefficient relative to the competing, error-prone non-homologous end joining pathway. Further, HDR is tightly restricted to the G1 and S phases of

the cell cycle, preventing precise repair of DSBs in post-mitotic cells. As a result, it has proven difficult or impossible to alter genomic sequences in a user-defined, programmable manner with high efficiencies in these populations.

NUCLEOBASE EDITOR

[0178] Disclosed herein is a base editor or a nucleobase editor for editing, modifying or altering a target nucleotide sequence of a polynucleotide. Described herein is a nucleobase editor or a base editor comprising a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (*e.g.*, adenosine deaminase). A polynucleotide programmable nucleotide binding domain, when in conjunction with a bound guide polynucleotide (*e.g.*, gRNA), can specifically bind to a target polynucleotide sequence (*i.e.*, via complementary base pairing between bases of the bound guide nucleic acid and bases of the target polynucleotide sequence) and thereby localize the base editor to the target nucleic acid sequence desired to be edited. In some embodiments, the target polynucleotide sequence comprises single-stranded DNA or double-stranded DNA. In some embodiments, the target polynucleotide sequence comprises RNA. In some embodiments, the target polynucleotide sequence comprises a DNA-RNA hybrid.

Polynucleotide Programmable Nucleotide Binding Domain

[0179] It should be appreciated that polynucleotide programmable nucleotide binding domains can also include nucleic acid programmable proteins that bind RNA. For example, the polynucleotide programmable nucleotide binding domain can be associated with a nucleic acid that guides the polynucleotide programmable nucleotide binding domain to an RNA. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, though they are not specifically listed in this disclosure.

[0180] A polynucleotide programmable nucleotide binding domain of a base editor can itself comprise one or more domains. For example, a polynucleotide programmable nucleotide binding domain can comprise one or more nuclease domains. In some embodiments, the nuclease domain of a polynucleotide programmable nucleotide binding domain can comprise an endonuclease or an exonuclease. Herein the term "exonuclease" refers to a protein or polypeptide capable of digesting a nucleic acid (*e.g.*, RNA or DNA) from free ends, and the term "endonuclease" refers to a protein or polypeptide capable of catalyzing (*e.g.*, cleaving) internal regions in a nucleic acid (*e.g.*, DNA or RNA). In some embodiments, an endonuclease can

cleave a single strand of a double-stranded nucleic acid. In some embodiments, an endonuclease can cleave both strands of a double-stranded nucleic acid molecule. In some embodiments a polynucleotide programmable nucleotide binding domain can be a deoxyribonuclease. In some embodiments a polynucleotide programmable nucleotide binding domain can be a ribonuclease.

[0181] In some embodiments, a nuclease domain of a polynucleotide programmable nucleotide binding domain can cut zero, one, or two strands of a target polynucleotide. In some embodiments, the polynucleotide programmable nucleotide binding domain can comprise a nickase domain. Herein the term "nickase" refers to a polynucleotide programmable nucleotide binding domain comprising a nuclease domain that is capable of cleaving only one strand of the two strands in a duplexed nucleic acid molecule (*e.g.,* DNA). In some embodiments, a nickase can be derived from a fully catalytically active (*e.g.,* natural) form of a polynucleotide programmable nucleotide binding domain by introducing one or more mutations into the active polynucleotide programmable nucleotide binding domain. For example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can include a D10A mutation and a histidine at position 840. In such embodiments, the residue H840 retains catalytic activity and can thereby cleave a single strand of the nucleic acid duplex. In another example, a Cas9-derived nickase domain can comprise an H840A mutation, while the amino acid residue at position 10 remains a D. In some embodiments, a nickase can be derived from a fully catalytically active (*e.g.,* natural) form of a polynucleotide programmable nucleotide binding domain by removing all or a portion of a nuclease domain that is not required for the nickase activity. For example, where a polynucleotide programmable nucleotide binding domain comprises a nickase domain derived from Cas9, the Cas9-derived nickase domain can comprise a deletion of all or a portion of the RuvC domain or the HNH domain.

[0182] The amino acid sequence of an exemplary catalytically active Cas9 is as follows:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV

YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD.

[0183] A base editor comprising a polynucleotide programmable nucleotide binding domain comprising a nickase domain is thus able to generate a single-strand DNA break (nick) at a specific polynucleotide target sequence (*e.g.*, determined by the complementary sequence of a bound guide nucleic acid). In some embodiments, the strand of a nucleic acid duplex target polynucleotide sequence that is cleaved by a base editor comprising a nickase domain (*e.g.*, Cas9-derived nickase domain) is the strand that is not edited by the base editor (*i.e.*, the strand that is cleaved by the base editor is opposite to a strand comprising a base to be edited). In other embodiments, a base editor comprising a nickase domain (*e.g.*, Cas9-derived nickase domain) can cleave the strand of a DNA molecule which is being targeted for editing. In such embodiments, the non-targeted strand is not cleaved.

[0184] Also provided herein are base editors comprising a polynucleotide programmable nucleotide binding domain which is catalytically dead (*i.e.*, incapable of cleaving a target polynucleotide sequence). Herein the terms "catalytically dead" and "nuclease dead" are used interchangeably to refer to a polynucleotide programmable nucleotide binding domain which has one or more mutations and/or deletions resulting in its inability to cleave a strand of a nucleic acid. In some embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain base editor can lack nuclease activity as a result of specific point mutations in one or more nuclease domains. For example, in the case of a base editor comprising a Cas9 domain, the Cas9 can comprise both a D10A mutation and an H840A mutation. Such mutations

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

[0185] Also contemplated herein are mutations capable of generating a catalytically dead polynucleotide programmable nucleotide binding domain from a previously functional version of the polynucleotide programmable nucleotide binding domain. For example, in the case of catalytically dead Cas9 ("dCas9"), variants having mutations other than D10A and H840A are provided, which result in nuclease inactivated Cas9. Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain). Additional suitable nuclease-inactive dCas9 domains can be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure. Such additional exemplary suitable nuclease-inactive Cas9 domains include, but are not limited to, D10A/H840A, D10A/D839A/H840A, and D10A/D839A/H840A/N863A mutant domains (*See*, *e.g.*, Prashant *et al.*, CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature Biotechnology*. 2013; 31(9): 833-838, the entire contents of which are incorporated herein by reference).

[0186] Non-limiting examples of a polynucleotide programmable nucleotide binding domain which can be incorporated into a base editor include a CRISPR protein-derived domain, a restriction nuclease, a meganuclease, TAL nuclease (TALEN), and a zinc finger nuclease (ZFN). In some embodiments, a base editor comprises a polynucleotide programmable nucleotide binding domain comprising a natural or modified protein or portion thereof which via a bound guide nucleic acid is capable of binding to a nucleic acid sequence during CRISPR (*i.e.*, Clustered Regularly Interspaced Short Palindromic Repeats)-mediated modification of a nucleic acid. Such a protein is referred to herein as a "CRISPR protein." Accordingly, disclosed herein is a base editor comprising a polynucleotide programmable nucleotide binding domain comprising all or a portion of a CRISPR protein (*i.e.* a base editor comprising as a domain all or a portion of a CRISPR protein, also referred to as a "CRISPR protein-derived domain" of the base editor). A CRISPR protein-derived domain incorporated into a base editor can be modified compared to a wild-type or natural version of the CRISPR protein. For example, as described below a CRISPR

protein-derived domain can comprise one or more mutations, insertions, deletions, rearrangements and/or recombinations relative to a wild-type or natural version of the CRISPR protein.

[0187] CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, and then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA," or simply "gRNA") 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.

[0188] In some embodiments, the methods described herein can utilize an engineered Cas protein. A guide RNA (gRNA) is a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding and a user-defined ~20 nucleotide spacer that defines the genomic target to be modified. Thus, a skilled artisan can change the genomic target of the Cas protein specificity is partially determined by how specific the gRNA targeting sequence is for the genomic target compared to the rest of the genome.

[0189] In some embodiments, the gRNA scaffold sequence is as follows: GUUUUAGAGC UAGAAAUAGC AAGUUAAAAU AAGGCUAGUC CGUUAUCAAC UUGAAAAAGU GGCACCGAGU CGGUGCUUUU.

[0190] In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is an endonuclease (*e.g.*, deoxyribonuclease or ribonuclease) capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a nickase capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some

embodiments, a CRISPR protein-derived domain incorporated into a base editor is a catalytically dead domain capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a target polynucleotide bound by a CRISPR protein derived domain of a base editor is DNA. In some embodiments, a target polynucleotide bound by a CRISPR protein-derived domain of a base editor is RNA.

[0191] Cas proteins that can be used herein include class 1 and class 2. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 or Csx12), Cas10, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i, CARF, DinG, homologues thereof, or modified versions thereof. An unmodified CRISPR enzyme can have DNA cleavage activity, such as Cas9, which has two functional endonuclease domains: RuvC and HNH. A CRISPR enzyme can direct cleavage of one or both strands at a target sequence, such as within a target sequence and/or within a complement of a target sequence. For example, a CRISPR enzyme can direct cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence.

[0192] A vector that encodes a CRISPR enzyme that is mutated to with respect, to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence can be used. Cas9 can refer to a polypeptide with at least or at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild-type exemplary Cas9 polypeptide (*e.g.*, Cas9 from *S. pyogenes*). Cas9 can refer to a polypeptide with at most or at most about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild-type exemplary Cas9 polypeptide (*e.g.*, from *S. pyogenes*). Cas9 can refer to the wild-type or a modified form of the Cas9 protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation, fusion, chimera, or any combination thereof.

[0193] In some embodiments, a CRISPR protein-derived domain of a base editor can include all or a portion of Cas9 from *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1); *Spiroplasma*

syrphidicola (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquis* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1); *Listeria innocua* (NCBI Ref: NP_472073.1); *Campylobacter jejuni* (NCBI Ref: YP_002344900.1); *Neisseria meningitidis* (NCBI Ref: YP_002342100.1), *Streptococcus pyogenes*, or *Staphylococcus aureus*.

Cas9 domains of Nucleobase Editors

[0194] 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.*, 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., *et al.*, Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., *et al.*, Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences 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.

[0195] 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 (dCas9), or a Cas9 nickase (nCas9). In some embodiments, the Cas9 domain is a nuclease active domain. For example, the Cas9 domain may be a Cas9 domain that cuts both strands of a duplexed nucleic acid (*e.g.*, both strands of a duplexed DNA molecule). In some embodiments, the Cas9 domain comprises any one of the amino acid sequences as set forth herein. In some embodiments the Cas9 domain comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29,

30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas9 domain comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, or at least 1200 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth herein.

[0196] 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.

[0197] 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.

[0198] 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.

[0199] In some embodiments, wild type Cas9 corresponds to Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_017053.1). Exemplary nucleotide and amino acid sequences are as follows:

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGATCAC
TGATGATTATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACAGTATCA
AAAAAAATCTTATAGGGGCTCTTTTATTTGGCAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAA
CGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTC
AAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTTGGTGGAAG
AAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAG
AAATATCCAACTATCTATCATCTGCGAAAAAAATTGGCAGATTCTACTGATAAAGCGGATTTGCG
CTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGTCATTTTTTGATTGAGGGAGATT
TAAATCCTGATAATAGTGATGTGGACAAACTATTTATCCAGTTGGTACAAATCTACAATCAATTA
TTTGAAGAAAACCCTATTAACGCAAGTAGAGTAGATGCTAAAGCGATTCTTTCTGCACGATTGAG
TAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAGAAATGGCTTGTTTG
GGAATCTCATTGCTTTGTCATTGGGATTGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAA
GATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGCAAAT
TGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAG
ATATCCTAAGAGTAAATAGTGAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAGCGCTAC
GATGAACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTA
TAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCC
AAGAAGAATTTTATAAATTTATCAAACCAATTTTAGAAAAAATGGATGGTACTGAGGAATTATTG
GTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCA
TCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAA
AAGACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATTG
GCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGAA
TTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATCATTTATTGAACGCATGACAAACTTTG
ATAAAAATCTTCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTATGAGTATTTTACGGTT

TATAACGAATTGACAAAGGTCAAATATGTTACTGAGGGAATGCGAAAACCAGCATTTCTTTCAGG
TGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAAGTAACCGTTAAGCAAT
TAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAGTTGAAGAT
AGATTTAATGCTTCATTAGGCGCCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTT
GGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAAGATA
GGGGGATGATTGAGGAAAGACTTAAAACATATGCTCACCTCTTTGATGATAAGGTGATGAAACAG
CTTAAACGTCGCCGTTATACTGGTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGA
TAAGCAATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGCAATTTTA
TGCAGCTGATCCATGATGATAGTTTGACATTTAAAGAAGATATTCAAAAAGCACAGGTGTCTGGA
CAAGGCCATAGTTTACATGAACAGATTGCTAACTTAGCTGGCAGTCCTGCTATTAAAAAAGGTAT
TTTACAGACTGTAAAAATTGTTGATGAACTGGTCAAAGTAATGGGGCATAAGCCAGAAAATATCG
TTATTGAAATGGCACGTGAAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAGAGCGTATG
AAACGAATCGAAGAAGGTATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAA
TACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTACAAAATGGAAGAGACATGTATGTGG
ACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGTTTC
ATTAAAGACGATTCAATAGACAATAAGGTACTAACGCGTTCTGATAAAAATCGTGGTAAATCGGA
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AGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAACTT
GATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTGGCACA
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TGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCTATAAAGTACGT
GAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTTGAT
TAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTATGATGTTCGTA
AAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTAAT
ATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCTAAT
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AGTTCCTTTGAAAAAAATCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTTAAAAAAGA
CTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGG
CTAGTGCCGGAGAATTACAAAAAGGAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTA
TATTTAGCTAGTCATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGTT

TGTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTAAGCGTG
TTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTGCATATAACAAACATAGAGACAAACCA
ATACGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGC
TTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATG
CCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTAGGA
GGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQIYNQL
FEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNSEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDRGMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGHSLHEQIANLAGSPAIKKGILQTVKIVDELVKVMGHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF
IKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFS
KESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP
IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD (single underline: HNH domain; double underline: RuvC domain)

[0200] In some embodiments, wild type Cas9 corresponds to, or comprises the following nucleotide and/or amino acid sequences:

ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCACTAATTCCGTTGGATGGGCTGTCATAAC
CGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACACAGACCGTCATTCGATTA

AAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACGGCAGAGGCGACTCGCCTGAAA
CGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACCGAATATGTTACTTACAAGAAATTTTTAG
CAATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGTTTGGAAGAGTCCTTCCTTGTCGAAG
AGGACAAGAAACATGAACGGCACCCCATCTTTGGAAACATAGTAGATGAGGTGGCATATCATGAA
AAGTACCCAACGATTTATCACCTCAGAAAAAAGCTAGTTGACTCAACTGATAAAGCGGACCTGAG
GTTAATCTACTTGGCTCTTGCCCATATGATAAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATC
TAAATCCGGACAACTCGGATGTCGACAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTG
TTTGAAGAGAACCCTATAAATGCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTC
TAAATCCCGACGGCTAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCG
GTAACCTTATAGCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGCTGAA
GATGCCAAATTGCAGCTTAGTAAGGACACGTACGATGACGATCTCGACAATCTACTGGCACAAAT
TGGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAATCCTCCTATCTG
ACATACTGAGAGTTAATACTGAGATTACCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTAC
GATGAACATCACCAAGACTTGACACTTCTCAAGGCCCTAGTCCGTCAGCAACTGCCTGAGAAATA
TAAGGAAATATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTATATTGACGGCGGAGCGAGTC
AAGAGGAATTCTACAAGTTTATCAAACCCATATTAGAGAAGATGGATGGGACGGAAGAGTTGCTT
GTAAAACTCAATCGCGAAGATCTACTGCGAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACA
TCAAATCCACTTAGGCGAATTGCATGCTATACTTAGAAGGCAGGAGGATTTTTATCCGTTCCTCA
AAGACAATCGTGAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCTG
GCCCGAGGGAACTCTCGGTTCGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAA
TTTTGAGGAAGTTGTCGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTG
ACAAGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACAGTG
TACAATGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTTCTAAGCGG
AGAACAGAAGAAAGCAATAGTAGATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTAAGCAAT
TGAAAGAGGACTACTTTAAGAAAATTGAATGCTTCGATTCTGTCGAGATCTCCGGGGTAGAAGAT
CGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAGATAATTAAAGATAAGGACTTCCT
GGATAACGAAGAGAATGAAGATATCTTAGAAGATATAGTGTTGACTCTTACCCTCTTTGAAGATC
GGGAAATGATTGAGGAAAGACTAAAAACATACGCTCACCTGTTCGACGATAAGGTTATGAAACAG
TTAAAGAGGCGTCGCTATACGGGCTGGGGACGATTGTCGCGGAAACTTATCAACGGGATAAGAGA
CAAGCAAAGTGGTAAAACTATTCTCGATTTTCTAAAGAGCGACGGCTTCGCCAATAGGAACTTTA
TGCAGCTGATCCATGATGACTCTTTAACCTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGA
CAAGGGGACTCATTGCACGAACATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCAT
ACTCCAGACAGTCAAAGTAGTGGATGAGCTAGTTAAGGTCATGGGACGTCACAAACCGGAAAACA
TTGTAATCGAGATGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAGAGCGG

ATGAAGAGAATAGAAGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAGGAGCATCCTGTGGA
AAATACCCAATTGCAGAACGAGAAACTTTACCTCTATTACCTACAAAATGGAAGGGACATGTATG
TTGATCAGGAACTGGACATAAACCGTTTATCTGATTACGACGTCGATCACATTGTACCCCAATCC
TTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACACGCTCGGATAAGAACCGAGGGAAAAG
TGACAATGTTCCAAGCGAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGCAGCTCCTAAATG
CGAAACTGATAACGCAAAGAAAGTTCGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCTGAA
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AATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCCGGGACTTCGCGACGGTGA
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TCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCTCGTAAAAAGGACTGGGA
CCCGAAAAAGTACGGTGGCTTCGATAGCCCTACAGTTGCCTATTCTGTCCTAGTAGTGGCAAAAG
TTGAGAAGGGAAAATCCAAGAAACTGAAGTCAGTCAAAGAATTATTGGGGATAACGATTATGGAG
CGCTCGTCTTTTGAAAAGAACCCCATCGACTTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAA
GGATCTCATAATTAAACTACCAAAGTATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGT
TGGCTAGCGCCGGAGAGCTTCAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTC
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GAGTCATCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACAGGGATAAA
CCCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCTCCAGC
CGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTCTACCAAGGAGGTGCTAG
ACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAACTCGGATAGATTTGTCACAGCTT
GGGGGTGACGGATCCCCCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAGACCATGACGGTGA
TTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGGCTGCAGGA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE

DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD (single underline: HNH domain; double underline: RuvC domain).

[0201] 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):

ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCACAAATAGCGTCGGATGGGCGGTGATCAC
TGATGAATATAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAATACAGACCGCCACAGTATCA
AAAAAAATCTTATAGGGGCTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGACTCGTCTCAAA
CGGACAGCTCGTAGAAGGTATACACGTCGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTC
AAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATCGACTTGAAGAGTCTTTTTTGGTGGAAG
AAGACAAGAAGCATGAACGTCATCCTATTTTTGGAAATATAGTAGATGAAGTTGCTTATCATGAG
AAATATCCAACTATCTATCATCTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGGATTTGCG
CTTAATCTATTTGGCCTTAGCGCATATGATTAAGTTTCGTGGTCATTTTTTGATTGAGGGAGATT
TAAATCCTGATAATAGTGATGTGGACAAACTATTTATCCAGTTGGTACAAACCTACAATCAATTA
TTTGAAGAAAACCCTATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTCTGCACGATTGAG
TAAATCAAGACGATTAGAAAATCTCATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTATTTG
GGAATCTCATTGCTTTGTCATTGGGTTTGACCCCTAATTTTAAATCAAATTTTGATTTGGCAGAA

GATGCTAAATTACAGCTTTCAAAAGATACTTACGATGATGATTTAGATAATTTATTGGCGCAAAT
TGGAGATCAATATGCTGATTTGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTACTTTCAG
ATATCCTAAGAGTAAATACTGAAATAACTAAGGCTCCCCTATCAGCTTCAATGATTAAACGCTAC
GATGAACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGTTCGACAACAACTTCCAGAAAAGTA
TAAAGAAATCTTTTTTGATCAATCAAAAAACGGATATGCAGGTTATATTGATGGGGGAGCTAGCC
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GTGAAACTAAATCGTGAAGATTTGCTGCGCAAGCAACGGACCTTTGACAACGGCTCTATTCCCCA
TCAAATTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAGAAGACTTTTATCCATTTTTAA
AAGACAATCGTGAGAAGATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTTGGTCCATTG
GCGCGTGGCAATAGTCGTTTTGCATGGATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGAA
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TGAACAGAAGAAAGCCATTGTTGATTTACTCTTCAAAACAAATCGAAAAGTAACCGTTAAGCAAT
TAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTTGAAATTTCAGGAGTTGAAGAT
AGATTTAATGCTTCATTAGGTACCTACCATGATTTGCTAAAAATTATTAAAGATAAAGATTTTTT
GGATAATGAAGAAAATGAAGATATCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAAGATA
GGGAGATGATTGAGGAAAGACTTAAAACATATGCTCACCTCTTTGATGATAAGGTGATGAAACAG
CTTAAACGTCGCCGTTATACTGGTTGGGGACGTTTGTCTCGAAAATTGATTAATGGTATTAGGGA
TAAGCAATCTGGCAAAACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGCAATTTTA
TGCAGCTGATCCATGATGATAGTTTGACATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGA
CAAGGCGATAGTTTACATGAACATATTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAGGTAT
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AAATACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGACATGTATG
TGGACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATCACATTGTTCCACAAAGT
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GGATAACGTTCCAAGTGAAGAAGTAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACG
CCAAGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGTGAA
CTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACTAAGCATGTGGC
ACAAATTTTGGATAGTCGCATGAATACTAAATACGATGAAAATGATAAACTTATTCGAGAGGTTA
AAGTGATTACCTTAAAATCTAAATTAGTTTCTGACTTCCGAAAAGATTTCCAATTCTATAAAGTA
CGTGAGATTAACAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTTT

GATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTATGATGTTC
GTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCT
AATATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCT
AATCGAAACTAATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTTTGCCACAGTGC
GCAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGAAAACAGAAGTACAGACAGGCGGATTC
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CCAATACGTGAACAAGCAGAAAATATTATTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGC
TGCTTTTAAATATTTTGATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAG
ATGCCACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTA
GGAGGTGACTGA

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV

REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD (single underline: HNH domain; double underline: RuvC domain).

[0202] 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.

[0203] 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 active Cas9. In some embodiments, the Cas9 protein is a nuclease dead Cas9 (dCas9). In some embodiments, the Cas9 protein is a Cas9 nickase (nCas9).

[0204] 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. 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)).

[0205] 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. 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. In some embodiments, a nuclease-inactive Cas9 domain comprises the amino acid sequence set forth in Cloning vector pPlatTET-gRNA2 (Accession No. BAV54124). In some embodiments, the dCas9 comprises the amino acid sequence of dCas9 (D10A and H840A):

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME

RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD (single underline: HNH domain; double underline: RuvC domain).

[0206] In some embodiments, the amino acid sequence of an exemplary catalytically inactive Cas9 (dCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

(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).

[0207] In some embodiments, the amino acid sequence of an exemplary catalytically inactive Cas9 (dCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

[0208] 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.

[0209] 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).

[0210] 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, 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.

[0211] The amino acid sequence of an exemplary catalytically Cas9 nickase (nCas9) is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE

KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

[0212] 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, a nucleic acid programmable DNA binding protein may be a CasX or CasY protein, which have been described in, for example, Burstein *et al.*, "New CRISPR-Cas systems from uncultivated microbes." Cell Res. 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR-Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little- studied nanoarchaea as part of an active CRISPR-Cas system. In bacteria, two previously unknown systems were discovered, CRISPR-CasX and CRISPR-CasY, which are among the most compact systems yet discovered. In some embodiments, in a base editor system described herein Cas9 is replaced by CasX, or a variant of CasX. In some embodiments, in a base editor system described herein Cas9 is replaced by CasY, or a variant of CasY. It should be appreciated that other RNA-guided DNA binding proteins may

be used as a nucleic acid programmable DNA binding protein (napDNAbp), and are within the scope of this disclosure.

[0213] 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 least 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.

[0214] An exemplary CasX ((uniprot.org/uniprot/F0NN87; uniprot.org/uniprot/F0NH53) tr|F0NN87|F0NN87_SULIHCRISPR-associatedCasxprotein OS = *Sulfolobus islandicus* (strain HVE10/4) GN = SiH_0402 PE=4 SV=1) amino acid sequence is as follows:

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGKAKK
KKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAPSFVKPE
FYEFGRSPGMVERTRRVKLEVEPHYLIIAAAGWVLTRLGKAKVSEGDYVGVNVFTPTRGILYSLI
QNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVRIYTISDAVGQNPTTINGGFSIDLTKLLE
KRYLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTG SKRLEDLLYFANRDLIMNLNSDDG
KVRDLKLISAYVNGELIRGEG

[0215] An exemplary CasX (>tr|F0NH53|F0NH53_SULIR CRISPR associated protein, Casx OS = *Sulfolobus islandicus* (strain REY15A) GN=SiRe_0771 PE=4 SV=1) amino acid sequence is as follows:

MEVPLYNIFGDNYIIQVATEAENSTIYNNKVEIDDEELRNVLNLAYKIAKNNEDAAAERRGKAKK
KKGEEGETTTSNIILPLSGNDKNPWTETLKCYNFPTTVALSEVFKNFSQVKECEEVSAPSFVKPE
FYKFGRSPGMVERTRRVKLEVEPHYLIMAAAGWVLTRLGKAKVSEGDYVGVNVFTPTRGILYSLI
QNVNGIVPGIKPETAFGLWIARKVVSSVTNPNVSVVSIYTISDAVGQNPTTINGGFSIDLTKLLE
KRDLLSERLEAIARNALSISSNMRERYIVLANYIYEYLTGSKRLEDLLYFANRDLIMNLNSDDGK
VRDLKLISAYVNGELIRGEG

[0216] Deltaproteobacteria CasX

MEKRINKIRKKLSADNATKPVSRSGPMKTLLVRVMTDDLKKRLEKRRKKPEVMPQVISNNAANNL
RMLLDDYTKMKEAILQVYWQEFKDDHVGLMCKFAQPASKKIDQNKLKPEMDEKGNLTTAGFACSQ
CGQPLFVYKLEQVSEKGKAYTNYFGRCNVAEHEKLILLAQLKPVKDSDEAVTYSLGKFGQRALDF
YSIHVTKESTHPVKPLAQIAGNRYASGPVGKALSDACMGTIASFLSKYQDIIIEHQKVVKGNQKR
LESLRELAGKENLEYPSVTLPPQPHTKEGVDfAYNEVIARVRMWVNLNLWQKLKLSRDDAKPLLR
LKGFPSFPVVERRENEVDWWNTINEVKKLIDAKRDMGRVFWSGVTAEKRNTILEGYNYLPNENDH
KKREGSLENPKKPAKRQFGDLLLYLEKKYAGDWGKVFDEAWERIDKKIAGLTSHIEREEARNAED
AQSKAVLTDWLRAKASFVLERLKEMDEKEFYACEIQLQKWYGDLRGNPFAVEAENRVVDISGFSI
GSDGHSIQYRNLLAWKYLENGKREFYLLMNYGKKGRIRFTDGTDIKKSGKWQGLLYGGGKAKVID
LTFDPDDEQLIILPLAFGTRQGREFIWNDLLSLETGLIKLANGRVIEKTIYNKKIGRDEPALFVA
LTFERREVVDPSNIKPVNLIGVARGENIPAVIALTDPEGCPLPEFKDSSGGPTDILRIGEGYKEK
QRAIQAAKEVEQRRAGGYSRKFASKSRNLADDMVRNSARDLFYHAVTHDAVLVFANLSRGFGRQG
KRTFMTERQYTKMEDWLTAKLAYEGLTSKTYLSKTLAQYTSKTCSNCGFTITYADMDVMLVRLKK
TSDGWATTLNNKELKAEYQITYYNRYKRQTVEKELSAELDRLSEESGNNDISKWTKGRRDEALFL
LKKRFSHRPVQEQFVCLDCGHEVHAAEQAALNIARSWLFLNSNSTEFKSYKSGKQPFVGAWQAFY
KRRLKEVWKPNA

[0217] An exemplary CasY ((ncbi.nlm.nih.gov/protein/APG80656.1)>APG80656.1 CRISPR-associated protein CasY [uncultured Parcubacteria group bacterium]) amino acid sequence is as follows:

MSKRHPRISGVKGYRLHAQRLEYTGKSGAMRTIKYPLYSSPSGGRTVPREIVSAINDDYVGLYGL
SNFDDLYNAEKRNEEKVYSVLDFWYDCVQYGAVFSYTAPGLLKNVAEVRGGSYELTKTLKGSHLY
DELQIDKVIKFLNKKEISRANGSLDKLKKDIIDCFKAEYRERHKDQCNKLADDIKNAKKDAGASL
GERQKKLFRDFFGISEQSENDKPSFTNPLNLTCCLLPFDTVNNNRNRGEVLFNKLKEYAQKLDKN
EGSLEMWEYIGIGNSGTAFSNFLGEGFLGRLRENKITELKKAMMDITDAWRGQEQEEELEKRLRI
LAALTIKLREPKFDNHWGGYRSDINGKLSSWLQNYINQTVKIKEDLKGHKKDLKKAKEMINRFGE
SDTKEEAVVSSLLESIEKIVPDDSADDEKPDIPAIAIYRRFLSDGRLTLNRFVQREDVQEALIKE
RLEAEKKKKPKKRKKKSDAEDEKETIDFKELFPHLAKPLKLVPNFYGDSKRELYKKYKNAAIYTD
ALWKAVEKIYKSAFSSSLKNSFFDTDFDKDFFIKRLQKIFSVYRRFNTDKWKPIVKNSFAPYCDI
VSLAENEVLYKPKQSRSRKSAAIDKNRVRLPSTENIAKAGIALARELSVAGFDWKDLLKKEEHEE
YIDLIELHKTALALLLAVTETQLDISALDFVENGTVKDFMKTRDGNLVLEGRFLEMFSQSIVFSE
LRGLAGLMSRKEFITRSAIQTMNGKQAELLYIPHEFQSAKITTPKEMSRAFLDLAPAEFATSLEP
ESLSEKSLLKLKQMRYYPHYFGYELTRTGQGIDGGVAENALRLEKSPVKKREIKCKQYKTLGRGQ
NKIVLYVRSSYYQTQFLEWFLHRPKNVQTDVAVSGSFLIDEKKVKTRWNYDALTVALEPVSGSER

VFVSQPFTIFPEKSAEEEGQRYLGIDIGEYGIAYTALEITGDSAKILDQNFISDPQLKTLREEVK
GLKLDQRRGTFAMPSTKIARIRESLVHSLRNRIHHLALKHKAKIVYELEVSRFEEGKQKIKKVYA
TLKKADVYSEIDADKNLQTTVWGKLAVASEISASYTSQFCGACKKLWRAEMQVDETITTQELIGT
VRVIKGGTLIDAIKDFMRPPIFDENDTPFPKYRDFCDKHHISKKMRGNSCLFICPFCRANADADI
QASQTIALLRYVKEEKKVEDYFERFRKLKNIKVLGQMKKI

[0218] 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.

[0219] The "efficiency" of non-homologous end joining (NHEJ) and/or homology directed repair (HDR) can be calculated by any convenient method. For example, 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).

[0220] In some embodiments, 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 I cleaves mismatched heteroduplex DNA which arises from hybridization of wild-type and mutant DNA strands (NHEJ generates small random insertions or deletions (indels) at the site of the original break). More cleavage indicates a greater percent NHEJ (a greater efficiency of NHEJ). As an illustrative example, a fraction (percentage) of NHEJ can be calculated using the following equation: $(1-(1-(b+c)/(a+b+c))^{1/2}) \times 100$, where "a" is the band intensity of DNA substrate and "b" and "c" are the cleavage products (Ran *et. al.*, Cell. 2013 Sep. 12; 154(6):1380-9; and Ran *et al.*, Nat Protoc. 2013 Nov.; 8(11): 2281–2308).

[0221] 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 some embodiments, 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.

[0222] 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.

[0223] 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.

[0224] 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.

[0225] In some embodiments, 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 embodiments, 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."

[0226] In some embodiments, 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.

[0227] In some embodiments, 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).

[0228] In some embodiments, 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).

[0229] In some embodiments, 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 embodiments, 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).

[0230] As another non-limiting example, in some embodiments, 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).

[0231] As another non-limiting example, in some embodiments, 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).

[0232] As another non-limiting example, in some embodiments, 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 embodiments, 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).

[0233] As another non-limiting example, in some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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.

[0234] 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.

[0235] 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.

[0236] In some embodiments, a modified SpCas9 including amino acid substitutions D1135M, S1136Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R (SpCas9-MQKFRAER) and having specificity for the altered PAM 5′-NGC-3′ was used.

[0237] 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.

[0238] Furthermore, Cpf1, unlike Cas9, 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 that are more similar to types I and III than type II systems. Functional Cpf1 does not require 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 (approximately 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′ or 5′-TTN-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 having an overhang of 4 or 5 nucleotides.

[0239] In some embodiments, the Cas9 is a Cas9 variant having specificity for an altered PAM sequence. In some embodiments, the Additional Cas9 variants and PAM sequences are described in Miller, S.M., *et al.* Continuous evolution of SpCas9 variants compatible with non-G PAMs, Nat. Biotechnol. (2020), the entirety of which is incorporated herein by reference. in some embodiments, a Cas9 variate have no specific PAM requirements. In some embodiments, a Cas9 variant, e.g. a SpCas9 variant has specificity for a NRNH PAM, wherein R is A or G and H is A, C, or T. In some embodiments, the SpCas9 variant has specificity for a PAM sequence AAA, TAA, CAA, GAA, TAT, GAT, or CAC. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1134, 1135, 1137, 1139, 1151, 1180, 1188, 1211, 1218, 1219, 1221, 1249, 1256, 1264, 1290, 1318, 1317, 1320, 1321, 1323, 1332, 1333, 1335, 1337, or 1339 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1135, 1218, 1219,

1221, 1249, 1320, 1321, 1323, 1332, 1333, 1335, or 1337 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1134, 1135, 1137, 1139, 1151, 1180, 1188, 1211, 1219, 1221, 1256, 1264, 1290, 1318, 1317, 1320, 1323, 1333 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1131, 1135, 1150, 1156, 1180, 1191, 1218, 1219, 1221, 1227, 1249, 1253, 1286, 1293, 1320, 1321, 1332, 1335, 1339 as numbered in SEQ ID NO: 1 or a corresponding position thereof. In some embodiments, the SpCas9 variant comprises an amino acid substitution at position 1114, 1127, 1135, 1180, 1207, 1219, 1234, 1286, 1301, 1332, 1335, 1337, 1338, 1349 as numbered in SEQ ID NO: 1 or a corresponding position thereof. Exemplary amino acid substitutions and PAM specificity of SpCas9 variants are shown in **Tables 1A-1D**.

[0240] Table 1A

SpCas9	SpCas9 amino acid position												
	1114	1135	1218	1219	1221	1249	1320	1321	1323	1332	1333	1335	1337
	R	D	G	E	Q	P	A	P	A	D	R	R	T
AAA		N		V	H						G		
AAA		N		V	H						G		
AAA				V							G		
TAA	G	N		V							I		
TAA		N		V							I		A
TAA	G	N		V							I		A
CAA				V							K		
CAA		N		V							K		
CAA		N		V							K		
GAA				V	H		V				K		
GAA		N		V			V				K		
GAA				V	H		V				K		
TAT			S	V	H	S		S				L	
TAT			S	V	H	S		S				L	
TAT			S	V	H	S		S				L	
GAT				V							I		
GAT				V					D			Q	
GAT				V					D			Q	
CAC				V						N		Q	N
CAC		N		V								Q	N
CAC				V						N		Q	N

[0241] Table 1B

SpCas9	SpCas9 amino acid position																		
	1114	1134	1135	1137	1139	1151	1180	1188	1211	1219	1221	1256	1264	1290	1318	1317	1320	1323	1333
	R	F	D	P	V	K	D	K	K	E	Q	Q	H	V	L	N	A	A	R
GAA										V	H						V		K
GAA			N	S						V							V	D	K
GAA			N							V	H		Y				V		K
CAA			N							V	H		Y				V		K
CAA	G		N	S						V	H		Y				V		K
CAA			N					R		V	H						V		K
CAA			N				G		R	V	H		Y				V		K
CAA			N							V	H		Y				V		K
AAA			N				G			V	H	R	Y				V	D	K
CAA	G		N				G			V	H		Y				V	D	K
CAA		L	N				G			V	H		Y			T	V	D	K
TAA	G		N				G			V	H		Y	G	S		V	D	K
TAA	G		N			E	G			V	H		Y		S		V		K
TAA	G		N				G			V	H		Y		S		V	D	K
TAA	G		N				G		R	V	H						V		K
TAA			N				G		R	V	H		Y				V		K
TAA	G		N		A		G			V	H						V		K
TAA	G		N							V	H						V		K

[0242] Table 1C

SpCas9	SpCas9 amino acid position																		
	1114	1131	1135	1150	1156	1180	1191	1218	1219	1221	1227	1249	1253	1286	1293	1320	1321	1332	1335	1339
	R	Y	D	E	K	D	K	G	E	Q	A	P	E	N	A	A	P	D	R	T
SacB. TAT			N				N		V	H						V	S		L	
SacB. TAT			N					S	V	H		S					S	G	L	
AAT			N					S	V	H	V	S		K	T		S	G	L	I
TAT	G		N			G		S	V	H		S	K				S	G	L	
TAT	G		N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N			G		S	V	H		S					S	G	L	
TAT	G	C	N		E	G		S	V	H		S					S	G	L	
TAT	G	C	N	V		G		S	V	H		S					S	G	L	
TAT		C	N			G		S	V	H		S					S	G	L	

SpCas9	SpCas9 amino acid position																			
	1114	1131	1135	1150	1156	1180	1191	1218	1219	1221	1227	1249	1253	1286	1293	1320	1321	1332	1335	1339
	R	Y	D	E	K	D	K	G	E	Q	A	P	E	N	A	A	P	D	R	T
TAT	G	C	N			G		S	V	H		S					S	G	L	

[0243] Table 1D

SpCas9	SpCas9 amino acid position													
	1114	1127	1135	1180	1207	1219	1234	1286	1301	1332	1335	1337	1338	1349
	R	D	D	D	E	E	N	N	P	D	R	T	S	H
SacB.CAC			N			V				N	Q	N		
AAC	G		N			V				N	Q	N		
AAC	G		N			V				N	Q	N		
TAC	G		N			V				N	Q	N		
TAC	G		N			V		H		N	Q	N		
TAC	G		N		G	V	D	H		N	Q	N		
TAC	G		N			V				N	Q	N		
TAC	G	G	N	E		V		H		N	Q	N		
TAC	G		N			V		H		N	Q	N		
TAC	G		N			V				N	Q	N	T	R

[0244] In some embodiments, the Cas9 is a Neisseria menigitidis Cas9 (NmeCas9) or a variant thereof. In some embodiments, the NmeCas9 has specificity for a NNNNGAYW PAM, wherein Y is C or T and W is A or T. In some embodiments, the NmeCas9 has specificity for a NNNNGYTT PAM, wherein Y is C or T. In some embodiments, the NmeCas9 has specificity for a NNNNGTCT PAM. In some embodiments, the NmeCas9 is a Nme1 Cas9. In some embodiments, the NmeCas9 has specificity for a NNNNGATT PAM, a NNNNCCTA PAM, a NNNNCCTC PAM, a NNNNCCTT PAM, a NNNNCCTG PAM, a NNNNCCGT PAM, a NNNNCCGGPAM, a NNNNCCCA PAM, a NNNNCCCT PAM, a NNNNCCCC PAM, a NNNNCCAT PAM, a NNNNCCAG PAM, a NNNNCCAT PAM, or a NNNGATT PAM. In some embodiments, the Nme1Cas9 has specificity for a NNNNGATT PAM, a NNNNCCTA PAM, a NNNNCCTC PAM, a NNNNCCTT PAM, or a NNNNCCTG PAM. In some embodiments, the NmeCas9 has specificity for a CAA PAM, a CAAA PAM, or a CCA PAM. In some embodiments, the NmeCas9 is a Nme2 Cas9. In some embodiments, the NmeCas9 has specificity for a NNNNCC (N4CC) PAM, wherein N is any one of A, G, C, or T. in some

embodiments, the NmeCas9 has specificity for a NNNNCCGT PAM, a NNNNCCGGPAM, a NNNNCCCA PAM, a NNNNCCCT PAM, a NNNNCCCC PAM, a NNNNCCAT PAM, a NNNNCCAG PAM, a NNNNCCAT PAM, or a NNNGATT PAM. In some embodiments, the NmeCas9 is a Nme3Cas9. In some embodiments, the NmeCas9 has specificity for a NNNNCAAA PAM, a NNNNCC PAM, or a NNNNCNNN PAM. Additional NmeCas9 features and PAM sequences as described in Edraki et al. Mol. Cell. (2019) 73(4): 714-726 is incorporated herein by reference in its entirety.

