

CLAIMS

~~What is claimed is:~~

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:

~~3. The fusion protein of claim 2, wherein the first XTEN linker comprises from greater than 50 to about 864 amino acid residues, and the second XTEN linker comprises from about 5 to 50 amino acid residues, and a Krüppel-associated box domain.~~

~~4. The fusion protein of claim 3, wherein the first XTEN linker comprises from about 60 to about 864 amino acid residues, and the second XTEN linker comprises from about 10 to about 40 amino acid residues.~~

~~5. The fusion protein of claim 4, wherein the first XTEN linker comprises from about 70 to about 864 amino acid residues, and the second XTEN linker comprises from about 10 to about 30 amino acid residues.~~

6. ~~The fusion protein of claim 1, 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:

7. ~~The fusion protein of claim 6, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9.~~

8. ~~The fusion protein of claim 6, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCpf1 or ddCpf1.~~

9. ~~The fusion protein of claim 1, and optionally~~ wherein the DNA methyltransferase domain comprises a Dnmt3A domain:

10. ~~The fusion protein of claim 9, wherein the Dnmt3A domain is linked to a Dnmt3L domain (Dnmt3A-3L domain).~~

11. ~~The fusion protein of claim 1, further comprising an epitope tag, a 2A peptide, a~~

~~fluorescent protein tag, a nuclear localization signal peptide, or a combination of two or more thereof.~~

~~143.~~ The fusion protein of claim 14, 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.

~~134.~~ The fusion protein of claim 14, 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.

~~145.~~ The fusion protein of claim 14, 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.

~~156.~~ 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:



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.

~~16.—The fusion protein of claim 15 having at least 90% sequence identity to the amino acid sequence of Formula (A).~~

~~17.—The fusion protein of claim 16 having at least 95% sequence identity to the amino acid sequence of Formula (A).~~

~~187.~~ A fusion protein having at least 85% sequence identity to SEQ ID NO:97, 98, 99, 107, 108, 109, or 110.

~~19. The fusion protein of claim 18 having at least 90% sequence identity to SEQ ID NO:97, 98, 99, 107, 108, 109, or 110.~~

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

~~21. The cell of claim 20, wherein the cell is a eukaryotic cell, a mammalian cell, or a stem cell.~~

229. ~~A~~ 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 ~~a~~ 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.

~~23. The method of claim 22, wherein the target nucleic acid comprises a CpG island.~~

~~24. The method of claim 22, wherein the target nucleic acid does not comprise a CpG island.~~

~~25. The method of claim 22, wherein the second polynucleotide comprises sgRNA.~~

2610. 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.

2711. 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.

~~28. The method of claim 26, wherein the second polynucleotide comprises sgRNA.~~

~~29~~12. The ~~method~~ fusion protein of claim ~~26~~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:-

~~30. The method of claim 26, 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:-

~~31. The method of claim 30, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9.~~

~~32. The method of claim 30, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCpf1 or ddCpf1.~~

~~33. The method of claim 26, and optionally~~ wherein the DNA methyltransferase domain comprises a Dnmt3A domain:-

~~34. The method of claim 33, wherein the Dnmt3A domain is linked to a Dnmt3L domain (Dnmt3A-3L domain).~~

~~35. The method of claim 26, wherein the dCas9 is covalently linked to the Dnmt3A domain via a peptide linker and wherein the Dnmt3A domain is covalently linked to the Krüppel associated box domain via a peptide linker.~~

~~36. The method of claim 35, wherein the peptide linker is a XTEN linker.~~

~~37~~13. The ~~method~~ fusion protein of claim ~~26~~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:-

~~38. The method of claim 37, 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:-

~~39. The method of claim 38, wherein the nuclease-deficient RNA-guided DNA~~

endonuclease enzyme is dCas9.

