Claims

1. A composition comprising a first mRNA comprising a first open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase, and a second mRNA comprising a second open reading frame encoding a uracil glycosylase inhibitor (UGI), wherein the second mRNA is different from the first mRNA, optionally wherein the composition comprises lipid nanoparticles.

2. The composition of claim 1, wherein the first open reading frame does not comprise a sequence encoding a UGI.

3. A composition comprising a first lipid nanoparticle (LNP) and a second LNP, wherein the first LNP comprises a first mRNA comprising a first open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase, further wherein the first open reading frame does not comprise a sequence encoding a uracil glycosylase inhibitor (UGI), and the second LNP comprises a second mRNA comprising a second open reading frame encoding a UGI, further wherein the second mRNA is different from the first mRNA.

4. An *in vitro* or *ex vivo* method of modifying a target gene comprising delivering to a cell a first mRNA comprising a first open reading frame encoding a first polypeptide comprising a cytidine deaminase and an RNA-guided nickase, a second mRNA comprising a second open reading frame encoding a uracil glycosylase inhibitor (UGI), wherein the second mRNA is different from the first mRNA, and at least one guide RNA (gRNA).

5. The method of claim 4, wherein the first open reading frame does not comprise a sequence encoding a UGI.

6. The composition or method of any one of claims 1-5, wherein the molar ratio of the second mRNA to the first mRNA is from 1:1 to 30:1, from 2:1 to 30:1, or from 7:1 to 22:1.

7. An mRNA comprising an open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase, wherein the polypeptide does not comprise a uracil glycosylase inhibitor (UGI).

8. An in vitro or ex vivo method of modifying at least one cytidine within a target gene in a cell, comprising expressing in the cell or contacting the cell with: (i) a first polypeptide comprising a cytidine deaminase and an RNA-guided nickase, wherein the first polypeptide does not comprise a uracil glycosylase inhibitor (UGI); (ii) a UGI polypeptide; and (iii) at least one guide RNA

(gRNA) wherein the first polypeptide and gRNA form a complex with the target gene and modify the at least one cytidine in the target gene.

9. A cell, wherein (i) the mRNA or composition of any one of claims 1-3 and 6-7 has been introduced to the cell, wherein the cell has been modified after the introduction; or (ii) the cell has been altered by the method of claim 4-6 or 8.

10. An engineered cell comprising at least one base edit and/or indel, wherein the base edit and/or indel is made by contacting a cell with a composition comprising a first mRNA comprising a first open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase, and a second mRNA comprising a second open reading frame encoding a uracil glycosylase inhibitor (UGI), wherein the second mRNA is different from the first mRNA.

11. The engineered cell of claim 10, wherein the first open reading frame does not comprise a sequence encoding a UGI.

12. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-11, wherein the cytidine deaminase is

(i) an enzyme of APOBEC family, optionally an enzyme of APOBEC3 subgroup;

(ii) a cytidine deaminase comprising an amino acid sequence that is at least 80%, 85%, 87%, 90%, 95%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 40, 41, and 960-1023;

(iii) a cytidine deaminase comprising an amino acid sequence that is at least 80%, 85%, 87%, 90%, 95%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 40, 41, and 960-1013;

(iv) a cytidine deaminase comprising an amino acid sequence that is at least 80%, 85%, 87%, 90%, 95%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 40, 41, 976, 977, 979, 980, 984-987, 993-1006, and 1009; or

(v) a cytidine deaminase comprising an amino acid sequence that is at least 80%, 85%, 87%, 90%, 95%, 98%, 99%, or 100% identical to any one of SEQ ID NOs: 40, 976, 981, 984, 986, and 1014-1023.

13. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-12, wherein the cytidine deaminase is an APOBEC3A deaminase (A3A), optionally a human A3A, further optionally a wild-type A3A, or optionally wherein the A3A comprises an amino acid sequence of any one of SEQ ID NOs: 40, 976, 977, 993-1006, or 1009, or an amino acid sequence with at least 87%, at least 90%, at least 95%, at least 98%, at least 99% identity to any one of SEQ ID NOs: 40, 976, 977, 993-1006, or 1009.

14. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-13, wherein the UGI comprises an amino acid sequence of SEQ ID NO: 27 or an amino acid sequence with at least 80%, at least 90%, at least 95%, at least 98%, at least 99% identity to SEQ ID NO: 27.

15. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-14, wherein the RNA-guided nickase is (i) an *S. pyogenes* (Spy) Cas9 nickase, optionally a D10A SpyCas9 nickase; or (ii) an *N. meningitidis* (Nme) Cas9 nickase, optionally a D16A NmeCas9 nickase, further optionally D16A Nme2Cas9 nickase.

16. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-15, wherein the RNA-guided nickase comprises an amino acid sequence of SEQ ID NO: 70, 73, or 76, or an amino acid sequence having at least 90%, 95%, 98%, or 99% identity to SEQ ID NO: 70, 73, or 76; or wherein the sequence encoding the RNA-guided nickase comprises a nucleotide sequence of any one of SEQ ID NOs: 71, 72, 74, 75 or 77-90, or a nucleotide sequence having at least 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 71, 72, 74, 75 or 77-90.

17. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-16, wherein the mRNA comprises one or more of:

a. a 5’ UTR with at least 90% identity to any one of SEQ ID NOs: 91-98;

b. a 3’ UTR with at least 90% identity to any one of SEQ ID NOs: 99-106;

c. a 5’ cap selected from Cap0, Cap1, Cap2, and a cap added co-transcriptionally or post-transcriptionally, optionally wherein the co-transcriptionally added cap is selected from anti-reverse cap analog (ARCA), AG (m7G(5')ppp(5')(2'OMeA)pG, or GG (m7G(5')ppp(5')(2'OMeG)pG, a cap added post-transcriptionally; and

d. a poly-adenylated (poly-A) tail, optionally wherein the poly-A tail is added to the mRNA by PCR tailing or enzymatic tailing and optionally wherein the poly-A tail comprises a sequence of SEQ ID NO: 109.

18. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-17, wherein the cytidine deaminase is located N-terminal to the RNA-guided nickase in the polypeptide.

19. The mRNA, composition, method, or cell of any one of claims 1-18, wherein the polypeptide comprising the cytidine deaminase and the RNA-guided nickase further comprises a peptide linker between the cytidine deaminase and the RNA-guided nickase, wherein the peptide

linker comprises one or more sequences selected from SEQ ID NOs: 268, 46-59, 61, 211-267, and 269-272.

20. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-19, wherein the polypeptide comprising the cytidine deaminase and the RNA-guided nickase further comprises a nuclear localization signal (NLS) and wherein the NLS comprises the sequence of any one of SEQ ID NOs: 63 and 110-122, or a sequence having at least 80%, 85%, 90%, or 95% identity to any one of SEQ ID NOs: 63 and 110-122; or wherein the NLS is encoded by the sequence of any one of SEQ ID NOs: 123-135; or by a sequence having at least 80%, 85%, 90%, 95%, 98% or 100% identity to the sequence of any one of SEQ ID NOs: 123-135.

21. The mRNA, composition, method, cell, or engineered cell of claim 20, wherein

(i) the NLS is at the C-terminus of the RNA-guided nickase;

(ii) the NLS is at the N-terminus of the RNA-guided nickase;

(iii) the NLS is fused to both the N-terminus and C-terminus of the RNA-guided nickase; or

(iv) the cytidine deaminase is located N-terminal to the NLS in the polypeptide and the RNA-guided nickase is located N-terminal to the NLS in the polypeptide.

