

AAV MEDIATED INSULIN AND GLUCOKINASE GENE THERAPY FOR TREATING DIABETES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the priority benefit of U.S. Provisional Application No. 63/595,296, filed November 1, 2023, and U.S. Provisional Application No. 63/615,452, filed December 28, 2023; each of which is hereby incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing in .XML file (4525_129PC02_SequenceListing_ST26.xml; Size: 302,607 bytes; and Date of Creation: October 24, 2024) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND

[0003] The two main forms of diabetes mellitus are type 1 (T1DM) and type 2 (T2DM) (Diabetes care, 1997, 20-1183-1197).

[0004] T1DM is characterized by a severe lack of insulin production due to specific destruction of the pancreatic β -cells. β -cell loss in T1DM is the result of an autoimmune mediated process, in which a chronic inflammation called insulitis causes β -cell destruction (Eizirik D. L. et al, 2001, Diabetologia, 44:2115-2133 and Mathis D et al, 2001, Nature, 414: 792-798). T1DM is one of the most common endocrine and metabolic conditions of childhood, and incidence is rapidly increasing, especially among young children. T1DM is diagnosed when the autoimmune-mediated β -cell destruction is almost complete, causing patients to need insulin-replacement therapy to survive. T1DM in an adult may present itself similarly to T2DM, with a slow deterioration in metabolic control, and subsequent progression to insulin dependency. This form is called latent autoimmune diabetes mellitus in adults (LADA) (Diabetes Atlas 4th edition, 2009, International Diabetes Federation).

- [0005] T2DM is the most common form of diabetes mellitus and has been attributed to an interaction between genetic, environmental, and behavioral risk factors. T2DM is characterized by insulin insensitivity, declining insulin production, and eventual pancreatic β -cell failure (Olokoba, A. et al, 2012, Oman Med. J. 27(4):269-273).
- [0006] The reduction of hyperglycemia and maintenance of normoglycemia is a goal of any therapeutic approach to T1DM and T2DM. The current therapy for all T1DM and a large subset of T2DM patients is based on regular subcutaneous injections of both short-acting and long-acting insulin preparations.
- [0007] Lifelong insulin treatment is often the therapy of choice for both T1DM and T2DM. While lifelong treatment with exogenous insulin has been largely successful in managing diabetes, diabetic complications can still occur due to difficulties with maintaining tight glycemic control. States of prolonged hyperglycemia can lead to severe microvascular or macrovascular complications, most commonly presenting as retinopathies, neuropathies, nephropathies, cerebrovascular accidents, or myocardial infarctions. These devastating complications can be prevented with improvements in glycemic control. Of note, brittle diabetes, which is a particularly labile form, can be very difficult to manage even with lifelong exogenous insulin.
- [0008] AAV gene therapy strategies offer a new treatment paradigm for diabetes. One challenge with AAV gene therapy generally includes the risk of host immune responses to the AAV capsid or the transgene-encoded protein(s), which have been observed in various clinical and preclinical studies. Such immune responses can adversely impact patients' health, prevent viral transduction, prevent repeated dosing strategies, eliminate transduced cells, and pose a significant barrier to the potential effectiveness of AAV gene therapy. This challenge is particularly great in the diabetes patient population because these patients are more sensitive to the immunosuppressive regimens commonly used with gene therapy, like steroids.

FIELD OF DISCLOSURE

- [0009] The present disclosure pertains to the medical field, including AAV gene therapy compositions comprising nucleic acids encoding insulin and glucokinase as well as immunosuppressive regimens for use with the same for treatment of diabetes.

BRIEF SUMMARY

- [0010]** Certain aspects of the disclosure are directed to a method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, wherein the method comprises administering a therapy (e.g., a combination gene therapy) to the subject comprising: one or more adeno-associated virus (AAV) vectors comprising (a) a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein; and (b) a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein; wherein the therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.
- [0011]** In some aspects, the therapy (e.g., combination gene therapy) comprises:
- (a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and
 - (b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs);
- wherein the therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.
- [0012]** In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same composition (e.g., via the same IM injection).
- [0013]** In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition (e.g., via the same IM injection).
- [0014]** In some aspects, the one or more AAV vectors is an AAV vector genome comprising an single expression cassette comprising a promoter (e.g., a first and a second promoter), the polynucleotide encoding the hIns protein, and the polynucleotide encoding the hGck protein, wherein the single expression cassette is flanked by inverted terminal repeats (ITRs).
- [0015]** In some aspects, the combination therapy is administered intramuscularly (IM) to at least two, at least three, at least four, at least five, at least six, at least seven, at least

eight, at least nine, or at least ten different skeletal muscle groups. In some aspects, wherein the combination therapy is administered intramuscularly (IM) to 2-10, 3-10, 4-10, 5-10, 6-10, 7-10 or 8-10 different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine, or ten different skeletal muscle groups.

[0016] In some aspects, the combination therapy is administered intramuscularly (IM) to (i) 2-10, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, or 9-10 different skeletal muscle groups; (ii) 2-8, 3-8, 4-8, 5-8, 6-8, or 7-8 different skeletal muscle groups; (iii) 2-6, 3-6, 4-6, or 5-6 different skeletal muscle groups; (iv) 2-5, 3-5, or 4-5 different skeletal muscle groups; (v) 2-4 or 3-4 different skeletal muscle groups.

[0017] In some aspects, the combination therapy comprises (i) a first recombinant AAV (rAAV) particle comprising the first AAV vector genome comprising the insulin expression cassette comprising the promoter operably linked to the polynucleotide encoding the human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising the second AAV vector genome comprising the glucokinase expression cassette comprising the promoter operably linked to the polynucleotide encoding the human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).

[0018] In some aspects, the different skeletal muscle groups can comprise a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat), optionally wherein the two or more different skeletal muscle groups comprise any combination thereof. In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat).

[0019] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring, optionally wherein the two or more different skeletal muscle groups comprise any combination

thereof. In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, or a hamstring.

[0020] In some aspects, the skeletal muscle group is selected from i) a left quadriceps and a right quadriceps, ii) a left bicep and a right bicep, iii) a left triceps and a right triceps, iv) a left gastrocnemius and a right gastrocnemius, v) a left gluteus maximus and a right gluteus maximus, vi) a left hamstring and a right hamstring, vii) a left deltoid and a right deltoid, viii) a left trapezius and a right trapezius, ix) a left pectoral muscle and a right pectoral muscle, and x) a left latissimus dorsi and a right latissimus dorsi.

[0021] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius, a gluteus maximus, and a hamstring, optionally wherein the two or more different skeletal muscle groups comprise any combination thereof.

[0022] In some aspects, the administration comprises bilateral injections to one or more of the skeletal muscle groups (e.g., IM injections to the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings). In some aspects, the administration comprises bilateral injections to one or more of the skeletal muscle groups (e.g., IM injections to the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, the right and left triceps, the right and left hamstrings, and any combinations thereof).

[0023] In some aspects, the bilateral injections are divided equally for each skeletal muscle group (e.g., the right quadriceps and the left quadriceps receive approximately the same administration volume and/or dose).

[0024] In some aspects, the administration comprises a total of 20-150 IM injections of the therapy (e.g., combination gene therapy) to the at least two different skeletal muscle groups. In some aspects, the administration comprises a total of 20-120 IM injections, 20-110 IM injections, 20-100 IM injections, 20-80 IM injections, 20-60 IM injections, 25-150 IM injections, 25-120 IM injections, 25-110 IM injections, or 25-100 IM injections to the at least two different skeletal muscle groups.

[0025] In some aspects, the administration comprises 1-80 (e.g., 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80) IM injections to a first skeletal muscle group and 1-80 (e.g., 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80) IM injections to a second skeletal muscle group. In some aspects, the administration

comprises 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a first skeletal muscle group, 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a second skeletal muscle group, and 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a third skeletal muscle group.

[0026] In some aspects, the administration comprises (i) 1-80 IM injections to a first skeletal muscle group and 1-80 IM injections to a second skeletal muscle group; (ii) 1-60 IM injections to a first skeletal muscle group, 1-60 IM injections to a second skeletal muscle group, and 1-60 IM injections to a third skeletal muscle group; (iii) 1-40 IM injections to a first skeletal muscle group, 1-40 IM injections to a second skeletal muscle group, 1-40 IM injections to a third skeletal muscle group, and 1-40 IM injections to a fourth skeletal muscle group; (iv) 1-30 IM injections to a first skeletal muscle group, 1-30 IM injections to a second skeletal muscle group, 1-30 IM injections to a third skeletal muscle group, 1-30 IM injections to a fourth skeletal muscle group, and 1-30 IM injections to a fifth skeletal muscle group; and (v) 1-25 IM injections to a first skeletal muscle group, 1-25 IM injections to a second skeletal muscle group, 1-25 IM injections to a third skeletal muscle group, 1-25 IM injections to a fourth skeletal muscle group, 1-25 IM injections to a fifth skeletal muscle group, and 1-25 IM injections to a sixth skeletal muscle group.

[0027] In some aspects, the administration comprises: (i) 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80 IM injections to a first skeletal muscle group and 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80 IM injections to a second skeletal muscle group; (ii) 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a first skeletal muscle group; 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a second skeletal muscle group; and 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a third skeletal muscle group; (iii) 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a first skeletal muscle group; 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a second skeletal muscle group; 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a third skeletal muscle group; and 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a fourth skeletal muscle group; (iv) 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a first skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a second skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a third skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM

injections to a fourth skeletal muscle group; and 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a fifth skeletal muscle group; or (v) 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a first skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a second skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a third skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a fourth skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a fifth skeletal muscle group; and 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a sixth skeletal muscle group.

- [0028] In some aspects, the administration comprises 8-50 IM injections to the quadriceps (e.g., 4-25 IM injections bilaterally to each quadriceps), 8-50 IM injections to the biceps (e.g., 4-25 IM injections bilaterally to each bicep), and 8-50 IM injections to the hamstrings (e.g., 4-25 IM injections bilaterally to each hamstring).
- [0029] In some aspects, each IM injection is administered in a volume of about 100 μ L to about 1.5 mL (e.g., about 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, or about 400 μ L to about 800 μ L).
- [0030] In some aspects, each IM injection is administered in a volume of about 100 μ L to about 2 mL, 100 μ L to about 1.9 mL, 100 μ L to about 1.8 mL, 100 μ L to about 1.7 mL, 100 μ L to about 1.6 mL, 100 μ L to about 1.5 mL, 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, about 400 μ L to about 800 μ L, about 500 μ L to about 1.5 mL, about 600 μ L to about 1.4 mL, about 700 μ L to about 1.3 mL, about 800 μ L to about 1.2 mL, or about 900 μ L to about 1.1 mL.
- [0031] In some aspects, each IM injection is administered in a volume of about 0.5 mL to about 1 mL.
- [0032] In some aspects, the total cumulative volume administered for all IM injections is about 20 mL to about 70 mL, about 20 mL to about 50 mL, or about 30 mL to about 40 mL.
- [0033] In some aspects, the dose per IM injection comprises about 2×10^{13} to 5×10^{13} vgs per mL.

- [0034] In some aspects, each IM injection comprises about 8×10^{10} to 5×10^{13} vgs per mL. In some aspects, each IM injection comprises about 2×10^{13} to about 4×10^{13} vgs per mL.
- [0035] In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-5 cm. In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.
- [0036] In some aspects, the administration comprises IM injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep, at least 1.4 cm deep, at least 1.5 cm deep, at least 2.0 cm deep, at least 2.5 cm deep, at least 3.0 cm deep, at least 3.5 cm deep, at least 4.0 cm deep, at least 4.5 cm deep, or at least 5.0 cm deep. In some aspects, each of the IM injections is about 0.5 cm to 2 cm deep. In some aspects, each of the IM injections is between 1-2 cm deep.
- [0037] In some aspects, each of the IM injections is at least 0.5 cm below the surface of the injected muscle, regardless of the thickness of the subcutaneous fat.
- [0038] In some aspects, the first AAV vector genome and the second AAV vector genome are administered simultaneously or sequentially, optionally, wherein the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition. In some aspects, AAV particles comprising the first AAV vector genome and the second AAV vector genome are admixed prior to administration.
- [0039] In some aspects, the method further comprises administering to the subject an immunosuppressive regimen.
- [0040] Certain aspects of the disclosure are directed to a method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising administering to the subject (i) an immunosuppressive regimen and (ii) a therapy (e.g., combination gene therapy) comprising one or more adeno-associated virus (AAV) vectors comprising (a) a promoter (e.g., a first and a second promoter), (b) a polynucleotide encoding a human insulin (hIns) protein; and (c) a polynucleotide

encoding a human glucokinase (hGck) protein. In some aspects, the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.

- [0041] In some aspects, the method further comprises administering to the subject an immunosuppressive regimen comprising an immunosuppressive agent.
- [0042] Certain aspects of the disclosure are directed to a method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising administering to the subject (i) an immunosuppressive regimen comprising an immunosuppressive agent, and (ii) a therapy (e.g., combination gene therapy) comprising one or more adeno-associated virus (AAV) vectors comprising (a) a promoter (e.g., a first and a second promoter), (b) a polynucleotide encoding a human insulin (hIns) protein; and (c) a polynucleotide encoding a human glucokinase (hGck) protein. In some aspects, the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.
- [0043] In some aspects the therapy (e.g., combination gene therapy) comprises (a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).
- [0044] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same composition (e.g., via the same IM injection).
- [0045] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition (e.g., via the same IM injection).
- [0046] In some aspects, the one or more AAV vectors is an AAV vector genome comprising an single expression cassette comprising the first promoter operably linked to the polynucleotide encoding the hIns protein and a second promoter operably linked to the polynucleotide encoding the hGck protein, wherein the single expression cassette is flanked by inverted terminal repeats (ITRs).
- [0047] Certain aspects of the disclosure are directed to a dosing regimen comprising:
(a) a first immunosuppressive agent;

(b) a therapy (e.g., combination gene therapy) administered intramuscularly after (a) which comprises one or more adeno-associated virus (AAV) vectors comprising (a) a promoter (e.g., a first and a second promoter), (b) a polynucleotide encoding a human insulin (hIns) protein; and (c) a polynucleotide encoding a human glucokinase (hGck) protein; and

(c) a second immunosuppressive agent administered after (b).

[0048] In some aspects, the therapy comprises (i) a first recombinant AAV (rAAV) particle comprising a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).

[0049] In some aspects, the therapy of the dosing regimen is administered intramuscularly (IM) at least two different muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten different skeletal muscle groups. In some aspects, the combination is administered intramuscularly (IM) to 2-10, 3-10, 4-10, 5-10, 6-10, 7-10 or 8-10 different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine, or ten different skeletal muscle groups.

[0050] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition.

[0051] In some aspects, the immunosuppressive regimen comprises administering one or more immunosuppressive agents prior to (pre-AAV), at the same time as, and/or after (post-AAV) administering the combination therapy.

[0052] In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (pre-AAV) prior to administering the combination therapy.

[0053] In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (post-AAV) after administering the combination therapy.

- [0054] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.
- [0055] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
- [0056] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 2 days prior to administering the combination therapy.
- [0057] In some aspects, the post-AAV immunosuppressive agent is initially administered 1, 2, 3, or 4 weeks after administering the combination therapy.
- [0058] In some aspects, the immunosuppressive agent is initially administered about 3 weeks after administering the combination therapy.
- [0059] In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered 2 times per day, daily, or every other day.
- [0060] In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered for about 6 to 40, about 8 to 40, about 10 to 40, about 12 to 40, about 6 to 30, about 8 to 30, about 10 to 30, about 12 to 30, about 6 to 20, about 8 to 20, about 10 to 20, or about 12 to 20 weeks.
- [0061] In some aspects, the immunosuppressive regimen comprises administering the immunosuppressive agent prior to administering the combination therapy.
- [0062] In some aspects, the immunosuppressive regimen comprises administering the immunosuppressive agent after administering the combination therapy.
- [0063] In some aspects, the immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.
- [0064] In some aspects, the immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
- [0065] In some aspects, the immunosuppressive agent is administered 1 to 3 days prior to administering the combination therapy.
- [0066] In some aspects, the immunosuppressive agent is administered 3 to 7 days prior to administering the combination therapy.
- [0067] In some aspects, the immunosuppressive agent is further administered 1, 2, 3, or 4 weeks after administering the combination therapy.
- [0068] In some aspects, immunosuppressive agent is administered once about every 1-3 weeks, optionally once about every 1-2 weeks after administering the combination therapy.

- [0069] In some aspects, the immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the combination therapy.
- [0070] In some aspects, immunosuppressive regimen comprises a second immunosuppressive agent.
- [0071] In some aspects, the immunosuppressive regimen comprises administering the second immunosuppressive agent prior to administering the combination therapy.
- [0072] In some aspects, the immunosuppressive regimen comprises administering the second immunosuppressive agent after administering the combination therapy.
- [0073] In some aspects, the second immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.
- [0074] In some aspects, the second immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
- [0075] In some aspects, the second immunosuppressive agent is administered 1 to 3 days prior to administering the combination therapy.
- [0076] In some aspects, the second immunosuppressive agent is administered 3 to 7 days prior to administering the combination therapy.
- [0077] In some aspects, the second immunosuppressive agent is further administered 1, 2, 3, or 4 weeks after administering the combination therapy.
- [0078] In some aspects, second immunosuppressive agent is administered 2 times per day, daily, or every other day.
- [0079] In some aspects, the second immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the combination therapy.
- [0080] In some aspects, the immunosuppressive regimen comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor.
- [0081] In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent selected from the group consisting of an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, a calcineurin inhibitor, or any combination thereof.

- [0082] In some aspects, the pre-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, mycophenolate mofetil (MMF), a tyk2 inhibitor, rapamycin, and/or abatacept. In some aspects, the pre-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, mycophenolate mofetil (MMF), a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.
- [0083] In some aspects, the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, and/or abatacept.
- [0084] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof. In some aspects, the IL-6 antagonist is tocilizumab.
- [0085] In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof. In some aspects, the calcineurin inhibitor is tacrolimus.
- [0086] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, satralizumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof.
- [0087] In some aspects, the IL-6 antagonist is tocilizumab.
- [0088] In some aspects, the second immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, abatacept, thymoglobulin, etanercept, basiliximab, sirolimus, or any combination thereof.
- [0089] In some aspects, the immunosuppressive regimen comprises administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the combination therapy.
- [0090] In some aspects, the immunosuppressive regimen comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the combination therapy.
- [0091] In some aspects, the total administered dose of the first AAV vector genome comprising the insulin expression cassette comprises about 7×10^{10} to about 5×10^{13} vector genomes (vg)/kg.
- [0092] In some aspects, the total administered dose of the second AAV vector genome comprising the glucokinase expression cassette comprises about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg.

- [0093] In some aspects, the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:0.25-1.25, 1:1-20, 1:2-20, 1:3-20, 1:4-20, 1:5-20, 1:1-18, 1:2-18, 1:3-18, 1:4-18, 1:5-18, 1:1-16, 1:2-16, 1:3-16, 1:4-16, 1:5-16, 1:1-15, 1:2-15, 1:3-15, 1:4-15, 1:5-15, 1:1-14, 1:2-14, 1:3-14, 1:4-14, 1:5-14, 1:1-12, 1:2-12, 1:3-12, 1:4-12, 1:5-12, 1:1-10, 1:2-10, 1:3-10, 1:4-10, or 1:5-10.
- [0094] In some aspects, the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:1-15, 1:2-15, 1:3-15, 1:4-15, 1:5-15, 1:1-14, 1:2-14, 1:3-14, 1:4-14, 1:5-14, 1:1-12, 1:2-12, 1:3-12, 1:4-12, 1:5-12, 1:1-10, 1:2-10, 1:3-10, 1:4-10, or 1:5-10.
- [0095] In some aspects, the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:1-10, 1:2-10, 1:3-10, 1:4-10, or 1:5-10.
- [0096] In some aspects, the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, or about 1:15.
- [0097] In some aspects, the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:8, about 1:9, or about 1:10.
- [0098] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 5×10^{11} and about 1×10^{13} vg/kg.
- [0099] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 2×10^{12} and about 1×10^{13} vg/kg.
- [0100] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 4×10^{12} to about 1×10^{13} vg/kg.
- [0101] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 7×10^{10} to about 7×10^{12} vg/kg.
- [0102] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{11} to about 5×10^{12} vg/kg.

- [0103] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{11} to about 1×10^{12} vg/kg.
- [0104] In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 1×10^{12} and about 5×10^{12} vg/kg.
- [0105] In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 2×10^{12} and about 5×10^{12} vg/kg.
- [0106] In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2×10^{12} to about 3.5×10^{12} vg/kg.
- [0107] In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} to about 5×10^{12} vg/kg.
- [0108] In some aspects, within 15 days-6 months, 30-120 days, or 30-60 days after administration of the combination therapy: (i) glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in the subject; (ii) circulating ketones are reduced in the subject, (iii) triglycerides are reduced in the subject, (iv) exogenous insulin is reduced in the subject, (v) circulating C-peptide levels are increased in the subject, or (vi) any combination thereof.
- [0109] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's circulating insulin level in the blood, when measured in the absence of exogenous insulin treatment, is about 1-40 $\mu\text{U/mL}$, optionally about 5-40 $\mu\text{U/mL}$, about 5-25 $\mu\text{U/mL}$, about 5-10 $\mu\text{U/mL}$, about 10-20 $\mu\text{U/mL}$, about 10-15 $\mu\text{U/mL}$, about 15-20 $\mu\text{U/mL}$, or about 15-25 $\mu\text{U/mL}$.
- [0110] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's HbA1C level is reduced by about 0.5 to 1.5% compared to pre-treatment.
- [0111] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's HbA1C level is less than about 7.0%, optionally less than about 6.5%, about 6.0%, about 5.7%, optionally about 5.0% to 6.5%.
- [0112] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's circulating glucose in the blood is at least about 70 to 140 mg/dL.

- [0113] In some aspects, the subject is not insulin resistant.
- [0114] In some aspects, the subject is insulin resistant.
- [0115] Certain aspects of the disclosure are directed to a dosing regimen comprising: (a) a first immunosuppressive agent; (b) a combination therapy administered intramuscularly (IM) after (a) which comprises (i) a first recombinant AAV (rAAV) particle comprising a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs); and (c) a second immunosuppressive agent.
- [0116] In some aspects, the first immunosuppressive agent comprises an IL-6 antagonist selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, and any combination thereof.
- [0117] In some aspects, the second immunosuppressive agent is a calcineurin inhibitor selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof.
- [0118] In some aspects, the first immunosuppressive agent comprises tocilizumab and the second immunosuppressive agent comprises tacrolimus.
- [0119] In some aspects, the dosing regimen comprises administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the combination therapy.
- [0120] In some aspects, the immunosuppressive regimen comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the combination therapy.
- [0121] In some aspects, the first AAV vector genome and second AAV vector genome are each administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the first AAV vector genome and second AAV vector genome are administered at a dose between about 1×10^{12} to about 1×10^{13} vg/kg.
- [0122] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 2×10^{12} to about 1×10^{13} vg/kg. In some

aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 4×10^{12} to about 1×10^{13} vg/kg. In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 4.5×10^{12} to about 5×10^{12} vg/kg. In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{12} to about 9×10^{12} vg/kg.

[0123] In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 1×10^{12} to about 5×10^{12} vg/kg. In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 2×10^{12} to about 5×10^{12} vg/kg. In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2×10^{12} to about 2.8×10^{12} vg/kg. In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 4×10^{12} vg/kg to about 5×10^{12} vg/kg.

[0124] In some aspects, the first AAV vector genome comprises an insulin expression cassette is administered at a dose of about 7×10^{10} vg/kg to about 1.8×10^{12} vg/kg.

[0125] In some aspects, the first AAV vector genome comprises an insulin expression cassette is administered at a dose of about 7×10^{11} to about 1.8×10^{12} vg/kg.

[0126] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{11} to about 5×10^{12} vg/kg.

[0127] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{11} to about 1×10^{12} vg/kg.

[0128] In some aspects, the second AAV vector genome comprises a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} vg/kg.

[0129] In some aspects, the first AAV vector genome (comprising the expression cassette encoding hIns) and the second AAV vector genome (comprising the expression cassette encoding hGck) are administered at a ratio selected of 1:0.25-1.25. In some aspects, the ratio is about 1:1. In some aspects, the ratio is 1:0.35-0.75. In some aspects, the ratio is 1:0.4-0.6. In some aspects, the ratio is 1:0.45-0.55. In some aspects, the ratio is about 1:0.5.

[0130] In some aspects, the ratio is about 1:1-15. In some aspects, the ratio is about 1:3-5. In some aspects, the ratio is about 1:2. In some aspects, the ratio is about 1:3. In some aspects, the ratio is about 1:4. In some aspects, the ratio is about 1:5.

- [0131]** In some aspects, the polynucleotide encoding the hIns protein comprises an open reading frame (ORF) comprising: a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to any one of: (i) nucleic acids 73-330 of any of SEQ ID NOs: 43-57, or 110-116, nucleic acids 88-345 of any of SEQ ID NOs: 117-122, 152 or 156, or nucleic acids 79-336 of SEQ ID NO: 153; or (ii) SEQ ID NO: 43-57, or SEQ ID NO: 110-122; and/or the polynucleotide encoding the human glucokinase hGck protein comprises an ORF comprising (i) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of (a) nucleic acids 1-1398 of any of SEQ ID NO: 61-80 or 162; or (ii) SEQ ID NO: 61-80 and 162.
- [0132]** In some aspects, the polynucleotide encoding the hIns protein comprises an open reading frame (ORF) comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 121; and/or the polynucleotide encoding the human glucokinase hGck protein comprises an ORF comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 68.
- [0133]** In some aspects, the hIns protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, amino acids 25-110 of SEQ ID NO: 145, SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145.
- [0134]** In some aspects, the hIns protein comprises a signal peptide. In some aspects, the signal peptide is a wild-type preproinsulin signal sequence, an IL-6 signal sequence, a fibronectin signal sequence, or a non-wild-type preproinsulin signal sequence. In some aspects, the signal peptide comprises amino acids 1-24 of SEQ ID NO: 41, amino acids 1-24 of SEQ ID NO: 144, or amino acids 1-24 of SEQ ID NO: 145. In some aspects, the hIns protein further comprises a cleavage site.
- [0135]** In some aspects, the polynucleotide encoding the hIns protein further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.
- [0136]** In some aspects, the polynucleotide encoding the hIns protein further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, or SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171.

- [0137] In some aspects, the encoded hGck protein comprises the amino acid sequence of SEQ ID NO: 82.
- [0138] In some aspects, the polynucleotide encoding the hGck protein further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.
- [0139] In some aspects, the polynucleotide encoding the hGck protein further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171.
- [0140] In some aspects, the first promoter is a eukaryotic promoter. In some aspects, the first promoter is a constitutive promoter. In some aspects, the first promoter is a ubiquitous promoter. In some aspects, the first promoter is a muscle specific promoter. In some aspects, the first promoter is a CMV promoter. In some aspects, the second promoter is a eukaryotic promoter. In some aspects, the first promoter is a constitutive promoter. In some aspects, the first promoter is a ubiquitous promoter. In some aspects, the first promoter is a muscle specific promoter. In some aspects, the second promoter is a CMV promoter.
- [0141] In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a eukaryotic promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a constitutive promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a ubiquitous promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a CMV promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a muscle specific promoter.
- [0142] In some aspects, the insulin expression cassette and/or the glucokinase expression cassette further comprises a polyadenylation (polyA) element.
- [0143] In some aspects, a first recombinant AAV (rAAV) particle comprises the first AAV vector genome. In some aspects, a second recombinant AAV (rAAV) particle comprises the second vector genome. In some aspects, the AAV serotype of the first rAAV particle and/or the second rAAV particle is selected from the group consisting of

AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh9, AAV9, AAVrh10, AAV10, AAV11, AAV12, and AAVrh74. In some aspects, the AAV serotype of the first rAAV particle and/or the second rAAV particle is AAV1.

- [0144] In some aspects, the first rAAV particle and the second rAAV particle are formulated in a single composition.
- [0145] In some aspects, the first rAAV particle and the second rAAV particle are administered simultaneously or sequentially.
- [0146] In some aspects, the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM). In some aspects, the diabetes is diabetes mellitus type 1 (T1DM). In some aspects, the diabetes is diabetes mellitus type 2 (T2DM).
- [0147] In some aspects, within 15 days and 6 months, 30-120 days, or 30-60 days after administration of the combination therapy: (i) glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in the subject; (ii) circulating ketones are reduced in the subject, (iii) triglycerides are reduced in the subject, (iv) exogenous insulin is reduced in the subject, (v) circulating C-peptide levels are increased in the subject, or (vi) any combination thereof.
- [0148] Certain aspects of the disclosure are directed to a method of immunosuppression in a subject administered an AAV gene therapy for the treatment of diabetes, wherein the subject is administered an immunosuppressive regimen comprising administering (a) an immunosuppressive agent (pre-AAV) prior to administering the AAV gene therapy; and (b) an immunosuppressive agent (post-AAV) after administering the AAV gene therapy.
- [0149] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 14 days prior to administering the AAV gene therapy.
- [0150] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 7 days prior to administering the AAV gene therapy.
- [0151] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 2 days prior to administering the AAV gene therapy.
- [0152] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 3 days prior to administering the AAV gene therapy.
- [0153] In some aspects, the pre-AAV immunosuppressive agent is administered 3 to 7 days prior to administering the AAV gene therapy.

- [0154] In some aspects, the post-AAV immunosuppressive agent is further administered for 1, 2, 3, or 4 weeks after administering the AAV gene therapy.
- [0155] In some aspects, post-AAV immunosuppressive agent is administered once about every 1-3 weeks, optionally once about every 1-2 weeks after administering the combination therapy.
- [0156] In some aspects, the post-AAV immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the AAV gene therapy.
- [0157] In some aspects, the immunosuppressive agent is initially administered about 3 weeks after administering the AAV gene therapy.
- [0158] In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered 2 times per day, daily, or every other day.
- [0159] In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered for about 6 to 40, about 8 to 40, about 10 to 40, about 12 to 40, about 6 to 30, about 8 to 30, about 10 to 30, about 12 to 30, about 6 to 20, about 8 to 20, about 10 to 20, or about 12 to 20 weeks.
- [0160] Certain aspects of the disclosure are directed to a method of immunosuppression in a subject administered an AAV gene therapy for the treatment of diabetes, wherein the subject is administered an immunosuppressive regimen comprising administering (a) a first immunosuppressive agent prior to administering the AAV gene therapy; and, optionally, (b) a second immunosuppressive agent after administering the AAV gene therapy.
- [0161] In some aspects, the first immunosuppressive agent is administered 1 to 14 days prior to administering the AAV gene therapy.
- [0162] In some aspects, the first immunosuppressive agent is administered 1 to 7 days prior to administering the AAV gene therapy.
- [0163] In some aspects, the first immunosuppressive agent is administered 1 to 3 days prior to administering the AAV gene therapy.
- [0164] In some aspects, the second immunosuppressive agent is administered 1 to 14 days prior to administering the AAV gene therapy.
- [0165] In some aspects, the second immunosuppressive agent is administered 1 to 7 days prior to administering the AAV gene therapy.

- [0166] In some aspects, the second immunosuppressive agent is administered 3 to 7 days prior to administering the AAV gene therapy.
- [0167] In some aspects, after the initial administration, the second immunosuppressive agent is administered 2 times per day, daily, or every other day.
- [0168] In some aspects, the first immunosuppressive agent and/or the second immunosuppressive agent is administered for about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some aspects, the immunosuppressive regimen comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor.
- [0169] In some aspects, the pre-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, MMF, a tyk2 inhibitor, rapamycin, or abatacept.
- [0170] In some aspects, the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, and/or abatacept.
- [0171] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof. In some aspects, the IL-6 antagonist is tocilizumab.
- [0172] In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof. In some aspects, the calcineurin inhibitor is tacrolimus.
- [0173] In some aspects, the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent is administered for about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.
- [0174] In some aspects, the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, a calcineurin inhibitor, or any combination thereof.

- [0175] In some aspects, the pre-AAV immunosuppressive agent and/or post-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, MMF, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.
- [0176] In some aspects, the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.
- [0177] In some aspects, the method comprises administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the AAV gene therapy.
- [0178] In some aspects, the method comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the AAV gene therapy.

BRIEF DESCRIPTION OF FIGURES

- [0179] **FIGs. 1A-1J** show noted biomarker levels measured in a first cynomolgus macaque (NHP1) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 1A** shows daily pre- and post-prandial blood glucose levels (mg/dL) and the amount of exogenous long-acting insulin (insulin glargine, “Lantus”) and short-acting insulin (insulin lispro, “Humalog”) administered (U/kg/day) prior to and following administration of AAV1-hINS+AAV1-hGCK (at day 0), with timepoints up to day 180 following AAV dosing. The % reduction of exogenous insulin administered compared to the 7-day average of exogenous insulin administered prior to AAV administration (baseline) (post-STZ treatment, pre-day 0) is shown at the top of the graph. Blood samples were drawn throughout the in-life phase and analyzed for C-peptide levels (**FIG. 1B**; ng/mL), HbA1C levels (**FIG. 1C**; %), and creatine kinase (CK) levels (**FIG. 1D**; U/L) from day 0 up to day 182 following AAV1-hINS+AAV1-hGCK administration. **FIG. 1E** shows body weight (kg) before and after AAV dosing and up to day 182. **FIG. 1F** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP1 (i) prior to STZ administration (Pre STZ), (ii) after STZ and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ at 56 days (D+56), 92 days (D+92), or 176 days (D+176) after AAV1-hIns+AAV1-hGck dosing.

FIG. 1G shows C-peptide levels (ng/mL) during IVGTT of NHP1 (i) prior to STZ administration (Pre STZ), (ii) after STZ and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ at 56 days (D+56), 92 days (D+92), or 176 days (D+176) after AAV1-hIns+AAV1-hGck dosing. **FIG. 1H** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, and a negative control measured from 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 161 days following AAV administration. Positive responses are indicated by boxes. **FIG. 1I** shows anti-insulin IgG (dilution factor) measured at 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 182 days after. **FIG. 1J** shows anti-glucokinase IgG (ECLsignal) measured at 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 161 days after.

[0180] **FIGs. 2A-2I** show noted biomarker levels measured in a second cynomolgus macaque (NHP2) administered AAV1-hINS + AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 2A** shows daily pre- and post-prandial blood glucose levels (mg/dL) and the amount of exogenous long-acting insulin (insulin glargine, “Lantus”) and short-acting insulin (insulin lispro, “Humalog”) administered (U/kg/day) prior to and following administration of AAV1-hINS+AAV1-hGCK (at day 0), with timepoints up to day 132 following AAV dosing. The % reduction of exogenous insulin administered compared to the 7-day average of exogenous insulin administered prior to AAV administration (baseline) (pre-day 0) is shown at the top of the graph. Blood samples were drawn weekly and analyzed for preprandial, fasted, or stimulated C-peptide levels (**FIG. 2B**; ng/mL), Hb1A1C levels (**FIG. 2C**; %) levels, and creatine kinase (CK) levels (**FIG. 2D**; U/L) from day 0 up to day 132 following AAV1-hINS+AAV1-hGCK administration. **FIG. 2E** shows body weight (kg) before and after AAV dosing and up to day 132. **FIG. 2F** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP2 (i) prior to STZ administration (Pre STZ), after STZ and before administration of AAV1-hIns+AAV1-hGck (Post STZ) and (iii) after STZ at 58 days (D+58) or 91 days (D+91) after AAV1-hINS+AAV1-hGCK dosing. **FIG. 2G** shows C-peptide levels (ng/mL) during IVGTT of NHP2 (i) prior to STZ administration (Pre STZ), (ii) after STZ and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ at either 58 days (D+58) or 91 days (D+91) after AAV1-hIns+AAV1-hGck dosing. **FIG. 2H** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear

cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV administration (-14) up to 105 days following AAV administration. Positive test responses are indicated by boxes. **FIG. 2I** shows anti-insulin IgG (Anti-Insulin dilutional titer) and anti-glucokinase IgG (anti-glucokinase ECL signal) measured at -14 days prior to AAV administration up to 132 days after.

[0181] **FIGs. 3A-3I** show noted biomarker levels measured in a third cynomolgus macaque (NHP3) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 3A** shows daily pre- and post-prandial blood glucose levels (mg/dL) and the amount of exogenous long-acting insulin (insulin glargine, “Lantus”) and short-acting insulin (insulin lispro, “Humalog”) administered (U/kg/day) prior to and following administration of AAV1-hINS+AAV1-hGCK (at day 0), with timepoints up to day 90 following AAV dosing. The % reduction of exogenous insulin administered compared to the 7-day average of exogenous insulin administered prior to AAV administration (baseline) (pre-day 0) is shown at the top of the graph. Blood samples were drawn weekly and analyzed for preprandial, fasted, or stimulated C-peptide levels (**FIG. 3B**; ng/mL), Hb1A1C levels (**FIG. 3C**; %) levels, and creatine kinase (CK) levels (**FIG. 3D**; U/L) from day 0 up to day 90 following AAV1-hINS+AAV1-hGCK administration. **FIG. 3E** shows body weight (kg) before and after AAV dosing up to day 90. **FIG. 3F** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP3 (i) prior to STZ administration (Pre STZ), after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) or 84 days (D+84) after AAV1-hIns+AAV1-hGck dosing. **FIG. 3G** shows C-peptide levels (ng/mL) during IVGTT of NHP3 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) or 84 days (D+84) after AAV1-hIns+AAV1-hGck dosing. **FIG. 3H** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV administration (-14) up to 91 days following AAV administration. Positive test responses are indicated by boxes. **FIG. 3I** shows anti-insulin IgG (Anti-Insulin dilutional titer) and anti-glucokinase IgG (anti-glucokinase ECL signal) measured at 14 days prior to AAV administration up to 91 days after.

[0182] **FIGs. 4A-4I** show noted biomarker levels measured in a fourth cynomolgus macaque (NHP4) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 4A** shows daily pre- and post-prandial blood glucose levels (mg/dL) and the amount of exogenous long-acting insulin (insulin glargine, “Lantus”) and short-acting insulin (insulin lispro, “Humalog”) administered (U/kg/day) prior to and following administration of AAV1-hINS+AAV1-hGCK (at day 0), with timepoints up to day 90 following AAV dosing. The % reduction of exogenous insulin administered compared to the 7-day average of exogenous insulin administered prior to AAV administration (baseline) (pre-day 0) is shown at the top of the graph. Blood samples were drawn weekly and analyzed for random (sample taken without regard for fasted or fed status), fasted, or stimulated (sample taken as part of IVGTT) C-peptide levels (**FIG. 4B**; ng/mL), Hb1A1C levels (**FIG. 4C**; %) levels, and creatine kinase (CK) levels (**FIG. 4D**; U/L) from day 0 up to day 90 following AAV1-hINS+AAV1-hGCK administration. **FIG. 4E** shows body weight (kg) before and after AAV dosing and up to day 90. **FIG. 4F** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP4 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) and 84 days (D+84) after AAV1-hIns+AAV1-hGck dosing. **FIG. 4G** shows C-peptide levels (ng/mL) during IVGTT of NHP4 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) after AAV1-hIns+AAV1-hGck dosing. **FIG. 4H** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV administration (-14) up to 91 days following AAV administration. Positive test responses are indicated by boxes. **FIG. 4I** shows anti-insulin IgG (Anti-Insulin dilutional titer) and anti-glucokinase IgG (anti-glucokinase ECL signal) measured at 14 days prior to AAV administration up to 80 days after.

[0183] **FIGs. 5A-5K** show noted biomarker levels measured in a fifth cynomolgus macaque (NHP5) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 5A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days

prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 180 days following AAV1-hINS+AAV1-hGCK dosing. Blood samples were drawn weekly and analyzed for C-peptide levels (**FIG. 5B**; ng/mL) and Hb1A1C levels (**FIG. 5C**; %) from day 0 to day 180 following AAV1-hINS+AAV1-hGCK administration. Blood samples were drawn weekly and analyzed for creatine kinase (CK) levels (**FIG. 5D**; U/L) from day 0 through day 180 following AAV1-hINS+AAV1-hGCK administration. **FIG. 5E** shows body weight (kg) before and after AAV1-hINS+AAV1-hGCK dosing and through day 182. **FIG. 5F** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP5 prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56), 91 days (D+91), and 175 days (D+175) after AAV1-hIns+AAV1-hGck dosing. **FIG. 5G** shows C-peptide levels (ng/mL) during IVGTT of NHP5 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56), 91 days (D+91), and 175 days (D+175) after AAV1-hIns+AAV1-hGck dosing. **FIG. 5H** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg/day) following administration of AAV1-hINS+AAV1-hGCK (at day 0) and timepoints through day 180 following AAV1-hINS+AAV1-hGCK dosing. **FIG. 5I** shows the % reduction of exogenous insulin (change from baseline) administered compared to the 30-day average of exogenous insulin administered prior to AAV1-hINS+AAV1-hGCK administration (baseline). The 30 day average % reduction is shown at the top of the graph. **FIG. 5J** shows insulin (μ U/mL) measured either after fasting or at randomly selected times, from administration of AAV1-hINS+AAV1-hGCK (at day 0) through day 182. **FIG. 5K** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 182 days following AAV1-hINS+AAV1-hGCK administration. Positive test responses are indicated by boxes.

[0184] **FIGs. 6A-6L** show noted biomarker levels measured in a sixth cynomolgus macaque (NHP6) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 6A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 182 days following AAV1-

hINS+AAV1-hGCK dosing. Blood samples were drawn weekly and analyzed for C-peptide levels (**FIG. 6B**; ng/mL) and Hb1A1C levels (**FIG. 6C**; %) from day 0 through day 182 following AAV1-hINS+AAV1-hGCK administration. Blood samples were drawn weekly and analyzed for creatine kinase (CK) levels (**FIG. 6D**; U/L) from day 0 through day 182 following AAV1-hINS+AAV1-hGCK administration. **FIG. 6E** shows body weight (kg) before and after AAV1-hINS+AAV1-hGCK dosing and through day 182. **FIG. 6F** shows circulating tacrolimus levels ($\mu\text{g/L}$), beginning at day 21 following AAV1-hINS+AAV1-hGCK administration through day 182. **FIG. 6G** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP6 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hINS+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56), 91 days (D+91), and 175 days (D+175) after AAV1-hIns+AAV1-hGck dosing. **FIG. 6H** shows C-peptide levels (ng/mL) during IVGTT of NHP6 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56), 91 days (D+91), and 175 days (D+175) after AAV1-hIns+AAV1-hGck dosing. **FIG. 6I** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg/day) following administration of AAV1-hINS+AAV1-hGCK (at day 0) and timepoints through day 182 following AAV1-hINS+AAV1-hGCK dosing. **FIG. 6J** shows the % reduction of exogenous insulin administered (change from baseline) compared to the 30-day average of exogenous insulin administered prior to AAV1-hINS+AAV1-hGCK administration (baseline). The 30 day average % reduction is shown at the top of the graph. **FIG. 6K** shows insulin ($\mu\text{U/mL}$) measured either after fasting or at randomly selected times, from administration of AAV1-hINS+AAV1-hGCK (at day 0) through day 180. **FIG. 6L** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 182 days following AAV1-hINS+AAV1-hGCK administration. Positive test responses are indicated by boxes.

[0185] **FIGs. 7A-7L** show noted biomarker levels measured in a seventh cynomolgus macaque (NHP7) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 7A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days

prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 122 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 7B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg/day) following administration of AAV1-hINS+AAV1-hGCK (at day 0) and timepoints through day 122 following AAV1-hINS+AAV1-hGCK dosing. **FIG. 7C** shows the % reduction of exogenous insulin administered (change from baseline) compared to the 30-day average of exogenous insulin administered prior to AAV1-hINS+AAV1-hGCK administration (baseline). The 30 day average % reduction is shown at the top of the graph. **FIG. 7D** shows Hb1A1C levels (%) from day 0 through day 122 following AAV1-hINS+AAV1-hGCK administration. **FIG. 7E** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP7 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 64 days (D+64) and 91 days (D+91) after AAV1-hIns+AAV1-hGck dosing. **FIG. 7F** shows C-peptide levels (ng/mL) during IVGTT of NHP7 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 64 days (D+64) and 91 days (D+91) after AAV1-hIns+AAV1-hGck dosing. **FIG. 7G** shows creatine kinase (CK) levels (U/L) from day 0 through day 122 following AAV1-hINS+AAV1-hGCK administration. **FIG. 7H** shows circulating tacrolimus levels ($\mu\text{g/L}$), beginning at day 21 following AAV1-hINS+AAV1-hGCK administration through day 90. **FIG. 7I** shows levels of human insulin ($\mu\text{U/mL}$) measured from administration of AAV1-hIns+AAV1-hGck (day 0) until day 122. **FIG. 7J** shows C-peptide levels (ng/mL) from day 0 to day 122 following AAV1-hINS+AAV1-hGCK administration. **FIG. 7K** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 122 days following AAV1-hINS+AAV1-hGCK administration. Positive test responses are indicated by boxes. **FIG. 7L** shows body weight (kg) before and after AAV1-hINS+AAV1-hGCK dosing and through day 122.

[0186] **FIGs. 8A-8L** show noted biomarker levels measured in an eighth cynomolgus macaque (NHP8) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 8A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days

prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 122 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 8B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg/day) following administration of AAV1-hINS+AAV1-hGCK (at day 0) and timepoints through day 122 following AAV1-hINS+AAV1-hGCK dosing. **FIG. 8C** shows the % reduction of exogenous insulin administered (change from baseline) compared to the 30-day average of exogenous insulin administered prior to AAV1-hINS+AAV1-hGCK administration (baseline). The 30 day average % reduction is shown at the top of the graph. **FIG. 8D** shows Hb1A1C levels (%) from day 0 through day 122 following AAV1-hINS+AAV1-hGCK administration. **FIG. 8E** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP8 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) and 91 days (D+91) after AAV1-hIns+AAV1-hGck dosing. **FIG. 8F** shows C-peptide levels (ng/mL) during IVGTT of NHP8 (i) prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+64) and 91 days (D+91) after AAV1-hIns+AAV1-hGck dosing. **FIG. 8G** shows creatine kinase (CK) levels (U/L) from day 0 through day 122 following AAV1-hINS+AAV1-hGCK administration. **FIG. 8H** shows circulating tacrolimus levels ($\mu\text{g/L}$), beginning at day 21 following AAV1-hINS+AAV1-hGCK administration through termination. **FIG. 8I** shows levels of human insulin ($\mu\text{U/mL}$) measured from administration of AAV1-hIns+AAV1-hGck (day 0) until 122. **FIG. 8J** shows C-peptide levels (ng/mL) from day 0 to day 122 following AAV1-hINS+AAV1-hGCK administration. **FIG. 8K** shows interferon- γ response (spots/million cells) in peripheral blood mononuclear cells against AAV1, glucokinase (Gck), insulin, a positive control, and a negative control measured from 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14) up to 122 days following AAV1-hINS+AAV1-hGCK administration. Positive test responses are indicated by boxes. **FIG. 8L** shows body weight (kg) before and after AAV1-hINS+AAV1-hGCK dosing and through day 122.

[0187] **FIG. 9A** summarizes anti-insulin IgG (dilution factor) for NHP1-NHP4 measured at 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14). **FIG. 9B** shows anti-glucokinase IgG (ECLsignal) for NHP1-NHP6 measured at 14 days prior to AAV

administration (-14). **FIG. 9C** shows levels of anti-drug antibodies (ADA) for insulin (screening signal) measured for NHP1 and NHP2 starting from day -14 until day 182.

[0188] **FIGs. 10A-10I** show measures of human glucokinase protein (ng/mg) from muscles at or near the injection site of AAV1-hINS+AAV1-hGCK in NHP1-NHP8. **FIG. 10A** shows glucokinase (ng/mg) protein levels from various muscles samples taken at the injection site of AAV1-hINS+AAV1-hGCK or 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. NHP1-NHP8 are shown. **FIG. 10B** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP1 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10C** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP2 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10D** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP3 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10E** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP4 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10F** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP5 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10G** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP6 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10H** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP7 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 10I** specifically shows the glucokinase levels (ng/mg) in muscle samples of NHP8 taken from the injection sites of AAV1-hINS+AAV1-hGCK or samples 1 cm away. The lower limit of quantification (LLOQ) is displayed as a dashed line.

[0189] **FIG. 11A** shows the levels of vector genome (Vg; copies/ μ g gDNA) and insulin mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP1 and NHP2. **FIG. 11B** shows the levels of vector

genome (Vg; copies/ μ g gDNA) and glucokinase mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP1 and NHP2. **FIG. 11C** shows the levels of vector genome (Vg; copies/ μ g gDNA) and insulin mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP3 and NHP4. **FIG. 11D** shows the levels of vector genome (Vg; copies/ μ g gDNA) and glucokinase mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP3 and NHP4. **FIG. 11E** shows the levels of vector genome (Vg; copies/ μ g gDNA) and insulin mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP5 and NHP6. **FIG. 11F** shows the levels of vector genome (Vg; copies/ μ g gDNA) and glucokinase mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP5 and NHP6. **FIG. 11G** shows the levels of vector genome (Vg; copies/ μ g gDNA) and insulin mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP7 and NHP8. **FIG. 11H** shows the levels of vector genome (Vg; copies/ μ g gDNA) and glucokinase mRNA (copies/ μ g RNA) taken from muscle samples at or near the injection site (IS) of AAV1-hINS+AAV1-hGCK for NHP7 and NHP8.

[0190] **FIGs. 12A-12G** show metabolic data in an ongoing study from a group of cynomolgus macaques (NHP9-NHP11) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 12A** shows lactate levels (mol/L) measured from NHP9-NHP11 beginning on the day of AAV1-hINS+AAV1-hGCK injection (day 0). **FIG. 12B** shows HbA1C levels (%) measured for NHP9-NHP11 beginning on the day of AAV1-hINS+AAV1-hGCK injection (day 0). **FIG. 12C** shows c-peptide levels (ng/mL) measured for NHP9 and NHP10 beginning on the day of AAV1-hINS+AAV1-hGCK injection (day 0). The lower limit of quantification (LLOQ) is displayed as a dashed line. **FIG. 12D** shows human insulin levels (μ U/mL) measured for NHP9 and NHP10 beginning on the day of AAV1-hINS+AAV1-hGCK injection (day 0). **FIG. 12E** shows glucagon levels (pmol/L) measured for NHP9 and NHP10 beginning on the day of AAV1-hINS+AAV1-hGCK injection (day 0). **FIG. 12F** shows triglyceride levels (mg/dL) measured for NHP9-NHP11 beginning on the day of AAV1-hINS+AAV1-hGCK injection (day 0). **FIG. 12G** shows HbA1c at days 0 and 28 for NHPs 9-14.

- [0191] **FIGs. 13A-13D** show noted biomarker levels measured in a ninth cynomolgus macaque (NHP9) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 13A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 184 days following AAV1-hINS+AAV1-hGCK dosing. An arrow indicates the day that the dose of tacrolimus was reduced. **FIG. 13B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg) administered from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 184 days following AAV1-hINS+AAV1-hGCK dosing. For each 30-day window beginning at day 0, the percent change compared to the pre-AAV 30-day window for glargine and lispro is displayed on top of the graph. An arrow indicates the day that the dose of tacrolimus was reduced due to an observed anemic event on Day 120 unrelated to the test article. **FIG 13C** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP9 prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) and 91 days (D+91) after AAV1-hIns+AAV1-hGck dosing. **FIG 13D** displays the quantification for **FIG. 13C** as AUC (area under the curve).
- [0192] **FIGs. 14A-14D** show noted biomarker levels measured in a tenth cynomolgus macaque (NHP10) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 14A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 184 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 14B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg) administered from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 184 days following AAV1-hINS+AAV1-hGCK dosing. For each 30-day window beginning at day 0, the percent change compared to the pre-AAV 30-day window for glargine and lispro is displayed on top of the graph. **FIG 14C** shows blood glucose levels (mg/dL) during an intravenous glucose tolerance test (IVGTT) of NHP10 prior to STZ administration (Pre STZ), (ii) after STZ administration and before administration of AAV1-hIns+AAV1-hGck (Post STZ), and (iii) after STZ administration at 56 days (D+56) and 91 days (D+91) after

AAV1-hIns+AAV1-hGck dosing. **FIG 14D** displays the quantification for FIG. 14C as AUC (area under the curve).

[0193] **FIGs. 15A-15B** show noted biomarker levels measured in an eleventh cynomolgus macaque (NHP11) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 15A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 15B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg) administered from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. For each window beginning at day 0, the percent change compared to the pre-AAV 30-day window for glargine and lispro is displayed on top of the graph.

[0194] **FIGs. 16A-16B** show noted biomarker levels measured in a twelfth cynomolgus macaque (NHP12) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 16A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 16B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg) administered from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. For each window beginning at day 0, the percent change compared to the pre-AAV 30-day window for glargine and lispro is displayed on top of the graph.

[0195] **FIGs. 17A-17B** show noted biomarker levels measured in a thirteenth cynomolgus macaque (NHP13) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 17A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 17B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg) administered from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. For each window beginning at day 0,

the percent change compared to the pre-AAV 30-day window for glargine and lispro is displayed on top of the graph.

[0196] **FIGs. 18A-18B** show noted biomarker levels measured in a fourteenth cynomolgus macaque (NHP14) administered AAV1-hINS+AAV1-hGCK following streptozotocin (STZ) treatment in a STZ-induced non-human primate (NHP) model of Type 1 diabetes. **FIG. 18A** shows daily pre- and post-prandial blood glucose levels (mg/dL) from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. **FIG. 18B** shows the amount of exogenous long-acting insulin (glargine) and short-acting insulin (lispro) (U/kg) administered from 30 days prior to injection of AAV1-hINS+AAV1-hGCK (-30) until 43 days following AAV1-hINS+AAV1-hGCK dosing. For each window beginning at day 0, the percent change compared to the previous 30-day window for glargine and lispro is displayed on top of the graph.

[0197] **FIGs. 19A-19B** show schematics of muscles and tendons in a leg from an anterior view (**FIG. 19A**) and a posterior view (**FIG. 19B**).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0198] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present application, including the definitions, will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0199] Throughout this disclosure, the term “a” or “an” entity refers to one or more of that entity; for example, “a polynucleotide,” is understood to represent one or more polynucleotides. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0200] Furthermore, “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects:

A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0201] The term “about” is used herein to mean approximately, roughly, around, or in the regions of. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower), unless indicated otherwise.

[0202] The term “at least” prior to a number or series of numbers is understood to include the number adjacent to the term “at least,” and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, “at least 18 nucleotides of a 21-nucleotide nucleic acid molecule” means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that “at least” can modify each of the numbers in the series or range. “At least” is also not limited to integers (e.g., “at least 5%” includes 5.0%, 5.1%, 5.18% without consideration of the number of significant figures).

[0203] Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with, 37 CFR §1.822 and established usage.

[0204] “Polynucleotide” or “nucleic acid” as used herein means a sequence of nucleotides connected by phosphodiester linkages. Polynucleotides are presented herein in the direction from the 5' to the 3' direction. A polynucleotide of the present disclosure can be a deoxyribonucleic acid (DNA) molecule or ribonucleic acid (RNA) molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), inosine (I) and uracil (U).

[0205] As used herein, the term “polypeptide” encompasses both peptides and proteins, unless indicated otherwise.

[0206] The term “coding sequence” or “sequence encoding” is used herein to mean a DNA or RNA region (the transcribed region) which “encodes” a particular protein, e.g.,

such as an insulin or a glucokinase. A coding sequence is transcribed (DNA) and translated (RNA) into a polypeptide, *in vitro* or *in vivo*, when placed under the control of an appropriate regulatory region, such as a promoter. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from prokaryotes or eukaryotes, genomic DNA from prokaryotes or eukaryotes, and synthetic DNA sequences. A transcription termination sequence can be located 3' to the coding sequence.

[0207] In some aspects, an expression cassette can comprise several operably linked fragments, such as one or more of a promoter, a 5'-untranslated sequence, a leader sequence, an intron, a coding sequence and a 3'-untranslated sequence (e.g., comprising a polyadenylation site or a signal sequence).

[0208] As used herein, "expression of a gene" refers to the process wherein a gene is transcribed into an RNA and/or translated into an active protein.

[0209] An open reading frame (ORF) as used herein is the part of a reading frame that has the ability to be translated. An ORF is a continuous stretch of codons that begins with a start codon and ends at a stop codon. In some aspects, an ORF sequence can be shown or referenced with or without the start codon sequence and/or the stop codon sequence.

[0210] A Kozak consensus sequence, Kozak consensus or Kozak sequence, is known as a sequence which occurs on eukaryotic mRNA and has the consensus (gcc)gccRccAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another "G." In some aspects, the polynucleotide comprises a nucleic acid sequence having at least 95%, at least 99% sequence identity, or more to the Kozak consensus sequence. In some aspects, the polynucleotide comprises a Kozak consensus sequence.

[0211] The term "sequence identity" is used herein to mean a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In certain aspects, sequence identity is calculated based on the full length of two given SEQ ID NO or on a part thereof. Part thereof can mean at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of both SEQ ID NO, or any other specified percentage. The term "identity" can also mean the degree of sequence relatedness between two or more

amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences.

[0212] In certain aspects, methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs.

[0213] “Substantial homology” or “substantial similarity,” when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95% to 99% of the sequence.

[0214] As used herein, and unless otherwise indicated, the term “complementary,” when used to describe a first nucleic acid sequence in relation to a second nucleic acid sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleic acid sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleic acid sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C, or 70°C, for 12-16 hours followed by washing (see, e.g., “Molecular Cloning: A Laboratory Manual, Sambrook, et al. (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can be used. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[0215] The term “promoter” is used herein to mean a nucleic acid sequence or fragment that functions to control the transcription of one or more genes (or coding sequences), located upstream with respect to the direction of transcription of the transcription initiation site of the gene (or coding sequence). In some aspects, the promoter is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and, optionally, any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. A “constitutive” promoter is a promoter that is active under most physiological and developmental conditions. An “inducible” promoter is a promoter that is regulated

depending on physiological or developmental conditions, or in some aspects an inducible promoter can be induced by an exogenous molecule (e.g., a chemical or drug) or other exogenous stimulus (e.g., light, or radiation). A “tissue specific” promoter is preferentially active in specific types of differentiated cells/tissues.

[0216] As used herein, the term “enhancer” is a cis-acting element that stimulates or inhibits transcription of adjacent genes. An enhancer that inhibits transcription is also referred to as a “silencer.” Enhancers can function (e.g., can be associated with a coding sequence) in either orientation, over distances of up to several kilobase pairs (kb) from the coding sequence and from a position downstream of a transcribed region.

[0217] The terms “operatively linked,” “operatively inserted,” “operatively positioned,” “under control” or “under transcriptional control” means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene (or coding sequence). DNA sequence and a regulatory sequence(s) are considered operably linked if they are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s). DNA can be operably inserted if the DNA of interest is positioned adjacent a DNA sequence which directs transcription and translation of the DNA of interest in a cell (i.e., facilitates the production of, e.g., a polypeptide encoded by a DNA of interest).

