## Summary

**[005]** The present disclosure relates to pharmaceutical compositions comprising novel, non-naturally occurring fusion molecules and one or more pharmaceutically acceptable carriers, formulated for oral delivery, and designed to provide for improved, effective therapies for treatment of, e.g., inflammatory diseases and/or autoimmune diseases and/or cancers.

**[006]** The present disclosure is based in part on the inventors' unique insight that oral delivery of a pharmaceutical composition comprising a fusion molecule which comprises a modified Cholix toxin coupled to a biologically active cargo may, among other things, provide the following advantages: a) in examples wherein the modified Cholix toxin is coupled to the biologically active cargo without a linker, or with a non-cleavable linker, the anchoring effect of the modified Cholix toxin by its receptor(s) at the surface of, e.g., immune cells that also express the receptor for the biologically active cargo, can allow for greater exposure of the biologically active cargo at the surface of the targeted cells and provide a synergistic effect by binding to both the Cholix receptor and the biologically active cargo receptor; b) in examples wherein the modified Cholix toxin is coupled to the biologically active cargo with a linker that is cleavable by an enzyme present at a basolateral membrane of an epithelial cell, or an enzyme present in the plasma of the subject, such cleavage will allow the biologically active cargo to be released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane c) the direct delivery of the biologically active cargo to the submucosal-GI space and hepatic-portal system may reduce the systemic toxicity observed when the cargo are administered by parenteral routes, as well as enabling access to the submucosal target biology that was difficult to target via non-oral or GI routes; d) once transported across the GI epithelium, the fusion molecules of the disclosure will exhibit extended half-life in serum, that is, the biologically active cargo of the fusion molecules will exhibit an extended serum half-life compared to the biologically active cargo in its non-fused state; e) oral administration of the fusion molecule can deliver a higher effective concentration of the delivered biologically active cargo to the liver of the subject than is observed in the subject's plasma; and f) the ability to deliver the biologically active cargo to a subject without using a needle to puncture the skin of the subject, thus improving such subjects' quality of life by avoiding pain or potential complications associated therewith, in addition to improved patient/care-giver convenience and compliance. Thus, in one aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically

active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is an interleukin-10 having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof. In another aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo selected from interleukin-19 (SEQ ID NO: 83), interleukin-20 (SEQ ID NO: 84), interleukin-22 (SEQ ID NO: 85), interleukin-24 (SEQ ID NO: 86), and interleukin-26 (SEQ ID NO: 87). In another aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a growth hormone having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 95, or a fragment thereof. In another aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a glucose-lowering agent selected from a GLP-1 agonist peptide having the amino acid sequence set forth in SEQ ID NO: 93, and a GLP-1 agonist peptide having the amino acid sequence set forth in SEQ ID NO: 94. In another aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a tumor necrosis factor (TNF) inhibitor having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 92, or a fragment thereof. In another aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a

biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a TNF inhibitor is selected from an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 88 and light chain variable region amino acid sequence set forth in SEQ ID NO: 89, and an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 90 and light chain variable region amino acid sequence set forth in SEQ ID NO: 91. In another aspect, there is provided a pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is an enzyme selected from the group consisting of hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, and PGE-adenosine deaminase.

**[007]** In, in one example, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more

pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a biologically active cargo to be delivered to a subject, wherein the Cholix toxin is non-toxic.

**[008]** In one example, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a biologically active cargo to be delivered to a subject, wherein the Cholix toxin is non-toxic, and wherein the fusion molecule has the ability to activate the receptor for the biologically active cargo, or to enable the catalytic process of a catalytically-active material.

**[009]** In various examples, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin truncated at an amino acid residue within Cholix toxin domain II. In various examples, the fusion molecules comprise a truncated Cholix toxin having the amino acid sequence set forth in, *e.g.,* SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40 or SEQ ID NO: 41.

**[010]** In various examples, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin truncated at an amino acid residue within Cholix toxin domain Ib. In various examples, the fusion molecules comprise a truncated Cholix toxin having the amino acid sequence set forth in, *e.g.,* SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO:48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, or SEQ ID NO: 80.

**[011]** In various examples, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin wherein domain III has been truncated or mutated. In various

examples, the fusion molecules comprise a mutated Cholix toxin having the amino acid sequence set forth in SEQ ID NO: 81 wherein the amino acid residue E581 of SEQ ID NO: 1 has been deleted (designated herein as "Cholix ΔE581").