[0245] An exemplary amino acid sequence of a Nme1Cas9 is provided below:

type II CRISPR RNA-guided endonuclease Cas9 [Neisseria meningitidis] WP_002235162.1

```
   1 maafkpnpin yilgldigia svgwamveid edenpiclid lgvrvferae vpktgdslam
  61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
 121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvadnahalq tgdfrtpael
 181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
 241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
 301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
 361 ekeglkdkks plnlspelqd eigtafslfk tdeditgrlk driqpeilea llkhisfdkf
 421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
 481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
 541 fpnfvgepks kdilklrlye qqhgkclysg keinlgrlne kgyveidhal pfsrtwddsf
 601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
 661 gfkernlndt ryvnrflcqf vadrmrltgk gkkrvfasng qitnllrgfw glrkvraend
 721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgevlhqkt hfpqpweffa
 781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
 841 qghmetvksa krldegvsvl rvpltqlklk dlekmvnrer epklyealka rleahkddpa
 901 kafaepfyky dkagnrtqqv kavrveqvqk tgvwvrnhng iadnatmvrv dvfekgdkyy
 961 lvpiyswqva kgilpdravv qgkdeedwql iddsfnfkfs lhpndlvevi tkkarmfgyf
1021 aschrgtgni nirihdldhk igkngilegi gvktalsfqk yqidelgkei rpcrlkkrpp
1081 vr
```

[0246] An exemplary amino acid sequence of a Nme2Cas9 is provided below:

