

CLAIMS:

1. A polynucleotide encoding a polypeptide (*e.g.*, mRNA), wherein the polynucleotide comprises:

- (a) a 5'-UTR comprising the sequence of SEQ ID NO: 1 or a variant or fragment thereof;
- (b) a coding region comprising a stop element (*e.g.*, as described herein); and
- (c) a 3'-UTR (*e.g.*, as described herein).

2. The polynucleotide of claim 1, wherein the 5' UTR comprises a nucleic acid sequence of Formula A:

G G A A A U C G C A A A A (N₂)_x (N₃)_x C U (N₄)_x (N₅)_x C G C G U U A G A U U U C U U
U U A G U U U U C U N₆ N₇ C A A C U A G C A A G C U U U U G U U C U C G C C (N₈
C C)_x (SEQ ID NO: 46),

wherein:

(N₂)_x is a uracil and x is an integer from 0 to 5, *e.g.*, wherein x =3 or 4;

(N₃)_x is a guanine and x is an integer from 0 to 1;

(N₄)_x is a cytosine and x is an integer from 0 to 1;

(N₅)_x is a uracil and x is an integer from 0 to 5, *e.g.*, wherein x =2 or 3;

N₆ is a uracil or cytosine;

N₇ is a uracil or guanine; and/or

N₈ is an adenine or guanine and x is an integer from 0 to 1.

3. The polynucleotide of claim 1, wherein the variant of SEQ ID NO: 1 comprises a sequence with at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1, or a fragment thereof (*e.g.*, a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of SEQ ID NO: 1).

4. The polynucleotide of claim 1, wherein the 5'UTR comprises the sequence of SEQ ID NO: 41 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 41, or a fragment thereof (*e.g.*, a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of SEQ ID NO: 41).

5. The polynucleotide of claim 1, wherein the 5'UTR comprises the sequence of SEQ ID NO: 42 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 42, or a fragment thereof (e.g., a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of SEQ ID NO: 42).
6. The polynucleotide of any one of claims 1-5, wherein the variant of the 5'-UTR comprises a uridine content of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80%.
7. The polynucleotide of claim 6, wherein the variant of the 5'-UTR comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 consecutive uridines (e.g., a polyuridine tract).
8. The polynucleotide of claim 7, wherein the variant of the 5'-UTR comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 polyuridine tracts.
9. The polynucleotide of claim 8, wherein one or more of the polyuridine tracts are adjacent to a different polyuridine tract.
10. The polynucleotide of claim 9, wherein each of, e.g., all, the polyuridine tracts are adjacent to each other, e.g., all of the polyuridine tracts are contiguous.
11. The polynucleotide of claim 10, wherein one or more of the polyuridine tracts are separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or 60 nucleotides.
12. The polynucleotide of claim 10, wherein each of, e.g., all of, the polyuridine tracts are separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or 60 nucleotides.
13. The polynucleotide of any one of the preceding claims wherein the 5' UTR comprises a Kozak sequence, e.g., a GCCRCC nucleotide sequence (SEQ ID NO: 43) wherein R is an adenine or guanine.

14. The polynucleotide of claim 1, wherein the 5'UTR comprises the sequence of SEQ ID NO:1.

15. The polynucleotide of any one of the preceding claims, wherein the 5'UTR results in:

(i) an increased half-life of the polynucleotide, *e.g.*, about 1.5-20-fold increase in half-life of the polynucleotide, and/or

(ii) an increased level and/or activity, *e.g.*, output, of the polypeptide encoded by the polynucleotide (*e.g.*, about 1.5-20-fold increase in level and/or activity).

16. The polynucleotide of claim 15, wherein the increase in half-life and/or increase in activity of the polynucleotide is compared to an otherwise similar polynucleotide which does not have a 5' UTR, has a different 5' UTR, or does not have a 5' UTR of any one of claims 1-14.

