

**We claim:**

1. A polynucleotide comprising an open reading frame (ORF), the ORF comprising:
  - a nucleotide sequence encoding an *N. meningitidis* (Nme) Cas9 polypeptide, wherein the nucleotide sequence is at least 90% identical to any one of SEQ ID NOs: 264, 267, 268, 250, 253, 252, 254, 259-261, 266, 273-275, 224-226, 231-233, 238-240, 245-247, 280-282, 287-289, 294-296, or 301-303, wherein the Nme Cas9 is an Nme2 Cas9, an Nme1 Cas9, or Nme3 Cas9; and
  - a nucleotide sequence encoding a first nuclear localization signal (NLS).
2. The polynucleotide of claim 1, wherein the ORF further comprises a nucleotide sequence encoding a second NLS, optionally wherein the first and second NLS are independently selected from SEQ ID NOs: 383, 422, 384-386, 388 and 410-421.
3. The polynucleotide of claim 1, wherein the polynucleotide further comprises a poly-A sequence or a polyadenylation signal sequence, optionally wherein the poly-A sequence comprises non-adenine nucleotides, optionally wherein the poly-A sequence comprises 100-400 nucleotides.
4. The polynucleotide of claim 3, wherein the poly-A sequence comprises a sequence of SEQ ID NO: 409.
5. The polynucleotide of claim 2, wherein the ORF further comprises (i) a nucleotide sequence encoding a linker sequence between the first NLS and the second NLS, and/or (ii) a nucleotide sequence encoding a linker sequence between the Nme Cas9 coding sequence and the NLS proximal to the Nme Cas9 coding sequence, optionally wherein the linker comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.
6. The polynucleotide of claim 5, wherein the linker sequence comprises an amino acid sequence GGG or GGGS.
7. The polynucleotide of claim 5, wherein the linker sequence comprises an amino acid sequence of any one of SEQ ID NOs: 61-122.
8. The polynucleotide of claim 1, wherein the ORF further comprises one or more additional heterologous functional domains.
9. The polynucleotide of claim 1, wherein the Nme Cas9 has double stranded endonuclease activity.
10. The polynucleotide of claim 1, wherein the Nme Cas9 has nickase activity.
11. The polynucleotide of claim 1, wherein the Nme Cas9 comprises a dCas9 DNA binding domain.

12. The polynucleotide of claim 1, wherein the NmeCas9 polypeptide comprises (i) an amino acid sequence with at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to any one of SEQ ID NOs: 262, 11, 248, 255, 269, 1, 4-10, 12-13, 220, 227, 234, 241, 276, 283, 290, 297, or 310-315; or (ii) an amino acid sequence of SEQ ID NOs: 262, 11, 248, 255, 269, 1, 4-10, 12-13, 220, 227, 234, 241, 276, 283, 290, 297, or 310-315.
13. The polynucleotide of claim 1, wherein the sequence encoding the NmeCas9 polypeptide: comprises (i) a nucleotide sequence having at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the nucleotide sequence of any one of SEQ ID NOs: 264, 267, 268, 39, 250, 249, 251-254, 256-261, 263, 265, 266, 270-275, 15, 18-27, 29, 32-38, 40-41, 221-226, 228-233, 235-240, 242-247, 277-282, 284-289, 291-296, 298-303, 304-309, or 316-321; or (ii) a nucleotide sequence of any one of SEQ ID NOs: 264, 267, 268, 39, 250, 249, 251-254, 256-261, 263, 265, 266, 270-275, 15, 18-24, 26, 27, 29, 32-38, 40-41, 221-226, 228-233, 235-240, 242-247, 277-282, 284-289, 291-296, 298-303, 304-309, or 316-321.
14. The polynucleotide of claim 1, wherein the NmeCas9 polypeptide comprises an amino acid sequence with at least 95% identity to any one of SEQ ID NOs: 262, 11, 248, 255, or 269; and/or wherein the polynucleotide encoding the NmeCas9 polypeptide comprises a nucleotide sequence having at least 95% identity to the nucleotide sequence of any one of SEQ ID NOs: 264, 267, 268, 39, 250, 249, 251-254, 256-261, 263, 265, 266, or 270-275.
15. A polynucleotide comprising an open reading frame (ORF) comprising a nucleotide sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 39 or 250, or the nucleotide sequence of SEQ ID NO: 39 or 250.
16. A polynucleotide comprising an open reading frame (ORF) encoding a polypeptide comprising:
  - 1) a cytidine deaminase, which is optionally an APOBEC3A deaminase;
  - 2) an *N. meningitidis* (Nme) Cas9 nickase comprising an amino acid sequence at least 90% identical to any one of SEQ ID NOs: 262, 269, 234, 241, 290, or 297, wherein the Nme Cas9 nickase is an Nme2 Cas9 nickase, an Nme1 Cas9 nickase, or an Nme3 Cas9 nickase; and
  - 3) a first nuclear localization signal (NLS);
 wherein the polypeptide does not comprise a uracil glycosylase inhibitor (UGI).
17. The polynucleotide of claim 16, wherein the ORF further comprises a nucleotide sequence encoding a second NLS.