22. The mRNA, composition, method, cell, or engineered cell of claim 20 or 21, wherein the polypeptide comprising a cytidine deaminase and the RNA-guide nickase further comprises two NLSs located at the N-terminal of the polypeptide, optionally an SV40 and nucleoplasmin NLS at the N-terminal of the polypeptide, optionally wherein the SV40 NLS comprises the sequence of SEQ ID NO: 63 and the nucleoplasmin NLS comprises the sequence of SEQ ID NO: 122.

23. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-22, wherein the polypeptide comprising the cytidine deaminase and the RNA-guided nickase comprises:

a. the cytidine deaminase that is an APOBEC cytidine deaminase, optionally an APOBEC3A deaminase, optionally wherein the cytidine deaminase comprises an amino acid sequence having at least 87% identity to SEQ ID NO: 40; or at least 80% identity to SEQ ID NO: 41;

b. the nickase, wherein the nickase is an *N. meningitidis* (Nme) Nme2Cas9 nickase, an Nme1Cas9 nickase, or an Nme3Cas9 nickase, optionally wherein the nickase comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 387; and

c. a first nuclear localization signal (NLS), optionally wherein the first NLS comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 63;

wherein the polypeptide does not comprise a uracil glycosylase inhibitor (UGI);

wherein the polypeptide optionally comprises a second NLS, optionally wherein the second NLS comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 122;

optionally wherein the cytidine deaminase is located C-terminal to the first NLS and the second NLS in the polypeptide.

24. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-23, wherein the open reading frame encoding the polypeptide comprising the cytidine deaminase and the RNA-guided nickase comprises, from 5' to 3':

a. a nucleotide sequence encoding a first nuclear localization signal (NLS), optionally wherein the first NLS comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 63; and optionally, a nucleotide sequence encoding a second NLS, optionally wherein the second NLS comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 122;

b. a nucleotide sequence encoding a cytidine deaminase comprising an amino acid sequence having at least 87% identity to SEQ ID NO: 40 or at least 80% identity to SEQ ID NO: 41; and

c. a nucleotide sequence (i) encoding an *N. meningitidis* (Nme) Cas9 nickase polypeptide comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 387, optionally an amino acid sequence that is at least 99% identical to SEQ ID NO: 387; or (ii) that is at least 95% identical to SEQ ID NO: 389, optionally at least 99% identical to SEQ ID NO: 389,

optionally wherein the ORF does not comprise a coding sequence for an NLS C-terminal to the sequence encoding the NmeCas9, optionally wherein the ORF does not comprise a coding sequence C-terminal to the ORF encoding the NmeCas9.

25. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-24, (i) wherein the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the amino acid sequence of any one of SEQ ID NOs: 303, 306, 309, 312, 313, or 321-337, or an amino acid sequence having at least 80%, 85%, 88%, 90%, 95%, 98%, or 99%

identity to any one of SEQ ID NOs: 303, 306, 309, 312, 313, or 321-337; or where the open reading frame encoding the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the sequence of any one of SEQ ID NOs: 302, 305, 308, 311, or 361-377, or a sequence having at least 80%, 85%, 88%, 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 302, 305, 308, 311, or 361-377; or

(ii) the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the amino acid sequence of SEQ ID NO: 3, 6, 15, 21, and 33, or an amino acid sequence having at least 80%, 85%, 88%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 3, 6, 15, 21, and 33; or where the open reading frame encoding the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the sequence of SEQ ID NO: 2, 5, 14, 20, and 32 or a sequence having at least 80%, 85%, 88%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 2, 5, 14, 20, and 32.

26. The mRNA, composition, method, or engineered cell of any one of claims 1-25, wherein the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the amino acid sequence of SEQ ID NO: 312, or an amino acid sequence having at least 80%, 85%, 88%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 312; or where the open reading frame encoding the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the sequence of SEQ ID NO: 311, or a sequence having at least 80%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 311.

