

CLAIMS

1. A fusion protein comprising, from N-terminus to C-terminus, a DNA methyltransferase domain, a first XTEN linker, a nuclease-deficient RNA-guided endonuclease enzyme, a second XTEN linker, and a Krüppel-associated box domain.
2. The fusion protein of claim 1, wherein the first XTEN linker comprises from about 5 to about 864 amino acid residues, and the second XTEN linker comprises from about 5 to about 864 amino acid residues, optionally wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is a CRISPR-associated protein, a leucine zipper domain, a winged helix domain, a helix-turn-helix motif, a helix-loop-helix domain, an HMB-box domain, a Wor3 domain, an OB-fold domain, an immunoglobulin domain, or a B3 domain, and optionally wherein the DNA methyltransferase domain comprises a Dnmt3A domain linked to a Dnmt3L domain (Dnmt3A-3L domain).
3. The fusion protein of claim 1, comprising, from N-terminus to C-terminus, the DNA methyltransferase domain, the first XTEN linker, the nuclease-deficient RNA-guided endonuclease enzyme, a nuclear localization signal peptide, and the Krüppel-associated box domain.
4. The fusion protein of claim 1, comprising, from N-terminus to C-terminus, the DNA methyltransferase domain, the first XTEN linker, the nuclease-deficient RNA-guided endonuclease enzyme, a nuclear localization signal peptide, the second XTEN linker, and the Krüppel-associated box domain.
5. The fusion protein of claim 1, comprising, from N-terminus to C-terminus, the DNA methyltransferase domain, the first XTEN linker, the nuclease-deficient RNA-guided endonuclease enzyme, an epitope tag, a nuclear localization signal peptide, the second XTEN linker, the Krüppel-associated box domain, a 2A cleavable peptide, and a fluorescent protein tag.
6. A fusion protein having at least 85% sequence identity to the amino acid sequence of Formula (A); where the amino acid sequence of Formula (A) is, from N-terminus to C-terminus:

$$C_1-R^3-C_2-R^2-A-R^1-R^4-B \text{ (A)},$$

wherein:

C₁ comprises SEQ ID NO:26 or SEQ ID NO:106;

R³ is absent or R³ comprises SEQ ID NO:27;

C₂ comprises SEQ ID NO:28;

R² is absent or R² comprises SEQ ID NO:32;

A comprises SEQ ID NO:23;

R¹ is absent or R¹ comprises SEQ ID NO:25;

R⁴ is absent or R⁴ comprises SEQ ID NO:31; and

B comprises SEQ ID NO:16, SEQ ID NO:103, SEQ ID NO:104, or SEQ ID NO:105.

7. A fusion protein having at least 85% sequence identity to SEQ ID NO:97, 98, 99, 107, 108, 109, or 110.

8. An isolated cell comprising the fusion protein of claim 1.

9. The fusion protein of claim 1 for use in a method of silencing a target nucleic acid sequence in a cell, the method comprising:

- (i) delivering a first polynucleotide encoding the fusion protein of claim 1 to a cell containing the target nucleic acid; and
- (ii) delivering to the cell a second polynucleotide comprising sgRNA or cr:tracrRNA; thereby silencing the target nucleic acid sequence.

10. A fusion protein for use in a method of silencing a target nucleic acid sequence in a cell, the method comprising:

- (i) delivering a first polynucleotide encoding a fusion protein to a cell containing the target nucleic acid, wherein the target nucleic acid does not comprise a CpG island; wherein the fusion protein comprises a nuclease-deficient RNA-guided DNA endonuclease enzyme, a Krüppel associated box domain, and a DNA methyltransferase domain; and
- (ii) delivering to the cell a second polynucleotide comprising sgRNA or cr:tracrRNA; thereby silencing the target nucleic acid sequence in the cell.

11. A fusion protein for use in a method of treating Angelman syndrome, an infectious disease, a tau pathology, or a neurodegenerative disease in a subject in need thereof, the method comprising:

- (i) delivering to the subject an effective amount of a first polynucleotide encoding a fusion protein comprising a nuclease-deficient RNA-guided DNA endonuclease enzyme, a Krüppel associated box domain, and a DNA methyltransferase domain; and
- (ii) delivering to the subject an effective amount of a second polynucleotide comprising sgRNA or cr:tracrRNA; thereby treating Angelman syndrome, the infectious disease, the tau pathology, or the neurodegenerative

disease.

