

CLAIMS

~~What is claimed is:~~

1. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, an XTEN linker, and a nuclease-deficient RNA-guided DNA endonuclease enzyme-

~~2. The fusion protein of claim 1~~, wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof.

~~3. The fusion protein of claim 2, wherein the demethylation domain is a TET1 domain.~~

~~4.~~ The fusion protein of claim ~~2~~1, wherein the TET1 domain comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:86, or SEQ ID NO:97.

~~5. The fusion protein of claim 1, optionally~~ wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9, dCas12a, dCpf1, Cas-phi, 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.

~~6. The fusion protein of claim 5, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9.~~

~~7.~~ The fusion protein of claim 1, wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues-

~~8. The fusion protein of claim 7, optionally~~ wherein the XTEN linker comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:98.

~~9. The fusion protein of claim 1, 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.~~

~~10.~~ A fusion protein comprising from N-terminus to C-terminus, an RNA-binding sequence, an XTEN linker, and at least one transcriptional activator-

~~11. The fusion protein of claim 10, optionally~~ wherein the transcriptional activator is

VP64, p65, Rta, or a combination of two or more thereof.

~~12. The fusion protein of claim 11, optionally wherein p65 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:13, SEQ ID NO:14, or SEQ ID NO:100.~~

~~13. The fusion protein of claim 11, optionally wherein Rta comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:15 or SEQ ID NO:16.~~

~~14. The fusion protein of claim 11, and optionally wherein VP64 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:17 or SEQ ID NO:18.~~

~~15. The fusion protein of claim 10, wherein the RNA-binding sequence is an MS2 RNA-binding sequence.~~

~~16. The fusion protein of claim 15, wherein the MS2 RNA-binding sequence comprises comprising the amino acid sequence of SEQ ID NO:21.~~

~~17. The fusion protein of claim 10, optionally wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues.~~

~~18. The, optionally wherein the fusion protein of claim 10 having has an amino acid sequence with at least 90% sequence identity to SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, or SEQ ID NO:110.~~

~~19. The fusion protein of claim 10, 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.~~

~~20. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, a first XTEN linker, a nuclease-deficient RNA-guided DNA endonuclease enzyme, a second XTEN linker, and a transcriptional activator.~~

~~21. The fusion protein of claim 20, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof.~~

~~22. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, an XTEN linker, and a nuclease-deficient RNA-guided DNA endonuclease enzyme.~~

~~23. The fusion protein of claim 20, further comprising a nuclear localization~~

sequence

24. ~~The fusion protein of claim 20, optionally~~ wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof.

25. ~~The fusion protein of claim 24, wherein the demethylation domain is a TET1 domain.~~

26. ~~The fusion protein of claim 20, optionally~~ wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9, dCas12a, dCpf1, Cas-phi, 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.

27. ~~The fusion protein of claim 26, wherein the nuclease-deficient RNA-guided DNA endonuclease enzyme is dCas9.~~

28. ~~The fusion protein of claim 20, and optionally~~ wherein the first XTEN linker and the second XTEN linker each independently comprise from about 10 amino acid residues to about 864 amino acid residues.

7. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, an XTEN linker, and a nuclease-deficient RNA-guided DNA endonuclease enzyme, optionally wherein the fusion protein further comprises a nuclear localization sequence.

29. ~~The fusion protein of claim 20, wherein the fusion protein further comprising an epitope tag, a 2A peptide, a fluorescent protein tag, or a combination of two or more thereof.~~

30. A fusion protein comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:111, SEQ ID NO:112, or SEQ ID NO:113.

31. ~~The fusion protein of claim 30, comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:111, SEQ ID NO:112, or SEQ ID NO:113.~~

32. ~~The, optionally wherein the fusion protein of claim 31 comprising comprises~~ SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:111, SEQ ID NO:112, or SEQ ID NO:113.

339. A polynucleotide encoding the fusion protein of claim 1 for use in a method of activating or reactivating 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: (a) a sgRNA or (b) a cr:tracrRNA;

thereby activating or reactivating the target nucleic acid sequence in the cell.

~~3410.~~ The ~~method~~polynucleotide of claim ~~329~~, wherein the target nucleic acid sequence comprises a CpG island.

~~35.~~—The ~~method of claim 32, or~~ wherein the target nucleic acid sequence comprises a non-CpG island.

