CLAIMS:

1. A composition comprising a nucleoprotein cargo for intracellular delivery and a synthetic peptide shuttle agent that is independent from, or is not covalently linked to, said nucleoprotein 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 nucleoprotein cargo in eukaryotic cells as compared to in the absence of the synthetic peptide shuttle agent.

2. The composition of claim 1, wherein the nucleoprotein cargo is:

(a) a deoxyribonucleoprotein (DNP) or ribonucleoprotein (RNP) cargo;

(b) an RNA-guided nuclease, a Cas nuclease, or a variant thereof that lacking nuclease activity, a base editor, or a prime editor, a CRISPR-associated transposase, Cas-recombinase, Cpf1-RNP, or Cas9-RNP;

(c) not covalently linked or pre-complexed with a cell-penetrating peptide; or

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

~~3. The composition of claim 1 or 2, wherein the nucleoprotein cargo is an RNA-guided nuclease, a Cas nuclease, such as a Cas type I, II, III, IV, V, or VI nuclease, or a variant thereof that lacking nuclease activity, a base editor, or a prime editor, a CRISPR-associated transposase, or a Cas-recombinase (e.g., recCas9).~~

~~4. The composition of any one of claims 1 to 3, the nucleoprotein cargo is Cpf1-RNP.~~

~~5. The composition of any one of claims 1 to 3, the nucleoprotein cargo is Cas9-RNP.~~

~~6. The composition of any one of claims 1 to 5, wherein the nucleoprotein cargo is not covalently linked or pre-complexed with a cell-penetrating peptide.~~

3.~~7.~~ The composition of claim 1 or 2, ~~any one of claims 1 to 6,~~ wherein 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.

4.~~8.~~ The composition of claim 3, ~~7,~~ 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).

5.~~9.~~ The composition of claim 3 or 4, ~~7 or 8,~~ wherein said shuttle agent respects at least one, at least two, at least three, at least four, at least five, at least six, 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.

6.~~10.~~ The composition of any one of claims 1 to 5, ~~9,~~ wherein said shuttle agent comprises a histidine-rich domain, optionally 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).

7.~~11.~~ The composition of any one of claims 1 to 6, ~~10,~~ wherein said shuttle agent comprises a flexible linker domain rich in serine and/or glycine residues ~~(e.g.,~~ separating N-terminal and a C-terminal segments of the shuttle agent; or positioned N- and/or C-terminal of a central amphipathic cationic alpha helical domain~~)~~.

~~12. The composition of any one of claims 1 to 11, 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);~~

(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] is 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.~~

8.~~13.~~ The composition of any one of claims 1 to 7, ~~12,~~ wherein the shuttle agent comprises or consists of:

(i) the amino acid sequence any one of **SEQ ID NOs: 285, 1 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 284, ~~285,~~ 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, 346, 348, 352, 355, 356, 358 to 360, 362, 363, 366, 369, 370, or 379**;

(ii) an amino acid sequence that differs from any one of **SEQ ID NOs: 285, 1 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 284, ~~285,~~ 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, 346, 348, 352, 355, 356, 358 to 360, 362, 363, 366, 369, 370 or 379** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids; (e.g., excluding any linker domains);

(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: 285, 1 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 284, ~~285,~~ 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, 346, 348, 352, 355, 356, 358 to 360, 362, 363, 366, 369, 370 or 379** (e.g., calculated excluding any linker domains);

(iv) an amino acid sequence that differs from any one of **SEQ ID NOs: 285, 1 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 284, ~~285,~~ 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, 344, 346, 348, 352, 355, 356, 358 to 360, 362, 363, 366, 369, 370 or 379** by only conservative amino acid substitutions (e.g., by no more than no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions, preferably excluding any linker domains), 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).

9.~~14.~~ The composition of any one of claims 1 to 8, ~~13,~~ wherein the shuttle agent comprises or consists of:

(a) a fragment of a parent shuttle agent as defined in any one of claims 3 to 8, ~~7 to 13,~~ wherein the fragment retains cargo transduction activity and comprises an amphipathic alpha-helical motif having both a positively-charged hydrophilic outer face and a hydrophobic outer face, or

(b) a variant of a parent shuttle agent as defined in any one of claims 3 to 8, ~~7 to 13,~~ wherein the variant retains cargo transduction activity and differs (or differs only) from the parent shuttle agent by having a reduced C-terminal positive charge density relative to the parent shuttle agent (e.g., by substituting one or more cationic residues, such as K/R, with non-cationic residues, preferably non-cationic hydrophilic residues; and/or by engineering hydrophobic residues (e.g., A, V, L, I, F, or W) between two proximal cationic residues;

wherein the fragment or variant has increased resistance to inhibition by the nucleoprotein cargo and/or to the presence of DNA and/or RNA, and/or has increased transduction activity for the nucleoprotein cargo.