type II CRISPR RNA-guided endonuclease Cas9 [Neisseria meningitidis] WP_002230835.1

```
1 maafkpnpin yilgldigia svgwamveid eeenpirlid lgvrvferae vpktgdslam
61 arrlarsvrr ltrrrahrll rarrllkreg vlqaadfden glikslpntp wqlraaaldr
121 kltplewsav llhlikhrgy lsqrkneget adkelgallk gvannahalq tgdfrtpael
181 alnkfekesg hirnqrgdys htfsrkdlqa elillfekqk efgnphvsgg lkegietllm
241 tqrpalsgda vqkmlghctf epaepkaakn tytaerfiwl tklnnlrile qgserpltdt
301 eratlmdepy rkskltyaqa rkllgledta ffkglrygkd naeastlmem kayhaisral
361 ekeglkdkks plnlsselqd eigtafslfk tdeditgrlk drvqpeilea llkhisfdkf
421 vqislkalrr ivplmeqgkr ydeacaeiyg dhygkkntee kiylppipad eirnpvvlra
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481 lsqarkving vvrrygspar ihietarevg ksfkdrkeie krqeenrkdr ekaaakfrey
541 fpnfvgepks kdilklrlye qqhgkclysg keinlvrlne kgyveidhal pfsrtwddsf
601 nnkvlvlgse nqnkgnqtpy eyfngkdnsr ewqefkarve tsrfprskkq rillqkfded
661 gfkecnlndt ryvnrflcqf vadhilltgk gkrrvfasng qitnllrgfw glrkvraend
721 rhhaldavvv acstvamqqk itrfvrykem nafdgktidk etgkvlhqkt hfpqpweffa
781 qevmirvfgk pdgkpefeea dtpeklrtll aeklssrpea vheyvtplfv srapnrkmsg
841 ahkdtlrsak rfvkhnekis vkrvwlteik ladlenmvny kngreielye alkarleayg
901 gnakqafdpk dnpfykkggq lvkavrvekt qesgvllnkk naytiadngd mvrvdvfckv
961 dkkgknqyfi vpiyawqvae nilpdidckg yriddsytfc fslhkydlia fqkdekskve
1021 fayyincdss ngrfylawhd kgskeqqfri stqnlvliqk yqvnelgkei rpcrlkkrpp
1081 vr
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Cas12 domains of Nucleobase Editors

[0247] Typically, microbial CRISPR-Cas systems are divided into Class 1 and Class 2 systems. Class 1 systems have multisubunit effector complexes, while Class 2 systems have a single protein effector. For example, Cas9 and Cpf1 are Class 2 effectors, albeit different types (Type II and Type V, respectively). In addition to Cpf1, Class 2, Type V CRISPR-Cas systems also comprise Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i). See, *e.g.*, Shmakov et al., "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems," Mol. Cell, 2015 Nov. 5; 60(3): 385-397; Makarova et al., "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?" CRISPR Journal, 2018, 1(5): 325-336; and Yan et al., "Functionally Diverse Type V CRISPR-Cas Systems," Science, 2019 Jan. 4; 363: 88-91; the entire contents of each is hereby incorporated by reference. Type V Cas proteins contain a RuvC (or RuvC-like) endonuclease domain. While production of mature CRISPR RNA (crRNA) is generally tracrRNA-independent, Cas12b/C2c1, for example, requires tracrRNA for production of crRNA. Cas12b/C2c1 depends on both crRNA and tracrRNA for DNA cleavage.

[0248] Nucleic acid programmable DNA binding proteins contemplated in the present disclosure include Cas proteins that are classified as Class 2, Type V (Cas12 proteins). Non-limiting examples of Cas Class 2, Type V proteins include Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i, homologues thereof, or modified versions thereof. As used herein, a Cas12 protein can also be referred to as a Cas12 nuclease, a Cas12 domain, or a Cas12 protein domain. In some embodiments, the Cas12 proteins of the present disclosure comprise an amino acid sequence interrupted by an internally fused protein domain such as a deaminase domain.

[0249] In some embodiments, the Cas12 domain is a nuclease inactive Cas12 domain or a Cas12 nickase. In some embodiments, the Cas12 domain is a nuclease active domain. For example, the Cas12 domain may be a Cas12 domain that nicks one strand of a duplexed nucleic acid (*e.g.*, duplexed DNA molecule). In some embodiments, the Cas12 domain comprises any one of the amino acid sequences as set forth herein. In some embodiments the Cas12 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 Cas12 domain comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more mutations compared to any one of the amino acid sequences set forth herein. In some embodiments, the Cas12 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.

[0250] In some embodiments, proteins comprising fragments of Cas12 are provided. For example, in some embodiments, a protein comprises one of two Cas12 domains: (1) the gRNA binding domain of Cas12; or (2) the DNA cleavage domain of Cas12. In some embodiments, proteins comprising Cas12 or fragments thereof are referred to as "Cas12 variants." A Cas12 variant shares homology to Cas12, or a fragment thereof. For example, a Cas12 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 Cas12. In some embodiments, the Cas12 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 Cas12. In some embodiments, the Cas12 variant comprises a fragment of Cas12 (*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 Cas12. 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 Cas12. 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.

[0251] In some embodiments, Cas12 corresponds to, or comprises in part or in whole, a Cas12 amino acid sequence having one or more mutations that alter the Cas12 nuclease activity. Such mutations, by way of example, include amino acid substitutions within the RuvC nuclease domain of Cas12. In some embodiments, variants or homologues of Cas12 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 to a wild type Cas12. In some embodiments, variants of Cas12 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.

[0252] In some embodiments, Cas12 fusion proteins as provided herein comprise the full-length amino acid sequence of a Cas12 protein, *e.g.*, one of the Cas12 sequences provided herein. In other embodiments, however, fusion proteins as provided herein do not comprise a full-length Cas12 sequence, but only one or more fragments thereof. Exemplary amino acid sequences of suitable Cas12 domains are provided herein, and additional suitable sequences of Cas12 domains and fragments will be apparent to those of skill in the art.

[0253] Generally, the class 2, Type V Cas proteins have a single functional RuvC endonuclease domain (See, *e.g.*, Chen et al., "CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity," Science 360:436-439 (2018)). In some cases, the Cas12 protein is a variant Cas12b protein. (See Strecker et al., Nature Communications, 2019, 10(1): Art. No.: 212). In one embodiment, a variant Cas12 polypeptide has an amino acid sequence that is different by 1, 2, 3, 4, 5 or more amino acids (*e.g.*, has a deletion, insertion, substitution, fusion) when compared to the amino acid sequence of a wild type Cas12 protein. In some instances, the variant Cas12 polypeptide has an amino acid change (*e.g.*, deletion, insertion, or substitution) that reduces the

activity of the Cas12 polypeptide. For example, in some instances, the variant Cas12 is a Cas12b polypeptide that 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 nickase activity of the corresponding wild-type Cas12b protein. In some cases, the variant Cas12b protein has no substantial nickase activity.

[0254] In some cases, a variant Cas12b protein has reduced nickase activity. For example, a variant Cas12b 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 nickase activity of a wild-type Cas12b protein.

[0255] In some embodiments, the Cas12 protein includes RNA-guided endonucleases from the Cas12a/Cpf1 family that displays 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, unlike Cas9, 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 are more similar to types I and III than type II systems. Functional Cpf1 does not require 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 (approximately 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′ or 5′-TTTN-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 having an overhang of 4 or 5 nucleotides.

[0256] In some aspects of the present disclosure, a vector encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence can be used. Cas12 can refer to a polypeptide with at least or at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild type exemplary Cas12 polypeptide (*e.g.*, Cas12 from Bacillus hisashii). Cas12 can refer to a polypeptide with at most or at most about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity and/or sequence homology to a wild type exemplary Cas12 polypeptide (*e.g.*, from Bacillus hisashii (BhCas12b), Bacillus sp. V3-13 (BvCas12b), and Alicyclobacillus acidiphilus (AaCas12b)). Cas12 can refer to the wild type or a modified form of the Cas12 protein that can comprise an amino acid change such as a deletion, insertion, substitution, variant, mutation, fusion, chimera, or any combination thereof.

[0257] Nucleic acid programmable DNA binding proteinsSome aspects of the disclosure provide fusion proteins comprising domains that act as nucleic acid programmable DNA binding proteins, which may be used to guide a protein, such as a base editor, to a specific nucleic acid (*e.g.*, DNA or RNA) sequence. In particular embodiments, a fusion protein comprises a nucleic acid programmable DNA binding protein domain and a deaminase domain. Non-limiting examples of nucleic acid programmable DNA binding proteins include, Cas9 (*e.g.*, dCas9 and nCas9), Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, and Cas12i. Non-limiting examples of Cas enzymes include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (also known as Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Csy1 , Csy2, Csy3, Csy4, Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, homologues thereof, or modified or engineered versions thereof. Other nucleic acid programmable DNA binding proteins are also within the scope of this disclosure, although they may not be specifically listed in this disclosure. See, *e.g.*, Makarova *et al.* "Classification

and Nomenclature of CRISPR-Cas Systems: Where from Here?" CRISPR J. 2018 Oct;1:325-336. doi: 10.1089/crispr.2018.0033; Yan *et al.*, "Functionally diverse type V CRISPR-Cas systems" Science. 2019 Jan 4;363(6422):88-91. doi: 10.1126/science.aav7271, the entire contents of each are hereby incorporated by reference.

[0258] One example of a nucleic acid programmable DNA-binding protein that has different PAM specificity than Cas9 is Clustered Regularly Interspaced Short Palindromic Repeats from *Prevotella* and *Francisella* 1 (Cpf1). Similar to Cas9, Cpf1 is also a class 2 CRISPR effector. It has been shown that Cpf1 mediates robust DNA interference with features distinct from Cas9. Cpf1 is a single RNA-guided endonuclease lacking tracrRNA, and it utilizes a T-rich protospacer-adjacent motif (TTN, TTTN, or YTN). Moreover, Cpf1 cleaves DNA via a staggered DNA double-stranded break. Out of 16 Cpf1-family proteins, two enzymes from Acidaminococcus and Lachnospiraceae are shown to have efficient genome-editing activity in human cells. Cpf1 proteins are known in the art and have been described previously, for example Yamano *et al.*, "Crystal structure of Cpf1 in complex with guide RNA and target DNA," *Cell* (165) 2016, p. 949-962; the entire contents of which is hereby incorporated by reference.

[0259] Useful in the present compositions and methods are nuclease-inactive Cpf1 (dCpf1) variants that may be used as a guide nucleotide sequence-programmable DNA-binding protein domain. The Cpf1 protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9 but does not have a HNH endonuclease domain, and the N-terminal of Cpf1 does not have the alfa-helical recognition lobe of Cas9. It was shown in Zetsche *et al.*, *Cell,* 163, 759-771, 2015 (which is incorporated herein by reference) that, the RuvC-like domain of Cpf1 is responsible for cleaving both DNA strands and inactivation of the RuvC-like domain inactivates Cpf1 nuclease activity. For example, mutations corresponding to D917A, E1006A, or D1255A in *Francisella novicida* Cpf1 inactivate Cpf1 nuclease activity. In some embodiments, the dCpf1 of the present disclosure comprises mutations corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/E1006A/D1255A. It is to be understood that any mutations, *e.g.*, substitution mutations, deletions, or insertions that inactivate the RuvC domain of Cpf1, may be used in accordance with the present disclosure.

[0260] In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a Cpf1 protein. In some embodiments, the Cpf1 protein is a Cpf1 nickase (nCpf1). In some embodiments, the Cpf1 protein is a nuclease inactive Cpf1 (dCpf1). In some embodiments, the Cpf1, the nCpf1, or the dCpf1 comprises an

amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a Cpf1 sequence disclosed herein. In some embodiments, the dCpf1 comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a Cpf1 sequence disclosed herein, and comprises mutations corresponding to D917A, E1006A, D1255A, D917A/E1006A, D917A/D1255A, E1006A/D1255A, or D917A/E1006A/D1255A. It should be appreciated that Cpf1 from other bacterial species may also be used in accordance with the present disclosure.

[0261] Wild type *Francisella novicida* Cpf1 (D917, E1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**D**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0262] *Francisella novicida* Cpf1 D917A (A917, E1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI

LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0263] *Francisella novicida* Cpf1 E1006A (D917, A1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND

VHILSI**D**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0264] *Francisella novicida* Cpf1 D1255A (D917, E1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**D**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0265] *Francisella novicida* Cpf1 D917A/E1006A (A917, A1006, and D1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL

DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**D**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0266] *Francisella novicida* Cpf1 D917A/D1255A (A917, E1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**E**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF

KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0267] *Francisella novicida* Cpf1 E1006A/D1255A (D917, A1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF
KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**D**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0268] *Francisella novicida* Cpf1 D917A/E1006A/D1255A (A917, A1006, and A1255 are bolded and underlined)

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKYHQFFIEEI
LSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQNLIDA
KKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDI
PTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSL
DEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLF

KQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSK
IYFKNDKSLTDLSQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLET
IKLALEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQASAE
DDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELANIVPLYNKIRNY
ITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENK
GEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIE
DCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQ
GKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPA
KEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKAND
VHILSI**A**RGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKDWKKIN
NIKEMKEGYLSQVVHEIAKLVIEYNAIVVF**A**DLNFGFKRGRFKVEKQVYQKLEKMLIEKLNYLVF
KDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP
TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVAD
VNGNFFDSRQAPKNMPQDA**A**ANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

[0269] In some embodiments, one of the Cas9 domains present in the fusion protein may be replaced with a guide nucleotide sequence-programmable DNA-binding protein domain that has no requirements for a PAM sequence.

[0270] In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) is a single effector of a microbial CRISPR-Cas system. Single effectors of microbial CRISPR-Cas systems include, without limitation, Cas9, Cpf1, Cas12b/C2c1, and Cas12c/C2c3. Typically, microbial CRISPR-Cas systems are divided into Class 1 and Class 2 systems. Class 1 systems have multisubunit effector complexes, while Class 2 systems have a single protein effector. For example, Cas9 and Cpf1 are Class 2 effectors. In addition to Cas9 and Cpf1, three distinct Class 2 CRISPR-Cas systems (Cas12b/C2c1, and Cas12c/C2c3) have been described by Shmakov *et al.*, "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems", *Mol. Cell,* 2015 Nov. 5; 60(3): 385-397, the entire contents of which is hereby incorporated by reference. Effectors of two of the systems, Cas12b/C2c1, and Cas12c/C2c3, contain RuvC-like endonuclease domains related to Cpf1. A third system, contains an effector with two predicated HEPN RNase domains. Production of mature CRISPR RNA is tracrRNA-independent, unlike production of CRISPR RNA by Cas12b/C2c1. Cas12b/C2c1 depends on both CRISPR RNA and tracrRNA for DNA cleavage.

[0271] The crystal structure of *Alicyclobaccillus acidoterrastris* Cas12b/C2c1 (AacC2c1) has been reported in complex with a chimeric single-molecule guide RNA (sgRNA). See *e.g.*, Liu *et al.*, "C2c1-sgRNA Complex Structure Reveals RNA-Guided DNA Cleavage Mechanism", *Mol. Cell,* 2017 Jan. 19; 65(2):310-322, the entire contents of which are hereby incorporated by reference. The crystal structure has also been reported in *Alicyclobacillus acidoterrestris* C2c1 bound to target DNAs as ternary complexes. See *e.g.*, Yang *et al.*, "PAM-dependent Target DNA Recognition and Cleavage by C2C1 CRISPR-Cas endonuclease", *Cell,* 2016 Dec. 15; 167(7):1814-1828, the entire contents of which are hereby incorporated by reference. Catalytically competent conformations of AacC2c1, both with target and non-target DNA strands, have been captured independently positioned within a single RuvC catalytic pocket, with Cas12b/C2c1-mediated cleavage resulting in a staggered seven-nucleotide break of target DNA. Structural comparisons between Cas12b/C2c1 ternary complexes and previously identified Cas9 and Cpf1 counterparts demonstrate the diversity of mechanisms used by CRISPR-Cas9 systems. In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) of any of the fusion proteins provided herein may be a Cas12b/C2c1, or a Cas12c/C2c3 protein. In some embodiments, the napDNAbp is a Cas12b/C2c1 protein. In some embodiments, the napDNAbp is a Cas12c/C2c3 protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to a naturally-occurring Cas12b/C2c1 or Cas12c/C2c3 protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12b/C2c1 or Cas12c/C2c3 protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at ease 99.5% identical to any one of the napDNAbp sequences provided herein. It should be appreciated that Cas12b/C2c1 or Cas12c/C2c3 from other bacterial species may also be used in accordance with the present disclosure.

[0272] A Cas12b/C2c1 ((uniprot.org/uniprot/T0D7A2#2) sp|T0D7A2|/C2C1_ALIAG CRISPR-associated endo- nuclease C2c1 OS = = *Alicyclobacillus acido- terrestris* ((strain ATCC 49025 / DSM 3922/ CIP 106132 / NCIMB 13137/GD3B) GN=c2c1 PE=1 SV=1)) amino acid sequence is as follows:

MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENLYRRSPNGDGEQECDKTA
EECKAELLERLRARQVENGHRGPAGSDDELLQLARQLYELLVPQAIGAKGDAQQIARKFLSPLAD
KDAVGGLGIAKAGNKPRWVRMREAGEPGWEEEKEKAETRKSADRTADVLRALADFGLKPLMRVYT

DSEMSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQEYAKLVEQKNRFEQKNFVGQ
EHLVHLVNQLQQDMKEASPGLESKEQTAHYVTGRALRGSDKVFEKWGKLAPDAPFDLYDAEIKNV
QRRNTRRFGSHDLFAKLAEPEYQALWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDATAHPI
WTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDDVTVPISMSEQLDNLLPRDP
NEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQLAHMHRRRGARDVYLNVSVRVQSQSEARGERRP
PYAAVFRLVGDNHRAFVHFDKLSDYLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVA
RKDELKPNSKGRVPFFFPIKGNDNLVAVHERSQLLKLPGETESKDLRAIREERQRTLRQLRTQLA
YLRLLVRCGSEDVGRRERSWAKLIEQPVDAANHMTPDWREAFENELQKLKSLHGICSDKEWMDAV
YESVRRVWRHMGKQVRDWRKDVRSGERPKIRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKV
SGQVIRAEKGSRFAITLREHIDHAKEDRLKKLADRIIMEALGYVYALDERGKGKWVAKYPPCQLI
LLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELINQAQVHDLLVGTMYAAFSSRFDARTGAPGI
RCRRVPARCTQEHNPEPFPWWLNKFVVEHTLDACPLRADDLIPTGEGEIFVSPFSAEEGDFHQIH
ADLNAAQNLQQRLWSDFDISQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFYTNTGVTYYE
RERGKKRRKVFAQEKLSEEEAELLVEADEAREKSVVLMRDPSGIINRGNWTRQKEFWSMV NQRI
EGYLVKQIRSRVPLQDSACENTGDI

[0273] BhCas12b (*Bacillus hisashii*) NCBI Reference Sequence: WP_095142515

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAIYEH
HEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRELYEELVPSSVEKKGEAN
QLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDPLAKILGKLA
EYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNLKVKEEYEKV
EKEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLRGWREIIQKWLKMDEN
EPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPYLYATFCEIDKKKKDAKQQ
ATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKLTVQLDRLIYPTESGGWEEKGK
VDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGTLGGARVQFDRDHLRRYPHKVESGN
VGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKPKELTEWIKDSKGKKLKSGIESLEIGLR
VMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIKGTELYAVHRASFNIKLPGETLVKSREVLRK
AREDNLKLMNQKLNFLRNVLHFQQFEDITEREKRVTKWISRQENSDVPLVYQDELIQIRELMYKP
YKDWVAFLKQLHKRLEVEIGKEVKHWRKSLSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEP
GEVRRLEPGQRFAIDQLNHLNALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFED
LSNYNPYEERSRFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSV
VTKEKLQDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADINAAQNL
QKRFWTRTHGFYKVYCKAYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIK
KGSSKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISK
LTNQYSISTIEDDSSKQSMKRPAATKKAGQAKKKK

[0274] In some embodiments, the Cas12b is BvCas12b V4, which is a variant of BhCas12b and comprises the following changes relative to BhCas12B: S893R, K846R, and E837G. BhCas12b (V4) is expressed as follows: 5′ mRNA Cap---5′UTR---bhCas12b---STOP sequence --- 3′UTR --- 120polyA tail

5′UTR:

GGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC

3′ UTR (TriLink standard UTR)

GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCT
GCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGA

Nucleic acid sequence of bhCas12b (V4)

[0275] ATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGCCACCAG
ATCCTTCATCCTGAAGATCGAGCCCAACGAGGAAGTGAAGAAAGGCCTCTGGAAAACCCACGAGG
TGCTGAACCACGGAATCGCCTACTACATGAATATCCTGAAGCTGATCCGGCAAGAGGCCATCTAC
GAGCACCACGAGCAGGACCCCAAGAATCCCAAGAAGGTGTCCAAGGCCGAGATCCAGGCCGAGCT
GTGGGATTTCGTGCTGAAGATGCAGAAGTGCAACAGCTTCACACACGAGGTGGACAAGGACGAGG
TGTTCAACATCCTGAGAGAGCTGTACGAGGAACTGGTGCCCAGCAGCGTGGAAAAGAAGGGCGAA
GCCAACCAGCTGAGCAACAAGTTTCTGTACCCTCTGGTGGACCCCAACAGCCAGTCTGGAAAGGG
AACAGCCAGCAGCGGCAGAAAGCCCAGATGGTACAACCTGAAGATTGCCGGCGATCCCTCCTGGG
AAGAAGAGAAGAAGAAGTGGGAAGAAGATAAGAAAAAGGACCCGCTGGCCAAGATCCTGGGCAAG
CTGGCTGAGTACGGACTGATCCCTCTGTTCATCCCCTACACCGACAGCAACGAGCCCATCGTGAA
AGAAATCAAGTGGATGGAAAAGTCCCGGAACCAGAGCGTGCGGCGGCTGGATAAGGACATGTTCA
TTCAGGCCCTGGAACGGTTCCTGAGCTGGGAGAGCTGGAACCTGAAAGTGAAAGAGGAATACGAG
AAGGTCGAGAAAGAGTACAAGACCCTGGAAGAGAGGATCAAAGAGGACATCCAGGCTCTGAAGGC
TCTGGAACAGTATGAGAAAGAGCGGCAAGAACAGCTGCTGCGGGACACCCTGAACACCAACGAGT
ACCGGCTGAGCAAGAGAGGCCTTAGAGGCTGGCGGGAAATCATCCAGAAATGGCTGAAAATGGAC
GAGAACGAGCCCTCCGAGAAGTACCTGGAAGTGTTCAAGGACTACCAGCGGAAGCACCCTAGAGA
GGCCGGCGATTACAGCGTGTACGAGTTCCTGTCCAAGAAAGAGAACCACTTCATCTGGCGGAATC
ACCCTGAGTACCCCTACCTGTACGCCACCTTCTGCGAGATCGACAAGAAAAAGAAGGACGCCAAG
CAGCAGGCCACCTTCACACTGGCCGATCCTATCAATCACCCTCTGTGGGTCCGATTCGAGGAAAG
AAGCGGCAGCAACCTGAACAAGTACAGAATCCTGACCGAGCAGCTGCACACCGAGAAGCTGAAGA
AAAAGCTGACAGTGCAGCTGGACCGGCTGATCTACCCTACAGAATCTGGCGGCTGGGAAGAGAAG
GGCAAAGTGGACATTGTGCTGCTGCCCAGCCGGCAGTTCTACAACCAGATCTTCCTGGACATCGA
GGAAAAGGGCAAGCACGCCTTCACCTACAAGGATGAGAGCATCAAGTTCCCTCTGAAGGGCACAC

TCGGCGGAGCCAGAGTGCAGTTCGACAGAGATCACCTGAGAAGATACCCTCACAAGGTGGAAAGC
GGCAACGTGGGCAGAATCTACTTCAACATGACCGTGAACATCGAGCCTACAGAGTCCCCAGTGTC
CAAGTCTCTGAAGATCCACCGGGACGACTTCCCCAAGGTGGTCAACTTCAAGCCCAAAGAACTGA
CCGAGTGGATCAAGGACAGCAAGGGCAAGAAACTGAAGTCCGGCATCGAGTCCCTGGAAATCGGC
CTGAGAGTGATGAGCATCGACCTGGGACAGAGACAGGCCGCTGCCGCCTCTATTTTCGAGGTGGT
GGATCAGAAGCCCGACATCGAAGGCAAGCTGTTTTTCCCAATCAAGGGCACCGAGCTGTATGCCG
TGCACAGAGCCAGCTTCAACATCAAGCTGCCCGGCGAGACACTGGTCAAGAGCAGAGAAGTGCTG
CGGAAGGCCAGAGAGGACAATCTGAAACTGATGAACCAGAAGCTCAACTTCCTGCGGAACGTGCT
GCACTTCCAGCAGTTCGAGGACATCACCGAGAGAGAGAAGCGGGTCACCAAGTGGATCAGCAGAC
AAGAGAACAGCGACGTGCCCCTGGTGTACCAGGATGAGCTGATCCAGATCCGCGAGCTGATGTAC
AAGCCTTACAAGGACTGGGTCGCCTTCCTGAAGCAGCTCCACAAGAGACTGGAAGTCGAGATCGG
CAAAGAAGTGAAGCACTGGCGGAAGTCCCTGAGCGACGGAAGAAAGGGCCTGTACGGCATCTCCC
TGAAGAACATCGACGAGATCGATCGGACCCGGAAGTTCCTGCTGAGATGGTCCCTGAGGCCTACC
GAACCTGGCGAAGTGCGTAGACTGGAACCCGGCCAGAGATTCGCCATCGACCAGCTGAATCACCT
GAACGCCCTGAAAGAAGATCGGCTGAAGAAGATGGCCAACACCATCATCATGCACGCCCTGGGCT
ACTGCTACGACGTGCGGAAGAAGAAATGGCAGGCTAAGAACCCCGCCTGCCAGATCATCCTGTTC
GAGGATCTGAGCAACTACAACCCCTACGAGGAAAGGTCCCGCTTCGAGAACAGCAAGCTCATGAA
GTGGTCCAGACGCGAGATCCCCAGACAGGTTGCACTGCAGGGCGAGATCTATGGCCTGCAAGTGG
GAGAAGTGGGCGCTCAGTTCAGCAGCAGATTCCACGCCAAGACAGGCAGCCCTGGCATCAGATGT
AGCGTCGTGACCAAAGAGAAGCTGCAGGACAATCGGTTCTTCAAGAATCTGCAGAGAGAGGGCAG
ACTGACCCTGGACAAAATCGCCGTGCTGAAAGAGGGCGATCTGTACCCAGACAAAGGCGGCGAGA
AGTTCATCAGCCTGAGCAAGGATCGGAAGTGCGTGACCACACACGCCGACATCAACGCCGCTCAG
AACCTGCAGAAGCGGTTCTGGACAAGAACCCACGGCTTCTACAAGGTGTACTGCAAGGCCTACCA
GGTGGACGGCCAGACCGTGTACATCCCTGAGAGCAAGGACCAGAAGCAGAAGATCATCGAAGAGT
TCGGCGAGGGCTACTTCATTCTGAAGGACGGGGTGTACGAATGGGTCAACGCCGGCAAGCTGAAA
ATCAAGAAGGGCAGCTCCAAGCAGAGCAGCAGCGAGCTGGTGGATAGCGACATCCTGAAAGACAG
CTTCGACCTGGCCTCCGAGCTGAAAGGCGAAAAGCTGATGCTGTACAGGGACCCCAGCGGCAATG
TGTTCCCCAGCGACAAATGGATGGCCGCTGGCGTGTTCTTCGGAAAGCTGGAACGCATCCTGATC
AGCAAGCTGACCAACCAGTACTCCATCAGCACCATCGAGGACGACAGCAGCAAGCAGTCTATGAA
AAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAG

[0276] In some embodiments, the Cas12b is BvCas12B. In some embodiments, the Cas12b comprises amino acid substitutions S893R, K846R, and E837G as numbered in the BvCas12b exemplary sequence provided below.

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

MAIRSIKLKMKTNSGTDSIYLRKALWRTHQLINEGIAYYMNLLTLYRQEAIGDKTKEAYQAELIN
IIRNQQRNNGSSEEHGSDQEILALLRQLYELIIPSSIGESGDANQLGNKFLYPLVDPNSQSGKGT
SNAGRKPRWKRLKEEGNPDWELEKKKDEERKAKDPTVKIFDNLNKYGLLPLFPLFTNIQKDIEWL
PLGKRQSVRKWDKDMFIQAIERLLSWESWNRRVADEYKQLKEKTESYYKEHLTGGEEWIEKIRKF
EKERNMELEKNAFAPNDGYFITSRQIRGWDRVYEKWSKLPESASPEELWKVVAEQQNKMSEGFGD
PKVFSFLANRENRDIWRGHSERIYHIAAYNGLQKKLSRTKEQATFTLPDAIEHPLWIRYESPGGT
NLNLFKLEEKQKKNYYVTLSKIIWPSEEKWIEKENIEIPLAPSIQFNRQIKLKQHVKGKQEISFS
DYSSRISLDGVLGGSRIQFNRKYIKNHKELLGEGDIGPVFFNLVVDVAPLQETRNGRLQSPIGKA
LKVISSDFSKVIDYKPKELMDWMNTGSASNSFGVASLLEGMRVMSIDMGQRTSASVSIFEVVKEL
PKDQEQKLFYSINDTELFAIHKRSFLLNLPGEVVTKNNKQQRQERRKKRQFVRSQIRMLANVLRL
ETKKTPDERKKAIHKLMEIVQSYDSWTASQKEVWEKELNLLTNMAAFNDEIWKESLVELHHRIEP
YVGQIVSKWRKGLSEGRKNLAGISMWNIDELEDTRRLLISWSKRSRTPGEANRIETDEPFGSSLL
QHIQNVKDDRLKQMANLIIMTALGFKYDKEEKDRYKRWKETYPACQIILFENLNRYLFNLDRSRR
ENSRLMKWAHRSIPRTVSMQGEMFGLQVGDVRSEYSSRFHAKTGAPGIRCHALTEEDLKAGSNTL
KRLIEDGFINESELAYLKKGDIIPSQGGELFVTLSKRYKKDSDNNELTVIHADINAAQNLQKRFW
QQNSEVYRVPCQLARMGEDKLYIPKSQTETIKKYFGKGSFVKNNTEQEVYKWEKSEKMKIKTDTT
FDLQDLDGFEDISKTIELAQEQQKKYLTMFRDPSGYFFNNETWRPQKEYWSIVNNIIKSCLKKKI
LSNKVEL.

[0277] In some embodiments, the Cas12b is BTCas12b.BTCas12b (*Bacillus thermoamylovorans*) NCBI Reference Sequence: WP_041902512

MATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAIYEHHEQDPKNPKKV
SKAEIQAELWDFVLKMQKCNSFTHEVDKDVVFNILRELYEELVPSSVEKKGEANQLSNKF
LYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDPLAKILGKLAE
YGLIPLFIPFTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNLKVKEE
YEKVEKEHKTLEERIKEDIQAFKSLEQYEKERQEQLLRDTLNTNEYRLSKRGLRGWREII
QKWLKMDENEPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPYLYAT
FCEIDKKKKDAKQQATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKLTV
QLDRLIYPTESGGWEEKGKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGT
LGGARVQFDRDHLRRYPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKFVNF
KPKELTEWIKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVDQKPDIEGKLF
FPIKGTELYAVHRASFNIKLPGETLVKSREVLRKAREDNLKLMNQKLNFLRNVLHFQQFE
DITEREKRVTKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAFLKQLHKRLEVEIGK

EVKHWRKSLSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQ
LNHLNALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPYEERS
RFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVTKEKL
QDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLSKDRKLVTTHADINAAQNLQ
KRFWTRTHGFYKVYCKAYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWGNAGK
LKIKKGSSKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFG
KLERILISKLTNQYSISTIEDDSSKQSM

[0278] In some embodiments, a napDNAbp refers to Cas12c. In some embodiments, the Cas12c protein is a Cas12c1 or a variant of Cas12c1. In some embodiments, the Cas12 protein is a Cas12c2 or a variant of Cas12c2. In some embodiments, the Cas12 protein is a Cas12c protein from Oleiphilus sp. HI0009 (i.e., OspCas12c) or a variant of OspCas12c. These Cas12c molecules have been described in Yan et al., "Functionally Diverse Type V CRISPR-Cas Systems," Science, 2019 Jan. 4; 363: 88-91; the entire contents of which is hereby incorporated by reference. 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 least 99.5% identical to a naturally-occurring Cas12c1, Cas12c2, or OspCas12c protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12c1, Cas12c2, or OspCas12c 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 Cas12c1, Cas12c2, or OspCas12c protein described herein. It should be appreciated that Cas12c1, Cas12c2, or OspCas12c from other bacterial species may also be used in accordance with the present disclosure.

[0279] Cas12c1

MQTKKTHLHLISAKASRKYRRTIACLSDTAKKDLERRKQSGAADPAQELSCLKTIKFKLEVPEGS
KLPSFDRISQIYNALETIEKGSLSYLLFALILSGFRIFPNSSAAKTFASSSCYKNDQFASQIKEI
FGEMVKNFIPSELESILKKGRRKNNKDWTEENIKRVLNSEFGRKNSEGSSALFDSFLSKFSQELF
RKFDSWNEVNKKYLEAAELLDSMLASYGPFDSVCKMIGDSDSRNSLPDKSTIAFTNNAEITVDIE
SSVMPYMAIAALLREYRQSKSKAAPVAYVQSHLTTTNGNGLSWFFKFGLDLIRKAPVSSKQSTSD
GSKSLQELFSVPDDKLDGLKFIKEACEALPEASLLCGEKGELLGYQDFRTSFAGHIDSWVANYVN
RLFELIELVNQLPESIKLPSILTQKNHNLVASLGLQEAEVSHSLELFEGLVKNVRQTLKKLAGID
ISSSPNEQDIKEFYAFSDVLNRLGSIRNQIENAVQTAKKDKIDLESAIEWKEWKKLKKLPKLNGL
GGGVPKQQELLDKALESVKQIRHYQRIDFERVIQWAVNEHCLETVPKFLVDAEKKKINKESSTDF

AAKENAVRFLLEGIGAAARGKTDSVSKAAYNWFVVNNFLAKKDLNRYFINCQGCIYKPPYSKRRS
LAFALRSDNKDTIEVVWEKFETFYKEISKEIEKFNIFSQEFQTFLHLENLRMKLLLRRIQKPIPA
EIAFFSLPQEYYDSLPPNVAFLALNQEITPSEYITQFNLYSSFLNGNLILLRRSRSYLRAKFSWV
GNSKLIYAAKEARLWKIPNAYWKSDEWKMILDSNVLVFDKAGNVLPAPTLKKVCEREGDLRLFYP
LLRQLPHDWCYRNPFVKSVGREKNVIEVNKEGEPKVASALPGSLFRLIGPAPFKSLLDDCFFNPL
DKDLRECMLIVDQEISQKVEAQKVEASLESCTYSIAVPIRYHLEEPKVSNQFENVLAIDQGEAGL
AYAVFSLKSIGEAETKPIAVGTIRIPSIRRLIHSVSTYRKKKQRLQNFKQNYDSTAFIMRENVTG
DVCAKIVGLMKEFNAFPVLEYDVKNLESGSRQLSAVYKAVNSHFLYFKEPGRDALRKQLWYGGDS
WTIDGIEIVTRERKEDGKEGVEKIVPLKVFPGRSVSARFTSKTCSCCGRNVFDWLFTEKKAKTNK
KFNVNSKGELTTADGVIQLFEADRSKGPKFYARRKERTPLTKPIAKGSYSLEEIERRVRTNLRRA
PKSKQSRDTSQSQYFCVYKDCALHFSGMQADENAAINIGRRFLTALRKNRRSDFPSNVKISDRLL
DN

[0280] Cas12c2

MTKHSIPLHAFRNSGADARKWKGRIALLAKRGKETMRTLQFPLEMSEPEAAAINTTPFAVAYNAI
EGTGKGTLFDYWAKLHLAGFRFFPSGGAATIFRQQAVFEDASWNAAFCQQSGKDWPWLVPSKLYE
RFTKAPREVAKKDGSKKSIEFTQENVANESHVSLVGASITDKTPEDQKEFFLKMAGALAEKFDSW
KSANEDRIVAMKVIDEFLKSEGLHLPSLENIAVKCSVETKPDNATVAWHDAPMSGVQNLAIGVFA
TCASRIDNIYDLNGGKLSKLIQESATTPNVTALSWLFGKGLEYFRTTDIDTIMQDFNIPASAKES
IKPLVESAQAIPTMTVLGKKNYAPFRPNFGGKIDSWIANYASRLMLLNDILEQIEPGFELPQALL
DNETLMSGIDMTGDELKELIEAVYAWVDAAKQGLATLLGRGGNVDDAVQTFEQFSAMMDTLNGTL
NTISARYVRAVEMAGKDEARLEKLIECKFDIPKWCKSVPKLVGISGGLPKVEEEIKVMNAAFKDV
RARMFVRFEEIAAYVASKGAGMDVYDALEKRELEQIKKLKSAVPERAHIQAYRAVLHRIGRAVQN
CSEKTKQLFSSKVIEMGVFKNPSHLNNFIFNQKGAIYRSPFDRSRHAPYQLHADKLLKNDWLELL
AEISATLMASESTEQMEDALRLERTRLQLQLSGLPDWEYPASLAKPDIEVEIQTALKMQLAKDTV
TSDVLQRAFNLYSSVLSGLTFKLLRRSFSLKMRFSVADTTQLIYVPKVCDWAIPKQYLQAEGEIG
IAARVVTESSPAKMVTEVEMKEPKALGHFMQQAPHDWYFDASLGGTQVAGRIVEKGKEVGKERKL
VGYRMRGNSAYKTVLDKSLVGNTELSQCSMIIEIPYTQTVDADFRAQVQAGLPKVSINLPVKETI
TASNKDEQMLFDRFVAIDLGERGLGYAVFDAKTLELQESGHRPIKAITNLLNRTHHYEQRPNQRQ
KFQAKFNVNLSELRENTVGDVCHQINRICAYYNAFPVLEYMVPDRLDKQLKSVYESVTNRYIWSS
TDAHKSARVQFWLGGETWEHPYLKSAKDKKPLVLSPGRGASGKGTSQTCSCCGRNPFDLIKDMKP
RAKIAVVDGKAKLENSELKLFERNLESKDDMLARRHRNERAGMEQPLTPGNYTVDEIKALLRANL
RRAPKNRRTKDTTVSEYHCVFSDCGKTMHADENAAVNIGGKFIADIEK

[0281] OspCas12c

MTKLRHRQKKLTHDWAGSKKREVLGSNGKLQNPLLMPVKKGQVTEFRKAFSAYARATKGEMTDGR
KNMFTHSFEPFKTKPSLHQCELADKAYQSLHSYLPGSLAHFLLSAHALGFRIFSKSGEATAFQAS
SKIEAYESKLASELACVDLSIQNLTISTLFNALTTSVRGKGEETSADPLIARFYTLLTGKPLSRD
TQGPERDLAEVISRKIASSFGTWKEMTANPLQSLQFFEEELHALDANVSLSPAFDVLIKMNDLQG
DLKNRTIVFDPDAPVFEYNAEDPADIIIKLTARYAKEAVIKNQNVGNYVKNAITTTNANGLGWLL
NKGLSLLPVSTDDELLEFIGVERSHPSCHALIELIAQLEAPELFEKNVFSDTRSEVQGMIDSAVS
NHIARLSSSRNSLSMDSEELERLIKSFQIHTPHCSLFIGAQSLSQQLESLPEALQSGVNSADILL
GSTQYMLTNSLVEESIATYQRTLNRINYLSGVAGQINGAIKRKAIDGEKIHLPAAWSELISLPFI
GQPVIDVESDLAHLKNQYQTLSNEFDTLISALQKNFDLNFNKALLNRTQHFEAMCRSTKKNALSK
PEIVSYRDLLARLTSCLYRGSLVLRRAGIEVLKKHKIFESNSELREHVHERKHFVFVSPLDRKAK
KLLRLTDSRPDLLHVIDEILQHDNLENKDRESLWLVRSGYLLAGLPDQLSSSFINLPIITQKGDR
RLIDLIQYDQINRDAFVMLVTSAFKSNLSGLQYRANKQSFVVTRTLSPYLGSKLVYVPKDKDWLV
PSQMFEGRFADILQSDYMVWKDAGRLCVIDTAKHLSNIKKSVFSSEEVLAFLRELPHRTFIQTEV
RGLGVNVDGIAFNNGDIPSLKTFSNCVQVKVSRTNTSLVQTLNRWFEGGKVSPPSIQFERAYYKK
DDQIHEDAAKRKIRFQMPATELVHASDDAGWTPSYLLGIDPGEYGMGLSLVSINNGEVLDSGFIH
INSLINFASKKSNHQTKVVPRQQYKSPYANYLEQSKDSAAGDIAHILDRLIYKLNALPVFEALSG
NSQSAADQVWTKVLSFYTWGDNDAQNSIRKQHWFGASHWDIKGMLRQPPTEKKPKPYIAFPGSQV
SSYGNSQRCSCCGRNPIEQLREMAKDTSIKELKIRNSEIQLFDGTIKLFNPDPSTVIERRRHNLG
PSRIPVADRTFKNISPSSLEFKELITIVSRSIRHSPEFIAKKRGIGSEYFCAYSDCNSSLNSEAN
AAANVAQKFQKQLFFEL

[0282] In some embodiments, a napDNAbp refers to Cas12g, Cas12h, or Cas12i, which have been described in, for example, Yan et al., "Functionally Diverse Type V CRISPR-Cas Systems," Science, 2019 Jan. 4; 363: 88-91; the entire contents of each is hereby incorporated by reference. By aggregating more than 10 terabytes of sequence data, new classifications of Type V Cas proteins were identified that showed weak similarity to previously characterized Class V protein, including Cas12g, Cas12h, and Cas12i. In some embodiments, the Cas12 protein is a Cas12g or a variant of Cas12g. In some embodiments, the Cas12 protein is a Cas12h or a variant of Cas12h. In some embodiments, the Cas12 protein is a Cas12i or a variant of Cas12i. It should be appreciated that other RNA-guided DNA binding proteins may be used as a napDNAbp, and are within the scope of this disclosure. 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 least 99.5% identical to a naturally-occurring Cas12g, Cas12h, or Cas12i protein. In some embodiments, the

napDNAbp is a naturally-occurring Cas12g, Cas12h, or Cas12i 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 Cas12g, Cas12h, or Cas12i protein described herein. It should be appreciated that Cas12g, Cas12h, or Cas12i from other bacterial species may also be used in accordance with the present disclosure. In some embodiments, the Cas12i is a Cas12i1 or a Cas12i2.

[0283] Cas12g1

MAQASSTPAVSPRPRPRYREERTLVRKLLPRPGQSKQEFRENVKKLRKAFLQFNADVSGVCQWAI
QFRPRYGKPAEPTETFWKFFLEPETSLPPNDSRSPEFRRLQAFEAAAGINGAAALDDPAFTNELR
DSILAVASRPKTKEAQRLFSRLKDYQPAHRMILAKVAAEWIESRYRRAHQNWERNYEEWKKEKQE
WEQNHPELTPEIREAFNQIFQQLEVKEKRVRICPAARLLQNKDNCQYAGKNKHSVLCNQFNEFKK
NHLQGKAIKFFYKDAEKYLRCGLQSLKPNVQGPFREDWNKYLRYMNLKEETLRGKNGGRLPHCKN
LGQECEFNPHTALCKQYQQQLSSRPDLVQHDELYRKWRREYWREPRKPVFRYPSVKRHSIAKIFG
ENYFQADFKNSVVGLRLDSMPAGQYLEFAFAPWPRNYRPQPGETEISSVHLHFVGTRPRIGFRFR
VPHKRSRFDCTQEELDELRSRTFPRKAQDQKFLEAARKRLLETFPGNAEQELRLLAVDLGTDSAR
AAFFIGKTFQQAFPLKIVKIEKLYEQWPNQKQAGDRRDASSKQPRPGLSRDHVGRHLQKMRAQAS
EIAQKRQELTGTPAPETTTDQAAKKATLQPFDLRGLTVHTARMIRDWARLNARQIIQLAEENQVD
LIVLESLRGFRPPGYENLDQEKKRRVAFFAHGRIRRKVTEKAVERGMRVVTVPYLASSKVCAECR
KKQKDNKQWEKNKKRGLFKCEGCGSQAQVDENAARVLGRVFWGEIELPTAIP

[0284] Cas12h1

MKVHEIPRSQLLKIKQYEGSFVEWYRDLQEDRKKFASLLFRWAAFGYAAREDDGATYISPSQALL
ERRLLLGDAEDVAIKFLDVLFKGGAPSSSCYSLFYEDFALRDKAKYSGAKREFIEGLATMPLDKI
IERIRQDEQLSKIPAEEWLILGAEYSPEEIWEQVAPRIVNVDRSLGKQLRERLGIKCRRPHDAGY
CKILMEVVARQLRSHNETYHEYLNQTHEMKTKVANNLTNEFDLVCEFAEVLEEKNYGLGWYVLWQ
GVKQALKEQKKPTKIQIAVDQLRQPKFAGLLTAKWRALKGAYDTWKLKKRLEKRKAFPYMPNWDN
DYQIPVGLTGLGVFTLEVKRTEVVVDLKEHGKLFCSHSHYFGDLTAEKHPSRYHLKFRHKLKLRK
RDSRVEPTIGPWIEAALREITIQKKPNGVFYLGLPYALSHGIDNFQIAKRFFSAAKPDKEVINGL
PSEMVVGAADLNLSNIVAPVKARIGKGLEGPLHALDYGYGELIDGPKILTPDGPRCGELISLKRD
IVEIKSAIKEFKACQREGLTMSEETTTWLSEVESPSDSPRCMIQSRIADTSRRLNSFKYQMNKEG
YQDLAEALRLLDAMDSYNSLLESYQRMHLSPGEQSPKEAKFDTKRASFRDLLRRRVAHTIVEYFD
DCDIVFFEDLDGPSDSDSRNNALVKLLSPRTLLLYIRQALEKRGIGMVEVAKDGTSQNNPISGHV
GWRNKQNKSEIYFYEDKELLVMDADEVGAMNILCRGLNHSVCPYSFVTKAPEKKNDEKKEGDYGK

RVKRFLKDRYGSSNVRFLVASMGFVTVTTKRPKDALVGKRLYYHGGELVTHDLHNRMKDEIKYLV
EKEVLARRVSLSDSTIKSYKSFAHV

[0285] Cas12i1

MSNKEKNASETRKAYTTKMIPRSHDRMKLLGNFMDYLMDGTPIFFELWNQFGGGIDRDIISGTAN
KDKISDDLLLAVNWFKVMPINSKPQGVSPSNLANLFQQYSGSEPDIQAQEYFASNFDTEKHQWKD
MRVEYERLLAELQLSRSDMHHDLKLMYKEKCIGLSLSTAHYITSVMFGTGAKNNRQTKHQFYSKV
IQLLEESTQINSVEQLASIILKAGDCDSYRKLRIRCSRKGATPSILKIVQDYELGTNHDDEVNVP
SLIANLKEKLGRFEYECEWKCMEKIKAFLASKVGPYYLGSYSAMLENALSPIKGMTTKNCKFVLK
QIDAKNDIKYENEPFGKIVEGFFDSPYFESDTNVKWVLHPHHIGESNIKTLWEDLNAIHSKYEED
IASLSEDKKEKRIKVYQGDVCQTINTYCEEVGKEAKTPLVQLLRYLYSRKDDIAVDKIIDGITFL
SKKHKVEKQKINPVIQKYPSFNFGNNSKLLGKIISPKDKLKHNLKCNRNQVDNYIWIEIKVLNTK
TMRWEKHHYALSSTRFLEEVYYPATSENPPDALAARFRTKTNGYEGKPALSAEQIEQIRSAPVGL
RKVKKRQMRLEAARQQNLLPRYTWGKDFNINICKRGNNFEVTLATKVKKKKEKNYKVVLGYDANI
VRKNTYAAIEAHANGDGVIDYNDLPVKPIESGFVTVESQVRDKSYDQLSYNGVKLLYCKPHVESR
RSFLEKYRNGTMKDNRGNNIQIDFMKDFEAIADDETSLYYFNMKYCKLLQSSIRNHSSQAKEYRE
EIFELLRDGKLSVLKLSSLSNLSFVMFKVAKSLIGTYFGHLLKKPKNSKSDVKAPPITDEDKQKA
DPEMFALRLALEEKRLNKVKSKKEVIANKIVAKALELRDKYGPVLIKGENISDTTKKGKKSSTNS
FLMDWLARGVANKVKEMVMMHQGLEFVEVNPNFTSHQDPFVHKNPENTFRARYSRCTPSELTEKN
RKEILSFLSDKPSKRPTNAYYNEGAMAFLATYGLKKNDVLGVSLEKFKQIMANILHQRSEDQLLF
PSRGGMFYLATYKLDADATSVNWNGKQFWVCNADLVAAYNVGLVDIQKDFKKK

[0286] Cas12i2

MSSAIKSYKSVLRPNERKNQLLKSTIQCLEDGSAFFFKMLQGLFGGITPEIVRFSTEQEKQQQDI
ALWCAVNWFRPVSQDSLTHTIASDNLVEKFEEYYGGTASDAIKQYFSASIGESYYWNDCRQQYYD
LCRELGVEVSDLTHDLEILCREKCLAVATESNQNNSIISVLFGTGEKEDRSVKLRITKKILEAIS
NLKEIPKNVAPIQEIILNVAKATKETFRQVYAGNLGAPSTLEKFIAKDGQKEFDLKKLQTDLKKV
IRGKSKERDWCCQEELRSYVEQNTIQYDLWAWGEMFNKAHTALKIKSTRNYNFAKQRLEQFKEIQ
SLNNLLVVKKLNDFFDSEFFSGEETYTICVHHLGGKDLSKLYKAWEDDPADPENAIVVLCDDLKN
NFKKEPIRNILRYIFTIRQECSAQDILAAAKYNQQLDRYKSQKANPSVLGNQGFTWTNAVILPEK
AQRNDRPNSLDLRIWLYLKLRHPDGRWKKHHIPFYDTRFFQEIYAAGNSPVDTCQFRTPRFGYHL
PKLTDQTAIRVNKKHVKAAKTEARIRLAIQQGTLPVSNLKITEISATINSKGQVRIPVKFDVGRQ
KGTLQIGDRFCGYDQNQTASHAYSLWEVVKEGQYHKELGCFVRFISSGDIVSITENRGNQFDQLS
YEGLAYPQYADWRKKASKFVSLWQITKKNKKKEIVTVEAKEKFDAICKYQPRLYKFNKEYAYLLR
DIVRGKSLVELQQIRQEIFRFIEQDCGVTRLGSLSLSTLETVKAVKGIIYSYFSTALNASKNNPI
SDEQRKEFDPELFALLEKLELIRTRKKKQKVERIANSLIQTCLENNIKFIRGEGDLSTTNNATKK

KANSRSMDWLARGVFNKIRQLAPMHNITLFGCGSLYTSHQDPLVHRNPDKAMKCRWAAIPVKDIG
DWVLRKLSQNLRAKNIGTGEYYHQGVKEFLSHYELQDLEEELLKWRSDRKSNIPCWVLQNRLAEK
LGNKEAVVYIPVRGGRIYFATHKVATGAVSIVFDQKQVWVCNADHVAAANIALTVKGIGEQSSDE
ENPDGSRIKLQLTS

[0287] Representative nucleic acid and protein sequences of the base editors follow:

BhCas12b GGSGGS-ABE8-Xten20 at P153

GCCACCATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGCCACCAG
ATCCTTCATCCTGAAGATCGAGCCCAACGAGGAAGTGAAGAAAGGCCTCTGGAAAACCCACGAGG
TGCTGAACCACGGAATCGCCTACTACATGAATATCCTGAAGCTGATCCGGCAAGAGGCCATCTAC
GAGCACCACGAGCAGGACCCCAAGAATCCCAAGAAGGTGTCCAAGGCCGAGATCCAGGCCGAGCT
GTGGGATTTCGTGCTGAAGATGCAGAAGTGCAACAGCTTCACACACGAGGTGGACAAGGACGAGG
TGTTCAACATCCTGAGAGAGCTGTACGAGGAACTGGTGCCCAGCAGCGTGGAAAAGAAGGGCGAA
GCCAACCAGCTGAGCAACAAGTTTCTGTACCCTCTGGTGGACCCCAACAGCCAGTCTGGAAAGGG
AACAGCCAGCAGCGGCAGAAAGCCCAGATGGTACAACCTGAAGATTGCCGGCGATCCCggaggct
ctggaggaagcTCCGAAGTCGAGTTTTCCCATGAGTACTGGATGAGACACGCATTGACTCTCGCA
AAGAGGGCTCGAGATGAACGCGAGGTGCCCGTGGGGGCAGTACTCGTGCTCAACAATCGCGTAAT
CGGCGAAGGTTGGAATAGGGCAATCGGACTCCACGACCCCACTGCACATGCGGAAATCATGGCCC
TTCGACAGGGAGGGCTTGTGATGCAGAATTATCGACTTTATGATGCGACGCTGTACGTCACGTTT
GAACCTTGCGTAATGTGCGCGGGAGCTATGATTCACTCCCGCATTGGACGAGTTGTATTCGGTGT
TCGCAACGCCAAGACGGGTGCCGCAGGTTCACTGATGGACGTGCTGCATCATCCAGGCATGAACC
ACCGGGTAGAAATCACAGAAGGCATATTGGCGGACGAATGTGCGGCGCTGTTGTGTCGTTTTTTT
CGCATGCCCAGGCGGGTCTTTAACGCCCAGAAAAAAGCACAATCCTCTACTGACGGCTCTTCTGG
ATCTGAAACACCTGGCACAAGCGAGAGCGCCACCCCTGAGAGCTCTGGCTCCTGGGAAGAAGAGA
AGAAGAAGTGGGAAGAAGATAAGAAAAAGGACCCGCTGGCCAAGATCCTGGGCAAGCTGGCTGAG
TACGGACTGATCCCTCTGTTCATCCCCTACACCGACAGCAACGAGCCCATCGTGAAAGAAATCAA
GTGGATGGAAAAGTCCCGGAACCAGAGCGTGCGGCGGCTGGATAAGGACATGTTCATTCAGGCCC
TGGAACGGTTCCTGAGCTGGGAGAGCTGGAACCTGAAAGTGAAAGAGGAATACGAGAAGGTCGAG
AAAGAGTACAAGACCCTGGAAGAGAGGATCAAAGAGGACATCCAGGCTCTGAAGGCTCTGGAACA
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TCTGGAACAGTATGAGAAAGAGCGGCAAGAACAGCTGCTGCGGGACACCCTGAACACCAACGAGT
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GAGAACGAGCCCTCCGAGAAGTACCTGGAAGTGTTCAAGGACTACCAGCGGAAGCACCCTAGAGA
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AAGCGGCAGCAACCTGAACAAGTACAGAATCCTGACCGAGCAGCTGCACACCGAGAAGCTGAAGA
AAAAGCTGACAGTGCAGCTGGACCGGCTGATCTACCCTACAGAATCTGGCGGCTGGGAAGAGAAG
GGCAAAGTGGACATTGTGCTGCTGCCCAGCCGGCAGTTCTACAACCAGATCTTCCTGGACATCGA
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TCGGCGGAGCCAGAGTGCAGTTCGACAGAGATCACCTGAGAAGATACCCTCACAAGGTGGAAAGC
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CAAGTCTCTGAAGATCCACCGGGACGACTTCCCCAAGGTGGTCAACTTCAAGCCCAAAGAACTGA
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ACTGACCCTGGACAAAATCGCCGTGCTGAAAGAGGGCGATCTGTACCCAGACAAAGGCGGCGAGA
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TCCAGGCATGAACCACCGGGTAGAAATCACAGAAGGCATATTGGCGGACGAATGTGCGGCGCTGT
TGTGTCGTTTTTTTCGCATGCCCAGGCGGGTCTTTAACGCCCAGAAAAAAGCACAATCCTCTACT
GACGGCTCTTCTGGATCTGAAACACCTGGCACAAGCGAGAGCGCCACCCCTGAGAGCTCTGGCGG
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GGCCTCCGAGCTGAAAGGCGAAAAGCTGATGCTGTACAGGGACCCCAGCGGCAATGTGTTCCCCA
GCGACAAATGGATGGCCGCTGGCGTGTTCTTCGGAAAGCTGGAACGCATCCTGATCAGCAAGCTG
ACCAACCAGTACTCCATCAGCACCATCGAGGACGACAGCAGCAAGCAGTCTATGAAAAGGCCGGC
GGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAGGGATCC*TACCCATACGATGTTCCAGATT*
*ACGCTTATCCCTACGACGTGCCTGATTATGCATACCCATATGATGTCCCCGACTATGCC*TAA

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QKRFWTRTHGFYKVYCKAYQVDGGSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNN
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FGVRNAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDG
SSGSETPGTSESATPESSGGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIKKGS
SKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISKLTN
QYSISTIEDDSSKQSMKRPAATKKAGQAKKKKGSYPYDVPDYAYPYDVPDYAYPYDVPDYA

BhCas12b GGSGGS-ABE8-Xten20 at K1019

GCCACCATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCGCCACCAG
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GAGCACCACGAGCAGGACCCCAAGAATCCCAAGAAGGTGTCCAAGGCCGAGATCCAGGCCGAGCT
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AAGCGGCAGCAACCTGAACAAGTACAGAATCCTGACCGAGCAGCTGCACACCGAGAAGCTGAAGA
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CTTCGACAGGGAGGGCTTGTGATGCAGAATTATCGACTTTATGATGCGACGCTGTACGTCACGTT
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GGCCTCCGAGCTGAAAGGCGAAAAGCTGATGCTGTACAGGGACCCCAGCGGCAATGTGTTCCCCA
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*ACGCTTATCCCTACGACGTGCCTGATTATGCATACCCATATGATGTCCCCGACTATGCC*TAA

MAPKKKRKVGIHGVPAAATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAIYEH
HEQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILRELYEELVPSSVEKKGEAN
QLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEEEKKKWEEDKKKDPLAKILGKLA
EYGLIPLFIPYTDSNEPIVKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNLKVKEEYEKV
EKEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLRGWREIIQKWLKMDEN
EPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNHPEYPYLYATFCEIDKKKKDAKQQ
ATFTLADPINHPLWVRFEERSGSNLNKYRILTEQLHTEKLKKKLTVQLDRLIYPTESGGWEEKGK
VDIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGTLGGARVQFDRDHLRRYPHKVESGN
VGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKPKELTEWIKDSKGKKLKSGIESLEIGLR
VMSIDLGQRQAAAASIFEVVDQKPDIEGKLFFPIKGTELYAVHRASFNIKLPGETLVKSREVLRK
AREDNLKLMNQKLNFLRNVLHFQQFEDITEREKRVTKWISRQENSDVPLVYQDELIQIRELMYKP
YKDWVAFLKQLHKRLEVEIGKEVKHWRKSLSDGRKGLYGISLKNIDEIDRTRKFLLRWSLRPTEP
GEVRRLEPGQRFAIDQLNHLNALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFED
LSNYNPYEERSRFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSV
VTKEKLQDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLSKDRKCVTTHADINAAQNL
QKRFWTRTHGFYKVYCKAYQVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKGGSG
GSSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALR
QGGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHR
VEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDGSSGSETPGTSESATPESSGLKIKKGS
SKQSSSELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFGKLERILISKLTN
QYSISTIEDDSSKQSMKRPAATKKAGQAKKKKGSYPYDVPDYAYPYDVPDYAYPYDVPDYA

[0288] For the sequences above, the Kozak sequence is bolded and underlined; ..... marks the N-terminal nuclear localization signal (NLS); lower case characters denote the GGGSGGS

linker; _ _ _ _ _marks the sequence encoding ABE8, unmodified sequence encodes BhCas12b; double underling denotes the Xten20 linker; single underlining denotes the C-terminal NLS; GGATCC denotes the GS linker; and italicized characters represent the coding sequence of the 3x hemagglutinin (HA) tag.

[0289] 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.

[0290] In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SaCas9 domain, the SaCas9d domain, or the SaCas9n domain can bind to a nucleic acid sequence having a NNGRRT or a NNNRRT 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.

[0291] 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.

[0292] Exemplary SaCas9 sequence

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQR
VKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVEEDTGNE
LSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQS
FIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALND
LNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNL
KVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTG
THNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQ
SIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLI
EKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEE**N**SKKGNR

TPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRY
ATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIFK
EWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNR
ELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQ
YGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKP
YRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKING
ELYRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEV
KSKKHPQIIKKG

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

[0293] Exemplary SaCas9n sequence

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQR
VKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVEEDTGNE
LSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQS
FIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALND
LNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNL
KVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTG
THNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQ
SIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLI
EKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEE<u>A</u>SKKGNR
TPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRY
ATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIFK
EWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNR
ELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQ
YGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKP
YRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFYNNDLIKING
ELYRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEV
KSKKHPQIIKKG

[0294] Residue A579 above, which can be mutated from N579 to yield a SaCas9 nickase, is underlined and in bold.

[0295] Exemplary SaKKH Cas9

KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQR
VKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVEEDTGNE

LSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQS
FIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALND
LNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNL
KVYHDIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTG
THNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQ
SIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLI
EKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKVLVKQEE**A**SKKGNR
TPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRY
ATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIFK
EWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNR
*K*LINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQ
YGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLSLKP
YRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQAEFIASFY*K*NDLIKING
ELYRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRPP*H*IIKTIASKTQSIKKYSTDILGNLYEV
KSKKHPQIIKKG.

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.

[0296] A polynucleotide programmable nucleotide binding domain of a base editor can itself comprise one or more domains. For example, a polynucleotide programmable nucleotide binding domain can comprise one or more nuclease domains. In some embodiments, the nuclease domain of a polynucleotide programmable nucleotide binding domain can comprise an endonuclease or an exonuclease. Herein the term "exonuclease" refers to a protein or polypeptide capable of digesting a nucleic acid (*e.g.*, RNA or DNA) from free ends, and the term "endonuclease" refers to a protein or polypeptide capable of catalyzing (*e.g.*, cleaving) internal regions in a nucleic acid (*e.g.*, DNA or RNA). In some embodiments, an endonuclease can cleave a single strand of a double-stranded nucleic acid. In some embodiments, an endonuclease can cleave both strands of a double-stranded nucleic acid molecule. In some embodiments a polynucleotide programmable nucleotide binding domain can be a deoxyribonuclease. In some embodiments a polynucleotide programmable nucleotide binding domain can be a ribonuclease.

[0297] In some embodiments, a nuclease domain of a polynucleotide programmable nucleotide binding domain can cut zero, one, or two strands of a target polynucleotide. In some embodiments, the polynucleotide programmable nucleotide binding domain can comprise a

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

[0298] A base editor comprising a polynucleotide programmable nucleotide binding domain comprising a nickase domain is thus able to generate a single-strand DNA break (nick) at a specific polynucleotide target sequence (*e.g.*, determined by the complementary sequence of a bound guide nucleic acid). In some embodiments, the strand of a nucleic acid duplex target polynucleotide sequence that is cleaved by a base editor comprising a nickase domain (*e.g.*, Cas9-derived nickase domain) is the strand that is not edited by the base editor (*i.e.*, the strand that is cleaved by the base editor is opposite to a strand comprising a base to be edited). In other embodiments, a base editor comprising a nickase domain (*e.g.*, Cas9-derived nickase domain) can cleave the strand of a DNA molecule which is being targeted for editing. In such embodiments, the non-targeted strand is not cleaved.

[0299] Also provided herein are base editors comprising a polynucleotide programmable nucleotide binding domain which is catalytically dead (*i.e.*, incapable of cleaving a target polynucleotide sequence). Herein the terms "catalytically dead" and "nuclease dead" are used interchangeably to refer to a polynucleotide programmable nucleotide binding domain which has one or more mutations and/or deletions resulting in its inability to cleave a strand of a nucleic acid. In some embodiments, a catalytically dead polynucleotide programmable nucleotide

binding domain base editor can lack nuclease activity as a result of specific point mutations in one or more nuclease domains. For example, in the case of a base editor comprising a Cas9 domain, the Cas9 can comprise both a D10A mutation and an H840A mutation. Such mutations inactivate both nuclease domains, thereby resulting in the loss of nuclease activity. In other embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain can comprise one or more deletions of all or a portion of a catalytic domain (*e.g.*, RuvC1 and/or HNH domains). In further embodiments, a catalytically dead polynucleotide programmable nucleotide binding domain comprises a point mutation (*e.g.*, D10A or H840A) as well as a deletion of all or a portion of a nuclease domain.

[0300] Also contemplated herein are mutations capable of generating a catalytically dead polynucleotide programmable nucleotide binding domain from a previously functional version of the polynucleotide programmable nucleotide binding domain. For example, in the case of catalytically dead Cas9 ("dCas9"), variants having mutations other than D10A and H840A are provided, which result in nuclease inactivated Cas9. Such mutations, by way of example, include other amino acid substitutions at D10 and H840, or other substitutions within the nuclease domains of Cas9 (*e.g.*, substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain).

[0301] Additional suitable nuclease-inactive dCas9 domains can be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure. Such additional exemplary suitable nuclease-inactive Cas9 domains include, but are not limited to, D10A/H840A, D10A/D839A/H840A, and D10A/D839A/H840A/N863A mutant domains (See, *e.g.*, Prashant *et al.*, CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature Biotechnology*. 2013; 31(9): 833-838, the entire contents of which are incorporated herein by reference). 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.

[0302] Non-limiting examples of a polynucleotide programmable nucleotide binding domain which can be incorporated into a base editor include a CRISPR protein-derived domain, a restriction nuclease, a meganuclease, TAL nuclease (TALEN), and a zinc finger nuclease (ZFN). In some embodiments, a base editor comprises a polynucleotide programmable nucleotide binding domain comprising a natural or modified protein or portion thereof which via a bound guide nucleic acid is capable of binding to a nucleic acid sequence during CRISPR (*i.e.*, Clustered Regularly Interspaced Short Palindromic Repeats)-mediated modification of a nucleic acid. Such a protein is referred to herein as a "CRISPR protein." Accordingly, disclosed herein is a base editor comprising a polynucleotide programmable nucleotide binding domain comprising all or a portion of a CRISPR protein (*i.e.* a base editor comprising as a domain all or a portion of a CRISPR protein, also referred to as a "CRISPR protein-derived domain" of the base editor). A CRISPR protein-derived domain incorporated into a base editor can be modified compared to a wild-type or natural version of the CRISPR protein. For example, as described below a CRISPR protein-derived domain can comprise one or more mutations, insertions, deletions, rearrangements and/or recombinations relative to a wild-type or natural version of the CRISPR protein.

[0303] In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is an endonuclease (*e.g.*, deoxyribonuclease or ribonuclease) capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a nickase capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a CRISPR protein-derived domain incorporated into a base editor is a catalytically dead domain capable of binding a target polynucleotide when in conjunction with a bound guide nucleic acid. In some embodiments, a target polynucleotide bound by a CRISPR protein derived domain of a base editor is DNA. In some embodiments, a target polynucleotide bound by a CRISPR protein-derived domain of a base editor is RNA.

[0304] In some embodiments, a CRISPR protein-derived domain of a base editor can include all or a portion of Cas9 from *Corynebacterium ulcerans* (NCBI Refs: NC_015683.1, NC_017317.1); *Corynebacterium diphtheria* (NCBI Refs: NC_016782.1, NC_016786.1);

Spiroplasma syrphidicola (NCBI Ref: NC_021284.1); *Prevotella intermedia* (NCBI Ref: NC_017861.1); *Spiroplasma taiwanense* (NCBI Ref: NC_021846.1); *Streptococcus iniae* (NCBI Ref: NC_021314.1); *Belliella baltica* (NCBI Ref: NC_018010.1); *Psychroflexus torquis* (NCBI Ref: NC_018721.1); *Streptococcus thermophilus* (NCBI Ref: YP_820832.1); *Listeria innocua* (NCBI Ref: NP_472073.1); *Campylobacter jejuni* (NCBI Ref: YP_002344900.1); *Neisseria meningitidis* (NCBI Ref: YP_002342100.1), *Streptococcus pyogenes*, or *Staphylococcus aureus*.

[0305] In some embodiments, a Cas9-derived domain of a base editor is a Cas9 domain from *Staphylococcus aureus* (SaCas9). In some embodiments, the SaCas9 domain is a nuclease active SaCas9, a nuclease inactive SaCas9 (SaCas9d), or a SaCas9 nickase (SaCas9n). In some embodiments, the SaCas9 domain comprises a N579X mutation. In some embodiments, the SaCas9 domain comprises a N579A mutation. 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.

[0306] A base editor can comprise a domain derived from all or a portion of a Cas9 that is a high fidelity Cas9. In some embodiments, high fidelity Cas9 domains of a base editor are engineered Cas9 domains comprising one or more mutations that decrease electrostatic interactions between the Cas9 domain and the sugar-phosphate backbone of a DNA, relative to a corresponding wild-type Cas9 domain. High fidelity Cas9 domains that have decreased electrostatic interactions with the sugar-phosphate backbone of DNA can have less off-target effects. In some embodiments, the Cas9 domain (*e.g.*, a wild type Cas9 domain) comprises one or more mutations that decrease the association between the Cas9 domain and the sugar-phosphate backbone of a DNA. In some embodiments, a Cas9 domain comprises one or more mutations that decreases the association between the Cas9 domain and the sugar-phosphate backbone of DNA by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or more.

[0307] In some embodiments, the modified Cas9 is a high fidelity Cas9 enzyme. In some embodiments, the high fidelity Cas9 enzyme is SpCas9 (K855A), eSpCas9(1.1), SpCas9-HF1, or hyper accurate Cas9 variant (HypaCas9). The modified Cas9 eSpCas9(1.1) contains alanine substitutions that weaken the interactions between the HNH/RuvC groove and the non-target

DNA strand, preventing strand separation and cutting at off-target sites. Similarly, SpCas9-HF1 lowers off-target editing through alanine substitutions that disrupt Cas9's interactions with the DNA phosphate backbone. HypaCas9 contains mutations (SpCas9 N692A/M694A/Q695A/H698A) in the REC3 domain that increase Cas9 proofreading and target discrimination. All three high fidelity enzymes generate less off-target editing than wildtype Cas9. An exemplary high fidelity Cas9 is provided below.

[0308] High Fidelity Cas9 domain mutations relative to Cas9 are shown in bold and underlines

MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT**A**FDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWG**A**LSRKLINGIRDKQSGKTILDFLKSDGFANRNFM**A**LIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETR**A**ITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

Guide Polynucleotides

[0309] In an embodiment, the guide polynucleotide is a guide RNA. As used herein, the term "guide RNA (gRNA)" and its grammatical equivalents can refer to an RNA which can be specific

for a target DNA and can form a complex with Cas protein. An RNA/Cas complex can assist in "guiding" Cas protein to a target DNA. Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA" or simply "gRNA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. See, *e.g.*, Jinek M. *et al.*, Science 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. Cas9 nuclease sequences and structures are well known to those of skill in the art (see *e.g.*, "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti, J.J. *et al.*, Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E. *et al.*, Nature 471:602-607(2011); and "Programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M.*et al*, Science 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and S. *thermophilus*. Additional suitable Cas9 nucleases and sequences can be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 nuclease has an inactive (*e.g.*, an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase.

[0310] In some embodiments, the guide polynucleotide is at least one single guide RNA ("sgRNA" or "gRNA"). In some embodiments, the guide polynucleotide is at least one tracrRNA. In some embodiments, the guide polynucleotide does not require PAM sequence to guide the polynucleotide-programmable DNA-binding domain (*e.g.*, Cas9 or Cpf1) to the target nucleotide sequence.

[0311] The polynucleotide programmable nucleotide binding domain (*e.g.*, a CRISPR-derived domain) of the base editors disclosed herein can recognize a target polynucleotide sequence by associating with a guide polynucleotide. A guide polynucleotide (*e.g.*, gRNA) is typically single-stranded and can be programmed to site-specifically bind (*i.e.*, via complementary base pairing) to a target sequence of a polynucleotide, thereby directing a base editor that is in conjunction with

the guide nucleic acid to the target sequence. A guide polynucleotide can be DNA. A guide polynucleotide can be RNA. As will be appreciated by one having skill in the art, in a guide polynucleotide sequence uracil (U) replaces thymine (T) in the sequence. In some embodiments, the guide polynucleotide comprises natural nucleotides (*e.g.*, adenosine). In some embodiments, the guide polynucleotide comprises non-natural (or unnatural) nucleotides (*e.g.*, peptide nucleic acid or nucleotide analogs). In some embodiments, the targeting region of a guide nucleic acid sequence can be at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. A targeting region of a guide nucleic acid can be between 10-30 nucleotides in length, or between 15-25 nucleotides in length, or between 15-20 nucleotides in length. In some embodiments, a guide polynucleotide may be truncated by 1, 2, 3, 4, etc. nucleotides, particularly at the 5′ end. By way of nonlimiting example, a guide polynucleotide of 20 nucleotides in length may be truncated by 1, 2, 3, 4, etc. nucleotides, particularly at the 5′ end.

[0312] In some embodiments, a guide polynucleotide comprises two or more individual polynucleotides, which can interact with one another via for example complementary base pairing (*e.g.*, a dual guide polynucleotide). For example, a guide polynucleotide can comprise a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA). For example, a guide polynucleotide can comprise one or more trans-activating CRISPR RNA (tracrRNA).

[0313] In type II CRISPR systems, targeting of a nucleic acid by a CRISPR protein (*e.g.*, Cas9) typically requires complementary base pairing between a first RNA molecule (crRNA) comprising a sequence that recognizes the target sequence and a second RNA molecule (trRNA) comprising repeat sequences which forms a scaffold region that stabilizes the guide RNA-CRISPR protein complex. Such dual guide RNA systems can be employed as a guide polynucleotide to direct the base editors disclosed herein to a target polynucleotide sequence.

[0314] In some embodiments, the base editor provided herein utilizes a single guide polynucleotide (*e.g.*, sgRNA). In some embodiments, the base editor provided herein utilizes a dual guide polynucleotide (*e.g.*, dual gRNAs). In some embodiments, the base editor provided herein utilizes one or more guide polynucleotide (*e.g.*, multiple gRNA). In some embodiments, a single guide polynucleotide is utilized for different base editors described herein. For example, a single guide polynucleotide can be utilized for an adenosine base editor.

[0315] In other embodiments, a guide polynucleotide can comprise both the polynucleotide targeting portion of the nucleic acid and the scaffold portion of the nucleic acid in a single molecule (*i.e.*, a single-molecule guide nucleic acid). For example, a single-molecule guide polynucleotide can be a single guide RNA (sgRNA or gRNA). Herein the term guide

polynucleotide sequence contemplates any single, dual or multi-molecule nucleic acid capable of interacting with and directing a base editor to a target polynucleotide sequence.