[0218] The term “transgene” is used herein to mean a gene or a nucleic acid molecule that is introduced into a cell. An example of a transgene is a nucleic acid encoding a therapeutic polypeptide (e.g., a gene encoding an insulin and/or a gene encoding a glucokinase). In some embodiments, the gene can be present but in some cases normally not expressed or expressed at an insufficient level in the cell. In this context, “insufficient” means that although said gene, e.g., insulin and/or glucokinase, is normally expressed in a cell, a condition and/or disease as disclosed herein (e.g., diabetes) could still develop. In certain aspects, the transgene allows for the increased expression or over-expression of the gene, e.g., an insulin and/or a glucokinase. The transgene can comprise sequences that are native to the cell, comprise sequences that do not naturally occur in the cell, or it can comprise combinations of both. In certain aspects, the transgene can comprise modified sequences coding for an insulin, a glucokinase, both an insulin and a glucokinase, and/or additional protein(s) that can be operably linked to appropriate regulatory sequences for expression of the sequences coding for an insulin, a glucokinase,

or both an insulin and a glucokinase in the cell. In some aspects, the transgene is not integrated into the host cell's genome.

[0219] The terms “modified genes”, “modified nucleic acids”, and the like are used interchangeably herein to mean the introduction of one or more modifications or changes relative to the in the natural sequence of the genes or nucleic acid sequence. Such modifications may or may not result in mutations to the encoded protein sequence. In some embodiments, the modified nucleic acid encodes a wild-type or mutant protein or fragment thereof.

[0220] The term “derived from,” as used herein, refers to a component that is isolated from or made using a specified molecule or organism, or information (e.g., amino acid or nucleic acid sequence) from the specified molecule or organism. For example, a nucleic acid sequence (e.g., a modified human insulin gene) that is derived from a second nucleic acid sequence (e.g., a wild-type human insulin gene) can include a nucleotide sequence or portion thereof that is identical or substantially similar to the nucleotide sequence of the second nucleic acid sequence. In some aspects, mutants, analogs or derivatives can be derived from a wild-type sequence.

[0221] In the case of a polynucleotide, the derived species can be obtained by, for example, naturally occurring mutagenesis, artificial directed mutagenesis or artificial random mutagenesis. The mutagenesis used to derive polynucleotides can be intentionally directed or intentionally random, or a mixture of each.

[0222] “Vector” as used herein typically refers to a recombinant plasmid or virus that comprises a polynucleotide to be delivered into a host cell, either *in vitro* or *in vivo*.

[0223] As used herein, the term “delivery vector” or “vector” includes any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, artificial chromosome, virus, virion, etc., which is capable of replication when associated with the proper control elements and which can transfer gene or nucleic acid sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors. In some aspects, useful vectors are contemplated to be those vectors in which the nucleic acid segment to be transcribed is positioned under the transcriptional control of a promoter. In some aspects, the delivery vector is selected from the group consisting of a viral vector, a plasmid, lipid, and a lysosome.

- [0224] The term “expression vector”, “expression construct” or “expression cassette” means any type of genetic construct containing a nucleic acid in which part or all of the nucleic acid encoding sequence is capable of being transcribed.
- [0225] In some aspects, the biological vectors include viruses, particularly attenuated and/or replication-deficient viruses. In some embodiments, chemical vectors include lipid complexes and naked DNA constructs.
- [0226] As used herein, the term “naked DNA” or “naked nucleic acid” and the like refers to a nucleic acid molecule that is not contained within a viral particle, bacterial cell, or other encapsulating or encapsidating means that facilitates delivery of nucleic acid into the cytoplasm of the target cell. Naked nucleic acid can be associated with means for facilitating delivery of the nucleic acid to the site of the target cell (e.g., to facilitate travel into the target cell of the nucleic acid through the alimentary canal, protect the nucleic acid from stomach acid, and/or serve to penetrate intestinal mucus) and/or to the surface of the target epithelial cell.
- [0227] A “viral genome” or “viral vector” can refer to a viral sequence that comprises one or more polynucleotide regions encoding or comprising a molecule of interest, e.g., a protein, a peptide, and a polynucleotide or a plurality thereof. Viral vectors can be used to deliver genetic materials into cells. Viral vectors can be modified for specific applications. In some aspects, the delivery vector of the disclosure is a viral vector selected from the group consisting of an adeno-associated viral (AAV) vector, an adenoviral vector, a lentiviral vector, or a retroviral vector.
- [0228] The term “adeno-associated virus vector” or “AAV vector” as used herein refers to any vector which comprises or derives from components of an adeno-associated virus and is suitable to infect mammalian cells, preferably human cells. The term AAV vector can designate an AAV-type viral particle or virion comprising a payload. The AAV vector can be derived from various serotypes, including combinations of serotypes (i.e., “pseudotyped” AAV) or from various genomes (e.g., single stranded or self-complementary). In addition, the AAV vector can be replication defective and/or targeted. As used herein, the term “adeno-associated virus” (AAV), includes but is not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, AAVrh8, AAVrh10, AAVrh.74, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, those

AAV serotypes and clades disclosed by Gao et al. (J. Virol. 78:6381 (2004)) and Moris et al. (Virol. 33:375 (2004)), and any other AAV. See, e.g., FIELDS et al. VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). In some aspects, an “AAV vector” includes a derivative of a known AAV vector. In some aspects, an “AAV vector” includes a modified or an artificial AAV vector. In some aspects, the AAV vector is modified or mutated relative to the wild-type AAV serotype sequence. In some aspects, the terms “AAV genome” and “AAV vector” can be used interchangeably.

- [0229] As used herein, a “recombinant AAV particle” or “rAAV particle” is an AAV virus that comprises a capsid protein and an AAV vector having at least one payload region (e.g., an expression cassette including a polynucleotide encoding insulin and/or Gck) and at least one inverted terminal repeat (ITR) region. In some aspects, the terms “AAV vectors of the present disclosure” or “AAV vectors” refer to AAV vectors comprising a polynucleotide encoding an insulin, a GcK, or a combination thereof, e.g., encapsulated in an AAV capsid.
- [0230] “Transduction” of a cell by a virus means that there is transfer of a nucleic acid from the virus particle to the cell. In some aspects, transduction refers to the delivery of a nucleic acid or nucleic acids encoding an insulin and/or a glucokinase into a recipient host cell by a viral vector. For example, transduction of a target cell by a rAAV vector of the disclosure leads to transfer of the rAAV genome (e.g., comprising a polynucleotide of the disclosure) contained in that vector into the transduced cell.
- [0231] “Transfection” of a cell means that genetic material is introduced into a cell for the purpose of genetically modifying the cell. Transfection can be accomplished by a variety of means known in the art, e.g., transduction or electroporation.
- [0232] The term “host cell” or “target cell” is used herein to mean the cell into which the polynucleotide delivery takes place, either *in vitro* or *in vivo*. In some aspect, AAV vectors are able to transduce both dividing and non-dividing cells.
- [0233] “Recombinant” means distinct from that generally found in nature.
- [0234] “Serotype” with respect to vector or virus capsid is defined by a distinct immunological profile based on the capsid protein sequences and capsid structure.
- [0235] “AAV Cap” means AAV Cap proteins, VP1, VP2 and VP3 and analogs thereof.
- [0236] “AAV Rep” means AAV Rep proteins and analogs thereof.
- [0237] “Flanked,” with respect to a sequence that is flanked by other elements, indicates the presence of one or more the flanking elements upstream and/or downstream, i.e., 5'

and/or 3', relative to the sequence. The term "flanked" is not intended to indicate that the sequences are necessarily contiguous. For example, there may be intervening sequences between the nucleic acid encoding the transgene and a flanking element. A sequence (e.g., a transgene) that is "flanked" by two other elements (e.g., ITRs), indicates that one element is located 5' to the sequence and the other is located 3' to the sequence; however, there may be intervening sequences between.

[0238] As used herein, the terms "effective amount," "therapeutically effective amount," and a "sufficient amount" of, e.g., a gene therapy composition comprising a rAAV particle and/or a polynucleotide disclosed herein, refer to a quantity sufficient to, when administered to the subject, including a human, effect beneficial or desired results, including clinical results, and, as such, an "effective amount" or synonym thereto depends on the context in which it is being applied. In some aspects, the amount of a given therapeutic agent or composition can vary depending upon various factors, such as the given agent, the pharmaceutical formulation, the route of administration, the type of disease or disorder, the identity of the subject (e.g., age, sex, and/or weight) or host being treated, and the like.

[0239] As used herein, the term "administration" refers to the delivery of a molecule or composition of the present disclosure (e.g., polynucleotides, expression cassettes, delivery vectors, expression constructs, rAAV, combination gene therapy, immunosuppressive agent, etc.) to a subject or system. In some aspects, the administration can include one or more doses delivered by an appropriate route.

[0240] As used herein, the term "skeletal muscle group" refers to muscles that can be identified using nomenclature based their anatomical location or relationship to a particular bone in a vertebrate body. Typically, a subject has a right and a left (bilateral) muscle for each skeletal muscle group. In some aspects, administration can be to a single (right or left) muscle of a skeletal muscle group. In some aspects, administration can be to both the right and left muscle of a skeletal muscle group, i.e., bilateral administration.

[0241] As used herein, the terms "bilateral" or "bilaterally" in the context of intramuscular administration refers to administering to the same muscle or muscle group on both sides of a subject (e.g., to both the right and left side quadriceps, both the right and left side biceps, and/or both the right and left side hamstring muscles). In contrast, "unilateral" refers to administration to a single side of a subject, e.g., the right or the left side.

- [0242] As used herein, the terms “immunosuppression” or “immunosuppressive” refers to a temporary or permanent reduction in or limit on an immune response in a subject. An “immunosuppressor” or an “immunomodulator” is a substance capable of inducing a temporary or permanent reduction in or limit to an immune response in a subject, or a change in the type of immune response. For example, an immunomodulator may direct T cell fate from a TH1 proinflammatory T cell, to a TH2 or Treg wound healing or regulatory T cell. In some aspects, the immunosuppression can be to reduce or limit an immune-mediated response to a viral vector (e.g., rAAV). In some aspects, the immune-mediated response can be an innate and/or an adaptive immune response.
- [0243] The term “ratio” refers to the comparison of two or more numbers that indicates their quantitative relation to each other. In some aspects, a ratios can be used to compare two portions within a whole or total amount.
- [0244] As used herein, the term “vector ratio” refers to the amount, in vector genomes (vg), of one AAV vector compared to the amount, in vector genomes, of another AAV vector. In some aspects, the vector ratio refers to the amount of AAV1-hINS vg to the amount of AAV1-hGCK vg. For example, equal number of AAV1-hINS vg and AAV1-hGCK vg can also be understood as a one-to-one vector ratio or a 1:1 vector ratio.
- [0245] As used herein, the term “gene therapy” is the insertion of nucleic acid sequences (e.g., a nucleic acid comprising a promoter operably linked to a polynucleotide encoding a therapeutic molecule) into an individual’s cells and/or tissues to treat a disease or condition. Gene therapy can also include insertion of a transgene that is inhibitory in nature, i.e., that inhibit, decrease or reduce expression, activity or function of an endogenous gene or protein, such as an undesirable or aberrant (e.g., pathogenic) gene or protein. Such transgenes can be exogenous. An exogenous molecule or sequence is understood to be molecule or sequence not normally occurring in the cell, tissue and/or individual to be treated. Both acquired and congenital diseases can be amenable to gene therapy.
- [0246] In some aspects, the disclosure provides polynucleotides encoding wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof. The disclosure also provides nucleic acid constructs that include as part of their sequence the polynucleotides encoding wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof. For example, the disclosure includes expression cassettes, plasmids and/or other vectors that include the polynucleotides along with other elements,

such as regulatory elements. In some aspects, the disclosure provides a packaged gene delivery vehicle, such as a viral capsid (e.g., AAV capsid), including the polynucleotides encoding wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof.

[0247] The disclosure also includes methods of expressing wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof by delivering the polynucleotides into a cell along with elements required to promote expression in the cell. The disclosure also provides gene therapy methods in which the polynucleotides encoding wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof is/are administered to a subject, e.g., as a component of one or more vectors and/or packaged as a component of one or more viral gene delivery vehicles (e.g., AAV particles).

[0248] The disclosure also provides gene therapy methods in which the polynucleotides encoding wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof is/are intramuscularly administered to a subject, e.g., as a component of one or more vectors and/or packaged as a component of one or more viral gene delivery vehicles (e.g., AAV particles), in particular, where the intramuscular administration is to at least two different skeletal muscle groups (e.g., quadriceps, biceps, and hamstrings), preferably, as bilateral administrations.

[0249] The disclosure also provides an immunosuppressive regimen (e.g., administration of an IL-6 antagonist such as tocilizumab prior to the gene therapy administration and/or administration of a calcineurin inhibitor such as tacrolimus for a time period after the gene therapy administration) to accompany the gene therapy methods in which the polynucleotides encoding wild-type or mutant insulin and/or wild-type glucokinase or a functional fragment thereof is/are intramuscularly administered to a subject, e.g., as a component of one or more vectors and/or packaged as a component of one or more viral gene delivery vehicles (e.g., AAV particles), in particular, where the intramuscular administration is to at least two different skeletal muscle groups (e.g., quadriceps, biceps, and hamstrings), preferably, as bilateral administrations. Treatment can, for example, be used to treat or reduce the symptoms of diabetes in a subject in need thereof.

[0250] Furthermore, certain aspects of the disclosure, e.g., disclosed methods of intramuscular administration (e.g., two or more skeletal muscle groups, preferably, bilaterally) and/or use of an immunosuppressive regimen (e.g., administration of an IL-6

antagonist such as tocilizumab prior to the gene therapy administration and/or administration of a calcineurin inhibitor such as tacrolimus for a time period after the gene therapy administration) can provide improvements in the safety and efficacy of the gene therapy treatments disclosed herein.

[0251] Additional aspects of the disclosure are directed to a method of immunosuppression in a subject administered an AAV gene therapy for the treatment of diabetes, wherein the subject is administered an immunosuppressive regimen comprising administering (a) an immunosuppressive agent (pre-AAV) prior to administering the AAV gene therapy; and (b) an immunosuppressive agent (post-AAV) after administering the AAV gene therapy.

[0252] In some aspects, the methods disclosed herein result in (i) a reduction in glycated blood hemoglobin (HbA1c) levels in the treated subject; (ii) a reduction in circulating ketones in the treated subject, (iii) a reduction in triglycerides in the treated subject, (iv) a reduction in exogenous insulin in the treated subject, (v) an increase in circulating C-peptide levels the treated subject, (vi) reduction in immune response to exogenous AAV, insulin protein, and/or glucokinase protein, or (vii) any combination thereof.

[0253] Each of these aspects of the disclosure is discussed in further detail herein.

Insulin and Glucokinase Gene Therapy Combination and Methods of Administration

[0254] Certain aspects of the disclosure are directed to a therapy, e.g., an AAV combination gene therapy, comprising intramuscular (IM) delivery of a polynucleotide encoding an insulin (Ins) protein and a polynucleotide encoding a glucokinase (Gck) protein. In preferred aspects, the combination gene therapy is administered to at least two different skeletal muscle groups. In some aspects, the combination therapy includes IM administration of the insulin and glucokinase encoding polynucleotides in a single pharmaceutical composition, e.g., (i) two separate rAAV particles comprising the insulin and glucokinase encoding polynucleotides, respectively, in the same pharmaceutical composition or (ii) a rAAV particle comprising both the insulin and glucokinase encoding polynucleotides in the pharmaceutical composition. In some aspects, the combination therapy of the disclosure is administered intramuscularly (IM) to 2-10, 3-10, 4-10, 5-10, 6-10, 7-10 or 8-10 different skeletal muscle groups.

[0255] In some aspects, the combination therapy is administered intramuscularly (IM) to (i) 2-10, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, or 9-10 different skeletal muscle groups; (ii)

2-8, 3-8, 4-8, 5-8, 6-8, or 7-8 different skeletal muscle groups; (iii) 2-6, 3-6, 4-6, or 5-6 different skeletal muscle groups; (ii) 2-5, 3-5, or 4-5 different skeletal muscle groups; (iv) 2-4 or 3-4 different skeletal muscle groups.

[0256] Certain aspects are directed to administering a combination therapy of the disclosure intramuscularly (IM) to at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten different skeletal muscle groups. In some aspects, the combination therapy of the disclosure is administered intramuscularly (IM) to 3-10, 4-10, 5-10, 6-10, 7-10 or 8-10 different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine or ten different skeletal muscle groups.

[0257] There are three (3) types of muscle: skeletal muscle, cardiac muscle, and smooth muscle. Each type of muscle has a different structure and function. Inside each skeletal muscle, muscle fibers are organized into bundles, called fascicles, surrounded by a middle layer of connective tissue. Skeletal muscles in vertebrates are typically attached to bones and are responsible for skeletal movements. Skeletal muscles also have a role in sustaining body posture and position, maintaining body temperature, storing nutrients, and stabilizing joints. Skeletal muscle groups of the upper body include, but are not limited to, biceps, triceps, abdominal, pectoral, deltoid, trapezius, latissimus dorsi, and erector spinae. Skeletal muscle groups of the lower body include, but are not limited to, quadriceps, hamstrings, gastrocnemius (e.g., calf), soleus, and gluteus (e.g., gluteus maximus).

[0258] In some aspects, the different skeletal muscle groups can comprise a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat), or any combination thereof. In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring, or any combination thereof. In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, and a hamstring, or any combination thereof.

[0259] In some aspects, the different skeletal muscle groups can comprise a quadriceps, a bicep (e.g., the biceps brachii or the biceps femoris), a triceps, a gastrocnemius (e.g., a

calf muscle), a gluteus maximus, a hamstring (e.g., the semitendinosus, the semimembranosus, or the biceps femoris), a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), a thigh muscle (e.g., vastus intermedius, vastus lateralis, or vastus medialis), a hip muscle (gluteus medius), and a latissimus dorsi (lat).

[0260] In some aspects, the different skeletal muscle groups can comprise a iliopsoas muscle, a tensor fasciae latae muscle, a vastus lateralis muscle, the iliotibial tract, a pectineus muscle, an adductor longus muscle, a gracilis muscle, a rectus femoris muscle, a vastus medialis muscle, a gluteus maximus muscle, an adductor magnus muscle, a semitendinosus muscle, a gracilis muscle, a semimembranosus muscle, a sartorius muscle, a gluteus medius muscle, a biceps femoris muscle, a plantaris muscle, or a gastrocnemius muscle. In some aspects, the different skeletal muscle groups can comprise any of the muscles disclosed in FIGs. **19A-19B**.

[0261] In some aspects, the administration comprises bilateral injections to one or more of the skeletal muscle groups (e.g., IM injections to the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, the right and left triceps, and/or the right and left hamstrings).

[0262] In some aspects, the administration comprises bilateral injections to one or more of the skeletal muscle groups (e.g., IM injections to the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0263] In some aspects, the bilateral injections are divided equally for each skeletal muscle group (e.g., the right quadriceps and the left quadriceps receive approximately the same administration volume and/or dose).

[0264] In some aspects, the different skeletal muscle groups can comprise a iliopsoas muscle, a tensor fasciae latae muscle, a vastus lateralis muscle, the iliotibial tract, a pectineus muscle, an adductor longus muscle, a gracilis muscle, a rectus femoris muscle, a vastus medialis muscle, a gluteus maximus muscle, an adductor magnus muscle, a semitendinosus muscle, a gracilis muscle, a semimembranosus muscle, a sartorius muscle, a gluteus medius muscle, a biceps femoris muscle, a plantaris muscle, or a gastrocnemius muscle. In some aspects, the different skeletal muscle groups can comprise any of the muscles disclosed in FIGs. **19A-19B**.

[0265] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid,

a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat).

[0266] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring. In some aspects, the two or more different skeletal muscle groups comprises any combination of two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, or a hamstring.

[0267] In some aspects, the administration comprises a total of 20-150 IM injections of the therapy (e.g., combination gene therapy) to the at least two different skeletal muscle groups. In some aspects, the administration comprises a total of 20-120 IM injections, 20-110 IM injections, 20-100 IM injections, 20-80 IM injections, 20-60 IM injections, 25-150 IM injections, 25-120 IM injections, 25-110 IM injections, or 25-100 IM injections.

[0268] In some aspects, the administration comprises 1-80 (e.g., 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80) IM injections to a first skeletal muscle group and 1-80 (e.g., 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80) IM injections to a second skeletal muscle group. In some aspects, the administration comprises 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a first skeletal muscle group, 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a second skeletal muscle group, and 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a third skeletal muscle group.

[0269] In some aspects, the administration comprises 8-50 IM injections to the quadriceps (e.g., 4-25 IM injections bilaterally to each quadriceps), 8-50 IM injections to the biceps (e.g., 4-25 IM injections bilaterally to each bicep), and 8-50 IM injections to the hamstrings (e.g., 4-25 IM injections bilaterally to each hamstring).

[0270] In some aspects, the administration comprises (i) 1-80 IM injections to a first skeletal muscle group and 1-80 IM injections to a second skeletal muscle group; (ii) 1-60 IM injections to a first skeletal muscle group, 1-60 IM injections to a second skeletal muscle group, and 1-60 IM injections to a third skeletal muscle group; (iii) 1-40 IM injections to a first skeletal muscle group, 1-40 IM injections to a second skeletal muscle group, 1-40 IM injections to a third skeletal muscle group, and 1-40 IM injections to a

fourth skeletal muscle group; (iv) 1-30 IM injections to a first skeletal muscle group, 1-30 IM injections to a second skeletal muscle group, 1-30 IM injections to a third skeletal muscle group, 1-30 IM injections to a fourth skeletal muscle group, and 1-30 IM injections to a fifth skeletal muscle group; and (v) 1-25 IM injections to a first skeletal muscle group, 1-25 IM injections to a second skeletal muscle group, 1-25 IM injections to a third skeletal muscle group, 1-25 IM injections to a fourth skeletal muscle group, 1-25 IM injections to a fifth skeletal muscle group, and 1-25 IM injections to a sixth skeletal muscle group.

[0271] In some aspects, the administration comprises: (i) 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80 IM injections to a first skeletal muscle group and 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80 IM injections to a second skeletal muscle group; (ii) 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a first skeletal muscle group; 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a second skeletal muscle group; and 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a third skeletal muscle group; (iii) 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a first skeletal muscle group; 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a second skeletal muscle group; 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a third skeletal muscle group; and 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a fourth skeletal muscle group; (iv) 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a first skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a second skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a third skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a fourth skeletal muscle group; and 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a fifth skeletal muscle group; or (v) 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a first skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a second skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a third skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a fourth skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a fifth skeletal muscle group; and 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a sixth skeletal muscle group.

[0272] In some aspects, the administration comprises a total of 20-70 (e.g., 20-70, 25-70, 30-70, 35-70, 40-70, 45-70, 50-70, 50-55, 55-60, 60-65, 65-70, 50-70, 50-60, 60-70, 55-

65, or 55-70) IM injections of the therapy (e.g., combination gene therapy) to the at least two different skeletal muscle groups. In some aspects, the administration comprises 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a first skeletal muscle group and 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a second skeletal muscle group. In some aspects, the administration comprises 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a first skeletal muscle group, 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a second skeletal muscle group, and 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a third skeletal muscle group.

[0273] In some aspects, each IM injection is administered in a volume of about 100 μ L to about 2 mL, 100 μ L to about 1.9 mL, 100 μ L to about 1.8 mL, 100 μ L to about 1.7 mL, 100 μ L to about 1.6 mL, 100 μ L to about 1.5 mL, 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, about 400 μ L to about 800 μ L, about 500 μ L to about 1.5 mL, about 600 μ L to about 1.4 mL, about 700 μ L to about 1.3 mL, about 800 μ L to about 1.2 mL, or about 900 μ L to about 1.1 mL.

[0274] In some aspects, each IM injection is administered in a volume of about 100 μ L to about 1.5 mL (e.g., about 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, or about 400 μ L to about 800 μ L).

[0275] In some aspects, each IM injection is administered in a volume of about 500 μ L to about 1 mL. In some aspects, each IM injection is administered in a volume of about 500 μ L. In some aspects, each IM injection is administered in a volume of about 1 mL

[0276] In some aspects, the total volume of the therapy administered is about 25 mL, about 30 mL, about 35 mL, about 40 mL, about 45 mL, about 50 mL, about 55 mL, about 60 mL, about 65 mL, or about 70 mL. In some aspects, the total volume of therapy administered is about 20 mL to about 70 mL, about 25 mL to about 50 mL, about 25 mL to about 35 mL, about 35 mL to about 45 mL, about 30 mL to about 35 mL, or about 35 mL to about 40 mL.

- [0277] In some aspects, the dose per IM injection comprises about 2×10^{13} to about 5×10^{13} vgs per mL.
- [0278] In some aspects, each IM injection comprises about 8×10^{10} to about 5×10^{13} vgs per mL. In some aspects, each IM injection comprises about 8×10^{10} to 2×10^{13} vgs per mL. In some aspects, each IM injection comprises about 2×10^{13} to about 4×10^{13} vgs per mL.
- [0279] In some aspects, each IM injection comprises about 8×10^{10} to about 8×10^{12} vgs per mL, about 8×10^{10} to about 8×10^{11} vgs per mL, about 8×10^{10} to about 2×10^{12} vgs per mL, about 8×10^{10} to about 4×10^{12} vgs per mL, about 2×10^{12} to about 8×10^{12} vgs per mL, or about 8×10^{11} to about 4×10^{12} vgs per mL.
- [0280] In some aspects, each IM injection comprises about 1×10^{11} to about 1×10^{13} vgs per mL, about 1×10^{11} to about 1×10^{12} vgs per mL, about 1×10^{11} to about 2.6×10^{12} vgs per mL, about 1×10^{11} to about 5×10^{12} vgs per mL, about 2.6×10^{12} to about 1×10^{13} vgs per mL, or about 1×10^{12} to about 5×10^{12} vgs per mL.
- [0281] In some aspects, each IM injection comprises about 1.2×10^{11} to about 1.2×10^{13} vgs per mL, about 1.2×10^{11} to about 1.2×10^{12} vgs per mL, about 1.2×10^{11} to about 3.1×10^{12} vgs per mL, about 1.2×10^{11} to about 6×10^{12} vgs per mL, about 3.1×10^{12} to about 1.2×10^{13} vgs per mL, or about 1.2×10^{12} to about 6×10^{12} vgs per mL.
- [0282] In some aspects, each IM injection comprises about 1.4×10^{11} to about 1.4×10^{13} vgs per mL, about 1.4×10^{11} to about 1.4×10^{12} vgs per mL, about 1.4×10^{11} to about 3.6×10^{12} vgs per mL, about 1.4×10^{11} to about 7×10^{12} vgs per mL, about 3.6×10^{12} to about 1.4×10^{13} vgs per mL, or about 1.4×10^{12} to about 7×10^{12} vgs per mL.
- [0283] In some aspects, each IM injection comprises about 1.6×10^{11} to about 1.6×10^{13} vgs per mL, about 1.6×10^{11} to about 1.6×10^{12} vgs per mL, about 1.6×10^{11} to about 4.1×10^{12} vgs per mL, about 1.6×10^{11} to about 8×10^{12} vgs per mL, about 4.1×10^{12} to about 1.6×10^{13} vgs per mL, or about 1.6×10^{12} to about 8×10^{12} vgs per mL.
- [0284] In some aspects, each IM injection comprises about 1.8×10^{11} to about 1.8×10^{13} vgs per mL, about 1.8×10^{11} to about 1.8×10^{12} vgs per mL, about 1.8×10^{11} to about 4.6×10^{12} vgs per mL, about 1.8×10^{11} to about 9×10^{12} vgs per mL, about 4.6×10^{12} to about 1.8×10^{13} vgs per mL, or about 1.8×10^{12} to about 9×10^{12} vgs per mL.
- [0285] In some aspects, each IM injection comprises about 2×10^{11} to about 2×10^{13} vgs per mL, about 2×10^{11} to about 2×10^{12} vgs per mL, about 2×10^{11} to about 5.1×10^{12} vgs per mL, about 2×10^{11} to about 1×10^{13} vgs per mL, about 5.1×10^{12} to about 2×10^{13} vgs per mL, or about 2×10^{12} to about 1×10^{13} vgs per mL.

- [0286] In some aspects, each IM injection comprises about 8×10^{10} to about 2×10^{11} vgs per mL, about 8×10^{10} to about 1×10^{11} vgs per mL, about 8×10^{10} to about 1.2×10^{11} vgs per mL, about 8×10^{10} to about 1.4×10^{11} vgs per mL, about 8×10^{10} to about 1.6×10^{11} vgs per mL, about 8×10^{10} to about 1.8×10^{11} vgs per mL, about 1.4×10^{11} to about 2×10^{11} vgs per mL, about 1×10^{11} to about 1.8×10^{11} vgs per mL, or about 1.2×10^{11} to about 1.6×10^{11} vgs per mL.
- [0287] In some aspects, each IM injection comprises about 8×10^{11} to about 2×10^{12} vgs per mL, about 8×10^{11} to about 1×10^{12} vgs per mL, about 8×10^{11} to about 1.2×10^{12} vgs per mL, about 8×10^{11} to about 1.4×10^{12} vgs per mL, about 8×10^{11} to about 1.6×10^{12} vgs per mL, about 8×10^{11} to about 1.8×10^{12} vgs per mL, about 1.4×10^{12} to about 2×10^{12} vgs per mL, about 1×10^{12} to about 1.8×10^{12} vgs per mL, or about 1.2×10^{12} to about 1.6×10^{12} vgs per mL.
- [0288] In some aspects, each IM injection comprises about 2×10^{12} to about 5.1×10^{12} vgs per mL, about 2×10^{12} to about 2.6×10^{12} vgs per mL, about 2×10^{12} to about 3.1×10^{12} vgs per mL, about 2×10^{12} to about 3.6×10^{12} vgs per mL, about 2×10^{12} to about 4.1×10^{12} vgs per mL, about 2×10^{12} to about 4.6×10^{12} vgs per mL, about 3.6×10^{12} to about 5.1×10^{12} vgs per mL, about 2.6×10^{12} to about 4.6×10^{12} vgs per mL, or about 3.1×10^{12} to about 4.1×10^{12} vgs per mL.
- [0289] In some aspects, each IM injection comprises about 4×10^{12} to about 1×10^{13} vgs per mL, about 4×10^{12} to about 5×10^{12} vgs per mL, about 4×10^{12} to about 6×10^{12} vgs per mL, about 4×10^{12} to about 7×10^{12} vgs per mL, about 4×10^{12} to about 8×10^{12} vgs per mL, about 4×10^{12} to about 9×10^{12} vgs per mL, about 7×10^{12} to about 1×10^{13} vgs per mL, about 5×10^{12} to about 9×10^{12} vgs per mL, or about 6×10^{12} to about 8×10^{12} vgs per mL.
- [0290] In some aspects, each IM injection comprises about 8×10^{12} to about 2×10^{13} vgs per mL, about 8×10^{12} to about 1×10^{13} vgs per mL, about 8×10^{12} to about 1.2×10^{13} vgs per mL, about 8×10^{12} to about 1.4×10^{13} vgs per mL, about 8×10^{12} to about 1.6×10^{13} vgs per mL, about 8×10^{12} to about 1.8×10^{13} vgs per mL, about 1.4×10^{13} to about 2×10^{13} vgs per mL, about 1×10^{13} to about 1.8×10^{13} vgs per mL, or about 1.2×10^{13} to about 1.6×10^{13} vgs per mL.
- [0291] In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-7 cm. In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 5 cm. In some aspects,

each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.

[0292] In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 6 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.

[0293] In some aspects, each of the IM injection sites on the subject are separated by a distance of about 7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 6 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 1 cm.

[0294] In some aspects, the administration comprises IM injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep, at least 1.4 cm deep, at least 1.5 cm deep, or at least 2.0 cm deep. In some aspects, each of the IM injections is between 1-2 cm deep.

[0295] In some aspects, each of the IM injections is at least 0.5 cm below the surface of the injected muscle, regardless of the thickness of the subcutaneous fat.

[0296] In some aspects, the administration comprises IM injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep,

at least 1.4 cm deep, at least 1.5 cm deep, at least 2.0 cm deep, at least 2.5 cm deep, at least 3.0 cm deep, at least 3.5 cm deep, at least 4.0 cm deep, at least 4.5 cm deep, or at least 5.0 cm deep.

[0297] In some aspects, the administration comprises IM injections that are about 0.5 cm deep, about 0.6 cm deep, about 0.7 cm deep, about 0.8 cm deep, about 0.9 cm deep, about 1.0 cm deep, about 1.1 cm deep, about 1.2 cm deep, about 1.3 cm deep, about 1.4 cm deep, about 1.5 cm deep, about 2.0 cm deep, about 2.5 cm deep, about 3.0 cm deep, about 3.5 cm deep, about 4.0 cm deep, about 4.5 cm deep, or about 5.0 cm deep.

[0298] In some aspects, each of the IM injections is between about 3.5 to about 5 cm deep.

[0299] In some aspects, the combination therapy (e.g. either a single formulation or separate formulations) is administered IM to at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine, or ten different skeletal muscle groups. In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring. In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, and a hamstring. In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius, a gluteus maximus, and a hamstring, optionally wherein the two or more different skeletal muscle groups comprise any combination thereof.

[0300] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat).

- [0301] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring. In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, or a hamstring.
- [0302] In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).
- [0303] In some aspects, the present disclosure is directed to a combination therapy formulated for IM administration comprising a) an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs), and b) a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by ITRs, wherein the combination therapy is administered to at least two different skeletal muscle groups.
- [0304] In some aspects, a first AAV vector genome comprising the insulin expression cassette and a second AAV vector genome comprising the glucokinase expression cassette are administered. In some aspects, a AAV vector genome comprising both the insulin expression cassette and the glucokinase expression cassette is administered.
- [0305] In some aspects, the present disclosure is directed to a combination therapy formulated for IM administration comprising a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs), and b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by ITRs, wherein the first AAV vector genome and the second AAV vector genome are in a vector ratio selected from the group consisting of 1:0.25-0.75, 1:0.75-1.25, 1:1.75-2.25, and 1:3.75-1:4.25 (e.g., about 1:0.5, about 1:1, about 1:2, or about 1:4).

- [0306] In some aspects, the vector ratio is about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, or 1:0.25-0.5. In some aspects, the vector ratio is about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, 1:0.5-0.75, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, or 1:0.45-0.5. In some aspects, the vector ratio is about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.35-0.75, 1:0.4-0.60, or 1:0.45-0.55.
- [0307] In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20, 1:0.85-1.15, 1:0.9-1.1, and 1:0.95-1.05.
- [0308] In some aspects, the vector ratio is about 1:0.25-0.75.
- [0309] In some aspects, the vector ratio is about 1:0.4-0.6.
- [0310] In some aspects, the vector ratio is about 1:0.5.
- [0311] In some aspects, the vector ratio is about 1:0.9-1.1.
- [0312] In some aspects, the vector ratio is about 1:1.
- [0313] In some aspects, the vector ratio is about 1:2.
- [0314] In some aspects, the vector ratio is about 1:3.
- [0315] In some aspects, the vector ratio is about 1:4.
- [0316] In some aspects, the vector ratio is about 1:5.
- [0317] In some aspects, the vector ratio is about 1:10.
- [0318] In some aspects, the vector ratio is about 1:20.
- [0319] In some aspects, the vector ratio is between about 1:3 and about 1:5.
- [0320] In some aspects, the vector ratio is between about 1:1 to about 1:15, about 1:1 to about 1:10, about 1:1 to about 1:5, about 1:1 to about 1:4, about 1:1 to about 1:3, about 1:3 to about 1:5, about 1:3 to about 1:10, about 1:3 to about 1:15, about 1:5 to about 1:10, about 1:5 to about 1:15, or about 1:10 to about 1:15.
- [0321] In some aspects, the first AAV vector genome comprising an insulin expression cassette has a dosage that produces between about 1 $\mu\text{U/mL}$ to about 3 $\mu\text{U/mL}$, about 1 $\mu\text{U/mL}$ to about 5 $\mu\text{U/mL}$, about 1 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 4 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$,

about 6 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 7 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 8 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 9 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 6 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 5 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 6 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, or about 6 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 1 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 10 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 15 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 20 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 25 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 30 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 35 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 20 $\mu\text{U/mL}$ to about 30 $\mu\text{U/mL}$, about 10 $\mu\text{U/mL}$ to about 30 $\mu\text{U/mL}$, or about 10 $\mu\text{U/mL}$ to about 20 $\mu\text{U/mL}$ of insulin in the serum of a subject.

[0322] In some aspects, the combination therapy comprise a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups. In some aspects, the skeletal muscle groups comprise a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups are selected from two or more of a quadriceps, a bicep, a hamstring, or any combination thereof. In some aspects, the at least two different skeletal muscle groups are comprise a quadriceps, a bicep, a hamstring, or any combination thereof.

[0323] In some aspects, the combination therapy comprise a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:1-15 (e.g., about 1:5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups. In some aspects, the skeletal muscle groups comprise a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat).

- [0324] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat).
- [0325] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring. In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, or a hamstring.
- [0326] In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).
- [0327] In some aspects, the combination therapy comprises a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least three different skeletal muscle groups. In some aspects, the different skeletal muscle groups comprise three or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat).
- [0328] In some aspects, the combination therapy comprises a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:1-15 (e.g., about 1:5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least three different skeletal muscle groups.
- [0329] In some aspects, the combination therapy comprises (i) a first recombinant AAV (rAAV) particle comprising the first AAV vector genome comprising the insulin

expression cassette comprising the promoter operably linked to the polynucleotide encoding the human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising the second AAV vector genome comprising the glucokinase expression cassette comprising the promoter operably linked to the polynucleotide encoding the human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).

[0330] In some aspects, the at least three different skeletal muscle groups are selected from a quadriceps, a bicep, and a hamstring, or any combination thereof. In some aspects, the at least three different skeletal muscle groups comprise a quadriceps, a bicep, and/or a hamstring.

[0331] In some aspects, the administration comprises bilateral injections into one or more of the at least three different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0332] In some aspects, the combination therapy comprise a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.9-1.1 (e.g., about 1:1.0), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups. In some aspects, the at least two different skeletal muscle groups comprise at least two of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups comprise at least two of a quadriceps, a bicep, and a hamstring. In some aspects, the combination therapy comprise a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:1-15 (e.g., about 1:5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups.

- [0333] In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).
- [0334] In some aspects, the combination therapy comprises a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.9-1.1 (e.g., about 1:1), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least three different skeletal muscle groups comprising a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups comprise at least two of a quadriceps, a bicep, and/or a hamstring.
- [0335] In some aspects, the combination therapy comprises a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:1-15 (e.g., about 1:5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least three different skeletal muscle groups comprising a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat).
- [0336] In some aspects, the administration comprises bilateral injections into one or more of the at least three different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).
- [0337] In some aspects, each IM injection is administered in a volume of about 100 μ L to about 1.5 mL (e.g., about 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, or about 400 μ L to about 800 μ L).

- [0338] In some aspects, the dose per IM injection comprises about 2×10^{13} to 5×10^{13} vg per mL.
- [0339] In some aspects, the dose per IM injection comprises about 7×10^{10} to 7×10^{12} vg/kg of the first AAV vector genome (e.g., hIns). In some aspects, the dose per IM injection comprises about 7×10^{10} to 7×10^{11} vg/kg of the first AAV vector genome (e.g., hIns). In some aspects, the dose per IM injection comprises about 7×10^{10} to 1.8×10^{12} vg/kg of the first AAV vector genome (e.g., hIns). In some aspects, the dose per IM injection comprises about 7×10^{10} to 3.5×10^{12} vg/kg of the first AAV vector genome (e.g., hIns). In some aspects, the dose per IM injection comprises about 1.8×10^{12} to 7×10^{12} vg/kg of the first AAV vector genome (e.g., hIns). In some aspects, the dose per IM injection comprises about 1.8×10^{12} to 3.5×10^{12} vg/kg of the first AAV vector genome (e.g., hIns). In some aspects, the dose per IM injection comprises about 3.5×10^{12} to 7×10^{12} vg/kg of the first AAV vector genome (e.g., hIns).
- [0340] In some aspects, the dose per IM injection comprises about 3.2×10^{12} to 3.5×10^{12} vg/kg of the second AAV vector genome (e.g., hGck). In some aspects, the dose per IM injection comprises about 2×10^{12} to 5×10^{12} vg/kg of the second AAV vector genome (e.g., hGck). In some aspects, the dose per IM injection comprises about 2×10^{12} to 3.5×10^{12} vg/kg of the second AAV vector genome (e.g., hGck). In some aspects, the dose per IM injection comprises about 3.5×10^{12} to 5×10^{12} vg/kg of the second AAV vector genome (e.g., hGck).
- [0341] In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.
- [0342] In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.

- [0343] In some aspects, each of the IM injection sites on the subject are separated by a distance of about 7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 6 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 1 cm.
- [0344] In some aspects, the administration comprises IM injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep, at least 1.4 cm deep, at least 1.5 cm deep, or at least 2.0 cm deep. In some aspects, each of the IM injections is between 1-2 cm deep.
- [0345] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 5×10^{11} to about 1×10^{14} vector genomes vg/kg. In some aspects, the first AAV vector genome and second AAV vector genome are each administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 1×10^{12} to about 1×10^{13} vg/kg.
- [0346] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 1×10^{12} vg/kg to about 1×10^{14} vg/kg, about 2×10^{12} vg/kg to about 1×10^{14} vg/kg, about 3×10^{12} vg/kg to about 1×10^{14} vg/kg, about 4×10^{12} vg/kg to about 1×10^{14} vg/kg, about 5×10^{12} vg/kg to about 1×10^{14} vg/kg, about 6×10^{12} vg/kg to about 1×10^{14} vg/kg, about 7×10^{12} vg/kg to about 1×10^{14} vg/kg, about 8×10^{12} vg/kg to about 1×10^{14} vg/kg, or about 9×10^{12} vg/kg to about 1×10^{14} vg/kg.
- [0347] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 7×10^{10} vg/kg to about 1×10^{14} vg/kg, about 8×10^{10} vg/kg to about 1×10^{14} vg/kg, about 9×10^{10} vg/kg to about 1×10^{14} vg/kg, about 1×10^{11} vg/kg to

about 1×10^{14} vg/kg, about 2×10^{11} vg/kg to about 1×10^{14} vg/kg, about 3×10^{11} vg/kg to about 1×10^{14} vg/kg, about 4×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{13} vg/kg, about 7×10^{10} vg/kg to about 1×10^{12} vg/kg, about 7×10^{10} vg/kg to about 1×10^{11} vg/kg, about 7×10^{10} vg/kg to about 7×10^{12} vg/kg, about 7×10^{10} vg/kg to about 7×10^{11} vg/kg, about 7×10^{10} vg/kg to about 1.8×10^{12} vg/kg, about 7×10^{10} vg/kg to about 3.5×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 7×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 3.5×10^{12} vg/kg, about 3.5×10^{12} vg/kg to about 7×10^{12} vg/kg, about 2×10^{12} vg/kg to about 5×10^{12} vg/kg, about 2×10^{12} vg/kg to about 3.5×10^{12} vg/kg, about 3.5×10^{12} vg/kg to about 5×10^{12} vg/kg, about 5×10^{11} vg/kg to about 1×10^{14} vg/kg, about 6×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{11} vg/kg to about 1×10^{14} vg/kg, about 8×10^{11} vg/kg to about 1×10^{14} vg/kg, or about 9×10^{11} vg/kg to about 1×10^{14} vg/kg.

[0348] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 2×10^{12} vg/kg to about 1×10^{14} vg/kg, about 3×10^{12} vg/kg to about 1×10^{14} vg/kg, about 4×10^{12} vg/kg to about 1×10^{14} vg/kg, about 5×10^{12} vg/kg to about 1×10^{14} vg/kg, about 6×10^{12} vg/kg to about 1×10^{14} vg/kg, about 7×10^{12} vg/kg to about 1×10^{14} vg/kg, or about 8×10^{12} vg/kg to about 1×10^{14} vg/kg.

[0349] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 5×10^{11} vg/kg to about 1×10^{14} vg/kg, about 6×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{11} vg/kg to about 1×10^{14} vg/kg, about 8×10^{11} vg/kg to about 1×10^{14} vg/kg, or about 9×10^{11} vg/kg to about 1×10^{14} vg/kg.

[0350] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 1×10^{13} vg/kg to about 1×10^{14} vg/kg, about 2×10^{13} vg/kg to about 1×10^{14} vg/kg, about 3×10^{13} vg/kg to about 1×10^{14} vg/kg, about 4×10^{13} vg/kg to about 1×10^{14} vg/kg, about 5×10^{13} vg/kg to about 1×10^{14} vg/kg, about 6×10^{13} vg/kg to about 1×10^{14} vg/kg, about 7×10^{13} vg/kg to about 1×10^{14} vg/kg, about 8×10^{13} vg/kg to about 1×10^{14} vg/kg, or about 8×10^{13} vg/kg to about 1×10^{14} vg/kg.

[0351] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly at a dose of about 2×10^{13} vg/kg to about 1×10^{14} vg/kg, about 3×10^{13} vg/kg to about 1×10^{14} vg/kg, about 4×10^{13} vg/kg to about 1×10^{14} vg/kg, about 5×10^{13}

vg/kg to about 1×10^{14} vg/kg, about 6×10^{13} vg/kg to about 1×10^{14} vg/kg, about 7×10^{13} vg/kg to about 1×10^{14} vg/kg, or about 8×10^{13} vg/kg to about 1×10^{14} vg/kg.

[illegible]

[0353] In some aspects, the first AAV vector genome is administered at a dose of about 3×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 1.5×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 4×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 2×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 5×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 2.5×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 6×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 3×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 7×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 8×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 4×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 9×10^{13} vg/kg and the second AAV vector genome is administered at a dose of about 4.5×10^{13} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 1×10^{14} vg/kg and the second AAV vector genome is administered at a dose of about 5×10^{13} vg/kg.

[0354] In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 2×10^{12} to about 1×10^{13} vg/kg. In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 4×10^{12} to about 1×10^{13} vg/kg. In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 4.5×10^{12} to about 5×10^{12} vg/kg. In some aspects, the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{12} to about 9×10^{12} vg/kg.

[0355] In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 1×10^{12} to about 5×10^{12} vg/kg (e.g., 3.5×10^{12} vg/kg). In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 2×10^{12} to about 5×10^{12} vg/kg. In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2×10^{12} to about 2.8×10^{12} vg/kg. In some aspects, the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 4×10^{12} vg/kg to about 5×10^{12} vg/kg.

[0356] In some aspects, the combination therapy comprise a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), wherein the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly (IM) at a dose of about 1×10^{12} to about 1×10^{14} vg/kg, wherein the IM administration is to at least two different skeletal muscle groups. In some aspects, the different skeletal muscle groups comprise a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and/or a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups comprise at least two of a quadriceps, a bicep, and/or a hamstring. In some aspects, the administration comprises bilateral injections into one or more of the at least two different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0357] In some aspects, the IM administration is to at least three different skeletal muscle groups skeletal muscle groups selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and/or a latissimus dorsi (lat). In some aspects, the at least three different skeletal muscle groups comprise a quadriceps, a bicep, and/or a hamstring. In some aspects, the administration comprises bilateral injections into one or more of the at least three different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings). In some aspects, the IM administration comprises multiple injections with an individual injection volume of between 100 μ L and 1.5 mL. In some aspects, an individual injection volume is about 100 μ L to about 1.5 mL (e.g., about 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, or about 400 μ L to about 800 μ L). In

some aspects, an individual injection volume is between 200 μL and 1.0 mL. In some aspects the individual injection volume is about 500 μL . In some aspects, the individual injection volume is between 1 μL and 500 μL . In some aspects, the individual injection volume is between 10 μL and 300 μL . In some aspects, the individual injection volume is between 50 μL and 250 μL . In some aspects, the individual injection volume is about 100 μL .

[0358] In some aspects, the IM administration comprises multiple injections with an individual injection dose of between 1×10^{11} vg/injection site and 5×10^{13} vg/injection site. In some aspects, an individual injection dose of comprises about 2×10^{13} to 5×10^{13} vg/injection site. In some aspects, the individual injection dose is about 1×10^{11} vg/injection site to about 1×10^{13} vg/injection site. In some aspects, the individual injection dose is about 1.25×10^{11} vg/injection site to about 1.75×10^{12} vg/injection site. In some aspects, the individual injection dose is about 1.5×10^{11} vg/injection site to about 1.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 1.75×10^{11} vg/injection site to about 1.25×10^{12} vg/injection site. In some aspects, the individual injection dose is about 1×10^{12} vg/injection site.

[0359] In some aspects, the IM administration comprises multiple injections with a minimum distance between each injection site. In some aspects the distance between each injection site is at least 0.5 cm, at least 0.6 cm, at least 0.7 cm, at least 0.8 cm, at least 0.9 cm, at least 1.0 cm, at least 1.1 cm, at least 1.2 cm, at least 1.3 cm, at least 1.4 cm, at least 1.5 cm, at least 2.0 cm, at least 3.0 cm. In some aspects, the distance between each injection site is between 0.5 cm and 3 cm, between 0.75 cm and 2.5 cm, between 1 cm and 2 cm, between .75 cm and 1.25 cm, or about 1 cm.

[0360] In some aspects, the IM administration comprises injections that are separated by a distance of at least 1-3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.

[0361] In some aspects, the IM administration comprises injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep, at least 1.4 cm deep, at least 1.5 cm deep, or at least 2.0 cm deep. In some aspects, each of the IM injections is between 1-2 cm deep.

- [0362]** In some aspects, the combination therapy is accompanied by an immunosuppressive regimen. In some aspects, the combination therapy is accompanied by an immunosuppressive regimen comprising an immunosuppressive agent. In some aspects, the immunosuppressive regimen comprises administering one or more immunosuppressive agents prior to, at the same time as, and/or after administering the combination therapy. In some aspects, the immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor. In some aspects, the immunosuppressive agent comprises an IL-6 antagonist and/or a calcineurin inhibitor.
- [0363]** In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (e.g., an IL-6 antagonist) prior to administering the combination therapy. In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (e.g., a calcineurin inhibitor) after administering the combination therapy. In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (e.g., an IL-6 antagonist) prior to administering the combination therapy and administering an immunosuppressive agent (e.g., a calcineurin inhibitor) after administering the combination therapy.
- [0364]** In some aspects, an immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 14 days (e.g., 1 to 3 days) prior to administering the combination therapy. In some aspects, an immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy. In some aspects, an immunosuppressive agent is administered 3 to 7 days prior to administering the combination therapy.
- [0365]** In some aspects, an immunosuppressive agent (e.g., a calcineurin inhibitor) is administered starting at 1, 2, 3, or 4 weeks (e.g., 3 weeks) after administering the combination therapy.
- [0366]** In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 2 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered starting at 1, 2, 3, or 4 weeks (e.g., 3 weeks) after administering the combination therapy.

- [0367] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered 1 to 14 days (e.g., 1 to 3 days) prior to administering the combination therapy.
- [0368] In some aspects, the first immunosuppressive agent is administered once every one week, two weeks, three weeks, or four weeks.
- [0369] In some aspects, the first immunosuppressive agent is administered for about 3 months, about 4 months, about 5 months, or about 6 months.
- [0370] In some aspects, immunosuppressive agent is administered once about every 1-3 weeks, optionally once about every 1-2 weeks after administering the combination therapy.
- [0371] In some aspects, the immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the combination therapy.
- [0372] In some aspects, immunosuppressive regimen comprises a second immunosuppressive agent.
- [0373] In some aspects, the immunosuppressive regimen comprises administering the second immunosuppressive agent prior to administering the combination therapy.
- [0374] In some aspects, the immunosuppressive regimen comprises administering the second immunosuppressive agent after administering the combination therapy.
- [0375] In some aspects, the second immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.
- [0376] In some aspects, the second immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
- [0377] In some aspects, the second immunosuppressive agent is administered 1 to 3 days prior to administering the combination therapy.
- [0378] In some aspects, the second immunosuppressive agent is administered 3 to 7 days prior to administering the combination therapy.
- [0379] In some aspects, the second immunosuppressive agent is further administered 1, 2, 3, or 4 weeks after administering the combination therapy.
- [0380] In some aspects, second immunosuppressive agent is administered 2 times per day, daily, or every other day.
- [0381] In some aspects, the second immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the combination therapy.

- [0382] In some aspects, the second immunosuppressive agent is administered once or twice daily for 4 to 52 weeks.
- [0383] In some aspects, the second immunosuppressive agent is administered once daily for 4 to 24 weeks, 4 to 16 weeks, or 4 to 8 weeks.
- [0384] In some aspects, the second immunosuppressive agent is administered twice daily for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some aspects, the second immunosuppressive agent is administered twice daily for about 1 to about 6 months, about 2 months to about 6 months, about 3 months to about 6 months, about 4 months to about 6 months, about 5 months to about 6 months, about 2 months to about 4 months, or about 3 months to about 4 months.
- [0385] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, and levilimab. In some aspects, the IL-6 antagonist is tocilizumab. In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof. In some aspects, the IL-6 antagonist is tocilizumab.
- [0386] In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, and voclosporin. In some aspects, the calcineurin inhibitor is tacrolimus.
- [0387] In some aspects, the second immunosuppressive agent is selected from the group consisting of thymoglobulin, etanercept, basiliximab, tacrolimus, sirolimus, and mycophenolate mofetil.
- [0388] In some aspects, the combination therapy is accompanied by an immunosuppressive regimen (e.g., administration of an IL-6 antagonist such as tocilizumab prior to the gene therapy administration and/or administration of a calcineurin inhibitor such as tacrolimus for a time period after the gene therapy administration) to accompany the gene therapy methods comprising intramuscular administration of a combination therapy comprising a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.9-1.1 (e.g., about 1:1), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups. In some aspects, the different skeletal muscle groups can comprise a quadriceps, a

bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and/or a latissimus dorsi (lat). In some aspects, the different skeletal muscle groups are selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and/or a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups are selected from a quadriceps, a bicep, a hamstring, or any combination thereof.

[0389] In some aspects, the combination therapy comprises a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising a glucokinase expression cassette in a vector ratio of about 1:0.9-1.1 (e.g., about 1:1.1), wherein the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly (IM) at a dose of about 1×10^{12} to about 1×10^{14} vg/kg (e.g., about 1×10^{13} to about 1×10^{14} vg/kg). In some aspects, the single intramuscular formulation is administered to at least three different skeletal muscle groups selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and/or a latissimus dorsi (lat). In some aspects, the at least three different skeletal muscle groups comprise a quadriceps, a bicep, and/or a hamstring.

[0390] In some aspects, the combination therapy is accompanied by an immunosuppressive regimen (e.g., administration of an IL-6 antagonist such as tocilizumab prior to the gene therapy administration and/or administration of a calcineurin inhibitor such as tacrolimus for a time period after the gene therapy administration) to accompany the gene therapy methods comprising intramuscular administration of a combination therapy comprising a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups are selected from a quadriceps, a bicep, and/or a hamstring.

[0391] In some aspects, the combination therapy comprises a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising a glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), wherein the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly (IM) at a dose of about 1×10^{12} to about 1×10^{14} vg/kg (e.g., about 1×10^{13} to about 1×10^{14} vg/kg). In some aspects, the single intramuscular formulation is administered to at least three different skeletal muscle groups selected from three or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the at least three different skeletal muscle groups comprise a quadriceps, a bicep, and a hamstring, or any combination thereof.

[0392] In some aspects of the disclosure, the methods comprising intramuscular administration (e.g., to quadriceps, biceps, and hamstrings, preferably, bilaterally) and use of an immunosuppressive regimen (e.g., administration of an IL-6 antagonist such as tocilizumab prior to the gene therapy administration and/or administration of a calcineurin inhibitor such as tacrolimus for a time period after the gene therapy administration), can provide improvements to the safety, efficacy and durability of the combination gene therapy disclosed herein. In some aspects, the methods result in (i) a reduction in glycated blood hemoglobin (HbA1c) levels in the treated subject; (ii) a reduction in circulating ketones in the treated subject, (iii) a reduction in triglycerides in the treated subject, (iv) a reduction in exogenous insulin in the treated subject, (v) an increase in circulating C-peptide levels in the treated subject, (vi) reduction in immune response to exogenous AAV, insulin protein, and/or glucokinase protein, and/or (vii) any combination thereof.

[0393] In some aspects, the polynucleotide encoding a hIns is a modified polynucleotide (e.g., preproinsulin or proinsulin, a mutant, an analogue, or a variant thereof). In some aspects, the polynucleotide encoding a hGck is a modified polynucleotide (e.g., Gck, a mutant, an analogue, or variant thereof). In some aspects, the modifications to the coding sequence preserve the wild-type or mutant amino acid sequence for insulin and/or glucokinase. In some aspects, the encoded human Ins protein comprises a signal sequence and a proinsulin polypeptide. In some aspects, the encoded human Ins protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids

25-110 of SEQ ID NO: 144, or amino acids 25-110 of SEQ ID NO: 145. In some aspects, the modified nucleic acid sequence encodes a human preproinsulin (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145). In some aspects, the modified nucleic acid sequence encodes a human Gck (e.g., SEQ ID NO: 82).

[0394] In some aspects, the modified nucleic acids are codon optimized. In some aspects, the codon optimization includes modifying codons in the open reading frame of the nucleic acid encoding insulin or glucokinase. In some aspects, the modified nucleic acids comprise reduced CpG content relative to the corresponding wild-type sequence and/or unmodified sequence.

[0395] In some aspects, the modified nucleic acid has reduced innate immunogenicity relative to the corresponding wild-type sequence and/or unmodified sequence. In some aspects, the modified nucleic acid has increased expression relative to the corresponding wild-type sequence and/or unmodified sequence. In some aspects, the modified nucleic acid has decreased expression relative to the corresponding wild-type sequence and/or unmodified sequence. In some aspects, the modified sequences are developed through *in silico* methods followed by manual sequence examination. Nucleic acids of the disclosure can be produced using molecular biology techniques, e.g., modified cDNAs encoding insulin or glucokinase can be obtained by PCR amplification or cDNA cloning techniques.

[0396] In some aspects, the nucleic acid sequences are modified to reduce CpG content, e.g., to minimize the inflammatory response through TLR9 dimerization and related pathways. In some aspects, certain CpG motifs are inhibitory or neutralizing for their inflammatory effects. In some embodiments, one or more of these motifs can be preserved. In some aspects, such CpG motifs can be introduced into a nucleic acid sequence for inhibition of the downstream effects of TLR9 dimerization.

[0397] In some aspects, the codon modifications can reduce the immunogenicity of the insulin and/or glucokinase encoding polynucleotides relative to a corresponding wild-type polynucleotide and/or unmodified polynucleotide. In some aspects, the codon modifications improve the expression of the insulin or glucokinase encoding polynucleotide relative to a corresponding wild-type and/or unmodified polynucleotide. In some aspects, the codon modifications can reduce the immunogenicity of the glucokinase encoding polynucleotides relative to a corresponding wild-type Gck polynucleotide and/or unmodified Gck polynucleotide.

[0398] The polynucleotides and modified nucleic acids of the disclosure can be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. The polynucleotides and modified nucleic acids can be isolated.

[0399] As used herein, a polynucleotide or nucleic acid is “isolated” or “rendered substantially pure” when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art, see e.g. F. Ausubel, *et al.*, ed. (1987) Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York.

[0400] In some aspects, a polynucleotide or modified nucleic acid of the disclosure can be, for example, DNA or RNA and may or may not contain intron sequences. In some aspects, the nucleic acid can be a cDNA molecule.