10.~~15.~~ The composition of claim 9, ~~14,~~ wherein:

(a) the fragment or variant comprises or consists of a C-terminal truncation of the parent shuttle agent;

(b) the fragment or variant comprises an amphipathic alpha-helical motif having both a positively-charged hydrophilic outer face and a hydrophobic outer face, which is flanked by or at least by 1, 2, 3, 4, 5, 6, 7, 8, 9, 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 non-cationic hydrophilic residues, such that the fragment or variant retains cargo transduction activity and/or has increased resistance to inhibition by the nucleoprotein cargo; or

(c) both (a) and (b).

~~16. The composition of claim 14 or 15, wherein the fragment or variant comprises an amphipathic alpha-helical motif having both a positively-charged hydrophilic outer face and a hydrophobic outer face, which is flanked by or at least by 1, 2, 3, 4, 5, 6, 7, 8, 9, 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 non-cationic hydrophilic residues, such that the fragment or variant retains cargo transduction activity and/or has increased resistance to inhibition by the nucleoprotein cargo.~~

11.~~17.~~ The composition of any one of claims 1 to 10, ~~16,~~ wherein shuttle agent comprises or consists of a peptide less than 20 amino acids in length and wherein the shuttle agent comprises a helical region comprising an amphipathic helix harboring:

- a cluster of hydrophobic amino acid residues on one side of the helix defining a hydrophobic angle of 140° to 280° in Schiffer-Edmundson's wheel representation, and
- a cluster of positively charged residues on the other side of the helix defining a positively charged angle of 40° to 160° in Schiffer-Edmundson's wheel representation.

~~18. The composition of any one of claims 1 to 17, wherein the shuttle agent comprises a helical region comprising an amphipathic helix harboring:~~

- ~~a cluster of hydrophobic amino acid residues on one side of the helix defining a hydrophobic angle of 140° to 280° in Schiffer-Edmundson's wheel representation, and~~
- ~~a cluster of positively charged residues on the other side of the helix defining a positively charged angle of 40° to 160° in Schiffer-Edmundson's wheel representation.~~

12.~~19.~~ The composition of claim 11, ~~18,~~ wherein:

(a) the hydrophobic angle is 160° to 260° or 180° to 240° in Schiffer-Edmundson's wheel representation; or

(b) the positively charged angle is 40° to 140°, 60° to 140°, or 60° to 120° in Schiffer-Edmundson's wheel representation;

(c) at least 20%, 30%, 40%, or 50% of the residues in the hydrophobic cluster are hydrophobic residues

(d) the hydrophobic residues are selected from the group consisting of:

(1) phenylalanine, isoleucine, tryptophan, leucine, valine, methionine, tyrosine, cysteine, glycine, and alanine; or

(2) phenylalanine, isoleucine, tryptophan, and leucine;

(e) at least 20%, 30%, 40%, or 50% of the residues in the positively charged cluster are positively charged residues;

(f) the positively charged residues are selected from the group consisting of lysine, arginine, and histidine;

(g) the positively charged residues are selected from the group consisting of lysine and arginine;

(h) the synthetic peptide shuttle agent is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids in length;

(i) the synthetic peptide shuttle agent has a hydrophobic moment (μH) of at least 3.0, 3.1, 3.2, 3.3, 3.4, 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, or 5.5; or

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

~~20. The composition of claim 18, wherein the hydrophobic angle is 180° to 240° in Schiffer-Edmundson's wheel representation.~~

~~21. The composition of any one of claims 18 to 20, wherein the positively charged angle is 40° to 140° in Schiffer-Edmundson's wheel representation.~~

~~22. The composition of any one of claims 18 to 20, wherein the positively charged angle is 60° to 140° in Schiffer-Edmundson's wheel representation.~~