**[012]** In various examples, the fusion molecules of the pharmaceutical compositions comprise a modified Cholix toxin wherein domain Ia has been mutated.

**[013]** In various examples, the biologically active cargo is selected from *e.g.,* a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material.

**[014]** In various examples, the biologically active cargo is an enzyme selected from hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, or PGE-adenosine deaminase.

**[015]** In various examples, the biologically active cargo is a polypeptide that is a modulator of inflammation in the GI tract selected from, *e.g.,* interleukin-10, interleukin-19, interleukin-20, interleukin-22, interleukin-24, or interleukin-26. In various examples, the biologically active polypeptide is interleukin-10 having the amino acid sequence set forth is SEQ ID NO: 82. In various examples, the biologically active polypeptide is interleukin-19 having the amino acid sequence set forth is SEQ ID NO: 83. In various examples, the biologically active polypeptide is interleukin-20 having the amino acid sequence set forth is SEQ ID NO: 84. In various examples, the biologically active polypeptide is interleukin-22 having the amino acid sequence set forth is SEQ ID NO: 85. In various examples, the biologically active polypeptide is interleukin-24 having the amino acid sequence set forth is SEQ ID NO: 86. In various examples, the biologically active polypeptide is interleukin-26 having the amino acid sequence set forth is SEQ ID NO: 87. In various examples, the biologically active cargo is a modulator of inflammation in the GI tract that is a small molecule. In various examples, the biologically active cargo is a modulator of inflammation in the GI tract that is an antisense or siRNA molecule.

**[016]** In various examples, the biologically active cargo is a TNFSF inhibitor that is an antibody, or a fragment thereof, or an artificial construct comprising an antibody or fragment thereof, or an artificial construct designed to mimic the binding of an antibody or fragment thereof to its antigen. In various examples, the biologically active cargo is a TNFSF inhibitor that is a soluble TNFSF receptor fusion protein. In various examples, the biologically active cargo is a TNFSF inhibitor that is a small molecule. In various examples, the biologically active cargo is a TNFSF inhibitor that is an antisense or siRNA molecule.

**[017]** In various examples, the biologically active cargo is an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 88 and light chain

variable region amino acid sequence set forth in SEQ ID NO: 89. In various examples, the biologically active cargo is an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 90 and light chain variable region amino acid sequences set forth in SEQ ID NO: 91. In various examples, the biologically active cargo is a soluble TNFSF receptor fusion protein dimer comprising the amino acid sequence set forth in SEQ ID NO: 92.

**[018]** In one example, the present disclosure relates to pharmaceutical compositions comprising novel, non-naturally occurring fusion molecules and one or more pharmaceutically acceptable carriers, formulated for oral delivery, and designed to provide for improved, effective therapies for treatment of metabolic disorders, e.g., Type 1 Diabetes and Type 2 Diabetes. Oral delivery of biologically active polypeptides (referring to a polymer composed of amino acid residues; typically also defined as proteins or peptides) has been a long-standing goal of the pharmaceutical industry. Unfortunately, the numerous physical, physiological, and biological barriers of the gastrointestinal (GI) tract are designed to inhibit uptake of proteins and peptides until they can be sufficiently degraded for absorption through amino acid and di- or tri-peptide transporters; and/or to traffic the proteins and peptides intracellularly to destructive lysosome compartments after endosomal uptake at the luminal surface. As such, the feasibility of polypeptide uptake from the intestine in a manner similar to that achievable with, e.g., small molecules, has been limited and low oral bioavailability continues to be a problem for most polypeptides and proteins.

**[019]** In various examples, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a glucose-lowering agent to be delivered to a subject.

**[020]** In various examples, the present disclosure is based in part on that oral delivery of a pharmaceutical composition comprising a fusion molecule which comprises a modified Cholix toxin coupled to a glucose-lowering agent may, among other things, provide the following advantages: a) in examples wherein the modified Cholix toxin is coupled to the glucose-lowering agent without a linker, the anchoring effect of the modified Cholix toxin by its receptor(s) at the surface of cells that also express the receptor for the glucose-lowering agent, can allow for greater exposure of the glucose-lowering agent at the surface of the targeted cells;

b) in examples wherein the modified Cholix toxin is coupled to the glucose-lowering agent with a linker that is cleavable by an enzyme present at a basal-lateral membrane of an epithelial cell, or an enzyme present in the plasma of the subject, such cleavage will allow the glucose-lowering agent to be released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane; c) the direct delivery of the glucose-lowering agent to the submucosal-GI space and hepatic-portal system may reduce the systemic toxicity observed when the glucose-lowering agents are administered by parenteral routes, as well as enabling access to the submucosal target biology that was difficult to target via non-oral or GI routes; d) the direct delivery of the glucose-lowering agent to the submucosal-GI space and hepatic-portal system may provide for improved dosing regimens, including less frequent insulin injections; and e) the ability to deliver the glucose-lowering agent to a subject without using a needle to puncture the skin of the subject, thus improving such subjects' quality of life by avoiding pain or potential complications associated therewith.

**[021]** In various examples, the glucose-lowering agent is selected from *e.g.,* a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material. In various examples, the glucose-lowering agent is an incretin or incretin mimetic. In various examples, the glucose-lowering agent is a GLP-1. In various examples, the glucose-lowering agent is a GLP-1 agonist. In various examples, the glucose-lowering agent is an exendin. In various examples, the glucose-lowering agent is a glucose inhibitory protein receptor (GIPR) agonist.

**[022]** In various examples, the glucose-lowering agent is a GLP-1 agonist that is a peptide. In various examples, the glucose-lowering agent is a GLP-1 agonist that is a small molecule. In various examples, the glucose-lowering agent is a GLP-1 agonist that is an antisense or siRNA molecule. In various examples, the glucose-lowering agent is a GLP-1 agonist that is an antibody, or a fragment thereof, or an artificial construct comprising an antibody or fragment thereof, or an artificial construct designed to mimic the binding of an antibody or fragment thereof to its antigen.

**[023]** In various examples, the biologically active cargo is a glucose-lowering agent that is a GLP-1 agonist peptide comprising the amino acid sequence set forth in SEQ ID NO: 93. In various examples, the biologically active cargo is a glucose-lowering agent that is a GLP-1 agonist peptide comprising the amino acid sequence set forth in SEQ ID NO: 94.

**[024]** In one example, the present disclosure relates to pharmaceutical compositions comprising novel, non-naturally occurring fusion molecules and one or more pharmaceutically

acceptable carriers, formulated for oral delivery, and designed to provide for improved, effective therapies for treatment of growth hormone deficiency, and like disorders.

**[025]** In various examples, the present disclosure relates to pharmaceutical compositions comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, formulated for oral delivery, wherein the fusion molecule comprises a modified Cholix toxin coupled to a growth hormone (GH) to be delivered to a subject.

**[026]** In various examples, the present disclosure is based in part on the inventors' unique insight that oral delivery of a pharmaceutical composition comprising a fusion molecule which comprises a modified Cholix toxin coupled to a growth hormone may, among other things, provide the following advantages: a) in examples wherein the modified Cholix toxin is coupled to the growth hormone with a linker that is cleavable by an enzyme present at a basolateral membrane surface of an epithelial cell, or an enzyme present in the plasma of the subject, such cleavage will allow the growth hormone to be released from the remainder of the fusion molecule soon after transcytosis across the epithelial membrane; b) the direct delivery of the growth hormone to the submucosal-GI space and hepatic-portal system may reduce systemic toxicities observed when the growth hormones are administered by parenteral routes, as well as enabling access to the submucosal target biology that was difficult to target via non-oral or GI routes (e,g, provide a more efficient induction of IGF-1 relative to systemic delivery via subcutaneous (sc) injection); c) the direct delivery of the growth hormone to the submucosal-GI space and hepatic-portal system may provide for improved dosing regimens; d) oral delivery will achieve a brief pulse of growth hormone to the liver that is more consistent with serum level observed in growing children, and this pulse profile is not achievable by sc injection; and e) the ability to deliver the growth hormone to a subject without using a needle to puncture the skin of the subject, thus improving such subjects' quality of life by avoiding pain or potential complications associated therewith, in addition to improved patient/care-giver convenience and compliance.

**[027]** In various examples, the growth hormone is selected from *e.g.,* a macromolecule, small molecule, peptide, polypeptide, nucleic acid, mRNA, miRNA, shRNA, siRNA, antisense molecule, antibody, DNA, plasmid, vaccine, polymer nanoparticle, or catalytically-active material. In various examples, the growth hormone is human growth hormone (or a variant thereof), growth hormone 2, or growth hormone-releasing hormone. In various examples, the growth hormone is human growth hormone (somatotropin) comprising the amino acid sequence set forth in SEQ ID NO: 95.

**[028]** In various examples, the fusion molecules comprise a modified Cholix toxin directly coupled to a biologically active cargo. In various embodiments, the biologically active cargo is directly coupled to the C-terminus of the Cholix toxin.

**[029]** In various examples, the fusion molecules comprise a modified Cholix toxin chemically coupled to a biologically active cargo.

**[030]** In various examples, the fusion molecules comprise a Cholix toxin coupled to a biologically active cargo by a non-cleavable linker. In various examples, the non-cleavable linker comprises the amino acid sequence of, *e.g.*, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98 or SEQ ID NO: 99.

**[031]** In various examples, the fusion molecules comprise a Cholix toxin coupled to a biologically active cargo by a cleavable linker. In various examples, the linker is cleavable by an enzyme that is present at a basolateral membrane of a polarized epithelial cell of the subject. In various examples, the linker is cleavable by an enzyme that is present in the plasma of said subject. In various examples, the cleavable linker comprises the amino acid sequence of, *e.g.*, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 119, or SEQ ID NO: 120.

**[032]** In various examples, the fusion molecules comprise a Cholix toxin coupled to a biologically active cargo by a cleavable linker, wherein the cleavable linker comprises an amino acid sequence that is known to be a substrate for tobacco etch virus (TEV) protease. In various examples, the cleavable linker comprises the amino acid sequence of, *e.g.*, SEQ ID NO: 121.

**[033]** In various examples, the fusion molecule comprises the amino acid sequence set forth in SEQ ID NO: 122. (this is Cholix$^{415}$–TEV-IL-10)

**[034]** In various examples, the fusion molecule comprises the amino acid sequence set forth in SEQ ID NO: 123. (this is Cholix$^{415}$–$(G_4S)_3$-IL-10)

**[035]** In another example, the present disclosure provides a method of treating an inflammatory disease in a subject, comprising orally administering a pharmaceutical composition of the present disclosure to the subject. In various examples, the inflammatory disease is selected from an inflammatory bowel disease, psoriasis or bacterial sepsis. In various examples, the inflammatory bowel disease is Crohn's disease, ulcerative colitis, collagenous

colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's syndrome or indeterminate colitis.

**[036]** In another example, the present disclosure provides a method of treating an autoimmune disease in a subject, comprising orally administering a pharmaceutical composition of the present disclosure to the subject. In various examples, the autoimmune disease is systemic lupus erythematosus (SLE), pemphigus vulgaris, myasthenia gravis, hemolytic anemia, thrombocytopenia purpura, Grave's disease, Sjogren's disease, dermatomyositis, Hashimoto's disease, polymyositis, inflammatory bowel disease, multiple sclerosis (MS), diabetes mellitus, rheumatoid arthritis, or scleroderma.

**[037]** In another example, the present disclosure provides a method of treating a cancer in a subject, comprising orally administering a pharmaceutical composition of the present disclosure to the subject. In various examples, the cancer to be treated includes, but is not limited to, non-Hodgkin's lymphomas, Hodgkin's lymphoma, chronic lymphocytic leukemia, hairy cell leukemia, acute lymphoblastic leukemia, multiple myeloma, carcinomas of the bladder, kidney ovary, cervix, breast, lung, nasopharynx, malignant melanoma and rituximab resistant NHL and leukemia.

**[038]** In another example, the present disclosure provides a method of treating a subject having a metabolic disorder, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disorder, wherein said metabolic disorder is diabetes, obesity, diabetes as a consequence of obesity, hyperglycemia, dyslipidemia, hypertriglyceridemia, syndrome X, insulin resistance, impaired glucose tolerance (IGT), diabetic dyslipidemia, or hyperlipidemia.

**[039]** In another example, the present disclosure provides a method of treating a subject having a fatty liver disease (e.g., nonalcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH)), a gastrointestinal disease, or a neurodegenerative disease, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disease.

**[040]** In another example, the present disclosure provides a method of treating a subject having a GH deficient growth disorder, said method comprising orally administering a fusion molecule of the present disclosure in an amount sufficient to treat said disorder, wherein said disorder is growth hormone deficiency (GHD), Turner syndrome (TS), Noonan syndrome, Prader-Willi syndrome, short stature homeobox-containing gene (SHOX) deficiency, chronic renal insufficiency, and idiopathic short stature short bowel syndrome, GH deficiency due to rare pituitary tumors or their treatment, and muscle-wasting disease associated with HIV/AIDS.

**[041]** In another example, the present disclosure relates to the use of a non-naturally occurring fusion molecule as disclosed herein for the preparation of a medicament for treatment, prophylaxis and/or prevention of an inflammatory disease in a subject in need thereof.

**[042]** In another example, the present disclosure relates to the use of a non-naturally occurring fusion molecule as described herein for the preparation of a medicament for treatment, prophylaxis and/or prevention of an autoimmune disease in a subject in need thereof.

**[043]** In another example, the present disclosure relates to the use of a non-naturally occurring fusion molecule as described herein for the preparation of a medicament for treatment, prophylaxis and/or prevention of a cancer in a subject in need thereof.

**[044]** In another example, the present disclosure relates to the use of a non-naturally occurring fusion molecule as described herein for the preparation of a medicament for treatment, prophylaxis and/or prevention of a metabolic disorder in a subject in need thereof.

**[045]** In another example, the present disclosure relates to the use of a non-naturally occurring fusion molecule as described herein for the preparation of a medicament for treatment, prophylaxis and/or prevention of a fatty liver disease in a subject in need thereof.

**[046]** In another example, the present disclosure relates to the use of a non-naturally occurring fusion molecule as described herein for the preparation of a medicament for treatment, prophylaxis and/or prevention of GH deficient growth disorder in a subject in need thereof.

**[047]** In other examples, the present disclosure provides polynucleotides that encode the non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the present disclosure; vectors comprising polynucleotides encoding non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the disclosure; optionally, operably-linked to control sequences recognized by a host cell transformed with the vector; host cells comprising vectors comprising polynucleotides encoding non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecules of the disclosure; a process for producing a non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecule of the disclosure comprising culturing host cells comprising vectors comprising polynucleotides encoding non-naturally occurring modified Cholix toxin-biologically active cargo

fusion molecules of the disclosure such that the polynucleotide is expressed; and, optionally, recovering the non-naturally occurring modified Cholix toxin-biologically active cargo fusion molecule from the host cell culture medium.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is an interleukin-10 having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 82, or a fragment thereof.

2. The pharmaceutical composition of claim 1, wherein the interleukin-10 has at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 82.

3. The pharmaceutical composition of claim 1, wherein the interleukin-10 has at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 82.

4. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo selected from interleukin-19 (SEQ ID NO: 83), interleukin-20 (SEQ ID NO: 84), interleukin-22 (SEQ ID NO: 85), interleukin-24 (SEQ ID NO: 86), and interleukin-26 (SEQ ID NO: 87).

5. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a growth hormone having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 95, or a fragment thereof.

6. The pharmaceutical composition of claim 5, wherein the growth hormone has at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 95.

7. The pharmaceutical composition of claim 5, wherein the growth hormone has at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 95.

8. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a glucose-lowering agent selected from a GLP-1 agonist peptide having the amino acid sequence set forth in SEQ ID NO: 93, and a GLP-1 agonist peptide having the amino acid sequence set forth in SEQ ID NO: 94.

9. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a tumor necrosis factor (TNF) inhibitor having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 92, or a fragment thereof.

10. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is a TNF inhibitor is selected from an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 88 and light chain variable region amino acid sequence set forth in SEQ ID NO: 89, and an antibody comprising the heavy chain variable region amino acid sequence set forth in SEQ ID NO: 90 and light chain variable region amino acid sequence set forth in SEQ ID NO: 91.

11. A pharmaceutical composition comprising a non-naturally occurring fusion molecule and one or more pharmaceutically acceptable carriers, for use in a method of orally delivering a biologically active cargo to a subject, wherein the fusion molecule comprises a non-toxic Cholix

toxin having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1, or fragment thereof, conjugated to a biologically active cargo that is an enzyme selected from the group consisting of hyaluronidase, streptokinase, tissue plasminogen activator, urokinase, and PGE-adenosine deaminase.

12. The pharmaceutical composition according to any one of claims 1-11, wherein the non-toxic Cholix toxin has at least 95% sequence identity to the amino acid set forth in SEQ ID NO: 1.

13. The pharmaceutical composition according to any one of claims 1-11, wherein the non-toxic Cholix toxin has at least 99% sequence identity to the amino acid set forth in SEQ ID NO: 1.

14. The pharmaceutical composition according to any one of claims 1-13, wherein the non-toxic Cholix toxin is covalently coupled to the biologically active cargo.

15. The pharmaceutical composition according to any one of claims 1-13, wherein the non-toxic Cholix toxin is non-covalently coupled to the biologically active cargo.