

CLAIMS~~What is claimed is:~~

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 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 a 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).

~~46. The polynucleotide of claim 1 or 3~~any one of claims 1-5, wherein the variant of ~~SEQ ID NO: 1~~the 5'-UTR comprises a uridine content of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, or 80%.

~~57. The polynucleotide of any one of claims 1, or 3-4~~claim 6, wherein the variant of the 5'-UTR of SEQ ID NO: 1 comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 consecutive uridines (e.g., a polyuridine tract).

~~6. The polynucleotide of claims 5, wherein the polyuridine tract in the variant of SEQ ID NO: 1 comprises at least at least 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-12, 4-12, 5-12, 6-12, 7-12, 8-12, 9-12, 10-12, 11-12, 2-6, or 3-5 consecutive uridines.~~

~~7. The polynucleotide of any one of claims 1 or 3-6, wherein the polyuridine tract in the variant of SEQ ID NO: 1 comprises 4 consecutive uridines.~~

~~8. The polynucleotide of any one of claims 1 or 3-7, wherein the polyuridine tract in the variant of SEQ ID NO: 1 comprises 5 consecutive uridines.~~

~~98. The polynucleotide of any one of claims 1 or 3-8~~claim 7, wherein the variant of the 5'-UTR of SEQ ID NO: 1 comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 polyuridine tracts.

~~10. The polynucleotide of claim 9, wherein the variant of SEQ ID NO: 1 comprises 3 polyuridine tracts.~~

~~11. The polynucleotide of claim 9, wherein the variant of SEQ ID NO: 1 comprises 4 polyuridine tracts.~~

~~12. The polynucleotide of claim 9, wherein the variant of SEQ ID NO: 1 comprises 5 polyuridine tracts.~~

~~13~~9. The polynucleotide of ~~any one of claims 1 or 3-12~~claim 8, wherein one or more of the polyuridine tracts are adjacent to a different polyuridine tract.

~~14~~10. The polynucleotide of ~~any one of claims 1 or 3-13~~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.

~~15~~11. The polynucleotide of ~~any one of claims 1 or 3-14~~claim 10, wherein one or more of the polyuridine tracts are separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 2, 13, 14, 15, 16, 17, 18. 19, 20, 30, 40, 50 or 60 nucleotides.

~~16~~2. The polynucleotide of ~~any one of claims 1 or 3-15~~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, 2, 13, 14, 15, 16, 17, 18. 19, 20, 30, 40, 50 or 60 nucleotides.

~~17. The polynucleotide of any one of claims 1 or 3-16, wherein a first polyuridine tract and a second polyuridine tract are adjacent to each other.~~

~~18~~3. 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.

~~19~~14. The polynucleotide of ~~any one of the preceding claims~~claim 1, wherein the 5'UTR comprises the sequence of SEQ ID NO: 1 or a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1.

~~20. The polynucleotide of any one of the preceding claims, 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).~~

~~21. The polynucleotide of any one of the preceding claims, 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).~~

1522. 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).

2316. The polynucleotide of claim 2215, 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 1-1424.

~~24. The polynucleotide of any one of claims 22-23, wherein the increase in half-life of the polynucleotide is measured according to an assay that measures the half-life of a polynucleotide, e.g., an assay described in any one of Examples disclosed herein.~~

~~25. The polynucleotide of any one of the preceding claims, wherein the 5'UTR results in an increased level and/or activity, e.g., output, of the polypeptide encoded by the polynucleotide.~~

~~26. The polynucleotide of claim 25, wherein the 5'UTR results in about 1.5-20 fold increase in level and/or activity, e.g., output, of the polypeptide encoded by the polynucleotide.~~

27. The polynucleotide of claim 26, wherein the increase in activity is compared to an otherwise similar polynucleotide which does not have a 5' UTR, has a different 5' UTR, or does not have the 5' UTR of claim 1.

28. The polynucleotide of any one of the preceding claims, 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, 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.

29. The polynucleotide of any one of the preceding claims, wherein 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.