18. The polynucleotide of claim 17, wherein the cytidine deaminase is located C-terminal to the first NLS and the second NLS in the polypeptide.
19. The polynucleotide of claim 16, wherein the ORF does not comprise a coding sequence for an NLS C-terminal to the sequence encoding the Nme Cas9, optionally wherein the ORF does not comprise a coding sequence C-terminal to the ORF encoding the NmeCas9.
20. The polynucleotide of claim 16, wherein the cytidine deaminase comprises
  - 1) an amino acid sequence with at least 87% identity to SEQ ID NO: 151; and/or
  - 2) an amino acid sequence with at least 80% identity to any one of SEQ ID NOs: 152-216.
21. The polynucleotide of claim 16, wherein the polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 14 or the ORF encoding the polypeptide comprises a nucleotide sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 42.
22. A polynucleotide comprising an open reading frame (ORF) comprising a nucleotide sequence having at least 99% identity to SEQ ID NO: 42.
23. A polynucleotide comprising an open reading frame (ORF) comprising, from 5' to 3'
  - 1) a nucleotide sequence encoding a first nuclear localization signal (NLS);
  - 2) a nucleotide sequence encoding a cytidine deaminase comprising an amino acid sequence at least 87% identical to SEQ ID NO: 151; and
  - 3) a nucleotide sequence (i) encoding an *N. meningitidis* (Nme) Cas9 nickase polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO: 262; and/or (ii) that is at least 95% identical to SEQ ID NO: 264.
24. The polynucleotide of any one of claims 1, 15, 16, 22, and 23, wherein the ORF comprises, from 5' to 3':
  - 1) a nucleotide sequence encoding a first nuclear localization signal (NLS) comprising an amino sequence at least 90% identical to SEQ ID NO: 383;
  - 2) a nucleotide sequence encoding a second NLS comprising an amino sequence at least 90% identical to SEQ ID NO: 422;
  - 3) a nucleotide sequence encoding a cytidine deaminase comprising an amino acid sequence at least 87% identical to SEQ ID NO: 151; and
  - 4) a nucleotide sequence encoding an Nme2 Cas9 nickase, wherein (i) the nucleotide sequence encodes an amino acid sequence at least 95% identical to

SEQ ID NO: 262; and/or (ii) the nucleotide sequence is at least 95% identical to SEQ ID NO: 264.

25. The polynucleotide of claim 24, further comprising one or both of (i) a nucleotide sequence encoding first peptide linker between the second NLS and the cytidine deaminase, optionally wherein the first peptide linker comprises GGG or GGGS; and (ii) a nucleotide sequence encoding a second peptide linker between the cytidine deaminase and the Nme2Cas9 nickase, optionally wherein the second peptide linker is selected from SEQ ID NOs: 118, 61-117, and 119-122.
26. The polynucleotide of claim 24, comprising, from 5' to 3'
- 1) a nucleotide sequence encoding a first NLS comprising the amino acid sequence of SEQ ID NO: 383;
  - 2) a nucleotide sequence encoding a second NLS comprising the amino acid sequence of SEQ ID NO: 422;
  - 3) a nucleotide sequence encoding a cytidine deaminase comprising an amino acid sequence that is at least 99% identical to SEQ ID NO: 151; and
  - 4) a nucleotide sequence (i) encoding an Nme2 Cas9 nickase that is at least 99% identical to SEQ ID NO: 262; and/or (ii) that is at least 99% identical to SEQ ID NO: 264.
27. The polynucleotide of claim 26, further comprising one or both of (i) a nucleotide sequence encoding a first peptide linker between the second NLS and the cytidine deaminase, the first peptide linker comprising GGG or GGGS; and/or (ii) a nucleotide sequence encoding a second peptide linker between the cytidine deaminase and the Nme2Cas9 nickase, the second peptide linker comprising the amino acid sequence of SEQ ID NO: 118.
28. A polypeptide comprising, from N-terminus to C-terminus:
- 1) a first nuclear localization signal (NLS);
  - 2) a cytidine deaminase comprising an amino acid sequence at least 87% identical to SEQ ID NO: 151; and
  - 3) an *N. meningitidis* (Nme) Cas9 nickase polypeptide comprising an amino acid sequence at least 90% identical to SEQ ID NO: 262.
29. The polypeptide of claim 28, comprising, from N-terminus to C-terminus:

