**CLAIMS:**

1. A composition comprising a non-anionic polynucleotide analog cargo for intracellular delivery and a synthetic peptide shuttle agent that is independent from, or is not covalently linked to, said non-anionic polynucleotide analog cargo, the synthetic peptide shuttle agent being a peptide comprising an amphipathic alpha-helical motif having both a positively-charged hydrophilic outer face and a hydrophobic outer face, wherein synthetic peptide shuttle agent increases cytosolic/nuclear delivery of said non-anionic polynucleotide analog cargo in eukaryotic cells as compared to in the absence of the synthetic peptide shuttle agent, wherein:

(A) the shuttle agent is:

(1) a peptide at least 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length comprising

(2) an amphipathic alpha-helical motif having

(3) a positively-charged hydrophilic outer face, and a hydrophobic outer face,

wherein at least five of the following parameters (4) to (15) are respected:

(4) the hydrophobic outer face comprises a highly hydrophobic core consisting of spatially adjacent L, I, F, V, W, and/or M amino acids representing 12 to 50% of the amino acids of the peptide, based on an open cylindrical representation of the alpha-helix having 3.6 residues per turn;

(5) the peptide has a hydrophobic moment (μ) of 3.5 to 11;

(6) the peptide has a predicted net charge of at least +3 or +4 at physiological pH;

(7) the peptide has an isoelectric point (pI) of 8 to 13;

(8) the peptide is composed of 35% to 65% of any combination of the amino acids: A, C, G, I, L, M, F, P, W, Y, and V;

(9) the peptide is composed of 0% to 30% of any combination of the amino acids: N, Q, S, and T;

(10) the peptide is composed of 35% to 85% of any combination of the amino acids: A, L, K, or R;

(11) the peptide is composed of 15% to 45% of any combination of the amino acids: A and L, provided there being at least 5% of L in the peptide;

(12) the peptide is composed of 20% to 45% of any combination of the amino acids: K and R;

(13) the peptide is composed of 0% to 10% of any combination of the amino acids: D and E;

(14) the difference between the percentage of A and L residues in the peptide (% A+ L), and the percentage of K and R residues in the peptide (% K + R), is less than or equal to 10%; and

(15) the peptide is composed of 10% to 45% of any combination of the amino acids: Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T and H; or

(B) the shuttle agent comprises an endosome leakage domain (ELD) operably linked to a cell penetrating domain (CPD).

2. The composition of claim 1, wherein the non-anionic polynucleotide analog cargo is a charge-neutral or cationic antisense synthetic oligonucleotide (ASO).

3. The composition of claim 1 or 2, wherein the non-anionic polynucleotide analog cargo is:

(a) a charge-neutral polynucleotide analog cargo having a phosphorodiamidate backbone, an amide backbone, a methylphosphonate backbone, a neutral phosphotriester backbone, a sulfone backbone, or a triazole backbone; or

(b) a cationic polynucleotide analog cargo having an aminoalkylated phosphoramidate backbone, a guanidinium backbone, an S-methylthiourea backbone, or a nucleosyl amino acid (NAA) backbone.

4. The composition of any one of claims 1 to 3, wherein the non-anionic polynucleotide analog cargo is a phosphorodiamidate morpholino oligomer (PMO), a peptide nucleic acid (PNA), a methylphosphonate oligomer, or a short interfering ribonucleic neutral oligonucleotide (siRNN).

5. The composition of any one of claims 1 to 4, wherein the non-anionic polynucleotide analog cargo is a 5- to 50-mer, a 5-mer to 75-mer, or a 5-mer to 100-mer.

6. The composition of any one of claims 1 to 5, wherein the non-anionic polynucleotide analog cargo is not covalently linked to a cell-penetrating peptide, octa-guanidine dendrimer, or other intracellular delivery moiety.

