~~WHAT IS CLAIMED IS:~~CLAIMS

1. A system for modifying a chromosomal sequence in a eukaryotic cell, the system comprising: (a) an engineered *Bacillus smithii* Cas9 protein; and (b) an engineered guide RNA, wherein the engineered guide RNA is designed to complex with the engineered Cas9 protein and the engineered guide RNA comprises a 5' guide sequence designed to hybridize with a target sequence in a double-stranded sequence, wherein the target sequence is 5' to a protospacer adjacent motif (PAM) and the PAM ~~has a~~ comprises the sequence ~~as listed in Table A~~ 5'-NNNNCAAA-3', wherein N is A, C, G, or T.

2. The system of claim 1, wherein the engineered Cas9 protein comprises at least one modification relative to its wild-type counterpart.

3. The system of claim 2, wherein the at least one modification comprises:

   (a) the addition of at least one heterologous domain; or

   (b) a substitution of one or more amino acids, an insertion of one or more amino acids, a deletion of one or more amino acids, or combination thereof, wherein the at least one modification is within a RuvC domain, HNH domain, REC domain, PAM interacting domain, or combination thereof.

4. The system of claim~~s 2 or~~ 3, wherein the at least one heterologous domain is a nuclear localization signal, a cell-penetrating domain, a marker domain, a chromatin modulating motif, an epigenetic modification domain, a transcriptional regulation domain, an RNA aptamer binding domain, or combination thereof.

~~5. The system of claim 2, wherein the at least one modification comprises a substitution of one or more amino acids, an insertion of one or more amino acids, a deletion of one or more amino acids, or combination thereof.~~

~~6. The system of claim 5, wherein the at least one modification is within a RuvC domain, HNH domain, REC domain, PAM interacting domain, or combination thereof.~~

~~7.~~5. The system of any one of claims 1 to ~~6~~4, wherein the engineered Cas9 protein is a nuclease and cleaves both strands of a double-stranded sequence, is a nickase and cleaves one strand of a double-stranded sequence, or has no nuclease or nickase activity.

~~8.~~6. The system of any one of claims 1 to ~~7~~5, wherein the engineered guide RNA is a single molecule.

~~9.~~7. The system of any one of claims 1 to ~~8~~6, wherein the engineered guide RNA sequence is optimized to facilitate base-paring within the engineered guide RNA, minimize base-paring within the engineered guide RNA, increase stability of the engineered guide RNA, facilitate transcription of the engineered guide RNA in a eukaryotic cell, or a combination thereof.

~~10. The system of any one of claims 1 to 9, wherein the engineered Cas9 protein is from *Bacillus smithii*, *Lactobacillus rhamnosus*, *Parasutterella excrementihominis*, *Mycoplasma canis*, *Mycoplasma gallisepticum*, *Akkermansia glycaniphila*, *Akkermansia muciniphila*, *Oenococcus kitaharae*, *Bifidobacterium bombi*, *Acidothermus cellulolyticus*, *Alicyclobacillus hesperidum*, *Wolinella succinogenes*, *Nitratifractor salsuginis*, *Ralstonia syzygii*, or *Corynebacterium diphtheria*.~~

~~11. The system of any one of claims 1 to 10, wherein the engineered Cas9 protein is from *Bacillus smithii* and the PAM sequence it recognizes is 5'-NNNNCAAA-3', the engineered Cas9 protein is from *Lactobacillus rhamnosus* and the PAM sequence it recognizes is 5'-NGAAA-3', the engineered Cas9 protein is from *Parasutterella excrementihominis* and the PAM sequence it recognizes is 5'-NGG-3', the engineered Cas9 protein is from *Mycoplasma canis* and the PAM sequence it recognizes is 5'-NNGG-3', the engineered Cas9 protein is from~~

~~*Mycoplasma gallisepticum* and the PAM sequence it recognizes is 5'-NNAAT-3', the engineered Cas9 protein is from *Akkermansia glycaniphila* and the PAM sequence it recognizes is 5'-NNNRTA-3', the engineered Cas9 protein is from *Akkermansia muciniphila* and the PAM sequence it recognizes is 5'-MMACCA-3', the engineered Cas9 protein is from *Oenococcus kitaharae* and the PAM sequence it recognizes is 5'-NNG-3', the engineered Cas9 protein is from *Bifidobacterium bombi* and the PAM sequence it recognizes is 5'-NNNNGRY-3', the engineered Cas9 protein is from *Acidothermus cellulolyticus* and the PAM sequence it recognizes is 5'-NGG-3', the engineered Cas9 protein is from *Alicyclobacillus hesperidum* and the PAM sequence it recognizes is 5'-NGG-3', the engineered Cas9 protein is from *Wolinella succinogenes* and the PAM sequence it recognizes is 5'-NGG-3', the engineered Cas9 protein is from *Nitratifractor salsuginis* and the PAM sequence it recognizes is 5'-NRGNK-3', the engineered Cas9 protein is from *Ralstonia syzygii* and the PAM sequence it recognizes is 5'-GGGRG-3' or the engineered Cas9 protein is from *Corynebacterium diphtheria* and the PAM sequence it recognizes is 5'-NNAMMMC-3', wherein K is G or T; M is A or C: N is A, C, G, or T; R is A or G; and Y is C or T.~~