27. The mRNA, composition, method, or engineered cell of any one of claims 1-25, wherein the polypeptide comprising a cytidine deaminase and an RNA-guided nickase comprises the amino acid sequence of SEQ ID NO: 313, or an amino acid sequence having at least 80%, 90%, 95%, 98%, or 99% identity to SEQ ID NO: 313.

28. The mRNA, composition, method, cell, or engineered cell of any one of claims 1-27, wherein at least 10%, 20%, 30%, 80%, 90%, or 100% of the uridine in the mRNA is substituted with a modified uridine, optionally wherein the modified uridine is one or more of N1-methyl-pseudouridine, pseudouridine, 5-methoxyuridine, or 5-iodouridine.

29. A polypeptide encoded by any one of the mRNAs of any one of claims 1-13 and 15-28.

30. A polypeptide comprising, from N-terminus to C-terminus,

a. a first NLS comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 63 or the amino acid sequence of SEQ ID NO: 63;

b. a second NLS comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 122 or the amino acid sequence of SEQ ID NO: 122;

c. a cytidine deaminase, optionally an APOBEC3A cytidine deaminase, comprising an amino acid sequence that is at least 87% identical to SEQ ID NO: 40 or the amino acid sequence of SEQ ID NO: 40, excluding an N-terminal methionine relative to SEQ ID NO: 40;

d. an Nme2Cas9 nickase comprising an amino acid sequence that is at least 90%, identical to SEQ ID NO: 387 or the amino acid of SEQ ID NO: 387, excluding an N-terminal methionine relative to SEQ ID NO: 387.

31. The polypeptide of claim 30, 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 GGS; and (ii) a second peptide linker between the cytidine deaminase and the Nme2Cas9 nickase, optionally wherein the second peptide linker comprises the amino acid sequence of SEQ ID NO: 268.

32. A polypeptide comprising an amino acid sequence that is at least 95%, 98%, or 99% identical to SEQ ID NO: 313 or comprising the sequence of SEQ ID NO: 313.

33. A polynucleotide encoding the polypeptide of any one of claims 30-32.

34. A polynucleotide comprising an open reading frame (ORF), the ORF comprising, from 5' to 3':

1) a nucleotide sequence encoding a first nuclear localization signal (NLS), wherein (i) the first NLS comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 63 or the amino acid sequence of SEQ ID NO: 63; or (ii) the nucleotide sequence encoding the first NLS comprises a nucleotide sequence having at least 90% identity to SEQ ID NO: 62; and
2) a nucleotide sequence encoding a second NLS, wherein the second NLS comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 122 or the amino acid sequence of SEQ ID NO: 122;

3) a nucleotide sequence encoding a cytidine deaminase comprising an amino acid sequence having at least 87% identity to SEQ ID NO: 40 or the amino acid sequence of SEQ ID NO: 40, excluding an N-terminal methionine relative to SEQ ID NO: 40; and

4) a nucleotide sequence encoding an Nme2Cas9 nickase, (1) wherein the Nme2Cas9 nickase comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 387 or the amino acid of SEQ ID NO: 387, excluding an N-terminal methionine relative to SEQ ID NO: 387; or (2) wherein the nucleotide sequence encoding the Nme2Cas9 nickase is at least 95% identical to SEQ ID NO: 389,

optionally wherein the ORF does not comprise a coding sequence encoding a uracil glycosylase inhibitor (UGI).

35. The polynucleotide of claim 34, further comprising one or both of (i) a nucleotide sequence encoding a first peptide linker between the second NLS and the cytidine deaminase, optionally wherein the first peptide linker comprises GGG or GGS; and (ii) a nucleotide sequence encoding a second peptide linker between the cytidine deaminase and the Nme2Cas9 nickase, optionally wherein the second peptide linker comprises the amino acid sequence of SEQ ID NO: 268.

36. A polynucleotide comprising an open reading frame (ORF) comprising a nucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 311, or the nucleotide sequence of SEQ ID NO: 311.

