POLYCISTRONIC EXPRESSION OF GUT PEPTIDES

FIELD

[0001] The disclosure relates to compositions and methods in the field of molecular biology. Specifically, the disclosure relates to polycistronic expression constructs for the expression of peptides as well as methods of using these polycistronic expression constructs.

BACKGROUND

[0002] Current treatments for obesity involve stimulant medications which are mildly effective and can have detrimental side effects, particularly if used long-term. Other current treatment methods involve invasive bariatric surgery which, while sometimes effective, can involve a variety of serious complications. More recently, satiation gut peptides (also referred to as satiation peptides or gut peptides) have been investigated as potential treatments for obesity.

[0003] Satiation gut peptides are chemical messengers that regulate gastrointestinal (GI) functions such as secretion, motility, absorption, digestion, and cell proliferation. These polypeptides are produced by endocrine cells in the stomach, pancreas, or intestine and act locally through autocrine or paracrine mechanisms, or at distant sites in a classical endocrine manner. Penetrating from plasma through the blood-brain barrier, they act by activating specific receptors in the satiety center of the hypothalamus, thus inducing satiation.

[0004] Acute supplemental therapy with satiation gut peptides reduces food intake and body weight in obese animal models as well as in lean and obese human subjects.

[0005] It is widely acknowledged that satiation gut peptides would not be effective through ingested oral administration since enzymes and acids in the gut would degrade them prior to reaching the blood. Accordingly, novel mechanisms for the expression of satiation gut peptides are urgently needed.

SUMMARY

[0006] Provided herein are expression constructs for the expression of gut peptides and methods of using such expression constructs.

[0007] In one aspect, the present disclosure provides for a bicistronic expression construct encoding a polyprotein, wherein: the polyprotein comprises a signal peptide, a first gut peptide, and a second gut peptide; and the polyprotein encoding sequence comprises: a sequence encoding the signal peptide; a sequence encoding the first gut peptide; a sequence encoding a first protease cleavage site, wherein the first protease cleavage site allows for the separation of the first gut peptide and the second gut peptide; and a sequence encoding the second gut peptide. In one example, provided is a bicistronic expression construct encoding a polyprotein, wherein:

a. the polyprotein comprises a signal peptide, a first gut peptide, and a second gut peptide; and
b. the polyprotein encoding sequence comprises:
    i. a sequence encoding the signal peptide;
    ii. a sequence encoding the first gut peptide; and
    iii. a sequence encoding the second gut peptide.

[0008] In some examples, the first gut peptide and/or the second gut peptide comprises a sequence selected from human glucagon like peptide 1 (hGLP-1) peptide, human glucose dependent insulinotropic (hGIP) peptide, human oxyntomodulin (hOXM) peptide, peptide YY (PYY), human glucagon (hGlucagon) peptide, and amylin peptide. In examples, the hGLP-1 peptide is the hGLP-1_{7-37} peptide. In examples, the hGIP peptide is the $hGIP_{1-42}$ peptide. In some examples, the first gut peptide and/or the second gut peptide comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:1-5. In some examples, the first gut peptide and/or the second gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS: 1-5. In some examples, the first gut peptide gut peptide and/or the second gut peptide comprises a sequence selected from SEQ ID NOS: 1-5.

[0009] In some examples, the sequence encoding the first gut peptide and/or the second gut peptide comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:6-12. In some examples, the sequence encoding the first gut peptide gut peptide and/or the second gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:6-12. In some examples, the sequence encoding the first gut peptide gut peptide and/or the second gut peptide comprises a sequence that is selected from SEQ ID NOS:6-12.

[0010] In some examples, the first gut peptide and the second gut peptide are the same gut peptide. In some examples, the sequence encoding the first gut peptide and the sequence encoding the second gut peptide are different. In some examples, at least one of the sequence encoding the first gut peptide and the sequence encoding the second gut peptide is codon-optimized. In some examples, the sequence encoding the first gut peptide and the sequence encoding the second gut peptide are codon-optimized. In some examples, the first gut peptide and the second gut peptide is hGLP-1. In some examples, the first gut peptide and the second gut peptide each comprise a sequence that is at least 80% identical to SEQ ID NO:1. In some examples, the first gut peptide and the second gut peptide each comprise a sequence that is at least 90% identical to SEQ ID NO:1. In some examples, the first gut peptide and the second gut peptide each comprise SEQ ID NO:1.