7. The composition of any one of claims 1 to 6, wherein:

(a) the shuttle agent respects at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or respects all of parameters (4) to (15);

(b) the shuttle agent is a peptide having a minimum length of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids, and a maximum length of 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 60, 65, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids;

(c) said amphipathic alpha-helical motif has a hydrophobic moment (μ) between a lower limit of 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and an upper limit of 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, or 11.0;

(d) said amphipathic alpha-helical motif comprises a positively-charged hydrophilic outer face comprising: (i) at least two, three, or four adjacent positively-charged K and/or R residues upon helical wheel projection; and/or (ii) a segment of six adjacent residues comprising three to five K and/or R residues upon helical wheel projection, based on an alpha helix having angle of rotation between consecutive amino acids of 100 degrees and/or an alpha-helix having 3.6 residues per turn;

(e) said amphipathic alpha-helical motif comprises a hydrophobic outer face comprising: (i) at least two adjacent L residues upon helical wheel projection; and/or (ii) a segment of ten adjacent residues comprising at least five hydrophobic residues selected from: L, I, F, V, W, and M, upon helical wheel projection, based on an alpha helix having angle of rotation between consecutive amino acids of 100 degrees and/or an alpha-helix having 3.6 residues per turn;

(f) said hydrophobic outer face comprises a highly hydrophobic core consisting of spatially adjacent L, I, F, V, W, and/or M amino acids representing from 12.5%, 13%, 13.5%, 14%, 14.5%, 15%, 15.5%, 16%, 16.5%, 17%, 17.5%, 18%, 18.5%, 19%, 19.5%, or 20%, to 25%, 30%, 35%, 40%, or 45% of the amino acids of the shuttle agent;

(g) the shuttle agent has a hydrophobic moment (μ) between a lower limit of 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and an upper limit of 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, or 10.5;

(h) the shuttle agent has a predicted net charge of between +3, +4, +5, +6, +7, +8, +9, to +10, +11, +12, +13, +14, or +15;

(i) the shuttle agent has a predicted pI of 10 to 13; or

(j) any combination of (a) to (i).

8. The composition of any one of claims 1 to 7, wherein said shuttle agent respects at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or all of the following parameters:

(8) the shuttle agent is composed of 36% to 64%, 37% to 63%, 38% to 62%, 39% to 61%, or 40% to 60% of any combination of the amino acids: A, C, G, I, L, M, F, P, W, Y, and V;

(9) the shuttle agent is composed of 1% to 29%, 2% to 28%, 3% to 27%, 4% to 26%, 5% to 25%, 6% to 24%, 7% to 23%, 8% to 22%, 9% to 21%, or 10% to 20% of any combination of the amino acids: N, Q, S, and T;

(10) the shuttle agent is composed of 36% to 80%, 37% to 75%, 38% to 70%, 39% to 65%, or 40% to 60% of any combination of the amino acids: A, L, K, or R;

(11) the shuttle agent is composed of 15% to 40%, 20% to 40%, 20 to 35%, or 20 to 30% of any combination of the amino acids: A and L;

(12) the shuttle agent is composed of 20% to 40%, 20 to 35%, or 20 to 30% of any combination of the amino acids: K and R;

(13) the shuttle agent is composed of 5 to 10% of any combination of the amino acids: D and E;

(14) the difference between the percentage of A and L residues in the shuttle agent (% A+ L), and the percentage of K and R residues in the shuttle agent (% K + R), is less than or equal to 9%, 8%, 7%, 6%, or 5%; and

(15) the shuttle agent is composed of 15 to 40%, 20% to 35%, or 20% to 30% of any combination of the amino acids: Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T, and H.

9. The composition of any one of claims 1 to 8, wherein said shuttle agent comprises a histidine-rich domain, wherein the histidine-rich domain is:

(i) positioned towards the N terminus and/or towards the C terminus of the shuttle agent;

(ii) is a stretch of at least 3, at least 4, at least 5, or at least 6 amino acids comprising at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% histidine residues; and/or comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 consecutive histidine residues; or

(iii) both (i) and (ii).

10. The composition of any one of claims 1 to 9, wherein said shuttle agent comprises a flexible linker domain rich in serine and/or glycine residues.