Insulin Nucleic Acids

[0401] In some aspects, the polynucleotide encoding the human insulin comprises a sequence encoding a wild-type human insulin (SEQ ID NO: 147) and/or a human Ins mutant or analogue (e.g., SEQ ID NO: 110 or SEQ ID NO: 111). In some aspects, the polynucleotide encoding the human insulin is modified relative to a wild-type (SEQ ID NO: 147) and/or an unmodified human insulin (Ins) or a human Ins mutant or analogue (e.g., SEQ ID NO: 110 or SEQ ID NO: 111). In some aspects, the polynucleotide encoding the human insulin comprises a sequence including a 5' UTR, an ORF, and/or a 3' UTR, e.g., corresponding to SEQ ID NO: 1, SEQ ID NO: 127, SEQ ID NO: 138, or SEQ ID NO: 170. In some aspects, the polynucleotide encoding the human insulin comprises a sequence including a 5' UTR (e.g., nucleic acids 5-329 of SEQ ID NO: 42), an ORF (e.g., SEQ ID NO: 121), and/or a 3' UTR (e.g., SEQ ID NO: 171), e.g., a polynucleotide comprising SEQ ID NO: 170. In some aspects, the polynucleotide encoding the human insulin is modified relative to a sequence corresponding to SEQ ID NO: 1, SEQ ID NO: 127, SEQ ID NO: 138, or SEQ ID NO: 170. In some aspects, the polynucleotide encoding the human insulin is modified relative to a sequence corresponding to SEQ ID NO: 170. In some aspects, the polynucleotide encoding the human insulin encodes wild-type human insulin (SEQ ID NO: 41), variants or mutants thereof (e.g., SEQ ID NO: 144 or SEQ ID NO: 145) or a functional fragment thereof.

[0402] Insulin includes two polypeptide chains, the A- and B- chains, linked together by disulfide bonds. It is first synthesized as a single polypeptide called preproinsulin. “Preproinsulin” is the primary translational product of the insulin gene. It is a peptide that is 110 amino acids in length. Preproinsulin includes a proinsulin molecule with a signal peptide attached to its N-terminus. Part of the N-terminus including the signal peptide of the preproinsulin is cleaved off, leaving the remaining amino acids as “proinsulin”. Amino acids 1-30 of the resulting cleaved sequence is the “B chain”, and here “B10” corresponds to position 34 of preproinsulin. Thus, for example, a “B10” proinsulin mutation corresponds to a H34 mutation in preproinsulin. In certain aspects, as referenced herein, “B10H” refers the wild-type histidine amino acid at the B10 position (also referenced as H34 in the wild-type preproinsulin sequence). The preproinsulin and proinsulin also include a C-peptide between the A- and B- chains. In the mature insulin protein, the C-peptide is proteolytically cleaved and the A- and B- chains are linked by disulfide bonds.

[0403] In some aspects, the polynucleotide encoding the human insulin disclosed herein encodes a preproinsulin mutant comprising one or more mutations at position(s) H34, P52, K53, R55, and/or L86 relative to the corresponding position in wild-type preproinsulin (SEQ ID NO: 41). In some aspects, the polynucleotide encoding the human insulin encodes a preproinsulin mutant comprising one or more of the following mutations H34D, H34I, H34V, P52D, K53R, R55K, and/or L86R relative to the corresponding position in SEQ ID NO: 41. In some aspects, the polynucleotide encoding the human insulin encodes a preproinsulin mutant comprising mutations H34D, H34I, H34V, P52D, K53R, R55K, and/or L86R relative to the corresponding position in SEQ ID NO: 41. In some aspects, the polynucleotide encoding the human insulin encodes a preproinsulin mutant comprising mutations P52D, K53R, R55K, and/or L86R relative to the corresponding position in SEQ ID NO: 41. In some aspects, the polynucleotide encoding the human insulin encodes an amino acids sequence at least 90%, 95%, 99% or 100% similar to an amino acid sequence selected from SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145. In some aspects, the polynucleotide encoding the human insulin encodes an amino acids sequence at least 90%, 95%, 99% or 100% similar to an amino acid sequence selected from SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145, wherein the amino acid sequence comprises one or more of the following mutations H34D, H34I, H34V, P52D, K53R, R55K, and/or L86R relative to the corresponding

position in SEQ ID NO: 41. In some aspects, the polynucleotide encoding the human insulin encodes an amino acids sequence that does not include a H34 mutation relative to the corresponding position in SEQ ID NO: 41.

- [0404] In some aspects, the polynucleotide encoding the human insulin comprises a cleavage site, e.g., a furin endoprotease cleavage site.
- [0405] In some aspects, the polynucleotide encoding the human insulin comprises a nucleic acid encoding a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence). In some aspects, the preproinsulin comprises a wild-type insulin signal sequence (e.g., MALWMRLLPLLALLALWGPDPAAA (SEQ ID NO: 165) or amino acids 1-24 of SEQ ID NO: 41). In some aspects, the signal sequence of wild-type preproinsulin is replaced with a non-insulin secretion peptide, e.g., an IL-6 signal sequence (e.g., MNSFSTSAFGPVAFSLGLLLVLPAAFPAP (SEQ ID NO: 166)) or a fibronectin signal sequence (e.g., MLRGPGPGLLLAVQCLGTAVPSTGA (SEQ ID NO: 167)).
- [0406] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising an amino acid modification selected from H34D, H34I, or H34V corresponding to wild-type preproinsulin amino acid positions (or a histidine (H) to aspartic acid (D), isoleucine (I) or valine (V) at position B10 of the proinsulin B-chain). In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising an amino acid modification H34D corresponding to wild-type preproinsulin amino acid positions (or a histidine (H) to aspartic acid (D) at position B10 of the proinsulin B-chain). In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising an amino acid modification selected from H34D, H34I, or H34V corresponding to wild-type preproinsulin amino acid positions (or a histidine (H) to aspartic acid (D), isoleucine (I) or valine (V) at position B10 of the proinsulin B-chain), wherein the human insulin optionally comprises a cleavage site, e.g., a furin cleavage site, and a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence).
- [0407] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32). In some aspects, the polynucleotide

encoding the human insulin encodes a human insulin comprising amino acid modifications K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32), wherein the human insulin optionally comprises a cleavage site, e.g., a furin cleavage site, and a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence).

[0408] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications H34D, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a histidine (H) to aspartic acid (D) at position B10, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32). In some aspects, the modified nucleic acid encodes a human insulin comprising amino acid modifications H34D, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a histidine (H) to aspartic acid (D) at position B10, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32), wherein the human insulin optionally comprises a cleavage site, e.g., a furin cleavage site, and a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence).

[0409] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications H34I, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a histidine (H) to isoleucine (I) at position B10, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32). In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications H34I, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a histidine (H) to isoleucine (I) at position B10, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32), wherein the human insulin optionally comprises a cleavage site, e.g., a furin cleavage site, and a signal peptide (e.g., a wild-

type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence).

[0410] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications H34V, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a histidine (H) to valine (V) at position B10, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32). In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications H34V, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a histidine (H) to valine (V) at position B10, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32), wherein the human insulin optionally comprises a cleavage site, e.g., a furin cleavage site, and a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence).

[0411] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications P49D, K53R, R55K, and L86R corresponding to wild-type preproinsulin amino acid positions (or modifications corresponding to the proinsulin a proline (P) to aspartic acid (D) at position B28, lysine (K) to arginine (R) at position B29, arginine (R) to lysine (K) at position C1, and leucine (L) to arginine (R) at position C32). In some aspects, the polynucleotide encoding the human insulin encodes a human insulin comprising amino acid modifications P49D, K53R, R55K, and L86R (corresponding to wild-type preproinsulin amino acid positions), wherein the human insulin optionally comprises a cleavage site, e.g., a furin cleavage site, and a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence).

[0412] In some aspects, the polynucleotide encoding the human insulin encodes a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof), wherein the nucleic acid comprises: (i) a nucleotide sequence encoding a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence) and (ii) a nucleotide sequence encoding a proinsulin polypeptide comprising an amino acid modification at a position selected from amino acid B10, B28, and/or B29 of the

human insulin B-chain, C1 and/or C32 of the human insulin C-chain, or any combination thereof relative to the corresponding amino acid in wild-type proinsulin (or an amino acid modification at a position selected from amino acid H34, P52, K53, R55, L86, or any combination thereof relative to the corresponding amino acid in wild-type preproinsulin). In some aspects, the signal peptide is not a wild-type preproinsulin signal sequence, e.g., the wild-type preproinsulin sequence is replaced with an IL-6 signal sequence or fibronectin signal sequence). In some aspects, the polynucleotide encoding the human insulin further comprises a cleavage site (e.g., a furin cleavage site). In some aspects, the encoded human Ins protein (e.g., a preproinsulin or variant thereof) comprises an amino acid modification selected from (i) H34D, H34I, or H34V (or a histidine (H) to aspartic acid (D), isoleucine (I) or valine (V) at position B10 of the proinsulin B-chain), and/or (ii) one or more amino acid modifications at P52, K53, R55, and/or L86 relative to the wild-type preproinsulin sequence (or positions B28 and/or B29 of the proinsulin B-chain or positions C1 and/or C32 of the proinsulin C-chain). In some aspects, the one or more amino acid modifications at P52, K53, R55, and/or L86 comprise P52D, K53R, R55K, L86R, or any combination thereof (or the one or more modifications in the proinsulin B-chain or C-chain comprise a proline (P) to aspartic acid (D) at position B28 of the proinsulin B-chain, a lysine (K) to arginine (R) at position B29 of the proinsulin B-chain, arginine (R) to lysine (K) at position C1 of the proinsulin C-chain, leucine (L) to arginine (R) at position C32 of the proinsulin C-chain, or any combination thereof).

[0413] In some aspects, the polynucleotide encoding the human insulin encodes a variant or mutant human insulin protein or a functional fragment thereof. In some aspects, the human insulin protein comprises an amino acid sequence having least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, or amino acids 25-110 of SEQ ID NO: 145. In some aspects, the human insulin protein comprises an amino acid sequence having least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145. In some aspects, the human insulin protein comprises an insertion, a deletion, a substitution, or combinations thereof relative to wild-type human insulin. In some aspects, the human insulin protein comprises

at least one substitution. In some aspects, the at least one substitution is a conservative substitution. In some aspects, the at least one substitution is a non-conservative substitution.

[0414] In some aspects, a polynucleotide of the disclosure comprises an open reading frame (ORF) encoding a human insulin comprising a nucleic acid sequence having a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, or nucleic acids 88-345 of any of SEQ ID NOs: 117-122. In some aspects, a polynucleotide of the disclosure comprises an open reading frame (ORF) encoding a human insulin comprising a nucleic acid sequence having a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 121. In some aspects, the ORF further comprises a nucleic acid sequence encoding a signal peptide.

[0415] In some aspects, a polynucleotide of the disclosure comprises an open reading frame (ORF) encoding a human insulin comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122, wherein the polynucleotide encodes a human insulin protein (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145) or a functional fragment thereof.

[0416] In some aspects, a polynucleotide of the disclosure comprises an open reading frame (ORF) comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 121. In some aspects, a polynucleotide of the disclosure comprises an open reading frame (ORF) comprising a nucleic acid sequence having the sequence of SEQ ID NO: 121.

- [0417] In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises an open reading frame comprising a nucleic acid having the sequence of 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122. In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises an open reading frame comprising a nucleic acid having the sequence of SEQ ID NO: 121. In some aspects, the polynucleotide encoding the human insulin comprises an ORF sequence present or referenced in **Table 8**.
- [0418] In some aspects, a polynucleotide of the disclosure comprises two or more ORFs. In some aspects, the two or more ORFs are operably linked. In some aspects, the ORFs are operably linked by an IRES.
- [0419] In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure further comprises a 5' UTR nucleic acid sequence. In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure further comprises a modified 5' UTR nucleic acid sequence. In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure further comprises a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, or SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, 5' UTR comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-329 of SEQ ID NO: 42.
- [0420] In some aspects, the polynucleotide comprising an ORF encoding the human insulin comprises a Kozak consensus sequence (Kozak consensus or Kozak sequence). In some aspects, the 5' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ

ID NO: 148. In some aspects, the polynucleotide comprising an ORF encoding the human insulin comprises a 5' UTR nucleic acid sequence present or referenced in **Table 8**.

[0421] In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure further comprises a modified 3' UTR nucleic acid sequence. In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure further comprises a 3' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171. In some aspects, the 3' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 171. In some aspects, the 3' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171. In some aspects, the 3' UTR comprises a restriction site selected from the group consisting of *Bam*HI, *Eco*RI, *Nde*I, *Eco*RV, *Spe*I, *Xba*I, *Nhe*I, *Vsp*I, *Nsi*I, *Sca*I, *Kpn*I, *Ssp*I, and *Pac*I, and any combination thereof. In some aspects, the polynucleotide comprising an ORF encoding the human insulin comprises a 3' UTR nucleic acid sequence present or referenced in **Table 8**.

[0422] In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 160, SEQ ID NO: 161, or SEQ ID NO: 170, wherein the nucleic acid sequence encodes a

human insulin protein (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145) or a functional fragment thereof. In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 138. In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 170.

[0423] In some aspects, the polynucleotide comprising an ORF encoding the human insulin of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-957 of a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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, or SEQ ID NO: 16. In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises a nucleic acid having the sequence of SEQ ID NO: 1, SEQ ID NO: 2, 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: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 160, SEQ ID NO: 161, or SEQ ID NO: 170. In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises a nucleic acid having the sequence of SEQ ID NO: 138. In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises a nucleic acid having the sequence of SEQ ID NO: 170. In some aspects, the polynucleotide encoding the human insulin of the disclosure comprises nucleic acids 5-957 of SEQ ID NO: 1, SEQ ID NO: 2, 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, or SEQ ID NO: 16. In some aspects, the polynucleotide comprising an ORF encoding the human insulin comprises a nucleic acid comprising a 5' UTR, an ORF, and a 3' UTR presented in **Table 8**.

[0424] In some aspects, the polynucleotide of the disclosure encodes a human insulin comprising a wild-type preproinsulin secretion signal peptide. In some aspects, the polynucleotide encoding the human insulin of the disclosure does not encode a wild-type preproinsulin secretion signal peptide. In some aspects, the wild-type preproinsulin is replaced by a non-insulin secretion signal. In some aspects, the polynucleotide encoding the human insulin of the disclosure encodes a human preproinsulin comprising an interleukin 6 (IL-6) secretion signal peptide. In some aspects, the polynucleotide encoding the human insulin of the disclosure encodes a human preproinsulin comprising a fibronectin secretion signal peptide.

Glucokinase Nucleic Acids

[0425] In some aspects, the polynucleotide encoding a human glucokinase comprises a sequence encoding wild-type human glucokinase (SEQ ID NO: 82) or a functional fragment thereof. In some aspects, the polynucleotide encoding the human glucokinase comprises a modified nucleic acid sequence which encodes wild-type human glucokinase (SEQ ID NO: 82) or a functional fragment thereof. In some aspects, the polynucleotide comprising a sequence encoding the human glucokinase disclosed herein is modified relative to the wild-type and/or unmodified, e.g., including a 5' UTR (e.g., SEQ ID NO: 42), an ORF (e.g., SEQ ID NO: 68), and/or a 3' UTR (e.g., SEQ ID NO: 60 or 171), nucleic acid sequence. In some aspects, the polynucleotide encoding a human Gck comprises a 5' UTR (e.g., SEQ ID NO: 42), an ORF (e.g., SEQ ID NO: 68), and/or a 3' UTR (e.g., SEQ ID NO: 60 or 171), e.g., a polynucleotide comprising SEQ ID NO: 19, SEQ ID NO: 27, or SEQ ID NO: 172.

[0426] In some aspects, the polynucleotide of the disclosure comprises an ORF encoding the human glucokinase comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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, SEQ ID NO: 80, or SEQ ID NO: 162, wherein the nucleic acid sequence encodes a human glucokinase protein (SEQ ID NO: 82) or a functional fragment thereof.

[0427] In some aspects, the polynucleotide of the disclosure comprises an ORF encoding the human glucokinase comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 68. In some aspects, the polynucleotide of the disclosure comprises an open reading frame encoding the human glucokinase comprising a nucleic acid having the sequence of 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, SEQ ID NO: 80, or SEQ ID NO: 162. In some aspects, the polynucleotide of the disclosure comprises an open reading frame encoding the human glucokinase comprising a nucleic acid having the sequence of SEQ ID NO: 68. In some aspects, the polynucleotide encoding the human glucokinase comprises an ORF sequence present or referenced in **Table 9**.

[0428] In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase of the disclosure further comprises a modified 5' UTR nucleic acid sequence. In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase of the disclosure further comprises a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase of the disclosure further comprises a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at

least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42.

[0429] In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase further comprises a Kozak consensus sequence (Kozak consensus or Kozak sequence). In some aspects, the 5' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase comprises a 5' UTR sequence present or referenced in **Table 9**.

[0430] In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase of the disclosure further comprises a modified 3' UTR nucleic acid sequence. In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase of the disclosure further comprises a 3' UTR comprising a nucleic acid sequence at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171. In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase of the disclosure further comprises a 3' UTR comprising a nucleic acid sequence at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 171. In some aspects, the 3' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171. In some aspects, the 3' UTR comprises a restriction site selected from the group consisting of *Bam*HI, *Eco*RI, *Nde*I, *Eco*RV, *Spe*I, *Xba*I, *Nhe*I, *Vsp*I, *Nsi*I, *Sca*I, *Kpn*I, *Ssp*I, and *Pac*I, and any combination thereof. In some aspects, the polynucleotide comprising an ORF encoding the human glucokinase comprises a 3' UTR sequence present or referenced in **Table 9**.

[0431] In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least

87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, or SEQ ID NO: 172, wherein the nucleic acid sequence encodes a human glucokinase protein (e.g., SEQ ID NO: 82) or functional fragment thereof. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 168. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 172. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-2025 of a sequence selected from 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid having the

sequence of 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, or SEQ ID NO: 172. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises a nucleic acid having the sequence of SEQ ID NO: 168. In some aspects, the polynucleotide encoding the human glucokinase of the disclosure comprises nucleic acids 5-2025 of 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39. In some aspects, the polynucleotide encoding the human glucokinase is a nucleic acid present or referenced in **Table 9**.

Expression cassettes

- [0432]** Certain aspects of the disclosure are directed to a combination gene therapy, e.g., a combination AAV gene therapy for delivery of an expression cassette (or expression construct) comprising a polynucleotide encoding an insulin (Ins) protein and an expression cassette comprising a polynucleotide encoding a glucokinase (Gck) protein. In some aspects, the combination therapy includes separate administration of the Ins and Gck expression cassettes. In some aspects, the combination therapy includes administration of the insulin and glucokinase expression cassettes in a single formulation, e.g., two separate rAAV particles in the same pharmaceutical composition.
- [0433]** In some aspects, the combination therapy comprises an insulin expression cassette comprising a promoter operably linked to a polynucleotide encoding a human insulin protein, wherein the insulin expression cassette is flanked by inverted terminal repeats. In some aspects, the combination therapy comprises a glucokinase expression cassette comprising a promoter operably linked to a polynucleotide encoding a human glucokinase protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats. In some aspects the first and second promoter are the same or

substantially the same promoter; or in some aspects the first and second promoters are different promoters that drive substantially equivalent levels of transgene expression.

[0434] An expression cassette comprising a eukaryotic promoter operably linked to a DNA of interest (e.g., a DNA encoding insulin or a DNA encoding glucokinase) can be used in the disclosure. In some aspects, the expression cassette containing the DNA sequence (or the corresponding RNA sequence), which can be used in accordance with the disclosure, can be any eukaryotic expression cassette containing the DNA or the RNA sequence of interest. For example, a plasmid or viral construct (e.g., an AAV vector) can be cleaved to provide linear DNA having ligatable termini. These termini can be bound to exogenous DNA having complementary, like ligatable termini to provide a biologically functional recombinant DNA molecule having an intact replicon and a desired phenotypic property. In some aspects, the expression cassette is capable of replication in both eukaryotic and prokaryotic hosts.

[0435] In some aspects, the exogenous DNA used in the disclosure is obtained from suitable cells, and the constructs prepared using techniques known in the art. Likewise, techniques for obtaining expression of exogenous DNA or RNA sequences in a genetically altered host cell are known in the art (see e.g., Kormal et al., *Proc. Natl. Acad. Sci. USA*, 84:2150-2154 (1987); Sambrook et al. *Molecular Cloning: a Laboratory Manual*, 2nd Ed., 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; each of which are hereby incorporated by reference with respect to methods and compositions for eukaryotic expression of a DNA of interest).

[0436] In some aspects, the DNA expression construct comprises a promoter to facilitate expression of the DNA of interest (e.g., polynucleotide encoding an insulin or a polynucleotide encoding a glucokinase) within a secretory cell. In some aspects, the promoter is a strong, eukaryotic promoter such as a promoter from cytomegalovirus (CMV), mouse mammary tumor virus (MMTV), Rous sarcoma virus (RSV), or adenovirus. Exemplary promoters include, but are not limited to the promoter from the immediate early gene of human CMV (Boshart et al., *Cell* 41:521-530 (1985) and the promoter from the long terminal repeat (LTR) of RSV (Gorman et al., *Proc. Natl. Acad. Sci. USA* 79:6777-6781 (1982)). In some aspects, the promoter is used together with an intronic sequence. In some aspects, the CMV promoter is a mini CMV promoter. Alternatively, the promoter used can be a tissue-specific promoter. In some aspects, the insulin expression cassette comprises a CMV promoter. In some aspects, the glucokinase

expression cassette comprises a CMV promoter. In some aspects, the insulin expression cassette comprises a constitutive promoter. In some aspects, the glucokinase expression cassette comprises a constitutive promoter. In some aspects, the insulin expression cassette comprises a ubiquitous promoter. In some aspects, the glucokinase expression cassette comprises a ubiquitous promoter. In some aspects, the insulin expression cassette comprises a muscle specific promoter. In some aspects, the glucokinase expression cassette comprises a muscle specific promoter.

[0437] The expression cassettes of the disclosure can also include other components such as a marker (e.g., an antibiotic resistance gene (such as an ampicillin resistance gene) or β -galactosidase) to aid in selection of cells containing and/or expressing the construct, an origin of replication for stable replication of the construct in a bacterial cell (preferably, a high copy number origin of replication), a nuclear localization signal, or other elements which facilitate production of the DNA expression construct, the protein encoded thereby, or both.

[0438] For eukaryotic expression, the expression cassette can contain at a minimum a eukaryotic promoter operably linked to a DNA of interest (e.g., a polynucleotide encoding an insulin or a glucokinase), which is in turn operably linked to a polyadenylation sequence. The polyadenylation signal sequence can be selected from any of a variety of polyadenylation signal sequences known in the art. In some aspects, the polyadenylation signal sequence is the SV40 early polyadenylation signal sequence. In some aspects, the polyadenylation signal sequence is a growth hormone polyadenylation signal sequence (e.g., a bovine growth hormone polyA or a human growth hormone polyA). In some aspects, the glucokinase expression cassette comprises an SV40 polyadenylation signal sequence. In some aspects, the insulin expression cassette comprises a bovine or human growth hormone polyadenylation signal sequence.

[0439] In some aspects, the expression cassette can also include one or more introns, which can increase levels of expression of the DNA of interest, particularly where the DNA of interest is a cDNA (e.g., contains no introns of the naturally-occurring sequence). Any of a variety of introns known in the art can be used (e.g., the human β -globin intron, which is inserted in the construct at a position 5' to the DNA of interest).

[0440] The DNA of interest (e.g., a polynucleotide encoding an insulin or a polynucleotide encoding a glucokinase) can be inserted into an expression cassette so that the therapeutic molecule (e.g., a protein) is expressed as a fusion protein (e.g., a fusion

protein having β -galactosidase or a portion thereof at the N-terminus and the therapeutic protein at the C-terminal portion). Production of a fusion protein can facilitate identification of transformed cells expressing the protein (e.g., by enzyme-linked immunosorbent assay (ELISA) using an antibody which binds to the fusion protein).

[0441] The vectors for delivery of the DNA of interest (e.g., the polynucleotide encoding an insulin, the polynucleotide encoding a glucokinase can be either viral or non-viral, or can be composed of naked DNA admixed with an adjuvant such as viral particles (e.g., AAV particle) or cationic lipids or liposomes. An “adjuvant” is a substance that does not by itself produce the desired effect, but acts to enhance or otherwise improve the action of the active compound. The precise vector and vector formulation used will depend upon several factors such as the cell and/or organ targeted for gene transfer.

[0442] Examples of suitable promoters include cytomegalovirus (CMV) intermediate early promoter, viral long terminal repeat promoters (LTRs), such as those from murine moloney leukaemia virus (MMLV) rous sarcoma virus, or HTLV-1, the simian virus 40 (SV 40) early promoter, RSV promoter, and the herpes simplex virus thymidine kinase promoter. In some aspects, the promoter is a constitutive promoter. In some aspects, the promoter is a ubiquitous promoter. In some aspects, the promoter is a cell-specific and/or a tissue-specific promoter. In some aspects, the promoter is a muscle specific promoter. In some aspects, the promoter is used together with an intronic sequence. In some aspects, the promoter is tissue specific. In some aspects, the first promoter is a CMV promoter. In some aspects, the second promoter is a CMV promoter. In some aspects, the CMV promoter is a mini CMV promoter. In some aspects, the insulin expression cassette comprises a CMV promoter. In some aspects, the glucokinase expression cassette comprises a CMV promoter. In some aspects the first and second promoter are the same or substantially the same promoter; or in some aspects the first and second promoters are different promoters that drive substantially equivalent levels of transgene expression.

[0443] In some aspects, the insulin expression cassette comprises a constitutive promoter. In some aspects, the glucokinase expression cassette comprises a constitutive promoter. In some aspects, the insulin expression cassette comprises a ubiquitous promoter. In some aspects, the glucokinase expression cassette comprises a ubiquitous promoter. In some aspects, the insulin expression cassette comprises a muscle specific promoter. In some aspects, the glucokinase expression cassette comprises a muscle specific promoter.

[0444] In some aspects, the expression cassette comprises a promoter operably linked to polynucleotide comprising an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122, wherein the polynucleotide encodes a human insulin protein (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145) or a functional fragment thereof. In some aspects, the expression cassette comprises a promoter operably linked to a polynucleotide encoding the human insulin protein comprises an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 121 or SEQ ID NO: 122. In some aspects, the polynucleotide encoding the human insulin protein of the disclosure comprises an open reading frame comprising a nucleic acid having the sequence of 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122. In some aspects, the polynucleotide encoding the human insulin protein of the disclosure comprises an open reading frame comprising a nucleic acid having the sequence of SEQ ID NO: 122. In some aspects, the polynucleotide encoding the human insulin protein of the disclosure comprises an open reading frame comprising a nucleic acid having the sequence of SEQ ID NO: 121. In some aspects, the polynucleotide encoding the human insulin protein comprises an ORF sequence present or referenced in **Table 8**.

[0445] In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin comprising a wild-type preproinsulin secretion signal peptide. In some

aspects, the polynucleotide encoding the human insulin protein of the disclosure does not encode a wild-type preproinsulin secretion signal peptide. In some aspects, the wild-type preproinsulin is replaced by a non-insulin secretion signal. In some aspects, the expression cassette comprises a polynucleotide encoding a human preproinsulin comprising an interleukin 6 (IL-6) secretion signal peptide. In some aspects, the expression cassette comprises a polynucleotide encoding a human preproinsulin comprising a fibronectin secretion signal peptide.

[0446] In some aspects, the expression cassette comprises the polynucleotide encoding the human insulin protein further comprises a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, or SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the 5' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the polynucleotide encoding the human insulin protein comprises a 5' UTR sequence present or referenced in **Table 8**.

[0447] In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein further comprising a 3' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171. In some aspects, the 3' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, SEQ ID NO: 171. In some aspects, the 3' UTR comprises a restriction site selected from the group consisting of *Bam*HI, *Eco*RI, *Nde*I, *Eco*RV, *Spe*I, *Xba*I, *Nhe*I, *Vsp*I, *Nsi*I, *Sca*I, *Kpn*I, *Ssp*I, and *Pac*I, and any combination thereof. In some aspects, the polynucleotide encoding the human insulin protein comprises a 3' UTR sequence present or referenced in **Table 8**.

[0448] In some aspects, the expression cassette comprises a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99%, or 100% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 160, SEQ ID NO: 161, or SEQ ID NO: 170, wherein the polynucleotide encodes a human insulin protein (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145) or a functional fragment thereof. In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 138 or SEQ ID NO: 171. In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-957 of a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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, or SEQ ID NO: 16. In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein having the sequence of SEQ ID NO: 1, SEQ ID NO: 2, 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: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 160, SEQ ID NO: 161, or SEQ ID NO: 170. In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein having the sequence of SEQ ID NO: 138 or SEQ ID NO: 170. In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein having at least 85%, at least 86%, at

least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-957 of a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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, or SEQ ID NO: 16. In some aspects, the expression cassette comprises a polynucleotide encoding a human insulin protein comprising a 5' UTR, an ORF, and a 3' UTR present or referenced in **Table 8**.

[0449] In some aspects, the expression cassette comprises a promoter operably linked to a polynucleotide encoding a human glucokinase protein sequence comprising an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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, SEQ ID NO: 80, or SEQ ID NO: 162. In some aspects, the expression cassette comprises a promoter operably linked to a polynucleotide encoding a human glucokinase protein sequence comprising an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 68. In some aspects, the expression cassette comprises a promoter operably linked to a polynucleotide encoding a human glucokinase protein having the sequence of 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, SEQ ID NO: 80, or SEQ ID NO: 162. In some aspects, the expression cassette comprises a promoter operably linked to a polynucleotide encoding a human glucokinase protein having the sequence of SEQ ID NO: 68. In some aspects, the polynucleotide encoding a human glucokinase protein comprises an ORF sequence present or referenced in **Table 9**.

- [0450] In some aspects, the expression cassette comprises a polynucleotide encoding a human glucokinase protein further comprising a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the 5' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the polynucleotide encoding a human glucokinase protein comprises a 5' UTR sequence present or referenced in **Table 9**.
- [0451] In some aspects, the expression cassette comprises a polynucleotide encoding a human glucokinase protein further comprising a 3' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60, 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: 149, or SEQ ID NO: 169. In some aspects, the 3' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171. In some aspects, the 3' UTR comprises a restriction site selected from the group consisting of *Bam*HI, *Eco*RI, *Nde*I, *Eco*RV, *Spe*I, *Xba*I, *Nhe*I, *Vsp*I, *Nsi*I, *Sca*I, *Kpn*I, *Ssp*I, and *Pac*I, or any combination thereof. In some aspects, the polynucleotide encoding a human glucokinase protein comprises a 3' UTR sequence present or referenced in **Table 9**.
- [0452] In some aspects, the expression cassette comprises a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 89, SEQ ID

NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, or SEQ ID NO: 172, wherein the nucleic acid sequence encodes a human glucokinase protein (e.g., SEQ ID NO: 82) or functional fragment thereof. In some aspects, the expression cassette comprises a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 168, wherein the nucleic acid sequence encodes a human glucokinase protein (e.g., SEQ ID NO: 82) or functional fragment thereof. In some aspects, the expression cassette comprises a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 172, wherein the nucleic acid sequence encodes a human glucokinase protein (e.g., SEQ ID NO: 82) or functional fragment thereof. In some aspects, the expression cassette comprises a polynucleotide encoding a human glucokinase protein having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-2025 of a sequence selected from 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39. In some aspects, the expression cassette comprises a polynucleotide encoding a human glucokinase protein having the sequence of 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 163, SEQ ID NO: 164, or SEQ ID NO: 168. In some aspects, the expression cassette comprises a polynucleotide encoding a human glucokinase protein comprising nucleic acids 5-2025 of 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39. In some aspects, the expression cassette comprises polynucleotide encoding a human glucokinase protein comprising a 5' UTR, an ORF, and a 3' UTR present or referenced in **Table 9**.

[0453] In some aspects, the combination therapy comprises administering an insulin expression cassette comprising a polynucleotide encoding a human insulin comprising a ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122 and administering a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase comprising a ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or 100% sequence identity to 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, SEQ ID NO: 80, or SEQ ID NO: 162. In some aspects, the combination therapy comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin comprising a ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 121 and glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase comprising a ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or 100% sequence identity to SEQ ID NO: 68.

- [0454]** In some aspects, the insulin expression cassette and the glucokinase expression cassette of the combination therapy are administered at a ratio of about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, 1:0.25-0.5, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, or 1:0.45-0.5. In some aspects, the insulin expression cassette and the glucokinase expression cassette of the combination therapy are administered at a ratio of about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, or 1:0.5-0.75. In some aspects, the insulin expression cassette and the glucokinase expression cassette of the combination therapy are administered at a ratio of about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.35-0.75, 1:0.4-0.60, or 1:0.45-0.55. In some aspects, the insulin expression cassette and the glucokinase expression cassette of the combination therapy are administered at a ratio of about 1:0.5, about 1:1, about 1:2, or about 1:4. In some aspects, the first AAV vector genome and the second AAV vector genome are in a vector ratio selected from the group consisting of 1:0.25-0.75, 1:0.75-1.25, 1:1.75-2.25, and 1:3.75-1:4.25 (e.g., about 1:0.5, about 1:1, about 1:2, and about 1:4). In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:0.4-0.6. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:0.5.
- [0455]** In some aspects, the insulin expression cassette and the glucokinase expression cassette of the combination therapy are administered at a ratio of about 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20; 1:0.85-1.15, 1:0.90-1.10, and 1:0.95-1.05. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:1.
- [0456]** In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:2. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is

1:3. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:4. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:5. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:10. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is 1:15. In some aspects, the vector ratio (i.e., first AAV vector comprising the hIns expression cassette to second AAV vector comprising the hGck expression cassette) is between about 1:1 to about 1:15, about 1:1 to about 1:10, about 1:1 to about 1:5, about 1:1 to about 1:4, about 1:1 to about 1:3, about 1:3 to about 1:5, about 1:3 to about 1:10, about 1:3 to about 1:15, about 1:5 to about 1:10, about 1:5 to about 1:15, or about 1:10 to about 1:15.

[0457] In some aspects, the polynucleotide encoding a human insulin protein encodes a human insulin comprising a wild-type preproinsulin secretion signal peptide. In some aspects, the polynucleotide encoding a human insulin protein does not encode a wild-type preproinsulin secretion signal peptide. In some aspects, the wild-type preproinsulin is replaced by a non-insulin secretion signal. In some aspects, the polynucleotide encoding a human insulin protein encodes a human preproinsulin comprising an interleukin 6 (IL-6) secretion signal peptide. In some aspects, the polynucleotide encoding a human insulin protein encodes a human preproinsulin comprising a fibronectin secretion signal peptide.

[0458] In some aspects, the insulin expression cassette comprising a polynucleotide encoding a human insulin protein comprises a 5' UTR, an ORF, and a 3' UTR present or referenced in **Table 8** and the glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein comprises a 5' UTR, an ORF, and a 3' UTR present or referenced in **Table 9**.

[0459] Certain aspects of the disclosure are directed to vector, e.g., a viral vector, comprising an expression construct. In some aspects, the expression construct comprises an expression cassette. In some aspects, the expression construct further comprises a genome that is able to stabilize and remain episomal in a cell. Within the context of the disclosure, in some aspects, a cell or host cell can encompass a cell used to make the construct or a cell to which the construct is administered. In some aspects, a construct is

capable of integrating into a cell's genome, e.g. through homologous recombination or otherwise. In some aspects, the expression construct is one wherein a nucleotide sequence encoding an insulin and/or a glucokinase as disclosed herein, is operably linked to a promoter as provided herein wherein the promoter is capable of directing expression of the nucleotide sequence(s) (i.e. coding sequence(s)) in a cell. In some aspects, an expression cassette as used herein comprises or consists of a nucleotide sequence encoding an insulin and/or a nucleotide sequence encoding a glucokinase, in each case the nucleotide sequence is operably linked to a promoter wherein the promoter is capable of directing expression of said nucleotide sequences. In some aspects, a viral expression construct is an expression construct that is intended to be used in gene therapy. It can be designed to comprise part of a viral genome as disclosed herein.

[0460] In some aspects, the expression construct further comprises one or more of: an ITR sequence (e.g., AAV2 ITRs), a polyA sequence (e.g., a SV40 polyadenylation signal, a bGH polyadenylation signal), and an enhancer sequence (e.g., a SV40 enhancer sequence).

[0461] In some aspects, expression constructs disclosed herein are prepared using recombinant techniques in which nucleic acid sequences encoding an insulin and/or a glucokinase are expressed in a suitable cell, e.g. cultured cells or cells of a multicellular organism, such as described in Ausubel et al., "Current Protocols in Molecular Biology", Greene Publishing and Wiley-Interscience, New York (1987) and in Sambrook and Russell (2001, supra); both of which are incorporated herein by reference in their entirety. Also see, Kunkel (1985) Proc. Natl. Acad. Sci. 82:488 (describing site directed mutagenesis) and Roberts et al. (1987) Nature 328:731-734 or Wells, J. A., et al. (1985) Gene 34: 315 (describing cassette mutagenesis).

Delivery Vectors

[0462] The present disclosure also provides vectors comprising any of the polynucleotides, expression cassettes, or constructs described herein. In some aspects, the delivery vector is a viral vector, a non-viral vectors, a plasmid, a lipid, or a lysosome. In some aspects, the delivery vector is a viral vector. In some aspects, the viral vector is an adeno-associated virus (AAV) expression vector.

[0463] In some aspects, a polynucleotide encoding an insulin and/or a glucokinase are used in an expression construct or expression vector. The phrase "expression vector"

generally refers to a nucleotide sequence that is capable of effecting expression of a gene in a host compatible with such sequences. These expression vectors can include at least suitable promoter sequences and optionally, transcription termination signals. An additional factor necessary or helpful in effecting expression can also be used as disclosed herein. A polynucleotide encoding an insulin and/or a glucokinase can be incorporated into an expression vector capable of introduction into and expression in an *in vitro* cell culture. In some aspects, the expression vector is suitable for replication in a prokaryotic host, such as bacteria, e.g., *E. coli*, or can be introduced into a cultured mammalian, plant, insect, (e.g., Sf9), yeast, fungi or other eukaryotic cell lines. In some aspects, the expression construct is suitable for expression *in vivo*.

[0464] In some aspects, the delivery vector comprises an insulin expression cassette comprising a promoter operably linked to a polynucleotide comprising an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122, wherein the polynucleotide encodes a human insulin protein (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145) or a functional fragment thereof. In some aspects, the delivery vector comprises an insulin expression cassette comprising a promoter operably linked to a polynucleotide comprising an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 122. In some aspects, the delivery vector comprises an insulin expression cassette comprising a promoter operably linked to a polynucleotide comprising an ORF having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 121. In some aspects, the polynucleotide comprises an ORF having the sequence of SEQ ID NOs: 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: 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, SEQ ID NO: 120, SEQ ID NO: 121, or SEQ ID NO: 122. In some aspects, the polynucleotide comprises an ORF having the sequence of SEQ ID NO: 122. In some aspects, the polynucleotide comprises an ORF having the sequence of SEQ ID NO: 121. In some aspects, the polynucleotide encoding a human insulin protein comprises an ORF sequence present or referenced in **Table 8**.

[0465] In some aspects, delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein encodes a human insulin comprising a wild-type preproinsulin secretion signal peptide. In some aspects, delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein does not encode a wild-type preproinsulin secretion signal peptide. In some aspects, the wild-type preproinsulin is replaced by a non-insulin secretion signal. In some aspects, delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein encodes a human preproinsulin comprising an interleukin 6 (IL-6) secretion signal peptide. In some aspects, delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein sequence encodes a human preproinsulin comprising a fibronectin secretion signal peptide.

[0466] In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein further comprising a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, 100% sequence identity to SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the 5' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the polynucleotide encoding a human insulin protein comprises a 5' UTR sequence present or referenced in **Table 8**.

[0467] In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein further comprising a 3' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171. In some aspects, the 3' UTR comprises a restriction site selected from the group consisting of *Bam*HI, *Eco*RI, *Nde*I, *Eco*RV, *Spe*I, *Xba*I, *Nhe*I, *Vsp*I, *Nsi*I, *Sca*I, *Kpn*I, *Ssp*I, and *Pac*I, and any combination thereof. In some aspects, the 3' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171. In some aspects, the polynucleotide encoding a human insulin protein comprises a 3' UTR sequence present or referenced in **Table 8**.

[0468] In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 160, SEQ ID NO: 161, or SEQ ID NO: 170, wherein the polynucleotide encodes a human insulin protein (e.g., SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145) or a functional fragment thereof. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 138 or SEQ ID NO: 170. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein having at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-957 of a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, 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, or SEQ ID NO: 16. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein having the sequence of SEQ ID NO: 1, SEQ ID NO: 2, 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: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 123, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 160, SEQ ID NO: 161, or SEQ ID NO: 170. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein having the sequence of SEQ ID NO: 138. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein having the sequence of SEQ ID NO: 170. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein comprising nucleic acids 5-957 of SEQ ID NO: 1, SEQ ID NO: 2, 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, or SEQ ID NO: 16. In some aspects, the delivery vector comprises an insulin expression cassette comprising a polynucleotide encoding a human insulin protein comprising a 5' UTR, an ORF, and a 3' UTR present or referenced in

Table 8.

[0469] In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a promoter operably linked to a polynucleotide encoding a human glucokinase protein comprising an ORF sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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, SEQ ID NO: 80, or SEQ ID NO: 162. In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a promoter operably linked to a polynucleotide encoding a human glucokinase protein having the sequence of 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, SEQ ID NO: 80, or SEQ ID NO: 162. In some aspects, the polynucleotide encoding a human glucokinase protein comprises an ORF sequence present or referenced in **Table 9**.

[0470] In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein further comprising a 5' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the 5' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 42, nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the polynucleotide encoding a human glucokinase protein comprises a 5' UTR sequence present or referenced in **Table 9**.

[0471] In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein further comprising a 3' UTR comprising a nucleic acid sequence having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171. In some aspects, the 3' UTR comprises a restriction site selected from the group consisting of *Bam*HI, *Eco*RI, *Nde*I, *Eco*RV, *Spe*I, *Xba*I, *Nhe*I, *Vsp*I, *Nsi*I, *Sca*I, *Kpn*I, *Ssp*I, and *Pac*I, and any

combination thereof. In some aspects, the 3' UTR comprises a nucleic acid having the sequence of SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, SEQ ID NO: 171. In some aspects, the polynucleotide encoding a human glucokinase protein comprises a 3' UTR sequence present or referenced in **Table 9**.

[0472] In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a polynucleotide having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to a sequence selected from 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 172, wherein the nucleic acid sequence encodes a human glucokinase protein (e.g., SEQ ID NO: 82) or functional fragment thereof. In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to nucleic acids 5-2025 of a sequence selected from 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39. In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein having the sequence of 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91,

SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, or SEQ ID NO: 172. In some aspects, the delivery vector comprises a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein comprising nucleic acids 5-2025 of 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: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39. In some aspects, the delivery vector comprises a glucokinase expression cassette comprising polynucleotide encoding a human glucokinase protein comprising a 5' UTR, an ORF, and a 3' UTR present or referenced in **Table 9**.

[0473] In some aspects, the delivery vectors can comprise sequences encoding a protein (e.g., insulin and/or Gck) operably linked with control or regulatory sequences, selectable markers, any fusion partners, and/or additional elements. In certain aspects, the modified nucleic acid is placed into a functional relationship with another nucleic acid sequence. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the protein. Such regulatory sequences are described, for example, in Goeddel (Gene Expression Technology, Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). In some aspects, the expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleic acid encoding the protein, and are typically appropriate to the host cell used to express the protein. In general, the transcriptional and translational regulatory sequences may include promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. As is also known in the art, expression vectors can contain a selection gene or marker to allow the selection of transformed host cells containing the expression vector. Selection genes are known in the art and will vary with the host cell used. For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. In some aspects, selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

- [0474] In some aspects, the delivery vector is a viral vector or a gene therapy vector comprising a viral expression construct. In certain aspects, the viral vector or a gene therapy vector is a vector that is suitable for gene therapy.
- [0475] In certain aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.25-0.75 and about 1:0.75-1.25. In certain aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.4-0.6 and about 1:0.9-1.1. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, 1:0.25-0.5, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, and 1:0.45-0.5. In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, and 1:0.5-0.75. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.4-0.60, and 1:0.45-0.55.
- [0476] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:1 to 1:15. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:2. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:3. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery

vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:4. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:5. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:10. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio of 1:15.

[0477] In certain aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.25-0.75 and about 1:0.75-1.25 (e.g., about 1:0.5 and about 1:1). In some aspects, the vector ratio (i.e., AAV vector comprising the hIns expression cassette to AAV vector comprising the hGck expression cassette) is 1:0.4-0.6. In some aspects, the delivery vectors (i.e., AAV vector comprising the hIns expression cassette to AAV vector comprising the hGck expression cassette) are delivered in a ratio of 1:0.5.

[0478] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a ratio selected from the group consisting of about 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20; 1:0.85-1.15, 1:0.90-1.10, and 1:0.95-1.05. In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:1 ratio.

[0479] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:2 ratio.

[0480] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:3 ratio.

[0481] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:4 ratio.

[0482] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:5 ratio.

[0483] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:10 ratio.

[0484] In some aspects, the delivery vector comprising the expression cassette encoding the human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are delivered in a 1:15 ratio.

[0485] In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 5×10^{11} to about 5×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 1×10^{12} to about 1×10^{14} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 1×10^{13} to about 1×10^{14} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , or about 9×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , or about 8×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 1×10^{13} , about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , about 8×10^{13} , or about 8×10^{13} vg/kg. In some aspects, the delivery

vector comprising the expression cassette encoding human insulin and the delivery vector comprising the expression cassette encoding human glucokinase are administered at a dose of about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , or about 8×10^{13} vg/kg.

[0486] In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 7×10^{10} vg/kg to about 1×10^{14} vg/kg, about 8×10^{10} vg/kg to about 1×10^{14} vg/kg, about 9×10^{10} vg/kg to about 1×10^{14} vg/kg, about 1×10^{11} vg/kg to about 1×10^{14} vg/kg, about 2×10^{11} vg/kg to about 1×10^{14} vg/kg, about 3×10^{11} vg/kg to about 1×10^{14} vg/kg, about 4×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{13} vg/kg, about 7×10^{10} vg/kg to about 1×10^{12} vg/kg, about 7×10^{10} vg/kg to about 1×10^{11} vg/kg, about 5×10^{11} vg/kg to about 1×10^{14} vg/kg, about 6×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{11} vg/kg to about 1×10^{14} vg/kg, about 8×10^{11} vg/kg to about 1×10^{14} vg/kg, about 9×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{14} vg/kg, about 8×10^{10} vg/kg to about 1×10^{14} vg/kg, about 9×10^{10} vg/kg to about 1×10^{14} vg/kg, about 1×10^{11} vg/kg to about 1×10^{14} vg/kg, about 2×10^{11} vg/kg to about 1×10^{14} vg/kg, about 3×10^{11} vg/kg to about 1×10^{14} vg/kg, about 4×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{13} vg/kg, about 7×10^{10} vg/kg to about 1×10^{12} vg/kg, about 7×10^{10} vg/kg to about 1×10^{11} vg/kg, about 7×10^{10} vg/kg to about 7×10^{12} vg/kg, about 7×10^{10} vg/kg to about 7×10^{11} vg/kg, about 7×10^{10} vg/kg to about 1.8×10^{12} vg/kg, about 7×10^{10} vg/kg to about 3.5×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 7×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 7×10^{12} vg/kg.

[0487] In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 5×10^{11} to about 5×10^{12} vg/kg.

[0488] In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 1×10^{11} to about 1×10^{12} vg/kg.

[0489] In some aspects, the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 2×10^{12} vg/kg to about 5×10^{12} vg/kg, about 2×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 5×10^{12} vg/kg,

[0490] In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 7×10^{10} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose

[illegible]

a dose of about 3.5×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 9×10^{11} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 1×10^{12} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 1.8×10^{12} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 2×10^{12} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 3.5×10^{12} vg/kg.

[0491] In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 3×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 1.5×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 4×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 2×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 5×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 2.5×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 6×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 3×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 7×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 3.5×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 8×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is

administered at a dose of about 4×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 9×10^{13} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 4.5×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 1×10^{14} vg/kg and the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 5×10^{13} vg/kg.

[0492] In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 2×10^{12} and about 1×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 4×10^{12} and about 1×10^{13} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 4.8×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human insulin is administered at a dose of about 9.6×10^{12} vg/kg.

[0493] In some aspects, the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 1×10^{12} and about 5×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 2×10^{12} and about 5×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 2.4×10^{12} vg/kg. In some aspects, the delivery vector comprising the expression cassette encoding human glucokinase is administered at a dose of about 4.8×10^{12} vg/kg. In some aspects, the gene therapy vector includes Adeno-associated virus (AAV) vector. These vectors infect a wide number of dividing and non-dividing cell types including synovial cells and liver cells. The episomal nature of the AAV vectors after cell entry makes these vectors suited for therapeutic applications. (Russell, 2000, J. Gen. Virol. 81: 2573-2604; Goncalves, 2005, Virol J. 2(1):43) as indicated above. AAV vectors can result in very stable long term expression

of transgene expression (up to 9 years in dog (Niemeyer et al, Blood. 2009 Jan. 22; 113(4):797-806) and up to 2 years in human (Nathwani et al, N Engl J Med. 2011 Dec. 22; 365(25):2357-65, Simonelli et al, Mol Ther. 2010 March; 18(3):643-50. Epub 2009 Dec. 1.)). Method for gene therapy using AAV vectors are described by Wang et al., 2005, J Gene Med. March 9 (Epub ahead of print), Mandel et al., 2004, Curr Opin Mol Ther. 6(5):482-90, and Martin et al., 2004, Eye 18(11):1049-55, Nathwani et al, N Engl J Med. 2011 Dec. 22; 365(25):2357-65, Apparailly et al, Hum Gene Ther. 2005 April; 16(4):426-34.

Adeno Associated Virus Vector (AAV vector)

- [0494] In some aspects, the polynucleotides (e.g., polynucleotide encoding a human insulin protein or polynucleotide encoding a human glucokinase protein) or expression cassettes (e.g., insulin expression cassette or glucokinase expression cassette) disclosed herein can be administered as a component of a packaged viral vector. In general, packaged viral vectors include a viral vector packaged in a capsid.
- [0495] In some aspects, the viral vector is an AAV vector. In some aspects, an AAV vector as used herein can comprise a recombinant AAV vector (rAAV). A “rAAV vector” as used herein refers to a recombinant vector comprising part of an AAV genome encapsidated in a protein shell of capsid protein derived from an AAV serotype as disclosed herein. Part of an AAV genome can contain the inverted terminal repeats (ITR) derived from an adeno-associated virus serotype, such as AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAVrh74 and others. In some aspects, the ITR is derived from AAV2.
- [0496] In some aspects, the combination therapy comprises an AAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein and an AAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein.
- [0497] In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio selected from 1:0.25-0.75 or 1:0.75-1.25. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio selected from 1:0.4-0.6 or 1:0.9-1.1. In some aspects, the AAV vector genome comprising

an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, 1:0.25-0.5, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, or 1:0.45-0.5.

[0498] In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:2, about 1:3, about 1:4, about 1:5, about 1:10, or about 1:15. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:1 to about 1:15, about 1:1 to about 1:10, about 1:1 to about 1:5, about 1:1 to about 1:4, about 1:1 to about 1:3, about 1:3 to about 1:5, about 1:3 to about 1:10, about 1:3 to about 1:15, about 1:5 to about 1:10, about 1:5 to about 1:15, or about 1:10 to about 1:15.

[0499] In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, or 1:0.5-0.75. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.35-0.75, 1:0.4-0.60, or 1:0.45-0.55. In some aspects, the first AAV vector genome and the second AAV vector genome are in a vector ratio selected from the group consisting of 1:0.25-0.75 and 1:0.75-1.25 (e.g., about 1:0.5, and about 1:1). In some aspects, the vector ratio is 1:0.4-0.6. In some aspects, the vector ratio is 1:0.5.

[0500] In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20, 1:0.85-1.15, 1:0.90-1.10, and 1:0.95-1.05. In some aspects, the vector ratio is 1:0.4-0.6. In some aspects, the AAV vector genome comprising an insulin

expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a 1:1 ratio. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.5, 1:2, or 1:4. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a ratio of 1:0.4-0.6 (e.g., about 1:0.5).

[0501] In some aspects, the first AAV vector genome and second AAV vector genome are each administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{12} to about 1×10^{14} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{13} to about 1×10^{14} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , or about 9×10^{12} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , or about 8×10^{12} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{13} , about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , about 8×10^{13} , or about 8×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , or about 8×10^{13} vg/kg.

[0502] In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 3×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 1.5×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression

cassette is administered at a dose of about 4×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2.5×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 6×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 7×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 8×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 4×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 9×10^{13} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 4.5×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{14} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 5×10^{13} vg/kg.

[0503] In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose between about 2×10^{12} and about 1×10^{13} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose between about 4×10^{12} and about 1×10^{13} vg/kg.

[0504] In some aspects, the AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 1×10^{12} and about 5×10^{12} vg/kg. In some aspects, the AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 2×10^{12} and about 5×10^{12} vg/kg.

- [0505] In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 7×10^{10} vg/kg to about 1×10^{14} vg/kg, about 8×10^{10} vg/kg to about 1×10^{14} vg/kg, about 9×10^{10} vg/kg to about 1×10^{14} vg/kg, about 1×10^{11} vg/kg to about 1×10^{14} vg/kg, about 2×10^{11} vg/kg to about 1×10^{14} vg/kg, about 3×10^{11} vg/kg to about 1×10^{14} vg/kg, about 4×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{13} vg/kg, about 7×10^{10} vg/kg to about 1×10^{12} vg/kg, about 7×10^{10} vg/kg to about 1×10^{11} vg/kg, about 5×10^{11} vg/kg to about 1×10^{14} vg/kg, about 6×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{11} vg/kg to about 1×10^{14} vg/kg, about 8×10^{11} vg/kg to about 1×10^{14} vg/kg, about 9×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 7×10^{12} vg/kg, about 7×10^{10} vg/kg to about 7×10^{11} vg/kg, about 7×10^{10} vg/kg to about 1.8×10^{12} vg/kg, about 7×10^{10} vg/kg to about 3.5×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 7×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 7×10^{12} vg/kg.
- [0506] In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{11} to about 5×10^{12} vg/kg.
- [0507] In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{11} to about 1×10^{12} vg/kg.
- [0508] In some aspects, the AAV vector genome comprising the expression cassette encoding human glucokinase is administered at a dose of about 2×10^{12} vg/kg to about 5×10^{12} vg/kg, about 2×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 5×10^{12} vg/kg.
- [0509] In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 7×10^{10} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 8×10^{10} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 9×10^{10} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the AAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{11} vg/kg and the AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} vg/kg.

[illegible]

comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} vg/kg.

- [0510] Typically, a vector genome requires the use of flanking 5' and a 3' ITR sequences to allow for efficient packaging of the vector genome into the rAAV capsid. In some aspects, the rAAV genome present in a rAAV vector comprises at least the nucleotide sequences of the inverted terminal repeat regions (ITR) of one of the AAV serotypes (e.g., of serotype AAV2 as disclosed earlier herein), or nucleotide sequences substantially identical thereto, and a modified nucleic acid sequence encoding an insulin and/or a glucokinase under control of a suitable regulatory element (e.g., a promoter), wherein the regulatory element and modified nucleic acid sequence(s) are inserted between the two ITRs.
- [0511] The complete genome of several AAV serotypes and corresponding ITR has been sequenced (Chiorini et al. 1999, J. of Virology Vol. 73, No. 2, p 1309-1319). They can be either cloned or made by chemical synthesis as known in the art, using for example an oligonucleotide synthesizer as supplied e.g. by Applied Biosystems Inc. (Fosters, Calif., USA) or by standard molecular biology techniques. The ITRs can be cloned from the AAV viral genome or excised from a vector comprising the AAV ITRs. The ITR nucleotide sequences can be either ligated at either end to the nucleotide sequence encoding one or more therapeutic proteins using standard molecular biology techniques, or the wild type AAV sequence between the ITRs can be replaced with the desired nucleotide sequence.
- [0512] The viral capsid component of the packaged viral vectors can be a parvovirus capsid, e.g., AAV Cap and/or chimeric capsids. Examples of suitable parvovirus viral capsid components are capsid components from the family Parvoviridae, such as an autonomous parvovirus or a Dependovirus. For example, the viral capsid may be an AAV capsid (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrH8, AAV9, AAV10, AAVrH10, AAV11, AAV12, or AAVrh74 capsid; one skilled in the art would know there are likely other variants not yet identified that perform the same or similar function), or may include components from two or more AAV capsids. A full complement of AAV Cap proteins includes VP1, VP2, and VP3. The ORF comprising nucleotide sequences encoding AAV VP capsid proteins can comprise less than a full complement AAV Cap proteins or the full complement of AAV Cap proteins can be provided. In some aspects, the viral capsid is an AAV1 capsid. In some aspects the

serotype of the viral vector delivering the insulin expression cassette is the same as the serotype of the viral vector delivering the glucokinase expression cassette.

[0513] One or more of the AAV Cap proteins can be a chimeric protein, including amino acid sequences AAV Caps from two or more viruses, preferably two or more AAVs. For example, the chimeric virus capsid can include an AAV1 Cap protein or subunit and at least one AAV2 Cap or subunit. In some aspects, the rAAV genome as present in a rAAV vector does not comprise any nucleotide sequences encoding viral proteins, such as the rep (replication) or cap (capsid) genes of AAV. This rAAV genome may further comprise a marker or reporter gene, such as a gene for example encoding an antibiotic resistance gene, a fluorescent protein (e.g. gfp) or a gene encoding a chemically, enzymatically or otherwise detectable and/or selectable product (e.g. lacZ, aph, etc.) known in the art.

[0514] In some aspects, the rAAV genome as present in said rAAV vector further comprises a promoter sequence operably linked to the polynucleotide encoding an insulin and/or a glucokinase. In some aspects, the promoters are ubiquitous promoters. In some aspects, the promoters are constitutive promoters. In some aspects, the promoter sequences are promoters which confer expression in muscle cells and/or muscle tissues. Examples of such promoters include a CMV and a RSV promoters as disclosed herein. In some aspects, the promoter is a CMV promoter. In some aspects, the promoter is used together with an intronic sequence. In some aspects, the CMV promoter is a mini CMV promoter.

[0515] In some aspects, suitable 3' untranslated sequence can also be operably linked to the polynucleotide encoding an insulin or a glucokinase. Suitable 3' untranslated regions can be those naturally associated with the nucleotide sequence or can be derived from different genes, such as for example the human growth hormone (hGH) or bovine growth hormone (bGH) 3' untranslated region (e.g., hGH or bGH polyadenylation signal, SV40 polyadenylation signal, SV40 polyadenylation signal and enhancer sequence).

[0516] In some aspects, additional nucleotide sequences can be operably linked to the polynucleotides encoding an insulin and/or a glucokinase, such as nucleotide sequences encoding signal sequences, nuclear localization signals, expression enhancers, and the like.

