

WHAT IS CLAIMED IS: CLAIMS

1. Use of (i) a polynucleotide, an expression cassette, a vector, or a rAAV particle comprising a nucleotide sequence encoding a human insulin (Ins) protein, wherein the nucleotide sequence is at least 85%, 90%, 95%, 99%, or 100% identical to any one of: (a) nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, 150-151, 154-155 or 157-159, 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 (b) SEQ ID NO: 43-57, SEQ ID NO: 110-122, or SEQ ID NO: 150-159; and (ii) a polynucleotide, an expression cassette, a vector, or a rAAV particle comprising a nucleotide sequence encoding a human glucokinase (Gck) protein, wherein the nucleotide sequence is 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 (b) SEQ ID NO: 61-80 and 162 in the manufacture of a medicament for treating or ameliorating the symptoms associated with diabetes.
2. The use of claim 1, wherein 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, amino acids 25-110 of SEQ ID NO: 145, SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145.
3. The use of claim 2 or 3, wherein the human Ins protein comprises a signal peptide.
4. The use of claim 3 wherein the signal peptide is a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence.
5. The use of any one of claims 1-4, wherein the polynucleotide, the expression cassette, the vector, or the rAAV particle further comprises a heterologous expression control sequence operably linked to the nucleotide sequence encoding the human Ins protein.
6. The use of claim 5, wherein the heterologous expression control sequence is a promoter, optionally a CMV promoter.
7. The use of any one of claims 1-6, wherein the vector comprising the nucleotide sequence encoding the human Ins protein is an adeno-associated virus (AAV) vector.

8. The use of any one of claims 1-7, wherein the encoded human Gck protein comprises the amino acid sequence of SEQ ID NO: 82.
9. The use of any one of claims 1-8, wherein the polynucleotide, the expression cassette, the vector, or the rAAV particle comprising the nucleotide sequence encoding the human Gck comprises a sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of SEQ ID NO: 20-39 or any combination thereof.
10. The use of any one of claims 1-9, wherein the polynucleotide, the expression cassette, the vector, or the rAAV particle further comprises a heterologous expression control sequence operably linked to the nucleotide sequence encoding the human Gck protein.
11. The use of claim 10, wherein the heterologous expression control sequence is a promoter, optionally a CMV promoter.
12. The use of any one of claims 1-11, wherein (i) the polynucleotide, the expression cassette, the vector, or the rAAV particle comprising the nucleotide sequence encoding the human Ins protein is at least 95%, 99%, or 100% identical to SEQ ID NO: 121; and (ii) the polynucleotide, an expression cassette, a vector, or a rAAV particle comprising the nucleotide sequence encoding the human Gck protein is at least 95%, 99%, or 100% identical to SEQ ID NO: 68.
13. The use of any one of claims 1-12, wherein the AAV serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVRH8, AAVrh9, AAV9, AAVrh10, AAV10, AAVRH10, AAV11, AAV12.
14. The use of any one of claims 1-13, wherein the AAV serotype of the AAV particles is AAV1.
15. The use of any one of claims 1-14, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).
- 4.16. A polynucleotide encoding a human insulin (Ins) protein 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 furin cleavage site.

- 2.17. 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%, 90%, 95%, 99%, or 100% identical to any one of: (a) nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, 150-151, 154-155 or 157-159, 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 (b) SEQ ID NO: 43-57, SEQ ID NO: 110-122, or SEQ ID NO: 150-159.
- 3.18. The polynucleotide of claim 2.17, wherein 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, amino acids 25-110 of SEQ ID NO: 145, SEQ ID NO: 41, SEQ ID NO: 144, or SEQ ID NO: 145.
4. ~~The polynucleotide claim 2.17 or 3.18, wherein the human Ins protein comprises a signal peptide.~~
- 5.19. ~~The polynucleotide of any of claims 1 or 4 wherein, optionally~~ the signal peptide is a wild-type preproinsulin signal sequence, an IL-6 signal sequence, or a fibronectin signal sequence.
- 6.20. The polynucleotide of claim 5.19, wherein the signal peptide comprises amino acids ~~25-110~~1-24 of SEQ ID NO: 41, ~~amino acids 25-110 of~~ SEQ ID NO: ~~144~~166, or ~~amino acids 25-110 of~~ SEQ ID NO: ~~145~~167.
- 7.21. The polynucleotide of any of claims ~~1-6~~17-20, wherein the human Ins protein further comprises a cleavage site.

