

CLAIMS

What is claimed is:

- 1 1. A fusion protein comprising a nuclease-deficient RNA-guided DNA
2 endonuclease enzyme, a Krüppel associated box domain, and a DNA methyltransferase domain.
- 1 2. The fusion protein of claim 1, wherein said nuclease-deficient RNA-
2 guided DNA endonuclease enzyme is dCas9, ddCpf1, a nuclease-deficient Cas9 variant, or a
3 nuclease-deficient Class II CRISPR endonuclease.
- 1 3. The fusion protein of claim 1, wherein said DNA methyltransferase
2 domain is a Dnmt3A-3L domain.
- 1 4. The fusion protein of claim 1, wherein the fusion protein comprises, from
2 N-terminus to C-terminus, the DNA methyltransferase domain, the nuclease-deficient RNA-
3 guided DNA endonuclease enzyme, and the Krüppel associated box domain.
- 1 5. The fusion protein of claim 4, wherein said nuclease-deficient RNA-
2 guided DNA endonuclease enzyme is dCas9 and said DNA methyltransferase domain is a
3 Dnmt3A-3L domain.
- 1 6. The fusion protein of claim 5, wherein said dCas9 is covalently linked to
2 said Dnmt3A-3L domain via a peptide linker and wherein said Dnmt3A-3L domain is covalently
3 linked to said Krüppel associated box domain via a peptide linker.
- 1 7. The fusion protein of claim 6, wherein said peptide linker is a XTEN
2 linker.
- 1 8. The fusion protein of claim 1, wherein the fusion protein comprises, from
2 N-terminus to C-terminus, the Krüppel associated box, the nuclease-deficient RNA-guided DNA
3 endonuclease enzyme, and the DNA methyltransferase domain.
- 1 9. The fusion protein of claim 8, wherein said nuclease-deficient RNA-
2 guided DNA endonuclease enzyme is dCas9 and said DNA methyltransferase domain is a
3 Dnmt3A-3L domain.
- 1 10. The fusion protein of claim 9, wherein said dCas9 is covalently linked to
2 said Dnmt3A-3L domain via a peptide linker and wherein said Krüppel associated box domain
3 is covalently linked to said dCas9 via a peptide linker.
- 1 11. The fusion protein of claim 10, wherein said peptide linker is a XTEN

2 linker.

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

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

1 14. The fusion protein of claim 1, wherein said Krüppel associated box
2 domain is covalently linked to said DNA methyltransferase domain via a peptide linker.

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

1 16. The fusion protein of claim 15, wherein said XTEN linker comprises
2 about 16 to 80 amino acid residues.

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

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

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

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

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

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

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

1 24. The complex of claim 21, wherein said target polynucleotide sequence is

2 part of a promoter, enhancer, or silencer.

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

1 26. A vector comprising said nucleic acid sequence of claim 19.

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

1 28. A cell comprising the fusion protein of claim 1; the nucleic acid of claim
2 19; the complex of claim 21, or the vector of claim 26.

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

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

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

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

1 33. The method of claim 31, wherein said first polynucleotide is contained
2 within a first vector.

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

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

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

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

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

1 39. A method of silencing a target nucleic acid sequence in a cell, the method
2 comprising delivering the complex of claim 21 to a cell containing said target nucleic acid.

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

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

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