40.—~~The method of claim 38, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCpf1 or ddCpf1.~~

41.—~~The method of claim 37, and optionally wherein the DNA methyltransferase domain comprises a Dnmt3A domain.~~

42.—~~The method of claim 41, wherein the Dnmt3A domain is linked to a Dnmt3L domain (Dnmt3A-3L domain).~~

43.—~~The method of claim 39, wherein the dCas9 is covalently linked to the Dnmt3A domain via a peptide linker and wherein the Krüppel-associated box domain is covalently linked to the dCas9 via a peptide linker.~~

44.—~~The method of claim 43, wherein the peptide linker is a XTEN linker.~~

45.—~~The method of claim 37, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is covalently linked to the Krüppel-associated box domain via a peptide linker.~~

46.—~~The method of claim 37, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is covalently linked to the DNA methyltransferase domain via a peptide linker.~~

47.—~~The method of claim 37, wherein the Krüppel-associated box domain is covalently linked to the DNA methyltransferase domain via a peptide linker.~~

48.—~~The method of claim 26, wherein the fusion protein further comprises an epitope tag, a 2A peptide, a fluorescent protein tag, a nuclear localization signal peptide, or a combination of two or more thereof.~~

49.—~~The method of claim 26, wherein the fusion protein further comprises a nuclear localization signal peptide.~~

5014. ~~The method~~ fusion protein of claim 2610, wherein the fusion protein ~~having~~ 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:

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.

~~51. The method of claim 50, wherein the fusion protein has at least 90% sequence identity to the amino acid sequence of Formula (A).~~

~~52. The method of claim 51, wherein the fusion protein has at least 95% sequence identity to the amino acid sequence of Formula (A).~~

5315. 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.

~~54. The method of claim 26, wherein the infectious disease is a viral infections disease.~~

~~55. The method of claim 54, wherein the infectious disease is a Flavivirus infectious disease.~~

5616. 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:

~~57. The fusion protein of claim 56, 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.~~

~~58. The fusion protein of claim 57, wherein the first XTEN linker comprises from greater than 50 to about 864 amino acid residues, and the second XTEN linker comprises from about 5 to 50 amino acid residues.~~

~~59. The fusion protein of claim 58, wherein the first XTEN linker comprises from about 60 to about 864 amino acid residues, and the second XTEN linker comprises from about 10 to about 40 amino acid residues~~

~~60. The fusion protein of claim 59, wherein the first XTEN linker comprises from about 70 to about 864 amino acid residues, and the second XTEN linker comprises from about 10 to about 30 amino acid residues.~~

61. The fusion protein of claim 56, optionally wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain or a transcription activator-like effector.

~~62. The fusion protein of claim 62, wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain.~~

~~63. The fusion protein of claim 62, wherein the nuclease-deficient DNA endonuclease enzyme is transcription activator like effector.~~

~~64. The fusion protein of claim 56, and optionally wherein the DNA methyltransferase domain comprises a Dnmt3A domain.~~

~~65. The fusion protein of claim 64, wherein the Dnmt3A domain is linked to a Dnmt3L domain (Dnmt3A-3L domain).~~

~~66. The fusion protein of claim 56, further comprising an epitope tag, a 2A peptide, a fluorescent protein tag, a nuclear localization signal peptide, or a combination of two or more thereof.~~

~~67. The fusion protein of claim 56, further comprising a nuclear localization signal peptide.~~

68~~17~~. The fusion protein of claim 56~~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.

69~~18~~. An isolated cell comprising the fusion protein of claim 56~~16~~.

~~70. The cell of claim 69, wherein the cell is a eukaryotic cell, a mammalian cell, or a stem cell.~~

71~~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 a first said polynucleotide ~~encoding a fusion protein of claim 56~~ to a cell containing the target nucleic acid;

thereby silencing the target nucleic acid sequence.

~~72. The method of claim 71, wherein the target nucleic acid comprises a CpG island.~~

~~73. The method of claim 71, wherein the target nucleic acid does not comprise a CpG island.~~

~~74~~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 a first said polynucleotide encoding a fusion protein of claim 56; thereby treating Angelman syndrome, the infectious disease, the tau pathology, or the neurodegenerative disease.

~~75. The method of claim 74, wherein the infectious disease is a viral infectious disease.~~

~~76. The method of claim 75, wherein the viral infectious disease is a Flavivirus infectious disease.~~