[0011] In some examples, the sequence encoding the first gut peptide and the sequence encoding the second gut peptide each comprise a sequence that is at least 80% identical to a sequence selected from SEQ ID NOS:6-8. In some examples, the sequence encoding the first gut peptide and the sequence encoding the second gut peptide each comprise a sequence that is at least 90% identical to a sequence selected from SEQ ID NOS:6-8. In some examples, the sequences encoding the first and the second gut peptide are selected from SEQ ID NOS:6-8.

[0012] In some examples, the bicistronic expression construct comprises a sequence encoding a polypeptide that is at least 80% identical to SEQ ID NO:45 or SEQ ID NO:55. In some examples, the bicistronic expression construct comprises a sequence encoding a polypeptide that is at least 90% identical to SEQ ID NO:45 or SEQ ID NO:55. In some examples, the bicistronic expression construct encodes a polypeptide comprising SEQ ID NO:45 or SEQ ID NO:55.

[0013] In some examples, the bicistronic expression construct comprises a sequence that is at least 80% identical to SEQ ID NO:50 or SEQ ID NO:57. In some examples, the bicistronic expression construct comprises a sequence that is at least 90% identical to SEQ ID NO:50 or SEQ ID NO:57. In some examples, the bicistronic expression construct comprises SEQ ID NO:50 or SEQ ID NO:57.

[0014] In some examples, the first gut peptide and the second gut peptide are different gut peptides. In some examples, the first gut peptide and the second gut peptide are selected from the group consisting of hGLP-1 and hGIP. In examples, the hGLP-1 peptide is the hGLP-1_{7-37} peptide. In examples, the hGIP peptide is the $hGIP_{1-42}$ peptide.

[0015] In some examples, the bicistronic expression construct encodes a sequence comprising a sequence that is at least 80% identical to any one of SEQ ID NOS:46-49 or SEQ ID NO:56. In some examples, the bicistronic expression construct encodes a sequence comprising a sequence that is at least 90% identical to any one of SEQ ID NOS: 46-49 or SEQ ID NO:56. In some examples, the bicistronic expression construct encodes a sequence comprising any one of SEQ ID NOS:46-49 or SEQ ID NO:56.

[0016] In some examples, the bicistronic expression construct comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:51-54 or SEQ ID NO:58. In some examples, the bicistronic expression construct comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:51-54 or SEQ ID NO:58. In some examples, the bicistronic expression construct comprises a sequence selected from SEQ ID NOS:51-54 or SEQ ID NO:58.

[0017] In another aspect, the present disclosure provides for a tricistronic expression construct encoding a polyprotein, wherein: the polyprotein comprises a signal peptide, a first gut peptide, a second gut peptide, and a third gut peptide; and the polyprotein encoding sequence comprises: a sequence encoding the signal peptide; a sequence encoding the first gut peptide; a sequence encoding a first protease cleavage site, wherein the first protease cleavage site allows for the separation of the first gut peptide and the second gut peptide; a sequence encoding the second gut peptide; a sequence encoding a second protease cleavage site allowing for the separation of the second gut peptide and the third gut peptide; and a sequence encoding the third gut peptide. In one example, provided is a tricistronic expression construct encoding a polyprotein, wherein:

a. the polyprotein comprises a signal peptide, a first gut peptide, a second gut peptide, and a third gut peptide; and
b. the polyprotein encoding sequence comprises:
    i. a sequence encoding the signal peptide;
    ii. a sequence encoding the first gut peptide;
    iii. a sequence encoding the second gut peptide; and
    iv. a sequence encoding the third gut peptide.

[0018] In some examples, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence selected from the group consisting of hGLP-1 peptide, hGIP peptide, hOXM peptide, peptide YY (PYY), hGlucagon peptide, and amylin peptide. In examples, the hGLP-1 peptide is the $hGLP\text{-}1_{7\text{-}37}$ peptide. In examples, the hGIP peptide is the $hGIP_{1\text{-}42}$ peptide. In some examples, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:1-5. In some examples, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:1-4. In some examples, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence selected from SEQ ID NOS:1-5.

[0019] In some examples, the sequence encoding first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:6-12. In some examples, the sequence encoding the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:6-12. In some examples,

the sequence encoding first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is selected from SEQ ID NOS:6-12.

[0020] In some examples, the first gut peptide, the second gut peptide, and the third gut peptide are the same gut peptide. In some examples, the sequence encoding the first gut peptide, the sequence encoding the second gut peptide, and the sequence encoding the third gut peptide are different. In some examples, at least one of the sequence encoding the first gut peptide, the sequence encoding the second gut peptide, and the sequence encoding the third gut peptide is codon-optimized. In some examples, the sequence encoding the sequence encoding the first gut peptide, the sequence encoding the second gut peptide, and the sequence encoding the third gut peptide are codon-optimized.