30. The polynucleotide of claim 29, 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 3' UTR comprises a micro RNA (miRNA) binding site, *e.g.*, as described herein, *e.g.*, a sequence of any one of SEQ ID NOs: 38-40, optionally wherein the 3' UTR comprises a TENT recruiting sequence, *e.g.*, as described herein, *e.g.*, a sequence of SEQ ID NO: 91 or 92,

~~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.~~

~~31~~17. The polynucleotide of any one of claims 1-~~27~~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, *e.g.*, 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.~~

~~183~~2. The polynucleotide of claim ~~31~~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 (e.g., a fragment that lacks the first one, two, three, four, five, six, or more nucleotides of any of the aforesaid sequences).

~~33. The polynucleotide of claim 32 or 33, wherein the 3' UTR comprises a micro RNA binding site, e.g., as described herein, e.g., a sequence of any one of SEQ ID NOs: 38-40, and/or wherein the 3' UTR comprises a TENT recruiting sequence, e.g., as described herein, e.g., a sequence of SEQ ID NO: 91 or 92.~~

3419. 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).

~~3520.~~ The polynucleotide of claim 3419, 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.

~~3621.~~ The polynucleotide of claim 3419, 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 X₋₁ or X₋₃ is each independently C or G;

X₉ is G or U; and/or

X₂ is A or U.

~~37~~22. The polynucleotide of claim ~~34~~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.

~~38~~23. The polynucleotide of claim ~~19~~34, 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.

~~39. The polynucleotide of any one of claims 36-39, wherein the consensus sequence has a high GC content, e.g., GC content of about 50%, 60%, 70%, 80%, 90% or 99%.~~

~~40~~24. The polynucleotide of any one of claims ~~34-39~~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).~~

~~41-25.~~ The polynucleotide of claim ~~40-24~~, 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 ~~34-37~~19-24.

~~42.~~ The polynucleotide of claim 40 or 41, wherein the increase in half life of the polynucleotide is measured according to an assay which measures the half life of a polynucleotide, *e.g.*, an assay described in any one of Examples disclosed herein.

~~43.~~ The polynucleotide of any one of claims 34-42, wherein the stop element results in an increased level and/or activity, *e.g.*, output or duration of expression, of the polypeptide encoded by the polynucleotide.

~~44.~~ The polynucleotide of claim 43, wherein the increase in level and/or activity, *e.g.*, output or duration of expression, of the polypeptide is measured according to an assay which measures the level and/or activity, *e.g.*, output or duration of expression of a polypeptide, *e.g.*, an assay described in any one of Examples disclosed herein.

~~45.~~ The polynucleotide of claim 44, wherein the stop element results in about 1.5-20 fold increase in level and/or activity, *e.g.*, output, of the polypeptide encoded by the polynucleotide.

~~46.~~ The polynucleotide of claim 44, wherein the stop element results in about 1.5-20 fold increase in level and/or activity, *e.g.*, detectable level or activity, of the polypeptide encoded by the polynucleotide for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 14 days.

~~47.~~ The polynucleotide of claim 46 wherein the stop element results in detectable level or activity of the polypeptide encoded by the polynucleotide for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 14 days.

~~48.~~ The polynucleotide of any one of claims 38-47, wherein the increase 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 34-39.

~~4926.~~ The polynucleotide of any one of claims ~~34-48~~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.;~~

~~5027.~~ The polynucleotide of claim ~~4926~~, 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).

~~51. The polynucleotide of any one of claims 34-50, wherein 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.~~

~~5228.~~ The polynucleotide of claim ~~2651~~, 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.

~~53. The polynucleotide of claim 52, wherein the 3' UTR comprises a micro RNA binding site, e.g., as described herein, e.g., a sequence of any one of SEQ ID NOs: 38-40, and/or wherein the 3' UTR comprises a TENT recruiting sequence, e.g., as described herein, e.g., a sequence of SEQ ID NO: 91 or 92.~~

~~54. The polynucleotide of any one of claims 34-53, 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 3' 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~~

~~—— (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.

