CLAIMS

~~What is claimed is:~~

1. A fusion protein comprising, from N-terminus to C-terminus:

(i) a DNA methyltransferase domain, a nuclease-deficient RNA-guided DNA endonuclease enzyme, and a Krüppel associated box domain~~, and a DNA methyltransferase domain~~; or

(ii) a Krüppel associated box domain, a nuclease-deficient RNA-guided DNA endonuclease enzyme, and a DNA methyltransferase domain.

2. The fusion protein of claim 1, wherein ~~said~~ the nuclease-deficient RNA-guided DNA endonuclease enzyme is ~~dCas9, ddCpf1, a nuclease-deficient Cas9 variant, or~~ a nuclease-deficient Class II CRISPR endonuclease.

3. The fusion protein of claim 1, wherein ~~said~~ the DNA methyltransferase domain ~~is~~ comprises a Dnmt3A protein and a Dnmt3L protein (a Dnmt3A-3L domain).

4. The fusion protein of claim 1, 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.

5. The fusion protein of claim 4, wherein ~~said~~ the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9 and ~~said~~ the DNA methyltransferase domain ~~is~~ comprises a Dnmt3A protein and a Dnmt3L protein (a Dnmt3A-3L domain); wherein the dCas9 is covalently linked to the Dnmt3A-3L domain via an XTEN linker.

~~6. The fusion protein of claim 5, wherein said dCas9 is covalently linked to said Dnmt3A-3L domain via a peptide linker and wherein said Dnmt3A-3L domain is covalently linked to said Krüppel associated box domain via a peptide linker.~~

~~7. The fusion protein of claim 6, wherein said peptide linker is a XTEN linker.~~

6~~8~~. The fusion protein of claim 1, wherein the fusion protein comprises, from N-terminus to C-terminus, the Krüppel associated box, the nuclease-deficient RNA-guided DNA endonuclease enzyme, an XTEN linker, and the DNA methyltransferase domain.

7~~9~~. The fusion protein of claim ~~8~~1, wherein ~~said~~ the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9 and ~~said~~ the DNA methyltransferase domain ~~is~~

comprises a Dnmt3A protein and a Dnmt3L protein (a Dnmt3A-3L domain).

8~~10~~. The fusion protein of claim ~~9~~7, wherein ~~said~~ the dCas9 is covalently linked to ~~said~~ the Dnmt3A-3L domain via a ~~peptide~~ XTEN linker and wherein ~~said~~ the Krüppel associated box domain is covalently linked to ~~said~~ the dCas9 via a peptide linker.

~~11. The fusion protein of claim 10, wherein said peptide linker is a XTEN linker.~~

9~~12~~. The fusion protein of claim 1, wherein ~~said~~ the nuclease-deficient RNA-guided DNA endonuclease enzyme is covalently linked to ~~said~~ the Krüppel associated box domain via a peptide linker.

10~~13~~. The fusion protein of claim 1, wherein ~~said~~ the nuclease-deficient RNA-guided DNA endonuclease enzyme is covalently linked to ~~said~~ the DNA methyltransferase domain via a peptide linker.

11~~14~~. The fusion protein of claim 1, wherein ~~said~~ the Krüppel associated box domain in (ii) is covalently linked to ~~said~~ the DNA methyltransferase domain via ~~a peptide linker~~ an XTEN linker.

~~15. The fusion protein of claim 12, wherein said peptide linker is a XTEN linker.~~

12~~16~~. The fusion protein of claim ~~15~~ 5, wherein ~~said~~ the XTEN linker comprises about 16 to 80 amino acid residues.

13~~17~~. The fusion protein of claim 1, further comprising a nuclear localization signal peptide.

14~~18~~. The fusion protein of claim 1, wherein ~~said~~ the fusion protein comprises the amino acid sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 12, 13, 14, or 15.

15~~19~~. A nucleic acid sequence encoding ~~said~~ the fusion protein of claim 1.

~~20. The nucleic acid sequence of claim 19, wherein the nucleic acid sequence is messenger RNA.~~

16~~21~~. A complex comprising: (i) a fusion protein of claim 1; and (ii) a polynucleotide comprising: (a) a DNA-targeting sequence that is complementary to a target polynucleotide sequence; and (b) a binding sequence for ~~said~~ the nuclease-deficient RNA-guided DNA endonuclease enzyme, wherein ~~said~~ the nuclease-deficient RNA-guided DNA

endonuclease enzyme is bound to ~~said~~ the polynucleotide via ~~said~~ the binding sequence.

~~22. The complex of claim 21, wherein said target polynucleotide sequence is part of a gene.~~

~~23. The complex of claim 21, wherein said target polynucleotide sequence is part of a transcriptional regulatory sequence.~~

~~24. The complex of claim 21, wherein said target polynucleotide sequence is part of a promoter, enhancer, or silencer.~~

~~25. The complex of claim 21, wherein said target polynucleotide sequence is within about 3000 bp flanking a transcription start site.~~

17~~26~~. A vector comprising ~~said~~ a nucleic acid sequence encoding the fusion protein of claim ~~19~~ 1.

~~27. The vector of claim 26, further comprising a polynucleotide, wherein said polynucleotide comprises: (a) a DNA-targeting sequence that is complementary to a target polynucleotide sequence; and (b) a binding sequence for said nuclease-deficient RNA-guided DNA endonuclease enzyme.~~

18~~28~~. A cell comprising the fusion protein of claim 1~~; the nucleic acid of claim 19; the complex of claim 21, or the vector of claim 26~~.

