

We claim:

1. A fusion protein comprising an insulin polypeptide and an Fc fragment, wherein the insulin polypeptide and the Fc fragment are connected by a linker, wherein the fusion protein comprises the following sequence:

FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYCGGGGGA
GGGGAGGGGAGGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 87).

2. A fusion protein comprising an insulin polypeptide and an Fc fragment, wherein the insulin polypeptide and the Fc fragment are connected by a linker, wherein the Fc fragment is of human origin and comprises the following sequence:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYX₁STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 77) wherein X₁ is S, D, A, or R,
wherein the insulin polypeptide consists of an insulin B-chain analog linked to an insulin A-chain analog
via a C-chain, wherein the 16th amino acid from the N-terminus of the insulin B-chain analog (i.e., B16) of
the insulin polypeptide is alanine (i.e., B16A).

3. The fusion protein of claim 2, wherein the insulin polypeptide comprises the following sequence:

FVNQHLCGSX₁LVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCX₂STCSLDQLENYC (SEQ ID
NO: 9), wherein X₁ is not D, and X₂ is not H.

4. The fusion protein of claim 3, wherein the insulin polypeptide comprises the following sequence:

FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYC (SEQ ID
NO: 10).

5. The fusion protein of claim 2, wherein the linker comprises the following sequence:

GGGGGAGGGGAGGGGAGGGGG (SEQ ID NO: 67).

6. The fusion protein of claim 2, wherein the linker comprises the following sequence:

GGGGGQGGGGQGGGGQGGGGG (SEQ ID NO: 13).

7. The fusion protein of claim 6, wherein the fusion protein comprises the following sequence:

FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYCGGGGGQ
GGGGQGGGGQGGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 78).

8. The fusion protein of claim 6, wherein the fusion protein comprises the following sequence:
 FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYCGGGGGQ
 GGGGQGGGGQGGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYDSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 80).
9. The fusion protein of claim 6, wherein the fusion protein comprises the following sequence:
 FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYCGGGGGQ
 GGGGQGGGGQGGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 82).
10. The fusion protein of claim 6, wherein the fusion protein comprises the following sequence:
 FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYCGGGGGQ
 GGGGQGGGGQGGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
 PEVKFNWYVDGVEVHNAKTKPREEQYRSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
 SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 84).
11. The fusion protein of claim 2, wherein the linker comprises the following sequence:
 GGGGAGGGG (SEQ ID NO: 11)
12. The fusion protein of claim 11, wherein the fusion protein comprises the following sequence:
 FVNQHLCGSHLVEALALVCGERGFHYGGGGGGSGGGGGIVEQCCTSTCSLDQLENYCGGGGAG
 GGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
 VEVHNAKTKPREEQYSSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 89).
13. The fusion protein of claim 2 comprising domains in the following orientation from N- to C- terminus:
 (N-terminus)—insulin polypeptide—linker—Fc fragment—(C-terminus).
14. The fusion protein of claim 2, wherein the fusion protein is a homodimer.
15. The fusion protein of any of claim 1, claim 7, claim 8, claim 9, claim 10, of claim 12, wherein the fusion protein is a homodimer.
16. The fusion protein of claim 14 or claim 15, wherein the percentage homodimer of the fusion protein is greater than 90%.