17. The polynucleotide of any one of claims 1-16, wherein:

(i) the coding region of (b) comprises a stop element chosen from a stop element provided in Table 3, *e.g.*, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO:36, SEQ ID NO: 37, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 62, SEQ ID NO: 93 or SEQ ID NO: 96; and/or

(ii) the 3' UTR of (c) comprises a 3' UTR sequence provided in Table 2 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a 3' UTR sequence provided in Table 2, or a fragment thereof (*e.g.*, a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of the 3' UTR sequence provided in Table 2)

optionally wherein the polynucleotide comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence provided in Table 4.

18. The polynucleotide of claim 17, wherein the 3' UTR comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 45, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID

NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 94 or SEQ ID NO: 95, or a fragment thereof (e.g., a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of any of the aforesaid sequences),

optionally wherein the polynucleotide comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any of SEQ ID NOs: 47, 48, 49, 50, 122, 52, 53, 54, 55, 59, 60, 61, 126, 127, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, or 120, or a variant or fragment thereof .

19. A polynucleotide encoding a polypeptide, wherein the polynucleotide comprises:

(a) a 5'-UTR (*e.g.*, as described herein);

(b) a coding region comprising a stop element chosen from a stop element provided in Table 3; and

(c) a 3'-UTR (*e.g.*, as described herein).

20. The polynucleotide of claim 19, wherein the stop element comprises the sequence of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 62, SEQ ID NO: 93 or SEQ ID NO: 96.

21. The polynucleotide of claim 19, wherein the coding region of (b) comprises a stop element comprising a consensus sequence of Formula B:

X₃-X₂-X₁-U-A-A-X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂ (SEQ ID NO: 37)

wherein:

X₁ is a G or A;

X₂, X₄, X₅ X₆ or X₇ is each independently C or U;

X₃ is C or A;

X₈, X₁₀, X₁₁, X₁₂ X₁ or X₃ is each independently C or G;

X₉ is G or U; and/or

X₂ is A or U.

22. The polynucleotide of claim 19, wherein the coding region of (b) comprises a stop element comprising a consensus sequence of Formula C:

X₃-X₂-X₁-U-G-A-X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂ (SEQ ID NO: 56)

wherein:

X₃, X₁, X₂, X₅, X₆, X₇, X₈, X₉, or X₁₂ is each independently G or C;

X₂, X₃, or X₄ is each independent A or C;

X₁ is A or G; and/or

X₁₀ or X₁₁ is each independently C or U.

23. The polynucleotide of claim 19, wherein the coding region of (b) comprises a stop element comprising a consensus sequence of Formula D

X₃-X₂-X₁-U-A-G-X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂ (SEQ ID NO: 57)

wherein:

X₃, X₁, X₂, X₃, X₁₀ is each independently G or C;

X₂ or X₉ is each independently A or U;

X₁ or X₄ is each independently A or G;

X₅ or X₈ is each independently A or C; and/or

X₆, X₇, X₁₁ or X₁₂ is each independently C or U.

24. The polynucleotide of any one of claims 19-23, wherein the stop element results in:

(i) an increased half-life of the polynucleotide, *e.g.*, about 1.5-20-fold increase in half-life of the polynucleotide, and/or

(ii) an increased level and/or activity, *e.g.*, output, of the polypeptide encoded by the polynucleotide (*e.g.*, about 1.5-20-fold increase in level and/or activity).

25. The polynucleotide of claim 24, wherein the increase in half-life of the polynucleotide is compared to an otherwise similar polynucleotide which does not have a stop element, has a different stop element, or does not have the stop element of any one of claims 19-24.

26. The polynucleotide of any one of claims 19-25, wherein:

(i) the 5' UTR of (a) comprises a 5' UTR sequence provided in Table 1 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a 5' UTR sequence provided in Table 1, or a variant or a fragment thereof (e.g., a fragment that lacks the first one, two, three, four, five, or six nucleotides of the 5' UTR sequence provided in Table 1); and/or

(ii) the 3' UTR of (c) comprises a 3' UTR sequence provided in Table 2 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a 3' UTR sequence provided in Table 2, or a fragment thereof (e.g., a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of the 3' UTR sequence provided in Table 2)

optionally wherein the polynucleotide comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence provided in Table 4.