11. The composition of any one of claims 1 to 10, wherein said shuttle agent comprises or consists of the amino acid sequence of:

(a) **[X1]-[X2]-[linker]-[X3]-[X4]** (Formula 1);

(b) **[X1]-[X2]-[linker]-[X4]-[X3]** (Formula 2);

(c) **[X2]-[X1]-[linker]-[X3]-[X4]** (Formula 3);

(d) **[X2]-[X1]-[linker]-[X4]-[X3]** (Formula 4);

(e) **[X3]-[X4]-[linker]-[X1]-[X2]** (Formula 5);

(f) **[X3]-[X4]-[linker]-[X2]-[X1]** (Formula 6);

(g) **[X4]-[X3]-[linker]-[X1]-[X2]** (Formula 7); or

(h) **[X4]-[X3]-[linker]-[X2]-[X1]** (Formula 8),

(i) **[linker]-[X1]-[X2]-[linker]** (Formula 9);

(j) **[linker]-[X2]-[X1]-[linker]** (Formula 10);

(k) **[X1]-[X2]-[linker]** (Formula 11);

(l) **[X2]-[X1]-[linker]** (Formula 12);

(m) **[linker]-[X1]-[X2]** (Formula 13);

(n) **[linker]-[X2]-[X1]** (Formula 14);

(o) **[X1]-[X2]** (Formula 15); or

(p) **[X2]-[X1]** (Formula 16),

wherein:

**[X1]** is selected from: 2[Φ]-1[+]-2[Φ]-1[ζ]-1[+]- ; 2[Φ]-1[+]-2[Φ]-2[+]- ; 1[+]-1[Φ]-1[+]-2[Φ]-1[ζ]-1[+]- ; and 1[+]-1[Φ]-1[+]-2[Φ]-2[+]- ;

**[X2]** is selected from: -2[Φ]-1[+]-2[Φ]-2[ζ]- ; -2[Φ]-1[+]-2[Φ]-2[+]- ; -2[Φ]-1[+]-2[Φ]-1[+]-1[ζ]- ; -2[Φ]-1[+]-2[Φ]-1[ζ]-1[+]- ; -2[Φ]-2[+]-1[Φ]-2[+]- ; -2[Φ]-2[+]-1[Φ]-2[ζ]- ; -2[Φ]-2[+]-1[Φ]-1[+]-1[ζ]- ; and -2[Φ]-2[+]-1[Φ]-1[ζ]-1[+]- ;

**[X3] i**s selected from: -4[+]-A- ; -3[+]-G-A- ; -3[+]-A-A- ; -2[+]-1[Φ]-1[+]-A- ; -2[+]-1[Φ]-G-A- ; -2[+]-1[Φ]-A-A- ; or -2[+]-A-1[+]-A ; -2[+]-A-G-A ; -2[+]-A-A-A- ; -1[Φ]-3[+]-A- ; -1[Φ]-2[+]-G-A- ; -1[Φ]-2[+]-A-A- ; -1[Φ]-1[+]-1[Φ]-1[+]-A ; -1[Φ]-1[+]-1[Φ]-G-A ; -1[Φ]-1[+]-1[Φ]-A-A ; -1[Φ]-1[+]-A-1[+]-A ; -1[Φ]-1[+]-A-G-A ; -1[Φ]-1[+]-A-A-A ; -A-1[+]-A-1[+]-A ; -A-1[+]-A-G-A ; and -A-1[+]-A-A-A ;

**[X4]** is selected from: -1[ζ]-2A-1[+]-A ; -1[ζ]-2A-2[+] ; -1[+]-2A-1[+]-A ; -1[ζ]-2A-1[+]-1[ζ]-A-1[+] ; -1[ζ]-A-1[ζ]-A-1[+] ; -2[+]-A-2[+] ; -2[+]-A-1[+]-A ; -2[+]-A-1[+]-1[ζ]-A-1[+] ; -2[+]-1[ζ]-A-1[+] ; -1[+]-1[ζ]-A-1[+]-A ; -1[+]-1[ζ]-A-2[+] ; -1[+]-1[ζ]-A-1[+]-1[ζ]-A-1[+] ; -1[+]-2[ζ]-A-1[+] ; -1[+]-2[ζ]-2[+] ; -1[+]-2[ζ]-1[+]-A ; -1[+]-2[ζ]-1[+]-1[ζ]-A-1[+] ; -1[+]-2[ζ]-1[ζ]-A-1[+] ; -3[ζ]-2[+] ; -3[ζ]-1[+]-A ; -3[ζ]-1[+]-1[ζ]-A-1[+] ; -1[ζ]-2A-1[+]-A ; -1[ζ]-2A-2[+] ; -