~~55. The polynucleotide of claim 54, 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);~~

~~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.~~

~~56. The polynucleotide of claim 54 or 55, 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);~~

~~57. The polynucleotide of claim 56, wherein the 3' UTR comprises a micro RNA binding site, e.g., as described herein, e.g., a sequence of any one of SEQ ID NOs: 38-40, and/or wherein the 3' UTR comprises a TENT recruiting sequence, e.g., as described herein, e.g., a sequence of SEQ ID NO: 91 or 92.~~

~~5289. 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.

~~5309.~~ The polynucleotide of claim ~~5829~~, wherein the 3' UTR core sequence is disposed immediately downstream of the stop element of (b).

~~6031.~~ The polynucleotide of claim ~~5829~~, wherein the 3' UTR core sequence is disposed at the C terminus end of the polynucleotide.

~~6432.~~ The polynucleotide of any one of claims ~~5829-6031~~, wherein the 3' UTR comprising a core sequence comprises a first flanking sequence and/or a -

~~62.~~ The polynucleotide of any one of claims ~~58-61~~, wherein the 3' UTR comprising a core sequence comprises a second flanking sequence.

~~63.~~ The polynucleotide of claim ~~61 or 62~~, wherein the 3' UTR comprising a core sequence comprises a first flanking sequence and a second flanking sequence.

~~64.~~ The polynucleotide of any one of claims ~~61-63~~, wherein the first flanking sequence comprises a sequence of about ~~5-25, about 5-20, about 5-15, about 5-10, about 10-25, about 15-25, about 20-25~~ nucleotides.

~~65.~~ The polynucleotide of any one of claims ~~61-64~~, wherein the first flanking sequence comprises a sequence of about ~~5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25~~ nucleotides, *e.g.*, 11 nucleotides.

~~66.~~ The polynucleotide of any one of claims ~~61-65~~, wherein the second flanking sequence comprises a sequence of about ~~20-80, about 20-75, about 20-70, about 20-65, about 20-60, about 20-55, about 20-50, about 20-45, about 20-40, about 20-35, about 20-30, about 20-25, about 25-~~

~~80, about 30-80, about 35-80, about 40-80, about 45-80, about 50-80, about 55-80, about 60-80, about 65-80, about 70-80 or about 75-80 nucleotides.~~

~~67. The polynucleotide of any one of claims 61-66, wherein the second flanking sequence comprises a sequence of about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, or 80 nucleotides, e.g., 39 nucleotides.~~

~~68. The polynucleotide of any one of claims 61-67, wherein the first flanking sequence is upstream or downstream of the core sequence.~~

~~69. The polynucleotide of any one of claims 61-67, wherein the second flanking sequence is upstream or downstream of the core sequence.~~

~~7033. The polynucleotide of any one of claims 58-6929-32, 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.~~

~~7134. The polynucleotide of any one of claims 58-7033, wherein the 3' UTR comprises a 15-25 nt fragment comprising a 60 nt fragment of SEQ ID NO: 11.~~

~~7235. The polynucleotide of any one of claims 58-7129-34, 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).~~

~~73. The polynucleotide of any one of claims 58-71, wherein the 3' UTR comprises the sequence of SEQ ID NO: 11 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: 11).~~

~~7436. The polynucleotide of any one of claims 58-7329-35, 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) e.g., as measured by an assay that measures the half life of a polynucleotide, e.g., an assay of any one of Examples disclosed herein.~~

~~75. The polynucleotide of claim 74, wherein the 3' UTR results in a polynucleotide with a mean half life score of greater than 10.~~

~~76. The polynucleotide of any one of claims 58-73, wherein the 3' UTR results in an increased level and/or activity, e.g., output, of the polypeptide encoded by the polynucleotide.~~

~~77. The polynucleotide of any one of claims 74-76, wherein the increase is compared to an otherwise similar polynucleotide which does not have a 3' UTR, has a different 3' UTR, or does not have the 3' UTR of claim 76.~~

~~78. The polynucleotide of any one of claims 58-77, wherein the 3' UTR comprises a micro RNA (miRNA) binding site, e.g., as described herein, and/or a TENT recruiting sequence, e.g., as described herein.~~

~~79. The polynucleotide of claim 78, 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.~~

~~80. The polynucleotide of claim 78 or 79, wherein the 3' UTR comprises a plurality of miRNA binding sites, e.g., 2, 3, 4, 5, 6, 7 or 8 miRNA binding sites.~~

~~81. The polynucleotide of claim 80, wherein the plurality of miRNA binding sites comprises the same or different miRNA binding sites.~~

~~8237~~. The polynucleotide of any one of claims ~~58-81~~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.~~

~~8338~~. The polynucleotide of claim ~~8237~~, 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).