[0316] Typically, a guide polynucleotide (*e.g.*, crRNA/trRNA complex or a gRNA) comprises a "polynucleotide-targeting segment" that includes a sequence capable of recognizing and binding to a target polynucleotide sequence, and a "protein-binding segment" that stabilizes the guide polynucleotide within a polynucleotide programmable nucleotide binding domain component of a base editor. In some embodiments, the polynucleotide targeting segment of the guide polynucleotide recognizes and binds to a DNA polynucleotide, thereby facilitating the editing of a base in DNA. In other embodiments, the polynucleotide targeting segment of the guide polynucleotide recognizes and binds to an RNA polynucleotide, thereby facilitating the editing of a base in RNA. Herein a "segment" refers to a section or region of a molecule, *e.g.*, a contiguous stretch of nucleotides in the guide polynucleotide. A segment can also refer to a region/section of a complex such that a segment can comprise regions of more than one molecule. For example, where a guide polynucleotide comprises multiple nucleic acid molecules, the protein-binding segment of can include all or a portion of multiple separate molecules that are for instance hybridized along a region of complementarity. In some embodiments, a protein-binding segment of a DNA-targeting RNA that comprises two separate molecules can comprise (i) base pairs 40-75 of a first RNA molecule that is 100 base pairs in length; and (ii) base pairs 10-25 of a second RNA molecule that is 50 base pairs in length. The definition of "segment," unless otherwise specifically defined in a particular context, is not limited to a specific number of total base pairs, is not limited to any particular number of base pairs from a given RNA molecule, is not limited to a particular number of separate molecules within a complex, and can include regions of RNA molecules that are of any total length and can include regions with complementarity to other molecules.

[0317] A guide RNA or a guide polynucleotide can comprise two or more RNAs, *e.g.*, CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA). A guide RNA or a guide polynucleotide can sometimes comprise a single-chain RNA, or single guide RNA (sgRNA) formed by fusion of a portion (*e.g.*, a functional portion) of crRNA and tracrRNA. A guide RNA or a guide polynucleotide can also be a dual RNA comprising a crRNA and a tracrRNA. Furthermore, a crRNA can hybridize with a target DNA.

[0318] As discussed above, a guide RNA or a guide polynucleotide can be an expression product. For example, a DNA that encodes a guide RNA can be a vector comprising a sequence coding for the guide RNA. A guide RNA or a guide polynucleotide can be transferred into a cell

by transfecting the cell with an isolated guide RNA or plasmid DNA comprising a sequence coding for the guide RNA and a promoter. A guide RNA or a guide polynucleotide can also be transferred into a cell in other way, such as using virus-mediated gene delivery.

[0319] A guide RNA or a guide polynucleotide can be isolated. For example, a guide RNA can be transfected in the form of an isolated RNA into a cell or organism. A guide RNA can be prepared by *in vitro* transcription using any *in vitro* transcription system known in the art. A guide RNA can be transferred to a cell in the form of isolated RNA rather than in the form of plasmid comprising encoding sequence for a guide RNA.

[0320] A guide RNA or a guide polynucleotide can comprise three regions: a first region at the 5′ end that can be complementary to a target site in a chromosomal sequence, a second internal region that can form a stem loop structure, and a third 3′ region that can be single-stranded. A first region of each guide RNA can also be different such that each guide RNA guides a fusion protein to a specific target site. Further, second and third regions of each guide RNA can be identical in all guide RNAs.

[0321] A first region of a guide RNA or a guide polynucleotide can be complementary to sequence at a target site in a chromosomal sequence such that the first region of the guide RNA can base pair with the target site. In some embodiments, a first region of a guide RNA can comprise from or from about 10 nucleotides to 25 nucleotides (*i.e.*, from 10 nucleotides to nucleotides; or from about 10 nucleotides to about 25 nucleotides; or from 10 nucleotides to about 25 nucleotides; or from about 10 nucleotides to 25 nucleotides) or more. For example, a region of base pairing between a first region of a guide RNA and a target site in a chromosomal sequence can be or can be about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, or more nucleotides in length. In some embodiments, a first region of a guide RNA can be or can be about 19, 20, or 21 nucleotides in length.

[0322] A guide RNA or a guide polynucleotide can also comprise a second region that forms a secondary structure. For example, a secondary structure formed by a guide RNA can comprise a stem (or hairpin) and a loop. A length of a loop and a stem can vary. For example, a loop can range from or from about 3 to 10 nucleotides in length, and a stem can range from or from about 6 to 20 base pairs in length. A stem can comprise one or more bulges of 1 to 10 or about 10 nucleotides. The overall length of a second region can range from or from about 16 to 60 nucleotides in length. For example, a loop can be or can be about 4 nucleotides in length and a stem can be or can be about 12 base pairs.

[0323] A guide RNA or a guide polynucleotide can also comprise a third region at the 3′ end that can be essentially single-stranded. For example, a third region is sometimes not complementarity to any chromosomal sequence in a cell of interest and is sometimes not complementarity to the rest of a guide RNA. Further, the length of a third region can vary. A third region can be more than or more than about 4 nucleotides in length. For example, the length of a third region can range from or from about 5 to 60 nucleotides in length.

[0324] A guide RNA or a guide polynucleotide can target any exon or intron of a gene target. In some embodiments, a guide can target exon 1 or 2 of a gene; in other embodiments, a guide can target exon 3 or 4 of a gene. A composition can comprise multiple guide RNAs that all target the same exon, or in some embodiments, multiple guide RNAs that can target different exons. An exon and an intron of a gene can be targeted.

[0325] A guide RNA or a guide polynucleotide can target a nucleic acid sequence of about 20 nucleotides. A target nucleic acid can be less than about 20 nucleotides. A target nucleic acid can be at least or at least about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, or anywhere between 1-100 nucleotides in length. A target nucleic acid can be at most or at most about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, or anywhere between 1-100 nucleotides in length. A target nucleic acid sequence can be or can be about 20 bases immediately 5′ of the first nucleotide of the PAM. A guide RNA can target a nucleic acid sequence. A target nucleic acid can be at least or at least about 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, or 1-100 nucleotides.

[0326] A guide polynucleotide, for example, a guide RNA, can refer to a nucleic acid that can hybridize to another nucleic acid, for example, the target nucleic acid or protospacer in a genome of a cell. A guide polynucleotide can be RNA. A guide polynucleotide can be DNA. The guide polynucleotide can be programmed or designed to bind to a sequence of nucleic acid site-specifically. A guide polynucleotide can comprise a polynucleotide chain and can be called a single guide polynucleotide. A guide polynucleotide can comprise two polynucleotide chains and can be called a double guide polynucleotide. A guide RNA can be introduced into a cell or embryo as an RNA molecule. For example, a RNA molecule can be transcribed *in vitro* and/or can be chemically synthesized. An RNA can be transcribed from a synthetic DNA molecule, *e.g.*, a gBlocks® gene fragment. A guide RNA can then be introduced into a cell or embryo as an RNA molecule. A guide RNA can also be introduced into a cell or embryo in the form of a non-RNA nucleic acid molecule, *e.g.*, a DNA molecule. For example, a DNA encoding a guide RNA can be operably linked to promoter control sequence for expression of the guide RNA in a cell or

embryo of interest. A RNA coding sequence can be operably linked to a promoter sequence that is recognized by RNA polymerase III (Pol III). Plasmid vectors that can be used to express guide RNA include, but are not limited to, px330 vectors and px333 vectors. In some embodiments, a plasmid vector (*e.g.*, px333 vector) can comprise at least two guide RNA-encoding DNA sequences.

[0327] Methods for selecting, designing, and validating guide polynucleotides, *e.g.*, guide RNAs and targeting sequences, are described herein and known to those skilled in the art. For example, to minimize the impact of potential substrate promiscuity of a deaminase domain in the nucleobase editor system (*e.g.*, an AID domain), the number of residues that could unintentionally be targeted for deamination (*e.g.*, off-target C residues that could potentially reside on ssDNA within the target nucleic acid locus) may be minimized. In addition, software tools can be used to optimize the gRNAs corresponding to a target nucleic acid sequence, *e.g.*, to minimize total off-target activity across the genome. For example, for each possible targeting domain choice using *S. pyogenes* Cas9, all off-target sequences (preceding selected PAMs, *e.g.*, NAG or NGG) may be identified across the genome that contain up to certain number (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of mismatched base-pairs. First regions of gRNAs complementary to a target site can be identified, and all first regions (*e.g.*, crRNAs) can be ranked according to its total predicted off-target score; the top-ranked targeting domains represent those that are likely to have the greatest on-target and the least off-target activity. Candidate targeting gRNAs can be functionally evaluated by using methods known in the art and/or as set forth herein.

[0328] As a non-limiting example, target DNA hybridizing sequences in crRNAs of a guide RNA for use with Cas9s may be identified using a DNA sequence searching algorithm. gRNA design may be carried out using custom gRNA design software based on the public tool cas-offinder as described in Bae S., Park J., & Kim J.-S. Cas-OFFinder: A fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases, Bioinformatics 30, 1473-1475 (2014). This software scores guides after calculating their genome-wide off-target propensity. Typically matches ranging from perfect matches to 7 mismatches are considered for guides ranging in length from 17 to 24. Once the off-target sites are computationally-determined, an aggregate score is calculated for each guide and summarized in a tabular output using a web-interface. In addition to identifying potential target sites adjacent to PAM sequences, the software also identifies all PAM adjacent sequences that differ by 1, 2, 3, or more than 3 nucleotides from the selected target sites. Genomic DNA sequences for a target nucleic acid sequence, *e.g.*, a target gene may be obtained and repeat elements may be screened

using publicly available tools, for example, the RepeatMasker program. RepeatMasker searches input DNA sequences for repeated elements and regions of low complexity. The output is a detailed annotation of the repeats present in a given query sequence.

[0329] Following identification, first regions of guide RNAs, *e.g.*, crRNAs, may be ranked into tiers based on their distance to the target site, their orthogonality and presence of 5′ nucleotides for close matches with relevant PAM sequences (for example, a 5′ G based on identification of close matches in the human genome containing a relevant PAM *e.g.*, NGG PAM for *S. pyogenes*, NNGRRT or NNGRRV PAM for *S. aureus*). As used herein, orthogonality refers to the number of sequences in the human genome that contain a minimum number of mismatches to the target sequence. A "high level of orthogonality" or "good orthogonality" may, for example, refer to 20-mer targeting domains that have no identical sequences in the human genome besides the intended target, nor any sequences that contain one or two mismatches in the target sequence. Targeting domains with good orthogonality may be selected to minimize off-target DNA cleavage.

[0330] In some embodiments, a reporter system may be used for detecting base-editing activity and testing candidate guide polynucleotides. In some embodiments, a reporter system may comprise a reporter gene based assay where base editing activity leads to expression of the reporter gene. For example, a reporter system may include a reporter gene comprising a deactivated start codon, *e.g.*, a mutation on the template strand from 3′-TAC-5' to 3′-CAC-5′. Upon successful deamination of the target C, the corresponding mRNA will be transcribed as 5′-AUG-3′ instead of 5'-GUG-3′, enabling the translation of the reporter gene. Suitable reporter genes will be apparent to those of skill in the art. Non-limiting examples of reporter genes include gene encoding green fluorescence protein (GFP), red fluorescence protein (RFP), luciferase, secreted alkaline phosphatase (SEAP), or any other gene whose expression are detectable and apparent to those skilled in the art. The reporter system can be used to test many different gRNAs, *e.g.*, in order to determine which nucleotide residue(s) with respect to the target DNA sequence the respective deaminase will target. sgRNAs that target non-template strand nucleotide residues can also be tested in order to assess off-target effects of a specific base editing protein, *e.g.*, a Cas9 deaminase fusion protein. In some embodiments, such gRNAs can be designed so that the mutated start codon will not be base-paired with the gRNA. The guide polynucleotides can comprise standard nucleotides, modified nucleotides (*e.g.*, pseudouridine), nucleotide isomers, and/or nucleotide analogs. In some embodiments, the guide polynucleotide can comprise at least one detectable label. The detectable label can be a fluorophore (*e.g.*, FAM,

TMR, Cy3, Cy5, Texas Red, Oregon Green, Alexa Fluors, Halo tags, or any other suitable fluorescent dye), a detection tag (*e.g.*, biotin, digoxigenin, and the like), quantum dots, or gold particles.

[0331] The guide polynucleotides can be synthesized chemically and/or enzymatically. For example, the guide RNA can be synthesized using standard phosphoramidite-based solid-phase synthesis methods. Alternatively, the guide RNA can be synthesized in vitro by operably linking DNA encoding the guide RNA to a promoter control sequence that is recognized by a phage RNA polymerase. Examples of suitable phage promoter sequences include T7, T3, SP6 promoter sequences, or variations thereof. In embodiments in which the guide RNA comprises two separate molecules (*e.g.*., crRNA and tracrRNA), the crRNA can be chemically synthesized and the tracrRNA can be enzymatically synthesized.

[0332] In some embodiments, a base editor system may comprise multiple guide polynucleotides, *e.g.*, gRNAs. For example, the gRNAs may target the base editor to one or more target loci (*e.g.*, at least one (1) gRNA, at least 2 gRNA, at least 5 gRNA, at least 10 gRNA, at least 20 gRNA, at least 30 g RNA, or at least 50 gRNA). In some embodiments, multiple gRNA sequences can be tandemly arranged iand are preferably separated by a direct repeat.

[0333] A DNA sequence encoding a guide RNA or a guide polynucleotide can also be part of a vector. In some embodiments, a vector comprises additional expression control sequences (*e.g.*, enhancer sequences, Kozak sequences, polyadenylation sequences, transcriptional termination sequences, etc.), selectable marker sequences (*e.g.*, GFP or antibiotic resistance genes such as puromycin), origins of replication, and the like. A DNA molecule encoding a guide RNA or guide polynucleotide can also be linear or circular.

[0334] In some embodiments, one or more components of a base editor system may be encoded by DNA sequences. Such DNA sequences may be introduced into an expression system, *e.g.*, a cell, together or separately. For example, DNA sequences encoding a polynucleotide programmable nucleotide binding domain and a guide RNA may be introduced into a cell, each DNA sequence can be part of a separate molecule (*e.g.*, one vector containing the polynucleotide programmable nucleotide binding domain coding sequence and a second vector containing the guide RNA coding sequence) or both can be part of a same molecule (*e.g.*, one vector containing coding (and regulatory) sequence for both the polynucleotide programmable nucleotide binding domain and the guide RNA).

[0335] A guide polynucleotide can comprise one or more modifications to provide a nucleic acid with a new or enhanced feature. A guide polynucleotide can comprise a nucleic acid affinity

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

[0336] In some embodiments, a gRNA or a guide polynucleotide can comprise modifications. A modification can be made at any location of a gRNA or a guide polynucleotide. More than one modification can be made to a single gRNA or guide polynucleotide. A gRNA or a guide polynucleotide can undergo quality control after a modification. In some embodiments, quality control can include PAGE, HPLC, MS, or any combination thereof.

[0337] A modification of a gRNA or a guide polynucleotide can be a substitution, insertion, deletion, chemical modification, physical modification, stabilization, purification, or any combination thereof.

[0338] A gRNA or a guide polynucleotide can also be modified by 5′adenylate, 5′guanosine-triphosphate cap, 5′N7-Methylguanosine-triphosphate cap, 5′triphosphate cap, 3′phosphate, 3′thiophosphate, 5′phosphate, 5′thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC spacer, rSpacer, Spacer 18, Spacer 9,3′-3′ modifications, 5′-5′ modifications, abasic, acridine, azobenzene, biotin, biotin BB, biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3′DABCYL, black hole quencher 1, black hole quencer 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2'-deoxyribonucleoside analog purine, 2'-deoxyribonucleoside analog pyrimidine, ribonucleoside analog, 2'-O-methyl ribonucleoside analog, sugar modified analogs, wobble/universal bases, fluorescent dye label, 2'-fluoro RNA, 2'-O-methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5′-triphosphate, 5′-methylcytidine-5′-triphosphate, or any combination thereof.

[0339] In some embodiments, a modification is permanent. In other embodiments, a modification is transient. In some embodiments, multiple modifications are made to a gRNA or guide polynucleotide. A gRNA or guide polynucleotide modification can alter physiochemical properties of a nucleotide, such as their conformation, polarity, hydrophobicity, chemical reactivity, base-pairing interactions, or any combination thereof.

[0340] A modification can also be a phosphorothioate substitute. In some embodiments, a natural phosphodiester bond can be susceptible to rapid degradation by cellular nucleases and; a modification of internucleotide linkage using phosphorothioate (PS) bond substitutes can be more stable towards hydrolysis by cellular degradation. A modification can increase stability in a gRNA or a guide polynucleotide. A modification can also enhance biological activity. In some

embodiments, a phosphorothioate enhanced RNA gRNA can inhibit RNase A, RNase T1, calf serum nucleases, or any combinations thereof. These properties can allow the use of PS-RNA gRNAs to be used in applications where exposure to nucleases is of high probability *in vivo* or *in vitro*. For example, phosphorothioate (PS) bonds can be introduced between the l3-5 nucleotides at the 5′- or 3′-end of a gRNA which can inhibit exonuclease degradation. In some embodiments, phosphorothioate bonds can be added throughout an entire gRNA to reduce attack by endonucleases.

Protospacer Adjacent Motif

[0341] 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. The PAM sequence can be any PAM sequence known in the art. Suitable PAM sequences include, but are not limited to, NGG, NGA, NGC, NGN, NGT, NGTT, NGCG, NGAG, NGAN, NGNG, NGCN, NGCG, NGTN, NNGRRT, NNNRRT, NNGRR(N), TTTV, TYCV, TYCV, TATV, NNNNGATT, NNAGAAW, or NAAAAC. Y is a pyrimidine; N is any nucleotide base; W is A or T.

[0342] 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.

[0343] 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.

[0344] In some embodiments, the PAM is an "NRN" PAM where the "N" in "NRN" is adenine (A), thymine (T), guanine (G), or cytosine (C), and the R is adenine (A) or guanine (G); or the PAM is an "NYN" PAM, wherein the "N" in NYN is is adenine (A), thymine (T), guanine (G), or cytosine (C), and the Y is cytidine (C) or thymine (T), for example, as described in R.T. Walton et al., 2020, *Science*, 10.1126/science.aba8853 (2020), the entire contents of which are incorporated herein by reference.

[0345] Several PAM variants are described in **Table 2** below.

Table 2. Cas9 proteins and corresponding PAM sequences

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

[0346] 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").

[0347] In some embodiments, the PAM is NGT. In some embodiments, the NGT PAM is recognized by a Cas9 variant. In some embodiments, the NGT PAM variant is generated through targeted mutations at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219. In some embodiments, the NGT PAM variant is created through targeted mutations at one or more residues 1219, 1335, 1337, 1218. In some embodiments, the NGT PAM variant is created

through targeted mutations at one or more residues 1135, 1136, 1218, 1219, and 1335. In some embodiments, the NGT PAM variant is selected from the set of targeted mutations provided in **Table 3** and **Table 4** below.

Table 3: 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 4: NGT PAM Variant Mutations at residues 1135, 1136, 1218, 1219, and 1335

	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			

[0348] In some embodiments, the NGT PAM variant is selected from variant 5, 7, 28, 31, or 36 in Tables 3 and 4. In some embodiments, the variants have improved NGT PAM recognition.

[0349] 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 5 below.

Table 5: 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

[0350] In some embodiments, base editors with specificity for NGT PAM may be generated as provided in **Table 6** below.

Table 6. 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

[0351] In some embodiments the NGTN variant is variant 1. In some embodiments, the NGTN variant is variant 2. In some embodiments, the NGTN variant is variant 3. In some embodiments, the NGTN variant is variant 4. In some embodiments, the NGTN variant is variant 5. In some embodiments, the NGTN variant is variant 6.

[0352] In some embodiments, the Cas9 domain is a Cas9 domain from *Streptococcus pyogenes* (SpCas9). In some embodiments, the SpCas9 domain is a nuclease active SpCas9, a nuclease inactive SpCas9 (SpCas9d), or a SpCas9 nickase (SpCas9n). In some embodiments, the SpCas9 comprises a D9X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid except for D. In some embodiments, the SpCas9 comprises a D9A mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the

SpCas9n domain can bind to a nucleic acid sequence having a non-canonical PAM. In some embodiments, the SpCas9 domain, the SpCas9d domain, or the SpCas9n domain can bind to a nucleic acid sequence having an NGG, a NGA, or a NGCG PAM sequence. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135E, R1335Q, and T1337R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135E, a R1335Q, and a T1337R mutation, or corresponding mutations in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135V, a R1335Q, and a T1337R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a R1335Q, and a T1337R mutation, or corresponding mutations in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises one or more of a D1135X, a G1218X, a R1335X, and a T1337X mutation, or a corresponding mutation in any of the amino acid sequences provided herein, wherein X is any amino acid. In some embodiments, the SpCas9 domain comprises one or more of a D1135V, a G1218R, a R1335Q, and a T1337R mutation, or a corresponding mutation in any of the amino acid sequences provided herein. In some embodiments, the SpCas9 domain comprises a D1135V, a G1218R, a R1335Q, and a T1337R mutation, or corresponding mutations in any of the amino acid sequences provided herein.

[0353] 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.

[0354] 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.

[0355] In an embodiment, *S. pyogenes* Cas9 (SpCas9) can be used as a CRISPR endonuclease for genome engineering. However, others can be used. In some embodiments, a different endonuclease can be used to target certain genomic targets. In some embodiments, synthetic SpCas9-derived variants with non-NGG PAM sequences can be used. Additionally, other Cas9 orthologues from various species have been identified and these "non-SpCas9s" can bind a variety of PAM sequences that can also be useful for the present disclosure. For example, the relatively large size of SpCas9 (approximately 4kb coding sequence) can lead to plasmids carrying the SpCas9 cDNA that cannot be efficiently expressed in a cell. Conversely, the coding sequence for *Staphylococcus aureus* Cas9 (SaCas9) is approximately 1 kilobase shorter than SpCas9, possibly allowing it to be efficiently expressed in a cell. Similar to SpCas9, the SaCas9 endonuclease is capable of modifying target genes in mammalian cells *in vitro* and in mice *in vivo*. In some embodiments, a Cas protein can target a different PAM sequence. In some embodiments, a target gene can be adjacent to a Cas9 PAM, 5′-NGG, for example. In other embodiments, other Cas9 orthologs can have different PAM requirements. For example, other PAMs such as those of *S. thermophilus* (5′-NNAGAA for CRISPR1 and 5′-NGGNG for CRISPR3) and *Neisseria meningiditis* (5′-NNNNGATT) can also be found adjacent to a target gene.

[0356] 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:

[0357] The amino acid sequence of an exemplary PAM-binding SpCas9 is as follows:

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE

KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

[0358] The amino acid sequence of an exemplary PAM-binding SpCas9n is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS

FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

[0359] The amino acid sequence of an exemplary PAM-binding SpEQR Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGF**E**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQL
GGD

[0360] In the above 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.

[0361] The amino acid sequence of an exemplary PAM-binding SpVQR Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDLSQL
GGD

[0362] Residues V1135, Q1335, and R1337 above, which can be mutated from D1135, R1335, and T1337 to yield a SpVQR Cas9, are underlined and in bold.

[0363] The amino acid sequence of an exemplary PAM-binding SpVRER Cas9 is as follows:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL

VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**E**Y**R**STKEVLDATLIHQSITGLYETRIDLSQL
GGD.

[0364] Residues V1135, R1218, E1335, and R1337 above, which can be mutated from D1135, G1218, R1335, and T1337 to yield a SpVRER Cas9, are underlined and in bold.

[0365] The amino acid sequence of an exemplary PAM-binding SpVRQR Cas9 is as follows:Exemplary SpVRQR Cas9

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS

FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQSITGLYETRIDLSQL
GGD.

[0366] Residues V1135, R1218, Q1335, and R1337 above, which can be mutated from D1135, G1218, R1335, and T1337 to yield a SpVRQR Cas9, are underlined and in bold.

[0367] In some embodiments, engineered SpCas9 variants are capable of recognizing protospacer adjacent motif (PAM) sequences flanked by a 3′ H (non-G PAM) (*see* **Tables 1A-1D**). In some embodiments, the SpCas9 variants recognize NRNH PAMs (where R is A or G and H is A, C or T). In some embodiments, the non-G PAM is NRRH, NRTH, or NRCH (*see e.g.*, Miller, S.M., *et al*. Continuous evolution of SpCas9 variants compatible with non-G PAMs, Nat. Biotechnol. (2020), the contents of which is incorporated herein by reference in its entirety).

[0368] 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.

[0369] The sequence of an exemplary Cas9 A homolog of Spy Cas9 in *Streptococcus macacae* with native 5′-NAAN-3′ PAM specificity is known in the art and described, for example, by Jakimo *et al.*, (www.biorxiv.org/content/biorxiv/early/2018/09/27/429654.full.pdf), and is provided below.

[0370] Exemplary SpyMacCas9

MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFGSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLADSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQIYNQL
FEENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGLFGNLIALSLGLTPNFKSNFDLAE

DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNSEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGAYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDRGMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGHSLHEQIANLAGSPAIKKGILQTVKIVDELVKVMGHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF
IKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVR
EINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEIQTVGQN
GGLFDDNPKSPLEVTPSKLVPLKKELNPKKYGGYQKPTTAYPVLLITDTKQLIPISVMNKKQFEQ
NPVKFLRDRGYQQVGKNDFIKLPKYTLVDIGDGIKRLWASSKEIHKGNQLVVSKKSQILLYHAHH
LDSDLSNDYLQNHNQQFDVLFNEIISFSKKCKLGKEHIQKIENVYSNKKNSASIEELAESFIKLL
GFTQLGATSPFNFLGVKLNQKQYKGKKDYILPCTEGTLIRQSITGLYETRVDLSKIGED.

[0371] In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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.

[0372] In some embodiments, a CRISPR protein-derived domain of a base editor can comprise all or a portion of a Cas9 protein with a canonical PAM sequence (NGG). In other embodiments, a Cas9-derived domain of a base editor can employ a non-canonical PAM sequence. Such sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" Nature 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition" Nature Biotechnology 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference.

Cas9 Domains with Reduced PAM Exclusivity

[0373] 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.

High fidelity Cas9 domains

[0374] 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 least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70%.

[0375] 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.

[0376] In some embodiments, the modified Cas9 is a high fidelity Cas9 enzyme. In some embodiments, the high fidelity Cas9 enzyme is SpCas9(K855A), eSpCas9(1.1), SpCas9-HF1, or hyper accurate Cas9 variant (HypaCas9). The modified Cas9 eSpCas9(1.1) contains alanine substitutions that weaken the interactions between the HNH/RuvC groove and the non-target DNA strand, preventing strand separation and cutting at off-target sites. Similarly, SpCas9-HF1 lowers off-target editing through alanine substitutions that disrupt Cas9's interactions with the

DNA phosphate backbone. HypaCas9 contains mutations (SpCas9 N692A/M694A/Q695A/H698A) in the REC3 domain that increase Cas9 proofreading and target discrimination. All three high fidelity enzymes generate less off-target editing than wildtype Cas9.

[0377] An exemplary high fidelity Cas9 is provided below. High Fidelity Cas9 domain mutations relative to Cas9 are shown in bold and underlined.

MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK
RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHE
KYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQL
FEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAE
DAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL
VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMT**A**FDKNLPNEKVLPKHSLLYEYFTV
YNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED
RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWG**A**LSRKLINGIRDKQSGKTILDFLKSDGFANRNFM**A**LIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
LDKAGFIKRQLVETR**A**ITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV
REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS
NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGF
SKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIME
RSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL
GGD.

Fusion proteins comprising a nuclear localization sequence (NLS)

[0378] 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, an NLS comprises an amino

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

[0379] In some embodiments, the NLS is present in a linker or the NLS is flanked by linkers, for example, the linkers described herein. In some embodiments, the N-terminus or C-terminus NLS is a bipartite NLS. A bipartite NLS comprises two basic amino acid clusters, which are separated by a relatively short spacer sequence (hence bipartite - 2 parts, while monopartite NLSs are not). The NLS of nucleoplasmin, KR[PAATKKAGQA]KKKK, is the prototype of the ubiquitous bipartite signal: two clusters of basic amino acids, separated by a spacer of about 10 amino acids. The sequence of an exemplary bipartite NLS follows:
PKKKRKVEGADKRTADGSEFESPKKKRKV.

[0380] In some embodiments, the fusion proteins as described herein do not comprise a linker sequence. In some embodiments, linker sequences between one or more of the domains or

proteins are present. In some embodiments, the general architecture of exemplary Cas9 fusion proteins with an adenosine deaminase and a Cas9 domain comprises any one of the following structures, where NLS is a nuclear localization sequence (e.g., any NLS provided herein), NH2 is the N-terminus of the fusion protein, and COOH is the C-terminus of the fusion protein:
NH2-NLS-[adenosine deaminase]-[Cas9 domain]-COOH;
NH2-NLS-[Cas9 domain]-[adenosine deaminase]-COOH;
NH2-[adenosine deaminase]-[Cas9 domain]-NLS-COOH; or
NH2-[Cas9 domain]-[adenosine deaminase]-NLS-COOH.

[0381] 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.

[0382] A vector that encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLSs) can be used. For example, there can be or be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 NLSs used. A CRISPR enzyme can comprise the NLSs at or near the amino-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 NLSs at or near the carboxy-terminus, or any combination thereof (*e.g.*, one or more NLS at the amino-terminus and one or more NLS at the carboxy terminus). When more than one NLS is present, each can be selected independently of others, such that a single NLS can be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies.

[0383] CRISPR enzymes used in the methods can comprise about 6 NLSs. An NLS is considered near the N- or C-terminus when the nearest amino acid to the NLS is within about 50 amino acids along a polypeptide chain from the N- or C-terminus, *e.g.*, within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, or 50 amino acids.

Cas9 Domains with Reduced Exclusivity

[0384] Typically, Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a canonical NGG PAM sequence to bind a particular nucleic acid region, where the "N" in "NGG" is adenosine (A), thymidine (T), or cytosine (C), and the G is guanosine. This may limit the ability to edit desired bases within a genome. In some embodiments, the base editing fusion proteins provided herein may need to be placed at a precise location, for example a region comprising a target base that is upstream of the PAM. See *e.g.*, Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" *Nature* 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference. Accordingly, in some embodiments, any of the fusion proteins provided herein may contain a Cas9 domain that is capable of binding a nucleotide sequence that does not contain a canonical (*e.g.,* NGG) PAM sequence. Cas9 domains that bind to non-canonical PAM sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" *Nature* 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition" *Nature Biotechnology* 33, 1293-1298 (2015); Nishimasu, H., *et al.*, "Engineered CRISPR-Cas9 nuclease with expanded targeting space" *Science*. 2018 Sep 21;361(6408):1259-1262, Chatterjee, P., *et al.*, Minimal PAM specificity of a highly similar SpCas9 ortholog" *Sci Adv*. 2018 Oct 24;4(10):eaau0766. doi: 10.1126/sciadv.aau0766, the entire contents of each are hereby incorporated by reference.

Fusion proteins with Internal Insertions

[0385] Provided herein are fusion proteins comprising a heterologous polypeptide fused to a nucleic acid programmable nucleic acid binding protein, for example, a napDNAbp. A heterologous polypeptide can be a polypeptide that is not found in the native or wild-type napDNAbp polypeptide sequence. The heterologous polypeptide can be fused to the napDNAbp at a C-terminal end of the napDNAbp, an N-terminal end of the napDNAbp, or inserted at an internal location of the napDNAbp. In some embodiments, the heterologous polypeptide is inserted at an internal location of the napDNAbp.

[0386] In some embodiments, the heterologous polypeptide is a deaminase or a functional fragment thereof. For example, a fusion protein can comprise a deaminase flanked by an N-terminal fragment and a C-terminal fragment of a Cas9 or Cas12 (*e.g.*, Cas12b/C2c1),

polypeptide. The deaminase in a fusion protein can be an adenosine deaminase. In some embodiments, the adenosine deaminase is a TadA (*e.g.*, TadA7.10 or TadA*8). In some embodiments, the TadA is a TadA*8. TadA sequences (*e.g.*, TadA7.10 or TadA*8) as described herein are suitable deaminases for the above-described fusion proteins.

[0387] The deaminase can be a circular permutant deaminase. For example, the deaminase can be a circular permutant adenosine deaminase. In some embodiments, the deaminase is a circular permutant TadA, circularly permutated at amino acid residue 116 as numbered in the TadA reference sequence. In some embodiments, the deaminase is a circular permutant TadA, circularly permutated at amino acid residue 136 as numbered in the TadA reference sequence. In some embodiments, the deaminase is a circular permutant TadA, circularly permutated at amino acid residue 65 as numbered in the TadA reference sequence.

[0388] The fusion protein can comprise more than one deaminase. The fusion protein can comprise, for example, 1, 2, 3, 4, 5 or more deaminases. In some embodiments, the fusion protein comprises one deaminase. In some embodiments, the fusion protein comprises two deaminases. The two or more deaminases can be homodimers. The two or more deaminases can be heterodimers. The two or more deaminases can be inserted in tandem in the napDNAbp. In some embodiments, the two or more deaminases may not be in tandem in the napDNAbp.

[0389] In some embodiments, the napDNAbp in the fusion protein is a Cas9 polypeptide or a fragment thereof. The Cas9 polypeptide can be a variant Cas9 polypeptide. In some embodiments, the Cas9 polypeptide is a Cas9 nickase (nCas9) polypeptide or a fragment thereof. In some embodiments, the Cas9 polypeptide is a nuclease dead Cas9 (dCas9) polypeptide or a fragment thereof. The Cas9 polypeptide in a fusion protein can be a full-length Cas9 polypeptide. In some cases, the Cas9 polypeptide in a fusion protein may not be a full length Cas9 polypeptide. The Cas9 polypeptide can be truncated, for example, at a N-terminal or C-terminal end relative to a naturally-occurring Cas9 protein. The Cas9 polypeptide can be a circularly permuted Cas9 protein. The Cas9 polypeptide can be a fragment, a portion, or a domain of a Cas9 polypeptide, that is still capable of binding the target polynucleotide and a guide nucleic acid sequence.

[0390] In some embodiments, the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), or fragments or variants thereof.

[0391] The Cas9 polypeptide of a fusion protein can comprise an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring Cas9 polypeptide.

[0392] The Cas9 polypeptide of a fusion protein can comprise an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the Cas9 amino acid sequence set forth below (called the "Cas9 reference sequence" below):

[0393] MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE
ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ
TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSN
FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDG
TEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY
EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEI
SGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDD
KVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQK
AQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQK
NSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDH
IVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER
GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDF
QFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAK
YFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLG
ITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPS
KYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYN
KHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRI
DLSQLGGD (single underline: HNH domain; double underline: RuvC domain).

[0394] Fusion proteins comprising a heterologous catalytic domain flanked by N- and C-terminal fragments of a Cas9 polypeptide are also useful for base editing in the methods as described herein. Fusion proteins comprising Cas9 and one or more deaminase domains, *e.g.*, adenosine deaminase, or comprising an adenosine deaminase domain flanked by Cas9 sequences are also useful for highly specific and efficient base editing of target sequences. In an embodiment, a

chimeric Cas9 fusion protein contains a heterologous catalytic domain (*e.g.*, adenosine deaminase) inserted within a Cas9 polypeptide. In some embodiments, the fusion protein comprises an adenosine deaminase domain and an adenosine deaminase domain inserted within a Cas9. In some embodiments, an adenosine deaminase is fused within a Cas9 and an adenosine deaminase is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas9 and an adenosine deaminase fused to the N-terminus. In some embodiments, an adenosine deaminase is fused within Cas9 and an adenosine deaminase is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas9 and an adenosine deaminase fused to the N-terminus.

[0395] In various embodiments, the catalytic domain has DNA modifying activity (*e.g.*, deaminase activity), such as adenosine deaminase activity. In some embodiments, the adenosine deaminase is a TadA (*e.g.*, TadA7.10). In some embodiments, the TadA is a TadA*8. In some embodiments, a TadA*8 is fused within Cas9 and an adenosine deaminase is fused to the C-terminus. In some embodiments, a TadA*8 is fused within Cas9 and an adenosine deaminase fused to the N-terminus. In some embodiments, an adenosine deaminase is fused within Cas9 and a TadA*8 is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas9 and a TadA*8 fused to the N-terminus. Exemplary structures of a fusion protein with a TadA*8 and an adenosine deaminase and a Cas9 are provided as follows:

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

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

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

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

[0396] In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

[0397] The heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp (*e.g.*, Cas9 or Cas12 (*e.g.*, Cas12b/C2c1)) at a suitable location, for example, such that the napDNAbp retains its ability to bind the target polynucleotide and a guide nucleic acid. A deaminase (*e.g.*, adenosine deaminase) can be inserted into a napDNAbp without compromising function of the deaminase (*e.g.*, base editing activity) or the napDNAbp (*e.g.*, ability to bind to target nucleic acid and guide nucleic acid). A deaminase (*e.g.*, adenosine deaminase) can be inserted in the napDNAbp at, for example, a disordered region or a region comprising a high temperature factor or B-factor as shown by crystallographic studies. Regions of a protein that are less ordered, disordered, or unstructured, for example solvent exposed regions and loops, can be used for

insertion without compromising structure or function. A deaminase (*e.g.*, adenosine deaminase)can be inserted in the napDNAbp in a flexible loop region or a solvent-exposed region. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted in a flexible loop of the Cas9 or the Cas12b/C2c1 polypeptide.

[0398] In some embodiments, the insertion location of a deaminase (*e.g.*, adenosine deaminase) is determined by B-factor analysis of the crystal structure of Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted in regions of the Cas9 polypeptide comprising higher than average B-factors (*e.g.*, higher B factors compared to the total protein or the protein domain comprising the disordered region). B-factor or temperature factor can indicate the fluctuation of atoms from their average position (for example, as a result of temperature-dependent atomic vibrations or static disorder in a crystal lattice). A high B-factor (*e.g.*, higher than average B-factor) for backbone atoms can be indicative of a region with relatively high local mobility. Such a region can be used for inserting a deaminase without compromising structure or function. A deaminase (*e.g.*, adenosine deaminase) can be inserted at a location with a residue having a Cα atom with a B-factor that is 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, or greater than 200% more than the average B-factor for the total protein. A deaminase (*e.g.*, adenosine deaminase) can be inserted at a location with a residue having a Cα atom with a B-factor that is 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% or greater than 200% more than the average B-factor for a Cas9 protein domain comprising the residue. Cas9 polypeptide positions comprising a higher than average B-factor can include, for example, residues 768, 792, 1052, 1015, 1022, 1026, 1029, 1067, 1040, 1054, 1068, 1246, 1247, and 1248 as numbered in the above Cas9 reference sequence. Cas9 polypeptide regions comprising a higher than average B-factor can include, for example, residues 792-872, 792-906, and 2-791 as numbered in the above Cas9 reference sequence.

[0399] A heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue selected from the group consisting of: 768, 791, 792, 1015, 1016, 1022, 1023, 1026, 1029, 1040, 1052, 1054, 1067, 1068, 1069, 1246, 1247, and 1248 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the heterologous polypeptide is inserted between amino acid positions 768-769, 791-792, 792-793, 1015-1016, 1022-1023, 1026-1027, 1029-1030, 1040-1041, 1052-1053, 1054-1055, 1067-1068, 1068-1069, 1247-1248, or 1248-1249 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the

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

[0400] A heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue selected from the group consisting of: 768, 792, 1022, 1026, 1040, 1068, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the heterologous polypeptide is inserted between amino acid positions 768-769, 792-793, 1022-1023, 1026-1027, 1029-1030, 1040-1041, 1068-1069, or 1247-1248 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the heterologous polypeptide is inserted between amino acid positions 769-770, 793-794, 1023-1024, 1027-1028, 1030-1031, 1041-1042, 1069-1070, or 1248-1249 as numbered in the above Cas9 reference sequence or corresponding amino acid positions thereof. In some embodiments, the heterologous polypeptide replaces an amino acid residue selected from the group consisting of: 768, 792, 1022, 1026, 1040, 1068, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0401] A heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue as described herein, or a corresponding amino acid residue in another Cas9 polypeptide. In an embodiment, a heterologous polypeptide (*e.g.*, deaminase) can be inserted in the napDNAbp at an amino acid residue selected from the group consisting of: 1002, 1003, 1025, 1052-1056, 1242-1247, 1061-1077, 943-947, 686-691, 569-578, 530-539, and 1060-1077 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The deaminase (*e.g.*, adenosine deaminase) can be inserted at the N-

terminus or the C-terminus of the residue or replace the residue. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of the residue.

[0402] In some embodiments, an adenosine deaminase (*e.g.*, TadA) is inserted at an amino acid residue selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, an adenosine deaminase (*e.g.*, TadA) is inserted in place of residues 792-872, 792-906, or 2-791 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the adenosine deaminase is inserted at the N-terminus of an amino acid selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the adenosine deaminase is inserted at the C-terminus of an amino acid selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the adenosine deaminase is inserted to replace an amino acid selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0403] In some embodiments, a CBE (*e.g.*, APOBEC1) is inserted at an amino acid residue selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the N-terminus of an amino acid selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted at the C-terminus of an amino acid selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the ABE is inserted to replace an amino acid selected from the group consisting of: 1016, 1023, 1029, 1040, 1069, and 1247 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0404] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 768 as numbered in the above Cas9 reference sequence, or a corresponding amino

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

[0405] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 791 or is inserted at amino acid residue 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 791 or is inserted at the N-terminus of amino acid 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of amino acid 791 or is inserted at the N-terminus of amino acid 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted to replace amino acid 791, or is inserted to replace amino acid 792, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0406] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted to replace amino acid residue 1016 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0407] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1022, or is inserted at amino acid residue 1023, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some

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

[0408] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1026, or is inserted at amino acid residue 1029, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 1026 or is inserted at the N-terminus of amino acid residue 1029, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of amino acid residue 1026 or is inserted at the C-terminus of amino acid residue 1029, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted to replace amino acid residue 1026, or is inserted to replace amino acid residue 1029, as numbered in the above Cas9 reference sequence, or corresponding amino acid residue in another Cas9 polypeptide.

[0409] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted to replace amino acid residue 1040 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0410] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1052, or is inserted at amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 1052 or is inserted at the N-terminus of amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of amino acid residue 1052 or is inserted at the C-terminus of amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted to replace amino acid residue 1052, or is inserted to replace amino acid residue 1054, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0411] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1067, or is inserted at amino acid residue 1068, or is inserted at amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 1067 or is inserted at the N-terminus of amino acid residue 1068 or is inserted at the N-terminus of amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the C-terminus of amino acid residue 1067 or is inserted at the C-terminus of amino acid residue 1068 or is inserted at the C-terminus of amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted to replace amino acid residue 1067, or is inserted to replace amino acid residue 1068, or is inserted to replace amino acid residue 1069, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0412] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at amino acid residue 1246, or is inserted at amino acid residue 1247, or is inserted at amino acid residue 1248, as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deaminase (*e.g.*, adenosine deaminase) is inserted at the N-terminus of amino acid residue 1246 or is inserted at the N-terminus of amino

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

[0413] In some embodiments, a heterologous polypeptide (*e.g.*, deaminase) is inserted in a flexible loop of a Cas9 polypeptide. The flexible loop portions can be selected from the group consisting of 530-537, 569-570, 686-691, 943-947, 1002-1025, 1052-1077, 1232-1247, or 1298-1300 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The flexible loop portions can be selected from the group consisting of: 1-529, 538-568, 580-685, 692-942, 948-1001, 1026-1051, 1078-1231, or 1248-1297 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0414] A heterologous polypeptide (*e.g.*, adenine deaminase) can be inserted into a Cas9 polypeptide region corresponding to amino acid residues: 1017-1069, 1242-1247, 1052-1056, 1060-1077, 1002 – 1003, 943-947, 530-537, 568-579, 686-691, 1242-1247, 1298 – 1300, 1066-1077, 1052-1056, or 1060-1077 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0415] A heterologous polypeptide (*e.g.*, adenine deaminase) can be inserted in place of a deleted region of a Cas9 polypeptide. The deleted region can correspond to an N-terminal or C-terminal portion of the Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 792-872 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 792-906 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the deleted region corresponds to residues 2-791 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. In some embodiments, the

deleted region corresponds to residues 1017-1069 as numbered in the above Cas9 reference sequence, or corresponding amino acid residues thereof.

[0416] Exemplary internal fusions base editors are provided in **Table 7A** below:

Table 7A: Insertion loci in Cas9 proteins

BE ID	Modification	Other ID
IBE001	Cas9 TadA ins 1015	ISLAY01
IBE002	Cas9 TadA ins 1022	ISLAY02
IBE003	Cas9 TadA ins 1029	ISLAY03
IBE004	Cas9 TadA ins 1040	ISLAY04
IBE005	Cas9 TadA ins 1068	ISLAY05
IBE006	Cas9 TadA ins 1247	ISLAY06
IBE007	Cas9 TadA ins 1054	ISLAY07
IBE008	Cas9 TadA ins 1026	ISLAY08
IBE009	Cas9 TadA ins 768	ISLAY09
IBE020	delta HNH TadA 792	ISLAY20
IBE021	N-term fusion single TadA helix truncated 165-end	ISLAY21
IBE029	TadA-Circular Permutant116 ins1067	ISLAY29
IBE031	TadA- Circular Permutant 136 ins1248	ISLAY31
IBE032	TadA- Circular Permutant 136ins 1052	ISLAY32
IBE035	delta 792-872 TadA ins	ISLAY35
IBE036	delta 792-906 TadA ins	ISLAY36
IBE043	TadA-Circular Permutant 65 ins1246	ISLAY43
IBE044	TadA ins C-term truncate2 791	ISLAY44

[0417] A heterologous polypeptide (*e.g.*, deaminase) can be inserted within a structural or functional domain of a Cas9 polypeptide. A heterologous polypeptide (*e.g.*, deaminase) can be inserted between two structural or functional domains of a Cas9 polypeptide. A heterologous polypeptide (*e.g.*, deaminase) can be inserted in place of a structural or functional domain of a Cas9 polypeptide, for example, after deleting the domain from the Cas9 polypeptide. The structural or functional domains of a Cas9 polypeptide can include, for example, RuvC I, RuvC II, RuvC III, Rec1, Rec2, PI, or HNH.

[0418] In some embodiments, the Cas9 polypeptide lacks one or more domains selected from the group consisting of: RuvC I, RuvC II, RuvC III, Rec1, Rec2, PI, or HNH domain. In some embodiments, the Cas9 polypeptide lacks a nuclease domain. In some embodiments, the Cas9 polypeptide lacks an HNH domain. In some embodiments, the Cas9 polypeptide lacks a portion of the HNH domain such that the Cas9 polypeptide has reduced or abolished HNH activity. In some embodiments, the Cas9 polypeptide comprises a deletion of the nuclease domain, and the deaminase is inserted to replace the nuclease domain. In some embodiments, the HNH domain is

deleted and the deaminase is inserted in its place. In some embodiments, one or more of the RuvC domains is deleted and the deaminase is inserted in its place.

[0419] A fusion protein comprising a heterologous polypeptide can be flanked by a N-terminal and a C-terminal fragment of a napDNAbp. In some embodiments, the fusion protein comprises a deaminase flanked by a N- terminal fragment and a C-terminal fragment of a Cas9 polypeptide. The N terminal fragment or the C terminal fragment can bind the target polynucleotide sequence. The C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment can comprise a part of a flexible loop of a Cas9 polypeptide. The C-terminus of the N terminal fragment or the N-terminus of the C terminal fragment can comprise a part of an alpha-helix structure of the Cas9 polypeptide. The N- terminal fragment or the C-terminal fragment can comprise a DNA binding domain. The N-terminal fragment or the C-terminal fragment can comprise a RuvC domain. The N-terminal fragment or the C-terminal fragment can comprise an HNH domain. In some embodiments, neither of the N-terminal fragment and the C-terminal fragment comprises an HNH domain.

[0420] In some embodiments, the C-terminus of the N terminal Cas9 fragment comprises an amino acid that is in proximity to a target nucleobase when the fusion protein deaminates the target nucleobase. In some embodiments, the N-terminus of the C terminal Cas9 fragment comprises an amino acid that is in proximity to a target nucleobase when the fusion protein deaminates the target nucleobase. The insertion location of different deaminases can be different in order to have proximity between the target nucleobase and an amino acid in the C-terminus of the N terminal Cas9 fragment or the N-terminus of the C terminal Cas9 fragment. For example, the insertion position of an ABE can be at an amino acid residue selected from the group consisting of: 1015, 1022, 1029, 1040, 1068, 1247, 1054, 1026, 768, 1067, 1248, 1052, and 1246 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0421] The N-terminal Cas9 fragment of a fusion protein (*i.e.* the N-terminal Cas9 fragment flanking the deaminase in a fusion protein) can comprise the N-terminus of a Cas9 polypeptide. The N-terminal Cas9 fragment of a fusion protein can comprise a length of at least about: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 amino acids. The N-terminal Cas9 fragment of a fusion protein can comprise a sequence corresponding to amino acid residues: 1-56, 1-95, 1-200, 1-300, 1-400, 1-500, 1-600, 1-700, 1-718, 1-765, 1-780, 1-906, 1-918, or 1-1100 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The N-terminal Cas9 fragment can comprise a sequence

comprising at least: 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to amino acid residues: 1-56, 1-95, 1-200, 1-300, 1-400, 1-500, 1-600, 1-700, 1-718, 1-765, 1-780, 1-906, 1-918, or 1-1100 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0422] The C-terminal Cas9 fragment of a fusion protein (*i.e.* the C-terminal Cas9 fragment flanking the deaminase in a fusion protein) can comprise the C-terminus of a Cas9 polypeptide. The C-terminal Cas9 fragment of a fusion protein can comprise a length of at least about: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, or 1300 amino acids. The C-terminal Cas9 fragment of a fusion protein can comprise a sequence corresponding to amino acid residues: 1099-1368, 918-1368, 906-1368, 780-1368, 765-1368, 718-1368, 94-1368, or 56-1368 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide. The N-terminal Cas9 fragment can comprise a sequence comprising at least: 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity to amino acid residues: 1099-1368, 918-1368, 906-1368, 780-1368, 765-1368, 718-1368, 94-1368, or 56-1368 as numbered in the above Cas9 reference sequence, or a corresponding amino acid residue in another Cas9 polypeptide.

[0423] The N-terminal Cas9 fragment and C-terminal Cas9 fragment of a fusion protein taken together may not correspond to a full-length naturally occurring Cas9 polypeptide sequence, for example, as set forth in the above Cas9 reference sequence.

[0424] The fusion protein described herein can effect targeted deamination with reduced deamination at non-target sites (*e.g.*, off-target sites), such as reduced genome wide spurious deamination. The fusion protein described herein can effect targeted deamination with reduced bystander deamination at non-target sites. The undesired deamination or off-target deamination can be reduced by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% compared with, for example, an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of a Cas9 polypeptide. The undesired deamination or off-target deamination can be reduced by at least one-fold, at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least tenfold, at least fifteen fold, at least twenty fold, at least thirty fold, at least forty fold, at least fifty fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, or at least hundred fold, compared

with, for example, an end terminus fusion protein comprising the deaminase fused to a N terminus or a C terminus of a Cas9 polypeptide.

[0425] In some embodiments, the deaminase (*e.g.*, adenosine deaminase) of the fusion protein deaminates no more than two nucleobases within the range of an R-loop. In some embodiments, the deaminase of the fusion protein deaminates no more than three nucleobases within the range of the R-loop. In some embodiments, the deaminase of the fusion protein deaminates no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleobases within the range of the R-loop. An R-loop is a three-stranded nucleic acid structure including a DNA:RNA hybrid, a DNA:DNA or an RNA: RNA complementary structure and the associated with single-stranded DNA. As used herein, an R-loop may be formed when a target polynucleotide is contacted with a CRISPR complex or a base editing complex, wherein a portion of a guide polynucleotide, *e.g.* a guide RNA, hybridizes with and displaces with a portion of a target polynucleotide, *e.g.* a target DNA. In some embodiments, an R-loop comprises a hybridized region of a spacer sequence and a target DNA complementary sequence. An R-loop region may be of about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleobase pairs in length. In some embodiments, the R-loop region is about 20 nucleobase pairs in length. It should be understood that, as used herein, an R-loop region is not limited to the target DNA strand that hybridizes with the guide polynucleotide. For example, editing of a target nucleobase within an R-loop region may be to a DNA strand that comprises the complementary strand to a guide RNA, or may be to a DNA strand that is the opposing strand of the strand complementary to the guide RNA. In some embodiments, editing in the region of the R-loop comprises editing a nucleobase on non-complementary strand (protospacer strand) to a guide RNA in a target DNA sequence.

[0426] The fusion protein described herein can effect target deamination in an editing window different from canonical base editing. In some embodiments, a target nucleobase is from about 1 to about 20 bases upstream of a PAM sequence in the target polynucleotide sequence. In some embodiments, a target nucleobase is from about 2 to about 12 bases upstream of a PAM sequence in the target polynucleotide sequence. In some embodiments, a target nucleobase is from about 1 to 9 base pairs, about 2 to 10 base pairs, about 3 to 11 base pairs, about 4 to 12 base pairs, about 5 to 13 base pairs, about 6 to 14 base pairs, about 7 to 15 base pairs, about 8 to 16 base pairs, about 9 to 17 base pairs, about 10 to 18 base pairs, about 11 to 19 base pairs, about 12 to 20 base pairs, about 1 to 7 base pairs, about 2 to 8 base pairs, about 3 to 9 base pairs, about 4 to 10 base pairs, about 5 to 11 base pairs, about 6 to 12 base pairs, about 7 to 13 base pairs, about 8 to 14 base