- ~~8. The polynucleotide of any of claims 1-7, wherein the nucleic acid 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.~~
- ~~9.22. The polynucleotide of any of claims 1-817-21, wherein 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, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.~~
- ~~10. The polynucleotide or expression cassette of any of claims 1-9, wherein 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, or SEQ ID NO: 101, or SEQ ID NO: 149.~~
- ~~11. The polynucleotide of any of claims 1-10, wherein the 3' UTR further 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.~~
- ~~12. The polynucleotide of any one of claims 1-11, which comprises at least two open reading frames (ORFs) that encode human Ins protein.~~
- ~~13. The polynucleotide of claim 12, wherein the at least two ORFs are operably linked by an IRES sequence.~~
- ~~14. The polynucleotide of claim 13, wherein the IRES sequence comprises a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to SEQ ID NO: 142 or SEQ ID NO: 143.~~
- ~~15. The polynucleotide of any of claims 1-10, wherein the nucleic acid comprises:
(a) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of SEQ ID NO: 1-16 or any combination thereof.~~
- ~~16. The polynucleotide of any one of claims 1-11, wherein the nucleic acid comprises:
(a) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of SEQ ID NO: 84-88 or any combination thereof.~~
- ~~17. The polynucleotide of any of claims 12-14, wherein the nucleic acid comprises:~~

~~(a) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of SEQ ID NO: 124-126, 130-132, and 139-141 or any combination thereof.~~

~~18.23.~~ The polynucleotide of any of claims ~~1-17-22~~, wherein the nucleic acid is operably linked to a promoter.

~~19.~~—An expression cassette comprising the polynucleotide of any one of claims ~~1-18~~ and a heterologous expression control sequence operably linked to the nucleic acid sequence.
The

~~20.~~—The polynucleotide or the expression cassette of claim ~~19~~, ~~wherein the heterologous expression control sequence is a promoter.~~

~~21.24.~~ ~~The polynucleotide or the expression cassette of claim 2023~~, wherein the promoter is a eukaryotic promoter.

~~22.25.~~ The polynucleotide or expression cassette of claim ~~2124~~, wherein the promoter is a CMV promoter.

~~23.26.~~ The polynucleotide or expression cassette of any of the previous claims, wherein the nucleic acid is operably linked to a polyadenylation (polyA) element.

~~24.27.~~ A vector comprising the polynucleotide or expression cassette of any of the previous claims, wherein the vector is a viral vector, a non-viral vector, a plasmid, a lipid, or a lysosome.

~~25.~~—The vector of claim ~~24~~, ~~wherein the vector is a viral vector, a non-viral vector, a plasmid, a lipid, or a lysosome.~~

~~26.28.~~ ~~The vector of claim 2427~~, wherein the vector is an adeno-associated virus (AAV) vector or a Lentivirus vector.

~~27.29.~~ A recombinant AAV (rAAV) particle, comprising an AAV capsid and a vector genome comprising the polynucleotide or the expression cassette of any of claims ~~1-2317-26~~.

- ~~28.30.~~ The vector or rAAV particle of claim ~~2628~~ or ~~2729~~, wherein the AAV serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVRH8, AAVrh9, AAV9, AAVrh10, AAV10, AAVRH10, AAV11, AAV12.
- ~~29.31.~~ The polynucleotide, the expression cassette, the vector, or the rAAV particle of any of the previous claims, wherein the signal peptide is an IL-6 signal peptide or a fibronectin signal peptide.
- ~~30.32.~~ A host cell comprising the polynucleotide, the expression cassette, the vector, or the rAAV particle of any of the previous claims.
- ~~31.33.~~ The host cell of claim ~~3032~~, wherein the host cell is a mammalian cell.
- ~~32.34.~~ A method of producing human Ins protein in a cell, comprising contacting and/or transforming the cell with the polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims ~~1-2917-31~~, thereby producing the human Ins protein in the cell.
- ~~33. — A method of producing human Ins protein in a subject, comprising administering to the subject the~~The polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims ~~1-29, thereby producing the human Ins protein17-31 for use~~ in the ~~subject.~~
- ~~34.35. A methodtreatment of treating or ameliorating the symptoms associated with diabetes in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of the subject the polynucleotide, the expression cassette, the vector, or the rAAV particle of any of any of claims 1-29, thereby treating diabetes in the subject.~~
- ~~35. — The method of claim 34, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).~~
36. A polynucleotide comprising a nucleic acid encoding a human glucokinase (Gck) protein, wherein the nucleic acid comprises an ORF comprising:
- (a) 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 (b) SEQ ID NO: 61-80 and 162, or any combination thereof.