~~84~~. The polynucleotide of any one of claims ~~58-83~~, wherein the coding region of (b) comprises a stop element sequence provided in Table 3.

~~8539~~. The polynucleotide of claim ~~8437~~, 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.

86. The polynucleotide of any one of claims 58-81, 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 3' 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
- (ii) the stop element of (b) comprises a stop element sequence provided in Table 3.

87. The polynucleotide of claim 86, 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).

88. The polynucleotide of any one of claims 58-81 or 86, 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, 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.

8942. 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.

~~90. The polynucleotide of claim 89, wherein the therapeutic payload or prophylactic payload comprises a secreted protein, a membrane bound protein; or an intercellular protein.~~

~~91. The polynucleotide of claim 90, wherein the therapeutic payload or prophylactic payload is chosen from a cytokine, an antibody, a vaccine (e.g., an antigen, an immunogenic epitope), a receptor, an enzyme, a hormone, a transcription factor, a ligand, a membrane transporter, a structural protein, a nuclease, or a component, variant or fragment (e.g., a biologically active fragment) thereof.~~

~~92. The polynucleotide of any one of claims 89-91, wherein the therapeutic payload or prophylactic payload comprises a protein or peptide.~~

~~93~~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.

~~94. The polynucleotide of claim 93, wherein the 5' cap structure comprises a sequence of GG, GA, or GGA, wherein the underlined, italicized G is an inverted G nucleotide followed by a 5'-5' triphosphate group.~~

~~95~~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.

~~96. The polynucleotide of claim 95, wherein the 3' stabilizing region comprises a poly A tail, e.g., a poly A tail comprising 80-150, e.g., 120, adenine (SEQ ID NO: 123), optionally wherein the poly A tail comprises one or more non-adenosine residues, e.g., one or more guanosines.~~

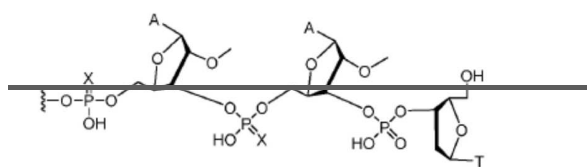
~~97. The polynucleotide of claim 95 or 96, wherein the poly A tail comprises a UCUAG sequence (SEQ ID NO: 44).~~

98. The polynucleotide of claim 97, wherein the poly A tail comprises about 80–120, *e.g.*, 100, adenines upstream of SEQ ID NO: 44.

99. The polynucleotide of claim 97 or 98, wherein the poly A tail comprises about 1–40, *e.g.*, 20, adenines downstream of SEQ ID NO: 44.

100. The polynucleotide of any one of claims 95–99, wherein the 3' stabilizing region comprises at least one alternative nucleoside, optionally wherein the alternative nucleoside is an inverted thymidine (idT).

101. The polynucleotide of any one of claims 95–100, wherein the 3' stabilizing region comprises a structure of Formula VII:



or a salt thereof, wherein each X is independently O or S, and A represents adenine and T represents thymine.

10245. The polynucleotide of any one of the preceding claims, wherein the polynucleotide comprises an mRNA.

10346. The polynucleotide of claim 10245, wherein the mRNA comprises at least one chemical modification.

104. The polynucleotide of claim 102 or 103, wherein the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza pseudouridine, 2-thio-1-methyl pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-

~~pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methoxyuridine, and 2'-O-methyl-uridine.~~

~~105~~47. A lipid nanoparticle (LNP) composition comprising a polynucleotide of any one of the preceding claims.