1[ζ]-2A-1[+]-1[ζ]-A-1[+] ; -2[+]-A-1[+]-A ; -2[+]-1[ζ]-1[+]-A ; -1[+]-1[ζ]-A-1[+]-A ; -1[+]-2A-1[+]-1[ζ]-A-1[+] ; and -1[ζ]-A-1[ζ]-A-1[+] ; and

**[linker]** is selected from: -Gn- ; -Sn- ; -(GnSn)n- ; -(GnSn)nGn- ; -(GnSn)nSn- ; -(GnSn)nGn(GnSn)n- ; and -(GnSn)nSn(GnSn)n- ;

wherein:

**[Φ]** is an amino acid which is: Leu, Phe, Trp, Ile, Met, Tyr, or Val, preferably Leu, Phe, Trp, or Ile;

**[+]** is an amino acid which is: Lys or Arg;

**[ζ]** is an amino acid which is: Gln, Asn, Thr, or Ser;

**A** is the amino acid Ala;

**G** is the amino acid Gly;

**S** is the amino acid Ser; and

**n** is an integer from 1 to 20, 1 to 19, 1 to 18, 1 to 17, 1 to 16, 1 to 15, 1 to 14, 1 to 13, 1 to 12, 1 to 11, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 1 to 4, or 1 to 3.

12. The composition of any one of claims 1 to 11, wherein the shuttle agent comprises or consists of:

(i) the amino acid sequence any one of **SEQ ID NOs: 35, 1 to 34, 36 to 50, 58 to 78, 80 to 107, 109 to 139, 141 to 146, 149 to 161, 163 to 169, 171, 174 to 234, 236 to 240, 242 to 260, 262 to 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, or 353 to 364**;

(ii) an amino acid sequence that differs from any one of **SEQ ID NOs: 35, 1 to 34, 36 to 50, 58 to 78, 80 to 107, 109 to 139, 141 to 146, 149 to 161, 163 to 169, 171, 174 to 234, 236 to 240, 242 to 260, 262 to 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, or 353 to 364** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids;

(iii) an amino acid sequence that is at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any one of **SEQ ID NOs: 35, 1 to 34, 36 to 50, 58 to 78, 80 to 107, 109 to 139, 141 to 146, 149 to 161, 163 to 169, 171, 174 to 234, 236 to 240, 242 to 260, 262 to 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, or 353 to 364**;

(iv) an amino acid sequence that differs from any one of **SEQ ID NOs: 35, 1 to 34, 36 to 50, 58 to 78, 80 to 107, 109 to 139, 141 to 146, 149 to 161, 163 to 169, 171, 174 to 234, 236 to 240, 242 to 260, 262 to 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326**

**to 332, 338 to 342, 344, or 353 to 364** by only conservative amino acid substitutions, wherein each conservative amino acid substitution is selected from an amino acid within the same amino acid class, the amino acid class being: Aliphatic: G, A, V, L, and I; Hydroxyl or sulfur/selenium-containing: S, C, U, T, and M; Aromatic: F, Y, and W; Basic: H, K, and R; Acidic and their amides: D, E, N, and Q; or

(v) any combination of (i) to (iv).

13. The composition of any one of claims 1 to 12, wherein the shuttle agent comprises:

(a) at least one synthetic amino acid;

(b) a cyclic peptide;

(c) one or more D-amino acids;

(d) a chemical modification to one or more amino acids, wherein the chemical modification does not destroy the transduction activity of the synthetic peptide shuttle agent; or

(e) any combination of (a) to (d).

14. The composition of any one of claims 1 to 13, wherein the shuttle agent is as defined in (A) and does not comprise a cell penetrating domain (CPD), a cell-penetrating peptide (CPP), or a protein transduction domain (PTD).