37. A ribonucleoprotein complex (RNP) comprising (i) a polypeptide encoded by any one of the mRNAs or polynucleotides of any one of claims 1-7, 12-28, and 33-36, or the polypeptide of any one of claims 29-32; and (ii) a guide RNA.

38. A vector comprising a nucleotide sequence encoding the mRNA or polynucleotide of any one of claims 1-7, 12-28, and 33-36.

39. An expression construct, or a plasmid comprising the expression construct, comprising a promoter operably linked to a sequence encoding any one of the mRNAs or polynucleotides of any one of claims 1-7, 12-28, and 33-36.

40. A host cell comprising the vector of claim 38, or the expression construct or plasmid of claim 39.

41. Use of the mRNA, composition, polypeptide, or polynucleotide according to any one of claims 1-8 and 12-36 for the manufacture of a medicament for modifying a target gene in a cell.

42. The mRNA, composition, or polynucleotide of any one of claims 1-7, 12-28, and 33-36, wherein the mRNA, composition, or polynucleotide is formulated as a lipid nanoparticle (LNP) composition.

43. A lipid nanoparticle (LNP) composition comprising an mRNA comprising an open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase, wherein the polypeptide does not comprise a uracil glycosylase inhibitor (UGI).

44. One or more lipid nanoparticle (LNP) compositions, comprising:

(a) a first mRNA comprising a first open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase;

(b) a second mRNA comprising a second open reading frame encoding a uracil glycosylase inhibitor (UGI); and

(c) one or more guide RNAs.

45. An *in vitro* or *ex vivo* method of modifying a target gene in a cell, comprising delivering to the cell one or more lipid nanoparticle (LNP) compositions, comprising:

(a) a first mRNA comprising a first open reading frame encoding a polypeptide comprising a cytidine deaminase and an RNA-guided nickase;

(b) a second mRNA comprising a second open reading frame encoding a uracil glycosylase inhibitor (UGI); and

(c) one or more guide RNAs.

46. The LNP composition or method of any one of claims 42-45, wherein

(i) parts (a), (b), and (c) are each in separate LNPs;

(ii) parts (a), (b), and (c) are in the same LNPs; or

(iii) parts (a) and (c) are in the same LNPs and part (b) is in a separate LNP;

further optionally the one or more guide RNAs are each in separate LNP.

47. The LNP composition or method of any one of claims 42-46, wherein the LNP composition comprises (i) an amine lipid; (ii) a helper lipid; (iii) a stealth lipid; (iv) a neutral lipid; or combinations of two or more of (i)-(iv), optionally wherein (i) the amine lipid is Lipid A

(9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3(diethylamino)propoxy)carbonyl)oxy)methyl)propyloctadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate); (ii) the helper lipid is cholesterol; (iii) the stealth lipid is PEG-DMG; (iv) the neutral lipid is DSPC; or combinations of two or more of (i)-(iv).

48. The LNP composition or method of any one of claims 42-47, wherein the LNP composition comprises about 35 mol-% amine lipid such as Lipid A; about 15 mol-% neutral lipid such as DSPC; about 2.5 mol-% of stealth lipid such as a PEG lipid, such as PEG-DMG, and the remainder of the lipid component is helper lipid such as cholesterol wherein the N/P ratio of the is about 6.

49. A polypeptide comprising a cytidine deaminase and an RNA-guided nickase, wherein the polypeptide does not comprise a uracil glycosylase inhibitor (UGI).

50. A composition comprising a first polypeptide comprising a cytidine deaminase and an RNA-guided nickase, wherein the first polypeptide does not comprise a uracil glycosylase inhibitor (UGI), and a second polypeptide comprising a UGI, wherein the second polypeptide is different from the first polypeptide.

51. A pharmaceutical composition or kit comprising the mRNA, RNP, composition, polypeptide, polynucleotide, or cell of any one of claims 1-3, 6-37, 4-44, and 46-50, and optionally a pharmaceutically acceptable carrier.