

CLAIMS

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, wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof.
2. The fusion protein of claim 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, 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.
3. The fusion protein of claim 1, wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues, 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.
4. A fusion protein comprising from N-terminus to C-terminus, an RNA-binding sequence, an XTEN linker, and at least one transcriptional activator, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof, 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, optionally wherein Rta comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:15 or SEQ ID NO:16, 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.
5. The fusion protein of claim 4, wherein the RNA-binding sequence is an MS2 RNA-binding sequence comprising the amino acid sequence of SEQ ID NO:21, optionally wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues, optionally wherein the fusion protein 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.
6. 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, optionally wherein the transcriptional

activator is VP64, p65, Rta, or a combination of two or more thereof, optionally wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof, 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, 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.

8. 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, optionally wherein the fusion protein 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.

9. 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 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.

10. The polynucleotide of claim 9, wherein the target nucleic acid sequence comprises a CpG island, or wherein the target nucleic acid sequence comprises a non-CpG island, optionally wherein the second polynucleotide comprises a sgRNA comprising at least one MS2 stem loop, optionally wherein the second polynucleotide encodes a transcriptional activator, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof, optionally wherein the second polynucleotide further encodes an MS2 RNA-binding sequence, and optionally wherein the MS2 RNA-binding sequence comprises the amino acid sequence of SEQ ID NO:21.

11. The polynucleotide of claim 9, wherein the method further comprises delivering

to the cell a third polynucleotide encoding a second fusion protein which comprises a transcriptional activator, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof, optionally wherein the second fusion protein further comprises an MS2 RNA-binding sequence, and optionally wherein the MS2 RNA-binding sequence comprises the amino acid sequence of SEQ ID NO:21.

12. A fusion protein comprising from N-terminus to C-terminus, a demethylation domain, an XTEN linker, and a nuclease-deficient DNA endonuclease enzyme, optionally wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof.

13. The fusion protein of claim 12, wherein the demethylation domain is a TET1 domain, 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, optionally wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain, or a TALE, optionally wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues, and 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.

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, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof, optionally wherein the fusion protein further comprises a nuclear localization sequence, optionally wherein the demethylation domain is a TET1 domain, a TET2 domain, a TET3 domain, or a combination of two or more thereof, optionally wherein the nuclease-deficient DNA endonuclease enzyme is a zinc finger domain, or a TALE, optionally wherein the XTEN linker comprises from about 10 amino acid residues to about 864 amino acid residues, and optionally wherein the fusion protein further comprises an epitope tag, a 2A peptide, a fluorescent protein tag, or a combination of two or more thereof.

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 said polynucleotide to a cell containing the target nucleic acid; thereby activating or reactivating the target nucleic acid sequence in the cell, optionally wherein the transcriptional activator is VP64, p65, Rta, or a combination of two or more thereof.