~~23. The composition of any one of claims 18 to 20, wherein the positively charged angle is 60° to 120° in Schiffer-Edmundson's wheel representation.~~

~~24. The composition of any one of claims 18 to 23, wherein at least 20%, 30%, 40%, or 50% of the residues in the hydrophobic cluster are hydrophobic residues.~~

~~25. The composition of claim 24, wherein the hydrophobic residues are selected from the group consisting of phenylalanine, isoleucine, tryptophan, leucine, valine, methionine, tyrosine, cysteine, glycine, and alanine.~~

~~26. The composition of claim 24, wherein the hydrophobic residues are selected from the group consisting of phenylalanine, isoleucine, tryptophan, and leucine.~~

~~27. The composition of any one of claims 18 to 26, wherein at least 20%, 30%, 40%, or 50% of the residues in the positively charged cluster are positively charged residues.~~

~~28. The composition of claim 27, wherein the positively charged residues are selected from the group consisting of lysine, arginine, and histidine.~~

~~29. The composition of claim 28, wherein the positively charged residues are selected from the group consisting of lysine and arginine.~~

~~30. The composition of any one of 18 to 29, wherein the synthetic peptide shuttle agent is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids in length.~~

~~31. The composition of any one of claims 1 to 30, wherein the synthetic peptide shuttle agent has a hydrophobic moment (μH) of at least 3.0, 3.1, 3.2, 3.3, 3.4, 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, or 5.5.~~

13.~~32.~~ The composition of any one of claims 1 to 12, ~~31,~~ 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).

~~or consists of a variant of the synthetic peptide shuttle agent, the variant being identical to the synthetic peptide shuttle agent as defined in any one of claims 1 or 7 to 31, except having at least one amino acid being replaced with a corresponding synthetic amino acid having a side chain of similar physiochemical properties (e.g., structure, hydrophobicity, or charge) as the amino acid being replaced, wherein the variant increases cytosolic/nuclear delivery of said cargo in eukaryotic cells as compared to in the absence of the synthetic peptide shuttle agent, preferably wherein the synthetic amino acid replacement:~~

~~(a) replaces a basic amino acids with any one of: α-aminoglycine, α,γ-diaminobutyric acid, ornithine, α,β-diaminopropionic acid, 2,6-diamino-4-hexynoic acid, β-(1-piperazinyl)-alanine, 4,5-dehydro-lysine, δ-hydroxylysine, ω,ω-dimethylarginine, homoarginine, ω,ω'-dimethylarginine, ω-methylarginine, β-(2-quinolyl)-alanine, 4-aminopiperidine-4-carboxylic acid, α-methylhistidine, 2,5-diiodohistidine, 1-methylhistidine, 3-methylhistidine, spinacine, 4-aminophenylalanine, 3-aminotyrosine, β-(2-pyridyl)-alanine, or β-(3-pyridyl)-alanine;~~

~~(b) replaces a non-polar (hydrophobic) amino acid with any one of: dehydro-alanine, β-fluoroalanine, β-chloroalanine, β-Iodoalanine, α-aminobutyric acid, α-aminoisobutyric acid, β-cyclopropylalanine,~~

~~azetidine-2-carboxylic acid, α-allylglycine, propargylglycine, tert-butylalanine , β-(2-thiazolyl)-alanine, thiaproline, 3,4-dehydroproline, tert-butylglycine, β-cyclopentylalanine, β-cyclohexylalanine, α-methylproline, norvaline, α-methylvaline, penicillamine, β, β-dicyclohexylalanine, 4-fluoroproline, 1-aminocyclopentanecarboxylic acid, pipecolic acid, 4,5-dehydroleucine, allo-isoleucine, norleucine, α-methylleucine, cyclohexylglycine, cis-octahydroindole-2-carboxylic acid, β-(2-thienyl)-alanine, phenylglycine, α-methylphenylalanine, homophenylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, β-(3-benzothienyl)-alanine, 4-nitrophenylalanine, 4-bromophenylalanine, 4-tert-butylphenylalanine, α-methyltryptophan, β-(2-naphthyl)-alanine, β-(1-naphthyl)-alanine, 4-iodophenylalanine, 3-fluorophenylalanine, 4-fluorophenylalanine, 4-methyltryptophan, 4-chlorophenylalanine, 3,4-dichloro-phenylalanine, 2,6-difluoro-phenylalanine, n-in-methyltryptophan, 1,2,3,4-tetrahydronorharman-3-carboxylic acid, β,β-diphenylalanine, 4-methylphenylalanine, 4-phenylphenylalanine, 2,3,4,5,6-pentafluoro-phenylalanine, or 4-benzoylphenylalanine;~~

~~(c) replaces a polar, uncharged amino acid with any one of: β-cyanoalanine, β-ureidoalanine, homocysteine, allo-threonine, pyroglutamic acid, 2-oxothiazolidine-4-carboxylic acid, citrulline, thiocitrulline, homocitrulline, hydroxyproline, 3,4-dihydroxyphenylalanine, β-(1,2,4-triazol-1-yl)-alanine, 2-mercaptohistidine, β-(3,4-dihydroxyphenyl)-serine, β-(2-thienyl)-serine, 4-azidophenylalanine, 4-cyanophenylalanine, 3-hydroxymethyltyrosine, 3-iodotyrosine, 3-nitrotyrosine, 3,5-dinitrotyrosine, 3,5-dibromotyrosine, 3,5-diiodotyrosine, 7-hydroxy-1,2,3,4-tetrahydroiso-quinoline-3-carboxylic acid, 5-hydroxytryptophan, thyronine, ß-(7-methoxycoumarin-4-yl)-alanine, or 4-(7-hydroxy-4-coumarinyl)-aminobutyric acid; and/or~~

~~(d) replaces an acidic amino acid with any one of: γ-hydroxyglutamic acid, γ-methyleneglutamic acid, γ-carboxyglutamic acid, α-aminoadipic acid, 2-aminoheptanedioic acid, α-aminosuberic acid, 4-carboxyphenylalanine, cysteic acid, 4-phosphonophenylalanine, or 4-sulfomethylphenylalanine.~~

<u>14.</u>~~33.~~ The composition of any one of claims 1 to <u>13, </u>~~32,~~ wherein the shuttle agent:

- does not comprise a cell penetrating domain (CPD), a cell-penetrating peptide (CPP), or a protein transduction domain (PTD);~~ or~~
- <u>does not comprise a CPD fused to an endosome leakage domain (ELD); or</u>
- <u>comprises an endosome leakage domain (ELD) and/or a cell penetrating domain (CPD)</u>.

~~34. The composition of any one of claims 1 to 32, wherein the shuttle agent comprises an endosome leakage domain (ELD) and/or a cell penetrating domain (CPD).~~

~~35. The composition of any one of claims 33 or 34, 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).~~

~~36. The composition of any one of claims 1 to 35, wherein the shuttle agent is a cyclic peptide and/or comprises one or more D-amino acids.~~

~~37. The composition of any one of claims 1 to 36, wherein the shuttle agent increases the transduction efficiency and/or total amount of nucleoprotein cargo delivered intracellularly in the eukaryotic cells by at least 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold over a corresponding negative control lacking said shuttle agent.~~

~~38. The composition of any one of claims 1 to 37, wherein the shuttle agent further comprises 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.~~

~~39. The composition of claim 38, wherein the chemical modification is at the N and/or C terminus of the shuttle agent.~~

~~40. The composition of claim 38 or 39, wherein the chemical modification is the addition of an acetyl group (e.g., an N-terminal acetyl group), a cysteamide group (e.g., a C-terminal cysteamide group), or a fatty acid (e.g., C4-C16 fatty acid, preferably N-terminal).~~

15.~~41.~~ The composition of any one of claims 1 to 14, ~~40,~~ wherein the concentration of the nucleoprotein cargo and/or the synthetic peptide shuttle agent in the composition is at least 1, 1.5, 2, 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.