17. The fusion protein of claim 14 or claim 15, wherein the fusion protein is made using one of HEK293 cells or CHO cells, and the resulting homodimer titer after purification using Protein A beads or a Protein A column is greater than 150 mg/L.
18. The fusion protein of claim 14 or claim 15, wherein the insulin receptor IC50 for the fusion protein is less than or equal to 5000 nM.
19. The fusion protein of claim 14 or 15, wherein the insulin receptor IC50 for the fusion protein is less than or equal to 2400 nM.
20. The fusion protein of claim 14 or 15, wherein the human FcRn receptor EC50 for the fusion protein is less than or equal to 1000 ng/mL.
21. The fusion protein of claim 14 or 15, wherein the human Fc(gamma)RI receptor assay OD450 Ratio at a biotinylated-Fc(gamma)RI concentration of 3000 ng/mL of the fusion protein is less than or equal to 0.50.
22. The fusion protein of claim 14 or 15, wherein the human C1q assay OD450 Ratio at a biotinylated-C1q concentration of 1000 ng/mL is less than or equal to 0.35.
23. The fusion protein of claim 14 or 15, wherein the fusion protein is formulated as a pharmaceutical composition.
24. The pharmaceutical composition of claim 23, wherein the fusion protein is present in the pharmaceutical composition at a concentration of about 3 mg/mL or greater.
25. The pharmaceutical composition of claim 23, wherein the composition is suitable for subcutaneous administration.
26. A method for lowering the blood glucose level of a patient, the method comprising administering a physiologically effective amount of the fusion protein of claim 14 or 15 or a pharmaceutical composition thereof to the patient.
27. The method of claim 26 in which the patient is diagnosed with diabetes.
28. The method of claim 26, wherein the fusion protein is administered subcutaneously.
29. The method of claim 26, wherein the fusion protein is administered daily, twice weekly, or once weekly to the patient.
30. The method of claim 26, wherein the fusion protein is administered once weekly to the patient at a dose between 0.025 and 0.5 mg/kg/week.
31. A cell engineered to express the fusion protein of any of claim 1, claim 7, claim 8, claim 9, claim 10, of claim 12.
32. The cell of claim 31, wherein the cell is transfected with a nucleic acid encoding the fusion protein.
33. The cell of claim 31, wherein the cell is a HEK293 cell or a CHO cell.
34. A cDNA encoding the fusion protein of claim 2.
35. A cDNA encoding the fusion protein of SEQ ID NO: 87 comprising the following nucleic acid

sequence:

atggaatggagctgggtctttcttcttctgtcagtaacgactggtgtccactccttctgaaccagcacctgtgcggctcccacctgggtggaagctctggc
actcgtgtgcggcgagcggggcttccactacgggggtggcggaggaggttctggtggcggcgaggcatcgtggaacagtgtgcacctccacctgct
ccctggaccagctggaaaactactgcggtggcggaggtggtcaggaggcgggtggagccggtggaggtggggctggaggaggcgggggagacaa
aactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttcttcttccccccaaaaccaaggacacctcatgatctcccg
accctgaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagtcaactgttacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgaggaggagcagtagcagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagt
caaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccat
ccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggttctatcccagcgacatcgccgtggagtgggagagcaatgggcag
ccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctctacagcaagctaccgtggacaagagcaggtggcagcag
gggaacgttctctatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggttag (SEQ ID NO: 88).

36. A cDNA encoding the fusion protein of SEQ ID NO: 78 comprising the following nucleic acid sequence:

atggaatggagctgggtctttcttcttctgtcagtaacgactggtgtccactccttctgaaccagcacctgtgcggctcccacctgggtggaagctctggc
actcgtgtgcggcgagcggggcttccactacgggggtggcggaggaggttctggtggcggcgaggcatcgtggaacagtgtgcacctccacctgct
ccctggaccagctggaaaactactgcggtggcggaggtggtcaaggaggcgggtggacagggtggaggtgggcaggaggaggcgggggagacaa
aactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttcttcttccccccaaaaccaaggacacctcatgatctcccg
accctgaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagtcaactgttacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgaggaggagcagtagcagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagt
caaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccat
ccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggttctatcccagcgacatcgccgtggagtgggagagcaatgggcag
ccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctctacagcaagctaccgtggacaagagcaggtggcagcag
gggaacgttctctatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggttag (SEQ ID NO: 79).