27. The polynucleotide of claim 26, wherein the 5' UTR comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 89 or SEQ ID NO: 90, or a fragment thereof (e.g., a fragment that lacks the first one, two, three, four, five, or six nucleotides of any of the aforesaid sequences).

28. The polynucleotide of claim 26, wherein the 3' UTR comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 45, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 94 or SEQ ID NO: 95, or a fragment thereof (e.g., a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of any of the aforesaid sequences),

optionally wherein the polynucleotide comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any of SEQ ID NOs: 47, 48, 49, 50, 122, 52, 53, 54, 55, 59, 60, 61, 126, 127, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, or 120, or a variant or fragment thereof.

29. A polynucleotide encoding a polypeptide, wherein the polynucleotide comprises:

- (a) a 5'-UTR (*e.g.*, as described herein);
- (b) a coding region comprising a stop element (*e.g.*, as described herein); and
- (c) a 3' UTR comprising a core sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 11 or a fragment thereof.

30. The polynucleotide of claim 29, wherein the 3' UTR core sequence is disposed immediately downstream of the stop element of (b).

31. The polynucleotide of claim 29, wherein the 3' UTR core sequence is disposed at the C terminus end of the polynucleotide.

32. The polynucleotide of any one of claims 29-31, wherein the 3' UTR comprising a core sequence comprises a first flanking sequence and/or a second flanking sequence.

33. The polynucleotide of any one of claims 29-32, wherein the 3' UTR comprises the sequence of SEQ ID NO: 11 or a fragment of SEQ ID NO: 11, *e.g.*, a 5 nucleotide (nt), 10 nt, 15 nt, 20 nt, 25 nt, 30 nt, 35 nt, 40 nt, 45 nt, 50 nt, 55 nt, 60 nt, 65 nt, or 70 nt fragment of SEQ ID NO: 11.

34. The polynucleotide of any one of claims 33, wherein the 3' UTR comprises a 60 nt fragment of SEQ ID NO: 11.

35. The polynucleotide of any one of claims 29-34, wherein the 3' UTR comprises the sequence of SEQ ID NO: 45 or a fragment thereof (*e.g.*, a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of SEQ ID NO: 45).

36. The polynucleotide of any one of claims 29-35, wherein the 3' UTR results in:

(i) an increased half-life of the polynucleotide, *e.g.*, about 1.5-10 fold increase in half-life of the polynucleotide, and/or

(ii) an increased level and/or activity, *e.g.*, output, of the polypeptide encoded by the polynucleotide (*e.g.*, about 1.5-20-fold increase in level and/or activity).

37. The polynucleotide of any one of claims 29-36, wherein:

(i) the 5' UTR of (a) comprises a 5' UTR sequence provided in Table 1 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a 5' UTR sequence provided in Table 1, or a fragment thereof (*e.g.*, a fragment that lacks the first one, two, three, four, five, or six nucleotides of the 5' UTR sequence provided in Table 1); and/or

(ii) the coding region of (b) comprises a stop element sequence provided in Table 3.

38. The polynucleotide of claim 37, wherein the 5' UTR comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 89 or SEQ ID NO: 90, or a fragment thereof (*e.g.*, a fragment that lacks the first one, two, three, four, five, or six nucleotides of any of the aforesaid sequences).

39. The polynucleotide of claim 37, wherein the coding region of (b) comprises a stop element chosen from a stop element provided in Table 3, *e.g.*, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36 or SEQ ID NO: 37.

40. The polynucleotide of any one of the preceding claims, wherein the 3' UTR comprises a microRNA (miRNA) binding site, *e.g.*, as described herein, and/or a TENT recruiting sequence, *e.g.*, as described herein.