[0021] In some examples, the first gut peptide, the second gut peptide and the third gut peptide is hGLP-1.

[0022] In some examples, the tricistronic expression construct encodes a sequence comprising a sequence that is at least 80% identical to any one of SEQ ID NOS:59-61 or SEQ ID NO:75. In some examples, the tricistronic expression construct encodes a sequence comprising a sequence that is at least 90% identical to any one of SEQ ID NOS:59-61 or SEQ ID NO:75. In some examples, the tricistronic expression construct encodes a sequence comprising a sequence selected from SEQ ID NOS:59-61 or SEQ ID NO:75.

[0023] In some examples, the tricistronic expression construct comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:67-69 or SEQ ID NO:78. In some examples, the tricistronic expression construct comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:67-69 or SEQ ID NO:78. In some examples, the tricistronic expression construct comprises a sequence selected from SEQ ID NOS:67-69 or SEQ ID NO:78.

[0024] In some examples, the first gut peptide, and the second gut peptide are different gut peptides. In some examples, the first gut peptide, the second gut peptide, and the third gut peptide are different gut peptides.

[0025] In some examples, the first gut peptide, the second gut peptide, and the third gut peptide are selected from the group consisting of (1) hGLP-1 peptide, hOXM peptide, and PYY or (2) hGLP-1 peptide, hGlucagon peptide, and hGIP peptide. In examples, the hGLP-1 peptide is the hGLP-1_{7-37} peptide. In examples, the hGIP peptide is the $hGIP_{1-42}$ peptide.

[0026] In some examples, the tricistronic expression construct encodes a sequence comprising a sequence that is at least 80% identical to any one of SEQ ID NOS:62-66 or SEQ ID NOS:76-77. In some examples, the tricistronic expression construct encodes a sequence comprising a sequence that is at least 90% identical to any one of SEQ ID NOS:62-66 or SEQ ID NOS:76-77. In some examples, the tricistronic expression construct encodes a sequence comprising any one of SEQ ID NOS:62-66 or SEQ ID NOS:76-77.

[0027] In some examples, the tricistronic expression construct comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:70-74 or SEQ ID NOS:79-80. In some examples, the tricistronic expression construct comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:70-74 or SEQ ID NOS:79-80. In some examples, the tricistronic expression construct comprises any one of SEQ ID NOS:70-74 or SEQ ID NOS:79-80.

[0028] In some examples, the bicistronic or the tricistronic expression construct encodes a polyprotein, wherein the polyprotein comprises a signal peptide. In some examples, the signal peptide is selected from the group consisting of an immunoglobulin M (IgM) signal peptide, human insulin (hInsul) signal peptide, murine Igh protein (mIgh) protein signal peptide, human growth hormone (hGH) signal peptide, murine erythropoietin (mEpo) signal peptide, murine growth hormone-releasing hormone (mGHRH) signal peptide, human albumin signal peptide, and human factor IX (FIX) signal peptide. In some examples, the signal peptide comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:13-20. In some examples, the signal peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:13-20. In some examples, the signal peptide comprises a sequence selected from SEQ ID NOS:13-20. In some examples, the sequence encoding the signal peptide comprises a sequence that is at least 80% identical to any one of SEQ ID NOS:21-28. In some examples, the sequence encoding the signal peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:21-28. In some examples, the sequence encoding the signal peptide comprises a sequence selected from SEQ ID NOS:21-28.

[0029] In some examples, the bicistronic or the tricistronic expression construct further comprises a promoter sequence. In some examples, the promoter is a CMV or a CASI promoter.

[0030] In some examples, the bicistronic or the tricistronic expression construct encodes a polyprotein comprising a protease cleavage site positioned between the first gut peptide and the second gut peptide. In some examples, the tricistronic expression construct encodes a polyprotein wherein the polyprotein further comprises a protease cleavage site that allows release of the first gut peptide, second gut peptide, and/or the third peptide from the polyprotein.

[0031] In some examples, at least one of the protease cleavage sites is a furin cleavage site.

[0032] In some examples, the bicistronic expression construct or the tricistronic expression construct comprises a riboswitch comprising an aptamer, wherein the aptamer binds to a small molecule.

[0033] In some examples, the bicistronic expression construct or the tricistronic expression construct comprises a gene regulation cassette comprising an aptamer, wherein the aptamer binds to a small molecule.