- 1) a first nuclear localization signal (NLS) comprising an amino sequence at least 90% identical to SEQ ID NO: 383;
  - 2) a second NLS comprising an amino sequence at least 90% identical to SEQ ID NO: 422;
  - 3) a cytidine deaminase comprising an amino acid sequence at least 87% identical to SEQ ID NO: 151; and
  - 4) an Nme2 Cas9 nickase comprising an amino acid sequence at least 90% identical to SEQ ID NO: 262.
30. The polypeptide of claim 29, further comprising one or both of (i) a first peptide linker between the second NLS and the cytidine deaminase, optionally wherein the first peptide linker comprises GGG or GGGS; and (ii) a second peptide linker between the cytidine deaminase and the Nme2Cas9 nickase, optionally wherein the second peptide linker is selected from SEQ ID NOs: 118, 61-117, and 119-122.
31. The polypeptide of claim 29, comprising, from N-terminus to C-terminus:
- 1) a first NLS comprising the amino acid sequence of SEQ ID NO: 383;
  - 2) a second NLS comprising the amino acid sequence of SEQ ID NO: 422;
  - 3) a cytidine deaminase comprising an amino acid sequence that is at least 99% identical to SEQ ID NO: 151; and
  - 4) an Nme2 Cas9 nickase comprising an amino acid sequence that is at least 99% identical to SEQ ID NO: 262.
32. The polypeptide of claim 31, further comprising one or both of (i) a first peptide linker between the second NLS and the cytidine deaminase, the first peptide linker comprising GGG or GGGS; and/or (ii) a second peptide linker between the cytidine deaminase and the Nme2Cas9 nickase, the second peptide linker comprising the amino acid sequence of SEQ ID NO: 118.
33. A polynucleotide comprising an open reading frame (ORF) encoding the polypeptide of any one of claims 28-32.
34. The polynucleotide of any one of claims 1, 15, 16, 22, 23, and 33, wherein the polynucleotide comprises
- 1) a 5' cap, optionally wherein the 5' cap is Cap0, Cap1, or Cap2;
  - 2) a 5' UTR (i) with at least 90% identity to any one of SEQ ID NOs: 393, 391, 392, 398; or (ii) comprising any one of SEQ ID NOs: 391-398; or

3) a 3' UTR (i) with at least 90% identity to any one of SEQ ID NOs: 401, 399-406; or (ii) comprising any one of SEQ ID NOs: 401, 399, 400, 402-406, optionally wherein the polynucleotide comprises a 5' UTR and a 3' UTR from the same source.

35. The polynucleotide of any one of claims 1, 15, 16, 22, 23, and 33, wherein the polynucleotide is an mRNA.

36. The polynucleotide of claim 35, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the uridine in the mRNA is substituted with a modified uridine.

37. A composition comprising the polynucleotide according to any one of claims 1, 15, 16, 22, 23, and 33, and at least one guide RNA (gRNA).

38. A composition comprising the polynucleotide according to any one of claims 1, 15, 16, 22, 23, and 33, further comprising a single guide RNA, wherein the single guide RNA comprises a guide region and a conserved region, (A) wherein the conserved region comprises one or more of:

(a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 2-24 nucleotides, wherein

(i) one or more of nucleotides 37-48 and 53-64 is deleted and optionally one or more of nucleotides 37-64 is substituted relative to SEQ ID NO: 500; and

(ii) nucleotide 36 is linked to nucleotide 65 by at least 2 nucleotides; and/or

(b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2-10, optionally 2-8 nucleotides, wherein