41. The polynucleotide of claim 40, wherein the 3' UTR comprises a miRNA binding site of SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40 or a combination thereof, and/or a TENT recruiting sequence comprising the sequence of SEQ ID NO: 91 or 92.
42. The polynucleotide of any one of the preceding claims, wherein the coding region of the polynucleotide comprises a sequence encoding a therapeutic payload or a prophylactic payload.
43. The polynucleotide of any one of the preceding claims, further comprising at least one 5' cap structure, *e.g.*, as described herein, and/or a poly A tail, *e.g.*, as described herein, optionally wherein the poly A tail comprises one or more non-adenosine residues, *e.g.*, one or more guanosines.
44. The polynucleotide of any one of the preceding claims, further comprising a 3' stabilizing region, *e.g.*, a stabilized tail *e.g.*, as described herein.
45. The polynucleotide of any one of the preceding claims, wherein the polynucleotide comprises an mRNA.
46. The polynucleotide of claim 45, wherein the mRNA comprises at least one chemical modification.
47. A lipid nanoparticle (LNP) composition comprising a polynucleotide of any one of the preceding claims.
48. A pharmaceutical composition comprising the LNP composition of claim 47.
49. A cell comprising the LNP composition of claim 47 or 48.
50. A method of increasing expression of a payload, *e.g.*, a therapeutic payload or a prophylactic payload in a cell, comprising administering to the cell the LNP composition of claim 47 or 48.

51. A method of delivering the LNP composition of claim 47 or 48, to a cell.
52. Use of an LNP composition of claim 47 or 48 in the manufacture of a medicament for the treatment or prevention of a disease or disorder, or symptom thereof, in a subject, *e.g.*, as described herein.
53. Use of an LNP composition of claim 47 or 48 in the manufacture of a medicament for modulating an immune response in a subject.
54. The use, or the LNP composition of any one of claims 47, 48, 52, or 53, wherein the LNP composition comprises: (i) an ionizable lipid, *e.g.*, an amino lipid; (ii) a sterol or other structural lipid; (iii) a non-cationic helper lipid or phospholipid; and (iv) a PEG-lipid.
55. The use, or the LNP composition of any one of claims 47, 48, or 52-54, wherein the ionizable lipid comprises a compound of Formula (I), a compound of Formula (IIa), or a compound of Formula (IIe).
56. The use, or the LNP composition of any one of claims 47, 48, or 52-55, wherein the non-cationic helper lipid or phospholipid comprises a compound selected from the group consisting of DSPC, DPPC, or DOPC.
57. The use, or the LNP composition of any one of claims 47, 48, or 52-56, wherein the phospholipid is DSPC, *e.g.*, a variant of DSPC, *e.g.*, a compound of Formula (IV).
58. The use, or the LNP composition of any one of claims 47, 48, or 52-57, wherein the structural lipid is chosen from alpha-tocopherol, β -sitosterol or cholesterol.
59. The use, or the LNP composition of any one of claims 47, 48, or 52-58, wherein the PEG lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-

modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, a PEG-modified dialkylglycerol, and mixtures thereof.

60. The use, or the LNP composition of any one of claims 47, 48, or 52-59, wherein the PEG lipid is selected from the group consisting of PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC and PEG-DSPE lipid.

61. The use, or the LNP composition of any one of claims 47, 48, or 52-60, wherein the PEG lipid is a compound chosen from: Formula (V), Formula (VI-A), Formula (VI-B), Formula (VI-C) or Formula (VI-D).

62. The use, or the LNP composition of any one of claims 47, 48, or 52-61, wherein the LNP comprises a molar ratio of about 20-60% ionizable lipid: 5-25% phospholipid: 25-55% cholesterol; and 0.5-15% PEG lipid.

63. The use, or the LNP composition of any one of claims 47, 48, or 52-62, wherein the LNP is formulated for intravenous, subcutaneous, intramuscular, intranasal, intraocular, rectal, pulmonary or oral delivery.

64. The use, or the LNP composition of any one of claims 52-63 wherein the subject is a mammal, *e.g.*, a human.

65. An LNP composition of claim 47 or 48, for use in therapy.