[0034] In a further aspect, the present disclosure provides for a vector comprising the bicistronic expression construct or the tricistronic expression construct as disclosed herein. Provided herein is a vector comprising a bicistronic expression or a tricistronic expression construct disclosed herein. In some examples, the vector is an adeno-associated virus (AAV) vector.

[0035] Provided herein is a cell comprising a vector disclosed herein. In some examples, the cell is isolated.

[0036] In a further aspect, the present disclosure provides for a pharmaceutical composition comprising the vector as disclosed herein and a pharmaceutically acceptable excipient. Provided herein is a pharmaceutical composition comprising a vector disclosed herein and a pharmaceutically acceptable excipient.

[0037] In a further aspect, the present disclosure provides for a use of a pharmaceutical composition comprising (a) the bicistronic expression construct as disclosed herein or a vector comprising the bicistronic expression construct as disclosed herein in the manufacture of a medicament for inducing satiation in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient. In one example, provided is a method of inducing satiation in a subject in need thereof, the method comprising administering to the subject an expression construct, a vector, or a pharmaceutical composition disclosed herein.

[0038] In a further aspect, the present disclosure provides for a use of a pharmaceutical composition comprising (a) the bicistronic expression construct as disclosed herein or a vector comprising the bicistronic expression construct as disclosed herein in the manufacture of a medicament for treating obesity in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient. In one example, provided is a method of treating obesity in a subject in need thereof, the method comprising administering to the subject an expression construct, a vector, or a pharmaceutical composition disclosed herein.

[0039] In a further aspect, the present disclosure provides for a use of a pharmaceutical composition comprising (a) the bicistronic expression construct as disclosed herein or a vector comprising the bicistronic expression construct as disclosed herein in the manufacture of a medicament for suppressing appetite in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient. In one example, provided

is a method of suppressing appetite in a subject in need thereof, the method comprising administering to the subject an expression construct, a vector, or a pharmaceutical composition disclosed herein.

[0040] In a further aspect, the present disclosure provides for a use of a pharmaceutical composition comprising (a) the bicistronic expression construct as disclosed herein or a vector comprising the bicistronic expression construct as disclosed herein in the manufacture of a medicament for reducing weight gain in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient. In one example, provided is a method of reducing of reducing weight or reducing weight gain in a subject in need thereof, the method comprising administering to the subject an expression construct, a vector, or a pharmaceutical composition disclosed herein.

[0041] In a further aspect, the present disclosure provides for a use of a pharmaceutical composition comprising (a) the bicistronic expression construct as disclosed herein or a vector comprising the bicistronic expression construct as disclosed herein in the manufacture of a medicament for improving glucose tolerance in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient. In one example, provided is a method of improving glucose tolerance in a subject in need thereof, the method comprising administering to the subject an expression construct, a vector, or a pharmaceutical composition disclosed herein.

[0042] In one example, provided is a method of inducing insulin release in a subject in need thereof, the method comprising administering to the subject an expression construct, a vector, or a pharmaceutical composition disclosed herein.

BRIEF DESCRIPTION OF THE FIGURES

[0043] **Figs. 1A, 1B,** and **1C** illustrate the expression of gut peptides using monocistronic expression constructs. **Fig. 1A.** Expression of hGLP-1_{7-37} peptide, a gut peptide, as determined by ELISA. See **Tables 5** and **6** for the nomenclature of hGLP-1 expression constructs. **Fig. 1B.** Expression of hGLP-1_{7-37} peptide, a gut peptide, as determined by ELISA. See **Tables 5** and **6** for the nomenclature of hGLP-1 expression constructs. **Fig. 1C.** Expression of $hGIP_{1-42}$ peptide,

[0050] **Expression constructs**

[0051] Provided herein are monocistronic, bicistronic, tricistronic and other polycistronic expression constructs for the expression of gut peptides.

[0052] In embodiments, the expression construct is a monocistronic expression construct for the expression of a single polypeptide.

[0053] In embodiments, the expression construct is a bicistronic expression construct for the expression of two polypeptides. The two polypeptides may be expressed as a polyprotein and the individual polypeptides maybe be released from the polyprotein after proteolytic cleavage.

[0054] In embodiments, the expression construct is a tricistronic expression construct for the expression of three polypeptides. The three polypeptides may be expressed as a polyprotein and the individual polypeptides maybe be released from the polyprotein after proteolytic cleavage.

[0055] In embodiments, the expression construct is a polycistronic expression construct for the expression of two or more polypeptides. The two or more polypeptides may be expressed as a polyprotein and the individual polypeptides maybe be released from the polyprotein after proteolytic cleavage. In some embodiments, the polycistronic expression construct expresses two, three, four, five, six, seven, eight, nine, or ten polypeptides. The two or more polypeptides may be the same or different polypeptides.

[0056] In one aspect, the expression constructs provided herein encode one or more gut peptides.

[0057] *Monocistronic expression constructs*

[0058] In some embodiments, the gut peptide is human glucagon-like peptide 1 (hGLP-1) peptide, human gastric inhibitory peptide (hGIP) peptide, human oxyntomodulin (hOXM) peptide, peptide YY or peptide tyrosine tyrosine (PYY), human glucagon (hGlucagon) peptide, or amylin peptide (also called insulinoma amyloid polypeptide (IAPP)). In embodiments, the hGLP-1 peptide is the hGLP-1_{7-36} peptide. In embodiments, the hGIP peptide is the $hGIP_{1-42}$ peptide. In some embodiments, the gut peptide is a gut peptide disclosed in **Table 1** or a portion of one of the gut peptides disclosed in **Table 1**.

[0059] In some embodiments, provided is an expression construct that encodes for a polypeptide comprising a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 91%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:1. In some embodiments, the expression construct encodes for a polypeptide comprising SEQ ID NO:1.

peptide, PYY, hGlucagon peptide, and amylin peptide. In embodiments, the hGLP-1 peptide is the hGLP-1_{7-37} peptide. In embodiments, the hGIP peptide is the $hGIP_{1-42}$ peptide. In some embodiments, the first gut peptide and/or the second gut peptide comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 91%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NOS:1-5. In some embodiments, the first gut peptide and/or the second gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS: 1-5. In some embodiments, the first gut peptide gut peptide and/or the second gut peptide comprises a sequence selected from SEQ ID NOS: 1-5.

[0099] In some embodiments, the sequence encoding the first gut peptide gut peptide and/or the second gut peptide comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 91%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NOS:6-12. In some embodiments, the sequence encoding the first gut peptide gut peptide and/or the second gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:6-12. In some embodiments, the sequence encoding the first gut peptide gut peptide and/or the second gut peptide comprises a sequence that is selected from SEQ ID NOS:6-12.

[0100] In some embodiments, the first gut peptide and the second gut peptide are the same gut peptide. In some embodiments, the first gut peptide and the second gut peptide are the same gut peptide, but the sequence encoding the first gut peptide and the sequence encoding the second gut peptide are different. In some embodiments, at least one of the sequence encoding the first gut peptide and the sequence encoding the second gut peptide is codon-optimized. In some embodiments, the sequence encoding the first gut peptide and the sequence encoding the second gut peptide are codon-optimized. In some embodiments, the first gut peptide and the second gut peptide is hGLP-1.

[0101] In some embodiments, the first gut peptide and the second gut peptide comprise a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 91%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:1. In some embodiments, the first gut peptide and the second gut peptide comprise a sequence that is at least 90% identical to SEQ ID NO:1. In some embodiments, the first gut peptide and the second gut peptide comprise SEQ ID NO:1.

[0102] In some embodiments, the sequence encoding the first gut peptide and the sequence encoding the second gut peptide comprise a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

[0109] *Tricistronic expression constructs*

[0110] In one aspect, provided is a tricistronic expression construct encoding a polyprotein, wherein:

a. the polyprotein comprises a signal peptide, a first gut peptide, a second gut peptide, and a third gut peptide; and
b. the polyprotein encoding sequence comprises:
    i. a sequence encoding the signal peptide
    ii. a sequence encoding the first gut peptide;
    iii. a sequence encoding the second gut peptide; and
    iv. a sequence encoding the third gut peptide.

[0111] In some embodiments, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence selected from the group consisting of hGLP-1 peptide, hGIP peptide, hOXM peptide, PYY, hGlucagon peptie, and amylin peptide. In embodiments, the hGLP-1 peptide is the hGLP-1_{7-37} peptide. In embodiments, the hGIP peptide is the $hGIP_{1-42}$ peptide. In some embodiments, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 91%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NOS:1-5. In some embodiments, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:1-5. In some embodiments, the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence selected from SEQ ID NOS:1-5.

[0112] In some embodiments, the sequence encoding first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 91%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NOS:6-12. In some embodiments, the sequence encoding the first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:6-12. In some embodiments, the sequence encoding first gut peptide, the second gut peptide, and/or the third gut peptide comprises a sequence that is selected from SEQ ID NOS:6-12.