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

[0427] The fusion protein can comprise more than one heterologous polypeptide. For example, the fusion protein can additionally comprise one or more UGI domains and/or one or more nuclear localization signals. The two or more heterologous domains can be inserted in tandem. The two or more heterologous domains can be inserted at locations such that they are not in tandem in the NapDNAbp.

[0428] A fusion protein can comprise a linker between the deaminase and the napDNAbp polypeptide. The linker can be a peptide or a non-peptide linker. For example, the linker can be an XTEN, (GGGS)n, (GGGGS)n, (G)n, (EAAAK)n, (GGS)n, SGSETPGTSESATPES. In some embodiments, the fusion protein comprises a linker between the N-terminal Cas9 fragment and the deaminase. In some embodiments, the fusion protein comprises a linker between the C-terminal Cas9 fragment and the deaminase. In some embodiments, the N-terminal and C-terminal fragments of napDNAbp are connected to the deaminase with a linker. In some embodiments, the N-terminal and C-terminal fragments are joined to the deaminase domain without a linker. In some embodiments, the fusion protein comprises a linker between the N-terminal Cas9 fragment and the deaminase, but does not comprise a linker between the C-terminal Cas9 fragment and the deaminase. In some embodiments, the fusion protein comprises

a linker between the C-terminal Cas9 fragment and the deaminase, but does not comprise a linker between the N-terminal Cas9 fragment and the deaminase.

[0429] In some embodiments, the napDNAbp in the fusion protein is a Cas12 polypeptide, *e.g.*, Cas12b/C2c1, or a fragment thereof. The Cas12 polypeptide can be a variant Cas12 polypeptide. In other embodiments, the N- or C-terminal fragments of the Cas12 polypeptide comprise a nucleic acid programmable DNA binding domain or a RuvC domain. In other embodiments, the fusion protein contains a linker between the Cas12 polypeptide and the catalytic domain. In other embodiments, the amino acid sequence of the linker is GGSGGS or GSSGSETPGTSESATPESSG. In other embodiments, the linker is a rigid linker. In other embodiments of the above aspects, the linker is encoded by GGAGGCTCTGGAGGAAGC or GGCTCTTCTGGATCTGAAACACCTGGCACAAGCGAGAGCGCCACCCCTGAGAGCTCTGGC.

[0430] Fusion proteins comprising a heterologous catalytic domain flanked by N- and C-terminal fragments of a Cas12 polypeptide are also useful for base editing in the methods as described herein. Fusion proteins comprising Cas12 and one or more deaminase domains, *e.g.*, adenosine deaminase, or comprising an adenosine deaminase domain flanked by Cas12 sequences are also useful for highly specific and efficient base editing of target sequences. In an embodiment, a chimeric Cas12 fusion protein contains a heterologous catalytic domain (*e.g.*, adenosine deaminase) inserted within a Cas12 polypeptide. In some embodiments, the fusion protein comprises an adenosine deaminase domain and an adenosine deaminase domain inserted within a Cas12. In some embodiments, an adenosine deaminase is fused within Cas12 and an adenosine deaminase is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas12 and an adenosine deaminase fused to the N-terminus. In some embodiments, an adenosine deaminase is fused within Cas12 and an adenosine deaminase is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas12 and an adenosine deaminase fused to the N-terminus. Exemplary structures of a fusion protein with an adenosine deaminase and an adenosine deaminase and a Cas12 are provided as follows:

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

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

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

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

[0431] In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

[0432] In various embodiments, the catalytic domain has DNA modifying activity (*e.g.*, deaminase activity), such as adenosine deaminase activity. In some embodiments, the adenosine deaminase is a TadA (*e.g.*, TadA7.10). In some embodiments, the TadA is a TadA*8. In some embodiments, a TadA*8 is fused within Cas12 and an adenosine deaminase is fused to the C-terminus. In some embodiments, a TadA*8 is fused within Cas12 and an adenosine deaminase fused to the N-terminus. In some embodiments, an adenosine deaminase is fused within Cas12 and a TadA*8 is fused to the C-terminus. In some embodiments, an adenosine deaminase is fused within Cas12 and a TadA*8 fused to the N-terminus. Exemplary structures of a fusion protein with a TadA*8 and an adenosine deaminase and a Cas12 are provided as follows:
N-[Cas12(TadA*8)]-[adenosine deaminase]-C;
N-[adenosine deaminase]-[Cas12(TadA*8)]-C;
N-[Cas12(adenosine deaminase)]-[TadA*8]-C; or
N-[TadA*8]-[Cas12(adenosine deaminase)]-C.

[0433] In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker.

[0434] In other embodiments, the fusion protein contains one or more catalytic domains. In other embodiments, at least one of the one or more catalytic domains is inserted within the Cas12 polypeptide or is fused at the Cas12 N- terminus or C-terminus. In other embodiments, at least one of the one or more catalytic domains is inserted within a loop, an alpha helix region, an unstructured portion, or a solvent accessible portion of the Cas12 polypeptide. In other embodiments, the Cas12 polypeptide is Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the Cas12 polypeptide has at least about 85% amino acid sequence identity to *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b. In other embodiments, the Cas12 polypeptide has at least about 90% amino acid sequence identity to *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b. In other embodiments, the Cas12 polypeptide has at least about 95% amino acid sequence identity to *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b. In other embodiments, the Cas12 polypeptide contains or consists essentially of a fragment of *Bacillus hisashii* Cas12b, *Bacillus thermoamylovorans* Cas12b, *Bacillus sp. V3-13* Cas12b, or *Alicyclobacillus acidiphilus* Cas12b.

[0435] In other embodiments, the catalytic domain is inserted between amino acid positions 153-154, 255-256, 306-307, 980-981, 1019-1020, 534-535, 604-605, or 344-345 of BhCas12b or a

corresponding amino acid residue of Cas12a, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the catalytic domain is inserted between amino acids P153 and S154 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids K255 and E256 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids D980 and G981 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids K1019 and L1020 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids F534 and P535 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids K604 and G605 of BhCas12b. In other embodiments, the catalytic domain is inserted between amino acids H344 and F345 of BhCas12b. In other embodiments, catalytic domain is inserted between amino acid positions 147 and 148, 248 and 249, 299 and 300, 991 and 992, or 1031 and 1032 of BvCas12b or a corresponding amino acid residue of Cas12a, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the catalytic domain is inserted between amino acids P147 and D148 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids G248 and G249 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids P299 and E300 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids G991 and E992 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acids K1031 and M1032 of BvCas12b. In other embodiments, the catalytic domain is inserted between amino acid positions 157 and 158, 258 and 259, 310 and 311, 1008 and 1009, or 1044 and 1045 of AaCas12b or a corresponding amino acid residue of Cas12a, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In other embodiments, the catalytic domain is inserted between amino acids P157 and G158 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids V258 and G259 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids D310 and P311 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids G1008 and E1009 of AaCas12b. In other embodiments, the catalytic domain is inserted between amino acids G1044 and K1045 at of AaCas12b.

[0436] In other embodiments, the fusion protein contains a nuclear localization signal (*e.g.*, a bipartite nuclear localization signal). In other embodiments, the amino acid sequence of the nuclear localization signal is MAPKKKRKVGIHGVPAA. In other embodiments of the above aspects, the nuclear localization signal is encoded by the following sequence:

[0437] ATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCC. In other embodiments, the Cas12b polypeptide contains a mutation that silences the catalytic

activity of a RuvC domain. In other embodiments, the Cas12b polypeptide contains D574A, D829A and/or D952A mutations. In other embodiments, the fusion protein further contains a tag (*e.g.*, an influenza hemagglutinin tag).

[0438] In some embodiments, the fusion protein comprises a napDNAbp domain (*e.g.*, Cas12-derived domain) with an internally fused nucleobase editing domain (*e.g.*, all or a portion of a deaminase domain, *e.g.*, an adenosine deaminase domain). In some embodiments, the napDNAbp is a Cas12b. In some embodiments, the base editor comprises a BhCas12b domain with an internally fused TadA*8 domain inserted at the loci provided in **Table 7B** below.

[0439] Table 7B: Insertion loci in Cas12b proteins

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

[0440] By way of nonlimiting example, an adenosine deaminase (*e.g.*, ABE8.13) may be inserted into a BhCas12b to produce a fusion protein (*e.g.*, ABE8.13-BhCas12b) that effectively edits a nucleic acid sequence.

[0441] In some embodiments, the base editing system described herein comprises an ABE with TadA inserted into a Cas9. Sequences of relevant ABEs with TadA inserted into a Cas9 are provided.

[0442] 101 Cas9 TadAins 1015

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

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0443] 102 Cas9 TadAins 1022

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

LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0444] 103 Cas9 TadAins 1029

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

LGGD

[0445] 103 Cas9 TadAins 1040

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

[0446] 105 Cas9 TadAins 1068

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

[0447] 106 Cas9 TadAins 1247

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

[0448] 107 Cas9 TadAins 1054

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

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

[0449] 108 Cas9 TadAins 1026

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR

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

[0450] 109 Cas9 TadAins 768

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD

LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQGSSGSETPGTSESATPESSGSEVEFSHEYWMR
HALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMAL
RQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGA
AGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRTTQKGQKNSR
ERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKK
MKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQIT
KHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREI
NNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQE
IGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGR
DFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDP
KKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSK
RVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFD
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[0451] 110.1 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
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LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP

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

[0452] 110.2 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI

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

[0453] 110.3 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
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NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY

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

[0454] 110.4 Cas9 TadAins 1250

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
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LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD

LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMRREDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0455] 110.5 Cas9 TadAins 1249

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

LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSGS
SGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMRRPEDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0456] 110.5 Cas9 TadAins delta 59-66 1250

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

MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSSGSETPGTSESATPESGSSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFE
PCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEG
ILADECAALLCYFFRMPRQVFNAQKKAQSSTDEDNEQKQLFVEQHKHYLD
EIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTN
LGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGG
D

[0457] 110.6 Cas9 TadAins 1251

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

VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
GSSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARDE
REVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRL
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG
MNHRVEITEGILADECAALLCYFFRMRRDNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0458] 110.7 Cas9 TadAins 1252

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

TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
DGSSGSSGSETPGTSESATPESGSSSGSEVEFSHEYWMRHALTLAKRARD
EREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYR
LIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYP
GMNHRVEITEGILADECAALLCYFFRMRRNEQKQLFVEQHKHYLDEIIEQ
ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0459] 110.8 Cas9 TadAins delta 59-66 C-truncate 1250

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

YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPG
SSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMC
AGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADE
CAALLCYFFRMPRQEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADA
NLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
YTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0460] 111.1 Cas9 TadAins 997

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

KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGSSGSETPGTSESATPESSGIKKYPKLESEFVYGDYKVYDVR
KMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGET
GEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKL
IARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM
ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGE
LQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEI
IEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLG
APAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0461] 111.2 Cas9 TadAins 997

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGSSGSSGSETPGTSESATPESSGGSSIKKYPKLESEFVYGDY

KVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI
ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPK
RNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKEL
LGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK
HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLF
TLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS
QLGGD

[0462] 112 delta HNH TadA

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSEVEFSHE
YWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAE
IMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNA
KTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND
KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAL
IKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFK
TEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKK
TEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVA
KVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIK

LPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG
SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATL
IHQSITGLYETRIDLSQLGGD

[0463] 113 N-term single TadA helix trunc 165-end

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIG
LHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIG
RVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFR
MPRSGGSSGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSV
GWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR
TARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERH
PIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRG
HFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARL
SKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQL
SKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAP
LSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG
ASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHL
GELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMT
RKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYE
YFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKE
DYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL
EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKL
INGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQ
GDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARE
NQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL
QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGK
SDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGF
IKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDF
RKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVY
DVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETN
GETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLAS
AGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYL

DEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT
NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG
GD

[0464] 114 N-term single TadA helix trunc 165-end delta 59-65

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVR
NAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRSGGS
SGGSSGSETPGTSESATPESSGGSSGGSDKKYSIGLAIGTNSVGWAVITD
EYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIV
DEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGD
LNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLE
NLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDD
DLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIK
RYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
KFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL
RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETI
TPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNE
LTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE
CFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTL
TLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDK
QSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEH
IANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKG
QKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSE
EVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVE
TRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFY
KVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA
KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK
KDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS
FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQI
SEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAA

FKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0465] 115.1 Cas9 TadAins1004

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREV
PVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDA
TLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNH
RVEITEGILADECAALLCYFFRMPRQLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0466] 115.2 Cas9 TadAins1005

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDERE
VPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLID
ATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMPRQESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0467] 115.3 Cas9 TadAins1006

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR

LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLEGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDER
EVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLI
DATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGM
NHRVEITEGILADECAALLCYFFRMPRQSEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0468] 115.4 Cas9 TadAins1007

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP

INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDE
REVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRL
IDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPG
MNHRVEITEGILADECAALLCYFFRMPRQEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0469] 116.1 Cas9 TadAins C-term truncate2 792

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI

LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGGSSGSETP
GTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNR
VIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVM
CAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILAD
ECAALLCYFFRMPRQSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE
LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI
TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVRE
INNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0470] 116.2 Cas9 TadAins C-term truncate2 791

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSSGSETPG
TSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRV
IGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMC
AGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADE
CAALLCYFFRMPRQGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE
LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI
TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVRE
INNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0471] 116.3 Cas9 TadAins C-term truncate2 790

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK

NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADEC
AALLCYFFRMPRQLGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQE
LDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI
TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVRE
INNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN
PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA
LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYF
DTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0472] 117 Cas9 delta 1017-1069

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

IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYSSGSEVEFSHEYWMRHALTLAKRARDEREVPVGA
VLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYV
TFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEI
TEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGEIVWDKGRDFATVR
KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF
DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEA
KGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVN
FLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILAD
ANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK
RYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

[0473] 118 Cas9 TadA-CP116ins 1067

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

MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNMNHRVEITEGILADECAALLCYFFRMPRQVFNAQ
KKAQSSTDGSSGSETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRAR
DEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNY
RLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHY
PGGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0474] 119 Cas9 TadAins 701

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

YVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDLTFKEDIQKAQVS
GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMA
RENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLY
YLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNR
GKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKA
GFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVS
DFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK
VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0475] 120 Cas9 TadACP136ins 1248

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

SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSMN
HRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0476] 121 Cas9 TadACP136ins 1052

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

TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLAMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGS
ETPGTSESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVL
NNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEP
CVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGNGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0477] 122 Cas9 TadACP136ins 1041

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

REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSMNHRVEITEG
ILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGTSESATPES
SGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI
GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRI
GRVVFGVRNAKTGAAGSLMDVLHYPGNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0478] 123 Cas9 TadACP139ins 1299

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

YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
DNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRMN
HRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDGSSGSETPGT
SESATPESSGSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVI
GEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCA
GAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0479] 124 Cas9 delta 792-872 TadAins

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

DFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK
VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE
TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML
ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0480] 125 Cas9 delta 792-906 TadAins

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

VEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKL
PKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS
PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLI
HQSITGLYETRIDLSQLGGD

[0481] 126 TadA CP65ins 1003

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

ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKH
YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0482] 127 TadA CP65ins 1016

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

YLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT
LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0483] 128 TadA CP65ins 1022

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

LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQ
LGGD

[0484] 129 TadA CP65ins 1029

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

LGGD

[0485] 130 TadA CP65ins 1041

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

[0486] 131 TadA CP65ins 1054

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

[0487] 132 TadA CP65ins 1246

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

[0488] In some embodiments, adenosine deaminase base editors were generated to insert TadA or variants thereof into the Cas9 polypeptide at the identified positions.

[0489] Exemplary, yet nonlimiting, fusion proteins are described in International PCT Application Nos. PCT/US2020/016285 and U.S. Provisional Application Nos. 62/852,228 and 62/852,224, the contents of which are incorporated by reference herein in their entireties.

Nucleobase Editing Domain

[0490] Described herein are base editors comprising a fusion protein that includes a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (*e.g.*, deaminase domain). The base editor can be programmed to edit one or more bases in a target polynucleotide sequence by interacting with a guide polynucleotide capable of recognizing the target sequence. Once the target sequence has been recognized, the base editor is anchored on the polynucleotide where editing is to occur and the deaminase domain components of the base editor can then edit a target base.

[0491] In some embodiments, the nucleobase editing domain includes a deaminase domain. In some embodiments, a deaminase domain can be an adenine deaminase or an adenosine deaminase. In some embodiments, the terms "adenine deaminase" and "adenosine deaminase" can be used interchangeably. Details of nucleobase editing proteins are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

A to G Editing

[0492] In some embodiments, a base editor described herein can comprise a deaminase domain which includes an adenosine deaminase. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. Adenosine deaminas is capable of deaminating (*i.e.*, removing an amine group) adenine of a deoxyadenosine residue in deoxyribonucleic acid (DNA).

[0493] In some embodiments, the nucleobase editors provided herein can be made by fusing together one or more protein domains, thereby generating a fusion protein. In certain embodiments, the fusion proteins provided herein comprise one or more features that improve the base editing activity (*e.g.*, efficiency, selectivity, and specificity) of the fusion proteins. For example, the fusion proteins provided herein can comprise a Cas9 domain that has reduced nuclease activity. In some embodiments, the fusion proteins provided herein can have a Cas9 domain that does not have nuclease activity (dCas9), or a Cas9 domain that cuts one strand of a duplexed DNA molecule, referred to as a Cas9 nickase (nCas9). Without wishing to be bound by any particular theory, the presence of the catalytic residue (*e.g.*, H840) maintains the activity of the Cas9 to cleave the non-edited (*e.g.*, non-deaminated) strand containing a T opposite the targeted A. Mutation of the catalytic residue (*e.g.*, D10 to A10) of Cas9 prevents cleavage of the edited strand containing the targeted A residue. Such Cas9 variants are able to generate a single-strand DNA break (nick) at a specific location based on the gRNA-defined target sequence, leading to repair of the non-edited strand, ultimately resulting in a T to C change on the non-edited strand. In some embodiments, an A-to-G base editor further comprises an inhibitor of inosine base excision repair, for example, a uracil glycosylase inhibitor (UGI) domain or a catalytically inactive inosine specific nuclease. Without wishing to be bound by any particular theory, the UGI domain or catalytically inactive inosine specific nuclease can inhibit or prevent base excision repair of a deaminated adenosine residue (*e.g.*, inosine), which can improve the activity or efficiency of the base editor.

[0494] A base editor comprising an adenosine deaminase can act on any polynucleotide, including DNA, RNA and DNA-RNA hybrids. In certain embodiments, a base editor comprising an adenosine deaminase can deaminate a target A of a polynucleotide comprising RNA. For example, the base editor can comprise an adenosine deaminase domain capable of deaminating a target A of an RNA polynucleotide and/or a DNA-RNA hybrid polynucleotide. In an embodiment, an adenosine deaminase incorporated into a base editor comprises all or a portion of adenosine deaminase acting on RNA (ADAR, *e.g.*, ADAR1 or ADAR2). In another embodiment, an adenosine deaminase incorporated into a base editor comprises all or a portion of adenosine deaminase acting on tRNA (ADAT). A base editor comprising an adenosine deaminase domain can also be capable of deaminating an A nucleobase of a DNA polynucleotide. In an embodiment an adenosine deaminase domain of a base editor comprises all or a portion of an ADAT comprising one or more mutations which permit the ADAT to deaminate a target A in DNA. For example, the base editor can comprise all or a portion of an

ADAT from *Escherichia coli* (EcTadA) comprising one or more of the following mutations: D108N, A106V, D147Y, E155V, L84F, H123Y, I156F, or a corresponding mutation in another adenosine deaminase.

[0495] The adenosine deaminase can be derived from any suitable organism (*e.g.*, *E. coli*). 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 some embodiments, the adenine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). The corresponding residue in any homologous protein can be identified by, *e.g.*, sequence alignment and determination of homologous residues. The mutations in any naturally-occurring adenosine deaminase (*e.g.*, having homology to ecTadA) that correspond to any of the mutations described herein (*e.g.*, any of the mutations identified in ecTadA) can be generated accordingly.

Adenosine deaminases

[0496] In some embodiments, fusion proteins described herein can comprise a deaminase domain which includes an adenosine deaminase. Such an adenosine deaminase domain of a base editor can facilitate the editing of an adenine (A) nucleobase to a guanine (G) nucleobase by deaminating the A to form inosine (I), which exhibits base pairing properties of G. Adenosine deaminase is capable of deaminating (*i.e.*, removing an amine group) adenine of a deoxyadenosine residue in deoxyribonucleic acid (DNA).

[0497] In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine. In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine in a deoxyadenosine residue of DNA. In some embodiments, the adenine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (*e.g.*, mutations in ecTadA). One of skill in the art will be able to identify the corresponding residue in any homologous protein, *e.g.*, by sequence alignment and determination of homologous residues. Accordingly, one of skill in the art would be able to generate mutations in any naturally-occurring adenosine deaminase (*e.g.*, having homology to ecTadA) that corresponds to any of the mutations described herein, *e.g.*, any of the mutations identified in ecTadA. In some embodiments, the adenosine

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

[0498] The disclosure provides adenosine deaminase variants that have increased efficiency (>50-60%) and specificity. In particular, the adenosine deaminase variants described herein are more likely to edit a desired base within a polynucleotide, and are less likely to edit bases that are not intended to be altered (*i.e.*, "bystanders").

[0499] In some embodiments, the adenosine deaminase is a TadA deaminase. In particular embodiments, the TadA is any one of the TadA described in PCT/US2017/045381 (WO 2018/027078), which is incorporated herein by reference in its entirety.

[0500] In some embodiments, the nucleobase editors of the disclosure are adenosine deaminase variants comprising an alteration in the following sequence:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD (also termed TadA*7.10).

[0501] In particular embodiments, the fusion proteins comprise a single (*e.g.*, provided as a monomer) TadA*8 variant. In some embodiments, the TadA*8 is linked to a Cas9 nickase. In some embodiments, the fusion proteins of the disclosure comprise as a heterodimer of a wild-type TadA (TadA(wt)) linked to a TadA*8 variant. In other embodiments, the fusion proteins of the disclosure comprise as a heterodimer of a TadA*7.10 linked to a TadA*8 variant. In some embodiments, the base editor is ABE8 comprising a TadA*8 variant monomer. In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant and a TadA(wt). In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant and TadA*7.10. In some embodiments, the base editor is ABE8 comprising a heterodimer of a TadA*8 variant. In some embodiments, the TadA*8 variant is selected from **Table 9**. In some embodiments, the ABE8 is selected from **Table 8, 9, 10,** or **11**. The relevant sequences follow:
Wild-type TadA (TadA(wt)) or "the TadA reference sequence"

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

TadA*7.10:

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

[0502] 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.

[0503] 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: MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDP TAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMD VLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD.

[0504] It should be appreciated, however, that additional adenosine deaminases useful in the present application would be apparent to the skilled artisan and are within the scope of this disclosure. For example, the adenosine deaminase may be a homolog of adenosine deaminase acting on tRNA (ADAT). Without limitation, the amino acid sequences of exemplary AD AT homologs include the following:

[0505] *Staphylococcus aureus* TadA:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAHAEHIAIER
AAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGS
LMNLLQQSNFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

[0506] *Bacillus subtilis* TadA:

MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEMLVIDEACKA
LGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPKGGCSGTLMNLLQEERFNHQAEVVS
GVLEEECGGMLSAFFRELRKKKKAARKNLSE

[0507] *Salmonella typhimurium* (*S. typhimurium*) TadA:

MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNHRVIGEGWNRPIGRHDP
TAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAMVHSRIGRVVFGARDAKTGAAGSLID
VLHHPGMNHRVEIIEGVLRDECATLLSDFFRMRRQEIKALKKADRAEGAGPAV

[0508] *Shewanella putrefaciens* (*S. putrefaciens*) TadA:

MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEILCLRSAGKKLE
NYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGTVVNLLQHPAFNHQVEVTSGV
LAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE

[0509] *Haemophilus influenzae* F3031 (*H. influenzae*) TadA:

MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDPTAHAEII
ALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYKTGAIGSRFHFFDDYKM
NHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKALLKSLSDK

[0510] *Caulobacter crescentus* (*C. crescentus*) TadA:

MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAHDPTAHAEIA
AMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGRVVFGADDPKGGAVVHGPKFFAQPTC
HWRPEVTGGVLADESADLLRGFFRARRKAKI

[0511] *Geobacter sulfurreducens* (*G. sulfurreducens*) TadA:

MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSNDPSAHAEMI
AIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVVFGCYDPKGGAAGSLYDLSADPRL
NHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAKATPALFIDERKVPPEP

[0512] An embodiment of *E. Coli* TadA (ecTadA) includes the following:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

[0513] 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*.

[0514] In one embodiment, a fusion protein of the disclosure comprises a wild-type TadA linked to TadA*7.10, which is linked to Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*7.10 domain (*e.g.*, provided as a monomer). In other embodiments, the ABE7.10 editor comprises TadA*7.10 and TadA(wt), which are capable of forming heterodimers.

[0515] 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.

[0516] It should be appreciated that any of the mutations provided herein (*e.g.*, based on the TadA reference sequence) can be introduced into other adenosine deaminases, such as *E. coli* TadA (ecTadA), *S. aureus* TadA (saTadA), or other adenosine deaminases (*e.g.*, bacterial

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

[0517] In some embodiments, the adenosine deaminase comprises a D108X mutation in the TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108G, D108N, D108V, D108A, or D108Y mutation, or a corresponding mutation in another adenosine deaminase.

[0518] In some embodiments, the adenosine deaminase comprises an A106X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A106V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* wild-type TadA or ecTadA).

[0519] In some embodiments, the adenosine deaminase comprises a E155X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a E155D, E155G, or E155V mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

[0520] In some embodiments, the adenosine deaminase comprises a D147X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D147Y, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA).

[0521] In some embodiments, the adenosine deaminase comprises an A106X, E155X, or D147X, mutation in the TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA), where X indicates any amino acid other than the

corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E155D, E155G, or E155V mutation. In some embodiments, the adenosine deaminase comprises a D147Y.

[0522] For example, an adenosine deaminase can contain a D108N, a A106V, a E155V, and/or a D147Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.,* ecTadA). In some embodiments, an adenosine deaminase comprises the following group of mutations (groups of mutations are separated by a ";") in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.,* ecTadA): D108N and A106V; D108N and E155V; D108N and D147Y; A106V and E155V; A106V and D147Y; E155V and D147Y; D108N, A106V, and E155V; D108N, A106V, and D147Y; D108N, E155V, and D147Y; A106V, E155V, and D147Y; and D108N, A106V, E155V, and D147Y. It should be appreciated, however, that any combination of corresponding mutations provided herein can be made in an adenosine deaminase (*e.g.,* ecTadA).

[0523] In some embodiments, the adenosine deaminase comprises one or more of a H8X, T17X, L18X, W23X, L34X, W45X, R51X, A56X, E59X, E85X, M94X, I95X, V102X, F104X, A106X, R107X, D108X, K110X, M118X, N127X, A138X, F149X, M151X, R153X, Q154X, I156X, and/or K157X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.,* ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H8Y, T17S, L18E, W23L, L34S, W45L, R51H, A56E, or A56S, E59G, E85K, or E85G, M94L, I95L, V102A, F104L, A106V, R107C, or R107H, or R107P, D108G, or D108N, or D108V, or D108A, or D108Y, K110I, M118K, N127S, A138V, F149Y, M151V, R153C, Q154L, I156D, and/or K157R mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.,* ecTadA).

[0524] In some embodiments, the adenosine deaminase comprises one or more of a H8X, D108X, and/or N127X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.,* ecTadA), where X indicates the presence of any amino acid. In some embodiments, the adenosine deaminase comprises one or more of a H8Y, D108N, and/or N127S mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.,* ecTadA).

[0525] In some embodiments, the adenosine deaminase comprises one or more of H8X, R26X, M61X, L68X, M70X, A106X, D108X, A109X, N127X, D147X, R152X, Q154X, E155X,

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

[0526] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, D108X, N127X, D147X, R152X, and Q154X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, M61X, M70X, D108X, N127X, Q154X, E155X, and Q163X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, D108X, N127X, E155X, and T166X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.,* ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

[0527] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8X, A106X, D108X, mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8X, R26X, L68X, D108X, N127X, D147X, and E155X, or a corresponding mutation or mutations in another adenosine deaminase, where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, D108X, A109X, N127X, and E155X

in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

[0528] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, D108N, N127S, D147Y, R152C, and Q154H in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, M61I, M70V, D108N, N127S, Q154R, E155G and Q163H in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, D108N, N127S, E155V, and T166P in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of H8Y, A106T, D108N, N127S, E155D, and K161Q in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, seven, or eight mutations selected from the group consisting of H8Y, R26W, L68Q, D108N, N127S, D147Y, and E155V in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, D108N, A109T, N127S, and E155G in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA).

[0529] Any of the mutations provided herein and any additional mutations (*e.g.*, based on the ecTadA amino acid sequence) can be introduced into any other adenosine deaminases. Any of the mutations provided herein can be made individually or in any combination in TadA reference sequence or another adenosine deaminase (*e.g.*, ecTadA).

[0530] Details of A to G nucleobase editing proteins are described in International PCT Application No. PCT/2017/045381 (WO2018/027078) and Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature, 551, 464-471 (2017), the entire contents of which are hereby incorporated by reference.

[0531] In some embodiments, the adenosine deaminase comprises one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a D108N, D108G, or D108V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a A106V and D108N mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises R107C and D108N mutations in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and Q154H mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, N127S, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a D108N, D147Y, and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a H8Y, D108N, and N127S mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises a A106V, D108N, D147Y and E155V mutation in TadA reference sequence, or corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

[0532] In some embodiments, the adenosine deaminase comprises one or more of a S2X, H8X, I49X, L84X, H123X, N127X, I156X and/or K160X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of S2A, H8Y, I49F, L84F, H123Y, N127S, I156F and/or K160S mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

[0533] In some embodiments, the adenosine deaminase comprises an L84X mutation adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an L84F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0534] In some embodiments, the adenosine deaminase comprises an H123X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H123Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0535] In some embodiments, the adenosine deaminase comprises an I156X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an I156F mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0536] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84X, A106X, D108X, H123X, D147X, E155X, and I156X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2X, I49X, A106X, D108X, D147X, and E155X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8X, A106X, D108X, N127X, and K160X in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA), where X indicates the presence of any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase.

[0537] In some embodiments, the adenosine deaminase comprises one, two, three, four, five, six, or seven mutations selected from the group consisting of L84F, A106V, D108N, H123Y, D147Y, E155V, and I156F in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one, two, three, four, five, or six mutations selected from the group consisting of S2A, I49F, A106V, D108N, D147Y, and E155V in TadA reference sequence.

[0538] In some embodiments, the adenosine deaminase comprises one, two, three, four, or five, mutations selected from the group consisting of H8Y, A106T, D108N, N127S, and K160S in TadA reference sequence, or a corresponding mutation or mutations in another adenosine deaminase (*e.g.*, ecTadA).

[0539] In some embodiments, the adenosine deaminase comprises one or more of a E25X, R26X, R107X, A142X, and/or A143X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of E25M, E25D, E25A, E25R, E25V, E25S, E25Y, R26G, R26N, R26Q, R26C, R26L, R26K, R107P, R107K, R107A, R107N, R107W, R107H, R107S, A142N, A142D, A142G, A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA). In some embodiments, the adenosine deaminase comprises one or more of the mutations described herein corresponding to TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

[0540] In some embodiments, the adenosine deaminase comprises an E25X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an E25M, E25D, E25A, E25R, E25V, E25S, or E25Y mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0541] In some embodiments, the adenosine deaminase comprises an R26X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises R26G, R26N, R26Q, R26C, R26L, or R26K mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0542] In some embodiments, the adenosine deaminase comprises an R107X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R107P,

R107K, R107A, R107N, R107W, R107H, or R107S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0543] In some embodiments, the adenosine deaminase comprises an A142X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A142N, A142D, A142G, mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0544] In some embodiments, the adenosine deaminase comprises an A143X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A143D, A143G, A143E, A143L, A143W, A143M, A143S, A143Q and/or A143R mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0545] In some embodiments, the adenosine deaminase comprises one or more of a H36X, N37X, P48X, I49X, R51X, M70X, N72X, D77X, E134X, S146X, Q154X, K157X, and/or K161X mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA), where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises one or more of H36L, N37T, N37S, P48T, P48L, I49V, R51H, R51L, M70L, N72S, D77G, E134G, S146R, S146C, Q154H, K157N, and/or K161T mutation in TadA reference sequence, or one or more corresponding mutations in another adenosine deaminase (*e.g.*, ecTadA).

[0546] In some embodiments, the adenosine deaminase comprises an H36X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an H36L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0547] In some embodiments, the adenosine deaminase comprises an N37X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an N37T, or

N37S mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0548] In some embodiments, the adenosine deaminase comprises an P48X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an P48T, or P48L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0549] In some embodiments, the adenosine deaminase comprises an R51X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an R51H, or R51L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0550] In some embodiments, the adenosine deaminase comprises an S146X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an S146R, or S146C mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0551] In some embodiments, the adenosine deaminase comprises an K157X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a K157N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0552] In some embodiments, the adenosine deaminase comprises an P48X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a P48S, P48T, or P48A mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0553] In some embodiments, the adenosine deaminase comprises an A142X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a A142N mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0554] In some embodiments, the adenosine deaminase comprises an W23X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a W23R, or W23L mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0555] In some embodiments, the adenosine deaminase comprises an R152X mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA), where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a R152P, or R52H mutation in TadA reference sequence, or a corresponding mutation in another adenosine deaminase (*e.g.*, ecTadA).

[0556] In one embodiment, the adenosine deaminase may comprise the mutations H36L, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, E155V, I156F, and K157N. In some embodiments, the adenosine deaminase comprises the following combination of mutations relative to TadA reference sequence, where each mutation of a combination is separated by a "_" and each combination of mutations is between parentheses:

(A106V_D108N),
(R107C_D108N),
(H8Y_D108N_N127S_D147Y_Q154H),
(H8Y_D108N_N127S_D147Y_E155V),
(D108N_D147Y_E155V),
(H8Y_D108N_N127S),
(H8Y_D108N_N127S_D147Y_Q154H),
(A106V_D108N_D147Y_E155V),
(D108Q_D147Y_E155V),
(D108M_D147Y_E155V),

(D108L_D147Y_E155V),
(D108K_D147Y_E155V),
(D108I_D147Y_E155V),
(D108F_D147Y_E155V),
(A106V_D108N_D147Y),
(A106V_D108M_D147Y_E155V),
(E59A_A106V_D108N_D147Y_E155V),
(E59A cat dead_A106V_D108N_D147Y_E155V),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156Y),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(D103A_D104N),
(G22P_D103A_D104N),
(D103A_D104N_S138A),
(R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(E25G_R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(E25D_R26G_L84F_A106V_R107K_D108N_H123Y_A142N_A143G_D147Y_E155V_I156F),
(R26Q_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(E25M_R26G_L84F_A106V_R107P_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(R26C_L84F_A106V_R107H_D108N_H123Y_A142N_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_A142N_A143L_D147Y_E155V_I156F),
(R26G_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(E25A_R26G_L84F_A106V_R107N_D108N_H123Y_A142N_A143E_D147Y_E155V_I156F),
(R26G_L84F_A106V_R107H_D108N_H123Y_A142N_A143D_D147Y_E155V_I156F),
(A106V_D108N_A142N_D147Y_E155V),
(R26G_A106V_D108N_A142N_D147Y_E155V),
(E25D_R26G_A106V_R107K_D108N_A142N_A143G_D147Y_E155V),
(R26G_A106V_D108N_R107H_A142N_A143D_D147Y_E155V),
(E25D_R26G_A106V_D108N_A142N_D147Y_E155V),
(A106V_R107K_D108N_A142N_D147Y_E155V),

(A106V_D108N_A142N_A143G_D147Y_E155V),
(A106V_D108N_A142N_A143L_D147Y_E155V),
(H36L_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F _K157N),
(N37T_P48T_M70L_L84F_A106V_D108N_H123Y_D147Y_I49V_E155V_I156F),
(N37S_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K161T),
(H36L_L84F_A106V_D108N_H123Y_D147Y_Q154H_E155V_I156F),
(N72S_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F),
(H36L_P48L_L84F_A106V_D108N_H123Y_E134G_D147Y_E155V_I156F),
(H36L_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K157N),
(H36L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(N37S_R51H_D77G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R51L_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_K157N),
(D24G_Q71R_L84F_H96L_A106V_D108N_H123Y_D147Y_E155V_I156F_K160E),
(H36L_G67V_L84F_A106V_D108N_H123Y_S146T_D147Y_E155V_I156F),
(Q71L_L84F_A106V_D108N_H123Y_L137M_A143E_D147Y_E155V_I156F),
(E25G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_Q159L),
(L84F_A91T_F104I_A106V_D108N_H123Y_D147Y_E155V_I156F),
(N72D_L84F_A106V_D108N_H123Y_G125A_D147Y_E155V_I156F),
(P48S_L84F_S97C_A106V_D108N_H123Y_D147Y_E155V_I156F),
(W23G_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(D24G_P48L_Q71R_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F_Q159L),
(L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(H36L_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_I156F
_K157N), (N37S_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F_K161T),
(L84F_A106V_D108N_D147Y_E155V_I156F),
(R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K160E_K161T),
(L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N_K160E),
(R74Q_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(R74A_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),

(R74Q_L84F_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_R98Q_A106V_D108N_H123Y_D147Y_E155V_I156F),
(L84F_A106V_D108N_H123Y_R129Q_D147Y_E155V_I156F),
(P48S_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F),
(P48S_A142N),
(P48T_I49V_L84F_A106V_D108N_H123Y_A142N_D147Y_E155V_I156F_L157N),
(P48T_I49V_A142N),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(H36L_P48S_R51L_L84F_A106V_D108N_H123Y_S146C_A142N_D147Y_E155V_I156F
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F
_K157N),
(H36L_P48T_I49V_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_
I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_E155V_I156F
_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_A142N_D147Y_E155V_I156F
_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F
_K157N),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_E155V_I156F
_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F
_K161T),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152H_E155V_I156F
_K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F
_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V
_I156F _K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S146C_D147Y_E155V
_I156F _K157N),

(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142A_S146C_D147Y_R152P_E155V_I156F_K157N),
(W23L_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146R_D147Y_E155V_I156F_K161T),
(W23R_H36L_P48A_R51L_L84F_A106V_D108N_H123Y_S146C_D147Y_R152P_E155V_I156F _K157N),
(H36L_P48A_R51L_L84F_A106V_D108N_H123Y_A142N_S146C_D147Y_R152P_E155V_I156F _K157N).

[0557] 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).

[0558] In some embodiments, the adenosine deaminase is TadA*7.10. In some embodiments, TadA*7.10 comprises at least one alteration. In particular embodiments, TadA*7.10 comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R. The alteration Y123H is also referred to herein as H123H (the alteration H123Y in TadA*7.10 reverted back to Y123H (wt)). In other embodiments, the TadA*7.10 comprises a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R. In particular embodiments, an adenosine deaminase variant comprises a deletion of the C terminus beginning at residue 149, 150, 151, 152, 153, 154, 155, 156, and 157, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

[0559] In other embodiments, a base editor of the disclosure is a monomer comprising an adenosine deaminase variant (*e.g.*, TadA*8) comprising one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, V82S, T166R, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In other embodiments, the adenosine deaminase variant (TadA*8) is a monomer comprising a combination of alterations selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S +

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

[0560] In one embodiment, an adenosine deaminase is a TadA*8 that comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD

[0561] In some embodiments, the TadA*8 is a truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

[0562] In some embodiments the TadA*8 is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

[0563] In one embodiment, a fusion protein of the disclosure comprises a wild-type TadA is

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

[0564] TadA(wt) or "the TadA reference sequence":

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD

[0565] TadA*7.10:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD

[0566] TadA*8:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

[0567] 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.

[0568] In particular embodiments, a TadA*8 comprises one or more mutations at any of the following positions shown in bold. In other embodiments, a TadA*8 comprises one or more mutations at any of the positions shown with underlining:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG 50
LHDPTAHAEI MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG 100
RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCYFFR 150
MPRQVFNAQK KAQSSTD

[0569] For example, the TadA*8 comprises alterations at amino acid position 82 and/or 166 (*e.g.*, V82S, T166R) alone or in combination with any one or more of the following Y147T, Y147R, Q154S, Y123H, and/or Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA. In particular embodiments, a combination of alterations is selected from the group of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R, relative to TadA*7.10, the TadA reference sequence, or a corresponding mutation in another TadA.

[0570] In some embodiments, the adenosine deaminase is TadA*8, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG
RVVFGVRNAK TGAAGSLMDV LHYPGMNHRV EITEGILADE CAALLCTFFR
MPRQVFNAQK KAQSSTD

[0571] In some embodiments, the TadA*8 is truncated. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length TadA*8. In some embodiments, the truncated TadA*8 is missing 1, 2, 3, 4, 5 ,6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length TadA*8. In some embodiments the adenosine deaminase variant is a full-length TadA*8.

[0572] In one embodiment, a fusion protein of the disclosure comprises a wild-type TadA is linked to an adenosine deaminase variant described herein (*e.g.*, TadA*8), which is linked to

Cas9 nickase. In particular embodiments, the fusion proteins comprise a single TadA*8 domain (*e.g.*, provided as a monomer). In other embodiments, the base editor comprises TadA*8 and TadA(wt), which are capable of forming heterodimers.

Additional Domains

[0573] A base editor described herein can include any domain which helps to facilitate the nucleobase editing, modification or altering of a nucleobase of a polynucleotide. In some embodiments, a base editor comprises a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9), a nucleobase editing domain (*e.g.*, deaminase domain), and one or more additional domains. In some embodiments, the additional domain can facilitate enzymatic or catalytic functions of the base editor, binding functions of the base editor, or be inhibitors of cellular machinery (*e.g.*, enzymes) that could interfere with the desired base editing result. In some embodiments, a base editor can comprise a nuclease, a nickase, a recombinase, a deaminase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain.

[0574] In some embodiments, a base editor can comprise an uracil glycosylase inhibitor (UGI) domain. In some embodiments, cellular DNA repair response to the presence of U: G heteroduplex DNA can be responsible for a decrease in nucleobase editing efficiency in cells. In such embodiments, uracil DNA glycosylase (UDG) can catalyze removal of U from DNA in cells, which can initiate base excision repair (BER), mostly resulting in reversion of the U:G pair to a C:G pair. In such embodiments, BER can be inhibited in base editors comprising one or more domains that bind the single strand, block the edited base, inhibit UGI, inhibit BER, protect the edited base, and /or promote repairing of the non-edited strand. Thus, this disclosure contemplates a base editor fusion protein comprising a UGI domain.

[0575] In some embodiments, a base editor comprises as a domain all or a portion of a double-strand break (DSB) binding protein. For example, a DSB binding protein can include a Gam protein of bacteriophage Mu that can bind to the ends of DSBs and can protect them from degradation. See Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire content of which is hereby incorporated by reference.

[0576] Additionally, in some embodiments, a Gam protein can be fused to an N terminus of a base editor. In some embodiments, a Gam protein can be fused to a C-terminus of a base editor.

The Gam protein of bacteriophage Mu can bind to the ends of double strand breaks (DSBs) and protect them from degradation. In some embodiments, using Gam to bind the free ends of DSB can reduce indel formation during the process of base editing. In some embodiments, 174-residue Gam protein is fused to the N terminus of the base editors. See. Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017). In some embodiments, a mutation or mutations can change the length of a base editor domain relative to a wild-type domain. For example, a deletion of at least one amino acid in at least one domain can reduce the length of the base editor. In another case, a mutation or mutations do not change the length of a domain relative to a wild-type domain. For example, substitution(s) in any domain does/do not change the length of the base editor.

[0577] In some embodiments, a base editor can comprise as a domain all or a portion of a nucleic acid polymerase (NAP). For example, a base editor can comprise all or a portion of a eukaryotic NAP. In some embodiments, a NAP or portion thereof incorporated into a base editor is a DNA polymerase. In some embodiments, a NAP or portion thereof incorporated into a base editor has translesion polymerase activity. In some embodiments, a NAP or portion thereof incorporated into a base editor is a translesion DNA polymerase. In some embodiments, a NAP or portion thereof incorporated into a base editor is a Rev7, Rev1 complex, polymerase iota, polymerase kappa, or polymerase eta. In some embodiments, a NAP or portion thereof incorporated into a base editor is a eukaryotic polymerase alpha, beta, gamma, delta, epsilon, gamma, eta, iota, kappa, lambda, mu, or nu component. In some embodiments, a NAP or portion thereof incorporated into a base editor comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to a nucleic acid polymerase (*e.g.*, a translesion DNA polymerase).