16.~~42.~~ The composition as defined in any one of claims 1 to 15:~~41.~~

(a) for use in increasing the transduction efficiency of the nucleoprotein cargo to the cytosolic/nuclear compartment of eukaryotic cells;

(b) for use in genome editing, base editing, or prime editing in eukaryotic cells;

(c) for use in modulating gene expression in the eukaryotic cells;

(d) for use in therapy, wherein the nucleoprotein cargo binds to a therapeutic target in the eukaryotic cells;

(e) for use in delivering a non-therapeutic nucleoprotein cargo as a diagnostic agent;

(f) for use in the manufacture of a medicament or diagnostic agent;

(g) for use in treating cancer (e.g., skin cancer, basal cell carcinoma, nevoid basal cell carcinoma syndrome), inflammation or an inflammation-related disease (e.g., psoriasis, atopic dermatitis, ulcerative colitis, urticaria, dry eye disease, dry or wet age-related macular degeneration, digital ulcers, actinic keratosis, idiopathic pulmonary fibrosis), pain (e.g., chronic or acute), or a disease affecting the lungs (e.g., cystic fibrosis, asthma, chronic obstructive pulmonary disease (COPD), or idiopathic pulmonary fibrosis); or

(h) any combination of (a) to (g).

17.~~43.~~ The composition of any one of claims 1 to 15, ~~41,~~ or the composition for use of claim 16, ~~42,~~ 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.~~44.~~ An *in vitro* method for the use as defined in claim 16, ~~42,~~ the method comprising:

(a) providing a nucleoprotein cargo for intracellular delivery in a population of eukaryotic cells;

(b) providing a synthetic peptide shuttle agent that is independent from, or is not covalently linked to, said nucleoprotein cargo;

(c) contacting the eukaryotic cells with the nucleoprotein 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 nucleoprotein cargo, as compared to in the absence of said synthetic peptide shuttle agent,

wherein the nucleoprotein cargo binds to an intracellular target, thereby effecting said use.

~~45. The method of claim 44, which is an *in vitro* method (e.g., for a therapeutic and/or diagnostic purpose).~~

~~46. The method of claim 44, which is an *in vivo* method (e.g., for therapeutic and/or diagnostic purpose).~~

19.~~47.~~ The method of claim 18, ~~any one of claims 44 to 46,~~ wherein:

(i) the nucleoprotein cargo is as defined in claim 1 or 2;~~any one of claims 1 to 6;~~

(ii) the synthetic peptide shuttle agent is as defined in any one of claims 1 or 3 to 14;~~1 or 7 to 23;~~

(iii) the eukaryotic cells are contacted a concentration of the cargo and/or the synthetic peptide shuttle agent as defined in claim 15;~~24;~~

(iv) the method is for a use as defined in claim 16;~~25;~~

(v) the eukaryotic cells are as defined in claim 17;~~26;~~ or

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

20.~~48.~~ An *in vitro* method for cargo transduction, the method comprising contacting target eukaryotic cells with said cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of said cargo, as compared to in the absence of said synthetic peptide shuttle agent, wherein said synthetic peptide shuttle agent is a peptide less than 20 amino acids in length, and wherein the shuttle agent and cargo are not covalently bound at the time of transduction across the plasma membrane.

21.~~49.~~ The method of claim 20, ~~48,~~ wherein:

(a) the synthetic peptide shuttle agent is as defined in any one of claims 11 or 12;

(b) the cargo is a polypeptide, peptide, a nucleoprotein as defined in claim 1 or 2, small molecule, oligonucleotide, or oligonucleotide analog (e.g., non-anionic oligonucleotide analog); or

(c) both (a) and (b).~~comprises a helical region comprising an amphipathic helix harboring:~~

~~a cluster of hydrophobic amino acid residues on one side of the helix defining a hydrophobic angle of 140° to 280° in Schiffer-Edmundson's wheel representation, and~~

~~a cluster of positively charged residues on the other side of the helix defining a positively charged angle of 40° to 160° in Schiffer-Edmundson's wheel representation.~~

~~50. The method of claim 49, wherein the hydrophobic angle is 160° to 260° in Schiffer-Edmundson's wheel representation.~~

~~51. The method of claim 49, wherein the hydrophobic angle is 180° to 240° in Schiffer-Edmundson's wheel representation.~~

~~52. The method of any one of claims 49 to 51, wherein the positively charged angle is 40° to 140° in Schiffer-Edmundson's wheel representation.~~

~~53. The method of any one of claims 49 to 51, wherein the positively charged angle is 60° to 140° in Schiffer-Edmundson's wheel representation.~~

~~54. The method of any one of claims 49 to 51, wherein the positively charged angle is 60° to 120° in Schiffer-Edmundson's wheel representation.~~

~~55. The method of any one of claims 49 to 54, wherein at least 20%, 30%, 40%, or 50% of the residues in the hydrophobic cluster are hydrophobic residues.~~