8. The system of any one of claims 1 to 7, wherein the engineered guide RNA comprises the nucleotide sequence of SEQ ID NO:31.

~~12.~~9. The system of any one of claims 1 to ~~11~~8, wherein the engineered Cas9 protein has an amino acid sequence having at least about 90% sequence identity to SEQ ID NO:2, ~~4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 117, 118, 119, 120, 121, 122, 123, or 124~~ 119, or 120.

~~13.~~10. The system of any one of claims 1 to ~~12~~9, wherein the engineered Cas9 protein has an amino acid sequence as set forth in SEQ ID NO:2, ~~4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 117, 118, 119, 120, 121, 122, 123, or 124~~ 119, or 120.

~~14.~~11. A plurality of nucleic acids encoding the system of any one of claims 1 to ~~13~~10, the plurality of nucleic acid comprising at least one nucleic acid encoding the engineered Cas9 protein, and at least one nucleic acid encoding the engineered guide RNA.

~~15.~~12. The plurality of nucleic acids of claim ~~14~~11, wherein the at least one nucleic acid encoding the engineered Cas9 protein is RNA.

~~16.~~13. The plurality of nucleic acids of claim ~~14~~11, wherein the at least one nucleic acid encoding the engineered Cas9 protein is DNA.

~~17.~~14. The plurality of nucleic acids of any one of claims ~~14~~11 to ~~16~~13, wherein the at least one nucleic acid encoding the engineered Cas9 protein is codon optimized for expression in a eukaryotic cell.

~~18.~~15. The plurality of nucleic acids of claim ~~17~~14, wherein the eukaryotic cell is a human cell, a non-human mammalian cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism.

~~19.~~16. The plurality of nucleic acids of claim ~~14~~11, wherein the at least one nucleic acid encoding the engineered guide RNA is DNA.

~~20.~~17. The plurality of nucleic acids of any one of claims ~~14~~11 to ~~19~~16, wherein the at least one nucleic acid encoding the engineered Cas9 protein is operably linked to a phage promoter sequence for *in vitro* RNA synthesis or protein expression in a bacterial cell, and the at least one nucleic acid encoding the engineered guide RNA is operably linked to a phage promoter sequence for *in vitro* RNA synthesis.

~~21.~~18. The plurality of nucleic acids of any one of claims ~~14~~11 to ~~19~~16, wherein the at least one nucleic acid encoding the engineered Cas9 protein is operably linked to a eukaryotic promoter sequence for expression in a eukaryotic cell, and

the at least one nucleic acid encoding the engineered guide RNA is operably linked to a eukaryotic promoter sequence for expression in a eukaryotic cell.

~~22.~~At least one vector comprising the plurality of nucleic acids of any one of claims ~~14~~11 to ~~21~~18~~.~~

~~23.~~19. ~~The at least one vector of claim 22, which is~~, optionally wherein the vector is selected from: a plasmid vector, a viral vector, or a self-replicating viral RNA replicon.

~~24.~~A eukaryotic cell comprising at least one system comprising an engineered Cas9 protein and an engineered guide RNA as defined in claims 1 to ~~13~~10, at least one nucleic acid as defined in claims ~~14~~11 to ~~21~~18, or at least one vector as defined in claim~~s 22 or 23~~ 19~~.~~

~~25. The eukaryotic cell of claim 24, which is~~, optionally wherein the eukaryotic cell is selected from: a human cell, a non-human mammalian cell, a plant cell, a non-mammalian vertebrate cell, an invertebrate cell, or a single cell eukaryotic organism~~.~~

~~26.~~20. ~~The eukaryotic cell of claims 24 or 25, which is~~, optionally wherein the eukaryotic cell is *in vivo*, *ex vivo*, or *in vitro*.