BASE EDITOR SYSTEM

[0578] Use of the base editor system provided herein comprises the steps of: (a) contacting a target nucleotide sequence of a polynucleotide (*e.g.*, double- or single stranded DNA or RNA) of a subject with a base editor system comprising a nucleobase editor (*e.g.*, an adenosine base editor) and a guide polynucleic acid (*e.g.*, gRNA), wherein the target nucleotide sequence comprises a targeted 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; and (d) cutting no more than one strand of said target region, where a

third nucleobase complementary to the first nucleobase base is replaced by a fourth nucleobase complementary to the second nucleobase. It should be appreciated that in some embodiments, step (b) is omitted. In some embodiments, said targeted nucleobase pair is a plurality of nucleobase pairs in one or more genes. In some embodiments, the base editor system provided herein is capable of multiplex editing of a plurality of nucleobase pairs in one or more genes. In some embodiments, the plurality of nucleobase pairs is located in the same gene. In some embodiments, the plurality of nucleobase pairs is located in one or more genes, wherein at least one gene is located in a different locus.

[0579] 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 Cas9 domain. In some embodiments, the first base is adenine, and the second base is not a G, C, A, or T. In some embodiments, the second base is inosine.

[0580] Base editing system as provided herein provides a new approach to genome editing that uses a fusion protein containing a catalytically defective *Streptococcus pyogenes* Cas9, an adenosine deaminase, and an inhibitor of base excision repair to induce programmable, single nucleotide (A→G) changes in DNA without generating double-strand DNA breaks, without requiring a donor DNA template, and without inducing an excess of stochastic insertions and deletions.

[0581] Provided herein are systems, compositions, and methods for editing a nucleobase using a base editor system. In some embodiments, the base editor system comprises (1) a base editor (BE) comprising a polynucleotide programmable nucleotide binding domain and a nucleobase editing domain (*e.g.*, a deaminase domain) for editing the nucleobase; and (2) a guide polynucleotide (*e.g.*, guide RNA) in conjunction with the polynucleotide programmable nucleotide binding domain. In some embodiments, the base editor system comprises an adenosine base editor (ABE). In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable DNA binding domain. In some embodiments, the polynucleotide programmable nucleotide binding domain is a polynucleotide programmable RNA binding domain. In some embodiments, the nucleobase editing domain is a deaminase domain. In some embodiments, a deaminase domain can be an adenine deaminase or an adenosine deaminase.

[0582] In some embodiments, the adenosine base editor can deaminate adenine in DNA. In some embodiments, ABE comprises an evolved TadA variant.

[0583] Details of nucleobase editing proteins are described in International PCT Application Nos. PCT/2017/045381 (WO2018/027078) and PCT/US2016/058344 (WO2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

[0584] In some embodiments, a single guide polynucleotide may be utilized to target a deaminase to a target nucleic acid sequence. In some embodiments, a single pair of guide polynucleotides may be utilized to target different deaminases to a target nucleic acid sequence.

[0585] 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.

[0586] 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.

[0587] 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.

[0588] In some embodiments, the base editor inhibits base excision repair (BER) of the edited strand. In some embodiments, the base editor protects or binds the non-edited strand. In some embodiments, the base editor comprises UGI activity. In some embodiments, the base editor

comprises a catalytically inactive inosine-specific nuclease. In some embodiments, the base editor comprises nickase activity. In some embodiments, the intended edit of base pair is upstream of a PAM site. In some embodiments, the intended edit of 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 edit of 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.

[0589] In some embodiments, the method does not require a canonical (*e.g.*, NGG) PAM site. In some embodiments, the nucleobase editor comprises a linker or a spacer. In some embodiments, the linker or spacer is 1-25 amino acids in length. In some embodiments, the linker or spacer is 5-20 amino acids in length. In some embodiments, the linker or spacer is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length.

[0590] In some embodiments, the base editing fusion proteins provided herein need to be positioned at a precise location, for example, where a target base is placed within a defined region (*e.g.*, a "deamination window"). In some embodiments, a target can be within a 4 base region. In some embodiments, such a defined target region can be approximately 15 bases upstream of the PAM. See Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

[0591] 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, 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 edit of base pair is within the target window. In some embodiments, the target window comprises the intended edit of base pair. In some embodiments, the method is performed using any of the base editors provided herein. In some embodiments, a target window is a deamination window. A deamination window can be the defined region in which a base editor acts upon and deaminates a target nucleotide. In some embodiments, the deamination window is within a 2, 3,

4, 5, 6, 7, 8, 9, or 10 base regions. In some embodiments, the deamination window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases upstream of the PAM.

[0592] The base editors of the present disclosure can comprise any domain, feature or amino acid sequence which facilitates the editing of a target polynucleotide sequence. For example, in some embodiments, the base editor comprises a nuclear localization sequence (NLS). In some embodiments, an NLS of the base editor is localized between a deaminase domain and a polynucleotide programmable nucleotide binding domain. In some embodiments, an NLS of the base editor is localized C-terminal to a polynucleotide programmable nucleotide binding domain.

[0593] Other exemplary features that can be present in a base editor as disclosed herein are localization sequences, such as 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.

[0594] Non-limiting examples of protein domains which can be included in the fusion protein include deaminase domains (*e.g.*, adenosine deaminase), a uracil glycosylase inhibitor (UGI) domain, epitope tags, and reporter gene sequences.

[0595] Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-5-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta-galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). Additional protein sequences can include amino acid sequences that bind DNA molecules or bind other cellular molecules, including, but not limited to, maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

[0596] In some embodiments, the adenosine base editor (ABE) can deaminate adenine in DNA. In some embodiments, ABE is generated by replacing APOBEC1 component of BE3 with natural or engineered *E. coli* TadA, human ADAR2, mouse ADA, or human ADAT2. In some embodiments, ABE comprises evolved TadA variant. In some embodiments, the ABE is ABE 1.2 (TadA*-XTEN-nCas9-NLS). In some embodiments, TadA* comprises A106V and D108N mutations.

[0597] In some embodiments, the ABE is a second-generation ABE. In some embodiments, the ABE is ABE2.1, which comprises additional mutations D147Y and E155V in TadA* (TadA*2.1). In some embodiments, the ABE is ABE2.2, ABE2.1 fused to catalytically inactivated version of human alkyl adenine DNA glycosylase (AAG with E125Q mutation). In some embodiments, the ABE is ABE2.3, ABE2.1 fused to catalytically inactivated version of *E. coli* Endo V (inactivated with D35A mutation). In some embodiments, the ABE is ABE2.6 which has a linker twice as long (32 amino acids, $(SGGS)_2$-XTEN-$(SGGS)_2$) as the linker in ABE2.1. In some embodiments, the ABE is ABE2.7, which is ABE2.1 tethered with an additional wild-type TadA monomer. In some embodiments, the ABE is ABE2.8, which is ABE2.1 tethered with an additional TadA*2.1 monomer. In some embodiments, the ABE is ABE2.9, which is a direct fusion of evolved TadA (TadA*2.1) to the N-terminus of ABE2.1. In some embodiments, the ABE is ABE2.10, which is a direct fusion of wild-type TadA to the N-terminus of ABE2.1. In some embodiments, the ABE is ABE2.11, which is ABE2.9 with an inactivating E59A mutation at the N-terminus of TadA* monomer. In some embodiments, the ABE is ABE2.12, which is ABE2.9 with an inactivating E59A mutation in the internal TadA* monomer.

[0598] In some embodiments, the ABE is a third generation ABE. In some embodiments, the ABE is ABE3.1, which is ABE2.3 with three additional TadA mutations (L84F, H123Y, and I156F).

[0599] In some embodiments, the ABE is a fourth generation ABE. In some embodiments, the ABE is ABE4.3, which is ABE3.1 with an additional TadA mutation A142N (TadA*4.3).

[0600] In some embodiments, the ABE is a fifth generation ABE. In some embodiments, the ABE is ABE5.1, which is generated by importing a consensus set of mutations from surviving clones (H36L, R51L, S146C, and K157N) into ABE3.1. In some embodiments, the ABE is ABE5.3, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to an internal evolved TadA*. In some embodiments, the ABE is ABE5.2, ABE5.4, ABE5.5, ABE5.6, ABE5.7, ABE5.8, ABE5.9, ABE5.10, ABE5.11, ABE5.12, ABE5.13, or ABE5.14, as shown in

Table 8 below. In some embodiments, the ABE is a sixth generation ABE. In some embodiments, the ABE is ABE6.1, ABE6.2, ABE6.3, ABE6.4, ABE6.5, or ABE6.6, as shown in **Table 8** below. In some embodiments, the ABE is a seventh generation ABE. In some embodiments, the ABE is ABE7.1, ABE7.2, ABE7.3, ABE7.4, ABE7.5, ABE7.6, ABE7.7, ABE7.8, ABE 7.9, or ABE7.10, as shown in **Table 8** below.

Table 8. Genotypes of ABEs

	23	26	36	37	48	49	51	72	84	87	106	108	123	125	142	146	147	152	155	156	157	161
ABE0.1	W	R	H	N	P		R	N	L	S	A	D	H	G	A	S	D	R	E	I	K	K
ABE0.2	W	R	H	N	P		R	N	L	S	A	D	H	G	A	S	D	R	E	I	K	K
ABE1.1	W	R	H	N	P		R	N	L	S	A	N	H	G	A	S	D	R	E	I	K	K
ABE1.2	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	D	R	E	I	K	K
ABE2.1	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.2	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.3	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.4	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.5	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.6	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.7	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.8	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.9	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.10	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.11	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE2.12	W	R	H	N	P		R	N	L	S	V	N	H	G	A	S	Y	R	V	I	K	K
ABE3.1	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.2	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.3	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.4	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.5	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.6	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.7	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE3.8	W	R	H	N	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE4.1	W	R	H	N	P		R	N	L	S	V	N	H	G	N	S	Y	R	V	I	K	K
ABE4.2	W	G	H	N	P		R	N	L	S	V	N	H	G	N	S	Y	R	V	I	K	K
ABE4.3	W	R	H	N	P		R	N	F	S	V	N	Y	G	N	S	Y	R	V	F	K	K
ABE5.1	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.2	W	R	H	S	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	T
ABE5.3	W	R	L	N	P		L	N	I	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.4	W	R	H	S	P		R	N	F	S	V	N	Y	G	A	S	Y	R	V	F	K	T

	23	26	36	37	48	49	51	72	84	87	106	108	123	125	142	146	147	152	155	156	157	161
ABE5.5	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.6	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.7	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.8	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.9	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.10	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.11	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.12	W	R	L	N	P		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE5.13	W	R	H	N	P		L	D	F	S	V	N	Y	A	A	S	Y	R	V	F	K	K
ABE5.14	W	R	H	N	S		L	N	F	C	V	N	Y	G	A	S	Y	R	V	F	K	K
ABE6.1	W	R	H	N	S		L	N	F	S	V	N	Y	G	N	S	Y	R	V	F	K	K
ABE6.2	W	R	H	N	T	V	L	N	F	S	V	N	Y	G	N	S	Y	R	V	F	N	K
ABE6.3	W	R	L	N	S		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE6.4	W	R	L	N	S		L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
ABE6.5	W	R	L	N	T	V	L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE6.6	W	R	L	N	T	V	L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
ABE7.1	W	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE7.2	W	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
ABE7.3	L	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE7.4	R	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	R	V	F	N	K
ABE7.5	W	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	H	V	F	N	K
ABE7.6	W	R	L	N	A		L	N	I	S	V	N	Y	G	A	C	Y	P	V	F	N	K
ABE7.7	L	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	P	V	F	N	K
ABE7.8	L	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	R	V	F	N	K
ABE7.9	L	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	P	V	F	N	K
ABE7.10	R	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	P	V	F	N	K

[0601] In some embodiments, the base editor is an eighth generation ABE (ABE8). In some embodiments, the ABE8 contains a TadA*8 variant. In some embodiments, the ABE8 has a monomeric construct containing a TadA*8 variant ("ABE8.x-m"). In some embodiments, the ABE8 is ABE8.1-m, which has a monomeric construct containing TadA*7.10 with a Y147T mutation (TadA*8.1). In some embodiments, the ABE8 is ABE8.2-m, which has a monomeric construct containing TadA*7.10 with a Y147R mutation (TadA*8.2). In some embodiments, the ABE8 is ABE8.3-m, which has a monomeric construct containing TadA*7.10 with a Q154S mutation (TadA*8.3). In some embodiments, the ABE8 is ABE8.4-m, which has a monomeric construct containing TadA*7.10 with a Y123H mutation (TadA*8.4). In some embodiments, the

ABE8 is ABE8.5-m, which has a monomeric construct containing TadA*7.10 with a V82S mutation (TadA*8.5). In some embodiments, the ABE8 is ABE8.6-m, which has a monomeric construct containing TadA*7.10 with a T166R mutation (TadA*8.6). In some embodiments, the ABE8 is ABE8.7-m, which has a monomeric construct containing TadA*7.10 with a Q154R mutation (TadA*8.7). In some embodiments, the ABE8 is ABE8.8-m, which has a monomeric construct containing TadA*7.10 with Y147R, Q154R, and Y123H mutations (TadA*8.8). In some embodiments, the ABE8 is ABE8.9-m, which has a monomeric construct containing TadA*7.10 with Y147R, Q154R and I76Y mutations (TadA*8.9). In some embodiments, the ABE8 is ABE8.10-m, which has a monomeric construct containing TadA*7.10 with Y147R, Q154R, and T166R mutations (TadA*8.10). In some embodiments, the ABE8 is ABE8.11-m, which has a monomeric construct containing TadA*7.10 with Y147T and Q154R mutations (TadA*8.11). In some embodiments, the ABE8 is ABE8.12-m, which has a monomeric construct containing TadA*7.10 with Y147T and Q154S mutations (TadA*8.12). In some embodiments, the ABE8 is ABE8.13-m, which has a monomeric construct containing TadA*7.10 with Y123H (Y123H reverted from H123Y), Y147R, Q154R and I76Y mutations (TadA*8.13). In some embodiments, the ABE8 is ABE8.14-m, which has a monomeric construct containing TadA*7.10 with I76Y and V82S mutations (TadA*8.14). In some embodiments, the ABE8 is ABE8.15-m, which has a monomeric construct containing TadA*7.10 with V82S and Y147R mutations (TadA*8.15). In some embodiments, the ABE8 is ABE8.16-m, which has a monomeric construct containing TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y) and Y147R mutations (TadA*8.16). In some embodiments, the ABE8 is ABE8.17-m, which has a monomeric construct containing TadA*7.10 with V82S and Q154R mutations (TadA*8.17). In some embodiments, the ABE8 is ABE8.18-m, which has a monomeric construct containing TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y) and Q154R mutations (TadA*8.18). In some embodiments, the ABE8 is ABE8.19-m, which has a monomeric construct containing TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y), Y147R and Q154R mutations (TadA*8.19). In some embodiments, the ABE8 is ABE8.20-m, which has a monomeric construct containing TadA*7.10 with I76Y, V82S, Y123H (Y123H reverted from H123Y), Y147R and Q154R mutations (TadA*8.20). In some embodiments, the ABE8 is ABE8.21-m, which has a monomeric construct containing TadA*7.10 with Y147R and Q154S mutations (TadA*8.21). In some embodiments, the ABE8 is ABE8.22-m, which has a monomeric construct containing TadA*7.10 with V82S and Q154S mutations (TadA*8.22). In some embodiments, the ABE8 is ABE8.23-m, which has a monomeric construct containing

TadA*7.10 with V82S and Y123H (Y123H reverted from H123Y) mutations (TadA*8.23). In some embodiments, the ABE8 is ABE8.24-m, which has a monomeric construct containing TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y), and Y147T mutations (TadA*8.24).

[0602] In some embodiments, the ABE8 has a heterodimeric construct containing wild-type *E. coli* TadA fused to a TadA*8 variant ("ABE8.x-d"). In some embodiments, the ABE8 is ABE8.1-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a Y147T mutation (TadA*8.1). In some embodiments, the ABE8 is ABE8.2-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a Y147R mutation (TadA*8.2). In some embodiments, the ABE8 is ABE8.3-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a Q154S mutation (TadA*8.3). In some embodiments, the ABE8 is ABE8.4-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a Y123H mutation (TadA*8.4). In some embodiments, the ABE8 is ABE8.5-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a V82S mutation (TadA*8.5). In some embodiments, the ABE8 is ABE8.6-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a T166R mutation (TadA*8.6). In some embodiments, the ABE8 is ABE8.7-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with a Q154R mutation (TadA*8.7). In some embodiments, the ABE8 is ABE8.8-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y147R, Q154R, and Y123H mutations (TadA*8.8). In some embodiments, the ABE8 is ABE8.9-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y147R, Q154R and I76Y mutations (TadA*8.9). In some embodiments, the ABE8 is ABE8.10-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y147R, Q154R, and T166R mutations (TadA*8.10). In some embodiments, the ABE8 is ABE8.11-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y147T and Q154R mutations (TadA*8.11). In some embodiments, the ABE8 is ABE8.12-d, which has heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y147T and Q154S mutations (TadA*8.12). In some embodiments, the ABE8 is ABE8.13-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y123H (Y123H reverted from H123Y), Y147R, Q154R and I76Y mutations (TadA*8.13). In some embodiments, the ABE8 is ABE8.14-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with I76Y and

V82S mutations (TadA*8.14). In some embodiments, the ABE8 is ABE8.15-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S and Y147R mutations (TadA*8.15). In some embodiments, the ABE8 is ABE8.16-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y) and Y147R mutations (TadA*8.16). In some embodiments, the ABE8 is ABE8.17-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S and Q154R mutations (TadA*8.17). In some embodiments, the ABE8 is ABE8.18-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y) and Q154R mutations (TadA*8.18). In some embodiments, the ABE8 is ABE8.19-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y), Y147R and Q154R mutations (TadA*8.19). In some embodiments, the ABE8 is ABE8.20-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with I76Y, V82S, Y123H (Y123H reverted from H123Y), Y147R and Q154R mutations (TadA*8.20). In some embodiments, the ABE8 is ABE8.21-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with Y147R and Q154S mutations (TadA*8.21). In some embodiments, the ABE8 is ABE8.22-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S and Q154S mutations (TadA*8.22). In some embodiments, the ABE8 is ABE8.23-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S and Y123H (Y123H reverted from H123Y) mutations (TadA*8.23). In some embodiments, the ABE8 is ABE8.24-d, which has a heterodimeric construct containing wild-type *E. coli* TadA fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y), and Y147T mutations (TadA*8.24).

[0603] In some embodiments, the ABE8 has a heterodimeric construct containing TadA*7.10 fused to a TadA*8 variant ("ABE8.x-7"). In some embodiments, the ABE8 is ABE8.1-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a Y147T mutation (TadA*8.1). In some embodiments, the ABE8 is ABE8.2-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a Y147R mutation (TadA*8.2). In some embodiments, the ABE8 is ABE8.3-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a Q154S mutation (TadA*8.3). In some embodiments, the ABE8 is ABE8.4-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a Y123H mutation (TadA*8.4). In some embodiments, the ABE8 is ABE8.5-7,

which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a V82S mutation (TadA*8.5). In some embodiments, the ABE8 is ABE8.6-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a T166R mutation (TadA*8.6). In some embodiments, the ABE8 is ABE8.7-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with a Q154R mutation (TadA*8.7). In some embodiments, the ABE8 is ABE8.8-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y147R, Q154R, and Y123H mutations (TadA*8.8). In some embodiments, the ABE8 is ABE8.9-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y147R, Q154R and I76Y mutations (TadA*8.9). In some embodiments, the ABE8 is ABE8.10-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y147R, Q154R, and T166R mutations (TadA*8.10). In some embodiments, the ABE8 is ABE8.11-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y147T and Q154R mutations (TadA*8.11). In some embodiments, the ABE8 is ABE8.12-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y147T and Q154S mutations (TadA*8.12). In some embodiments, the ABE8 is ABE8.13-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y123H (Y123H reverted from H123Y), Y147R, Q154R and I76Y mutations (TadA*8.13). In some embodiments, the ABE8 is ABE8.14-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with I76Y and V82S mutations (TadA*8.14). In some embodiments, the ABE8 is ABE8.15-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S and Y147R mutations (TadA*8.15). In some embodiments, the ABE8 is ABE8.16-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y) and Y147R mutations (TadA*8.16). In some embodiments, the ABE8 is ABE8.17-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S and Q154R mutations (TadA*8.17). In some embodiments, the ABE8 is ABE8.18-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y) and Q154R mutations (TadA*8.18). In some embodiments, the ABE8 is ABE8.19-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y), Y147R and Q154R mutations (TadA*8.19). In some embodiments, the ABE8 is ABE8.20-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with I76Y, V82S, Y123H (Y123H reverted from H123Y), Y147R and Q154R mutations (TadA*8.20). In some embodiments, the ABE8 is ABE8.21-7,

which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with Y147R and Q154S mutations (TadA*8.21). In some embodiments, the ABE8 is ABE8.22-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S and Q154S mutations (TadA*8.22). In some embodiments, the ABE8 is ABE8.23-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S and Y123H (Y123H reverted from H123Y) mutations (TadA*8.23). In some embodiments, the ABE8 is ABE8.24-7, which has a heterodimeric construct containing TadA*7.10 fused to TadA*7.10 with V82S, Y123H (Y123H reverted from H123Y), and Y147T mutations (TadA*8.24

[0604] In some embodiments, the ABE is ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, or ABE8.24-d as shown in **Table 9** below.

Table 9: Adenosine Deaminase Base Editor 8 Variants

ABE8	Adenosine Deaminase	Adenosine Deaminase Description
ABE8.1-m	TadA*8.1	Monomer_TadA*7.10 + Y147T
ABE8.2-m	TadA*8.2	Monomer_TadA*7.10 + Y147R
ABE8.3-m	TadA*8.3	Monomer_TadA*7.10 + Q154S
ABE8.4-m	TadA*8.4	Monomer_TadA*7.10 + Y123H
ABE8.5-m	TadA*8.5	Monomer_TadA*7.10 + V82S
ABE8.6-m	TadA*8.6	Monomer_TadA*7.10 + T166R
ABE8.7-m	TadA*8.7	Monomer_TadA*7.10 + Q154R
ABE8.8-m	TadA*8.8	Monomer_TadA*7.10 + Y147R_Q154R_Y123H
ABE8.9-m	TadA*8.9	Monomer_TadA*7.10 + Y147R_Q154R_I76Y
ABE8.10-m	TadA*8.10	Monomer_TadA*7.10 + Y147R_Q154R_T166R
ABE8.11-m	TadA*8.11	Monomer_TadA*7.10 + Y147T_Q154R
ABE8.12-m	TadA*8.12	Monomer_TadA*7.10 + Y147T_Q154S
ABE8.13-m	TadA*8.13	Monomer_TadA*7.10 + Y123H_Y147R_Q154R_I76Y
ABE8.14-m	TadA*8.14	Monomer_TadA*7.10 + I76Y_V82S
ABE8.15-m	TadA*8.15	Monomer_TadA*7.10 + V82S_Y147R
ABE8.16-m	TadA*8.16	Monomer_TadA*7.10 + V82S_Y123H_Y147R
ABE8.17-m	TadA*8.17	Monomer_TadA*7.10 + V82S_Q154R
ABE8.18-m	TadA*8.18	Monomer_TadA*7.10 + V82S_Y123H_Q154R

ABE8	Adenosine Deaminase	Adenosine Deaminase Description
ABE8.19-m	TadA*8.19	Monomer_TadA*7.10 + V82S_Y123H_Y147R_Q154R
ABE8.20-m	TadA*8.20	Monomer_TadA*7.10 + I76Y_V82S_Y123H_Y147R_Q154R
ABE8.21-m	TadA*8.21	Monomer_TadA*7.10 + Y147R_Q154S
ABE8.22-m	TadA*8.22	Monomer_TadA*7.10 + V82S_Q154S
ABE8.23-m	TadA*8.23	Monomer_TadA*7.10 + V82S_Y123H
ABE8.24-m	TadA*8.24	Monomer_TadA*7.10 + V82S_Y123H_Y147T
ABE8.1-d	TadA*8.1	Heterodimer_(WT) + (TadA*7.10 + Y147T)
ABE8.2-d	TadA*8.2	Heterodimer_(WT) + (TadA*7.10 + Y147R)
ABE8.3-d	TadA*8.3	Heterodimer_(WT) + (TadA*7.10 + Q154S)
ABE8.4-d	TadA*8.4	Heterodimer_(WT) + (TadA*7.10 + Y123H)
ABE8.5-d	TadA*8.5	Heterodimer_(WT) + (TadA*7.10 + V82S)
ABE8.6-d	TadA*8.6	Heterodimer_(WT) + (TadA*7.10 + T166R)
ABE8.7-d	TadA*8.7	Heterodimer_(WT) + (TadA*7.10 + Q154R)
ABE8.8-d	TadA*8.8	Heterodimer_(WT) + (TadA*7.10 + Y147R_Q154R_Y123H)
ABE8.9-d	TadA*8.9	Heterodimer_(WT) + (TadA*7.10 + Y147R_Q154R_I76Y)
ABE8.10-d	TadA*8.10	Heterodimer_(WT) + (TadA*7.10 + Y147R_Q154R_T166R)
ABE8.11-d	TadA*8.11	Heterodimer_(WT) + (TadA*7.10 + Y147T_Q154R)
ABE8.12-d	TadA*8.12	Heterodimer_(WT) + (TadA*7.10 + Y147T_Q154S)
ABE8.13-d	TadA*8.13	Heterodimer_(WT) + (TadA*7.10 + Y123H_Y147T_Q154R_I76Y)
ABE8.14-d	TadA*8.14	Heterodimer_(WT) + (TadA*7.10 + I76Y_V82S)
ABE8.15-d	TadA*8.15	Heterodimer_(WT) + (TadA*7.10 + V82S_ Y147R)
ABE8.16-d	TadA*8.16	Heterodimer_(WT) + (TadA*7.10 + V82S_Y123H_Y147R)
ABE8.17-d	TadA*8.17	Heterodimer_(WT) + (TadA*7.10 + V82S_Q154R)
ABE8.18-d	TadA*8.18	Heterodimer_(WT) + (TadA*7.10 + V82S_Y123H_Q154R)
ABE8.19-d	TadA*8.19	Heterodimer_(WT) + (TadA*7.10 + V82S_Y123H_Y147R_Q154R)
ABE8.20-d	TadA*8.20	Heterodimer_(WT) + (TadA*7.10 + I76Y_V82S_Y123H_Y147R_Q154R)
ABE8.21-d	TadA*8.21	Heterodimer_(WT) + (TadA*7.10 + Y147R_Q154S)
ABE8.22-d	TadA*8.22	Heterodimer_(WT) + (TadA*7.10 + V82S_Q154S)
ABE8.23-d	TadA*8.23	Heterodimer_(WT) + (TadA*7.10 + V82S_Y123H)
ABE8.24-d	TadA*8.24	Heterodimer_(WT) + (TadA*7.10 + V82S_Y123H_Y147T)

[0605] In some embodiments, base editors (*e.g.*, ABE8) are generated by cloning an adenosine deaminase variant (*e.g.*, TadA*8) into a scaffold that includes a circular permutant Cas9 (*e.g.*, CP5 or CP6) and a bipartite nuclear localization sequence. In some embodiments, the base editor (*e.g.*, ABE7.9, ABE7.10, or ABE8) is an NGC PAM CP5 variant (*S. pyrogenes* Cas9 or spVRQR Cas9). In some embodiments, the base editor (*e.g.*, ABE7.9, ABE7.10, or ABE8) is an AGA PAM CP5 variant (*S. pyrogenes* Cas9 or spVRQR Cas9). In some embodiments, the base editor (*e.g.*, ABE7.9, ABE7.10, or ABE8) is an NGC PAM CP6 variant (*S. pyrogenes* Cas9 or spVRQR Cas9).

In some embodiments, the base editor (*e.g.* ABE7.9, ABE7.10, or ABE8) is an AGA PAM CP6 variant (*S. pyrogenes* Cas9 or spVRQR Cas9).

[0606] In some embodiments, the ABE has a genotype as shown in **Table 10** below.

Table 10. Genotypes of ABEs

	23	26	36	37	48	49	51	72	84	87	105	108	123	125	142	145	147	152	155	156	157	161
ABE7.9	L	R	L	N	A		L	N	F	S	V	N	Y	G	N	C	Y	P	V	F	N	K
ABE7.10	R	R	L	N	A		L	N	F	S	V	N	Y	G	A	C	Y	P	V	F	N	K

[0607] As shown in **Table 11** below, genotypes of 40 ABE8s are described. Residue positions in the evolved *E. coli* TadA portion of ABE are indicated. Mutational changes in ABE8 are shown when distinct from ABE7.10 mutations. In some embodiments, the ABE has a genotype of one of the ABEs as shown in **Table 11** below.

Table 11. Residue Identity in Evolved TadA

	23	36	48	51	76	82	84	106	108	123	146	147	152	154	155	156	157	166
ABE7.10	**R**	**L**	**A**	**L**	**I**	**V**	**F**	**V**	**N**	**Y**	**C**	**Y**	**P**	**Q**	**V**	**F**	**N**	**T**
ABE8.1-m												T						
ABE8.2-m												R						
ABE8.3-m														S				
ABE8.4-m										H								
ABE8.5-m						S												
ABE8.6-m																		R
ABE8.7-m														R				
ABE8.8-m										H		R		R				
ABE8.9-m					Y							R		R				
ABE8.10-m												R		R				R
ABE8.11-m												T		R				
ABE8.12-m												T		S				
ABE8.13-m					Y					H		R		R				
ABE8.14-m					Y	S												
ABE8.15-m						S						R						
ABE8.16-m						S				H		R						
ABE8.17-m						S								R				
ABE8.18-m						S				H				R				
ABE8.19-m						S				H		R		R				
ABE8.20-m					Y	S				H		R		R				
ABE8.21-m												R		S				
ABE8.22-m						S								S				
ABE8.23-m						S				H								

ABE8.24-m						S				H		T						
ABE8.1-d												T						
ABE8.2-d												R						
ABE8.3-d														S				
ABE8.4-d										H								
ABE8.5-d						S												
ABE8.6-d																		R
ABE8.7-d														R				
ABE8.8-d										H		R		R				
ABE8.9-d					Y							R		R				
ABE8.10-d												R		R				R
ABE8.11-d												T		R				
ABE8.12-d												T		S				
ABE8.13-d					Y					H		R		R				
ABE8.14-d					Y	S												
ABE8.15-d						S						R						
ABE8.16-d						S				H		R						
ABE8.17-d						S								R				
ABE8.18-d						S				H				R				
ABE8.19-d						S				H		R		R				
ABE8.20-d					Y	S				H		R		R				
ABE8.21-d												R		S				
ABE8.22-d						S								S				
ABE8.23-d						S				H								
ABE8.24-d						S				H		T						

[0608] In some embodiments, the base editor is ABE8.1, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

[0609] ABE8.1_Y147T_CP5_NGC PAM_monomer

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD***SGGSSGGSSGSETPGTSESATPESSGGS***
SGGSEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEKGK
SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAK
FLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILA
DANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYRSTKEVLDATLI
HQSITGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM*DKKYSIGLAIGTNSVGWAVITDEY
KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEM

AKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY
LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR
RLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQ
YADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIH
LGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQK
KAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNE
ENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQS
GKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQT
VKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQ
LQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV
PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL
DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

[0610] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

[0611] In some embodiments, the base editor is ABE8.1, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

[0612] pNMG-B335 ABE8.1_Y147T_CP5_NGC PAM_monomer

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTDSGGSSG*GSSGSETPGTSESATPESSGGS*
*SGGS*EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVEKGK
SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAK
FLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILA
DANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYRSTKEVLDATLI
HQSITGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM*DKKYSIGLAIGTNSVGWAVITDEY
KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEM
AKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY
LALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR

RLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQ
YADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIH
LGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEE
VVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQK
KAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNE
ENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQS
GKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQT
VKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQ
LQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV
PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL
DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

[0613] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

[0614] In some embodiments, the base editor is ABE8.14, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

[0615] pNMG-357_ABE8.14 with NGC PAM CP5

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDGGSSGGS*SGSETPGTSESATPESSGGSS*
*GGS*MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESS*
*GGSSGGS*EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRK
VLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFMQPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLA
SAKFLQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRV
ILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPRAFKYFDTTIARKEYRSTKEVLDA
TLIHQSITGLYETRIDLSQLGGD*GGSGGSGGSGGSGGSGGSGGM*DKKYSIGLAIGTNSVGWAVIT
DEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS
NEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLR

LIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLS
KSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQI
GDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKY
KEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPH
QIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWN
FEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSG
EQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFL
DNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD
KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGI
LQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVE
NTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS
DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVA
QILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAL
IKKYPKLESEFVYGDYKVYDVRKMIAKSEQEGADKRTADGSEFESPKKKRKV*

[0616] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, and the underlined sequence denotes a bipartite nuclear localization sequence.

[0617] In some embodiments, the base editor is ABE8.8-m, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.8-m

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS***DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT**
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV

MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV*

[0618] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0619] In some embodiments, the base editor is ABE8.8-d, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.8-d

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLH<u>H</u>PGMN
HRVEITEGILADECAALLC<u>R</u>FFRMPR<u>R</u>VFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESS*
*GGSSGGS*DKKYSIGL<u>A</u>IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLV
QTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKS
NFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMD
GTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIP
YYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL

YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVE
ISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD
DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQ
KAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD
HIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALP
SKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY
NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETR
IDLSQLGGDEGADKRTADGSEFESPKKKRKV*

[0620] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0621] In some embodiments, the base editor is ABE8.13-m, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.13-m

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG

VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV*

[0622] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0623] In some embodiments, the base editor is ABE8.13-d, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.13-d

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMN
HRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESS*
*GGSSGGS*DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLV
QTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKS
NFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMD
GTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIP

YYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVE
ISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD
DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQ
KAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD
HIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALP
SKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY
NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETR
IDLSQLGGDEGADKRTADGSEFESPKKKRKV*

[0624] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0625] In some embodiments, the base editor is ABE8.17-m, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.17-m

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRRVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY

FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV*

[0626] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0627] In some embodiments, the base editor is ABE8.17-d, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.17-d

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLIDATLY<u>S</u>TFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMN
HRVEITEGILADECAALLCYFFRMPR<u>R</u>VFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESS*
*GGSSGGS*DKKYSIGL<u>A</u>IGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLV
QTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKS
NFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA
SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMD

GTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIP
YYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVE
ISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD
DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQ
KAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD
HIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALP
SKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY
NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETR
IDLSQLGGDEGADKRTADGSEFESPKKKRKV*

[0628] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0629] In some embodiments, the base editor is ABE8.20-m, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.20-m

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLYDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV

GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV*

[0630] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0631] In some embodiments, the base editor is ABE8.20-d, which comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

ABE8.20-d

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESSGGS*
*SGGS*SEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMA
LRQGGLVMQNYRLYDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMN
HRVEITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTD*SGGSSGGSSGSETPGTSESATPESS*
*GGSSGGS*DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD
EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLV
QTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKS
NFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSA

SMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMD
GTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIP
YYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL
YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVE
ISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD
DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQ
KAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ
KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD
HIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL
GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALP
SKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY
NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETR
IDLSQLGGD*EGADKRTADGSEFESPKKKRKV**

[0632] In the above sequence, the plain text denotes an adenosine deaminase sequence, bold sequence indicates sequence derived from Cas9, the italicized sequence denotes a linker sequence, underlined sequence denotes a bipartite nuclear localization sequence, and double underlined sequence indicates mutations.

[0633] In some embodiments, an ABE8 of the disclosure is selected from the following sequences:

[0634] 01. monoABE8.1_bpNLS + Y147T

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE

ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0635] 02. monoABE8.1_bpNLS + Y147R

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCRFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG

LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0636] 03. monoABE8.1_bpNLS + Q154S

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRSVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0637] 04. monoABE8.1_bpNLS + Y123H

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0638] 05. monoABE8.1_bpNLS + V82S

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD

LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0639] 06. monoABE8.1_bpNLS + T166R

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRQVFNAQKKAQSSRDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS

RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0640] 07. monoABE8.1_bpNLS + Q154R

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
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EITEGILADECAALLCYFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH

RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0641] 08. monoABE8.1_bpNLS + Y147R_Q154R_Y123H

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
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EITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0642] 09. monoABE8.1_bpNLS + Y147R_Q154R_I76Y

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA

YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0643] 10. monoABE8.1_bpNLS + Y147R_Q154R_T166R

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
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SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV

MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0644] 11. monoABE8.1_bpNLS + Y147T_Q154R

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SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT

IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0645] 12. monoABE8.1_bpNLS + Y147T_Q154S

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCTFFRMPRSVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0646] 13. monoABE8.1_bpNLS + H123Y123H_Y147R_Q154R_I76Y

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLYDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHHPGMNHRV
EITEGILADECAALLCRFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS

SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0647] 14. monoABE8.1_bpNLS + V82S + Q154R

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQ
GGLVMQNYRLIDATLYSTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRV
EITEGILADECAALLCYFFRMPRRVFNAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGS
SGGSDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMI
KRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV
GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY

FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
PQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF
YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYF
FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQT
GGFSKESILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGIT
IMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASARELQKGNELALPSKY
VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH
RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVLDATLIHQSITGLYETRIDL
SQLGGDEGADKRTADGSEFESPKKKRKV

[0648] In some embodiments, the base editor is a fusion protein comprising a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9-derived domain) fused to a nucleobase editing domain (*e.g.*, all or a portion of a deaminase domain). 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).

[0649] In some embodiments, the base editor further comprises a domain comprising all or a portion of a uracil glycosylase inhibitor (UGI). In some embodiments, the base editor comprises a domain comprising all or a portion of a uracil binding protein (UBP), such as a uracil DNA glycosylase (UDG). In some embodiments, the base editor comprises a domain comprising all or a portion of a nucleic acid polymerase. In some embodiments, a nucleic acid polymerase or portion thereof incorporated into a base editor is a translesion DNA polymerase.

[0650] In some embodiments, a domain of the base editor can comprise multiple domains. For example, the base editor comprising a polynucleotide programmable nucleotide binding domain derived from Cas9 can comprise an REC lobe and an NUC lobe corresponding to the REC lobe and NUC lobe of a wild-type or natural Cas9. In another example, the base editor can comprise

one or more of a RuvCI domain, BH domain, REC1 domain, REC2 domain, RuvCII domain, L1 domain, HNH domain, L2 domain, RuvCIII domain, WED domain, TOPO domain or CTD domain. In some embodiments, one or more domains of the base editor comprise a mutation (*e.g.*, substitution, insertion, deletion) relative to a wild type version of a polypeptide comprising the domain. For example, an HNH domain of a polynucleotide programmable DNA binding domain can comprise an H840A substitution. In another example, a RuvCI domain of a polynucleotide programmable DNA binding domain can comprise a D10A substitution.

[0651] Different domains (*e.g.*, adjacent domains) of the base editor disclosed herein can be connected to each other with or without the use of one or more linker domains (*e.g.*, an XTEN linker domain). In some embodiments, a linker domain can be a bond (*e.g.*, covalent bond), chemical group, or a molecule linking two molecules or moieties, *e.g.*, two domains of a fusion protein, such as, for example, a first domain (*e.g.*, Cas9-derived domain) and a second domain (*e.g.*, an adenosine deaminase domain). In some embodiments, a linker is a covalent bond (*e.g.*, a carbon-carbon bond, disulfide bond, carbon-hetero atom bond, etc.). In certain embodiments, a linker is a carbon nitrogen bond of an amide linkage. In certain embodiments, a linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, a linker is polymeric (*e.g.*, polyethylene, polyethylene glycol, polyamide, polyester, etc.). In certain embodiments, a linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In some embodiments, a linker comprises an aminoalkanoic acid (*e.g.*, glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, etc.). In some embodiments, a linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, a linker is based on a carbocyclic moiety (*e.g.*, cyclopentane, cyclohexane). In other embodiments, a linker comprises a polyethylene glycol moiety (PEG). In certain embodiments, a linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. A linker can include functionalized moieties to facilitate attachment of a nucleophile (*e.g.*, thiol, amino) from the peptide to the linker. Any electrophile can 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, 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 second domain (*e.g.*, UGI, etc.).

[0652] Typically, a 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, a linker is an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, a linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, a linker is 2-100 amino acids in length, for example, 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, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 amino acids in length. In some embodiments, the linker is about 3 to about 104 (*e.g.*, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100) amino acids in length. Longer or shorter linkers are also contemplated. In some embodiments, a linker domain comprises the amino acid sequence SGSETPGTSESATPES, which can also be referred to as the XTEN linker. Any method for linking the fusion protein domains can be employed (*e.g.,* ranging from very flexible linkers of the form (SGGS)n, (GGGS)n, (GGGGS)n, and (G)n, to more rigid linkers of the form (EAAAK)n, (GGS)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), or $(XP)_n$ motif, 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, a linker comprises a plurality of proline residues and is 5-21, 5-14, 5-9, 5-7 amino acids in length, *e.g.*, PAPAP, PAPAPA, PAPAPAP, PAPAPAPA, $P(AP)_4$, $P(AP)_7$, $P(AP)_{10}$ (see, *e.g.*, Tan J, Zhang F, Karcher D, Bock R. Engineering of high-precision base editors for site-specific single nucleotide replacement. Nat Commun. 2019 Jan 25;10(1):439; the entire contents are incorporated herein by reference). Such proline-rich linkers are also termed "rigid" linkers.

[0653] A fusion protein as described herein comprises a nucleic acid editing domain. In some embodiments, the deaminase is an adenosine deaminase. 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.

Linkers

[0654] In certain embodiments, linkers may be used to link any of the peptides or peptide domains as described herein. The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (*e.g.*, a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, *etc.*). In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, the linker is polymeric (*e.g.*, polyethylene, polyethylene glycol, polyamide, polyester, *etc.*). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (*e.g.*, glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, *etc.*). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, the linker is based on a carbocyclic moiety (*e.g.*, cyclopentane, cyclohexane). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In other embodiments, the linker comprises amino acids. In certain embodiments, the linker comprises a peptide. In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. The linker may include functionalized moieties to facilitate attachment of a nucleophile (*e.g.*, thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated amides, Michael acceptors, alkyl halides, aryl halides, acyl halides, and isothiocyanates.

[0655] In some embodiments, the linker is an amino acid or a plurality of amino acids (*e.g.*, a peptide or protein). In some embodiments, the linker is a bond (*e.g.*, a covalent bond), an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is about 3 to about 104 (*e.g.*, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100) amino acids in length.

[0656] In some embodiments, the adenosine deaminase and the napDNAbp are fused via a linker that is 4, 16, 32, or 104 amino acids in length. In some embodiments, the linker is about 3 to about 104 amino acids in length. In some embodiments, any of the fusion proteins provided

herein, comprise an adenosine deaminase and a Cas9 domain that are fused to each other via a linker. Various linker lengths and flexibilities between the deaminase domain (*e.g.*, an engineered ecTadA) and the Cas9 domain 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 adenosine deaminase and the Cas9 domain of any of the fusion proteins provided herein are fused via a linker (*e.g.*, an XTEN linker) comprising the amino acid sequence SGSETPGTSESATPES.

Cas9 complexes with guide RNAs

[0657] Some aspects of this disclosure provide complexes comprising any of the fusion proteins provided herein, and a guide RNA (*e.g.*, a guide that targets an Mecp2 allele bearing RTT targetable mutations) bound to a CAS9 domain (*e.g.*, a dCas9, a nuclease active Cas9, or a Cas9 nickase) of fusion protein. These complexes are also termed ribonucleoproteins (RNPs). Any method for linking the fusion protein domains can be employed (*e.g.*, ranging from very flexible linkers of the form $(GGGS)_n$, $(GGGGS)_n$, and $(G)_n$ to more rigid linkers of the form $(EAAAK)_n$, $(SGGS)_n$, SGSETPGTSESATPES (see, *e.g.*, Guilinger JP, Thompson DB, Liu DR. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 2014; 32(6): 577-82; the entire contents are incorporated herein by reference) and $(XP)_n$) in order to achieve the optimal length for activity for the nucleobase editor. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the linker comprises a $(GGS)_n$ motif, wherein n is 1, 3, or 7. In some embodiments, the Cas9 domain of the fusion proteins provided herein are fused via a linker comprising the amino acid sequence SGSETPGTSESATPES..

[0658] 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 or 5′-NAA-3′). In some embodiments, the guide nucleic acid (*e.g.*, guide RNA) is complementary to a sequence in Mecp2 allele bearing RTT targetable mutations.

[0659] 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, NAA, NGCG, NGN, NNGRRT, NNNRRT, NGCG, NGCN, NGTN, NGTN, NGTN, or 5′ (TTTV) sequence.

[0660] 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 differ, *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.

[0661] It will be apparent to those of skill in the art that in order to target any of fusion protein comprising a Cas9 domain and an adenosine deaminase variant (*e.g.*, TadA*8), 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.

[0662] The domains of the base editor disclosed herein can be arranged in any order. Non-limiting examples of a base editor comprising a fusion protein comprising *e.g.*, a polynucleotide-programmable nucleotide-binding domain and a deaminase domain can be arranged as following:
NH_2-[nucleobase editing domain]-Linker1-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, adenosine deaminase]-Linker1-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, adenosine deaminase]-Linker1-[*e.g.*, Cas9 derived domain]-Linker2-[UGI]-COOH;
NH_2-[*e.g.*, TadA*7.10]-Linker1-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, adenosine deaminase]-Linker1-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, TadA*7.10]-Linker1-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, TadA*7.10]-Linker1-[*e.g.*, Cas9 derived domain]-Linker2-[UGI]-COOH
NH_2-[*e.g.*, adenosine deaminase]-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, Cas9 derived domain]-[*e.g.*, adenosine deaminase]-COOH;
NH_2-[*e.g.*, adenosine deaminase]-[*e.g.*, Cas9 derived domain]-[inosine BER inhibitor]-COOH;
NH_2-[*e.g.*, adenosine deaminase]-[inosine BER inhibitor]-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[inosine BER inhibitor]-[*e.g.*, adenosine deaminase]-[*e.g.*, Cas9 derived domain]-COOH;
NH_2-[*e.g.*, Cas9 derived domain]-[*e.g.*, adenosine deaminase]-[inosine BER inhibitor]-COOH;
NH_2-[*e.g.*, Cas9 derived domain]-[inosine BER inhibitor]-[*e.g.*, adenosine deaminase]-COOH; or
NH_2-[inosine BER inhibitor]-[*e.g.*, Cas9 derived domain]-[*e.g.*, adenosine deaminase]-COOH.

[0663] Additionally, in some embodiments, a Gam protein can be fused to an N terminus of a base editor. In some embodiments, a Gam protein can be fused to a C terminus of a base editor. The Gam protein of bacteriophage Mu can bind to the ends of double strand breaks (DSBs) and protect them from degradation. In some embodiments, using Gam to bind the free ends of DSB can reduce indel formation during the process of base editing. In some embodiments, 174-residue Gam protein is fused to the N terminus of the base editors. See Komor, A.C., *et al.*,

"Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017). In some embodiments, a mutation or mutations can change the length of a base editor domain relative to a wild type domain. For example, a deletion of at least one amino acid in at least one domain can reduce the length of the base editor. In another case, a mutation or mutations do not change the length of a domain relative to a wild type domain. For example, substitutions in any domain does not change the length of the base editor.

[0664] In some embodiments, the base editing fusion proteins provided herein need to be positioned at a precise location, for example, where a target base is placed within a defined region (*e.g.*, a "deamination window"). In some embodiments, a target can be within a 4-base region. In some embodiments, such a defined target region can be approximately 15 bases upstream of the PAM. See Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

[0665] A defined target region can be a deamination window. A deamination window can be the defined region in which a base editor acts upon and deaminates a target nucleotide. In some embodiments, the deamination window is within a 2, 3, 4, 5, 6, 7, 8, 9, or 10 base regions. In some embodiments, the deamination window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases upstream of the PAM.

[0666] The base editors of the present disclosure can comprise any domain, feature or amino acid sequence which facilitates the editing of a target polynucleotide sequence. For example, in some embodiments, the base editor comprises a nuclear localization sequence (NLS). In some embodiments, an NLS of the base editor is localized between a deaminase domain and a polynucleotide programmable nucleotide binding domain. In some embodiments, an NLS of the base editor is localized C-terminal to a polynucleotide programmable nucleotide binding domain.

[0667] Non-limiting examples of protein domains which can be included in the fusion protein include a deaminase domain (*e.g.*, adenosine deaminase), a uracil glycosylase inhibitor (UGI) domain, epitope tags, reporter gene sequences, and/or protein domains having one or more of the following activities: methylase activity, demethylase activity, transcription activation activity,

transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity, and nucleic acid binding activity. Additional domains can be a heterologous functional domain. Such heterologous functional domains can confer a function activity, such as DNA methylation, DNA damage, DNA repair, modification of a target polypeptide associated with target DNA (*e.g.*, a histone, a DNA-binding protein, etc.), leading to, for example, histone methylation, histone acetylation, histone ubiquitination, and the like.

[0668] Other functions conferred can include methyltransferase activity, demethylase activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, remodeling activity, protease activity, oxidoreductase activity, transferase activity, hydrolase activity, lyase activity, isomerase activity, synthase activity, synthetase activity, and demyristoylation activity, or any combination thereof.

[0669] Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-5-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta-galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). Additional protein sequences can include amino acid sequences that bind DNA molecules or bind other cellular molecules, including but not limited to maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

Cas12 complexes with guide RNAs

[0670] Some aspects of this disclosure provide complexes comprising any of the fusion proteins provided herein, and a guide RNA (*e.g.*, a guide that targets a target polynucleotide for editing).

[0671] 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. In some embodiments, the 3′ end of the target sequence is immediately adjacent to a non-canonical PAM sequence.

[0672] 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 *e.g.*, TTN, DTTN, GTTN, ATTN, ATTC, DTTNT, WTTN, HATY, TTTN, TTTV, TTTC, TG, RTR, or YTN PAM site.

[0673] 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.

[0674] 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 Cas12 binding, and a guide sequence, which confers sequence specificity to the Cas12: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 Cas12: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.

[0675] The domains of the base editor disclosed herein can be arranged in any order as long as the deaminase domain is internalized in the Cas12 protein. Non-limiting examples of a base editor comprising a fusion protein comprising *e.g.*, a Cas12 domain and a deaminase domain can be arranged as following:

NH2-[Cas12 domain]-Linker1-[ABE8]-Linker2-[Cas12 domain]-COOH;
NH2-[Cas12 domain]-Linker1-[ABE8]-[Cas12 domain]-COOH;
NH2-[Cas12 domain]-[ABE8]-Linker2-[Cas12 domain]-COOH;
NH2-[Cas12 domain]-[ABE8]-[Cas12 domain]-COOH;
NH2-[Cas12 domain]-Linker1-[ABE8]-Linker2-[Cas12 domain]-[inosine BER inhibitor]-COOH;
NH2-[Cas12 domain]-Linker1-[ABE8]-[Cas12 domain]-[inosine BER inhibitor]-COOH;
NH2-[Cas12 domain]-[ABE8]-Linker2-[Cas12 domain]-[inosine BER inhibitor]-COOH;
NH2-[Cas12 domain]-[ABE8]-[Cas12 domain]-[inosine BER inhibitor]-COOH;
NH2-[inosine BER inhibitor]-[Cas12 domain]-Linker1-[ABE8]-Linker2-[Cas12 domain]-COOH;
NH2-[inosine BER inhibitor]-[Cas12 domain]-Linker1-[ABE8]-[Cas12 domain]-COOH;
NH2-[inosine BER inhibitor]-[Cas12 domain]-[ABE8]-Linker2-[Cas12 domain]-COOH;
NH2-[inosine BER inhibitor]NH2-[Cas12 domain]-[ABE8]-[Cas12 domain]-COOH;

[0676] Additionally, in some cases, a Gam protein can be fused to an N terminus of a base editor. In some cases, a Gam protein can be fused to a C terminus of a base editor. The Gam protein of bacteriophage Mu can bind to the ends of double strand breaks (DSBs) and protect them from degradation. In some embodiments, using Gam to bind the free ends of DSB can reduce indel formation during the process of base editing. In some embodiments, 174-residue Gam protein is fused to the N terminus of the base editors. See. Komor, A.C., et al., "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017). In some cases, a mutation or mutations can change the length of a base editor domain relative to a wild type

domain. For example, a deletion of at least one amino acid in at least one domain can reduce the length of the base editor. In another case, a mutation or mutations do not change the length of a domain relative to a wild type domain. For example, substitution(s) in any domain does/do not change the length of the base editor.

[0677] In some embodiments, the base editing fusion proteins provided herein need to be positioned at a precise location, for example, where a target base is placed within a defined region (*e.g.*, a "deamination window"). In some cases, a target can be within a 4-base region. In some cases, such a defined target region can be approximately 15 bases upstream of the PAM. See Komor, A.C., et al., "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., et al., "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., et al., "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

[0678] A defined target region can be a deamination window. A deamination window can be the defined region in which a base editor acts upon and deaminates a target nucleotide. In some embodiments, the deamination window is within a 2, 3, 4, 5, 6, 7, 8, 9, or 10 base regions. In some embodiments, the deamination window is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases upstream of the PAM.