~~36.~~—The ~~method of claim 32, optionally~~ wherein the second polynucleotide comprises ~~the~~a sgRNA.

~~37.~~—The ~~method of claim 32, wherein the sgRNA comprises~~ comprising at least one MS2 stem loop.

~~38.~~—The ~~method of claim 37, wherein the sgRNA comprises two MS2 stem loops.~~

~~39.~~—The ~~method of claim 32, optionally~~ wherein the second polynucleotide encodes a transcriptional activator.

~~40.~~—The ~~method of claim 39, optionally~~ wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof.

~~41.~~—The ~~method of claim 32, optionally~~ wherein the second polynucleotide further encodes an MS2 RNA-binding sequence.

~~42.~~—The ~~method of claim 41, and optionally~~ wherein the MS2 RNA-binding sequence comprises the amino acid sequence of SEQ ID NO:21.

~~43.~~—The ~~method of claim 32, wherein the second polynucleotide further encodes for an XTEN linker, an epitope tag, a 2A peptide, a fluorescent protein tag, a nuclear localization signal peptide, or a combination of two or more thereof.~~

~~4411.~~ The ~~method~~polynucleotide of claim ~~329~~, wherein the method further comprising

comprises delivering to the cell a third polynucleotide encoding a second fusion protein which comprises a transcriptional activator-

~~45. The method of claim 44, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof-~~

~~46. The method of claim 44, optionally wherein the second fusion protein further comprises an MS2 RNA-binding sequence-~~

~~47. The method of claim 46, and optionally wherein the MS2 RNA-binding sequence comprises the amino acid sequence of SEQ ID NO:21.~~

~~48. The method of claim 44, wherein the second fusion protein further comprises an XTEN linker, an epitope tag, a 2A peptide, a fluorescent protein tag, a nuclear localization signal peptide, or a combination of two or more thereof-~~

4912. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, an XTEN linker, and a nuclease-deficient DNA endonuclease enzyme-

~~50. The fusion protein of claim 49, optionally wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof.~~

5113. The fusion protein of claim 4912, wherein the demethylation domain is a TET1 domain-

~~52. The fusion protein of claim 51, optionally wherein the TET1 domain comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO:86, or SEQ ID NO:97-~~

~~53. The fusion protein of claim 49, optionally wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain-~~

~~54. The fusion protein of claim 49, wherein the nuclease deficient DNA endonuclease enzyme is or a TALE-~~

~~55. The fusion protein of claim 49, optionally wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues-~~

~~56. The fusion protein of claim 55, and optionally wherein the XTEN linker comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:5, SEQ~~

~~57. The fusion protein of claim 49, 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.~~

~~58~~14. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, a first XTEN linker, a nuclease-deficient DNA endonuclease enzyme, a second XTEN linker, and a transcriptional activator:-

~~59. The fusion protein of claim 58, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof.~~

~~60. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, an XTEN linker, and a nuclease-deficient DNA endonuclease enzyme.~~

~~61. The, optionally wherein the fusion protein of claim 58, further comprising comprises a nuclear localization sequence.~~

~~62. The fusion protein of claim 58, optionally wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof.~~

~~63. The fusion protein of claim 62, wherein the demethylation domain is a TET1 domain.~~

~~64. The fusion protein of claim 58, optionally wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain.~~

~~65. The fusion protein of claim 58, wherein the nuclease-deficient DNA endonuclease enzyme is or a TALE.~~

~~66. The fusion protein of claim 58, optionally wherein the ~~first~~ XTEN linker ~~and the second XTEN linker~~ each independently comprises from about 10 amino acid residues to about 864 amino acid residues.~~

~~67. The fusion protein of claim 58, and optionally wherein the fusion protein further ~~comprising~~ comprises an epitope tag, a 2A peptide, a fluorescent protein tag, or a combination of two or more thereof.~~

~~68~~15. A polynucleotide encoding the fusion protein of claim 14 for use in a method of

activating or reactivating a target nucleic acid sequence in a cell, the method comprising delivering a said polynucleotide ~~encoding a fusion protein of claim 58~~ to a cell containing the target nucleic acid; thereby activating or reactivating the target nucleic acid sequence in the cell.

~~69. The method of claim 68, optionally~~ wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof.