PEPTIDE-BASED NON-PROTEINACEOUS CARGO DELIVERY

The present description relates to the intracellular delivery of non-proteinaceous cargoes. More specifically, the present description relates to the use of synthetic peptide shuttle agents for the intracellular delivery of small molecules and other non-proteinaceous cargoes, as well as improved synthetic peptide shuttle agents having transduction activity for both proteins and small molecules.

The present description refers to a number of documents, the contents of which are herein incorporated by reference in their entirety.

BACKGROUND

Most drugs have traditionally been small molecule organic compounds that are sufficiently small and lipophilic to pass through cellular membranes to engage intracellular targets. During conventional drug discovery processes, small molecule drug candidates are routinely selected based not only on their affinity for their biological targets, but also on their drug-like physicochemical properties that, amongst other things, govern their ability to be delivered intracellularly and reach their biological targets. Thus, under conventional drug development ideologies, compounds identified in large-scale screening efforts as showing high target binding affinity and specificity may be ultimately discarded as clinical drug candidates because of their diminished ability to be delivered intracellularly. Furthermore, even cell membrane-permeable compounds may benefit from improved intracellular/cytosolic delivery, for example to increase speed of uptake and/or reduce the concentration administered to obtain the desired biological effect. There is therefore a need for technologies that can facilitate the intracellular/cytosolic delivery of small molecule cargoes to provide greater flexibility in terms of drug design and perhaps open the door for the use of novel therapeutic compounds that may otherwise have been disregarded based on traditional small molecule drug design.

SUMMARY

Synthetic peptide shuttle agents represent a recently defined family of peptides previously reported to quickly and efficiently transduce proteinaceous cargoes to the cytosol and/or nucleus of a wide variety of target eukaryotic cells. The first generation of such peptide shuttle agents were described in WO/2016/161516, wherein the peptide shuttle agents comprise an endosome leakage domain (ELD) operably linked to a cell penetrating domain (CPD). WO/2018/068135 subsequently described further synthetic peptide shuttle agents rationally-designed based on a set of fifteen design parameters for the purpose of improving the transduction of proteinaceous cargoes, while reducing toxicity of the first generation peptide shuttle agents. The present disclosure relates to the discovery that such synthetic peptide shuttle agents, previously reported to transduce large proteinaceous cargoes, also generally have

the ability of quickly and efficiently transducing smaller, non-proteinaceous cargoes (e.g., small molecule organic compounds). The experimental results presented in **Example 2** show that synthetic peptide shuttle agents, including representative members of the shuttle agents described in WO/2016/161516 and WO/2018/068135, as well as additional rationally-designed shuttle agents, are able to transduce the membrane impermeable fluorescent dye propidium iodide (PI), which can be considered as a small molecule organic compound cargo. Strikingly, negative control peptides that fail to respect key rational-design parameters described in WO/2018/068135 for the delivery of proteinaceous cargoes also failed to transduce PI, suggesting that the rational-design parameters of WO/2018/068135 for proteinaceous cargo delivery may also generally apply to the design of peptide shuttle agents for the delivery of non-proteinaceous cargoes. In **Example 3**, it is shown that a representative synthetic peptide shuttle agent not only enables intracellular delivery of structurally unrelated small molecule inhibitors of the HedgeHog signalling pathway into cultured cells, but that the delivered inhibitors are free to bind to their intracellular targets and exert their inhibitory activity. In **Example 4**, it is shown that a representative synthetic peptide shuttle agent enables *in vivo* delivery and activity of small molecule inhibitors of HedgeHog signalling following topical application in shaved mice. In **Example 5**, it is shown that a different representative synthetic peptide shuttle agent enables intracellular delivery of a membrane-impermeable small molecule compound that is a sodium channel inhibitor (QX-314), resulting in an associated reduction in evoked current amplitudes as measured by patch-clamping. Finally, **Examples 6 and 7** show the results of a large-scale screening of over 300 candidate peptide shuttle agents for PI and GFP-NLS transduction activity, and reveal a striking correlation between PI transduction efficiency and GFP-NLS transduction efficiency, suggesting that robust PI transduction predicts shuttle agents having proteinaceous cargo transduction activity.

In some aspects, described herein is a method for non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with a non-proteinaceous cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of said non-proteinaceous cargo, as compared to in the absence of said synthetic peptide shuttle agent.

In some aspects, described herein is a composition for use in transducing a non-proteinaceous cargo into target eukaryotic cells, the composition comprising a synthetic peptide shuttle agent formulated with a pharmaceutically suitable excipient, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the non-proteinaceous cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

In some aspects, describe herein is a composition for use in therapy, the composition comprising a synthetic peptide shuttle agent formulated with a non-proteinaceous cargo (e.g., therapeutically or

biologically active non-proteinaceous cargo) to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the non-proteinaceous cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

In some aspects, described herein is a synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes, the shuttle agent comprising or consisting of the amino acid sequence any one of **SEQ ID NOs: 1 to 50**. In some aspects, described herein is a synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes, the shuttle agent comprising or consisting of an amino acid sequence that differs from any one of **SEQ ID NOs: 1 to 50** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., excluding any linker domains, such as flexible serine/glycine-rich linker domains). In some aspects, described herein is a synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes, the shuttle agent comprising or consisting of 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: 1 to 50** (e.g., calculated excluding any linker domains, such as flexible serine/glycine-rich linker domains).

In some aspects, described herein is a synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, the shuttle agent being:

(1) a peptide at least 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 +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,

wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

In some aspects, described herein is a synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, wherein the shuttle agent comprises or consists of: (a) the amino acid sequence any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344**; or (b) an amino acid sequence that differs from (a) 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, such as flexible serine/glycine-rich linker domains), wherein the shuttle agent: increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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; and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

In some aspects, described herein is a synthetic peptide shuttle agent having proteinaceous cargo transduction activity in target eukaryotic cells, wherein the shuttle agent comprises or consists of: (a) the amino acid sequence any one of **SEQ ID NOs: 52, 57, 79, 108, 140, 147, 148, 173, 241, 261, 286, 295, 301, 309, 312, 325, 333-337, or 343**; or (b) an amino acid sequence that differs from (a) 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, such as flexible serine/glycine-rich linker domains), wherein the shuttle agent: increases the transduction efficiency of GFP-NLS 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, and/or enables a transduction efficiency of at least 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% (e.g., as determined by flow cytometry) of GFP-NLS in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

In some aspects, described herein is a synthetic peptide shuttle agent variant having transduction activity for proteinaceous and/or non-proteinaceous cargoes in target eukaryotic cells, the synthetic peptide shuttle agent variant being identical to any one of the synthetic peptide shuttle agents as defined herein, 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 shuttle agent variant increases the transduction efficiency of said cargo in target eukaryotic cells, as compared to in the absence of the shuttle agent variant.

In some aspects, described herein is an *in vitro* or *in vivo* method for proteinaceous and/or non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with the cargo and a concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined herein sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of said synthetic peptide shuttle agent.

In some aspects, described herein is a composition for use in therapy, the composition comprising the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined herein formulated with a proteinaceous and/or non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant in the composition is sufficient to increase the transduction

efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

In some aspects, described herein is a kit comprising the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined herein, and a proteinaceous and/or non-proteinaceous cargo to be transduced by the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant.

In some aspects, described herein a process for producing a candidate synthetic peptide shuttle agent expected to have transduction activity for a cargo of interest in target eukaryotic cells, the method comprising synthesizing a peptide which is: (1) a peptide at least 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 parameters (4) to (15) defined herein are respected, wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

In some aspects, described herein an *in vitro* or *in vivo* method for identifying, qualifying, or selecting a synthetic peptide shuttle agent expected to have transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, the method comprising: providing model eukaryotic cells or a model organism suitable for assessing cargo transduction in the target eukaryotic cells; providing a candidate synthetic peptide shuttle agent (e.g., as defined herein); and measuring the transduction activity (e.g., transduction efficiency, such as by flow cytometry) of the candidate synthetic peptide shuttle agent to transduce propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent into the model eukaryotic cells or model organism, wherein the candidate shuttle agent is expected to have transduction activity for both proteinaceous and non-proteinaceous cargoes in the target eukaryotic cells when the transduction activity (e.g., transduction efficiency) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent is increased 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 the candidate synthetic peptide shuttle agent, and/or a transduction efficiency of at least 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%, 50%,

51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of the propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent occurs, in the model eukaryotic cells or model organism.

General Definitions

Headings, and other identifiers, e.g., (a), (b), (i), (ii), etc., are presented merely for ease of reading the specification and claims. The use of headings or other identifiers in the specification or claims does not necessarily require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

The use of the word "**a**" or "**an**" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one" but it is also consistent with the meaning of "one or more", "at least one", and "one or more than one".

The term "**about**" is used to indicate that a value includes the standard deviation of error for the device or method being employed in order to determine the value. In general, the terminology "about" is meant to designate a possible variation of up to 10%. Therefore, a variation of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% of a value is included in the term "about". Unless indicated otherwise, use of the term "about" before a range applies to both ends of the range.

As used in this specification and claim(s), the words "**comprising**" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

As used herein, "**protein**" or "**polypeptide**" or "**peptide**" means any peptide-linked chain of amino acids, which may or may not comprise any type of modification (e.g., chemical or post-translational modifications such as acetylation, phosphorylation, glycosylation, sulfatation, sumoylation, prenylation, ubiquitination, etc.). For further clarity, protein/polypeptide/peptide modifications are envisaged so long as the modification does not destroy the cargo transduction activity of the shuttle agents described herein. For example, shuttle agents described herein may be linear or circular, may be synthesized with one or more D- or L-amino acids, and/or may be conjugated to a fatty acid (e.g., at their N terminus). Shuttle agents described herein may also have 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.

As used herein, a "**domain**" or "**protein domain**" generally refers to a part of a protein having a particular functionality or function. Some domains conserve their function when separated from the rest of the

protein, and thus can be used in a modular fashion. The modular characteristic of many protein domains can provide flexibility in terms of their placement within the shuttle agents of the present description. However, some domains may perform better when engineered at certain positions of the shuttle agent (e.g., at the N- or C-terminal region, or therebetween). The position of the domain within its endogenous protein is sometimes an indicator of where the domain should be engineered within the shuttle agent and of what type/length of linker should be used. Standard recombinant DNA techniques can be used by the skilled person to manipulate the placement and/or number of the domains within the shuttle agents of the present description in view of the present disclosure. Furthermore, assays disclosed herein, as well as others known in the art, can be used to assess the functionality of each of the domains within the context of the shuttle agents (e.g., their ability to facilitate cell penetration across the plasma membrane, endosome escape, and/or access to the cytosol). Standard methods can also be used to assess whether the domains of the shuttle agent affect the activity of the cargo to be delivered intracellularly. In this regard, the expression "**operably linked**" as used herein refers to the ability of the domains to carry out their intended function(s) (e.g., cell penetration, endosome escape, and/or subcellular targeting) within the context of the shuttle agents of the present description. For greater clarity, the expression "operably linked" is meant to define a functional connection between two or more domains without being limited to a particular order or distance between same.

As used herein, the term "**synthetic**" used in expressions such as "synthetic peptide", synthetic peptide shuttle agent", or "synthetic polypeptide" is intended to refer to non-naturally occurring molecules that can be produced *in vitro* (e.g., synthesized chemically and/or produced using recombinant DNA technology). The purities of various synthetic preparations may be assessed by, for example, high-performance liquid chromatography analysis and mass spectroscopy. Chemical synthesis approaches may be advantageous over cellular expression systems (e.g., yeast or bacteria protein expression systems), as they may preclude the need for extensive recombinant protein purification steps (e.g., required for clinical use). In contrast, longer synthetic polypeptides may be more complicated and/or costly to produce via chemical synthesis approaches and such polypeptides may be more advantageously produced using cellular expression systems. In some embodiments, the peptides or shuttle agents of the present description may be chemically synthesized (e.g., solid- or liquid phase peptide synthesis), as opposed to expressed from a recombinant host cell. In some embodiments, the peptides or shuttle agent of the present description may lack an N-terminal methionine residue. A person of skill in the art may adapt a synthetic peptide or shuttle agent of the present description by using one or more modified amino acids (e.g., non-naturally-occurring amino acids), or by chemically modifying the synthetic peptide or shuttle agent of the present description, to suit particular needs of stability or other needs.

As used herein, the term "**independent**" is generally intended refer to molecules or agents which are not covalently bound to one another. For example, the expression "independent cargo" is intended to

refer to a cargo to be delivered intracellularly (transduced) that is not covalently bound (e.g., not fused) to a shuttle agent of the present description. In some aspects, having shuttle agents that are independent of (not fused to) a cargo may be advantageous by providing increased shuttle agent versatility – e.g., being able to readily vary the ratio of shuttle agent to cargo (as opposed to being limited to a fixed ratio in the case of a covalent linkage between the shuttle agent and cargo).

As used herein, the expression "**is or is from**" or "**is from**" comprises functional variants of a given protein domain (e.g., CPD or ELD), such as conservative amino acid substitutions, deletions, modifications, as well as variants or function derivatives, which do not abrogate the activity of the protein domain.

Other objects, advantages and features of the present description will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the appended drawings:

Fig. 1A-1D show delivery and viability results of HeLa cells co-incubated for 1 minute with different categories of synthetic peptide shuttle agents combined with a non-proteinaceous cargo (propidium iodide, PI; Fig. 1A and1B) or a proteinaceous cargo (GFP-NLS protein; Fig. 1C and 1D). Results were acquired by flow cytometry two hours after cargo delivery and expressed as percentages of fluorescent cells (% PI+ cells or % GFP+ cells). Categories of peptides shown (from left to right): Synthetic peptide shuttle agents comprising an endosome leakage domain (ELD) operably linked to a cell penetrating domain (CPD) described in WO/2016/161516; Rationally-designed synthetic peptide shuttle agents described in WO/2018/068135; additional rationally-designed synthetic peptide shuttle agents described herein; Cyclic peptides described herein; and Negative control peptides that fail to respect several rational-design parameters set forth in WO/2018/068135. **In Fig. 1A**, "FS then PI" indicates that PI was added 1 hour after the treatment with the synthetic peptide shuttle agents, ensuring that PI-positive signal is not due to cell death. "Negative control" are cells incubated with cargo alone ("PI" in **Fig. 1A and 1B** or "GFP-NLS" in **Fig. 1C and 1D**), or untreated cells that were not exposed to the cargo or peptide shuttle agents ("NT", **Fig. 1A-1D**).

Fig. 2 is a table summarizing the results in Fig. 1A-1D.

Fig. 3 shows the activity of small molecule inhibitors of HedgeHog signalling (Gant61, HPI-4, Itraconazole, or ATO) transduced into NIH3T3 Gli-luciferase reporter cells by the peptide shuttle agent FSD250D. Successful small molecule transduction in the presence of the peptide shuttle agent ("+ FSD250D"; **SEQ ID NO: 36**) resulted in reduced luminescence intensity of the NIH3T3 Gli-

luciferase reporter cells stimulated with recombinant mouse Sonic HedgeHog protein (+ mShh), as compared to in the absence of the peptide shuttle agent ("- FSD250D").

Fig. 4 shows the successful *in vivo* transduction of small molecule inhibitors of HedgeHog signalling (Gant61 and Itraconazole) in skin cells of shaved mice by the peptide shuttle agent FSD250D. Depilation of mouse skin induces hair growth associated with a strong induction of the HedgeHog pathway. This experiment consisted of activating the HedgeHog pathway in mice by depilation, and then measuring the delay in hair regrowth by delivering in the skin cells small molecule HedgeHog pathway inhibitors (Gant61 or Itraconazole) that bind to intracellular targets. The results show that mice treated with the small molecule HedgeHog inhibitors Gant61 or Itraconazole in the presence of FSD250D ("FSD250D+Gant61 100 μM" and "FSD250D+Itraconazole 100 μM") showed delayed hair regrowth at 10 days post-treatment (*), as compared to in the absence of FSD250D ("Gant61 100 μM" and "Itraconazole 100 μM"), or in the presence of the shuttle peptide alone ("FSD250D").

Fig. 5A-5C shows representative patch-clamp electrophysiology whole-cell current traces of HEK293 cells stably expressing the sodium channel Nav1.7 upon exposure to the membrane impermeable sodium channel inhibitor QX-314 with or without FSD194. Reduction of the current amplitude was observed when cells were transiently exposed to QX-314 and GFP-NLS in the presence of FSD194 (i.e., 1 mM QX-314 + 15 μM GFP-NLS + 5 μM FSD194), consistent with the presence of QX-314 inside the cells (**Fig. 5C**). This same current amplitude reduction was not observed in the absence of QX-314 (i.e., 15 μM GFP-NLS + 5 μM FSD194 +; **Fig. 2A**) or in the absence of FSD194 (i.e., 2.5 mM QX-314 + 15 μM GFP-NLS; **Fig. 2B**). Furthermore, GFP-NLS-positive cells were identified in the QX-314 + GFP-NLS + FSD194 and in the FSD194 + GFP-NLS conditions, but not in the QX-314 + GFP-NLS conditions, indicating that GFP-NLS was indeed co-transduced along with the QX-314 by the peptide shuttle agent.

Fig. 6 and **Fig. 7** show the results of a large-scale screening of over 300 candidate peptide shuttle agents for PI and GFP-NLS transduction activity. **Fig. 6** shows results of all candidate peptide shuttle agents screened that had a mean PI transduction efficiency of 10% or higher, sorted based on their level of mean PI transduction efficiency. **Fig. 7** shows results of all candidate peptide shuttle agents screened that had a mean PI transduction efficiency of under 10% and a mean GFP-NLS transduction efficiency of at least 7%, sorted based on their level of mean GFP-NLS transduction efficiency.

SEQUENCE LISTING

This application contains a Sequence Listing in computer readable form created April 15, 2020 having a size of about 122 kb. The computer readable form is incorporated herein by reference.

SEQ ID NO:	Description
1	CM18-Penetratin-cys
2	TAT-KALA
3	His-CM18-PTD4
4	His-LAH4-PTD4
5	PTD4-KALA
6	EB1-PTD4
7	His-CM18-PTD4-6Cys
8	CM18-PTD4
9	CM18-PTD4-6His
10	His-CM18-PTD4-His
11	TAT-CM18
12	FSD5
13	FSD10
14	FSD12
15	FSD18
16	FSD19
17	FSD21
18	FSD23
19	FSD120*
20	FSD127*
21	FSD129*
22	FSD131*
23	FSD134*
24	FSD146*
25	FSD155*
26	FSD156*
27	FSD157*
28	FSD159*
29	FSD162*
30	FSD168*
31	FSD173*
32	FSD174*
33	FSD194*
34	FSD220*
35	FSD250*
36	FSD250D*
37	FSD253*
38	FSD258*
39	FSD262*
40	FSD263*
41	FSD264*
42	FSD265*
43	FSD268*
44	FSD286*
45	FSD271*
46	FSD272*
47	FSD273*
48	FSD276*
49	FSD268 Cyclic Amide*
50	FSD268 Cyclic Disulfide*
51	FSD10 Scramble
52	FSD268 Scramble*
53	FSD174 Scramble*
54	FSN3
55	FSN4
56	FSN7
57	FSN8
58	FSD117
59	FSD118
60	FSD119
61	FSD121
62	FSD122
63	FSD123
64	FSD124
65	FSD125
66	FSD126
67	FSD127
68	FSD128
69	FSD130
70	FSD132
71	FSD133
72	FSD135
73	FSD137
74	FSD138
75	FSD139
76	FSD140
77	FSD141
78	FSD142
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140	FSD214
141	FSD215
142	FSD216
143	FSD217
144	FSD218
145	FSD219
146	FSD221
147	FSD222
148	FSD223
149	FSD224
150	FSD225
151	FSD226
152	FSD227

153	FSD228
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155	FSD230
156	FSD231
157	FSD232
158	FSD233
159	FSD234
160	FSD235
161	FSD236
162	FSD237
163	FSD238
164	FSD239
165	FSD240
166	FSD241
167	FSD243
168	FSD244
169	FSD246
170	FSD247
171	FSD248
172	FSD250 Scramble
173	FSD250E
174	FSD251
175	FSD254
176	FSD255
177	FSD256
178	FSD257
179	FSD259
180	FSD260
181	FSD261
182	FSD266
183	FSD267
184	FSD269
185	FSD270
186	FSD274
187	FSD275
188	FSD276
189	FSD277
190	FSD278
191	FSD279
192	FSD280
193	FSD281
194	FSD282
195	FSD283
196	FSD284
197	FSD285
198	FSD287
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323	FSD415
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334	FSD427
335	FSD428
336	FSD429
337	FSD430
338	FSD431
339	FSD432
340	FSD433
341	FSD434
342	FSD435
343	FSD436
344	FSD438

* Peptide names changed from those used in CA 3,040,645.

DETAILED DESCRIPTION

In some aspects, described herein are methods for non-proteinaceous and/or proteinaceous cargo transduction. The methods generally comprise contacting target eukaryotic cells with a non-proteinaceous and/or proteinaceous cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of the cargo, as compared to in the absence of the synthetic peptide shuttle agent. Also described herein are versatile synthetic peptide shuttle agents having dual transduction activity for both proteinaceous and non-proteinaceous cargoes, as well as the use of PI or other membrane-impermeable fluorescent DNA intercalating agent as a "surrogate" cargo for selecting synthetic peptide shuttle agents having such dual transduction activity.

Non-proteinaceous cargoes

In some embodiments, the non-proteinaceous cargo may be a compound (e.g., organic compound) having a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da. In some embodiments, the non-proteinaceous cargo may be a compound (e.g., organic compound) having a molecular weight of between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da. In some embodiments, the non-proteinaceous cargo may be a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target. In some embodiments, the non-proteinaceous cargo is not a biopolymer, such as a polynucleotide or a polysaccharide, particularly a biopolymer having a uniform negative charge such as a polynucleotide greater than 50, 60, 70, 80, 90, 100, 150, or 200 nucleotides in length. In some embodiments, the non-proteinaceous cargo may have a cationic net charge in aqueous solution. In some embodiments, the non-proteinaceous cargo is not covalently bound to (i.e., is independent from) the synthetic peptide shuttle agent (e.g., at the moment of transduction).

In some embodiments, the non-proteinaceous cargo may be a cargo that is cell membrane-impermeable or that has low membrane permeability (e.g., due to the physicochemical properties of the cargo precluding it from freely diffusing across the cell membrane), wherein the peptide shuttle agents described herein facilitate or

increase its intracellular delivery and/or access to the cytosol. In some embodiments, the non-proteinaceous cargo may be a cargo that is cell membrane-permeable, wherein peptide shuttle agents described herein nevertheless increase its intracellular delivery and/or access to the cytosol. In some embodiments, peptide shuttle agents described herein may reduce the amount or concentration of the cargo that is required to be administered to achieve its intended biological effect, as compared to administration of the cargo alone.

In some embodiments, the non-proteinaceous cargo to be transduced may be a drug for treating any disease or condition having an intracellular biological or therapeutic target. In some embodiments, the non-proteinaceous cargo may be a drug for 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).

In particular embodiments, the non-proteinaceous cargo to be transduced may be or comprise a HedgeHog inhibitor (e.g., itraconazole, posaconazole, arsenic trioxide (ATO), Gant61, PF-4708671, HPI-1, HPI-4). In particular embodiments, the non-proteinaceous cargo to be transduced may be or comprise a pain inhibitor, such as a voltage-gated sodium (Nav) channel inhibitor (e.g., QX-314). In particular embodiments, the non-proteinaceous cargo to be transduced may be or comprise an inhibitor of inflammation, such as an inhibitor of a pathway leading to production of inflammatory cytokines (e.g., an NF-kappa B pathway inhibitor).

In some embodiments, the shuttle agents described herein may possess the ability to transduce both non-proteinaceous and proteinaceous cargoes to the cytosol of target eukaryotic cells.

Rational design parameters and peptide shuttle agents

In some aspects, the shuttle agents described herein may be a peptide having transduction activity for proteinaceous cargoes, non-proteinaceous cargoes, or both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells. In some embodiments, the shuttle agents described herein preferably satisfy one or more of the following fifteen rational design parameters.

(1) In some embodiments, the shuttle agent is a peptide at least 17, 18, 19, or 20 amino acids in length. For example, the peptide may comprise a minimum length of 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acid residues, and a maximum length of 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 amino acid residues. In some embodiments, shorter peptides (e.g., in the 17-50 or 20-50 amino acid range) may be particularly advantageous because they may be more easily synthesized and purified by chemical synthesis approaches, which may be more suitable for clinical use (as opposed to recombinant proteins that must be purified from cellular expression systems). While numbers and ranges in the present description are often listed as multiples of 5, the present description should not be so limited. For example, the maximum length described herein should be understood as also encompassing a

length of 56, 57, 58…61, 62, etc., in the present description, and that their non-listing herein is only for the sake of brevity. The same reasoning applies to the % of identities listed herein.

(2) In some embodiments, the peptide shuttle agent comprises an amphipathic alpha-helical motif. As used herein, the expression "**alpha-helical motif**" or "**alpha-helix**", unless otherwise specified, refers to a right-handed coiled or spiral conformation (helix) having angle of rotation between consecutive amino acids of 100 degrees and/or an alpha-helix having 3.6 residues per turn. As used herein, the expression "**comprises an alpha-helical motif**" or "an amphipathic alpha-helical motif" and the like, refers to the three-dimensional conformation that a peptide (or segment of a peptide) of the present description is predicted to adopt when in a biological setting based on the peptide's primary amino acid sequence, regardless of whether the peptide actually adopts that conformation when used in cells as a shuttle agent. Furthermore, the peptides of the present description may comprise one or more alpha-helical motifs in different locations of the peptide. For example, the shuttle agent FSD5 in WO/2018/068135 is predicted to adopt an alpha-helix over the entirety of its length (see Figure 49C of WO/2018/068135), while the shuttle agent FSD18 of WO/2018/068135 is predicted to comprise two separate alpha-helices towards the N and C terminal regions of the peptide (see Figure 49D of WO/2018/068135). In some embodiments, the shuttle agents of the present description are not predicted to comprise a beta-sheet motif, for example as shown in Figures 49E and 49F of WO/2018/068135. Methods of predicting the presence of alpha-helices and beta-sheets in proteins and peptides are well known in the art. For example, one such method is based on 3D modeling using PEP-FOLD™, an online resource for de novo peptide structure prediction (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) (Lamiable et al., 2016; Shen et al., 2014; Thévenet et al., 2012). Other methods of predicting the presence of alpha-helices in peptides and protein are known and readily available to the skilled person.

As used herein, the expression "**amphipathic**" refers to a peptide that possesses both hydrophobic and hydrophilic elements (e.g., based on the side chains of the amino acids that comprise the peptide). For example, the expression "**amphipathic alpha helix**" or "**amphipathic alpha-helical motif**" refers to a peptide predicted to adopt an alpha-helical motif having a non-polar hydrophobic face and a polar hydrophilic face, based on the properties of the side chains of the amino acids that form the helix.

(3) In some embodiments, peptide shuttle agents of the present description comprise an amphipathic alpha-helical motif having a positively-charged hydrophilic outer face, such as one that is rich in R and/or K residues. As used herein, the expression "**positively-charged hydrophilic outer face**" refers to the presence of at least three lysine (K) and/or arginine (R) residues clustered to one side of the amphipathic alpha-helical motif, based on alpha-helical wheel projection (e.g., see Figure 49A, left panel of WO/2018/068135). Such helical wheel projections may be prepared using a variety of programs, such as the online helical wheel projection tool available at: http://rzlab.ucr.edu/scripts/wheel/wheel.cgi. In some embodiments, the amphipathic alpha-helical motif may comprise a positively-charged hydrophilic outer face that comprises: (a) at least two, three, or four adjacent positively-charged K and/or R residues upon helical wheel projection; and/or (b) 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.

In some embodiments, peptide shuttle agents of the present description comprise an amphipathic alpha-helical motif comprising a hydrophobic outer face, the hydrophobic outer face comprising: (a) at least two adjacent L residues upon helical wheel projection; and/or (b) 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.

(4) In some embodiments, peptide shuttle agents of the present description comprise an amphipathic alpha-helical motif having a highly hydrophobic core composed of spatially adjacent highly hydrophobic residues (e.g., L, I, F, V, W, and/or M). In some embodiments, the highly hydrophobic core may consist of spatially adjacent L, I, F, V, W, and/or M amino acids representing 12 to 50% of the amino acids of the peptide, calculated while excluding any histidine-rich domains (see below), based on an open cylindrical representation of the alpha-helix having 3.6 residues per turn, as shown for example in Figure 49A, right panel of WO/2018/068135. In some embodiments, the highly hydrophobic core may consist 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 peptide. More particularly, highly hydrophobic core parameter may be calculated by first arranging the amino acids of the peptide in an opened cylindrical representation, and then delineating an area of contiguous highly hydrophobic residues (L, I, F, V, W, M), as shown in Figure 49A, right panel of WO/2018/068135. The number of highly hydrophobic residues comprised in this delineated highly hydrophobic core is then divided by the total amino acid length of the peptide, excluding any histidine-rich domains (e.g., N- and/or C-terminal histidine-rich domains). For example, for the peptide shown in Figure 49A of WO/2018/068135, there are 8 residues in the delineated highly hydrophobic core, and 25 total residues in the peptide (excluding the terminal 12 histidines). Thus, the highly hydrophobic core is 32% (8/25).

(5) **Hydrophobic moment** relates to a measure of the amphiphilicity of a helix, peptide, or part thereof, calculated from the vector sum of the hydrophobicities of the side chains of the amino acids (Eisenberg et al., 1982). An online tool for calculating the hydrophobic moment of a polypeptide is available from: http://rzlab.ucr.edu/scripts/wheel/wheel.cgi. A high hydrophobic moment indicates strong amphiphilicity, while a low hydrophobic moment indicates poor amphiphilicity. In some embodiments, peptide shuttle agents of the present description may consist of or comprise a peptide or alpha-helical domain having have a hydrophobic moment (μ) of 3.5 to 11. In some embodiments, the shuttle agent may be a peptide comprising an amphipathic alpha-helical motif having a hydrophobic moment 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. In some embodiments, the shuttle agent may be a peptide having a hydrophobic moment 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. In some embodiments, the hydrophobic moment is calculated excluding any histidine-rich domains that may be present in the peptide.

(6) In some embodiments, peptide shuttle agents of the present description may have a predicted net charge of at least +4 at physiological pH, calculated from the side chains of K, R, D, and E residues. For example, the net charge of the peptide may be at least +5, +6, +7, at least +8, at least +9, at least +10, at least +11, at least +12, at least +13, at least +14, or at least +15 at physiological pH. These positive charges are generally conferred by the greater presence of positively-charged lysine and/or arginine residues, as opposed to negatively charged aspartate and/or glutamate residues.

(7) In some embodiments, peptide shuttle agents of the present description may have a predicted isoelectric point (pI) of 8 to 13, preferably from 10 to 13. Programs and methods for calculating and/or measuring the isoelectric point of a peptide or protein are known in the art. For example, pI may be calculated using the Prot Param software available at: http://web.expasy.org/protparam/

(8) In some embodiments, peptide shuttle agents of the present description may be composed of 35 to 65% of hydrophobic residues (A, C, G, I, L, M, F, P, W, Y, V). In particular embodiments, the peptide shuttle agents may be 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) In some embodiments, peptide shuttle agents of the present description may be composed of 0 to 30% of neutral hydrophilic residues (N, Q, S, T). In particular embodiments, the peptide shuttle agents may be 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) In some embodiments, peptide shuttle agents of the present description may be composed of 35 to 85% of the amino acids A, L, K and/or R. In particular embodiments, the peptide shuttle agents may be 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) In some embodiments, peptide shuttle agents of the present description may be composed of 15 to 45% of the amino acids A and/or L, provided there being at least 5% of L in the peptide. In particular embodiments, the peptide shuttle agents may be 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, provided there being at least 5% of L in the peptide.

(12) In some embodiments, peptide shuttle agents of the present description may be composed of 20 to 45% of the amino acids K and/or R. In particular embodiments, the peptide shuttle agents may be composed of 20% to 40%, 20 to 35%, or 20 to 30% of any combination of the amino acids: K and R.

(13) In some embodiments, peptide shuttle agents of the present description may be composed of 0 to 10% of the amino acids D and/or E. In particular embodiments, the peptide shuttle agents may be composed of 5 to 10% of any combination of the amino acids: D and E.

(14) In some embodiments, the absolute difference between the percentage of A and/or L and the percentage of K and/or R in the peptide shuttle agent may be less than or equal to 10%. In particular embodiments, the absolute difference between the percentage of A and/or L and the percentage of K and/or R in the peptide shuttle agent may be less than or equal to 9%, 8%, 7%, 6%, or 5%.

(15) In some embodiments, peptide shuttle agents of the present description may be composed of 10% to 45% of the amino acids Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T, or H (i.e., not A, L, K, or R). In particular embodiments, the peptide shuttle agents may be 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.

In some embodiments, peptide shuttle agents of the present description respect at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at leave thirteen, at least fourteen, or all of parameters **(1)** to **(15)** described herein. In particular embodiments, peptide shuttle agents of the present description respect all of parameters **(1) to (3)**, and at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or all of parameters **(4)** to **(15)** described herein.

In some embodiments, where a peptide shuttle agent of the present description comprises only one histidine-rich domain, the residues of the one histidine-rich domain may be included in the calculation/assessment of parameters (1) to (15) described herein. In some embodiments, where a peptide shuttle agent of the present description comprises more than one histidine-rich domain, only the residues of one of the histidine-rich domains may be included in the calculation/assessment of parameters (1) to (15) described herein. For example, where a peptide shuttle agent of the present description comprises two histidine-rich domains: a first histidine-rich domain towards the N terminus, and a second histidine-rich domain towards the C terminus, only the first histidine-rich domain may be included in the calculation/assessment of parameters (1) to (15) described herein.

In some embodiments, a machine-learning or computer-assisted design approach may be implemented to generate peptides that respect one or more of parameters (1) to (15) described herein. Some parameters, such as parameters (1) and (5)-(15), may be more amenable to implementation in a computer-assisted design approach, while structural parameters, such as parameters (2), (3) and (4), may be more amenable to a manual design approach. Thus, in some embodiments, peptides that respect one or more of parameters (1) to (15) may be generated by combining computer-assisted and manual design approaches. For example, multiple sequence alignment analyses of a plurality of peptides shown herein (and others) to function as effective shuttle agents revealed the presence of some consensus sequences – i.e., commonly found patterns of alternance of hydrophobic, cationic, hydrophilic, alanine and glycine amino acids. The presence of these consensus sequences are likely to give rise to structural parameters (2), (3) and (4) being respected (i.e., amphipathic alpha-helix formation, a positively-charged face, and a highly hydrophobic core of 12%-50%). Thus, these and other consensus sequences may be employed in machine-learning and/or computer-assisted design approaches to generate peptides that respect one or of parameters (1)-(15).

Accordingly, in some embodiments, peptide shuttle agents described herein may comprise or consist 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),

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.

In some embodiments, peptide shuttle agents of the present description may comprise or consist of a peptide which 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 the amino

acid sequence of any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344**, or to the amino acid sequence of any one of SEQ ID NOs: 104, 105, 107, 108, 110-131, 133-135, 138, 140, 142, 145, 148, 151, 152, 169-242, and 243-10 242 as disclosed in WO/2018/068135, or a functional variant thereof. In some embodiments, peptide shuttle agents of the present description may comprise the amino acid sequence motifs of SEQ ID NOs: 158 and/or 159 of WO/2018/068135, which were found in each of peptides FSD5, FSD16, FSD18, FSD19, FSD20, FSD22, and FSD23. In some embodiments, peptide shuttle agents of the present description may comprise the amino acid sequence motif of SEQ ID NO: 158 of WO/2018/068135 operably linked to the amino acid sequence motif of SEQ ID NO: 159 of WO/2018/068135. As used herein, a "**functional variant**" refers to a peptide having cargo transduction activity, which differs from the reference peptide by one or more conservative amino acid substitutions. As used herein in the context of functional variants, a "**conservative amino acid substitution**" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been well defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, and optionally proline), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

In some embodiments, peptide shuttle agents of the present description do not comprise one or more of the amino acid sequences of any one of SEQ ID NOs: 57-59, 66-72, or 82-102 of WO/2018/068135. In some embodiments, peptide shuttle agents of the present description do not comprise one or more of the amino acid sequences of any one of SEQ ID NOs: 104, 105, 107, 108, 110-131, 133-135, 138, 140, 142, 145, 148, 151, 152, 169-242, and 243-10 242 as disclosed in WO/2018/068135. Rather, in some embodiments, peptide shuttle agents of the present description may relate to variants of such previously described shuttle agent peptides, wherein the variants are further engineered for improved dual transduction activity (i.e., capable of more robustly transducing proteinaceous and non-proteinaceous cargoes).

In some embodiments, peptide shuttle agents of the present description may have a minimal threshold of transduction efficiency and/or cargo delivery score for a "surrogate" cargo as measured in a eukaryotic cell model system (e.g., an immortalized eukaryotic cell line) or in a model organism. The expression "**transduction efficiency**" refers to the percentage or proportion of a population of target cells into which a cargo of interest is delivered intracellularly, which can be determined for example by flow cytometry, immunofluorescence microscopy, and other suitable methods may be used to assess cargo transduction efficiency (e.g., as described in WO/2018/068135). In some embodiments, transduction efficiency may be expressed as a percentage of cargo-positive cells. In some embodiments, transduction efficiency may be expressed as a fold-increase (or fold-decrease) over a suitable negative control assessed under identical conditions except for in the absence of cargo and shuttle agent ("no treatment"; NT)

or in the absence of shuttle agent ("cargo alone").

As used herein, the expression **"surrogate cargo"** refers to any proteinaceous or non-proteinaceous cargo that can be transduced by a shuttle agent having known cargo transduction activity whose level of intracellular delivery and endosomal escape (i.e., cytosolic and/or nuclear delivery) can be readily measured and/or tracked (e.g., via fluorescence or a functional assay), wherein the surrogate cargo is intended to assess the suitability of a given shuttle agent for transducing a cargo of interest (e.g., proteinaceous or non-proteinaceous cargo, such as a therapeutically active cargo binding to an intracellular target) that is different from the surrogate cargo. Examples of suitable surrogate cargoes may include fluorescent cargoes (e.g., PI or other membrane-impermeable fluorescent DNA intercalating agents, GFP, GFP-NLS or other fluorescent proteins, fluorescent dextran, etc.). Non-proteinaceous cargoes such as PI or other membrane-impermeable fluorescent DNA intercalating agents may be particularly advantageous because they are relatively inexpensive and exhibit enhanced fluorescence only after being bound to genomic DNA – a property that makes them particularly suitable to distinguish endosomally-trapped cargo from endosomally-escaped cargo (i.e., cargoes gaining access to the cytosolic/nuclear compartment). As used herein, any suitable model system (e.g., immortalized cell lines, *ex vivo* cells, model laboratory organisms) may be used to assess shuttle agent transduction activity for the surrogate cargo. Conveniently, a eukaryotic cell line model may be selected as a suitable model system, wherein the cell line is selected to be informative for assessing transduction activity in the target eukaryotic cells that will ultimately be transduced. Indeed, a plurality of cell cultures and model organisms are commercially available as model system to study various diseases.

In some embodiments, peptide shuttle agents of the present description increase the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent in a suitable eukaryotic cell model system (e.g., in HeLa or other suitable immortalized cell line). In some embodiments, peptide shuttle agents of the present description increase the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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 ("cargo alone"), in HeLa cells or other suitable eukaryotic cell line model for assessing cargo transduction in the target eukaryotic cells of interest. In some embodiments, peptide shuttle agents of the present description enable a transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) in HeLa cells or other suitable eukaryotic cell line model for assessing cargo transduction in the target eukaryotic cells of interest.

In some embodiments, peptide shuttle agents of the present description increase the transduction efficiency of GFP-NLS or other suitable proteinaceous surrogate cargo in a suitable eukaryotic cell model system (e.g., in

HeLa or other suitable immortalized cell line). In some embodiments, peptide shuttle agents of the present description increase the transduction efficiency of GFP-NLS or other suitable proteinaceous surrogate cargo by at least 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 15, 20, 25, 30, 35, 40, 45 or 50-fold over a corresponding negative control lacking said shuttle agent ("cargo alone"), in HeLa cells or other suitable eukaryotic cell line model for assessing cargo transduction in the target eukaryotic cells of interest. In some embodiments, peptide shuttle agents of the present description enable a transduction efficiency of GFP-NLS or other suitable proteinaceous surrogate cargo of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) in HeLa cells or other suitable eukaryotic cell line model for assessing cargo transduction in the target eukaryotic cells of interest.

In some embodiments, peptide shuttle agents of the present description may comprise or consist of the shuttle agents listed in **Fig. 6** having a mean PI transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%. In some embodiments, peptide shuttle agents of the present description may comprise or consist of a shuttle agent listed in **Fig. 6** having a normalized mean PI delivery score of at least 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, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, or 60.

In some embodiments, peptide shuttle agents of the present description may comprise or consist of the shuttle agents listed in **Fig. 6** having a mean GFP-NLS transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%. In some embodiments, peptide shuttle agents of the present description may comprise or consist of the shuttle agents listed in **Fig. 6** having a normalized mean GFP-NLS delivery score of at least 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, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, or 200.

In some embodiments, peptide shuttle agents of the present description may comprise or consist of the

shuttle agents listed in **Fig. 7** having a mean GFP-NLS transduction efficiency of at least 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30%, or a normalized mean GFP-NLS delivery score of at least 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, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, or 30.

In some embodiments, the shuttle agents of the present description may comprise shuttle agent variants that differ from the shuttle agents defined herein by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. Preferably, linker domains (e.g., flexible serine/glycine-rich linker domains) are excluded from the differing amino acid consideration, as the lengths and/or amino acid composition of the linker domains may greatly vary without affecting transduction activity. In some embodiments, peptide shuttle agents of the present description may comprise or consist of an amino acid sequence that differs from any one of the shuttle agents described herein 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 shuttle agent: increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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; and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells. In some embodiments, 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.

Chemical modifications and synthetic amino acids

In some embodiments, shuttle agents of the present description may comprise oligomers (e.g., dimers, trimers, etc.) of peptides described herein. Such oligomers may be constructed by covalently binding the same or different types of shuttle agent monomers (e.g., using disulfide bridges to link cysteine residues introduced into the monomer sequences). In some embodiments, shuttle agents of the present description may comprise an N-terminal and/or a C-terminal cysteine residue.

In some embodiments, shuttle agents of the present description may comprise or consist of a cyclic peptide. In some embodiments, the cyclic peptide may be formed via a covalent link between a first residue positioned towards the N terminus of the shuttle agent and a second residue positioned towards the C terminus of the shuttle agent. In some embodiments, the first and second residues are flanking residues positioned at the N and

the C termini of the shuttle agent. In some embodiments, the first and second residues may be linked via an amide linkage to form the cyclic peptide. In some embodiments, the cyclic peptide may be formed by a disulfide bond between two cysteine residues within the shuttle agent, wherein the two cysteine residues are positioned towards the N and C termini of the shuttle agent. In some embodiments, the shuttle agent may comprise, or be engineered to comprise, flanking cysteine residues at the N and C termini, which are linked via a disulfide bond to form the cyclic peptide. In some embodiments, the cyclic shuttle agents described herein may be more resistant to degradation (e.g., by proteases) and/or may have a longer half-life than a corresponding linear peptide.

In some embodiments, the shuttle agents of the present description may comprise one or more D-amino acids. In some embodiments, the shuttle agents of the present description may comprise a D-amino acid at the N and/or C terminus of the shuttle agent. In some embodiments, the shuttle agents maybe comprised entirely of D-amino acids. In some embodiments, the shuttle agents described herein having one or more D-amino acids may be more resistant to degradation (e.g., by proteases) and/or may have a longer half-life than a corresponding peptide comprised of only L-amino acids.

In some embodiments, the shuttle agents of the present description may comprise 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. As used herein in this context, the term "**destroy**" means that the chemical modification irreversibly abolishes the cargo transduction activity of a peptide shuttle agent described herein. Chemical modifications that may transiently inhibit, attenuate, or delay the cargo transduction activity of a peptide shuttle agent described herein may be included in the chemical modifications to the shuttle agents of the present description. In some embodiments, the chemical modification to any one of the shuttle agents described herein may be at the N and/or C terminus of the shuttle agent. Examples of chemical modifications include 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, C6-C14, C6-C12, C6-C8, or C8 fatty acid, preferably being N-terminal).

In some embodiments, the shuttle agents of the present description comprise shuttle agent variants having transduction activity for proteinaceous and/or non-proteinaceous cargoes in target eukaryotic cells, the variants being identical to any shuttle agent of the present description, except having at least one amino acid being replaced with a corresponding synthetic amino acid or amino acid analog having a side chain of similar physiochemical properties (e.g., structure, hydrophobicity, or charge) as the amino acid being replaced. In some embodiments, 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-lodoalanine, 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.

Histidine-rich domains

In some embodiments, peptide shuttle agents of the present description may further comprise one or more histidine-rich domains. In some embodiments, the histidine-rich domain may be a stretch of at least 2, at least 3, at least 4, at least 5, or at least 6 amino acids comprising at least 30%, at least 35%, at least 40%, at least 45%, 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. In some embodiments, the histidine-rich domain may comprise 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. Without being bound by theory, the

histidine-rich domain in the shuttle agent may act as a proton sponge in the endosome through protonation of their imidazole groups under acidic conditions of the endosomes, providing another mechanism of endosomal membrane destabilization and thus further facilitating the ability of endosomally-trapped cargoes to gain access to the cytosol. In some embodiments, the histidine-rich domain may be located at or towards the N and/or C terminus of the peptide shuttle agent.

Linkers

In some embodiments, peptide shuttle agents of the present description may comprise one or more **suitable linkers** (e.g., flexible polypeptide linkers). In some embodiments, such linkers may separate two or more amphipathic alpha-helical motifs (e.g., see the shuttle agent FSD18 in Figure 49D of WO/2018/068135). In some embodiments, linkers can be used to separate two more domains (CPDs, ELDs, or histidine-rich domains) from one another. In some embodiments, linkers may be formed by adding sequences of small hydrophobic amino acids without rotatory potential (such as glycine) and polar serine residues that confer stability and flexibility. Linkers may be soft and allow the domains of the shuttle agents to move. In some embodiments, prolines may be avoided since they can add significant conformational rigidity. In some embodiments, the linkers may be serine/glycine-rich linkers (e.g., GS, GGS, GGSGGGS, GGSGGGSGGGS, or the like). In some embodiments, the use shuttle agents comprising a suitable linker may be advantageous for delivering a cargo to suspension cells, rather than to adherent cells. In some embodiments, the linker may comprise or consist of: -Gn- ; -Sn- ; -(GnSn)n- ; -(GnSn)nGn- ; -(GnSn)nSn- ; -(GnSn)nGn(GnSn)n- ; or -(GnSn)nSn(GnSn)n- , wherein G is the amino acid Gly; S is the amino acid Ser; and n is an integer from 1 to 5.

Domain-based peptide shuttle agents

In some aspects, the shuttle agents described herein may be a shuttle agent as described in WO/2016/161516, comprising an endosome leakage domain (ELD) operably linked to a cell penetrating domain (CPD).

Endosome leakage domains (ELDs)

In some aspects, peptide shuttle agents of the present description may comprise an endosome leakage domain (ELD) for facilitating endosome escape and access to the cytoplasmic compartment. As used herein, the expression "**endosome leakage domain**" refers to a sequence of amino acids which confers the ability of endosomally-trapped cargoes to gain access to the cytoplasmic compartment. Without being bound by theory, endosome leakage domains are short sequences (often derived from viral or bacterial peptides), which are believed to induce destabilization of the endosomal membrane and liberation of the endosome contents into the cytoplasm. As used herein, the expression "**endosomolytic peptide**" is intended to refer to this general class of peptides having endosomal membrane-destabilizing properties. Accordingly, in some embodiments, synthetic peptide or polypeptide-based shuttle agents of

the present description may comprise an ELD which is an endosomolytic peptide. The activity of such peptides may be assessed for example using the calcein endosome escape assays described in Example 2 of WO/2016/161516.

In some embodiments, the ELD may be a peptide that disrupts membranes at acidic pH, such as pH-dependent membrane active peptide (PMAP) or a pH-dependent lytic peptide. For example, the peptides GALA and INF-7 are amphiphilic peptides that form alpha helixes when a drop in pH modifies the charge of the amino acids which they contain. More particularly, without being bound by theory, it is suggested that ELDs such as GALA induce endosomal leakage by forming pores and flip-flop of membrane lipids following conformational change due to a decrease in pH (Kakudo, Chaki et al., 2004, Li, Nicol et al., 2004). In contrast, it is suggested that ELDs such as INF-7 induce endosomal leakage by accumulating in and destabilizing the endosomal membrane (El-Sayed, Futaki et al., 2009). Accordingly, in the course of endosome maturation, the concomitant decline in pH causes a change in the conformation of the peptide and this destabilizes the endosome membrane leading to the liberation of the endosome contents. The same principle is thought to apply to the toxin A of *Pseudomonas* (Varkouhi, Scholte et al., 2011). Following a decline in pH, the conformation of the domain of translocation of the toxin changes, allowing its insertion into the endosome membrane where it forms pores (London 1992, O'Keefe 1992). This eventually favors endosome destabilization and translocation of the complex outside of the endosome. The above described ELDs are encompassed within the ELDs of the present description, as well as other mechanisms of endosome leakage whose mechanisms of action may be less well defined.

In some embodiments, the ELD may be an antimicrobial peptide (AMP) such as a linear cationic alpha-helical antimicrobial peptide (AMP). These peptides play a key role in the innate immune response due to their ability to strongly interact with bacterial membranes. Without being bound by theory, these peptides are thought to assume a disordered state in aqueous solution, but adopt an alpha-helical secondary structure in hydrophobic environments. The latter conformation thought to contribute to their typical concentration-dependent membrane-disrupting properties. When accumulated in endosomes at certain concentrations, some antimicrobial peptides may induce endosomal leakage.

In some embodiments, the ELD may be an antimicrobial peptide (AMP) such as Cecropin-A/Melittin hybrid (CM) peptide. Such peptides are thought to be among the smallest and most effective AMP-derived peptides with membrane-disrupting ability. Cecropins are a family of antimicrobial peptides with membrane-perturbing abilities against both Gram-positive and Gram-negative bacteria. Cecropin A (CA), the first identified antibacterial peptide, is composed of 37 amino acids with a linear structure. Melittin (M), a peptide of 26 amino acids, is a cell membrane lytic factor found in bee venom. Cecropin-melittin hybrid peptides have been shown to produce short efficient antibiotic peptides without cytotoxicity for eukaryotic cells (i.e., non-hemolytic), a desirable property in any antibacterial agent. These chimeric peptides were constructed from various combinations of the hydrophilic N-terminal domain of Cecropin A with the hydrophobic N-terminal domain of Melittin, and have been tested on bacterial model systems. Two 26-mers, CA(1-13)M(1-13) and CA(1-8) M(1-18) (Boman et al., 1989), have been

shown to demonstrate a wider spectrum and improved potency of natural Cecropin A without the cytotoxic effects of melittin.

In an effort to produce shorter CM series peptides, the authors of Andreu et al., 1992 constructed hybrid peptides such as the 26-mer (CA(1-8)M(1-18)), and compared them with a 20-mer (CA(1-8)M(1-12)), a 18-mer (CA(1-8)M(1-10)) and six 15-mers ((CA(1-7)M(1-8), CA(1-7)M(2-9), CA(1-7)M(3-10), CA(1-7)M(4-11), CA(1-7)M(5-12), and CA(1-7)M(6-13)). The 20 and 18-mers maintained similar activity comparatively to CA(1-8)M(1-18). Among the six 15-mers, CA(1-7)M(1-8) showed low antibacterial activity, but the other five showed similar antibiotic potency compared to the 26-mer without hemolytic effect. Accordingly, in some embodiments, synthetic peptide or polypeptide-based shuttle agents of the present description may comprise an ELD which is or is from CM series peptide variants, such as those described above.

In some embodiments, the ELD may be the CM series peptide CM18 composed of residues 1–7 of Cecropin-A (KWKLFKKIGAVLKVLTTG) fused to residues 2–12 of Melittin (YGRKKRRQRRR), [C(1–7)M(2–12)]. When fused to the cell penetrating peptide TAT, CM18 was shown to independently cross the plasma membrane and destabilize the endosomal membrane, allowing some endosomally-trapped cargoes to be released to the cytosol (Salomone et al., 2012). However, the use of a CM18-TAT11 peptide fused to a fluorophore (atto-633) in some of the authors' experiments, raises uncertainty as to the contribution of the peptide versus the fluorophore, as the use of fluorophores themselves have been shown to contribute to endosomolysis -- e.g., via photochemical disruption of the endosomal membrane (Erazo-Oliveras et al., 2014).

In some embodiments, the ELD may be CM18 having the amino acid sequence of SEQ ID NO: 1 of WO/2016/161516, or a variant thereof having at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 90%, 91%, 92%, 93%, 94%, or 95% identity to SEQ ID NO: 1 of WO/2016/161516 and having endosomolytic activity.

In some embodiments, the ELD may be a peptide derived from the N terminus of the HA2 subunit of influenza hemagglutinin (HA), which may also cause endosomal membrane destabilization when accumulated in the endosome.

In some embodiments, synthetic peptide or polypeptide-based shuttle agents of the present description may comprise an ELD which is or is from an ELD set forth in **Table I**, or a variant thereof having endosome escape activity and/or pH-dependent membrane disrupting activity.

Table I: Examples of endosome leakage domains

Name	SEQ ID NO of WO/2016/161516	Reference(s)
CM18	1	Salomone, Cardarelli et al., 2012
Diphtheria toxin T domain (DT)	2	Uherek, Fominaya et al., 1998, Glover, Ng et al., 2009
GALA	3	Parente, Nir et al., 1990 Li, Nicol et al., 2004
PEA	4	Fominaya and Wels 1996

INF-7	5	El-Sayed, Futaki et al., 2009
LAH4	6	Kichler, Mason et al., 2006 Kichler et al., 2003
HGP	7	Kwon et al., 2010
H5WYG	8	Midoux, Kichler et al., 1998
HA2	9	Lorieau, Louis et al., 2010
EB1	10	Amand, Norden et al., 2012
VSVG	11	Schuster, Wu et al., 1999
Pseudomonas toxin	12	Fominaya, Uherek et al., 1998
Melittin	13	Tan, Chen et al., 2012
KALA	14	Wyman, Nicol et al., 1997
JST-1	15	Gottschalk, Sparrow et al., 1996
$C(LLKK)_3C$	63	Luan et al., 2015
$G(LLKK)_3G$	64	Luan et al., 2015

In some embodiments, shuttle agents of the present description may comprise one or more ELD or type of ELD. More particularly, they can comprise at least 2, at least 3, at least 4, at least 5, or more ELDs. In some embodiments, the shuttle agents can comprise between 1 and 10 ELDs, between 1 and 9 ELDs, between 1 and 8 ELDs, between 1 and 7 ELDs, between 1 and 6 ELDs, between 1 and 5 ELDs, between 1 and 4 ELDs, between 1 and 3 ELDs, etc.

In some embodiments, the order or placement of the ELD relative to the other domains (CPD, histidine-rich domains) within the shuttle agents of the present description may be varied provided the shuttling ability of the shuttle agent is retained.

In some embodiments, the ELD may be a variant or fragment of any one those listed in **Table I**, and having endosomolytic activity. In some embodiments, the ELD may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 1-15, 63, or 64 of WO/2016/161516, or a sequence which is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 90%, 91%, 92%, 93%, 94%, or 95% identical to any one of SEQ ID NOs: 1-15, 63, or 64 of WO/2016/161516, and having endosomolytic activity.

In some embodiments, shuttle agents of the present description do not comprise one or more of the amino acid sequences of any one of SEQ ID NOs: 1-15, 63, or 64 of WO/2016/161516.

Cell penetration domains (CPDs)

In some aspects, the shuttle agents of the present description may comprise a cell penetration domain (CPD). As used herein, the expression "**cell penetration domain**" refers to a sequence of amino acids which confers the ability of a macromolecule (e.g., peptide or protein) containing the CPD to be transduced into a cell.

In some embodiments, the CPD may be (or may be from) a cell-penetrating peptide or the protein transduction domain of a cell-penetrating peptide. Cell-penetrating peptides can serve as carriers to successfully deliver a variety of cargoes intracellularly (e.g., polynucleotides, polypeptides, small molecule compounds or other macromolecules/compounds that are otherwise membrane-impermeable). Cell-penetrating peptides often include short peptides rich in basic amino acids that, once fused (or otherwise operably linked) to a macromolecule, mediate its internalization inside cells (Shaw, Catchpole et al., 2008). The first cell-penetrating peptide was identified by analyzing the cell penetration ability of the HIV-1 trans-activator of transcription (Tat) protein (Green and Loewenstein 1988, Vives, Brodin et al., 1997). This protein contains a short hydrophilic amino acid sequence, named "TAT", which promotes its insertion within the plasma membrane and the formation of pores. Since this discovery, many other cell-penetrating peptides have been described. In this regard, in some embodiments, the CPD can be a cell-penetrating peptide as listed in **Table II**, or a variant thereof having cell-penetrating activity.

Table II: Examples of cell-penetrating peptides

Name	SEQ ID NO of WO/2016/161516	Reference(s)
SP	16	Mahlum, Mandal et al., 2007
TAT	17	Green and Loewenstein 1988, Fawell, Seery et al., 1994, Vives, Brodin et al., 1997
Penetratin (Antennapedia)	18	Perez, Joliot et al., 1992
pVEC	19	Elmquist, Lindgren et al., 2001
M918	20	El-Andaloussi, Johansson et al., 2007
Pep-1	21	Morris, Depollier et al., 2001
Pep-2	22	Morris, Chaloin et al., 2004
Xentry	23	Montrose, Yang et al., 2013
Arginine stretch	24	Zhou, Wu et al., 2009
Transportan	25	Hallbrink, Floren et al., 2001
SynB1	26	Drin, Cottin et al., 2003
SynB3	27	Drin, Cottin et al., 2003
PTD4	65	Ho et al, 2001

Without being bound by theory, cell-penetrating peptides are thought to interact with the cell plasma membrane before crossing by pinocytosis or endocytosis. In the case of the TAT peptide, its hydrophilic nature and charge are thought to promote its insertion within the plasma membrane and the formation of a pore (Herce and Garcia 2007). Alpha helix motifs within hydrophobic peptides (such as SP) are also thought to form pores within plasma membranes (Veach, Liu et al., 2004).

In some embodiments, shuttle agents of the present description may comprise one or more CPD or type of CPD. More particularly, they may comprise at least 2, at least 3, at least 4, or at least 5 or more CPDs. In some embodiments, the shuttle agents can comprise between 1 and 10 CPDs, between 1 and 6 CPDs, between 1 and 5 CPDs, between 1 and 4 CPDs, between 1 and 3 CPDs, etc.

In some embodiments, the CPD may be TAT having the amino acid sequence of SEQ ID NO: 17 of WO/2016/161516., or a variant thereof having at least 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%, or 95% identity to SEQ ID NO: 17 of WO/2016/161516 and having cell penetrating activity; or Penetratin having the amino acid sequence of SEQ ID NO: 18 of WO/2016/161516, or a variant thereof having at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81% 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, or 95% identity to SEQ ID NO: 18 of WO/2016/161516 and having cell penetrating activity.

In some embodiments, the CPD may be PTD4 having the amino acid sequence of SEQ ID NO: 65 of WO/2016/161516, or a variant thereof having at least 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%, or 95% identity to SEQ ID NO: 65 of WO/2016/161516.

In some embodiments, the order or placement of the CPD relative to the other domains (ELD, histidine-rich domains) within the shuttle agents of the present description may be varied provided the transduction ability of the shuttle agent is retained.

In some embodiments, the CPD may be a variant or fragment of any one those listed in **Table II**, and having cell penetrating activity. In some embodiments, the CPD may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 16-27 or 65 of WO/2016/161516, or a sequence which is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 85%, 90%, 91%, 92%, 93%, 94%, or 95% identical to any one of SEQ ID NOs: 16-27 or 65 of WO/2016/161516., and having cell penetrating activity.

In some embodiments, shuttle agents of the present description do not comprise any one of the amino acid sequences of SEQ ID NOs: 16-27 or 65 of WO/2016/161516.

Methods, kits, uses, compositions, and cells

In some embodiments, the present description relates to methods for delivering a proteinaceous and/or non-proteinaceous cargo from an extracellular space to the cytosol and/or nucleus of a target eukaryotic cell. The methods comprise contacting the target eukaryotic cell with the cargo in the presence of a shuttle agent at a concentration sufficient to increase the transduction efficiency of said cargo, as compared to in the absence of said shuttle agent. In some embodiments, contacting the target eukaryotic cell with the cargo in the presence of the shuttle agent results in an increase in the transduction efficiency of said non-proteinaceous cargo by at least 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, or 100-fold, as compared to in the absence of said shuttle agent.

In some embodiments, the present description relates to a method for increasing the transduction efficiency of a proteinaceous and/or non-proteinaceous cargo to the cytosol and/or nucleus of target eukaryotic cells. As used herein, the expression "**increasing transduction efficiency**" refers to the ability of a shuttle agent of the present description to improve the percentage or proportion of a population of target cells into which a cargo of interest (e.g., non-proteinaceous cargo) is delivered intracellularly. Immunofluorescence microscopy, flow cytometry, and other suitable methods may be used to assess cargo transduction efficiency. In some embodiments, a shuttle agent of the

present description may enable a transduction efficiency of at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85%, for example as measured by immunofluorescence microscopy, flow cytometry, FACS, and other suitable methods. In some embodiments, a shuttle agent of the present description may enable one of the aforementioned transduction efficiencies together wish a cell viability of at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, for example as measured by the assay described in Example 3.3a of WO/2018/068135, or by another suitable assay known in the art.

In addition to increasing target cell transduction efficiency, shuttle agents of the present description may facilitate the delivery of a cargo of interest (e.g., a proteinaceous and/or non-proteinaceous cargo) to the cytosol and/or nucleus of target cells. In this regard, efficiently delivering an extracellular cargo to the cytosol and/or nucleus of a target cell using peptides can be challenging, as the cargo often becomes trapped in intracellular endosomes after crossing the plasma membrane, which may limit its intracellular availability and may result in its eventual metabolic degradation. For example, use of the protein transduction domain from the HIV-1 Tat protein has been reported to result in massive sequestration of the cargo into intracellular vesicles. In some aspects, shuttle agents of the present description may facilitate the ability of endosomally-trapped cargo to escape from the endosome and gain access to the cytoplasmic compartment. In this regard, the expression "**to the cytosol**" for example in the phrase "increasing the transduction efficiency of a non-proteinaceous cargo to the cytosol," is intended to refer to the ability of shuttle agents of the present description to allow an intracellularly delivered cargo of interest to escape endosomal entrapment and gain access to the cytoplasmic and/or nuclear compartment. After a cargo of interest has gained access to the cytosol, it may be free to bind to its intracellular target (e.g., nucleus, nucleolus, mitochondria, peroxisome). In some embodiments, the expression "to the cytosol" is thus intended to encompass not only cytosolic delivery, but also delivery to other subcellular compartments that first require the cargo to gain access to the cytoplasmic compartment.

In some embodiments, the methods of the present description are *in vitro* methods (e.g., such as for therapeutic and/or diagnostic purpose). In other embodiments, the methods of the present description are *in vivo* methods (e.g., such as for therapeutic and/or diagnostic purpose). In some embodiments, the methods of the present description comprise topical, enteral/gastrointestinal (e.g., oral), or parenteral administration of the non-proteinaceous cargo and the synthetic peptide shuttle agent. In some embodiments, described herein are compositions formulated for topical, enteral/gastrointestinal (e.g., oral), or parenteral administration of the non-proteinaceous cargo and the synthetic peptide shuttle agent.

In some embodiments, the methods of the present description may comprise contacting the target eukaryotic cell with the shuttle agent, or composition as defined herein, and the proteinaceous and/or non-proteinaceous cargo. In some embodiments, the shuttle agent, or composition may be pre-incubated with the cargo to form a mixture, prior to exposing the target eukaryotic cell to that mixture. In some embodiments, the type of shuttle agent may be selected based on the identity and/or physicochemical properties of the cargo to be delivered intracellularly. In other

embodiments, the type of shuttle agent may be selected to take into account the identity and/or physicochemical properties of the cargo to be delivered intracellularly, the type of cell, the type of tissue, etc.

In some embodiments, the method may comprise multiple treatments of the target cells with the shuttle agent, or composition (e.g., 1, 2, 3, 4 or more times per day, and/or on a pre-determined schedule). In such cases, lower concentrations of the shuttle agent, or composition may be advisable (e.g., for reduced toxicity). In some embodiments, the cells may be suspension cells or adherent cells. In some embodiments, the person of skill in the art will be able to adapt the teachings of the present description using different combinations of shuttles, domains, uses and methods to suit particular needs of delivering a proteinaceous and/or non-proteinaceous cargo to particular cells with a desired viability.

In some embodiments, the methods of the present description may apply to methods of delivering a proteinaceous and/or non-proteinaceous cargo intracellularly to a cell *in vivo*. Such methods may be accomplished by parenteral administration or direct injection into a tissue, organ, or system.

In some aspects, the synthetic peptide shuttle agents of the present description may be for use in an *in vitro* or *in vivo* method for increasing the transduction efficiency of a proteinaceous and/or non-proteinaceous cargo (e.g., a therapeutically or biologically active proteinaceous and/or non-proteinaceous cargo) into target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used or is formulated for use at a concentration sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant.

In some embodiments, synthetic peptide shuttle agents of the present description maybe for use in therapy, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant transduces a therapeutically or biologically active proteinaceous and/or non-proteinaceous cargo to the cytosol and/or nucleus of target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used (or is formulated for use) at a concentration sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent.

In some aspects, described herein is a composition for use in transducing a proteinaceous and/or non-proteinaceous cargo into target eukaryotic cells, the composition comprising a synthetic peptide shuttle agent formulated with a pharmaceutically suitable excipient, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent. In some embodiments, the composition further comprises the cargo. In some embodiments, the composition may be mixed with the cargo prior to administration or therapeutic use.

In some aspects, described herein is a composition for use in therapy, the composition comprising a synthetic peptide shuttle agent formulated with a proteinaceous and/or non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic

peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

In some embodiments, the shuttle agent, or composition, and the proteinaceous and/or non-proteinaceous cargo may be exposed to the target cell in the presence or absence of serum. In some embodiments, the method may be suitable for clinical or therapeutic use.

In some embodiments, the present description relates to a kit for delivering a proteinaceous and/or non-proteinaceous cargo from an extracellular space to the cytosol and/or nucleus of a target eukaryotic cell. In some embodiments, the present description relates to a kit for increasing the transduction efficiency of a proteinaceous and/or non-proteinaceous cargo to the cytosol of a target eukaryotic cell. The kit may comprise the shuttle agent, or composition as defined herein, and a suitable container.

In some embodiments, the target eukaryotic cells may be an animal cell, a mammalian cell, or a human cell. In some embodiments, the target eukaryotic cells may be stem cells (e.g., embryonic stem cells, pluripotent stem cells, induced pluripotent stem cells, neural stem cells, mesenchymal stem cells, hematopoietic stem cells, peripheral blood stem cells), primary cells (e.g., myoblast, fibroblast), immune cells (e.g., NK cell, T cell, dendritic cell, antigen presenting cell), epithelial cells, skin cells, gastrointestinal cells, mucosal cells, or pulmonary cells. In some embodiments, target cells comprise those having the cellular machinery for endocytosis (i.e., to produce endosomes).

In some embodiments, the present description relates to an isolated cell comprising a synthetic peptide shuttle agent as defined herein. In some embodiments, the cell may be a protein-induced pluripotent stem cell. It will be understood that cells that are often resistant or not amenable to DNA transfection may be interesting candidates for the synthetic peptide shuttle agents of the present description.

In some embodiments, the present description relates to a method for producing a synthetic peptide shuttle agent that delivers a proteinaceous and/or non-proteinaceous cargo from an extracellular space to the cytosol and/or nucleus of a target eukaryotic cell, the method comprising synthesizing a peptide which is: (1) a peptide at least 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 parameters (4) to (15) defined herein are respected.

In some embodiments, the present description relates to a method for identifying or selecting a shuttle agent that delivers a proteinaceous and/or non-proteinaceous cargo from an extracellular space to the cytosol and/or nucleus of a target eukaryotic cell, the method comprising: (a) synthesizing a peptide which is the peptide as defined herein; (b) contacting the target eukaryotic cell with the cargo in the presence of said peptide; (c) measuring the transduction efficiency of the cargo in the target eukaryotic cell; and (d) identifying or selecting the peptide as being a shuttle agent that transduces the cargo, when an increase in transduction activity (e.g., transduction efficiency) of said cargo in the target eukaryotic cell is observed.

In some aspects, the present description relates to a composition for use in transducing a proteinaceous and/or non-proteinaceous cargo into target eukaryotic cells, the composition comprising a synthetic peptide shuttle agent formulated with a pharmaceutically suitable excipient, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent. In some embodiments, the composition further comprises the cargo.

In some embodiments, the present description relates to oral formulations comprising the shuttle agents described herein and a cargo as described herein, for example an enterically-coated oral dosage form.

In some embodiments, applications of the shuttle agents described herein in food, farming, and/or agricultural industries may be envisaged. In some embodiments, the shuttle agents described herein may be formulated as a feed additive to aid in weight gain and/or the absorption of nutrients. In some embodiments, the shuttle agents described herein may be formulated as a feed additive to aid in weight gain and/or the absorption of nutrients.

In some aspects, described herein is a process for producing a candidate synthetic peptide shuttle agent expected to have transduction activity for a proteinaceous and/or non-proteinaceous cargo of interest in target eukaryotic cells, the method comprising synthesizing a peptide which is: (1) a peptide at least 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 parameters (4) to (15) as defined herein are respected, and wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

In some aspects, described herein is an *in vitro* or *in vivo* method for identifying or selecting a synthetic peptide shuttle agent expected to have transduction activity for proteinaceous and/or non-proteinaceous cargoes in target eukaryotic cells, the method comprising: providing model eukaryotic cells or a model organism suitable for assessing cargo transduction in the target eukaryotic cells; providing a candidate synthetic peptide shuttle agent (e.g., any shuttle agent as defined herein); and measuring the transduction activity (e.g., cargo transduction efficiency, such as by flow cytometry) of the candidate synthetic peptide shuttle agent to transduce propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent into the eukaryotic cell line model, wherein the candidate shuttle agent is expected to have transduction activity for both proteinaceous and non-

proteinaceous cargoes in the target eukaryotic cells when the transduction activity (e.g., transduction efficiency) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent is increased 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 the candidate synthetic peptide shuttle agent, and/or a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of the propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent occurs, in the model eukaryotic cells or model organism.

ITEMS I

In some aspects, described here are one or more of the following items:

1. A method for non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with a non-proteinaceous cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of said non-proteinaceous cargo, as compared to in the absence of said synthetic peptide shuttle agent.
2. The method of item 1, wherein the non-proteinaceous cargo: (a) is an organic compound; (b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da; (c) is a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target; (d) is not a biopolymer, such as a polynucleotide or a polysaccharide; (e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or (f) any combination of (a) to (e).
3. The method of item 1 or 2, wherein non-proteinaceous cargo is a drug for 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).
4. The method of any one of items 1 to 3, wherein non-proteinaceous cargo is or comprises a HedgeHog inhibitor (e.g., itraconazole, posaconazole, arsenic trioxide (ATO), Gant61, PF-4708671, HPI-1, HPI-4), a pain inhibitor such as a voltage-gated sodium (Nav) channel inhibitor (e.g., QX-314), and/or an inhibitor of inflammation (e.g., an inhibitor of inflammatory cytokine production, or an NF-kappa B pathway inhibitor).
5. The method of any one of items 1 to 4, wherein the shuttle agent is: (1) a peptide at least 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 +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.

6. The method of item 5, 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 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 +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).

7. The method of any one of items 1 to 6, 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.

8. The method of any one of items 1 to 7, 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).

9. The method of any one of items 1 to 8, wherein said shuttle agent comprises a flexible linker domain rich in serine and/or glycine residues.

10. The method of any one of items 1 to 9, 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), 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.

11. The method of any one of items 1 to 10, wherein the shuttle agent comprises or consists of a peptide which is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to the amino acid sequence of any one of **SEQ ID NOs: 19-50.**

12. The method of any one of items 1 to 11, wherein the shuttle agent comprises an endosome leakage domain (ELD), and/or a cell penetrating domain (CPD).

13. The method of any one of items 1 to 12, 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).

14. The method of any one of items 1 to 13, wherein the shuttle agent is a cyclic peptide and/or comprises one or more D-amino acids.

15. The method of any one of items 1 to 14, which is an *in vitro* method, such as for therapeutic and/or diagnostic purpose.

16. The method of any one of items 1 to 14, which is an *in vivo* method, such as for therapeutic and/or diagnostic purpose.
17. The method of item 16 comprising topical, enteral/gastrointestinal (e.g., oral), or parenteral administration of the non-proteinaceous cargo and the synthetic peptide shuttle agent.
18. A composition for use in transducing a non-proteinaceous cargo into target eukaryotic cells, the composition comprising a synthetic peptide shuttle agent formulated with a pharmaceutically suitable excipient, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the non-proteinaceous cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.
19. The composition of item 17, further comprising the non-proteinaceous cargo.
20. The composition of item 18 or 19, wherein: (a) the synthetic peptide shuttle agent is as defined in any one of items 1 or 5 to 14; (b) the non-proteinaceous cargo is as defined in any one of items 2 to 4; (c) the composition is for use in the *in vitro* or *in vivo* method as defined in any one of items 15 to 17; or (d) any combination of (a) to (c).
21. A kit for use in the method of any one of items 1 to 17, the kit comprising the synthetic peptide shuttle agent is as defined in any one of items 1 or 5 to 14, and the non-proteinaceous cargo is as defined in any one of items 2 to 4.
22. The method of any one of items 1 to 17, the composition of any one of items 18 to 20, or the kit of item 21, wherein the target 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, or gastrointestinal cells.
23. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes, the shuttle agent comprising an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to any one of **SEQ ID NOs: 19-50**.
24. The synthetic peptide shuttle agent of item 23, which is the shuttle agent as defined in any one of items 5 to 13.

ITEMS II

In some aspects, described here are one or more of the following items:

1. A method for non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with a non-proteinaceous cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of said non-proteinaceous cargo, as compared to in the absence of said synthetic peptide shuttle agent.
2. The method of item 1, wherein the non-proteinaceous cargo: (a) is an organic compound; (b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or

between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da; (c) is a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target; (d) is not a biopolymer, such as a polynucleotide or a polysaccharide; (e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or (f) any combination of (a) to (e).

3. The method of item 1 or 2, wherein non-proteinaceous cargo is a drug for 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).

4. The method of any one of items 1 to 3, wherein non-proteinaceous cargo is or comprises a HedgeHog inhibitor (e.g., itraconazole, posaconazole, arsenic trioxide (ATO), Gant61, PF-4708671, HPI-1, HPI-4), a pain inhibitor such as a voltage-gated sodium (Nav) channel inhibitor (e.g., QX-314), and/or an inhibitor of inflammation (e.g., an inhibitor of inflammatory cytokine production, or an NF-kappa B pathway inhibitor).

5. The method of any one of items 1 to 4, wherein the shuttle agent is: (1) a peptide at least 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 +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.

6. The method of item 5, 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 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 +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).

7. The method of any one of items 1 to 6, 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.

8. The method of any one of items 1 to 7, 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).

9. The method of any one of items 1 to 8, wherein said shuttle agent comprises a flexible linker domain rich in serine and/or glycine residues.

10. The method of any one of items 1 to 9, 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), 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.

11. The method of any one of items 1 to 10, wherein the shuttle agent comprises or consists of: the amino acid sequence any one of **SEQ ID NOs: 1 to 50;** an amino acid sequence that differs from any one of **SEQ ID NOs: 1 to 50** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., excluding any linker domains); or 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: 1 to 50** (e.g., calculated excluding any linker domains).

12. The method of any one of items 1 to 10, wherein the shuttle agent comprises or consists of: the amino acid sequence any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344**; an amino acid sequence that differs from any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., excluding any linker domains); or 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: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344** (e.g., calculated excluding any linker domains).

13. The method of any one of items 1 to 12, wherein the shuttle agent comprises an endosome leakage domain (ELD), and/or a cell penetrating domain (CPD).

14. The method of any one of items 1 to 13, 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).

15. The method of any one of items 1 to 14, wherein the shuttle agent is a cyclic peptide and/or comprises one or more D-amino acids.

16. The method of any one of items 1 to 15, wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.
17. The method of any one of items 1 to 16, wherein the shuttle agent increases the transduction efficiency of GFP-NLS 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of GFP-NLS, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.
18. The method of any one of items 1 to 17, 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.
19. The method of item 18, wherein the chemical modification is at the N and/or C terminus of the shuttle agent.
20. The method of item 18 or 19, 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).
21. The method of any one of items 1 to 20, which is an *in vitro* method, such as for therapeutic and/or diagnostic purpose.
22. The method of any one of items 1 to 20, which is an *in vivo* method, such as for therapeutic and/or diagnostic purpose.
23. The method of item 22 comprising topical, enteral/gastrointestinal (e.g., oral), or parenteral administration of the non-proteinaceous cargo and the synthetic peptide shuttle agent.
24. A composition for use in transducing a non-proteinaceous cargo into target eukaryotic cells, the composition comprising a synthetic peptide shuttle agent formulated with a pharmaceutically suitable excipient, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the non-proteinaceous cargo

into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

25. The composition of item 24, further comprising the non-proteinaceous cargo.

26. A composition for use in therapy, the composition comprising a synthetic peptide shuttle agent formulated with a non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the non-proteinaceous cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

27. The composition of any one of items 24 to 26, wherein: (a) the synthetic peptide shuttle agent is as defined in any one of items 1 or 5 to 20; (b) the non-proteinaceous cargo is as defined in any one of items 2 to 4; (c) the composition is for use in the *in vitro* or *in vivo* method as defined in any one of items 21 to 23; or (d) any combination of (a) to (c).

28. A kit for use in the method of any one of items 1 to 23, the kit comprising the synthetic peptide shuttle agent as defined in any one of items 1 or 5 to 20, and the non-proteinaceous cargo is as defined in any one of items 2 to 4.

29. The method of any one of items 1 to 23, the composition of any one of items 24 to 27, or the kit of item 28, wherein the target 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, or gastrointestinal cells.

30. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes, the shuttle agent comprising or consisting of: the amino acid sequence any one of **SEQ ID NOs: 19 to 50**; an amino acid sequence that differs from any one of **SEQ ID NOs: 19 to 50** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., excluding any linker domains); or 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: 19 to 50** (e.g., calculated excluding any linker domains).

31. The synthetic peptide shuttle agent of item 30, which is the shuttle agent as defined in any one of items 5 to 20.

32. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, the shuttle agent being: (1) a peptide at least 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 +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, wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

33. The synthetic peptide shuttle agent of item 32, wherein the shuttle agent increases the transduction efficiency of GFP-NLS 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of GFP-NLS, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

34. The synthetic peptide shuttle agent of item 32 or 33, 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 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 +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).

35. The synthetic peptide shuttle agent of any one of items 32 to 34, 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.

36. The synthetic peptide shuttle agent of any one of items 32 to 35, wherein said shuttle agent: (i) comprises a histidine-rich domain as defined in item 8; (ii) comprises a flexible linker domain as defined in item 9; (iii) is the shuttle agent as defined in any one of items 10 to 14; or (iv) any combination of (i) to (iii).
37. The synthetic peptide shuttle agent of any one of items 32 to 36, further comprising 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.
38. The synthetic peptide shuttle agent of item 37, wherein the chemical modification is at the N and/or C terminus of the shuttle agent.
39. The synthetic peptide shuttle agent of item 37 or 38, 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).
40. The synthetic peptide shuttle agent of any one of items 32 to 39, wherein the shuttle agent comprises or consists of: the amino acid sequence any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344**; an amino acid sequence that differs from any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids (e.g., excluding any linker domains); or 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: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344** (e.g., calculated excluding any linker domains).
41. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, wherein the shuttle agent comprises or consists of: (a) the amino acid sequence any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344**; or (b) an amino acid sequence that differs from any one of **SEQ ID NOs: 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 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344** 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 shuttle agent: increases the transduction efficiency of propidium iodide or other

membrane-impermeable fluorescent DNA intercalating agent 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; and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

42. The synthetic peptide shuttle agent of item 41, which is the synthetic peptide shuttle agent as defined in any one of items 32 to 39.

43. A synthetic peptide shuttle agent having proteinaceous cargo transduction activity in target eukaryotic cells, wherein the shuttle agent comprises or consists of: (a) the amino acid sequence any one of **SEQ ID NOs: 52, 57, 79, 108, 140, 147, 148, 173, 241, 261, 286, 295, 301, 309, 312, 325, 333-337, or 343**; or (b) an amino acid sequence that differs from any one of **SEQ ID NOs: 52, 57, 79, 108, 140, 147, 148, 173, 241, 261, 286, 295, 301, 309, 312, 325, 333-337, or 343** 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 shuttle agent: increases the transduction efficiency of GFP-NLS 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, and/or enables a transduction efficiency of at least 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, or 30% (e.g., as determined by flow cytometry) of GFP-NLS in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

44. The synthetic peptide shuttle agent of any one of items 41 to 43, 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.

45. A synthetic peptide shuttle agent variant having transduction activity for proteinaceous and/or non-proteinaceous cargoes in target eukaryotic cells, the synthetic peptide shuttle agent variant being identical to the synthetic peptide shuttle agent as defined in any one of items 32 to 44, 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 shuttle agent variant increases the transduction efficiency of said cargo in the target eukaryotic cells, as compared to in the absence of the shuttle agent variant.

46. The synthetic peptide shuttle agent variant of item 45, 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-lodoalanine, 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.

47. The synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of items 32 to 46 for use in an *in vitro* or *in vivo* method for increasing the transduction efficiency of a proteinaceous and/or non-proteinaceous cargo (e.g., a therapeutically active proteinaceous and/or non-proteinaceous cargo) into target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic

peptide shuttle agent variant is used at a concentration sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant.

48. The synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of items 32 to 47 for use in therapy, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant transduces a therapeutically active proteinaceous and/or non-proteinaceous cargo to the cytosol and/or nucleus of target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used at a concentration sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent.

49. An *in vitro* or *in vivo* method for proteinaceous and/or non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with the cargo and a concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of items 32 to 46 sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of said synthetic peptide shuttle agent.

50. The *in vitro* or *in vivo* method of item 49, which is a method for therapeutic and/or diagnostic purpose.

51. A composition for use in therapy, the composition comprising the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of items 32 to 46 formulated with a proteinaceous and/or non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

52. The composition of item 51, which is formulated for topical, enteral/gastrointestinal (e.g., oral), or parenteral administration.

53. A kit comprising the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of items 32 to 46, and a proteinaceous and/or non-proteinaceous cargo to be transduced by the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant.

54. The synthetic peptide shuttle agent or synthetic peptide shuttle agent variant of any one of items 32 to 48, the *in vitro* or *in vivo* method of item 49 or 50, the composition of item 51 or 52, or the kit of item 53, wherein the target 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, or gastrointestinal cells.

55. The synthetic peptide shuttle agent or synthetic peptide shuttle agent variant of any one of items 32 to 48 or 54, the *in vitro* or *in vivo* method of item 49 or 50 or 54, the composition of item 51, 52, or 54, or the kit of item 53 or 54, wherein the non-proteinaceous cargo is as defined in any one of items 2 to 4.

56. A process for producing a candidate synthetic peptide shuttle agent expected to have transduction activity for a cargo of interest in target eukaryotic cells, the method comprising synthesizing a peptide which is: (1) a peptide at least 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 +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, wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model (e.g., HeLa) suitable for assessing cargo transduction in said target eukaryotic cells.

57. The process of item 56, wherein the candidate synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of items 32 to 46.

58. An *in vitro* or *in vivo* method for identifying, selecting, or qualifying a synthetic peptide shuttle agent expected to have transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, the method comprising: providing model eukaryotic cells or a model organism suitable for assessing cargo transduction in the target eukaryotic cells; providing a candidate synthetic peptide shuttle agent (e.g., as defined in any one of items 5 to 20 or 32 to 46); and measuring the transduction activity (e.g., cargo transduction efficiency, such as by flow cytometry) of the candidate synthetic peptide shuttle

agent to transduce propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent into the model eukaryotic cells or model organism, wherein the candidate shuttle agent is expected to have transduction activity for both proteinaceous and non-proteinaceous cargoes in the target eukaryotic cells when the transduction activity (e.g., transduction efficiency) of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent is increased 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 the candidate synthetic peptide shuttle agent, and/or a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% (e.g., as determined by flow cytometry) of the propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent occurs, in the model eukaryotic cells or model organism.

EXAMPLES

Example 1: Materials and Methods

All materials and methods not described or specified herein were generally as performed in WO/2016/161516 and/or WO/2018/068135.

1.1 Materials and reagents

Material	Company	City, Province-State, Country
RPMI 1640 media	Sigma-Aldrich	Oakville, ON, Canada
DMEM	Sigma-Aldrich	Oakville, ON, Canada
Alpha MEM	Stem Cell Technology	Oakville, ON, Canada
Fetal bovine serum (FBS)	NorthBio	Toronto, ON, Canada
Geneticin	VWR/100218-044	Ville Mont-Royal, QC, Canada
Non-essential amino acids	VWR/10128-762	Ville Mont-Royal, QC, Canada
Na-pyruvate	VWR/CAAAJ61840-18	Ville Mont-Royal, QC, Canada
HEPES	VWR/CA97061-824	Ville Mont-Royal, QC, Canada
L-glutamine-Penicillin-Streptomycin	Sigma-Aldrich	Oakville, ON, Canada
Trypsin-EDTA solution	Sigma-Aldrich	Oakville, ON, Canada
Dexamethasone	Sigma-Aldrich	Oakville, ON, Canada
CytoTox-ONE	Promega	Madison, Wisconsin, United States
DMSO	Sigma-Aldrich/D2650-100ml	Oakville, ON, Canada
Itraconazole	VWR/10188-660	Ville Mont-Royal, QC, Canada
Gant61	Santa Cruz Biotechnology/SC-202630	Dallas, Texas, United States
HPI4	Cedarlane/A16349-10	Burlington, ON, Canada
Arsenic trioxide (ATO)	VWR/CAAA33289-14	Ville Mont-Royal, QC, Canada
Recombinant mouse Sonic HedgeHog (mShh)	Genscript/Z03050	Piscataway, NJ, United States
ONE-Step Luciferase Assay kit	BPS Bioscience/ 60690-1	San Diego, CA
Propidium iodide (PI)	Sigma-Aldrich/P4170-10MG	Oakville, ON, Canada

HisPrep™ column	GE Healthcare	Baie d'Urfe, QC, Canada
Q Sepharose™	GE Healthcare	Baie d'Urfe, QC, Canada
Amicon Ultra centrifugal filters	EMD Millipore	Etobicoke, ON Canada
Resazurin sodium salt	Sigma-Aldrich/R7017-1G	Oakville, ON, Canada
PES syringe filter 0.2um	VWR/28145-501	Ville Mont-Royal, QC, Canada
Alexa™-594 Anti-Mouse	Abcam #150116	Toronto, ON, Canada
Fluoroshield™ with DAPI	Sigma #F6057	Oakville, ON, Canada
Phusion™ High-Fidelity DNA polymerase	(NEB #M0530S)	Whitby, ON, Canada
Opti-MEM™	Sigma-Aldrich	Oakville, ON, Canada
QX-314	Sigma Aldrich/L5783-250MG	Oakville, ON, Canada

1.3 Cell lines and culture conditions

Cells were cultured following the manufacturer's instructions.

Cell lines	Description	ATCC/others	Culture media	Serum	Additives
HeLa	Human cervical carcinoma cells	ATCC™ CCL-2	DMEM	10% FBS	L-glutamine 2 mM Penicillin 100 units Streptomycin 100µg/mL
NIH3T3 Gli-luciferase cells	Mouse Swiss NIH embryo fibroblasts	BPS Bioscience/60409	DMEM	10% BCS	1% Pen/Strep 500 µg/ml Geneticin
			Opti-MEM	0.5% BCS	1% Non essential amino acids 1mM Na-pyruvate 10mM HEPES 1% Pen/Strep
HEK293 cells	Human embryonic kidney 293 cells modified to express Nav1.7	Thériault et al., 2015	DMEM	10% FBS	L-glutamine 2 mM penicillin 100 U/mL Streptomycin 10 mg/mL

FBS: Fetal bovine serum
BCS: Bovine calf serum

1.4 Propidium iodide transduction protocol

HeLa cells were plated (20 000 cells/well) in a 96 well-dish the day prior the experiment. Each delivery mix comprising a synthetic peptide shuttle agent (10 µM) and the propidium iodide (PI) (10 µg/mL) or the GFP-NLS (10 µM) were prepared and completed to 50 µL with phosphate-buffered saline (PBS). Cells were washed once with PBS and the Shuttle/PI or Shuttle/GFP-NLS added on cells for one minute. Then 100 µL DMEM containing 10% FBS was added to the mix and removed. Cells were washed once with PBS and incubated in DMEM containing 10% FBS. Cells were analyzed after 2-hour incubation by flow cytometry. For the condition "FS then PI", only the synthetic peptide shuttle agent (10 µM) was added on HeLa cells for 1 minute and one hour later PI (10 µg/mL) was added for one minute following the same washing step. Cells were analyzed one hour after PI or GFP-NLS treatment.

1.5 Hedgehog pathway inhibitors transduction protocol in Gli reporter NIH3T3 cells

Stock solutions of cargoes were prepared as follows: Gant61 stock (20 mM in DMSO); HPI4 stock (40 mM in DMSO); Itraconazole stock (4.8 mM (4 mg/mL) in DMSO); Arsenic trioxide (ATO) stock (40 mM in H_2O). Peptide shuttle agent (5 µM) and Hedgehog pathway inhibitor (100 µM) were mixed and volume was completed to 50 µL with PBS.

Hedgehog signaling pathway Gli Reporter NIH 3T3 cells were cultured in DMEM containing 10% calf serum. Cells were trypsinized, centrifuged and resuspended at 10 million cells/mL in PBS. 50 µL of cells (500 000 cells/well) were distributed in a round bottom non-treated 96-well plate. Resuspended cells were mixed with a delivery mix containing the peptide shuttle agent (5 µM) and Hedgehog pathway inhibitor (100 µM). Cells were incubated 90 seconds with the delivery mix at room temperature, 200 µL of DMEM containing 10% calf serum (200 µL) was added in each well, and cells were centrifuged (400g, 4 min.) and washed with 200 µL of PBS. Cells were then resuspended in 200 µL of DMEM and then transferred to a well of a 6-well plate containing 1 mL of DMEM containing 10% calf serum and incubated at 37°C for 2 hours. The media was gently removed and 1 ml of either control media (Opti-MEM™) or activating media (Opti-MEM with 5 µg/mL mShh) was added to each well. Cells were incubated at 37°C for 24-30 hours.

For analyses, cells were trypsinized and resuspended in each well with 200 µL of Opti-MEM™, and then split equally to two wells of a round bottom 96-well plate. Viability was assessed using flow cytometry analysis and ONE-Step Luciferase assay was used to measure luminescence following manufacturer's instructions.

1.6 Hedgehog pathway inhibitors transduction protocol *in vivo*

Cargoes were suspended as recommended: Gant61 stock 20 mM in DMSO; Itraconazole stock 4.8 mM (4 mg/mL) in DMSO. Female C57BL6 mice aged between 6 to 7 weeks were shaved and depilated using hair removal product (Nair™). Five days after depilation, 30 µL of a mix containing PBS, the synthetic peptide shuttle agent FSD250D (**SEQ ID NO: 36**), and/or the cargo were applied on 3 cm^2 of the depilated skin. Mice were imaged 3, 10 and 17 days after treatment.

1.7 QX-314 and GFP-NLS co-transduction and patch-clamp technique

Cell culture. HEK293 cells stably expressing Nav1.7 were grown in Dulbecco's minimal essential medium (DMEM, Gibco BRL Life Technologies) supplemented with fetal bovine serum (FBS, 10%), L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (10 mg/mL). The cells were incubated at 37ºC in a 5% CO_2 humidified atmosphere.

Delivery. Cells were seeded 24 hours prior to the experiment in 24-well plate. Cells were washed twice with PBS. A solution containing 1mM of QX-314, 5µM of FSD194 and 15µM of GFP-NLS protein was applied to cells for 90 seconds and removed by aspiration. Control was performed using 5µM of FSD194 or of 2.5mM

QX-314, in presence of GFP-NLS. Cells were washed with 800 µL of DMEM containing 10% FBS and transferred in the recording solution to perform the electrophysiology. GFP-positive cells were determined by microscopy and then selected for patch-clamp analysis.

Electrophysiology. Frequency protocol with QX-314 was recorded within 30 s after the whole-cell configuration was formed. Frequency protocol consists of a pulse of 10 ms at -20 mV from a holding potential of -140 mV at 10 Hz. Whole-cell Na+ currents in HEK293 cells were recorded at room temperature using an Axopatch 200B with the whole-cell configuration of the patch-clamp technique (Molecular Devices). pClamp v10.0 was used for the pulse stimulations and recordings (Molecular Devices). Currents were filtered at 5 kHz, digitized at 100 kHz using a Digidata 1550 AD converter (Molecular devices), and stored on a computer for subsequent analyses. Series resistance was compensated by 70-80%. When needed, linear leak current artifacts were removed using on-line leak subtraction. Fire-polished low-resistance electrodes (2MΩ) were pulled from 8161 glass (Corning).

Recording Solutions. Bath solution: 35 mM NaCl, 115 mM NMDG, 2 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES. The pH was adjusted to pH 7.3 with 1 M NaOH. Pipette solution: 35 mM NaCl, 105 mM CsF, 10 mM EGTA, and 10 mM HEPES. The pH was adjusted to pH 7.4 with 1 M CsOH.

Example 2: Synthetic peptide shuttle agents enable intracellular delivery of propidium iodide

Propidium iodide (PI) is a fluorescent DNA intercalating dye often used as a nuclear stain in fluorescence microscopy and flow cytometry applications. Binding of PI to DNA results in enhanced fluorescence by 20- to 30-fold, as well as a shift in its maximum excitation/emission spectra. Since PI is not normally able to cross the plasma membrane of live cells, it is routinely used to detect dead cells in a cell population. It was surprisingly found herein that synthetic peptide shuttle agents, including shuttle agent peptides described in WO/2016/161516 and WO/2018/068135 for the transduction of proteinaceous cargoes, are able to transduce PI as well as other non-proteinaceous cargoes.

HeLa cells were cultured as described in **Example 1.3** and subjected to the PI transduction protocol as described in **Example 1.4**, with the proteinaceous cargo GFP-NLS being transduced separately as a control in some experiments. Results were acquired by flow cytometry two hours after delivery and expressed as percentages of fluorescent cells (% PI+ cells or % GFP+ cells), as shown in **Figs. 1A-1D** and as summarized in the table shown in **Fig. 2**.

Figs. 1 and 2 show delivery and viability results of HeLa cells co-incubated for 1 minute with a synthetic peptide shuttle agent or control peptide, combined with either the non-proteinaceous cargo PI (**Fig. 1A and 1B**) or the proteinaceous cargo GFP-NLS (**Fig. 1C and 1D**). Multiple members of different families of peptide shuttle agents or control peptides were tested. The first group of synthetic peptide shuttle agents tested comprises an endosome leakage domain (ELD) operably linked to a cell penetrating domain (CPD), as previously described in WO/2016/161516 for their ability to transduce proteinaceous cargoes. The second and

third groups of synthetic peptide shuttle agents tested correspond to those rationally-designed and optimized for the delivery of proteinaceous cargoes, the second group being peptides previously described in WO/2018/068135. The fourth group of synthetic peptide shuttle agents tested correspond to cyclic peptides possessing either an amide bond between its C and N termini (e.g., "FSD268 cyclic amide"; **SEQ ID NO: 49**) or a disulfide bridge between two flanking cysteines added in N and C terminal positions (e.g., "FSD268 cyclic disulfide"; **SEQ ID NO: 50**). The fifth group of peptides are negative control peptides that do not respect several synthetic peptide shuttle agent rational-design parameters described in WO/2018/068135 (e.g., FSN3, FSN4 and FSN8; **SEQ ID NOs: 54, 55, and 57**, respectively). These negative control peptides also include "FSD10 scramble" (**SEQ ID NO: 51**), "FSD268 scramble" (**SEQ ID NO: 52**), and "FSD174 scramble" (**SEQ ID NO: 53**) peptides having the same amino acid compositions as the peptide shuttle agents FSD10, FSD268, and FSD174, respectively (**SEQ ID NOs: 13, 43, and 32**, respectively), but in which the order of the amino acids (i.e., the primary amino acid sequence) is changed to deviate from several of the rational-design parameters described in WO/2018/068135. In **Fig. 1A and 1B**, "FS then PI" indicates that PI was added 1 hour after the treatment with the synthetic peptide shuttle agents, ensuring that PI-positive signal is not due to cell death. Finally, the right-most bars in **Fig. 1A-1D** correspond to negative controls in which cells were incubated with cargo alone ("PI" in **Fig. 1A and 1B** or "GFP-NLS" in **Fig. 1C and 1D**), or untreated cells that were not exposed to the cargo or shuttle peptides ("NT", **Fig. 1A-1D**).

Collectively, the results reveal that members of the family of synthetic peptide shuttle agents comprising an ELD operably linked to a CPD (as described in WO/2016/161516), as well as those rationally-designed for the transduction of proteinaceous cargoes (as described in WO/2018/068135), are able to increase the transduction efficiency of a non-proteinaceous, relatively low molecular weight cargo such as PI (in addition to their protein transduction activity). Strikingly, several negative control peptides that fail to respect rational-design parameters described in WO/2018/068135 for the delivery of proteinaceous cargoes also failed to transduce PI, suggesting that the rational-design parameters of WO/2018/068135 may also apply to the design of peptide shuttle agents for the delivery of non-proteinaceous cargoes.

Furthermore, the same synthetic peptide shuttle in linear form (FSD268; **SEQ ID NO: 43**), in circularized form using amide (FSD268 cyclic amide; **SEQ ID NO: 49**) or disulfide (FSD268 Cyclic Disulfide; **SEQ ID NO: 50**) bonds, increased the delivery of PI, confirming that the synthetic shuttle peptides need not be linear to be functional.

Example 3: Synthetic peptide shuttle agents enable intracellular delivery of small molecule inhibitors of the HedgeHog signalling pathway

A rationally-designed peptide shuttle agent, FSD250D (**SEQ ID NO: 36**), having efficient transduction activity for proteinaceous cargoes, was evaluated for its ability to transduce small molecule inhibitors of the HedgeHog signalling pathway in cultured cells, as described in **Example 1.5**. The FSD250D peptide has the

same amino acid sequence as FSD250 (**SEQ ID NO: 35**), except that all the amino acids in FSD250D are D-amino acids. Results are shown in **Fig. 3** and in **Table 1**.

Table 1: Hedgehog pathway inhibitor delivery to GLI reporter NIH3T3 cells

	+ FSD250D		- FSD250D	
Conditions	**Mean luminescence intensity**	**Standard Deviation**	**Mean luminescence intensity**	**Standard Deviation**
Ctrl - mShh	-	-	**0**	626
Ctrl + mShh	-*	-	**6461**	773
Gant61	**4216**	240	**6770**	647
HPI-4	**1993**	318	**6370**	981
Itraconazole	**1519**	682	**5612**	682
ATO	**4686**	216	**5967**	562

* Previous experiments affirmed that the presence of the peptide FSD250D together with mShh did not significantly result in a change in luminescence intensity.

Briefly, the NIH3T3 Gli-luciferase reporter cell line is designed to monitor the activity of the HedgeHog signaling pathway and contains the firefly luciferase gene under the control of Gli responsive elements stably integrated into NIH3T3 cells. As shown in **Fig. 3** and **Table 1**, exposure of the NIH3T3 Gli-luciferase reporter cells to recombinant mouse Sonic HedgeHog protein as a positive control ("Ctrl+ mShh") results in an increase in luminescence intensity that is not observed in the negative control cells which were not exposed to mShh ("Ctrl – mShh"). The presence of the peptide shuttle agent FSD250D had no effect on cellular luminescence intensity following mShh stimulation (data not shown), which was expected given that the receptor for mShh (Patched) is at the cell surface (not intracellular). However, exposure of the reporter cells to structurally different small molecule inhibitors of the HedgeHog signalling pathway that bind to intracellular targets (Gant61, HPI-4, Itraconazole, or ATO) resulted in significantly reduced cellular luminescence intensity in the presence of FSD250D as compared to in the absence of FSD250D, suggesting successful transduction of the small molecules by the peptide shuttle agent. Similar results were observed using the peptide FSD19 (data not shown).

Example 4: Synthetic peptide shuttle agents enable intracellular delivery of small molecule inhibitors of the HedgeHog signalling pathway

A rationally-designed peptide shuttle agent, FSD250D (**SEQ ID NO: 36**), having efficient transduction activity for proteinaceous cargoes, was evaluated for its ability to transduce small molecule inhibitors of the HedgeHog signalling pathway in a depilated mouse model, as described in **Example 1.6**.

Briefly, depilation of mouse skin induces hair growth associated with a strong induction of the HedgeHog pathway and increased expression of Gli1. This experiment consisted of activating the HedgeHog pathway in mice by depilation, and then measuring the delay in hair regrowth by delivering in the skin cells small molecule HedgeHog pathway inhibitors that bind to intracellular targets (Gant61 or Itraconazole). The results in **Fig. 4** show that mice treated with the small molecule HedgeHog inhibitors Gant61 or Itraconazole

(100 μM) in the presence of FSD250D showed delayed hair regrowth at 10 days post-treatment (*), as compared to in the absence of FSD250D.

Example 5: Synthetic peptide shuttle agents enable co-intracellular delivery of small molecule sodium channel inhibitor (QX-314) and GFP-NLS in HEK293 cells

The small molecule compound QX-314 (Lidocaine N-ethyl bromide) is a quaternary derivative of lidocaine. QX-314 is not membrane permeable. When delivered to the cell cytoplasm, the QX-314 blocks both fast Na+-dependent action potentials and voltage-dependent, non-inactivating Na+ conductance (Ilfeld and Yaksh, 2009). To evaluate the simultaneous co-transduction of a small molecule and a proteinaceous cargo by peptide shuttle agents, HEK293 cells stably expressing the sodium channel Nav1.7 were exposed to a mixture of QX-314 and GFP-NLS in the presence or absence of the peptide shuttle agent FSD194 (**SEQ ID NO: 33**). As a control, cells were also treated with GFP-NLS and the peptide shuttle agent FSD194 in the absence of QX-314. Results were evaluated using the patch-clamp technique as described in **Example 1.7** and representative whole-cell Na+ currents of the treated HEK293 cells are shown in **Fig. 5A-5C**. Currents were evoked with a 10 ms depolarizing pulse at 10 Hz. Reduction of the current amplitude was observed when cells were incubated for 90 seconds with QX-314 and GFP-NLS in the presence of the peptide shuttle agent FSD194 (i.e., 1 mM QX-314 + 15 μM GFP-NLS + 5 μM FSD194), consistent with the presence of QX-314 inside the cells (**Fig. 5C**). In contrast, the same current amplitude reduction was not observed when the cells were incubated without QX-314 (i.e., 15 μM GFP-NLS + 5 μM FSD194 +; **Fig. 5A**) or with QX-314 but in the absence of FSD194 (i.e., 2.5 mM QX-314 + 15 μM GFP-NLS; **Fig. 5B**). Furthermore, GFP-NLS-positive cells were identified in the QX-314 + GFP-NLS + FSD194 and in the FSD194 + GFP-NLS conditions, but not in the QX-314 + GFP-NLS conditions, indicating that GFP-NLS was indeed co-transduced along with the QX-314 by the peptide shuttle agent.

Example 6: Robust PI transduction predicts shuttle agents having proteinaceous cargo transduction activity

High-throughput screening efforts to identify, select, and/or qualify novel peptide shuttle agents having protein transduction activity can rapidly become prohibitively expensive due to the high cost of manufacturing and purifying large quantities of recombinant proteins as cargoes, particularly for complex proteins such as recombinant immunoglobulins. The use of GFP or GFP-NLS as a proteinaceous cargo is advantageous, as it enables rapid screening by flow cytometry to assess intracellular delivery. However, the use of GFP-NLS requires verification by microscopy for each peptide shuttle agent, in parallel to flow cytometry measurements, to ensure that the candidate shuttle agent enabled the GFP-NLS cargo to avoid endosomal entrapment and gain access the cytosol/nucleus, which is resource- and time-consuming. Thus, a more cost effective "surrogate" cargo that could reliably predict protein transduction activity and endosomal escape would be highly desirable.

The results in **Example 2** demonstrate that synthetic peptide shuttle agents having validated transduction activity for GFP (and other proteinaceous cargoes) can also transduce small molecules such as PI.

This raises the intriguing possibility of the converse being true: whether PI can be used as a reliable "surrogate" cargo to screen for and identify/select/qualify novel shuttle agents that possess robust transduction activity for proteinaceous cargoes. Commercially, PI is widely available and relatively inexpensive. Furthermore, PI exhibits 20- to 30-fold enhanced fluorescence and a detectable shift in maximum excitation/emission spectra *only after being bound to genomic DNA* – a property that makes it particularly suitable to distinguish endosomally-trapped cargo from endosomally-escaped cargo having access to the cytosolic/nuclear compartment. Thus, intracellular delivery and endosomal escape could both be measurable by flow cytometry since any PI that remained trapped in endosomes would not reach the nucleus and would exhibit neither the enhanced fluorescence nor the spectra shift.

To evaluate the suitability of PI as a "surrogate" cargo for novel shuttle agents, a proprietary library of over 300 candidate peptide shuttle agents was screened in parallel for both PI and GFP-NLS transduction activity in HeLa cells using flow cytometry as generally described in **Example 1.4**. Aside from the concentrations of the cargoes (i.e., 10 μg/mL for PI vs 10 μM for GFP-NLS), the transduction protocols were otherwise the same.

Due to the large number of peptides screened, negative controls were performed in parallel for each experimental batch and included a "no treatment" (NT) control in which the cells were not exposed to shuttle peptide or cargo, as well as a "cargo alone" control in which cells were exposed to the cargo in the absence of shuttle agent. Results are shown in **Figs. 6 and 7**, in which "transduction efficiency" refers to the percentage of all viable cells that are positive for the cargo (PI or GFP-NLS). "Mean Delivery score" provides a further indication of the total amount of cargo that was delivered per cell, amongst all cargo-positive cells. Mean PI or GFP-NLS delivery score was calculated by multiplying the mean fluorescence intensity (of at least duplicate samples) measured for the viable PI+ or GFP+ cells by the mean percentage of viable PI+ or GFP+ cells, divided by 100,000 for GFP delivery or by 10,000 for PI delivery. The Mean Delivery Scores for PI and GFP-NLS for each candidate shuttle agent was then normalized by dividing by the Mean Delivery Score for the "cargo alone" negative control performed in parallel for each experimental batch. Thus, the "Norm. Mean Delivery Score" in **Fig. 6 and 7** represents the fold-increase in Mean Delivery Score over the "cargo alone" negative control.

The batch-to-batch variation observed for the negative controls was relatively small for GFP-NLS but was appreciably higher with PI as cargo. For example, the variation in transduction efficiency for the "cargo alone" negative control ranged from 0.4% to 1.3% for GFP-NLS and from 0.9% to 6.3% for PI. Furthermore, transduction efficiencies for several negative control peptides (i.e., peptides known to have low or no GFP transduction activity) tested in parallel (e.g., FSD174 Scramble; data not shown) sometimes gave lower transduction efficiencies for PI (but not for GFP-NLS) than the "cargo alone" negative control, in some cases by as much as 5%, perhaps due to non-specific interactions between PI and the peptides. This phenomenon was not observed for GFP-NLS transduction experiments. The foregoing suggested that the shuttle agent transduction

efficiencies at least for PI may be more appropriately compared to that of a negative control peptide rather than to the "cargo alone" condition.

The screening of over 300 candidate peptide shuttle agents for PI and GFP-NLS transduction activity revealed that shuttle agents showing robust transduction efficiency for PI generally correlated with robust transduction efficiency for GFP-NLS. Strikingly, progressively higher PI transduction efficiencies were generally associated with progressively higher GFP-NLS transduction efficiencies. This is illustrated by grouping all the candidate shuttle agents screened into increment windows according to their PI transduction efficiencies and then calculating the average GFP transduction efficiency for all shuttle agents falling within that %PI window, as shown in the table below.

Mean PI transduction efficiency (%PI+) window	**Mean GFP transduction efficiency (%GFP+) within %PI+ window**
less than 10%	12%
10-14%	21%
15-19%	30%
20-29%	40%
30-39%	48%
40-49%	53%
50-59%	60%
60-69%	69%
70-79%	77%
at least 80%	80%

Fig. 6 shows results of all candidate peptide shuttle agents screened that had a mean PI transduction efficiency of 10% or higher, sorted based on their level of mean PI transduction efficiency. Strikingly, of the 306 candidate peptide shuttle agents having a mean PI transduction efficiency of at least 10%, 96% of the candidate peptide shuttle agents exhibited GFP transduction efficiencies of 10% or higher. Thresholds of at least 15% and 20% PI transduction efficiency correspond to values of at least 2.5- and 3-fold higher than the highest PI transduction efficiency for the "cargo alone" negative control observed (about 6%) in all experimental batches. Of the 273 candidate peptide shuttle agents listed in **Fig. 6** having a mean PI transduction efficiency of at least 15%, 97% of the candidate peptide shuttle agents exhibited GFP transduction efficiencies of 15% or higher. Moreover, of the 256 candidate peptide shuttle agents listed in **Fig. 6** having a mean PI transduction efficiency of at least 20%, 99.6% of the candidate peptide shuttle agents exhibited GFP transduction efficiencies of 10% or higher, and 96% of the candidate peptide shuttle agents exhibited GFP transduction efficiencies of 20% or higher.

These results strongly suggest that robust PI delivery predicts peptide shuttle agents having robust proteinaceous cargo transduction activity, and thus that PI can indeed be used as a "surrogate" cargo to screen for and identify/select/qualify novel peptide shuttle agents having dual cargo transducing activity (i.e., for small molecules and proteins).

Included amongst the candidate peptide shuttle agents in **Fig. 6** having a mean PI transduction efficiency of at least 20% were peptides having lengths of less than 20 residues: FSD390 (17 aa), FSD367 (19 aa), and FSD366 (18 aa). Also included amongst the candidate peptide shuttle agents in **Fig. 6** having a mean PI transduction efficiency of at least 20% were peptides comprising either non-physiological amino acid analogs (e.g., FSD435, which corresponds to FSD395 except for lysine residues (K) being replaced with L-2,4-diaminobutyric acid residues) or chemical modifications (e.g., FSD438, which corresponds to FSD10 except for an N-terminal octanoic acid modification; FSD436, which corresponds to FSD222 except for phenylalanine residues (F) being replaced with (2-naphthyl)-L-alanine residues; FSD171, which corresponds to FSD168 except having an N-terminal acetyl group and a C-terminal cysteamide group. These results confirm the robustness of the peptide shuttle agent platform technology to tolerate the use of non-physiological amino acids or analogs thereof in place of physiological amino acids and/or chemical modifications.

Example 7: Lower levels of PI delivery are less predictive of peptide shuttle agents having proteinaceous cargo transduction activity

The results of the over 300 candidate peptide shuttle agents screened in **Example 6** having a mean PI transduction efficiency of less than 10% but a mean GFP-NLS transduction efficiency of at least 7% are shown in **Fig. 7**, this time sorted according to their level of mean GFP transduction efficiency.

For candidate peptides having PI transduction efficiencies less than 10%, the large-scale nature of the screening approach employed herein may preclude any firm conclusions as to their potential lack of cargo transduction activity. Indeed, WO/2016/161516 and WO/2018/068135 disclose that shuttle agent peptides function in a concentration-dependent manner and that multiple elements such as shuttle agent concentration, cargo concentration, exposure time, and cell-type may influence shuttle agent performance in transduction assays. The large-scale screening of candidate peptide shuttle agents described herein imposed a "blanket" single shuttle agent concentration, a single cargo concentration, a single exposure time/protocol to each and every peptide tested. Thus, it is difficult to make any firm conclusions as to the non-proteinaceous cargo transduction activity based solely on a low PI transduction efficiency observed in this large-scale screening.

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Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Thr Gln Gly Gly
1 5 10 15

Ser Pro Pro Pro Ser Gly Arg Arg Leu Lys Ala Lys Arg Ala Lys Ala
20 25 30

<210> 25
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD155

<400> 25

Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Thr Gln Gly Gly
1 5 10 15

Ser Gly Glu Gly Ser Gly Arg Arg Leu Lys Ala Lys Arg Ala Lys Ala
20 25 30

<210> 26
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD156

<400> 26

Trp Ile Arg Leu Phe Thr Lys Leu Trp Arg Ile Phe Gln Gln Gly Lys
1 5 10 15

Arg Ile Lys Ala Lys Arg Ala
20

<210> 27
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD157

<400> 27

Trp Ile Arg Leu Phe Thr Lys Leu Trp Arg Ile Phe Gln Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Lys Arg Ile Lys Ala Lys Arg Ala
20 25

<210> 28
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD159

<400> 28

Trp Ile Arg Leu Phe Thr Lys Leu Trp Arg Ile Phe Arg Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Lys Arg Ile Lys Ala Lys Ala Ala
20 25

<210> 29
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD162

<400> 29

Ile Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Lys Lys Ala Gln Ala Ala Lys Arg
20 25

<210> 30
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD168

<400> 30

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25

<210> 31
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD173

<400> 31

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 32
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD174

<400> 32

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala
20 25 30

Arg

<210> 33
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD194

<400> 33

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Lys Ala Lys Arg Ala Lys Ala
20 25 30

<210> 34
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD220

<400> 34

Trp Ala Arg Ala Phe Ala Lys Ala Trp Arg Ile Phe Gln Gln Gly Lys
1 5 10 15

Arg Ile Lys Ala Lys Arg Ala
20

<210> 35
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD250

<400> 35

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 36
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD250D

<220>
<221> MUTAGEN
<222> (1)..(30)
<223> All D-amino acids

<400> 36

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 37
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD253

<400> 37

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Arg Gly Gly Arg Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 38
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD258

<400> 38

Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 39
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD262

<400> 39

Lys Trp Lys Leu Leu Arg Leu Trp Ser Arg Leu Leu Arg Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 40
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD263

<400> 40

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Ala Arg Gln Ala Arg
20 25 30

<210> 41
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD264

<400> 41

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Ala Arg Ala Ala Arg
20 25 30

<210> 42
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD265

<400> 42

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Ala Ala Arg Gln Ala Arg
20 25 30

<210> 43
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD268

<400> 43

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 44
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD286

<400> 44

Lys Trp Lys Leu Leu Arg Ala Leu Ala Arg Leu Leu Lys Leu Ala Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Arg Arg Leu Gly Ala Arg Ala Gln Ala
20 25 30

Arg

<210> 45
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD271

<400> 45

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Arg
1 5 10 15

Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 46
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD272

<400> 46

Lys Trp Lys Leu Ala Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 47
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD273

<400> 47

Lys Trp Lys Leu Leu Arg Ala Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 48
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD276

<400> 48

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Arg Ala Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 49
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD268 Cyclic Amide

<220>
<221> MOD_RES
<222> (1)..(32)
<223> Cyclic peptide: covalent link between K1 and R32

<400> 49

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 50
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD268 Disulfide

<220>
<221> DISULFID
<222> (1)..(34)
<223> Cyclic peptide: disulfiude bond between C1 and C34

<400> 50

Cys Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp
1 5 10 15

Gly Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala
20 25 30

Arg Cys

<210> 51
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD10 Scarmble

<400> 51

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln Ala Arg
20 25 30

Thr Gly

<210> 52
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD268 Scramble

<400> 52

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 53
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD174 Scramble

<400> 53

Leu Gly Arg Ser Gly Arg Ile Lys Ile Gly Gly Trp Ser Ala Leu Ala
1 5 10 15

Ser Arg Ala Arg Gln Ala Arg Gly Leu Lys Ile Trp Thr Gln Gly Arg
20 25 30

Leu

<210> 54
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSN3

<400> 54

His His His His His His Gln Phe Leu Cys Phe Trp Leu Asn Lys Met
1 5 10 15

Gly Lys His Asn Thr Val Trp His Gly Arg His Leu Lys Cys His Lys
20 25 30

Arg Gly Lys Gly
35

<210> 55
<211> 35
<212> PRT
<213> Artificial Sequence

<220>
<223> FSN4

<400> 55

His His His His His His Leu Leu Tyr Leu Trp Arg Arg Leu Leu Lys
1 5 10 15

Phe Trp Cys Ala Gly Arg Arg Val Tyr Ala Lys Cys Ala Lys Ala Tyr
20 25 30

Gly Cys Phe
35

<210> 56
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSN7

<400> 56

Leu Ile Lys Leu Trp Ser Arg Phe Ile Lys Phe Trp Thr Gln Gly Arg
1 5 10 15

Arg Ile Lys Ala Lys Leu Ala Arg Ala Gly Gln Ser Trp Phe Gly
20 25 30

<210> 57
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> FSN8

<400> 57

His His His His His His Phe Arg Lys Leu Trp Leu Ala Ile Val Arg
1 5 10 15

Ala Lys Lys

<210> 58
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD117

<400> 58

His His His His His His Phe Leu Lys Phe Trp Ser Arg Leu Phe Lys
1 5 10 15

Phe Trp Thr Gln Gly Arg Arg Lys Gly Ala Gln Ala Ala Phe Arg
20 25 30

<210> 59
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD118

<400> 59

His His His His His His Ile Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Ile Trp Thr Gln Gly Arg Arg Lys Gly Ala Gln Ala Ala Ile Arg
20 25 30

<210> 60
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD119

<400> 60

His His His His His His Phe Leu Lys Ile Trp Ser Arg Ala Leu Ile
1 5 10 15

Lys Ile Trp Thr Gln Gly Leu Arg Lys Gly Ala Gln Ala Ala Lys Arg
20 25 30

<210> 61
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD121

<400> 61

His His His His His His Val Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Ile Trp Thr Gln Gly Arg Arg Lys Gly Ala Gln Ala Ala Val Arg
20 25 30

<210> 62
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD122

<400> 62

His His His His His His Phe Leu Lys Val Trp Ser Arg Leu Val Lys
1 5 10 15

Val Trp Thr Gln Gly Arg Arg Lys Gly Ala Gln Ala Ala Phe Arg
20 25 30

<210> 63
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD123

<400> 63

His His His His His His Val Leu Lys Val Trp Ser Arg Leu Val Lys
1 5 10 15

Val Trp Thr Gln Gly Arg Arg Lys Gly Ala Gln Ala Ala Val Arg
20 25 30

<210> 64
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD124

<400> 64

His His His His His His Phe Leu Lys Ile Trp Gln Arg Leu Ile Lys
1 5 10 15

Ile Trp Gln Gln Gly Arg Arg Lys Gly Ala Gln Ala Ala Phe Arg
20 25 30

<210> 65
<211> 31
<212> PRT

<213> Artificial Sequence

<220>
<223> FSD125

<400> 65

His His His His His His Phe Leu Lys Ile Trp Asn Arg Leu Ile Lys
1 5 10 15

Ile Trp Asn Asn Gly Arg Arg Lys Gly Ala Asn Ala Ala Phe Arg
20 25 30

<210> 66
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD126

<400> 66

His His His His His His Phe Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Ile Trp Thr Gln Gly Trp Arg Thr Gly Ala Gln Ala Gly Phe
20 25 30

<210> 67
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD127

<400> 67

His His His His His His Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Gly Trp Thr Gln Gly Trp Arg Thr Ile Ala Gln Ala Leu Gly
20 25 30

<210> 68
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD128

<400> 68

His His His His His His Phe Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Ile Trp Pro Gln Pro Arg Arg Lys Gly Ala Gln Ala Ala Phe Arg
20 25 30

<210> 69
<211> 31

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD130

<400> 69

Leu Ile Lys Ile Trp Thr Gln Phe Leu Lys Ile Trp Ser Arg Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 70
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD132

<400> 70

His His His His His His Arg Phe Ala Ala Gln Ala Gly Lys Arg Arg
1 5 10 15

Gly Gln Thr Trp Ile Lys Ile Leu Arg Ser Trp Ile Lys Leu Phe
20 25 30

<210> 71
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD133

<400> 71

His His His His Phe Leu His His Ser Trp Ile Lys Lys Ile Leu Arg
1 5 10 15

Thr Trp Ile Arg Arg Gly Gln Gln Ala Gly Lys Phe Ala Ala Arg
20 25 30

<210> 72
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD135

<400> 72

Leu Ile Arg Lys Trp Ile His Leu Ile His Ser Trp Phe Gln Asn Leu
1 5 10 15

Arg Arg Leu Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 73

<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD137

<400> 73

Leu Leu Arg Lys Trp Ser His Leu Leu His Ile Trp Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 74
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD138

<400> 74

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Arg Arg Leu Lys Ala Lys Arg Ala Lys
20 25 30

Ala

<210> 75
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD139

<400> 75

His His His His His His Leu Ile Arg Leu Trp Ser His Leu Ile His
1 5 10 15

Ile Trp Phe Gln Asn Arg Arg Leu Lys Trp Lys Lys Lys Tyr Ala Arg
20 25 30

Ala Ala Ala Arg Gln Ala Arg Ala
35 40

<210> 76
<211> 46
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD140

<400> 76

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His His His His His His Leu Ile Arg Leu Trp Ser His Leu Ile His
1               5                   10                  15


Ile Trp Phe Gln Asn Arg Arg Leu Lys Trp Lys Lys Lys Tyr Ala Arg
            20                  25                  30


Ala Ala Ala Arg Gln Ala Arg Ala His His His His His His
        35                  40                  45


<210>  77
<211>  41
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD141

<400>  77

Leu Ile Arg Leu Trp Ser His Leu Ile His Ile Trp Phe Gln Asn Arg
1               5                   10                  15


Arg Leu Lys Trp Lys Lys Lys Gly Gly Ser Gly Gly Gly Ser Tyr Ala
            20                  25                  30


Arg Ala Ala Ala Arg Gln Ala Arg Ala
        35                  40


<210>  78
<211>  25
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD142

<400>  78

Phe Leu Lys Ile Trp Ser His Leu Ile His Ile Trp Thr Gln Gly Arg
1               5                   10                  15


Arg Leu Lys Ala Lys Arg Ala Lys Ala
            20                  25


<210>  79
<211>  22
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD143

<400>  79

Leu Ile Arg Lys Trp Ile His Leu Ile His Ser Trp Phe Gln Gly Arg
1               5                   10                  15


Arg Leu Gly Ala Arg Ala
            20


<210>  80
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<211> 49
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD144

<400> 80

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His His His His His His Lys Lys Ala Leu Leu Ala His Ala Leu His
1               5                   10                  15


Leu Leu Ala Leu Leu Ala Leu His Leu Ala His Ala Leu Lys Lys Ala
            20                  25                  30


Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg His His His His His
        35                  40                  45


His
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<210> 81
<211> 52
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD145

<400> 81

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His His His His His His Lys Lys His Leu Leu Ala His Ala Leu His
1               5                   10                  15


Leu Leu Ala Leu Leu Ala Leu His Leu Ala His Ala Leu Ala His Leu
            20                  25                  30


Lys Lys Ala Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg His His
        35                  40                  45


His His His His
    50
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<210> 82
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD147

<400> 82

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Leu Leu Lys Leu Trp Thr Gln Leu Leu Lys Leu Trp Ser Arg Gly Gly
1               5                   10                  15


Ser Gly Gly Gly Ser Arg Arg Leu Lys Ala Lys Arg Ala Lys Ala
            20                  25                  30
```

<210> 83
<211> 28

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD148

<400> 83

His His His His His His Met Val Thr Val Leu Phe Arg Arg Leu Arg
1 5 10 15

Ile Arg Arg Ala Cys Gly Pro Pro Arg Val Arg Val
20 25

<210> 84
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD149

<400> 84

His His His His His His Met Val Arg Val Leu Thr Arg Phe Leu Arg
1 5 10 15

Ile Gly Ala Arg Cys Arg Arg Pro Pro Val Val Arg
20 25

<210> 85
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD150

<400> 85

His His His His His His Trp Ile Thr Trp Leu Phe Lys Arg Leu Lys
1 5 10 15

Ile Arg Arg Ala Ala Gly Gln Ser Lys Phe Arg Ile Ala Gly
20 25 30

<210> 86
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD151

<400> 86

His His His His His His Trp Ile Thr Trp Leu Arg Lys Ile Leu Lys
1 5 10 15

Arg Phe Arg Lys Ala Ala Gln Ser Gly Phe Arg Ile Ala Gly
20 25 30

<210> 87

<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD152

<400> 87

His His His His His His Trp Ile Thr Trp Leu Arg Lys Ile Leu Lys
1 5 10 15

Arg Phe Gly Lys Ala Ala Gln Ser Gly Phe Arg Ile Ala Arg
20 25 30

<210> 88
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD153

<400> 88

His His His His His His Trp Ile Thr Trp Leu Arg Lys Ile Leu Lys
1 5 10 15

Arg Leu Gly Gly Ala Ala Gln Ser Ile Ile Thr Gly Gly Gln
20 25 30

<210> 89
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD154

<400> 89

His His His His His His Trp Ile Thr Trp Leu Phe Lys Arg Leu Lys
1 5 10 15

Ile Arg Arg Ala Ala Gly Gly Ser Gly Gly Gly Ser Gln Ser Lys Phe
20 25 30

Arg Ile Ala Gly
35

<210> 90
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD158

<400> 90

Trp Ile Arg Leu Phe Thr Lys Leu Trp Arg Ile Phe Arg Gln Gly Lys
1 5 10 15

Arg Ile Lys Ala Lys Ala Ala
            20

<210> 91
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD160

<400> 91

Ile Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1   5   10   15

Arg Leu Gly Ala Gln Ala Ala Leu Arg
            20   25

<210> 92
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD161

<400> 92

Ile Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1   5   10   15

Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Gln Ala Ala Leu Arg
            20   25   30

<210> 93
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD163

<400> 93

Ile Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1   5   10   15

Ser Gly Gly Gly Ser Arg Arg Lys Lys Ala Gln Ala Ala Lys Arg
            20   25   30

<210> 94
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD164

<400> 94

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1   5   10   15

```
Arg Leu Gly Ala Arg Ala Ala Arg Ala
            20                  25


<210>  95
<211>  31
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD165

<400>  95

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1               5                   10                  15


Ser Gly Gly Gly Ser Arg Arg Lys Lys Ala Arg Ala Ala Arg Ala
            20                  25                  30


<210>  96
<211>  26
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD166

<400>  96

Leu Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Thr Lys Gly Arg
1               5                   10                  15


Arg Lys Lys Ala Arg Ala Ala Gln Ala Arg
            20                  25


<210>  97
<211>  32
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD167

<400>  97

Leu Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Thr Lys Gly Gly
1               5                   10                  15


Ser Gly Gly Gly Ser Arg Arg Lys Lys Ala Arg Ala Ala Gln Ala Arg
            20                  25                  30


<210>  98
<211>  31
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  FSD169

<400>  98

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1               5                   10                  15
```

Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
            20   25   30

<210> 99
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD170

<400> 99

Leu Ile Lys Ile Trp Thr Gln Leu Leu Lys Ile Trp Ser Arg Gly Arg
1   5   10   15

Arg Leu Gly Ala Arg Ala Gln Ala Arg
            20   25

<210> 100
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD171

<220>
<221> MOD_RES
<222> (1)..(1)
<223> Acetyl

<220>
<221> MOD_RES
<222> (25)..(25)
<223> Amide

<220>
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<223> Cysteamide

<400> 100

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1   5   10   15

Arg Leu Gly Ala Arg Ala Gln Ala Arg
            20   25

<210> 101
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD172

<400> 101

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1   5   10   15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Gln Ala Arg
20 25 30

<210> 102
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD175

<400> 102

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 103
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD176

<400> 103

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Gly Gly Ser Ala Arg Ala Ala
20 25 30

Arg Gln Ala Arg
35

<210> 104
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD177

<400> 104

Lys Leu Lys Ile Trp Ser Arg Leu Ile Arg Lys Trp Thr Lys Gly Leu
1 5 10 15

Arg Leu Gly Ala Gln Ala Gln Ala Arg
20 25

<210> 105
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD178

<400> 105

Lys Leu Lys Ile Trp Ser Arg Leu Ile Arg Lys Trp Thr Lys Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Leu Arg Leu Gly Ala Gln Ala Gln Ala Arg
20 25 30

<210> 106
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD179

<400> 106

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Gly Arg Glu Ser Arg Lys Pro Arg Lys Ser Arg Gln
20 25

<210> 107
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD180

<400> 107

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Arg Gly Arg Glu Ser Arg Lys Pro Arg Lys Ser
20 25 30

Arg Gln

<210> 108
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD181

<400> 108

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Leu
1 5 10 15

Gly Leu Leu Val Leu Arg Val Arg Ala Gly Lys Arg
20 25

<210> 109
<211> 34
<212> PRT

<213> Artificial Sequence

<220>
<223> FSD182

<400> 109

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Leu Gly Leu Leu Val Leu Arg Val Arg Ala Gly
20 25 30

Lys Arg

<210> 110
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD183

<400> 110

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala
20

<210> 111
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD184

<400> 111

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Ala Arg
20

<210> 112
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD185

<400> 112

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Ala Arg Gln
20 25

<210> 113
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD186

<400> 113

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Gly Leu Glu Ala Arg Ala Pro Arg Lys Ala Arg
20 25

<210> 114
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD187

<400> 114

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Lys Pro Arg Lys Ser Arg Gln
20 25

<210> 115
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD188

<400> 115

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Gly Arg Glu Ser Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 116
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD189

<400> 116

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Arg Ala Gln Arg Ala Gln Arg Ala
32

20 25

<210> 117
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD190

<400> 117

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Ala Gln Arg Ala Gln Arg Ala
20

<210> 118
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD191

<400> 118

His His His His His His Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Ile Trp Thr Gln Gly Thr Arg Ser Lys Arg Ala Gly Leu Gln Phe Pro
20 25 30

<210> 119
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD192

<400> 119

His His His His His His Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys
1 5 10 15

Ile Trp Thr Gln Gly Val Gly Arg Val His Arg Leu Leu Arg Lys
20 25 30

<210> 120
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD193

<400> 120

Lys Trp Lys Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Arg
1 5 10 15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala
20 25 30

Arg

<210> 121
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD195

<400> 121

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Lys Ala Arg Ala Gln Ala Arg
20 25

<210> 122
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD196

<400> 122

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Ala Ala Arg
20 25

<210> 123
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD197

<400> 123

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Lys Ala Arg Ala Ala Ala Arg
20 25

<210> 124
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD198

<400> 124

Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly Ser Gly Gly Gly
1 5 10 15

Ser Arg Arg Lys Gly Ala Gln Ala Ala Phe Arg
20 25

<210> 125
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD199

<400> 125

Trp Ser Arg Leu Ile Thr Lys Ile Trp Arg Ile Phe Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Ala
20

<210> 126
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD200

<400> 126

Trp Ser Arg Leu Ile Thr Lys Ile Trp Arg Ile Phe Thr Gln Gly Arg
1 5 10 15

Arg Leu Lys Ala Arg Ala Ala
20

<210> 127
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD201

<400> 127

Trp Ser Arg Leu Ile Lys Leu Trp Thr Gln Gly Arg Arg Leu Lys Ala
1 5 10 15

Arg Ala Ala

<210> 128
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD202

<400> 128

Trp Ile Arg Leu Phe Lys Leu Trp Gln Gln Gly Lys Arg Ile Lys Ala
1 5 10 15

Lys Arg Ala

<210> 129
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD203

<400> 129

Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg Arg Leu Gly Ala
1 5 10 15

Arg Ala Gln Ala Arg
20

<210> 130
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD204

<400> 130

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg
20

<210> 131
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD205

<400> 131

Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg Arg Leu Gly Ala
1 5 10 15

Arg

<210> 132
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD206

<400> 132

Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg Arg Leu
1 5 10 15

Gly Ala Arg Ala Gln
20

<210> 133
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD207

<400> 133

Leu Ala Lys Ala Trp Ala Arg Ala Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25

<210> 134
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD208

<400> 134

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 135
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD209

<400> 135

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln Ala Arg Thr
20 25 30

Gly

<210> 136
<211> 32

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD210

<400> 136

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Lys Ala Lys Arg Ala Lys Ala
20 25 30

<210> 137
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD211

<400> 137

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln Ala Arg
20 25 30

Thr Gly

<210> 138
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD212

<400> 138

Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser Arg
1 5 10 15

Arg Leu Lys Ala Lys Arg Ala Lys Ala
20 25

<210> 139
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD213

<400> 139

Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Arg Arg Leu Lys Ala Lys

20 25

<210> 140
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD214

<400> 140

Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser Arg
1 5 10 15

Arg Leu Lys Ala Lys
20

<210> 141
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD215

<400> 141

Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly
1 5 10 15

Ser Arg Arg Leu Lys Ala Lys Arg Ala
20 25

<210> 142
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD216

<400> 142

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Gly Arg Ser Arg Lys Pro Arg Lys Ser Arg Gln
20 25

<210> 143
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD217

<400> 143

Lys Trp Lys Leu Lys Leu Trp Arg Leu Lys Gly Gly Ser Gly Gly Gly
1 5 10 15

Ser Arg Arg Ala Lys Ala
20

<210> 144
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD218

<400> 144

Lys Trp Lys Leu Lys Leu Trp Arg Leu Lys Ser Arg Leu Lys Leu Trp
1 5 10 15

Arg Leu Lys Gly Gly Ser Gly Gly Gly Ser Arg Arg Ala Lys Ala
20 25 30

<210> 145
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD219

<400> 145

Trp Ile Arg Leu Trp Thr His Leu Trp His Ile Trp Gln Gln Gly Lys
1 5 10 15

Arg Ile Lys Ala Lys Arg Ala
20

<210> 146
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD221

<400> 146

Trp Lys Leu Ile Arg Leu Phe Thr Arg Leu Ile Lys Ile Trp Gly Gln
1 5 10 15

Arg Arg Leu Lys Ala Lys Arg Ala
20

<210> 147
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD222

<400> 147

Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly Gln Arg Arg
1 5 10 15

Leu Lys Ala Lys Arg Ala
20

<210> 148
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD223

<400> 148

Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gln Gly Gly Ser
1 5 10 15

Gly Gly Gly Ser Arg Arg Leu Lys Ala Lys Arg Ala
20 25

<210> 149
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD224

<400> 149

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gln Arg Arg Leu Lys Ala Lys Arg Ala
20 25

<210> 150
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD225

<400> 150

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Arg Arg Leu Lys Ala Lys Arg Ala
20 25 30

<210> 151
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD226

<400> 151

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gln Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25

<210> 152
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD227

<400> 152

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Arg Arg Leu Gly Ala Arg Ala Gln Ala
20 25 30

Arg

<210> 153
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD228

<400> 153

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gln Arg Arg Leu Lys Ala Lys Arg Ala Lys Ala
20 25

<210> 154
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD229

<400> 154

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Arg Arg Leu Lys Ala Lys Arg Ala Lys
20 25 30

Ala

<210> 155
<211> 26
<212> PRT

<213> Artificial Sequence

<220>
<223> FSD230

<400> 155

Lys Trp Lys Leu Ala Lys Ala Trp Ala Arg Ala Leu Lys Leu Trp Gly
1 5 10 15

Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25

<210> 156
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD231

<400> 156

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Lys Ala Lys Arg Ala Leu Lys
20 25 30

Ala

<210> 157
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD232

<400> 157

Lys Trp Lys Trp Ala Arg Ala Trp Ala Arg Ala Trp Lys Lys Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 158
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD233

<400> 158

Lys Leu Lys Leu Ala Arg Ala Leu Ala Arg Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 159
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD234

<400> 159

Lys Ile Lys Ile Ala Arg Ala Ile Ala Arg Ala Ile Lys Lys Ile Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 160
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD235

<400> 160

Lys Phe Lys Phe Ala Arg Ala Phe Ala Arg Ala Phe Lys Lys Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 161
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD236

<400> 161

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Ser
1 5 10 15

Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser Arg Arg Leu
20 25 30

Gly Ala Arg Ala Gln Ala Arg
35

<210> 162
<211> 39
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD237

<400> 162

Lys Trp Lys Leu Leu Lys Leu Trp Thr Gln Leu Leu Lys Leu Trp Thr

1 5 10 15

Gln Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser Arg Arg Leu
20 25 30

Gly Ala Arg Ala Gln Ala Arg
35

<210> 163
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD238

<400> 163

Lys Trp Lys Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 164
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD239

<400> 164

Lys Trp Lys Leu Leu Lys Ile Trp Thr Gln Leu Ile Lys Ile Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Gln Ala Arg Gln Ala Arg
20 25 30

<210> 165
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD240

<400> 165

Lys Trp Lys Ala Leu Leu Ala Leu Ala Leu His Leu Ala His Leu Ala
1 5 10 15

Leu His Leu Lys Lys Ala Gly Arg Arg Lys Gly Ala Gln Ala Ala Phe
20 25 30

Arg

<210> 166
<211> 33

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD241

<400> 166

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Ala Ala Arg Gln Ala Arg
20 25 30

Ala

<210> 167
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD243

<400> 167

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Ala Ala Arg
20 25 30

Gln Ala Arg Ala
35

<210> 168
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD244

<400> 168

Lys Trp Lys Leu Ala Lys Ala Trp Ala Arg Ala Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Ala Ala Arg Lys Ala Lys
20 25 30

Arg Ala

<210> 169
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD246

<400> 169

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Ala Ala Arg Lys Ala Lys
20 25 30

Arg Ala

<210> 170
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD247

<400> 170

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Ala Ala Arg
20 25 30

Lys Ala Lys Arg Ala
35

<210> 171
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD248

<400> 171

Lys Trp Lys Leu Ala Lys Ala Trp Ala Arg Ala Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 172
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD250 Scramble

<400> 172

Arg Gly Lys Leu Trp Ser Leu Ser Lys Leu Lys Gly Trp Gly Gly Ala
1 5 10 15

Arg Ala Ser Lys Ala Gln Leu Ala Arg Leu Gly Leu Trp Arg
20 25 30

<210> 173
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD250E

<400> 173

Lys Trp Lys Leu Leu Glu Leu Trp Ser Glu Leu Leu Glu Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 174
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD251

<400> 174

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Glu Ala Ala Glu Gln Ala Glu
20 25 30

<210> 175
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD254

<400> 175

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Arg Gly Gly Arg Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 176
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD255

<400> 176

Lys Trp Lys Leu Leu Lys Leu Trp Gly Gly Ser Arg Leu Leu Lys Leu
1 5 10 15

Trp Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg

20 25 30

<210> 177
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD256

<400> 177

Lys Trp Lys Leu Leu Lys Leu Gly Arg Trp Ser Arg Leu Gly Leu Lys
1 5 10 15

Leu Trp Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala
20 25 30

Arg

<210> 178
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD257

<400> 178

Lys Trp Lys Leu Leu Lys Leu Trp Ala Ala Ser Arg Leu Leu Lys Leu
1 5 10 15

Trp Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 179
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD259

<400> 179

Lys Trp Lys Leu Leu Lys Leu Ala Arg Trp Ser Arg Leu Ala Leu Lys
1 5 10 15

Leu Trp Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala
20 25 30

Arg

<210> 180
<211> 30
<212> PRT
<213> Artificial Sequence

<220>

<223> FSD260

<400> 180

Arg Trp Arg Leu Leu Arg Leu Trp Ser Arg Leu Leu Arg Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 181
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD261

<400> 181

Gly Gly Ser Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 182
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD266

<400> 182

Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 183
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD267

<400> 183

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Ala Arg Tyr Ala Arg
20 25 30

<210> 184
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD269

<400> 184

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Tyr Ala Arg Tyr Ala Arg
20 25 30

<210> 185
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD270

<400> 185

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Ala Ala Ala Glu Lys
20 25

<210> 186
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD274

<400> 186

Lys Trp Lys Leu Ala Arg Ala Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 187
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD275

<400> 187

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Ala Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 188
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD276

<400> 188

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Arg Ala Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 189
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD277

<400> 189

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Ala Arg Ala Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 190
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD278

<400> 190

Lys Trp Lys Leu Ala Arg Ala Trp Ser Arg Leu Ala Arg Ala Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 191
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD279

<400> 191

Lys Trp Lys Leu Ala Arg Ala Leu Ala Arg Ala Trp Ser Arg Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 192
<211> 30
<212> PRT

<213> Artificial Sequence

<220>
<223> FSD280

<400> 192

Lys Trp Lys Leu Leu Lys Leu Trp Lys Arg Leu Leu Lys Lys Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 193
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD281

<400> 193

Lys Trp Ser Leu Leu Lys Leu Trp Ser Ala Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 194
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD282

<400> 194

Lys Trp Lys Leu Trp Lys Leu Leu Ser Arg Leu Trp Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 195
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD283

<400> 195

Lys Trp Lys Leu Ala Arg Lys Phe Lys Arg Ala Ile Lys Lys Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 196
<211> 30

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD284

<400> 196

Lys Trp Ala Leu Ala Arg Ala Phe Ala Arg Ala Ile Ala Ile Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 197
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD285

<400> 197

Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Arg Arg Leu Gly Ala Arg Ala Gln Ala Arg
20 25 30

<210> 198
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD287

<400> 198

Lys Trp Lys Leu Leu Arg Ala Leu Ala Arg Leu Leu Lys Ala Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Arg Arg Leu Gly Ala Arg Ala Gln Ala
20 25 30

Arg

<210> 199
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD288

<400> 199

Lys Trp Lys Leu Leu Lys Trp Trp Ser Arg Leu Leu Lys Trp Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg

20 25 30

<210> 200
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD289

<400> 200

Lys Trp Lys Leu Leu Lys Phe Trp Ser Arg Leu Leu Lys Phe Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 201
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD290

<400> 201

Lys Trp Lys Leu Leu Lys Leu Tyr Ser Arg Leu Leu Lys Leu Tyr Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 202
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD291

<400> 202

Lys Trp Lys Leu Leu Lys Leu Phe Ser Arg Leu Leu Lys Leu Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 203
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD292

<400> 203

Lys Trp Lys Leu Leu Ser Leu Trp Ser Ser Leu Leu Ser Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 204
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD293

<400> 204

Lys Trp Lys Leu Leu Ser Leu Trp Ser Arg Leu Leu Ser Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 205
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD2294

<400> 205

Lys Trp Lys Leu Leu Lys Leu Trp Ser Ser Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 206
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD295

<400> 206

Lys Trp Lys Leu Leu Lys Leu Trp Ser Leu Leu Lys Leu Trp Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 207
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD296

<400> 207

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gln
1 5 10 15

Gln Gly Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln
20 25 30

Ala Arg

<210> 208
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD297

<400> 208

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Asn
1 5 10 15

Asn Gly Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln
20 25 30

Ala Arg

<210> 209
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD298

<400> 209

Ser Trp Ser Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 210
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD299

<400> 210

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Ile
1 5 10 15

Lys Ile Phe Gly Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 211
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD300

<400> 211

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Trp
1 5 10 15

Arg Ile Phe Gly Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 212
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD301

<400> 212

Gly Gly Ser Gly Gly Gly Ser Lys Trp Lys Leu Leu Lys Leu Trp Ser
1 5 10 15

Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 213
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD302

<400> 213

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Gly Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 214
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD303

<400> 214

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 215
<211> 25
<212> PRT

<213> Artificial Sequence

<220>
<223> FSD304

<400> 215

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gln
1 5 10 15

Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 216
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD305

<400> 216

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Gly Gly Gly Gly Gly Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 217
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD306

<400> 217

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Gln Ala Arg
20 25

<210> 218
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD307

<400> 218

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg
20 25

<210> 219
<211> 32

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD308

<400> 219

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gly Ala Arg Ala Gly Ala Arg Gly Ala Arg
20 25 30

<210> 220
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD309

<400> 220

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Gly Ala Gln Ala Gly Gln Ala Gly
20 25 30

<210> 221
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD310

<400> 221

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Gly Arg Gly Gln Gly Arg Gln Gly Arg
20 25 30

<210> 222
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD311

<400> 222

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Gly Gly Arg Gly Gly Gly Arg
20 25 30

<210> 223

<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD312

<400> 223

Trp Ile Arg Leu Phe Thr Lys Leu Trp Ile Phe Gln Gln Gly Gly Ser
1 5 10 15

Gly Gly Gly Ser Lys Arg Ile Lys Ala Lys Arg Ala
20 25

<210> 224
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD313

<400> 224

Trp Ile Arg Leu Phe Ser Arg Leu Trp Arg Ile Phe Gln Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Lys Arg Ile Lys Ala Lys Arg Ala
20 25

<210> 225
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD314

<400> 225

Lys Trp Lys Trp Ile Arg Leu Phe Ser Arg Leu Trp Arg Ile Phe Gln
1 5 10 15

Gln Gly Gly Ser Gly Gly Gly Ser Lys Arg Ile Lys Ala Lys Arg Ala
20 25 30

<210> 226
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD315

<400> 226

Trp Ile Arg Leu Phe Ser Arg Leu Trp Arg Ile Phe Gln Gln Gly Gly
1 5 10 15

Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 227
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD316

<400> 227

Lys Trp Lys Trp Ile Arg Leu Phe Ser Arg Leu Trp Arg Ile Phe Gln
1 5 10 15

Gln Gly Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln
20 25 30

Ala Arg

<210> 228
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD317

<400> 228

Trp Ile Arg Leu Phe Thr Lys Leu Trp Gln Ile Phe Gln Gln Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 229
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD318

<400> 229

Trp Ile Arg Leu Phe Thr Lys Leu Trp Arg Ile Phe Gln Gln Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 230
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD319

<400> 230

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Ala Ala Ala Gln Lys
20 25

<210> 231
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD320

<400> 231

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Ala Ala Ala Gln Gln
20 25

<210> 232
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD321

<400> 232

Lys Trp Lys Leu Ala Lys Ala Trp Ser Arg Ala Ile Lys Ile Trp Gly
1 5 10 15

Ala Arg Ala Gln Ala Arg Gln Ala
20

<210> 233
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD322

<400> 233

Lys Trp Lys Leu Ala Lys Ala Trp Ser Arg Ala Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Gln Ala Arg Gln Ala
20 25 30

<210> 234
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD323

<400> 234

Trp Ile Arg Leu Phe Thr Arg Leu Ile Lys Ile Trp Gly Gln Arg Arg
1 5 10 15

Leu Lys Ala Lys Arg Ala
20

<210> 235
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD324

<400> 235

Trp Ala Arg Ala Phe Ala Arg Ala Trp Arg Ile Phe Gln Gln Arg Arg
1 5 10 15

Leu Lys Ala Lys Arg Ala
20

<210> 236
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD325

<400> 236

Trp Ala Arg Ala Phe Ala Arg Ala Trp Arg Ile Phe Gln Gln Arg Arg
1 5 10 15

Leu Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 237
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD326

<400> 237

Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly Gln Ala Arg
1 5 10 15

Ala Gln Ala Arg Gln Ala Arg
20

<210> 238
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD327

<400> 238

Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly Arg Arg Leu

1 5 10 15

Lys Ala Lys Arg Ala Lys Ala
20

<210> 239
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD328

<400> 239

Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly Arg Arg Leu
1 5 10 15

Gly Ala Arg Ala Gln Ala Arg
20

<210> 240
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD330

<400> 240

Leu Ala Arg Ala Phe Ala Arg Ala Leu Leu Lys Leu Trp Gly Gln Arg
1 5 10 15

Arg Leu Lys Ala Lys Arg Ala
20

<210> 241
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD331

<400> 241

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Gly Gln Arg Arg Leu
1 5 10 15

Lys Ala Lys Arg Ala
20

<210> 242
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD332

<400> 242

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Gly Arg Arg Leu Gly
1 5 10 15

Ala Arg Ala Gln Ala Arg
20

<210> 243
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD333

<400> 243

Lys Trp Lys Leu Leu Arg Leu Leu Leu Arg Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 244
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD334

<400> 244

Lys Trp Lys Leu Leu Arg Trp Leu Trp Arg Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 245
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD335

<400> 245

Lys Trp Lys Leu Ala Arg Leu Leu Leu Arg Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 246
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD336

<400> 246

Lys Trp Lys Leu Leu Arg Leu Phe Leu Arg Leu Phe Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 247
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD337

<400> 247

Lys Trp Lys Leu Ala Arg Trp Leu Trp Arg Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 248
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD338

<400> 248

Lys Trp Lys Leu Leu Arg Trp Phe Trp Arg Leu Phe Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 249
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD339

<400> 249

Lys Trp Lys Leu Ala Arg Leu Phe Leu Arg Ala Phe Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 250
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD340

<400> 250

Lys Trp Lys Leu Ala Arg Trp Phe Trp Arg Ala Phe Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 251
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD341

<400> 251

Leu Leu Lys Ile Trp Ser Arg Leu Ile Lys Ile Trp Thr Gln Gly Arg
1 5 10 15

Arg Leu Gly Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg
20 25 30

Gln Ala Arg Thr Gly
35

<210> 252
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD342

<400> 252

Lys Trp Lys Leu Ala Arg Trp Phe Trp Arg Ala Phe Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln Ala Arg
20 25 30

Thr Gly

<210> 253
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD343

<400> 253

Lys Trp Lys Leu Leu Gln Leu Trp Ser Arg Leu Leu Gln Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 254
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD344

<400> 254

Gln Trp Gln Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 255
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD345

<400> 255

Lys Leu Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 256
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD346

<400> 256

Lys Phe Lys Leu Leu Lys Leu Phe Ser Arg Leu Leu Lys Leu Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 257
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD347

<400> 257

Lys Trp Lys Leu Leu Lys Leu Leu Ser Arg Leu Leu Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 258
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD348

<400> 258

Lys Trp Lys Leu Leu Lys Leu Leu Ser Arg Leu Leu Lys Leu Leu Gly
1 5 10 15

Gly Gly Gly Gly Gly Gly Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 259
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD349

<400> 259

Lys Trp Lys Trp Leu Lys Leu Trp Ser Arg Leu Trp Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 260
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD350

<400> 260

Lys Trp Lys Leu Leu Lys Phe Trp Ser Arg Leu Leu Lys Phe Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 261
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD351

<400> 261

Lys Trp Lys Leu Leu Lys Leu Phe Ser Arg Leu Phe Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
70

20 25 30

<210> 262
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD352

<400> 262

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Ile Lys Ile Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 263
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD353

<400> 263

Lys Trp Lys Leu Leu Lys Leu Gln Ser Arg Leu Leu Lys Leu Gln Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 264
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD354

<400> 264

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Gly Arg
20 25

<210> 265
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD355

<400> 265

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gly Ala Arg
20 25

<210> 266
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD356

<400> 266

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Gly
20 25

<210> 267
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD357

<400> 267

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Arg Arg Arg
20 25

<210> 268
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD358

<400> 268

Lys Trp Lys Leu Leu His Leu Trp Ser Arg Leu Leu His Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 269
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD359

<400> 269

Lys Trp Lys Leu Leu Lys Leu Trp Ser Lys Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Gly Gly Gly Gly Gly Ala Lys Ala Ala Lys Gln Ala Lys
20 25 30

<210> 270
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD360

<400> 270

Arg Trp Arg Leu Leu Arg Leu Trp Ser Arg Leu Leu Arg Leu Trp Gly
1 5 10 15

Gly Gly Gly Gly Gly Gly Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 271
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD361

<400> 271

Leu Leu Lys Leu Trp Ser Lys Leu Leu Lys Leu Trp Gly Gly Gly Gly
1 5 10 15

Gly Gly Gly Ala Lys Ala Ala Lys Gln Ala Lys
20 25

<210> 272
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD362

<400> 272

Leu Leu Arg Leu Trp Ser Arg Leu Leu Arg Leu Trp Gly Gly Gly Gly
1 5 10 15

Gly Gly Gly Ala Arg Ala Ala Arg Gln Ala Arg
20 25

<210> 273
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD363

<400> 273

Leu Leu Lys Leu Trp Ser Lys Leu Leu Lys Leu Trp Gly Gly Gly Ala
1 5 10 15

Lys Ala Ala Lys Gln Ala Lys
20

<210> 274
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD364

<400> 274

Leu Leu Arg Leu Trp Ser Arg Leu Leu Arg Leu Trp Gly Gly Gly Ala
1 5 10 15

Arg Ala Ala Arg Gln Ala Arg
20

<210> 275
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD365

<400> 275

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly
1 5 10 15

Gly Gly Gln Ala Arg
20

<210> 276
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD366

<400> 276

Lys Trp Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gly Gly Gly Gln
1 5 10 15

Ala Arg

<210> 277
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD367

<400> 277

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Gly Gly Gly

1 5 10 15

Gln Ala Arg

<210> 278
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD368

<400> 278

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ser Arg Leu Leu Lys
1 5 10 15

Leu Trp Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala
20 25 30

Arg

<210> 279
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD369

<400> 279

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Leu Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 280
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD370

<400> 280

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 281
<211> 32
<212> PRT
<213> Artificial Sequence

<220>

<223> FSD371

<400> 281

Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys Leu Trp Gln
1 5 10 15

Gln Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 282
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD372

<400> 282

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Asn
1 5 10 15

Asn Gly Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln
20 25 30

Ala Arg Thr Gly
35

<210> 283
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD373

<400> 283

Gly Gly Ser Gly Gly Gly Ser Leu Leu Lys Leu Trp Ser Arg Leu Leu
1 5 10 15

Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 284
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD374

<400> 284

Gly Gly Ser Gly Gly Gly Ser Leu Leu Lys Ile Trp Ser Arg Leu Ile
1 5 10 15

Lys Ile Trp Thr Gln Gly Arg Arg Leu Gly Gly Ser Gly Gly Gly Ser
20 25 30

<210> 285

<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD375

<400> 285

Gly Gly Ser Gly Gly Gly Ser Lys Trp Lys Leu Ala Arg Ala Phe Ala
1 5 10 15

Arg Ala Ile Lys Lys Leu Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 286
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD376

<400> 286

Gly Gly Ser Gly Gly Gly Ser Leu Ala Arg Ala Phe Ala Arg Ala Ile
1 5 10 15

Lys Ile Phe Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 287
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD377

<400> 287

Gly Gly Gly Lys Trp Lys Leu Leu Lys Leu Trp Ser Arg Leu Leu Lys
1 5 10 15

Leu Trp Gly Gly Gly
20

<210> 288
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD378

<400> 288

Gly Gly Ser Gly Gly Gly Ser Lys Trp Lys Trp Ile Arg Leu Phe Ser
1 5 10 15

Arg Trp Ile Arg Leu Phe Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 289
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD379

<400> 289

Lys Trp Lys Leu Ser Lys Leu Trp Ser Lys Leu Ser Lys Leu Trp Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Arg Ala Ala Arg Gln Ala Arg
20 25 30

<210> 290
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD381

<400> 290

Leu Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25 30

<210> 291
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD382

<400> 291

Leu Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Leu Leu Gly
1 5 10 15

Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 292
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD383

<400> 292

Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Leu Leu Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 293
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD384

<400> 293

Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Leu Leu Gly Gln Ala Arg
1 5 10 15

Ala Gln Ala Arg Gln Ala Arg
20

<210> 294
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD385

<400> 294

Leu Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Ala Arg Ala Gln Ala Arg Gln Ala Arg
20 25

<210> 295
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD386

<400> 295

Leu Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Leu Leu Gly
1 5 10 15

Gly Gly Gly Lys Gly Gly Gly Lys Gly Gly Lys
20 25

<210> 296
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD387

<400> 296

Gln Leu Gln Leu Leu Arg Leu Leu Leu Arg Leu Leu Lys Lys Leu Gln
1 5 10 15

Leu Gln

<210> 297
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD388

<400> 297

Lys Trp Lys Leu Ala Arg Ala Phe Ser Arg Ala Ile Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln Ala Arg
20 25 30

Thr Gly

<210> 298
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD389

<400> 298

Lys Trp Lys Leu Ala Lys Ala Phe Ser Lys Ala Ile Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Lys Ala Leu Lys Lys Gln Ala Lys
20 25 30

Thr Gly

<210> 299
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD390

<400> 299

Lys Trp Lys Leu Trp Ser Lys Leu Leu Lys Leu Trp Ser Lys Leu Trp
1 5 10 15

Lys

<210> 300
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD391

<400> 300

Gly Gly Lys Gly Gly Lys Gly Gly Lys Trp Lys Leu Leu Lys Leu Trp
1 5 10 15

Ser Arg Leu Leu Lys Leu Trp Gly Gly Lys Gly Gly Lys Gly Gly
20 25 30

<210> 301
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD392

<400> 301

Gly Gly Trp Gly Gly Trp Gly Gly Lys Trp Lys Leu Leu Lys Leu Trp
1 5 10 15

Ser Arg Leu Leu Lys Leu Trp Gly Gly Trp Gly Gly Trp Gly Gly
20 25 30

<210> 302
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD393

<400> 302

Arg Ala Gln Arg Ala Ala Arg Ala Ser Gly Gly Gly Ser Gly Gly Trp
1 5 10 15

Leu Lys Leu Leu Arg Ser Trp Leu Lys Leu Leu Lys Trp Lys
20 25 30

<210> 303
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD394

<400> 303

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Ile Phe Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gly Gly Gly Lys Trp Lys Leu Ala Arg Ala
20 25 30

Phe Ala Arg Ala Ile Lys Ile Phe
35 40

<210> 304
<211> 32

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD395

<400> 304

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 305
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD396

<400> 305

Lys Leu Lys Leu Ala Lys Leu Leu Leu Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 306
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD397

<400> 306

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 307
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD398

<400> 307

Lys Leu Lys Leu Leu Lys Ala Leu Ala Lys Leu Leu Lys Lys Ala Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 308

<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD399

<400> 308

Lys Leu Lys Leu Ala Lys Ala Leu Leu Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 309
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD400

<400> 309

Lys Leu Lys Ala Ala Lys Ala Leu Ala Lys Ala Leu Lys Ala Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 310
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD401

<400> 310

Gly Gly Ser Gly Gly Gly Ser Lys Trp Lys Leu Leu Lys Leu Trp Ser
1 5 10 15

Arg Leu Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser Ala Arg Ala
20 25 30

Ala Arg Gln Ala Arg
35

<210> 311
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD402

<400> 311

Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25

<210> 312
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD403

<400> 312

Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25

<210> 313
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD404

<400> 313

Lys Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25

<210> 314
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD406

<400> 314

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Lys Ala Gln Ala Lys Gln Ala
20 25 30

<210> 315
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD407

<400> 315

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Ala Lys Ala Ala Lys Gln Ala Lys
20 25 30

<210> 316
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD408

<400> 316

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Gly
20 25

<210> 317
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD409

<400> 317

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Gly
20 25

<210> 318
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD410

<400> 318

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Leu Ala Lys Ala Leu Ala Lys Leu Ala Lys
20 25 30

<210> 319
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD411

<400> 319

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15
85

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Leu Ala Lys Gln Ala Lys
20 25 30

<210> 320
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD412

<400> 320

Lys Leu Lys Leu Leu Lys Leu Leu Leu Lys Leu Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Leu Ala Gly
20 25

<210> 321
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD413

<400> 321

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Leu Ala Lys Gln Ala Lys
20 25 30

<210> 322
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD414

<400> 322

Leu Leu Lys Lys Leu Leu His Leu Leu His Ser Leu Leu Gln Asn Leu
1 5 10 15

Lys Lys Leu Gly Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala
20 25 30

Lys Gln Ala Lys
35

<210> 323
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD415

<400> 323

Leu Ile Arg Lys Trp Ile His Leu Ile His Ser Trp Phe Gln Asn Leu
1 5 10 15

Arg Arg Leu Gly Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala
20 25 30

Lys Gln Ala Lys
35

<210> 324
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD416

<400> 324

Gly Gly Ser Gly Gly Gly Ser Lys Trp Lys Leu Ala Lys Ala Trp Ser
1 5 10 15

Arg Ala Leu Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 325
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD417

<400> 325

Gly Gly Ser Gly Gly Gly Ser Leu Ala Lys Ala Trp Ser Arg Ala Leu
1 5 10 15

Lys Leu Trp Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 326
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD418

<400> 326

Gly Gly Ser Gly Gly Gly Ser Lys Leu Lys Leu Leu Lys Leu Leu Leu
1 5 10 15

Lys Leu Leu Lys Lys Leu Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 327
<211> 29

<212> PRT
<213> Artificial Sequence

<220>
<223> FSD419

<400> 327

Gly Gly Ser Gly Gly Gly Ser Lys Leu Lys Leu Ala Lys Ala Leu Ala
1 5 10 15

Lys Ala Leu Lys Lys Leu Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 328
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD421

<400> 328

Gly Gly Ser Gly Gly Gly Ser Leu Leu Lys Lys Leu Leu His Leu Leu
1 5 10 15

His Ser Leu Leu Gln Asn Leu Lys Lys Leu Gly Gly Ser Gly Gly Gly
20 25 30

Ser

<210> 329
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD422

<400> 329

His His His His His His Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg
1 5 10 15

Ala Ile Lys Lys Leu His His His His His His
20 25

<210> 330
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD423

<400> 330

His His His His His His Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys
1 5 10 15

Ile Phe His His His His His His

20

<210> 331
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD424

<400> 331

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser
20 25

<210> 332
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD425

<400> 332

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 333
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD426

<400> 333

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Lys Lys Leu Lys Ala Lys Lys Ala Leu Lys
20 25 30

Ala

<210> 334
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD427

<400> 334

Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly Gly Ser Gly
1 5 10 15

Gly Gly Ser Lys Lys Leu Lys Ala Lys Lys Ala Leu Lys Ala
20 25 30

<210> 335
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD428

<400> 335

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Lys Lys Leu Lys Ala Lys Lys Ala
20 25 30

<210> 336
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD429

<400> 336

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Lys Lys Leu Lys Ala Lys
20 25

<210> 337
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD430

<400> 337

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Leu Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Lys Lys Leu Lys Ala Lys Leu Ala Leu Lys
20 25 30

Ala

<210> 338
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD431

<400> 338

Lys Trp Lys Leu Ala Lys Ala Phe Ala Lys Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Lys Ala Leu Lys Lys Gln Ala Lys
20 25 30

Thr Gly

<210> 339
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD432

<400> 339

Lys Trp Lys Leu Ala Arg Ala Phe Ala Arg Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Lys Ala Gln Ala Lys Gln Ala Lys
20 25 30

<210> 340
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD433

<400> 340

Lys Leu Lys Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Tyr Ala Arg Ala Leu Arg Arg Gln Ala Arg
20 25 30

Thr Gly

<210> 341
<211> 34
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD434

<400> 341

Lys Trp Lys Leu Ala Lys Ala Phe Ala Lys Ala Ile Lys Lys Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gly Gly Lys Gly Gly Lys Lys Gln Gly Lys
20 25 30

Thr Gly

<210> 342
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD435

<220>
<221> MUTAGEN
<222> (1)..(32)
<223> Xaa is L-2,4-diaminobutyric acid

<400> 342

Xaa Leu Xaa Leu Leu Xaa Leu Leu Leu Xaa Leu Leu Xaa Xaa Leu Gly
1 5 10 15

Gly Ser Gly Gly Gly Ser Gln Ala Xaa Ala Gln Ala Xaa Gln Ala Xaa
20 25 30

<210> 343
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> FSD436

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Thr Gly
```

CLAIMS:

1. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, the shuttle agent being:

(1) a peptide at least 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 +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,

wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells;
and/or

wherein the shuttle agent increases the transduction efficiency of GFP-NLS 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of GFP-NLS, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells; and

wherein the shuttle agent comprises or consists of:

(a) the amino acid sequence of **SEQ ID NO: 285**;
(b) an amino acid sequence that differs from the amino acid sequence of **SEQ ID NO: 285** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids; or
(c) an amino acid sequence that is at least 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 the full-length amino acid sequence of **SEQ ID NO: 285**.

2. The synthetic peptide shuttle agent of claim 1, 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 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 +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).

3. The synthetic peptide shuttle agent of claim 1 or 2, 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.

4. The synthetic peptide shuttle agent of any one of claims 1 to 3, wherein said shuttle agent comprises (a) a histidine-rich domain positioned towards the N terminus and/or towards the C terminus of the shuttle agent; (b) 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 (c) both (a) and (b).

5. The synthetic peptide shuttle agent of any one of claims 1 to 4, wherein the synthetic peptide: is a cyclic peptide; comprises one or more D-amino acids; and/or 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.

6. The synthetic peptide shuttle agent of claim 5, wherein the chemical modification is at the N and/or C terminus of the shuttle agent; and/or wherein the chemical modification is the addition of an acetyl group, a cysteamide group, or a fatty acid.

7. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, wherein the shuttle agent comprises or consists of:

- the amino acid sequence as defined in claim 1(a); or
- an amino acid sequence that differs from the amino acid sequence as defined in claim 1(a) by only conservative amino acid substitutions;

wherein shuttle agent: increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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; and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells.

8. The synthetic peptide shuttle agent of claim 7, 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.

9. A synthetic peptide shuttle agent variant having transduction activity for proteinaceous and/or non-proteinaceous cargoes in target eukaryotic cells, the synthetic peptide shuttle agent variant being identical to the synthetic peptide shuttle agent as defined in any one of claims 1 to 8, except having at least one amino acid being replaced with a corresponding synthetic amino acid having a side chain of similar physiochemical properties as the amino acid being replaced, wherein the shuttle agent variant increases the transduction efficiency of said cargo in the target eukaryotic cells, as compared to in the absence of the shuttle agent variant, 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-lodoalanine, 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.

10. The synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9:

- when used in an *in vitro* or *ex vivo* method for increasing the transduction efficiency of a proteinaceous and/or non-proteinaceous cargo into target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used at a concentration sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant; or
- for use in therapy, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant transduces a therapeutically active proteinaceous and/or non-proteinaceous cargo to the

cytosol and/or nucleus of target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used at a concentration sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent.

11. An *in vitro* or *ex vivo* method for proteinaceous and/or non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with the cargo and a concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9 sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of said synthetic peptide shuttle agent.

12. A composition for use in therapy, the composition comprising the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9 formulated with a therapeutically active proteinaceous and/or non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

13. The composition for use of claim 12, which comprises a non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent.

14. A process for producing a candidate synthetic peptide shuttle agent expected to have transduction activity for a cargo of interest in target eukaryotic cells, the method comprising synthesizing a peptide which is:

(1) a peptide at least 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 +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,

wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells;
and/or

wherein the shuttle agent increases the transduction efficiency of GFP-NLS 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, and/or enables a transduction efficiency of at least 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%, 50%, 51%,

52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of GFP-NLS, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells; and

wherein the shuttle agent comprises or consists of:

(a) the amino acid sequence of **SEQ ID NO: 285**;

(b) an amino acid sequence that differs from the amino acid sequence of **SEQ ID NO: 285** by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids; or

(c) an amino acid sequence that is at least 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 the full-length amino acid sequence of **SEQ ID NO: 285**.

15. An *in vitro* or *ex vivo* method for non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with a non-proteinaceous cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of said non-proteinaceous cargo, as compared to in the absence of said synthetic peptide shuttle agent, wherein the non-proteinaceous cargo:

(a) is an organic compound;

(b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;

(c) is a small molecule;

(d) is not a polynucleotide or a polysaccharide;

(e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or

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

wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.

16. A synthetic peptide shuttle agent:

– when used in an *in vitro* or *ex vivo* method for increasing the transduction efficiency of a non-proteinaceous cargo into target eukaryotic cells, wherein the synthetic peptide shuttle agent is used at a concentration sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent; or

– when used in therapy, wherein the synthetic peptide shuttle agent transduces a therapeutically active non-proteinaceous cargo to the cytosol and/or nucleus of target eukaryotic cells, wherein the synthetic peptide shuttle agent is used at a concentration sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent;

wherein the non-proteinaceous cargo:

(a) is an organic compound;

(b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;

(c) is a small molecule;

(d) is not a polynucleotide or a polysaccharide;

(e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or

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

wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.

17. A composition for use in therapy, the composition comprising a synthetic peptide shuttle agent formulated with a therapeutically active non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent, and wherein the non-proteinaceous cargo:

(a) is an organic compound;

(b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;

(c) is a small molecule;

(d) is not a polynucleotide or a polysaccharide;

(e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or

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

wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.

18. Use of a composition in the manufacture of a medicament for treating a disease treatable by intracellular delivery of a therapeutically active non-proteinaceous cargo, wherein the composition comprises a synthetic peptide shuttle agent formulated with the therapeutically active non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent, and wherein the non-proteinaceous cargo:

(a) is an organic compound;
(b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;
(c) is a small molecule;
(d) is not a polynucleotide or a polysaccharide;
(e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or
(f) any combination of (a) to (e),

wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.