37. The polynucleotide of ~~claims~~claim 36, wherein the encoded human Gck protein comprises the amino acid sequence of SEQ ID NO: 82.
38. The polynucleotide of claim 36 or claim 37, wherein the nucleic acid 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.
39. The polynucleotide of any of claims 36-38, wherein 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, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.
40. The polynucleotide of any of claims 36-39, wherein 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: 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, or SEQ ID NO: 149.
41. The polynucleotide of any of claims 36-40, wherein the ~~3' UTR further 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 *Pae*I.~~nucleic acid comprises:
- ~~42. The polynucleotide of any of claims 36-40, wherein the nucleic acid comprises:~~
- (a) a nucleotide sequence at least 85%, 90%, 95%, 99%, or 100% identical to a sequence selected from any of SEQ ID NO: 20-39 or any combination thereof.
- ~~43. The nucleotide of any one of claims 36-40, wherein the nucleic acid comprises~~
- ~~(a) a nucleotide sequence at least 85%, 90%, 95%, 99%, The polynucleotide or 100% identical to a sequence selected from any of SEQ ID NO: 89-96 and 163-164 or any combination thereof.~~
- ~~44.~~42. The polynucleotide~~expresson cassette~~ of any of claims 36-~~43~~41, wherein the nucleic acid is operably linked to a promoter.
- ~~45. An expression cassette comprising the polynucleotide of any one of claims 36-44 and a heterologous expression control sequence operably linked to the nucleic acid sequence.~~

- ~~46. The polynucleotide or the~~ expression cassette of claim ~~45, wherein the heterologous expression control sequence is a promoter.~~
- ~~47. The polynucleotide or the expression cassette of claim 46~~42, wherein the promoter is a eukaryotic promoter.
- ~~48.43. The polynucleotide or expression cassette of claim 46, wherein the promoter is, optionally~~
a CMV promoter.
- ~~49.44. The polynucleotide or expression cassette of any of claims 36-48~~43, wherein the nucleic acid is operably linked to a polyadenylation (polyA) element.
- ~~50.45. A vector comprising the polynucleotide or expression cassette of any of claims 36-49~~44,
~~wherein the vector is a viral vector, a non-viral vector, a plasmid, a lipid, or a lysosome.~~
- ~~51. The vector of claim 50, wherein the vector is a viral vector, a non-viral vector, a plasmid, a lipid, or a lysosome.~~
- ~~52.46. The vector of claim 51~~45, wherein the vector is an adeno-associated virus (AAV) vector
~~or a Lentivirus vector.~~
- ~~53.47. A recombinant AAV (rAAV) particle, comprising an AAV capsid and a vector genome comprising the polynucleotide or the expression cassette of any of claims 36-49~~46.
- ~~54.48. The vector or rAAV particle of claim 52~~46 or ~~53~~47, wherein the AAV serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVRH8, AAVrh9, AAV9, AAVrh10, and AAV10, AAV11, AAV12.
- ~~55.49. A host cell comprising the polynucleotide, the expression cassette, the vector, or the rAAV particle of any of claims 36-54~~48.
- ~~56.50. The host cell of claim 55~~49, wherein the host cell is a mammalian cell.
- ~~57.51. A method of producing human Gck protein in a cell, comprising contacting and/or transforming the cell with the polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims 36-54~~48, thereby producing the human Gck protein in the cell.

~~58. — A method of producing human Gck protein in a subject, comprising administering to the subject the~~The polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims 36-~~54~~, thereby producing the human Gck protein~~48 for use~~ in the subject.

~~59.52. A method~~treatment of ~~treating or ameliorating the symptoms associate with diabetes in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of the subject the~~ polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims 36-~~54~~, thereby treating diabetes in the subject.

~~60. — The method of claim 59, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).~~

~~61. — A method of treating or ameliorating the symptoms associate with diabetes in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of (i) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims 1-29 and (ii) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims 36-54, thereby treating diabetes in the subject.~~

~~62.53. A methods of producing human Ins protein and human Gck protein in a subject in need thereof and/or treating or ameliorating the symptoms associate with diabetes in a subject in need thereof comprising administering to the subject a plurality of polynucleotides, a plurality of expression cassettes, a plurality of vectors, or a plurality of rAAV particles comprising~~Use of the (i) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims ~~1-29~~17-31 and/or (ii) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims 36-~~54~~, thereby producing human Ins protein and human Gck protein and/or treating diabetes in the subject.~~48, in the manufacture of a medicament for the treatment of diabetes.~~

~~63. — The method of claim 61 or 62, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).~~

~~64.54. The method of any of claims 61-63, wherein~~ Use of the (i) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims ~~1-29~~17-31 and (ii) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any

of claims ~~36-54~~ are administered simultaneously or sequentially, ~~48~~, in the manufacture of a medicament for the treatment of diabetes.

~~55. The method~~ Use of the polynucleotide, the expression cassette, the vector, or rAAV particle of claim 53 or 54, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).

~~56. Use of the polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims 32-35, 57-60, 53-55, wherein (i) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims 17-31 and 61-64, wherein the delivery~~ (ii) the polynucleotide, the expression cassette, the vector, or rAAV particle of any of any of claims 36-48 are to be administered simultaneously or sequentially.

~~65.57. Use of the polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims 35 and/or 52-56, wherein the~~ administration is intramuscular.

~~66.58. The method~~ Use of the polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims ~~32-35, 57-60, and 61-65~~ 52-57, wherein (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, or (iv) any combination thereof.