15. The composition of any one of claims 1 to 14, wherein:

(i) said ELD is or is from: an endosomolytic peptide; an antimicrobial peptide (AMP); a linear cationic alpha-helical antimicrobial peptide; a Cecropin-A/Melittin hybrid (CM) peptide; pH-dependent membrane active peptide (PAMP); a peptide amphiphile; a peptide derived from the N terminus of the HA2 subunit of influenza hemagglutinin (HA); CM18; Diphtheria toxin T domain (DT); GALA; PEA; INF-7; LAH4; HGP; H5WYG; HA2; EB1; VSVG; *Pseudomonas* toxin; melittin; KALA; JST-1; $C(LLKK)_3C$; $G(LLKK)_3G$; or any combination thereof;

(ii) said CPD is or is from: a cell-penetrating peptide or the protein transduction domain from a cell-penetrating peptide; TAT; PTD4; Penetratin; pVEC; M918; Pep-1; Pep-2; Xentry; arginine stretch; transportan; SynB1; SynB3; or any combination thereof; or

(iii) both (i) and (ii).

16. The composition of any one of claims 1 to 15, wherein the concentration of the non-anionic polynucleotide analog cargo and/or the synthetic peptide shuttle agent in the composition is at least 2.5, 3,

3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 μM.

17. The composition of any one of claims 1 to 16, wherein the eukaryotic cells are animal cells, mammalian cells, human cells, stem cells, primary cells, immune cells, T cells, NK cells, dendritic cells, epithelial cells, skin cells, gastrointestinal cells, lung cells, or ocular cells.

18. The composition of any one of claims 1 to 17, wherein the non-anionic polynucleotide analog cargo is:

(a) a non-anionic antisense oligonucleotide targeting a gene of the Hedgehog pathway;
(b) a non-anionic antisense oligonucleotide that targets Gli1 for knockdown; or
(c) a non-anionic antisense oligonucleotide hybridizes to the polynucleotide sequence of any one of **SEQ ID NOs: 365-368**.

19. The composition as defined in any one of claims 1 to 18 for use in therapy, wherein the non-anionic polynucleotide analog cargo modulates gene expression of a therapeutically relevant target RNA in the eukaryotic cells.

20. Use of the composition as defined in any one of claims 1 to 18 in the manufacture of a medicament for treating a disease treatable by intracellular delivery of the non-anionic polynucleotide analog cargo, wherein the non-anionic polynucleotide analog cargo modulates gene expression of a therapeutically relevant target RNA in the eukaryotic cells.

21. The use of claim 20, wherein the non-anionic polynucleotide analog cargo is a non-anionic antisense oligonucleotide that targets Gli1 for knockdown and the disease is Gorlin's syndrome and/or basal cell carcinoma.

22. An *in vitro* method for modifying gene expression in eukaryotic cells, the method comprising:

(a) providing a non-anionic polynucleotide analog cargo for intracellular delivery, the non-anionic polynucleotide analog cargo being designed to hybridize to an RNA of interest in the eukaryotic cells;
(b) providing a synthetic peptide shuttle agent that is independent from, or is not covalently linked to, said non-anionic polynucleotide analog cargo;

(c) contacting the eukaryotic cells with the non-anionic polynucleotide analog cargo in the presence of the synthetic peptide shuttle agent at a concentration sufficient to increase the transduction efficiency and/or cytosolic/nuclear delivery of the non-anionic polynucleotide analog cargo, as compared to in the absence of said synthetic peptide shuttle agent,

wherein the non-anionic polynucleotide analog cargo hybridizes to the RNA of interest upon cytosolic/nuclear delivery, thereby effecting gene expression modification, and wherein the shuttle agent is as defined in any one of claims 1 or 7 to 16.

23. A synthetic peptide shuttle agent comprising or consisting of:

(i) the amino acid sequence any one of **SEQ ID NOs: 357 to 364**;

(ii) an amino acid sequence that differs from any one of **SEQ ID NOs: 357 to 364** by no more than 5, 6, 7, 8, 9, or 10 amino acids;

(iii) an amino acid sequence that is at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any one of **SEQ ID NOs: 357 to 364**;

(iv) an amino acid sequence that differs from any one of **SEQ ID NOs: 357 to 364** by only conservative amino acid substitutions, wherein each conservative amino acid substitution is selected from an amino acid within the same amino acid class, the amino acid class being: Aliphatic: G, A, V, L, and I; Hydroxyl or sulfur/selenium-containing: S, C, U, T, and M; Aromatic: F, Y, and W; Basic: H, K, and R; Acidic and their amides: D, E, N, and Q; or

(v) any combination of (i) to (iv).