~~29. The cell of claim 28, wherein said cell is a eukaryotic cell.~~

~~30. The cell of claim 28, wherein said cell is a mammalian cell.~~

19~~31~~. A method of silencing a target nucleic acid sequence in a harvested cell, comprising: (i) delivering a first polynucleotide encoding a fusion protein of claim 1 to a harvested cell containing ~~said~~ the target nucleic acid; and (ii) delivering to ~~said~~ the cell a second polynucleotide comprising: (a) a DNA-targeting sequence that is complementary to ~~said~~ the target nucleic acid sequence; and (b) a binding sequence for ~~said~~ the nuclease-deficient RNA-guide DNA endonuclease enzyme.

~~32. The method of claim 31, wherein said fusion protein silences said target nucleic acid sequence in said cell by methylating a chromatin containing said target nucleic acid sequence and/or by introducing repressive chromatin marks to a chromatin containing said target nucleic acid sequence.~~

~~33. The method of claim 31, wherein said first polynucleotide is contained~~

~~within a first vector.~~

~~34. The method of claim 31, wherein said first polynucleotide is contained within a second vector.~~

~~35. The method of claim 34, wherein said first vector and the second vector are the same.~~

~~36. The method of claim 34, wherein said first vector is different from said second vector.~~

~~37. The method of claim 31, wherein said cell is a mammalian cell.~~

~~38. The method of claim 31, wherein said method has a specificity that is 2-fold higher than a specificity to a non-target nucleic acid sequence.~~

20~~39~~. A method of silencing a target nucleic acid sequence in a harvested cell, the method comprising delivering ~~the~~ a complex ~~of claim 21~~ to a harvested cell containing ~~said~~ the target nucleic acid; wherein the complex comprises: (i) a fusion protein of claim 1; and (ii) a polynucleotide comprising: (a) a DNA-targeting sequence that is complementary to a target polynucleotide sequence; and (b) a binding sequence for the nuclease-deficient RNA-guided DNA endonuclease enzyme, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is bound to the polynucleotide via the binding sequence.

21. The fusion protein of claim 1, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9 which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:23; ddCas12a which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:34; dLbCfp1 which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:35; or dFnCfp1 which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:36.

22. The fusion protein of claim 1, wherein the Krüppel associated box domain comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:16.

23. The fusion protein of claim 3, wherein the Dnmt3A protein comprises an amino acid sequence with at least 85% sequence identity to SEQ ID NO:26, and the Dnmt3L protein comprises an amino acid sequence with at least 85% sequence identity to SEQ ID NO:28.

24. The fusion protein of claim 1, wherein the fusion protein comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:15.

25. The fusion protein of claim 1, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is selected from the group consisting of dCas9, ddCpf1, ddCas12a, ddLbCpf1 and ddFnCpf1.

26. A fusion protein comprising, a DNA methyltransferase domain, a nuclease-deficient RNA-guided DNA endonuclease enzyme, and a Krüppel associated box domain; wherein

(1) the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9 which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:23; ddCas12a which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:34; dLbCfp1 which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:35; or dFnCfp1 which comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:36;

(2) the Krüppel associated box domain comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO:16; and

(3) the DNA methyltransferase domain comprises a Dnmt3A protein, a Dnmt3L protein, or a Dnmt3A protein and a Dnmt3L protein; wherein the Dnmt3A protein comprises an amino acid sequence with at least 85% sequence identity to SEQ ID NO:26, and the Dnmt3L protein comprises an amino acid sequence with at least 85% sequence identity to SEQ ID NO:28.

27. The fusion protein of claim 26, wherein the Krüppel associated box domain comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:16.

28. The fusion protein of claim 27, wherein the Krüppel associated box domain comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:16.

29. The fusion protein of claim 28, wherein the Krüppel associated box domain comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO:16.

30. The fusion protein of claim 26, wherein the Dnmt3A protein comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO:26.

31. The fusion protein of claim 30, wherein the Dnmt3A protein comprises an amino acid sequence with at least 95% sequence identity to SEQ ID NO:26.

32. The fusion protein of claim 31, wherein the Dnmt3A protein comprises an amino acid sequence with at least 99% sequence identity to SEQ ID NO:26.

33. The fusion protein of claim 26, wherein the Dnmt3L protein comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO:28.

34. The fusion protein of claim 33, wherein the Dnmt3L protein comprises an amino acid sequence with at least 95% sequence identity to SEQ ID NO:28.

35. The fusion protein of claim 34, wherein the Dnmt3L protein comprises an amino acid sequence with at least 99% sequence identity to SEQ ID NO:28.

36. The fusion protein of claim 26, wherein the dCas9 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:23.

37. The fusion protein of claim 36, wherein the dCas9 comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:23.

38. The fusion protein of claim 37, wherein the dCas9 comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO:23.

39. The fusion protein according to claim 25, wherein the Krüppel associated box domain comprises an amino acid sequence of SEQ ID NO: 16, the Dnmt3A protein comprises an amino acid sequence of SEQ ID NO: 26, the Dnmt3L protein comprises an amino acid sequence of SEQ ID NO: 28 and the dCas9 comprises an amino acid sequence of SEQ ID NO: 23.

40. The fusion protein according to claim 39, wherein the fusion protein comprises, from N-terminus to C-terminus, a DNA methyltransferase domain, a nuclease-deficient RNA-guided DNA endonuclease enzyme, and a Krüppel associated box domain.

~~40. The method of claim 39, wherein said complex silences said target nucleic acid sequence in said cell by methylating a chromatin containing said target nucleic acid sequence and/or by introducing repressive chromatin marks to a chromatin containing said target nucleic acid sequence.~~

~~41. The method of claim 39, wherein said cell is a mammalian cell.~~

~~42. The method of claim 39, wherein said method has a specificity that is 2-fold higher than a specificity to a non-target nucleic acid sequence.~~