12. The fusion protein of claim 10, wherein the fusion protein comprises, from N-terminus to C-terminus, the DNA methyltransferase domain, the nuclease-deficient RNA-guided DNA endonuclease enzyme, and the Krüppel associated box domain, optionally wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is a CRISPR-associated protein, a leucine zipper domain, a winged helix domain, a helix-turn-helix motif, a helix-loop-helix domain, an HMB-box domain, a Wor3 domain, an OB-fold domain, an immunoglobulin domain, or a B3 domain, and optionally wherein the DNA methyltransferase domain comprises a Dnmt3A domain linked to a Dnmt3L domain (Dnmt3A-3L domain).

13. The fusion protein of claim 10, wherein the fusion protein comprises, from N-terminus to C-terminus, the Krüppel associated box, the nuclease-deficient RNA-guided DNA endonuclease enzyme, and the DNA methyltransferase domain, optionally wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is a CRISPR-associated protein, a leucine zipper domain, a winged helix domain, a helix-turn-helix motif, a helix-loop-helix domain, an HMB-box domain, a Wor3 domain, an OB-fold domain, an immunoglobulin domain, or a B3 domain, and optionally wherein the DNA methyltransferase domain comprises a Dnmt3A domain linked to a Dnmt3L domain (Dnmt3A-3L domain).

14. The fusion protein of claim 10, wherein the fusion protein has at least 85% sequence identity to the amino acid sequence of Formula (A); where the amino acid sequence of Formula (A) is, from N-terminus to C-terminus:



wherein:

C_1 comprises SEQ ID NO:26 or SEQ ID NO:106;

R^3 is absent or R^3 comprises SEQ ID NO:27;

C_2 comprises SEQ ID NO:28;

R^2 is absent or R^2 comprises SEQ ID NO:32;

A comprises SEQ ID NO:23;

R^1 is absent or R^1 comprises SEQ ID NO:25;

R^4 is absent or R^4 comprises SEQ ID NO:31; and

B comprises SEQ ID NO:16, SEQ ID NO:103, SEQ ID NO:104, or SEQ ID NO:105.

15. A polynucleotide encoding the fusion protein of claim 1 for use in a method of treating Angelman syndrome, an infectious disease, a tau pathology, or a neurodegenerative disease in a subject in need thereof, the method comprising:

- (i) delivering to the subject an effective amount of a first polynucleotide encoding a fusion protein of claim 1; and
- (ii) delivering to the subject an effective amount of second polynucleotide comprising sgRNA or cr:tracrRNA; thereby treating Angelman syndrome, the infectious disease, the tau pathology, or the neurodegenerative disease in the subject.

16. A fusion protein comprising, from N-terminus to C-terminus, a DNA methyltransferase domain, a first XTEN linker, a nuclease-deficient endonuclease enzyme, a second XTEN linker, and a Krüppel-associated box domain, optionally wherein the first XTEN linker comprises from about 5 to about 864 amino acid residues, and the second XTEN linker comprises from about 5 to about 864 amino acid residues, optionally wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain or a transcription activator-like effector, and optionally wherein the DNA methyltransferase domain comprises a Dnmt3A domain linked to a Dnmt3L domain (Dnmt3A-3L domain).

17. The fusion protein of claim 16, comprising, from N-terminus to C-terminus, the DNA methyltransferase domain, the first XTEN linker, the nuclease-deficient endonuclease enzyme, an epitope tag, a nuclear localization signal peptide, the second XTEN linker, the Krüppel-associated box domain, a 2A cleavable peptide, and a fluorescent protein tag.

18. An isolated cell comprising the fusion protein of claim 16.

19. A polynucleotide encoding the fusion protein of claim 16 for use in a method of silencing a target nucleic acid sequence in a cell, the method comprising delivering said polynucleotide to a cell containing the target nucleic acid; thereby silencing the target nucleic acid sequence.

20. A polynucleotide encoding the fusion protein of claim 16 for use in a method of treating Angelman syndrome, an infectious disease, a tau pathology, or a neurodegenerative disease in a subject in need thereof, the method comprising delivering to the subject an effective amount of said polynucleotide; thereby treating Angelman syndrome, the infectious disease, the tau pathology, or the neurodegenerative disease.