[0113] In some embodiments, the tricistronic expression construct encodes a sequence comprising a sequence disclosed in **Table 9** or a portion of a sequence disclosed in **Table 9.** In

We claim:

1. A bicistronic expression construct encoding a polyprotein, wherein:
    a. the polyprotein comprises a signal peptide, a first gut peptide, and a second gut peptide; and
    b. the polyprotein encoding sequence comprises:
        i. a sequence encoding the signal peptide;
        ii. a sequence encoding the first gut peptide;
        iii. a sequence encoding a first protease cleavage site, wherein the first protease cleavage site allows for the separation of the first gut peptide and the second gut peptide; and
        iv. a sequence encoding the second gut peptide.

2. A tricistronic expression construct encoding a polyprotein, wherein:
    a. the polyprotein comprises a signal peptide, a first gut peptide, a second gut peptide, and a third gut peptide; and
    b. the polyprotein encoding sequence comprises:
        i. a sequence encoding the signal peptide;
        ii. a sequence encoding the first gut peptide;
        iii. a sequence encoding a first protease cleavage site, wherein the first protease cleavage site allows for the separation of the first gut peptide and the second gut peptide;
        iv. a sequence encoding the second gut peptide;
        v. a sequence encoding a second protease cleavage site allowing for the separation of the second gut peptide and the third gut peptide; and
        vi. a sequence encoding the third gut peptide.

3. The bicistronic expression construct of claim 1 or the tricistronic expression construct of claim 2, wherein the signal peptide is selected from the group consisting of an immunoglobulin M (IgM) signal peptide, human insulin (hInsul) signal peptide, murine Igh protein (Igh) signal peptide, human growth hormone (hGH) signal peptide, murine

erythropoietin (mEpo) signal peptide, murine growth hormone-releasing hormone (mGHRH) signal peptide, human albumin signal peptide, and human factor IX (FIX) signal peptide.

4. The bicistronic expression construct or the tricistronic expression construct of claim 3, wherein (a) the signal peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:13-20 or (b) the sequence encoding the signal peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:21-28.

5. The bicistronic expression construct or the tricistronic expression construct of claim 4, wherein (a) the signal peptide comprises any one of SEQ ID NOS:13-20 or (b) the sequence encoding the signal peptide comprises any one of SEQ ID NOS:21-28.

6. The bicistronic expression construct of any one of claims 1 or 3-5 or the tricistronic expression construct of any one of claims 2-5, wherein (a) the first gut peptide and/or the second gut peptide encoded by the bicistronic or the tricistronic expression construct independently comprises a sequence selected from the group consisting of human protein glp-1 (hGLP-1), human glucose dependent insulinotropic peptide (hGIP), human oxyntomodulin (hOXM), peptide YY (PYY), human glucagon, and amylin, and/or (b) the third gut peptide encoded by the tricistronic expression construct independently comprises a sequence selected from the group consisting of human hGLP-1, hGIP, hOXM, PYY, human glucagon, and amylin.

7. The bicistronic expression construct or the tricistronic expression construct of claim 6, wherein (a) the first gut peptide, the second gut peptide, and/or the third gut peptide independently comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:1-5 and/or (b) the sequence encoding the first gut peptide, the second gut peptide, and/or the third gut peptide independently comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:6-12.

8. The bicistronic expression construct or the tricistronic expression construct of claim 7, wherein (a) the first gut peptide gut peptide, the second gut peptide, and/or the third gut

peptide independently comprises any one of SEQ ID NOS:1-5 and/or (b) the sequence encoding the first gut peptide, the second gut peptide, and/or the third gut peptide independently comprises any one of SEQ ID NOS:6-12.

9. The bicistronic expression construct of any one of claims 1 or 3-8 or the tricistronic expression construct of any one of claims 2-8, wherein the sequence encoding the first gut peptide, the second gut peptide, and/or the third gut peptide is codon-optimized.

10. The bicistronic expression construct of any one of claims 1, 3-6, or 9, wherein the first gut peptide and the second gut peptide are hGLP-1.

11. The bicistronic expression construct of claim 10, wherein (a) the first gut peptide and the second gut peptide each comprise a sequence that is at least 90% identical to SEQ ID NO:1 or (b) the sequences encoding the first and the second gut peptide each comprise a sequence that is at least 90% identical to any one of SEQ ID NOS:6-8.

12. The bicistronic expression construct of claim 11, wherein (a) the first gut peptide and the second gut peptide each comprise SEQ ID NO:1 or (b) the sequences encoding the first and the second gut peptide each comprise any one of SEQ ID NOS:6-8.

13. The bicistronic expression construct of claim 10, wherein the bicistronic expression construct comprises (a) a sequence encoding a polypeptide that is at least 90% identical to SEQ ID NO:45 or SEQ ID NO:55 or (b) a sequence that is at least 90% identical to SEQ ID NO:50 or SEQ ID NO:57.

14. The bicistronic expression construct of claim 13, wherein the bicistronic expression construct comprises (a) a sequence encoding a polypeptide comprising SEQ ID NO:45 or SEQ ID NO:55 or (b) SEQ ID NO:50 or SEQ ID NO:57.

15. The bicistronic expression construct of any one of claims 1, 3-6, or 9, wherein the first gut peptide and the second gut peptide are different and are independently selected from the group consisting of hGLP-1 and hGIP.

16. The bicistronic expression construct of claim 15, wherein (a) the first gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:1 and the second gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:2 or (b) the sequence encoding the first gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOs:6-8 and the sequence encoding the second gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:9.

17. The bicistronic expression construct of claim 16, wherein (a) the first gut peptide comprises SEQ ID NO:1 and the second gut peptide comprises SEQ ID NO:2 or (b) the sequence encoding the first gut peptide any one of SEQ ID NOs:6-8 and the sequence encoding the second gut peptide comprises SEQ ID NO:9.

18. The bicistronic expression construct of claim 15, wherein the bicistronic expression construct comprises (a) a sequence encoding a polypeptide that is at least 90% identical to any one of SEQ ID NOS:46-49 or SEQ ID NO:56 or (b) a sequence that is at least 90% identical to any one of SEQ ID NOS:51-54 or SEQ ID NO:58.

19. The bicistronic expression construct of claim 18, wherein the bicistronic expression construct comprises (a) a sequence encoding a polypeptide comprising any one of SEQ ID NOS:46-49 or SEQ ID NO:56 or (b) any one of SEQ ID NOS:51-54 or SEQ ID NO:58.

20. The tricistronic expression construct of any one of claims 2-6 or 9, wherein the first gut peptide, the second gut peptide, and the third gut peptide are different and independently selected from the group consisting of:
    a. hGLP-1, hOXM, and PYY; or
    b. hGLP-1, hGlucagon, and hGIP.

21. The tricistronic expression construct of claim 20, wherein:

a. the first gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:1, the second gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:3, and the third gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:4; or
b. the sequence encoding the first gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOs:6-8, the sequence encoding the second gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:10, and the sequence encoding the third gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:11.

22. The tricistronic expression construct of claim 21, wherein:
a. the first gut peptide SEQ ID NO:1, the second gut peptide comprises SEQ ID NO:3, and the third gut peptide comprises SEQ ID NO:4; or
b. the sequence encoding the first gut peptide comprises any one of SEQ ID NOs:6-8, the sequence encoding the second gut peptide comprises SEQ ID NO:10, and the sequence encoding the third gut peptide comprises SEQ ID NO:11.

23. The tricistronic expression construct of claim 20, wherein:
a. the first gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:1, the second gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:5, and the third gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:2; or
b. the sequence encoding the first gut peptide comprises a sequence that is at least 90% identical to any one of SEQ ID NOs:6-8, the sequence encoding the second gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:12, and the sequence encoding the first gut peptide comprises a sequence that is at least 90% identical to SEQ ID NO:9.

24. The tricistronic expression construct of claim 23, wherein:
a. the first gut peptide comprises SEQ ID NO:1, the second gut peptide comprises SEQ ID NO:5, and the third gut peptide comprises SEQ ID NO:2; or

b. the sequence encoding the first gut peptide comprises any one of SEQ ID NOs:6-8, the sequence encoding the second gut peptide comprises SEQ ID NO:12, and the sequence encoding the third gut peptide comprises SEQ ID NO:9.

25. The tricistronic expression construct of claim 20, wherein the tricistronic expression construct comprises (a) a sequence encoding a polypeptide that is at least 90% identical to any one of SEQ ID NOS:62-66 or SEQ ID NOS:76-77 or (b) comprises a sequence that is at least 90% identical to any one of SEQ ID NOS:70-74 or SEQ ID NOS:79-80.

26. The tricistronic expression construct of claim 25, wherein the tricistronic expression construct comprises (a) a sequence encoding a polypeptide comprising any one of SEQ ID NOS:62-66 or SEQ ID NOS:76-77 or (b) any one of SEQ ID NOS:70-74 or SEQ ID NOS:79-80.

27. The tricistronic expression construct of claim 25, wherein the tricistronic expression construct comprises (a) a sequence encoding a polypeptide that is at least 90% identical to SEQ ID NO:66 or (b) comprises a sequence that is at least 90% identical to SEQ ID NO:77.

28. The tricistronic expression construct of claim 27, wherein the tricistronic expression construct comprises (a) a sequence encoding a polypeptide comprising SEQ ID NO:66 or (b) SEQ ID NO:77.

29. The tricistronic expression construct of any one of claims 2-6 or 9, wherein the first gut peptide, the second gut peptide and the third gut peptide are hGLP-1.

30. The tricistronic expression construct of claim 29, wherein (a) the first gut peptide, the second gut peptide, and the third gut peptide each comprise a sequence that is at least 90% identical to SEQ ID NO:1 or (b) the sequences encoding the first gut peptide, the second gut peptide, and the third gut peptide each comprise a sequence that is at least 90% identical to any one of SEQ ID NOS:6-8.

31. The tricistronic expression construct of claim 30, wherein (a) the first gut peptide, the second gut peptide, and the third gut peptide each comprise SEQ ID NO:1 or (b) the sequences encoding the first gut peptide, the second gut peptide, and the third gut peptide each comprise any one of SEQ ID NOS:6-8.

32. The tricistronic expression construct of claim 29, wherein the tricistronic expression construct comprises (a) a sequence encoding a polypeptide a sequence that is at least 90% identical to any one of SEQ ID NOS:59-61 or SEQ ID NO:75 or (b) a sequence that is at least 90% identical to any one of SEQ ID NOS:67-69 or SEQ ID NO:78.

33. The tricistronic expression construct of claim 32, wherein the tricistronic expression construct comprises (a) a sequence encoding a polypeptide comprising any one of SEQ ID NOS:59-61 or SEQ ID NO:75 or (b) SEQ ID NOS:67-69 or SEQ ID NO:78.

34. The bicistronic expression construct of any one of claims 1, 3-19 or the tricistronic expression construct of any one of claims 2-9 or 20-33, wherein the bicistronic expression construct or the tricistronic expression construct further comprises a promoter sequence.

35. The bicistronic expression construct or the tricistronic expression construct of claim 34, wherein the promoter is CMV promoter or a CASI promoter.

36. The bicistronic expression construct of any one of claims 1, 3-19, 34, or 35 or the tricistronic expression construct of any one of claims 2-9 or 20-35, wherein the first protease cleavage site or the second protease cleavage site is a furin cleavage site.

37. The bicistronic expression construct of any one of claims 1, 3-19, or 34-36 or the tricistronic expression construct of any one of claims 2-9 or 20-36, wherein the polyprotein encoding sequence comprises a riboswitch comprising an aptamer, wherein the aptamer binds to a small molecule.

38. A vector comprising the bicistronic expression construct of any one of claims 1, 3-19, or 34-37 or the tricistronic expression construct of any one of claims 2-9, or 20-37.

39. The vector of claim 38, wherein the vector is an AAV vector.

40. A pharmaceutical composition comprising the vector of claims 38 or 39 and a pharmaceutically acceptable excipient.

41. Use of a pharmaceutical composition comprising (a) the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 or a vector comprising the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 in the manufacture of a medicament for inducing satiation in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient.

42. Use of a pharmaceutical composition comprising (a) the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 or a vector comprising the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 in the manufacture of a medicament for treating obesity in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient.

43. Use of a pharmaceutical composition comprising (a) the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 or a vector comprising the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 in the manufacture of a medicament for suppressing appetite in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient.

44. Use of a pharmaceutical composition comprising (a) the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 or a vector comprising the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 in the manufacture of a medicament for reducing weight gain in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient.

45. Use of a pharmaceutical composition comprising (a) the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 or a vector comprising the bicistronic expression construct of any one of claims 1, 3-19 or 34-37 in the manufacture of a medicament for improving glucose tolerance in a subject in need thereof, wherein the medicament further comprises (b) a pharmaceutically acceptable excipient.

46. The use of any one of claims 41-45, wherein the bicistronic expression construct further comprises a sequence encoding a third gut peptide, wherein the polyprotein further comprises a third gut peptide, and wherein the polyprotein further comprises a second protease cleavage site allowing for the separation of the second gut peptide and the third gut peptide.