[0679] The base editors of the present disclosure can comprise any domain, feature or amino acid sequence which facilitates the editing of a target polynucleotide sequence. For example, in some embodiments, the base editor comprises a nuclear localization sequence (NLS). In some embodiments, an NLS of the base editor is localized between a deaminase domain and a napDNAbp domain. In some embodiments, an NLS of the base editor is localized C-terminal to a napDNAbp domain.

[0680] Protein domains included in the fusion protein can be a heterologous functional domain. Non-limiting examples of protein domains which can be included in the fusion protein include a deaminase domain (*e.g.*, adenosine deaminase), a uracil glycosylase inhibitor (UGI) domain, epitope tags, and reporter gene sequences. Protein domains can be a heterologous functional domain, for example, having one or more of the following activities: transcriptional activation activity, transcriptional repression activity, transcription release factor activity, gene silencing activity, chromatin modifying activity, epigenetic modifying activity, histone modification

activity, RNA cleavage activity, and nucleic acid binding activity. Such heterologous functional domains can confer a function activity, such as modification of a target polypeptide associated with target DNA (*e.g.*, a histone, a DNA binding protein, etc.), leading to, for example, histone methylation, histone acetylation, histone ubiquitination, and the like. Other functions and/or activities conferred can include transposase activity, integrase activity, recombinase activity, ligase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylation activity, deSUMOylation activity, or any combination of the above.

[0681] A domain may be detected or labeled with an epitope tag, a reporter protein, other binding domains. Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-5-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta-galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). Additional protein sequences can include amino acid sequences that bind DNA molecules or bind other cellular molecules, including but not limited to maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

[0682] In some embodiments, BhCas12b guide polynucleotide has the following sequence:

BhCas12b sgRNA scaffold (underlined) + 20nt to 23nt guide sequence (denoted by N_n)

5′GUUCUGTCUUUUGGUCAGGACAACCGUCUAGCUAUAAGUGCUGCAGGGUGUGAGAAACUCCUA
UUGCUGGACGAUGUCUCUUACGAGGCAUUAGCACNNNNNNNNNNNNNNNNNNNN-3′

[0683] In some embodiments, BvCas12b and AaCas12b guide polynucleotides have the following sequences:

BvCas12b sgRNA scaffold (underlined) + 20nt to 23nt guide sequence (denoted by N_n)

5′GACCUAUAGGGUCAAUGAAUCUGUGCGUGUGCCAUAAGUAAUUAAAAAUUACCCACCACAGGA
GCACCUGAAAACAGGUGCUUGGCACNNNNNNNNNNNNNNNNNNNN-3′

AaCas12b sgRNA scaffold (underlined) + 20nt to 23nt guide sequence (denoted by N_n)

5′GUCUAAAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCCAGGUGGCAAAGCCCGU
UGAACUUCUCAAAAAGAACGAUCUGAGAAGUGGCACNNNNNNNNNNNNNNNNNNNN-3′

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

[0684] Some aspects of this disclosure provide methods of using the fusion proteins, or complexes provided herein. For example, some aspects of this disclosure provide methods comprising contacting a DNA molecule encoding a mutant form of a protein 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.

[0685] In some embodiments, a fusion protein of the disclosure is used for mutagenizing a target of interest. In particular, an adenosine deaminase nucleobase editor (*e.g.*, ABE8) described herein is capable of making multiple mutations within a target sequence. These mutations may affect the function of the target. For example, when an adenosine deaminase nucleobase editor (*e.g.*, ABE8) is used to target a regulatory region the function of the regulatory region is altered and the expression of the downstream protein is reduced.

[0686] 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.

[0687] 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 an adenosine deaminase variant (*e.g.*, ABE8), 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

[0688] CRISPR-Cas9 nucleases have been widely used to mediate targeted genome editing. In most genome editing applications, Cas9 forms a complex with a guide polynucleotide (*e.g.*, single guide RNA (sgRNA)) and induces a double-stranded DNA break (DSB) at the target site specified by the sgRNA sequence. Cells primarily respond to this DSB through the non-homologous end-joining (NHEJ) repair pathway, which results in stochastic insertions or deletions (indels) that can cause frameshift mutations that disrupt the gene. In the presence of a donor DNA template with a high degree of homology to the sequences flanking the DSB, gene correction can be achieved through an alternative pathway known as homology directed repair (HDR). Unfortunately, under most non-perturbative conditions HDR is inefficient, dependent on cell state and cell type, and dominated by a larger frequency of indels. As most of the known genetic variations associated with human disease are point mutations, methods that can more efficiently and cleanly make precise point mutations are needed. Base editing systems, as provided herein, provide a new way to genome edit without generating double-stranded DNA breaks, without requiring a donor DNA template, and without inducing an excess of stochastic insertions and deletions.

[0689] The base editors provided herein are capable of modifying a specific nucleotide base encoding a protein comprising a mutation without generating a significant proportion of indels. An "indel" as used herein, refers to the insertion or deletion of a nucleotide base within a nucleic acid. Such insertions or deletions can lead to frame shift mutations within a coding region of a gene. In some embodiments, it is desirable to generate base editors that efficiently modify (*e.g.*, mutate) a specific nucleotide within a nucleic acid, without generating a large number of insertions or deletions (*i.e.*, indels) in the target nucleotide sequence. In certain embodiments,

any of the base editors provided herein are capable of generating a greater proportion of intended modifications (*e.g.*, point mutations or deaminations) versus indels.

[0690] In some embodiments, any of base editor systems provided herein result in less than 50%, less than 40%, less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.9%, less than 0.8%, less than 0.7%, less than 0.6%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, less than 0.09%, less than 0.08%, less than 0.07%, less than 0.06%, less than 0.05%, less than 0.04%, less than 0.03%, less than 0.02%, or less than 0.01% indel formation in the target polynucleotide sequence.

[0691] In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein result in less than 50%, less than 40%, less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.9%, less than 0.8%, less than 0.7%, less than 0.6%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, less than 0.09%, less than 0.08%, less than 0.07%, less than 0.06%, less than 0.05%, less than 0.04%, less than 0.03%, less than 0.02%, or less than 0.01% indel formation in the target polynucleotide sequence.

[0692] In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein result in less than 0.8% indel formation in the target polynucleotide sequence. In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein result in at most 0.8% indel formation in the target polynucleotide sequence. In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein result in less than 0.3% indel formation in the target polynucleotide sequence. In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described results in lower indel formation in the target polynucleotide sequence compared to a base editor system comprising one of ABE7 base editors. In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein results in lower indel formation in the target polynucleotide sequence compared to a base editor system comprising an ABE7.10.

[0693] In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein has reduction in indel frequency compared to a base editor system comprising one of the ABE7 base editors. In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein has at least 0.01%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% reduction in indel frequency compared to a base editor system comprising one of the ABE7 base editors. In some embodiments, a base editor system comprising one of the ABE8 base editor variants described herein has at least 0.01%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% reduction in indel frequency compared to a base editor system comprising an ABE7.10.

[0694] The disclosure provides adenosine deaminase variants (*e.g.*, ABE8 variants) that have increased efficiency and specificity. In particular, the adenosine deaminase variants described herein are more likely to edit a desired base within a polynucleotide, and are less likely to edit bases that are not intended to be altered (*e.g.*, "bystanders").

[0695] In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced bystander editing or mutations. In some embodiments, an unintended editing or mutation is a bystander mutation or bystander editing, for example, base editing of a target base (*e.g.*, A or C) in an unintended or non-target position in a target window of a target nucleotide sequence. In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced bystander editing or mutations compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10. In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced bystander editing or mutations by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10. In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced bystander editing or mutations by at least 1.1

fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, or at least 3.0 fold compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10.

[0696] In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced spurious editing. In some embodiments, an unintended editing or mutation is a spurious mutation or spurious editing, for example, non-specific editing or guide independent editing of a target base (*e.g.*, A or C) in an unintended or non-target region of the genome. In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced spurious editing compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10. In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced spurious editing by at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10. In some embodiments, any of the base editing system comprising one of the ABE8 base editor variants described herein has reduced spurious editing by at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, or at least 3.0 fold compared to a base editor system comprising an ABE7 base editor, *e.g.*, ABE7.10.

[0697] 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, such as a point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations *i.e.*, mutation of bystanders). In some embodiments, any of the base editors provided herein are capable of generating at least 0.01% of intended mutations (*i.e.* at least 0.01% base editing efficiency). In some embodiments, any of the base editors provided herein are capable of generating at least 0.01%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of intended mutations.

[0698] In some embodiments, any of the ABE8 base editor variants described herein have at least 0.01%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% base editing efficiency. In some embodiments, the base editing efficiency may be measured by calculating the percentage of edited nucleobases in a population of cells. In some embodiments, any of the ABE8 base editor variants described herein have base editing efficiency of at least 0.01%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% as measured by edited nucleobases in a population of cells.

[0699] In some embodiments, any of the ABE8 base editor variants described herein has higher base editing efficiency compared to the ABE7 base editors. In some embodiments, any of the ABE8 base editor variants described herein have at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 105%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 155%, at least 160%, at least 165%, at least 170%, at least 175%, at least 180%, at least 185%, at least 190%, at least 195%, at least 200%, at least 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, at least 300%, at least 310%, at least 320%, at least 330%, at least 340%, at least 350%, at least 360%, at least 370%, at least 380%, at least 390%, at least 400%, at least 450%, or at least 500% higher base editing efficiency compared to an ABE7 base editor, *e.g.*, ABE7.10.

[0700] In some embodiments, any of the ABE8 base editor variants described herein has at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3.0 fold, at least 3.1 fold, at least 3.2, at least 3.3 fold, at least 3.4 fold, at least 3.5 fold, at least 3.6 fold, at least 3.7 fold, at least 3.8 fold, at least 3.9 fold, at least 4.0 fold, at least 4.1 fold, at least 4.2 fold, at least 4.3 fold, at least 4.4 fold, at least 4.5 fold, at

least 4.6 fold, at least 4.7 fold, at least 4.8 fold, at least 4.9 fold, or at least 5.0 fold higher base editing efficiency compared to an ABE7 base editor, *e.g.*, ABE7.10.

[0701] In some embodiments, any of the ABE8 base editor variants described herein have at least 0.01%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% on-target base editing efficiency. In some embodiments, any of the ABE8 base editor variants described herein have on-target base editing efficiency of at least 0.01%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% as measured by edited target nucleobases in a population of cells.

[0702] In some embodiments, any of the ABE8 base editor variants described herein has higher on-target base editing efficiency compared to the ABE7 base editors. In some embodiments, any of the ABE8 base editor variants described herein have at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 105%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 155%, at least 160%, at least 165%, at least 170%, at least 175%, at least 180%, at least 185%, at least 190%, at least 195%, at least 200%, at least 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, at least 300%, at least 310%, at least 320%, at least 330%, at least 340%, at least 350%, at least 360%, at least 370%, at least 380%, at least 390%, at least 400%, at least 450%, or at least 500% higher on-target base editing efficiency compared to an ABE7 base editor, *e.g.*, ABE7.10.

[0703] In some embodiments, any of the ABE8 base editor variants described herein has at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3.0 fold, at least 3.1 fold, at least 3.2 fold, at least 3.3 fold, at least 3.4 fold, at least 3.5 fold, at least 3.6 fold, at least 3.7 fold, at least 3.8 fold, at least 3.9 fold, at

least 4.0 fold, at least 4.1 fold, at least 4.2 fold, at least 4.3 fold, at least 4.4 fold, at least 4.5 fold, at least 4.6 fold, at least 4.7 fold, at least 4.8 fold, at least 4.9 fold, or at least 5.0 fold higher on-target base editing efficiency compared to an ABE7 base editor, *e.g.*, ABE7.10.

[0704] The ABE8 base editor variants described herein may be delivered to a host cell via a plasmid, a vector, a LNP complex, or an mRNA. In some embodiments, any of the ABE8 base editor variants described herein is delivered to a host cell as an mRNA. In some embodiments, an ABE8 base editor delivered via a nucleic acid based delivery system, *e.g.*, an mRNA, has on-target editing efficiency of at least at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% as measured by edited nucleobases. In some embodiments, an ABE8 base editor delivered by an mRNA system has higher base editing efficiency compared to an ABE8 base editor delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 105%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 155%, at least 160%, at least 165%, at least 170%, at least 175%, at least 180%, at least 185%, at least 190%, at least 195%, at least 200%, at least 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, at least 300% higher, at least 310%, at least 320%, at least 330%, at least 340%, at least 350%, at least 360%, at least 370%, at least 380%, at least 390%, at least 400%, at least 450%, or at least 500% on-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3.0 fold, at least 3.1 fold, at least 3.2 fold, at least 3.3 fold, at least 3.4 fold, at least 3.5 fold, at least 3.6 fold, at least 3.7 fold, at least 3.8 fold, at least 3.9 fold, at least 4.0 fold, at least 4.1 fold, at least 4.2 fold, at least 4.3 fold, at least 4.4 fold, at least 4.5 fold, at least 4.6 fold, at least 4.7 fold, at least 4.8 fold, at least 4.9 fold, or at least 5.0

fold higher on-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system.

[0705] In some embodiments, any of base editor systems comprising one of the ABE8 base editor variants described herein result in less than 50%, less than 40%, less than 30%, less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.9%, less than 0.8%, less than 0.7%, less than 0.6%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, less than 0.09%, less than 0.08%, less than 0.07%, less than 0.06%, less than 0.05%, less than 0.04%, less than 0.03%, less than 0.02%, or less than 0.01% off-target editing in the target polynucleotide sequence.

[0706] In some embodiments, any of the ABE8 base editor variants described herein has lower guided off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% lower guided off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, or at least 3.0 fold lower guided off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least about 2.2 fold decrease in guided off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system.

[0707] In some embodiments, any of the ABE8 base editor variants described herein has lower guide-independent off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at

least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% lower guide-independent off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, any of the ABE8 base editor variants described herein has at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3.0 fold, at least 5.0 fold, at least 10.0 fold, at least 20.0 fold, at least 50.0 fold, at least 70.0 fold, at least 100.0 fold, at least 120.0 fold, at least 130.0 fold, or at least 150.0 fold lower guide-independent off-target editing efficiency when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, ABE8 base editor variants described herein has 134.0 fold decrease in guide-independent off-target editing efficiency (*e.g.*, spurious RNA deamination) when delivered by an mRNA system compared to when delivered by a plasmid or vector system. In some embodiments, ABE8 base editor variants described herein does not increase guide-independent mutation rates across the genome.

[0708] 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, such as a point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations (*e.g.*, spurious off-target editing or bystander editing). In some embodiments, an intended mutation is a mutation that is generated by a specific base editor bound to a gRNA, specifically designed to alter or correct a mutation in a target gene. Some aspects of the disclosure are based on the recognition that any of the base editors provided herein are capable of efficiently generating an intended mutation in a nucleic acid (*e.g.* a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor bound to a gRNA, specifically designed to alter or correct an 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).

[0709] In some embodiments, the base editors provided herein are capable of generating a ratio of intended point mutations to indels (*i.e.*, unintended mutations) 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 8.5:1, at least 9:1, at least 10:1, at least 11:1, at least 12:1, at least 13:1, at least 14:1, 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. It should be appreciated that the characteristics of the base editors described herein may be applied to any of the fusion proteins, or methods of using the fusion proteins provided herein.

[0710] The number of intended mutations and indels can be determined using any suitable method, for example, as described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632); Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017); the entire contents of which are hereby incorporated by reference.

[0711] In some embodiments, to calculate indel frequencies, sequencing reads are scanned for exact matches to two 10-bp sequences that flank both sides of a window in which indels can occur. If no exact matches are located, the read is excluded from analysis. If the length of this indel window exactly matches the reference sequence the read is classified as not containing an indel. If the indel window is two or more bases longer or shorter than the reference sequence, then the sequencing read is classified as an insertion or deletion, respectively. In some embodiments, the base editors provided herein can limit 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.

[0712] The number of indels formed at a target nucleotide region can 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, the 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 the target nucleotide sequence (*e.g.*, a nucleic acid within the genome of a cell) to a base editor. It should be appreciated that the characteristics of the base editors as described herein can be applied to any of the fusion proteins, or methods of using the fusion proteins provided herein.

[0713] 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, any 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.

Multiplex Editing

[0714] In some embodiments, the base editor system provided herein is capable of multiplex editing of a plurality of nucleobase pairs in one or more genes. In some embodiments, the plurality of nucleobase pairs is located in the same gene. In some embodiments, the plurality of nucleobase pairs is located in one or more gene, wherein at least one gene is located in a different locus. In some embodiments, the multiplex editing can comprise one or more guide polynucleotides. In some embodiments, the multiplex editing can comprise one or more base editor systems. In some embodiments, the multiplex editing can comprise one or more base editor systems with a single guide polynucleotide. In some embodiments, the multiplex editing can comprise one or more base editor systems with a plurality of guide polynucleotides. In some embodiments, the multiplex editing can comprise one or more guide polynucleotides with a single base editor system. In some embodiments, the multiplex editing can comprise at least one guide polynucleotide that does not require a PAM sequence to target binding to a target

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

[0715] In some embodiments, the plurality of nucleobase pairs is in one or more genes. In some embodiments, the plurality of nucleobase pairs are in the same gene. In some embodiments, at least one gene in the one or more genes is located in a different locus. In some embodiments, the editing is editing of the plurality of nucleobase pairs in at least one protein coding region. In some embodiments, the editing is editing of the plurality of nucleobase pairs in at least one protein non-coding region. In some embodiments, the editing is editing of the plurality of nucleobase pairs in at least one protein coding region and at least one protein non-coding region.

[0716] In some embodiments, the editing is in conjunction with one or more guide polynucleotides. In some embodiments, the base editor system can comprise one or more base editor system. In some embodiments, the base editor system can comprise one or more base editor systems in conjunction with a single guide polynucleotide. In some embodiments, the base editor system can comprise one or more base editor system in conjunction with a plurality of guide polynucleotides. In some embodiments, the editing is in conjunction with one or more guide polynucleotide with a single base editor system. In some embodiments, the editing is in conjunction with at least one guide polynucleotide that does not require a PAM sequence to target binding to a target polynucleotide sequence. In some embodiments, the editing is in conjunction with at least one guide polynucleotide that require a PAM sequence to target binding to a target polynucleotide sequence. In some embodiments, the editing is in conjunction with a mix of at least one guide polynucleotide that does not require a PAM sequence to target binding to a target polynucleotide sequence and at least one guide polynucleotide that require a PAM sequence to target binding to a target polynucleotide sequence. It should be appreciated that the characteristics of the multiplex editing using any of the base editors as described herein can be

applied to any of combination of the methods of using any of the base editors provided herein. It should also be appreciated that the editing can comprise a sequential editing of a plurality of nucleobase pairs.

[0717] In some embodiments, the base editor system capable of multiplex editing of a plurality of nucleobase pairs in one or more genes comprises one of the ABE8 base editor variants described herein. In some embodiments, the base editor system capable of multiplex editing of a plurality of nucleobase pairs in one or more genes comprises one of ABE7 base editors. In some embodiments, the base editor system capable of multiplex editing comprising one of the ABE8 base editor variants described herein has higher multiplex editing efficiency compared the base editor system capable of multiplex editing comprising one of ABE7 base editors. In some embodiments, the base editor system capable of multiplex editing comprising one of the ABE8 base editor variants described herein has at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100%, at least 105%, at least 110%, at least 115%, at least 120%, at least 125%, at least 130%, at least 135%, at least 140%, at least 145%, at least 150%, at least 155%, at least 160%, at least 165%, at least 170%, at least 175%, at least 180%, at least 185%, at least 190%, at least 195%, at least 200%, at least 210%, at least 220%, at least 230%, at least 240%, at least 250%, at least 260%, at least 270%, at least 280%, at least 290%, at least 300% higher, at least 310%, at least 320%, at least 330%, at least 340%, at least 350%, at least 360%, at least 370%, at least 380%, at least 390%, at least 400%, at least 450%, or at least 500% higher multiplex editing efficiency compared the base editor system capable of multiplex editing comprising one of ABE7 base editors. In some embodiments, the base editor system capable of multiplex editing comprising one of the ABE8 base editor variants described herein has at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.1 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3.0 fold, at least 3.1 fold, at least 3.2 fold, at least 3.3 fold, at least 3.4 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, or at least 6.0 fold higher multiplex editing efficiency compared the base editor system capable of multiplex editing comprising one of ABE7 base editors.

METHODS FOR EDITING NUCLEIC ACIDS

[0718] Some aspects of the disclosure provide methods for editing a nucleic acid. In some embodiments, the method is a method for editing a nucleobase of a nucleic acid molecule encoding a protein (*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.

[0719] 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.

[0720] In some embodiments, the disclosure provides methods for editing a nucleotide (*e.g.*, SNP in a gene encoding a protein). 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

[0721] Fusion proteins of the disclosure comprising an adenosine deaminase variant may be expressed in virtually any host cell of interest, including but not limited to bacteria, yeast, fungi, insects, plants, and animal cells using routine methods known to the skilled artisan. For example, a DNA encoding an adenosine deaminase of the disclosure can be cloned by designing suitable primers for the upstream and downstream of CDS based on the cDNA sequence. The cloned DNA may be directly, or after digestion with a restriction enzyme when desired, or after addition of a suitable linker and/or a nuclear localization signal ligated with a DNA encoding one or more additional components of a base editing system. The base editing system is translated in a host cell to form a complex.

[0722] A DNA encoding a protein domain described herein can be obtained by chemically synthesizing the DNA, or by connecting synthesized partly overlapping oligoDNA short chains by utilizing the PCR method and the Gibson Assembly method to construct a DNA encoding the

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

[0723] An expression vector containing a DNA encoding a nucleic acid sequence-recognizing module and/or a nucleic acid base converting enzyme can be produced, for example, by linking the DNA to the downstream of a promoter in a suitable expression vector.

[0724] When the expression vectors are *Escherichia coli*-derived plasmids (*e.g.*, pBR322, pBR325, pUC12, pUC13); *Bacillus subtilis*-derived plasmids (*e.g.*, pUB110, pTP5, pC194); yeast-derived plasmids (*e.g.*, pSH19, pSH15); insect cell expression plasmids (*e.g.*, pFast-Bac); animal cell expression plasmids (*e.g.*, pA1-11, pXT1, pRc/CMV, pRc/RSV, pcDNAI/Neo); bacteriophages such as .lamda.phage and the like; insect virus vectors such as baculovirus and the like (*e.g.*, BmNPV, AcNPV); animal virus vectors such as retrovirus, vaccinia virus, adenovirus and the like are used.

[0725] In some embodiments, any promoter appropriate for a host to be used for gene expression can be used. In a conventional method using DSB, since the survival rate of the host cell sometimes decreases markedly due to the toxicity, it is desirable to increase the number of cells by the start of the induction by using an inductive promoter. However, since sufficient cell proliferation can also be afforded by expressing the nucleic acid-modifying enzyme complex of the present disclosure, a constitution promoter can also be used without limitation.

[0726] For example, when the host is an animal cell, SRα promoter, SV40 promoter, LTR promoter, CMV (cytomegalovirus) promoter, RSV (*Rous sarcoma* virus) promoter, MoMuLV (*Moloney* mouse leukemia virus) LTR, HSV-TK (simple herpes virus thymidine kinase) promoter and the like are used. Of these, CMV promoter, SRα promoter and the like are preferable.

[0727] When the host is *Escherichia coli*, trp promoter, lac promoter, recA promoter, λP_L promoter, lpp promoter, T7 promoter and the like are preferable.

[0728] When the host is genus *Bacillus*, SPO1 promoter, SPO2 promoter, penP promoter and the like are preferable.

[0729] When the host is a yeast, Gal1/10 promoter, PHO5 promoter, PGK promoter, GAP promoter, ADH promoter and the like are preferable.

[0730] When the host is an insect cell, polyhedrin promoter, P10 promoter and the like are preferable.

[0731] When the host is a plant cell, CaMV35S promoter, CaMV19S promoter, NOS promoter and the like are preferable.

[0732] As the expression vector, besides those mentioned above, one containing enhancer, splicing signal, terminator, polyA addition signal, a selection marker such as drug resistance gene, auxotrophic complementary gene and the like, replication origin and the like on demand can be used.

[0733] An RNA encoding a protein domain described herein can be prepared by, for example, transcription to mRNA in a vitro transcription system known per se by using a vector encoding DNA encoding the above-mentioned nucleic acid sequence-recognizing module and/or a nucleic acid base converting enzyme as a template.

[0734] A fusion protein of the disclosure can be intracellularly expressed by introducing an expression vector containing a DNA encoding a nucleic acid sequence-recognizing module and/or a nucleic acid base converting enzyme into a host cell, and culturing the host cell.

[0735] As the host, genus *Escherichia*, genus *Bacillus*, yeast, insect cell, insect, animal cell and the like are used.

[0736] As the genus *Escherichia*, *Escherichia coli* K12.cndot.DH1 (Proc. Natl. Acad. Sci. USA, 60, 160 (1968)), *Escherichia coli* JM103 (Nucleic Acids Research, 9, 309 (1981)), *Escherichia coli* JA221 (Journal of Molecular Biology, 120, 517 (1978)), *Escherichia coli* HB101 (Journal of Molecular Biology, 41, 459 (1969)), *Escherichia coli* C600 (Genetics, 39, 440 (1954)) and the like are used.

[0737] As the genus *Bacillus*, *Bacillus subtilis* M1114 (Gene, 24, 255 (1983)), *Bacillus subtilis* 207-21 (Journal of Biochemistry, 95, 87 (1984)) and the like are used.

[0738] As the yeast, *Saccharomyces cerevisiae* AH22, AH22R$^-$, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, *Pichia pastoris* KM71 and the like are used.

[0739] As the insect cell when the virus is AcNPV, cells of cabbage armyworm larva-derived established line (*Spodoptera frugiperda* cell; Sf cell), MG1 cells derived from the mid-intestine of *Trichoplusia ni*, High Five™ cells derived from an egg of *Trichoplusia ni*, *Mamestra brassicae*-derived cells, *Estigmena acrea*-derived cells and the like are used. When the virus is BmNPV, cells of *Bombyx mori*-derived established line (*Bombyx mori* N cell; BmN cell) and the like are used as insect cells. As the Sf cell, for example, Sf9 cell (ATCC CRL1711), Sf21 cell (all above, In Vivo, 13, 213-217 (1977)) and the like are used.

[0740] As the insect, for example, larva of *Bombyx mori*, *Drosophila*, cricket and the like are used (Nature, 315, 592 (1985)).

[0741] As the animal cell, cell lines such as 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.

[0742] As the plant cell, suspend cultured cells, callus, protoplast, leaf segment, root segment and the like prepared from various plants (*e.g.*, grain such as rice, wheat, corn and the like, product crops such as tomato, cucumber, eggplant and the like, garden plants such as carnation, Eustoma russellianum and the like, experiment plants such as tobacco, *arabidopsis thaliana* and the like) are used.

[0743] All the above-mentioned host cells may be haploid (monoploid), or polyploid (*e.g.*, diploid, triploid, tetraploid and the like). In the conventional mutation introduction methods, mutation is, in principle, introduced into only one homologous chromosome to produce a hetero gene type. Therefore, desired phenotype is not expressed unless dominant mutation occurs, and homozygousness inconveniently requires labor and time. In contrast, according to the present disclosure, since mutation can be introduced into any allele on the homologous chromosome in the genome, desired phenotype can be expressed in a single generation even in the case of recessive mutation, which is extremely useful since the problem of the conventional method can be solved.

[0744] An expression vector can be introduced by a known method (*e.g.*, lysozyme method, competent method, PEG method, $CaCl_2$ coprecipitation method, electroporation method, the microinjection method, the particle gun method, lipofection method, Agrobacterium method and the like) according to the kind of the host.

[0745] *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.

[0746] The genus *Bacillus* can be introduced into a vector according to the methods described in, for example, Molecular & General Genetics, 168, 111 (1979) and the like.

[0747] A yeast can be introduced into a vector according to the methods described in, for example, Methods in Enzymology, 194, 182-187 (1991), Proc. Natl. Acad. Sci. USA, 75, 1929 (1978) and the like.

[0748] An insect cell and an insect can be introduced into a vector according to the methods described in, for example, Bio/Technology, 6, 47-55 (1988) and the like.

[0749] An animal cell can be introduced into a vector according to the methods described in, for example, Cell Engineering additional volume 8, New Cell Engineering Experiment Protocol, 263-267 (1995) (published by Shujunsha), and Virology, 52, 456 (1973).

[0750] A cell introduced with a vector can be cultured according to a known method according to the kind of the host.

[0751] For example, when *Escherichia coli* or genus *Bacillus* is cultured, a liquid medium is preferable as a medium to be used for the culture. The medium preferably contains a carbon source, nitrogen source, inorganic substance and the like necessary for the growth of the transformant. Examples of the carbon source include glucose, dextrin, soluble starch, sucrose and the like; examples of the nitrogen source include inorganic or organic substances such as ammonium salts, nitrate salts, corn steep liquor, peptone, casein, meat extract, soybean cake, potato extract and the like; and examples of the inorganic substance include calcium chloride, sodium dihydrogen phosphate, magnesium chloride and the like. The medium may contain yeast extract, vitamins, growth promoting factor and the like. The pH of the medium is preferably about 5 to about 8.

[0752] As a medium for culturing *Escherichia coli*, for example, M9 medium containing glucose, casamino acid (Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York 1972) is preferable. Where necessary, for example, agents such as 3β-indolylacrylic acid may be added to the medium to ensure an efficient function of a promoter. *Escherichia coli* is cultured at generally about 15 to about 43°C. Where necessary, aeration and stirring may be performed.

[0753] The genus *Bacillus* is cultured at generally about 30°C to about 40°C. Where necessary, aeration and stirring may be performed.

[0754] Examples of the medium for culturing yeast include Burkholder minimum medium (Proc. Natl. Acad. Sci. USA, 77, 4505 (1980)), SD medium containing 0.5% casamino acid (Proc. Natl. Acad. Sci. USA, 81, 5330 (1984)) and the like. The pH of the medium is preferably about 5 to about 8. The culture is performed at generally about 20°C to about 35°C. Where necessary, aeration and stirring may be performed.

[0755] As a medium for culturing an insect cell or insect, for example, Grace's Insect Medium (Nature, 195, 788 (1962)) containing an additive such as inactivated 10% bovine serum and the like as appropriate and the like are used. The pH of the medium is preferably about 6.2 to about 6.4. The culture is performed at generally about 27°C. Where necessary, aeration and stirring may be performed.

[0756] As a medium for culturing an animal cell, for example, 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 are used. The pH of the medium is preferably about 6 to about 8. The culture is performed at generally about 30°C to about 40°C. Where necessary, aeration and stirring may be performed.

[0757] 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.

[0758] When a higher eukaryotic cell, such as animal cell, insect cell, plant cell and the like is used as a host cell, a DNA encoding a base editing system of the present disclosure (*e.g.*, comprising an adenosine deaminase variant) is introduced into a host cell under the regulation of an inducible promoter (*e.g.*, metallothionein promoter (induced by heavy metal ion), heat shock protein promoter (induced by heat shock), Tet-ON/Tet-OFF system promoter (induced by addition or removal of tetracycline or a derivative thereof), steroid-responsive promoter (induced by steroid hormone or a derivative thereof) etc.), the induction substance is added to the medium (or removed from the medium) at an appropriate stage to induce expression of the nucleic acid-modifying enzyme complex, culture is performed for a given period to carry out a base editing and, introduction of a mutation into a target gene, transient expression of the base editing system can be realized.

[0759] Prokaryotic cells such as *Escherichia coli* and the like can utilize an inducible promoter. Examples of the inducible promoter include, but are not limited to, lac promoter (induced by IPTG), cspA promoter (induced by cold shock), araBAD promoter (induced by arabinose) and the like.

[0760] Alternatively, the above-mentioned inductive promoter can also be utilized as a vector removal mechanism when higher eukaryotic cells, such as animal cell, insect cell, plant cell and the like are used as a host cell. That is, a vector is mounted with a replication origin that functions in a host cell, and a nucleic acid encoding a protein necessary for replication (*e.g.*, SV40 on and large T antigen, oriP and EBNA-1 etc. for animal cells), of the expression of the nucleic acid encoding the protein is regulated by the above-mentioned inducible promoter. As a result, while the vector is autonomously replicatable in the presence of an induction substance, when the induction substance is removed, autonomous replication is not available, and the vector naturally falls off along with cell division (autonomous replication is not possible by the addition of tetracycline and doxycycline in Tet-OFF system vector).

METHODS OF USING BASE EDITORS

[0761] The correction of point mutations in disease-associated genes and alleles provides new strategies for gene correction with applications in therapeutics and basic research.

[0762] The present disclosure provides methods for the treatment of a subject diagnosed with a disease associated with or caused by a point mutation 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 genetic mutation, an effective amount of a nucleobase editor (*e.g.*, an adenosine deaminase base editor) that corrects the point mutation in the disease associated gene. The present disclosure provides methods for the treatment of RTT that are associated with or caused by a point mutation that can be corrected by deaminase mediated gene editing. 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.

[0763] Provided herein are methods of using a base editor or base editor system for editing a nucleobase in a target nucleotide sequence associated with a disease or disorder. In some embodiments, the activity of the base editor (*e.g.*, comprising an adenosine deaminase and a Cas9 domain) results in a correction of the point mutation. In some embodiments, the target DNA sequence comprises a G→A point mutation associated with a disease or disorder, and

deamination of the mutant A base results in a sequence that is not associated with a disease or disorder. In some embodiments, the target DNA sequence comprises a T→C point mutation associated with a disease or disorder, and the deamination of the mutant C base results in a sequence that is not associated with a disease or disorder.

[0764] In some embodiments, the target DNA sequence encodes a protein, and the point mutation is in a codon and results in a change in the amino acid encoded by the mutant codon as compared to the wild-type codon. In some embodiments, the deamination of the mutant A results in a change of the amino acid encoded by the mutant codon. In some embodiments, the deamination of the mutant A results in the codon encoding the wild-type amino acid. In some embodiments, the deamination of the mutant C results in a change of the amino acid encoded by the mutant codon. In some embodiments, the deamination of the mutant C results in the codon encoding the wild-type amino acid. In some embodiments, the subject has or has been diagnosed with a disease or disorder.

[0765] In some embodiments, the adenosine deaminases provided herein are capable of deaminating a deoxyadenosine residue of DNA. Other aspects of the disclosure provide fusion proteins that comprise an adenosine deaminase (*e.g.*, an adenosine deaminase that deaminates deoxyadenosine in DNA as described herein) and a domain (*e.g.*, a Cas9 or a Cpf1 protein) capable of binding to a specific nucleotide sequence. For example, the adenosine can be converted to an inosine residue, which typically base pairs with a cytosine residue. Such fusion proteins are useful *inter alia* for targeted editing of nucleic acid sequences. Such fusion proteins can be used for targeted editing of DNA *in vitro*, *e.g.*, for the generation of mutant cells or animals; for the introduction of targeted mutations, *e.g.*, for the correction of genetic defects in cells *ex vivo*, *e.g.*, in cells obtained from a subject that are subsequently re-introduced into the same or another subject; and for the introduction of targeted mutations *in vivo*, *e.g.*, the correction of genetic defects or the introduction of deactivating mutations in disease-associated genes in a G to A, or a T to C to mutation can be treated using the nucleobase editors provided herein. The present disclosure provides deaminases, fusion proteins, nucleic acids, vectors, cells, compositions, methods, kits, systems, etc. that utilize the deaminases and nucleobase editors.

Use of Nucleobase Editors to Target Nucleotides in the Mecp2 gene

[0766] The suitability of nucleobase editors that target a nucleotide in the *Mecp2* gene is evaluated as described herein. In one embodiment, a single cell of interest is transfected, transduced, or otherwise modified with a nucleic acid molecule or molecules encoding a

nucleobase editor described herein together with a small amount of a vector encoding a reporter (*e.g.*, GFP). These cells can be immortalized human cell lines, such as 293T, K562 or U20S. Alternatively, primary human cells may be used. Cells may also be obtained from a subject or individual, such as from tissue biopsy, surgery, blood, plasma, serum, or other biological fluid. Such cells may be relevant to the eventual cell target,

[0767] Delivery may be performed using a viral vector as further described below. 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.

[0768] The activity of the nucleobase editor is assessed as described herein, *i.e.*, by sequencing the target gene to detect alterations in the 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).

[0769] The fusion proteins that induce the greatest levels of target specific alterations in initial tests can be selected for further evaluation.

[0770] In particular embodiments, the nucleobase editors are used to target polynucleotides of interest. In one embodiment, a nucleobase editor as described herein is delivered to cells (*e.g.*, a neuron) in conjunction with a guide RNA that is used to target a nucleic acid sequence, *e.g.*, a *Mecp2* polynucleotide harboring RTT-associated mutations, thereby altering the target gene, *i.e.*, *Mecp2.*

[0771] In some embodiments, a base editor is targeted by a guide RNA to introduce one or more edits to the sequence of a gene of interest. In some embodiments, the one or more alterations introduced into the *Mecp2* gene are as presented in Table 10 *infra.*

Generating an Intended Mutation

[0772] In some embodiments, the purpose of the methods provided herein is to restore the function of a dysfunctional gene via gene editing. In some embodiments, the function of a

dysfunctional gene is restored by introducing an intended mutation. In some embodiments, the methods provided herein can be used to disrupt the normal function of a gene product. The nucleobase editing proteins provided herein can be validated for gene editing-based human therapeutics *in vitro*, *e.g.*, by correcting a disease-associated mutation in human cell culture. It will be understood by the skilled artisan that the nucleobase editing proteins provided herein, *e.g.*, the fusion proteins comprising a polynucleotide programmable nucleotide binding domain (*e.g.*, Cas9) and a nucleobase editing domain (*e.g.*, an adenosine deaminase domain) can be used to correct any single point A to G or C to T mutation. In the first case, deamination of the mutant A to I corrects the mutation, and in the latter case, deamination of the A that is base-paired with the mutant T, followed by a round of replication, corrects the mutation.

[0773] In some embodiments, the present disclosure provides base editors that efficiently generate an intended mutation, such as a point mutation, in a nucleic acid (*e.g.*, a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations. In some embodiments, an intended mutation is a mutation that is generated by a specific base editor (*e.g.*, adenosine base editor) bound to a guide polynucleotide (*e.g.*, gRNA), specifically designed to generate the intended mutation. In some embodiments, the intended mutation is a mutation associated with a disease or disorder, e.g., RETT syndrome. In some embodiments, the intended mutation is an adenine (A) to guanine (G) point mutation (e.g., SNP) associated with a disease or disorder, e.g., RETT syndrome. In some embodiments, the intended mutation is an adenine (A) to guanine (G) point mutation within the coding region or non-coding region of a gene (e.g., regulatory region or element). In some embodiments, the intended mutation is a point 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.

[0774] 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 point mutations : unintended point 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 (*e.g.*, intended point mutations : unintended point 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

[0775] Details of base editor efficiency are described in International PCT Application Nos. PCT/2017/045381 (WO 2018/027078) and PCT/US2016/058344 (WO 2017/070632), each of which is incorporated herein by reference for its entirety. Also see Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); and Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), the entire contents of which are hereby incorporated by reference.

[0776] In some embodiments, editing of a plurality of nucleobase pairs in one or more genes using the methods provided herein results in formation of at least one intended mutation. In some embodiments, said formation of said at least one intended mutation results in a precise correction of a disease-causing mutation. It should be appreciated that multiplex editing can be accomplished using any method or combination of methods provided herein.

Precise Correction of Pathogenic Mutations

[0777] In some embodiments, the intended mutation is a precise correction of a pathogenic mutation or a disease-causing mutation. The pathogenic mutation can be a pathogenic single nucleotide polymorphism (SNP) or be caused by a SNP. For example, the pathogenic mutation can be an amino acid change in a protein encoded by a gene. In another example, the pathogenic mutation can be a pathogenic SNP in a gene. The precise correction can be to revert the pathogenic mutation back to its wild-type state. In some embodiments, the pathogenic mutation is a G→A point mutation associated with a disease or disorder, and wherein the deamination of the mutant A base with an A-to-G base editor (ABE) results in a sequence that is not associated with a disease or disorder. In some embodiments, the pathogenic mutation is a C→T point mutation. The C→T point mutation can be corrected, for example, by targeting an A-to-G base editor (ABE) to the opposite strand and editing the complement A of the pathogenic T nucleobase. A base editor can be targeted to a pathogenic SNP, or to the complement of the pathogenic SNP. The nomenclature of the description of pathogenic or disease-causing mutations and other sequence variations are described in den Dunnen, J.T. and Antonarakis, S.E., "Mutation Nomenclature Extensions and Suggestions to Describe Complex Mutations: A Discussion." Human Mutation 15:712 (2000), the entire contents of which is hereby incorporated by reference.

[0778] In a particular embodiment, the disease or disorder is Rett Syndrome (RTT). In some embodiments, the pathogenic mutation is in the *Mecp2* gene.

DELIVERY SYSTEM

Nucleic Acid-Based Delivery of a Nucleobase Editors and gRNAs

[0779] Nucleic acids encoding base editing systems according to the present disclosure can be administered to subjects or delivered into cells *in vitro* or *in vivo* by art-known methods or as described herein. In one embodiment, nucleobase editors can be delivered by, *e.g.*, vectors (*e.g.*, viral or non-viral vectors), non-vector based methods (*e.g.*, using naked DNA, DNA complexes, lipid nanoparticles), or a combination thereof.

[0780] Nucleic acids encoding nucleobase editors can be delivered directly to cells (*e.g.*, hematopoietic cells or their progenitors, hematopoietic stem cells, and/or induced pluripotent stem cells) as naked DNA or RNA, for instance by means of transfection or electroporation, or can be conjugated to molecules (*e.g.*, N-acetylgalactosamine) promoting uptake by the target cells. Nucleic acid vectors, such as the vectors described herein can also be used.

[0781] 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 an adenosine deaminase variant (*e.g.*, TadA*8).

[0782] The nucleic acid vector can also include any suitable number of regulatory/control elements, *e.g.*, promoters, enhancers, introns, polyadenylation signals, Kozak consensus sequences, or internal ribosome entry sites (IRES). These elements are well known in the art. For hematopoietic cells suitable promoters can include IFNbeta or CD45.

[0783] Nucleic acid vectors according to this disclosure include recombinant viral vectors. Exemplary viral vectors are set forth herein. Other viral vectors known in the art can also be used. In addition, viral particles can be used to deliver base editing system components in nucleic acid and/or peptide form. For example, "empty" viral particles can be assembled to contain any suitable cargo. Viral vectors and viral particles can also be engineered to incorporate targeting ligands to alter target tissue specificity.

[0784] 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 12** (below).

Table 12

Lipids Used for Gene Transfer

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

Lipids Used for Gene Transfer

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

[0785] **Table 13** lists exemplary polymers for use in gene transfer and/or nanoparticle formulations.

Table 13

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

Polymers Used for Gene Transfer	
Polymer	Abbreviation
Poly(2-aminoethyl propylene phosphate)	PPE-EA
Chitosan	
Galactosylated chitosan	
N-Dodacylated chitosan	
Histone	
Collagen	
Dextran-spermine	D-SPM

[0786] **Table 14** summarizes delivery methods for a polynucleotide encoding a fusion protein described herein.

Table 14

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

Delivery	Vector/Mode	Delivery into Non-Dividing Cells	Duration of Expression	Genome Integration	Type of Molecule Delivered
	Mammalian Virus-like Particles	YES	Transient	NO	Nucleic Acids
	Biological liposomes: Erythrocyte Ghosts and Exosomes	YES	Transient	NO	Nucleic Acids

[0787] In another aspect, the delivery of genome editing system components or nucleic acids encoding such components, for example, a nucleic acid binding protein such as, for example, Cas9 or variants thereof, and a gRNA targeting a genomic nucleic acid sequence of interest, may be accomplished by delivering a ribonucleoprotein (RNP) to cells. The RNP comprises the nucleic acid binding protein, *e.g.*, Cas9, in complex with the targeting gRNA. RNPs may be delivered to cells using known methods, such as electroporation, nucleofection, or cationic lipid-mediated methods, for example, as reported by Zuris, J.A. *et al.*, 2015, *Nat. Biotechnology*, 33(1):73-80. RNPs are advantageous for use in CRISPR base editing systems, particularly for cells that are difficult to transfect, such as primary cells. In addition, RNPs can also alleviate difficulties that may occur with protein expression in cells, especially when eukaryotic promoters, *e.g.*, CMV or EF1A, which may be used in CRISPR plasmids, are not well-expressed. Advantageously, the use of RNPs does not require the delivery of foreign DNA into cells. Moreover, because an RNP comprising a nucleic acid binding protein and gRNA complex is degraded over time, the use of RNPs has the potential to limit off-target effects. In a manner similar to that for plasmid based techniques, RNPs can be used to deliver binding protein (*e.g.*, Cas9 variants) and to direct homology directed repair (HDR).

[0788] A promoter used to drive base editor coding nucleic acid molecule expression can include AAV ITR. This can be advantageous for eliminating the need for an additional promoter element, which can take up space in the vector. The additional space freed up can be used to drive the expression of additional elements, such as a guide nucleic acid or a selectable marker. ITR activity is relatively weak, so it can be used to reduce potential toxicity due to over expression of the chosen nuclease.

[0789] Any suitable promoter can be used to drive expression of the base editor and, where appropriate, the guide nucleic acid. For ubiquitous expression, promoters that can be used include

CMV, CAG, CBh, PGK, SV40, Ferritin heavy or light chains, etc. For brain or other CNS cell expression, suitable promoters can include: SynapsinI for all neurons, CaMKIIalpha for excitatory neurons, GAD67 or GAD65 or VGAT for GABAergic neurons, etc. For liver cell expression, suitable promoters include the Albumin promoter. For lung cell expression, suitable promoters can include SP-B. For endothelial cells, suitable promoters can include ICAM. For hematopoietic cells suitable promoters can include IFNbeta or CD45. For Osteoblasts suitable promoters can include OG-2.

[0790] In some embodiments, a base editor of the present disclosure is of small enough size to allow separate promoters to drive expression of the base editor and a compatible guide nucleic acid within the same nucleic acid molecule. For instance, a vector or viral vector can comprise a first promoter operably linked to a nucleic acid encoding the base editor and a second promoter operably linked to the guide nucleic acid.

[0791] The promoter used to drive expression of a guide nucleic acid can include: Pol III promoters such as U6 or H1 Use of Pol II promoter and intronic cassettes to express gRNA Adeno Associated Virus (AAV).

Viral Vectors

[0792] A base editor described herein can therefore be delivered with viral vectors. In some embodiments, a base editor disclosed herein can be encoded on a nucleic acid that is contained in a viral vector. In some embodiments, one or more components of the base editor system can be encoded on one or more viral vectors. For example, a base editor and guide nucleic acid can be encoded on a single viral vector. In other embodiments, the base editor and guide nucleic acid are encoded on different viral vectors. In either case, the base editor and guide nucleic acid can each be operably linked to a promoter and terminator. The combination of components encoded on a viral vector can be determined by the cargo size constraints of the chosen viral vector.

[0793] The use of RNA or DNA viral based systems for the delivery of a base editor takes advantage of highly evolved processes for targeting a virus to specific cells in culture or in the host and trafficking the viral payload to the nucleus or host cell genome. Viral vectors can be administered directly to cells in culture, patients (*in vivo*), or they can be used to treat cells *in vitro*, and the modified cells can optionally be administered to patients (*ex vivo*). Conventional viral based systems could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long

term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

[0794] Viral vectors can include lentivirus (*e.g.*, HIV and FIV-based vectors), Adenovirus (*e.g.*, AD100), Retrovirus (*e.g.*, Maloney murine leukemia virus, MML-V), herpesvirus vectors (*e.g.*, HSV-2), and Adeno-associated viruses (AAVs), or other plasmid or viral vector types, in particular, using formulations and doses from, for example, U.S. Patent No. 8,454,972 (formulations, doses for adenovirus), U.S. Patent No. 8,404,658 (formulations, doses for AAV) and U.S. Patent No. 5,846,946 (formulations, doses for DNA plasmids) and from clinical trials and publications regarding the clinical trials involving lentivirus, AAV and adenovirus. For example, for AAV, the route of administration, formulation and dose can be as in U.S. Patent No. 8,454,972 and as in clinical trials involving AAV. For Adenovirus, the route of administration, formulation and dose can be as in U.S. Patent No. 8,404,658 and as in clinical trials involving adenovirus. For plasmid delivery, the route of administration, formulation and dose can be as in U.S. Patent No. 5,846,946 and as in clinical studies involving plasmids. Doses can be based on or extrapolated to an average 70 kg individual (*e.g.* a male adult human), and can be adjusted for patients, subjects, mammals of different weight and species. Frequency of administration is within the ambit of the medical or veterinary practitioner (*e.g.*, physician, veterinarian), depending on usual factors including the age, sex, general health, other conditions of the patient or subject and the particular condition or symptoms being addressed. The viral vectors can be injected into the tissue of interest. For cell-type specific base editing, the expression of the base editor and optional guide nucleic acid can be driven by a cell-type specific promoter.

[0795] The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof (*See, e.g.*, Buchscher *et al.*, J. Virol. 66:2731-2739 (1992); Johann *et al.*, J. Virol. 66:1635-1640

(1992); Sommnerfelt *et al.*, Virol. 176:58-59 (1990); Wilson *et al.*, J. Virol. 63:2374-2378 (1989); Miller *et al.*, J. Virol. 65:2220-2224 (1991); PCT/US94/05700).

[0796] Retroviral vectors, especially lentiviral vectors, can require polynucleotide sequences smaller than a given length for efficient integration into a target cell. For example, retroviral vectors of length greater than 9 kb can result in low viral titers compared with those of smaller size. In some aspects, a base editor of the present disclosure is of sufficient size so as to enable efficient packaging and delivery into a target cell via a retroviral vector. In some embodiments, a base editor is of a size so as to allow efficient packing and delivery even when expressed together with a guide nucleic acid and/or other components of a targetable nuclease system.

[0797] In applications where transient expression is preferred, adenoviral based systems can be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors can also be used to transduce cells with target nucleic acids, *e.g.*, in the *in vitro* production of nucleic acids and peptides, and for *in vivo* and *ex vivo* gene therapy procedures (*See, e.g.*, West *et al.*, Virology 160:38-47 (1987); U.S. Patent No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994). The construction of recombinant AAV vectors is described in a number of publications, including U.S. Patent No. 5,173,414; Tratschin *et al.*, Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, *et al.*, Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski *et al.*, J. Virol. 63:03822-3828 (1989).

[0798] 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.

[0799] 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 disclosure 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.

[0800] Viral vectors can be selected based on the application. For example, for *in vivo* gene delivery, AAV can be advantageous over other viral vectors. In some embodiments, AAV allows low toxicity, which can be due to the purification method not requiring ultra-centrifugation of cell particles that can activate the immune response. In some embodiments, AAV allows low probability of causing insertional mutagenesis because it doesn't integrate into the host genome. Adenoviruses are commonly used as vaccines because of the strong immunogenic response they induce. Packaging capacity of the viral vectors can limit the size of the base editor that can be packaged into the vector.