[0517] Except as otherwise indicated, methods known to those skilled in the art may be used for the construction of recombinant parvovirus and AAV (rAAV) constructs, packaging vectors expressing the parvovirus Rep and/or Cap sequences, and transiently

and stably transfected packaging cells. Such techniques are known to those skilled in the art. See, e.g., SAMBROOK et al., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, N.Y., 1989); AUSUBEL et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley Sons, Inc., New York).

[0518] Certain aspects of the disclosure are directed to a method of administering a combination therapy comprising a rAAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein and a rAAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein.

[0519] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from 1:0.25-0.75, or 1:0.75-1.25. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from 1:0.4-0.6, or 1:0.9-1.1.

[0520] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, 1:0.25-0.5, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, or 1:0.45-0.5.

[0521] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, or 1:0.5-0.75. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.35-0.75, 1:0.4-0.60, or 1:0.45-0.55. In some aspects, the first AAV vector genome and the second AAV vector genome are in a vector ratio selected from the group consisting of 1:0.25-0.75, and 1:0.75-1.25 (e.g., about 1:0.5, and about 1:1). In some aspects, the vector ratio is 1:0.4-0.6. In some aspects, the vector ratio is about 1:0.5. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector

genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.5, 1:2, 1:4, 1:5, 1:10, or 1:15. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.5.

[0522] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20, 1:0.85-1.15, 1:0.90-1.10, and 1:0.95-1.05. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a 1:1 ratio.

[0523] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{12} to about 1×10^{14} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{13} to about 1×10^{14} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , or about 9×10^{12} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , or about 8×10^{12} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered at a dose of about 1×10^{13} , about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , about 8×10^{13} , or about 8×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector

genome comprising a glucokinase expression cassette are administered at a dose of about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , or about 8×10^{13} vg/kg.

[0524] In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 3×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 1.5×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 4×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2.5×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 6×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 7×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 8×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 4×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 9×10^{13} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 4.5×10^{13} vg/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{14} vg/kg and the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 5×10^{13} vg/kg.

[0525] In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose between about 2×10^{12} and about 1×10^{13} vg/kg. In some

aspects, the rAAV vector genome comprising an insulin expression cassette is administered at a dose between about 4×10^{12} and about 1×10^{13} vg/kg.

[0526] In some aspects, the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 1×10^{12} and about 5×10^{12} vg/kg. In some aspects, the rAAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 2×10^{12} and about 5×10^{12} vg/kg.

Host cells

[0527] In some aspects, the present disclosure also provides host cells comprising the polynucleotides expression cassettes, vectors, or expression constructs disclosed herein. In some aspects, the host cell is a mammalian cell.

[0528] A construct prepared for introduction into a particular host can include a replication system recognized by the host, an intended DNA segment encoding a desired polypeptide, and transcriptional and translational initiation and termination regulatory sequences operably linked to the polypeptide-encoding segment. The term “operably linked” has already been defined herein. For example, a promoter or enhancer is operably linked to a coding sequence if it stimulates the transcription of the sequence. DNA for a signal sequence is operably linked to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of a polypeptide. Generally, a DNA sequence that is operably linked are contiguous, and, in the case of a signal sequence, both contiguous and in reading frame. However, enhancers need not be contiguous with a coding sequence whose transcription they control. Linking is accomplished by ligation at convenient restriction sites or at adapters or linkers inserted in lieu thereof, or by gene synthesis.

[0529] The selection of an appropriate promoter sequence generally depends upon the host cell selected for the expression of a DNA segment. Examples of suitable promoter sequences include prokaryotic, and eukaryotic promoters well known in the art (see, e.g. Sambrook and Russell, 2001, supra). A transcriptional regulatory sequence typically includes a heterologous enhancer or promoter that is recognized by the host. The selection of an appropriate promoter depends upon the host, but promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters are known and

available (see, e.g. Sambrook and Russell, 2001, supra). An expression vector includes the replication system and transcriptional and translational regulatory sequences together with the insertion site for the polypeptide encoding segment can be employed. In most cases, the replication system is only functional in the cell that is used to make the vector (bacterial cell as *E. coli*). Most plasmids and vectors do not replicate in the cells infected with the vector. Examples of workable combinations of cell lines and expression vectors are described in Sambrook and Russell (2001, supra) and in Metzger et al. (1988) Nature 334: 31-36. For example, suitable expression vectors can be expressed in, yeast, e.g. *S. cerevisiae*, e.g., insect cells, e.g., Sf9 cells, mammalian cells, e.g., CHO cells and bacterial cells, e.g., *E. coli*. A cell may thus be a prokaryotic or eukaryotic host cell. A cell may be a cell that is suitable for culture in liquid or on solid media.

[0530] The methods of introducing exogenous nucleic acid into host cells are well known in the art, and will vary with the host cell used. Techniques include but are not limited to dextran-mediated transfection, calcium phosphate precipitation, calcium chloride treatment, polyethylenimine mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, viral or phage infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In the case of mammalian cells, transfection may be either transient or stable.

[0531] Host cells may be yeast, e.g. *S. cerevisiae*, e.g., insect cells, e.g., Sf9 cells, mammalian cells, e.g., CHO cells, and bacterial cells, e.g., *E. coli*. A cell may thus be a prokaryotic or eukaryotic host cell. A cell may be a cell that is suitable for culture in liquid or on solid media. Alternatively, a host cell is a cell that is part of a multicellular organism such as a transgenic plant or animal. In some aspects, the host cell is a mammalian cell.

[0532] In some aspects, methods of introducing the viral vectors comprising the polynucleotides disclosed herein into a cellular host for replication and packaging can be employed, including but not limited to, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal. In embodiments wherein the viral vector functions are provided by transfection using a virus vector, standard methods for producing viral infection may be used.

[0533] In some aspects, packaging functions can include genes for viral vector replication and packaging. Thus, for example, the packaging functions may include, as needed,

functions necessary for viral gene expression, viral vector replication, rescue of the viral vector from the integrated state, viral gene expression, and packaging of the viral vector into a viral particle. The packaging functions can be supplied together or separately to the packaging cell using a genetic construct such as a plasmid or an amplicon. The packaging functions can exist extrachromosomally within the packaging cell, or can be integrated into the cell's chromosomal DNA. Examples include genes encoding AAV Rep and Cap proteins.

[0534] In some aspects, helper functions can include helper virus elements needed for establishing active infection of the packaging cell, which is required to initiate packaging of the viral vector. Examples include functions derived from adenovirus, baculovirus and/or herpes virus sufficient to result in packaging of the viral vector. For example, adenovirus helper functions will typically include adenovirus components E1a, E1b, E2a, E4, and VA RNA. The packaging functions can be supplied by infection of the packaging cell with the required virus. The packaging functions can be supplied together or separately to the packaging cell using a genetic construct such as a plasmid or an amplicon. The packaging functions can exist extrachromosomally within the packaging cell, or can be integrated into the cell's chromosomal DNA.

[0535] Any suitable helper virus functions may be employed. For example, where the packaging cells are insect cells, baculovirus can serve as a helper virus. Herpes virus can also be used as a helper virus in AAV packaging methods.

[0536] Any method of introducing the nucleotide sequence carrying the helper functions into a cellular host for replication and packaging can be employed, including but not limited to, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal. In embodiments wherein the helper functions are provided by transfection using a virus vector or infection using a helper virus; standard methods for producing viral infection may be used.

[0537] Any suitable permissive or packaging cell known in the art can be employed in the production of the packaged viral vector. Mammalian cells or insect cells are preferred. Examples of cells useful for the production of packaging cells in the practice of the invention include, for example, human cell lines or primate cells, such as VERO, WI38, MRC5, A549, 293 cells, B-50 or any other HeLa cells, HepG2, Saos-2, HuH7, and HT1080 cell lines.

[0538] In some aspects, the cell lines for use as packaging cells are insect cell lines. Any insect cell which allows for replication of AAV and which can be maintained in culture can be used in accordance with the present invention. Examples include *Spodoptera frugiperda*, such as the Sf9 or Sf21 cell lines, *Drosophila* spp. cell lines, or mosquito cell lines, e.g., *Aedes albopictus* derived cell lines. A preferred cell line is the *Spodoptera frugiperda* Sf9 cell line. The following references are incorporated herein for their teachings concerning use of insect cells for expression of heterologous polypeptides, methods of introducing nucleic acids into such cells, and methods of maintaining such cells in culture: *Methods in Molecular Biology*, ed. Richard, Humana Press, NJ (1995); O'Reilly et al., *Baculovirus Expression Vectors: A Laboratory Manual*, Oxford Univ. Press (1994); Samulski et al., *J. Vir.* 63:3822-8 (1989); Kajigaya et al., *Proc. Nat'l. Acad. Sci. USA* 88: 4646-50 (1991); Ruffing et al., *J. Vir.* 66:6922-30 (1992); Kimbauer et al., *Vir.* 219:37-44 (1996); Zhao et al., *Vir.* 272:382-93 (2000); and Samulski et al., U.S. Pat. No. 6,204,059.

[0539] During production, the packaging cells can include one or more viral vector functions along with helper functions and packaging functions sufficient to result in replication and packaging of the viral vector. These various functions can be supplied together or separately to the packaging cell using a genetic construct such as a plasmid or an amplicon, and they can exist extrachromosomally within the cell line or integrated into the cell's chromosomes.

[0540] The cells can be supplied with any one or more of the functions already incorporated, e.g., a cell line with one or more vector functions incorporated extrachromosomally or integrated into the cell's chromosomal DNA, a cell line with one or more packaging functions incorporated extrachromosomally or integrated into the cell's chromosomal DNA, or a cell line with helper functions incorporated extrachromosomally or integrated into the cell's chromosomal DNA.

Pharmaceutical compositions

[0541] In some aspects, the present disclosure also provides pharmaceutical compositions comprising the polynucleotides, expression cassettes, vectors, or expression constructs disclosed herein, which is formulated for intramuscular administration. In some aspects, the pharmaceutical compositions are formulated for administration to at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at

least ten different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine, or ten different skeletal muscle groups.

[0542] In some aspects, the different skeletal muscle groups are selected from two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and/or a latissimus dorsi (lat). In some aspects, the different skeletal muscle groups are selected from two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and/or a hamstring. In some aspects, the different skeletal muscle groups are selected from a quadriceps, a bicep, a hamstring, or any combination thereof.

[0543] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the two or more different skeletal muscle groups is any combination of two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), or a latissimus dorsi (lat).

[0544] In some aspects, the skeletal muscle group is selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring. In some aspects, the two or more different skeletal muscle groups comprises any combination of two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, or a hamstring.

[0545] In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0546] In some aspects, the pharmaceutical compositions are formulated for administration to at least three different skeletal muscle groups comprising a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat), or any combination thereof.

[0547] In some aspects, the at least three different skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and

a hamstring, or any combination thereof. In some aspects, the at least three different skeletal muscle groups are selected from a quadriceps, a bicep, and a hamstring, or any combination thereof.

[0548] In some aspects, the administration comprises bilateral injections into one or more of the at least three different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0549] In some aspects, each IM injection is administered in a volume of about 100 μ L to about 1.5 mL (e.g., about 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, or about 400 μ L to about 800 μ L). In some aspects, dose per IM injection comprises about 2×10^{13} to 5×10^{13} vgs per mL.

[0550] In some aspects there is provided a composition comprising an expression cassette or a delivery vector (e.g., a viral vector packaged in an AAV capsid) comprising a polynucleotide encoding an insulin or glucokinase as disclosed herein. In some aspects, a composition is a gene therapy composition. In some aspects, the composition is a pharmaceutical composition said pharmaceutical composition comprising a pharmaceutically acceptable carrier, adjuvant, diluents, solubilizer, filler, preservative and/or excipient.

[0551] Such pharmaceutically acceptable carrier, filler, preservative, solubilizer, diluent and/or excipient may for instance be found in Remington: The Science and Practice of Pharmacy, 20th Edition. Baltimore, Md.: Lippincott Williams & Wilkins, 2000.

[0552] In some aspects, the composition is for use as a medicament. In some aspects, the medicament is used for preventing, reducing or ameliorating the symptoms of, delaying, curing, reverting and/or treating a diabetes. In some aspects, diabetes can be Diabetes Type 1, Diabetes Type 2 or Monogenic Diabetes. In some aspects, the subject treated is a mammal, e.g. cats, rodent, (mice, rats, gerbils, guinea pigs, mice or rats), dogs, or human beings. In some aspects, the diabetes is diabetes mellitus type 1 (T1DM). In some aspects, the diabetes is diabetes mellitus type 2 (T2DM).

- [0553] In some aspects, the polynucleotide, expression cassette, expression construct, delivery vector and/or composition is used for preventing, reducing or ameliorating the symptoms of, delaying, reverting, curing and/or treating a diabetes, when said the polynucleotides, expression cassettes, expression constructs, delivery vector and/or composition is able to exhibit an anti-diabetes effect. An anti-diabetes effect can be reached when glucose disposal in blood is increased and/or when glucose tolerance is improved. This can be assessed using techniques known to the skilled person. In this context, “increase” (respectively “improvement”) means at least a detectable increase (respectively a detectable improvement) using an assay known to the skilled person or using assays as carried out in the experimental part.
- [0554] In some aspects, polynucleotide, expression cassette, expression construct, delivery vector and/or composition is used for immunosuppression in a subject administered an AAV gene therapy for the treatment of diabetes.
- [0555] An anti-diabetes effect can also be observed when the progression of a typical symptom (i.e. insulinitis, beta cell loss) has been slowed down as assessed by a physician. A decrease of a typical symptom associated with diabetes can mean a slowdown in progression of symptom development or a complete disappearance of symptoms. Symptoms, and also a decrease in symptoms, can be assessed using a variety of methods, to a large extent the same methods as used in diagnosis of diabetes, including clinical examination and routine laboratory tests. Such methods include both macroscopic and microscopic methods, as well as molecular methods, biochemical, immunohistochemical and others.
- [0556] In some aspects, the gene therapy combination methods disclosed herein are accompanied by an immunosuppression regimen that comprises one or more immunosuppressive agents. In some aspects, the one or more immunosuppressive agents comprise an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor. In some aspects, the one or more immunosuppressive agents comprises an IL-6 antagonist and/or a calcineurin inhibitor. In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, and levilimab. In some aspects, the IL-6 antagonist is

tocilizumab. In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, and voclosporin. In some aspects, the calcineurin inhibitor is tacrolimus.

[0557] In some aspects, the immunosuppression regimen comprises administering a pharmaceutical composition comprising tocilizumab prior to the combination gene therapy disclosed herein. In some aspects, the immunosuppression regimen comprises administering a pharmaceutical composition comprising tacrolimus after to the combination gene therapy disclosed herein. In some aspects, the immunosuppression regimen comprises administering a pharmaceutical composition comprising tocilizumab prior to the combination gene therapy disclosed herein and administering a pharmaceutical composition comprising tacrolimus after to the combination gene therapy disclosed herein.

[0558] A medicament as defined herein (polynucleotide, expression cassette, expression construct, delivery vector, composition, gene therapy combination (with or without an immunosuppression regimen), etc.) is preferably able to alleviate one symptom or one characteristic of a patient or of a cell, tissue or organ of said diabetes patient if after at least one week, one month, six month, one year or more of treatment using the polynucleotide, expression cassette, viral expression construct, viral vector, or composition disclosed herein, said symptom or characteristic is decreased or no longer detectable.

[0559] A polynucleotide, expression cassette, expression construct, delivery vector, composition, or gene therapy combination as disclosed herein for use in preventing, reducing or ameliorating the symptoms of, delaying, reverting, curing and/or treating a diabetes can be suitable for administration to a cell, tissue and/or an organ in vivo of individuals affected by or at risk of developing a diabetes, and may be administered in vivo. Said combination and/or composition can be directly or indirectly administrated to a cell, tissue and/or an organ in vivo of an individual affected by or at risk of developing a diabetes.

[0560] In some aspects, the mode of administration of the combination gene therapy is intramuscular. In some aspects, the combination gene therapy is administered to skeletal muscle. In some aspects, the administration is to at least two different skeletal muscle groups comprising two or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g.,

a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), a latissimus dorsi (lat), or any combination thereof.

[0561] In some aspects, the at least two different skeletal muscle groups are selected from two or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, or any combination thereof. In some aspects, the at least two different skeletal muscle groups are selected from two or more of a quadriceps, a bicep, a hamstring, or any combination thereof.

[0562] In some aspects, the administration comprises bilateral injections into one or more of the at least two different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0563] In some aspects, the administration is to at least three different skeletal muscle groups comprising three or more of a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), a latissimus dorsi (lat), or any combination thereof. In some aspects, the at least three different skeletal muscle groups are selected from three or more of a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, or any combination thereof. In some aspects, the at least three different skeletal muscle groups comprise a quadriceps, a bicep, a hamstring, or any combination thereof.

[0564] In some aspects, the administration comprises bilateral injections into one or more of the at least three different skeletal muscle groups. In some aspects, the administration comprises bilateral injections into the quadriceps, bilateral injections into the biceps, and bilateral injections into the hamstrings.

[0565] In some aspects, immunosuppression regimen as disclosed herein can be directly or indirectly administered using suitable means known in the art. In some aspects, one or more of the immunosuppressive agent is administered intravenously, subcutaneously, intramuscularly, intrathecally, intraarticularly and/or intraventricularly.

[0566] In some aspects, the immunosuppression regimen as disclosed herein can be delivered as is to an individual, a cell, tissue or organ of said individual. Depending on the disease or condition, a cell, tissue or organ of said individual may be as earlier defined

herein. In some aspects, polynucleotide, expression cassette, expression construct, delivery vector, composition, combination gene therapy, and/or immunosuppression regimen as disclosed herein is dissolved in a solution that is compatible with the corresponding delivery method. For intravenous, subcutaneous, intramuscular, intrathecal, intraarticular and/or intraventricular administration the solution may be a physiological salt solution.

[0567] In some aspects, administration is intramuscular administration. In some aspects, intramuscular administration is carried out using a multineedle. In some aspects, a therapeutically effective dose of the polynucleotide, expression cassette, expression construct, the vector, or the composition as described herein is administered in a single and unique dose hence avoiding repeated periodical administration. In some aspects, the single dose is administered to muscle tissue. In some aspects, the single dose is administered to skeletal muscle tissue. In some aspects, the single dose comprise multiple injections (e.g., two, three, four, or five) to one or more skeletal muscles (e.g., multiple skeletal muscle groups). In some aspects, the single dose comprises a total of 20-150 injections of the combination therapy to the at least two different skeletal muscle groups. In some aspects, the administration comprises a total of 20-120 IM injections, 20-110 IM injections, 20-100 IM injections, 20-80 IM injections, 20-60 IM injections, 25-150 IM injections, 25-120 IM injections, 25-110 IM injections, or 25-100 IM injections. In some aspects, the administration comprises 1-80 (e.g., 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80) IM injections to a first skeletal muscle group and 1-80 (e.g., 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80) IM injections to a second skeletal muscle group.

[0568] In some aspects, the administration comprises a total of 35-70 (e.g., 35-70, 40-70, 45-70, 50-70, 50-55, 55-60, 60-65, 65-70, 50-70, 50-60, 60-70, 55-65, or 55-70) IM injections of the therapy (e.g., combination gene therapy) to the at least two different skeletal muscle groups. In some aspects, the administration comprises 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a first skeletal muscle group and 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a second skeletal muscle group. In some aspects, the administration comprises 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a first skeletal muscle group, 10-35 (e.g., 10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a second skeletal muscle group, and 10-35 (e.g.,

10-15, 15-20, 20-25, 25-30, 10-20, 20-30, 15-25, or 30-35) IM injections to a third skeletal muscle group.

[0569] In some aspects, the different skeletal muscle groups comprise a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), a latissimus dorsi (lat), or any combination thereof. In some aspects, the different skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, or any combination thereof. In some aspects, the different skeletal muscle groups are selected from a quadriceps, a bicep, a hamstring, or any combination thereof. In some aspects, the single dose comprises at least two of 8-50 IM injections to the quadriceps (e.g., 4-25 IM injections bilaterally to each quadriceps), 8-50 IM injections to the biceps (e.g., 4-25 IM injections bilaterally to each bicep), and 8-50 IM injections to the hamstrings (e.g., 4-25 IM injections bilaterally to each hamstring).

[0570] In some aspects, the single dose is administered to at least three different skeletal muscle groups. In some aspects, the administration comprises 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a first skeletal muscle group, 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a second skeletal muscle group, and 1-50 (e.g., 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50) IM injections to a third skeletal muscle group. In some aspects, the single dose comprises 8-50 IM injections to the quadriceps (e.g., 4-25 IM injections bilaterally to each quadriceps), 8-50 IM injections to the biceps (e.g., 4-25 IM injections bilaterally to each bicep), and 8-50 IM injections to the hamstrings (e.g., 4-25 IM injections bilaterally to each hamstring).

[0571] In some aspects, the administration to each of the skeletal muscle groups is bilateral. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0572] In some aspects, a compound can be present in a pharmaceutical composition of the disclosure. In some aspects, said compound can help in delivery of the polynucleotide, expression cassette, or composition comprising the same. In some aspects, the compound is a compound capable of forming complexes, nanoparticles, micelles, liposomes that deliver each constituent as defined herein, complexed or trapped in a vesicle or liposome

through a cell membrane, or combinations thereof. Many of these compounds are known in the art. In some aspects, the further compound is polyethylenimine (PEI), or similar cationic polymers, including polypropyleneimine or polyethylenimine copolymers (PECs) and derivatives, synthetic amphiphiles (SAINT-18), Lipofectin™, DOTAP, or combinations thereof.

Methods of Use

[0573] The present disclosure also provides a method for preventing, reducing or ameliorating the symptoms of, delaying, reverting, curing and/or treating diabetes comprising intramuscularly administering to a subject in need thereof a combination therapy comprising the polynucleotides, the expression cassettes, the delivery vectors, expression constructs, or combination gene therapy disclosed herein, wherein the combination therapy is administered intramuscularly (IM) at least two different sites, optionally, wherein the method further comprises administering an immunosuppressive regimen. In some aspects, the diabetes can be T1DM. In some aspects, the diabetes can be T2DM. In certain aspects, the methods of the disclosure comprise administration (e.g., intramuscular administration) a combination therapy comprising the polynucleotides, the expression cassettes, a delivery vectors, or expression construct, optionally, wherein the method further comprises administering immunosuppressive regimen (e.g., one or more immunosuppressive agents) disclosed herein to a cell, tissue, or subject in need thereof.

[0574] In certain aspects, the methods comprise administration of a combination therapy comprising (i) a polynucleotide encoding a human insulin protein, an insulin expression cassette, a delivery vectors, or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) a polynucleotide encoding a human glucokinase protein, a glucokinase expression cassette, a delivery vectors, or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein, wherein the combination therapy is administered intramuscularly (IM) at least two different skeletal muscle groups. In some aspects, the at least two different skeletal muscle groups comprise two or more selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat). In some aspects, the at least two different skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, or any

combination thereof. In some aspects, the at least two different skeletal muscle groups comprise a quadriceps, a bicep, a hamstring, or any combination thereof.

[0575] In some aspects, the subject is not insulin resistant. In some aspects, the subject is insulin resistant.

[0576] In some aspects, the administration comprises bilateral injections into one or more of the different skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0577] In certain aspects, the methods comprise administration of combination therapy comprising: one or more adeno-associated virus (AAV) vectors comprising (a) a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein; and (b) a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein; wherein the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups. In some aspects, the method further comprises administering an immunosuppressive regimen.

[0578] In some aspects, the combination therapy comprises:

(a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and

(b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs);

wherein the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.

[0579] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same composition (e.g., via the same IM injection).

[0580] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition (e.g., via the same IM injection).

- [0581] In some aspects, the one or more AAV vectors is an AAV vector genome comprising an single expression cassette comprising the first promoter operably linked to the polynucleotide encoding the hIns protein and the second promoter operably linked to the polynucleotide encoding the hGck protein, wherein the single expression cassette is flanked by inverted terminal repeats (ITRs).
- [0582] Certain aspects of the disclosure are directed to a method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising administering to the subject (i) an immunosuppressive regimen and (ii) a combination therapy comprising one or more adeno-associated virus (AAV) vectors comprising (a) a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein; and (b) a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.
- [0583] In some aspects the combination therapy comprises (a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).
- [0584] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same composition (e.g., via the same IM injection).
- [0585] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition (e.g., via the same IM injection).
- [0586] In some aspects, the one or more AAV vectors is an AAV vector genome comprising an single expression cassette comprising the first promoter operably linked to the polynucleotide encoding the hIns protein and promoter operably linked to the polynucleotide encoding the hGck protein, wherein the single expression cassette is flanked by inverted terminal repeats (ITRs).
- [0587] Certain aspects of the disclosure are directed to a dosing regimen comprising:
(a) a first immunosuppressive agent;

(b) a combination gene therapy administered intramuscularly after (a) which comprises (i) a first recombinant AAV (rAAV) particle comprising a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs); and

(c) a second immunosuppressive agent administered after (b), optionally, wherein the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.

[0588] In certain aspects, the methods comprise administration of an immunosuppressive regimen and a combination therapy comprising (i) a polynucleotide encoding a human insulin protein, an insulin expression cassette, a delivery vectors, or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) a polynucleotide encoding a human glucokinase protein, a glucokinase expression cassette, a delivery vectors, or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein, wherein the combination therapy is administered intramuscularly (IM) at least two different sites. In certain aspects, the administration of (i) and (ii) is simultaneous or sequential. In some aspects, the present disclosure provides a method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising administering a combination therapy to the subject comprising: (a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs); wherein the combination therapy is administered intramuscularly (IM) at least two different sites.

[0589] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same composition (e.g., via the same IM injection).

- [0590] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition (e.g., via the same IM injection).
- [0591] In some aspects, the one or more AAV vectors is an AAV vector genome comprising an single expression cassette comprising the first promoter operably linked to the polynucleotide encoding the hIns protein and the second promoter operably linked to the polynucleotide encoding the hGck protein, wherein the single expression cassette is flanked by inverted terminal repeats (ITRs).
- [0592] In some aspects, the method further comprises administering to the subject an immunosuppressive regimen.
- [0593] In some aspects, the present disclosure provides a method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising administering an immunosuppressive regimen and a combination therapy to the subject comprising: (a) a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (b) a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs). In some aspects, the combination therapy is administered intramuscularly (IM) at three different sites. In some aspects, the combination therapy is administered into the quadriceps, biceps, and hamstrings bilaterally.
- [0594] In some aspects, the combination therapy is administered intramuscularly (IM) to at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine, or ten different skeletal muscle groups.
- [0595] In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat), or any combination thereof. In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a

hamstring, or any combination thereof. In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep, and a hamstring, or any combination thereof.

[0596] In some aspects, the skeletal muscle groups are selected from a quadriceps, a bicep (e.g., the biceps brachii or the biceps femoris), a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring (e.g., the semitendinosus, the semimembranosus, or the biceps femoris), a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), a thigh muscle (e.g., vastus intermedius, vastus lateralis, or vastus medialis), a hip muscle (gluteus medius), and a latissimus dorsi (lat).

[0597] In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0598] In some aspects, the pharmaceutical formulation comprising AAV vectors is administered in multiple injections with an individual injection volume of between 100 μ L and 1.5 mL. In some aspects, an individual injection volume is about 100 μ L to about 1.5 mL (e.g., about 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, or about 400 μ L to about 800 μ L). In some aspects, an individual injection volume is between 200 μ L and 1.0 mL. In some aspects the individual injection volume is about 500 μ L. In some aspects, the individual injection volume is between 1 μ L and 500 μ L. In some aspects, the individual injection volume is between 10 μ L and 300 μ L. In some aspects, the individual injection volume is between 50 μ L and 250 μ L. In some aspects, the individual injection volume is about 100 μ L.

[0599] In some aspects, the pharmaceutical formulation comprising AAV vectors is administered in multiple injections with an individual injection volume of between about 500 μ L to about 1 mL. In some aspects, the pharmaceutical formulation comprising AAV vectors is administered in multiple injections with an individual injection volume of about 500 μ L. In some aspects, the pharmaceutical formulation comprising AAV vectors is administered in multiple injections with an individual injection volume of about 1 mL.

[0600] In some aspects, the pharmaceutical formulation comprising AAV vectors is administered in multiple injections with an individual injection dose of between 1×10^{11} vg/injection site and 5×10^{13} vg/injection site. In some aspects, an individual injection

dose of comprises about 2×10^{13} to 5×10^{13} vg/injection site. In some aspects, the individual injection dose is about 1×10^{11} vg/injection site to about 1×10^{13} vg/injection site. In some aspects, the individual injection dose is about 1.25×10^{11} vg/injection site to about 1.75×10^{12} vg/injection site. In some aspects, the individual injection dose is about 1.5×10^{11} vg/injection site to about 1.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 1.75×10^{11} vg/injection site to about 1.25×10^{12} vg/injection site. In some aspects, the individual injection dose is about 1×10^{12} vg/injection site.

[0601] In some aspects, the individual injection site is about 7×10^{10} vg/kg to about 1×10^{14} vg/kg, about 8×10^{10} vg/kg to about 1×10^{14} vg/kg, about 9×10^{10} vg/kg to about 1×10^{14} vg/kg, about 1×10^{11} vg/kg to about 1×10^{14} vg/kg, about 2×10^{11} vg/kg to about 1×10^{14} vg/kg, about 3×10^{11} vg/kg to about 1×10^{14} vg/kg, about 4×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{13} vg/kg, about 7×10^{10} vg/kg to about 1×10^{12} vg/kg, about 7×10^{10} vg/kg to about 1×10^{11} vg/kg, about 5×10^{11} vg/kg to about 1×10^{14} vg/kg, about 6×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{11} vg/kg to about 1×10^{14} vg/kg, about 8×10^{11} vg/kg to about 1×10^{14} vg/kg, about 9×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 7×10^{12} vg/kg, about 7×10^{10} vg/kg to about 7×10^{11} vg/kg, about 7×10^{10} vg/kg to about 1.8×10^{12} vg/kg, about 7×10^{10} vg/kg to about 3.5×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 7×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 7×10^{12} vg/kg.

[0602] In some aspects, the individual injection site is about 8×10^{10} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 7×10^{10} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 9×10^{10} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 1×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 2×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 3×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 4×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 5×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 6×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 7×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some

aspects, the individual injection site is about 8×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 9×10^{11} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 1×10^{12} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 1.5×10^{12} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 2×10^{12} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 2.5×10^{12} vg/injection site to about 3.5×10^{12} vg/injection site. In some aspects, the individual injection site is about 3×10^{12} vg/injection site to about 3.5×10^{12} vg/injection site.

- [0603] In some aspects, the pharmaceutical formulation is administered in multiple injections with a minimum distance between each injection site. In some aspects the distance between each injection site is at least 0.5 cm, at least 0.6 cm, at least 0.7 cm, at least 0.8 cm, at least 0.9 cm, at least 1.0 cm, at least 1.1 cm, at least 1.2 cm, at least 1.3 cm, at least 1.4 cm, at least 1.5 cm, at least 2.0 cm, at least 3.0 cm. In some aspects, the distance between each injection site is between 0.5 cm and 3 cm, between 0.75 cm and 2.5 cm, between 1 cm and 2 cm, between .75 cm and 1.25 cm, or about 1 cm.
- [0604] In some aspects, the IM administration comprises injections that are separated by a distance of at least 1-3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.
- [0605] In some aspects, the administration comprises IM injections that are separated by a distance of at least 1-7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 7 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 6 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of at least 1 cm.
- [0606] In some aspects, each of the IM injection sites on the subject are separated by a distance of about 7 cm. In some aspects, each of the IM injection sites on the subject are

separated by a distance of about 6 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 5 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 4 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 3 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 2 cm. In some aspects, each of the IM injection sites on the subject are separated by a distance of about 1 cm.

[0607] In some aspects, the IM administration comprises injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep, at least 1.4 cm deep, at least 1.5 cm deep, or at least 2.0 cm deep. In some aspects, each of the IM injections is between 1-2 cm deep.

[0608] Certain aspects of the disclosure are directed to a method of immunosuppression in a subject administered an AAV gene therapy for the treatment of diabetes, wherein the subject is administered an immunosuppressive regimen comprising administering (a) an immunosuppressive agent (pre-AAV) prior to administering the AAV gene therapy; and (b) an immunosuppressive agent (post-AAV) after administering the AAV gene therapy. In some aspects, the AAV gene therapy is administered by intramuscular (IM) injection.

[0609] In some aspects, the immunosuppressive regimen comprises administering one or more immunosuppressive agents prior to, together with, and/or at any point after administering the first AAV vector genome and the second AAV vector genome. In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent prior to administering the first AAV vector genome and the second AAV vector genome. In some aspects, the immunosuppressive regimen further comprises administering a second immunosuppressive agent after administering the first AAV vector genome and the second AAV vector genome.

[0610] In some aspects, the immunosuppressive agent is administered 1 to 14 days prior to administering the first AAV vector genome and the second AAV vector genome. In some aspects, the immunosuppressive agent is administered 1 to 7 days prior to administering the first AAV vector genome and the second AAV vector genome. In some aspects, the immunosuppressive agent is administered 1 day prior to administering the first AAV vector genome and the second AAV vector genome.

- [0611] In some aspects, the immunosuppressive agent is initially administered 1, 2, 3, or 4 weeks after administering the first AAV vector genome and the second AAV vector genome. In some aspects, the immunosuppressive agent is initially administered 3 weeks after administering the first AAV vector genome and the second AAV vector genome. In some aspects, after the initial administration, the immunosuppressive agent is administered every 2 times per day, daily, or every other day.
- [0612] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 2 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered starting at 1, 2, 3, or 4 weeks (e.g., 3 weeks) after administering the combination therapy.
- [0613] In some aspects, the second immunosuppressive agent is administered once or twice daily for 4 to 52 weeks. In some aspects, the second immunosuppressive agent is administered once daily for 4 to 24 weeks, 4 to 16 weeks, or 4 to 8 weeks.
- [0614] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 14 days prior to administering the AAV gene therapy. In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 7 days prior to administering the AAV gene therapy. In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 2 days prior to administering the AAV gene therapy.
- [0615] In some aspects, the post-AAV immunosuppressive agent is initially administered 1, 2, 3, or 4 weeks after administering the AAV gene therapy. In some aspects, the immunosuppressive agent is initially administered about 3 weeks after administering the AAV gene therapy.
- [0616] In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered 2 times per day, daily, or every other day. In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered for about 6 to 40, about 8 to 40, about 10 to 40, about 12 to 40, about 6 to 30, about 8 to 30, about 10 to 30, about 12 to 30, about 6 to 20, about 8 to 20, about 10 to 20, or about 12 to 20 weeks.
- [0617] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered 1 to 14 days (e.g., 1 to 3 days) prior to administering the combination therapy.

- [0618] In some aspects, the first immunosuppressive agent is administered once every one week, two weeks, three weeks, or four weeks.
- [0619] In some aspects, the first immunosuppressive agent is administered for about 3 months, about 4 months, about 5 months, or about 6 months.
- [0620] In some aspects, the second immunosuppressive agent is administered twice daily for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some aspects, the second immunosuppressive agent is administered twice daily for about 1 to about 6 months, about 2 months to about 6 months, about 3 months to about 6 months, about 4 months to about 6 months, about 5 months to about 6 months, about 2 months to about 4 months, or about 3 months to about 4 months.
- [0621] In some aspects, the immunosuppressive regimen comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor (for example, rapamycin), mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor.
- [0622] In some aspects, the pre-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, MMF, a tyk2 inhibitor, rapamycin (also known as Sirolimus), or abatacept.
- [0623] In some aspects, the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, or abatacept.
- [0624] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof. In some aspects, the IL-6 antagonist is tocilizumab. In some aspects, the IL-6 antagonist targets IL-6. In some aspects, the IL-6 antagonist targets IL-6 receptor.
- [0625] In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof. In some aspects, the calcineurin inhibitor is tacrolimus.
- [0626] In some aspects, the immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor (for example, rapamycin), mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor. In some aspects,

the immunosuppressive agent comprises an IL-6 antagonist or a calcineurin inhibitor. In some aspects, the immunosuppressive agent administered prior to administering the first AAV vector genome and the second AAV vector genome is an IL-6 antagonist. In some aspects, the immunosuppressive agent administered after administering the first AAV vector genome and the second AAV vector genome is a calcineurin inhibitor. In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, satralizumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, and levilimab. In some aspects, the IL-6 antagonist is tocilizumab. In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, and voclosporin. In some aspects, the calcineurin inhibitor is tacrolimus.

- [0627] In some aspects, the immunosuppressive agent comprises thymoglobulin, etanercept, basiliximab, tacrolimus, sirolimus, or mycophenolate mofetil.
- [0628] In some aspects, the tacrolimus is administered orally.
- [0629] In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a ratio selected from the group consisting of about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, 1:0.25-0.5, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, and 1:0.45-0.5.
- [0630] In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a ratio selected from the group consisting of about 1:1 to about 1:15, about 1:1 to about 1:10, about 1:1 to about 1:5, about 1:1 to about 1:4, about 1:1 to about 1:3, about 1:3 to about 1:5, about 1:3 to about 1:10, about 1:3 to about 1:15, about 1:5 to about 1:10, about 1:5 to about 1:15, or about 1:10 to about 1:15.

- [0631]** In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a ratio selected from the group consisting of about 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20, 1:0.85-1.15, 1:0.90-1.10, and 1:0.95-1.05. In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a 1:1 ratio.
- [0632]** In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a ratio selected from the group consisting of about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, and 1:0.5-0.75.
- [0633]** In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a ratio selected from the group consisting of about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.4-0.60, and 1:0.45-0.55.
- [0634]** In some aspects, (i) the polynucleotide encoding a human insulin protein, the insulin expression cassette, the delivery vector or expression construct encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) and/or (ii) the polynucleotide

encoding a human glucokinase protein, the glucokinase expression cassette, the delivery vector or expression construct comprising a nucleic acid encoding a human glucokinase (Gck) protein are administered in a ratio selected from the group consisting of 1:0.25-0.75, and 1:0.75-1.25 (e.g., about 1:0.5, and about 1:1).

[0635] Certain aspects of the disclosure are directed methods of use comprising administering combination therapy a polynucleotide encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof) comprising (i) a nucleotide sequence encoding a signal peptide, optionally wherein the signal peptide is not a wild-type preproinsulin signal sequence, and (ii) a nucleotide sequence encoding a proinsulin polypeptide comprising an amino acid modification at a position selected from amino acid B10, B28, and/or B29 of the human insulin B-chain, C1 and/or C32 of the human insulin C-chain, or any combination thereof relative to the corresponding amino acid position in wild-type proinsulin, and optionally the polynucleotide further comprises a cleavage site. In some aspects, the signal peptide is a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence. In some aspects, the cleavage site between the signal sequence and the circulating protein is a furin cleavage site.

[0636] Certain aspects of the disclosure are directed to methods of use comprising administering a combination therapy comprising a polynucleotide comprising a nucleic acid encoding a human insulin (Ins) protein, wherein the nucleic acid comprises an open reading frame (ORF) comprising: (i) a nucleotide sequence encoding a signal peptide and (ii) a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, or nucleic acids 88-345 of any of SEQ ID NOs: 117-122. In some aspects, the encoded human Ins protein comprises (i) a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence) and (ii) amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, or amino acids 25-110 of SEQ ID NO: 145. In some aspects, the encoded human insulin protein further comprises a cleavage site (e.g., a furin cleavage site).

[0637] Certain aspects of the disclosure are directed to a method of use comprising administering combination therapy comprising a polynucleotide comprising a nucleic acid encoding a human insulin (Ins) protein, wherein the nucleic acid comprises an open reading frame (ORF) comprising: a nucleotide sequence at least 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NOs: 43-57, or 110-122. In some aspects, the encoded human Ins protein comprises a signal sequence and a proinsulin polypeptide. In some aspects, the encoded human Ins protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, or amino acids 25-110 of SEQ ID NO: 145. In some aspects, the encoded human Ins protein is a preproinsulin. In some aspects, the encoded human Ins protein comprises the amino acid sequence of SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145. In some aspects, the polynucleotide or nucleic acid sequence further comprises a 5' UTR and/or a 3' UTR. In some aspects, the polynucleotide or nucleic acid comprises: a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NOs: 1-16, 84-88, 123, 127-129, 133-138, 160-161, or 170.

[0638] Certain aspects of the disclosure are directed to a method of use comprising administering a combination therapy comprising a polynucleotide comprising a nucleic acid encoding a human insulin (Ins) protein (e.g., a preproinsulin or variant thereof), wherein the nucleic acid comprises: (i) a nucleotide sequence encoding a signal peptide (e.g., a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence) and (ii) a nucleotide sequence encoding a proinsulin polypeptide comprising an amino acid modification at a position selected from amino acid B10, B28, and/or B29 of the human insulin B-chain, C1 and/or C32 of the human insulin C-chain, or any combination thereof relative to the corresponding amino acid in wild-type proinsulin (or an amino acid modification at a position selected from amino acid H34, P52, K53, R55, L86, or any combination thereof relative to the corresponding amino acid in wild-type preproinsulin). In some aspects, the signal peptide is not a wild-type preproinsulin signal sequence (e.g., the wild-type preproinsulin sequence is replaced with an IL-6 signal sequence or fibronectin signal sequence). In some aspects, the proinsulin polypeptide comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, or amino acids 25-110 of SEQ ID NO: 145. In some aspects, the polynucleotide further comprises a cleavage site (e.g., a furin cleavage site).

[0639] Certain aspects of the disclosure are directed to a method of use comprising administering combination therapy comprising a polynucleotide comprising a nucleic acid encoding a human glucokinase (Gck) protein, wherein the nucleic acid comprises an

ORF comprising: a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of nucleic acids 1-1398 of any of SEQ ID NO: 61-80 or 162; or SEQ ID NO: 61-80 and 162. In some aspects, the encoded human Gck protein comprises the amino acid sequence of SEQ ID NO: 82. In some aspects, the polynucleotide or nucleic acid sequence encoding a Gck protein further comprises a 5' UTR and/or a 3' UTR. In some aspects, the nucleic acid further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 42, 5-329 of SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148. In some aspects, the nucleic acid further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 169. In some aspects, the polynucleotide or nucleic acid comprises: a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NOs: 20-39 and 89-96, 163-164, or 168. In some aspects, the nucleic acid is operably linked to a promoter (e.g., a eukaryotic promoter). Certain aspects of the disclosure are directed to an expression cassette comprising a polynucleotide of the disclosure and a heterologous expression control sequence operably linked to the nucleic acid sequence. In some aspects, the nucleic acid is operably linked to a polyadenylation (polyA) element.

[0640] Certain aspects of the disclosure are directed to a method of use comprising administering combination therapy comprising a polynucleotide comprising a nucleic acid encoding a human insulin (Ins) protein, wherein the nucleic acid comprises an open reading frame (ORF) comprising: a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NOs: 43-57, or 110-122 and a polynucleotide comprising a nucleic acid encoding a human glucokinase (Gck) protein, wherein the nucleic acid comprises an ORF comprising: a nucleotide sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of nucleic acids 1-1398 of any of SEQ ID NO: 61-80 or 162; or SEQ ID NO: 61-80 and 162.

[0641] Certain aspects of the disclosure are directed to a method of use comprising administering combination therapy comprising a polynucleotide or nucleic acid encoding a human insulin protein wherein the polynucleotide comprises a sequence at least 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NOs: 1-16, 84-88, 123, 127-129, 133-138, 160-161, or 170 and polynucleotide or nucleic acid encoding a human glucokinase protein wherein the polynucleotide comprises a sequence at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any of SEQ ID NOs: 20-39 and 89-96, 163-164, and 168.

[0642] Certain aspects of the disclosure are directed to methods of use comprising administering a combination therapy comprising a vector (e.g., viral vector, a non-viral vector, a plasmid, a lipid, or a lysosome) comprising a polynucleotide or an expression cassette of the disclosure. In some aspects, the vector is an adeno-associated virus (AAV) vector.

[0643] Certain aspects of the disclosure are directed to a method of administering a combination therapy comprising an AAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein and a AAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein. In some aspects, the serotype of the AAV vector delivering the insulin expression cassette is the same as the serotype of the AAV vector delivering the glucokinase expression cassette. In some aspects, the serotype of the AAV vector delivering the insulin expression cassette is different from the serotype of the AAV vector delivering the glucokinase expression cassette.

[0644] In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio selected from 1:0.25-0.75, or 1:0.75-1.25. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio selected from 1:0.4-0.6, or 1:0.9-1.1. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio of about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, or 1:0.25-0.5. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio of about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, or 1:0.5-0.75. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV

vector genome comprising a glucokinase expression cassette are administered in a vector ratio of about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.35-0.75, 1:0.4-0.60, or 1:0.45-0.55. In some aspects, the first AAV vector genome and the second AAV vector genome are in a vector ratio selected from the group consisting of 1:0.25-0.75, and 1:0.75-1.25 (e.g., about 1:0.5, and about 1:1). In some aspects, the vector ratio is 1:0.4-0.6. In some aspects, the vector ratio is 1:0.5. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio of about 1:0.5, or 1:1. In some aspects, the AAV vector genome comprising an insulin expression cassette and the AAV vector genome comprising a glucokinase expression cassette are administered in a vector ratio of about 1:0.5.

[0645] In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 1×10^{12} to about 1×10^{14} vg/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 1×10^{13} to about 1×10^{14} vg/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , or about 9×10^{12} vg/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , or about 8×10^{12} vg/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 1×10^{13} , about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , about 8×10^{13} , or about 8×10^{13} vg/kg. In some aspects, the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered at a dose of about 2×10^{13} , about 3×10^{13} , about 4×10^{13} , about 5×10^{13} , about 6×10^{13} , about 7×10^{13} , or about 8×10^{13} vg/kg.

- [0646] In some aspects, the first AAV vector genome is administered at a dose of about 7×10^{10} vg/kg to about 1×10^{14} vg/kg, about 8×10^{10} vg/kg to about 1×10^{14} vg/kg, about 9×10^{10} vg/kg to about 1×10^{14} vg/kg, about 1×10^{11} vg/kg to about 1×10^{14} vg/kg, about 2×10^{11} vg/kg to about 1×10^{14} vg/kg, about 3×10^{11} vg/kg to about 1×10^{14} vg/kg, about 4×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 1×10^{13} vg/kg, about 7×10^{10} vg/kg to about 1×10^{12} vg/kg, about 7×10^{10} vg/kg to about 1×10^{11} vg/kg, about 5×10^{11} vg/kg to about 1×10^{14} vg/kg, about 6×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{11} vg/kg to about 1×10^{14} vg/kg, about 8×10^{11} vg/kg to about 1×10^{14} vg/kg, about 9×10^{11} vg/kg to about 1×10^{14} vg/kg, about 7×10^{10} vg/kg to about 7×10^{12} vg/kg, about 7×10^{10} vg/kg to about 7×10^{11} vg/kg, about 7×10^{10} vg/kg to about 1.8×10^{12} vg/kg, about 7×10^{10} vg/kg to about 3.5×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 7×10^{12} vg/kg, about 1.8×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 7×10^{12} vg/kg.
- [0647] In some aspects, the first AAV vector genome is administered at a dose of about 5×10^{11} to about 5×10^{12} vg/kg.
- [0648] In some aspects, the first AAV vector genome is administered at a dose of about 1×10^{11} to about 1×10^{12} vg/kg.
- [0649] In some aspects, the second AAV vector genome is administered at a dose of about 2×10^{12} vg/kg to about 5×10^{12} vg/kg, about 2×10^{12} vg/kg to about 3.5×10^{12} vg/kg, or about 3.5×10^{12} vg/kg to about 5×10^{12} vg/kg.
- [0650] In some aspects, the first AAV vector genome is administered at a dose of about 7×10^{10} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 8×10^{10} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 9×10^{10} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 1×10^{11} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 2×10^{11} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of about 3×10^{11} vg/kg and the second AAV vector genome is administered at a dose of about 3.5×10^{12} vg/kg. In some aspects, the first AAV vector genome is administered at a dose of

about 1×10^{14} vg/kg and the second AAV vector genome is administered at a dose of about 5×10^{13} vg/kg.

[0652] In some aspects, the first AAV vector genome is administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the first AAV vector genome is administered at a dose between about 2×10^{12} and about 1×10^{13} vg/kg. In some aspects, the first AAV vector is administered at a dose between about 4×10^{12} and about 1×10^{13} vg/kg.

[0653] In some aspects, the second AAV vector genome is administered at a dose between about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg. In some aspects, the second AAV vector genome is administered at a dose between about 1×10^{12} and about 5×10^{12} vg/kg. In some aspects, the second AAV vector genome is administered at a dose between about 2×10^{12} and about 5×10^{12} vg/kg.

[0654] In some aspects, the total administered dose of the first AAV vector genome comprising the insulin expression cassette comprises about 7×10^{10} to about 5×10^{13} vector genomes (vg)/kg.

[0655] In some aspects, the total administered dose of the second AAV vector genome comprising the glucokinase expression cassette comprises about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg.

[0656] Certain aspects of the disclosure are directed to a method of administering combination therapy comprising a recombinant AAV (rAAV) particle, comprising an AAV capsid and a vector genome comprising the polynucleotide or the expression cassette of the disclosure. Certain aspects of the disclosure are directed to a method of administering a combination therapy comprising a recombinant AAV (rAAV) particle comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein and a rAAV particle comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein. In some aspects, the AAV serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh9, AAV9, AAVrh10, AAV10, AAV11, AAV12, and AAVrh74. In some aspects, the AAV serotype is AAV1.

[0657] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from 1:0.25-0.75, or 1:0.75-1.25. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector

genome comprising a glucokinase expression cassette are administered in a ratio selected from 1:0.4-0.6, or 1:0.9-1.1.

[0658] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from about 1:0.25-0.75, 1:0.25-0.7, 1:0.25-0.65, 1:0.25-0.60, 1:0.25-0.55, or 1:0.25-0.5. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, or 1:0.5-0.75. In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from about 1:0.25-0.75, 1:0.3-0.70, 1:0.35-0.65, 1:0.35-0.75, 1:0.4-0.60, or 1:0.45-0.55.

[0659] In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio selected from 1:0.25-0.75, or 1:0.75-1:1.25 (e.g., about 1:0.5, and about 1:1). In some aspects, the rAAV vector genome comprising an insulin expression cassette and the rAAV vector genome comprising a glucokinase expression cassette are administered in a ratio of about 1:0.5.

[0660] In some aspects, the combination therapy is prepared as a dosage form for administration to a subject by combining a composition comprising a AAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein disclosed herein and a composition comprising a AAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein disclosed herein, wherein each composition is in an amount sufficient to provide a vector ratio selected from the group consisting of 1:0.25-0.75, and 1:0.75-1.25. In some aspects, the vector ratio is selected from 1:0.4-0.6, or 1:0.9-1.1. In some aspects, the ratio is about 1:0.25-0.75, 1:0.3-0.75, 1:0.35-0.75, 1:0.40-0.75, 1:0.45-0.75, 1:0.5-0.75, 1:0.3-0.7, 1:0.3-0.65, 1:0.3-0.6, 1:0.3-0.55, 1:0.3-0.5, 1:0.35-0.7, 1:0.35-0.65, 1:0.35-0.6, 1:0.35-0.55, 1:0.35-0.5, 1:0.4-0.7, 1:0.4-0.65, 1:0.4-0.6, 1:0.4-0.55, 1:0.4-0.5, 1:0.45-0.7, 1:0.45-0.65, 1:0.45-0.6, 1:0.45-0.55, or 1:0.45-0.5.

[0661] In some aspects, the combination therapy is prepared as a dosage form for administration to a subject by combining a composition comprising a AAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human

insulin protein disclosed herein and a composition comprising a AAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein disclosed herein, wherein each composition in an amount sufficient to provide a vector ratio selected from the group consisting of about 1:1 to about 1:15, about 1:1 to about 1:10, about 1:1 to about 1:5, about 1:1 to about 1:4, about 1:1 to about 1:3, about 1:3 to about 1:5, about 1:3 to about 1:10, about 1:3 to about 1:15, about 1:5 to about 1:10, about 1:5 to about 1:15, or about 1:10 to about 1:15.

[0662] In some aspects, the combination therapy is prepared as a dosage form for administration to a subject by combining a composition comprising a AAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein disclosed herein and a composition comprising a AAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein disclosed herein, wherein each composition in an amount sufficient to provide a vector ratio selected from the group consisting of 1:0.75-1.25, 1:0.8-1.25, 1:0.85-1.25, 1:0.9-1.25, 1:0.95-1.25, and 1:1.0-1.25. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.75-1.20, 1:0.75-1.15, 1:0.75-1.10, 1:0.75-1.05, and 1:0.75-1.00. In some aspects, the vector ratio is selected from the group consisting of 1:0.75-1.25, 1:0.80-1.20, 1:0.85-1.15, 1:0.90-1.10, and 1:0.95-1.05. In some aspects, the combination therapy is prepared as a dosage form for administration to a subject by combining a composition comprising a AAV vector comprising an insulin expression cassette comprising a polynucleotide encoding a human insulin protein disclosed herein and a composition comprising a AAV vector comprising a glucokinase expression cassette comprising a polynucleotide encoding a human glucokinase protein disclosed herein, wherein each composition in an amount sufficient to provide a vector ratio of 1:1.

[0663] In some aspects, the vector ratio is about 1:0.5, about 1:2, and about 1:4. In some aspects, the vector ratio is 1:0.4-0.6. In some aspects, the vector ratio is about 1:0.5. In some aspects, the combination therapy comprises a mixture of an AAV vector comprising an insulin expression cassette and an AAV vector comprising a glucokinase expression cassette in the same pharmaceutical formulation (e.g., with a vector ratio of 1:0.25-0.75, e.g., about 1:0.5). In some aspects, the combination therapy comprises an AAV vector comprising an insulin expression cassette and an AAV vector comprising a glucokinase

expression cassette in separate pharmaceutical formulations administered simultaneously or sequentially according to a ratio disclosed herein.

[0664] Certain advantages for the gene therapy methods disclosed herein include the potential for administration of the polynucleotides, the expression cassettes, the delivery vectors, or expression constructs disclosed herein that provides the therapeutic gene expression through the lifetime of the diabetic subject. WO 2012/007458 discloses the generation of two viral vectors, one expressing the insulin gene and one expressing the glucokinase gene as a treatment of diabetes. Furthermore, WO 2016/110518 discloses single-vector gene constructs comprising insulin and glucokinase genes. In certain aspects, the present disclosure provides improved nucleic acid sequences, expression constructs, and/or delivery vectors for diabetes treatment or prevention having increased expression of insulin and glucokinase, decreased adverse immune reaction, and/or allowing for administration of a lower dose of viral vector.

[0665] In some aspects, the methods of the disclosure alleviates or reduces one or more symptom(s) of diabetes in an individual, in a cell, tissue or organ of said individual or alleviates or reduces one or more characteristic(s) or symptom(s) of a cell, tissue or organ of said individual, the method comprising administering to said individual one or more of the polynucleotides, expression cassettes, vectors, or expression constructs disclosed herein.

[0666] Treatment recommendations for adults with diabetes generally target a HbA1c <7.0% without significant hypoglycemia. In some aspects, the 'normal range' for HbA1c is <7.0%, e.g., <6.5%, <6.0%, <5.7%, e.g., between about 5.0% and about 6.5%. Most marketed products lower HbA1c between 0.5% and 1.50%. In some aspects, the methods of the disclosure normalize HbA1c levels in a treated diabetic subject to HbA1c levels in a non-diabetic subject, e.g., within 8 weeks. In some aspects, the methods of the disclosure allow for reduction and/or regulation of glycated blood hemoglobin (HbA1c) levels in the subject. In some aspects, the HbA1c levels in a subject after treatment are <7.0% (e.g., <6.5%, <6.0%, <5.7%, e.g., between 5.0% and 6.5%), e.g., within 8 weeks after treatment.

[0667] Insulin plays a central role in the regulation of lipid metabolism in liver, adipose, and gut (Vergès B. Insulin sensitivity and lipids. *Diabetes Metab.* 2001 Apr;27(2 Pt 2):223-7. PMID: 11452214.). In uncontrolled Type 1 diabetes, patients are unable to utilize glucose, requiring an alternative fuel source. In adipose tissue, insulin inhibits

hormone-sensitive lipase normally promoting storage of triglycerides in the adipocytes and reducing release of free fatty acids from adipose tissue in the circulation. When circulating insulin levels are low, there is a profound reduction of lipoprotein catabolism (Taskinen MR. Lipoprotein lipase in diabetes. *Diabetes Metab Rev.* 3:551-570. 1987 doi: 10.1002/dmr.5610030208. 1987). Lipolysis, resulting in an increased level of circulating triglyceride-rich lipoproteins (chylomicrons, VLDLs), occurs leading to hypertriglyceridemia. In some aspects, the methods of the disclosure reduce the level of a triglyceride-rich lipoprotein (e.g., chylomicrons or VLDLs) in a subject (e.g., a subject suffering from diabetes), in a cell, tissue or organ of said subject, the method comprising administering to the subject one or more of the polynucleotides, expression cassettes, vectors, or expression constructs disclosed herein.

[0668] In the liver, ketone bodies (β -hydroxybutyrate (β -HB) and acetoacetate (AcAc)) are produced by the β -oxidation of fatty acids. During fasting or dietary carbohydrate restriction, ketones serve as a source of alternative energy in glucose limiting conditions and can provide up to 80% of the brain's energy requirements. While useful in the short term, a chronic elevation of circulating ketones can produce unwanted effects in the brain, kidney, liver, and microvasculature (Kanikarla-Marie P, Jain SK. Hyperketonemia and ketosis increase the risk of complications in type 1 diabetes. *Free Radic Biol Med.* 95:268-277, 2016. doi:10.1016/j.freeradbiomed.2016.03.020) and a resulting ketoacidosis which can be fatal. In some aspects, the methods of the disclosure reduce the level of a ketones in a subject (e.g., a subject suffering from diabetes), in a cell, tissue or organ of said subject, the method comprising administering to the subject one or more of the polynucleotides, expression cassettes, vectors, or expression constructs disclosed herein

[0669] In some aspects, the methods of the disclosure provide (i) reduction and/or regulation of glycated blood hemoglobin (HbA1c) levels in the subject; (ii) reduction in circulating ketones in the subject, (iii) reduction in triglycerides in the subject, (iv) reduction in exogenous insulin in the subject, (v) increased circulating C-peptide levels in the subject, (vi) reduction in immune response to exogenous AAV, insulin protein, and/or glucokinase protein, and/or (vii) any combination thereof.

[0670] In some aspects, the methods of the disclosure provide reduction and/or regulation of glycated blood hemoglobin (HbA1c) levels in the subject. In some aspects, the HbA1c

levels in a subject after treatment are <7.0% (e.g., <6.5%, <6.0%, <5.7%, e.g., between 5.0% and 6.5%), e.g., within 8 weeks after treatment.

[0671] In some aspects, the methods of the disclosure provide reduction in exogenous insulin in the subject. In some aspects, the AAV vector genome, expression cassette, or polynucleotide encoding human insulin has a dosage that produces between about 1 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 4 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 7 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 8 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 9 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 6 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 5 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 6 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, or about 6 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 1 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 10 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 15 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 20 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 25 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 30 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 35 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 20 $\mu\text{U/mL}$ to about 30 $\mu\text{U/mL}$, about 10 $\mu\text{U/mL}$ to about 30 $\mu\text{U/mL}$, or about 10 $\mu\text{U/mL}$ to about 20 $\mu\text{U/mL}$ of insulin in the serum of a subject.

[0672] In some aspects, the methods of the disclosure provide reduction and/or regulation of blood glucose in the subject. In some aspects, the methods of disclosure do not reduce blood glucose below 70 mg/dL.

[0673] In some aspects, the method or use is performed *in vitro*, for instance using a cell culture. In some aspects, the method or use is performed *in vivo*. In some aspects, a polynucleotide, an expression cassette, a delivery vectors, or expression construct disclosed herein is combined with an additional compound known to be used for treating diabetes in an individual. In some aspects, a polynucleotide, an expression cassette, a delivery vectors, or expression construct disclosed herein is combined with an immunosuppressive agent. In some aspects, the method further comprises administering recombinant insulin, e.g., via regular injections.

[0674] In some aspects, the method disclosed herein is not repeated. In some aspects, the method disclosed herein is repeated each year or each 2, 3, 4, 5, 6, 7, 8, 9, or 10 years.

- [0675] In some aspects, the method comprises administering a therapeutically effective dose of the combination therapy comprising the polynucleotides, expression constructs, the vectors, or the compositions as described herein, wherein the administration is a single, e.g., avoiding repeated periodical administration. In some aspects, the single dose is administered to muscle tissue. In some aspects, the single dose is administered to skeletal muscle tissue. In some aspects, the single dose comprise multiple injections (e.g., two, three, four, or five) to one or more muscles (e.g., multiple skeletal muscle groups). In some aspects, the single dose comprises a total of 20-150 injections of the combination therapy to the at least two different skeletal muscle groups. In some aspects, the single dose is administered to the quadriceps, biceps, and hamstrings bilaterally. In some aspects, each of the quadriceps, biceps, and hamstrings receive multiple injections bilaterally. In some aspects, the single dose comprises at least two of 8-50 IM injections to the quadriceps (e.g., 4-25 IM injections bilaterally to each quadriceps), 8-50 IM injections to the biceps (e.g., 4-25 IM injections bilaterally to each bicep), and 8-50 IM injections to the hamstrings (e.g., 4-25 IM injections bilaterally to each hamstring).
- [0676] In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (e.g., an IL-6 antagonist) prior to administering the combination therapy. In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (e.g., a calcineurin inhibitor) after administering the combination therapy. In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (e.g., an IL-6 antagonist) prior to administering the combination therapy and administering an immunosuppressive agent (e.g., a calcineurin inhibitor) after administering the combination therapy.
- [0677] In some aspects, an immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 14 days (e.g., 1 to 2 days) prior to administering the combination therapy. In some aspects, an immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 2 days) prior to administering the combination therapy.
- [0678] In some aspects, an immunosuppressive agent (e.g., a calcineurin inhibitor) is administered starting at 1, 2, 3, or 4 weeks (e.g., 3 weeks) after administering the combination therapy.
- [0679] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 2 days) prior to administering the combination therapy

and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered starting at 1, 2, 3, or 4 weeks (e.g., 3 weeks) after administering the combination therapy.

- [0680] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy.
- [0681] In some aspects, the first immunosuppressive agent is administered once every one week, two weeks, three weeks, or four weeks.
- [0682] In some aspects, the first immunosuppressive agent is administered for about 3 months, about 4 months, about 5 months, or about 6 months.
- [0683] In some aspects, the second immunosuppressive agent is administered once or twice daily for 4 to 52 weeks.
- [0684] In some aspects, the second immunosuppressive agent is administered once daily for 4 to 24 weeks, 4 to 16 weeks, or 4 to 8 weeks.
- [0685] In some aspects, the second immunosuppressive agent is administered twice daily for 4 to 24 weeks, 4 to 16 weeks, or 4 to 8 weeks.
- [0686] In some aspects, the second immunosuppressive agent is administered twice daily for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some aspects, the second immunosuppressive agent is administered twice daily for about 1 to about 6 months, about 2 months to about 6 months, about 3 months to about 6 months, about 4 months to about 6 months, about 5 months to about 6 months, about 2 months to about 4 months, or about 3 months to about 4 months.
- [0687] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, and levilimab. In some aspects, the IL-6 antagonist is tocilizumab.
- [0688] In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, and voclosporin. In some aspects, the calcineurin inhibitor is tacrolimus.
- [0689] In some aspects, the second immunosuppressive agent is selected from the group consisting of thymoglobulin, etanercept, basiliximab, tacrolimus, sirolimus, and mycophenolate mofetil.
- [0690] In some aspects, the combination therapy is accompanied by an immunosuppressive regimen (e.g., administration of an IL-6 antagonist such as

tocilizumab prior to the gene therapy administration and/or administration of a calcineurin inhibitor such as tacrolimus for a time period after the gene therapy administration) to accompany the gene therapy methods comprising intramuscular administration of a combination therapy comprising a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), optionally, wherein the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least two different skeletal muscle groups. In some aspects, the different skeletal muscle groups are selected from a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), a latissimus dorsi (lat), and any combination thereof. In some aspects, the at least two different skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, or any combination thereof. In some aspects, the at least two different skeletal muscle groups are selected from a quadriceps, a bicep, a hamstring, or any combination thereof. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0691] In some aspects, the different skeletal muscle group is selected from i) a left quadriceps and a right quadriceps, ii) a left bicep and a right bicep, iii) a left triceps and a right triceps, iv) a left gastrocnemius and a right gastrocnemius, v) a left gluteus maximus and a right gluteus maximus, vi) a left hamstring and a right hamstring, vii) a left deltoid and a right deltoid, viii) a left trapezius and a right trapezius, ix) a left pectoral muscle and a right pectoral muscle, and x) a left latissimus dorsi and a right latissimus dorsi.

[0692] In some aspects, the first and second vector genomes are combined in a single intramuscular formulation, which is administered to at least three different skeletal muscle groups comprising a quadriceps, a bicep, a triceps, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle (e.g., pectoralis major), and a latissimus dorsi (lat), or any combination thereof. In some aspects, the at least three different skeletal muscle groups are selected from a quadriceps, a bicep, a gastrocnemius (e.g., a calf muscle), a gluteus maximus, and a hamstring, or any

combination thereof. In some aspects, the at least three different skeletal muscle groups are selected from a quadriceps, a bicep, and a hamstring, or any combination thereof. In some aspects, the administration comprises bilateral injections into one or more of the skeletal muscle groups (e.g., IM injections into the right and left quadriceps, the right and left biceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings).

[0693] In some aspects, combination therapy comprise a first AAV vector genome comprising an insulin expression cassette and a second AAV vector genome comprising an glucokinase expression cassette in a vector ratio of about 1:0.4-0.6 (e.g., about 1:0.5), wherein the first AAV vector genome, the second AAV vector genome, or both the first AAV vector genome and second AAV vector genome are administered intramuscularly (IM) at a dose of about 1×10^{12} to about 1×10^{14} vg/kg (e.g., about 1×10^{13} to about 1×10^{14} vg/kg).

[0694] Certain aspects of the disclosure are directed to a dosing regimen comprising:

- (a) a first immunosuppressive agent;
- (b) a combination gene therapy administered intramuscularly after (a) which comprises (i) a first recombinant AAV (rAAV) particle comprising a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AA V (rAAV) particle comprising a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs); and
- (c) a second immunosuppressive agent administered after (b).

[0695] In some aspects, the combination therapy is administered intramuscularly (IM) at least two different skeletal muscle groups. In some aspects, the combination therapy is administered intramuscularly (IM) at least three different skeletal muscle groups.

[0696] In some aspects, the first immunosuppressive agent comprises an IL-6 antagonist selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, and any combination thereof.

- [0697] In some aspects, the second immunosuppressive agent is a calcineurin inhibitor selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof.
- [0698] In some aspects, the first immunosuppressive agent comprises tocilizumab and the second immunosuppressive agent comprises tacrolimus.
- [0699] In some aspects, the dosing regimen comprises administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the combination therapy.
- [0700] In some aspects, the immunosuppressive regimen comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the combination therapy.
- [0701] In some aspects, the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition.
- [0702] In some aspects, the immunosuppressive regimen comprises administering one or more immunosuppressive agents prior to (pre-AAV), at the same time as, and/or after (post-AAV) administering the combination therapy.
- [0703] In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (pre-AAV) prior to administering the combination therapy.
- [0704] In some aspects, the immunosuppressive regimen comprises administering an immunosuppressive agent (post-AAV) after administering the combination therapy.
- [0705] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy. In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
- [0706] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 2 days prior to administering the combination therapy.
- [0707] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.
- [0708] In some aspects, the pre-AAV immunosuppressive agent is administered 1 to 3 days prior to administering the AAV gene therapy.
- [0709] In some aspects, the pre-AAV immunosuppressive agent is administered 3 to 7 days prior to administering the AAV gene therapy.

- [0710] In some aspects, the post-AAV immunosuppressive agent is initially administered 1, 2, 3, or 4 weeks after administering the combination therapy.
- [0711] In some aspects, the post-AAV immunosuppressive agent is further administered for 1, 2, 3, or 4 weeks after administering the AAV gene therapy.
- [0712] In some aspects, post-AAV immunosuppressive agent is administered once about every 1-3 weeks, optionally once about every 1-2 weeks after administering the combination therapy.
- [0713] In some aspects, the post-AAV immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the AAV gene therapy.
- [0714] In some aspects, the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent is administered for about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.
- [0715] In some aspects, the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, a calcineurin inhibitor, or any combination thereof.
- [0716] In some aspects, the pre-AAV immunosuppressive agent and/or post-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, MMF, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.
- [0717] In some aspects, the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.
- [0718] In some aspects, the method comprises administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the AAV gene therapy.
- [0719] In some aspects, the method comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the AAV gene therapy.
- [0720] In some aspects, the immunosuppressive agent is initially administered about 3 weeks after administering the combination therapy. In some aspects, after the initial

administration, the post-AAV immunosuppressive agent is administered 2 times per day, daily, or every other day. In some aspects, after the initial administration, the post-AAV immunosuppressive agent is administered for about 6 to 40, about 8 to 40, about 10 to 40, about 12 to 40, about 6 to 30, about 8 to 30, about 10 to 30, about 12 to 30, about 6 to 20, about 8 to 20, about 10 to 20, or about 12 to 20 weeks.

[0721] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 2 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered starting at 1, 2, 3, or 4 weeks (e.g., 3 weeks) after administering the combination therapy.

[0722] In some aspects, a first immunosuppressive agent (e.g., an IL-6 antagonist) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy and a second immunosuppressive agent (e.g., a calcineurin inhibitor) is administered 1 to 7 days (e.g., 1 to 3 days) prior to administering the combination therapy.

[0723] In some aspects, the second immunosuppressive agent is administered 3 to 7 days prior to administering the AAV gene therapy.

[0724] In some aspects, the first immunosuppressive agent is administered once every one week, two weeks, three weeks, or four weeks.

[0725] In some aspects, the first immunosuppressive agent is administered for about 3 months, about 4 months, about 5 months, or about 6 months.

[0726] In some aspects, the second immunosuppressive agent is administered twice daily for 4 to 24 weeks, 4 to 16 weeks, or 4 to 8 weeks.

[0727] In some aspects, the second immunosuppressive agent is administered twice daily for about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months. In some aspects, the second immunosuppressive agent is administered twice daily for about 1 to about 6 months, about 2 months to about 6 months, about 3 months to about 6 months, about 4 months to about 6 months, about 5 months to about 6 months, about 2 months to about 4 months, or about 3 months to about 4 months.

[0728] In some aspects, the second immunosuppressive agent is administered once or twice daily for 4 to 52 weeks.

[0729] In some aspects, the second immunosuppressive agent is administered once daily for 4 to 24 weeks, 4 to 16 weeks, or 4 to 8 weeks.

[0730] In some aspects, the immunosuppressive regimen comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate

mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, and/or a calcineurin inhibitor. In some aspects, the immunosuppressive regimen comprises an IL-6 antagonist and/or a calcineurin inhibitor.

[0731] In some aspects, the pre-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, mycophenolate mofetil (MMF), a tyk2 inhibitor, rapamycin, or abatacept. In some aspects, the pre-AAV immunosuppressive agent comprises an IL-6 antagonist.

[0732] In some aspects, the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, mycophenolate mofetil (MMF), methotrexate, a tyk2 inhibitor, rapamycin, or abatacept. In some aspects, the post-AAV immunosuppressive agent comprises a calcineurin inhibitor.

[0733] In some aspects, the post-AAV immunosuppressive agent comprises thymoglobulin, etanercept, basiliximab, tacrolimus, sirolimus, and mycophenolate mofetil.

[0734] In some aspects, the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof. In some aspects, the IL-6 antagonist is tocilizumab.

[0735] In some aspects, the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof. In some aspects, the calcineurin inhibitor is tacrolimus.

[0736] In some aspects, tacrolimus is administered orally to the subject. In some aspects, tacrolimus is administered orally at a dose of 0.1 mg/kg/day beginning on 3 days prior to AAV administration and ending 12 weeks after AAV administration. In some aspects, tacrolimus trough levels are monitored. In some aspects, the target trough levels are between about 5 ng/mL to about 15 ng/mL. In some aspects, the target trough levels are between about 10 ng/mL to about 15 ng/mL. In some aspects, the target trough levels are between about 5 ng/mL to about 10 ng/mL. In some aspects, the target trough levels is about 5 ng/mL, about 6 ng/mL, about 7 ng/mL, about 8 ng/mL, about 9 ng/mL, about 10 ng/mL, about 11 ng/mL, about 12 ng/mL, about 13 ng/mL, about 14 ng/mL or about 15 ng/mL.

- [0737] In some aspects, tocilizumab is administered subcutaneously. In some aspects, tocilizumab is subcutaneously administration at a dose of 162 mg every other week for patients less than 100 kg in weight. In some aspects, tocilizumab is subcutaneously administration at a dose of 162 mg every week for patients greater than 100 kg in weight. In some aspects, administration of tocilizumab begins 3 days prior to AAV administration and ends 12 weeks after AAV administration.
- [0738] In some aspects, the first AAV vector genome and second AAV vector genome are each administered at a dose between about 1×10^{13} to about 1×10^{14} vector genomes (vg)/kg.
- [0739] In some aspects, the first AAV vector genome and the second AAV vector genome are administered at a ratio selected of 1:0.25-0.75. In some aspects, the ratio is 1:0.4-0.6. In some aspects, the ratio is 1:0.45-0.55. In some aspects, the ratio is about 1:0.5.
- [0740] In some aspects, the polynucleotide encoding the hIns protein comprises an open reading frame (ORF) comprising: a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to any one of: (i) nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, or nucleic acids 88-345 of any of SEQ ID NOs: 117-122, 152 or 156; or (ii) SEQ ID NO: 43-57, or SEQ ID NO: 110-122; and/or the polynucleotide encoding the human glucokinase hGck protein comprises an ORF comprising (i) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of (a) nucleic acids 1-1398 of any of SEQ ID NO: 61-80 or 162; or (ii) SEQ ID NO: 61-80 and 162.
- [0741] In some aspects, the polynucleotide encoding the hIns protein comprises an open reading frame (ORF) comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 121; and/or the polynucleotide encoding the human glucokinase hGck protein comprises an ORF comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 68.
- [0742] In some aspects, the hIns protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, amino acids 25-110 of SEQ ID NO: 145, SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145.
- [0743] In some aspects, the hIns protein comprises a signal peptide. In some aspects, the signal peptide is a wild-type preproinsulin signal sequence, an IL-6 signal sequence, a fibronectin signal sequence, or a non-wild-type preproinsulin signal sequence. In some

aspects, the signal peptide comprises amino acids 1-24 of SEQ ID NO: 41, amino acids 1-24 of SEQ ID NO: 144, or amino acids 1-24 of SEQ ID NO: 145. In some aspects, the hIns protein further comprises a cleavage site.

[0744] In some aspects, the polynucleotide encoding the hIns protein further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.

[0745] In some aspects, the polynucleotide encoding the hIns protein further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, or SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171.

[0746] In some aspects, the encoded hGck protein comprises the amino acid sequence of SEQ ID NO: 82.

[0747] In some aspects, the polynucleotide encoding the hGck protein further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.

[0748] In some aspects, the polynucleotide encoding the hGck protein further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171.

[0749] In some aspects, the first promoter is a eukaryotic promoter. In some aspects, the first promoter is a CMV promoter. In some aspects, the second promoter is a eukaryotic promoter. In some aspects, the second promoter is a CMV promoter.

[0750] In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a eukaryotic promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a constitutive promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a ubiquitous promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a CMV promoter. In some aspects, the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a muscle specific promoter.

- [0751] In some aspects, the insulin expression cassette and/or the glucokinase expression cassette further comprises a polyadenylation (polyA) element.
- [0752] In some aspects, a first recombinant AAV (rAAV) particle comprises the first AAV vector genome. In some aspects, a second recombinant AAV (rAAV) particle comprises the second vector genome. In some aspects, the AAV serotype of the first rAAV particle and/or the second rAAV particle is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh9, AAV9, AAVrh10, AAV10, AAV11, AAV12, and AAVrh74. In some aspects, the AAV serotype of the first rAAV particle and/or the second rAAV particle is AAV1.
- [0753] In some aspects, the first rAAV particle and the second rAAV particle are formulated in a single composition.
- [0754] In some aspects, the first rAAV particle and the second rAAV particle are administered simultaneously or sequentially.
- [0755] In some aspects, the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM). In some aspects, the diabetes is diabetes mellitus type 1 (T1DM). In some aspects, the diabetes is diabetes mellitus type 2 (T2DM).
- [0756] In some aspects, within 15 days-6 months, 30-120 days, or 30-60 days after administration of the combination therapy: (i) glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in the subject; (ii) circulating ketones are reduced in the subject, (iii) triglycerides are reduced in the subject, (iv) exogenous insulin is reduced in the subject, (v) circulating C-peptide levels are increased in the subject, or (vi) any combination thereof.
- [0757] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's circulating insulin level in the blood, when measured in the absence of exsanguinous insulin treatment, is about 1-40 $\mu\text{U/mL}$, optionally about 5-40 $\mu\text{U/mL}$, about 5-25 $\mu\text{U/mL}$, about 5-10 $\mu\text{U/mL}$, about 10-20 $\mu\text{U/mL}$, about 10-15 $\mu\text{U/mL}$, about 15-20 $\mu\text{U/mL}$, or about 15-25 $\mu\text{U/mL}$.
- [0758] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's HbA1C level is reduced by about 0.5 to 1.5% compared to pre-treatment.
- [0759] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's HbA1C level is less than

about 7.0%, optionally less than about 6.5%, about 6.0%, about 5.7%, optionally about 5.0% to 6.5%.

[0760] In some aspects, within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's circulating glucose in the blood is at least about 70 to 140 mg/dL.

[0761] In some aspects, after administration of the combination therapy, glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in the subject. In some aspects, the AAV vector genome, expression cassette, or pharmaceutical composition has a dosage that produces HbA1c levels in a subject after treatment <7.0% (e.g., <6.5%, <6.0%, <5.7%, e.g., between 5.0% and 6.5%), e.g., within 8 weeks after treatment.

[0762] In some aspects, after administration of the combination therapy, exogenous insulin is reduced in the subject. In some aspects, the AAV vector genome, expression cassette, or pharmaceutical composition has a dosage that produces between about 1 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 4 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 7 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 8 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 9 $\mu\text{U/mL}$ to about 10 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 6 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 5 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 6 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 9 $\mu\text{U/mL}$, about 6 $\mu\text{U/mL}$ to about 8 $\mu\text{U/mL}$, or about 6 $\mu\text{U/mL}$ to about 7 $\mu\text{U/mL}$, about 1 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 3 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 5 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 10 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 15 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 20 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 25 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 30 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 35 $\mu\text{U/mL}$ to about 40 $\mu\text{U/mL}$, about 20 $\mu\text{U/mL}$ to about 30 $\mu\text{U/mL}$, about 10 $\mu\text{U/mL}$ to about 30 $\mu\text{U/mL}$, or about 10 $\mu\text{U/mL}$ to about 20 $\mu\text{U/mL}$ of insulin in the serum of a subject (e.g., 30 days after administration), thereby reducing reliance on exogenous insulin.

[0763] In some aspects, the AAV vector genome, expression cassette, or pharmaceutical composition has a dosage that produces between about 1 ng/mg to about 50 ng/mg, about 1 ng/mg to about 40 ng/mg, about 1 ng/mg to about 30 ng/mg, about 1 ng/mg to about 20 ng/mg, about 1 ng/mg to about 10 ng/mg, about 1 ng/mg to about 5 ng/mg, about 5 ng/mg to about 10 ng/mg, about 5 ng/mg to about 20 ng/mg, about 5 ng/mg to about 30

ng/mg, about 5 ng/mg to about 40 ng/mg, about 5 ng/mg to about 50 ng/mg, about 10 ng/mg to about 50 ng/mg, about 20 ng/mg to about 50 ng/mg, about 30 ng/mg to about 50 ng/mg, about 40 ng/mg to about 50 ng/mg, about 20 ng/mg to about 40 ng/mg, or about 25 ng/mg to about 50 ng/mg of glucokinase in a muscle tissue sample taken at the site of injection in a subject (e.g., 30 days after administration).

[0764] In some aspects, the AAV vector genome, expression cassette, or pharmaceutical composition has a dosage that produces about 1 ng/mg, about 5 ng/mg, about 10 ng/mg, about 20 ng/mg, about 25 ng/mg, about 30 ng/mg, about 40 ng/mg, or about 50 ng/mg of glucokinase in a muscle tissue sample taken at the site of injection in a subject (e.g., 30 days after administration).

[0765] In some aspects, after administration of the combination therapy, blood glucose is reduced in the subject (e.g., about 30 days after administration of the combination therapy). In some aspects, blood glucose is reduced to between about 70 mg/dL to about 140 mg/dL, about 70 mg/dL to about 130 mg/dL, about 70 mg/dL to about 120 mg/dL, about 70 mg/dL to about 110 mg/dL, about 70 mg/dL to about 100 mg/dL, about 70 mg/dL to about 90 mg/dL, about 70 mg/dL to about 80 mg/dL, about 80 mg/dL to about 140 mg/dL, about 90 mg/dL to about 140 mg/dL, about 100 mg/dL to about 140 mg/dL, about 110 mg/dL to about 140 mg/dL, about 120 mg/dL to about 140 mg/dL, about 130 mg/dL to about 140 mg/dL, about 80 mg/dL to about 120 mg/dL, about 90 mg/dL to about 130 mg/dL, or about 75 mg/dL to about 134 mg/dL.

[0766] In some aspects, blood glucose is reduced to about 70 mg/dL, about 80 mg/dL, about 90 mg/dL, about 100 mg/dL, about 110 mg/dL, about 120 mg/dL, about 130 mg/dL, or about 140 mg/dL.

[0767] In some aspects, blood glucose is reduced to at least 70 mg/dL, at least 80 mg/dL, at least 90 mg/dL, at least 100 mg/dL, at least 110 mg/dL, at least 120 mg/dL, at least 130 mg/dL, or at least 140 mg/dL.

[0768] In some aspects, the AAV vector genome, expression cassette, or pharmaceutical composition has a dosage that produces blood glucose is not reduced below 70 mg/dL (e.g., 30 days after administration).

[0769] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections may set forth one or more but not all exemplary embodiments of the

present invention as contemplated by the inventor(s), and thus, are not intended to limit the present invention and the appended claims in any way.

[0770] The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined in accordance with the following claims and their equivalents.

[0771] Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLES

Example 1: Insulin Nucleic Acids

[0772] The following human insulin nucleic acid sequences (shown in **Table 8**) corresponding to SEQ ID Nos: 1-16, 84-88, 123, 127-129, 133-138, 160-161, and 170 were designed *in silico*. 5' UTR sequences (SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148) are in BOLD, ORF sequences are underlined (SEQ ID NOs: 43-57, 110-122), and 3' UTR sequences are italicized (SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171). The 5' UTRs having the sequence of SEQ ID NO: 42 was further modified to remove the CTAG at positions 1-4. Accordingly, in certain constructs the 5' UTR included nucleic acids 5-329 of SEQ ID NO: 42.

[0773] In some aspects, the nucleic acids include codon optimized and CpG reduced sequences relative to wild-type and/or unmodified human insulin nucleic acid sequences (e.g., SEQ ID NO: 1). The modified nucleic acids were chemically synthesized, prepared in expression cassettes including a CMV promoter, and cloned into an expression plasmid. The modified sequences were confirmed by Sanger sequencing.

Example 2: Gck Nucleic Acids

[0774] The following human glucokinase (Gck) nucleic acid sequences (shown in **Table 9**) corresponding to SEQ ID NOs: 20-39 89-96, 163-164, 168, and 172 were designed *in silico*. 5' UTR sequences (SEQ ID NO: 42 or SEQ ID NO: 83) are in BOLD, ORF sequences are underlined (SEQ ID NOs: 61-80 and 162), and 3' UTR are italicized (SEQ ID NO: 60, 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: 149, or SEQ ID NO: 171). The 5' UTRs having the sequence of SEQ ID NO: 42 was further modified to remove the CTAG at positions 1-4. Accordingly, in certain constructs the 5' UTR included nucleic acids 5-329 of SEQ ID NO: 42.

[0775] In some aspects, the nucleic acids include codon optimized and CpG reduced sequences relative to wild-type and/or unmodified human Gck nucleic acid sequences (e.g., SEQ ID NO: 19). The modified nucleic acids were chemically synthesized, prepared in expression cassettes including a CMV promoter, and cloned into an expression plasmid. The modified sequences were confirmed by Sanger sequencing.

Example 3: *In Vivo* Evaluation of Insulin and Glucokinase Combination Therapy in Non-Human Primates

[0776] The efficacy of the AAV1-hIns and AAV1-hGck vectors in a streptozotocin (STZ)-induced non-human primate (NHP) model of Type 1 diabetes was assessed. Type 1 diabetes was induced using STZ in non-human primates (cynomolgus macaques) that tested seronegative for AAV1 neutralizing antibodies. Pharmaceutical-grade STZ was infused via a surgically implanted vascular access port based on body weight (BW) (100 mg/kg). STZ was administered immediately after preparation to avoid decomposition. (Graham ML, Mutch LA, Kittredge JA, Rieke EF, Robinson NA, Zolondek EK, Faig AW, Dufour TA, Munson JW, Schuurman HJ. Management of adverse side-effects after chemotherapy in macaques as exemplified by streptozotocin: case studies and recommendations. Lab Anim. 46(3):178-92 (July 2012). doi: 10.1258/la.2012.011077. Epub 2012 May 10. PMID: 22577153.)

[0777] The diabetic state was confirmed by a fasting C-peptide below 0.5 ng/mL or the absence of a stimulated C-peptide response during an intravenous glucose tolerance test (IVGTT), in combination with persistent hyperglycemia (blood glucose (BG) > 200 mg/dL) prior to the initiation of exogenous insulin. Diabetes induction was only considered successful if the diabetic state was documented, and there were no complications that prevented enrollment onto the study.

[0778] After STZ infusion, animals were monitored at least twice daily for BG, appetite, urine or stool type and frequency, and general appearance. Long-acting and short-acting insulin (glargine; Lantus, Sanofi-Aventis, Bridgewater, NJ, USA and lispro; Humalog, Eli Lilly, Indianapolis, IN, USA, respectively) was initiated after three consecutive BG

readings exceeding 300 mg/dL or after 48 h, whichever occurred first, and given twice daily per sliding scale targeting BG levels of 100–200 mg/dL.

[0779] Throughout the experimental period, the animals were regularly assessed for BW, blood hematological parameters (white blood cell counts [WBC], hemoglobin [Hb], hematocrit, platelet counts and lymphocyte counts) and serum chemistry (creatinine, blood urea nitrogen [BUN], alanine aminotransferase [ALT], alkaline phosphatase [ALP], aspartate aminotransferase [AST], bilirubin, albumin, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol and triglycerides). The incidence of relevant STZ-induced clinical or laboratory AEs is low, and careful monitoring with supportive intervention usually results in recovery from any AEs. Following diabetes induction, animals were treated with AAV1-hIns and AAV1-hGck vectors intramuscularly (IM). Two cynomolgus macaques (NHP1 and NHP2) were dosed with a 1:0.5 ratio of AAV1-hIns:AAV1-hGck (admixed). Each animal received 28 injections (100 μ L each) distributed bilaterally to the quadriceps (8 injections per side) and biceps (6 injections per side). Injection sites were spaced approximately 1 cm apart. Each animal received a total dose of $4.8\text{E}+12$ vg/kg (vg = vector genome) ($3.6\text{E}+12$ vg/kg as measured by the gene of interest) of AAV1-hIns and $2.4\text{E}+12$ vg/kg ($1.8\text{E}+12$ vg/kg as measured by the gene of interest) of AAV1-hGck.

[0780] A third cynomolgus macaque (NHP3) was dosed with a 1:0.5 ratio of AAV1-hIns:AAV1-hGck (admixed), and received 28 injections (100 μ L each) distributed bilaterally to the quadriceps (6 injections per side), biceps (4 injections per side), and hamstrings (4 injections per side) with a total dose of $4.8\text{E}+12$ vg/kg ($3.6\text{E}+12$ vg/kg as measured by the gene of interest) of AAV1-hIns and $2.4\text{E}+12$ vg/kg ($1.8\text{E}+12$ vg/kg as measured by the gene of interest) of AAV1-hGck. The dosing regimen is shown in **Table 1**.

[0781] A fourth cynomolgus macaque (NHP4) was dosed with a 1:0.5 ratio of AAV1-hIns:AAV1-hGck (admixed), and received 56 injections (100 μ L each) distributed bilaterally to the quadriceps (12 injections per side), biceps (Biceps Brachii) (8 injections per side), and hamstrings (8 injections per side) with a total dose of $7.1\text{E}+12$ vg/kg of AAV1-hIns and $3.2\text{E}+12$ vg/kg of AAV1-hGck.

Table 1. AAV1-hIns and AAV1-hGck Dosing Regimen

NHP	Dose	Injections	Muscle groups (# injections per side)	Immune suppression regimen	Necropsy Day Post-Dose
1	3.6E12 vg/kg AAV1-hINS 1.8E12 vg/kg AAV1-hGCK	100 µL x 28	Quadriceps (8) Bicep (6)	None	182
2	3.6E12 vg/kg AAV1-hINS 1.8E12 vg/kg AAV1-hGCK	100 µL x 28	Quadriceps (8) Bicep (6)	None	132
3	3.6E12 vg/kg AAV1-hINS 1.8E12 vg/kg AAV1-hGCK	100 µL x 28	Quadriceps (6) Bicep (4) Hamstring (4)	None	91
4	7.1E12 vg/kg AAV1-hINS 3.2E12 vg/kg AAV1-hGCK	100 µL x 56	Quadriceps (12) Bicep (8) Hamstring (8)	None	91

3.1 Blood Glucose and Exogenous Insulin Requirements

[0782] Pre-prandial and post-prandial capillary blood glucose was monitored daily in all study animals. Insulin was administered to STZ-induced diabetic animals prior to and following administration of AAV1-hIns and AAV1-hGck as needed. For most diabetic NHPs, fasting blood sugars of 40-150 mg/dL and less than 200 mg/dL were targeted. If a diabetic NHP had hypoglycemic risk factors (social stress, injury, recent or recurrent hypoglycemia, etc.), then goal fasting glucose values of 60-180 mg/dL and less than 250 mg/dL were targeted.

[0783] Daily measurements of pre- and post-prandial blood glucose (mg/dL) and the amount of exogenous insulin administered (U/Kg/day) for NHP1-NHP4 are shown in FIGs. 1A, 2A, 3A, and 4A, respectively. Baseline insulin levels were calculated as the average amounts of insulin administered in the 30 days prior to AAV treatment.

[0784] NHP1 was evaluated for 6 months post-AAV administration. The amount of exogenous insulin administered was reduced by about 77% from baseline amounts at

~60-90 days after AAV treatment and remained about ~33% lower through 180 days (FIG. 1A).

[0785] NHP2 was evaluated for 4 months post AAV administration. The amount of exogenous insulin administered was reduced by about ~29% from baseline amounts at ~60-90 days after AAV treatment and remained about 17% reduced through 130 days (FIG. 2A).

[0786] NHP3 was evaluated for 3 months post AAV administration. The amount of exogenous insulin administered was reduced by about 19% from baseline amounts at ~30-60 days after AAV treatment and increased to about +8% from 60-90 days after administration (FIG. 3A). Immunological analysis of NHP3 revealed a significant cellular immune response to human insulin pre-AAV treatment which may explain the lack of effect of the AAV treatment.

[0787] NHP4 was evaluated for 3 months post AAV-treatment. The amount of exogenous insulin administered was reduced by about 19% from day 0 to day 30, about 48% from day 30 to day 60, and remained about ~35% lower through days 60-90 after AAV treatment (FIG. 4A). NHP4 achieved a substantial reduction in exogenous insulin until Day 45, followed by a partial rebound resulting in a concurrent increase in administered exogenous insulin.

3.2 Circulating C-peptide, Creatine Kinase, and HbA1C

[0788] Blood samples were drawn prior to STZ treatment, post-STZ treatment, at the time of AAV administration (Study day 0) and then weekly until necropsy, and analyzed for C-peptide levels (FIGs. 1B, 2B, 3B, and 4B). C-peptide is produced during the conversion of preproinsulin to proinsulin to insulin, in a 1:1 molar ratio with expressed insulin, and this serves as a surrogate measurement of insulin expression (where, as here, direct measurement of insulin expression is confounded by treatment with exogenous insulin). The absence of an increase in circulating C-peptide in response to IVGTT after STZ treatment demonstrates that pancreatic beta cells have been ablated by STZ.

[0789] In NHP1, C-peptide levels increased through approximately 105 days after AAV administration and subsequently receded but remained well above baseline (FIG. 1B). In NHP2, C-peptide levels peaked between 60 and 90 days post AAV administration and returned to near baseline after approximately 4 months. (FIG. 2B). In NHP3 C-peptide levels were negligible at any time post AAV administration, consistent with the observed

preexisting immunity to human insulin observed in this animal (FIG. 3B). In NHP4, C-peptide levels were low but above baseline through necropsy at 90 days after AAV administration (FIG. 4B).

[0790] HbA1C levels were analyzed at day 0, 28, 56, 80, 94, 116, 144 and 180 days (NHP1), 0, 28, 56, 70, 92, 120 and 135 days (NHP2), or 0, 28, 45, 56, 70, 80 and 90 days (NHP3 and NHP4) after administration of AAV1-hIns and AAV1-hGck (FIGs. 1C, 2C, 3C, and 4C, respectively). HbA1c is a form of glycated hemoglobin (Hb). HbA1c levels serve as an integrated signal of post-prandial glucose exposure over time, with elevated levels indicating poor glycemic control, which has been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy.

[0791] Creatine kinase levels were measured pre-STZ treatment, on the day of AAV treatment (study day 0) and then weekly until necropsy (FIGs. 1D, 2D, 3D, and 4D). A spike in creatine kinase levels was observed at the terminal measurement for all animals, which is believed to be a consequence of the euthanasia procedure. Creatine kinase levels remained relatively even through all but the last time point in NHP1, NHP2, and NHP3 (FIGs. 1D, 2D, and 3D, respectively). In NHP4, creatine kinase levels rose from about 30-60 days after AAV administration, and then again at necropsy (FIG. 4D).

[0792] In humans, HbA1c is assumed to be a three-month average of blood glucose levels (see, e.g., Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomark Insights*. 2016 Jul 3;11:95-104. doi: 10.4137/BMI.S38440. PMID: 27398023; PMCID: PMC4933534). The lifespan of a red blood cell in cynomolgus NHPs is much less than that of humans and trends can be observed much more rapidly (see, e.g., Glomski, Chester A., Alessandra Pica, and Jessica F. Greene. *Erythrocytes of the rhesus and cynomolgus monkeys*. CRC Press, 2015). In all NHP, HbA1C decreased around 30-60 days after AAV administration and then rose around 90 days after AAV administration. In NHP1, HbA1C levels remained elevated through 180 days after AAV administration (FIG. 1C). In NHP2, HbA1C levels remained elevated through 130 days after AAV administration (FIG. 2C).

3.3 Weight

[0793] Body weight was measured weekly after administration of AAV1-hIns and AAV1-hGck in NHP1-NHP4 (FIGs. 1E, 2E, 3E, and 4E, respectively). All animals

showed a slight increase in weight throughout the last time point consistent with the anabolic effects of insulin.

3.4 Intravenous Glucose Tolerance Test

- [0794] The intravenous glucose tolerance test (IVGTT) is used more often than oral glucose tolerance test (OGTTs) in NHPs, since most monkeys are not trained to take oral dosing or repeated blood samplings and since inter-animal differences in intestinal glucose absorption may complicate interpretation.
- [0795] Glucose disposal is estimated from the elimination rate, which allows a glucose elimination constant (K_g) to be calculated. The glucose elimination after intravenous glucose administration follows an exponential function. After logarithmic transformation of the data, the elimination becomes linear. K_g is calculated as the slope for the logarithmic transformation of the individual glucose values and is calculated from the formula $kg = (0.693 \times 100) / t_{1/2}$ where $t_{1/2}$ is the half-time of glucose elimination (in minutes). The unit for kg is percentage of glucose decay per minute. The slopes of the glucose elimination curve show a strong linear correlation with first and second phase glucose disposal. However, STZ destroys the β -cells, thus eliminating the first phase response making the slope primarily a second phase event.
- [0796] FIGs. 1F, 2F, 3F, and 4F depict the blood glucose levels during IVGTT of NHP1-NPH4, respectively, prior to STZ administration, 7 days before IM administration of AAV1-hIns+AAV1-hGck, and at multiple timepoints after AAV dosing. Prior to administration of STZ, the K_g of NHP1 and NHP2 was calculated to be 6.24 and 6.91 respectively (FIGs. 1F and 2F, respectively). After STZ administration, and before AAV dosing, K_g fell significantly to 0.66 and 0.42, respectively. At day 56 (NHP1) and 58 (NHP2) following administration of AAV1-hIns+AAV1-hGck, K_g significantly improved in both animals to 1.49 and 1.01. K_g fell again to 0.74 and 0.78 in NHP1 at days 92 and 176, respectively, and 0.74 in NHP2 at day 91 following AAV dosing.
- [0797] Prior to administration of STZ, the K_g of NHP3 was 4.48 and fell significantly to 0.51 after STZ administration (FIG. 3F). At day 56 following administration of AAV1-hIns+AAV1-hGck, K_g improved to 1.33 and decreased to 0.99 at day 84 after AAV dosing. Prior to administration of STZ, the K_g of NHP4 was 5.94 and fell significantly to 0.71 after STZ administration (FIG. 4F). At days 56 and 84 following administration of AAV1-hIns+AAV1-hGck, K_g improved to 1.15 and 1.14, respectively. The improved

glucose elimination rate in the NHPs administered AAV1-hIns+AAV1-hGck suggested improved insulin function. FIGs. 1G, 2G, 3G and 4G depict the C-peptide levels during IVGTT of NHP1-NHP4, respectively, 3 to 5 days prior to STZ administration, 7 days before IM administration of AAV1-hIns+AAV1-hGck, and at multiple timepoints after AAV dosing. In normal (STZ-untreated) animals, insulin and C-peptide are stored together in granules and released together from the beta cells upon glucose exposure. In this experiment, intramuscular injection of AAV vector expressing insulin under the control of a constitutive promoter is believed to result in continuous expression and release of both insulin and C-peptide, which would not be expected to respond to a glucose challenge.

[0798] C-peptide levels in NHP1 after administration of STZ were significantly lower than prior to administration of STZ (FIG. 1G). C-peptide levels improved significantly beginning at day 75 and achieving a plateau from day 120 to 178 days after AAV1-hIns+AAV1-hGck administration. C-peptide levels in NHP2 after administration of STZ were significantly lower than prior to administration of STZ (FIG. 2G). C-peptide levels improved significantly from day 68 to day 92 following AAV1-hIns+AAV1-hGck administration. The recovery of C-peptide levels in the NHPs administered AAV1-hIns+AAV1-hGck suggested improved insulin production and function following AAV dosing, thereby reversing the effects of STZ.

[0799] These results indicate that IM administration of AAV1-hIns and AAV1-hGck lead to improved insulin function in a STZ-induced diabetic NHP system. The improved blood glucose control and insulin function seen in NHP4 further indicate that expanding the region of administration to include the quadriceps, biceps, and hamstrings may be beneficial. Additionally, the higher total dose of AAV1-hIns+AAV1-hGck administered to NHP4 suggest a dose-dependent response in the therapeutic benefits of this combination therapy in the NHP model system.

3.5 Capsid and Transgene Immunogenicity

[0800] Peptide libraries for in vitro stimulation of lymphocytes:

[0801] All peptide libraries was synthesized as 15-mers with 10 amino acid overlap with the previous peptide (Mimotopes, Victoria, Australia: AAV1 lot #30946, Insulin lot #34345, glucokinase lot #34343). The libraries were reconstituted in DMSO following

GTP SOP 6013 and pooled into peptide groups at a concentration of approximately 2 µg/mL per peptide in the ELISpot assay.

[0802] ELISpot:

[0803] T-cell responses to AAV1 capsid as well as the Insulin and Glucokinase transgenes were analyzed by IFN-γ ELISPOT per GTP SOP 6002. ELISPOT data were imaged and analyzed on a CTL Immunospot S6 Core Analyzer per GTP SOPs 6002 and 8502. Sample was positivity for IFN-γ production if the sample met both of the following criteria: 1) sample average value > 55 SFU/million cells and at least three times (3x) greater than the DMSO negative control value and 2) DMSO negative control value: Average value ≤ 55 SFU/million cells.

[0804] Assay wells were stimulated with DMSO as a negative control, individual AAV1 capsid, Insulin and Glucokinase transgene peptide pools, as well as PHA, Anti-CD3, and PMA/ION as positive controls. The following cell plating strategy was employed: for individual peptide pools, 2.0E+5 cells were plated per well, respectively, with data calculated for and presented as the average spot forming units (SFU) per million (1.0E+6) cells; For the PHA, Anti-CD3, and PMA/ION controls, 2.0E+5 cells, 2.0E+4 cells, and 2.0E+3 cells were plated per well, respectively, with the data reported as SFU per the original tested cell number. Values for these controls were not extrapolated to SFU per million cells. Medium with DMSO was used as the negative control.

[0805] Results:

[0806] NHP1-NHP4 respectively were negative for anti-AAV1 nAbs prior to AAV administration and mounted an expected anti-AAV1 nAb response post AAV administration. NHP1-NHP4 were also assessed for IFNγ cellular ELISpot response in FIGs. 1H, 2H, 3H, and 4H, respectively. NHP1 had a low, transiently positive IFNγ cellular ELISpot response to AAV1 capsid peptides that appeared at Day 71 and Day 161. NHP3 had a sustained cellular IFNγ response to capsid that was likely adjuvated by a preexisting cellular response raised to exogenous insulin. NHP4 had a low, transiently positive IFNγ cellular ELISpot response to AAV1 capsid peptides at Day 42 that resolved by the next tested time point and did not return through Day 91. For NHP1, NHP2, and NHP4, there were no positive IFNγ cellular responses against vectorized human insulin or glucokinase. NHP3 had a sustained IFNγ cellular response against insulin that was detectable prior to administration of AAV1-hIns and AAV-hGck vectors. Therefore, the response was initiated by administration of exogenous insulin.

- [0807] Humoral immune responses against human proteins are common in response to both biologics and human transgenes expressed in NHPs.
- [0808] The antibody response to human insulin and glucokinase was measured for NHP1 (FIGs. 1I and 1J, respectively) along with NHP2 (FIG. 2I), NHP3 (FIG. 3I), and NHP4 (FIG. 4I). NHP1, NHP2, and NHP3 mounted antibody responses to human insulin. These responses were variable in magnitude and non-neutralizing as evidenced by the sustained reduction in required exogenous insulin post AAV administration. There was no humoral immune response detected against human insulin in NHP4.
- [0809] FIG. 9A summarizes anti-insulin IgG (dilution factor) for NHP1-NHP4 measured at 14 days prior to AAV1-hINS+AAV1-hGCK administration (-14). FIG. 9B shows anti-glucokinase IgG (ECLsignal) for NHP1-NHP6 measured at 14 days prior to AAV administration (-14). FIG. 9C shows levels of anti-drug antibodies (ADA) for insulin (screening signal) measured for NHP1 and NHP2 starting from day -14 until day 182.
- [0810] NHP1, NHP2, and NHP4 mounted antibody responses to human glucokinase. For NHP3, no antibody response to human glucokinase was detected.

3.6 Tissue Expression of Insulin and Glucokinase

- [0811] Muscle samples were taken from NHP1-NHP4 either at the site of injection of AAV1-hIns and AAV-hGck vectors or randomly 1 cm away from the injection site at necropsy. Four punches per every dosed skeletal muscle type (Quadriceps, Gastrocnemius, Tibialis Anterior, and hamstring) bilateral were harvested for every animal. A randomly selected pair of injection site punch and 1 cm away from injection site punch per muscle type, bilateral were homogenized and analyzed as described below.
- [0812] Harvested muscle tissue punches were flash frozen and shipped on dry ice and were stored at -80°C (Nominal) upon receipt. Frozen muscle tissue punches were weighed, added to an appropriate volume of lysis buffer (MSD Tris lysis buffer supplemented with 5 mM EDTA and 1X protease and phosphatase inhibitor cocktail, and homogenized to lysates on Precellys (Bertin Technologies) homogenizer. Lysates were clarified by centrifugation at 4°C (Nominal), quantified for total protein using detergent compatible (DC) protein assay (Bio-Rad), aliquoted and stored at -80°C (Nominal). The stored lysates were diluted appropriately and used for quantification of human GcK and insulin.

- [0813]** AAV derived human GCK protein in muscle was measured using a qualified assay utilizing Abcam Human GCK ELISA kit (ab125967) and recombinant human GcK calibrator provided in the kit. Qualified QC samples were prepared by diluting stock calibrator to concentrations of 20 ng/mL, 5 ng/mL, and 2 ng/mL with sample diluent N or naïve NHP muscle lysate diluted to 1 mg/mL total protein. On the day of the assay, study sample lysates were diluted to a total protein concentration of 1 mg/mL in sample Diluent N before addition to the plate precoated with antibody specific to human GcK protein. The plates were incubated with shaking for 2 Hours at room temperature. The plate was washed, a biotinylated GCK specific detection antibody was then added to the plate and incubated with shaking for 1 hour at room temperature. The plate was washed, and a streptavidin-peroxidase conjugate was added and incubated with shaking for 0.5 hour at room temperature. Following incubation, chromogen substrate was added to each well and incubated in ambient light with shaking at room temperature until the optimal blue color density developed. The reaction was stopped, and the plate was read at 450 nm using a SpectraMax plate reader. The OD values obtained from the calibration standards were fit to a 4-parameter logistic (4PL) fit equation with fixed weighting (*i.e.*, no weighting applied to curve fit, Softmax Pro 7 software) to calculate the GCK protein concentrations in QCs and study samples.
- [0814]** AAV derived human insulin protein in muscle was measured using a qualified assay utilizing Abcam Human Insulin ELISA kit (ab278123) and recombinant human Insulin calibrator provided in the kit. Qualified QC samples were prepared by diluting stock calibrator to concentrations of 351 pmol/L, 125.9 pmol/L, and 74.7 pmol/mL with kit sample diluent and naïve NHP muscle lysate diluted to 0.5 mg/mL total protein. Study sample lysates diluted to a total protein concentration of 0.5 mg/mL in kit sample diluent, prepared standards and QCs, were added to the assay plate followed by capture and detection antibody cocktail and incubated with shaking overnight at 2-4°C. The plate was washed, a chromogen substrate TMB was added to each well and incubated in ambient light with shaking at room temperature until the optimal blue color density developed. The reaction was stopped, and the plate was read at 450 nm using a SpectraMax plate reader. The OD values obtained from the calibration standards were fit to a 4-parameter logistic (4PL) fit equation with fixed weighting (*i.e.*, no weighting applied to curve fit, Softmax Pro 7 software) to calculate insulin protein concentrations.

- [0815] Each sample was analyzed for glucokinase protein, shown in FIGs. 10A-10E. FIG. 10A displays the glucokinase levels measured from every muscle sample for each of NHP1-NHP8. Detectable human glucokinase was measured in at least one sample for each NHP in the study. The glucokinase levels for each individual NHP1-NHP4 is displayed in FIGs. 10B-10E, respectively. All samples analyzed were Below Limit of Quantitation for human insulin in muscle lysates (data not shown).

3.7 Vector mRNA Quantification

- [0816] Tissue Homogenization:
- [0817] Harvested tissues from the animal study site were preserved in RNAlater for 18-24 hours at 4°C before decanting the RNAlater solution and storing the preserved tissue at -60°C to -80°C. Frozen tissues were shipped on dry ice and were stored at -60°C to -80°C upon receipt at our partner CRO. Tissues were homogenized in lysis buffer according to the CRO SOPs and a portion of the homogenate was used for total RNA isolations. Remaining tissue homogenate was returned to the freezer for storage.
- [0818] RNA Isolations:
- [0819] Total RNA was isolated from study sample tissue homogenates using the QIAasympphony RNA kit (Qiagen) on the automated QIAasympphony instrument (Qiagen) according to the CRO SOPs. Isolated RNA stock concentrations were quantified using a NanoDrop 8000 (ThermoFisher).
- [0820] Vector mRNA (RT-qPCR) Copy Quantifications:
- [0821] hInsulin vector mRNA copies in study tissues were determined using an RT-qPCR assay to target the open reading frame of the vector transgene and the untranslated region located at the 3' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing. hGcK vector mRNA copies in study tissues were determined using an RT-qPCR assay to target the untranslated region and open reading frame of the vector transgene located at the 5' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing.
- [0822] Linearized plasmids containing either the hInsulin or the hGcK target sequences were serially diluted to use as the assay standards for quantification purposes. The appropriate standards, quality controls, and diluted RNA samples were added to a PCR

plate containing a reaction mixture of the hInsulin or hGcK primers and probes. Targets were amplified using set RT-qPCR thermocycling parameters in a QuantStudio 7 Flex Real-Time PCR System instrument (Applied Biosystems) (**Table 2**). Data was captured in the QuantStudio 6 and 7 Flex Real-Time PCR System software (Applied Biosystems), target cycle thresholds were set, and the data was exported for further analysis in Microsoft Excel.

Table 2 - RT-qPCR Thermocycling Parameters:

Cycle Step	Temperature (°C)	Time	40 cycles
1	50	10 minutes	
2	95	2 minutes	
3	95	15 seconds	
4	60	60 seconds	

[0823] AAV vector mRNA copies for hInsulin and hGcK were reported as single stranded copies/μg total RNA (ss copies/μg RNA).

3.8 Vector Genome Quantification

[0824] Tissue Homogenization:

[0825] Harvested tissues from the animal study site were preserved in RNAlater for 18-24 hours at 4°C before decanting the RNAlater solution and storing the preserved tissue at -60°C to -80°C. Frozen tissues were shipped on dry ice and were stored at -60°C to -80°C upon receipt at our partner CRO. Tissues were homogenized in lysis buffer according to the CRO SOPs and a portion of the homogenate was used for genomic DNA isolations. Remaining tissue homogenate was returned to the freezer for storage.

[0826] DNA Isolations:

[0827] Genomic DNA was isolated from study sample tissue homogenates using the QIAasympy DSP DNA mini kit (Qiagen) on the automated QIAasympy instrument (Qiagen) according to the CRO SOPs. Isolated DNA stock concentrations were quantified using a NanoDrop 8000 (ThermoFisher).

[0828] Vector Genome DNA (qPCR) Copy Quantifications:

[0829] hInsulin vector genome copies in study tissues were determined using a qPCR assay to target the open reading frame of the vector transgene and the untranslated region located at the 3' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed

in qualification testing. hGcK vector genome copies in study tissues were determined using a qPCR assay to target the untranslated region and open reading frame of the vector transgene located at the 5' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing.

[0830] Linearized plasmids containing either the hInsulin or the hGcK target sequences were serially diluted to use as the assay standards for quantification purposes. The appropriate standards, quality controls, and diluted DNA samples were added to a PCR plate containing a reaction mixture of the hInsulin or hGcK primers and probes. Targets were amplified using set qPCR thermocycling parameters in a QuantStudio 7 Flex Real-Time PCR System instrument (Applied Biosystems) (**Table 3 above**). Data was captured in the QuantStudio 6 and 7 Flex Real-Time PCR System software (Applied Biosystems), target cycle thresholds were set, and the data was exported for further analysis in Microsoft Excel.

Table 3 - qPCR Thermocycling Parameters:

Cycle Step	Temperature (°C)	Time	40 cycles
1	95	5 minutes	
2	95	1 second	
3	60	20 seconds	

[0831] AAV vector genome copies for hInsulin and hGcK were reported as double stranded copies/μg host genomic DNA (ds copies/μg gDNA).

[0832] The expression level of human insulin, human glucokinase, and both AAV vectors was measured. NHP1 and NHP2 displayed widespread expression of human insulin and the AAV1-hIns vector (FIG. 11A), along with human glucokinase and AAV-hGck vector (FIG. 11B), in all but one tested sample, including those 1 cm away from the injection site. NHP3 and NHP4 displayed expression of human insulin and AAV1-hIns vector (FIG. 11C), and human glucokinase and AAV-hGck vector (FIG. 11D). While NHP4 had widespread expression similar to NHP1 and NHP2, NHP3 had less widespread detection of AAV vectors and their cargo.

Example 4: *In Vivo* Evaluation of Insulin and Glucokinase Combination Therapy with Immunosuppressive Regimen in Non-Human Primates

- [0833] Given the reduction in exogenous insulin administration observed following dosing with the AAV1-hIns and AAV-hGck vectors in the four STZ-induced diabetic cynomolgus macaques of Example 3, additional therapeutic efficacy was investigated with the introduction of immunosuppressive therapy. Type 1 diabetes was induced in four cynomolgus macaques by treatment STZ as detailed in Example 3. Following diabetes induction, animals were treated with the AAV1-hIns and AAV1-hGck vectors administer IM.
- [0834] The four cynomolgus macaques (NHP5-NHP8) were dosed with a 1:0.5 ratio of AAV1-hIns:AAV1-hGck (admixed).
- [0835] NHP 5 and NHP6 received 56 injections (100 µL each) distributed bilaterally to the quadriceps (12 injections per side), biceps (8 injections per side), and hamstrings (8 injections per side) with a total dose of 9.6E12 vg/kg (7.1E12 vg/kg as measured by gene of interest) of AAV1-hIns and 4.8E12 vg/kg (3.2E12 as measured by gene of interest) of AAV1-hGck. NHP 7 and NHP 8 each received 56 injections (100 µL each) distributed bilaterally to the quadriceps (12 injections per side), biceps (8 injections per side), and hamstrings (8 injections per side) with a total dose of 7.0E12 vg/kg of AAV1-hIns and 3.5E12 vg/kg of AAV1-hGck, as measured by gene of interest.
- [0836] NHP 5 received upfront immunosuppression with tocilizumab (8 mg/kg) administered intravenously on day -1 relative to AAV administration. NHP6-NHP 8 each received upfront immunosuppression with tocilizumab (8 mg/kg) administered intravenously on day -1 relative to AAV administration. Tocilizumab is a humanized monoclonal antibody targeting the IL-6 receptor (IL-6R). The dosing regimen in shown in **Table 4**.
- [0837] Additionally, NHP6-NHP8 were administered tacrolimus IM beginning on day 21 (0.01-0.05 mg/kg), twice a day, to termination, with a target drug level of 10-18 ng/mL. NHP7 had tacrolimus discontinued on day 90 due to hypoglycemia. Tacrolimus targets FKBP12, leading to inhibition of calcineurin, thereby inhibiting both T-cell signal transduction and IL-2 transcription (Liu J et al. Cell. 1991; 66(4): 807-15). Circulating tacrolimus levels (µg/L), beginning at day 21 following AAV1-hINS+AAV1-hGCK

administration through day 180 are shown for NHP6 in FIG. 6F, through day 122 for NHP7 in FIG. 7H, and through day 122 for NHP8 in FIG. 8H.

Table 4. AAV1-hIns and AAV1-hGck + Immunosuppressant Dosing Regimen

NHP	Dose	Injections	Muscle groups (# injections per side)	Immune suppression regimen	Necropsy Day Post-Dose
5	7.1E12 vg/kg AAV1-hINS 3.2E12 vg/kg AAV1-hGCK	100 µL x 56	Quadriceps (12) Bicep (8) Hamstring (8)	Tocilizumab 8 mg/kg IV (Day - 1)	182
6	7.1E12 vg/kg AAV1-hINS 3.2E12 vg/kg AAV1-hGCK	100 µL x 56	Quadriceps (12) Bicep (8) Hamstring (8)	Tocilizumab, 8 mg/kg IV (Day - 1) Tacrolimus 0.01- 0.05 mg/kg IM (BID beginning day +21)	182
7	7.0E12 vg/kg AAV1-hINS 3.5E12 vg/kg AAV1-hGCK	100 µL x 56	Quadriceps (12) Bicep (8) Hamstring (8)	Tocilizumab, 8 mg/kg IV (Day - 1) Tacrolimus 0.01- 0.05 mg/kg IM (BID beginning day +21)*	122
8	7.0E12 vg/kg AAV1-hINS 3.5E12 vg/kg AAV1-hGCK	100 µL x 56	Quadriceps (12) Bicep (8) Hamstring (8)	Tocilizumab, 8 mg/kg IV (Day - 1) Tacrolimus 0.01- 0.05 mg/kg IM (BID beginning day +21)	122

*Tacrolimus ended on day 90.

4.1 Blood Glucose and Exogenous Insulin Requirements

[0838] Insulin was administered to diabetic animals following administration of AAV1-hIns and AAV1-hGck as needed. For most diabetic NHPs fasting blood sugars of 40-150 mg/dL and less than 200 mg/dL were targeted. If a diabetic NHP had hypoglycemic risk factors (social stress, injury, recent or recurrent hypoglycemia, etc.), then goal fasting glucose values of 60-180 mg/dL and less than 250 mg/dL were targeted.

[0839] Daily measurements of pre- and post-prandial blood glucose (mg/dL) for NHP5-NHP8 are shown in FIGs. 5A, 6A, 7A, and 8A, respectively. The amount of exogenous insulin administered (U/kg/day) is shown for NHP5 in FIG. 5H, for NHP6 in FIG. 6I, for NHP7 in FIG. 7B, and for NHP8 in FIG. 8B. Furthermore, the percent decrease in exogenous insulin administration is shown for NHP5 in FIG. 5I, for NHP6 in FIG. 6J, for NHP7 in FIG. 7C, and for NHP8 in FIG. 8C. From day 30 to day 60 following the combined AAV1-hIns and AAV1-hGck administration (and immunosuppressive agent), exogenous insulin was reduced about 36% (NHP5) and about 45% (NHP6) compared to baseline. Significantly, by day 40 for NHP5, by day 50 for NHP6, by day 30 for NHP7, and by day 30 for NHP8, each subject demonstrated insulin independence without hypoglycemia, with no additional exogenous insulin administered. NHP5 lost insulin independence after approximately 1 day. Insulin reduction then decreased to 28% by around day 110, before rising again to 62% by day 180. From day 40 until day 70, NHP5 had an almost complete withdrawal of long-acting insulin. NHP6 remained insulin independent through day 70, thereafter maintaining a roughly 50% reduction in exogenous insulin through day 182. NHP7 and NHP8 each remained insulin independent through day 122.

4.2 Circulating C-peptide, Creatine Kinase, HbA1C, and Insulin Levels

[0840] Blood samples were drawn weekly and analyzed for C-peptide levels and are shown for NHP5 in FIG. 5B, for NHP6 in FIG. 6B, for NHP7 in FIG. 7J, and for NHP8 in FIG. 8J. In both NHP5 and NHP6, C-peptide levels steadily increased from day 7 achieving a plateau at day 45-56 following the combined AAV1-hIns and AAV1-hGck administration (and immunosuppressive agent). C-peptide levels in NHP5 then decrease until day 90, thereafter increasing again to a plateau through 180 days. The C-peptide levels in NHP6 gradually drop throughout the 60-182 day timecourse. For both NHP7 and NHP8, C-peptide levels were low and stable throughout the 122 day timecourse. Creatine kinase levels were also analyzed weekly and are shown for NHP5 in FIG. 5D, for NHP6 in FIG. 6D, for NHP7 in FIG. 7G, and for NHP8 in FIG. 8G. Creatine kinase levels remained relatively unchanged through all time points for each NHP.

[0841] HbA1C levels were analyzed 0, 28, 45, 56, 80, 91, 161, 168, 175, and 182 days after administration of AAV1-hIns and AAV1-hGck in NHP5 and NHP6 (FIGs. 5C and 6C, respectively). HbA1C levels were also analyzed 0, 28, 56, 91, 119, and 122 days after

administration of AAV1-hIns and AAV1-hGck in NHP7 and NHP8 (FIGs. 7D and 8D, respectively). In both NHP5 and NHP6, HbA1C decreased steadily to day 45 then increased at day 56 before stabilizing until day 180 after administration of AAV1-hIns and AAV1-hGck, indicating improved blood glucose control. NHP7 showed a small decrease in HbA1c until 56 before stabilizing through to 122 days after administration of AAV1-hIns and AAV1-hGck. NHP8 displayed a gradual decrease in HbA1C level throughout the 122 day timecourse.

[0842] Insulin levels were measured for NHP5 and NHP6 and are shown in FIGs. 5J and 6K, respectively. For both NHP5 and NHP6, insulin levels peaked close to day 90 before decreasing to a low stable value through to day 180.

4.3 Weight

[0843] Body weight was measured weekly after administration of AAV1-hIns and AAV1-hGck in NHP5–NHP8 (FIGs. 5E, 6E, 7L, and 8L, respectively). All animals showed a slight increase in weight throughout the last time point, consistent with the anabolic effects of insulin.

4.4 Intravenous Glucose Tolerance Test

[0844] FIGs. 5F and 6G depict the blood glucose levels during IVGTT of NHP5 and NHP6, respectively, 7 days prior to STZ administration, 4-7 days before IM administration of AAV1-hIns+AAV1-hGck, 56 days after IM administration of AAV1-hIns+AAV1-hGck, 91 days after IM administration of AAV1-hIns+AAV1-hGck, and 175 days after IM administration of AAV1-hIns+AAV1-hGck. FIG 7E shows bloods glucose levels during IVGTT for NHP7 at 7 days prior to STZ, 4-7 days before IM administration of AAV1-hIns+AAV1-hGck, and 64 or 91 days after IM administration of AAV1-hIns+AAV1-hGck. FIG 8E shows bloods glucose levels during IVGTT for NHP8 at 7 days prior to STZ, 4-7 days before IM administration of AAV1-hIns+AAV1-hGck, and 56 or 91 days after IM administration of AAV1-hIns+AAV1-hGck. Prior to administration of STZ, the Kg of NHP5, NHP6, NHP7, and NHP8 were calculated to be 3.22, 10.1, 8.54, and 3.96 respectively (FIGs. 5F, 6G, 7E, and 8E, respectively). After STZ administration and before AAV dosing, Kg fell significantly to 0.90, 0.0, 0.5, and 0.33, respectively, similar to the decrease seen following administration of STZ in NHP1-NHP4 in Example 3.

[0845] At Day 56, for NHP5, NHP6, and NHP8, and Day 64 for NHP7, all subjects had improved fasting blood glucose levels and IVGTT profiles that more closely resembled those from before STZ administration. At Day 91, NHP5 had a fasting blood glucose level and IVGTT profile approximating those observed post-STZ but before AAV administration, consistent with its increased reliance on exogenous insulin pre- and post-prandially. At Day 175, NHP5 blood glucose removal was decreased compared to Day 91, but still better than Day 56. NHP6 showed a gradual decrease in blood glucose removal capacity from Day 56 through Day 175. For NHP7 and NHP8, blood glucose removal returned to Pre STZ levels from the earliest measurement (Day 64 for NHP7 and Day 56 for NHP8) to the end last measurement on Day 91.

[0846] FIGs. 5G, 6H, 7F, and 8F depict the C-peptide levels during IVGTT of NHP5, NHP6, NHP7, and NHP8, respectively, 7 days prior to STZ administration and 4-7 days before IM administration of AAV1-hIns+AAV1-hGck. As observed in NHP1-NHP4, administration of STZ resulted in a lack of C-peptide response to IVGTT, and as expected, this lack of response was not affected by IM administration of AAV1-hIns+AAV1-hGCK in NHP5-NHP8 (FIGs. 5G, 6H, 7F, and 8F, respectively), demonstrating that STZ treatment successfully ablated pancreatic beta cells.

[0847] These results indicate that immunosuppression prior to administration of AAV1-hIns+AAV1-hGck may result in improved therapeutic efficacy of the AAV vectors. In comparison with NHP1-NHP4 of Example 3, NHP5-NHP8 showed significant improvements in blood glucose control, and a sharp reduction in the need for exogenous insulin administration. These results indicate that the efficacy of the AAV1-hIns+AAV1-hGck combination therapy may be enhanced by an initial immunosuppressive treatment prior to AAV dosing (e.g., tocilizumab at day -1) and a maintenance immunosuppressive regimen (e.g., tacrolimus beginning about 1-3 weeks after AAV dosing (e.g., around day 21)).

4.5 Capsid and Transgene Immunogenicity

[0848] Peptide libraries for in vitro stimulation of lymphocytes:

[0849] All peptide libraries was synthesized as 15-mers with 10 amino acid overlap with the previous peptide (Mimotopes, Victoria, Australia: AAV1 lot #30946, Insulin lot #34345, glucokinase lot #34343). The libraries were reconstituted in DMSO following

GTP SOP 6013 and pooled into peptide groups at a concentration of approximately 2 µg/mL per peptide in the ELISpot assay.

[0850] ELISpot:

[0851] T-cell responses to AAV1 capsid as well as the Insulin and Glucokinase transgenes were analyzed by IFN-γ ELISPOT per GTP SOP 6002. ELISPOT data were imaged and analyzed on a CTL Immunospot S6 Core Analyzer per GTP SOPs 6002 and 8502. Sample was positivity for IFN-γ production if the sample met both of the following criteria: 1) sample average value > 55 SFU/million cells and at least three times (3x) greater than the DMSO negative control value and 2) DMSO negative control value: Average value ≤ 55 SFU/million cells.

[0852] Assay wells were stimulated with DMSO as a negative control, individual AAV1 capsid, Insulin and Glucokinase transgene peptide pools, as well as PHA, Anti-CD3, and PMA/ION as positive controls. The following cell plating strategy was employed: for individual peptide pools, 2.0E+5 cells were plated per well, respectively, with data calculated for and presented as the average spot forming units (SFU) per million (1.0E+6) cells; For the PHA, Anti-CD3, and PMA/ION controls, 2.0E+5 cells, 2.0E+4 cells, and 2.0E+3 cells were plated per well, respectively, with the data reported as SFU per the original tested cell number. Values for these controls were not extrapolated to SFU per million cells. Medium with DMSO was used as the negative control.

[0853] Results:

[0854] NHP5-NHP8 were negative for anti-AAV1 nAbs prior to AAV administration and mounted an expected anti-AAV1 nAb response post AAV administration. NHP5-NHP8 were also assessed for IFNγ cellular ELISpot response in FIGs. 5K, 6L, 7K, and 8K, respectively. Each of NHP5-NHP8 displayed a response to AAV1 at some point in the timecourse. These responses were typically low and transient, although most recurred. Only NHP5 showed an AAV1 response below the highest dose of virus (FIG. 5K).

[0855] FIG. 9B also shows anti-glucokinase IgG (ECLsignal) for NHP5 and NHP6 measured at 14 days prior to AAV administration (-14), with low levels of antibody seen initially prior to declining after day 42.

[0856] None of NHP5-NHP8 had a cellular immune response to human insulin or glucokinase (FIGs. 5K, 6L, 7K, and 8K, respectively).

4.6 Tissue Expression of Insulin and Glucokinase

- [0857] Muscle samples were taken from NHP5-NHP8 either at the site of injection of AAV1-hIns and AAV-hGck vectors or randomly 1 cm away from the injection site at necropsy. Four punches per every dosed skeletal muscle type (Quadriceps, Gastrocnemius, Tibialis Anterior, and hamstring) bilateral were harvested for every animal. A randomly selected pair of injection site punch and 1 cm away from injection site punch per muscle type, bilateral were homogenized and analyzed as described below.
- [0858] Harvested muscle tissue punches were flash frozen and shipped on dry ice and were stored at -80°C (Nominal) upon receipt. Frozen muscle tissue punches were weighed, added to an appropriate volume of lysis buffer (MSD Tris lysis buffer supplemented with 5 mM EDTA and 1X protease and phosphatase inhibitor cocktail, and homogenized to lysates on Precellys (Bertin Technologies) homogenizer. Lysates were clarified by centrifugation at 4°C (Nominal), quantified for total protein using detergent compatible (DC) protein assay (Bio-Rad), aliquoted and stored at -80°C (Nominal). The stored lysates were diluted appropriately and used for quantification of human GcK and insulin.
- [0859] AAV derived human GCK protein in muscle was measured using a qualified assay utilizing Abcam Human GCK ELISA kit (ab125967) and recombinant human GcK calibrator provided in the kit. Qualified QC samples were prepared by diluting stock calibrator to concentrations of 20 ng/mL, 5 ng/mL, and 2 ng/mL with sample diluent N or naïve NHP muscle lysate diluted to 1 mg/mL total protein. On the day of the assay, study sample lysates were diluted to a total protein concentration of 1 mg/mL in sample Diluent N before addition to the plate precoated with antibody specific to human GcK protein. The plates were incubated with shaking for 2 Hours at room temperature. The plate was washed, a biotinylated GCK specific detection antibody was then added to the plate and incubated with shaking for 1 hour at room temperature. The plate was washed, and a streptavidin-peroxidase conjugate was added and incubated with shaking for 0.5 Hour at room temperature. Following incubation, chromogen substrate was added to each well and incubated in ambient light with shaking at room temperature until the optimal blue color density developed. The reaction was stopped, and the plate was read at 450 nm using a SpectraMax plate reader. The OD values obtained from the calibration standards were fit to a 4-parameter logistic (4PL) fit equation with fixed weighting (*i.e.*, no

weighting applied to curve fit, Softmax Pro 7 software) to calculate the GCK protein concentrations in QCs and study samples.

[0860] AAV derived human insulin protein in muscle was measured using a qualified assay utilizing Abcam Human Insulin ELISA kit (ab278123) and recombinant human Insulin calibrator provided in the kit. Qualified QC samples were prepared by diluting stock calibrator to concentrations of 351 pmol/L, 125.9 pmol/L, and 74.7 pmol/mL with kit sample diluent and naïve NHP muscle lysate diluted to 0.5 mg/mL total protein. Study sample lysates diluted to a total protein concentration of 0.5 mg/mL in kit sample diluent, prepared standards and QCs, were added to the assay plate followed by capture and detection antibody cocktail and incubated with shaking overnight at 2-4°C. The plate was washed, a chromogen substrate TMB was added to each well and incubated in ambient light with shaking at room temperature until the optimal blue color density developed. The reaction was stopped, and the plate was read at 450 nm using a SpectraMax plate reader. The OD values obtained from the calibration standards were fit to a 4-parameter logistic (4PL) fit equation with fixed weighting (*i.e.*, no weighting applied to curve fit, Softmax Pro 7 software) to calculate insulin protein concentrations.

[0861] Each sample was analyzed for glucokinase protein, shown in FIGs. 10A and 10F-10I. FIG. 10A displays the glucokinase levels measured from every muscle sample for each of NHP1-NHP8. Detectable human glucokinase was measured in at least one sample for each NHP in the study. The glucokinase levels for each individual NHP5-NHP8 is displayed in FIGs. 10F-10I, respectively. All samples analyzed were Below Limit of Quantitation for human insulin in muscle lysates (data not shown).

4.7 Vector mRNA Quantification

[0862] Tissue Homogenization:

[0863] Harvested tissues from the animal study site were preserved in RNAlater for 18-24 hours at 4°C before decanting the RNAlater solution and storing the preserved tissue at -60°C to -80°C. Frozen tissues were shipped on dry ice and were stored at -60°C to -80°C upon receipt at our partner CRO. Tissues were homogenized in lysis buffer according to the CRO SOPs and a portion of the homogenate was used for total RNA isolations. Remaining tissue homogenate was returned to the freezer for storage.

[0864] RNA Isolations:

- [0865] Total RNA was isolated from study sample tissue homogenates using the QIAasympphony RNA kit (Qiagen) on the automated QIAasympphony instrument (Qiagen) according to the CRO SOPs. Isolated RNA stock concentrations were quantified using a NanoDrop 8000 (ThermoFisher).
- [0866] Vector mRNA (RT-qPCR) Copy Quantifications:
- [0867] hInsulin vector mRNA copies in study tissues were determined using an RT-qPCR assay to target the open reading frame of the vector transgene and the untranslated region located at the 3' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing. hGcK vector mRNA copies in study tissues were determined using an RT-qPCR assay to target the untranslated region and open reading frame of the vector transgene located at the 5' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing.
- [0868] Linearized plasmids containing either the hInsulin or the hGcK target sequences were serially diluted to use as the assay standards for quantification purposes. The appropriate standards, quality controls, and diluted RNA samples were added to a PCR plate containing a reaction mixture of the hInsulin or hGcK primers and probes. Targets were amplified using set RT-qPCR thermocycling parameters in a QuantStudio 7 Flex Real-Time PCR System instrument (Applied Biosystems) (**Table 2 above**). Data was captured in the QuantStudio 6 and 7 Flex Real-Time PCR System software (Applied Biosystems), target cycle thresholds were set, and the data was exported for further analysis in Microsoft Excel.
- [0869] AAV vector mRNA copies for hInsulin and hGcK were reported as single stranded copies/ μ g total RNA (ss copies/ μ g RNA).

4.8 Vector Genome Quantification

- [0870] Tissue Homogenization:
- [0871] Harvested tissues from the animal study site were preserved in RNAlater for 18-24 hours at 4°C before decanting the RNAlater solution and storing the preserved tissue at -60°C to -80°C. Frozen tissues were shipped on dry ice and were stored at -60°C to -80°C upon receipt at our partner CRO. Tissues were homogenized in lysis buffer

according to the CRO SOPs and a portion of the homogenate was used for genomic DNA isolations. Remaining tissue homogenate was returned to the freezer for storage.

[0872] DNA Isolations:

[0873] Genomic DNA was isolated from study sample tissue homogenates using the QIASymphony DSP DNA mini kit (Qiagen) on the automated QIASymphony instrument (Qiagen) according to the CRO SOPs. Isolated DNA stock concentrations were quantified using a NanoDrop 8000 (ThermoFisher).

[0874] Vector Genome DNA (qPCR) Copy Quantifications:

[0875] hInsulin vector genome copies in study tissues were determined using a qPCR assay to target the open reading frame of the vector transgene and the untranslated region located at the 3' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing. hGcK vector genome copies in study tissues were determined using a qPCR assay to target the untranslated region and open reading frame of the vector transgene located at the 5' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing.

[0876] Linearized plasmids containing either the hInsulin or the hGcK target sequences were serially diluted to use as the assay standards for quantification purposes. The appropriate standards, quality controls, and diluted DNA samples were added to a PCR plate containing a reaction mixture of the hInsulin or hGcK primers and probes. Targets were amplified using set qPCR thermocycling parameters in a QuantStudio 7 Flex Real-Time PCR System instrument (Applied Biosystems) (**Table 3 above**). Data was captured in the QuantStudio 6 and 7 Flex Real-Time PCR System software (Applied Biosystems), target cycle thresholds were set, and the data was exported for further analysis in Microsoft Excel.

[0877] Reported Results:

[0878] AAV vector genome copies for hInsulin and hGcK were reported as double stranded copies/ μ g host genomic DNA (ds copies/ μ g gDNA).

[0879] The expression level of human insulin, human glucokinase, and both AAV vectors was measured. NHP5 and NHP6 displayed widespread expression of human insulin and the AAV1-hIns vector (FIG. 11E), along with human glucokinase and AAV-hGck vector (FIG. 11F). However, for NHP6 did not display expression of human glucokinase or the

presence of AAV-hGck vector in some samples. NHP7 and NHP8 displayed expression of human insulin and AAV1-hIns vector (FIG. 11G), and human glucokinase and AAV-hGck vector (FIG. 11H). Similar to NHP6, some samples of NHP7 and NHP8 did not display the presence of human glucokinase mRNA or the AAV-hGck vector.

Example 5: *In Vivo* Evaluation of Insulin and Glucokinase Combination Therapy with Immunosuppressive Regimen in a Predictive Model of Type-1 diabetes in Non-Human Primates

- [0880] Six cynomolgus macaques (NHP9-NHP14) were dosed with a varying ratios of AAV1-hIns:AAV1-hGck (admixed) as described in **Table 5**.
- [0881] NHP9-NHP14 received 56 injections (100 µL each) distributed bilaterally to the quadriceps (12 injections per side), biceps (8 injections per side), and hamstrings (8 injections per side) with a total dose of AAV1-hIns and AAV1-hGck as shown in **Table 5**. NHP9-NHP14 received upfront immunosuppression with tocilizumab (8 mg/kg) administered intravenously on day -3 relative to AAV administration. Tocilizumab is a humanized monoclonal antibody targeting the IL-6 receptor (IL-6R). Additionally, NHP9-NHP14 were administered tacrolimus IM beginning on day -3 (0.05 mg/kg) and weekly thereafter, to termination. The dosing regimen is shown in **Table 5**.

Table 5. AAV1-hIns and AAV1-hGck + Immunosuppressant Dosing Regimen

NHP	Dose	Ratio	Injections	Immune suppression regimen
NHP9-NHP11	1.8E12 vg/kg AAV1-hINS	1:2	100 µL x 56	Tocilizumab 8 mg/kg IV (Day -3)
	3.5E12 vg/kg AAV1-hGCK			Tacrolimus 0.05 mg/kg IM (Day -3, then twice daily)
NHP12-NHP14	7E11 vg/kg AAV1-hINS	1:5	100 µL x 56	Tocilizumab 8 mg/kg IV (Day -3)
	3.5E12 vg/kg AAV1-hGCK			Tacrolimus 0.05 mg/kg IM (Day -3, then twice daily)

5.1 Blood Glucose and Exogenous Insulin Requirements

- [0882] Insulin was administered to diabetic animals following administration of AAV1-hIns and AAV1-hGck as needed. For most diabetic NHPs fasting blood sugars of 40-150 mg/dL and less than 200 mg/dL were targeted. If a diabetic NHP had hypoglycemic risk

factors (social stress, injury, recent or recurrent hypoglycemia, etc.), then goal fasting glucose values of 60-180 mg/dL and less than 250 mg/dL were targeted.

[0883] Daily measurements of pre- and post-prandial blood glucose (mg/dL) for NHP9-NHP14 are shown in FIGs. 13A, 14A, 15A, 16A, 17A, and 18A, respectively. The amount of exogenous insulin administered (U/kg/day) and the percent decrease in exogenous insulin administration over a 30 day rolling average is shown for NHP9-NHP14 are shown in FIGs. 13B, 14B, 15B, 16B, 17B, and 18B, respectively. For all animals, blood glucose range trends upward slightly. Furthermore, glargine and lispro administration go back up past Day 90 relative to AAV administration.

5.2 Intravenous 5.2 Glucose Tolerance Test

[0884] FIGs. 13C-13D and 14C-14D depict the blood glucose levels during IVGTT of NHP9 and NHP10, respectively, 7 days prior to STZ administration, 4-7 days before IM administration of AAV1-hIns+AAV1-hGck, 56 days after IM administration of AAV1-hIns+AAV1-hGck, and 91 days after IM administration of AAV1-hIns+AAV1-hGck.

[0885] At Day 56, for NHP9 and NHP10, all subjects had improved fasting blood glucose levels and IVGTT profiles less than Post-STZ.

5.3 Circulating Lactate, C-peptide, HbA1C, Glucagon, Triglyceride and Insulin Levels

[0886] Blood samples were drawn weekly and analyzed for C-peptide levels and are shown for NHP9-NHP14 for FIG. 12C. In NHP9 and NHP10, C-peptide levels steadily increased from day 7 achieving a plateau at day 28-119 following the combined AAV1-hIns and AAV1-hGck administration (and immunosuppressive agent). C-peptide levels in NHP9 and NHP10 then decrease until day 1472, thereafter increasing again to a plateau through 161 days (BLQ is 0.32, anything less than that was graphed at 0.16).

[0887] HbA1C levels were analyzed at day 0, 28, 56, 77, 91, 119 (NHP10), and 126 (NHP9), 147, 182 days (NHP9 and NHP10), or 0 and 28 days (NHP11, NHP12, NHP13, and NHP14) after administration of AAV1-hIns and AAV1-hGck (FIGs. 12B and 12G, respectively). HbA1c is a form of glycated hemoglobin (Hb). HbA1c levels serve as an integrated signal of post-prandial glucose exposure over time, with elevated levels indicating poor glycemic control, which has been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy.

- [0888] Lactate levels were analyzed at day 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 90, 106, 119, 133, 147, 161, 175 and 182 days (NHP9 and NHP10; Days 133 and 147 were not measured in NHP9 due to an anemic event that was not related to the test article), or 0 and 28 days (NHP11) (FIG. 12A). Lactate levels were stable for NHP9, NHP10, and NHP11 throughout time points analyzed.
- [0889] Insulin levels were measured for NHP9-NHP14 and are shown in FIG. 12D. For NHP9, insulin levels increased until day 28, then decreased and plateaued to day 161. For NHP10, insulin levels increased until day 28, then decreased and plateaued until day 106, after which there was a slight increase through day 161.
- [0890] Glucagon levels were analyzed at day 0, 28, 42, 56, 70, 84, 91, 119, and 133 days (NHP9 and NHP10; Day 133 was not measured for NHP9 due to reasons above) after administration of AAV1-hIns and AAV1-hGck (FIG. 12E). Glucagon levels slightly decrease after AAV administration, then remained stable throughout the analyzed data points.
- [0891] Triglyceride levels were analyzed at day 0, 7, 14, 21, 28, 35, 42, 49, 56, 70, 84, 91, 106, 119, 133, 147, 161, 175 and 182 days (NHP9 and NHP10) after administration of AAV1-hIns and AAV1-hGck (FIG. 12F). Triglyceride levels slightly peaked after day 50, but otherwise remained stable throughout the analyzed data points.

5.4 Vector mRNA Quantification

- [0892] Tissue Homogenization:
- [0893] Harvested tissues from the animal study site were preserved in RNAlater for 18-24 hours at 4°C before decanting the RNAlater solution and storing the preserved tissue at -60°C to -80°C. Frozen tissues were shipped on dry ice and were stored at -60°C to -80°C upon receipt at our partner CRO. Tissues were homogenized in lysis buffer according to the CRO SOPs and a portion of the homogenate was used for total RNA isolations. Remaining tissue homogenate was returned to the freezer for storage.
- [0894] RNA Isolations:
- [0895] Total RNA was isolated from study sample tissue homogenates using the QIAasympyphony RNA kit (Qiagen) on the automated QIAasympyphony instrument (Qiagen) according to the CRO SOPs. Isolated RNA stock concentrations were quantified using a NanoDrop 8000 (ThermoFisher).
- [0896] Vector mRNA (RT-qPCR) Copy Quantifications:

[0897] hInsulin vector mRNA copies in study tissues were determined using an RT-qPCR assay to target the open reading frame of the vector transgene and the untranslated region located at the 3' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing. hGcK vector mRNA copies in study tissues were determined using an RT-qPCR assay to target the untranslated region and open reading frame of the vector transgene located at the 5' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing.

[0898] Synthesized RNA oligos containing either the hInsulin or the hGcK target sequences were serially diluted to use as the assay standards for quantification purposes. The appropriate standards, quality controls, and diluted RNA samples were added to a PCR plate containing a reaction mixture of the hInsulin or hGcK primers and probes. Targets were amplified using set RT-qPCR thermocycling parameters in a QuantStudio 7 Flex Real-Time PCR System instrument (Applied Biosystems) (See **Table 6**). Data was captured in the QuantStudio 6 and 7 Flex Real-Time PCR System software (Applied Biosystems), target cycle thresholds were set, and the data was exported for further analysis in Microsoft Excel.

[0899] **Table 6 - RT-qPCR Thermocycling Parameters:**

Cycle Step	Temperature (°C)	Time	40 cycles
1	55	10 minutes	
2	95	20 seconds	
3	95	15 seconds	
4	60	60 seconds	

[0900] AAV vector mRNA copies for hInsulin and hGcK were reported as single stranded copies/μg total RNA (ss copies/μg RNA).

5.5 Vector Genome Quantification

[0901] Tissue Homogenization:

[0902] Harvested tissues from the animal study site were preserved in RNAlater for 18-24 hours at 4°C before decanting the RNAlater solution and storing the preserved tissue at -60°C to -80°C. Frozen tissues were shipped on dry ice and were stored at -60°C to -80°C upon receipt at our partner CRO. Tissues were homogenized in lysis buffer

according to the CRO SOPs and a portion of the homogenate was used for genomic DNA isolations. Remaining tissue homogenate was returned to the freezer for storage.

- [0903] DNA Isolations:
- [0904] Genomic DNA was isolated from study sample tissue homogenates using the QIASymphony DSP DNA mini kit (Qiagen) on the automated QIASymphony instrument (Qiagen) according to the CRO SOPs. Isolated DNA stock concentrations were quantified using a NanoDrop 8000 (ThermoFisher).
- [0905] Vector Genome DNA (qPCR) Copy Quantifications:
- [0906] hInsulin vector genome copies in study tissues were determined using a qPCR assay to target the open reading frame of the vector transgene and the untranslated region located at the 3' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing. hGcK vector genome copies in study tissues were determined using a qPCR assay to target the untranslated region and open reading frame of the vector transgene located at the 5' end of the transgene found within the test article vector. Primers and probe unique to this region were designed and the specificity of the assay was confirmed in qualification testing.
- [0907] Linearized plasmids containing either the hInsulin or the hGcK target sequences were serially diluted to use as the assay standards for quantification purposes. The appropriate standards, quality controls, and diluted DNA samples were added to a PCR plate containing a reaction mixture of the hInsulin or hGcK primers and probes. Targets were amplified using set qPCR thermocycling parameters in a QuantStudio 7 Flex Real-Time PCR System instrument (Applied Biosystems) (Table 7). Data was captured in the QuantStudio 6 and 7 Flex Real-Time PCR System software (Applied Biosystems), target cycle thresholds were set, and the data was exported for further analysis in Microsoft Excel.

Table 7 - qPCR Thermocycling Parameters:

Cycle Step	Temperature (°C)	Time	40 cycles
1	95	5 minutes	
2	95	1 second	
3	60	20 seconds	

- [0908] Reported Results:

[0909] AAV vector genome copies for hInsulin and hGcK were reported as double stranded copies/ μ g host genomic DNA (ds copies/ μ g gDNA).

Table 8: Nucleic Acid Sequences Encoding Human Insulin

SEQ ID NOs	Name	Sequence
Full: 1 5' UTR: 148 ORF: 110 3' UTR: 149	Unmodified hIns - 1 (B10D)	AAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAG TGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGACAGAAAGTTGGTCGTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTC GAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTACTTAATACGACTCACTATAGGCTA GCCTCGAGAATTCTGCCATGGCCCTGTGGATGCGCCTCTGCCCTTGCT GGCGCTGCTGGCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACC AACACCTGTGCGGCTCAGATCTGGTGGAAGCTCTCTACCTAGTGTGCGGG GAACGAGGCTTCTTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACC TGCAGGTGGGGCAGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCT GCAGCCCTTGCCCTGGAGGGGGTCGCGACAGAAGCGTGGCATTGTGGAA CAATGCTGTACCAGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAA CTAGACGCAGCTGCTAGCTAATCGATACCGTCGACCTCGAGGAATTCACGCGT GGTACCTCTAGAGTCGACCCGGGCGGCCGCTCCCTTTAGTGAGGGTTAATG CTTCGAGCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAGTAGA ATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGT AACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAATTTATG TTTCAGGTTACAGGGGAGATGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTAC AAATGTGGTAAATCCGATAAGGGACTAGAGCAGAAGCTTTTG
Full: 2 5' UTR: 42 ORF: 43 3' UTR: 60	Modified hIns - 2	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTC TCTTAAGGTAGCCTTGACAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA TGGCCCTGTGGATGAGACTCCTGCCCCTGCTGGCACTGCTGGCCCTCTGG GGACCTGACCCAGCAGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACA CACCCAGGACCAAGAGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG AGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA GGGGTCCAGACAGAAGAGAGGCATTGTGGAACAATGCTGTACCAGCATC TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC CTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT

		GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACACA ACAATTGCATTTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT AAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG AAGCTTTTG
Full: 3 5' UTR: 42 ORF: 44 3' UTR: 60	Modified hIns - 3	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTC TCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA <u>TGGCCCTGTGGATGAGGCTCCTGCCCCTGCTGGCCCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGGGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGAGAGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGAGCAGGCAGAAGAGAGGGGCATTGTGGAACAATGCTGTACCAGCATC</u> <u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAG</u> <u>AGCCAGCTGCCTTCC</u> CTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACACA ACAATTGCATTTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT AAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG AAGCTTTTG
Full: 4 5' UTR: 42 ORF: 45 3' UTR: 60	Modified hIns - 4	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTC TCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA <u>TGGCCCTGTGGATGAGGCTCCTGCCCCTGCTGGCTCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCAGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGGGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGAGAGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGAGCAGGCAGAAGAGAGGGCATTGTGGAACAATGCTGTACCAGCATC</u> <u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAG</u> <u>AGCCAGCTGCCTTCC</u>

		CTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT GATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAAACAAGTTAACAACA ACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT AAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG AAGCTTTTG
Full: 5 5' UTR: 42 ORF: 46 3' UTR: 60	Modified hIns - 5	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA <u>TGGCCCTGTGGATGAGACTGCTCCCCCTCCTGGCTTTGCTGGCCCTGTGGG</u> <u>GACCAGATCCTGCAGCTGCCTTTGTGAATCAGCACCTGTGTGGCTCAGAT</u> <u>CTGGTTGAGGCACTCTACCTGGTGTGTGGGGAGAGAGGCTTCTTTACAC</u> <u>ACCAAGGACCAAGAGGGAGGCAGAGGATCTGCAGGTAGGTCAGGTGGA</u> <u>GCTGGGAGGTGGCCCTGGAGCAGGATCCCTGCAGCCCTTGGCACTGGAG</u> <u>GGCTCCAGACAGAAGAGGGGCATTGTGGAACAGTGTTGCACTAGCATCT</u> <u>GCAGCCTGTACCAGCTGGAGAAGTACTGCAACTAAAGCCAGCTGCCTTCC</u> CTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT GATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAAACAAGTTAACAACA ACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT AAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG AAGCTTTTG
Full: 6 5' UTR: 42 ORF: 47 3' UTR: 60	Modified hIns - 6	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA <u>TGGCCCTGTGGATGCGCCTCCTGCCCCCTGCTGGCGCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAAAGAGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGTCGCGACAGAAGCGGGGCATTGTGGAACAATGCTGTACCAGCATC</u>

		<u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC</u> <u>CTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT</u> <u>GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT</u> <u>GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACACA</u> <u>ACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT</u> <u>AAAGCAAGTAAAACTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 7 5' UTR: 42 ORF: 48 3' UTR: 60	Modified hIns - 7	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTTAAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA <u>TGGCCCTGTGGATGCGCCTCCTGCCCCCTGCTGGCGCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCCGACGCTTTGTGAACCAACACCTGTGCGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAAAGAGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGTCGCGACAGAAGAGAGGCATTGTGGAACAATGCTGTACCAGCATC</u> <u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC</u> <u>CTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT</u> <u>GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT</u> <u>GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACACA</u> <u>ACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT</u> <u>AAAGCAAGTAAAACTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 8 5' UTR: 42 ORF: 49 3' UTR: 60	Modified hIns -8	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTTAAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA <u>TGGCCCTGTGGATGAGACTCCTGCCCCCTGCTGGCTCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGAGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u>

		GGGGTCTAGACAGAAGAGGGGCATTGTGGAACAATGCTGTACCAGCATC TGCTCCCTCTACCAGCTGGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC CTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT GATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAACAAGTTAACAACA ACAATTGCATTTCATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT AAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAG AAGCTTTTG
Full: 9 5' UTR: 42 ORF: 50 3' UTR: 60	Modified hIns - 9	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTC TCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCCTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA TGGCCCTGTGGATGAGACTCCTGCCCCTGCTGGCCCTGCTGGCCCTCTGG GGACCTGACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACA CACCCAGGACCAAGAGAGAGGCGAGAGGACCTGCAGGTGGGGCAGGTGG AGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA GGGGAGCAGACAGAAGAGAGGCAATTGTGGAACAATGCTGTACCAGCATC TGCTCCCTCTACCAGCTGGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC CTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT GGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT GATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAACAAGTTAACAACA ACAATTGCATTTCATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT AAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAG AAGCTTTTG
Full: 10 5' UTR: 42 ORF: 51 3' UTR: 60	Modified hIns - 10	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTC TCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCCTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA TGGCCCTGTGGATGAGACTCCTGCCCCTGCTGGCTCTGCTGGCCCTCTGG GGACCTGACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACA CACCCAGGACCAAGAGGGGAGGCGAGAGGACCTGCAGGTGGGGCAGGTGG

		<u>AGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGAGTAGACAGAAGAGGGGCATTGTGGAACAATGCTGTACCAGCATC</u> <u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC</u> <u>CTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT</u> <u>GGACAAACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT</u> <u>GATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTAACAACA</u> <u>ACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT</u> <u>AAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 11 5' UTR: 42 ORF: 52 3' UTR: 60	Modified hIns - 11	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTTAAAGGTAGCCTTGCAGAAAGTTGGTCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA <u>TGGCCCTGTGGATGAGACTCCTGCCCCTGCTGGCTCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGAGAGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGTGGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGTCTAGACAGAAGAGAGGCATTGTGGAACAATGCTGTACCAGCATC</u> <u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC</u> <u>CTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT</u> <u>GGACAAACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT</u> <u>GATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTAACAACA</u> <u>ACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTT</u> <u>AAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 12 5' UTR: 42 ORF: 53 3' UTR: 60	Modified hIns -12	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTTAAAGGTAGCCTTGCAGAAAGTTGGTCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA <u>TGGCCCTGTGGATGCGGCTCTTGCCCCTTCTGGCTTTACTGGCACTATGGG</u> <u>GCCCTGACCCAGCGGCCGCTTTCGTGAACCAGCACCTCTGCGGAAGCGAT</u> <u>CTGGTCGAGGCCCTGTACCTGGTTTGTGGGGAAAGAGGTTTTTTCTATACC</u>

		CCGAGGACAAAGCGCGAGGCAGAAAGACCTTCAAGTGGGCCAGGTAGAGC TCGGAGGGGGCCCCGGTGCCGGATCCCTGCAGCCTCTGGCTCTCGAAGGG TCTCGACAAAAACGTGGCATCGTGGAGCAGTGCTGTACTTCAATTTGCAG TTTGTACCAGCTTGAGAATTATTGTAAGTGAAGCCAGCTGCCTTCCCTTTAC TGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAA ACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTA TTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAACAATTG CATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAA GTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAGAAGCTTT TG
Full: 13 5' UTR: 42 ORF: 54 3' UTR: 60	Modified hIns - 13	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGAAGTCT TCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA TGGCCCTGTGGATGCGGCTCTTGCCCTTTCTGGCTTTACTGGCACTATGGG GCCCTGACCCAGCGGCCGCTTTTGTGAACCAGCACCTCTGCGGATCTGAT CTGGTTGAGGCCTTGTACCTGGTTTGTGGGGAAAGAGGTTTTTCTATACC CCAAGGACAAAGCGCGAGGCAGAAAGACCTTCAAGTGGGCCAGGTAGAGC TGGGAGGGGGCCCCGGTGACAGGATCCCTGCAGCCTCTGGCTCTAGAAGG GTCTAGACAAAAAAGGGGCATAGTGGAGCAGTGCTGTACTTCAATTTGCA GTTTGTACCAGCTTGAGAATTATTGTAAGTGAAGCCAGCTGCCTTCCCTTTA CTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACA AACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCT ATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAACAATT GCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCA AGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAGAAGCTT TTG
Full: 14 5' UTR: 42 ORF: 55 3' UTR: 60	Modified hIns - 14	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGAAGTCT TCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA TGGCCCTGTGGATGCGGCTCTTGCCCTTTCTGGCTTTACTGGCACTATGGG GCCCTGACCCAGCGGCCGCTTTTGTGAACCAGCACCTCTGCGGATCTGAT

		CTGGTGGAGGCCTTGACCTGGTTTGTGGGGAAAGAGGTTTTTCTATAC CCCCAGGACAAAGCGCGAGGCAGAAGACCTTCAAGTGGGCCAGGTAGAG CTGGGAGGGGGCCCGGTGCAGGATCCCTGCAGCCTCTGGCTCTGGAAG GGTCTAGACAAAAAAGGGGCATAGTGGAGCAGTGCTGTACTTCAATTTGC AGTTTGTACCAGCTTGAGAATTATTGTAAGTGAAGCCAGCTGCCTTCCCTTT ACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTGGAC AAACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGC TATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTAACAACAACAATT GCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCA AGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAGAAGCTT TTG
Full: 15 5' UTR: 42 ORF: 56 3' UTR: 60	Modified hIns - 15	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAAGTTAAGCTGCAGTGAAGTCT TCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCCTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA TGGCCCTGTGGATGAGACTCTTGCCCTTCTGGCTTTACTGGCACTATGGG GCCCTGACCCAGCAGCTGCTTTTGTGAACCAGCACCTCTGTGGATCTGAT CTGGTTGAGGCCTTGACCTGGTTTGTGGGGAAAGAGGTTTTTCTATACC CCAAGGACAAAGAGGGGAGGCAGAAGACCTTCAAGTGGGCCAGGTAGAG CTGGGAGGGGGCCCTGGTGCAGGATCCCTGCAGCCTCTGGCTCTAGAAGG GTCTAGACAAAAAAGGGGCATAGTGGAGCAGTGCTGTACTTCAATTTGCA GTTTGTACCAGCTTGAGAATTATTGTAAGTGAAGCCAGCTGCCTTCCCTTTA CTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTGGACA AACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGC ATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTAACAACAACAATT GCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCA AGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAGAAGCTT TTG
Full: 16 5' UTR: 42 ORF: 57 3' UTR: 60	Modified hIns - 16	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAAGTTAAGCTGCAGTGAAGTCT TCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCCTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA TGGCCCTGTGGATGAGACTCTTGCCCTTCTGGCTTTACTGGCACTATGGG

		<u>GCCCTGACCCAGCAGCTGCTTTTGTGAACCAGCACCTCTGTGGATCTGAT</u> <u>CTGGTGGAGGCCCTGTACCTGGTTTGTGGGGAAAGAGGTTTTTCTATAC</u> <u>CCCCAGGACAAAGAGAGAGAGGCAGAAAGACCTTCAAGTGGGCCAGGTAGAG</u> <u>CTGGGAGGGGGCCCAGGTGCAGGATCCCTGCAGCCTCTGGCTCTGGAAG</u> <u>GGTCTAGACAAAAAAGAGGCATAGTGGAGCAGTGCTGTACTTCAATTTGC</u> <u>AGTTTGTACCAGCTTGAGAATTATTGTAAGTGAAGCCAGCTGCCTTCCCTTT</u> <u>ACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGAC</u> <u>AAACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGC</u> <u>TATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAACAATT</u> <u>GCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCA</u> <u>AGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCAGAAGCTT</u> <u>TTG</u>
Full: 84 5' UTR: 83 ORF: 43 3' UTR: 97	Modified hIns – 17	AAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAG TGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAGTTGGTCGTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTC GAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGAGACTCCTGCCCTGCTGGCACTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCAGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGATCTGGTG</u> <u>GAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGAGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>AGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCC</u> <u>AGACAGAAGAGAGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAAGTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC</u> <u>CACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT</u> <u>GCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCA</u> <u>TTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAAGT</u> <u>AAAACCTCTACAAATGTGGTAAAATAAGGGACTAGAGCAGGGATCCAAGCTT</u>
Full: 85 5' UTR: nucleic acids 5-329 of 42 ORF: 43 3' UTR: 98	Modified hIns – 18	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTC GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGAGACTCCTGCCCTGCTGGCACTGCTGGCCCTCTGGGGACCTG</u>

		<u>ACCCAGCAGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGATCTGGTG</u> <u>GAAAGCTCTCTACCTAGTGTGTGGGAAAGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGAGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>AGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCC</u> <u>AGACAGAAGAGAGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC</u> <u>CACAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT</u> <u>GCTTTATTTGTAACCAATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCA</u> <u>TTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAAGT</u> <u>AAAACCTCTACAAATGTGGTAAAATAAGGGACTAGAGCAGGAATTCAAGCTT</u>
Full: 86 5' UTR: nucleic acids 5-329 of 42 ORF: 46 3' UTR: 99	Modified hIns – 19	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGACAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCATGGCCCT <u>GTGGATGAGACTGCTCCCCCTCCTGGCTTTGCTGGCCCTGTGGGGACCAG</u> <u>ATCCTGCAGCTGCCTTTGTGAATCAGCACCTGTGTGGCTCAGATCTGGTTG</u> <u>AGGCACTCTACCTGGTGTGTGGGAGAGAGGCTTCTTTTACACACCAAGG</u> <u>ACCAAGAGGGAGGCAGAGGATCTGCAGGTAGGTCAGGTGGAGCTGGGAG</u> <u>GTGGCCCTGGAGCAGGATCCCTGCAGCCCTTGGCACTGGAGGGCTCCAGA</u> <u>CAGAAGAGGGGCATTGTGGAACAGTGTTGCACTAGCATCTGCAGCCTGTA</u> <u>CCAGCTGGAGAACTACTGCAACTAAAGCCAGCTGCCTTCCCTTTACTGTGG</u> <u>GTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAC</u> <u>AACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCT</u> <u>TTATTTGTAACCAATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTC</u> <u>ATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAAGTAAA</u> <u>ACCTCTACAAATGTGGTAAAATAAGGGACTAGAGCAGCATATGAAGCTT</u>
Full: 87 5' UTR: nucleic acids 5-329 of 42 ORF: 47 3' UTR: 100	Modified hIns – 20	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGACAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCATGGCCCT <u>GTGGATGCGCCTCCTGCCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCAGATCTGGTG</u>

		<u>GAAGCTCTCTACCTAGTGTGCGGGGAAAGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>CGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCTG</u> <u>CGACAGAAGCGGGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC CACAAC TAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT GCTTTATTTGTAACCATTAAGCTGCAATAAA CAAGTTAACAACAACAATTGCA TTCATTTTATGTTTCAGGTT CAGGGGAGATGTGGGAGGTTTTTAAAGCAAGT AAAACCTCTACAAATGTGGTAAAAAAGGGACTAGAGCAGGATATCAAGCTT
Full: 88 5' UTR: nucleic acids 5-329 of 42 ORF: 49 3' UTR: 101	Modified hIns – 21	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGAGACTCCTGCCCTTGCTGGCTCTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCAGATCTGGTG</u> <u>GAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGAGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>AGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCT</u> <u>AGACAGAAGAGGGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC CACAAC TAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT GCTTTATTTGTAACCATTAAGCTGCAATAAA CAAGTTAACAACAACAATTGCA TTCATTTTATGTTTCAGGTT CAGGGGAGATGTGGGAGGTTTTTAAAGCAAGT AAAACCTCTACAAATGTGGTAAAAAAGGGACTAGAGCAGACTAGTAAGCTT
Full: 123 5' UTR: nucleic acids 5-329 of 42 ORF: 110 3' UTR: 60	Modified hIns – 22	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGCGCCTCCTGCCCTTGCTGGCGCTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCCGAGCCTTTGTGAACCAACACCTGTGCGGCTCAGATCTGGTG</u> <u>GAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAG</u>

		<u>GACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>CGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCTG</u> <u>CGACAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC</u> <u>CACAACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT</u> <u>GCTTTATTTGTAACCAATTATAAGCTGCAATAAAACAAGTTAACACAACAATTGCA</u> <u>TTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTTAAAGCAAGT</u> <u>AAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAGAAAGCTTTTG</u>
Full: 127 5' UTR: nucleic acids 5-329 of 42 ORF: 111 3' UTR: 60	Unmodified hIns – 2 (B10H)	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGACAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTG</u> <u>GAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>CGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCTG</u> <u>CGACAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC</u> <u>CACAACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT</u> <u>GCTTTATTTGTAACCAATTATAAGCTGCAATAAAACAAGTTAACACAACAATTGCA</u> <u>TTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTTAAAGCAAGT</u> <u>AAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAGAAAGCTTTTG</u>
Full: 128 5' UTR: nucleic acids 5-329 of 42 ORF: 112 3' UTR: 60	Modified hIns – 27	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGACAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTG</u> <u>GAAGCTCTCTACCTAGTGTGCGGGGAAGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u>

		<u>CGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCTG</u> <u>CGACAGAAGCGGGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC</u> <u>CACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT</u> <u>GCTTTATTTGTAACCAATTATAAGCTGCAATAAAACAAGTTAACAACAACAATTGCA</u> <u>TTCAITTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAAGT</u> <u>AAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAGAAGCTTTTTG</u>
Full: 129 5' UTR: nucleic acids 5-329 of 42 ORF: 113 3' UTR: 60	Modified hIns – 28	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTC GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGGCCCT <u>GTGGATGAGACTCCTGCCCCCTGCTGGCTCTGCTGGCCCTCTGGGGACCTG</u> <u>ACCCAGCTGCAGCCTTTGTGAACCAACACCTGTGTGGCTCACACCTGGTG</u> <u>GAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTCTTCTACACACCCAG</u> <u>GACCAAGAGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGGAGCTGGG</u> <u>AGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGAGGGGTCT</u> <u>AGACAGAAGAGGGGCATTGTGGAACAATGCTGTACCAGCATCTGCTCCCT</u> <u>CTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAAC</u> <u>CACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT</u> <u>GCTTTATTTGTAACCAATTATAAGCTGCAATAAAACAAGTTAACAACAACAATTGCA</u> <u>TTCAITTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAAGT</u> <u>AAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAGAAGCTTTTTG</u>
Full: 133 5' UTR: nucleic acids 5-329 of 42 ORF: 117 3' UTR: 60	Modified hIns – 32	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTC GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA <u>GCTTCAGCACAAAGCGCCTTCGGCCCCGTGGCTTTCTCTCTGGGACTGCTGC</u> <u>TGGTGCTGCCTGCCGCTTTCCCTGCCCCCTTTGTGAACCAACACCTGTGCG</u> <u>GCTCAGATCTGGTGGAAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTC</u> <u>TTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGC</u> <u>AGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC</u>

		<u>CCTGGAGGGGTCGCGACAGAAGCGTGGCATTGTGGAACAATGCTGTACC</u> <u>AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCT</u> <u>GCCTTCCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGA</u> <u>TGAGTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGA</u> <u>AATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTA</u> <u>ACAACAACAATTGCATTCATTTTATGTTTCAGGTT CAGGGGGAGATGTGGGAGG</u> <u>TTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 134 5' UTR: nucleic acids 5-329 of 42 ORF: 118 3' UTR: 60	Modified hIns – 33	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAAC TTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA <u>GCTTCAGCACAAAGCGCCTTTGGCCCAGTGGCTTTCTCTCTGGGACTGCTGC</u> <u>TGGTGCTGCCTGCCGCTTTCCCTGCCCCTTTTGTGAACCAACACCTGTGCG</u> <u>GCTCAGATCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAAAGAGGCTTC</u> <u>TTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGC</u> <u>AGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC</u> <u>CCTGGAGGGGTCGCGACAGAAGCGGGGCATTGTGGAACAATGCTGTACC</u> <u>AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCT</u> <u>GCCTTCCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGA</u> <u>TGAGTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGA</u> <u>AATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTA</u> <u>ACAACAACAATTGCATTCATTTTATGTTTCAGGTT CAGGGGGAGATGTGGGAGG</u> <u>TTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 135 5' UTR: nucleic acids 5-329 of 42 ORF: 119 3' UTR: 60	Modified hIns – 34	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAAC TTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA <u>GCTTCAGCACAAAGTGCCTTTGGCCCAGTGGCTTTCTCTCTGGGACTGCTGC</u> <u>TGGTGCTGCCTGCAGCTTTCCCTGCCCCTTTTGTGAACCAACACCTGTGTG</u> <u>GCTCAGATCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTC</u> <u>TTCTACACACCCAGGACCAAGAGAGAGGCAGAGGACCTGCAGGTGGGGC</u>

		<u>AGGTGGAGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC</u> <u>CCTGGAGGGGTCCAGGCAGAAGAGAGGCATTGTGGAACAATGCTGTACC</u> <u>AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCT</u> <u>GCCTTCCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGA</u> <u>TGAGTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGA</u> <u>AATTTGTGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTA</u> <u>ACAACAACAATTGCATTCAATTTATGTTTCAGGTT CAGGGGGAGATGTGGGAGG</u> <u>TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 136 5' UTR: nucleic acids 5-329 of 42 ORF: 120 3' UTR: 60	Modified hIns – 35	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTG CAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA <u>GCTTCAGCACAAAGCGCCTTCGGCCCCGTGGCTTTCTCTCTGGGACTGCTGC</u> <u>TGGTGCTGCCTGCCGCTTTCCCTGCCCCCTTTGTGAACCAACACCTGTGCG</u> <u>GCTCACACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTC</u> <u>TTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGC</u> <u>AGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC</u> <u>CCTGGAGGGGTGCGGACAGAAGCGTGGCATTGTGGAACAATGCTGTACC</u> <u>AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCT</u> <u>GCCTTCCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGA</u> <u>TGAGTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGA</u> <u>AATTTGTGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTA</u> <u>ACAACAACAATTGCATTCAATTTATGTTTCAGGTT CAGGGGGAGATGTGGGAGG</u> <u>TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 137 5' UTR: nucleic acids 5-329 of 42 ORF: 121 3' UTR: 60	Modified hIns – 36	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTG CAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA <u>GCTTCAGCACAAAGCGCCTTTGGCCCAAGTGGCTTTCTCTCTGGGACTGCTGC</u> <u>TGGTGCTGCCTGCCGCTTTCCCTGCCCCCTTTGTGAACCAACACCTGTGCG</u> <u>GCTCACACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAAAGAGGCTTC</u>

		TTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGC AGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC CCTGGAGGGGTGCGGACAGAAGCGGGGCATTGTGGAACAATGCTGTACC AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCT GCCTTCCCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGA TGAGTTTGGACAAACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGA AATTTGTGATGCTATTGCTTTATTTGTAACCAATTATAAGCTGCAATAAACAAGTTA ACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGG TTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAG AGCAGAAGCTTTTG
Full: 138 5' UTR: nucleic acids 5-329 of 42 ORF: 122 3' UTR: 60	Modified hIns – 37	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA GCTTCAGCACAAAGTGCCCTTTGGCCCAAGTGGCTTTCTCTCTGGGACTGCTGC TGGTGCTGCCTGCAGCTTTCCCTGCCCCCTTTGTGAACCAACACCTGTGTG GCTCACACCTGGTGGAAGCTCTCTACCTAGTGTGTGGGGAAAGAGGCTTC TTCTACACACCCAGGACCAAGAGAGAGAGGCAGAGGACCTGCAGGTGGGGC AGGTGGAGCTGGGAGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC CCTGGAGGGGTCCAGGCAGAAGAGAGGCATTGTGGAACAATGCTGTACC AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCT GCCTTCCCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGA TGAGTTTGGACAAACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGA AATTTGTGATGCTATTGCTTTATTTGTAACCAATTATAAGCTGCAATAAACAAGTTA ACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGG TTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAG AGCAGAAGCTTTTG
Full: 160 5' UTR: 148 ORF: 110	Unmodified hIns_2 (B10D)	AAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAG TGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGTGACTCTCTTAA GGTAGCCTTGCAGAAAGTTGGTCGTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTACTTAATACGACTCACTATAGGCTA GCCTCGAGAATTCTGCCATGGCCCTGTGGATGCGCCTCTGCCCTGCT GGCGCTGCTGGCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACC

		<u>AACACCTGTGCGGCTCAGATCTGGTGGAAGCTCTCTACCTAGTGTGCGGG</u> <u>GAACGAGGCTTCTTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACC</u> <u>TGCAGGTGGGGCAGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCT</u> <u>GCAGCCCTTGGCCCTGGAGGGGTCGCGACAGAAGCGTGGCATTGTGGAA</u> <u>CAATGCTGTACCAGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAA</u> <u>CTAG</u>
Full: 161 5' UTR: 42 ORF – 110 3' UTR - 60	Unmodified hIns_3 (B10D)	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA TCTTAAGGTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTA AGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGG CTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTG GTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA <u>TGGCCCTGTGGATGCGCCTCCTGCCCCCTGCTGGCGCTGCTGGCCCTCTGG</u> <u>GGACCTGACCCAGCCGACGCCCTTTGTGAACCAACACCTGTGCGGCTCAGA</u> <u>TCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACA</u> <u>CACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGCAGGTGG</u> <u>AGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGCCCTGGA</u> <u>GGGGTCGCGACAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATC</u> <u>TGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAGAGCCAGCTGCCTTCC</u> <u>CTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT</u> <u>GGACAAACCACA</u> <u>ACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT</u> <u>GATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAACAAGTTAACAACA</u> <u>ACAATTGCATTCA</u> <u>TTTTATGTTTCAGGTT</u> <u>CAGGGG</u> <u>GAGATGTGGGAGGTTTTTT</u> <u>AAAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 170 5' UTR: nucleic acids 5-329 of 42 ORF: 121 3' UTR: 171	Modified hIns_41	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCAG TGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA GCTAGCCTTGCAGAAAGTTGGTCCTGAGGCACTGGGCAGGTAAGTATC AAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCTTGTG GAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGGTCTTA CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCA ATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCATGAACA <u>GCTTCAGCACAAAGCGCCTTTGGCCCAGTGGCTTTCTCTCTGGGACTGCTGC</u> <u>TGGTGCTGCCTGCCGCTTTCCCTGCCCCCTTTGTGAACCAACACCTGTGCG</u> <u>GCTCACACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAAAGAGGCTTC</u> <u>TTCTACACACCCAGGACCAAGCGGGAGGCAGAGGACCTGCAGGTGGGGC</u> <u>AGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCTGCAGCCCTTGGC</u>

		<u>CCTGGAGGGGTCGCGACAGAAGCGGGGCATTGTGGAACAATGCTGTACC</u> <u>AGCATCTGCTCCCTCTACCAGCTGGAGAACTACTGCAACTAG</u> <i>AGCCAGCT</i> <i>GCCTTCCCTT</i> <u>ACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGA</u> <i>TGAGTTTGGACAAACCACA</i> <u>ACTAGAATGCAGTGAAAAAATGCTTTATTTGTGA</u> <i>AATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTA</i> <i>ACAACAACAATTGCATTCA</i> <u>TTTTATGTTTCAGGTT</u> <i>CAGGGGGAGATGTGGGAGG</i> <i>TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAG</i> <i>AGCATGGCTACGTA</i>
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Table 9: Nucleic Acid Sequences Encoding Glucokinase

SEQ ID NOs	Name	Sequence
Full: 19 5' UTR: 148 ORF: 162 3' UTR: 149	Wildtype hGck	AAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCA GTGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGTGAAGTCTCTT AAGGTAGCCTTGACAGAGTTGGTCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCT TGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGT CTTACTGACATCCACTTTGCGTTTCTCTCCACAGGTGTCCACTCCCA GTTCAATTACAGCTCTTAAGGCTAGAGTACTTAATACGACTCACTAT AGGCTAGCCTCGAGAATTCTGCCATGGCGATGGATGTCACAAGGAGC CAGGCCCAGACAGCCTTGACTCTGGTAGAGCAGATCCTGGCAGAGTTCC AGCTGCAGGAGGAGGACCTGAAGAAGGTGATGAGACGGATGCAGAAGG AGATGGACCGCGGCTGAGGCTGGAGACCCATGAAGAGGCCAGTGTGA AGATGCTGCCACCTACGTGCGCTCCACCCAGAAGGCTCAGAAGTCGG GGACTTCCTCTCCCTGGACCTGGGTGGCACTAACTTCAGGGTGATGCTGG TGAAGGTGGGAGAAGGTGAGGAGGGGAGTGGAGCGTGAAGACCAAAC ACCAGATGTACTCCATCCCCGAGGACGCCATGACCGGCACTGCTGAGAT GCTCTTCGACTACATCTCTGAGTGCATCTCCGACTTCCTGGACAAGCATC AGATGAAACACAAGAAGCTGCCCCTGGGCTTCACCTTCTCCTTTCCTGTG AGGCACGAAGACATCGATAAGGGCATCCTTCTCAACTGGACCAAGGGCT TCAAGGCCTCAGGAGCAGAAGGGAACAATGTCGTGGGGCTTCTGCGAG ACGCTATCAAACGGAGAGGGGACTTTGAAATGGATGTGGTGGCAATGGT GAATGACACGGTGGCCACGATGATCTCCTGCTACTACGAAGACCATCAG TGCGAGGTCGGCATGATCGTGGGCACGGGCTGCAATGCCTGCTACATGG AGGAGATGCAGAATGTGGAGCTGGTGGAGGGGACGAGGGCCGCATGT GCGTCAATACCGAGTGGGGCGCCTTCGGGGACTCCGGCGAGCTGGACGA GTTCTCTGCTGGAGTATGACCGCCTGGTGGACGAGAGCTCTGCAAACCCC GGTCAGCAGCTGTATGAGAAGCTCATAGGTGGCAAGTACATGGGCGAGC TGGTGCGGCTTGTGCTGCTCAGGCTCGTGGACGAAAACCTGCTCTTCCAC GGGAGGGCTCCGAGCAGCTGCGCACACGCGGAGCCTTCGAGACGCGCT TCGTGTCGCAGGTGGAGAGCGACACGGGCGACCGCAAGCAGATCTACA ACATCCTGAGCACGCTGGGGCTGCGACCCTCGACCACCGACTGCGACAT CGTGCGCCGCGCCTGCGAGAGCGTGTCTACGCGCGCTGCGCACATGTGC TCGGCGGGGCTGGCGGGCGTCATCAACCGCATGCGCGAGAGCCGCAGC GAGGACGTAATGCGCATCACTGTGGGCGTGGATGGCTCCGTGTACAAGC TGACCCCCAGCTTCAAGGAGCGGTTCCATGCCAGCGTGCGCAGGCTGAC GCCCAGCTGCGAGATCACCTTCATCGAGTCGAGGAGGGCAGTGGCCGG GGCGCGGCCCTGGTCTCGGCGGTGGCCTGTAAGAAGGCCTGTATGCTGG

		<u>GCCAGTGAACGCAGCTGCTAGCTAATCGATACCGTCGACCTCGAGGAATTCA</u> <u>CGCGTGGTACCTCTAGAGTCGACCCGGGCGGCCGCTTCCCTTTAGTGAGGG</u> <u>TTAATGCTTCGAGCAGACATGATAAGATACATTGATGAGTTTGGACAAACCAC</u> <u>AACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGC</u> <u>TTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAAACAATTGCATT</u> <u>CATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCAAGTA</u> <u>AAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAGAAGCTTTTG</u>
Full: 20 5' UTR: 42 ORF: 61 3' UTR: 60	Modified hGck - 2	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC <u>CACCATGGCCATGGATGTCACAAGGAGCCAGGCCAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG</u> <u>GTCCACCCCAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTCACCTTCTCATTTCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCCTCAGGAGCAGAA</u> <u>GGGAACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACCA</u> <u>TGATCTCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGT</u> <u>GGGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGATGAGGGCAGGATGTGTGTCAATACAGAGTGGGG</u> <u>AGCCTTTGGGGACTCTGGGGAGCTGGATGAGTTCTGCTGGAGTATGAC</u> <u>AGGCTGGTGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGGCTTGTGCTGCT</u> <u>CAGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAG</u> <u>CTGAGGACAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCAGGTGGAGA</u> <u>GTGACACAGGAGACAGGAAGCAGATCTACAACATCCTGAGCACCTGG</u> <u>GGCTGAGACCTCCACCACAGACTGTGACATTGTGAGGAGGGCTTGTGA</u> <u>GAGTGTGTCTACCAGGGCTGCCACATGTGCTCTGCTGGGCTGGCTGGG</u> <u>GTCATCAACAGGATGAGGGAGAGCAGGAGTGAGGATGTAATGAGGATC</u>

		<u>ACTGTGGGAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGG</u> <u>AGAGGTTCCATGCCAGTGTGAGGAGGCTGACCCCAGCTGTGAGATCAC</u> <u>CTTTATTGAGTCTGAGGAGGGCAGTGGCAGGGGAGCAGCCCTGGTCTCT</u> <u>GCTGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <i>AGCCAGCTGC</i> <i>CTTCCCTTACTGTGGGTAAATGCTTG</i> CAGCAGACATGATAAGATACATTGATG <i>AGTTTGGACAAACCACA</i> ACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAA <i>TTTGTGATGCTATTGCTTTATTTGTA</i> ACCATTATAAGCTGCAATAAACAAAGTTAA <i>CAACAACAATTGCATTCA</i> TTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGG <i>TTTTTAAAGCAAGTAA</i> ACCTCTACAAATGTGGTAAATCCGATAAGGGACTA <i>GAGCAGAAGCTTTTG</i>
Full: 21 5' UTR: 42 ORF: 62 3' UTR: 60	Modified hGck-3	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCA ACTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGT CCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC CACC <u>ATGGCCATGGATGTCACAAGGAGCCAGGCC</u> CAGACAGCCTTGACT <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG</u> <u>GTCCACCC</u> CAGAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTG <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGTCTGTGAAGACCAAAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTCACCTTCTCATTTCCCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAA</u> <u>GGGAACAATGTGGTGGGGCTTCTGAGGGATGCTATCAAAAGGAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACAA</u> <u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGT</u> <u>GGGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGATGAGGGCAGAATGTGTGTCAATACAGAGTGGGG</u> <u>AGCCTTTGGG</u> ACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGAC <u>AGACTGGTGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGACTTGTGCTGCT</u> <u>CAGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAG</u> <u>CTGAGAACAAGAGGAGCCTTTGAGACCAGATTTGTGTCCAGGTGGAGT</u> <u>CTGACACAGGAGACAGGAAGCAGATCTACAACATCCTGAGCACCCCTGG</u>

		<u>GGCTGAGGCCCTCCACCACAGACTGTGACATTGTGAGAAGAGCCTGTGA</u> <u>GTCTGTGTCTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCTGGGG</u> <u>TCATCAACAGAATGAGGGAGAGCAGGTCTGAGGATGTAATGAGAATCA</u> <u>CTGTGGGAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGA</u> <u>GAGATTCCATGCCTCTGTGAGAAGGCTGACCCCCAGCTGTGAGATCACC</u> <u>TTTATTGAGTCTGAGGAGGGCAGTGGCAGAGGAGCTGCCCTGGTCTCTG</u> <u>CTGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCT</u> <u>TCCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGA</u> <u>GTTTGGACAAACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAAT</u> <u>TTGTGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAACAAAGTTAAC</u> <u>AACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGT</u> <u>TTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 22 5' UTR: 42 ORF: 63 3' UTR: 60	Modified hGck-4	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTCTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCCTGCAGAATTGC <u>CACCATGGCCATGGATGTCACAAGGAGCCAGGCCAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCACCTATGTGAG</u> <u>GTCCACCCAGAAAGGCTCAGAAGTGGGGGACTTCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGTGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACTGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAG</u> <u>TGCATCTCAGACTTCTTGACAAGCATCAGATGAAACACAAGAAGCTGC</u> <u>CCCTGGGCTTCACCTTCTCCTTTCCTGTGAGGCATGAAGACATTGATAAG</u> <u>GGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAG</u> <u>GGAACAATGTTGTGGGGCTTCTGAGGGATGCTATCAAAAGGAGAGGGG</u> <u>ACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACTAT</u> <u>GATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTAGGCATGATTGTG</u> <u>GGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGC</u> <u>TGGTGGAGGGGGATGAGGGCAGAATGTGTGTCAATACTGAGTGGGGTG</u> <u>CCTTTGGGGACTCAGGGGAGCTGGATGAGTTCCTGCTGGAGTATGACAG</u> <u>ACTGGTGGATGAGAGCTCTGCAAACCTGGTCAGCAGCTGTATGAGAAG</u> <u>CTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGACTTGTGCTGCTCA</u>

		<u>GGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGCT</u> <u>GAGAACAAGAGGAGCCTTTGAGACCAGATTTGTGTCCCAGGTGGAGTCT</u> <u>GACACTGGTGACAGGAAGCAGATCTACAACATCCTGAGCACACTGGGGC</u> <u>TGAGGCCCTCCACCACAGACTGTGACATTGTGAGAAGAGCTTGTGAGAG</u> <u>TGTGTCTACCAGGGCTGCTCACATGTGCAGTGCTGGGCTGGCTGGGGTC</u> <u>ATCAACAGAATGAGGGAGAGCAGGTCTGAGGATGTAATGAGAATCACT</u> <u>GTGGGTGTGGATGGCAGTGTGTACAAGCTGCACCCCTCATTCAAGGAGA</u> <u>GATTCCATGCCAGTGTGAGAAGGCTGACCCCCAGCTGTGAGATCACCTT</u> <u>CATTGAGTCTGAGGAGGGCAGTGGCAGGGGTGCAGCCCTGGTCAGTGCA</u> <u>GTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <u>AGCCAGCTGCCCTTC</u> <u>CCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTT</u> <u>TGGACAAACCACA</u> <u>ACTAG</u> <u>AATGCAGTG</u> <u>AAAAAAATGCTTTATTTGTG</u> <u>AAATTTG</u> <u>TGATGCTATTGCTTTATTTGTA</u> <u>ACCATTA</u> <u>AGCTGCA</u> <u>ATAAA</u> <u>CAAGTTA</u> <u>ACAAC</u> <u>AACAATTGCATT</u> <u>CATTTATGTTT</u> <u>CAGGTT</u> <u>CAGGGG</u> <u>GAGATGTGGG</u> <u>AGGTTTTT</u> <u>TAAAGCAAGT</u> <u>AAAACCTCT</u> <u>ACAAATGTGGT</u> <u>AAAATCCGATAAGGG</u> <u>ACTAGAGC</u> <u>AGAAGCTTTTG</u>
Full: 23 5' UTR: 42 ORF: 64 3' UTR: 60	Modified hGck-5	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCA ACTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGT CCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTGCAGAATTGC CACC <u>ATGGCCATGGATGTCACAAGGAGCCAGGCC</u> <u>CAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG</u> <u>GTCCACCC</u> <u>CAGAAGGCTCAGAAGTGGGGGACTTCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGTCTGTGAAGACCAAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACTGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAG</u> <u>TGCATCTCAGACTTCTTGACAAGCATCAGATGAAACACAAGAAGCTGC</u> <u>CCCTGGGCTTCACCTTCTCCTTTCTGTGAGGCATGAAGACATAGATAAG</u> <u>GGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAG</u> <u>GGAACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGGG</u> <u>ACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACTAT</u> <u>GATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATAGTG</u> <u>GGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGC</u> <u>TGGTGGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGGAG</u>

		<u>CCTTTGGGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGACAG</u> <u>GCTGGTGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGAAG</u> <u>CTCATAGGTGGCAAGTACATGGGGGAGCTGGTGAGGCTTGTGCTGCTCA</u> <u>GGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGCT</u> <u>GAGAACAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGTCT</u> <u>GACACTGGGGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGG</u> <u>CTGAGGCCCTCCACCACTGACTGTGACATAGTGAGGAGAGCCTGTGAGT</u> <u>CTGTGTCTACCAGGGCTGCCACATGTGCTCTGCTGGGCTGGCAGGGGT</u> <u>CATCAACAGGATGAGGGAGAGCAGATCTGAGGATGTAATGAGGATCAC</u> <u>TGTGGGTGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAG</u> <u>AGGTTCATGCCTCTGTGAGGAGGCTGACCCCAGCTGTGAGATCACCT</u> <u>TTATAGAGTCTGAGGAGGGCAGTGGCAGAGGGGCAGCCCTGGTCTCTGC</u> <u>AGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <i>AGCCAGCTGCCTT</i> <i>CCCTTTACTGTGGGTTAATGCTTG CAGCAGACATGATAAGATACATTGATGAG</i> <i>TTTGGACAAACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATT</i> <i>TGTGATGCTATTGCTTTATTTGTAAACCATTATAAGCTGCAATAAAACAAGTTAACA</i> <i>ACAACAATTGCATTCAATTTATGTTTCAGGTT CAGGGGAGATGTGGGAGGTT</i> <i>TTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGA</i> <i>GCAGAAGCTTTTG</i>
Full: 24 5' UTR: 42 ORF: 65 3' UTR: 60	Modified hGck-6	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTG CAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC CACCATGGCCATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACT <u>CTGGTAGAGCAGATCCTGGCAGAGTTCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCACCTATGTGAG</u> <u>GTCCACCCAGAAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAAAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTCACCTTCTCATTTCCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAA</u> <u>GGGAACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACCA</u>

		<u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATAGT</u> <u>GGGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGG</u> <u>AGCCTTTGGGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGAC</u> <u>AGGCTGGTGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGGGAGCTGGTGAGGCTTGTGCTGCT</u> <u>CAGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCAGAGCAG</u> <u>CTGAGAACAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGA</u> <u>GTGACACAGGTGACAGGAAGCAGATCTACAACATCCTGAGCACCCCTGGG</u> <u>GCTGAGACCCCTCCACCACAGACTGTGACATTGTGAGGAGGGCCTGTGAG</u> <u>AGTGTGTCTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCAGGAG</u> <u>TCATCAACAGGATGAGGGAGAGCAGGAGTGAGGATGTAATGAGGATCA</u> <u>CTGTGGGAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTC AAGGA</u> <u>GAGGTTCCATGCCAGTGTGAGAAGGCTGACCCCCAGCTGTGAGATCACC</u> <u>TTTATAGAGTCTGAGGAGGGCAGTGGCAGGGGAGCAGCCCTGGTCTCTG</u> <u>CTGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><u>AGCCAGCTGCCT</u> <u>TCCCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGA</u> <u>GTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAAT</u> <u>TTGTGATGCTATTGCTTTATTTGTAACCATATAAGCTGCAATAACAAGTTAAC</u> <u>AACAACAATTGCATTTCATTTATGTTTCAGGTT CAGGGGGAGATGTGGGAGGT</u> <u>TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 25 5' UTR: 42 ORF: 66 3' UTR: 60	Modified hGck-7	<u>[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC</u> <u>AGTCAGTGCTTCTGACACAACAGTCTCCAAC TTAAGCTGCAGTGACT</u> <u>CTCTTAAGGTAGCCTTG CAGAAGTTGGTCCTGAGGCACTGGGCAGG</u> <u>TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT</u> <u>GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT</u> <u>ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA</u> <u>CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC</u> <u>CACC</u><u>ATGGCCATGGATGTCACAAGGAGCCAGGCC CAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG</u> <u>GTCCACCC CAGAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTACCTTCTCATTTCCCTGTGAGGCATGAAGACATTGATAA</u>

		<u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAA</u> <u>GGGAACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACAA</u> <u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATAGT</u> <u>GGGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGATGAGGGCAGAATGTGTGTCAATACAGAGTGGGG</u> <u>AGCCTTTGGGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGAC</u> <u>AGACTGGTGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGGCTTGTGCTGCT</u> <u>CAGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAG</u> <u>CTGAGGACAAGAGGAGCCTTTGAGACCAGATTTGTGTACAGGTGGAGT</u> <u>CTGACACTGGAGACAGAAAGCAGATCTACAACATCCTGAGCACACTGGG</u> <u>GCTGAGGCCCTCCACCACTGACTGTGACATAGTGAGGAGAGCTTGTGAG</u> <u>AGTGTGTCTACCAGAGCTGCCCACATGTGCTCTGCTGGGCTGGCTGGGG</u> <u>TCATCAACAGGATGAGAGAGAGCAGGAGTGAGGATGTAATGAGAATCA</u> <u>CTGTGGGGGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGA</u> <u>GAGGTTCCATGCCTCTGTGAGGAGGCTGACCCCAGCTGTGAGATCACC</u> <u>TTTATTGAGTCTGAGGAGGGCAGTGGAAGGGGAGCAGCCCTGGTCTCTG</u> <u>CAGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><i>AGCCAGCTGCC</i> <i>TTCCCTTACTGTGGGTTAATGCTTG</i>CAGCAGACATGATAAGATACATTGATGA <i>GTTTGGACAAACCACA</i>ACTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAAT <i>TTGTGATGCTATTGCTTTATTTGTA</i>ACCATTATAAGCTGCAATAAACAAAGTTAAC <i>AACAACAATTGCATT</i>CATTTATGTTTCAGGTTCAGGGGGAGATGTGGGAGGT <i>TTTTTAAAGCAAGTAA</i>ACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAG <i>AGCAGAAGCTTTTG</i>
Full: 26 5' UTR: 42 ORF: 67 3' UTR: 59	Modified hGck-8	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC CACCATGGCCATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACT <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG</u> <u>GTCCACCCCAGAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGTGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAAAACACCAGATGTACTCCATCCCTG</u>

		<u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTCACCTTCTCATTTCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCCTCAGGAGCAGAA</u> <u>GGGAACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTA</u> <u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGT</u> <u>GGGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAG</u> <u>CTGGTGGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGGA</u> <u>GCCTTTGGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGACA</u> <u>GGCTGGTGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGAA</u> <u>GCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGACTTGTGCTGCTC</u> <u>AGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCCTCAGAGCAGC</u> <u>TGAGGACAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGAG</u> <u>TGACACTGGAGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGG</u> <u>CTGAGGCCCTCTACCACAGACTGTGACATAGTGAGAAGGGCCTGTGAGT</u> <u>CTGTGTCTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCTGGAGTC</u> <u>ATCAACAGAATGAGGGAGAGCAGAAGTGAGGATGTAATGAGGATCACT</u> <u>GTGGGAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAGA</u> <u>GGTTCCATGCCAGTGTGAGGAGGCTGACCCCCAGCTGTGAGATCACCTT</u> <u>TATTGAGTCTGAGGAGGGCAGTGGCAGGGGAGCTGCCCTGGTCTCTGCT</u> <u>GTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCCTT</u> <u>CCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTT</u> <u>TGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTG</u> <u>TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAAACAAC</u> <u>AACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTTTTT</u> <u>TAAAGCAAGTAAAACTCTACAAATGTGGTAAATCCGATAAGGGACTAGAGC</u> <u>AGAAGCTTTTG</u>
Full: 27 5' UTR: 42 ORF: 68 3' UTR: 60	Modified hGck-9	<u>[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC</u> <u>AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT</u> <u>CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG</u> <u>TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT</u> <u>GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCCTGATAGGCACCT</u> <u>ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA</u> <u>CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC</u> <u>CACCATGGCTATGGATGTCACAAGGAGCCAGGCCAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACCGGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCACCTATGTGCG</u>

		<u>CTCCACCCCAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTCACCTTCTCCTTTCCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAA</u> <u>GGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTA</u> <u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTG</u> <u>GGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAG</u> <u>CTGGTGGAGGGGGATGAGGGCCGCATGTGTGTCAATACTGAGTGGGGCG</u> <u>CCTTTGGGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACCG</u> <u>CCTGGTGGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAA</u> <u>CTCATAGGTGGCAAGTACATGGGAGAGCTGGTGC GGCTTGTGCTGCTCA</u> <u>GGTTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAGCT</u> <u>GCGCACACGCGGAGCCTTTGAGACGCGCTTTGTGTCACAAGTGGAGTCT</u> <u>GACACAGGAGACCGCAAGCAGATCTACAACATCCTGAGCACACTGGGG</u> <u>CTGCGGCCCTCCACCACTGACTGTGACATTGTGCGCCGCGCTGTGAGA</u> <u>GTGTGTCTACGCGCGCTGCGCACATGTGCTCAGCGGGGCTGGCGGGAGT</u> <u>CATCAACCGCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACT</u> <u>GTGGGAGTGGATGGCAGTGTGTACAAGCTGCACCCAGCTTCAAGGAGC</u> <u>GGTTCATGCCTCAGTGCGCAGGCTGACCCCACTGTGAGATCACCTTT</u> <u>ATTGAGTCTGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCAGCGCG</u> <u>GTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><u>AGCCAGCTGCCTTC</u> <u>CCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGATT</u> <u>TGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG</u> <u>TGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAAACAAGTTAACAA</u> <u>ACAATTGCATTCAATTTATGTTTCAGGTTCAAGGGGAGATGTGGGAGGTTTTT</u> <u>TAAAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAGAGC</u> <u>AGAAGCTTTTG</u>
Full: 28 5' UTR: 42 ORF: 69 3' UTR: 60	Modified hGck-10	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCCTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTCTGCAGAATTGC CACCATGGCCATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACT

		<u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGCG</u> <u>CTCCACCCCAGAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTTCACCTTCTCCTTTCCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCCTCAGGAGCAGAA</u> <u>GGGAACAATGTGGTGGGGCTTCTGCGGGATGCTATCAAAAGAAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACCA</u> <u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGT</u> <u>GGGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAG</u> <u>CTGGTGGAGGGGGATGAGGGCCGCATGTGTGTCAATACAGAGTGGGGC</u> <u>GCCTTTGGGGACAGCGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACC</u> <u>GCCTGGTGGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAA</u> <u>GCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGCGGCTTGTGCTGCTC</u> <u>AGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGC</u> <u>TGCGCACACGCGGAGCCTTTGAGACGCGCTTTGTGAGCCAGGTGGAGAG</u> <u>TGACACAGGAGACCGCAAGCAGATCTACAACATCCTGAGCACCTGGGG</u> <u>CTGCGGCCCAGCACCACTGACTGTGACATTGTGCGCCGCGCCTGTGAGT</u> <u>CAGTGTCTACGCGCGCTGCGCACATGTGCAGCGCGGGGCTGGCGGGAGT</u> <u>CATCAACCGCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACT</u> <u>GTGGGAGTGGATGGCAGTGTGTACAAGCTGCACCCCAGCTTCAAGGAGC</u> <u>GGTTCCATGCCTCAGTGCGCAGGCTGACCCCCAGCTGTGAGATCACCTTT</u> <u>ATTGAGTCTGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCAGCGCG</u> <u>GTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCTTC</u> <u>CCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTT</u> <u>TGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTG</u> <u>TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAAC</u> <u>AACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTT</u> <u>TAAAGCAAGTAAAACTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGC</u> <u>AGAAGCTTTTG</u>
Full: 29 5' UTR: 42 ORF: 70 3' UTR: 60	Modified hGck-11	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTCTGTATAGGCACCT

		ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC CACCATGGCTATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACT CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCCTGAGGC TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGCG CTCCACCCCAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG GAGGGGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCCG GAGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTG AGTGCATCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCT GCCCCTGGGCTTCACCTTCTCCTTTCTGTGAGGCATGAAGACATTGATA AGGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGA AGGGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGG GGACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACT ATGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTAGGCATGATAG TGGGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA GCTGGTGGAGGGGGATGAGGGCCGCATGTGTGTCAATACTGAGTGGGGC GCCTTTGGGGACAGTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACC GCCTGGTGGATGAGAGCTCTGCAAACCCAGGTCAGCAGCTGTATGAGAA GCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGCGGCTTGTGCTGCTC AGGCTAGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCAGAGCAGC TGCGCACACGCGGAGCCTTTGAGACGCGCTTTGTGTCTCAAGTGGAGAG TGACACAGGTGACCGCAAGCAGATCTACAACATCCTGAGCACTCTGGGG CTGCGGCCCTCAACCACAGACTGTGACATTGTGCGCCGCGCCTGTGAGT CTGTGTCTACGCGCGCTGCGCACATGTGCAGTGCGGGGCTGGCGGGAGT CATCAACCGCATGCGCGAGAGCCGCTCAGAGGATGTAATGCGCATCACT GTGGGTGTGGATGGCTCTGTGTACAAGCTGCACCCCAGCTTCAAGGAGC GGTTCCATGCCAGTGTGCGCAGGCTGACTCCCAGCTGTGAGATCACCTTT ATAGAGTCAGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCTCCGCG GTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCTTC CCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTT TGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG TGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAACAAGTTAACAAC AACAATTGCATTATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTT TAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGC AGAAGCTTTTG
Full: 30 5' UTR: 42	Modified hGck-12	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACT

ORF: 71 3' UTR: 60	<u>CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG</u> <u>TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT</u> <u>GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT</u> <u>ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA</u> <u>CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC</u> <u>CACCATGGCTATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCACCTATGTGCG</u> <u>CTCCACCCAGAAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGTCTGTGAAGACCAAACACCAGATGTA</u> <u>CTCCATCCCTG</u> <u>AGGATGCCATGACTGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAG</u> <u>TGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGC</u> <u>CCCTGGGCTTCACCTTCTCATTTCTGTGAGGCATGAAGACATTGATAAG</u> <u>GGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCCTCAGGAGCAGAAG</u> <u>GGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGGG</u> <u>ACTTTGAAATGGATGTGGTGCCAATGGTGAATGACACTGTGGCCACTAT</u> <u>GATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTG</u> <u>GGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGC</u> <u>TGGTGGAGGGGGATGAGGGCCGCATGTGTGTCAATACTGAGTGGGGCGC</u> <u>CTTTGGGGACTCTGGTGAGCTGGATGAGTTCCTGCTGGAGTATGACCGC</u> <u>CTGGTGGATGAGAGCTCTGCAAACCCTGGTCAGCAGCTGTATGAGAAGC</u> <u>TCATAGGTGGCAAGTACATGGGTGAGCTGGTGCGGCTTGTGCTGCTCAG</u> <u>GTTAGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCCTCTGAGCAGCTG</u> <u>CGCACACGCGGAGCCTTTGAGACGCGCTTTGTGTCTCAAGTGGAGTCTG</u> <u>ACACTGGTGACCGCAAGCAGATCTACAACATCCTGAGCACTCTGGGGCT</u> <u>GAGACCCTCTACCACTGACTGTGACATTGTGCGCCGCGCCTGTGAGTCTG</u> <u>TGTCTACGCGCGCTGCGCACATGTGCTCTGCGGGGCTGGCGGGTGTGAT</u> <u>CAACCGCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACTGTG</u> <u>GGTGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAGCGGT</u> <u>TCCATGCCTCTGTGCGCAGGCTGACTCCCAGCTGTGAGATCACCTTTATT</u> <u>GAGTCTGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCTCTGCGGTGG</u> <u>CCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <u>AGCCAGCTGCCTTCCCTT</u> <u>TACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGG</u> <u>ACAAACCACA</u> <u>ACTAGAAATGCAGTGA</u> <u>AAAAAATGCTTTATTTGTGAAATTTGTGA</u> <u>TGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAACAAGTTAACAACAAC</u> <u>AATTGCATTCA</u> <u>TTTTATGTTTCAGGTT</u> <u>CAGGGGAGATGTGGGAGGTTTTTAA</u>
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		<i>AGCAAGTAAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGCAGA AGCTTTTG</i>
Full: 31 5' UTR: 42 ORF: 72 3' UTR: 60	Modified hGck-13	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC CACCATGGCTATGGATGTCACAAGGAGCCAGGCCAGACAGCCTTGACT CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGAGGCCTGAGGC TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG ATCCACCCAGAAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG GAGGGGCAGTGGAGTGTGAAGACCAAAACACCAGATGTACTCCATCCCTG AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA GTGCATCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG CCCCTGGGCTTACCTTCTCCTTTCCTGTGAGGCATGAAGACATTGATAA GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAA GGGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGG GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTA TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTG GGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAG CTGGTGGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGGA GCCTTTGGGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACA GACTGGTGGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGA AGCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGGCTTGTGCTGCT CAGGTTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAG CTGAGAACAAGGGGAGCCTTTGAGACAAGATTTGTGTACAGGTGGAGT CTGACACAGGAGACAGGAAGCAGATCTACAACATCCTGAGCACCCCTGG GGCTGAGACCCTCCACCACTGACTGTGACATTGTGAGGAGAGCCTGTGA GAGTGTGTCTACCAGGGCTGCTCACATGTGCTCAGCAGGGCTGGCTGGA GTCATCAACAGAATGAGGGAGAGCAGATCTGAGGATGTAATGAGGATC ACTGTGGGTGTGGATGGCAGTGTGTACAAGCTGCACCCCAGCTTCAAGG AGAGATTCCATGCCTCAGTGAGGAGGCTGACCCCAGCTGTGAGATCAC CTTCATTGAGTCTGAGGAGGGCAGTGGCAGAGGAGCAGCCCTGGTCTCT GCTGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGC CTTCCCTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATG

		<i>AGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAA TTTGTGATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAAGTTAA CAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGG TTTTTTAAAGCAAGTAAAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTA GAGCAGAAGCTTTTG</i>
Full: 32 5' UTR: 42 ORF: 73 3' UTR: 60	Modified hGck-14	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGCACAAACAGTCTCCAACCTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTGCAGAATTGC CACCATGGCCATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACT CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGAGGCCTGAGGC TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG ATCCACCCCAGAAGGCTCAGAAGTGGGGGACTTCTCTCCCTGGACCTG GGTGGCACTAACTTCAGGGTGTGCTGGTGAAGGTGGGAGAAGGTGAG GAGGGGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTG AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA GTGCATCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTG CCCCTGGGCTTCACCTTCTCCTTTCTGTGAGGCATGAAGACATTGATAA GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCCTCAGGAGCAGAA GGGAACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGG GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACCA TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGT GGGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAG CTGGTGGAGGGGGATGAGGGCAGAATGTGTGTCAATACAGAGTGGGGA GCCTTTGGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACA GACTGGTGGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGA AGCTCATAGGTGGCAAGTACATGGGAGAGCTGGTGAGACTTGTGCTGCT CAGGCTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAG CTGAGAACAAGAGGAGCCTTTGAGACCAGATTTGTGAGCCAGGTGGAGT CAGACACAGGAGACAGAAAGCAGATCTACAACATCCTGAGCACCTGG GGCTGAGACCCAGCACCACTGACTGTGACATTGTGAGAAGAGCCTGTGA GTCTGTGTCTACCAGAGCTGCCCACATGTGCAGTGCTGGGCTGGCTGGA GTCATCAACAGAATGAGAGAGAGCAGATCAGAGGATGTAATGAGAATC ACTGTGGGTGTGGATGGCTCTGTGTACAAGCTGCACCCAGCTTCAAGG AGAGATTCCATGCCAGTGTGAGAAGGCTGACCCCACTGTGAGATCAC

		<u>CTTTATTGAGTCAGAGGAGGGCAGTGGCAGAGGAGCAGCCCTGGTCTCT</u> <u>GCTGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><u>AGCCAGCTGC</u> <u>CTTCCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATG</u> <u>AGTTTGGACAAACCACAAC</u><u>TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAA</u> <u>TTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAA</u> <u>CAACAACAATTGCATTCA</u><u>TTTTATGTTTCAGGTT</u><u>CAGGGGAGATGTGGGAGG</u> <u>TTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTA</u> <u>GAGCAGAAGCTTTTG</u>
Full: 33 5' UTR: 42 ORF: 74 3' UTR: 60	Modified hGck-15	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGTCTTGACACAACAGTCTCCA<u>ACTTAAGCTGCAGTGACT</u> CTCTTAAGGTAGCCTTG<u>CAGAAGTTGGTCCTGAGGCACTGGGCAGG</u> TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTCTGCAGAATTGC CACCATGGCTATGGATGTCACAAGGAGCCAGGCC<u>CAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGAGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG</u> <u>ATCCACCCCAAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAAAACACCAGATGTACTCCATCCCA</u> <u>GAGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTG</u> <u>AGTGCATCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCT</u> <u>GCCCCTGGGCTTCACCTTCTCCTTTCTGTGAGGCATGAAGACATTGATA</u> <u>AGGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGA</u> <u>AGGGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGG</u> <u>GGACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACT</u> <u>ATGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTAGGCATGATAG</u> <u>TGGGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGG</u> <u>AGCCTTTGGGGACAGTGGAGAGCTGGATGAGTTCTGCTGGAGTATGAC</u> <u>AGACTGGTGGATGAGAGCTCTGCAAACCCTGGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGTGAGCTGGTGAGGCTTGTGCTGCT</u> <u>CAGGCTAGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCAGAGCAG</u> <u>CTGAGAACAAGGGGAGCCTTTGAGACAAGATTTGTGTCTCAGGTGGAGA</u> <u>GTGACACTGGAGACAGGAAGCAGATCTACAACATCCTGAGCACACTGG</u> <u>GGCTGAGACCCTCAACCACTGACTGTGACATTGTGAGGAGAGCCTGTGA</u> <u>GTCTGTGTCTACAAGGGCTGCACACATGTGCAGTGTGGGCTGGCAGGT</u>

		<u>GTCATCAACAGAATGAGGGAGAGCAGATCAGAGGATGTAATGAGGATC</u> <u>ACTGTGGGAGTGGATGGCTCTGTGTACAAGCTGCACCCCAGCTTCAAGG</u> <u>AGAGATTCCATGCCAGTGTGAGGAGGCTGACCCCAGCTGTGAGATCAC</u> <u>CTTTATAGAGTCAGAGGAGGGCAGTGGCAGAGGTGCTGCCCTGGTCTCT</u> <u>GCAGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGC</u> <u>CTTCCCTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATG</u> <u>AGTTTGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAA</u> <u>TTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTAA</u> <u>CAACAACAATTGCATTCATTTTATGTTTCAGGTT CAGGGGAGATGTGGGAGG</u> <u>TTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTA</u> <u>GAGCAGAAGCTTTTG</u>
Full: 34 5' UTR: 42 ORF: 75 3' UTR: 60	Modified hGck-16	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAAC TTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTG CAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTCTGCAGAATTGC CACCATGGCTATGGATGTCACAAGGAGCCAGGCC CAGACAGCCTTGACT CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACAGAGGCCTGAGGC TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAG ATCCACCC CAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG GAGGGGCAGTGGTCTGTGAAGACCAAACACCAGATGTACTCCATCCCTG AGGATGCCATGACTGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAG TGCATCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGC CCCTGGGCTTCACCTTCTCATTTCTGTGAGGCATGAAGACATTGATAAG GGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAG GGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGGG ACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACTAT GATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTG GGCACTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGC TGGTGGAGGGGGATGAGGGCAGAATGTGTGTCAATACTGAGTGGGGTG CCTTTGGGGACTCTGGTGAGCTGGATGAGTTCTCTGCTGGAGTATGACAG ACTGGTGGATGAGAGCTCTGCAAACCCTGGTCAGCAGCTGTATGAGAAG CTCATAGGTGGCAAGTACATGGGTGAGCTGGTGAGACTTGTGCTGCTCA GGTTAGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGCT GAGAACAAGAGGAGCCTTTGAGACTAGATTTGTGTCTCAGGTGGAGTCT

		<u>GACACTGGTGACAGAAAGCAGATCTACAACATCCTGAGCACTCTGGGGC</u> <u>TGAGACCTCTACCACTGACTGTGACATTGTGAGAAGAGCCTGTGAGTC</u> <u>TGTGTCTACTAGAGCTGCTCACATGTGCTCTGCTGGGCTGGCTGGTGTCA</u> <u>TCAACAGAATGAGAGAGAGCAGATCTGAGGATGTAATGAGAATCACTG</u> <u>TGGGTGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAGAG</u> <u>ATTCCATGCCTCTGTGAGAAGGCTGACTCCCAGCTGTGAGATCACCTTCA</u> <u>TTGAGTCTGAGGAGGGCAGTGGCAGAGGTGCTGCCCTGGTCTCTGCTGT</u> <u>GGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <u>AGCCAGCTGCCTTCCC</u> <u>TTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTG</u> <u>GACAAACCACA</u> <u>ACTAGAATGCAGTGA</u> <u>AAAAAATGCTTTATTTGTGAAATTTGTG</u> <u>ATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAACAA</u> <u>CAATTGCATTCA</u> <u>TTTTATGTTTCAGGTT</u> <u>CAGGGG</u> <u>GAGATGTGGGAGGTTTTTA</u> <u>AAGCAAGTAAACCTCTACA</u> <u>AATGTGGTAA</u> <u>AATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 35 5' UTR: 42 ORF: 76 3' UTR: 60	Modified hGck-17	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCA ACTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGT CCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTGCAGAATTGC CACC <u>ATGGCCATGGACGTGACCCGGAGCCAGGCTCAAAACAGCACTGAC</u> <u>TCTCGTCGAGCAGATCTTGGCGGAATTCCAGCTTCAAGAGGAAGATCTG</u> <u>AAGAAAGTTATGAGAAGGATGCAGAAGGAGATGGACCGCGGCTTACGA</u> <u>CTGGAACGCACGAGGAGGCCTCCGTGAAAATGCTACCCACCTACGTGC</u> <u>GTTCTACACCTGAAGGATCAGAGGTGGGGGATTTTCTCAGTCTGGACTT</u> <u>GGGTGGCACTAACTTCCGGGTCATGCTGGTGAAGGTTGGAGAAGGGGAG</u> <u>GAAGGCCAGTGGTCGGTGAAAACCAAGCATCAGATGTATAGCATTCCAG</u> <u>AGGATGCTATGACAGGCACCGCCGAGATGCTTTTTGACTACATATCCGA</u> <u>ATGCATCTCTGATTTCTCGACAAGCACCAAATGAAACATAAGAACTG</u> <u>CCGCTGGGATTTACTTTCTCATTTCCCGTGAGACACGAGGATATTGACAA</u> <u>GGGGATCCTCTTGAATTGGACGAAAGGCTTCAAGGCAAGTGGCGCCGAA</u> <u>GGAAACAATGTAGTGGGGCTTCTGAGGGATGCTATTAAGCGCCGGGGTG</u> <u>ACTTTGAGATGGACGTTGTGGCAATGGTCAACGATACAGTGGCCACAAT</u> <u>GATCAGCTGTTATTACGAGGACCATCAGTGCGAAGTCGGCATGATAGTG</u> <u>GGA</u> <u>ACTGGGTGTAATGCGTGCTATATGGAGGAAATGCAGAACGTTGAGT</u> <u>TAGTGGAAGGCGATGAGGGTAGAATGTGTGTAAATACCGAGTGGGGAG</u> <u>CTTT</u> <u>CGGGGACTCCGGCGAACTGGATGAGTTCTACTCGAATACGACAG</u> <u>GCTGGTGGATGAGTCTAGCGCCAACCCTGGCCAGCAATTGTATGAAAAA</u>

		<u>CTGATCGGAGGGAAGTACATGGGTGAGCTTGTCCGCCTCGTTCTGCTGC</u> <u>GATTAGTGGACGAGAATCTCCTGTTTCACGGCGAAGCATCCGAGCAGTT</u> <u>GCGTACACGGGGAGCCTTCGAAACCAGATTTGTCTCACAAGTGGAGAGT</u> <u>GATACTGGGGACAGGAAACAGATTTACAACATCCTTAGCACACTGGGCC</u> <u>TACGCCCATCTACGACCGATTGCGACATTGTGCGGCGAGCTTGTGAGTC</u> <u>CGTTTCGACTAGAGCCGCACACATGTGCTCAGCTGGTCTCGCCGGAGTA</u> <u>ATCAATAGGATGCGCGAAAGCCGGAGTGAGGACGTGATGAGAATAACA</u> <u>GTCGGCGTGATGGGTCTGTCTATAAGCTGCATCCCTCCTTCAAGGAAA</u> <u>GGTTTCACGCGAGCGTGCGCCGCTGACCCCTTCATGTGAGATTACTTTC</u> <u>ATCGAATCCGAGGAGGGCAGTGGACGGGGGCGAGCCTTGGTTTCTGCTG</u> <u>TGGCCTGCAAAAAGGCATGCATGCTTGGCCAATGA</u><i>AGCCAGCTGCCTTCC</i> <i>CTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTT</i> <i>GGACAAACCACA</i>ACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT <i>GATGCTATTGCTTTATTTGTA</i>ACCATTATAAGCTGCAATAACAAGTTAACACA <i>ACAATTGCATT</i>CATTTTATGTTTCAGGTTCAGGGGGAGATGTGGGAGGTTTTTT <i>AAAGCAAGTAAA</i>ACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCA <i>GAAGCTTTTG</i>
Full: 36 5' UTR: 42 ORF: 77 3' UTR: 60	Modified hGck-18	[CTAG]AAGCTTTATTGTGGTAGTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACTTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGC <u>CACCATGGCCATGGATGTGACCAGAAGCCAGGCTCAAACAGCACTGAC</u> <u>TCTGGTAGAGCAGATCTTGGCGGAATTCAGCTTCAAGAGGAAGATCTG</u> <u>AAGAAAGTTATGAGAAGGATGCAGAAGGAGATGGACCGCGGCTTAAGG</u> <u>CTGGAAACCCATGAGGAGGCCAGTGTGAAAATGCTACCCACCTATGTAA</u> <u>GATCTACACCTGAAGGATCAGAGGTGGGGGATTTTCTCAGTCTGGACTT</u> <u>GGGTGGCACTAACTTCAGGGTCATGCTGGTGAAGGTTGGAGAAGGGGA</u> <u>GGAAGGCCAGTGGTCAGTGAAAACCAAGCATCAGATGTATAGCATTCCA</u> <u>GAGGATGCTATGACAGGTACCGCTGAGATGCTTTTTGACTACATATCTG</u> <u>AATGCATCTCTGATTTCTAGACAAGCACCAAATGAAACATAAGAACT</u> <u>GCCGCTGGGATTTACTTTCTCATTTCTGTGAGACATGAGGATATTGACA</u> <u>AGGGGATCCTCTTGAATTGGACAAAAGGCTTCAAGGCAAGTGGCGCAGA</u> <u>AGGAAACAATGTAGTGGGGCTTCTGAGGGATGCTATTAAGCGCAGAGGT</u> <u>GACTTTGAGATGGATGTTGTGGCAATGGTCAATGATACAGTGGCCACAA</u> <u>TGATCAGCTGTTATTATGAGGACCATCAGTGTGAAGTGGGCATGATAGT</u> <u>GGGAACTGGGTGTAATGCCTGCTATATGGAGGAAATGCAGAATGTTGAG</u>

		<u>TTAGTGGAAGGTGATGAGGGTAGAATGTGTGTAAATACAGAGTGGGGA</u> <u>GCTTTTGGGGACAGCGGAGAACTGGATGAGTTCCTATTAGAATATGACA</u> <u>GGCTGGTGGATGAGTCTAGCGCCAACCCTGGCCAGCAATTGTATGAAAA</u> <u>ACTGATTGGAGGGAAGTACATGGGTGAGCTTGTCGCCCTTGTTCTGCTGC</u> <u>GGTTAGTGGATGAGAATCTCCTGTTTCATGGGGAAGCAAGTGAGCAGTT</u> <u>GCGGACAAGGGGAGCCTTTGAAACCAGATTTGTCTCACAAGTGGAGAGT</u> <u>GATACTGGGGACAGGAAACAGATTTACAACATCCTTAGCACACTGGGCC</u> <u>TAAGACCATCTACCACTGATTGTGACATTGTGCGCGGGCTTGAGTGC</u> <u>AGTTTCCACTAGAGCCGCACACATGTGCTCAGCTGGTTTGGCTGGAGTA</u> <u>ATCAATAGGATGCGCGAAAGCAGGAGTGAGGATGTGATGAGAATAACA</u> <u>GTTGGTGTGGATGGGTCTGTCTATAAGCTGCATCCCTCCTTCAAGGAAAG</u> <u>GTTTCACGCGTCTGTGCGCAGACTGACCCCTTCATGTGAGATTACTTTCA</u> <u>TAGAATCTGAGGAGGGCAGTGGAAGGGGGGCAGCCTTGGTTTCTGCTGT</u> <u>GGCCTGCAAAAAGGCATGCATGCTTGGCCAATGAAGCCAGCTGCCTTCCC</u> <u>TTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTG</u> <u>GACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTG</u> <u>ATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACAACAA</u> <u>CAATTGCATTCATTTTATGTTTCAGGTTCAAGGGGAGATGTGGGAGGTTTTTA</u> <u>AAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAGAGCAG</u> <u>AAGCTTTTG</u>
Full: 37 5' UTR: 42 ORF: 78 3' UTR: 60	Modified hGck-19	<u>[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC</u> <u>AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT</u> <u>CTCTTAAGGTAGCCTTGCAAGATTGGTCTGAGGCACTGGGCAGG</u> <u>TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT</u> <u>GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCCTGATAGGCACCT</u> <u>ATTGGTCTTACTGACATCCACTTTGCCTTTCCTCTCCACAGGTGTCCA</u> <u>CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTGCAGAATTGC</u> <u>CACCATGGCCATGGATGTGACCAGAAGCCAGGCTCAAACAGCACTGAC</u> <u>TCTGGTGGAGCAGATCTTGGCGGAATTCCAGCTTCAAGAGGAAGATCTG</u> <u>AAGAAAGTTATGAGAAGGATGCAGAAGGAGATGGACCGCGGCTTAAGG</u> <u>CTGGAAACCCATGAGGAGGCCAGTGTGAAAATGCTACCCACCTATGTAA</u> <u>GATCTACACCTGAAGGATCAGAGGTGGGGGATTTTCTCAGTCTGGACTT</u> <u>GGGTGGCACTAACTTCAGGGTCATGCTGGTGAAGGTTGGAGAAGGGGA</u> <u>GGAAGGCCAGTGGTCAGTGAAAACCAAGCATCAGATGTATAGCATTCCA</u> <u>GAGGATGCTATGACAGGTACCGCTGAGATGCTTTTTGACTACATATCTG</u> <u>AATGCATCTCTGATTTCTGGACAAGCACCAAATGAAACATAAGAACT</u> <u>GCCGCTGGGATTTACTTTCTCATTTCTGTGAGACATGAGGATATTGACA</u> <u>AGGGGATCCTCTTGAATTGGACCAAAGGCTTCAAGGCAAGTGGCGCAGA</u> <u>AGGAAACAATGTAGTGGGGCTTCTGAGGGATGCTATTAAGCGCAGAGGT</u>

		<u>GACTTTGAGATGGATGTTGTGGCAATGGTCAATGATACAGTGGCCACAA</u> <u>TGATCAGCTGTTATTATGAGGACCATCAGTGTGAAGTGGGCATGATAGT</u> <u>GGGAACTGGGTGTAATGCCTGCTATATGGAGGAAATGCAGAATGTTGAG</u> <u>TTAGTGGAAGGTGATGAGGGTAGAATGTGTGTAAATACAGAGTGGGGA</u> <u>GCTTTTGGGGACAGCGGAGAACTGGATGAGTTCCTACTGGAATATGACA</u> <u>GGCTGGTGGATGAGTCTAGCGCCAACCCTGGCCAGCAATTGTATGAAAA</u> <u>ACTGATTGGAGGGAAGTACATGGGTGAGCTTGTCCGCCTGGTTCCTGCTG</u> <u>CGGTTAGTGGATGAGAATCTCCTGTTTCATGGGGAAGCAAGTGAGCAGT</u> <u>TGCGGACAAGGGGAGCCTTTGAAACCAGATTTGTCTCACAAGTGGAGAG</u> <u>TGATACTGGGGACAGGAAACAGATTTACAACATCCTTAGCACACTGGGC</u> <u>CTAAGACCATCTACCACAGATTGTGACATTGTGCGGCGGGCTTGTGAGT</u> <u>CAGTTAGCACTAGAGCCGCACACATGTGCTCAGCTGGTCTGGCTGGAGT</u> <u>AATCAATAGGATGCGCGAAAGCAGGAGTGAGGATGTGATGAGAATAAC</u> <u>AGTGGGTGTGGATGGGTCTGTCTATAAGCTGCATCCCTCCTTCAAGGAA</u> <u>AGGTTTCACGCGTCTGTGCGCAGACTGACCCCTTCATGTGAGATTACTTT</u> <u>CATAGAATCTGAGGAGGGCAGTGGAAGGGGGGCAGCCTTGGTTTCTGCT</u> <u>GTGGCCTGCAAAAAGGCATGCATGCTTGGCCAATGA</u><u>AGCCAGCTGCCTTC</u> <u>CCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTT</u> <u>TGGACAAACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG</u> <u>TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTACAAC</u> <u>AACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTT</u> <u>TAAAGCAAGTAAAACTCTACAAATGTGGTAAAAATCCGATAAGGGACTAGAGC</u> <u>AGAAGCTTTTG</u>
Full: 38 5' UTR: 42 ORF: 79 3' UTR: 60	Modified hGck-20	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCAACCTAAGCTGCAGTGACT CTCTTAAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGG TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTCTGATAGGCACCT ATTGGTCTTACTGACATCCACTTTGCCTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTCTGCAGAATTGC CACCATGGCCATGGATGTGACCAGAAGCCAGGCTCAAACAGCACTGAC TCTGGTAGAGCAGATCTTGGCTGAATTCCAGCTTCAAGAGGAAGATCTG AAGAAAGTTATGAGAAGGATGCAGAAGGAGATGGACAGGGGCTTAAGA CTGGAAACCCATGAGGAGGCCAGTGTGAAAATGCTACCCACCTATGTAA GGTCTACACCTGAAGGATCAGAGGTGGGGGATTTTCTCAGTCTGGACTT GGGTGGCACTAACTTCAGAGTCATGCTGGTGAAGGTTGGAGAAGGGGA GGAAGGCCAGTGGTCAGTGAAAACCAAGCATCAGATGTATAGCATTCCA GAGGATGCTATGACAGGCACAGCAGAGATGCTTTTTGACTACATATCTG AATGCATCTCTGATTTCTAGACAAGCACCAAATGAAACATAAGAACT

		<u>GCCACTGGGATTTACTTTCTCATTTCTGTGAGACATGAGGATATTGACA</u> <u>AGGGGATCCTCTTGAATTGGACCAAAGGCTTCAAGGCAAGTGGTGCTGA</u> <u>AGGAAACAATGTAGTGGGGCTTCTGAGGGATGCTATTAAGAGGAGAGG</u> <u>TGACTTTGAGATGGATGTTGTGGCAATGGTCAATGATACAGTGGCCACA</u> <u>ATGATCAGCTGTTATTATGAGGACCATCAGTGTGAAGTGGGCATGATAG</u> <u>TGGGAACTGGGTGTAATGCCTGCTATATGGAGGAAATGCAGAATGTTGA</u> <u>GTTAGTGGAAGGAGATGAGGGTAGAATGTGTGTAAATACAGAGTGGGG</u> <u>AGCTTTTGGGGACAGTGGGGAAGTGGATGAGTTCCTATTAGAATATGAC</u> <u>AGGCTGGTGGATGAGTCTTCAGCCAACCCTGGCCAGCAATTGTATGAAA</u> <u>AACTGATTGGAGGGAAGTACATGGGTGAGCTTGTGAGGCTTGTTCTGCT</u> <u>GAGATTAGTGGATGAGAATCTCCTGTTTCATGGTGAAGCATCTGAGCAG</u> <u>TTGAGGACAAGAGGAGCCTTTGAAACCAGATTTGTCTCACAAGTGGAGA</u> <u>GTGATACTGGGGACAGGAAACAGATTTACAACATCCTTAGCACACTGGG</u> <u>CCTAAGGCCATCTACCACTGATTGTGACATTGTGAGAAGGGCTTGAG</u> <u>AGTGTTTCCACTAGAGCAGCACACATGTGCTCAGCTGGTTTGGCTGGAG</u> <u>TAATCAATAGGATGAGAGAAAAGCAGGAGTGAGGATGTGATGAGAATAA</u> <u>CAGTTGGAGTGGATGGGTCTGTCTATAAGCTGCATCCCTCCTTCAAGGA</u> <u>AAGGTTTCATGCCTCTGTGAGAAGGCTGACCCCTTCATGTGAGATTACTT</u> <u>TCATAGAATCAGAGGAGGGCAGTGGAAGAGGGGCAGCCTTGTTTCTGC</u> <u>TGTGGCCTGCAAAAAGGCATGCATGCTTGGCCAATGAAGCCAGCTGCCTT</u> <u>CCCTTTACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAG</u> <u>TTTGGACAAACCACAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATT</u> <u>TGTGATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAAACAAGTTAACA</u> <u>ACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTT</u> <u>TTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAGA</u> <u>GCAGAAGCTTTTG</u>
Full: 39 5' UTR: 42 ORF: 80 3' UTR: 60	Modified hGck-21	<u>[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC</u> <u>AGTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACT</u> <u>CTCTTAAGGTAGCCTTGCGAAGTTGGTCCTGAGGCACTGGGCAGG</u> <u>TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT</u> <u>GGGCTTGTGGAGACAGAGAAGACTCTTGGCTTCTGATAGGCACCT</u> <u>ATTGGTCTTACTGACATCCACTTTGCCTTCTCTCCACAGGTGTCCA</u> <u>CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCTGCGAATTGC</u> <u>CACCATGGCCATGGATGTGACCAGAAGCCAGGCTCAAACAGCACTGAC</u> <u>TCTGGTGGAGCAGATCTTGGCTGAATTCCAGCTTCAAGAGGAAGATCTG</u> <u>AAGAAAGTTATGAGAAGGATGCAGAAGGAGATGGACAGAGGCTTAAGA</u> <u>CTGGAAACCCATGAGGAGGCCAGTGTGAAAATGCTACCCACCTATGTAA</u> <u>GATCTACACCTGAAGGATCAGAGGTGGGGGATTTTCTCAGTCTGGACTT</u> <u>GGGTGGCACTAACTTCAGAGTCATGCTGGTGAAGGTTGGAGAAGGGGA</u>

		<u>GGAAGGCCAGTGGTCAGTGAAAACCAAGCATCAGATGTATAGCATTCCA</u> <u>GAGGATGCTATGACAGGCACAGCAGAGATGCTTTTTGACTACATATCTG</u> <u>AATGCATCTCTGATTTCTGGACAAGCACCAAAATGAAACATAAGAAACT</u> <u>GCCCCTGGGATTTACTTTCTCATTTCCCTGTGAGACATGAGGATATTGACA</u> <u>AGGGGATCCTCTTGAATTGGACCAAAGGCTTCAAGGCAAGTGGTGCTGA</u> <u>AGGAAACAATGTAGTGGGGCTTCTGAGGGATGCTATTAAGAGAAGAGG</u> <u>TGACTTTGAGATGGATGTTGTGGCAATGGTCAATGATACAGTGGCCACA</u> <u>ATGATCAGCTGTTATTATGAGGACCATCAGTGTGAAGTGGGCATGATAG</u> <u>TGGGAACTGGGTGTAATGCCTGCTATATGGAGGAAATGCAGAATGTTGA</u> <u>GTTAGTGGAAGGAGATGAGGGTAGAATGTGTGTAAATACAGAGTGGGG</u> <u>AGCTTTTGGGGACAGTGGGGAAGTGGATGAGTTCCTACTGGAATATGAC</u> <u>AGGCTGGTGGATGAGTCTTCAGCCAACCCTGGCCAGCAATTGTATGAAA</u> <u>AACTGATTGGAGGGAAGTACATGGGTGAGCTTGTGAGACTGGTTCTGCT</u> <u>GAGATTAGTGGATGAGAATCTCCTGTTTCATGGTGAAGCATCTGAGCAG</u> <u>TTGAGAACAAAGAGGAGCCTTTGAAACCAGATTTGTCTCACAAGTGGAGA</u> <u>GTGATACTGGGGACAGGAAACAGATTTACAACATCCTTAGCACACTGGG</u> <u>CCTAAGACCATCTACCACTGATTGTGACATTGTGAGAAGAGCTTGTGAG</u> <u>AGTGTTAGCACTAGAGCAGCACACATGTGCTCAGCTGGTCTGGCTGGAG</u> <u>TAATCAATAGGATGAGAGAAAGCAGAAGTGAGGATGTGATGAGAATAA</u> <u>CAGTGGGAGTGGATGGGTCTGTCTATAAGCTGCATCCCTCCTTCAAGGA</u> <u>AAGGTTTCATGCCTCTGTGAGAAGACTGACCCCTTCATGTGAGATTACTT</u> <u>TCATAGAATCAGAGGAGGGCAGTGGAAGAGGGGCAGCCTTGGTTTCTGC</u> <u>TGTGGCCTGCAAAAAGGCATGCATGCTTGGCCAATGA</u><u>AGCCAGCTGCCTT</u> <u>CCCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAG</u> <u>TTTGGACAAACCACAACCTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATT</u> <u>TGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACA</u> <u>ACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTT</u> <u>TTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAATCCGATAAGGGACTAGA</u> <u>GCAGAAGCTTTTG</u>
Full: 89 5' UTR: 83 ORF: 61 3' UTR: 102	Modified hGck-22	AAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCA GTGCTTCTGACACAACAGTCTCGAACTTAAGCTGCAGTGACTCTCTT AAGGTAGCCTTGCAGAAGTTGGTTCGTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCT TGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGT CTTACTGACATCCACTTTGCCTTTTCTCTCCACAGGTGTCCACTCCCA GTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCAT <u>GGCCATGGATGTCA</u><u>AAGGAGCCAGGCCAGACAGCCTTGACTCTGGTA</u> <u>GAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAAG</u> <u>GTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCTGAGGCTGGAG</u>

		<u>ACCCATGAAGAGGCCAGTGTGAAGATGCTGCCACCTATGTGAGGTCCA</u> <u>CCCCAGAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTGGGTGG</u> <u>CACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGGG</u> <u>GCAGTGGAGTGTGAAGACCAAACACCAGATGTACTCCATCCCTGAGGAT</u> <u>GCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCAT</u> <u>CTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCCTG</u> <u>GGCTTCACCTTCTCATTTCCTGTGAGGCATGAAGACATTGATAAGGGCAT</u> <u>CCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGAAC</u> <u>AATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGGGACTTTG</u> <u>AAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACCATGATCTC</u> <u>CTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGTGGGCACA</u> <u>GGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGTGG</u> <u>AGGGGGATGAGGGCAGGATGTGTGTCAATACAGAGTGGGGAGCCTTTG</u> <u>GGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGACAGGCTGGT</u> <u>GGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGAAGCTCATA</u> <u>GGTGGCAAGTACATGGGAGAGCTGGTGAGGCTTGTGCTGCTCAGGCTGG</u> <u>TGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGCTGAGGAC</u> <u>AAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGAGTGACACA</u> <u>GGAGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGAGA</u> <u>CCCTCCACCACAGACTGTGACATTGTGAGGAGGGCTTGTGAGAGTGTGT</u> <u>CTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCTGGGGTCATCAA</u> <u>CAGGATGAGGGAGAGCAGGAGTGAGGATGTAATGAGGATCACTGTGGG</u> <u>AGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAGAGGTTT</u> <u>CATGCCAGTGTGAGGAGGCTGACCCCCAGCTGTGAGATCACCTTTATTG</u> <u>AGTCTGAGGAGGGCAGTGGCAGGGGAGCAGCCCTGGTCTCTGCTGTGGC</u> <u>CTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <i>AGCCAGCTGCCTTCCCTT</i> <i>ACTGTGGGTAAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGG</i> <i>CAAAACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGAT</i> <i>GCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAACACAACA</i> <i>ATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTTTTTAAA</i> <i>GCAAGTAAACCTCTACAAATGTGGTAAAAATAAGGGACTAGAGCAGTCTAGAA</i> <i>AGCTT</i>
Full: 90 5' UTR: nucleic acids 5-329 of 42 ORF: 61 3' UTR: 103	Modified hGck-23	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA GTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTCTTAAAGGTAGCCTTGAGAAAGTTGGTCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCT TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA

		<u>TGGCCATGGATGTCA</u><u>AAGGAGCCAGGCC</u><u>CAGACAGCCTTGACTCTGGT</u> <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAGGTCC</u> <u>ACCCCAAGAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG</u> <u>GGCAGTGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u> <u>TCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCCCT</u> <u>GGGCTTCACCTTCTCATTTCCCTGTGAGGCATGAAGACATTGATAAGGGC</u> <u>ATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGA</u> <u>ACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGGGACTT</u> <u>TGAAATGGATGTGGTGGCAATGGTGAATGACACTGTGGCCACCATGATC</u> <u>TCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGTGGGCA</u> <u>CAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGT</u> <u>GGAGGGGGATGAGGGCAGGATGTGTGTCAATACAGAGTGGGGAGCCTT</u> <u>TGGGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGACAGGCTG</u> <u>GTGGATGAGAGCTCAGCAAACCTGGTCAGCAGCTGTATGAGAAGCTCA</u> <u>TAGGTGGCAAGTACATGGGAGAGCTGGTGAGGCTTGTGCTGCTCAGGCT</u> <u>GGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGCTGAGG</u> <u>ACAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGAGTGACA</u> <u>CAGGAGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGA</u> <u>GACCCTCCACCACAGACTGTGACATTGTGAGGAGGGCTTGTGAGAGTGT</u> <u>GTCTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCTGGGGTCATC</u> <u>AACAGGATGAGGGAGAGCAGGAGTGAGGATGTAATGAGGATCACTGTG</u> <u>GGAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAGAGGT</u> <u>TCCATGCCAGTGTGAGGAGGCTGACCCCCAGCTGTGAGATCACCTTTATT</u> <u>GAGTCTGAGGAGGGCAGTGGCAGGGGAGCAGCCCTGGTCTCTGCTGTGG</u> <u>CCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><i>AGCCAGCTGCCTTCCCTT</i> <i>TACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGG</i> <i>ACAAACCACA</i><i>ACTAG</i><i>AATGCAGTG</i><i>AAAAAAATGCTTTATTTGTG</i><i>AAATTTGTGA</i> <i>TGCTATTGCTTTATTTGTA</i><i>ACCATTATAAGCTGCAATAAA</i><i>CAAGTTAACAACAAC</i> <i>AATTGCATTCA</i><i>TTTTATGTTTCAGGTT</i><i>CAGGGGGAGATGTGGGAGGTTTTTAA</i> <i>AGCAAGTAAA</i><i>ACCTCTACA</i><i>AAATGTGGTAAAATAAGGGACTAGAGCAGGCTAGC</i> <i>AAGCTT</i>
Full: 91 5' UTR: nucleic acids 5-329 of 42	Modified hGck-24	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA GTGCTTCTGACACAACAGTCTCCAACTTAAGCTGCAGTGACTCTCTT AAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCT

ORF: 65 3' UTR: 104	<u>TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG</u> <u>TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC</u> <u>AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA</u> <u>TGGCCATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACTCTGGT</u> <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAGGTCC</u> <u>ACCCAGAAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG</u> <u>GGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u> <u>TCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCT</u> <u>GGGCTTCACCTTCTCATTTCTGTGAGGCATGAAGACATTGATAAGGGC</u> <u>ATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGA</u> <u>ACAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGGGACTT</u> <u>TGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACCATGATC</u> <u>TCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATAGTGGGCA</u> <u>CAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGT</u> <u>GGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGGAGCCTTT</u> <u>GGGGACTCTGGGGAGCTGGATGAGTTCCTGCTGGAGTATGACAGGCTGG</u> <u>TGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGAAGCTCAT</u> <u>AGGTGGCAAGTACATGGGGGAGCTGGTGAGGCTTGTGCTGCTCAGGCTG</u> <u>GTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCAGAGCAGCTGAGAA</u> <u>CAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGAGTGACAC</u> <u>AGGTGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGAG</u> <u>ACCCTCCACCACAGACTGTGACATTGTGAGGAGGGCCTGTGAGAGTGTG</u> <u>TCTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCAGGAGTCATCA</u> <u>ACAGGATGAGGGAGAGCAGGAGTGAGGATGTAATGAGGATCACTGTGG</u> <u>GAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAGGAGAGGTT</u> <u>CCATGCCAGTGTGAGAAGGCTGACCCCCAGCTGTGAGATCACCTTTATA</u> <u>GAGTCTGAGGAGGGCAGTGGCAGGGGAGCAGCCCTGGTCTCTGCTGTGG</u> <u>CCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCTTCCCTT</u> <u>TACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGG</u> <u>ACAAACCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGA</u> <u>TGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAACAAGTTAACAACAAC</u> <u>AATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAA</u> <u>AGCAAGTAAAACCTCTACAAATGTGGTAAAAATAAGGGACTAGAGCAGATTAAT</u> <u>AAGCTT</u>
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Full: 92 5' UTR: nucleic acids 5-329 of 42 ORF: 67 3' UTR: 105	Modified hGck-25	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA GTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGACTCTCTT AAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCT TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA TGGCCATGGATGTCAAGGAGCCAGGCCAGACAGCCTTGACTCTGGT AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA GGTGATGAGAAGGATGCAGAAGGAGATGGACAGGGGCCCTGAGGCTGGA GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAGGTCC ACCCAGAAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTGGGTG GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG GGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA TCTCTGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCT GGGCTTCACCTTCTCATTTCTGTGAGGCATGAAGACATTGATAAGGGC ATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGA ACAAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGGAGAGGGGACTT TGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTATGATC TCCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGTGGGCA CTGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGT GGAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGGAGCCTTT GGGGACTCTGGGGAGCTGGATGAGTTCTCTGCTGGAGTATGACAGGCTGG TGGATGAGAGCTCAGCAAACCCTGGTCAGCAGCTGTATGAGAAGCTCAT AGGTGGCAAGTACATGGGAGAGCTGGTGAGACTTGTGCTGCTCAGGCTG GTGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCAGAGCAGCTGAGGA CAAGGGGAGCCTTTGAGACCAGGTTTGTGTCCCAGGTGGAGAGTGACAC TGGAGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGAG GCCCTCTACCACAGACTGTGACATAGTGAGAAGGGCCTGTGAGTCTGTG TCTACCAGGGCTGCCCACATGTGCTCTGCTGGGCTGGCTGGAGTCATCA ACAGAATGAGGGAGAGCAGAAGTGAGGATGTAATGAGGATCACTGTGG GAGTGGATGGCTCTGTGTACAAGCTGCACCCCTCATTCAAGGAGAGGTT CCATGCCAGTGTGAGGAGGCTGACCCCCAGCTGTGAGATCACCTTTATT GAGTCTGAGGAGGGCAGTGGCAGGGGAGCTGCCCTGGTCTCTGCTGTGG CCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCTTCCCTT TACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGG ACAAACCACAAGTGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGA TGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAACAAGTTAACAACAAC
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		<i>AATTGCATTCAATTTATGTTTCAGGTTCAAGGGGAGATGTGGGAGGTTTTTAA</i> <i>AGCAAGTAAAACCTCTACAAATGTGGTAAAATAAGGGACTAGAGCAGATGCAT</i> <i>AAGCTT</i>
Full: 93 5' UTR: nucleic acids 5-329 of 42 ORF: 68 3' UTR: 106	Modified hGck-26	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA GTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA AAGGTAGCCTTGACAGAGTTGGTCCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCT TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATGCCACCA <u>TGGCTATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACTCTGGT</u> <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGCGCTCC</u> <u>ACCCGAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG</u> <u>GGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u> <u>TCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCCCT</u> <u>GGGCTTCACCTTCTCCTTTCCCTGTGAGGCATGAAGACATTGATAAGGGCA</u> <u>TCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGAA</u> <u>CAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAAGAAGAGGGGACTTT</u> <u>GAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTATGATCT</u> <u>CCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTGGGCAC</u> <u>AGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAAATGTGGAGCTGGTG</u> <u>GAGGGGGATGAGGGCCGCATGTGTGTCAATACTGAGTGGGGCGCCTTTG</u> <u>GGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACCGCCTGGT</u> <u>GGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAAGCTCATA</u> <u>GGTGGCAAGTACATGGGAGAGCTGGTGCGGCTTGTGCTGCTCAGGTTGG</u> <u>TGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAGCTGCGCAC</u> <u>ACGCGGAGCCCTTTGAGACGCGCTTTGTGTACAAAGTGAGTCTGACACA</u> <u>GGAGACCGCAAGCAGATCTACAACATCCTGAGCACACTGGGGCTGCGGC</u> <u>CCTCCACCACTGACTGTGACATTGTGCGCCGCGCCTGTGAGAGTGTGTCT</u> <u>ACGCGCGCTGCGCACATGTGCTCAGCGGGGCTGGCGGGAGTCATCAACC</u> <u>GCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACTGTGGGAGT</u> <u>GGATGGCAGTGTGTACAAGCTGCACCCAGCTTCAAGGAGCGGTTCCAT</u> <u>GCCTCAGTGCGCAGGCTGACCCCCAGCTGTGAGATCACCTTTATTGAGTC</u> <u>TGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCAGCGCGGTGGCCTG</u> <u>TAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCTTCCTTTACTG</u>

		<i>TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAA CCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTA TTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAACAATTG CATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCA AGTAAAACCTCTACAAATGTGGTAAAATAAGGGACTAGAGCAGAGTACTAAGC TT</i>
Full: 94 5' UTR: nucleic acids 5-329 of 42 ORF: 69 3' UTR: 107	Modified hGck-27	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA GTGCTTCTGACACAACAGTCTCCAAC TTAAGCTGCAGTGACTCTCTT AAGGTAGCCTTGCAGAAGTTGGTCCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCT TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA TGGCCATGGATGTCAAGGAGCCAGGCCCAGACAGCCTTGACTCTGGT <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCACCTATGTGCGCTCC</u> <u>ACCCAGAAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG</u> <u>GGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u> <u>TCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCTT</u> <u>GGGCTTCACCTTCTCCTTTTCTGTGAGGCATGAAGACATTGATAAGGGCA</u> <u>TCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGAA</u> <u>CAATGTGGTGGGGCTTCTGCGGGATGCTATCAAAAGAAGAGGGGACTTT</u> <u>GAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACCATGATCT</u> <u>CCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGTGGGCAC</u> <u>TGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGTG</u> <u>GAGGGGGATGAGGGCCGCATGTGTGTCAATACAGAGTGGGGCGCCTTTG</u> <u>GGGACAGCGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACCGCCTGGT</u> <u>GGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAAGCTCATA</u> <u>GGTGGCAAGTACATGGGAGAGCTGGTGCGGCTTGTGCTGCTCAGGCTGG</u> <u>TGGATGAAAACCTGCTCTTCCATGGGGAGGCCTCTGAGCAGCTGCGCAC</u> <u>ACGCGGAGCCTTTGAGACGCGCTTTGTGAGCCAGGTGGAGAGTGACACA</u> <u>GGAGACCGCAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGCGGC</u> <u>CCAGCACCACTGACTGTGACATTGTGCGCCGCGCCTGTGAGTCAGTGTCT</u> <u>ACGCGCGCTGCGCACATGTGCAGCGCGGGGCTGGCGGGAGTCATCAACC</u> <u>GCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACTGTGGGAGT</u> <u>GGATGGCAGTGTGTACAAGCTGCACCCAGCTTCAAGGAGCGGTTCCAT</u>

		<u>GCCTCAGTGCGCAGGCTGACCCCCAGCTGTGAGATCACCTTTATTGAGTC</u> <u>TGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCAGCGCGGTGGCCTG</u> <u>TAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <u>AGCCAGCTGCCTTCCCTTACTG</u> <u>TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAA</u> <u>CCACAAGTGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTA</u> <u>TTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAGTTAAACAACAACATTG</u> <u>CATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAAAGCA</u> <u>AGTAAACCTCTACAAATGTGGTAAAATAAGGGACTAGAGCAGGGTACCAAGC</u> <u>TT</u>
Full: 95 5' UTR: nucleic acids 5-329 of 42 ORF: 72 3' UTR: 108	Modified hGck-28	<u>AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA</u> <u>GTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA</u> <u>CTCTCTT</u> <u>AAGGTAGCCTTGACAGAGTTGGTCCTGAGGCACTGGGCAGGTAAGT</u> <u>ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCT</u> <u>TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG</u> <u>TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC</u> <u>AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAAATTGCCACCA</u> <u>TGGCTATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACTCTGGT</u> <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGAATGCAGAAGGAGATGGACAGAGGCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAGATCC</u> <u>ACCCGAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG</u> <u>GGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u> <u>TCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCT</u> <u>GGGCTTACCTTCTCCTTTCCCTGTGAGGCATGAAGACATTGATAAGGGCA</u> <u>TCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGAA</u> <u>CAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGGGACTTT</u> <u>GAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTATGATCT</u> <u>CCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTGGGCAC</u> <u>AGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGTG</u> <u>GAGGGGGATGAGGGCAGGATGTGTGTCAATACTGAGTGGGAGCCTTTG</u> <u>GGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACAGACTGGT</u> <u>GGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAAGCTCATA</u> <u>GGTGGCAAGTACATGGGAGAGCTGGTGAGGCTTGTGCTGCTCAGGTTGG</u> <u>TGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAGCTGAGAAC</u> <u>AAGGGGAGCCTTTGAGACAAGATTTGTGTACAGGTGGAGTCTGACACA</u> <u>GGAGACAGGAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGAGA</u> <u>CCCTCCACCACTGACTGTGACATTGTGAGGAGAGCCTGTGAGAGTGTGT</u>

		<u>CTACCAGGGCTGCTCACATGTGCTCAGCAGGGCTGGCTGGAGTCATCAA</u> <u>CAGAATGAGGGAGAGCAGATCTGAGGATGTAATGAGGATCACTGTGGG</u> <u>TGTGGATGGCAGTGTGTACAAGCTGCACCCCAGCTTCAAGGAGAGATT</u> <u>CATGCCTCAGTGAGGAGGCTGACCCCCAGCTGTGAGATCACCTTCATTG</u> <u>AGTCTGAGGAGGGCAGTGGCAGAGGAGCAGCCCTGGTCTCTGCTGTGGC</u> <u>CTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <i>AGCCAGCTGCCTTCCCTTT</i> <i>ACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGG</i> <i>CAAACCACAAC</i> <i>TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGAT</i> <i>GCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAACAACA</i> <i>ATTGCATTCATTTTATGTTTCAGGTT</i> <i>CAGGGGAGATGTGGGAGGTTTTTAAA</i> <i>GCAAGTAAACCTCTACAAATGTGGTAAATAAGGGACTAGAGCAGAATATTA</i> <i>AGCTT</i>
Full: 96 5' UTR: nucleic acids 5-329 of 42 ORF: 73 3' UTR: 109	Modified hGck-29	AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA GTGCTTCTGACACAACAGTCTCCAACTTAAGCTGCAGTGA CTCTCTT AAGGTAGCCTTG CAGAAGTTGGTCTGAGGCACTGGGCAGGTAAGT ATCAAGGTTACAAGACAGTTTAAGGAGACCAATAGAACTGGGCT TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA <u>A</u> <u>TGGCCATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACTCTGGT</u> <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGAATGCAGAAGGAGATGGACAGAGGCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGAGATCC</u> <u>ACCCAGAAAGGCTCAGAAGTGGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAAGGTGAGGAGG</u> <u>GGCAGTGGAGTGTGAAGACCAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u> <u>TCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCCT</u> <u>GGGCTTCACCTTCTCCTTTCCCTGTGAGGCATGAAGACATTGATAAGGGCA</u> <u>TCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGAA</u> <u>CAATGTGGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGGGACTTT</u> <u>GAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACCATGATCT</u> <u>CCTGCTACTATGAAGACCATCAGTGTGAGGTGGGCATGATTGTGGGCAC</u> <u>TGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGTG</u> <u>GAGGGGGATGAGGGCAGAATGTGTGTCAATACAGAGTGGGGAGCCTTT</u> <u>GGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACAGACTGG</u> <u>TGGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAAGCTCAT</u> <u>AGGTGGCAAGTACATGGGAGAGCTGGTGAGACTTGTGCTGCTCAGGCTG</u> <u>GTGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAGCTGAGAA</u>

		<u>CAAGAGGAGCCTTTGAGACCAGATTTGTGAGCCAGGTGGAGTCAGACAC</u> <u>AGGAGACAGAAAGCAGATCTACAACATCCTGAGCACCTGGGGCTGAG</u> <u>ACCCAGCACCCTGACTGTGACATTGTGAGAAGAGCCTGTGAGTCTGTG</u> <u>TCTACCAGAGCTGCCCACATGTGCAGTGCTGGGCTGGCTGGAGTCATCA</u> <u>ACAGAATGAGAGAGAGCAGATCAGAGGATGTAATGAGAATCACTGTGG</u> <u>GTGTGGATGGCTCTGTGTACAAGCTGCACCCCAGCTTCAAGGAGAGATT</u> <u>CCATGCCAGTGTGAGAAGGCTGACCCCAGCTGTGAGATCACCTTTATT</u> <u>GAGTCAGAGGAGGGCAGTGGCAGAGGAGCAGCCCTGGTCTCTGCTGTG</u> <u>GCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u> <u>AGCCAGCTGCCTTCCCT</u> <u>TTACTGTGGGTTAATGCTTGCA</u> <u>GACATGATAAGATACATTGATGAGTTTG</u> <u>GACAAACCACA</u> <u>ACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTG</u> <u>ATGCTATTGCTTTATTTGTA</u> <u>ACCATTATAAGCTGCAATAAACAGTTAACACAA</u> <u>CAATTGCATTCA</u> <u>TTTTATGTTTCAGGTT</u> <u>CAGGGGAGATGTGGGAGGTTTTTA</u> <u>AAGCAAGTAAACCTCTACA</u> <u>AATGTGGTAAATAAGGGACTAGAGCAGTTAAT</u> <u>TAAGCTT</u>
Full: 163 5' UTR: 42 ORF: 162 3' UTR: nucleotides 1- 285 of SEQ ID NO: 60	Unmodified hGck_2	CTAGAAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCA ACTTAAGCTGCAGTGA CTC TCTTAAGGTAGCCTTG CAGAAAGTTGGTCCTGAGGCACTGGGCAGGT AAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTG GGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTA TTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCAC TCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCC ACCATGGCGATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACTC <u>TGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGACGGATGCAGAAGGAGATGGACCGCGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTACGTGCG</u> <u>CTCCACCC</u> <u>CAGAAGGCTCAGAAGTCGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGCGTGAAGACCAAAACACCAGATGTACTCCATCCCC</u> <u>GAGGACGCCATGACCGGCACTGCTGAGATGCTCTTCGACTACATCTCTG</u> <u>AGTGCATCTCCGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCT</u> <u>GCCCCCTGGGCTTCACCTTCTCCTTTCCTGTGAGGCACGAAGACATCGATA</u> <u>AGGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGA</u> <u>AGGGAACAATGTCGTGGGGCTTCTGCGAGACGCTATCAAACGGAGAGG</u> <u>GGACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACGGTGGCCACG</u> <u>ATGATCTCCTGCTACTACGAAGACCATCAGTGCGAGGTCGGCATGATCG</u> <u>TGGGCACGGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGACGAGGGCCGCATGTGCGTCAATACCGAGTGGGG</u> <u>CGCCTTCGGGGACTCCGGCGAGCTGGACGAGTTCCTGCTGGAGTATGAC</u>

		<u>CGCCTGGTGGACGAGAGCTCTGCAAACCCCGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGCGAGCTGGTGCGGCTTGTGCTGCT</u> <u>CAGGCTCGTGGACGAAAACCTGCTCTTCCACGGGGAGGCCTCCGAGCAG</u> <u>CTGCGCACACGCGGAGCCTTCGAGACGCGCTTCGTGTCGCAGGTGGAGA</u> <u>GCGACACGGGCGACCGCAAGCAGATCTACAACATCCTGAGCACGCTGG</u> <u>GGCTGCGACCCTCGACCACCGACTGCGACATCGTGCGCCGCGCCTGCGA</u> <u>GAGCGTGTCTACGCGCGCTGCGCACATGTGCTCGGCGGGGCTGGCGGGC</u> <u>GTCATCAACCGCATGCGCGAGAGCCGAGCGAGGACGTAATGCGCATCA</u> <u>CTGTGGGCGTGGATGGCTCCGTGTACAAGCTGCACCCCAGCTTCAAGGA</u> <u>GCGGTTCCATGCCAGCGTGCGCAGGCTGACGCCCAGCTGCGAGATCACC</u> <u>TTCATCGAGTCGGAGGAGGGCAGTGGCCGGGGCGCGGCCCTGGTCTCGG</u> <u>CGGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><i>AGCCAGCTGCC</i> <i>TTCCCTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGA</i> <i>GTTTGGACAAACCACAAGTACAATGCAGTGAAAAAATGCTTTATTTGTGAAAT</i> <i>TTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAC</i> <i>AACAACAATTGCATTCAATTTATGTTTCAGGTTGAGGGGAGATGTGGGAGGT</i> <i>TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAG</i> <i>AGCAT</i>
Full: 164 5' UTR: 42 ORF: 162 3' UTR: 60	Unmodified hGck_3	CTAGAAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCA GTCAGTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCACTGACTC TCTTAAGGTAGCCTTGACAGAAGTTGGTCCTGAGGCACTGGGCAGGT AAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTG GGCTTGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTA TTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCAC TCCCAGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCC ACC<u>ATGGCGATGGATGTCACAAGGAGCCAGGCCCAGACAGCCTTGACTC</u> <u>TGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGACGGATGCAGAAGGAGATGGACCGCGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTACGTGCG</u> <u>CTCCACCCCAGAAGGCTCAGAAGTCGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGCGTGAAGACCAAAACACCAGATGTACTCCATCCCC</u> <u>GAGGACGCCATGACCGGCACTGCTGAGATGCTCTTCGACTACATCTCTG</u> <u>AGTGCATCTCCGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCT</u> <u>GCCCCTGGGCTTCACCTTCTCCTTTCCCTGTGAGGCACGAAGACATCGATA</u> <u>AGGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGA</u> <u>AGGGAACAATGTCGTGGGGCTTCTGCGAGACGCTATCAAACGGAGAGG</u> <u>GGACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACGGTGGCCACG</u> <u>ATGATCTCCTGCTACTACGAAGACCATCAGTGCGAGGTGCGCATGATCG</u>

		<u>TGGGCACGGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGA</u> <u>GCTGGTGGAGGGGGACGAGGGCCGCATGTGCGTCAATACCGAGTGGGG</u> <u>CGCCTTCGGGGACTCCGGCGAGCTGGACGAGTTCCTGCTGGAGTATGAC</u> <u>CGCCTGGTGGACGAGAGCTCTGCAAACCCCGGTCAGCAGCTGTATGAGA</u> <u>AGCTCATAGGTGGCAAGTACATGGGCGAGCTGGTGCGGCTTGTGCTGCT</u> <u>CAGGCTCGTGGACGAAAACCTGCTCTTCCACGGGGAGGCCTCCGAGCAG</u> <u>CTGCGCACACGCGGAGCCTTCGAGACGCGCTTCGTGTCGCAGGTGGAGA</u> <u>GCGACACGGGCGACCGCAAGCAGATCTACAACATCCTGAGCACGCTGG</u> <u>GGCTGCGACCCTCGACCACCGACTGCGACATCGTGCGCCGCGCCTGCGA</u> <u>GAGCGTGTCTACGCGCGCTGCGCACATGTGCTCGGCGGGGCTGGCGGGC</u> <u>GTCATCAACCGCATGCGCGAGAGCCGAGCGAGGACGTAATGCGCATCA</u> <u>CTGTGGGCGTGGATGGCTCCGTGTACAAGCTGCACCCCAGCTTCAAGGA</u> <u>GCGGTTCATGCCAGCGTGCGCAGGCTGACGCCAGCTGCGAGATCACC</u> <u>TTCATCGAGTCGGAGGAGGGCAGTGGCCGGGGCGCGGCCCTGGTCTCGG</u> <u>CGGTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGA</u><u>AGCCAGCTGCC</u> <u>TTCCCTTACTGTGGGTTAATGCTTGACAGACATGATAAGATACATTGATGA</u> <u>GTTTGGACAAACCACAAC</u><u>ACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAAT</u> <u>TTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTAAC</u> <u>AACAACAATTGCATTCA</u><u>TTTTATGTTTCAGGTT</u><u>CAGGGGGAGATGTGGGAGGT</u> <u>TTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAAATCCGATAAGGGACTAG</u> <u>AGCAGAAGCTTTTG</u>
Full: 168 5' UTR: 42 ORF: 68 3' UTR: 169	hGck	[CTAG]AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGC AGTCAGTGCTTCTGACACAACAGTCTCCA<u>ACTTAAGCTGCAGTGA</u><u>CT</u> CTCTTAAGGTAGCCTTGCAGAAGTTGGT<u>CCTGAGGCACTGGGCAGG</u> TAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACT GGGCTTGTGGAGACAGAGAAGACTCTTGGCTT<u>CTGATAGGCACCT</u> ATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCA CTCCCAGTTCAATTACAGCTCTTAAGGCTAGAGT<u>CCTGCAGAATTGC</u> CACCATGGCTATGGATGTCACAAGGAGCCAGGCC<u>CAGACAGCCTTGACT</u> <u>CTGGTAGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGA</u> <u>AGAAGGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCCTGAGGC</u> <u>TGGAGACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGCG</u> <u>CTCCACCC</u><u>CAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTG</u> <u>GGTGGCACTAACTTCAGGGT</u><u>GATGCTGGTGAAGGTGGGAGAAGGTGAG</u> <u>GAGGGGCAGTGGAGTGTGAAGACCAAAACACCAGATGTACTCCATCCCTG</u> <u>AGGATGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGA</u> <u>GTGCATCTCAGACTT</u><u>CCTGGACAAGCATCAGATGAAACACAAGAAGCTG</u> <u>CCCCTGGGCTT</u><u>CACCTTCTCCTTTCCTGTGAGGCATGAAGACATTGATAA</u> <u>GGGCATCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAA</u>

		<u>GGGAACAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGG</u> <u>GACTTTGAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTA</u> <u>TGATCTCCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTG</u> <u>GGCACAGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAG</u> <u>CTGGTGGAGGGGGATGAGGGCCGCATGTGTGTCAATACTGAGTGGGGCG</u> <u>CCTTTGGGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACCG</u> <u>CCTGGTGGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAAG</u> <u>CTCATAGGTGGCAAGTACATGGGAGAGCTGGTGC GGCTTGTGCTGCTCA</u> <u>GGTTGGTGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAGCT</u> <u>GCGCACACGCGGAGCCTTTGAGACGCGCTTTGTGTCACAAGTGGAGTCT</u> <u>GACACAGGAGACCGCAAGCAGATCTACAACATCCTGAGCACACTGGGG</u> <u>CTGCGGCCCTCCACCACTGACTGTGACATTGTGCGCCGCGCCTGTGAGA</u> <u>GTGTGTCTACGCGCGCTGCGCACATGTGCTCAGCGGGGCTGGCGGGAGT</u> <u>CATCAACCGCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACT</u> <u>GTGGGAGTGGATGGCAGTGTGTACAAGCTGCACCCCAGCTTCAAGGAGC</u> <u>GGTTCCATGCCTCAGTGCGCAGGCTGACCCCCAGCTGTGAGATCACCTTT</u> <u>ATTGAGTCTGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCAGCGCG</u> <u>GTGGCCTGTAAGAAGGCCTGTATGCTGGGCCAGTGAAGCCAGCTGCCTTC</u> <i>CCTTTACTGTGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTT</i> <i>TGGACAAACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG</i> <i>TGATGCTATTGCTTTATTTGTAACCATTAAGCTGCAATAAAACAAGTTAACAAC</i> <i>AATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTAA</i> <i>AGCAAGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCATG</i> <i>GCTACGTA</i>
Full: 172 5' UTR: 42 ORF: 68 3' UTR: 171	Modified hGck-30	<u>AAGCTTTATTGTGGTAGTTTATCACAGTTAAATTGCTAAGGCAGTCA</u> <u>GTGCTTCTGACACAACAGTCTCCAACCTTAAGCTGCAGTGA CTCTCTT</u> <u>AAGGTAGCCTTG CAGAAGTTGGTCCTGAGGCACTGGGCAGGTAAGT</u> <u>ATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCT</u> <u>TGTGGAGACAGAGAAGACTCTTGGCTTTCTGATAGGCACCTATTGG</u> <u>TCTTACTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCC</u> <u>AGTTCAATTACAGCTCTTAAGGCTAGAGTCCTGCAGAATTGCCACCA</u> <u>TGGCTATGGATGTCACAAGGAGCCAGGCCAGACAGCCTTGACTCTGGT</u> <u>AGAGCAGATCCTGGCAGAGTTCCAGCTGCAGGAGGAGGACCTGAAGAA</u> <u>GGTGATGAGAAGAATGCAGAAGGAGATGGACCGCGGCCTGAGGCTGGA</u> <u>GACCCATGAAGAGGCCAGTGTGAAGATGCTGCCCACCTATGTGCGCTCC</u> <u>ACCCCAGAAGGCTCAGAAGTTGGGGACTTCCTCTCCCTGGACCTGGGTG</u> <u>GCACTAACTTCAGGGTGATGCTGGTGAAGGTGGGAGAAGGTGAGGAGG</u> <u>GGCAGTGGAGTGTGAAGACCAAACACCAGATGTACTCCATCCCTGAGGA</u> <u>TGCCATGACAGGCACTGCTGAGATGCTCTTTGACTACATCTCTGAGTGCA</u>

	<u>TCTCAGACTTCCTGGACAAGCATCAGATGAAACACAAGAAGCTGCCCT</u> <u>GGGCTTCACCTTCTCCTTTCCTGTGAGGCATGAAGACATTGATAAGGGCA</u> <u>TCCTTCTCAACTGGACCAAGGGCTTCAAGGCCTCAGGAGCAGAAGGGAA</u> <u>CAATGTTGTGGGGCTTCTGAGAGATGCTATCAAAAGAAGAGGGGACTTT</u> <u>GAAATGGATGTGGTGGCAATGGTGAATGACACAGTGGCCACTATGATCT</u> <u>CCTGCTACTATGAAGACCATCAGTGTGAGGTTGGCATGATTGTGGGCAC</u> <u>AGGCTGCAATGCCTGCTACATGGAGGAGATGCAGAATGTGGAGCTGGTG</u> <u>GAGGGGGATGAGGGCCGCATGTGTGTCAATACTGAGTGGGGCGCCTTTG</u> <u>GGGACTCTGGAGAGCTGGATGAGTTCCTGCTGGAGTATGACCGCCTGGT</u> <u>GGATGAGAGCTCAGCAAACCCAGGTCAGCAGCTGTATGAGAAGCTCATA</u> <u>GGTGGCAAGTACATGGGAGAGCTGGTGCGGCTTGTGCTGCTCAGGTTGG</u> <u>TGGATGAAAACCTGCTCTTCCATGGGGAGGCCAGTGAGCAGCTGCGCAC</u> <u>ACGCGGAGCCTTTGAGACGCGCTTTGTGTCACAAGTGGAGTCTGACACA</u> <u>GGAGACCGCAAGCAGATCTACAACATCCTGAGCACACTGGGGCTGCGGC</u> <u>CCTCCACCACTGACTGTGACATTGTGCGCCGCGCCTGTGAGAGTGTGTCT</u> <u>ACGCGCGCTGCGCACATGTGCTCAGCGGGGCTGGCGGGAGTCATCAACC</u> <u>GCATGCGCGAGAGCCGCTCTGAGGATGTAATGCGCATCACTGTGGGAGT</u> <u>GGATGGCAGTGTGTACAAGCTGCACCCCAGCTTCAAGGAGCGGTTCCAT</u> <u>GCCTCAGTGCGCAGGCTGACCCCCAGCTGTGAGATCACCTTTATTGAGTC</u> <u>TGAGGAGGGCAGTGGCAGAGGCGCGGCCCTGGTCAAGCGGTGGCCCTG</u> <u>TAAGAAGGCCTGTATGCTGGGCCAGTGA</u><u>AGCCAGCTGCCTTCCCTTTACTG</u> <u>TGGGTTAATGCTTGCAGCAGACATGATAAGATACATTGATGAGTTTGGACAAA</u> <u>CCACAAGTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTA</u> <u>TTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAAACAACAATTG</u> <u>CATTCATTTTATGTTTCAGGTT</u><u>CAGGGGAGATGTGGGAGGTTTTTAAAGCA</u> <u>AGTAAAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCA</u>
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WHAT IS CLAIMED IS:

1. A method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, wherein the method comprises administering a combination therapy to the subject comprising: one or more adeno-associated virus (AAV) vectors comprising
 - (a) a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein; and
 - (b) a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein;wherein the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.
2. The method of claim 1, wherein the combination therapy comprises:
 - (a) a first AAV vector genome comprising an insulin expression cassette comprising the first promoter operably linked to the polynucleotide encoding a hIns protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and
 - (b) a second AAV vector genome comprising a glucokinase expression cassette comprising the second promoter operably linked to the polynucleotide encoding the hGck protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).
3. The method of claim 2, wherein the first AAV vector genome and the second AAV vector genome are administered simultaneously or sequentially, optionally, wherein the first AAV vector genome and the second AAV vector genome are administered in the same pharmaceutical composition.
4. The method of any one of claims 1-3, wherein the polynucleotide encoding a human insulin (hIns) protein and the polynucleotide encoding a human glucokinase (hGck) protein are administered in the same pharmaceutical composition.
5. The method of any one of claims 2-4, wherein the first AAV vector genome and the second AAV vector genome are admixed prior to IM injection.

6. The method of claim 1, wherein the one or more AAV vectors is an AAV vector genome comprising an expression cassette comprising the first promoter operably linked to the polynucleotide encoding the hIns protein and the second promoter operably linked to the polynucleotide encoding the hGck protein, wherein the expression cassette is flanked by inverted terminal repeats (ITRs).
7. The method of any one of claims 1-6, wherein the combination therapy comprises (i) a first recombinant AAV (rAAV) particle comprising the first AAV vector genome comprising the insulin expression cassette comprising the promoter operably linked to the polynucleotide encoding the human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising the second AAV vector genome comprising the glucokinase expression cassette comprising the promoter operably linked to the polynucleotide encoding the human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs).
8. The method of any one of claims 1-7, wherein the combination therapy is administered intramuscularly (IM) to at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten different skeletal muscle groups.
9. The method of any one of claims 1-8, wherein the combination therapy is administered intramuscularly (IM) to (i) 2-10, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, or 9-10 different skeletal muscle groups; (ii) 2-8, 3-8, 4-8, 5-8, 6-8, or 7-8 different skeletal muscle groups; (iii) 2-6, 3-6, 4-6, or 5-6 different skeletal muscle groups; (iv) 2-5, 3-5, or 4-5 different skeletal muscle groups; (v) 2-4 or 3-4 different skeletal muscle groups.
10. The method of any one of claims 1-9, wherein the combination therapy is administered intramuscularly (IM) to two, three, four, five, six, seven, eight, nine, or ten different skeletal muscle groups.
11. The method of any one of claims 1-10, wherein the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius, a gluteus maximus, a hamstring, a

deltoid, a trapezius, a pectoral muscle, and a latissimus dorsi (lat), optionally wherein the two or more different skeletal muscle groups comprise any combination thereof, optionally wherein the two or more different skeletal muscle group is selected from i) a left quadriceps and a right quadriceps, ii) a left bicep and a right bicep, iii) a left triceps and a right triceps, iv) a left gastrocnemius and a right gastrocnemius, v) a left gluteus maximus and a right gluteus maximus, vi) a left hamstring and a right hamstring, vii) a left deltoid and a right deltoid, viii) a left trapezius and a right trapezius, ix) a left pectoral muscle and a right pectoral muscle, and x) a left latissimus dorsi and a right latissimus dorsi.

12. The method of any one of claims 1-11, wherein the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius, a gluteus maximus, and a hamstring, optionally wherein the two or more different skeletal muscle groups comprise any combination thereof.
13. The method of any one of claims 1-12, wherein the skeletal muscle group is selected from a quadriceps, a bicep, a gastrocnemius, a gluteus maximus, and a hamstring, optionally wherein the two or more different skeletal muscle groups comprise any combination thereof.
14. The method of any one of claims 1-13, wherein the administration comprises bilateral injections into one or more of the different skeletal muscle groups, optionally wherein the bilateral injections are divided equally for each skeletal muscle group.
15. The method of claim 14, wherein the bilateral injections comprise IM injections to the right and left quadriceps, the right and left biceps, the right and left triceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings, and any combinations thereof.
16. The method of any one of claims 1-15, wherein the administration comprises a total of 20-150 IM injections of the combination therapy to the at least two different skeletal muscle groups.

17. The method of claim 16, wherein the administration comprises a total of 20-120 IM injections, 20-110 IM injections, 20-100 IM injections, 20-80 IM injections, 20-60 IM injections, 25-150 IM injections, 25-120 IM injections, 25-110 IM injections, or 25-100 IM injections to the at least two different skeletal muscle groups.
18. The method of any one of claims 1-17, wherein the administration comprises:
 - (i) 1-80 IM injections to a first skeletal muscle group and 1-80 IM injections to a second skeletal muscle group;
 - (ii) 1-60 IM injections to a first skeletal muscle group, 1-60 IM injections to a second skeletal muscle group, and 1-60 IM injections to a third skeletal muscle group;
 - (iii) 1-40 IM injections to a first skeletal muscle group, 1-40 IM injections to a second skeletal muscle group, 1-40 IM injections to a third skeletal muscle group, and 1-40 IM injections to a fourth skeletal muscle group;
 - (iv) 1-30 IM injections to a first skeletal muscle group, 1-30 IM injections to a second skeletal muscle group, 1-30 IM injections to a third skeletal muscle group, 1-30 IM injections to a fourth skeletal muscle group, and 1-30 IM injections to a fifth skeletal muscle group; and
 - (v) 1-25 IM injections to a first skeletal muscle group, 1-25 IM injections to a second skeletal muscle group, 1-25 IM injections to a third skeletal muscle group, 1-25 IM injections to a fourth skeletal muscle group, 1-25 IM injections to a fifth skeletal muscle group, and 1-25 IM injections to a sixth skeletal muscle group.
19. The method of any one of claims 1-18, wherein the administration comprises:
 - (i) 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80 IM injections to a first skeletal muscle group and 2-80, 4-80, 6-80, 8-80, 10-80, 20-80, 30-80, 40-80, 50-80, or 60-80 IM injections to a second skeletal muscle group;
 - (ii) 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a first skeletal muscle group; 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a second skeletal muscle group; and 2-60, 4-60, 6-60, 8-60, 10-60, 20-60, 30-60, 40-60, or 50-60 IM injections to a third skeletal muscle group;
 - (iii) 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a first skeletal muscle group; 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a second skeletal muscle group; 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a

third skeletal muscle group; and 2-40, 4-40, 6-40, 8-40, 10-40, 20-40, or 30-40 IM injections to a fourth skeletal muscle group;

(iv) 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a first skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a second skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a third skeletal muscle group; 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a fourth skeletal muscle group; and 2-30, 4-30, 6-30, 8-30, 10-30, or 20-30 IM injections to a fifth skeletal muscle group; or

(v) 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a first skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a second skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a third skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a fourth skeletal muscle group; 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a fifth skeletal muscle group; and 2-25, 4-25, 6-25, 8-25, 10-25, or 20-25 IM injections to a sixth skeletal muscle group.

20. The method of claim 19, wherein the administration comprises 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50 IM injections to a first skeletal muscle group, 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50 IM injections to a second skeletal muscle group, and 2-50, 4-50, 6-50, 8-50, 10-50, 20-50, 30-50, or 40-50 IM injections to a third skeletal muscle group.
21. The method of any one of claims 1-20, wherein the administration comprises 8-50 IM injections to the quadriceps, 8-50 IM injections to the biceps, and 8-50 IM injections to the hamstrings.
22. The method of claim 21, wherein the administration comprises 4-25 IM injections bilaterally to each quadriceps, 4-25 IM injections bilaterally to each bicep, and 4-25 IM injections bilaterally to each hamstring.
23. The method of any one of claims 1-22, wherein each IM injection is administered in a volume of about 100 μ L to about 1.5 mL, optionally about 0.5 mL to about 1 mL.

24. The method of claim 23, wherein each IM injection is administered in a volume of about 100 μ L to about 2 mL, 100 μ L to about 1.9 mL, 100 μ L to about 1.8 mL, 100 μ L to about 1.7 mL, 100 μ L to about 1.6 mL, 100 μ L to about 1.5 mL, 100 μ L to about 1.4 mL, about 100 μ L to about 1.35 mL, about 100 μ L to about 1.2 mL, about 100 μ L to about 1.0 mL, about 100 μ L to about 800 μ L, about 200 μ L to about 800 μ L, about 200 μ L to about 600 μ L, about 400 μ L to about 800 μ L, about 500 μ L to about 1.5 mL, about 600 μ L to about 1.4 mL, about 700 μ L to about 1.3 mL, about 800 μ L to about 1.2 mL, or about 900 μ L to about 1.1 mL.
25. The method of any one of claims 1-24, wherein the total cumulative volume administered for all IM injections is about 20 mL to about 70 mL, about 20 mL to about 50 mL, or about 30 mL to about 40 mL.
26. The method of any one of claims 1-25, wherein each IM injection comprises about 8×10^{10} to about 5×10^{13} vgs per mL, optionally wherein the IM injection comprises about 2×10^{13} to about 4×10^{13} vgs per mL.
27. The method of any one of claims 1-26, wherein the IM administration comprises injections that are separated by a distance of at least about 1-5 cm, optionally at least about 1-3 cm.
28. The method of any one of claims 1-27, wherein each of the IM injections are separated by a distance of at least 1 cm, at least 2 cm, at least 3 cm, at least 4 cm, or at least 5 cm.
29. The method of any one of claims 1-28, wherein the administration comprises IM injections that are at least 0.5 cm deep, at least 0.6 cm deep, at least 0.7 cm deep, at least 0.8 cm deep, at least 0.9 cm deep, at least 1.0 cm deep, at least 1.1 cm deep, at least 1.2 cm deep, at least 1.3 cm deep, at least 1.4 cm deep, at least 1.5 cm deep, at least 2.0 cm deep, at least 2.5 cm deep, at least 3.0 cm deep, at least 3.5 cm deep, at least 4.0 cm deep, at least 4.5 cm deep, or at least 5.0 cm deep..
30. The method of any one of claims 1-29, wherein each of the IM injections is about 0.5 cm to 2.0 cm deep, optionally about 1-2 cm deep.

31. The method of any one of claims 1-30, wherein each of the IM injections is at least 0.5 cm below the surface of the injected muscle, regardless of the thickness of the subcutaneous fat.
32. The method of any one of claims 1-31, further comprising administering to the subject an immunosuppressive regimen comprising an immunosuppressive agent.
33. A method of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising administering to the subject (i) an immunosuppressive regimen comprising an immunosuppressive agent, and (ii) a combination therapy comprising one or more adeno-associated virus (AAV) vectors comprising:
 - (a) a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein; and
 - (b) a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, optionally, wherein the combination therapy is administered intramuscularly (IM) to at least two different skeletal muscle groups.
34. The method of claim 32 or 33, wherein the immunosuppressive regimen comprises administering one or more immunosuppressive agents prior to (pre-AAV), at the same time as, and/or after (post-AAV) administering the combination therapy.
35. The method of any one of claims 32-34, wherein the immunosuppressive regimen comprises administering the immunosuppressive agent prior to administering the combination therapy.
36. The method of any one of claims 32-35, wherein the immunosuppressive regimen comprises administering the immunosuppressive agent after administering the combination therapy.
37. The method of any one of claims 32-36, wherein the immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.

38. The method of any one of claims 34-37, wherein the immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
39. The method of any one of claims 34-38, wherein the immunosuppressive agent is administered 1 to 3 days prior to administering the combination therapy.
40. The method of any one of claims 32-38, wherein the immunosuppressive agent is administered 3 to 7 days prior to administering the combination therapy.
41. The method of any one of claims 32-40, wherein the immunosuppressive agent is further administered 1, 2, 3, or 4 weeks after administering the combination therapy.
42. The method of any one of claims 32-41, wherein immunosuppressive agent is administered once about every 1-3 weeks, optionally once about every 1-2 weeks after administering the combination therapy.
43. The method of any one of claims 32-42, wherein the immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the combination therapy.
44. The method of any one of claims 32-43, wherein immunosuppressive regimen comprises a second immunosuppressive agent.
45. The method of claim 44, wherein the immunosuppressive regimen comprises administering the second immunosuppressive agent prior to administering the combination therapy.
46. The method of claim 44 or 45, wherein the immunosuppressive regimen comprises administering the second immunosuppressive agent after administering the combination therapy.
47. The method of any one of claims 44-46, wherein the second immunosuppressive agent is administered 1 to 14 days prior to administering the combination therapy.

48. The method of any one of claims 44-47, wherein the second immunosuppressive agent is administered 1 to 7 days prior to administering the combination therapy.
49. The method of any one of claims 44-48, wherein the second immunosuppressive agent is administered 1 to 3 days prior to administering the combination therapy.
50. The method of any one of claims 44-48, wherein the second immunosuppressive agent is administered 3 to 7 days prior to administering the combination therapy.
51. The method of any one of claims 44-50, wherein the second immunosuppressive agent is further administered 1, 2, 3, or 4 weeks after administering the combination therapy.
52. The method of any one of claims 44-51, wherein second immunosuppressive agent is administered 2 times per day, daily, or every other day.
53. The method of any one of claims 44-52, wherein the second immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the combination therapy.
54. The method of any one of claims 32-53, wherein the immunosuppressive regimen comprises administering an immunosuppressive agent selected from the group consisting of an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, a calcineurin inhibitor, or any combination thereof.
55. The method of any one of claims 32-54, wherein the immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, MMF, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.

56. The method of claim 54 or claim 55, wherein the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, satralizumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof.
57. The method of claim 56, wherein the IL-6 antagonist is tocilizumab.
58. The method of any one of claims 32-57, wherein the second immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, abatacept, thymoglobulin, etanercept, basiliximab, sirolimus, or any combination thereof.
59. The method of claim 58, wherein the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof.
60. The method of claim 59, wherein the calcineurin inhibitor is tacrolimus.
61. The method of any one of claims 32-60, wherein the immunosuppressive regimen comprises administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the combination therapy.
62. The method of any one of claims 32-61, wherein the immunosuppressive regimen comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the combination therapy.
63. The method of any one of claims 2-62, wherein the total administered dose of the first AAV vector genome comprising the insulin expression cassette comprises about 7×10^{10} to about 5×10^{13} vector genomes (vg)/kg.
64. The method of any one of claims 2-63, wherein the total administered dose of the second AAV vector genome comprising the glucokinase expression cassette comprises about 5×10^{11} to about 5×10^{13} vector genomes (vg)/kg.

65. The method of any one of claims 32-64, wherein the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:0.25-1.25, 1:1-20, 1:2-20, 1:3-20, 1:4-20, 1:5-20, 1:1-18, 1:2-18, 1:3-18, 1:4-18, 1:5-18, 1:1-16, 1:2-16, 1:3-16, 1:4-16, 1:5-16, 1:1-15, 1:2-15, 1:3-15, 1:4-15, 1:5-15, 1:1-14, 1:2-14, 1:3-14, 1:4-14, 1:5-14, 1:1-12, 1:2-12, 1:3-12, 1:4-12, 1:5-12, 1:1-10, 1:2-10, 1:3-10, 1:4-10, or 1:5-10.
66. The method of any one of claims 32-65, wherein the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:1-15, 1:2-15, 1:3-15, 1:4-15, 1:5-15, 1:1-14, 1:2-14, 1:3-14, 1:4-14, 1:5-14, 1:1-12, 1:2-12, 1:3-12, 1:4-12, 1:5-12, 1:1-10, 1:2-10, 1:3-10, 1:4-10, or 1:5-10.
67. The method of any one of claims 32-66, wherein the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:1-10, 1:2-10, 1:3-10, 1:4-10, or 1:5-10.
68. The method of any one of claims 32-66, wherein the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, or about 1:15.
69. The method of any one of claims 32-66, wherein the ratio of the first AAV vector genome comprising the insulin expression cassette to the second AAV vector genome comprising the glucokinase expression cassette is about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:8, about 1:9, or about 1:10.
70. The method of any one of claims 2-69, wherein the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 5×10^{11} and about 1×10^{13} vg/kg.

71. The method of any one of claims 2-70, wherein the first AAV vector genome comprising an insulin expression cassette is administered at a dose between about 2×10^{12} and about 1×10^{13} vg/kg.
72. The method of any one of claims 2-71, wherein the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 4×10^{12} to about 1×10^{13} vg/kg.
73. The method of any one of claims 2-69, wherein the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 7×10^{10} to about 7×10^{12} vg/kg.
74. The method of any one of claims 2-69, wherein the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 5×10^{11} to about 5×10^{12} vg/kg.
75. The method of any one of claims 2-69, wherein the first AAV vector genome comprising an insulin expression cassette is administered at a dose of about 1×10^{11} to about 1×10^{12} vg/kg.
76. The method of any one of claims 2-75, wherein the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 1×10^{12} and about 5×10^{12} vg/kg.
77. The method of any one of claims 2-76, wherein the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose between about 2×10^{12} and about 5×10^{12} vg/kg.
78. The method of any one of claims 2-77, wherein the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 2×10^{12} to about 3.5×10^{12} vg/kg.

79. The method of any one of claims 2-77, wherein the second AAV vector genome comprising a glucokinase expression cassette is administered at a dose of about 3.5×10^{12} to about 5×10^{12} vg/kg.
80. The method of any one of claims 1-79, wherein within 15 days-6 months, 30-120 days, or 30-60 days after administration of the combination therapy: (i) glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in the subject; (ii) circulating ketones are reduced in the subject, (iii) triglycerides are reduced in the subject, (iv) exogenous insulin is reduced in the subject, (v) circulating C-peptide levels are increased in the subject, or (vi) any combination thereof.
81. The method of any one of claims 1-80, wherein within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's circulating insulin level in the blood, when measured in the absence of exsanguinous insulin treatment, is about 1-40 $\mu\text{U/mL}$, optionally about 5-40 $\mu\text{U/mL}$, about 5-25 $\mu\text{U/mL}$, about 5-10 $\mu\text{U/mL}$, about 10-20 $\mu\text{U/mL}$, about 10-15 $\mu\text{U/mL}$, about 15-20 $\mu\text{U/mL}$, or about 15-25 $\mu\text{U/mL}$.
82. The method of any one of claims 1-81, wherein within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's HbA1C level is reduced by about 0.5 to 1.5% compared to pre-treatment.
83. The method of any one of claims 1-82, wherein within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's HbA1C level is less than about 7.0%, optionally less than about 6.5%, about 6.0%, about 5.7%, optionally about 5.0% to 6.5%.
84. The method of any one of claims 1-83, wherein within 15 days-6 months, 30-120 days, 30-60 days, or 60-90 days after administration of the combination therapy the subject's circulating glucose in the blood is at least about 70 to 140 mg/dL.
85. The method of any one of claims 1-84, wherein the diabetes is diabetes mellitus type 1 (T1DM).

86. The method of any one of claims 1-84, wherein the diabetes is diabetes mellitus type 2 (T2DM).
87. The method of any one of claims 1-86, wherein the subject is not insulin resistant.
88. The method of any one of claims 1-86, wherein the subject is insulin resistant.
89. A dosing regimen comprising:
- (a) a first immunosuppressive agent;
 - (b) a combination therapy administered intramuscularly (IM) after (a) which comprises (i) a first recombinant AAV (rAAV) particle comprising a first AAV vector genome comprising an insulin expression cassette comprising a first promoter operably linked to a polynucleotide encoding a human insulin (hIns) protein, wherein the insulin expression cassette is flanked by inverted terminal repeats (ITRs); and (ii) a second recombinant AAV (rAAV) particle comprising a second AAV vector genome comprising a glucokinase expression cassette comprising a second promoter operably linked to a polynucleotide encoding a human glucokinase (hGck) protein, wherein the glucokinase expression cassette is flanked by inverted terminal repeats (ITRs); and
 - (c) a second immunosuppressive agent.
90. The dosing regimen of claim 89, wherein the first immunosuppressive agent comprises an IL-6 antagonist selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, and any combination thereof.
91. The dosing regimen of claim 89 or 90, wherein the second immunosuppressive agent is a calcineurin inhibitor selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof.
92. The dosing regimen of any one of claims 89-91, wherein the first immunosuppressive agent comprises tocilizumab and the second immunosuppressive agent comprises tacrolimus.

93. The dosing regimen of any one of claims 89-92, comprising administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the combination therapy.
94. The dosing regimen of any one of claims 89-93, wherein the immunosuppressive regimen comprises administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the combination therapy
95. The method or dosing regimen of any one of claims 1-94, wherein:
- (a) the polynucleotide encoding the hIns protein comprises an open reading frame (ORF) comprising: a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to any one of: (i) nucleic acids 73-330 of any of SEQ ID NOs: 43-57, or 110-116, nucleic acids 88-345 of any of SEQ ID NOs: 117-122; or (ii) SEQ ID NO: 43-57, or SEQ ID NO: 110-122; and/or
 - (b) the polynucleotide encoding the human glucokinase hGck protein comprises an ORF comprising (i) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of (a) nucleic acids 1-1398 of any of SEQ ID NO: 61-80 or 162; or (ii) SEQ ID NO: 61-80 and 162.
96. The method or dosing regimen of any one of claims 1-95, wherein:
- (a) the polynucleotide encoding the hIns protein comprises an open reading frame (ORF) comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 121; and/or
 - (b) the polynucleotide encoding the human glucokinase hGck protein comprises an ORF comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 68.
97. The method or dosing regimen of any one of claims 1-96, wherein the hIns protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, amino acids 25-110 of SEQ ID NO: 145, SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145.

98. The method or dosing regimen of any one of claims 1-97, wherein the hIns protein comprises a signal peptide.
99. The method or dosing regimen of claim 98, wherein the signal peptide is a wild-type preproinsulin signal sequence, an IL-6 signal sequence, a fibronectin signal sequence, or a non-wild-type preproinsulin signal sequence.
100. The method or dosing regimen of claim 98 or 99, wherein the signal peptide comprises amino acids 1-24 of SEQ ID NO: 41, amino acids 1-24 of SEQ ID NO: 144, amino acids 1-24 of SEQ ID NO: 145, or SEQ ID NO: 165.
101. The method or dosing regimen of any one of claims 1-100, wherein the hIns protein further comprises a cleavage site.
102. The method or dosing regimen of any one of claims 1-101, wherein the polynucleotide encoding the hIns protein further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.
103. The method or dosing regimen of any one of claims 1-102, wherein the polynucleotide encoding the hIns protein further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, or SEQ ID NO: 101, SEQ ID NO: 149, or SEQ ID NO: 171.
104. The method or dosing regimen of any one of claims 1-103, wherein the encoded hGck protein comprises the amino acid sequence of SEQ ID NO: 82.
105. The method or dosing regimen of any one of claims 1-104, wherein the polynucleotide encoding the hGck protein further comprises a 5' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.

106. The method or dosing regimen of any one of claims 1-105, wherein the polynucleotide encoding the hGck protein further comprises a 3' UTR comprising a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 60, 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: 149, SEQ ID NO: 169, or SEQ ID NO: 171.
107. The method or dosing regimen of any one of claims 1-106, wherein the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a eukaryotic promoter.
108. The method or dosing regimen of any one of claims 1-106, wherein the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a constitutive promoter.
109. The method or dosing regimen of any one of claims 1-106, wherein the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a ubiquitous promoter.
110. The method or dosing regimen of any one of claims 1-109, wherein the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a CMV promoter.
111. The method or dosing regimen of any one of claims 1-107, wherein the insulin expression cassette promoter and/or the glucokinase expression cassette promoter is a muscle specific promoter.
112. The method or dosing regimen of any one of claims 1-111, wherein the insulin expression cassette further comprises a polyadenylation (polyA) element.
113. The method or dosing regimen of any one of claims 1-112, wherein the glucokinase expression cassette further comprises a polyadenylation (polyA) element.

114. The method or dosing regimen of any one of claims 2-113, wherein a first recombinant AAV (rAAV) particle comprises the first AAV vector genome and a second recombinant AAV (rAAV) particle comprises the second vector genome.
115. The method or dosing regimen of claim 114, wherein the AAV serotype of the first rAAV particle and/or the second rAAV particle is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh9, AAV9, AAVrh10, AAV10, AAV11, AAV12, and AAVrh74.
116. The method or dosing regimen of claim 114 or 115, wherein the AAV serotype of the first rAAV particle and/or the second rAAV particle is AAV1.
117. The method or dosing regimen of any one of claims 114-116, wherein the first rAAV particle and the second rAAV particle are formulated in a single pharmaceutical composition.
118. The method or dosing regimen of any one of claims 114-116, wherein the first rAAV particle and the second rAAV particle are administered simultaneously or sequentially.
119. A method of immunosuppression in a subject administered an AAV gene therapy for the treatment of diabetes, wherein the subject is administered an immunosuppressive regimen comprising administering (a) an immunosuppressive agent (pre-AAV) prior to administering the AAV gene therapy; and (b) an immunosuppressive agent (post-AAV) after administering the AAV gene therapy.
120. The method of claim 119, wherein the pre-AAV immunosuppressive agent is administered 1 to 14 days, 1 to 7 days, 1 to 3 days, or 3 to 7 days prior to administering the AAV gene therapy.
121. The method of claim 119 or 120, the post-AAV immunosuppressive agent is further administered for 1, 2, 3, or 4 weeks after administering the AAV gene therapy.

122. The method of any one of claims 119-121, wherein post-AAV immunosuppressive agent is administered once about every 1-3 weeks, optionally once about every 1-2 weeks after administering the combination therapy.
123. The method of any one of claims 119-122, wherein the post-AAV immunosuppressive agent is administered for about 1-6 months, optionally about 1-3 months after administering the AAV gene therapy.
124. The method of any one of claims 119-123, wherein the post-AAV immunosuppressive agent is administered for about 6 to 40, about 8 to 40, about 10 to 40, about 12 to 40, about 6 to 30, about 8 to 30, about 10 to 30, about 12 to 30, about 6 to 20, about 8 to 20, about 10 to 20, or about 12 to 20 weeks.
125. The method of any one of claims 119-124, wherein the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent is administered for about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months.
126. The method of any one of claims 119-125, wherein the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, a TNF α antagonist, an mTOR pathway inhibitor, mycophenolate mofetil (MMF), a tyk2 inhibitor, methotrexate, rapamycin, abatacept, antithymocyte globulin (ATG), a B cell depletion agent, a T cell costimulation antagonist, a T cell depletion agent, a corticosteroid, a calcineurin inhibitor, or any combination thereof.
127. The method of any one of claims 119-126, wherein the pre-AAV immunosuppressive agent and/or post-AAV immunosuppressive agent comprises an IL-6 antagonist, an IL-1 antagonist, MMF, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.
128. The method of any one of claims 119-127, wherein the pre-AAV immunosuppressive agent and/or the post-AAV immunosuppressive agent comprises a calcineurin inhibitor, MMF, methotrexate, a tyk2 inhibitor, rapamycin, abatacept, or any combination thereof.

129. The method of any one of claims 119-128, wherein the IL-6 antagonist is selected from the group consisting of tocilizumab, sarilumab, siltuximab, olokizumab, elsilimomab, clazakizumab, sirukumab, levilimab, or any combination thereof.
130. The method of claim 129, wherein the IL-6 antagonist is tocilizumab.
131. The method of any one of claims 126-130, wherein the calcineurin inhibitor is selected from the group consisting of cyclosporine, tacrolimus, voclosporin, and any combination thereof.
132. The method of claim 131, wherein the calcineurin inhibitor is tacrolimus.
133. The method of any one of claims 119-132, comprising administering tocilizumab, optionally subcutaneously, on about day -3 and about every week or about every other week through about 12 weeks relative to the AAV gene therapy.
134. The method of any one of claims 119-133, comprising administering tacrolimus, optionally orally, on about day -3 and every 1-3 days through about 3-12 weeks relative to the AAV gene therapy.
135. The method of claim 119-134, wherein the subject is administered the AAV gene therapy by intramuscular (IM) injection.
136. The method of claim 135, wherein the IM injection is to at least two different skeletal muscle groups.
137. The method of claim 136, wherein the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius, a gluteus maximus, a hamstring, a deltoid, a trapezius, a pectoral muscle, and a latissimus dorsi (lat).
138. The method of claim 136 or 137, wherein the skeletal muscle group is selected from a quadriceps, a bicep, a triceps, a gastrocnemius, a gluteus maximus, and a hamstring.

139. The method of any one of claims 119-137, wherein the administration comprises bilateral injections into one or more of the skeletal muscle groups.
140. The method of claim 139, wherein the bilateral injections comprise IM injections into the right and left quadriceps, the right and left biceps, the right and left triceps, the right and left gastrocnemius muscles, the right and left gluteus maximus muscles, and/or the right and left hamstrings.