37. A cDNA encoding the fusion protein of SEQ ID NO: 80 comprising the following nucleic acid sequence:

atggaatggagctgggtctttcttcttctgtcagtaacgactggtgtccactccttctgaaccagcacctgtgcggctcccacctgggtggaagctctggc
actcgtgtgcggcgagcggggcttccactacgggggtggcggaggaggttctggtggcggcgaggcatcgtggaacagtgtgcacctccacctgct
ccctggaccagctggaaaactactgcggtggcggaggtggtcaaggaggcgggtggacagggtggaggtgggcaggaggaggcgggggagacaa
aactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttcttcttccccccaaaaccaaggacacctcatgatctcccg
accctgaggtcacatgcgtggtggtggacgtgagccacgaagacctgaggtcaagtcaactgttacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgaggaggagcagtagcagcacgtaccgtgtggtcagcgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagt
caaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacacctgccccat
ccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggttctatcccagcgacatcgccgtggagtgggagagcaatgggcag
ccggagaacaactacaagaccacgctcccgtgctggactccgacggctccttcttctctacagcaagctaccgtggacaagagcaggtggcagcag
gggaacgttctctatgctccgtgatgcatgaggtctgcacaaccactacacgcagaagagcctctccctgtctccgggttag (SEQ ID NO: 81).

38. A cDNA encoding the fusion protein of SEQ ID NO: 82 comprising the following nucleic acid sequence:

atggaatggagctgggtctttcttcttctgtcagtaacgactggtgtccactccttctgtgaaccagcacctgtgcggctcccacctggtggaagctctggc
actcgtgtgcggcgagcggggcttccactacgggggtggcggaggaggttctggtggcggcgaggcatcgtggaacagtgtgcacctccacctgct
ccctggaccagctggaaaactactgcggtggcggaggtggtcaaggaggcgggtggacagggtggaggtgggcaggaggaggcgggggagacaa
aactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtcttcttccccccaaaaccaaggacaccctcatgatctccgg
accctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgggaggagcagtagccagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagt
caaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccat
ccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcag
ccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagtcaccgtggacaagagcaggtggcagcag
gggaacgttctcatgtccgtgatgatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggttag (SEQ ID NO: 83).

39. A cDNA encoding the fusion protein of SEQ ID NO: 84 comprising the following nucleic acid sequence:

atggaatggagctgggtctttcttcttctgtcagtaacgactggtgtccactccttctgtgaaccagcacctgtgcggctcccacctggtggaagctctggc
actcgtgtgcggcgagcggggcttccactacgggggtggcggaggaggttctggtggcggcgaggcatcgtggaacagtgtgcacctccacctgct
ccctggaccagctggaaaactactgcggtggcggaggtggtcaaggaggcgggtggacagggtggaggtgggcaggaggaggcgggggagacaa
aactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagtcttcttccccccaaaaccaaggacaccctcatgatctccgg
accctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaag
acaaagccgcgggaggagcagtagacaagcagtagcctgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagt
caaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccat
ccgggatgagctgaccaagaaccaggtcagcctgacctgctggtcaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcag
ccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttctctacagcaagtcaccgtggacaagagcaggtggcagcag
gggaacgttctcatgtccgtgatgatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggttag (SEQ ID NO: 85).

40. A cDNA encoding the fusion protein of SEQ ID NO: 89 comprising the following nucleic acid sequence:

atggaatggagctgggtctttcttcttctgtcagtaacgactggtgtccactccttctgtgaaccagcacctgtgcggctcccacctggtggaagctctggc
actcgtgtgcggcgagcggggcttccactacgggggtggcggaggaggttctggtggcggcgaggcatcgtggaacagtgtgcacctccacctgct
ccctggaccagctggaaaactactgcggtggcggaggtgccggaggcgggggagacaaaactcacacatgccaccgtgccagcacctgaactcct
ggggggaccgtcagtcttcttccccccaaaaccaaggacaccctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtgagccac
gaagaccctgaggtcaagtcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagcagtagacagcagcagtagc
gtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtctccaacaaagccctcccagccccatcgaga
aaaccatctccaaagccaaaggcagccccgagaaccacaggtgtacacctgccccatcccgggatgagctgaccaagaaccaggtcagcctgac
ctgcctggtcaaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctgg
actccgacggctccttcttctctacagcaagtcaccgtggacaagagcaggtggcagcaggggaacgttctctcatgtccgtgatgatgaggctctg
cacaaccactacacgcagaagagcctctccctgtctccgggttag (SEQ ID NO: 90).