(i) one or more of nucleotides 82-86 and 91-95 is deleted and optionally one or more of positions 82-96 is substituted relative to SEQ ID NO: 500; and

(ii) nucleotide 81 is linked to nucleotide 96 by at least 4 nucleotides; and/or

(c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 2-18, optionally 2-16 nucleotides, wherein

(i) one or more of nucleotides 113-121 and 126-134 is deleted and optionally one or more of nucleotides 113-134 is substituted relative to SEQ ID NO: 500; and

(ii) nucleotide 112 is linked to nucleotide 135 by at least 4 nucleotides;

wherein one or both nucleotides 144-145 are optionally deleted relative to SEQ ID NO: 500;

wherein at least 10 nucleotides are modified nucleotides; and/or

- (B) wherein the conserved region comprises one or more of:
- (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 2-24 nucleotides, wherein
    - (i) one or more of nucleotides 37-64 is deleted and optionally substituted relative to SEQ ID NO: 500; and
    - (ii) nucleotide 36 is linked to nucleotide 65 by (i) a first internal linker that alone or in combination with nucleotides substitutes for 4 nucleotides, or (ii) at least 4 nucleotides; and/or
  - (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2-10, optionally 2-8 nucleotides, wherein
    - (i) one or more of nucleotides 82-95 is deleted and optionally substituted relative to SEQ ID NO: 500; and
    - (ii) nucleotide 81 is linked to nucleotide 96 by (i) a second internal linker that alone or in combination with nucleotides substitutes for 4 nucleotides, or (ii) at least 4 nucleotides; and/or
  - (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 2-18, optionally 2-16 nucleotides, wherein
    - (i) one or more of nucleotides 113-134 is deleted and optionally substituted relative to SEQ ID NO: 500; and
    - (ii) nucleotide 112 is linked to nucleotide 135 by (i) a third internal linker that alone or in combination with nucleotides substitutes for 4 nucleotides, or (ii) at least 4 nucleotides;
- wherein one or both nucleotides 144-145 are optionally deleted as compared to SEQ ID NO: 500;
- wherein the gRNA comprises at least one of the first internal linker, the second internal linker, and the third internal linker.
39. A polypeptide encoded by the polynucleotide of any one of claims 1, 15, 16, 22, 23, and 33.
40. A vector comprising the polynucleotide of any one of claims 1, 15, 16, 22, 23, and 33.
41. An expression construct, or a plasmid comprising the expression construct comprising a promoter operably linked to a sequence encoding the polynucleotide of any one of claims 1, 15, 16, 22, 23, and 33, optionally wherein the promoter is an RNA polymerase promoter, optionally a bacterial RNA polymerase promoter.

42. The expression construct of claim 41, further comprising (i) a poly-A tail sequence, optionally wherein the poly-A tail sequence is an encoded poly-A tail sequence; and/or (ii) a polyadenylation signal sequence.
43. A host cell comprising the vector of claim 40 or the expression construct of claim 41.
44. A pharmaceutical composition comprising the polynucleotide, composition, or polypeptide of any one of claims 1, 15, 16, 22, 23, and 37 - 39 and a pharmaceutically acceptable carrier.
45. A kit comprising the polynucleotide, composition, or polypeptide of any one of claims 1, 15, 16, 22, 23 and 37 - 39.
46. The polynucleotide, composition, or polypeptide of any one of claims 1, 15, 16, 22, 23, and 37 - 39 for use as a medicament.
47. Use of the polynucleotide, composition, or polypeptide of any one of claims 1, 15, 16, 22, 23, 37 - 39 in the manufacture of a medicament for modifying a target gene in a cell.
48. The polynucleotide or composition of any one of claims 1, 15, 16, 22, and 37 - 38, wherein the polynucleotide or composition is formulated as a lipid nanoparticle (LNP) composition.
49. An *in vitro* method of modifying a target gene comprising delivering to a cell the polynucleotide, polypeptide, or composition of any one of claims 1, 15, 16, 22, 23, and 37 - 39.
50. An *in vitro* method of modifying a target gene, comprising delivering to the cell one or more lipid nanoparticle (LNP) compositions, comprising the polynucleotide according to any one of claims 1, 15, 16, 22, and 23, and one or more guide RNAs.
51. A method of producing a polynucleotide of any one of claims 1-50, comprising contacting the expression construct of claim 41 with an RNA polymerase and NTPs that comprise at least one modified nucleotide.