~~56. The method of claim 55, wherein the hydrophobic residues are selected from the group consisting of phenylalanine, isoleucine, tryptophan, leucine, valine, methionine, tyrosine, cysteine, glycine, and alanine.~~

~~57. The method of claim 55, wherein the hydrophobic residues are selected from the group consisting of phenylalanine, isoleucine, tryptophan, and/or leucine.~~

~~58. The method of any one of claims 49 to 57, wherein at least 20%, 30%, 40%, or 50% of the residues in the positively charged cluster are positively charged residues.~~

~~59. The method of any one of claims 49 to 58, wherein the positively charged residues are selected from the group consisting of lysine, arginine, and histidine.~~

~~60. The method of any one of claims 49 to 58, wherein the positively charged residues are selected from the group consisting of lysine and arginine.~~

~~61. The method of any one of claims 49 to 60, wherein the synthetic peptide shuttle agent is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids in length.~~

~~62. The method of any one of claims 49 to 61, wherein the synthetic peptide shuttle agent has a hydrophobic moment (μH) of at least 3.0, 3.1, 3.2, 3.3, 3.4, 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, or 5.5.~~

~~63. The method of any one of claims 49 to 62, wherein the cargo is a polypeptide, peptide, nucleoprotein (e.g., as defined in any one of claims 1 to 6), small molecule, oligonucleotide, or oligonucleotide analog (e.g., non-anionic oligonucleotide analog).~~

22.~~64.~~ A synthetic peptide which is the shuttle agent as defined in any one of claims 1 to 21.~~63.~~

~~65. The synthetic peptide of claim 64 for use in therapy and/or in cargo transduction (e.g., polypeptide, peptide, nucleoprotein (e.g., as defined in any one of claims 1 to 6), small molecule, oligonucleotide, or oligonucleotide analog (e.g., non-anionic oligonucleotide analog) in eukaryotic cells, wherein the shuttle agent is used at concentration sufficient to increase the transduction efficiency of said cargo, as compared to in the absence of said synthetic peptide shuttle agent, and wherein the synthetic peptide shuttle agent and cargo are not covalently bound.~~

~~66. Use of the synthetic peptide shuttle agent as defined in any one of claims 1 to 63 for the manufacture of a medicament (e.g., for treating a disease as defined in claim 42).~~

~~67. A composition comprising the synthetic peptide shuttle agent of claim 64 and a suitable excipient.~~

23.~~68.~~ A synthetic peptide shuttle agent for use in~~, or suitable for use in,~~ the delivery of non-anionic cargoes across mucus-producing membranes, ~~(e.g., airway epithelium),~~ the synthetic peptide shuttle agent comprising or consisting essentially of a central core amphipathic alpha helical region having shuttle agent activity, flanked N- and C-terminally by flexible linker domains, wherein one or both of the flexible linker domains comprises or consists essentially of a sufficient number of non-cationic hydrophilic residues such that cargo transduction activity across mucus-producing membranes of the synthetic peptide shuttle agent is increased relative to that of the central core amphipathic alpha helical region lacking the flexible linker domains.

24.~~69.~~ The synthetic peptide shuttle agent of claim 23, ~~68,~~ wherein the central core amphipathic alpha helical region:

(a) is an endosomolytic peptide;

(b) is at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids in length;

(c) is a fragment of a parent shuttle agent as defined in claim 9(a) or 10;~~14(a) or 15;~~

(d) is an amphipathic helix as defined in claim 11 or 12;~~any one of claims 18 to 29 or 49 to 60;~~

(e) has a hydrophobic moment (μH) of at least 3.0, 3.1, 3.2, 3.3, 3.4, 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, or 5.5; or

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

25.~~70.~~ The synthetic peptide shuttle agent of claim 23 or 24, ~~68 or 69,~~ wherein the non-cationic hydrophilic residues comprise or consist essentially of glycine, serine, aspartate, glutamate, histidine, tyrosine, threonine, cysteine, asparagine, glutamine, or any combination thereof.

~~71. The synthetic peptide shuttle agent of any one of claims 68 to 70, wherein the flexible linker domain is as defined in claim 12.~~

~~72. The synthetic peptide shuttle agent of any one of claims 68 to 71 for the use as defined in claim 42 or 43.~~