~~106~~48. A pharmaceutical composition comprising the LNP composition of claim ~~105~~47.

~~107~~49. A cell comprising the LNP composition of claim ~~105~~47 or ~~106~~48.

~~108~~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~~47 or 48~~105 or 106~~.

~~109~~51. A method of delivering the LNP composition of claim ~~47 or 48~~47 or 48~~105 or 106~~, to a cell.

~~110. The method of claim 109, comprising contacting the cell *in vitro*, *in vivo* or *ex vivo* with the LNP composition.~~

~~111~~52. Use of A method of delivering an LNP composition of claim 47 or 48 ~~105 or 106~~in the manufacture of a medicament for the treatment or prevention of, ~~to a subject having a disease or disorder, or symptom thereof, in a subject, e.g., as described herein.~~

~~112~~53. Use of an LNP composition of claim 47 or 48 in the manufacture of a medicament for A method of modulating an immune response in a subject, ~~comprising administering to the subject in need thereof an effective amount of an LNP composition of claim 105 or 106.~~

~~113. A method of treating, preventing, or preventing a symptom of, a disease or disorder comprising administering to a subject in need thereof an effective amount of an LNP composition of claim 105 or 106.~~

~~114~~54. The ~~method~~use, or the LNP composition of any one of claims ~~105-113~~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.

~~115. The method, or the LNP composition of claim 114, wherein the ionizable lipid comprises an amino lipid.~~

~~116. The method, or the LNP composition of claim 114 or 115, wherein the ionizable lipid comprises a compound of any of Formulae (I), (IA), (IB), (IC), (II), (IIa), (IIb), (IIc), (IIId), (IIE), (IIe), (IIg), (III), (IIIa1), (IIIa2), (IIIa3), (IIIa4), (IIIa5), (IIIa6), (IIIa7), or (IIIa8).~~

~~117~~55. The ~~use~~method, or the LNP composition of any one of claims ~~47, 48, or 52-54~~105-116, wherein the ionizable lipid comprises a compound of Formula (I), a compound of Formula (IIa), or a compound of Formula (IIE).

~~118~~56. The ~~use~~method, or the LNP composition of any one of claims ~~47, 48, or 52-55~~105-117, wherein the non-cationic helper lipid or phospholipid comprises a compound selected from the group consisting of DSPC, DPPC, or DOPC.

~~119~~57. The ~~use~~method, or the LNP composition of any one of claims ~~47, 48, or 52-56~~105-118, wherein the phospholipid is DSPC, *e.g.*, a variant of DSPC, *e.g.*, a compound of Formula (IV).

~~120~~58. The ~~use~~method, or the LNP composition of any one of claims ~~47, 48, or 52-57~~105-119, wherein the structural lipid is chosen from alpha-tocopherol, β -sitosterol or cholesterol.

~~121~~59. The ~~use~~method, or the LNP composition of any one of claims ~~47, 48, or 52-58~~105-120, 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.

~~12260~~. The ~~use~~method, or the LNP composition of any one of claims 47, 48, or 52-59~~105-121~~, 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.

~~12361~~. The ~~use~~method, or the LNP composition of any one of claims 47, 48, or 52-60~~105-121~~, wherein the PEG lipid is a compound chosen from: Formula (V), Formula (VI-A), Formula (VI-B), Formula (VI-C) or Formula (VI-D).

~~12462~~. The ~~use~~method, or the LNP composition of any one of claims 47, 48, or 52-61~~105-123~~, 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.

~~12563~~. The ~~method~~use, or the LNP composition of any one of claims 47, 48, or 52-62~~105-124~~, wherein the LNP is formulated for intravenous, subcutaneous, intramuscular, intranasal, intraocular, rectal, pulmonary or oral delivery.

~~12664~~. The ~~method~~use, or the LNP composition of any one of claims ~~105-125~~52-63 wherein the subject is a mammal, *e.g.*, a human.

~~127~~. The ~~method~~, or the LNP composition of any one of claims ~~105-126~~, wherein the subject has a disease or disorder disclosed herein.

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