[0801] AAV has a packaging capacity of about 4.5 Kb or 4.75 Kb including two 145 base inverted terminal repeats (ITRs). This means disclosed base editor as well as a promoter and transcription terminator can fit into a single viral vector. Constructs larger than 4.5 or 4.75 Kb can lead to significantly reduced virus production. For example, SpCas9 is quite large, the gene itself is over 4.1 Kb, which makes it difficult for packing into AAV. Therefore, embodiments of the present disclosure include utilizing a disclosed base editor which is shorter in length than conventional base editors. In some examples, the base editors are less than 4 kb. Disclosed base editors can be less than 4.5 kb, 4.4 kb, 4.3 kb, 4.2 kb, 4.1 kb, 4 kb, 3.9 kb, 3.8 kb, 3.7 kb, 3.6 kb, 3.5 kb, 3.4 kb, 3.3 kb, 3.2 kb, 3.1 kb, 3 kb, 2.9 kb, 2.8 kb, 2.7 kb, 2.6 kb, 2.5 kb, 2 kb, or 1.5 kb. In some embodiments, the disclosed base editors are 4.5 kb or less in length.

[0802] An AAV can be AAV1, AAV2, AAV5 or any combination thereof. One can select the type of AAV with regard to the cells to be targeted; *e.g.*, one can select AAV serotypes 1, 2, 5 or a hybrid capsid AAV1, AAV2, AAV5 or any combination thereof for targeting brain or neuronal cells; and one can select AAV4 for targeting cardiac tissue. AAV8 is useful for delivery to the liver. A tabulation of certain AAV serotypes as to these cells can be found in Grimm, D. et al, J. Virol. 82: 5887-5911 (2008)).

[0803] Lentiviruses are complex retroviruses that have the ability to infect and express their genes in both mitotic and post-mitotic cells. The most commonly known lentivirus is the human immunodeficiency virus (HIV), which uses the envelope glycoproteins of other viruses to target a broad range of cell types.

[0804] Lentiviruses can be prepared as follows. After cloning pCasES10 (which contains a lentiviral transfer plasmid backbone), HEK293FT at low passage (p=5) were seeded in a T-75 flask to 50% confluence the day before transfection in DMEM with 10% fetal bovine serum and without antibiotics. After 20 hours, media is changed to OptiMEM (serum-free) media and transfection was done 4 hours later. Cells are transfected with 10 μg of lentiviral transfer plasmid (pCasES10) and the following packaging plasmids: 5 μg of pMD2.G (VSV-g pseudotype), and 7.5 μg of psPAX2 (gag/pol/rev/tat). Transfection can be done in 4 mL OptiMEM with a cationic lipid delivery agent (50 μl Lipofectamine 2000 and 100 μl Plus reagent). After 6 hours, the media is changed to antibiotic-free DMEM with 10% fetal bovine serum. These methods use serum during cell culture, but serum-free methods are preferred.

[0805] Lentivirus can be purified as follows. Viral supernatants are harvested after 48 hours. Supernatants are first cleared of debris and filtered through a 0.45 μm low protein binding (PVDF) filter. They are then spun in an ultracentrifuge for 2 hours at 24,000 rpm. Viral pellets are resuspended in 50 μl of DMEM overnight at 4° C. They are then aliquoted and immediately frozen at -80°C.

[0806] In another embodiment, minimal non-primate lentiviral vectors based on the equine infectious anemia virus (EIAV) are also contemplated. In another embodiment, RetinoStat.RTM., an equine infectious anemia virus-based lentiviral gene therapy vector that expresses angiostatic proteins endostatin and angiostatin that is contemplated to be delivered via a subretinal injection. In another embodiment, use of self-inactivating lentiviral vectors are contemplated.

[0807] Any RNA of the systems, for example a guide RNA or a base editor-encoding mRNA, can be delivered in the form of RNA. Base editor-encoding mRNA can be generated using *in vitro* transcription. For example, nuclease mRNA can be synthesized using a PCR cassette containing the following elements: T7 promoter, optional kozak sequence (GCCACC), nuclease sequence, and 3′ UTR such as a 3′ UTR from beta globin-polyA tail. The cassette can be used for transcription by T7 polymerase. Guide polynucleotides (*e.g.*, gRNA) can also be transcribed using *in vitro* transcription from a cassette containing a T7 promoter, followed by the sequence "GG", and guide polynucleotide sequence.

[0808] To enhance expression and reduce possible toxicity, the base editor-coding sequence and/or the guide nucleic acid can be modified to include one or more modified nucleoside *e.g.* using pseudo-U or 5-Methyl-C.

[0809] 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. 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.

[0810] A fragment of a fusion protein of the disclosure 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.

[0811] In one embodiment, dual AAV vectors are generated by splitting a large transgene expression cassette in two separate halves (5′ and 3′ ends, or head and tail), where each half of the cassette is packaged in a single AAV vector (of <5 kb). The re-assembly of the full-length transgene expression cassette is then achieved upon co-infection of the same cell by both dual AAV vectors followed by: (1) homologous recombination (HR) between 5′ and 3′ genomes (dual AAV overlapping vectors); (2) ITR-mediated tail-to-head concatemerization of 5′ and 3′ genomes (dual AAV *trans*-splicing vectors); or (3) a combination of these two mechanisms (dual AAV hybrid vectors). The use of dual AAV vectors *in vivo* results in the expression of full-length proteins. The use of the dual AAV vector platform represents an efficient and viable gene transfer strategy for transgenes of >4.7 kb in size.

Inteins

[0812] In some embodiments, a portion or fragment of a nuclease (*e.g.*, Cas9) is fused to an intein. The nuclease can be fused to the N-terminus or the C-terminus of the intein. In some

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

[0813] Inteins (intervening protein) are auto-processing domains found in a variety of diverse organisms, which carry out a process known as protein splicing. Protein splicing is a multi-step biochemical reaction comprised of both the cleavage and formation of peptide bonds. While the endogenous substrates of protein splicing are proteins found in intein-containing organisms, inteins can also be used to chemically manipulate virtually any polypeptide backbone.

[0814] In protein splicing, the intein excises itself out of a precursor polypeptide by cleaving two peptide bonds, thereby ligating the flanking extein (external protein) sequences via the formation of a new peptide bond. This rearrangement occurs post-translationally (or possibly co-translationally). Intein-mediated protein splicing occurs spontaneously, requiring only the folding of the intein domain.

[0815] 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.

[0816] In some embodiments, an N-terminal fragment of a base editor (*e.g.*, ABE, CBE) is fused to a split intein-N and a C-terminal fragment is fused to a split intein-C. These fragments are then packaged into two or more AAV vectors. The use of certain inteins for joining heterologous protein fragments is described, for example, in Wood *et al.*, J. Biol. Chem. 289(21); 14512-9 (2014). For example, when fused to separate protein fragments, the inteins IntN and IntC recognize each other, splice themselves out and simultaneously ligate the flanking N- and C-terminal exteins of the protein fragments to which they were fused, thereby reconstituting a full-length protein from the two protein fragments. Other suitable inteins will be apparent to a person of skill in the art.

[0817] In some embodiments, an ABE was split into N- and C- terminal fragments at Ala, Ser, Thr, or Cys residues within selected regions of SpCas9. These regions correspond to loop regions identified by Cas9 crystal structure analysis. The N-terminus of each fragment is fused to an intein-N and the C- terminus of each fragment is fused to an intein C at amino acid positions S303, T310, T313, S355, A456, S460, A463, T466, S469, T472, T474, C574, S577, A589, and S590, which are indicated in bold capital letters in the sequence below.

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   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 llSdilrvnT eiTkaplsas mikrydehhq dltllkalvr qqlpekykei ffdqSkngya
 361 gyidggasqe efykfikpil ekmdgteell vklnredllr kqrtfdngsi phqihlgelh
 421 ailrrqedfy pflkdnreki ekiltfripy yvgplArgnS rfAwmTrkSe eTiTpwnfee
 481 vvdkgasaqs fiermtnfdk nlpnekvlpk hsllyeyftv yneltkvkyv tegmrkpafl
 541 sgeqkkaivd llfktnrkvt vkqlkedyfk kieCfdSvei sgvedrfnAS lgtyhdllki
 601 ikdkdfldne nedilediv ltltlfedre mieerlktya hlfddkvmkq lkrrrytgwg
 661 rlsrklingi rdkqsgktil dflksdgfan rnfmqlihdd sltfkediqk aqvsgqgdsl
 721 hehianlags paikkgilqt vkvvdelvkv mgrhkpeniv iemarenqtt qkgqknsrer
 781 mkrieegike lgsqilkehp ventqlqnek lylyylqngr dmyvdqeldi nrlsdydvdh
 841 ivpqsflkdd sidnkvltrs dknrgksdnv pseevvkkmk nywrqllnak litqrkfdnl
 901 tkaergglse ldkagfikrq lvetrqitkh vaqildsrmn tkydendkli revkvitlks
 961 klvsdfrkdf qfykvreinn yhhahdayln avvgtalikk ypklesefvy gdykvydvrk
1021 miakseqeig katakyffys nimnffktei tlangeirkr plietngetg eivwdkgrdf
1081 atvrkvlsmp qvnivkktev qtggfskesi lpkrnsdkli arkkdwdpkk yggfdsptva
1141 ysvlvvakve kgkskklksv kellgitime rssfeknpid fleakgykev kkdliiklpk
1201 yslfelengr krmlasagel qkgnelalps kyvnflylas hyeklkgspe dneqkqlfve
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1261 qhkhyldeii eqisefskrv iladanldkv lsaynkhrdk pireqaenii hlftltnlga
1321 paafkyfdtt idrkrytstk evldatlihq sitglyetri dlsqlggd

PHARMACEUTICAL COMPOSITIONS

[0818] Other aspects of the present disclosure relate to pharmaceutical compositions comprising any of the base editors, fusion proteins, or the fusion protein-guide polynucleotide complexes described herein. In some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises additional agents (*e.g.*, for specific delivery, increasing half-life, or other therapeutic compounds).

[0819] Some non-limiting examples of materials that can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Buffering agents, wetting agents, emulsifying agents, diluents, encapsulating agents, skin penetration enhancers, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. For example, carriers can include, but are not limited to, saline, buffered saline, dextrose, arginine, sucrose, water, glycerol, ethanol, sorbitol, dextran, sodium carboxymethyl cellulose, and combinations thereof.

[0820] 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, in some

embodiments, an agent that maintains the pH of the formulation at a predetermined level, such as in the range of about 5.0 to about 8.0, and that 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.

[0821] 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.

[0822] In some embodiments, the pharmaceutical composition is formulated for delivery to a subject, *e.g.*, for gene editing. In some embodiments, administration of the pharmaceutical compositions contemplated herein may be carried out using conventional techniques including, but not limited to, infusion, transfusion, or parenterally. In some embodiments, parenteral administration includes infusing or injecting intravascularly, intravenously, intramuscularly, intraarterially, intrathecally, intratumorally, intradermally, intraperitoneally, transtracheally, subcutaneously, subcuticularly, intraarticularly, subcapsularly, subarachnoidly and intrasternally. In some embodiments, suitable routes of administrating the pharmaceutical composition described herein include, without limitation: topical, subcutaneous, transdermal, intradermal, intralesional, intraarticular, intraperitoneal, intravesical, transmucosal, gingival, intradental, intracochlear, transtympanic, intraorgan, epidural, intrathecal, intramuscular, intravenous, intravascular, intraosseus, periocular, intratumoral, intracerebral, and intracerebroventricular administration.

[0823] 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.

[0824] 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 al.*, 1989, J. Neurosurg. 71: 105). Other controlled release systems are discussed, for example, in Langer, supra.

[0825] 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.

[0826] 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 al.*, 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.

[0827] 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.

[0828] Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing a compound of the disclosure 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 disclosure. 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.

[0829] In another aspect, an article of manufacture containing materials useful for the treatment of Rett Syndrome (RTT) 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 disclosure. 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.

[0830] 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.

[0831] In some embodiments, compositions provided herein are administered to a subject, for example, to a human subject, in order to effect a targeted genomic modification within the subject. In some embodiments, cells are obtained from the subject and contacted with any of the pharmaceutical compositions provided herein. In some embodiments, cells removed from a subject and contacted *ex vivo* with a pharmaceutical composition are re-introduced into the subject, optionally after the desired genomic modification has been effected or detected in the cells. Methods of delivering pharmaceutical compositions comprising nucleases are known, and are described, for example, in U.S. Patent Nos. 6,453,242; 6,503,717; 6,534,261; 6,599,692; 6,607,882; 6,689,558; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, the disclosures of which are incorporated by reference herein in their entireties. Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals or organisms of all sorts, for example, for veterinary use.

[0832] 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.

[0833] Formulations of the pharmaceutical compositions described herein can be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient(s) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit. Pharmaceutical formulations can additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated in its entirety herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. See also PCT application PCT/US2010/055131 (Publication number 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.

[0834] 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.

[0835] The compositions, as described above, can be administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being treated, and the desired outcome. It may also depend upon the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well-known to the medical practitioner. For therapeutic applications, it is that amount sufficient to achieve a medically desirable result.

Methods of Treating RETT

[0836] Provided also are methods of treating Rett Syndrome (RTT or RETT) and/or the genetic mutations in *Mecp2* that cause RETT that comprise administering to a subject (*e.g.*, a mammal, such as a human) a therapeutically effective amount of a pharmaceutical composition that comprises a polynucleotide encoding a base editor system (*e.g.*, ABE8 base editor and gRNA) described herein. In some embodiments, the base editor is a fusion protein that comprises a

polynucleotide programmable DNA binding domain and an adenosine deaminase domain. A cell of the subject is transduced with the base editor and one or more guide polynucleotides that target the base editor to effect an A•T to G•C alteration of a nucleic acid sequence containing mutations in the *Mecp2* gene.

[0837] The methods herein include administering to the subject (including a subject identified as being in need of such treatment, or a subject suspected of being at risk of disease and in need of such treatment) an effective amount of a composition described herein. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (*e.g.*, opinion) or objective (*e.g.*, measurable by a test or diagnostic method).

[0838] The therapeutic methods, in general, comprise administration of a therapeutically effective amount of a pharmaceutical composition comprising, for example, a vector encoding a base editor and a gRNA that targets the *Mecp2* gene of a subject (*e.g.*, a human patient) in need thereof. Such treatment will be suitably administered to a subject, particularly a human subject, suffering from, having, susceptible to, or at risk for RTT. The compositions herein may be also used in the treatment of any other disorders in which RTT may be implicated.

[0839] In one embodiment, a method of monitoring treatment progress is provided. The method includes the step of determining a level of diagnostic marker (Marker) (*e.g.*, SNP associated with RTT) or diagnostic measurement (*e.g.*, screen, assay) in a subject suffering from or susceptible to a disorder or symptoms thereof associated with RTT in which the subject has been administered a therapeutic amount of a composition herein sufficient to treat the disease or symptoms thereof. The level of Marker determined in the method can be compared to known levels of Marker in either healthy normal controls or in other afflicted patients to establish the subject's disease status. In preferred embodiments, a second level of Marker in the subject is determined at a time point later than the determination of the first level, and the two levels are compared to monitor the course of disease or the efficacy of the therapy. In certain preferred embodiments, a pre-treatment level of Marker in the subject is determined prior to beginning treatment according to the methods of the disclosure; this pre-treatment level of Marker can then be compared to the level of Marker in the subject after the treatment commences, to determine the efficacy of the treatment.

[0840] In some embodiments, cells are obtained from the subject and contacted with a pharmaceutical composition as 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 affected or detected in the

cells. Methods of delivering pharmaceutical compositions comprising nucleases are described, for example, in U.S. Patent Nos. 6,453,242; 6,503,717; 6,534,261; 6,599,692; 6,607,882; 6,689,558; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, the disclosures of 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, for example, for veterinary use.

Kits

[0841] Various aspects of this disclosure provide kits comprising a base editor system. In one embodiment, the kit comprises a nucleic acid construct comprising a nucleotide sequence encoding a nucleobase editor fusion protein. In one embodiment, the fusion protein comprises an adenine deaminase and a nucleic acid programmable DNA binding protein (napDNAbp). In some embodiments, the kit comprises at least one guide RNA capable of targeting a nucleic acid molecule of interest, *e.g.*, *Mecp2* RTT-associated mutations. In some embodiments, the kit comprises a nucleic acid construct comprising a nucleotide sequence encoding at least one guide RNA.

[0842] The kit provides, in some embodiments, instructions for using the kit to edit one or more *Mecp2* RTT-associated mutations. The instructions will generally include information about the use of the kit for editing nucleic acid molecules. In other embodiments, the instructions include at least one of the following: precautions; warnings; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container. In a further embodiment, a kit can comprise instructions in the form of a label or separate insert (package insert) for suitable operational parameters. In yet another embodiment, the kit can comprise one or more containers with appropriate positive and negative controls or control samples, to be used as standard(s) for detection, calibration, or normalization. The kit can further comprise a second container comprising a pharmaceutically-acceptable buffer, such as (sterile) phosphate-buffered saline, Ringer's solution, or dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0843] In certain embodiments, the kit is useful for the treatment of a subject having Rett Syndrome.

[0844] The practice of the present disclosure 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 disclosure, and, as such, may be considered in making and practicing the disclosure. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

EXAMPLES

[0845] 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 disclosure, and are not intended to limit the scope of what is described in the present disclosure and in the embodiments thereof.

EXAMPLE 1 A•T to G•C DNA base editing for correction of *Mecp2* RTT-associated mutations in cells

[0846] Six of the eight most prevalent RTT-causing Mecp2 mutations were targeted for reversion to wild-type sequence using A•T to G•C DNA base editors (ABEs) that employ Cas9 moieties with validated protospacer adjacent motif (PAM) sequence preferences (**FIG. 2**). To determine which guide RNA (gRNA) and ABE-Cas9 platform is able to most efficiently and precisely correct a targeted Mecp2 mutation, an *Mecp2* allele bearing RTT targetable mutations (Table 15), including R255X was genomically integrated in HEK293T cells by lentivirus transduction. Editing efficiencies of gRNAs and ABE-Cas9 editors for a given mutation were measured. Five days after transfection of DNA encoding ABE-Cas9 editors and gRNAs, cells were lysed and analyzed for base editing at the desired site by miSeq analysis.

Table 15: 6 RETT mutations.

Causal mutation	Target sequence	PAM		Cas9 PAM-enabling mutations	editing position
c.316C>T	CCATGTCCAGCCTTCAGGCA	GGG	SpCas9		3
(2.77%)	TCCATGTCCAGCCTTCAGGC	AGG	SpCas9		4
R106W	TTCCATGTCCAGCCTTCAGG	CAG	SpCas9	xCas9	5
	GCTTCCATGTCCAGCCTTCA	GGC	SpCas9	MQKSER/ICKSER/VRKSER; Nureki	7
	CTTCCATGTCCAGCCTTCAGG	CAGGGT	SaCas9		6
	AGCTTCCATGTCCAGCCTTCAGG	CAGGGT	SaCas9		8
	AGCTTCCATGTCCAGCCTTC	CTTA	Cpf1		8
	CTTAAGCTTCCATGTCCAGC	TTTG	Cpf1		12
c.397C>T	AGCAAAAGGCTTTTCCCTGG	GGA	SpCas9	VRQR	4
(4.56%)	GAGCAAAAGGCTTTTCCCTG	GGG	SpCas9		5
R133C	AGAGCAAAAGGCTTTTCCCT	GGG	SpCas9		6
	TAGAGCAAAAGGCTTTTCCC	TGG	SpCas9		7
	TAGAGCAAAAGGCTTTTCCCT	GGGGAT	SaCas9		7
	TTTAGAGCAAAAGGCTTTTCCCT	GGGGAT	SaCas9		9
c.473C>T	CCATGAAGTCAAAATCATTA	GGG	SpCas9		3
(8.81%)	ACCATGAAGTCAAAATCATT	AGG	SpCas9		4
T158M	TACCATGAAGTCAAAATCAT	TAG	SpCas9	xCas9; ScCas9	5
	TTACCATGAAGTCAAAATCAT	TAGGGT	SaCas9		6
	AGTTACCATGAAGTCAAAATCAT	TAGGGT	SaCas9		8
c.763C>T (6.68%) R255X	CTTTTCACTTCCTGCCGGGG	CGT	SpCas9	LRSVQL/LRKIQK/LRSVQK; Nureki	7
c.808C>T	TCAGCCCCGTTTCTTGGGAA	TGG	SpCas9		3
(5.8%)	GCTTTCAGCCCCGTTTCTTG	GGA	SpCas9	VRQR	7
R270X	CGGCTTTCAGCCCCGTTTCTT	GGGAAT	SaCas9		9
	CCCGGCTTTCAGCCCCGTTTCTT	GGGAAT	SaCas9		11
c.916C>T (5.17%)	GCACTTCTTGATGGGGAGTA	CGG	SpCas9		3
R306C	TCTTGCACTTCTTGATGGGG	AGT	SpCas9	LRSVQL/LRKIQK/LRSVQK	7
	CTTGCACTTCTTGATGGGGAG	TACGGT	SaCas9	KKH	6
	GTCTTGCACTTCTTGATGGGGAG	TACGGT	SaCas9	KKH	8

[0847] Particular target/spacer sequences for four of the most severe mutations, R255X, R106W, T158M, and R270X, are shown in Table 16 below:

Table 16: Rett Mutation Target and PAM Sequences

Mutation	Spacer	PAM
R255X	CTTTTC**A**CTTCCTGCCGGGG	NGTT
R106W	AGCTTCC**A**TGTCCAGCCTTC	NGG
T158M	ACC**A**TG**AA**GTCAAAATCATT	NGG
R270X	GCTTTC**A**GCCCCGTTTCTTG	NGG

The above target /spacer sequences show a targetable "a" nucleobase in bold and underlining. The R255X mutation may be targeted using a NGTT PAM variant and the R106W, T158M, and R270X mutations may be targeted using a NGG PAM.

EXAMPLE 2 Base editing for the Correction of the R106W Mutation

[0848] R106W is one of the four most severe mutations in Rett Syndrome. ABEs may be used for the correction of R106W by efficiently converting A>G at a targeted site. Base editing was tested in the MECP2-HEK293T cell line, which contains a single, genomically integrated copy of MECP2 containing RETT mutations as discussed in Example 1. Five (5) guide RNAs (gRNAs) were tested targeting the R106W mutation in RTT with ABE8 base editor variants (**FIG. 3**).

[0849] The gRNA encompasses the scaffold sequence and the spacer sequence (target sequence) for disease-associated genes as provided herein or as determined based on the knowledge of the skilled practitioner and as would be understood to the skilled practitioner in the art. (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); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1).

[0850] The five (5) DNA target sequences for the R106W mutation are provided below:

DNA target sequence 1: CCATGTCCAGCCTTCAGGCA**GGG**

DNA target sequence 2: TCCATGTCCAGCCTTCAGGC**AGG**

DNA target sequence 3: TTCCATGTCCAGCCTTCAGG**CAG**

DNA target sequence 4: GCTTCCATGTCCAGCCTTCAGGC

DNA target sequence 5: AGCTTCCATGTCCAGCCTTCAGG

PAM sequences are underlined and in bold font. As shown in Table 15 above, target sequences 1, 2 and 5 utilize a NGG PAM (*i.e.*, SpCas9). Target sequence 3 requires xCas9 (as described in Liu *et al.* WO2017070633, incorporated herein by reference). Target sequence 4 requires Cas9 PAM variants (as described in Joung *et al.* WO2019040650, incorporated herein by reference).

[0851] The five (5) guide RNA sequences, which hybridize to the complement of each of the above DNA target sequences, are as follows:

gRNA1: 5′-gCCAUGUCCAGCCUUCAGGCA-3′

gRNA2: 5′-gUCCAUGUCCAGCCUUCAGGC-3′

gRNA3: 5′-gUUCCAUGUCCAGCCUUCAGG-3′

gRNA4: 5′-GCUUCCAUGUCCAGCCUUCA-3′

gRNA5: 5′-gAGCUUCCAUGUCCAGCCUUC-3′

A lowercase "g" in both the DNA target sequences and the gRNA sequences indicates a mismatch in the sequence where a polymerase must initiate transcription. In some embodiments, a guide polynucleotide may be truncated by 1, 2, 3, 4, etc. nucleotides, particularly at the 5′ end.

[0852] For each of the gRNAs, the scaffold sequence is as follows: GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC CGAGTCGGTGCTTTTTTT. As will be appreciated by one having skill in the art, in a guide polynucleotide sequence (gRNA) uracil (U) replaces thymine (T) in the sequence.

[0853] The ABE base editors used include ABE8 heterodimer variants: ABE8.14, ABE8.15, ABE8.16, ABE8.17, ABE8.18, ABE8.19, ABE8.20, ABE8.21, ABE8.22, ABE8.23, ABE8.24, ABE8.25, and ABE8.26 (*see* Table 9). Positive control base editor ABE7.10 and a negative control were also used for comparison.

[0854] For plasmid transfections, HEK293T cells were transfected and plated with 250ng of gRNA and 750ng of ABE8 variant base editor expression plasmid using Opti-MEM media and Lipofectamine 2000. The ABE8 base editor variants were used having the NGG PAM sequence. The cells were lysed and prepped for sequencing 5 days post-transfection (with a media change at day 3 post-transfection).

[0855] Out of the five (5) gRNA sequences, gRNA1, gRNA2, and gRNA5 provided the best A to G editing (**FIG. 3**). The ABE8 base editor variants had increased base editing activity compared to the ABE7.10 control. As shown in **FIG. 3**, the amount of A to G gene editing achieved was about 35% using gRNA2.

EXAMPLE 3 Base editing for the Correction of an R133C Mutation

[0856] ABEs may be used for the correction of the R133C by efficiently converting A>G at a targeted site. (FIGS. 1 and 2). Base editing is tested in the MECP2-HEK293T cell line, which contains a single, genomically integrated copy of *MECP2* containing RETT mutations as discussed in Example 1. Four (4) guide RNAs (gRNAs) are tested targeting the R133C mutation in RETT with ABE8 base editor variants.

[0857] The gRNA encompasses the scaffold sequence and the spacer sequence (target sequence) for disease-associated genes as provided herein or as determined based on the knowledge of the skilled practitioner and as would be understood to the skilled practitioner in the art. (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); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1).

[0858] Four (4) DNA target sequences for the R133C mutation are provided below:

DNA target sequence 1: AGAGCAAAAGGCTTTTCCCT

DNA target sequence 2: TAGAGCAAAAGGCTTTTCCC

DNA target sequence 3: AGAGCAAAAGGCTTTTCCCT

DNA target sequence 4: TTTAGAGCAAAAGGCTTTTCCCT

The target sequences utilize PAM sequences as shown in Table 15 above. Target sequences 1 and 2 utilize SpCas9. Target sequences 3 and 4 utilize SaCas9.

[0859] Four (4) guide RNA sequences (gRNAs), which hybridize to the complement of each of the above DNA target sequences, are as follows:

gRNA1: 5'-gAGAGCAAAAGGCUUUUCCCU-3'

gRNA2: 5'-gUAGAGCAAAAGGCUUUUCCC-3'

gRNA3: 5'-gUAGAGCAAAAGGCUUUUCCCU-3'

gRNA4: 5'-gUUUAGAGCAAAAGGCUUUUCCCU-3'

A lowercase "g" in both the DNA target sequences and the gRNA sequences indicates a mismatch in the sequence where a polymerase must initiate transcription. In some embodiments, a guide polynucleotide may be truncated by 1, 2, 3, 4, etc. nucleotides, particularly at the 5′ end.

[0860] For each of the gRNAs, the scaffold sequence is as follows: GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCAC CGAGTCGGTGCTTTTTTT. As will be appreciated by one having skill in the art, in a guide polynucleotide sequence (gRNA) uracil (U) replaces thymine (T) in the sequence.

[0861] ABE base editors for use include ABE8 heterodimer variants: ABE8.14, ABE8.15, ABE8.16, ABE8.17, ABE8.18, ABE8.19, ABE8.20, ABE8.21, ABE8.22, ABE8.23, ABE8.24, ABE8.25, and ABE8.26 (*see* Table 9). Positive control base editor ABE7.10 and a negative control are also used for comparison.

[0862] For plasmid transfections, HEK293T cells are transfected and plated with 250ng of gRNA and 750ng of ABE8 variant base editor expression plasmid using Opti-MEM media and Lipofectamine 2000. The cells are lysed and prepped for sequencing 5 days post-transfection (with a change of medium at day 3 post-transfection).

EXAMPLE 4 Base editing for the Correction of an R306C Mutation

[0863] ABEs may be used for the correction of the R306C by efficiently converting A>G at a targeted site. (FIGS. 1 and 2). Base editing is tested in the MECP2-HEK293T cell line, which contains a single, genomically integrated copy of *MECP2* containing RETT mutations as discussed in Example 1. Three (3) guide RNAs (gRNAs) are tested targeting the R306C mutation in RETT with ABE8 base editor variants.

[0864] The gRNA encompasses the scaffold sequence and the spacer sequence (target sequence) for disease-associated genes as provided herein or as determined based on the knowledge of the skilled practitioner and as would be understood to the skilled practitioner in the art. (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); Gaudelli, N.M., *et al.*, "Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage" Nature 551, 464-471 (2017); Komor, A.C., *et al.*, "Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity" Science Advances 3:eaao4774 (2017), and Rees, H.A., *et al.*, "Base editing: precision chemistry on the genome and transcriptome of living cells." Nat Rev Genet. 2018 Dec;19(12):770-788. doi: 10.1038/s41576-018-0059-1).

[0865] Three (3) DNA target sequences for the R306C mutation are provided below:

DNA target sequence 1: TCTTGCACTTCTTGATGGGG

DNA target sequence 2: CTTGCACTTCTTGATGGGGAG

DNA target sequence 3: GTCTTGCACTTCTTGATGGGGAG

The target sequences utilize PAM sequences as shown in Table 15 above. Target sequence 1 utilizes SpCas9. Target sequences 2 and 3 utilize SaCas9.

[0866] Three (3) guide RNA sequences (gRNAs), which hybridize to the complement of each of the above DNA target sequences, are as follows:

gRNA1: 5′-gUCUUGCACUUCUUGAUGGGG-3′

gRNA2: 5′-gCUUGCACUUCUUGAUGGGGAG-3′

gRNA3: 5′-gGUCUUGCACUUCUUGAUGGGGAG-3′

A lowercase "g" in both the DNA target sequences and the gRNA sequences indicates a mismatch in the sequence where a polymerase must initiate transcription. In some embodiments, a guide polynucleotide may be truncated by 1, 2, 3, 4, etc. nucleotides, particularly at the 5′ end.

[0867] For each of the gRNAs, the scaffold sequence is as follows: GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT. As will be appreciated by one having skill in the art, in a guide polynucleotide sequence (gRNA) uracil (U) replaces thymine (T) in the sequence.

[0868] ABE base editors for use include ABE8 heterodimer variants: ABE8.14, ABE8.15, ABE8.16, ABE8.17, ABE8.18, ABE8.19, ABE8.20, ABE8.21, ABE8.22, ABE8.23, ABE8.24, ABE8.25, and ABE8.26 (*see* Table 9). Positive control base editor ABE7.10 and a negative control are also used for comparison.

[0869] For plasmid transfections, HEK293T cells are transfected and plated with 250ng of gRNA and 750ng of ABE8 variant base editor expression plasmid using Opti-MEM media and Lipofectamine 2000. The cells are lysed and prepped for sequencing 5 days post-transfection (with a change of medium at day 3 post-transfection).

CLAIMS

What is claimed is:

1. A method of editing a methyl CpG binding protein 2 (*MECP2*) gene or regulatory element thereof in a subject, the method comprising administering to a subject in need thereof (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 relative to a TadA reference sequence, or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in the *MECP2* gene or a regulatory element thereof, which comprises a SNP associated with Rett syndrome (RETT); wherein the A-to-G nucleobase alteration is at the SNP associated with RETT, which results in an R133C or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

2. The method of claim 1, wherein the TadA reference sequence comprises amino acid sequence
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG
SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

3. The method of claim 1 or claim 2, wherein the A-to-G nucleobase alteration changes the SNP associated with RETT to a wild type nucleobase.

4. The method of claim 1 or claim 2, wherein the A-to-G nucleobase alteration changes the SNP associated with RETT to a non-wild type nucleobase that results in one or more ameliorated symptoms of RETT.

5. The method of any one of claims 1-4, wherein the A-to-G nucleobase alteration at the SNP associated with RETT changes a cysteine to an arginine or stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide.

6. The method of any one of claims 1-5, wherein the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306.

7. The method of any one of claims 1-6, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT.

8. The method of any one of claims 1-7, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT.

9. The method of claim 8, wherein the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

10. A base editor system comprising (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 relative to a TadA reference sequence or a corresponding position thereof, and wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (*MECP2*) gene or regulatory element thereof, which comprises a SNP associated with Rett syndrome (RETT); wherein the A-to-G nucleobase alteration is at the SNP associated with RETT, which results in an R133C or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

11. The base editor system of claim 10, wherein the A-to-G nucleobase alteration changes the SNP associated with RETT to a wild type nucleobase.

12. The base editor system of claim 10, wherein the A-to-G nucleobase alteration changes the SNP associated with RETT to a non-wild type nucleobase that results in one or more ameliorated symptoms of RETT.

13. The base editor system of any one of claims 10-12, wherein the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306.

14. The base editor system of any one of claims 10-13, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT.

15. The base editor system of any one of claims 10-14, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT.

16. The base editor system of claim 15, wherein the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

17. A method of editing an *MECP2* polynucleotide comprising a single nucleotide polymorphism (SNP) associated with Rett Syndrome (RETT), the method comprising contacting the *MECP2* polynucleotide with an Adenosine Deaminase Base Editor 8 (ABE8) in a complex with one or more guide polynucleotides, wherein the ABE8 comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain, and wherein one or more of said guide polynucleotides target said base editor to effect an A•T to G•C alteration of the SNP in the *MECP2* polynucleotide associated with RETT, wherein the alteration is one or both of R133C or R306C.

18. The method of claim 17, wherein the contacting is in a cell, a eukaryotic cell, a mammalian cell, or human cell.

19. The method of claim 17 or claim 18, wherein the cell is *in vivo* or *ex vivo*.

20. The method of any one of claims 17-19, wherein the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, or stop codon to arginine in the methyl CpG binding protein 2 (Mecp2) polypeptide.

21. The method of any one of claims 17-20, wherein the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306.

22. The method of any one of claims 17-21, wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof.

23. The method of any one of claims 17-22, wherein the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM).

24. The method of claim 23, wherein the modified SpCas9 binds to a PAM comprising a nucleic acid sequence selected from 5′-NGT-3′ or 5′-NGG-3′.

25. The method of claim 23, wherein the modified SpCas9 binds to a NGT PAM variant.

26. The method of claim 24 or claim 25, wherein the NGT PAM variant comprises amino acid substitutions at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219 of the modified SpCas9, or corresponding amino acid substitutions thereof.

27. The method of any one of claims 23-25, wherein the modified SpCas9 comprises the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, , T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof.

28. The method of any one of claims 23-25, wherein the modified SpCas9 comprises the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, G1218R, E1219F, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, T1337L, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof.

29. The method of any one of claims 17-28, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant.

30. The method of claim 29, wherein the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

31. The method of any one of claims 17-30, wherein the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA).

32. The method of any one of claims 17-31, wherein the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

33. The method of claim 32, wherein the adenosine deaminase domain comprises alterations at amino acid position 82 and 166.

34. The method of claim 32, wherein the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration.

35. The method of any one of claims 32-34, wherein the adenosine deaminase domain further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R.

36. The method of any one of claims 32-35, wherein the adenosine deaminase domain comprises an alteration selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

37. The method of any one of claims 17-36, wherein the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations.

38. The method of any one of claims 17-37, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant.

39. The method of claim 37, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

40. The method of claim 17, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*8 domain and wild-type TadA domain.

41. The method of claim 37, wherein the adenosine deaminase monomer further comprises an alteration selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

42. The method of claim 17, wherein the ABE8 base editor comprises a heterodimer comprising a wild-type TadA domain and an adenosine deaminase variant comprising a combination of alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

43. The method of any one of claims 17-42, wherein the guide polynucleotide comprises a nucleic acid sequence selected from AGAGCAAAAGGCUUUUCCCU-3', 5'-

UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

44. The method of claim 17, wherein the adenosine deaminase is a TadA deaminase.

45. The method of claim 44, wherein the TadA deaminase is a TadA*8 variant.

46. The method of claim 45, wherein the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24.

47. The method of claim 17, wherein the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24.

48. The method of any one of claims 17-47, wherein the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

49. The method of any one of claims 17-48, wherein the ABE8 base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

50. The method of any one of claims 17-49, wherein the ABE8 base editor comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

51. A cell produced by introducing into the cell, or a progenitor thereof:

an ABE8 base editor, or a polynucleotide encoding said base editor, wherein said ABE8 base editor comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain; and

one or more guide polynucleotides that target the base editor to effect an A•T to G•C alteration of the SNP in an *MECP2* polynucleotide associated with RETT syndrome (RETT), wherein the alteration is one or both of R133C or R306C.

52. The cell of claim 51, wherein the cell is a neuron.

53. The cell of claim 51 or claim 52, wherein the neuron expresses an MECP2 polypeptide.

54. The cell of any one of claims 51-53, wherein the cell is from a subject having RETT.

55. The cell of any one of claims 51-54, wherein the cell is a mammalian cell or a human cell.

56. The cell of any one of claims 51-55, wherein the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide.

57. The cell of any one of claims 51-56, wherein the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306.

58. The cell of any one of claims 51-57, wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof.

59. The cell of any one of claims 51-58, wherein the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an alteredprotospacer-adjacent motif (PAM).

60. The cell of claim 59, wherein the modified SpCas9 binds to a PAM comprising a nucleic acid sequence selected from 5′-NGT-3′ or 5′-NGG-3′.

61. The cell of claim 60, wherein the modified SpCas9 binds to a NGT PAM variant.

62. The method of claim 61, wherein the NGT PAM variant comprises amino acid substitutions at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219 of the modified SpCas9, or corresponding amino acid substitutions thereof.

63. The cell of claim 59, wherein the modified SpCas9 comprises the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K,

D1332R, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, , T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof.

64. The cell of claim 59, wherein the modified SpCas9 comprises the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, G1218R, E1219F, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, T1337L, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof.

65. The cell of any one of claims 51-64, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant.

66. The cell of claim 65, wherein the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

67. The cell of any one of claims 51-66, wherein the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA).

68. The cell of any one of claims 51-67, wherein the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

69. The cell of claim 68, wherein the adenosine deaminase domain comprises alterations at amino acid position 82 and 166.

70. The cell of claim 69, wherein the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration.

71. The cell of any one of claims 68-70, wherein the adenosine deaminase further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R.

72. The cell of any one of claims 68-71, wherein the adenosine deaminase domain comprises a combination of alterations selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

73. The cell of any one of claims 68-72, wherein the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations.

74. The cell of any one of claims 68-72, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant.

75. The cell of claim 73, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

76. The cell of claim 51, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*7.10 domain and TadA*8 domain.

77. The cell of claim 73, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

78. The cell of claim 51, wherein the ABE8 base editor comprises a heterodimer comprising a TadA*7.10 domain and an adenosine deaminase variant comprising alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

79. The cell of claim 51, wherein the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

80. The cell of claim 51, wherein the adenosine deaminase is a TadA deaminase.

81. The cell of claim 80, wherein the TadA deaminase is a TadA*8 variant.

82. The cell of claim 81, wherein the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24.

83. The cell of claim 51, wherein the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24.

84. The cell of any one of claims 51-83, wherein the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

85. The cell of any one of claims 51-84, wherein the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

86. The cell of any one of claims 51-85, wherein the ABE8 base editor comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG
SLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD.

87. The cell of any one of claims 51-86, wherein the gRNA comprises a scaffold having the following sequence:
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGCACCGAGTCGGTGCTTTTTTT.

88. A method of treating RETT Syndrome (RETT) in a subject, comprising administering to said subject:

an ABE8 base editor, or a polynucleotide encoding said base editor, wherein said ABE8 base editor comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain; and

one or more guide polynucleotides that target the ABE8 base editor to effect an A•T to G•C alteration of the SNP in an MECP2 polynucleotide associated with RETT, wherein the alteration is one or both of R133C and/or R306C.

89. The method of claim 88, wherein the subject is a mammal or a human.

90. The method of claim 88 or claim 89, comprising delivering the ABE8 base editor, or polynucleotide encoding said ABE8 base editor, and said one or more guide polynucleotides to a cell of the subject, optionally, wherein the cell is a neuron.

91. The method of any one of claims 88-90, wherein the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, or stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide.

92. The method of any one of claims 88-91, wherein the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306.

93. The method of any one of claims 88-92, wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof.

94. The method of any one of claims 88-93, wherein the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM).

95. The method of claim 94, wherein the modified SpCas9 binds to a PAM comprising a nucleic acid sequence selected from 5′-NGT-3′ or 5′-NGG-3′.

96. The method of claim 94, wherein the modified SpCas9 binds to a NGT PAM variant.

97. The method of claim 96, wherein the NGT PAM variant comprises amino acid substitutions at one or more residues 1335, 1337, 1135, 1136, 1218, and/or 1219, of the modified SpCas9, or corresponding amino acid substitutions thereof.

98. The method of claim 94, wherein the modified SpCas9 comprises the amino acid substitutions L1111R, D1135V, G1218R, E1219F, A1322R, R1335V, T1337R and one or more of L1111, D1135L, S1136R, G1218S, E1219V, D1332A, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, and T1337M, or corresponding amino acid substitutions thereof.

99. The method of claim 94, wherein the modified SpCas9 comprises the amino acid substitutions D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337, and one or more of L1111R, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L, T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereof.

100. The method of any one of claims 88-99, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant.

101. The method of claim 100, wherein the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

102. The method of any one of claims 88-101, wherein the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA).

103. The method of any one of claims 88-102, wherein the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

104. The method of claim 103, wherein the adenosine deaminase domain comprises alterations at amino acid position 82 and 166.

105. The method of claim 103, wherein the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration.

106. The method of any one of claims 103-105, wherein the adenosine deaminase domain further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R.

107. The method of any one of claims 103-106, wherein the adenosine deaminase domain comprises an alteration selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

108. The method of any one of claims 88-107, wherein the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations.

109. The method of any one of claims 88-107, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant.

110. The method of claim 108, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

111. The method of claim 88, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*7.10 domain and TadA*8 domain.

112. The method of claim 108, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

113. The method of claim 88, wherein the ABE8 base editor comprises a heterodimer comprising a TadA7.10 domain and an adenosine deaminase variant comprising alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

114. The method of any one of claims 88-113, wherein the guide polynucleotide has a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

115. The method of claim 102, wherein the adenosine deaminase is a TadA deaminase.

116. The method of claim 115, wherein the TadA deaminase is a TadA*8 variant.

117. The method of claim 116, wherein the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24.

118. The method of claim 88, wherein the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d,

ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24.

119. The method of any one of claims 88-118, wherein the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

120. The method of any one of claims 88-119, wherein the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

121. A method of treating Rett syndrome (RETT) in a subject, the method comprising:

administering to a subject in need thereof (i) an adenosine base editor or a nucleic acid sequence encoding the adenosine base editor and (ii) a guide polynucleotide or a nucleic acid sequence encoding the guide polynucleotide, wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain, wherein the adenosine deaminase domain comprises an amino acid substitution at amino acid position 82 or 166 relative to a TadA reference sequence, or a corresponding position thereof,

wherein the guide polynucleotide directs the adenosine base editor to effect an A-to-G nucleobase alteration in a methyl CpG binding protein 2 (*MECP2*) gene or a regulatory element thereof comprising a SNP associated with RETT in the subject, thereby treating RETT in the subject, and wherein the SNP associated with RETT results in an R133C or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

122. The method of claim 121, wherein the administration ameliorates at least one symptom associated with RETT.

123. The method of claim 121, wherein the administration results in faster amelioration of at least one symptom related to RETT compared to treatment with a base editor without the amino acid substitution in the adenosine deaminase.

124. The method of any one of claims 121-123, wherein the A-to-G nucleobase alteration changes the SNP associated with RETT to a wild type nucleobase.

125. The method of any one of claims 121-124, wherein the A-to-G nucleobase alteration changes the SNP associated with Rett syndrome to a non-wild type nucleobase that results in ameliorated RETT symptoms.

126. The method of any one of claims 121-125, wherein the guide polynucleotide comprises a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT.

127. The method of any one of claims 121-126, wherein the adenosine base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to the *MECP2* gene or regulatory element thereof comprising the SNP associated with RETT.

128. The method of claim 127, wherein the guide polynucleotide comprises a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

129. The method of any one of claims 1-9 or 121-128, or the base editor of any one of claims 10-16, wherein the guide polynucleotide comprises a nucleic acid sequence comprising at least 10 contiguous nucleotides that are complementary to the *MECP2* gene or a regulatory element thereof.

130. The method of claim 129, wherein the guide polynucleotide comprises a nucleic acid sequence comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that are complementary to the *MECP2* gene ore a regulatory element thereof.

131. The method of any one of claims 17-50, or 88-120, or the cell of any one of claims 51-87, wherein the guide polynucleotide comprises a nucleic acid sequence comprising at least 10 contiguous nucleotides that are complementary to the *MECP2* polynucleotide.

132. The method of claim 131, wherein the guide polynucleotide comprises a nucleic acid sequence comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that are complementary to the *MECP2* polynucleotide.

133. The method of any one of claims 121-128, wherein the SNP associated with RETT results in an R133C and/or an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

134. The method of claim 133, wherein the SNP associated with RETT results in an R133C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

135. The method of claim 133, wherein the SNP associated with RETT results in an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

136. The base editor system of any one of claims 10-16, wherein the A-to-G nucleobase alteration at the SNP associated with RETT results in an R133C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

137. The base editor system of any one of claims 10-16, wherein the A-to-G nucleobase alteration at the SNP associated with RETT results in an R306C amino acid mutation in a MECP2 polypeptide, or a variant thereof, encoded by the *MECP2* gene.

138. The method of any one of claims 17-50, or 88-120, wherein alteration of the SNP associated with RETT comprises both R133C and R306C.

139. The method of any one of claims 17-50, or 88-120, wherein alteration of the SNP associated with RETT is R133C.

140. The method of any one of claims 17-50, or 88-120, wherein alteration of the SNP associated with RETT is R306C.

141. The cell of any one of claims 51-87, wherein the alteration of the SNP associated with RETT syndrome (RETT) is R133C.

142. The cell of any one of claims 51-87, wherein the alteration of the SNP associated with RETT syndrome (RETT) is R306C.

143. A guide polynucleotide or guide RNA comprising 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 contiguous nucleotides that are perfectly complementary to an *MECP2* gene that encodes an MECP2 protein.

144. A guide polynucleotide or guide RNA of claim 143 comprising a nucleic acid sequence selected from 5'-AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

145. The guide polynucleotide or guide RNA of claim 143 or claim 144, which further comprises a scaffold sequence, wherein the scaffold sequence is optionally as follows: GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTT.

146. A composition comprising an Adenosine Deaminase Base Editor 8 (ABE8) and a guide RNA, wherein the ABE8 comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain, and wherein the guide RNA targets the base editor to effect an A•T to G•C alteration of the SNP in an *MECP2* polynucleotide associated with RETT syndrome, and wherein the alteration is one or both of R133C or R306C.

147. The composition of claim 146, wherein the A•T to G•C alteration at the SNP associated with RETT changes a cysteine to an arginine, or stop codon to arginine in the methyl CpG binding protein 2 (MECP2) polypeptide.

148. The composition of claim 146 or claim 147, wherein the SNP associated with RETT results in expression of an MECP2 polypeptide comprising an arginine at amino acid position 133 and/or 306.

149. The composition of any one of claims 146-148, wherein the polynucleotide programmable DNA binding domain is a Cas9 selected from *Streptococcus pyogenes* Cas9 (SpCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus 1* Cas9 (St1Cas9), *Steptococcus canis* Cas9(ScCas9), or variant thereof.

150. The composition of any one of claims 146-149, wherein the polynucleotide programmable DNA binding domain comprises a modified SpCas9 that binds to an altered protospacer-adjacent motif (PAM).

151. The composition of any one of claims 146-149, wherein the polynucleotide programmable DNA binding domain is a nuclease inactive or nickase variant.

152. The composition of claim 151, wherein the nickase variant comprises an amino acid substitution D10A or a corresponding amino acid substitution thereof.

153. The composition of any one of claims 146-152, wherein the adenosine deaminase domain is capable of deaminating adenosine in deoxyribonucleic acid (DNA).

154. The composition of any one of claims 146-153, wherein the adenosine deaminase domain comprises an alteration at amino acid position 82 and/or 166 of MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG SLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKKAQSSTD.

155. The composition of claim 154, wherein the adenosine deaminase domain comprises an alteration selected from a V82S alteration, a T166R alteration, or both a V82S and an T166R alteration.

156. The composition of claim 154 or 155, wherein the adenosine deaminase domain further comprises one or more of the following alterations: Y147T, Y147R, Q154S, Y123H, and Q154R.

157. The composition of any one of claims 154-156, wherein the adenosine deaminase domain comprises an alteration selected from the group consisting of: Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R +

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

158. The composition of any one of claims 146-157, wherein the ABE8 comprises an adenosine deaminase variant monomer, wherein the adenosine deaminase monomer comprises V82S and T166R alterations.

159. The composition of any one of claims 146-158, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a wild-type adenosine deaminase domain and an adenosine deaminase variant.

160. The composition of claim 158, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

161. The composition of claim 146, wherein the ABE8 comprises an adenosine deaminase heterodimer comprising a TadA*8 domain and wild-type TadA domain.

162. The composition of claim 158, wherein the adenosine deaminase variant monomer further comprises one or more alterations selected from the group consisting of Y147T, Y147R, Q154S, Y123H, V82S, T166R, and Q154R.

163. The composition of claim 146, wherein the ABE8 base editor comprises a heterodimer comprising a wild-type TadA domain and an adenosine deaminase variant comprising a combination of alterations selected from the group consisting of Y147T + Q154R; Y147T + Q154S; Y147R + Q154S; V82S + Q154S; V82S + Y147R; V82S + Q154R; V82S + Y123H; I76Y + V82S; V82S + Y123H + Y147T; V82S + Y123H + Y147R; V82S + Y123H + Q154R; Y147R + Q154R +Y123H; Y147R + Q154R + I76Y; Y147R + Q154R + T166R; Y123H + Y147R + Q154R + I76Y; V82S + Y123H + Y147R + Q154R; and I76Y + V82S + Y123H + Y147R + Q154R.

164. The composition of any one of claims 146-163, wherein the guide RNA comprises a nucleic acid sequence selected from AGAGCAAAAGGCUUUUCCCU-3', 5'-UAGAGCAAAAGGCUUUUCCC-3', 5'-UAGAGCAAAAGGCUUUUCCCU-3', 5'-UUUAGAGCAAAAGGCUUUUCCCU-3', 5'-UCUUGCACUUCUUGAUGGGG-3', 5'-CUUGCACUUCUUGAUGGGGAG-3', or 5'-GUCUUGCACUUCUUGAUGGGGAG-3'.

165. The composition of claim 146, wherein the adenosine deaminase is a TadA deaminase.

166. The composition of claim 165, wherein the TadA deaminase is a TadA*8 variant.

167. The composition of claim 166, wherein the TadA*8 variant is selected from the group consisting of: TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, and TadA*8.24.

168. The composition of claim 146, wherein the ABE8 base editor is selected from the group consisting of: ABE8.1-m, ABE8.2-m, ABE8.3-m, ABE8.4-m, ABE8.5-m, ABE8.6-m, ABE8.7-m, ABE8.8-m, ABE8.9-m, ABE8.10-m, ABE8.11-m, ABE8.12-m, ABE8.13-m, ABE8.14-m, ABE8.15-m, ABE8.16-m, ABE8.17-m, ABE8.18-m, ABE8.19-m, ABE8.20-m, ABE8.21-m, ABE8.22-m, ABE8.23-m, ABE8.24-m, ABE8.1-d, ABE8.2-d, ABE8.3-d, ABE8.4-d, ABE8.5-d, ABE8.6-d, ABE8.7-d, ABE8.8-d, ABE8.9-d, ABE8.10-d, ABE8.11-d, ABE8.12-d, ABE8.13-d, ABE8.14-d, ABE8.15-d, ABE8.16-d, ABE8.17-d, ABE8.18-d, ABE8.19-d, ABE8.20-d, ABE8.21-d, ABE8.22-d, ABE8.23-d, and ABE8.24.

169. The composition of any one of claims 146-168, wherein the guide RNA comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

170. The composition of any one of claims 146-169, wherein the ABE8 base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an *MECP2* nucleic acid sequence comprising the SNP associated with RETT.

171. The composition of any one of claims 146-170, wherein the ABE8 base editor comprises or consists essentially of the following sequence or a fragment thereof having adenosine deaminase activity:

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFGVRNAKTGAAG
SLMDVLHYPGMNHRVEITEGILADECAALLCTFFRMPRQVFNAQKKAQSSTD

172. The composition of any one of claims 146-171, further comprising a lipid, optionally wherein the lipid is a cationic lipid.

173. The composition of any one of claims 146-172, which is a pharmaceutical composition comprising a pharmaceutically acceptable excipient or diluent.

174. The pharmaceutical composition of claim 173 for the treatment of RETT syndrome.

175. The pharmaceutical composition of claim 173, wherein the gRNA and the ABE8 base editor are formulated together or separately.

176. The pharmaceutical composition of any one of claims 172-175, further comprising a vector suitable for expression in a mammalian cell, wherein the vector comprises a polynucleotide encoding the ABE8 base editor.

177. The pharmaceutical composition of claim 176, wherein the vector is a viral vector.

178. The pharmaceutical composition of claim 177, wherein the viral vector is a retroviral vector, adenoviral vector, lentiviral vector, herpesvirus vector, or adeno-associated viral vector (AAV).

179. The pharmaceutical composition of any one of claims 172-178, further comprising a ribonucleoparticle suitable for expression in a mammalian cell.

180. A method of treating RETT syndrome, the method comprising administering to a subject in need thereof the pharmaceutical composition of any one of claims 172-179.

181. Use of the pharmaceutical composition of any one of claims 172-179 in the treatment of RETT syndrome in a subject.

182. The method of claim 180, or the use of claim 181, wherein the subject is a mammal or a human.

183. A composition comprising the cell of any one of claims 51-87.

184. The composition of claim 183, further comprising a pharmaceutically acceptable carrier or diluent.

185. A pharmaceutical composition comprising (i) a nucleic acid encoding an ABE8 base editor; and (ii) the guide polynucleotide or guide RNA of any one of claims 143-145.

186. The pharmaceutical composition of claim 185, further comprising a lipid.

187. The pharmaceutical composition of claim 186, wherein the lipid is a cationic lipid.

188. The pharmaceutical composition of claim 186 or claim 187, wherein the nucleic acid encoding the base editor is an mRNA.

FIG. 1

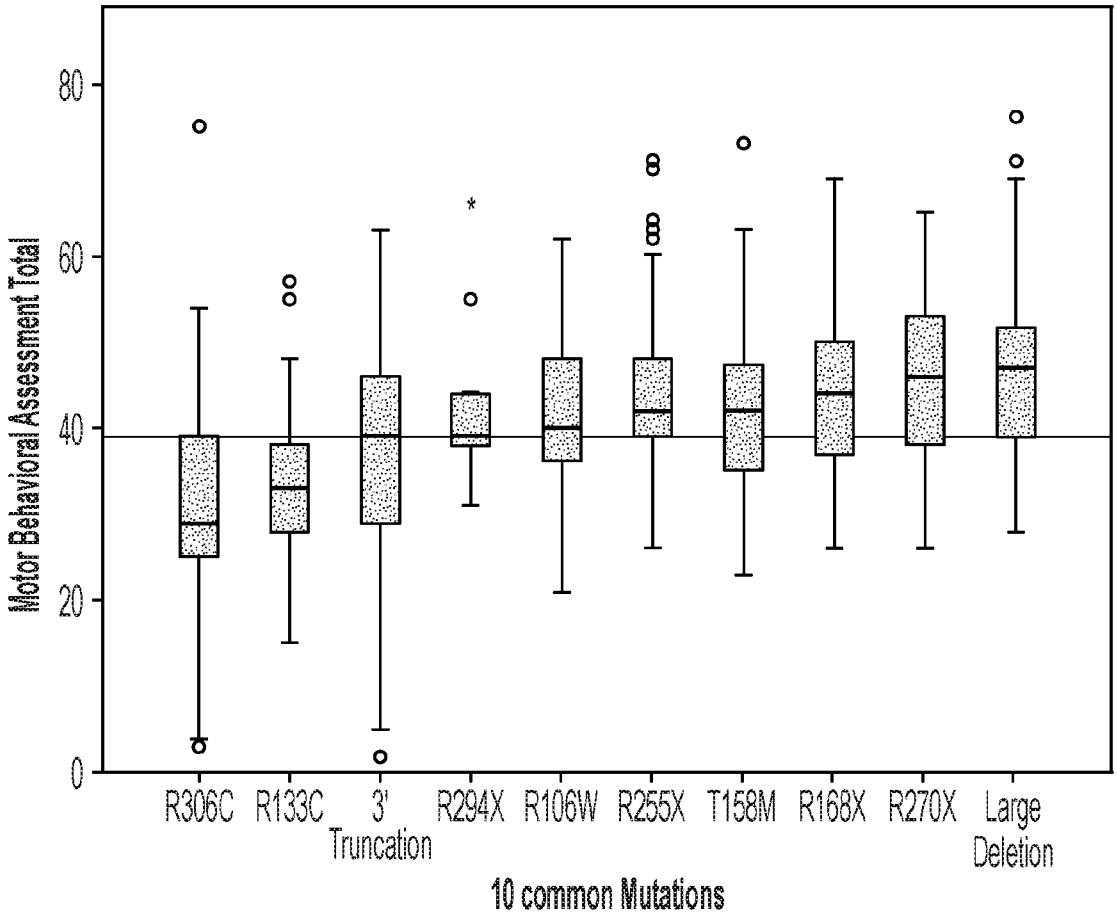

Motor Behavioral Assessment Total
80
60
40
20
0
R306C
R133C
3' Truncation
R294X
R106W
R255X
T158M
R168X
R270X
Large Deletion
10 common Mutations

FIG. 2

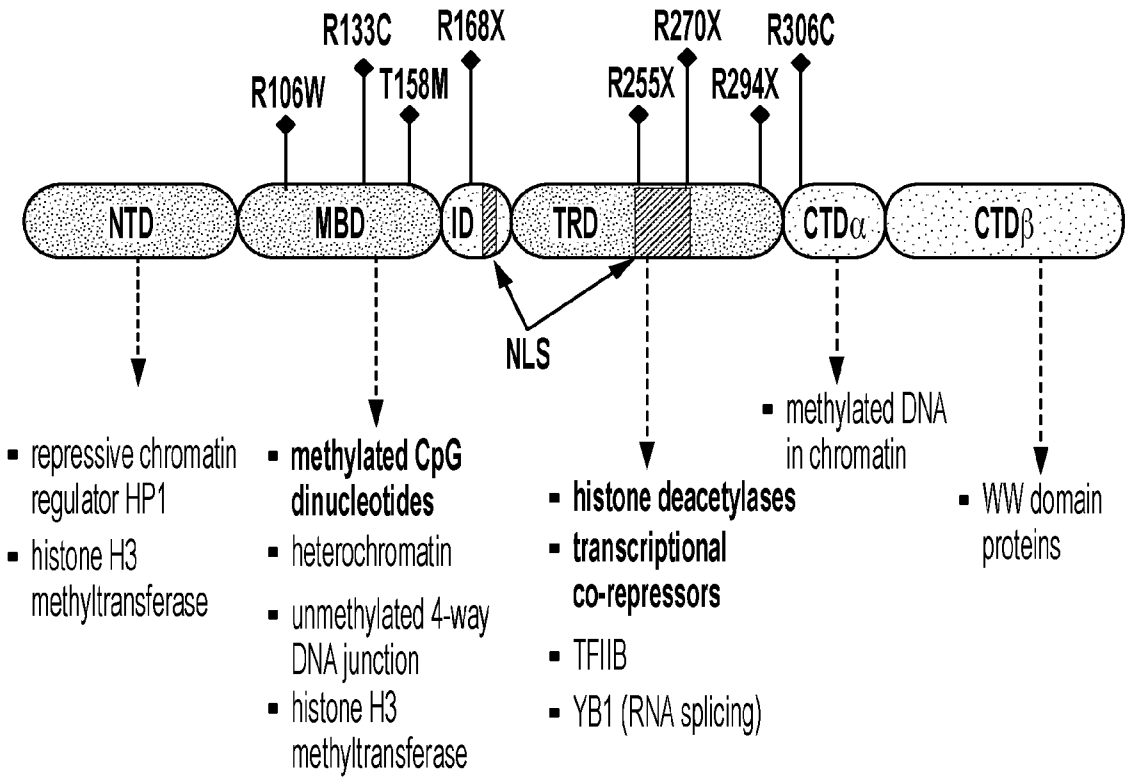
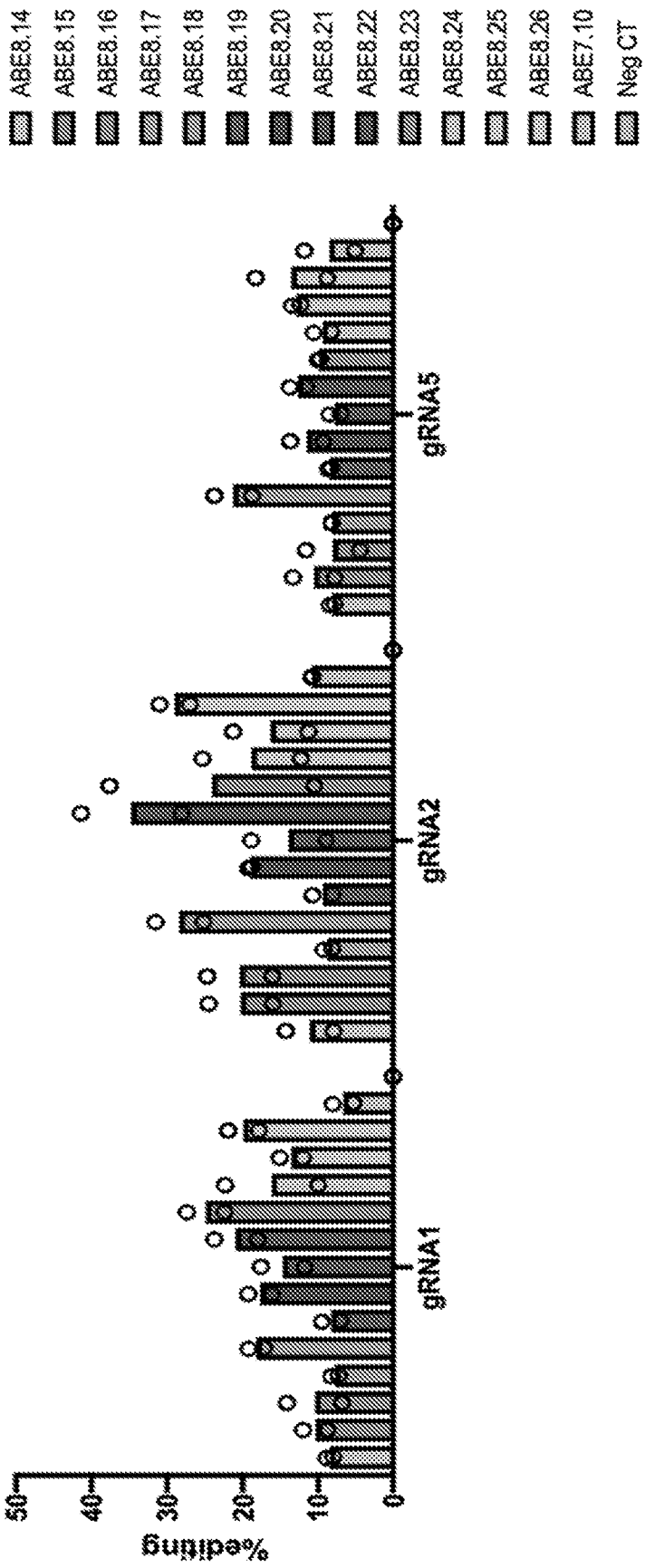

R133C
R168X
R270X
R306C
R106W
T158M
R255X
R294X
NTD
MBD
ID
TRD
CTDα
CTDβ
NLS
repressive chromatin regulator HP1
histone H3 methyltransferase
methylated CpG dinucleotides
heterochromatin
unmethylated 4-way DNA junction
histone H3 methyltransferase
histone deacetylases
transcriptional co-repressors
TFIIB
YB1 (RNA splicing)
methylated DNA in chromatin
WW domain proteins

FIG. 3


ABE8.14
ABE8.15
ABE8.16
ABE8.17
ABE8.18
ABE8.19
ABE8.20
ABE8.21
ABE8.22
ABE8.23
ABE8.24
ABE8.25
ABE8.26
ABE7.10
Neg CT
%editing
0
10
20
30
40
50
gRNA1
gRNA2
gRNA5