~~27. A method for modifying a chromosomal sequence in a eukaryotic cell, the method comprising introducing into the eukaryotic cell at least one system comprising an engineered Cas9 protein and an engineered guide RNA as defined in claims 1 to 13, at least one nucleic acid as defined in claims 14 to 21, or at least one vector as defined in claims 22 or 23, and optionally, at least one donor polynucleotide, wherein the at least one engineered guide RNA guides the at least one engineered Cas9 protein to the target site in the chromosomal sequence such that modification of the chromosomal sequence occurs.~~

~~28. The method of claim 27, wherein the modification comprises a substitution of at least one nucleotide, a deletion of at least one nucleotide, an insertion of at least one nucleotide, a conversion of at least one nucleotide, a modification of at least one nucleotide, a modification of at least associated histone protein, or combination thereof.~~

~~29. The method of claims 27 or 28, wherein the engineered Cas9 protein has nuclease or nickase activity, the at least one donor polynucleotide is not introduced into the cell, and the modification comprises at least one indel.~~

~~30. The method of claim 29, wherein the modification comprises inactivation of the chromosomal sequence.~~

~~31. The method of claims 27 or 28, wherein the engineered Cas9 protein has nuclease or nickase activity, the at least one donor polynucleotide is introduced into the cell, and the modification comprises a change of at least one nucleotide in the chromosomal sequence.~~

~~32. The method of claim 31, wherein the at least one donor polynucleotides a donor sequence that has at least one nucleotide change relative to sequence near the target site in the chromosomal sequence.~~

~~33. The method of claim 31, wherein the at least one donor polynucleotide comprises a donor sequence that corresponds to an exogenous sequence.~~

~~34. The method of claims 32 or 33, wherein the donor sequence is flanked by sequences having substantial sequence identity to sequences located upstream and downstream of the target site in the chromosomal sequence.~~

~~35. The method of claims 32 or 33, wherein the donor sequence is flanked by short overhangs that are compatible with overhangs generated by the at least one engineered Cas9 protein.~~

36. The method of any one of claims 27 to 35, wherein the eukaryotic cell is a human cell, a non-human mammalian cell, a plant cell, a non-mammalian vertebrate cell, an invertebrate cell, or a single cell eukaryotic organism.

37. The method of any one of claims 27 to 36, wherein the eukaryotic cell is *in vivo*, *ex vivo*, or *in vitro*.

38. A fusion protein comprising a Cas9 protein linked to at least one chromatin modulating motif, wherein the Cas9 protein is a *Bacillus smithii*, *Lactobacillus rhamnosus*, *Parasutterella excrementihominis*, *Mycoplasma canis*, *Mycoplasma gallisepticum*, *Akkermansia glycaniphila*, *Akkermansia muciniphila*, *Oenococcus kitaharae*, *Bifidobacterium bombi*, *Acidothermus cellulolyticus*, *Alicyclobacillus hesperidum*, *Wolinella succinogenes*, *Nitratifractor salsuginis*, *Ralstonia syzygii*, or *Corynebacterium diphtheria* Cas9 protein.

39. The fusion protein of claim 38, wherein the at least one chromatin modulating motif is a high mobility group (HMG) box (HMGB) DNA binding domain, a HMG nucleosome-binding (HMGN) protein, a central globular domain from a histone H1 variant, a DNA binding domain from a chromatin remodeling complex protein, or a combination thereof.

40. The fusion protein of claims 38 or 39, wherein the at least one chromatin modulating motif is HMGB1 box A domain, HMGN1 protein, HMGN2 protein, HMGN3a protein, HMGN3b protein, histone H1 central globular domain, imitation switch (ISWI) protein DNA binding domain, chromodomain-helicase-DNA protein 1 (CHD1) DNA binding domain, or a combination thereof.

41. The fusion protein of any one of claims 38 to 40, wherein the at least one chromatin modulating motif is linked to the Cas9 protein directly via a chemical bond, indirectly via a linker, or a combination thereof.

42. The fusion protein of any one of claims 38 to 41, wherein the at least one chromatin modulating motif is linked to the Cas9 protein at its N-terminus, C-terminus, an internal location, or a combination thereof.

43. The fusion protein of any one of claims 38 to 42, further comprising at least one nuclear localization signal.

44. The fusion protein of any one of claims 38 to 43, further comprising at least one at least one cell-penetrating domain, at least one marker domain, or a combination thereof.

45. The fusion protein of any one of claims 38 to 44, wherein the fusion protein has an amino acid sequence having at least 90% sequence identity with SEQ ID NO:117, 118, 119, 120, 121, 122, 123, or 124.

46. The fusion protein of any one of claims 38 to 45, wherein the fusion protein has an amino acid sequence as set forth in SEQ ID NO:117, 118, 119, 120, 121, 122, 123, or 124.